Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

for

Novel Glycoconjugated Squaraine Dyes for Selective Optical Imaging of Cancer Cells

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1. Materials and Methods

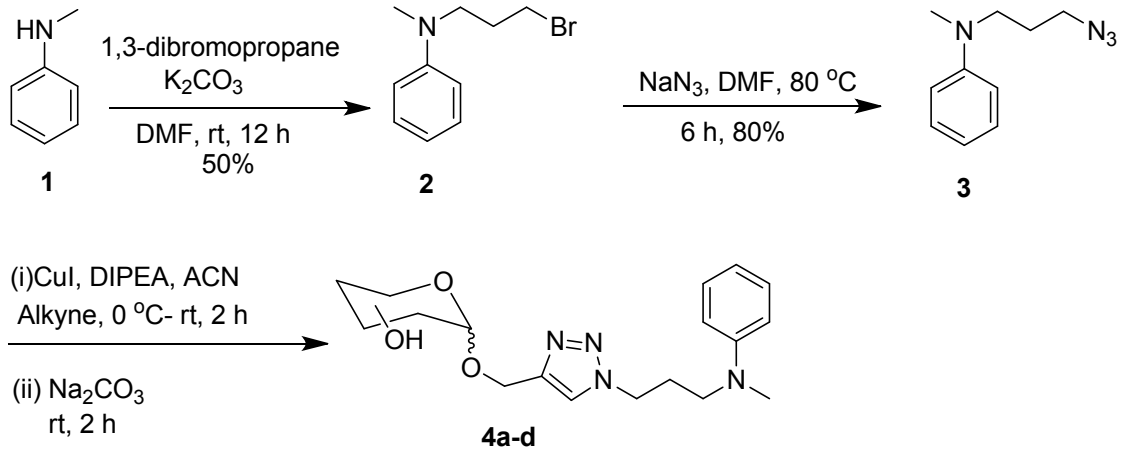
All the chemicals purchased from Sigma-Aldrich, Alfa Aesar, Merck and SDFCL were used without further purification. ^{1}H and ^{13}C were recorded on Bruker 500 MHz spectrometer and tetramethylsilane (TMS) used as the standard. IR spectra were recorded on Bruker FT-IR spectrometer. Mass spectra were recorded under EI/HRMS at 60,000 resolution using Thermo Scientific Exactive Mass Spectrometer and MALDI-TOF MS spectra were recorded using Shimadzu Axima CFR (Plus). Absorption spectra were measured on a Shimadzu UV-3101 PC NIR scanning spectrophotometer and emission recorded on SPEX Fluorolog F112X spectrofluorimeter. Temperature dependent studies were carried out with a thermostat directly attached to the wall of the cuvette holder. Fluorescence quantum yield(φ_f) were measured by relative method using squarylium III (φ_f = 0.65 in dichloromethane) as standard.[1]

HeLa cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India and also from Prof S. Murty Srinivasula of Indian Institute for Science Education and Research (IISER), Thiruvananthapuram, India. SW480 cell lines were obtained from NCCS and H9c2 cell lines were from American Type Culture Collection (ATCC), USA. For maintenance of cell lines, Dulbeccos Modified Eagle's Medium (DMEM) *(Sigma)* containing 10% fetal bovine serum (FBS) *(Gibco),* antibiotics (100 U/mL Penicillin and 100 µg/mL streptomycin) and amphotericin (0.25 µg/mL) *(HiMedia)* were employed. The cells were maintained in CO_2 incubators at 37 ˚C with 5% CO_2 in air and 99% humidity. Passaging of cells when confluent was carried out using 0.25% trypsin and 0.02% EDTA *(HiMedia)* in phosphate buffered saline (PBS). Experiments were carried out after 36 h of seeding the cells at appropriate density in suitable well plates.

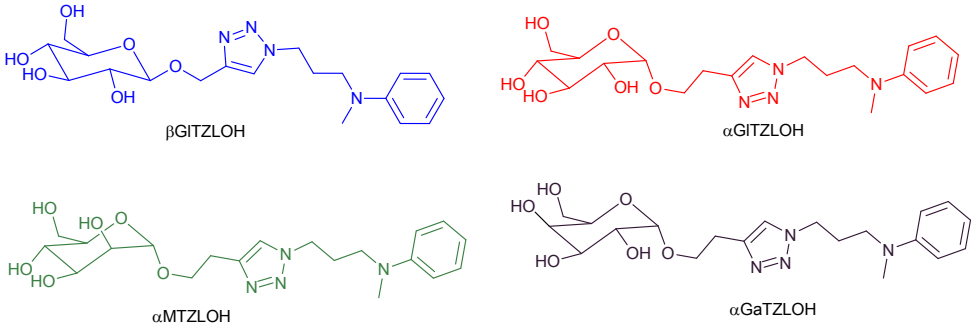
2. Synthesis and characterization

2.1. Synthetic scheme

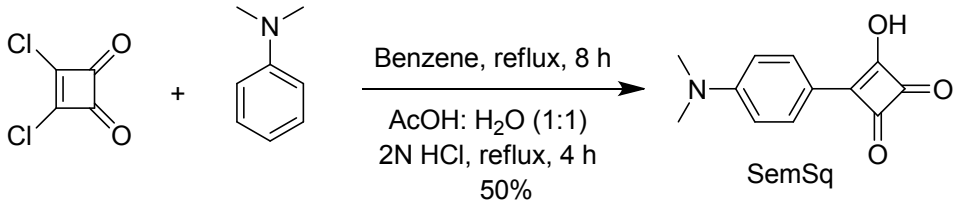
Scheme 1



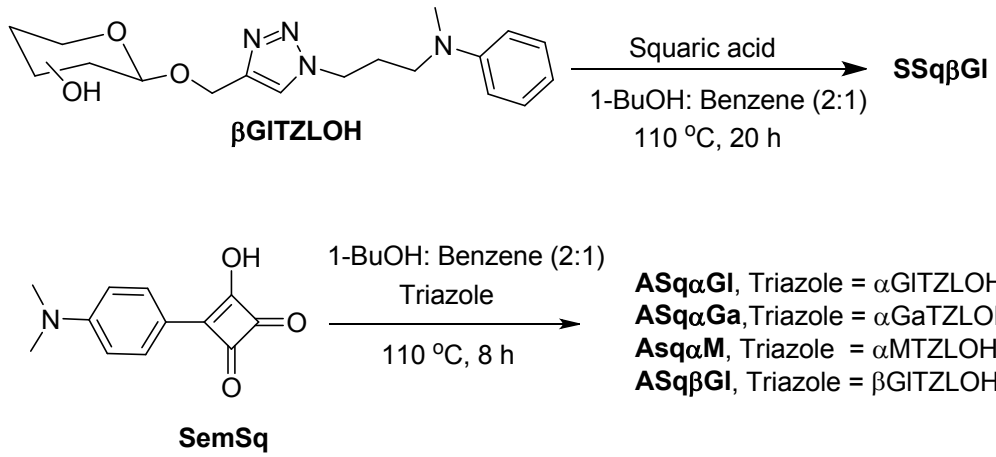
4a = **αGlTZLOH** (Alkyne= Propargylα-D-glucoside)
4b= βGlTZLOH (Alkyne = Propargylβ-D-glucoside)
4c = αGaTZLOH (Alkyne= Propargyα-D-galactoside)
4d = αMTZLOH (Alkyne = Propargylα-D-mannoside)



Scheme 2



Scheme 3



2.2. Experimental procedure and characterizations

2.2.1. Synthesis of N-(3-bromopropyl)-N-methylaniline (2): N-(3-bromopropyl)-N-methylaniline **(2)** was synthesized according to the conventional procedure. To a solution of N-methylaniline (5 g, 46.6 mmol) in dry DMF (20 mL) potassium carbonate (32 g, 233 mmol) was added and allowed to stir for 10 min. To this reaction mixture 1,3-dibromopropane was added slowly and then reaction mixture was allowed to stir for 12 h at room temperature under argon atmosphere. After the completion of

reaction, reaction mixture was filtered and compound was extracted with diethyl ether. Organic layer was washed with brine and solvent removed under reduced pressure. Residue was subjected to column purification on silica gel, eluting with hexane to yield the title compound **2** as yellow liquid (5.3 g, 50%). **^{1}H NMR** ($CDCl_3$, 500 MHz):δ 7.23 (t, *J* = 8.0 Hz, 2 H) 6.71 (m, 3 H), 3.48 (t, *J* = 7.0 Hz ,2 H,), 3.44 (t, *J* = 6.5 Hz, 2 H), 2.94 (s, 3 H), 2.150-2.09 (m, 2 H) ppm. **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 149.2, 129.5, 127.2, 112.7, 50.9, 38.9, 31.9, 30.2 ppm. HRMS (ESI) *m/z* calcd for $C_{10}H_{15}BrN$: 228.0388; found: 228.0385 $[M+H]^+$.

2.2.2. Synthesis of N-(3-azidopropyl)-N-methylaniline (3): Compound **2** (3 g, 23.3 mmol) and sodium azide (2.2 g, 35 mmol) was allowed to reflux in dry DMF for 8 h. Completion of reaction was monitored by thin layer chromatography (TLC). Reaction mixture was cooled to room temperature and diluted with diethylether (25 mL). Organic layer was washed with brine and dried over sodium sulphate. Solvent was evaporated under *vacuo*. Crude product obtained was subjected to silica gel column chromatography using hexane as eluent to afford the title compound **3** as yellow liquid (2 g, 80%). **^{1}H NMR** ($CDCl_3$, 500 MHz): δ 7.20 (t, *J* = 7.0 Hz, 2 H,), 6.68 (bs, 3 H), 3.35 (d, *J* = 7.0 Hz, 2 H), 3.281 (t, *J* = 6.0 Hz, 2 H), 2.878 (s, 3 H), 1.792 (t, *J* = 6.5 Hz, 2 H) ppm. **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 149.2, 129.4, 116.6, 112.4, 49.8. 49.2, 38.5, 26.3 ppm. HRMS (ESI) *m/z* calcd for $C_{10}H_{15}N_4$: 191.1297; found: 191.1294 $[M+H]^+$.

2.2.3. Synthesis of alkynes: The alkyne derivatives such as 2, 3, 4, 6-Tetra-*O*-acetyl-1-(2'-propargyl)-α-D-glucose, 2,3,4,6-Tetra-*O*-acetyl-1-(2'-propargyl)-α-D-galactose, 2,3,4,6-Tetra-*O*-acetyl-1-(2'-propargyl)- α-D-mannose and 2,3,4,6-Tetra-*O*-acetyl-1-(2'-propargyl)- β-D-glucose were obtained by following the reported procedures[2,3] and products formed were confirmed from HRMS analysis.

2.2.4. General procedure for the synthesis of 1,2,3-triazoles: Alkyne (1 equiv.) and azide (1 equiv.) were dissolved in acetonitrile. Copper iodide (1.5 equiv.) was added to this solution followed by the addition of N,N-diisopropylamine (3 equiv.) and the reaction mixture was allowed to stir for 2 h at room temperature. After the completion of reaction, reaction mixture was diluted with water and ammonium chloride. Extracted with ethyl acetate and the combined organic layer was washed with brine solution, dried over sodium sulphate. Solvent removed under reduced pressure and the residue obtained was directly used for next reaction. Crude product dissolved in methanol and allowed to stir in presence of sodium carbonate (5 equiv.) for 2 h at room temperature. After monitoring the completion of reaction with TLC, reaction mixture was filtered and solvent removed under reduced pressure. Residue obtained was purified by silica gel column chromatography using $CHCl_3$/MeOH (10:1) solvent system to afford corresponding 1,2,3-triazole derivatives.

2.2.4.1. Synthesis of 3-(4-(dimethylamino)phenyl)-4-hydroxycyclobut-3-ene-1,2-dione (SemSq): SemSq was synthesised according to the traditional procedure.[3] 3,4-Dichlorocyclobutene-1,2-dione (2) (1.5 g, 9.9 mmol), and N,N-dimethylaniline (1.2 g, 9.7 mmol), were dissolved in dry benzene (30 mL) and refluxed for 8 h. After cooling, the reaction mixture was poured into ice water (200 mL) and the two layers formed were separated. The organic layer was washed with water, and the crude product obtained was dissolved in a mixture of acetic acid (25 mL), water (25 mL) and 2N HCl was added (10 mL). The resulting mixture was then refluxed for 4 h at 120 ^{o}C. After cooling, this solution was added to crushed ice, the precipitated product was isolated by filtration, washed with diethylether, and dried to yield 2.0 g (50%) of the pure product as a brown coloured powder. **^{1}H NMR** (500 MHz, DMSO-d_6): 7.86 (d, *J* = 9 Hz, 2H,), 6.88 (d, *J* = 9 Hz, 2H,), 3.03 (s, 6H, -N-CH_3) ppm

2.2.4.2. Synthesis of 2-(hydroxymethyl)6((1(3(methyl(phenyl)amino)propyl)-1-H-1,2,3-triazol-4yl)methoxy)tetrahydro-2H-pyran-3,4,5-triol (αGlTZLOH): αGlTZLOH was synthesized by reacting 2,3,4,6-Tetra-*O*-acetyl-1-(2'-propargyl)-α-D-glucose and compound 3 to obtain acetylated derivative. Which then upon reaction with sodium carbonate yielded αGlTZLOH as colourless viscous liquid (1.2 g, 56%). **^{1}H NMR** (Methanol-d_4, 500 MHz): δ 8.01 (s, 1 H), 7.19-7.16 (m, 2 H), 6.71-6.64 (m, 3 H), 4.93

(d, *J* = 4.0 Hz, 1 H), 4.83 (bs, 1 H), 4.68 (d, *J* = 12.5 Hz, 1 H), 4.44 (t, *J* = 7.0 Hz, 2 H), 3.83 (d, *J* = 11.0 Hz, 1 H), 3.72-3.61 (m, 3 H), 3.45- 3.42 (m, 2 H), 3.38 (s, 1 H), 3.35-3.31 (m, 2 H), 2.89 (s, 3 H), 2.19-2.14 (m, 2 H) ppm. **^{13}C NMR** (MeOH-d_4, 125 MHz): δ 148.8, 128.3, 123.6, 116.1, 112.1, 97.6, 76.1, 73.1, 72.1, 71.6, 71.5, 69.9, 59.5, 53.6, 48.8, 36.9, 26.4 ppm. HRMS (ESI) *m/z* calcd for $C_{19}H_{28}N_4O_6Na$: 431.1907; found: 431.1916 $[M+Na]^+$.

2.2.4.2. Synthesis of 2-(hydroxymethyl)-6-((1-(3-(methyl(phenyl)amino)propyl)-1-H-1,2,3-triazol-4-yl)methoxy)tetrahydro-2H-pyran-3,4,5-triol (αGaTZLOH): αGaTZLOH was synthesized by reacting 2,3,4,6-Tetra-*O*-acetyl-1-(2′-propargyl)-α-D-galactose and compound 3 to obtain acetylated derivative. Which then reaction with sodium carbonate yielded αGaTZLOH as colourless viscous liquid (1.4 g, 58%). **^{1}H NMR** (CD_3CN, 500 MHz): δ 7.78 (s, 1 H), 7.21-7.09 (m, 2 H), 6.69-6.64 (m, 3 H), 5.01 (bs, 1 H), 4.75 (d, *J* = 12.5 Hz, 1 H), 4.61 (d, *J* = 12.5 Hz, 1 H), 4.38 (t, *J* = 7.0 Hz, 2 H), 3.99-3.98 (m, 3 H), 3.94 (s, 1 H), 3.75 (bs, 1 H), 3.60 (d, *J* = 6.0 Hz, 2 H), 3.34 (t, *J* = 7.0 Hz, 2 H), 2.13 (t, *J* = 7.0 Hz, 2 H) ppm. **^{13}C NMR** (CD_3CN, 125 MHz): δ 149.2, 144.2, 129.1, 123.7, 117.3, 116.3, 112.4, 107.4, 84.5, 81.3, 78.1, 63.3, 59.8, 49.2, 47.8, 37.7, 27.02 ppm. HRMS (ESI) *m/z* calcd for $C_{19}H_{28}N_4O_6Na$: 431.1907; found: 431.1910 $[M+Na]^+$.

2.2.4.3. Synthesis of 2-(hydroxymethyl)-6-((1-(3-(methyl(phenyl)amino)propyl)-1-H-1,2,3-triazol-4-yl)methoxy)tetrahydro-2H-pyran-3,4,5-triol (αMTZLOH): αMTZLOH was synthesized by reacting 2,3,4,6-Tetra-*O*-acetyl-1-(2′-propargyl)-α-D-mannose and compound **3** to obtain acetylated derivative. Which then reaction with sodium carbonate yielded αMTZLOH as colourless viscous liquid (1.3 g, 60%). **^{1}H NMR** (Methanol-d_4, 500 MHz): δ 8.00 (s, 1 H), 7.19-7.16 (m, 2 H), 6.71-6.65 (m, 3 H), 4.87 (bs, 1 H), 4.81 (d, *J* = 12.0 Hz, 1 H), 4.66 (d, *J* = 12.0 Hz, 1 H), 4.46 (t, , *J* = 7.0 Hz, 2 H), 3.88-3.86 (bs, 1 H), 3.86 (bs, 1 H), 3.75-3.87 (m, 2 H), 3.65 (d, 1 H), 3.59-3.57 (m, 1 H), 3.38 (t, *J* = 7.5 Hz, 2 H), 2.22-2.16 (m, 2 H) ppm. **^{13}C NMR** (Methanol-d_4, 125 MHz): δ 149.3, 143.9, 128.7,124.1, 116.5, 112.6, 99.4, 73.6, 70.6, 67.2, 61.6, 59.3, 49.2, 37.3, 26.9 ppm. HRMS (ESI) *m/z* calcd for $C_{19}H_{28}N_4O_6Na$: 431.1907; found: 431.1907 $[M+Na]^+$.

2.2.4.4. Synthesis of 2-(hydroxymethyl)-6-((1-(3-(methyl(phenyl)amino)propyl)-1H-1,2,3-triazol-4-yl)methoxy)tetrahydro-2H-pyran-3,4,5-triol (βGlTZLOH): βGlTZLOH was synthesized by reacting 2,3,4, 6-Tetra-*O*-acetyl-1-(2′-propargyl)-β-D-glucose and compound **3** to obtain benzoylated derivative. Which then reaction with sodium carbonate yielded βGlTZLOH as colourless viscous liquid (1.4 g, 54%). **^{1}H NMR** (CD_3CN, 500 MHz): δ 7.82 (s, 1 H), 7.19-7.16 (m, 2 H), 6.67-6.64 (m, 3 H), 4.91 (d, *J* = 12.5 Hz, 1 H), 4.43 (d, *J* = 8.9 Hz, 1 H), 4.35 (t, *J* = 7.0 Hz, 2 H), 3.82 (d, *J* = 11.5Hz, 1 H), 3.68-3.66 (m, 1 H), 3.44-3.40 (m, 1 H), 3.36-3.3 (m, 2 H), 3.25 (t, *J* = 8.0 Hz,1 H), 2.85 (s, 3 H), 2.10 (t, *J* = 7.5 Hz, 2 H) ppm. **^{13}C NMR** (CD_3CN, 125 MHz): δ 154.5, 149.2, 134.5, 129.4, 122.8, 121.6, 117.7, 107.3, 101.3, 83.3, 81.8, 78.8, 75.4, 67.2, 66.8, 54.5, 53.3, 43.2, 32.4 ppm. HRMS (ESI) *m/z* calcd for $C_{19}H_{28}N_4O_6Na$: 431.1907; found: 431.1916 $[M+Na]^+$.

2.2.5. Synthesis of SSqβGl: Symmetrical squaraine SSqβGl was synthesized by refluxing βGlTZLOH (500 mg, 1.22 mmol) and 3,4-dihydroxycyclobutene-1,2-dione (70 mg, 0.6 mmol) in 1-butanol/benzene (2:1) solvent mixture at 110 °C for 20 h accompanied by azeotropic removal of water using Dean Stark trap. After completion of the reaction, solvent was removed under reduced pressure. Residue obtained was purified by repeated precipitation from MeOH/EtOAc (1:10) solvent mixture and finally from methanol. The desired product was obtained as dark blue solid (160 mg, 15%) m p:165-170 °C (decomposing). **^{1}H NMR** (DMSO-d_6, 500 MHz): δ 8.18 (s, 2 H), 8.14 (d, *J* = 8.5 Hz, 4 H), 6.96 (d, *J* = 9.0 Hz, 4 H), 5.04-5.03 (m, 2 H), 4.94-4.91 (m, 4 H), 4.75 (d, *J* = 12.5 Hz, 4 H), 4.57 (bs, 2 H), 4.46 (t, *J* = 7.0 Hz, 2 H), 4.26 (d, *J* = 7.5Hz, 2 H), 3.72-3.69 (m, 2 H), 3.61 (m, 4 H), 3.47-3.44 (m, 2 H), 3.15 (s, 6 H), 3.13-3.12 (m, 3 H), 3.07-3.06 (m, 2 H), 2.99-2.98 (m, 2 H), 2.17 (t, 6.5Hz, 2H) ppm. **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 181.63, 154.04, 143.97, 131.67, 124.19, 118.83, 113.10, 102.09, 99.48, 76.94, 73.38, 61.52, 61.15, 46.93, 39.74, 39.58, 27.37 ppm; MALDI-TOF calculated for $C_{42}H_{54}N_8O_{14}$ ($[M]^+$) 894.3770 found 894.00.

2.2.6. General procedure for synthesis of unsymmetrical squaraines (SemSq): Unsymmetrical squaraines were synthesised by the condensation of 3-(4-(dimethylamino)phenyl)-4-hydroxycyclobut-3-ene-1,2-dione (1 equiv.) and corresponding triazole substrate (1 equiv.) in 1-butanol/benzene (1:2) solvent mixture at 110 °C for 8 h. Where 3-(4-(dimethylamino)phenyl)-4-hydroxycyclobut-3-ene-1,2-dione was obtained from the conventional synthetic procedure.[4] After monitoring completion of the reaction using TLC, solvent was removed under reduced pressure. The residue obtained was purified by repeated precipitation from EtOAC/MeOH solvent mixture and finally from methanol solvent to yield the unsymmetrical squaraines as blue solid.

2.2.6.1. ASqαGl (124 mg, 17%). m p: 220-224 °C. **^{1}H NMR** (DMSO-d_6, 500 MHz): δ 8.17 (s, 1 H) 8.15-8.12 (m, 4 H), 6.98-6.95 (m, 4 H), 4.78 (d, J = 3.5 Hz, 1 H), 4.71 (d, J = 12.5 Hz, 1 H), 4.53 (d, J = 12.5 Hz, 1 H), 4.46 (t, J = 7.0 Hz, 2 H), 3.65 (bs, 1 H), 3.63 (m, 4 H), 3.20 (s, 6 H), 3.17 (s, 3 H), 3.15 (bs, 4 H), 2.21 (m, 2 H). **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 187.6, 181.6, 173.6, 155.0, 153.6, 143.9, 131.5, 124.1, 124.1, 119.0, 113.1, 113.0, 97.93, 73.7, 71.8, 70.3, 60.92, 39.7, 25.3. HRMS (ESI): *m/z* calcd for $C_{31}H_{37}N_5O_8Na$: 630.2551; found: 630.2555 $[M+Na]^+$.

