DOI: 10.1002/ejoc.200500810

A Convenient Synthesis of An Original, Bifunctional Chelator Based on an ON_2S Ligand and its Coordination Chemistry with the Oxorhenium(V) Core

Julien Le Gal[a] and Eric Benoist*[a]

Keywords: Chelates / Conjugation / N,O ligands / Rhenium

A semi-rigid, tetradentate, bifunctional chelator (BFC) for the oxorhenium core has been synthesised in five steps with an overall yield of 46 %. An aromatic amine is available for conjugation to a peptide through an isothiocyanate function. This BFC is the first amine-functionalised tetradentate ligand containing both amido and thiol functionalities. Monooxorhenium complexes were readily prepared from both the bifunctional chelator and a β-alanine-conjugated system. These complexes were obtained in good yield and were fully characterised. Moreover, they were found to be stable to ligand-exchange reactions.

Introduction

The development of improved bifunctional chelators (BFCs) has gained widespread interest because of their usefulness in medical imaging and therapy, for example as MRI contrast agents and target-specific radiopharmaceuticals for diagnostic and therapy.[1,2] In the latter case, the chelators are used to connect a radionuclide to a targeting molecule to form a radiopharmaceutical. An ideal BFC should coordinate the radionuclide with a high yield and form a relatively stable complex. The ability of a BFC to form a metal chelate with a high thermodynamic stability and a high in vivo kinetic inertness is therefore crucial to allow localisation of the radioisotope at the target site (tumours or organs) and to minimise intoxication arising from the loss of the radioisotope. Consequently, there is an increasing requirement for the development of new, stable chelators for bioconjugation purposes.

Recent research efforts to design new BFCs for subsequent use in diagnostics and therapy have focused on the development of systems with a semi-rigid or rigid structure. These systems typically impart relatively high stability to their metal complexes compared to acyclic ones (pre-organisation concept).[3] In fact, the rigidity of the molecular skeleton reduces the freedom of donor atoms, and this spatial pre-organisation induces a more efficient complexation. Surprisingly, while chelating agents with cycloalkyl or macrocyclic frameworks have been intensively used and described,[4–10] semi-rigid systems with an aromatic backbone have been developed only recently.[11–13] However, polydentate ligands with an aromatic design have stable geometries and are also easily functionalisable, which is essential for bioconjugation purposes.

In recent works we developed the new family of monofunctionalised, unsymmetrical, semi-rigid, tetradentate ligands $PhXN_2S$ (X = O, N, S) for technetium(V) and rhenium(V) complexation. The presence of an aromatic cycle in the framework of these ligands favours and stabilises the chelate ring of the corresponding complexes.[14] Complexation of these ligands by a TcO or ReO core was shown to result in a single isomer.[15] Among these molecules, $PhON_2S$ has been shown to form stable, five-coordinate complexes with technetium and rhenium. This chelating core therefore appeared to be highly attractive for the radiolabelling of peptides with these radionuclides.

Consequently, the aim of this work was to produce a novel, bifunctional chelator based on the $PhON_2S$ framework in a simple manner. This BCF, $(NH_2)PhON_2S$, is the first example of an amine-functionalised, tetradentate, bifunctional chelator containing both amido and thiol functions. The functionalisation of multidentate ligands often needs time-consuming, multistep syntheses. Accordingly, we present in this paper a convenient and short synthesis of this BCF from inexpensive and commercially available starting materials, its conjugation to a biomolecule model and cysteine challenge experiments with the corresponding rhenium complexes.

Results and Discussion

While recent N_3O[13] or N_2S_2[16] BCFs based on an aromatic ring are functionalised with a carboxylic acid, we decided to functionalise the $PhON_2S$ framework by the addition of an amine moiety to the aromatic ring of the chela-

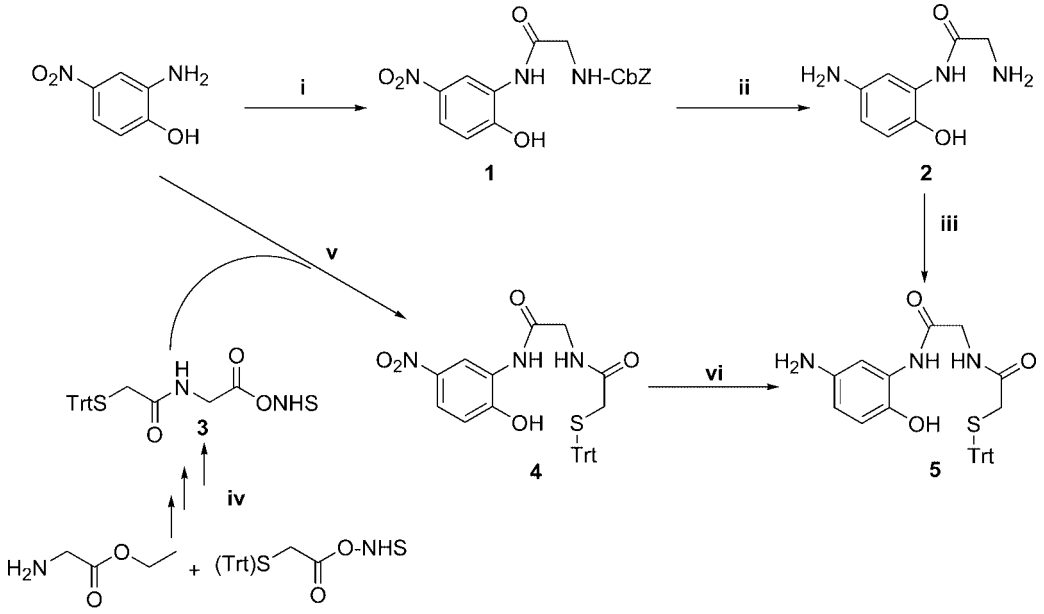
[a] Laboratoire de Chimie Inorganique, EA 807, Université Paul Sabatier, Bat. IIR1,
118, route de Narbonne, 31062 Toulouse, France
Fax: +33-5-6155-6118
E-mail: benoist@chimie.ups-tlse.fr

