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NICOTINAMIDE-SUBSTITUTED COMPLEXES AS REDOX MARKERS

1. SYNTHESIS AND UV INVESTIGATION OF RHENIUM AND TECHNETIUM MIXED- LIGAND SYSTEMS

T. Kniess*[1], H. Spies[1], W.Brandau[2], B.Johannsen[1]

[1] Forschungszentrum Rossendorf, Institut für Bioanorganische und Radiopharmazeutische Chemie,
PF 510119, D-01314 Dresden, Germany
[2] Klinik für Nuclearmedizin Universitätsklinikum Essen, Hufelandstr. 55, D-45147 Essen, Germany

SUMMARY

In order to develop a redox delivery system for Tc radiotracers the synthesis of new nicotinamide and pyridinium salt-substituted Re(V) mixed-ligand complexes of the '3+1' type is described. The complexation of $^{99/99m}$Tc with these ligands was studied by thin layer chromatography and UV-VIS spectroscopy. The reduction of the pyridinium salt with sodium dithionite to the 1,4-dihydropyridine was carried out on the free ligand and on the complex. The stability of the 1,4-dihydropyridines was observed by UV-VIS spectrometry.

Key words: nicotinamide, pyridinium-salt, 1,4-dihydropyridine, Re- and Tc-complexes radiopharmaceuticals

INTRODUCTION

Radiopharmaceutical research is aimed at present at tracers whose ultimate distribution is determined by specific biochemical or receptor-binding interactions (1). One of the challenging tasks is the development of radiotracers able to indicate and to image the various redox processes occurring in the organism. Such compounds whose tissue uptake and retention rely on enzymatic reduction are the 2-nitroimidazoles, among them the iodo-labeled misonidazole (2,3). The working principle of substituted 2-nitroimidazoles is, that they will be reduced in the organism enzymatically and accumulated and retained in hypoxic tissue and in tumour cells.

The availability of redox-active radiotracers based on technetium-99m would be an enormous enrichment of diagnostic nuclear medicine. Recent efforts led to technetium complexes of 2-nitroimidazoles, the first group of redox-active Tc radiotracers to be studied in vitro and in vivo (4,5).

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*) To whom all correspondence should be addressed.

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An alternative approach to important biological redox systems is based on the work of Bodor and co-workers (6,7). They used the NAD$^+$/NADH equilibrium as a model to develop a redox delivery system to move polar drugs through the blood-brain barrier (BBB) (8-10). The reduced lipophilic dihydropyridine linkèd with the drug penetrates the BBB. In the brain the reduced form will be oxidized to the pyridinium salt and after hydrolysis the free polar active compound is trapped .

To exploit this drug delivery system for the design of redox-active Tc tracers we looked for technetium and rhenium chelates suitable for coupling to a pyridinium salt or a dihydropyridine moiety - both of which are constituents of the NAD$^+$/NADH redox pair. To our knowledge such types of complexes are unknown .

In the present paper we report the synthesis of neutral rhenium and technetium chelates bearing either a pyridinium or a dihydropyridine functionality as potential metal-based redox tracers. Nicotinamide was converted into the N-(2-mercaptoethyl) derivative which directly or after quaternization to 3(2-mercaptoethyl-carbamoyl)-1-methyl-pyridinium iodide binds to an oxometal core. The remaining coordination sites were occupied by the tridentate bis(2-mercaptoethyl)-sulphide (HSSSH) resulting in neutral „3+1' mixed-ligand complexes. (11). Additionally, the reduction of the pyridinum salt to the dihydropyridine group with sodium dithionite is documented for the free ligand and for the complex . UV-VIS spectrometric investigations were carried out concerning the characterization and the stability of the dihydropyridines.

EXPERIMENTAL

General: Melting points were obtained on a Boetius apparatus and were uncorrected. Infrared spectra were determined on a Specord M 80 spectrophotometer and UV-VIS spectra provided by a Specord M 40 spectrometer (Carl Zeiss Jena). The NMR spectra were recorded on an FT-Spectrometer DC 300 (Bruker) in $CDCl_3$ or DMSO-d_6 . Elemental analyses were performed on a LECO CHNS932 Elemental Analyzer.

