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Synthesis of Oxorhenium(V) and Oxotechnetium(V) [SN(R)S/S] Mixed Ligand Complexes Containing a Phenothiazine Moiety on the Tridentate SN(R)S Ligand

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Abstract—Two oxorhenium and two oxotechnetium [SN(R)S/S] mixed ligand complexes bearing the phenothiazine moiety on the tridentate ligand SN(R)S have been synthesized and characterized. The corresponding complexes at tracer level (^{99m}Tc) have also been prepared.

Introduction

The needs of modern nuclear medicine for molecular probes of high specificity and selectivity prompt the development of new classes of radioligands aiming at specific receptor sites. Due to the fact that ^{99m}Tc is inexpensive, readily available, easy to image and has decay energies that minimize the dose burden to patients, many efforts are focusing on developing receptor imaging agents based on ^{99m}Tc.[1,2]

The '3 + 1' concept is an interesting approach for the preparation of neutral, lipophilic, small-size oxotechnetium mixed ligand complexes of the general type $TcOL^1L$.[3] The preparation of '3 + 1' complexes requires the simultaneous action of a dianionic tridentate ligand L^1H_2, containing the SSS, SOS or SN(R)S donor atom set and a monodentate thiol coligand, LH, on a suitable oxotechnetium(V) precursor. Analogous oxorhenium complexes are commonly synthesized since rhenium displays similar coordination chemistry with technetium. Very often rhenium is used as a non-radioactive alternative to technetium for structural characterization.

The '3 + 1' has been widely applied in the development of novel diagnostic radiopharmaceuticals.[3] A great number of ^{99m}TcO[SN(R)S][S] derivatives have been synthesized and evaluated in experimental animals as potential brain perfusion agents.[4,5] Furthermore, the incorporation of the appropriate receptor seeking group, mainly on the monodentate thiol, LH, led to the generation of receptor specific complexes, which were evaluated as SPECT imaging agents of the 5-HT_{2A} serotonin receptor[6,7] or the dopamine transporter system in brain.[8,9]

Phenothiazines are neuroleptic drugs and their clinical effect is exerted mainly through the antagonism of dopamine receptors in brain.[10] Thus, the synthesis of ^{99m}Tc complexes incorporating in their structure the phenothiazine moiety is of great interest for the development of potential agents for imaging dopamine receptors.

In this article, we report the synthesis of two novel SN(R)S tridentate ligands (**4** and **5**, Scheme 1) in which the phenothiazine and 2-chlorophenothiazine have been incorporated to the SN(R)S backbone.

Using the tridentate ligands **4** and **5** as well as *p*-methylthiophenol as a monodentate ligand, four novel mixed ligand complexes (**6**–**9**, Scheme 2) of the general

