**Supporting Information**

# Radiolabeling and Preclinical Evaluation of A new S-alkylated Cysteine Derivative conjugated to C-substituted Macrocycle for Positron Emission Tomography

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**Figure S1** $^{1}$HNMR spectrum of di-tert-butyl 2,2'-(12-amino-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetate(**2**)

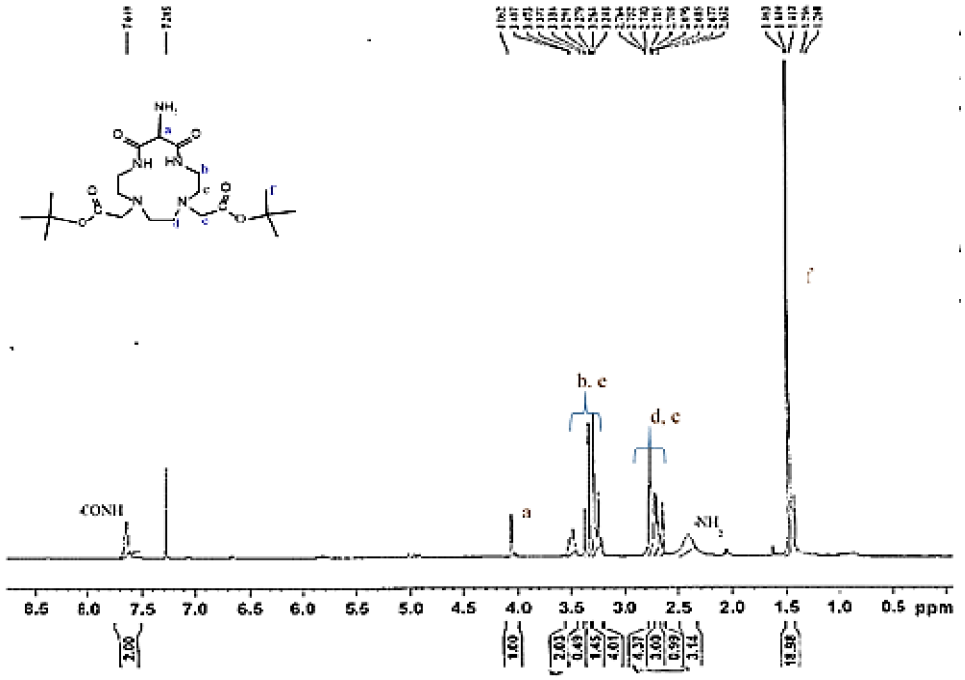



**Figure S2** $^{13}$CNMR spectrum of di-tert-butyl 2,2'-(12-amino-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetate(**2**)

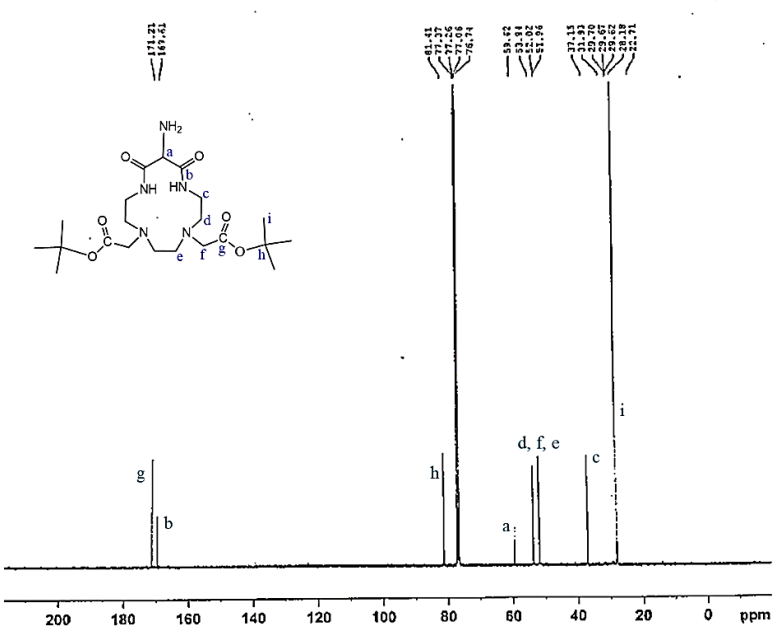


**Figure S3**LC-MS mass spectrum of di-tert-butyl 2,2'-(12-amino-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetate(**2**)