tor, as depicted in Scheme 1. In contrast to carboxylic acid derivatisation, amine functionalisation will provide selective conjugation with amino groups or carboxylic acid functions of a peptide through a thiourea or amide bond, respectively. The bifunctional chelator **5** was prepared according to two different synthetic routes from commercially available 2-amino-4-nitrophenol (Scheme 1).

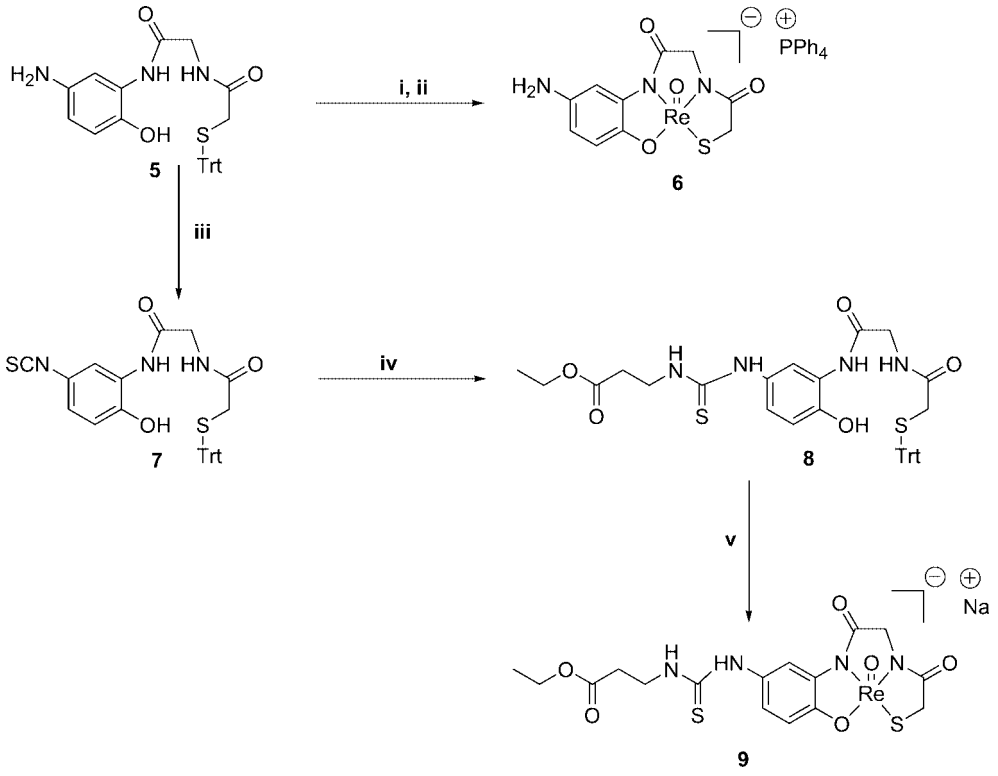
The intermediate **1** was first synthesised by a conventional carbodiimide–amide coupling of *N*-(benzyloxycarbonyl)glycine and the starting compound. Compared to the same reaction previously described by Bermejo and co-workers,[17] which uses 2-aminophenol as starting material, our yield was modest (44%) as the presence of the deactivating nitro group at the 4-position of the aromatic ring decreases the reactivity of the amine. In the second step, cleavage of the CbZ group and nitro-group reduction were both achieved by palladium-catalysed hydrogenation, which generates diamine **2** with a 95% yield. Unfortunately we have not been able to reproduce this step efficiently as most of our trials have been contaminated by side-products. Deniaud and co-workers have recently reported similar problems for the benzyloxycarbonyl group deprotection of analogous compounds in a neutral medium with palladium on charcoal as catalyst.[11b] Acylation of **2** with the activated ester of *S*-protected mercaptoacetic acid afforded the desired ligand **5** in 55% yield. The acylation reaction is regioselective under these conditions, and no condensation between the activated ester and the aromatic amino group of **2** was observed. Although this route produced BFC **5** in three steps with a reasonable overall yield (23%), the non-reproducibility of the deprotection/reduction step led us to propose another synthetic route in which the reduction of the nitro group is performed after the formation of the chelate cavity.

