The complexation of rhodium(III) with acyclic diaminedithioether (DADTE) ligands†

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^{103}Rhodium(III) complexes derived from seven acyclic tetradentate N_2S_2 ligands (one diaminedithiol and six diaminedithioether ligands) have been synthesized and characterized. Structural variations in the ligand include the length of carbon backbone between the coordinating atoms (222; 232; 323; 333), the presence or absence of *gem*-dimethyl groups α to sulfur, and the nature of the organic moiety on the sulfurs (hydrogen, *p*-methoxybenzyl and methyl). For each ligand, the formation of *cis* and/or *trans* dichloro isomeric complexes was assessed. Two complexes have been further characterized by single crystal X-ray diffraction. Preparation of the ^{103}Rhodium(III) complexes was conducted and overall radiochemical yields, *in vitro* stability and log $D_{7.4}$ values were measured. From these studies, the ligand with the 232 chain length, *gem*-dimethyl groups and the methyl thioether (**L4**) emerged as a preferred ligand for formation of rhodium complexes with *trans* geometry and highest radiochemical yields.

Introduction

For the past twenty years, there has been much interest in ^{105}Rh as a therapeutic radioisotope due to its nuclear properties. ^{105}Rh decays to ^{105}Pd by β^- emissions of 566 keV (75%), 248 keV (19.7%), 260 keV (5.2%) and 133 keV (0.042%). The presence of concomitant γ-ray emissions [Eγ = 306 keV (5.1%), Eγ = 319 keV (19%)] provides the possibility for *in vivo* tracking of the therapeutic dose. ^{105}Rh has a half-life of 35.36 h, which is sufficiently long to allow the radiopharmaceutical to be manufactured, delivered and used clinically worldwide.[1]

Various chemical classes, including nitrogen and sulfur-based cyclic and acyclic ligands (Fig. 1a),[2–10] have been evaluated for the preparation of a unique ^{105}Rh complex with maximum stability *in vivo*. Rh(III) chloride forms octahedral complexes with tetradentate ligands where two of the three chlorides are retained. The two chlorides can be found *trans* or *cis* to each other, and whereas one *trans* isomer can be formed, multiple *cis* isomers are possible (Fig. 1b). The extent to which geometric isomers result from a selected ligand is an important design criterion, because isomeric complexes can exhibit different biological and pharmacological characteristics. Characterization of the Rh(III) octahedral complexes from 1,4,7,10-tetraazadecane (222-tet) showed that the *cis*-dichloro configuration was strongly favored, while the 11-membered 1,4,8,11-tetraazaundecane (232-tet) exclusively favored the *trans*-dichloro- configuration.[2] In a later study, Rh(III) complexes of the 12- to 16-membered cyclic tetraamines, 1,4,7,10-tetraazacyclododecane (cyclen), 1,4,7,10-tetraazacyclotridecane, 1,4,8,11-tetraazacyclotetradecane (cyclam), 1,4,8,12-tetraazacyclopentadecane and 1,5,9,13-tetraazacyclohexadecane, respectively, were synthesized.[3–5] According to this study, the 12- and 13-membered N_4 macrocycles formed only *cis* complexes, the 14-membered structure formed both *cis* and *trans* complexes and the 15- and 16-membered N_4 macrocycles formed only the *trans* complex. Clearly, the macrocyclic ring size affected the isomeric outcome observed for the nitrogen-based ligands.

In 1989, Blake and co-workers investigated Rh(III) complexation reactions with 12-, 14-, and 16-membered macrocycles containing

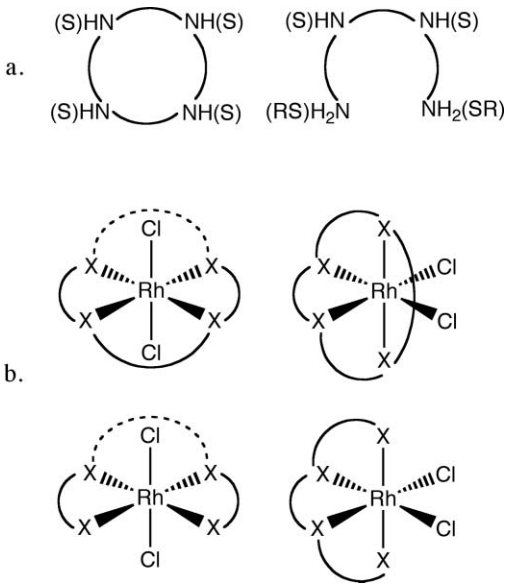

Fig. 1 a. Stylized representation of ligands investigated for complexation with Rh. R = $-CH_2CO_2H$, Benzyl. Actual structures are described within the text. b. Stylized *trans* and one of the possible *cis* Rh(III) complexes of macrocyclic (upper) and acylic (lower) nitrogen and sulfur-based tetradentate ligands. X = S, SR, NH, or NH_2.

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† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra of ligands and complexes as well as LC-MS results for final complexes. CCDC reference numbers 781961 (*trans*-[$RhCl_2$(**L3**)]PF_6) and 781962 (*trans*-[$RhCl_2$(**L6**)]PF_6). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0dt00813c

sulfur as the sole type of coordinating atom.[6,7] As found for the N_4 macrocycles, the 12-membered S_4 macrocycle formed only the *cis* complexes, while the 16-membered S_4 macrocycle formed the *trans* complex. However, in contrast to the 14-membered N_4 macrocycle, the *trans* isomer was not observed in the case of S_4 macrocycle. It was postulated that the larger atomic radius of sulfur *versus* nitrogen was a contributing factor in this result.

Goswami *et al.*[8] studied the Rh(III) complexation behavior of several acyclic tetrathioether ligands. Structural modifications included varying the number of carbons between the sulfur atoms and the moiety on the terminal sulfurs (–CH_2CO_2H "AcOH" and –Bz). The 222-S_4-diAcOH and 222-S_4-diBz ligand systems formed a *cis* Cl–Rh–Cl core. The larger 333-S_4-diAcOH, 323-S_4-diAcOH, and 333-S_4-diBz ligands made *trans* complexes, while 232-S_4-diAcOH and 232-S_4-diBz formed both *cis* and *trans* isomers in high yield. Radiolabeling studies were performed with 222-S_4-diAcOH, 232-S_4-diAcOH and 333-S_4-diAcOH and the radiolabeled complexes showed high *in vitro* stability of 98%, 96% and 89%, respectively, over 5 days. Biodistribution studies were performed with the complexes derived from the 222-S_4-diAcOH (pure *cis* complex) and 333-S_4-diAcOH (pure *trans* complex). Both complexes showed effective clearance from the blood and all tissues, primarily through the kidneys and the urine due to their +1 charge.[9]

Li *et al.*[10] further studied the Rh(III) complexation properties with 14-membered rings where either nitrogen or nitrogen/sulfur combinations were utilized as coordinating atoms ([14]aneNS_3, [14]aneN_2S_2 and [14]aneN_4). The reactions were performed on the radiochemical scale with ^{105}Rh(III)-chloride and analyzed by radio-TLC and paper electrophoresis. Complexation yields decreased with an increasing number of N atoms in the macrocycle (94% NS_3, 81% N_2S_2 and 59% N_4, respectively) and the results were consistent with formation of *cis* octahedral products when compared with non-radioactive standards.

In this study, seven acyclic N_2S_2 chelating ligands (one diaminedithiol "DADT" and six diaminedithioether "DADTE" ligands) (Fig. 2) have been synthesized and evaluated for their ability to incorporate Rh(III).[11,12] Characterization of the non-radioactive ^{103}Rh(III) complexes and the radioactive ^{105}Rh(III) complexes was conducted. The length of carbon chain between the coordinating atoms (222; 232; 323; 333) in the ligand backbone was modified. The presence or absence of *gem*-dimethyl groups as well as the organic moiety on the sulfur atom (*p*-methoxybenzyl or methyl) were investigated. The *p*-methoxy group or *gem*-dimethyl groups can contribute bulk and add to the lipophilicity of the resultant complex. This increase in lipophilicity can affect the extent of non-specific binding in fatty tissue or contribute to greater accumulation in non-target organs, such as the liver. Key outcomes for this study include the number of complexes formed, the radiochemical complexation yield, and *in vitro* stability of the radioactive complexes.

