Supplementary Information

In vivo targeting of DNA vaccines to dendritic cells using functionalized gold nanoparticles

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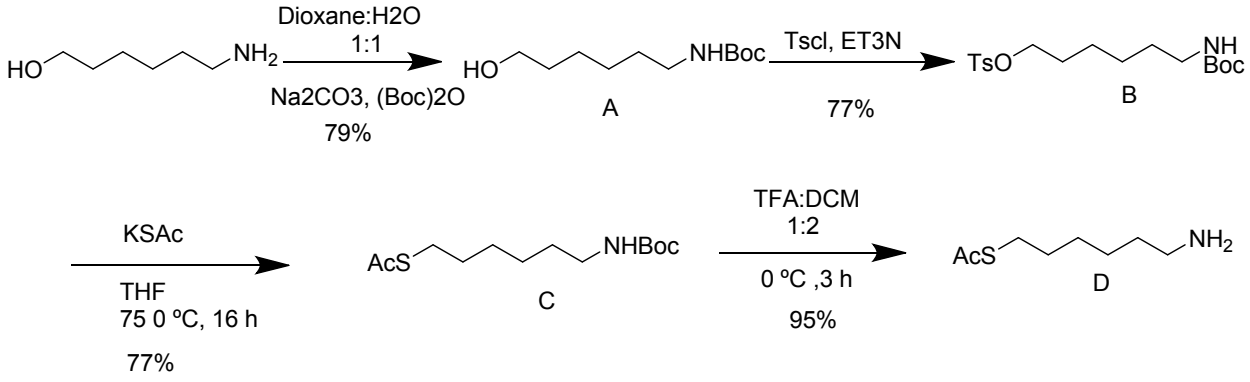
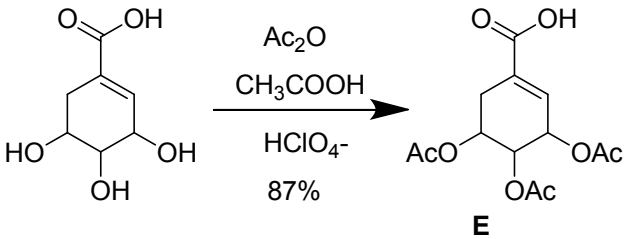
Synthesis of SGSH &GSH:

Synthesis of the thio ligand containing the mannose mimicking shikimoyl and transfection enhancing guanidinyl functionalities (SGSH) and its non shikimoylated control analog (GSH) were synthesized as shown below schematically in **scheme S1** and **scheme S2** respectively. Briefly, tosylation of the tert-butyl (6-hydroxyhexyl) carbamate (**A**) yielded tosylated intermediate (**B**) which upon treatment with potassium thio acetate yielded intermediate (**C**). N-tert-butoxy carbonyl deprotection of intermediate(**C**) afforded the amine intermediate (**D**). Shikimic acid was converted to its tri-O-acetyl derivative (**E**) by conventional acetylation with acetic anhydride/acetic acid followed by treatment with perchloric acid.

Synthesis of intermediate D:

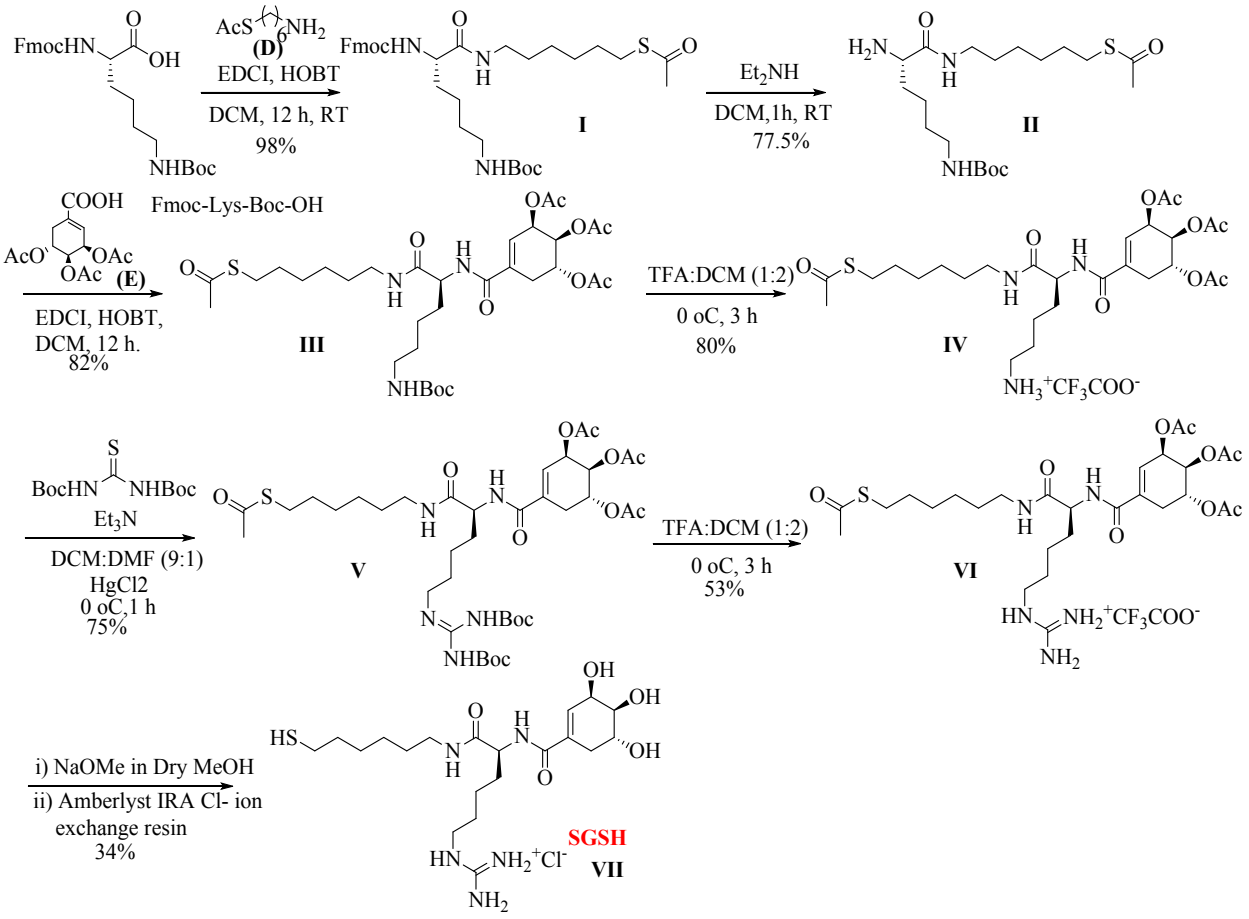


Synthesis of intermediate (E):



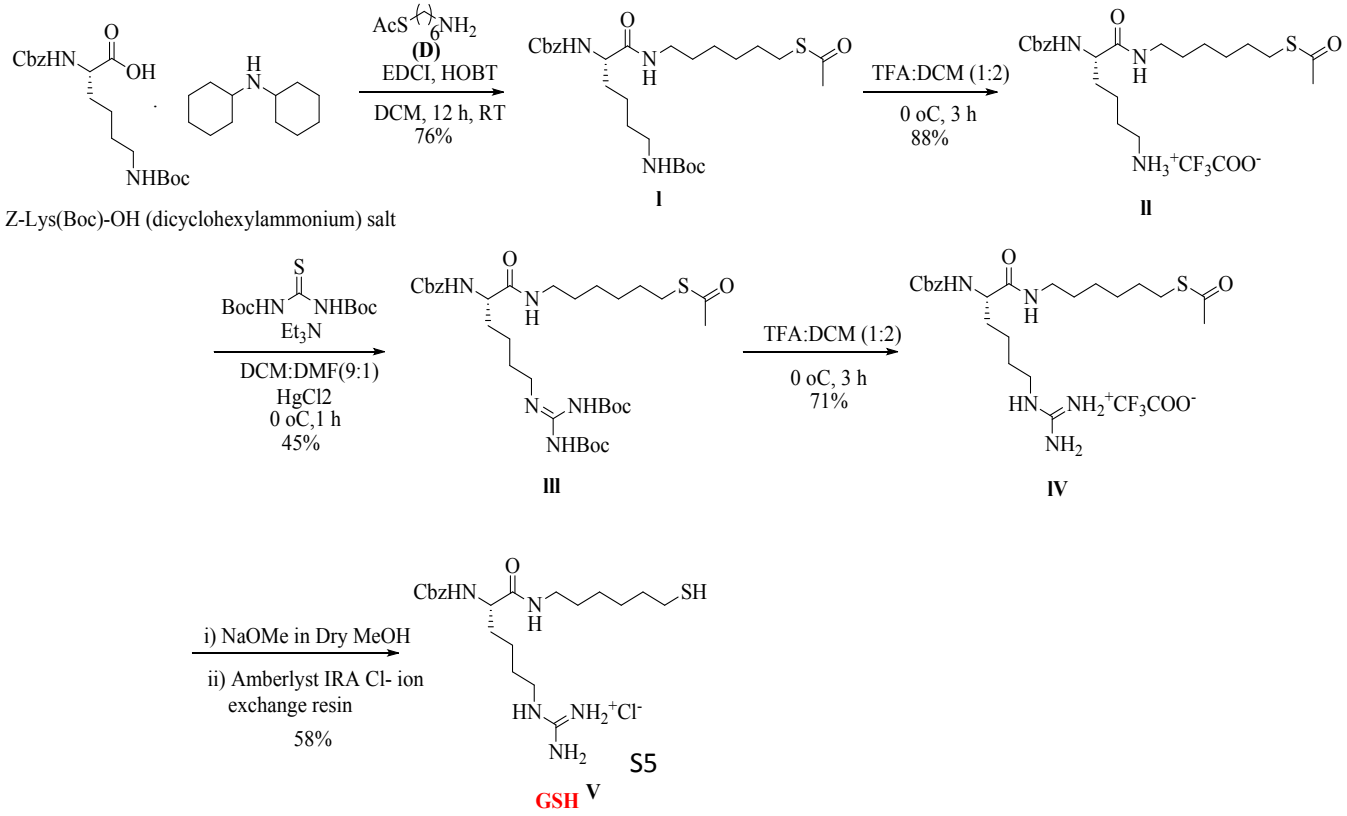
Scheme S1: Conventional peptide coupling of the intermediate **(D)** with Fmoc-Lys(Boc)-OH was afforded intermediate **I (Scheme S1)** which upon basic deprotection of Fmoc group followed by peptide coupling with the triacetyl shikimic acid **(E)** afforded intermediated **III (Scheme S1).** Acid deprotection of the side chain Boc group of intermediate **III** followed by guanidinylation of the resulting amine intermediate **(IV)** with N,N'-diBoc-thio urea/$HgCl_2$ yielded intermediate **(V).** Acid mediated deprotection of Boc followed by removal of all the four acetyl groups of intermediate **VI** with NaOMe/MeOH afforded the target ligand **SGSH (VII** in **Scheme S1)**

Scheme S1:



Scheme S2: the control ligand without the presence of shikimoyl group **(GSH, Scheme S2)** was synthesized by conventional peptide coupling of Z-Lys(Boc)-OH (as its dicyclohexylammonium salt) with intermediate **D** yielded intermediate **I (Scheme S2).** Intermediate I upon acid mediated Boc removal followed by guanidinylation with N,N'-diBoc-thio urea/$HgCl_2$ afforded intermediate **III.** Acid mediated Boc removal of intermediate **III** followed by removal of acetyl group with deprotection of the NaOMe/MeOH afforded the non-targetting control ligand **GSH (V, Scheme S2).**

Scheme S2:



Synthesis of tert-butyl (6-hydroxyhexyl) carbamate (A):

4 grams (1 eqv, 34.1 mmol) of 6-amino hexanol was taken in a 250 mL round bottomed flask and dissolved in 50 mL of dioxane, water mixture (1:1 v/v). Temperature of reaction mixture was cooled to 0 ℃ and 5.43 grams (1.5 eqv, 51.2 mmol) of sodium carbonate (Na_2CO_3) was slowly added in 3 portions. The reaction mixture was stirred for 30 min at room temperature and 8.1 grams (1.1 eqv, 37.6 mmol) of $(Boc)_2O$ was slowly added to the reaction mixture, and the mixture was stirred for overnight at room temperature. Reaction completion was monitored by TLC with 5% methanol in chloroform (R_f = 0.7). The dioxane from reaction mixture was removed by using rota-evaporator at 50 ℃, washed with n-hexane (2×100 mL) to remove excess $(Boc)_2O$. The resultant product was extracted with chloroform (2×200 mL) from aqueous layer and dried over anhydrous sodium sulphate, filtered and the solvent from the filtrate was removed by rota evaporator. The yield of resultant product **(A)** 79% (5.9 g). The obtained product was pure and was directly used for the next reaction without any further purification.

ESI-MS *m/z*: 240 $[M + Na]^+$ for $C_{13}H_{16}O_{18}$.

^{1}H NMR: (400 MHz, $CDCl_3$): δ/ppm = 1.25 (m, 4H, a+a□), 1.4 (m, 11H, b+c), 1.5 (m, 2H, d), 3.05 (m, 2H, e), 3.6 (m, 2H, f), 4.7 (s, 1H, g).

Synthesis of compound B (6-((tert-butoxycarbonyl)amino)hexyl 4-methylbenzenesulfonate):

Compound **A**, 4 g (1eqv, 18.5 mmol) was dissolved in 10 mL DCM in 100 mL RB flask, the reaction mixture was cooled to 0 ºC. 4.5 g of (1.3 eqv, 24.6 mmol) tosyl chloride was added and 3.5 mL (1.5 eqv, 127.5 mmol) of tri ethyl amine was slowly added for 10 min. The reaction mixture was allowed to stir for 3 h at RT. The resultant product upon purification with column chromatography with 60-120 mesh silica gel using 10-15% ethyl acetate in hexane (v/v) as eluent afforded 5.3 g (77% yield) of the pure intermediate **B.** (R_f = 0.4 using 30% ethyl acetate in hexane , v/v).

ESI-MS m/z: 394 $[M+Na]^+$ for $C_{18}H_{29}NO_5S$

^{1}H NMR: (400 MHz, $CDCl_3$): δ/ppm = 1.3 (m, 4H, a+a□), 1.4 (m, 11H, b+c), 1.65 (m, 2H, d), 2.5 (s, 3H, e), 3.1 (m, 2H, f), 4 (t, 3H, g), 4.5 (s, 1H, h), 7.35 (d, 2H, i), 7.8 (d, 2H, j).

Synthesis of S-(6-((tert-butoxycarbonyl)amino)hexyl) ethanethioate (C):

Compound B, 4 g (1 eqv, 10.7 mmol) was dissolved in 10 mL of dry THF and 2.4 g of potassium thioacetate (2 eqv, 21.6 mmol) was added slowly. The reaction mixture was allowed to stir under reflux at temperature 75 ℃ for 16 h. Resultant crude product was dissolved in chloroform and filtered through Whatman filter paper and filtrate was concentrated using rota evaporater under vacuum at 50 ℃. The residue C upon column chromatography using 60-120 mesh silica gel with 10% ethyl acetate in hexane (v/v) as eluent, afforded 3.0 g (77% yeild) of the pure intermediate C (R_f= 0.7, 30% ethyl acetate in hexane, v/v).

ESI-MS m/z: [M+1] 276.2 for $C_{13}H_{25}NO_3S$

^{1}H NMR: (400 MHz, $CDCl_3$): 1.3 (m, 4H, a+a□), 1.4 (m, 11H, b+c), 1.65 (m, 2H, d), 2.3 (s, 3H, e), 2.8 (m, 2H, f), 3.1 (t, 3H, g), 4.5 (s, 1H, h).

Synthesis of S-(6-aminohexyl) ethanethioate (Compound D):

Compound C (1.4 g) was dissolved in 2 mL of DCM in 25 mL RB flask at 0 ℃ temperature and 1 mL of TFA was added. The reaction mixture was stirred for 3 h at RT. Complete reaction was monitored by TLC. The reaction mixture was neutralized by adding 5 mL of NaHCO3 and extracted with chloroform. The organic layer was washed with saturated brine (Nacl) solution (2x20 mL) and dried over sodium sulphate. The filtered supernatant upon rotary evaporation,

afforded 892 mg (95% yield) of free amine as intermediate D (R_f = 0.3, 5% methanol in chloroform, v/v).

