Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information and Figures

A Disulfide Based Low Molecular Weight Gel for a Selective Sustained Release of Biomolecules

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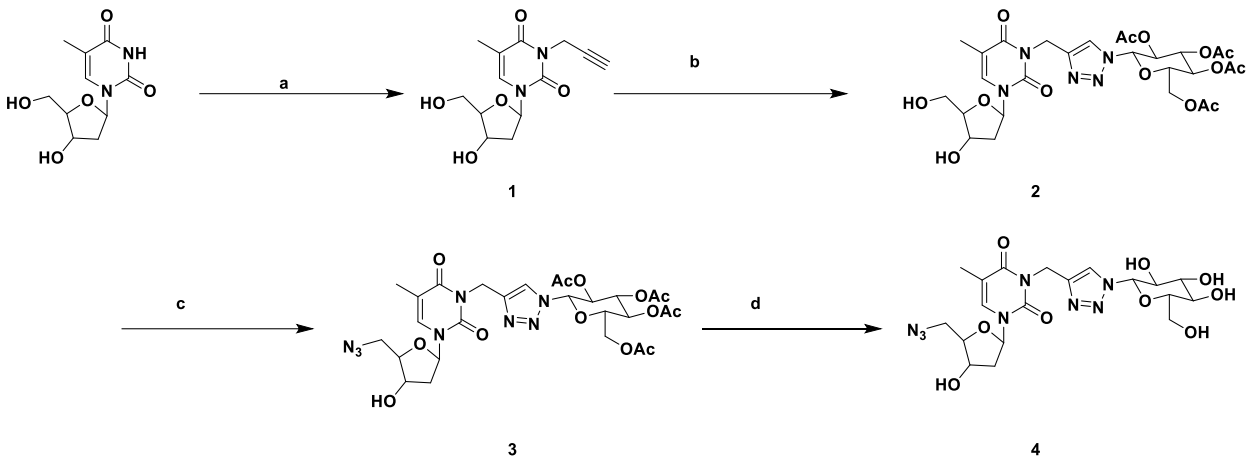
Contents

SUPPORTING INFORMATION SI 1

Synthesis of compounds

The compound **1** was synthesized by literature procedures[1]

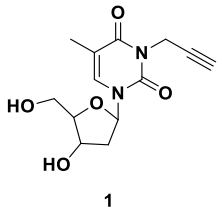
Scheme 1:



Scheme 1. Reagents and conditions: **(a)** K_2CO_3 (1.5 equiv), propargyl bromide (1.5 equiv), DMF, rt; (95%) **(b)** 1-azido-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (1 equiv), $CuSO_4.5H_2O$ (0.1 equiv), sodium ascorbate (0.2 equiv), *t*BuOH/water (1:1), 75°C, 20 h; (68%) **(c)** PPh_3 (1.2 equiv), CBr_4 (3 equiv), NaN_3 (5 equiv), DMF; (73%) **(d)** NaOMe 1M, MeOH (97%)

Synthesis of compound 4

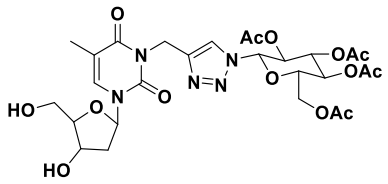
Synthesis of N-3-Propargylthymidine **(1)**



Propargyl bromide 3.68 g 80% soln. in toluene (2.94 mL, 31 mmol) was added to a mixture of thymidine (5 g, 20.65 mmol), potassium carbonate (4.28 g, 31 mmol) in anhydrous DMF (75 mL). The reaction mixture was stirred for two days at room temperature. DMF was removed to dryness under reduced pressure, water was added to the solid residue, and the product was

extracted with ethyl acetate. The organic layer was separated, washed with brine, dried (Na_2SO_4), evaporated and purified by eluting ethyl acetate: pet. ether (8:2 to 100:0) to give yellow oil 4.75 g (95%). **^{1}H-NMR ($CDCl_3$, 300 MHz)** δ 7.58-7.57 (d, J = 3 Hz, 1H, H-6 thymine), 6.29-6.25 (t, J = 6 Hz, 1H, H-1'), 4.68-4.67 (d, J = 3 Hz, 2H, Propaargyl), 4.56-4.52 (m, 1H, H-3'), 4.02-3.98 (m, 1H, H-4'), 3.92-3.80 (m, 2H, H-5'), 2.41-2.25 (m, 2H, H-2'), 2.20-2.18 (t, 1H, J = 3 Hz, CH-Propargyl), 1.93-192 (d, 3H, J = 3 Hz, CH_3-Thymine).

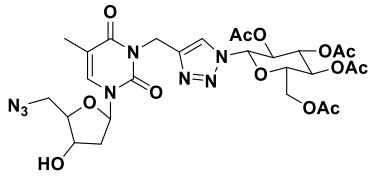
Synthesis of N-*3-[1-((β-D-Glucopyranosidetetraacetate)-1*H-*1,2,3-triazol-4-yl) methyl] thymidine **(2)***



2

Copper sulfate (0.677 g, 2.7 mmol) and sodium ascorbate (1.08 g, 5.43 mmol) were successively added to the suspension of compound **1** (7.6 g, 27.13 mmol) and 1-deoxy-1-azido-2, 3, 4, 6-tetra-*O*-acetyl glucopyranose (10.12 g, 27.13 mmol) in 160 mL of $_t$BuOH/H_2O (1:1). The mixture was stirred at 75 $^\circ$C overnight. Next day, after cooling to room temperature, the solvents were removed under reduced pressure and extracted with DCM and washed with water. Dried over Na_2SO_4 and concentrated. Crude product was purified by using MeOH:ethylacetate (2:98) obtain the desired compound **2 (**12 g, 68%) **^{1}H NMR ($CDCl_3$, 300 MHz) δ** 7.93 (s, 1H, H triazole), 7.63 (s, 1H, H-6 thymine), 6.28-6.24 (t, *J*= 6 Hz, 1H, H-1'), 5.89-5.86 (d, *J*= 9 Hz, 1H, H-1), 5.51-5.45 (t, *J*= 9 Hz, 1H, H-2), 5.44-5.38 (t, *J*= 9 Hz, 1H, H-3), 5.29-5.14 (m, 3H, H-4, NCH_2 triazole), 4.57-4.53 (m, 1H, H-3'), 4.31-4.12 (m, 2H, H-5' or H-6), 4.06-3.98 (m, 2H, H-4', H-5), 3.84-3.81 (m, 4H, H-5' or H-6), 2.39-2.23 (m, 2H, H-2'), 1.89 (s, 3H, CH_3 thymine), 2.07, 206, 2.01, 1.84 (s, 12H, 4 CH_3(C=O)); **^{13}C NMR ($CDCl_3$, 75 MHz) δ** 170.71, 170.03, 169.42, 168.98 (C=O acetate), 163.2, 150.69 (C=O thymine), 143.61 (Cq triazole), 135.25 (C-6 thymine), 122.88 (CH triazole), 110.01 (C-5 thymine), 87.17, 86.41 (C-4', C-1'), 85.56 (C-1), 74.96 (C-5), 72.68, 70.79, 70.21 (C-3', C-3, C-2), 67.63 (C-4), 61.92, 61.57 (C-6, C-5'), 40.20 (C-2'), 35.78 (triazole CH_2N thymine), 20.71-20.17 (**C**H_3(C=O)), 13.17 (CH_3 thymine).

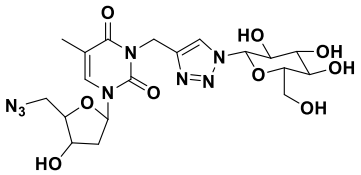
Synthesis of 5'-Deoxy-N-3-[1-((β-D-glucopyranosidetetraacetate)-1H-1,2,3-triazol-4-yl) methyl] azidothymidine **(3)**



3

The compound **2** (7.78 g, 11.90 mmol) was dissolved in dry DMF (40 mL). Triphenylphosphine (3.75 g, 14.28 mmol), sodium azide (3.87 g, 60 mmol), and carbon tetra-bromide (4.74 g, 14.28 mmol) were added in above reaction mixture. The reaction mixture was stirred at room temperature for 24 h. DMF was evaporated to dryness. The resulting residue was dissolved and extracted with dichloromethane. Combined organic layer was washed with water and dried over Na_2SO_4, and concentrated under a reduced pressure and was purified by column chromatography, eluting ethyl acetate and petroleum ether (7:3 to 100%) to obtain the compound **3** as white foam 5.85 g (73%) **$_1$H NMR (300 MHz, $CDCl_3$) δ** 7.89 (s, 1H, H triazole), 7.50-7.39 (m, 1H, H-6 thymine), 6.40-6.33 (m, 1H, H-1'), 5.88-5.85 (d, *J*= 9 Hz, 1H, H-1), 5.50-5.44 (t, *J*= 9 Hz, 1H, H-2), 5.44-5.37 (t, *J*= 9 Hz, 1H, H-3), 5.32-5.16 (m, 4H, OH(C3'), H-4, CH_2N triazole), 4.51-4.44 (m, 1H, H-5), 4.32-4.26 (m, 1H, H-3'), 4.21-3.98 (m, 3H, H-6, H4') 3.77- 3.58 (m, 2H, H-5'), 2.38-2.45 (m, 1H, H-2'b), 2.30-2.17 (m, 1H, H-2'a), 1.95 (s, 3H, CH_3 thymine), 2.09, 2.06, 2.02, 1.84 (s, 12H, 4 CH_3(C=O)); **$_{13}$C NMR (75 MHz, $CDCl_3$)** δ 170.64, 170.01, 169.38, 168.83 (C=O acetate), 162.97, 150.56 (C=O thymine), 143.65 (Cq triazole), 134.04 (C-6 thymine), 122.61 (CH triazole), 110.36 (C-5 thymine), 85.49, 85.44 (C-4', C-1'), 84.47 (C-1), 74.93 (C-5), 72.69, 71.20, 70.15 (C-3', C-3, C-2), 67.61(C-4), 61.54, 52.21 (C-6, C-5'), 40.19 (C-2'), 35.89 (triazole CH_2N thymine), 20.70-20.15 (**C**H_3(C=O)), 13.28 (CH_3 thymine).

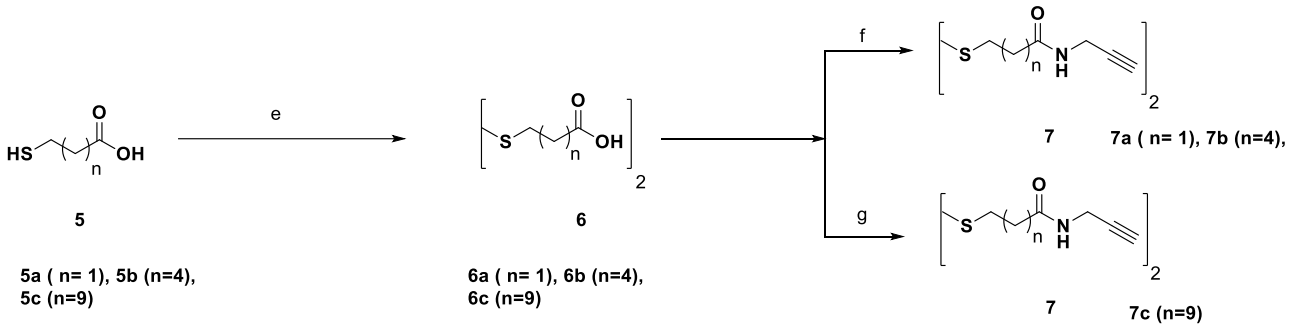
Synthesis of 5'-Deoxy-N-3-[1-((β-D-glucopyranoside)-1H-1,2,3-triazol-4-yl)methyl]azido thymidine **(4)**



4

The stirred solution of compound 3 (1.5 g, 2.21 mmol) in anhydrous methanol (20 mL) was treated with freshly prepared 1N sodium methoxide solution at room temperature. After 1h stirring, Amberlite IRC-50 ion exchange resin was added to the reaction mixture and stirred for 30 min and filtered. After filtration, the solvent was removed under reduced pressure. The solid residue was applied to a column of silica gel, and the product eluted with DCM:MeOH (100 to 8:2) to afford the title compound 4 as a white amorphous solid 1.1 g (97%).$_1$H NMR (300 MHz, D_2O) δ 8.06 (s, 1H, H triazole), 7.47 (s, 1H, H-6 thymine), 6.21-6.21 (m, 1H, H-1'), 5.61-5.57 (m, 1H, H-1), 5.11 (s, 2H, CH_2-triazole), 4.40–4.33 (m, 1H, H-3'), 4.0-3.92 (m,1H, H4'), 3.83-3.73 (m, 2H, H2, H6b), 3-68-3.39 (m, 5H, H-3, 4, 5 + H-5', H-6a), 2.43-2.20 (m, 2H, H-2'b, H-2'a), 1.80 (s, 3H, CH_3 thymine); $_{13}$C NMR (75 MHz, D_2O) δ 164.88, 151.37 (C=O thymine), 143.03 (Cq triazole), 135.82 (C-6 thymine), 123.74 (CH triazole), 110.76 (C-5 thymine), 87.40, 86.12 (C-4', C-1'), 84.44 (C-1), 78.75 (C-5), 75.76, 72.18, 70.72, (C-3', C-3, C-2), 68.81 (C-4), 60.30, 51.44, (C-6, C-5'), 37.74 (C-2'), 36.14 (triazole CH_2N thymine), 12.14 (CH_3 thymine).
$_1$H-NMR

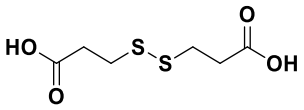
Scheme 2:



Scheme 2. Reagents and conditions: **(e)** SO_2Cl_2 (0.5 equiv), CH_2Cl_2, 0 °C, 1 h; **(f)** DCC (2.5), HOBt (1.5), propagylamine (2.5 equiv), DMF, rt, overnight **(g)** DIPEA (4 equiv), HATU (3 equiv), propagylamine (4 equiv), DMF, rt, overnight;

The compound **6** was synthesized by literature procedures[2]

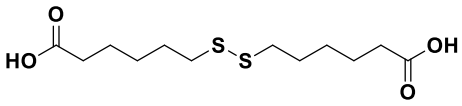
3, 3'-disulfanediyldipropionic acid **(6a)**



6a

To the stirred solution of 3-thiopropionic acid (1.2 g, 11.30 mmol) dissolved in 30 ml of dichloromethane (cooled at 0 °C) was added slowly 12 mL of dichloromethane containing sulfuryl chloride (0.762 g, 5.65 mmol) and the reaction mixture was stirred at 0 °C for 1h. After 60 min, the reaction was quenched by adding water and concentrated. The precipitation was filtered and washed three times with water, giving 3, 3'-disulfanediyldipropanoic acid **6a** as a white solid 0.908 g (38%). **$_1$H NMR (300 MHz, DMSO-d$_6$)** δ 12.35 (bs, 2H, -COOH), 2.90-2.85 (t, J = 9 Hz, 4H, -CH$_2$-C=O), 2.64-2.69 (t, J = 9 Hz, 4H, -CH$_2$-S-)

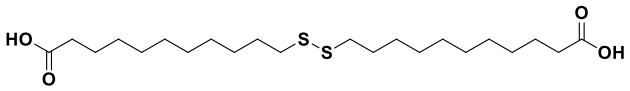
6, 6'-disulfanediyldihexanoic acid **(6b)**



6b

To a stirred solution of 6-mercaptohexanoic acid (0.5 g, 3.37 mmol) dissolved in 10 ml of dichloromethane (cooled at 0 °C) was added slowly a solution of sulfuryl chloride (0.23 g, 1.68 mmol) dissolved in dichloromethane (3 mL) and reaction mixture was stirred at 0 °C for 1h. After 60 min, the reaction was quenched by adding water and concentrated. The precipitation was filtered and washed three times with water, giving 6,6'-disulfanediyldihexanoic acid **6b** as a white solid 0.490 g (49%). **$_1$H-NMR (300 MHz, DMSO-d$_6$)** δ 11.99 (s, 2H, -COOH), 2.72-2.67 (t, 3H, J = 9 Hz, -CH$_2$-S-), 2.36 (t, 1H, J = 6Hz, **-CH$_2$**-S-), 2.22-2.18 (t, 4H, **-CH$_2$**-C=O), 1.66-1.29 (m, 12H, -S-CH$_2$-**CH$_2$-CH$_2$-CH$_2$-**CH$_2$-C=O).

