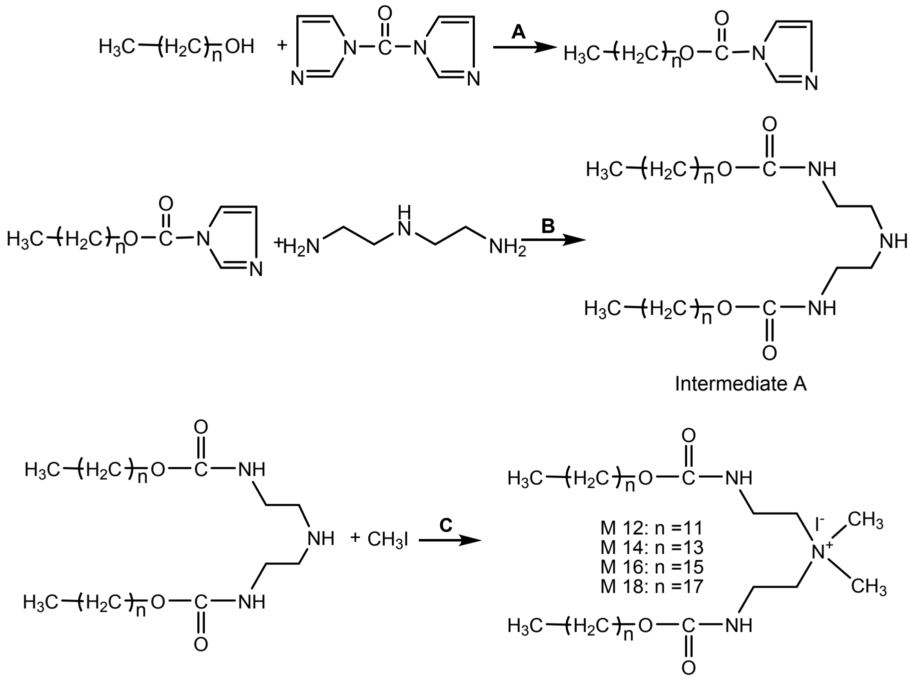
Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

1. The synthesis of mono-head quaternary ammoniums

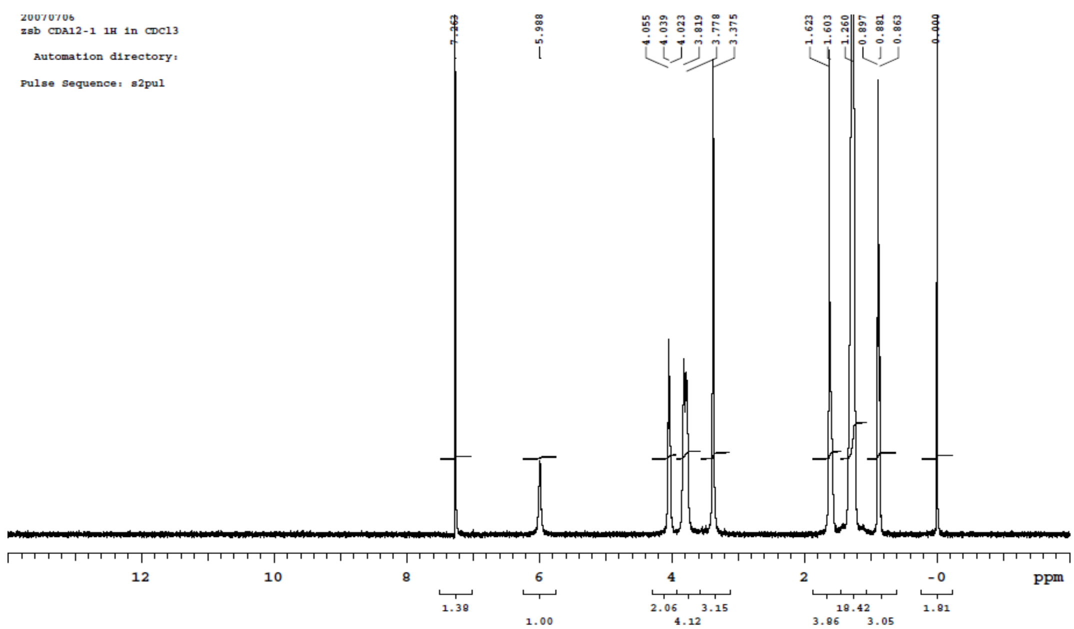
Scheme 1. Synthesis of Cationic Lipids (M12-M18). Reaction Conditions: (A) CH_2Cl_2, 40 ºC, 3 h; (B) THF, 40 ºC, 3 h; (C) 120 ~160 ºC, 40 h.



2. The Characterization of lipids

2.1. M12

A



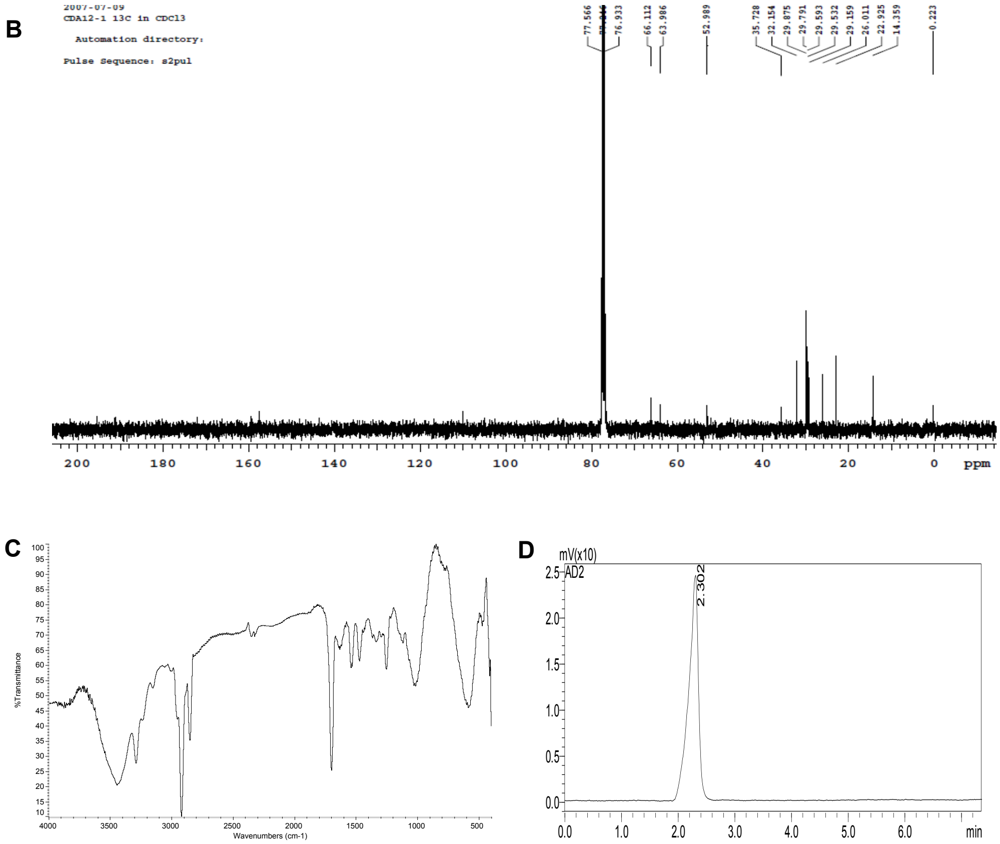
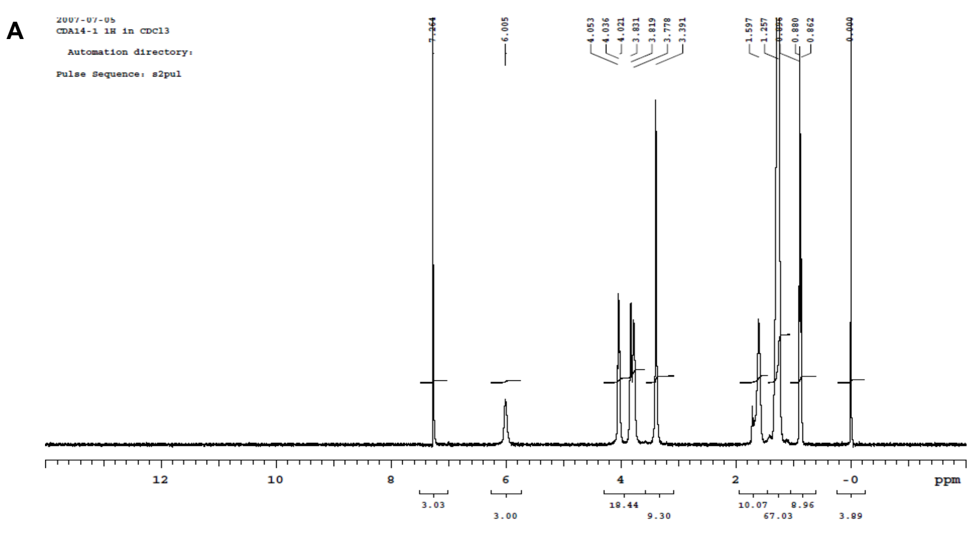