This improved route involves five steps also starting from 2-amino-4-nitrophenol, with the first three steps (synthesis of **3**) following a literature precedent.[14d] Treatment of **3** with 2-amino-4-nitrophenol in acetonitrile led to the formation of **4** in excellent yield (92%). The crucial step of this route is the reduction of the nitro group. Although a large number of conditions are known for the reduction of nitrobenzene to aniline derivatives, most of them are harsh and unsuitable for acid-labile functional groups such as the trityl group.[18] Catalytic transfer-hydrogenation using palladium on charcoal seemed inappropriate as the catalyst is usually poisoned by small amounts of sulfur such as thiols or sulfides. As expected, the reduction of the nitro group with H_2/Pd/C gave **5** in very low yield. Recently, mild and efficient reductions of nitroarene compounds have been achieved using hydrazine derivatives as hydrogen donor in the presence of a metal as catalyst (Fe, Ni, Zn...).[12,19] Thus, the nitro-group reduction in the presence of a sulfur atom was solved by using a synthetic protocol developed by Katzenellenbogen and co-workers.[20] The aromatic nitro group was readily reduced with hydrazine/Raney nickel to give the aniline **5** in excellent yield. Following this synthetic strategy, the bifunctional chelator **5** was obtained with an overall yield of 46%. We have therefore demonstrated that by using a convenient strategy it is possible to functionalise an ON_2S framework with an amine function. To the best of our knowledge, this BCF is the first amine-functionalised tetradentate ligand that contains both amido and thiol groups.

The rhenium complexation study was carried out using $[ReOCl_3(PPh_3)_2]$ as the $Re^{V}O$ starting material[21] in the presence of sodium acetate as deprotonating agent. Analytically pure complex **6** was obtained from bifunctional chelator **5**, in 58% yield, after a metathesis reaction in the presence of tetraphosphonium chloride (Scheme 2).



Scheme 1. Synthesis of ligand **5**. Reagents and conditions: (i) *N*-CbZ-glycine, DCC, THF, 44%; (ii) H_2, Pd/C, MeOH, 95%; (iii) (Trt)$SCH_2COONHS$, Et_3N, 5 h, 40 °C, ACN, 55%; (iv) ref.[14d], 64%; (v) **3**, DMAP, 4 h, 60 °C, ACN, 92%; (vi) Raney nickel, $NH_2NH_2{\cdot}H_2O$, 1,2-dichloroethane/EtOH, 78%.



Scheme 2. Complexation and coupling studies. Reagents and conditions: (i) $[ReOCl_3(PPh_3)_2]$, NaOAc, 4 h, 65 °C, MeOH; (ii) PPh_4Cl, 30 min, room temp., MeOH/CH_2Cl_2, 58%; (iii) Cl_2CS, 1 h, room temp., THF, 87%; (iv) β-alanine ethyl ester hydrochloride, NEt_3, 3 h, room temp., THF, 91%; (v) $[ReOCl_3(PPh_3)_2]$, NaOAc, 4 h, 65 °C, MeOH, 60%.

The coordination reaction led to a unique rhenium complex with the general formula $[PPh_4][ReO(PhON_2S)]$, as evident from the elemental analysis. As expected, this five-coordinate rhenium complex was characterised by (i) the presence of one single isomer, the sp^2 character of the nitrogen of the amine function preventing the formation of *syn*/*anti* isomers, (ii) an intense band in the IR spectrum, attributable to the Re=O stretching vibration, near 960 cm^{-1}, (iii) a non-equivalence of the two hydrogens of each methylene group (AB pattern) of the tetradentate ligand framework in the ^1H NMR spectrum, the downfield signal of both AB patterns being assigned to the *endo* protons (*syn* to the Re=O group) and the upfield signals to the *exo* ones (*anti* to the Re=O group),[15,22] and (iv) an overall charge on the rhenium atom –1. Thus, we could expect that these polar chelate moieties should promote efficient renal clearance of rhenium conjugates from the blood, like MAG_3 derivatives.[23,24] Biodistribution studies of the prototypic complex $[Na][^{99m}TcO\{(NH_2)PhON_2S\}]$ in rats showed that the complex is preferentially eliminated via the renal-urinary excretion route and presents a good clearance from the bloodstream, thus indicating its good stability against exchange reactions with blood proteins.[25] To sum up, the spectroscopic characteristics of the oxorhenium complex **6** are similar to those obtained for the non-functionalised complexes; therefore, even without an X-ray structure, complex **6** is likely to exhibit a distorted square-pyramidal geometry with the oxo ligand in the apical position.

For bioconjugation purposes, **5** was converted into the isothiocyanate derivative **7**, in 87% yield, by treatment with thiophosgene in dry THF. The reasonably good stability of this NCS derivative allowed us to purify it by column chromatography on silica gel. The presence of the NCS function was confirmed by the band at 2124 cm^{-1} in its IR spectrum. In order to demonstrate the high reactivity of **7** towards biologically relevant molecules, the β-alanine bioconjugate was formed.[26] A solution of **7** was stirred for one hour, at room temperature, with ethyl β-alanine ester to give the β-alanine bioconjugate **8** in 91% yield after purification by column chromatography. Complexation of **8** with $[ReOCl_3(PPh_3)_2]$ in the presence of sodium acetate led to the bioconjugate rhenium complex **9** in 60% yield as the sodium salt (Scheme 2). This complex exhibits similar spectroscopic properties to complex **6**.

To determine the applicability of this system for use as a therapeutic agent in vivo, the stability of the rhenium com-

Table 1. Stability of complexes **6** and **9** against ligand exchange with cysteine.