***Synthesis of (3R,4S,5R)-3,4,5-tri(acetyloxy)-1-cyclohexene-1-carboxylic acid* (E):**

1 g of (-) shikimic acid was taken in 50 mL RB flask and 2.5 mL of dry acetic anhydride and 5 mL of glacial acetic acid were added followed by addition of per chloric acid (3-4 drops) at 0 ℃. The reaction mixture stirred at room temperature for 12 h. The reaction mixture was then poured onto the crushed ice in glass beaker. The organic compound was extracted with chloroform (3 x 15 mL), washed with brine solution (2 x 15 mL), dried over anhydrous sodium sulphate, filtered and the solvent from the filtrate was removed by rota evaporator. The residue upon several times washings with ice cold pentane (to ensure complete removal of acetic acid) afforded intermediate **E** as gummy white solid compound (1.5 g, 87% yield, R_f = 0.4, 5% methanol in chloroform, v/v).

^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 2.04-2.24 [s, 9H, a+a+a]; 2.47 [dd, 1H, b]; 2.9 [dd, 1H,b']; 5.35 [m, 2H, c+d]; 5.8 [m, 1H, e]; 6.85 [d, 1H, f]

ESI-MS *m*/*z*: 323 $[M+Na]^+$ for $C_{13}H_{16}O_{18}$

Scheme S1

(S)-S-(6-(2-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino) hexanamido) hexyl) ethanethioate (Compound I):

9.78 g (1 eqv, 20.9 mmol) of Fmoc-Lys-Boc-OH was dissolved in 50 mL dichloromethane, and 6.01 g (1.5 eq, 31.35 mmol) of EDCI and 4.79 g (1.5 eq, 31.35 mmol) of HOBT was added at RT. The reaction mixture was stirred for 15 min, 3.68 g (1eqv, 20.9 mmol) of compound **D** was added. To maintain basic condition in reaction mixture, 3 mL of DIPEA was added. The reaction mixture was stirred in an inert atmosphere using N_2 gas for 12 h. The crude product was dissolved in 100 mL of chloroform and washed sequentially with $NaHCO_3$ (2×100 ml) solution and 0.5N HCl solution (2×100 mL), and saturated brine solution (2×100 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated using rotary evaporation. The resultant residue upon column chromatographic purification over 60-120 mesh silica gel and 1.5% methanol in chloroform as eluent afforded pure **(I)** 0.30 g (98% yield, R_f= 0.6, 5% methanol in chloroform, v/v).

ESI-MS: m/z = $[M+1]^+$ 626 for $C_{34}H_{47}N_3O_6S$

^{1}H NMR: (500 MHz, $CDCl_3$): δ/ppm = 1.3 (m, 4H, a+a□), 1.4 (m, 15H, b+c+d+e), 1.6 (m, 1H, f), 1.8 (m, 3H, f+g), 2.3 (s, 3H, h), 2.8 (m, 2H, i), 3.1 (m, 2H, j), 3.3 (t, 2H, k), 4.1 (m, 1H, l), 4.2 (t, 1H, m), 4.4 (d, 2H, n), 4.6 (broad peak, 1H, o), 5.6 (broad peak, 1H, p), 6.3 (broad peak, 1H, q), 7.3 (dd, 2H, r), 7.4 (dd, 2H, s), 7.6 (d, 2H, t), 7.8 (d, 2H,u).

(S)-S-(6-(2-amino-6-((tert-butoxycarbonyl)amino)hexanamido)hexyl) ethanethioate (Compound II):

4 g of compound **I** was dissolved in 5 mL DCM in 50 mL RB flask and reaction mass was cooled to 0 ℃. 1 mL diethyl amine was added slowly for 5 min and reaction mixture was allowed to stirr for 2 h, the reaction mixture was concentrated and the residue upon column chromatographic purification using 60-120 silica gel using 2-3% methanol in chloroform (v/v) as eluent afforded 2.58 g (77.5% yield), of pure the intermediate compound **II**. (R_f = 0.4, 5% methanol in chloroform, v/v).

(1R,2S,3R)-5-(((S)-2,2-dimethyl-4,11,20-trioxo-3-oxa-19-thia-5,12-diazahenicosan-10-yl)carbamoyl)cyclohex-4-ene-1,2,3-triyl triacetate (compound III):

Acetyl protected shikimic acid, 2.6 g (1.2 eqv, 8.9 mmol) was dissolved in 50 mL DCM and 2.13 g (1.5 eqv, 11.1 mmol) of EDCI and 1.7 g (1.5 eqv, 11.1 mmol) was added. The reaction mixture was then allowed to stir for 15 min. 3 g (1eqv, 7.4 mmol) of **compound II** was added. To maintain basic condition 3 mL of DIPEA was added and the reaction mixture was allowed to stir for overnight in inert (N_2) atmosphere. The crude product was dissolved in 100 mL of chloroform and washed sequentially with saturated $NaHCO_3$ (2×100 ml) solution, 0.5N HCl solution (2×100 mL), and saturated brine solution (2×100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated using rotary evaporation. The residue upon column chromatography over 60-120 mesh silica gel and 1% methanol in chloroform as eluent afforded compound **(III)** 4.2 g (82% yield, R_f = 0.5, 5% methanol in chloroform, v/v).

ESI-MS: m/z = $[M+1]^+$ 686, $[M+Na]^+$ 708 for $C_{32}H_{51}N_3O_{11}S$.

^{1}HNMR: (500 MHz, $CDCl_3$) δ/ppm = 1.4 (m, 6H, a+b+c), 1.5 (m, 11H, d+e), 1.6 (m, 4H, f+g), 1.7 (dt, 1H, h), 1.9 (dt, 1H, h□), 2.1 (s, 9H, i +i□+i□□), 2.3 (s, 3H, j), 2.4 (d, 1H, k), 2.8 (t, 2H, l), 2.9 (d, 1H, m), 3.3 (t, 2H, n), 3.4 (t, 2H, o), 4.4 (t, 1H, p), 5.3 (t,1H,q), 5.4 (dd, 1H, r), 5.7 (dd, 1H, s), 6.4 (broad peak, 2H, t+u), 6.6 (d, 1H, v).

Triflate salt of (1R,2S,3R)-5-(((S)-1-((6-(acetylthio)hexyl)amino)-6-amino-1-oxohexan-2-yl)carbamoyl)cyclohex-4-ene-1,2,3-triyl triacetate (compound IV):

Compound III (550 mg) was dissolved in 2 mL DCM and the reaction mixture was cooled to 0 ℃. 1 mL TFA was added and the solution was allowed to stir for 3 h at RT. Reaction completion was monitored by TLC. Reaction mixture was neutralized by 5 mL of $NaHCO_3$ and extracted with chloroform. The organic layer was washed with saturated brine solution (2x20 mL) and dried over sodium sulphate. The filtered supernatant upon concentration by rotary evaporation afforded 469 mg (80% yield) of free amine as intermediate **IV** (R_f = 0.2, 5% methanol in chloroform, v/v).

(1R,2S,3R)-5-(((S)-6-((tert-butoxycarbonyl)amino)-2,2-dimethyl-4,13,22-trioxo-3-oxa-21-thia-5,7,14-triazatricos-6-en-12-yl)carbamoyl)cyclohex-4-ene-1,2,3-triyl triacetate (Compound V):

469 mg (1eqv, 0.8 mmol) of compound IV was dissolved in 10 mL of 9:1 (DCM: DMF) solvent mixture and 1 mL of triethyl amine was added to maintain the basic condition. The reaction mixture allowed to stir for 15 min at RT. 331 mg (1.5 eqv, 1.2 mmol) of DBTU was added, reaction mixture cooled to 0 ℃ and added 325 mg (1.5eqv, 1.2 mmol) of mercuric chloride ($HgCl_2$) was added and the reaction mixture was allowed to stir for 1 h at 0 ℃, diluted with DCM (15 mL) and filtered through celite bed. The filtrate was sequentially washed with 0.5N HCl aqueous solution (2×50 mL) and saturated brine solution and dried over on anhydrous sodium sulphate. The organic layer was removed by rotary evaporation. The residue upon column chromatography over 60-120 mesh silica gel and 1% methanol in chloroform (v/v) as eluent afforded 500 mg (75%, yield) of the pure compound **V**. R_f = 0.5, 5% methanol in chloroform, v/v).

ESI-MS: m/z = $[M+1]^+$ 828, $[M+Na]^+$ 851 for $C_{38}H_{61}N_5O_{13}S$

^{1}HNMR: (500 MHz, $CDCl_3$) δ/ppm = 1.2 (m, 6H, a+b+c), 1.4 (m, 20H, d+e), 1.6 (m, 4H, f+g), 1.7 (dt, 1H, h), 1.9 (dt, 1H, h□), 2.1 (s, 9H, i +i□+i□□), 2.3 (s, 3H, j), 2.4 (d, 1H, k), 2.8 (t, 1H, l), 2.9 (d, 1H, m), 3.2 (t, 2H, n), 3.4 (t, 4H, o), 4.5 (t, 1H, p), 5.2 (dt, 1H, q), 5.4 (dd, 1H, r), 5.7 (dd, 1H, s), 6.3 (broad peak, 2H, t+u), 6.6 (d, 1H, v), 8.3 (broad peak, 1H, w), 11.5 (broad peak, 1H, w).

Triflate salt of (1R,2S,3R)-5-(((S)-1-((6-(acetylthio)hexyl)amino)-6-guanidino-1-oxohexan-2-yl)carbamoyl)cyclohex-4-ene-1,2,3-triyl triacetate (compound VI):

Compound V (1.4 g) was dissolved in 6 mL of TFA: DCM (1:2 v/v) and the solution was cooled to 0 ℃ and allowed to stir for 3 h at RT. Reaction completion was monitored by TLC. The reaction mixture was neutralized by 5 mL of sodium bicarbonate and extracted with chloroform. The organic layer was washed with saturated brine solution (2x20 mL) and dried over sodium sulphate. The organic layer was removed using rotary evaporation and The residue upon column chromatography over 60-120 mesh silica gel and 4% methanol in chloroform (v/v) as eluent, afforded 550 mg (53%, yield) of the pure compound **VI**. R_f = 0.4, 5% methanol in chloroform, v/v).

Amino(((S)-6-((6-mercaptohexyl)amino)-6-oxo-5-((3R,4S,5R)-3,4,5-trihydroxycyclohex-1-enecarboxamido)hexyl)amino)methaniminium chloride (Compound VII-SGSH):

Sodium methoxide (89.5 mg, 5eq, 1.5 mmol) was dissolved in 4 mL of dry methanol and the solution was cooled to 0 ℃, **compound V** (200 mg, 1 eqv, 0.3 mmol) was added and the reaction mixture was stirred for 1 h at RT. The resultant product was neutralized with Amberlite IR120 (H^+), filtered through Whatmann filter paper and the filtrate was dried over anhydrous sodium sulphate. The residue after concentrating over rota vapor was dissolved in minimum amount of methanol and passed through amberlyst A-26 chloride ion exchange resin. The methanol was removed by rota vapor and the residue upon recrystallization using 1:10 (v/v) methanol in acetone afforded 50 mg (34.2 % yield) of the pure target compound (**VII, SGSH**), as a white solid. (R_f = 0.3, 5% methanol in chloroform, v/v). **ESI-MS**: m/z = $[M+1]^+$ 460.13 for $C_{20}H_{38}ClN_5O_5S$

^{1}H NMR: (500 MHz, $CDCl_3$+CD_3OD): δ/ppm = 0.9-1.9 (m, 15H, a+b+c+d+e+f+g), 2.1(m, 1H,h), 2.4-2.8 (m, 3H, h,i), 3.1(m, 4H, j+k), 3.6 (m, 2H, m+n), 3.9-4.0 (m, 1H, l), 4.4(m, 1H,o), 6.4(d, 1H,p)

Scheme S2:

Synthesis of (S)-S-(6-(2-(((benzyloxy)carbonyl)amino)-6-((tert butoxycarbonyl)amino)hexanamido)hexyl) ethanethioate (compound I):

3.19 g (1 eqv, 5.68 mmol) of Z-Lys-Boc-OH(dicyclohexylammonium) salt was dissolved in 50 mL dichloromethane and 1.63 g (1.5 eqv, 85.2 mmol) of EDCI and 1.3 g (1.5 eq, 85.2 mmol) of HOBT were added to the solution at RT. The reaction mixture was stirred for 15 min and 1 g (1 eqv, 5.68 mmol) of compound **D** was added to the reaction mixture. To maintain basic condition in reaction mixture, 3 mL of DIPEA was added. The reaction mixture was stirred in an inert atmosphere using N_2 gas for 12 h. The crude residue obtained after evaporation of solvent was dissolved in 100 mL of chloroform and washed subsequently with saturated sodium bicarbonate (2×100 ml) solution, 0.5N HCl solution (2×100 mL) and saturated brine solution (2×100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated using rotary evaporation. The residue upon column chromatography over 60-120 mesh silica gel and 1.0% methanol in chloroform as eluent afforded 2.3 g of compound **(l)** (76% yield, R_f = 0.5, 5% methanol in chloroform, v/v).

ESI-MS: m/z = [M^++Na] 560 for $C_{27}H_{43}N_3O_6S$

^{1}H NMR: (500 MHz, $CDCl_3$): δ/ppm = 1.2-1.3(m, 4H, a+b), 1.35(s, 9H, c), 1.4(m, 4H, d+e,), 1.5(m,2H,f), 1.5-1.6(m, 1H,g), 1.5-1.6(m, 1H, g), 1.7-1.9(m, 3H, g+h), 2.3(s, 3H, i), 2.85 (m,2H,j), 3.05(m2H, k), 3.25(t,2H, l), 4.05(m, 1H,m), 4.6(broad, 1H, n), 5.1(s, 2H,o), 5.55(broad, 1H, p), 6.25(broad, 1H, q), 7.3(m, 5H, r +r+r)

Triflate salt of (S)-S-(6-(6-amino-2-(((benzyloxy)carbonyl)amino)hexanamido)hexyl) ethanethioate (compound lI)

Compound I (1.4 g) was dissolved in 2 mL DCM and the solution was cooled to 0 ℃. 1 mL TFA was added and the solution was allowed to stir for 3 h at RT. Reaction completion was monitored by TLC. Reaction mixture was neutralized by 5 mL of sodium bicarbonate and extracted with chloroform.and organic layer was washed with saturated brine solution (2×20 mL) followed by dried over on sodium sulphate. The organic layer removed using rotary evoparation and the residue upon column chromatography over 60-120 mesh silica gel and 8% methanol in chloroform (v/v) as eluent afforded 1.1 g (88%, yield) of the pure compound **II**.(R_f = 0.2, 5% methanol in chloroform, v/v).

Synthesis of (S)-S-(12-(((benzyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)-2,2-dimethyl-4,13-dioxo-3-oxa-5,7,14-triazaicos-6-en-20-yl) ethanethioate (compound III):

1 g, (1 eqv, 2.28 mmol) of **compound II** was dissolved in 10 mL of 9:1 (DCM: DMF) solvent mixture and 1.5 mL of triethyl amine was added to maintain the basic condition. The solution was allowed to stir for 15 min at RT and their after, 947 mg (1.5 eqv, 3.43 mmol) of DBTU was added and the reaction mixture was cooled to 0 ℃. 930 mg (1.5 eqv, 3.43 mmol) of mercuric chloride ($HgCl_2$) was added and the reaction mixture was allowed stir for 1 h at same temperature. The reaction mixture was diluted with DCM (15 mL) and filtered through celite bed. The filtrate was sequentially washed with 0.5N HCl aqueous solution (2×50 mL) saturated brine solution and dried over on anhydrous sodium sulphate. The organic solvent was removed using rotary evoparation and the residue upon column chromatography over 60-120 mesh silica gel and 0.5 % methanol in chloroform (v/v) as eluent afforded 685 mg (45%, yield) of the pure compound **III**. (R_f = 0.8, 5% methanol in chloroform, v/v).

ESI-MS: m/z = $[M^+$+Na] 702, $[M^+$+K] 718 for $C_{33}H_{53}N_5O_8S$

^{1}H NMR: (500 MHz, $CDCl_3$): δ/ppm = 1.2-1.3(m, 4H, a+b), 1.35(s, 9H, c), 1.45-1.5(m, 6H, d+e+f,), 1.6(m, 1H, g), 1.7(m, 1H, h), 1.8(m, 1H, g□), 2.3(s, 3H,i), 2.8(m,2H, j), 3.2(m, 2H,k), 3.4(t,2H, l), 4.1(m, 1H, m), 5.1(s, 2H, n), 5.4(broad, 1H, o), 6.2(broad, 1H, p), 7.25(m, 5H, q+q□+q□□), 8.3(broad, 1H, r), 11.45(broad, 1H, s).

Triflate salt of (S)-S-(6-(2-(((benzyloxy)carbonyl)amino)-6-guanidinohexanamido)hexyl) ethanethioate (Compound IV):

Compound III (200 mg) was dissolved in 2 mL DCM and the reaction mixture was cooled to 0 ℃. 1 mL TFA was added and the solution was allowed to stir for 3 h at RT. Completion of reaction was monitored by TLC. Reaction mixture was neutralized with 5 mL of sodium bicarbonate and extracted into chloroform. The organic layer was washed with saturated brine solution (2×20 mL) and dried over sodium sulphate. The filtered supernatant upon concentrating with rotary evaporation afforded 141 mg (70.9 % yield) of free amine as intermediate compound **IV** (R_f= 0.2, 5% methanol in chloroform, v/v).

