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(*S*)-(2-(2′-Pyridyl)ethyl)cysteamine and (*S*)-(2-(2′-Pyridyl)ethyl)-D,L-homocysteine as Ligands for the "*fac*-[M(CO)$_3$]$^+$" (M = Re, ^{99m}Tc) Core

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The reaction of *fac*-[NEt$_4$]$_2$[Re(CO)$_3$Br$_3$] with (*S*)-(2-(2′-pyridyl)ethyl)-cysteamine, **L$_1$**, in methanol leads to the formation of the cationic *fac*-[Re(CO)$_3$(NSN)][Br] complex, **1**, with coordination of the nitrogen of the pyridine, the sulfur of the thioether, and the nitrogen of the primary amine. When *fac*-[NEt$_4$]$_2$[Re(CO)$_3$Br$_3$] reacts with the homocysteine derivative (*S*)-(2-(2′-pyridyl)ethyl)-D,L-homocysteine, **L$_2$**, the neutral *fac*-Re(CO)$_3$(NSO) complex, **2**, is produced with coordination of the nitrogen of the primary amine, the sulfur of the thioether, and the oxygen of the carboxylate group, while the pyridine ring remains uncoordinated. The analogous technetium-99m complexes, **1′** and **2′**, were also prepared quantitatively by the reaction of **L$_1$** and **L$_2$** with the *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ precursor at 70 °C in water. Given that both (*S*)-(2-(2′-pyridyl)ethyl)cysteamine and homocysteine can be easily N- or S-derivatized by a bioactive molecule of interest, both the NSN or NSO ligand systems could be used to develop target-specific radiopharmaceuticals for diagnosis and therapy.

During the past two decades, much effort in the area of technetium and rhenium radiopharmaceuticals has been focused on the development of the chemistry of the MO(V)$^{3+}$ (M = Tc or Re) core.[1] The gentle reduction of Tc(VII) to Tc(I) under 1 atm of CO and the subsequent introduction of the low-valent [M(CO)$_3$(H$_2$O)$_3$]$^+$ (M = Tc or Re) synthons offered a new impetus in the development of diagnostic ^{99m}Tc(I) and therapeutic $^{186/188}$Re(I) radiopharmaceuticals,[2] and current literature is focusing on the chemistry and biology of M(I). The aqua ligands of the [M(CO)$_3$(H$_2$O)$_3$]$^+$ cation are labile and readily substituted by a variety of functional groups including amines, thioethers, imines, thiols, and phosphines.[3] Furthermore, the small size of the *fac*-[M(CO)$_3$]$^+$ core provides a convenient platform for the development of efficient radiopharmaceuticals.

Previous studies[4] on the coordination chemistry of the *fac*-[M(CO)$_3$]$^+$ core suggested that an ideal bifunctional chelating system should be tridentate because it forms complexes with more favorable pharmakokinetics compared to a bidentate one. It should also contain one or more amine functionalities, preferably aromatic N-heterocycles, in combination with a site suitable for further functionalization with biologically active molecules.

In the present Communication, we describe the products of the reaction of *fac*-[NEt$_4$]$_2$[Re(CO)$_3$Br$_3$] and *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ with the (*S*)-(2-(2′-pyridyl)ethyl) cysteamine, **L$_1$**, and (*S*)-(2-(2′-pyridyl)ethyl)-D,L-homocys-

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(1) Johannsen, B.; Spies, H. *Top. Curr. Chem.* **1996**, *176*, 77.

(2) (a) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, P. A.; Herrmann, W. A.; Artus, G.; Abram, U.; Kaden, T. A. *J. Organomet. Chem.* **1995**, *493*, 119. (b) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, A. P.; Abram, U.; Kaden, T. A. *J. Am. Chem. Soc.* **1998**, *120*, 7987. (c) Schibli, R.; Schwarzbach, R.; Alberto, R.; Ortner, K.; Schmalle, H.; Dumas, C.; Egli, A.; Schubiger, P. A. *Bioconjugate Chem.* **2002**, *13*, 750.

