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Small antibacterial molecules highly active against drug-resistant *Staphylococcus aureus*

Rajib Dey, [a] Kathakali De, [a] Riya Mukherjee, [a] Sreyan Ghosh [a] and Jayanta Haldar *[a,b]

[a]Antimicrobial Research Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bengaluru 560064, Karnataka, India

[b]School of Advanced Materials, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bengaluru 560064, Karnataka, India

Address correspondence to Jayanta Haldar, jayanta@jncasr.ac.in

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Materials and instrumentation

2-(methylamino)ethanol,1-Aminohexane, 1-aminooctane,1-aminodecane, 1-aminodododecane, bromoacetyl bromide, 1,3-dibromopropane,1,6-dibromohexane, 1,8-dibromooctane, 1,10-dibromodecane, 1,12-dibromododecane, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid(HEPES), and α-D(+)-glucose were purchased from Sigma-Aldrich and used as received. Anhydrous potassium carbonate (K_2CO_3), anhydrous sodium sulfate (Na_2SO_4), phosphorus pentaoxide (P_2O_5), calcium hydride (CaH_2), dichloromethane (DCM), chloroform, ethanol, acetonitrile, diethyl ether, acetone, and molecular sieves (4 Å) were purchased from SD Fine, India, and were of analytical grade. Acetonitrile, DCM, and chloroform were dried over P_2O_5, and stored over molecular sieves. The visualization of TLC plate was performed by using UV or iodine. Column chromatography was performed on alumina gel (60−120 mesh), purchased from SDFCL (India). Nuclear magnetic resonance (NMR) spectra were recorded using Bruker AMX-400 (400 MHz and 600MHz for ^{1}H NMR and ^{13}C NMR) spectrometer. The chemical shift (δ) values are reported in parts per million (ppm). Mass spectra were recorded on a 6538-UHD Accurate mass Q-TOF LC-MS high resolution mass spectrometer (HRMS). Infrared (IR) spectra were recorded by Bruker IFS66 V/s spectrometer on NaCl crystal for liquid compounds and KBr pellet for solid compounds. For optical density (OD) measurement, Tecan Infinite Pro series M200 microplate reader was employed. Staphylococcus aureus MTCC737 and Escherichia coli MTCC443 were purchased from MTCC (Chandigarh, India). Pseudomonas aeruginosa ATCC424, methicillin-resistant Staphylococcus aureus ATCC33591, and β-lactam-resistant Klebsiella pneumoniae ATCC700603 were obtained from ATCC (Rockville, MD, USA). MRSA R3545, MRSA R3889, MRSA R3890 were obtained from National Institute of Mental Health and Neurosciences, Bangalore, India. Vancomycin-resistant Staphylococcus aureus (VRSA-1 and VRSA-4) were obtained from Dr. Sidharth Chopra, CSIR-Central Drug Research Institute, Lucknow, India.

Synthesis and characterization

General procedure to synthesis the compounds 1a-1e

2-(methylamino)ethanol (10 mmol) was dissolved in dry acetonitrile (20mL) and dibromoalkane (4 mmol) was added to the reaction mixture. To this reaction mixture, solid K_2CO_3 (6 mmol) was added and it was stirred at 85° C for 48 hours. Once the reaction was completed, solid K_2CO_3 was filtered out. The filtrate was then concentrated using rotary evaporator and was further purified using column chromatography to obtain the pure product with a yield of 68-75%.

1a: ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.62-3.60 (t, -N^+CH_2*CH_2*OH, 4H), 2.51-2.45 (m, -N^+*CH_2*CH_2OH, and -N^+*CH_2*CH_2-, 8H), 2.25 (s, -N^+*(CH_3)*, 6H), 1.67-1.61 (m, -N^+CH_2*CH_2*CH_2N^+-, 2H), HRMS: calculated m/z 191.1681 $[M+H]^+$; observed m/z 191.1716$[M+H]^+$

1b: ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.592-3.566 (t, -N^+CH_2*CH_2*OH, 4H), 2.535-2.508 (t, -N^+*CH_2*CH_2OH, 4H), 2.401-2.382 (t, -N^+*CH_2*CH_2-, 4H), 2.42 (s, -N^+*(CH_3)*, 6H), 1.495-1.460 (m, -N^+CH_2*CH_2*CH_2-, 4H), 1.348-1.293 (m, -$N^+CH_2CH_2$*CH_2*-, 4H).HRMS: calculated m/z 233.2151 $[M+H]^+$; observed m/z 233.2217 $[M+H]^+$

1c: ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.618-3.592 (t, -N^+CH_2*CH_2*OH, 4H), 2.571-2.559 (t, -N^+*CH_2*CH_2OH, 4H), 2.450-2.414 (t, -N^+*CH_2*CH_2-, 4H), 2.83 (s, -N^+*(CH_3)*, 6H), 1.490 (bs, -N^+CH_2*CH_2*CH_2-, 4H), 1.305 (bs, -$N^+CH_2CH_2$*CH_2*-, 8H). HRMS: calculated m/z 261.2464 $[M+H]^+$; observed m/z 261.2614 $[M+H]^+$

1d: ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.527-3.592 (t, -N^+CH_2*CH_2*OH, 4H), 2.472-2.445 (t, -N^+*CH_2*CH_2OH, 4H), 2.350-2.313 (t, -N^+*CH_2*CH_2-, 4H), 2.180 (s, -N^+*(CH_3)*, 6H), 1.416-1.381 (m, -N^+CH_2*CH_2*CH_2-, 4H), 1.211 (bs, -$N^+CH_2CH_2$*CH_2*-, 12H). HRMS: calculated m/z 289.2777 $[M+H]^+$; observed m/z 289.2837 $[M+H]^+$

1e: ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.590-3.563 (t, -N^+CH_2*CH_2*OH, 4H), 2.536-2.509 (t, -N^+*CH_2*CH_2OH, 4H), 2.414-2.377 (t, -N^+*CH_2*CH_2-, 4H), 2.244 (s, -N^+*(CH_3)*, 6H), 1.480-1.445 (m, -N^+CH_2*CH_2*CH_2-, 4H), 1.273-1.260 (m, -$N^+CH_2CH_2$*CH_2*-, 18H). HRMS: calculated m/z 317.3090 $[M+H]^+$; observed m/z 317.3098 $[M+H]^+$.

General procedure to synthesis N-alkyl-1-bromoethanamide (2a-2d)

1-Aminoalkanes (60 mmol) was dissolved in dicholoromethane (DCM) (100 mL). To this reaction mixture, K_2CO_3 (90 mmol) solution in water (80 mL) was added. This two phases solution was then cooled to 4-5 °C. While maintaining the temperature at 4-5 °C,

bromoacetyl bromide (90 mmol) solution in DCM (50 mL) was added drop wise to the reaction mixture for about 30 min. The reaction mixture was then allowed to stir at room temperature for about 12 hours. Following the completion of reaction, the organic layer was separated from the aqueous layer. The aqueous solution was washed with DCM (3 × 50 mL). Then, the combined organic solution was washed with water (3 × 100 mL), passed through the anhydrous Na_2SO_4 and concentrated to give product with quantitative yields (95-100%).

N-Hexyl-1-bromoethanamide (**2a**): FT-IR (cm^{-1}): 3257 (amide NH str.), 2940 (CH_2 assym. str.), 2849 (CH_2 sym. str.), 1685 (amide I, C=O str.), 1542 (amide II, NH ben.), 1473 (CH_2 scissor); ^{1}H NMR: (400 MHz, $CDCl_3$): δ, 6.521 (br. s, amide –*NH*CO, 1H), 3.924 (s, -CO*CH₂*Br, 2H), 3.292 (m, -CONH*CH₂*-, 2H), 1.561 (m, -*CH₂*$(CH_2)_3CH_3$, 2H), 1.342 (m, -CH_2(*CH₂*$)_3CH_3$, 6H), 0.891 (t, terminal –*CH₃*, 3H).

N-Octyl-1-bromoethanamide (**2b**): FT-IR (cm^{-1}): 3254 (amide NH str.), 2949 (CH_2 assym. str.), 2864 (CH_2 sym. str.), 1668 (amide I, C=O str.), 1563 (amide II, NH ben.), 1462 (CH_2 scissor); ^{1}H NMR: (400 MHz, $CDCl_3$): δ6.458 (br. s, amide –*NH*CO, 1H), 3.890 (s, -CO*CH₂*Br, 2H), 3.281 (m, -CONH*CH₂*-, 2H), 1.532 (m, -*CH₂*$(CH_2)_5CH_3$, 2H), 1.295 (m, -CH_2(*CH₂*$)_5CH_3$, 10H), 0.879 (t, terminal –*CH₃*, 3H).

N-Decyl-1-bromoethanamide (**2c**): FT-IR (cm^{-1}): 3261 (amide NH str.), 2934 (CH_2 assym. str.), 2858 (CH_2 sym. str.), 1681 (amide I, C=O str.), 1559 (amide II, NH ben.), 1468 (CH_2 scissor). ^{1}H NMR: (400 MHz, $CDCl_3$): δ 6.454 (br. s, amide –*NH*CO, 1H), 3.892 (s, -CO*CH₂*Br, 2H), 3.285 (m, -CONH*CH₂*-, 2H), 1.543 (m, -*CH₂*$(CH_2)_7CH_3$, 2H), 1.268-1.307 (m, -CH_2(*CH₂*$)_7CH_3$, 14H), 0.891 (t, terminal –*CH₃*, 3H).

N-Dodecyl-1-bromoethanamide (**2d**). FT-IR (cm^{-1}): 3253 (amide NH str.), 2932 (CH_2 assym. str.), 2846 (CH_2 sym. str.), 1687 (Amide I, C=O str.), 1564 (Amide II, NH ben.), 1472 (CH_2 scissor); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 6.471 (br s, amide –*NH*CO, 2H), 3.889 (s, –CO*CH₂*Br, 2H), 3.272 (t, –CONH*CH₂*–, 2H), 1.526 (q, –*CH₂*$(CH_2)_9$–, 2H), 1.281 (m, –(*CH₂*$)_9$–, 18H), 0.852 (t, terminal –*CH₃*, 3H).

General scheme for the synthesis of small antibacterial molecules

To the solution of compound 1a-1e (15 mmol) in dry chloroform (15mL), N-Alkyl-1-bromoethanamide 2a-2d (45 mmol) were added separately and the reaction mixtures were

allowed to stir at 85°C for 48 hours. Afterwards, the reaction mixtures were cooled down to room temperature and transferred to round bottom flask. The total volume of the reaction mixtures was then further reduced to 1/10th of the original volume by using rotary evaporator. The products were precipitated using excess of diethyl ether (150mL). The excess organic solvent was then decanted off and the precipitates were washed repeatedly (3 times) using diethyl ether followed by evaporating the solvent using high vacuum pump to get the final products (**SAM-2** to **SAM-4**, **SAM-6** to **SAM-8**, **SAM-10** to **SAM-12**, **SAM-14** to **SAM-16**, **SAM-18** to **SAM-20**) with 95-98% yield. Whereas, compounds (**SAM-1**, **SAM-5**, **SAM-9**, **SAM-13** and **SAM-17**) were purified by using reversed phase-HPLC to obtain the yield in the range of 61-68%.

SAM-1: Yield-65%; FT-IR (cm^{-1}): 3255 (amide NH str.), 2935 (CH_2 assym. str.), 2852 (CH_2 sym. str.), 1678 (amide I, C=O str.), 1555 (amide II, NH ben.), 1468 (CH_2 scissor); ^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.611-8.562 (m, -CO*NH*CH_2-, 2H), 4.275-4.233 (dd, -N^+*CH_2*CONH-, 4H), 4.045 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.828-3.658 (bs, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H), 3.362 (s, -N^+(*CH_3*)$_2$, 6H) , 3.225-3.192 (m, -NH*CH_2*CH_2-, 4H) , 2.575-2.564 (m, -NCH_2*CH_2*CH_2N-, 2H), 1.507-1.484 (m, -$NHCH_2$*CH_2*CH_2-, 4H), 1.304-1.275 (m, -*CH_2*CH_3, 12H), 0.883-0.861 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.503, 64.558, 61.819, 60.699, 60.613, 55.749, 50.291, 50.234, 50.138, 39.883, 31.333, 28.815, 27.470, 26.560, 26.086, 22.481, 17.335, 13.945. HRMS: calculated m/z 587.7355 [M–CF_3COO^-]$^+$, 237.3598 [M–2CF_3COO^-]$^{2+}$; observed m/z 587.3823[M–CF_3COO^-]$^+$, 237.2007 [M–2CF_3COO^-]$^{2+}$.

SAM-2: Yield-99%; FT-IR (cm^{-1}): 3345 (OH str.), 3250 (amide NH str.), 2927 (CH_2 assym. str.), 2852 (CH_2 sym. str.), 1664 (amide I, C=O str.), 1541 (amide II, NH ben.), 1456 (CH_2 scissor);^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.312 (bs, -CO*NH*CH_2-, 2H), 4.530 (bs, -N^+*CH_2*CONH-, 4H), 4.153 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.952-3.828 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.510 (s, -N^+(*CH_3*)$_2$, 6H) , 3.213-3.101 (m, -NH*CH_2*CH_2-, 4H) , 2.653 (bs, -NCH_2*CH_2*CH_2N-, 2H), 1.549-1.514 (m, -$NHCH_2$*CH_2*CH_2-, 4H), 1.295-1.272 (m, -*CH_2*CH_3, 20H), 0.870-0.845 (t, terminal CH_3 ,6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.648, 65.311, 65.205, 62.065, 60.466, 60.399, 55.889, 51.121, 51.054, 40.119, 31.904, 29.357, 29.309, 29.118, 27.732, 22.732, 18.547, 14.181. HRMS: calculated m/z 609.3954 [M–Br^-]$^+$, 265.2380 [M–2Br^-]$^{2+}$; observed m/z 609.3616[M–Br^-]$^+$, 265.2241 [M–2Br^-]$^{2+}$.

SAM-3: Yield-96%; FT-IR (cm^{-1}): 3345 (OH str.) , 3250 (amide NH str.), 2909 (CH_2 assym. str.), 2852 (CH_2 sym. str.), 1664 (amide I, C=O str.), 1550 (amide II, NH ben.), 1456 (CH_2 scissor);^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.305 (m, -CO*NH*CH_2-, 2H), 4.530 (bs, -N^+*CH_2*CONH-, 4H), 4.148 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.947-3.823 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.561 (s, -N^+(*CH_3*)$_2$, 6H) , 3.208-3.201 (m, -NH*CH_2*CH_2-, 4H) , 2.650 (bs, -NCH_2*CH_2*CH_2N-, 2H), 1.533-1.522 (m, -$NHCH_2$*CH_2*CH_2-, 4H), 1.282-1.244 (m, -*CH_2*CH_3, 28H), 0.893-0.867 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.629, 65.291, 65.186, 62.065, 60.389, 55.870, 51.092, 51.025, 40.129, 32.000, 29.740, 29.712, 29.434, 29.386, 29.137, 27.232, 22.770, 18.567, 14.201. HRMS: calculated m/z 666.4676 $[M–Br^-]^+$, 293.2696 $[M–2Br^-]^{2+}$; observed m/z 666.4580$[M–Br^-]^+$, 293.2734 $[M–2Br^-]^{2+}$.

SAM-4: Yield-98%; FT-IR (cm^{-1}): 3325 (OH str.), 3213 (amide NH str.), 2909 (CH_2 assym. str.), 2841(CH_2 sym. str.), 1673 (amide I, C=O str.), 1541 (amide II, NH ben.), 1464 (CH_2 scissor);^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.290 (bs, -CO*NH*CH_2-, 2H), 4.520 (bs, -N^+*CH_2*CONH-, 4H), 4.148 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.944-3.820 (bs, -N^+*CH_2*CH_2OH, 4H and -N^+*CH_2*CH_2CH_2-, 8H) ,3.504 (s, -N^+(*CH_3*)$_2$, 6H) , 3.209 (bs, -NH*CH_2*CH_2-, 4H) , 2.644 (bs, -NCH_2*CH_2*CH_2N-, 2H), 1.536(bs, -$NHCH_2$*CH_2*CH_2-, 4H), 1.286-1.244 (m, -*CH_2*CH_3, 36H), 0.882-0.859 (t, terminal CH_3-6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.609, 65.378, 65.263, 62.141, 62.065, 60.533, 55.985, 51.255, 40.158, 32.019, 29.807, 29.779, 29.472, 29.415, 29.166, 27.251, 22.779, 18.643, 14.201. HRMS: calculated m/z 721.5206$[M–Br^-]^+$, 321.3006 $[M–2Br^-]^{2+}$; observed m/z 721.5304$[M–Br^-]^+$, 321.3062 $[M–2Br^-]^{2+}$.

SAM-5: Yield-62%; FT-IR (cm^{-1}): 3341 (OH str.), 3254 (amide NH str.), 2920 (CH_2 assym. str.), 2853 (CH_2 sym. str.), 1683 (amide I, C=O str.), 1554 (amide II, NH ben.), 1470 (CH_2 scissor); ^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.408 (bs, -CO*NH*CH_2-, 2H), 4.446 (bs, -N^+*CH_2*CONH-, 4H), 4.115-4.020 (m, -N^+CH_2*CH_2*OH, 4H) , 3.864-3.549 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.441 (s, -N^+(*CH_3*)$_2$, 6H) , 3.213 (bs, -NH*CH_2*CH_2-, 4H) , 1.973 (bs, -$NHCH_2$*CH_2*CH_2-, 4H), 1.544-1.481 (m, -N^+CH_2*CH_2*-, 8H), 1.263-1.256 (m, -*CH_2*CH_3, 12H), 0.871-0.851 (t, terminal CH_3- 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.934, 64.486, 64.361, 64.334, 61.259, 60.761, 55.961 50.679, 50.358, 39.946, 31.434, 28.834, 27.278, 26.522, 26.450, 24.435, 22.567, 21.959, 13.993. HRMS: calculated m/z 629.4459 $[M–CF_3COO^-]^+$, 258.2302 $[M–2CF_3COO^-]^{2+}$; observed m/z 629.4421 $[M–CF_3COO^-]^+$, 258.2294 $[M–2CF_3COO^-]^{2+}$.

SAM-6: Yield-95%; FT-IR (cm^{-1}): 3354 (OH str.), 3241 (amide NH str.), 2927 (CH_2 assym. str.), 2841 (CH_2 sym. str.), 1673 (amide I, C=O str.), 1550 (amide II, NH ben.), 1464 (CH_2 scissor);^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.401 (bs, -CO*NH*CH_2-, 2H), 4.439 (bs, -N^+*CH_2*CONH-, 4H), 4.108 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.857-3.705 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.434(s, -N^+(*CH_3*)$_2$, 6H) , 3.206 (bs, -NH*CH_2*CH_2-, 4H) , 1.967 (bs, -$NHCH_2$*CH_2*CH_2-, 4H), 1.537-1.474 (m, -N^+CH_2*CH_2*-, 8H), 1.256-1.249 (m, -*CH_2*CH_3, 20H), 0.865-0.844 (t, terminal CH_3-6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.983, 64.669,64.411,64.334, 61.845, 61.739, 56.004, 50.776, 50.728, 39.947, 31.895, 29.329, 29.281, 29.137, 27.146, 24.905, 22.722, 21.908, 14.191. HRMS: calculated m/z 651.4424 $[M–Br^-]^+$, 286.4528$[M–2Br^-]^{2+}$; observed m/z 651.4514$[M–Br^-]^+$, 286.2666 $[M–2Br^-]^{2+}$.

SAM-7: Yield-96%; FT-IR (cm^{-1}): 3336 (OH str.) , 3230 (amide NH str.), 2918 (CH_2 assym. str.), 2852 (CH_2 sym. str.), 1664 (amide I, C=O str.), 1541 (amide II, NH ben.), 1447 (CH_2 scissor);^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.418 (bs, -CO*NH*CH_2-,2H), 4.467 (bs, -N^+*CH_2*CONH-, 4H), 4.128 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.898-3.722 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) , 3.452-3.446 (m, -N^+(*CH_3*)$_2$, 6H) , 3.231 (bs, -NH*CH_2*CH_2-, 4H) , 1.927 (bs, -$NHCH_2$*CH_2*CH_2-, 4H), 1.552(bs , -N^+CH_2*CH_2*-, 8H), 1.303-1.253 (m, -*CH_2*CH_3, 28H), 0.889-0.867 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.973, 64.755, 64.688, 64.458, 64.382, 61.883, 56.033, 50.834, 39.966, 31.981, 29.683, 29.405, 29.388, 29.166, 27.174, 24.953, 22.760, 21.927, 14.201. HRMS: calculated m/z 707.5044 $[M–Br^-]^+$, 314.2928$[M–2Br^-]^{2+}$; observed m/z 707.5151$[M–Br^-]^+$, 314.2986 $[M–2Br^-]^{2+}$.

SAM-8: Yield-99%; FT-IR (cm^{-1}):3334 (OH str.), 3250 (amide NH str.), 2916 (CH_2 assym. str.), 2849 (CH_2 sym. str.), 1679 (amide I, C=O str.), 1550 (amide II, NH ben.), 1466 (CH_2 scissor); ^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.432-8.427 (m, -CO*NH*CH_2-, 2H), 4.872(bs, -OH, 2H) , 4.489-4.481(m, -N^+*CH_2*CONH-, 4H), 4.130(bs, -N^+CH_2*CH_2*OH, 4H) , 3.907-3.699 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.455-3.443(m, -N^+(*CH_3*)$_2$, 6H), 3.231-3.219 (m, -NH*CH_2*CH_2-, 4H) , 1.95 (bs, -$NHCH_2$*CH_2*CH_2-, 4H), 1.552-1.491 (m, - N^+CH_2*CH_2*-, 8H), 1.294-1.248 (m, -*CH_2*CH_3, 40H), 0.889-0.867 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.925, 64.650, 64.545, 64.487, 64.382, 55.956, 50.642, 50.585, 39.957, 32.000, 29.740, 29.702, 29.453, 29.348, 29.137, 27.165, 24.828, 22.770, 21.851, 21.793, 14.210. HRMS: calculated m/z 763.5670 $[M–Br^-]^+$, 342.3241$[M–2Br^-]^{2+}$; observed m/z 763.564$[M–Br^-]^+$, 342.3241 $[M–2Br^-]^{2+}$.

SAM-9: Yield-67%; FT-IR (cm^{-1}): 3329 (OH str.), 3254 (amide NH str.), 2922 (CH_2 assym. str.), 2856 (CH_2 sym. str.), 1668 (amide I, C=O str.), 1554 (amide II, NH ben.), 1460 (CH_2 scissor); ^{1}H-NMR (600 MHz, $CDCl_3$): δ8.444-8.428 (m, -CO*NH*CH_2-, 2H), 4.873(bs, -OH, 2H) , 4.484-4.427(m, -N^+*CH_2*CONH-, 4H), 4.113(bs, -N^+CH_2*CH_2*OH, 4H) , 3.813-3.733 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.438-3.413(m, -N^+(*CH_3*)$_2$, 6H), 3.219-3.209 (m, -NH*CH_2*CH_2-, 4H) , 1.887 (bs, -N^+CH_2*CH_2*CH_2-, 4H), 1.552-1.537 (m, -$NHCH_2$*CH_2*CH_2-, 4H), 1.473(bs, -N^+CH_2CH*CH_2*-, 8H), 1.278-1.238(m, -*CH_2*CH_3, 12H), 0.875-0.853 (t, terminal CH_3- 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.962, 64.821, 64.361, 55.892, 50.373, 39.946, 39.769, 31.362, 31.099, 28.892, 27.451, 27.077, 26.565, 26.062, 25.066, 25.505, 22.338, 21.600, 13.979, 13.859. HRMS: calculated m/z 657.4772 [M–CF_3COO^-]$^+$, 272.2458 [M–2CF_3COO^-]$^{2+}$; observed m/z 657.4578 [M–CF_3COO^-]$^+$, 272.2380 [M–2CF_3COO^-]$^{2+}$.

SAM-10: Yield-98%; FT-IR (cm^{-1}): 3345 (OH str.), 3230 (amide NH str.), 2927 (CH_2 assym. str.), 2852 (CH_2 sym. str.), 1673 (amide I, C=O str.), 155 (amide II, NH ben.), 1456 (CH_2 scissor); ^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.460-8.443 (m, -CO*NH*CH_2-, 2H), 4.844(bs, -OH, 2H), 4.483-4.476 (m, -N^+*CH_2*CONH-, 4H), 4.129 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.757-3.742 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.416(s, -N^+(*CH_3*)$_2$, 6H) , 3.242-3.209 (m, -NH*CH_2*CH_2-, 4H) , 1.900 (bs, -N^+CH_2*CH_2*CH_2-, 4H), 1.571-1.557 (m, -$NHCH_2$*CH_2*CH_2-, 4H), 1.483(bs, -N^+CH_2CH*CH_2*-, 8H), 1.290-1.258(m, -*CH_2*CH_3, 20H), 0.877-0.853 (t, terminal CH_3- 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.935, 64.908, 64.411, 61.892, 55.918, 50.585, 39.937, 31.885, 29.300, 29.271, 29.108, 27.404, 27.126, 25.384, 22.712, 22.042, 14.191. HRMS: calculated m/z 679.4731 [M–Br^-]$^+$, 300.2771[M–2Br^-]$^{2+}$; observed m/z 679.4831[M–Br^-]$^+$, 300.2827 [M–2Br^-]$^{2+}$.