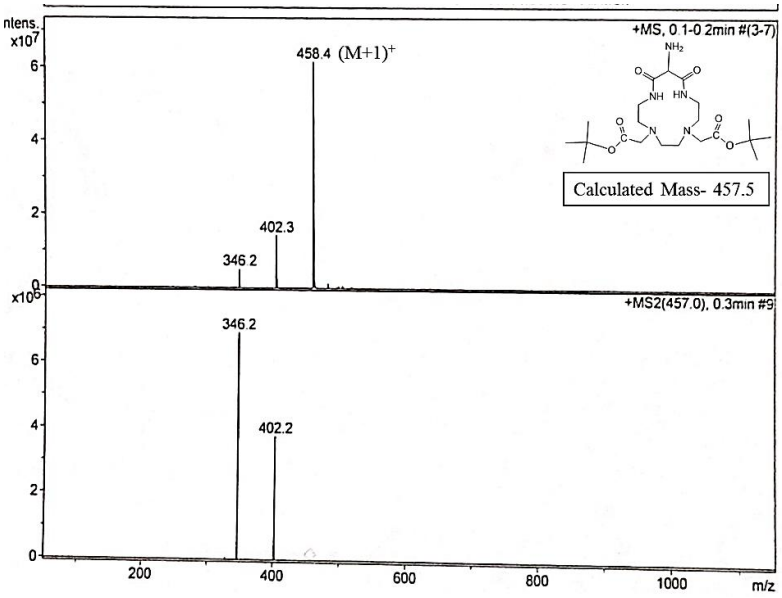



**Figure S4** HRMS spectrum of di-tert-butyl 2,2'-(12-amino-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetate(**2**)

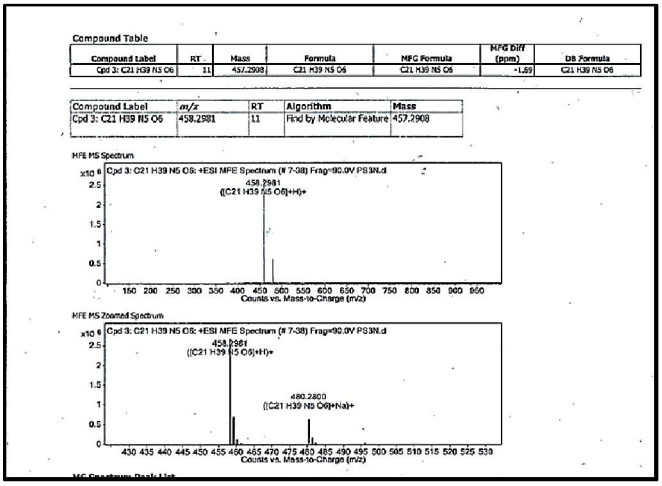


**Figure S5** $^{1}$HNMR spectrum of di-tert-butyl2,2'-(12-(2-chloroacetamido)-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetate(**3**)

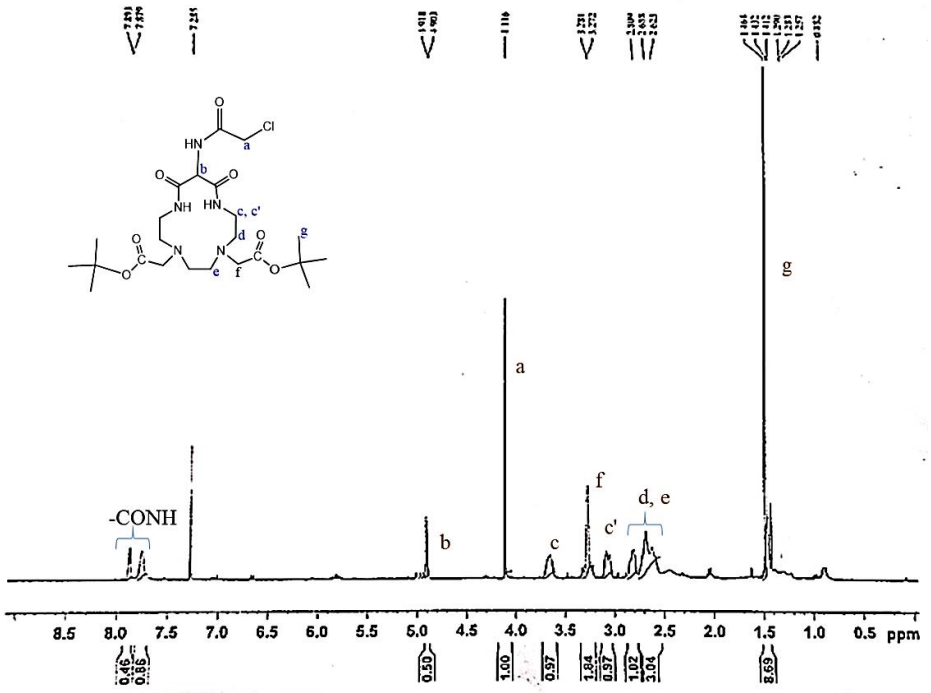



**Figure S6** $^{13}$CNMR spectrum of di-tert-butyl2,2'-(12-(2-chloroacetamido)-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetate(**3**)