(3) (a) Alberto, R.; Schibli, R.; Waibel, R.; Abram, U.; Schubiger, A. P. *Coord. Chem. Rev.* **1999**, *190*−*192*, 901. (b) Schibli, R.; La Bella, R.; Alberto, R.; Garcia-Garayoa, E.; Ortner, K.; Abram, U.; Schubiger, P. A. *Bioconjugate Chem.* **2000**, *11*, 345. (c) Banerjee, S. R.; Levadala, M. K.; Lazarova, N.; Wei, L.; Valliant, J. F.; Stephenson, K. A.; Babich, J. W.; Maresca, K. P.; Zubieta, J. *Inorg. Chem.* **2002**, *41*, 6417. (d) Pietzsch, H.-J.; Gupta, A.; Reisgys, M.; Drews, A.; Seifert, S.; Syhre, R.; Spies, H.; Alberto, R.; Abram, U.; Schubiger, P. A.; Johannsen, B. *Bioconjugate Chem.* **2000**, *11*, 414. (e) Kramer, D.; Davison, A.; Davis W.: Jones, A. G. *Inorg. Chem.* **2002**, *41*, 6181.

(4) (a) Schibli, R.; Netter, M.; Scapozza, L.; Birringer, M.; Schelling, P.; Dumas, C.; Schoch, J.; Schubiger, A. P. *J. Organomet. Chem.* **2003**, *668*, 67. (b) Pak, J.K.; Benny, P.; Spingler, B.; Ortner, K.; Alberto, R. *Chem. Eur. J.* **2003**, *9*, 2053. (c) Van Staveren, D.; Mundwiler, S.; Hoffmanns, U.; Pak, J.; Spingler, B.; Metzler-Nolte, N.; Alberto, R. *Org. Biomol. Chem.* **2004**, *2*, 2593. (d) Vitor, R.; Alves, S.; Correia, J.; Paulo, A.; Santos, I. *J. Organomet. Chem.*, in press.

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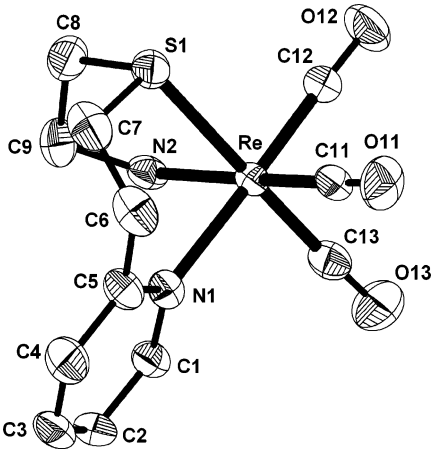
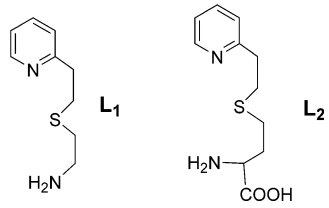


Figure 1. Molecular structure of **1** with the atomic labeling. Selected bond lengths (Å): Re−C(12) 1.912(9), Re−C(11) 1.919(11), Re−C(13) 1.922(10), Re−N(2) 2.204(7), Re−N(1) 2.223(6), Re−S(1) 2.474(2).

Chart 1. (*S*)-(2-(2′-Pyridyl)ethyl)cysteamine, $\mathbf{L_1}$, and (*S*)-(2-(2′-Pyridyl)ethyl)-D,L-homocysteine, $\mathbf{L_2}$



teine, $\mathbf{L_2}$, ligands (Chart 1). Both ligands have been prepared by pyridinethylation[5] of the corresponding thiol (cysteamine for $\mathbf{L_1}$ and homocysteine for $\mathbf{L_2}$) and characterized by spectroscopic methods.

The reaction of $[NEt_4]_2[Re(CO)_3Br_3]$ with the NSN tridentate $\mathbf{L_1}$ in methanol leads to the formation of the expected cationic $[Re(CO)_3(NSN)]Br$ complex, **1** (Figure 1).[6] Strong bands at 2025, 1934, and 1912 cm^{-1} in its IR spectrum indicate the presence of three carbonyl ligands in a facial arrangement. The ^1H NMR spectra of the complex (Table 1) show the typical differentiation of the chemical shifts of the geminal protons of the chelated NSN backbone, which become diastereotopic after coordination to the metal core. In addition, the chemical shift of the proton on C-1 shows the characteristic downfield shift of approximately 0.5 ppm, compared to the free ligand, induced by the coordination of the nitrogen of the pyridine ring. X-ray analysis[7] shows that the nitrogen of the aromatic amine, the sulfur of the thioether, and the nitrogen of the primary amine are facially coordinated to the metal, forming a cationic complex with one five-membered ring and one six-membered ring (Figure 1). At the technetium-99m level, the corresponding complex *fac*-$[^{99m}Tc(CO)_3(NSN)]^+$, **1′**, was obtained almost quantitatively (>90%) by heating the *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor at 70 °C in water and in the presence of a 10^{-5} M concentration of $\mathbf{L_1}$.[8] Its structure was established by chromatographic comparison with the prototype rhenium complex using high-performance liquid chromatographic techniques. The radioactive complex was stable against the histidine and cysteine challenge.[3b]

