FULL PAPER

Dalton
www.rsc.org/dalton

Design and synthesis of site directed maleimide bifunctional chelators for technetium and rhenium

Sangeeta Ray Banerjee,[a] Paul Schaffer,[b] John W. Babich,[c] John F. Valliant[b] and Jon Zubieta[a]

[a] *Department of Chemistry, Syracuse University, Syracuse, NY, 13244, USA*
[b] *Department of Chemistry, McMaster University, Hamilton, Ontario, Canada 13244*
[c] *Molecular Insight Pharmaceuticals, 160 Second Street, Cambridge, MA, 02142, USA*

Received 19th May 2005, Accepted 22nd August 2005
First published as an Advance Article on the web 8th September 2005

A new family of heterobifunctional linkers (**L1–L9**) containing a terminus consisting of a tridentate donor set for coordination of the $\{M(CO)_3\}^+$ core (M = Tc, Re), and a thiol reactive maleimide group has been prepared conveniently and in high yield under Mitsunobu reaction conditions by the coupling of an appropriate alcohol derivative with maleimide. The rhenium complexes $[Re(CO)_3(\mathbf{L}x)]Br$ (x = 1–9) were prepared in good yields from the reactions of the ligands and $(NEt_4)_2[Re(CO)_3Br_3]$ in refluxing methanol. The ligands and their Re complexes were characterized by ^1H and ^{13}C NMR, IR, and ESI-MS. Ligand **L4** and $[Re(CO)_3(\mathbf{L5})]Br$ have been structurally characterized by X-ray crystallography. Photoexcitation of solutions of the complexes $[Re(CO)_3(\mathbf{L}x)]Br$ (x = 4–6) gives rise to intense and prolonged luminescence at room temperature (fluorescence lifetimes of *ca.* 16 μs). The ligands and their Re complexes react smoothly at the maleimide linker with sulfhydryl groups of peptides and proteins at room temperature in phosphate-buffered saline (PBS, pH 7.4) to form stable thioether bioconjugates. The photoluminescence properties of the labeled conjugates are similar to those of the parent complexes, but with even longer lifetimes. The ligands can also be labeled at room temperature with ^{99m}Tc to give chemically robust complexes. The corresponding hydrazinonicotinamide derivative *N*-[5-(6′-hydrazinopyridine-3′-carbonyl)aminopentyl]-maleimide (**L10**) was also prepared. While coupling of **L10** to cysteine ethylester and synthesis of the rhenium derivative $[ReCl_3(HYNIC\text{-}maleimide)_2]$ were successfully accomplished, attempts to couple $[ReCl_3(HYNIC\text{-}maleimide)_2]$ to glutathione or BSA yielded intractable mixtures.

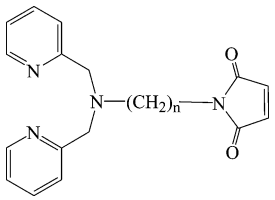
1 Introduction

The coordination chemistry of the group 7 congeners technetium and rhenium has been exploited in the design and synthesis of radiopharmaceuticals for nuclear medicine. Technetium-99m is the most prevalent radionuclide in nuclear medicine, with ideal nuclear properties that make it the radionuclide of choice for diagnostic imaging.[1–9] The two β-emitting isotopes of rhenium, ^{186}Re and ^{188}Re, have nuclear properties suitable for therapeutic applications.[10–13] Furthermore, the non-radioactive isotopes of rhenium, $^{185,187}Re$, provide model compounds for the radioactive analogues and materials which may serve as luminescent probes.[14–18]

In its radiopharmaceutical applications, the metal ion is bound by an appropriate ligand to provide a complex suitable for administration to the patient. In order to control the biodistribution of radiopharmaceuticals in the body, the current generation of radiopharmaceuticals relies on attachment of the radionuclide to biologically active molecules, whose localization depends upon specific receptor–binding interactions. One approach for linking radioactive metal cations to biomolecules exploits the use of bifunctional chelates.[19,20] The bifunctional chelate serves to bind the metal radionuclide securely without dissociation *in vivo* and to provide a structural appendage for the biomolecule linkage while maintaining maximal biomolecule integrity.

The design of the metal binding terminus of the bifunctional chelate depends upon the metal oxidation state and core structure to be incorporated.[9] Two commonly exploited metal cores in the design of ^{99m}Tc radiopharmaceuticals are the $\{Tc(V)O\}^{3+}$ core and the $\{Tc(organohydrazino)\}^{n+}$ core.[9] More recently, the *fac*-$\{Tc(I)(CO)_3\}^+$ moiety has been made accessible by Alberto and co-workers.[21–28] In this context, we have recently reported the design of a series of M(I) binding ligands (M = Tc, Re) based on amino acid derived bis(pyridyl)amine, referred to as a single amino acid chelate (SAAC), which forms inert complexes with the $\{M(CO)_3\}^+$ core, where M = ^{99m}Tc and Re.[9,29–31]

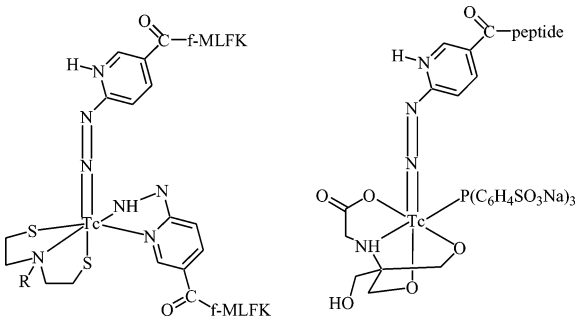
However, the application of this coordination chemistry primarily exploits acylation of the primary amine groups of lysine residues. There are limitations to this approach: since many biomolecules exhibit numerous free amino groups, heterogeneous products can be obtained; when biologically essential groups are derivatized, the labeling process can result in significant alterations in biological activity, receptor binding affinity or pharmacokinetics; many biologically significant peptides do not contain free amino groups. Consequently, there are significant impediments to the selective conjugation of specific amino groups in the protein or peptide of interest. On the other hand, the free thiol function is not very common in most peptides and proteins and is only present in cysteine residues. Consequently, this functional group can be labeled with high chemoselectivity, in contrast to carboxylate and amine reactive reagents, and thiol-reactive reagents provide a means of selectively modifying a biopolymer at a defined site.[32–34] In this respect, maleimide linkers possess an activated carbon-carbon double bond which undergoes specific alkylation reactions with the sulfhydryl group to form stable thioethers.[35] Consequently, the stoichiometry and site attachment are predictable and significant alterations in biological activity may be avoided. We recently communicated the syntheses of maleimide-dipicolylamine derivatives (Scheme 1) for ^{99m}Tc labeling and their $\{Re(CO)_3\}^+$ complexes.[36] In this work, we provide the complete details of the syntheses, characterizations and properties. The preparation and properties of the hydrazino-nicotinamide maleimide are also presented for



Scheme 1

DOI:10.1039/b507096a

comparison. The HYNIC moiety has been demonstrated to be a useful reagent for radiolabeling a variety of peptides and proteins with ^{99m}Tc,[37–44] albeit with a different core structure from that obtained with the $\{M(CO)_3\}^+$ subunit (Scheme 2).



Scheme 2 Coligand dependence of the technetium–organohydrazino core structures.

2 Experimental

2.1 General considerations

All reagents were weighed in air and the reactions were conducted under an atmosphere of air, unless otherwise indicated. Di-*tert*-butyl fumarate was prepared by the reported method[45] followed by ozonolysis to prepare tert-butyl glyoxalate in good yields.[46] Anhydrous DCE, THF, pyridine-2-carbaldehyde, quinoline-2-carbaldehyde, $NaBH(OAc)_3$, and amino alcohols were all obtained from Aldrich, whereas diethyl azodiester (97%) was obtained from Lancaster. All other reagents were purchased from commercial sources and used as received. Flash column chromatography was done with silica gel 60 (240–400 mesh). Analytical TLC was performed using Aldrich aluminum-backed 0.2 mm silica gel Z19, 329-1 plates and visualized by ultraviolet light, I_2 and 1% ninhydrin in EtOH. Melting points were determined with a Thomas-Hoover Capillary melting point apparatus and were uncorrected. Elemental analyses were performed by Oneida research services, Whitesboro, NY. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 300 spectrometer, and all peak positions are relative to TMS. HSQC spectra were acquired on a Bruker Avance 500 MHz spectrometer, using standard pulse programs with gradients. HSQC spectra were collected with 4 scans per increment, or 64 scans per increment (depending on whether the natural abundance peaks were desired), into 2048 × 512 points with no sample spinning and no zero-filling. The high resolution ESI mass spectra were obtained from the Ohio State mass spectrometry facility where samples were dissolved in 1 : 1 mixture of MeOH–THF (for cationic samples) or 1 : 1 : 1 MeOH–THF–NaCl (for neutral samples). High resolution masses are within 5 ppm of theoretical values.

2.2 Photophysical measurements

The UV-vis absorption spectra were taken on a Varian Cary (Model CARY-50 Bio) spectrophotometer. Steady state emission spectra were recorded in a PTI fluorimeter. The 321 nm output of the flash lamp was the excitation source for emission lifetime measurements. Luminescence decay signals were recorded on a PTI spectrophotometer. Data were processed and analyzed using a program for exponential fits on an IBM-compatible PC. Luminescence quantum yields were measured by the optical dilute method[47] using an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ ($\phi = 0.028$, excitation wavelength at 455 nm)[48] as the standard solution.

2.3 Synthesis of bis(picolyl)aminoethylmaleimide (L1)

DEAD (0.297 g, 1.71 mmol) was added dropwise over a period of 15 min to a solution of bis(picolyl)aminoethanol (0.340 g, 1.4 mmol), PPh_3 (0.437 g, 1.65 mmol) and maleimide (0.167 g, 1.72 mmol) in THF (15 mL) at 0 °C under nitrogen atmosphere. The mixture was then stirred at 0 °C for another 5–6 h and subsequently poured into water and extracted with ether. The ether extract was dried over Na_2SO_4 and concentrated. The residue was purified through flash column chromatography with 2% methanol–dichloromethane to give the product a yellow oil in 55% yield. NMR: ^{1}H (300 MHz, $CDCl_3$): 8.48 (d, $J = 6.0$ Hz, 2H), 7.68 (t, $J = 9.0$ Hz, 2H), 7.45 (d, $J = 7.8$ Hz, 2H), 7.03 (t, $J = 6.0$ Hz, 2H), 6.68 (s, 2H), 3.65 (s, 4H), 3.45 (t, $J = 5.1$ Hz, 2H), 2.58 (t, $J = 5.1$ Hz, 2H). ^{13}C (300 MHz, $CDCl_3$): 171.01, 158.98, 148.46, 136.21, 134.05, 122.78, 121.69, 59.82, 59.502, 53.13.

2.4 Syntheses of bis(picolyl)aminopropylmaleimide (L2) and bis(picolyl)aminohexylmaleimide (L3)

Ligands **L2** and **L3** were prepared in a similar fashion to **L1**, with bis(picolyl)aminopropanol and bis(picolyl)aminohexanol, respectively, in place of bis(picolyl)aminoethanol.

L2. NMR: ^{1}H (300 MHz, $CDCl_3$): 8.49 (d, $J = 8.4$ Hz, 2H), 7.61 (t, $J = 8.4$ Hz, 2H), 7.05 (d, $J = 9.0$ Hz, 2H), 6.53 (s, 1H), 3.77 (s, 4H), 3.46 (t, $J = 7.2$ Hz, 2H), 2.55 (t, $J = 6.9$ Hz, 2H), 1.75 (m, 2H). ^{13}C (300 MHz, MeOH-d_4): 170.85, 160.44, 147.70, 136.57, 134.09, 129.54, 129.28, 127.69, 126.35, 121.39, 61.53, 51.78, 36.20, 26.36. Mp 143–145 °C. HRMS (1 : 1 MeOH–THF): calc. for $C_{19}H_{20}N_4O_2Na^+$ 359.14978; found 359.14608.

