Supporting information to accompany:

Towards quantitative structure-activity correlation in transfection promoted by pyridinium cationic lipids

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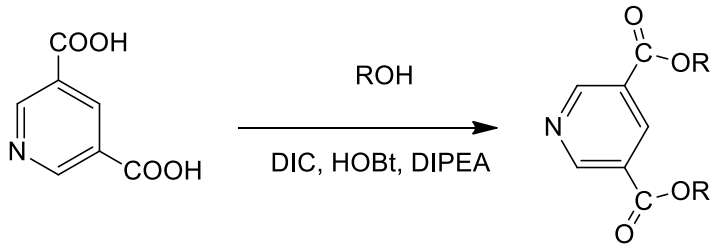
Contents

General procedures for synthesis

Most chemicals and solvents were used as received from known suppliers, except THF and DMF which were dried and distilled before use. Compounds were purified by column chromatography using silica gel 60.NMR spectra were collected on a Bruker AC300 (300 MHz) or Bruker Avance (500 MHz) instrument. Proton (^{1}H) chemical shift are reported in part per million (ppm, δ scale), referenced with residual proton shift of $CDCl_3$ (7.26). The ^{1}H NMR results are reported as shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiple, br = broad), integration and J value (Hz). ^{13}C NMR are reported in part per million and are referenced to carbon resonance of $CDCl_3$ (77.23). ESI-Mass spectra were recorded on a Waters MicroMass Q-TOF instrument running in positive ion mode. Elemental analysis was done at Canadian Microanalytical Service Ltd. Compounds diC12:0, diC14:0, diC16:0, diC18:0, and diC18:1 and their precursor pyridines are known (Pijper, D.; Bulten, E.; Smisterova, J.; Wagenaar, A.; Hoekstra, D.; Engberts, J.; Hulst, R. Novel biodegradable pyridinium amphiphiles for gene delivery *Eur. J. Org. Chem.* **2003**, *2003*, 4406. doi: 10.1002/ejoc.200300361); spectroscopic characterization data are provided for completeness.

Ester coupling:



To a solution of 1 equivalent of 3,5-pyridinedicarboxylic acid in relation to 2.4 equivalent of the alcohol in THF were added 2.4 equivalents of *N*,*N*-diisopropyl carbodiimide (DIC), hydroxybenzotriazole (HOBt) and *N*,*N*-diisopropylethyl amine (DIPEA). The reaction was sealed under an atmosphere of N_2, and stirred for 24 h at rt. Once complete, the reaction was filtered to remove DIU, and diluted with dichloromethane (DCM). The organic phase was extracted with phosphate buffer (pH = 3) (twice), water (twice) 10% NaCl (twice) and rinsed with sat. NaCl (once), dried with anhydrous sodium sulfate, and concentrated under vacuum. The crude product was purified by column chromatography on silica gel, using a gradient of ether in hexanes as eluent.

Synthesis of diC9:0 pyridine

Ester coupling conditions: 1 equivalent of 3,5-pyridinedicarboxylic acid (0.835 g, 5 mmol), 2.4 equivalents of DIC (1.514 g, 12 mmol), 2.4 equivalents of HOBt (1.620 g, 12 mmol), 2.4 equivalents of 1-nonaol (1.731 g, 12 mmol) and 2.4 equivalents of DIPEA (1.550 g, 12 mmol) were stirred in THF at rt for 24 h. Standard work-up and purification by silica gel chromatography, using 15% ether/hexanes as eluent, yields ***2-10*** as a white solid, 1.777 g (84%). NMR ($CDCl_3$) ^{1}H δ: 9.35 (d, J=3 Hz, 2H), 8.85 (t, J=6 Hz, 1H), 4.38 (t, J=15 Hz, 4H) , 1.82 (m, 4H), 1.25 (m, 28 H), 0.88 (m, 6H). ^{13}C δ: 164.4, 154.0, 137.8, 126.2, 65.9, 31.7, 29.5, 28.0, 25.9, 22.5, 14.1. MS (+ve ESI): calc'd for $C_{25}H_{42}NO_4^+$ = 420.311 amu, obtained = 420.315 amu.

