[^{99m}Tc]Tc-DTPA-bis(Cholineethylamine) as an Oncologic Tracer for Detection of Choline Transporter (ChT) and Choline Kinase (ChK) Expression in Cancer

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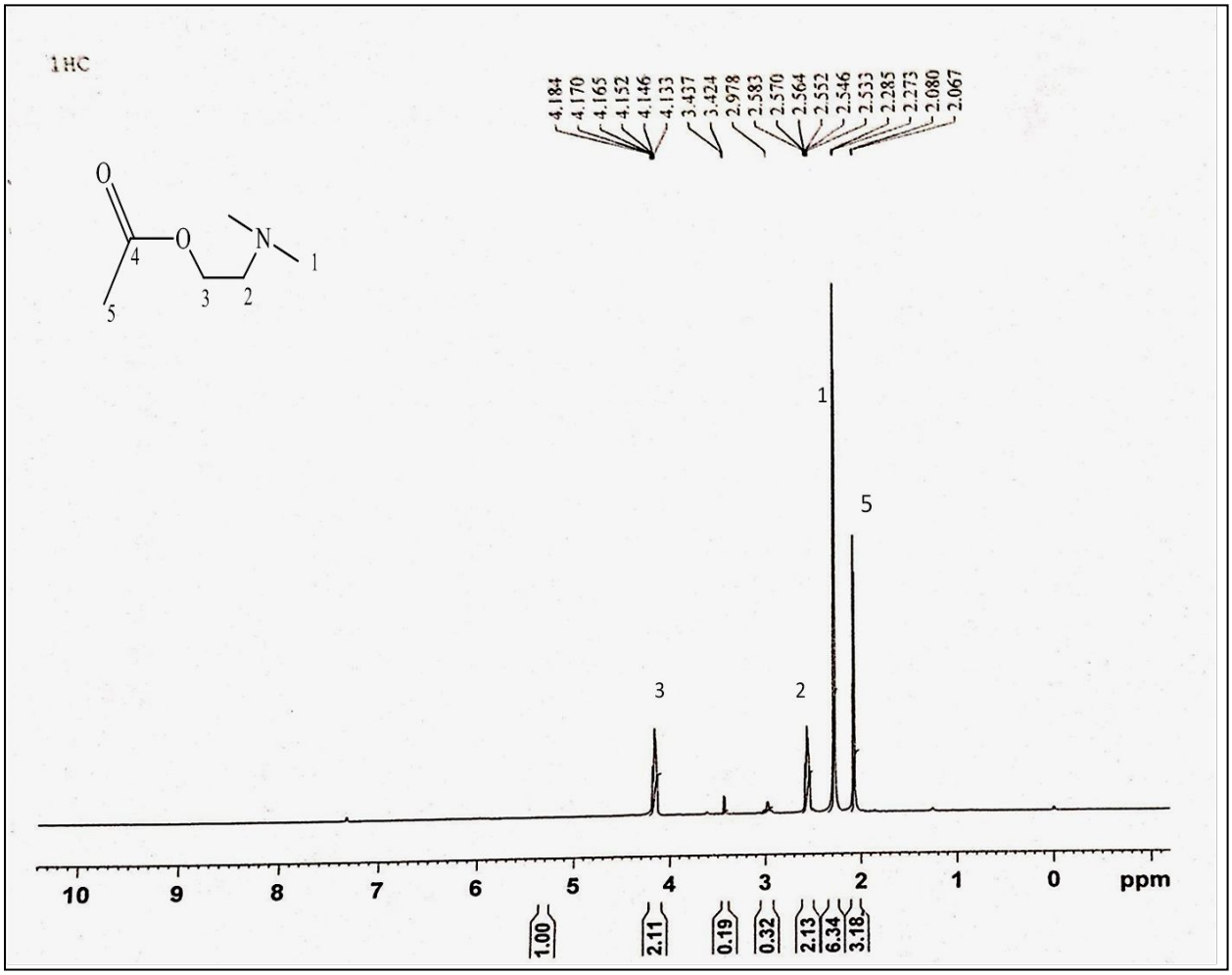
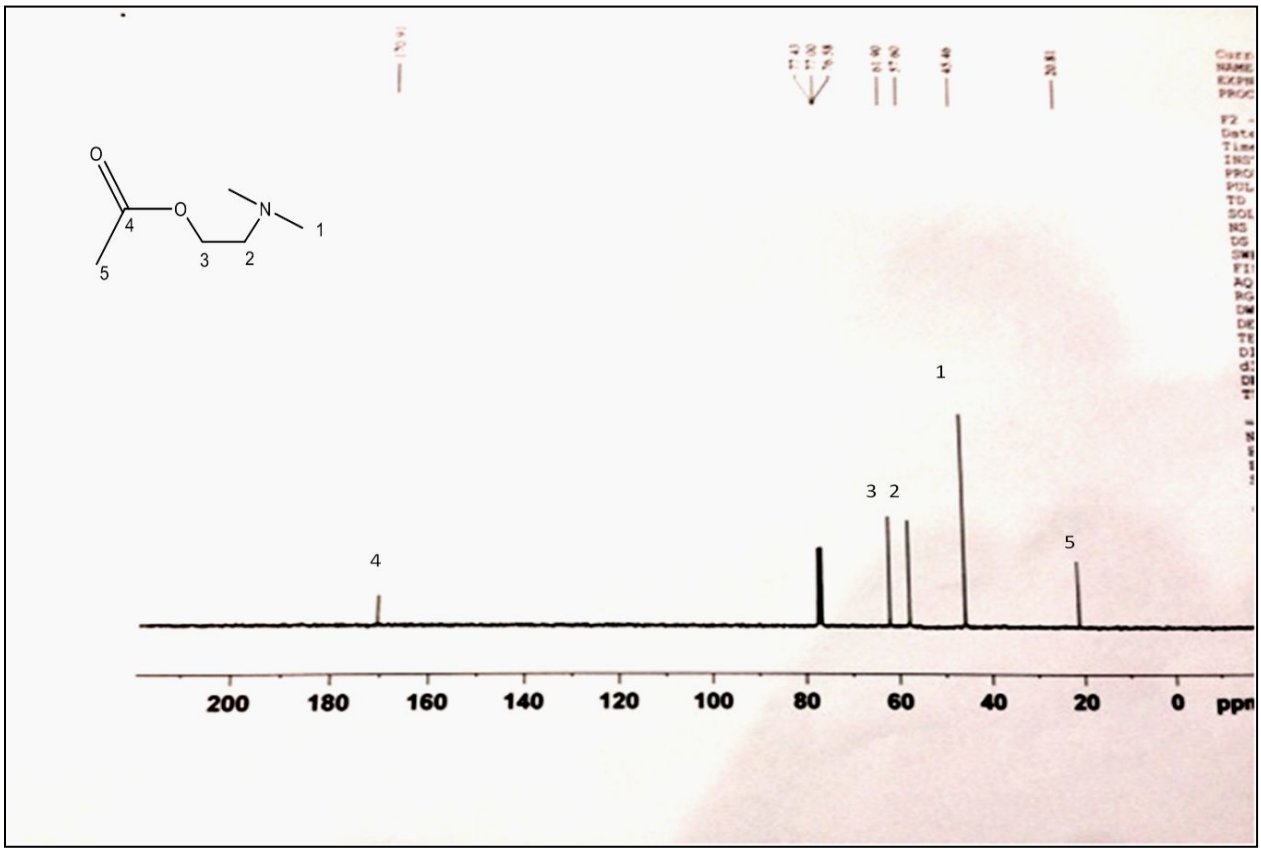
Figure S1: ^{1}H Spectrtum of 2-(dimethylamino)ethylacetate



