Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Cyclization and Unsaturation rather than Isomerization of Side Chains Governs the Selective Antibacterial Activity of Cationic-amphiphilic Polymers

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Experimental Methods

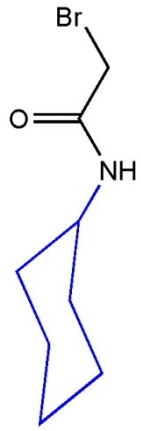
Materials

All the solvents were of reagent grade and dried prior to use wherever required. Poly(isobutylene-*alt*-maleic anhydride) (Mw ~ 6000 Da, Catalog no. 531278) purchased from Sigma-Aldrich and used as received. All other reagents were procured from the commercial suppliers and used as received. Culture media and the antibiotics were from HIMEDIA (India) and Sigma-Aldrich respectively. NMR spectra were recorded using Bruker AMX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer. The chemical shifts (δ) are reported in parts per million downfield from the peak for the internal standard TMS for ^{1}H-NMR. Infrared (IR) spectra of the solid compounds were recorded on Bruker IFS66 V/s spectrometer using KBr pellets. IR spectra of the compounds soluble in low-boiling solvents were recorded with the same instrument using NaCl crystal. Mass spectra were recorded on a Micromass Q-ToF micromass spectrometer. Optical density and absorbance were measured by Tecan InfinitePro series M200 Microplate Reader. Bacterial strains *S. aureus* (MTCC 737) and *E. coli* (MTCC 443 equivalent to ATCC 25922) were purchased from MTCC (Chandigarh, India). MRSA (ATCC 33591), vancomycin resistant *E. faecium* (VRE) ((OrlaJensen) Schleifer and Kilpper-Balz, ATCC 51559) were obtained from ATCC (Rockville, Md). *E. coli* was cultured in Luria Bertani broth (10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl in 1000 mL of sterile distilled water while*S. aureus* and MRSA were grown in nutrient broth (1 g of beef extract, 2 g of yeast extract, 5 g of peptone and 5 g of NaCl in 1000 mL of sterile distilled water). VRE was cultured in Brain Heart Infusion broth (BHI). For solid media, 5% agar was used along with above mentioned composition. The bacterial samples were freeze dried and stored at -80 °C. 5 µL of these stocks

were added to 3 mL of the nutrient broth and the culture was grown for 6 h at 37 °C prior to the experiments.

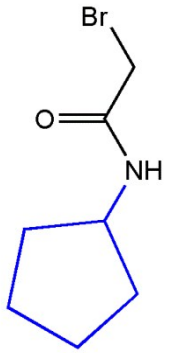
Synthesis of alkylating agents

***N-alkyl-1-bromoethanamide*:** Alkyl amine (118 mmol) was dissolved in dichloromethane (55 mL). Potassium carbonate, K_2CO_3 (24.55 g, 178 mmol) was dissolved in 60 mL of distilled water and the solution was added to the organic solution. The resulting two phase solution was cooled to 4 °C. A solution of bromoacetyl bromide (35.85 g, 178 mmol) in dichloromethane (55 mL) was carefully added drop wise to the cooled solution while maintaining the temperature at 4 °C for about 30 min. Then the reaction mixture was stirred at room temperature for 12 h. The aqueous solution was separated and washed with dichloromethane (2 × 25 mL). The organic solution was washed with water (2 × 50 mL) and passed over the anhydrous Na_2SO_4 and concentrated to yield a white solid quantitatively (100 % yield). All the compounds were characterized by AT-FTIR, ^{1}H NMR and mass spectrometry.

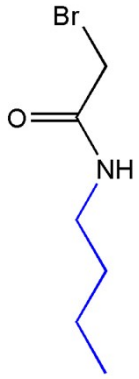


***N-(cyclohexyl)-1-bromoethanamide*:** FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 (C-H str.), 1680 cm^{-1} (Amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm 1.195 (m, cyCH_2, 2H), 1.383 (m,

cyCH_2, 2H), 1.621 (m, cyCH_2, 2H), 1.721 (m, cyCH_2, 2H), 1.917 (m, cyCH_2, 2H), 3.761 (m, cyCH, 1H), 3.861 (s, -COCH_2Br, 2H), 6.475 (br s, amide –NHCO, 1H)); HR-MS: *m/z* 220.03 (observed): 220.03 (calculated for $[M + H]^+$).

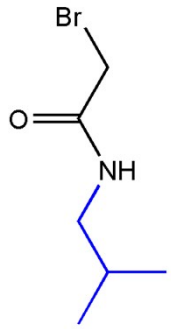


***N-(cyclopentyl)-1-bromoethanamide*:** FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1680 cm^{-1} (Amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm 1.428 (m, cyCH_2, 2H), 1.657 (m, cy$(CH_2)_2$, 4H), 2.013 (m, cyCH_2, 2H), 3.856 (s, -COCH_2Br, 2H), 4.19 (m, cyCH, 1H), 6.385 (br s, amide –NHCO, 1H)); HR-MS: *m/z* 206.01 (observed): 206.01 (calculated for $[M + H]^+$).

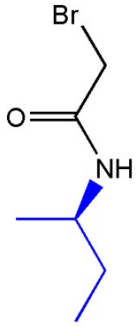


***N-(n-butyl)-1-bromoethanamide*:** FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1680 cm^{-1} (Amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm 0.878 (t, terminal –CH_3, 3H),

1.543 (m, CONHCH$_2$*CH$_2$ CH$_2$CH$_3$*, 4H), 3.278 (t, -CONH*CH$_2$*-, 2H), 3.881 (s, -CO*CH$_2$*Br, 2H), 6.475 (br s, amide –*NH*CO, 1H)); HR-MS: *m/z* 194.00 (observed): 194.01 (calculated for [M + H]$^+$).

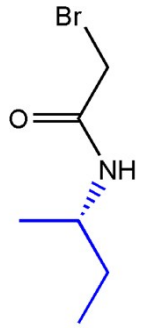


***N-(iso-butyl)-1-bromoethanamide*:** FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1680 cm^{-1} (Amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm 0.929 (d, terminal -*(CH$_3$)$_2$*, 6H), 1.813 (m, -CH$_2$*CH*(CH$_3$)$_2$, 1H), 3.113 (d, -*CH$_2$*CH(CH$_3$)$_2$, 2H), 3.887 (s, -CO*CH$_2$*Br, 2H), 6.475 (br s, amide –*NH*CO, 1H)); HR-MS: *m/z* 194.02 (observed): 194.01 (calculated for [M + H]$^+$).

