CHAPTER 4

Asymmetric [2+2] Charged complexes

4.1 Introduction

\[ [^{99m}\text{Tc}≡\text{N}]^{2+} \] core, as discussed previously, forms kinetically inert and thermodynamically stable complexes with a combination of \(\pi\)-donor and \(\pi\)-acceptor ligands.\(^{186,189,195}\) The addition of a long chain \(\pi\)-acceptor bi-dentate PNP ligand to the \([^{99m}\text{Tc}≡\text{N}]^{2+}\) core, generates a new versatile intermediate \([^{99m}\text{TcN(PNP)}]^2+\), that leads to complexes with varied biological characteristics. The \textit{in-vivo} pharmacokinetics of \([^{99m}\text{TcN(PNP)}]\) complexes for the same carrier biomolecule can be suitably altered by changing the lipophilicity and the overall charge of the final complex. The lipophilicity of \([^{99m}\text{TcN(PNP)}]\) complexes can be tailored by changing the alkyl substituents present on the phosphorus and nitrogen atoms in the PNP ligand. For example, the presence of phenyl substituent in the PNP ligand increases the lipophilicity leading to delayed clearance of the activity from the background organs. Also, the alkyl ether linkages present on the heteroatoms (P and N) increases the \textit{in-vivo} metabolic clearance from the non-target organs. Apart from this, by using different bi-dentate \(\pi\)-donor chelates such as dithiocarbamates, cysteine, etc. and introducing a hydrophilic or hydrophobic linker between the bi-functional chelating agent (BFCA) and the bio-molecule, the \textit{in-vivo} pharmacokinetic behavior of the complexes can be varied to a great extent.

In addition to lipophilicity, the charge on the final complex is another important parameter for determining the pharmacokinetic behavior of the final complexes inside the body. For example, the negatively charged complexes are known to clear rapidly from the
blood pool via the renal pathway\textsuperscript{250} whereas the positively charged lipophilic complexes accumulate in the myocardium for prolonged period due to coulombic attraction with the negatively charged mitochondrial membrane.\textsuperscript{201} Also, neutral complexes are desired for crossing the blood brain barrier.\textsuperscript{251}

In the case of $[^{99m}\text{TcN(PNP)}]^ {2+}$ complexes the overall charge can be varied by changing the nature of bi-dentate $\pi$-donor groups (Fig. 4.1).\textsuperscript{187,190,247} A di-anionic ligand (S’, COO’), as reported in the previous chapter, leads to neutral complexes whereas uni-positively ‘charged asymmetric [2+2] complexes’ are formed if the $\pi$-donor ligand is mono-anionic (NH$_2$, S’ or S, S’) in nature. The present chapter deals with the investigation on the effect of both charge and lipophilicity on the overall pharmacological behavior of the $[^{99m}\text{TcN(PNP)}]^ {2+}$ complexes.

The amino acid cysteine is a useful BFCA for $[^{99m}\text{TcN(PNP)}]^ {2+}$ intermediate, where a biomolecule, like fatty acid can be attached either at the -COOH or -NH$_2$ group, and the other two groups, SH and NH$_2$/ COOH, can be used for labeling with $[^{99m}\text{TcN(PNP)}]^ {2+}$ core.\textsuperscript{187,190,247} The overall charge of the complex depends on the groups in cysteine that are co-ordinated to the $[^{99m}\text{TcN(PNP)}]^ {2+}$ core. The overall charge will be uni-positive, if -SH and -NH$_2$ groups of cysteine are involved in co-ordination, or neutral, if -SH and -COOH groups are co-ordinated to the $[^{99m}\text{TcN(PNP)}]^ {2+}$ core.
In the previous chapter, a 16 carbon diacid was derivatized with a cysteine residue, leaving the cysteine SH and COOH groups available for complexation with the $[^{99m}\text{Tc}N\text{PNP}]^{2+}$ intermediate to give a neutral complex 7C [Fig. 4.2(a)]. This chapter presents the synthesis of a uni-positively charged structural analogue 9C [Fig. 4.2(b)] of the neutral complex and evaluation of its bio-distribution in a mice model. The complex was prepared by coupling a 16 carbon fatty acid to the cysteine residue leaving SH and NH$_2$ groups free (Fig. 4.3) for co-ordination with the same $[^{99m}\text{Tc}N\text{PNP}6]^{2+}$ intermediate. This complex 9C was evaluated and the bio-distribution results were compared with the neutral analogue to evaluate the effect of charge. The results were also compared with the $^{125}$I labeled standard agent IPPA to assess the potential of the prepared complex for use as a radiopharmaceutical to evaluate cardiac function.

![Fig. 4.2 Structural analogues (a) neutral 7C and (b) charged 9C 16 carbon fatty acid complexes](image)

The promising *in-vivo* biological results of the 16 carbon uni-positively charged complex 9C (as discussed later), infused interest in evaluating the other uni-positively charged complexes with different lipophilicities. The lipophilicity was varied by varying the carbon chain lengths of the long chain fatty acid. In this regard, three more fatty acid-cysteine conjugates (8, 10 and 11) were synthesized (Fig. 4.3) and used for the formation of $[^{99m}\text{Tc}N\text{PNP}]^{2+}$ fatty acid complexes, keeping the other chemical environment of the final complex *viz.* PNP ligand 6 and BFCA (cysteine) same. These complexes were then evaluated
in mice and the bio-distribution results compared together with the 9C, to assess the effect of lipophilicity on the target uptake and non-target clearance characteristics.

4.2. Experimental

4.2.1 Materials and Methods

The general experimental details are given in chapter 2, section 2.2.2.1.

4.2.2 Synthesis of fatty acid-cysteine conjugates (8-11)

Ethyl ester of ω-bromo fatty acids (8b/9b)

A solution of the ω-bromo fatty acid 8a or 9a (1.5 mmol) in EtOH (20 mL) and conc. H2SO4 (0.1 mL) was refluxed overnight. After cooling, EtOH was removed under vacuum, ice cold water (20 mL) was added to the oily residue and mixture extracted with chloroform (3 × 10 mL). The chloroform extracts were pooled, dried and evaporated to obtain the target compounds 8b and 9b respectively.

Ethyl 15-bromopentadecanoate (8b)

Yield: 95% (518 mg).

IR (neat, cm⁻¹): 2918 (s); 2850 (m); 1734 (s).

Ethyl 16-bromohexadecanoate (9b)

Yield: 97% (525 mg).