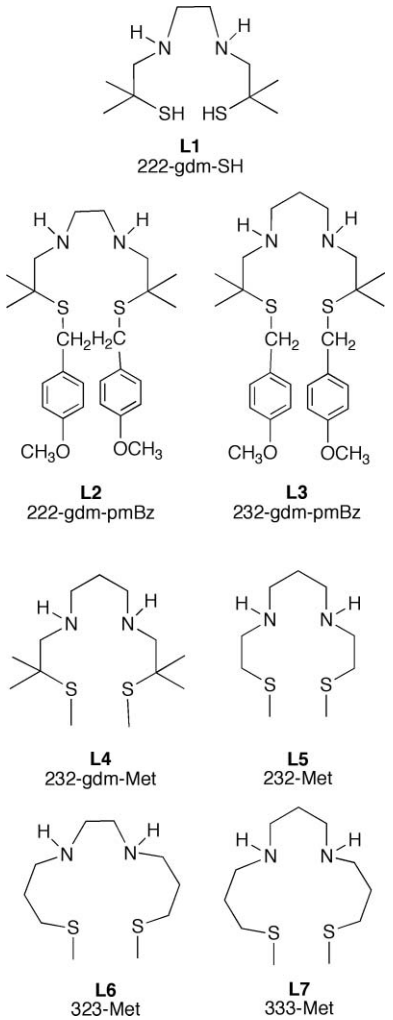


Fig. 2 Ligands synthesized and evaluated in this study.

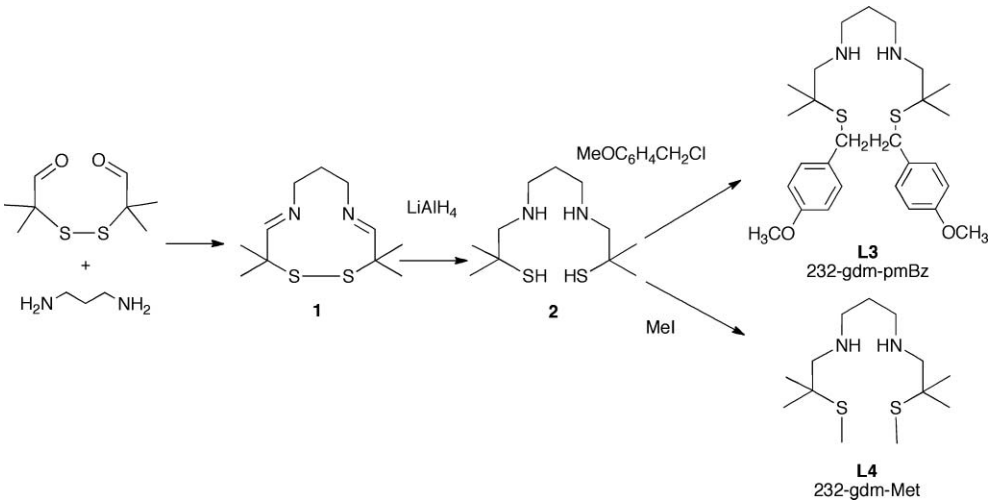
Results and discussion

Synthesis of ^{103}Rh(III) complexes. L1

L1 (222-gdm-SH) was chosen as the initial chelating agent for the Rh(III) complexation reaction. Several attempts to prepare [$RhCl_2$(**L1**)]PF_6, using several experimental variations (ligand concentration, pH, temperature, time of reaction and percent ethanol), were unsuccessful. Instead, compounds with very high molecular weight were observed by LC-MS suggesting the formation of aggregates due to thiol-bridged species[13] (data not shown).

In an iterative fashion, six additional ligands were synthesized, characterized and complexation reactions were performed. Ideally, the ligand identified from these studies would exhibit properties that could be extended to conjugation with peptides for use in radiotherapeutic applications *via* the bifunctional chelate approach. These qualities include, but are not limited to, the formation of one isomer upon complexation with rhodium(III), suitable lipophilicity, and stability of the complex *in vitro* over six days.

L2 (222-gdm-pmBz), where the free thiol groups were transformed into thioethers through reaction with *p*-methoxybenzyl chloride, was synthesized. The Rh(III) complexation reaction with **L2** was performed according to the general procedure and dark orange crystals were obtained. HPLC chromatograms showed two products eluting near to each other, in an approximately 50 : 50 ratio, presumably, the *cis* and *trans* isomers. Analysis by LC-MS supported this assignment, since the same molecular ions were observed for each peak with the expected isotopic distribution. The composite product was obtained in 30% yield.



Scheme 1

The complexity of the ^{1}H-NMR spectrum of [$RhCl_2$(**L2**)]PF_6, due to the presence of isomers, made interpretation difficult. Before the Rh(III) complexation, the symmetrical ligand exhibited first order splitting; one ^{1}H signal was observed for each unique –CH_2– and –CH_3 group on **L2**. After complexation, the peaks occurred downfield relative to the location of signals for each hydrogen in the ligand and additional ^{1}H signals were observed. For example, rather than one singlet corresponding to all four –CH_3 groups in the ligand, four singlets were obtained, reflecting the unique environment. However, the most characteristic peaks of the ^{1}H NMR spectrum for the ligand are the aromatic proton signals around 7 ppm, the methoxy signal at 3.7 ppm and the high-field methyl signal at 1.3 ppm. In addition to these signals, the characteristic singlet –CH_2– signal that was observed for the –S***H***$_2$$C_6H_4OCH_3$ on **L2** became a doublet after complexation because the two H's become non-equivalent. It proved difficult to identify the signals for the protons on the ethylene bridge due to the strong geminal and vicinal couplings of these protons. The trends observed for the ^{1}H NMR analysis were similar to those found in ^{13}C NMR analysis; the ^{13}C signals occurred further downfield than for the ligand, but overall, the ^{13}C NMR spectrum proved to be somewhat less complicated. In the case of the ligand itself, ten ^{13}C signals were observed for the twenty carbons on the symmetrical ligand. A total of thirty ^{13}C signals were observed for the isolated mixture. The ^{13}C NMR spectrum is consistent with the LC-MS results that support formation of two isomers. ^{1}H and ^{13}C spectra of ligands and complexes, as well as representative chromatograms, are found in the supplementary information.

It was hypothesized that increasing the cavity size of the ligand backbone would minimize the formation of geometric isomers formed by Rh(III) complexes with DADTE ligands. Therefore, **L3** (232-gdm-pmBz), which has a larger cavity due to the additional methylene group between the nitrogen atoms in the backbone chain, but retains the *gem*-dimethyl groups and the *p*-methoxybenzyl moieties on the sulfur atoms, was designed. **L3** was synthesized in a similar fashion as **L1** and **L2** *via* the macrocyclic diiminedisulfide, formed in turn through the condensation of 2, 2′-dithiobis(2-methylpropanal) with propylenediamine. Concomitant reductions of the imine functionalities and disulfide bond were achieved with lithium aluminium hydride (Scheme 1). Alkylation of the thiol groups afforded the ligand, **L3**.

Upon Rh(III) complexation with **L3**, dark orange crystals were obtained. LC-MS analysis showed a minor product eluting prior to one predominant product in a 5 : 95 ratio, each with the same positive-ion peaks consistent with [$RhCl_2$(**L3**)]PF_6 formulation. The ^{13}C NMR studies were performed with the pure major product in deuterated acetonitrile. Before the complexation, eleven ^{13}C signals were obtained for **L3**. After complexation with Rh(III), the major isomer was no longer symmetric. Each individual carbon atom, with the exception of the unsubstituted aromatic carbons, was seen in the ^{13}C NMR.

Single crystals of the major [$RhCl_2$(**L3**)]PF_6 isomer were prepared by slow evaporation of an ethanol–acetonitrile solution, and X-ray diffraction analysis confirmed the *trans* geometric assignment. Fig. 3a shows the molecular structure of the cation with the atomic labeling scheme. The ORTEP diagram shows the complex to have slightly distorted octahedral geometry, with the Rh(III) atom positioned in the center of the N_2S_2 cavity and the two chlorine atoms in axial sites. The *p*-methoxybenzyl group on the S(1) atom of the ligand lies above the N_2S_2 coordination plane while the one on the S(2) lies below the N_2S_2 coordination plane. Both N–H atoms are pointing toward Cl(2), and the lone pair of the S(1) donors are pointing toward Cl(1) where the lone pair of the S(2) donors are pointing toward Cl(2). The least-squares plane determination showed that the sulfur donor S(1) lies 0.0617 Å above the plane, while S(2) lies 0.0611 Å below the plane. Likewise, nitrogen donor N(1) lies 0.0689 Å below the plane while N(2) lies 0.0683 Å above the plain. The rhodium ion sits 0.0068 Å above the plane, in the direction of Cl(2). The values of the Rh–S and Rh–N bond lengths in the equatorial ligand plane average 2.3343(7) and 2.100(2) Å, respectively (Table 1). The distances between the rhodium atom and the two axial chlorine atoms differ only slightly, (2.3358(7) and 2.3461(6) Å), and compare well with the Rh–Cl distances found in other rhodium-chloride complexes (*e.g.*, [$RhCl_2$(232-S4-dibz)]PF_6); 2.349(2) Å).[9] In this complex, there is a central six-membered chelating ring with two five-membered chelating rings oriented on opposite sides. All angles around the rhodium center deviate from 90°. The S–Rh–N angles in the