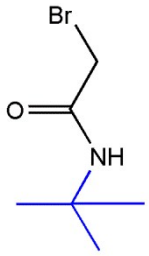


***N-((S)-(+)-sec-butyl)-1-bromoethanamide*:** FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1680 cm^{-1} (Amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm 0.921 (t, -CHCH$_2$*CH$_3$*, 3H), 1.162 (d, -CH$_2$CH*CH$_3$*, 3H), 1.511 (m, -CH*CH$_2$*CH$_3$, 2H), 3.905 (m, -*CH*CH$_2$CH$_3$, 1H),

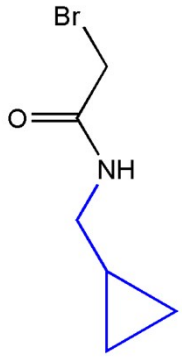
3.869 (s, -CO*CH*$_2$Br, 2H), 6.475 (br s, amide –*NH*CO, 1H)); HR-MS: *m/z* 194.01 (observed): 194.01 (calculated for $[M + H]^+$).



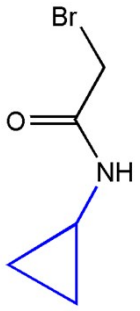
N-((R)-(-)-sec-butyl)-1-bromoethanamide*:** FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1680 cm^{-1} (Amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm 0.921 (t, -$CHCH_2$ CH_3***, 3H), 1.162 (d, -CH_2CH***CH_3***, 3H), 1.511 (m, -CH***CH_2***CH_3, 2H), 3.869 (s, -CO***CH_2***Br, 2H), 3.905 (m, -***CH***CH_2CH_3, 1H), 6.475 (br s, amide –*NH*CO, 1H)); HR-MS: *m/z* 194.01 (observed): 194.01 (calculated for $[M + H]^+$).



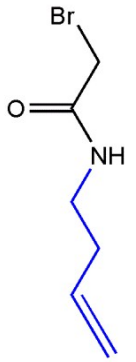
N-(t-butyl)-1-bromoethanamide*:** FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1680 cm^{-1} (Amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm 1.3 (s, terminal -C$(CH_3)_3$***, 9H), 3.845 (s, -CO***CH_2***Br, 2H), 6.475 (br s, amide –*NH*CO, 1H)); HR-MS: *m/z* 194.02 (observed): 194.01 (calculated for $[M + H]^+$).



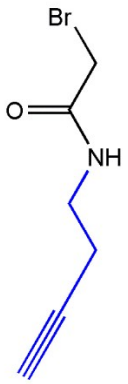
***N-(methylcyclopropyl)-1-bromoethanamide*:** FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1680 cm^{-1} (Amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm 0.236 (m, cyCH_2, 2H), 0.547 (m, cyCH_2, 2H), 0.986 (m, cyCH, 1H), 3.155 (dd, -CH_2CH$(CH_2)_2$, 2H), 3.891 (s, -COCH_2Br, 2H), 6.576 (br s, amide –NHCO, 1H)); HR-MS: *m/z* 192.00 (observed): 191.99 (calculated for $[M + H]^+$).



***N-(cyclopropyl)-1-bromoethanamide*:** FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1680 cm^{-1} (Amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm 0.565 (m, cyCH_2, 2H), 0.822 (m, cyCH_2, 2H), 2.734 (m, cyCH, 1H), 3.8 (s, -COCH_2Br, 2H), 6.475 (br s, amide –NHCO, 1H)); HR-MS: *m/z* 177.98 (observed): 177.98 (calculated for $[M + H]^+$).



***N-(but-3-enyl)-1-bromoethanamide*:** FT-IR: 3250 cm^{-1} (amide N-H str.), 3049 cm^{-1} (C-H str.), 1680 cm^{-1} (Amide I, C=O str.), 1642 cm^{-1} (C=C str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm 2.3 (m, -CONHCH$_2$*CH$_2$*-, 2H), 3.2 (t, -CONH*CH$_2$*CH$_2$-, 2H), 3.8 (s, -CO*CH$_2$*Br, 2H), 5.138 (dq, CH$_2$CH$_2$CH=*CH$_2$*, J_{cis} = 10.52 Hz, 1H), 5.192 (dq, CH$_2$CH$_2$CH=*CH$_2$*, J_{trans} = 17.27 Hz, 1H), 5.85 (m, CH$_2$CH$_2$*CH*=CH$_2$, 1H), 6.475 (br s, amide –*NH*CO, 1H) ; HR-MS: *m/z* 190.98 (observed); 190.99 (calculated for $[M + H]^+$).



***N-(but-3-ynyl)-1-bromoethanamide*:** FT-IR: 3250 cm^{-1} (amide N-H str.), 3310 cm^{-1} (≡C-H str.), 2857-2942 cm^{-1} (-C-H str.), 2119 cm^{-1} (C≡C str.), 1680 cm^{-1} (Amide I, C=O str.), 1642 cm^{-1} (C=C str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, $CDCl_3$): δ/ppm 1.82 (t, -CH$_2$C≡*CH*, 1H), 2.29 (m, -CONHCH$_2$*CH$_2$*-,

2H), 3.37 (t, -CONH*CH*$_2$CH$_2$-, 2H), 3.8 (s, -CO*CH*$_2$Br, 2H), 6.475 (br s, amide –*NH*CO, 1H)); HR-MS: *m/z* 188.98 (observed); 188.97 (calculated for $[M + H]^+$).

Synthesis of Polymeric Derivatives

Poly(isobutylene-alt-N-(N',N'-dimethylaminopropyl)-maleimide) (PIBMI)

To a solution of 10 g of poly(isobutylene-*alt*-maleic anhydride) (PIBMA) (Avg. M_w = 6000 g/mol) in 60 mL of dimethyl formamide (DMF), 7.96 g of 3-aminopropyldimethylamine (1.2 equivalents with respect to the monomer weight of the polymer (154 g/mol)) was added and stirred at 120 °C for 48 h in a screw-top pressure tube. The reaction mixture was cooled, precipitated with 200 mL of distilled water and was centrifuged at 10,000 rpm for 15 min. The polymer was dried at 55 °C for 24 h under vacuum to give a pale yellow solid with 100% yield (complete conversion of the anhydride to imide was confirmed by complete disappearance of peaks at 1850 cm^{-1} (C=O asym. str.) and 1785 (C=O sym. str.) for the anhydride ring and appearance of peaks 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.) for the imide ring by FT-IR).