Materials and methods: Methyl nicotinate, ethanolamine, thionyl chloride, 3-thia-1,5-pentanedithiol and sodium dithionite were obtained from Fluka (Buchs). $Na_3PO_3S \times 12H_2O$ was synthesized according to a literature procedure (12). Methyl iodide was obtained from Riedel de Haen (Seelze). The precursor chloro(3-thiapentane-1,5-dithiolato)oxorhenium(V) was prepared according to the

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literature (13). Stannous chloride dihydrate was obtained from Aldrich (Steinheim). Sodium pertechnetate in saline solution was eluted from a ^{99}Mo/^{99m}Tc generator purchased from Mallinkrodt (Petten).

Thin-layer chromatography (TLC) was performed on silufol plates Kavalier (Prag). Radioactive spots in TLC were scanned by a TRACEMASTER 20 (Berthold). The RP-18 glass-backed plates were obtained from Merck (Darmstadt).

Synthesis

3(2-hydroxyethyl-carbamoyl)pyridine 2 : 41.1 g (0.3 mol) methyl nicotinate 1 were heated with 18,3 g (0.3 mol) ethanolamine to a temperature of 150 - 160 °C and the resulting methanol was collected by distillation. After 3 hours the heating bath was removed and the product solidified as a yellowish mass. Recrystallization from 1,2-dichlorethane gave the pure compound but the crude product was used for the next step. yield: 49.8 g (0.29 mol, 98 %), M.p. 82 - 86 °C. ^{1}H-NMR ($CDCl_3$) : 3.62 (2H, q), 3.82 (1H, s), 3.83 (2H, t), 7.25 (1H, s), 7.38 (1H, m), 8.13 (1H, d), 8.66 (1H, d), 8.99 (1H, s). Elemental analysis $C_8H_{10}N_2O_2$; theory C: 57.83, H: 6.02, N: 16.86; found C: 57.79, H: 5.97, N: 16.49.

3(2-chlorethyl-carbamoyl)pyridine 3 : 49 g (0.295 mol) 2 were dissolved in 200 ml chloroform and cooled to 0°C. 42.8 g (0.36 mol) of thionyl chloride were transferred into the solution, while stirring vigorously (glass stirrer). The mixture was warmed up to room temperature and than refluxed for four hours until the oily product solidified. 200 Ml water and 45 g $NaHCO_3$ were added in several portions after cooling. The organic layer was separated and the residue extracted twice with 200 ml chloroform. The combined organic solutions were dried over $MgSO_4$ and evaporated. The resulting product was purified by column chromatography on aluminium oxide with chloroform/acetone (1:1) as the eluent to yield 3 as white needles. yield: 25 g (0.133 mol, 45 %), M.p. 89 91 °C,
^{1}H-NMR ($CDCl_3$) : 3.66 (2H, q), 3.77 (2H, t), 7.02 (1H, s), 7.33 (1H, m), 8.08 (1H, d), 8.66 (1H, d), 8.95 (1H, s). Elemental analysis $C_8H_9ClN_2O$; theory C: 52.03, H: 4.87, N: 15.17, Cl: 19.24; found C: 51.82, H: 4.92, N: 14.91, Cl: 18.61.

3(2-mercaptoethyl-carbamoyl)pyridine 4 : 3.7 g (20 mmol) 3 were dissolved in 20 ml water and stirred with a solution of 15.8 g (40 mmol) Na_3PO_3S x 12 H_2O in 150 ml water for 7 hours at 40 - 50 °C. After cooling to room temperature the pH was adjusted to 4.0 by adding 3.5 % HCl. The mixture was allowed to stand overnight and than extracted with CH_2Cl_2 (4 x 100 ml). The organic layer was dried over $MgSO_4$ and evaporated. Purification by column chromatography on silica gel with

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acetone/chloroform (6:1) yielded 4 as white crystals. yield: 1.8 g (9.6 mmol, 48 %), M.p. 96 - 98 °C. ^{13}C-NMR (DMSO-d_6) : 23.32 (CH_2), 42.80 (CH_2), 123.60 (C_{ar}), 129.89 (C_{ar}), 135.10 (C_{ar}), 148.38 (C_{ar}), 151.95 (C_{ar}), 165.00 (CO). Elemental analysis $C_8H_{10}N_2OS$; theory C: 52.74, H: 5.49, N: 15.38, S: 17.58; found C: 52.57, H: 5.58, N: 15.12, S: 16.93.

