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PREPARATION AND EVALUATION OF CATIONIC $^{99}Tc/^{99m}Tc$ DIMETHOXY- AND DIETHOXY-HEXANEDIONE DIOXIME COMPLEXES

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SUMMARY

The new ligands 1,6-dimethoxy-3,4-hexanedione dioxime (DMHD) and 1,6-diethoxy-3,4-hexanedione dioxime (DEHD) were synthesized and their boron containing $^{99}Tc/^{99m}Tc$ complexes, formed by reduction of pertechnetate with BH_4^-, were isolated, purified and characterized by IR/UV/VIS spectroscopy and FAB mass spectrometry. These rather stable cationic complexes differ significantly in lipophilicity as assessed by their octanol/saline partition coefficients and HPLC capacity factors. Unexpectedly the organ uptake and clearance of both complexes in mice proved to be very similar with only the uptake of ^{99m}Tc-DMHD in the kidneys being enhanced.

Key words: technetium, cationic dioxime complexes, lipophilicity, biodistribution.

1. INTRODUCTION

Numerous investigations have been carried out in order to develop and improve ^{99m}Tc radiopharmaceuticals for myocardial perfusion imaging, but only a few compounds have been shown to be suitable or promising, notably the cationic complex hexakis(2-methoxy-2-methylpropyl-1-isonitrile)-technetium(I) (MIBI) [1] and the neutral seven coordinate tris(cyclo-hexanedione dioxime)-methylboron-chloro-technetium(III) [2]. Very recently Lahiri et al. [3] synthesized a series of ^{99m}Tc complexes of novel functionalized diphosphines e.g. 1,3-bis(dimethylphosphino)-2,2-di(methoxymethyl)propane (PL37) and 1,2-bis(di(2-

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ethoxyethyl)phosphino)ethane (P53), which display excellent myocardial image quality.

In addition to the technetium dioxime complex mentioned above, Nunn and co-workers [4-8] investigated other neutral, seven-coordinate 1,2-cyclohexanedione- and 2,3-butanedione dioxime complexes of Tc(III) capped with a boronic acid derivative and containing an axial Cl, Br or OH ligand. The compounds were extensively evaluated for heart and brain perfusion imaging; their synthesis and characterization has been described very recently by Linder et al. [9]. In developing myocardial cationic technetium agents of balanced lipophilicity Schwochau et al. [10, 11] synthesized and characterized $^{99}Tc/^{99m}Tc$ dioxime complexes formed with various vicinal dioxime ligands and assessed systematically the effects of carbon chain length, position isomerism and introduction of a terminal methoxy group on the myocardial uptake. Structure distribution relationship studies of a homologous series of anionic ^{99m}Tc-2,3-dioxime complexes - without characterizing the corresponding ^{99g}Tc complexes - were pursued by Salako et al. [12].

The present work continues the investigations of Schwochau et al. [10,11] on cationic dioxime complexes by introducing two terminal methoxy- or ethoxy-groups into the symmetric 3,4-hexanedione dioxime ligand, because in view of the successful isonitrile (MIBI) and the functionalized diphosphine (PL37 and P53) complexes methoxy- and ethoxy-groups appeared to promote the myocardial uptake. Moreover it was the objective of this study to evaluate the effect of different lipophilicity on the biodistribution.

2. MATERIALS AND METHODS

2.1 General

Unless otherwise stated, all chemicals and reagents were of analytical grade or equivalent. NH_4TcO_4 was procured from ORNL, U.S.A. $Na^{99m}TcO_4$ was eluted from "Minitec 2000" model ^{99m}Tc generators of Squibb Diagnostic von Heyden GmbH, F.R.G. Prior to use, all organic solvents were dried over molecular sieves. Benzyl chloride, acrolein and triethylamine were freshly distilled. 5-(2-hydroxyethyl)-4-methyl-1,3-thiazol was used as purchased from Aldrich Chemical Company, F.R.G.

