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Structural characterisation and bioconjugation of an active ester containing oxorhenium(V) complex incorporating a thioether donor

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A simple new 2,3,5,6-tetrafluorophenyl ester containing diamide–thioether–thiol bifunctional chelating agent LH_3, $HS(CH_2)_2SCH_2C(O)NHCH_2C(O)NH(CH_2)_3C(O)OC_6HF_4$, has been synthesised. The key intermediates were prepared using standard peptide chemistry procedures. Reaction of LH_3 with $[Bu_4N][ReOCl_4]$ formed an uncharged oxorhenium(V) complex, which was characterised by X-ray structural analysis. The five-co-ordinate complex showed approximately square-pyramidal geometry with an apical oxo group and a basal ligand set comprising a deprotonated thiol group, two deprotonated amide groups, and a thioether group. A second complex of stoichiometry $[ReO(LH_2)_2]Cl$ was formed by reaction of LH_3 with a rhenium(V) gluconate intermediate in water at pH 4.7. The 1 : 1 complex [ReOL] was conjugated with the small protein N-TIMP-2 by aminolysis at a lysine residue, to form a 1 : 1 adduct as established by electrospray mass spectrometry.

Introduction

In the diagnosis and therapy of human diseases by use of target-specific delivery of radiolabelled biomolecules, the development of improved bifunctional chelating agents (BCA's) has an important role. These chelators should be capable of linking small peptides, proteins, antibodies or their fragments with a metallic radionuclide. Prominent among the metallic radionuclides suitable for these purposes are technetium-99m and rhenium-186/188. The benefits of these compared to other radionuclides are well known.[1] However, although several designs have been used in practice with success, BCA's for Tc and Re that meet all criteria of an ideal radiopharmaceutical do not yet exist. Some desirable properties are a high labelling efficiency under mild conditions, minimal isomerism, and good *in vivo* stability and pharmacokinetics. Also, the labelling procedure should be as simple as is practically feasible so that labelling can be performed quickly and with minimal handling and purification, because of the hazard and cost associated with the radionuclides.

Strategies for labelling a biological molecule using a bifunctional chelator may be classified as (1) "pre-formed chelate" approach (in which the metal is chelated first and the complex is then conjugated to the biomolecule) and (2) "post-labelling" or final-step-labelling (in which the metal is added to a biomolecule–chelator conjugate).[2] The latter is the theoretically preferred approach because the number of steps involving the radionuclide is minimised. Unfortunately, ligands which complex the metal with the required speed and specificity in the presence of the biomolecule (which itself contains strong chelating groups), are not yet available and the preformed chelate approach is required with the present generation of chelators. New ligands suitable for post-labelling are now being sought.

Several co-ordination systems have already been used in the design of BCA's for Tc and Re. These include the large group of N_2S_2 ligands such as diaminedithiols,[3] monoamide–monoaminedithiols,[4] diamidedithiols,[4,5] and the N_3S triamidethiols.[6] Other examples are propylene amines (PnAO),[7] tetraamines,[8] boronic adducts of technetium dioximes (BATO's),[9] and hydrazinonicotinamides (HYNIC).[10] Some comparative reviews are available.[2a,11]

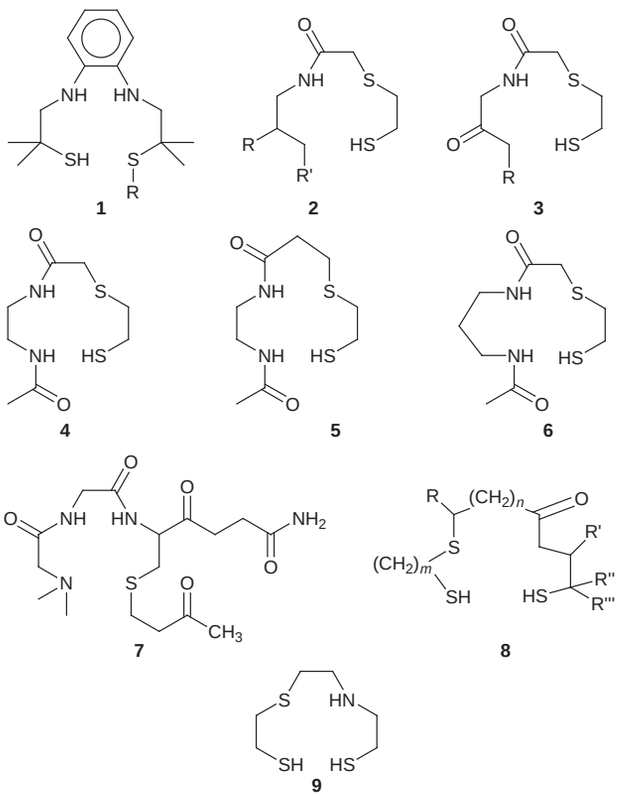
Tetradentate ligands containing a thioether donor have recently been considered as a new type of chelator for technetium or rhenium. Only a few examples (see below) have been described hitherto. McBride and co-workers described the synthesis and biological evaluation of a diamino–thioether–thiol chelator **1** for ^{99m}Tc.[12] Hom and co-workers developed an amino–amido–thioether–thiol ligand **2** and prepared the oxorhenium(V) complex that mimics the steroid 5α-dihydrotestosterone (DHT).[13] Scheunemann and co-workers[14] and Kelly and co-workers[15] synthesised N-terminal derivatised diamido–thioether–thiol ligand **3** and its structural variants **4**–**6**, though metal complexes of these have not been characterised. Technetium complexes of the amino–diamido–thioether type **7** have been described by Wong and co-workers.[2b] Recently Archer and co-workers[16] and Hilger and co-workers[17] prepared several amido–thioether–dithiol ligands **8** and an amino-analogue **9**,[16] and their Re and Tc complexes.