PIBMI: FT-IR: 2950-2850 (C-H str.), 1767 cm^{-1} (C=O asym. str.), 1696 cm^{-1} (C=O sym. str.), 1470-1410cm^{-1} (C-C str.), 1290-1110 (C-O str.); ^{1}HNMR (400 MHz, $CDCl_3$): δ/ppm 0.7–1.2 (br CH_2C*(CH$_3$)$_2$*, 6H), 1.7 (br *CH*$_2$C(CH$_3$)$_2$, 2H), 1.86 (br NCH$_2$*CH*$_2$CH$_2$N(CH$_3$)$_2$, 2H), 2.2-2.5 (br NCH$_2$CH$_2$*CH*$_2$N*(CH$_3$)$_2$*, 8H), 2.7–3.1 (br, *CHCH*, 2H), 3.6 (br N*CH*$_2$CH$_2$CH$_2$N(CH$_3$)$_2$, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 179.9, 179.7, 179.4, 177.4, 177.3, 177.2, 55.5, 45.9, 45.5, 44.1, 40.8, 40.6, 40.2, 40.0, 37.4, 26.2, 25.5, 24.8, 24.7, and 24.6.

Synthesis of quaternized polymers

To a solution of 0.5 g of PIBMI in 20 mL of dry DMF/dry $CHCl_3$ (1:1), 2 equivalents (with respect to the monomer weight of PIBMI) of *N*-alkyl-1-bromoethanamide was added and stirred at 75 °C for 96 h in a screw top pressure tube. The solution was cooled, precipitated with 40 mL of diethylether and filtered. The white solid was washed with diethylether (4 × 40 mL) and dried at 40 °C for 6 h under vacuum. All the polymeric derivatives were characterized by ^{1}H NMR and the data are provided in †ESI. The percentage of conversion given by the degree of quaternization was calculated from ^{1}H-NMR analysis as described in†ESI.

QCyHexAP: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.) 1680 cm^{-1} (amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, D_2O): δ/ppm 0.95–1.2 (br, $CH_2C\textit{(CH}_3\textit{)}_2$, 6H), 1.19 (br, cy$\textit{CH}_2$, 2H), 1.38 (br, cy$\textit{CH}_2$, 2H), 1.62 (br, cy$\textit{CH}_2$, 2H), 1.7 (br, $\textit{CH}_2C(CH_3)_2$, 2H), 1.72 (br, cy$\textit{CH}_2$, 2H), 1.82 (br, cy$\textit{CH}_2$, 2H), 2.0 (br, $NCH_2\textit{CH}_2CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.1-3.3 (br, $NCH_2CH_2CH_2N\textit{(CH}_3\textit{)}_2$, 6H), 3.5 (br, $NCH_2CH_2\textit{CH}_2N(CH_3)_2$, 2H), 3.6 (br, $N\textit{CH}_2CH_2CH_2N(CH_3)_2$, 2H), 3.6 (br, -$N(CH_3)_2\textit{CH}_2CO$, 2H), 4.04 (br, cy*CH*, 1H).

QCyPenAP: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.) 1680 cm^{-1} (amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, D_2O): δ/ppm 0.95–1.2 (br, $CH_2C\textit{(CH}_3\textit{)}_2$, 6H), 1.43 (br, cy$\textit{CH}_2$, 2H), 1.66 (br, cy$\textit{(CH}_2\textit{)}_2$, 4H), 1.7 (br, $\textit{CH}_2C(CH_3)_2$, 2H), 1.98 (br, cy$\textit{CH}_2$, 2H), 2.1 (br, $NCH_2\textit{CH}_2CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.1-3.3 (br, $NCH_2CH_2CH_2N\textit{(CH}_3\textit{)}_2$, 6H), 3.5 (br, $NCH_2CH_2\textit{CH}_2N(CH_3)_2$, 2H), 3.6 (br, $N\textit{CH}_2CH_2CH_2N(CH_3)_2$, 2H), 4.13 (br, -$N(CH_3)_2\textit{CH}_2CO$, 2H), 4.13 (m, cy*CH*, 1H).

QMeCyprAP: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.) 1680 cm^{-1} (amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, D_2O): δ/ppm 0.236 (br, cyCH_2, 2H), 0.547 (br, cyCH_2, 2H), 0.986 (br, cy*CH*, 1H), 0.92–1.4 (br, $CH_2C(CH_3)_2$, 6H), 1.7 (br, $CH_2C(CH_3)_2$, 2H), 2.1 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.8–3.2 (br, *CHCH*, 2H), 3.1 (br, -$CH_2CH(CH_2)_2$, 2H), 3.2-3.4 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 6H), 3.6 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 3.6 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 4.06 (br, -$N(CH_3)_2CH_2CO$, 2H).

QCyprAP: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.) 1680 cm^{-1} (amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, D_2O): δ/ppm 0.64 (br, cyCH_2, 2H), 0.86(br, cyCH_2, 2H), 0.92–1.4 (br, $CH_2C(CH_3)_2$, 6H), 1.7 (br, $CH_2C(CH_3)_2$, 2H), 2.1 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.73 (m, cy*CH*, 1H), 2.8–3.2 (br, *CHCH*, 2H), 3.2-3.4 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 6H), 3.6 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 3.6 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 4.1 (br, -$N(CH_3)_2CH_2CO$, 2H).

Q*n*-buAP: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.) 1680 cm^{-1} (amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, D_2O): δ/ppm 0.9 (br, terminal –CH_3, 3H), 0.96–1.27 (br, $CH_2C(CH_3)_2$, 6H), 1.53 (br, -$CONHCH_2CH_2$ CH_2CH_3, 4H), 1.7 (br, $CH_2C(CH_3)_2$, 2H), 2.1 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.1-3.3 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 8H), 3.6 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 3.6 (br, -$N(CH_3)_2CH_2CO$, 2H), 4.1 (br, -$CONHCH_2$-, 2H).

Q*iso*-buAP: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.) 1680 cm^{-1} (amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, D_2O): δ/ppm 0.92 (br, terminal -*(CH₃)₂*, 6H), 0.96–1.38 (br, CH_2C*(CH₃)₂*, 6H), 1.7 (br, *CH₂*$C(CH_3)_2$, 2H), 1.82 (br, -CH_2*CH*$(CH_3)_2$, 1H), 2.1 (br, NCH_2*CH₂*$CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.13 (br, -*CH₂*$CH(CH_3)_2$, 2H), 3.2-3.4 (br, $NCH_2CH_2CH_2N$*(CH₃)₂*, 6H), 3.6 (br, NCH_2CH_2*CH₂*$N(CH_3)_2$, 2H), 3.6 (br, N*CH₂*$CH_2CH_2N(CH_3)_2$, 2H), 4.1 (br, -$N(CH_3)_2$*CH₂*CO, 2H).

