Rhenium and Technetium-oxo Complexes with Thioamide Derivatives of Pyridylhydrazine Bifunctional Chelators Conjugated to the Tumour Targeting Peptides Octreotate and Cyclic-RGDfK

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ABSTRACT: This research aimed to develop new tumor targeted theranostic agents taking advantage of the similarities in coordination chemistry between technetium and rhenium. A γ-emitting radioactive isotope of technetium is commonly used in diagnostic imaging, and there are two β− emitting radioactive isotopes of rhenium that have the potential to be of use in radiotherapy. Variants of the 6-hydrazinonicotinamide (HYNIC) bifunctional ligands have been prepared by appending thioamide functional groups to 6-hydrazinonicotinamide to form pyridylisoemicarbazide ligands (SHYNIC). The new bidentate ligands were conjugated to the tumor targeting peptides Tyr3-octreotate and cyclic-RGD. The new ligands and conjugates were used to prepare well-defined {M═O}3+ complexes (where $M = {^{99m}Tc}$ or natRe or $^{188}$Re) that feature two targeting peptides attached to the single metal ion. These new SHYNIC ligands are capable of forming well-defined rhenium and technetium complexes and offer the possibility of using the $^{99m}$Tc imaging and $^{188}$/186Re therapeutic matched pairs.

INTRODUCTION

The technetium-99m isotope has excellent properties for detection with single photon emission computed tomography (SPECT) due to its low energy and nonparticulate gamma-ray emission ($t_{1/2} = 6.01$ h, $E_{max} = 141$ keV γ-ray emission, $\lambda < 10$ pm). Despite recent concerns over production related shortages and the Tumour Targeting Peptides Octreotate and Cyclic-RGDfK Derivatives of Pyridylhydrazine Bifunctional Chelators Conjugated to the single metal ion. These new SHYNIC ligands are capable of forming well-defined rhenium and technetium complexes and offer the possibility of using the $^{99m}$Tc imaging and $^{188}$/186Re therapeutic matched pairs.

and therapeutic ($^{186}$/188Re) matched pair using a single targeted ligand to form essentially isostructural complexes.2−6

One approach to targeted imaging and therapy is to incorporate appropriate metal radionuclides into coordination complexes that are attached to biological targeting vectors such as tumor targeting peptides, antibodies or antibody fragments. Peptides that feature the -RGD- (arginine-glycine-aspartic acid) fibronectin fragment such as the cyclic-RGDfK pentapeptide (cRGDK) bind to $\alpha_v\beta_3$ integrin receptors that are overexpressed in certain invasive tumors including osteosarcomas, glioblastoma, melanomas, and breast cancer, and can be used to selectively target tumor cells.7−16 Metabolically stabilized somatostatin analogues such as octreotide and octreotate bind to somatostatin subtype 2 receptors (sstr2) that are overexpressed in many types of neuroendocrine tumors compared to relatively low levels of expression in other tissues and organs.17−21

Tumor targeting technetium based imaging agents can be prepared using 6-hydrazinonicotinamide (HYNIC) derivatives conjugated to targeting molecules as ligands to form...
the technetium(III) diazenido complexes. The HYNIC ligand forms remarkably stable technetium complexes, and manipulation of the carboxylate functional group to attach a variety of targeting molecules is generally straightforward. HYNIC binds to technetium through the terminal hydrazide nitrogen but probably forms bidentate complexes through coordination to the pyridyl nitrogen.32,33 In all the crystallographically characterized technetium and rhenium complexes with one or more HYNIC-like ligands, such as 2-hydrazinopyridine, the pyridyl nitrogen is also coordinated to the metal center.33 A variety of coligands such as tricine, nicotinic acid, EDDA, and phosphines (TPPTS, TPPDS, TPPMS = tri/bi/sodium triphenylphosphine tri/di/monosulfonate) are required to complete the coordination sphere and stabilize the metal oxidation state, and this leads to a high degree of uncertainty in the exact nature of the primary coordination sphere as well as challenges in ensuring structural homogeneity in the formulated product. Variation of the coligand can modify the in vivo metabolism and excretion.34 A well-established “ternary ligand system” involves combining the HYNIC ligand with tetradentate tricine and monodentate trisodium 3,3′,3″-phosphanetriyltris(benzenesulfonate) (TPPTS) coligands, but the possibility of forming multiple isomers adds complications.33,35−39

Despite the superficial similarities in coordination chemistry between technetium and rhenium extrapolation of the HYNIC strategy to radioactive rhenium isotopes is challenging presumably due to their differences in kinetic lability and redox chemistry.33 Some of the difficulty in isolating pure Re-HYNIC-peptide conjugates can be understood by considering the reaction of [ReOCl₃(PPh₃)₂] o r [ReOCl₄] with the two equivalents of ligand (Scheme 1). This reaction results in relatively complex coordination chemistry due to the ability of the pyridylhydrazone derived ligands to coordinate as either monodentate or bidentate ligands and the existence of protic equilibria as well as the formation of complexes where two pyridylhydrazone derived units are coordinated to the rhenium (Figure 1).40−43

Modification of the terminal hydrazine nitrogen of hydrazinopyridine to incorporate an additional thioureia functional group results in a ligand system that is capable of forming well-defined, very stable complexes with [ReOCl₃(PPh₃)₂] o r [ReOCl₄] while retaining the bioconjugation possibilities well established for HYNIC.44,45 A preliminary communication reported the structural characterization of a ReOCl₃(PPh₃)₂ complex featuring two pyridylphenylthiocarbazide (SHYNIC) ligands (Figure 1).44 In this manuscript we extend this concept by synthesizing a family of different substituted pyridylthiocarbazide ligands with carboxylate or ester functional groups that were used to tether octreotride and cyclic-RGD peptides to the ligands. The new ligands were used to prepare {MO}³⁺ complexes (where M = Tc or Re) that feature two targeting peptides attached to the single metal ion. These modified HYNIC ligands are capable of forming well-defined rhenium and technetium complexes and offer the possibility of using the two radionuclides as imaging and therapeutic matched pairs.

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**RESULTS AND DISCUSSION**

**Synthesis of H₄L₁−₄, and Their Ester Derivatives, H₄L₁−₄(OMe) and [ReOCl₄]⁻ Complexes.** Synthesis of 6-hydrazinonicotinic acid (HYNIC), 2, required treatment of 6-chloronicotinic acid (1) with aqueous hydrazine. Ligands H₄L₁ to H₄L₄ were prepared by reaction of either the ethyl, tert-buty l, phenyl, or nitrophenyl isothiocyanate with 6-hydrazinonicotinic acid (1) in anhydrous N,N-dimethylacetamide (DMA) (Scheme 1).

The rhenium complexes of the methyl ester derivatives of H₄L₁−₃ complexes, [ReO(HL₁−₃(OMe))₂]⁺, can be prepared by reaction of the either trans-[ReOCl₃(PPh₃)₂] or [Bu₄N]-[ReOCl₄] with the two equivalents of ligand (Scheme 2). The IR spectra for the three complexes, [ReO(H₄L₁−₃(OMe))₂]⁺, display medium intensity bands at ν 960−963 cm⁻¹ characteristic of Re=O stretches.46 Bands, which occur between ν 1553 and 1557 cm⁻¹ due to carbonyl stretching of the ester functional group, shift approximately 150 cm⁻¹ lower in energy when compared to the metal-free ligands.

Analysis of the complexes by ¹H NMR data reveals that the two coordinated ligands are magnetically equivalent, with three resonances at δ 8.63, 8.25, and 7.86 ppm corresponding to the six pyridinyl CH protons for [ReO(H₄L₁(OMe))₂]⁺, and similar resonances for the phenyl and tert-butyl derivatives. The pyridine proton which is closest to the rhenium ion shifts from δ 6.55 ppm in free ligand to δ 7.86 ppm in the complex. The methyl ester functional group gives rise to singlets at δ 3.89 (DMSO-d₆), 3.86 (CHCl₃-d₂), and 3.92 (DMSO-d₆) for complexes [ReO((HL₁−₃(OMe))₂]⁺ respectively. The [ReO(H₄L₁(OMe))₂]⁺ complex was stable to cysteine and histidine challenge experiments with very little decomposition evident (<5%), as detected by analysis by HPLC and UV/vis spectroscopy, when incubated at 37 °C for 24 h in the presence of a 100-fold excess of cysteine and histidine.

Red crystals of [ReO(H₄L₁(OMe))₂]CF₃CO₂ suitable for X-ray crystallographic analysis were obtained by evaporation of a solution of the compound that had been purified by semipreparative HPLC using an aqueous/CH₃CN mobile phase with 0.1% trifluoroacetic acid (Figure 2a). The compound crystallizes in the triclinic space group, P1, and the rhenium ion is in a distorted square pyramidal environment with the oxo group in the apical position relative to the pseudo basal plane of two five-membered chelate rings. Each thiacarbonylhydrazide functional group is doubly deprotonated and serves as a

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Figure 1. (a) 6-Hydrazinonicotinic acid. (b) Metal complex (M = Tc or Re) with 6-hydrazinonicotinimide (HYNIC) acting as a monodentate ligand. It is necessary to complete the coordination sphere with coligands (L). (c) Metal complex (M = Tc or Re) with 6-hydrazinonicotinimide (HYNIC) acting as a bidentate ligand. (d) Pyridylphenylthiocarbazide (SHYNIC) ligand. (e) Re⁵⁺-oxo complex featuring two SHYNIC ligands.
The Re-O bond distance (1.679(3) Å) is typical for a five-coordinate, rhenium(V)-monoxo complex and consistent with IR spectroscopy (Re=O, ν = 963 cm⁻¹). The N2–C6 and N6–C14 bond lengths (1.287(6) Å) are significantly shorter than the other bonds within the chelate ring suggesting significant sp² hybridized C=N bond character. The Re=N bond distances average 2.05 Å and are marginally shorter than typical Re=N bonds (ca. 2.15–2.18 Å) suggesting some degree of multiple bond character. The Re−N1−N2 and Re−N5−N6 bond angles average 124°, suggesting an approximately sp² hybridized nitrogen. The Re−S bond distances average 2.29 Å and are similar to the Re=S bond distances in rhenium(V) complexes with amino thiolate ligands and Re=S bond distances in rhenium complexes with thiosemicarbazone ligands.