Synthesis of diC11:0 pyridine

Ester coupling conditions: 1 equivalent of 3,5-pyridinedicarboxylic acid (0.835 g, 5 mmol), 2.4 equivalents of DIC (1.564 g, 12 mmol), 2.4 equivalents of HOBt (1.840 g, 12 mmol), 2.4 equivalents of 1-undecanol (2.068 g, 12 mmol) and 2.4 equivalents of DIPEA (1.854 g, 12 mmol) were stirred in THF at rt for 24 h. Standard work-up and purification by silica gel chromatography, using 15% ether/hexanes as eluent, yields ***2-12*** as a white solid, 1.235 g (51%). NMR ($CDCl_3$) ^{1}H δ: 9.35 (d, J=3 Hz, 2H), 8.85 (t, J=6 Hz, 1H), 4.38 (t, J=15 Hz, 4H), 1.79 (m, 4H), 1.26 (m, 36H), 0.87 (br t, 6H). ^{13}C δ: 164.3, 154.50, 138.4, 126.6, 66.1, 31.8, 29.3, 28.5, 25.9, 22.6, 14.0. MS (+ve ESI): calc'd for $C_{29}H_{50}NO_4^+$ = 476.373 amu, obtained = 476.377 amu.

Synthesis of diC12:0 pyridine

Ester coupling conditions: 1 equivalent of 3,5-pyridinedicarboxylic acid (0.835 g, 5 mmol), 2.4 equivalents of DIC (1.564 g, 12 mmol), 2.4 equivalents of HOBt (1.840 g, 12 mmol), 2.4 equivalents of 1-dodecanol (2.238 g, 12 mmol) and 2.4 equivalents of DIPEA (1.854 g, 12 mmol) were stirred in THF at rt for 24 h. Standard work-up and purification by silica gel chromatography, using 15% ether/hexanes as eluent, yields ***2-14*** as a white solid, 1.01 g (40%). NMR ($CDCl_3$) ^{1}H δ: 9.36 (d, J=3 Hz, 2H), 8.85 (t, J=6 Hz, 1H), 4.39 (t, J=15 Hz, 4H), 1.80 (m, 4H), 1.27 (m, 36H), 0.88 (br t, 6H). ^{13}C δ: 164.5, 154.0, 137.9, 126.5, 65.9, 31.8, 29.3, 28.6, 25.9, 22.6, 14.0. MS (+ve ESI; low res): calc'd for $C_{31}H_{54}NO_4^+$ = 504.405 amu, obtained = 504.409 amu.

Synthesis of diC14:0 pyridine

Ester coupling conditions: 1 equivalent of 3,5-pyridinedicarboxylic acid (0.835 g, 5 mmol), 2.4 equivalents of DIC (1.564 g, 12 mmol), 2.4 equivalents of HOBt (1.840 g, 12 mmol), 2.4 equivalents of 1-tetradecanol (2.5750 g, 12 mmol) and 2.4 equivalents of DIPEA (1.854 g, 12 mmol) were stirred in THF at rt for 24 h. Standard work-up and purification by silica gel chromatography, using 15% ether/hexanes as eluent, yields ***2-16*** as a white solid, 0.732 g (26%). NMR ($CDCl_3$) ^{1}H δ: 9.36 (br s, 2H), 8.87 (t, J=3 Hz, 1H) , 4.39 (t, J=15 Hz, 4H) , 1.79 (m, 4H), 1.26 (m, 48H), 0.89 (br t, 6H). ^{13}C δ: 164.5, 154.0, 137.9, 126.3, 65.9, 31.8, 29.4, 28.6, 25.9, 22.6, 14.0. MS (+ve ESI; low res): calc'd for $C_{35}H_{62}NO_4^+$ = 560.4 amu, obtained = 560.4amu.

Synthesis of diC18:0 pyridine

Ester coupling conditions: 1 equivalent of 3,5-pyridinedicarboxylic acid (0.835 g, 5 mmol), 2.4 equivalents of DIC (1.564 g, 12 mmol), 2.4 equivalents of HOBt (1.840 g, 12 mmol), 2.4 equivalents of 1-octadecanol (3.245 g, 12 mmol) and 2.4 equivalents of DIPEA (1.854 g, 12 mmol) were stirred in THF at rt for 24 h. Standard work-up and purification by silica gel chromatography, using 15% ether/hexanes as eluent, yields ***2-18*** as a white solid, 1.09 g (32%). NMR ($CDCl_3$) ^{1}H δ: 9.34 (br s, 2H), 8.83 (t, J=3 Hz, 1H), 4.36 (t, J=12 Hz, 4H), 1.78 (m, 4H), 1.23 (m, 64H), 0.89(br t, 6H). ^{13}C δ: 164.5, 154.0, 137.9, 126.3, 65.9, 31.9, 29.4, 28.6, 25.9, 22.6, 14.0. MS (+ve ESI; low res): calc'd for $C_{43}H_{78}NO_4^+$ = 672.5 amu, obtained = 672.4 amu.