Q(S)-(+)-*s*-buAP: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.) 1680 cm^{-1} (amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, D_2O): δ/ppm 0.89 (br, -$CHCH_2$*CH₃*, 3H), 0.96–1.27 (br, CH_2C*(CH₃)₂*, 6H), 1.2 (br, -CH_2CH*CH₃*, 3H), 1.5 (br, -CH*CH₂*CH_3, 2H), 1.7 (br, *CH₂*$C(CH_3)_2$, 2H), 2.1 (br, NCH_2*CH₂*$CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.2-3.4 (br, $NCH_2CH_2CH_2N$*(CH₃)₂*, 6H), 3.6 (br, NCH_2CH_2*CH₂*$N(CH_3)_2$, 2H), 3.6 (br, N*CH₂*$CH_2CH_2N(CH_3)_2$, 2H), 3.8 (br, -*CH*CH_2CH_3, 1H), 4.09 (br, -$N(CH_3)_2$*CH₂*CO, 2H).

Q(R)-(-)-*s*-buAP: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.) 1680 cm^{-1} (amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, D_2O): δ/ppm 0.88 (br, -$CHCH_2$*CH₃*, 3H), 0.96–1.27 (br, CH_2C*(CH₃)₂*, 6H), 1.2 (br, -CH_2CH*CH₃*, 3H), 1.5 (br, -CH*CH₂*CH_3, 2H), 1.7 (br, *CH₂*$C(CH_3)_2$, 2H), 2.1 (br, NCH_2*CH₂*$CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.2-3.4 (br, $NCH_2CH_2CH_2N$*(CH₃)₂*, 6H),

3.6 (br, $NCH_2CH_2\mathit{CH_2}N(CH_3)_2$, 2H), 3.6 (br, $N\mathit{CH_2}CH_2CH_2N(CH_3)_2$, 2H), 3.8 (br, -$\mathit{CH}CH_2CH_3$, 1H), 4.09 (br, -$N(CH_3)_2\mathit{CH_2}CO$, 2H).

Q*t*-buAP: FT-IR: 3250 cm^{-1} (amide N-H str.), 2950-2850 cm^{-1} (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.), 1680 cm^{-1} (amide I, C=O str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, D_2O): δ/ppm 0.96–1.27 (br, $CH_2C\mathit{(CH_3)_2}$, 6H), 1.35 (br, terminal -$C\mathit{(CH_3)_3}$, 9H), 1.7 (br, $\mathit{CH_2}C(CH_3)_2$, 2H), 2.1 (br, $NCH_2\mathit{CH_2}CH_2N(CH_3)_2$, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.2-3.4 (br, $NCH_2CH_2CH_2N\mathit{(CH_3)_2}$, 6H), 3.6 (br, $NCH_2CH_2\mathit{CH_2}N(CH_3)_2$, 2H), 3.6 (br, $N\mathit{CH_2}CH_2CH_2N(CH_3)_2$, 2H), 3.98 (br, -$N(CH_3)_2\mathit{CH_2}CO$, 2H).

Q*n*-buenAP: FT-IR: 3250 cm^{-1} (amide N-H str.), 3049 cm^{-1} (C-H str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.),1680 cm^{-1} (Amide I, C=O str.), 1642 cm^{-1} (C=C str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, D_2O): δ/ppm 0.96–1.27 (br, $CH_2C\mathit{(CH_3)_2}$, 6H), 1.7 (br, $\mathit{CH_2}C(CH_3)_2$, 2H), 2.0 (br, $NCH_2\mathit{CH_2}CH_2N(CH_3)_2$, 2H), 2.3 (br, -$CONHCH_2\mathit{CH_2}$-, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.29 (br, $NCH_2CH_2CH_2N\mathit{(CH_3)_2}$, 6H), 3.35 (br, -$CONH\mathit{CH_2}CH_2$-, 2H), 3.6 (br, $NCH_2CH_2\mathit{CH_2}N(CH_3)_2$, 2H), 3.6 (br, $N\mathit{CH_2}CH_2CH_2N(CH_3)_2$, 2H), 4.10 (br, -$N(CH_3)_2\mathit{CH_2}CO$, 2H), 5.17 (br, $CH_2CH_2CH{=}\mathit{CH_2}$, 2H), 5.85 (m, $CH_2CH_2\mathit{CH}{=}CH_2$, 1H).

Q*n*-buynAP: FT-IR: 3250 cm^{-1} (amide N-H str.), 3310 cm^{-1} (≡C-H str.), 2857-2942 cm^{-1} (-C-H str.), 2119 cm^{-1} (C≡C str.), 1767 cm^{-1} (imide C=O asym. str.), 1696 cm^{-1} (imide C=O sym. str.), 1680 cm^{-1} (Amide I, C=O str.), 1642 cm^{-1} (C=C str.), 1560 cm^{-1} (Amide II, N-H ben.), 1470-1410 cm^{-1} (C-C str.), 1290-1110 cm^{-1} (C-O str.); ^{1}H-NMR (400 MHz, D_2O): δ/ppm 0.96–1.27 (br, $CH_2C\mathit{(CH_3)_2}$, 6H), 1.7 (br, $\mathit{CH_2}C(CH_3)_2$, 2H), 1.82 (br, -$CH_2C{\equiv}\mathit{CH}$, 1H), 2.1 (br, $NCH_2\mathit{CH_2}CH_2N(CH_3)_2$, 2H), 2.29 (br, -$CONHCH_2\mathit{CH_2}$-, 2H), 2.7–3.1 (br, *CHCH*, 2H), 3.2-3.4

(br, $NCH_2CH_2CH_2N(CH_3)_2$, 6H), 3.37 (br, -$CONHCH_2CH_2$-, 2H), 3.6 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 3.6 (br, $NCH_2CH_2CH_2N(CH_3)_2$, 2H), 4.16 (br, -$N(CH_3)_2CH_2CO$, 2H).

Antibacterial activity

Antibacterial activity of the polymers was measured and MIC was calculated as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibacterial activity of polymers was assayed in a modified micro-dilution broth format. Stock solutions were made by serially diluting the test compounds using autoclaved Millipore water. Bacteria, to be tested, grown for 6 h in the suitable media contained ~10^9 CFU mL^{-1} (determined by spread plating method), which was then diluted to ~10^5 CFU mL^{-1} using cation adjusted Mueller-Hinton broth (CAMHB) as per CLSI guidelines. 50 µL of serially diluted compound was added to a 96 well plate (Polystyrene) containing 150 µL bacterial solutions. Two controls were made; one containing 150 µL of media and 50 µL of compound and the other containing 150 µL of bacterial solution and 50 µL water . The plate was then incubated at 37 °C for a period of 24 h and the O.D. value was measured at 600 nm using a Tecan InfinitePro series M200 Microplate Reader. MIC value was determined by taking the average of triplicate O.D. values for each concentration and plotting it against concentration using Origin Pro 8.0 software. The data was then subjected to sigmoidal fitting. From the curve the MIC value was determined, as the point in the curve where the O.D. was similar to that of control having no bacteria. MIC curves for individual agents are representative data from the two independent experiments and each experiment was performed in triplicates.