Table 1. ^1H and ^{13}C NMR Chemical Shifts for Complexes **1** and **2** in DMSO-d_6 at 25 °C[a]

	1	2		1	2
H-1	9.00	8.53	C-1	155.95	149.00
H-2	7.57	7.26	C-2	125.56	121.56
H-3	8.13	7.75	C-3	141.11	136.54
H-4	7.73	7.36	C-4	127.62	123.25
H-6	3.45, 2.79	2.98	C-5	160.81	158.03
H-7	3.53, 2.32	3.87	C-6	27.38	37.42
H-8	2.91, 2.55	2.68, 2.60	C-7	38.90	30.07
H-9	3.17, 1.60	2.20, 2.02	C-8	37.90	27.60
H-10	5.23, 5.50	3.57	C-9	43.15	26.90
NH		5.91, 4.97	C-10		51.80
			C-11		182.92
			CO	191.70	190.43
			CO	191.64	193.84
			CO	191.12	194.11

[a] Numbering of the atoms is according to the structures shown in Figures 1 and 2.

The reaction, however, of *fac*-$[NEt_4]_2[Re(CO)_3Br_3]$ with the $\mathbf{L_2}$ ligand, which contains a donor-atom set similar to that of $\mathbf{L_1}$ along with a carboxylate group, did not lead to the expected NSN complex. Instead, the neutral NSO complex **2** (Figure 2) was isolated as a colorless crystalline product.[9,10] The IR spectrum of **2** shows the typical facial tricarbonyl pattern with intense bands at 2021, 1905, and 1884 cm^{-1}. Furthermore, a strong band at 1653 cm^{-1} indicates that the oxygen from the carboxylic acid participates in the coordination sphere. In the ^1H NMR spectrum of **2** (Table 1), the chemical shifts of the protons on C-6 and C-7 of the pyridylethyl moiety, as well as that of the pyridine ring, remain essentially unchanged compared to those of the free ligand. X-ray analysis[11] shows that the nitrogen of the

(5) (a) Fish, R. H.; Windle, J. J.; Gaffield, W.; Scherer, J. R. *Inorg. Chem.* **1973**, *12* 855. (b) Alsfasser, R.; vanEldik, R. *Inorg. Chem.* **1996**, *35*, 628.

(6) Synthesis of $[Re(CO)_3(NSN)]Br$, **1**: A solution of the ligand $\mathbf{L_1}$·HCl (0.021 mg, 0.1 mmol) in methanol (2 mL) and 0.1 mL of NaOH (1 N) were added to a solution of $[NEt_4]_2[Re(CO)_3Br_3]$ (0.077 g, 0.1 mmol) in methanol (15 mL). The mixture was refluxed for 2 h. The solution was cooled to room temperature, and the solvent was evaporated to dryness, affording a colorless residue. Colorless crystals suitable for X-ray crystallography were obtained by slow evaporation of a CH_2Cl_2/MeOH solution. Yield 47 mg (89%). IR (KBr, cm^{-1}): 3193 (s), 3114 (m), 3035 (m), 2933 (s), 2025 (vs, CO), 1934 (vs, CO), 1912 (vs, CO), 1648 (m), 1594 (m), 1486 (m), 1451 (m), 1416 (w), 1320 (m), 1293 (w), 1260 (w), 1174 (m), 1162 (m), 1103 (w), 1073 (w), 1050 (w), 1020 (w), 990 (m), 923 (m), 840 (m), 778 (s), 643 (w), 617 (w), 575 (w), 532 (m), 517 (w), 575 (w), 532 (w), 516 (w), 477 (w). Anal. Calcd for $C_{12}H_{14}BrN_2O_3ReS$: C, 27.07; H, 2.65; N, 5.26%. Found: C, 26.90; H, 2.31; N, 5.55%. Mass spectrum (ESI-MS) *m*/*z*: $[M + H]^+$ 453.1