Fig. 1. Structure characterization of M12, A: ^{1}H NMR; B: ^{13}C NMR; C: IR; D: HPLC

Data assignment: ^{1}H NMR (400MHz, $CDCl_3$) δ=6.00(2H, s, O(C=O)N***H***), 4.05 (4H, t, C$\boldsymbol{H_2}$O), 3.82 (8H, s, NHC$\boldsymbol{H_2}$C$\boldsymbol{H_2}$$N^+$), 3.36 (6H, s, N^+C$\boldsymbol{H_3}$), 1.59 (4H, s, C$\boldsymbol{H_2}$$CH_2$O), 1.28~1.20 (36H, s, (C$\boldsymbol{H_2}$)$_9$), 0.89~0.86 (6H, t, CH_2C$\boldsymbol{H_3}$). ^{13}C NMR (100MHz, $CDCl_3$) δ=157.3 (***C***=O), 66.0 (***C***H_2O), 63.9 (***C***H_2N^+), 52.9 (***C***H_3N^+), 35.6 (NH***C***H_2), 32.1 (***C***$H_2CH_2CH_3$), 29.7~29.1 ((***C***H_2)$_6$; ***C***H_2CH_2O), 25.9 (***C***$H_2CH_2CH_2$O), 22.8 (***C***H_2CH_3), 14.3 (***C***H_3). MS *m/z*: 556.5074 $[M-I]^+$ (Calc, 556.5053). IR ν/cm^{-1}: 3282.49 (ν_{NH}), 1698.16 ($\nu_{C=O}$), 1536.41 (δ_{NH}), 1250-1235 (ν_{COC}, ν_{CN}). HPLC purity: 99.5 %.

2.2. M14



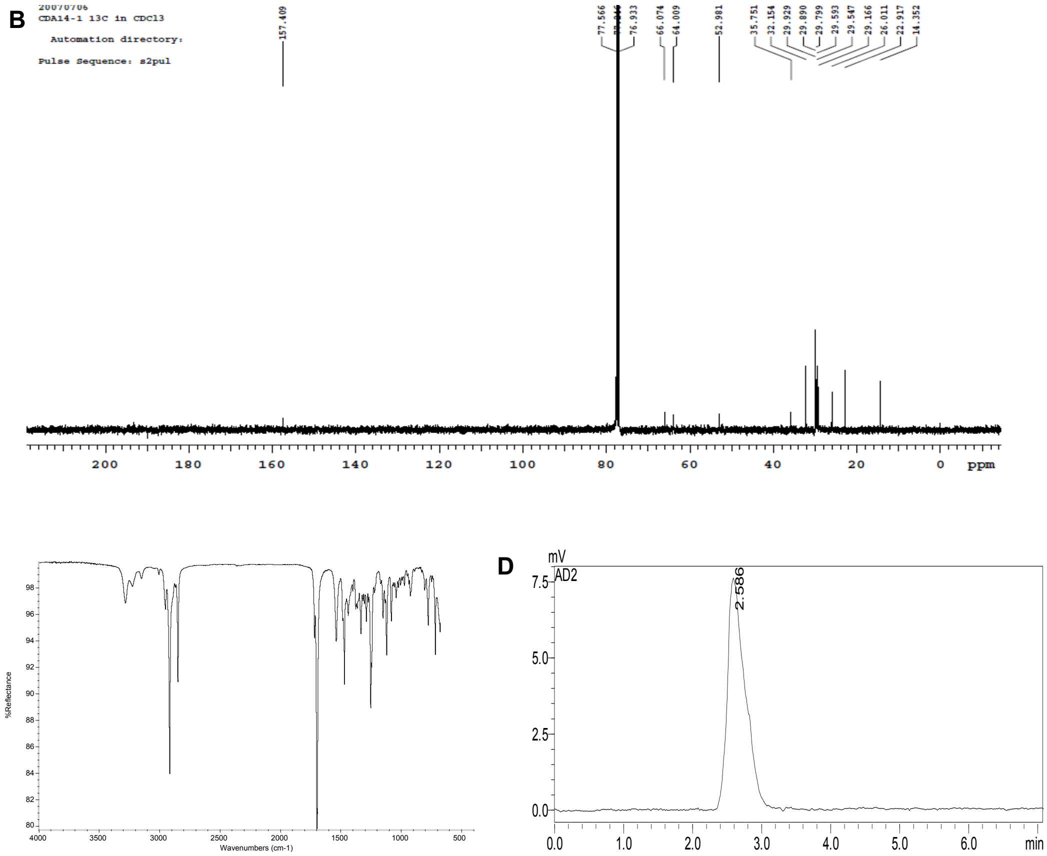
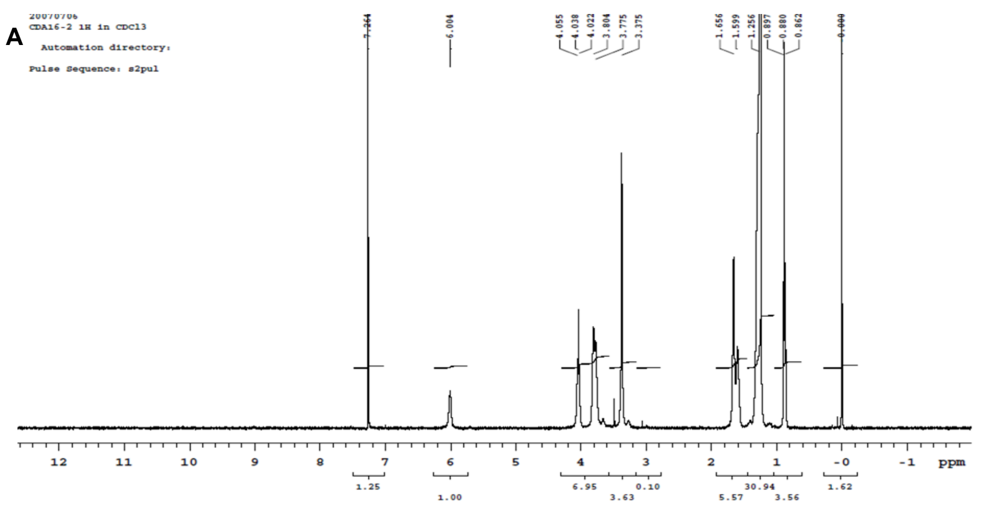


Fig. 2. Structure characterization of M14, A: ^{1}H NMR; B: ^{13}C NMR; C: IR; D: HPLC

Data assignment: ^{1}H NMR (400MHz, $CDCl_3$) δ=6.02 (2H, s, O(C=O)N***H***), 4.04 (4H, t, C$\boldsymbol{H_2}$O), 3.82 (8H, s, NHC$\boldsymbol{H_2}$C$\boldsymbol{H_2}$$N^+$), 3.38 (6H, s, N^+C$\boldsymbol{H_3}$), 1.59 (4H, s, C$\boldsymbol{H_2}$$CH_2$O), 1.25~1.21 (44H, s, $(C\boldsymbol{H_2})_{11}$), 0.89~0.86 (6H, t, CH_2C$\boldsymbol{H_3}$). ^{13}C NMR (100MHz, $CDCl_3$) δ=157.4 (***C***=O), 66.1 (***C***H_2O), 64.0 (***C***H_2N^+), 52.9 (***C***H_3N^+), 35.7 (NH***C***H_2), 32.1 (***C***$H_2CH_2CH_3$), 29.9~29.2 ($(CH_2)_8$; ***C***H_2CH_2O), 26.0 (***C***$H_2CH_2CH_2O$), 22.9 (***C***H_2CH_3), 14.4 (***C***H_3). MS *m/z*: 612.5680 [M-I]$^+$ (Calc, 612.5679). IR ν/cm^{-1}: 3283.15 (ν_{NH}), 1698.21 ($\nu_{C=O}$), 1537.55 (δ_{NH}), 1256-1240 (ν_{COC}, ν_{CN}). HPLC purity: 99.2 %.