Bacteria were diluted to ~5×10^5 CFU mL^{-1} in M9 media (*E. coli)* or minimal essential medium (MEM) *(S. aureus)*. 50 µL of polymers were added to a 96 well plate (Polystyrene)

containing 150 µL bacterial solutions. The plate was then incubated at 37 °C for a period of 24 h. After 24 h, the bacterial suspension in the well with no visual turbidity was spot-plated on agar plates and viable colonies were counted.

Hemolytic activity

The hemolytic activity was determined against human erythrocytes. Erythrocytes were isolated from freshly drawn, heparanized human blood and resuspended to 5 % v/v in PBS (pH 7.4). In a 96-well micro titer plate, 150 µL of erythrocyte suspension was added followed by 50 µL of serially diluted compound to give a final solution of 3.75 % v/v erythrocytes. PBS buffer was added instead of polymer solution as negative hemolysis control and Triton X-100 (1% v/v) was used as positive hemolysis control. The plate was incubated for 1 h at 37 °C and was then centrifuged at 3,500 rpm for 5 min. 100 µL of the supernatant was then transferred to a fresh micro titer plate and absorbance at 540 nm was measured using a Tecan InfinitePro series M200 Micro plate Reader. Percentage of hemolysis was determined as $(A - A_0)/ (A_{total} - A_0) \times 100$, where A is the absorbance of the test well, A_0 the absorbance of the negative controls, and A_{total} the absorbance of 100% hemolysis wells, all at 540 nm. Hemolysis was plotted as a function of polymer concentration and the HC_{50} was defined as the polymer concentration, which causes 50% hemolysis relative to the positive control. In some cases, hemolysis did not reach 50% up to the highest polymer concentration tested and the HC_{50} was not determined. Hemolysis curves for each polymer are representative data from two independent experiments and each experiment was performed in triplicates.

In-vitro mammalian cell toxicity

Cytotoxicity Assay by LDH measurement. CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega) kit was used for determining the cytotoxicity of the compounds. In brief, HEK293 cells that were maintained in complete DMEM media (Gibco) supplemented with 10% FBS (Gibco) and Penicillin-Streptomycin solution (Gibco) were seeded in 96 well plates at a concentration of 10^4 cells/well. They were allowed to adhere to the plate overnight. 0.5% Triton-X and media were used as positive and untreated controls respectively. The cells were treated with respective test compound solutions. After 24 hrs of treatment, the plates were centrifuged at 1100 rpm for 5 min. The supernatants from respective wells were transferred and the assay was performed according to the manufacturer's instructions. 100 µL of the supernatant was then transferred to a fresh micro titer plate and absorbance at 490 nm was measured using a Tecan InfinitePro series M200 Micro plate Reader. Percentage of cell death was determined as $(A - A_0)/(A_{total} - A_0) \times 100$, where A is the absorbance of the test well, A_0 the absorbance of the negative controls, and A_{total} the absorbance of triton-X treated wells, all at 490 nm. Percentage of LDH release was plotted as a function of polymer concentration and the IC_{50} was defined as the polymer concentration, which causes 50% LDH release relative to the positive control. In some cases, LDH release did not reach 50% up to the highest polymer concentration tested and the IC_{50} was not determined.

Cytoplasmic membrane depolarization assay

Bacteria were harvested, washed with 5 mM HEPES and 5 mM glucose and resuspended in 5 mM glucose, 5 mM HEPES buffer and 100 mM KCl solution in 1:1:1 ratio (~10^{8-9} CFU mL^{-1}). Measurements were made in a Corning 96 well black plate with clear bottom with 150 µl of

bacterial suspension and 2 μM of $DiSC_3(5)$. 0.2 mM of EDTA was used to permeabilize the outer membrane and allow the dye uptake. The fluorescence of the dye was monitored using a Tecan InfinitePro series M200 Micro plate Reader at excitation wavelength of 622 nm and emission wavelength of 670 nm. Dye uptake, and resultant self quenching, was modulated by the membrane potential. After reaching the maximum uptake of the dye by bacteria, which was indicated by a minimum in dye fluorescence, polymer solution was added to the cells, and the decrease in potential was monitored by increase in fluorescence for further 30 min. All the other test compounds were dissolved in water at 4 mg mL^{-1} and $DiSC_3(5)$ dissolved in DMSO were further diluted in the above 5 mM glucose, 5 mM HEPES buffer and 100 mM KCl solution in 1:1:1 ratio. A control without the polymers served as negative control.

Cytoplasmic membrane permeabilization assay

Bacteria were harvested, washed, and resuspended in 5 mM HEPES and 5 mM glucose buffer of pH 7.2 (~10^{8-9} CFU mL^{-1}). Then, 150 μl of bacterial suspension, 10 μM propidium iodide (PI) and polymer solution were added to the cells in a Corning 96 well black plate with clear bottom. Stock solutions of PI and the polymers were made in water and further diluted in HEPES. Excitation wavelength of 535 nm and emission wavelength of 617 nm were used. The uptake of PI was measured using a Tecan InfinitePro series M200 Microplate Reader by the increase in fluorescence of PI for 30 min as a measure of membrane permeabilization.

Release of ATP levels

Mid-log phase bacteria (~10^{8-9} CFU mL^{-1}) were harvested and washed twice with 10 mM TRIS buffer (pH = 7.5) and were resuspended in the same buffer. Then, 150 μl of bacterial suspension

and 50 µl of test drugs were added to the microcentrifuge tube and incubated at 37 °C for 15 min. After 15 min, the bacterial suspension was centrifuged and 50 µl of the supernatant was transferred into a Corning 96 well black plate with clear bottom to find out the released ATP levels using ATP Bioluminiscience Assay Kit (Sigma Aldrich) as per the manufacturer's instructions. A standard curve for ATP levels was generated using the ATP standards provided in the kit in the range of 1E-6 to 1E-11 moles of ATP levels. Polymers were used at 50 µg mL^{-1}. Water served as the untreated control. Stock solutions of the polymers were made in water and further diluted in water. Relative ATP levels both in the standard curve and the test sample measurement were measured by subtracting the background ATP levels from the test sample ATP levels as per the manufacturer's instructions. All measurements were performed in duplicates using Tecan InfinitePro series M200 Microplate Reader.

