Oxorhenium(V) and Oxotechnetium(V) Complexes of Cysteine

Mita Chatterjee,[†] Basudeb Achari,[‡] Satyabrata Das,[§] Rahul Banerjee,[§] Chandana Chakrabarti,[§] Jiban K. Dattagupta,[§] and Somenath Banerjee*,[†]

Departments of Nuclear Medicine and Medicinal Chemistry, Indian Institute of Chemical Biology, 4 Raja S. C. Mullick Road, Calcutta 700032, India, and Crystallography and Molecular Biology Division, Saha Institute of Nuclear Physics, 1/AF Bidhan Nagar, Calcutta 700064, India

Received May 15, 1997

Earlier attempts to obtain technetium complexes with cysteine always resulted in the formation of a product contaminated with polymeric species. A pure product, which could be chemically characterized and adopted for radiopharmaceutical preparation, has now been obtained by using cystine as the precursor of cysteine. This method has been extended to prepare the corresponding rhenium chelate, isolated as the tetraphenylphosphonium salt $[Ph_4P]^+[\{ReO(Cys)_2\}^-\{HReO(Cys)_2\}]\cdot 4H_2O$. The X-ray crystal structure of this compound revealed the presence of both neutral and anionic chelated species. In $[HReO(Cys)_2]$, the cysteine carboxylate moiety is unidentatedly bound to rhenium, while the carboxylic acid of the second cysteine remains as free COOH. The coordination environment around rhenium in the anionic species $[ReO(Cys)_2^-]$ is similar, the only difference being that the uncoordinated carboxylate moiety is present as a COO^- anion. The thiolate, amine coordination of the ligand with the metal is present in both the chelate units. The compound crystallized in an orthorhombic system with the space group $P2_12_12_1$, and having four formula units in each cell. The crystal data are $a = 9.700(2)$ Å, $b = 12.836(3)$ Å, and $c = 36.228(3)$ Å. The rhenium chelate has been structurally correlated with the technetium chelates through comparable spectroscopic and chromatographic data. The technetium-99m analogue of this rhenium chelate exhibited renal tubular transport and renal retention, which makes this radiopharmaceutical useful for evaluation of the clinical status of renal patients.

Introduction

A major impetus for the recent developments in the coordination chemistry of technetium and rhenium is due to the importance of the radioisotopes of these elements for diagnosis and therapy in nuclear medicine.[1−3] Technetium-99m (^{99m}Tc) is the radioisotope of choice for imaging in diagnostic nuclear medicine due to its ideal photon energy of 140 keV, lack of particulate radiation dose, half-life of 6 h, and wide availability.[4] Coordination compounds of the β-emitter rhenium are, on the other hand, of current interest in cancer therapy, since this cytotoxic radionuclide can be delivered to the affected portion of the organ after chelation and coupling with appropriate monoclonal antibody.[5] Correlation of rhenium and technetium chelate chemistry is also important since the well-developed chemistry of rhenium may be of valuable assistance in developing new ^{99m}Tc chelates with potential application in diagnostic nuclear medicine.[6]

Both technetium and rhenium, especially at their lower valency states, show good chelating abilities with a wide variety of ligands containing O, S, N, or P donor atoms. Several ^{99m}Tc chelates derived from these ligands have been evaluated as dynamic and static agents of various organ systems.[7] Of particular interest have been the tetradentate N_2S_2 ligands. For example, both *N*,*N*′-ethylene-L,L-dicystine (L,L-EC)[8] and its diethyl ester (ECD)[9] coupled with ^{99m}Tc have produced diagnostic agents useful in renal function and brain-imaging studies, respectively. It therefore appeared that cysteine itself might form a bis-bidentate chelate structurally comparable to ^{99m}Tc−L,L-EC and should be recognized by renal tubular transport protein in the same way. However, the ^{99m}Tc complex made from cysteine earlier in this laboratory, though exhibiting high renal specificity in animals, failed to exhibit transportation by the above transport protein.[10] Such results are not surprising since earlier experiments on chelation of cysteine with ^{99}Tc established that appreciable amounts of polymeric products are formed,[11−13] presumably due to the presence of excess thiolate ligand.[12]

* To whom correspondence should be addressed.
[†] Department of Nuclear Medicine, Indian Institute of Chemical Biology.
[‡] Department of Medicinal Chemistry, Indian Institute of Chemical Biology.
[§] Saha Institute of Nuclear Physics.

(1) Deutsch, E.; Libson, K.; Jurisson, S.; Lindoy, L. F. *Prog. Inorg. Chem.* **1983**, *30,* 75.
(2) Deutsch, E.; Libson, K. *Comments Inorg. Chem.* **1984**, *3*, 83.
(3) Clarke, M. J.; Podbielski, L. *Coord. Chem. Rev.* **1987**, *78*, 253.
(4) (a) Baum, S.; Bramlet, R. *Basic Nuclear Medicine*: Appleton-Century-Crofts: New York, 1975. (b) Subramanian, G., Rhodes, B. A., Cooper, J. F., Sodd, V. J., Eds. *Radiopharmaceuticals;* The Society of Nuclear Medicine: New York, 1975.
(5) Deutsch, E.; Libson, K.; Vanderheyden, J. L.; Ketring, A. R.; Moxon, H. R. *Nucl. Med. Biol.* **1986**, *13*, 465.
(6) Rao, T. N.; Adhikesavalu, D.; Camerman, A.; Fritzberg, A. R. *J. Am. Chem. Soc.* **1990**, *112*, 5798.
(7) Fritzberg, A. R., Ed. *Radiopharmaceuticals: Progress and Clinical Perspectives*; CRC Press Inc.: Boca Raton, FL, 1986; Vol. 1.
(8) Verbruggen, A. M.; Nosco, D. L.; Van Nerom, C. G.; Bormans, G. M.; Adriaens, P. J.; De Roo, M. J. *J. Nucl. Med.* **1992**, *33*, 551.
(9) Vallabhajosula, S.; Zimmerman, R.E.; Picard, M.; Stritzke, P.; Mena, I.; Hellman, R. S.; Tikofsky, R. S.; Stabin, M. G.; Morgan, R. A.; Goldsmith, S. J. *J. Nucl. Med.* **1989**, *30*, 599.
(10) Chattopadhyay, M.; Banerjee, S. N. *J. Inorg. Biochem.* **1988**, *34*, 25.
(11) Johannsen, B.; Syhre, R.; Spies, H.; M(nze, R. *J. Nucl. Med.* **1978**, *19*, 816.
(12) Bryson, N.; Dewan, J. C.; Lister-James, J.; Jones, A. G.; Davison, A. *Inorg. Chem.* **1988**, *27*, 2154.
(13) Bryson, N.; Lister-James, J.; Jones, A. G.; Davis, W. M.; Davison, A. *Inorg. Chem.* **1990**, *29*, 2948.

