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# SYNTHESIS OF NEW $N_2S$ LIGANDS, PREPARATION OF $^{99m}Tc$ COMPLEXES AND THEIR PRELIMINARY BIODISTRIBUTION IN MICE

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## SUMMARY

Two new $N_2S$ ligands of MPBDA (N-(2-mercapto-propyl)-1,2-benzenediamine) and MEBDA (N-(2-mercapto-ethyl)-1,2-benzenediamine) were synthesized, and their lipophilic complexes of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA were prepared in high yield (>92%) by stannous reduction of $^{99m}TcO_4^-$ . Effects of pH, reaction temperature and time on the formation of $^{99m}$Tc complexes were investigated. Potential use of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA in cerebral and myocardial imaging were evaluated in mice. High uptakes are demonstrated in both brain and heart, and residence times within heart are long. Two minutes following i.v. Administration, 1.85%ID of $^{99m}$Tc-MPBDA and 1.49%ID of $^{99m}$Tc-MEBDA appear in brain, and 1.64%ID of $^{99m}$Tc-MPBDA and 1.63%ID of $^{99m}$Tc-MEBDA are in heart. At 1hr after injection, 1.17%ID and 1.26%ID of $^{99m}$Tc-MPBDA remain respectively in brain and heart, and 0.67%ID and 1.18%ID of $^{99m}$Tc-MEBDA remain in brain and heart respectively. The activity in blood clears rapidly and the clearance half-life is less than 15 minutes. The properties of $^{99m}$Tc-MPBDA make it a potential useful agent for cerebral and myocardial perfusion imaging.

***Key Words***: *$N_2S$ ligands organic synthesis technetium complexes biodistribution*

## INTRODUCTION

A useful radiopharmaceutical for SPECT imaging rCBF must have a high cerebral uptake and a long residence time in the brain without redistribution during imaging acquisition.

Technetium-99m-labelled neutral lipophilic agents can cross the intact blood-brain barrier (BBB). In 1984, Volkert et al. showed that $^{99m}$Tc-PnAO

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{propylene amino oxime, 3,3'-(1,3-propanediyldiimino)bis-(3-methyl-2-butanone)-dioxime} can cross the intact BBB[1,2]. However, the residence time within the brain is not long enough to allow single-headed rotating SPECT imaging. Subsequently, Nowotnik et al. found that $^{99m}$Tc-d,l-hexamethylpropylene amine oxime (HMPAO) has a high cerebral uptake and long residence time to allow SPECT imaging[3]. However, the complex of $^{99m}$Tc-d,l-HMPAO is not stable in vitro, the brain/blood ratio is low[4], and quantitative SPECT images underestimate blood flow at high flow rates[5].

The neutral, lipid soluble Tc(V) oxo complexes based on the 3,6-diazaoctane-1,8-dithiol ($N_2S_2$) ligand system have shown significant uptake in the brain[6,7,8]. However, most of them were rapidly cleared from brain. In 1989, $^{99m}$Tc-l,l-ethyl cysteinate dimmer (ECD) was shown to cross the BBB in several species with significant retention only in higher species[9,10]. The cerebral retention of $^{99m}$Tc-l,l-ECD is caused by hydrolysis from a diethyl ester to the monoethyl ester by an enzyme in the brain[11]. The complex of $^{99m}$Tc-l,l-ECD is stable in vitro and has a high brain/blood ratio, but the SPECT images may not accurately reflect rCBF at high flow rates[12].

Neutral, lipophilic $^{99m}$TcO-MRP20 (N-(2(1H pyrolylmethyl))N'-(4-pentene-3-one-2))ethane-1,2-diamine) has shown significant cerebral uptake and long residence time to allow SPECT imaging[13]. The retention of $^{99m}$TcO-MRP20 in brain is caused by its decomposition.

Liu Boli et al [14]obtained the order of stability between $^{99m}$Tc-d,l-CBPAO (4,8-diaza-3,9-dimethyl-6,6-(trimethylene)-undecane-2,10-dionebisoxime) and