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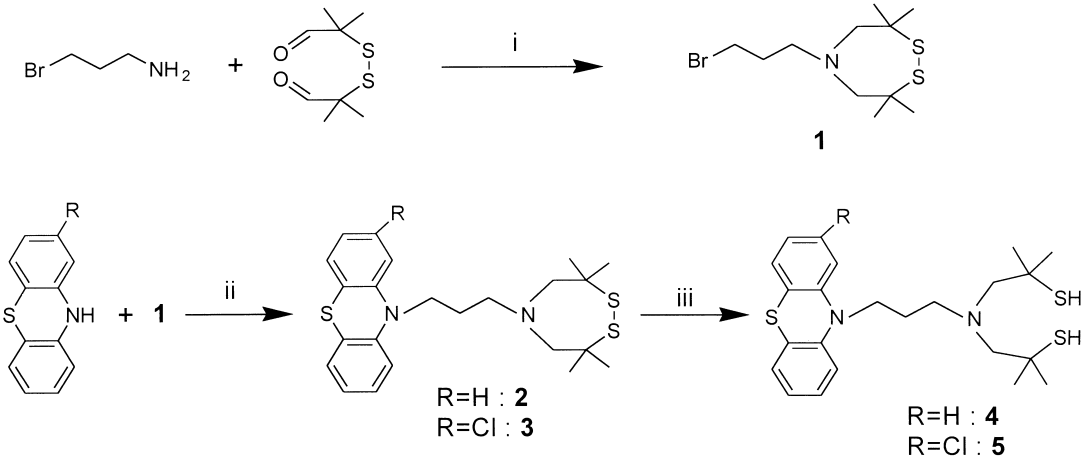
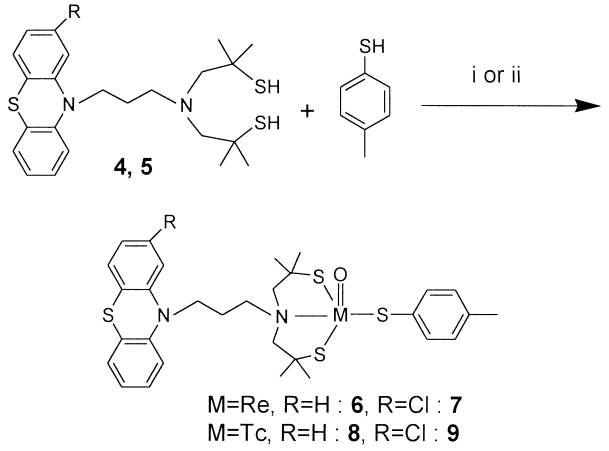


Scheme 1. (i) $NaCNBH_3$, MeOH, pH: 6 glacial CH_3COOH, rt, 24 h, (yield 70%); (ii) dry DMF, powder NaOH, rt, 1 h, (yield 80%); (iii) $LiAlH_4$, dry THF, reflux 18 h (yield 80%).



Scheme 2. Preparation and structures of complexes studied: (i) $ReOCl_3(PPh_3)_2$ for **6** and **7**; (ii) ^{99}TcO-gluconate for **8** and **9**.

formula MO[SN(R)S][S] (M = Re or ^{99}Tc) have been synthesized and fully characterized. The corresponding ^{99m}Tc complexes have also been prepared.

Results and Discussion

The synthesis of the tridentate ligands **4** and **5** is presented in Scheme 1. Reductive amination of 2,2′-dithiobis(2-methylpropanal) with 3-bromopropylamime in a molar ratio 1:1 in the presence of $NaCNBH_3$ at pH = 6 results in the heterocyclic bromide, 5-(3-bromopropyl)-3,3,7,7-tetramethyl[1,2,5]perhydrodithiazepine, **1**. Alkylation of phenothiazine or 2-chlorophenothiazine with **1** in DMF in the presence of solid NaOH gives the heterocyclic bisulfide, **2** or **3**, respectively. Finally, reduction of **2** or **3** with $LiAlH_4$ in dry THF yields the desired tridentate ligand, **4** or **5**. All the products were purified through flash column chromatography and characterized by IR and ^{1}H NMR spectroscopy.

The oxorhenium complexes **6** and **7** were prepared by ligand exchange reaction using $ReOCl_3(PPh_3)_2$ as precursor and equimolar quantities of **4** or **5** and *p*-methylthiophenol (Scheme 2).[11] The corresponding oxotechnetium complexes **8** and **9** were synthesized in a similar manner but using ^{99}TcO-gluconate as precursor (Scheme 2).[12] All complexes were extracted in dichloromethane and were isolated as crystalline products by slow evaporation from a solution of dichloromethane and methanol. The complexes were characterized by elemental analysis, IR, and NMR spectroscopy. ^{1}H and ^{13}C NMR data for complexes **6** and **7** are presented in Table 1. The complexes are neutral, lipophilic and stable in the solid state as well as in organic solutions as shown by HPLC and NMR.