10.1021/ic970577q CCC: $15.00
Published on Web 09/24/1998

In view of these findings we reasoned that it would be more appropriate to use cystine as the starting material for the preparation of technetium cysteinate because cystine can readily undergo metal-induced disulfide cleavage[14] and this would exclude the presence of excess thiolate in the reaction mixture. ^{99m}Tc−cysteine prepared in this way[15] has been found to interact with renal tubular transport protein and proved to be a valuable radiopharmaceutical for clinical evaluation of renal patients.[16] However, there is some subtle difference in the clinical properties of the L,L-EC and cysteine chelates. For example, ^{99m}Tc−cysteine, but not ^{99m}Tc−L,L-EC, shows appreciable kidney retention, which is quite useful for the evaluation of kidney morphology.[16] Again, though penicillamine closely resembles cysteine in terms of the arrangement of the donor groups, its ^{99m}Tc chelate shows appreciable kidney retention but lacks in ultrafast renal excretory kinetics exhibited by the aforementioned two chelates.[17]

It was anticipated that differences in biological properties of the above three ^{99m}Tc chelates are due to the different modes of attachment of the ligands to technetium. The structure of ^{99m}Tc−D-penicillamine has been established,[18] while a structure has been proposed for ^{99m}Tc−L,L-EC in the literature.[8] An acceptable structure of Tc-cysteinate which may explain its biological activity vis-à-vis the Tc chelates of penicillamine and L,L-EC, however, remained to be elucidated. Inasmuch as technetium and rhenium complexes with identical ligands have essentially identical coordination parameters,[19] we decided to synthesize and determine the structure of the cysteine complex of oxorhenium(V).

Experimental Section

L-Cystine, L-cysteine monohydrochloride monohydrate, and ammonium perrhenate were purchased from Aldrich Chemical Co. Ammonium ^{99}Tc-pertechnetate was purchased as an aqueous solution (0.38 mmol/mL) from Amersham, and its concentration was confirmed by UV spectroscopy. All other chemicals were of reagent grade and purchased locally. UV−visible spectra were recorded on a Hitachi U-2000 spectrophotometer. Infrared spectra were recorded as KBr pellets by a JASCO 700 spectrophotometer. 2D (^{1}H−^{1}H) NMR spectra of $HReO(Cys)_2$ were recorded on a 400 MHz Bruker VM-400 spectrometer, the ^{13}C NMR spectrum was measured with a Bruker AVANCE DPX-300 spectrometer, and all other spectra were recorded on a 100 MHz JEOL FX-100 spectrometer. HPLC analyses were performed isocratically on a C_{18} μbondapak column (3.9 × 300 mm) using a Waters Associates Model 501 solvent delivery system with an appropriate eluting solvent. The technetium and rhenium chelates were analyzed with a Model 481 UV detector of the same company, whereas the ^{99m}Tc chelate was analyzed with a Beckman Model 170 radioisotope detector. The radioactivities of various ^{99}Tc samples were measured with a Beckman Model LS-5000-TD counter using the ^{14}C counting channel.

Preparation of [H^{99}TcO(Cys)$_2$] (1). Method 1. To a stirred pale green solution of $[Bu_4N][TcOCl_4]$ (85 mg, 0.17 mmol) in methanol (5 mL) was slowly added an aqueous solution (3 mL) of cysteine monohydrochloride monohydrate (60 mg, 0.34 mmol), whereupon the color became bright yellow. After adjusting the pH to 7.4 with 1 M NH_4OAc, the solution was passed through a CM cellulose column (44 × 2.2 cm) eluting with methanol. The eluate was evaporated, dissolved in water (2 mL), and chromatographed over a Sephadex LH-20 column (56 × 2.2 cm). The aqueous eluate was concentrated (0.5 mL) and treated with an aqueous solution (0.5 mL) of $BaCl_2$ (50 mg, 0.24 mmol). The precipitated barium salt was purified by recrystallization from 50% MeOH/H_2O to furnish the analytical sample (25 mg, 35%). Anal. Calcd for $Ba(C_6H_{11}O_5N_2S_2Tc)_2\cdot 6H_2O$: C, 15.11; H, 3.56; N, 5.87. Found: C, 14.97; H, 3.29; N, 5.33.

Method 2. To a solution of cystine (240 mg, 1 mmol) in aqueous potassium hydroxide (1 M, 3 mL) was added an aqueous NH_4TcO_4 solution (0.45 mL, 0.17 mmol) followed by a solution (1 mL) of $Na_2S_2O_4$ (65 mg, 0.37 mmol); a dark red solution resulted, which on standing at room temperature for 4 h became yellow. The pH of the solution was adjusted to 7 with 1 N HCl, and the precipitated cystine was filtered off. The filtrate after purification on CM cellulose and Sephadex LH-20 columns, as stated above, furnished a brownish yellow solid (21 mg, 35%), which was further treated as in method 1 to obtain the analytical sample. Anal. Calcd: as above. Found: C, 15.45; H, 3.94; N, 5.29.

Preparation of $[Ph_4P]^+[\{ReO(Cys)_2\}^-\{HReO(Cys)_2\}]\cdot 4H_2O$ (2). The preceding method (method 2) for the preparation of **1** was slightly modified to produce the rhenium chelate. To a solution of cystine (250 mg, 1.04 mmol) in aqueous potassium hydroxide (1 M, 3.2 mL) was added an aqueous solution of NH_4ReO_4 (100 mg, 0.37 mmol) and $Na_2S_2O_4$ (600 mg, 3.4 mmol), and the reaction mixture was heated at 100 °C for 15 min, cooled, and acidified to pH 7 with 1 N HCl to remove most of the starting amino acid. Subsequent purification of the rhenium chelate on CM cellulose and Sephadex LH columns afforded a colored residue. It was recrystallized from 60% iPrOH/H_2O to yield a brown powder (35 mg) identified as $HReO(Cys)_2$ from spectroscopic studies (UV, IR, 2D NMR). This on treatment with an aqueous solution (1.5 mL) of tetraphenylphosphonium bromide (35 mg, 0.08 mmol) slowly deposited the crystals. Repeated trituration of the crystals with iPrOH and final recrystallization from aqueous EtOH (1:1) furnished the sample for elemental analysis and X-ray studies. Anal. Calcd for $C_{36}H_{41}N_4O_{10}PS_4Re_2\cdot 4H_2O$: C, 33.43; H, 3.79, N, 4.33. Found: C, 32.89; H, 3.58; N, 4.18.