Synthesis of (S)-amino((5-(((benzyloxy)carbonyl)amino)-6-((6-mercaptohexyl)amino)-6-oxohexyl)amino)methaniminium chloride (compound V):

Sodium methoxide (56 mg, 5 eqv, 1.04 mmol) was dissolved in 4 mL of dry methanol and the solution was cooled to 0 ℃. **Compound IV** (100 mg, 1 eqv, 0.20 mmol) and the reaction mixture was stirred for 1h at room temperature. The resultant product neutralized with Amberlite IR120 (H^+), filtered through Whatmann filter paper and the filtrate was dried over anhydrous Na_2SO_4. The solvent was removed by rotary evaporation and the residue was dissolved in minimum amount of methanol and passed through amberlyst A-26 chloride ion exchange resin. The methanol was evaporated with rota evaporator the resulting residue was upon recrystallization from 1:10 (v/v) of methanol in acetone afforded 53 mg (58 % yield) of the pure target compound (**V,** GSH) as a white solid. (R_f = 0.3, 20% methanol in chloroform, v/v).

ESI-MS: m/z = [M^+] 437 for $C_{21}H_{36}ClN_5O_3S$

^{1}H NMR: (500 MHz, $CDCl_3$ + CD3OD): δ/ppm = 1.1-1.8(m, 14H, a+b+c+d+e+f+g), 2.8(t, 2H,h), 3.0-3.2(m, 4H, i+j), 4.1(m, 1H,k),5.0(s, 2H, l), 7.15-7.3(m, 5H, m+n+o)

