# A NEW BATO COMPLEX $^{99m}$TcCl(4–MCDO)$_3$MeB FOR MYOCARDIAL IMAGING*

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## ABSTRACT

A new neutral complex,$^{99m}$TcCl(4–MCDO)$_3$MeB,((Bis[4-methyl-1,2-cyclohexanedionedioximato(1-)-O] - [4-methyl-1,2-cyclohexanedione - dioximato(2-)-O]methylborato(2-)-N, N′, N″, N‴, N⁗)-chlorotechnetium, generally called BATO (Boronic acid adducts of technetium dioximes), has been synthesized and evaluated for potential use in myocardial perfusion imaging. It has intrinsic affinity for the blood clearance. The uptake of heart, lung and blood in mice at 2 min separately are 1.12, 2.48 and 6.66%ID. The complex formation is rapid, simple and highly yielded ($\geq$ 93%). This process is easy to kit formation.

**Keywords** BATO complex, Myocardial imaging agent, Heart uptake, Labeling yield

## 1 INTRODUCTION

The research of myocardial imaging agents has been primarily focused on $^{99m}$Tc agents in the past decade[1~8]. The $^{99m}$Tc-labelled isonitriles, initially developed by Johns and Davison, have been used in clinical trial[1]. $^{99m}$Tc-TBI (t-butylisonitrile), the first agent of this series, showed very high myocardial uptake and retention reflecting regional perfusion. However, the initial lung uptake and subsequent high liver retention of $^{99m}$Tc-TBI limited its clinical use[2]. $^{99m}$Tc-MIBI and $^{99m}$Tc-CPI overcame its disadvantages, and $^{99m}$Tc-MIBI has been used widely as a myocardial imaging agent in clinical practice[3~5]. J. D. Kelly described a new type of $^{99m}$Tc-complex([$^{99m}$Tc(tetrofosmin)$_2$ O$_2$]$^+$) showing rapid clearance from blood and liver. Another group of Tc(Arene)$_2^+$ compounds being a "sandwich" complex was reported[8]. The initial studies in animals showed very high myocardial uptake and prolonged retention. With the developing of imaging techniques, a kind of rapid myocardial imaging agents generally called BATO (Boronic acid adducts of technetium of dioximes) were synthesized by A. D. Nunn *et al*[6]. It has higher uptake in heart and rapid clearance from blood and also negligible lung activity. One of these complexes, $^{99m}$TcCl(CDO)$_3$MeB(SQ30217) has been used as a myocardial imaging agent in clinical trial[9,10]. But the high cost of its ingredients in kit limits its wide use in clinical trials. A new BATO complex $^{99m}$TcCl(4-MCDO)$_3$MeB

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overcomes this disadvantage and has a similar biodistribution compared with the previous report[9]. Its assumed structure is shown in Fig.1 according to the structure of $TcCl(CDO)_3MeB$[11].

## 2 MATERIALS AND METHODS

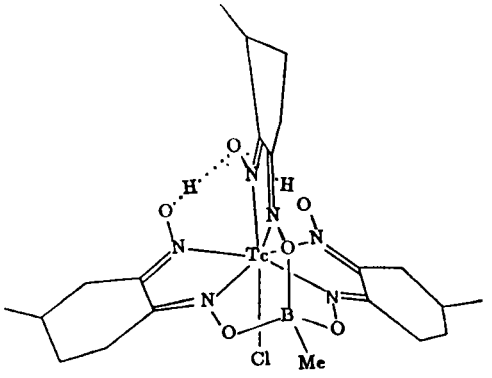


**Fig.1 Structure of $^{99m}TcCl(4\text{-}MCDO)_3MeB$**

### 2.1 Reagents

The complex $^{99m}TcCl(4\text{-}MCDO)_3MeB$, was prepared by a method reported previously[9]. What we adopted in this paper is that $\gamma$-cyclodextrin is eliminated from the kit and the reaction temperature is lower. Diethylenetriamine pentaacetic acid (DTPA) is purchased from Shanghai Chemical Agent Department. Sodium chloride (NaCl), DMG (dimethylglyoxime) are purchased from Beijing Chemical Industry Plant. Methylboronic acid ($MeB(OH)_2$) is obtained from Aldrich. $^{99m}TcO_4^-$ is obtained by eluting $^{99m}Mo$-$^{99m}Tc$ generator (China Institute of Atomic Energy) with normal saline.