L3. Oily product: NMR: ^{1}H (300 MHz, $CDCl_3$): 8.49 (d, $J = 6.0$ Hz, 2H), 7.67 (t, $J = 8.2$ Hz, 2H), 7.48 (t, $J = 6.0$ Hz, 2H), 7.11 (d, $J = 6.4$ Hz, 2H), 6.66 (s, 2H), 3.80 (s, 4H), 3.48 (t, $J = 6.6$ Hz, 2H), 2.50 (t, $J = 6.5$ Hz, 2H), 1.70–1.10 (m, 8H). ^{13}C (300 MHz, $CDCl_3$): 171.02, 160.25, 149.09. 136.53, 134.19, 122.99, 122.02, 60.65 54.39, 37.99, 28.69, 26.98, 26.72, 25.90.

2.5 Syntheses of bis(quinolinoylmethyl)aminoethylmaleimide (L4), bis(quinolinoylmethyl) aminonpropylmaleimide (L5) and bis(quinolinoylmethyl)aminohexylmaleimide (L6)

The ligands **L4–L6** were prepared in a similar fashion to that for **L1** with bis(quinolinoylmethyl)aminoethanol, bis(quinolinoylmethyl)propanol, and bis(quinolinoylmethyl)hexanol, respectively, in place of bis(picolyl)aminoethanol.

L4. NMR: ^{1}H (300 MHz, $CDCl_3$): 8.02 (d, $J = 8.4$ Hz, 4H), 7.71 (d, $J = 8.1$ Hz, 2H), 7.63 (t, $J = 6.9$ Hz, 2H), 7.52 (d, $J = 9.0$ Hz, 2H), 7.41 (t, $J = 6.0$ Hz, 2H), 6.44 (s, 1H), 3.99 (s, 4H), 3.66 (t, $J = 5.7$ Hz, 2H), 2.86 (t, $J = 5.7$ Hz, 2H). ^{13}C (300 MHz, MeOH-d_4): 170.44, 159.82, 148.17, 147.54, 136.32, 133.79, 129.42, 129.11, 127.49, 126.23, 121.13, 61.13, 52.14, 36.01. Mp 130–132 °C. IR (KBr): 1698, 1505, 1129. HRMS (1 : 1 MeOH–THF): calc. for $C_{26}H_{22}N_4O_2Na^+$ 445.163494, found 445.16418.

L5. NMR: ^{1}H (300 MHz, $CDCl_3$): 8.13 (d, $J = 8.4$ Hz, 2H), 8.05 (d, $J = 8.4$ Hz, 2H), 7.80–7.68 (m, 6H), 7.50 (t, $J = 9.0$ Hz, 2H), 6.53 (s, 1H), 4.00 (s, 4H), 3.51 (t, $J = 7.2$ Hz, 2H), 2.66 (t, $J = 6.9$ Hz, 2H), 1.82 (m, 2H). ^{13}C (300 MHz, MeOH-d_4): 170.85, 160.44, 147.70, 136.57, 134.09, 129.54, 129.28, 127.69, 126.35, 121.39, 61.53, 51.78, 36.20, 26.36. Mp 143–145 °C. HRMS (1 : 1 MeOH–THF): calc. for $C_{27}H_{24}N_4O_5Na^+$ 459.179144, found 459.17710.

L6. NMR: ^{1}H (300 MHz, $CDCl_3$): 8.12 (d, $J = 8.4$ Hz, 2H), 8.04 (d, $J = 8.7$ Hz, 2H), 7.81–7.71 (m, 4H), 7.65 (t, $J = 6.9$ Hz, 2H), 7.47 (t, $J = 6.9$ Hz, 2H), 6.59 (s, 1H), 3.95 (s, 4H), 3.39 (t, $J = 7.2$ Hz, 2H), 2.59 (t, $J = 6.0$ Hz, 2H), 1.50 (m, 4H), 1.42–1.08 (m, 4H). ^{13}C (300 MHz, MeOH-d_4): 170.92, 160.97, 147.68, 136.40, 134.11, 129.46, 129.16, 127.66, 127.48, 126.19, 121.17, 61.61, 54.60, 37.90, 28.63, 27.16, 27.16, 26.91, 26.64. HRMS (MeOH): calc. for $C_{30}H_{30}N_4O_2Na^+$ 501.226094, found 501.22545.

2.6 Syntheses of *N*,*N*-2-(pyridylmethyl)(carboxymethyl)-aminopropylmaleimide (L7), *N*,*N*-(quinolinoylmethyl)-(carboxymethyl)aminopropylmaleimide (L8) and bis(carboxymethyl)aminopropylmaleimide (L9)

The ligands **L7–L9** were prepared by similar methods to those used for **L1–L6**.

L7. NMR: ^{1}H (300 MHz, $CDCl_3$): 13.43 (br s, 1H), 8.57 (d, J = 7.5 Hz, 1H), 7.36 (d, J = 7.5 Hz, 1H), 7.295 (t, J = 7.5 Hz, 1H), 7.20 (t, J = 6.0, 1H), 6.49 (s, 2H), 4.02 (s, 2H), 3.48 (s, 2H), 3.33 (br t, 2H), 2.69 (br t, 2H), 1.89 (m, 2H). ^{13}C ($CDCl_3$): 172.64, 170.15, 157.82, 147.60, 136.10, 134.45, 125.26, 122.90, 59.36, 57.37, 53.87, 37.23, 28.50.

L8. NMR: ^{1}H (300 MHz, $CDCl_3$): 12.30 (br s), 8.12 (d, J = 8.1 Hz, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 9 Hz, 1H), 7.65 (t, J = 6 Hz, 1H), 7.49 (t, J = 7.5 Hz, 1H), 7.37 (d, J = 8.4 Hz), 6.52 (s, 2H), 4.37 (s, 2H), 3.62 (s, 2H), 3.45 (t, 2H), 2.97 (t, 2H), 1.84 (m, 2H). ^{13}C (300 MHz, $CDCl_3$): 173.07, 170.76, 146.22, 138.38, 134.12, 130.624, 127.82, 127.62, 127.23, 120.79, 59.5, 57.89, 53.04, 35.39, 25.94. HRMS (1 : 1 : 1 MeOH–THF–H_2O + NaCl): calc. for $C_{19}H_{19}N_3O_4Na^+$ 376.126775, found 376.127116.

L9. NMR: ^{1}H (300 MHz, $CDCl_3$): 6.65 (s, 2H), 3.49 (t, J = 6.3 Hz, 2H), 3.39 (s, 4H), 2.63 (t, J = 6.3 Hz, 2H), 1.57 (m, 2H). ^{13}C (300 MHz, $CDCl_3$): 171.1, 170.98, 134.48, 56.70, 54.01, 38.03, 26.70.

2.7 Representative synthesis of a $\{Re(CO)_3\}^+$ core complex: preparation of [Re(CO)$_3$(L2)]Br

A solution of $(NEt_4)_2[Re(CO)_3Br_3]$ (387 mg, 0.50 mmol in 20 ml methanol) was added to a solution of **L2** (168 mg, 0.50 mmol in 5 ml) and heated to reflux under an argon atmosphere for 3 h. After cooling the reaction mixture to room temperature, the solvent was removed under reduced pressure, and the solid residue was dissolved in 20 ml of methylene chloride. Extraction with water (3 × 30 ml) removed the tetraethylammonium bromide. The organic layer was dried over sodium sulfate, and the solvent volume was reduced to ~1 ml. Standard chromatographic purification on a silica gel column with 2 : 98 methanol–methylene chloride solution gave the purified product in 81% yield (278 mg). Alternatively, $[Re(CO)_3(H_2O)_3]Br$ was used as the starting material to achieve improved yields.[50] NMR: ^{1}H (300 MHz, MeOH-d_4): 8.84 (d, J = 6.0 Hz, 2H), 7.94 (t, J = 8.1 Hz, 2H), 7.66 (d, J = 9.0 Hz, 2H), 7.38 (t, J = 6.0 Hz, 2H), 6.87 (s, 2H), 4.85 (dd, J = 16.5 Hz, 4H), 3.89 (m, 2H), 3.69 (t, J = 6 Hz, 2H), 2.25 (m, 2H). ^{13}C (300 MHz, MeOH-d_4): 197.21, 196.59, 172.76, 162.12, 153.25, 141.81, 135.78, 127.13, 125.04, 69.52, 56.85, 36.14, 26.08. HRMS (MeOH): calc. for $C_{22}H_{20}N_4O_5Re^+$ 607.0985; found 607.0990.

2.8 Syntheses of [Re(CO)$_3$(L1)Br and [Re(CO)$_3$(L3)]Br

The complexes were prepared in an analogous fashion to [Re(CO)$_3$(**L2**)]Br in *ca.* 80% yields.

[Re(CO)$_3$(L1)]Br. NMR: ^{1}H (300 MHz, MeOH-d_4): 8.83 (d, J = 7.9 Hz, 2H), 7.66 (t, J = 9.1 Hz, 2H), 7.43 (d, J = 7.8 Hz, 2H), 7.34 (t, J = 6.0 Hz, 2H), 6.88 (s, 2H), 4.45 (dd, 16.2 Hz, 4H), 3.95 (t, J = 5.1 Hz, 2H), 3.79 (t, J = 5.1 Hz, 2H). ^{13}C (300 MHz, MeOH-d_4): 195.42, 194.99, 171.92, 160.39, 150.70, 140.11, 125.50, 123.69, 71.87, 67.52, 58.23.

[Re(CO)$_3$(L3)]Br. NMR: ^{1}H (300 MHz, MeOH-d_4): 8.85 (d, J = 6.2 Hz, 2H), 7.95 (t, J = 8.0 Hz, 2H), 7.66 (d, J = 9.0 Hz, 2H), 7.40 (t, J = 6.1 Hz, 2H), 6.87 (s, 2H), 4.87 (dd, J = 16.3 Hz, 4H), 3.82 (m, 2H), 3.56 (t, J = 6.3 Hz, 2H), 2.02 (m, 2H), 1.8–1.2 (m, 6H). ^{13}C (300 MHz, MeOH-d_4): 197.21, 196.60, 171.46, 163.24, 150.55, 141.65, 134.78, 127.13, 124.04, 68.75, 67.85, 37.01, 28.78, 27.08, 26.93, 25.97.

2.9 [Re(CO)$_3$(L4)]Br, [Re(CO)$_3$(L5)]Br and [Re(CO)$_3$(L8)]Br

The complexes were prepared in an analogous fashion to [Re(CO)$_3$(**L2**)]Br.

[Re(CO)$_3$(L4)]Br. NMR: ^{1}H (300 MHz, MeOH-d_4): 8.53 (d, J = 8.4 Hz, 2H), 8.51 (d, J = 8.7 Hz, 2H), 8.04 (d, J = 9.0 Hz, 2H), 7.88 (t, J = 8.1 Hz, 2H), 7.75–7.69 (m, 4H), 6.95 (s, 1H), 5.38 (m, 4H), 3.97 (m, 2H), 4.26–4.14 (m, 4H). ^{13}C (300 MHz, MeOH-d_4): 197.04, 195.32, 172.20, 166.20, 148.17, 143.15, 135.97, 134.20, 131.05, 129.92, 129.58, 121.21, 70.09, 66.13, 35.44. Mp 206–208 °C. IR (KBr): 2029, 1919, 1705, 1515. HRMS (1 : 1 MeOH–THF): calc. for $C_{29}H_{22}N_4O_5Re^+$ 693.119421, found 693.11502.

[Re(CO)$_3$(L5)]Br. NMR: ^{1}H (300 MHz, MeOH-d_4): 8.53 (d, J = 8.4 Hz, 2H), 8.45 (d, J = 8.7 Hz, 2H), 7.99 (d, J = 9.0 Hz, 2H), 7.83 (t, J = 9.0 Hz, 2H), 7.72–7.63 (m, 4H), 6.84 (s, 1H), 5.25 (m, 4H), 3.97 (m, 2H), 3.72 (m, 2H), 2.36 (m, 2H). ^{13}C (300 MHz, MeOH-d_4): 197.24, 195.50, 172.64, 166.55, 148.13, 143.03, 135.67, 134.11, 131.01, 129.84, 129.53, 121.19, 70.09, 67.23, 36.08, 26.95. MP 206–208 °C. IR (KBr): 2029, 1919, 1705, 1515. HRMS (1 : 1 MeOH–THF): calc. for $C_{30}H_{24}N_4O_5Re^+$ 707.129866, found 707.12556.