BCA's contain an activated group that is able to link specifically with corresponding reactive sites of the biomolecule. We chose the 2,3,5,6-tetrafluorophenyl ester function because of its well known behaviour towards peptides and proteins containing primary amine residues.[4,5b,5c] This type of functionalisation of multidentate ligands often needs time-consuming multistep syntheses. The aim in this work was to produce an easily synthesised BCA containing a thioether donor, and to characterise its suitability for complexing Tc and Re. Here we describe a simple synthesis (using standard procedures of peptide chemistry) of such a ligand (LH_3), the crystal structure of its complex with rhenium(V), and its bioconjugation with a small protein, N-TIMP-2 (the active domain of human tissue inhibitor of metalloproteinases-2).[18]

Results and discussion

Ligand synthesis

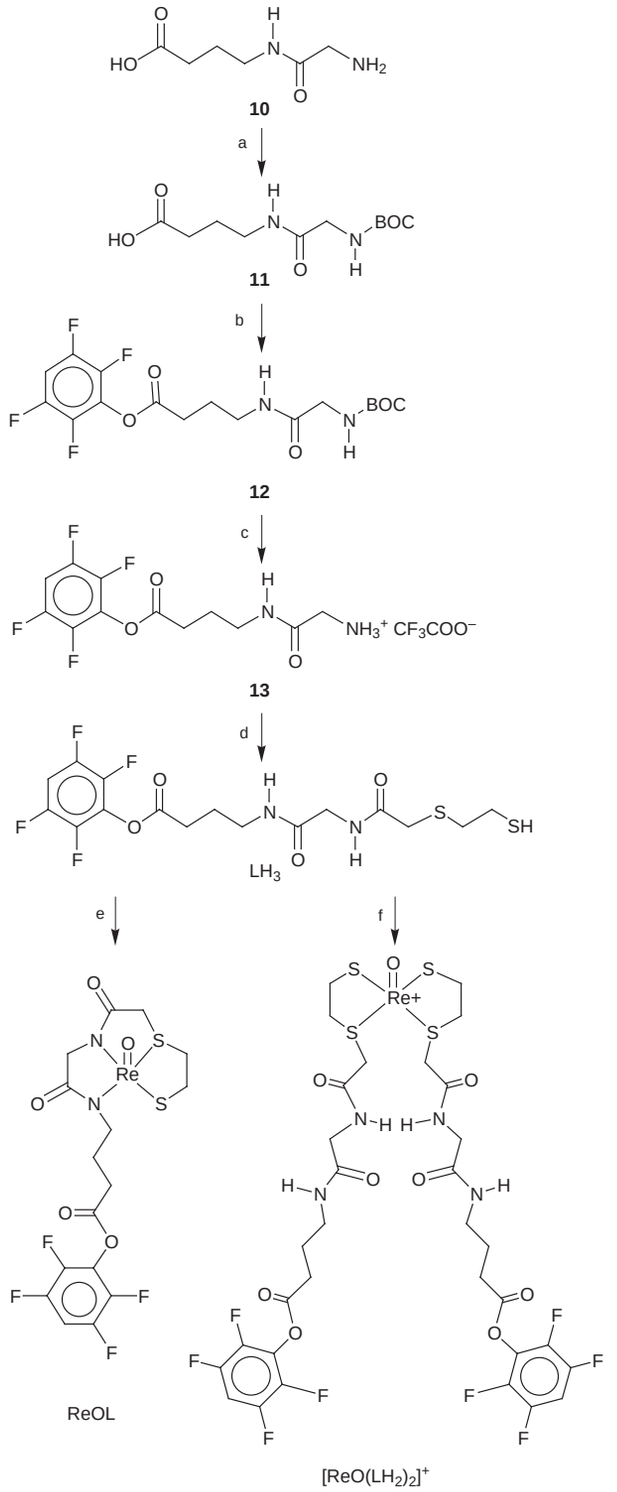
The commercially available dipeptide **10** was N-protected with di-*tert*-butyl dicarbonate to give the N-BOC product **11**