The potential of the substituted pyridylthiosemicarbazide (SHYNIC) ligands H₂L₁ to be modified with amino acids using standard solid phase peptide synthesis techniques was first accomplished by attaching L-lysine to H₂L₁ to give H₂L₁(Lys). The doubly N-protected lysine derivative, N₆-Fmoc-L-Lys, was immobilized on chlorotrityl resin, and the N₆-Fmoc group was removed by treatment with piperidine. The ligand, H₂L₁, was added to the resin in a mixture of DMF followed by the coupling agent HATU (HATU = 1H-1,2,3-triazolo[4,5-b]-pyridinium-3-oxid hexafluorophosphate) in the presence of N,N-diisopropylethylamine (DIPEA). The product was cleaved from the resin using trifluoroacetic acid that also resulted in the deprotection of the N₆-Fmoc group (Scheme 3). This lysine amino acid conjugate provides an amino acid with an appended chelator, which with appropriate protecting groups, could be incorporated into biological targeting molecules with total site specificity via solid-phase peptide synthesis.

The rhenium complex, [ReO(HL₁(Lys))₂]⁺, was prepared by adding either trans-[ReOCl₃(PPh₃)$_2$] or [BuN][ReOCl₄] suspended in DMF to the reaction mixture, while the ligand remained immobilized on the resin. Performing the complexation while the ligand remained immobilized on the resin and with the amino group still protected ensured that the functional groups of the lysine did not complicate the coordination chemistry. When green trans-[ReOCl₄(PPh₃)$_2$] is used as the starting material, the green colored suspension gradually changes to colorless, and the resin beads turn dark red indicative of the formation of [ReO(HL₁(Lys))]²⁺. After being stirred at room temperature, the resin was washed with dimethylformamide and dichloromethane and cleaved off the resin with a 10% trifluoroacetic acid/dichloromethane mixture (Scheme 3). Analysis of [ReO(HL₁(Lys))]²⁺ complex by electrospray ionization mass spectrometry (ESI-MS) reveals the expected peaks. Analysis by $^1$H NMR shows the singlet attributed to the aromatic proton on the pyridine ring (pyH) shifts upon coordination to the metal center from δ 8.48 in H₂L₁(Lys) to 8.58 ppm in [ReO(HL₁(Lys))]²⁺. The four downfield $^{13}$C(H) NMR signals in [ReO(HL₁(Lys))]²⁺, 173.0 (CO₂H), 169.0 (CONH), and 163.6 (pyC), were assigned using HSQC and HMBC techniques.

**Scheme 2. Synthesis of Rhenium Complexes**

[ReO(HL₃)(OMe)]$_2$⁻

N₆-Fmoc-L-Lys, was immobilized on chlorotrityl resin, and the N₆-Fmoc group was removed by treatment with piperidine. The ligand, H₂L₁, was added to the resin in a mixture of DMF followed by the coupling agent HATU (HATU = 1H-1,2,3-triazolo[4,5-b]-pyridinium-3-oxid hexafluorophosphate) in the presence of N,N-diisopropylethylamine (DIPEA). The product was cleaved from the resin using trifluoroacetic acid that also resulted in the deprotection of the N₆-Fmoc group (Scheme 3). This lysine amino acid conjugate provides an amino acid with an appended chelator, which with appropriate protecting groups, could be incorporated into biological targeting molecules with total site specificity via solid-phase peptide synthesis.

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DMF on the solid phase, followed by cleavage from the resin and cyclization using a small modification of published procedures. The ligands, H2L1−3, were conjugated to cRGDfK using standard peptide coupling conditions (HATU, DIPEA) to give H2L1−3(cRGDfK) (Scheme 4). The new conjugates were purified by semipreparative RP-HPLC and characterized by electrospray mass spectrometry and analytical HPLC. The reaction for the ethyl-SHYNIC derivative, H2L1−3(cRGDfK), resulted in a higher isolated yield (80%) than H2L2−3(cRGDfK) (24%) and H2L3−3(cRGDfK) (55%). Analysis of aqueous solutions of H2L1−3(cRGDfK) by RP-HPLC revealed no degradation over a 24 h.

The {ReO}3+ complexes of H2L1−3(cRGDfK) were prepared by adding [ReOCl4]− in DMF at ambient temperature followed by addition of H2L1−3(cRGDfK). The triﬂuoroacetate counterion and hydrogen atoms (except those bound to nitrogen) are omitted. (b) Representation of hydrogen bonded centrosymmetric dimer with the two remaining H-bond donors capped by water molecules.

| Table 1. Selected Bond Lengths (Å) and Angles (deg) for the Rhenium Complex for [ReO(HL1(OMe))2]CF3CO2 |
|---------------------------------------------------------------|
| Bond Lengths                                                      |
| Re−O(1)                                      1.679(3)       | Re−S(1)                 2.2820(11)                    | Re−S(2)                 2.2917(11)                    |
| N(1)−C(1)                                       1.35(6)            | Re−N(1)                 2.049(4)                        | Re−N(5)                 2.050(4)                        |
| C(6)−N(3)                                        1.34(6)            | N(1)−C(1)               1.28(7)                         | N(5)−N(6)               1.41(5)                        |
| C(14)−N(7)                                       1.35(6)            | N(3)−C(6)               1.28(7)                         | N(6)−C(14)              1.28(7)                        |
| N(5)−C(9)                                        1.36(6)            | C(6)−S(1)               1.784(5)                        | C(14)−S(2)              1.769(4)                        |
| Bond Angles                                         |
| O(1)−Re−S(1)                                     115.44(11)         | N(1)−Re−S(1)            80.74(11)                       | N(5)−Re−S(2)            80.23(11)                       |
| O(1)−Re−S(2)                                     116.75(11)         | N(5)−Re−S(2)            89.71(11)                       | C(1)−N(1)−Re            125.0(3)                        |
| O(1)−Re−N(1)                                     101.73(15)         | N(5)−Re−S(1)            87.98(11)                       | C(9)−N(5)−Re            126.2(3)                        |
| O(1)−Re−N(5)                                     102.61(15)         | N(1)−Re−S(2)            87.98(11)                       | N(1)−C(1)−N(1)−N(2)     169.5(3)                        |
| N(1)−Re−N(5)                                     155.64(15)         | C(1)−N(1)−Re            125.0(3)                        | N(2)−C(1)−N(1)−N(2)     167.1(4)                        |
| S(1)−Re−S(2)                                     127.80(4)          | C(9)−N(5)−Re            126.2(3)                        | C(10)−C(9)−N(5)−N(6)    168.4(4)                        |
| Torsion Angles                                      |
| N(4)−C(1)−N(1)−N(2)                                 | N(6)−C(14)−N(7)−C(15)  13.8(7)                           |
| N(8)−C(9)−N(5)−N(6)                                 | N(2)−C(6)−N(3)−C(7)     5.1(7)                           |
| N(4)−C(1)−N(1)−N(2)                                 | N(2)−C(6)−N(3)−C(7)     5.1(7)                           |
| N(8)−C(9)−N(5)−N(6)                                 | N(2)−C(6)−S(1)−Re       3.7(4)                           |
| N(4)−C(1)−N(1)−N(2)                                 | N(6)−C(14)−S(2)−Re      4.1(4)                           |
| C(2)−C(1)−N(1)−N(2)                                | N(3)−C(6)−S(1)−Re       -174.7(3)                       |
| C(10)−C(9)−N(5)−N(6)                                | N(7)−C(14)−S(2)−Re      -175.1(3)                       |

"Triﬂuoroacetate counterion bond lengths and angles are not provided."
by isolation and purification using semipreparative RP-HPLC (Scheme 4). Analysis of the complexes by ESI-MS revealed the 2+ molecular ion peaks at m/z 926.343, 954.372, and 974.371 for [ReO(HL1(cRGDfK))2]+, [ReO(HL2(cRGDfK))2]+, and [ReO(HL3(cRGDfK))2]+ respectively.

An intramolecular disulfide bridge between the second and seventh cysteine residues in Tyr3-octreotate improves the metabolic stability of the peptide, and this disulfide is often introduced by oxidation of the linear octapeptide with 2,2′-dithiodipyridine. Unfortunately the bioconjugation of ligands H2L1−3 to Tyr3-octreotate was complicated by degradation of the pyridylthiocarbazide (SHYNIC) ligands (H2L1−3) in the presence of the two cysteine thiol containing residues in the linear peptide, leading to loss of H2S identified in the ESI-MS by a loss of 34 atomic mass units. This loss of H2S results in the formation of a carbodiimide form of the SHYNIC ligands. The formation of carbodiimides from thioureas is well-known.65 This degradation and loss of sulfur were not observed for RGD-based conjugates, suggesting that in the case of the octreotate conjugates, thiocarbazide-thiol-disulfide interchange/scrambling promotes the loss of H2S from the ligands (Scheme 5).