isomers of $^{99m}$Tc-d,l-HMPAO by CNDO/2 method in 1993. They suggested that the brain retention ability of these complexes is relate to their stability in vitro. In 1995, based on the computed results of the solid angle factor (SAFv) of the vacancy trans to Tc(V)O-chelates, Liu Boli et al[15] inferred that the proper instability of Tc(V)O-chelates for brain imaging agent may be valuable to their high brain retention. In attempt to further increase stability in vitro, C.Cutler et al[16] have reported on three new PAO derivatives $^{99m}$Tc-OCBPAO (4,8-diaza-3,9-dimethyl-6,6-(3-oxacyclobutylene)-undecane-2,10-dionebisoxime), $^{99m}$Tc-EOCBPAO (4,8-diaza-3,9-diethyl-6,6-(3-oxacyclobutylene)-undecane-2,10-dionebisoxime), $^{99m}$Tc-IPOCBPAO (5,9-diaza-2,4,10,12-tetramethyl-7,7-(3-oxacyclobutylene)-tridecane-2,11-dionebisoxime). After animal distribution investigations and in vitro stability determinations of $^{99m}$Tc-d,l-PAO derivatives, they have suggested that the retention of these complexes within the brain depends on the in vitro instability. The in vitro stability of PAO complexes appears to determine the extent to which these complexes will be retained in the brain. In our previous work[17], based on crystal data of $^{99m}$Tc-d,l-HMPAO, the structures of $^{99m}$Tc-d,l-PAO complexes have been optimized by method of INDO/1. The computed results indicate that brain retention ability of $^{99m}$Tc-d,l-PAO complexes depends on the instability in vitro of complexes.

In this paper, based on the idea above, two new $N_2S$ ligands of MPBDA (N-(2-mercapto-propyl)-1,2-benzenediamine) and MEBDA (N-(2-mercapto-ethyl)-1,2-benzenediamine) have been synthesized and their lipophilic complexes of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA have been prepared. Preliminary

biodistribution of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA have been investigated in mice. Details on synthesis of MPBDA and MEBDA have been described in this paper.

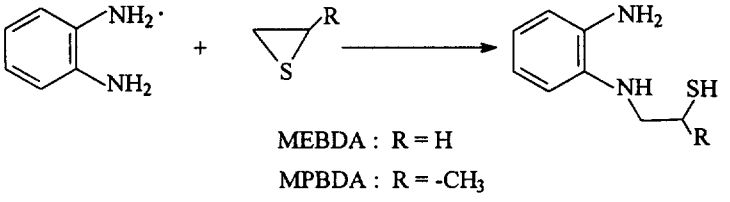


**Figure 1. Synthesis of MEBDA and MPBDA**

## MATERIALS AND METHODS

*Synthesis of MPBDA and MEBDA*

Organic compounds were characterized by melting point, $^{1}$HNMR, IR spectroscopy, elemental analysis and Mass spectroscopy. All chemicals were of reagent grade and were used as received. The ligands of MPBDA and MEBDA were synthesized as following procedure:

4.5g (0.06mol) of propylene sulfide was added dropwise into a solution of 6.6g (0.06mol) 1,2-phenylenediamine in 40 ml of absolute ethanol. The reaction mixture was refluxed under nitrogen for 8h. The solvent was removed under reduced pressure and the residue was purified though silica gel (40% EtOAc / hexane). Dry hydrogen chloride gas was bubbled into the solution to give 3.6g (26.7% yield) of white solid. The precipitated solid was collected under suction. Recrystallization from ethanol-ether provided MPBDA sample for analysis. Mp:

 

158-159 ℃. IR (KBr): 3372, 3284, 2955, 2921, 2862, 2608, 2536, 1624, 1583, 1520, 1460, 1379, 755cm$^{-1}$. MS (FAB$^+$, m/z): 183 ($M^+$), 150, 136, 121 (100), 109, 92. $^1$HNMR ($d_6$-DMSO): δ 1.34 (s, 3H), 3.02-3.20 (m, 5H), 6.60-6.80 (m, 2H), 7.10-7.30 (m, 2H). Anal. Calc. for $C_9H_{14}N_2S$·HCl: C, 49.53; H, 6.93; N, 12.84. Found: C, 49.54; H, 7.06; N, 12.52.

2.6g (21.2% yield) of white MEBDA sample was obtained in similar manner. Mp: 148-149 ℃. IR (KBr): 3310, 3120, 2832, 2536, 1624, 1530, 1480, 1454, 1325, 755cm$^{-1}$; MS (FAB$^+$, m/z): 169; $^1$HNMR ($d_6$-DMSO): δ 2.50 (s, 1H), 2.70-2.90 (m, 3H), 3.30-3.40 (m, 2H), 6.70-6.90 (m, 2H), 7.10-7.30 (m, 2H), 9.50 (w, 2H); Anal. Calc. for $C_8H_{12}N_2S$·HCl: C, 46.93; H, 6.41; N, 13.69. Found: C, 46.80; H, 6.55; N, 14.08.

