CHAPTER 2

Symmetric [2+2] Complexes

2.1 Introduction

As detailed in the previous chapter, radiopharmaceutical preparations with enhanced specific activity and stability are the key requirements when specific binding to the receptor or antigen on the targeted tissues is the mechanism of action. In the case of \(^{99m}\)Tc based radiopharmaceuticals, this has led to the development of novel technetium cores such as \([^{99m}\text{TcN}]^{2+}\), capable of labeling various molecules and forming high specific activity complexes.\(^7\) This core has high affinity for bi-dentate ligands having groups such as dithiocarbamates and xanthis (S donors) forming square pyramidal complexes of type \([^{99m}\text{TcNL}_2]\) [L = bi-dentate ligand] where Tc≡N occupies the apical position and the four S atoms span the basal plane. Since two molecules of the same ligand (L) are bound to the \([^{99m}\text{TcN}]^{2+}\) core and the complexes formed are symmetric along a plane passing through Tc≡N moiety, such class of complexes are termed ‘symmetric [2+2] complexes’ (Fig. 2.1).

![Fig. 2.1 General structure of symmetric [2+2] complexes](image)

The \([^{99m}\text{TcN}]^{2+}\) intermediate core has an overall charge of +2, which on complexation with two molecules of uni-negative anion (dithiocarbamate/ xanthate sodium salts) forms neutral symmetric [2+2] complexes. Also, the lateral alkyl groups present on
dithiocarbamates/xanthates provide lipophilic nature to the complexes. The neutral and lipophilic properties are necessary attributes of radiopharmaceutical preparations intended for myocardial perfusion imaging.\textsuperscript{223}

The present chapter has been divided into two parts where the first part deals with symmetric [2+2] complexes prepared using dithiocarbamate ligands and the latter with a xanthate ligand. The work involves synthesis of suitable ligands, radiolabeling with $[^{99m}\text{TcN}]^{2+}$ core and subsequent evaluation as a myocardial agent in suitable small animal.
2.2 \([^{99m}\text{Tc}N]-\text{Dithiocarbamate symmetric [2+2] complexes as myocardial perfusion tracers}\)

2.2.1 Introduction

The search for a new \(^{99m}\text{Tc}\) based myocardial imaging agent with optimum characteristics is a continuing and relevant field of research till date. \(^{201}\text{TlCl}\), the currently used gold standard for myocardial perfusion imaging is produced in a cyclotron and hence less accessible. In addition, it has other limitations due to its long half-life (73 h) and large radiation dose to the patient.\(^{224}\) The characteristics of \(^{99m}\text{Tc}\)-labeled myocardial imaging agents currently available \(v_i\)z. \(^{99m}\text{Tc}\)-methoxyisobutyl isonitrile (MIBI) and \(^{99m}\text{Tc}\)-tetrofosmin, are also far from being ideal.\(^{191}\) Unlike \(^{201}\text{TlCl}\), the aforementioned agents do not undergo redistribution in the myocardium\(^{225}\) warranting the unavoidable double injection protocol that imposes logistic problems. Moreover, these uni-positively charged complexes are taken up and retained in the heart for a longer time due to the negative membrane potential of the myocytes.\(^{226}\) This hinders the possibility of rest and stress studies, needed to distinguish between ischemic and infarcted regions of the myocardium, to be performed on the same day. Hence, currently these agents are injected at a small dose (typically 185 MBq or 5 mCi) initially for stress studies followed by a large dose (typically 740 MBq or 20 mCi) after a gap of 4-6 h. A logical approach therefore, constitutes the search for an agent that is neutral and sufficiently lipophilic to be taken up and retained in the heart for sufficient time, but not too long. In this direction, \(^{99m}\text{Tc}\)-teboroxime was the first of a class of neutral complexes that showed myocardial extraction, but had the serious limitation of being rapidly washed out from the target tissue.\(^{227}\) \(^{99m}\text{Tc}(\text{NOEt})_2\) complex, which makes use of the \([^{99m}\text{Tc=\text{N}}]^2\) core is one of the latest in this category, showing considerable promise as a myocardial perfusion imaging agent, and is currently in phase III clinical trials.\(^{182}\) This agent
shows good redistribution in heart like $^{201}$Tl, and can be administered at a higher activity in a single injection, leading to better image quality at a lower radiation dose to the patient, compared to that with $^{201}$TlCl. However, its use is limited owing to high lung and liver uptake, warranting a better formulation.

1-isocyano-2-methoxy-2-methylpropane (MIBI) labeled with $^{99m}$Tc is one of the predominant $^{99m}$Tc-labeled myocardial imaging agents in clinical use today. $^{99m}$Tc labeled 2-isocyano-2-methylpropane (TBI) has also shown good myocardial uptake, although high accumulation of activity in the liver and lung make it unattractive for regular clinical use. In an effort to develop a $^{99m}$Tc-based neutral agent that could strike a perfect balance between redistribution and hence optimum residence time to enable rest-stress study on the same day as well as achieving good target to non-target ratio, two dithiocarbamate ligands were synthesized in the present study, using t-butylamine and methoxyisobutyl amine as the respective precursors. These compounds structurally resemble TBI and MIBI ligands except for the co-ordinating group (Fig. 2.2), where a dithiocarbamate group has replaced the isonitrile group. Labeling was carried out using a $[^{99m}$TcN]$^{2+}$ core and the complexes prepared were studied with respect to

![Fig. 2.2 Structural similarity between (a) clinically used ligands and (b) synthesized ligands](image-url)
their myocardial uptake. The results obtained were compared with that of [$^{99m}$TcN(NOEt)$_2$] which is now undergoing phase III clinical trials.