HPLC of the Chelates. The retention times of $[HReO(Cys)_2]$, $[H^{99}TcO(Cys)_2]$, and $[H^{99m}TcO(Cys)_2]$ were measured using a reverse phase HPLC system with a μbondapak C_{18} column (3.9 × 300 mm). After injection of the chelate the column was eluted either with ammonium formate (0.01 M, pH 7)−acetonitrile (3:7 v/v) buffer or with tetrabutylammonium chloride (0.005M)−methanol (3:2 v/v) buffer at a flow rate of 1 mL/min. The UV detectors were set at 426 and 344 nm respectively for ^{99}Tc and Re chelates whereas ^{99m}Tc chelates were detected radiometrically.

Crystallography. The crystal was initially characterized using precession and Weissenberg photographs. The density was measured by floatation method in a carbon tetrachloride−bromoform mixture. Final cell parameters were obtained from high-angle reflections (θ = 9−12° on a CAD-4 Enraf-Nonius[20] four-circle diffractometer using Cu Kα (1.5418 Å) radiation. The intensities of three reflections were measured periodically to monitor crystal decay. The intensity data, collected in the $\omega-2\theta$ scan mode, were corrected for Lorentz and polarization factors. An empirical absorption correction was also applied. The parameters related to crystal data, data collection, and refinement are listed in Table 4.

The structure was solved by using a combination of Patterson and Fourier techniques. The two rhenium atoms were initially obtained by the Patterson method, and a subsequent heavy atom phased Fourier synthesis yielded the other heavier atoms, namely, phosphorus and sulfur. The rest of the structure was revealed by successive application of least-squares refinements followed by difference Fourier syntheses. Finally, four water oxygen atoms were included in the structure. The temperature factors were made anisotropic at this stage, and refinement

(14) Greenstein, J. P., Winitz, M., Eds. *Chemistry of the Amino Acids*; John Wiley and Sons Inc.: New York, 1961; Vol. 3, p 1879.
(15) Chattopadhyay, M.; Jalan, K. N.; Pal, A. K.; Banerjee, S. N. *Nucl. Med. Biol.* **1988**, *15*, 535.
(16) Misra, M.; Das, B. K.; Gambhir, S.; Sewatkar, A. B.; Ghosh, S.; Banerjee, S. N. *Clin. Nucl. Med.* **1994**, *19*, 314.
(17) Yokoyama, A.; Saji, H.; Tanaka, H.; Odori, T.; Morita, R.; Mori, T.; Torizuka, K. *J. Nucl. Med.* **1976**, *17*, 810.
(18) Franklin, K. J.; Howard-Lock, H. E.; Lock, C. J. L. *Inorg. Chem.* **1982**, *21*, 1941.
(19) Deutsch, E.; Libson, K.; Vanderheyden, J. L. In *Technetium and Rhenium in Chemistry and Nuclear Medicine*; Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Cortina International: Verona, Italy, 1990; Vol. 3, p 13.
(20) *CAD-4 Software*, Version 5.0; Enraf-Nonius: Delft, The Netherlands; 1989.

Table 1. Visible UV Spectrophotometric Data

compound	solvent	λ_{max}, nm (ϵ, M^{-1} cm^{-1})
$HReO(Cys)_2$	H_2O	495 (86), 344 (3503), 295 sh (1985)
$HPh_4P[ReO(Cys)_2]_2$	MeOH	488 (234), 344 (9789), 298 sh (509), 271 (6141), 222 (36 950)
$Ba[TcO(Cys)_2]_2$ method 1	H_2O	423 (4146), 305 (1587), 245 sh (3748)
$Ba[TcO(Cys)_2]_2$ method 2	H_2O	423 (4652), 305 (1745), 245 sh (4200)
$HTcO(Cys)_2$	H_2O	426 (4275), 304 (1154), 264 sh (4832)

Table 2. Infrared Spectral Data

compound	M═O stretch, cm^{-1}
$HReO(Cys)_2$	969
$HPh_4P[ReO(Cys)_2]_2$	977
$Ba[TcO(Cys)_2]_2$ (method 1)	926
$Ba[TcO(Cys)_2]_2$ (method 2)	929
$HTcO(Cys)_2$	940

Table 3. ^1H NMR Spectroscopic Data

compound	solvent	δ, ppm
$HReO(Cys)_2$	D_2O	4.26 (1H, d, $J = 4$), 3.59−3.51 (2H, m), 3.31−3.17 (2H, m), 2.77 (1H, d, $J = 17.2$)[a]
$HPh_4P[ReO(Cys)_2]_2$[b]	DMSO-d_6	4.02 (2H, d, $J = 4$), 3.80−2.92 (8H, m), 2.68 (2H, d, $J = 12$)
$Ba[TcO(Cys)_2]_2$[c]	D_2O	3.92−3.60 (4H, m), 3.34 (1H, d, $J = 14$)
$HTcO(Cys)_2$	D_2O	4.42 (1H, d, $J = 4$), 4.16−3.56 (m, 4H), 3.36 (1H, d, $J = 14$)

[a] J values are in hertz. [b] Additional signals at 8.28−7.60 (20H, m) for the Ph_4P^+ moiety. [c] The signal for one proton appears to have merged under the solvent signal at 4.4. An identical spectrum was obtained for chelates prepared by either method 1 or 2.