Electrophoreses were run on a horizontal Desaga Desaphor system. VIS/UV spectra were recorded with a Beckman Acta III C spectrophotometer, the IR spectra with a Shimadzu IR-

460 using KBr pellets. ^1H NMR measurements were performed on a Varian EM 390 90 MHz spectrometer with TMS as the internal standard. The Fast Atom Bombardment (FAB) mass spectra were measured at Hoechst AG, Frankfurt/M. using a Kratos MS 50 spectrometer with 3-nitrobenzylalcohol as matrix. The HPLC system employed was equipped with a Waters UV-Absorbance Model 440 detector set at 254 or 405 nm; an in-house designed system was used in series with the mass detector for the detection of radioactivity.

2.2 Preparation of Ligands

1,6-Dimethoxy- and 1,6-diethoxy-3,4-hexanedione dioxime were prepared by reaction of hydroxylamine with the corresponding diketones. The synthesis of the diketones was achieved in three steps (Fig. 1). Reaction of acrolein with methanol or ethanol according to Elderfield et al. [13] lead to the formation of ß-alkoxypropionaldehydes. Heating of these compounds in refluxing ethanol with triethylamine and 3-benzyl-5-(2-hydroxyethyl)-4-methyl-1,3-thiazolium-chloride (BzHMT-Cl) as a catalyst [14] yielded the acyloins 1,6-dimethoxy- and 1,6-diethoxy-3-hydroxy-4-oxo-hexane, respectively, which were oxidized with Bi_2O_3.

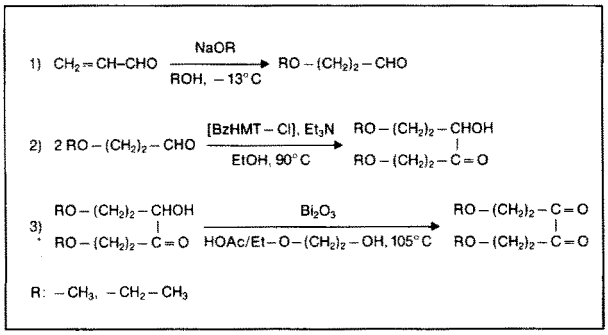

Fig. 1 Synthesis of 1,6-Dimethoxy- and 1,6-Diethoxy-3,4-hexanedione

2.2.1 1,6-Dimethoxy-3,4-hexanedione dioxime (DMHD)

3-Benzyl-5-(2-hydroxyethyl)-4-methyl-1,3-thiazolium-chloride (I): This compound was synthesized by reaction of benzyl chloride with 5-(2-hydroxyethyl)-4-methyl-1,3-thiazol and purified after Stetter et al. [14]; m.p. 141-141.5°C.

ß-Methoxypropionaldehyde (II): Sodium metal (2.0 g, 87 mmol) was dissolved in methanol

(576.0 g, 18.0 mol). The solution was cooled to -13°C and acrolein (224.0 g, 97%, 4.0 mol) was added dropwise over a period of 2 h. The reaction mixture was stirred for 2 h at -13°C, neutralized with acetic acid and immediately fractionated under reduced pressure (yield 236.0 g, 67%), b.p. 62-66°C/130-135 hPa, n_D^{21} 1.4016.

1,6-dimethoxy-3-hydroxy-4-oxo-hexane (III).: A mixture of freshly distilled aldehyde (II) (236.0 g, 2.7 mol), catalyst (I) (35.1 g, 0.134 mol) and ethanol (500 mL) was heated up to 90°C and triethylamine (78.8 g, 0.78 mol) added dropwise. After 2 h stirring at 90°C the alcohol was removed by distillation. The residue was diluted with 1,2-dichloroethane, washed with water and dried over Na_2SO_4. After filtration the liquid was fractionated under reduced pressure (yield 142.2 g, 62%), b.p. 115-119°C/16-17 hPa, n_D^{22} 1.4507.

