SUPPLEMENTARY INFORMATION

1. The Characterization of tri-peptide lipids

CDL12

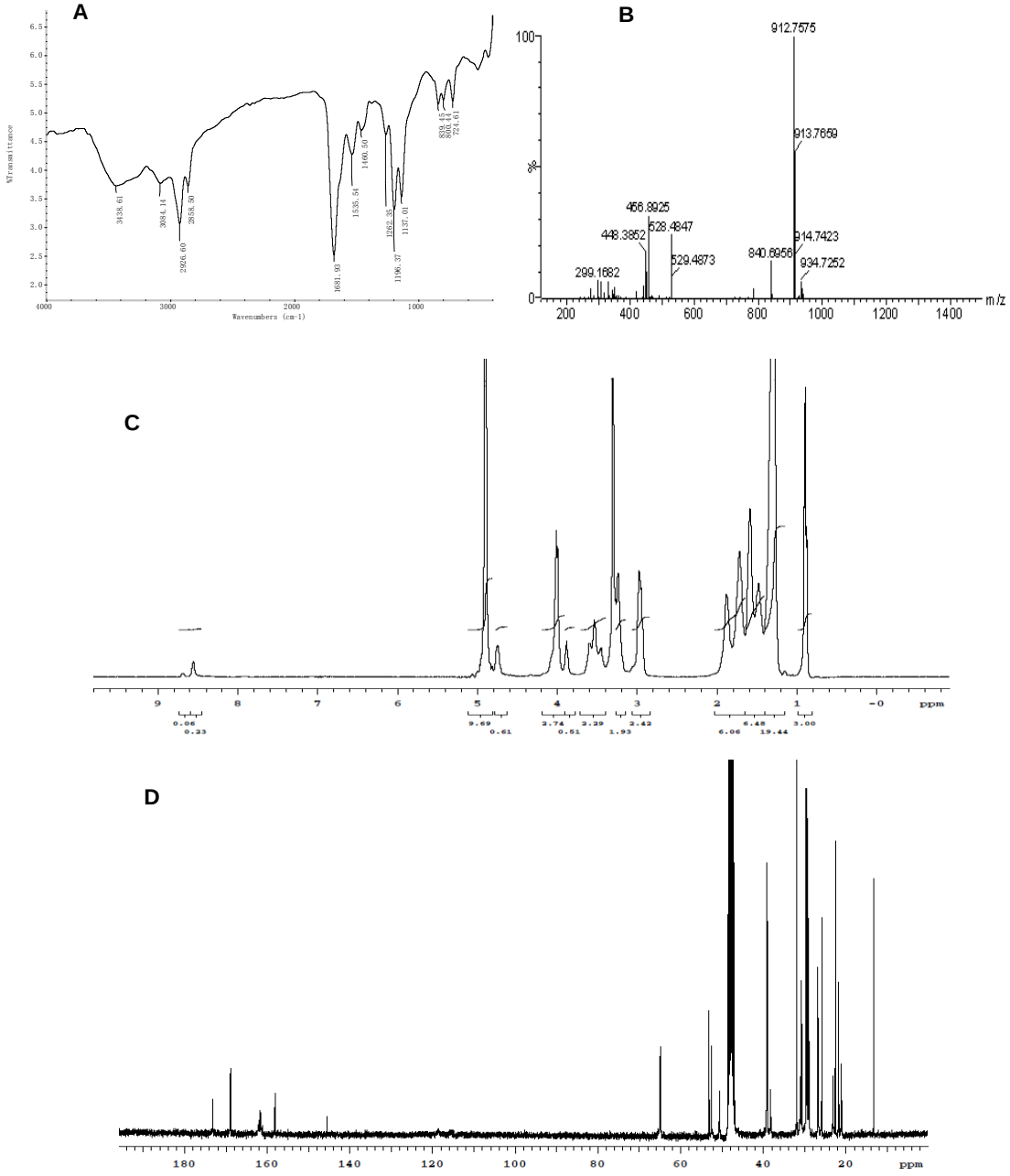

Fig. S1. Structure characterization of CDL12, A: IR; B: HR-ESI-MS; C: ^{1}H NMR; D: ^{13}C NMR.
Data assignment: ^{1}H NMR (400MHz, CD_3OD) δ: 0.901 (s,6H, C*H*$_3$); 1.294 (t, 36H, *J*= 6.8,CH_3CH_2); 1.603 (s, 4H, CH_2CH_2O); 4.009 (s, 4H, CH_2CH_2O); 3.256 (s, 6H,$NHCH_2$); 2.989 (s, 8H,CH_2N); 3.544 (t, 3H, *J* = 6.0, COC*H*); 1.893-1.911 (s, 6H, $CHCH_2CH2$); 1.536 (s, 6H, $CHCH_2CH_2CH_2$); 1.730 (s, 4H, CH_2CH_2NH). ^{13}C NMR (400MHz, CD_3OD) δ 161.317 (*C*=O), 172.85 (*C*OCH), 56.456 (*C*HNH), 27.108 (NHCH CH_2CH_2), 30.507 (CH_2CH_2NH), 42.167 ($NHCH_2$), 32.167 ($CH_2CH_2CH_3$), 33.532~32.984 ((CH_2)$_8$), 25.691-26.468 (CH_2CH_3), 17.178 (CH_3). MS *m/z*: 912.7575 $[M+H]^+$ (Calc, 913.7659). 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: 85.0 %.

