Supporting Information

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A stimuli-responsive anticancer drug delivery system with inherent antibacterial activities

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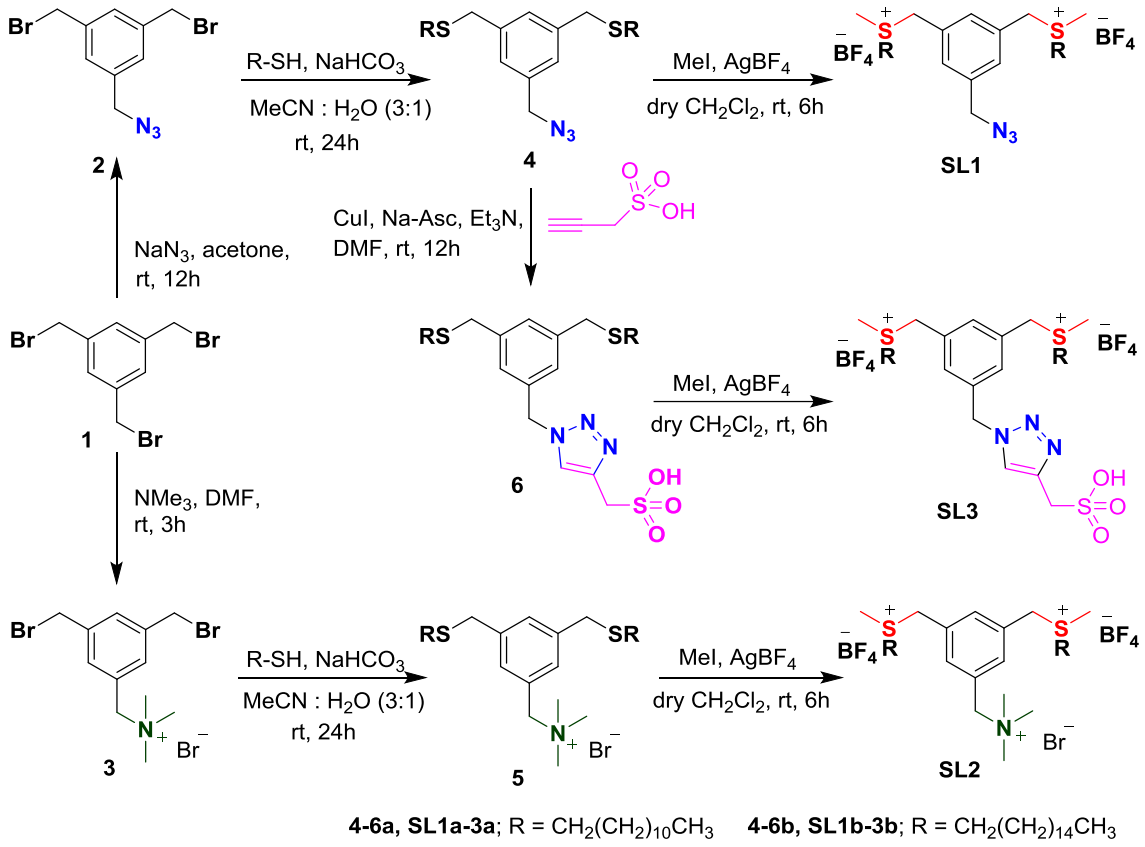
I. General information:

All reagents were purchased from Sigma-Aldrich, Merck, Himedia and other commercial sources and used directly without further purification. The column chromatography was performed using 60–120 mesh silica gels. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 (0.25 mm). The ^{1}H NMR and ^{13}C NMR were recorded at 400 or 600 and 100 or 151 MHz with Varian AS400 spectrometer and Brucker spectrometer, respectively. The chemical shifts were reported in parts per million (δ) using DMSO-d_6, $CDCl_3$ as internal solvent. The coupling constant (*J* values) and chemical shifts (δ_{ppm}) were reported in Hertz (Hz) and parts per million (ppm), respectively downfield from tetramethylsilane using residual chloroform (d = 7.24 for ^{1}H NMR, d = 77.23 for ^{13}C NMR) as an internal standard. Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), m (multiplet), and br (broadened). High-resolution mass spectra (HRMS) were recorded at Agilent Q-TOF mass spectrometer with Z-spray source using built-in software for analysis of the recorded data. The 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) was purchased from Avanti Polar Lipids (Alabaster, AL). Ultrapure water (Milli-Q system, Millipore, Billerica, MA) was used for the preparation of buffers. The stock solutions of compounds were prepared in gas chromatographic grade DMSO which also purchased from Sigma.

II. Synthesis and characterization of the compounds:



Scheme S1: Synthetic routes of the sulfonium lipids.

Synthesis of 1-(azidomethyl)-3,5-bis(bromomethyl)benzene (2)[1] — To stirring solution of 1,3,5-tris(bromomethyl)benzene (**1**) (1.40 mmol) in dry DMF solvent was slowly added sodium azide (1.2 mmol) and the resulting mixture was stirred for 3 hours at room temperature. The progress of the reaction was monitored by TLC. After maximum consumption of compound **1**, the solvent was evaporated under reduced pressure and CH_2Cl_2 (25 mL) was added. The salts were filtered off and the filtrate was concentrated under reduced pressure. The crude product was purified through silica gel column chromatography with a solvent gradient system using ethyl acetate and hexane (0-1.5%

EtOAc in hexanes) to obtain the pure product as a yellow clear oil (200 mg; 45% yield). The compound was characterized by ^{1}H and ^{13}C NMR which is in accordance with the literature report.[1]

Synthesis of 1-(3,5-bis(bromomethyl)phenyl)-N,N,N-trimethylmethanaminium (3) ─ To stirring solution of 1,3,5-tris(bromomethyl)benzene (**1**, 1.40 mmol) in acetone solvent was added trimethylamine (in 25% methanol solution; 1.2 mmol) and the resulting mixture was stirred for 12 hours at room temperature. A white precipitate was observed, which was filtered, and washed with acetone to afford the desired product as a white solid (380 mg, 80%). The compound was characterized by ^{1}H and ^{13}C NMR which is in accordance with the literature report.[2]

Synthesis of ((5-(azidomethyl)-1,3-phenylene)bis(methylene))bis(dodecylsulfane) (4a) ─ To a stirring solution of 1-(azidomethyl)-3,5-bis(bromomethyl)benzene (**2**, 0.31 mmol) in CH_3CN/H_2O (3:1 in volume) was slowly added (dropwise) a previously stirring solution of dodecanthiol and sodium bicarbonate in CH_3CN/H_2O (3:1 in volume) at room temperature. The resulting reaction mixture was stirred for 36 hours, after that the solvent was removed under reduced pressure. Then the reaction mixture was diluted with cold water and ethyl acetate. The organic layer was extracted with EtOAc (3 × 50 mL) and washed with brine, and dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure. The crude reaction mixture was purified through silica gel column chromatography with a solvent gradient system using ethyl acetate and hexane (0-10% EtOAc in hexanes) to afford compound **4a** as a colorless gummy liquid. **Characterization of the compound** : Colourless gummy liquid (yield – 87%) ^{1}H NMR (600 MHz, $CDCl_3$) δ_{ppm} 7.23 – 7.22 (m, 1H), 7.14 (s, 1H), 4.31 (s, 2H), 3.69 – 3.66 (m, 4H), 2.42 – 2.37 (m, 4H), 1.57 – 1.50 (m, 4H), 1.35 – 1.24 (m, 36H), 0.88 (t, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ_{ppm} 139.7, 135.8,

129.3, 127.2, 54.5, 36.0, 31.9, 31.4, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 22.7, 14.1; **HRMS (ESI)** calcd. for $C_{33}H_{63}N_4S_2$ $[M+NH_4]^+$: 579.4494, found: 579.4463.

Synthesis of ((5-(azidomethyl)-1,3-phenylene)bis(methylene))bis(hexadecylsulfane) (4b) ─ To a stirring solution of 1-(azidomethyl)-3,5-bis(bromomethyl)benzene (**2**, 0.31 mmol) in CH_3CN/H_2O (3:1 in volume) was slowly added (dropwise) a previously stirring solution of hexadecanthiol and sodium bicarbonate in CH_3CN/H_2O (3:1 in volume) at room temperature. The resulting reaction mixture was stirred for 36 hours, after that the solvent was removed under reduced pressure. Then the reaction mixture was diluted with cold water and ethyl acetate. The organic layer was extracted with EtOAc (3 × 50 mL) and washed with brine, and dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure. The crude reaction mixture was purified through silica gel column chromatography with a solvent gradient system using ethyl acetate and hexane (0-10% EtOAc in hexanes) to afford compound **4b** as a colorless gummy liquid. **Characterization of the compound**: Colorless gummy liquid (yield – 83%) ^{1}H NMR (600 MHz, $CDCl_3$) δ_{ppm} 7.31 (m, 1H), 7.23 (m, 2H), 4.60 (s, 2H), 4.35 (s, 2H), 3.73 – 3.70 (m, 4H), 2.42 – 2.39 (m, 4H), 1.58 – 1.52 (m, 4H), 1.34 – 1.31 (m, 4H), 1.28 – 1.25 (m, 48H), 0.88 (t, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ_{ppm} 140.2, 138.4, 136.3, 128.8, 128.5, 126.7, 54.4, 45.7, 36.0, 35.9, 31.9, 31.6, 31.5, 29.7, 29.6, 29.6, 29.5, 29.5, 29.3, 29.2, 28.9, 28.8, 22.6, 14.1; **HRMS (ESI)** calcd. for $C_{41}H_{79}N_4S_2$ $[M+NH_4]^+$: 691.5746, found: 691.5738.

Synthesis of 1-(3,5-bis((dodecylthio)methyl)phenyl)-N,N,N-trimethylmethanaminium (5a) ─ ─ To a stirring solution of 1-(3,5-bis(bromomethyl)phenyl)-N,N,N-trimethylmethanaminium (**3**, 0.14 mmol) in CH_3CN/H_2O (3:1 in volume) was slowly added (dropwise) a previously stirring

solution of dodecanthiol (0.30 mmol) and sodium bicarbonate (0.30 mmol) in CH_3CN/H_2O (3:1 in volume) at room temperature. The resulting reaction mixture was stirred for 36 hours, after that the solvent was removed under reduced pressure. Then the reaction mixture was diluted with cold water and ethyl acetate. The organic layer was extracted with EtOAc (3 × 50 mL) and washed with brine, and dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure. The crude reaction mixture was purified through silica gel column chromatography with a solvent gradient system using ethyl acetate and hexane (0.1-10% EtOAc in hexanes) to afford compound **5a** as a colorless gummy liquid. **Characterization of compound:** colorless gummy liquid (yield – 81%) ^{1}H NMR (600 MHz, $CDCl_3$) δ_{ppm} 7.41 (s, 3H), 4.92 (s, 2H), 3.70 (s, 4H), 3.41 (s, 9H), 2.42 (t, 4H), 1.58 – 1.50 (m, 4H), 1.35 – 1.24 (m, 39H), 0.87 (t, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ_{ppm} 140.5, 131.7, 127.7, 69.1, 52.9, 35.9, 32.1, 31.9, 29.7, 29.6, 29.6,29.5, 29.3, 29.2, 29.2, 28.9, 22.6, 14.0; **HRMS (ESI)** calcd. for $C_{44}H_{84}NS_2$ $[M]^+$: 578.4788, found: 578.4768.

Synthesis of 1-(3,5-bis((hexadecylthio)methyl)phenyl)-N,N,N-trimethylmethanaminium (5b) – To a stirring solution of 1-(3,5-bis(bromomethyl)phenyl)-N,N,N-trimethylmethanaminium (**3**, 0.14 mmol) in CH_3CN/H_2O (3:1 in volume) was slowly added (dropwise) a previously stirring solution of hexadecanthiol (0.30 mmol) and sodium bicarbonate (0.30 mmol) in CH_3CN/H_2O (3:1 in volume) at room temperature. The resulting reaction mixture was stirred for 36 hours, after that the solvent was removed under reduced pressure. Then the reaction mixture was diluted with cold water and ethyl acetate. The organic layer was extracted with EtOAc (3 × 50 mL) and washed with brine, and dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure. The crude reaction mixture was purified through silica gel column chromatography with a solvent gradient system using ethyl acetate and hexane (0.1-10% EtOAc in hexanes) to afford

compound **5b** as a colorless gummy liquid. **Characterization of compound:** colourless gummy liquid (yield – 81%) ^{1}H NMR (600 MHz, $CDCl_3$) δ_{ppm} 7.43 – 7.41 (m, 3H), 4.88 (m, 2H), 3.73 – 3.71 (s, 4H), 3.39 – 3.37 (m, 9H), 2.45 – 2.41 (m, 4H), 1.57 – 1.52 (m, 4H), 1.34 – 1.24 (m, 54H), 0.87 (t, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ_{ppm} 140.7, 131.9, 127.8, 69.3, 53.1, 36.1, 32.2, 32.1, 29.9, 29.8, 29.7, 29.5, 29.3, 29.1, 22.9, 14.3; **HRMS (ESI)** calcd. for $C_{44}H_{84}NS_2$ $[M]^+$: 690.6040, found:690.6029.

Synthesis of SL1a **—** To a stirring solution of ((5-(azidomethyl)-1,3-phenylene)bis(methylene))bis(dodecylsulfane) (**4a**, 0.09 mmol) in dry DCM solvent was added $AgBF_4$ (0.09 mmol) and methyl iodide (0.27 mmol) and the resulting mixture was stirred at room temperature for 5 hours. The progress of the reaction was monitored by TLC. After maximum consumption of compound **4a**, the solvent was removed under reduced pressure and the resulting mixture was filtered off using a pad of celite to remove silver salt. Then the crude mixture was washed with ether and CH_3CN to afford compound **SL1a** as a brown gummy liquid. **Characterization of the compound:** Brown gummy liquid (yield – 80%) ^{1}H NMR (600 MHz, DMSO-d_6) δ_{ppm} 7.63 (s, 1H), 7.54 – 7.29 (m, 2H), 5.48 – 5.36 (m, 2H), 4.90 – 4.60 (m, 4H), 3.28 – 3.26 (m, 6H), 2.82 – 2.80 (m, 4H), 1.72 – 1.59 (m, 4H), 1.24 – 1.15 (m, 38H), 0.85 (t, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ_{ppm} 141.0, 133.0, 130.2, 101.9, 53.1, 44.5, 40.9, 31.7, 29.4, 29.4, 29.2, 29.1, 29.1, 28.7, 28.2, 23.7, 22.5, 14.4; **ES-MS (ESI+) m/z:** $[(M + NH_4^+)/2]$ 305.2431.

Synthesis of SL1b **—** To a stirring solution of ((5-(azidomethyl)-1,3-phenylene)bis(methylene))bis(hexadecylsulfane) (**4b**, 0.09 mmol) in dry DCM solvent was added $AgBF_4$ (0.09 mmol) and methyl iodide (0.27 mmol) and the resulting mixture was stirred at room

temperature for 5 hours. The progress of the reaction was monitored by TLC. After maximum consumption of compound **4b**, the solvent was removed under reduced pressure and the resulting mixture was filtered off using a pad of celite to remove silver salt. Then the crude mixture was washed with ether and CH_3CN to afford compound **SL1b** as a brown gummy liquid. **Characterization of the compound :** Brown gummy liquid (yield – 72%) ^{1}H NMR (600 MHz, $CDCl_3$,) δ_{ppm} 8.09 – 7.77 (m, 2H), 7.63 – 7.41 (m, 1H), 4.75 – 4.64 (m, 4H), 4.57 – 4.40 (m, 2H), 3.43 – 3.19 (m, 6H), 2.87 – 2.73 (m, 4H), 1.84 – 1.63 (m, 4H), 1.37 – 1.20 (m, 51H), 0.86 (t, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ_{ppm} 130.4, 130.2, 101.9, 53.1, 44.5, 40.9, 31.7, 29.5, 29.4, 29.4, 29.2, 29.1, 28.7, 28.2, 23.7, 22.5, 14.4; **ES-MS (ESI+) m/z:** $[(M + 2BF_4^-)/2]$ 439.2758.