1,6-dimethoxy-3,4-hexanedione (IV).: A mixture of (III) (146.2 g, 0,8 mol), 2-ethoxyethanol (550 mL) and acetic acid (168.0 g, 97%) was heated to 105°C and Bi_2O_3 (104.4 g, 0.22 mol) was added in one batch. Within a few minutes a colour change of Bi_2O_3 from pale yellow to black occured. The reaction mixture was stirred for 2 h at 105°C then cooled and filtered. The filtrate was diluted with 1,2-dichloroethane, washed with water until neutral and dried over Na_2SO_4. After filtration the solution was fractionated under reduced pressure (yield 53.4 g, 37%), b.p. 77-80°C/16-17 hPa, n_D^{21} 1.4235.

1,6-dimethoxy-3,4-hexanedione dioxime (V): $NH_2OH{\cdot}HCl$ (15.7 g, 0.23 mol) and NaOH (9.0 g, 0.23 mol) were dissolved in 1:1 (v:v) water/ethanol and (IV) (16.4 g, 94 mmol) was added dropwise. The mixture was stirred at 50°C for 2 h, then the solvent was carefully removed by vacuum distillation. The product was extracted from the residue with ethanol, the alcohol distilled off and the remaining crude solid product recrystallised from 7/1 (v/v) water/ethanol. White crystals were obtained (yield 3.3 g, 17%), m.p. 140-140.2°C, Anal. calcd. for $C_8H_{16}N_2O_4$ (%): C, 47.06; H, 7.84; N, 13.73. Found: C, 47.01; H, 7.81; N, 13.8. IR spectrum (KBr): 3275 $cm^{-1}\nu$(O-H), 1618 $cm^{-1}\nu_{asym}$(C=N), 1096 $cm^{-1}\nu$(C-O), 1048 $cm^{-1}\nu$(N-O). ^{1}H-NMR spectrum (acetone-d_6): δ 2.91 (t, 4H), δ 3.27 (s, 6H), δ 3.46 (t, 4H), δ 10.50 (s, 2H).

2.2.2 1,6-Diethoxy-3,4-hexanedione dioxime (DEHD)

Unless otherwise stated the synthetic operations were the same as described for DMHD.

ß-Ethoxypropionaldehyde (VI): Sodium metal was dissolved in ethanol. The reaction required

4 h instead of 2 h in the case of II. (yield 83.0 g, 20%), b.p. 64-67°C/89-90 hPa, $n_D^{20.5}$ 1.4055.

1,6-diethoxy-3-hydroxy-4-oxo-hexane (VII): (yield 102.0 g, 52%), b.p. 127°C/12 hPa, $n_D^{19.5}$ 1.4405.

1,6-diethoxy-3-hexanedione (VIII): (yield 61.0 g, 61%), b.p. 135°C/23 hPa, $n_D^{19.5}$ 1.4388.

1,6-diethoxy-3,4-hexanedione dioxime (IX): After recrystallization from 1/2 (v/v) water/ethanol white needle-shaped crystals were obtained (yield 22.0 g, 30%), m.p. 118°C, Anal. calcd. for $C_{10}H_{20}N_2O_4$: C, 51.72; H, 8.62; N, 12.07. Found: C, 51.7; H, 8.91; N, 12.1. IR spectrum (KBr): 3300 $cm^{-1}\nu$(O-H), 1610 $cm^{-1}\nu_{asym}$(C=N), 1092 $cm^{-1}\nu$(C-O), 1052 $cm^{-1}\nu$(N-O). ^{1}H-NMR spectrum (chloroform-d_1): δ 1.22 (t, 6H), δ 3.50 (t, 4H), δ 3.61 (m, 8H), δ 8.72 (s, 2H).

