Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 673-681 (1999)

NICOTINAMIDE-SUBSTITUTED COMPLEXES AS REDOX MARKERS

2. SYNTHESIS OF A ^{99}TC-DIHYDROPYRIDINE MIXED-LIGAND COMPLEX AND INVESTIGATION OF THE STABILITY IN TISSUE HOMOGENATES

A. Rother [2], T. Kniess *[1], M. Pütz [2], H. Jungclas [2], H. Spies [1], B. Johannsen [1]

[1] Forschungszentrum Rossendorf, Institut für Bioanorganische und Radiopharmazeutische Chemie, PF 510119, D-01314 Dresden, Germany
[2] Fachbereich Chemie, Kernchemie, Philipps-Universität Marburg, D-35032 Marburg, Germany

SUMMARY

For developing a dihydropyridine/pyridinium salt redox delivery system that could be useful for SPECT investigations by ^{99m}Tc compounds the synthesis of a mixed-ligand complex of the long-lived isotope ^{99}Tc is described. The new compound bearing a pyridinium salt moiety was characterised by NMR-spectrometry and X-ray structure analysis. By reduction with sodium dithionite the corresponding 1,4-dihydropyridine complex was prepared and the stability in buffer, tissue homogenates, blood plasma and cerebrospinal fluid was investigated by UV-VIS spectrometry.

Key words: redox delivery system, pyridinium salt, 1,4-dihydropyridine, ^{99}Tc-complexes

INTRODUCTION

Diagnostic nuclear medicine provides valuable information on a variety of disease states. In recent years there has been an increasing interest in tracers able to visualise biochemical reactions in vivo. For imaging the various redox processes occurring in the organism the development of redox-active radiotracers is needed.

*) To whom all correspondence should be addressed.

CCC 0362–4803/99/070673–09$17.50

Received 7 January 1999
Revised 28 January 1999
Accepted 1 February 1999

The search for Tc tracers in this field is in its infancy. First technetium complexes with 2-nitroimidazoles that are enzymatically reducible in the organism and accumulate in hypoxic tissue and in tumour cells were studied in vivo some years ago (1,2). Recently ^{64}Cu-labeled complexes based on bis(thiosemicarbazone) and bis(salicylaldimine) ligands were prepared and tested for cell uptake under normoxic and hypoxic conditions (3). Looking for technetium chelates bearing a pyridinium salt/dihydropyridine moiety by analogy to the NAD^+/NADH redox system we developed a mixed-ligand rhenium pyridinium complex (4). Re serves as a surrogate for ^{99m}Tc and describes the reduction with sodium dithionite to the dihydropyridine compound. In the present paper we report the synthesis of a ^{99}Tc-pyridinium salt complex according the "3+1" principle and its characterisation by ^{1}H NMR-, ^{13}C NMR-spectroscopy and X-ray structure analysis. After conversion to the corresponding 1,4-dihydropyridine compound the stability of the ^{99}Tc-dihydropyridine complex in buffer, tissue homogenates, blood plasma and cerebrospinal fluid and its dependence on temperature was investigated.

EXPERIMENTAL

General: HPLC-investigations were carried out with an analytical RP18 column (Lichrospher 100 RP-18, 5µm, MERCK) and a semi-preparative RP18 column (Ultracarb 5 ODS, 20 , PHENOMENEX) using a 3:1 mixture of iso-propanol/phosphate buffer (10 mmol, pH = 7.0) as eluent with a flow rate of 0.2 ml/min or 0.8 ml/min respectively. The products were determined by UV absorbance at 254 nm and by ß-detection with a scintillation detector (Ramona 90, RAYTEST). The NMR spectra were recorded on an FT-Spectrometer ARX 500 (BRUKER) in DMSO-d_6. The UV/VIS spectroscopic measurements were carried out with a diode array spectrometer with 1024 diodes (J & M ANALYTISCHE MESS- UND REGELTECHNIK). The X-ray structure analysis was performed on an Image Plate Detector System (STOE) with MoK_α X-rays (71.07 pm) and calculated with the programs Stoe Expose, Stoe Cell and Stoe Integrate.