Synthesis of SL2a **—** To stirring solution of 1-(3,5-bis((dodecylthio)methyl)phenyl)-N,N,N-trimethylmethanaminium (**5a**, 0.05 mmol) in dry DCM solvent was added $AgBF_4$ (0.05 mmol) and methyl iodide (0.15 mmol) and the resulting mixture was stirred for 5 hours at room temperature. The progress of the reaction was monitored by TLC. After maximum consumption of maximum amount of compound **5a**, the solvent was removed under reduced pressure and the resulting mixture was filtered off using a pad of celite to remove silver salt. Then the crude mixture was washed with ether and CH_3CN to afford compound **SL2a** as a brown gummy liquid. **Characterization of compound:** Brown gummy liquid (yield – 77%); ^{1}H NMR (600 MHz, DMSO-d_6) δ_{ppm} 7.75- 7.56 (m, 3H), 4.86 – 4.73 (m, 4H), 4.60 – 4.54 (m, 2H), 3.07 – 3.04 (m, 9H), 2.85 – 2.80 (m, 6H), 1.74 – 1.67 (m, 4H), 1.36 - 1.24 (m, 40H), 0.85 (t, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ_{ppm} 136.4, 130.6, 129.7, 89.9, 70.1, 68.3, 52.6, 47.4, 44.6, 41.4, 32.1, 30.2, 30.0, 29.9, 29.7, 29.6, 28.6, 24.2, 22.9, 21.1, 14.3; **ES-MS (ESI+) m/z:** $[(M + H)^+/2]$ 305.2433.

Synthesis of SL2b **—** To stirring solution of 1-(3,5-bis((hexadecylthio)methyl)phenyl)-N,N,N-trimethylmethanaminium (**5b**, 0.05 mmol) in dry DCM solvent was added $AgBF_4$ (0.05 mmol) and methyl iodide (0.15 mmol) and the resulting mixture was stirred for 5 hours at room temperature. The progress of the reaction was monitored by TLC. After maximum consumption of compound **5b**, the solvent was removed under reduced pressure and the resulting mixture was filtered off using a pad of celite to remove silver salt. Then the crude mixture was washed with ether and CH_3CN to afford compound **SL2b** as a brown gummy liquid. **Characterization of compound:** brown gummy liquid (yield – 76%) ^{1}H NMR (600 MHz, $CDCl_3$) δ_{ppm} 7.73 – 7.49 (m, 3H), 4.60 – 4.44 (m, 4H), 3.46 – 2.95 (m, 9H), 2.81 – 2.73 (m, 6H), 1.85 - 1.71 (m, 5H), 1.49 – 1.40 (m, 2H), 1.31 – 1.17 (m, 52H), 0.88 (t, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ_{ppm} 136.4, 130.6, 129.7, 89.9, 70.1, 68.3, 52.6, 47.4, 44.6, 41.4, 32.1, 30.2, 30.0, 29.9, 29.7, 29.6, 28.6, 24.2, 22.9, 21.1, 14.3; **ES-MS (ESI+) m/z:** $[(M + BF_4^-)^+/2]$ 404.3398.

Synthesis of SL3a **—** To a stirring solution of ((5-(azidomethyl)-1,3-phenylene)bis(methylene))bis(dodecylsulfane) (**4a**, 0.09 mmol) in DMF (3 mL) was added prop-2-yne-1-sulfonic acid (0.13 mmol) and the mixture was stirred for 10 minutes. After that, sodium ascorbate (0.003 mmol) and CuI (0.002 mmol) were added to the reaction mixture and the solution was allowed to stir for 24 hours at room temperature. After maximum consumption of compound **4a,** the unused solvent was removed under reduced pressure and diluted with ethyl acetate. The organic layer was separated, washed with brine and dried over anhydrous Na_2SO_4. The solid crude product was washed with distilled hexane. The crude reaction mixture was purified through silica gel column chromatography with a solvent gradient system using methanol and DCM (0.1-10% methanol in DCM) to afford compound (1-(3,5-bis((dodecylthio)methyl)benzyl)-1H-1,2,3-triazol-

4-yl)methanesulfonic acid (**6a**) as a white solid which was used for next reaction without further characterization. To a stirring solution of compound **6a** in dry DCM was added $AgBF_4$ (0.06 mmol), and methyl iodide (0.18 mmol), and the resulting mixture was stirred for 5 hours at room temperature. The progress of the reaction was monitored by TLC. After maximum consumption of compound **6a**, the solvent was removed under reduced pressure and the resulting mixture was filtered off using a pad of celite to remove silver salt. Then the crude mixture was washed with ether and CH_3CN to afford compound **SL3a** as a brown gummy liquid. **Characterization of compound:** Dark Brown gummy liquid (yield – 71%) ^{1}H NMR (600 MHz, $CDCl_3$) δ_{ppm} 9.78 (s, 1H), 8.00 – 7.93 (m, 3H), 4.69 – 4.67 (m, 2H), 3.75 – 3.71 (m, 2H), 3.33 – 3.20 (m, 4H), 2.78 (s, 6H), 2.35 – 2.28 (m, 1H), 1.85 – 1.71 (m, 4H), 1.44 – 1.40 (m, 4H), 1.29 – 1.23 (m, 41H), 0.86 (t, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ_{ppm} 138.5, 133.2, 130.3, 129.2, 128.4, 125.5, 68.1, 47.4, 44.8, 41.6, 32.1, 29.9, 29.9, 29.8, 29.6, 29.2, 28.6, 25.8, 24.3, 22.9, 21.5, 14.3; **ES-MS (ESI+) m/z:** $[(M/2 + 2BF_4^- + Na^+]$ 552.0522.

Synthesis of SL3b **–** To a stirring solution of ((5-(azidomethyl)-1,3-phenylene)bis(methylene))bis(hexadecylsulfane) (**4b**, 0.07 mmol) in DMF (3 mL) was added prop-2-yne-1-sulfonic acid (0.11 mmol) and the mixture was stirred for 10 minutes. After that, sodium ascorbate (0.003 mmol) and CuI (0.002 mmol) were added to the reaction mixture and the solution was allowed to stir for 24 hours at room temperature. After maximum consumption of compound **4b**, the unused solvent was removed under reduced pressure and diluted with ethyl acetate. The organic layer was separated, washed with brine and dried over anhydrous Na_2SO_4. The solid crude product was washed with distilled hexane. The crude reaction mixture was purified through silica gel column chromatography with a solvent gradient system using methanol and

DCM (0.1-10% methanol in DCM) to afford compound (1-(3,5-bis((hexadecylthio)methyl)benzyl)-1H-1,2,3-triazol-4-yl)methanesulfonic acid (**6b)** as a white solid which was used for next reaction without further characterization. To a stirring solution of compound **6b** in dry DCM was added $AgBF_4$ (0.06 mmol), and methyl iodide (0.18 mmol), and the resulting mixture was stirred for 5 hours at room temperature. The progress of the reaction was monitored by TLC. After maximum consumption of compound **6b**, the solvent was removed under reduced pressure and the resulting mixture was filtered off using a pad of celite to remove silver salt. Then the crude mixture was washed with ether and CH_3CN to afford compound **SL3b** as a brown gummy liquid. **Characterization of compound:** Dark brown gummy solid (yield – 68%) ^{1}H NMR (600 MHz, $CDCl_3$) δ_{ppm} 9.80 – 9.76 (m, 1H), 8.00 – 7.99 (m, 2H), 7.96 (s, 1H), 4.73 – 4.63 (m, 2H), 3.70 (s, 4H), 2.92 – 2.67 (m, 6H), 2.52 – 2.51 (m, 2H), 1.79 – 1.72 (m, 4H), 1.64 – 1.57 (m, 4H), 1.45 – 1.40 (m, 4H), 1.31 – 1.25 (m, 56H), 0.87 (t, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ_{ppm} 138.6, 138.3, 133.1, 130.4, 67.2, 58.9, 47.2, 40.2, 34.2, 32.1, 29.9, 29.8, 29.7, 29.5, 29.2, 28.5, 24.8, 24.3, 22.8, 21.6, 19.8, 14.3; **ES-MS (ESI+) m/z:** [(M/3 + Na^+] 298.2419.

III. Vesicle preparation:

Vesicles were prepared by thin film hydration method in 20 mM HEPES buffer, pH 7.4, containing 100 mM KCl.[3, 4] First, 40-50 µL solution of the lipid was taken from 10 mM lipid stock and dried under reduced pressure for 3 hours to prepare the lipid film. Then 500 µL of the HEPES buffer pH 7.4 was added into the lipid film and heated to 60-70 °C for 10 min (final concentration of the liposome ~1 mM). After that the solution was vortexed well until the dry lipid film was mostly disappeared. Finally, the solution was sonicated for 10 times (30 s of sonication followed by 30 s of cooling on ice). A hand-held miniextruder (Avanti Polar Lipids, Alabaster, AL) with

polycarbonate membrane (diameter of 200 nm) was used to prepare small unilamellar vesicles (at room temperature) whenever required. We observed that hydration of the dried film of the **SL1a** lipids over 60-70 °C only resulted in the formation of soluble aggregates.

IV. Transmission electron microscopy:

The morphology of the aqueous soluble aggregates of the lipids were investigated by transmission electron microscope (TEM). For TEM imaging the lipid solution was prepared by the above mentioned method in HEPES buffer, pH 7.4.[3, 4] The prepared lipid solution (without extrusion) was diluted to half of its original concentration using HEPES buffer, pH 7.4. From that diluted solution only 10 μL of lipid solution was placed onto a carbon-coated copper grid and allowed to absorb for 1 minute. The grid was then carefully blotted with filter paper and only a trace amount of the solution in the middle of the grid was kept. After that, the grid was allowed to dry for 10 minutes at 30 °C. Finally, 5-10 μL of 2% uranyl acetate solution (in water) was added on the grid and allowed to dry for another 1 min. The excess uranyl acetate solution was wicked off and the grid was dried overnight at 30 °C. The images were collected using a JEOL JEM 2100 transmission electron microscope (operated at a maximum accelerating voltage of 200 kV).

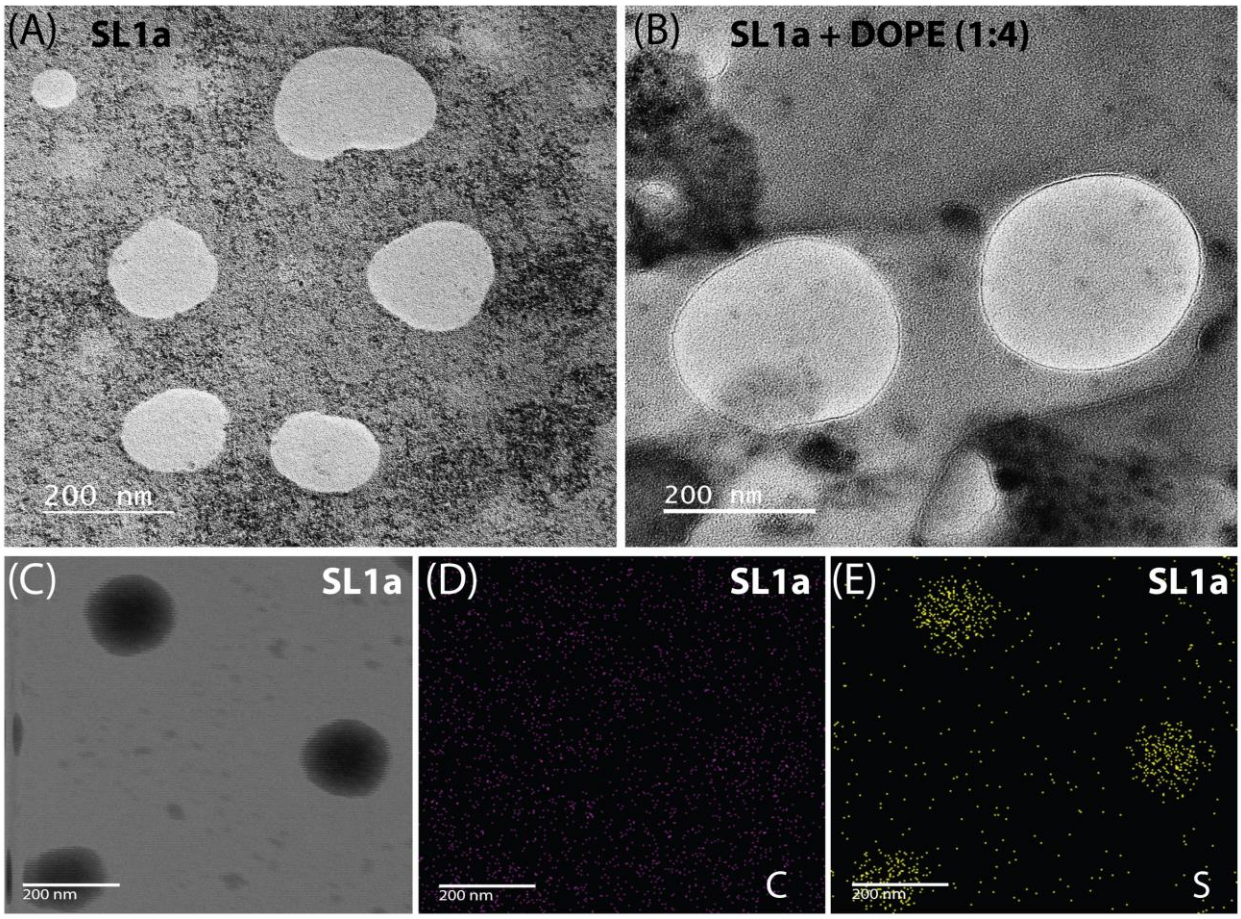


Fig. S1. TEM images of the vesicles generated from 100% **SL1a** lipid (A) and the lipid mixture of **SL1a**/DOPE (in the molar ration 1:4) (B). TEM-EDX bright field image of 100% **SL1a** lipid (C). Carbon TEM-EDX elemental mapping of 100% **SL1a** lipid (D). Sulphur TEM-EDX elemental mapping of 100% **SL1a** lipid (E). The carbon-coated copper grid was used for the TEM and TEM-EDX analysis.