### 2.2 Radiolabeling

The $^{99m}TcCl(4\text{-}MCDO)_3MeB$ is prepared to form a lypophilized kit by addition of 0.1 ml anhydrous ethanol and 0.9 ml pertechnetate (containing 37~370 MBq) to a 10 ml vial containing the 4-methyl-cyclohexane-(1,2-dione-dioxime(MCDO) (12.7 $\mu$mol), methyl boronic acid (30.0 $\mu$mol), $SnCl_2$(0.25 $\mu$mol) as a reduction agent, anhydrous citric acid (45 $\mu$mol). The mixture is stirred and heated at 80°C for 10 min. The percent labeling yield is measured by chromatography. A 1~5 $\mu$l sample is moved to silica gel paper (1cm×10cm, Shanghai). And then two same strips are separately developed with 0.9% NaCl and 0.9% NaCl/acetone (50:50 = $V$ : $V$) solution in small jars[9]. The first chromatography could separate BATO ($R_f$ = 0.1) from $^{99m}TcO_4^-$($R_f$ = 0.7) and other lipophilic impurities. The other could separate BATO ($R_f$ = 0.7) from reduced hydrolyzed and other radio impurities ($R_f$ = 0.1 and $R_f$ = 1.0). The radiochemical purity (RCP) is estimated as:

$$\%\ \text{of RCP(BATO)} = 100 - \text{impurity}\%\ \text{(1st strip)} - \text{impurity}\%\ \text{(2nd strip)}$$

where in the 1st strip, the impurity activities appeared in the front ($R_f$ = 0.7 and 1.0) and in the 2nd strip, the impurity activities are in the origin and front ($R_f$ = 0.1 and 1.0). The $R_f$ of BATO complex is 0.1 and 0.7 separately in the two developing solvent. The radiochemical purity is over 93%. This material, adjusted the pH to 6~7 by 1 mol/L NaOH, is then used for animal studies.

### 2.3 Biodistribution in mice

The myocardial uptake of $^{99m}$TcCl(4-MCDO)$_3$MeB is measured in female mice (18~22 g). Saline solution containing $^{99m}$TcCl(4-MCDO)$_3$MeB in a volume of 0.1 ml is injected directly into the tail vein. Mice are killed separately at 2, 15 and 30 min post injection by cardiac excision. The organs of interest are removed and counted using a well gamma counter. Percent dose per organ is calculated by tissue counts in comparison to suitably diluted aliquots of injected materials. Total activities of blood are calculated assuming that they are 7% of total body weight.

## 3 RESULTS

### 3.1 Effect of time

At 60°C, pH 2, the labeling yield is evaluated at various reaction times. The formation of the complex reaches a plateu at 10 min (see Fig.2). Prolonged reaction seems to have no significant effect to the labeling yield.

### 3.2 Biodistribution of mice

The biodistribution of $^{99m}$TcCl(4-MCDO)$_3$MeB in mice is shown in Table 1. The complex shows good heart uptake with rapid clearance from blood. And there is no significant uptake in lung. It also has a longer retention time in heart (15 min, 0.68%ID). The high stability of this complex is also improved by measuring its radiolabeling yield after labeling 8 h. The ratios of heart to blood, to lung and to liver are reported in Table 2. This complex has a similar biodistribution with $^{99m}$TcCl(CDO)$_3$MeB reported by Rama K Narra[9]. However, the uptake data in organs of interest cannot compare since mice (18~22 g) and rats (225~275 g) are used in animal studies, respectively.