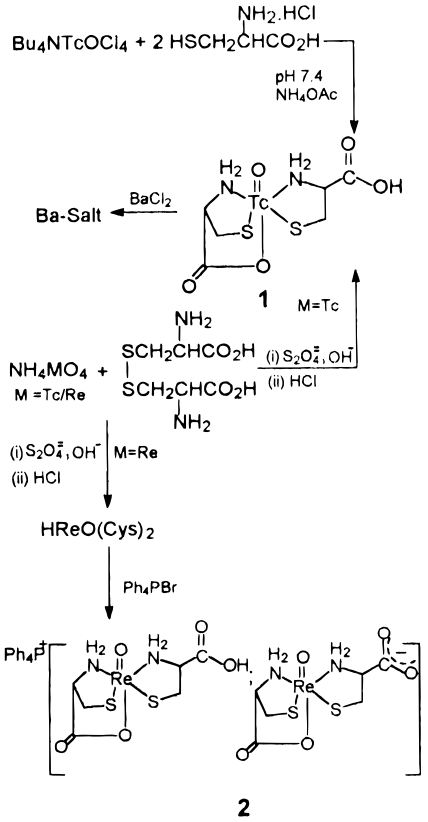
Table 4. Crystallographic Data for **2**

formula	$[\{ReO(C_6H_{11}N_2O_4S_2)\}\{ReO(C_6H_{10}N_2O_4S_2)\}]^-\cdot[P(C_6H_5)_4]^+\cdot 4H_2O$
fw	1293.40
space group	$P2_12_12_1$
a, Å	9.700(2)
b, Å	12.836(3)
c, Å	36.228(3)
V, Å^3	4511(2)
Z	4
ρ_{calcd}, gm cm^{-3}	1.905
ρ_{obsd}, gm cm^{-3}	1.94(2)
λ (Cu Kα), Å	1.54180
μ, mm^{-1}	3.544
T, °C	20
R indices (all data)	R1[a] = 0.0652, wR2[b] = 0.1694

[a] $R1 = [\sum||F_o| - |F_c||]/\sum|F_o|$. [b] $wR2 = [\sum\{w(F_o^2 - F_c^2)^2\}/\sum\{wF_o^2\}^2]^{1/2}$.

using full-matrix least squares was terminated when the maximum average shift/error became less than 0.520 for all parameters. There were indications of additional disordered water sites, but they could not be adequately modeled and showed irregular behavior during refinement. The residual index was $R = 0.065$ for all data. The positional parameters for non-hydrogen atoms are listed in Table 5. The chirality (L) of cysteines was confirmed. SHELXS86[21] was used for structure solution and SHELXL93[22] for refinement. The molecular graphics program ORTEX[23] was used to prepare all the figures.

Scheme 1



Results

The oxotechnetium chelate **1** of cysteine may be prepared (Scheme 1) from $Bu_4NTcOCl_4$ and cysteine under carefully controlled reaction conditions (method 1). After quick purification over CM cellulose and Sephadex LH-20 columns, it is precipitated as the barium salt, which furnishes the expected analytical and spectroscopic results. If not quickly processed, the yellow chelate solution slowly degenerates to a green solution within a few hours, or rapidly on addition of a few milligrams of cysteine. This oxotechnetium chelate **1** may be prepared (Scheme 1) more conveniently from NH_4TcO_4, $Na_2S_2O_4$, and cystine in aqueous alkali (to keep the ligand in solution) (method 2). After the usual chromatographic purification, the solution furnished a brown solid, which produced the same barium salt as obtained in method 1 (as evidenced by their analytical and spectroscopic properties). Attempts to grow crystals suitable for X-ray crystallography were unsuccessful. The corresponding rhenium chelate was prepared (Scheme 1) by a modification of method 2 to furnish a brown powder, on which detailed ^1H NMR data (Table 3) were obtained. This compound yielded the tetraphenylphosphonium salt **2**, which was subjected to X-ray crystallographic analysis. (See Table 6.)

Spectroscopy. The electronic spectral data of Tc chelate **1**, its barium salt, the Re chelate $HReO(Cys)_2$, and its tetraphenylphosphonium salt **2**, are given in Table 1. The absorptions are in the expected regions. The tetraphenylphosphonium salt **2** absorbed in same region as the free complex but with increased intensity. In the infrared region (Table 2), the Tc and Re chelates (as also their barium and tetraphenylphosphonium salts) respectively showed the characteristic M═O absorption around 940 and 970 cm^{-1}. The ^1H NMR spectral data of the above compounds are listed in Table 3. The patterns of the recorded spectra are quite similar and consistent with the assigned

(21) Sheldrick, G. M., *SHELXS86. Program for the Solution of Crystal Structures*: University of Göttingen: Göttingen, Germany, 1985.
(22) Sheldrick, G. M. *SHELXL93. Program for Crystal Structure Refinement*; University of Göttingen: Göttingen, Germany, 1993,
(23) McArdle, P. *J. Appl. Crystallogr.* **1993**, *26*, 752.

Table 5. Atomic Positional Parameters (Å × 10^4) and Equivalent Isotropic Displacement Parameters (Å^2 × 10^3) of **2**[a]