2.3. M16



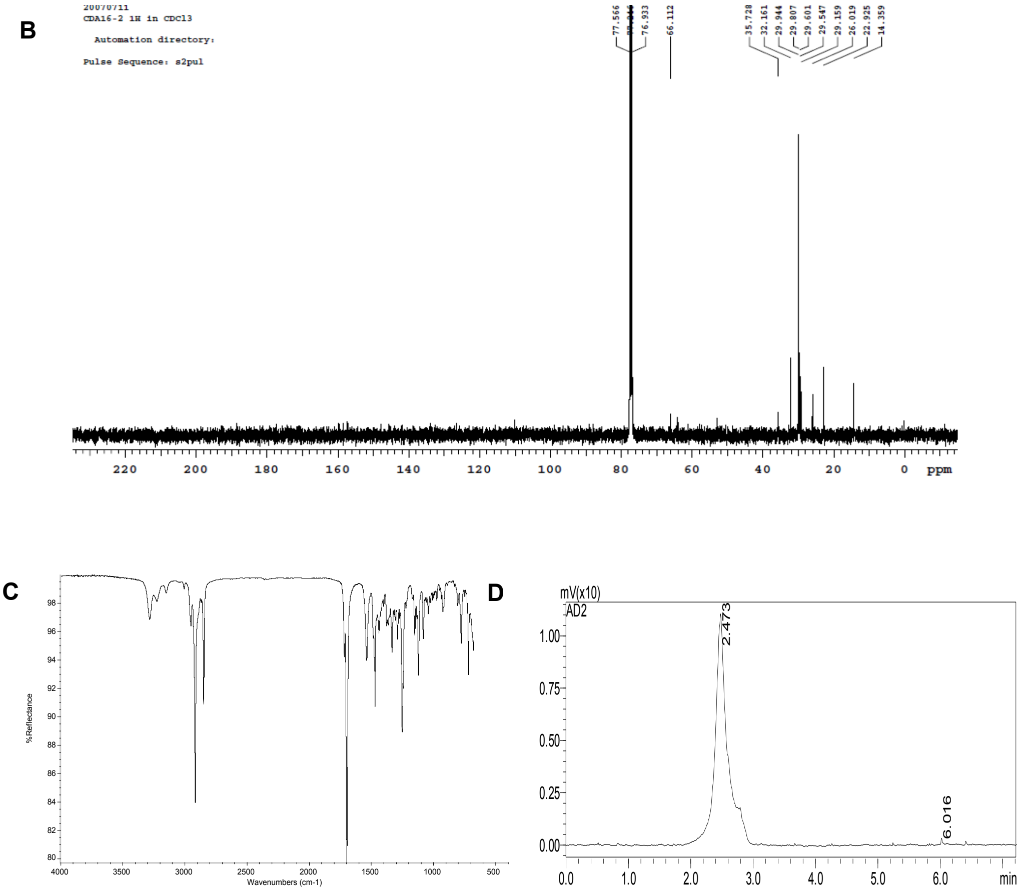
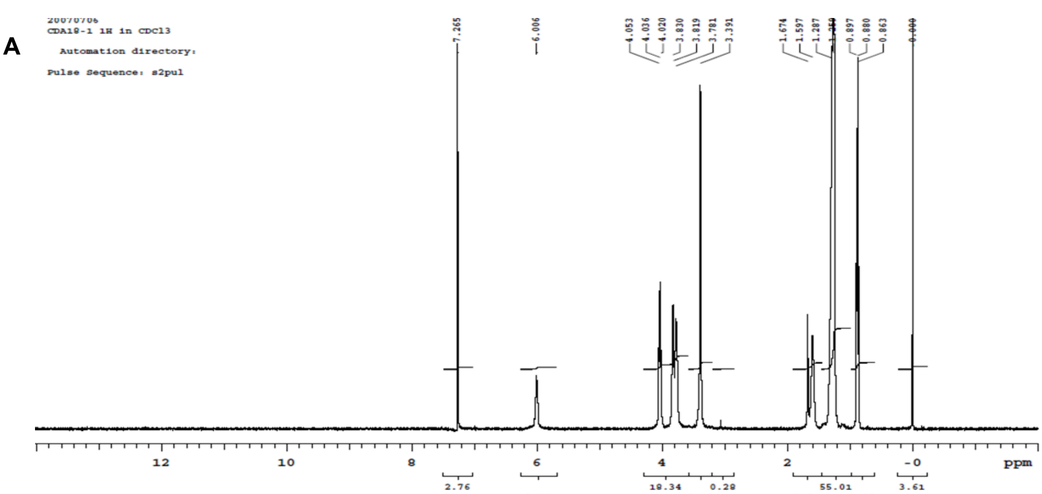


Fig. 3. Structure characterization of M16, A: ^{1}H NMR; B: ^{13}C NMR; C: IR; D: HPLC

Data assignment: ^{1}H NMR (400MHz, $CDCl_3$) δ=6.01 (2H, s, O(C=O)N***H***), 4.06 (4H, t, C$\boldsymbol{H_2}$O), 3.80 (8H, s, NHC$\boldsymbol{H_2}$C$\boldsymbol{H_2}$$N^+$), 3.38 (6H, s, N^+C$\boldsymbol{H_3}$), 1.60(4H, s, C$\boldsymbol{H_2}$$CH_2$O), 1.26 (52H, s, (C$\boldsymbol{H_2}$)$_{13}$), 0.89 ~0.86 (6H, t, CH_2C$\boldsymbol{H_3}$). ^{13}C NMR (100MHz, $CDCl_3$) δ=157.4 (***C***=O), 66.0 (***C***H_2O), 63.9 (***C***H_2N^+), 52.9 (***C***H_3N^+), 35.6 (NH***C***H_2), 32.1 (***C***$H_2CH_2CH_3$), 29.8~29.1 ((***C***H_2)$_{10}$; ***C***H_2CH_2O), 25.9 (***C***$H_2CH_2CH_2$O), 22.8 (***C***H_2CH_3), 14.2 (***C***H_3). MS *m/z*: 668.6324 $[M-I]^+$ (Calc, 668.6305). IR ν/cm^{-1}: 3290.78 (ν_{NH}), 1689.86 ($\nu_{C=O}$), 1540.55 (δ_{NH}), 1254-1240 (ν_{COC}, ν_{CN}). HPLC purity: 98.6 %.