[Re(CO)$_3$(L8)]Br. NMR: ^{1}H (300 MHz, MeOH-d_4: 8.67 (d, J = 8.5 Hz, 1H), 8.49 (d, J = 8.5 Hz, 1H), 8.08 (d, J = 9.1 Hz, 1H), 7.88 (t, J = 7.9 Hz, 1H), 7.63 (t, J = 8.1 Hz, 1H), 7.57 (d, J = 8.8 Hz), 6.87 (s, 2H), 4.85 (d, 2H), 4.05 (s, 2H), 3.91 (t, 2H), 3.32 (t, 2H), 1.99 (m, 2H). ^{13}C (300 MHz, MeOH-d_4): 196.45, 195.32, 174.07, 171.63, 167.21, 148.42, 147.38, 137.12, 134.24, 131.22, 129.67, 121.54, 69.89, 67.47, 58.04, 36.49, 27.43. HRMS (1 : 1 MeOH–THF + NaCl): calc. for $C_{22}H_{18}N_3O_7ReNa^+$ 646.059439, found 646.05816.

2.10 Synthesis of [Re(CO)$_3$(L6)]Br

A solution of $(NEt_4)_2[Re(CO)_3Br_3]$ (387 mg, 0.50 mmol in 20 ml methanol) was added to a solution of **L6** (240 mg, 0.50 mmol in 5 ml) and refluxed under argon atmosphere for 3 h. After cooling the reaction mixture at room temperature, the solvent was removed under reduced pressure and the solid residue was dissolved in 20 ml of methylene chloride and extracted with water (3 × 30 ml) to remove all the tetraethylammonium bromide. The organic layer was dried over sodium sulfate and the solvent was reduced to ~1 ml and was subjected to a standard chromatographic purification step using a silica gel column. The pure product was eluted with 2 : 98 methanol–methylene chloride solution. Yield: 340 mg, ~82%. NMR: ^{1}H (300 MHz, $CDCl_3$): 8.39 (d, J = 8.6 Hz, 2H), 8.31 (d, J = 8.7 Hz, 2H), 7.86 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 17 Hz, 2H), 7.65 (t, J = 8.7 Hz, 2H), 7.48 (t, J = 6.0 Hz, 2H), 6.68 (s, 1H), 6.03 (d, J = 17 Hz, 2H), 5.03 (d, J = 17 Hz, 2H), 3.83 (m, 2H), 3.51 (m, 2H), 2.07 (m, 2H), 1.67–1.37 (m, 6H). ^{13}C (300 MHz, $CDCl_3$): 195.91, 194.55, 172.06, 165.22, 147.03, 141.48, 134.27, 132.92, 129.74, 128.52, 128.24, 120.98, 69.13, 68.92, 37.57, 28.42, 26.89, 26.54, 26.29. HRMS (1 : 1 MeOH–THF): calc. for $C_{33}H_{30}N_4O_5Re^+$ 749.176816, found 749.17669.

2.11 Coupling of [Re(CO)$_3$(L6)]Br with glutathione

A solution of [Re(CO)$_3$(**L6**)]Br (24 mg, 0.029 mmol) in DMF (0.7 ml) was added dropwise to a solution of GSH (9.0 mg, 0.029 mmol) in phosphate-buffered saline (PBS, pH 7.5, 1 ml). The mixture was stirred at room temperature for 40 min. At this time TLC indicated the disappearance of [Re(CO)$_3$(**L6**)]Br (R_f 0.5; methanol–CH_2Cl_2 2 : 98) and the appearance of one of the major product (R_f 0.6; *n*-BuOH–methanol–H_2O–AcOH 4 : 2 : 1 : 0.5). After dilution with water (3.3 ml) the mixture was passed through a Waters Sep-Pak C-18 cartridge (1 ml). The cartridge was washed with water (3 × 5 ml) and the product was eluted with 2 : 1 methanol–water (5 ml). Evaporation of the eluate afforded the product as a colorless solid. Yield: 27 mg (~82%).

^{1}H NMR (300 MHz, MeOH-d_4): 8.78 (m), 8.24 (d, $J = 8.7$ Hz, 2H), 8.11 (d, $J = 6.9$ Hz, 2H), 7.90–7.76 (m, 4H), 5.30–4.60 (m, 5H), 3.95–3.81 (m, 3H), 3.80–3.72 (m, 2H), 3.69–3.63 (m, 1H), 3.62–3.48 (m, 3H), 3.33–2.81 (m, 2H), 2.72–2.41 (m, 3H), 2.28–1.94 (m, 4H), 1.75–1.32 (m, 8H). HRMS (1 : 1 MeOH–THF + NaCl): calc. for $C_{43}H_{47}N_7O_{11}ReSNa^+$ 1079.25039 and $C_{43}H_{47}N_7O_{11}ReSH^+$ 1057.268448, found 1079.24893 and 1057.27396.

2.12 Coupling reactions of [Re(CO)$_3$(L4)]Br with glutathione

The compound [Re(CO)$_3$(**L4**-glutathione)]$^+$ was prepared in an analogous fashion to [Re(CO)$_3$(**L6**-glutathione)]$^+$ from [Re(CO)$_3$(**L4**)]Br and glutathione. Yield: ~80%. ^{1}H NMR (300 MHz, D_2O): 8.29 (d, $J = 9.04$ Hz, 2H), 7.89–7.81 (m, 6H), 7.62 (m, 2H), 7.50 (d, $J = 7.8$ Hz, 2H), 4.7 (t, $J = 8.7$ Hz, 1H), 4.23–4.05 (m, 4H), 4.03–3.67 (m, 6H), 3.36–3.01 (m, 5H), 2.7–2.42 (m, 3H), 2.3–2.15 (m, 2H). HRMS (1 : 1 MeOH–THF + NaCl): calc. for $C_{39}H_{39}N_7O_{11}ReSNa^+$ and $C_{36}H_{39}N_7O_8ReSH^+$: 1023.1884 and 1001.2064, respectively; found: 1023.1873 and 1001.2081, respectively.

2.13 (a) Coupling reaction of L4 with glutathione

A solution of **L4** (13.9 mg, 0.033 mmol) in DMF (0.8 mL) was added to a solution of GSH (10.2 mg, 0.033 mmol) in phosphate-buffered saline (PBS, pH 7.5, 1.1 ml). The mixture was stirred for 1 h at room temperature, whereupon the solution was diluted with H_2O (3.5 ml) and passed through a Waters Sep-Pak cartridge. The product was eluted with 2 : 1 methanol–H_2O. Evaporation of the eluate provided a colorless product **L4**–glutathione in 91% yield (21.9 mg). ^{1}H NMR, 300 MHz, D_2O: 8.29 (d, $J = 9.04$ Hz, 2H), 7 89–7.81 (m, 6H), 7.62 (m, 2H), 7.50 (d, $J = 7.8$ Hz, 2H), 4.7 (t, $J = 8.7$ Hz, 1H), 4.23–4.05 (m, 4H), 4.03–3.67 (m, 6H), 3.36–3.01 (m, 5H), 2.7–2.42 (m, 3H), 2.3–2.15 (m, 2H), HRMS (1 : 1 MeOH–THF + NaCl): calc. for $C_{36}H_{39}N_7O_8SNa^+$ 752.2473 and $C_{36}H_{39}N_7O_8SH^+$ 730.26415, found 752.2464 and 730.26535.

(b) Synthesis of [Re(CO)$_3$ (L4-glutathione)]$^+$

A solution of $(NEt_4)_2[Re(CO)_3Br_3]$ (128 mg, 0.0166 mmol) in 20 ml of methanol was added to a solution of **L4**-glutathione (12.1 mg, 0.0166 mmol) in phosphate-buffered saline (PBS, pH 7.5, 1 ml). After stirring at room temperature for 2 h, the solution was diluted with 3.5 ml of H_2O and passed through a Waters Sep-Pak C-18 cartridge. The cartridge was washed with water (3 × 5 ml) and the product was eluted with 2 : 1 methanol–H_2O (1 ml). Evaporation of the eluate afforded [Re(CO)$_3$(**L4**-glutathione)]Br as a colorless solid. Yield ~90%. HRMS (1 : 1 MeOH–THF + NaCl): calc. for $C_{39}H_{39}N_7O_{11}SReNa^+$ 1023.1844 and $C_{39}H_{39}N_7O_{11}SReH^+$ 1001.2064; found: 1023.1869 and 1001.2112.

2.14 Labeling of bovine serum albumin (BSA) and human serum albumin (HSA) with complex [Re(CO)$_3$(L6)]Br

BSA or HSA (24 mg, 358 mmol) and [Re(CO)$_3$(**L6**)]Br (3.26 mg, 3.93 μmol, 11 molar equiv.) were incubated in 1 mL of phosphate buffer (pH 7.4) at room temperature for 8 h. The solid residue was removed by centrifugation. The supernatant was diluted to 3 mL using Tris-HCl buffer solution (pH 7.4), then eluted on a gel filtration column (PD-10, Amersham Bioscience) with the same buffer solution as the eluent. Elution was monitored spectroscopically at 280 nm in 0.5 ml fractions. Fractions containing the protein were collected and the solution was concentrated on the YM-30 (30 000 Mol. Wt. cut off filter) centricon. The Re : BSA ratio of the Re–BSA conjugate was determined to be 1.5 : 1. The concentration of Re(I) complex was determined by its absorbance at 320 nm, assuming the extinction coefficient was the same as that of the free dye.

2.15 Labeling of single-stranded DNA with the complex [Re(CO)$_3$(L6)]Br

A solution of the complex [Re(CO)$_3$(**L6**)]Br (10 mg, 1.3 μmol) was added to a solution of 5-S-ODN (15 nmol) in PBS buffer (pH 7.4) and was left at room temperature for 3 h. After dilution with water, the solution was eluted using a gel filtration column (PD-10, Amersham Bioscience) equilibrated with the same buffer solution. The eluate was fractionated (~1 ml each) and was monitored spectroscopically at 270 nm. Fractions containing the labeled product were collected, and the solution was concentrated using a YM-3 centricon. (3000 D molecular wt. cut off filter).

2.16 Synthesis of *N*-[5-(6′-hydrazinopyridine-3′-carbonyl)-aminopentyl]maleimide (L10)

Method 1. *N*-(5-(6′-Boc-hydrazinopyridine-3′-carbonyl)-maleimide (compound **A** of Scheme 5) was prepared by the literature method.[49] A mixture of compound **A** (3.0 g, 8.9 mmol), 5-aminopentane-1-ol (0.91 g, 8.8 mmol) and EDCI (0.92 g, 8.8 mmol) in 15 ml DMF at 0–4 °C was stirred for 1 h. *N*-(5-(6′-Boc-hydrazinopyridine-3′-carbonyl)aminopentyl-1-ol (**B**) was obtained in 80% yield after chromatographic purification. Triphenylphosphine (1.90 g, 7.26 mmol) was added to a solution of **B** (2.45 g, 5.9 mmol) in 20 ml of dry THF under argon. After cooling to 0–4 °C, maleimide (0.704 g, 7.26 mmol) was added to the reaction mixture. Diethyl diester DEAD (1.85 g, 7.20 mmol) in 10 ml THF was then added slowly to the reaction mixture over a period of 2 h. The solution was allowed to warm to room temperature and was stirred for 8 h. The mixture was then poured into water and extracted with ether. The ether extract was dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography with 0.5–1% methanol–dichloromethane to give the product as colorless solid, *N*′-{5-[5-(2,5-dioxo-2,5-dihydropyrrol-l-yl)pentylcarbamoyl]pyridin-2-yl}hydrazinecarboxylic acid *tert*-butyl ester (**C**). (Yield: *ca.* 0.98 g, 48%) Deprotection of **C** was accomplished by stirring a solution of **C** in 4 M HCl in dioxane solution to give a white precipitate of *N*-[5-(6′-hydrazinopyridine-3′-carbonyl)aminopentyl]maleimide (**L10**) in almost quantitative yield as the hydrogen chloride salt. NMR: ^{1}H (300 MHz, $CDCl_3$): 8.71 (s, 1H), 8.48 (d, 1H), 7.31 (d, 1H), 7.00 (s, 2H), 3.81–3.37 (br m, 4H), 1.81–1.41 (br m, 6H). ^{13}C (300 MH_3, $CDCl_3$): 171.02, 166.21, 161.48, 156.35, 147.30, 137.35, 134.09, 122.09, 105.36, 39.69, 37.47, 28.87, 28.19, 23.96. ESI-MS: *m/z* for $C_{15}H_{19}N_5O_3Na^+$, 340.138008 (calc.), 340.14073 (found).