3(2-mercaptoethyl-carbamoyl)-1-methyl-pyridinium-iodide 5 : 370 mg (2.03 mmol) 4 in 35 ml absolute acetone were refluxed with 6.0 g (40 mmol) methyl iodide for 20 minutes. After cooling, the mixture was concentrated by evaporation up to 5 ml and stored overnight at - 18°C. The resulting yellow needles were separated. The product 5 was characterized and used without further purification. yield: 490 mg (1.50 mmol, 74 %), M.p. 102 - 104 °C, ^{13}C-NMR (DMSO-d_6) : 23.07 (CH_2), 42.91 (CH_2), 48.24 (CH_3), 127.34 (C_{ar}), 133.19 (C_{ar}), 142.66 (C_{ar}), 145.43 (C_{ar}), 147.16 (C_{ar}), 161.39 (CO). Elemental analysis $C_9H_{13}N_2IOS$; theory C: 33.33, H: 4.01, N: 8.64, I: 39.19, S: 9.87; found C: 33.46, H: 3.83, N: 8.52, I: 38.37, S: 9.59.

(3-pyridinyl-ethylcarbamoyl-thiolato)(3-thiapentane-1,5-dithiolato)oxorhenium(V) 6 :
1.06 g (2.7 mmol) chloro(3-thiapentane-1,5-dithiolato)oxorhenium(V) were dissolved in 60 ml boiling acetonitrile. A solution of 0.975 g (5.4 mmol) 4 in 20 ml acetonitrile was added and the mixture was refluxed for one hour. The solid product was separated, dried and purified by column chromatography (Al_2O_3, methanol). The eluate was concentrated to a half of its original volume and allowed to stand overnight at - 18°C. The product 6 crystallized as fine red needles. yield: 1.03 g (1.92 mmol, 71 %), M.p. 186 - 188 °C. ^{13}C-NMR (DMSO-d_6) : 35.41 (CH_2), 42.42 (CH_2), 43.13 ($2xCH_2$), 45.61 ($2xCH_2$), 123.56 (C_{ar}), 129.95 (C_{ar}), 135.08 (C_{ar}), 148.44 (C_{ar}), 151.69 (C_{ar}), 164.62 (CO). Elemental analysis $C_{12}H_{17}N_2O_2S_4Re$; theory C: 26.91, H: 3.17, N: 5.23, S: 23.92; found C: 26.43, H: 3.02, N: 5.13, S:23.40. IR (KBr): 960 (Re=O), 1664 (C=O).

(1-methyl-3-pyridinyl-ethylcarbamoyl-thiolato)(3-thiapentane-1,5-dithiolato)-oxorhenium(V)-iodide 7 :
Route A: 535 mg (1mmol) 6 were refluxed with 1.42 g (10 mmol) methyl iodide in 150 ml absolute acetone. After 4 hours the solution was nearly discoloured and a light red product had separated. It was filtered and recrystallized from methanol. yield: 336 mg (0.5 mmol, 50 %)
Route B: 83 mg (211µmol) chloro(3-thiapentane-1,5-dithiolato)oxorhenium(V) were refluxed in 5 ml acetonitrile. 137 mg (422 µmol) 5 in 2 ml acetonitrile were added and the mixture was refluxed for 30 minutes. The solution was concentrated to 3 ml and after cooling 3 ml diethylether were added to the mixture. The solid product was separated and recrystallized. yield: 86 mg (126 µmol, 60 %).
M.p. 197 - 198 °C. ^{1}H-NMR (DMSO-d_6): 2.26 (2H, m), 3.04 (2H, t), 3.68 (2H, q), 3.87 (2H, t), 4.08