*Technetium complexation*

Sodium pertechnetate - $^{99m}Tc$ was obtained from $^{99}Mo$-$^{99m}Tc$ generator( China Institute of Atomic Energy, Beijing ) by eluting with normal saline. All other chemicals were of reagent grade and were used as received. The $^{99m}Tc$ complexes of MPBDA and MEBDA were prepared as following procedure:

4mg of MPBDA (or MEBDA) was dissolved in 0.2ml absolute ethyl alcohol in a 10ml glass vial. One drop of Tween-80, 1ml of distilled water and 0.1ml of stannous chloride solution ( formed by 1mg of stannous chloride dihydrate in 1ml of 2mol/L hydrochloride acid solution ) were injected into the vial. After

 

adjusting pH of mixture, 0.6 milliliters of sodium pertechnetate ( about 1-1.2mci ) was injected into the vial. The mixture reacted at room temperature (17 ℃) for 15 minutes. Complex radiochemical purity ( RCP ) was assayed by thin layer chromatography.

*Analysis of $^{99m}$Tc complexes*

The distribution of radioactivity was determined by method of thin layer chromatography. A 1-2μl sample was applied to Xinghua No.1 chromatography strip (1cm×10cm, Beijing, China). The chromatogram was developed by ascending chromatography in tanks containing methanol / chloroform (V:V=1:9) to a depth of 1cm. The chromatography separated $^{99m}$Tc-MPBDA or $^{99m}$Tc-MEBDA (Rf = 0.9) from $^{99m}TcO_4^-$ (Rf = 0.1) and other impurities (Rf = 0.1). The radiochemical purity of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA were respectively more than 95% and 92%. After adjusting pH, the solution was used for animal studying.

*Biodistribution of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA in mice*

Biodistribution studies were performed in Kunming mice of either sex weighing between 18-20g. Each animal was administered 100μl of the saline solution containing $^{99m}$Tc complex of MPBDA (or MEBDA) through the lateral tail vein. The radiochemical purity of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA were respectively 96.2% and 94.1%. Mice were killed separately at 2min, 5min,

 

15min, 30min and 60min after injection. The organs and tissues of interest were removed and assayed for radioactivity in a gamma counter. The various organs studied were perfused prior to measurement of $^{99m}Tc$. Suitable standards, representing 1/100 of the injected dose, were prepared from the injection material. Blood values were taken as 7% of the total body weight.

## RESULTS AND DISCUSSION

### *Synthesis of MPBDA and MEBDA, Preparation and Analysis of $^{99m}Tc$ complexes*

The route of synthesis of MPBDA and MEBDA are shown in Figure 1. One molecular equivalent of propylene sulfide (ethylene sulfide) with equal molecular equivalent of 1,2-phenylenediamine to provide MPBDA in 26.7% yield (MPBDA in 21.2% yield).

The solution of MPBDA (MEBDA) and stannous chloride as reductant allow the $^{99m}Tc$ complex to be prepared simply by adding generator eluate to the vial. Thin layer chromatography permits the quantitative determination of radioactive components following complex formation. The chromatography separated $^{99m}Tc$-MPBDA or $^{99m}Tc$-MEBDA (Rf = 0.9) from $^{99m}TcO_4^-$ (Rf = 0.1) and other impurities (Rf = 0.1). Addition of $^{99m}Tc$-pertechnetate to the vial contains MPBDA and stannous chloride provides lipophilic complex of $^{99m}Tc$-MPBDA in more than 95% labelling yield immediately after complex formation.

The radiochemical purity of $^{99m}$Tc-MEBDA is more than 92%.

*Effects of pH, reaction temperature and time on the formation of $^{99m}$Tc complexes*

The ligands of MPBDA and MEBDA readily form neutral, lipophilic complexes with $^{99m}$Tc to provide new radiopharmaceuticals for cerebral perfusion imaging. Effects of pH, reaction temperature and time were investigated, and the results are shown respectively in Figure 2, 3, 4. The complex reaction may be performed only on the condition of acid media. If pH is raised to 6-7, precipitate is occurred in the solution. It is shown that this reaction is not very sensitive to temperature over 17 ℃. Therefore, it is convenient to choose room temperature as optimal reaction temperature. At pH 2.5 and room temperature (17 ℃), the radiochemical purity of $^{99m}$Tc-MPBDA is more than 96% and the radiochemical purity of $^{99m}$Tc-MEBDA is more than 92% only when mixture have reacted for 5min, and the radiochemical purity of complexes do not increase significantly when reaction time prolong.