2.4. M18



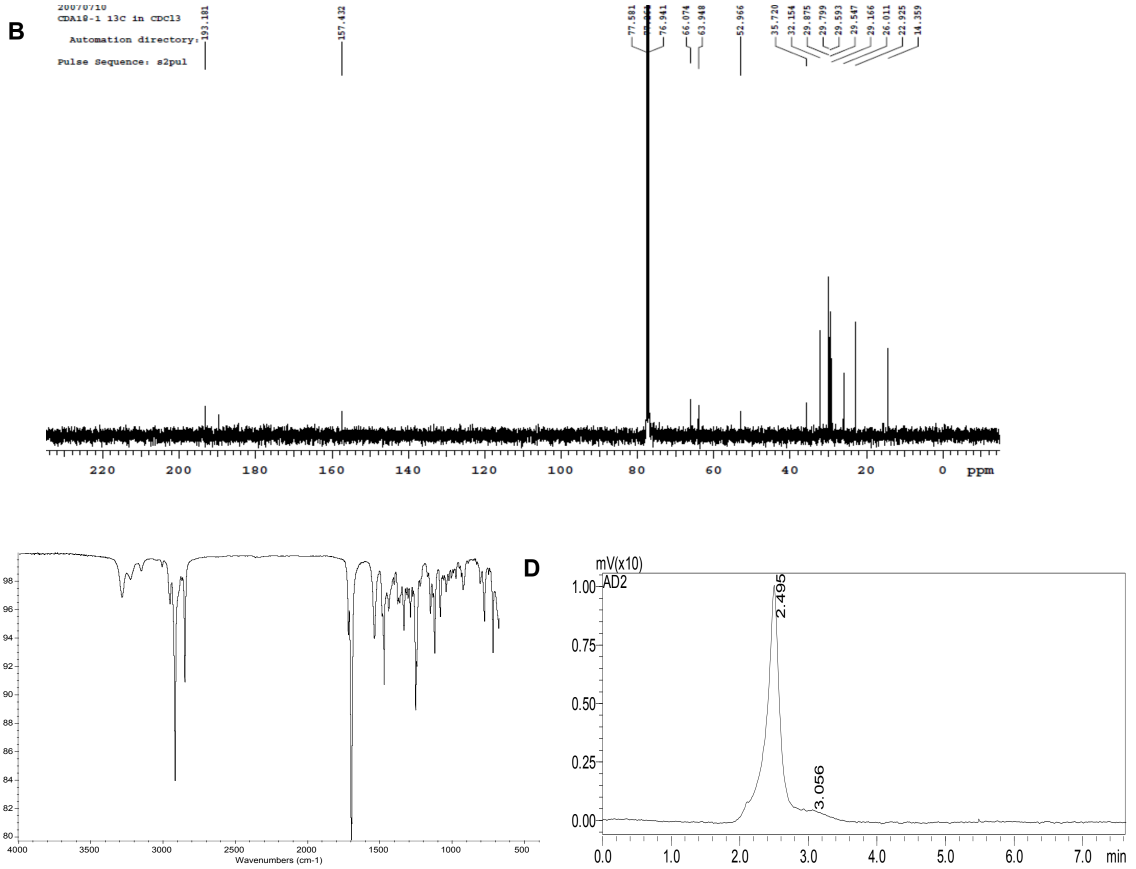
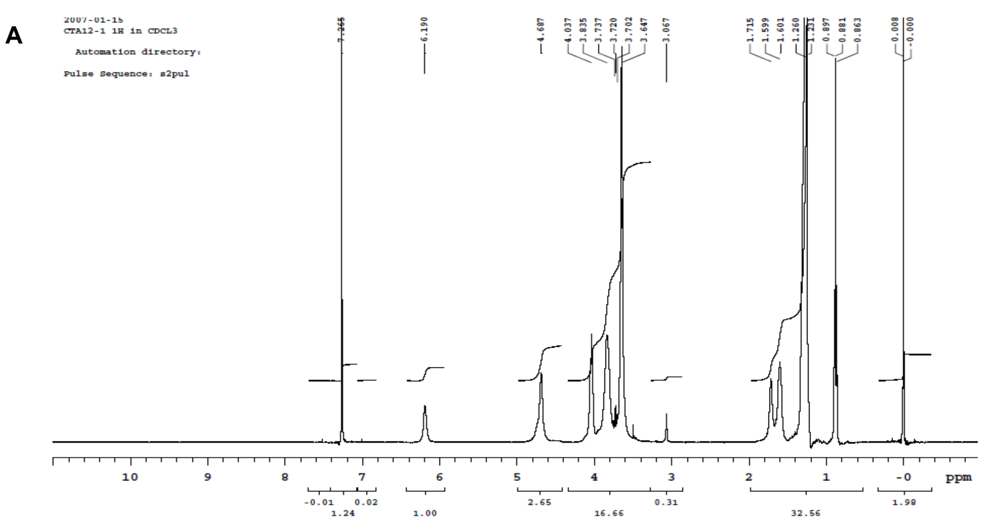


Fig. 4. Structure characterization of M18, A: [1]H NMR; B: [13]C NMR; C: IR; D: HPLC

Data assignment: ^{1}H NMR (400MHz, $CDCl_3$) δ=6.01 (2H, s, O(C=O)N***H***), 4.05(4H, t, C***H₂***O), 3.83 (8H, s, NHC***H₂***C***H₂***N^+), 3.39 (6H, s, N^+C***H₃***), 1.59 (4H, s, C***H₂***CH_2O), 1.28~1.26 (60H, s, (C***H₂***)$_{15}$), 0.89~0.86 (6H, t, CH_2C***H₃***). ^{13}C NMR (100MHz, $CDCl_3$) δ=157.3 (***C***=O), 65.9 (***C***H_2O), 63.8 (***C***H_2N^+), 52.8 (***C***H_3N^+), 35.6 (NH***C***H_2), 32.1 (***C***$H_2CH_2CH_3$), 29.8~29.1 ((***C***H_2)$_{12}$, ***C***H_2CH_2O), 25.9 (***C***$H_2CH_2CH_2O$), 22.8 (***C***H_2CH_3), 14.3 (***C***H_3). MS *m/z*: 724.6964 $[M-I]^+$ (Calc, 724.6931). IR ν/cm^{-1}: 3290.78 (ν_{NH}), 1698.16 ($\nu_{C=O}$), 1544.70 (δ_{NH}), 1254-1230 (ν_{COC}, ν_{CN}). HPLC purity: 98.5%.

2.5. G12



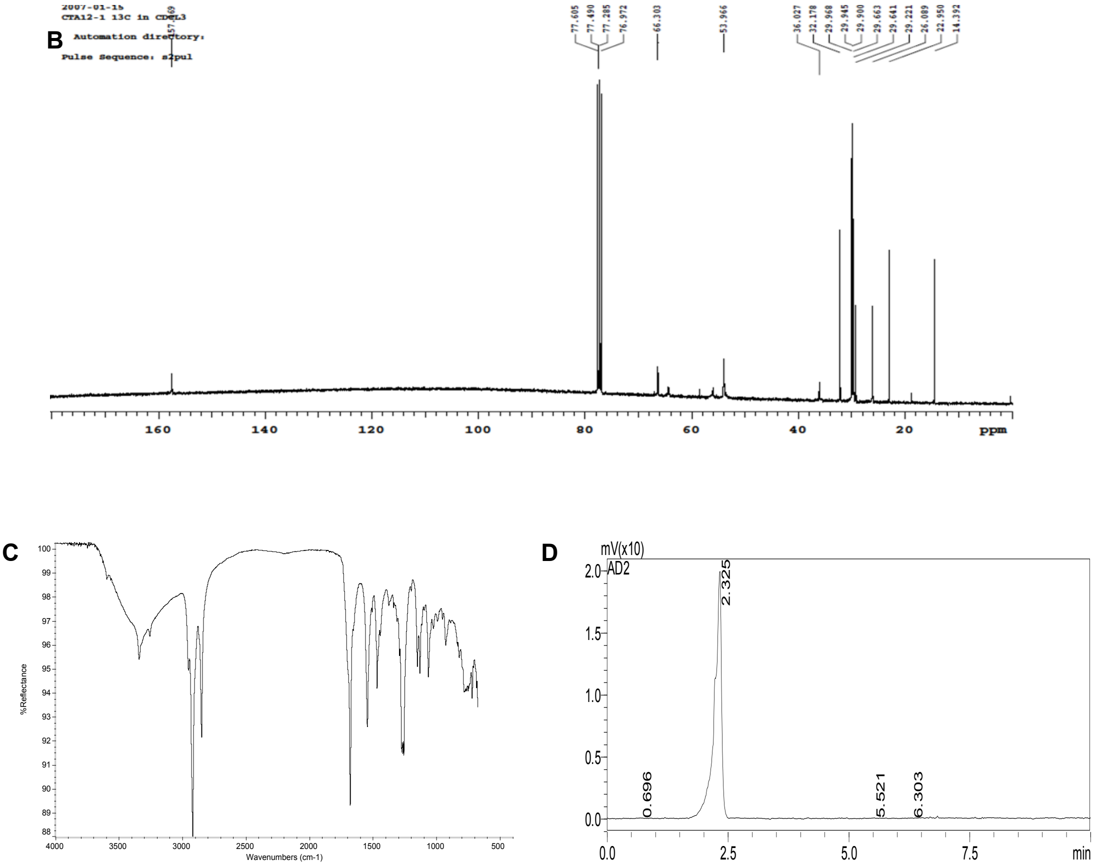
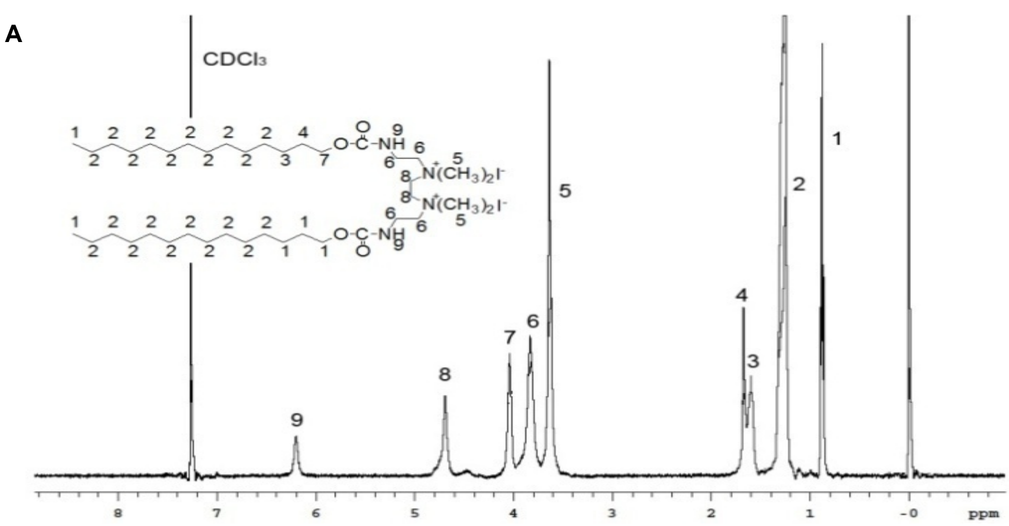