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(2H, q), 4.32 (2H, d), 4.39 (3H, s), 8.24 (1H, t), 8.89 (1H, d), 9.10 (1H, d), 9.34 (1H, t), 9,41 (1H, s). Elemental analysis $C_{13}H_{20}N_2IO_2S_4Re$; theory C: 23.04, H: 2.95, N: 4.13, I: 18.75, S: 18.90; found C: 22.86, H: 2.81, N: 4.08, I: 16.84, S: 18.65. IR (KBr): 960 (Re=O), 1668 (C=O).

3(2-mercaptoethyl-carbamoyl)-1-methyl-1,4-dihydropyridine **8** : 9.75 mg (30 µmol) **5** and 15 mg (180 µmol) $NaHCO_3$ were dissolved in 2 ml water. The mixture was cooled to almost 0°C and 10.5 mg (60 µmol) $Na_2S_2O_4$ were added when the solution turned light orange. When the reduction was complete the colourless solution was used for further reactions. UV: $\lambda_{max} = 361,8$ nm

Radiolabelling with $^{99/99m}Tc$

(1-methyl-3-pyridinyl-ethylcarbamoyl-thiolato)(3-thiapentane-1,5-dithiolato)oxotechnetium(V)-iodide **10** : 1 µmol $^{99}TcO_4^-$ and (5-10 MBq) $^{99m}TcO_4^-$ in 1 ml 0.1 M sodium gluconate was diluted with 1 ml 0.9 % NaCl and then reduced with 100 µl freshly prepared 0.01 M stannous chloride dihydrate in 0.1 M HCl. 1.95 mg (6 µmol) **5** in 200 µl water were added to this $^{99/99m}Tc(V)$-gluconate solution. 1.0 ml acetone was added to the yellow complex and then 0.3 mg (2 µmol) 3-thia-1,5-pentanedithiol in 300 µl acetone. The reaction process was controlled by TLC (RP18 plates, butanol/acetic acid/water = 2:1:1). **10** was determined as a peak with $R_f = 0.54$ in 73 % radiochemical purity.

[1-methyl-3-(1,4)dihydropyridinyl-ethylcarbamoyl-thiolato](3-thiapentane-1,5-dithiolato)oxo-technetium(V) **12** : 1µmol (5-10 Mbq) $^{99/99m}Tc(V)$ gluconate prepared as above was reacted with 1 ml of dihydropyridine solution (containing 15 µmol **8**). After 5 minutes 1 ml acetone and 0.3 mg (2 µmol) 3-thia-1,5-pentanedithiol in 300 µl acetone were added. The reaction was observed by UV-VIS spectroscopy.

RESULTS AND DISCUSSION

Synthesis of rhenium complexes

Coupling of the pyridine moiety to Tc or Re requires the presence of an appropriate donor group able to form a stable single bond to the metal. While mercaptide sulphur is the preferred donor atom for Tc and Re in the oxidation state +V, we aimed to introduce a 2-mercaptoethylgroup into the amide functionalty of nicotinamide. The synthesis of 3(2-mercaptoethyl-carbamoyl)-1-methylpyridine **4** is associated, as the literature shows, with some difficulties and by-products (14,15). We decided to start from methyl nicotinate **1** which was heated with ethanolamine at 150°C to eliminate methanol

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and form 3(2-hydroxyethyl-carbamoyl)pyridine 2 (Figure 1) . Treatment of the hydroxyl group with thionyl chloride led to the 3(2-chlorethyl-carbamoyl)pyridine 3. The introduction of the thiol group resulted in reacting 3 with sodium thiophosphate dodecahydrate in aqueous solution by modification of a literature procedure (16). Acid hydrolysis of the phosphoric acid thioester supplied the free thiol 4 in high purity. Thiol 4 was methylated with excess methyl iodide. Alkylation of sulphur could be prevented by application of an inert solvent. Both thiolates 4 and 5 were reacted with the precursor chloro(3-thiapentane-1,5-dithiolato)oxo-rhenium(V) according to the '3+1'-principle (11). In this way the new rhenium mixed-ligand complexes 6 and 7 were obtained. Complex 7 was also obtained by direct methylation of the complex 6 with excess methyl iodide in acetone (route A).