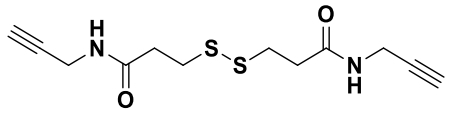
11, 11'-Disulfanediyldiundecanoic acid (6c)



6c

To the cold (0 °C) and a stirred solution of 11-mercaptoundecanoic acid (5 g, 22.90 mmol) dissolved in 50 ml of dichloromethane was added slowly 25 mL of dichloromethane containing (1.5 g, 11.45 mmol) of sulfuryl chloride. After 60 min, the reaction was quenched by adding water and concentrated. The precipitation was filtered and washed three times with water, giving 11, 11'-disulfanediyldiundecanoic acid **6c** as a white solid 4.9 g (98%). **^{1}H NMR (300 MHz, DMSO-d$_6$)** δ 2.70-2.65 (t, 4H, J = 9Hz, 4H, -CH$_2$-S-), 2.20-2.15 (t, J = 9Hz, 4H, -CH$_2$-C=O), 1.65–1.56 (m, 8H), 1.36–1.25 (m, 24H); **^{13}C NMR (75 MHz, DMSO-d$_6$)** δ 174.91 (C=O), 38.40 (-**C**H$_2$-S-), 34.11 (-**CH$_2$**-C=O), 29.35, 29.23, 29.05, 29.0 (-S-CH$_2$-CH$_2$-**CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-** CH$_2$-CH$_2$-C=O), 28.22 (-S-CH$_2$-**CH$_2$**-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-C=O), 24.96 (-S-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$- **CH$_2$**-CH$_2$-C=O),

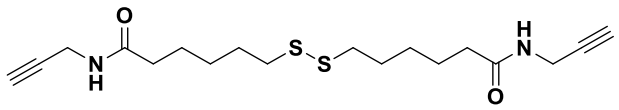
3,3'-disulfanediylbis(N-(prop-2-yn-1-yl)propanamide) **(7a)**



7a

Compound **6a** (0.722 g, 3.44 mmol), DCC (1.77 g, 8.6 mmol), and HOBt (0.7 g, 5.16 mmol) were dissolved in DMF (15 mL) and stirred for 10 min. Propargylamine (0.472 g, 8.6 mmol) was added dropwise to the reaction mixture and stirred overnight at room temperature. Next day, DCU was removed by filtration. The filtrate was concentrated under vacuum and the crude product was dissolved in ethyl acetate. The organic layer was washed with sat NaHCO$_3$, water and dried over sodium sulfate. The solvent was eliminated under vacuum and the crude product was purified by using ethyl acetate and petroleum benzine (7:3 to 100 % ethyl acetate) to afford the pure compound as a white powder **7a** (0.7 g, 71%).**^{1}H NMR (300 MHz, CD$_3$OD)** δ 3.98-3.97(d, J = 3 Hz, 4H, Propargyl), 2.98-2.93 (t, J = 9 Hz, 4H, -CH$_2$-C=O), 2.64-2.69 (m, 6H,CH-propargyl & -CH$_2$-S-); **^{13}C NMR (75 MHz, CD$_3$OD)** δ 171.91 (C=O), 79.06 (-CH$_2$-**C**≡CH), 70.80 (-CH$_2$-C≡**C**H),

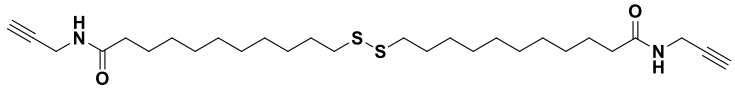
6,6'-disulfanediylbis(N-(prop-2-yn-1-yl)hexanamide) **(7b)**



7b

Compound **6b** (0.49 g, 1.66 mmol), DCC (0.86 g, 4.15 mmol) and HOBt (0.34 g, 2.49 mmol) were dissolved in DMF (22 mL) and stirred for 10 min. Propargylamine (0.23 g, 4.15 mmol) was added dropwise to the reaction mixture and stirred overnight at room temperature. Next day, DCU was removed by filtration. The filtrate was concentrated under vacuum and the crude product was dissolved in ethyl acetate. The organic layer was washed with sat $NaHCO_3$, water and dried over sodium sulfate. The solvent was evaporated under vacuum and the crude product was purified by using ethyl acetate and petroleum benzine (7:3 to 100 % ethyl acetate) to afford the pure compound as a white powder **7b** (0.450 g, 73%).**$_1$H-NMR (300 MHz, CD_3OD)** δ 3.96-3.95 (d, J = 3 Hz, 4H, Propargyl), 2.68-2.73 (t, J = 6 Hz, 4H, -CH_2-C=O), 2.60-2.58 (t, J = 3Hz, 2H,CH-propargyl), 2.25-2.20 (t, 4H, J = 6Hz, CH_2-S-), 1.77-1.60 (m, 8H, -S-CH_2-**CH_2**-CH_2-**CH_2**-CH_2-C=O), 1.49-1.39 (m, 4H, -S-CH_2-CH_2-**CH_2**-CH_2-CH_2-C=O), **$_{13}$C-NMR (75 MHz, CD_3OD)** δ 174.24 (C=O), 79.26 (-CH_2-**C**≡CH), 70.64 (-CH_2-C≡**CH**), 38.09 (-**CH_2**-C=O), 35.21 (-S-**CH_2**), 28.46 (-**CH_2**-C≡CH), 27.96 (-S-CH_2-**CH_2**-CH_2-CH_2-CH_2 C=O), 27.53 (-S-CH_2-CH_2-CH_2-**CH_2**-CH_2-C=O), 25.0 (-S-CH_2-CH_2-**CH_2**-CH_2-CH_2-C=O).

Bis-[N-(propynyl)-11,11'-disulphanediyl diundecanoic acid diamide **(7c)$_3$**

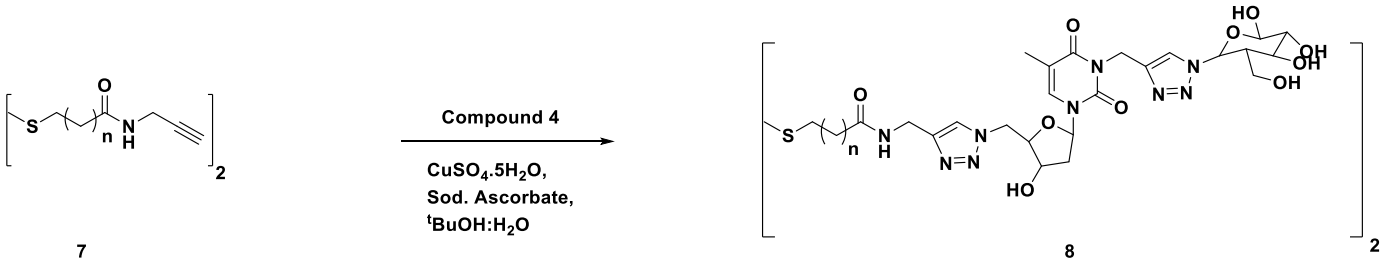


7c

To an ice-cold solution of dicarboxylic acid **6c** (2 g, 4.6 mmol) in dry DMF (50 mL) DIPEA (2.38 g, 18.4 mmol) was added and the reaction mixture stirred for 5 min. Afterwards, HBTU (5.25 g, 13.8 mmol) was added and after an additional 30 min of stirring, propargylamine (1.01 g 18.4 mmol), dissolved in 16 mL dry DMF, was added dropwise. Then the reaction mixture was stirred for overnight at room temperature. The precipitated product was subsequently filtered and washed with ice-cold DMF, dried to obtain the desired compound **7c** (1.2 g, 51%); **$_1$H NMR (300 MHz, DMSO-d_6)** δ 8.21 (s, 2H, 2 NH), 3.83 (d, 4H, J = 3 Hz, 4H, Propargyl), 3.07 (s, 2H,

-CH_2-C≡**CH**), 2.20-2.66 (t, 4H, J = 6 Hz, -S-CH_2), 2.08-2.06 (t, 4H, J = 9 Hz, -CH_2-C=O), 1.65 (m, 4H, -S-CH_2-**CH_2**), 1.49-1.45 (m, 4H, -**CH_2**-CH_2-C=O), 1.33- 1.23 (m, 24H, -S-CH_2-CH_2-**CH_2-CH_2-CH_2-CH_2-CH_2-CH_2**-CH_2-CH_2-C=O); **^{13}C-NMR (75 MHz, DMSO-d_6) δ** 172.36 (C=O), 81.83 (-CH_2-**C**≡CH), 72.95 (-CH_2-C≡**CH**), 38.62 (-S-CH_2), 35.58 (-**CH_2**-C=O), 29.28, 29.26, 29.15, 29.06, 28.98 (-S-CH_2-**CH_2**-CH_2-**CH_2-CH_2-CH_2-CH_2-CH_2**-CH_2-CH_2-C=O) 28.20 (-S-CH_2-CH_2-**CH_2**-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-C=O, -**CH_2**-C≡CH), 25.55 (-**CH_2**-CH_2-C=O).

Scheme 3:



7

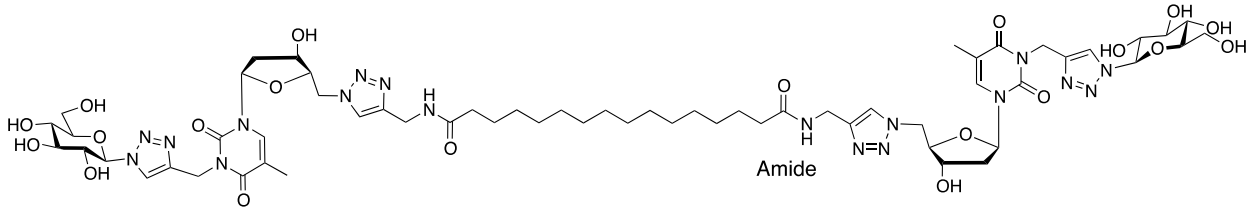
7a (n= 1), 7b (n=4),
7c (n=9)

8

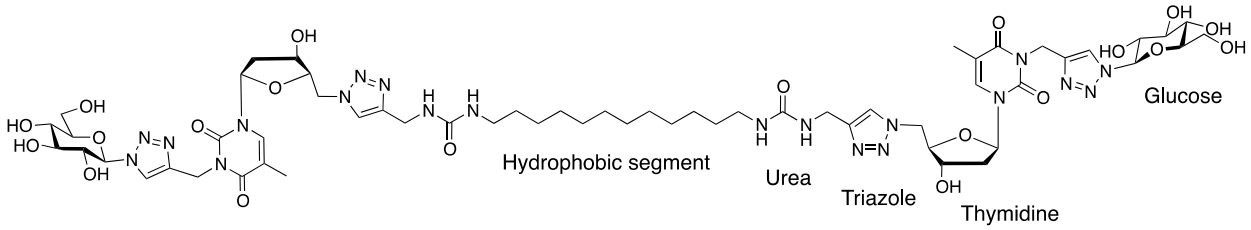
8a (n= 1), 8b (n=4),
8c (n=9)

Scheme 3. Reagents and conditions: (a) N,N'-dipropargyldisulfidediamide), $CuSO_{4.5}H_2O$ (0.2 equiv), sodium ascorbate (0.4 equiv), $_t$BuOH/water (1:1), 79°C, overnight

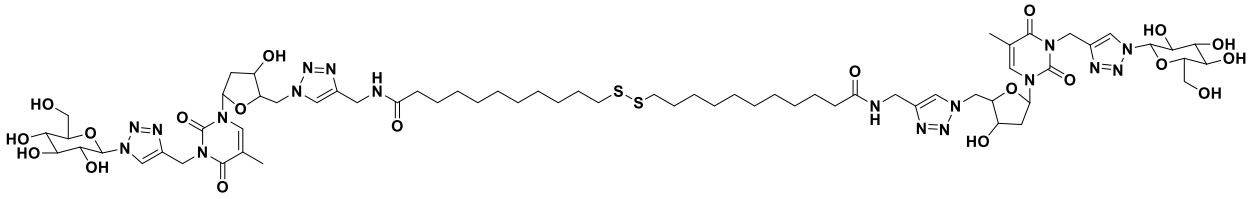
Scheme 4:



GNBA-3



GNBA-1

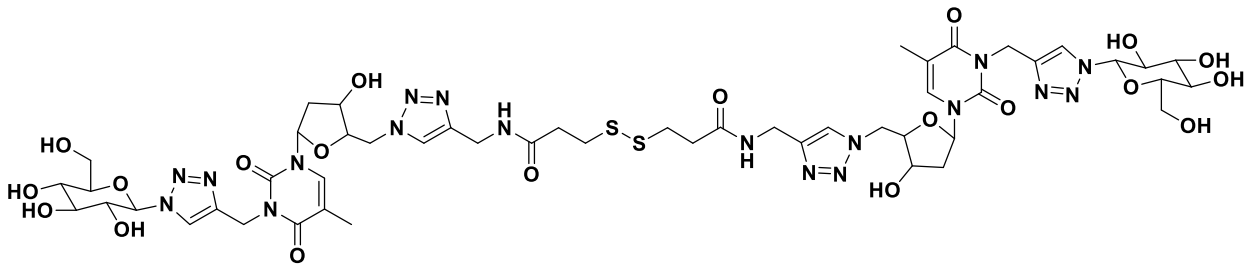


8c

SS-GNBA-3

Scheme 4. Chemical structures of the non-disulfide GNBA derivatives including bis amide MR73, bis Urea MR181 and disulfide GNBA **3**. The latter is a new compound (compound 8c), whereas the non-disulfide controls (MR 73 and MR181) have been reported by our group previously see reference SI1.

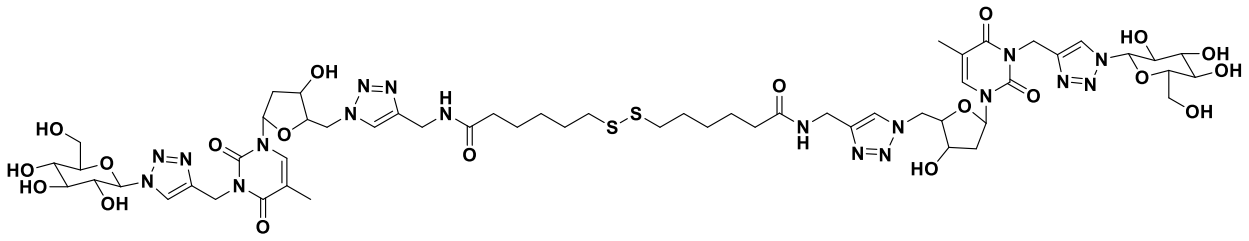
Synthesis of the compound (**8a) (SS-GNBA-1)**



8a

Calculated amount of Copper sulfate and sodium ascorbate, were successively added to a suspension of alkyne **7a** (*1*) and azide **4** in 22 mL of H_2O/^{t}BuOH (1:1). The mixture was stirred at 75 °C for 16h. Next day, after cooling the reaction mixture to room temperature; the solvents were removed under reduced pressure. The solid was dissolved in 50 ml of warm methanol and filtered to remove the insoluble byproduct. Filtrate was evaporated and dissolved in mixture of the methanol and dichloromethane (40%) and purified by eluting with MeOH/CH_2Cl_2 (3:7 to 4:6) to obtain compound **8a** as a white amorphous solid (0.2 g, 28%); **^{1}H NMR (300 MHz, D_2O)** **δ** 8.05 (s, 2H, 2 H triazole), 7.74 (s, 2H, 2 H triazole), 6.95 (s, 2H, 2 H-6 thymine), 6.08-6.04 (t, *J*= 6 Hz, 2H, 2 H-1'), 5.55-5.52 (d, *J*= 9 Hz, 2H, 2 H-1), 5.03 (s, 4H, 2 triazole CH_2N thymine), 4.67-4.51 (m, 4H, 2 H-5'), 4.34-4.18 (m, 6H, 2 H-3', 2 triazole CH_2 NH(C=O)), 4.18-4.08 (m, 2H, 2 H-4'), 3.86-3.73 (m, 4H, 2 H-2, 2 H-6B), 3.65-3.45 (m, 8H, 2 H-3, 2 H-4, 2 H-5, 2 H-6a), 2.62-2.58 (t, *J* = 6 Hz, 4H, CH_2-S), 2.38-2.34 (t, *J* = 6 Hz, 4H, -CH_2-C=O), 2.27-2.07 (m, 4H, 2 H-2'), 1.73 (s, 6H, 2 CH_3 thymine); **^{13}C NMR (75 MHz, D_2O)** δ 173.65 (C=O amide), 164.58, 151.07 (C=O thymine), 144.67, 142.87 (Cq triazole), 135.61 (C-6 thymine), 124.95, 123.90 (CH triazole), 110.56 (C-5 thymine), 87.42 (C-1), 86.0 (C-1'), 83.18 (C-4'), 78.77 (C-5), 75.80 (C-3), 72.19 (C-2), 70.30 (C-3'), 68.81 (C-4), 60.30 (C-6), 50.88 (C-5'), 37.53 (C-2'), 36.06, 34.40 (triazole-**CH_2**N-thymine, CH_2C=O), 34.31(triazole **CH_2**-NH(C=O)), 33.20 (CH_2-S-), 12.24 (CH_3 thymine); HRMS (m/z): Calculated $[M+Na]^+$ ($C_{50}H_{68}N_{18}O_{20}S_2Na$) 1327.4196, found 1327.4157.

