Supporting Information

.....

Sulfonium-based liposome-encapsulated antibiotics deliver a synergistic antibacterial activity

Anjali Patel, Subhasis Dey, Kamal Shokeen, Tomasz M. Karpiński, Senthilkumar Sivaprakasam, Sachin Kumar and Debasis Manna*

Email: dmanna@iitg.ac.in

I. General information:

All the required reagents were purchased from Sigma-Aldrich, Merck, and other commercial sources and bacterial media from Himedia, which were used directly without further purification. The bacterial culture was acquired from the Microbial Type Culture Collection (MTCC). The synthesized compound was purified by column chromatography 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 the internal solvent. The coupling constants (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 = 7.28 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 were recorded at Agilent Q-TOF mass spectrometer with Z-spray source using built-in software for analysis of the recorded data. Ultrapure water (Milli-Q system, Millipore, Billerica, MA) was used for the preparation of buffers.

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

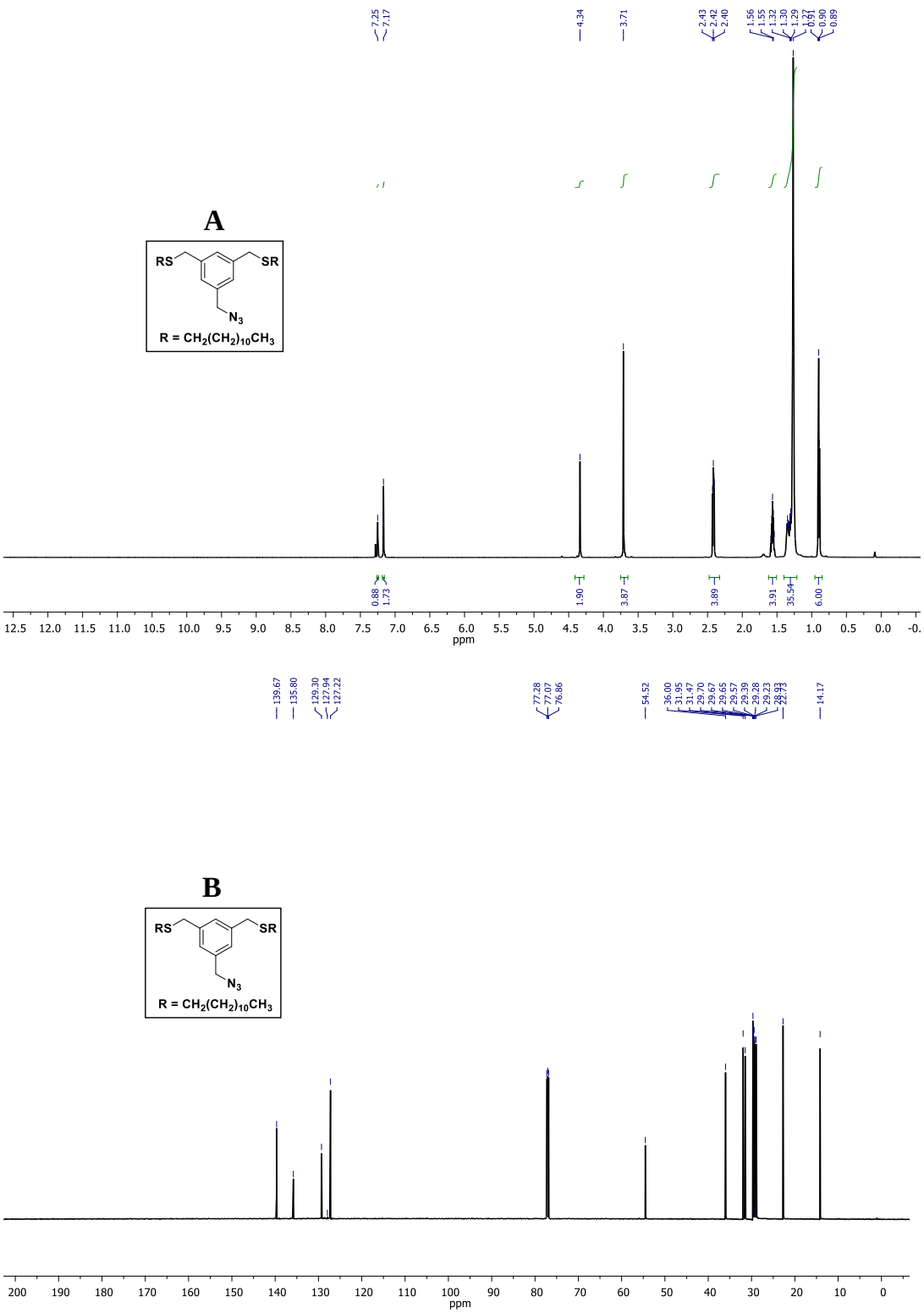

Fig. S1. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **4.**

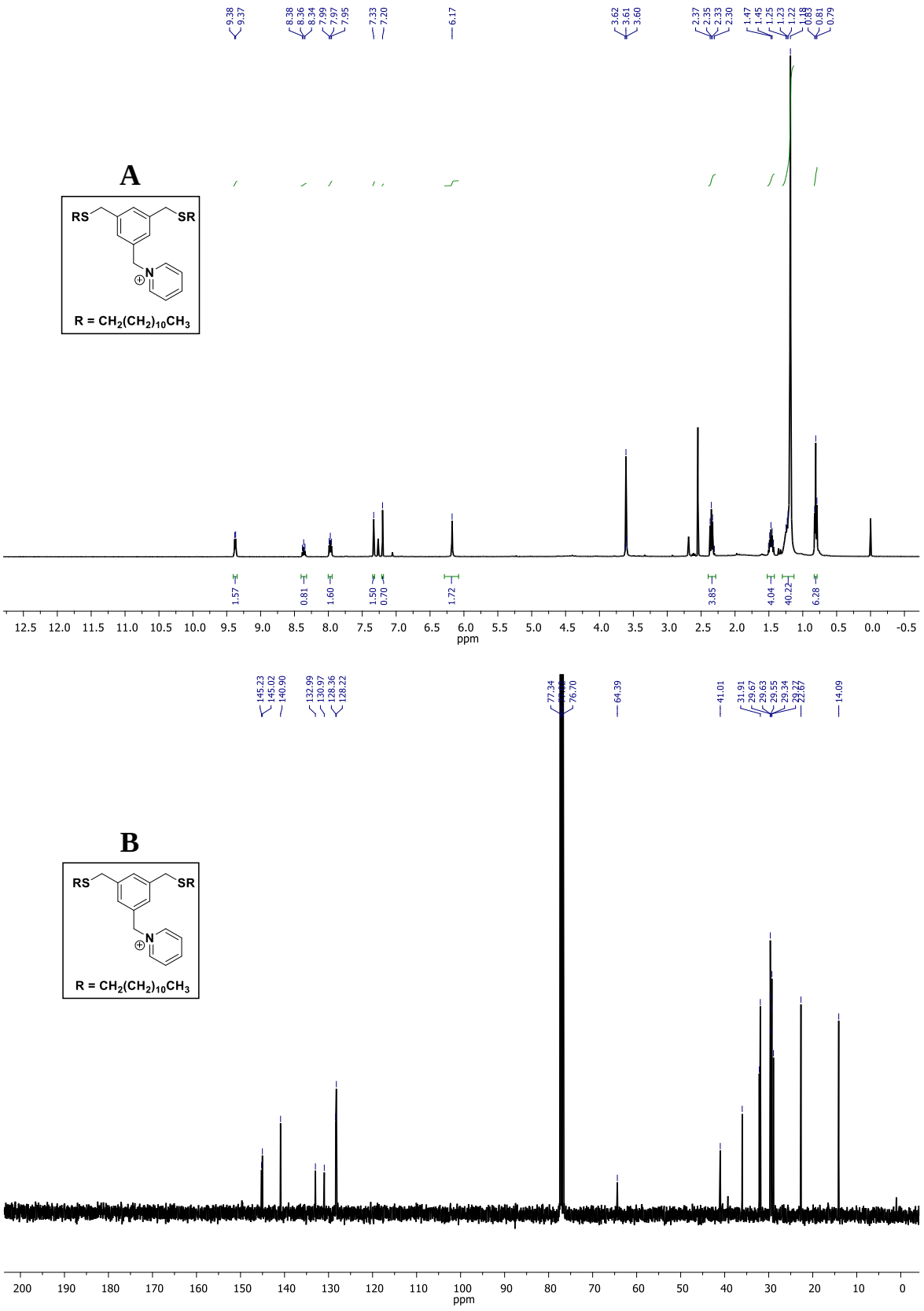