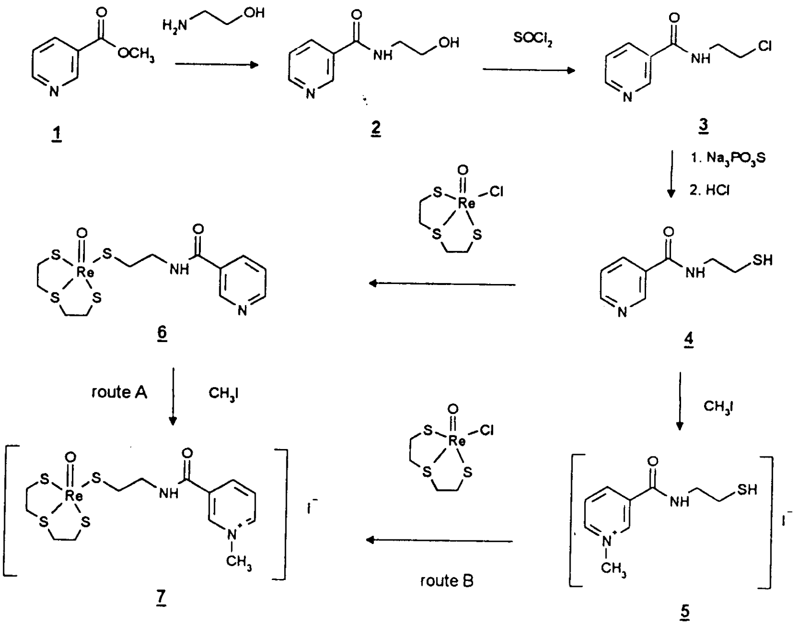

Figure 1. Reaction pathway for preparation of nicotinamide-substituted Re-complexes 6 and 7 .

A further important step is the conversion of the pyridinium salt into the corresponding dihydropyridine. The most convenient method to generate dihydropyridines from pyridinium salts is reduction with sodium dithionite in alkaline solution (17,18). In this way it should be possible to reduce the pyridinium salt '3+1'-complex 7 with $Na_2S_2O_4$ to the corresponding 1,4-dihydropyridine derivative. Because of the sensitivity to oxidation of the dihydropyridines it is difficult to detect them analytically. Nevertheless, a significant identifying mark is the characteristic UV-absorption at 360 nm (8,19).

By stirring 7 in alkaline aqueous $Na_2S_2O_4$ a brick-red solid separated (decomposition 105 - 115°C). Attempts at purification resulted in a viscous red substance. Figure 2 shows the UV-VIS spectrum of

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complex 7 and the alteration of the spectrum after reduction to the dihydropyridine. A new band at 299.3 nm appeared and the absorption at 366 nm increased. The latter may serve as an indication for the existence of a dihydropyridine complex.

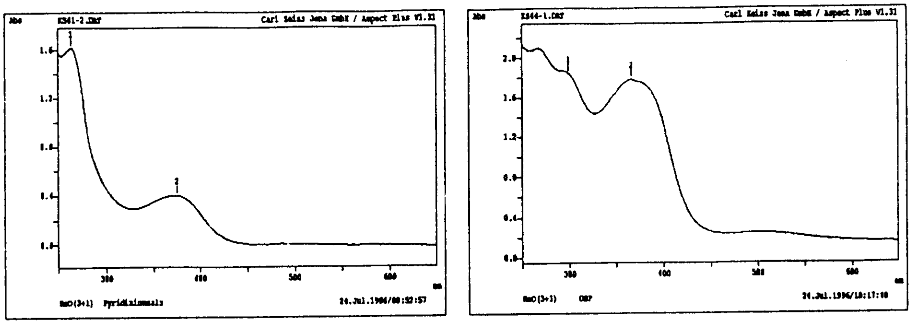

Figure 2. UV-VIS spectrum of the complex 7 in water (left) and after reduction with $Na_2S_2O_4$ (right).