Synthesis of diC20:0 pyridine

Ester coupling conditions: 1 equivalent of 3,5-pyridinedicarboxylic acid (0.334 g, 1.2 mmol), 2.4 equivalents of DIC (0.363 g, 2.88 mmol), 2.4 equivalents of HOBt (0.735 g, 2.88 mmol), 2.4 equivalents of 1-eicosanol (0.373 g, 2.88 mmol) and 2.4 equivalents of DIPEA (1.854 g, 2.88 mmol) were stirred in THF at rt for 24 h. Standard work-up and purification by silica gel chromatography, using 15% ether/hexanes as eluent, yields ***2-20*** as a white solid, 0.308 g (35%). NMR ($CDCl_3$) ^{1}H δ: 9.36 (d, J=3 Hz, 2H), 8.85 (t, J=6 Hz, 1H), 4.38 (t, J=12 Hz, 4H), 1.80 (m, 4H), 1.26 (m, 72 H), 0.88 (br t, 6H). ^{13}C δ: 164.5, 154.0, 137.9, 126.3, 65.9, 31.9, 29.4, 28.6, 25.9, 22.6, 14.0. MS (+ve ESI): calc'd for $C_{47}H_{86}NO_4^+$ = 728.655 amu, obtained = 728.359 amu.

Synthesis of diisoC9:0 pyridine

Ester coupling conditions: 1 equivalent of 3,5-pyridinedicarboxylic acid (0.835 g, 5 mmol), 2.4 equivalents of DIC (1.564 g, 12 mmol), 2.4 equivalents of HOBt (1.840 g, 12 mmol), 2.4 equivalents of 3,5,5-trimethylhexan-1-ol (1.731 g, 12 mmol) and 2.4 equivalents of DIPEA (1.854 g, 12 mmol) were stirred in THF at rt for 24 h. Standard work-up and purification by silica gel chromatography, using 15% ether/hexanes as eluent, yields ***2-22*** as a colorless oil, 1.233 g (58%). NMR ($CDCl_3$) ^{1}H δ: 9.34 (d, J=3 Hz, 2H), 8.83 (t, J=3 Hz, 1H), 4.41 (t, J=15 Hz, 4H), 1.83 (m, 6H), 0.90-1.63 (m, 30H). ^{13}C δ: 164.4, 154.0, 137.8, 126.2, 64.3, 50.9, 37.7, 31.0, 29.3, 27.2, 26.2, 22.5. MS (+ve ESI): calc'd for $C_{25}H_{42}NO_4^+$ = 420.311 amu, obtained = 420.286 amu.

Synthesis of dibrC20:0 pyridine

Ester coupling conditions: 1 equivalent of 3,5-pyridinedicarboxylic acid (0.835 g, 5 mmol), 2.4 equivalents of DIC (1.564 g, 12 mmol), 2.4 equivalents of HOBt (1.840 g, 12 mmol), 2.4 equivalents of nonadecan-9-ol (3.583 g, 12 mmol) and 2.4 equivalents of DIPEA (1.854 g, 12 mmol) were stirred in THF at rt for 24 h. Standard work-up and purification by silica gel chromatography, using 15% ether/hexanes as eluent, yields ***2-24*** as a colorless oil, 2.144 (67%). NMR ($CDCl_3$) ^{1}H δ: 9.35 (d, J=3 Hz, 2H), 8.84 (t, J=3 Hz, 1H), 4.28 (d, J=6 Hz, 4H), 1.80 (m, 2H), 1.27 (m, 64H), 0.87 (br t, 12H). ^{13}C δ: 164.5, 154.0, 137.8, 126.3, 68.5, 37.4, 31.8, 31.3, 29.5, 26.7, 22.6, 14.0. MS (+ve ESI): calc'd for $C_{47}H_{86}NO_4^+$ = 728.655 amu, obtained = 728.660 amu.