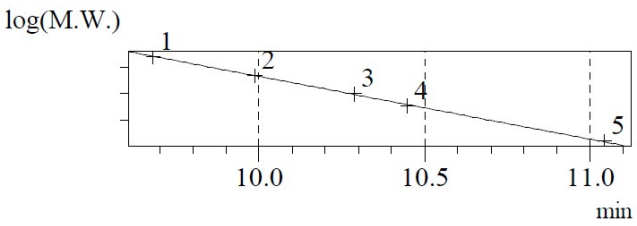
Gel Permeation Chromatography (GPC)

GPC of the water soluble Poly(isobutylene-alt-maleic acid) obtained after hydrolysis of Poly(isobutylene-alt-maleic anhydride) was performed. Experiments were carried out on a Shimadzu-LC 20AD instrument with refractive index (RI) detector using Polysep-GPC-P Linear (300×7.8 mm,Phenomenex, Catalogue no. 00H-3147-K0) column in sodium acetate buffer (0.2 M, pH = 5.3) with a flow rate of 0.8 mL min^{-1}. Pullulan standards were used for the experiment to determine the polydispersity index (PDI = M_w/M_n) of the polymer.

Reverse-phase high performance liquid chromatography (RP-HPLC)

Chromatographic profiles were analyzed by reverse phase HPLC using 0.1% trifluoroacetic acid (TFA) in water/acetonitrile (0−100%) as mobile phase. HPLC analysis was performed on a

Shimadzu-LC 8 Å liquid chromatography instrument (C18 column, 10 mm diameter, 250 mm length) with UV detector monitoring at 220 nm. The data was acquired from 0-40 min and presented from 5-30 min.



#	Time(min)	Molecular Weight
1	9.679	50000
2	9.987	22000
3	10.288	10000
4	10.448	6000
5	11.043	1300

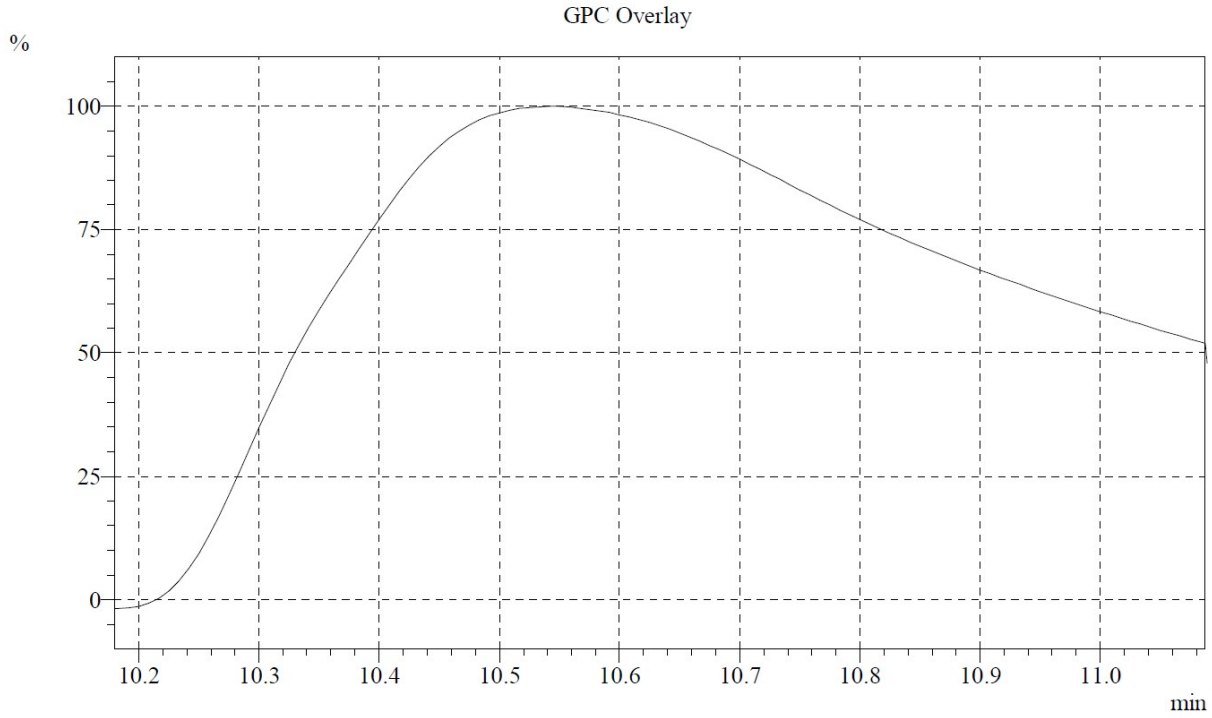


Fig. S1. GPC spectra of the polymer, PIBMA. Top panel shows the standard curve generated by using the Pullulan standards of different molecular weights and their retention times. Bottom panel shows the retention time curve for the polymer, PIBMA.

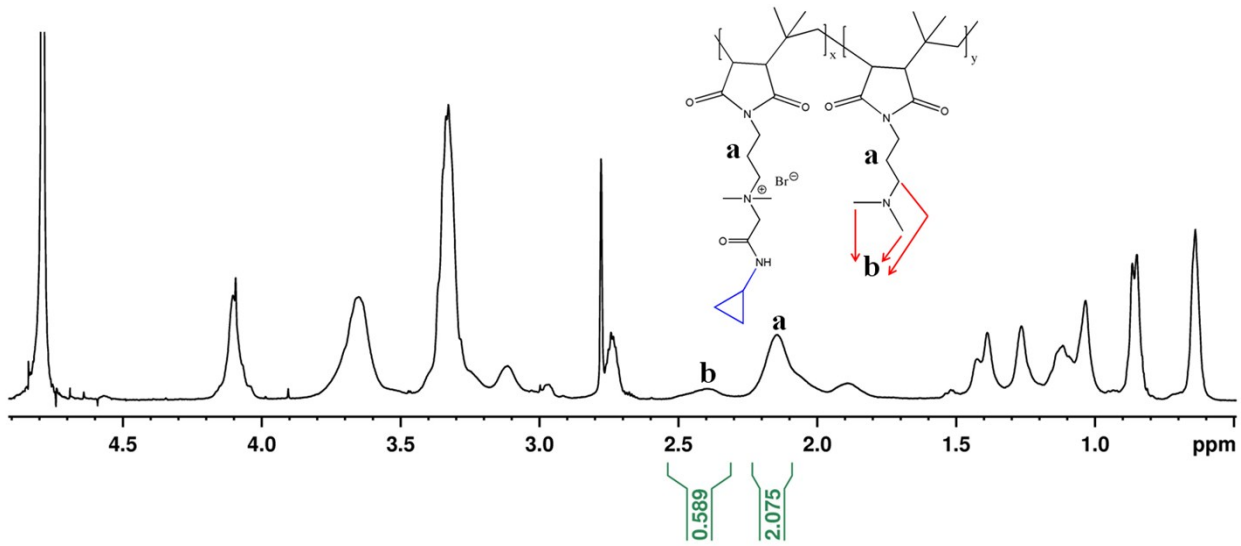


Fig. S2. ^{1}H NMR of QCyprAP (in D_2O) indicating the peaks used for the calculation of degree of quaternization (δ/ppm): (a) for 2.15 (br, -NCH_2***CH_2***CH_2-, 2H) and (b) for 2.2-2.5 (br, -NCH_2CH_2***CH_2***N***$(CH_3)_2$***, 8H).