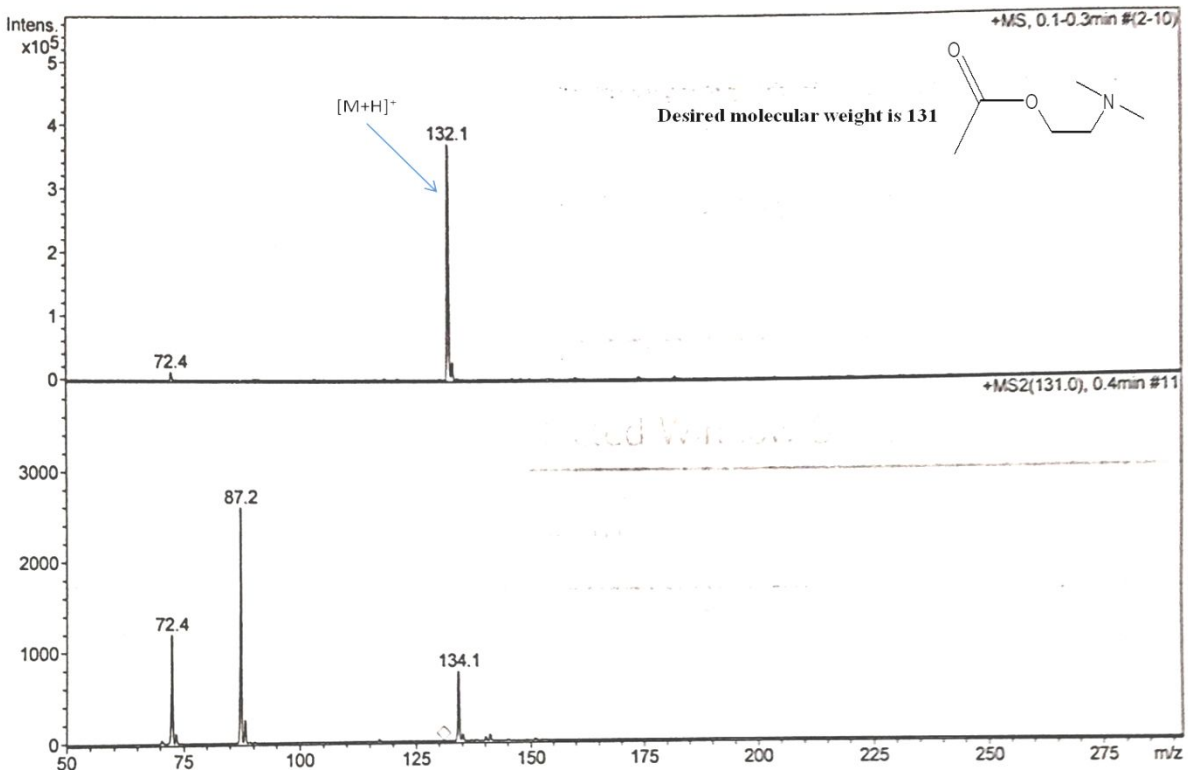
Step 1: ^{1}HNMR (400 MHz, $CDCl_3$) δ (ppm): 2.06 (s, 3H, -C**H**$_3$), 2.28 (s, 6H, -N(C**H**$_3$)$_2$), 2.56 (t, 2H, -C**H**$_2$-), 4.15 (t, 2H, -C**H**$_2$-).

Figure S2 : ^{13}C Spectrum of 2-(dimethylamino)ethylacetate



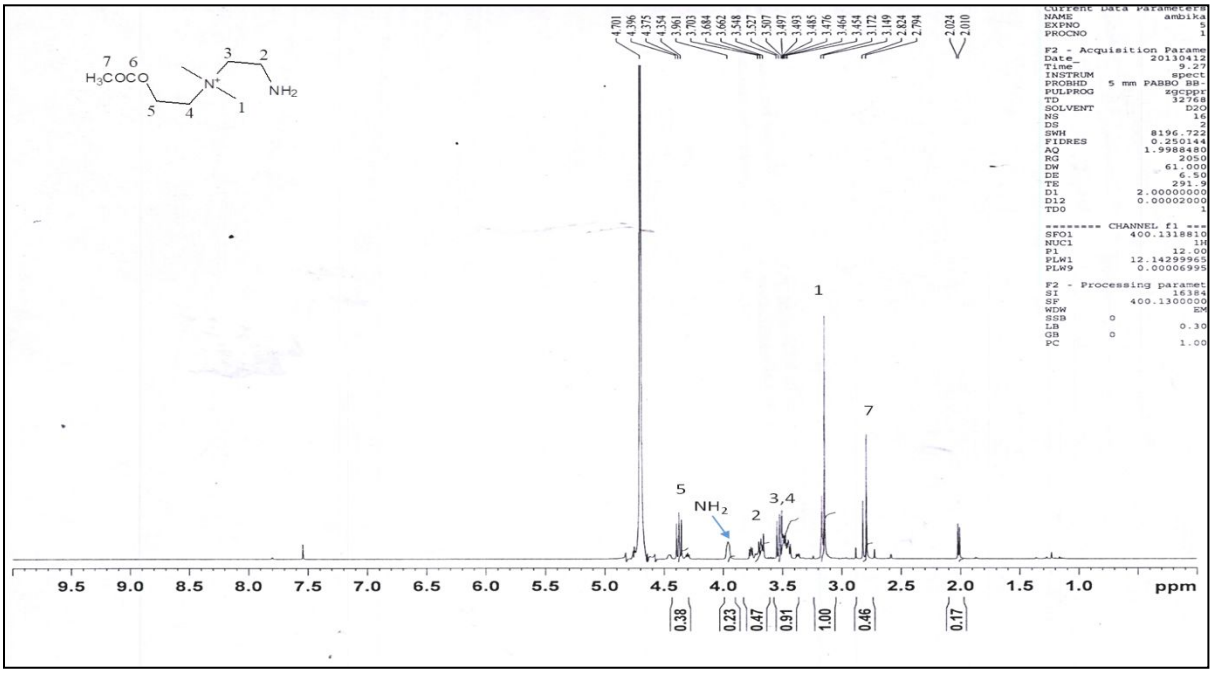
Step1:^{13}C NMR (100 MHz, CDCl3) δ (ppm):20.81 (-**C**H_3), 45.40 (-N(**C**H_3)$_2$), 57.60 (-**C**H_2-), 61.90 (-**C**H_2-), 170.91 (-**C**O-).

Figure S3: Mass Spectrum of 2-(dimethylamino)ethylacetate



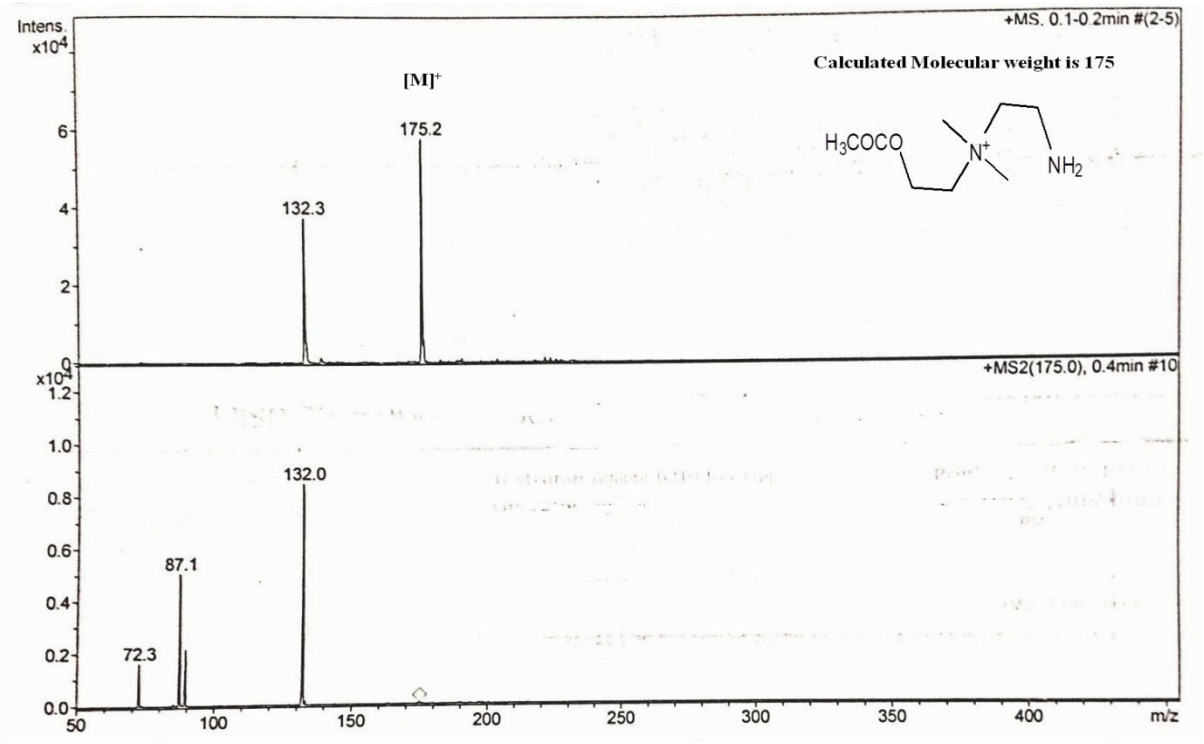
Step1:ESI-MS (m/z): $C_6H_{14}NO_2$: 131 Found 132.1 $[M+H]^+$.