2.2.6.2. ASqβGl (132 mg, 18%) m p: 238-242 °C. **^{1}H NMR** (DMSO-d_6, 500 MHz): δ 8.81 (s, 1 H), 8.14 (t, J = 13.0 Hz, 4 H), 6.96 (t, J = 9.0 Hz, 4 H), 5.03-4.92 (m, 2 H), 4.26 (d, J = 8.0 Hz, 1 H), 4.85 (d, J = 12.5 Hz, 1 H), 4.66 (d, J = 12.0 Hz, 1 H), 4.45 (t, J = 7.0 Hz, 2 H), 4.26 (d, J = 8.0 Hz, 1 H), 3.65 (d, J = 11.5 Hz, 1 H), 3.60 (t, J = 7.0 Hz, 2 H), 3.47-3.44 (m, 1 H), 3.20 (s, 6 H), 3.14 (s, 3 H), 3.12 (bs, 2 H), 3.07-3.03 (m, 1 H), 2.99 (t, J = 8.0 Hz, 1 H), 2.17 (t, J = 7.0 Hz, 2 H). **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 187.0, 182.2, 155.6, 144.4, 132.2, 132.0, 124.7, 119.4, 119.2, 113.7, 102.6, 77.5, 77.2, 73.9, 70.6, 62.04, 61.67, 49.65, 47.5, 40.8, 39.18, 27.9 ppm. HRMS (ESI): *m/z* calcd for $C_{31}H_{37}N_5O_8Na$: 630.2551; found: 630.2556 $[M+Na]^+$.

2.2.6.3. ASqαGa (122 mg, 16%). m p: 165-170 °C (decomposing). **^{1}H NMR** (DMSO-d_6, 500 MHz): δ 8.15-8.12 (m, 5 H), 6.98-6.95 (m, 4 H), 4.84 (bs, 1 H), 4.66 (d, J_1 = 12.0 Hz, 1 H), 4.52 (d, J = 12Hz , 1H), 4.45 (t, J = 7.0 Hz, 2 H), 3.86-3.83 (m, 1 H), 3.79-3.78 (m, 2 H), 3.61-3.58 (m, 2 H), 3.51-3.50 (m, 3 H), 3.36 (s, 6 H), 3.20 (s, 3 H), 2.17 (t, J = 7.0Hz, 2H) ppm. **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 186.3, 185.4, 154.8, 143.9, 131.6, 124.0, 118.8, 118.5, 113.1, 106.9, 94.5, 82.1, 76.7, 70.2, 62.6, 59.9, 49.1, 46.9, 38.9, 27.3. HRMS (ESI) *m/z* calcd for $C_{31}H_{37}N_5O_8Na$: 630.2551; found: 630.2549 $[M+Na]^+$.

2.2.6.4. ASqαM (112mg, 14%). m p: 228-232 ˚C. **^{1}H NMR** (DMSO-d_6, 500 MHz): δ 8.01 (s, 1H), 7.19-7.1 (m, 2H), 6.71-6.65 (m, 3H), 4.87 (bs, 1H), 4.81 (d, J = 12.0 Hz, 1 H), 4.66 (d, J = 12.0 Hz, 1 H), 4.46 (t, J = 7.0 Hz, 2H), 3.88 (bs, 1 H), 3.86 (bs, 1 H), 3.75-3.69 (m, 2 H), 3.65 (d, 1 H), 3.59-3.57 (m, 1 H), 3.38 (t, J = 7.5 Hz, 2 H), 2.22-2.17 (m, 2 H) ppm. **^{13}C NMR** (DMSO-d_6, 125 MHz): δ 149.3, 143.9, 128.7, 124.1, 116.5, 112.6, 99.3, 73.5, 71.1, 70.6, 67.2, 61.6, 59.3, 49.2, 37.3, 26.9 ppm. HRMS (ESI): *m/z* calcd for $C_{31}H_{37}N_5O_8Na$: 630.2551; found: 630.2555 $[M+Na]^+$.

3. Normalised absorption and emission spectra of ASqβGl and SSqβGl dyes

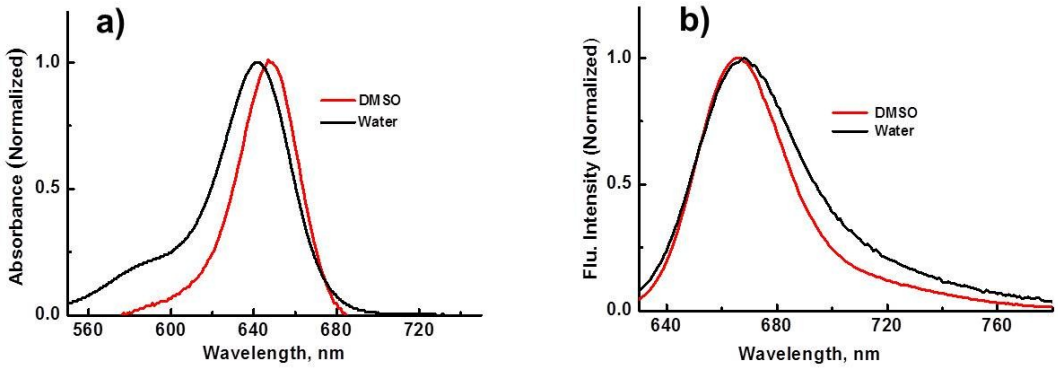
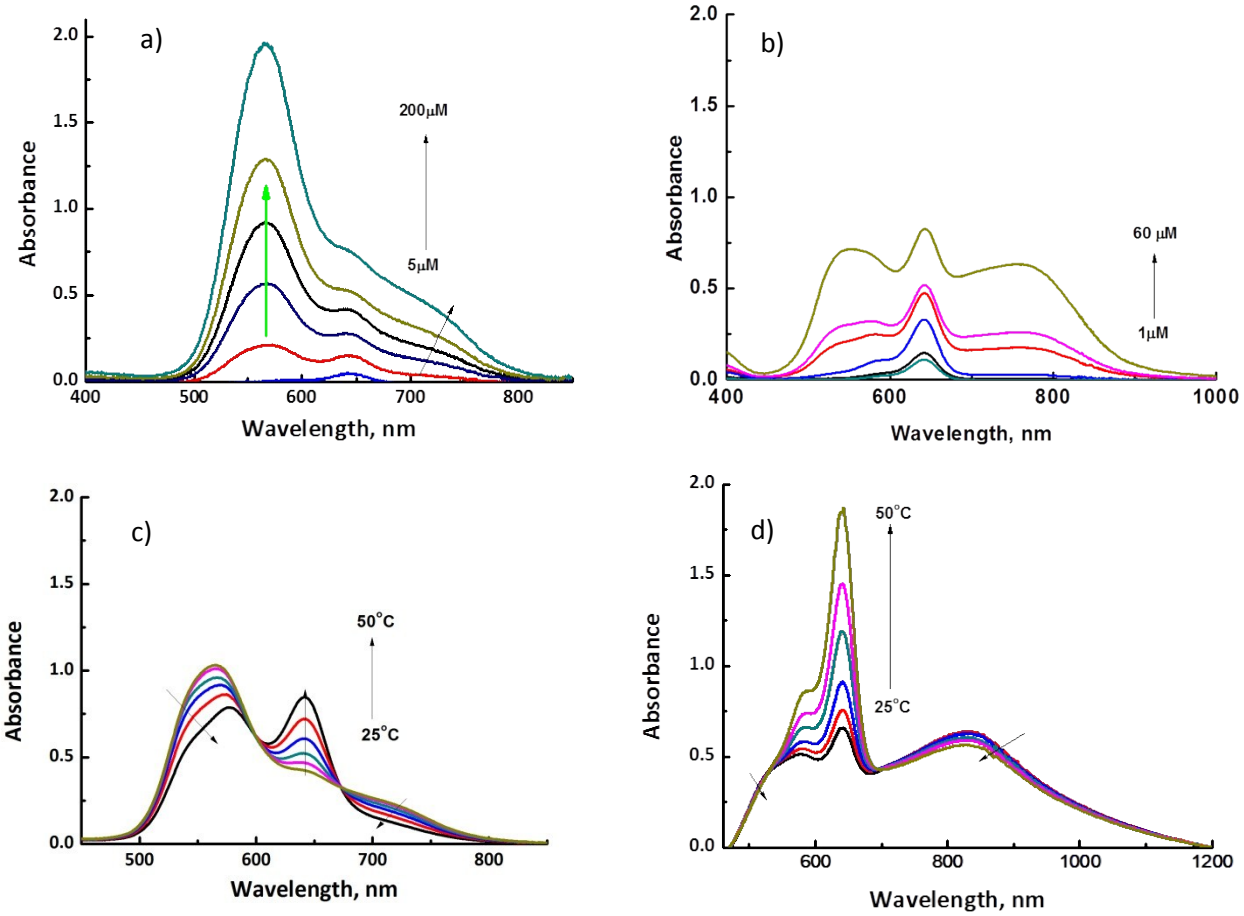


Figure S1. Normalized UV-Vis absorption and emission spectra of ASqβGl (a & b) and SSqβGl (c & d) in DMSO and water.

4. Concentration and temperature dependent absorption spectral changes of SSqβGl and ASqβGl in water



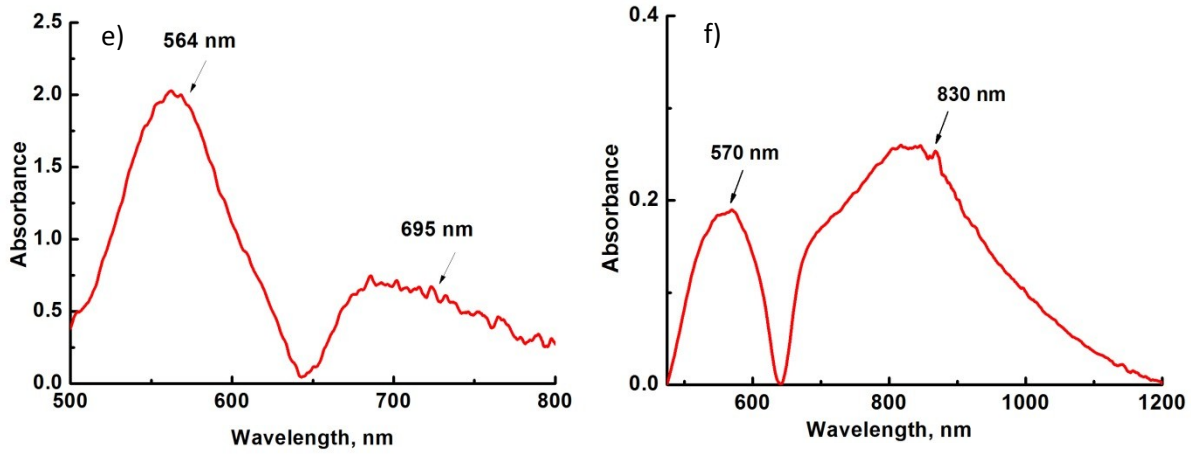


Figure S2. Absorption spectral changes of (a) SSqβGl [5, 20, 40, 80, 100, 200 μM, pathlength 10 mm] (b) ASqβGl [1, 5, 10, 20, 30 & 60 μM, Path length 1 mm] in water with increase in concentration from and temperature dependent absorption spectral changes of (c) SSqβGl (20 μM) and (d) ASqβGl (30 μM) with increase in temperature from 25, 30, 35, 40, 45 and 50 °C (e) Difference spectra obtained by subtracting the absorption spectra of the aggregate normalized at λ, 649 nm, from that of monomer (at lower concentration) of SSqβGl (f) Difference spectra obtained by subtracting the normalized absorption spectra of the aggregate from that of monomer (at lower concentration) of ASqβGl.

5. Concentration dependent emission spectral changes of ASqβGl and SSqβGl in water

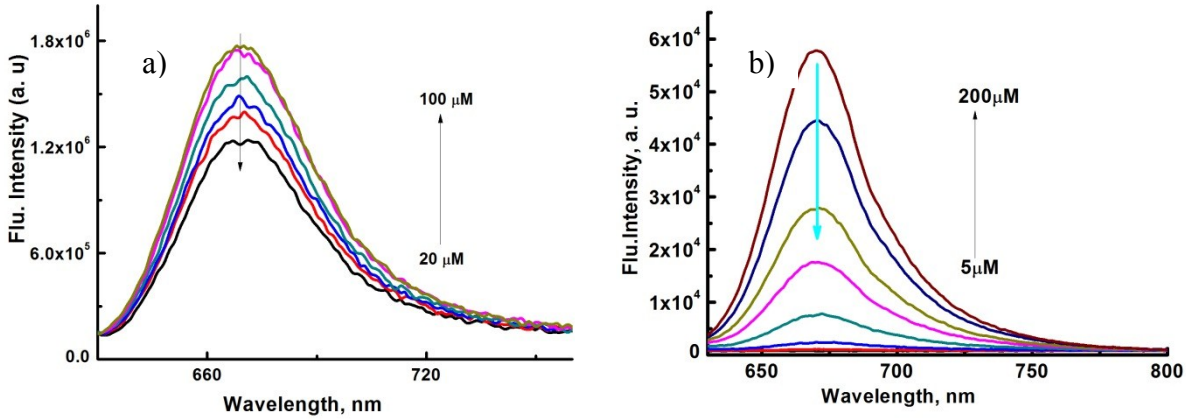


Figure S3. Changes in emission spectra of (a) ASqβGl [20, 30, 40, 60, 80 and 100 μM**]** and (b) SSqβGl [5, 10, 20, 40, 60, 80, 100 and 200 μM] in water solution.

6. Excitation spectra of ASqβGl and SSqβGl in water

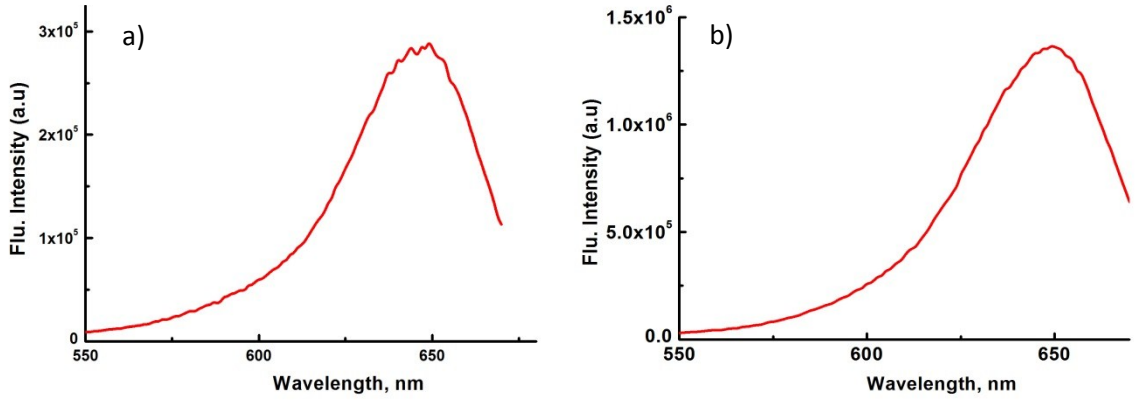


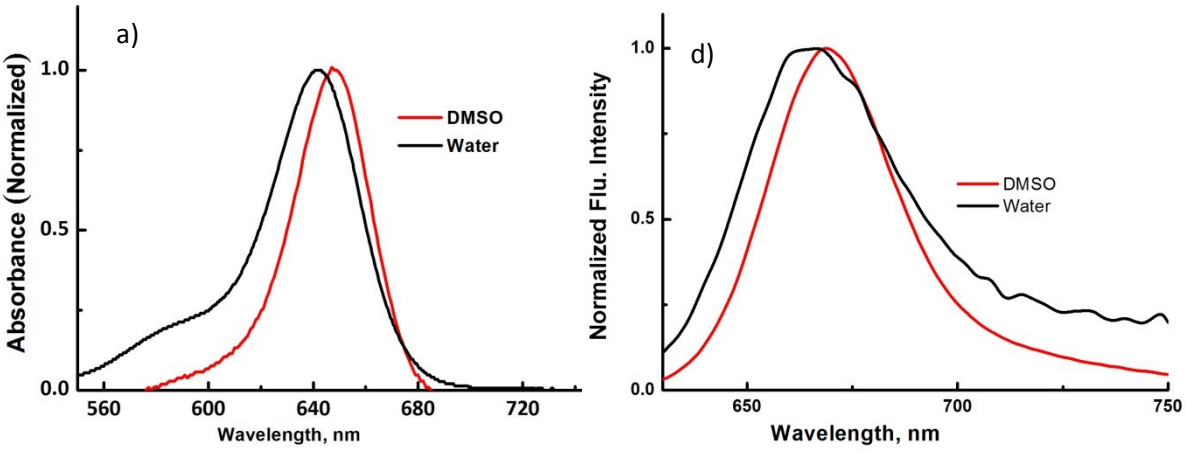
Figure S4. Excitation spectra of (a) ASqβGl [5.5×10^{-5} M concentration, emission collected at 670 nm] and (b) SSqβGl [5.5×10^{-5} M concentration, emission collected at 669 nm]

Table S1. Photophysical data of squaraine dyes ASqαGl, ASqαGa & ASqαM

Solvent	Abs λ_{max} (nm)	Em λ_{max} (nm)	Stokes shift (cm^{-1})	ε ($M^{-1}cm^{-1}$)	Φ_f (%)
		ASqαGl			
DMSO	648	669	484.4	$1.8 \pm 0.1 \times 10^5$	0.26
Water	642	667	583.8	-	0.01
		ASqαGa			
DMSO	649	669	460.6	$1.7 \pm 0.2 \times 10^5$	0.28
Water	642	667	583.8	-	0.03
		ASqαM			
DMSO	648	668	462.0	$1.6 \pm 0.1 \times 10^5$	0.25
Water	642	667	583.8	-	0.02

(Accuracy of fluorescence quantum yield= 0.05)

7. Normalized absorption and emission spectra of ASqαGl, ASqαGa & ASqαM



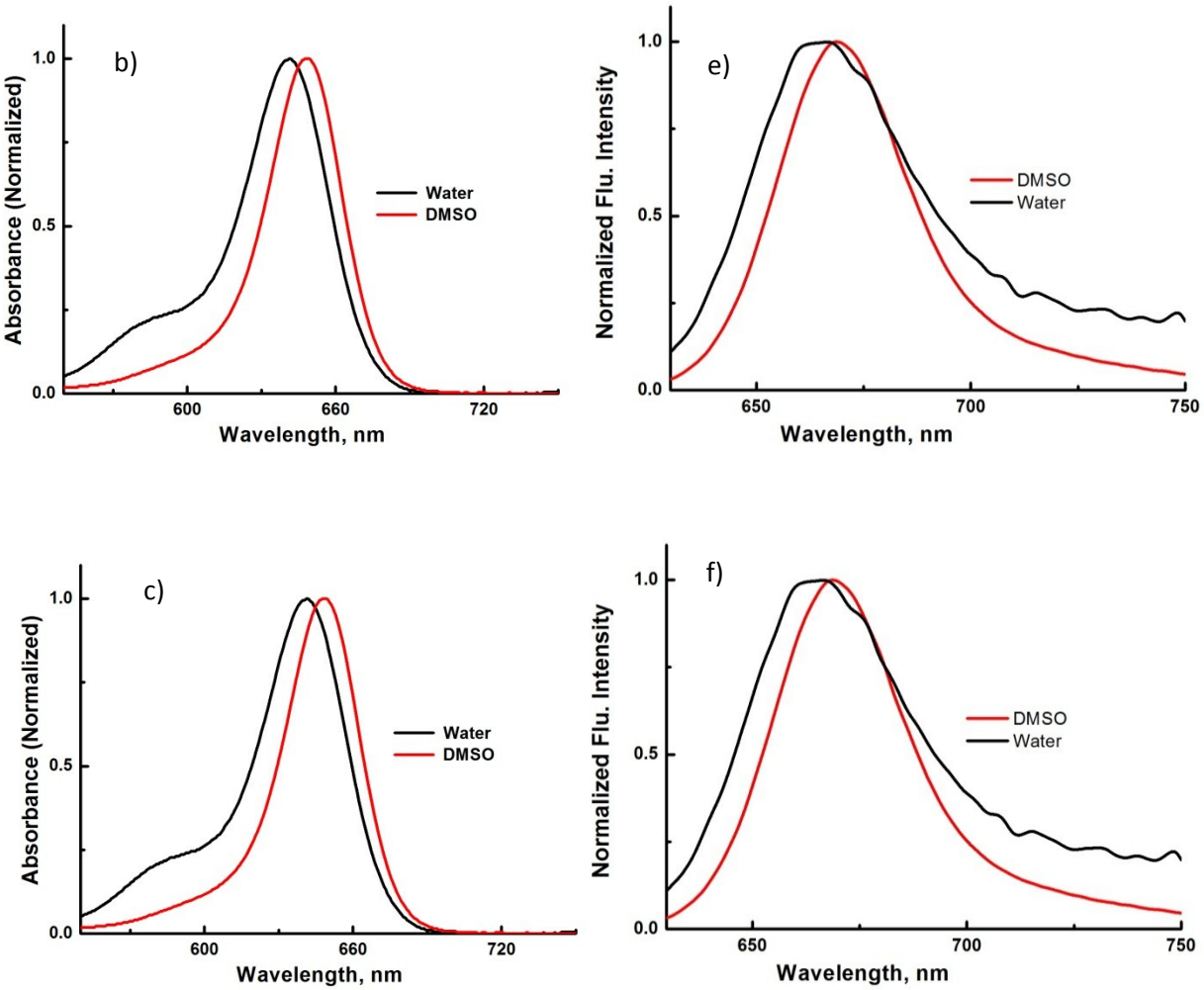
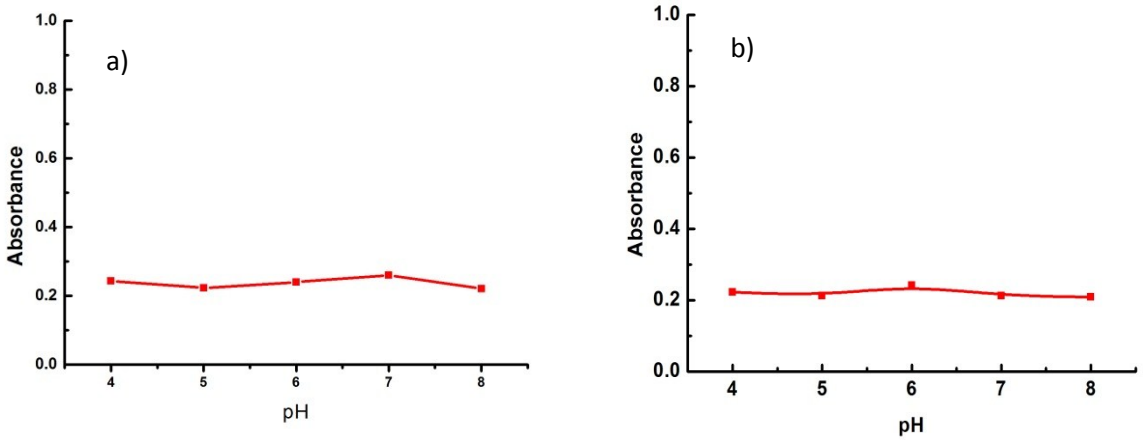


Figure S5. Normalized absorption and emission spectra of ASqαGl (a and b) (b) ASqαGa (c) ASqαM and normalized emission spectra of (d) ASqαGl (e) ASqαGa (f) ASqαM in DMSO and water solvents.