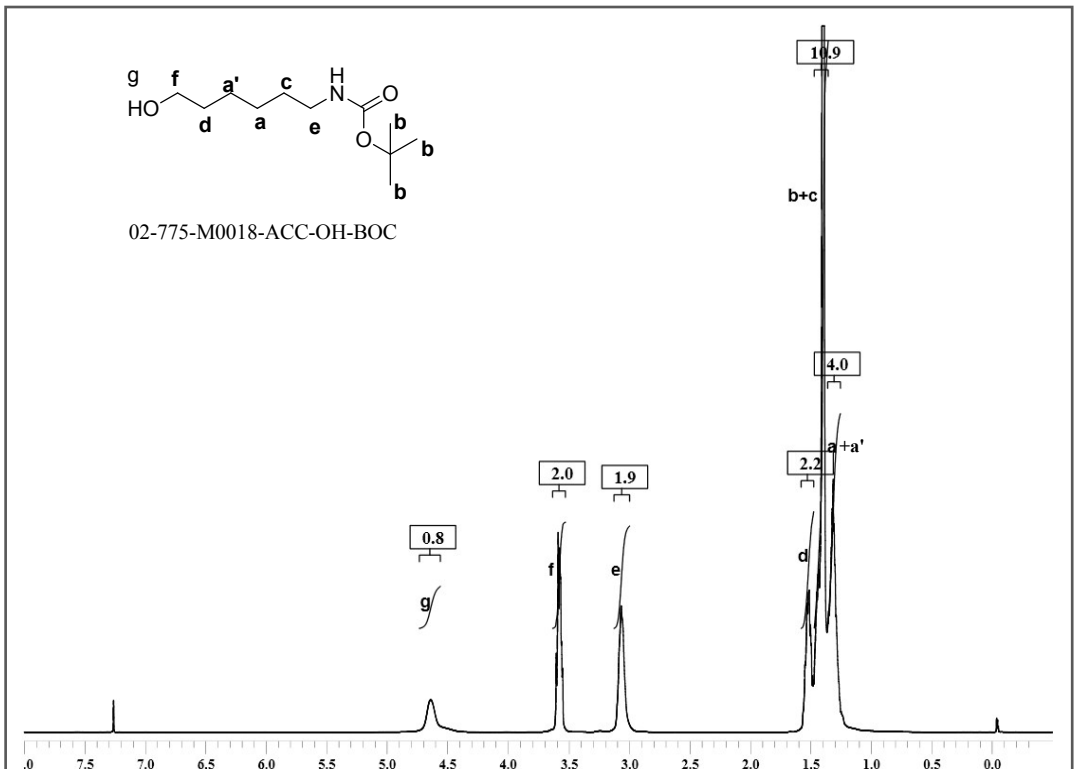


Fig. S1. ^{1}H NMR (400MHz) of compound **A** in $CDCl_3$

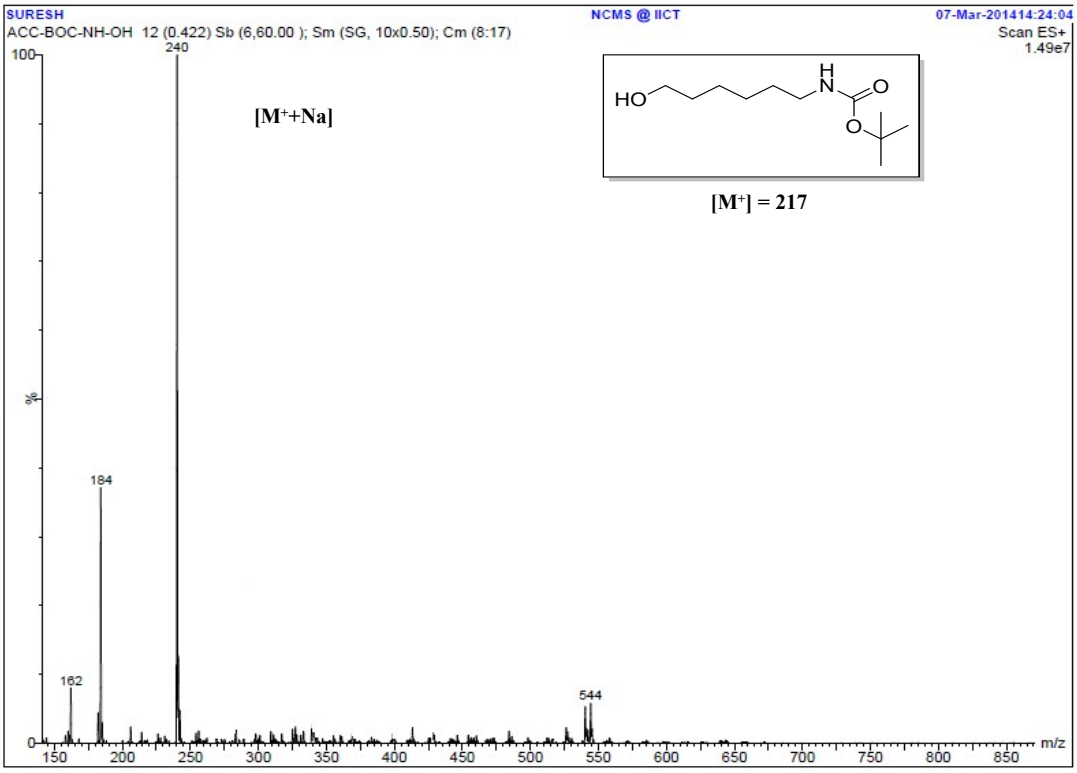


Fig. S2. ESI Mass spectrum of compound **A**

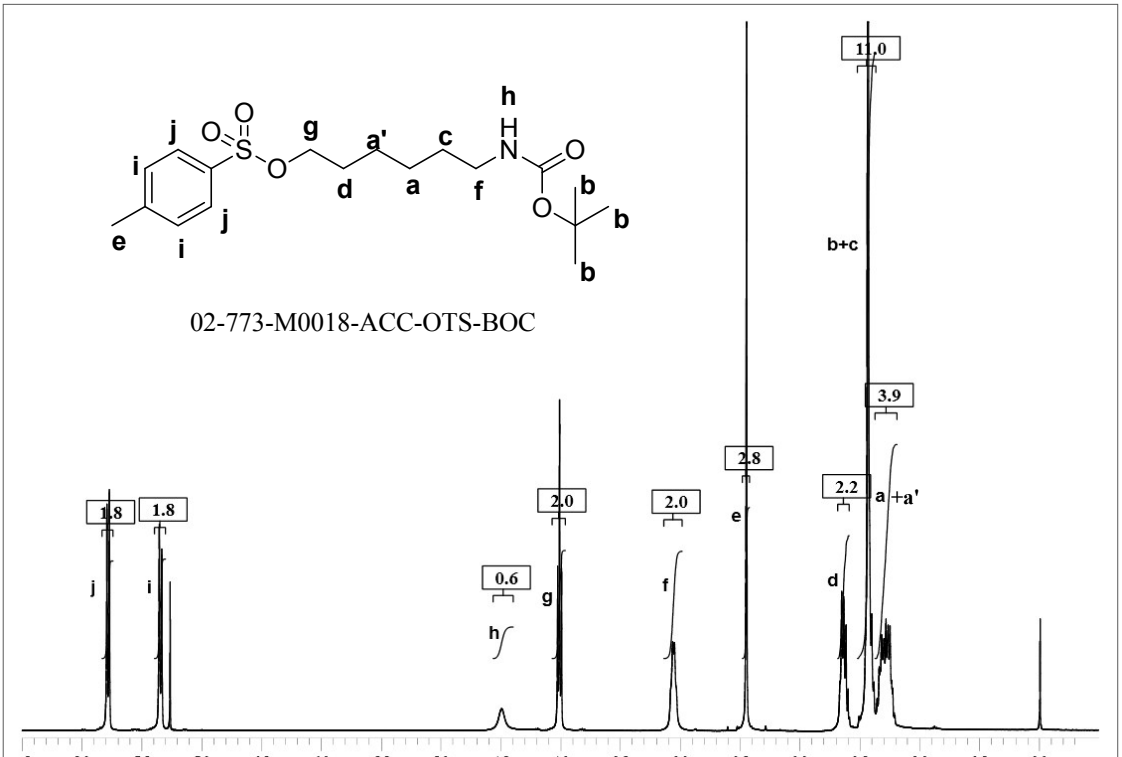


Fig. S3. ^{1}H NMR (400MHz) of compound **B** in $CDCl_3$

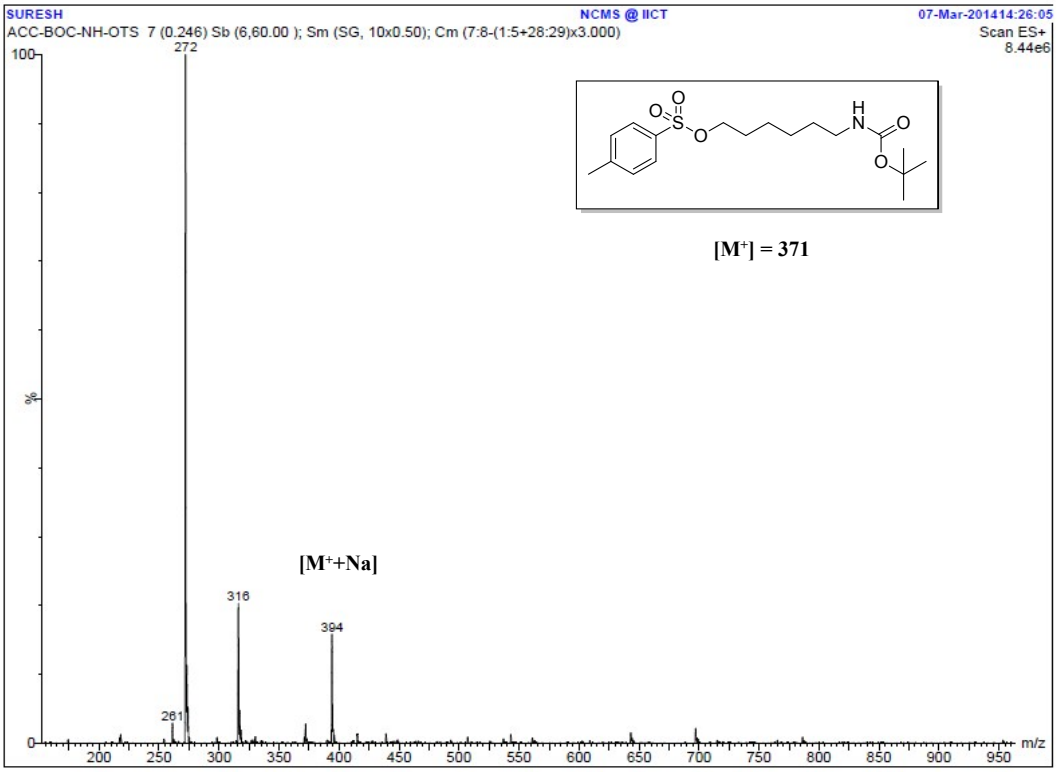


Fig. S4. ESI Mass spectrum of compound **B**

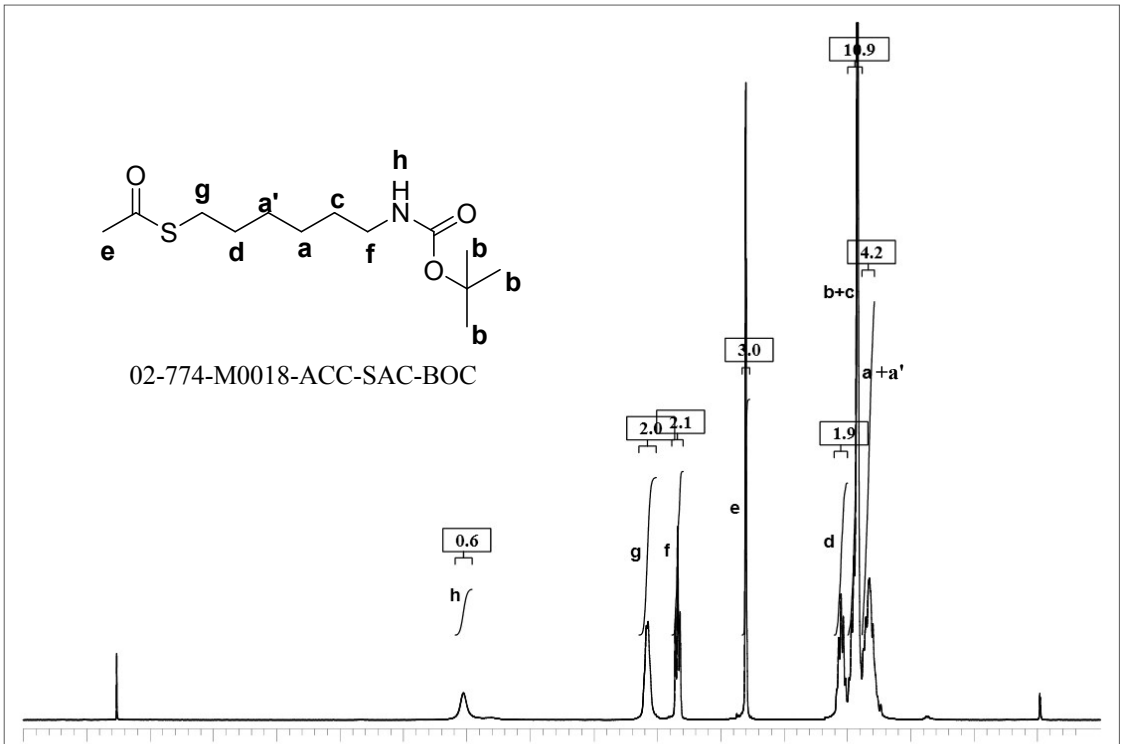


Fig. S5. ^{1}H NMR (400MHz) of compound **C** in $CDCl_3$

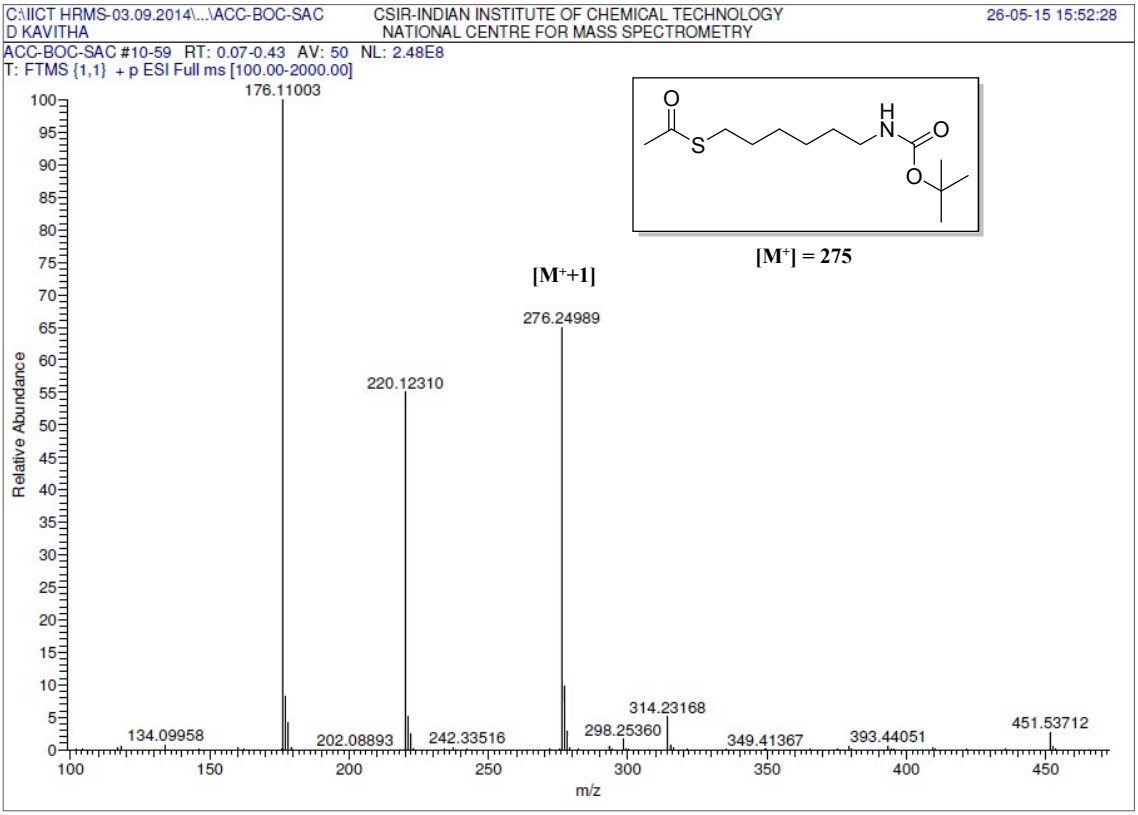


Fig. S6. ESI Mass spectrum of compound **C**

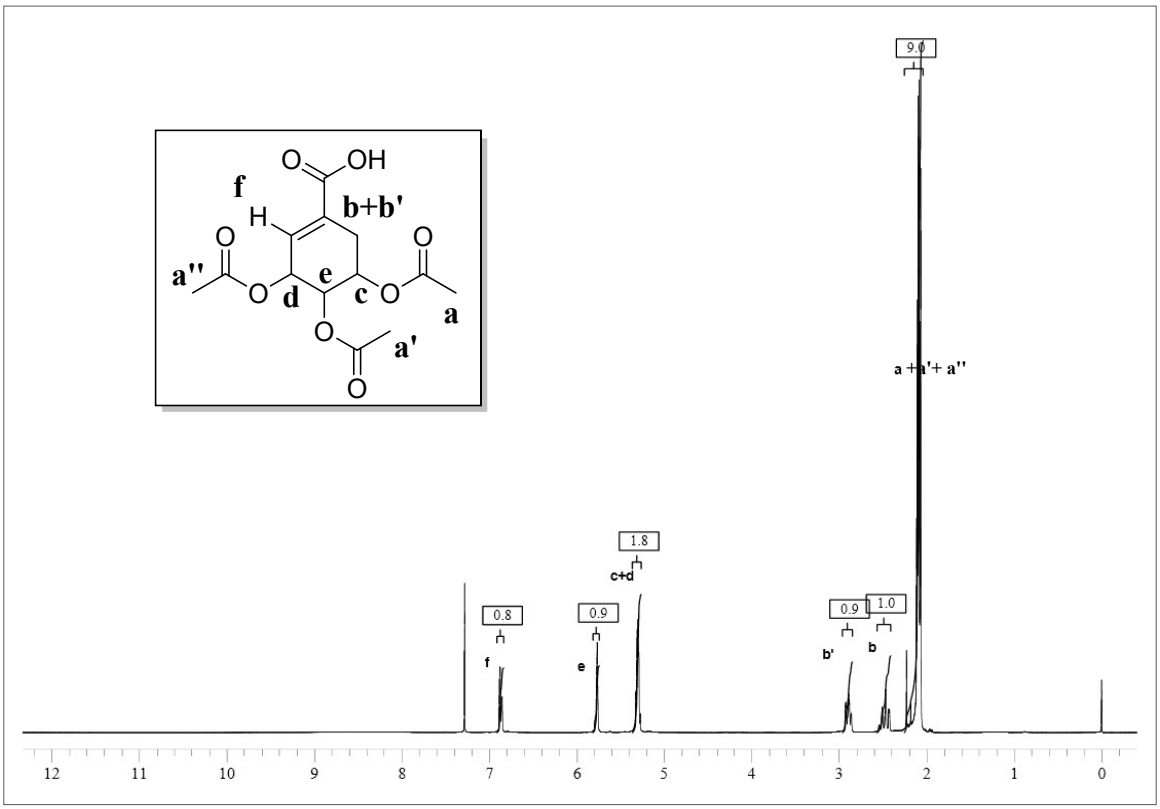


Fig. S7. ^{1}H NMR (500MHz) of compound **E** in $CDCl_3$

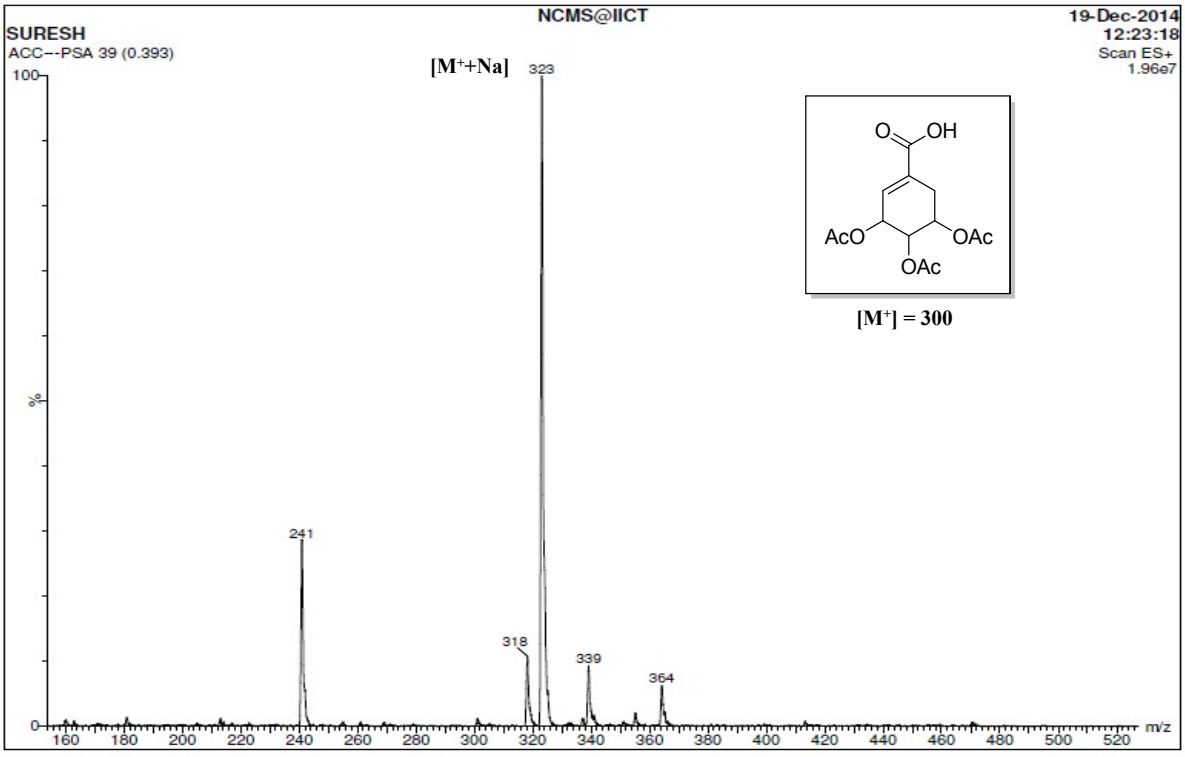


Fig. S8. ESI Mass spectrum of compound **E**

