DOI: 10.1002/ejic.201100796

$^{99m}Tc^{I}/Re^{I}$ Tricarbonyl Complexes with Tridentate Cysteamine Based Ligands: Synthesis, Characterization and in vitro/in vivo Evaluation

Carolina Moura,[a] Lurdes Gano,[a] Isabel C. Santos,[a] Isabel Santos,[a] and António Paulo*[a]

Keywords: Medicinal chemistry / Radiopharmaceuticals / Antitumor agents / Technetium / Rhenium / Carbonyl complexes / Bifunctional chelators / Melanin binders

Tricarbonyl M^{I} (M = Re, ^{99m}Tc) complexes of a new tridentate cysteamine based chelator have been synthesized and fully characterized. To gain an insight into the usefulness of this new chelator for labeling biomolecules with a *fac*-$[M(CO)_3]^+$ core, the ligand framework has been functionalized with a *N*-(dialkylaminoalkyl) pharmacophore. The corresponding $^{99m}Tc^{I}$ complexes showed moderate affinity for melanin and moderate cell uptake by B16F1 murine melanoma cells, and therefore deserve further evaluation in vivo as radioactive probes for the detection of melanomas.

Introduction

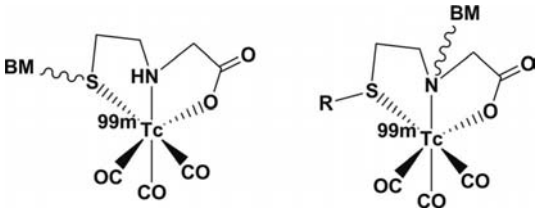
The group 7 elements Tc and Re can be seen as a unique pair that enable the design of radiopharmaceuticals for diagnostic (^{99m}Tc) and therapeutic applications ($^{186/188}$Re) in nuclear medicine by profiting from the similitude of their chemistry.[1] Moreover, nonradioactive Re complexes are often used as reference compounds for the assignment of the chemical identity of the ^{99m}Tc congeners. For radiopharmaceutical research, Re and Tc offer the possibility of exploring a large variety of oxidation states, metallic cores, and bifunctional chelators.[2–6] In this field, the so-called tricarbonyl approach has gained considerable attention in the last few years, following the introduction by Alberto and coworkers of a convenient and fully aqueous based kit preparation method for the organometallic precursors *fac*-$[M(OH_2)_3(CO)_3]^+$ (M = Tc, Re).[7,8]

With the tricarbonyl approach, it is possible to use uni-, bi- and tridentate ligands but tridentate chelators usually provide complexes with a higher chemical robustness and enhanced in vivo stability. For this reason, tridentate ligands are considered the most appropriate ligands for stabilizing the *fac*-$[M(CO)_3]^+$ (M = Tc, Re) core in biological media. The study of new bifunctional chelators (BFCs) for the labeling of biomolecules with the *fac*-$[M(CO)_3]^+$ moiety is still an active area of research. In particular, it is still quite important to find BFCs that form M^{I} (M = Tc, Re) tricarbonyl complexes that, after functionalization with a given pharmacophore, are able to diffuse freely through cell membranes to reach intracellular targets. Tripodal/tridentate (N,S,O)-donor ligands of the cysteine type have received considerable attention as BFCs for the labeling of different biomolecules with the *fac*-$[^{99m}Tc(CO)_3]^+$ core,[9–12] in contrast to related linear/tridentate ligands that have the same donor atom set. Nevertheless, the different topologies of structurally related chelators (e.g. linear vs. tripodal) might influence the in vitro and in vivo behavior of the corresponding complexes, namely their capability to cross biological membranes.

In this work, we introduce a new linear (N,S,O)-donor ligand framework derived from cysteamine that offers the possibility of functionalization with biomolecules at two different positions while filling all the three vacant coordination sites of the *fac*-$[M(CO)_3]^+$ fragment (Scheme 1). To assess the adequacy of this new ligand framework for stabilizing the *fac*-$[M(CO)_3]^+$ core, we have studied the reaction of the newly synthesized 2-[2-(ethylthio)ethylamino]acetic acid (**L^1H**) with the aqua-tricarbonyl precursors *fac*-$[M(OH_2)_3(CO)_3]^+$ (M = Re, ^{99m}Tc). Herein, we report the synthesis and characterization of the organometallic complexes *fac*-$[M(CO)_3(L^1)]$ [M = Re (**1**), ^{99m}Tc (**1a**)], and the in vitro and in vivo stability of **1a**. To gain a further insight into the usefulness of the new ligand framework for labeling small pharmacophores by the tricarbonyl approach, **L^1H** has been functionalized with a *N*,*N*-diethylethylamine group with ethylenic and propylenic linkers to give 2-{[2-(diethylamino)ethyl][2-(ethylthio)ethyl]amino}acetic acid (**L^2H**) and 2-{[3-(diethylamino)propyl][2-(ethylthio)ethyl]-amino}acetic acid (**L^3H**), respectively. We also report the preparation of the corresponding complexes *fac*-$[M(\kappa^3\text{-}L)(CO)_3]$ [M = Re, L = L^2 (**2**), L^3 (**3**); M = ^{99m}Tc, L = L^2 (**2a**), L^3 (**3a**)]. By including the *N*-(dialkylaminoalkyl) structural element in the ligand we expected to obtain complexes with affinity for melanin and with relevance for the design of radioactive probes for the detection of melanotic melanoma.

[a] Unidade de Ciências Químicas e Radiofarmacêuticas, ITN, Estrada Nacional 10, 2686-953 Sacavém, Portugal
E-mail: apaulo@itn.pt

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

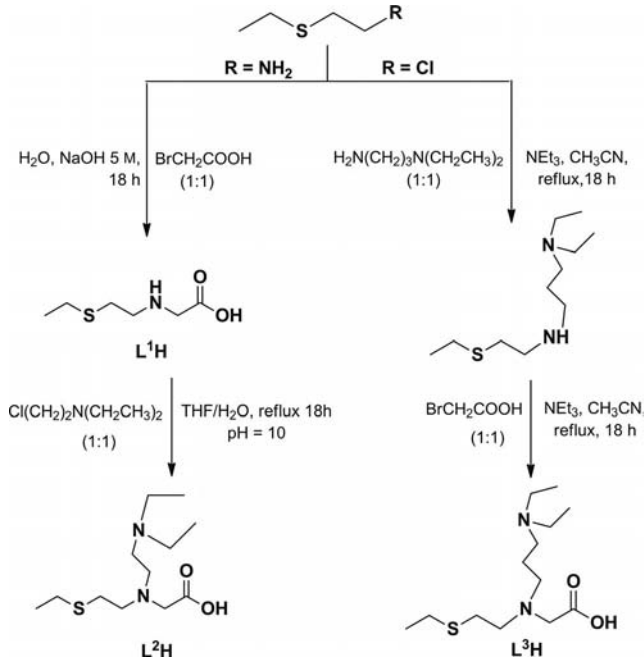


Scheme 1. Molecular structures of the $^{99m}Tc^{I}$ tricarbonyl complexes prepared with a new cysteamine-based bifunctional chelator (BM = biomolecule).