(7) Crystal data for **1**: $C_{12}H_{14}BrN_2O_3ReS$, $M = 532.42$, monoclinic, space group $P2_1/n$, $a = 9.981(3)$ Å, $b = 14.230(3)$ Å, $c = 12.264(3)$ Å, $\beta = 112.75(1)°$, $V = 1606.4(7)$ Å^3, $Z = 4$, μ(Mo Kα) = 10.188 mm^{-1}, 2965 reflections measured, 2829 unique ($R_{int} = 0.0399$) that were used in all calculations, 223 parameters refined. Final *R* indices for 2527 reflections with $I > 2\sigma(I)$ R1 = 0.0399, wR2 = 0.1069; for all data R1 = 0.0456, wR2 = 0.1117. Data were measured at 298 K on a Crystal Logic Dual Goniometer diffractometer using graphite-monochromated Mo radiation. Structure solution was effected with SHELXS-97 and refinement with SHELXL-97 using WinGX.[14] Hydrogen atoms were located by difference maps and were refined isotropically, except those on C2 and C6, which were introduced at calculated positions as riding on bonded atoms. All non-H atoms were refined anisotropically.

(8) Preparation of *fac*-$[^{99m}Tc(CO)_3(NSN)]^+$, **1'**: Two hundred microliters of a 2×10^{-5} M stock solution of $\mathbf{L_1}$ was added to a solution of $[^{99m}Tc(H_2O)_3(CO)_3]$ (200 μL, 3−5 mCi). The vial was sealed and heated at 70 °C for 30 min. The formation of the complex was checked by RP-HPLC (yield 90%).

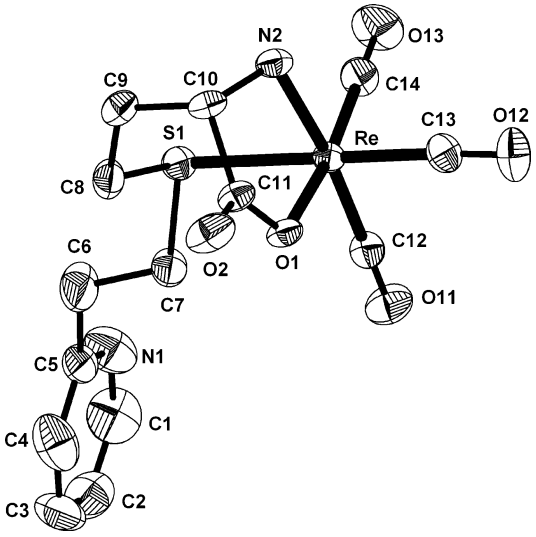


Figure 2. Molecular structure of **2** with the atomic labeling. Selected bond lengths (Å): Re−C(14) 1.910(5), Re−C(13) 1.919(5), Re−C(12) 1.924(5), Re−O(1) 2.158(3), Re−N(2) 2.199(4), Re−S(1) 2.506(1).

primary amine, the sulfur of the thioether, and the oxygen of the carboxylate group are facially coordinated to the metal, forming one five-membered ring (Re−O1−C11−C10−N2), one six-membered ring (Re−S1−C8−C9−C10−N2), and one seven-membered ring (Re−S1−C8−C9−C10−C11−O1), while the nitrogen of the pyridine ring remains free (Figure 2). The corresponding $^{99m}Tc(CO)_3(NSO)$ complex, **2′**, was obtained in a radiochemical yield of over 90% in the presence of a 10^{-4} M concentration of $\mathbf{L_2}$.[12] As in the case of **1′**, the structure of **2′** was established by HPLC comparison to the prototype rhenium complex. Complex **2′** was also stable against the histidine and cysteine challenge.[3b]

This unexpected product belongs to the newly introduced NSO class of complexes in the field of technetium and rhenium radiopharmaceuticals.[13] Our data demonstrate that the donor-atom system (N-primary amine)(S-thioether)-(O-carboxylate) system is preferred over the (N-primary amine)(S-thioether)(N-pyridine) system because, at both the rhenium and technetium-99m levels, only the $M(CO)_3(NSO)$ complex is generated and pyridine never coordinates under the conditions studied. Because the pyridine nitrogen is considered to be a very efficient donor atom for the $M(CO)_3^+$ core, it becomes obvious that NSO coordination leads to more stable complexes. Higher stability could be rendered by the formation of three (five-, six-, and seven-membered) bridged chelate rings in complex **2**, as well as by the neutrality of the overall complex.