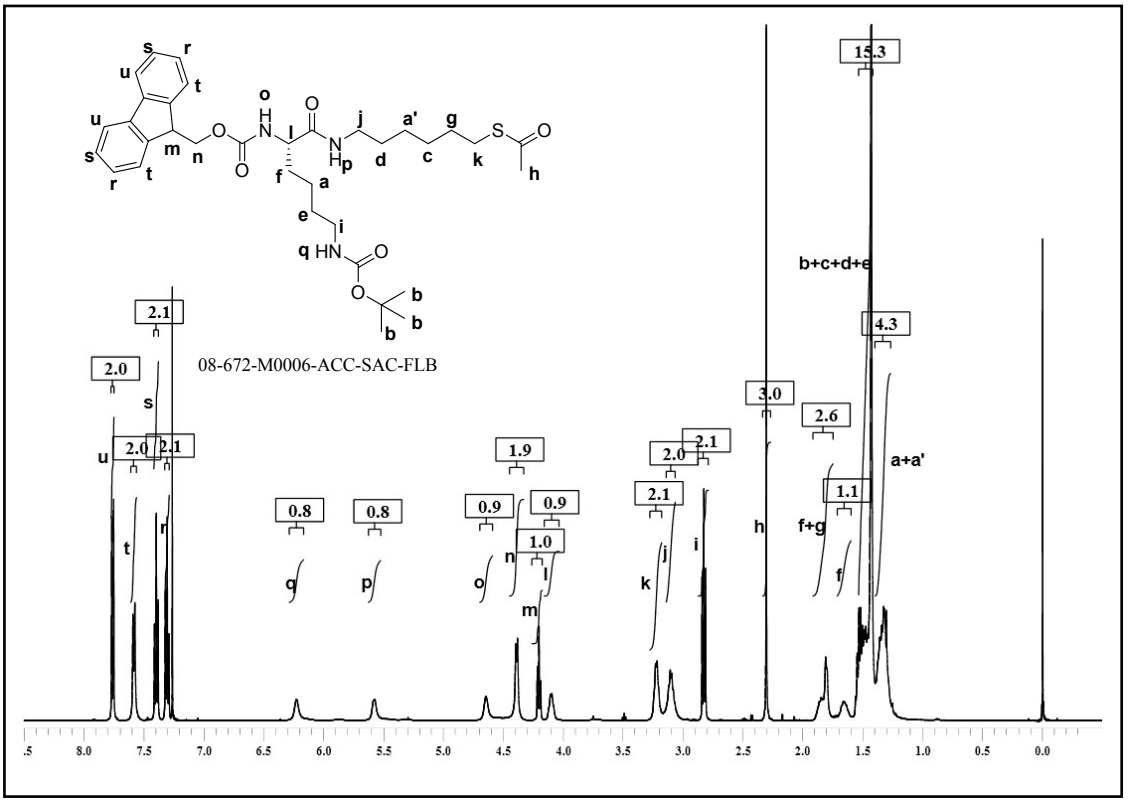


Fig. S9. ^{1}H NMR (500MHz) of compound **I (**Scheme **S1)** in $CDCl_3$

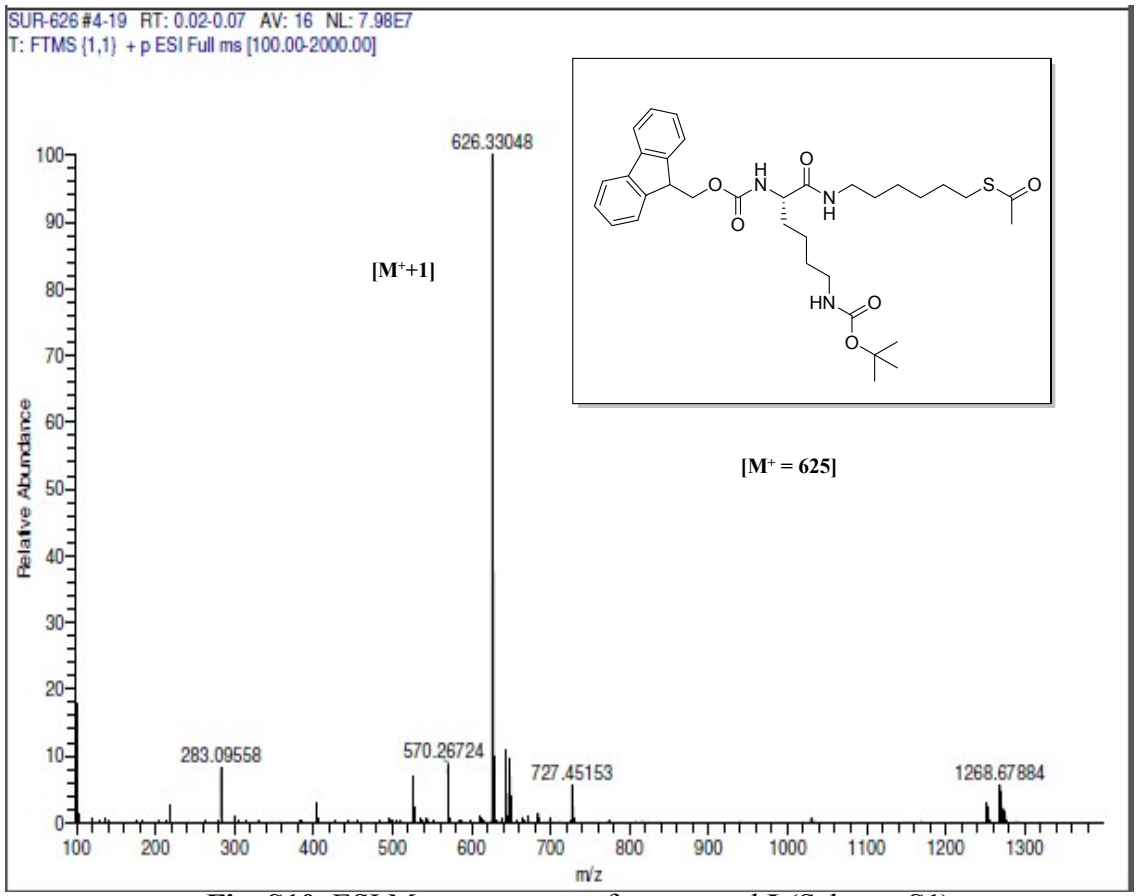


Fig. S10. ESI Mass spectrum of compound **I (**Scheme **S1)**

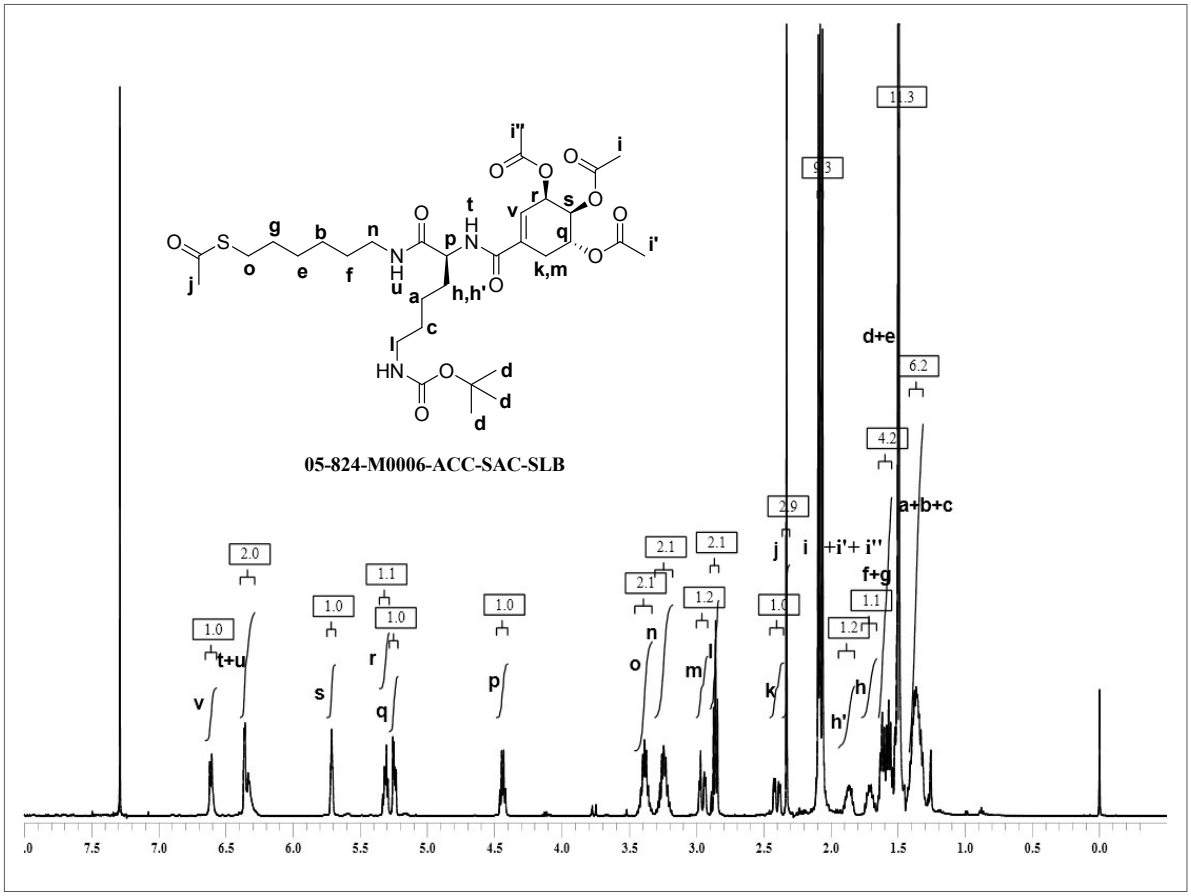


Fig. S11. ^{1}H NMR (500MHz) of compound **III (**Scheme **S1)** in $CDCl_3$

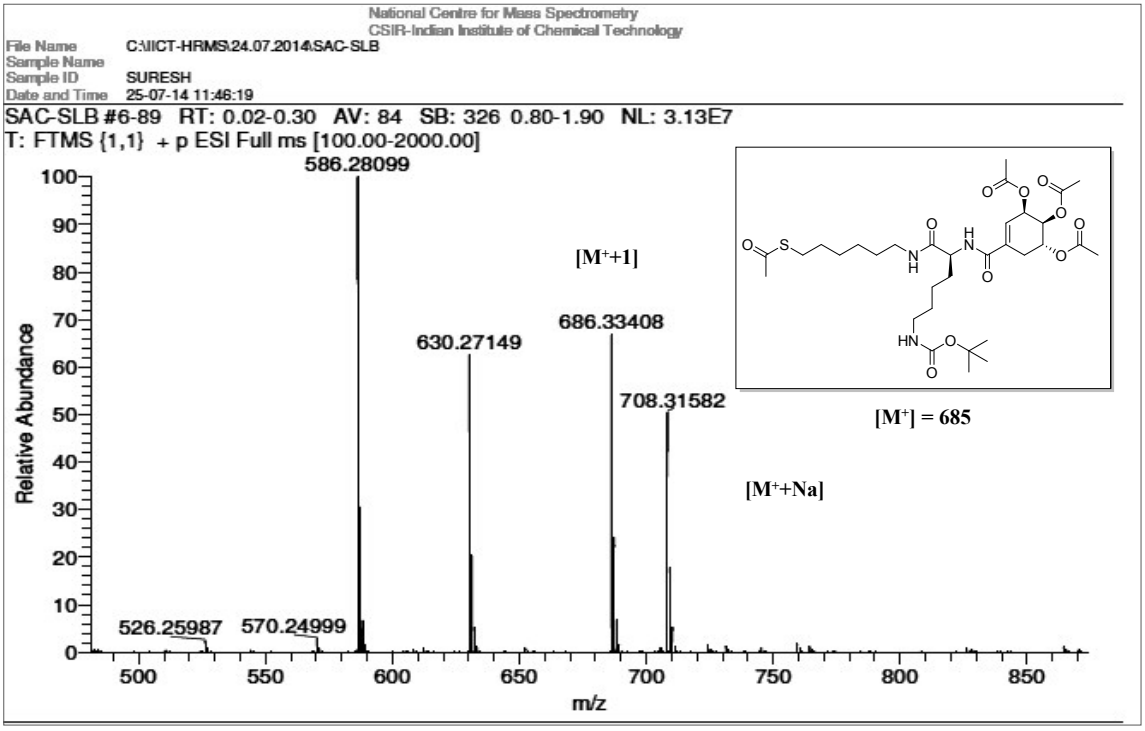


Fig. S12. ESI Mass spectrum of compound **III (**Scheme **S1)**

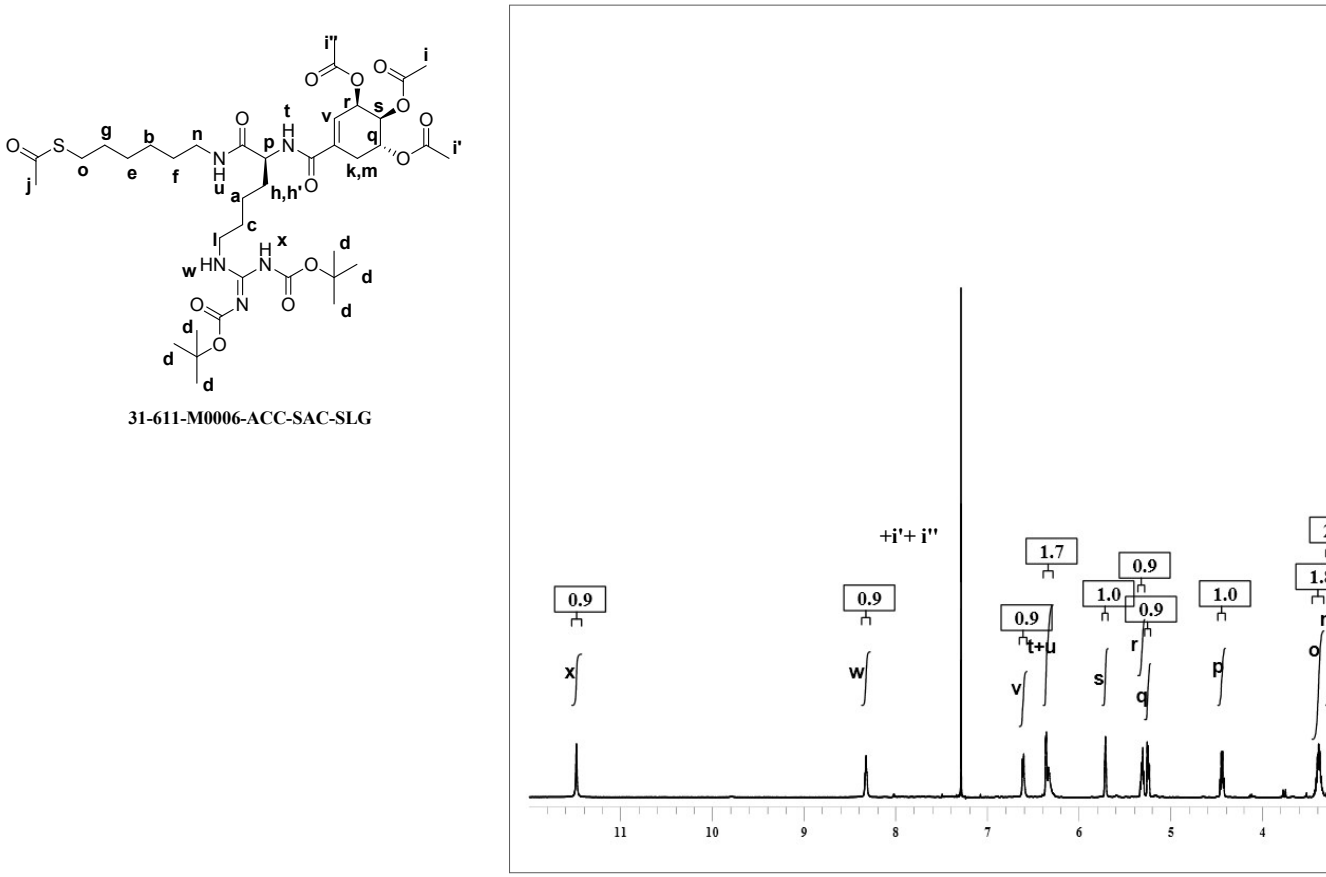


Fig. S13. ^{1}H NMR (500MHz) of compound **V (**Scheme **S1)** in $CDCl_3$

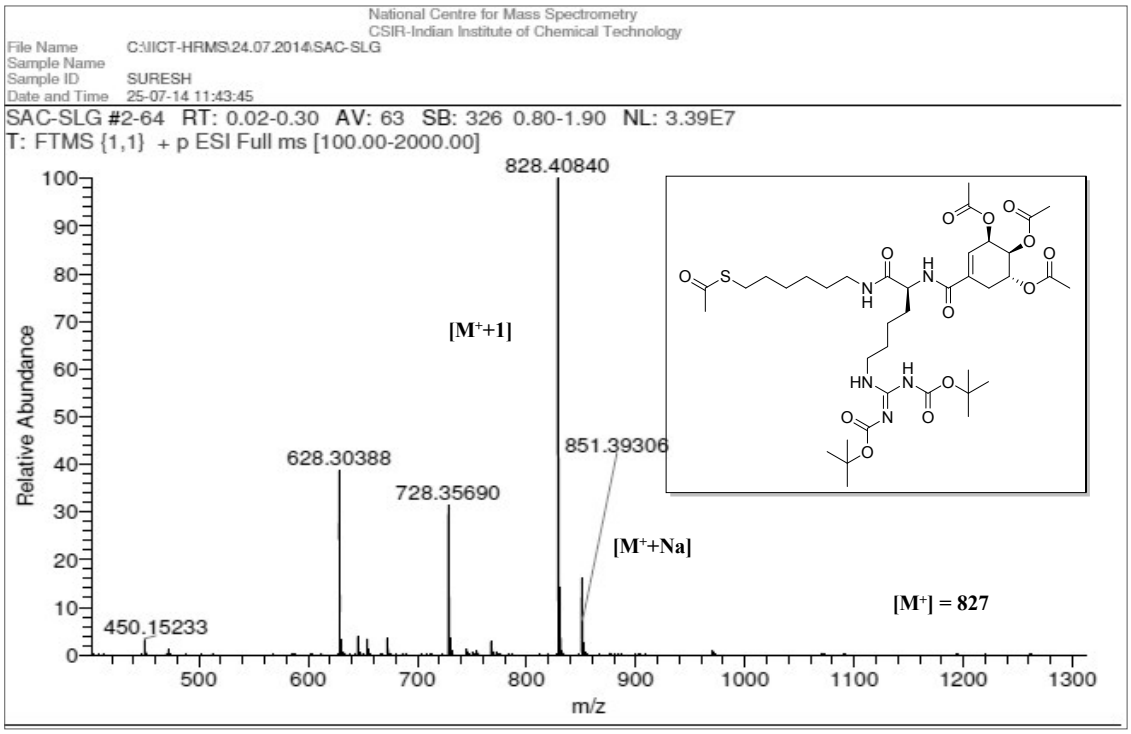


Fig. S14. ESI Mass spectrum of compound **V (**Scheme **S1)**

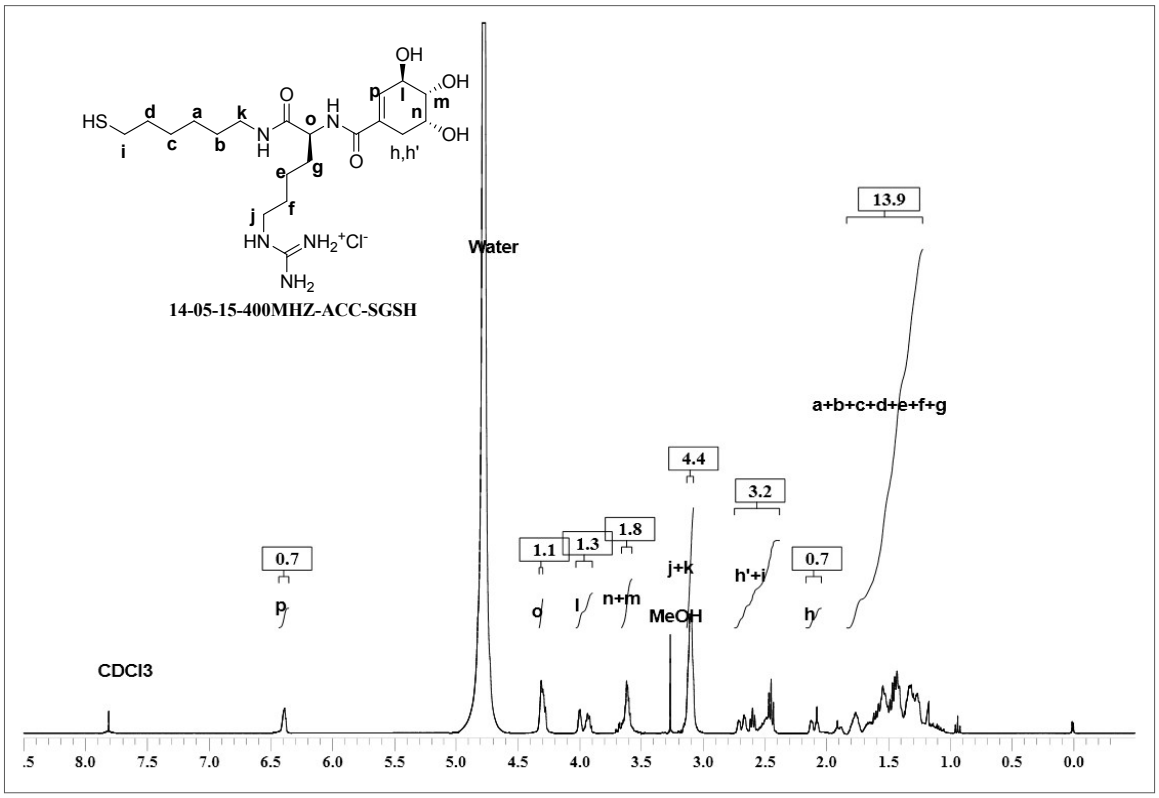


Fig. S15. ^{1}H NMR (500MHz) of compound **VII (**Scheme **S1)** in $CDCl_3$ +CD_3OD