IR (neat, cm⁻¹): 2919 (s); 2849 (m); 1740 (s).
**ω-phthalimido fatty esters (8c/9c)**

A mixture of compound 8b/9b (1.37 mmol), phthalimide (203 mg, 1.37 mmol) and anhydrous potassium carbonate (209 mg, 1.5 mmol) in CH$_3$CN (15 mL) was refluxed overnight. Upon completion of the reaction (cf. TLC), the reaction mixture was cooled, filtered and concentrated under vacuum to obtain the crude product, which on silica gel column chromatography [EtOAc/ hexane (1:9 v/v)] furnished the pure compounds 8c and 9c respectively.

**Ethyl 15-phthalimidopentadecanoate (8c)**

Yield: 56% (318 mg), $R_f = 0.7$ [EtOAc/ hexane (1:9 v/v)].

IR (neat, cm$^{-1}$): 2918 (s); 2850 (m); 1734 (s); 1702 (s); 1462 (m); 1405 (m); 1170 (m); 1060 (m); 712 (s).

$^1$H NMR (CDCl$_3$, δ ppm): 7.83-7.86 (m, 2H, C$_6$H$_4$C-); 7.69-7.72 (m, 2H, C$_6$H$_4$C-); 4.12 (q, 2H, -COOCH$_2$CH$_3$, $J = 7.2$ Hz); 3.67 (t, 2H, -CH$_2$CH$_2$N(CO)$_2$, $J = 7.2$ Hz); 2.28 (t, 2H, -CH$_2$CH$_2$COOEt, $J = 7.5$ Hz); 1.59-1.67 (m, 4H, -CH$_2$CH$_2$COOEt & -CH$_2$CH$_2$N(CO)$_2$); 1.18-1.32 (m, 23H, (C$_6$H$_2$)$_{10}$ & -COOCH$_2$CH$_3$) [Fig. 4.4].

**Ethyl 16-phthalimidohexadecanoate (9c)**

Yield: 87% (511 mg), $R_f = 0.8$ [EtOAc/ hexane (1:9 v/v)].

IR (neat, cm$^{-1}$): 2919 (s); 2849 (m); 1740 (s); 1704 (s); 1464 (m); 1406 (m); 1174 (m); 1064 (m); 714 (s).

$^1$H NMR (CDCl$_3$, δ ppm): 7.79-7.85 (m, 2H, C$_6$H$_4$C-); 7.67-7.73 (m, 2H, C$_6$H$_4$C-); 4.11 (q, 2H, -COOCH$_2$CH$_3$, $J = 7.2$ Hz); 3.66 (t, 2H, -CH$_2$CH$_2$N(CO)$_2$, $J = 7.0$ Hz); 2.27 (t, 2H, -CH$_2$CH$_2$COOEt, $J = 7.4$ Hz); 1.62 (m, 4H, -CH$_2$CH$_2$COOEt & -CH$_2$CH$_2$N(CO)$_2$); 1.20-1.27 (m, 25H, (CH$_2$)$_{11}$ & -COOCH$_2$CH$_3$) [Fig. 4.5].
Ethyl esters of ω-amino acid (8d/ 9d/ 10b/ 11b)

8d/ 9d

A mixture of compound 8c/ 9c (0.69 mmol), 80% hydrazine hydrate (219 µL, 3.49 mmol) in EtOH (15 mL) was refluxed for 3 h. The reaction mixture was brought to room temperature, and excess hydrazine hydrate and EtOH were removed under vacuum. The precipitate obtained was re-dissolved in EtOH (15 mL), treated with aqueous 2N HCl (5 mL) and the mixture refluxed overnight. On cooling, solvent was removed under vacuum to obtain white residue, which was re-dissolved in EtOH (15 mL) and conc. H₂SO₄ (0.1 mL) and refluxed overnight. The reaction mixture was cooled to room temperature to obtain a white precipitate (phthalhydrazide) and filtered. The filtrate was evaporated under vacuum to obtain oily residue, to which cold water was added and the pH of the resulting solution adjusted above 7 with aqueous 5% NaHCO₃. The aqueous solution was extracted with chloroform (3 × 10 mL), pooled, dried and concentrated under vacuum to give the pure products 8d and 9d respectively.

Ethyl 15-aminopentadecanoate (8d)

Yield: 97% (191 mg).

IR (neat, cm⁻¹): 3370 (w); 2918 (s); 2850 (s); 1734 (s); 1656 (w); 1599 (w); 1466 (s); 1375 (m); 1180 (s); 1114 (w); 1034 (w); 736 (m).

Ethyl 16-aminohexadecanoate (9d)

Yield: quantitative (230 mg).

IR (neat, cm⁻¹): 3197 (w); 2916 (s); 2848 (m); 1738 (s); 1577 (m); 1518 (s); 1463 (m); 1182 (s); 721 (m).

¹H NMR (CDCl₃, δ ppm): 4.05-4.16 (q, 2H, -COOCH₂CH₃, J = 7.0 Hz); 3.00 (m, 2H, -CH₂CH₂NH₂); 2.27 (t, 2H, -CH₂CH₂COOEt, J = 7.4 Hz); 1.89 (m, 4H, -CH₂CH₂NH₂ and -
A solution of ω-amino fatty acid 10a/11a (2.3 mmol) in EtOH (20 mL) and conc. H₂SO₄ (0.1 mL) was refluxed overnight. After cooling, solvent was removed under vacuum, ice cold water (20 mL) added and the pH of the solution was brought to 8 using aqueous 5% NaHCO₃. The resultant solution was extracted with CHCl₃ (3 × 10 mL), dried and evaporated under vacuum to give the products 10b and 11b respectively.

11-amino fatty acid ester (10b)
Yield: Quantitative (525 mg).
IR (neat, cm⁻¹): 3370 (b); 2918 (s); 2850 (s); 1735 (s); 1599 (w); 1466 (m); 1375 (m); 1180 (s); 1114 (m); 1034 (m); 917 (w); 859 (w); 726 (m).

12-amino fatty acid ester (11b)
Yield: 96% (540 mg).
IR (neat, cm⁻¹): 3365 (b); 2922 (s); 2851 (s); 1737 (s); 1594 (m); 1466 (m); 1373 (m); 1181 (s); 1113 (m); 1034 (m); 917 (w); 860 (w); 722 (m).