Method 2. 5-Aminocaproic acid (6.72 g, 51.23 mmol) was added to a stirred solution of maleic anhydride (5.02 g, 51.19 mmol) in acetic acid. A white solid began to precipitate immediately and stirring was continued for 3 h at room temperature. The solid was collected by filtration (yield: 10.91 g, 47.59 mmol, 93%). Without further purification, the product was mixed with 45 ml of acetic anhydride (45 ml). Sodium acetate (2.239 g, 0.027 mmol) was added and the reaction mixture was heated to 90 °C for 2 h. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (100 ml). The solution was washed with water (100 ml) followed by brine (100 ml) and the organic layer was dried over sodium sulfate. The solvent was removed by evaporation to provide an off-white solid, which was purified by short column chromatography (EtOAc) to give 5-maleimideocaproic acid (**D**) as a white solid (yield: 7.42 g, 35.12 mmol, 74%). MP 88–89 °C. ^{1}H NMR ($CDCl_3$): 6.68 (2H, s), 3.51 (2H, t, $J = 7.7$ Hz), 2.34 (2H, t, $J = 7.7$ Hz), 1.56–1.69 (4H, m), 1.29–1.37 (2H, m). ^{13}C NMR ($CDCl_3$): 179.4, 170.8, 134.0, 37.6, 33.7, 28.1, 26.1, 24.1. HRMS-EI (M^+): $C_{10}H_{13}NO_4$: 211.0845 (calc.); 211.0844 (found).

Triethylamine (1.65 ml, 11.84 mmol) and diphenoxyl phosphoryl azide (DPPA, 3.26 g, 11.84 mmol) were added to a stirred

solution of **D** (2.50 g, 11.84 mmol) in *tert*-butanol (30 ml) and the reaction mixture was heated at reflux for 8 h. The solvent was removed by evaporation, and the residue was purified by column chromatography (25% EtOAc–hexane) to give *N*-[5-(*tert*-butoxylcarbonyl)aminopentyl]maleimide (**E**).

Trifluoroacetic acid (10 ml) was added to a solution of compound **E** (520 mg, 1.84 mmol) in methylene chloride (10 ml) and the mixture was stirred at room temperature for 1 h. The solvent was removed by evaporation to yield a brown oil of the *N*-(5-aminopentyl)malemide salt of trifluoroacetic acid (**F**).

6-Boc-hydrazinopyridine-3-carboxylic acid (188 mg, 0.74 mmol) was added to a solution of *N*-(5-aminopentyl)-maleimide trifluoroacetic acid salt (**F**) (220 mg, 0.74 mmol) in 4 ml DMF (4 ml). This was followed by the sequential addition of BtOH (113 mg, 0.74 mmol), EDCl (142 mg, 0.74 mmol) and triethylamine (103 ml, 0.74 mmol). The reaction mixture was stirred at room temperature overnight and then poured into water and extracted with ethyl acetate (30 ml × 2). The organic extracts were combined, washed with brine (30 ml) and the solvent was evaporated to give the crude product, which was further purified by column chromatography (5% MeOH–CH_2Cl_2) to give *N*-[5-(6′-Boc-hydrazinopyridine-3′-carbonyl)aminopentyl]maleimide (**G**). Yield 162 mg (0.39 mmol, 52%).

Mp 139–141 °C; ^{1}H NMR ($CDCl_3$) 8.50 (1H, s), 7.99 (1H, d, J = 9.2 Hz), 6.73 (1H, d, J = 9.2 Hz), 6.68 (s, 2H), 3.55 (2H, t, J = 7.7 Hz), 3.43 (2H, m), 1.46 (9H, s), 1.20–1.65 (6H, m); HSMS-FAB: calc. 418.2090 (M + H); found 418.2104.

A solution of HCl in dioxane was prepared by bubbling HCl into dioxane at a moderate rate for 10 min. Compound **G** (200 mg, 0.48 mmol) was combined with HCl–dioxane 92 ml) and the reaction mixture was stirred at room temperature for 1 h. The solvent was removed by evaporation to furnish an unstable product, HYNIC–maleimide·2HCl (**L10**·2HCl) which was used for subsequent reactions without further purification (yield: 144 mg, 0.41 mmol, 85%). High resolution MS-FAB: calc. 354.1333 (M + H); found 354.1334.

2.17 Coupling reaction of Boc-HYNIC-maleimide (G) with cysteine ethylester (Boc-H)

Cysteine ethylester (4.6 mg, 0.0311 mmol) and Boc-HYNIC-maleimide (10.3 mg, 0.0248 mmol) were dissolved in sodium phosphate buffer (0.4 ml, pH = 7.1) and stirred. After several minutes, a yellow solid was observed, and stirring was continued for 2 h. The solid was collected by filtration, washed thoroughly with ether and dried under vacuum to afford 5.0 mg of product. The product was purified by reverse phase HPLC on a 2.5 × 50 cm Whatman ODS-3 column eluted with a gradient of acetonitrite in 0.1% TFA. Fractions containing the major component were combined, and the solvent was removed to yield 2.7 mg of product. HRMS (1 : 1 MeOH–THF + NaCl): calc. for $C_{25}H_{38}N_6O_7SNa^+$, 589.241489; found, 589.236736.

2.18 Synthesis of [$ReCl_3$(HYNIC-maleimide)$_2$]

A solution of $(NH_4)[ReO_4]$ (0.10 g, 0.37 mmol) and **L10**·2HCl (0.581 g, 1.49 mmol) in 10 ml of MeOH was stirred at room temperature for 6 h. The resultant dark brown precipitate was collected by filtration, washed with diethyl ether (3 × 15 ml) and methanol and dried to give the product (yield: 0.25 g, 72%).

^{1}H NMR (DMSO-d_6): 9.44 (s, 1H, py(1)), 9.13 (s, 1H, py(2)), 8.22 (d, 1H, NH(1))), 8.72 (d, 1H, NH(2)), 8.46 (d, 1H, py(1)), 8.41 (d, 1H, py(2)), 7.88 (d, 1H, py(1)), 7.58 (d, 1H, py(2)), 7.17 (s, 4H, mal)). ESI-MS: m/z for $C_{30}H_{34}Cl_3N_{10}O_6ReNa^+$, 945.11808 (found), 945.117798 (calc.).

2.19 Synthesis of [^{99m}Tc(mannitol)(HYNIC-maleimide)$_2$]

A solution of Sn(II) mannitol was prepared by dissolving 800 mg of mannitol (Aldrich) in dilute HCl followed by the dropwise addition of stannous chloride (1 mg in 0.1 cm^3 6 M NCl). The solutions were purged with nitrogen prior to and during manipulation. The pH of the final Sn(II) mannitol solution was adjusted to between 5 and 6 with NaOH. The final ligand concentration was *ca.* 80 mg mL^{-1} and the Sn(II) concentration [as Sn(II)Cl_2] was 100 mg mL^{-1}. The solution was filtered using a 0.22 mm Millex GS filter, and 0.5 mL aliquots were transferred to sterile rubber-stoppered glass vials, purged with N_2, and stored at −70 °C until use.

^{99m}Tc-mannitol was prepared by adding 2.0 mL of {$^{99m}TcO_4$}$^-$ in saline to a 0.5 mL aliquot of Sn(II) mannitol solution. The mixture was vortexed briefly and incubated at room temperature for 5 min before radiochemical analysis. The final concentration of radioactivity was 5–10 mCi mL^{-1}. Radiochemical analysis was performed by ITLC-sg with acetone and saline as the mobile phases. The R_fs of ^{99m}Tc-mannitol were 0.0 and 1.0 in acetone and saline, respectively. The potential impurities, unreduced TcO_4^- and reduced hydrolyzed TcO_2 have R_fs of 1.0 and 0.0 in these solvent systems.

^{99m}Tc-mannitol solution (500 μL) was added to HYNIC-maleimide (0.25 mg) in 0.5 mL dimethyl sulfoxide and 0.1 mL H_2O buffered with acetate (pH 5.2). The reaction solution was vortexed briefly; subsequently, the reaction mixture was incubated at 100 °C for 45 min. Labeling was monitored using a Vydac C18 column (300 A, 4.6 mm × 25 cm × 5 μm); methanol–H_2O was used to elute the products. C18 HPLC indicated >95% radiochemical purity, with labeling yields >80%.

2.20 Synthesis of [^{99m}Tc(mannitol) (HYNIC-maleimide-cysteine ethylester)]

Method 1. ^{99m}Tc-mannitol solution (100 μL) was added to HYNIC-maleimide-cysteine ethylester (H) (2 mg in acetonitrile). The reaction was stirred and incubated at 70 °C for 30 min. Labeling was monitored using a Vydac C18 column as noted above. C18 HPLC indicated >90% radiochemical purity. However, the product decomposed slowly over a period of several hours to give uncharacterized materials.

Method 2. [^{99m}Tc(mannitol)(HYNIC-maleimide)$_2$] solution (100 μL) from above and cysteine ethylester (1.15 mg, 0.008 mmol) in sodium phosphate buffer (0.05 mL, pH = 7.1) were stirred and then incubated at 40 °C for 45 min. The product was eluted on a Vydac C18 column using methanol–H_2O. Radiochemical purity was monitored as noted previously. Radiochemical purity was >90%. However, labeling yields were *ca.* 70%.

2.21 Synthesis of [^{99m}Tc(CO)$_3$(Lx)]$^+$ derivatives

Labeling with {^{99m}Tc(I)$(CO)_3$}$^+$ was accomplished in two steps using readily prepared IsoLink™ kits (Mallinckrodt) to form the [^{99m}Tc$(CO)_3(H_2O)_3$]$^+$ (100 μl), which was reacted with the appropriate maleimide derivative (0.25 mg) in 0.5 ml of methanol. The sealed vial was heated at 100 °C for 30 min. After cooling, the reaction was checked for purity *via* HPLC using a Vydac C18 column (4.6 mm × 25 cm × 5 μm); methanol was used to elute the products. The purity, analyzed *via* C18 HPLC, showed >95% radiochemical purity. The labeling yields were all >80%, with >60% achievable at levels as low as 1 μg ml^{-1}.

2.22 Synthesis of [^{99m}Tc(CO)$_3$(L5-cysteine ethylester)]

Method 1. [^{99m}Tc$(CO)_3(H_2O)_3$]$^+$ (Mallinckrodt, 100 μL) was heated at 100 °C for 30 min with **L5**-cysteine ethylester (0.20 mg) in 5 mL of methanol. Column chromatography under the conditions outlined above gave the product in >95% radiochemical purity. The labeling yields were >85%.

Method 2. A solution of [^{99m}Tc$(CO)_3$(**L5**)]$^+$ (200 μL, methanol) was added to cysteine ethylester (0.45 mg, 0.0031 mmol) in 0.5 mL of water. After heating at 85 °C for 45 min, the solution was eluted from a Vydac C18 column as described above. Radiochemical purity >95%; labeling yield >75%.

Table 1 Crystallographic data for the structures of **L4**·$CHCl_3$ and $[Re(CO)_3(\mathbf{L5})]Br$

	L4·$CHCl_3$	$[Re(CO)_3(\mathbf{L5})]Br$
Empirical formula	$C_{27}H_{23}Cl_3N_4O_2$	$C_{30}H_{24}BrN_4O_5Re$
M_r	541.84	786.64
Space group	$P\bar{1}$	$P\bar{1}$
T/K	81(2)	82(2)
a/Å	9.2492(8)	11.639(2)
b/Å	11.175(1)	11.829(2)
c/Å	13.096(1)	13.249(2)
α/°	101.983(2)	65.300(3)
β/°	90.707(2)	77.198(3)
γ/°	101.991(2)	72.582(3)
V/Å^3	1293.1(2)	1571.3(5)
Z	2	2
D_c/Mg cm^{-3}	1.392	1.663
μ/mm^{-1}	0.387	5.181
$R1^a$ (all data)	0.0577	0.0602
$wR2^b$	0.1250	0.1299

^{a} $R1 = \sum\|F_o| - |F_c\|/\sum|F_o|$. ^{b} $wR2 = [\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2]^{1/2}$.