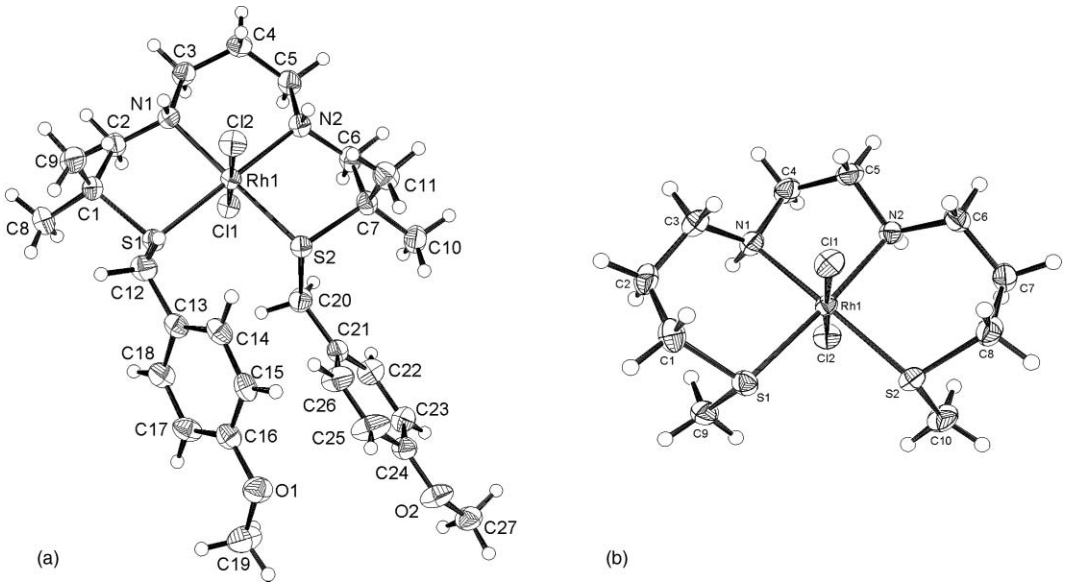


Fig. 3 a. *ORTEP* drawing of *trans*-[$RhCl_2$(232-gdm-pmBz)]$^+$ and b. *trans*-[$RhCl_2$(323-Met)]$^+$. Probability ellipsoids are shown at the 50% level.

Table 1 Selected bond lengths (Å) and angles (°) for *trans*-[$RhCl_2$(232-gdm-pmBz)]PF_6 (*trans*-[$RhCl_2$(**L3**)]PF_6) and *trans*-[$RhCl_2$(323-Met)]PF_6 (*trans*-[$RhCl_2$(**L6**)]PF_6)

	trans-[$RhCl_2$(232-gdm-pmBz)]PF_6 *trans*-[$RhCl_2$(**L3**)]PF_6	*trans*-[$RhCl_2$(323-Met)]-PF_6 *trans*-[$RhCl_2$(**L6**)]PF_6
Rh–N1	2.101(2)	2.1071(15)
Rh–N2	2.099(2)	2.0955(15)
Rh–S1	2.3259(7)	2.3339(5)
Rh–S2	2.3427(7)	2.3329(5)
Rh–Cl1	2.3461(6)	2.3489(5)
Rh–Cl2	2.3358(7)	2.3353(5)
N2–Rh–N1	94.83(9)	84.44(6)
N2–Rh–S1	176.93(7)	174.86(4)
N1–Rh–S1	84.75(6)	92.17(4)
N2–Rh–Cl2	88.24(7)	87.88(4)
N1–Rh–Cl2	84.72(7)	84.12(4)
S1–Rh–Cl2	94.78(2)	95.633(17)
N2–Rh–S2	85.35(6)	96.48(4)
N1–Rh–S2	176.27(7)	179.07(4)
S1–Rh–S2	95.27(2)	86.918(17)
Cl2–Rh–S2	88.56(2)	95.815(19)
N2–Rh–Cl1	89.81(7)	89.19(4)
N1–Rh–Cl1	89.24(7)	95.43(5)
S1–Rh–Cl1	87.14(2)	87.275(17)
Cl2–Rh–Cl1	176.24(2)	177.069(17)
S2–Rh–Cl1	94.48(2)	84.689(18)

five-membered rings are close to the expected value of about 82° (range 84.75° and 85.35°), whereas the N–Rh–N angle formed by the six-membered ring is 94.83°. These large values allow the metal atom to lie in the plane of the N_2S_2 ligand.

The two nitrogen and two sulfur atoms on the N_2S_2 ligand system become chiral stereocenters *S(1)*, *S(2)*, *N(1)*, and *N(2)* upon coordination with Rh(III). Each stereogenic center has two possible stereoisomers, *R* or *S*. Thus, all possible combinations of the four centers should have produced eight pairs of enantiomers; however, due to the plane of symmetry in the N_2S_2 Rh(III) complex, 6 pairs of enantiomers (*RRRR/SSSS*, *RRRS/RSSS*, *SSSR/SRRR*, *SRSS/RRSR*, *RSRR/SSRS* and *SRRS/RSSR*) and two pairs of *meso* complexes (*SSRR/RRSS* and *SRSR/RSRS*) were formed for each *cis* and *trans* isomer. Based on crystal structure analysis, it is found that *trans*-[$RhCl_2$(**L3**)]PF_6 represents an *SRSS* stereoisomer. Since the structure is centrosymmetric, the mirror image is also present but not shown. Complete data and structure refinement for *trans*-[$RhCl_2$(**L3**)]PF_6 are found in supplementary information.

Shifting the ratio of *cis*:*trans* isomers found in the product distribution from 50 : 50 to 5 : 95 in [$RhCl_2$(**L2**)]PF_6 and [$RhCl_2$(**L3**)]PF_6, respectively, supported the hypothesis that a larger cavity was required for formation of a sole product; however, **L3** was not deemed the optimum ligand. **L4** (232-gdm-Met) was synthesized as an analog of **L3** where compound **4** was alkylated with methyl iodide rather than *p*-methoxybenzyl chloride to afford a less bulky Rh(III) complex (Scheme 1). It was anticipated that this structural modification would further improve the possibility of obtaining only the *trans* isomer. The complexation reaction with **L4** was performed according to the general procedure, and dark orange crystals were obtained in 42% yield. The product was analyzed by HPLC and LC-MS. The LC-MS spectrum displayed only one peak with the expected isotopic distribution, supporting that a single isomer of [RhCl2(**L4**)PF_6] is formed. For the solid product obtained from the complexation reaction with **L4**, the ^{13}C NMR lent support to the isolation of a single isomer.

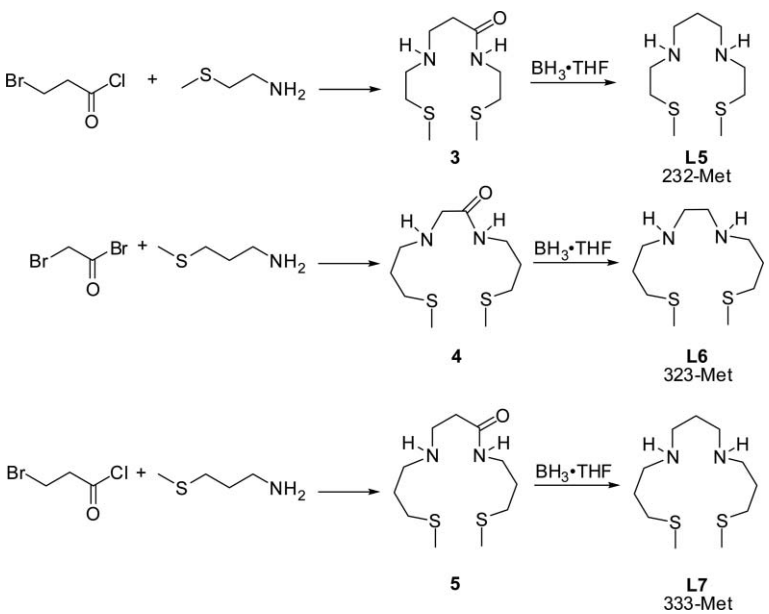
Based upon these results, the preferred backbone appeared to be the 232 ligand system. Replacement of the bulky *p*-methoxybenzyl groups with methyl groups also achieved one of the major objectives of the study; *i.e.*, synthesis of a ligand that would form only one Rh(III) complex. However, the presence of the *gem*-dimethyl groups on the backbone contributes to the lipophilicity of the ligand. We were interested in reducing the lipophilicity of the final complex without jeopardizing formation of a sole *trans* product; thus, **L5** (232-Met), a direct analog of **L4** without the *gem*-dimethyl groups, was synthesized. The route of synthesis (Scheme 2) required the step-wise construction of the N_2S_2 core. 3-bromopropionyl chloride was used as the bifunctional

ligand to attach two molecules of 2-(methylthio)ethylamine to form intermediate **3**, followed by reduction of the adduct by the borane tetrahydrofuran complex to yield **L5**. The non-radioactive rhodium complex was prepared and the reaction mixture was analyzed by HPLC and LC-MS, ^{1}H NMR and ^{13}C NMR spectroscopy. A single or at least one predominant, geometric isomer was anticipated upon complexation, since **L5** has the same backbone chain as **L3** and **L4**. However, according to the LC-MS results, the formation of at least two isomers was observed, each consistent with the molecular formulation of [$RhCl_2$(**L5**)]PF_6. This complex was not pursued further.