8. Stability measurement

Stability of Sq dyes in phosphate buffer saline solution over a pH range of 4-8 is confirmed by measuring the absorption and emission spectrum the probe at varying pH levels. Optical properties remains undisturbed indicate the stability of the dyes at different pH levels.



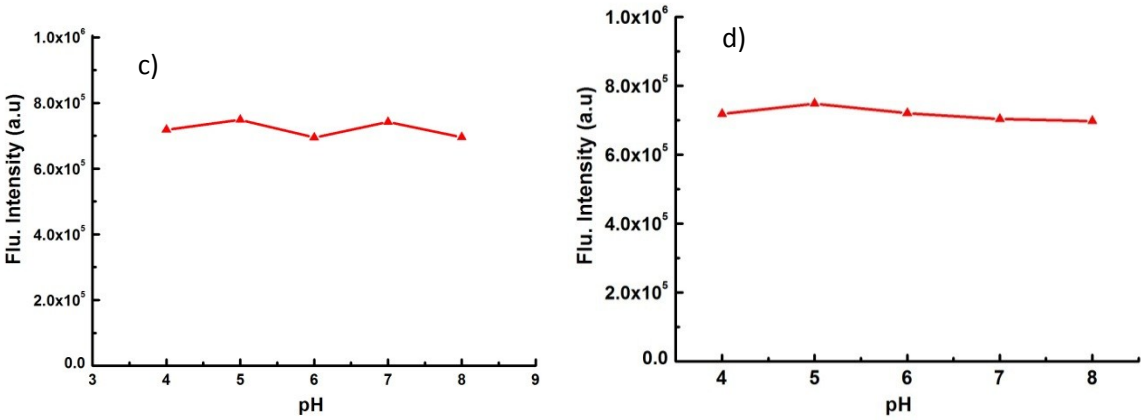


Figure S6. Changes in the absorption spectra with varying pH, (a) ASqβGl ($4.4x10^{-6}$ M) (b) ASqαGl ($4.4x10^{-6}$ M) and changes in the emission spectra with varying pH (c) **ASqβGl** [Emission max. 668 nm] and (d) ASqαGl [Emission max. 667 nm, λ_{ex} 630 nm.]
[Path length 10 mm. Absorbance monitored at 641 nm for ASqβGl and 642 nm for ASqαGl were plotted against pH.]

9. Cytotoxicity assay

Cell viability after incubating the cells with different concentrations of squaraine dyes were determined by methyl thiazolyl tetrazolium (MTT) assay. It is a colorimetric assay based on the ability of live, but not dead cells to reduce MTT (yellow) to a purple formazan product. The cells were spread in 96-well plates at 10^4 cells/well. After 36 h of seeding, they were incubated with different concentrations of squaraine dyes individually for 24 h. Subsequently, the cells were exposed to MTT at a concentration of 50 µg/well for 2.5 to 3 h at 37 °C in CO_2 incubator. The working solution of MTT was prepared in Hanks balanced salt solution (HBSS). After viewing formazan crystals under the microscope, the crystals were solubilised by treating the cells with DMSO: isopropanol solvent mixture at a ratio of 1:1 for 20 min at 37 °C. Plate was read at an absorbance of 570 nm. The relative cell viability in percent was calculated using the following equation and cell viability of control cells were kept as 100%.

$$Relative\ cell\ viability = \frac{Absorbance\ of\ treated}{Absorbance\ of\ control} \times 100$$

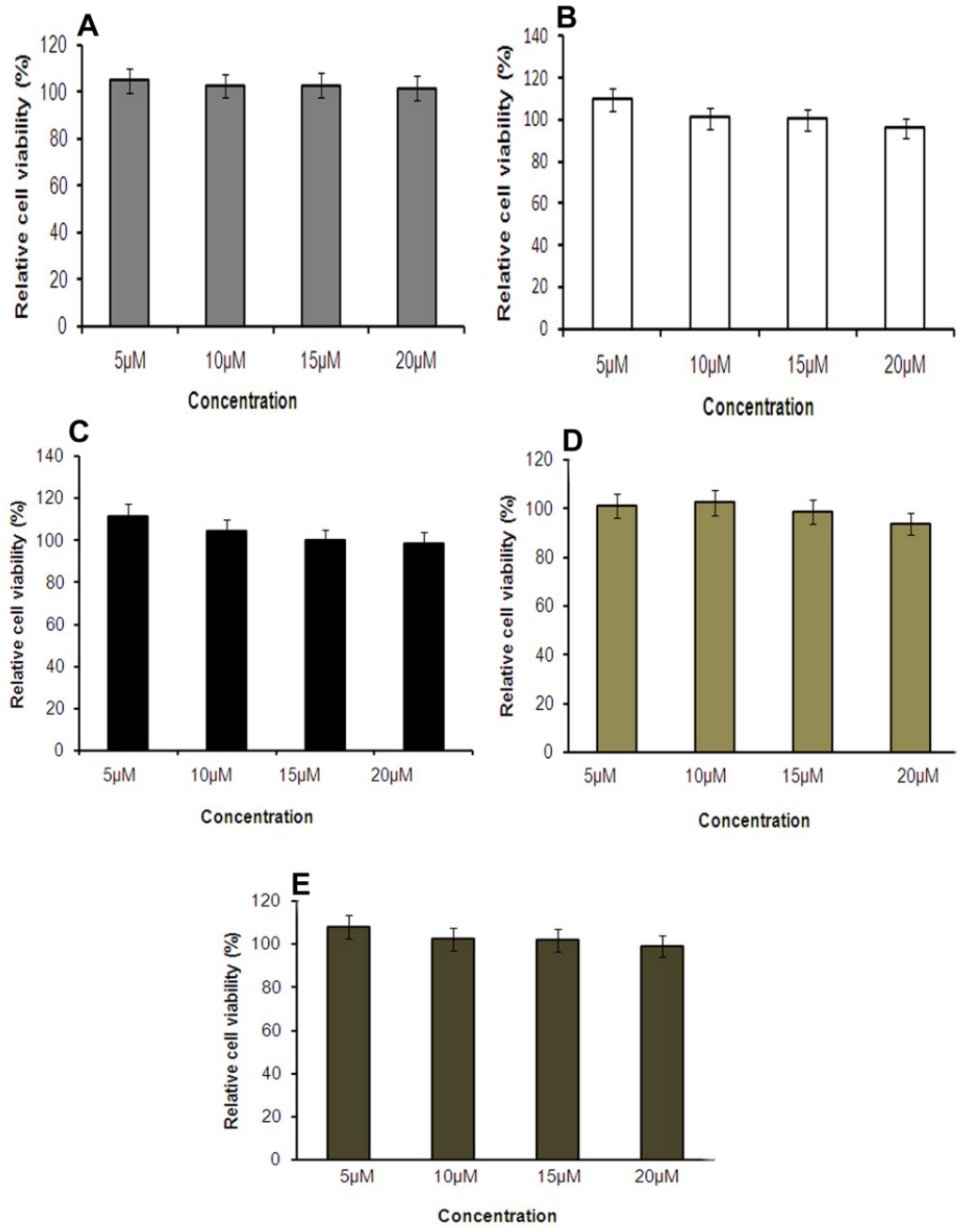


Figure S7. Relative cell viabilities of HeLa cells incubated with (**A**) SSqβGl (**B**) ASqβGl (**C**) ASqαGl, (**D**) ASqαGa and (**E**) ASqαM for a 24 h time period.

10. Optimum concentration

To obtain the optimum concentration of ASqαGl, ASqβGl and SSqβGl, we have collected the fluorescence images (by high-content spinning disk facility) of HeLa cells incorporated with different concentrations (5, 10, 20, 30 μM) of the dyes. Incubation time was 30 minutes. Based on the intensity of images obtained from repeated trials of imaging as well as by fluorimetric analysis of adherent cells, we have chosen the minimum concentration which yielded clear fluorescent images as optimum concentration. Accordingly 20, 15 and 10 μM were the optimum concentration for SSqβGl, ASqβGl and ASqαGl respectively. The dyes used for all the biological studies were dissolved in PBS containing 0.1% DMSO.

11. Cellular uptake studies of squaraine dyes by fluorescence imaging

Cellular uptake studies of squaraine dyes were executed by fluorescence imaging of adherent cells. The cells were seeded at a density of 10^4 cells/well of 96 well black plates *(BD Biosciences, USA)* for the purpose. After 36 h of seeding, the cells were incubated with different concentrations of squaraine dyes in serum deprived low glucose medium (5.5 mM glucose) for 30 min. Subsequently, the cells were washed twice with PBS solution. Nuclear staining was done by Hoechst. Images of the cells were collected by high-content spinning disk facility *(BD Pathway 855; BD Biosciences)* using *AttoVision 1.5.3 software.* For imaging cellular uptake of squaraine dyes, B635/20 excitation filter was used. Solution of the Sq dye used for imaging studies contains 1% DMSO in PBS solution.

12. Fluorescence images of HeLa cells incubated with ASqβGl & SSqβGl

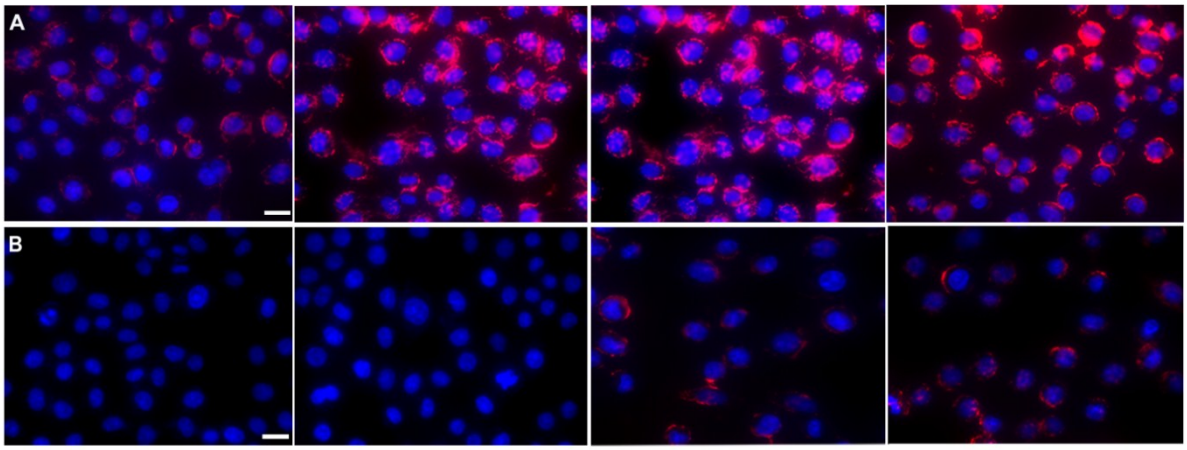


Figure S8. Fluorescence images of HeLa cells incubated with 10, 15, 20 & 30 µM concentrations of A) ASqβGl, B) SSqβGl. Nuclear staining using Hoechst dye. (Incubation time: 30 min., Scale bar: 20 µm)

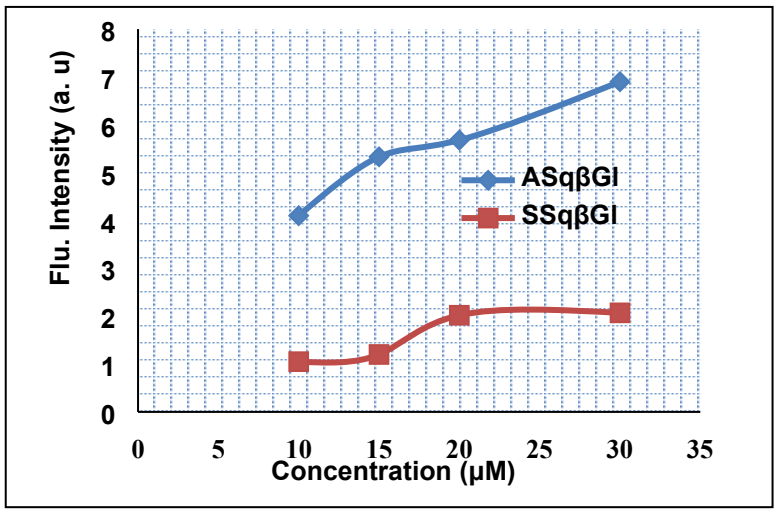
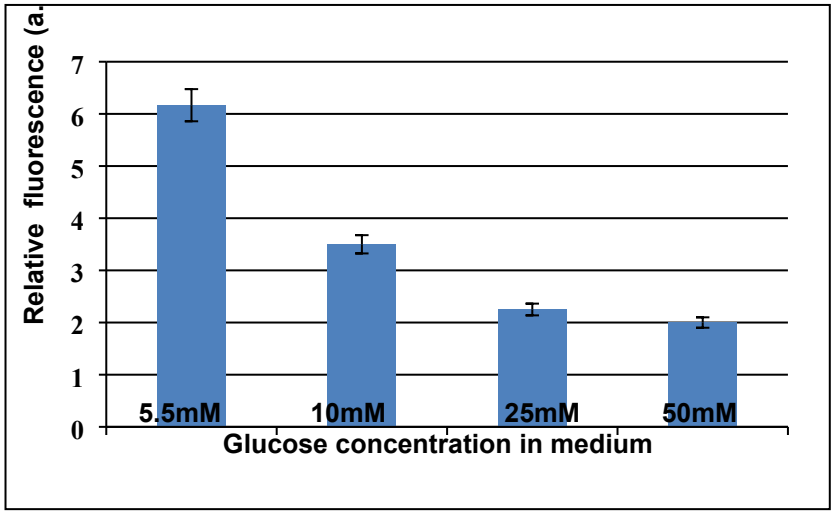


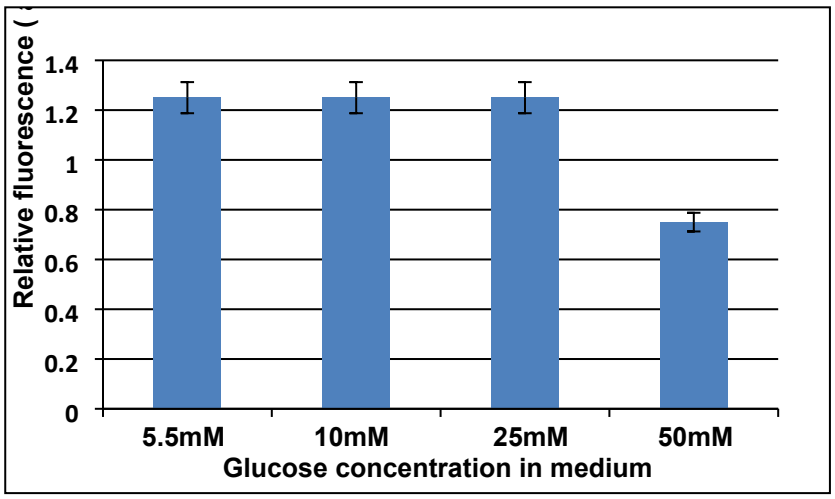
Figure S9. Chart representing the fluorescence intensity from the cells incubated with ASqβGl and SSqβGl.

13. Inhibiton of the dye uptake in the presence of D-glucose

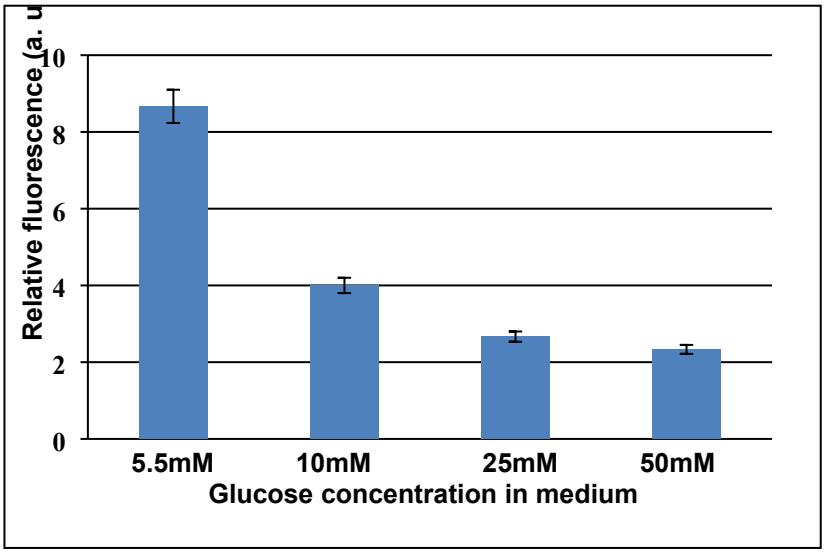
a)



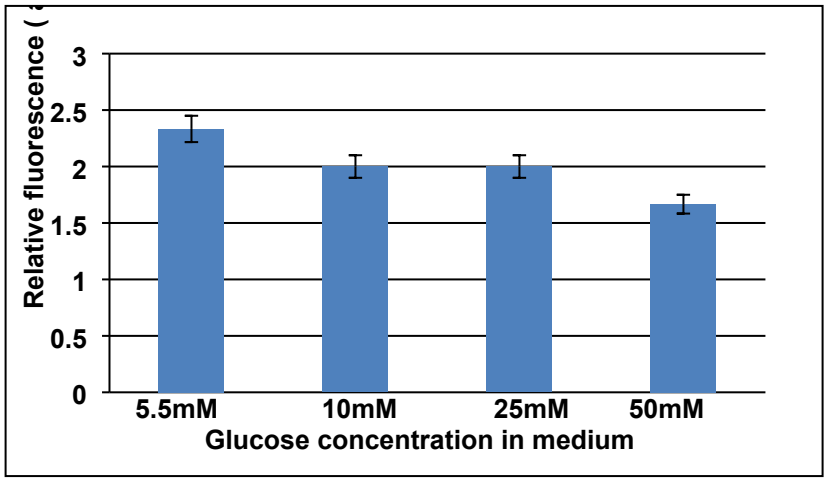
b)



c)



d)



e)

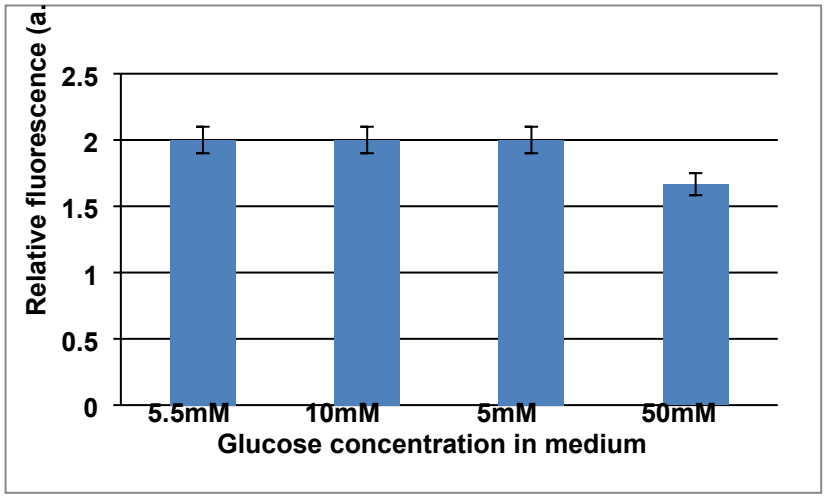


Figure S10. Relative fluorescence intensities for HeLa cells incorporated with (a) ASqβGl (15 µM), (b) SSqβGl (20 µM), (c) ASqαGl (10 µM), (d) ASqαGa (15 µM) and (e) ASqαM (15 µM) at different glucose concentrations.