2.2.2 Experimental

2.2.2.1 Materials and methods

2-methylpropan-2-amine and 2-hydroxy-2-methylpropanenitrile were purchased from M/s Lancaster, England. Sodium N-ethoxy, N-ethyl dithiocarbamate (NOEt), dimethyl cyclodextrin (DMC) and [$^{99m}$TcN]$^{2+}$ intermediate kit vial were obtained as a gift from Schering Cis Bio International, France through an IAEA coordinated research project. Lithium aluminium hydride (LAH), 15-hydroxypentadecanoic acid, methyl chloroethanamine hydrochloride, 2-bromoethyl methyl ether, ethyl p-toluene sulphonate, n-butyl lithium (2.5 M in hexane), S-trityl cysteine, hexadecanedioic acid, 11-amino undecanoic acid, 16-bromo hexadecanoic acid, triethyl silane, succinic dihydrazide, stannous chloride and cannula (two tips flexible steel needle) were obtained from M/s Aldrich, USA. Potassium diphenyl phosphide solution in THF (0.5 M), 12-amino dodecanoic acid, 15-bromo pentadecanoic acid, N-Boc, S-trityl cysteine, anhydrous K$_2$CO$_3$ and 1-(3-dimethylaminopropyl)-3-carbodiimide (EDCI) were purchased from M/s Fluka, Germany. Hydrazine hydrate (80%) was purchased from M/s Riedel-de-Haën, Germany. Phthalimide was obtained from M/s Loba Chemicals, India. Sodium hydroxide, anhydrous zinc chloride, carbon disulphide, diethanolamine and thionyl chloride were purchased from M/s S.D. Fine chemicals, Mumbai, India. Iodophenyl pentadecanoic acid (IPPA) was purchased from Emka Chemicals, Germany. All other reagents used were of analytical or HPLC grade. All the organic extracts were dried over anhydrous Na$_2$SO$_4$. The solvents were dried following standard protocols. The solvents used for HPLC were filtered through 0.22 μm Millipore filter paper. Bis[(diethoxypropylphosphanyl)ethyl]ethoxyethylamine was obtained as a gift from Prof.
Adriano Duatti, Italy. Na$^{125}$I was obtained from Radiochemicals section, Radiopharmaceuticals Division, BARC, India. Sodium pertechnetate (Na$^{99m}$TcO$_4$) was eluted with saline just before use from a $^{99}$Mo-$^{99m}$Tc gel generator supplied by Board of Radiation and Isotope Technology, India. Silica gel plates (Silica Gel 60 F$_{254}$) were obtained from M/s Merck, India. Whatman chromatography paper (Whatman 3 mm Chr, 20 mm width, Maidstone, England) was used for paper electrophoresis.

**Instrumentation methods**

The radioactivity profile of TLC and Paper electrophoresis strips were determined using GINA-Star TLC chromatography evaluation system, Germany. Chromatograms of the prepared complexes were obtained with either a JASCO PU 1580 or JASCO PU 2080 Plus dual pump HPLC system, with a JASCO 1575 or JASCO 2075 Plus tunable absorption detector and a radiometric detector system, using a C18 reversed phase HiQ Sil (5 µm, 4 × 250 mm) column. Elemental analyses were performed with a C, H, N, S elemental analyzer, Thermofinnigan, Flash EA 1112 series, Italy. The IR spectra of the compounds were recorded with a JASCO FT-IT/420 spectrophotometer, Japan. The $^1$H NMR spectra were recorded with either 200 or 300 MHz Bruker spectrophotometer or 300 MHz Varian spectrophotometer, USA. $^{31}$P NMR spectra were recorded with a 300 MHz Bruker spectrophotometer. Mass spectra were recorded with either QTOF micromass instrument using electron spray ionization (ESI) in positive mode or Varian Prostar mass spectrometer using ESI in negative mode.

**2.2.2.2 Synthesis**

2.2.2.2.1 Synthesis of sodium tert-butylcarbamodithioate (TBDTC) (1)

In a typical procedure, 2-methylpropan-2-amine (0.5 mL, 4.73 mmol) and crushed sodium hydroxide (385 mg, 9.6 mmol) were stirred vigorously in diethyl ether (15 mL) cooled in an ice bath. To the stirred solution, carbon disulphide (0.3 mL, 4.73 mmol) was
added dropwise and stirring continued for 30 min. The reaction mixture was brought to room temperature, stirring continued for another 2 h, the white precipitate was collected by filtration, washed with dry ether and dried under vacuum.

Yield: quantitative (808 mg).

Elemental analysis (C,H,N,S): Observed (Calculated) 35.10 (35.07), 6.23 (5.89), 8.05 (8.18), 37.33 (37.45).

2.2.2.2 Synthesis of sodium 2-methoxy-2-methylpropylcarbamodithioate (MIBDTC) (2)

2-methoxy-2-methylpropanitrile (2a)

2-hydroxy-2-methylpropanitrile (1 mL, 11 mmol) was added to a solution of freshly fused zinc chloride (1.47 g, 11 mmol) in excess of anhydrous methanol. The reaction mixture was refluxed overnight in an oil bath. After cooling to room temperature, the reaction mixture was poured into ice water and extracted with ether. The ether layer was washed with brine (saturated NaCl solution) and dried. Ether was removed by simple distillation and the pure product was collected by distilling at 120°C.

Yield: 96% (1.04 g).

1H NMR (CDCl₃, δ ppm): 3.25 (s, 3H, -OCH₃); 1.31 (s, 6H, (CH₃)₂C-) [Fig. 2.3]

2-methoxy-2-methylpropan-1-amine (2b)

In a three-necked flask, fitted with a nitrogen inlet, dropping funnel and reflux condenser, LAH (0.8 g, 20 mmols) was taken and suspended in dry diethyl ether (25 mL). The mixture was kept under nitrogen and cooled in an ice bath. Compound 2a (1 g, 10 mmol) dissolved in dry ether (2 mL), was added dropwise to the well stirred reaction mixture. After 30 min, the reaction mixture was brought to room temperature and stirred overnight. The unreacted LAH was quenched by dropwise addition of aqueous saturated Na₂SO₄ solution. The precipitate formed was filtered through a sintered funnel and washed with ether (2 × 15
The ether layer was washed with brine and dried. The ether was removed by distillation and the pure compound 2b isolated by distillation at 110 °C.

Yield: 80% (824 mg).

1H NMR (CDCl₃, δ ppm): 3.26 (s, 3H, -OCH₃); 2.65 (s, 2H, -CH₂); 1.6 (s, 2H, -NH₂); 1.16 (s, 6H, (CH₃)₂C-) [Fig. 2.4].

Sodium 2-methoxy-2-methylpropylcarbamodithioate (MIBDTC) (2)

The procedure followed for the synthesis of 2 was similar to that followed for the synthesis of 1 using 2b as the starting material.

Yield: 95% (800 mg).