atom	*x*	*y*	*z*	U_{eq}
Re1	9207(1)	623(1)	8387(1)	36(1)
O1	8893(15)	1852(10)	8380(4)	54(4)
Re2	3126(1)	−5842(1)	8626(1)	41(1)
O2	3511(14)	−4669(10)	8787(3)	51(4)
S1	7407(5)	−130(4)	−8689(1)	47(1)
C11	6150(18)	−355(15)	8318(5)	49(5)
C12	6945(17)	562(13)	7956(4)	39(4)
C13	5989(19)	−639(15)	7627(5)	48(5)
N11	7917(15)	271(11)	7911(4)	41(4)
O11	6045(19)	30(13)	7375(4)	75(6)
O12	5095(17)	−1387(15)	7625(5)	82(6)
S2	10662(6)	359(5)	8882(1)	66(2)
C21	12296(22)	273(24)	8630(7)	82(9)
C22	12057(27)	−27(25)	8249(8)	84(10)
C23	11243(29)	−991(27)	8232(8)	89(10)
N21	1148(16)	783(14)	8087(4)	55(5)
O21	9903(16)	−935(12)	8209(4)	61(5)
O22	11803(27)	−1923(20)	8238(7)	32(10)
S3	4386(5)	−6992(4)	8980(1)	51(1)
C31	5837(24)	−7231(19)	8673(5)	64(7)
C32	5469(23)	−6885(18)	8285(5)	61(6)
C33	4267(23)	−7496(18)	8145(5)	58(6)
N31	4995(16)	−5779(14)	8288(4)	52(5)
O31	3072(15)	−7114(12)	8240(3)	57(4)
O32	4389(19)	−8325(13)	7981(4)	73(5)
S4	1105(5)	−6335(4)	8891(1)	49(1)
C41	−142(19)	−5802(15)	8576(5)	52(6)
C42	371(20)	−5854(19)	8190(5)	57(6)
C43	−563(32)	−5344(28)	7907(7)	92(11)
N41	1760(16)	−5301(14)	8190(4)	56(5)
O41	−77(19)	−4661(18)	7675(5)	97(8)
O42	−1797(25)	−5606(24)	7928(9)	150(13)
P1	6954(4)	−4794(3)	9821(1)	33(1)
C51	7998(17)	−4424(12)	10207(4)	36(4)
C52	8074(24)	−3387(14)	10307(5)	54(6)
C53	8892(24)	−3084(17)	10596(5)	62(7)
C54	9682(22)	3815(16)	10769(5)	53(6)
C55	9666(23)	−4811(17)	10673(5)	56(6)
C56	8783(18)	−5154(14)	10393(4)	43(5)
C61	7182(16)	−6136(12)	9732(4)	35(4)
C62	8065(21)	−6490(14)	9467(4)	47(5)
C63	8268(23)	−7562(14)	9411(5)	52(5)
C64	7548(25)	−8278(15)	9623(7)	68(8)
C65	6624(26)	−7920(16)	9892(7)	70(8)
C66	6459(22)	−6874(17)	9949(6)	59(6)
C71	5176(16)	−4531(11)	9910(4)	33(4)
C72	4771(19)	−4055(14)	10238(5)	42(4)
C73	3340(18)	−3901(13)	10299(5)	44(5)
C74	2396(18)	−4165(13)	10036(5)	45(5)
C75	2814(17)	−4641(14)	9709(5)	45(5)
C76	4175(19)	−4824(14)	9652(5)	43(5)
C81	7589(18)	−4063(13)	9435(5)	41(5)
C82	8967(23)	−3819(17)	9415(6)	55(6)
C83	9527(25)	−3278(17)	9130(6)	64(7)
C84	8679(26)	−2956(17)	8829(6)	67(7)
C85	7304(23)	−3234(17)	8837(6)	60(6)
C86	6764(21)	−3805(14)	9134(5)	52(5)
O1W	7582(21)	2374(16)	7534(5)	85(6)
O2W	14290(21)	−2892(14)	8329(5)	86(7)
O3W	−852(42)	−2657(30)	7659(8)	197(18)
O4W	4002(29)	1530(16)	7278(5)	151(10)

[a] $U_{eq} = (1/3)\sum_i\sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i \cdot \mathbf{a}_j$.

structure. However, the signals appear highly superimposed and a better resolution of the spectral pattern was achieved by recording the spectrum of $HReO(Cys)_2$ at 400 MHz, where four sets of signals could be clearly observed. The ^{13}C NMR spectrum of **2** showed the following peaks: (δ 42.4, 50.9 (CH_2), 66.5, 67.8 (CH), 119.9 (d, J_{CP} = 89.1 Hz), 132.1 (d, J_{CCCP} = 12.8 Hz), 136.4 (d, J_{CCP} = 10.5 Hz), 137.2 (d, J_{CCCCP} = 3 Hz), 177.0, 179.6 (C=O).

Table 6. Selected Bond Lengths (Å) and Angles (deg) of **2**

Re1−O1	1.607(12)	Re2−O2	1.659(13)
Re1−N11	2.179(13)	Re2−O31	2.150(13)
Re1−N21	2.180(20)	Re2−N41	2.180(20)
Re1−O21	2.208(14)	Re2−N31	2.190(14)
Re1−S1	2.275(4)	Re2−S4	2.273(5)
Re1−S2	2.305(5)	Re2−S3	2.307(5)
O1−Re1-N11	94.7(6)	N41−Re2-N31	94.9(6)
O1−Re1-N21	93.6(7)	O2−Re2-S4	107.3(5)
N11−Re1-N21	96.9(5)	O31−Re2-S4	92.4(4)
O1−Re1-O21	160.4(6)	N41-Re2S4	82.5(4)
N11−Re1-O21	75.9(6)	N31−Re2-S4	163.9(5)
N21−Re1-O21	71.1(6)	O2-Re2-S3	105.4(5)
O1−Re1-S1	106.3(5)	O31-Re2-S3	83.7(4)
N11−Re1-S1	81.5(4)	N41-Re2-S3	158.6(5)
N21−Re1-S1	160.2(5)	N31-Re2-S3	84.1(4)
O21−Re1-S1	89.5(4)	S4−Re2-S3	92.5(2)
O1−Re1-S2	105.9(5)	C11−S1-Re1	103.0(6)
N11−Re1-S2	159.4(4)	C12−N11-Re1	116.2(9)
N21−Re1-S2	82.7(4)	C21−S2-Re1	98.7(7)
O21−Re1-S2	84.7(4)	C22−N21-Re1	104.4(13)
S1−Re1-S2	92.0(2)	C23−O21-Re1	110.0(20)
O2−Re2-O31	157.6(6)	C31−S3-Re2	100.2(7)
O2−Re2-N41	95.9(7)	C32−N31-Re2	102.9(11)
O31−Re2-N41	75.8(6)	C33−O31-Re2	115.8(12)
O2−Re2-N31	88.7(7)	C41−S4-Re2	101.9(6)
O31−Re2-N31	71.6(6)	C42−N41-Re2	112.9(11)

HPLC of the Chelates. The cysteine complexes of both technetium and rhenium were very polar and could not be retained significantly on a C_{18} column with the usual buffer modifier eluting system. In a typical experiment with an ammonium formate (0.1 N, pH 7) and acetonitrile (3:7 v/v) mixture, both technetium and rhenium chelates eluted at the void time. Elution with aqueous $Bu_4N^+Cl^-$ in methanol significantly increased their retention times; with a 3:2 v/v mixture, the retention times for $H^{99}TcO(Cys)_2$, $HReO(Cys)_2$, and $H^{99m}TcO(Cys)_2$ were 6.82, 6.33, and 6.60 min, respectively. All of these chelates eluted as single components without any appreciable amount of contamination (<5%). The minor difference observed in the retention times of ^{99}Tc and ^{99m}Tc chelates is consistent with the entry to response time difference associated with the two detection systems.