Fig. S2. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of lipid **5a.**

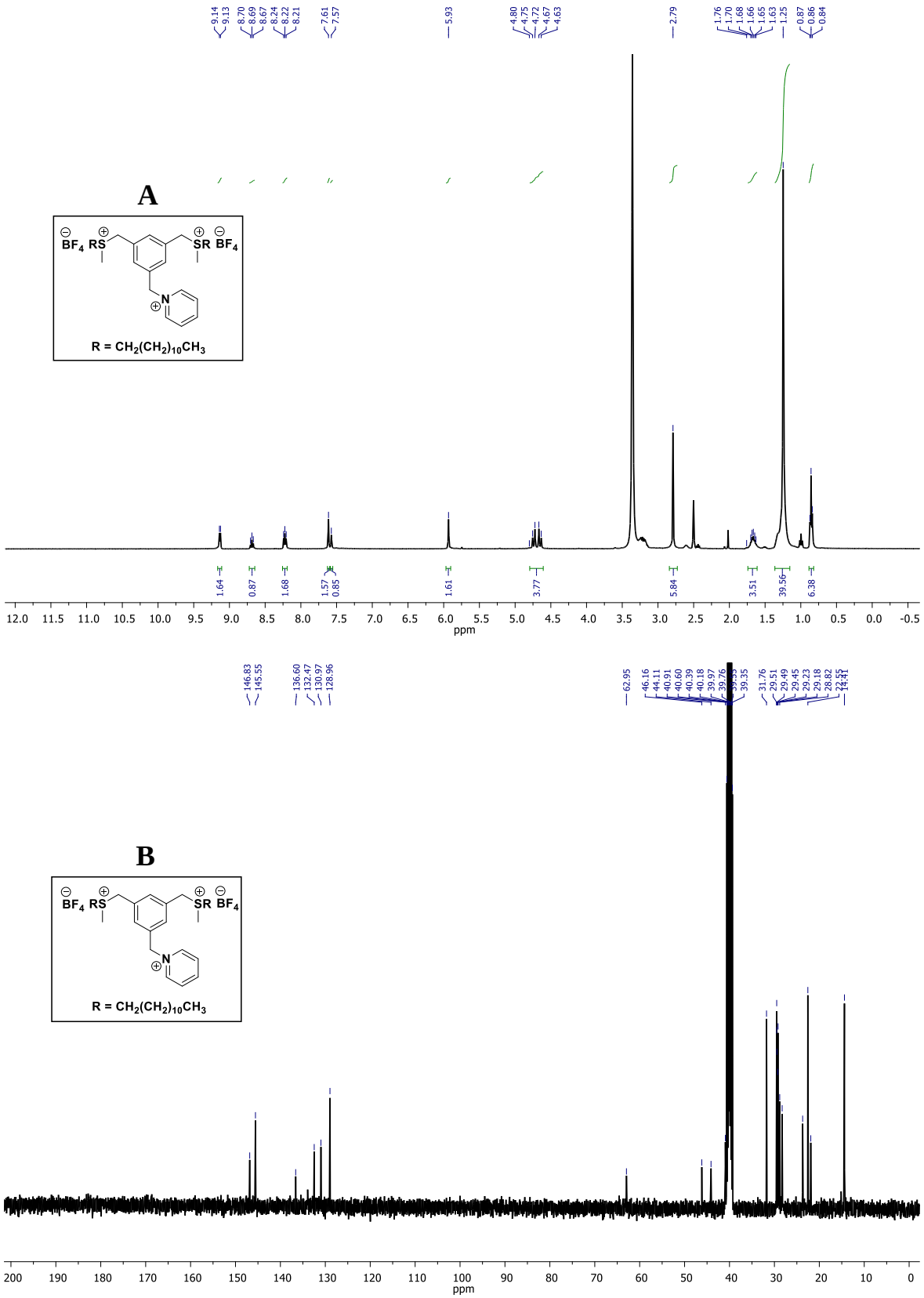

Fig. S3. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of lipid **7a.**

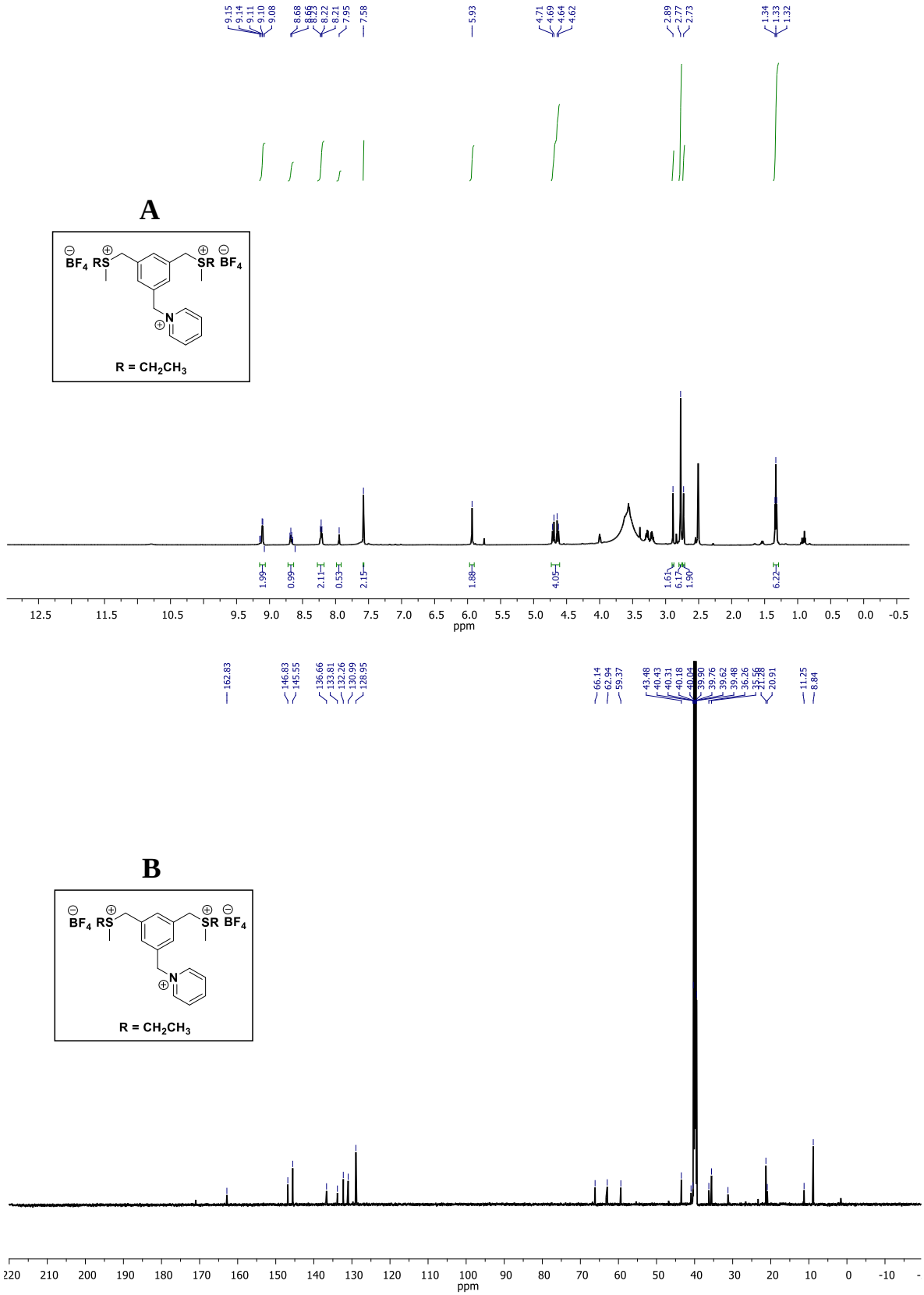

Fig. S4. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **7b.**

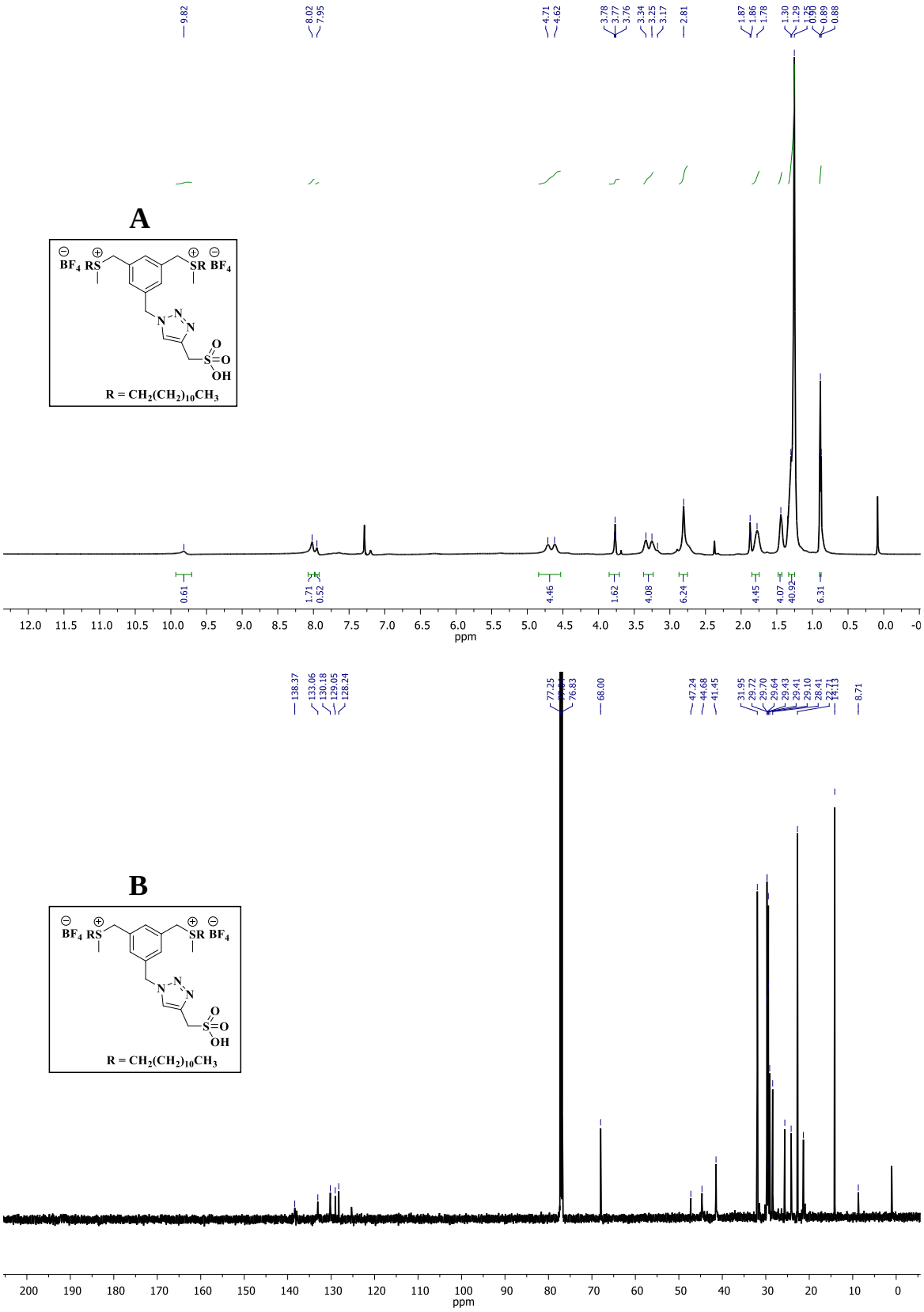

Fig. S5. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of lipid **8.**

III. Stock and working compound preparation- The stock solutions of the compounds were prepared in chloroform and stored at -20˚C. For working standard, the compounds were taken in a separate vial, and chloroform was removed under reduced pressure. The required concentration of the compound was prepared by adding a buffer to the dried compound and kept for 12 hours for hydration, and vortexed with intermittent sonication to form liposomes.