CDL14

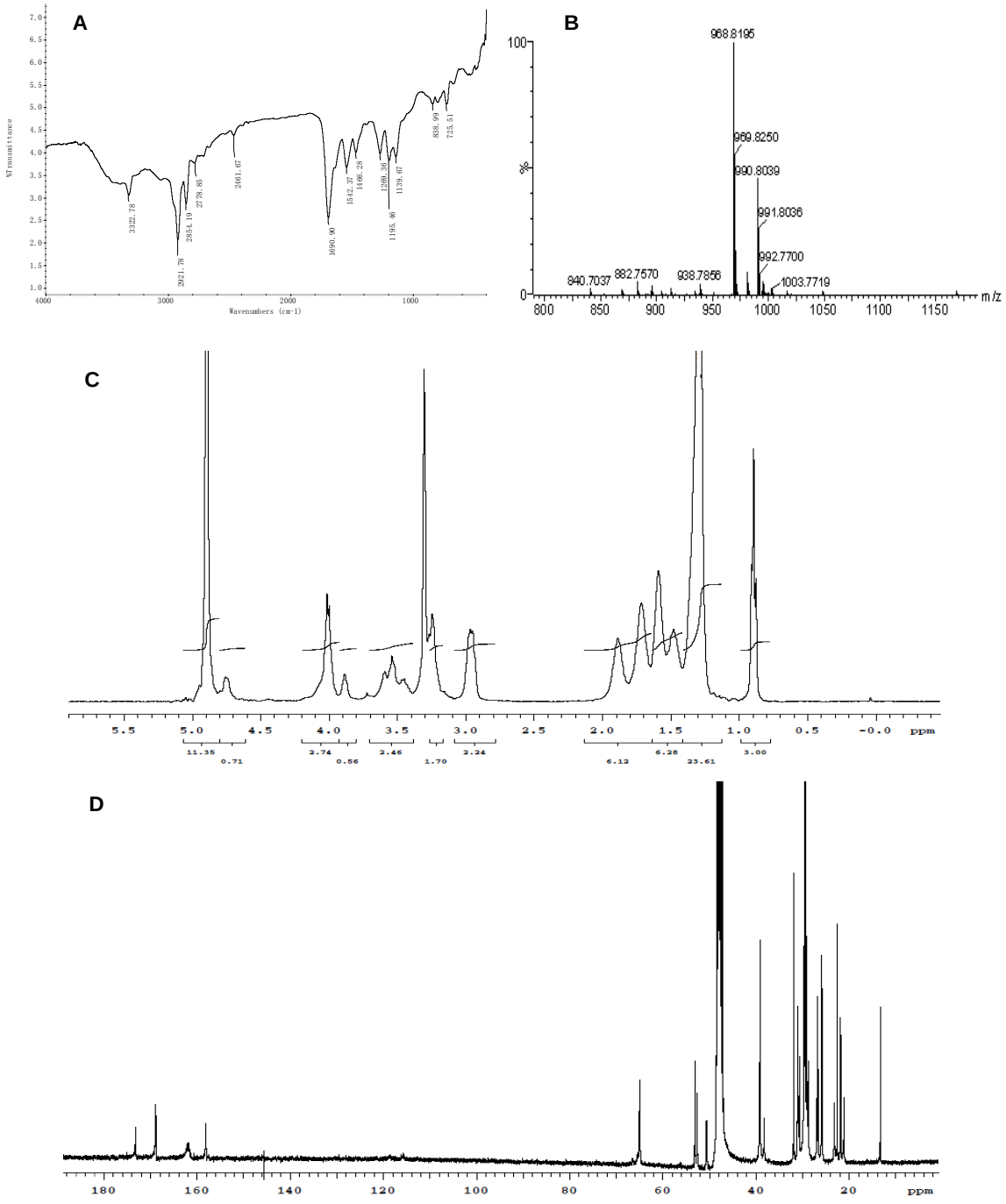

Fig. S2. Structure characterization of CDL14, A: IR; B: HR-ESI-MS; C: ^{1}H NMR; D: ^{13}C NMR.
Data assignment: ^{1}H NMR (400MHz, CD_3OD) δ: 0.902 (s,6H, CH_3); 1.293 (t, 44H, *J*= 6.8,CH_3CH_2); 1.612(s, 4H, CH_2CH_2O); 4.013 (s, 4H, CH_2CH_2O); 3.256 (s, 6H,$NHCH_2$); 2.980 (s, 8H,CH_2N); 3.544 (t, 3H, *J* = 6.0, COC*H*); 1.893-1.911 (s, 6H, $CHCH_2CH2$); 1.536 (s, 6H, $CHCH_2CH_2CH_2$); 1.724 (s, 4H, CH_2CH_2NH). ^{13}C NMR (400MHz, CD_3OD) δ 161.662 (*C*=O), 173.20 (*C*OCH), 64.999 (*C*HNH), 26.658 (NHCH CH_2CH_2), 30.69 (CH_2CH_2NH), 48.243 ($NHCH_2$), 31.879 ($CH_2CH_2CH_3$), 30.063~28.785 ($(CH_2)_8$), 26.872-25.843 (CH_2CH_3), 13.253 (CH_3). MS *m/z*: 968.8195 $[M+H]^+$ (Calc, 969.8250). IR ν/cm^{-1}: 3322.78 (ν_{NH}), 2921.78 (ν_{CH}), 1690.90 ($\nu_{C=O}$), 1542.37 (δ_{NH}), 1269.35-1195.36 (ν_{COC}, ν_{CN}). HPLC purity: 96.07 %.