As conventional off-resin oxidative cyclization methodologies were inadequate for this synthesis, H2L1−3(Tyr3-Oct) was prepared entirely on solid support, where intramolecular oxidation/cyclization preceded bioconjugation of H2L1−3. The eight-residue peptide was synthesized by sequential addition of the amino acid residues via solid-phase peptide synthesis, using acetamidomethyl (Acm) protected cysteine residues followed by in situ Acm removal and simultaneous disulfide bond formation using thallium(III) trifluoracetate.66 Following cyclization, the preactivated SHYNIC derivative (H2L1−3) is

Table 2. Summary of Crystal Data and Structure Refinement for [ReO(HL1(OMe))2]CF3CO2 a

| data collection | compound details | data collection | compound details |
|-----------------|------------------|----------------|------------------|
| empirical formula | C20H26N8O5ReS2.H2O.CF3CO2 | Z (Å³) | 1551.05(13) |
| formula weight | 839.84 | μ (mm⁻³) | 5.999 |
| crystal size (mm³) | 0.26 × 0.10 × 0.022 | Dcrys (Mg m⁻³) | 1.797 |
| crystal system | triclinic | μ (mm⁻³) | 9.599 |
| space group | P1 | F(000) | 828 |
| T (K) | 130.00(10) | reflections measured | 10363 |
| λ (Å) | 1.54184 | independent reflections | 5568 [Rint = 0.0449] |
| a (Å) | 10.9154(4) | final R indices [I > 2σ(I)] | R1 = 0.0329 |
| b (Å) | 11.1201(6) | final R indices (all data) | R1 = 0.0398 |
| c (Å) | 13.2651(7) | | wR(F²) = 0.0794 |
| α (deg) | 86.355(4) | goodness-of fit on F² | 1.027 |
| β (deg) | 74.965(4) | | wR(F²) = 0.0831 |
| γ (deg) | 87.273(4) |

“Crystals were grown from a concentrated solution of the complex in methanol.

Scheme 3. "On-Resin" Formation of H2L1(Lys) and the Corresponding Rhenium-oxo Complex, [ReO(HL1(Lys))2]+
reacted at the deprotected α-phenylalanine N-terminus. Cleavage and deprotection of remaining protecting groups are achieved by treatment with trifluoroacetic acid (Scheme 6).

The rhenium complexes of H2L1-3(Tyr3-Oct) could be prepared on-resin or in-solution by treatment with [tBu4N][ReOCl4] in methanol (Scheme 6). The on-resin approach is potentially of interest in producing radioactive complexes in high specific activity as unreacted [ReO4]− and other impurities such as colloidal rhenium could be readily removed by filtration of the resin. The pure complex can be cleaved from the resin with 50% trifluoroacetic acid and is stable to this relatively high concentration of acid. Analysis by HPLC and ESI-MS confirmed the identity of the complexes, with the [ReO-(HL1-3(Tyr3-Oct))2]+ complexes showing signals in the ESI-MS that could be attributed to the 3+ molecular ion with expected rhenium isotope peak patterns (Figure 3).

**Preparation of [188Re(HL1(Tyr3-Oct))2]3+.** Preliminary radiolabeling of H2L1(Tyr3-Oct) with radioactive 188Re was performed using generator-produced [188ReO4]−. A solution of [188ReO4]−, in an aqueous mixture of sodium chloride (0.9% w/v concentration) and sodium tartrate, was reduced with stannous chloride. This mixture was then reacted with H2L1(Tyr3-Oct) at 100 °C, leading to the formation of [188ReO(HL1(Tyr3-Oct))2]+ in ca. 67% radiochemical yield. The compound was characterized by analytical reversed phased HPLC (Figure 4), where [188ReO(HL1(Tyr3-Oct))2]+ (retention time = 11.6 min, detected using a NaI(Tl)) elutes with a similar retention time to the nonradioactive analogue [natReO-(HL1(Tyr3-Oct))2]+ (retention time = 11.3 min, detected at λ220), where natRe refers to naturally abundant Re isotopes. The small difference in retention times is due to the different configurations of the radioactivity and UV detectors. This elution profile of [ReO(HL1(Tyr3-Oct))2]+ is distinct from that of the free ligand, H2L1(Tyr3-Oct) that elutes at 9.7 min under the same conditions. Unreacted 188Re species, presumably [188ReO4]−, elute with the solvent front at 2.1 min (Figure 4).

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**Scheme 4. Synthesis of H2L1-3(cRGDfK) and [ReO(HL1-3(cRGDfK))2]+**

**Scheme 5. Suggested Mechanism for the Formation of a Carbodiimide from H2L1-3(Tyr3-Oct) Resulting in Loss of H2S**
Preparation of $^{99m}$Tc-Labeled Complexes, $[99mTcO(HL_{1-3}(cRGDK))_2]^+$ and $[99mTcO(HL_{1-3}(Tyr_3-Oct))_2]^+$. The technetium-99m complexes $[99mTcO(HL_{1-3}(cRGDK))_2]^+$ and $[99mTcO(HL_{1-3}(Tyr_3-Oct))_2]^+$ were prepared in ca. 60—80% radiocchemical yield using mild conditions and relatively simple procedures (Supporting Information, Figures S1 and S2).
Radiolabeled 99mTc complexes were characterized by analysis by butyl, and L3 (phenyl) Compounds (H

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The new pyridylthiocarbazide ligands (SHYNIC, H 2

CONCLUDING REMARKS

Table 3. RP-HPLC Retention Times (min) for Ligands and [MO(HL)2]+ Complexes (M = Re, 99mTc)

Table 3. RP-HPLC Retention Times (min) for Ligands and [MO(HL)2]+ Complexes (M = Re, 99mTc) | H2L(Tyr3-Oct) | [99mTcO(HL3)](Tyr3-Oct))2| [99mTcO(HL3)](Tyr3-Oct))2| [188ReO(HL3)](Tyr3-Oct))2| [188ReO(HL3)](Tyr3-Oct))2|
|----------------|----------------|----------------|----------------|----------------|----------------|
| compound | H2L(Tyr3-Oct) | technetium complex | technetium complex | technetium complex | technetium complex |
| L1-cRGDfK | 10.0 | 11.4 | 11.5 | 11.5 | 11.5 |
| L3-cRGDfK | 9.3 | 10.3 | 10.9 | 10.9 | 10.9 |
| L3-cRGDfK | 10.9 | 12.0 | 12.7 | 12.7 | 12.7 |
| L3-(Tyr3-Oct) | 10.1 | 11.4 | 11.9 | 11.9 | 11.9 |
| L3-(Tyr3-Oct) | 10.9 | 11.7 | 12.1 | 12.1 | 12.1 |
| L3-(Tyr3-Oct) | 11.0 | 12.9 | 13.0 | 13.0 | 13.0 |

*Linear gradient from 0 to 90% Buffer B to A. Buffer A: 0.1% TFA in Milli-Q water. Buffer B: 0.1% TFA in CH3CN.

times between the traces for 99mTc and Re complexes is, in part, due to the detector configurations but could also reflect the difference in polarity between the oxorhenium(V) and oxotechnetium(V) cores.

The stability of [99mTcO(HL3(Tyr3-Oct))2] was assessed by incubation in human plasma at 37 °C. The complex was stable for at least 2 h with only small amounts of degradation products (<5%) evident that, based on their retention times in analytical HPLC, are most likely due to degradation of the peptide.

**CONCLUDING REMARKS**

The new pyridylthiocarbazide ligands (SHYNIC, H2L1−3) described here offer a useful alternative to the standard HYNIC system. While HYNIC has proved a very successful and versatile bifunctional ligand for 99mTc coligands are required to complete the coordination sphere of the metal ion and extrapolation to radioactive rhenium isotopes has been challenging.3,63 This family of bidentate ligands form stable complexes with the [ReO3] core with two ligands coordinated to a single metal ion. A rhenium complex with a methyl ester functional group has been characterized by X-ray crystallography and features the rhenium ion in a distorted square pyramidal environment with the oxo group in the apical position relative to the pseudo basal plane of two five-membered chelate rings with a NNSSSS trans configuration about the Re-oxo core. The basic ligands have been decorated with the tumor targeting peptides cyclic-RGD and Tyr3-octreotate, and these conjugates form complexes with rhenium to give well-defined single species, [ReO((HL1−3)(cRGDfK))2] and [ReO((HL1−3)(Tyr3-octreotate))2], without having to add coligands resulting in the formation of a single structural and geometrical isomer. It is possible to form the rhenium complexes using either standard solution chemistry or "on-resin", and the latter approach may prove useful in isolating radioactive 188/186Re analogues in high specific activity. These complexes feature two targeting peptides separated by 14 chemical bonds, and there is evidence that molecules containing more than one targeting peptide, sometimes referred to as bivalent, can display enhanced receptor binding due to simultaneous binding to more than one receptor on the surface on any given cell.6,7,22 It is possible to prepare [188ReO((HL1−3)(Tyr3-Oct))2] in ~67% yield from generator produced [188ReO2], and improved yields should be possible by optimizing the reaction conditions. The analogous technetium complexes, [TcO((HL1−3)(cRGDfK))2] and [TcO((HL1−3)(Tyr3-octreotate))2], were prepared directly from [99mTcO3] with tin chloride acting as a reducing agent. Comparison of HPLC profiles suggests the rhenium and technetium complexes are isostructural. The complexes described in this manuscript have two ligands coordinated to a single metal ion, whereas conventional HYNIC systems involve one HYNIC ligand binding to one metal ion. It is likely the two different systems will exhibit quite different biodistribution in vivo. These new systems warrant further investigation as potential theranostic agents employing an imaging [99mTc] and therapeutic [186/188Re] matched pair for a single targeted agent.