Degree of Quaternization:

Degree of quaternization of the polymeric derivatives was calculated using ^{1}H NMR analysis following a literature procedure.[1-3]

Degree of quaternization (x) = (1- y) × 100 %

Wherein y = {([***CH_2***N***$(CH_3)_2$***]/8)/ ([***CH_2***CH_2]/2)}

y = {(m/8)/ (n/2)}, m = [***CH_2***N***$(CH_3)_2$***] and n = [***CH_2***CH_2]

For e.g. QCyprAP, Degree of quaternization (x) = 1-{(0.589/8) / (2.075/2)} × 100 %

= 93%

Wherein, [***CH_2***CH_2] and [***CH_2***N***$(CH_3)_2$***] are the integrals of the hydrogens, a and b respectively that are bold and italicized.

The molecular weight (number average molecular weight, M_n) (Table 1) of all the derivatives is calculated based on the molecular weight of the precursor polymer, poly(isobutylene-*alt*-maleic

anhydride) (average M_w~ 6000 Da, monomer weight is 154 g/mol and n~39) and the obtained degree of quaternization (% of DQ) as described above. [1-3]

For e.g. QCyprAP

M_n $= [(m_x \times 0.93) + \{m_y \times (1\text{-}0.93)\}] n$

$= [(415.16 \times 0.93) + (238.18 \times 0.07)] 39$

$= 15.68$ kDa

Where m_x and m_y are the molecular weights of the quaternized and non-quaternized repeating units respectively.

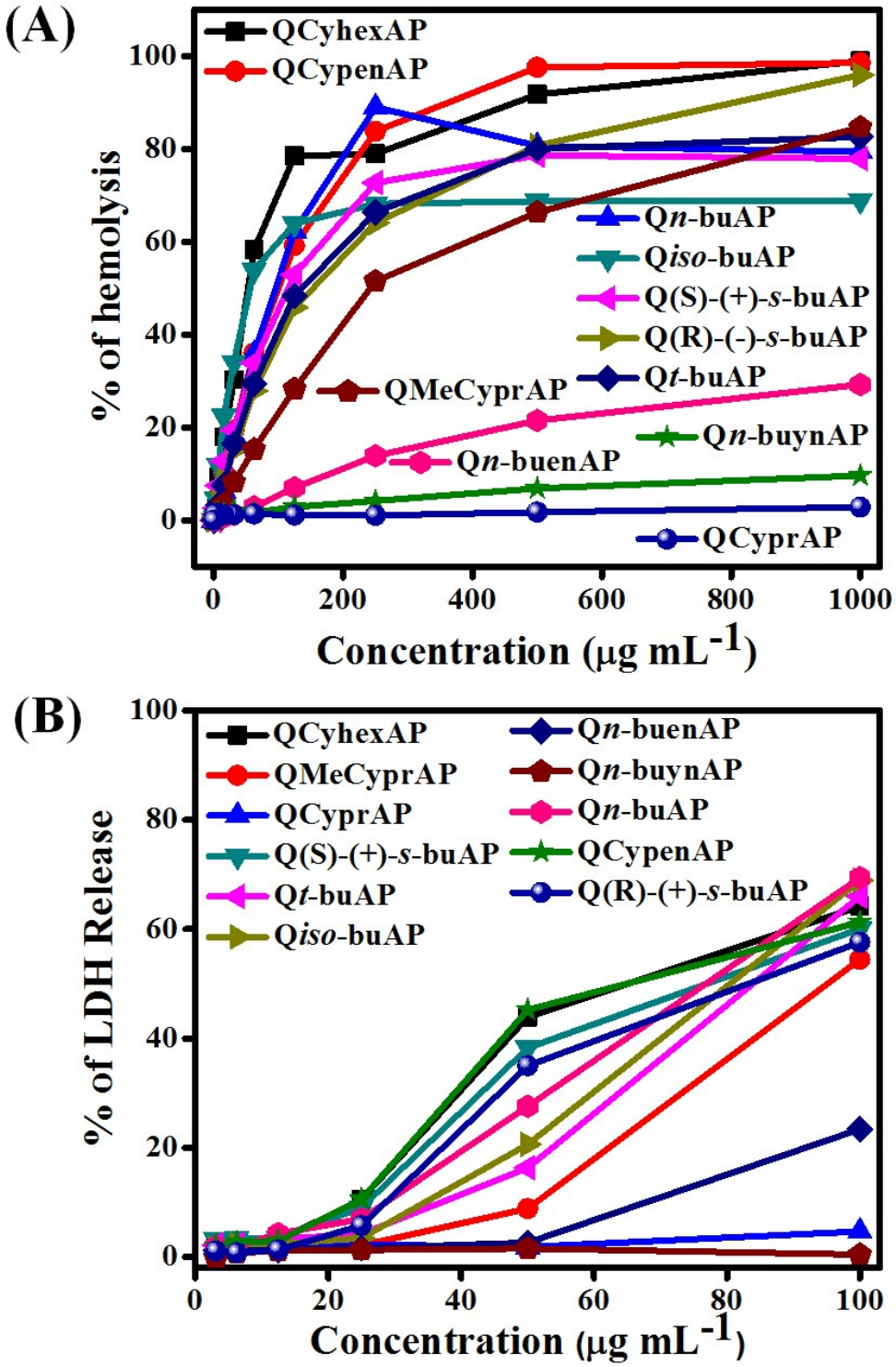


Fig. S3. Mammalian toxicity profiles of cationic-amphiphilic polymers. (A) % of hemolysis against human red blood cells (hRBCs) and (B) % of LDH (lactate dehydrogenase) release against HEK (human endothelial kidney) cells upon treatment with various concentrations of the polymers.