Figure S4: ^{1}H spectrum of 2-acetoxy-N-(2-aminoethyl)-N,N-dimethylethanaminium

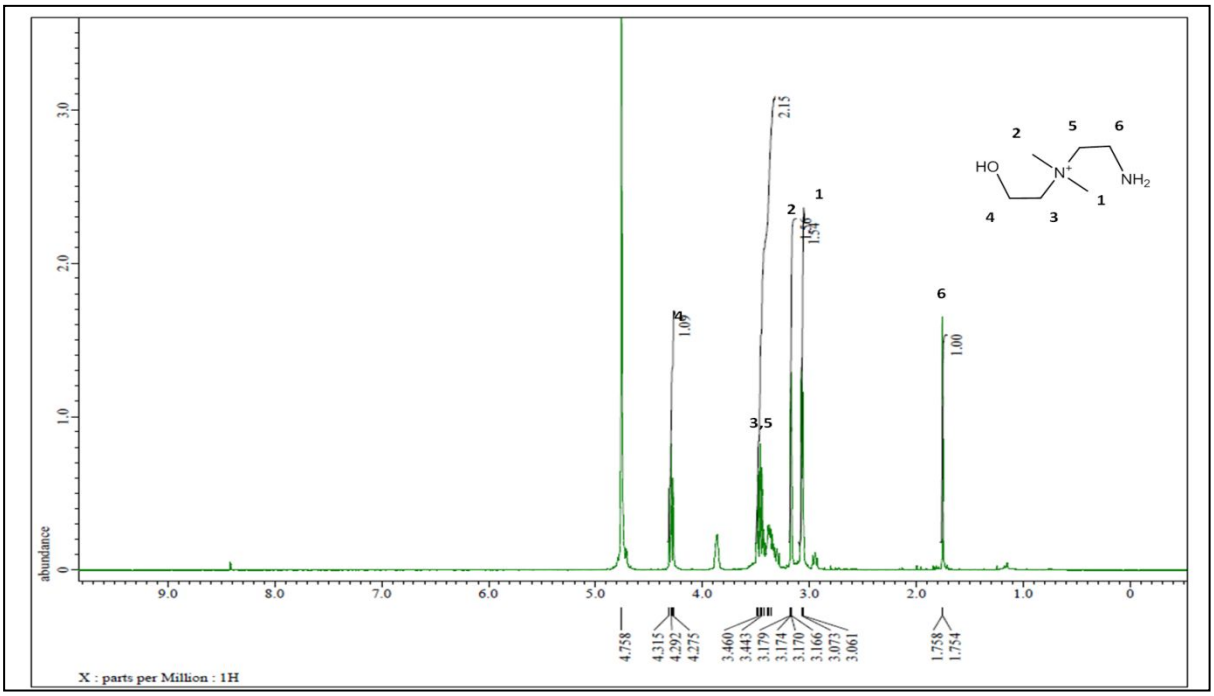


Step2: ^{1}HNMR (400 MHz, $CDCl_3$) δ (ppm) : 2.8 (s, 3H, COOC**H**3), 3.17 (s, 6H, -N^{+}(C**H**3)2), 3.49 (m, 4H, 2×-C**H**2-), 3.54 (t, 2H, -C**H**2-NH2), 3.96 (brs, 2H, -N**H**2), 4.35 (t, 2H, -C**H**2-)

Figure S5: Mass spectrum of Synthesis of 2-acetoxy-N-(2-aminoethyl)-N,N-dimethylethanaminium

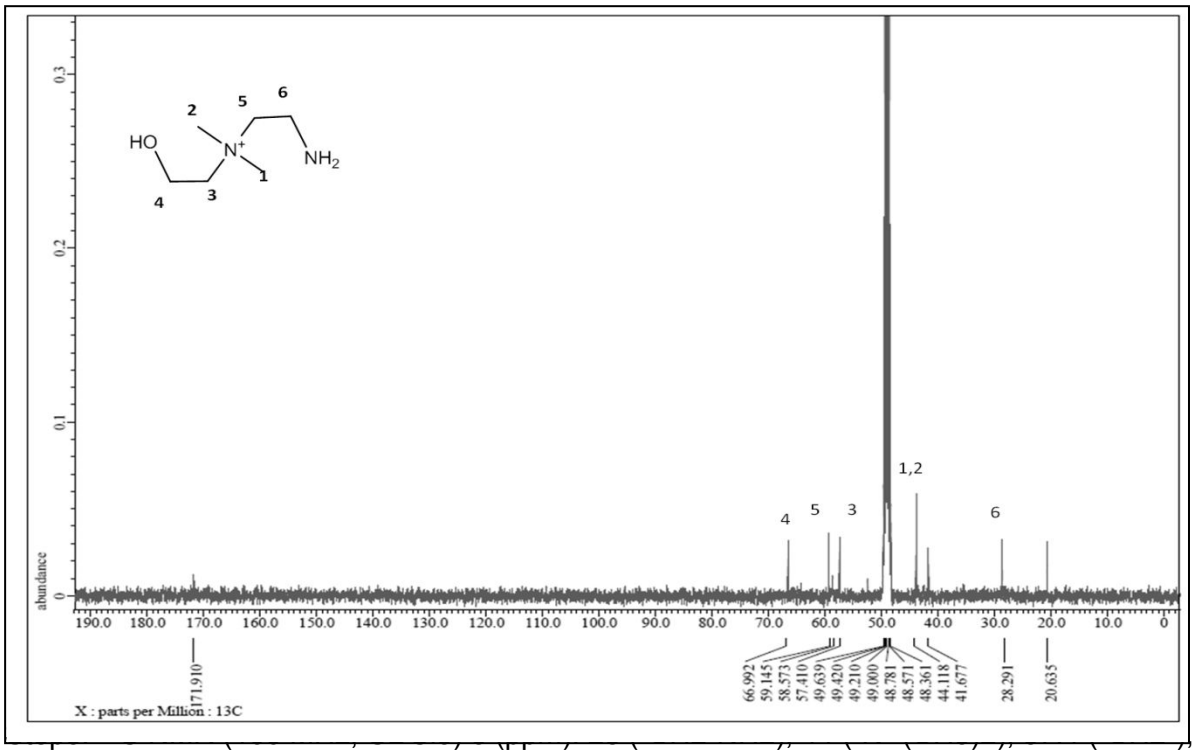


FigureS6: ^{1}H Spectrum of 2-amino-N-(2-hydroxyethyl)-N, N-dimethylethanaminium



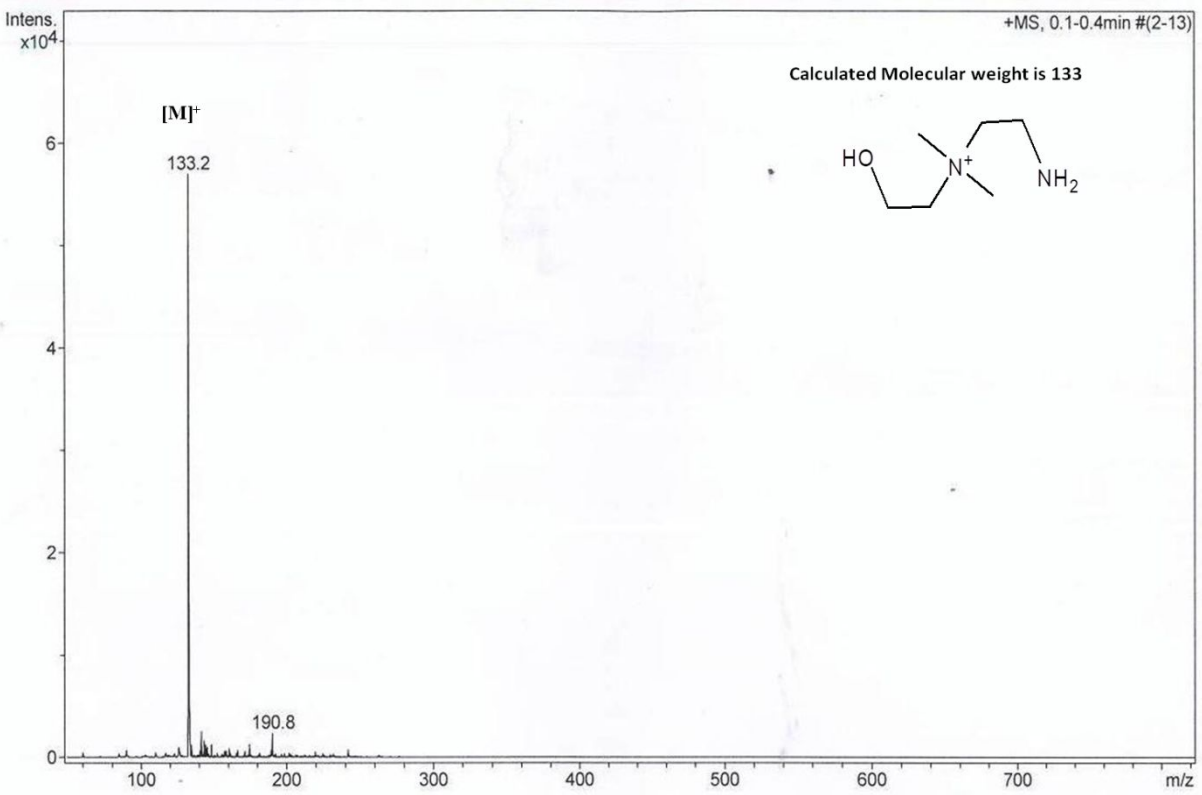
Step3: ^{1}HNMR (400 MHz, $CDCl_3$) δ (ppm): 1.71 (s, 2H, -C**H**2-NH2), 3.06 (s, 3H, -N^+ (C**H**3)), 3.17 (3H, -N^+ (C**H**3)), 3.4-3.6 (m, 4H, 2× -C**H**2-), 3.78 (brs, 2H, -N**H**2), 4.3 (T, 2H, -C**H**2-).