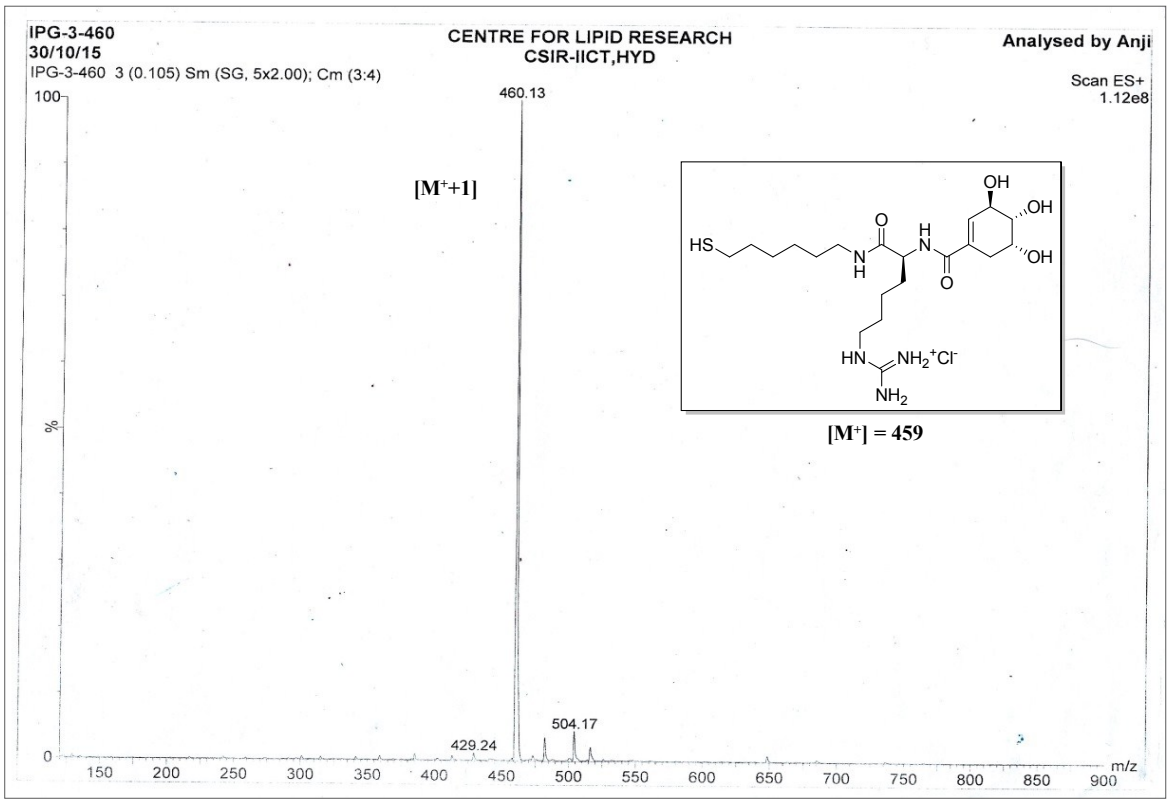


Fig. S16. ESI Mass spectrum of compound **VII (**Scheme **S1)**

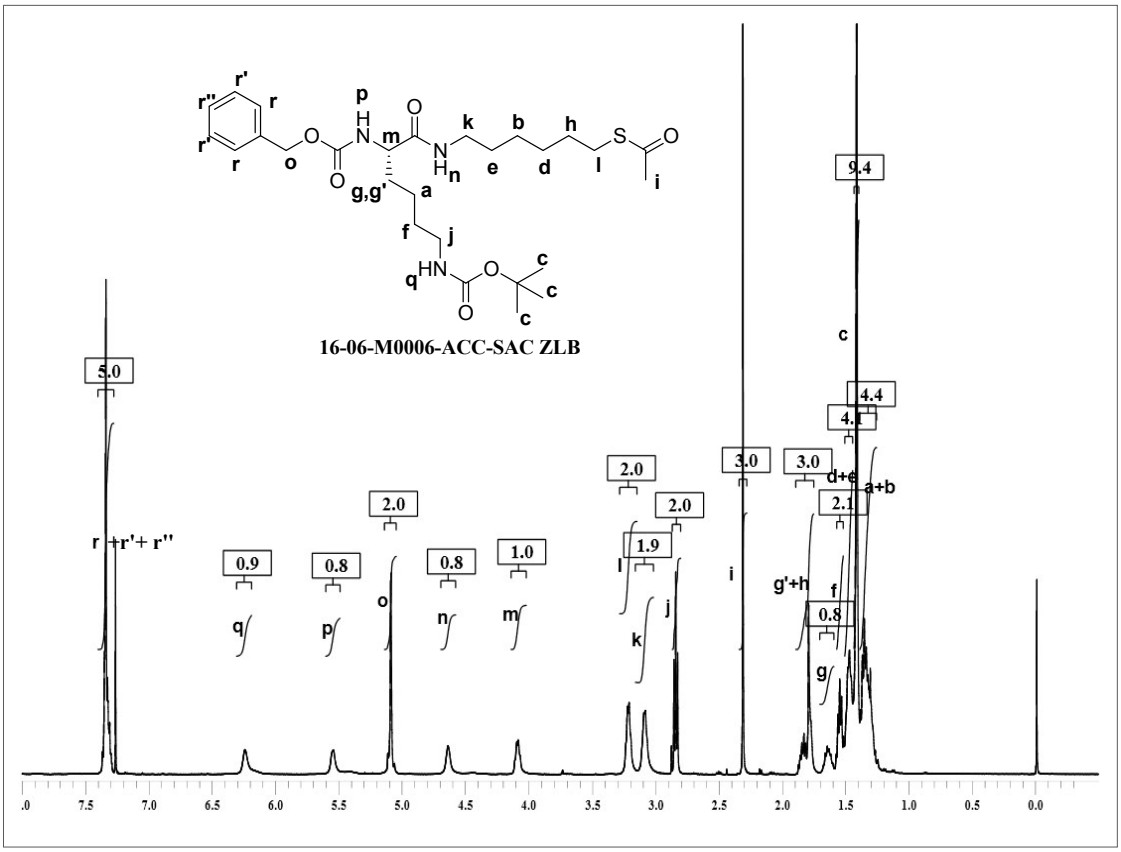


Fig. S17. ^{1}H NMR (500MHz) of compound **I (**Scheme **S2)** in $CDCl_3$

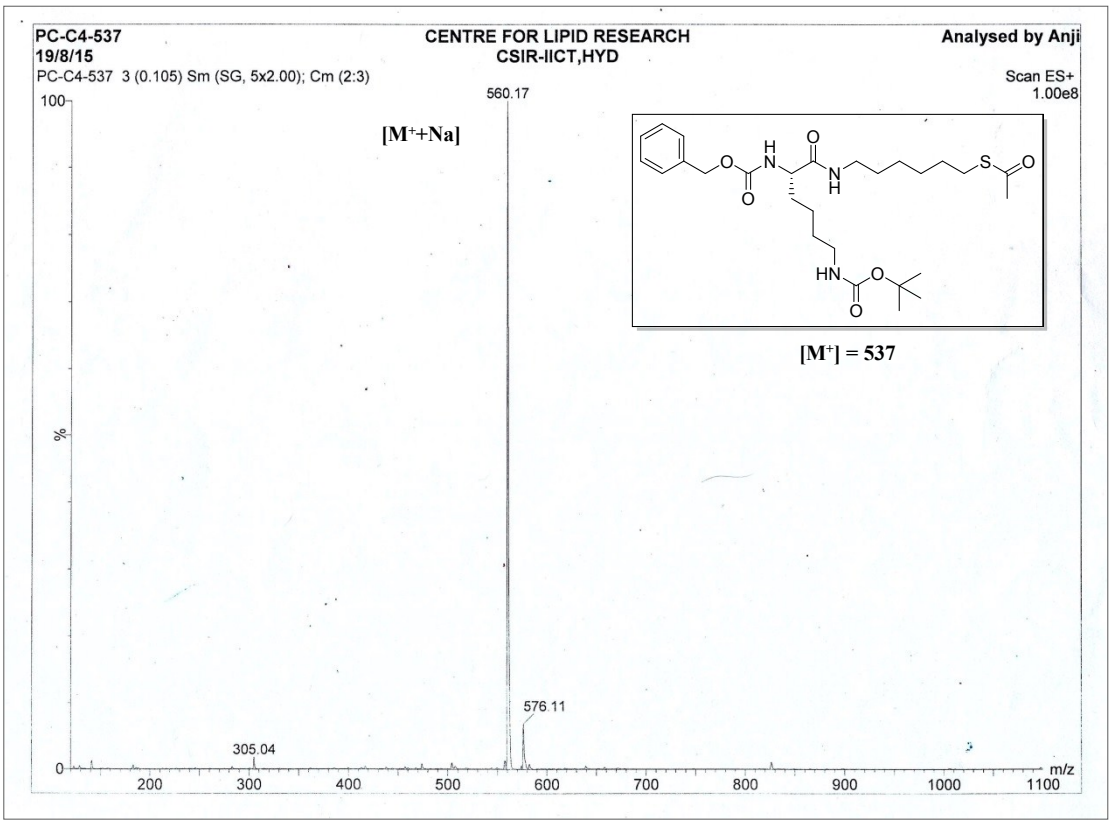


Fig. S18. ESI Mass spectrum of compound **I (**Scheme **S2)**

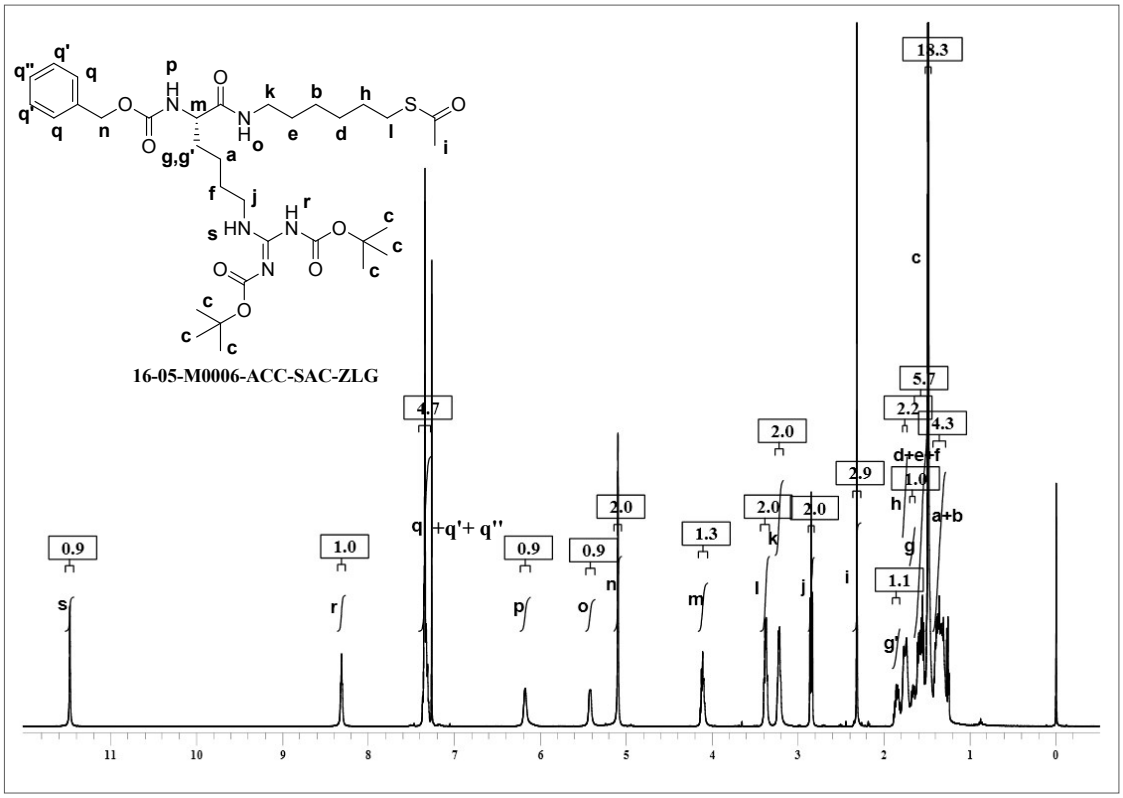


Fig. S19. ^{1}H NMR (500MHz) of compound **III (**Scheme **S2)** in $CDCl_3$

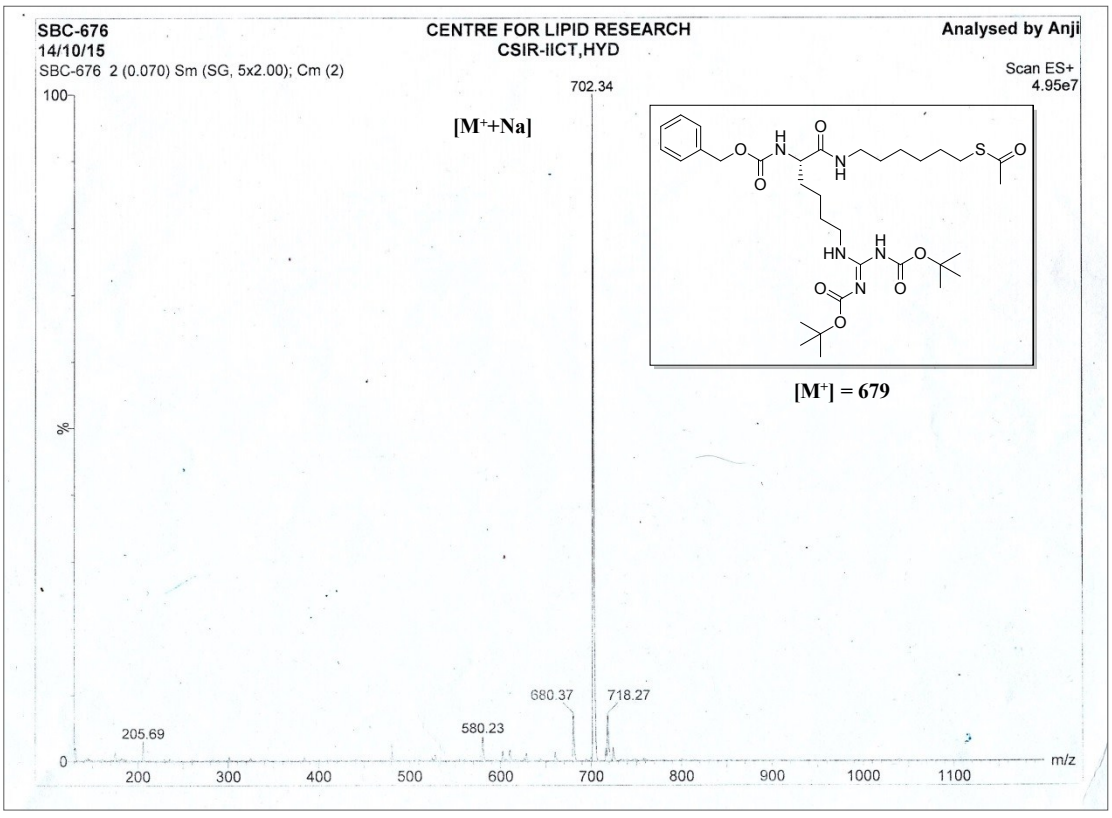


Fig. S20. ESI Mass spectrum of compound **III (**Scheme **S2)**

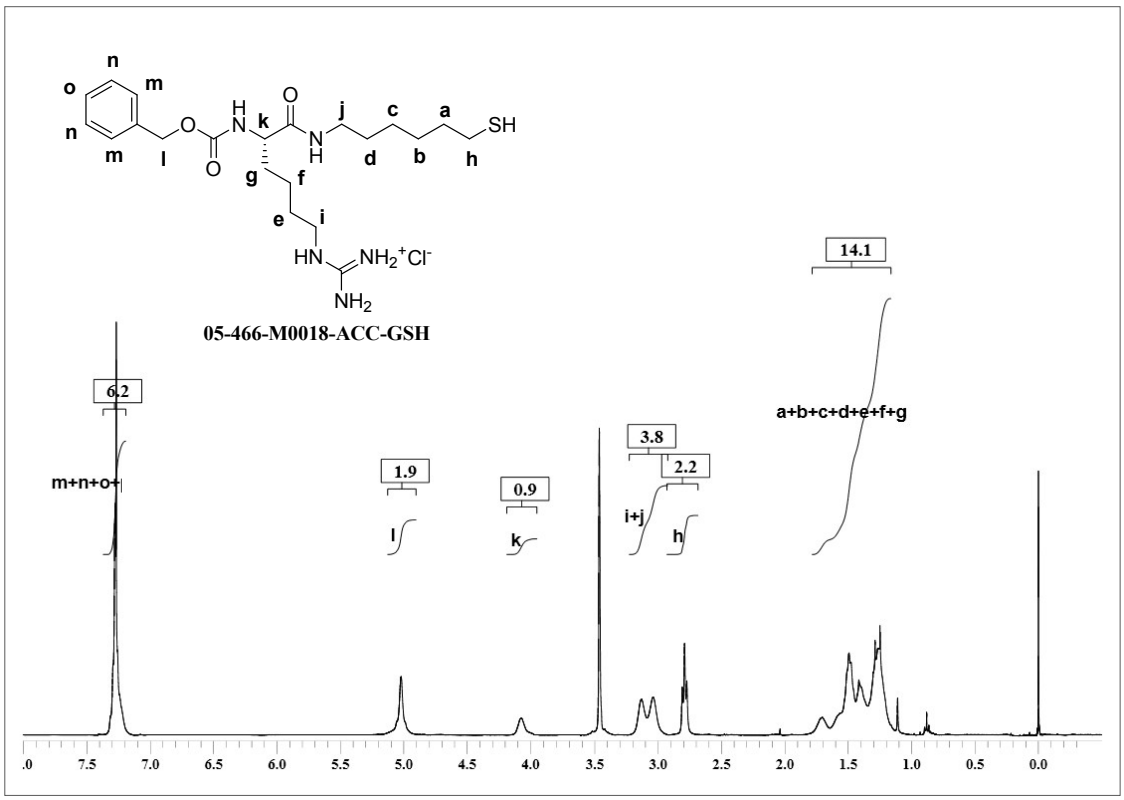


Fig. S21. ^{1}H NMR (500MHz) of compound **V (**Scheme **S2)** in $CDCl_3$ + CD_3OD

