Site directed maleimide bifunctional chelators for the $M(CO)_3^+$ core ($M = {}^{99m}Tc$, Re)†

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A series of 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 has been prepared and their $Re(CO)_3^+$ complexes and glutathione conjugates structurally characterized. The flexibility of design allows preparation of ligands suitable for both fluorescence imaging, radioimaging and radiotherapeutic studies of proteins and peptides as well as other biopolymers using site specific conjugation.

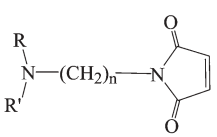
The significant development in recent years of the chemistry of the Group 7 congeners technetium and rhenium reflects the applications of the radionuclides ^{99m}Tc and $^{186,188}Re$ in the development of diagnostic and therapeutic radiopharmaceuticals, respectively.[1,2] Technetium-99m is the most widely used radionuclide in diagnostic medicine owing to its ideal nuclear properties, low cost and widespread availability.[3,4] In addition to its important radioisotopes, the nonradioactive isotopes of rhenium ($^{185,187}Re$) provide both model chemistry for the radioactive analogues and materials which may serve as luminescent probes.[5] We have recently reported the synthesis of a series of M(I) binding ligands (M = Tc, Re) based on a lysine derived bis(pyridyl)amine, referred to as a single amino acid chelate (SAAC), which forms inert complexes with the chemically robust $M(CO)_3^+$ core ($M = {}^{99m}Tc$, Re). SAAC can be readily incorporated into peptides using conventional solid phase synthetic methods as if it were a natural amino acid.[6] Furthermore, we have prepared the Re(I) complex of the bis-quinolinyl amine analog of SAAC that exhibits attractive fluorescence properties.[7]

We now have turned our attention to the application of this coordination chemistry to site directed modification of peptides and proteins. Many bifunctional chelates can be labeled with a variety of radiometals,[8] primarily through acylation of the primary amine groups of lysine residues. While a generally useful approach, there are significant limitations to selective conjugation of specific amino groups in the protein or peptide of interest. Because the free thiol functional group is not very common in most proteins or peptides and can be labeled with high chemoselectivity, thiol-reactive reagents provide a means of selectively modifying a biopolymer at a defined site. Additionally, using solid phase peptide synthesis or protein engineering, it is possible to incorporate a cysteine residue at a defined position in peptides and proteins, respectively. Furthermore, methods for effective PEG conjugation of proteins have been described in which PEG is activated with a maleimide group capable of specifically forming a covalent bond with free thiols on the surface of proteins.[9] For these reasons, maleimide linkers[10] are of particular interest to bioconjugate chemistry as the stoichiometry and site attachment are predictable and significant alterations in biological activity may be avoided. In this study, we report the syntheses and characterizations of novel maleimide-dpa and maleimide-dqa (dpa = dipicolylamine; dqa = diquinolinoyl methylamine) derivatives for ^{99m}Tc labeling of peptides and of their $Re(CO)_3^+$ complexes.

The bifunctional chelate **L1** was prepared from the reaction of bis(picolyl)aminopropanol, prepared as previously described,[6c] with maleimide using Mitsunobu conditions.[10]‡ Appropriate modifications allowed the synthesis of a family of bifunctional chelates of the type maleimide-$(CH_2)_n$NRR′ [n = 2, 3, 6, R = R′ = quinolinoylmethyl, (**L2–L4**); n = 3, R = R′, $-CH_2CO_2H$ (**L5**); R = $-CH_2CO_2H$, R′ = pyridylmethyl and quinolinoylmethyl, respectively- (**L6**, **L7**)] (Scheme 1) providing a range of effective tridentate donors for the $M(CO)_3^+$ unit and allowing the isolation of neutral, cationic or anionic complexes $[M(CO)_3(\mathbf{L})]^n$, depending on the donor group identity. Ligand **L2** has been structurally characterized after recrystallization from chloroform as **L2**·$HCCl_3$.§ Furthermore, the bifunctional chelates **L2–L4** provide ligands whose Re(I) complexes should exhibit fluorescence.[7]

The $Re(CO)_3^+$ complexes of **L1–L4** were prepared by the reaction of $(NEt_4)_2[Re(CO)_3Br_3]$[11] with the appropriate ligand. As shown in Fig. 1, the structure of $[Re(CO)_3(\mathbf{L3})]Br$ consists of discrete $[Re(CO)_3(\mathbf{L3})]^+$ cations and Br^- anions.§ The Re(I) site exhibits distorted octahedral geometry through coordination to three carbonyl groups in a *meridional* arrangement, the amine nitrogen and the nitrogen donors of the two quinoline units.