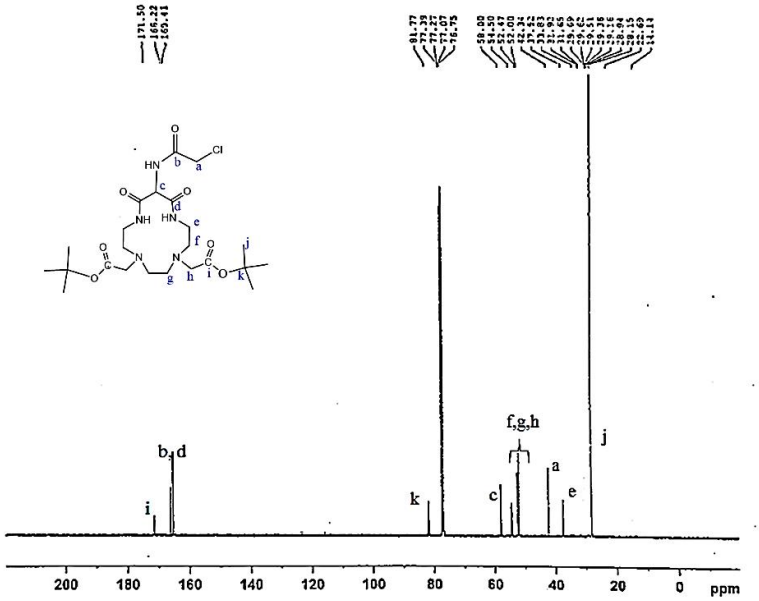


**Figure S7** LC-MS spectrum of di-tert-butyl 2,2'-(12-(2-chloroacetamido)-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetate(**3**)

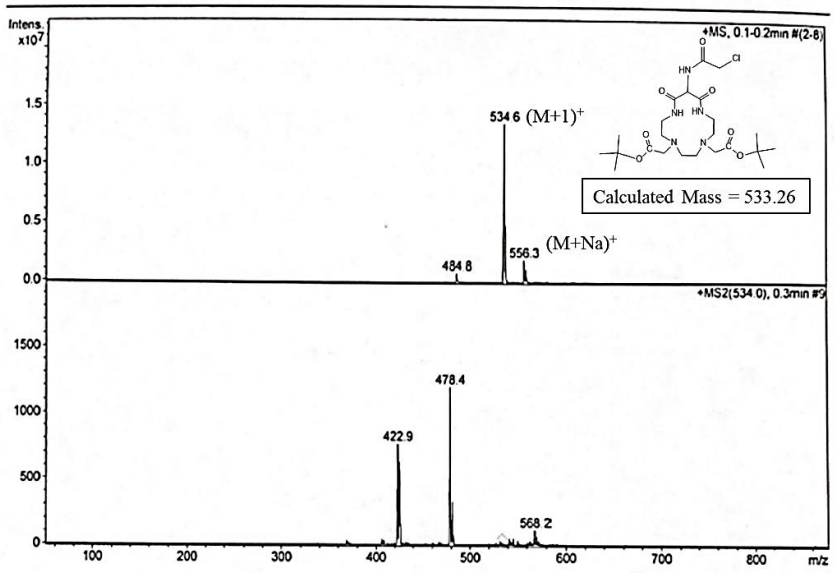



**Figure S8** HRMS spectrum of di-tert-butyl 2,2'-(12-(2-chloroacetamido)-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetate(**3**)

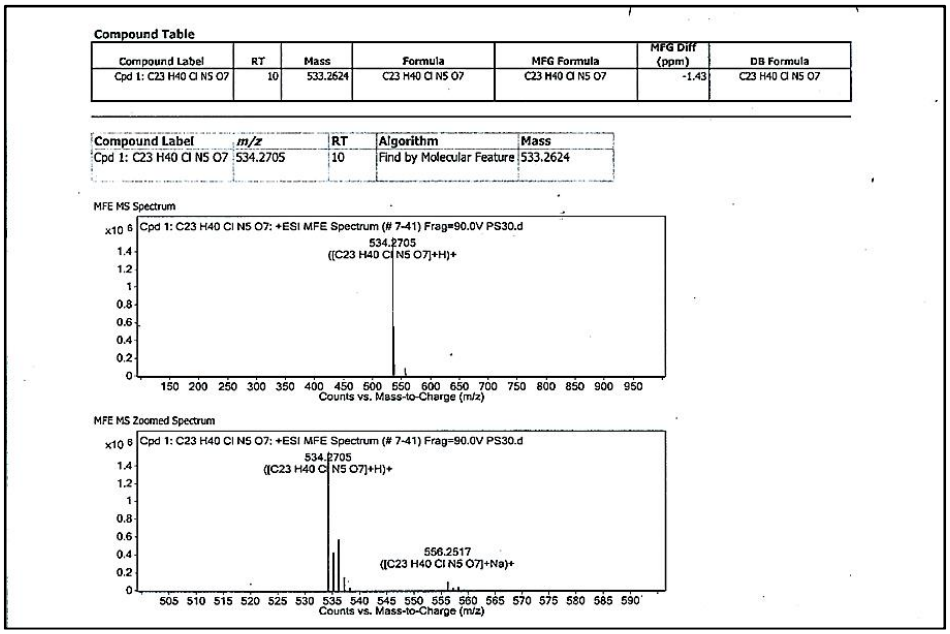


**Figure S9** [1]HNMR spectrum of 2-acetamido-3-((2-((4,7-bis(2-(tert-butoxy)-2-oxoethyl)-11,13-dioxo-1,4,7,10-tetraazacyclotridecan-12-yl)amino)-2-oxoethyl)thio)propanoic acid(**4**)