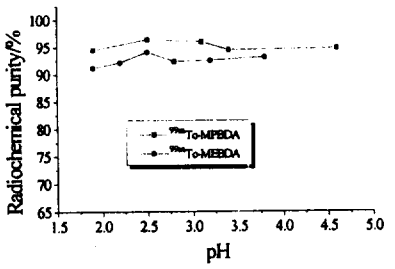


**Figure 2. Effect of pH on the formation of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA**

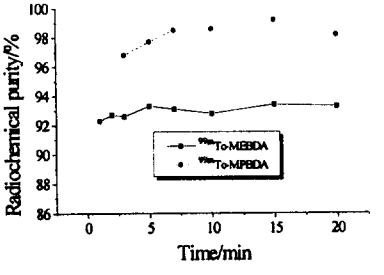


**Figure 3. Effect of reaction time on the formation of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA**

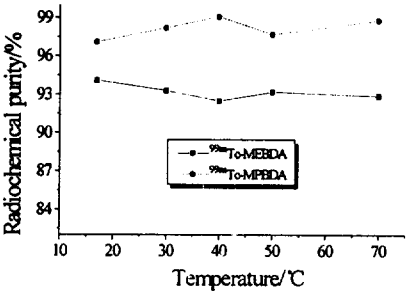


**Figure 4. Effect of reaction temperature on the formation of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA**

*Biodistributions of $^{99m}Tc$-MPBDA and $^{99m}Tc$-MEBDA complexes in mice*

The biodistributions of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA in mice are shown in Table 1 and 2. Two minutes following i.v. administration of these complexes in mice, 1.85%ID of $^{99m}$Tc-MPBDA and 1.49%ID of $^{99m}$Tc-MEBDA appear in the brain. Retention of $^{99m}$Tc-MPBDA is high, 1.17% ID of $^{99m}$Tc-MPBDA remain in the brain at 60min after injection. It is shown that about more than 10%ID of activities remain in blood at 2min postinjection. However, the activities clearance from blood are rapid, and the clearance half-lives are less than 15 minutes. The ratios of brain/blood of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA in mice are 1.42 and 0.85 respectively at 1hr postinjection.

1.64% of $^{99m}$Tc-MPBDA and 1.69% of $^{99m}$Tc-MEBDA of the injected dose are extracted into heart at 2min after injection and retention of activities are high. In comparison with the activity at 2min postinjection, more than 75% of both complexes activities remain in the heart at 1hr after injection. The

 

heart/blood, heart/liver, heart/lungs ratios of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA respectively are 7.31 and 10.01, 1.05 and 0.41, 1.78 and 0.73 at 1hr postinjection. 5 minutes following i.v. Administration of $^{99m}$Tc-MPBDA, the liver contains its peak activity of more than 15%ID. 15 minutes following i.v. Administration of $^{99m}$Tc-MEBDA, the liver contains its peak activity of more than 45%ID. The structure and retention mechanism in brain and heart of these $^{99m}$Tc complexes need to be determined by further research work.

**Table 1. Biodistribution of $^{99m}$Tc-MPBDA in mice (x±s.d., n=3)**
**Expressed as % Injected Dose / Total Tissue and % Injected Dose / g Tissue**

| | 2min | | 5min | | 15min | | 30min | | 60min | |
|---|---|---|---|---|---|---|---|---|---|---|
| tissues | %ID/organ | %ID/g | %ID/organ | %ID/g | %ID/organ | %ID/g | %ID/organ | %ID/g | %ID/organ | %ID/g |
| blood | 14.44±0.35 | 10.86±1.09 | 8.72±0.06 | 6.56±0.20 | 6.19±0.07 | 4.66±0.13 | 4.07±0.06 | 3.06±0.10 | 2.77±0.09 | 2.08±0.28 |
| brain | 1.85±0.38 | 4.80±0.99 | 1.80±0.02 | 4.95±0.30 | 1.32±0.12 | 3.67±0.29 | 1.16±0.03 | 3.08±0.12 | 1.17±0.05 | 2.95±0.26 |
| heart | 1.64±0.33 | 22.69±4.16 | 1.49±0.03 | 22.71±3.35 | 1.40±0.12 | 19.76±2.05 | 1.30±0.06 | 18.72±0.35 | 1.26±0.06 | 15.21±1.31 |
| liver | 14.78±1.72 | 21.10±2.71 | 15.77±0.41 | 23.68±2.41 | 14.43±0.11 | 22.46±0.75 | 14.90±0.32 | 19.51±1.09 | 10.17±1.08 | 14.50±1.91 |
| kidneys | 3.79±0.29 | 17.83±2.92 | 3.53±0.16 | 17.88±1.96 | 3.65±0.22 | 18.73±0.43 | 3.38±0.36 | 16.24±0.60 | 3.01±0.16 | 12.80±0.61 |
| spleen | 0.42±0.05 | 5.79±1.59 | 0.49±0.05 | 5.74±0.24 | 0.50±0.13 | 6.16±0.39 | 0.36±0.05 | 5.15±0.15 | 0.37±0.05 | 4.31±0.62 |
| lungs | 3.71±0.37 | 20.74±3.66 | 2.99±0.09 | 23.27±3.93 | 2.33±0.31 | 18.31±1.15 | 1.71±0.23 | 11.37±0.44 | 1.30±0.06 | 8.53±1.22 |