Elemental analysis (C,H,N,S): Observed (Calculated) 34.90 (35.80), 6.50 (6.01), 6.80 (6.96), 31.50 (31.86).
Fig. 2.3 $^1$H NMR spectrum of compound 2a
Fig. 2.4 $^1$H NMR spectrum of compound 2b
2.2.2.3 Radiolabeling

2.2.2.3.1 Preparation of $[^{99m}\text{TcN}]^{+2}$ core

The kit vial for the preparation of $[^{99m}\text{TcN}]^{+2}$ core stored at 4 °C was allowed to attain ambient temperature. To this kit vial containing succinic dihydrazide (5 mg) and stannous chloride (0.1 mg), freshly eluted $^{99m}\text{TcO}_4^-$ (1 mL, 37 MBq or 1 mCi) was added, vortexed for 1 min and allowed to stand at room temperature for 20 min. The $^{99m}\text{Tc}$-nitrido core, thus prepared was characterized by TLC, paper electrophoresis and HPLC.

2.2.2.3.2 Preparation of $^{99m}\text{TcN}$-Dithiocarbamate complexes

The synthesized dithiocarbamate ligand (1 or 2, 0.1-1 mg) was dissolved in saline (0.5 mL). To this, freshly prepared $[^{99m}\text{TcN}]^{+2}$ core (0.5 mL) was added, vortexed for 1 min and the reaction vial incubated for 10 min at room temperature to give desired complexes 1C and 2C respectively. The complexes formed were characterized by paper electrophoresis and HPLC.

2.2.2.3.3 Preparation of $^{99m}\text{TcN(NOEt)}_2$

The $[^{99m}\text{TcN}]^{+2}$ core (0.5 mL) was added to a vial, containing NOEt (10 mg) and DMC (10 mg) ligands dissolved in saline (0.5 mL), and vortexed for 1 min and incubated at room temperature (~25 °C) for 10 min. The complex formed was characterized by TLC.

2.2.2.4.4 Optimization studies

Parameters such as ligand concentration and reaction time were optimized to obtain maximum complexation yield. Variation in complexation yield was studied by changing the ligand concentration from 0.001 mg/mL to 1 mg/mL.

2.2.2.4 Quality control techniques

2.2.2.4.1 Thin layer chromatography (TLC)

TLC was carried out using 11 cm long flexible silica gel plates. About, 1-2 μL of the test solution was spotted at 1.5 cm from the bottom of the TLC strip. The TLC strip was
developed in a suitable solvent, dried, cut into 1 cm segments and the radioactivity associated with each segment was measured using a well-type NaI(Tl) detector. For the characterization of $^{99m}$Tc-nitrido intermediate species, the plates were developed in ethanol: chloroform: toluene: 0.5M ammonium acetate (6:3:3:0.5 v/v) as well as in saline. The analysis of $^{99m}$TcN(NOEt)$_2$ complex was carried out using ethyl acetate as the developing solvent.

2.2.2.4.2 *Paper electrophoresis*

Paper electrophoresis was done using a Whatman No.3 chromatography paper and 0.05 M phosphate buffer (pH 7.4). About 2 µL of the test solution was applied at the middle of the strip and the strip was developed under a potential gradient of 10 V/cm for 1 h. The strip was dried, cut into 1 cm segments and the radioactivity associated with these was determined using a well-type NaI(Tl) detector.

2.2.2.4.3 *High performance liquid chromatography (HPLC)*

The $[^{99m}TcN]^{2+}$ core and the labeled complexes were analyzed by HPLC using about 25 µL (925 KBq or 25 µCi) of the test solution. Acetonitrile: water (80:20 v/v) mixture was used as the mobile phase, under isocratic condition, at a flow rate of 1 mL/ min.

2.2.2.4.4 *Partition coefficient*

Lipophilicity (LogP) of the complexes was determined from octanol/ saline partition coefficient (P) following a reported procedure.$^{229}$ For the assay, solvent extraction was performed by mixing 1 mL of the reaction mixture with 1 mL of octanol on a vortex mixer for about a minute. The two phases were allowed to separate. Equal aliquots of the organic and aqueous layers were withdrawn and measured for the radioactivity. The organic extract was back-extracted repeatedly with saline to estimate the distribution ratio.

2.2.2.4.5 *Bio-distribution studies*

Normal adult Swiss mice (20-25g body weight) were used for the bio-distribution studies. The radiolabeled preparation (3.7 MBq or 0.1 mCi in 100µL) was injected
intronvenously via the tail vein. Individual sets of animals \((n = 3)\) were utilized for studying the bio-distribution at different time points (5 min, 10 min, 30 min and 60 min). The animals were sacrificed immediately at the end of the respective time point and the relevant organs and tissue were excised. The organs were weighed and the radioactivity associated with each was measured with a flat-bed type NaI (Tl) counter using a suitable energy window for \(^{99m}\text{Tc}\) (140 keV \(\pm 10\%\)). For the sake of comparison, the activity retained in each organ/ tissue was expressed as percent value of the injected dose per gram (\% ID/ g). All procedures performed herein were in strict compliance with the national laws governing conduct of animal experiments.

2.2.3 Results and Discussion

2.2.3.1 Synthesis of the ligands

The literature procedure was followed for the preparation of \(1\) and \(2\).\(^{181}\) The general scheme for the synthesis of dithiocarbamate ligand is shown in Fig. 2.5. The sodium salt of dithiocabamate was precipitated on reaction of \(\text{CS}_2\) with respective amines in presence of sodium hydroxide base. The amine precursor \(2b\) used for the synthesis of \(2\) was synthesized following the reaction scheme shown in Fig. 2.6. The intermediate \(2b\) was obtained on \(O\)-methylation of acetone cyanohydrin using anhydrous zinc chloride and MeOH followed by reduction of the intermediate \(2a\) using LAH. Both the intermediates, compound \(2a\) and \(2b\), were characterized by \(^1\text{H}\) NMR spectroscopy. The final dithiocarbamate ligands (\(1\) and \(2\)) were characterized by elemental analyses.