Results and Discussion

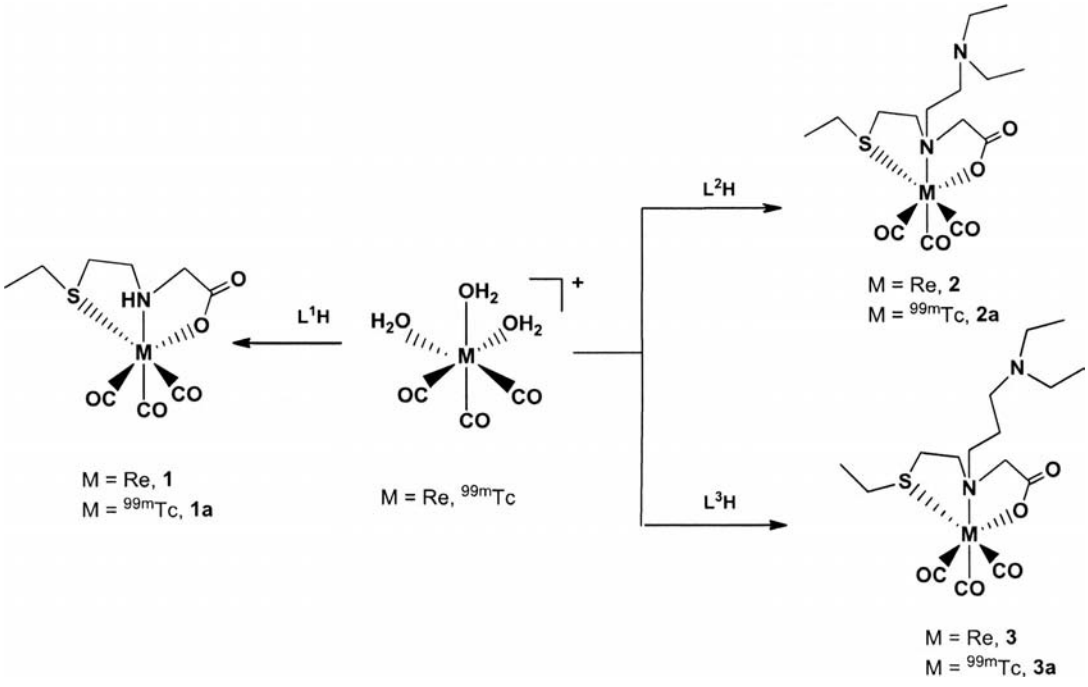
Synthesis of the Ligands and Their Respective Re Complexes

As shown in Scheme 2, the newly synthesized acid **L^{1}H** was prepared by *N*-alkylation of 2-(ethylthio)ethanamine with bromoacetic acid. After purification of the reaction products by column chromatography, **L^{1}H** was recovered as a microcrystalline white solid with a moderate yield of 63%. **L^{1}H** was functionalized with a *N*,*N*-diethylethylamine group with ethylenic and propylenic linkers to give **L^{2}H** and **L^{3}H**, respectively. Two different strategies were used to obtain the BFCs **L^{2}H** and **L^{3}H** (Scheme 2). **L^{2}H** was synthesized by *N*-alkylation of an appropriate chloride derivative with **L^{1}H**. The synthesis of **L^{3}H** was performed in two steps. The first step involved the reaction of the commercially available 2-chloroethyl ethyl sulfide and 3-(diethylamino)propylamine, which gave a secondary amine derivative. Then, in the second step, this derivative was treated with bromoacetic acid to afford the final ligand.



Scheme 2. Synthesis scheme for the ligands.

The *N*,*N*-diethylamine structural element incorporated into the structures of **L^{2}H** and **L^{3}H** is also present in several benzamide derivatives that have affinity for melanin and accumulate selectively in melanotic melanoma cells.[13–14] For this reason, several research groups have designed and evaluated ^{99m}Tc complexes functionalized with *N*-(dialkylaminoalkyl) groups to aid in the search of radioactive probes for in vivo targeting of melanoma.[15–19] In this field, our research group reported recently $^{99m}Tc^{I}$ tricarbonyl



Scheme 3. Synthesis scheme for the Re^{I} and $^{99m}Tc^{I}$ complexes.

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

complexes of tridentate pyrazolyl containing chelators functionalized with *N*-(2-aminoethyl)diethylamine and 4-amino-*N*-(2-diethylaminoethyl)benzamide groups that act as melanin binders.[20] This class of complexes exhibited a high in vitro affinity for melanin but showed, in general, hydrophilic character, which certainly justified their poor uptake by murine melanoma cells. We anticipated that the framework of **L^{1}H** will provide smaller and more lipophilic complexes than these previously reported complexes, while retaining affinity for melanin upon functionalization with a *N*-(dialkylaminoalkyl) pharmacophore.

The synthesis of the Re^I organometallic complexes **1**–**3** was performed by ligand exchange reactions of *fac*-[Re(H_2O)$_3$(CO)$_3$]Br with **L^{1}H**–**L^{3}H** in refluxing methanol (Scheme 3). Complexes **1**–**3** were purified by column chromatography or by reverse phase high performance liquid chromatography (RP-HPLC), and were recovered in low to moderate yields (25%-45%). All these complexes are stable towards aerial oxidation or hydrolysis, either in the solid state or in solution. Their characterization was done by common spectroscopic techniques (IR, ^{1}H and ^{13}C NMR), by ESI-MS, and elemental analyses, and also in the case of **1** by X-ray diffraction analysis.

NMR Studies

As can be seen in Figure 1, the ^{1}H NMR spectrum of complex **1** in CD_3OD recorded at 20 °C showed two relatively broad signals for the N–H proton and another two broad signals for one of the methylenic protons, CH_2(a). The corresponding ^{13}C NMR recorded at the same temperature also presented two signals for each of the magnetically nonequivalent methylenic carbon nuclei. These findings indicated the presence of two different species in solution that are undergoing dynamic processes. To gain a better insight into such dynamic processes, we have studied the effect of temperature, from –10 to 60 °C, on the ^{1}H NMR spectra of **1**, and we have recorded the respective ^{13}C NMR spectrum at 60 °C.

When lowering the temperature to –10 °C, the peaks become sharper and this effect is most evident for the above mentioned N–H and CH_2(a) methylenic proton signals, which give rise, respectively, to two singlets at δ = 7.18 and 7.37 ppm and to two multiplets at δ = 2.31 and 2.46 ppm, with approximate relative intensities of 1:2. When the temperature was raised, these signals became broader and then at 60 °C each set of two signals merged to give single well defined peaks, which appear at δ = 7.25 and 2.44 ppm, respectively. At –10 °C, the remaining diastereotopic methylenic protons are manifest as a complex set of multiplets between 2.8 and 3.8 ppm. No further changes were observed in the ^{1}H NMR spectra of **1** when the temperature was lowered to below –10 °C. The several CH_2 resonances in the spectra of **1** were assigned based on 2D homonuclear [^{1}H,^{1}H] gradient COSY (gCOSY) and heteronuclear [^{1}H,^{13}C] gradient heteronuclear single quantum correlation (gHSQC) experiments, which were run at –10 °C and 60 °C. The ^{13}C NMR spectrum of **1** recorded at 60 °C also showed only one peak for each CH_2 carbon, although they are relatively broad and the CH_2(a) and CH_2(d) peaks overlap. Altogether, these data indicate that the two isomers interconvert rapidly as the temperature is raised, a fast exchange regime was not completely reached even when the temperature was 60 °C.

Most probably the dynamic behavior observed for **1** in solution is due to the pyramidal inversion of the coordinated sulfur atom, as invoked for other Re^I tricarbonyl complexes with thioether containing ligands.[10] Such inversion leads to the formation of two interconverting diastereoisomers that have different orientations of their terminal ethyl substituent groups and sulfur lone pairs, which can be either *endo* or *exo* relatively to their carbonyl groups.

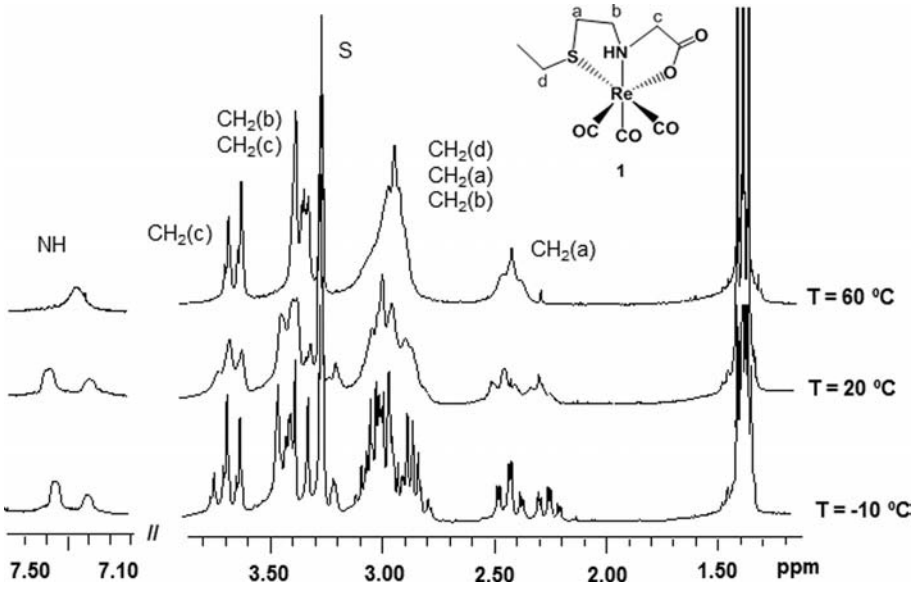


Figure 1. ^{1}H NMR spectra of complex **1** in CD_3OD recorded at various temperatures.