To understand if the unexpected multiple isomer formation with **L5** occurred due to the absence of the *gem*-dimethyl groups on the backbone, and if it can be overcome by increasing the length of the backbone chain even further, ligands **L6** (323-Met) and **L7** (333-Met) were synthesized. In the case of **L6**, the number of carbons between nitrogen and sulfur was increased to three, with two carbons between the nitrogens; in the case of **L7** (333-Met), three carbons were placed between all coordinating atoms. These two ligands were synthesized by analogy to the synthesis of **L5**; *viz.*, reaction of the appropriate haloalkylated acyl halide with 3-(methylthio)propylamine (Scheme 2). Rh(III) complexation reactions with **L6** and **L7** were performed according to the general procedure and the dark orange [$RhCl_2$(**L6**)]PF_6 and [$RhCl_2$(**L7**)]PF_6 crystals were obtained with 44% and 40% yield, respectively. In each case, LC-MS spectroscopy indicated the presence of at least five isomers with two major and three minor positive-ion peaks with the correct isotopic distribution, approximately in a 47 : 47 : 2 : 2 : 2 ratio for both [$RhCl_2$(**L6**)]PF_6 and [$RhCl_2$(**L7**)]PF_6.

^{1}H and ^{13}C NMR studies were performed with the isolated products [$RhCl_2$(**L6**)]PF_6 and [$RhCl_2$(**L7**)]PF_6 in deuterated acetonitrile. Other than the central carbon signal contained in the propylene bridge, all ^{13}C signals for [$RhCl_2$(**L6**)]PF_6 occur further downfield than the ligand. The ^{1}H spectrum was very difficult to interpret, since the hydrogens on each methylene become nonequivalent and adopt an individual chemical shift. However, six signals were observed corresponding to the methyl groups on the complex (two signals for the *trans*-isomer and one signal for each *cis*-pair). This result was consistent with the ^{13}C findings confirming the observation of five isomers. For example, six unique signals were observed for the S-methyl group.

Based on the LC-MS and ^{13}C NMR results it was found that Rh(III) forms at least 5 isomers with **L6**; however, only the *trans* isomer of [$RhCl_2$(**L6**)]PF_6 was crystallized. Crystals of complex **L6** suitable for an X-ray diffraction study were grown by diffusion of methanol into an acetonitrile solution of the complex. The molecular structure of *trans*-[$RhCl_2$(**L6**)]PF_6, showed a slightly distorted octahedral geometry (Fig. 3b). Crystal structure analysis confirmed the expected octahedral coordination geometry of the complex cation, *trans*-[$RhCl_2$(**L6**)]$^{+}$. The tetradentate ligand coordinates the rhodium atom in a planar fashion, with two chloride atoms occupying the axial positions of the complex (Table 1). The octahedral coordination polyhedron is slightly distorted. The least-squares plane determination showed that the sulfur donor S(1) lies 0.0352 Å above the plane, while S(2) lies 0.0352 Å below the plane. Likewise, nitrogen donor N(1) lies 0.0413 Å below the plane, while N(2) lies 0.0401 Å above the plane. The rhodium ion sits 0.0359 Å above the plane, in the direction of Cl(2). Both methyl groups of the ligand lie on the same side of the N_2S_2 coordination plane, with the N–H atoms pointing toward Cl(2) and the lone pair on each of the S donors pointing toward Cl(1). Thus, the donor stereocenters S(l), S(2), N(l), and N(2) in the complex have an *RSRS* absolute configuration, respectively. The values of the Rh–S and Rh–N bond lengths in the equatorial ligand plane are 2.3339(5) and 2.1071(15) Å, respectively. The distances between the rhodium atom and the two axial chlorides differ only slightly (2.3353(5) and 2.3489(5) Å) and compare well with the Rh–Cl distances found in other rhodium(III)-chloride complexes (*e.g.*, [$RhCl_2$(232-S4-dibz)]PF_6) (Rh–Cl) 2.349(2) Å).[5] All angles around the rhodium center deviate from 90°. The S–Rh–N angles in the equatorial plane are larger than 90° (96.48 and 92.17°), and the S–Rh–S and N–Rh–N angles are smaller than 90°



Scheme 2

Table 2 Selected results of coordination reactions with $^{103/105}$Rh(III)

	Chemistry with ^{103}Rh(III)Cl$_3$		Radiochemistry with ^{105}Rh(III)Cl$_3$	
Compound [$^{103/105}$RhCl$_2$(L)]Cl	Number of isomers	*cis*:*trans* HPLC peak ratios	% Overall Labeling Efficiency	Log D$_{7.4}$
L2	2	50 : 50	46	ND
L3	2	5 : 95	46	1.90 ± 0.07
L4	1	0 : 100	61	0.46 ± 0.48
L5	5	~ 47 : 47:[*cis* –2:2 : 2]	53	–0.26 ± 0.50
L6	5	~ 47 : 47:[*cis* –2:2 : 2]	56	ND
L7	5	~ 47 : 47:[*cis* –2:2 : 2]	48	ND

(86.92 and 84.44°, respectively). In contrast to **L2**, this complex has a central five-membered chelating ring with two six-membered chelating rings oriented on the opposite sides. We note that smaller central bite angles are observed only when the complex has a five-membered chelate ring. The bite angle for the five-member chelating ring is 84.44° and for six-member chelating rings are 92.17° and 96.48°.

Before Rh(III) complexation with **L7** six ^{13}C signals were observed, but after coordination to the Rh(III), the ^{13}C NMR spectrum indicated the formation of five isomers. A similar trend, as seen with the complex derived from **L6**, was observed from the ^{13}C NMR spectrum, where six peaks were observed for each of the symmetric carbon groups and five peaks were observed for the central methylene group.

These results were surprising, because increasing the distance between coordinating atoms was expected to decrease the formation of multiple isomers. However, a possible rationale for the number of isomers could be due to increased structural fluidity from the absence of the *gem*-dimethyl groups on the backbone chain.

Radiolabeling reactions with ^{105}Rh

To investigate the similarities and the differences between the macroscopic and the radiotracer level in Rh(III) complexation reactions, the radiolabeling reactions with ^{105}Rh and **L1–L7** were performed parallel to the Rh(III) complexation reactions. The identity of [^{105}RhCl$_2$(**L**)]Cl was verified by co-injecting radiolabeled with the non-radioactive complexes onto the radio-HPLC system. Determination of the yields were based on the area of the intact [^{105}RhCl$_2$(**L**)]Cl complexes detected on the radio-HPLC chromatogram. Similar to the results obtained in the Rh(III) complexation reaction at the macroscopic level with **L1**, no ^{105}Rh radiolabeling was observed at the tracer level with **L1**. However, in the case of ligands **L2–L7** the ^{105}Rh labeling was successfully accomplished. The labeling efficiencies for the DADTE ligands ranged from 46–61% (Table 2).

Stability studies of [^{105}RhCl$_2$(L3)]Cl, [^{105}RhCl$_2$(L4)]Cl [^{105}RhCl$_2$(L5)]Cl

The stability of HPLC-purified [^{105}RhCl$_2$(**L3**)]Cl (peak 1 (*cis*) and peak 2 (*trans*)) [^{105}RhCl$_2$(**L4**)]Cl and [^{105}RhCl$_2$(**L5**)]Cl (peak 1 (*cis*) and peak 2 (*trans*)) were performed in phosphate-buffered saline (PBS) buffer (pH = 7.5) over 3 to 7 days at 37° C in the presence of gentisic acid (GA). In the case of [^{105}RhCl$_2$(**L3**)]Cl, the percent intact complex in peak 1, decreased from 97% to 76%, and the percent intact complex in peak 2 decreased from 99% to 76%, in 168 h. This result suggests that both isomers, were ~76% stable over 7 days. In the case of [^{105}RhCl$_2$(**L4**)]Cl, the percent intact complex decreased from 98% to 88% in 144 h, indicating that the radiolabeled complex was 90% stable over 6 days.