Figure S7: ^{13}C Spectrum of 2-amino-N-(2-hydroxyethyl)-N, N-dimethylethanaminium



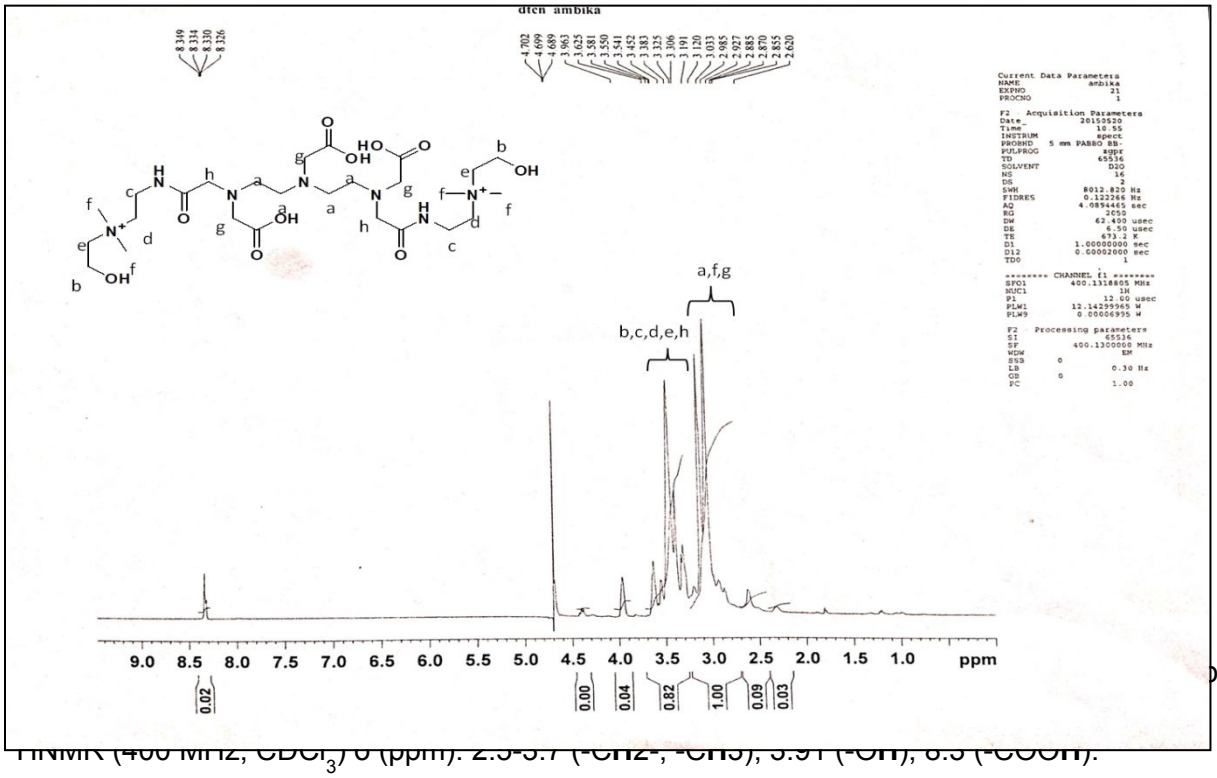
59.2 (-**C**H2-), 66.3 (-**C**H2-).

FigureS8 : Mass Spectrum of 2-amino-N-(2-hydroxyethyl)-N, N-dimethylethanaminium



Step3: ESI-MS (m/z): $C_6H_{17}N_2O^+$: calculated mass: 133; Found at 133.2 $[M]^+$

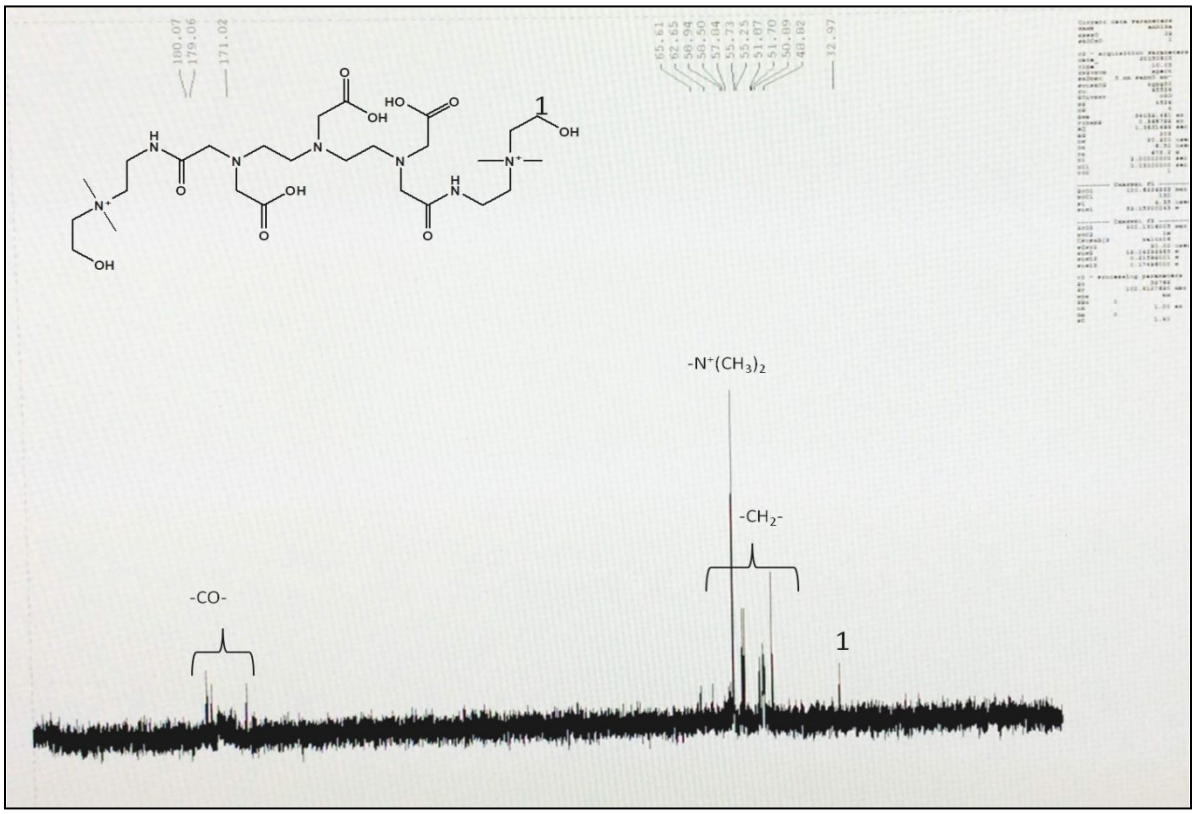
FigureS9: ^{1}H Spectrum of 6,9,12-tris(carboxymethyl)-N1,N17-bis(2-hydroxyethyl)-N1,N1,N17,N17-tetramethyl-4,14-dioxo-3,6,9,12,15-pentaazaheptadecane-1,17-diaminium



)4:

^{1}HNMR (400 MHz, $CDCl_3$) δ (ppm): 2.5-3.7 (-CH2-, -CH3), 3.91 (-OH), 8.5 (-COOH).

Figure S10: ^{13}C Spectrum of 6,9,12-tris(carboxymethyl)-N1,N17-bis(2-hydroxyethyl)-N1,N1,N17,N17-tetramethyl-4,14-dioxo-3,6,9,12,15-pentaazaheptadecane-1,17-diaminium

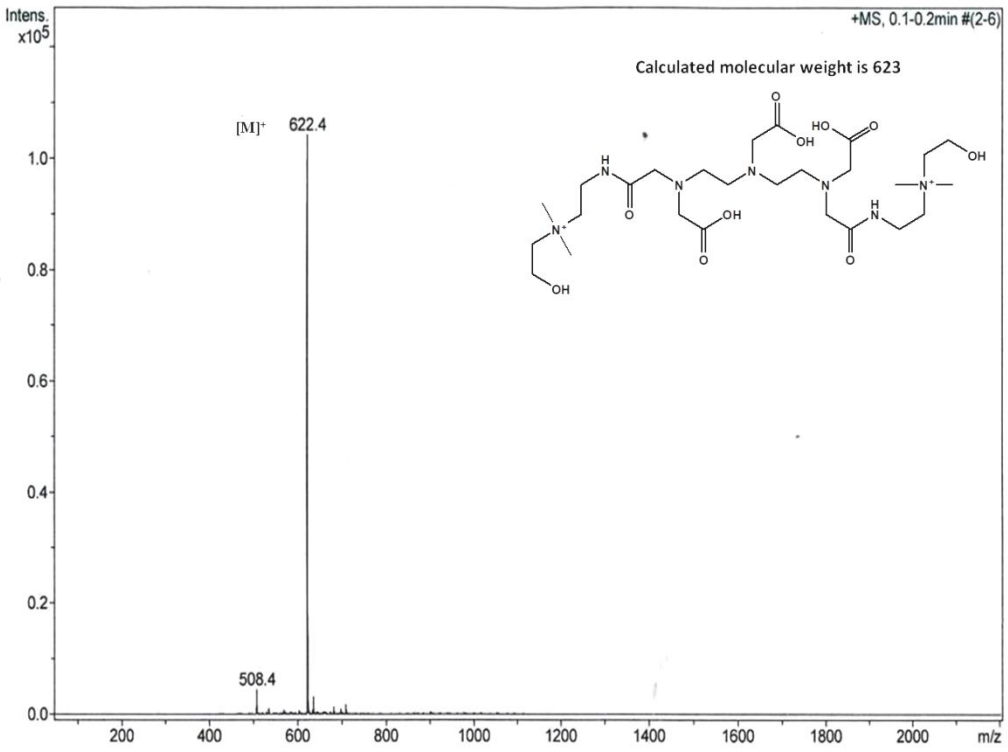


180 (-**C**OOH).