In conclusion, two new high-affinity tridentate NSN ($\mathbf{L_1}$) and NSO ($\mathbf{L_2}$) ligands that form stable cationic and neutral complexes, respectively, with the $[M(CO)_3(H_2O)_3]^+$ (M = Tc or Re) synthons, even at very low concentration, are presented. These ligands can be easily conjugated with a target-specific bioactive molecule: $\mathbf{L_1}$ through the nitrogen of the primary amine and $\mathbf{L_2}$, being a homocysteine derivative, through the sulfur of the thioether. This conjugation can be either direct or through the mediation of an appropriate linker bearing a terminal amine or carboxylic acid, suitable for further derivatization. Thus, both new NSN and NSO donor-atom systems are promising model compounds for the development of target-specific radiopharmaceuticals for diagnosis and therapy.

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Supporting Information Available: Synthesis and complete characterization of ligands $\mathbf{L_1}$ and $\mathbf{L_2}$, a table with selected bond distances and angles for compounds **1** and **2**, and full crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Synthesis of $[Re(CO)_3(NSO)]$, **2**: A solution of the ligand $\mathbf{L_2}$ (0.024 g, 0.1 mmol) in methanol (2 mL) and 0.1 mL of NaOH (1 N) were added, with stirring, to a solution of $[NEt_4]_2[Re(CO)_3Br_3]$ (0.077 g, 0.1 mmol) in methanol (15 mL). The mixture was refluxed for 2 h. The solution was cooled to room temperature, and the solvent was evaporated to dryness, affording a colorless residue. Colorless crystals suitable for X-ray crystallography were obtained by slow evaporation of a MeOH/H_2O solution. Yield 29 mg (57%). IR (KBr, cm^{-1}): 3240 (m), 3158 (m), 2021 (vs, CO), 1905 (vs, CO), 1884 (vs, CO), 1656 (s, C═O), 1591 (m), 1470 (m), 1433 (m), 1390 (m), 1322 (m), 1195 (m), 995 (m), 916 (m), 795 (m), 747 (m), 650 (m), 597 (w), 526 (w), 502 (w). mp: 204 °C. Anal. Calcd for $C_{14}H_{15}N_2O_5ReS\cdot 2MeOH$: C, 33.50; H, 4.04; N, 4.88%. Found: C, 33.92; H, 4.37; N, 5.37%. Mass spectrum (ESI-MS) *m/z*: $[M + H]^+$ 511.3. It should be noted that the same product was obtained when the reaction was carried out in the absence of NaOH and that no change in product composition was observed after prolonged reflux of the reaction mixture.

(10) Because D,L-homocysteine was used for the preparation of $\mathbf{L_2}$ and the thioether group is a prochiral center, more than one stereoisomers are expected after the coordination of the metal. The existence of one single product peak in HPLC can be explained either by the identical retention times of the stereoisomers or by the preferential formation of one.

(11) Crystal data for **2**: $C_{14}H_{15}N_2O_5ReS$, $M = 509.54$, monoclinic, space group $P2_1/a$, $a = 11.066(5)$ Å, $b = 8.394(3)$ Å, $c = 17.927(8)$ Å, $\beta = 97.56(2)°$, $V = 1651(1)$ Å^3, $Z = 4$, μ(Mo Kα) = 7.513 mm^{-1}, 3067 reflections measured, 2899 unique ($R_{int} = 0.0201$) that were used in all calculations, 268 parameters refined. Final *R* indices for 2697 reflections with $I > 2\sigma(I)$ R1 = 0.0247, wR2 = 0.0656; for all data R1 = 0.0270, wR2 = 0.0671. Data were measured at 298 K on a Crystal Logic Dual Goniometer diffractometer using graphite-monochromated Mo radiation. Structure solution was effected with SHELXS-97 and refinement with SHELXL-97 using WinGX.[14] Hydrogen atoms were located by difference maps and were refined isotropically. All non-H atoms were refined anisotropically.

(12) **2′** was synthesized as described above for **1′** in ref 8.

(13) Alberto, R.; Pak, J. K,; vanStaveren, D.; Mundwiler, S.; Benny, P. *Biopolymers* **2004**, *76*, 324.

(14) (a) Sheldrick, G. M. *SHELXS-97: Program for Crystal Structure Solution*; University of Gottingen: Gottingen, Germany, 1997. (b) Sheldrick, G. M. *SHELXL-97: Program for Crystal Structure Refinement*; University of Gottingen: Gottingen, Germany, 1997. (c) Farrugia, L. J. *J. Appl. Crystallogr.* **1999**, *32*, 837.