![Fig. 2.5 General scheme for the synthesis of dithiocarbamate ligands](image-url)
2.2.3.2 Radiolabeling

The two synthesized dithiocarbamate derivatives act as bi-dentate ligands to coordinate with $^{99m}\text{Tc}$ via the $[^{99m}\text{TcN}]^{2+}$ intermediate core. The scheme for the formation of $[^{99m}\text{TcN}]^{2+}$ intermediate core is shown in Fig. 2.7(a). The radiolabeling strategy involved prior preparation of $[^{99m}\text{TcN}]^{2+}$ intermediate using a kit vial containing succinic dihydrazide (SDH) which acts as a nitride donor ($\text{N}_3^-$) and stannous chloride as reducing agent. To the freshly prepared $[^{99m}\text{TcN}]^{2+}$ intermediate, the respective dithiocarbamate ligand (1 or 2) was added to obtain the desired complexes [1C or 2C, Fig. 2.7(b)].

The $^{99m}\text{Tc}$-nitrido intermediate was characterized by TLC and paper electrophoresis. The TLC was carried out using ethanol: chloroform: toluene: 0.5M ammonium acetate mixture (6:3:3:0.5 v/v) as well as saline as developing solvents. In the former solvent system, $^{99m}\text{Tc}$-nitrido intermediate species remained at the point of application ($R_f = 0–0.1$) with

![Fig. 2.6 Scheme for the synthesis of methoxyisobutyl amine](image)

![Fig. 2.7 Syntheses of (a) $[^{99m}\text{TcN}]^{2+}$ intermediate and (b) $[^{99m}\text{TcN}]$-dithiocarbamate complexes](image)
insignificant activity corresponding to $^{99m}$TcO$_4^-$ ($R_f = 0.4-0.6$). In the latter solvent system, most of the activity moved with the solvent front ($R_f = 0.8$-1). The small amount of activity, observed at the point of sample application was attributable to the possible presence of reduced technetium, which does not move in any solvent. Thus, knowing the percentage of unreacted pertechnetate remaining in the reaction mixture from the former solvent system and percentage of reduced technetium in the reaction mixture from the latter solvent system, the extent of formation of $^{99m}$Tc-nitrido intermediate was determined and found to be >98%. In paper electrophoresis, the $^{99m}$Tc-nitrido intermediate showed a movement of 5 cm/h towards the anode, thus, confirming the negative charge on the technetium nitrido intermediate [Fig. 2.8(a)].

Both the $^{99m}$TcN-dithiocarbamate complexes 1C and 2C were prepared in >95% yield at a low ligand concentration of 0.01 mg/mL [5.8x10$^{-5}$ M] for 1 and 1 mg/mL [4.8x10$^{-3}$ M] for 2. The complexes 1C and 2C were characterized by paper electrophoresis and HPLC. In paper electrophoresis almost all the activity was found at the point of application indicating the formation of neutral complexes [Fig. 2.8(b) & 2.8(c)]. The HPLC chromatograms of the $^{99m}$TcN-intermediate species, 1C and 2C are shown in Fig. 2.9. It was observed that the retention time of $^{99m}$TcN-intermediate species was 2.9 ± 0.2 min, while that of radiolabeled complexes 1C and 2C were 4.5 ± 0.2 min and 4.1 ± 0.1 min respectively.

The procedure followed for the synthesis of $^{99m}$TcN-(NOEt) complex was similar to that of the other two dithiocarbamate complexes. The formation of the complex was confirmed by TLC analysis using EtOAc as the mobile phase. The complex was formed in >98% yield and showed a movement of $R_f = 0.7$-0.8, whereas the nitrido intermediate remained at the point of spotting ($R_f = 0$-0.1).
Fig. 2.8 Electrophoresis patterns of (a) $^{99m}$TcN intermediate, (b) complex 1C and (c) complex 2C
Fig. 2.9 HPLC profiles of (a) $^{99m}$TcN intermediate, (b) complex 1C and (c) complex 2C
Dithiocarbamates are known to complex with $[^{99m}\text{Tc}]^2+\text{core}$ leading to neutral complexes of $^{99m}\text{TcN}_2\text{L}_2$ type\textsuperscript{171} having square pyramidal geometry with an apical $^{99m}\text{Tc}≡\text{N}$ bond and four sulphur atoms spanning the basal plane. Since the two ligands (1 and 2) used for complexation via $[^{99m}\text{TcN}]^2+\text{core}$ belongs to the same class of dithiocarbamates, the two neutral complexes formed could be envisaged to possess the formula $^{99m}\text{TcN(TBDTC)}_2$ and $^{99m}\text{TcN(MIBDTC)}_2$ respectively and form square pyramidal complexes.

In the case of complex 1C, more than 95% complexation yield was observed at a concentration as low as 0.01 mg/mL, whereas in the case of complex 2C, the yield was more than 90% at 0.1 mg/mL concentration. The formations of the two $^{99m}\text{TcN}$-complexes 1C and 2C were instantaneous at room temperature and the preparations were stable for over a period of 20 h at room temperature with ~90% retention of radiochemical purity.

2.2.3.3 Biological studies

The prepared complexes 1C and 2C were bio-evaluated, to evaluate their potential as myocardial agent. Some of the essential characteristics of a $^{99m}\text{Tc}$ formulation as myocardial agent are

(i) *in-vivo* kinetic inertness, so that it does not get degraded inside the body by potential chelates present, such as cysteine, glutathione, serum proteins, etc.

(ii) lipophilic nature, so as to cross the lipid bi-layer membrane of the cell

(iii) high uptake in the normal myocardium with a long enough retention to carry out SPECT imaging

(iv) rapid clearance from background tissue/ organs such as blood, liver and lungs so as to get a clear image of the target organ. Hence, target/ non-target ratios such as heart/ blood, heart/ liver and heart/ lungs are critical in evaluating the final quality of the image obtained by a myocardial agent.

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For the complexes 1C and 2C, \textit{in-vitro} challenge experiments, to determine the kinetic stability were not carried out due to the known stability of such category of complexes from the literature.\textsuperscript{181} In view of the other parameters, the lipophilicity of the prepared complexes was determined. The Log P values of 1C, 2C and \textsuperscript{99m}TcN(NOEt)\textsubscript{2} complexes in octanol/ saline partition system were found to be 1.7, 1.9 and 1.98 respectively indicating their high lipophilicity.