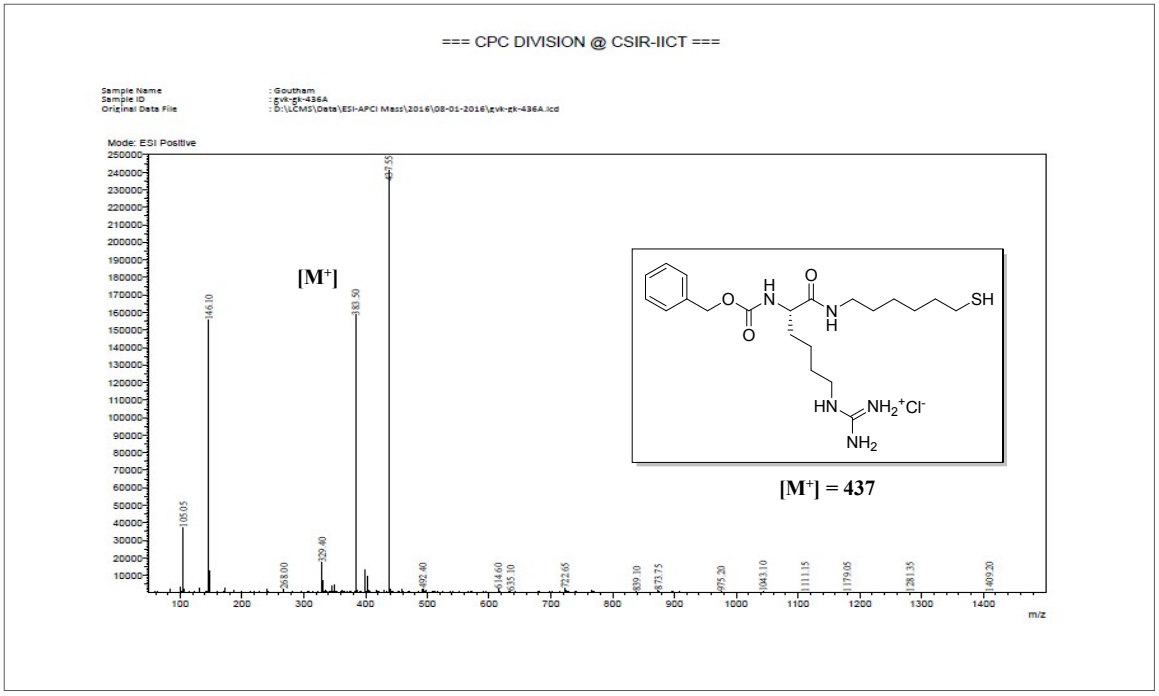


Fig. S22. ESI Mass spectrum of compound **V (**Scheme **S2)**

HPLC profiles of purified SGSH and GSH ligands using two different mobile phases

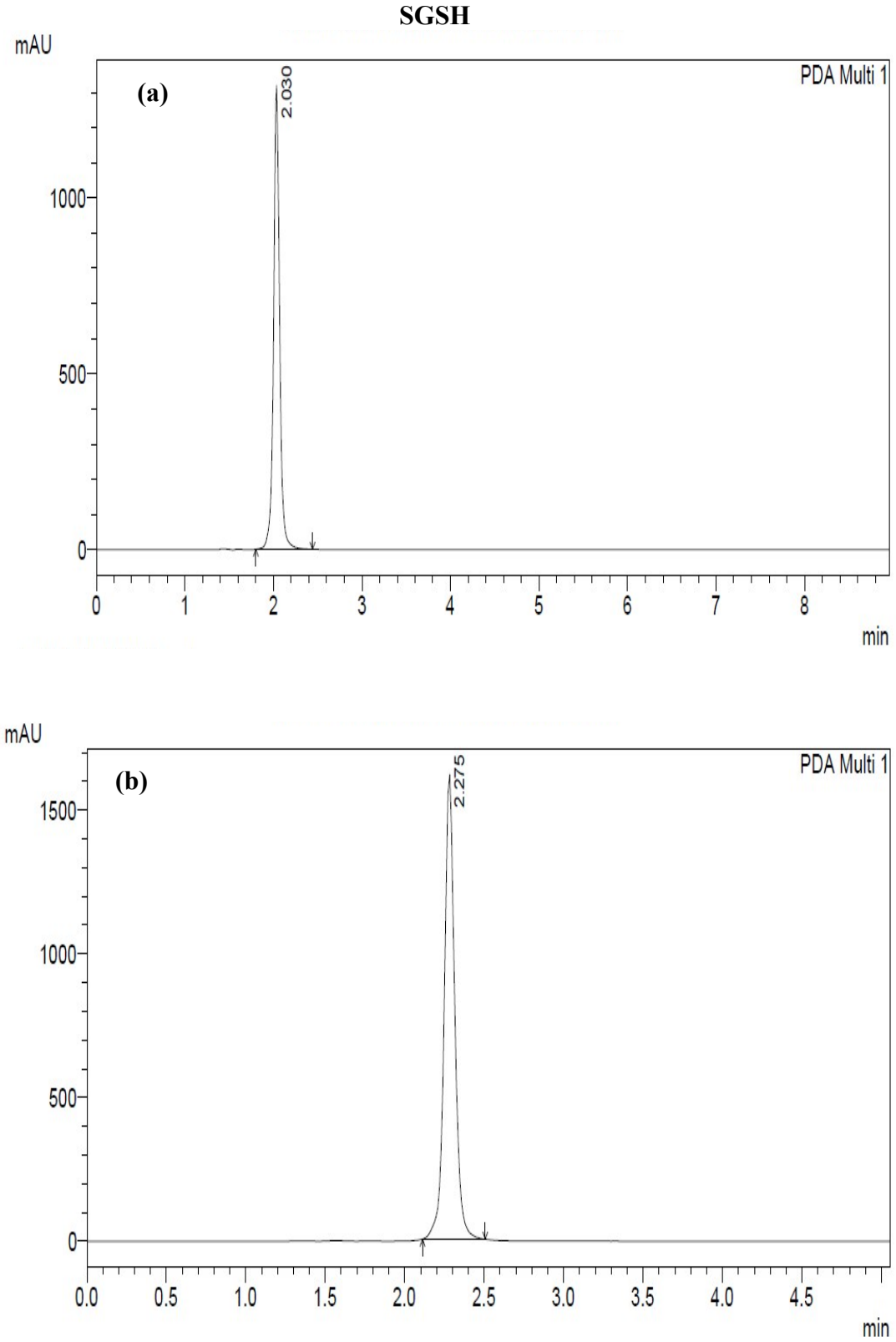


Fig. S23. Representative HPLC Chromatograms for compound **VII (**Scheme **S1)** using pure methanol as mobile phase(A) and using 95:5 methanol:water, v/v, as the mobile phase (B).

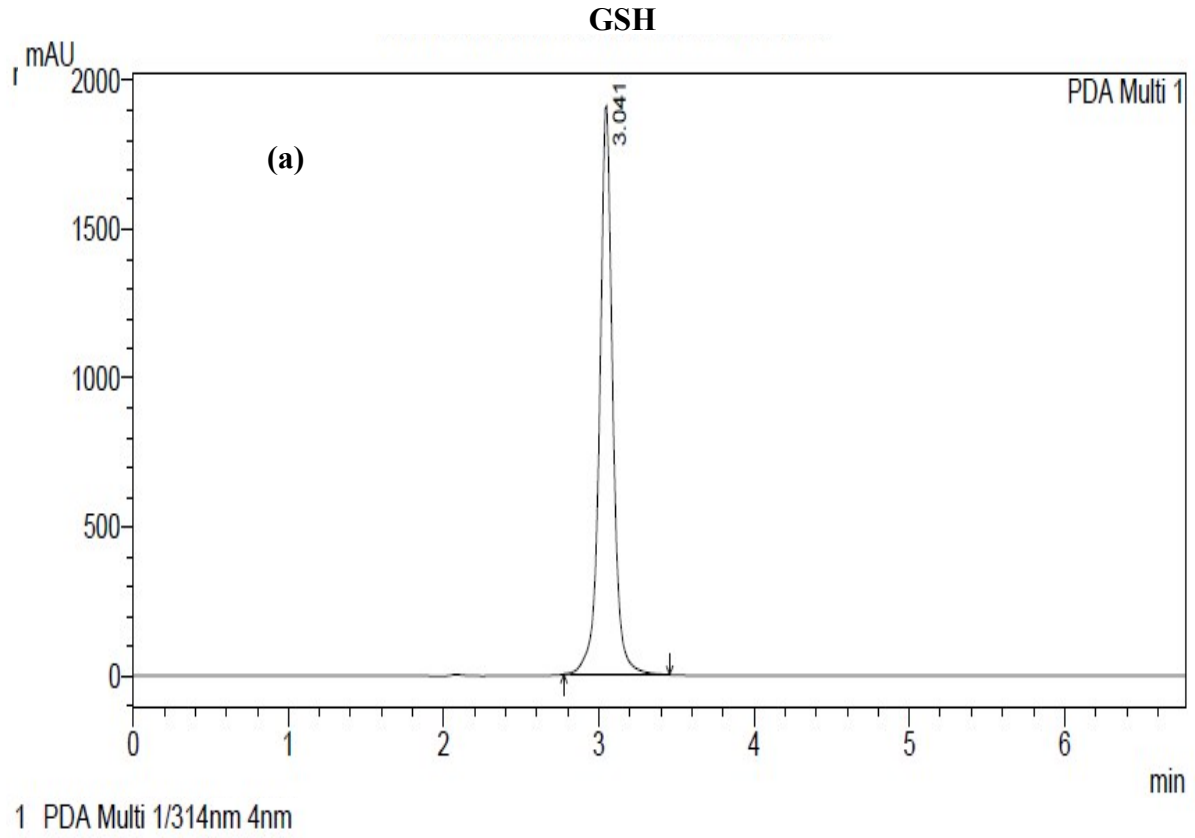


Fig. S24. Representative HPLC Chromatograms for compound **V (**Scheme **S2)** using pure methanol as mobile phase(A) and using 95:5 methanol:water, v/v, as the mobile phase (B).

HPLC conditions:

Mobile phases: Pure Methanol (**a**); Methanol:Water, 95:5 (v/v) (**b**).

System: Agilent HPLC system (1100 series, Waldbronn, Germany)

Column: on C18 (250mm x 4.6mm i.d, 5µm)

Flow Rate: 1.0 mL/min (0-20 min.)

Typical Column Pressure: 60-65 Bars

Temperature: 25 ^{0}C; Detection: UV at 260 nm.

Table S1. AuNP preparation and its conjugation to SGSH and GSH.

Total volume of reaction: 15 mL

Quantity		
$HAuCl_4$ (10^{-2}M)	PEI (1%)	Water
500 µL	250 µL	14 µL

AuNPs

SGSH 40 μg/mL — After 2 h reaction, Centrifuged at 17000 rpm — GSH 40 μg/mL

AuNP-SGSH | AuNP-GSH

Table S2. Sizes and Zeta potentials (ξ) of nanoconjugates.

Sample	Hydrodynamic Diameter (nm)	Zeta Potential (mV)
AuNPs	24 ± 3	+20 ± 3
AuNP-GSH	49 ± 5	+21 ± 3
AuNP-SGSH	45 ± 2	+22 ± 4
AuNP -DNA	89 ± 8	+18 ± 2
AuNP-GSH-DNA	73 ± 6	+11 ± 2.6
AuNP-SGSH-DNA	64 ± 5	+12 ± 2.8

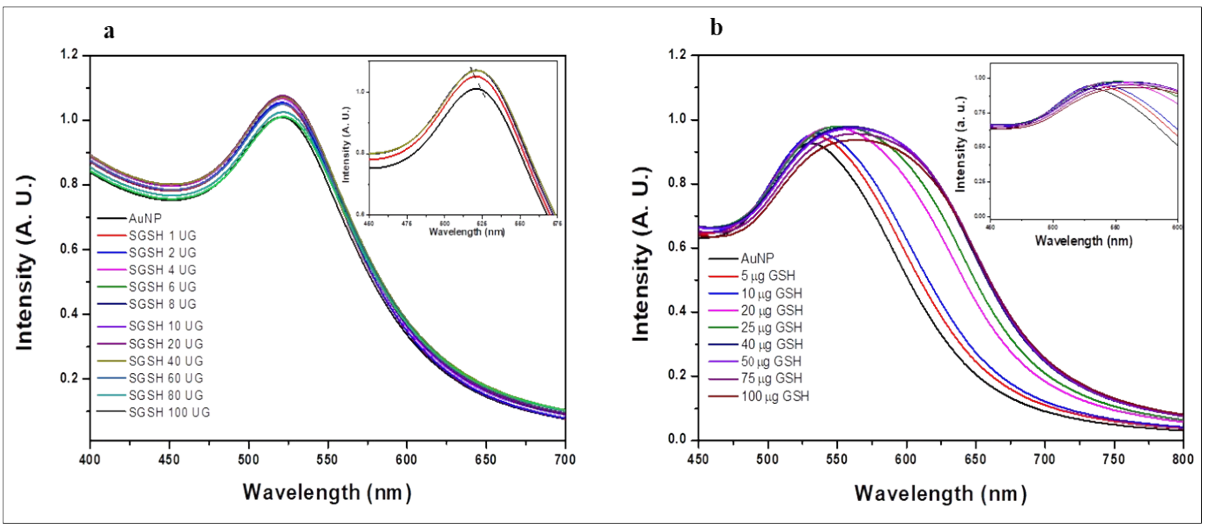


Fig. S25. UV-visible spectroscopy of AuNP-SGSH and AuNP-GSH

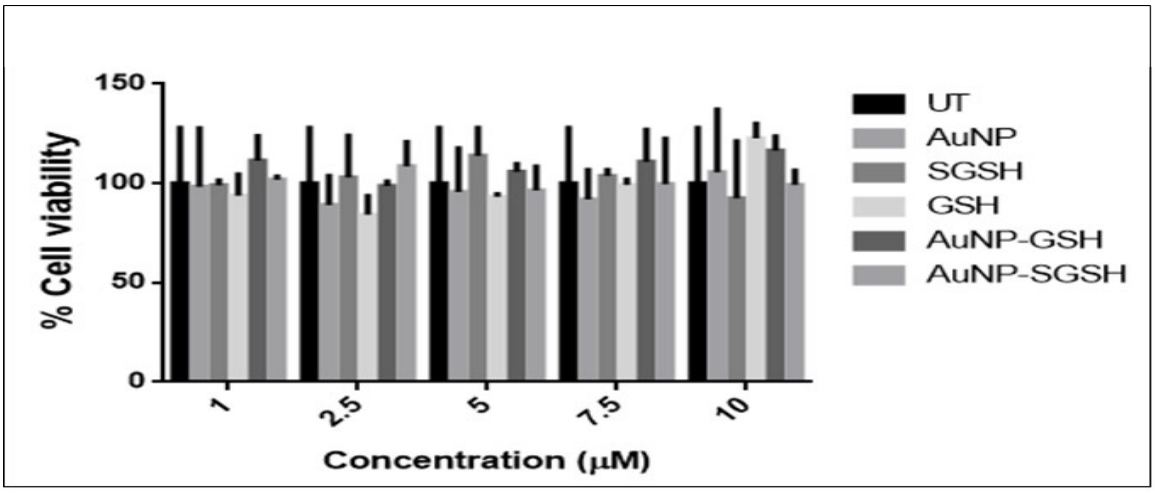


Fig. S26. cellular cytoxicity profiles of AuNP nanoconjugates: The cellular cytotoxicity profiles by conventional MTT assay in dendritic cells revealed non-cytotoxic nature of the Au-nanoconjugates up to 10 µL added nanoconjugtes (nanoconjugate stock 3 mg/mL of gold).

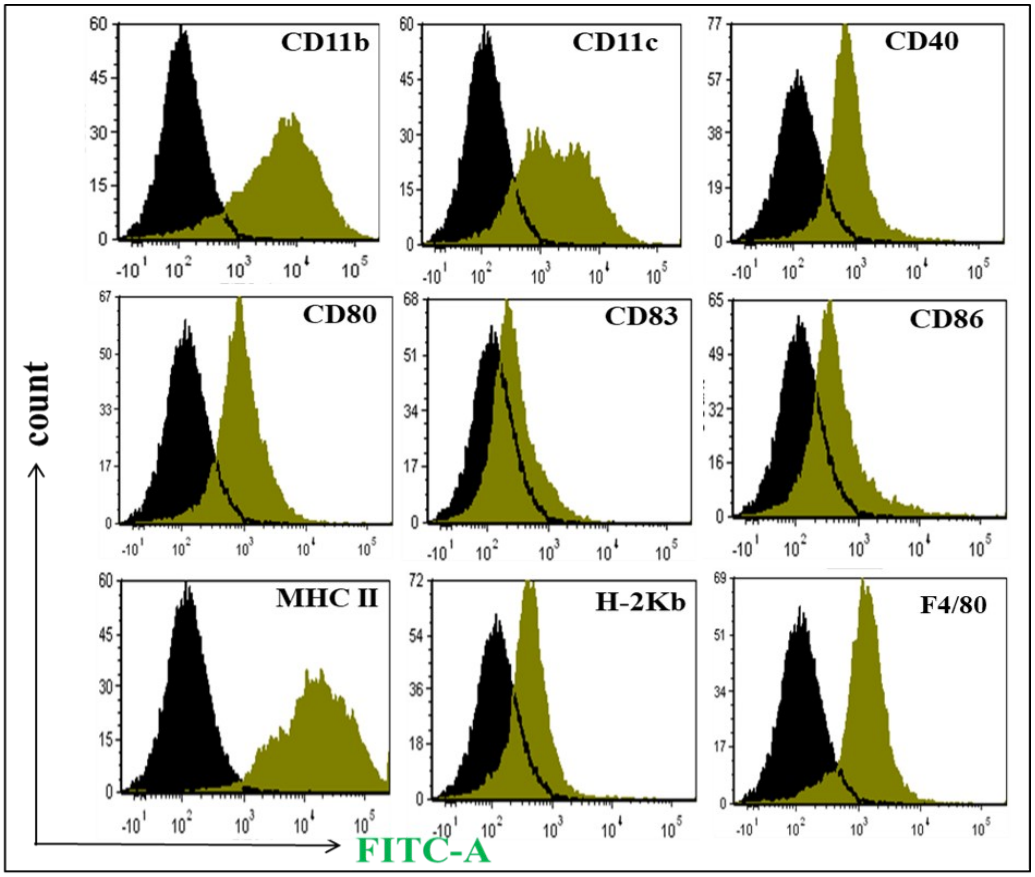


Fig. S27. Dendritic cells isolated from mouse bone marrow express several DC cell surface markers. mbmDCs were stained with FITC conjugated monoclonal antibodies specific for the DC cell surface markers including CD11b, CD11c, CD40, CD80, CD83, CD86, MHC II, H-2Kb, and F4/80 the stained cells were analysed using flow cytometry. ~1 x 10^6 mbmDCs were stained with FITC -conjugated monoclonal antibodies. Each experiment was repeated three times and similar markers profiles were observed in each time.