**General Experimental.** All reagents were purchased from standard commercial sources. Nuclear magnetic resonance (NMR) spectra were acquired on either an Agilent 400-MR (1H NMR at 400 MHz and 13C{1H} NMR at 101 MHz) or a standard commercial sources. Nuclear magnetic resonance (NMR) spectra were acquired on either an Agilent 400-MR (1H NMR at 400 MHz and 13C{1H} NMR at 101 MHz) or a Varian FT-NMR 500 spectrometer (1H NMR at 500 MHz and 13C{1H} NMR at 126 MHz) at 298 K. Chemical shifts were referenced to residual solvent peaks and are quoted in ppm relative to TMS.

Fmoc-L-amino acids, Fmoc-D-amino acids, Nvoc-Cl, HATU, DIC, Wang resin, 2-chlorotrityl, Fmoc-Lys(ivDde)−OH, and Fmoc-Cys(Acm)−OH were purchased from standard commercial sources.

Linear protected RGDfK peptide (Arg(Pbf)-Gly(tBoc)-Asp(OtBu)-dPhe-Lys(tBoc)) was synthesized manually using standard Fmoc solid phase peptide synthesis (SPPS) procedures on the 2-chlorotrityl chloride resin. The linear pentapeptide was cleaved from the resin (with retention of protecting groups) using 1% TFA in dichloromethane and shaking for 40 min. The mixture was filtered and the filtrate was reduced in volume to afford crude linear product. Cyclization involved reacting the crude material in a mixture of dichloromethane (1 mg mL−1), HATU (0.9 equiv), and DIPEA (6 equiv) at RT for 2 h, then evaporation to dryness.
in vacuo. A solution of TFA (97.5%) and Milli-Q water (2.5%) was added to the crude material to deprotect the peptide, followed by removal of the trifluoroacetic acid by sparging with a stream of N₂. The peptide was precipitated with diethyl ether, isolated by centrifugation (3 min, 3600 rpm) and dissolved in Milli-Q water (5 mL), and finally purified by semiprep RP-HPLC (Column 1); Gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 0 to 40% B to A, over 40 min (1.0% min⁻¹), UV detection at λ 220 nm with a flow rate of 5 mL min⁻¹.

Linear protected Tyr-occteptide, dPhe-Cys(Trr)-Tyr(tBu)-dTrp(tBoc)-Lys(tBoc)-Thr(tBu)-Cys(Trr)-Thr-(tBu)-OH, was synthesized using standard automated Fmoc SPPS procedures on a 2-chorotriyl chloride or Wang resin unless otherwise specified.

Analytical reversed phase high performance liquid chromatography (RP-HPLC) was undertaken using an Agilent 1100 Series HPLC system at a flow rate of 1 mL min⁻¹ with either Column 1: A Zorbax Eclipse XDB-C18 column (150 mm × 4.6 mm, 5.0 μm) or Column 2: A Phenomenex Aeris Peptide XB-C18 column (250 mm × 4.6 mm, 3.6 μm). Solvent gradients for analytical analysis were either using System A: Gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 0 to 100% B over 25 min and UV detection at λ 214, 254, 280, and 280 nm, System B: Gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 0 to 60% B over 30 min and UV detection at λ 214, 220, 254, 280, and 350 nm or System E: Gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 20 to 80% B, over 30 min and UV detection at λ 220, 254, and 350 nm with a flow rate of 1 mL min⁻¹.

Semi preparative reversed phase high performance liquid chromatography (semiprep RP-HPLC) was performed using an Agilent 1200 series preparative HPLC unit with variable wavelength detector. An automated Agilent 1200 fraction collector collected 0.5–4 mL fractions. Peak separation was achieved using either Column 3: Kinexet C18 100 Å, AXIA column (150 mm × 21.2 mm, 5 μm), Column 4: Phenomenex Synergi Hydro-RP 80 Å (50 mm × 21.2 mm, 4 μm), Column 5: Varian Pursuit XRs C18 100 Å (150 × 21.2 mm, 5 μm) or Column 6: SGE ProteCol C18 120 Å (250 mm × 10 mm, 5 μm). Gradient elution, flow rate, and wavelength detection are compound specific and are detailed under the Experimental Section of a particular compound. Each fraction collected above 400 mA was analyzed using ESI-MS and analytical HPLC.

Analytical HPLC traces of radiolabeled ¹⁸⁶Re compounds were acquired using an Agilent 1200 LC system with in-line UV and gamma detection (Flow-Count, LabLogic). Peak separation was achieved using an Agilent Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm), with column 1 and system F: Gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 0 to 100% B over 20 min and UV detection at λ 220 nm.

Analytical HPLC traces of radiolabeled ⁹⁹ᵐTc compounds were acquired using a Shimadzu 10 AVP UV–visible spectrophotometer (Shimadzu, Kyoto, Japan) and a sodium iodide scintillation detector with two LC-10ATVP solvent delivery systems for solvents A and B. Peak separation was achieved using Column 7: Nacalai Tesque Cosomosil SC18-AR Waters column (4.6 × 150 mm, 5 μm) (Kyoto, Japan) at a flow rate of 1 mL min⁻¹. Gradient elution followed System C: Gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 0 to 100% B over 20 min and UV detection at λ 254 nm.

X-ray structure determination and refinement was obtained for [ReO(HL¹(OMe))₂]TFA on an Oxford Diffraction SuperNova CCD diffractometer using Cu–Kα radiation, and the temperature during data collection was maintained at 130.0(1) using an Oxford Cryosystems cooling device. The structure was solved by direct methods using SHELXT and refined using least-squares methods using SHELXL.⁷³,⁷⁵ Thermal ellipsoid plots were generated using ORTEP-3 integrated within the WINGX suite of programs.⁷⁶ The trifluoroacetate counterion, although recognizable from the difference electron density maps, was badly disordered and could not be modeled satisfactorily. Application of the Squeeze procedure gave a void volume of 272 Å³ containing 127 electrons, consistent with the presence of two trifluoroacetate anions per unit cell.⁷⁷ The charge on the complex is unambiguously (+1) given the presence of the two pyridinium protons which are involved in intramolecular hydrogen bonds and the ethylamino protons which are also involved in hydrogen bonds. The crystallographic data has been deposited in the Cambridge Structural Database (CCDC 1543360).

**Ligand Synthesis.** Note: The designation of H₄L¹–⁴ refers to the structure with a carboxylic acid-substituted, pyridylhydrazine with the different thiocarbahydrazide functional groups (Et, tBu, and Ph respectively). Further derivatization of the carboxylate functional group is represented by placing the substituted group at the carboxylic carbon in replacement of the OH group (e.g., H₄L¹–⁴–(OMe)) denotes the substitution of a methoxy at the carboxyl carbon to give a methyl ester.

6-[2-Ethylcarbamothioyi]hazidinyl]-3-pyrindcarboxylic acid, H₄L¹. To a suspension of 6-hydrizino-3-pyrindcarboxylic acid (0.45 g, 2.9 mmol) in anhydrous ethanol (5 mL) was added ethyl isothiocyanate (0.51 mL, 5.8 mmol) under an atmosphere of nitrogen. The suspension was heated to reflux for 16 h then cooled to RT, and the precipitate that formed was collected by filtration, washed with cold ethanol (20 mL), and diethyl ether (20 mL) to give H₄L¹ as a colorless powder (0.67 g, 95%).¹ Hydrogen NMR [DMSO-d₆, 500 MHz]: δ (ppm) 9.39 (1H, s, NH), 9.01 (1H, s, NH), 8.63 (1H, d, J = 1.7 Hz, pyH⁻), 8.19 (1H, t, J = 4.8 Hz, NH) 8.04 (1H, d, J = 8.2 Hz, pyH), 6.53 (1H, d, J = 8.8 Hz, pyH), 3.45 (2H, dt, J = 13.3, 6.8 Hz, CH₂), 1.19 (3H, t, J = 7.1 Hz, CH₃). ¹³C{¹H} NMR [DMSO-d₆, 125.7 MHz]: δ (ppm) 181.5 (C, NCS), 166.5 (C, COH), 160.2 (C, pyC), 150.4 (C, pyC), 138.8 (C, pyC), 117.8 (C, pyC), 105.6 (C, pyC), 38.4 (C, CH₃), 146.4 (C, CH₂); IR: νmax (cm⁻¹) 2981 (s/sh, N=H), 1605 (s/sh, C=O), 1539 (s/sh), 1319 (m/sh), 1282 (s/sh), 1245 (s/sh), 782 (s/sh); ESI-MS (+): m/z calc'd for C₄H₇NO₂S₂ 421.0759, found 421.0817 [M + H]⁺, 100%; RP-HPLC (Column 1, System A): Rₚ (min) 7.2.