Complexes **6** and **7** were also characterized by X-ray crystallography.[13] For both complexes the coordination geometry about rhenium can be described as trigonally distorted square pyramidal ($\tau = 0.48$ and $\tau = 0.31$ for **6** and **7**, respectively).[14] The basal plane is defined by the SNS donor atoms of the tridentate ligand and the sulfur of the *p*-methylthiophenol while the oxygen atom of the oxorhenium core occupies the apical position (Fig. 1). It is worth noting that the propyl chain attached to the nitrogen of the tridentate ligand adopts its extended configuration in **7** (C5–C6–C7–N2 = 176°) while in **6** it exists in its compressed form (C5–C6–C7–N2 = 69°). The configuration of the propyl chain is *syn* with respect to the oxygen of the oxorhenium core. The NMR studies demonstrate that **8** and **9** adopt the *syn* configuration as well.

The synthesis of the complexes was successfully transferred at the technetium tracer level (^{99m}Tc) using ^{99m}TcO-glucoheptonate as precursor.[15] Due to the coordinating power of the SN(R)S/S system, the reaction was fast and nearly quantitative as determined by organic solvent extraction of the aqueous reaction mixture. Aliquots of the organic extracts were analyzed by HPLC and in all cases recovery from the column was monitored and found to be quantitative.

The structural analogy between the ^{99m}Tc complexes prepared at tracer level with the respective oxorhenium and oxotechnetium-99 complexes prepared in macroscopic amounts was established by comparison of the

Table 1. ^{1}H and ^{13}C NMR chemical shifts ($CDCl_3$, 25 °C) for complexes **6** and **7**. Numbering of the atoms is according to the crystallographic structure shown in Figure 1

	6	7		6	7
H-2 (H-3) *endo*[a]	3.36	3.37	C-1 (C-4)	61.45	61.47
H-2 (H-3) *exo*[a]	2.31	2.32	C-2 (C-3)	76.29	76.30
H-5	4.09	4.08	C-5	63.34	63.17
H-6	2.45	2.43	C-6	21.92	21.67
H-7	3.95	3.92	C-7	44.25	44.41
H-9	6.87	6.83	C-8	144.75	146.01
H-10	7.23	—	C-9	115.44	115.84
H-11	6.99	7.03	C-10	128.00	133.36
H-12	7.24	7.11	C-11	123.19	123.62
H-15	7.24	7.24	C-12	127.33	128.44
H-16	6.99	6.97	C-13	126.55	124.95
H-17	7.23	7.25	C-14	126.55	126.17
H-18	6.87	6.88	C-15	127.33	128.05
H-21 (H-25)	7.50	7.50	C-16	123.19	123.02
H-22 (H-24)	7.18	7.18	C-17	128.00	127.56
H-26	2.40	2.40	C-18	115.44	115.72
H-27 (H-29)	1.68	1.68	C-19	144.75	144.03
H-28 (H-30)	1.45	1.47	C-20	148.66	148.63
			C-21 (C-25)	133.21	133.19
			C-22 (C-24)	128.74	128.74
			C-23	136.14	136.15
			C-26	21.20	21.19
			C-27 (C-29)	30.85	30.85
			C-28 (C-30)	31.30	31.31

[a]*endo* Protons are those directed towards the oxygen of the oxorhenium core; *exo* are those directed away from the oxygen of the oxorhenium core.

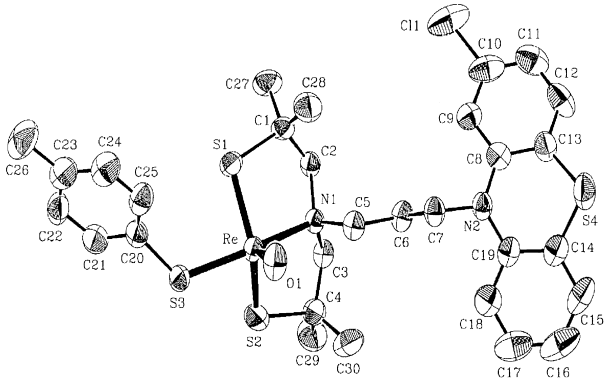


Figure 1. ORTEP drawing of **7**, showing with 50% probability thermal ellipsoids. Selected bond lengths (Å) and angles (°): Re–O(1) 1.682(6), Re–N(1) 2.234(5), Re–S(1) 2.273(2), Re–S(2) 2.290(3), Re–S(3) 2.287(2), O(1)–Re–N(1) 102.4(2), O(1)–Re–S(1) 112.1(3), N(1)–Re–S(1) 83.7(2), O(1)–Re–S(3) 105.3(2), N(1)–Re–S(3) 151.5(2), S(1)–Re–S(3) 92.1(1), O(1)–Re–S(2) 114.9(3), N(1)–Re–S(2) 82.7(2), S(1)–Re–S(2) 132.9(1), S(3)–Re–S(2) 79.7(1).