[^{99}Tc](1-methyl-3-pyridinyl-ethylcarbamoyl-thiolato)-(3-thiapentan-1,5-dithiolato)oxotechnetium(V) iodide **2** : 54 ml of an aqueous [^{99}Tc]NH_4TcO_4 solution (c=4.64 mmol/l, 250 µmol) was added to 5.45 g (25 mmol) sodium-d-gluconate dissolved in 10 ml water. Reduction to ^{99}Tc-gluconate was carried out by addition of 15 ml 0.02 M solution of stannous chloride (300 µmol) in 0.1M HCl. After complete reduction 486 mg (1.5 mmol) 3(2-mercaptoethyl-carbamoyl)-1-methyl-pyridinium iodide **1** (4) dissolved in 5 ml water was added followed by 25 ml acetonitrile after 25 minutes and 39 mg (250 µmol) 3-thia-1,5-pentanedithiol dissolved in 5 ml acetonitrile. The reaction mixture was evaporated to 5 ml and the product was purified by preparative HPLC. yield: 96 mg (163 µmol, 65 %), M.p. 190-194°C,

^{1}H NMR (DMSO-d_6) δ[ppm]: 2.26 (2H, td), 3.03 (2H, td), 3.67 (2H, q), 3.86 (2H, t), 4.08 (2H, dd), 4.30 (2H, dd), 4.39 (3H, s), 8.22 (1H, t), 8.88 (1H, d), 9.09 (1H, d), 9.30 (1H, t), 9.39 (1H, s) ;^{13}C NMR (DMSO-d_6) δ[ppm]: 34.94 (CH_2), 42.64 (CH_2), 43.04 (2xCH_2), 45.74 (2xCH_2), 48.22 (CH_3), 127.33 (C_{ar}), 133.29 (C_{ar}), 142.63 (C_{ar}), 145.45 (C_{ar}), 147.07 (C_{ar}), 161.19 (CO).

[^{99}Tc](1-methyl-3-(1,4)dihydropyridinyl-ethylcarbamoyl-thiolato)(3-thiapentan-1,5-dithiolato)oxo technetium(V) **3** : 8.0 ml diethyl ether was added to 0.6 g (3.45 mmol) sodium dithionite and 0.48 g (3.45 mmol) sodium carbonate dissolved in 2.0 ml water. To this biphasic system 10 mg (17 µmol) [^{99}Tc](1-methyl-3-pyridinyl-ethylcarbamoyl-thiolato)(3-thiapentan-1,5-dithiolato)-oxo-technetium(V) iodide **2** dissolved in 0.5 ml water was added and the mixture was refluxed for 20 minutes with vigorous stirring. After cooling the organic layer was separated and at -18°C concentrated in vacuum to 1.0 ml. This ethereal solution of the product **3** is stable for one day at -18°C and was used for the UV-VIS spectrometric investigations without purification.

UV-VIS spectrometric investigations of kinetic and stability of the [^{99}Tc](1-methyl-3-(1,4)dihydropyridinyl-ethylcarbamoyl-thiolato)(3-thiapentan-1,5-dithiolato)oxotechnetium(V) **3**:

For investigation of the kinetics of **3** in different media 0.1 M phosphate buffers at pH 6.7 - 7.6 were used. The homogenates of kidney, liver and brain were diluted with phosphate buffer pH = 7.4. Blood plasma and cerebrospinal fluid were used without dilution. The measurements were performed at 20 °C and at 37 °C with 1.0 cm standard cuvettes in a temperature-controlled cuvette holder. For the kinetic measurements 100 µl of the ethereal solution of **3** was added to 2.0 ml buffer in the cuvette and the diethyl ether was removed by passing a stream of argon. The samples were measured at regular intervals of 10 seconds over a period of 15 minutes. The decrease in the absorption at 365 nm served as the criterion for the decay of the dihydropyridine complex.

RESULTS AND DISCUSSION

The synthesis of the [^{99}Tc](1-methyl-3-pyridinyl-ethylcarbamoyl-thiolato)(3-thiapentan-1,5-dithiolato)oxotechnetium(V) iodide **2** was carried out according to the "3+1" principle by reaction of ^{99}Tc gluconate with the monodentate ligand 3(2-mercaptoethyl-carbamoyl)-1-methyl-pyridinium iodide **1** (4) and the tridentate 3-thia-1,5-pentanedithiol (Fig.1). The corresponding (4:1)-complex of monodentate ligand and technetium is observed as an intermediate (4). After addition of the tridentate ligand the resulting complex **2** was purified by HPLC.