Synthesis of the compound (**8b) (SS-GNBA-2)**

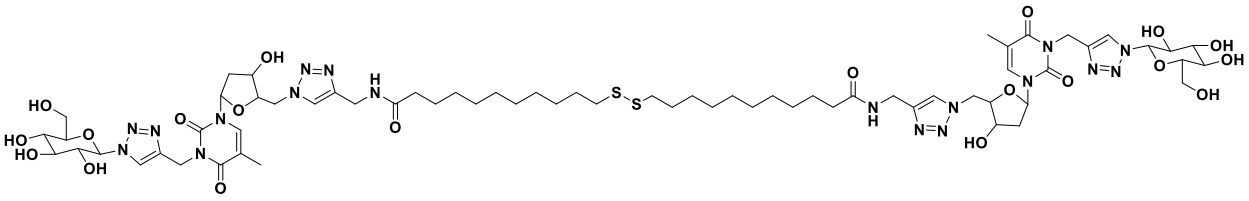


8b

Calculated amount of Copper sulfate (0.027 g, 0.108 mmol) and sodium ascorbate (0.043 g, 0.22 mmol were successively added to a suspension of alkyne **7b** (0.2 g, 0.54 mmol) and azide **4** (0.56 g, 1.08) in 20 mL of H_2O/^{t}BuOH (1:1). The mixture was stirred at 75 °C for 16h. Next day, after cooling the reaction mixture to room temperature; the solvents were removed under reduced

pressure. The solid was dissolved in 50 ml of warm methanol and filtered to remove the insoluble byproduct. Filtrate was evaporated and dissolved in mixture of the methanol and dichloromethane (40%) and purified by eluting with MeOH/CH_2Cl_2 (3:7 to 4:6) to obtain compound **8b** as a white amorphous solid (0.35 g, 46%). **$_1$H NMR (300 MHz, DMSO-d$_6$) δ** 8.31-8.28 (t, *J* = 6, 2H, 2 NH-amide), 8.12 (s, 2H, 2 H triazole), 7.91 (s, 2H, 2 H triazole), 7.50 (s, 2H, 2 H-6 thymine), 6.26-6.21 (t, *J* = 6 Hz, 2H, 2 H-1'), 5.76-5.00 (m, 6H, 2 H-1, 4H, 2 triazole CH_2N thymine), 4.28-4.07 (m, 4H, 2 H-5'), 3.78-3.64 (m, 4H, 2 triazole CH_2 NH(C=O)), 3.78-3.64 (m, 4H, 2 H-3', 2 H-4'), 3.47-3.33 (m, 8H, 2H-2, 2 H-6B, 2 H-3), 3.24-3.17 (m, 4H, 2 H-4, 2 H-5, 2 H-6a), 2.70-2.65 (t, *J* = 6 Hz, 4H, -CH_2-C=O), 2.27-2.14 (m, 4H, 2 H-2'), 2.11-2.06 (t, *J* = 9 Hz, 4H, CH_2-S), 1.89 (s, 6H, 2 CH_3 thymine), 1.65-145 (m, 8H, -S-CH_2-**CH_2**-CH_2-**CH_2**-CH_2-C=O), 1.36-1.24 (m, 4H, -S-CH_2-CH_2-**CH_2**-CH_2-CH_2-C=O); **$_{13}$C NMR (75 MHz, DMSO-d$_6$)** δ 172.44 (C=O amide), 162.75, 150.71 (C=O thymine), 145.55, 143.05 (Cq triazole), 135.52 (C-6 thymine), 123.94, 122.93 (CH triazole), 109.56 (C-5 thymine), 87.87 (C-1), 85.60 (C-1'), 84.65 (C-4'), 80.41 (C-5), 77.45 (C-3), 72.34 (C-2), 71.18 (C-3'), 69.98 (C-4), 61.13 (C-6), 51.55 (C-5'), 38.09 (C-2'), 35.50, 34.53 (triazole CH_2N-thymine, **C**H_2C=O), 28.76 (triazole CH_2 NH(C=O)), 37.89 (CH_2-S-), 25.25 (-S-CH_2-**CH_2**-CH_2-**CH_2**-CH_2-C=O), 19.59 (-S-CH_2-CH_2-**CH_2**-CH_2-CH_2-C=O), 13.18 (CH_3 thymine); HRMS (m/z): Calculated $[M+Na]^+$ ($C_{56}H_{80}N_{18}O_{20}S_2Na$) 1411.5135, found 1411.5071

Synthesis of the compound (**8c) (SS-GNBA-3)**



8c

Calculated amount of Copper sulfate (0.013 g, 0.05 mmol) and sodium ascorbate (0.020 g, 0.1 mmol), were successively added to suspension of alkyne **7c** (0.126 g, 0.25 mmol) and azide (0.253g 0.50 mmol) in 10 mL of H_2O/tBuOH (1:1) in the 50 mL of RBF. The mixture was stirred at 78 ℃ for 16 h. Next day, after cooling the reaction mixture to room temperature; the solvents

were removed under reduced pressure. The solid was dissolved in 20 ml of warm methanol and filtered to remove the insoluble byproduct. Filtrate was evaporated and dissolved in 40% (MeOH:DCM). Compound was purified by using column chromatography eluting with CH_2Cl_2/MeOH (7:3 to 6:4) to obtain compound as a white amorphous solid 8c (0.16 g, 42%);**^{1}H NMR (300 MHz, DMSO-d$_6$) δ** 8.28-8.25 (t, *J* = 3, 2H, 2 NH-amide), 8.11 (s, 2H, 2 H triazole), 7.89 (s, 2H, 2 H triazole), 7.49 (s, 2H, 2 H-6 thymine), 6.25-6.21 (t, *J* = 6 Hz, 2H, 2 H-1'), 5.54-5.00 (m, 14H, 2 H-1, 2 triazole CH_2N thymine, 8 OH $_{sugar}$), 4.73-4.57 (m, 6H, 2 H-5', 2 3'-OH), 3.78-3.64 (m, 4H, 2 triazole CH_2 NH(C=O)), 3.78-3.64 (m, 4H, 2 H-3', 2 H-4'), 3.47-3.32 (m, 8H, 2H-2, 2 H-6B, 2 H-3), 3.24-3.17 (m, 4H, 2 H-4, 2 H-5, 2 H-6a), 2.71-2.66 (t, *J* = 6 Hz, 4H, -CH_2-C=O), 2.27-2.14 (m, 4H, 2 H-2'), 2.09-2.09 (t, *J* = 9 Hz, 4H, CH_2-S), 1.88 (s, 6H, 2 CH_3 thymine), 1.65-143 (m, 8H, -S-CH_2-**CH_2**, -**CH_2**-CH_2-C=O), 1.35-1.23 (m, 24H, 12 - CH_2-); **^{13}C NMR (75 MHz, DMSO-d$_6$)** δ 172.59 (C=O amide), 162.75, 150.71 (C=O thymine), 145.58, 143.05 (Cq triazole), 135.52 (C-6 thymine), 123.92, 122.93 (CH triazole), 109.56 (C-5 thymine), 87.88 (C-1), 85.63 (C-1'), 84.57 (C-4'), 80.42 (C-5), 77.45 (C-3), 72.35 (C-2), 71.18 (C-3'), 69.98 (C-4), 61.14 (C-6), 51.56 (C5'), 38.34 (C-2'), 35.57, 34.54 (triazole CH_2N-thymine, **CH_2**C=O), 28.20 (triazole CH_2 NH(C=O)), 36.59 (CH_2-S-), 25.56 (-S-CH_2-**CH_2**, **CH_2**-CH_2-C=O), 29-37-28.99, 28.20, 19.59 (CH_2), 13.15 (CH_3 thymine); HRMS (m/z): Calculated $[M+Na]^+$ ($C_{66}H_{100}N_{18}O_{20}S_2Na$) 1551.6700, found 1551.6620.

SUPPORTING INFORMATION SI 2

Gelation Test

The newly synthesized molecules were tested for the hydrogel information by dissolving the compounds SS-GNBA-1 (**8a**), -2 (**8b**) (2% w/v) and -3 (**8c**) **(0.5% w/v)** individually in microtubes using ultrapure water. Further, the solution was heated at 80°C with 1000 rpm until dissolution. Once the compound was completely dissolved, the solution was allowed to cool at room temperature to form the hydrogel. The formation of the hydrogel was confirmed by the inversion test (the sample does not flow under its own weight when the tube is turned upside-

down). The gel formation was not observed for compound SS-GNBA-1 (**8a**) nor for SS-GNBA-2 (**8b**)**,** which remained in solution.

For biological studies the molecule was dissolved in 1x PBS (phosphate buffer saline, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4) **and sterilized using 0.2 µm pore size filter before injection subcutaneously in mice.**

Mechanical property measurement of hydrogels

Rheological measurements were carried out on a Malvern Kinexus Proþ rheometer with steel plate-plate geometry (diameter: 20 mm). The lower plate is equipped with a Peltier temperature control system, and all samples were studied at 25 ± 0.01 °C unless otherwise indicated. All measurements were conducted with a 0.3 mm gap distance between the plates. A solvent trap was also used to ensure homogeneous temperature and to prevent water evaporation. The hydrogels were heated at 85 °C and the resulting liquid was immediately placed into the rheometer and subjected to sinusoidal oscillations. All experiments were carried out within the linear viscoelastic regime (LVER), in which the measured shear moduli (G', G'') are independent of the applied strain (i.e. without disruption of the gel structure). For this purpose, an amplitude strain sweep (from 0.01 to 100% at an angular frequency of 6.28 rad s^{-1}) was performed for each sample.

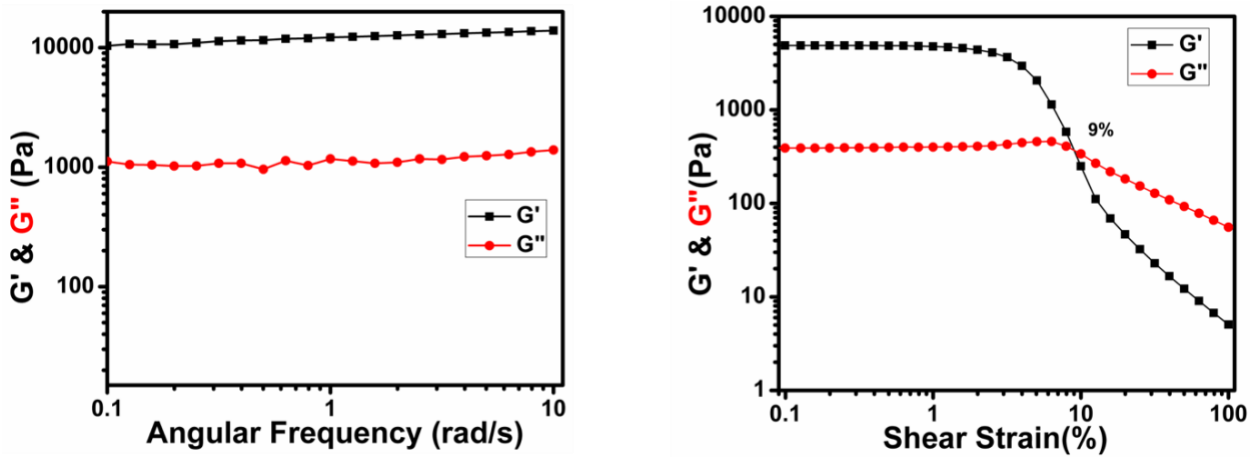


Figure S1. Oscillatory rheological properties of the SS-GNBA-3 hydrogel at 2% w/v. Left: frequency sweep results. Right: amplitude sweep results.

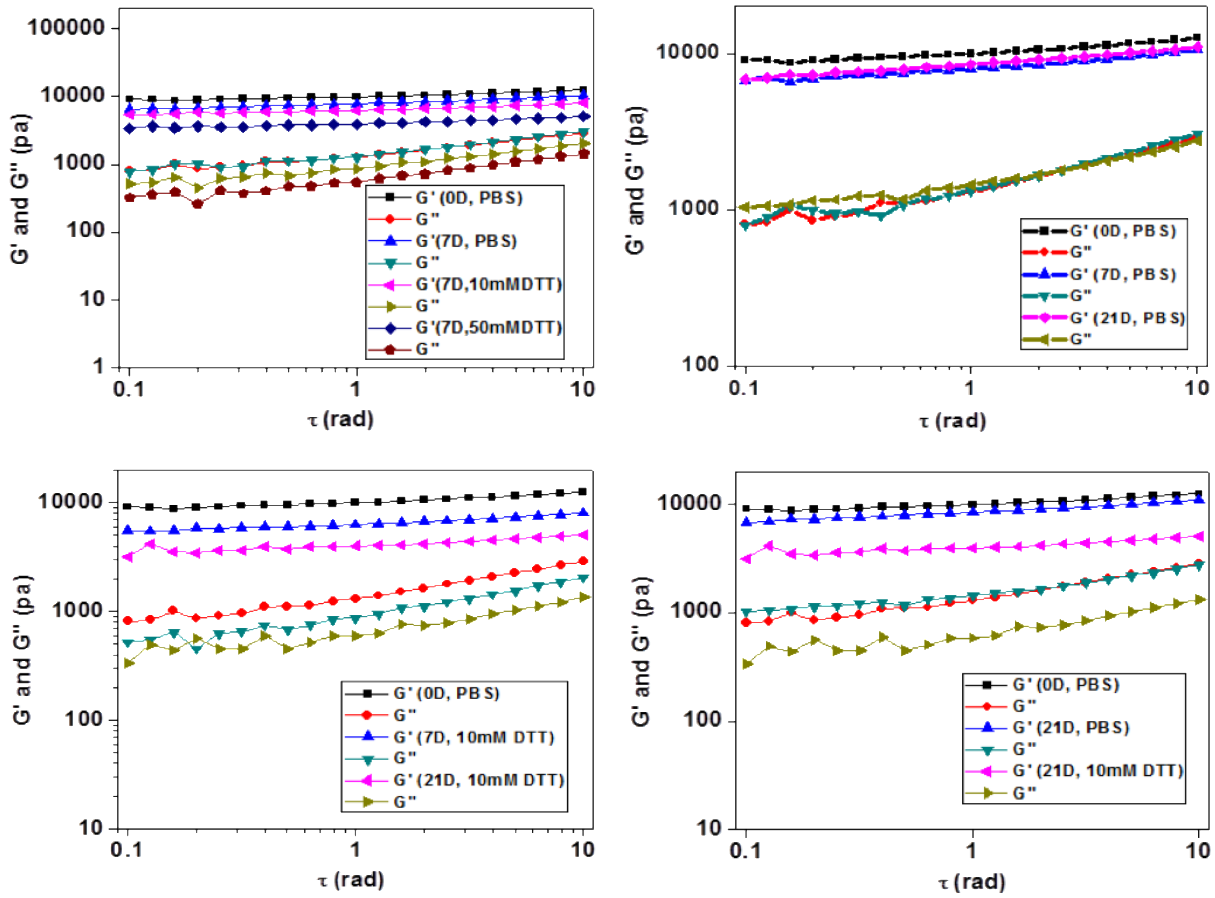
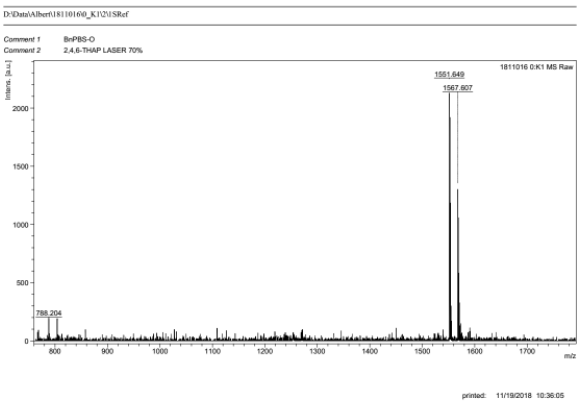
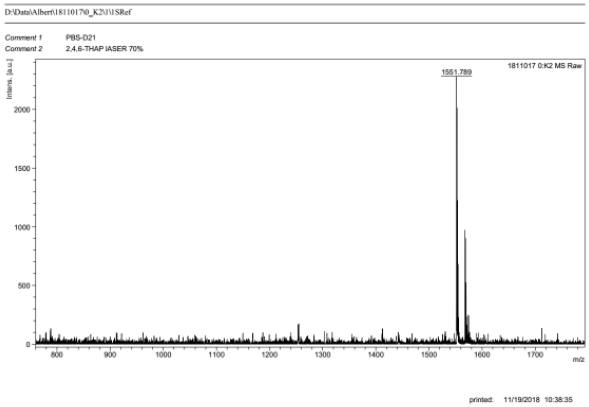