ω-(N-Boc, S-trityl cysteinyl amido) fatty ester (8e/9e/10c/11c)
A mixture of ω-amino ester 8d/9d/10b/11b (0.5 mmol) and N-Boc, S-trityl cysteine (233 mg, 0.5 mmol) in CH₂Cl₂ (15 mL) was cooled to 0 °C in an ice bath. To this, EDCI (106 mg, 0.55 mmol) was added and the mixture stirred at 0°C for 1 h, after which it was brought to room temperature and stirred overnight. Upon completion of the reaction (cf. TLC), the reaction mixture was washed with water (3 × 10 mL), dried and concentrated to yield the crude product, which was purified by silica gel column chromatography [EtOAc/ CHCl₃ (1:19 v/v)] to give pure 8e, 9e, 10c and 11c respectively.
11-(N-Boc, S-trityl cysteiny1 amido) undecanoic ester (10c)

Yield: 88% (297 mg), R_f = 0.6 [EtOAc/ CHCl_3 (1:19 v/v)].

IR (neat, cm⁻¹): 3131 (b); 3057 (w); 2924 (s); 2852 (s); 1734 (s); 1713 (s); 1656 (s); 1530 (b); 1491 (m); 1444 (m); 1366 (m); 1248 (w); 1167 (s); 1033 (w); 742 (s); 699 (s).

¹H NMR (CDCl₃, δ ppm): 7.14-7.41 (m, 15H, (C₆H₅)₃C-); 6.12 (s, 1H, -CHCONHC₆H₄-); 4.92-4.96 (m, 1H, -NHCHCH₂S-); 4.09 (q, 2H, -COOCH₂CH₃, J = 7.2 Hz); 3.80-3.83 (m, 1H, -NHCHCH₂S-); 3.09-3.15 (m, 2H, -CH₂CH₃NHCO-); 2.62-2.68 (m, 1H, -CHCH₆CH₅S-); 2.44-2.54 (m, 1H, -CHCH₆CH₅S-); 2.26 (t, 2H, -CH₂CH₃COOEt, J = 7.4 Hz); 1.49-1.55 (m, 4H, -CH₂CH₂COOEt & -CH₂CH₃NHCO-); 1.39 (s, 9H, (CH₃)₃C-); 1.19-1.26 (m, 15H, (CH₂)₆ & -COOCH₂CH₃) [Fig. 4.7].

12-(N-Boc, S-trityl cysteiny1 amido) dodecanoic ester (11c)

Yield: 82% (282 mg), R_f = 0.6 [EtOAc/ CHCl₃ (1:19 v/v)].

IR (neat, cm⁻¹): 3309 (b); 3057 (w); 2925 (s); 2852 (s); 1736 (s); 1713 (s); 1656 (s); 1530 (b); 1494 (m); 1444 (m); 1366 (m); 1167 (s); 1033 (w); 742 (s); 699 (s).

¹H NMR (CDCl₃, δ ppm): 7.14-7.41 (m, 15H, (C₆H₅)₃C-); 6.09 (s, 1H, -CHCONHC₆H₄-); 4.89-4.93 (m, 1H, -NHCHCH₂S-); 4.09 (q, 2H, -COOCH₂CH₃, J = 7.2 Hz); 3.80-3.83 (m, 1H, -NHCHCH₂S-); 3.09-3.18 (m, 2H, CH₂CH₅NHCO-); 2.62-2.65 (m, 1H, -CHCH₆CH₅S-); 2.44-2.53 (m, 1H, -CHCH₆CH₅S-); 2.22-2.29 (t, 2H, CH₂CH₃COOEt, J = 7.6 Hz); 1.49-1.59 (m, 4H, -CH₂CH₂COOEt & -CH₂CH₃NHCO-); 1.39 (s, 9H, (CH₃)₃C-); 1.12-1.26 (m, 17H, (CH₂)₆ & -COOCH₂CH₃) [Fig. 4.8].

Ethyl 15-(N-Boc, S-trityl cysteiny1 amido)pentadecanoate (8e)

Yield: 68% (248 mg), R_f = 0.6 [EtOAc/ CHCl₃ (1:19 v/v)].

IR (neat, cm⁻¹): 3131 (b); 3054 (w); 2972 (w); 2920 (s); 2848 (s); 1711 (bs); 1680 (m); 1489 (m); 1362 (m); 1162 (s); 1030 (w); 742 (s); 698 (s).
1H NMR (CDCl3, δ ppm): 7.21-7.43 (m, 15H, (C6H5)3C-); 6.02 (s, 1H, -CHCONHCH2-); 4.84 (m, 1H, -NHCHCH2S-); 4.12 (q, 2H, -COOCCH2CH3, J = 7.2 Hz); 3.81 (m, 1H, -NHCHCH2S-); 3.15-3.17 (m, 2H, -CH2CH2NHCO-); 2.68-2.70 (m, 1H, -CHCH2HbS-); 2.48-2.52 (m, 1H, -CHCH2HbS-); 2.29 (t, 2H, -CH2CH2COOEt, J = 7.5 Hz); 1.61 (m, 4H, -CH2CH2COOEt & -CH2CH2NHCO-); 1.41 (s, 9H, -C(CH3)3); 1.23-1.25 (m, 23H, (CH2)10 & -COOCH2CH3) [Fig. 4.9].

Ethyl 16-(N-Boc, S-trityl cysteinyl amido)hexadecanoate (9e)

Yield: 72% (267 mg), Rf = 0.6 [EtOAc/ CHCl3 (1:19 v/v)].

IR (neat, cm⁻¹): 3316 (b); 3057 (w); 2975 (w); 2925 (s); 2853 (s); 1713 (bs); 1681 (m); 1488 (m); 1366 (m); 1168 (s); 1032 (w); 743 (s); 700 (s).

1H NMR (CDCl3, δ ppm): 7.22-7.43 (m, 15H, (C6H5)3C-); 5.99 (m, 1H, -CHCONHCH2-); 4.82 (m, 1H, -NHCHCH2S-); 4.11 (q, 2H, -COOCCH2CH3, J = 7.2 Hz); 3.81 (m, 1H, -NHCHCH2S-); 3.14-3.19 (m, 2H, -CH2CH2NHCO-); 2.67-2.74 (m, 1H, -CHCH2HbS-); 2.45-2.53 (m, 1H, -CHCH2HbS-); 2.29 (t, 2H, -CH2CH2COOEt, J = 7.5 Hz); 1.61 (m, 4H, -CH2CH2COOEt & -CH2CH2NHCO-); 1.41 (s, 9H, -C(CH3)3); 1.15-1.35 (m, 25H, (CH2)11 & -COOCH2CH3) [Fig. 4.10].