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

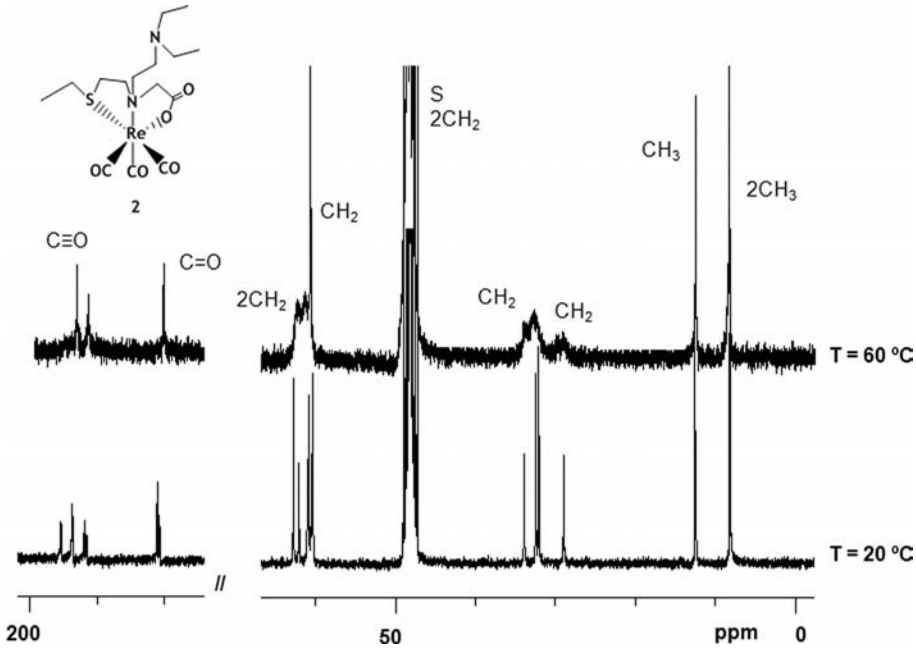


Figure 2. ^{13}C NMR spectra of complex **2** in CD_3OD recorded at 20 °C and 60 °C.

At 20 °C, the rate of the fluxional process is slow enough to distinguish the two invertomers of **1** by ^1H and ^{13}C NMR spectroscopy.

In the case of complexes **2** and **3** that contain the same S,N,O-donor set but also bear a *N*,*N*-diethylamine substituent, the ^1H and ^{13}C NMR spectra obtained at room temperature also indicated the presence of two interconverting species for each of these complexes, which are due to the pyramidal inversion at the sulfur atom. The ^1H NMR spectra of these compounds are quite complicated due to the presence of the additional CH_2 resonances associated with the linkers and *N*,*N*-diethylamine substituents. For this reason, we could not follow properly the effect of temperature on the ^1H resonances of **2** and **3**. However, the effect of the temperature on the ^{13}C NMR spectra of **2** and **3** was similar to that found for complex **1**. As an example, the spectra of complex **2** recorded at 20 °C and 60 °C are presented in Figure 2. At 20 °C, each methylenic carbon of **2** and **3** produces two relatively narrow resonances, which is consistent with the presence of two diastereoisomeric forms, as discussed above for **1**. When the temperature was raised to 60 °C, the signals of the two diastereoisomers merged giving raise to relatively narrow resonances for the CH_2 groups of the linker and *N*-diethylamine substituent, and to rather broad resonances for the carbon atoms linked to the coordinating atoms. These findings indicate that a fast exchange limit spectrum could not be obtained at this temperature, and show that inversion of sulfur coordination geometry most strongly effects the chemical environment of the atoms that are close to the metallic center.

X-ray Diffraction Studies

The molecular structure of **1** was confirmed by X-ray diffraction analysis. The asymmetric unit of the analyzed crystal of **1** contains two independent molecules that have the same chirality at the sulfur atom, with the ethyl groups in *endo* positions relatively to the CO ligands coordinated *trans* to the carboxylate oxygen atoms of the $\mathbf{L^1}$ ligands. Therefore, these two independent molecules do not correspond to the two diastereoisomers that were found in solution. A selection of bond lengths and angles for the two independent molecules of **1** is given in Table 1. An ORTEP diagram of one of the independent molecules of **1** is shown

Table 1. Selected bond lengths [Å] and angles (°) for the molecules in the crystallographic asymmetric unit of **1**.

Molecule 1			
Re(1)–C(2)	1.921(5)	Re(1)–O(4)	2.135(4)
Re(1)–C(1)	1.924(6)	Re(1)–N(1)	2.210(4)
Re(1)–C(3)	1.891(6)	Re(1)–S(1)	2.4980(12)
C(3)–Re(1)–C(2)	87.5(2)	C(1)–Re(1)–N(1)	173.32(19)
C(3)–Re(1)–C(1)	89.6(2)	O(4)–Re(1)–N(1)	76.83(15)
C(2)–Re(1)–C(1)	90.0(2)	C(3)–Re(1)–S(1)	95.87(15)
C(3)–Re(1)–O(4)	171.64(18)	C(2)–Re(1)–S(1)	174.20(17)
C(2)–Re(1)–O(4)	96.93(18)	C(1)–Re(1)–S(1)	94.68(16)
C(1)–Re(1)–O(4)	97.47(19)	O(4)–Re(1)–S(1)	79.14(10)
C(3)–Re(1)–N(1)	95.84(19)	N(1)–Re(1)–S(1)	80.92(11)
C(2)–Re(1)–N(1)	94.08(19)		
Molecule 2			
Re(2)–C(11)	1.904(6)	Re(2)–O(9)	2.142(4)
Re(2)–C(10)	1.915(6)	Re(2)–N(2)	2.200(5)
Re(2)–C(12)	1.933(7)	Re(2)–S(2)	2.4806(14)
C(11)–Re(2)–C(10)	86.9(2)	C(12)–Re(2)–N(2)	171.9(2)
C(11)–Re(2)–C(12)	89.3(2)	O(9)–Re(2)–N(2)	76.49(15)
C(10)–Re(2)–C(12)	89.9(3)	C(11)–Re(2)–S(2)	95.36(17)
C(11)–Re(2)–O(9)	173.42(19)	C(10)–Re(2)–S(2)	174.9(2)
C(10)–Re(2)–O(9)	97.3(2)	C(12)–Re(2)–S(2)	94.62(18)
C(12)–Re(2)–O(9)	95.74(19)	O(9)–Re(2)–S(2)	80.01(10)
C(11)–Re(2)–N(2)	98.3(2)	N(2)–Re(2)–S(2)	81.99(13)
C(10)–Re(2)–N(2)	93.2(2)		