14. Fluorescence images of HeLa cells incubated with ASqαGa & ASqαM

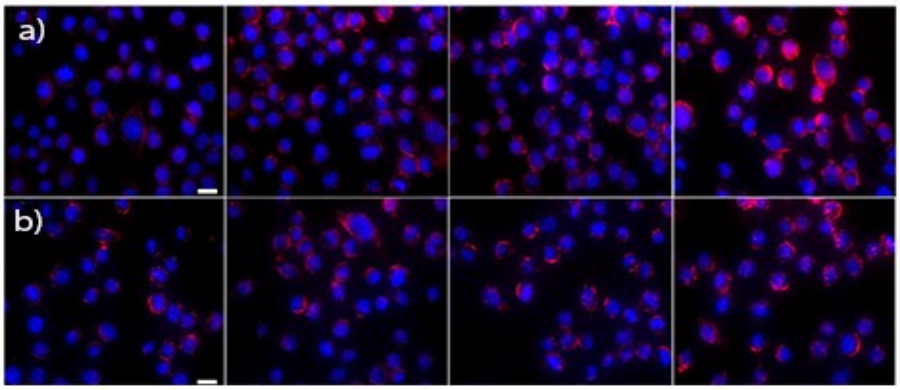
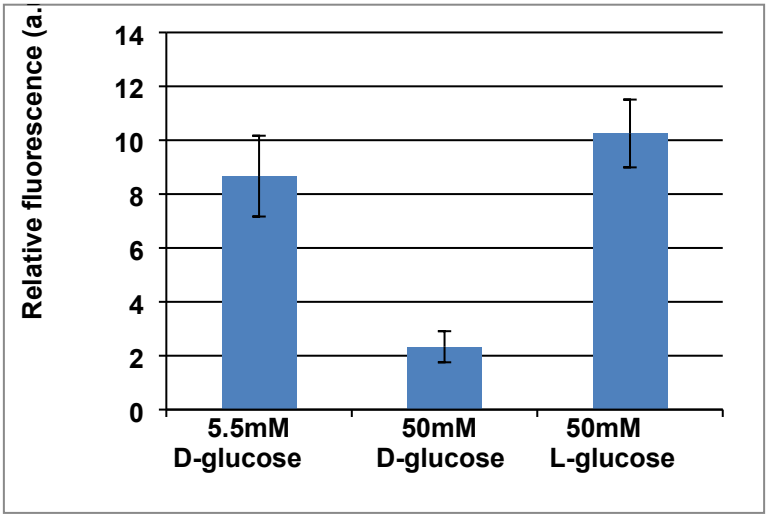


Figure S11. Fluorescence images of HeLa cells incubated with 10, 15, 20 & 30 µM concentrations of (a) ASqαGa and (b) ASqαM. Nuclear staining using Hoechst dye. (Incubation time: 30 min., Scale bar 20 µm)

15. Inhibition of dye uptake in presence of L-glucose



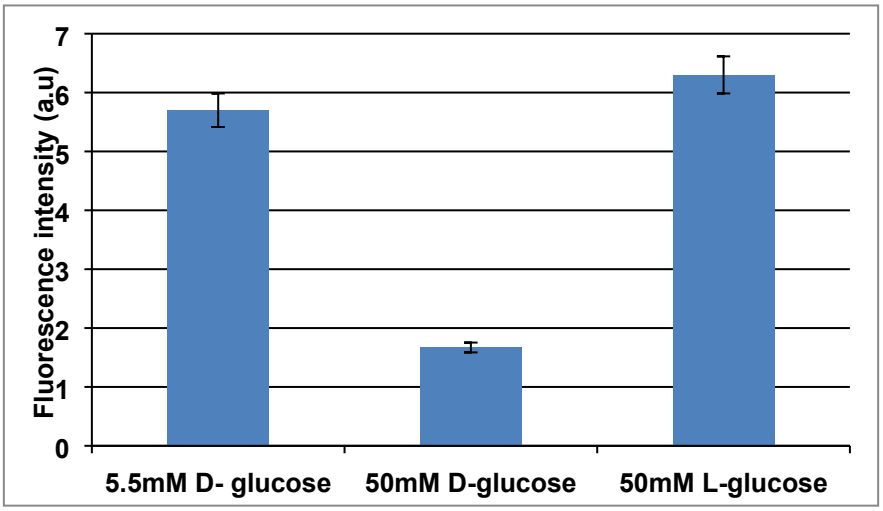


Figure S12. Relative fluorescence intensities for HeLa cells incroporated with 15 µM concentration of the Sq dye (a) ASqαGl and (b) ASqβGl.

16. Fluorescence images of HeLa and H9c2 cell lines incubated with ASqαGl: a comparative analysis

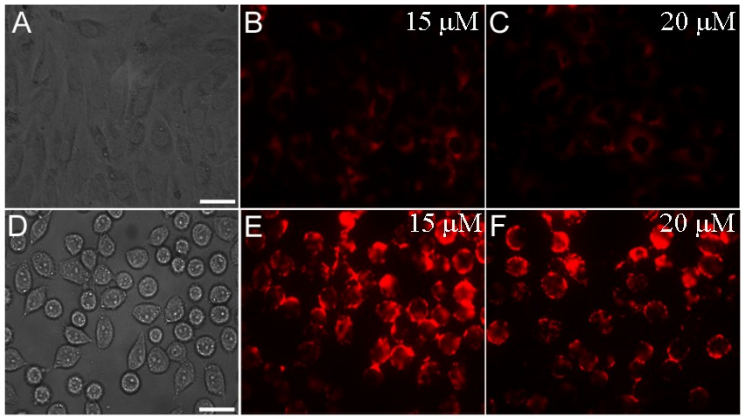


Figure S13. Transmitted light images of H9c2 (A) and HeLa (D) and the corresponding fluorescence images of H9c2 (B & C) and HeLa (E & F) incubated with ASqαGl : 15 and 20 µM respectively. The images were obtained by high-content spinning disk facility. Scale bar: 20 µm

17. Preferential uptake of Sq probe, ASqαGl in cancer cell lines (HeLa) over normal cell lines (H9C2): Analysis by flow cytometry

The cells were seeded at a density of 5x 10^4 cells in a 24-well plate. After attaining confluence, the cells were washed twice with PBS and incubated with ASqαGl for 30 min. The cells were subsequently washed with PBS and subjected to trypsinization followed by trypsin inactivation using 10% FBS containng PBS. The cells were then centrifuged at 750 rpm for 4 min and the pellets were resuspended in PBS for flow cytometric analysis

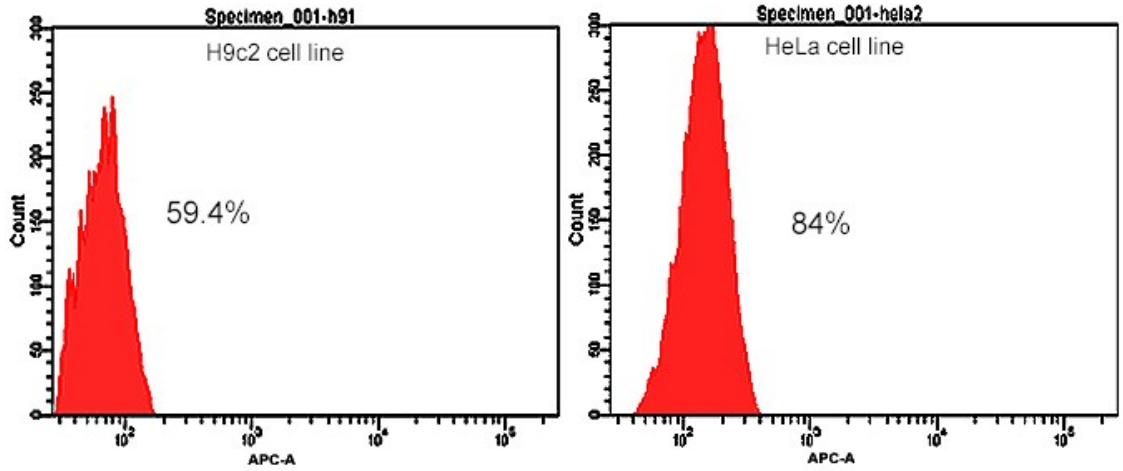


Figure S14. Representative histograms showing cellular uptake of ASqαGl demonstrated by mean cell fluorescence levels in APC-A histograms

18. Fluorescence image of SW480 cells incubated with ASqαGl

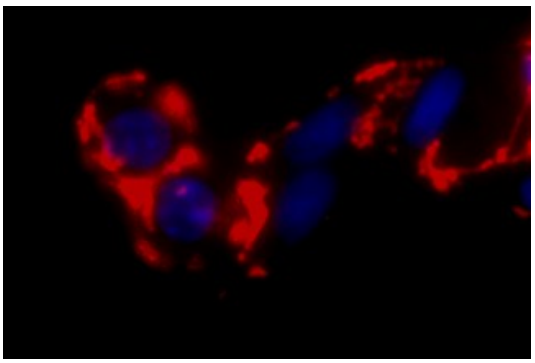


Figure S15. Fluorescence image of SW480 cells incubated with 10 µM concentration of ASqαGl. Nuclear staining using Hoechst dye. (Incubation time: 30 min., Scale bar: 20 µm)

19. Fluorescence intensity of ASqαGl in HeLa cells compared to 2-NBDG

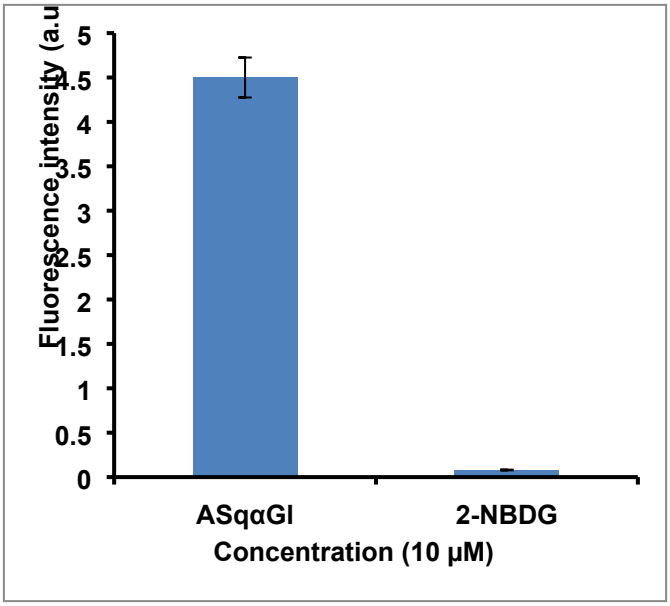


Figure S16. Fluorescent intensity of ASqαGl (10 μM) in HeLa cells compared with the commercially available glucose conjugtae 2-NBDG (10 μM) under similar experimental conditions.

20. *In vitro* stability assay of the Sq probe ASqβGl

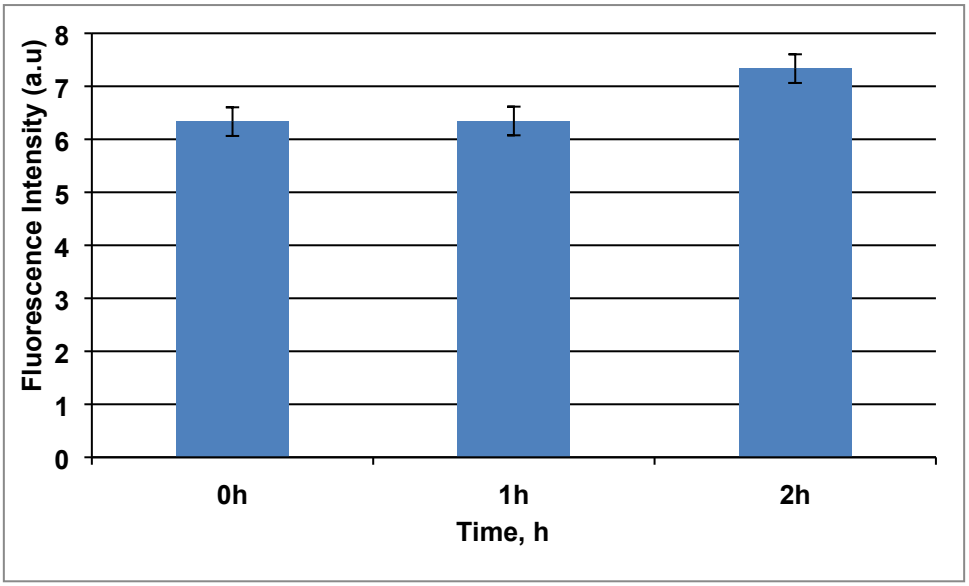


Figure S17. Graph representing the stability of the probe inside the cells demonstrated by the fluorescent intensities recorded from the cells . Fluorescence intensity of the probe AsqαGl (15 μM) was monitored for 2 hrs**.**

21. Emission intensity of the Sq probe, ASqαGl before and after internalization in Hela cells

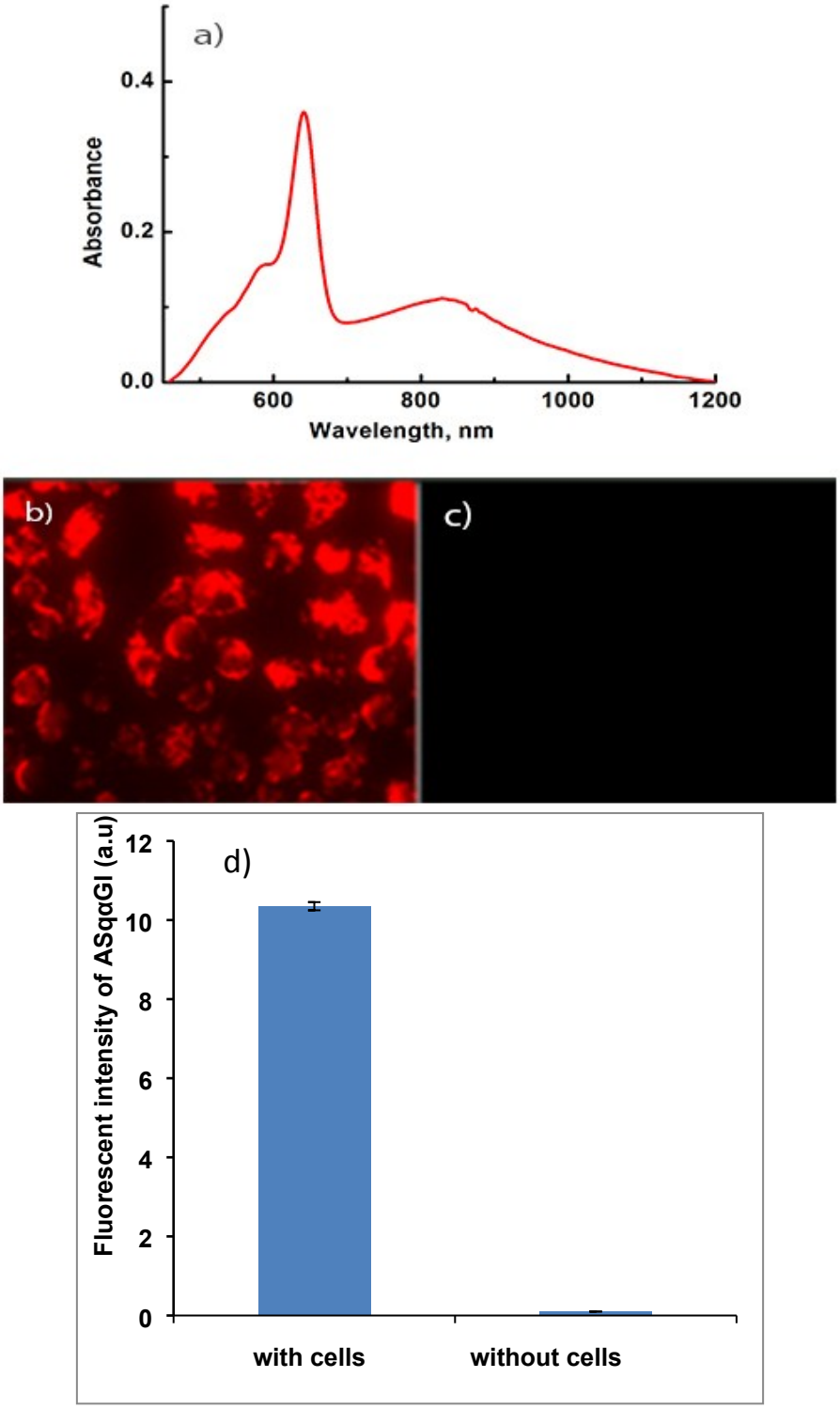


Figure S18. (a) UV visible absorption spectra of ASqαGl (15 μM) in PBS solution b) ASqαGl (15 μM) showing fluorescence inside the cells after incubation for 30 min c) ASqαGl not showing fluorescence in the absence of cells under similar experimental conditions

22. MALDI-TOF experiment.

HeLa cells were incubated with ASqαGl (30 μM) for 1 hour in low glucose medium. The cells were then washed twice with PBS, trypsinized using 0.25% trypsin and 0.02% EDTA in PBS to detach the cells from the substratum, trypsin-inactivated using 10% serum-containing PBS, and centrifuged at 1500 rpm for 3 minutes to pellet the cells. The supernatant was removed and the pelleted cells were resuspended in PBS and centrifuged at 1200 rpm for 1 minute. The supernatant was removed and the cell pellet was resuspended in deionized water and again subjected to centrifugation at 1200 rpm for 1 min. The supernatant was removed and to the pellet, 1 ml of DMSO was added that gave a light blue colour. This solution was subjected to MALDI-TOF experiment.

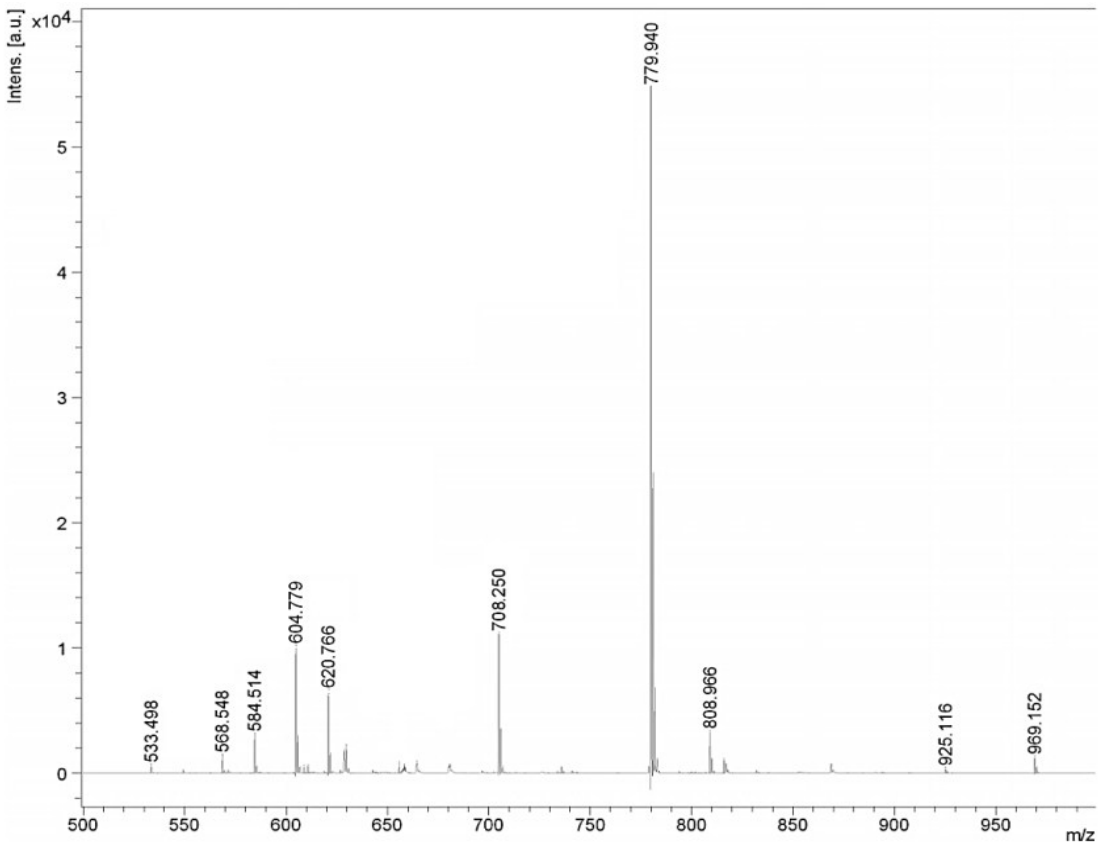


Figure S19. MALDI-TOF spectrum of phosphorylated ASqαGl (glucose-6-phosphate-ASqαGl)

23. NMR spectra

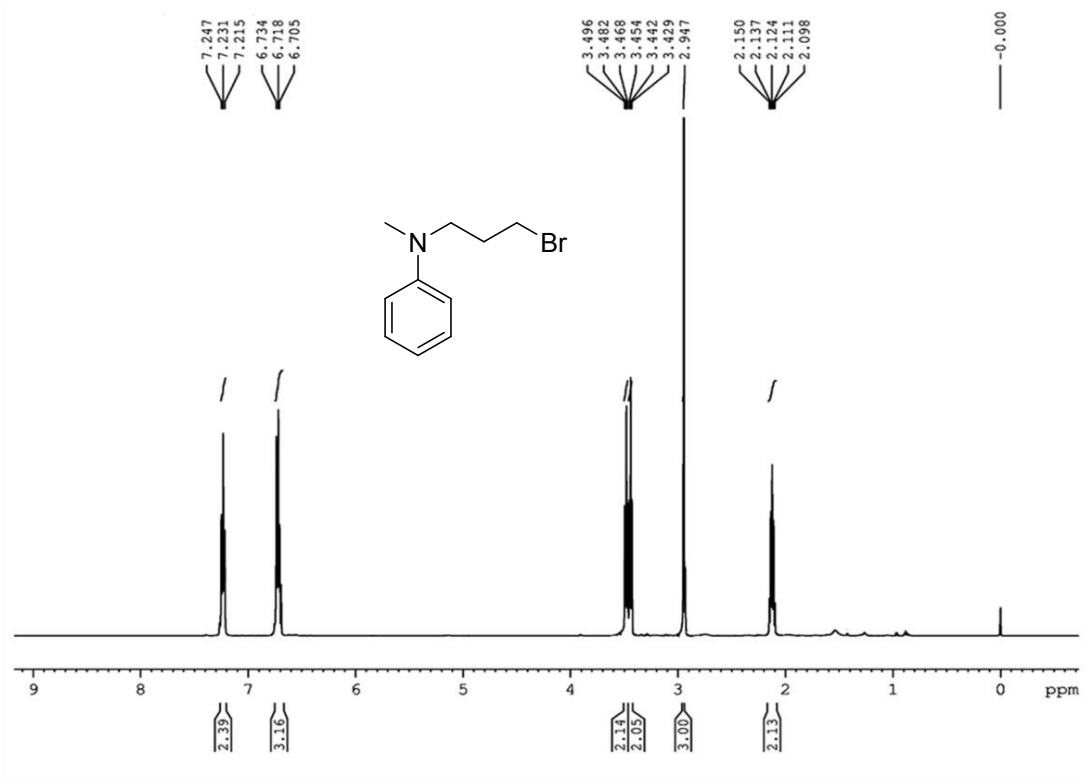


Figure S20. ^{1}H NMR spectrum of compound N-(3-bromopropyl)-N-methylaniline (2)

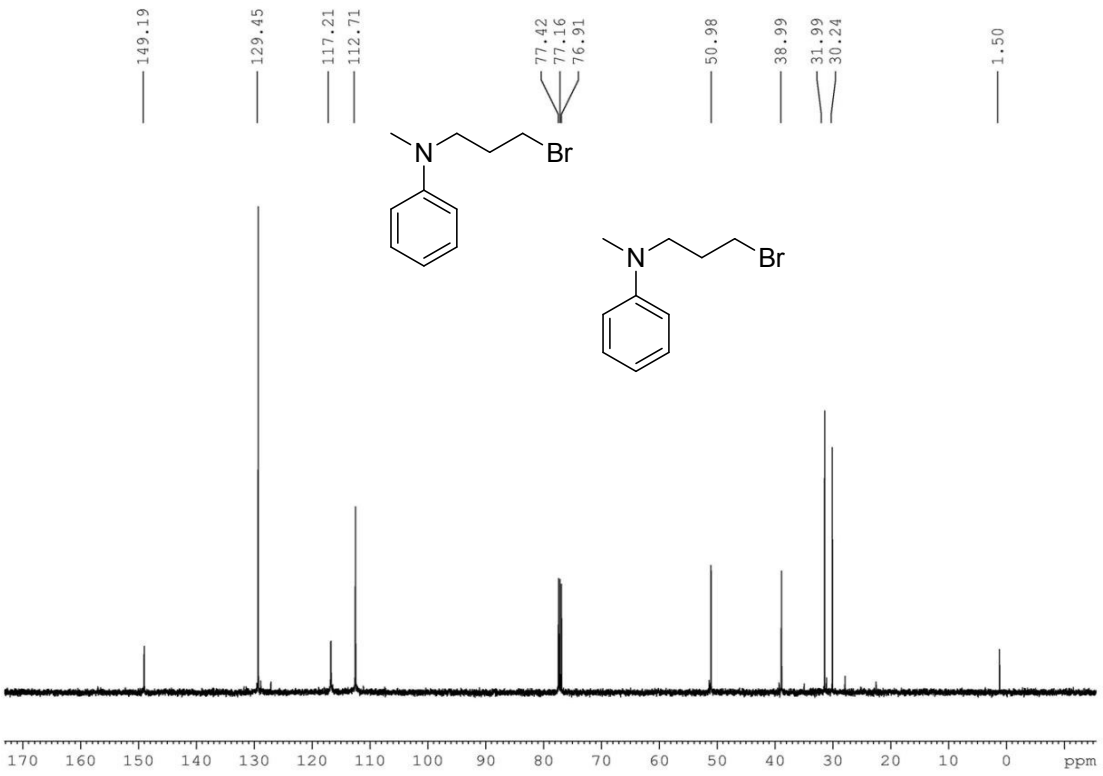


Figure S21. ^{13}C NMR spectrum of compound N-(3-bromopropyl)-N-methylaniline (2)