V. Lipid phase transition temperature measurements:

To determine the phase transition temperature (T_m) from the ordered gel to liquid disordered phase of the lipids the temperature dependent steady-state anisotropy measurements were performed.[3, 4] The environment sensitive dye 1, 6 -diphenyl-1, 3, 5-hexatriene (DPH) was used as the

fluorescence probe to measure the change in anisotropy values. All the vesicles were prepared according to the method described in the previous section (the hydration was done by using 20 mM HEPES buffer, pH 7.4, containing 100 mM KCl). After that to the lipid solution (1000 µL) and DPH solution (10 µL of 1 mM stock solution in THF) were taken in a microcentrifuge tube (final concentration of DPH was 10 µM; DPH < 1% v/v in vesicle solution). The solution was kept at room temperature under tumbling condition for overnight to ensure the maximum incorporation of DPH molecules inside the hydrophobic core of the lipid bilayer. Then steady-state fluorescence anisotropy measurements were performed with a refrigerated system using a Peltier temperature controller connected to a fluoromax-4 spectrofluorometric (Horiba Scientific). The degree (r) of anisotropy in the DPH fluorescence (λ_{ex} = 350 nm; λ_{em} = 429 nm) was calculated using the following equation (eq. 1) at the peak of the fluorescence spectrum. The I_{VV} and I_{VH} are the fluorescence intensities of the emitted light polarized parallel and perpendicular to the excited light, respectively, and $G = I_{VH}/I_{HH}$ is the instrumental grating factor.

$$r = \frac{(IVV - GIVH)}{(IVV + 2GIVH)} \qquad \text{(eq. 1)}$$

At every 3-4 °C temperature differences the anisotropy values were recorded, apart from the near to the T_m value of the lipid, in that case the anisotropy values were collected at every 1 or 2 °C. All anisotropy values of the DPH probe are the mean values of three individual measurements. Finally, to determine the T_m value of the lipid plots of degree of anisotropy (r) of the DPH probe as a function of temperature were investigated.

VI. pH dependent particle size and surface charge of the liposome:

The vesicles were prepared according to the method as described in earlier section in 25 mM HEPES buffer, pH 7.4, containing 100 mM KCl.[3, 4] Different buffer solution were freshly prepared

using isosmotic buffers consisting of 10 mM buffering agent and 10 mM salt to vary the pH range (pH 3 to pH 8.5) and used for the measurements. Citric acid and trisodium-citrate for pH 3.0–6.5; 3 (nmorpholino) propanesulfonic acid (MOPS) for pH 7.0 and tris(hydroxymethyl)aminomethane (Tris)–HCl were used for pH 7.5–8.5. Both the zeta potential and hydrodynamic diameter of the vesicles at different pH were measured using a Zetasizer Nano ZS90 (Malvern, Westborough, MA) instrument, at 25 °C.

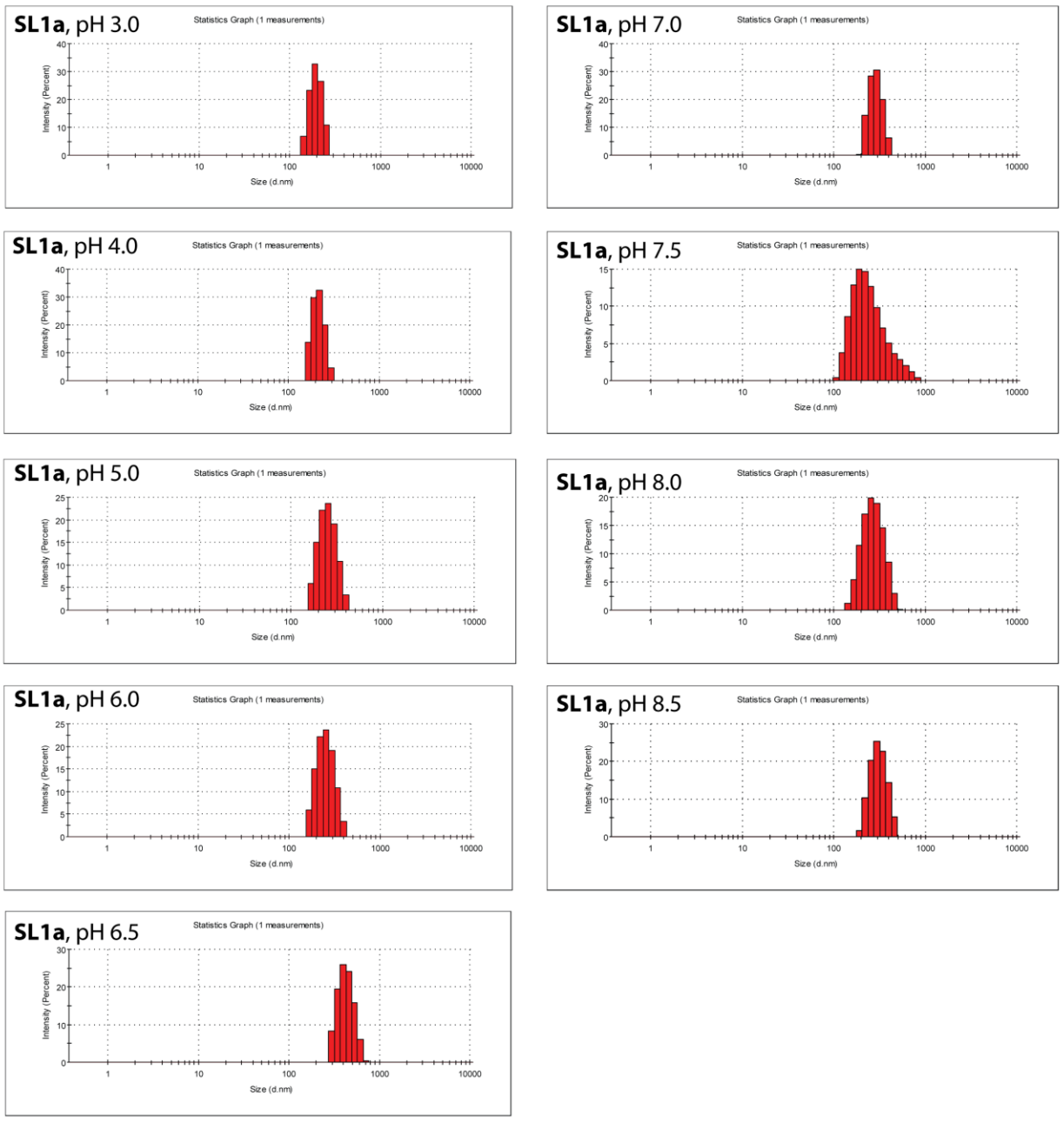


Fig. S2. Dynamic light scattering (DLS) measurements. Size distribution of the extruded vesicles (200 nm) generated from **SL1a** lipid measured at 25 °C. Content is given in percentage distribution of different sized vesicles at different pH.

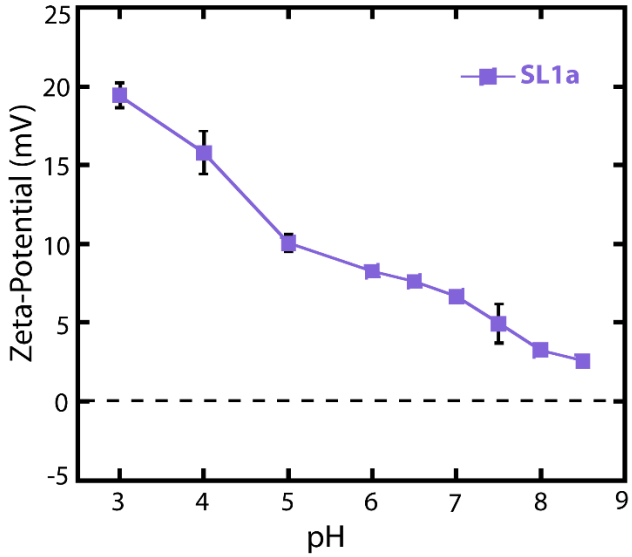


Fig. S3. Surface potential of the extruded vesicles (200 nm) generated from **SL1a** lipid at different pH.

VII. Dealkylation of SL1a lipid in the presence of glutathione:

The lipid **SL1a** (1 mM) was dissolved in 25 mM HEPES buffer, pH 7.4 containing 10 mM reduced glutathione (GSH) and the mixture was incubated in 37 °C. At different time intervals same amount of aliquot of the reaction solution was taken out and HPLC analysis was performed. The aliquot was dissolved in methanol before its injection to the analytical HPLC (Waters 600E HPLC) system. The Hypersil GOLD™ C18 Selectivity LC Column and a UV-detector (254 nm) was used for the analysis. Acetonitrile/water isocratic gradient (95/5 (v/v)) was used as the mobile phase at a flow rate of 1.0 mL/minute for 10 minutes run time. Fragmented product was collected and the identity of the product was confirmed by ES-MS (ESI+) analysis.

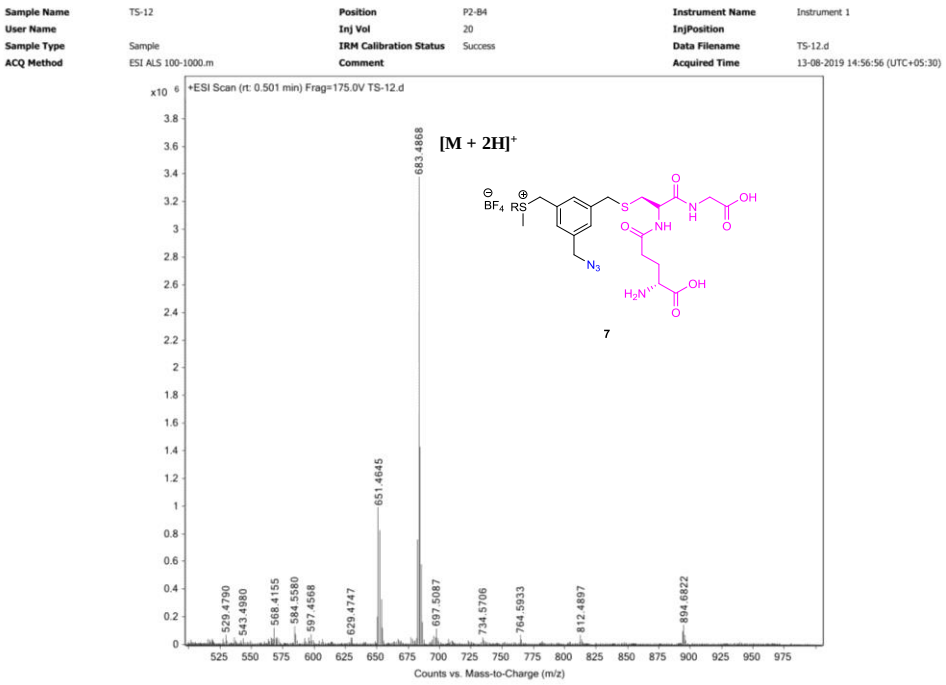


Fig. S4. Mass spectral signals of compounds **7** (in water) after separation by HPLC analysis.

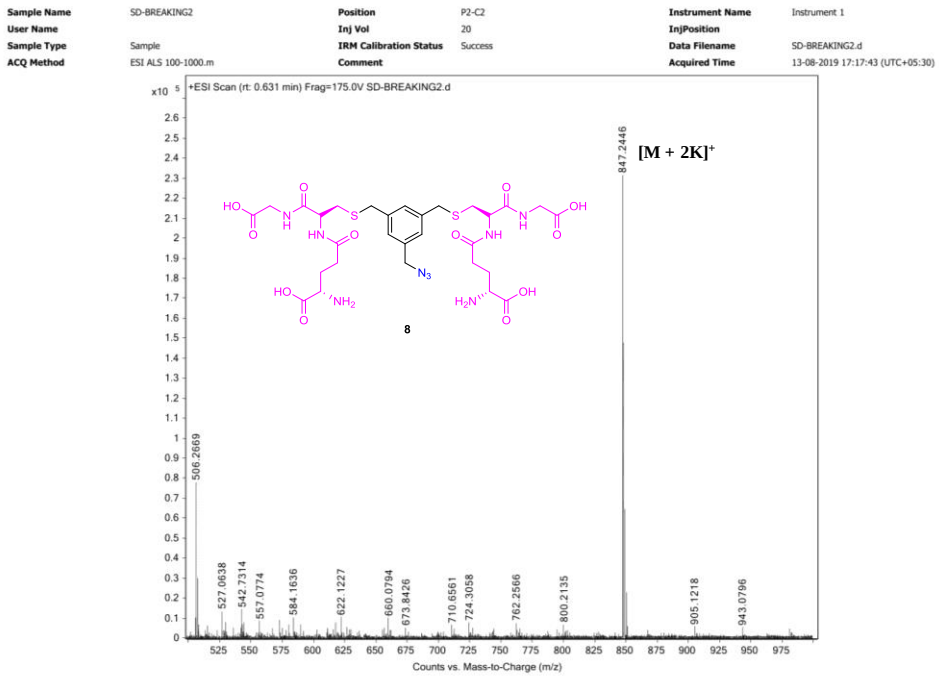


Fig. S5. Mass spectral signals of compounds **8** (in water) after separation by HPLC analysis.

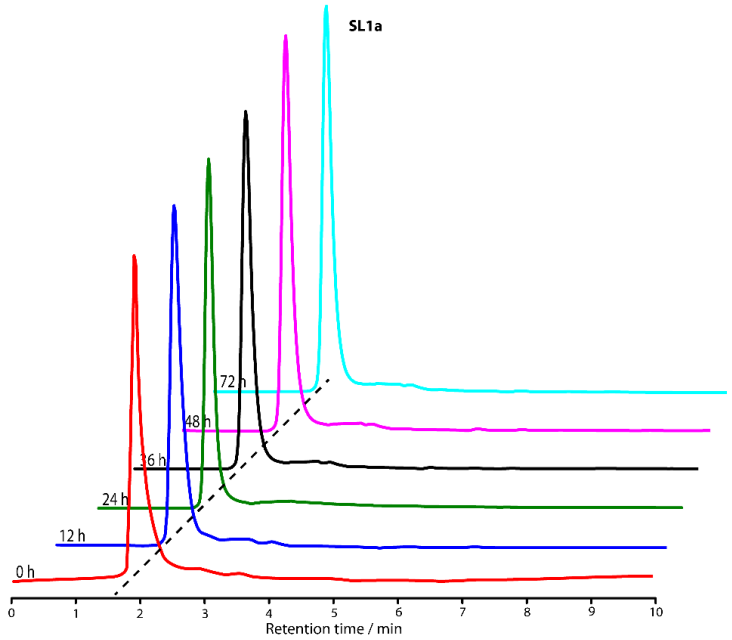


Fig. S6. The HPLC traces for the dealkylation of lipid **SL1a** in the absence of GSH at different time intervals.

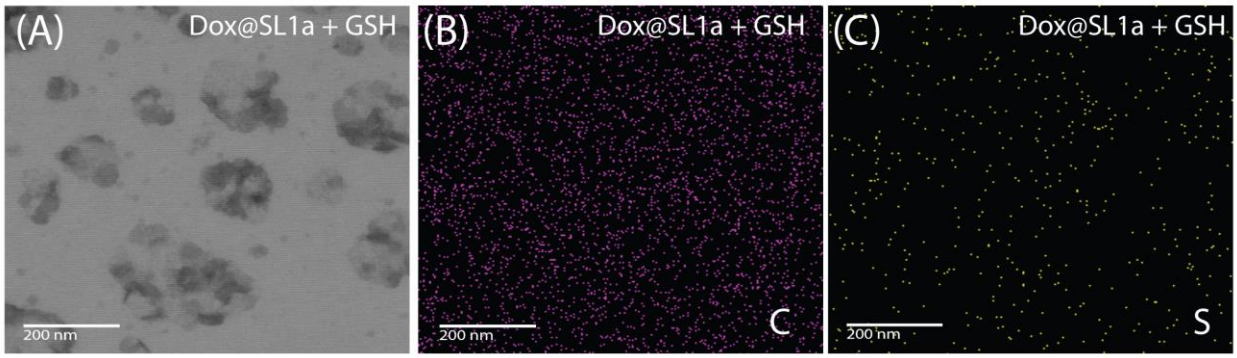


Fig. S7. TEM-EDX bright field image of the **SL1a** lipid in the presence of GSH (10 mmol) after 36 h of incubation (A). Carbon TEM-EDX elemental mapping of the **SL1a** lipid in the presence of GSH (10 mmol) after 36 h of incubation (B). Sulphur TEM-EDX elemental mapping of of the **SL1a** lipid in the presence of GSH (10 mmol) after 36 h of incubation (C). The carbon-coated copper grid was used for the TEM and TEM-EDX analysis.