Figure S2. Rheological properties in reductive conditions. Comparison of G' and G'' of the hydrogel after incubation of PBS (pH7.2) and DTT (10mM and 50mM) solution for 0, 7 and 21 days (*quantity of the leftover hydrogel after incubating in the 50 mM DTT was negligible).

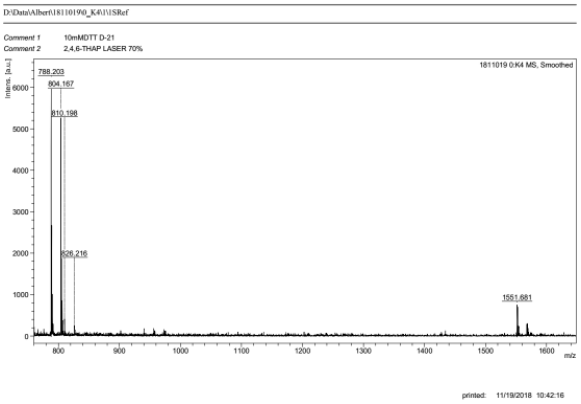
A) Day 0



B) Day 21, PBS



C) Day 21, 10 mM DTT



D) Day 21, 50 mM DTT

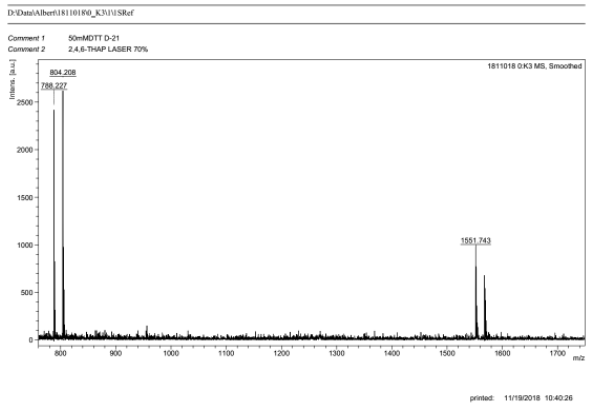


Figure S3. **MALDI-TOF analysis of products released from hydrogel SS-GNBA-3 after incubation in reductive conditions.** After the 21-day incubation in PBS **(B)**, 10 mM DTT **(C)** or 50 mM DTT **(D)**, mass spectrometry was performed and compared with the spectrum of the initial compound **(A)**.

SUPPORTING INFORMATION SI 3

In vitro studies

In vitro cytotoxicity assays.

Cell viability and metabolic activity were assessed by indirect contact with immortalized L929 cells. Hydrogel fragments of SS-GNBA-3 (10x100 µL) were incubated in 2.9 mL of α-MEM (Gibco, A10490-01, France). The medium containing compounds released from the hydrogel was recovered during a short washing ("24h(0)"), during the first 24 hours ("24h(1)"), and then during the two following 24 hours "24(2)" and "24(3)", or during 3 days ("72h"). L929 cells were then cultured during 24h at 37°C under 5% CO_2 atmosphere, in the presence of 100 µL of these media either pure ("100%") or diluted 2 times ("50%"), 10 times ("10%") or 100 times ("1%") in α-MEM, with a final concentration of 10% fetal calf serum (FCS). As a positive control of toxicity, cells were also grown with the medium containing 0.1 % (w/v) Triton-X100 ("toxic"). Viability (neutral red assay) and metabolic activity (MTT assay) are shown relative to cells grown in α-MEM containing 10% FCS. All the data are presented as mean ± s.d. (n=6). No toxicity with the media containing released compounds from the hydrogel was observed (viability and metabolic activity > 80%).

In vitro degradation studies

The *in vitro* degradation of the disulfide hydrogel was assessed by incubating the samples in 1x PBS, 10mM and 50mM DTT as a function of time. DTT was used as it accelerates the *in vitro* degradation by reducing the disulfide bonds. The di-sulfide hydrogel (2% w/v) was prepared as mentioned before. The hydrogel disks of 100 µL were incubated at 37°C for 21 days under a mild shaking condition (50rpm) in 1x PBS, whereas for DTT solution, incubation was at 25 °C. The hydrogel was weighed prior to the incubation (initial weight), and at the different time points the hydrogel was removed and weighed immediately after removing the excess water using KIM wipes. The final weight of the hydrogel was compared with the initial weight of hydrogel for the determination of percentage weight remaining.

In vitro release study

The hydrogel was prepared as mentioned before. The hydrogel was loaded with FITC-BSA and FITC-Dextran (Fisher Scientific) before hydrogel formation at a concentration of 1mg/mL.

150µL of the hydrogel containing FITC-BSA and FITC-dextran were pipetted into the inserts. The inserts were placed in 24 well plates containing 1x PBS at 37 °C in shaking incubator at 50 rpm. The medium was replaced with the fresh medium at predetermined times (1h, 2h, 4h, 20h, 24h, 48h). The amount of FITC-BSA and FITC-dextran in the release medium was measured by a plate reader at 491 nm (Victor, Perkin Elmer, USA). The cumulative release was calculated based on the initial release. The influence of disulfide during the release was assessed by comparing the release kinetics with Bolo bis amide without disulfide.

In vivo subcutaneous implantation or injection

For the measurement of *in vivo* degradation, the hydrogel was prepared in accordance with the protocol mentioned in our previous publication [1]. The formed hydrogel (100µL per implantation or injection) was implanted or injected subcutaneously at the bilateral position of anaesthetized (4% isoflurane) OF-1 female mice (10-week-old, from Charles River, France). Six implantations were performed for each condition. All animal experiments were approved by the central animal facility of the University of Bordeaux (Bordeaux, France) (accreditation number: A33-063-917) with the animal experimentation permission APAFIS#4375-2016030408537165. The animals were sacrificed after one week and three weeks of implantation. The subcutaneous tissue with the hydrogel was harvested and fixed in 4% PFA overnight at 4℃. The fixed tissue was dehydrated, embedded in paraffin and sectioned at 7 µm thickness. The slides were stained with Masson`s trichrome to analyze the inflammation, cell infiltration, and collagen deposition.

In vivo release of BSA

The hydrogels were prepared as described above (concentration 1.5 %) and loaded with BSA-Texas red (Fischer scientific, France) at 1 mg/mL before the gelation process. 100 µL of the formed hydrogels containing BSA-Texas red were injected subcutaneously on the right side. The amount of BSA-Texas red remaining in the hydrogel was visualized by IVIS Lumina LT optical imaging system (Perkin Elmer, USA) at the excitation wavelength of 605 nm and emission 695-770 nm,. The remaining BSA-Texas red in the hydrogel was quantified by measuring the fluorescent signal in the ROIs designed at day 0.

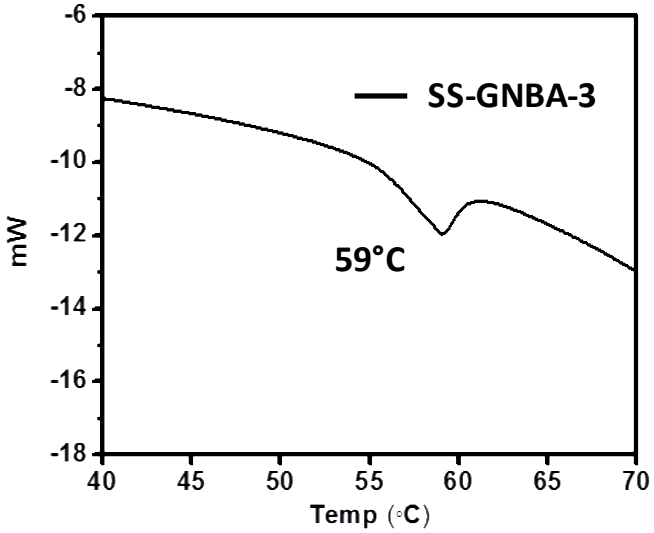
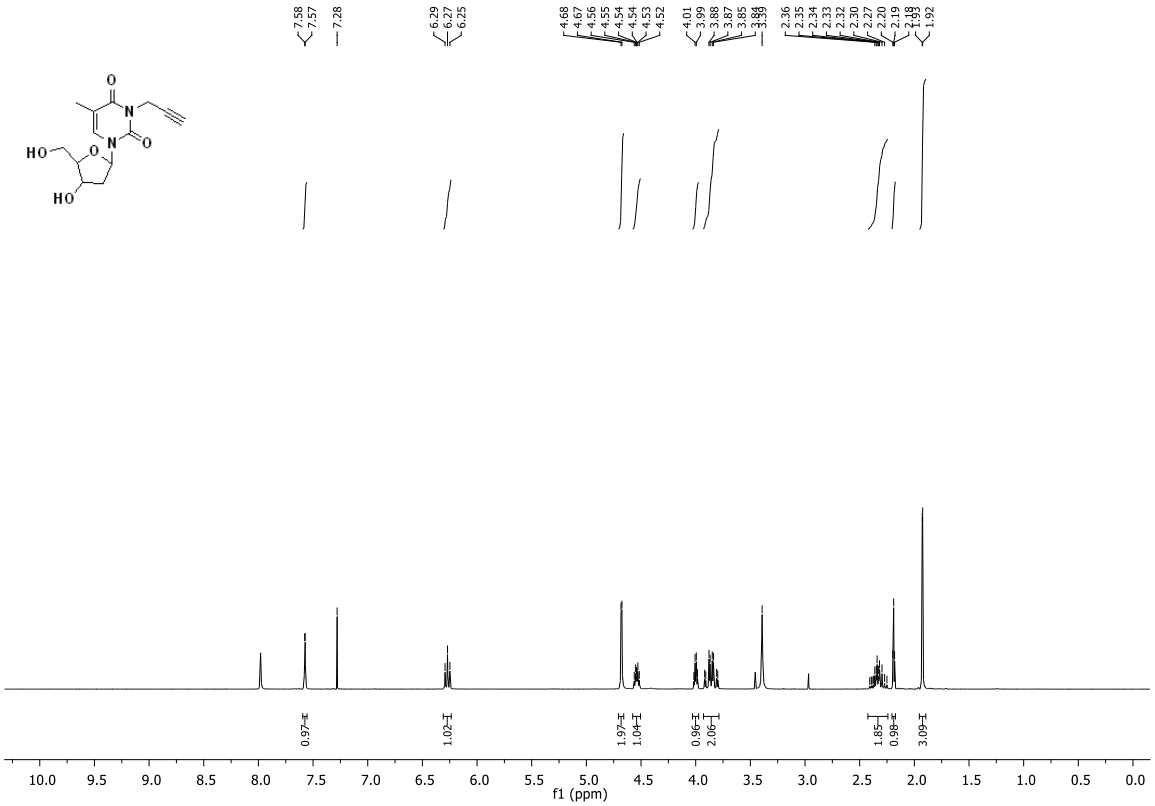


Figure S4. DSC heating curve for hydrogels obtained from SS-GNBA-3.

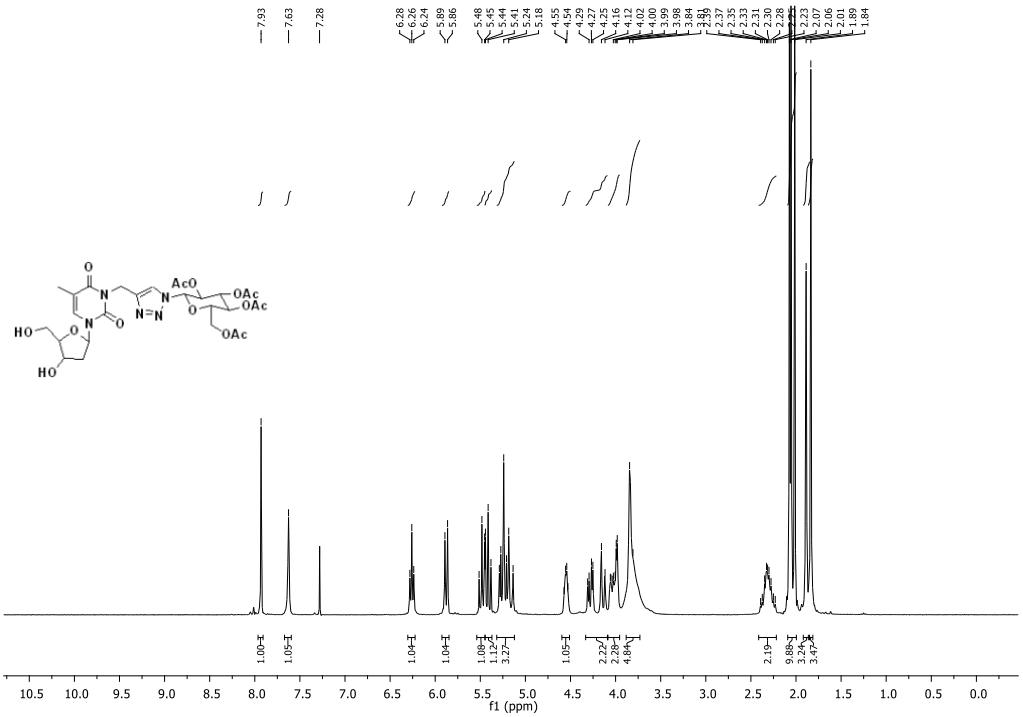
SUPPORTING INFORMATION SI 4

Characterization of the compounds by ^{1}H and ^{13}C-NMR

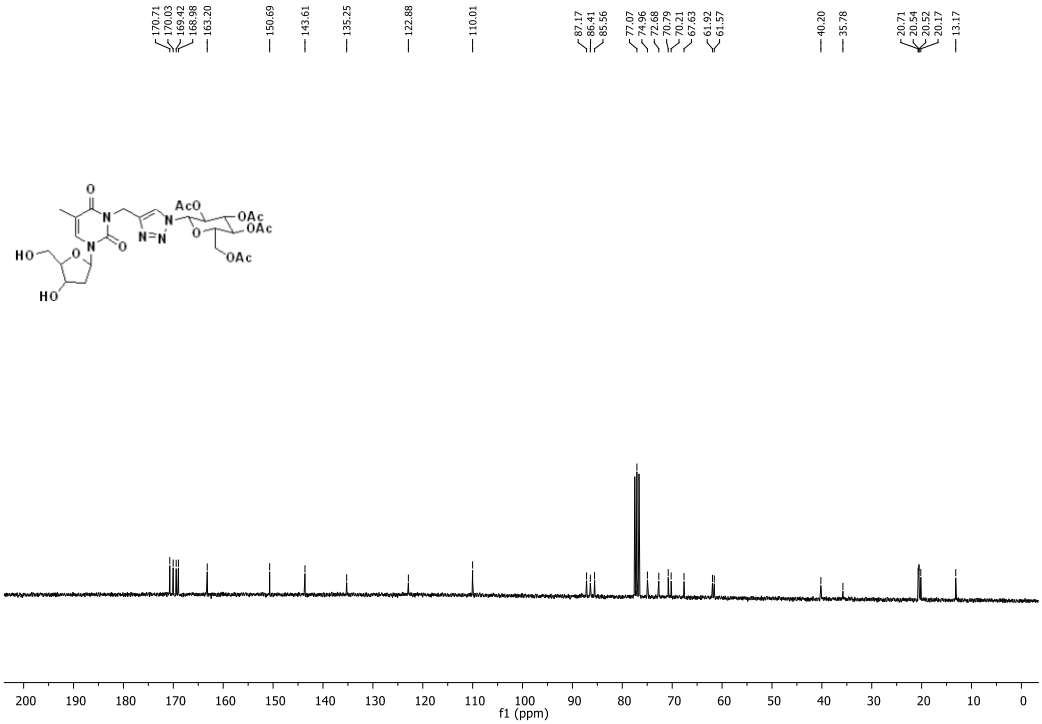
^{1}H-NMR of compound (1)