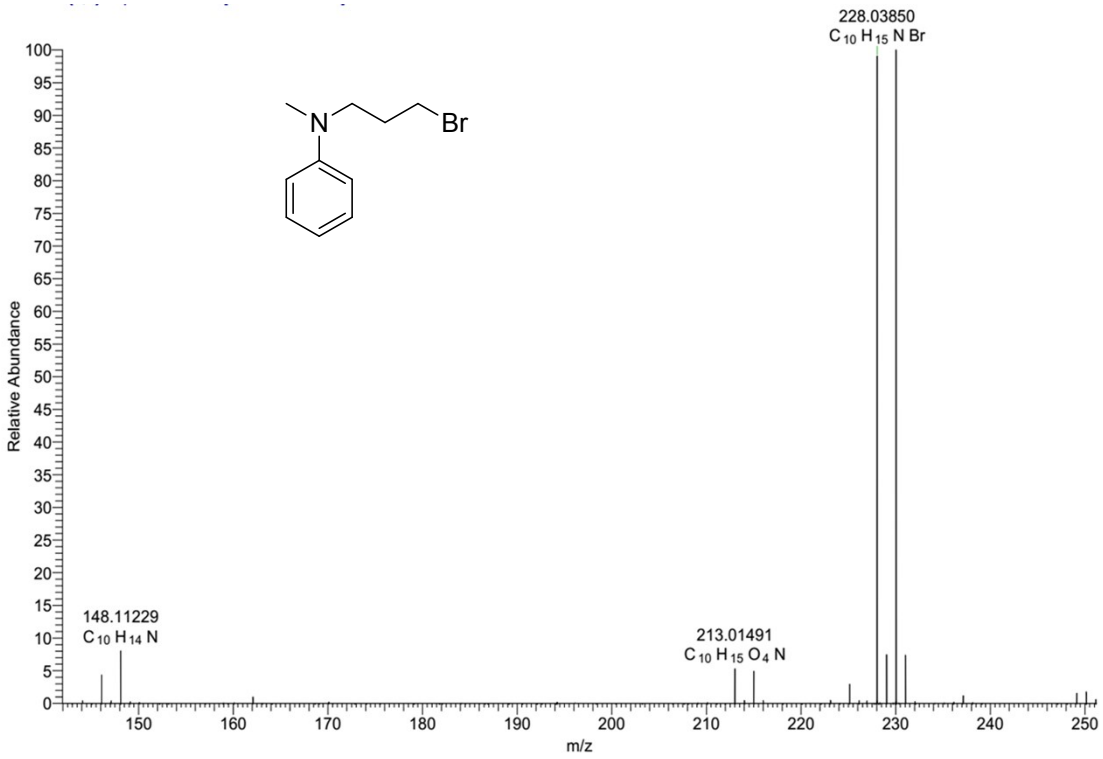


Figure S22. High resolution mass spectrum of compound N-(3-bromopropyl)-N-methylaniline (2)

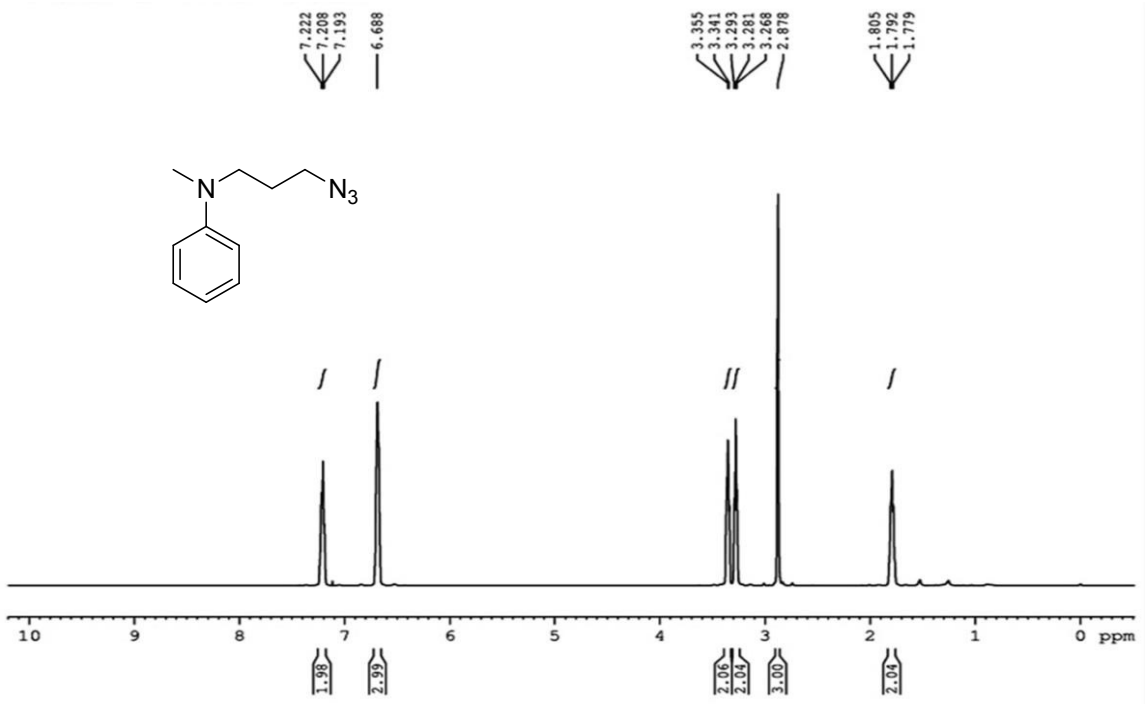


Figure S23. ^{1}H NMR spectrum of compound N-(3-azidopropyl)-N-methylaniline (3)

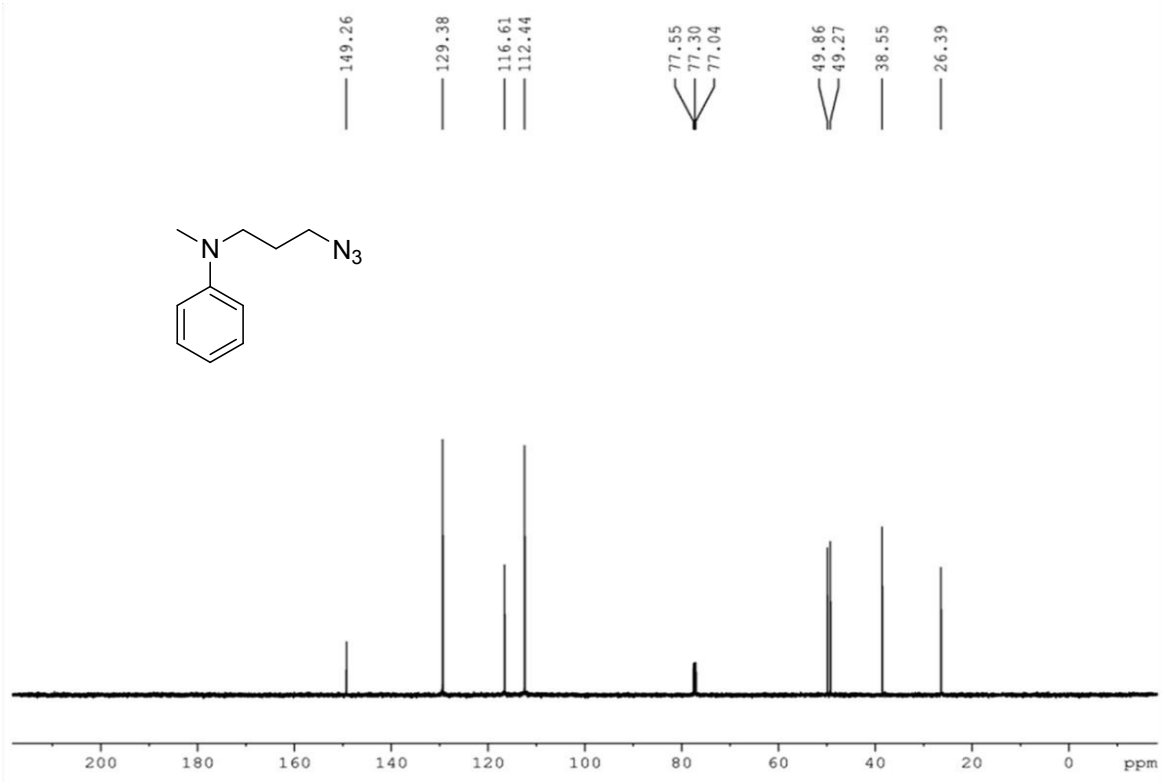


Figure S24. ^{13}C NMR spectrum of compound N-(3-azidopropyl)-N-methylaniline (3)

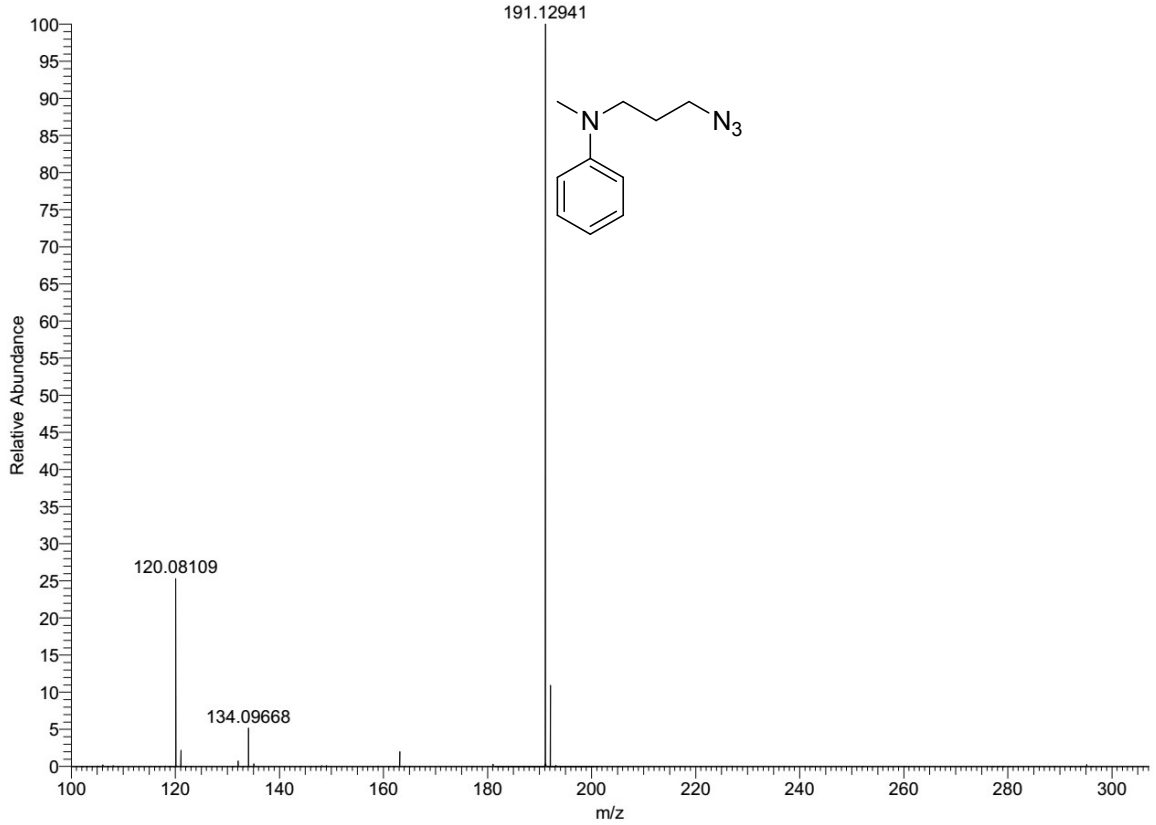


Figure S25. High resolution mass spectrum of compound N-(3-azidopropyl)-N-methylaniline (3)