ω-(N-Boc, S-trityl cysteinyl amido) fatty acid (8f/ 9f/ 10d/ 11d)

A mixture of 8e/ 9e/ 10c/ 11c (0.15 mmol) and aqueous 1M KOH solution (300 µL, 0.3 mmole) in MeOH (600 µL) was stirred at room temperature for 2 d. Upon completion of the reaction (cf. TLC), MeOH was removed under vacuum, water (5 mL) was added and the pH of the reaction mixture was adjusted to 3 using aqueous 2N HCl to give compound 8f, 9f, 10d and 11d respectively as white precipitate which was filtered and dried under vacuum.

11-(N-Boc, S-trityl cysteinyl amido) undecanoic acid (10d)

Yield: 80% (78 mg), Rf = 0.2 [EtOAc/ CHCl3 (1:9 v/v)].
IR (neat, cm⁻¹): 3313 (b); 3057 (w); 2924 (s); 2852 (s); 1685 (s); 1656 (s); 1530 (b); 1491 (m); 1444 (m); 1366 (m); 1248 (w); 1167 (s); 1033 (w); 742 (s); 699 (s).

¹H NMR (CDCl₃, δ ppm): 7.14-7.41 (m, 15H, (C₆H₅)₃C-); 6.32 (s, 1H, -CHCONHCH₂-); 5.22 (bs, 1H, -NHCH₂CH₂S-); 3.80-3.83 (m, 1H, -NHCH₂CH₂S-); 3.09-3.15 (m, 2H, -CH₂CH₂NHCO-); 2.62-2.68 (m, 1H, -CHCH₃CH₃S-); 2.44-2.54 (m, 1H, -CHCH₃CH₃S-); 2.22-2.29 (m, 2H, -CH₂CH₂COOH); 1.49-1.55 (m, 4H, -CH₂CH₂COOH & -CH₂CH₂NHCO-); 1.39 (s, 9H, -C(CH₃)₃); 1.23 (s, 12H, (CH₂₆) [Fig. 4.11].

MS (ESI, -ve mode): Mass (calculated) C₃₈H₄₀N₂O₅S 646.8; m/z (observed) 645.5 [Fig. 4.12].

12-(N-Boc, S-trityl cysteiny1 amido) dodecanoic acid (11d)

Yield: 75% (74 mg), Rₐ = 0.2 [EtOAc/ CHCl₃ (1:9 v/v)].

IR (neat, cm⁻¹): 3309 (b); 3057 (w); 2925 (s); 2852 (s); 1685 (s); 1656 (s); 1530 (b); 1494 (m); 1444 (m); 1366 (m); 1248 (w); 1167 (s); 1033 (w); 742 (s); 699 (s).

¹H NMR (CDCl₃, δ ppm): 7.14-7.42 (m, 15H, (C₆H₅)₃C-); 6.22 (bs, 1H, -CHCONHCH₂-); 5.12 (bs, 1H, -NHCH₂CH₂S-); 3.80-3.83 (m, 1H, -NHCH₂CH₂S-); 3.09-3.18 (m, 2H, -CH₂CH₂NHCO-); 2.62-2.65 (m, 1H, -CHCH₃CH₃S-); 2.44-2.53 (m, 1H, -CHCH₃CH₃S-); 2.22-2.29 (m, 2H, -CH₂CH₂COOH); 1.49-1.59 (m, 4H, -CH₂CH₂COOH & -CH₂CH₂NHCO-); 1.39 (s, 9H, -C(CH₃)₃); 1.19 (s, 14H, (CH₂₆) [Fig. 4.13].

MS (ESI, -ve mode): Mass (calculated) C₃₈H₄₂N₂O₅S 660.9; m/z (observed) 659.5 [Fig. 4.14].

15-(N-Boc, S-trityl cysteiny1 amido) pentadecanoic acid (8f)

Yield: 80% (84 mg), Rₐ = 0.2 [EtOAc/ CHCl₃ (1:9 v/v)].

IR (neat, cm⁻¹): 3312 (b); 3053 (w); 2923 (s); 2850 (s); 1681 (s); 1655 (s); 1528 (b); 1488 (m); 1443 (m); 1366 (m); 1247 (w); 1167 (s); 1032 (w); 740 (s); 696 (s).

¹H NMR (CDCl₃, δ ppm): 7.18-7.42 (m, 15H, (C₆H₅)₃C-); 6.16 (m, 1H, -CHCONHCH₂-); 5.06 (m, 1H, -NHCH₂CH₂S-); 3.82 (m, 1H, -NHCH₂CH₂S-); 3.14-3.16 (m, 2H, -CH₂CH₂NHCO-); 2.64-2.70 (m, 1H, -CHCH₃CH₃S-); 2.47-2.52 (m, 1H, -CHCH₃CH₃S-); 2.31
(m, 2H, -CH₂CH₂COOH); 1.59-1.60 (m, 4H, -CH₂CH₂COOH & -CH₂CH₂NHCO-); 1.40 (s, 9H, -C(CH₃)₃); 1.25 (s, 20H, (CH₂)₁₀) [Fig. 4.15].

MS (ESI, -ve mode): Mass (calculated) C₄₂H₅₈N₂O₅S 702.4; m/z (observed) 701.7 [Fig. 4.16].

16-(N-Boc, S-trityl cysteiny1 amido) hexadecanoic acid (9f)

Yield: 80% (86 mg). R₇ = 0.2 [EtOAc/CHCl₃ (1:9 v/v)].

IR (neat, cm⁻¹): 3313 (b); 3057 (w); 2924 (s); 2852 (s); 1685 (s); 1656 (s); 1530 (b); 1491 (m); 1444 (m); 1366 (m); 1248 (w); 1167 (s); 1033 (w); 742 (s); 699 (s).

¹H NMR (CDCl₃, δ ppm): 7.21-7.43 (m, 15H, (C₆H₅)₃C-); 6.18 (s, 1H, -CHCONHCH₂-); 5.09 (m, 1H, -NHCHCH₂S-); 3.85 (m, 1H, -NHCHCH₂S-); 3.08-3.17 (m, 2H, -CH₂CH₂NHCO-); 2.67 (m, 1H, -CHCH₂H₈S-); 2.52 (m, 1H, -CHCH₂H₈S-); 2.32-2.34 (m, 2H, -CH₂CH₂COOH); 1.62 (m, 4H, -CH₃CH₂COOH & -CH₃CH₂NHCO-); 1.40 (s, 9H, (CH₃)₃C-); 1.26 (s, 22H, (CH₂)₁₁) [Fig. 4.17].