IV. Dynamic light scattering measurement

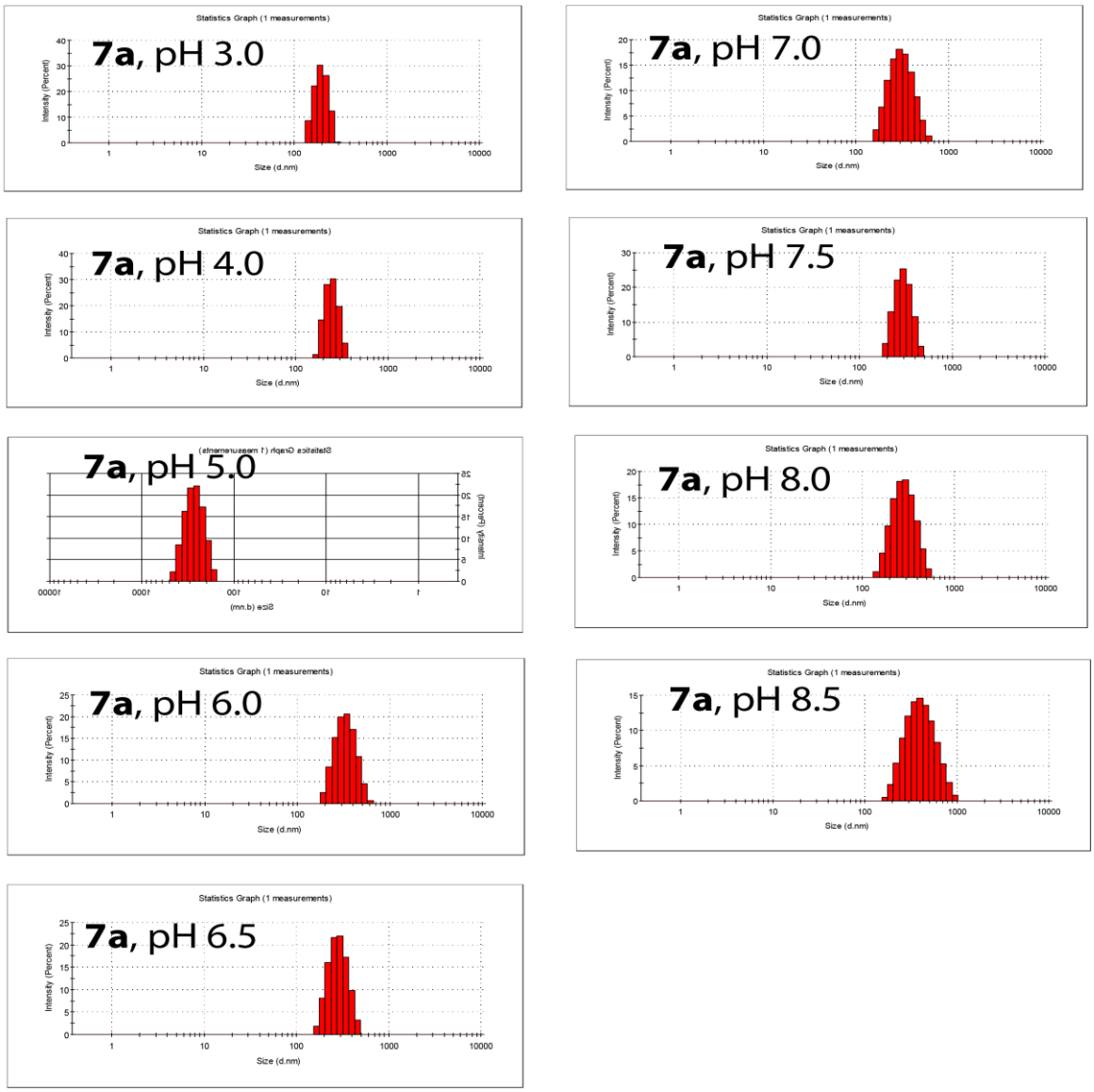

Fig S6. Size measurement of **7a** vesicles at various pH.

V. Antibiotic encapsulation efficiency measurements