To assess the coupling of the maleimide derivatized ligands, reactions between **L1**, **L3**, **L4** and glutathione (glu-cys-gly) were performed by reacting **L** and glutathione in a 1:1 molar ratio in PBS buffer at pH 7.4, followed by reverse phase column chromatographic (Sep-Pak) purification. Yields of greater than



n = 3; R = R' = 2-pyridylmethyl (**L1**)
n = 2, 3, 6; R = R' = quinolinoylmethyl (**L2-L4**)
n = 3; R = R' = -CH_2CO_2H (**L5**)
n = 3; R = 2-pyridylmethyl, R' = -CH_2CO_2H (**L6**)
n = 3; R = 2-quinolylmethyl, R' = -CH_2CO_2H (**L7**)

Scheme 1

† Electronic supplementary information (ESI) available: A: Labeling of **L1**–**L4** with $^{99m}Tc(CO)_3^+$. B: Absorption and fluorescence studies. C: X-Ray structure of **L2**. See http://www.rsc.org/suppdata/cc/b4/b417588c/

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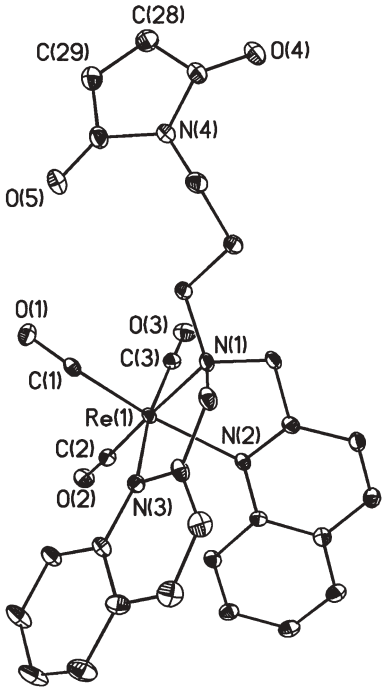
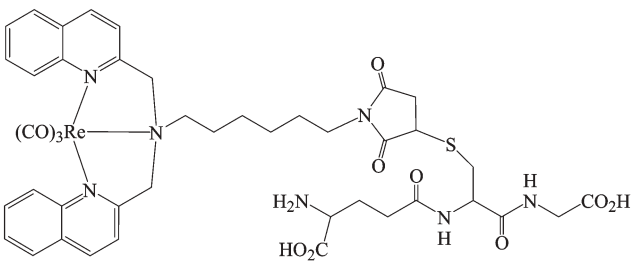


Fig. 1 A view of the structure of $[Re(CO)_3(\mathbf{L3})]Br$, showing the atom-labeling scheme and 50% probability ellipsoids.

90% of the peptide-ligand-maleimide conjugate were achieved. Alternatively, the $[Re(CO)_3(\mathbf{L})]Br$ complexes could be directly coupled to the glutathione under conditions similar to those described above to produce the metalated conjugates (Scheme 2) in 80–90% yield.

Radiolabeling of the maleimide derivatized ligands may be achieved by reacting the ligands with $[^{99m}Tc(CO)_3(H_2O)_3]^+$,[12] at 90 °C for 20 min. The labeling yields were greater than 85%, with greater than 95% radiochemical purity before HPLC purification, based on radiochromatograms (ESI, Fig. S1†). The products are stable for at least 24 h, and the procedure allows labeling to 1 μg ml^{-1}.

The flexibility of the ligand design permits the introduction of chelates, as noted for ligands **L2–L4**, which would allow the preparation of fluorescent and radioactive chelate complexes, which are isostructural. The long emission lifetime and large Stokes' shift of fluorescent Re complexes are particularly attractive in this application. The complex $[Re(CO)_3(\mathbf{L4})]Br$ exhibits a strong UV absorbance with a maximum at 321 nm. Upon photoexcitation, long-lived fluorescence emission at 550 nm is produced with a lifetime of 15.9 μsec in ethylene glycol under Ar, assigned to a



Scheme 2

^{3}MLCT [dπ(Re) → π*(ligand)] transition. The measured quantum yield of the complex (Φ = 0.015) in ethylene glycol under argon atmosphere is low but not significantly different from the fluorescent quantum yields reported for other transition metal based complexes currently employed as fluorescent probes.[7,13] Furthermore, the fluorescence lifetime of the complex of *ca.* 16 μs renders cellular imaging practical. Peptide and protein conjugates of $[Re(CO)_3(\mathbf{L4})]^+$ also exhibit similar photophysical properties as the parent complex. For example, $[Re(CO)_3(\mathbf{L4}$-glutathione$)]^+$ exhibits a strong absorbance at *ca.* 322 nm in ethylene glycol, giving rise to peak fluorescence intensity at 555 nm, with a lifetime of *ca.* 14 μs under Ar atmosphere.