CDO12

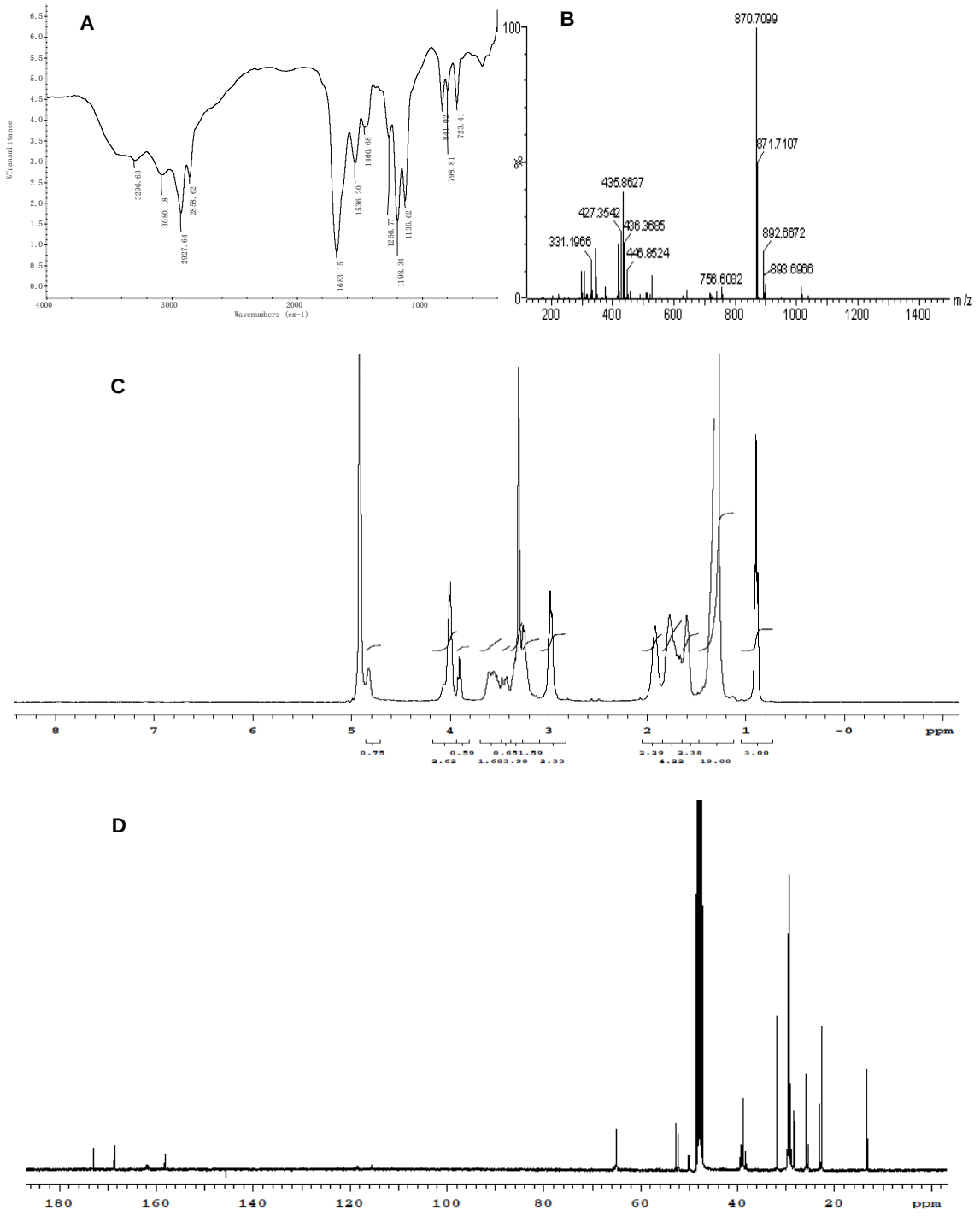

Fig. S3. Structure characterization of CDO12, A: IR; B: HR-ESI-MS; C: ^{1}H NMR; D: ^{13}C NMR.
Data assignment: ^{1}H NMR (400MHz, CD_3OD) δ: 0.895 (s,6H, C*H*$_3$); 1.286 (t, 36H, *J*= 6.8,CH_3CH_2); 1.599 (s, 4H, CH_2CH_2O); 4.010 (s, 4H, CH_2CH_2O); 3.258 (s, 6H,$NHCH_2$); 2.984 (s, 8H,CH_2N); 3.525 (t, 3H, *J* = 6.0, COC*H*); 1.917-1.930 (s, 6H, $CHCH_2CH2$); 1.286 (s, 6H, $CHCH_2CH_2CH_2$); 1.729 (s, 4H, CH_2CH_2NH). ^{13}C NMR (400MHz, CD_3OD) δ 167.368 (*C*=O), 172.176 (*C*OCH), 64.174 (*C*HNH), 27.403 (NHCH CH_2CH_2), 31.069 (CH_2CH_2NH), 37.478 ($NHCH_2$), 31.069 ($CH_2CH_2CH_3$), 27.563~28.79 ($(CH_2)_8$), 22.175-25.033 (CH_2CH_3), 12.428 (CH_3). MS *m/z*: 870.7099 $[M+H]^+$ (Calc, 871.7107). IR ν/cm^{-1}: 3296.63 (ν_{NH}), 2927.64-2858.62 (ν_{CH}), 1683.15 ($\nu_{C=O}$), 1536.20 (δ_{NH}), 1266.77-1198.34 (ν_{COC}, ν_{CN}). HPLC purity: 92.2 %.