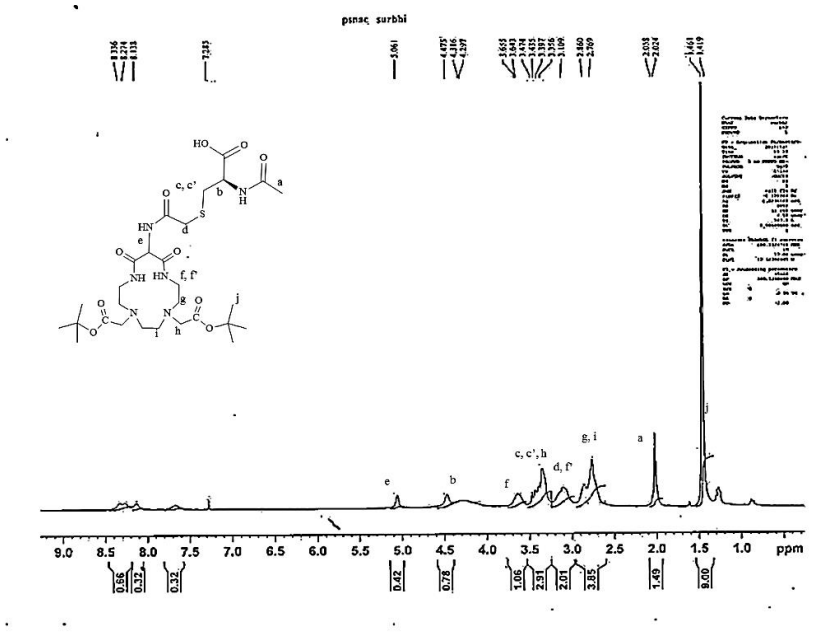



**Figure S10** [13]CNMR spectrum of 2-acetamido-3-((2-((4,7-bis(2-(tert-butoxy)-2-oxoethyl)-11,13-dioxo-1,4,7,10-tetraazacyclotridecan-12-yl)amino)-2-oxoethyl)thio)propanoic acid(**4**)

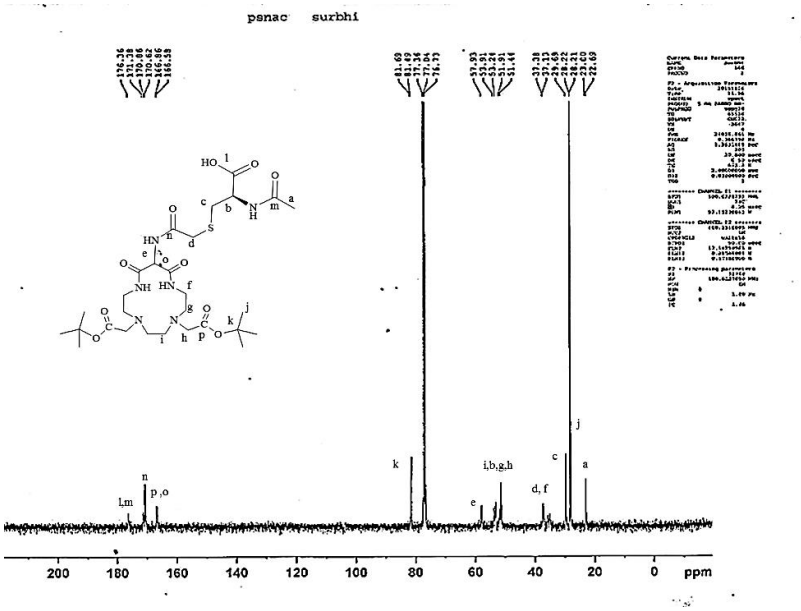


**Figure S11** LC-MS spectrum of 2-acetamido-3-((2-((4,7-bis(2-(tert-butoxy)-2-oxoethyl)-11,13-dioxo-1,4,7,10-tetraazacyclotridecan-12-yl)amino)-2-oxoethyl)thio)propanoic acid(**4**)