To evaluate the other characteristics, \textit{in-vivo} bio-distribution of 1C and 2C were carried out with normal Swiss mice and the results are shown in Tables 2.1 and 2.2 respectively. The bio-evaluation of the complex 1C and 2C warranted comparison with a known standard agent, hence, \textsuperscript{99m}TcN(NOEt)\textsubscript{2} which belongs to similar category of complexes and is currently in phase III clinical trials was taken as a standard. Table 2.3 shows the bio-distribution results of the standard agent \textsuperscript{99m}TcN(NOEt)\textsubscript{2} in Swiss mice. Fig. 2.10 shows the myocardial uptake and clearance pattern of 1C, 2C and the standard agent. The two complexes showed significant uptake in the myocardium. Among the two, 1C showed high initial myocardial uptake [8.97 ± 0.51% ID/g at 5 min post injection (p.i.)],

![Graph showing myocardial uptake pattern of complexes 1C and 2C in comparison with \textsuperscript{99m}TcN(NOEt)\textsubscript{2}](image)

\textbf{Fig. 2.10} Myocardial uptake pattern of complexes 1C and 2C in comparison with \textsuperscript{99m}TcN(NOEt)\textsubscript{2}
### Table 2.1 Bio-distribution pattern of $^{99m}$TcN(TBDTC)$_2$ in Swiss mice [% ID/g (1SD), n=3]

<table>
<thead>
<tr>
<th>Organ</th>
<th>5 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>14.43 (1.97)</td>
<td>13.86 (1.00)</td>
<td>16.52 (0.95)</td>
<td>18.91 (4.71)</td>
</tr>
<tr>
<td>Heart</td>
<td>8.97 (0.51)</td>
<td>6.59 (2.01)</td>
<td>4.17 (0.86)</td>
<td>1.96 (0.91)</td>
</tr>
<tr>
<td>Lungs</td>
<td>9.87 (1.75)</td>
<td>6.5 (0.86)</td>
<td>5.5 (0.9)</td>
<td>5.68 (1.85)</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.33 (2.57)</td>
<td>7.7 (3.51)</td>
<td>5.56 (0.68)</td>
<td>4.64 (1.12)</td>
</tr>
<tr>
<td>Blood</td>
<td>1.51 (0.09)</td>
<td>1.36 (0.11)</td>
<td>0.82 (0.09)</td>
<td>0.94 (0.32)</td>
</tr>
<tr>
<td>Heart/ blood</td>
<td>5.92</td>
<td>5.35</td>
<td>5.11</td>
<td>2.01</td>
</tr>
<tr>
<td>Heart/ liver</td>
<td>0.63</td>
<td>0.47</td>
<td>0.25</td>
<td>0.1</td>
</tr>
<tr>
<td>Heart/ lung</td>
<td>0.92</td>
<td>1.0</td>
<td>0.75</td>
<td>0.34</td>
</tr>
</tbody>
</table>

### Table 2.2 Bio-distribution pattern of $^{99m}$TcN(MIBDTC)$_2$ in Swiss mice [% ID/g (1SD), n=3]

<table>
<thead>
<tr>
<th>Organ</th>
<th>5 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>11.41 (2.24)</td>
<td>25.13 (1.65)</td>
<td>33.86 (6.14)</td>
<td>25.15 (1.73)</td>
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<tr>
<td>Heart</td>
<td>6.41 (0.75)</td>
<td>2.86 (0.16)</td>
<td>2.88 (0.82)</td>
<td>1.76 (0.72)</td>
</tr>
<tr>
<td>Lungs</td>
<td>7.69 (2.56)</td>
<td>4.24 (0.37)</td>
<td>3.97 (0.36)</td>
<td>3.25 (0.17)</td>
</tr>
<tr>
<td>Kidney</td>
<td>9.49 (0.23)</td>
<td>4.75 (0.22)</td>
<td>4.94 (0.68)</td>
<td>3.83 (0.21)</td>
</tr>
<tr>
<td>Blood</td>
<td>2.67 (0.46)</td>
<td>1.73 (0.03)</td>
<td>2.13 (0.23)</td>
<td>2.07 (0.77)</td>
</tr>
<tr>
<td>Heart/ blood</td>
<td>2.4</td>
<td>1.63</td>
<td>1.35</td>
<td>0.85</td>
</tr>
<tr>
<td>Heart/ liver</td>
<td>0.56</td>
<td>0.11</td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td>Heart/ lung</td>
<td>0.83</td>
<td>0.67</td>
<td>0.73</td>
<td>0.54</td>
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</tbody>
</table>

### Table 2.3 Bio-distribution pattern of $^{99m}$TcN(NOEt)$_2$ in Swiss mice [% ID/g (1SD), n=3]

<table>
<thead>
<tr>
<th>Organ</th>
<th>5 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>11.30 (1.96)</td>
<td>9.52 (0.89)</td>
<td>10.22 (0.62)</td>
<td>12.63 (1.86)</td>
</tr>
<tr>
<td>Heart</td>
<td>11.08 (0.81)</td>
<td>6.64 (0.45)</td>
<td>4.74 (0.66)</td>
<td>3.7 (0.58)</td>
</tr>
<tr>
<td>Lungs</td>
<td>21.48 (3.08)</td>
<td>14.87 (0.89)</td>
<td>14.21 (1.99)</td>
<td>14.18 (0.35)</td>
</tr>
<tr>
<td>Kidney</td>
<td>25.97 (2.21)</td>
<td>22.36 (0.35)</td>
<td>18.23 (2.11)</td>
<td>17.08 (1.19)</td>
</tr>
<tr>
<td>Blood</td>
<td>2.05 (0.34)</td>
<td>1.25 (0.12)</td>
<td>1.05 (0.13)</td>
<td>0.83 (0.1)</td>
</tr>
<tr>
<td>Heart/ blood</td>
<td>5.56</td>
<td>5.36</td>
<td>4.52</td>
<td>4.51</td>
</tr>
<tr>
<td>Heart/ liver</td>
<td>1.03</td>
<td>0.71</td>
<td>0.46</td>
<td>0.3</td>
</tr>
<tr>
<td>Heart/ lung</td>
<td>0.52</td>
<td>0.45</td>
<td>0.34</td>
<td>0.26</td>
</tr>
</tbody>
</table>
which was slightly lower than that of $^{99m}$TcN(NOEt)$_2$ (11.41 ± 2.24% ID/g) at the same time point. Similar to the standard agent, the initial accumulated activity for the two complexes in the myocardium was not retained and cleared off with time. The washout kinetics of 1C was observed to be similar to that $^{99m}$TcN(NOEt)$_2$, but was significantly different when compared to 2C complex. The latter complex showed a delayed clearance from the myocardium between 10 and 30 min which may be due to slow washout of the radioactivity from the blood pool (Fig. 2.11).