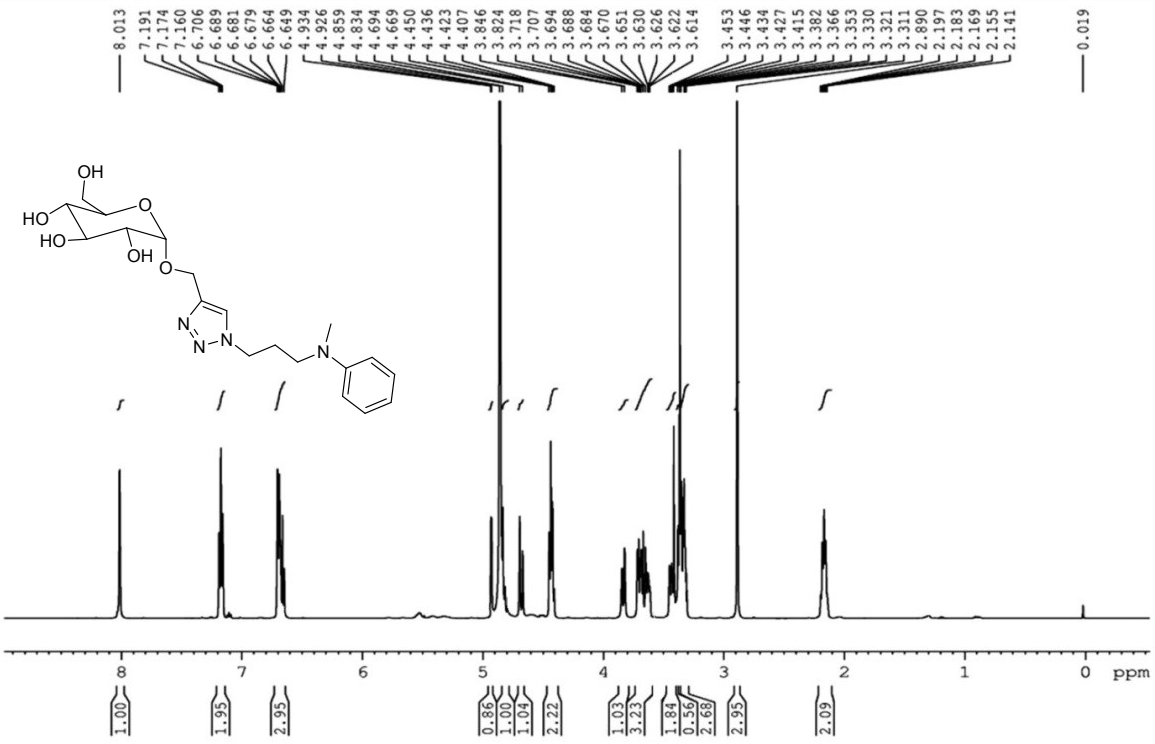


Figure S26. ^{1}H NMR spectrum of compound αGlTZLOH

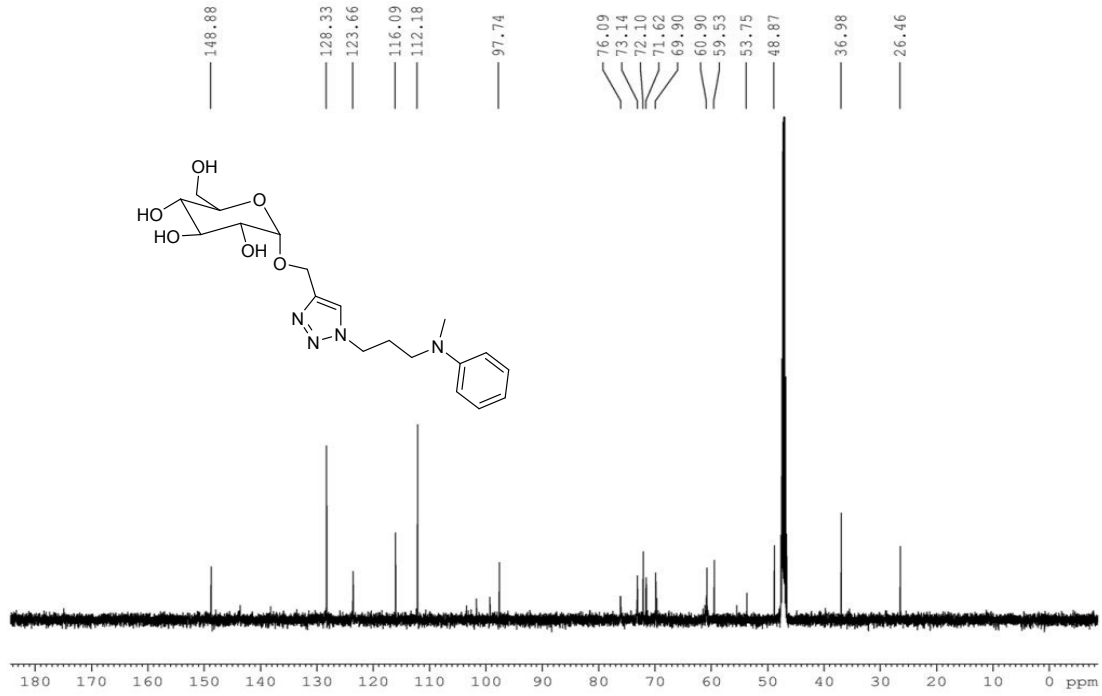


Figure S26. ^{13}C NMR spectrum of compound αGlTZLOH

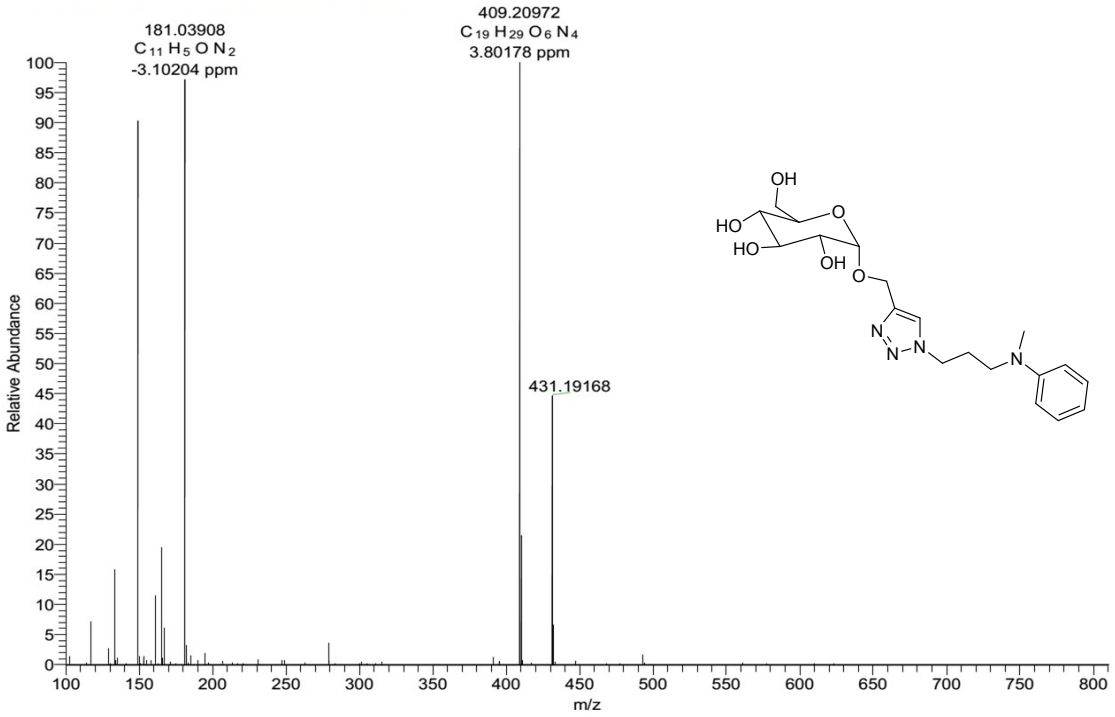


Figure S27. High resolution mass spectrum of compound αGlTZLOH

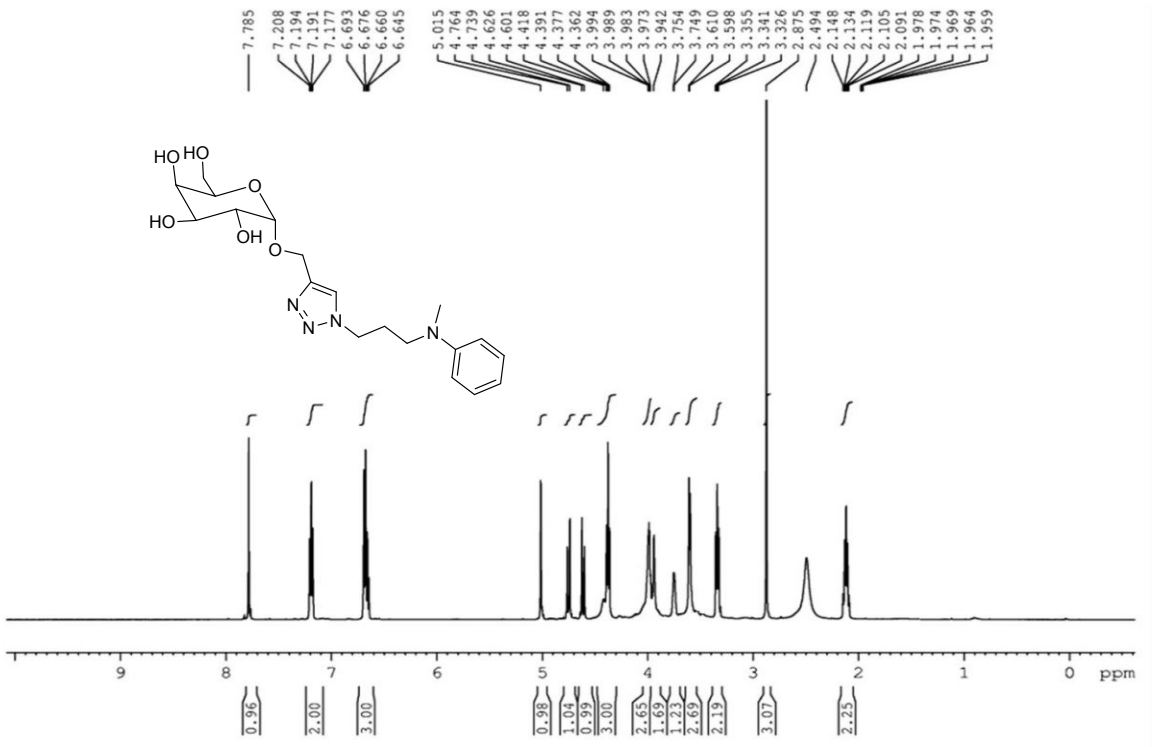


Figure S28.[1]H NMR spectrum of compound αGaTZLOH

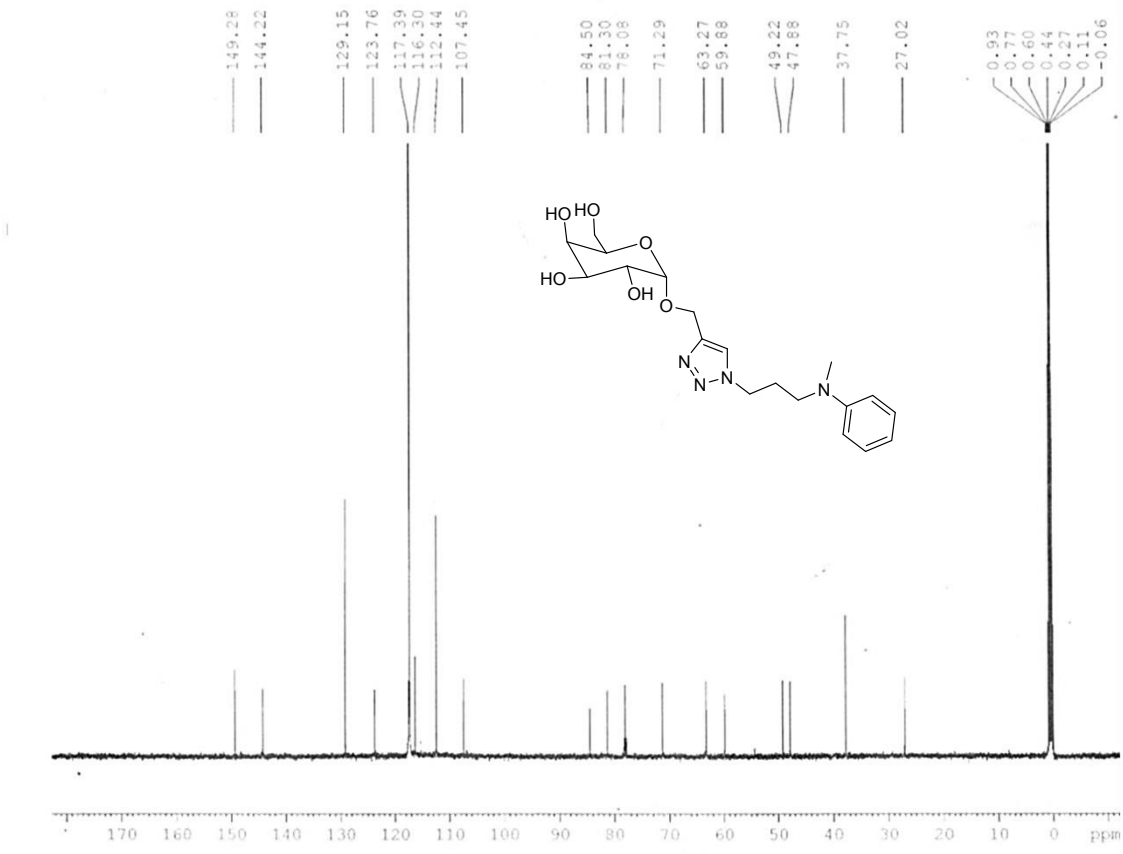


Figure S29. [13]C NMR spectrum of compound αGaTZLOH

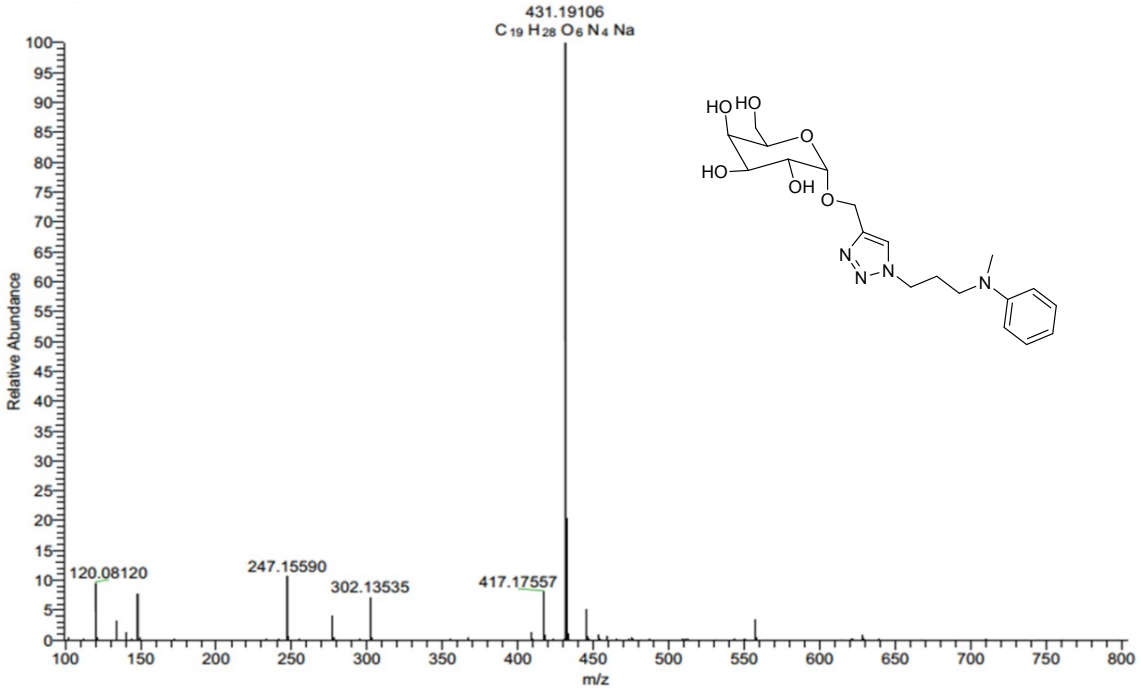


Figure S30. High resolution mass spectrum of compound αGaTZLOH

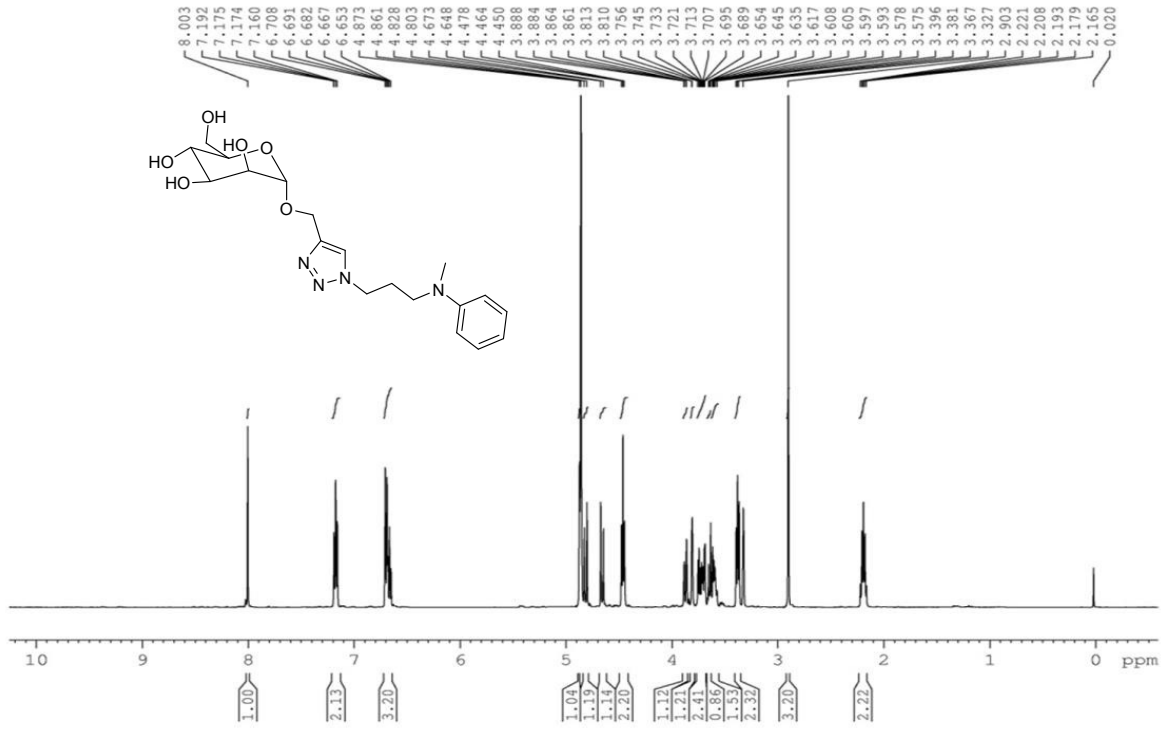
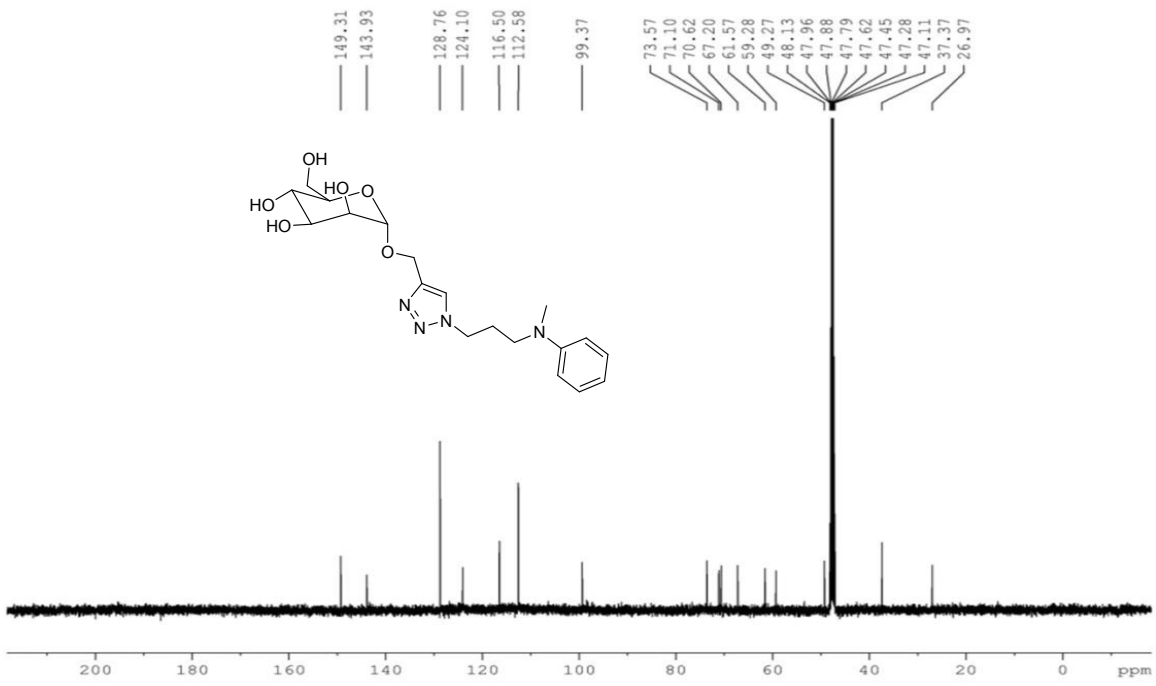