Crystal Structure. The structure consists of two distorted octahedral oxorhenium−cysteine chelate complexes [Re1(O1)-$(Cys)_2]^-$ (**Re1**) and [HRe2(O2)$(Cys)_2$] (**Re2**), one tetra phenyl phosphonium ion $[Ph_4P]^+$, and four water molecules in the asymmetric unit (Figure 1). The structure of **Re1** is shown in Figure 1 and selected geometric parameters are given in Table 4. The rhenium-oxo bond distances are 1.61(1) Å for **Re1** and 1.66(1) Å for **Re2**. The two cysteine moieties are ligated to ReO^{3+} through N and S in a cis arrangement in the equatorial plane, and the sixth coordination site is occupied by one of the carboxylate oxygens (see Figure 1). In the case of **Re2**, significant deviation from planarity is noticed for the four equatorial atoms, whereas this does not occur in **Re1**. In both cases, rhenium atoms are displaced out of the plane toward the oxo group, the distance being 0.39 and 0.36 Å for **Re1** and **Re2**, respectively. The metal−sulfur distances range from 2.273(5) to 2.307(5) Å in both units. All of the oxo−Re−S angles are about 106°, deviating from the normal octahedral value. The Re−N distances range from 2.18(2) to 2.19(2) Å and the metal to carboxylate oxygen bond distances are 2.15(1) and 2.21(2) Å. The values of the torsion angles O11−C13−C12−N11 and O41−C43−C42−N41 are −5(2)° and 10(3)°, putting both in the eclipsed conformation. There are no significant differences in the structures and conformations

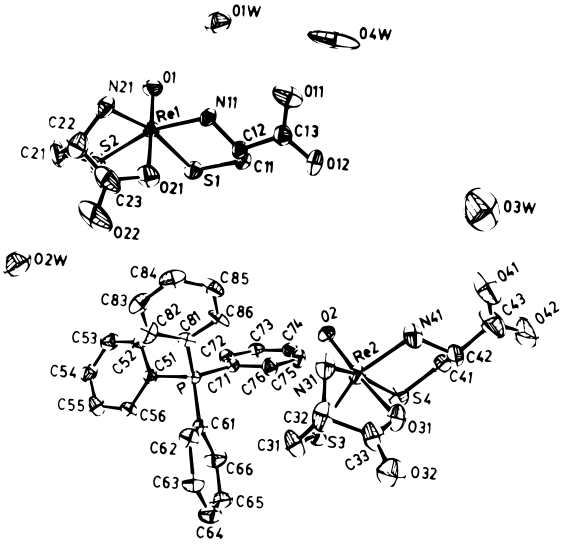


Figure 1. Molecular structure of **2** with the atom-numbering scheme, showing 40% probability displacement ellipsoids.

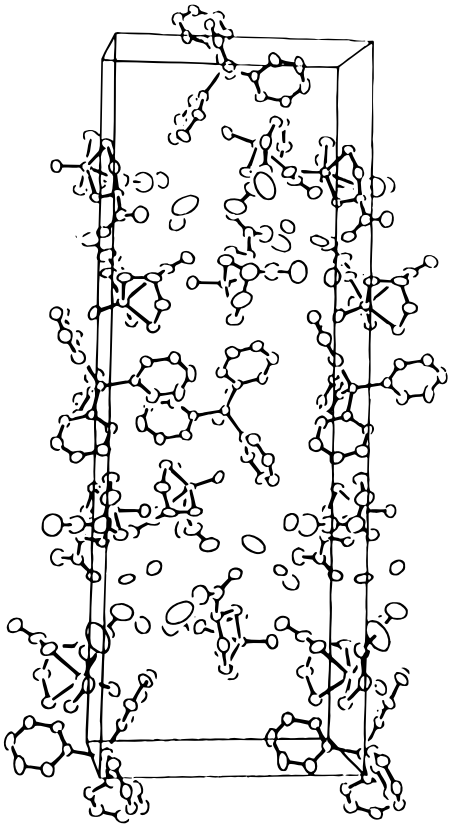


Figure 2. Packing diagram of the title complex **2** looking down the *a* axis.

of **Re1** and **Re2**. Geometrical parameters of the tetraphenylphosphonium ions are well within the range of standard values.

The values of the important torsion angles S1−C11−C12−N11, S2−C21−C22−N21, S3−C31−C32−N31, and S4−C41−C42−N41 are −52(2)°, 59(2)°, 54(2)°, and −55(2)°, respectively.

The crystal is packed (Figure 2) in alternating layers of hydrophobic and hydrophilic interactions. The primary feature of the packing is the disposition of **Re1** and **Re2** in the form of a spiral about a 2_1 screw axis located at (0, *y*, $^3/_4$). A pair of hydrogen bonds relate both **Re1** and **Re2** with their respective symmetry-related partners. The hydrogen-bonding parameters

Table 7. Hydrogen-Bonding Parameters for **Re1** and **Re2**

D−H···A[a]	bond length, Å H···A	bond length, Å D···A	torsion angle deg D−H···A
N11−H···O41^i	2.14(2)	2.98(2)	157(2)
N21−H···O41^i	2.14(2)	3.01(2)	162(2)
N41−H···O11^{ii}	2.21(2)	2.99(2)	144(2)
N31−H···O11^{ii}	1.93(2)	2.81(2)	165(2)

[a] Symmetry codes: (i) $1-x$, $^1/_2+y$, $^3/_2-z$; (ii) $1-x$, $-^1/_2+y$, $^3/_2-z$.

are provided in Table 7. On either side of the spiral is a column of tetraphenylphosphonium ions which provides the hydrophobic interactions to sustain the structure. The interactions within the column are primarily between the benzene rings of tetraphenyl phosphonium ions, while those between **Re1**, **Re2**, and the tetraphenylphosphonium ions are predominantly of the van der Waals type.

Four water molecules have been found in the structure (their coordination with other atoms and among themselves is given in the Supporting Information). All of them are sandwiched between **Re1** and **Re2** about the 2_1 screw axis. These highly localized water molecules form a water channel along the same axis. They have strong interactions with the carboxylate oxygens. Other than these, O1W and O2W also have strong interactions with N11 and O2, respectively. All of the carboxylate oxygens of **Re1** and **Re2** are oriented toward the screw axis. A network of hydrogen bonds is formed predominantly between these oxygens and the water molecules along this axis. Water−water contacts also contribute to this network. Two of the water molecules (O3W, O4W) show high temperature factors. There are strong indications of other disordered water sites, but they could not be adequately modeled.