	Complex **6**[a]			Complex **9**[a]		
Conditions	1 h	6 h	18 h	1 h	6 h	18 h
Aqueous	>99%	>99%	>99%	>99%	>99%	>99%
Cysteine	>99%	98%	94%	99%	96%	91%

[a] Percent of complex remaining at the indicated time. Conditions tested: aqueous medium at room temperature; ligand exchange with cysteine at 37 °C.

plexes **6** and **9** to cysteine-exchange experiments was studied (Table 1). Both complexes proved to be very stable and inert in the presence of an excess of cysteine. There is no significant loss in complex stability over 18 h − the half-life of ^{188}Re. Moreover, both complexes are stable for more than 48 h in aqueous medium under neutral conditions.

Conclusion

In conclusion, a simplified route has been developed for the synthesis of a new, semi-rigid, tetradentate, bifunctional chelator, the first amine-functionalised tetradentate ligand that contains both amido and thiol functionalities. (NH_2)-$PhON_2S$ was obtained in five steps, from inexpensive starting materials, with an overall yield of 46%. This approach has been validated by its conjugation to a model peptide followed by complexation with rhenium. The high stability and inertness of these rhenium complexes are promising and suggest that this bifunctional chelator could be useful for the development of new target-specific radiotherapeutic agents. Further experiments with rhenium-188 will be starting soon to confirm these initial results.

Experimental Section

NMR spectra were recorded with a Bruker AC 250 (250 MHz) or Bruker 400 (400 MHz) spectrometer. Chemical shifts are given in δ values (ppm) downfield from internal TMS, and coupling constants (J) are given in Hertz (Hz). Infrared spectra were recorded as KBr pellets on a Bruker Vector 22 spectrophotometer in the range 4000–400 cm^{-1}. Negative and positive electrospray or DCI mass spectra were obtained with a NERMAG R 10-10 mass spectrometer. Microanalysis was performed by the microanalytical department of the Ecole Nationale Supérieure des Arts Chimiques et Technologiques de Toulouse (ENSIACET). Melting points recorded with a Stuart SMP3 melting point apparatus are uncorrected. HPLC analysis was performed with a Waters 600E gradient chromatography with a Waters Lambda Max UV detector at 270 nm and a Satisfaction RP18AB column using MeOH/H_2O/TFA (50:50:0.1) as eluent (flow rate: 1 mL min^{-1}).

Chromatographic purification was conducted with "gravity" silica gel obtained from Merck. TLC was performed on silica oxide plates (Merck) coated with fluorescent indicator. Solvents were dried with suitable reagents and were freshly distilled under nitrogen before use. The intermediate **3**[14d] and $[ReOCl_3(PPh_3)_2]$[21] were prepared according to literature protocols.

***N*-(2-Hydroxy-5-nitrophenyl)-2-[(phenylmethoxy)carbonylamino]-ethanamide (1):** 2-Amino-4-nitrophenol (4.67 g, 30.0 mmol) and a slight excess of dicyclohexylcarbodiimide (8.04 g, 39.0 mmol) were added to a solution of *N*-(benzyloxycarbonyl)glycine (7.01 g, 33.0 mmol) in THF (100 mL). The mixture was then stirred under N_2 at room temperature overnight. The insoluble dicyclohexyl urea was removed by filtration and the solvent evaporated to dryness. The crude mixture was purified by column chromatography on silica gel (eluent: CH_2Cl_2/AcOEt, 80:20) to yield **1** as a grey powder (4.52 g, 44%). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 3.90 (d, J = 6.0 Hz, 2 H, CH_2N), 5.06 (s, 2 H, CH_2O), 7.02 (d, J = 8.9 Hz, 1 H, H_{Ar}), 7.36 (s, 5 H, $H_{Ar\ bz}$), 7.67 (t, J = 6.0 Hz, 1 H, NH), 7.88 (dd, J = 8.9 and 2.7 Hz, 1 H, H_{Ar}), 8.98 (d, J = 2.7 Hz, 1 H, H_{Ar}), 9.45 (s, 1 H, NH) ppm. ^{13}C{^{1}H} NMR (62.9 MHz, [D$_6$]DMSO): δ = 44.4 (CH_2N), 65.6 (CH_2O), 114.5, 115.8, 120.5, 127.7, 127.8, 128.4 (8 CH_{Ar}), 126.5, 136.9, 139.2, 153.4 (4C_{Ar}), 156.7, 168.9 (2 CO) ppm. MS (DCI/NH_3): m/z = 346 [M + H$^+$], 363 [M + NH_4^+]. IR (KBr): ν_{NO_2} = 1533, 1293; $\nu_{C=O}$ = 1683 cm^{-1}.

2-Amino-*N*-(5-amino-2-hydroxyphenyl)ethanamide (2): Catalytic hydrogenation of **1** (1.00 g, 2.90 mmol) in methanol (40 mL) over 10% Pd/C (25% w/w, 0.25 g) was carried out at atmospheric pressure. The mixture was stirred overnight then the catalyst was filtered off through Celite and washed with ethanol (60 mL). The solvent was then removed under reduced pressure to give **2** as a green solid (0.50 g, 95%). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 3.22 (s, 2 H, CH_2N), 6.10 (dd, J = 8.6 and 2.7 Hz, 1 H, H_{Ar}), 6.54 (d, J = 8.6 Hz, 1 H, H_{Ar}), 7.52 (d, J = 2.7 Hz, 1 H, H_{Ar}) ppm. ^{13}C{^{1}H} NMR (62.9 MHz, [D$_6$]DMSO): δ = 45.1 (CH_2N), 106.1, 109.3, 115.5 (3 CH_{Ar}), 126.8, 137.3, 141.0 (3 C_{Ar}), 171.0 (CO) ppm. MS (DCI/NH_3): m/z = 182 [M + H$^+$], 199 [M + NH_4^+]. IR (KBr): ν_{NH_2} = 3267, 3319; $\nu_{C=O}$ = 1665 cm^{-1}.