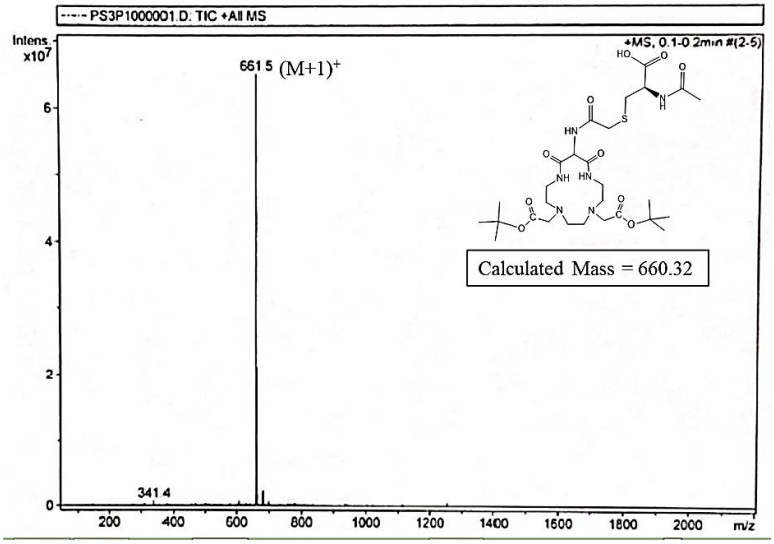



**Figure S12** HRMS spectrum of 2-acetamido-3-((2-((4,7-bis(2-(tert-butoxy)-2-oxoethyl)-11,13-dioxo-1,4,7,10-tetraazacyclotridecan-12-yl)amino)-2-oxoethyl)thio)propanoic acid(**4**)

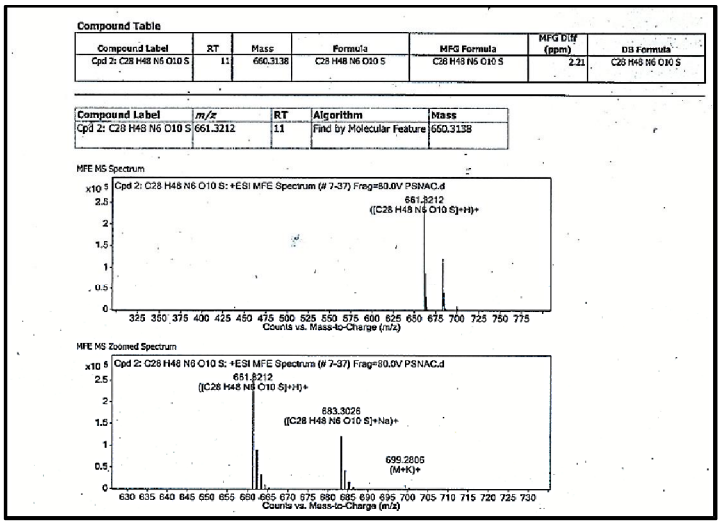


**Figure S13** [1]HNMR spectrum of 2,2'-(12-(2-((2-acetamido-2-carboxyethyl)thio)acetamido)-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetic acid (**5**)

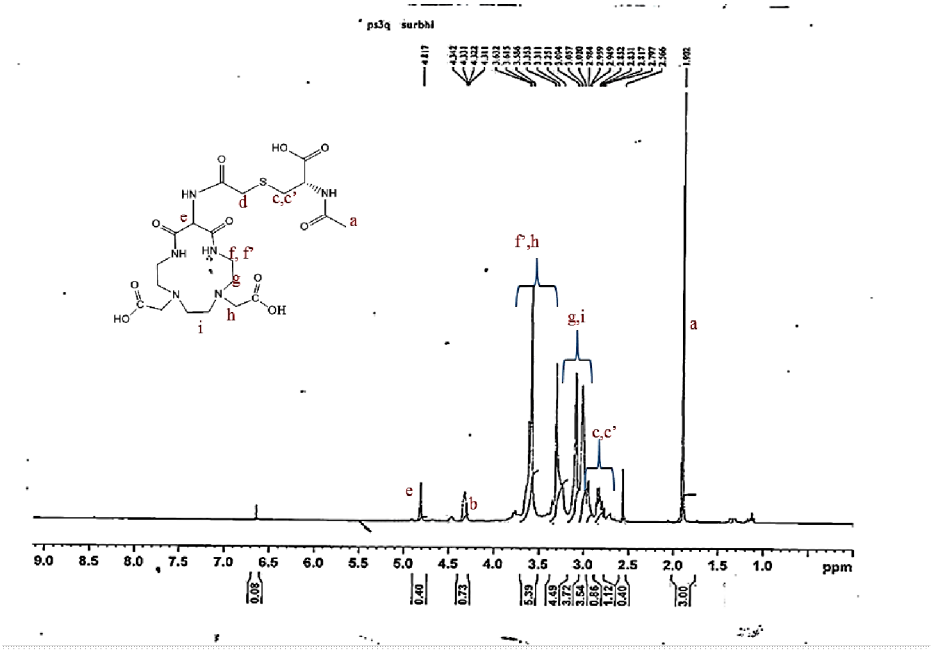



**Figure S14** [13]CNMR spectrum of 2,2'-(12-(2-((2-acetamido-2-carboxyethyl)thio)acetamido)-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetic acid (**5**)