VIII. Doxorubicin encapsulation and release efficiency measurement:

To a glass vial the **SL1a** lipid (50 µL from a stock solution of 28 mM in chloroform) was taken and dried under N_2 atmosphere for 4 hours to form multilayers. Then, 10 µL of doxorubicin (Dox) hydrochloride (sock solution of 2 mM in water) was added into the same vial and hydrated with 1 mL of 25 mM HEPES buffer, pH 7.4, containing 100 mM KCl. After that, the solution was vortexed well for 15-20 minutes and then sonicated as mentioned earlier.[3, 4] The unencapsulated free Dox was separated by centrifuge followed by three times washing with same buffer HEPES buffer, pH 7.4. After that, the spectroscopic measurements were performed and finally the vesicles were lysed with 2 mM triton X-100 to release the Dox molecules. From the UV-Vis spectra the concentration of Dox in free, encapsulated (inside the vesicles) and released (from the vesicles) state was calculated. From the calibration curve, extinction coefficient (ε) was found 0.00781 $\mu M^{-1}cm^{-1}$for Dox.

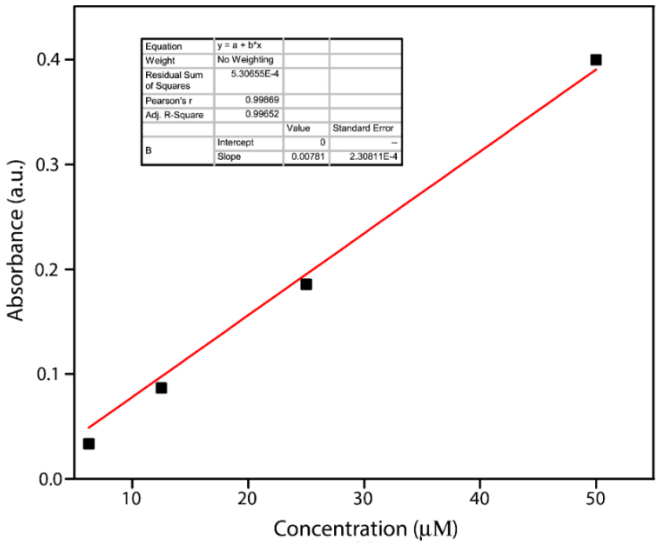


Fig. S8. Calculation of extinction coefficient (ε) for Dox.

According to the Lambert-beer's law, $A = \varepsilon.C_{f1}.l$.

Where C_{f1} is the concentration of Dox after complete release from the **SL1a** lipid vesicles at pH 7.4. Initial concentration of Dox (C_i) was 20 µM. Path length (l) = 1 cm.

So, $C_{f1} = A/\varepsilon.l = 0.055094/0.00781 \times 1 = 7.054$ µM.

Thus, Dox encapsulation efficiency at pH 7.4 = $(C_{f1}/C_i) \times 100 = 35.3$ %.

To measure the Dox release profile the vesicles was prepared by the same thin film hydration method as previously mentioned in 25 mM HEPES buffer, pH 7.4, containing 100 mM KCl, in the presence of Dox. After that, the free Dox molecules were separated by centrifuge followed by three times washing with same buffer. Then the loaded Dox loaded vesicle was diluted to 1 mM in the presence and absence of GSH (10 mM) containing same buffer solution. The release of Dox molecules from the vesicles was measured by monitoring the emission signals at 516 nm (λ_{ex} = 485 nm) in Fluoromax-4 spectrofluorometer at room temperature. Finally, the vesicles were lysed using Triton X-100 surfactant to measure the fluorescence of total encapsulated Dox.

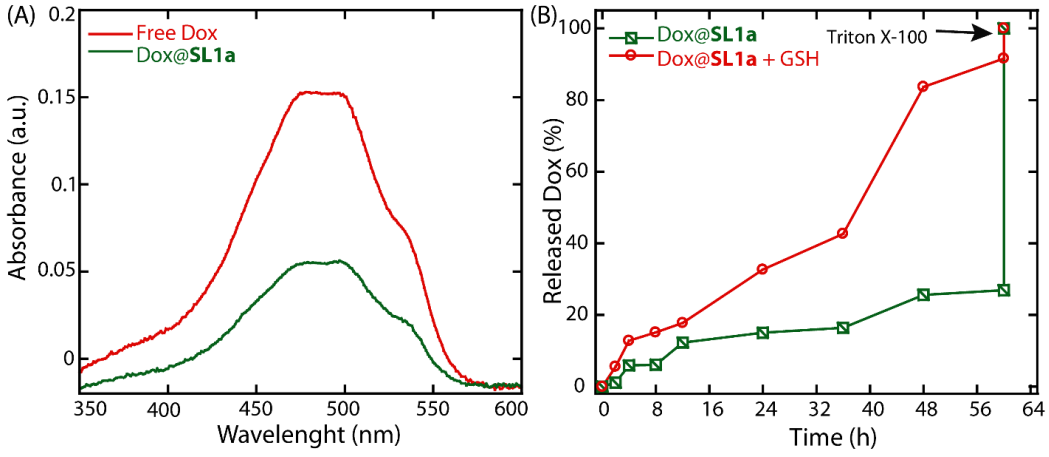


Fig. S9. UV-Vis spectra of free-Dox, Dox-encapsulated vesicles of **SL1a** lipid at pH 7.4. Dox release profile in the absence and presence of GSH at pH 7.4 (B).

VIII. Cell culture:

The MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, GIBCO) supplemented with 10% Fetal Bovine Serum (FBS, GIBCO) and penicillin-streptomycin solution (GIBCO; 100 U/ml penicillin and 100 µg/ml streptomycin). BJ normal human fibroblasts (ATCC catalog CRL-2522) were also cultured in DMEM supplemented with 10% FBS, 1% non-essential amino acids (NEAA; GIBCO) and penicillin-streptomycin solution (100 U/ml penicillin and 100 µg/ml streptomycin) Cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C in a CO_2 incubator. In all experiments, medium was changed daily with complete media.

IX. Determination of cell viability using MTT assay:

The MDA-MB-231 and BJ cells were cultured as mentioned earlier. For cell viability study, both the cell lines were seeded in a 96-well flat-bottomed plates at a density of 4×10^3 cells per well and incubated for 24 hours in complete media in a CO_2 incubator. Thereafter, cells were treated with different concentrations of **SL1a**, free Dox and Dox@**SL1a** for 48 or 72 hours (as per the experimental design mentioned in the figure legend) in 1% FBS containing media. The cell viability was then assessed using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] (Sigma-Aldrich) assays. Briefly, media was discarded and cells were treated with MTT (final concentration: 0.5 mg/ml) for 2 hours at 37°C in a CO_2 incubator and the resulting purple colored formazan crystals formed were dissolved in 200 µL of DMSO. Cell viability was then measured using Multiscan GO (Thermo Scientific) at 540 nm. Cell viability (%) = (fluorescent intensity (sample)) / (fluorescence intensity (control)) × 100%.

X. Determination of cellular uptake using microscopy analysis:

The MDA-MB-231 cells were cultured as mentioned earlier. For microscopy analysis, cells were seeded in 6-well plates at a density of 1.5×10^5 cells per well, each well containing a sterile 22 mm square cover slip (pre-coated with 0.1% gelatin) in the bottom and incubated for overnight in complete media in a CO_2 incubator. Cells were then treated with Dox@**SL1a** at their respective IC_{50} concentration for 8 hours in 1% FBS containing media. Post-treatment, cells were washed with PBS buffer and fixed with 4% formaldehyde at 37 °C for 10 minutes. Cover slips were then washed thrice with PBS to wash out formaldehyde. Further, cells were treated with 0.1% triton X-100 for 5 minutes at 37°C. Cells were again washed thrice with PBS buffer to remove triton X-100. To counterstain nuclei, DAPI (5 µg/mL) was added and incubated for 5 minutes. Cells were then washed thrice with PBS. Subsequently, cover slips were taken out from the wells and mounted over a drop of FluorPreserve Reagent (Sigma) over a glass slide. The slides were then allowed to dry for 15 mins. Finally, microscopy was performed using confocal laser scanning microscope (Leica TCS SP8; λ_{ex} = 488 nm, λ_{em} = 560–630 nm for Dox and λ_{ex} = 405 nm, λ_{em} = 440–480 nm for DAPI).

XI. Determination of cellular uptake using flow cytometry:

The MDA-MB-231 cells were cultured as mentioned earlier. Cells were seeded in 6-well plates at a density of 1.5×10^5 cells per well and incubated for 24 hours in complete media. The cells were then treated with Dox@**SL1a** and free Dox were treated with their respective IC_{50} concentration in 1% FBS containing media for 4 and 8 hours at 37°C. Next, the cells were washed twice with PBS (GIBCO), trypsinised (0.05%; GIBCO) for 2 minutes at 37°C followed by 5 min centrifugation at 800 rpm at 4°C. The supernatant was discarded and cell pellets were re-suspended

in 300uL of PBS (supplemented with 2% FBS). Finally, the solutions were kept on ice and drug uptake by the MDA-MB-321 cells was analyzed by flow cytometry analysis using BD FACS Calibur Flow Cytometer (BD Biosciences). Data analysis was performed by the FCS Express 5 Flow Cytometry software.

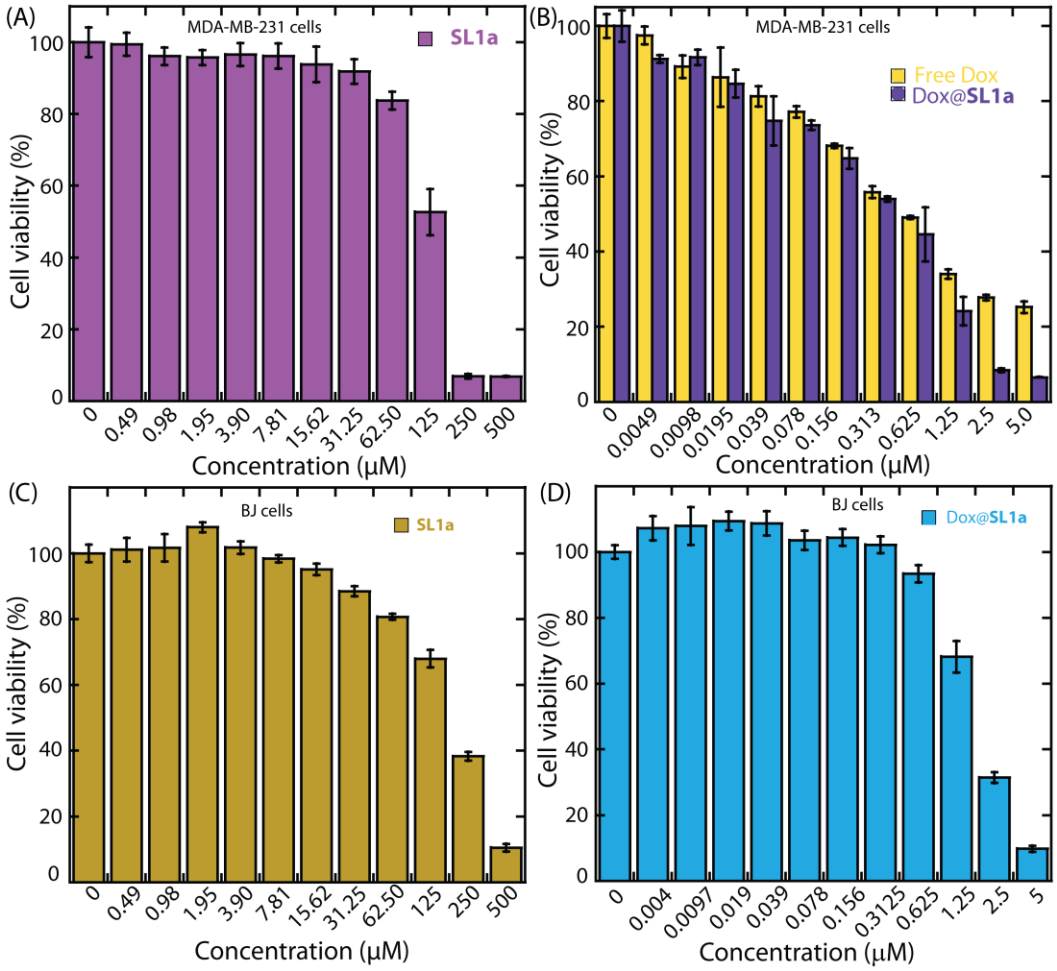


Fig. S10. Viability of MDA-MB-231 cells in the presence of **SL1a** (A), free Dox and Dox@**SL1a** (B) at different concentration. Viability of BJ cells in the presence of **SL1a** (C) and Dox@**SL1a** (D) at different concentration. The cell viabilities were measured by MTT assay after 48 hours of compound treatment.

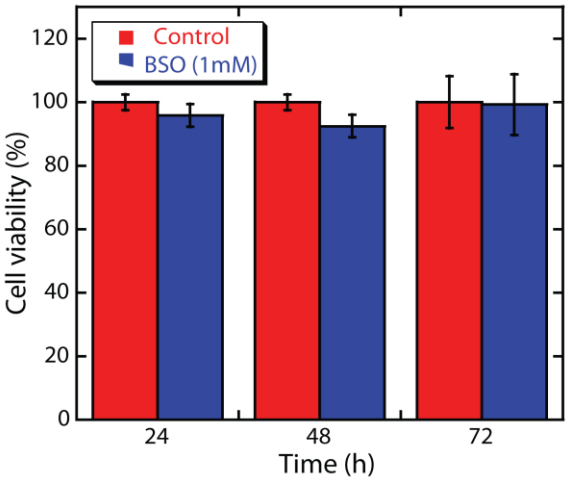


Fig. S11. Viability of MDA-MB-231 cells in the presence of BSO (1 mM) at different time.