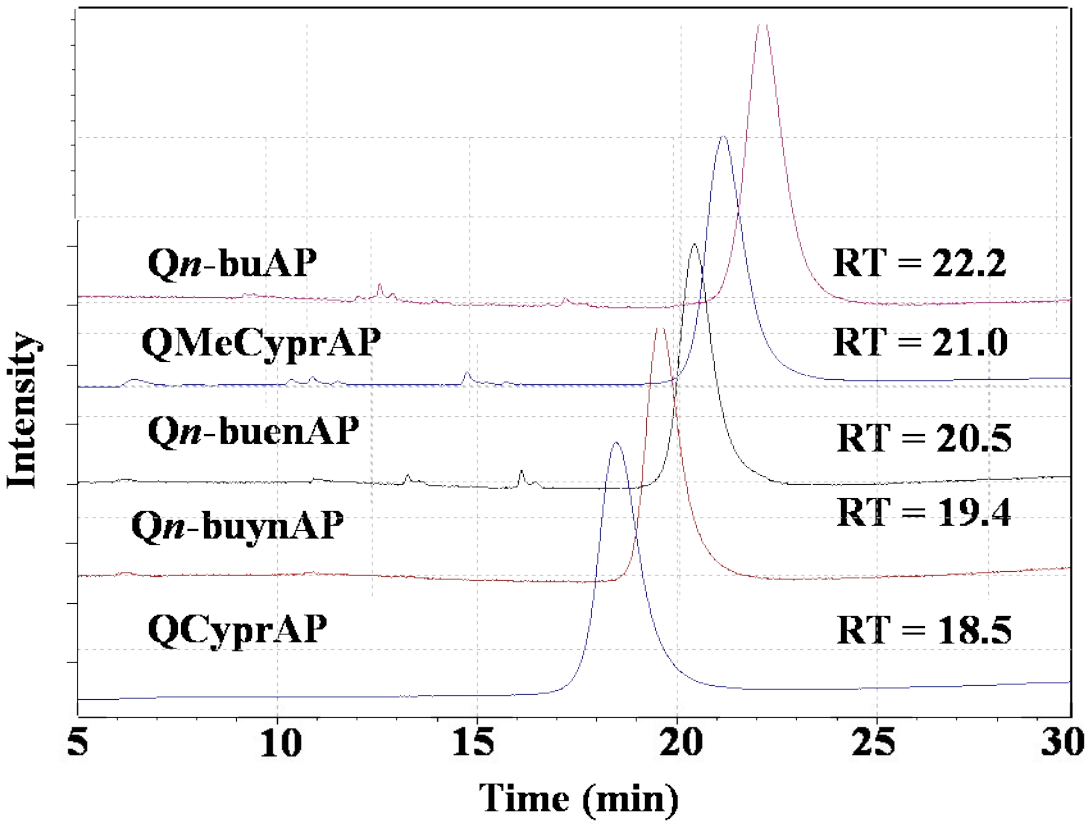


Fig. S4. Hydrophobicity profiles of cationic-amphiphilic polymers measured using the reverse-phase high performance liquid chromatography (RP-HPLC).

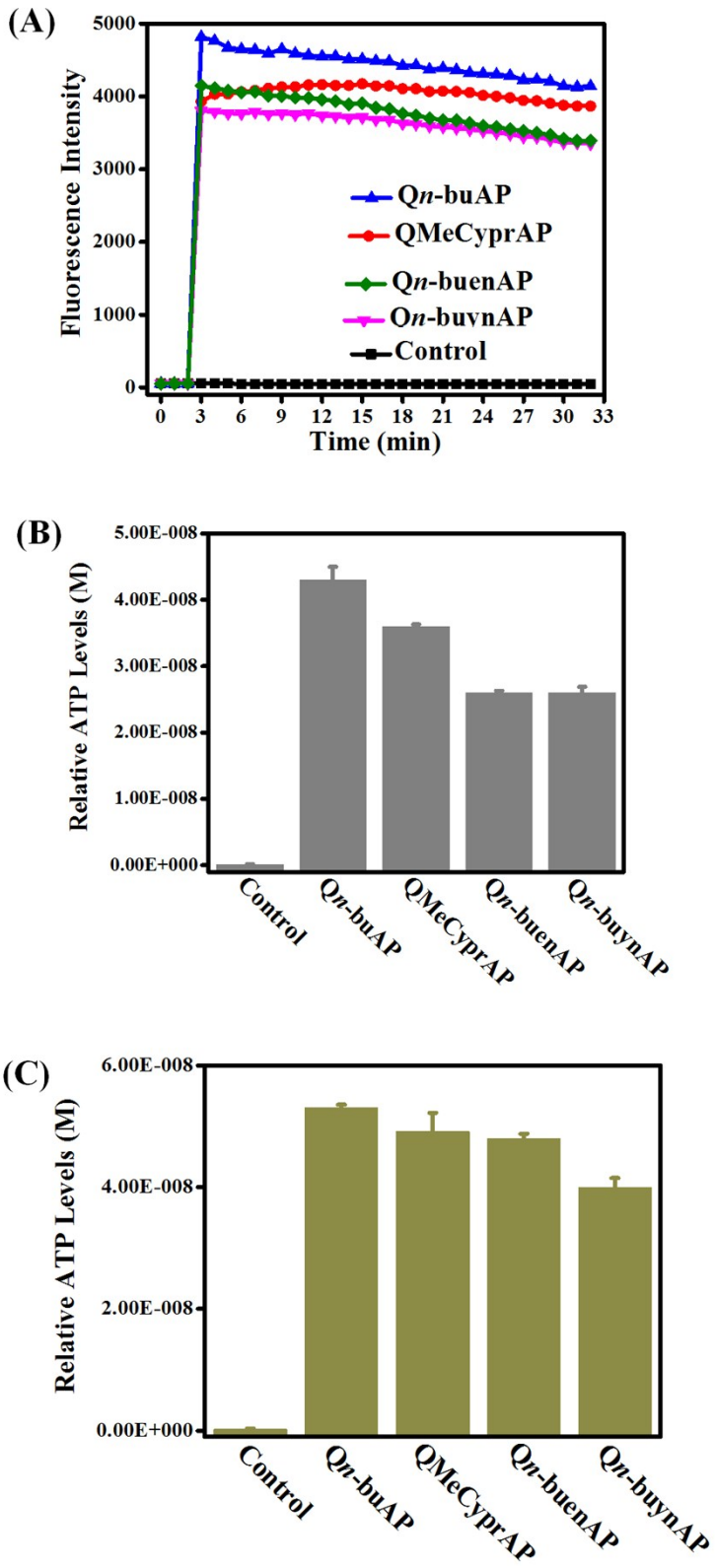


Fig. S5. Membrane active properties of cationic-amphiphilic polymers. (A) Membrane depolarization against *S. aureus* (A), Leakage of ATP levels against *E. coli* (B) and *S. aureus* (C).

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1. R. J. Verheul, S. Wal, and W. E. Hennink, *Biomacromolecules*, 2010, **11***,* 1965.
2. J. Bohrisch, T. Schimmel, H. Engelhardt, and W. Jaeger, *Macromolecules*, 2002, **35***,* 4143.
3. C. A. J. Lin, R. A. Sperling, J. K. Li, T. Y. Yang, P.Y. Li, M. Zanella, W. H. Chang, and W. J. Parak, *Small*, 2008, **4***,* 334.