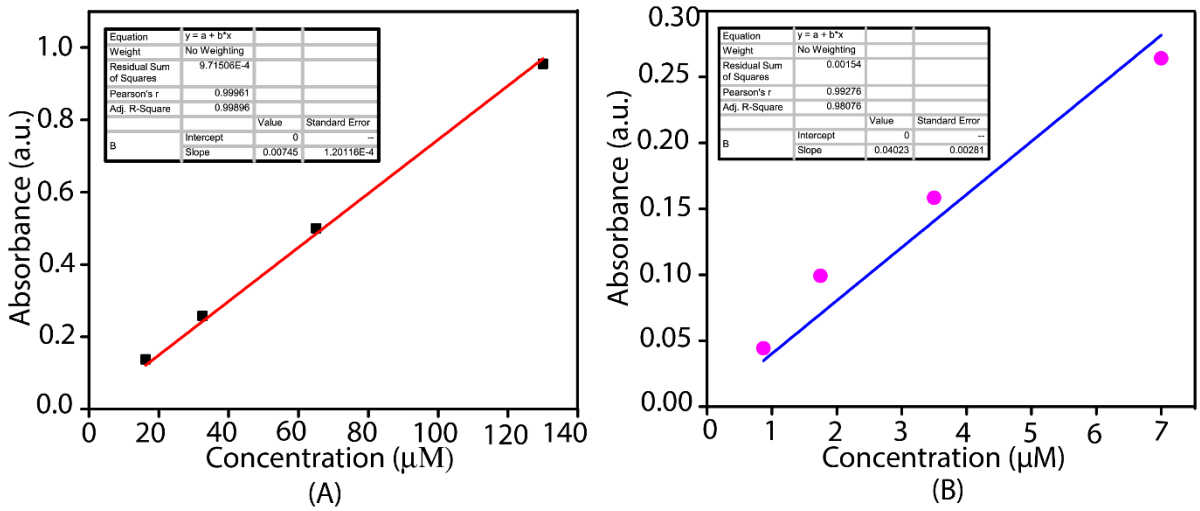

Fig. S7. Standard plot for the calculation of extinction coefficient (ε) for Tetracycline (A) and Amoxicillin (B).