2.23 X-Ray crystallography

Crystallographic data for the compounds were collected with a Bruker P4 diffractometer equipped with a SMART CCD system[51] and using Mo-Kα radiation (λ = 0.71073 Å). The data were collected at 90 K and corrected for Lorentz and polarization effects.[52] Absorption corrections were made using SADABS.[53] The structure solution and refinement were carried out using the SHELXTL[54] crystallographic software package. The structures were solved using direct methods, and all of the nonhydrogen atoms were located from the initial solution. After locating all of the nonhydrogen atoms in each structure, the model was refined against F^2, initially using isotropic then anisotropic thermal displacement parameters, until the final value of Δ/σ_{max} was less than 0.001. Crystal data for the compounds are summarized in Table 1. Selected bond lengths and angles for the compounds are collected in Tables 2 and 3.

CCDC reference numbers 253367 and 258469.

See http://dx.doi.org/10.1039/b507096a for crystallographic data in CIF or other electronic format.

3 Results and discussion

3.1 Syntheses and characterization

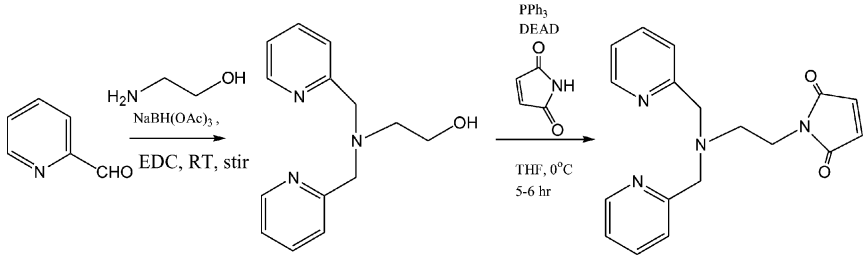
Most methods for the preparation of N-substituted maleimides rely on the reaction of an amine with maleic anhydride followed by dehydration of the intermediate maleamic acid, generally by acid promotion. However, two recently described procedures offer convenient one-step methods.[55,56] Mitsunobu reaction conditions, exploiting the reaction of an alcohol with maleimide, allow facile preparation of alcohol derivatives incorporating the tridentate donor set that effectively coordinates the *fac*-$\{M(CO)_3\}^+$ core. Furthermore, the starting materials for this method are readily available and inexpensive. As shown in Scheme 3, the synthetic procedure involves two steps: the first is the reductive alkylation of an amino alcohol with two

Table 2 Selected bond lengths (Å) and angles (°) for **L4**·$CHCl_3$

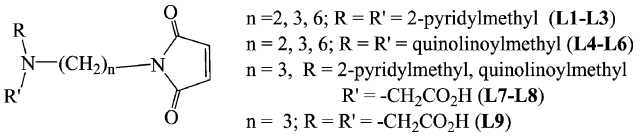
O1–C23	1.2112(18)	C5–C6	1.369(3)
O2–C26	1.2120(18)	C6–C7	1.406(3)
N1–C11	1.4642(17)	C7–C8	1.372(2)
N1–C21	1.4648(17)	C8–C9	1.416(2)
N1–C10	1.4682(18)	C11–C12	1.5055(19)
N3–C12	1.3184(17)	C13–C14	1.364(2)
N3–C20	1.3746(17)	C14–C15	1.4169(19)
N2–C1	1.3180(17)	C15–C16	1.416(2)
N2–C9	1.3730(18)	C15–C20	1.4179(19)
N4–C26	1.3860(17)	C16–C17	1.370(2)
N4–C23	1.3876(18)	C17–C18	1.410(2)
N4–C22	1.4540(18)	C18–C19	1.3734(19)
C1–C2	1.421(2)	C19–C20	1.4149(18)
C1–C10	1.5074(19)	C21–C22	1.525(2)
C2–C3	1.363(2)	C23–C24	1.493(2)
C3–C4	1.417(2)	C24–C25	1.329(2)
C4–C9	1.417(2)	C25–C26	1.493(2)
C4–C5	1.418(2)		
C11–N1–C21	110.28(11)	N3–C20–C19	118.50(12)
C11–N1–C10	111.31(11)	N3–C20–C15	122.28(12)
C21–N1–C10	111.73(11)	C19–C20–C15	119.20(12)
C12–N3–C20	118.14(12)	N1–C21–C22	111.20(11)
C1–N2–C9	118.25(12)	N4–C22–C21	111.05(11)
C26–N4–C23	110.31(12)	O1–C23–N4	125.52(14)
C26–N4–C22	124.35(12)	O1–C23–C24	127.96(14)
C23–N4–C22	125.34(12)	N4–C23–C24	106.52(12)
N2–C1–C2	122.99(13)	C25–C24–C23	108.16(13)
N2–C1–C10	117.51(12)		

Table 3 Selected bond lengths (Å) and angles (°) for $[Re(CO)_3(\mathbf{L5})]Br$

Re1–C1	1.911(5)	N1–C13	1.491(6)
Re1–C2	1.925(5)	N1–C14	1.494(7)
Re1–C3	1.930(5)	N1–C24	1.516(6)
Re1–N1	2.217(4)	N2–C4	1.340(6)
Re1–N3	2.220(4)	N2–C12	1.391(6)
Re1–N2	2.226(4)	N3–C15	1.331(6)
O1–C1	1.159(6)	N3–C23	1.404(6)
O2–C2	1.146(6)	N4–C27	1.383(7)
O3–C3	1.141(7)	N4–C30	1.400(7)
O4–C27	1.213(6)	N4–C26	1.450(7)
O5–C30	1.205(7)		
C1–Re1–C2	87.1(2)	C14–N1–C24	109.4(4)
C1–Re1–C3	87.9(2)	C13–N1–Re1	105.1(3)
C2–Re1–C3	83.8(2)	C14–N1–Re1	110.5(3)
C1–Re1–N1	95.48(17)	C24–N1–Re1	113.5(3)
C2–Re1–N1	176.57(17)	C4–N2–C12	117.6(4)
C3–Re1–N1	93.93(19)	C4–N2–Re1	112.7(3)
C1–Re1–N3	98.10(18)	C12–N2–Re1	129.7(3)
C2–Re1–N3	103.51(18)	C15–N3–C23	119.1(4)
C3–Re1–N3	170.67(18)	C15–N3–Re1	112.1(3)
N1–Re1–N3	78.45(16)	C23–N3–Re1	126.7(3)
C1–Re1–N2	171.15(17)	C27–N4–C30	110.2(5)
C2–Re1–N2	101.74(17)	C27–N4–C26	124.4(4)
C3–Re1–N2	92.13(18)	C30–N4–C26	124.8(5)
N1–Re1–N2	75.69(14)	O1–C1–Re1	176.1(4)
N3–Re1–N2	80.84(15)	O2–C2–Re1	176.7(5)
C13–N1–C14	110.2(4)	O3–C3–Re1	178.3(5)
C13–N1–C24	108.1(4)		



Scheme 3 Representative synthesis of ligands **L1–L9**



Scheme 4 Ligands **L1–L9** of this study.

equivalents of pyridine-2-aldehyde or quinoline-2-aldehyde in dichloroethane under argon to provide the N,N-disubstituted aminoalcohol in greater than 80% yield; this is followed by a Mitsunobu reaction to substitute the alcohol group with the maleimide moiety. As shown in Scheme 4, the general method provides a range of tridentate ligands with a variety of donor groups and tether lengths between the chelating terminus and the maleimide group. Yields are dependent on the alkyl tether and the functional groups at the chelate terminus. Thus, yields are moderately higher for spacers $n = 2$ and 6 compared to $n = 3$. No products were isolated for spacer lengths $n = 4$ and 5, possibly due to the juxtaposition of the –OH and $–NH_2$ groups resulting in cyclization under the Mitsunobu conditions.[57] Yields fall in the range of 50–55% for the bis-picolyl derivatives (**L1–L3**) and in the range of 70–75% for the bis-quinoline compounds (**L4–L6**). Ligands **L1–L3** are obtained as colorless oils, which are unstable at room temperature but which may be stored in air-tight containers at −20 °C for several months. In contrast, **L4–L6** are obtained as crystalline compounds which are indefinitely stable. The rhenium tricarbonyl derivatives $[Re(CO)_3(\mathbf{L}x)]Br$ (x = 1–9) were obtained in good yields from the reactions of $(NEt_4)_2[Re(CO)_3Br_3]$ and the appropriate ligand in refluxing methanol for 3–4 h.

The ligands and their corresponding rhenium compounds were characterized by NMR, IR, and mass spectrometry. The ^{13}C NMR spectra were unambiguously assigned by ^1H–^1H COSY and ^1H–^{13}C HSQC experiments. The spectra of a representative example, $[Re(CO)_3(\mathbf{L6})]Br$, are shown in Fig. 1 and 2. In the ^{13}C NMR, peaks assigned to the *fac*-$Re(CO)_3$ unit were observed at 195.91 and 194.55 ppm with the characteristic 1 : 2 peak height, consistent with the local mirror symmetry through one carbonyl group, the rhenium site and the amine nitrogen. The singlet at 6.68 ppm in the ^1H NMR was assigned to the maleimide proton, corresponding to the ^{13}C NMR singlet at 171.01 ppm. The doublets at 8.31 and 7.86 ppm are correlated and assigned to protons at positions 1 and 2, respectively, of the quinoline unit. The related carbon peaks are found at 141.48 and 120.98 ppm, respectively, in the ^{13}C NMR spectrum. The doublet at 8.39 ppm

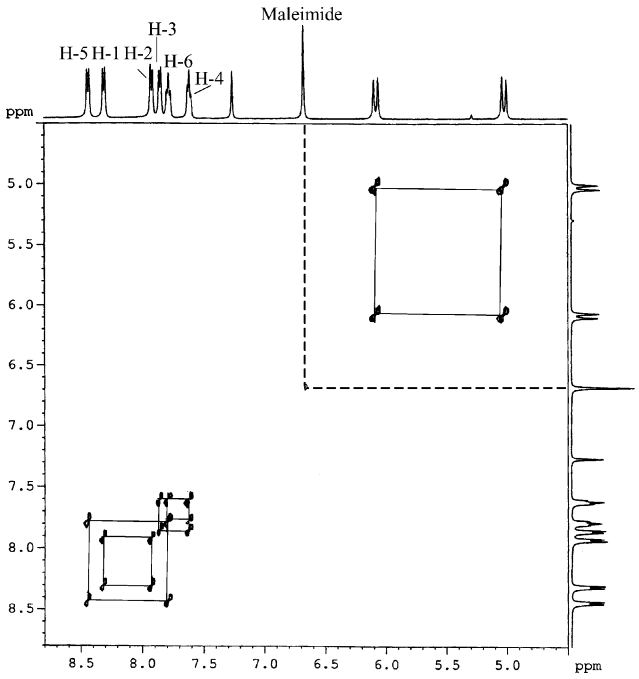


Fig. 2 ^1H–^1H correlation NMR spectrum of $[Re(CO)_3(\mathbf{L6})]Br$ in $CDCl_3$ at room temperature.

correlates with the triplet at 7.86 ppm, ascribing these resonances to the protons at positions 5 and 6. The corresponding carbon peaks occur at 132.92 and 128.64 ppm, respectively. The triplet at 7.65 ppm correlates with the H-5 triplet and with the doublet at 7.86 ppm and is consequently assigned as the H-4 proton, while the doublet is assigned to the H-3 proton. The corresponding ^{13}C NMR peaks are observed at 128.64 and 129.74 ppm, respectively. The vicinal protons adjacent to the quinoline ring were observed as a well separated doublet at 6.03 and 5.03 ppm due to the different environments of the endo and exo protons with respect to the $\{Re(CO)_3\}$ core and the presence of the bulky maleimide group. The correlated carbon resonance appears at 69.13 ppm. The peak at 68.92 ppm was assigned to the carbon atom adjacent to the secondary nitrogen. Other peak assignments for the aliphatic tether were made in the same way.