![Figure 2.11](image_url)

**Fig. 2.11** Clearance pattern of complexes 1C, 2C and $^{99m}$TcN(NOEt)$_2$ from the non-target organs

The heart/ blood, heart/ lung and heart/ liver ratios of the two complexes 1C, 2C and the standard agent $^{99m}$TcN(NOEt)$_2$ are shown in Tables 2.1-2.3. The heart/ lung ratios of the two complexes were better than that of the standard agent at all the time points of the study. Similarly, heart/ blood ratio of 1C was found to be better than that of $^{99m}$TcN(NOEt)$_2$ up to 30 min p.i. thereafter there was a sharp decline, leading to a lower value at 60 min p.i. The heart/ liver ratios of the two complexes remained below 1 throughout the period of study, and were lower than that of $^{99m}$TcN(NOEt)$_2$.
The clearance of activity from different organs exhibited by the two radiolabeled compounds 1C, 2C and the standard agent $^{99m}$TcN(NOEt)$_2$ is shown in Fig. 2.11. The two $^{99m}$Tc-complexes showed significant liver accumulation with increase in liver activity up to 30 min, after which a definite decline was observed. This may be due to the lipophilic nature of the two complexes which showed clearance via the hepatobiliary system. The clearance of activity from the liver was faster with 2C, possibly due to the presence of metabolizable ether linkage. The lung uptake observed with both the synthesized $^{99m}$TcN-complexes was lower than that of $^{99m}$TcN(NOEt)$_2$ throughout the period of study, with faster clearance in the case of 2C. The latter complex however showed significant blood pool activity, which may be due to association of the complex with the serum proteins.

Although the in-vivo behavior of the currently studied $^{99m}$Tc complexes showed promising myocardial uptake, their rapid myocardial washout and high liver activity, in comparison to the standard agent $^{99m}$TcN(NOEt)$_2$, limited their potential for the aforementioned application.

2.2.4 Conclusion

The dithiocarbamate ligand analogues of 2-methylpropan-2-amine (TBI) and 2-hydroxy-2-methylpropanenitrile (MIBI) were synthesized and labeled with $[^{99m}\text{TcN}]^{2+}$ core to give high specific activity complexes. The bio-distribution studies, carried out in Swiss mice, showed reasonable myocardial uptake and better heart/ lung ratios compared to $^{99m}$TcN(NOEt)$_2$. Also, the heart/ blood ratio of $^{99m}$TcN(TBDTC)$_2$ was better than the standard agent. But poor myocardial retention of the complexes and slow liver clearance limited their potential for myocardial perfusion imaging. Nevertheless, this study has given an insight into the easy derivatization of carrier molecules in the form of dithiocarbamate desired for the incorporation of $^{99m}$Tc activity via $[^{99m}\text{TcN}]^{2+}$ core and their general in-vivo behavior.
2.3 A $^{99m}$TcN-fatty acid xanthate symmetric [2+2] complex as a myocardial metabolic tracer

2.3.1 Introduction

Fatty acids are taken up in the myocardium and form the source of energy for normoxic cells. With the carboxylic acid termini free to retain the biochemical properties of the molecule, the fatty acid chain length is the major governing determinant for its uptake in the myocardium.\(^\text{216}\) Once inside the normoxic cells, fatty acids are rapidly metabolized by β-oxidation. The differential fatty acid metabolism in normal and ischemic cells becomes a tool for detection of cardiovascular diseases using Single Photon Emission Computed Tomography (SPECT) imaging. Several $^{123}$I-labeled straight chain fatty acid derivatives have been prepared earlier, and tested \textit{in-vivo}.\(^\text{230,231}\) However, \textit{in-vivo} instability and rapid metabolic washout were the limiting factors rendering it unsuitable for SPECT imaging, which is the preferred modality for clear delineation of the myocardium. To overcome the above shortcomings, $^{123}$I labeled iodophenylpentadecanoic acid (IPPA) and beta-methyl iodo phenyl pentadecanoic acid (BMIPP)\(^\text{210,217,218,232-233}\) were introduced. These agents showed good myocardial uptake with slow clearance making them suitable for SPECT imaging and convenient for use in patients who cannot undergo stress studies using $^{201}$TlCl and $^{99m}$Tc-MIBI.\(^\text{234}\) These two $^{123}$I radiolabeled fatty acids have therefore served as gold standards in myocardial functional imaging. However, the inherent drawbacks in the use of cyclotron-produced $^{123}$I (limited availability and short half-life) restrict their clinical use. Hence, intense efforts are on to develop suitable $^{99m}$Tc-based agents for SPECT imaging. In this regard, a number of $^{99m}$Tc-labeled fatty acid derivatives have been prepared, but all of these exhibited poor myocardial extraction. This could be attributed to improper chemical modification in the parent molecule leading to altered biological behavior. The earlier reported $^{99m}$Tc-fatty acid
derivatives used the common $[^{99m}\text{Tc}]=\text{O}]^{3+}$ core with N$_2$S$_2$ as the chelating atoms that were incorporated into the fatty acid chain. But these complexes showed poor myocardial extraction.$^{235-237}$ A modified “3+1” approach$^{216}$ using the same core was followed but the product lacked in-vivo stability. Also, $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ core$^{238}$ has been used for labeling a fatty acid derivative via histidine moiety. But, this also did not improve myocardial extraction, due to the stereochemical changes in the molecule.

In the present work, a xanthate derivative of 15-hydroxy pentadecanoic acid (HPDA) (Fig. 2.12) was prepared and labeled using the $[^{99m}\text{Tc}]=\text{N}]^{2+}$ core. The prepared complex was evaluated in Swiss mice for its biological behavior.

![Fig. 2.12 Structure of synthesized fatty acid xanthate](image)

**2.3.2 Experimental**

**2.3.2.1 Materials and Methods**

The general experimental details are given in section 2.2.2.1.