MS (ESI, -ve mode): Mass (calculated) C₄₃H₆₀N₂O₅S 716.4; m/z (observed) 715.6 [Fig. 4.18].

ω (cysteiny1 amido) fatty acid (8f/9f/10d/11d)

The compound 8f/9f/10d/11d (0.08 mmoles) was stirred with TFA (2 mL) for 2 h at room temperature. To the resultant yellow solution, Et₃SiH was added dropwise until the solution turned colorless. Stirring was continued for another 15 min. The solvent was removed under vacuum to give the desired products 8, 9, 10 and 11 respectively and used as such for radiolabeling without further characterization.
Fig. 4.4  H NMR spectrum of compound 8c
Fig. 4.5 $^1$H NMR spectrum of compound 9c
Fig. 4.6 $^1$H NMR spectrum of compound 9d
Fig. 4.7 $^1$H NMR spectrum of compound 10c
Fig. 4.8 $^1$H NMR spectrum of compound 11c
Fig. 4.9 H NMR spectrum of compound 8e
Fig. 4.10 H NMR spectrum of compound 9e
Fig. 4.11: 1H NMR spectrum of compound 10d
Fig. 4.12 Mass spectrum of compound 10d
Fig. 4.13 H NMR spectrum of compound 11d
Fig. 4.14 Mass spectrum of compound 11d
Fig. 4.15 ¹H NMR spectrum of compound 8f
Fig. 4.16 Mass spectrum of compound 8f
Fig. 4.17: H NMR spectrum of compound 9f
Fig. 4.18 Mass spectrum of compound 9f
### 4.2.3 Radiolabeling

To the freshly prepared $[^{99m}\text{TcN}]^{2+}$ intermediate core (750 μL, 1.85 GBq or 50 mCi), was added PNP ligand 6 (~2.5 mg) along with compound 8/9/10/11 (~5 mg) each dissolved in nitrogen-purged ethanol (250 μL) and the reaction mixture heated at 90 °C for 30 min. The prepared final complexes 8C, 9C, 10C and 11C respectively were characterized by HPLC.

### 4.2.4 Quality control techniques

#### 4.2.4.1 HPLC

The radiochemical purities of the $[^{99m}\text{TcN}(\text{PNP})]^+2$ complexes were assessed by HPLC using a C18 reversed phase column. Water (A) and methanol (B) were used as the mobile phase and the following gradient elution technique was adopted for the separation (0 min 50% A, 15 min 0% A, 50 min 0% A). Flow rate was maintained at 1 mL/min. The test solution (25 μL) was injected into the column and elution was monitored by observing the radioactivity profile.

The same C18 reversed phase analytical column was used for the purification of complexes. On purification through HPLC, around 18.5 MBq (500 μCi) of the radiolabeled fatty acid product was obtained in MeOH-water medium which was removed under vacuum and reformulated in aqueous 10% ethanol solution. This was used for carrying out the *in-vitro* and *in-vivo* evaluation studies.

#### 4.2.4.2 Partition coefficient (Log $P_{o/w}$)

The HPLC purified labeled compound (0.1 mL, 185 KBq or 5 μCi) was mixed with water (0.9 mL) and octanol (1 mL) on a vortex mixer for about 1 min and then centrifuged for 5 min to effect the separation of the two layers. Equal aliquots of the two layers were withdrawn and measured for the radioactivity. The readings thus obtained were used to calculate the Log $P_{o/w}$ value of the complex.
4.2.4.3 Stability studies

4.2.4.3.1 Cysteine challenge

A mixture of purified fatty acid complex 8C/ 9C/ 10C/ 11C (50 µL, 370 KBq or 10 µCi) and aqueous 10 mM cysteine solution (50 µL) in saline (400 µL) was incubated at 37 °C for 30 min. Thereafter, the respective samples were analyzed by TLC to determine the radiochemical integrity of the complexes [8C: $R_f = 0.0-0.1$, 9C: $R_f = 0.0-0.1$, 10C: $R_f = 0.1-0.2$, 11C: $R_f = 0.1-0.2$ [EtOH/ CHCl₃/ C₆H₆/ 0.5 M CH₃COONH₄ (1.5:2:1.5:0.5 v/v)].

4.2.4.3.2 Serum stability

The purified fatty acid complex 8C/ 9C/ 10C/ 11C (50 µL, 370 KBq or10 µCi) was incubated with human serum (450 µL) at 37 °C for 30 min. Thereafter, the serum proteins were precipitated by addition of EtOH (500 µL), the solution centrifuged and the supernatant analyzed by TLC.

4.2.4.3 Bio-distribution studies

All procedures performed herein were in accordance with the national laws pertaining to the conduct of animal experiments. Normal Swiss mice (20–25 g body weight) were used for the in-vivo distribution studies. All the mice involved in the study were kept under fasting condition for 6-7 h prior to the experiment, with water given ad libitum. The HPLC purified radiolabeled preparation (100 µL, 740 K bq or 20 µCi) was administered intravenously through tail vein of each animal. Individual sets of animals (n=3) were utilized for studying the bio-distribution at different time points (2 min, 5 min, 10 min and 30 min). The animals were sacrificed immediately at the end of the respective time point and the relevant organs and tissue were excised for measurement of associated activity. The organs were weighed and the activity associated with each was measured in a flat-bed type NaI(Tl) counter with suitable energy window for $^{99m}$Tc (140keV ± 10%). For the sake of comparison, the activity
4.3 Results and discussion

4.3.1 Synthesis

Four fatty acids, having chain lengths of 11, 12, 15 and 16 carbon atoms respectively, were modified with cysteine at ω-position following a four (10 and 11) and six (8 and 9) step synthetic procedures respectively (Fig. 4.19 and Fig. 4.20). All the derivatives 8-11 were prepared after attaching ω-amino group of long chain fatty acid to acid residue of cysteine. The ω-amino esters 8d and 9d were prepared from ω-bromo acids in additional three steps. The first step involved the acid group protection of 8a and 9a followed by conversion of the bromo group to amine via the Gabriel phthalimide synthesis to yield 8d and 9d respectively. The other two amino esters 10b and 11b were directly obtained upon acid protection of 10a and 11a respectively. The free amino group of 8d/9d/10b/11b was coupled with N-Boc, S-trityl cysteine using EDCI as a coupling agent. The ethyl ester group of the coupled derivative 8c/9c/10c/11c was hydrolyzed under alkaline conditions to give 8f/9f/10d/11d. The intermediates were characterized using FT-IR, 1H NMR and Mass spectroscopies. Finally, the target compounds, 8-11 were obtained respectively by simultaneous deprotection of Boc and trityl groups using TFA-Et3SiH. The compounds 8-11 contained free SH, NH2 groups of the cysteine residue in suitable stereochemical orientations for labeling with the [99mTcN(PNP)]2+ core.