The pattern of C–O stretching frequencies in the IR spectra of one sharp and intense absorption in the 2029–2019 cm^{-1}

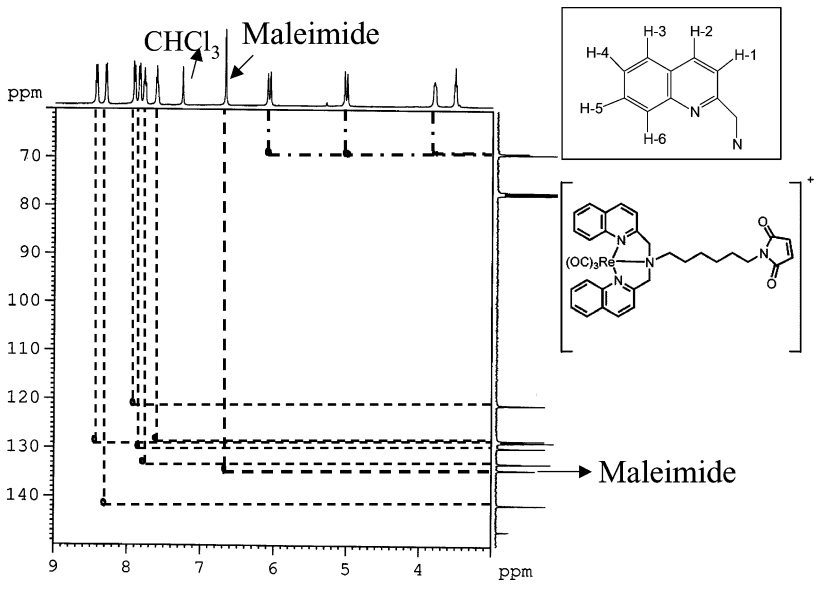


Fig. 1 ^1H–^{13}C correlation NMR spectrum of $[Re(CO)_3(\mathbf{L6})]Br$ in $CDCl_3$ at room temperature. The inset shows the atom-labeling for the quinoline protons.

range and a second broad, intense band in the 1880–1919 cm^{-1} region confirms the facial arrangement of the CO ligands in the complexes. An additional strong absorption at *ca.* 1700 cm^{-1} was ascribed to the maleimide carbonyl group. ESI-MS mass spectra were obtained for the complexes. All exhibit the characteristic isotopic distribution ratio (2 : 3) corresponding to a single rhenium atom (isotopic composition ^{185}Re 40% and ^{187}Re 60%). An additional peak at M − 96 mass units was assigned to the cleavage of the maleimide group from the aliphatic tether.

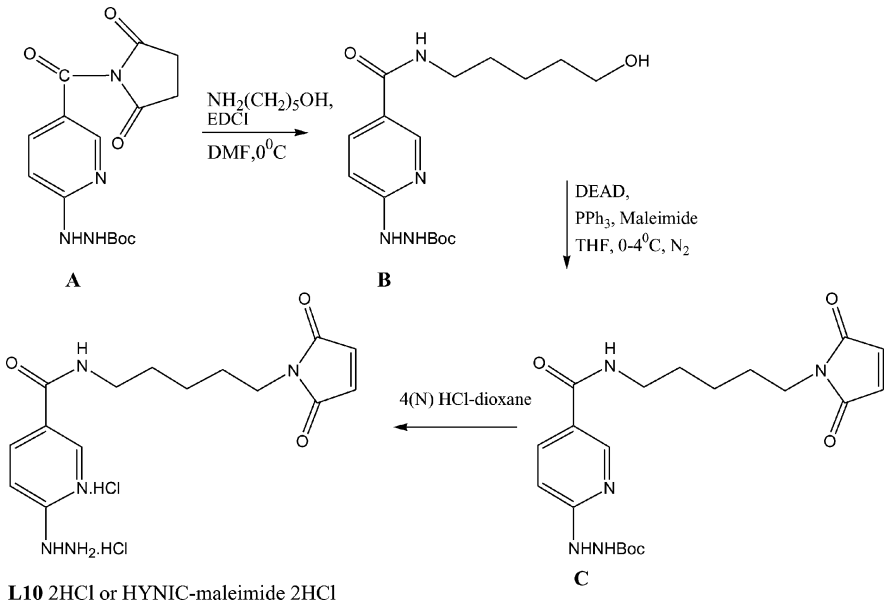
The corresponding HYNIC-maleimide bifunctional chelate was also prepared. The more direct procedure, shown in Scheme 5, proceeds from the reaction of *N*-[5-(6′-Boc-hydrazinopyridine-3′-carbonyl) maleimide[49] with 5-aminopentane-1-ol to give *N*-[5-(6′-Boc-hydrazinopyridine-3′-carbonyl)aminopentyl-1-al (**B**). Reaction of **B** with maleimide in the presence of DEAD and PPh_3 provides Boc-HYNIC-aminopentyl maleimide **C** which upon deprotection in HCl–dioxane provides *N*-[5-(6′-hydrazinopyridine-3′-carbonyl)aminopentyl]-maleimide as the hydrochloride salt. (HYNIC-maleimide·2HCl or **L10**·2HCl) in moderate yield.

Alternatively, the *N*-(5-aminopentyl)maleimide salt of trifluoroacetic acid (compound **F** of Scheme 6) may be prepared from 5-aminocaproic acid in three steps. Reaction of **F** with 6-Boc-hydrazino-pyridine-3-carboxylic acid provides *N*-[5-(6′-Boc-hydrazinopyridine-3′-carbonyl)aminopentyl] maleimide (**G**), which may be deprotected in HCl–dioxane to provide HYNIC maleimide·2HCl (**L10**·2HCl).

The reaction of **L10**·2HCl with $(NH_4)[ReO_4]$ in methanol yields the {rhenium(III)–bishydrazino} core complex $[ReCl_3$-(HYNIC-maleimide)$_2$]. The infrared spectrum of the complex exhibits medium intensity bands at 1550 and 1500 cm^{-1} which are attributed to ν(N=N), consistent with multiple bond character of the organohydrazino ligands. The spectrum also contains a series of bands in the 1600 to 1400 cm^{-1} range assigned to aromatic stretching modes ν(C=C) and ν(C=N). The ^1H NMR spectrum exhibits the multiple ligand peaks between 7.17 and 9.44 ppm, which are characteristic for two inequivalent pyridine moieties of the prototypical $\{Re(\mu_2\text{-}HNNC_5H_3NR)(\mu_1\text{-}NNC_5H_3NHR)\}^{3+}$ core (Scheme 7) which is common for $[MCl_3$(organohydrazine)$_2]$ and related compounds.[44]

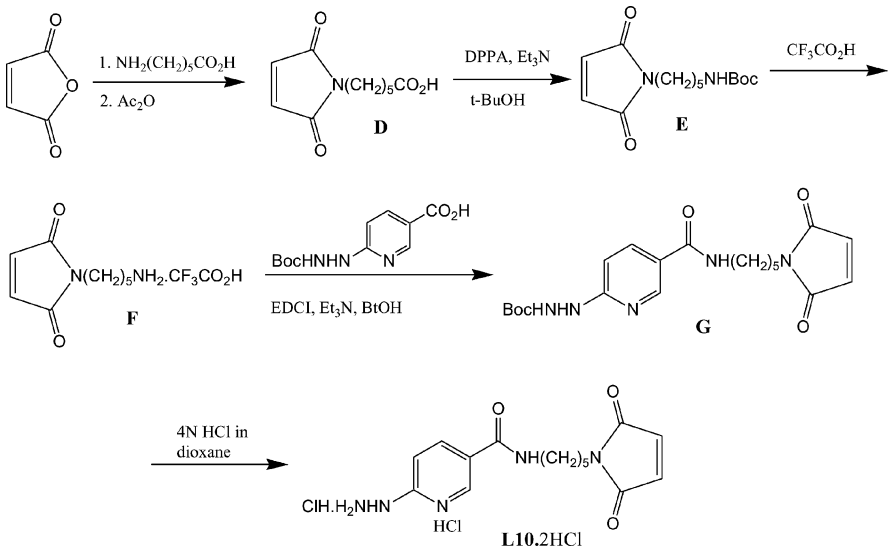
3.2 Crystal structure determinations

The bis-quinolinemaleimide derivatives **L4–L6** were obtained as crystalline, air-stable solids, and the structure of **L4** was obtained, as shown in Fig. 3. The relevant metrical parameters

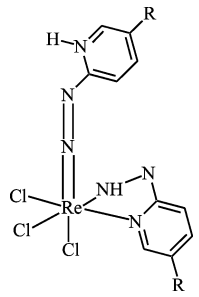


L10 2HCl or HYNIC-maleimide 2HCl

Scheme 5 Preparation of **L10**·2HCl or HYNIC-maleimide·2HCl.



Scheme 6 Alternative synthesis of **L10**·2HCl.



Scheme 7 The $\{Re(\mu_1\text{-}NNC_5H_3NHR)(\mu_2\text{-}NHNC_5H_3NR)\}$ core in $[ReCl_3(NNC_5H_3NHR)(NHNC_5H_3NR)]$.

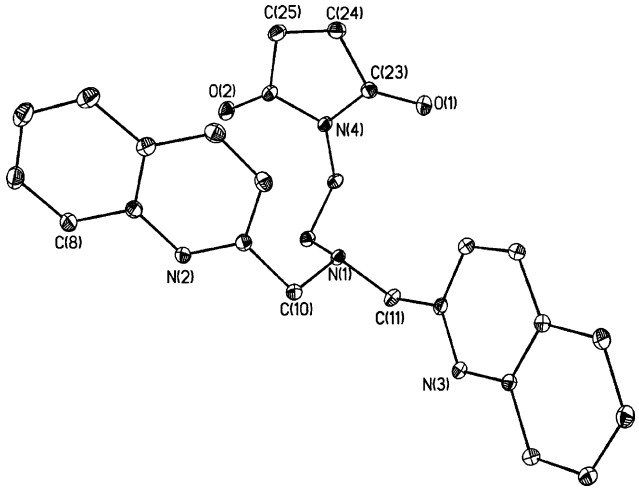


Fig. 3 A view of the structure of **L4**, showing the atom-labeling scheme and 50% probability ellipsoids.

are the C=C bond length of 1.329(2) Å and C=O bond distances of 1.211(1) Å. The C=C bond length is unexceptional and within the expected range for conjugated C=C bonds in a dione system [1.317(8)–1.332(9) Å].[58]

The rhenium compounds $[Re(CO)_3(\mathbf{Lx})]Br$ were obtained as colorless crystalline materials. Crystals of $[Re(CO)_3(\mathbf{L5})]Br$ were isolated from saturated methanol–methylene chloride solution at −20 °C. As shown in Fig. 4, the Re(I) site exhibits distorted octahedral geometry defined by three facially bound carbonyl groups and the amine and quinoline nitrogen donors of the ligand. The crystallographic mirror plane passes through the maleimide plane, the aliphatic backbone and the Re–N1 and

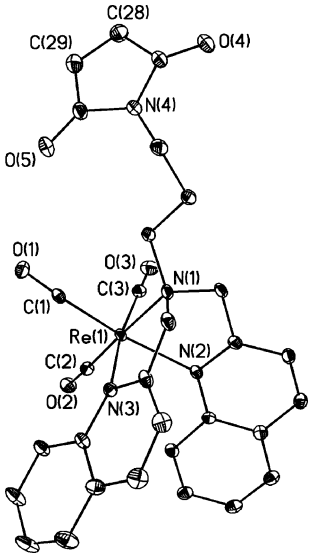


Fig. 4 A view of the structure of $[Re(CO)_3(\mathbf{L5})]^+$, showing the atom-labeling scheme and 50% probability ellipsoids.