Synthesis of diC18:1 pyridine

Ester coupling conditions: 1 equivalent of 3,5-pyridinedicarboxylic acid (0.835 g, 5 mmol), 2.4 equivalents of DIC (1.564 g, 12 mmol), 2.4 equivalents of HOBt (1.840g, 12 mmol), 2.4 equivalents of oleyl alcohol (3.220 g, 12 mmol) and 2.4 equivalents of DIPEA (1.854 g, 12 mmol) were stirred in THF at rt for 24 h. Standard work-up and purification by silica gel chromatography, using 15%ether/hexanes as eluent, yields ***2-26*** as a colorless oil, 0.537g (16%). NMR ($CDCl_3$) ^{1}H δ: 9.35 (d, J=3 Hz, 2H), 8.85 (t, J=6 Hz, 1H, 6), 5.36 (m, 4H), 4.38 (t, J=12 Hz, 4H), 2.00 (m, 8H), 1.79 (m, 4H), 1.27 (m, 48H), 0.90 (br t, 6H). ^{13}C δ: 164.5, 154.0, 137.9, 129.9, 129.7, 126.3, 65.9, 32.5, 31.8, 29.3, 28.6, 27.1, 25.9, 22.6, 14.0. MS (+ve ESI; low res): calc'd for $C_{47}H_{74}NO_4^+$ = 668.5 amu, obtained = 668.4 amu.

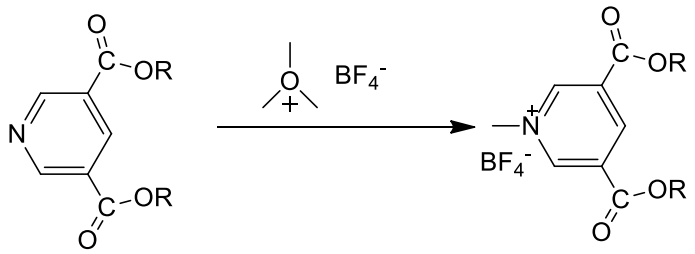
Synthesis of diC11:1 pyridine

Ester coupling conditions: 1 equivalent of 3,5-pyridinedicarboxylic acid (0.835 g, 5 mmol), 2.4 equivalents of DIC (1.564 g, 12 mmol), 2.4 equivalents of HOBt (1.840 g, 12 mmol), 2.4 equivalents of 10-undecen-1-ol (2.043 g, 12 mmol) and 2.4 equivalents of DIPEA (1.854 g, 12 mmol) were stirred in THF at rt for 24 h. Standard work-up and purification by silica gel chromatography, using 15% ether/hexanes as eluent, yields ***2-28*** as a colorless oil, 1.069 g (45%). NMR ($CDCl_3$) ^{1}H δ: 9.36 (d, J=3 Hz, 2H), 8.85 (t, J=6 Hz, 1H), 5.76 (m, 2H), 4.97 (m, 4H), 4.38 (t, J=15 Hz, 4H), 2.03 (m, 4H), 1.82 (m, 4H), 1.31 (m, 28 H). ^{13}C δ: 164.5, 154.0, 139.1, 137.9, 128.3, 114.1, 65.9, 33.7, 29.4, 29.3, 29.2, 29.0, 28.8, 28.5, 25.9. MS (+ve ESI): calc'd for $C_{29}H_{46}NO_4^+$ = 472.342 amu, obtained = 472.346 amu.

Synthesis of (C16:0)(C11:1) pyridine

Ester coupling conditions: 1 equivalent of 3,5-pyridinedicarboxylic acid (0.835 g, 5 mmol), 2.4 equivalents of DIC (1.564 g, 12 mmol), 2.4 equivalents of HOBt (1.840 g, 12 mmol), undecen-1-ol (1.022 g, 6 mmol), 1.2 equivalents of 1-hexadecanol (1.4593 g, 6 mmol) and 2.4 equivalents of DIPEA (1.854 g, 12 mmol) were stirred in THF at rt for 24 h. Standard work-up and purification by silica gel chromatography, using 15% ether/hexanes as eluent, yields 1.0395 g of white solid which was later determined via ESI mass spectroscopy of methylated analogues to contain 22% of ***2-4***, 28% of ***2-28*** and 50% of ***4-1***. NMR ($CDCl_3$) ^{1}H δ: 9.35 (d, J=3 Hz, 2H), 8.84 (t, J=6 Hz, 1H), 5.79 (m, 1H), 4.95 (m, 2H), 4.37 (t, J=15 Hz, 4H), 2.00 (m, 2H), 1.79 (m, 4H), 1.27 (m, 38H), 0.87 (br t, 3H). ^{13}C δ: 164.5, 149.2, 154.0, 139.1, 137.9, 126.3, 114.1, 65.9, 33.7, 31.8, 29.3, 28.8, 25.9, 22.9, 14.0. MS (+ve ESI; low res): calc'd for (2-4) $C_{39}H_{70}NO_4^+$ = 616.5 amu, obtained = 616.7 amu. calc'd for (2.28) $C_{29}H_{46}NO_4^+$ = 472.3 amu, obtained = 472.5 amu, calc'd for (4-1) $C_{34}H_{58}NO_4^+$ = 544.8 amu, obtained = 544.8 amu.