**2.3.2.2 Synthesis of 15-HPDA xanthate ligand (3)**

In a typical procedure, a mixture of 15-HPDA (0.5 g, 1.94 mmol) and crushed sodium hydroxide (0.78 g, 5.81 mmol) were stirred vigorously in THF (20 mL) for 5 min at room temperature. To the stirred solution, carbon disulphide (0.14 mL, 2.13 mmol) was added. Stirring was continued overnight at room temperature. After completion of the reaction THF was removed under vacuum, the residue was washed with ether and re-crystallized from methanol-ether to obtain the product 3 as a yellow solid.

Yield: 30% (220 mg)

C, H, N, S: Observed (Calculated) 43.96 (44.06), 4.30 (4.52), 5.66 (5.71), 26.45 (26.14).
MS (ESI, +ve mode): Mass (M) (calculated) C₁₆H₂₈O₅S₂Na₂ 378.1275; m/z (observed) (M-2Na) 333.1656 [Fig. 2.13].

2.3.2.3 Radiolabeling

To a solution of ligand 3 (~5 mg) in saline (0.5 mL), freshly prepared [⁹⁹ᵐTcN]²⁺ intermediate (0.5 mL) (as reported in Section 2.2.2.4.1) was added and the pH of the resultant mixture kept around 7-8. The reaction mixture was vortexed for 1 min and incubated at room temperature for 30 min to give the desired complex 3C.

2.3.2.4 Quality Control techniques

The radiochemical purity of the [⁹⁹ᵐTcN]²⁺ intermediate was checked by TLC, while that of 3C by paper electrophoresis and HPLC.

2.3.2.4.1 TLC

The [⁹⁹ᵐTcN]²⁺ intermediate was characterized by TLC using ethanol: chloroform: toluene: 0.5M ammonium acetate (6:3:3:0.5 v/v) as the developing solvent.

2.3.2.4.2 Paper electrophoresis

Paper electrophoresis was done using a 37 cm long strip of Whatman No.3 chromatography paper and 0.05 M phosphate buffer (pH 7.4). About 2 μL of the test solution was spotted at the middle of the strip and the electrophoresis was carried out for 1 h under a potential gradient of ~10 V/cm. The strip was dried and the radioactivity profile determined using a TLC chromatography evaluation system.

2.3.2.4.3 HPLC

About 25 μL of the test solution was injected into the column and elution was monitored by observing the radioactivity profile. Water (A) and acetonitrile (B) each containing 0.1% trifluoroacetic acid were used as the mobile phase and the following gradient elution technique was adopted for the separation (0 min 98% A, 2 min 98% A, 3 min 2% A, 10 min 2% A).
Fig. 2.13 Mass spectrum of compound 3

BARC-FA-XAN 4 (0.075) AM (Cen,4, 80.00, Hf,0.0,0.0,0.00); Sm (Md, 4.00); Sb (1,20.00 ); Cm (2:28)

Ionisation Mode
TOF MS ES+
1.50e3
2.3.2.4.4 Biological studies

2.3.2.4.4.1 Serum stability studies

Stability of the complex 3C was assessed in-vitro in human serum. About 50 μL of the radiolabeled preparation was added to 500 μL serum and the mixture was incubated at 37 °C. Aliquots were taken at the intervals of 30 and 60 min. The serum proteins were precipitated with equal volume of cold ethanol, and the precipitate was removed after centrifugation at 10000 g for 20 min. The respective supernatants were subjected to HPLC as above to quantitate the protein unbound complex.

2.3.2.4.4.2 Bio-distribution studies

Normal Swiss mice (20-25g body weight) were used for the bio-distribution studies. Each animal was intravenously injected with complex 3C (~2.5 MBq, 0.1 mL) via the tail vein. Four different sets (3 each) of animals were kept under normal conditions for various time periods (5, 10, 30 and 60 min). The animals were sacrificed immediately after the respective incubation periods and the relevant organs and tissues were excised for measuring the associated radioactivity, using a flat-bed type NaI(Tl) scintillation counter with a suitable energy window for $^{99m}$Tc. The accumulated activity per gram of the specific organ/tissue was expressed in terms of percentage of the total injected dose. All the procedures were performed in accordance with the national laws pertaining to the conduct of animal experiments.

2.3.3 Results and discussion

2.3.3.1 Synthesis of 15-HPDA xanthate

The fatty acid-xanthate (3) was synthesized in a single step (Fig. 2.14) by reacting 15-HPDA with CS$_2$ in presence of sodium hydroxide as the base. The product was obtained in moderate yield and was characterized by elemental and mass spectral analyses.
2.3.3.2 Radiolabeling

2.3.3.2.1 $[^{99m}\text{TcN}]^{2+}$ intermediate core

The schematic representation for the formation of $[^{99m}\text{TcN}]^{2+}$ core is shown in Fig. 2.7(a). The distinction between the $[^{99m}\text{TcN}]^{2+}$ core and its precursor $^{99m}\text{TcO}_4^-$ was achieved by TLC, using EtOH/CHCl$_3$/toluene/0.5M NH$_4$Ac (6:3:3:0.5 v/v) as the solvent system. The $^{99m}\text{TcN}$-core (~99%) was found to be concentrated near the point of application ($R_f = 0-0.25$), while $^{99m}\text{TcO}_4^-$ in the same solvent system showed a $R_f = 0.4-0.6$.

2.3.3.2.2 $[^{99m}\text{TcN}]-15$-HPDA xanthate complex (3C)

The synthetic scheme of 3C is shown in Fig. 2.15. The desired complex was obtained on adding freshly prepared $[^{99m}\text{TcN}]^{2+}$ core to the synthesized ligand 3. The final complex 3C was characterized by paper electrophoresis and HPLC. Electrophoresis pattern of the nitrido intermediate and the complex 3C, visualised using a radioactive scanner is shown in Fig. 2.16. More than 95% of the radioactivity corresponding to 3C was seen with the band having an $R_f = 0.26$. Under identical conditions, the $^{99m}\text{TcN}$-core had ~98% activity at $R_f = 0.62$. The HPLC chromatograms of $^{99m}\text{TcN}$-intermediate and 3C are shown in Fig. 2.17. The retention times of the complex 3C was found to be $7.5 \pm 0.1$ min while that of $^{99m}\text{TcN}$-core was $2.8 \pm 0.1$ min.