Figure S31. ^{1}H NMR spectrum of compound αMTZLOH



Fi

gure S32. ^{13}C NMR spectrum of compound αMTZLOH

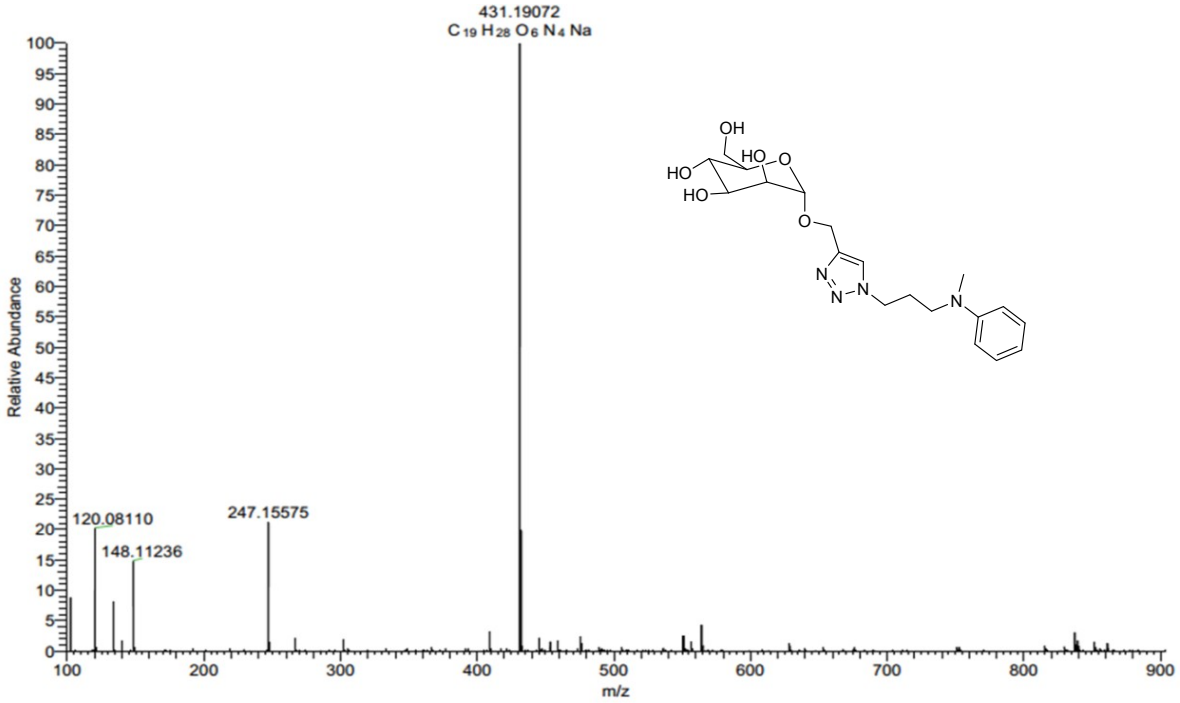


Figure S33. High resolution mass spectrum of compound αMTZLOH

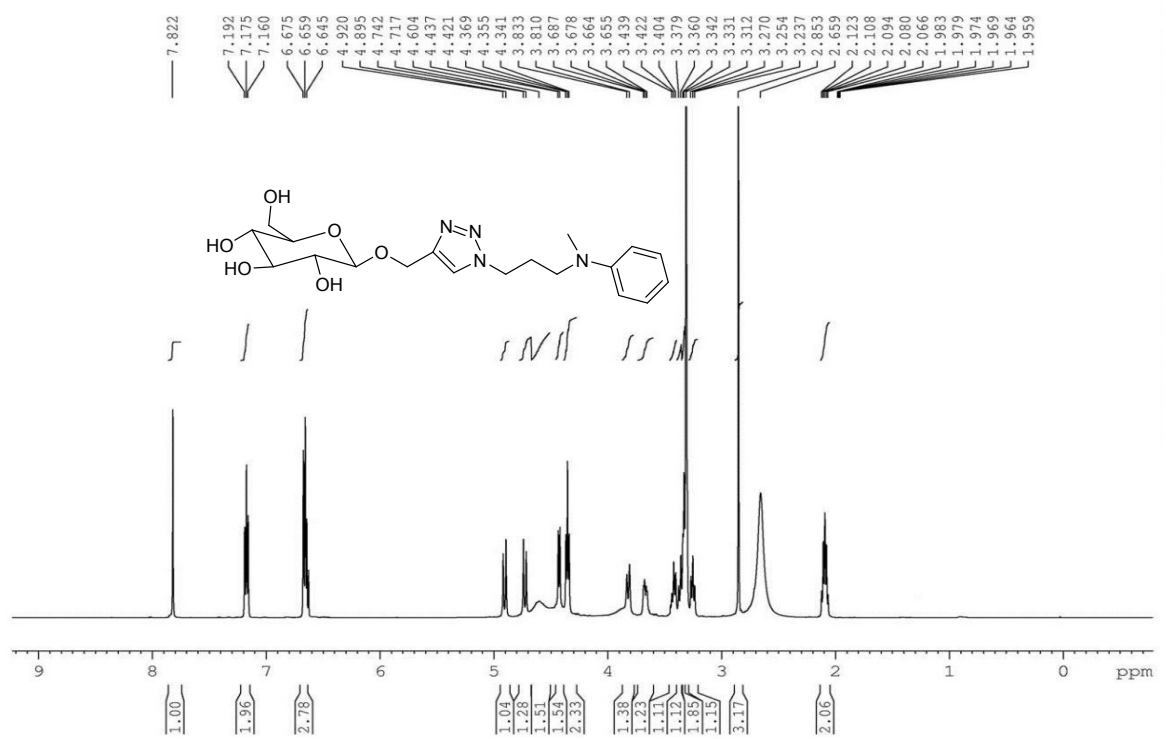


Figure S34. ^{1}H NMR spectrum of compound βGlTZLOH

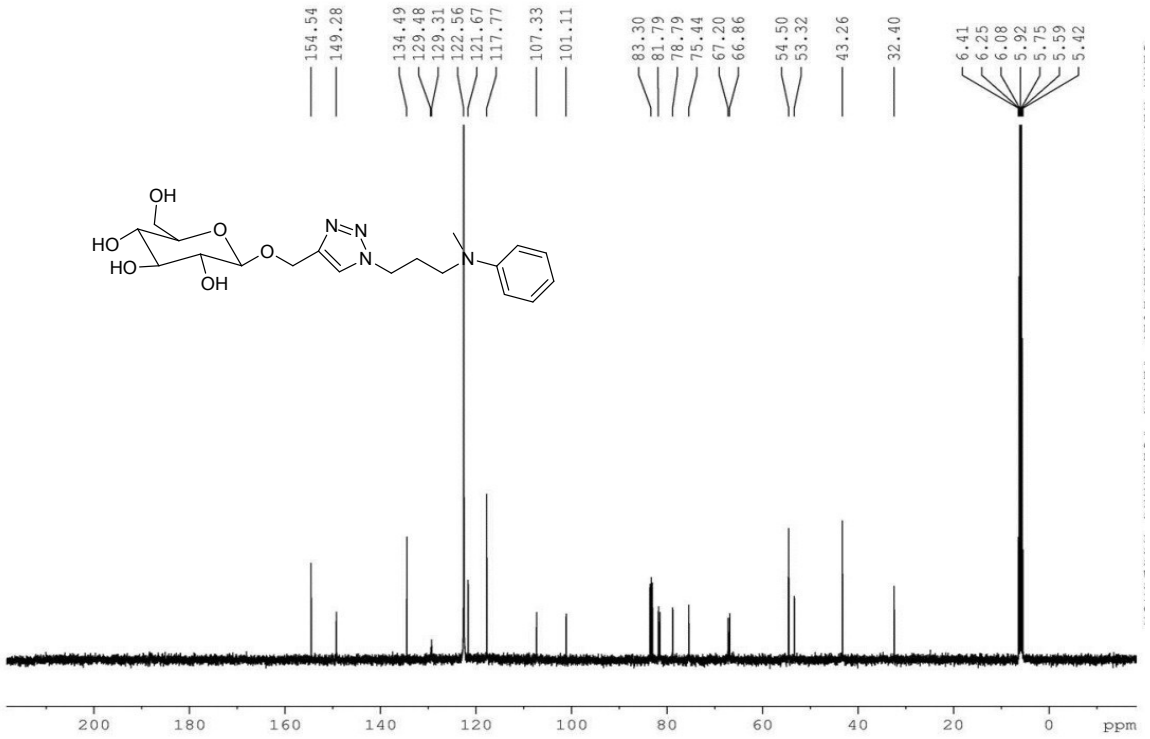


Figure S35. ^{13}C NMR spectrum of compound βGlTZLOH

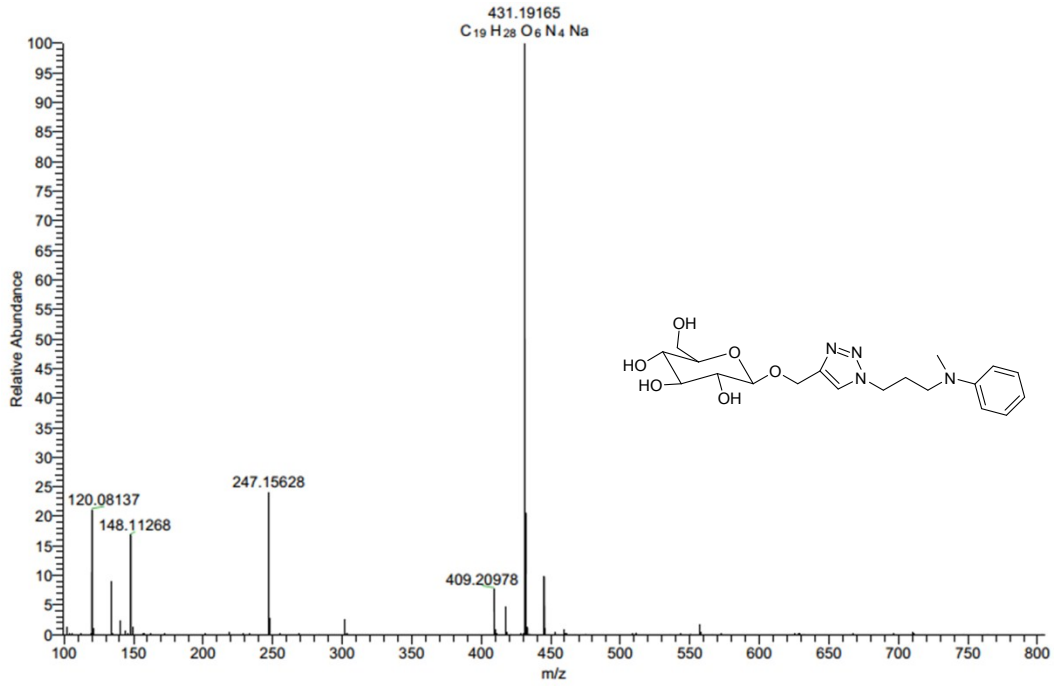


Figure S36. High resolution mass spectrum of compound βGlTZLOH

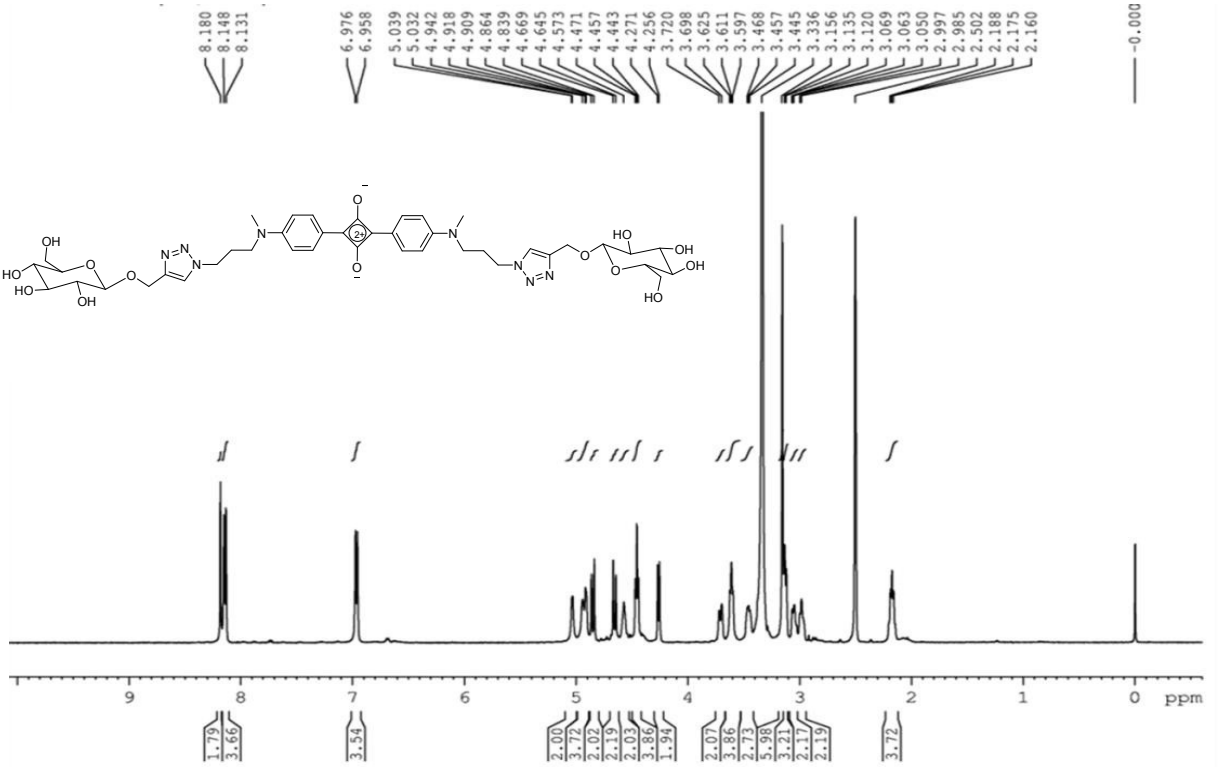


Figure S37. ^{1}H NMR spectrum of compound SSqβGl

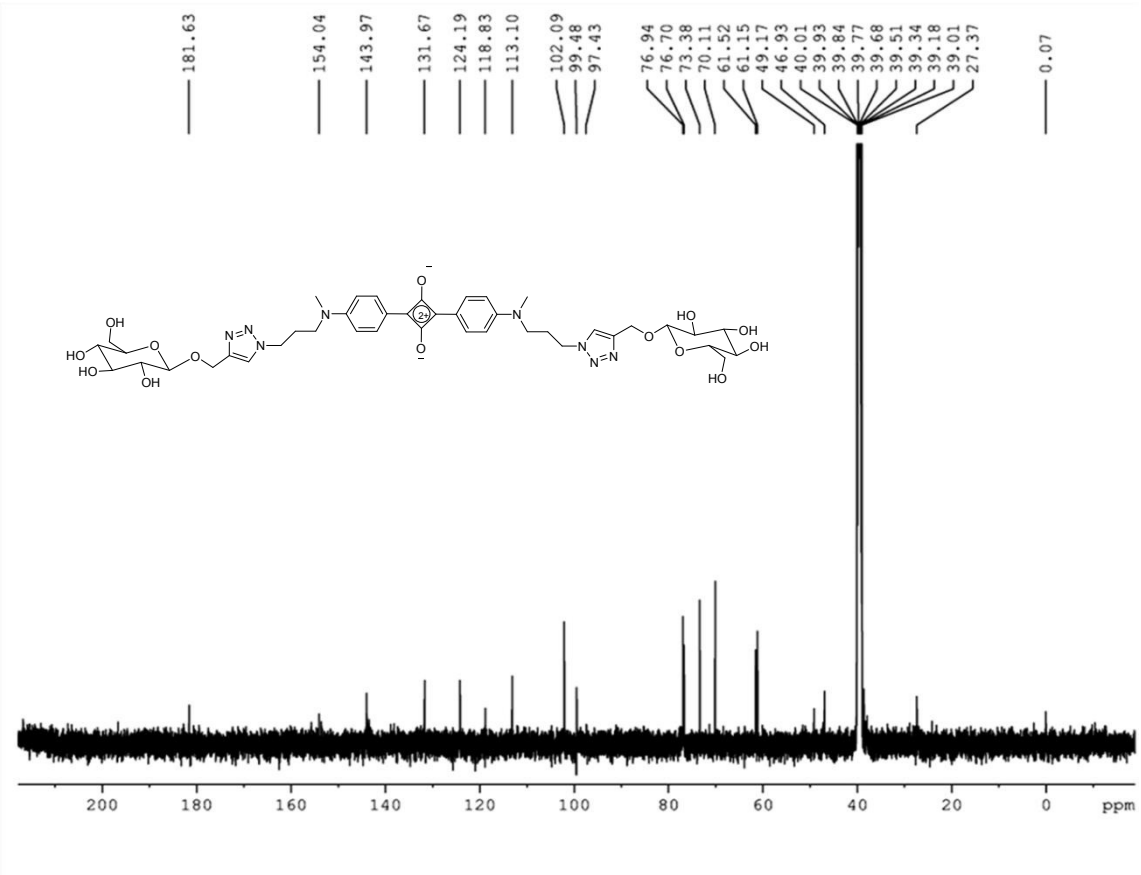


Figure S38. ^{13}C NMR spectrum of compound SSqβGl

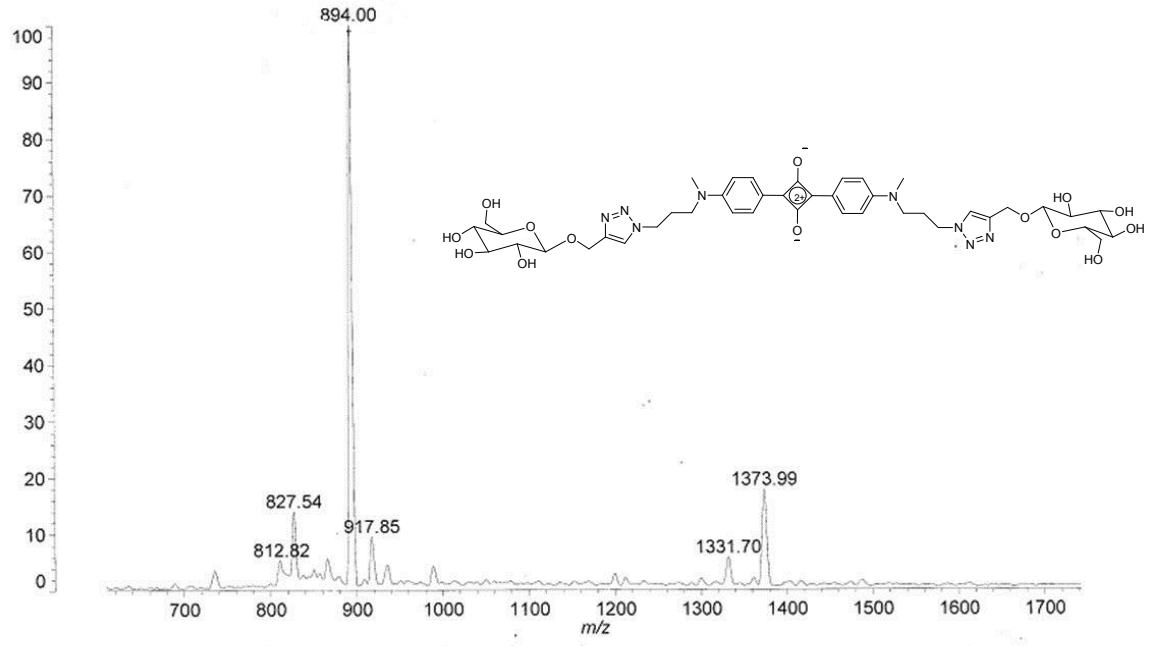


Figure S39. MALDI-TOF spectrum of compound SSqβGl

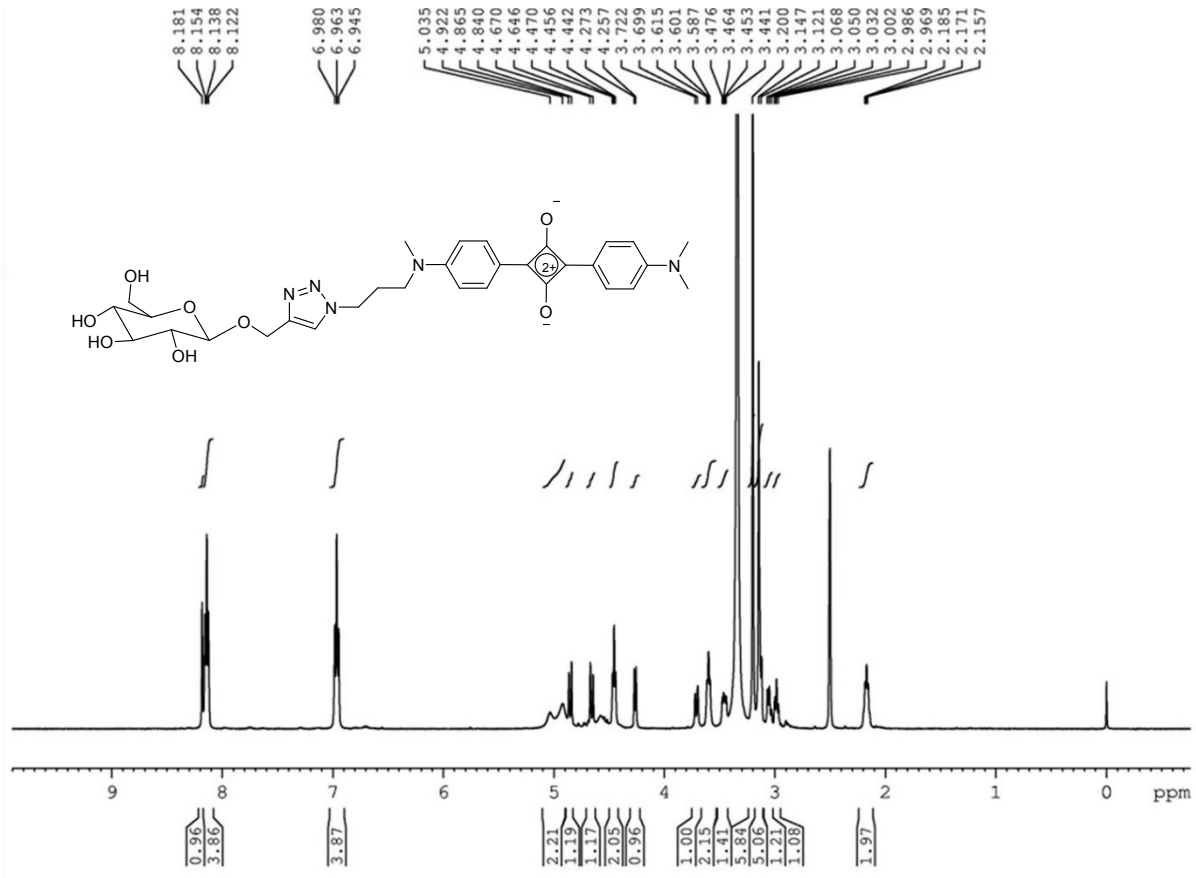


Figure S40. ^{1}H NMR spectrum of compound ASqβGl

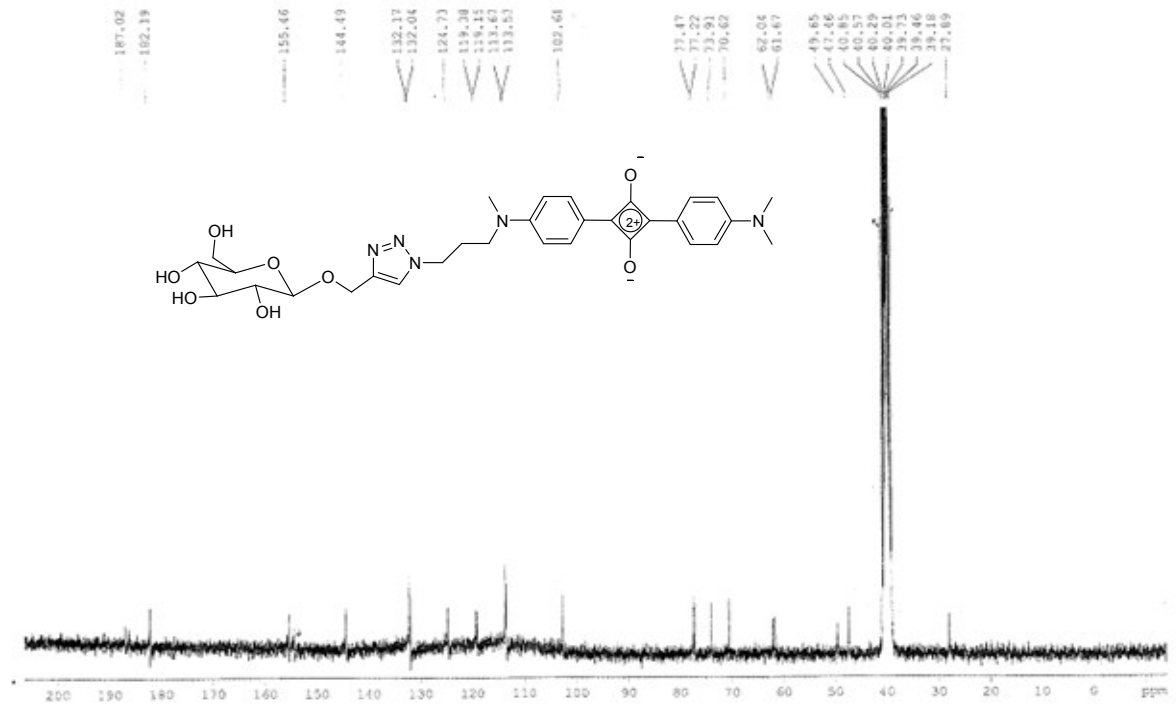


Figure S41. ^{13}C NMR spectrum of compound ASqβGl

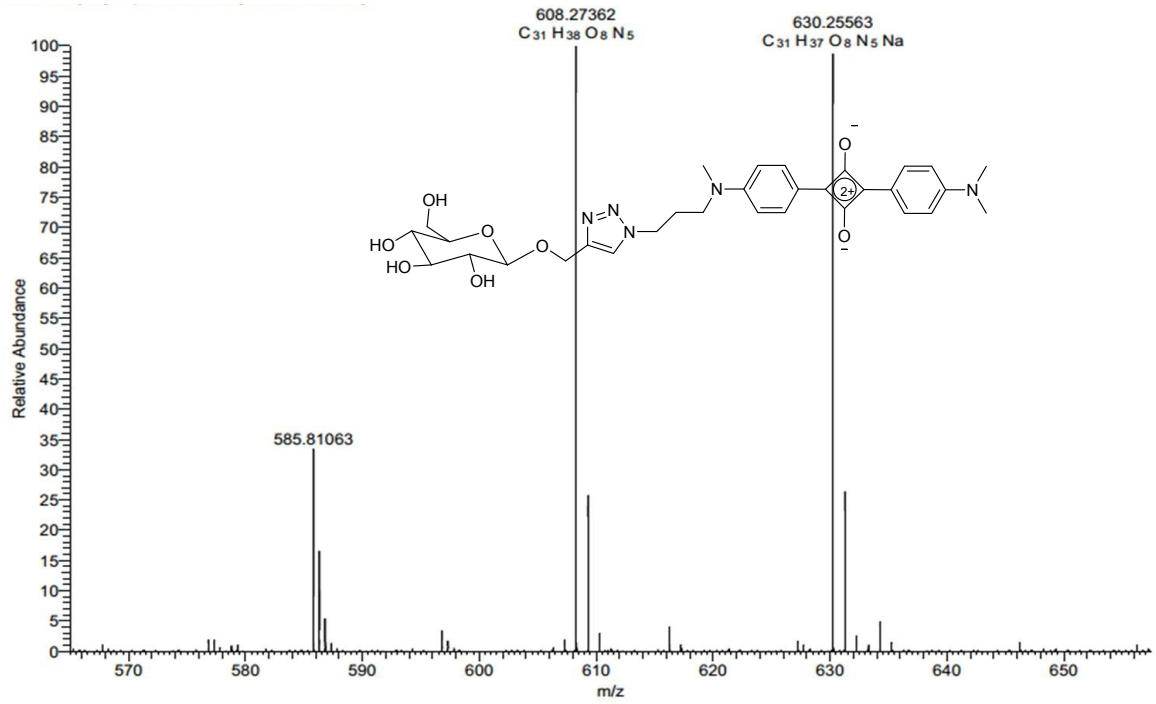


Figure S42. High resolution mass spectrum of compound ASqβGl