SAM-11: Yield-95%; FT-IR (cm^{-1}): 3336 (OH str.), 3241 (amide NH str.), 2927 (CH_2 assym. str.), 2852 (CH_2 sym. str.), 1684(amide I, C=O str.), 1541 (amide II, NH ben.), 1447(CH_2 scissor); ^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.457-8.441 (m, -CO*NH*CH_2-, 2H), 4.886(bs, -OH, 2H), 4.497-4.440 (m, -N^+*CH_2*CONH-, 4H), 4.125 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.825-3.745 (bs, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.427(s, -N^+(*CH_3*)$_2$, 6H) , 3.233-3.221 (m, -NH*CH_2*CH_2-, 4H) , 1.900 (bs, -N^+CH_2*CH_2*CH_2-, 4H), 1.600-1.565 (m, -$NHCH_2$*CH_2*CH_2-, 4H), 1.473(bs, -N^+CH_2CH*CH_2*-, 8H), 1.301-1.251 (m, -*CH_2*CH_3, 28H), 0.887-0.864 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.945, 64.861, 64.430, 61.864, 55.918, 50.594, 39.947, 31. 981, 29. 673, 29.329, 29.128, 27.442, 27.146, 25.403, 22.760, 22.052, 14.210. HRMS: calculated m/z 735.5363 [M–Br^-]$^+$, 328.5325 [M–2Br^-]$^{2+}$; observed m/z 735.5463[M–Br^-]$^+$, 328.3145 [M–2Br^-]$^{2+}$.

SAM-12: Yield-96%; FT-IR (cm^{-1}): 3354 (OH str.), 3250 (amide NH str.), 2918 (CH_2 assym. str.), 2854 (CH_2 sym. str.), 1664 (amide I, C=O str.), 1550 (amide II, NH ben.), 1456 (CH_2 scissor); ^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.421 (bs, -CO*NH*CH_2-, 2H), 4.843(bs, -OH, 2H), 4.446(bs, -N^+*CH_2*CONH-, 4H), 4.103 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.801-3.728 (bs, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.412-3.392(m, -N^+(*CH_3*)$_2$, 6H) , 3.202 (bs, -NH*CH_2*CH_2-, 4H) , 1.878 (bs, -N^+CH_2*CH_2*CH_2-, 4H), 1.527 (bs, -$NHCH_2$*CH_2*CH_2-, 4H), 1.398 (bs, -$N^+CH_2CH_2$*CH_2*-, 8H), 1.236-1.215 (m, -*CH_2*CH_3, 36H), 0.851 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.954, 64.822, 64.420, 61.845, 55.918, 50.594, 39.947, 32.000, 29.740, 29.692, 29.453, 29.358, 29.137, 27.414, 27.155, 25.374, 22.779, 22.042, 14.220. HRMS: calculated m/z 791.5983 [M–Br^-]$^+$, 356.3397[M–2Br^-]$^{2+}$; observed m/z 791.5951[M–Br^-]$^+$, 356.3400 [M–2Br^-]$^{2+}$.

SAM-13: Yield-61%; FT-IR (cm^{-1}): 3335 (OH str.), 3234 (amide NH str.), 2922 (CH_2 assym. str.), 2856 (CH_2 sym. str.), 1677 (amide I, C=O str.), 1554 (amide II, NH ben.), 1460 (CH_2 scissor);^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.825-8.814 (m, -CO*NH*CH_2-, 2H) 4.378-4.242 (dd,-N^+*CH_2*CONH-, 4H), 4.094- 4.060 (m, -N^+CH_2*CH_2*OH, 4H) , 3.650-3.642 (bs, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.338-3.336 (m , -N^+(*CH_3*)$_2$, 6H) , 3.226-3.205 (bs, -NH*CH_2*CH_2-, 4H) , 1.780-1.751(m, -N^+CH_2*CH_2*CH_2-, 4H), 1.524-1.500 (m, -$NHCH_2$*CH_2*CH_2-, 4H), 1.335-1.256 (m, -$N^+CH_2CH_2$*CH_2*and -*CH_2*CH_3, 24H), 0.882-0.860 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.957, 64.869, 64.414, 61.202, 55.883, 50.487, 39.759, 31.357, 28.882, 28.035, 27.843, 26.560, 25.579, 22.496, 22.003, 13.974. HRMS: calculated m/z 685.5085 [M–CF_3COO^-]$^+$, 286.2665 [M–2CF_3COO^-]$^{2+}$; observed m/z 685.4881[M–CF_3COO^-]$^+$, 286.2531 [M–2CF_3COO^-]$^{2+}$.

SAM-14: Yield-95%; FT-IR (cm^{-1}): 3299 (OH str.), 3230 (amide NH str.), 2927 (CH_2 assym. str.), 2841 (CH_2 sym. str.), 1673 (amide I, C=O str.), 1541 (amide II, NH ben.), 1464 (CH_2 scissor);^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.476-8.468 (bs, -CO*NH*CH_2-, 2H), 4.870(bs, -OH, 2H), 4.462-4.450 (m, -N^+*CH_2*CONH-, 4H), 4.119 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.780-3.724(bs, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.404 (s, -N^+(*CH_3*)$_2$, 6H) , 3.266-3.195 (bs, -NH*CH_2*CH_2-, 4H) , 1.829 (bs, -N^+CH_2*CH_2*CH_2-, 4H), 1.548-1.526 (m, -$NHCH_2$*CH_2*CH_2-, 4H), 1.366-1.249 (m, -$N^+CH_2CH_2$*CH_2* and -*CH_2*CH_3, 32H), 0.890-0.865 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.964, 64.707, 64.516, 61.730, 55.889, 50.719, 39.928, 31.885, 29.300, 29.271, 29.108, 28.256, 28.093, 28.017, 27.136, 25.786, 22.712, 22.368, 14.191. HRMS: calculated

m/z 707.5044 $[M–Br^-]^+$, 314.2928$[M–2Br^-]^{2+}$; observed m/z 707.5021$[M–Br^-]^+$, 314.2931 $[M–2Br^-]^{2+}$.

SAM-15: Yield-95%; FT-IR (cm^{-1}): 3354 (OH str.), 3230 (amide NH str.), 2927 (CH_2 assym. str.), 2852 (CH_2 sym. str.), 1673 (amide I, C=O str.), 1541 (amide II, NH ben.), 1464 (CH_2 scissor); ^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.468 (bs, -CO*NH*CH_2-, 2H), 4.858 (bs, -OH, 2H), 4.497-4.459 (m, -N^+*CH_2*CONH-, 4H), 4.119 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.777-3.732 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.405-3.400(s, -N^+(*CH_3*)$_2$, 6H) , 3.215 (bs, -NH*CH_2*CH_2-, 4H) , 1.834(bs,-N^+CH_2*CH_2*CH_2-, 4H), 1.536 (bs, -$NHCH_2$*CH_2*CH_2-, 4H), 1.371-1.238(m, -$N^+CH_2CH_2$*CH_2* and -*CH_2*CH_3, 40H), 0.866-0.845 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$):162.945, 64.784, 64.478, 61.749, 55.879, 50.690, 39.937, 31.971, 29.664, 29.329, 29.118, 28.161, 27.998, 27.146, 25.738, 22.760, 22.329, 14.201. HRMS: calculated m/z 763.5670 $[M–Br^-]^+$, 342.3241$[M–2Br^-]^{2+}$; observed m/z 763.5647$[M–Br^-]^+$, 342.3241 $[M–2Br^-]^{2+}$.

SAM-16: Yield-99%; FT-IR (cm^{-1}): 3325 (OH str.), 3230 (amide NH str.), 2918 (CH_2 assym. str.), 2852 (CH_2 sym. str.), 1673 cm−1(amide I, C=O str.), 1550 (amide II, NH ben.), 1456 (CH_2 scissor); ^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.491-8.475 (m, -CO*NH*CH_2-, 2H), 4.871 (bs, -OH, 2H), 4.527-4.451 (dd, -N^+*CH_2*CONH-, 4H), 4.143-4.134 (m, -N^+CH_2*CH_2*OH, 4H) , 3.791-3.736(m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.418(s, -N^+(*CH_3*)$_2$, 6H) , 3.251-3.217(m, -NH*CH_2*CH_2-, 4H) , 1.857(bs, -N^+CH_2*CH_2*CH_2-, 4H), 1.557 (bs, -$NHCH_2$*CH_2*CH_2-, 4H), 1.390-1.251(m, -$N^+CH_2CH_2$*CH_2* and -*CH_2*CH_3, 48H), 0.892-0.869 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.945, 64.832, 64.458, 61.768, 55.889, 50.690, 39.947, 32.000, 29.731, 29.683, 29.444, 29.348, 29.128, 28.103, 27.921, 27.155, 25.709, 22.770, 22.301, 14.210. HRMS: calculated m/z 819.6302 $[M–Br^-]^+$, 370.6214 $[M–2Br^-]^{2+}$; observed m/z 819.6255$[M–Br^-]^+$, 370.3553$[M–2Br^-]^{2+}$.

SAM-17: Yield-68%; FT-IR (cm^{-1}): 3332 (OH str.), 3206 (amide NH str.), 2913 (CH_2 assym. str.), 2845 (CH_2 sym. str.), 1677 (amide I, C=O str.), 1545 (amide II, NH ben.), 1468 (CH_2 scissor); ^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.792-8.776 (m, -CO*NH*CH_2-, 2H), 4.352-4.259 (dd, -N^+*CH_2*CONH-, 4H), 4.079-4.044 (m, -N^+CH_2*CH_2*OH, 4H) , 3.662-3.601 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.327(s, -N^+(*CH_3*)$_2$, 6H) , 3.221-3.210 (m, -NH*CH_2*CH_2-, 4H) , 1.857(bs, -N^+CH_2*CH_2*CH_2-, 4H), 1.508-1.496 (m, -$NHCH_2$*CH_2*CH_2-, 4H), 1.334-1.265(m, -$N^+CH_2CH_2$*CH_2* and -*CH_2*CH_3, 28H), 0.881-0.858 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.957,

64.912, 64.519, 55.873, 50.645, 40.961, 39.950,39.773, 31.362, 31.118, 28.882,28.375, 28.250, 26.579,25.775,22.500,22.223, 13.984. HRMS: calculated m/z 713.5398 [M–CF_3COO^-]$^+$, 300.2771 [M–$2CF_3COO^-$]$^{2+}$; observed m/z 713.5189 [M–CF_3COO^-]$^+$, 300.2687 [M–$2CF_3COO^-$]$^{2+}$.

SAM-18: Yield-98%; FT-IR (cm^{-1}): 3338 (OH str.), 3224 (amide NH str.), 2920 (CH_2 assym. str.), 2863 (CH_2 sym. str.), 1686 (amide I, C=O str.), 1552 (amide II, NH ben.), 1458 (CH_2 scissor); ^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.488-8.470 (m, -CO*NH*CH_2-, 2H), 4.828 (bs, -OH, 2H), 4.487-4.460 (m,-N^+*CH_2*CONH-, 4H), 4.116 (bs,-N^+CH_2*CH_2*OH, 4H) , 3.746-3.738 (bs, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.392 (s, -N^+(*CH_3*)$_2$, 6H) , 3.228-3.194 (m, -NH*CH_2*CH_2-, 4H) , 1.857(bs, -N^+CH_2*CH_2*CH_2-, 4H), 1.549-1.526 (m, -$NHCH_2$*CH_2*CH_2-, 4H), 1.344-1.243 (m, -$N^+CH_2CH_2$*CH_2* and -*CH_2*CH_3, 36H), 0.861-0.838 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.916, 64.803, 64.593,61.643,55.879, 50.814, 39.937, 31.885,29.300, 29.271, 29.099, 28.697, 28.553, 27.136, 26.006, 22.712, 22.588, 14.191. HRMS: calculated m/z 735.5357 [M–Br^-]$^+$, 328.3084 [M–$2Br^-$]$^{2+}$; observed m/z 735.5571[M–Br^-]$^+$, 328.3198[M–$2Br^-$]$^{2+}$.

SAM-19: Yield-96%; FT-IR (cm^{-1}): 3307 (OH str.) , 3202 (amide NH str.), 2918 (CH_2 assym. str.), 2841(CH_2 sym. str.), 1673 cm−1(amide I, C=O str.), 1541 (amide II, NH ben.), 1456 (CH_2 scissor); ^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.500-8.482 (m, -CO*NH*CH_2-, 2H), 4.527-4.450 (m, -N^+*CH_2*CONH-, 4H), 4.131-4.124 (m, -N^+CH_2*CH_2*OH, 4H) , 3.759-3.750 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.406 (s, -N^+(*CH_3*)$_2$, 6H) , 3.239-3.205 (m, -NH*CH_2*CH_2-, 4H) , 1.840-1.799 (bs, -N^+CH_2*CH_2*CH_2-, 4H), 1.549 (bs, -$NHCH_2$*CH_2*CH_2-, 4H), 1.358-1.240 (m, -$N^+CH_2CH_2$*CH_2* and -*CH_2*CH_3, 44H), 0.877-0.854 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.916, 64.794, 61.643, 55.870, 50.805, 39.947, 31.981, 29.664, 29.396, 29.329,2 9.108, 28.639, 28.515, 27.155, 25.977, 22.760, 22.559, 14.210. HRMS: calculated m/z 791.5989 [M–Br^-]$^+$, 356.5944 [M–$2Br^-$]$^{2+}$; observed m/z 791.5960[M–Br^-]$^+$, 356.3403[M–$2Br^-$]$^{2+}$.

SAM-20: Yield-97%; FT-IR (cm^{-1}):3316 (OH str.), 3202 (amide NH str.), 2909 (CH_2 assym. str.), 2841(CH_2 sym. str.), 1673 (amide I, C=O str.), 1541 (amide II, NH ben.), 1464 (CH_2 scissor);^{1}H-NMR: (600 MHz, $CDCl_3$): δ8.481 (bs, -CO*NH*CH_2-, 2H), 4.836 (bs, -OH, 2H) 4.524-4.448 (m, -N^+*CH_2*CONH-, 4H), 4.123 (bs, -N^+CH_2*CH_2*OH, 4H) , 3.747-3.690 (m, -N^+*CH_2*CH_2OH and -N^+*CH_2*CH_2CH_2-, 8H) ,3.402 (s, -N^+(*CH_3*)$_2$, 6H) , 3.215 (bs, -NH*CH_2*CH_2-, 4H) , 1.808 (bs, -

$N^+CH_2\mathit{CH_2}CH_2$-, 4H), 1.546-1.464 (m, -$NHCH_2\mathit{CH_2}CH_2$-, 4H), 1.349-1.234(m, -$N^+CH_2CH_2\mathit{CH_2}$ and -$\mathit{CH_2}CH_3$, 52H), 0.866-0.845 (t, terminal CH_3, 6H). ^{13}C-NMR (600MHz, $CDCl_3$): 162.897, 64.794, 64.573, 61.643, 55.860, 50.795, 39.947, 31.990, 29.731, 29.444, 29.118, 28.515, 27.155, 25.977, 22.770, 22.559, 14.210. HRMS: calculated m/z 847.6609 $[M–Br^-]^+$, 384.3710 $[M–2Br^-]^{2+}$; observed m/z 847.6475 $[M–Br^-]^+$, 384.3670$[M–2Br^-]^{2+}$.

Supplementary figures

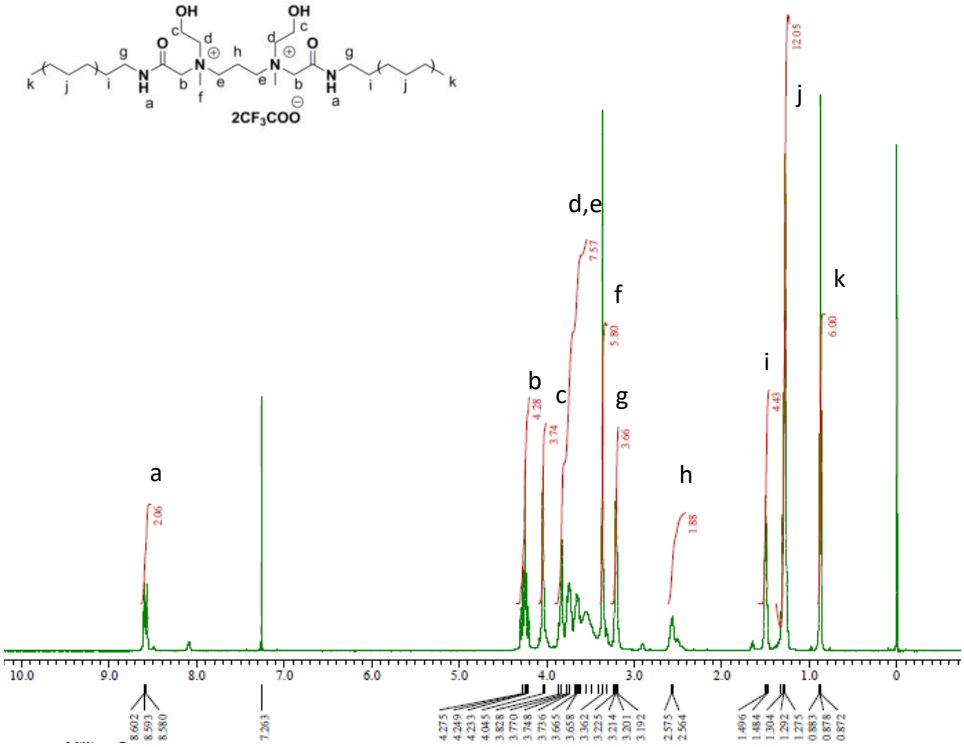

Figure S1. ^{1}HNMR of **SAM-1**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

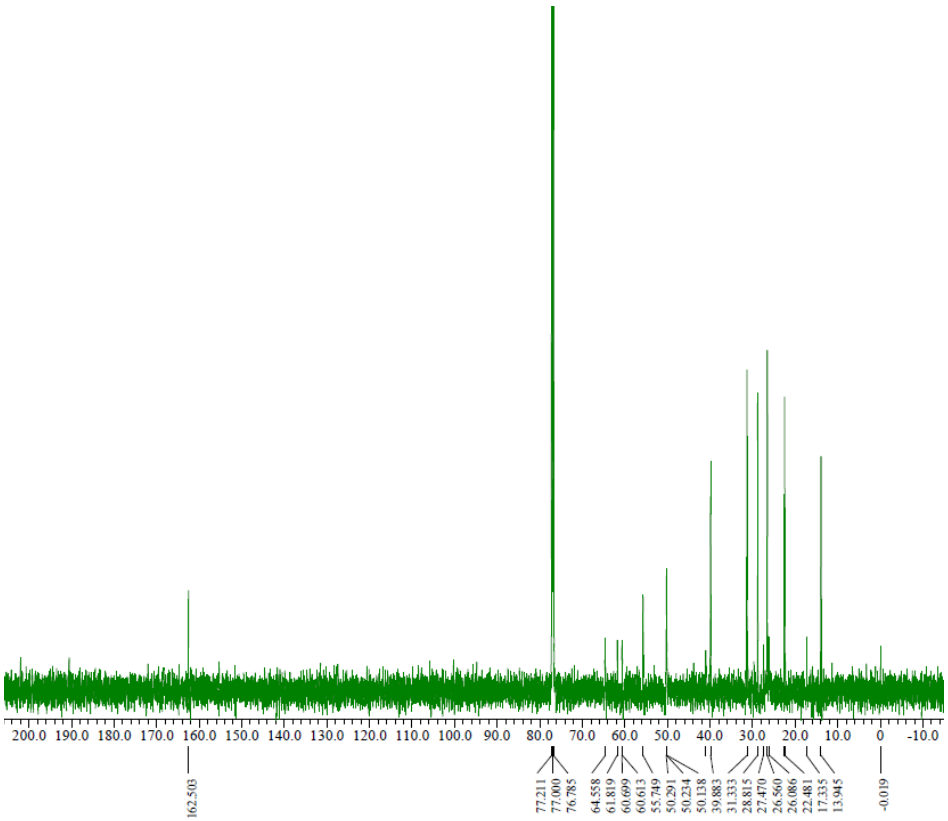

Figure S2. ^{13}CNMR of **SAM-1.** NMR spectrum was recorded in $CDCl_3$ at 600MHz

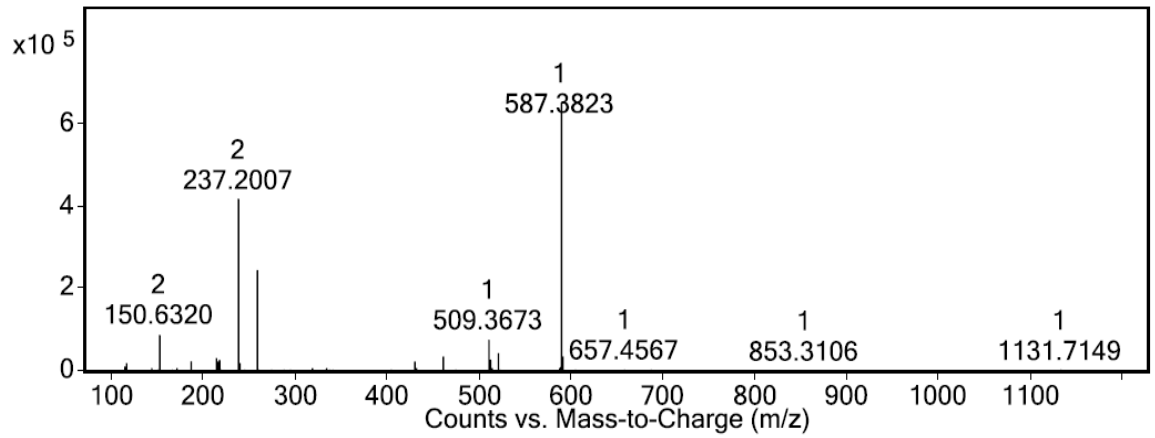

Figure S3. HRMS spectra of **SAM-1**

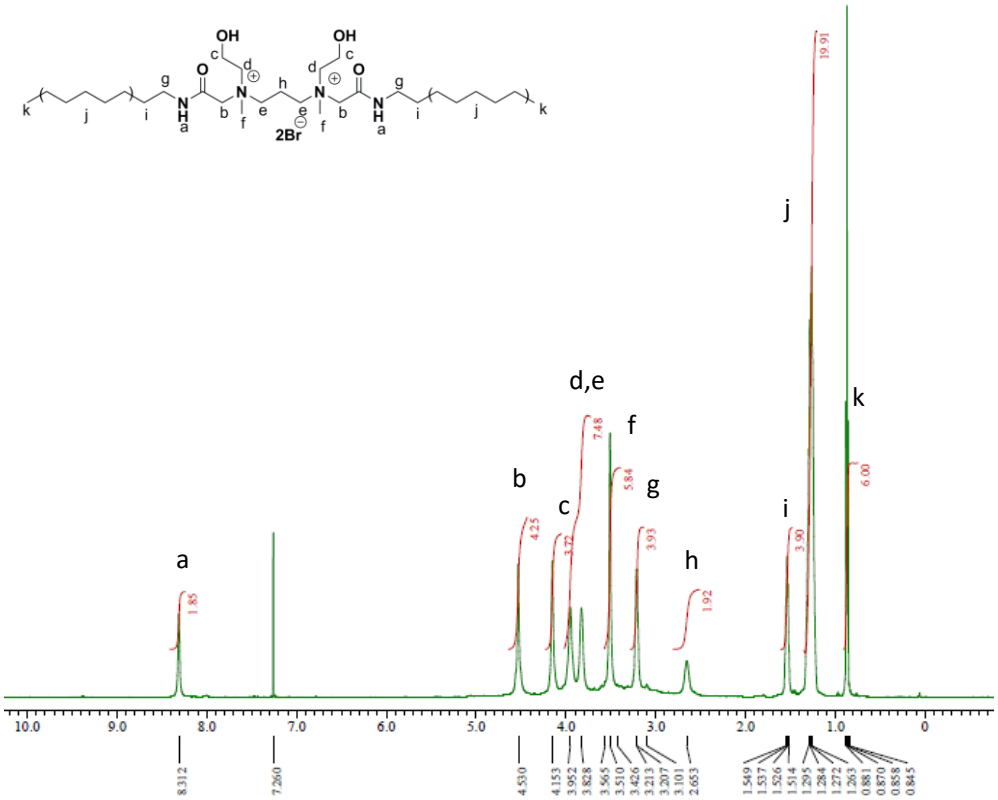

Figure S4. ^{1}HNMR of **SAM-2**. NMR spectrum was recorded in $CDCl_3$ at 600MHz

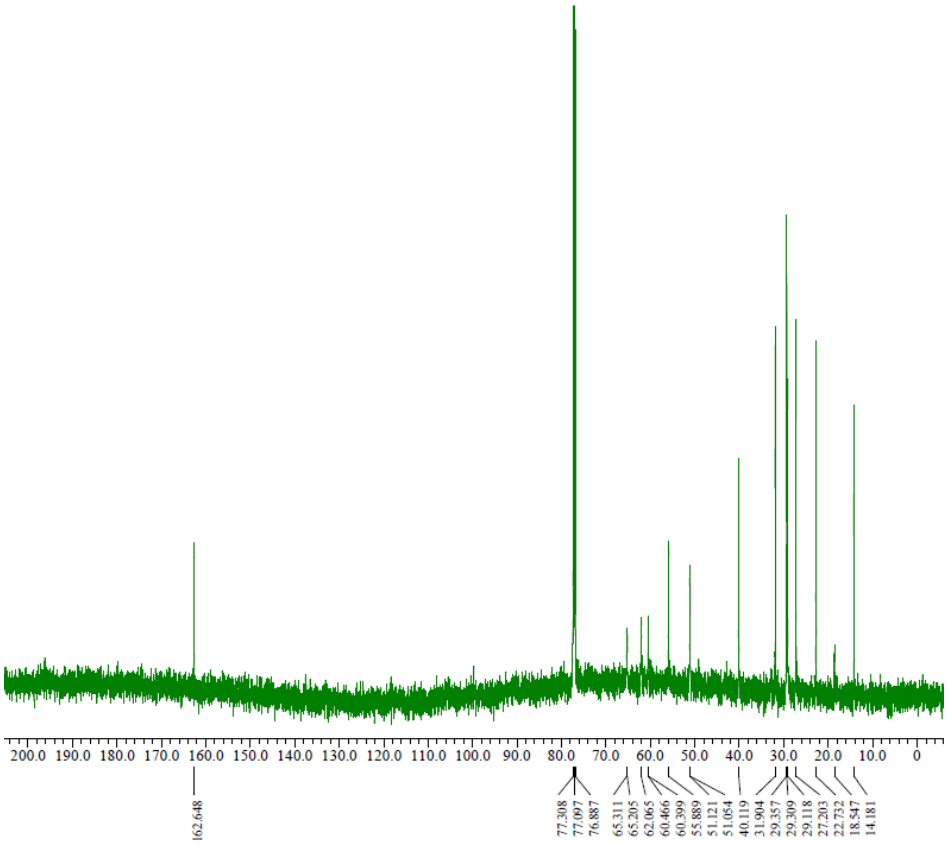

Figure S5. ^{13}C NMR of **SAM-2.** NMR spectrum was recorded in $CDCl_3$ at 600MHz.