Re–C2–O2 bond vectors. As a result of steric demands, the plane of the bulky maleimide group is nearly parallel to the planes of the quinoline groups. As shown in Table 3, the metrical parameters are unexceptional in comparison to unsubstituted maleimide structures[59] and to Re(I)–maleimide compounds.[60] Intermolecular π-stacking interactions are observed between quinoline rings of adjacent molecules, with distances between ring planes of 3.18–3.22 Å with a dihedral angle of 1°.

3.3 Electronic spectroscopy

The ligands **L4–L6** may allow the preparation of isostructural fluorescent $\{Re(CO)_3\}^+$ core complexes and radioactive $\{^{99m}Tc(CO)_3\}^+$ core complexes. Consequently, the fluorescence properties of $[Re(CO)_3(\mathbf{L6})]Br$ were investigated to determine whether the rhenium-based complexes possess suitable characteristics for use as biological probes. The electronic spectrum of $[Re(CO)_3(\mathbf{L6})]Br$ exhibits absorbances at 321 and 412 nm with extinction coefficients of 17 200 and 1250 M^{-1}, respectively (Fig. 5). The absorbance at 321 nm is assigned to a spin-allowed metal-to-ligand charge transfer (MLCT)[dπ(Re) → π*(ligand).[61–63] Excitation of the complex at λ_{ex} = 321 nm gives rise to an intense fluorescence emission at 550 nm. The large Stokes shift is characteristic of this class of luminophores.[64] The emission peak is assigned to a ^{3}MLCT [dπ(Re) → π*(ligand)] excited state on the basis of previous spectroscopic studies of Re(I) tricarbonyl complexes.[65–82] The fluorescence lifetime for $[Re(CO)_3(\mathbf{L6})]Br$ is 16 μs (λ_{em} = 550 nm) in ethylene glycol under Ar atmosphere, which is sufficiently long to overcome the effects of endogenous fluorescence. Cellular autofluorescence can complicate in vitro imaging studies; however, since it occurs on the nanosecond time scale, it can be eliminated using time-gating techniques so long as the probe under investigation has a sufficiently long lifetime. The fluorescence quantum yield of $[Re(CO)_3(\mathbf{L6})]Br$ of 0.015 in ethylene glycol under argon is low but comparable to those reported for other transition-metal based fluorescence probes.[83,84]

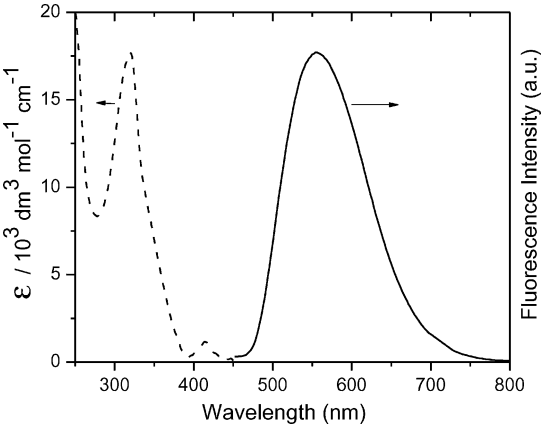
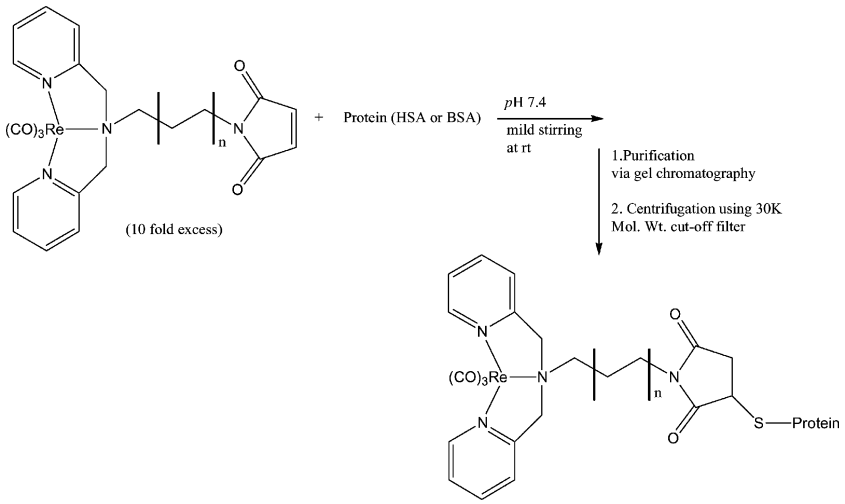


Fig. 5 Electronic absorption (---) and emission (—) spectra of $[Re(CO)_3(\mathbf{L6})]^+$, in ethylene glycol at room temperature.

3.4 Modeling studies: labeling of glutathione, bovine serum albumin and human serum albumin

A preliminary study of the labeling ability of the $Re(CO)_3$-maleimide conjugates was undertaken using the tripeptide glutathione as a model for thiol containing/functionalized biomolecules. The labeling experiments were carried out in PBS (pH 7.4) at room temperature for 10 min. The labeled products were obtained in greater than 95% purity after Sep-pak (C-18) purification and gel-filtration, without the use of HPLC. The thiol containing proteins BSA and HSA were subsequently successfully labeled with the complex conjugates (Scheme 8).

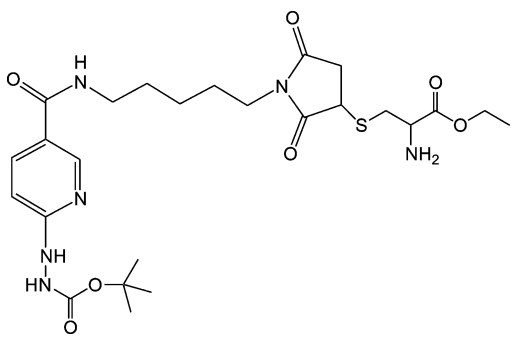


Scheme 8 Labeling of BSA and HSA with the complex conjugates.

The bifunctional chelators can also be used in a "post-labeling" approach in which the chelator is first attached to the biomolecule and then labeled with the $\{M(CO)_3\}^+$ subunit. Thus, the reaction of **L4** with glutathione provides **L4**-glutathione, which upon reaction with $(NEt_4)_2[Re(CO)_3(H_2O)_3]$ yields [Re(CO)$_3$(**L4**-glutathione)]$^+$.

The electronic spectra of the [Re(CO)$_3$(**L6**)]-biomolecule conjugates in tris buffer exhibited transitions at 321 nm and 412 nm, characteristic of ^{1}MLCT [dπ(Re) $\rightarrow$ π*(quin)]. By absorption spectroscopy, the rhenium: protein ratios were 1.56 : 1 and 1.83 : 1 for Re-HSA and Re-BSA, respectively. Upon excitation, the conjugates display intense and long-lived luminescence in tris buffer at room temperature. The luminescence quantum yields of the bioconjugates are similar to those of other biomolecules labeled with luminescent rhenium(I) compounds ($\phi \approx 0.01$–0.02).[85] The emission-lifetime curve was fit with a mono-exponential function to give an estimated lifetime of 13.9 μs for [Re(CO)$_3$(**L6**)]–glutathione and 21.95 and 22.78 μs for [Re(CO)$_3$(**L6**)]–HSA and [Re(CO)$_3$(**L6**)]–BSA, respectively. The luminescence of the conjugates is quenched by oxygen, due to the triplet character of the emission states.

The metal complexes of HYNIC-maleimide did not label biomolecules as cleanly as the Re(CO)$_3$-maleimide conjugates. HYNIC-maleimide was successfully coupled to cysteine ethylester to provide the compound of Scheme 9, as confirmed by HRMS. However, attempts to couple [ReCl$_3$(HYNIC-maleimide)$_2$] to glutathione or BSA yielded intractable mixtures. This observation suggests that the labile chloride ligands must be replaced by a suitable tridentate ligand to fix these coordination sites and prevent complex degradation. This issue has been addressed in a recent report.[86]



Scheme 9

The single stranded DNA 5′-S-ODN has also been labeled with [Re(CO)$_3$(**L6**)]$^+$ in over 85% purity before HPLC purification. The coupled [Re(CO)$_3$(**L6**-DNA primer)] is soluble in water and aqueous buffer solution and exhibits long-lived luminescence. The emission wavelength and emission lifetime are similar to those of the free complex at room temperature in tris-HCl, pH 7.4 ($\tau_F = 17.8$ μs, $\lambda_{em} = 550$ nm).

3.5 Radiolabeling of the ligands L*x*, of L5-cysteine ethylester and of HYNIC-cysteine ethylester

Radiolabeling of the maleimide derivatized ligands was achieved by reacting the ligands with [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^{+1}$ at 90 °C for 20 min. The labeling yields exceeded 85%, with greater than 95% radiochemical purity post HPLC purification, based on radiochromatograms. The products are stable for at least 24 h, and the procedure allows labeling to 1 μg ml^{-1}.

The prelabeled [^{99m}Tc(CO)$_3$(**L5**)]$^+$ readily coupled to the model compound cysteine ethylester in >95% radiochemical purity and >85% labeling yield. Alternatively, in a postlabeling approach, [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ was reacted with (**L5**-cysteine ethylester) to provide the radiolabeled complex in high radiochemical purity and >75% labeling yield.

Similarly, [^{99m}Tc(mannitol) (HYNIC-maleimide)$_2$] was coupled to cysteine ethylester in a prelabeling approach, and [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ was reacted with HYNIC-cysteine ethylester in a postlabeling experiment. While both provided product in >90% radiochemical purity, the labeling yields were lower than those exploiting the tricarbonyl and tridentate chelate technology. Furthermore, the product is considerably more prone to decomposition over time.

4 Conclusions

Bifunctional chelates containing a tridentate donor set for complexation of the $\{M(CO)_3\}^+$ core and a maleimide group for site specific coupling to peptides and proteins containing free thiol groups can be conveniently prepared by exploiting the Mitsunobu reaction. The ligands, as well as their complexes with the $\{M(CO)_3\}^+$ core, react selectively with the sulfhydryl groups of glutathione and proteins to provide the metal-labeled complex-biopolymer conjugates.

The rhenium complexes of the *N*,*N*-bis(quinolinoylmethyl)-amine derivatives [Re(CO)$_3$(**L***x*-maleimide)]Br ($x = 4$–6) exhibit luminescence properties which make these reagents suitable for in vitro fluorescence microscopy studies. The peptide and protein conjugates of [Re(CO)$_3$(**L6**)]$^+$ also exhibit similar photophysical properties as those of the parent compound, but with enhanced fluorescence lifetimes.

Both the *N*,*N*-(bispicolyl) (**L1–L3**) and *N*,*N*-bis(quinolinoylmethyl) (**L4–L6**) based bifunctional chelates can be radiolabeled with ^{99m}Tc in high yield to provide chemically robust complexes. Consequently, this series of bifunctional chelates form both fluorescent Re complexes and the analogous stable ^{99m}Tc complexes. These complexes are readily linked to biopolymers, allowing images obtained on a fluorescent microscope to be directly correlated with *in vivo* radioimaging studies because the structures of the two probes are effectively identical. Preliminary investigations indicate that the rhenium complexes are chemically robust and remain intact during extra- and intracellular processing.