2.3 Preparation of the ^{99}Tc complexes

NH_4TcO_4 (50 mg, 0.13 mmol) and DMHD (846.0 mg, 4.1 mmol) or DEHD (951.0 mg, 4.1 mmol) were dissolved in 2/1 (v/v) isopropanol/water (30 mL). A solution of $NaBH_4$ (50 mg) in 0.15 M NaCl (10 mL), was added dropwise and immediately the mixture turned brown-red. To improve the yield of the complex formation, the alkaline solution was neutralized with 0.2 M HCl and kept at 50°C for 1 h. The solvent was distilled off under reduced pressure and the residue dissolved in water and the excess ligand extracted with diethylether. Excess borate and pertechnetate were removed by anion exchange. The complex was separated by cation exchange HPLC on sulphonated silica gel (Nucleosil 10 SA) followed by reversed phase HPLC on RP-2 (Multospher 5C2). Mixtures of 40/60 and 60/40 (v/v) of methanol/0.01 M NaCl were used as mobile phases for cation exchange HPLC of ^{99}Tc-DMHD and ^{99}Tc-DEHD, respectively, while mixtures of 40/60 and 55/45 (v/v) methanol/water were employed for the reversed phase HPLC of the complexes.

^{99}Tc-DMHD complex: IR spectrum (KBr): 2352 $cm^{-1}\nu$(O···H···O), 1560 $cm^{-1}\nu_{asym}$(C=N), 1180 $cm^{-1}\nu$(B-O), 1110 $cm^{-1}\nu$(N-O), 1008 $cm^{-1}\nu$'(N-O); UV/VIS spectrum (methanol): 420 nm, 327 nm, 212 nm; FAB-MS spectrum: m/z$^+$ 751.2 (M^+).

^{99}Tc-DEHD complex: IR spectrum (KBr): 2352 $cm^{-1}\nu$(O···H···O), 1560 $cm^1\nu_{asym}$(C=N), 1180 $cm^{-1}\nu$(B-O), 1110 $cm^{-1}\nu$(N-O), 1008 $cm^{-1}\nu$'(N-O); UV/VIS spectrum (methanol): 420 nm, 327 nm, 212 nm.

2.3.1 Electrophoresis

Electrophoreses of the complexes were run under an argon atmosphere at pH 8 in a borate buffer with paper (Schleicher/Schüll Nr. 0860) as a supporting medium. At an electric field strength of about 20 volts·cm^{-1} both complexes migrated towards the cathode.

2.3.2 Lipophilicity

The lipophilicity of the complexes was assessed by both reversed phase HPLC capacity factors and partition coefficients between n-octanol and physiological saline.

The retention times t_c of the isolated complexes were measured at 25°C by HPLC on a Waters Bondapak 5C8 (250 x 4 mm) analytical column using a 55/45 (v/v) methanol/water mixture as the mobile phase. The capacity factors k' were calculated taking TcO_4^- as an unretained standard.

The determination of partition coefficients K_d was performed in distributing about 1 mg of the ^{99}Tc complex in 4 mL 50/50 (v/v) n-octanol/0.15 M NaCl by a shake flask method, using a Vibrofix VF1 for 3 minutes at ambient temperature. The two phases were separated by centrifuging the emulsion. 60 μL of each phase were diluted with 0.15 M NaCl or n-octanol to 10 mL. The contents of ^{99}Tc were assayed by β^--scintillation measurements under standard geometry. Each K_d-value is the average of 3 determinations.

2.4 Preparation of the ^{99m}Tc complexes and biodistribution studies

$Na^{99m}TcO_4$ ($\approx$ 3.02 GBq) in 0.15 M NaCl solution (5 mL) was mixed with a solution of the ligand (2.5 mg) in isopropanol (5 mL) and reduced with $NaBH_4$ (1.0 mg) dissolved in 0.15 M NaCl (0.1 mL). The consecutive operations were almost identical to those described for the preparation of the ^{99g}Tc complexes. The HPLC purification was performed on a reversed phase RP-2 column under the conditions stated in section 2.3.

The separated complexes were injected into groups of 3 to 5 NMRI-mice, about 100 kBq per

mouse. The animals were sacrificed 1 min (5 mice), 10 min (4 mice) and 100 min (3 mice) post injection. The organs of interest were isolated, weighed and their radioactivity determined in an automated gamma counter.

3. RESULTS AND DISCUSSION

Both complexes proved to be quite stable cations. In repeating analytical HPLC of methanolic ^{99}Tc-DMHD or ^{99}Tc-DEHD solutions on a reversed phase RP-2 column at intervals of 24 h, almost no changes in retention times and peak intensities could be observed.