Discussion

Despite the good affinity of the thiolate group for technetium, formation of undesired products while chelating technetium with polyfunctional ligands having thiolate function is well-known.[13] The literature contains reports of numerous examples of the formation of multiple complexes during chelating of technetium-99m with penicillamine (D-pen),[17] dimercaptosuccinic acid (DMSA),[24] and *S*-benzoyl mercaptoacetyl triglycine (BZ-MAG_3).[25] Similar results were obtained in technetium-99 chemistry during attempts to chelate technetium-99 with amido thiolate derivatives.[12,13] It was postulated that the presence of excess ligand has a degrading effect on the chelate initially formed. This can be avoided by using suitable S-protecting groups that can undergo metal-induced deprotection, thus avoiding the presence of excess thiolate in the chelate mixture.[13] Useful S-protecting groups used for this purpose are benzyl, acetyl aminomethyl, benzoyl amino methyl, etc.[12,13] Thus S-protection of cysteine could be a possible solution to avoid the formation of polymeric products in the attempted chelation of ^{99}Tc with cysteine reported earlier.[11] It was felt that the use of the corresponding disulfide as cysteine precursor could be a useful and convenient alternative. Utilizing this idea, cystine was successfully used to prepare the cysteine chelate of technetium by reducing $NH_4{}^{99}TcO_4$ with sodium dithionite in the presence of cystine. The same chelate could be prepared by condensing cysteine with $Bu_4NTcOCl_4$. The identity of these two preparations was established by the usual spectroscopic and analytical data. However, when cysteine is used as a ligand,

(24) Ikeda, I.; Inoue, O.; Kurata, K. *J. Nucl. Med.* **1977**, *18*, 1222.
(25) Brandau, W.; Bubeck, B.; Eisenhut, M.; Taylor, D. M. *Appl. Radiat. Isot.* **1988**, *39*, 121.

its amount in the reaction mixture must be carefully controlled since the formation of the polymeric product cannot be prevented otherwise. Therefore, it is not possible to extend this method to prepare ^{99m}Tc cysteine where the presence of a large amount of cysteine with respect to this isotope of technetium is unavoidable because of the low concentration of the tracer (10^{-8} M) used for such preparation. However, under the above condition the disulfide (cystine) can be used to produce the desired cysteine complex of ^{99m}Tc in nearly quantitative yield.[15] The clinical utility of this chelate[16] has been described.

Rhenium chelates have good structural relevance to the corresponding ^{99m}Tc chelates and are often used as structural models for understanding the biological properties of ^{99m}Tc-based radiopharmaceuticals.[19] Therefore $HReO(Cys)_2$ has also been prepared, and the preparative method extends the utility of disulfides as thiolate replacement in rhenium chelate preparations.

The UV−visible spectra of $HTcO(Cys)_2$ and its barium salts prepared by different procedures were quite similar to that of $HTcO(D\text{-pen})_2$.[18] The weak bands observed in the visible region for $HReO(Cys)_2$ and $HPh_4P[ReO(Cys)_2]_2$ are characteristic of several low-spin d^2 rhenium chelates as observed in oxorhenium amido thiolates.[26] The infrared spectra of technetium chelates show the Tc═O stretching in the region 926−940 cm^{-1}, consistent with the results reported for compounds containing the TcO^{3+} core.[26,27] The Re═O stretching frequency of $HReO(Cys)_2$ and its tetraphenyl phosphonium salt are observed at somewhat higher wavenumbers (969 and 977 cm^{-1}), in agreement with reported values for some well-characterized rhenium complexes having the ReO^{3+} core.[26]

The ^{1}H NMR spectra (Table 3) of technetium and rhenium chelates are similar except that the signals for the protons in the rhenium chelate appeared in general somewhat upfield in comparison to its technetium analogue. The high-resolution (400 MHz) spectra of $HReO(Cys)_2$ showed clearly the presence of four sets of signals. In the ^{1}H−^{1}H COSY spectrum, the downfield doublet at δ 4.26 was found to be coupled to the proton signal at δ 3.57, which in turn was correlated with the most upfield proton peak (δ 2.77), representing the signals for a $CH-CH_2$ unit. The signals corresponding to the other $CH-CH_2$ unit are therefore at δ 3.54 (1H) and 3.17−3.31 (2H). The δ 4.26 doublet with a small coupling constant can be safely assigned to the bridged ring methine proton; the C−H bond of the other methine shows a dihedral angle approximating 180° with one of the C−H's of the vicinal methylene group, and the corresponding coupling constant must be large.

The ^{13}C NMR spectral assignments are based on DEPT experimental results and comparison of the observed J_{C-P} values for the carbons belonging to the tetraphenylphosphonium unit with values reported for similar compounds in the literature.[28] The shifts are in the expected regions.

The HPLC result shows that the desired chelate is obtainable in high yield by reduction of pertechnetate in the presence of cystine over a concentration range 10^{-3} M (for ^{99}Tc) to less than 10^{-8} M (for ^{99m}Tc) of technetium. The elution patterns and retention times of these two technetium chelates on C_{18} columns were quite similar. Since $H^{99}TcO(Cys)_2$ had been correlated to the corresponding rhenium compound through their spectroscopic results, the structural data of the latter established from crystallographic results may be used to explain the biological property of ^{99m}Tc chelate.

The crystal structure of the rhenium chelate (Figure 1) shows the presence of two oxorhenium bis-cysteinate units **Re1** and **Re2** which are closely similar in their chemical structure. The metal−oxygen bond distances are 1.607(12) and 1.659(13) Å in these two units, within the range established for several well-characterized mono-oxo complexes of technetium and rhenium.[29,30] The metal−sulfur bond distances are in the range 2.275(4)−2.305(5) Å, also consistent with other technetium and rhenium thiolate complexes.[29−31]

The Re−N bond lengths [2.18(2)−2.19(2)Å] are similar to Tc−N bond lengths recorded for $HTcO(D\text{-Pen})_2$.[18] The latter have been considered to be unusually long due to the steric effect caused by the sixth coordinated oxygen atoms.[18] However, similar Tc−N bond lengths without sixth coordination site donation have been reported recently for some molecules. An interesting example is oxotechnetium *N*,*N*′-ethylene-bis-L,L-cysteine diethyl ester,[32] which shows two different Tc−N bond lengths arising out of the coordination of technetium with nondeprotonated amine (2.168 Å) and deprotonated amine (1.924 Å). Therefore, it may be concluded that there is nothing unusual in the Re−N bond lengths in $HReO(Cys)_2$ and the observed length is characteristic of technetium or rhenium coordination with nondeprotonated amines.