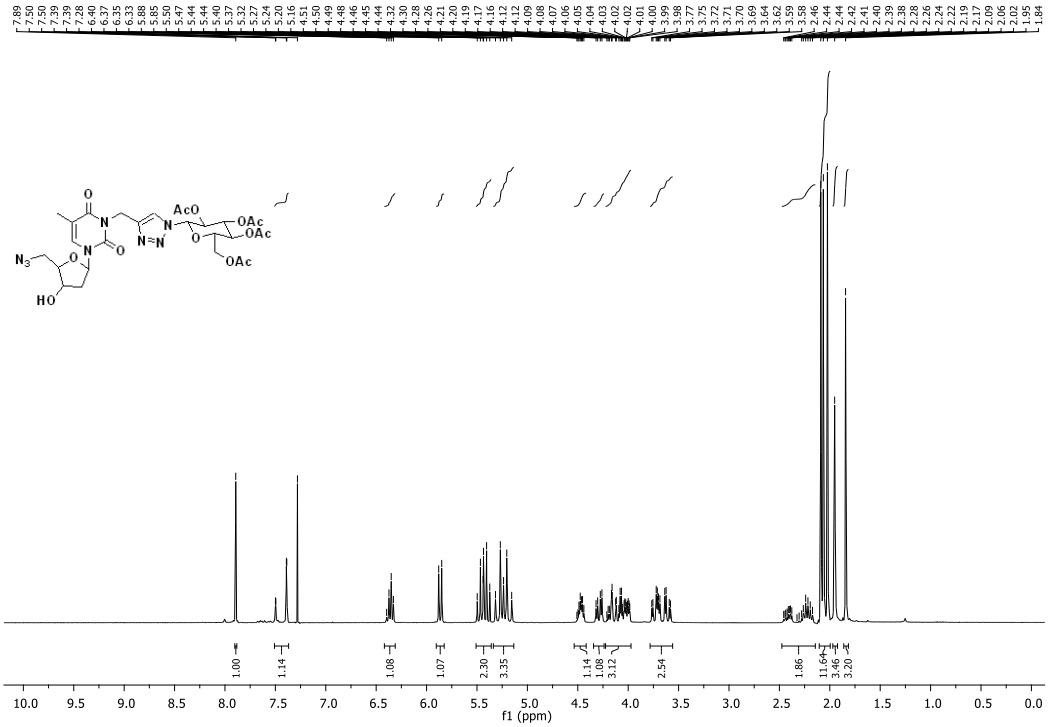
$_{1}$H-NMR compound (2)



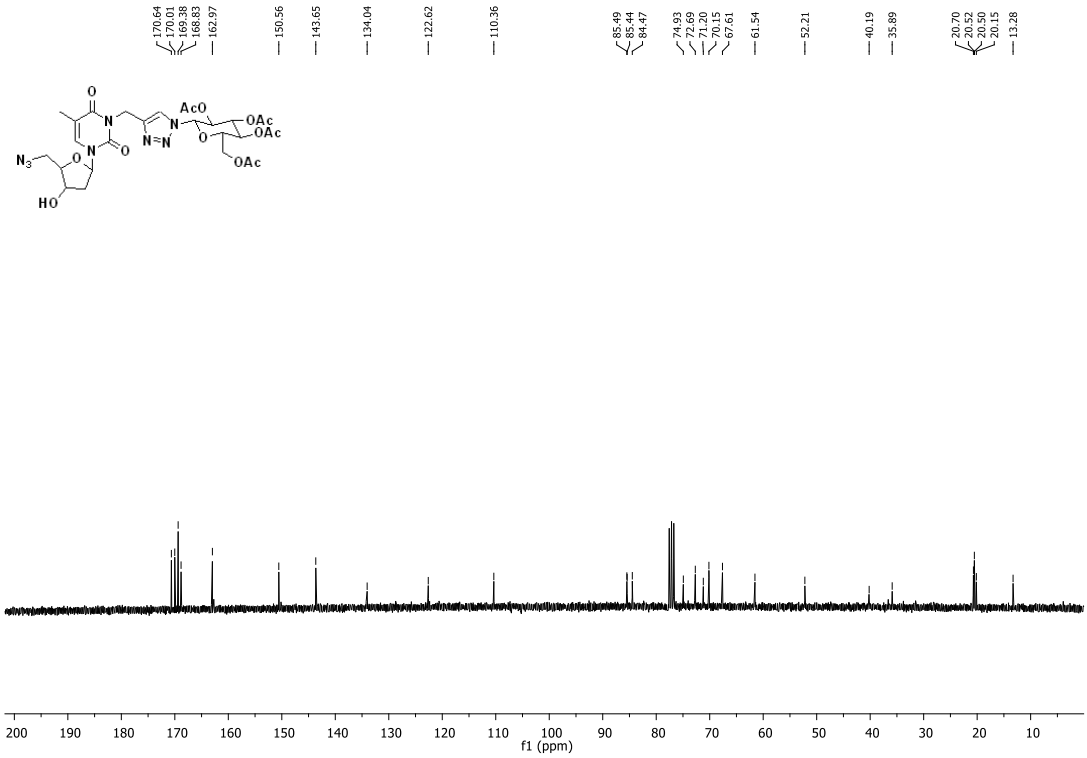
$_{13}$C-NMR of compound (2)



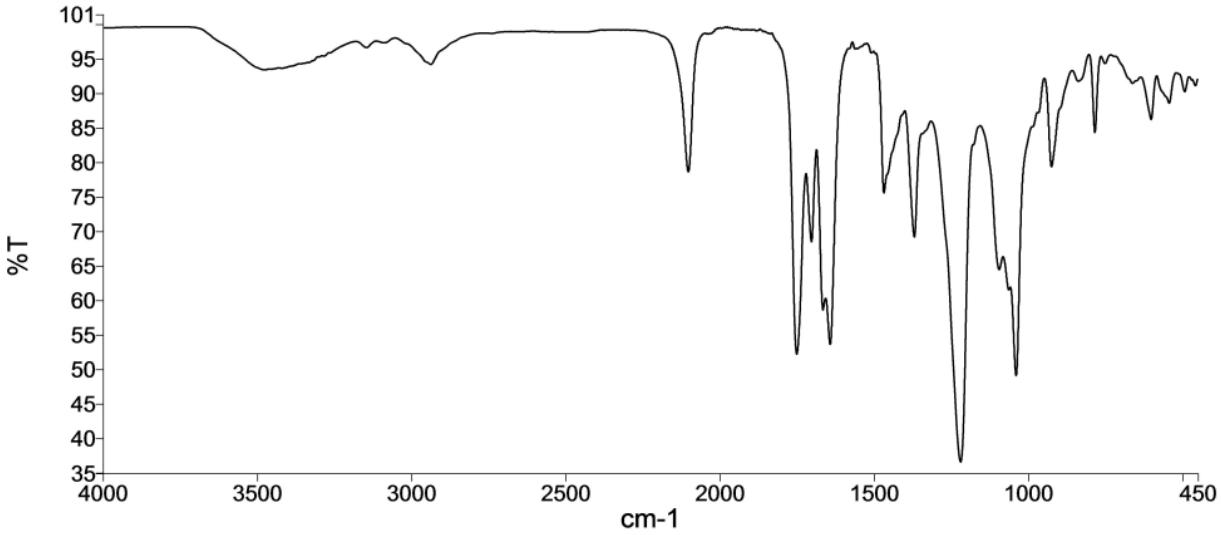
$_{1}$H-NMR compound (3)



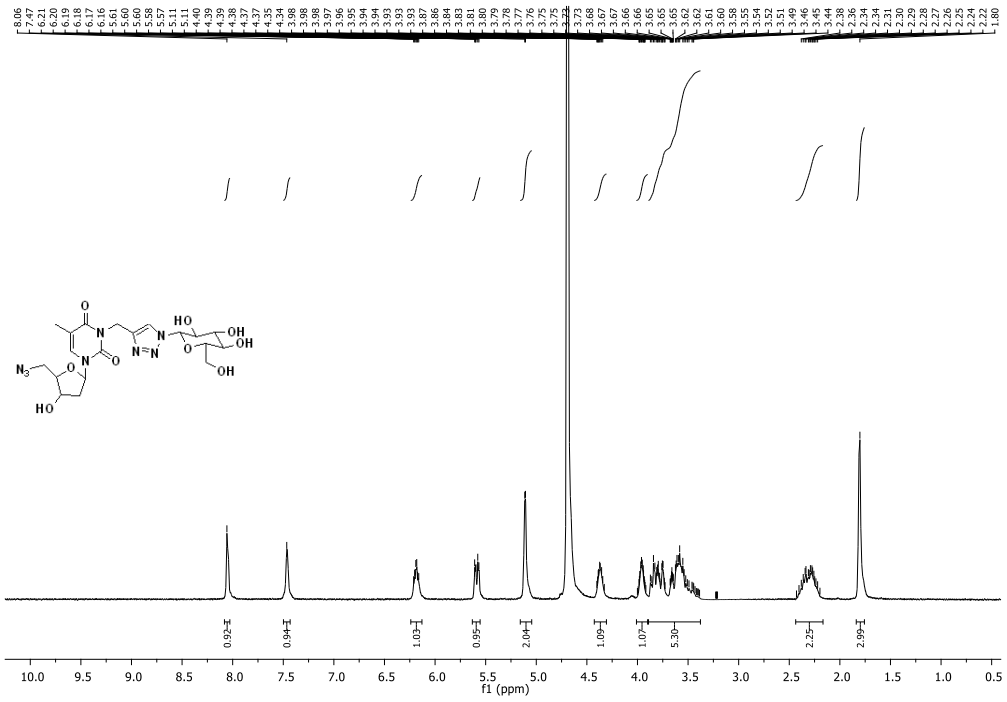
$_{13}$C-NMR compound (3)



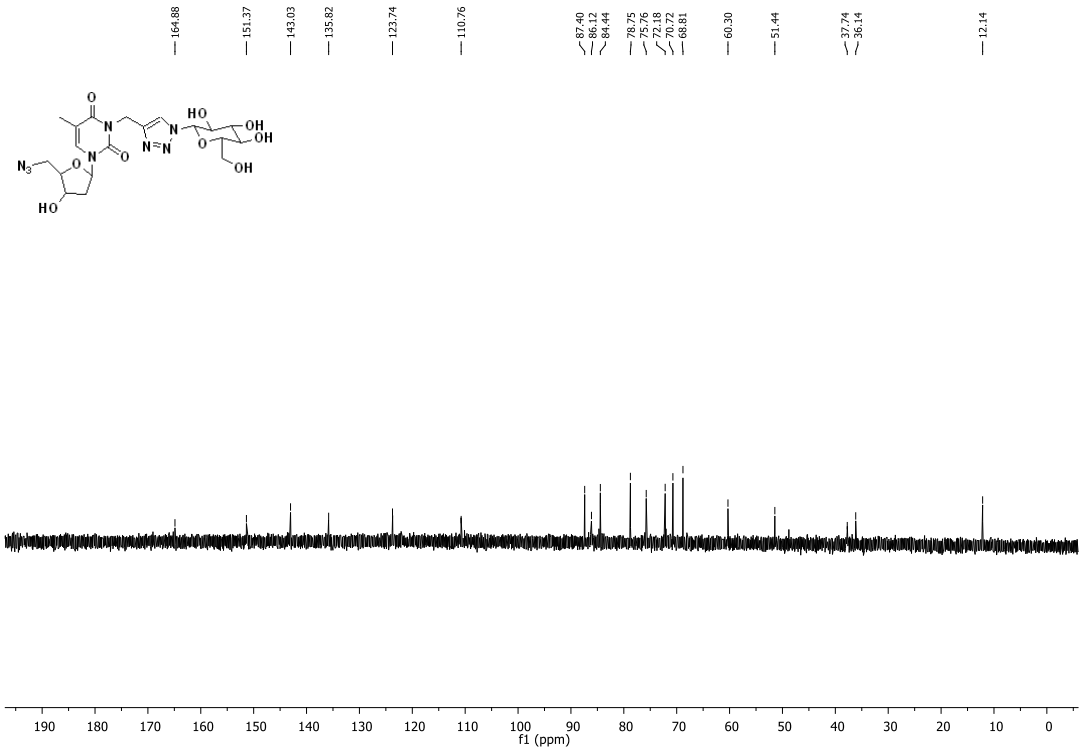
IR compound of compound (3)



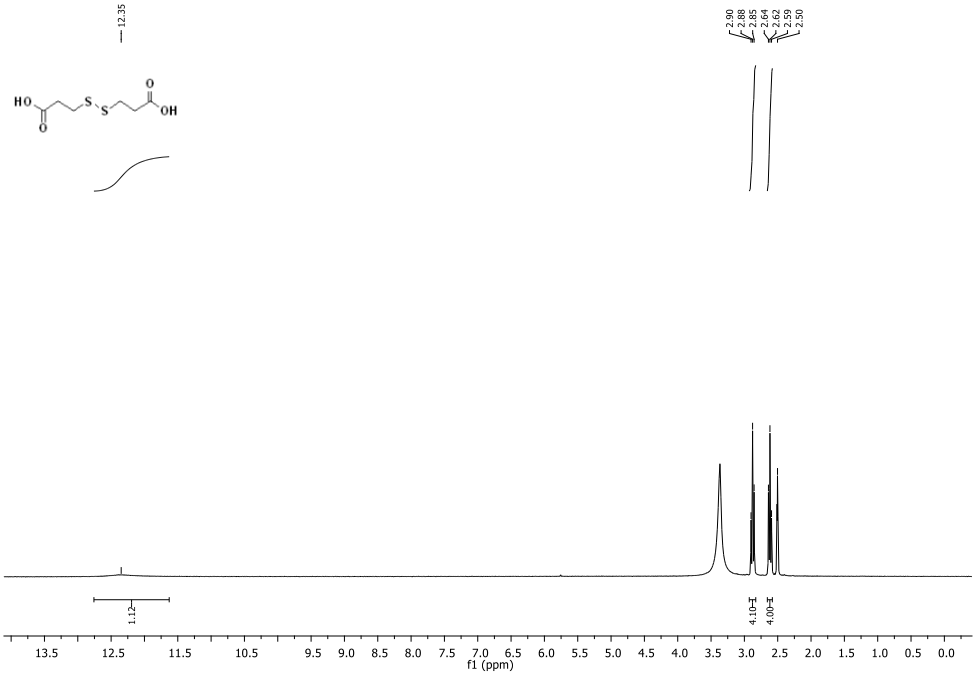
$_1$H-NMR of compound (4)