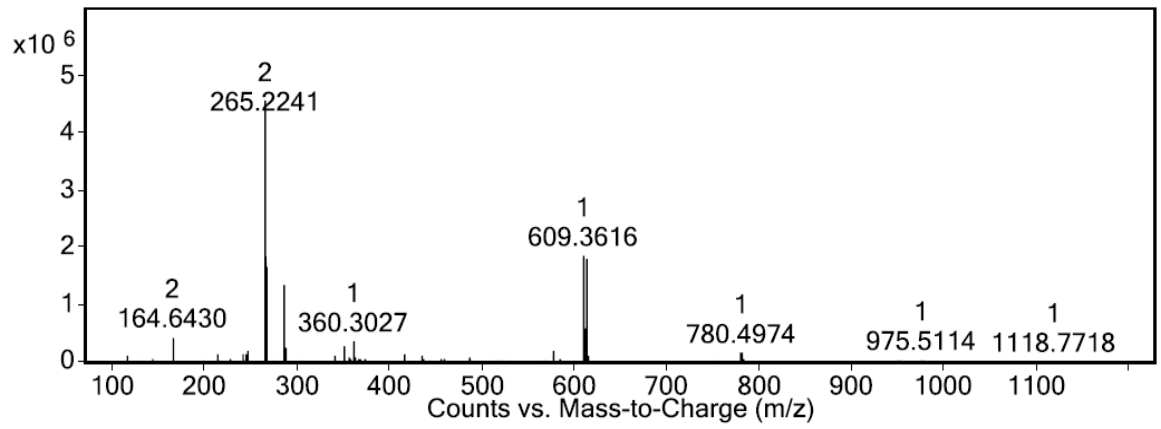

Figure S6. HRMS spectra of **SAM-2**

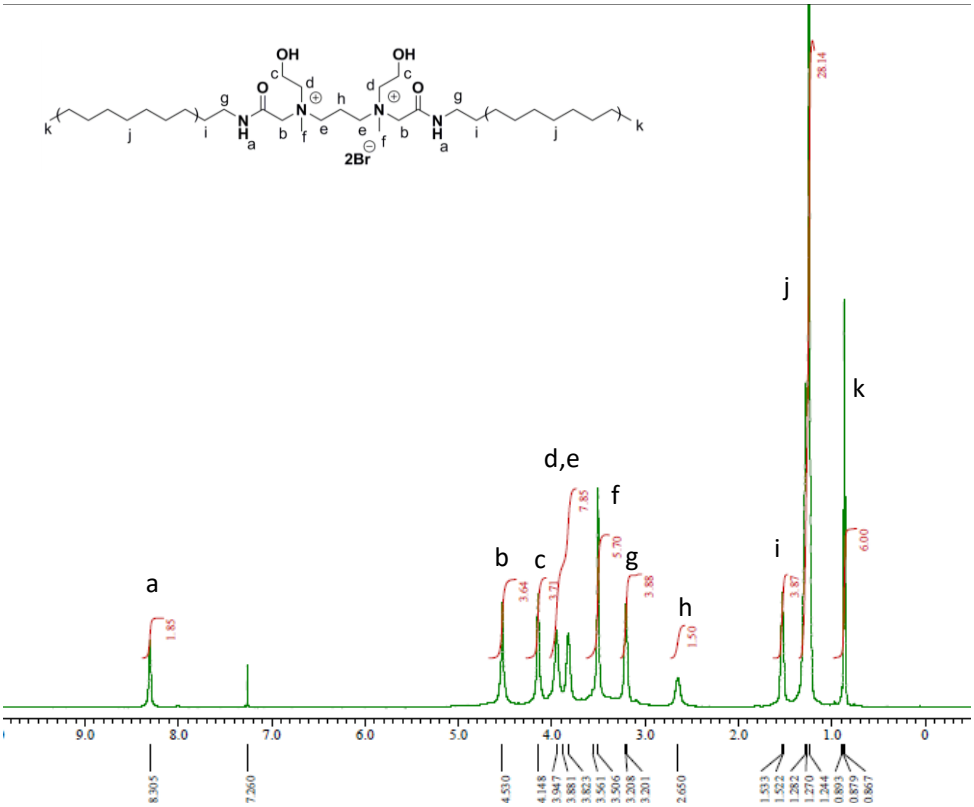

Figure S7. [1]HNMR of **SAM-3**. NMR spectrum was recorded in $CDCl_3$ at 600MHz

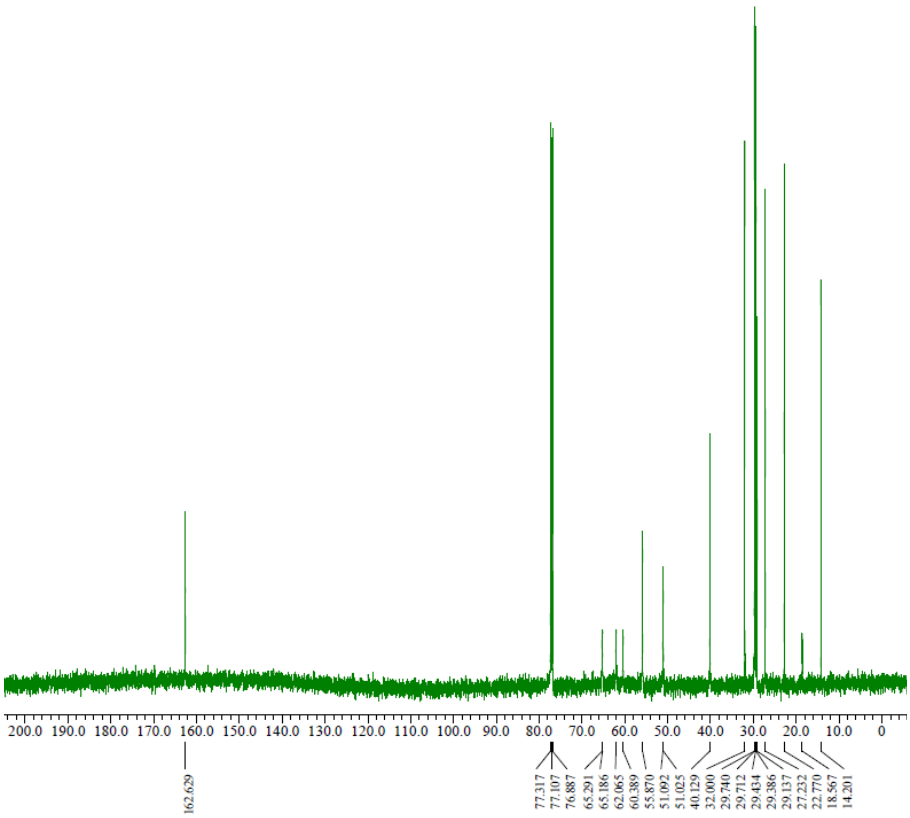

Figure S8. [13]CNMR of **SAM-3**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

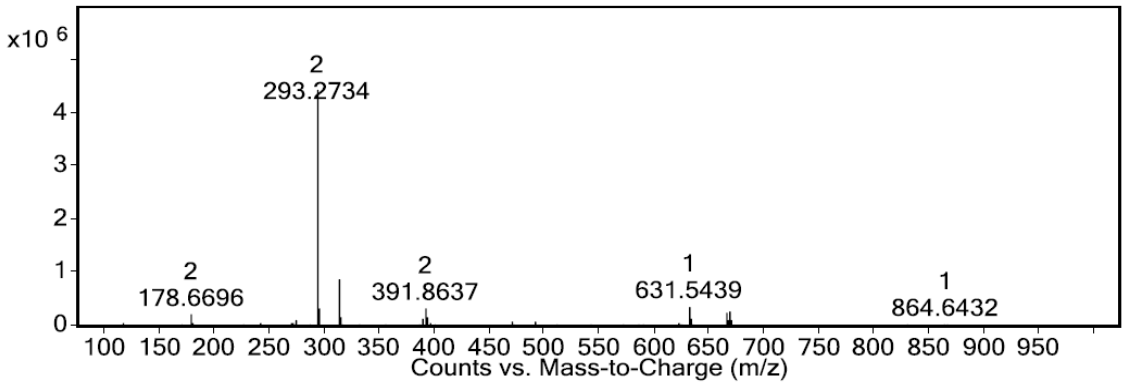

Figure S9. HRMS spectra of **SAM-3**.

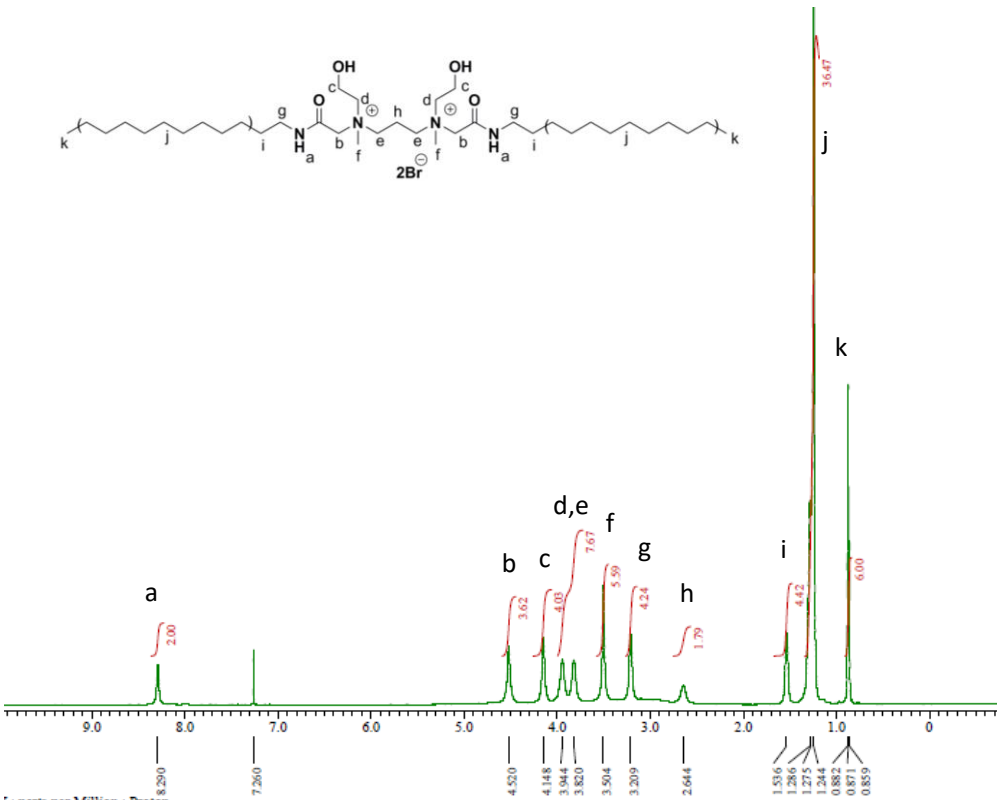

Figure S10. ^{1}HNMR of **SAM-4**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

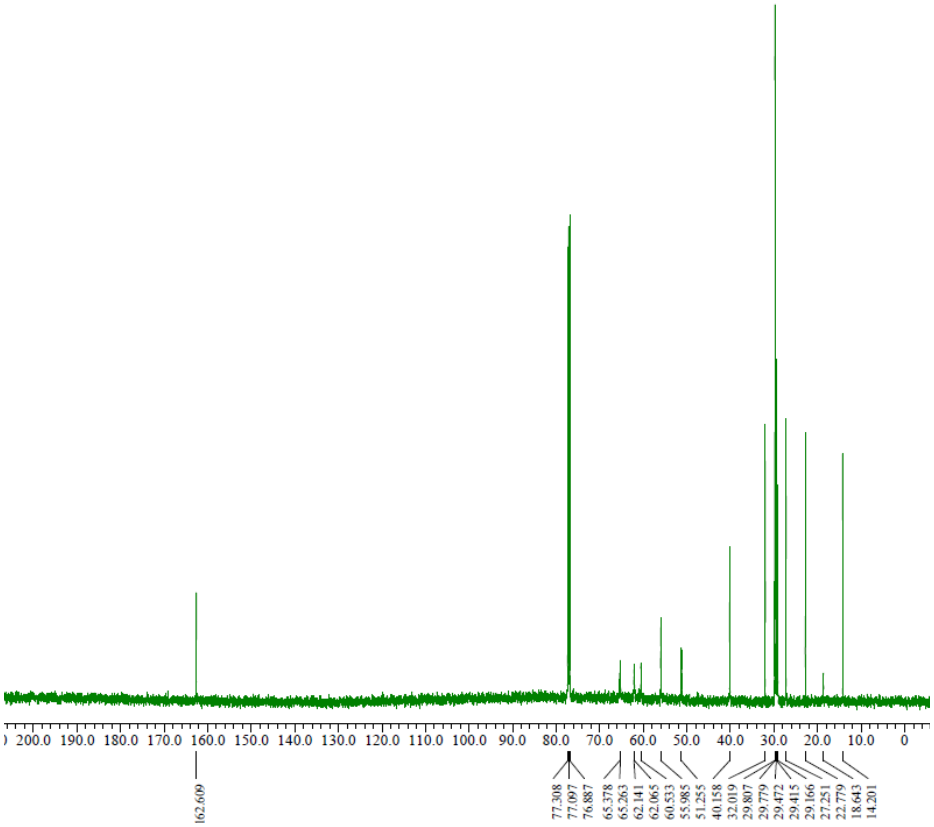

Figure S11. ^{13}CNMR of **SAM-4**. NMR spectrum was recorded in $CDCl_3$ at 600MHz

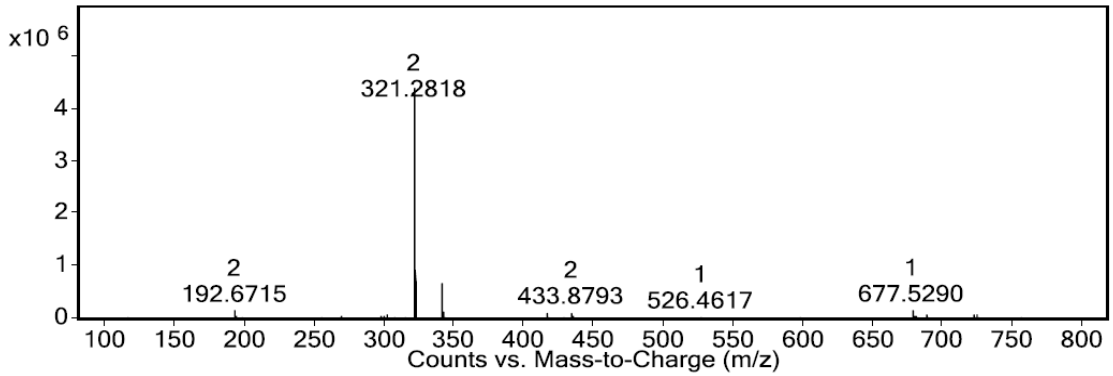

Figure S12. HRMS spectra of **SAM-4**.

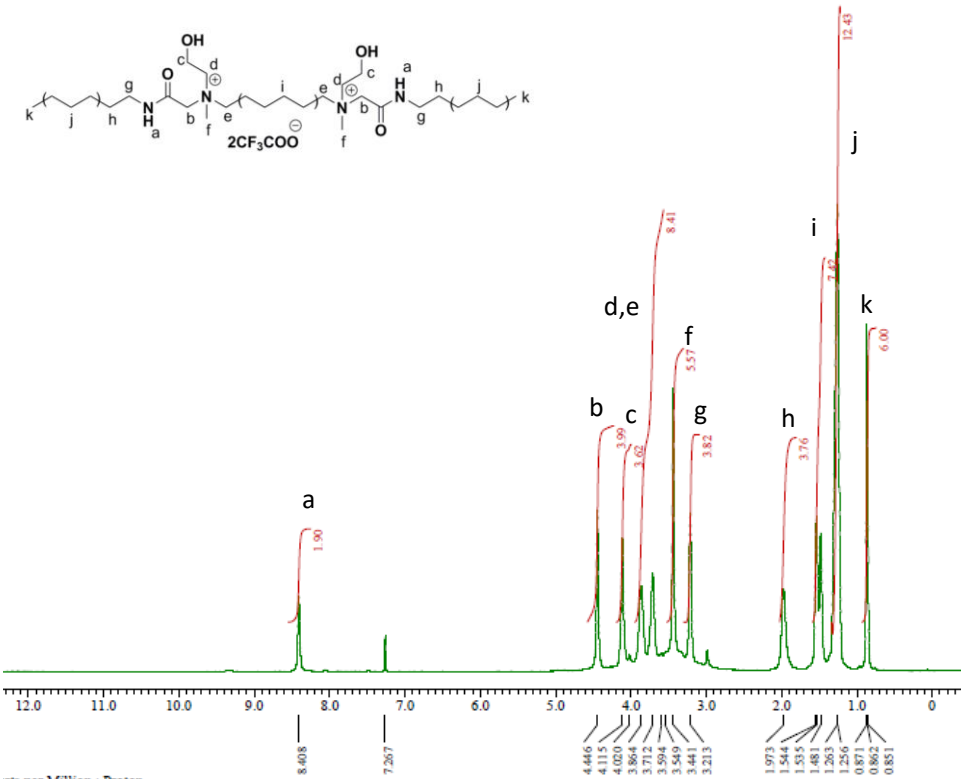

Figure S13. ^{1}HNMR of **SAM-5**. NMR spectrum was recorded in $CDCl_3$ at 600MHz

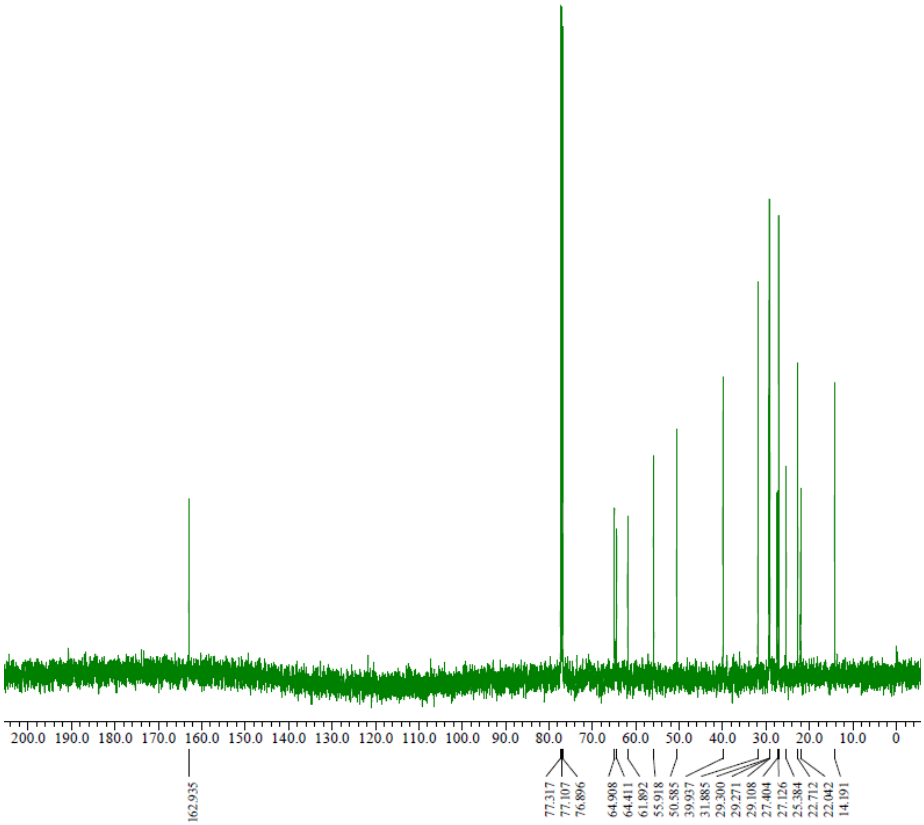

Figure S14. ^{13}CNMR of **SAM-5**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

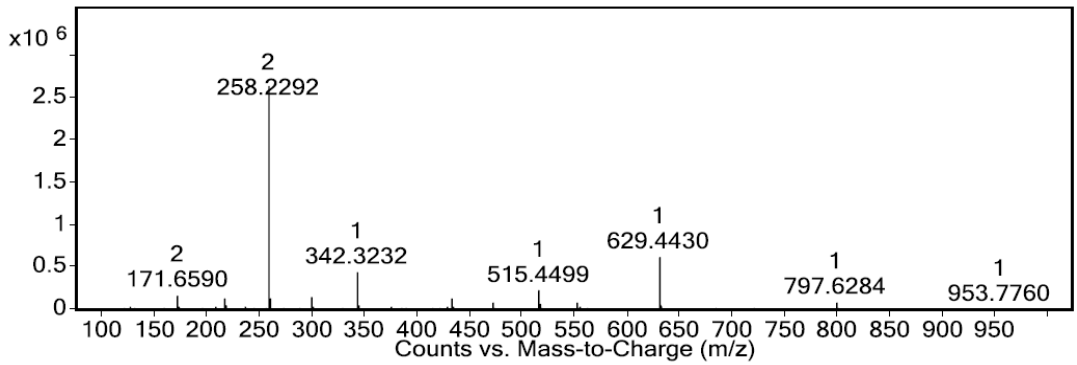

Figure S15. HRMS spectra of **SAM-5.**

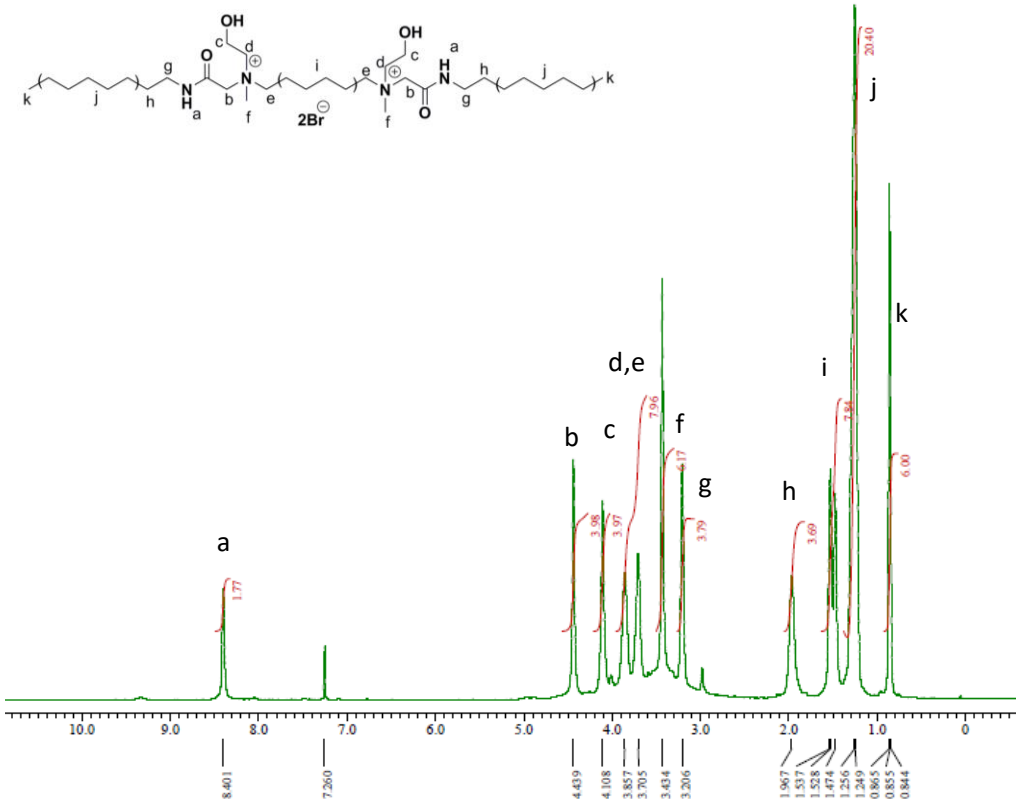

Figure S16. ^{1}HNMR of **SAM-6**. NMR spectrum was recorded in $CDCl_3$ at 600MHz**.**