In the case of [^{105}RhCl$_2$(**L5**)]Cl, the percent intact complex in peak 1, decreased from 84% to 77%, and the percent intact complex in peak 2 decreased from 82% to 75%, in 72 h. This result suggests that both peaks, *cis*/*trans* isomers, were 91% stable over 3 days.

Overall, greater than 90% stability was observed for [^{105}RhCl$_2$(**L4**)]Cl and the isomeric formulations of [^{105}RhCl$_2$(**L5**)]Cl, while greater than 76% stability was observed for each isomer of [^{105}RhCl$_2$(**L3**)]Cl. From the previously published literature, it has been shown that *p*-methoxybenzyl groups on the ligand are not as stable as alkyl groups. In addition, they are bulkier, which may increase the chances of exposure to self-radiolysis. Therefore, these structural features may result in a less stable radiolabeled complex.

The distribution coefficients (log D)

The distribution coefficients between octanol and water at pH 7.4 were determined with the shake-flask method for [^{105}RhCl$_2$(**L3**)]Cl, [^{105}RhCl$_2$(**L4**)]Cl and [^{105}RhCl$_2$(**L5**)]Cl. These ^{105}Rh complexes were found to be very hydrophilic due to the overall charge on the complex (Table 2). As expected, for complexes [^{105}RhCl$_2$(**L4**)]Cl and [^{105}RhCl$_2$(**L5**)]Cl, a decrease in log D values was found relative to [^{105}RhCl$_2$(**L3**)]Cl. Substituting the *p*-methoxy group with a methyl group decreased the log D value by 1.44 units, and in the case of [^{105}RhCl$_2$(**L5**)]Cl, removal of the *gem*-dimethyl groups on the backbone chain decreased the log D value by an additional 0.72 units. Therefore, an effect on the resulting log D values was observed as moieties were removed and replaced with smaller groups or hydrogen atoms. However, these complexes all proved to be quite hydrophilic; thus, the presumption that the *gem*-dimethyl groups on the ligand would adversely contribute to the overall lipophilicity of the complexes proved less important.

Experimental

Materials and methods

All chemicals were obtained from commercial sources (Aldrich Chemical Co.) and used as received unless otherwise stated. Trifluoroacetic acid (TFA) was obtained from VWR Scientific Products (St. Louis, MO). Tetrahydrofuran was freshly distilled from sodium benzophenone ketyl.

HPLC analysis of the non-radioactive complexes was performed on a Beckmann Coulter System Gold HPLC equipped with a 168 diode array detector, a 507e autoinjector and the 32 KARAT software package (Beckmann Coulter, Fullerton, CA). A C-18 Kromosil column (150 × 4.6 mm, 5 μm, 100 Å, Keystone Scientific Inc, San Jose, CA) was used for the analytical HPLC.

ESI-MS analyses were performed on a Finnigan TSQ7000 mass spectrometer (Thermo Finnigan, San Jose, CA). A NovaPak column (3.9 × 300 mm, Waters Corp, Milford, MA) was used for the LC-MS analysis. In all HPLC experiments, eluents used in all runs were water (A), and acetonitrile (B), each containing 0.1% TFA at 1 cm^3 min^{-1} while being monitored at 214/280 nm.

Elemental analyses were determined by Atlantic Microlab (Norcross, GA). ^1H NMR (250, 300 and/or 500 MHz) and ^{13}C NMR spectra were obtained with Bruker instruments (Bruker BioSpin, Westmont, IL). *J* values are given in Hz. Radiolabeling analyses were performed on a Varian Pro Star HPLC (Model 240 pump) equipped with a Phenomenex Jupiter C-18 (4.1 × 250 mm, 5 mm) column with water (A), and acetonitrile (B) eluents, each containing 0.1% TFA at 1 cm^3 min^{-1} while being monitored at 280 nm.

Sep-Pak Vac (0.1 g) cartridges were obtained from Waters Inc, Milford, MA. Thin layer chromatography plates (MKC-18F Silica gel 60 Å 2.5 × 7.5 cm, 200 μm thickness) were obtained from Whatman International Ltd, Madison, England. Quantitation of the radioactivity on the TLC strips was performed using a radiochromatographic strip scanner (BioScan System 200); the radioactivity in the extracts was quantitated by counting aliquots of the solutions in a Na(Tl)I well counter. Paper chromatographic strips were developed with acetonitrile containing NH_4PF_6.

Chemistry

Ligand synthesis. 2,2,9,9-tetramethyl-4,7-diaza-1,10-decanedithiol, (**L1**, 222-gdm-SH) and *N*,*N*′-bis-(2-(4-methoxybenzylthio)-2-methylpropyl)ethane-1,2-diamine (**L2**, 222-gdm-pmBz) were synthesized as previously described.[14]

1,2-Dithia-5,9-diaza-3,3,11,11-tetramethyl-cycloundeca-4,9-diene (1). 2,2′-dithiobis(2-methylpropanal) **11**[14] (10.0 g, 0.0485 mol) and 1,3-diaminopropane (4.05 cm^3, 0.0485 mol) were refluxed in benzene (100 mL) in a reflux apparatus equipped with a Dean Stark trap. After ~ 0.57 cm^3 (0.097 mol) of H_2O was collected (8 h), the reaction was cooled and concentrated to afford a yellow oil. The oil was dissolved in ethyl acetate and passed through silica. Removal of the solvent gave the crude product, which was recrystallized to yield **1** (4.0 g, 34%) as white crystals: mp 103 °C (from EtOAc). Found: C, 54.1; H, 8.2; N 11.6. Calc. for $C_{11}H_{20}N_2S_2$: C, 54.05; H, 8.25; N, 11.5%. δ_H(250 MHz; $CDCl_3$) 7.54 (2 H, s, 2 X N=C*H*), 3.80–3.73 (2 H, m, NCH_2–CH_2–CH_2N), 3.60–3.52 (2 H, m, NCH_2–CH_2–CH_2N), 2.07 (2 H, apparent p, NCH_2–CH_2–CH_2N), 1.39 (6 H, s, 2 X CH_3); 1.30 (6 H, s, 2 X CH_3); δ_C(62.5 MHz; $CDCl_3$) 168.41, 60.81, 50.71, 24.75, 24.65 and 24.16.

2,2,10,10-Tetramethyl-4,8-diaza-1,11-undecanedithiol (2). The reduction was modeled after a literature procedure[15] for a similar compound. Excess $LiAlH_4$ (51.2 mmol) was added carefully to a solution of **1** (3.2 g, 12.8 mmol) in dry THF (100 cm^3), and the mixture was heated at reflux for 12 h. The resulting reaction mixture was cooled to −78 °C, quenched with 20 mL of saturated aqueous Na_2SO_4, diluted with 50 cm^3 of diethyl ether, and then allowed to warm to room temperature with vigorous stirring. The pH of the resulting paste was lowered to ~9 by the addition of 1 M HCl. The remaining white precipitate was removed by filtration, and the filtrate was washed with water (2 × 100 cm^3), dried over anhydrous Na_2SO_4, and then concentrated to afford **2** as a pale yellow oil (2.12 g, 60%). δ_H(300 MHz; $CDCl_3$) 2.75 (4 H, t, *J* 7, NCH_2–CH_2–CH_2N), 2.60 (4 H, s, 2 X C$(CH_3)_2$CH_2), 1.69 – 1.75 (8 H, m, *J* 7, 2 X NCH_2–CH_2–CH_2N, S*H*, N*H*), 1.38 (12 H, s, 2 X C(CH_3)$_2$; δ_C(75 MHz; $CDCl_3$) 63.34, 48.60, 44.97, 30.41 and 28.33. This material was immediately used in the next reaction.