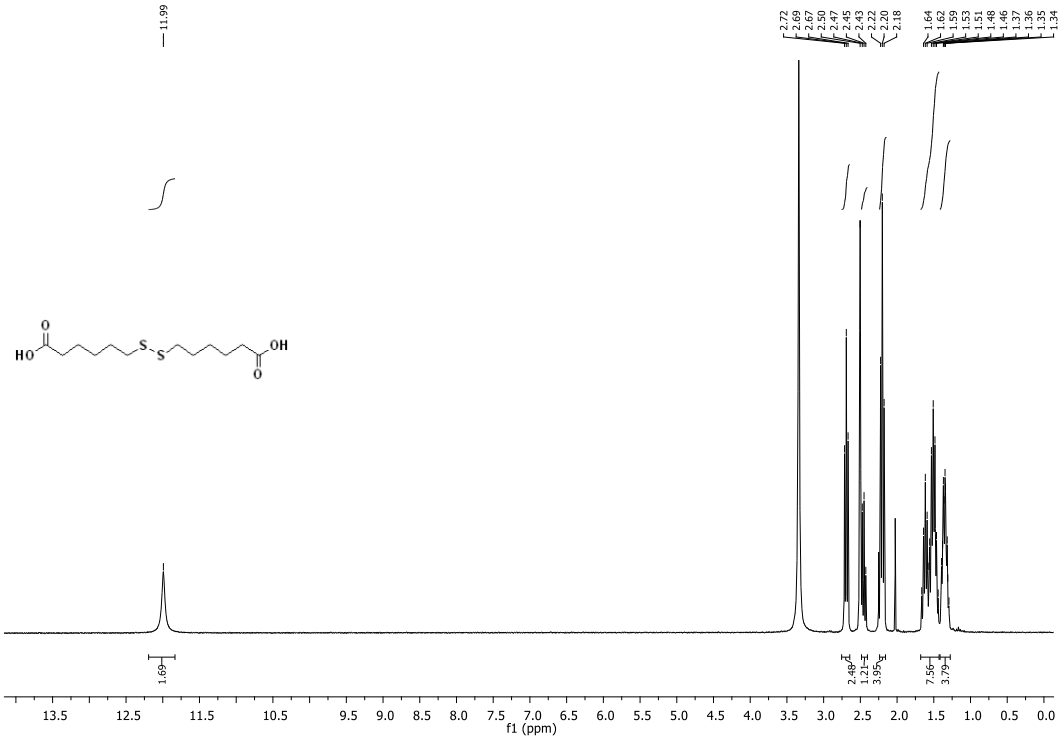
^{13}C-NMR of compound (4)



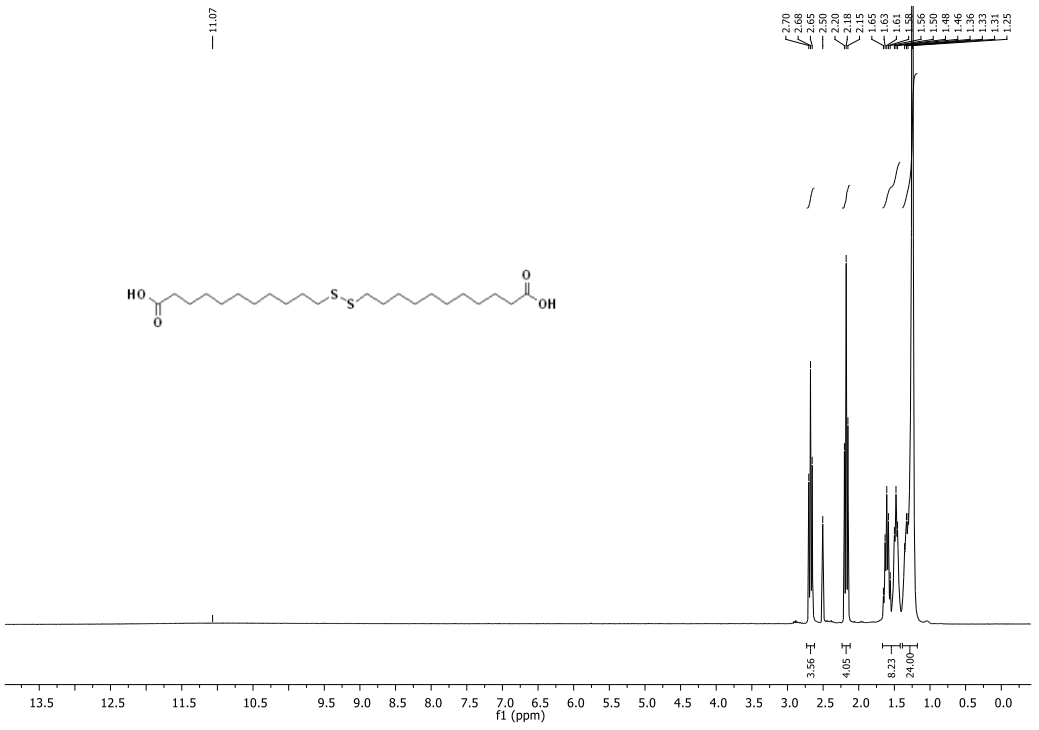
^{1}H-NMR of compound (6a)



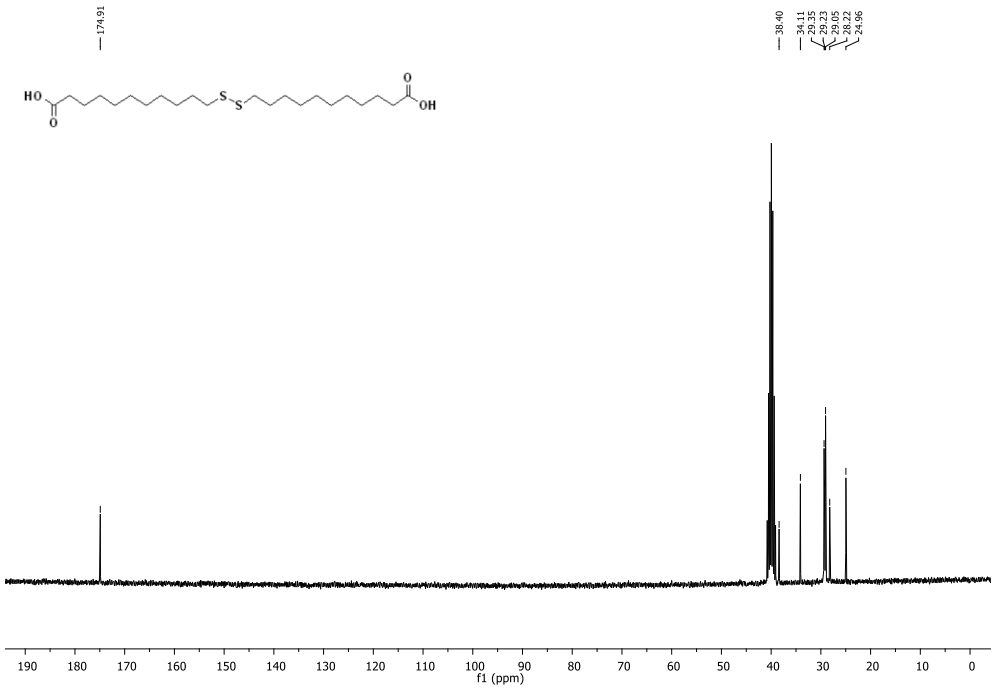
$_1$H-NMR of compound (6b)



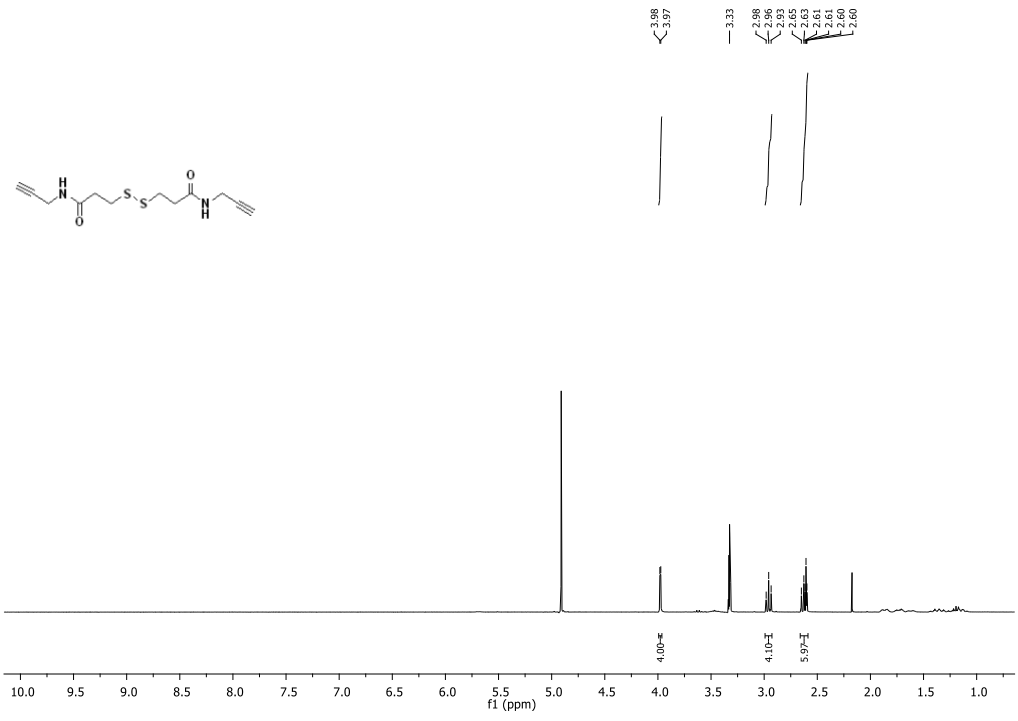
$_1$H-NMR of compound (6c)



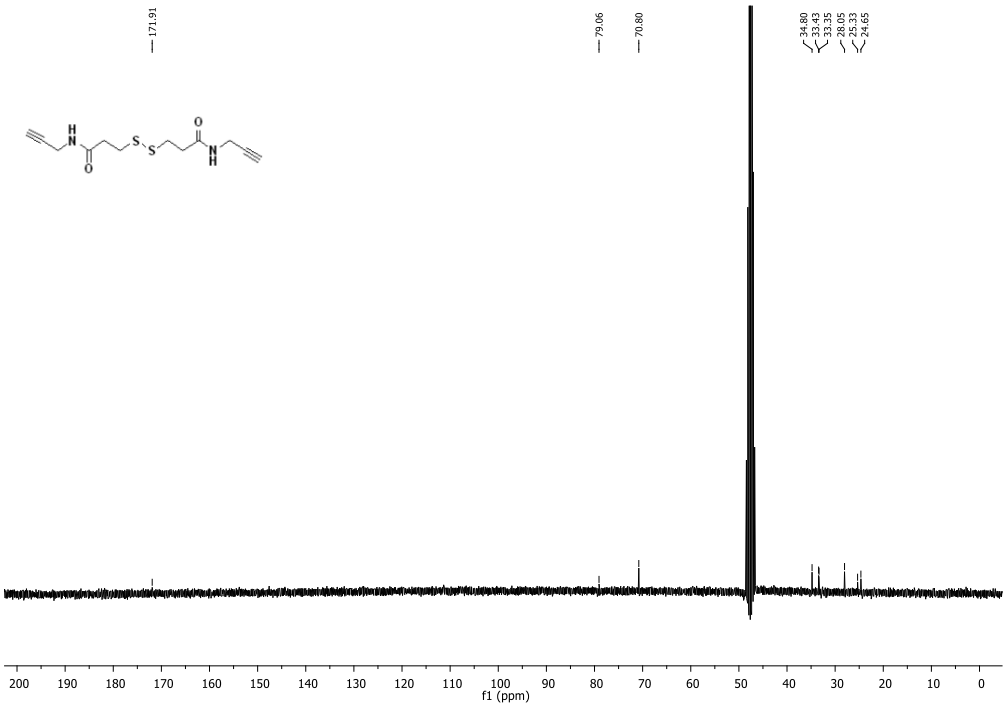
^{13}C-NMR of compound (6c)



^{1}H-NMR of compound (7a)



^{13}C-NMR of compound (7a)



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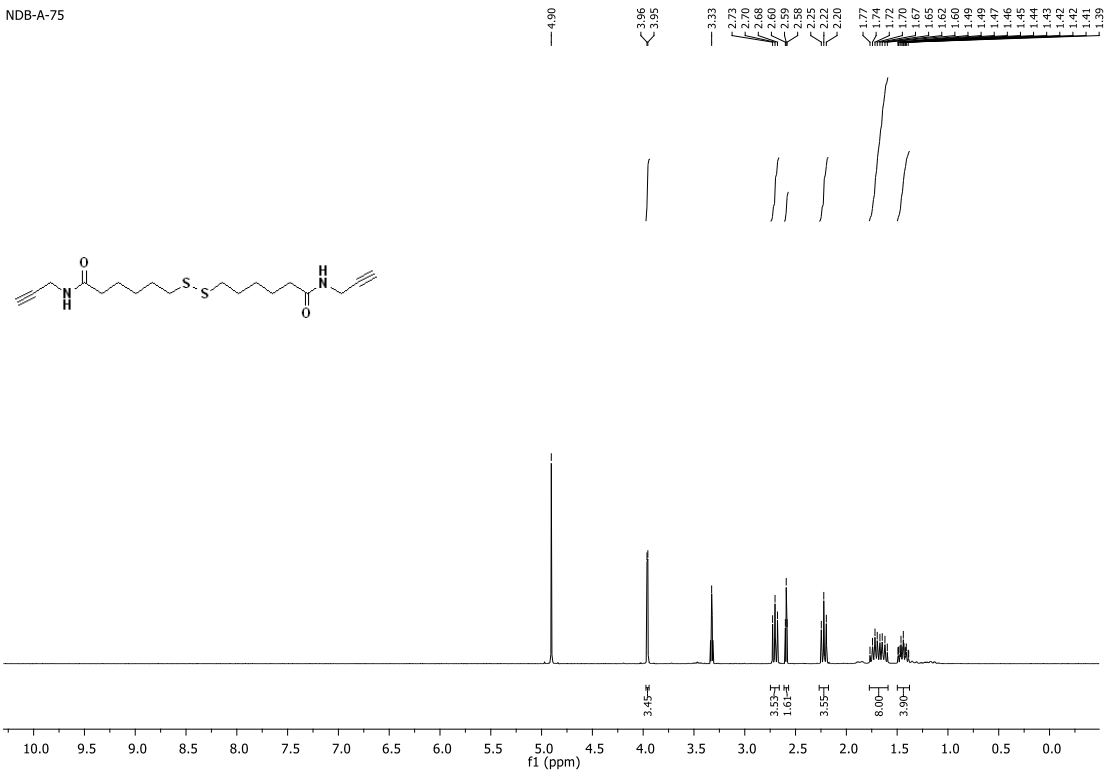