6-[2-(tert-Butylcarbamothioyi)hazidinyl]-3-pyrindcarboxylic acid, H₄L². To a suspension of 6-hydrizino-3-pyrindcarboxylic acid (0.45 g, 2.9 mmol) in anhydrous DMA (5 mL) was added tert-butyl isothiocyanate (0.56 mL, 4.4 mmol) under an atmosphere of nitrogen. The suspension was heated to 85 °C and after 30 min became a yellow mixture, which was heated at 85 °C for a further 3 h. The mixture was concentrated by evaporation under reduced pressure to a volume of approximately 1 mL and cold diethyl ether (15 mL) was added. The suspension was stirred vigorously overnight at RT. The precipitate was collected via filtration and washed with copious amounts of cold diethyl ether to afford an off-white
solid (0.68 g, 86%). 1H NMR [MeOH-d₄, 500 MHz]: δ (ppm) 8.73 (1H, d, J = 2.2 Hz, pyF²), 8.18 (1H, dd, J = 8.7, 2.2 Hz, pyF²), 6.76 (1H, dd, J = 8.7, 0.7 Hz, pyH⁵), 1.51 (9H, s); 13C{1H} NMR [MeOH-d₄, 125.7 MHz]: δ (ppm) 181.0 (C, N=C), 166.1 (C, CO₂H), 162.0 (C, pyC⁴), 138.8 (C, pyC⁵), 117.8 (C, pyC⁶), 105.6 (C, pyC⁴), 54.7 (C, -C(CH₃)_3), 29.0 (3C, -C(CH₃)_3); IR: v_{max} (em⁻¹) 1604 (s, NHCS), 1533 (s), 1295 (s/sh), 1254 (s/sh), 1240 (m/sh), 1119 (w/sh), 781 (s); HRMS (ESI⁺): m/z calc’d for C₁₃H₁₆N₄O₅S 289.0759, found 289.0749 ([M + H]+, 100%); RP-HPLC (Column 1, System A): Rr (min) 9.2.

**Methyl 6-Chloropyridine-3-carboxylate, 3.** A suspension of 6-chloronicotinic acid (5.0 g, 32 mmol) in methanol (100 mL) was cooled to 0 °C, and H₂SO₄ (0.5 mL) was added dropwise. The mixture was heated at 60 °C for 8 h. The crude reaction mixture was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with sat. NaCl. The organic layer was collected and dried over Na₂SO₄ filtered, and concentrated to afford a chalky, yellow powder (0.08 g, 81%). 1H NMR [CHCl₃-d₆, 600 MHz]: δ (ppm) 8.95 (1H, d, J = 2.4 Hz, pyF²), 8.21 (1H, dd, J = 8.3, 2.4 Hz, pyF²), 7.40 (1H, d, J = 8.3 Hz, pyF²), 3.92 (3H, s, OCH₃); 13C{1H} NMR [CHCl₃-d₆, 151 MHz]: δ (ppm) 165.0 (C, CO₂Me), 155.7 (C, pyC⁴), 139.7 (C, pyC⁵), 125.1 (C, pyC⁶), 124.3 (C, pyC⁷), 52.7 (C, OCH₃); HRMS (ESI⁺): m/z calc’d for C₆H₅NO₂ 168.0773, found 168.0777 ([M + H]+, 100%); RP-HPLC (Column 2, System A): Rr (min) 12.9.

**Methyl 6-[(2-Ethylcarbamothioyl)hydrazinyl]-3-pyridinecarboxylate, H₂L₄(OMe).** The compound 6-[(2-tert-Butylcarbamothioyl)hydrazinyl]-3-pyridinecarboxylate, 4, (approximately 0.06 g, 0.67 mmol), was added to anhydrous ethanol (8 mL) and heated until entirely dissolved. Ethyl isothiocyanate (60 μL, 0.67 mmol) was added to the solution and stirred at reflux for 3 h. The solution was cooled to RT. A stream of N₂ was blown over the solution, and the resulting precipitate was collected by filtration and washed with copious amounts of diethyl ether to afford a colorless crystalline solid (0.05 g, 81%), 1H NMR [DMSO-d₆, 400 MHz]: δ (ppm) 9.41 (1H, s, -NHNH₂), 9.10 (1H, s, -NHNH₂), 8.66 (1H, d, J = 1.8 Hz, pyF²), 8.21 (1H, br, J = 6.5 Hz, -NHCH₂CH₂CH₃), 8.06 (1H, dd, J = 8.8, 2.1 Hz, pyF²), 6.55 (1H, d, J = 8.8 Hz, pyF²), 3.80 (3H, s, OCH₃), 3.45 (2H, quin, J = 6.6 Hz, CH₂CH₂CH₃), 1.03 (3H, t, J = 7.1 Hz, CH₂CH₃); 13C{1H} NMR [DMSO-d₆, 151 MHz]: δ (ppm) 181.3 (C, N=C), 165.4 (C, CO₂CH₃), 162.2 (C, pyC⁵), 153.8 (C, pyC⁶), 116.7 (C, pyC⁴), 105.9 (C, pyC⁷), 51.9 (C, CO₂CH₃), 38.4 (C, CH₂), 14.6 (C, CH₃); IR: v_{max} (em⁻¹) 3143 (m, br, N–H), 2977 (m, br), 1710 (s, CH=O), 1533 (m, br), 1295 (s, CH₃), 1240 (m, CH₃), 781 (m, s); HRMS (ESI⁺): m/z calc’d for C₁₃H₁₇N₂O₄S 255.0916, found 255.0938 ([M + H]+, 100%); RP-HPLC (Column 2, System A): Rr (min) 9.2.

**Methyl 6-[(2-tert-Butylcarbamothioyl)hydrazinyl]-3-pyridinecarboxylate, H₂L₄(OMe).** The compound 6-[(2-tert-butylcarbamothioyl)hydrazinyl]-3-pyridinecarboxylic acid (H₄L₂) (0.2 g, 0.74 mmol), was added to deoxygenated and dried CH₃OH (20 mL). Sulfuric acid (0.10 mL, 1 M) was added dropwise, and the reaction was heated to 50–60 °C for 3 h. Addition of aqueous NaOH (0.1 M) caused a precipitate to form (pH 7–8). The precipitate was collected by filtration as a gray powder (0.13 g, 63%). 1H NMR [CDCl₃-d₆, 500 MHz]: δ (ppm) 8.74 (1H, d, J = 1.7 Hz, pyF²), 8.16 (1H, dd, J = 8.7, 2.2 Hz, pyF²), 6.76 (1H, dd, J = 8.7, 0.3 Hz, pyF²), 3.86 (3H, s, CO₂CH₃), 1.47 (9H, s, (CH₃)₃); 13C{1H} NMR [CDCl₃-d₆, 125.7 MHz]: δ (ppm) 180.9 (C, N=C), 165.6 (C, CO₂CH₃), 161.4 (C, pyC⁴), 150.6 (C, pyC⁵), 140.1 (C, pyC⁶), 119.8 (C, pyC⁷), 106.1 (C, pyC⁸), 53.8 (C, OCH₃), 52.1 (C, (CH₃)₃), 28.3 (3C, -CH(CH₃)₂); IR: v_{max} (em⁻¹) 1721 (s, CH=O), 1523 (m, s), 1285 (s, s), 1250 (s, m), 1133 (m, s), 775 (s, m); ESI-MS (+): m/z calc’d for C₂₀H₂₄N₂O₄S 383.1229, found 383.0434 ([M + H]+, 100%); RP-HPLC (Column 2, System A): Rr (min) 8.7.
was centrifuged (3 min, 3600 rpm) and the supernatant was detected, 165.0 (C, py2C5), 138.8 (C, ArC5), 137.7 (C, pyC5), 128.0 (2C, ArC=C5), 127.0 (C, ArC5), 124.6 (2C, ArC=C5), 116.6 (C, pyC5), 105.3 (C, pyC5), 51.8 (C, CO2CH3): IR: \( \nu_{\text{max}} \) (cm\(^{-1}\)) 3262 (m/sh, N=C), 1707 (s/sh, C=O), 1618 (m/sh), 1537 (s/sh), 1274 (s/sh), 1133 (m/sh), 742 (m/sh): ESI-MS (+): \( m/z \) calc’d for C\(_{38}\)H\(_{41}\)N\(_2\)O\(_2\)S 303.0916, found 303.1274 \{[M + H]+, 100\%\}; RP-HPLC (Column 2, System A): \( R_f \) (min) 13.1.

**H\(_2\)L\(_1\)**(lys). Dichloromethane (10 mL) was added to 2-chlorotriyl resin (1.00 mmol g\(^{-1}\) loading) (3.1 g, 3.1 mmol) swelling the resin. A mixture of \( N_e\)-tBoc-N\(_e\)-Fmoc-c-Lys (1.7 g, 3.6 mmol) and DIPEA (2.1 mL, 12 mmol) in dichloromethane (15 mL) was added to the resin, which was then shaken in ambient temperature for 2 h. The resin was filtered and the filtrate was discarded. The resulting resin was washed with dichloromethane (3 mL), DMF (3 mL), dichloromethane (3 mL), and finally diethyl ether (3 mL). After several hours of suction drying the resin was weighed and loading determined (0.77 mmol g\(^{-1}\)). To the dried resin-bound fBoc-Lys(Fmoc)–OH (0.51 g, 0.39 mmol) was added a DMF/piperidine (80:20 \( v/v \)) solution (20 mL), which was manually stirred. After 20 min, the supernatant was removed by filtration through a sintered frit, and the remaining resin was washed with DMF (3 mL). A positive TNBSA assay (2,4,6-trinitrobenzenesulfonic acid in methanol (5% \( v/v \)) was used to indicate deprotection of the Fmoc protected epsilon (\( \epsilon \)) amine. A mixture of HATU (285 mg, 0.75 mmol), H\(_2\)L\(_1\) (176 mg, 0.75 mmol), and DIPEA (261 \( \mu \)L, 1.5 mmol) in DMF (4 mL) was added to the resin and reacted for 4 h at ambient temperature. The liquid was drained and the resin was washed successively with dichloromethane (3 mL), then DMF (3 mL), and again with dichloromethane (3 mL), a TFA/H\(_2\)O/dichloromethane (18:2:80 \( v/v \)) solution was added to half the resin (approximately 0.19 mmol), and the mixture shaken for 1 h and then filtered. The filtrate was concentrated in vacuo and diethyl ether (40 mL) was added to form a suspension, which was centrifuged (3 min, 3600 rpm) and the supernatant was discarded. The crude material was dissolved in H\(_2\)O/CH\(_3\)CN (90:10 \( v/v \)), filtered (Millipore 0.45 \( \mu \)m porosity syringe filter), and then purified by semipreparative RP-HPLC (Column S).