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

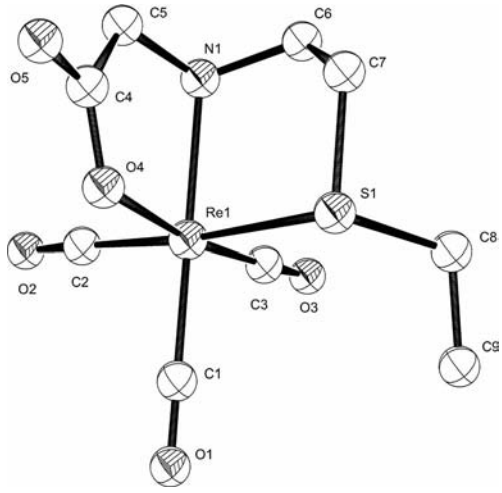


Figure 3. ORTEP view of one of the crystallographically independent molecules of complex **1**.

in Figure 3. As can be seen in Figure 3, the coordination geometry around the six coordinate rhenium atom is a distorted octahedron. One of the faces of this octahedron is occupied by the three carbonyl ligands, and the remaining coordination sites are occupied by the N,S,O-donor ligand. The Re–C, Re–O, Re–N, and Re–S bond lengths found for **1** are comparable to those reported for other octahedral complexes containing the *fac*-$[Re(CO)_3]^+$ unit and the same donor atoms.[9–12,21]

Synthesis, Characterization and Biological Evaluation of ^{99m}Tc Complexes

The synthesis of the congener ^{99m}Tc complexes **1a**–**3a** was achieved in aqueous solution by reaction of *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ with $\mathbf{L^1H}$–$\mathbf{L^3H}$ (10^{-4} M) at 100 °C for 30 min (Scheme 3). Under these conditions, all complexes were obtained in a high radiochemical yield (≥ 95%). Complex **1a** could be obtained with high radiochemical purity when the reaction solution was at pH 7.4. However, the use of acidic conditions (pH 3.0) was necessary to avoid the formation of radiochemical impurities in the case of **2a** and **3a**. The formation of such radiochemical impurities results most probably from the hydrolytic cleavage of the pendant arm used to couple the *N*,*N*-diethylamine group to the chelator, and is almost certainly a metal assisted process, as previously reported for other ^{99m}Tc complexes.[22,23]

The chemical identifications of **1a**–**3a** have been done by comparison of their HPLC profiles with those of the corresponding rhenium complexes **1**–**3**. At room temperature, only a single peak was observed in the HPLC chromatograms for all ^{99m}Tc and Re complexes, despite of the formation of diastereoisomers in the case of the rhenium complexes, indicating that the interconversion of the diastereosomers is faster than the HPLC timescale. However, it may happen that both diastereoisomers have coincident retention times.

The resistance of **1** against transchelation has been studied with a 10^3-fold excess of two potentially metal binding amino acids, cysteine and histidine. Complex **1** did not undergo significant transchelation with cysteine or histidine, even after 6 h of incubation at 37 °C. The in vivo metabolic stability of complex **1** has also been assessed by HPLC analysis of the urine and blood from CD1 mice injected with this complex collected 1 h post injection (p.i.) (Figure 4). These studies have shown that the intact complex was the main radioactive species detected in these biological samples. Altogether, these data have shown that the new S,N,O-donor ligand, $\mathbf{L^1H}$, forms a $^{99m}Tc^I$ complex with high in vitro and in vivo stability.

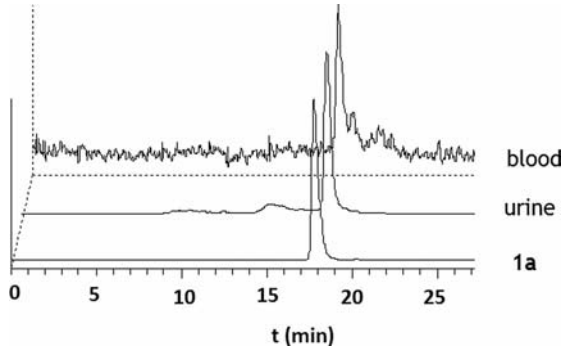


Figure 4. HPLC data (radiometric detection) for blood and urine obtained 60 min p.i from mice injected with **1a**.

The in vitro evaluation of **1a**–**3a** also involved the measurement of their lipophilicity (Table 2). The lipophilicity, expressed as the distribution coefficient ($\log D$) in octanol/0.1 M phosphate buffer (pH 7.4), was determined by the multiple back extraction method.[24] At pH 7.4, **1a** and **2a** are moderately lipophilic with $\log D_{o/w}$ values of 0.36 and 0.53, respectively. In contrast, complex **3a** is hydrophilic ($\log D_{o/w}$ = –0.72) at the same pH. The observed lipophilicity results for complexes **2a** and **3a** were unexpected. In fact, one could expect, by taking into consideration the additive effect on lipophilicity of the methylenic substituents of the linkers, that **3a** would be more lipophilic than **2a**. Complexes **2a** and **3a** are ionizable compounds and, therefore, we hypothesize that their unexpected $\log D_{7.4}$ values could reflect different pK_a values for their basic pendant *N*,*N*-diethylamine groups. Different pK_a values would lead to significantly different degrees of ionization (α) of the compounds and will affect their distribution coefficients. We have determined the pK_a values of **2a** and **3a** using

Table 2. In vitro characterization data for complexes **1a**–**3a**.

	r.t. (min)[a,b]	$\log D_{7.4}$	pK_a[b]	% Bound to melanin
1a	18.5 (18.4)	0.36 ± 0.03	–	<1
2a	17.8 (17.8)	0.53 ± 0.01	7.21 (6.97)	23 ± 2
3a	17.90 (17.88)	–0.72 ± 0.02	8.27 (8.27)	31 ± 6

[a] Retention times (r.t.) determined with a gradient of aqueous 0.1% CF_3COOH and methanol as the solvent. [b] The values in parentheses are for the Re congeners **1**–**3**. The concentration of melanin was 0.5 mg/10 mL, and samples were collected after 1 h of incubation.

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

HPLC techniques. The obtained values are presented in Table 2, together with the pK_a's of the fully characterized Re congeners (**2** and **3**), determined with the same methodology. The observed pK_a's of 7.21 and 8.27 show that roughly 60% and 10% of **2a** and **3a**, respectively, are in their neutral and unprotonated forms at pH 7.4. This difference justifies why **2a** presents a lower log $D_{7.4}$ value than **3a**, despite the presence of an additional methylenic group in the structure of the later. At high pH both complexes must be almost fully unprotonated, therefore **3a** is expected to be more lipophilic than **2a** under these conditions. This was confirmed by measurements of the distribution coefficients of **2a** and **3a** at pH 10.6, which gave log $D_{10.6}$ values of 1.01 and 1.23, respectively.

As already mentioned, the molecular target of complexes **2a** and **3a** is the intracellular melanin present in the cytosol of melanotic melanoma cells. Hence, we have evaluated the melanin affinity of **2a** and **3a** by incubation of the compounds with synthetic melanin in distilled water, and we have assessed the percentage of bound complexes that formed using a methodology described elsewhere.[20] The percentage of the complexes that bound to melanin was 23 and 31% for **2a** and **3a**, respectively. Under the same conditions, complex **1a** has shown negligible binding to melanin (less than 1%), showing that the introduction of the *N*-diethylethylamine substituent contributes to an increase in the melanin affinity of the complexes. Melanin is a polymer rich in negatively charged groups, and melanin binders interact with this pigment mainly through ionic interactions. Due to its highest pK_a, the fraction of complex **3a** in the protonated form is higher than that of complex **2a**, which most probably justifies the higher binding affinity of **3a** for melanin relative to **2a**.

Cellular uptake studies were performed for **2a** and **3a** with murine B16F1 melanoma cells to obtain an insight into the ability of the complexes to accumulate in melanotic melanoma cells. As can be seen in Figure 5, the cell uptake at 37 °C was time dependent and showed plateau values of 16.5 and 10.4% after 4 h of incubation for **2a** and **3a**, respectively, when expressed as a percentage of total activity per million cells. Compound **2a** has shown faster uptake kinetics and a higher cell accumulation than **3a**, which seems to indicate that the cell uptake and accumulation of these complexes is ruled by their lipophilicity at physiologic pH and not by their affinity for melanin.