(Scheme 1). Coupling with 2,3,5,6-tetrafluorophenol to form **12** was done using 1,3-dicyclohexylcarbodiimide in tetrahydrofuran. Deprotection of the amine with trifluoroacetic acid produced the trifluoroacetate salt **13** in high yield. For this deprotection the use of HCl in diethyl ether was also investigated but the yields were unsatisfactory. The thioether–thiol moiety was incorporated using the ring-opening reaction of the amine group with 2-oxo-1,4-dithiane, an elegant method previously used to prepare a multidentate thioether ligand under mild conditions in one step.[14] In this case generation of the free amine and ring opening of 2-oxo-1,4-dithiane leading to the desired thiol LH_3 produced the best results by a one-pot synthesis in a mixture of phosphate buffer (pH 6) and chloroform. Attempts to reduce the amide carbonyl group with BH_3 to obtain an alternative, potentially more reactive chelator containing an amine donor have not been successful. The unprotected thiol LH_3 could be stored under argon at 0 °C for several months without decomposition, but in concentrated dimethyl sulfoxide solution ^{1}H NMR spectroscopy revealed formation of 2,3,5,6-tetrafluorophenol over a period of hours as a result of nucleophilic attack by the thiol group.

Preparation of rhenium(v) complexes

The complexation behaviour of LH_3 towards rhenium was investigated by preparing the neutral oxorhenium(v) complex ReOL using $[Bu_4N][ReOCl_4]$ and sodium acetate in methanol at room temperature. TLC indicated the occurrence of two purple species, a major one with R_f 0.5 and a minor one with R_f 0.4. The latter has not been characterised but we surmise that it may be a transesterified analogue of ReOL containing a methyl group instead of the tetrafluorophenyl group. After recrystallisation from ethyl acetate only one species (R_f 0.5) was found by TLC and HPLC. Microanalysis, FAB$^+$–MS and ^{1}H NMR spectroscopy of this major product are consistent with a mononuclear 1:1 complex ReOL, in which the reactive ester group is retained. Assignments of ^{1}H NMR peaks were made using two-dimensional correlated spectroscopy ^{1}H/^{1}H-COSY. However, this analysis did not unequivocally allow assignment of the signals for protons attached to C^1,C^2,C^3 and C^5 (see



Scheme 1 Synthesis of the ligand LH_3 and its complex ReOL. Reagents and yields (a) $(BOC)_2O$, 67%; (b) 2,3,5,6-tetrafluorophenol, 1,3-dicyclohexylcarbodiimide, 78%; (c) trifluoroacetic acid, 91%; (d) 2-oxo-1,4-dithiane, phosphate buffer (pH 6, 1.0 M), 47%; (e) $[Bu_4N][ReOCl_4]$, NaO_2CMe, 22%; (f) oxorhenium(v) gluconate, 70%.

Fig. 1 for atom numbering scheme). Co-ordination of the ligand to rhenium(v) caused considerable downfield shifts of the non-equivalent methylene hydrogen atoms of the five-membered chelate rings.

Reaction of LH_3 with an equimolar amount of oxorhenium(v) gluconate[19] in water (pH 4.7, room temperature) afforded a brown product within 5 min. This has not been fully characterised but elemental analysis and electrospray mass spectrometry revealed a compound containing two ligand units with the constitution $[ReO(LH_2)_2]Cl$ (see Scheme 1). The

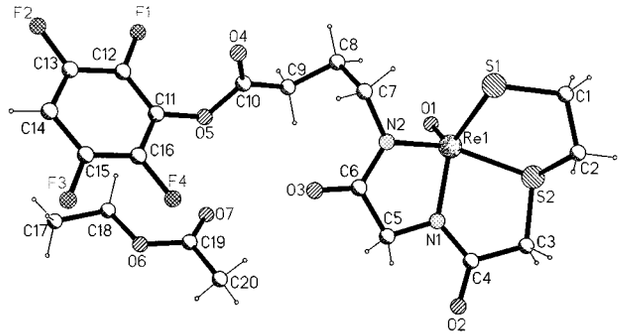


Fig. 1 Structure of complex ReOL showing the atom labelling scheme.