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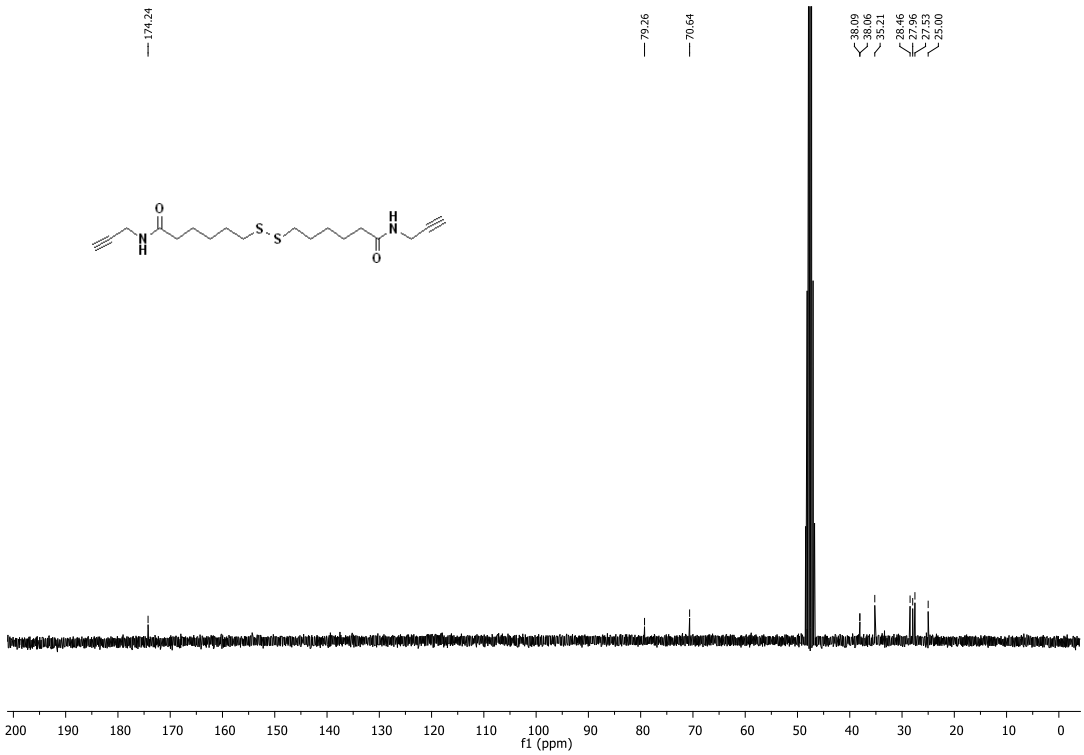
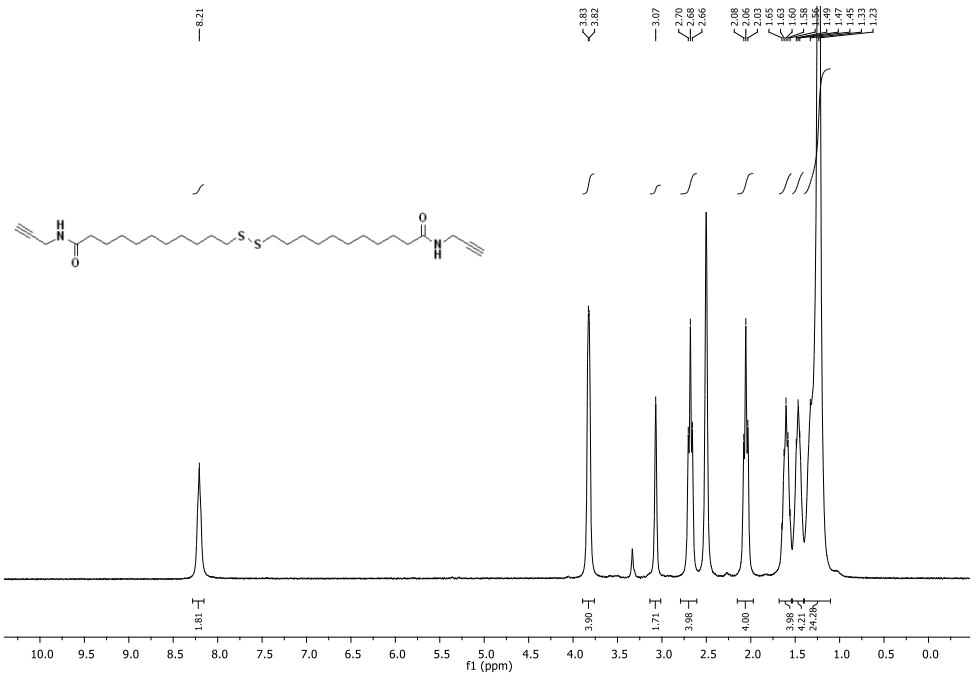


1H-NMR of compound (7c)



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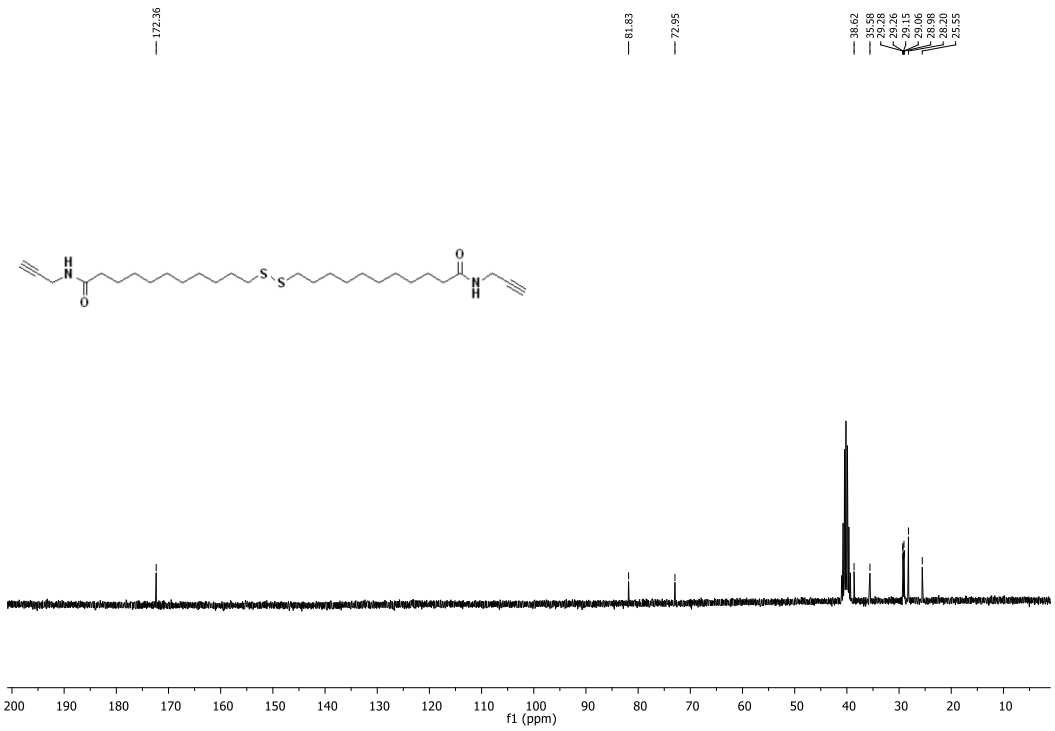

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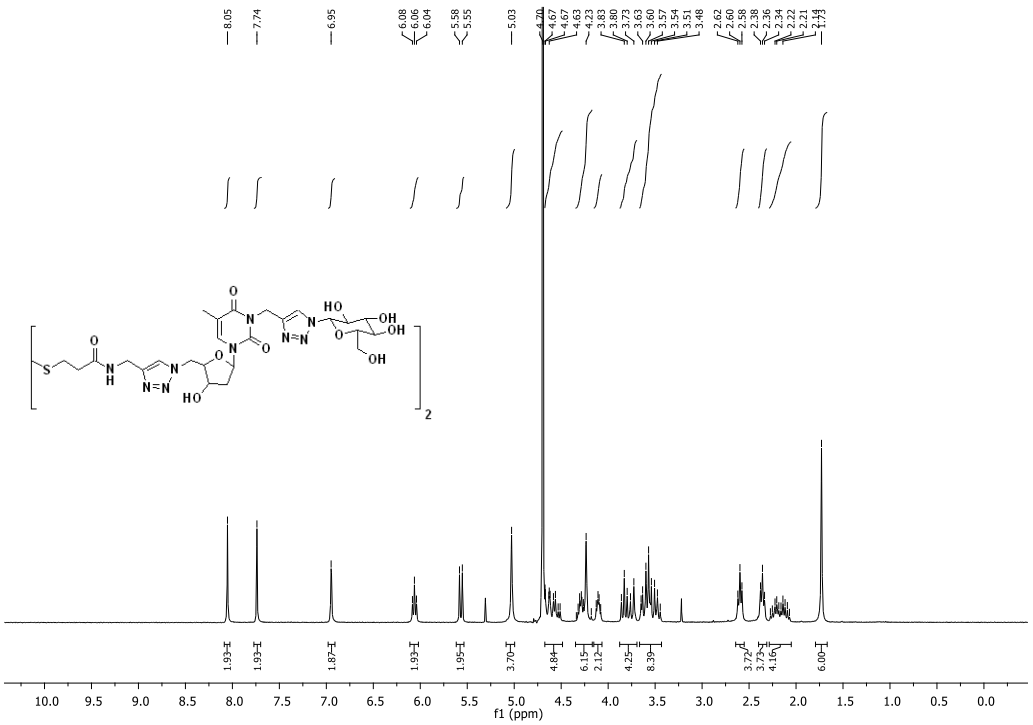

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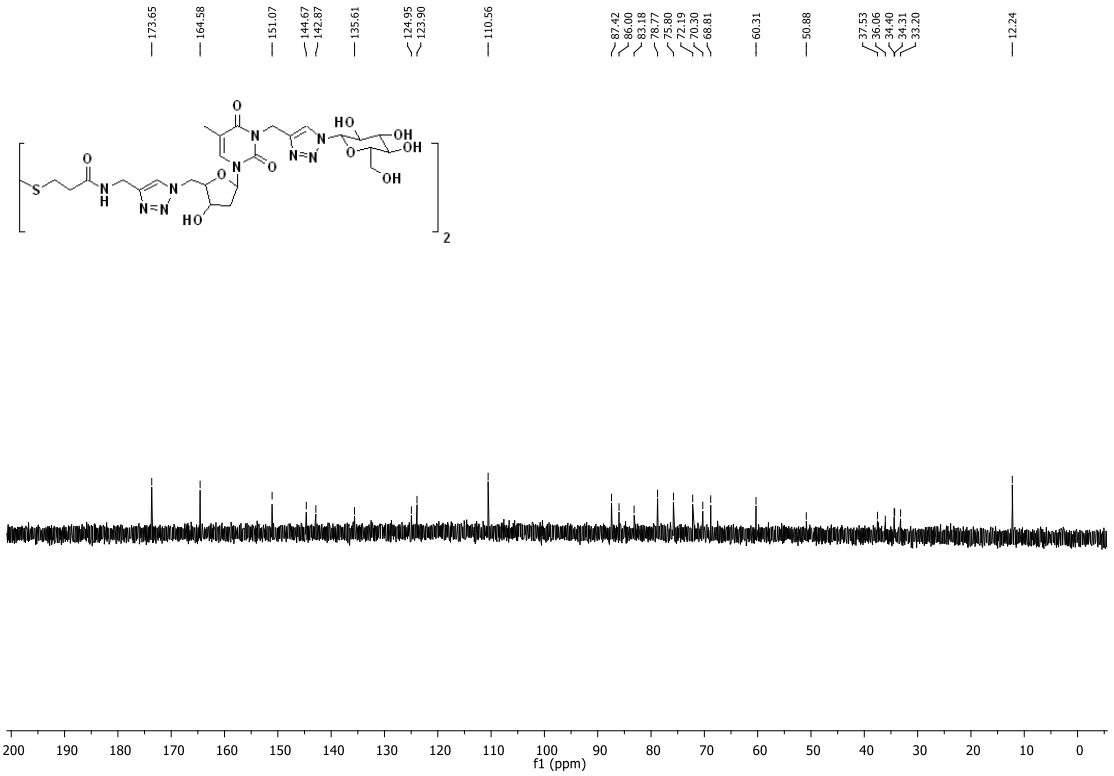

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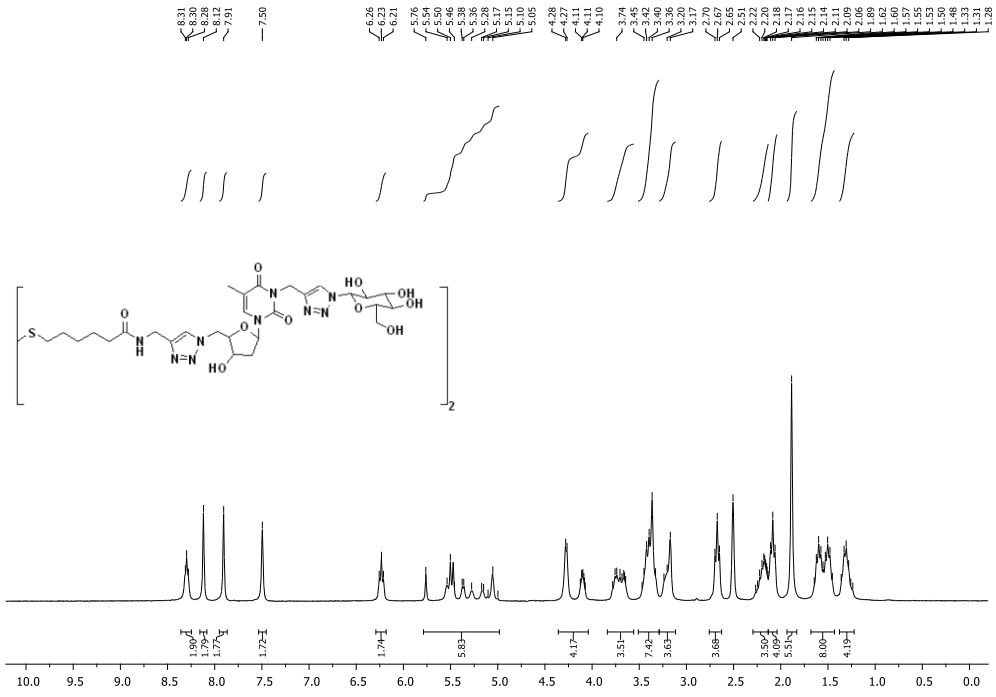

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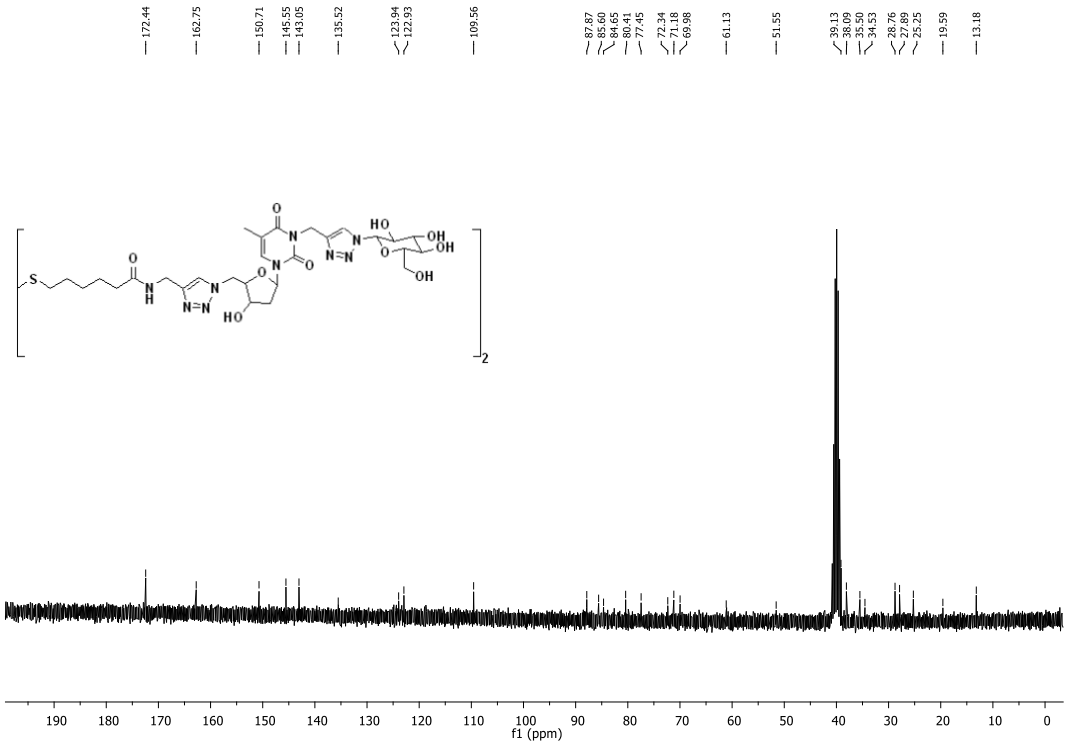