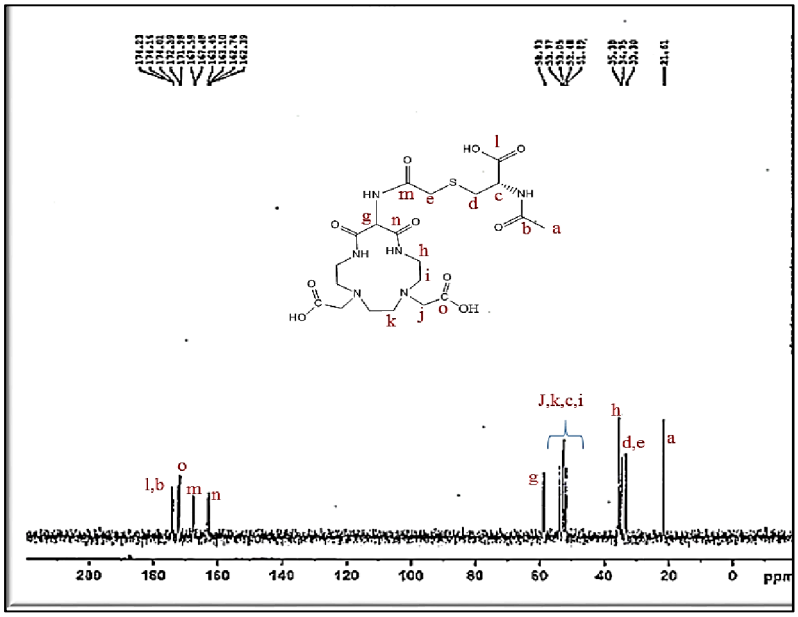


**Figure S15** LC-MS spectrum of 2,2'-(12-(2-((2-acetamido-2-carboxyethyl)thio)acetamido)-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetic acid (**5**)

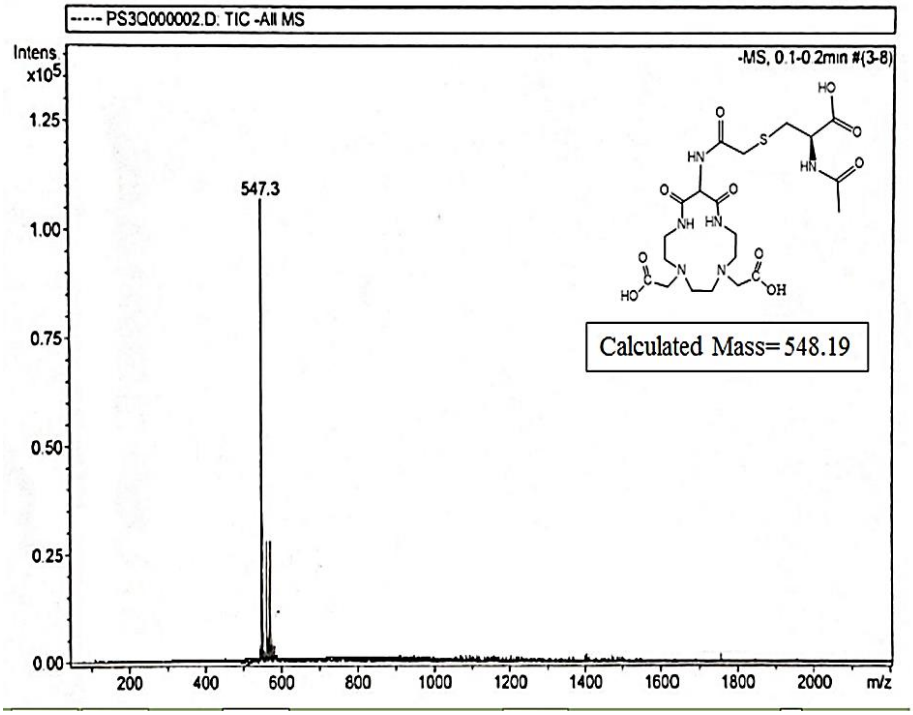



**Figure S16** HRMS spectrum of 2,2'-(12-(2-((2-acetamido-2-carboxyethyl)thio)acetamido)-11,13-dioxo-1,4,7,10-tetraazacyclotridecane-4,7-diyl)diacetic acid (**5**)