Fig. 5. Structure characterization of G12, A: ^{1}H NMR; B: ^{13}C NMR; C: IR; D: HPLC

Data assignment: ^{1}H NMR (400MHz, $CDCl_3$) δ= 6.19 (2H, s, O(C=O)N***H***), 4.68 (4H, s, $N^+C\boldsymbol{H_2}C\boldsymbol{H_2}N^+$), 4.03 (4H, t, $C\boldsymbol{H_2}O$), 3.83 (8H, s, $NHC\boldsymbol{H_2}C\boldsymbol{H_2}N^+$), 3.72 (12H, s, $C\boldsymbol{H_3}N^+$), 1.71 (4H, s, $C\boldsymbol{H_2}CH_2O$), 1.59 (4H, s, $C\boldsymbol{H_2}CH_2CH_2O$), 1.28~1.23 (32H, s, $(C\boldsymbol{H_2})_8$), 0.89~0.86 (6H, t, $CH_2C\boldsymbol{H_3}$). ^{13}C NMR (100MHz, $CDCl_3$) δ=157.4 (***C***=O), 66.3 ($\boldsymbol{C}H_2O$), 64.4 ($\boldsymbol{C}H_2N^+$), 55.9 ($N^+\boldsymbol{C}H_2\boldsymbol{C}H_2N^+$), 53.9 ($\boldsymbol{C}H_3N^+$), 36.0 ($NH\boldsymbol{C}H_2$), 32.2 ($\boldsymbol{C}H_2CH_2CH_3$), 29.9~29.2 ($((\boldsymbol{C}H_2)_6$; $\boldsymbol{C}H_2CH_2O$), 26.1 ($\boldsymbol{C}H_2CH_2CH_2O$), 22.9 ($\boldsymbol{C}H_2CH_3$), 14.4 ($\boldsymbol{C}H_3$). MS *m/z*: 755.4941 $[M-I]^+$ (Calc, 755.4911). IR ν/cm^{-1}: 3303.23 (ν_{NH}), 1685.71 ($\nu_{C=O}$), 1544.70 (δ_{NH}), 1250-1230 (ν_{COC}, ν_{CN}). HPLC purity: 98.5 %.

2.6. G14



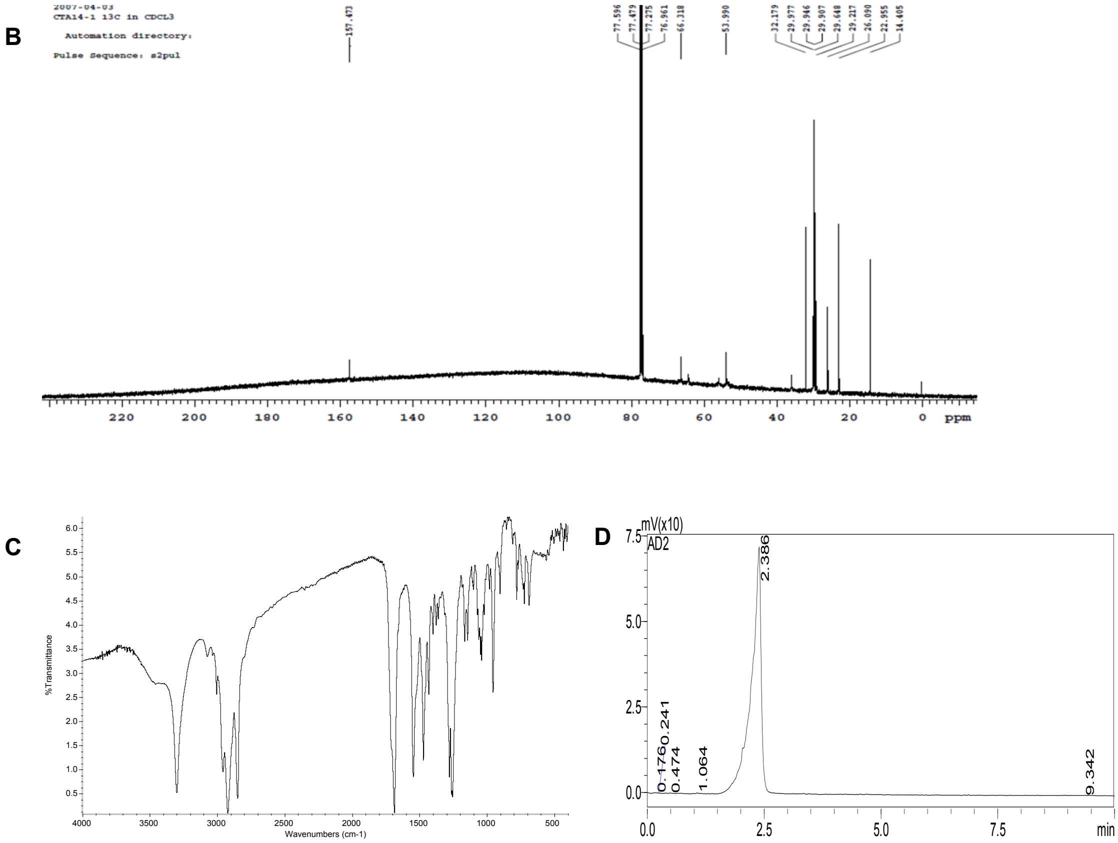
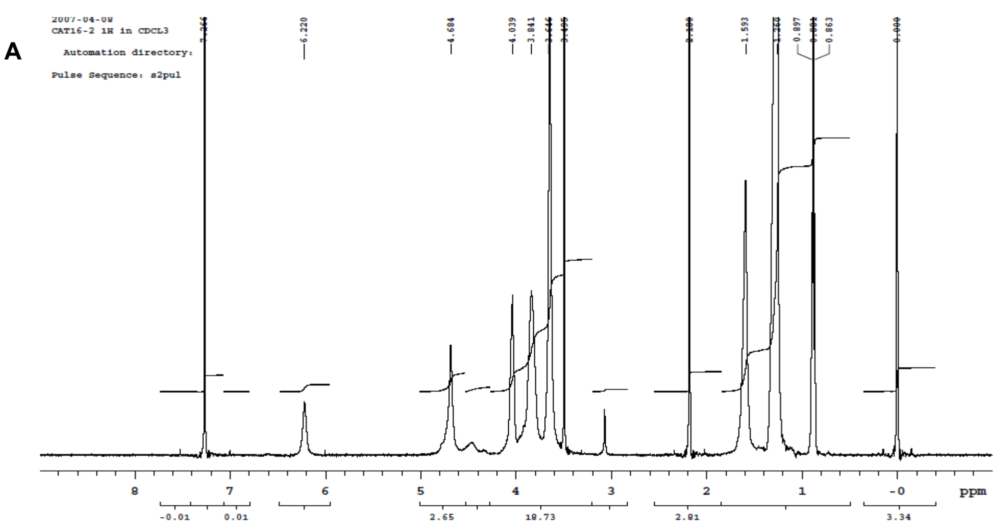