HPLC retention times, adopting parallel radiometric and photometric detection. Thus, by co-injection of the oxorhenium (**6** and **7**), oxotechnetium (**8** and **9**) and the corresponding oxotechnetium-99m complexes, identical retention times are exhibited revealing their structural analogy.

In this study, the synthesis of stable, neutral, and lipophilic oxorhenium and oxotechnetium complexes carrying the phenothiazine moiety was successfully demonstrated using the '3 + 1' concept. The in vivo and in vitro pharmacological evaluation of the technetium-99m complexes is in progress.

References and Notes

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11. Synthesis of oxorhenium complexes **6** and **7**. Exemplified for **7**: To a 0.5 M AcONa solution in MeOH (4 mL), $ReOCl_3(PPh_3)_2$ (166.6 mg, 0.2 mmol), tridentate ligand, **5**, 1-[3-(2-chloro-10*H*-10-phenothiazinyl)propyl] (2-methyl-2-sulfanylpropyl)amine-2-methyl-propanethiol (93 mg, 0.2 mmol) and *p*-methylthiophenol (24.8 mg, 0.2 mmol) are suspended under stirring. The mixture is refluxed until a clear solution forms. After addition of CH_2Cl_2 the organic phase is washed with H_2O, dried, and purified through column chromatography. Dark green crystals separate by slow evaporation from CH_2Cl_2/MeOH.

Complex **6**. IR (KBr, cm^{-1}): 957 Re=O, 807. Anal. calcd for: $C_{30}H_{37}N_2S_4ORe$: C, 47.66; H, 4.93; N, 3.71; S, 16.96. Found: C, 47.52; H, 5.17; N, 3.37; S, 17.19. NMR data are given in Table 1.

Complex **7**. IR (KBr, cm^{-1}): 955 Re=O, 807. Anal. calcd for: $C_{30}H_{36}N_2S_4ClORe$: C, 45.58; H, 4.59; N, 3.54; S, 16.22. Found: C, 45.51; H, 5.05; N, 3.38; S, 16.59. NMR data are given in Table 1.

12. Synthesis of oxotechnetium complexes **8** and **9**. Exemplified for **9**: General method. A solution of stannous chloride (45 mg, 0.24 mmol) in HCl (1.0 mL, 1 N) was added dropwise to an aqueous solution of $NH_4{}^{99}TcO_4$ (36.2 mg, 0.2 mmol) containing $^{99m}TcO_4^-$ (0.1 mL, 0.5 mCi) and sodium gluconate (200 mg) to obtain the ^{99}Tc-gluconate. The pH of the solution was adjusted to 7.5 with NaOH (1 N). This solution was added to a mixture of the tridentate ligand 1-[3-(2-chloro-10*H*-10-phenothiazinyl)propyl](2-methyl-2-sulfanylpropyl)amine-2-methyl-propanethiol (93 mg, 0.2 mmol) and the monodentate ligand *p*-methylthiophenol (24.8 mg, 0.2 mmol) dissolved in 3 mL ethanol. The solution was stirred for 60 min at 55 °C, and extracted with dichloromethane (3×10 mL). The organic phase was separated, dried over $MgSO_4$ and filtered. The volume of the solution was reduced to 5 mL and then 5 mL of methanol were added. Slow evaporation of the solvents at room temperature afforded the product as dark red crystals.