**Table 1**

**Biodistribution of $^{99m}$TcCl(4-MCDO)$_3$MeB in mice expressed as % injected dose/total tissue and % injected dose/g tissue ($n$=9)**

| | 2 min | | 15 min | | 30 min | |
|---|---|---|---|---|---|---|
| | %ID/organ | %ID/g organ | %ID/organ | %ID/g organ | %ID/organ | %ID/g organ |
| blood | 6.66±0.06 | 4.76±0.30 | 2.39±0.02 | 1.74±0.04 | 2.11±0.12 | 1.53±0.04 |
| brain | 0.10±0.01 | 0.28±0.03 | 0.06±0.01 | 0.17±0.03 | 0.07±0.02 | 0.20±0.08 |
| heart | 1.12±0.18 | 16.61±1.17 | 0.68±0.08 | 14.40±4.35 | 0.48±0.05 | 6.40±1.02 |
| liver | 16.01±1.01 | 19.68±3.01 | 12.85±1.40 | 17.45±0.36 | 10.44±0.28 | 15.52±5.40 |
| kidneys | 2.30±0.02 | 9.59±0.46 | 1.96±0.21 | 10.24±0.54 | 1.55±0.24 | 6.72±0.74 |
| spleen | 0.55±0.11 | 5.97±0.50 | 0.38±0.01 | 7.15±2.56 | 0.28±0.02 | 4.68±1.81 |
| lungs | 2.48±0.05 | 17.17±3.12 | 1.63±0.05 | 12.88±2.33 | 1.42±0.08 | 12.73±3.82 |

**Table 2**

**Ratios of heart to tissues for $^{99m}$TcCl(4-MCDO)$_3$MeB in mice**

| | 2 min | 15 min | 30 min |
|---|---|---|---|
| Heart/Blood | 3.49 | 8.28 | 4.18 |
| Heart/lung | 1.10 | 1.12 | 0.50 |
| Heart/liver | 0.96 | 0.82 | 0.41 |

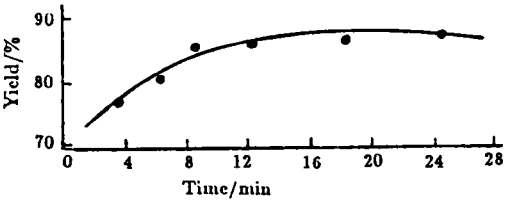


**Fig.2 Effects of time on the formation of $^{99m}$TcCl(4-MCDO)$_3$MeB**

## 4 DISCUSSION

The formation of this complex is easy, rapid and stable. This process is amable to kit formation. Compared with the previously reported method[12], the method adopted in this paper has two advantages: one is lower cost, the other is simple and convenient reaction conditions. These characteristics can promote BATO complex wide use in clinical trials, especially in the developing country.

The labeling reaction was completed at pH 1~2. If pH was raised into 5~6, the solution will precipitate and reaction cannot continue. The optimum pH is that of the kit solution. This reaction is sensitive to temperature, radiolabeling yield increases with the temperature increase. However, it is unfavorable to this labeling reaction over 90°C. Generally, 80~85°C was chosen as optimum reaction temperature.

The net charge of $^{99m}TcCl(4\text{-}MCDO)_3MeB$ should be zero according to the previous report. However, it has a significant heart uptake (2 min, 1.12%ID). This complex and many other neutral complexes have good heart uptake in reported literature. It has improved that the complex with +1 net charge will be no longer an essential condition if it will be a myocardial imaging agent. This complex also shows rapid blood clearance and low lung uptake; at 15 min post injection the heart/blood and heart/lung ratios reach 8.28 and 1.12, respectively. The biologic behavior of $^{99m}TcCl(4\text{-}MCDO)_3MeB$ clearly suggests that this agent is potentially useful for myocardial perfusion imaging.

In addition, further studies on dog, monkey and person are needed. Especially, there is no myocardial imaging agent which is designed and prepared by our Chineses used in clinical trials at present. Preparing a new, not mimic myocardial agent with Chinese characterization will be very significant and important now and in the future.

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