***N*-(2-Hydroxy-5-nitrophenyl)-2-[(triphenylmethylthio)methylcarbonylamino]ethanamide (4):** DMAP (1.22 g, 10 mmol) was added to a solution of 2-amino-4-nitrophenol (1.54 g, 10 mmol) and compound **3** (4.88 g, 10 mmol) in acetonitrile (150 mL). The solution was heated at 60 °C under nitrogen for 4 h. After cooling, the product was left to precipitate overnight at –18 °C. It was then filtered and the precipitate was washed with cold acetonitrile and dried under vacuum to give **4** as a yellow powder (4.84 g, 92%). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 2.88 (s, 2 H, CH_2S), 3.90 (d, J = 5.0 Hz, 2 H, CH_2N), 6.99 (d, J = 8.8 Hz, 1 H, H_{Ar}), 7.29 (m, 16 H, H_{Ar} + 15 $H_{Ar\ Trt}$), 7.90 (dd, J = 8.8 and 2.7 Hz, 1 H, H_{Ar}), 8.39 (s, 1 H, NH), 8.94 (s, 1 H, NH), 9.46 (s, 1 H, OH) ppm. ^{13}C{^{1}H} NMR (100.6 MHz, [D$_6$]DMSO): δ = 36.3 (CH_2S), 43.8 (CH_2N), 66.6 (C_{Trt}), 115.1, 116.4, 121.2 (3 CH_{Ar}), 127.0 (C_{Ar}), 127.3, 128.6, 129.6 (15 $CH_{Ar\ Trt}$), 139.3 (C_{Ar}), 144.5 (3 $C_{Ar\ Trt}$), 154.6 (C_{Ar}), 168.5, 168.7 (2 CO) ppm. MS (ES$^+$): m/z = 550 [M + H$^+$]. IR (KBr): $\nu_{C=O}$ = 1675, 1656 cm^{-1}.

***N*-(5-Amino-2-hydroxyphenyl)-2-[(triphenylmethylthio)methylcarbonylamino]ethanamide (5). Method 1:** *O*-(*N*-hydroxysuccinimido)-2-(triphenylmethylthio)ethanoate[27] (1.19 g, 10 mmol) and NEt_3 (280 mg, 10 mmol) were added to **2** (500 mg, 2.76 mmol) in acetonitrile (40 mL). The solution was stirred at 40 °C under nitrogen for 5 h. After cooling, the solution was concentrated to dryness and the crude product was purified by column chromatography on silica gel (eluent: $CHCl_3$/MeOH, 95:5) to afford compound **5** as a white powder (755 mg, 55%).

Method 2: Monohydrate hydrazine (1.0 mL) and 50% Raney nickel slurry (0.2 mL) were added to compound **4** (600 mg, 1.14 mmol) dissolved in 1,2-dichloroethane/EtOH solution (50 mL, 50:50 v/v). These additions were repeated twice until the reaction was complete (by TLC, 2 h). The slurry was filtered off through Celite then the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (eluent: $CHCl_3$/MeOH, 95:5) to afford compound **5** as a white powder (442 mg, 78%). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 2.88 (s, 2 H, CH_2S), 3.79 (d, J = 5.7 Hz, 2 H, CH_2N), 4.51 (s, 2 H, NH_2), 6.19 (dd, J = 2.4 and 8.4 Hz, 1 H, H_{Ar}), 6.56 (d, J = 8.4 Hz, 1 H, H_{Ar}), 7.13 (d, J = 2.4 Hz, 1 H, H_{Ar}), 7.30 (m, 15 H, $H_{Ar\ Trt}$), 8.33 (s, 1 H, NH), 8.65 (s, 1 H, NH), 8.96 (s, 1 H, OH) ppm. ^{13}C{^{1}H} NMR (100.6 MHz, [D$_6$]DMSO): δ = 36.3 (CH_2S), 43.7 (CH_2N), 66.6 (C_{Trt}), 108.4, 110.9, 116.6 (3 CH_{Ar}), 126.7 (C_{Ar}), 127.3, 128.6, 129.6 (15 $CH_{Ar\ Trt}$), 138.6, 141.6 (2 C_{Ar}), 144.5 (3 $C_{Ar\ Trt}$), 167.7, 168.4 (2 CO) ppm. MS (DCI/NH_3): m/z = 498 [M + H$^+$], 515 [M + NH_4^+]. IR (KBr): $\nu_{C=O}$ = 1640 cm^{-1}. $C_{29}H_{27}N_3O_3S$ (497.61): calcd. C 70.00, H 5.47, N 8.44; found C 69.84, H 5.01, N 8.25.