***N*,*N*′-bis(2-(4-Methoxybenzylthio)-2-methylpropyl)propane-1,3-diamine, L3 (232-gdm-pmBz).** To a stirred solution of 2 (2.0 g, 8.0 mmol) in ethanol (50 cm^3), aqueous NaOH (50 cm^3, 2.5 M) was added followed by neat *p*-methoxybenzyl chloride (4.3 cm^3, 32 mmol). Stirring was continued for 1 h and most of the ethanol was distilled under reduced pressure. The mixture was then extracted (3 × 20 cm^3) with diethyl ether. The combined ethereal solutions were dried with anhydrous Na_2SO_4. After filtration, the diethyl ether was evaporated and ethanol saturated in HCl(g) was added until the pH was 2–3. The warm mixture was cooled to room temperature and the product was precipitated with ether. The precipitate was filtered and washed with ether to yield **L3** as a white di-HCl salt (6.99 g, 76%), mp 285 °C. Found: C, 57.2; H, 7.9; N, 4.9. Calc. for $C_{27}H_{42}N_2O_2S_2$·2HCl: C, 57.5; H, 7.9; N, 5.0%. δ_H(300 MHz; D_2O) 7.28 (4 H, d, *J* 8.7, aromatic H), 6.88 (4 H, d, *J* 8.7, aromatic H), 3.76 (4 H, s, 2 X SCH_2), 3.70 (6 H, s, 2 X OCH_3), 3.26 (4 H, s, 2 X C$(CH_3)_2$CH_2), 3.01 (4 H, m), 2.62 (2 H, m), 1.31 (12 H, s, 2 X C(CH_3)$_2$); δ_C(75 MHz; D_2O) 156.0, 130.6, 130.1, 114.4, 55.8, 55.3, 45.1, 44.0, 31.5, 25.7 and 21.6. ESI-MS: *m*/*z* 490.27 (M^+, 100%), 491.27 (31.6); found: $(M+H)^+$ 491.2, 492.3.

***N*,*N*′-bis(2-Methyl-2-(methylthio)propyl)propane-1,3-diamine, L4 (232-gdm-Met).** **L4** was prepared from 2 (1 g, 4 mmol) and methyl iodide (0.75 cm^3, 12 mmol) using the procedure described for the preparation of **L3**. After purification, **L4** was obtained as the white di-HCl salt (0.6 g, 44%). Found: C, 44.4; H, 9.2; N, 8.0. Calc. for $C_{13}H_{30}N_2S_2$·2HCl: C, 44.1; H, 9.15; N, 7.9%. δ_H(500 MHz; D_2O) 3.18–3.14 (4 H, m, NCH_2–CH_2–CH_2N), 3.11 (4 H, s, 2 X C$(CH_3)_2$CH_2), 2.18–2.14 (2 H, m, NCH_2–CH_2–CH_2N), 1.98 (6 H, s, 2 X SCH_3), 1.30 (12 H, s, 2 X C(CH_3)$_2$); δ_C(125 MHz; D_2O) 54.74, 45.30, 41.70, 25.19, 21.62 and 9.86. ESI-MS: *m*/*z* 278.19 (M^+, 100%); found: $(M+H)^+$ 279.10.

***N*-(2-(methylthio)ethyl)-3-(2-(methylthio)ethylamino)propanamide (3).** To a stirred solution of 3-bromopropionyl chloride (4.6 g, 27 mmol) in CH_2Cl_2 (35 cm^3) at −78 °C, 2-(methylthio)ethylamine (4.9 g, 54 mmol) and triethylamine (19 mL, 136 mmol) in 35 cm^3 of CH_2Cl_2 were added dropwise over 15 min. After the addition, the reaction mixture was warmed to room temperature and stirring was continued for 24 h. Water was added (20 cm^3) and the layers were separated. The organic portion was washed with 1M HCl, saturated $NaHCO_3$ and brine (20 cm^3 each). The organic layer was dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. For purification, the crude product was loaded onto a short-path silica column and washed with $CHCl_3$. The pure product was eluted with MeOH to afford a white solid (1.27 g, 23%). δ_H(250 MHz;

MeOD) 3.35 (2 H, t, *J* 6.9, NC(O)CH_2CH$_2$N), 2.97 (2 H, t, *J* 6.6, C(O)NCH_2CH$_2$S), 2.91 (2 H, t, *J* 6.7, NCH_2CH$_2$S), 2.66 (2 H, t, *J* 6.7, NCH$_2$CH_2S), 2.57 (2 H, t, *J* 6.9, NC(O)CH$_2$CH_2N), 2.46 (2 H, t, *J* 6.6, C(O)NCH$_2$CH_2S), 2.07 (3 H, s, SCH_3), 2.06 (3 H, s, SCH_3); δ_C(62.5 MHz; MeOD) 172.5, 47.1, 45.0, 37.4, 35.6, 34.2, 33.7, 15.1, 14.8.

***N*,*N*-bis(2-(Methylthio)ethyl)propane-1,3-diamine, L5 (232-Met).** Borane-THF complex (1M, 20 mL) was added to a solution of 3 (0.67 g, 2.86 mmol) in anhydrous THF (20 cm^3). The reaction was refluxed overnight under N_2. The reaction was cooled to 0 °C and 10 cm^3 of concentrated HCl were added dropwise. The THF was removed by distillation under atmospheric pressure, and 20 cm^3 of water were added to increase the solvent volume. NaOH pellets were added to adjust the pH to >12, and the resulting mixture was extracted with diethyl ether (3 × 5 cm^3). The combined ethereal extracts were dried with anhydrous Na_2SO_4 and filtered. The ether was evaporated under reduced pressure to afford the product as a white solid (0.38 g, 60%). δ_H(300 MHz; D_2O) 3.23 (4 H, t, *J* 6.8, 2 X NCH_2CH$_2$S), 3.10 (4 H, m, NCH_2CH$_2$CH_2N), 2.76 (4 H, t, *J* 6.8, 2 X NCH$_2$CH_2S), 2.06 (2 H, m, NCH$_2$CH_2CH$_2$N), 2.04 (6 H, s, 2 X SCH_3); δ_C(75 MHz; D_2O) 45.83, 44.27, 28.88, 22.45, 13.85. ESI-MS: *m*/*z* 222.96 (M$^+$, 100%); found: (M$^+$) 222.12.

***N*-(3-(Methylthio)propyl)-2-(3-(methylthio)propylamino)acetamide, (4).** In a procedure identical with that used in the preparation of **3**, **4** was prepared from 3-(methylthio)propylamine (3 g, 28.5 mmol) and 2.9 g (14.3 mmol) of bromoacetyl bromide. After purification, the desired product was obtained as a colorless oil (3.2 g, 89%). δ_H(250 MHz; $CDCl_3$) 7.81 (1 H, br s, C(O)N*H*), 3.39 (1 H, t, *J* 6.6, C(O)NCH_2CH$_2$CH$_2$S), 3.36 (1 H, t, *J* 6.6, C(O)NCH_2CH$_2$CH$_2$S), 3.31 (2 H, s, NCH_2C(O)N), 3.17 (< 1 H, s, N*H*), 2.75 (2 H, t, *J* 7, NCH_2CH$_2$CH$_2$S), 2.58 (2 H, t, *J* 7, NCH$_2$CH$_2$CH_2S), 2.54 (2 H, t, *J* 7, NCH$_2$CH$_2$CH_2S), 2.10 (6 H, s, 2 X SCH_3), 1.83 (4 H, m, NCH$_2$CH_2CH$_2$S); δ_C(62.5 MHz; $CDCl_3$) 172.93, 52.05, 48.50, 37.79, 31.63, 31.43, 28.67, 28.49, 15.32, 15.30.

***N*,*N*′-bis(3-(Methylthio)propyl)ethane-1,2-diamine, L6 (323-Met).** The same procedure as described for **L5** was applied to starting material 4 (3.2 g, 12.8 mmol). The product was obtained as a colorless oil (2.3 g, 75%). Found: C, 38.95; H, 8.25; N, 9.0. Calc. for $C_{10}H_{24}N_2S_2$·2HCl: C, 38.8; H, 8.5; N, 9.1%. δ_H(300 MHz; $CDCl_3$) 2.72 (4 H, s rising out of m, NCH_2CH_2N), 2.71 (4 H, t, *J* 7, 2 X NCH_2CH$_2$CH$_2$S), 2.55 (4 H, t, *J* 7, 2 X NCH$_2$CH$_2$CH_2S), 2.10 (6 H, s, 2 X SCH_3), 1.78 (4 H, apparent p, *J* 7, 2 X NCH$_2$CH_2CH$_2$S), 1.56 (2H, s, 2 X N*H*); δ_C(75 MHz; $CDCl_3$) 48.74, 48.34, 31.59, 28.88, 15.05. ESI-MS: *m*/*z* 236.14, 237.14; found: (M+H)$^+$ 236.9, 238.9.