The HPLC system involved a gradient elution of Buffer A (0.1% TFA in H\(_2\)O) and Buffer B (0.1% TFA in CH\(_3\)CN) from 20 to 40% B to A, over 20 min (1.0% min\(^{-1}\)) then 40–60% over 40 min (0.5% min\(^{-1}\)) and UV detection at 220 nm with a flow rate of 5 mL min\(^{-1}\). Fractions containing the desired compound were identified by HPLC-MS, consolidated and lyophilized to afford an off-white, fluffy compound (10 mL, 14%). ESI-MS (+): \( m/z \) calc’d for C\(_{38}\)H\(_{41}\)N\(_2\)O\(_2\) 369.1709, found 369.1798 \{[M + H]+, 100\%; \} RP-HPLC (Column 2, System A): \( R_f \) (min) 6.00, (Column 2, System B) 24.0.

**H\(_2\)L\(_1\)**(N\(_o\)-Ac-Lys-OMe). A solution of HATU (0.23 g, 0.56 mmol) and DIPEA (0.29 mL, 1.1 mmol) in DMSO (1 mL) was added to H\(_2\)L\(_2\) (0.15 g, 0.56 mmol) and shaken for 2 min. \( N_e\)-Acetyl-L-lysine methyl ester hydrochloride (0.10 g, 0.42 mmol) in DMSO (1 mL) was added and shaken for 2 h. Diethyl ether (40 mL) was added and the mixture was centrifuged. The supernatant was discarded and the remaining yellow oil was purified by semipreparative RP-HPLC (Column S). The HPLC system involved a gradient elution of Buffer A (0.1% TFA in H\(_2\)O) and Buffer B (0.1% TFA in CH\(_3\)CN) from 0 to 40% B to A, over 80 min (0.5% min\(^{-1}\)) and UV detection at 220, 254, 275, and 305 nm with a flow rate of 8 mL min\(^{-1}\). Fractions containing the desired compound were identified by HPLC-MS, consolidated and lyophilized to afford a colorless solid (10 mg, 80%). ESI-MS (+): \( m/z \) calc’d for [C\(_{38}\)H\(_{42}\)N\(_2\)O\(_2\)S\(_2\)\]\(^{+}\) 826.378, found 826.376, calc’d for [C\(_{38}\)H\(_{42}\)N\(_2\)O\(_2\)S\(_2\)]\(^{+}\) 413.693, found 413.692; RP-HPLC (Column 2, System A): \( R_f \) (min) 11.3.

**H\(_2\)L\(_1\)**(cRGDK). The same procedure was used as for H\(_2\)L\(_1\)**(cRGDK), except H\(_2\)L\(_1\) was replaced with 6-\{(2-tert-butylcarbamothioyl)hydroxazinyl]-3-pyridinecarboxylic acid, (H\(_2\)L\(_1\))\(^{+}\) (5.3 mg, 19 \( \mu \)mol), and other reagent quantities were adapted accordingly; HATU (6.5 mg, 17 \( \mu \)mol), DIPEA (45 \( \mu \)L, 0.13 mmol), and cRGDK (10 mg, 17 \( \mu \)mol). Semi-preparative RP-HPLC purification (Column 4) involved a gradient elution of Buffer A (0.1% TFA in H\(_2\)O) and Buffer B (0.1% TFA in CH\(_3\)CN) from 0 to 40% B to A, over 80 min (0.5% min\(^{-1}\)) and UV detection at 220, 254, 275, and 350 nm with a flow rate of 8 mL min\(^{-1}\). Fractions containing the desired compound were identified by HPLC-MS, consolidated and lyophilized to afford a colorless solid (10 mg, 80%). ESI-MS (+): \( m/z \) calc’d for [C\(_{38}\)H\(_{42}\)N\(_2\)O\(_2\)S\(_2\)]\(^{+}\) 854.4096, found 854.4097, calc’d for [C\(_{38}\)H\(_{42}\)N\(_2\)O\(_2\)S\(_2\)]\(^{+}\) 427.7087, found 427.7083; RP-HPLC (Column 2, System A): \( R_f \) (min) 12.9.
µL, 0.13 mmol), and crGDK (10 mg, 17 µmol). Semi-preparative RP-HPLC purification (Column 4) involved a gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 0 to 40% B to A, over 80 min (0.5% min⁻¹) and UV detection at λ 214, 220, and 254 nm with a flow rate of 8 mL min⁻¹. Fractions containing the desired compound were identified by HPLC-MS, consolidated and lyophilized to afford a pale yellow solid (56 mg, 18%). HRMS (⁺) m/z calcd for [C₃₂H₃₆N₁₂O₇S]⁺ 527.2505, found 527.2488, calcd for [C₃₂H₃₆N₁₂O₇S]⁺ 527.2488, found 527.2488; RP-HPLC (Column 2, System A): Rₜ (min) 13.0.

H₄L²(Tyr³-Oct). The same procedure was used as for H₄L¹(Tyr³-Oct), except H₄L¹ was replaced with H₄L² (0.20 g, 0.75 mmol, 3 equiv), and all other reagents were used according to their reported equivalencies. The precipitate after lyophilization was dissolved in CH₃CN/H₂O (8 mL, 50:50 v/v) and filtered (Millipore 0.45 µm porosity syringe filter). The compound was purified by semi preparative RP-HPLC (Column 5) with an isocratic step gradient system of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN). The elution method involved 28% B for 10 min then 32% B for 60 min (0.5% min⁻¹), with UV detection at λ 214, 220, 254, 280, and 350 nm with a flow rate of 8 mL min⁻¹. Fractions containing the desired compound were identified by HPLC-MS, consolidated, and lyophilized to afford a fluffly colorless compound (18 mg, 5.5%). HRMS (⁺) m/z calcd for [C₃₂H₃₆N₁₂O₇S]⁺ 1299.5113, found 1299.5100; RP-HPLC (Column 2, System A): Rₜ (min) 14.4.

H₄L¹(Tyr²-Oct). The same procedure was used as for H₄L¹(Tyr³-Oct), except H₄L¹ was replaced with H₄L² (0.22 g, 0.75 mmol, 3 equiv). The precipitate after lyophilization was dissolved in CH₃CN/H₂O (10 mL, 50:50 v/v) and filtered (Millipore 0.45 µm porosity syringe filter). The compound was purified by semi preparative RP-HPLC (Column 5) with a linear gradient system of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 20% to 50% B to A over 60 min (0.5% min⁻¹) (or isocratic elution at 29% Buffer B) and UV detection at λ 254 nm with a flow rate of 7 mL min⁻¹. Fractions containing the desired compound were identified by HPLC-MS, consolidated, and lyophilized to afford a fluffy white solid (14 mg, 4.2%). HRMS (⁺) m/z calcd for [C₃₂H₃₆N₁₂O₇S]⁺ 1319.4800, found 1319.4849, calcd for [C₃₂H₃₆N₁₂O₇S]⁺ 1319.4849, found 1319.4849; RP-HPLC (Column 2, System A): Rₜ (min) 14.1.

Synthesis of Rhenium Complexes. Note: Unless specified Re represents ‘rhenium with natural isotope abundance’.

[ReO(HL¹(Ome))₂]Cl. To H₄L¹(Ome) (90 mg, 0.35 mmol) and (Bu₄N)[ReOCl₄] (103 mg, 0.18 mmol) was added anhydrous MeOH (13 mL). The mixture immediately turned deep red and was stirred at room temperature for 4 h. The reaction mixture was filtered, diethyl ether (ca. 15 mL) was added, and the resulting precipitate was collected by filtration to give [ReO(HL¹(Ome))₂]Cl as a dark-red, microcrystalline solid (0.11 g, 84%). ^1H NMR [DMSO-d₆, 400 MHz]: δ (ppm) 8.63 (2H, s, pyH), 8.26 (2H, dd, J = 9.3, 1.8 Hz, pyH), 7.87 (2H, d, J = 9.4 Hz, pyH), 7.43 (2H, br m, NH), 3.89 (6H, s, OCH₃), 3.37 (4H, d, J = 13.6, 7.0 Hz, CH₂CH₃), 1.16 (6H, t, J = 7.5 Hz, CH₂CH₃), 1.33 [13^¹¹H] NMR [CHCl₃-d₆, 150.9 MHz]: δ (ppm) 173.7 (2C, CO₂H), 165.1 (2C, N≡CS), 165.2 (2C, pyC), 140.5 (2C, pyC), 139.9 (2C, pyC), 123.9 (2C, pyC), 122.0 (2C, pyC), 52.3 (2C, CO₂CH₃), 42.6 (2C, CH₂CH₃), 14.5 (2C, CH₂CH₃); IR: νmax (cm⁻¹) 1553 (s/sh, C=O), 1287 (s/sh), 963 (s/sh, Re=O), 764 (m/sh); HRMS (ESI⁻): m/z calcd for ReC₈₀H₅₃N₁₄O₅S₂ 709.0916, found 709.0898 ([M⁻]⁻, 100%); RP-HPLC (Column 2, System A): Rₜ (min) 14.8.