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Notes and references

‡ *Representative syntheses*: *Bis(picolyl)aminopropylmaleimide* (***L1***): DEAD (0.297 g, 1.71 mmol) was added dropwise over a period of 15 min to a solution of bis(picolyl)aminopropanol (0.364 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 diethyl ether. The ether extract was dried over Na_2SO_4 and concentrated. The residue was purified through flash column chromatography with 2% MeOH–CH_2Cl_2 to give the product as a yellow oil in 55% yield. ^{1}H NMR (300 MHz, MeOH-d_4): δ 8.49 (d, 2H), 7.61 (t, 2H), 7.46 (t, 2H), 7.05 (d, 2H), 6.66 (s, 2H), 3.77 (s, 4H), 3.48 (t, 2H), 2.5 (t. 2H), 1.75 (t, 2H). ^{13}C NMR: δ 170.69, 159.57, 148.95, 136.42, 134.04, 123.05, 121.98, 60.23, 51.44, 36.04, 26.17. HRMS (1:1 MeOH–THF + NaCl): Calc. for $C_{19}H_{20}N_4O_2Na^+$ *m/z* 359.1478; found 359.14608. *Bis(qinolinoylmethyl)aminopropylmaleimide* (***L3***): this was prepared in a similar fashion to **L1**, with bis(quinolinolymethyl)aminopropanol in place of bis(picolyl)aminopopanol. ^{1}H NMR (300 MHz, $CDCl_3$): δ 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 NMR (300 MHz, MeOH-d_4): δ 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 $C_{27}H_{24}N_4O_5Na^+$ *m/z* 459.179144; found 459.17710. *[Re(CO)$_3$(**L1**)]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 **L1** (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 CH_2Cl_2. 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 MeOH–CH_2Cl_2 solution gave the purified product in 81% yield (278 mg). ^{1}H NMR (300 MHz, MeOH-d_4): δ 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 NMR (300 MHz, MeOH-d_4): δ 197.21, 196.59, 172.76, 162.12, 153.25 141.81, 135.78, 127.13, 125.04, 69.52, 86.85, 36.14, 26.08. HRMS (MeOH): Calc. for $C_{22}H_{20}N_4O_5Re^+$ *m/z* 607.0985; found 607.099. $[Re(CO)_3(\mathbf{L3})]Br$ and $[Re(CO)_3(\mathbf{L4})]Br$ were prepared in an analogous fashion to $[Re(CO)_3(\mathbf{L1})]Br$. Yields: ~80%. *[Re(CO)$_3$(**L3**)]Br*: ^{1}H NMR (300 MHz, MeOH-d_4): δ 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 NMR (300 MHz, MeOH-d_4): δ 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 $C_{30}H_{24}N_4O_5Re^+$ *m/z* 707.129866; found 707.12556.*[Re(CO)$_3$(**L4**)]Br*: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.28–8.23 (m, 4H), 7.79 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.1 Hz, 2H), 7.60 (d,

J = 8,7 Hz, 2H), 7.48 (t, J = 6.0 Hz, 2H), 6.55 (s, 1H), 5.25 (m, 4H), 3.97 (m, 2H), 3.72 (m, 2H), 2.36 (m, 2H). ^{13}C NMR, 300 MHz, MeOH-d_4): δ 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 °C. IR (KBr): 2023, 1924, 1700, 1513. HRMS (1:1 MeOH–THF): Calc. for $C_{33}H_{30}N_4O_5Re^+$ m/z 749.176816, found 749.17669. *Coupling of [Re(CO)$_3$(**L4**)]Br with glutathione*: A solution of [Re(CO)$_3$(**L4**)]Br (24 mg, 0.029 mmol) in DMF (0.7 ml) was added dropwise to a solution of glutathione (9 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)$_3$(**L4**)]Br (R_f 0.5; MeOH–CH_2Cl_2 2:98) and the appearance of one of the major product (R_f 0.6; n-BuOH–MeOH–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 MeOH–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): δ 8.78 (m, 4H), 8.24 (d, J = 8.7 Hz, 2H), 8.11 (t, J = 6.9 Hz, 2H), 7.9–7.76 (m, 4H), 5.30–4.6 (m, 5H), 3.95–3.81 (m, 3H), 3.81–3.72 (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 $C_{43}H_{47}N_7O_{11}ReSNa^+$ m/z 1079.25039 and $C_{43}H_{47}N_7O_{11}ReSH^+$ m/z 1057.268448, found 1079.24893 and 1057.27396.

§ *Crystal data*: **L2**·$HCCl_3$, $C_{27}H_{23}Cl_3N_4O_2$, triclinic, $P\bar{1}$, a = 9.2492(8), b =11.175(1), c = 13.096(1) Å, α = 101.983(2), β = 90.707(2), γ = 101.991(2)°, V = 1293.1(2) Å^3, Z = 2, D_c = 1.392 g cm^{-3}; structure solution and refinement converged at R_1 = 0.0577 and wR_2 = 0.1250 for 8452 reflections (all data to θ = 31.50°; ESI, Fig. S4). [Re(CO)$_3$(**L3**)]Br, $C_{30}H_{24}BrN_4O_5Re$, triclinic, $P\bar{1}$, a = 11.639(2), b = 11.829(2), c = 13.249(3) Å, α = 65.300(3), β = 77.198(3), γ = 72.582(3)°, V = 1571.3(5) Å^3, Z = 2, D_c = 1.663 g cm^{-3}; structure solution and refinement converged at R_1 = 0.0602 and wR_2 = 0.1299 for 10184 reflections (all data to θ = 31.50°). CCDC 253367 and 258469. See http://www.rsc.org/suppdata/cc/b4/b417588c/ for crystallographic data in .cif or other electronic format.

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