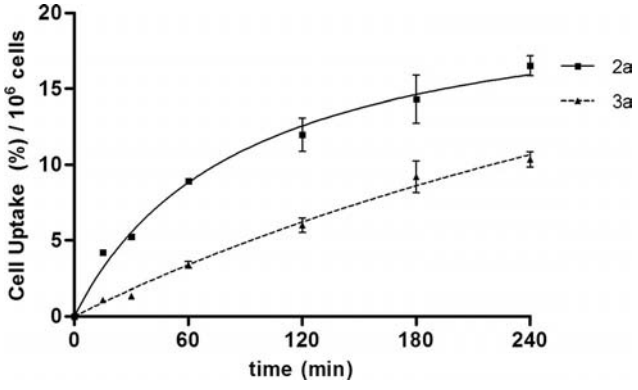


Figure 5. In vitro cell uptake of complexes **2a** and **3a** by murine B16F1 melanoma cells.

Conclusions

The ligand $\mathbf{L^1H}$ allowed for the formation of M^I (M = Re, ^{99m}Tc) tricarbonyl complexes (**1** and **1a**) that have high in vitro and in vivo stability, therefore $\mathbf{L^1H}$ appeared to be a new S,N,O-donor framework suitable for further exploration in the design of bifunctional chelators for labeling biomolecules with the *fac*-$[M(CO)_3]^+$ core. The new ligand framework has been functionalized with a *N*-(dialkylaminoalkyl) fragment, introduced at the central amine with different spacers. The introduction of this small pharmacophore did not affect the binding mode and coordination capability of the resulting functionalized (N,S,O)-donor ligands ($\mathbf{L^2H}$ and $\mathbf{L^3H}$). However, surprisingly, the length of the linker markedly influenced the pK_a values of the corresponding ^{99m}Tc complexes **2a** and **3a** and their lipophilicity at physiologic pH. Complexes **2a** and **3a** have a moderate affinity for melanin and have shown a moderate cell uptake by B16F1 murine melanoma cells. These findings indicate that **2a** and **3a** deserve to be evaluated further in vivo as radioactive probes for the detection of melanoma. A study of the biodistribution of **2a** and **3a** in melanoma-bearing mice is currently underway.

Experimental Section

Materials and Methods: Unless otherwise stated the synthesis and work-up of the ligands and complexes were performed in air. The starting material *fac*-$[Re(H_2O)_3(CO)_3]Br$ was synthesized by a literature method.[25] Na[$^{99m}TcO_4$] was eluted from a commercial ^{99}Mo/^{99m}Tc generator with 0.9% saline. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 300 MHz spectrometer; ^{1}H and ^{13}C chemical shifts are given in ppm and are referenced to the residual solvent resonances that are relative to $SiMe_4$. IR spectra were recorded with KBr pellets on a Bruker Tensor 27 spectrometer. Electrospray ionisation mass spectrometry (ESI-MS) was performed at the Instituto Tecnológico e Nuclear (ITN) on a QITMS instrument in positive ion mode. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates. Column chromatography was performed with silica gel 60 (Merck). HPLC analysis of the Re and ^{99m}Tc complexes was performed on a Perkin–Elmer LC pump 200 coupled to a LC 290 tunable UV/Vis detector and to a Berthold LB-507A radiometric detector, and with an analytical Macherey–Nagel C18 reversed phase column (Nucleosil 100–10, 250 × 3 mm) and at a flow rate of 1 mL min^{-1}. Elution was with a gradient of aqueous 0.1% CF_3COOH (A) and methanol (B) method: 0–3 min, 100% A; 3–3.1 min, 100–75% A; 3.1–9 min, 75% A; 9–9.1 min 75–66% A; 9.1–20 min, 66–0% A; 20–25 min, 0% A; 25–25.1 min, 0–100% A; 25.1–30 min, 100% A.

2-[2-(Ethylthio)ethylamino]acetic Acid ($\mathbf{L^1H}$): To a solution of 2-(ethylthio)ethanamine (600 μL, 5.40 mmol) in H_2O (12 mL), at 0 °C, was added dropwise a solution of bromoacetic acid (250 mg, 1.80 mmol) in H_2O (12 mL), and a 8 N NaOH solution (900 μL, 7.20 mmol). The reaction mixture was left to stir at room temperature overnight. Then, the pH was adjusted to 5–6 by addition of

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

1 N HCl, and the solvent was removed under vacuum. The residue was applied to the top of a silica-gel column, and eluted with a gradient method starting from $CHCl_3$/MeOH/NH_4OH (70:28:2) and changing to MeOH/NH_4OH (98:2). Compound **L^1H** in the form of an ammonium salt was recovered from the collected fractions as a white solid after removal of the solvent under reduced pressure. Yield 200 mg (63%). R_f [SiO_2, $CHCl_3$/MeOH/NH_4OH (70:28:2)] = 0.28. $C_6H_{12}NO_2SNH_4$ (180.29): calcd. C 39.98, H 8.95, N 15.54, S 17.78; found C 40.00, H 8.38, N 15.32, S 17.82. ESI-MS (*m*/*z*): calcd. for $[M + H]^+$, 164.1; found 164.1. IR (KBr pellets): $\tilde{\nu}_{max}$ = 1582 (C=O) cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ_H = 1.26 (t, 3 H, CH_3), 2.60 (q, 2 H, CH_2), 2.85 (t, 2 H, CH_2), 3.25 (t, 2 H, CH_2), 3.55 (s, 2 H, CH_2) ppm. ^{13}C NMR (300 MHz, CD_3OD): δ_c = 15.13 (CH_3), 26.39 (CH_2), 28.21 (CH_2), 47.84 (CH_2), 50.60 (CH_2), 171.15 (C=O) ppm.

2-{[2-(Diethylamino)ethyl][2-(ethylthio)ethyl]amino}acetic Acid (L^2H): To a solution of **L^1H** (250 mg, 1.50 mmol) in H_2O (6 mL) was added a solution of 2-chloro-*N*,*N*-diethylethanamine (258 mg, 1.50 mmol) in THF (9 mL). The pH was adjusted to 10–12 by the addition of 5 N NaOH, and the mixture was refluxed overnight. The solvent was removed under vacuum and the solid purified by silica gel column chromatography with $CHCl_3$/MeOH/NH_4OH (80:18:2) as the eluent. Compound **L^2H** in the form of an ammonium salt was recovered from the collected fractions after removal of the solvent under reduced pressure. Yield 140.0 mg (34%). R_f [SiO_2, $CHCl_3$/MeOH/NH_4OH (80:18:2)] = 0.77. $C_{12}H_{25}N_2O_2SNH_4$ (279.20): calcd. C 51.58, H 10.46, N 15.04, S 11.47; found C 51.63, H 10.03, N 14.85, S 11.55. ESI-MS (*m*/*z*): calcd. for $[M + H]^+$, 263.2; found 263.1. IR: $\tilde{\nu}_{max}$ = 1578 (C=O) cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ_H = 1.21 (t, 3 H, CH_3), 1.28 (t, 6 H, 2 CH_3), 2.55 (q, 2 H, CH_2), 2.65 (t, 2 H, CH_2), 2.81 (m, 4 H, 2 CH_2), 3.06 (m, 2 H, CH_2), 3.10 (m, 6 H, 3 CH_2) ppm. ^{13}C NMR (300 MHz, CD_3OD): δ_c = 8.98 (CH_3), 15.30 (CH_3), 27.02 (CH_2), 29.99 (CH_2), 48.25 (CH_2), 52.25 (CH_2), 53.52 (CH_2), 56.71 (CH_2), 58.45 (CH_2), 179.95 (C=O) ppm.