Histidine and Cysteine Challenge Experiments. A 50-fold excess of histidine or cystine was added to a solution of
The mixture was heated at reflux for 16 h, then filtered, and diethyl ether was added to the filtrate. The resulting precipitate was collected by filtration, washed with cold diethyl ether, and then dissolved in MeOH. Addition of excess NaBH₄ resulted in the precipitation of a red-brown precipitate that was collected, and washed with hot hexane (10 mL) to afford [ReO(HL₂(Ome))₂]BPH₄ (72 mg, 39%). ¹H NMR [CDCl₃, 600 MHz]: δ ppm) 8.10 (2H, d, J = 9.5 Hz, pyF²), 7.75 (2H, d, J = 9.4 Hz, pyF¹), 7.62 (8H, m, ArBAr), 7.33 (2H, m, pyF¹), 6.93 (8H, t, J = 7.1 Hz, BArH), 6.72 (4H, t, J = 7.1 Hz, BArH), 3.86 (6H, s, CO₂(CH₃)₃), 1.39 (18H, s, (CH₃)₃), ²³C¹H NMR [CDCl₃, 150.9 MHz]: δ ppm) 164.5, 142.5, 135.8, 85.2 (8C, BArC), 133.9–133.8, 128.9, 128.6, 127.5, 126.2–126.1 (8C, BArC), 122.0 (4C, BArC), 54.9 (2C, -OCH₂), 52.6 (2C, -C(CH₃)₃), 29.0 (6C, -C(CH₃)₃); IR: v max (cm⁻¹) 1555 (s/sh, C=O), 1284 (s/sh), 960 (m/sh, Re=O), 756 (m/sh); HRMS (ESI⁺) m/z calc’d for [Re₂C₄H₄N₂O₉S₂]⁺ 1105.3762, found 1105.4079, calc’d for [Re₂C₄H₄N₂O₉S₂]³⁺ 553.1920, found 553.2094; RP-HPLC (Column 2, System A): Rₚ (min) 16.9.

To resin-bound H₃L₄(Lys)₄ (approximately 0.19 mmol) was added trans-[ReOCl₆(PPh₃)₂] (78 mg, 0.09 mmol) suspended in DMF (5 mL). The slurry was stirred for 2 h. The green suspension turned colorless and the resin turned dark red. The resin was isolated by filtration and washed with DMF (3 mL), dichloromethane (3 mL), and again with DMF (3 mL). The rhenium complex was cleaved from the resin by treatment with TFA/dichloromethane (50:50 v/v) solution (30 mL) for 3 h. The mixture was sparged with N₂ to reduce the volume to 10% of the original volume. Cold diethyl ether (40 mL) was added, the mixture was centrifuged (3 min, 3600 rpm), and the supernatant was discarded. This process was repeated twice. The precipitate was dissolved in CH₃CN (1.0 mL) and then Milli-Q water (2.0 mL) was added. The complex was purified by semipreparative RP-HPLC (Column S) involved a gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 10 to 70% B to A, over 120 min (0.5% min⁻¹) and UV detection at λ 214, 220, and 254 nm, with a flow rate of 8 mL min⁻¹. Fractions containing the desired compound were identified by HPLC-MS, consolidated, and lyophilized to afford a red solid (22 mg, 23%, assuming [ReO(HL₂(Ome))₂]-[CF₃CO₂])⁻. ¹H NMR [MeOH-d₄, 600 MHz]: δ ppm) 8.58 (2H, d, J = 9.5 Hz, pyF¹), 8.36 (2H, d, J = 9.6 Hz, pyF²), 8.03 (2H, d, J = 9.5 Hz, pyF²), 7.43–7.40 (2H, m, pyCH₂), 7.29 (4H, t, J = 9.5 Hz, ArF), 7.16 (4H, s, ArF), 6.95 (2H, t, J = 7.3 Hz, ArF), 3.92 (6H, s, CO₂(CH₃)₃); IR: v max (cm⁻¹) 1555 (s/sh, C=O), 1282 (s/sh), 960 (m/sh, Re=O), 752 (m/sh); HRMS (ESI⁺) m/z calc’d for [Re₂C₄H₄N₂O₉S₂]⁺ 1105.3762, found 1105.3754, calc’d for [Re₂C₄H₄N₂O₉S₂]³⁺ 553.1920, found 553.2094; RP-HPLC (Column 2, System A): Rₚ (min) 15.4.

To H₃L₄(N₆-Ac-Lys-Ome) (15 mg, 33 μmol in MeOH (1 mL) was added a solution of (iBu₄N)[ReOCl₆] (0.9 mg, 15 μmol) in MeOH (0.3 mL). The mixture was stirred at ambient temperature for 2 h, then diluted with Milli-Q water and purified by semipreparative RP-HPLC (Column 4). The HPLC system involved a gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 10 to 20% B to A, over 10 min (1.0 min⁻¹), then 20 to 40% B to A over 40 min (0.5% min⁻¹) and UV detection at λ 214, 220, 254, and 280 nm with a flow rate of 8 mL min⁻¹. Fractions containing the desired compound were identified by HPLC-MS, consolidated, and lyophilized to afford a red solid (5 mg, 27%, assuming [ReO(HL₂(N₆-Ac-Lys-Ome)]CF₃CO₂). ¹H NMR [MeOH-d₄, 600 MHz]: δ ppm) 8.68 (2H, s, pyF²), 8.36 (2H, d, J = 9.6 Hz, pyF²), 8.03 (2H, d, J = 9.5 Hz, pyF²), 4.43–4.40 (2H, m, acCH), 3.72 (6H, s, CO₂CH₂), 3.43 (4H, q, J = 6.6 Hz, eCH₂), 1.99 (6H, s, COCH₃), 1.92–1.86 (4H, m, βCH₂), 1.78–1.72 (4H, m, δCH₂), 1.71–1.66 (4H, m, γCH₂), 1.50 (18H, s, (CH₃)₃); ³¹P¹H NMR [MeOH-d₄, 150.9 MHz]: δ ppm) 174.2 (2C, COOH), 173.4 (2C, COCH₃), 167.2 (2C, N = CS), 161.5 (2C, CONH), 163.3 (2C, pyC), 140.7 (2C, d, pyC), 139.1 (2C, d, pyC), 124.5 (2C, pyC), 122.6 (2C, pyC), 55.3 (2C, -C(CHO)), 53.7 (3C, αCH₂), 52.7 (3C, -CO₂CH₃), 40.8 (2C, eCH₂), 32.1 (2C, βCH₂), 29.9 (2C, δCH₂), 28.9 (6C, -C(CH₃)₃), 24.3 (2C, COCH₃), 22.3 (2C, γCH₂); HRMS (ESI⁺) m/z calc’d for [ReC₂H₄N₂O₉S₂]⁺ 937.2574, found 937.2574, calc’d for [ReC₂H₄N₂O₉S₂]³⁺ 469.1354, found 469.1355, calc’d for [ReC₂H₄N₂O₉S₂]⁵⁺ 313.0256, found 313.0399; RP-HPLC (Column 2, System A): Rₚ (min) 10.1.

A solution of H₃L₄(Lys)[CRGDIK] (5 mg, 6.1 μmol) in MeOH (250 μL) was added to a mixture of (iBu₄N)[ReOCl₆] (1.8 mg, 3.0 μmol) in MeOH (250 μL). The mixture was shaken for 2.5 h, then diluted with Milli-Q water (5 mL), filtered (Millipore 0.45 μm porosity syringe filter), and purified by semipreparative RP-HPLC (Column 6). The HPLC system involved isocratic elution of 77% Buffer A (0.1% TFA in H₂O) and 23% Buffer B (0.1% TFA in CH₃CN) (0.5% min⁻¹) and UV detection at λ 214, 220, 254, 280, and 350 nm with a flow rate of 5 mL min⁻¹. Fractions containing the desired compound were identified by HPLC-MS, consolidated, and lyophilized to afford a white solid (3.1 mg, 53%, assuming...
[ReO(HL₁(cRGDK))₂][CF₃CO₂]. HRMS (ESI⁺) m/z calc’d for [ReC₇₀H₁₀₁N₂₆O₁₇S₂]²⁺ 926.3413, 926.3433, calc’d for [ReC₇₂H₁₁₀N₂₆O₁₇S₂]²⁺ 917.8968, found 917.9939; RP-HPLC (Column 2, System A): Rₜ (min) 11.0.

[ReO(HL₂(cRGDK))₃]⁺. The same procedure was used as for [ReO(HL(cRGDK))₂][CF₃CO₂], except H₂L₁(cRGDK) was replaced with H₂L₂(cRGDK) (8.0 mg, 9.4 μmol) in MeOH (250 μL); [tBu₄N][ReOCl₄] (2.7 mg, 4.7 μmol) in MeOH (500 μL). The compound was purified by semipreparative RP-HPLC purification (Column 4) involved gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 0 to 20% over 20 min (1% min⁻¹), then 20 to 60% B to A over 40 min (0.5% min⁻¹) and UV detection at 214, 220, 254, 280, and 350 nm with a flow rate of 8 mL min⁻¹. Fractions containing the desired compound were identified by HPLC-MS, consolidated and lyophilized to afford a white solid (5.5 mg, ~54%, assuming [ReO(HL₂(cRGDK))₃][CF₃CO₂]). HRMS (ESI⁺) m/z calc’d for [ReC₇₂H₁₀₁N₂₆O₁₇S₂]³⁺ 954.3732, found 954.3715, calc’d for [ReC₇₂H₁₁₀N₂₆O₁₇S₂]³⁺ 945.8972, found 946.8972; RP-HPLC (Column 2, System A): Rₜ (min) 12.9.