Fig. 6. Structure characterization of G14, A: ^{1}H NMR; B: ^{13}C NMR; C: IR; D: HPLC

Data assignment: ^{1}H NMR (400MHz, $CDCl_3$) δ=6.19 (2H, s, O (C=O) N***H***), 4.69 (4H, s, $N^+C\boldsymbol{H_2}C\boldsymbol{H_2}N^+$), 4.04 (4H, t, C***H***$_2$O), 3.83 (8H, s, $NHC\boldsymbol{H_2}C\boldsymbol{H_2}N^+$), 3.64 (12H, s, $C\boldsymbol{H_3}N^+$), 1.67 (4H, s, $C\boldsymbol{H_2}CH_2O$), 1.60 (4H, s, $2C\boldsymbol{H_2}CH_2\ CH_2O$), 1.26 (40H, s, $(C\boldsymbol{H_2})_{10}$), 0.89~0.86 (6H, t, $CH_2C\boldsymbol{H_3}$). ^{13}C NMR (100MHz, $CDCl_3$) δ=157.5 (***C***=O), 66.3 (***C***H_2O), 64.3 ($NHCH_2\boldsymbol{C}H_2N^+$), 55.9 ($N^+\boldsymbol{C}H_2\boldsymbol{C}H_2N^+$), 53.9 ($\boldsymbol{C}H_3N^+$), 36.0 (NH***C***$H_2$), 32.2 ($\boldsymbol{C}H_2CH_2CH_3$), 29.9~26.2 (($\boldsymbol{C}H_2)_8$; $\boldsymbol{C}H_2CH_2O$), 26.1 ($\boldsymbol{C}H_2CH_2CH_2O$), 22.9 ($\boldsymbol{C}H_2CH_3$), 14.4 ($\boldsymbol{C}H_3$). MS *m/z*: 811.5563 $[M\text{-}I]^+$ (Calc, 811.5537) and 342.3250 $[M\text{-}I]^{2+}/2$ (Calc, 342.3246). IR ν/cm^{-1}: 3299.08 (ν_{NH}), 1681.57 ($\nu_{C=O}$), 1548.85 (δ_{NH}), 1250-1230 (ν_{COC}, ν_{CN}). HPLC purity: 99.3 %.

2.7. G16



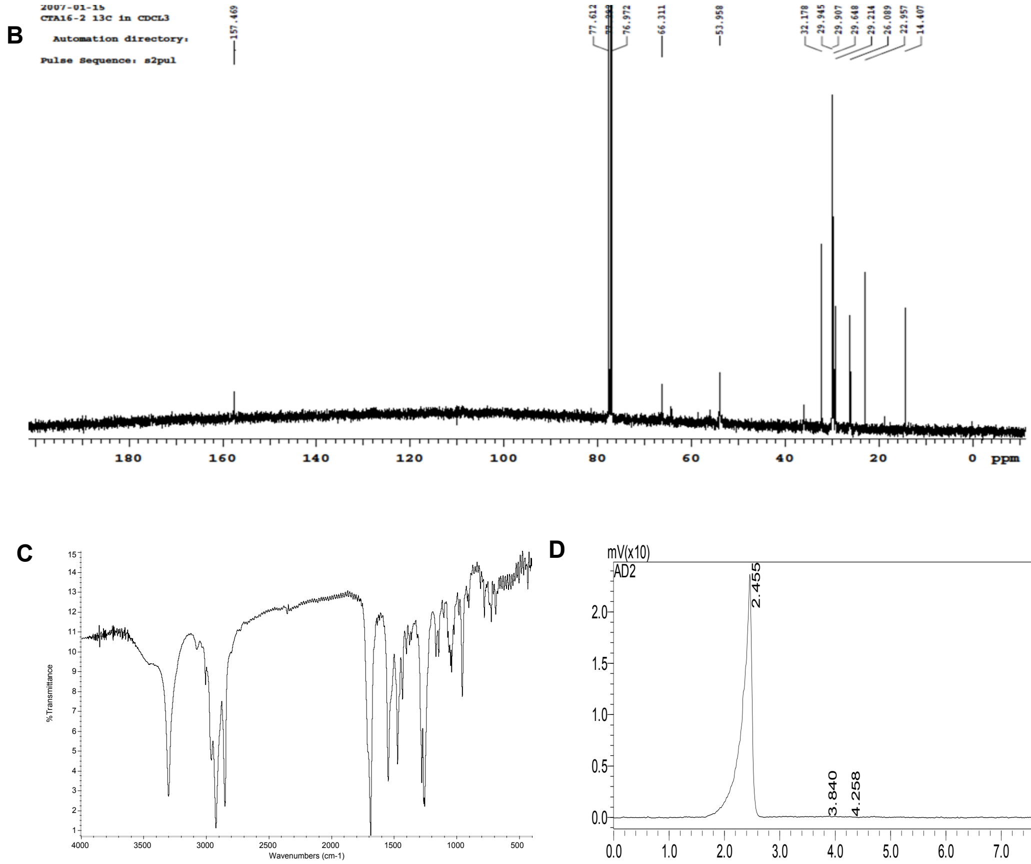
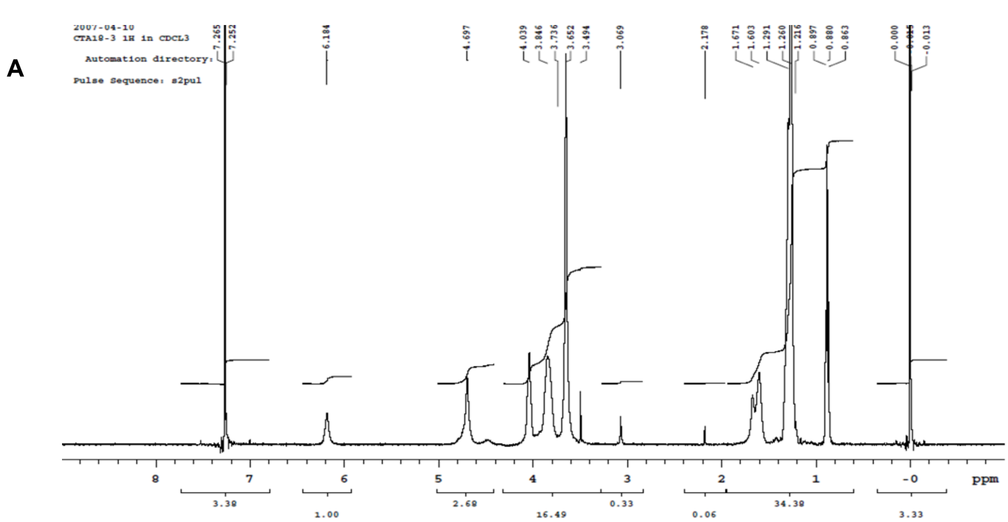


Fig. 7. Structure characterization of G16, A: [1]H NMR; B: [13]C NMR; C: IR; D: HPLC

Data assignment: [1]H NMR (400MHz, $CDCl_3$) δ=6.22 (2H, s, O(C=O)N***H***), 4.68(4H, s, $N^+C\boldsymbol{H_2}C\boldsymbol{H_2}N^+$), 4.04 (4H, s, $C\boldsymbol{H_2}O$), 3.83 (8H, s, $NHC\boldsymbol{H_2}C\boldsymbol{H_2}N^+$), 3.64 (12H, s, $C\boldsymbol{H_3}N^+$), 1.59 (4H, s, $C\boldsymbol{H_2}CH_2O$), 1.26 (48H, s, $(C\boldsymbol{H_2})_{12}$), 0.89~0.86 (6H, t, $CH_2C\boldsymbol{H_3}$). [13]C NMR (100MHz, $CDCl_3$) δ=157.3 (***C***=O), 66.2 ($\boldsymbol{C}H_2O$), 64.3 ($NHCH_2\boldsymbol{C}H_2N^+$), 55.9 ($N^+CH_2\boldsymbol{C}H_2N^+$), 53.8 ($\boldsymbol{C}H_3N^+$), 36.0 ($NH\boldsymbol{C}H_2$), 32.0 ($\boldsymbol{C}H_2CH_2CH_3$), 29.8~29.1 ($(\boldsymbol{C}H_2)_{10}$; $\boldsymbol{C}H_2CH_2O$), 25.9($\boldsymbol{C}H_2CH_2CH_2O$), 22.8 ($\boldsymbol{C}H_2CH_3$), 14.3 ($\boldsymbol{C}H_3$). MS *m/z*: 867.6172 $[M-I]^+$ (Calc, 867.6163) and 370.3546 $[M-I]^{2+}/2$ (Calc, 370.3559). IR ν/cm^{-1}: 3265.90 (ν_{NH}), 1706.45 ($\nu_{C=O}$), 1523.96 (δ_{NH}), 1250-1230 (ν_{COC}, ν_{CN}). HPLC purity: 99.1 %.