Table 1 Crystal data and structure refinement for complex ReOL·$MeCO_2Et$

Empirical formula	$C_{16}H_{15}F_4N_2O_5ReS_2 \cdot C_4H_8O_2$
Formula weight	729.72
Crystal system	Monoclinic
Space group	$P2_1/n$
a/Å	14.093(2)
b/Å	11.506(2)
c/Å	17.288(3)
β/°	112.390(10)
U/Å^3	2592.0(7)
Z	4
D_c/Mg m^{-3}	1.870
μ/mm^{-1}	4.920
F(000)	1424
Crystal size/mm, colour and habitus	0.20 × 0.20 × 0.10, red needle
θ Range for data collection/°	2.18–25.34
h, k, l Ranges	−16 to 16, −13 to 13, −20 to 20
Reflections collected	15143
Independent reflections	4704
Reflections observed with $F > 4\sigma F$	3416
Standard decay correction (%)	0
Weighting scheme, w^{-1}*	$\sigma^2(F_o^2) + (0.005P)^2$
Data, restraints, parameters	4704, 0, 326
Goodness of fit F^2	0.971
$wR2$ (all data)	0.0965
$R1$ (all data)	0.0629
$R1$ (observed data)	0.0393
Largest difference peak and hole/e Å^{-3}	1.730, −1.170

* $P = [\max(F_o^2,0) + 2F_c^2]/3$.

cationic character of this complex was confirmed by its binding to a cation exchange column. Poor solubility properties prevented full characterisation by NMR spectroscopy. It is not possible to conclude with the present data whether this complex has a *cis*- (as shown in Scheme 1) or *trans*-structure. Formation of such 2:1 complexes has been hypothesised before for ligands of the related type **4**.[16] Higher pH conditions may be needed for efficient 1:1 complex formation as amide groups have to be deprotonated. This is a general problem with chelators containing amido–thiol groups. Inclusion of a thioether donor may thus prove advantageous, allowing efficient complexation of Re/Tc even at low pH to form an intermediate 2:1 complex conjugate in the presence of protein, which may then be converted to the 1:1 complex by raising the pH.

Crystallography

Complex ReOL crystallises in the monoclinic space group $P2_1/n$ with the asymmetric unit containing one molecule of the rhenium complex and one ethyl acetate of crystallisation (Fig. 1). The complex is racemic with both enantiomers in the crystal structure (centric space group). The geometry is best described as distorted square-pyramidal with the oxo group apical. In the least-squares plane defined by the nitrogen and sulfur atoms, N1 and S1 lie respectively 0.234(6) Å and 0.171(2) Å below the plane, while N2 and S2 lie 0.218(5) Å and 0.187(2) Å above it. The rhenium atom lies 0.739(1) Å above the plane. Bond lengths and angles are in the expected ranges with the Re1–O1 distance at 1.683(4) Å and, unsurprisingly, the Re–thioether linkage (Re1–S2) has the longest interaction at 2.359(2) Å. This distance is in accordance with other examples of similar bonds.[2b,16,20] The co-ordinated amido groups are close to planar and are not likely to give rise to isomeric forms in solution. The crystal data and methods of the structure determination are summarised in Table 1. Selected bond lengths and angles are given in Table 2.

Table 2 Selected bond lengths (Å) and angles (°) for complex ReOL

Re(1)–O(1)	1.683(4)	O(2)–C(4)	1.211(8)
Re(1)–N(1)	1.988(5)	O(3)–C(6)	1.220(7)
Re(1)–N(2)	2.024(5)	O(4)–C(10)	1.185(9)
Re(1)–S(1)	2.285(2)	O(5)–C(11)	1.387(10)
Re(1)–S(2)	2.359(2)	O(5)–C(10)	1.369(9)
S(2)–C(2)	1.820(7)	C(1)–C(2)	1.511(10)
S(2)–C(3)	1.817(7)	C(3)–C(4)	1.506(9)
S(1)–C(1)	1.839(7)	C(5)–C(6)	1.517(9)
O(1)–Re(1)–N(1)	118.6(2)	C(2)–S(2)–Re(1)	104.2(2)
N(1)–Re(1)–N(2)	78.5(2)	C(7)–N(2)–Re(1)	124.4(4)
N(1)–Re(1)–S(1)	127.2(2)	C(4)–N(1)–Re(1)	125.7(4)
O(1)–Re(1)–S(2)	102.5(2)	C(3)–S(2)–Re(1)	101.3(2)
N(2)–Re(1)–S(2)	150.9(2)	C(1)–S(1)–Re(1)	106.2(3)
O(1)–Re(1)–N(2)	105.8(2)	C(6)–N(2)–Re(1)	118.3(4)
O(1)–Re(1)–S(1)	114.2(2)	C(5)–N(1)–Re(1)	118.0(4)
N(2)–Re(1)–S(1)	89.1(2)	C(2)–C(1)–S(1)	109.9(5)
N(1)–Re(1)–S(2)	82.3(2)	C(4)–C(3)–S(2)	112.8(5)
S(1)–Re(1)–S(2)	85.20(7)	C(1)–C(2)–S(2)	106.7(5)