4.3.2 Radiolabeling

The radiolabeling strategy involved prior preparation of [99mTcN]2+ intermediate, to which PNP ligand 6 and respective fatty acid derivative 8-11 were added, to yield the desired
Fig. 4.22 HPLC profiles of (a) $[^{99m}\text{Tc}](\text{PNP}6)$ core and complexes (b) 10C (c) 11C (d) 8C (e) 9C
complexes 8C-11C (Fig. 4.21). These were characterized by HPLC [Fig. 4.22(b)-(e)] and the peak area measurements indicated the radiolabeling yields >80%.

The syn and anti isomers of the complexes 8C-11C are possible due to the presence of chiral carbon in cysteine residue. However, the two isomers could be observed only if they are sufficiently resolved in the HPLC column. Thus, while two closely spaced peaks, accounting for the diastereomeric pair with the predominance of one diastereomer, could be observed in the HPLC elution profile of 8C, 9C and 11C complex, such a pattern was not visible in the case of complex 10C. Stereochemistry of the isomers can be unambiguously ascertained only after preparing the complex in macroscopic level using the long lived $^{99}$Tc or inactive rhenium analogue and subsequent characterization. However, this was not attempted considering our major objective to examine the potential of the radiolabeled complexes as a myocardial agent. The major isomer was isolated by HPLC and used for bio-evaluation studies.

In the present study, fatty acid derivatives 8-11 have been prepared with the cysteine residue having free thiol and amino groups. The respective addition of these ligands to the intermediate $[^{99m}\text{TcN}(\text{PNP})]^{2+}$ yields a uni-positively charged [2+2] asymmetric pseudo-octahedral complex. Since the complexes 8C-11C are similar to the complexes reported earlier, therefore these were envisaged to exhibit similar geometry with an overall uni-positive charge.

4.3.3 Biological studies

4.3.3.1 Stability and hydrophobicity of the complexes 8C-11C

As the first step of bio-evaluation, the in-vitro stability of the HPLC purified complexes 8C-11C was studied in presence of challenging ligand cysteine. The complexes upon incubation with excess of cysteine were found to be stable and showed no transchelation as analyzed by TLC. Also, the stability of the complexes in serum was
assessed. The complexes 10C and 11C were found to be stable in serum, however, complexes 8C and 9C showed degradation to an extent of 20% and 10% respectively. The Log P<sub>o/w</sub> values of the four complexes 8C-11C as determined using octanol-water system were found to be 0.80, 0.83, 1.13 and 1.20 respectively.

4.3.3.2 Bio-distribution studies

4.3.4.1 Charged complex 9C in comparison with neutral analogue 7C and 125I-IPPA

To evaluate the effect of charge on the in-vivo pharmacokinetic behavior, in terms of target uptake and non-target clearance characteristics, the bio-distribution results of charged 9C and its neutral analogue 7C compared. Also, to evaluate the potential of charged complex 9C as myocardial agent, its bio-distribution results were compared with the standard agent 125I-IPPA. The Fig. 4.23 shows the myocardial uptake and clearance pattern of the charged complex 9C under evaluation. The results obtained with 125I-IPPA as well as earlier reported neutral analogue 7C are also shown for comparison. The initial uptake shown by 9C (9.88 ± 2.99% ID/g) in the myocardium at 2 min p.i. is similar to that of 125I-IPPA. However, initially accumulated activity was not retained and gets cleared from the myocardium. This shows that the [99mTcN(PNP)]-cysteine moiety is not functioning like the 125I-iodophenyl ring in 125I-IPPA. Though the initial myocardial uptake value of neutral complex 7C was far below 9C, however, the washout kinetics from the myocardium was similar.

![Fig. 4.23 Myocardial uptake and retention of charged complex 9C in comparison with 125I-IPPA and neutral complex 7C](image-url)
The time dependent variation in heart/ blood, heart/ lung and heart/ liver ratios of 9C, 7C and 125-I-IPPA are shown in Fig. 4.24(a)-(c). There is no significant difference in heart/ lung and heart/ liver ratios among the three radiolabeled compounds. 125-I-IPPA, however, showed better heart/ blood ratio compared to the other two complexes 9C and 7C. Unlike the neutral complex 7C, charged analogue 9C showed increased radioactivity uptake in non-target organs with slow washout, accounting for the similarity in the heart/ liver, heart/ lung and heart/ blood ratios of the two complexes.

The clearance of activity from different organs exhibited by 9C, 7C and 125-I-IPPA is shown in Fig. 4.25. Both 9C and 7C cleared via the hepatobiliary route. The rapid increase in the intestinal radioactivity with time indicates fast clearance of the radioactivity from liver. This may be due to metabolizable ether residues present on the final [99mTcN(PNP)] complex which facilitates early clearance of the radioactivity from the liver. Also, a different
mechanism based on P-glycoproteins (Pgp) or multidrug resistance-associated protein (MDR)-Pgp may also be responsible for rapid elimination of $[^{99m}TcN(PNP)]$ complex from the tissues. Contrary to $9C$ and $7C$, $^{125}\text{I}-\text{IPPA}$ shows slow clearance of liver activity which results in low heart/liver ratios. The effectiveness of $[^{99m}\text{TcN}(\text{PNP})]^{2+}$ intermediate in achieving good in-vivo pharmacokinetic behavior is evident from the rapid non-target clearance pattern of $[^{99m}\text{TcN}(\text{PNP})]^{2+}$ complexes compared to $^{125}\text{I}-\text{IPPA}$.

Thus, charged complexes seem to be better molecules than their neutral counterparts in terms of target uptake (initial value similar to the standard agent), however, show inferior

Fig. 4.25 Activity distribution pattern of (a) charged complex $9C$ (b) $^{125}\text{I}-\text{IPPA}$ and (c) neutral complex $7C$ in different organs in Swiss mice.
behavior in terms of non-target clearance kinetics. Both the complexes show identical behavior in terms of washout kinetics of the radioactivity from the myocardium and in target/non-target ratios.