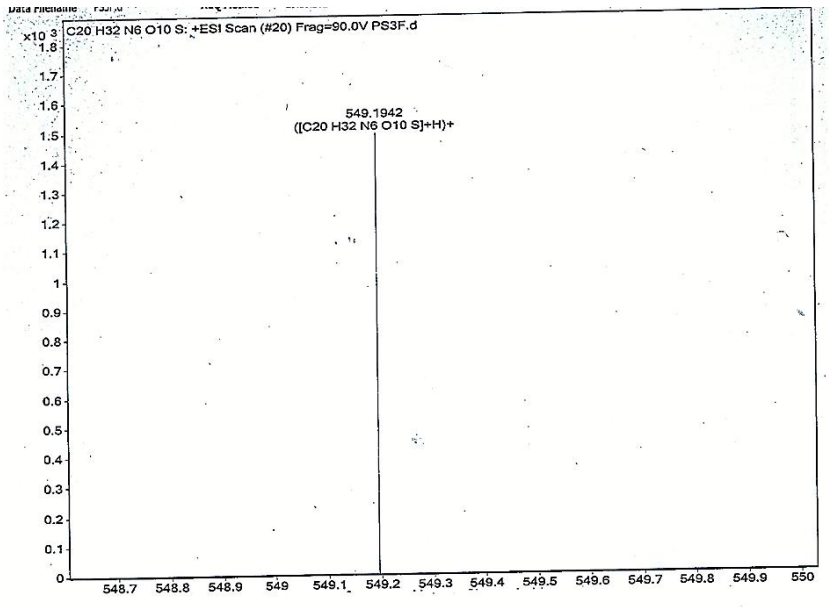


**Figure S17** HPLC profile ofGa-ATRIDAT-NAC at 230 nm.

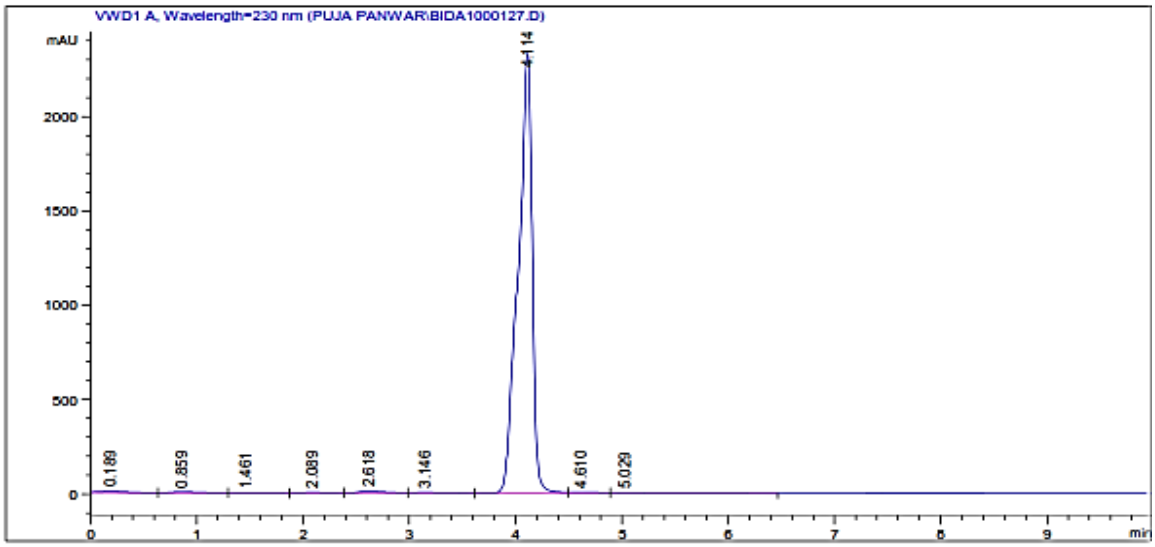
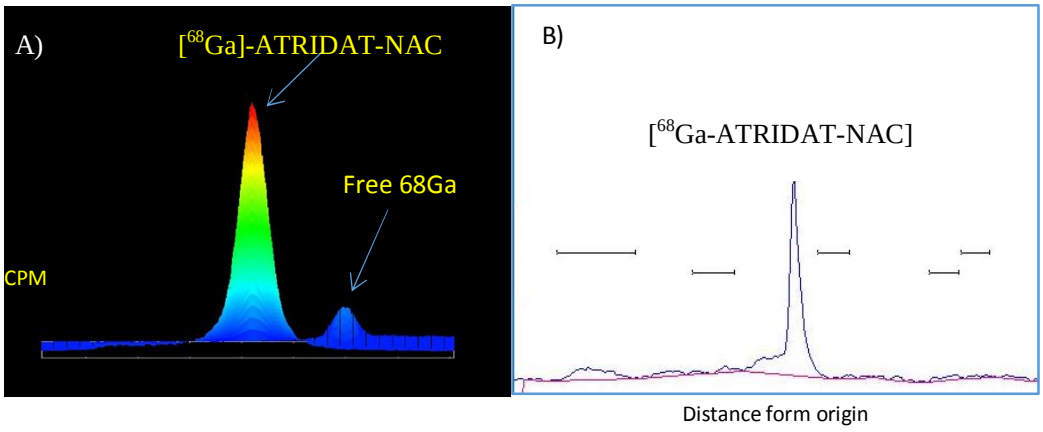
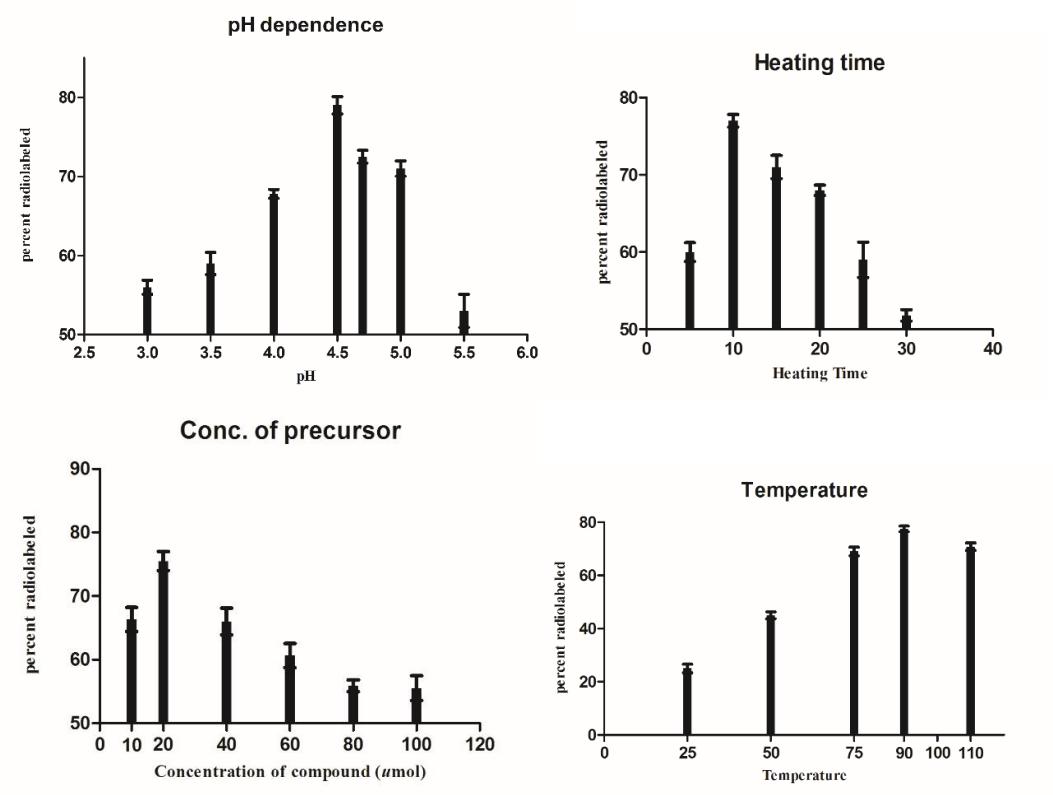