Conjugation reaction of complex [ReOL] with N-TIMP-2

To obtain preliminary confirmation of the ability of this complex to form bioconjugates by aminolysis of the active ester group, purified ReOL was conjugated to the protein N-TIMP-2. This protein is the active N-terminal domain of human tissue inhibitor of metalloproteinases-2 consisting of 127 amino acid residues.[18] Fig. 2 shows the electrospray mass spectrum (ES-MS) of a "labelling" experiment where equimolar amounts of ReOL and protein were mixed at pH 8 for 1 h. The parent protein is detected together with a second ion corresponding to an additional 475 mass units consistent with 1:1 conjugate formation with elimination of tetrafluorophenol. This second peak confirms that the conjugate contains the intact chelating group bound to the ReO^{3+} core as expected. Assuming that the parent protein and the conjugate ionise similarly in the mass spectrometer it is estimated that about 17% of the protein is conjugated. Increasing the complex: protein ratio produced multiple labellings since N-TIMP-2 contains several reactive lysine residues.[18] It should be noted that the 1:1 ratio of protein to chelate complex in the present reaction conditions is unrealistic as a model for radiolabelling reactions with ^{99m}Tc or ^{188}Re, where in practice the concentration of protein will greatly exceed the concentration of complex since the metal radionuclide is present at no-carrier-added levels.

Conclusion

We have described a convenient synthesis of a new bifunctional diamide–thioether–thiol ligand LH_3 containing a tetrafluorophenyl ester group. LH_3 forms an uncharged oxorhenium(v) complex ReOL, which may be used to form bioconjugates with proteins by aminolysis at a lysine residue. Under acidic conditions a stable 2:1 complex ion $[ReO(LH_2)_2]^+$ can also be formed. Further investigation of ligands of this type will examine and optimise their use with ^{99m}Tc and ^{188}Re using both pre-formed chelate and post-labelling approaches.

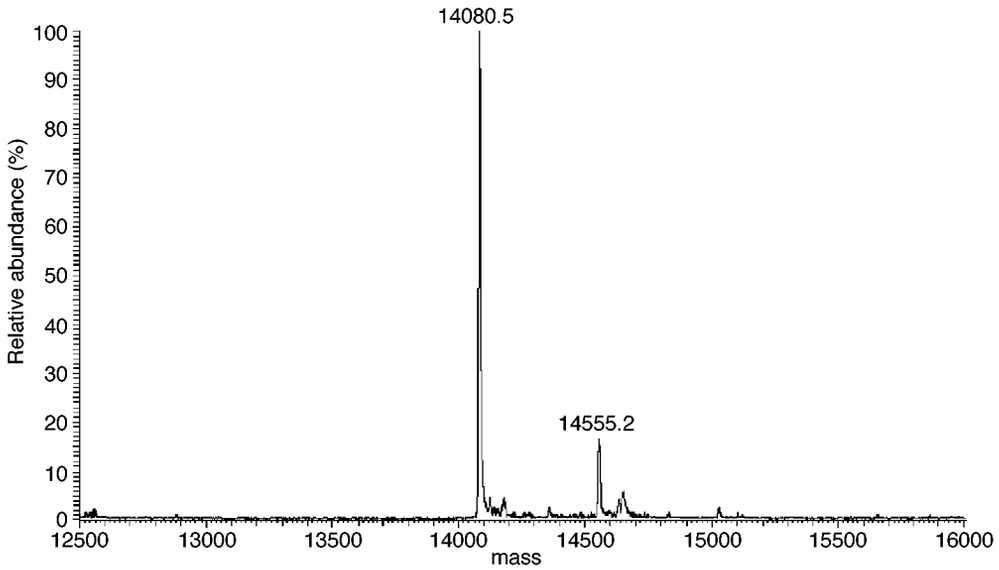


Fig. 2 Deconvoluted electrospray mass spectrum of the products obtained from the conjugation reaction of ReOL with N-TIMP-2. Unmodified N-TIMP-2 appears at $m = 14080.5$ and the 1 : 1 conjugate appears at $m = 14555.2$.