As expected both the UV/VIS spectra and the IR spectra of the homologous complexes were found to be nearly identical. The UV/VIS spectra exhibited Tc central atom bands around 420 nm, giving rise to their yellow color and ligand bands at 327 and 212 nm. After complexation the C=N vibration frequencies of the free ligands at 1618 cm^{-1} for DMHD and 1610 cm^{-1} for DEHD were shifted to 1560 cm^{1}, due to the formation of Tc-N=C bonds. Infrared absorptions at 2352 cm^{-1} belong to the O-H stretching vibrations of intramolecular hydrogen bonds. A strong band at 1180 cm^{-1} can be assigned to a B-O stretching vibration, indicating boron as a complex constituent.

In spite of repeated HPLC purification, the dioxime complexes could not be obtained in a pure crystalline state. Thus, their composition could not exactly be confirmed by elemental analysis. However, the analysis data allow at least the conclusion that the Tc-central atom is surrounded by three dioxime ligands and that the complexes contain boron as a constituent.

The positive FAB mass spectra of the chlorides of ^{99}Tc-DMHD and ^{99}Tc-DEHD were recorded. The FAB mass spectrum of the DMHD complex cation quoted for example in Fig. 2 showed the highest intensity mass peak at m/z$^+$ 751. No indications for chlorine as a complex constituent could be observed. Summarizing the analytical data described above, the M$^+$ peak can be attributed to the six coordinate complex cation depicted in Fig. 2. The structure requires the oxidation state of +5 for technetium and is in agreement with the structure of cationic technetium dioxime complexes described by Schwochau et al. [10,11].

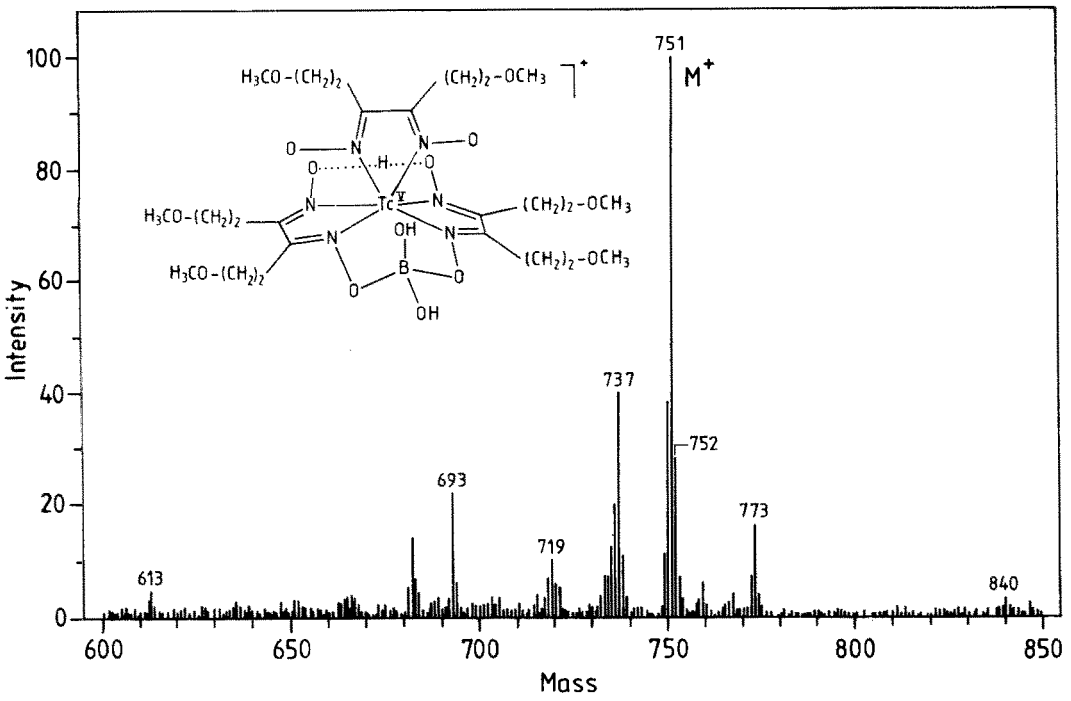

Fig. 2 Positive FAB Mass Spectra of the Cationic ^{99}Tc-1,6-Dimethoxy-3,4-hexanedione Dioxime (DMHD) Complex.