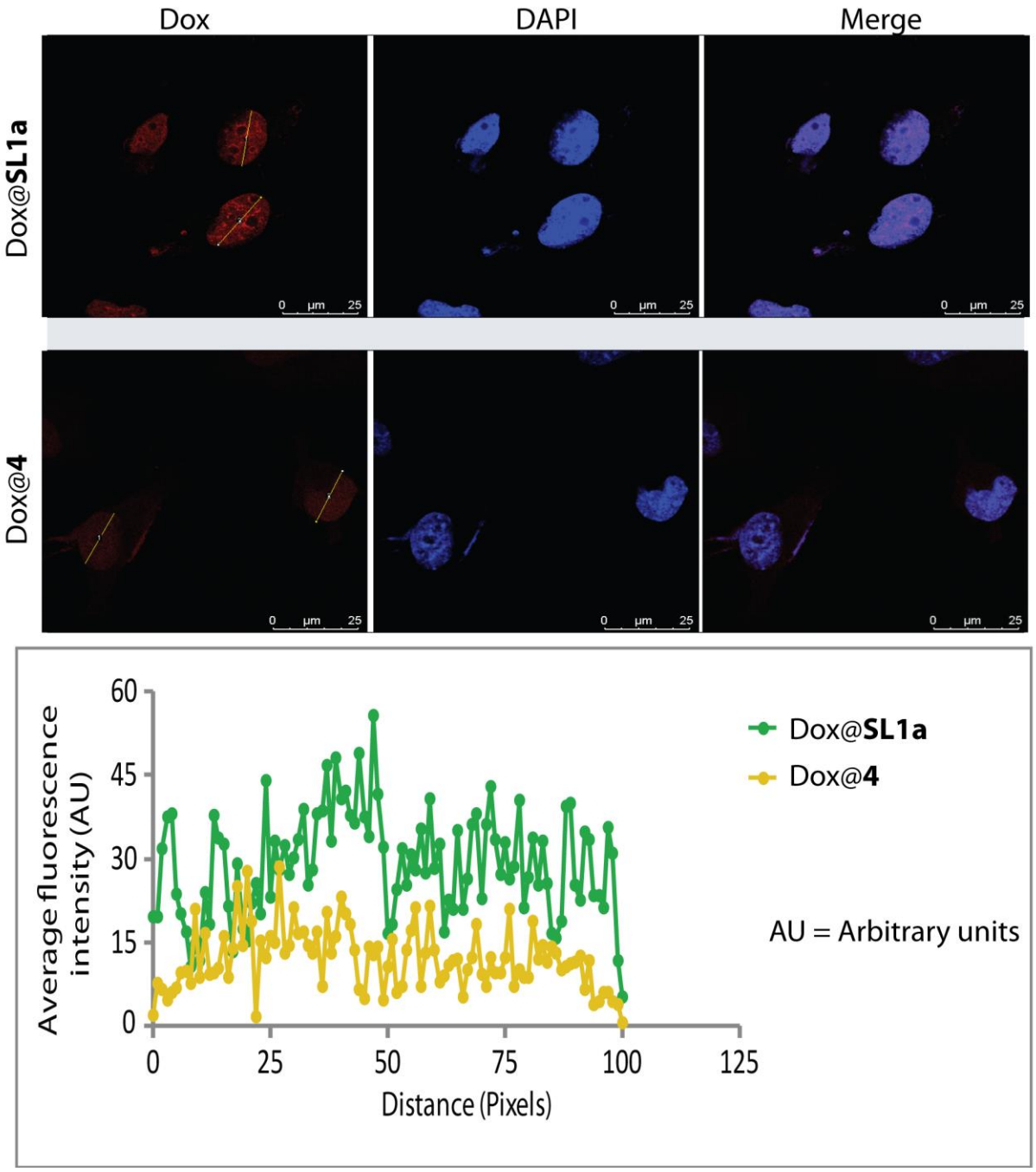


Fig. S12. CLSM images of the MDA-MB-231 cells treated with Dox@**SL1a** and Dox@**4a** (control lipid without the sulfonium moieties) for 8 h. Red channel (Dox), blue channel (DAPI), merge of red and blue channels illustrate the Dox delivery. Scale bar 25 µm. Concentration = 0.49 µM. The average fluorescence intensity was measured across the nucleus using Image J software (1.46r).

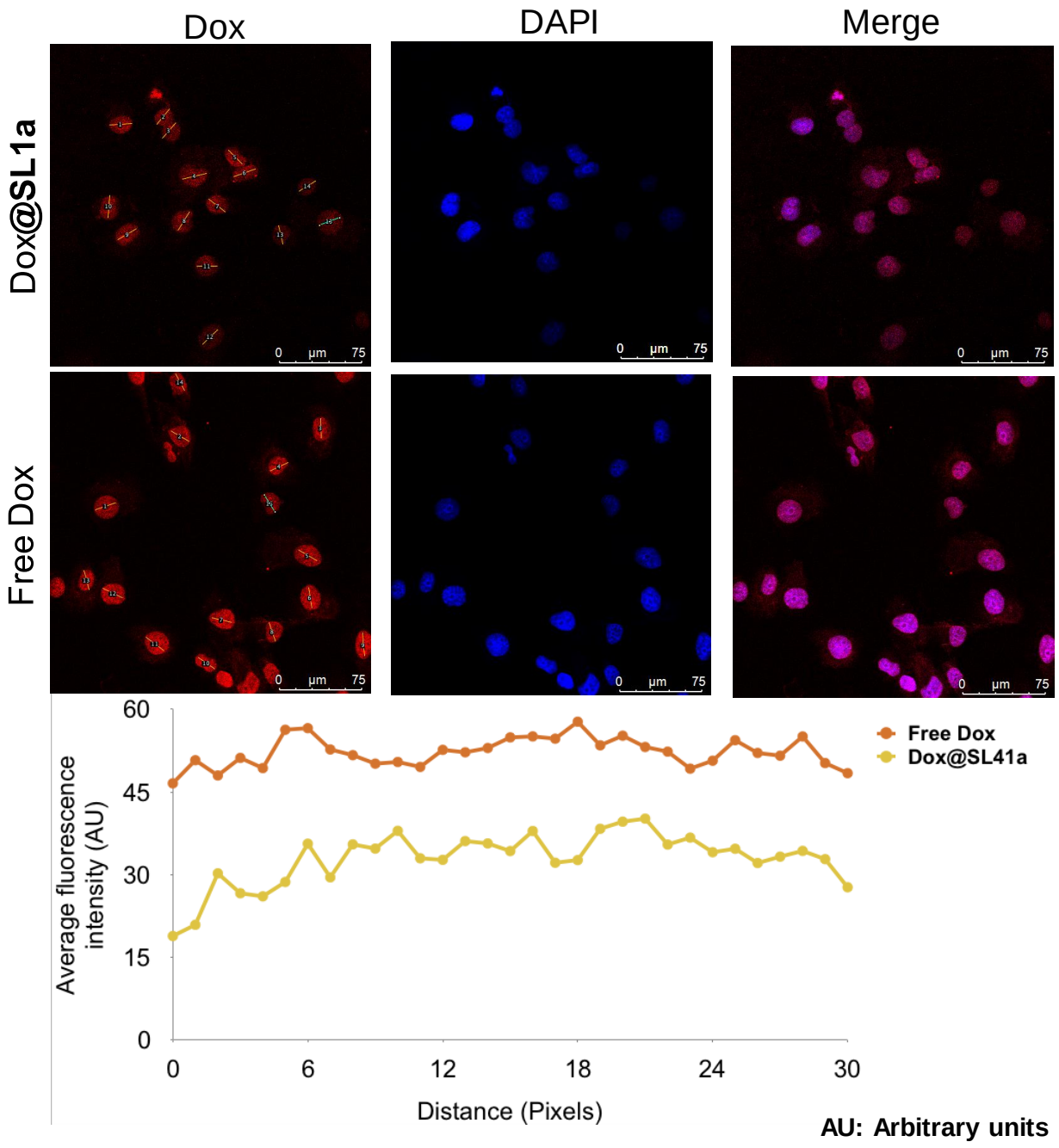


Fig. S13. CLSM images of the MDA-MB-231 cells treated with Dox@**SL1a** and free Dox for 8 hours. Red channel (Dox) illustrates the Dox delivery. Scale bar 75 µm. Concentration = 0.49 µM. The average fluorescence intensity was measured across the nucleus (number of cells counted: 15) using Image J software (1.46r).

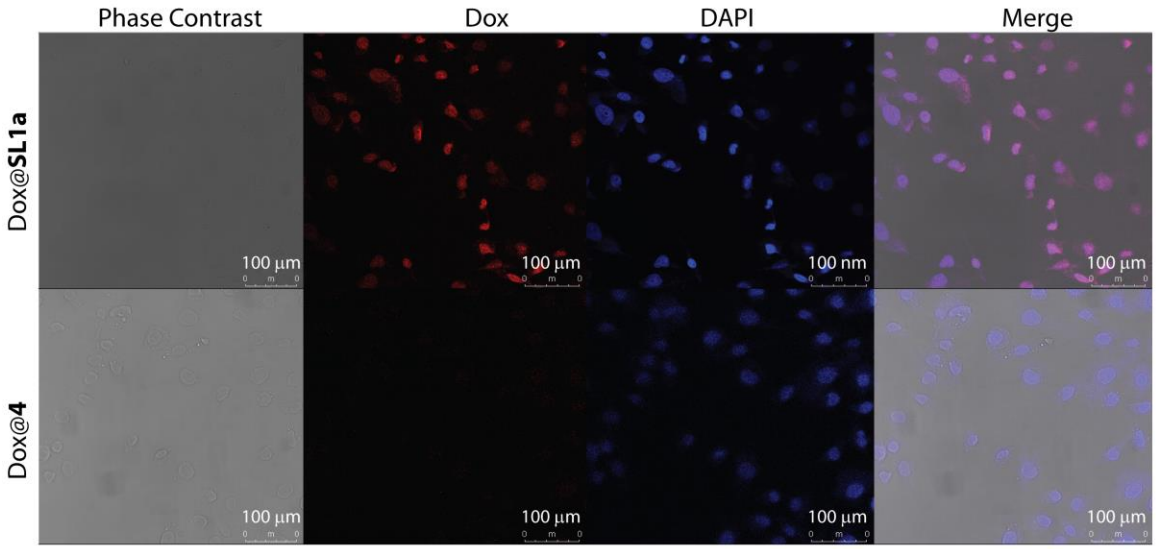


Fig. S14. CLSM images of the MDA-MB-231 cells treated with Dox@**SL1a** and Dox@**4a** (control lipid without the sulfonium moieties) for 8 h. Phase contrast, Red channel (Dox), blue channel (DAPI), merge of phase contrast, red and blue channels illustrate the Dox delivery. Scale bar 100 µm. Concentration = 0.49 µM.

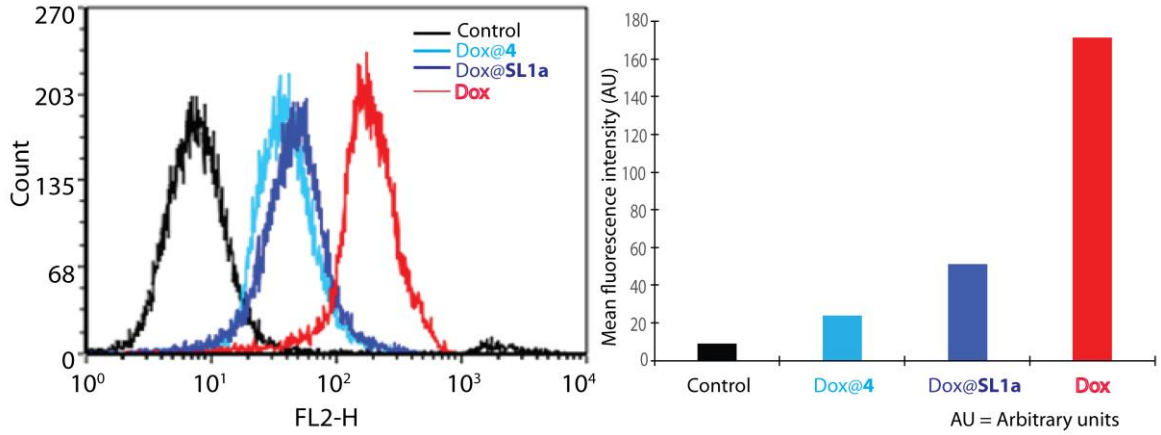


Fig. S15. Flow cytometry histogram profiles of MDA-MB-231 cells after 8 h of the treatment with Dox@**4a**, Dox@**SL1a** and free Dox, and their mean fluorescence intensity. Inherent fluorescence signal of Dox molecules was used for the measurement (FL2-H channel). Only cells were used as control. Concentration = 0.49 µM.

XII. Measurement of antimicrobial activity:

The minimum inhibitory concentration (MIC) of **SL1a** lipid was measured by micro broth dilution method against both gram-negative (*E. coli*; MTCC-1687) and gram-positive (*S. aureus*; MTCC-96) bacteria. The *E. coli* and *S. aureus* microbes were grown in Luria Bertani (LB) and Brain Heart Infusion (BHI) broth media, respectively at 37˚C and 180 rpm in a shaker for overnight and a fresh media was inoculated from the overnight grown culture till the OD reached up to 0.5 to 0 .6. The cells were then harvested by centrifugation, added to the media at a cell concentration of 10^6 CFU/mL. Then, the cells were added to the wells containing serially diluted sulfonium lipid (**SLa1**) in 96 well plate. Plate was incubated for 14 to 16 h at 37˚C and the absorbance of the plate was measured by using Tecan infinite M200 Plate reader at 600 nm. The MIC was considered as the concentration at which absorbance was close to the control (wells containing only media and compound) or the wells had no visible growth. This assay was repeated at various concentration ranges to get the most accurate MIC values. The MIC values of the Dox and Dox@**SL1a** treated bacterial cells were also measured using the similar method.

XIII. Morphological analysis of the bacterial cells.

The morphology of the bacterial cells in the absence or the presence of the chemicals were investigated by field emission scanning electron microscopy (FESEM) analysis**.** The *E. coli* cells were grown in media as mentioned in the above experimental section and cells were treated by the lipid at a concentration of its MIC value along with control and incubated for 5 h at 37˚C. The bacterial cells were collected by centrifugation and washed with PBS pH 7.4 buffer followed by Mili-Q water, and then it was drop casted on to the aluminum foil wrapped on a glass grid. The

sample was dried under laminar airflow. Glass grid was stacked on a metal grid by using carbon tape and double gold coating was performed before the analysis.

Table S1. The calculated MIC values of the lipid against both gram-positive and gram negative bacteria.

Bacterial strain	MIC (μM)			
	SL1a	Only Dox	Dox@**SL1a** (With respect to effective Dox concentration)	Dox@**SL1a** (With respect to **SL1a** concentration)
E. coli; MTCC-1687	32.00 ± 1.00	>72.00	0.19 ± 0.04	18.75 ± 4.00
S. aureus; MTCC-96	14.00 ± 1.00	36.00 ± 5.00	0.10 ± 0.03	10.00 ± 3.00

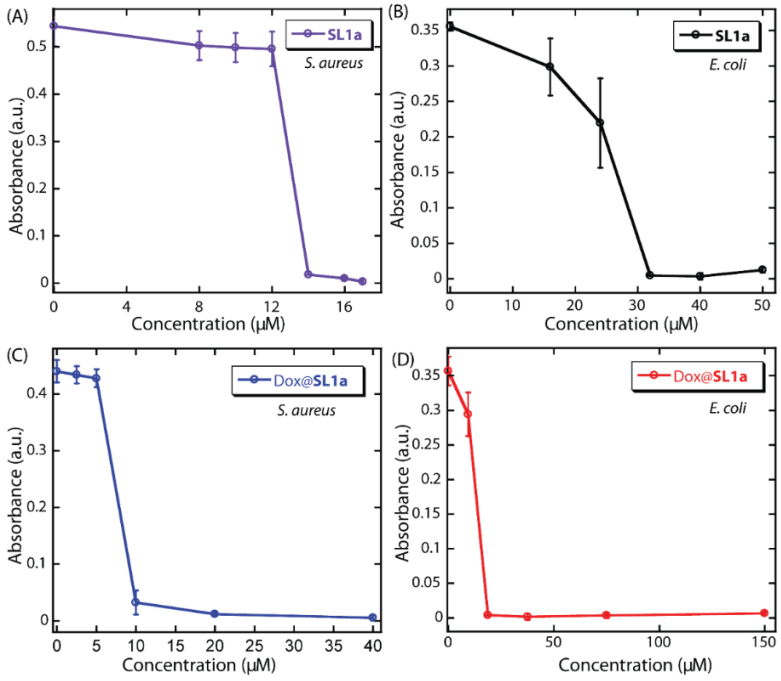


Fig. S16. Plot of absorbance at different concentration of **SL1a** and Dox@**SL1a** in *S. aureus* (A and C) *and E. coli* (B and D) cells. All plots were prepared with respect to **SL1a** lipid concentrations.