Experimental

General

N-Glycyl-4-aminobutyric acid **1** was obtained from Sigma. 2-Oxo-1,4-dithiane,[21] oxorhenium(V) gluconate[19] and N-TIMP-2[22] were prepared according to the literature. All other reagents and solvents were purchased from Aldrich, Fisher, or Merck and were used as supplied. Tetrahydrofuran was distilled from sodium–benzophenone ketyl immediately prior to use. Thin-layer chromatography (TLC) was performed using sheets from Riedel-de Haën (Silica gel 60 F 254). Thiol groups were detected on TLC plates using Ellman's reagent.[23] Visualisation was also achieved by iodine vapour or UV illumination. For column chromatography Silica gel 60 from Fluka (0.035–0.07 mm) was used. Solid phase extraction was performed with Sep-Pak Vac *t*C2 (6 cm^3, 1 g) cartridges (Waters). Ion exchange chromatography was done using SAX and SCX disposable columns (Isolute SPE, 0.5 cm diameter × 0.7 cm). Melting points were determined with a Gallenkamp apparatus. IR spectra were recorded on a 2020 Galaxy Series FT-IR spectrometer (Mattson Instruments) using KBr pellets. ^1H and ^{13}C NMR spectroscopy were performed on a JEOL GSX (270 MHz) except for the $^1H/^1H$ double quantum filtered COSY spectrum (DQF-COSY[24]) for which a Varian UNITY Inova (600 MHz) spectrometer was used. The standards were tetramethylsilane and 2,2-dimethyl-2-silapentane-5-sulfonate. Fast atom bombardment mass spectra (FAB^+-MS) were obtained using 3-nitrobenzyl alcohol as matrix by the EPSRC Mass Spectrometry Centre, Swansea, UK. Electrospray mass spectra were recorded on a Finnigan MAT LCQ ion trap mass spectrometer by the Wellcome Trust Protein Science Facility, University of Kent. Samples were desalted on-line by reverse-phase HPLC on a C4 column (2.1 × 50 mm) running on a Hewlett Packard 1100 HPLC system. Mass spectra were acquired with the ion trap operating in the high mass mode and spectra deconvoluted using Bio-Explore to give protein masses. Microanalyses were performed by the Microanalytical Service at the University of Kent.

Preparations

N-BOC-Glycyl-4-aminobutyric acid 11. The preparation of **11** was done by a modification of a known synthesis.[25] To a mixture of **10** (5.0 g, 31.21 mmol), NaOH (34 cm^3, 1.0 N) and water (25 cm^3), a solution of di-*tert*-butyl dicarbonate (6.81 g, 31.21 mmol in 25 cm^3 Bu^tOH) was added at 0 °C. After stirring at room temperature for 16 h $KHSO_4$ (7.01 g, 51.50 mmol) dissolved in water (50 cm^3) was added. TLC (ethyl acetate, iodine) revealed a yellow spot at $R_f = 0.5$. The reaction solution was extracted with ethyl acetate (3 × 50 cm^3) and then with ethyl acetate–brine (50 cm^3 : 50 cm^3). The combined organic extracts were washed with brine and dried over $MgSO_4$. After evaporation of the solvent at 30–40 °C *in vacuo* the residue was solidified by addition of diethyl ether (50 cm^3) and storing at −20 °C for 1 h to give a white powder which was collected by filtration. Yield: 5.42 g (67%), mp 75–78 °C (Found: C, 50.75; H, 8.00; N, 10.74. $C_{11}H_{20}N_2O_5$ requires C, 50.76; H, 7.75; N, 10.76%); IR: 1732, 1711, 1683, 1633, 1537, 1175 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$): δ 1.45 (9 H, s, 3 CH_3), 1.85 (2 H, m, CH_2), 2.40 (2 H, m, CH_2,), 3.30 (2 H, m, CH_2), 3.80 (2 H, m, CH_2), 5.30–6.75 (1 H, m, NH), 7.01–7.20 (1 H, m, NH), 10.95 (1 H, m, OH).

2,3,5,6-Tetrafluorophenyl N-BOC-glycyl-4-aminobutyrate 12. To a stirred solution of **11** (3.0 g, 11.53 mmol) and 2,3,5,6-tetrafluorophenol (2.91 g, 17.54 mmol) in thf (40 cm^3) 1,3-dicyclohexylcarbodiimide (3.09 g, 14.98 mmol) dissolved in thf (70 cm^3) was added. After stirring for 22 h at room temperature the precipitate was filtered off and washed with thf (2 × 10 cm^3). The filtrate was concentrated *in vacuo* at 40–45 °C. TLC (ethyl acetate–*n*-hexane 2 : 3) showed ester **12** at $R_f = 0.1$. The crude product was isolated by column chromatography (silica gel). After elution with ethyl acetate–*n*-hexane (2 : 3) to remove unreacted starting material ester **12** was obtained by elution with ethyl acetate. Removal of the solvent *in vacuo* gave a white solid immediately. Yield: 3.65 g (78%), mp 78–82 °C (Found: C, 50.03; H, 5.13; N, 6.91. $C_{17}H_{20}F_4N_2O_5$ requires: C, 50.00; H, 4.97; N, 6.86%); IR: 1773, 1676, 1652, 1528 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$): δ 1.45 (9 H, s, 3 CH_3), 2.00 (2 H, m, CH_2), 2.70 (2 H, m, CH_2), 3.40 (2 H, m, CH_2), 3.80 (2 H, m, CH_2), 5.70 (1 H, m, NH), 6.50 (1 H, m, NH), 7.00 (1 H, m, aryl H).