2.8. G18



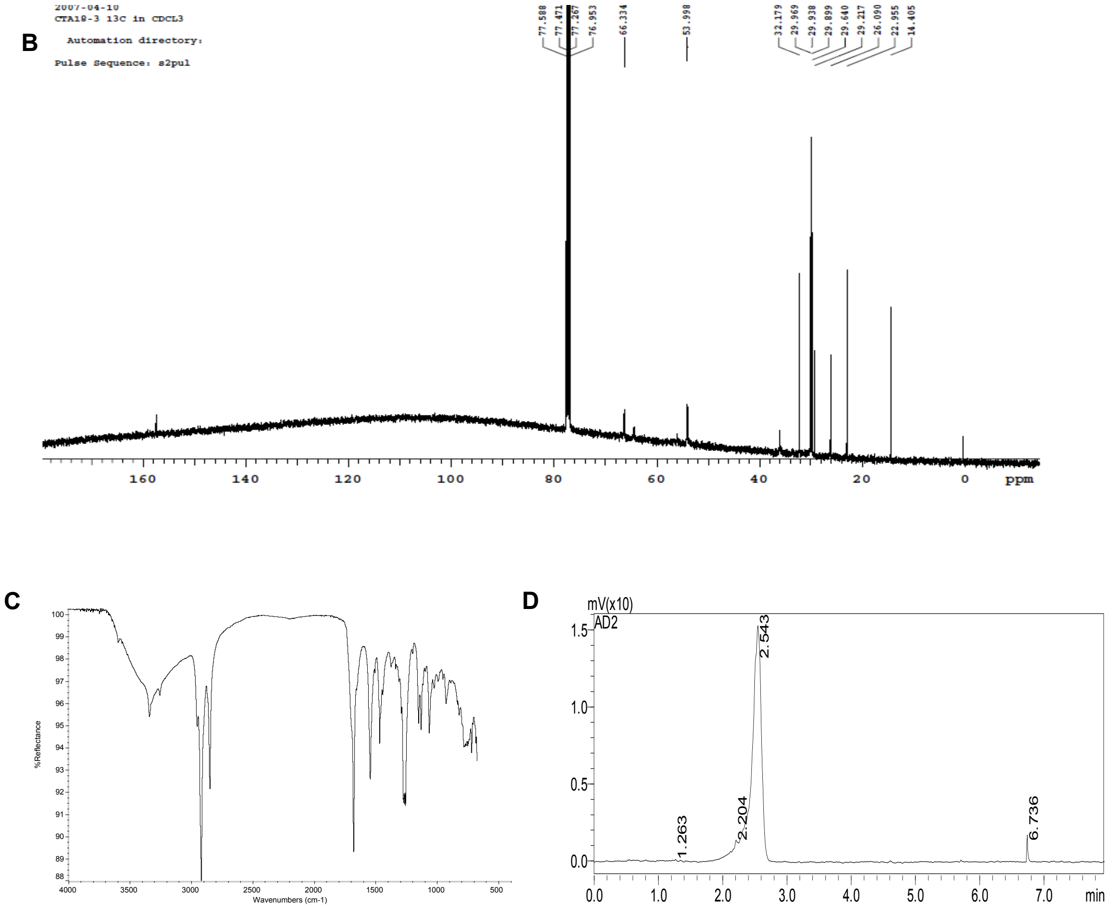


Fig. 8. Structure characterization of G18, A: ^{1}H NMR; B: ^{13}C NMR; C: IR; D: HPLC

Data assignment: ^{1}H NMR (400MHz, $CDCl_3$) δ= 6.18 (2H, s, O(C=O)N***H***), 4.69 (4H, s, $N^+C\boldsymbol{H_2}C\boldsymbol{H_2}N^+$), 4.04 (4H, s, $C\boldsymbol{H_2}O$), 3.84 (8H, s, $NHC\boldsymbol{H_2}C\boldsymbol{H_2}N^+$), 3.65 (12H, s , $C\boldsymbol{H_3}N^+$), 1.67 (4H, s, $C\boldsymbol{H_2}CH_2O$), 1.60 (4H, s, $C\boldsymbol{H_2}\ CH_2CH_2O$), 1.29~1.22 (56H, s, $(CH_2)_{14}$), 0.89~0.86 (6H, t, $CH_2C\boldsymbol{H_3}$). ^{13}C NMR (100MHz, $CDCl_3$) δ=157.6 (*C*=O), 66.3 (CH_2O), 64.3 ($NHCH_2\boldsymbol{C}H_2N^+$), 55.9 ($N^+\boldsymbol{C}H_2\boldsymbol{C}H_2N^+$), 53.9 ($\boldsymbol{C}H_3N^+$), 36.0 ($NH\boldsymbol{C}H_2$), 32.2 ($\boldsymbol{C}H_2CH_2CH_3$), 29.9~29.2 ($(\boldsymbol{C}H_2)_{12}$; $\boldsymbol{C}H_2CH_2O$), 22.5 ($\boldsymbol{C}H_2CH_3$), 14.4 ($\boldsymbol{C}H_3$). MS *m/z*: 923.6747 $[M-I]^+$ (Calc, 923.6789). IR ν/cm^{-1}: 3365.44 (ν_{NH}), 1718.85 ($\nu_{C=O}$), 1523.96 (δ_{NH}), 1254-1240 (ν_{COC}, ν_{CN}). HPLC purity: 98.4 %.

3. Lipoplex formation confirmed by gel electrophoresis

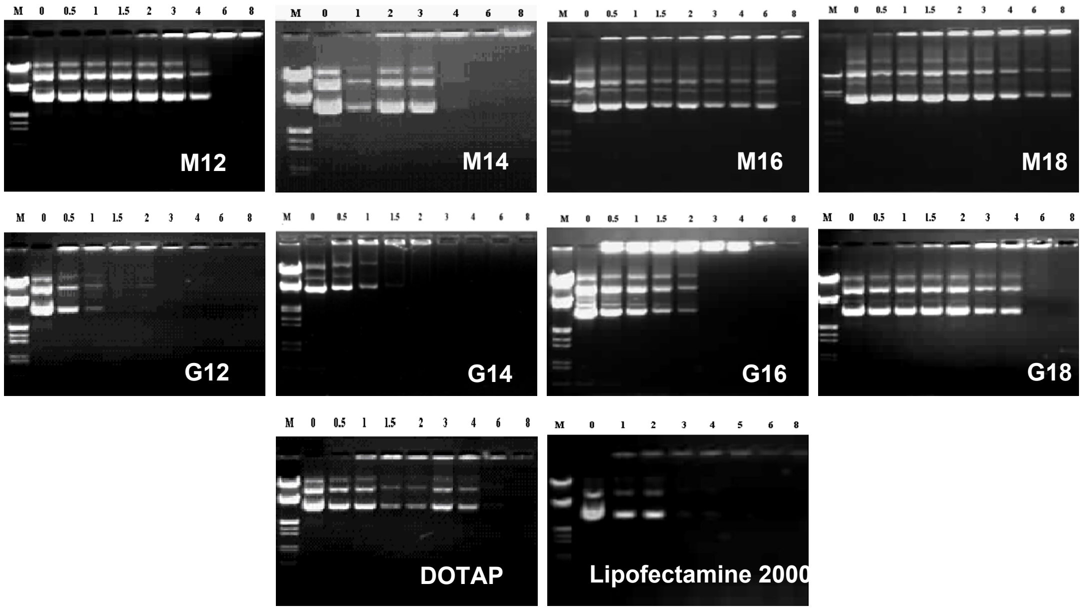


Fig. 9. Complex formation of liposomes/pGFP-N2 at various charge ratios, determined by gel electrophoresis using 1.0% agarose in tris-acetate running buffer. Lane 1: marker (λ DNA/*EcoR* I + *Hind* III Markers from SABC); lane 2: naked pDNA (0.2 µg); lanes 3-10 pDNA (0.2 µg) with N/P ratios of 0.5, 1, 1.5, 2, 3, 4, 6 and 8. For M14 lanes 3-8 correspond to ratios of 1, 2, 3, 4, 6 and 8; for Lipofectamine 2000, lanes 3-9 correspond to ratios of 1, 2, 3, 4, 5, 6, and 8.