Synthesis of technetium complexes

Further investigations refer to the preparation of pyridinium/ dihydropyridine-substituted technetium complexes and on the stability of the dihydropyridine species. The reaction pattern for the synthesis of the nicotinamide substituted $^{99/99m}$Tc mixed-ligand complexes is shown in figure 3.

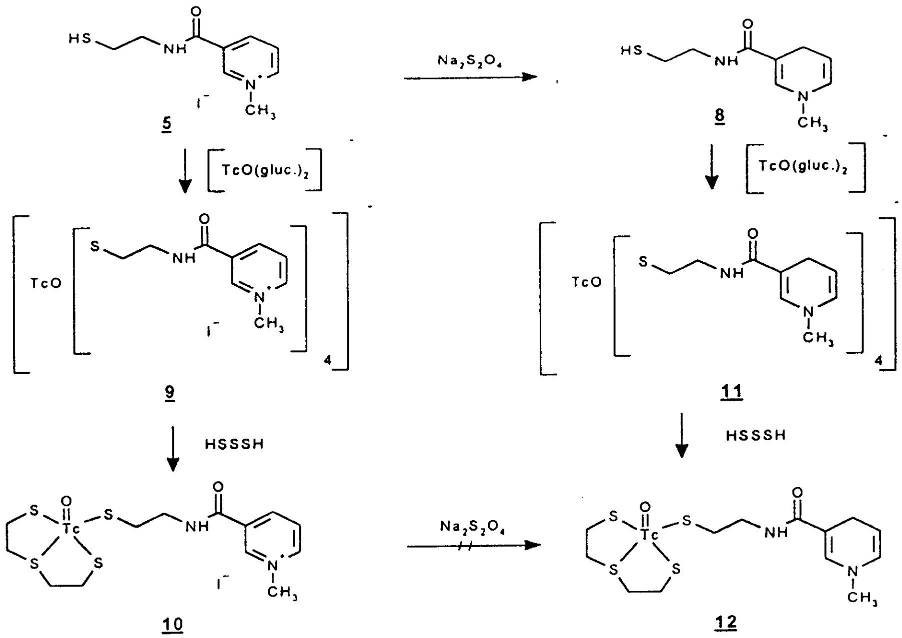

Figure 3. Reaction pathway for preparation of pyridinium salt and dihydropyridine-substituted Tc-complexes 10 and 12 .

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Complex 10 is available in a two-step procedure. First the pyridinium salt 5 was reacted with $^{99/99m}$Tc gluconate to the supposed intermediate 9. Subsequently 3-thia-1,5-pentanedithiol (HSSSH) was added and three monodentate ligands were displaced resulting in 10 .

The progress of the reaction sequence was observed by TLC as shown in figure 4. In the first step after reaction of 5 with $^{99/99m}$Tc-gluconate the intermediate 9 is detectable with an R_F= 0.17. When the tridentate HSSSH was added, the peak of 9 disappeared and the new '3+1' complex 10 was found as the major product (R_F=0.49). An investigation by capillary electrophoresis proved that 10 is a cationic complex.

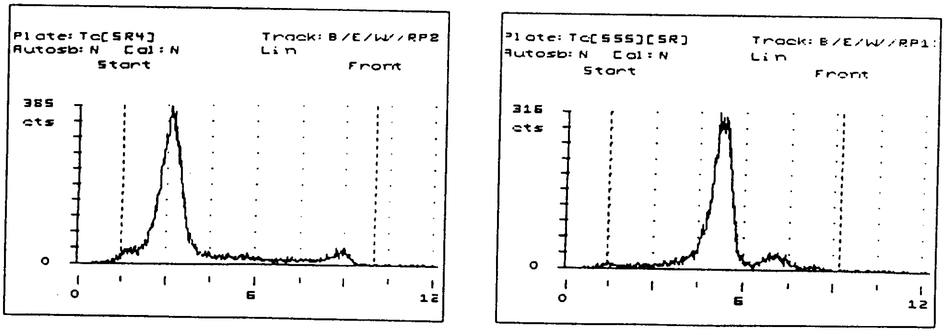

Figure 4. TLC of reaction of $^{99/99m}$Tc-gluconate with 5 before (left) and after (right) addition of HSSSH