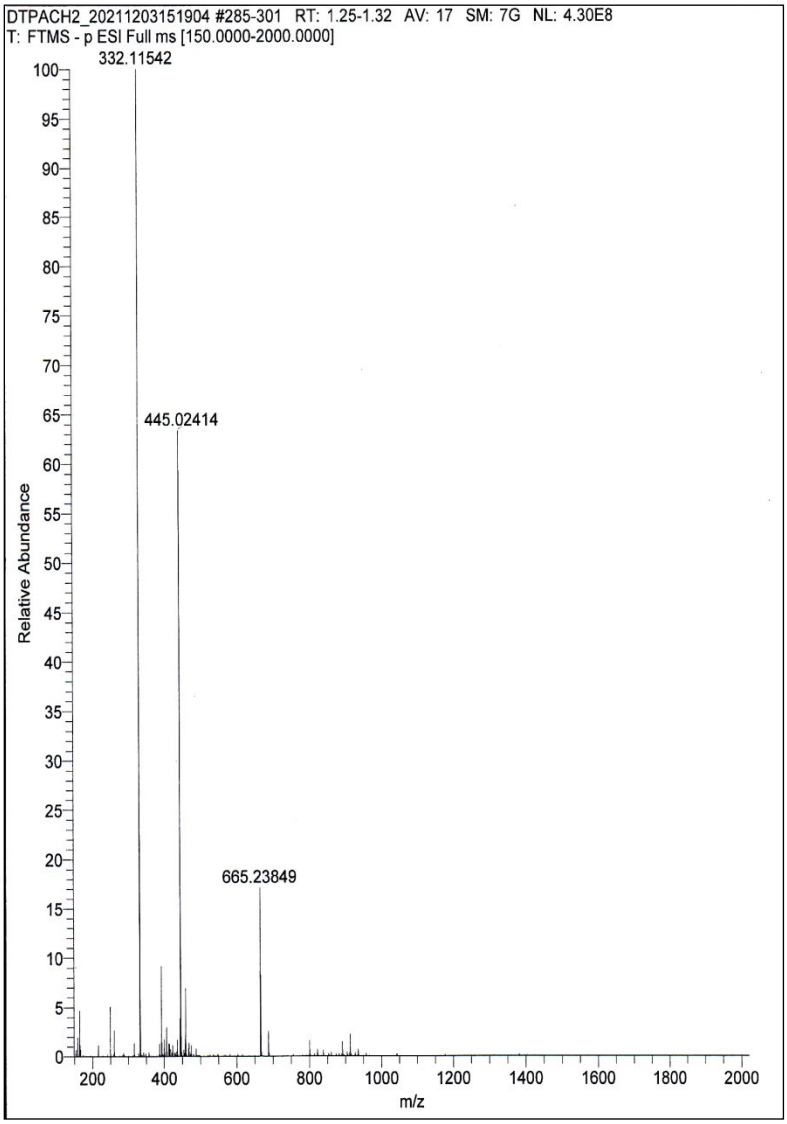
Figure S11 Mass Spectra of 6,9,12-tris(carboxymethyl)-N1,N17-bis(2-hydroxyethyl)-N1,N1,N17,N17-tetramethyl-4,14-dioxo-3,6,9,12,15-pentaazaheptadecane-1,17-diaminium

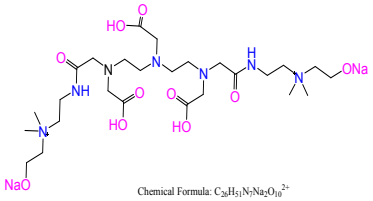
a) LCMS Spectrum b) HRAMS Spectrum

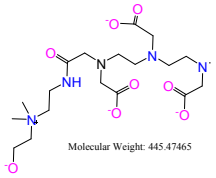
a)



b)


DTPACH2_20211203151904 #285-301 RT: 1.25-1.32 AV: 17 SM: 7G NL: 4.30E8
T: FTMS - p ESI Full ms [150.0000-2000.0000]
332.11542
445.02414
665.23849
Relative Abundance
m/z


Chemical Formula: C26H51N7Na2O10 2+
m/z: 333.67409


Molecular Weight: 445.47465

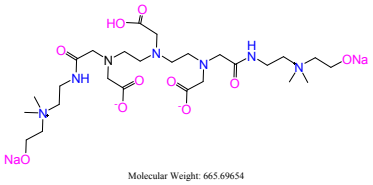
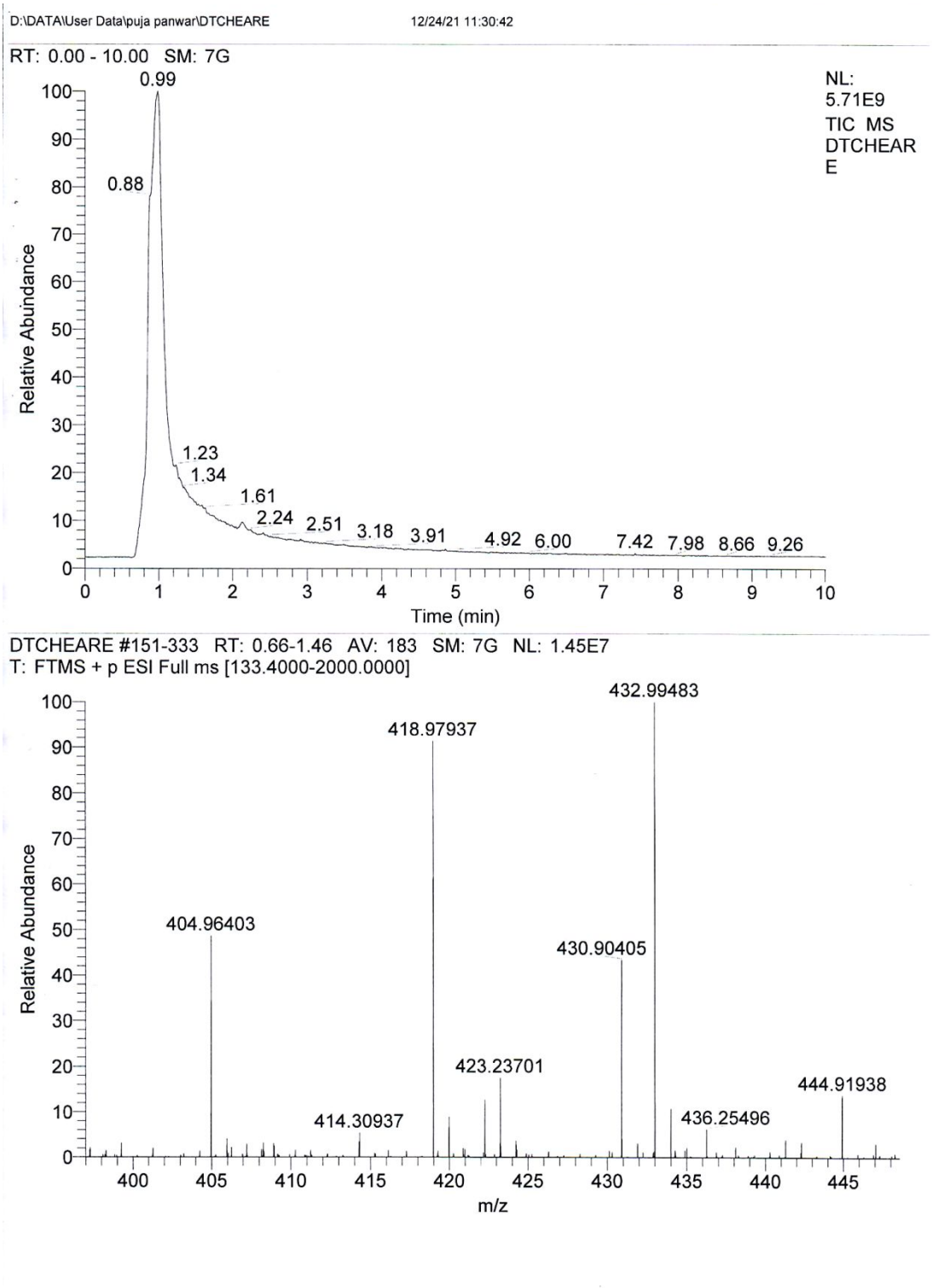

Molecular Weight: 665.69654

Figure S12: HRMS spectra of Re- DTPA-bis(ChoEA) complex



TableS1: Table showing calculated and observed mass in HRMS spectra of plausible Re-DTPA-bis(ChoEA) Complex.