[PPh$_4$][ReO{(NH$_2$)ON$_2$S}] (6): [ReOCl$_3$(PPh$_3$)$_2$] (324.5 mg, 0.39 mmol) was added to a mixture of **5** (149.1 mg, 0.3 mmol) and sodium acetate (163.2 mg, 1.2 mmol) in dry methanol (40 mL). After refluxing for 4 h, the solution was cooled, filtered, then evaporated to dryness. The residue was dissolved in a mixture of MeOH (15 mL) and CH_2Cl_2 (15 mL), then tetraphenylphosphonium chloride (122.7 mg, 0.33 mmol) was added. After stirring for 30 min the solvent was removed and the crude product was purified by column chromatography on silica gel (eluent: CH_2Cl_2/MeOH, 98:2 then 95:5 then 90:10) to yield complex **6** as a red powder (137.8 mg, 58%). M.p. 184 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.30 (m, 2 H, NH_2), 3.63 (d, J = 17.1 Hz, 1 H, CH_2S), 3.93 (d, J = 17.1 Hz, 1 H, CH_2S), 4.35 (d, J = 18.3 Hz, 1 H, CH_2N), 5.29 (d, J = 18.3 Hz, 1 H, CH_2N), 6.14 (dd, J = 8.2 and 2.4 Hz, 1 H, H_{Ar}), 6.72 (d, J = 8.2 Hz, 1 H, H_{Ar}), 7.48 (m, 9 H, H_{Ar} + $H_{Ar\ PPh_4}$), 7.67 (m, 8 H, $H_{Ar\ PPh_4}$), 7.81 (m, 4 H, $H_{Ar\ PPh_4}$) ppm. ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$): δ_C(ppm) = 40.4 (CH_2S), 61.2 (CH_2N), 106.6, 109.6, 114.6 (3 CH_{Ar}), 116.8, 118.0 (4 $C_{Ar\ PPh_4}$), 130.9, 131.1, 134.3, 134.4, 135.7, 135.8 (20 $CH_{Ar\ PPh_4}$), 140.6, 164.7 (2 C_{Ar}), 186.9, 193.4 (2 CO) ppm. MS (ES$^-$): m/z (%) = 452 (60), 454 (100) [M$^-$]. IR (KBr): $\nu_{Re=O}$ = 950 cm^{-1}. $C_{34}H_{29}N_3O_4PReS$ (792.86): calcd. C 51.51, H 3.69, N 5.30; found C 51.08, H 3.43, N 5.14.

***N*-(4-Isothiocyanato-2-hydroxyphenyl)-2-[(triphenylmethylthio)methylcarbonylamino]ethanamide (7):** Thiophosgene (540 μL, 7.0 mmol) was added to a solution of **5** (350 mg, 0.7 mmol) in THF (10 mL). The solution was stirred for one hour at room temperature under nitrogen, and the solvent was then removed under reduced pressure. The residue was purified by column chromatography on silica gel (eluent: $CHCl_3$ then $CHCl_3$/MeOH, 95:5) to give compound **7** as a white powder (330 mg, 87%). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 2.86 (s, 2 H, CH_2S), 3.85 (d, J = 5.6 Hz, 2 H, CH_2N), 6.88 (d, J = 8.6 Hz, 1 H, H_{Ar}), 7.01 (dd, J = 8.6 and 2.6 Hz, 1 H, H_{Ar}), 7.31 (m, 15 H, $H_{Ar\ Trt}$), 7.99 (d, J = 2.6 Hz, 1 H, H_{Ar}), 8.34 (s, 1 H, NH), 9.27 (s, 1 H, NH), 10.59 (s, 1 H, OH) ppm. ^{13}C{^{1}H} NMR (100.6 MHz, [D$_6$]DMSO): δ = 36.3 (CH_2S), 43.7 (CH_2N), 66.6 (C_{Trt}), 116.0, 118.8 (2 CH_{Ar}), 120.9 (CS), 122.1 (CH_{Ar}), 127.4 (C_{Ar}), 127.3, 128.6, 129.6 (15 $CH_{Ar\ Trt}$), 132.6 (C_{Ar}), 144.5 (3 $C_{Ar\ Trt}$), 147.5 (C_{Ar}), 168.4 (2 CO) ppm. MS (DCI/NH$_3$): m/z = 540 [M + H$^+$], 557 [M + NH$_4^+$]. IR (KBr): $\nu_{C=O}$ = 1657, 1698; ν_{NCS} = 2124 cm^{-1}. $C_{30}H_{25}N_3O_3S_2$ (539.67): calcd. C 66.77, H 4.67, N 7.79; found C 66.58, H 4.37, N 7.70.

(β-ala)ON$_2$S(Trt) (8): Compound **7** (301.8 mg, 0.56 mmol) was added, under nitrogen, to a solution of β-alanine ethyl ester hydrochloride (85.5 mg, 0.56 mmol) and NEt_3 (79.3 μL, 0.56 mmol) in THF (10 mL). The mixture was stirred for three hours at room temperature and the solvent was then removed under reduced pressure. The residue was purified by column chromatography on silica gel (eluent: $CHCl_3$ then $CHCl_3$/MeOH, 95:5) to give compound **8** as a white powder (334.2 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19 (t, J = 7.1 Hz, 3 H, CH_3), 2.63 (t, J = 5.8 Hz, 2 H, CH_2), 3.19 (s, 2 H, CH_2S), 3.74 (d, J = 5.1 Hz, 2 H, CH_2N), 3.83 (t, J = 5.8 Hz, 2 H, CH_2), 4.06 (q, J = 7.1 Hz, 2 H, OCH_2), 6.74 (s, 1 H, NH), 6.83 (m, 3 H, H_{Ar}), 6.95 (s, 1 H, NH), 7.28 (m, 9 H, $H_{Ar\ Trt}$), 7.41 (m, 6 H, $H_{Ar\ Trt}$), 8.09 (s, 1 H, NH), 8.75 (m, 2 H, OH + NH) ppm. ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$): δ = 14.3 (CH_3), 33.8 (CH_2), 35.7 (CH_2S), 40.7 (CH_2), 44.3 (CH_2N), 61.1 (OCH_2), 68.1 (C_{Trt}), 118.3, 119.4, 123.5 (3 CH_{Ar}), 126.5 (C_{Ar}), 127.4, 128.5, 129.7 (15 $CH_{Ar\ Trt}$), 130.4 (C_{Ar}), 144.0 (3 $C_{Ar\ Trt}$), 146.5 (C_{Ar}), 167.9, 170.4, 172.9 (3 CO), 180.9 (CS) ppm. MS (DCI/NH$_3$): m/z = 657 [M + H$^+$]. IR (KBr): $\nu_{C=O}$ = 1720, 1654 cm^{-1}. $C_{35}H_{36}N_4O_5S_2$ (656.82): calcd. C 64.00, H 5.52, N 8.53; found C 63.18, H 5.40, N 8.12.