[ReO(HL₃(Tyr⁻Oct))]⁺. A solution of H₂L₃(Tyr⁻Oct) (9.0 mg, 7.1 μmol) in MeOH (50 μL) was added to a mixture of [tBu₄N][ReOCl₄] (2.1 mg, 3.5 μmol) and MeOH (100 μL). The reaction mixture was shaken vigorously for 2 h. Milli-Q water was added (4 mL) and the reaction was filtered (Millipore 0.45 μm pore size filter). The compound was purified by semipreparative RP-HPLC purification (Column 6) with a gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 10 to 80% B to A, over 70 min (1% min⁻¹) and UV detection at λ 214, 220, and 254 nm, with a flow rate of 5 mL min⁻¹. Fractions containing the desired compound were identified by HPLC-MS, consolidated, and lyophilized to afford a white solid (2.2 mg, ~42%, assuming [ReO(HL₃(cRGDK))₃][CF₃CO₂]). HRMS (ESI⁺) m/z calc’d for [ReC₇₆H₁₄₈N₂₈O₃₇S₆]⁺ 1371.9470, found 1371.9470; RP-HPLC (Column 2, System A): Rₜ (min) 15.4.

[ReO(HL₂(Tyr⁻Oct))]⁺. The same procedure was used as for [ReO(HL(Tyr⁻Oct))]⁺, except H₂L₁(Tyr⁻Oct) was replaced with H₂L₂(Tyr⁻Oct) (8.0 mg, 6.2 μmol) in MeOH (200 μL) and other reaction quantities were adapted accordingly; [tBu₄N][ReOCl₄] (1.8 mg, 3.1 μmol) in MeOH (100 μL). The compound was purified by semipreparative RP-HPLC (Column 6) with isocratic elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) at 38% Buffer B, over 60 min (1.0% min⁻¹) and UV detection at λ 214, 220, and 254 nm, with a flow rate of 4 mL min⁻¹. Fractions containing the desired compound were consolidated and lyophilized to afford a red, fluffy material (5.5 mg, ~61%, assuming [ReO(HL₂(Tyr⁻Oct))]₂[CF₃CO₂]). ESI-MS (⁺) m/z calc’d for [ReC₁₁₆H₁₈₆N₂₈O₄₇S₆]²⁺ 1399.9784, found 1399.9720, calc’d for [ReC₁₁₆H₁₈₆N₂₈O₄₇S₆]²⁺ 1393.6548, found 1393.6460; RP-HPLC (Column 2, System A): Rₜ (min) 16.2.

[ReO(HL₃(Tyr⁻Oct))]⁺. Preloaded, resin-bound (Wang) ligand, H₂L₁(Tyr⁻Oct) (approximately 6.0 μmol), was swelled in CH₃Cl₂ and drained twice. Anhydrous DMF (2 × 10 mL) was added to the resin mixture was stirred and drained. DMF (1 mL) was added to the washed resin and [tBu₄N]-[ReOCl₄] (2.1 mg, 2.9 μmol) in DMF (50 μL) was added to the resin. The resin mixture was reacted for 2 h at RT. The solution was drained and the resin was washed with copious amounts of DMF (5 × 10 mL), CH₃Cl₂ (3 × 10 mL), and diethyl ether (10 mL). The crude material was cleaved off the resin by addition of TFA/CH₃Cl₂/TIPS/H₂O (50:46:1:3 v/v) (20 mL). The mixture was shaken for 1.5 h and filtered and the filtrate sparged with a stream of N₂ until the red mixture had reduced to approximately 0.5 mL. Diethyl ether (2 × 10 mL) was added to the crude material, and the resulting precipitate was collected via centrifugation (3 min, 3600 rpm). The compound was purified by semipreparative RP-HPLC purification (Column 6) by gradient elution of Buffer A (0.1% TFA in H₂O) and Buffer B (0.1% TFA in CH₃CN) from 10 to 80% B to A, over 70 min (1.0% min⁻¹) and UV detection at λ 214, 220, and 254 nm, with a flow rate of 4 mL min⁻¹. Fractions containing the desired compound were consolidated and lyophilized to afford a red, fluffy material (5.5 mg, ~58%, assuming [ReO(HL₃(Tyr⁻Oct))]₂[CF₃CO₂]). ESI-MS (⁺) m/z calc’d for [ReC₁₂₈H₁₈₆N₂₈O₄₇S₆]²⁺ 1419.9470, found 1419.9281, calc’d for [ReC₁₂₈H₁₈₆N₂₈O₄₇S₆]²⁺ 1414.9673, found 1414.9660; RP-HPLC (Column 2, System A): Rₜ (min) 16.2.

Synthesis of [¹⁸⁸ReO(HL')(Tyr⁻Oct)]⁺. Rhenium-188 was produced from an ITG ¹⁸⁸W/¹⁸⁸Re generator (ITG Isotope Technologies, Garching, Germany). Generator-produced ¹⁸⁸ReO₄⁻ (86 MBq) in aqueous sodium chloride solution (200 μL, 0.9% w/v) was added to a sealed, N₂-purged vial containing sodium tartrate (0.2 mg) and stannous chloride (0.1 mg) in water (400 μL), followed by heating at 80 °C for 30 min. An aliquot of this solution (50 μL) was added to a separate sealed, N₂-purged vial containing H₂L₁(Tyr⁻Oct) (100 μg) dissolved in water (100 μL). The reaction was left for 30 min at ambient temperature, after which an aliquot was removed for HPLC analysis, revealing that there was no reaction between ¹⁸⁸Re and peptide. The reaction vial was then heated at 100 °C for 1 h, after which an aliquot was analyzed by HPLC (column 1, system F). Radiochemical yield: 67%, retention time of [¹⁸⁸ReO(HL'(Tyr⁻Oct))]⁺ = 11.6 min, compared to retention time of [¹⁸⁸ReO(HL'(Tyr⁻Oct))]⁺ = 11.3 min and H₂L₁(Tyr⁻Oct) = 9.7 min.

Synthesis of Technetium-99m Complexes. Sodium pertechnetate, Na₉⁹⁹mTcO₄⁻ (1000 MBq), was eluted from a Gentech 99 Mo/⁹⁹mTc sterile generator (Austin Health: Nuclear Medicine and Centre for PET, Australia, via ANSTO Health) as a 1.0 mL saline solution (0.9% v/v). A solution of SnCl₂ in 0.1 M HCl (0.5 mg mL⁻¹) was prepared and purged with nitrogen. Disodium tartrate dihydrate was dissolved in water (1
mg mL\(^{-1}\) in a separate evacuated vial, and a 0.5 mL aliquot was taken from both solutions and mixed together. To the solution was added \(99m\text{TcO}_4^−\) (0.1 mL in 0.9% saline, 108 MBq). The conjugated peptide, H\(_2\)L\(^{1−3}\)(cRGDK) or H\(_2\)L\(^{1−3}\)(Tyr\(^3\)-Oct), was dissolved in degassed Milli-Q water (1 mg mL\(^{-1}\)), and 100 μL of this mixture was added to the technetium solution. The sample was neutralized with NaHCO\(_3\) (pH 6.5, approximately 55 μL) then filtered or allowed to react without neutralizing at ambient temperature for 30 to 120 min. The samples were filtered (Supelco, Iso-disc Filter, 4 mm x 0.45 μm). Radiochemical yields were evaluated by reverse-phase high-performance liquid chromatography (Column 7, System C).

Stability Studies in Human Serum. Human blood samples were centrifuged with a Heraeus Labofuge 6000 centrifuge at 3000g for 10 min (Heraeus, Hanau, Germany). Radioactivity readings for serum stability studies were taken with a Capintec CRC-35R dose calibrator (Capintec, New Jersey, USA) and were measured in MBq. Centrifugation of radioactive compounds was undertaken using an Eppendorf 5415 D (PerkinElmer, Massachusetts, USA) automatic γ counter, which measured the radioactive decay of each sample in counts per minute (cpm).

For serum stability studies, blood from a healthy male (20 mL) was centrifuged (10 min, 3000 rpm) to separate blood plasma and red blood cells. The plasma was transferred to a separate vial and the red blood cells were discarded. An aliquot of plasma (0.6 mL) was added to labeled compound, \(99m\text{Tc}([\text{HL}2\text{L}^{−3}\text{(Tyr}^{3}\text{-Oct})]_2)^+\) (0.15 mL), the radioactivity was monitored and the mixture was then incubated at 37 °C. Aliquots (0.1 mL) of the mixture were removed from heating at 10 min and after 2 h. Acetonitrile (0.1 mL) was added to the serum/tracer mix to precipitate serum proteins. The suspension was shaken for 5 min and then centrifuged (5 min, 13 200 rpm). The radioactivity of the supernatant and pellet was recorded. The supernatant (20 μL) was analyzed by analytical RP-HPLC (Column 7, System C) for UV and radioactivity analysis and the pellet was washed with acetonitrile (3 × 0.1 mL), and radioactivity levels were again recorded (radioactivity levels of the pellet were negligible).

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.7b01247.

Representative RP-HPLC chromatograms (PDF)

Accession Codes
CCDC 1543360 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes
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