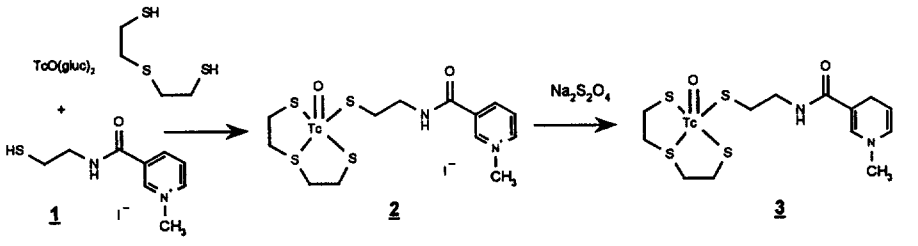

Figure 1. Reaction pathway for the synthesis of the ^{99}Tc-complexes **2** and **3**.

This step is necessary to remove unreacted ^{99}Tc gluconate, intermediate and ligands. Fig. 2 shows the radio-HPLC chromatograms of the intermediate ^{99}Tc (4:1)-complex with a retention time of 18 min (Fig.2a) and of the product **2** after purification with a retention time of 24.4 min (Fig.2b).

The molecular structure of complex **2** was established by crystallographic means. The X-ray structure reveals that the monodentate and the tridentate ligand form a square pyramid with distorted basal surface and the oxygen in an axial position (Fig.3). The Tc-O binding distance was found to be 168 Å, the average Tc-S binding distance is 232 Å whilst the thioether-metal binding has a distance of 237 Å because of the coordinative character of this bond.

The reduction of **2** to the complex **3** with sodium dithionite in pure 0.1 M potassium carbonate was not a successful way to prepare the dihydropyridine because the basic conditions lead to rapid decomposition of the complex. So we looked for an alternative procedure that was realised by using a biphasic system diethyl ether/water according to a literature procedure (6). After vigorous stirring the lipophilic dihydropyridine complex **3** accumulated in the organic phase and could be separated. UV-VIS investigations showed that the dihydropyridine in ethereal solution is stable for 24 hours whereas in alkaline solution it rapidly decomposes.

For the kinetic investigations of the stability of the dihydropyridine complex **3** the UV spectrum was recorded in phosphate buffer at various pH's. The samples

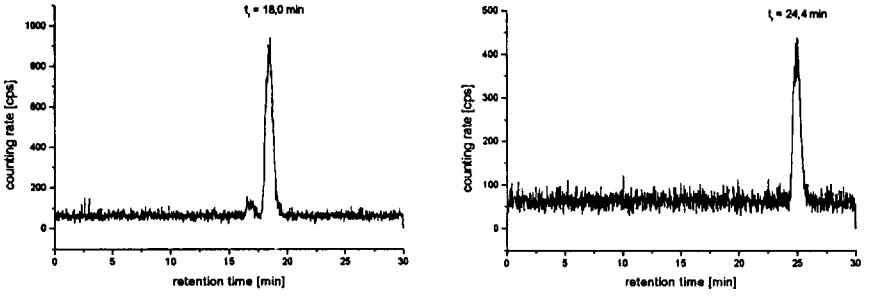

Figure 2. Radiochromatogram of the reaction of ^{99}TcO(gluc)$_2$ with **1** (Fig.2a - left) and of the purified ^{99}Tc complex **2** (Fig.2b - right) (Lichrospher 100 RP-18; iso-propanol/phosphate buffer 10mM pH:7.0 = 3:1; 0.2 ml/min)

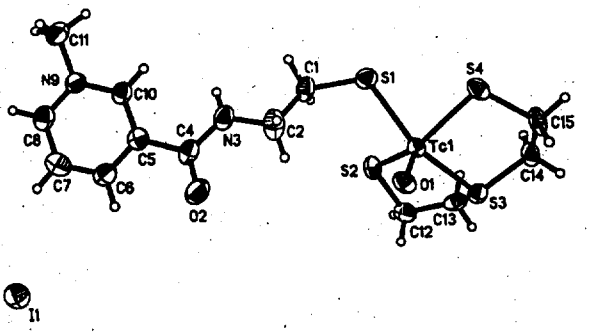

Figure 3 . Molecular structure of 2. Selected bond length [Å] and angles [°]: Tc-O1 1.680(6), Tc-S1 2.320(3), Tc-S2 2.288(3), Tc-S3 2.372(3), Tc-S4 2.294(3), O1-Tc-S1 105.3(3), O1-Tc-S2 114,6(3), O1-Tc-S3 101.8(3), O1-Tc-S4 114,8(3)

were measured at regular intervals of 10 seconds over a period of 15 minutes. Dihydropyridines are characterised by a strong UV-absorption at 360 nm (7) that decrease with oxidation to the pyridinium salt. Figure 4a shows a typical UV-VIS spectrum of 3 at pH = 6.97 and 20°C. The declining absorption at 360 nm was used as a basis for the decay of the dihydropyridine complex. The increasing band at 270 nm is characteristic of the pyridinium salt that had a maximum absorption at 265 nm. The appearance of an isosbestic point at 315 nm may serve as proof of a pure two-compound system. The drop in the absorption at 360 nm over time described an exponential function and in this way the half life of the re-oxidation was established as a criterion for the stability.