The lipophilicity of ^{99}Tc-DMHD and ^{99}Tc-DEHD differs significantly in terms of both the n-octanol/physiological saline partition coefficients K_d and the reversed phase HPLC capacity factors k' (Table 1) by factors of 62 and 16, respectively, indicating the less pronounced hydrophilic effect of the terminal ethoxy groups compared to the methoxy groups.

Table 1. Partition Coefficients K_d and Capacity Factors k' of the ^{99}Tc Dioxime Complexes

Complex	K_d	k'
^{99}Tc-DMHD	0.59	1.13
^{99}Tc-DEHD	36.80	18.16

The organ distribution of the complexes in NMRI-mice is shown in Fig. 3. Table 2 presents the heart/blood, heart/liver and heart/lungs distribution ratios.

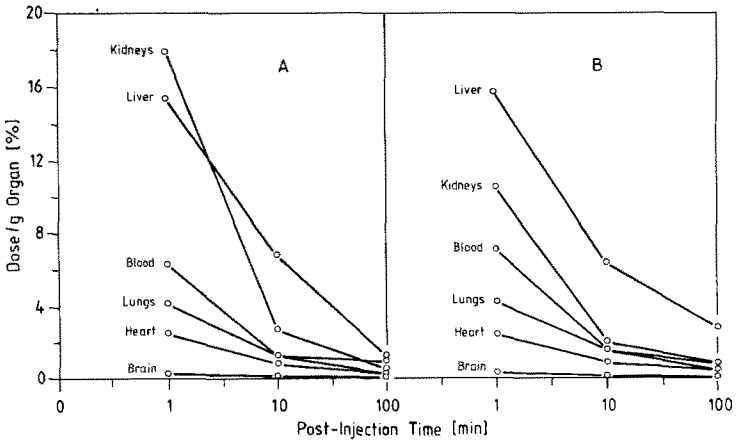

Fig. 3 Biodistribution of the ^{99m}Tc-1,6-Dimethoxy-3,4-hexanedione Dioxime (A) and ^{99m}Tc-Diethoxy-3,4-hexanedione Dioxime (B) Complexes in Mice.

In spite of the remarkably different lipophilicity of Tc-DMHD and Tc-DEHD the time dependent organ distributions of both complexes are surprisingly similar, only the kidney uptake of ^{99m}Tc-DMHD at 1 min p.i. is enhanced by 8 % dose/g as compared to ^{99m}Tc-DEHD. However, the myocardial uptake of around 2.4 % dose/g at 1 min p.i. for both complexes proves to be low, demonstrating the negligible effect of the methoxy- or ethoxy-groups introduced into 3,4-hexanedione dioxime. The ^{99m}Tc-complex of 3,4-hexanedione dioxime displayed a maximum uptake in the myocardium of about 3 % dose/g [10].

Table 2. Distribution Ratios of the DMHD- and DEHD Complexes in Mice.

Ligand	Heart/Blood			Heart/Liver			Heart/Lungs		
	1 min	10 min	100 min	1 min	10 min	100 min	1 min	10 min	100 min
DMHD	0.39	0.69	1.00	0.16	0.12	0.17	0.60	0.62	0.23
DEHD	0.34	0.54	0.56	0.15	0.13	0.15	0.57	0.56	1.00

The rate of organ clearance for ^{99m}Tc-DMHD and ^{99m}Tc-DEHD is rather fast including the clearance from the heart. Realizing the poor heart/blood, heart/liver and heart/lungs distribution ratios (Table 2) both complexes do not appear to be suitable for myocardial imaging.

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