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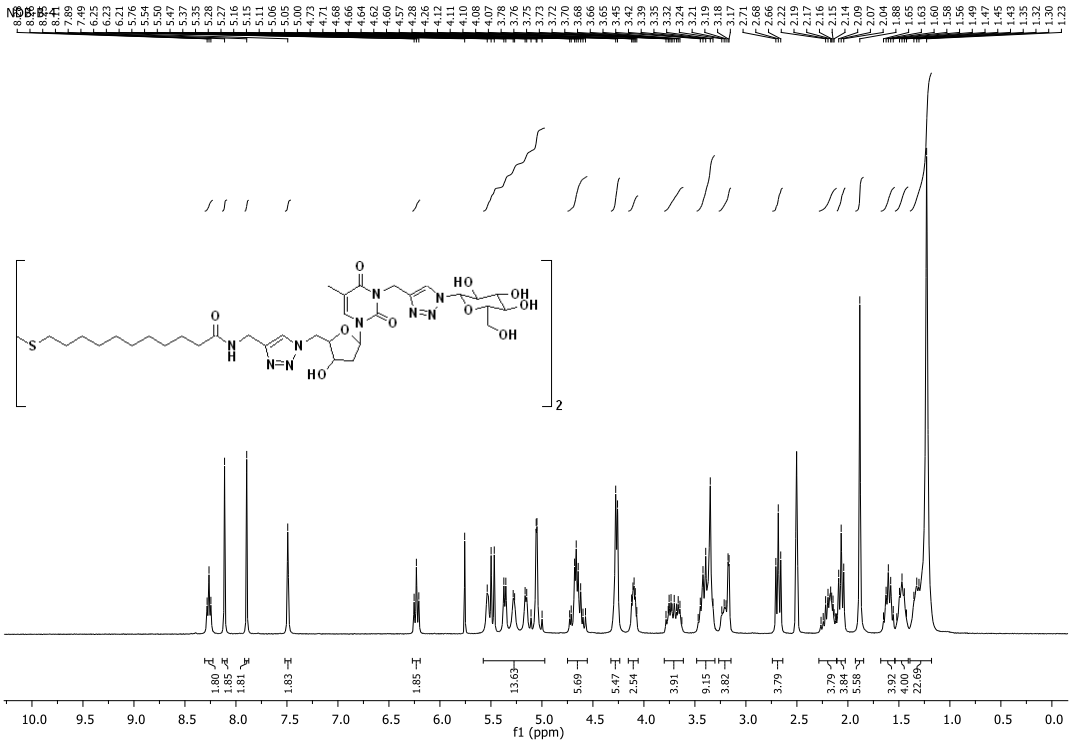

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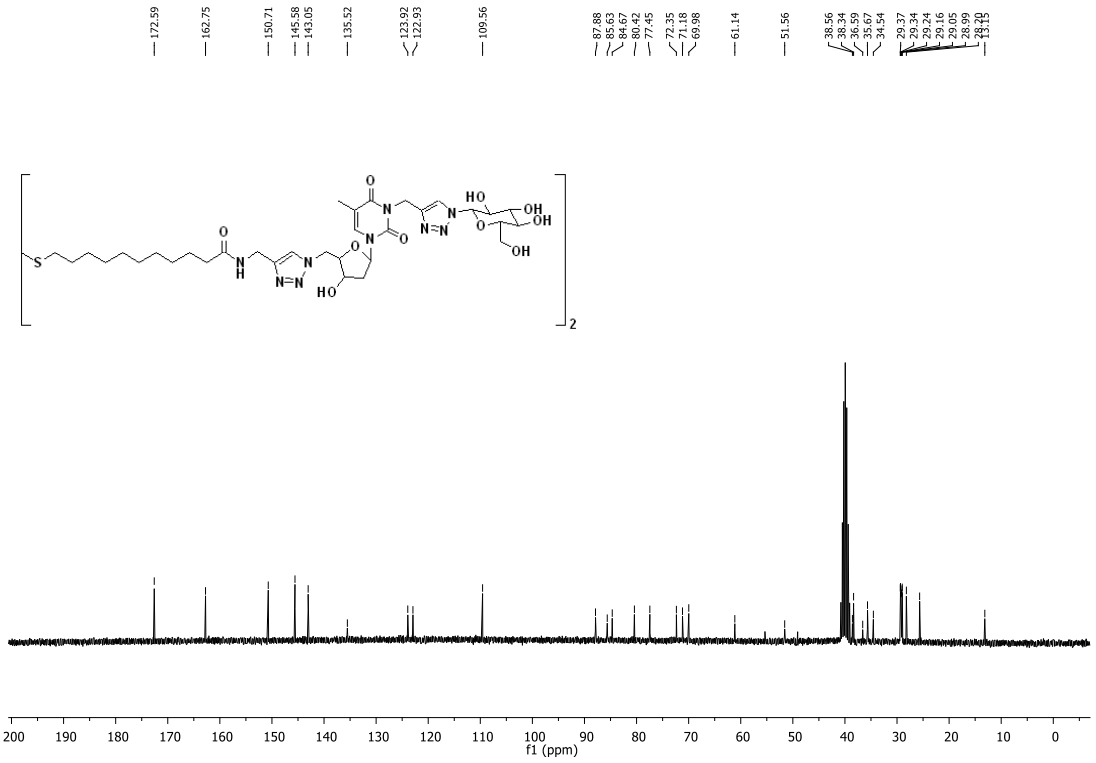

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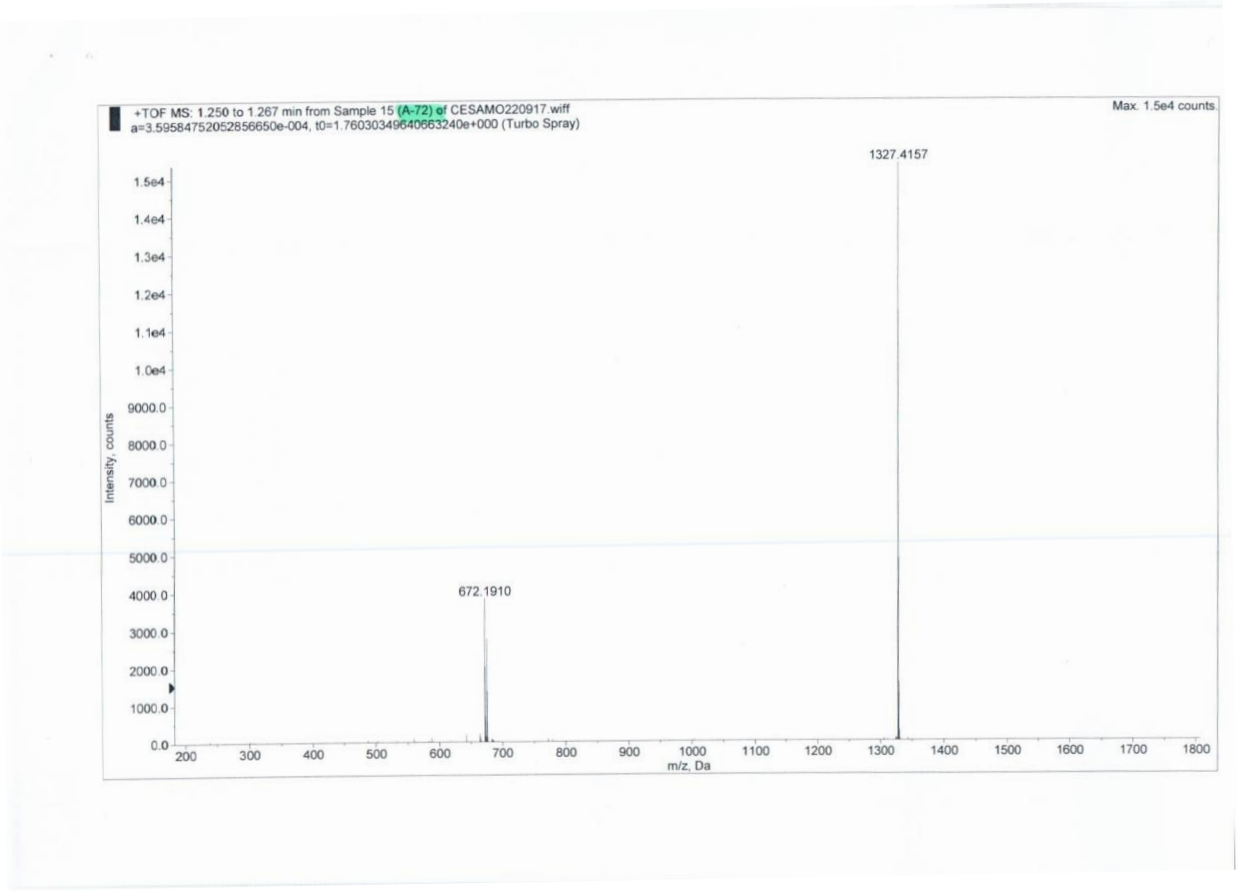
HRMS of compound (8a)



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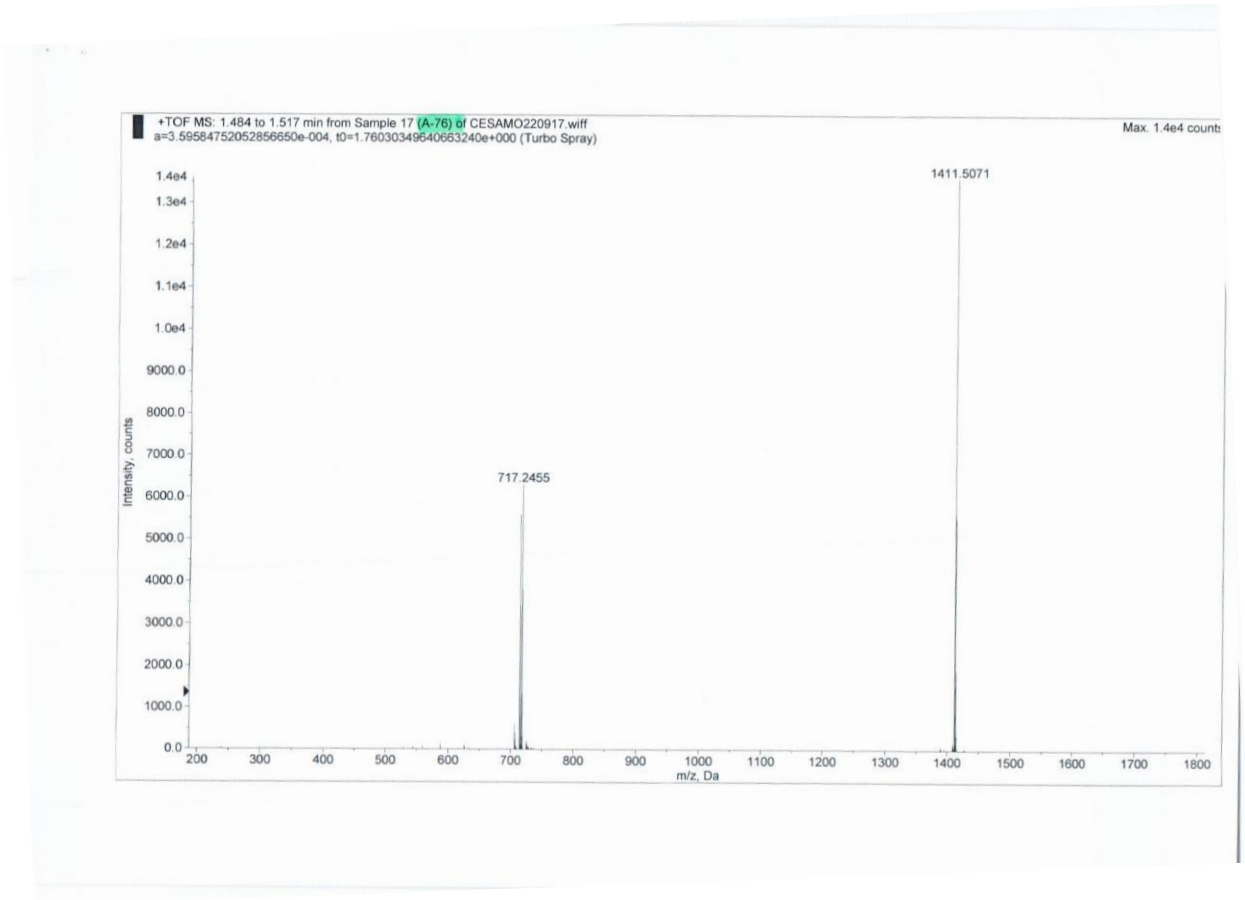
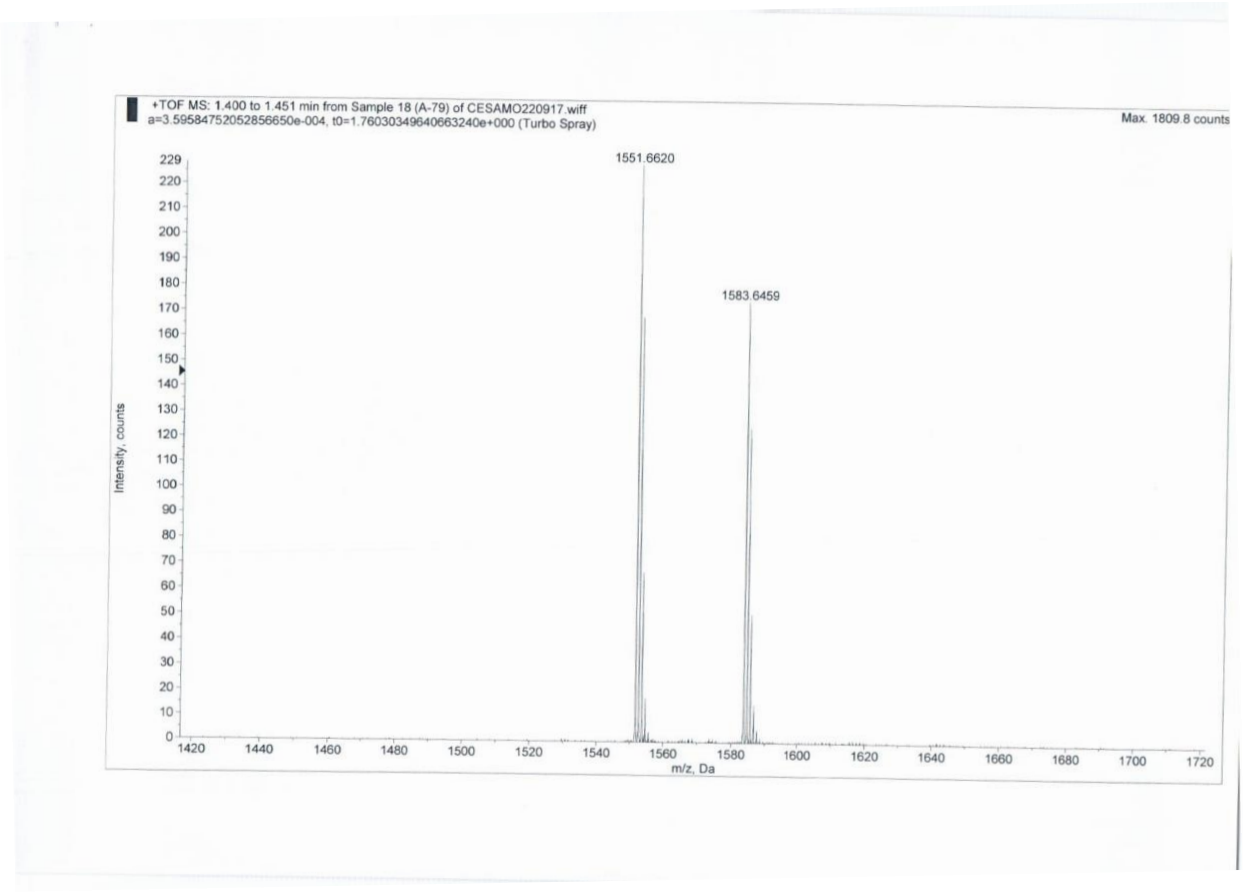


HRMS of compound (8c)



SUPPORTING INFORMATION SI 6

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