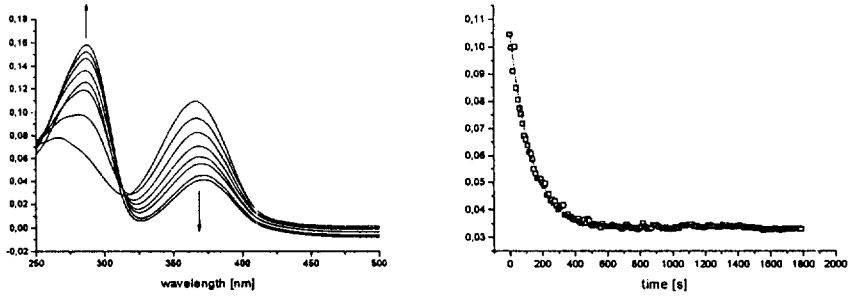

Figure 4a (left): UV-VIS spectrum of complex 3 at pH = 6.97 and 20°C over 10 minutes, the arrows show the course of measurement. Figure 4b (right): exponential decrease in the absorption of 3 at 360 nm at pH = 6.97 and 20°C over time.

In figure 4b the decay of the dihydropyridine complex **3** as a function of time is shown and it is obvious that 600 seconds after start of the measurement most of the dihydropyridine has decomposed.

To investigate the stability of **3** as a function of pH and temperature 0.1M phosphate buffers were used with physiologically relevant values between pH = 6.7 - 7.6 at 20 °C and at 37 °C. The tissue homogenates of kidney, liver and brain were diluted with 0.1M phosphate buffer (pH=7.4) and tested at 37°C. Because of the turbidity of the biological materials caused by suspended particles that could not be removed by centrifugation, the samples had a high background UV-absorption. The absorbance at 360 nm was near 2.0 and the decreasing values can only show tendencies.

Table 1 gives an overview of the half-lives of the complex **3** in various media and selected temperatures. Considering the half-life times it is remarkable that at 37 °C the stability decreases with increasing pH, whereas at room temperature the relationship is inverted. In liver homogenate a significantly faster

pH	temperature [°C]	media	half-life time [s]
6.72	37	phosphate buffer	178
6.79	20	phosphate buffer	53
6.94	37	phosphate buffer	134
6.97	20	phosphate buffer	117
7.15	37	phosphate buffer	107
7.17	20	phosphate buffer	160
7.34	37	phosphate buffer	80
7.60	37	phosphate buffer	41
7.60	20	phosphate buffer	394
7.40	37	kidney homogenate	102
7.40	37	liver homogenate	45
7.40	37	brain homogenate	144
7.40	37	blood plasma	133

Table 1. Half-lives of complex **3** in various media and selected temperatures.

decomposition than in other tissue homogenates was observed and in blood plasma the dihydropyridine is more stable which is in accordance with previous work by Bodor et.al. (8). In cerebrospinal fluid the complex is notably more stable than in all other media. No exponential course of decomposition was observed here, the concentration of **3** declines linearly.

SUMMARY AND CONCLUSION

For developing a redox delivery system a ^{99}Tc complex with a pyridinium salt moiety was synthesised and characterised by NMR-spectrometry and X-ray structure analysis. The corresponding 1,4-dihydropyridine compound was generated by reduction with sodium dithionite and investigated by UV-VIS spectrometry. However as a result of the studies of the stability of the dihydropyridine complex **3** in buffer and tissue homogenate it must be concluded that the dihydropyridine ^{99}Tc-mixed-ligand complex **3** with half-lives between 40 and 400 seconds is not stable enough for further biological experiments and preparation of the corresponding ^{99m}Tc derivative is not useful. Future work will focus on improvements to the stability of the dihydropyridine by introduction of electron withdrawing substituents such as benzyl or isopropyl at the pyridinum nitrogen. Bromine substitution in the 5-position of the pyridinium ring and the utilisation of the chinolinium salt/dihydrochinoline system also has a stabilising effect (5). Another option might be to employ a tetradentate ligand system where a higher stability of the chelate against the reducing media sodium dithionite/alkali is expected.

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