Methylation reaction:



To a solution of 1 equivalent of pyridine in DCM, 1 equivalent of trimethyloxoniumtetrafluoroborate was added and stirred at room temperature overnight. Then it was concentrated in vacuum to give the tetrafluoroborate salt of compound in quantative yield.

Synthesis of diC9:0

Methylation conditions: To a solution of ***diC9:0 pyridine*** (100 mg, 0.238 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0352 g, 0.238 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave white solid in quantitative yield. NMR ($CDCl_3$) ^{1}H δ: 9.37 (m, 3H), 4.62 (s, J=15 Hz, 3H), 4.43 (t, 4H) 1.80 (m, 4H), 1.27 (br s, 28H), 0.87 (br t, 6H). ^{13}C δ: 160.5, 149.3, 144.3, 131.1, 67.4, 49.9, 33.8, 29.4, 28.4, 25.7, 22.6, 14.0. MS (+ve ESI): calc'd for $C_{26}H_{44}NO_4^+$ = 434.326 amu, obtained = 434.330 amu.

Synthesis of diC11:0

Methylation conditions: To a solution of ***diC11:0 pyridine*** (100 mg, 0.210 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0311 g, 0.210 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave white solid in quantitative yield. NMR ($CDCl_3$) ^{1}H δ: 9.37 (m, 3H), 4.68 (s, 3H), 4.51 (t, J=15 Hz, 4H) 1.80 (m, 4H), 1.27 (br s, 36H), 0.87(br t, 6H). ^{13}C δ: 160.8, 149.5, 147.7, 144.6, 131.3, 67.7, 50.2, 29.5, 28.5, 25.9, 22.6, 14.0. MS (+ve ESI): calc'd for $C_{30}H_{52}NO_4^+$ = 490.389 amu, obtained = 490.393 amu.

Synthesis of diC12:0

Methylation conditions: To a solution of ***diC12:0 pyridine*** (200 mg, 0.398 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0587 g, 0.398 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave white solid of in quantitative yield. NMR ($CDCl_3$) ^{1}H δ: 9.29 (m, 3H), 4.64 (s, 3H), 4.43 (t, J=12 Hz, 4H), 1.81 (m, 4H), 1.26 (br s, 36H), 0.88 (br t, 6H). ^{13}C δ: 160.3, 149.2, 144.7, 131.4, 67.9, 50.1, 31.8, 29.6, 28.3, 25.7, 22.6, 14.0. MS (+ve ESI; low res): calc'd for $C_{32}H_{56}NO_4^+$ = 518.4 amu, obtained = 518.6 amu.

Synthesis of diC14:0

Methylation conditions: To a solution of ***diC14:0 pyridine*** (200 mg, 0.358 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0529 g, 0.358 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave white solid in quantitative yield. NMR ($CDCl_3$) ^{1}H δ: 9.34 (m, 3H), 4.87 (s, 3H), 4.45 (t, J=15 Hz, 4H), 1.83 (m, 4H), 1.27 (br s, 48H), 0.89 (br t, 6H). ^{13}C δ: 160.4, 149.2, 144.6, 131.3, 67.8, 50.0, 31.9, 29.6, 28.3, 25.7, 22.6, 14.0. MS (+ve ESI; low res): calc'd for $C_{36}H_{64}NO_4^+$ = 574.4 amu, obtained = 574.4 amu.

Synthesis of diC18:0

Methylation conditions: To a solution of ***diC18:0 pyridine*** (100 mg, 0.149 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0220 g, 0.149 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave white solid in quantitative yield. NMR ($CDCl_3$) ^{1}H δ: 9.38 (m, 3H), 4.63 (s, 3H), 4.42 (t, 4H), 1.80 (m, 4H), 1.27 (br s, 64H), 0.88 (br t, 6H). ^{13}C δ: 160.5, 149.3, 144.4, 131.2, 67.7, 49.9, 31.9, 29.3, 28.4, 25.7, 22.6, 14.0. MS (+ve ESI; low res): calc'd for $C_{44}H_{80}NO_4^+$ = 686.6 amu, obtained = 686.5 amu.