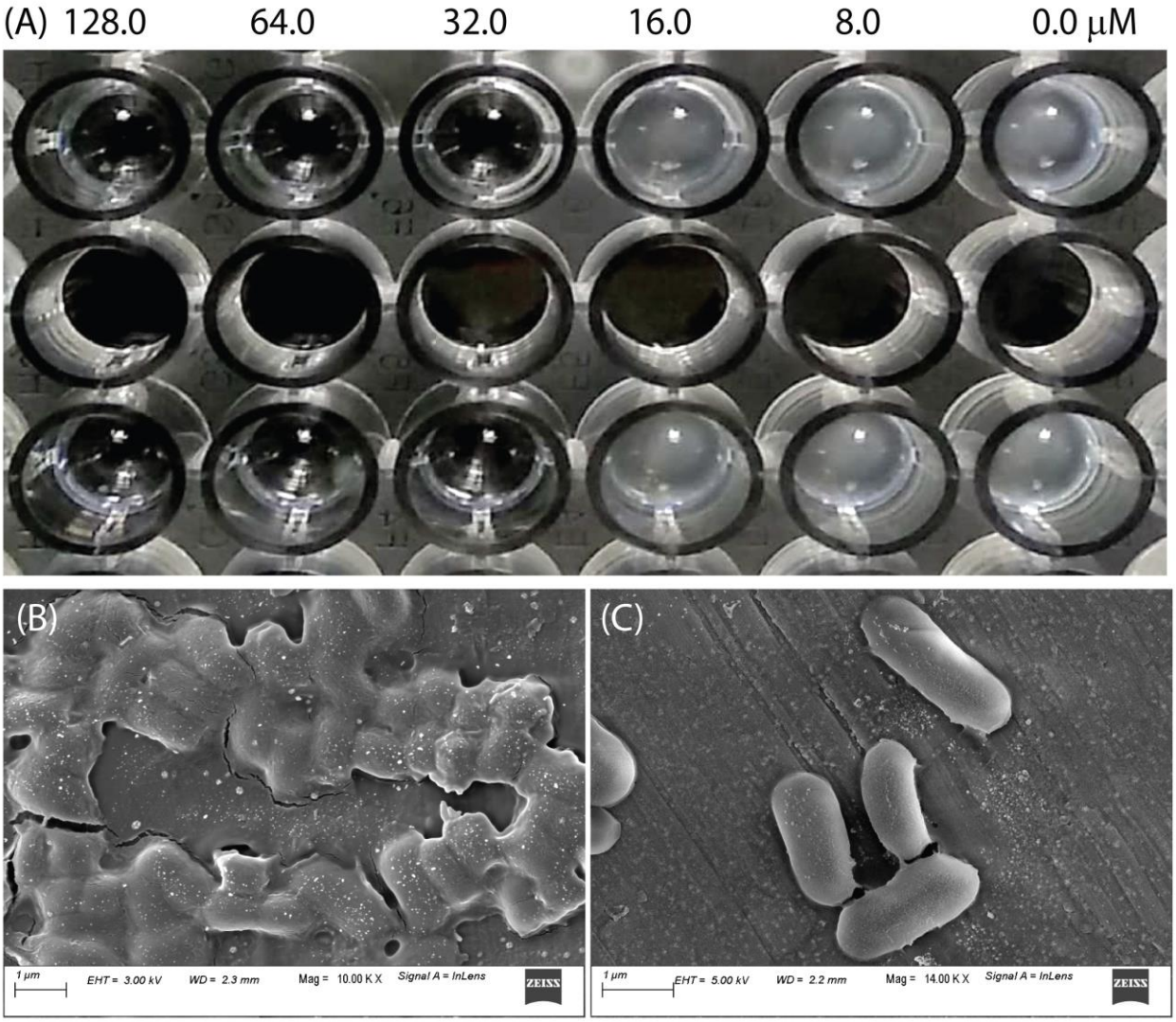
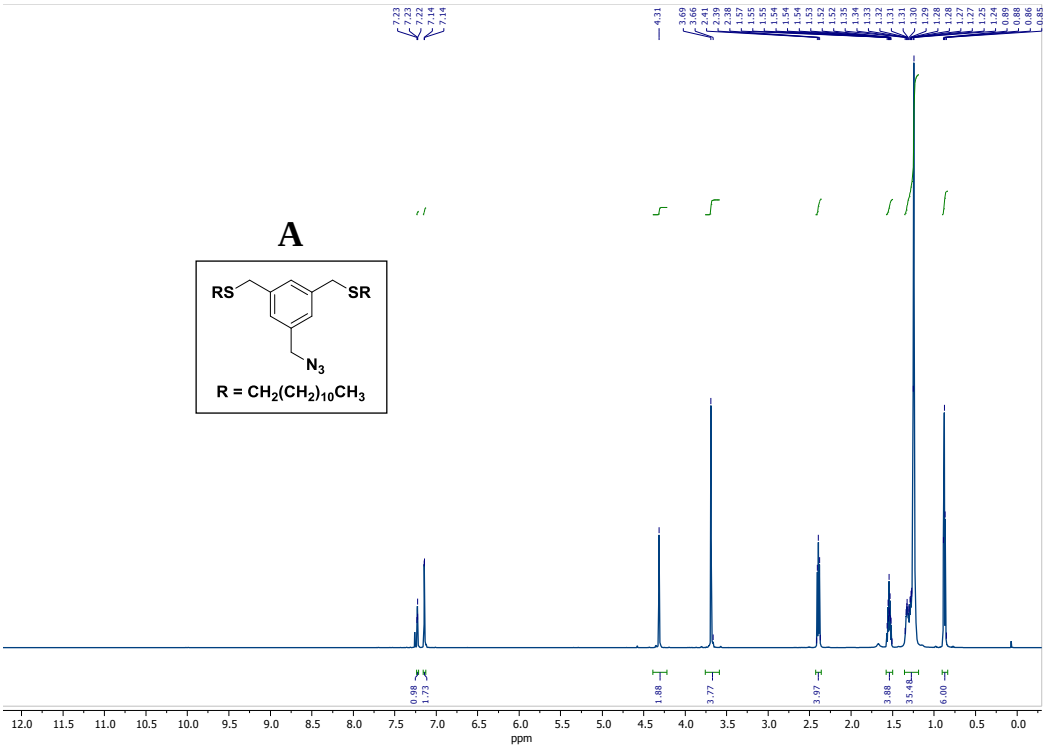


Fig. S17. The 96 well plate bacterial assay of **SL1a** lipid (first and third row; with respect to **SL1a** lipid concentrations) against *E. coli* (MTCC-1687*S*). The clear wells specify inhibition of bacterial growth, while cloudy wells indicate unconstrained bacterial growth. FESEM image of *E. coli* (MTCC-1687*S)* treated with only **SL1a** lipid (B, at MIC value). FESEM image of *E. coli* (MTCC-1687*S*, at MIC value) (C).

XIV. ^{1}H NMR and ^{13}C NMR spectra of synthesized compounds:



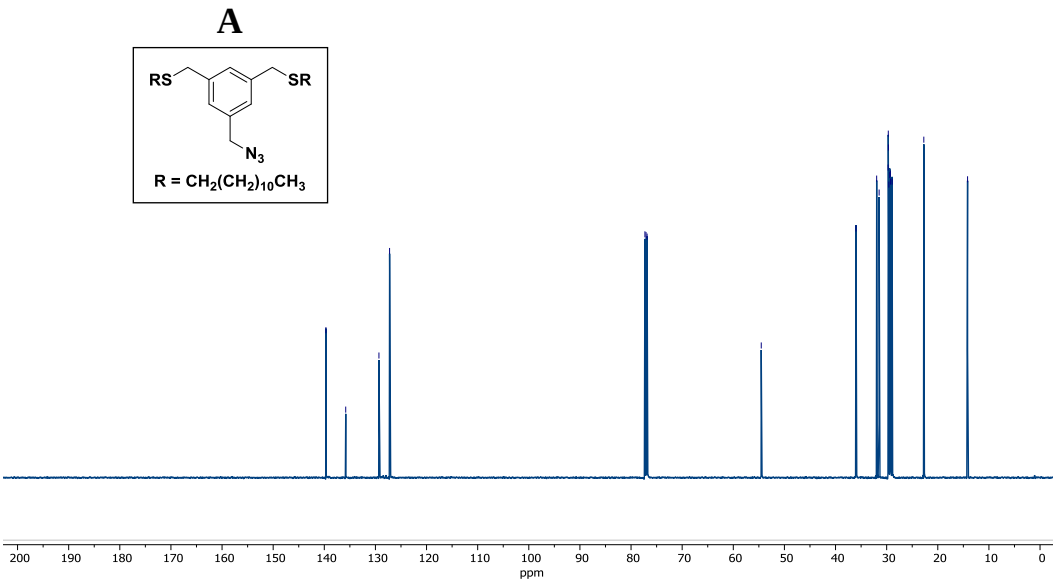
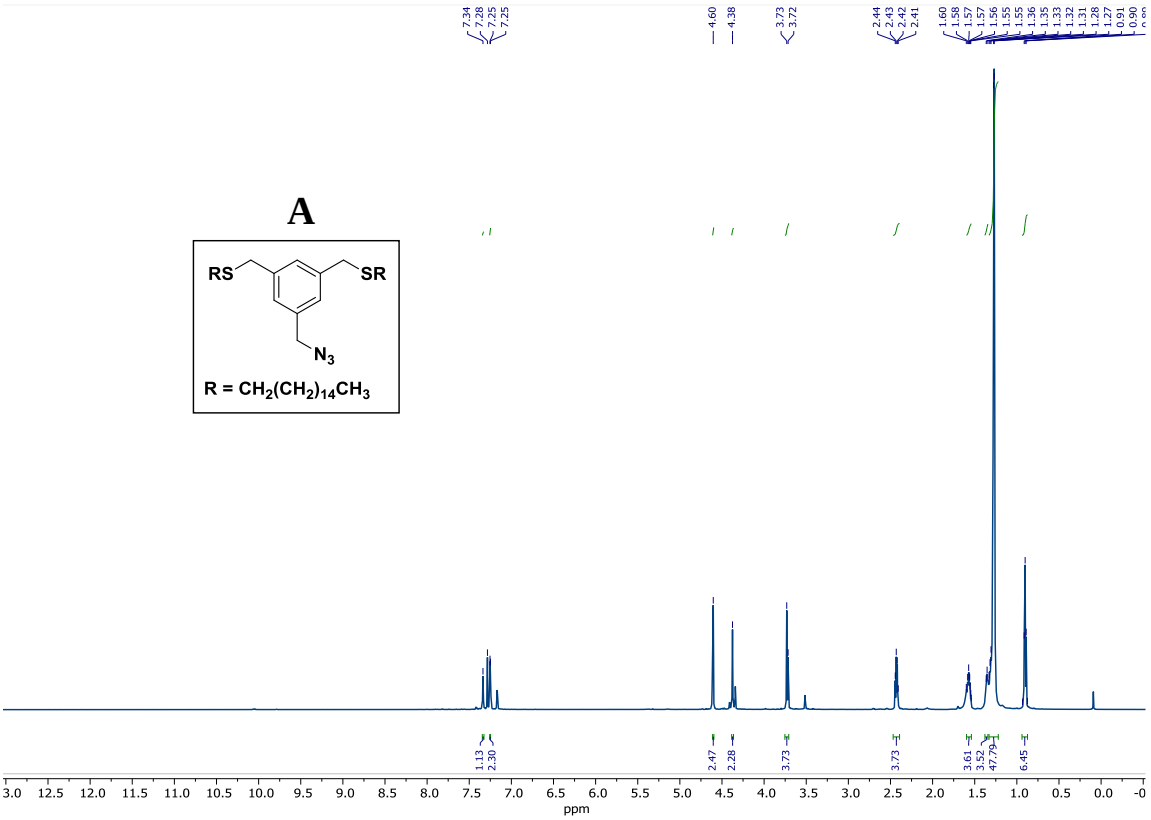


Fig. S15. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of lipid **4a**.



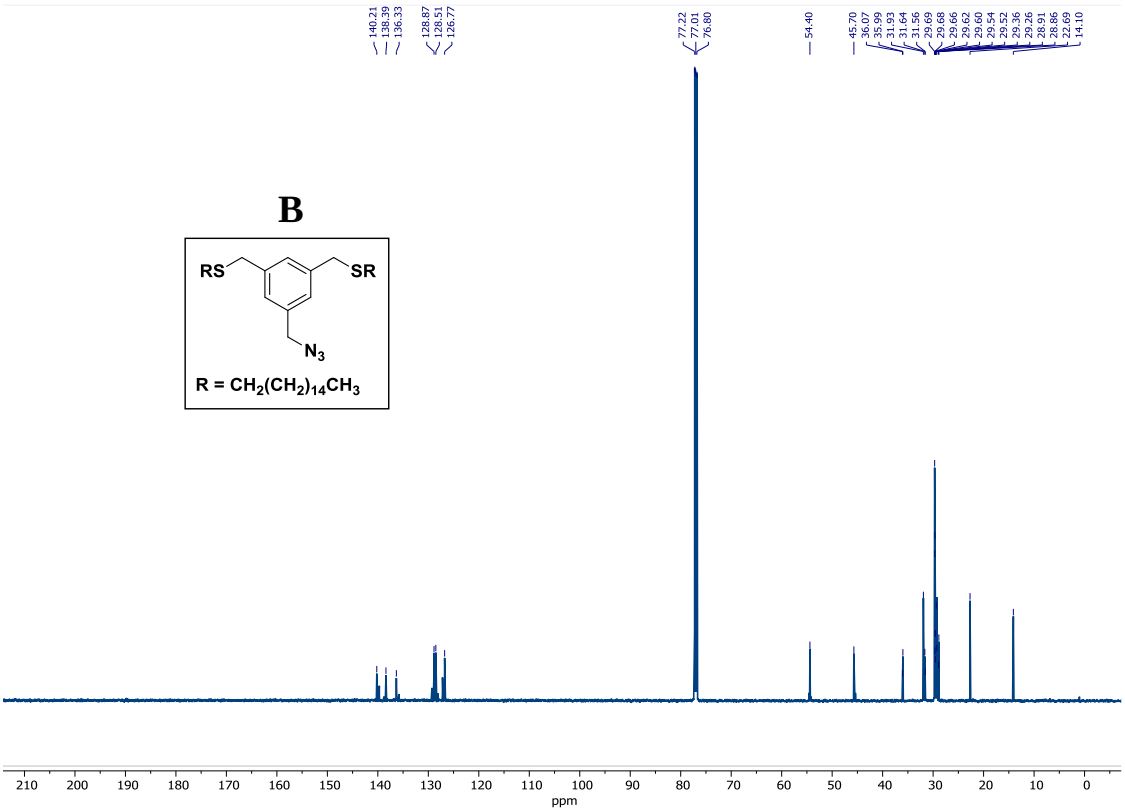


Fig. S16. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of lipid **4b**.