CDO14

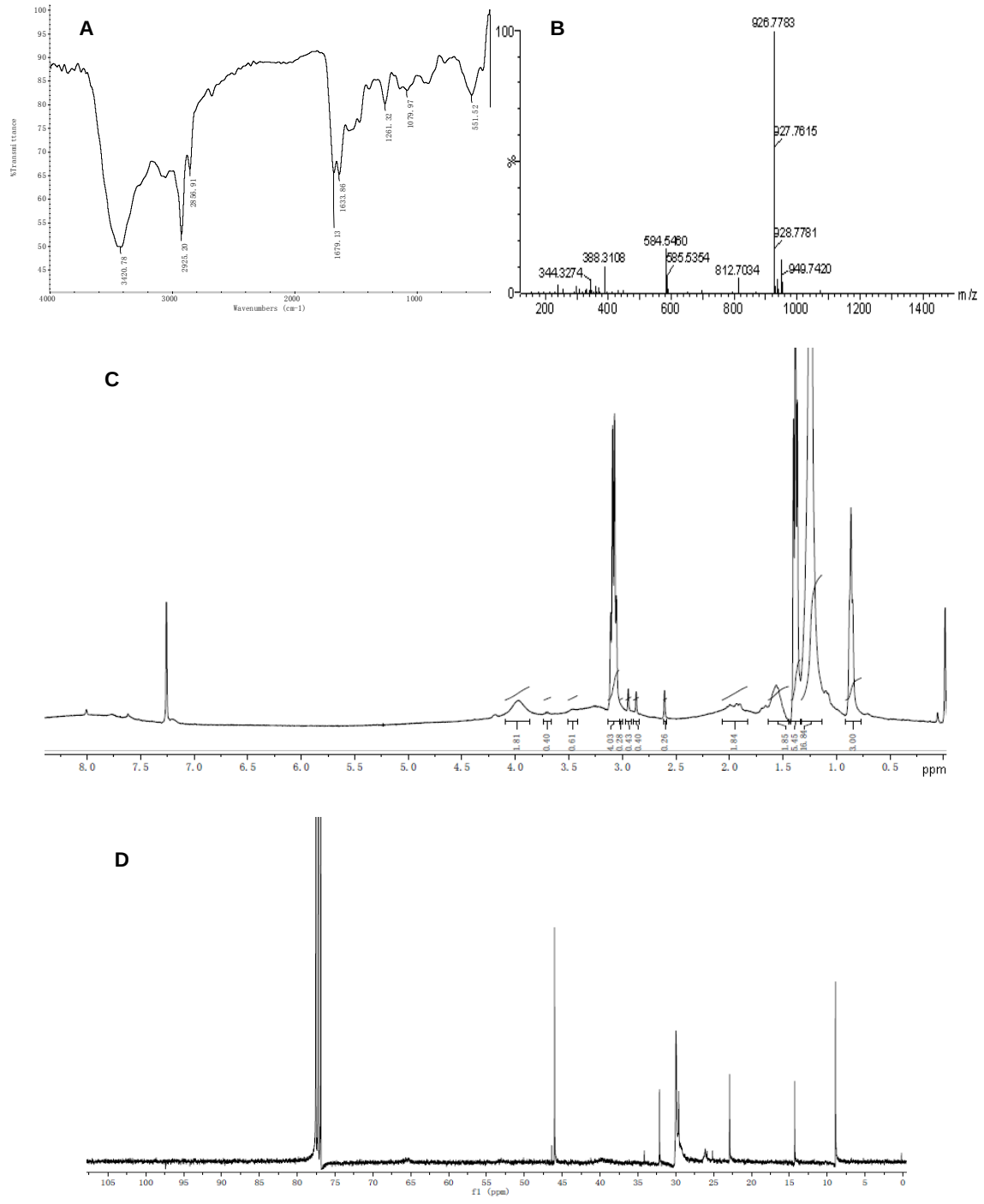

Fig. S4. Structure characterization of CDO14, A: IR; B: HR-ESI-MS; C: ^{1}H NMR; D: ^{13}C NMR.
Data assignment: ^{1}H NMR (400MHz, $CDCl_3$) δ: 0.866 (s,6H, CH_3); 1.244 (t, 44H, *J*= 6.8,CH_3CH_2); 1.564 (s, 4H, CH_2CH_2O); 3.969 (s, 4H, CH_2CH_2O); 2.945 (s, 8H,CH_2N); 2.871 (t, 3H, *J* = 6.0, COC*H*); 1.906-1.998 (s, 6H, $CHCH_2CH2$); 1.564 (s, 6H, $CHCH_2CH_2CH_2$); 1.660 (s, 4H, CH_2CH_2NH). ^{13}C NMR (400MHz, $CDCl_3$) δ 46.011 (*C*HNH), 22.888 (NHCH CH_2CH_2), 14.285 (CH_2CH_2NH), 32.146 ($CH_2CH_2CH_3$), 29.594~29.971 ((CH_2)$_8$), 25.146-26.038 (CH_2CH_3), 8.898 (CH_3). MS *m/z*: 926.7783 $[M+H]^+$ (Calc, 927.7615). IR ν/cm^{-1}: 3420.78 (ν_{NH}), 2925.20 (ν_{CH}), 1679.13 ($\nu_{C=O}$), 1261.32(ν_{CN}). HPLC purity: 92.3 %.