***N*,*N*-Diethyl-6-(ethylthio)hexan-1-amine:** To a solution of 2-chloroethyl ethyl sulfide (467 μL, 4.02 mmol) in dry CH_3CN (20 mL) was added [3-(diethylamino)propyl]amine (634 μL, 4.02 mmol), NEt_3 (1.69 mL, 12.06 mmol) and KI. The reaction mixture was refluxed overnight and, after cooling to room temperature, the solvent was removed under vacuum. The residue was purified by silica-gel column chromatography with a mixture of $CHCl_3$/MeOH/NH_4OH (85:13:2) as the eluent. The desired product was recovered as a yellow oil from the collected fractions after removal of the solvent under reduced pressure. Yield 348 mg (40%). R_f [SiO_2, $CHCl_3$/MeOH/NH_4OH (85:13:2)] = 0.78. $C_{11}H_{26}N_2S$ (218.40): calcd. C 60.49, H 12.00, N 12.83, S 14.68; found C 60.39, H 11.68, N 12.94, S 14.54. ESI-MS: calcd. for $[M + H]^+$, 219.2; found 219.0. ^1H NMR (300 MHz, $CDCl_3$): δ_H = 0.93 (t, 6 H, 2 CH_3), 1.17 (t, 3 H, CH_3–S), 1.57 (m, 2 H, CH_2), 2.47 (m, 8 H, 4 CH_2), 2.59 (m, 4 H, 2 CH_2), 2.71 (m, 2 H, CH_2) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ_c = 11.77 (CH_3), 15.03 (CH_3–S), 25.92 (CH_2), 27.30 (CH_2), 31.95 (CH_2), 46.97 (CH_2), 48.55 (CH_2), 48.76 (CH_2), 51.28 (CH_2) ppm.

2-{[3-(Diethylamino)propyl][2-(ethylthio)ethyl]amino}acetic Acid (L^3H): To a solution of *N*,*N*-diethyl-6-(ethylthio)hexan-1-amine (348 mg, 1.59 mmol) in H_2O (20 mL), kept at 0 °C, was added dropwise a solution of bromoacetic acid (221 mg, 1.59 mmol) in H_2O (20 mL), this was followed by the addition of a 8 N NaOH (800 μL) solution. The reaction mixture was left to stir at room temperature overnight. The pH of the reaction mixture was then adjusted to 5, and the solvent was removed under vacuum. The residue was purified by silica-gel column chromatography with a mixture of $CHCl_3$/MeOH/NH_4OH (80:18:2) as the eluent. **L^3H** was recovered as a yellow oil from the collected fractions after removal of the solvent under reduced pressure. Yield 109 mg (23%). R_f [SiO_2, $CHCl_3$/MeOH/NH_4OH (80:18:2)] = 0.52. $C_{13}H_{27}N_2O_2SNH_4$ (293.47): calcd. C 53.21, H 10.65, N 14.32, S 10.92; found C 53.36, H 10.28, N 14.24, S 10.82. ESI-MS (*m*/*z*): calcd. for $[M + H]^+$, 277.1; found 277.2. IR (KBr): $\tilde{\nu}$ = 1581 (C=O) cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ_H = 1.21 (t, 3 H, CH_3–S), 1.27 (t, 6 H, 2 CH_3), 1.88 (m, 2 H, CH_2), 2.55 (m, 4 H, 2 CH_2), 2.61 (s, 4 H, 2 CH_2), 3.07 (s, 2 H, CH_2), 3.23 (m, 6 H, 3 CH_2) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ_c = 8.43 (CH_3), 15.28 (CH_3–S), 21.59 (CH_2), 27.07 (CH_2), 29.32 (CH_2), 46.96 (CH_2), 51.61 (CH_2), 52.25 (CH_2), 54.49 (CH_2), 59.00 (CH_2), 179.26 (C=O) ppm.

General Procedure for the Synthesis of the Rhenium(I) Complexes: $[Re(H_2O)_3(CO)_3]Br$ was treated with equimolar amounts of **L^1H**–**L^3H** in refluxing methanol for 18 h. After this time, the solvent was removed under vacuum and the desired complexes were purified by column chromatography or by RP-HPLC. The RP-HPLC purification was achieved with a preparative Waters μ Bondapak C18 (150 × 19 mm) column, with a flow rate of 5.0 mL min^{-1}, and with the methods described below for each compound (A: 0.1% CF_3COOH; B: MeOH).

***fac*-[Re(CO)$_3$(k^3-L^1)] (1):** Yield 28.0 mg (25%). Complex **1** was purified by RP-HPLC with the following method: 0–5 min, 75% A; 5–30 min, 75–50% A; 30–30.1 min, 50–0% A; 30.1–38 min, 0% A. $ReC_9H_{12}NO_5S{\cdot}2CF_3COOH$ (660.51): calcd. C 23.64, H 2.14, N 2.12; found C 23.41, H 3.06, N 2.29. ESI-MS (*m*/*z*): calcd. for $[M + H]^+$, 434.0; found 433.9. IR: $\tilde{\nu}_{max}$ = 1637 (C=O), 1876, 1910, 2027 (C≡O) cm^{-1}. ^1H NMR (20 °C, 300 MHz, CD_3OD): δ_H = 1.39 (t, 3 H, CH_3); 2.31, 2.46 [1 H, br., CH_2(a)]; 2.90–3.06 [m, 4 H, CH_2(a), CH_2(b), CH_2(d)], 3.28–3.46 [m, 2 H, CH_2(b), CH_2(c)], 3.64–3.71 [m, 1 H, CH_2(c)], 7.18, 7.37 (1 H, br., NH) ppm. *T* = 60 °C: ^1H NMR (CD_3OD): δ_H = 1.40 (t, 3 H, CH_3); 2.44 [m, 1 H, CH_2(a)]; 2.97 [m, 4 H, CH_2(a), CH_2(b), CH_2(d)]; 3.36–3.41 [m, 2 H, CH_2(b), CH_2(c)]; 3.72 [m, 1 H, CH_2(c)]; 7.25 (1 H, br., NH) ppm. ^{13}C NMR (20 °C, 300 MHz, CD_3OD): δ_c = 13.56 (CH_3), 30.65, 31.05 [CH_2(a)], 33.00, 33.74 [CH_2(d)], 54.68, 55.30 [CH_2(c)], 56.16, 56.84 [CH_2(b)], 116.35 (C_{TFA}), 164.01 (C_{TFA}), 184.02 (C=O), 192.66, 194.89, 196.04 (C≡O). ^{13}C NMR (60 °C, 300 MHz, CD_3OD): δ_c = 13.47 (CH_3), 30.91 [br., CH_2(a), CH_2(d)], 55.06 [CH_2(c)], 56.39 [CH_2(b)], 183.62 (C=O), 192.61, 195.87 (C≡O).

***fac*-[Re(CO)$_3$(k^3-L^2)] (2):** Yield 35.0 mg (26%). Complex **2** was purified by RP-HPLC with the following method: 0–5 min, 75% A; 5–30 min, 75–50% A; 30–30.1 min, 50–0% A; 30.1–38 min, 0% A. $ReC_{15}H_{25}N_2O_5S{\cdot}CF_3COOH$ (645.66): calcd. C 31.62, H 4.06, N 4.34; found C 31.63, H 5.03, N 4.30. ESI-MS (*m*/*z*): calcd. for $[M + H]^+$, 533.1; found 533.0. IR: $\tilde{\nu}_{max}$ = 1687 (C=O), 1866, 2037 (C≡O) cm^{-1}. ^1H NMR (20 °C, 300 MHz, CD_3OD): δ_H = 1.31–1.47 (m, 9 H, CH_3), 2.66 (m, 1 H, CH_2), 2.95 (m, 1 H, CH_2), 3.14–4.02 (m, 14 H, CH_2) ppm. ^{13}C NMR (20 °C, 300 MHz, CD_3OD): δ_c = 9.05, 13.29, 13.44 (CH_3), 29.88, 32.99, 33.35, 34.81, 48.92, 49.37, 61.28, 61.59, 61.82, 61.95, 63.03, 63.72 (CH_2), 117.52 (C_{TFA}), 161.72 (C_{TFA}), 181.84, 182.17 (C=O), 192.65, 193.00, 194.74, 196.47 (C≡O) ppm. ^{13}C NMR (60 °C, 300 MHz, CD_3OD): δ_c = 9.13, 13.33 (CH_3), 29.96, 30.86 (br., CH_2), 33.58, 34.75 (br., CH_2), 49.15 (CH_2), 49.51 (CH_2), 61.58 (CH_2), 62.35 (br., CH_2), 63.52 (br., CH_2), 119.34 (C_{TFA}), 162.34 (C_{TFA}), 181.54 (C=O), 192.78, 194.47 (C≡O) ppm.