***N*-(3-(Methylthio)propyl)-3-(3-(methylthio)propylamino)propanamide, 5.** Compound **5** was prepared from 3-(methylthio)-propylamine (4.69 g, 44.5 mmol) and 3-bromopropionyl chloride (4.50 g, 22.3 mmol) in a procedure identical with that used in the preparation of **4**. The product was obtained as a clear oil (1.20 g, 23%). δ_H(250 MHz; $CDCl_3$) 7.81 (1 H, br s, N(CO)*H*), 3.34 (1 H, t, *J* 6, SCH$_2$CH$_2$CH_2C(O)N), 3.32 (1 H, t, *J* 6, SCH$_2$CH$_2$CH_2C(O)N), 3.19 (1 H, br s, N*H*), 2.92 (2 H, t, *J* 5, N(CO)CH$_2$CH_2N), 2.77 (2 H, t, *J* 6, NCH_2CH$_2$CH$_2$S), 2.57 (2 H, t, *J* 6, NCH$_2$CH$_2$CH_2S), 2.54 (2 H, t, *J* 6, NCH$_2$CH$_2$CH_2S), 2.42 (2 H, t, *J* 5, N(CO)CH_2CH$_2$N), 2.11 (3 H, s, SCH_3), 2.10 (3 H, s, SCH_3), 1.82 (4 H, m, NCH$_2$CH_2CH$_2$S); δ_C(62.5 MHz; $CDCl_3$) 172.26, 49.95, 49.65, 47.87, 45.27, 37.92, 34.80, 31.65, 31.36, 31.23, 28.52, 28.30, 15.27, 15.23.

***N*,*N*′-bis(2-(Methylthio)propyl)propane-1,3-diamine, L7 (333-Met).** The same procedure as described for the preparation of **L5** was applied to **5** (1.2 g, 5.16 mmol). The product was obtained as a colorless oil (0.75 g, 58%). Found: C, 39.8; H, 8.4; N, 8.4. Calc. for $C_{11}H_{26}N_2S_2$·2HCl: C, 40.85; H, 8.7; N, 8.7%. δ_H(300 MHz; $CDCl_3$) 2.85 (2 H, br s, NH), 2.64 (8 H, t, *J* 6, 2 X NCH_2CH$_2$CH$_2$S, NCH_2CH$_2$CH_2N), 2.47 (4 H, t, *J* 6, 2 X NCH$_2$CH$_2$CH_2S), 2.03 (6 H, s, 2 X SCH_3), 1.73 – 1.64 (6 H, m, NCH$_2$CH_2CH$_2$S, NCH$_2$CH_2CH$_2$N); δ_C(75 MHz; $CDCl_3$) 48.47, 48.25, 31.78, 29.58, 28.93, 15.23. ESI-MS: *m*/*z* 250.15 (100.0%): found: (M+H)$^+$ 250.91.

General procedure for the preparation of ^{103}Rh(III) complexes [^{103}RhCl$_2$(L)]PF$_6$. A solution of 0.15 mmol of ^{103}Rh(III)Cl$_3$·xH$_2$O (Rh(III) 40% by weight) in 10 cm^3 of 20% aqueous EtOH was added to a stirring solution of 0.15 mmol of ligand in 40 cm^3 of distilled water. The pH of the reaction mixture was adjusted to ~5 by dropwise addition of 2.5 M NaOH. The resulting deep red solution was stirred and heated at 80 °C for 1 h. During this period of time, the color of the reaction mixture turned to bright yellow. Any insoluble material was removed by centrifugation, and the volume of the solution was reduced by distillation under reduced pressure. A solid product with a light yellow color was precipitated from the solution with the addition of saturated aqueous NH_4PF_6. The precipitate was isolated by centrifugation and washed several times with water, EtOH and diethyl ether. In each case, the color of the isolated product turned dark orange after drying under high vacuum.

***cis*/*trans*-[Dichloro (*N*,*N*-bis-(2-(4-methoxybenzylthio)-2-methylpropyl)ethane-1,2-diamine) rhodium(III)] hexafluorophosphate. *cis*/*trans*-[RhCl$_2$(222-gdm-pmBz)]PF$_6$, [RhCl$_2$(L2)]PF$_6$.** Yield: 30% (36 mg). A gradient mobile phase protocol (98% to 80% in 54 min, and from 80% to 20% in the last 6 min) was utilized for analysis by HPLC and LC-MS. ESI-MS: *m*/*z* for $C_{26}H_{40}Cl_2N_2O_2RhS_2$: 649.1 (100%), 650.1 (30) and 651 (70); found: (M)$^+$ 649 (100%), 650 (30) and 651 (70).

***cis*/*trans*-[Dichloro (*N*,*N*-bis-(2-(4-methoxybenzylthio)-2-methylpropyl)propane-1,3-diamine) rhodium(III)] hexafluorophosphate. *cis*/*trans*-[RhCl$_2$(232-gdm-pmBz)]PF$_6$, [RhCl$_2$(L3)]PF$_6$.** Yield: 35% (43 mg, 0.052 mmol). Found: C, 40.4; H, 5.3; N, 3.5. Calc. for $C_{27}H_{42}Cl_2F_6N_2O_2PRhS_2$: C, 40.1; H, 5.2; N, 3.5%. A gradient mobile phase protocol (98% to 80% in 5 min, 80% for 5 min then from 80% to 60% in the next 20 min and from 60% to 20% in the last 5 min) was utilized for analysis by HPLC and LC-MS. ESI-MS: *m*/*z* 663.1 (100%), 664.1 (30) and 665 (70); found: (M)$^+$ 663.1 (100%), 664 (30) and 665 (70). Single crystals were prepared by slow evaporation of an ethanol–acetonitrile solution, and analyzed by X-ray diffraction methods (*vide infra*).

***cis*/*trans*-[Dichloro (*N*,*N*-bis-(2-methyl-2-methylthio)propyl) propane-1,3-diamine) rhodium(III)] hexafluorophosphate. *cis*/*trans*-[RhCl$_2$(232-gdm-Met)]PF$_6$, *cis*/*trans*-[RhCl$_2$(L4)]PF$_6$.** Yield: 42% (38 mg, 0.063 mmol). A gradient mobile phase protocol (98% to 57% in 5 min, 57% for 5 min then from 57% to 52% in 30 min

and from 52% to 10% in the last 2 min) was utilized for analysis by HPLC and LC-MS. ESI-MS: *m/z* for $C_{13}H_{30}Cl_2N_2RhS_2$: 451.03 (100%), 452.03 (16.7) and 453.03 (65.2); found: (M^+) 451 (100%), 452 (17) and 453 (70).

***cis/trans*-[Dichloro (*N*,*N*-bis-(methylthio)ethyl) propane-1,3-diamine) rhodium(III)] hexafluorophosphate. *cis/trans*-[$RhCl_2$(232-Met)]PF_6, *cis/trans*-[$RhCl_2$(L5)]PF_6.** Yield: 30% (24 mg, 0.045 mmol). A gradient mobile phase protocol (98% to 76% in 32 min and 76% to 20% in the last 3 min) was utilized for analysis by LC-MS. ESI-MS: *m/z* for $C_9H_{22}Cl_2N_2RhS_2$: 394.97 (100.0%), 396.96 (73.0); found: (M^+) 394.75, 396.73.

***cis/trans*-[Dichloro (*N*,*N*-bis-(3-methylthio)propyl) ethane-1,2-diamine) rhodium(III)] hexafluorophosphate. *cis/trans*-[$RhCl_2$(323-Met)]PF_6, *cis/trans*-[$RhCl_2$(L6)]PF_6.** Yield: 44% (36 mg, 0.66 mmol). mp 295 °C (dec). Found: C, 21.9; H, 4.3; N 5.2. Calc. for $C_{10}H_{24}Cl_2N_2RhS_2{\cdot}PF_6$: C, 21.6; H, 4.4; N, 5.05. ESI-MS: *m/z* for $C_{10}H_{24}Cl_2N_2RhS_2$ 408.98 (100%), 410.98 (73), 412.98 (10.5); found (M^+) 408.82, 410.8, 412.81. Crystals suitable for an X-ray diffraction study were grown by diffusion of methanol into an acetonitrile solution of the complex (*vide infra*).

***cis/trans*-[Dichloro (*N*,*N*-bis-(3-methylthio)propyl) propane-1,3-diamine) rhodium(III)] hexafluorophosphate. *cis/trans*-[$RhCl_2$(333-Met)]PF_6, *cis/trans*-[$RhCl_2$(L7)]PF_6.** Yield: 40%. ESI-MS: *m/z* for $C_{11}H_{26}Cl_2N_2RhS_2$ 423.00 (100.0%), 424.99 (73.0); found: (M^+) 422.87, 424.88.

X-ray structure determinations and refinements

Intensity data were obtained at minus 100 °C on a Bruker SMART CCD Area Detector system using the ω scan technique with Mo-Kα radiation from a graphite monochromator. Intensities were corrected for Lorentz and polarization effects. Equivalent reflections were merged, and absorption corrections[16] were made using the multi-scan method. Structures were solved by direct methods with full-matrix least-squares refinement, using the SHELX package.[17,18] All non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were placed at calculated positions and included in the refinements using a riding model, with fixed isotropic *U*, except those except those on the carbons of a disordered ethanol of crystallization in the structure of [$RhCl_2$(**L3**)]PF_6, which were omitted from the final model. Final difference maps contained no features of chemical significance.