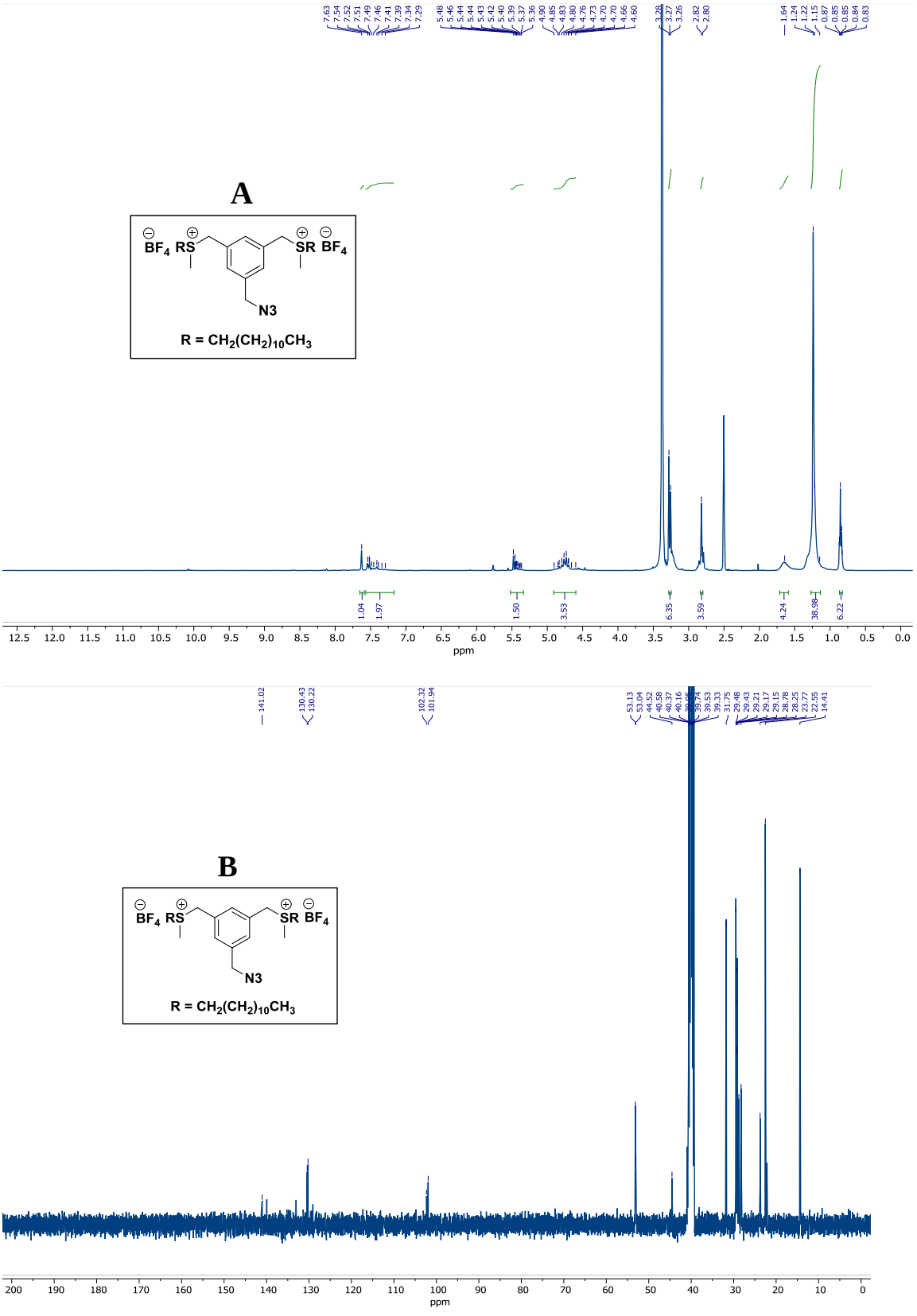


Fig. S17. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of lipid **SL1a**.

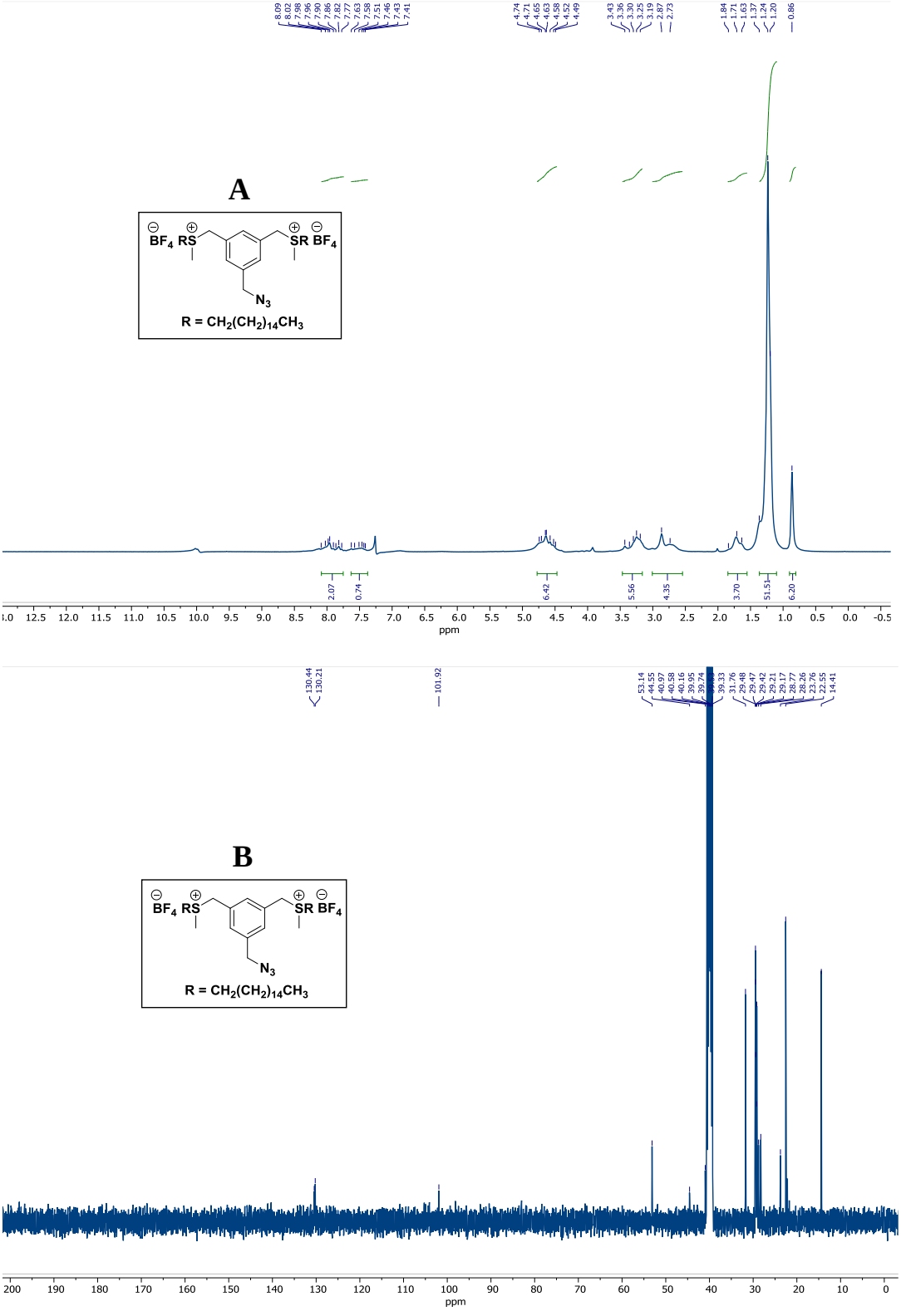


Fig. S18. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of lipid **SL1b**.