Synthesis of *fac*-[Re(CO)$_3$(κ^3-L^3)] (3): Yield 42 mg (0.077 mmol, 45%). Complex **3** was purified by silica-gel column chromatography with a mixture of $CHCl_3$/MeOH/NH_4OH (75:23:2) as the eluent. R_f [SiO_2, $CHCl_3$/MeOH/aq.NH_3 (75:23:2)] = 0.82.

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

$ReC_{16}H_{27}N_2O_5S$ (545.66): calcd. C 35.22, H 4.99, N 5.13; found C 34.99, H 4.66, N 4.98. ESI-MS (*m*/*z*): calcd. for $[M + H]^+$, 547.1; found 547.1. IR: $\tilde{\nu}_{max}$ = 1656 (C=O), 1880, 1935, 2027 (C≡O) cm^{-1}. ^1H NMR (20 °C, 300 MHz, CD_3OD): δ_H = 1.15 (m, 6 H, CH_3), 1.40 (m, 3 H, CH_3), 1.70 (m, 2 H, CH_2), 2.12 (m, 2 H, CH_2), 2.65 (m, 1 H, CH_2), 2.94 (t, 2 H, CH_2), 3.08–3.97 (m, 11 H, CH_2) ppm. ^{13}C NMR (20 °C, 300 MHz, CD_3OD): δ_c = 11.43, 13.23, 13.44 (CH_3), 22.87, 23.17, 29.57, 32.93, 33.43, 35.04, 47.90, 50.70, 50.76, 61.28, 61.39, 62.32, 63.43, 67.33, 69.23 (CH_2), 182.70, 182.98 (C=O), 193.14, 193.42, 195.28, 197.01 (C≡O) ppm. ^{13}C NMR (60 °C, 300 MHz, CD_3OD): δ_c = 11.64 (CH_3), 13.29 (CH_3), 23.23 (CH_2), 29.51 (br., CH_2), 33.15, 33.76, 34.97 (br., CH_2), 48.08 (CH_2), 51.16 (CH_2), 61.52 (CH_2), 63.16 (br., CH_2), 68.90, 69.34 (br., CH_2), 182.46 (C=O), 193.26, 195.11 (C≡O) ppm.

X-ray Diffraction Analysis: Single crystals of **1** adequate for X-ray diffraction analysis were obtained from a saturated solution of the complex in CH_3CN. The X-ray diffraction analysis was performed on a Bruker AXS APEX CCD area detector diffractometer, with graphite monochromated Mo-K_α radiation (0.71073 Å). The crystal data are summarized in Table 3.

Table 3. Crystallographic data for complex **1**.

Empirical formula	$Re_4C_{36}H_{48}NO_4S{\cdot}H_2O$
Molecular weight	1747.84
Crystal system	triclinic
Crystal size [mm^3]	$0.20 \times 0.12 \times 0.10$
Space group	$P\bar{1}$
a [Å]	9.9662(2)
b [Å]	11.8823(2)
c [Å]	12.9285(2)
α [°]	98.9870(10)
β [°]	111.4410(10)
γ [°]	108.2200(10)
V [$Å^3$]	1288.70(4)
T [K]	150(2)
Z	1
D_c [$g\,cm^{-3}$]	2.252
Reflections collected	11391
Independent reflections	4511 [*R*(int) = 0.0256]
μ(Mo-K_α) [mm^{-1}]	9.601
σ range for data collection [°]	3.30–25.03
Number of data	4511
Number of parameters	318
R_1 [$I > 2\sigma(I)$]	0.0243
wR_2 [$I > 2\sigma(I)$]	0.0547
R_1 (all data)	0.0274
wR_2 (all data)	0.0559
GOF	1.071

An empirical absorption correction was applied to the data with SADABS.[26] Data collection and data reduction were done with the SMART and SAINT programs.[27] The structure was solved by direct methods with sir97[28] and refined by full-matrix least-squares analysis with shelxl97[29] within the WINGX[30] suite of programs. Non-hydrogen atoms were refined with anisotropic thermal parameters, whereas all hydrogen atoms were placed in the model at idealized positions and allowed to refine by riding on the parent C atom. Molecular graphics were prepared with ORTEP3.[31]

CCDC-835282 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

General Method for the Synthesis of the $^{99m}Tc^I$ Complexes 1a–3a: To a nitrogen-purged glass vial containing a 4.4×10^{-3} M aqueous solution of $\mathbf{L^1H}$–$\mathbf{L^3H}$ (40 μL) was added a solution of the organometallic precursor *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ (400 μL, 1–2 mCi) in saline at pH 7.4 (**1a**) or at pH 3.0 (**2a** and **3a**). The reaction mixture was then heated to 100 °C for 30 min, cooled to room temperature, and the pH adjusted to 7.4 in the case of complexes **2a** and **3a**. The final solutions were analyzed by RP-HPLC by the method described above.

Challenge Experiments: To a nitrogen-purged glass vial were added either cysteine or histidine (900 μL, 1.11×10^{-3} M) in Phosphate-Buffered Saline (PBS) (pH 7.4) and complex **1a** (100 μL). The resulting solutions were incubated at 37 °C, and aliquots were removed after 1, 3 and 6 h and analyzed by HPLC.

Partition Coefficient Measurements: The $\log D_{o/w}$ values of complexes **1a**–**3a** were determined by the"shake flask" method preformed under physiological conditions (*n*-octanol/0.1 M PBS, pH 7.4).[24]

Determination of pK_a Values: The pK_a values for the Re complexes **1**–**3** and ^{99m}Tc complexes **1a**–**3a** were determined by previously described HPLC methods.[15,32] The pK_a values were determined on a Perkin–Elmer LC pump 200 coupled to a LC 290 tunable UV/Vis detector and a Berthold LB-507A radiometric detector, with a PR$P\bar{1}$ (250 × 4.1 mm, 10 μm, Hamilton) reversed phase column run under isocratic conditions with a flow rate of 1.0 mL/min and at room temperature. The mobile phase was acetonitrile:phosphate buffer (0.01 M), 60:40. The pK_a's were determined with buffers with pH values between 3 and 10. The pK_{HPLC} values were obtained from the fitted points of inflection of the sigmoidal $\log D_{HPLC}$/pH profiles. The aqueous ionization constants pK_a were calculated from the pK_{HPLC} values after correction with a predetermined correction factor obtained from analysis of standard amine compounds.[32]

In vitro Binding to Melanin: The melanin binding affinity of **1a**–**3a** was assessed with synthetic tyrosine–melanin (Sigma). The general procedure was as follows: an aliquot of the radioactive preparations of **1a**–**3a** (100 μL) was added to a melanin suspension (0.5 mg/10 mL) in distilled water. The reaction mixture was incubated at room temperature for 1 h with stirring. After incubation, the tubes were centrifuged at 30 000 g for 10 min, and aliquots of the supernatant were measured in a gamma counter. Control tubes that contained the radioactive preparation without melanin were also measured. The difference between the activity of aliquots taken from the supernatants of the test tubes (with melanin) and the control tubes (without melanin) allowed for the calculation of the percentage of the complexes that remained unbound.

Cell Culture: B16F1 murine melanotic melanoma cells (ECACC, UK) were grown in Dulbecco's Modified Eagle Medium (DMEM) containing GlutaMax I supplemented with 10% heat inactivated fetal bovine serum and 1% penicillin/streptomycin antibitiotic solution (all from Gibco, Alfagene, Lisbon). Cells were cultured at 37 °C in a humidified atmosphere comprising 95% air and 5% CO_2 (Heraeus, Germany), and the medium was changed every other day. The cells adhered in monolayers and, when confluent, were harvested from the cell culture flasks with trypsin–ethylenediaminetetraacetic acid (EDTA) (Gibco, Alfagene, Lisbon) and seeded farther apart.