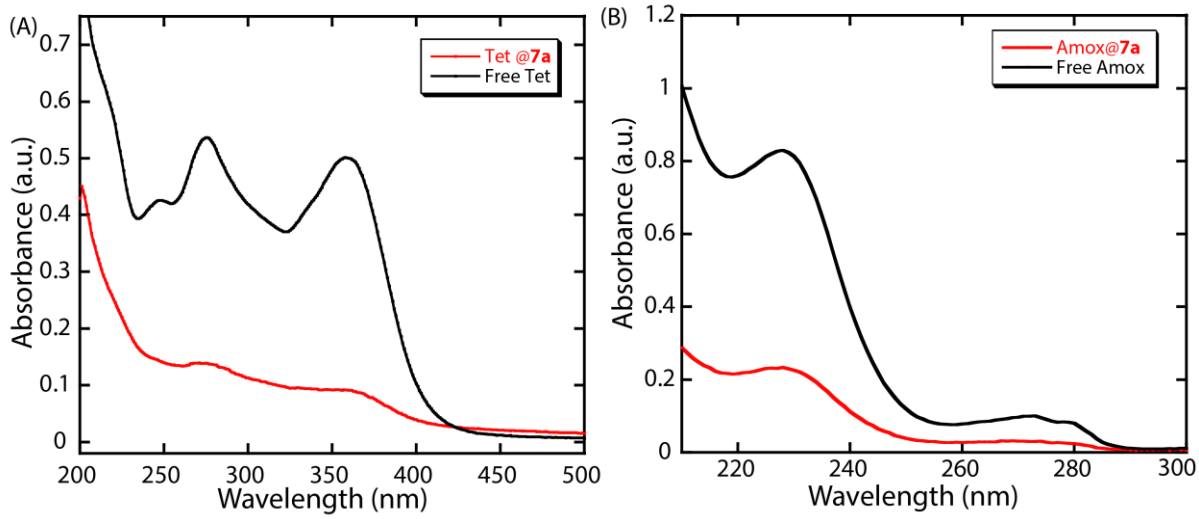

Fig. S8. UV-Vis spectra of free antibiotics, antibiotic encapsulated vesicles of **7a** at pH 7.4 tetracycline (A) amoxicillin (B)

VI. Synergy calculation

Synergy measurement of the antibiotic encapsulated **7a** lipid was calculated by checkerboard analysis. To quantify the synergistic effect of antibiotic encapsulated **7a** following equation was used-

$$\frac{MIC\ of\ \mathbf{7a}\ loaded\ with\ antibiotic\ (\mu\boldsymbol{M})}{MIC\ of\ \mathbf{7a}(\mu\boldsymbol{M})} + \frac{MIC\ of\ antibiotic\ loaded\ in\ \mathbf{7a}(\mu\boldsymbol{M})}{MIC\ of\ antibiotic(\mu\boldsymbol{M})} = FIC\ Index$$

A fractional inhibitory concentration (FIC) value less than 0.5 indicates the synergy.

Table S1: Fractional inhibitory concentration (FIC) value of antibiotic encapsulated **7a** vesicles.

Compound name	Bacterial strain	MIC of **7a** loaded with antibiotic (μM)	MIC of antibiotic loaded in **7a** (μM)	FIC value
7a@Tetracycline	S. *aureus*	1.0	0.7	0.31
	E. *coli*	1.4	2.0	0.41
7a@Amoxiciline	S. *aureus*	1.0	1.2	0.30
	E. *coli*	1.3	1.7	0.24

VII. Hemolytic assay

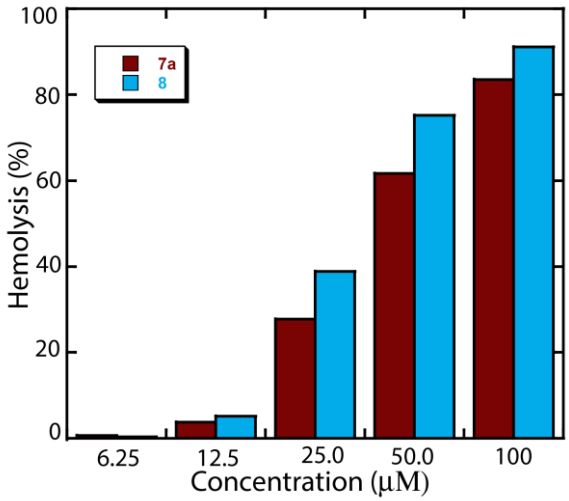

Fig S9. Hemolytic activity of **7a** and **8** in the human red blood cell.

Table S2. Calculation of the therapeutic index (TI) of the compound and composites.

	7a		Tetracycline@**7a**		amoxicillin@**7a**	
	E. coli	S. aureous	E. coli	S. aureous	E. coli	S. aureous
IC_{50} (μM)	27	27	8	8	29.4	29.4
MIC (μM)	7.5	6.3	1.4	0.7	1.0	1.2
TI (IC_{50}/MIC)	3.6	4.3	5.7	11.4	29.4	24.5