***N*-Glycyl-4-amino-*N*-butyric acid 2,3,5,6-tetrafluorophenyl ester trifluoroacetate 13.** Selective acidolytic removal of an *N*-*tert*-butyloxycarbonyl group in the presence of an ester function using trifluoroacetic acid has been described elsewhere.[26] To a stirred solution of **12** (3.65 g, 8.94 mmol) in dichloromethane (60 cm^3) trifluoroacetic acid (6 cm^3) was added during 10 min at room temperature. After continued stirring at room

temperature for 4 h completion of the reaction was indicated by TLC (ethyl acetate–*n*-hexane 2:3). The solvent was evaporated *in vacuo* to give a yellow oil that was solidified by addition of diethyl ether (60 cm^3) and stirring for 5 min. The resulting white solid was collected by filtration. Yield: 3.44 g (91%), mp 95–100 °C (Found: C, 39.60; H, 3.09; N, 6.47. $C_{14}H_{13}F_7N_2O_5$ requires: C, 39.82; H, 3.10; N, 6.63%); IR: 1780, 1674, 1652, 1523 cm^{-1}; ^{1}H NMR (270 MHz, D_2O): δ 2.00 (2 H, quintet, J = 7.2, $CH_2CH_2CH_2$), 2.85 (2 H, t, J = 7.2, CH_2), 3.40 (2 H, t, J = 7.2 Hz, CH_2), 3.80 (2 H, s, $CH_2NH_3^+$), 7.33 (1 H, m, aryl H).

***N*-(*N*-5-sulfanyl-3-thiapentanoyl)glycyl-4-aminobutyric acid 2,3,5,6-tetrafluorophenyl ester LH_3.** To a well-stirred solution of 2-oxo-1,4-dithiane (2 g, 14.90 mmol) in chloroform (100 cm^3), phosphate buffer (32 cm^3, pH 6, 1.0 M) and water (18 cm^3), the trifluoroacetate salt **13** (2 g, 4.74 mmol) was added. Stirring at room temperature was continued for 8 h. After addition of water (50 cm^3) and separation of the organic phase the aqueous phase was extracted with chloroform (2 × 20 cm^3). The combined organic fractions were dried over $MgSO_4$. The crude product was concentrated *in vacuo* at 35 °C to give an oil and purified by flash chromatography (ethyl acetate). Fractions containing the thiol LH_3 were combined. Evaporation of the solvent *in vacuo* at 35 °C afforded a white solid that was stored under argon at 5 °C. Yield: 979 mg (47%), mp 95–97 °C (Found: C, 43.90; H, 4.22; N, 6.42. $C_{16}H_{18}F_4N_2O_4S_2$ requires: C, 43.44; H, 4.10; N, 6.33%); IR: 1778, 1655, 1639, 1523 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.71 (1 H, t, J = 7.6, SH), 2.01 (2 H, quintet, J = 6.6, $CH_2CH_2CH_2$), 2.75 (6 H, m, CH_2), 3.28 [2 H, s, $SCH_2C(O)$], 3.42 (2 H, q, J = 6.6, CH_2CH_2NH), 3.99 [2 H, d, J = 5.6 Hz, $C(O)CH_2NH$], 6.72 (1 H, m, NH), 7.01 (1 H, m, aryl H), 7.58 (1 H, m, NH); ^{13}C NMR (270 MHz, $CDCl_3$): δ 24.2, 24.5, 30.8, 35.6, 36.9, 38.7, 43.4, 103.0, 103.3, 103.6, 168.8, 169.1, 169.8; FAB$^+$MS: m/z = 443 (M+H), 465 (M+Na).