Cellular Uptake Studies: Cellular uptake assays of the ^{99m}Tc complexes **2a** and **3a** were performed with B16F1 cells seeded at a density of 0.2 million/0.5 mL culture medium per well in 24 well tissue culture plates, and allowed to attach overnight. After that period,

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

the medium was removed and replaced by fresh medium containing approximately 2×10^5cpm/0.5 mL of each ^{99m}Tc complex. The cells were incubated again in a humidified 5% CO_2 atmosphere, at 37 °C for a period of 15 min to 4 h. After 0.25, 0.5, 1, 2, 3 and 4 h incubation periods the cells were washed twice with cold PBS, lysed with 0.1 N NaOH and the cellular extracts were counted for radioactivity. Each experiment was performed in quadruplicate. Cellular uptake data were expressed as an average value plus the standard deviation.

Evaluation of the In Vivo Stabilities: All animal experiments were performed in compliance with Portuguese regulations for animal treatment. The animals were housed in a temperature and humidity controlled room with a 12 h light/12 h dark schedule. Blood and urine samples from the mice injected with complex **1a**, which were collected at sacrifice time, were analyzed by HPLC to check the in vivo stability of the complex. Prior to HPLC analysis, the urine samples were centrifuged, and the serum was separated from the blood samples and treated with ethanol to precipitate the proteins present. The supernatant from these biological samples was analyzed by the method referred to above for the HPLC analysis of the ^{99m}Tc complexes.

Acknowledgments

C. M. thanks the Fundação do Ministério de Ciência e Tecnologia (FCT) for a Doctoral research grant (SFRH/BD/38469/2007). J. Marçalo is acknowledged for the ESI-MS analyses which were run on a QITMS instrument acquired with the support of the Programa Nacional de Reequipamento Científico (Contract REDE/1503/REM/2005 - ITN) of FCT and is part of RNEM - Rede Nacional de Espectrometria de Massa.

[1] J. Blower, S. Prakash, *Perspect. Bioinorg. Chem.* **1999**, *4*, 91–143.
[2] S. Liu, *Chem. Soc. Rev.* **2004**, *33*, 445–461.
[3] U. Abram, R. Alberto, *J. Braz. Chem. Soc.* **2006**, *17*, 1486–1500.
[4] R. Alberto, *Eur. J. Inorg. Chem.* **2009**, *18*, 21–31.
[5] J. D. G. Correia, A. Paulo, I. Santos, *Curr. Radiopharm.* **2009**, *2*, 277–294.
[6] M. D. Bartholom, A. S. Louie, J. F. Valliant, J. Zubieta, *Chem. Rev.* **2010**, *110*, 2903–2920.
[7] R. Alberto, R. Schibli, A. Egli, A. P. Schubiger, *J. Am. Chem. Soc.* **1998**, *120*, 7987–7988.
[8] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A. P. Schubiger, *J. Am. Chem. Soc.* **2001**, *123*, 3135–3136.
[9] D. R. van Staveren, P. D. Benny, R. Waibel, P. Kurz, J.-K. Pak, R. Alberto, *Helv. Chim. Acta* **2005**, *88*, 447–460.
[10] H. He, M. Lipowska, X. Xu, A. T. Taylor, M. Carlone, L. G. Marzilli, *Inorg. Chem.* **2005**, *44*, 5437–5446.
[11] O. Karagiorgou, G. Patsis, M. Pelecanou, C. P. Raptopoulou, A. Terzis, T. Siatra-Papastaikoudi, R. Alberto, I. Pirmettis, M. Papadopoulos, *Inorg. Chem.* **2005**, *44*, 4118–4120.
[12] H. He, J. E. Morley, B. Twamley, R. H. Groeneman, D.-K. Bucar, L. R. MacGillivray, P. D. Benny, *Inorg. Chem.* **2009**, *48*, 10625–10634.
[13] J. M. Michelot, M. F. C. Moreau, A. J. Veyre, J. F. Bonafous, F. J. Bacin, J. C. Madelmont, F. Bussiere, P. A. Souteyrand, L. P. Mauclaire, F. M. Chossat, J. M. Papon, P. G. Labarre, P. Kauffmann, R. J. Plagne, *J. Nucl. Med.* **1993**, *34*, 1260–1266.
[14] I. Greguric, S. R. Taylor, D. Denoyer, P. Ballantyne, P. Berghofer, P. Roselt, T. Q. Pham, F. Mattner, T. Bourdier, O. C. Neels, D. S. Dorow, C. Lo Loc'h, R. J. Hicks, A. Katsifis, *J. Med. Chem.* **2009**, *52*, 5299–5302.
[15] M. Friebe, A. Mahmood, H. Spies, R. Berger, B. Johannsen, A. Mohammed, M. Eisenhut, C. Bolzati, A. Davison, A. G. Jones, *J. Med. Chem.* **2000**, *43*, 2745–2752.
[16] M. Friebe, A. Mahmood, C. Bolzati, A. Drews, B. Johannsen, M. Eisenhut, D. Kraemer, A. Davison, A. G. Jones, *J. Med. Chem.* **2001**, *44*, 3132–3140.
[17] M. Eisenhut, A. Mohammed, W. Mier, F. Schonsiegel, M. Friebe, A. Mahmood, A. G. Jones, U. Haberkorn, *J. Med. Chem.* **2002**, *45*, 5802–5805.
[18] Z. Cheng, A. Mahmood, H. Li, A. Davison, A. G. Jones, *Cancer Res.* **2005**, *65*, 4979–4986.
[19] H. W. Peindy N'Dongo, P. D. Raposinho, C. Fernandes, I. Santos, D. Can, P. Schmutz, B. Spingler, R. Alberto, *Nucl. Med. Biol.* **2010**, *37*, 255–264.
[20] C. Moura, T. Esteves, L. Gano, P. D. Raposinho, A. Paulo, I. Santos, *New J. Chem.* **2010**, *34*, 2564–2578.
[21] C. Moura, C. Fernandes, L. Gano, A. Paulo, I. C. Santos, I. Santos, M. J. Calhorda, *J. Organomet. Chem.* **2009**, *694*, 950–958.
[22] C. Fernandes, I. C. Santos, I. Santos, H. J. Pietzsch, J. U. Kunstler, W. Kraus, A. Rey, N. Margaritis, A. Bourkoula, A. Chiotellis, M. Paravatou-Pestsotas, I. Pirmettis, *Dalton Trans.* **2008**, 3215–3225.
[23] S. Mundwiler, L. Candreia, P. Häfliger, K. Ortner, R. Alberto, *Bioconjugate Chem.* **2004**, *15*, 195–202.
[24] D. E. Troutner, W. A. Volkert, T. J. Hoffman, R. A. Holmes, *Int. J. Appl. Radiat. Isot.* **1984**, *35*, 467–470.
[25] N. Lazarova, S. James, J. Babich, J. Zubieta, *Inorg. Chem. Commun.* **2004**, *7*, 1023–1026.
[26] *SADABS*, Area-Detector Absorption Correction, Bruker AXS Inc., Madison, WI, USA, **2004**.
[27] *SAINT*, Area-Detector Integration Software, version 7.23, Bruker AXS Inc., Madison, WI, USA, **2004**.
[28] A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori, R. Spagna, *J. Appl. Crystallogr.* **1999**, *32*, 115–119.
[29] G. M. Sheldrick, *SHELXL-97: Program for the refinement of Crystal Structure*, University of Göttingen, Germany, **1997**.
[30] L. Farrugia, *J. Appl. Crystallogr.* **1999**, *32*, 837–838.
[31] L. Farrugia, *J. Appl. Crystallogr.* **1997**, *30*, 565.
[32] C. Stylli, A. E. Theobald, *Appl. Radiat. Isot.* **1987**, *38*, 701–708.

Received: July 29, 2011
Published Online: October 25, 2011

 © 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim