Supporting information

Excellent synergistic activity of designed membrane acting pyridinium containing antimicrobial cationic N-acylethanolamine with isoniazid against mycobacterium

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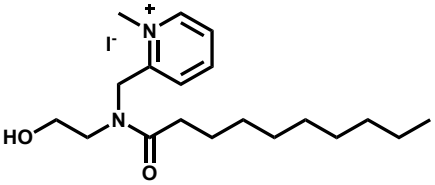
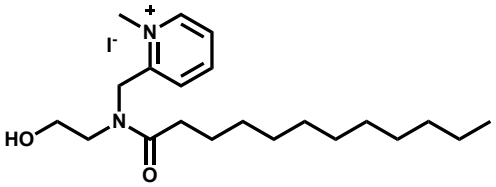
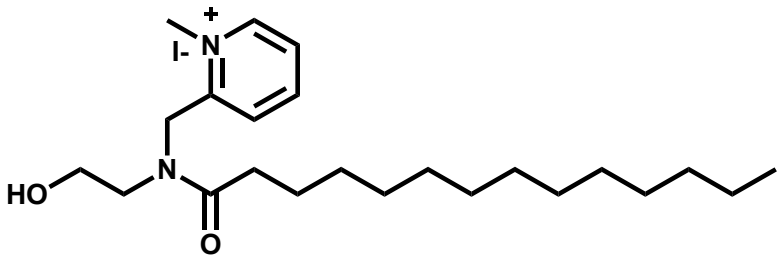
2-((N-(2-hydroxyethyl)decanamido)methyl)-1-methylpyridin-1-ium iodide: ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm) 9.02 (1H, d, J = 6.0 Hz), 8.40 (1H, t, J = 8.0 Hz), 7.90-7.85 (2H, m), 5.15 (2H,s), 4.45 (3H, s), 3.84-3.81 (4H, m), 2.52 (2H, t, J = 7.5 Hz), 1.65-1.60 (2H, m), 1.31-1.24 (12H, m), 0.88 (3H, t, J = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm) 175.1, 156.1, 146.2, 145.2, 126.6, 125.9, 60.6, 51.2, 49.1, 47.1, 33.2, 31.9, 29.5, 29.4, 29.3, 25.1, 22.7, 14.1. HRMS, Calculated: 471.1484 (M+Na); found: 471.1485.



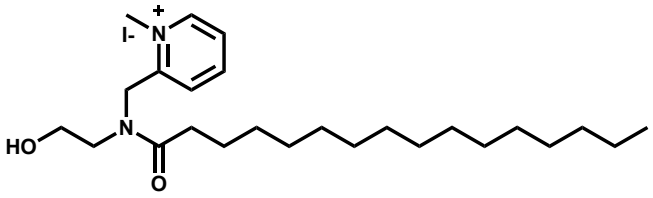
2-((N-(2-hydroxyethyl)dodecanamido)methyl)-1-methylpyridin-1-ium iodide: ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm) 9.03 (1H, d, J = 6.0 Hz), 8.40 (1H, t, J = 8.0 Hz), 7.91-7.85 (2H, m), 5.12 (2H, s), 4.49 (3H, s), 3.83-3.80 (4H, m), 2.51 (2H, t, J = 7.5 Hz), 2.36 (1H, br), 1.62-1.58 (2H, m), 1.33-1.28 (16H, m), 0.88 (3H, t, 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm). 175.1, 156.0, 146.3, 145.2, 126.6, 126.0, 60.5, 51.2, 49.1, 47.1, 33.2, 31.9, 29.7, 29.6, 29.55, 29.51, 29.4, 29.3, 14.1. HRMS, Calculated: 499.1797 (M+Na); found: 499.1797



2-((N-(2-hydroxyethyl)tetradecanamido)methyl)-1-methylpyridin-1-ium iodide: ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm) 9.02 (1H, d, J = 6.0 Hz), 8.37 (1H, t, J = 7.5 Hz), 7.89-7.86 (2H, m), 5.17 (2H, s), 4.51 (3H, m), 3.86-3.81 (4H, m), 2.51 (2H, t, J = 7.5 Hz), 1.65-1.59 (2H, m), 1.30-1.22 (20H, m), 0.88 (3H, t, J = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm) 175.0, 156.2, 146.2, 145.2, 126.7, 125.9, 60.6, 51.2, 49.2, 47.1, 33.1, 31.9, 29.7, 29.65, 29.63, 29.5, 29.47, 29.42, 29.3, 25.1, 22.7, 14.1. HRMS, Calculated: 377.3163 (M-I); found: 377.3323.

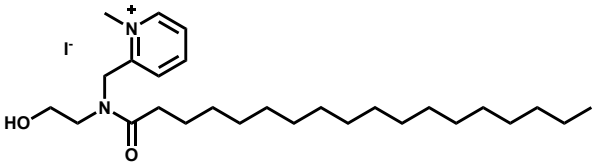


2-((N-(2-hydroxyethyl)palmitamido)methyl)-1-methylpyridin-1-ium iodide:



^{1}H NMR (400 MHz, $CDCl_3$, δ ppm); 8.86 (1H, d, J = 5.9 Hz); 8.30 (1H, t, J = 7.96 Hz); 7.76 -7.79 (2H, m); 5.13 (2H, s); 4.44 (3H, s), 3.75-3.82 (4H, m); 2.43 (2H, t, J = 7.4 Hz); 1.54 – 1.58 (2H, m); 1.15-1.25 (24H, m); 0.81 (3H, t, J = 6.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, δ ppm): 175.11, 156.4, 146.1, 145.2, 126.8, 125.9, 60.8, 51.2, 49.3, 47.2, 33.2, 32.0, 29.9, 29.7, 29.6, 29.56, 29.51, 29.4, 25.2, 22.8, 14.2;HRMS, Calculated: 405.3476 (M-Iodine); found: 405.3737.

2-((N-(2-hydroxyethyl)stearamido)methyl)-1-methylpyridin-1-ium iodide: ^{1}H NMR (500 MHz, DMSO-d6, δ ppm) 9.00 (1H, d, J = 6.0 Hz), 8.45 (1H, t, J = 8.0 Hz), 7.98 (1H, t, J = 7.0 Hz), 7.80 (1H, d, J = 8.0 Hz), 4.89 (2H, s), 4.30 (3H, s), 3.58-3.54 (4H, m), 2.56 (2H, t, J = 7.0 Hz), 1.53-1.51 (2H, m), 1.43-1.20 (28H, m), 0.86 (3H, t, J = 7.0 Hz); ^{13}C NMR (125 MHz, DMSO-d6, δ ppm) 174.3, 155.9, 147.1, 145.5, 125.9, 125.8, 59.4, 50.9, 47.4, 45.6, 39.5, 32.5, 31.7, 29.5, 29.4, 29.42, 29.3, 29.2, 25.0, 25.6, 14.4. HRMS, Calculated: 433.3783 (M-Iodine); found: 433.3851.



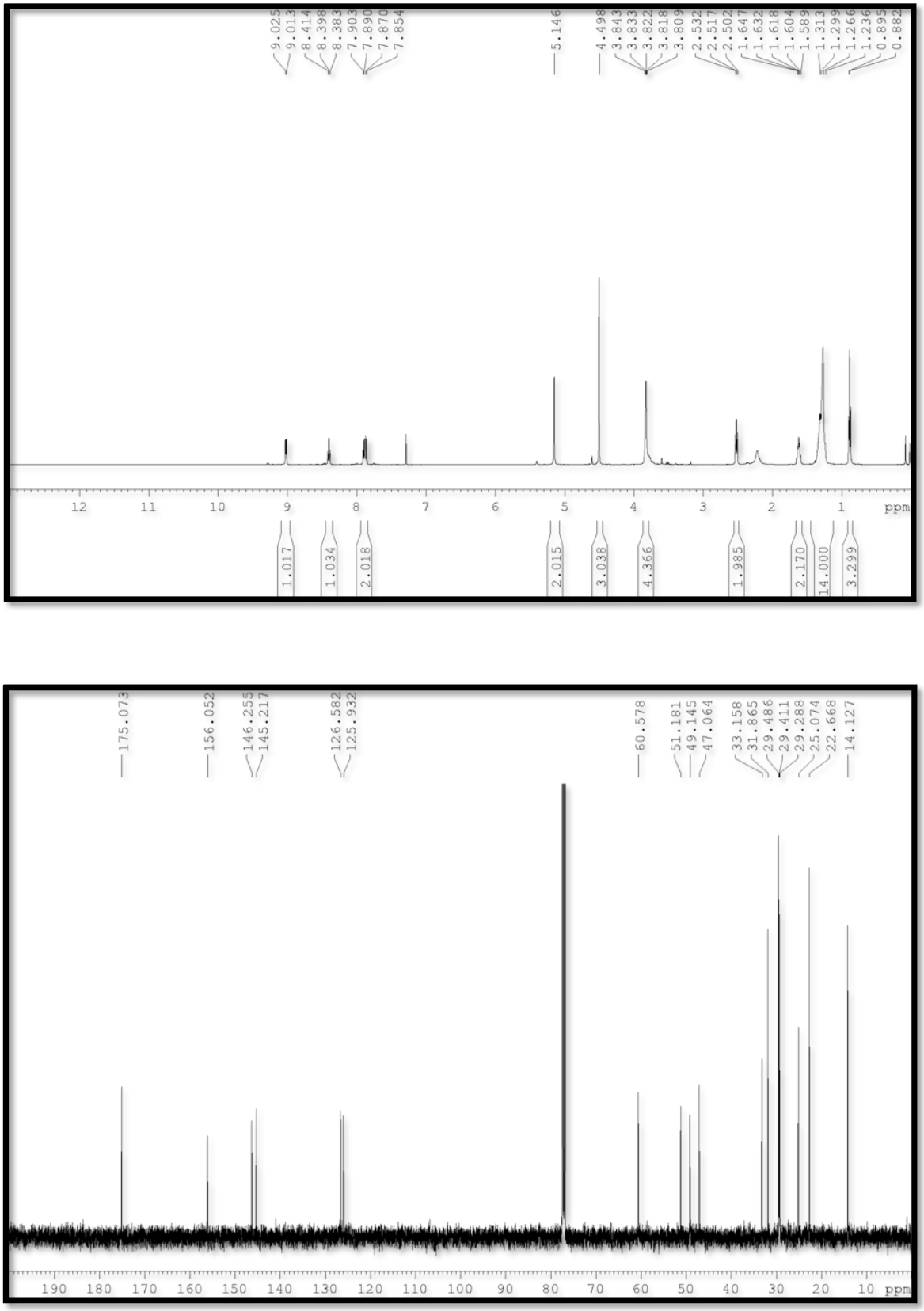


Fig. S1: ^{1}H and ^{13}C NMR of 2-((N-(2-hydroxyethyl)decanamido)methyl)-1-methylpyridin-1-ium iodide (**5a**).

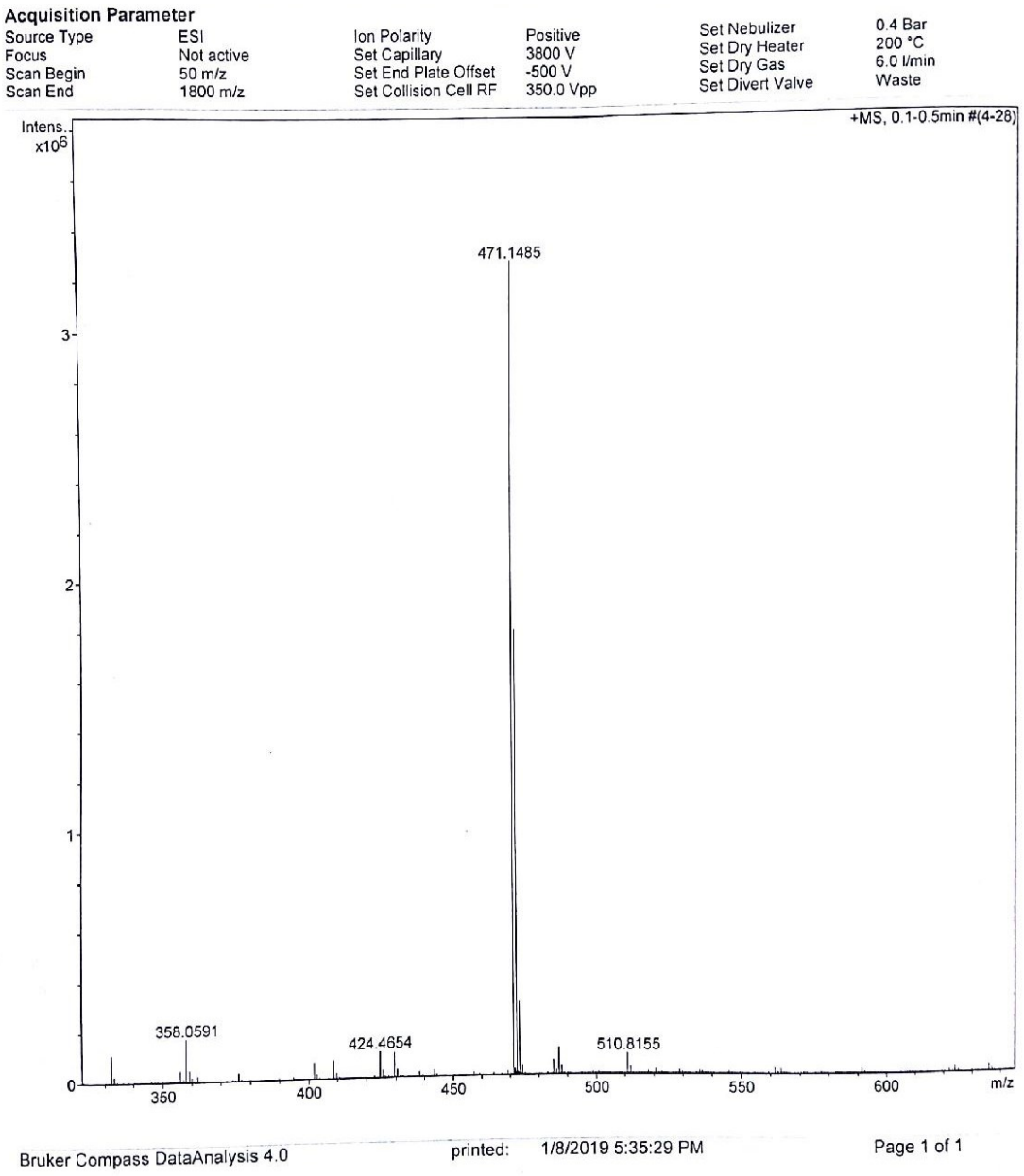


Fig. S2: HRMS of 2-((N-(2-hydroxyethyl)decanamido)methyl)-1-methylpyridin-1-ium iodide (**5a**)

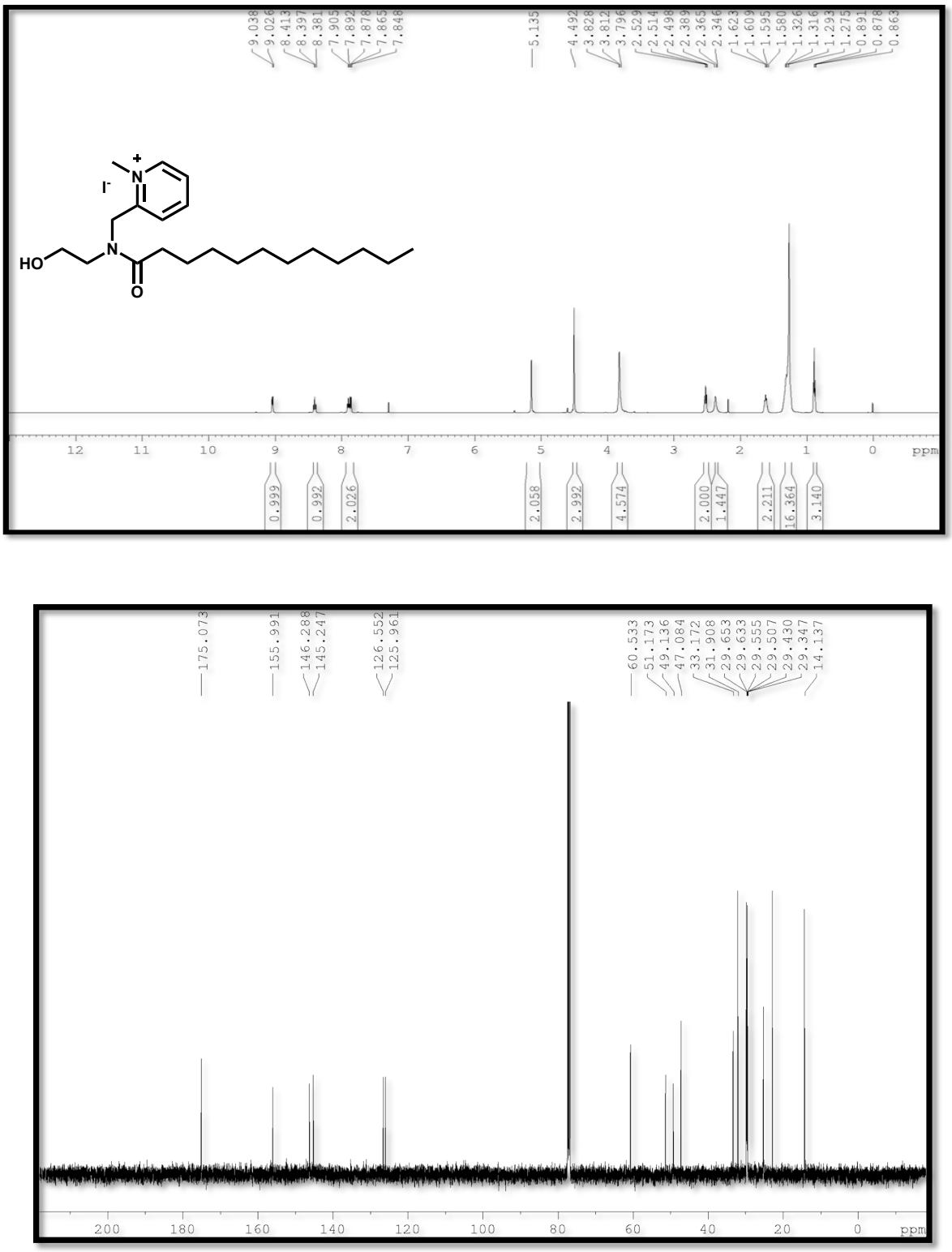


Fig. S3: ^{1}H and ^{13}C NMR of 2-((N-(2-hydroxyethyl)dodecanamido)methyl)-1-methylpyridin-1-ium iodide (**5b**)

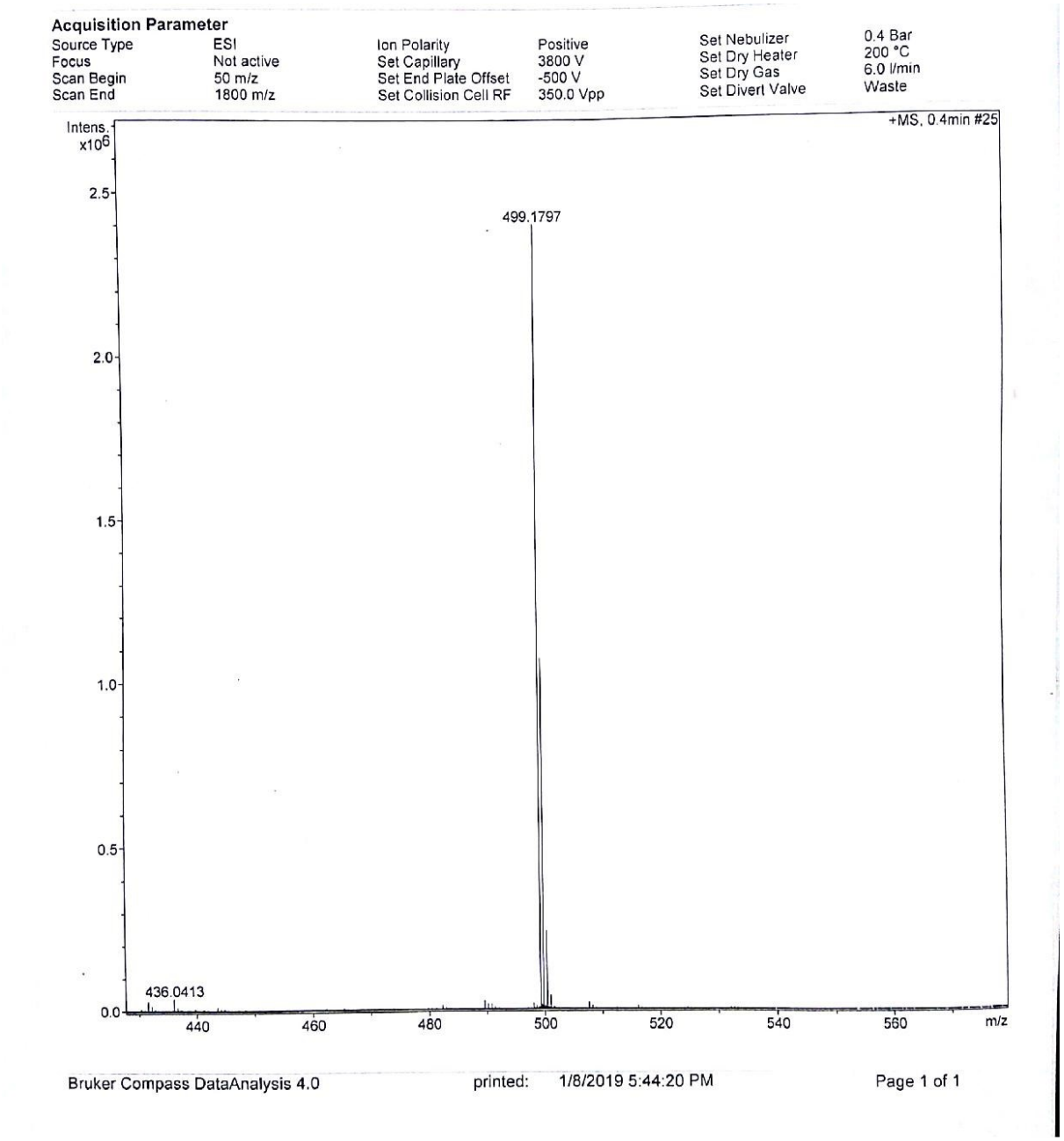


Fig. S4: HRMS of 2-((N-(2-hydroxyethyl)dodecanamido)methyl)-1-methylpyridin-1-ium iodide(**5b**)

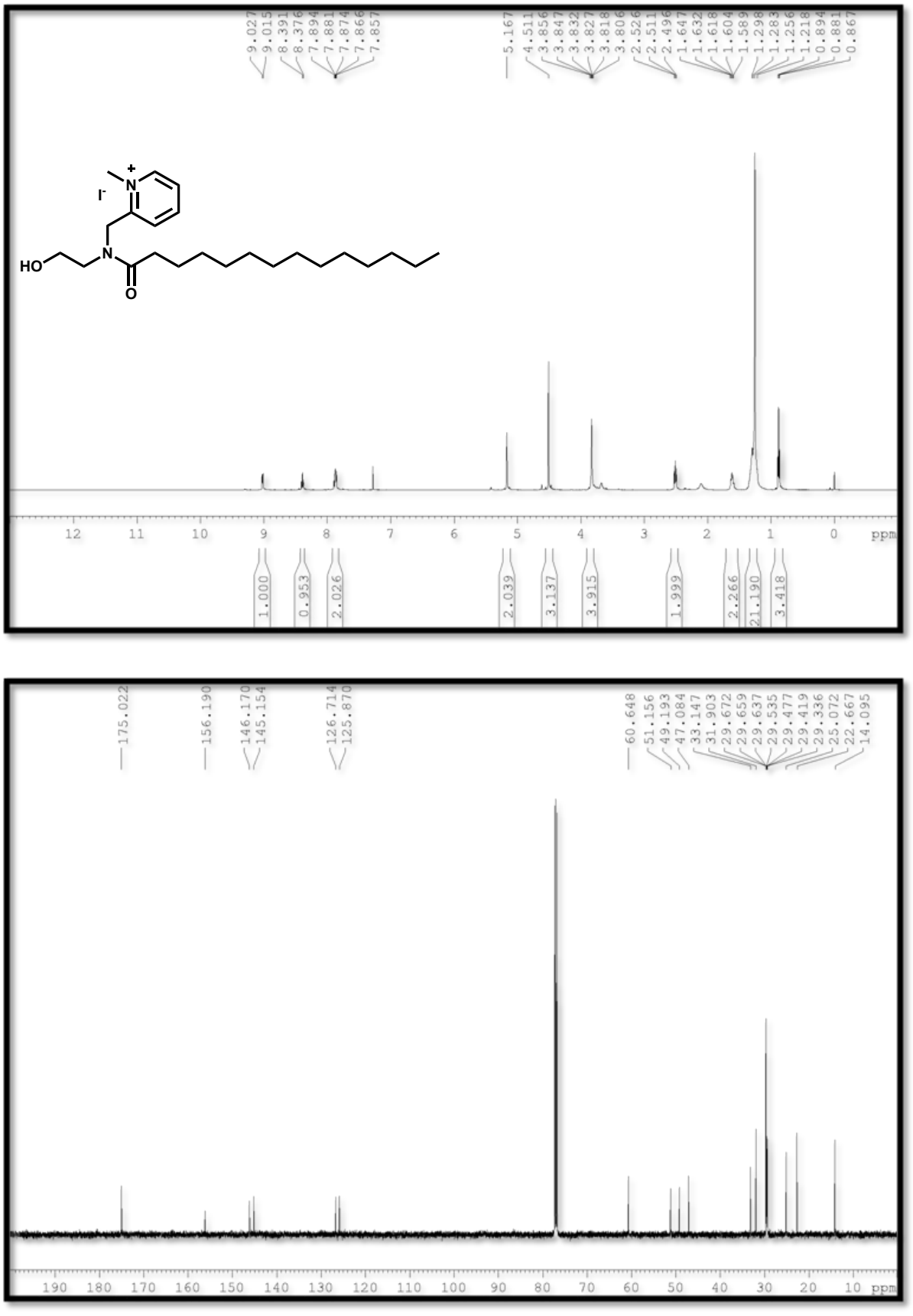


Fig. S5: ^{1}H and ^{13}C NMR of 2-((N-(2-hydroxyethyl)tetradecanamido)methyl)-1-methylpyridin-1-ium iodide (**5c**)

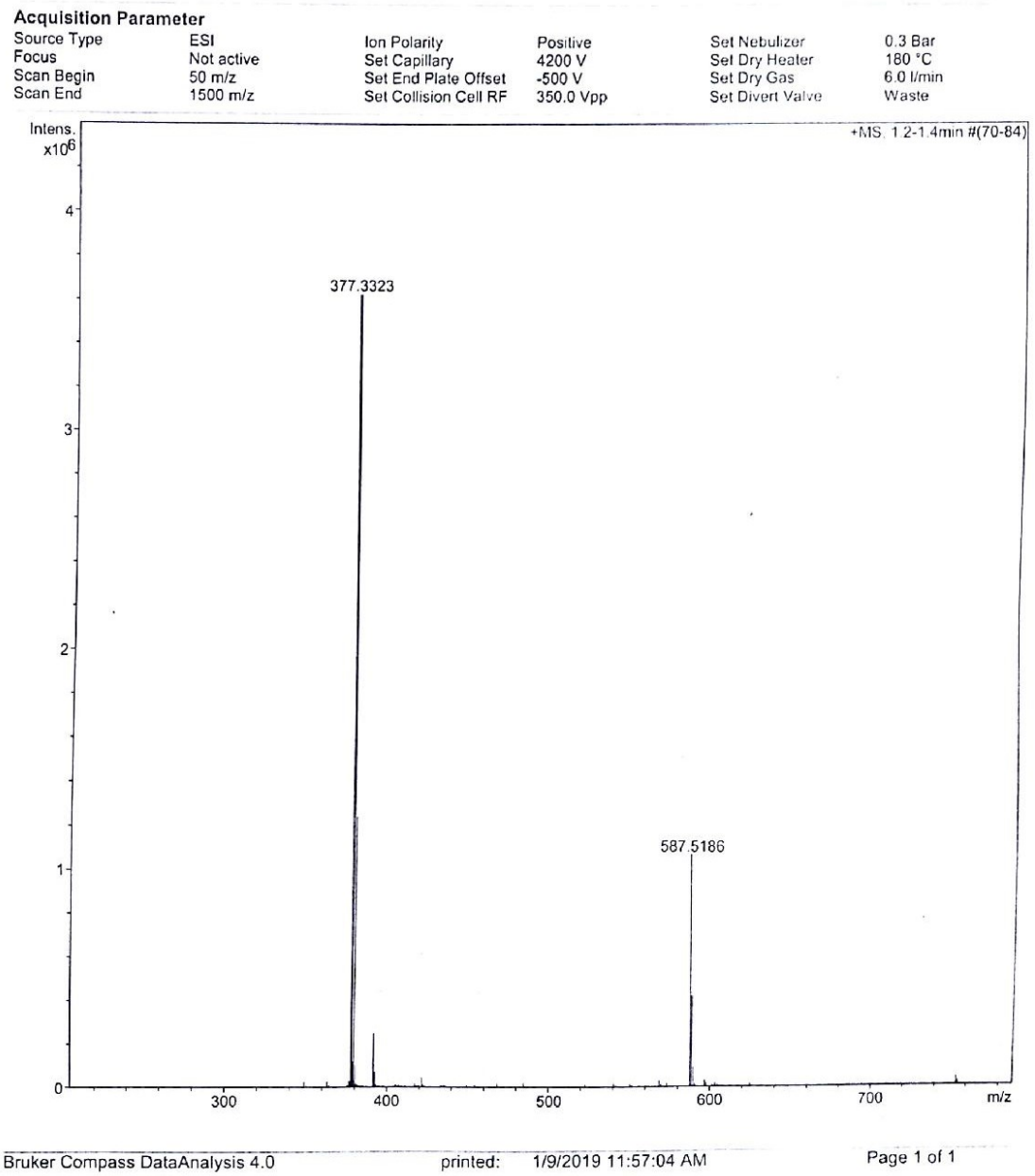


Fig. S6: HRMS of 2-((N-(2-hydroxyethyl)tetradecanamido)methyl)-1-methylpyridin-1-ium iodide (**5c**)

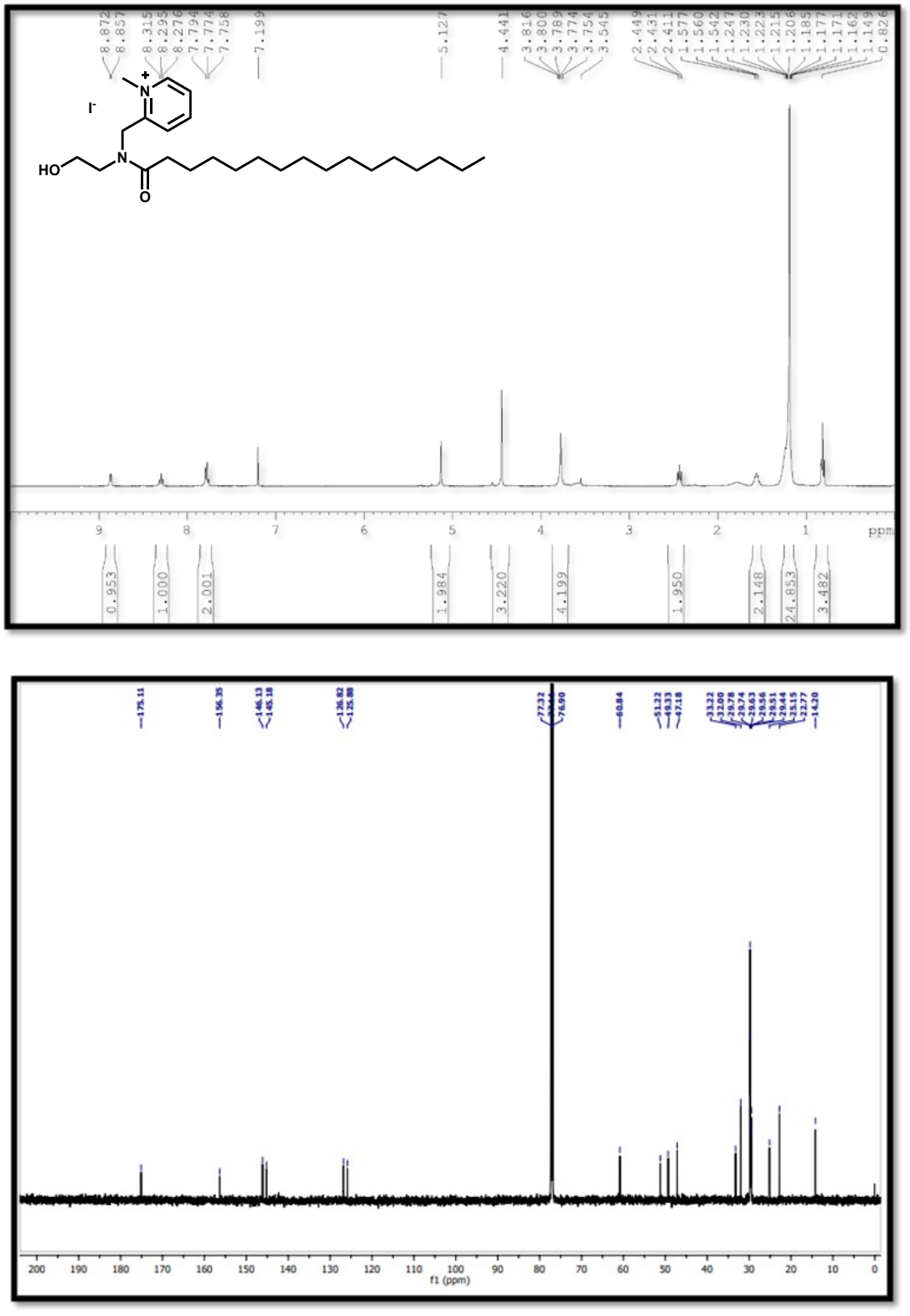


Fig. S7: ^{1}H of 2-((N-(2-hydroxyethyl)palmitamido)methyl)-1-methylpyridin-1-ium iodide (**5d**)

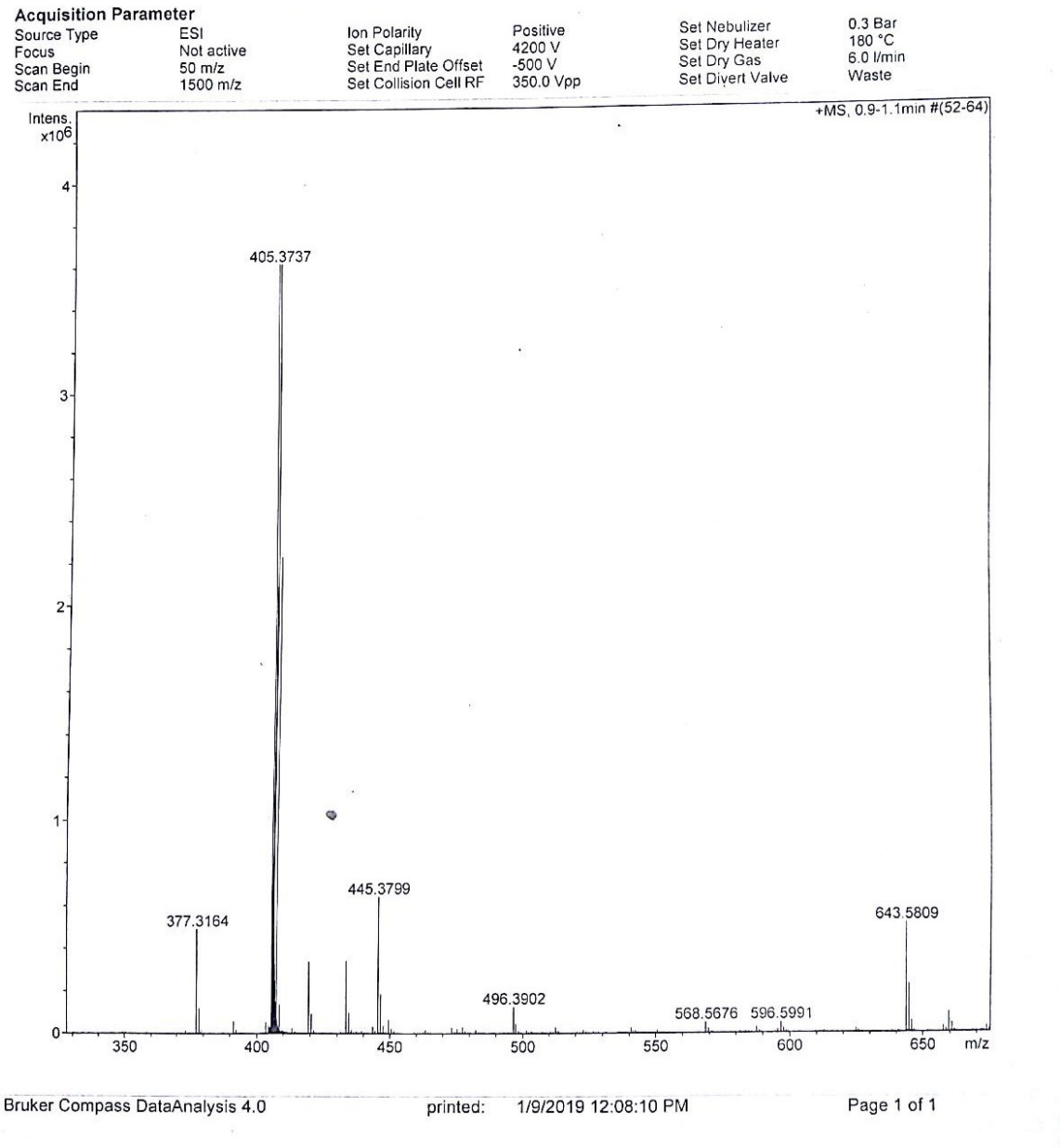


Fig. S8: HRMS of 2-((N-(2-hydroxyethyl)palmitamido)methyl)-1-methylpyridin-1-ium iodide (**5d**)

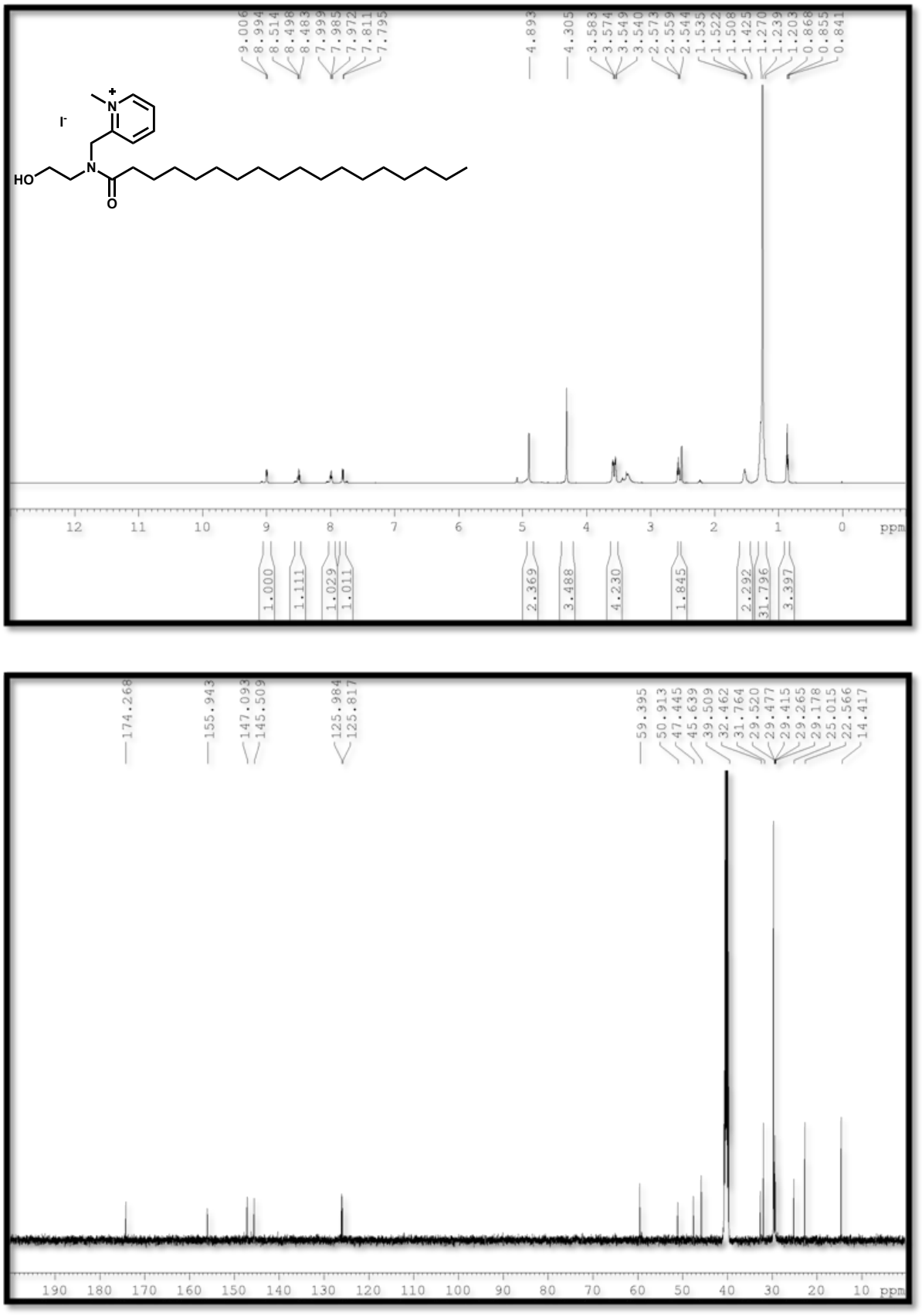


Fig. S9: ^{1}H and ^{13}C NMR of 2-((N-(2-hydroxyethyl)stearamido)methyl)-1-methylpyridin-1-ium iodide (**5e**)

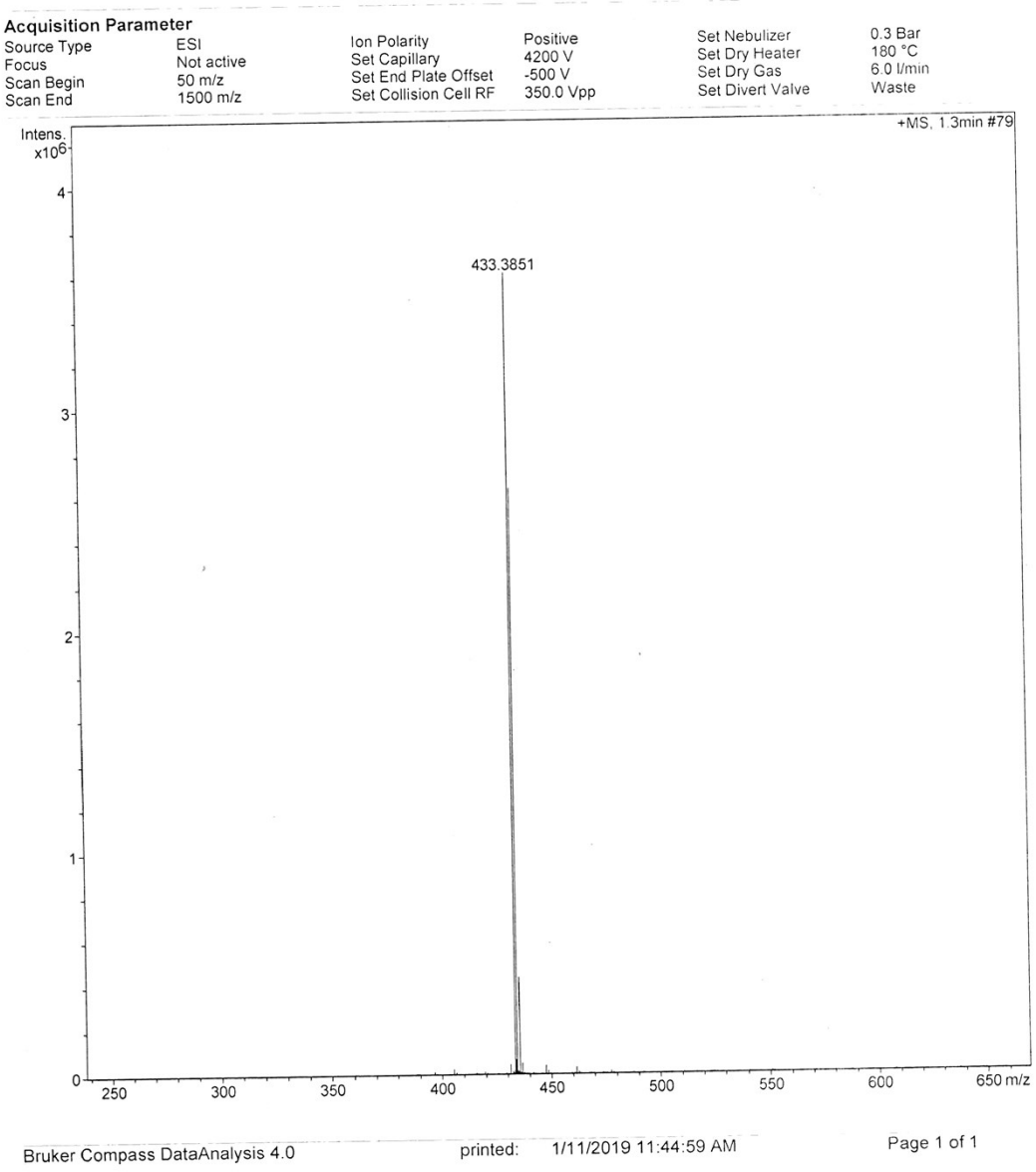


Fig. S10: HRMS of 2-((N-(2-hydroxyethyl)stearamido)methyl)-1-methylpyridin-1-ium iodide (**5e**)

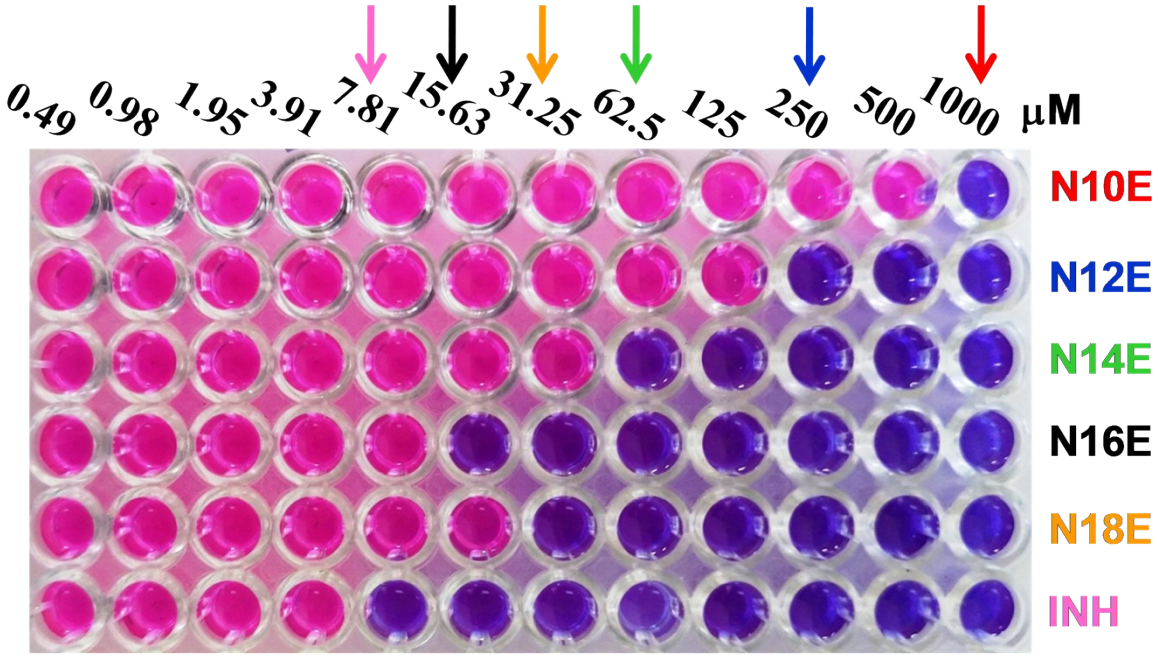


Fig. S11: Minimum inhibitory concentration by REMA. cNAEs were serial diluted at the represented concentration in the 96 well plate. To each well, 100 μL of 1×10^5 cfu/mL *M. smegmatis* were added and cultured at 37°C. After 72 h, 30 μL of a 0.01% (wt/vol) resazurin solution was added to each well and incubated for 2 h. Pink color indicates respiratory cells and blue color indicates non-respiratory cells. The colour change from blue to pink was marked as MIC, which was denoted by an arrow. Isoniazid (INH) was used as a standard drug control.

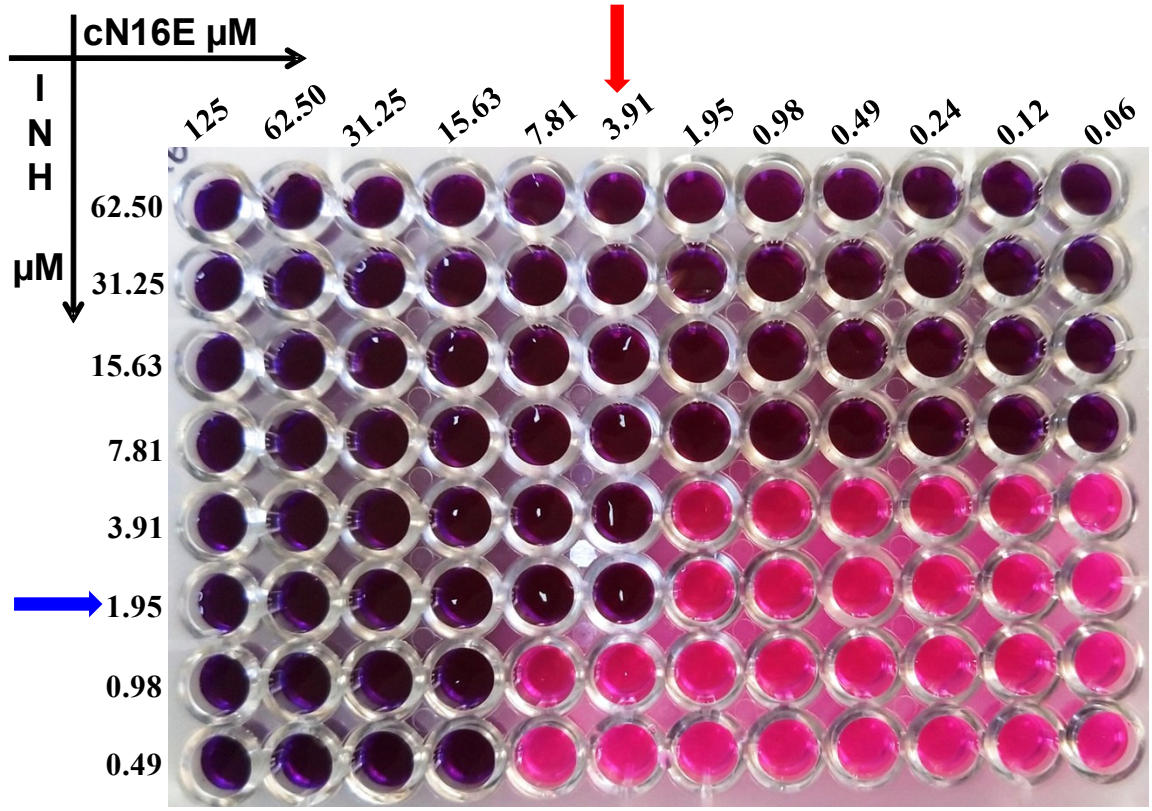


Figure S12: Representative checker board assay. Two fold serial dilutions of cN16Es and INH was prepared separately in a 96-well plate and mixed together to obtain the represented concentration. To each well, 100 µL of 1×10^5 cfu/mL *M. smegmatis* were added and cultured at 37°C. After 72 h, 30 µL of a 0.01% (wt/vol) resazurin solution was added to each well and incubated for 2 h. The colour change from blue to pink was marked as MIC. Red arrow indicates the MIC of cN16E when combined with INH. Blue arrow indicates the MIC of INH when combined with cN16E.