The analogous HYNIC-maleimide was also prepared. However, reactions of the [$ReCl_3$(HYNIC-maleimide)$_2$] complex with appropriate biomolecules yielded intractable mixtures. This observation is consistent with the persistent difficulties with the macroscale purification of Re and Tc HYNIC complexes and with the characterization of ^{99m}Tc-HYNIC reagents on the tracer level.[86]

References

1 K. Schwochau, *Angew. Chem., Int. Ed.*, 1994, **33**, 2258.
2 S. S. Jurisson and J. D. Lydon, *Chem. Rev.*, 1999, **99**, 2205.
3 D. L. Nosco and B. Beaty-Nosco, *Coord. Chem. Rev.*, 1999, **184**, 91.
4 G. Bandoli, A. Dolmella, M. Porchia, F. Refosco and F. Tisato, *Coord. Chem. Rev.*, 2001, **214**, 43.
5 S. Banerjee, M. R. Pillai and N. Ramamoorthy, *Semin. Nucl. Med.*, 2001, **31**, 260.
6 R. Schibli and P. A. Schubiger, *Eur. J. Nucl. Med.*, 2002, **29**, 1529.
7 R. Alberto and U. Abram, *Handb. Nucl. Chem.*, 2003, **4**, 211.
8 R. Alberto, in *Comprehensive Coordination Chemistry II*, ed. J. McCleverty and T. Meyer, Elsevier Ltd., Oxford, UK, 2004, vol. 5, p. 127.
9 S. R. Banerjee, L. Francesconi, J. F. Valliant, J. W. Babich and J. Zubieta, *J. Nucl. Med. Biol.*, 2005, **32**, 1.
10 W. A. Volkert and S. Jurisson, *Top. Curr. Chem.*, 1996, **176**, 123.
11 J. R. Dilworth and S. J. Parrott, *Chem. Soc. Rev.*, 1998, **27**, 43.
12 K. Hashimoto and K. Yoskihara, *Top. Curr. Chem.*, 1996, **176**, 275.
13 W. A. Volkert and T. J. Hoffman, *Chem. Rev.*, 1999, **99**, 2269.
14 X. Guo, F. N. Castellano, L. Li and J. R. Lakowicz, *Anal. Chem.*, 1998, **70**, 632.
15 X. guo, F. N. Castellano, L. Li, H. Szmacinski, J. R. Lakowicz and J. Sipior, *Anal. Biochem.*, 1997, **254**, 179.
16 Y. Shen, B. P. Malival and J. R. Lakowicz, *J. Fluoresc.*, 2001, **11**, 315.
17 K. K.-W. Lo and W.-K. Hui, *Inorg. Chem.*, 2005, **44**, 1992.
18 K. A. Stephenson, S. R. Banerjee, T. Besanger, O. O. Sogebin, M. K. Levadala, N. McFarlane, J. A. Lemon, D. R. Boreham, K. P. Maresca, J. D. Brennan, J. W. Babich, J. Zubieta and J. F. Valliant, *J. Am. Chem. Soc.*, 2004, **126**, 8598.
19 W. M. Yeh, D. G. Sherman and C. F. Meares, *Anal. Biochem.*, 1979, **100**, 152.
20 C. F. Meares and T. G. Wensel, *Acc. Chem. Res.*, 1984, **17**, 202.
21 R. Alberto, R. Schibli, D. Angst, P. A. Schubiger, U. Abram, S. Abram and T. L. A. Kaden, *Transition Met. Chem.*, 1997, **22**, 597.
22 R. Alberto, A. Schibli, A. Egli and P. A. Schubiger, *J. Am. Chem. Soc.*, 1998, **120**, 7987.
23 R. Alberto, R. Schibli and P. A. Schubiger, *J. Am. Chem. Soc.*, 1999, **121**, 6076.
24 R. Wabei, R. Alberto, J. Wielude, R. Finneru, R. Schibli, A. Stichelberger, A. Egli, U. Abram, J. P. Mach, A. Pluckthern and P. A. Schubiger, *Nat. Biotechnol.*, 1999, **17**, 897.
25 A. Amman, C. Decristoforo, I. Ott, M. Wenger, D. Bader, R. Alberto and G. Putz, *Nucl. Med. Biol.*, 2001, **28**, 243.
26 J. Wald, R. Alberto, K. Ortner and L. Andreai, *Angew. Chem., Int. Ed.*, 2001, **40**, 3062.
27 R. Alberto, A. Egli, K. Hegetschweiler, V. Gamlich and P. A. Schubiger, *J. Chem. Soc., Dalton Trans.*, 1994, 2815.
28 R. Alberto, K. Ortner, N. Wheatley, R. Schibli and P. A. Schubiger, *J. Am. Chem. Soc.*, 2001, **123**, 3135.
29 K. A. Stephenson, J. Zubieta, S. R. Banerjee, M. K. Levadala, L. Taggart, L. Ryan, N. McFarlane, D. R. Boreham, K. P. Maresca, J. W. Babich and J. F. Valliant, *Bioconjugate Chem.*, 2004, **15**, 128.
30 S. R. Banerjee, M. K. Levadala, N. Lazarova, L. Wei, J. F. Valliant, K. A. Stephenson, J. W. Babich, K. P. Maresca and J. Zubieta, *Inorg. Chem.*, 2002, **41**, 6417.
31 M. K. Levadala, S. R. Banerjee, K. P. Maresca, J. W. Babich and J. Zubieta, *Synthesis*, 2004, 1159.
32 B. de Bruin, B. Kuhnast, F. Hinnen, L. Yaouaneq, M. Amessou, L. Johannes, A. Samson, R. Boisgard, B. Tavitian and F. Dollé, *Bioconjugate Chem.*, 2005, **16**, 406.
33 M. Brinkley, *Bioconjugate Chem.*, 1992, **3**, 2.
34 D. S. Wilbur, *Bioconjugate Chem.*, 1992, **3**, 433.
35 D. G. Smyth, A. Nagamatur and J. S. Fruton, *J. Am. Chem. Soc.*, 1960, **82**, 4600.
36 S. R. Banerjee, J. W. Babich and J. Zubieta, *Chem. Commun.*, 2005, 1784.
37 S. Liu and D. S. Edwards, *Chem. Rev.*, 1999, **99**, 2235, and references therein.
38 S. Liu, D. S. Edwards and J. A. Barrett, *Bioconjugate Chem.*, 1997, **8**, 621.
39 D. A. Schwartz, M. J. Abrams, M. M. Hansen, F. E. Gul, S. K. Larsen, D. Rauh and J. Zubieta, *Bioconjugate Chem.*, 1991, **2**, 333.
40 M. J. Abrams, J. Juweid, C. I. ten Kate, D. A. Schwartz, M. M. Hauser, F. E. Gaul, A. J. Fucello, R. H. Rubin, H. W. Strauss and A. J. Fischman, *J. Nucl. Med.*, 1993, **34**, 1964.
41 J. W. Babich and A. J. Fischman, *Nucl, Med. Biol.*, 1995, **22**, 25.
42 Z.-F. Su, G. Liu, S. Gupta, Z. Zhu, M. Rusckowski and D. J. Hnatowich, *Bioconjugate Chem.*, 2002, **13**, 561.
43 S. Liu, A. R. Harris, N. E. Williams and D. S. Edwards, *Bioconjugate Chem.*, 2002, **13**, 881.
44 D. J. Rose, K. P. Maresca, T. Nicholson, A. Davison, A. G. Jones, J. Babich, A. Fischman, W. Graham, J. R. D. DeBord and J. Zubieta, *Inorg. Chem.*, 1998, **37**, 2701, and references therein.
45 J. Blake, J. R. Tretter, G. J. Jusahz, W. Bonthrone and H. Rapoport, *J. Am. Chem. Soc.*, 1966, **88**, 4061.
46 J. E. Bishop, J. F. O'Connell and H. Rapoport, *J. Org. Chem.*, 1991, **56**, 5079.
47 J. N. Demas and G. A. Crosby, *J. Phys. Chem.*, 1971, **75**, 991.
48 K. Nakamaru, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2697.
49 A. J. Fischman, M. F. Solomon, J. W. Babich, M. J. Abrams, R. J. Callahan, H. W. Strauss and R. H. Rubin, *J. Nucl. Med.*, 1994, **34**, 1964.
50 N. Lazarova, S. James, J. W. Babich and J. Zubieta, *Inorg. Chem. Commun.*, 2004, **7**, 1023.
51 *SMART, Data Collection Software, version 4.050*, Siemens Analytical Instruments Inc., Madison, WI, 1996.
52 *SAINT, Data Reduction Software, Version 4.050*, Siemens Analytical Instruments Inc., Madison, WI, 1996.
53 G. M. Sheldrick, *SADABS*, University of Göttingen, 1996.
54 *SHELXTL PC*, Siemens Analytical X-Ray Instruments Inc., Madison, WI, 1993.
55 M. A. Walker, *J. Org. Chem.*, 1995, **60**, 5352.
56 R. Y. Reddy, S. Kondo, T. Toru and U. Ueno, *J. Org. Chem.*, 1996, **61**, 2652.
57 H. D. King, G. M. Dubowchik and M. A. Walker, *Tetrahedron Lett.*, 2002, **43**, 1987.
58 F. H. Allen, O. Kennard, D. G. Watson, L. Brammer, A. G. Orpen and R. Taylor, *J. Chem. Soc., Perkin Trans. 2*, 1987, S1–S19.
59 P. J. Cox and S. F. Parker, *Acta Crystallogr., Sect. C*, 1996, **52**, 2578.
60 K. K.-W. Lo, W.-Ki. Hui, C.-M. Ng and K.-K. Cheung, *Inorg. Chem.*, 2002, **41**, 40.
61 J. N. Demas and G. A. Crosby, *J. Phys. Chem.*, 1971, **75**, 991.
62 K. Nakamaru, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2697.
63 W. Bannwarth, *Helv. Chim. Acta*, 1988, **71**, 1517.
64 J. D. Dattelbaum, O. O. Abugo and J. R. Lakowicz, *Bioconjugate Chem.*, 2000, **11**, 533.
65 K. K. W. Lo, W. K. Hui, D. C. M. Ng and K. K. Cheung, *Inorg. Chem.*, 2002, **41**, 40.
66 M. Wrighton and D. L. Morse, *J. Am. Chem. Soc.*, 1974, **96**, 998.
67 B. P. Sullivan, *J. Phys. Chem.*, 1989, **93**, 24.
68 G. Tapolsky, R. Duesing and T. J. Meyer, *Inorg. Chem.*, 1990, **29**, 2285.
69 A. P. Zipp, L. Sacksteder, J. Streich, A. Cook, J. N. Demas and B. A. DeGraff, *Inorg. Chem.*, 1993, **32**, 5629.
70 D. J. Stufkens and A. Vlcek, *Coord. Chem. Rev.*, 1998, **177**, 127.
71 P. Chen, M. Curry and T. J. Meyer, *Inorg. Chem.*, 1989, **28**, 2771.
72 V. W. W. Yam, V. C. Y. Lau and K. K. Cheung, *J. Chem. Soc., Chem. Commun.*, 1995, 259.
73 L. Wallace, C. Woods and D. P. Rillema, *Inorg. Chem.*, 1995, **34**, 2875.
74 L. A. Lucia, K. Abbous and K. S. Schanze, *Inorg. Chem.*, 1997, **36**, 6224.
75 V. W. W. Yam, K. K. W. Lo, K. K. Cheung and R. Y. C. Kong, *J. Chem. Soc., Dalton Trans.*, 1997, 2067.

76 H. Kunkely and A. Vogler, *Inorg. Chem. Commun.*, 2000, **3**, 188.
77 V. W. W. Yam, K. K. W. Lo, K. K. Cheung and R. Y. C. Kong, *J. Chem. Soc., Chem. Commun.*, 1995, 1191.
78 N. B. Thornton and K. S. Schanze, *Inorg. Chem.*, 1993, **32**, 4994.
79 H. D. Stoeffler, N. B. Thornton, S. L. Temkin and K. S. Schanze, *J. Am. Chem. Soc.*, 1995, **117**, 7119.
80 A. J. DiBilio, B. R. Crane, W. A. Wehbi, C. N. Kiser, M. M. Abu-Omar, R. M. Carlos, J. H. Richards, J. R. Winkler and H. B. Gray, *J. Am. Chem. Soc.*, 2001, **123**, 3181.
81 X. Q. Guo, F. N. Castellano, L. Li, H. Szmacinski, J. R. Lakowicz and J. Sipior, *Anal. Biochem.*, 1997, **254**, 179.
82 X. Q. Guo, F. N. Castellano, L. Li and J. R. Lakowicz, *Anal. Chem.*, 1998, **70**, 632.
83 J. D. Dattelbaum, O. O. Abugo and J. R. Lakowicz, *Bioconjugate Chem.*, 2000, **11**, 533.
84 K. K. W. Lo, K. H. K. Tsang, W. K. Hui and N. Zhu, *Chem. Commun.*, 2003, 2704.
85 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Kluwer Academic/Plenum Publishers, New York, 2nd edn, 1999.
86 S. R. Banerjee, K. P. Maresca, J. W. Babich, W. A. Graham, M. Barzane, Q. Dong, A. J. Fischman and J. Zubieta, *Bioconjugate Chem.*, 2005, **16**, 885.