[Na][ReO{(β-ala)ON$_2$S}] (9): [ReOCl$_3$(PPh$_3$)$_2$] (103.8 mg, 0.126 mmol) was added to **8** (63 mg, 0.096 mmol) and sodium acetate (52.2 mg, 0.384 mmol) dissolved in dry methanol (20 mL). After refluxing for 4 h, the solution turned brown. After cooling, the solution was filtered and the solvents evaporated to dryness. The residue was purified twice by column chromatography on silica gel (eluent: $CHCl_3$/MeOH: 95:15 then 85:15) to yield complex **9** as a dark-red powder (37.0 mg, 60%). M.p. 172 °C. ^{1}H NMR (400 MHz, MeOD): δ = 1.21 (t, J = 7.2 Hz, 3 H, CH_3), 2.66 (t, J = 6.6 Hz, 2 H, CH_2), 3.81 (d, J = 17.4 Hz, 1 H, CH_2S), 3.84 (t, J = 6.6 Hz, 2 H, CH_2), 4.10 (q, J = 7.2 Hz, 2 H, OCH_2), 4.15 (d, J = 17.4 Hz, 1 H, CH_2S), 4.53 (d, J = 18.3 Hz, 1 H, CH_2N), 5.47 (d, J = 18.3 Hz, 1 H, CH_2N), 6.83 (dd, J = 8.5 and 2.3 Hz, 1 H, H_{Ar}), 7.08 (d, J = 8.5 Hz, 1 H, H_{Ar}), 8.16 (d, J = 2.3 Hz, 1 H, H_{Ar}) ppm. ^{13}C{^{1}H} NMR (100.6 MHz, MeOD): δ = 14.2 (CH_3), 34.0 (CH_2), 40.8 (CH_2S), 41.0 (CH_2), 61.1 (OCH_2), 61.7 (CH_2N), 118.6, 119.4, 122.3 (3 CH_{Ar}), 127.5, 131.4, 146.2 (3 C_{Ar}), 172.0 (CO), 179.6 (CS), 187.4, 193.1 (2 CO) ppm. MS (ES$^-$): m/z (%) = 611 (60), 613 (100) [M$^-$]. IR (KBr): $\nu_{Re=O}$ = 968 cm^{-1}. $C_{16}H_{18}N_4NaO_6S_2Re$ (636.67). calcd. C 30.18, H 3.01, N 8.80; found C 31.00, H 3.05, N 9.00.

Stability vs. Cysteine: A freshly prepared 50 mM buffered saline solution of cysteine was added to a 1-mM methanolic solution of the rhenium complex (**6** or **9**). The vial was sealed with a Teflon-lined cap and the solution was stirred and incubated at 37 °C for various time intervals (1, 6 and 18 h). Aliquots were removed periodically and analysed by HPLC.

[1] *The Chemistry of Contrast Agents in Medicinal Magnetic Resonance Imaging* (Eds.: A. E. Merbach, E. Tohl), Wiley, Chichester, **2001**.

[2] a) D. Parker, *Chem. Soc. Rev.* **1990**, *19*, 271–291; b) S. Liu, *Chem. Soc. Rev.* **2004**, *33*, 445–461.

[3] a) R. D. Hancock, A. E. Martell, *Chem. Rev.* **1989**, *89*, 1875–1914; b) A. S. De Sousa, G. J. B. Croft, C. A. Wagner, J. P. Mickael, R. D. Hancock, *Inorg. Chem.* **1991**, *30*, 3525–3529; c) A. S. De Sousa, R. D. Hancock, *J. Chem. Soc., Chem. Commun.* **1995**, 415–416.

[4] a) A. Loussouarn, M. Duflos, E. Benoist, J.-F. Chatal, G. Le Baut, J.-F. Gestin, *J. Chem. Soc., Perkin Trans. 1* **1998**, 237–241; b) A. Loussouarn, A. Ouadi, L. Morandeau, P. Remaud, R. Giles, J. F. Gestin, J. Webb, *Tetrahedron Lett.* **2003**, *44*, 3539–3541.

[5] S. G. Gouin, J.-F. Gestin, A. Reliquet, J. C. Meslin, D. Deniaud, *Tetrahedron Lett.* **2002**, *43*, 3003–3005.