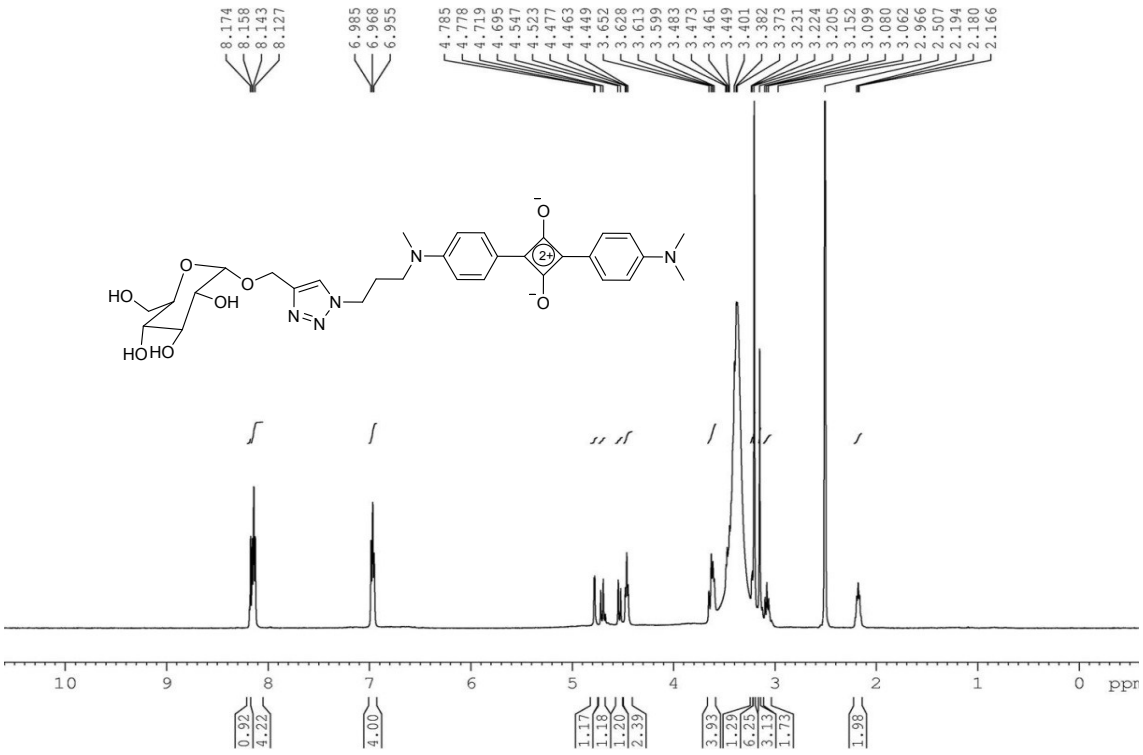


Figure S43. ^{1}H NMR spectrum of compound ASqαGl

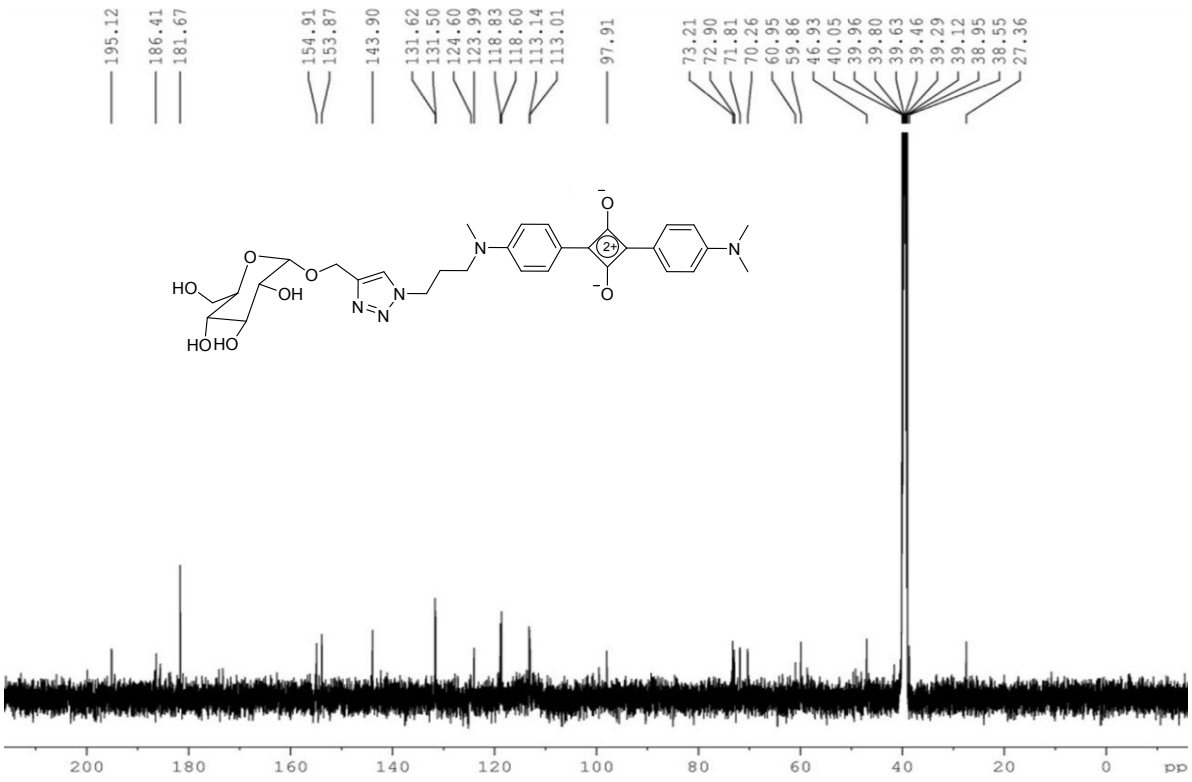


Figure S44. ^{13}C NMR spectrum of compound ASqαGl

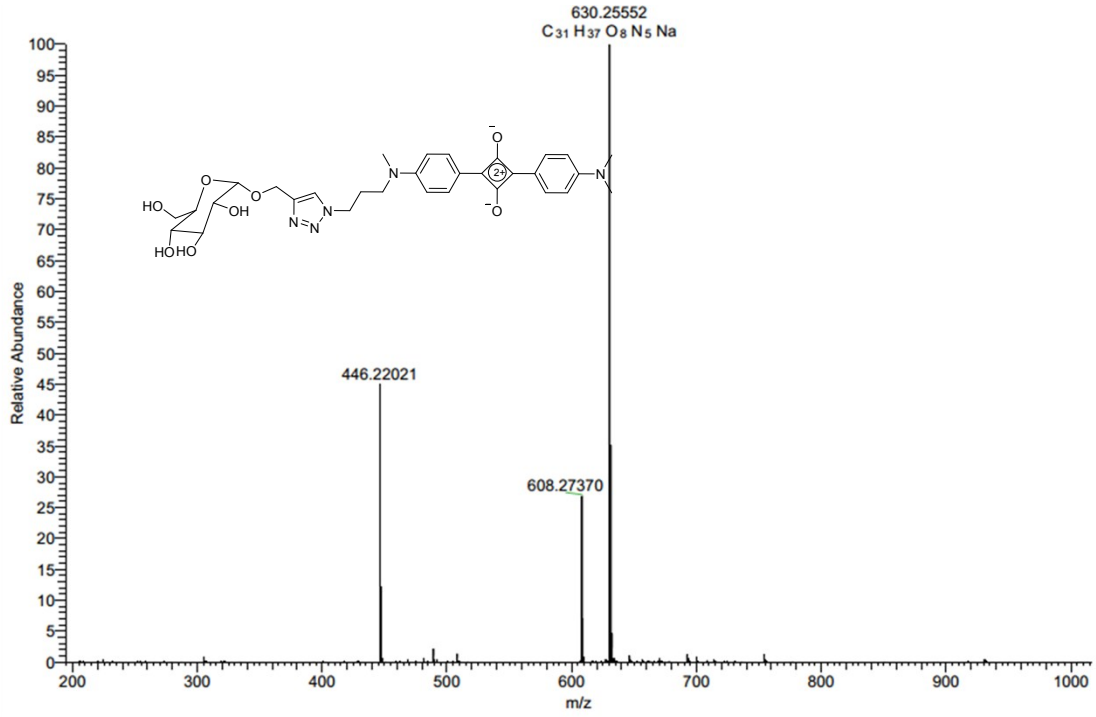


Figure S45. High resolution mass spectrum of compound ASqαGl

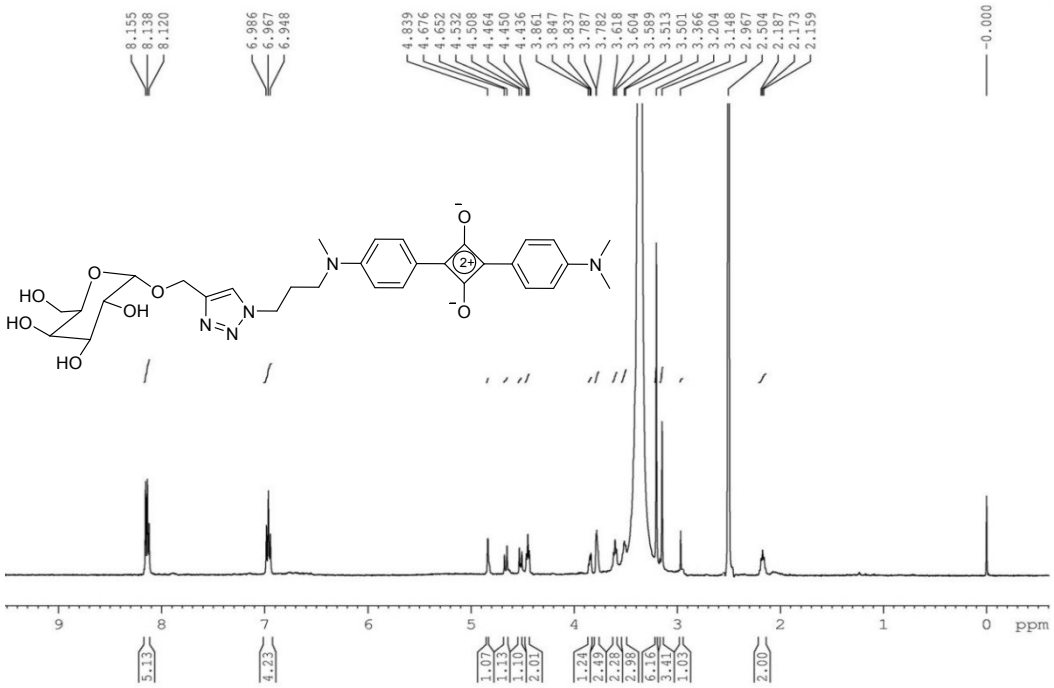


Figure S46. ^{1}H NMR spectrum of compound ASqαGa

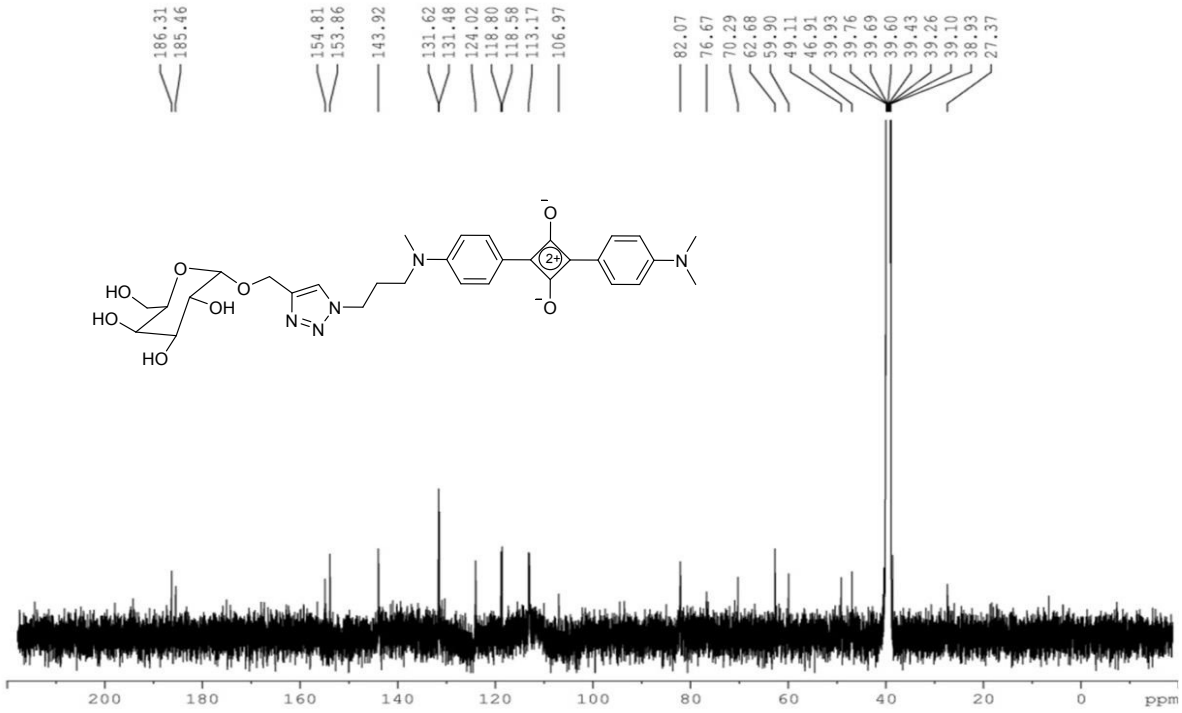


Figure S47. ^{13}C NMR spectrum of compound ASqαGa

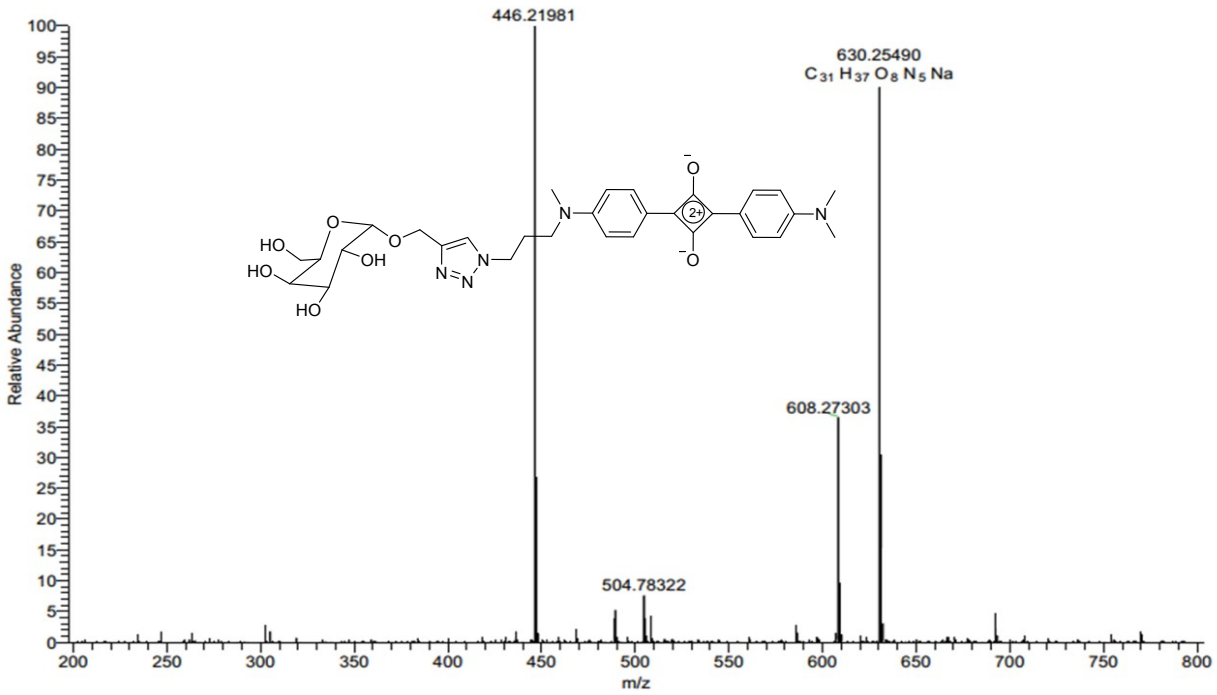


Figure S48. High resolution mass spectrum of compound ASqαGa

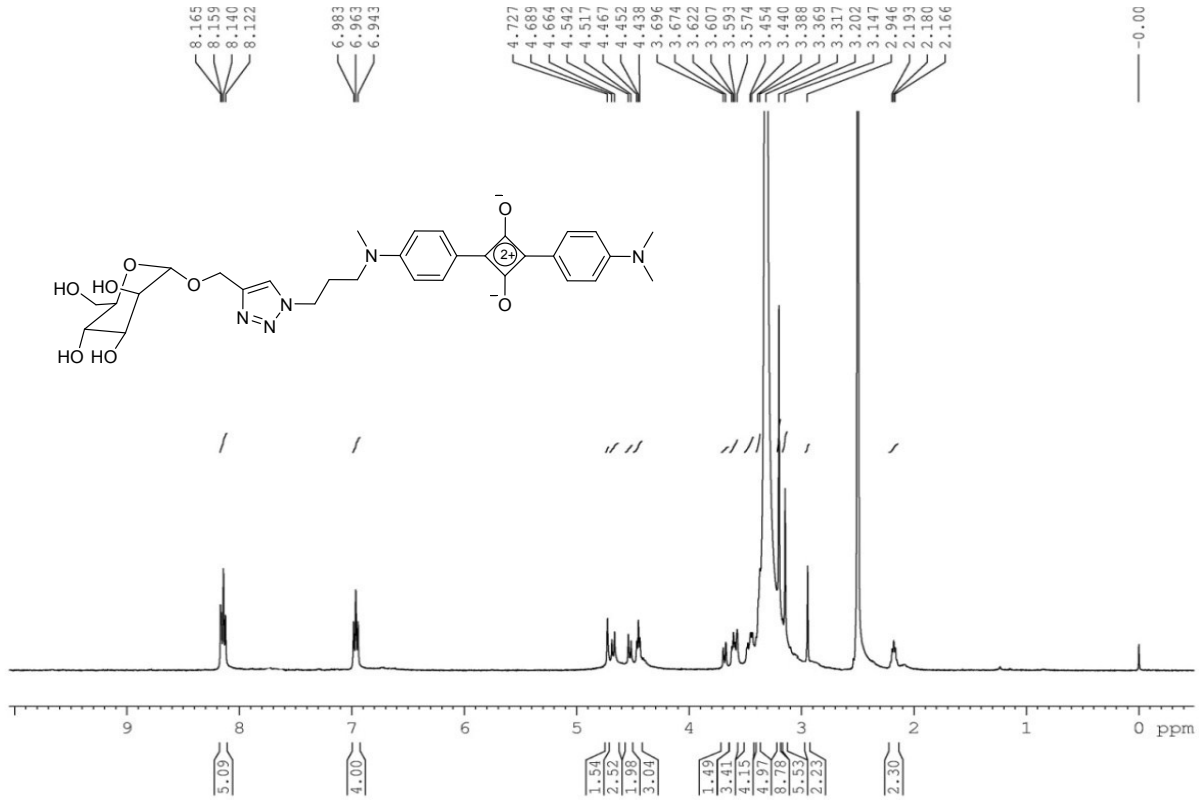


Figure S49. ^{1}H NMR spectrum of compound ASqαM

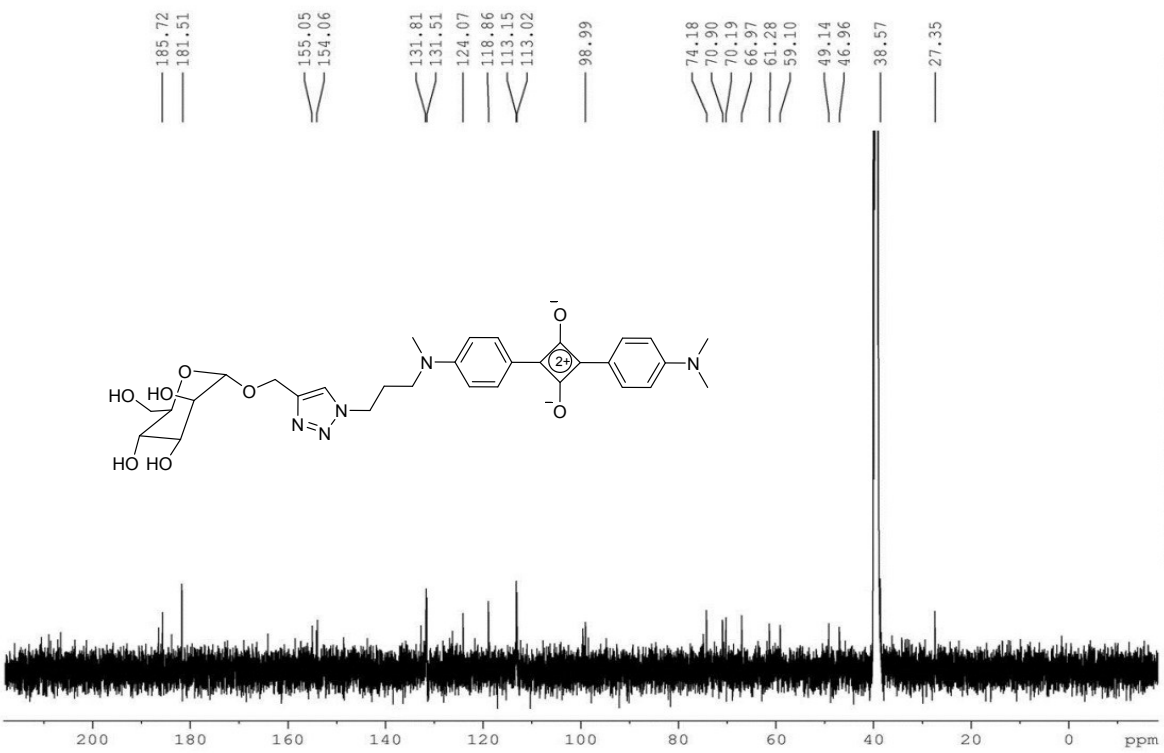


Figure S50. ^{13}C NMR spectrum of compound ASqαM

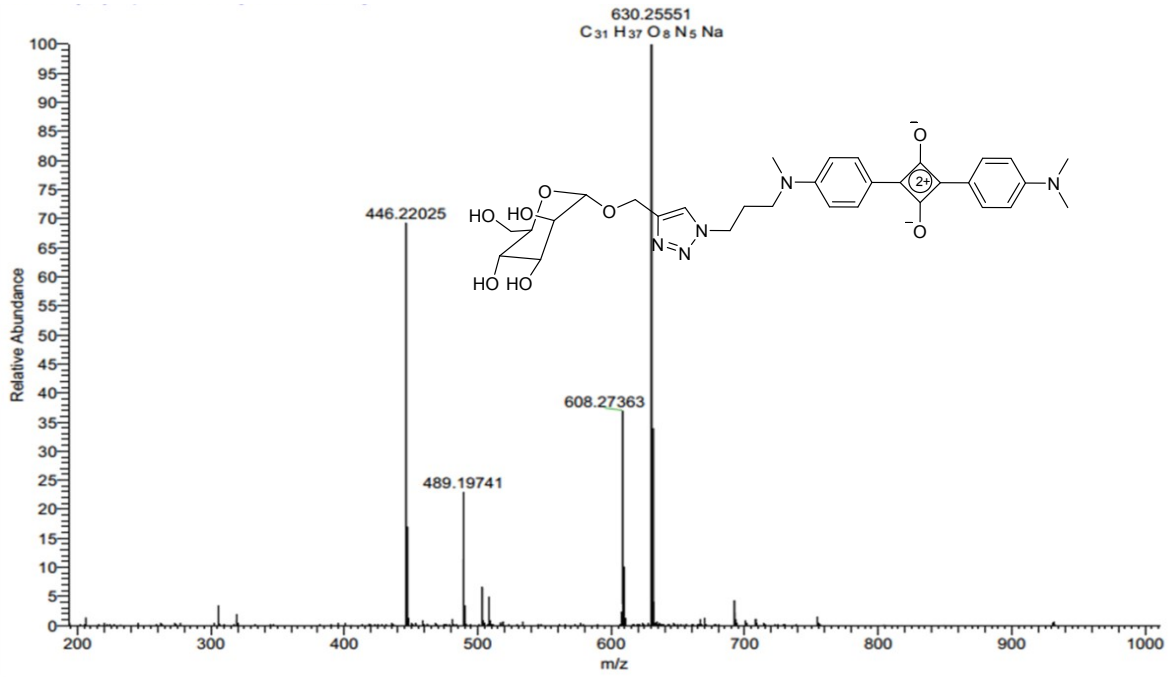


Figure S51. High resolution mass spectrum of compound ASqαM

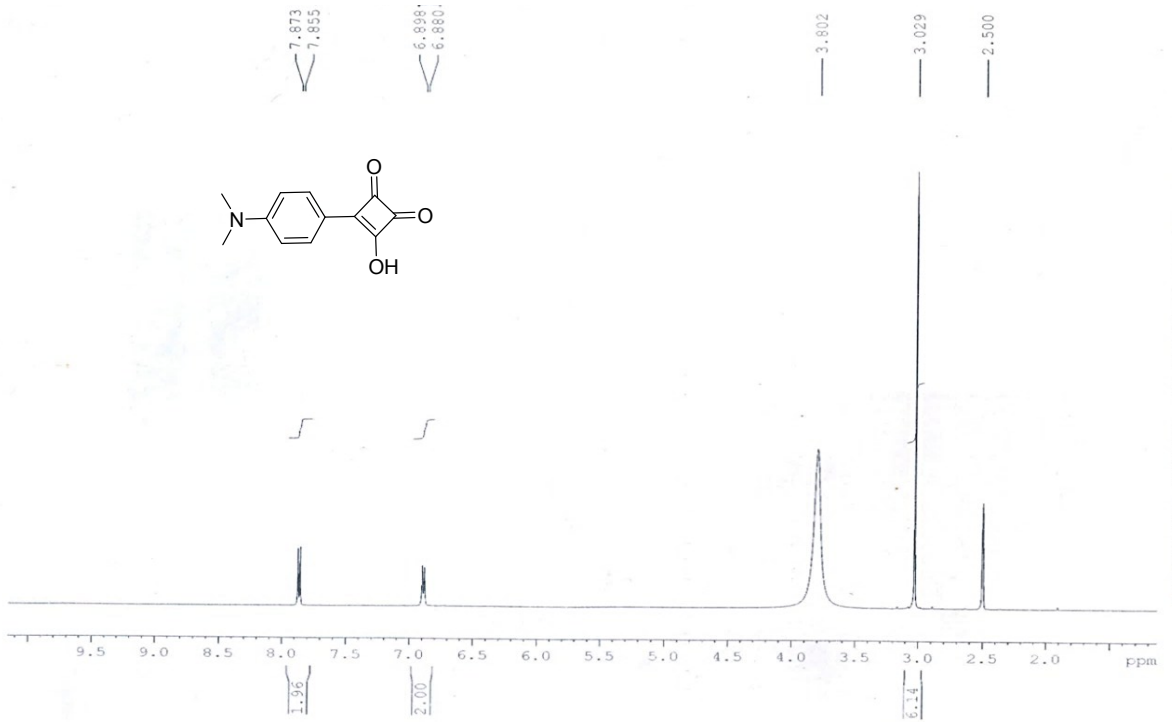


Figure S52. ^{1}H NMR spectrum of compound SemSq

24. References

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