4.3.4.2 Other fatty acid complexes

The superior target uptake pattern of charged complex over its neutral analogue infused interest in exploring the different charged complexes of varying chain lengths. In this view, the bio-distribution of purified complexes 8C, 10C and 11C were carried in Swiss mice and their results compared alongwith 9C. Also, to evaluate their potential as myocardial agent, the comparison with ¹²⁵I-IPPA has been carried out. The bio-distribution results of 8C-11C are shown in Tables 4.1-4.4. All four complexes showed uptake in the myocardium (Fig. 4.26). A steady rise in the myocardial uptake values was observed on increasing the fatty acid chain length (lipophilicity), however, the initial uptake was not retained. The clearance of radioactivity from the myocardium was non-uniform with the initial rapid clearance phase up to 10 min p.i. was followed by slow phase upto 30 min p.i.

![Fig. 4.26 Uptake and retention characteristics of different fatty acid complexes (8C-11C) in the myocardium of Swiss mice](image-url)
Table 4.1 Bio-distribution of complex 10C in Swiss mice [% ID/ g (1SD), (n=3)]

<table>
<thead>
<tr>
<th>Organs</th>
<th>2 min</th>
<th>5 min</th>
<th>10 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>84.03 (11.46)</td>
<td>73.78 (10.72)</td>
<td>63.23 (19.69)</td>
<td>23.87 (3.68)</td>
</tr>
<tr>
<td>Int + GB</td>
<td>5.10 (1.63)</td>
<td>16.99 (6.35)</td>
<td>34.01 (5.14)</td>
<td>55.82 (10.22)</td>
</tr>
<tr>
<td>Kidney</td>
<td>9.83 (0.75)</td>
<td>5.95 (1.48)</td>
<td>3.17 (0.40)</td>
<td>2.38 (0.93)</td>
</tr>
<tr>
<td>Heart</td>
<td>4.40 (1.38)</td>
<td>1.80 (0.19)</td>
<td>1.19 (0.36)</td>
<td>0.85 (0.12)</td>
</tr>
<tr>
<td>Lungs</td>
<td>12.24 (4.68)</td>
<td>4.40 (0.76)</td>
<td>1.89 (0.70)</td>
<td>1.15 (0.31)</td>
</tr>
<tr>
<td>Muscles</td>
<td>0.67 (0.05)</td>
<td>0.60 (0.09)</td>
<td>0.46 (0.02)</td>
<td>0.22 (0.05)</td>
</tr>
<tr>
<td>Blood</td>
<td>6.77 (3.07)</td>
<td>1.22 (0.16)</td>
<td>0.85 (0.16)</td>
<td>0.25 (0.01)</td>
</tr>
<tr>
<td>Heart/ blood</td>
<td>0.8 (0.3)</td>
<td>1.48 (0.12)</td>
<td>1.36 (0.18)</td>
<td>3.44 (0.76)</td>
</tr>
<tr>
<td>Heart/ liver</td>
<td>0.06 (0.02)</td>
<td>0.02 (0.00)</td>
<td>0.02 (0.00)</td>
<td>0.04 (0.01)</td>
</tr>
<tr>
<td>Heart/ lungs</td>
<td>0.4 (0.15)</td>
<td>0.42 (0.07)</td>
<td>0.69 (0.18)</td>
<td>0.8 (0.2)</td>
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</tbody>
</table>

Table 4.2 Bio-distribution of complex 11C in Swiss mice [% ID/ g (1SD), (n=3)]

<table>
<thead>
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<th>10 min</th>
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</thead>
<tbody>
<tr>
<td>Liver</td>
<td>35.71 (5.24)</td>
<td>38.94 (4.09)</td>
<td>30.04 (4.24)</td>
<td>15.57 (3.03)</td>
</tr>
<tr>
<td>Int + GB</td>
<td>7.65 (3.05)</td>
<td>20.33 (2.93)</td>
<td>15.44 (1.59)</td>
<td>33.10 (6.10)</td>
</tr>
<tr>
<td>Kidney</td>
<td>12.06 (0.69)</td>
<td>9.39 (1.15)</td>
<td>6.02 (1.26)</td>
<td>4.91 (0.57)</td>
</tr>
<tr>
<td>Heart</td>
<td>3.65 (0.74)</td>
<td>3.59 (0.23)</td>
<td>1.51 (0.27)</td>
<td>1.08 (0.08)</td>
</tr>
<tr>
<td>Lungs</td>
<td>8.66 (1.75)</td>
<td>2.67 (1.56)</td>
<td>2.02 (1.12)</td>
<td>1.09 (0.46)</td>
</tr>
<tr>
<td>Muscles</td>
<td>0.96 (0.05)</td>
<td>0.98 (0.07)</td>
<td>0.55 (0.15)</td>
<td>0.50 (0.15)</td>
</tr>
<tr>
<td>Blood</td>
<td>10.40 (1.29)</td>
<td>7.34 (1.65)</td>
<td>3.98 (0.52)</td>
<td>2.64 (0.24)</td>
</tr>
<tr>
<td>Heart/ blood</td>
<td>0.35 (0.08)</td>
<td>0.52 (0.12)</td>
<td>0.38 (0.03)</td>
<td>0.41 (0.00)</td>
</tr>
<tr>
<td>Heart/ liver</td>
<td>0.1 (0.03)</td>
<td>0.09 (0.01)</td>
<td>0.05 (0.00)</td>
<td>0.07 (0.02)</td>
</tr>
<tr>
<td>Heart/ lungs</td>
<td>0.42 (0.02)</td>
<td>0.33 (0.03)</td>
<td>0.33 (0.03)</td>
<td>0.3 (0.09)</td>
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</table>
Table 4.3 Bio-distribution of complex 8C in Swiss mice [% ID/ g (1SD), (n=3)]