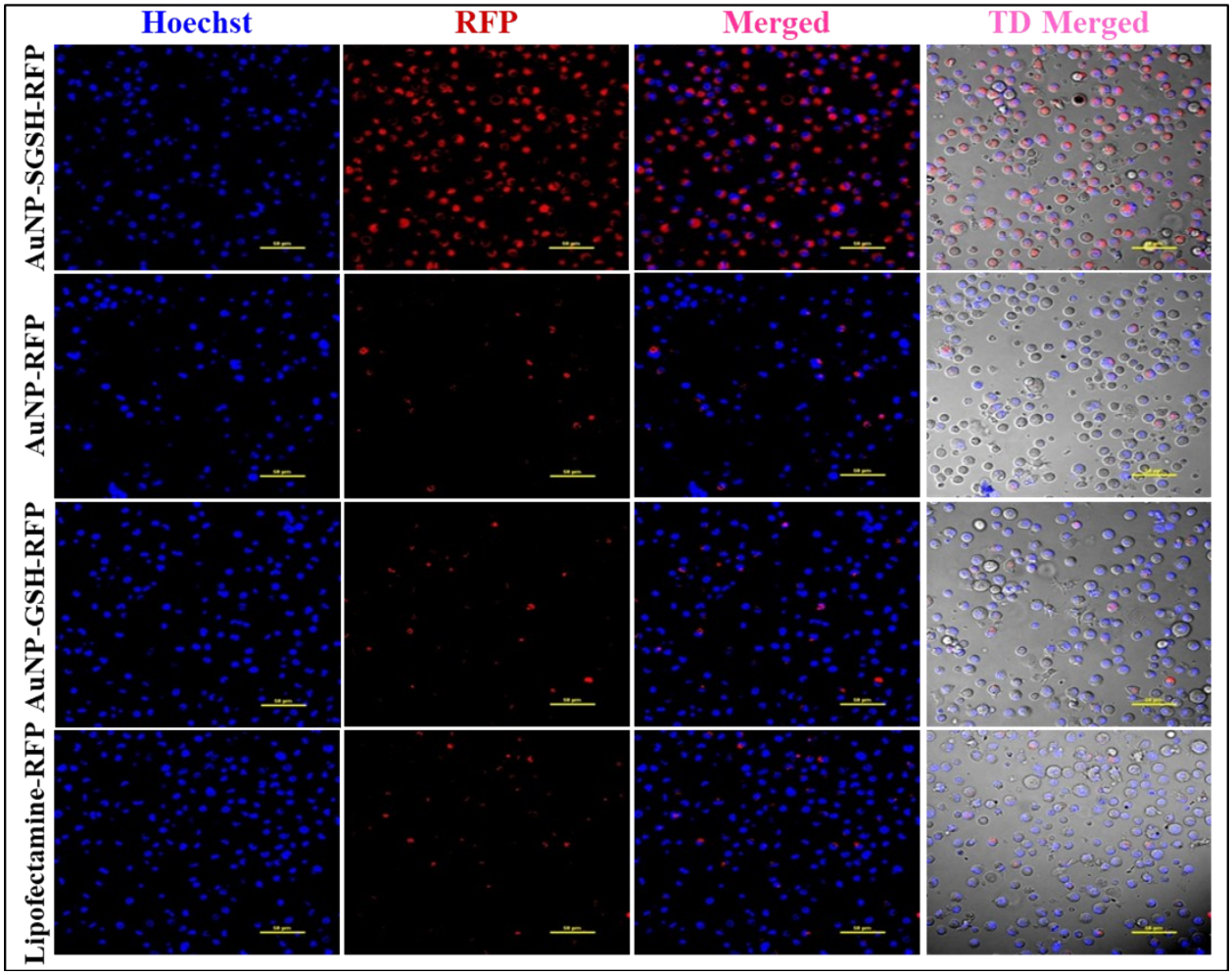


Fig. S28. Confocal images of mbmDCs transfected with the nanoplexes of AuNP-SGSH-RFP, AuNP-RFP, AuNP-GSH-RFP, and Lipofectamine-RFP . Transfection experiments ~1 x 10^6 cells were used, the degrees of RFP expression in transfected mbmDCs were visualized using confocal microscope. Transfection efficiencies of DCs were also measured using RFP lipoplexes of the commercially available transfection kit, Lipofectamine 2000. Red fluorescence from RFP protein was monitored using excitation and emission wavelengths at 650 nm and 670 nm, respectively. Cell nucleus was labeled with Hoechst- 33258 (Ex_λ 352 nm/Em_λ 461 nm). Magnification: 40x, scale bar: 50 µm.

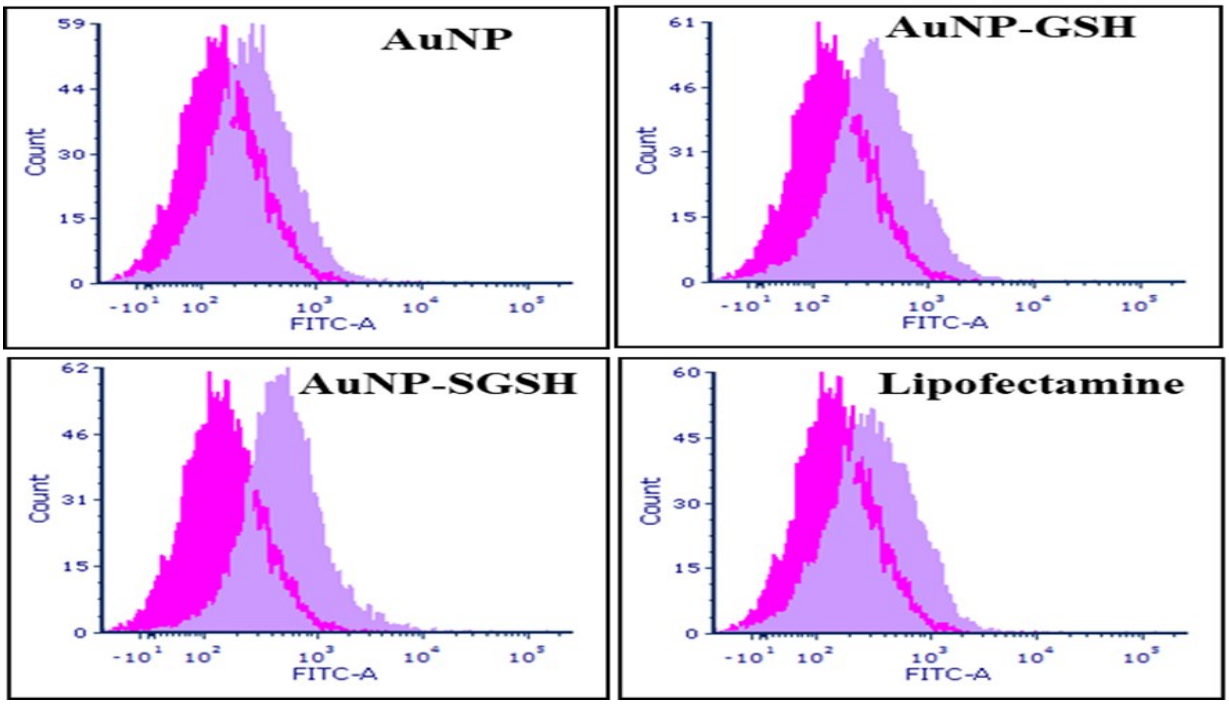


Fig. S29. FACS profiles for GFP expression in mbmDCs (~1 x 10^6 cells) transfected with nanoplexes of gold nanoconjugates (AuNP-pGFP, AuNP-GSH-pGFP and AuNP-SGSH-pGFP) and lipoplex of lipofectamine+pGFP. The histograms were recorded 24 h post transfection. Untreated mbmDCs served as a control. Dark pink histogram refers to fluorescence from untreated cells; pale pink histogram refers to fluorescence from treated cells. Each experiment was repeated three times and the transfection profiles were found to be similar in each time.

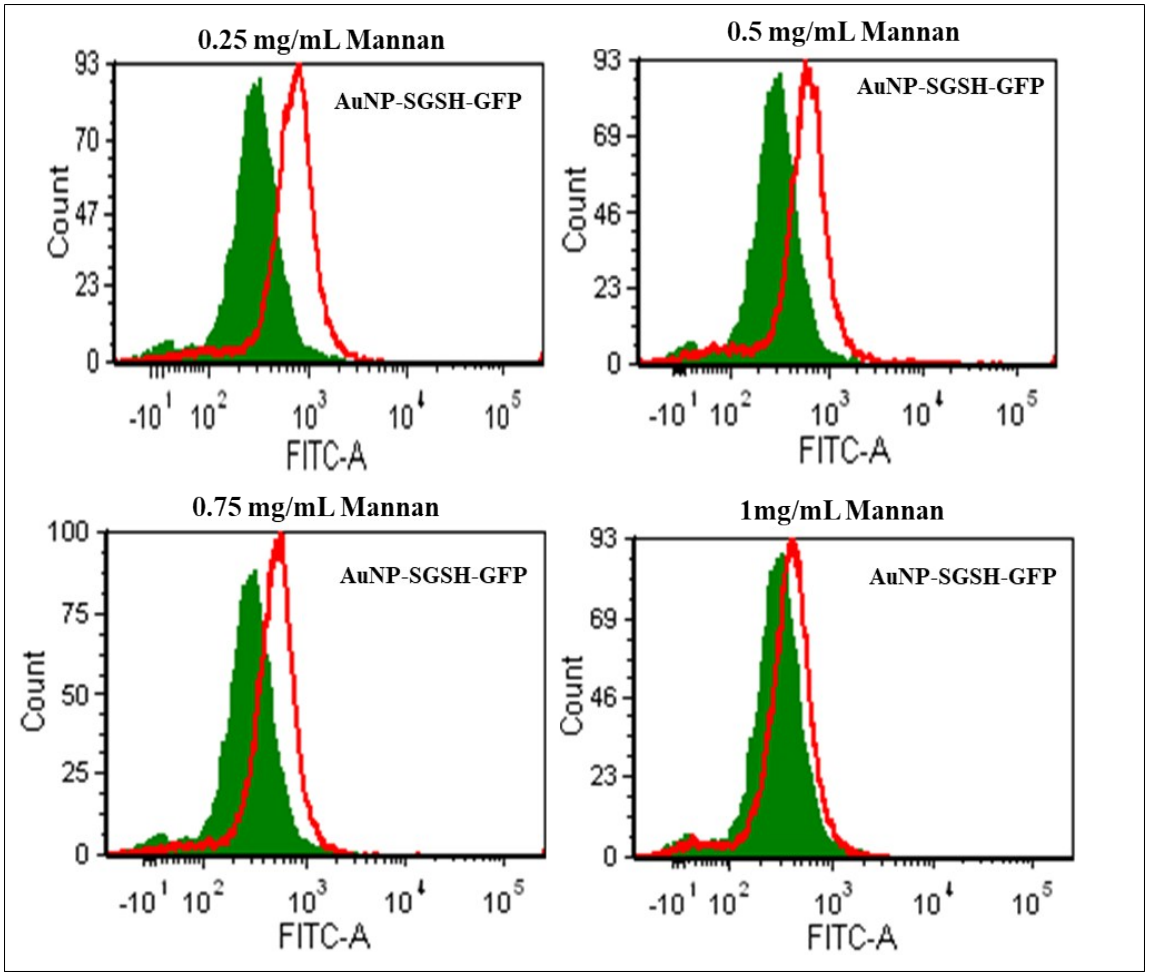


Fig. S30. The transfection efficiencies of nanoplex (AuNP-SGSH-pGFP) in mbmDCs pre-incubated with mannan in the range of 0.25-1 mg/mL. The degrees of GFP expression in transfected mbmDCs were quantified by flow cytometry. Green histogram refers to fluorescence from untreated cells; Red histogram refers to fluorescence from treated cells.

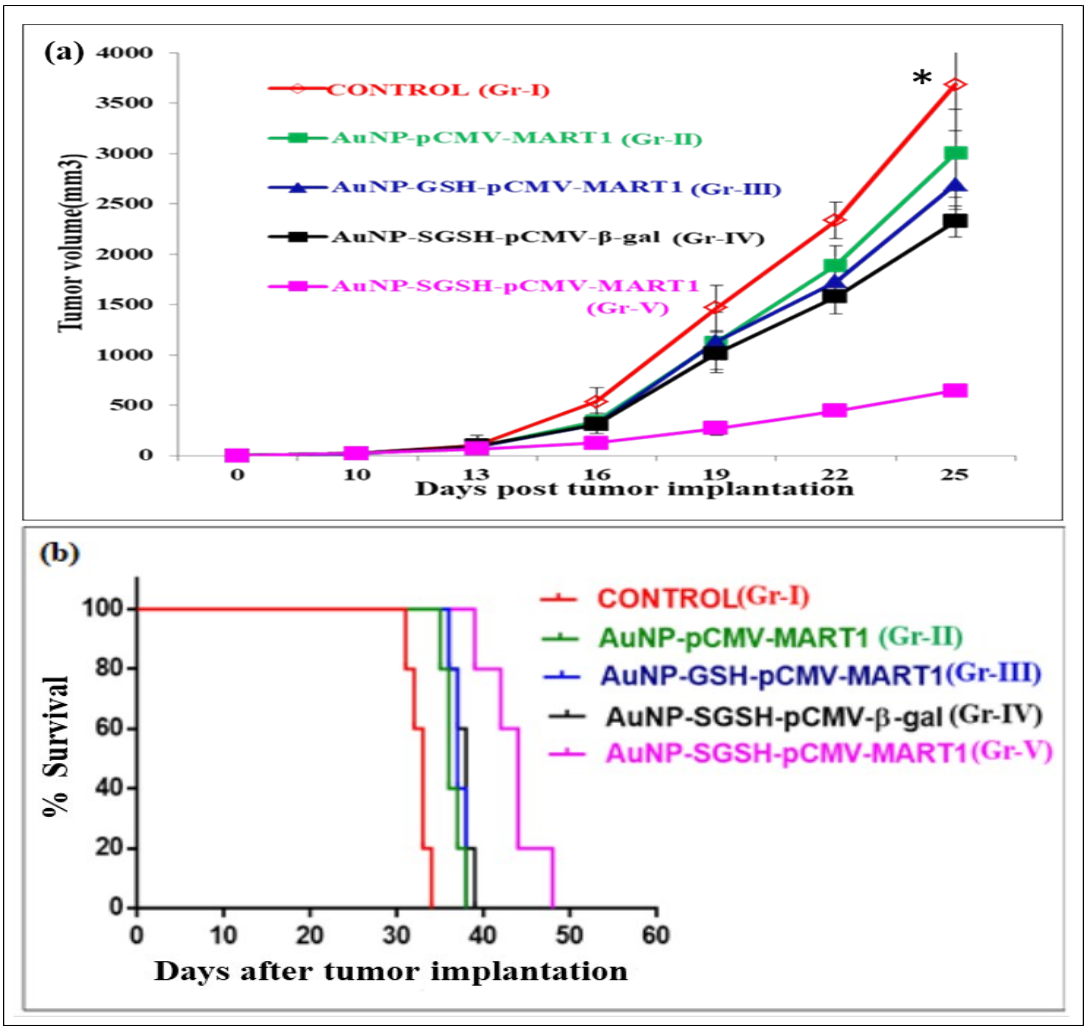


Fig. S31. (**a**) 6-8 weeks old female C57BL/6J mice (each weighing 20-22 g) were injected with ~1 x 10^5 B16F10 cells subcutaneously in the right flank. On day 12, mice were randomly sorted into four groups (n = 5) and each group was immunized subcutaneously with one of the following: nanoplexes of AuNP & pCMV-MART1 (green, Gr- **II**); AuNP-GSH & pCMV-MART1 (blue, Gr- **III**); AuNP-SGSH & pCMV-β-gal (black, Gr- **IV**); and AuNP-SGSH & p-CMV-MART1 (pink, Gr- **IV**). One group was injected with 5% glucose (vehicle control, red, Gr- **I**). This treatment was repeated on days 15 and 18 post tumor cell implantation. Tumor volumes (V = 1/2.ab^2 where, a = maximum length of the tumor and b = minimum length of t

tumor measured perpendicular to each other) were measured with a slide caliper for up to 28 days (*P< 0.05 vs. AuNP-SGSH&p-CMV-MART1). (**b**) Survival study graph.