Except for one carboxylic acid group of one particular cysteine, those of the other three are deprotonated, and this explains the overall charge difference between the two chelate units. The protonated carboxyl group shows carbon−oxygen bond distances to be 1.31(3) and 1.22(3) Å for C33−O31 and C33−O32, and this clearly indicates the single- and double-bond characters of carbon−oxygen bonds of that carboxylic acid. Such a difference in bond length (0.09 Å) is not observed in the deprotonated carboxylates where it ranged between 0.01 and 0.05 Å.

The ^{99m}Tc analogue of oxorhenium bis-cysteinate exhibits ultrafast renal excretion[15] and inhibition of such excretion in the presence of hippurate analogues,[10] thereby indicating the presence of a pharmacophore consistent with Despopoulos model.[33] One of the requirements of such a model is that the reactive groups (oxo and carboxylates) should be on the same side of the molecule.[6] Considering the case of $HReO(Cys)_2$, the torsion angle O1−Re1−C13−O11 and O2−Re2−C43−O41 in the **Re1** and **Re2** units are +42(1)° and +57(2)°, comparable to the O1−C7−C9−O3 torsion angle (46°) present in *p*-amino hippurate,[34] which is the standard Despopoulos model.[33]

The other requirement for a substrate to behave physiologically as in the Despopoulos model is that the above reactive groups (oxo and carboxylates) should be separated by 3−4 Å or its simple multiples to interact with the renal anion transport system which has periodically recurrent recognition sites placed 3−4 Å apart.[33] The oxo−carboxylate distances in the **Re1**

(26) Davison, A.; Orvig, C.; Trop, H. S.; Sohn, M.; Depamphilis, B. V.; Jones, A. G. *Inorg. Chem.* **1980**, *19*, 1988.
(27) Davison, A.; Jones, A. G.; Orvig, C.; Sohn, M. *Inorg. Chem.* **1981**, *20*, 1629.
(28) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York, 1972; p 376.
(29) Bandoli, G.; Mazzi, U.; Roncari, E.; Deutsch, E. *Coord. Chem. Rev.* **1982**, *44*, 191.
(30) Melnick, M.; Van Lier, J. E. *Coord. Chem. Rev.* **1987**, *77*, 275.
(31) (a) Smith, J. E.; Byrne, E. F.; Cotton, F. E.; Sekutowski, J. C. *J. Am. Chem. Soc.* **1978**, *100*, 5571. (b) DePamphilis, B. V.; Jones, A. G.; Davis, M. A.; Davison, A. *J. Am. Chem. Soc.* **1978**, *100*, 5570.
(32) Edwards, D. S.; Cheesman, E. H.; Watson, M. W.; Maheu, L. J.; Nguyen, S. A.; Dimitre, L.; Nason, T.; Watson, A. D.; Walovitch, R. In *Technetium and Rhenium in Chemistry and Nuclear Medicine*; Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Cortina International: Verona, Italy, 1990; p 433.
(33) Despopoulos, A. *J. Theor. Biol.* **1965**, *8*, 163.
(34) Chakravarty, C.; Duttagupta, J. K. *Z. Kristallogr.* **1993**, *207*, 53.

[5.13(2)−6.19(2) Å] and **Re2** [5.32(2)-6.14(3) Å] units are approximately 2 times the carbonyl−carboxylate distance present in the hippurate side chain in the completely folded form (3 Å).[33] Therefore, the two rhenium units qualify as Despopoulos substrates from the above two considerations; a similar argument has recently been advanced to explain the renal properties of several ^{99m}Tc chelates from structural data of their ^{99}Tc and Re analogues.[35,36]

Another important biological property of the ^{99m}Tc analogue of oxorhenium bis-cysteinate is its fixation to kidney,[15] which is useful for the diagnosis of the morphological status of that organ. In this regard it resembles mercury chloromerodrin,[37] another Despopoulos model which can fix itself to thiol-rich kidney cell enzymes through ready ionization of its mercury center. A similar ionizability of the Tc−carboxylate bond is indicated by the longer than normal metal−carboxylate bond length [2.208(14) and 2.150(13) Å vs 1.750−1.985[30] Å] observed in the rhenium analogue, possibly because of the trans disposition of these bonds to the ReO core. A comparable arrangement of atoms resulted in an elongated Tc−O bond length [2.214(4) Å] in $HTcO(D\text{-Pen})_2$,[18] the ^{99m}Tc analogue of which also shows kidney retention like $H^{99m}TcO(Cys)_2$. On the other hand, the closely related renal agent ^{99m}Tc−*N*,*N*′-ethylenebiscysteinate, in which none of the carboxyl groups are coordinated to the trans TcO core, shows ultrafast renal excretion only but no renal retention.[8]

Acknowledgment. We thank Prof. K. Nag, Indian Association for Cultivation of Science, Calcutta, India, for many useful discussions. X-ray diffraction data were collected at All India Institute of Medical Sciences, New Delhi, India, and we are grateful to the authorities of that Institute for the same. Financial help from the Department of Atomic Energy and Department of Science and Technology, Government of India, is gratefully acknowledged.

Supporting Information Available: Tables of crystal data and structure refinement (S1), anisotropic thermal parameters (S2), complete list of bond lengths and angles (S3), and water contacts (up to 3.25 Å) (S4) (12 pages). X-ray crystallographic data for **2**, in CIF format, are available on the Internet only. Ordering and access information is given on any current masthead page.

IC970577Q

(35) Hansen, L.; Cini, R.; Taylor, A.; Marzilli, L. G. *Inorg. Chem.* **1992**, *31*, 2801.
(36) Hansen, L.; Marzilli, L. G.; Eshima, D.; Malveaux, E. J.; Folks, R.; Taylor, A. *J. Nucl. Med.* **1994**, *35*, 1198.
(37) Sprague, J. M. *Ann. N.Y. Acad. Sci.* **1958**, *71*, 328.