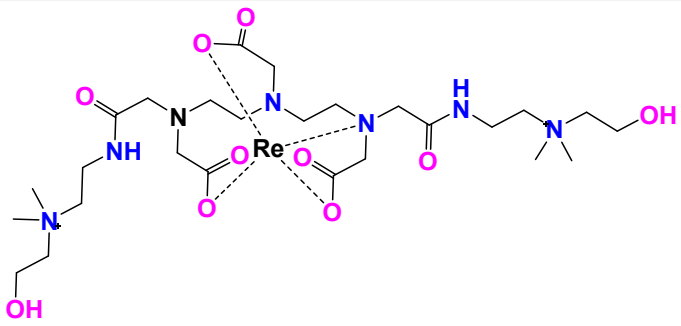
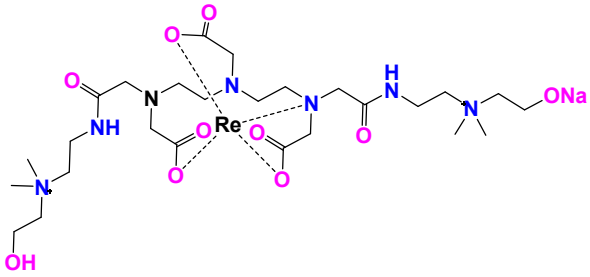
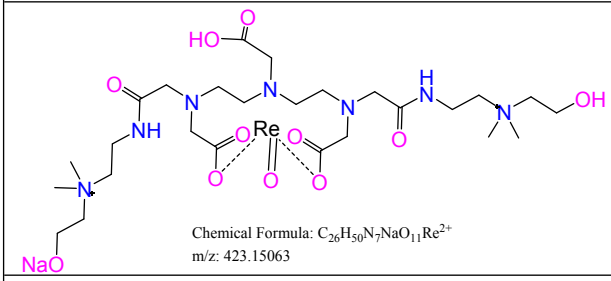
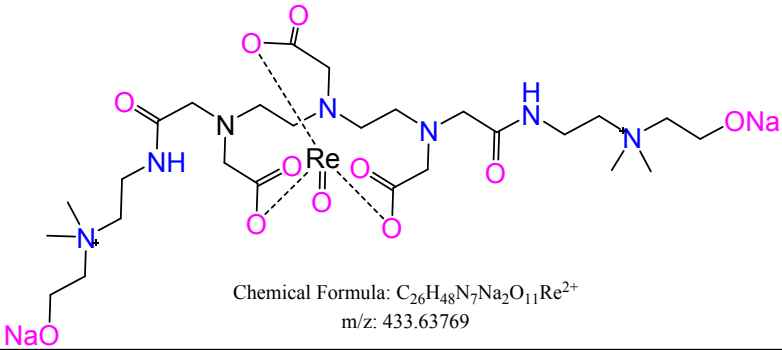
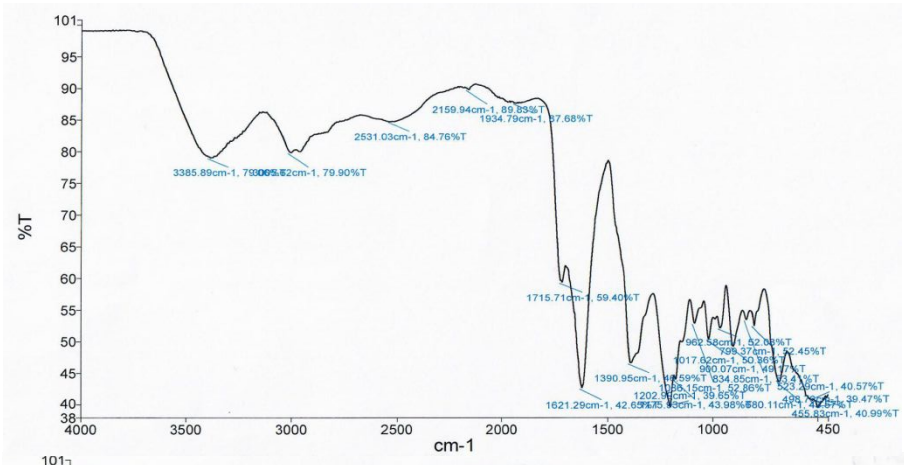
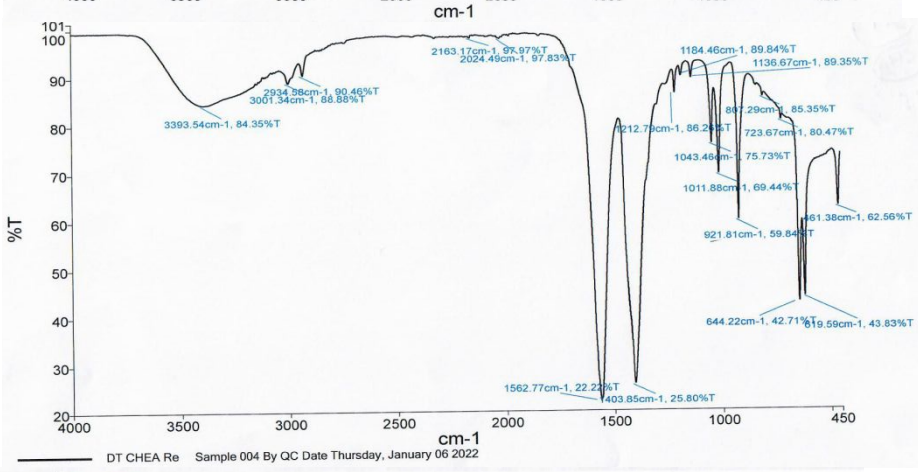
 Chemical Formula: $C_{26}H_{50}N_7O_{10}Re^{2+}$ m/z: 403.65828	Found: 404.96403
 Chemical Formula: $C_{26}H_{49}N_7NaO_{10}Re^{2+}$ m/z: 414.64926	Found:414.30937
 Chemical Formula: $C_{26}H_{50}N_7NaO_{11}Re^{2+}$ m/z: 423.15063	Found: 423.23701
 Chemical Formula: $C_{26}H_{48}N_7Na_2O_{11}Re^{2+}$ m/z: 433.63769	Found 432.99483

Figure S13: IR spectra of a) DTPA-bis(ChoEA) b)Re-DTPA-bis(ChoEA) complex c) Overlay of IR Spectra

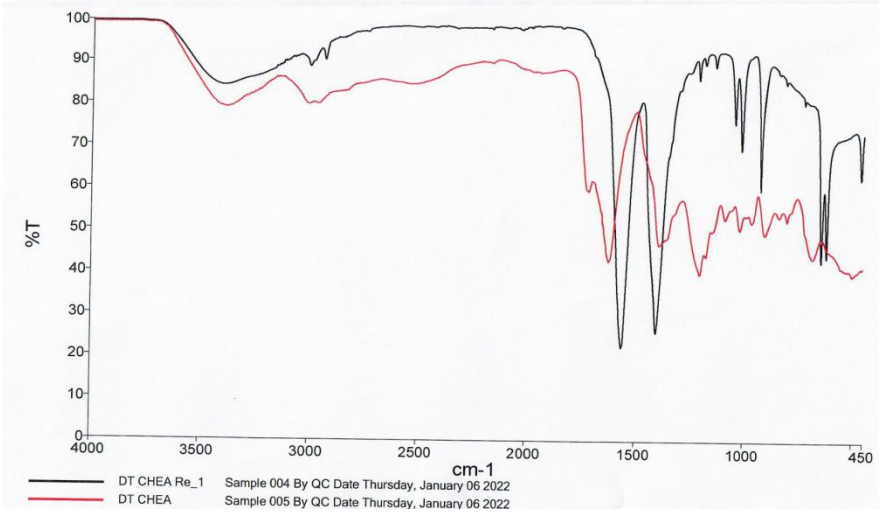
a)



b)



c)



Methods

In vivo toxicity Assay

***3-(4, 5-dimethylthiazol-2-Yl)-2, 5-Diphenyltetrazolium Bromide* (MTT) Assay/ Cell viability assay**

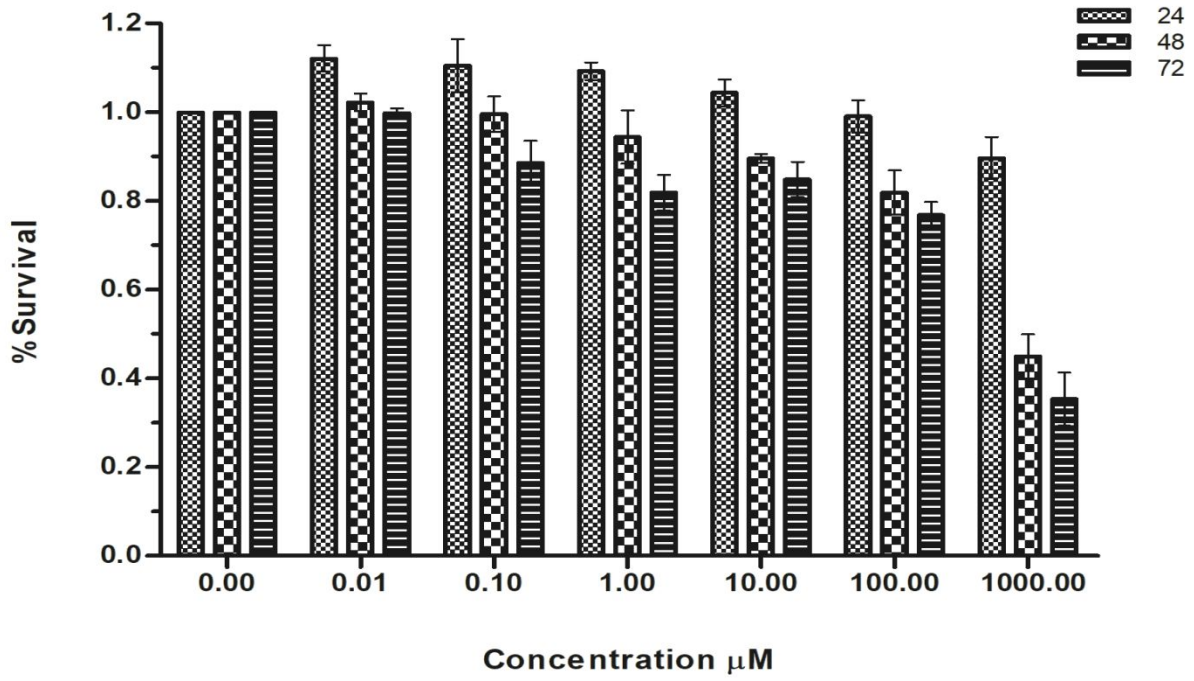
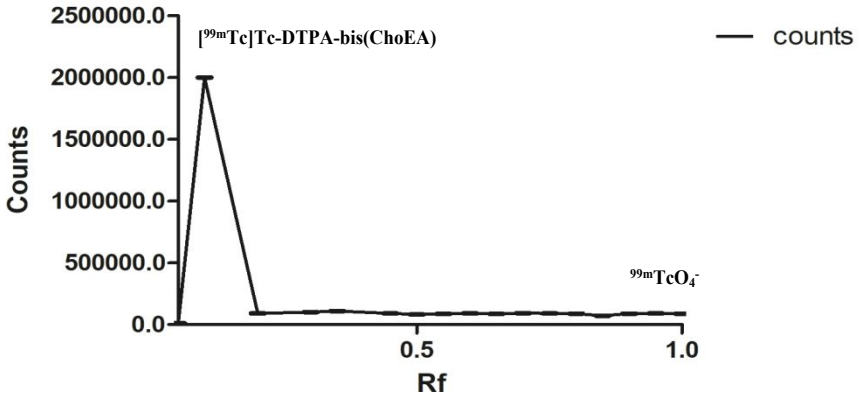