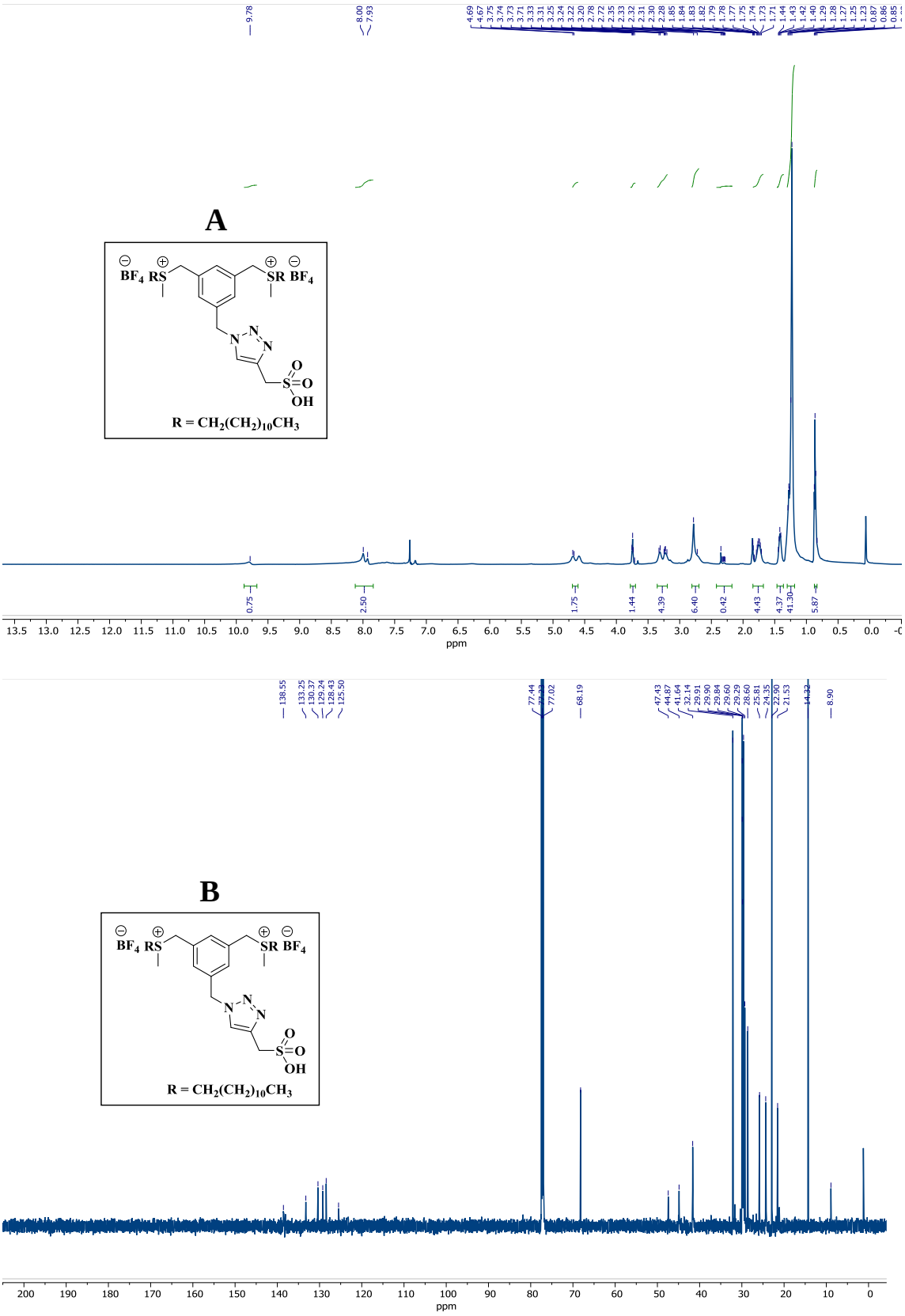


Fig. S19. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of lipid **SL3a.**

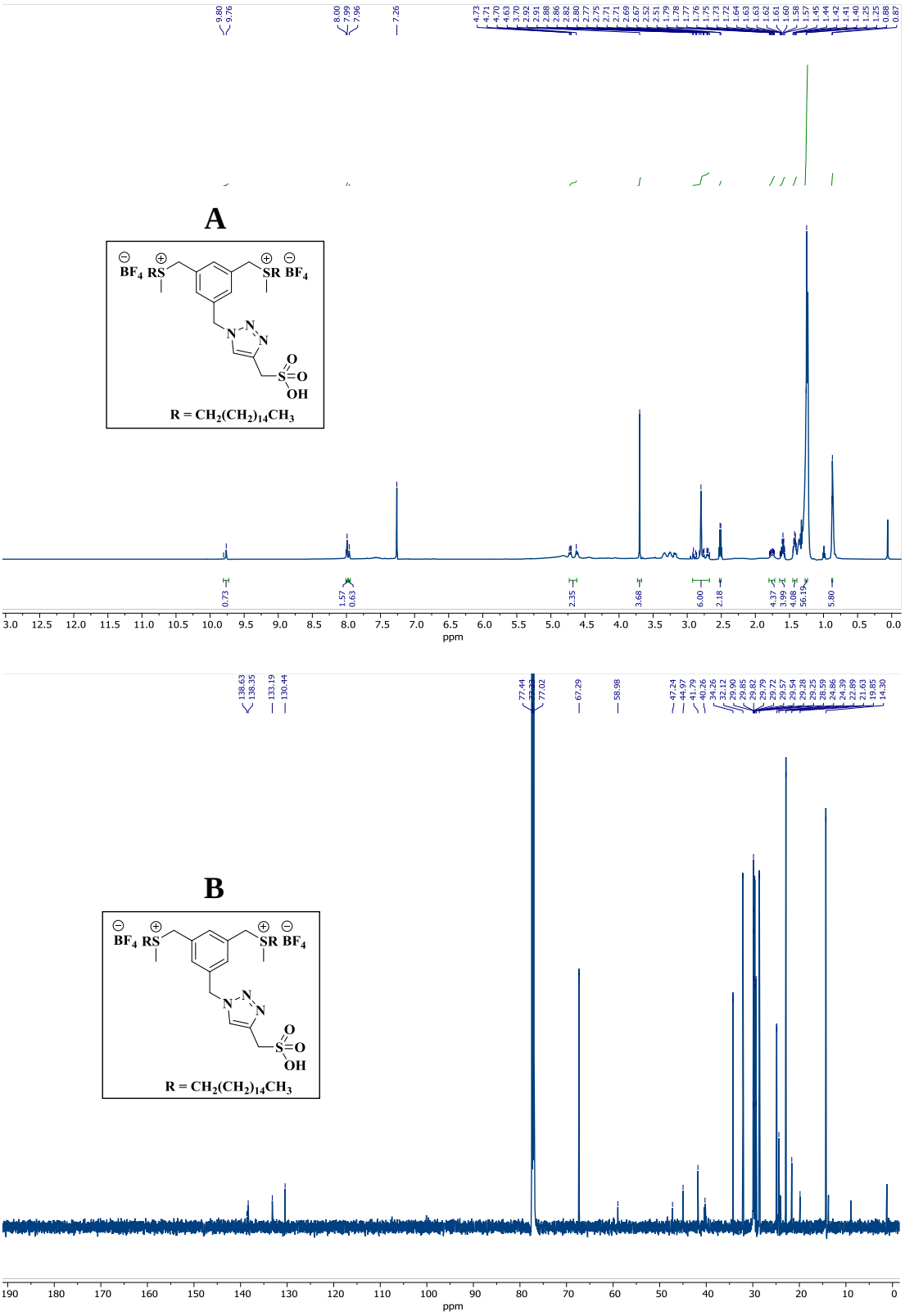


Fig. S20. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of lipid **SL3b.**

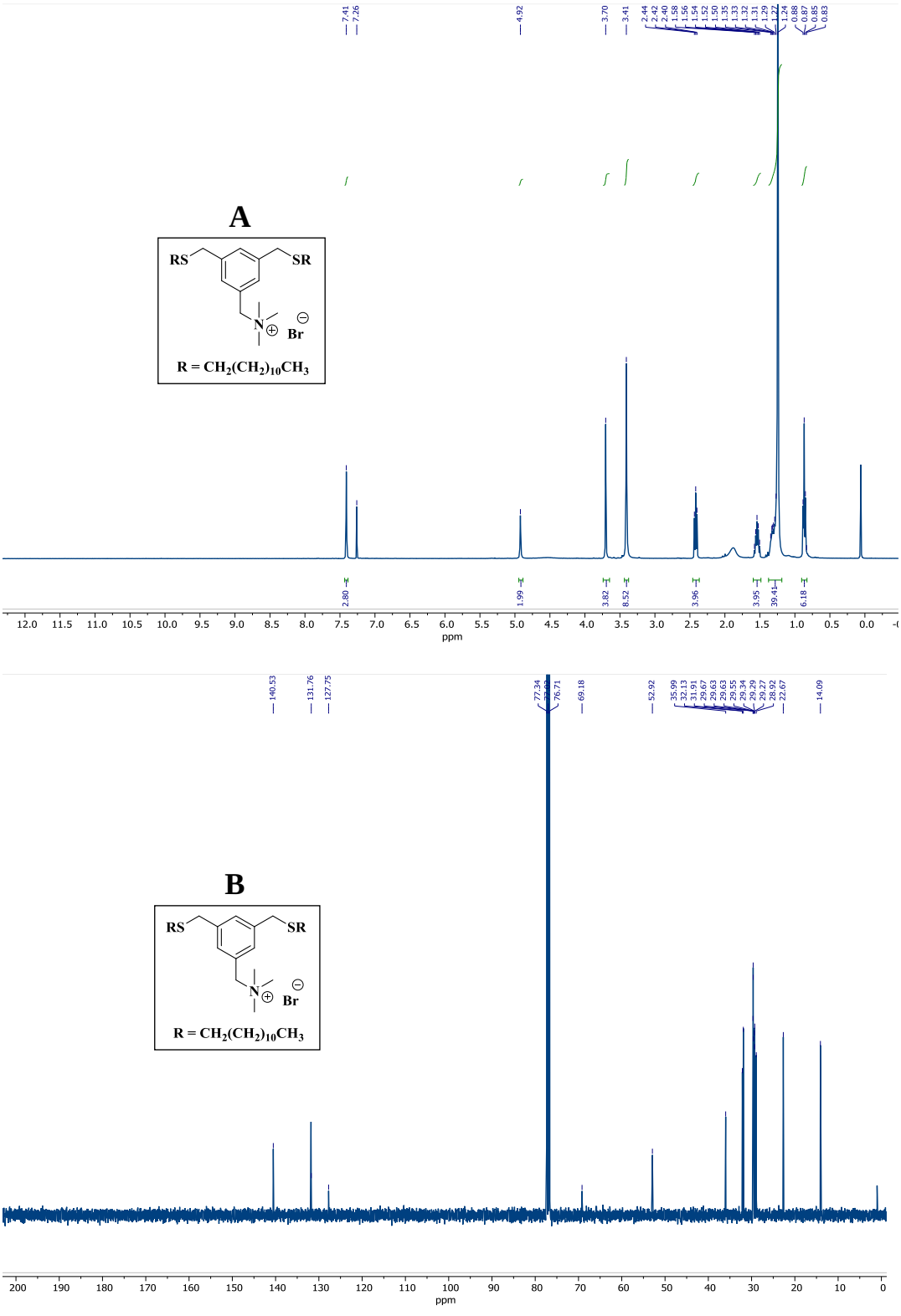


Fig. S21. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of **5a**

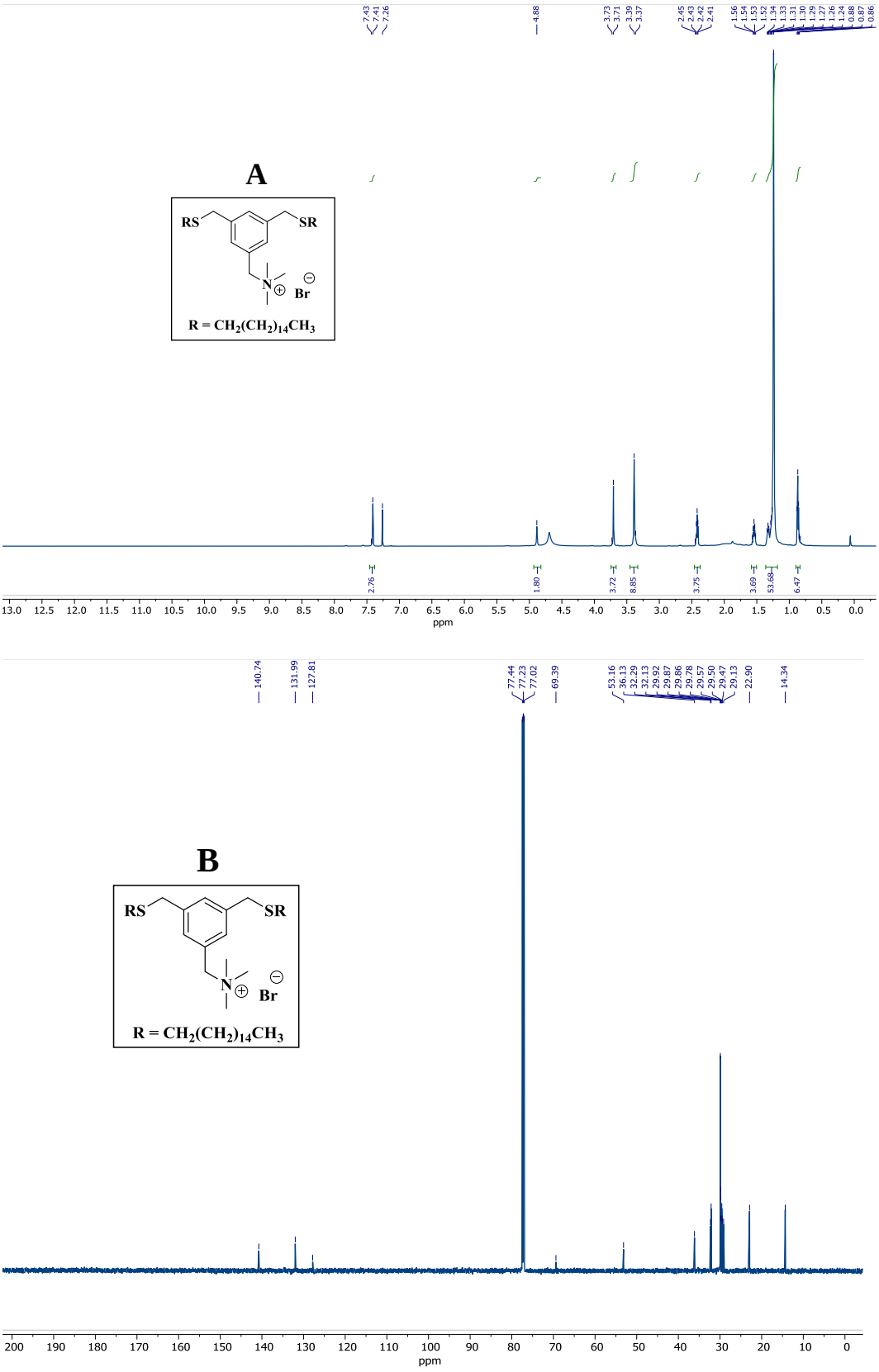


Fig. S22. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of **5b**

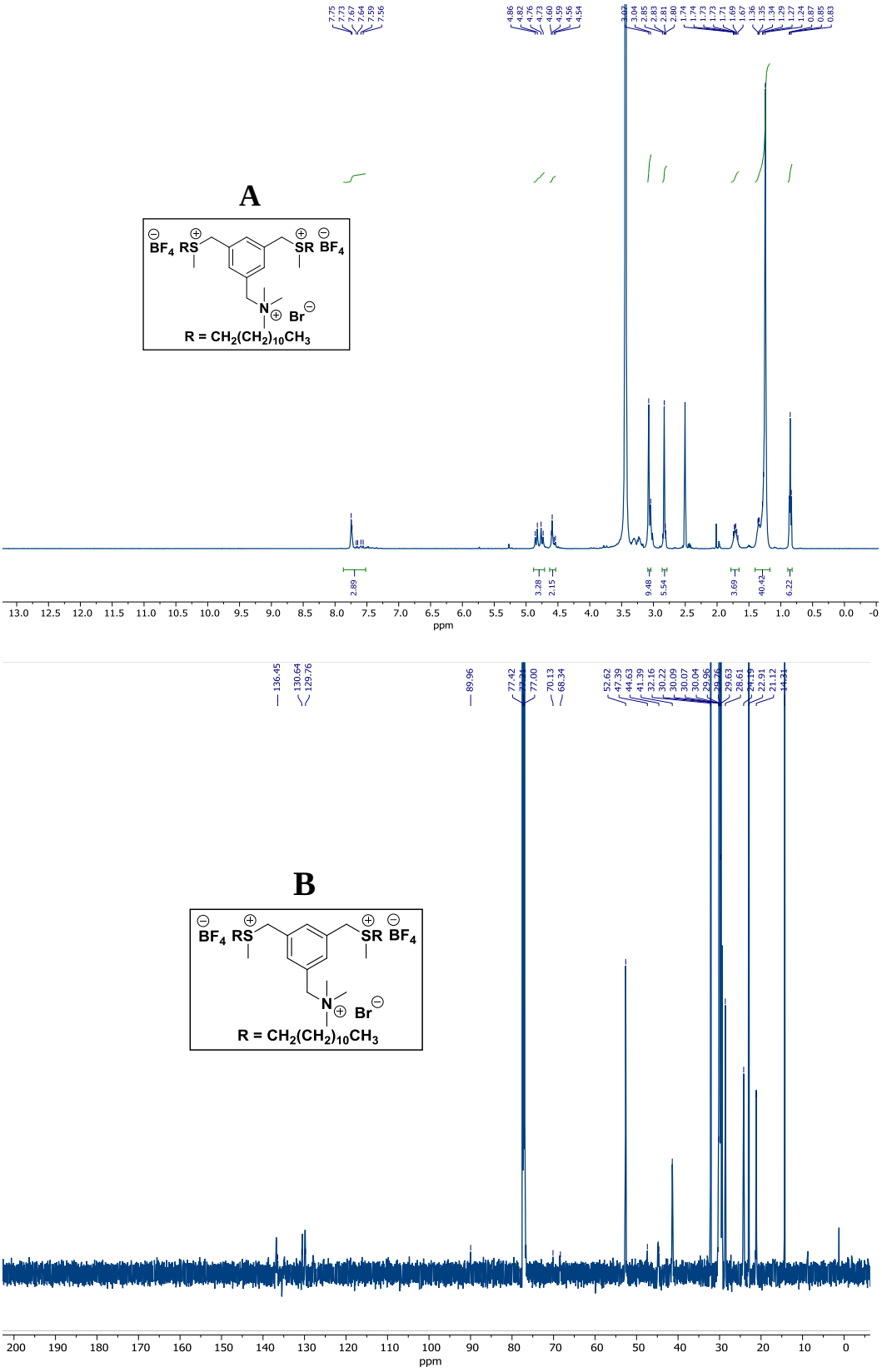


Fig. S23. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of **SL2a.**

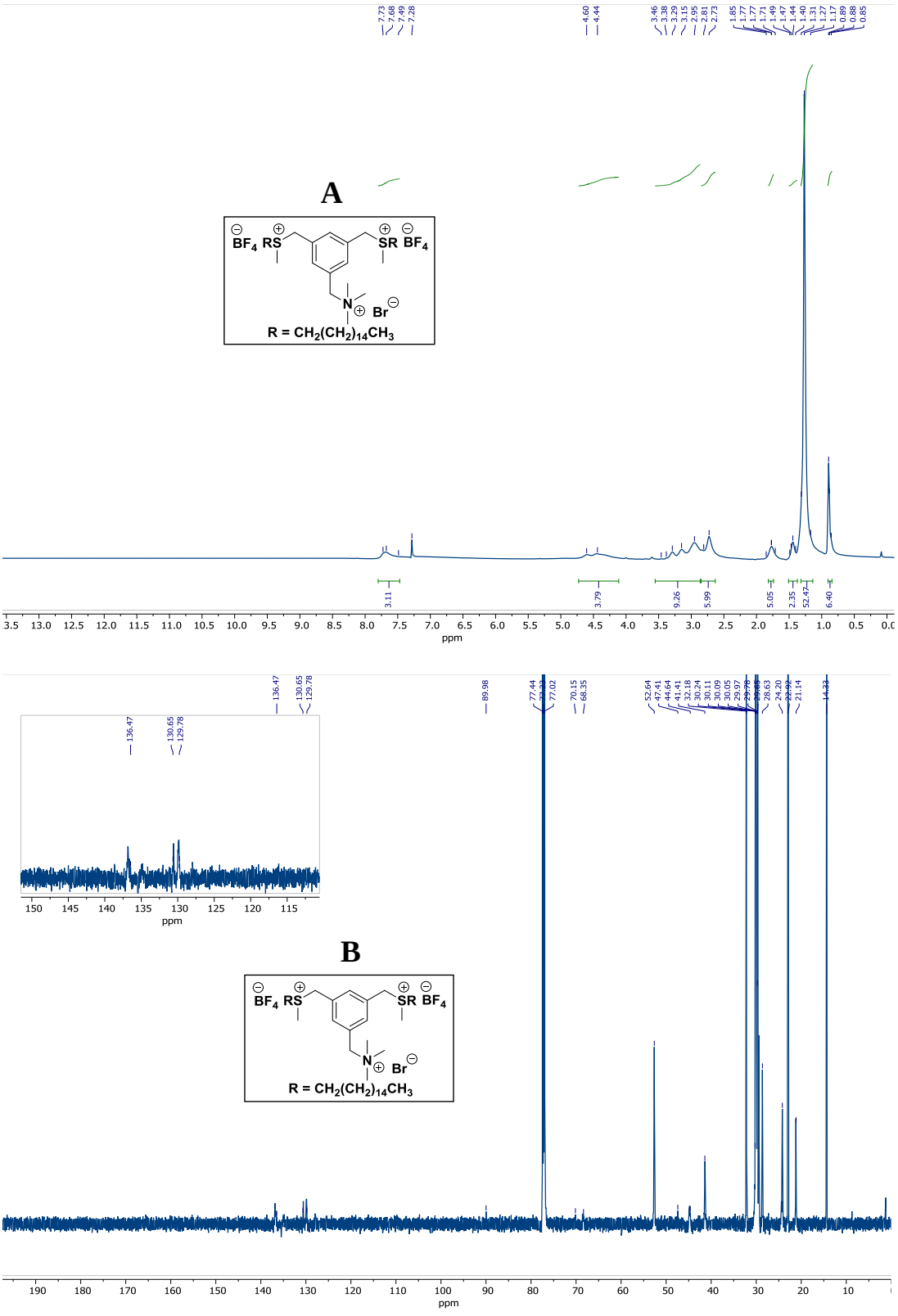


Fig. S24. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of **SL2b.**

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