<table>
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<tr>
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<th>10 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>33.97 (14.45)</td>
<td>26.48 (8.03)</td>
<td>29.43 (4.58)</td>
<td>17.73 (5.13)</td>
</tr>
<tr>
<td>Int + GB</td>
<td>3.10 (1.66)</td>
<td>4.30 (2.19)</td>
<td>8.12 (2.10)</td>
<td>15.57 (3.23)</td>
</tr>
<tr>
<td>Kidney</td>
<td>9.75 (3.50)</td>
<td>6.85 (0.74)</td>
<td>4.73 (2.96)</td>
<td>2.62 (1.10)</td>
</tr>
<tr>
<td>Heart</td>
<td>5.57 (1.67)</td>
<td>3.37 (0.09)</td>
<td>0.68 (0.24)</td>
<td>0.39 (0.08)</td>
</tr>
<tr>
<td>Lungs</td>
<td>16.84 (2.88)</td>
<td>8.12 (1.92)</td>
<td>2.11 (0.28)</td>
<td>1.09 (0.36)</td>
</tr>
<tr>
<td>Muscles</td>
<td>0.97 (0.3)</td>
<td>0.58 (0.12)</td>
<td>0.32 (0.10)</td>
<td>0.14 (0.08)</td>
</tr>
<tr>
<td>Blood</td>
<td>16.83 (3.00)</td>
<td>10.94 (3.74)</td>
<td>2.11 (0.93)</td>
<td>1.09 (0.36)</td>
</tr>
<tr>
<td>Heart/ blood</td>
<td>0.36 (0.18)</td>
<td>0.32 (0.06)</td>
<td>0.35 (0.12)</td>
<td>0.38 (0.05)</td>
</tr>
<tr>
<td>Heart/ liver</td>
<td>0.18 (0.08)</td>
<td>0.13 (0.02)</td>
<td>0.03 (0.00)</td>
<td>0.02 (0.00)</td>
</tr>
<tr>
<td>Heart/ lungs</td>
<td>0.34 (0.1)</td>
<td>0.42 (0.05)</td>
<td>0.42 (0.13)</td>
<td>0.21 (0.05)</td>
</tr>
</tbody>
</table>

Table 4.4 Bio-distribution of complex 9C in Swiss mice [% ID/ g (1SD), (n=3)]

<table>
<thead>
<tr>
<th>Organs</th>
<th>2 min</th>
<th>5 min</th>
<th>10 min</th>
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</thead>
<tbody>
<tr>
<td>Liver</td>
<td>17.92 (9.15)</td>
<td>35.63 (14.71)</td>
<td>33.80 (14.53)</td>
<td>21.82 (9.34)</td>
</tr>
<tr>
<td>Int + GB</td>
<td>3.92 (3.49)</td>
<td>20.53 (12.16)</td>
<td>16.60 (7.73)</td>
<td>26.46 (2.12)</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.81 (3.90)</td>
<td>10.27 (4.21)</td>
<td>6.32 (1.52)</td>
<td>3.33 (0.60)</td>
</tr>
<tr>
<td>Heart</td>
<td>9.88 (2.99)</td>
<td>3.56 (2.28)</td>
<td>2.31 (0.97)</td>
<td>1.39 (0.36)</td>
</tr>
<tr>
<td>Lungs</td>
<td>28.38 (5.89)</td>
<td>9.70 (4.13)</td>
<td>6.87 (3.98)</td>
<td>3.87 (0.84)</td>
</tr>
<tr>
<td>Muscles</td>
<td>1.36 (0.24)</td>
<td>1.03 (0.11)</td>
<td>0.76 (0.08)</td>
<td>0.24 (0.00)</td>
</tr>
<tr>
<td>Blood</td>
<td>18.46 (5.77)</td>
<td>6.77 (1.63)</td>
<td>4.00 (0.23)</td>
<td>2.37 (0.18)</td>
</tr>
<tr>
<td>Heart/ blood</td>
<td>0.54 (0.05)</td>
<td>0.5 (0.16)</td>
<td>0.52 (0.17)</td>
<td>0.59 (0.2)</td>
</tr>
<tr>
<td>Heart/ liver</td>
<td>0.90 (0.75)</td>
<td>0.1 (0.01)</td>
<td>0.07 (0.00)</td>
<td>0.07 (0.03)</td>
</tr>
<tr>
<td>Heart/ lungs</td>
<td>0.35 (0.16)</td>
<td>0.35 (0.06)</td>
<td>0.28 (0.13)</td>
<td>0.35 (0.05)</td>
</tr>
</tbody>
</table>
For the complex 10C, the heart/blood ratio improved with time attaining a maximum of 3.44 ± 0.76 at 30 min p.i., which was observed to be better than the value obtained with 125I-IPPA [Fig. 4.27 (a)] at same interval of time. However, for complexes 8C, 9C and 11C the ratio did not improve above 1 throughout the period of study. The heart/lung and heart/liver ratios also did not exceed 1 and similar trend was obtained with 125I-IPPA [Fig. 4.27 (b) & 4.27 (c)].

**Fig. 4.27** Time dependent changes in the (a) heart/ blood (b) heart/ lung (c) heart/ liver ratios of different radiolabeled fatty acids (8C-11C and 125I-IPPA)
The time dependent radioactivity profile of the complexes 8C, 10C and 11C in other non-target organs is shown in Fig. 4.28. The washout pattern of radioactivity from blood, liver and lungs shows a decreasing trend with increase in the fatty acid chain length (lipophilicity). The complexes cleared mainly via the hepatobiliary route. The radioactivity from other non-target organs was also found to clear with time.

**Fig. 4.28** Activity distribution pattern of complexes (a) 10C (b) 11C and (c) 8C in different organs in Swiss mice
4.4 Conclusion

The present chapter is aimed at depicting the versatility of $[^{99mTc} \text{N(PNP)}]^{2+}$ intermediate by varying the charge and lipophilicity of $[^{99mTc} \text{N(PNP)}]^{2+}$ complexes. In this regard, a series of four uni-positively charged fatty acid complexes of varying chain lengths (11, 12, 15 and 16 carbon) were synthesized. The bio-distribution studies of uni-positively charged 16 carbon fatty acid complex in Swiss mice showed high initial myocardial extraction similar to the standard agent $^{125\text{I}}$-IPPA at 2 min p.i., however with poor retention subsequently. The complex showed better uptake characteristics than the neutral analogue. Thus, positively charged fatty acid derivatives, prepared using $[^{99mTc} \text{N(PNP)}]^{2+}$ core seem to be better candidates for the development of myocardial metabolic tracers than their neutral counterparts reported in the previous chapter.

The increase in the fatty acid chain length from 11 to 16 showed steady improvement in the myocardial uptake values. However, all the complexes showed rapid washout from the myocardium limiting their utility for external imaging. The effect of lipophilicity increase on the clearance pattern from the non-target organs, on the contrary, showed a decreasing trend. Thus, a proper balance of chain length, which shows an optimum uptake and clearance from the non-target organs so as to maximize target/ non-target ratio, and a modification in the lead molecule to affect prolonged retention in the myocardium, is desired for the development of a new myocardial metabolic tracer.