Complex **8**. IR (KBr, cm^{-1}): 930 Tc=O, 807, 751. Anal. calcd for: $C_{30}H_{37}N_2S_4OTc$: C, 53.95; H, 5.58; N, 4.19; S, 19.20. Found: C, 54.11; H, 5.35; N, 4.35; S, 19.52. ^1H NMR (ppm, $CDCl_3$): 7.49, 7.15 (4H, m, 4-CH_3-*phenylthiol)*, 7.23–6.88 (8H, m, *phenothiazine*), 4.18 (2H, m, $NCH_2CH_2CH_2$phenothiazine),

3.97 (2H, m, $NCH_2CH_2CH_2$phenothiazine), 3.42, 2.27 (4H, d, $SC(CH_3)_2CH_2N$), 2.44 (2H, m, $NCH_2CH_2CH_2$phenothiazine), 2.41 (3H, s, 4-CH_3-phenylthiol), 1.73, 1.55 (6H, s, $SC(CH_3)_2CH_2N$).

Complex **9**. IR (KBr, cm^{-1}): 928 Tc=O, 807. Anal. calcd for: $C_{30}H_{36}N_2S_4ClOTc$: C, 51.31; H, 5.17; N, 3.99; S, 18.26. Found: C, 51.58; H, 5.01; N, 3.75; S, 18.51. ^1H NMR (ppm, $CDCl_3$): 7.49, 7.15 (4H, m, 4-CH_3-*phenylthiol)*, 7.24–6.84 (8H, m, *phenothiazine*), 4.18 (2H, m, $NCH_2CH_2CH_2$phenothiazine), 3.96 (2H, m, $NCH_2CH_2CH_2$phenothiazine), 3.43, 2.28 (4H, d, $SC(CH_3)_2CH_2N$), 2.44 (2H, m, $NCH_2CH_2CH_2$phenothiazine), 2.39 (3H, s, 4-CH_3-phenylthiol), 1.72, 1.36 (6H, s, $SC(CH_3)_2CH_2N$).

13. Crystal data for **6**: $C_{30}H_{37}N_2OReS_4$, monoclinic, space group $P2_1$, $a=12.974(5)$, $b=12.621(5)$, $c=9.924(4)$ Å, $\beta=90.06(1)^\circ$, $V=1625(1)$ Å^3, and $Z=2$; 5422 measured reflections, 4940 with $I>2\sigma(I)$, 435 refined parameters, $R1=0.0299$, $wR2=0.0776$. Intensity measurement: Crystal Logic Dual Goniometer diffractometer, MoK_α radiation, graphite monochromator, ω–2θ scan, $2\theta_{max}=48^\circ$, $T=25\,^\circ$C. Crystal data for **7**: $C_{30}H_{36}ClN_2OReS_4$, monoclinic, space group $P2_1/n$, $a=14.202(7)$, $b=13.957(7)$, $c=16.646(9)$ Å, $\beta=103.42(1)^\circ$, $V=3209(3)$ Å^3, and $Z=4$; 5668 measured reflections, 4653 with $I>2\sigma(I)$, 446 refined parameters, $R1=0.0464$, $wR2=0.1133$. Intensity measurement: Crystal Logic Dual Goniometer diffractometer, MoK_α radiation, graphite monochromator, ω–2θ scan, $2\theta_{max}=50^\circ$, $T=25\,^\circ$C. The structures were solved by direct methods using SHELXS-86 and refined by full-matrix least-squares techniques using SHELXL-93. Non-hydrogen atoms were refined anisotropically while H-atoms (except those on methyl groups which were located on calculated positions as riding on bonded atoms) were located by difference maps and were refined isotropically. Atomic coordinated, bond angles, bond lengths and thermal parameters have been deposited at the Cambridge Crystallographic Data Center in CIF file. CCDC number: 153161 and 153162.

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15. Labelling with ^{99m}Tc was carried out by reacting equimolar quantities (0.02 mmol) of the tridentate and monodentate ligands with a ^{99m}Tc-glucoheptonate precursor solution in 50:50 EtOH/H_2O. Yields: 85%.