2. TEM images of liposome and lipoplex with DNA

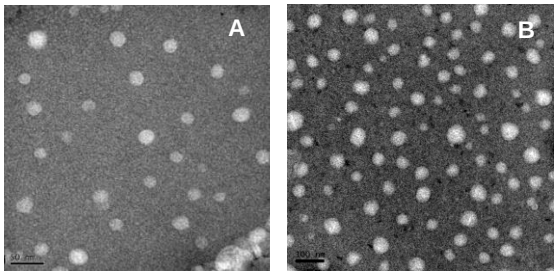

Fig. S5. TEM images of CDO14 liposome and CDO14/DNA lipoplex. (A) liposome, 30 K, bar: 50 nm; (B) Lipoplex, N/P = 3:1, 10 K, bar: 100 nm.

3. DNA-binding assay

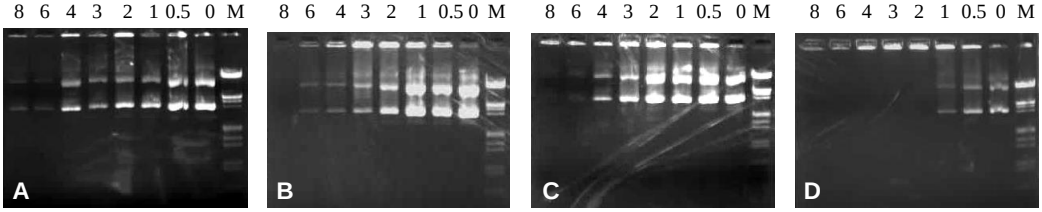

Fig. S6. Gel electrophoresis of liposomes/pDNA complexes at various weight ratios (A: CDL12D; B: CDL14D; C: CDO12D; D: CDO14D). Lane M: marker (λ DNA/*EcoR* I + *Hind* III Markers), lane 0: naked pDNA and lanes 0.5-8: pDNA with progressively increasing proportions of liposome, 0.5/1, 1/1, 2/1, 3/1, 4/1, 6/1, 8/1.