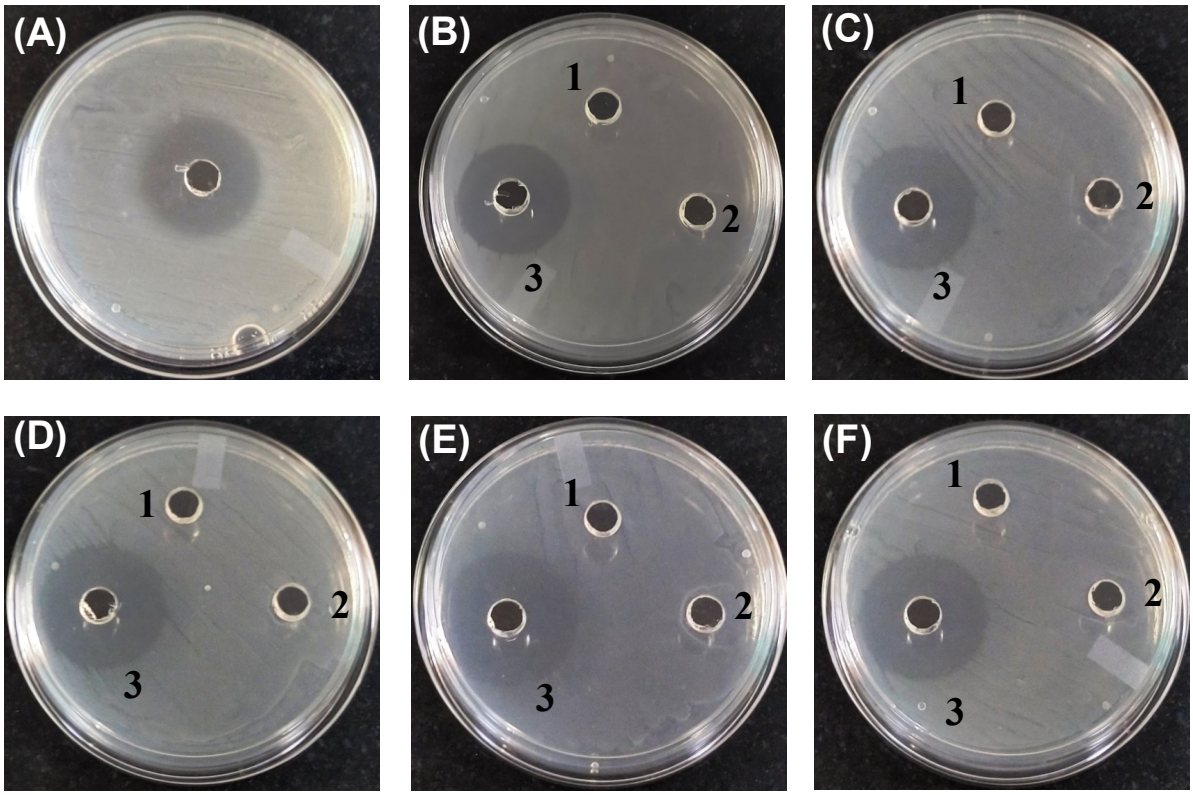


Figure S13: Zone of inhibition assay. (A) Control. 7.81 µM INH, the concentration corresponds to 1X MIC. (B) **1** - 500 µM cN10E, the concentration corresponds to 0.5X MIC, **2** – 1.95 µM INH, the concentration corresponds to 0.25X MIC, **3** – combination of 500 µM cN10E and 1.95 µM INH; (C) **1** – 125 µM cN12E the concentration corresponds to 0.5X MIC, **2** – 1.95 µM INH, the concentration corresponds to 0.25X MIC, **3** – combination of 125 µM cN12E and 1.95 µM INH; (D) **1** – 31.25 µM cN14E, the concentration corresponds to 0.5X MIC, **2** – 1.95 µM INH, the concentration corresponds to 0.25X MIC, **3** – combination of 31.25 µM cN14E and 1.95 µM INH; (E) **1** – 3.91 µM cN16E, the concentration corresponds to 0.25X MIC, **2** – 1.95 µM INH, the concentration corresponds to 0.25X MIC, **3** – combination of 3.91 µM cN16E and 1.95 µM INH; (F) **1** – 7.81 µM cN18E the concentration corresponds to 0.25X MIC, **2** – 1.95 µM INH, the concentration corresponds to 0.25X MIC, **3** – combination of 7.81 µM cN18E and 1.95 µM INH. About 50 µL of the described drug was added to the well. The appearance of zone indicates that only combinations are effective than the individual.

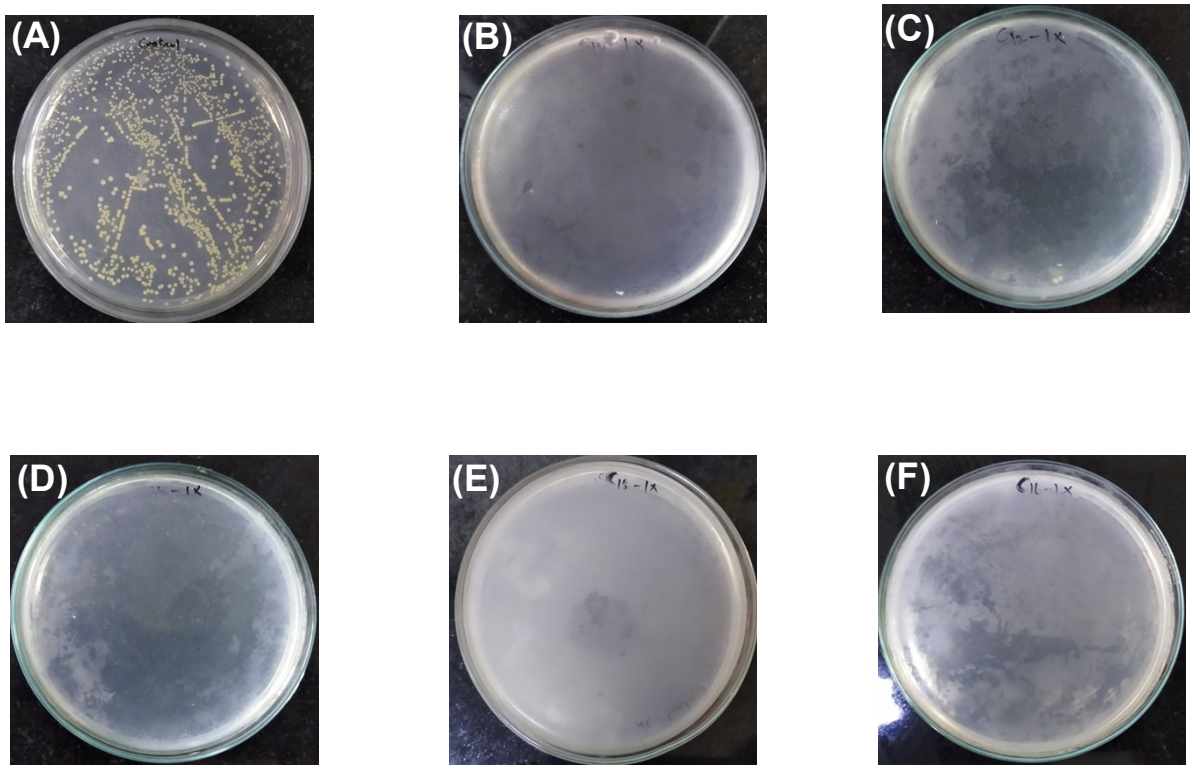


Figure S14: Bacterial colony count assay. *M. smegmatis* was treated with combination of cNAEs and INH, and then, plated on nutrient agar plate. (A) Control. *M. smegmatis* was grown without drugs, (B) treated with the combination of 500 µM cN10E and 1.95 µM INH, (C) treated with the combination of 125 µM cN12E and 1.95 µM INH, (D) treated with the combination of 31.25 µM cN14E and 1.95 µM INH, (E) treated with the combination of 3.91 µM cN16E and 1.95 µM INH, (F) treated with the combination of 7.81 µM cN10E and 1.95 µM INH. The absence of colonies suggested that the bacterium was killed by treatment with the combination drugs.