Attempts to prepare the dihydropyridine derivative 12 by direct reduction of complex 10 with $Na_2S_2O_4$ suffered from the instability of the chelate in alkaline medium. After addition of the reducing agent to an alkaline solution of 10 a brown discoloration of the yellow complex was observed and within three hours a brown precipitate appeared. TLC showed that after 15 minutes part of the starting complex was already decomposed and insoluble material was found at the start that did not run on the TLC mobile solvent. Three hours later only the insoluble precipitate was visible. It can be assumed that the brown insoluble compound is reduced hydrolyzed technetium. A reduction of the metal has obviously occurred. This competitive reaction is not surprising because the ligand 5 as well as the sodium dithionite have been used in great excess over the technetium. To avoid reduction of the metal, the free dihyropyridine ligand 8 was prepared starting from 5 followed by complexation as an additional step (Fig. 3). 5 was reduced with sodium dithionite within 15 minutes. Because of the sensitivity of the dihydropyridine to air oxidation, the product was not isolated . The solution containing fresh reduced 8 was analysed by UV-VIS spectrometry where a characteristic dihydropyridine absorption at 361,8 nm was observed that disappeared within 45 minutes (Fig. 5). If a solution of freshly reduced

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dihydropyridine 8 is given to $^{99/99m}$Tc-gluconate, a complex of structure 11 is expected. The UV-VIS spectrum of the resulting species 11 again showed the significant dihydropyridine absorption at 360.4 nm that disappeared over 45 minutes (Fig. 5).

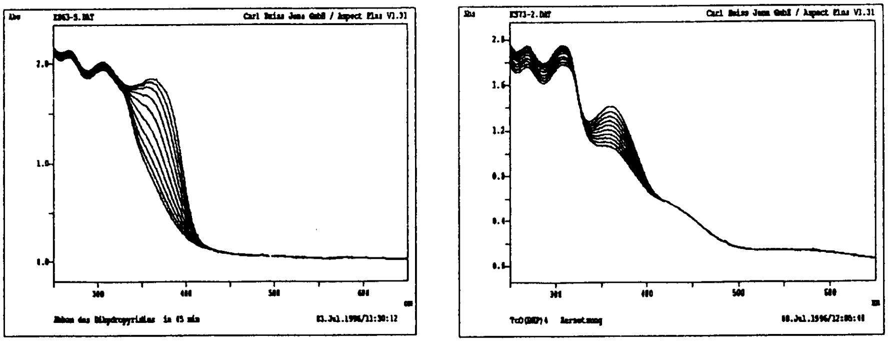

Figure 5. UV-VIS spectrum of 8 (left) and 11 (right) in water over 45 minutes.

By immediate addition of tridentate ligand HSSSH to 11 , the desired dihydropyridine '3+1'-complex 12 becomes available. In the UV-VIS spectrum of 12 a strong absorption at 365.6 nm was observed . Further work on the separation and stability of the Tc-complexes 10 and 12 by HPLC- and UV-methods is in progress and will be published in a subsequent paper.

SUMMARY AND CONCLUSION

Synthesis and the properties of new pyridinium-salt complexes of rhenium and technetium of the '3+1' type are described. By reduction of the pyridinium salt with sodium dithionite mixed-ligand dihydropyridine-metal complexes are available. Investigation of the stability of the dihydropyridines by UV-VIS spectrometry shows that decomposition occurs in a short time.

Further efforts will be made to obtain complexes of a higher stability, suitable for in vivo tests. Improvements in the stability of the dihydropyridine complexes may be achieved by introduction of various substituents on the pyridinium ring and modification of the tridentate ligand. In addition the search for other ways of reduction by chemical, biochemical or electrochemical methods should be continued.

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