Figure S14 Cytotoxicity evaluation of DTPA-bis(ChoEA) on HEK-293 cell line. Data is plotted for survival fraction versus concentration of compound for different time intervals.

Result: Cell viability for DTPA-bis(ChoEA) was evaluated by calculating total cell surviving fraction for a range of concentrations (0.001μM-1000μM). Toxicity was evaluated for HEK-293 cell line for unlabeled compound. The derivative was not able to induce cytotoxicity at lower concentrations. Both time and concentration dependent toxicity in the form of time dependent bar graph are shown in supplementary file section 2.1. The conjugate was found to be relative less toxic up to 100 μM concentrations but a fall in cell survival was observed at higher

concentrations. The compound was observed to be non-toxic at 24 and 48 h at ≤ 100μM concentration. A surviving fraction of 0.81 ± 0.05 was observed at 100 μM, 48 h; with increasing treatment concentration to 1000 μM, 48 h surviving fraction observed was 0.44 ± 0.03. The compound did show toxicity at 1000 μM concentration at 48 and 72 h. IC_{50} was observed to be 500 μM.

Radiolabeling and serum stability



FigureS15: Radiochromatogram showing formation of single species.

Result: Radiolabeling yield of 98.5±0.7% was obtained within 30 minutes with minimal colloidal and hydrolyzed fraction. ^{99m}Tc radiolabeling was carried out for DTPA-bis(ChoEA) at room temperature using $SnCl_2$ as reducing agent at pH 7. More than 98% radiolabeling yield with minimal free, hydrolyzed and colloidal fraction was observed for DTPA-conjugate without purification.

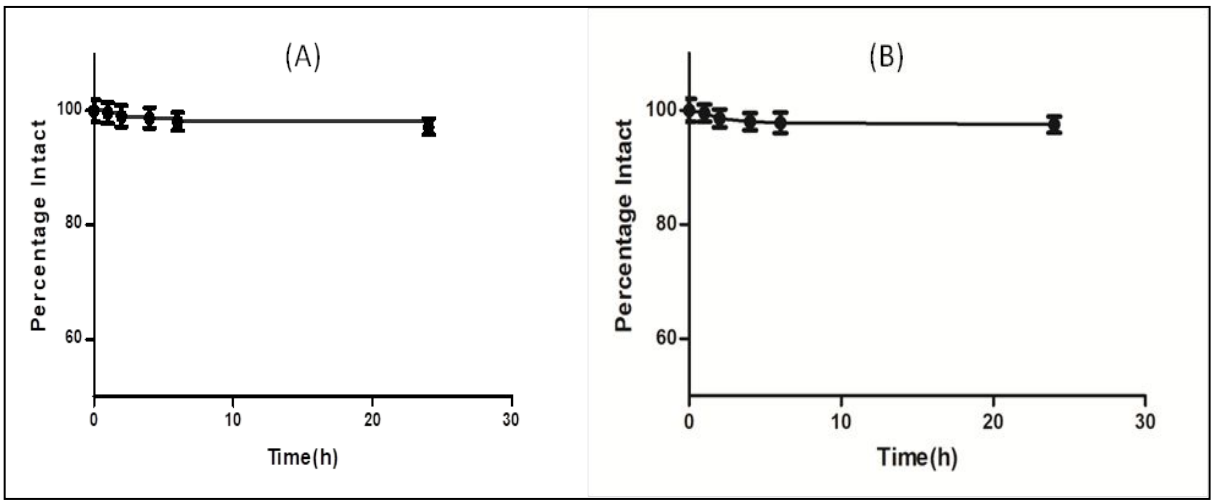


Figure S16 Serum stability of [^{99m}Tc]Tc-DTPA-bis(ChoEA) (A) human serum (B) PBS

Result: The radiolabeled DTPA-bis(ChoEA) showed high kinetic inertness under physiological conditions. Very slow degradation of the complex was observed at 24 h. Approximately 97% of the radioconjugate was found intact at 24 h both in human serum and PBS. The results were suggestive of minimal transcomplexation of the chelate.

In Silico docking studies

The docking study was performed with autodockvina and was analysed using biovia discovery studio visualizer. Target molecule used was crystal structure of choline kinase complexed with phosphocholine (PDB id: 2ckq) with a resolution of 2.4 A. The protein was prepared through autodock tools 4.2. The ligand energy minimization and 3D coordinates were generated through open Babel software. The best pose was generated rank based.

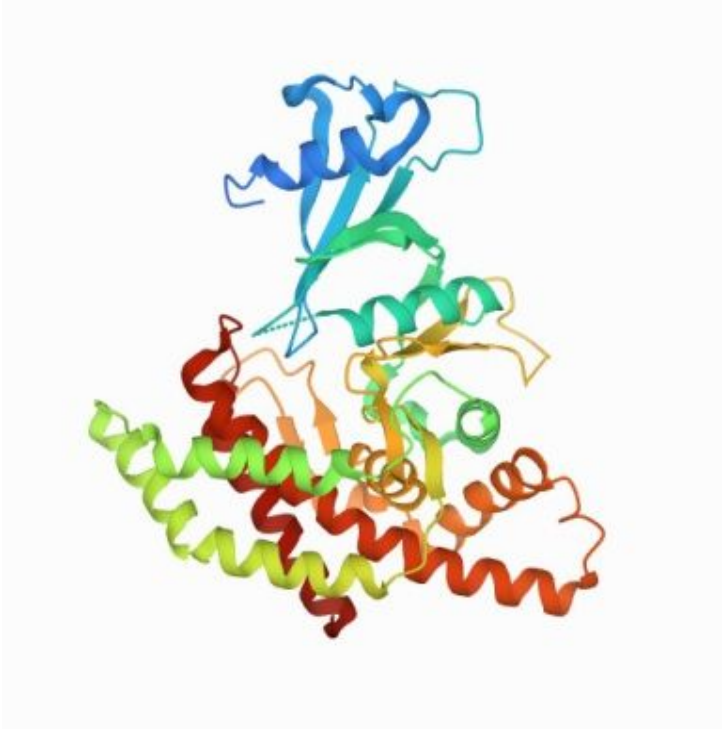
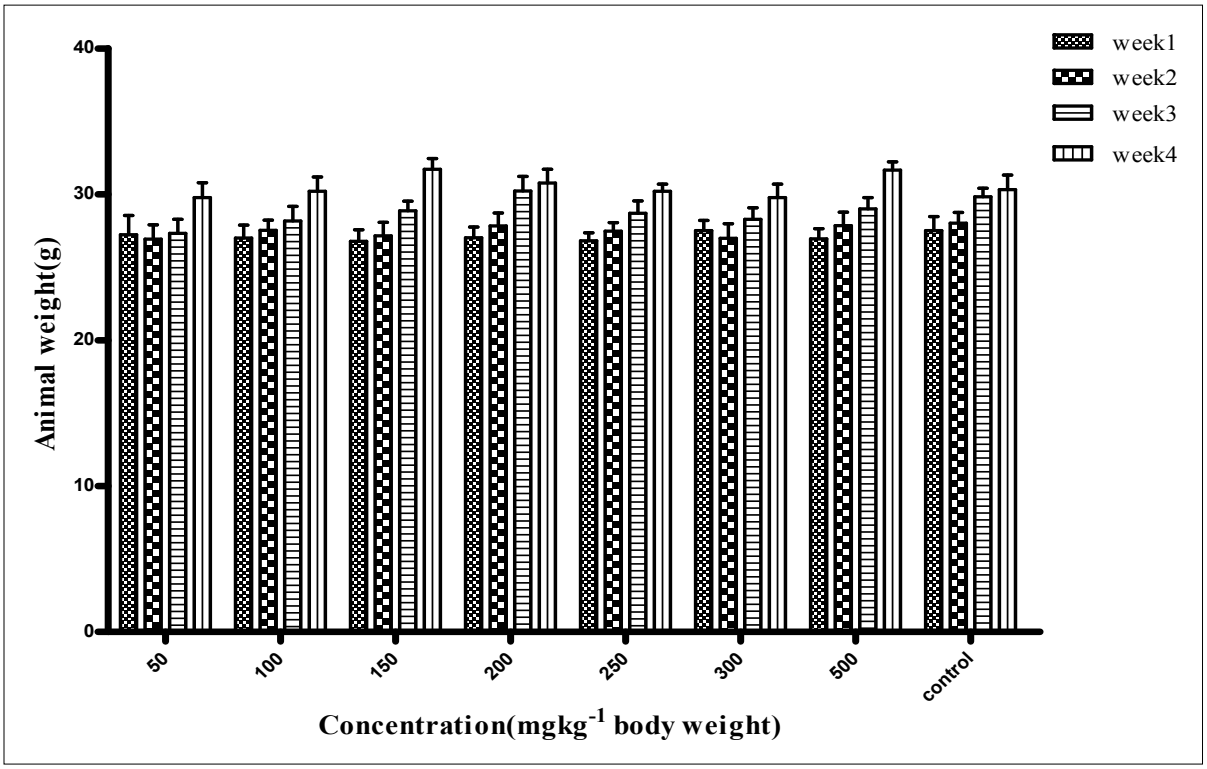