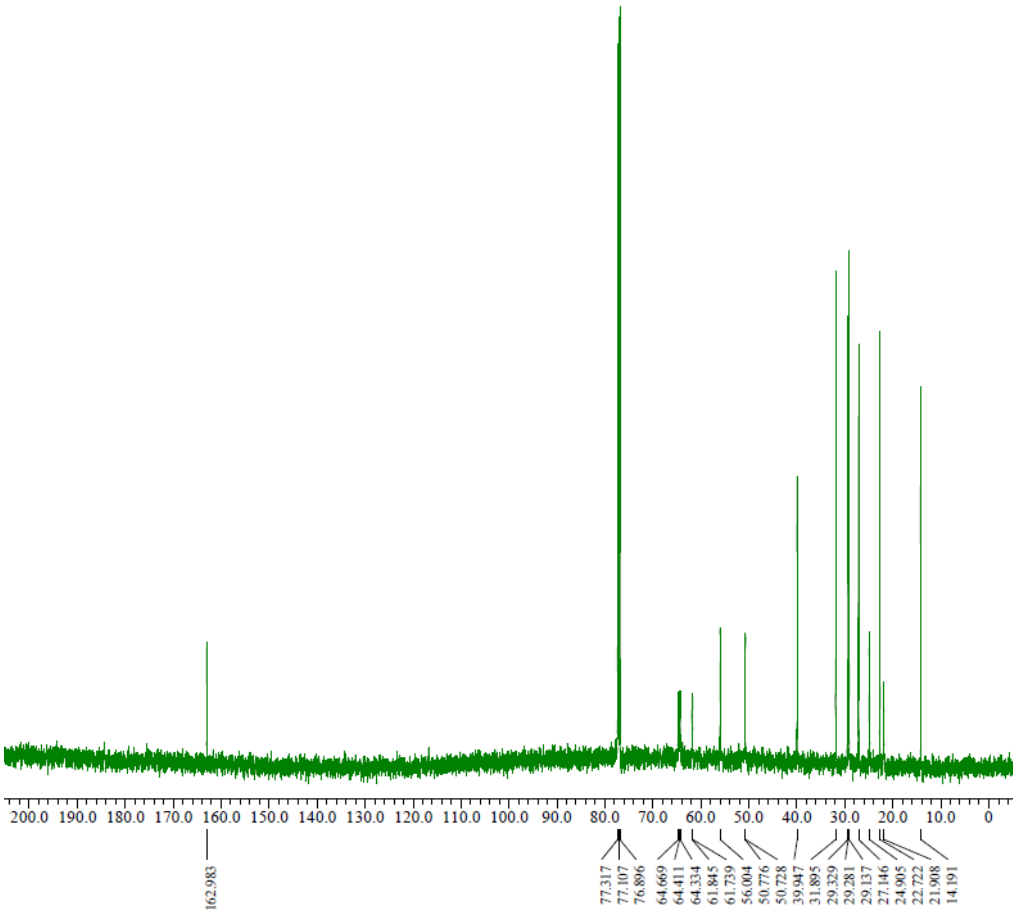

Figure S17. ^{13}CNMR of **SAM-6** NMR spectrum was recorded in $CDCl_3$ at 600MHz**.**

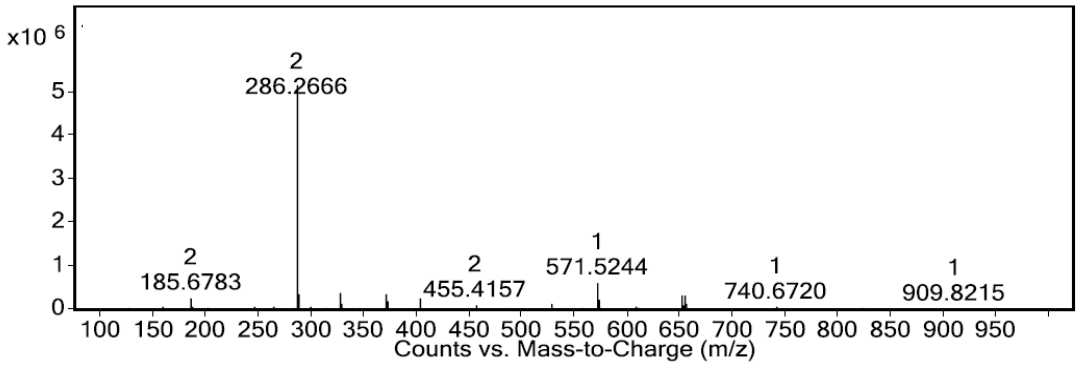

Figure S18. HRMS spectra of **SAM-6**.

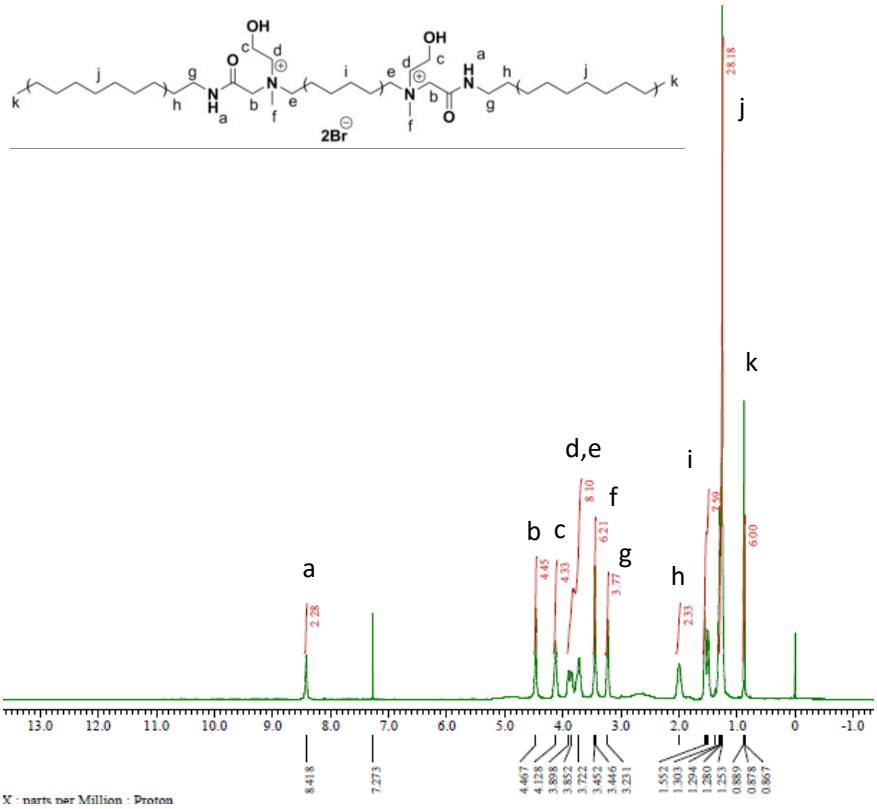

Figure S19. ^{1}HNMR of **SAM-7**. NMR spectrum was recorded in $CDCl_3$ at 600MHz**.**

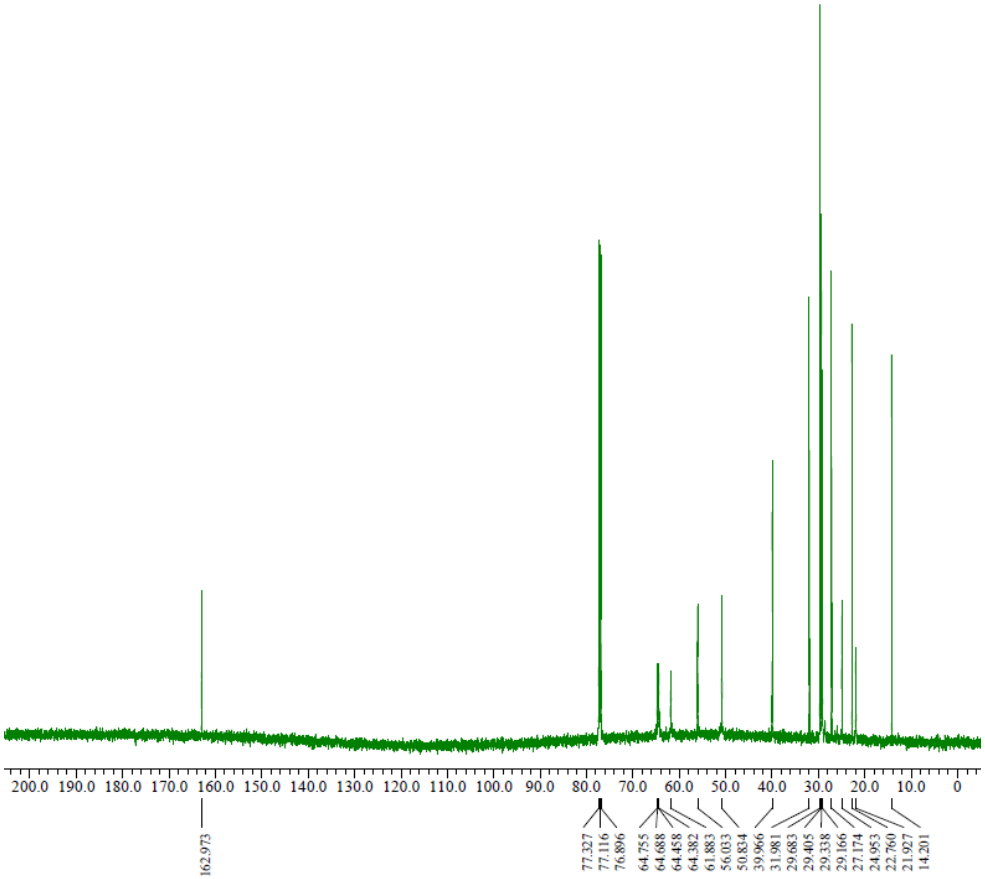

Figure S20. ^{13}CNMR of **SAM-7**. NMR spectrum was recorded in $CDCl_3$ at 600MHz

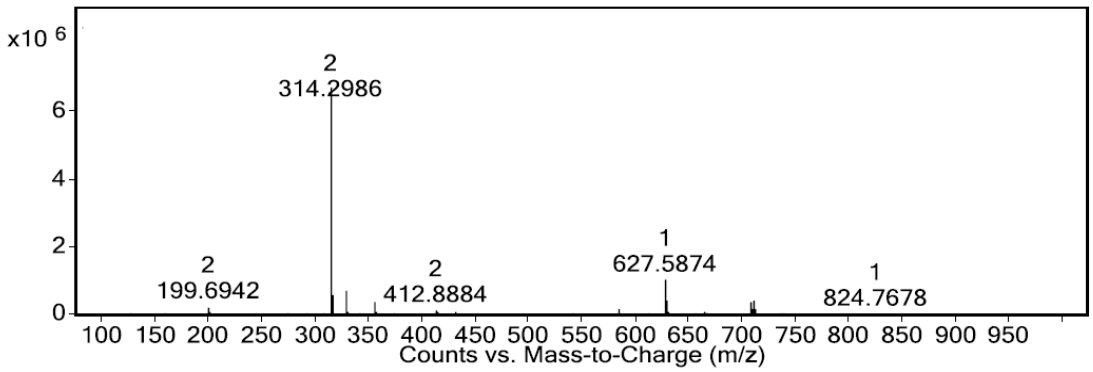

Figure S21. HRMS spectra of **SAM-7.**

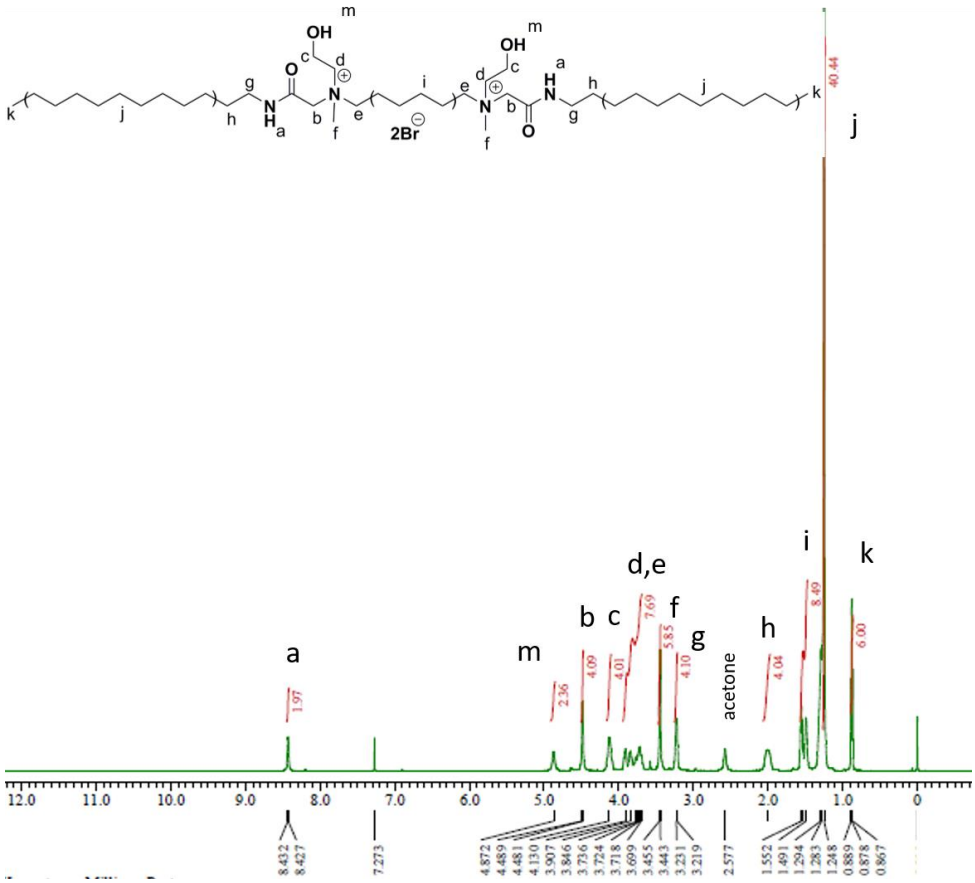

Figure S22. ^{1}HNMR of **SAM-8**. NMR spectrum was recorded in $CDCl_3$ at 600MHz**.**

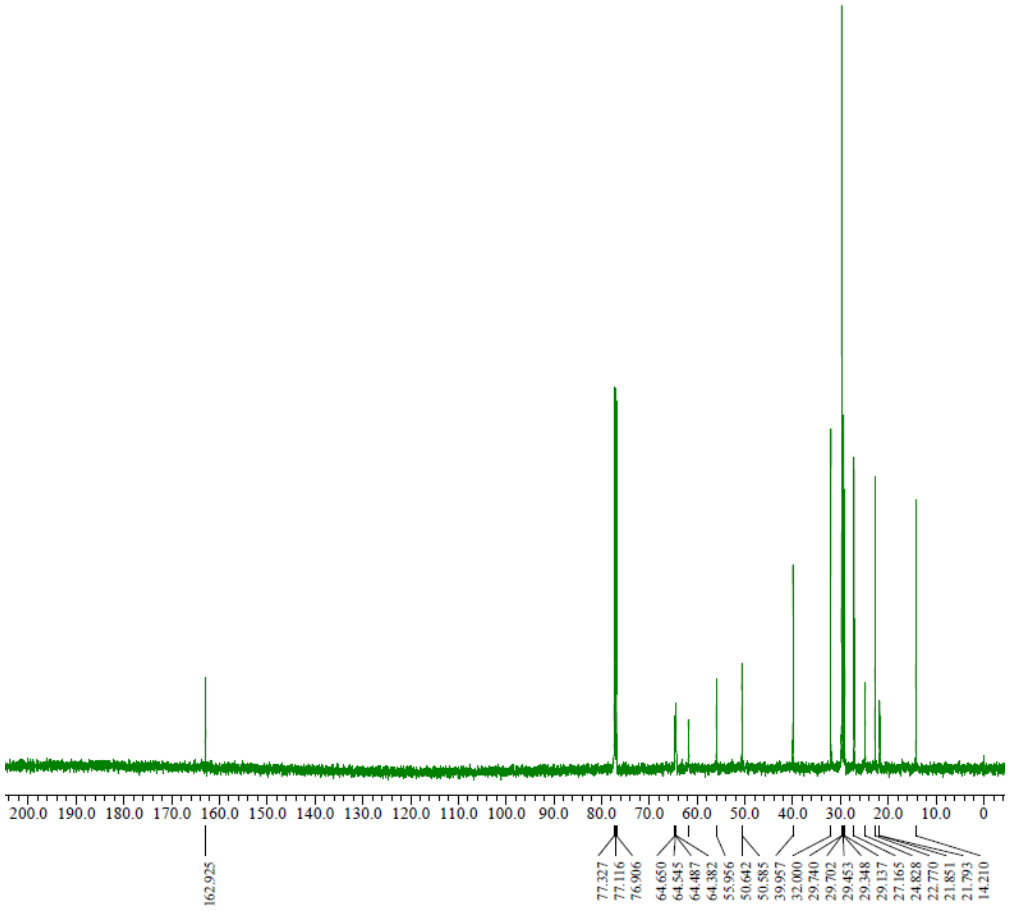

Figure S23. ^{13}CNMR of **SAM-8**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

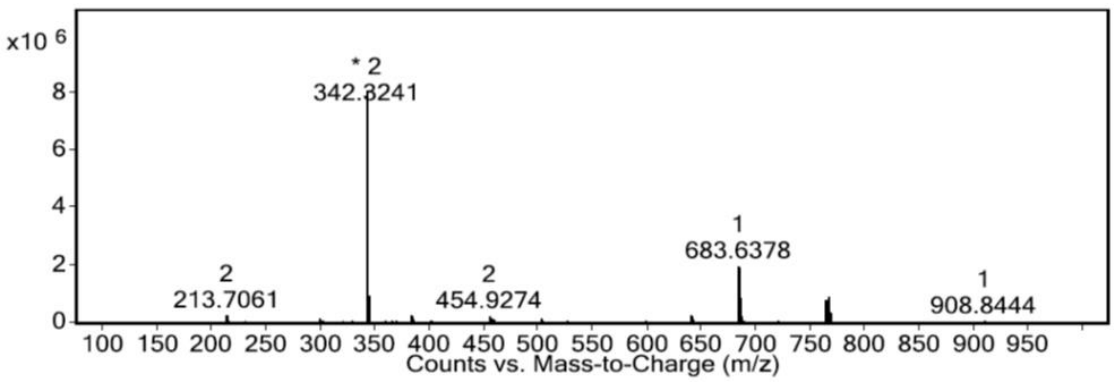

Figure S24. HRMS spectra of **SAM-8**.

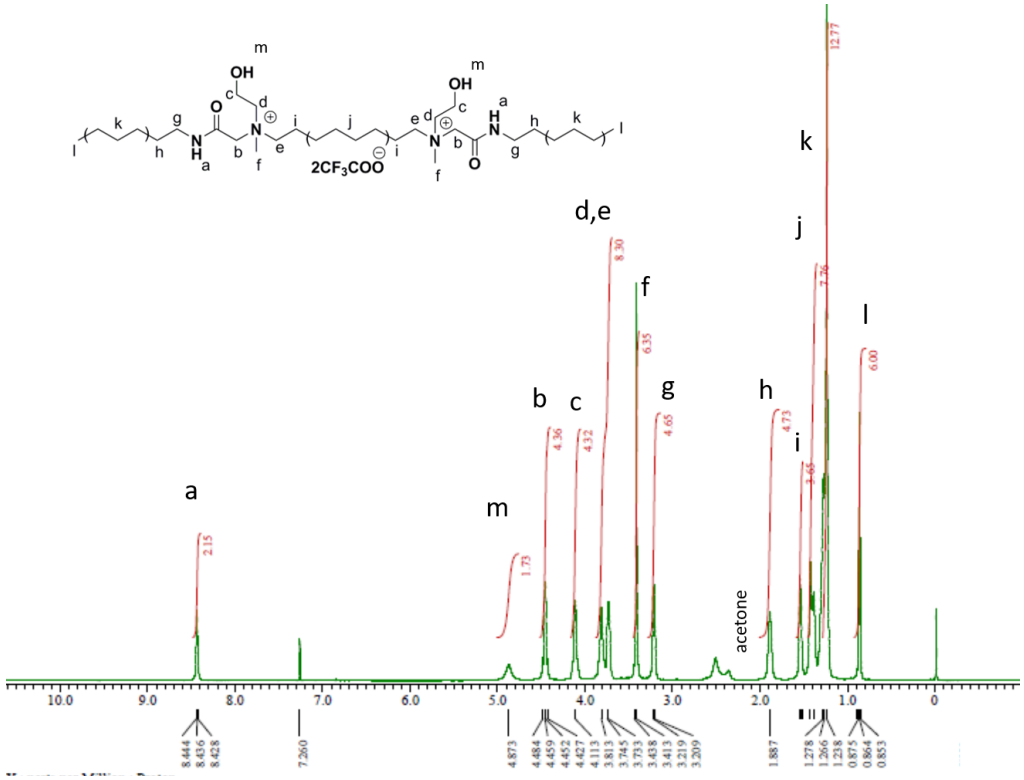

Figure S25. ^{1}HNMR of **SAM-9**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

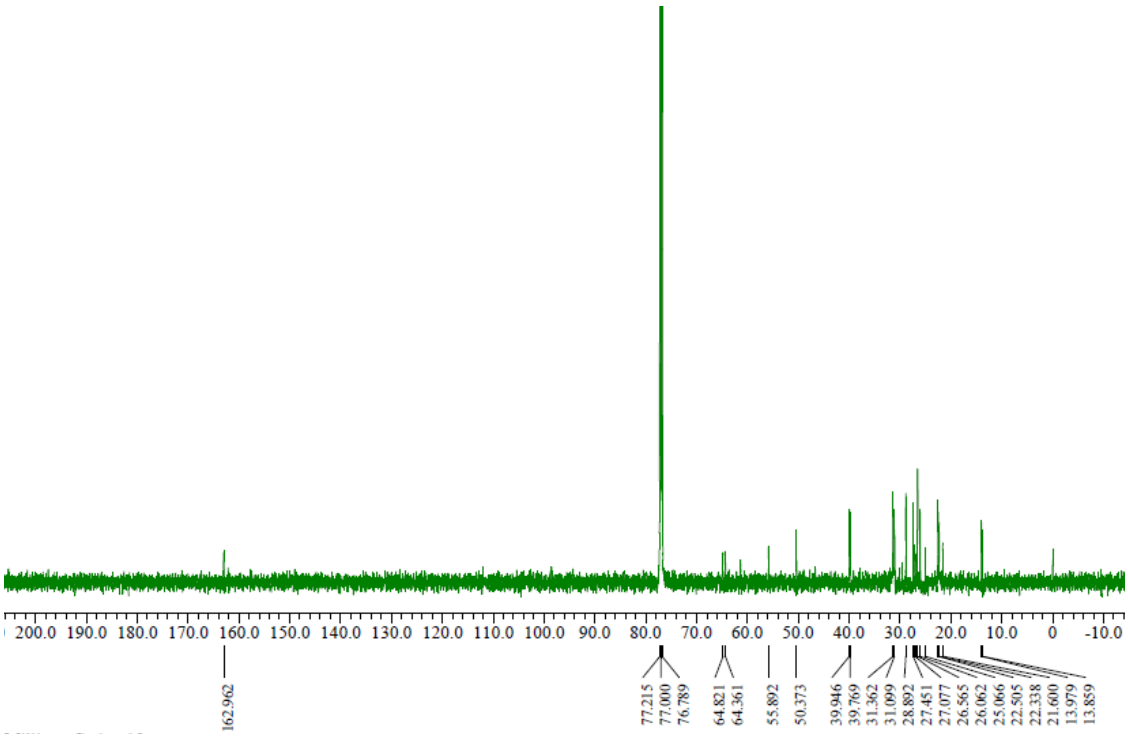

Figure S26. ^{13}CNMR of **SAM-9**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

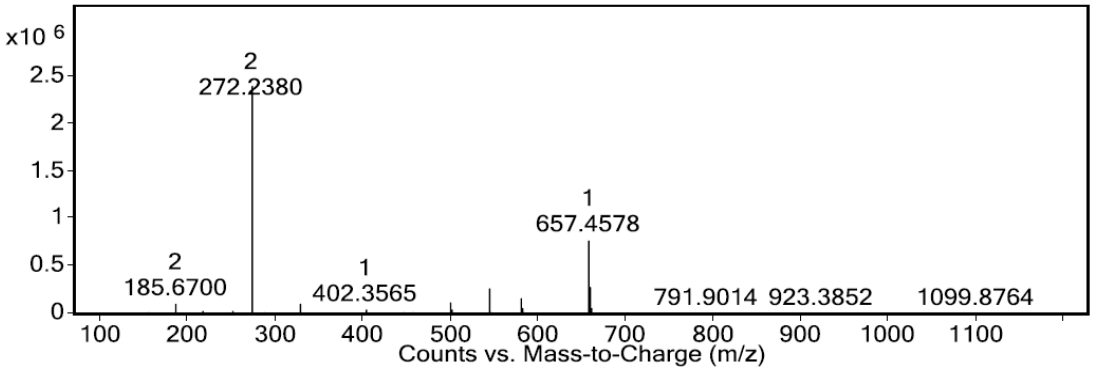

Figure S27. HRMS spectra of **SAM-9**.

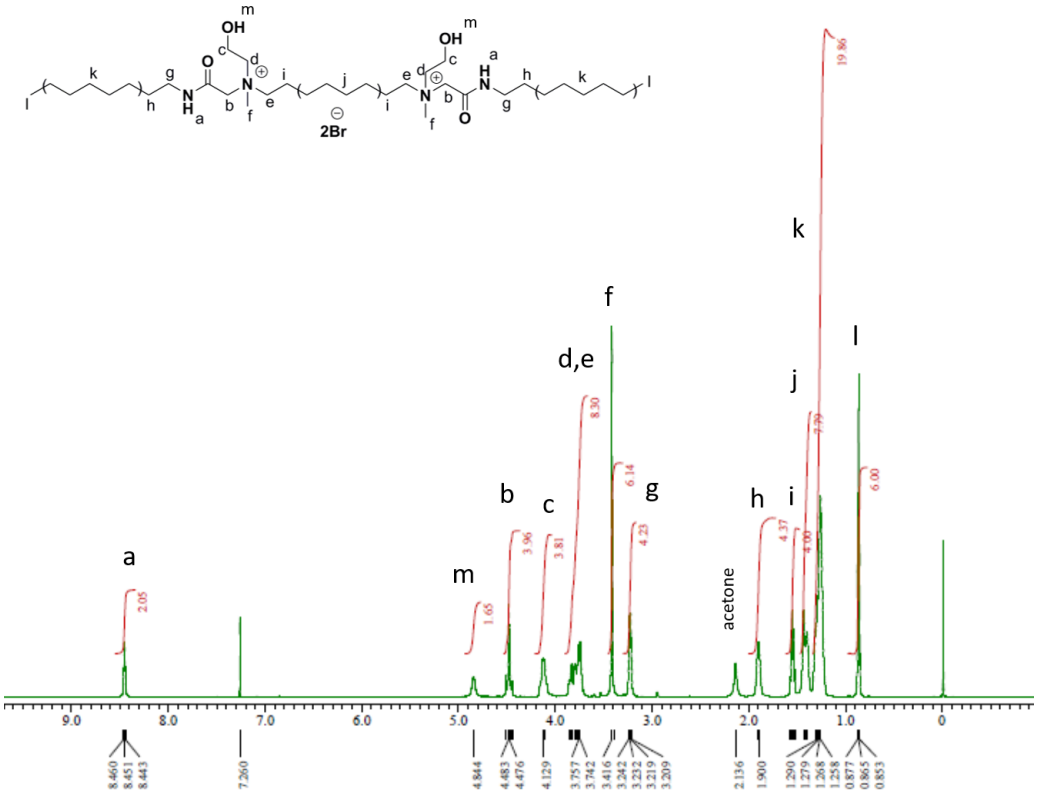

Figure S28. ^{1}HNMR of **SAM-10**. NMR spectrum was recorded in $CDCl_3$ at 600MHz

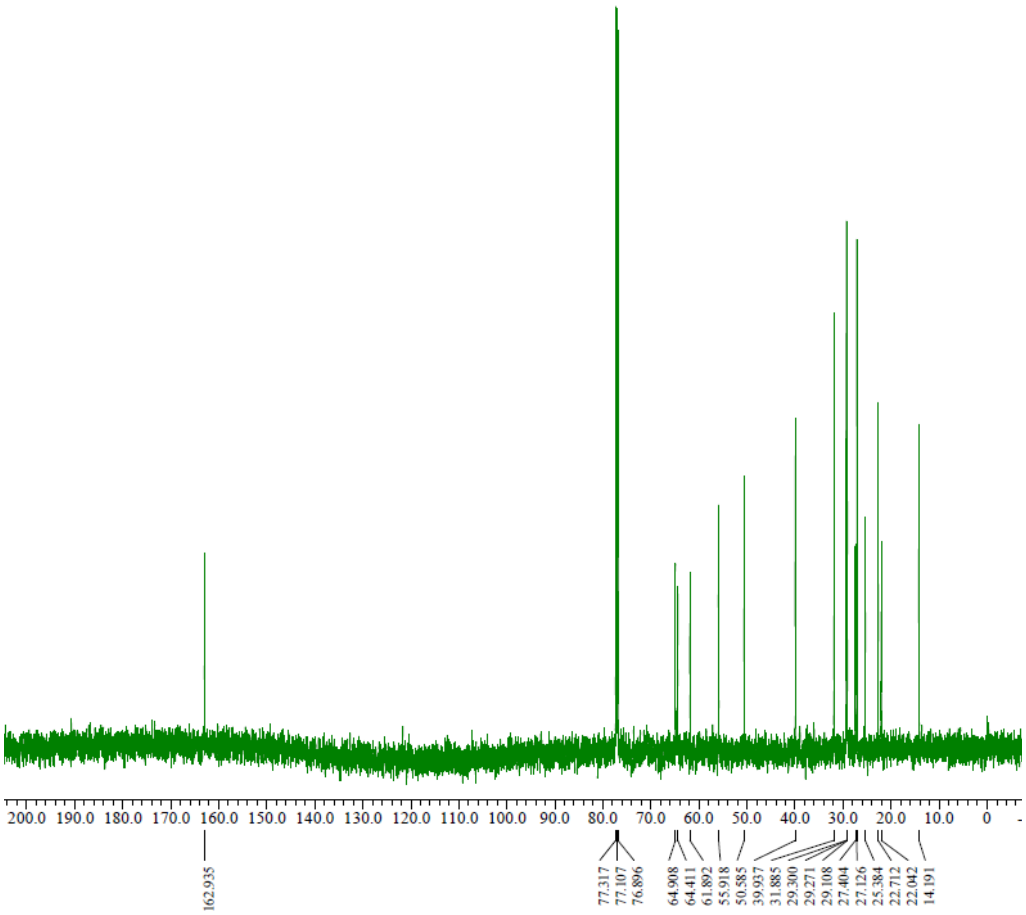

Figure S29. ^{13}CNMR of **SAM-10**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

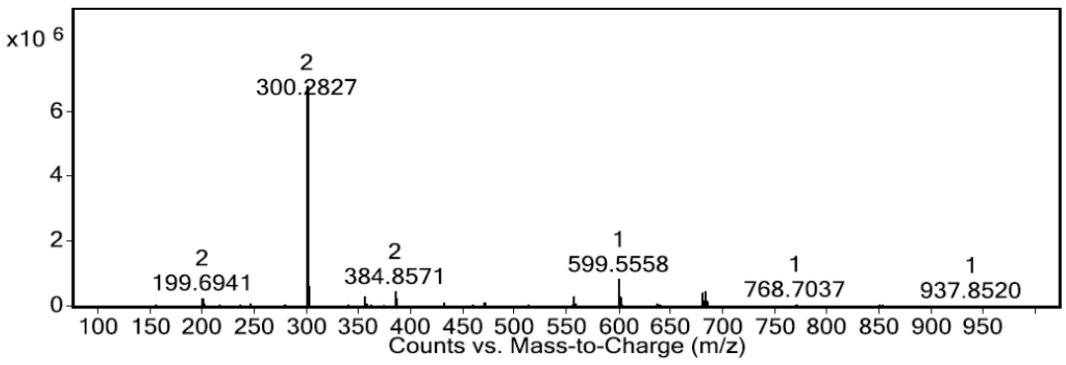

Figure S30. HRMS spectra of **SAM-10**.

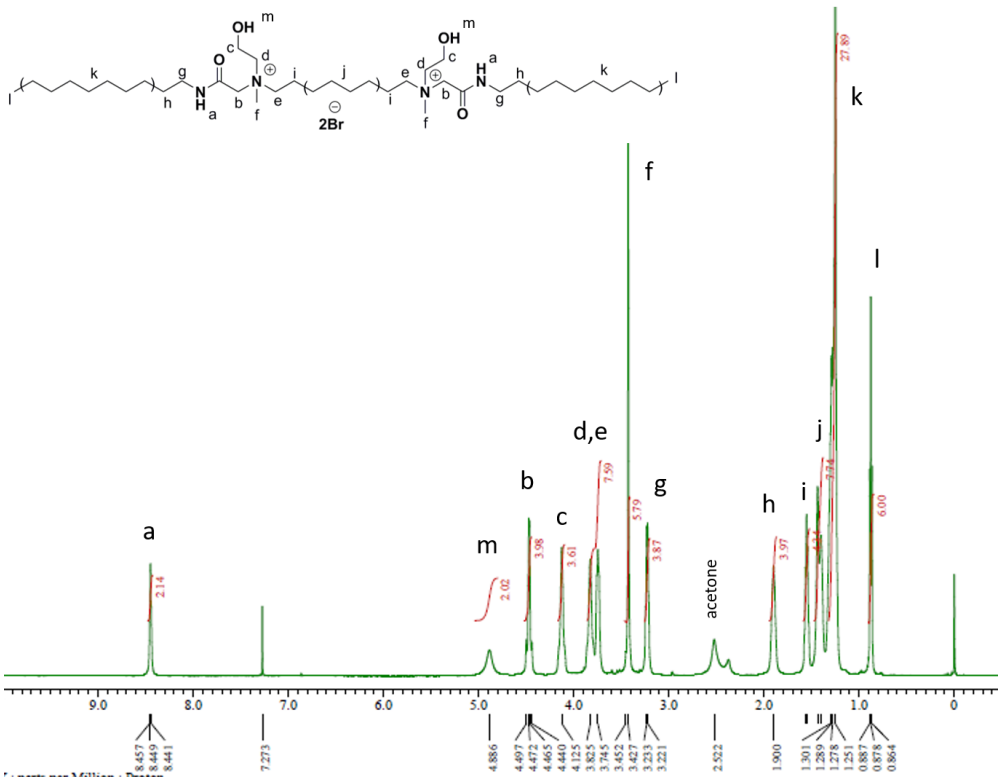

Figure S31. [1]HNMR of **SAM-11**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

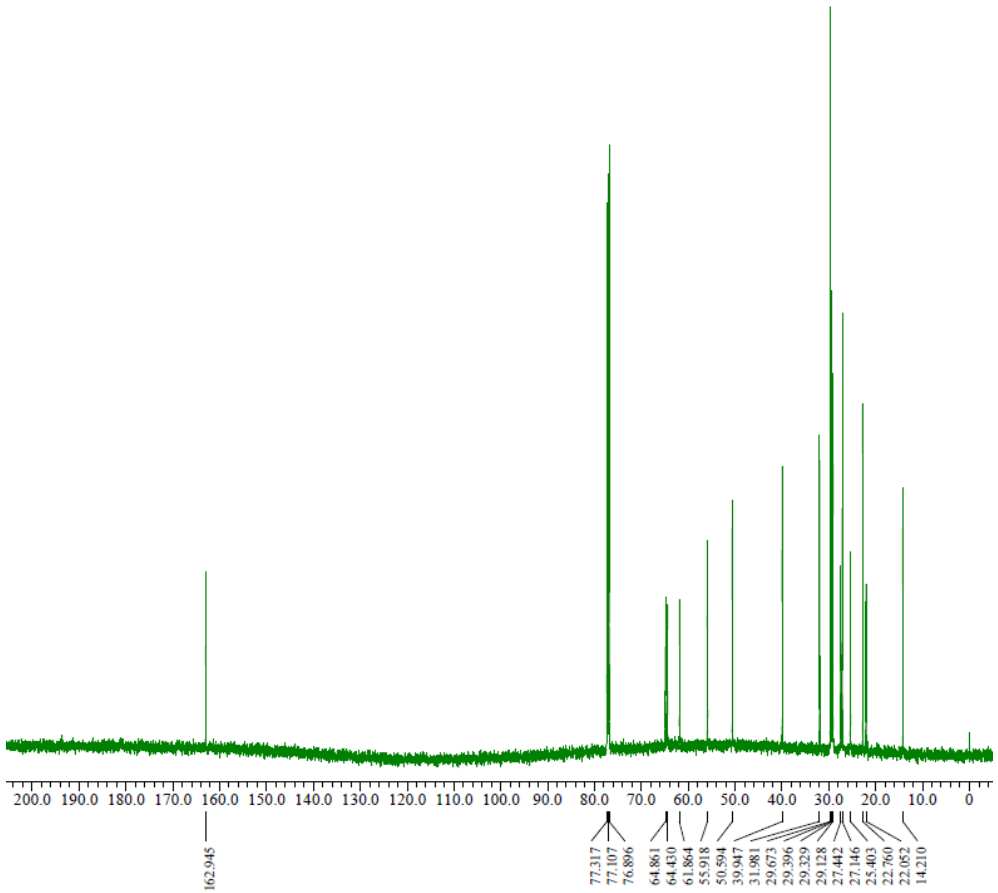

Figure S32. [13]CNMR of **SAM-11**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

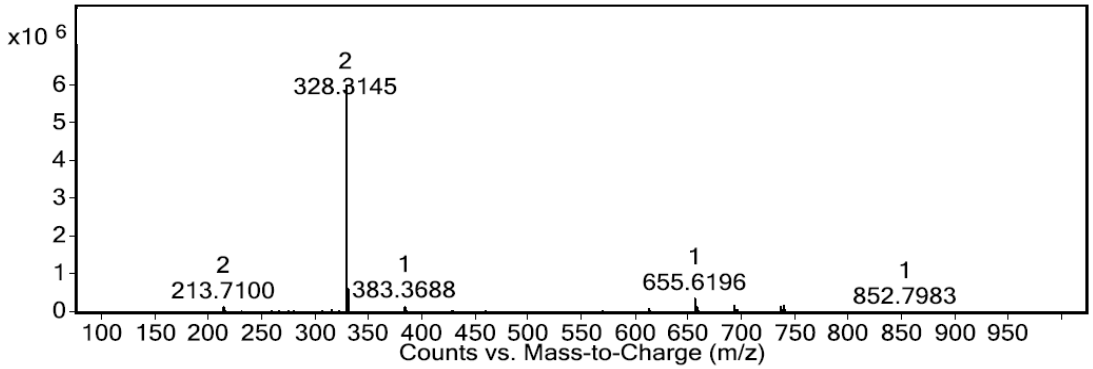

Figure S33. HRMS spectra of **SAM-11**

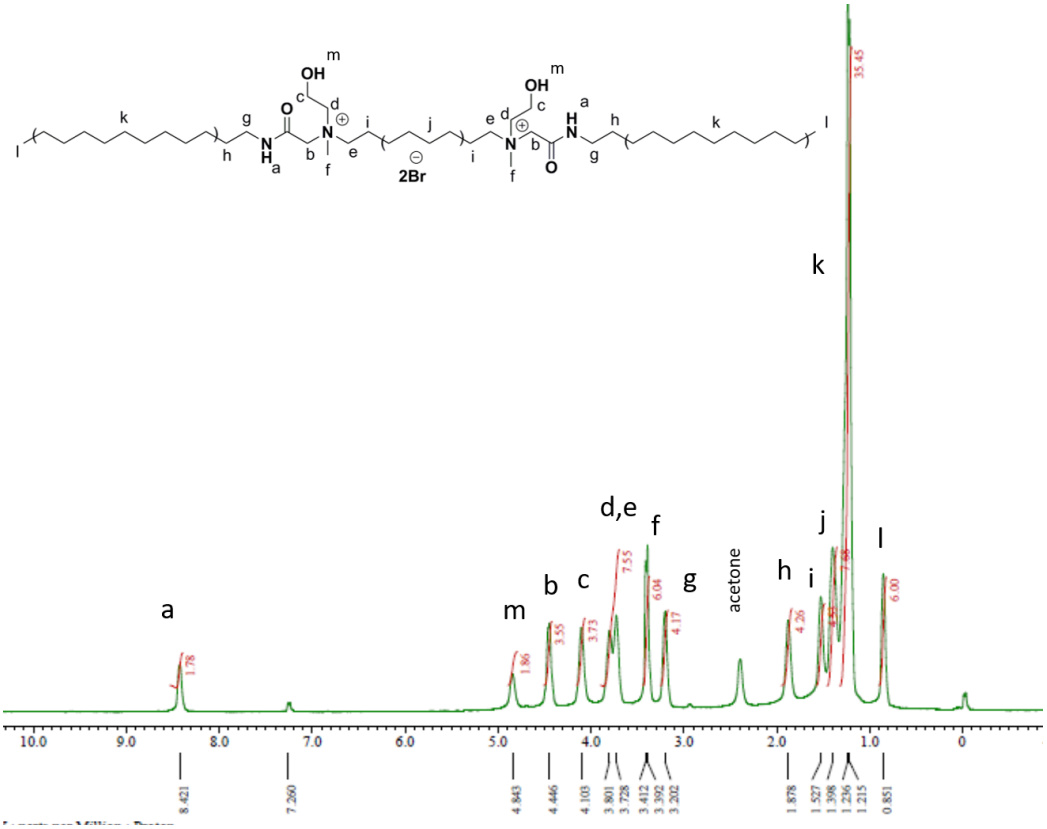

Figure S34. ^{1}HNMR of **SAM-12**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

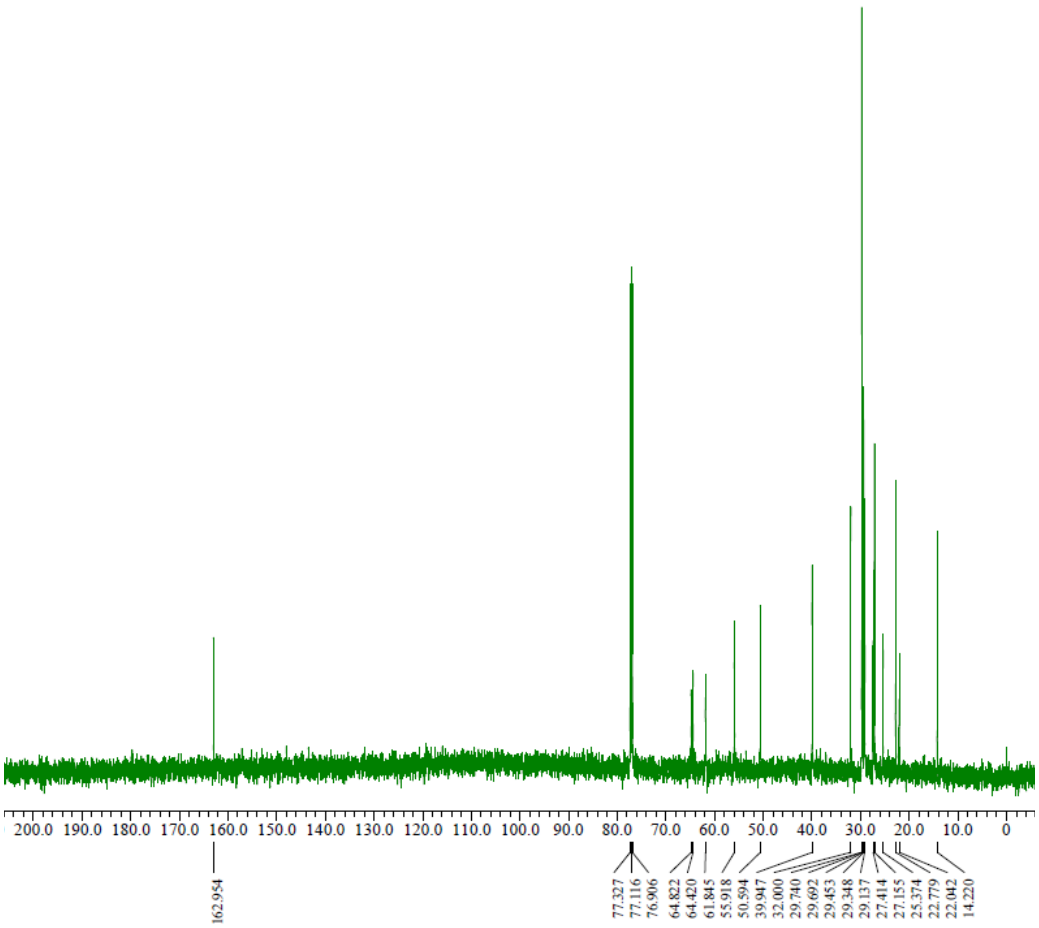

Figure S35. ^{13}CNMR of **SAM-12**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

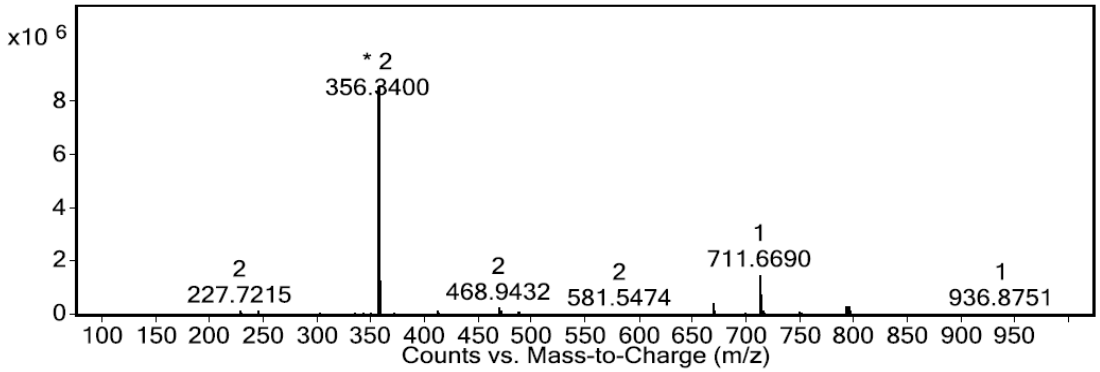

Figure S36. HRMS spectra of **SAM-12**

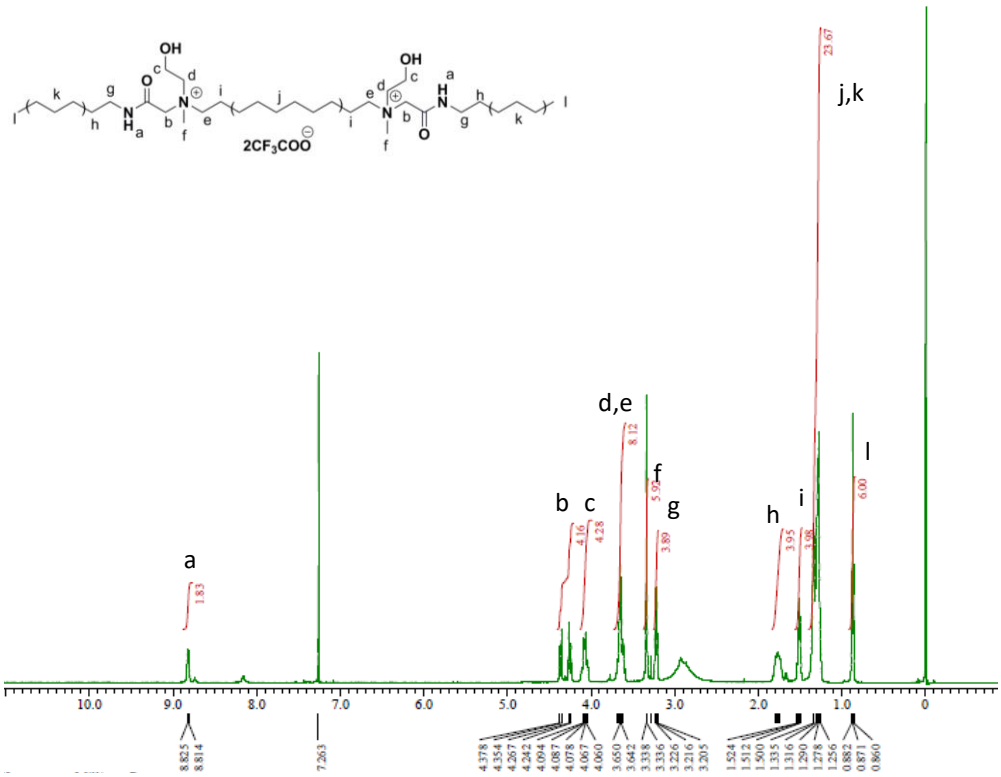

Figure S37. ^{1}HNMR of **SAM-13**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

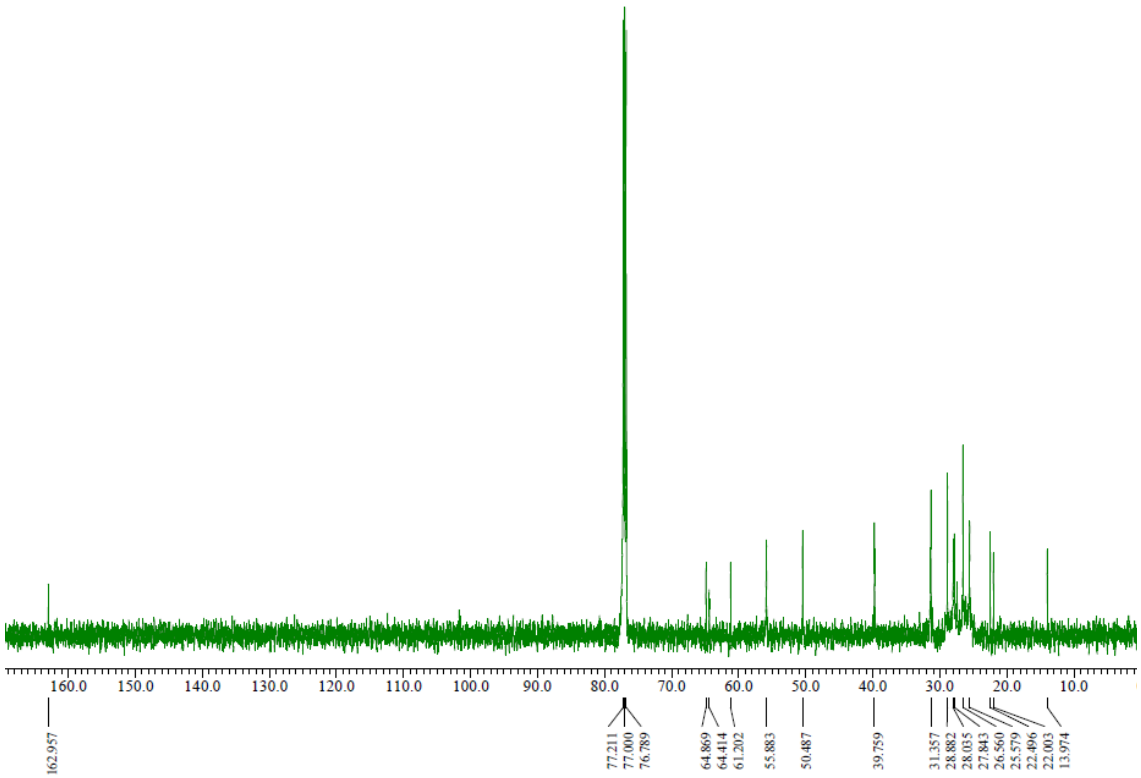

Figure S38. ^{13}CNMR of **SAM-13**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

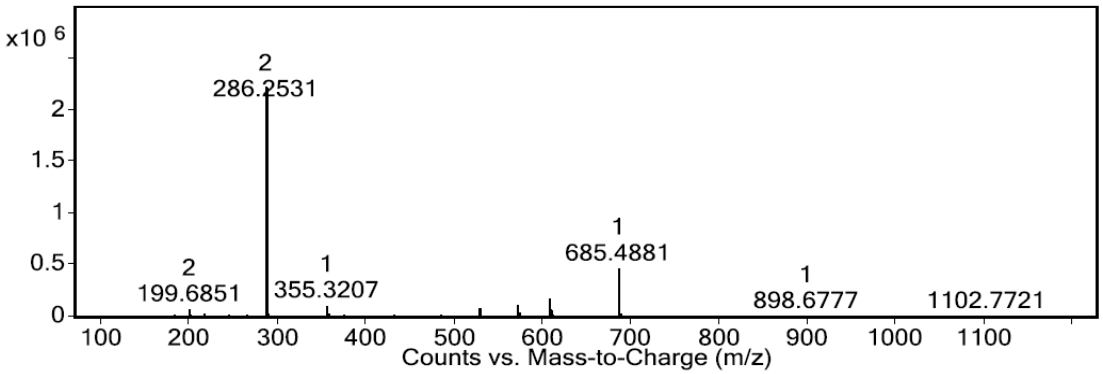

Figure S39. HRMS spectra of **SAM-13**.

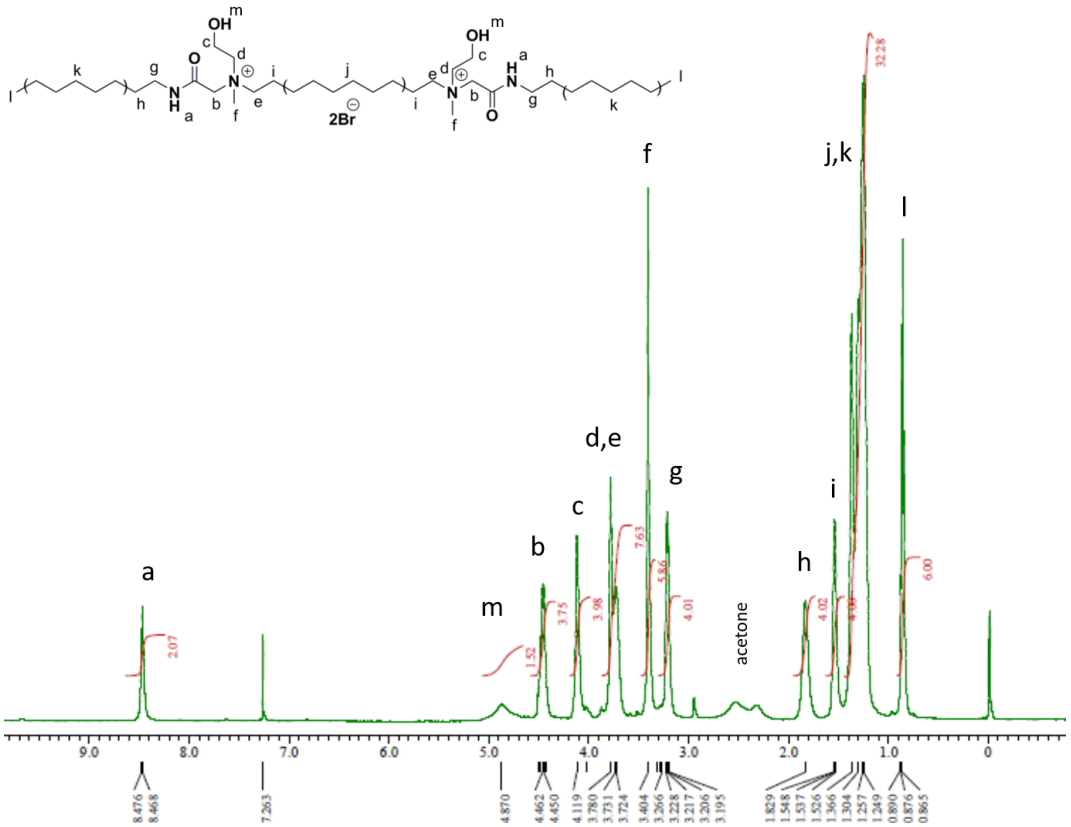

Figure S40. ^{1}HNMR of **SAM-14**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

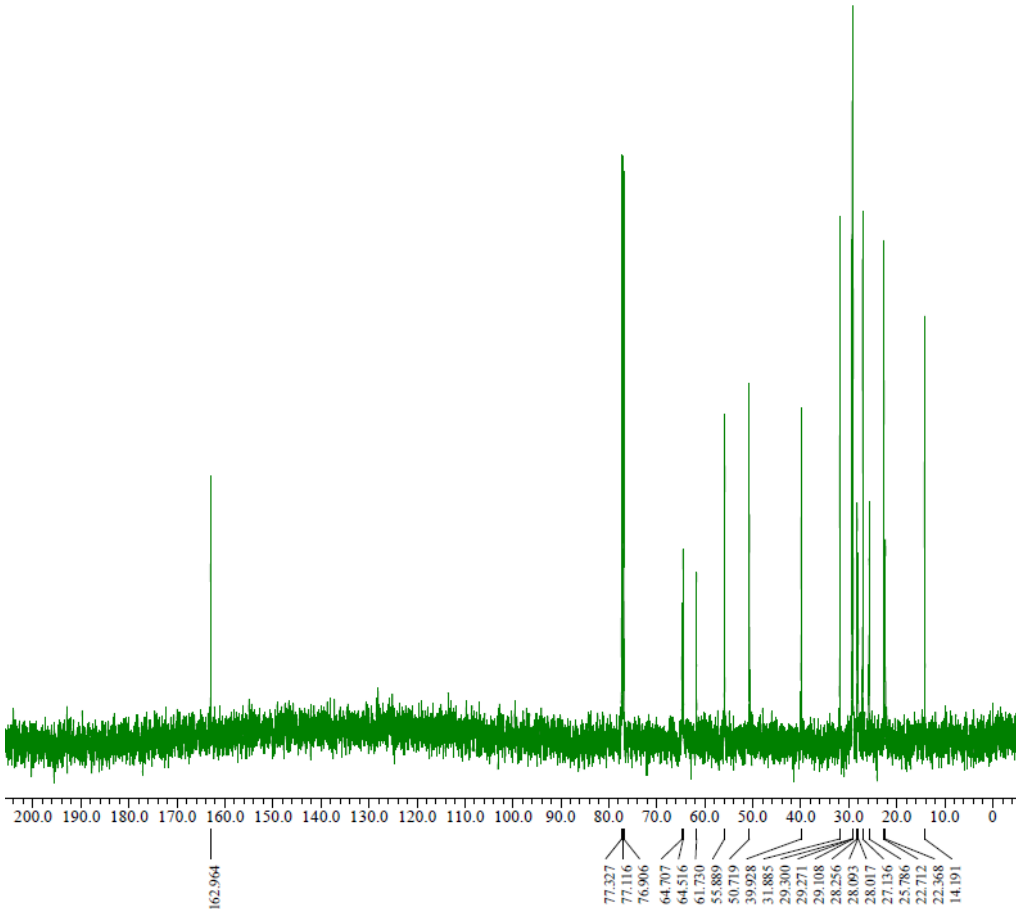

Figure S41. ^{13}CNMR of **SAM-14**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

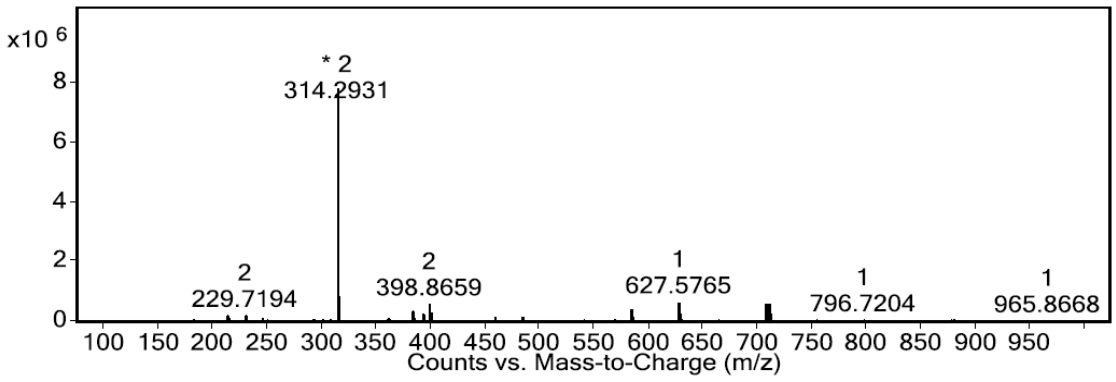

Figure S42. HRMS spectra of **SAM-14**.

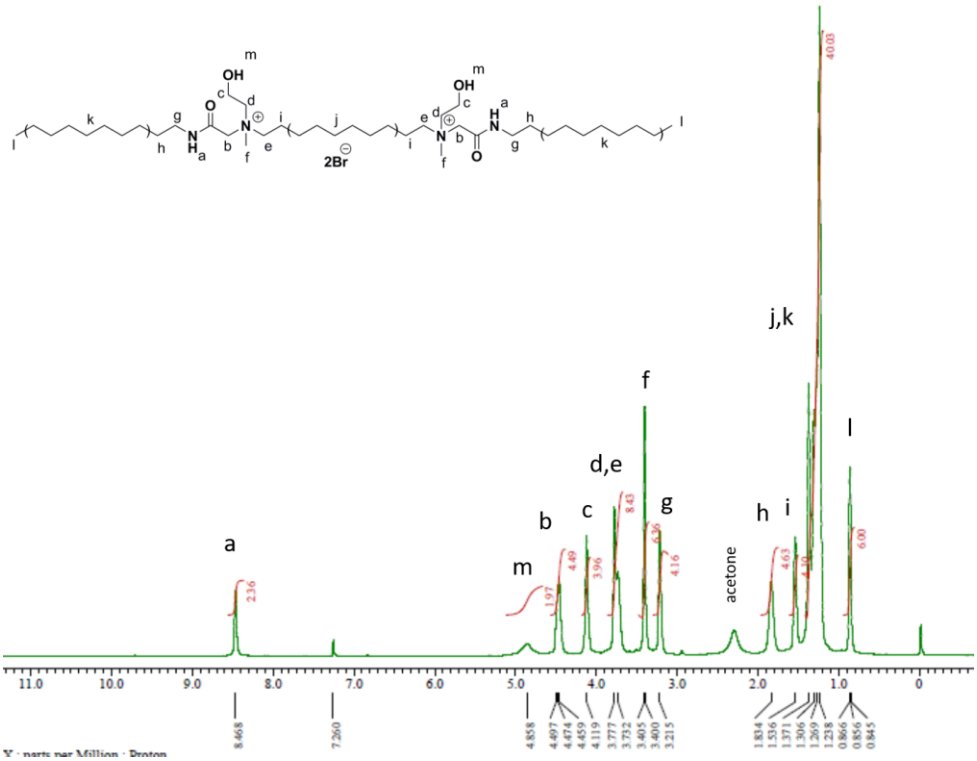

Figure S43. ^{1}HNMR of **SAM-15**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

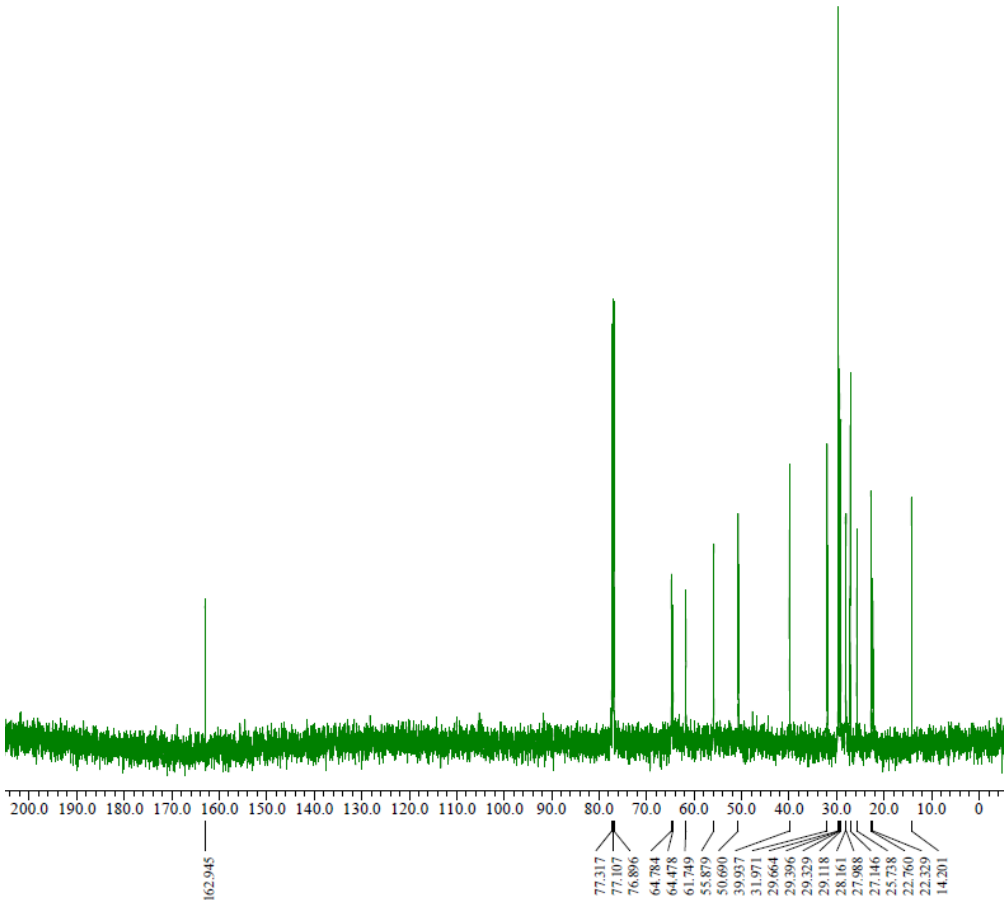

Figure S44. ^{13}CNMR of **SAM-15**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

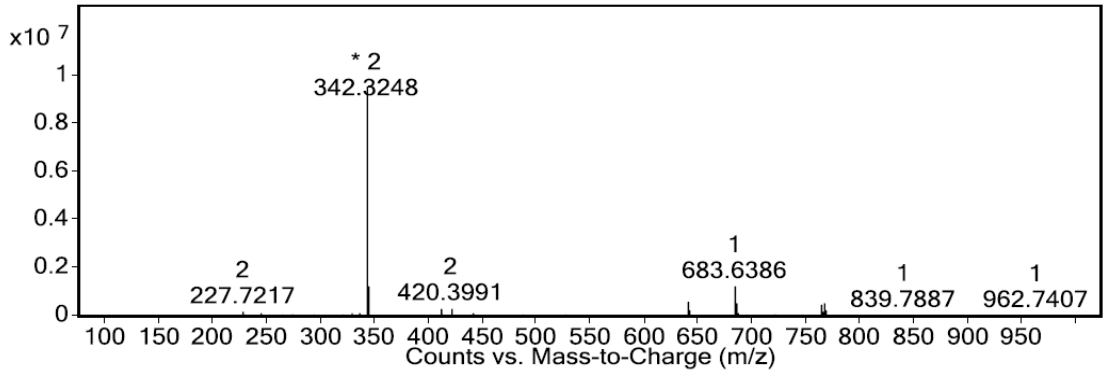

Figure S45. HRMS spectra of **SAM-15**.

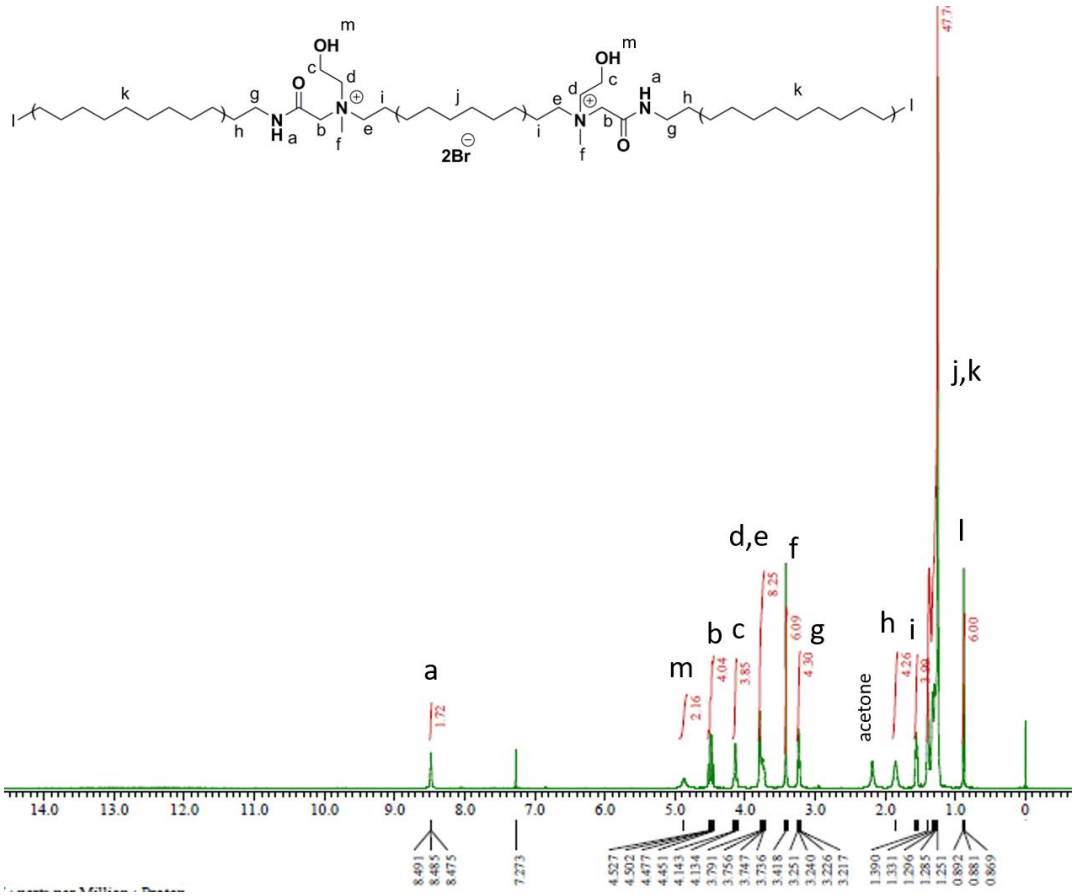

Figure S46. ^{1}HNMR of **SAM-16**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

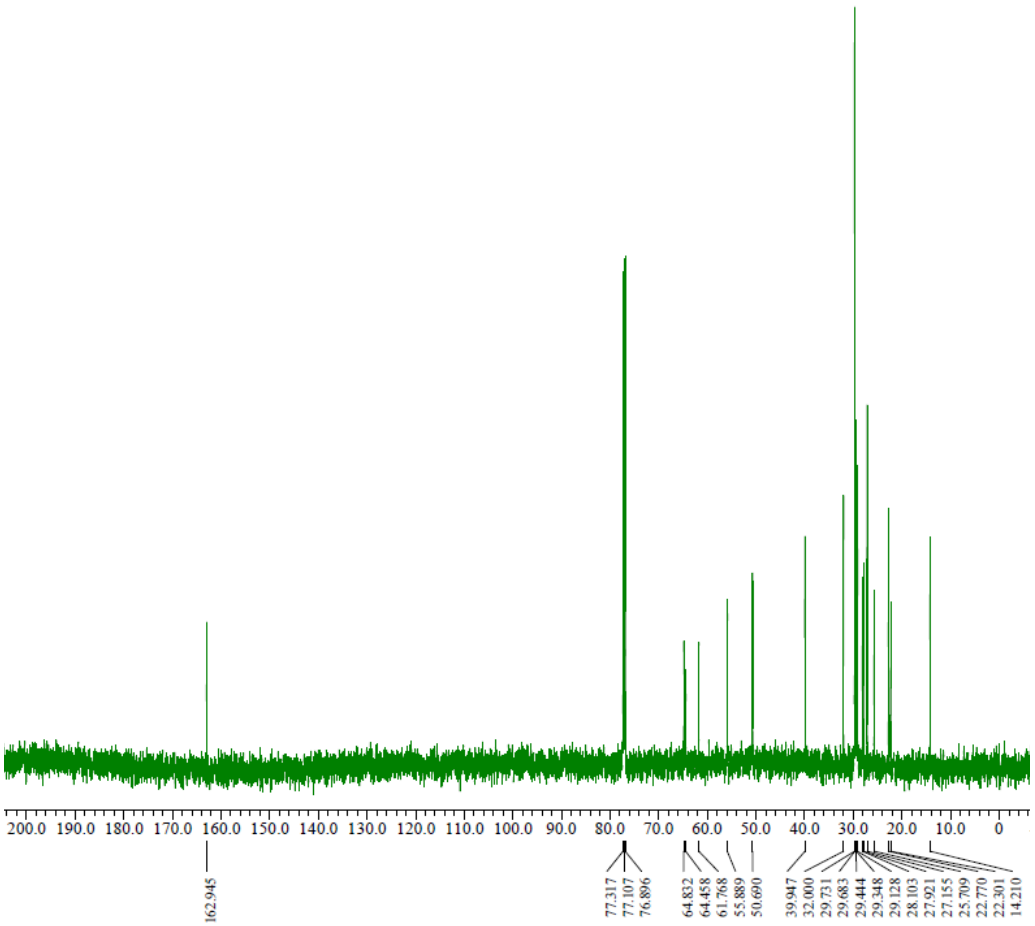

Figure S47. ^{13}CNMR of **SAM-16**. NMR spectrum was recorded in $CDCl_3$ at 600MHz

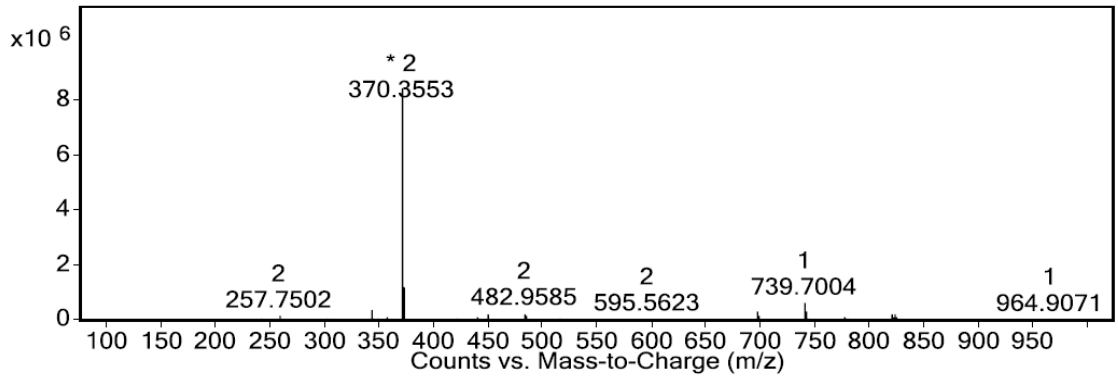

Figure S48. HRMS spectra of **SAM-16**.

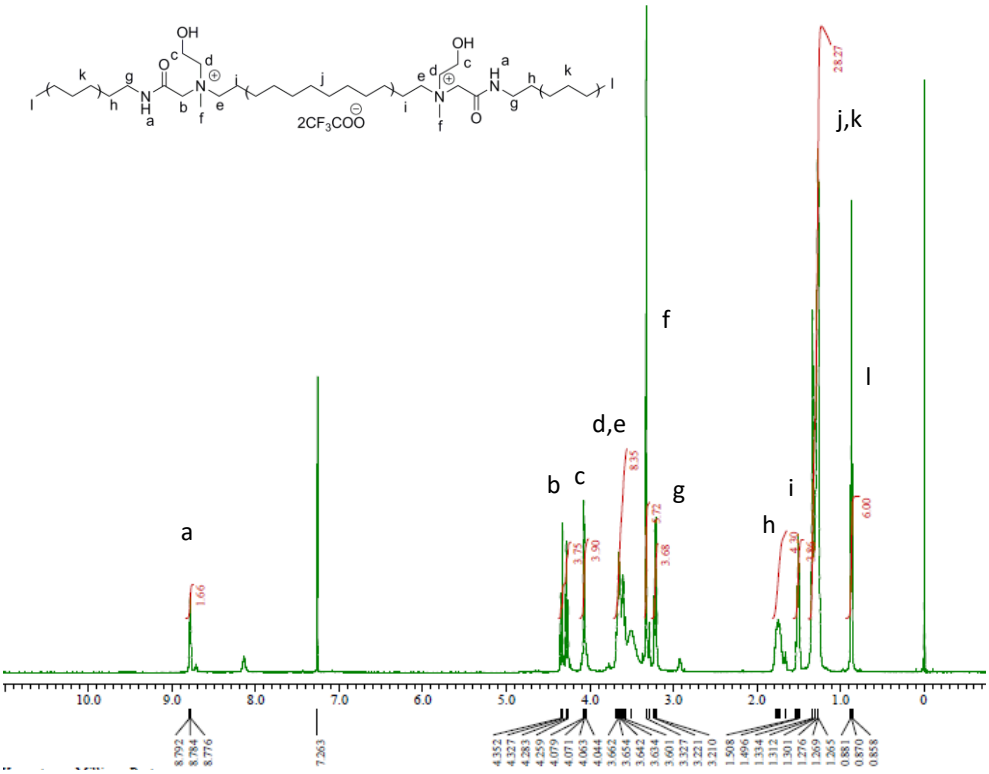

Figure S49. ^{1}HNMR of **SAM-17**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

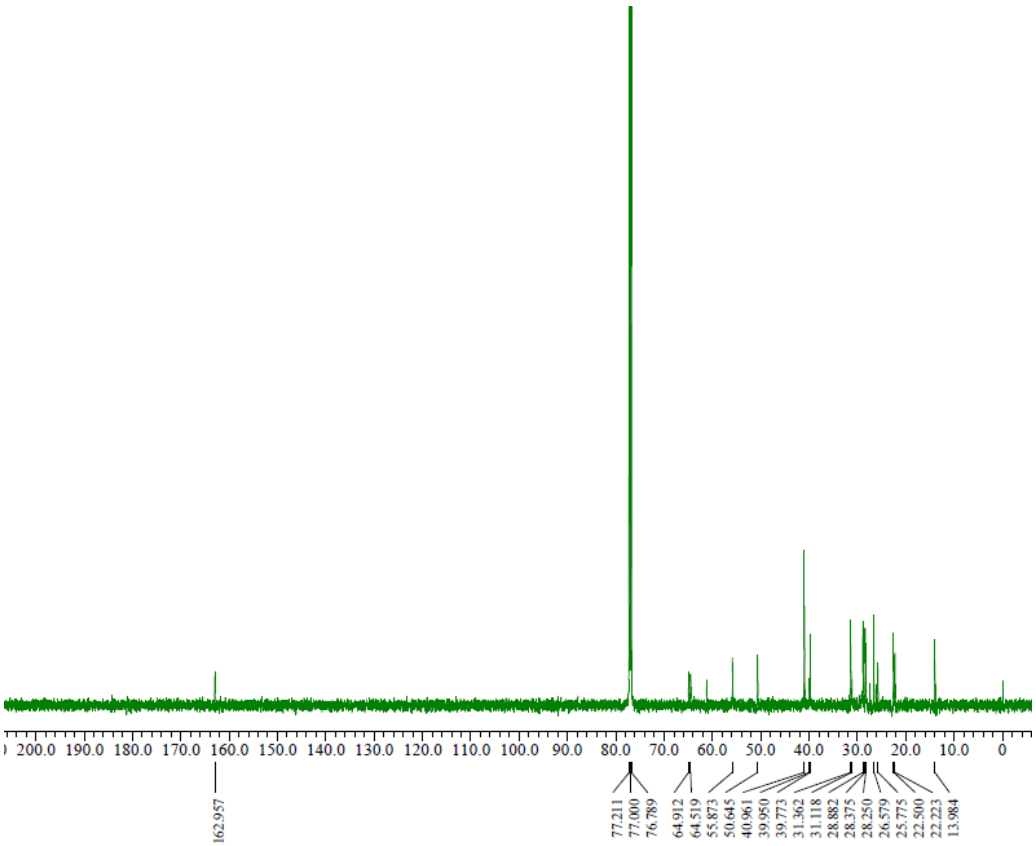

Figure S50. ^{13}CNMR of **SAM-17.** NMR spectrum was recorded in $CDCl_3$ at 600MHz.

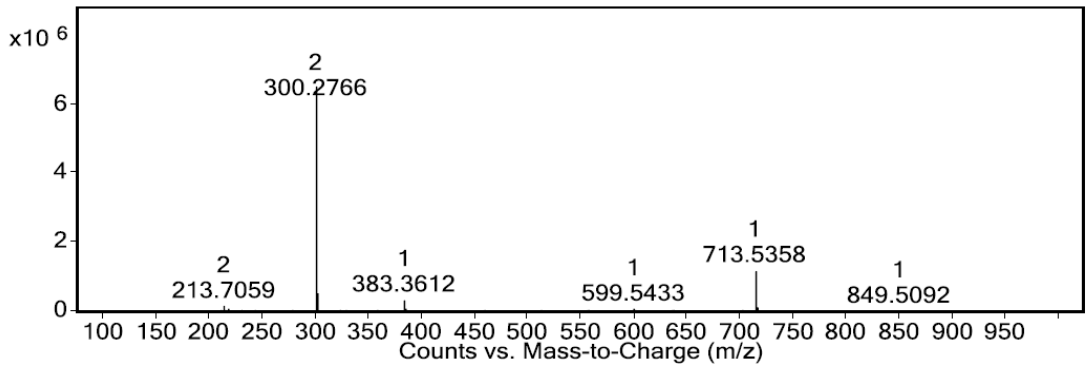

Figure S51. HRMS spectra of **SAM-17**.

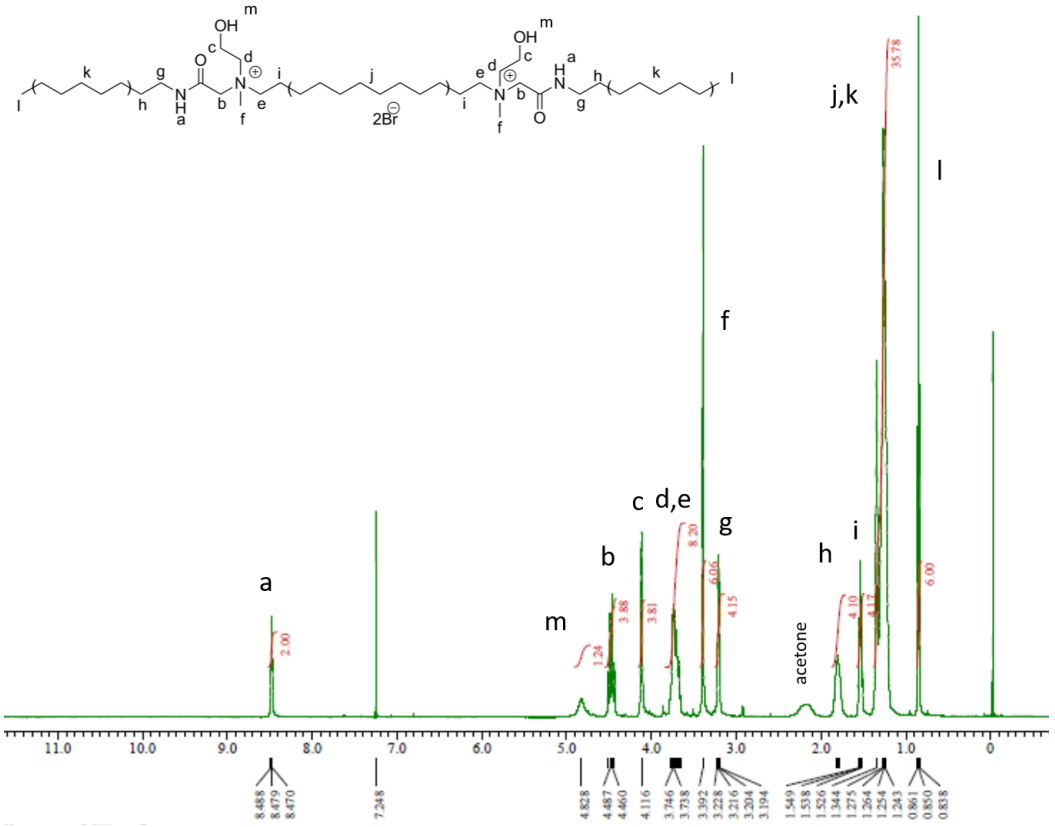

Figure S52. ^{1}HNMR of **SAM-18**. NMR spectrum was recorded in $CDCl_3$ at 600 MHz.

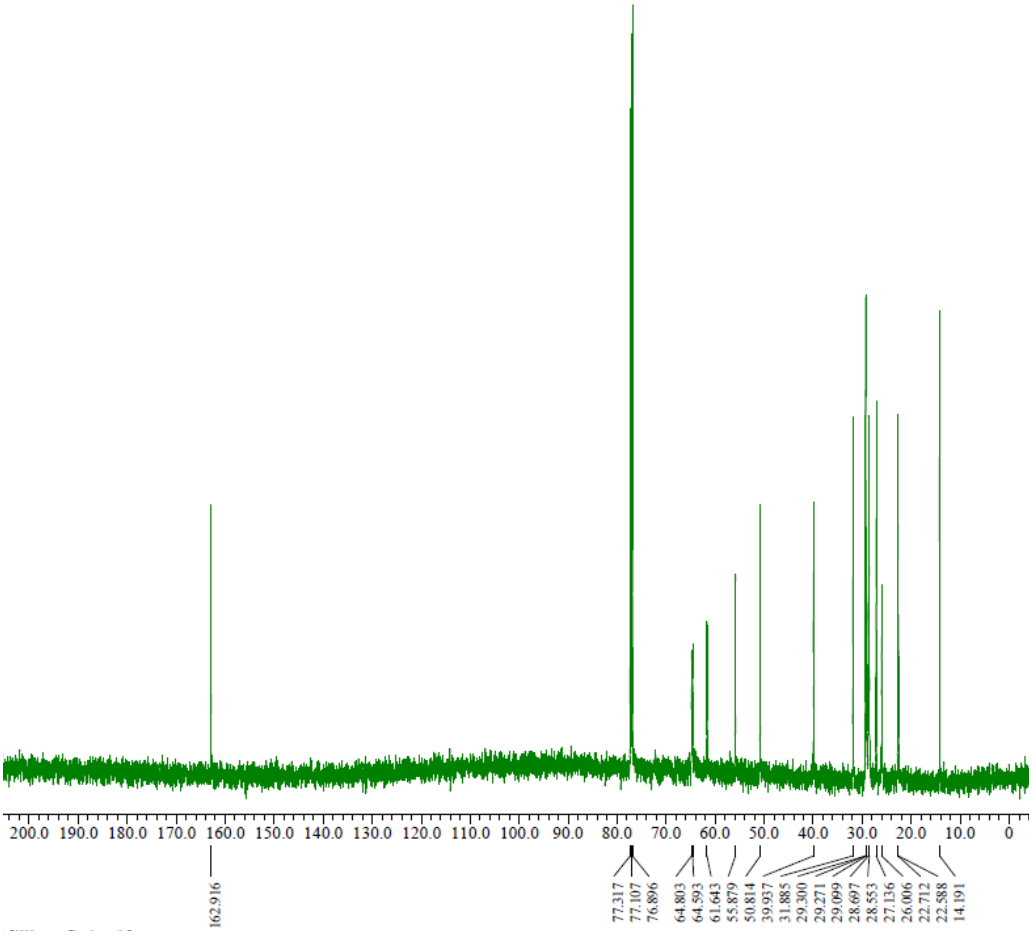

Figure S53. ^{13}CNMR of **SAM-18**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

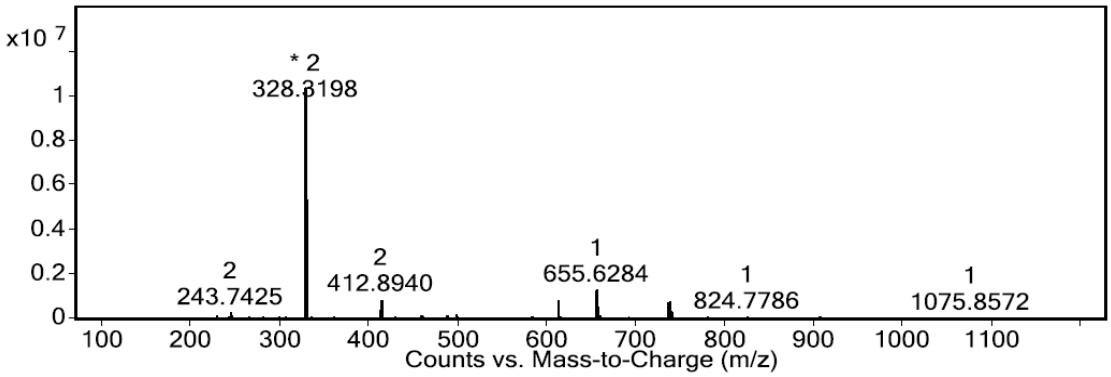

Figure S54. HRMS spectra of **SAM-18**.

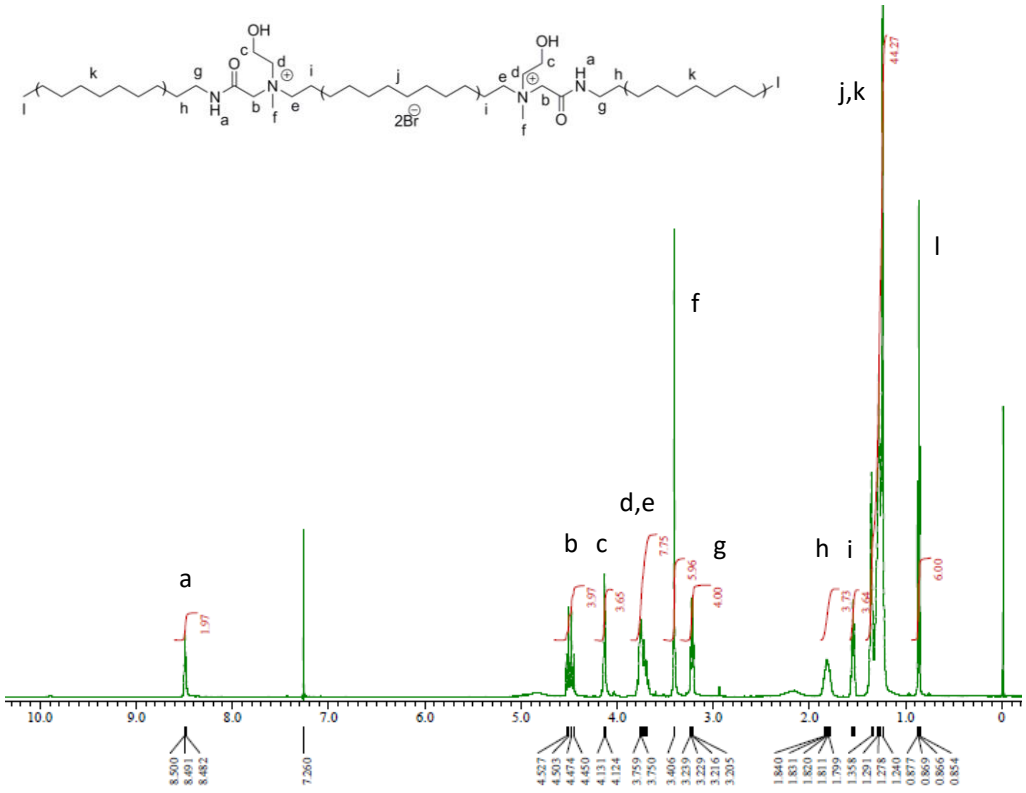

Figure S55. [1]HNMR of **SAM-19**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

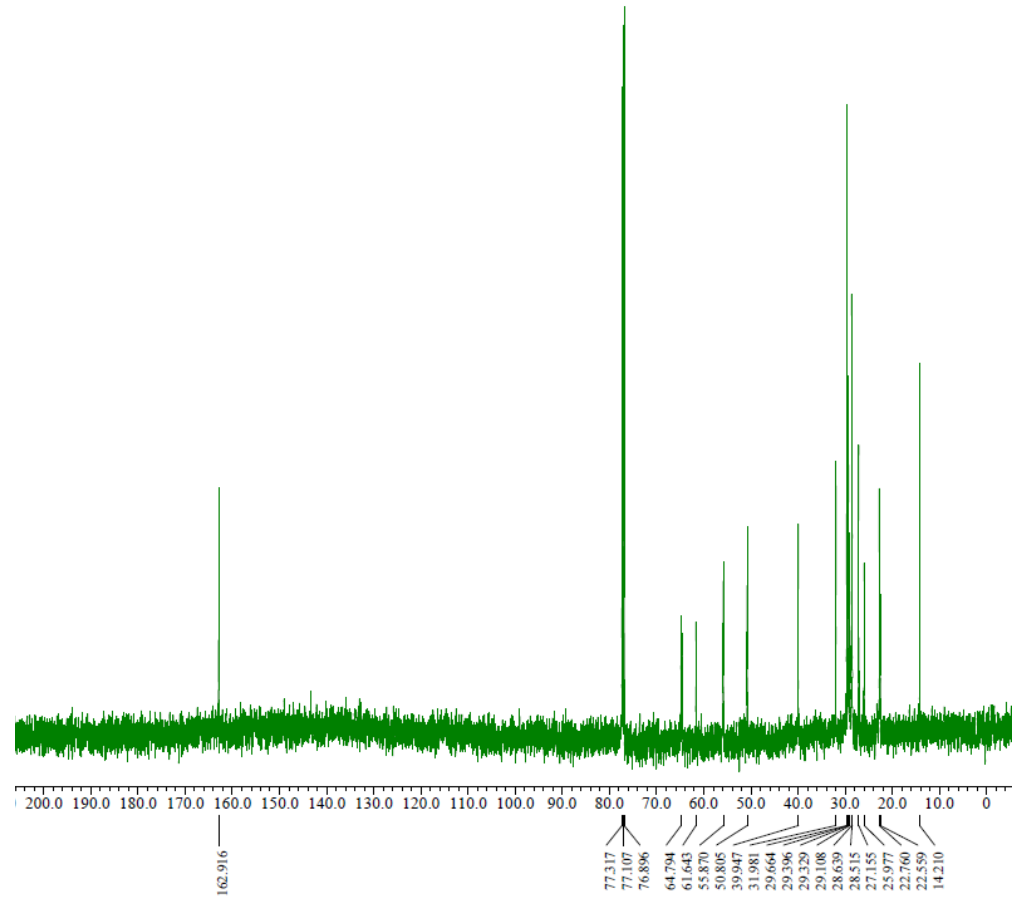

Figure S56. [13]CNMR of **SAM-19**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

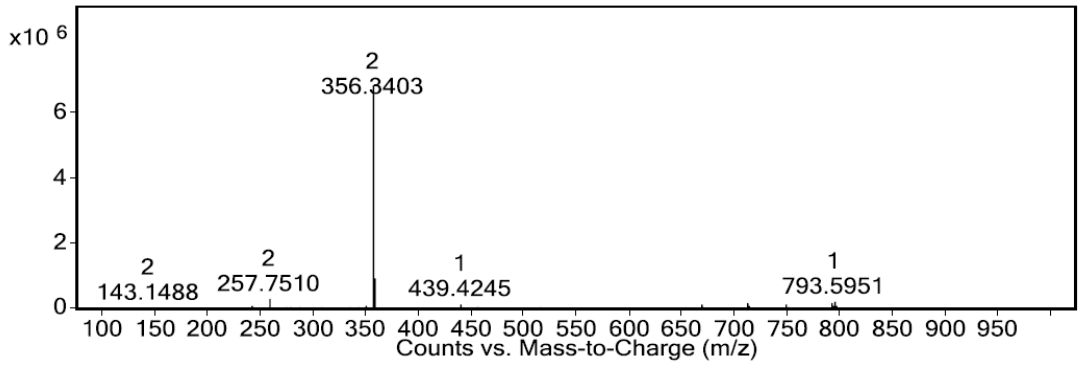

Figure S57. HRMS spectra of **SAM-19**.

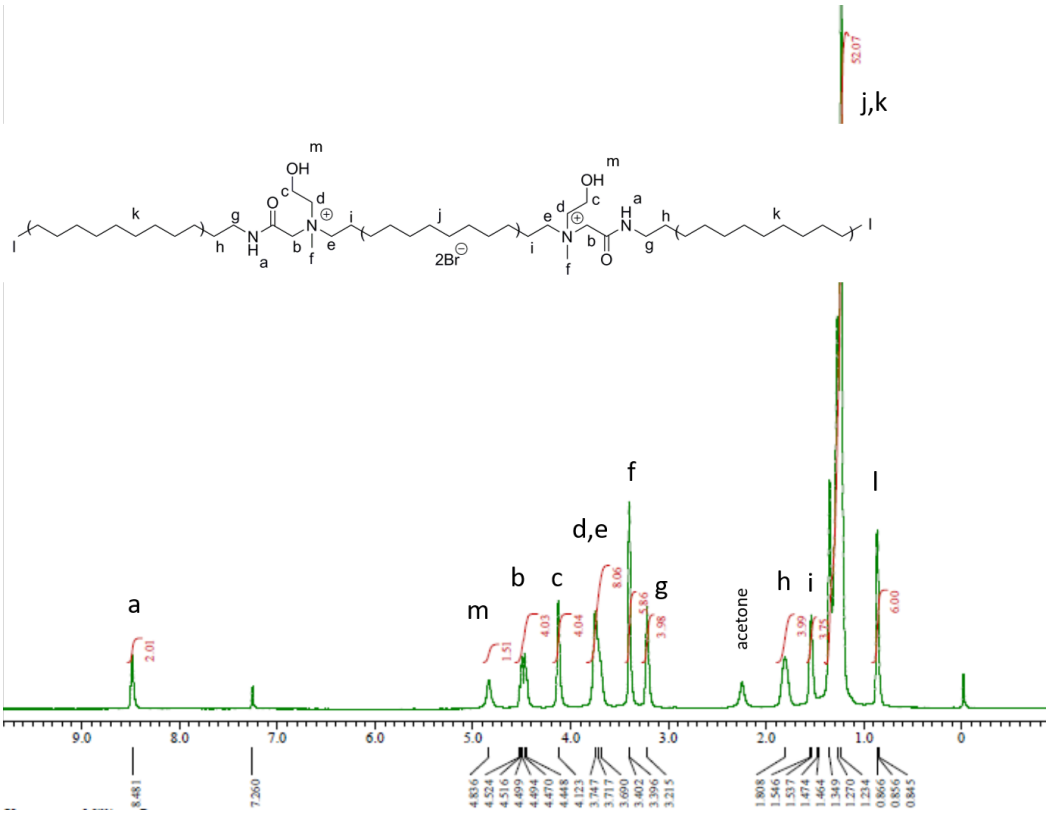

Figure S58. ^{1}HNMR of **SAM-20**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

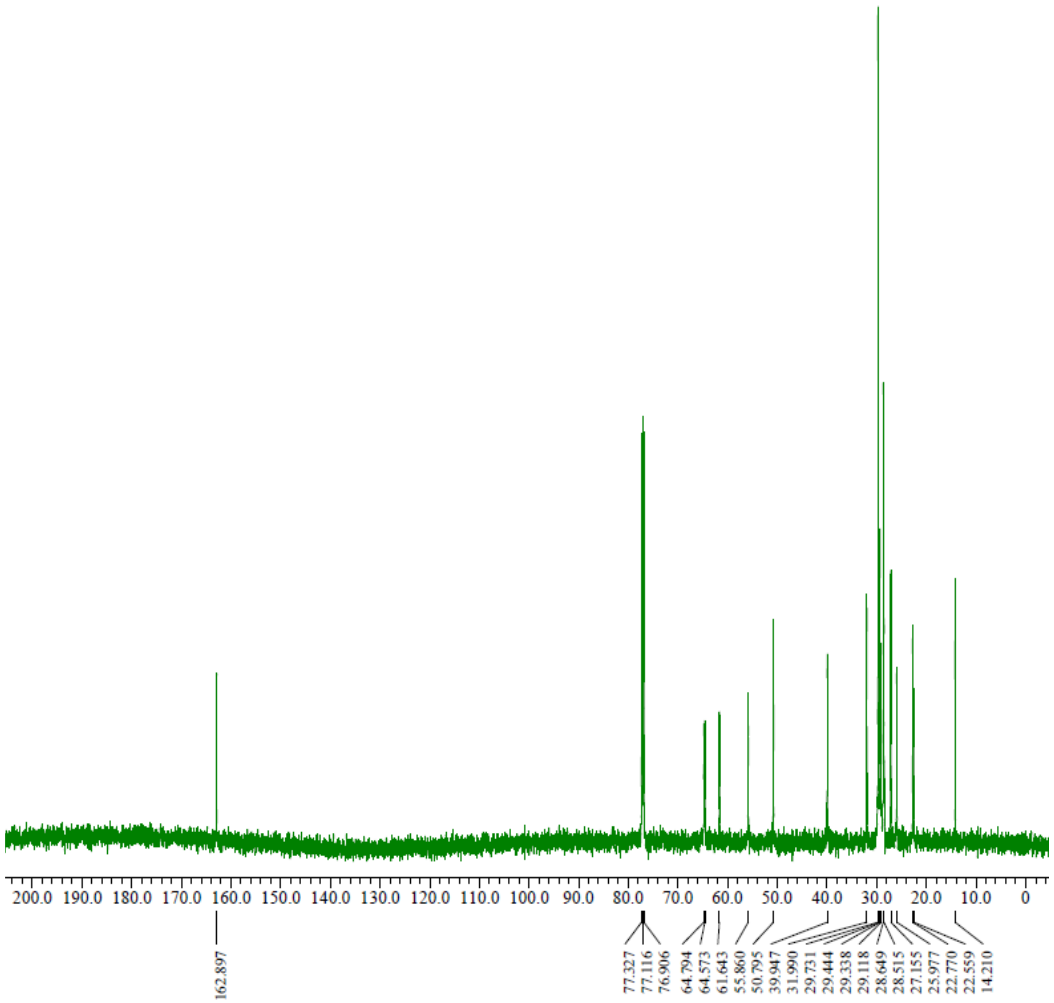

Figure S59. ^{13}CNMR of **SAM-20**. NMR spectrum was recorded in $CDCl_3$ at 600MHz.

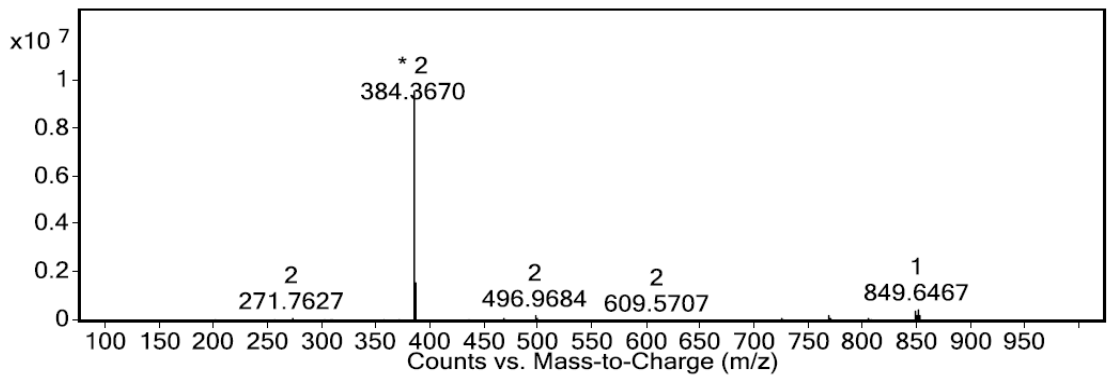

Figure S60. HRMS spectra of **SAM-20**.

Antibacterial activity[1,2]

A previously reported protocol was followed to perform the antibacterial assay. Initially, all bacterial strains were preserved at -80°C in nutrient broth (NB) supplemented with 15% (v/v) glycerol. 5µL of this bacterial stock was grown for 24 h on NB agar plate and MacConkey agar plate for Gram-positive and Gram-negative bacteria respectively by incubating at 37° C. One bacterial colony was then allowed to grow in nutrient broth medium for 6 hours (mid-log phase) at 37° C before starting the experiments. Following the drop plating method, the mid-log phase culture was found to give 10^8 CFU/mL counting for all bacteria. This grown culture was then diluted to 10^5 CFU/mL in Muller Hinton Broth (MHB) medium. Then, all compounds were serially diluted by two-fold in 96 well plate in MHB medium. After that, 180µL of bacterial suspension was added to the 96 well plate containing 20µL of compound solution. The plates were then kept for incubation for 24h at 37°C. The OD was recorded at 600 nm using TECAN (Infinite series, M200 pro) plate reader. Each concentration was maintained to have triplicate values and the entire experiment was performed twice. The final minimum inhibitory concentration (MIC) value was in the range of triplicate OD values for each of the concentration.

Hemolytic assay[1,2]

Briefly, all the compounds were serially diluted in sterile milipore water by two fold in 96 well plate. Then, blood was taken from a healthy donor. Human erythrocytes were then harvested from the heparinized blood, washed thrice with 1×PBS and finally resuspended to 5 vol % in 1×PBS. 150 µL of this suspension was then added in the wells of a 96-well plate containing 50 µL solutions of compounds at various concentration. As a negative control, only 50 µL water without any compound was taken and 50 µL 0.1 vol % solution of Triton-X was used as a positive control. The plates were then incubated at 37 °C for 1 h. Afterwards, the plates were centrifuged at 3500 rpm for 5 min and 100 µL of supernatant from each well was transferred to a new 96-well plate to record the absorbance at 540 nm wavelegth. The percentage of hemolysis was calculated as $[(A-A_o)/(A_t-A_o)]\times100$, where A indicates the absorbance for the test samples, A_o is the absorbance for the wells containing only water and erthrocytes suspension, and A_t is the absorbance of TritonX containing cells, all at 540 nm.

Cytotoxicity

Cytotoxicity of compounds was determined against two different cell lines Madin-Darby Canine Kidney Epithelial Cells (MDCK) and adenocarcinomic human alveolar basal epithelial cells (A549) cells by using Alamer Blue assay. All cells were firstly seeded onto the wells of a tissue culture treated 96-well plate (~10^4 cells/well) in DMEM media (supplemented with 10% fetal bovine serum and 5% penicillin-streptomycin). Then the tested compounds were serially diluted by 2-fold in 96 well plate. The media from the plate containing the cells were discarded and each concentration of compounds was then added to the respective wells. Without any compound nontreated cells was taken as a positive control and the cells treated with 0.1% (v/v) Triton-X solution was taken as a negative control. The plates were then incubated at 37 °C for 24h under 5% CO_2 atmosphere. Next, 10 µL of 10X solution of Alamar blue was added to each well. It was then kept for further incubation for 4 h at 37 °C under 5% CO_2 atmosphere. Afterwards, the absorbance was recorded at 570 nm wavelength and 600 nm wavelength was used as the reference. The percentage of cell viability was calculated using the following equation: cell viability (%) = $(A_c - A_t)/(A_0 - A_t) \times 100$, where A_c indicates the absorbance for cells treated with compound, A_t is the absorbance for the cells treated with 0.1% (v/v) Triton-X and A_0 is the absorbance of the untreated cells, all at 570 nm. Each concentration had triplicate values, and the average of triplicate absorbance values was plotted against concentration followed by fitting with a sigmoidal plot. From the curve the values were determined corresponding to 50% cell viability.

MDCK cells were seeded onto the wells of a tissue culture treated 96-well plate (~10^4 cells/well). The cells were then treated with **SAM-17** at 32 µg/mL. As a positive control 0.1 vol % Triton-X was used and untreated cells were used for negative control. All plates were then incubated at 37 °C for 24h under 5% CO_2 atmosphere. Next, both the treated and untreated cells were stained with calcein AM (2 µM, Fluka) and propidium iodide (PI, 4.5 µM) (Sigma-Aldrich) at 37 °C for 15 min under 5% CO_2 atmosphere. The cells were then washed with PBS and Live/Dead images of cells were captured with a 50× objective in a Leica DM2500 fluorescence microscope. For imaging, the band-pass filter for calcein AM was at 500−550 nm wavelength and a long-pass filter for PI was at 590−800 nm.

Bactericidal time-kill kinetics[1,2,3]

The bactericidal activity was determined for the compounds by carrying out the time kill kinetics assay against MRSA ATCC33591 and MRSA R3545 strain. The 6h grown mid-log phase culture of these bacteria were diluted to 10^5 CFU/mL in MHB media. Bacterial suspensions were then incubated at 37°C with the optimized compound by maintaining the working concentration at 32µL, 16µL, 8µL and 4µL. As a negative control, 0.9% saline (same volume) was used instead of the compound. A volume of 20µL aliquot from that solution for the time interval corresponding 1, 2, 4, 6, 8 and 12 hours were serially diluted by ten-fold in 0.9% saline. 20µL from each dilution at different time interval were plated on the NB agar plates and incubated for 24 h at 37°C. The bacterial colonies were counted and the result was shown in logarithmic scale.

Antibacterial activity against stationary phase MRSA[1]

For the stationary phase culture, 3µL of 6h grown mid-log phase bacteria were added to 3 mL of nutrient broth and allowed to grow for 16 h at 37°C. Then, this stationary phase culture was diluted to ~10^6 CFU/mL in 1×PBS and 180 µL of these bacterial suspensions were added to 96 well plate containing 20 µL of various concentrations of **SAM-17** and vancomycin in sterile milipore water. Sterile milpore water of same volume was used as negative control instead of compound. The plate was then incubated for 6h at 37°C. Afterwards, 20 µL of the treated bacterial suspensions from each concentration were serially diluted by 10-fold in 1×PBS and 20 µL from each dilution were spot-plated on NB agar plate. The plates were incubated at 37°C for 24 h. The bacterial colonies were then counted visually. The results are expressed in logarithmic scale by taking the average of two individual experiment. The detection limit for this experiment is 50 CFU/mL.

Biofilm disruption assay[1,4]

The 6 h grown mid log phase culture of MRSA ATCC33591 and MRSA R3545 (~10^8 CFU/mL) were diluted to 10^5 CFU/mL in nutrient broth medium supplemented with 1% glucose and 1% NaCl. A sterile 18mm glass coverslip was kept in each of the wells of 6 well plates and 2 mL of the bacterial suspension was added on each coverslip. The plates were then kept for incubation at 37°C for 24h in static condition. After the formation of preformed biofilms on

coverslip, these were washed with 1×PBS carefully, transferred to a new 6 well plate followed by treatment with 2 mL of the compounds diluted at various concentration in biofilm medium and incubated at 37°C for 24h in static condition. As a control experiment, the same volume of the respective medium was used instead of the compounds. After 24 h, the compound treated coverslips were washed with 1× PBS carefully and placed into another 6 well plate. To this, 2 mL of trypsin-EDTA solution diluted in saline (1:4) was added and was allowed for incubation for 15 min. The suspension of cells was then serially diluted by 10 fold in 96 well plate. 20 µL of this concentration was plated on NB agar plate and incubated for 24h at 37°C. Finally, the plates were counted and the cell viability was determined comparing with the untreated control.

In order to quantify the reduction of biomass, firstly, the biofilm grown cover slips were carefully washed with 1×PBS and dried under air for 10-15 min. These coverslips were then stained with 2mL of crystal-violet solution (0.1% w/v) for 5-10 min. After that, the stained biofilms were again washed with 1×PBS and these CV-stained biofilms coverslips were then scratched carefully with 2 mL of 95% ethanol in water. By taking 100 µL of this CV stained biofilm solution, the O.D. (at 522 nm) was recorded by using plate reader. These experiments were performed twice and the average data with standard deviation were presented in the figure.

Membrane active mechanism of action

Membrane permeabilization assay[2,3,5]

MRSA ATCC33591 and MRSA R3545 bacteria were harvested from 6h grown mid-log phase culture (~10^8 CFU/mL) by centrifugation at 3500 rpm for 5 min. It was then washed with 5 mM HEPES buffer followed by resuspension in 1:1 mixture of 5 mM HEPES buffer and 5 mM glucose solution. Next, 30 µL of 1 mM propidium iodide (PI) was mixed with 3 mL of bacterial suspension to get a final concentration of 10 µM of dye. Afterwards, 190 µL of these bacterial suspensions was placed in each well of black and clear bottom 96-well plate and fluorescence intensity was measured for 4 minutes at 617 nm emission wavelength by exciting at a wavelength of 535 nm. Then 10 µL of compounds at various concentration (diluted in sterile milipore water) was mixed with this suspension and fluorescence intensity was determined upto 28 minutes. Same volume of milipore water without any compound

was used as a negative control. Average value of triplicate data for this experiment was presented in the figure.

Cytoplasmic membrane depolarization assay[1]

6h grown mid-log phase culture of MRSA ATCC33591 and MRSA R3545 (~10^8 CFU/mL) were centrifuged at 3500 rpm for 5 min, followed by washing with 5 mM HEPES buffer (pH=7.4) and resuspended in 1:1:1 ratio of 5 mM HEPES buffer, 5 mM glucose and 100 mM KCl solution. Then, 3 µL of 2 mM DiSC3 (5) (3,3'-Dipropylthiadicarbocyanine iodide) dye was added to 3 mL of bacterial suspension to have a final concentration of 2 µM. This dye containing bacterial suspensions were then incubated in darkness for 30 minutes. The volume of 190 µL of these suspensions was then placed into black and clear bottom 96-well plate and the fluorescence intensity was monitored for 4 min. The emission intensity at 670 nm was recorded by exciting at a wavelength of 622 nm. Afterwards, 10 µL of compounds at various concentrations was added to the dye mixed bacterial suspension and fluorescence intensity of the treated wells were monitored up to 28 minutes. As a negative control, 10 µL of milipore water was added instead of compound. These experiments were performed in triplicates and the average data were presented in the figure.

Live/dead assay of bacteria[4]

Briefly, 1 mL of mid-log phase bacterial culture (~10^8 CFU/mL) was centrifuged at 3500 rpm for 5 minutes and resuspended using 1mL of 0.9% saline. **SAM-17** was added to the same bacterial suspension with a final concentration of 32 µg/mL and incubated for 2h at 37°C. Next, the compound containing bacterial suspension was subjected to centrifugation followed by resuspension in 0.9% saline. Afterwards, 20 µL of a fluorescent probe mixture containing 3 µM of SYTO9 (Invitrogen, USA) and 15 µM of PI (Sigma-Aldrich, USA) was added to the previous bacterial suspension. The mixture was then allowed to incubate in darkness for 15 min. Subsequently, the unbound dyes were removed through centrifugation and it was further resuspended in saline. Finally, 5 µL of this solution was used for fluorescence microscopy. The excitation wavelengths were at 450-490 nM and 515-560 nM for SYTO9 and PI respectively. Fluorescence emission was monitored using a band gap filter at 450-490 nM for SYTO9 and at 515-560 nM for PI. All images were captured by using 100× objective with a Leica DM 2500 fluorescence microscope.

Antibacterial activity upon incubation with mammalian fluid and mice liver homogenate[5-7]

Activity of **SAM-17** against MRSA ATCC33591 was determined after individual incubation with 50% plasma, 50% serum and 50% mice liver homogenate. For this, firstly, 100 µL of compound was separately mixed with 100 µL of human blood plasma, serum and liver homogenate, followed by incubation for 3 hours at 37°C. For control MIC was also determined after incubation of compound in saline and 1X PBS. Afterwards, these mixtures were serially diluted by 2 folds in 0.9% saline for plasma and serum and in 1X PBS (phosphate buffer saline) for mice liver homogenate. Next 180 µL of bacterial suspension in MHB media (~10^5 CFU/mL) was mixed with 20 µL of compound at different concentrations and incubated for 24 hours at 37°C. Next, the MIC was determined by following the previously described protocol.

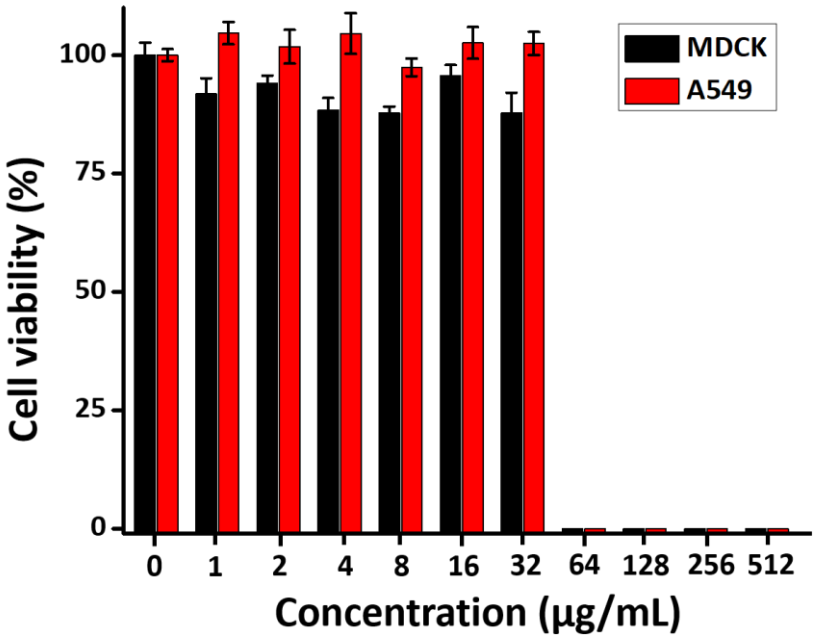

Figure S61. Cell viability of MDCK and A549 cells against **SAM-6** at different concentrations through Alamar blue assay.

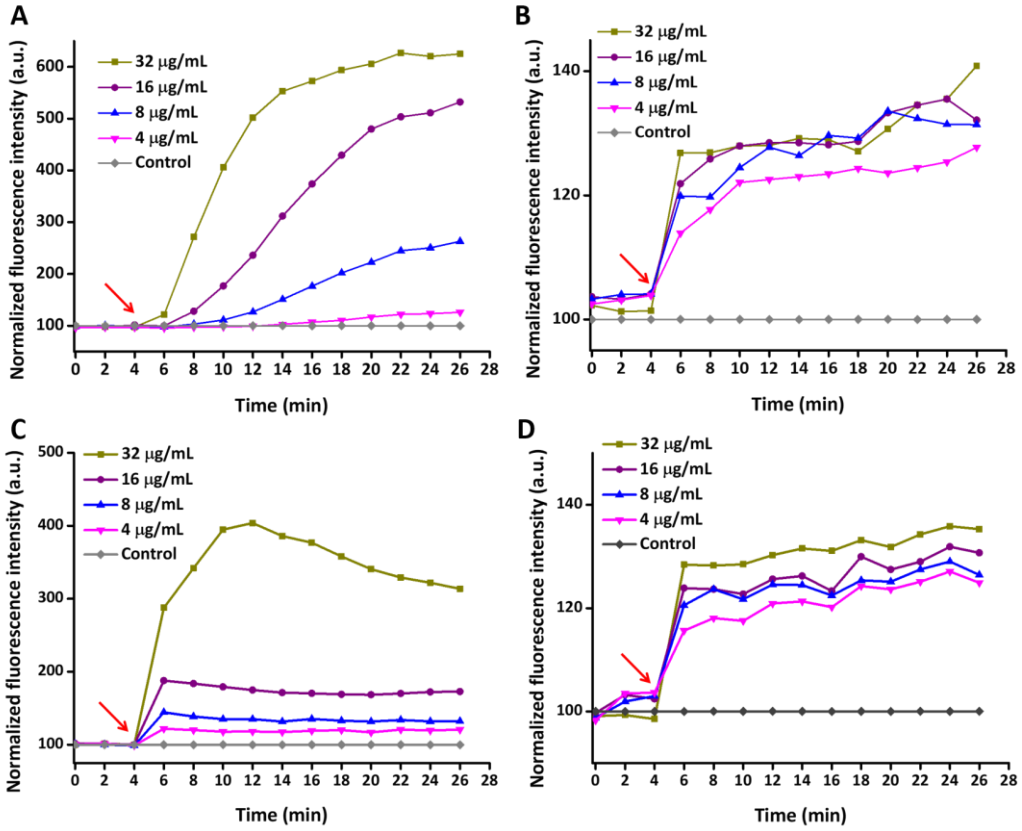

Figure S62. Membrane active mechanism of action. Membrane permeabilization of MRSA ATCC33591 by A) **SAM-6** and B) **SAM-17**. Membrane depolarization of MRSA ATCC33591 by C) **SAM-6** and D) **SAM-17**. Red arrow indicates compound addition.

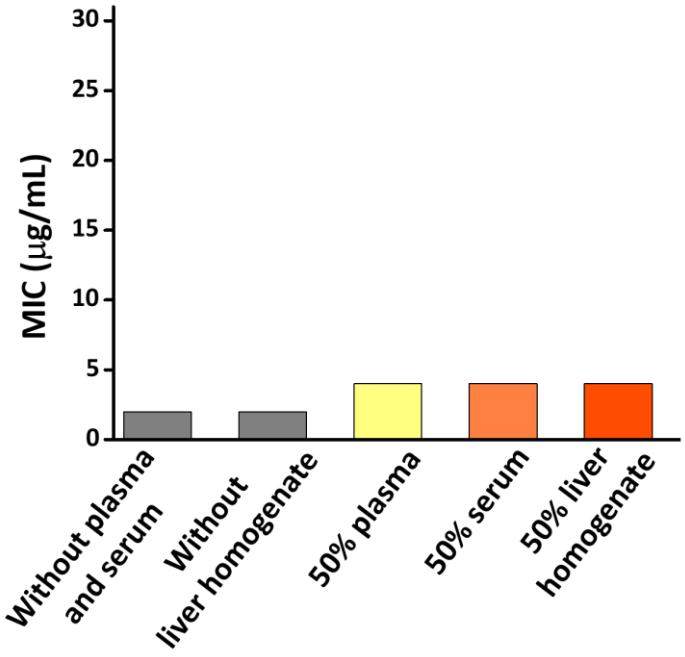

Figure S63. Antibacterial activity of **SAM-17** against MRSA ATCC33591 after 3h incubation with plasma, serum and mice liver homogenate.

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