Xanthates are known to complex with $[^{99m}\text{TcN}]^{2+}$ core leading to neutral complexes of $^{99m}\text{TcN}L_2$ type having square pyramidal geometry with an apical $^{99m}\text{Tc}=\text{N}$ bond and four sulphur atoms occupying the basal plane. It may be presumed that the neutral fatty acid xanthate complex prepared above would have a similar structure.
\[
\text{\( \text{Tc} \equiv \text{N} \)}^{2+} \xrightarrow{3} \text{R} = \text{COOH(CH}_2\text{)}_{13}\text{CH}_2
\]

**Fig. 2.15** Synthesis of \(^{99m}\text{TcN}\)-15-HPDA xanthate complex

**Fig. 2.16** Paper electrophoresis patterns of (a) \(^{99m}\text{TcN}\)-intermediate and (b) complex 3C

**Fig. 2.17** HPLC profiles of (a) \(^{99m}\text{TcN}\)-intermediate and (b) complex 3C
2.3.3.3 Biological studies

In view of the essential parameters (i-iv) required for a myocardial agent as mentioned in section 2.2.3.3, the in-vitro stability of the complex was carried out to determine the kinetic inertness of the complex 3C. The complex was found to be stable in serum up to 1 h as confirmed by HPLC wherein the retention of radioactive peak was observed.

To evaluate the complex 3C as a potential myocardial agent in terms of its in-vivo parameters, bio-distribution studies were carried in Swiss mice. From the bio-distribution studies (Table 2.4) it was observed that the maximum myocardial uptake of this preparation was $3.10 \pm 0.08\% \text{ID/g}$ at 5 min p.i., with a rapid wash out leading to a meagre $0.79 \pm 0.18\% \text{ID/g}$ of the activity remaining in the heart at 60 min p.i. The blood pool activity was initially high ($8.21 \pm 1.10\% \text{ID/g}$ at 5 min p.i.), but declined with time. The heart/ blood ratio remained low within a reasonably narrow range throughout the period of the study. The complex showed high liver and lung uptake with slow clearance. Marginal hepatic clearance of the complex was evident from the gradually increased radioactivity in the intestinal region. An appreciable fraction of activity was seen in the kidneys, which could be possibly due to metabolism of the complex 3C in the liver.

As is well known from the literature, straight chain fatty acids having 15-21 carbon show maximum extraction in the myocardium. But the present complex 3C, being a symmetric complex may behave as a straight chain of 30 carbon acid and thus have an altered in-vivo pharmacokinetic behavior. However, it was thought that the fatty acid (<15 carbon) on complexation with $[^{99m}\text{TcN}]^{2+}$ core may not behave as a single straight chain, and subsequently affect the myocardial uptake. Thus, it was decided to use a 15 carbon acid, the minimum chain for favorable myocardial extraction, for the preparation of the complex.
However, as the experiments show, the proposed molecule exhibited limited uptake with rapid clearance from myocardium, decreasing its utility for the intended application.

<table>
<thead>
<tr>
<th>Organs</th>
<th>5 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>48.16 (0.02)</td>
<td>29.02 (2.57)</td>
<td>27.07 (1.03)</td>
<td>29.69 (5.67)</td>
</tr>
<tr>
<td>Intestine + GB</td>
<td>1.23 (0.08)</td>
<td>0.83 (0.13)</td>
<td>3.66 (0.31)</td>
<td>5.23 (0.64)</td>
</tr>
<tr>
<td>Kidney</td>
<td>21.57 (3.89)</td>
<td>17.06 (1.60)</td>
<td>16.29 (1.38)</td>
<td>16.89 (4.29)</td>
</tr>
<tr>
<td>Heart</td>
<td>3.10 (0.08)</td>
<td>1.38 (0.19)</td>
<td>0.92 (0.06)</td>
<td>0.79 (0.18)</td>
</tr>
<tr>
<td>Lungs</td>
<td>5.84 (0.28)</td>
<td>2.99 (0.67)</td>
<td>2.03 (0.05)</td>
<td>2.96 (1.14)</td>
</tr>
<tr>
<td>Blood</td>
<td>8.21 (1.10)</td>
<td>3.06 (0.38)</td>
<td>1.96 (0.23)</td>
<td>1.85 (0.28)</td>
</tr>
<tr>
<td>Heart/ blood</td>
<td>0.38</td>
<td>0.45</td>
<td>0.43</td>
<td>0.47</td>
</tr>
<tr>
<td>Heart/ lung</td>
<td>0.53</td>
<td>0.46</td>
<td>0.45</td>
<td>0.27</td>
</tr>
<tr>
<td>Heart/ liver</td>
<td>0.06</td>
<td>0.05</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**2.3.4 Conclusion**

The xanthate derivative of 15-HPDA could be synthesized and labeled with the $[^{99m}\text{TcN}]^{2+}$ core yielding more than 95% complexation. Neutral $^{99m}\text{Tc}$ complex could be obtained in high yields and adequate stability. The study indicated that a xanthate derivative of a fatty acid makes a feasible route for labeling biomolecules with $^{99m}\text{Tc}$ tracer via $[^{99m}\text{TcN}]^{2+}$ core under mild conditions. Bio-distribution study carried out in Swiss mice showed desirable extent of uptake in myocardium, albeit with significant uptake in the surrounding organs leading to unfavorable target to non-target ratios, which would affect the quality of the images. However, these studies have opened a new route for radiolabeling fatty acids via $[^{99m}\text{TcN}]^{2+}$ core.
2.4 Conclusion

The present chapter evaluates the usefulness of $[^{99m}\text{TcN}]^{2+}$ core in terms of its ease of preparation and the stability of the intermediate core. Also, it highlights the simple derivatization, in the form of dithiocarbamates and xanthates, required in the lead molecule for the easy incorporation of $^{99m}\text{Tc}$ metal via $[^{99m}\text{TcN}]^{2+}$ core. The suitability of this core for the attachment of biomolecules, has been represented by taking an example of fatty acid. Apart from this, the chapter also presents the mild reaction conditions required for final complex formation and \textit{in-vivo} inertness of the final $[^{99m}\text{TcN}]$-complexes, which are desirable parameters for final radiopharmaceutical preparations.