4. Particle size, and ζ-potential of M14

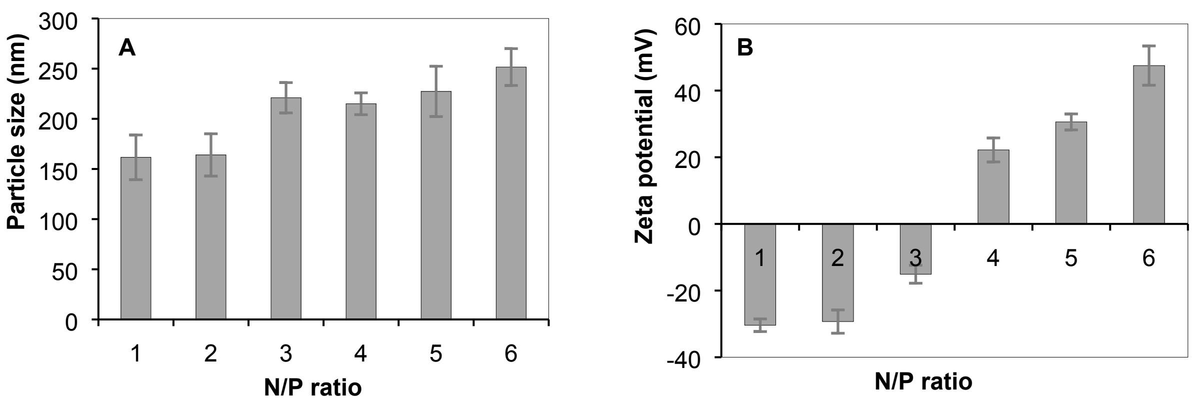


Fig. 10. Zeta potential and particle size of M14 lipoplexes at different N/P weight ratios. Lipoplexes (20 µL) were diluted in 1 mL distilled water. Zeta potential (A) and particle size (B) were measured using a Malvern ZetaSizer. The PDI at an N/P ratios of 1, 2, 3, 4, 6, and 8 was 0.196, 0.206, 0.301, 0.269, 0.304and 0.332, respectively.

5. Particle size of G series liposomes over a period of 3 months

Tab. 1. Particle size and Zeta potential of liposomes from the G series over a period of 3 months.

Liposomes	Size (nm)	PDI	Zeta potential (mV)
G12	122.3	0.212	55.1
G14	132.7	0.198	44.8
G16	141.5	0.183	51.7
G18	150.1	0.226	54.7

6. *In vitro* pDNA transfection

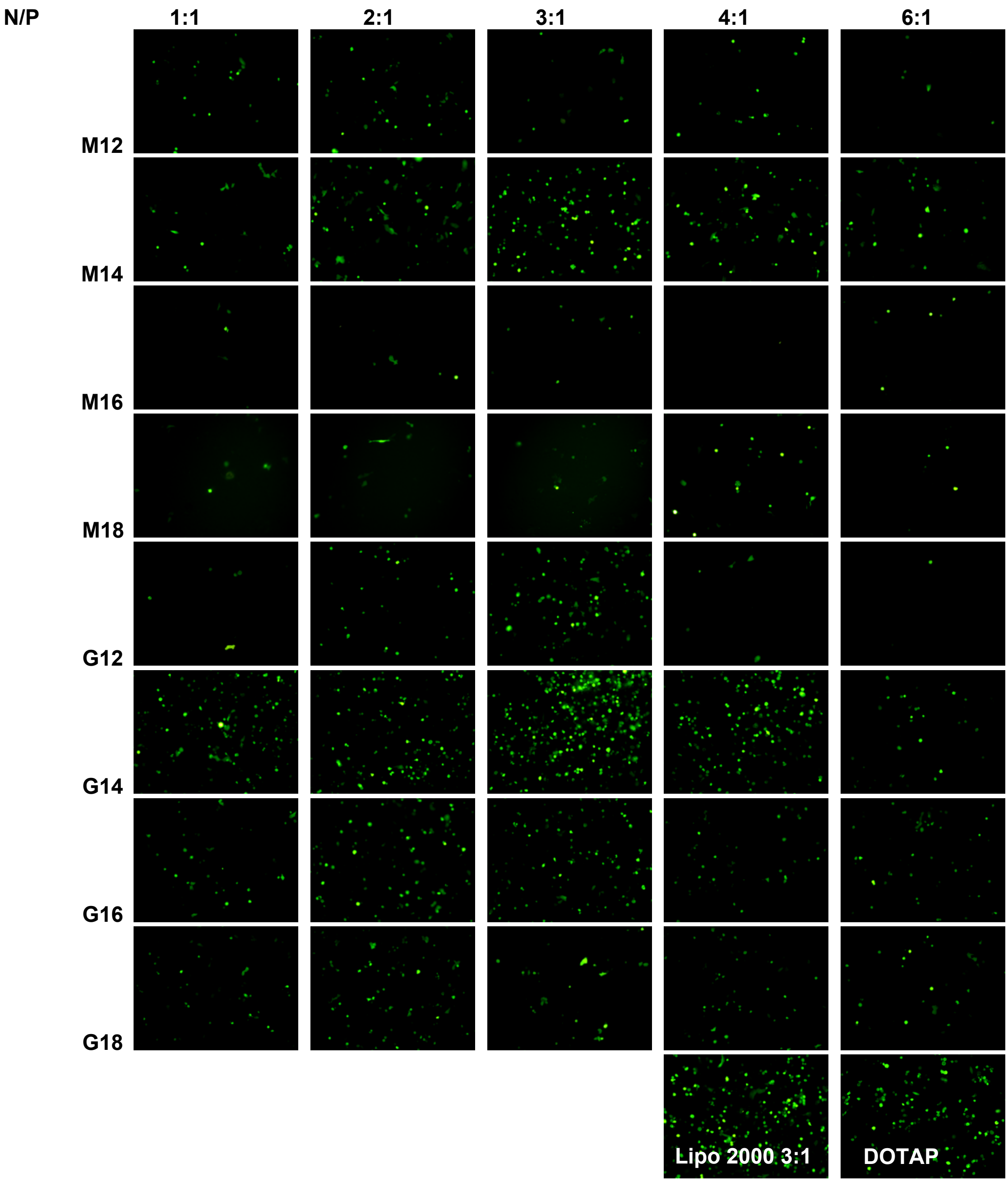


Fig. 11. Fluorescence microscopic images (20×10) of GFP gene expression in Hep-2 cells using M and G liposomes at different N/P ratios. As controls, DOTAP and Lipofectamine 2000 were used at the N/P ratio of 3:1. Images were taken 48 h after transfection.

7. *In vitro* pDNA transfection of DOTAP

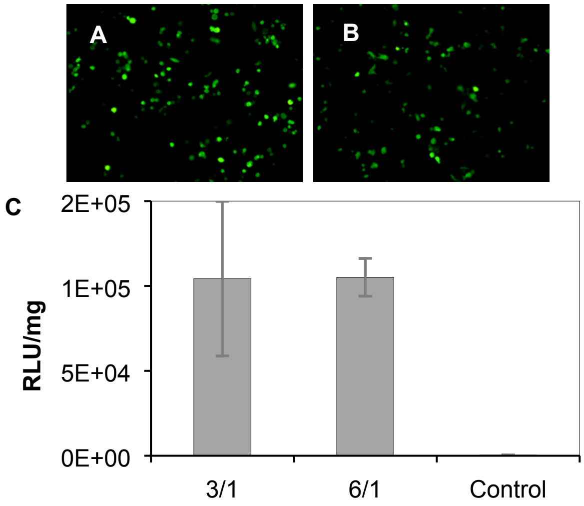


Fig. 12. Gene expression in Hep-2 cells in the presence of DOTAP at the N/P ratios of 3:1 and 6:1. (A, B: Fluorescence microscopic images (20 × 10) of GFP, A: N/P=3:1; B: N/P=6:1; C: Luciferase expression of pGL3.)