(*trans*-[$RhCl_2$(232-gdm-pmBz)]PF_6, *trans*-[$RhCl_2$(L3)] PF_6). $C_{28}H_{45}N_2O_{2.50}S_2Cl_2Rh{\cdot}PF_6$, M = 832.56, a = 17.8256(11) Å, b = 10.4632(6) Å, c = 18.7671(11) Å, α = 90°, β = 94.3150(10)°, γ = 90°, V = 3490.4(4) Å^3, T = 173(2) K, Crystal system, Monoclinic, space group $P2_1/c$, λ source = 0.71073 Å, Z = 4, D_c = 1.584 g cm^{-3}, $F(000)$ = 1708, dimensions 0.50 × 0.30 × 0.05 mm^3, semi-empirical absorption correction from equivalents, maximum and minimum transmission factors of 0.96 and 0.64, respectively, data collection range 2.18 to 27.14°, $-22 \le h \le 22$, $-10 \le k \le 13$, $-24 \le l \le 24$, 24240 reflections were measured, and 7684 were unique (R_{int} = 0.0271), $R(F)$ = 0.0325, $R_W(F)^2$ = 0.0819, GoF = 1.030.

(*trans*-[$RhCl_2$(323-Met)]PF_6, *trans*-[$RhCl_2$(L6)] PF_6). $C_{10}H_{24}N_2S_2Cl_2Rh{\cdot}PF_6$, M = 555.21, a = 12.4677(12) Å, b = 9.5972(9) Å, c = 16.3961(15) Å, α = 90°, β = 103.783(2)°, γ = 90°, V = 1905.4(3) Å^3, T = 173(2) K, Crystal system, Monoclinic, space group $P2_1/c$, λ source = 0.71073 Å, Z = 4, D_c = 1.935 g cm^{-3}, $F(000)$ = 1112, dimensions 0.55 × 0.50 × 0.25 mm^3, semi-empirical absorption correction from equivalents, maximum and minimum transmission factors of 0.70 and 0.53, respectively, data collection range 1.68 to 27.11°, $-15 \le h \le 15$, $-12 \le k \le 12$, $-20 \le l \le 20$, 12833 reflections were measured, and 4168 were unique (R_{int} = 0.0169), $R(F)$ = 0.0203, $R_W(F)^2$ = 0.0507, GoF = 1.094.

Radiolabeling reactions with ^{105}Rh. ***Caution!*** ^{105}Rh is a moderate energy beta emitter with a low abundance gamma emission. Proper radiation safety procedures and adequate shielding were used when handling this radionuclide in laboratories approved for radioisotope use.

^{105}Rh production. ^{105}Rh was prepared at the University of Missouri Research Reactor Center (MURR) by bombardment of an enriched ^{104}Ru metal target encapsulated in T21 Quartz for 155 h at a thermal neutron flux of 3 ′ 10^{14} n/cm^2 s^{-1}. The Ru metal was oxidized to RuO_4 and separated from ^{105}Rh by the method previously described.[19] This involved breaking the quartz vial and transferring the irradiated Ru metal into an impinger containing NaOH (3 mL, 2 M) through which Cl_2 gas (50 s @ 30 cm^3 min^{-1}) had been bubbled to generate NaOCl *in situ*. The reaction mixture was heated for 1 h at 40 °C with air flowing through the impinger followed by the bubbling of additional Cl_2 gas (12 min @ 30 cm^3 min^{-1}), followed by heating at 90 °C for 1 h with airflow, during which time the RuO_4 was distilled off and trapped in HCl (3 M, 400 cm^3). The reaction mixture was filtered to remove any undissolved Ru and heated for 30 min at 90 °C, and then treated with HCl (1 M, 0.25 cm^3) and heated at 90 °C without airflow for 30 min. Approximately 20–25 mCi (740–925 MBq) of ^{105}Rh(III)-chloride in an HCl solution were typically obtained at the end of processing. The ^{105}Rh(III)-chloride reagent is considered to be a mixture of primarily three or four different chloro-aquo species (*i.e.*, $^{105}RhCl_3(OH_2)_3$, $^{105}RhCl_4(OH_2)_2^{1-}$, $^{105}RhCl_5(OH_2)^{2-}$, and $^{105}RhCl_6^{3-}$),[1] although electrophoresis analysis generally shows the presence of only the three anionic species.[20]

^{105}Rh radiolabeling to form [$^{105}RhCl_2$(L)]Cl complexes. An aliquot of stock ligand solution (1 × 10^{-2} M, 0.100 cm^3) was added to a vial containing 1.6 mg of gentisic acid (GA), and the mixture was vortexed. After addition of 0.100 cm^3 of ^{105}Rh (~ 3.2 mCi), the pH of the solution was adjusted to ~ 4.5 with the addition of 2.5 M and 0.1 M NaOH consecutively. After the pH adjustment, 0.100 cm^3 of EtOH was added, and the volume of the reaction mixture was brought to 1 cm^3 by addition of water. Reaction mixtures were heated at 80 °C for 1h. After radiolabeling, the resulting solution was analyzed by radio-HPLC, and fractions were collected as a function of peaks observed by the radiodetector. The radioactivity of the collected fractions was compared to the amount of radioactivity injected to confirm that, in all cases, none of the activity was retained on the column. The [$^{105}RhCl_2$(**L**)]Cl peak was purified by radio-HPLC and recovered from a reverse-phase SepPak light cartridge. The SepPak cartridge was first activated with 4 cm^3 of EtOH and washed with 2 × 4 cm^3 of water. In the cases of [$^{105}RhCl_2$(**L2**)]Cl, [$^{105}RhCl_2$(**L3**)Cl and [$^{105}RhCl_2$(**L4**)]Cl, the collected HPLC eluent (~2–3 cm^3) was diluted with water to attain a 1 : 9 CH_3CN–H_2O

ratio (total 10 cm^3), and then was passed through the SepPak. The column was then washed with 2 × 4 cm^3 of water and the [$^{105}RhCl_2$(**L**)]Cl was eluted with 0.02–0.04 cm^3 μL of EtOH. In the case of [$^{105}RhCl_2$(**L5**)]Cl, due to its high solubility in water, instead of Sep-Pak purification, evaporation of the solvent under argon gas was performed.

Stability studies for [$^{105}RhCl_2$(L)]Cl (L = 3, 4, 5). Purified ^{105}Rh complexes were first prepared according to the optimized reaction conditions. To the vials containing 0.02 cm^3 (0.5 mCi) of purified [$^{105}RhCl_2$(**L3**)]Cl (*cis* and *trans*), [$^{105}RhCl_2$(**L4**)Cl (*trans*) and [$^{105}RhCl_2$(**L5**)]Cl (*cis* and *trans*), respectively, 0.500 cm^3 of phosphate-buffered saline (PBS) and 10 mg of GA were added.

Distribution coefficient (log D). The log D values were determined by the classic shake-flask method.[21,22] 100 μCi (0.001–0.005 cm^3) of the purified radiolabeled complexes ([$^{105}RhCl_2$(**L3**)]Cl, [$^{105}RhCl_2$(**L4**)Cl and [$^{105}RhCl_2$(**L5**)]Cl) were added to a pre-mixed suspension of 3.5 cm^3 of buffer-equilibrated octanol and 3.5 cm^3 of octanol-equilibrated PBS buffer at pH 7.4. The resultant solution was vortexed for two min and then centrifuged for 2 min at 2000 rpm. A 0.10 cm^3 aliquot of the octanol layer and a 1.0 cm^3 aliquot from the aqueous layer were counted in a NaI(Tl) scintillation well counter. The log D was calculated according to the following equation:

$$\text{Log D} = \log\left[\frac{(10 \times \text{average of organic counts-BKG})}{[\text{average buffer count-BKG}]}\right]$$

Then 3.0 cm^3 of the remaining octanol phase was transferred to another tube containing 0.5 cm^3 of octanol and 3.5 cm^3 of PBS. The last three steps were repeated until multiple, reproducible measurements of constant log D were obtained.

Conclusion

Of the seven ligands investigated for the coordination of rhodium(III), one emerged as a preferred ligand. The ligand with the 232 chain length, *gem*-dimethyl groups and methyl thioether (**L4**) forms *trans* rhodium complexes with highest radiochemical yields and *in vitro* stability. One caveat from the results obtained is that the temperature used for radiolabeling (80 °C) may not be compatible with all biological constructs. If a construct were stable to the radiolabeling conditions, this ligand could serve as a suitable carrier for ^{105}Rh in biological targets designed for radiotherapeutic studies.

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