**Figure S18** Radio-tlc scan of [$^{68}$Ga-ATRIDAT-NAC] from ez-tlc scanner




**A)** Before cartridge purification **B)** After cartridge purification [$^{68}$Ga-ATRIDAT-NAC] peak

**Figure S19** Optimization of Radiolabeling with respect to heating time, pH, and precursor concentration used and temperature.




When pH was changed from 3 to 4.5, the activity increased from 56 to 79 % and then further decreased to 53 % on further increasing the pH to 5.5. When radiolabeling was performed at room temperature, 25 % radiolabeling was achieved which increased to 77.5 % at 90 °C and did not increased on further increasing the temperature. Best radiolabeling yield was obtained by heating for 10 min. On further heating, the radiolabeling yield got reduced to nearly 50 %. 20 $\mu$mol concentration of radiolabeled compound gave highest radiolabeling yield of 75.5 % which decreased on further increasing the concentration.

**Figure S20** Graph showing clearance of radiolabeled compound from the blood of female Wistar rat with time injected intravenously.

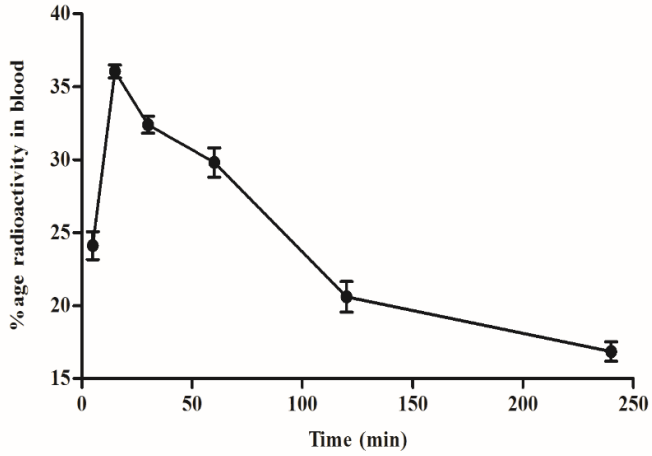



**Figure S21** Graph showing stability of radiolabeled compound in human serum