4. *In vitro* pDNA transfection

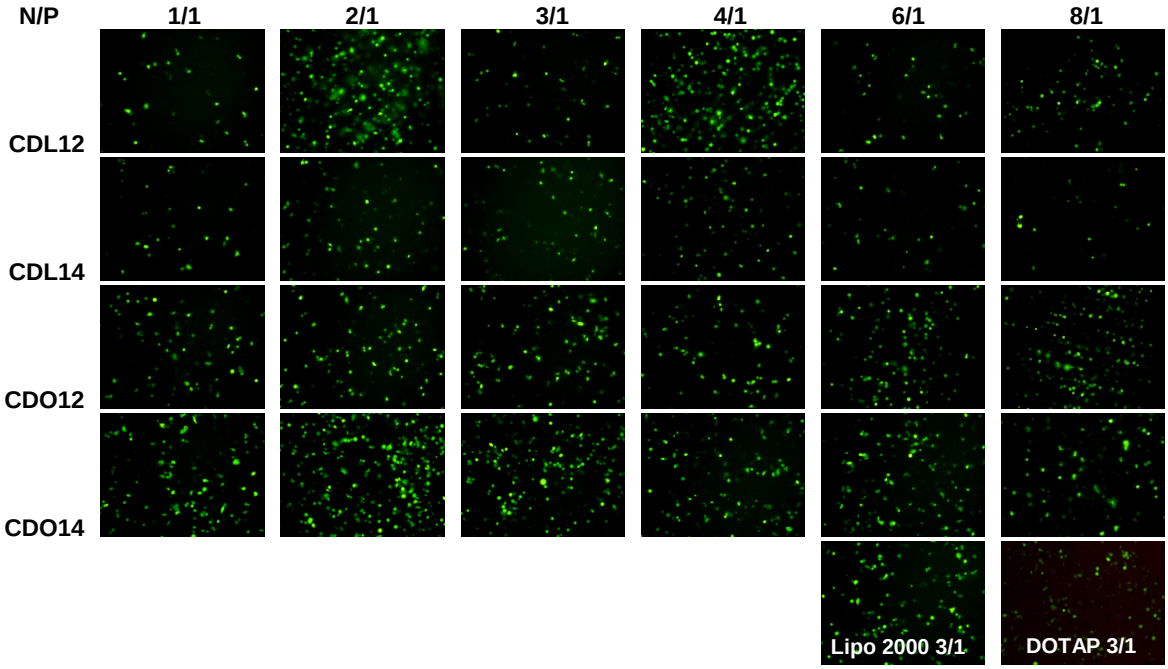

Fig. S7. Fluorescent images of NCI-H460 cells at 48 h after treatment with tri-peptide liposomes, compared to Lipofectamine 2000 and DOTAP at the N/P ratio of 3/1.

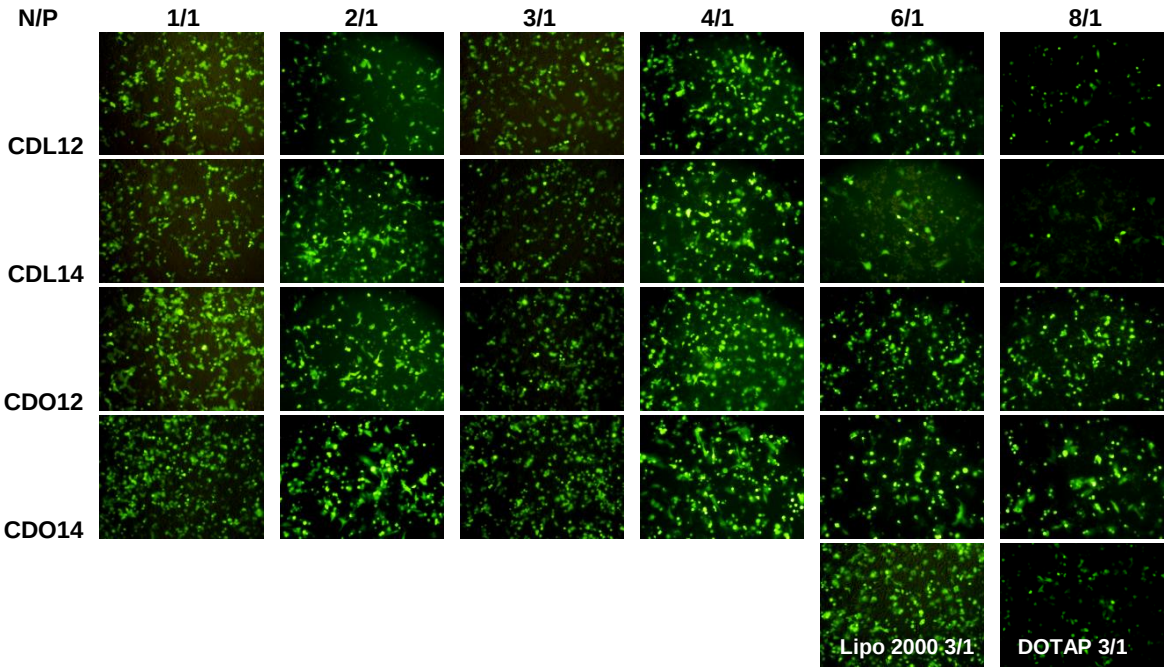

Fig. S8. Fluorescent images of Hep-2 cells at 48 h after treatment with tri-peptide liposomes, compared to Lipofectamine 2000 and DOTAP at the N/P ratio of 3/1.