[6] L. J. Charbonnière, N. Weibel, R. F. Ziessel, *J. Org. Chem.* **2002**, *67*, 3933–3936.

[7] A. K. Mishra, J.-F. Chatal, *New J. Chem.* **2001**, *25*, 336–339.

[8] C. Wu, H. Kobayashi, B. Sun, M. Yoo, C. H. Paik, O. A. Gansow, J. A. Carrasquillo, I. Pastan, M. W. Brechbiel, *Bioorg. Med. Chem.* **1997**, *5*, 1925–1934.

[9] a) M. W. Brechbiel, O. A. Gansow, *J. Chem. Soc., Perkin Trans. 1* **1992**, 1173–1178; b) M. W. Brechbiel, O. A. Gansow, C. G. Pippin, R. D. Rogers, R. P. Planalp, *Inorg. Chem.* **1996**, *35*, 6343–6348.

[10] E. A. Lewis, C. C. Allan, R. W. Boyle, S. J. Archibald, *Tetrahedron Lett.* **2004**, *45*, 3059–3062.

[11] a) S. G. Gouin, E. Benoist, J.-F. Gestin, J.-C. Meslin, D. Deniaud, *Eur. J. Org. Chem.* **2004**, 878–885; b) S. G. Gouin, J.-F. Gestin, L. Morandeau, F. Segat-Dioury, J.-C. Meslin, D. Deniaud, *Org. Biomol. Chem.* **2005**, *3*, 454–461.

[12] B. S. Chhikara, N. Kumar, V. Tandon, A. K. Mishra, *Bioorg. Med. Chem.* **2005**, *13*, 4713–4720.

[13] M. Papachristou, I. Permettis, T. Siatra-Papastaikoudi, M. Pelecanou, C. Tsoukalas, C. P. Raptopoulou, A. Terzis, E. Chiotellis, M. Papadopoulos, *Eur. J. Inorg. Chem.* **2003**, 3826–3830.

[14] a) L. Fourteau, E. Benoist, M. Dartiguenave, *Synlett* **2001**, *1*, 126–128; b) J. Le Gal, E. Benoist, M. Gressier, Y. Coulais, M.

Dartiguenave, *Tetrahedron Lett.* **2002**, *43*, 9295–9297; c) J. Le Gal, PhD thesis, University of Toulouse, **2004**; d) J. Le Gal, L. Latapie, M. Gressier, Y. Coulais, M. Dartiguenave, E. Benoist, *Org. Biomol. Chem.* **2004**, *2*, 876–883.
[15] J. Le Gal, F. Tisato, G. Bandoli, M. Gressier, J. Jaud, S. Michaud, E. Benoist, *Dalton Trans.* **2005**, 3800–3807.
[16] S. Liu, D. S. Edwards, in *Technetium and Rhenium in Chemistry and Nuclear Medicine 4* (Eds.: M. Nicolini, U. Mazzi), SGEditoriali, Padova, Italy, **1994**, pp. 383–386.
[17] M. R. Bermejo, A. M. Gonzalez, M. Fondo, A. Garcia-Deibe, M. Maneiro, J. Sanmartin, O. L. Hoyos, M. Watkinson, *New J. Chem.* **2000**, *24*, 235–241.
[18] a) R. C. Larock, *Comprehensive Organic Transformations*, VCH Publishers, New York, **1989**, pp 411; b) J. P. Adams, *J. Chem. Soc., Perkin Trans. 1* **2002**, 2586–2597.
[19] a) A. R. Boothroyd, M. A. Kerr, *Tetrahedron Lett.* **1995**, *36*, 2411–2414; b) P. Haquette, S. Dagorne, R. Welter, G. Jaouen, *J. Organomet. Chem.* **2003**, *682*, 240–247; c) P. Selvam, S. K. Mojapatra, S. U. Sonavane, R. V. Jayaram, *Tetrahedron Lett.* **2004**, *45*, 2003–2007.
[20] L. G. Luyt, J. A. Katzenellenbogen, *J. Labelled Compd. Radiopharm.* **2001**, *44*, S773–S774.
[21] J. Chatt, G. A. Rowe, *J. Chem. Soc.* **1962**, 4019–4033.
[22] R. Visentin, R. Rossin, M. C. Giron, A. Dolmella, G. Bandoli, U. Mazzi, *Inorg. Chem.* **2003**, *42*, 950–959.
[23] C. J. Smith, T. J. Hoffman, C. Higginbotham, E. Wong, D. Esihmia, J. R. Thornback, W. A. Volkert, *J. Nucl. Med.* **1998**, *39*, 215P.
[24] P. A. Schubiger, R. Alberto, A. Smith, *Bioconjugate Chem.* **1996**, *7*, 165–179.
[25] Unpublished work realized on two batches of three male rats. %(Injected Dose)/organ (5 min p.i./30 min p.i.): liver: 22.36/9.17%; blood: 31.79/11.23%; kidneys: 10.50/6.55%; urine: 1.08/10.50%.
[26] The ethyl β-alanine ester was used to model peptide conjugation because this compound has been introduced generally as a spacer between the biomolecule and the bifunctional chelator to minimize interference from the chelator upon binding to the peptide/receptor.
[27] R. A. Bell, D. W. Hughes, C. J. L. Lock, J. F. Valliant, *Can. J. Chem.* **1996**, *74*, 1503–1511.

Received: October 17, 2005
Published Online: January 12, 2006