Figure S17 3D Structure of choline kinase alpha (RCSB PDB:2ckq)

Ligand	Glide score (kcal/mol)	Hydrophobic interaction	pi-stacking	Hydrogen bond and salt bridge	Charged interaction
DTPA-bis-cholineethylamine	-7.1	Trp423, Trp420, Asn311, Asp330, Arg444, Tyr354,	Gln308	Ile116, Asn122, Leu120	Glu349, Asp306
Fluroethylcholine	-4.3	Gly119, Glu349, Tyr333, Tyr354, Trp420, Trp423, Tyr440,	Gln308	Ser121, Leu120	Asp306
99m-Tc DTPA-bis-cholineethylamine	-6.1	Leu214, Arg213, Thr216, Tyr354, Ser355, Gly118,	Glu434, Glu357	Gly119	Glu349, Asp353
Choline	-3.7	Tyr333,Tyr354, Ser416, Trp423, Tyr440, Asn305, Cys307, Asn311, Glu349	Gln308	Ser121,Leu120,	Asp306

Table S2 Table showing Glide Score and corresponding interactions of DTPA-bis-cholineethylamine , Fluoroethylcholine , [^{99m}Tc]Tc-DTPA-bis(ChoEA), and Choline.

Animal Toxicity studies



FigureS18 Bar graph showing variation in animal weight with respect to varying concentrations of DTPA-bis(ChoEA) over a period of 4 weeks.

Result: It was observed that doses upto 500mg/kg body weights were well tolerated by subjects without any visible side effect such as tremors and death. No adverse effects such as weight loss or mortality were observed in all groups up to 4 weeks after intravenous administration of unlabeled conjugates. Long term toxicity was not evaluated.

ROI Analysis of SPECT images

Semi quantitative volumetric VOI analyses were done using AMIRA software. The analyses were suggestive of high target to non-target ratio at the tumor site with minimal background activity.

Ratio	PC3	A549	HCT116
Tumor to Muscle (T/M)	36.57±4.51	29.90±5.13	34.21±5.02
Tumor to Liver (T/L)	2.96±0.57	2.45±0.83	4.45±1.74

Table S3. Table showing T/M, T/L values from PC3, A549, HCT116 imaging studies calculated by semiquantitative analysis of MicroSPECT images.

First in-human study of [^{99m}Tc]Tc- DTPA-bis(ChoEA)

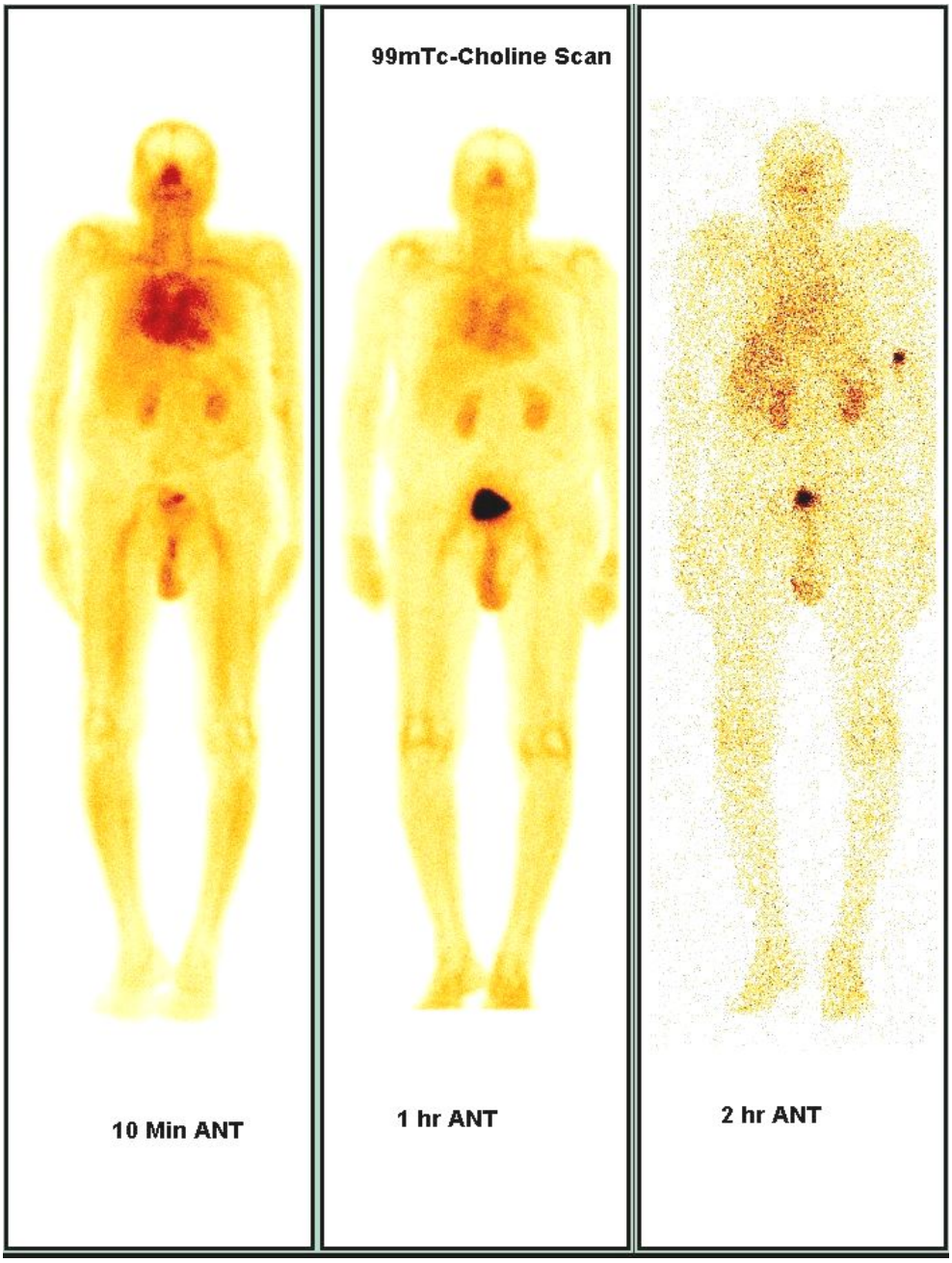


Figure S19 First in-human clinical study in untreated subject of prostate cancer after intravenous injection of [^{99m}Tc]Tc- DTPA-bis(ChoEA) **without bladder irrigation.**

Case report

Presented case is first in human whole body scan of [^{99m}Tc]Tc-DTPA-bis(ChoEA) in 60 yr old patient. The patient had history of LUTS with Haemoturia from one month. However the patient never had any trauma or fracture. The patient have not been subjected to any previous chemotherapy and radiotherapy. The PSA levels were 71.87ng/mL . Whole body SPECT images were acquired post 10 mCi injection of radiolabeled [^{99m}Tc]Tc-DTPA-bis(ChoEA). Early 10 minute and delayed 1, 2 h anterior, posterior images were acquired. Maximum activity was observed in kidneys, liver, heart and no radioactivity was found in bone, neither in the bone marrow nor in the epiphysis in Whole body SPECT scan. The region of prostate appeared to be obscured by renal tracer activity.

The mean and standard deviation of the administered mass of DTPA-bis(ChoEA) was 1.2 ± 0.5 mg (range, 1–5 mg; 0.016 mg/Kg Body weight). The mean administered activity was 430 ± 25 MBq (range, 370-555 MBq). There were no adverse or clinically detectable pharmacologic effects in the subjects. No significant changes in vital signs or the results of laboratory studies or electrocardiograms were observed.