[2,3,5,6-Tetrafluorophenyl *N*-(*N*-5-sulfanyl-3-thiapentanoyl)-glycyl-4-aminobutyrate(3−)]oxorhenium(V) ReOL. LH_3 (100 mg, 0.226 mmol) and sodium acetate (93 mg, 1.13 mmol) were dissolved in methanol (50 cm^3) under argon and cooled to 0 °C. After addition of $[Bu_4N][ReOCl_4]$ (130 mg, 0.226 mmol) in methanol (5 cm^3) during 5 min the mixture was allowed to warm up and stir for 28 h at room temperature. A brownish solution was obtained. The solvent was removed *in vacuo* and the crude product purified using a small column of silica gel and ethyl acetate containing 10% Pr^iOH as eluent. A purple product was collected and the solvent evaporated *in vacuo*. Thin-layer chromatography at this point revealed two purple spots, R_f 0.5 and 0.4. The crude product was dissolved in acetonitrile (0.5 cm^3) and applied to a *t*C2 cartridge equilibrated with water. The column was eluted twice with water containing MeCN (5 cm^3, 10% MeCN; 5 cm^3, 30% MeCN). Elution with MeCN (5 cm^3) and evaporation of the solvent *in vacuo* gave the pure complex (TLC R_f 0.5). Yield: 32 mg (22%), mp 157–160 °C (decomp.) (Found: C, 30.45; H, 2.86; N, 4.20. $C_{16}H_{15}Fe_4N_2O_5ReS_2$ requires: C, 29.95; H, 2.36; N, 4.37%); IR: 1786, 1659, 1639, 1523 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$; see Fig. 1 for atom numbering scheme): δ 1.98–2.04 + 3.99–4.08 (2 H, mm, C^1H or C^2H), 3.07 + 3.85 (1 H, dd, ^2J = 14.4; 1 H dd, ^2J = 10.2, ^3J = 3.6; C^1H or C^2H), 3.57 (1 H, d, ^2J = 18.1, C^3H or C^5H), 4.47 (1 H, d, ^2J = 18.1, C^3H or C^5H), 4.29 (1 H, d, ^2J = 18.7, C^3H or C^5H), 4.46 (1 H, d, ^2J = 18.7, C^3H or C^5H), 4.93–5.00 (1 H, m, ^2J = 13.4, ^3J = 7.7, C^7H), 3.99–4.08 [1 H (2 H), m, C^7H], 2.12–2.28 (2 H, m, C^8H), 2.78 (2 H, t, ^3J = 7.6, C^9H), 6.98 [1 H, tt, ^3J(H,F) = 9.7, ^4J(H,F) = 3.5 Hz; $C^{14}H$]; ^{13}C NMR (270 MHz, $CDCl_3$): δ 25.5, 30.8, 37.9, 44.2, 48.0, 51.0, 55.8, 102.7, 103.0, 103.3, 169.2, 187.4, 188.6; FAB$^+$MS: m/z = 643 (M+H), 665 (M+Na). Crystals suitable for X-ray analysis were obtained from ethyl acetate.

Reaction of LH_3 with oxorhenium(V) gluconate

A stirred solution of ligand LH_3 (40 mg, 0.0904 mmol) in Pr^iOH (8 cm^3) was degassed with Ar for 10 min. An aqueous (pH 4.7) solution of oxorhenium(V) gluconate[19] (2.52 cm^3, 0.0904 mmol) was added and the mixture stirred for 5 min at room temperature. After addition of water (10 cm^3) and ethyl acetate (20 cm^3) the orange-brown organic phase was separated, washed twice with brine and dried over $MgSO_4$. Evaporation of the solvent and treatment with diethyl ether afforded a brown solid insoluble in water and poorly soluble in chloroform. Yield: 38 mg (70%), mp 95–100 °C (Found: C, 36.38; H, 3.43; N, 5.19. $C_{36}H_{42}ClF_8N_4O_{11}ReS_4$ {for $[ReO(LH_2)_2]Cl\cdot EtOAc$} requires: C, 35.78; H, 3.50; N, 4.64%); IR: 1786, 1643, 1523 cm^{-1}. ES-MS 1085.3 (M^+), 100%; 1107.3 ($M^+ - H + Na$), 25%. The complex dissolved in acetonitrile (0.3 cm^3) was trapped by an SCX cation exchange column after elution with acetonitrile (1 cm^3) but was eluable using an SAX anion exchange column and the same amount of eluting solvent.

Labelling of N-TIMP-2

In a typical experiment a solution of ReOL in dimethyl sulfoxide (0.001 cm^3, 0.001 mmol) was added to buffered N-TIMP-2 (0.1 cm^3, 0.001 mmol, borate buffer, pH 8.0, 100 mM). After incubation for 1 h at room temperature the mixture was stored at −80 °C until mass spectrometry could be performed. Investigation by ES-MS revealed signals due to unmodified N-TIMP-2 at m = 14080.5 (predicted mass 14084, 100%) and 1:1 conjugate at m = 14555.2 (protein + 475, 18%).

Crystallography

Data collection. Intensity data were collected at 293(2) K on a MSC/Rigaku Raxis-IIc diffractometer with monochromated Mo-Kα radiation (0.71073 Å) using MSC data collection software. Cell constants were obtained from least-squares refinement of the setting angles of 349 centred reflections (θ = 3.5–25°).

Structure analysis and refinement. The structure of ReOL was solved by direct methods (SHELXS 86)[27] and refined on F_o^2 by full-matrix least squares (SHELXL 93).[28] All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were fixed on idealised positions.

CCDC reference number 186/1114.

See http://www.rsc.org/suppdata/dt/1998/3087/ for crystallographic files in .cif format.

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