**Table 2. Biodistribution of $^{99m}$Tc-MEBDA in mice (x±s.d., n=3)**
**Expressed as % Injected Dose / Total Tissue and % Injected Dose / g Tissue**

| | 2min | | 5min | | 15min | | 30min | | 60min | |
|---|---|---|---|---|---|---|---|---|---|---|
| tissues | %ID/organ | %ID/g | %ID/organ | %ID/g | %ID/organ | %ID/g | %ID/organ | %ID/g | %ID/organ | %ID/g |
| blood | 11.01±0.88 | 8.74±0.70 | 6.62±1.26 | 5.26±1.00 | 4.77±1.09 | 3.78±0.87 | 4.65±0.17 | 3.69±0.14 | 2.86±0.05 | 2.27±0.04 |
| brain | 1.49±0.27 | 4.40±0.75 | 1.22±0.38 | 3.69±1.18 | 0.82±0.08 | 2.50±0.21 | 0.69±0.01 | 2.20±0.03 | 0.67±0.02 | 1.93±0.02 |
| heart | 1.63±0.19 | 26.42±1.63 | 1.38±0.16 | 18.77±2.30 | 1.26±0.07 | 20.07±0.61 | 1.16±0.03 | 24.02±0.64 | 1.18±0.07 | 22.92±4.15 |
| liver | 32.62±1.76 | 75.45±13.4 | 43.31±5.58 | 69.42±7.92 | 47.54±7.36 | 67.17±6.07 | 37.97±3.81 | 64.72±5.16 | 23.05±1.24 | 55.82±3.24 |
| kidneys | 3.02±0.26 | 16.71±1.18 | 2.73±0.19 | 12.74±0.12 | 3.25±0.31 | 15.07±1.79 | 3.29±0.25 | 17.98±1.85 | 5.89±0.13 | 23.75±0.53 |
| spleen | 0.79±0.03 | 26.93±0.46 | 1.67±0.05 | 18.48±0.64 | 1.53±0.13 | 20.16±0.08 | 1.48±0.15 | 22.46±1.57 | 1.29±0.08 | 18.02±2.48 |
| lungs | 10.67±0.38 | 51.25±4.12 | 5.29±0.18 | 38.03±1.26 | 5.24±0.11 | 36.78±0.14 | 5.88±0.12 | 35.62±0.17 | 3.03±0.18 | 31.56±0.24 |

**Table 3. The brain/blood and heart/tissue ratios of $^{99m}$Tc-MPBDA and $^{99m}$Tc-MEBDA**

| | $^{99m}$Tc-MPBDA | | | | | $^{99m}$Tc-MEBDA | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2min | 5min | 15min | 30min | 60min | 2min | 5min | 15min | 30min | 60min |
| brain/blood | 0.44 | 0.75 | 0.79 | 1.01 | 1.42 | 0.51 | 0.70 | 0.66 | 0.60 | 0.85 |
| heart/blood | 2.09 | 3.46 | 4.24 | 6.12 | 7.31 | 3.02 | 3.57 | 5.31 | 6.51 | 10.01 |
| heart/liver | 1.08 | 0.96 | 0.88 | 0.96 | 1.05 | 0.35 | 0.27 | 0.30 | 0.37 | 0.41 |
| heart/lungs | 1.09 | 0.98 | 1.08 | 1.65 | 1.78 | 0.52 | 0.49 | 0.55 | 0.67 | 0.73 |

## CONCLUSIONS

The rapid blood clearance and significant cerebral and myocardial uptake coupled with a long clearance time in mice brain and heart of $^{99m}$Tc-MPBDA suggest that it will be a potential cerebral and myocardial perfusion imaging agent in high species animals.

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