Synthesis of diC20:0

Methylation conditions: To a solution of ***diC20:0 pyridine*** (100 mg, 0.134 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0199 g, 0.134 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave white solid in quantitative yield. NMR ($CDCl_3$) ^{1}H δ: 9.39 (m, 3H), 4.64 (s, 3H), 4.43 (t, J=15 Hz, 4H), 1.82 (m, 4H), 1.26 (br s, 72H), 0.88 (br t, 6H). ^{13}C δ: 160.4, 149.2, 144.4, 131.3, 67.8, 49.8, 31.9, 29.3, 28.3, 25.7, 22.6, 14.0. MS (+ve ESI): calc'd for $C_{48}H_{88}NO_4^+$ = 742.671 amu, obtained = 742.675amu.

Synthesis of diisoC9:0

Methylation conditions: To a solution of ***diisoC9:0 pyridine*** (100 mg, 0.238 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0353 g, 0.238 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave colorless oil in quantitative yield. NMR ($CDCl_3$) ^{1}H δ: 9.37 (m, 3H), 4.61 (s, 3H), 4.44 (t, J=12 Hz, 4H), 1.83 (m, 6H), 1.0-1.30 (m, 30H). ^{13}C δ: 160.6, 149.3, 144.1, 131.1, 66.3, 50.9, 49.9, 37.3, 31.0, 29.3, 26.3, 22.4. M S (+ve ESI): calc'd for $C_{26}H_{44}NO_4^+$ = 434.326 amu, obtained = 434.329 amu.

Synthesis of dibrC20:0

Methylation conditions: To a solution of ***dibrC20:0 pyridine*** (200 mg, 0.247 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0406 g, 0.247 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave colorless oil in quantitative yield. NMR ($CDCl_3$) ^{1}H δ: 9.37 (m, 3H), 4.68 (s, 3H), 4.36 (d, J=6 Hz, 4H), 1.84 (m, 2H), 1.26 (br s, 64H), 0.87 (br t, 12H). ^{13}C δ: 160.5, 149.3, 144.2, 147.4, 131.2, 129.9, 70.0, 50.3, 37.3, 31.8, 31.2, 29.5, 26.6, 22.4, 14.0. MS (+ve ESI): calc'd for $C_{48}H_{88}NO_4^+$ = 742.671 amu, obtained = 742.675 amu.

Synthesis of diC18:1

Methylation conditions: To a solution of ***diC18:1 pyridine*** (124 mg, 0.185 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0274 g, 0.185 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave colorless oil in quantitative yield. NMR ($CDCl_3$) ^{1}H δ: 9.42 (m, 3H), 5.34 (m, 4H), 4.62 (s, 3H), 4.42 (t, J=12 Hz, 4H), 2.00 (m, 8H), 1.80 (m, 4H), 1.30 (br s, 48H), 0.87 (br t, 6H). ^{13}C δ: 160.5, 149.3, 144.4, 131.2, 129.9, 129.7, 126.3, 67.4, 49.9, 32.5, 31.8, 29.2, 28.4, 27.2, 26.7, 25.7, 22.6, 14.0. MS (+ve ESI; low res): calc'd for $C_{44}H_{76}NO_4^+$ = 682.5 amu, obtained = 682.4 amu.

Synthesis of diC11:1

Methylation conditions: To a solution of ***diC11:1 pyridine*** (217 mg, 0.460 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0680 g, 0.460 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave colorless oil in quantitative yield. NMR ($CDCl_3$) ^{1}H δ: 9.39 (m, 3H), 5.80 (m, 2H), 4.94 (m, 4H), 4.65 (s, 3H), 4.41 (t, J=12 Hz, 4H) , 2.03 (m, 4H), 1.81 (m, 4H), 1.30 (m, 28H). ^{13}C δ: 160.7, 149.5, 144.7, 139.4, 131.5, 114.3, 67.6, 50.2, 34.0, 29.3, 28.5, 28.5, 25.9. MS (+ve ESI): calc'd for $C_{30}H_{48}NO_4^+$ = 486.358 amu, obtained = 486.362amu.

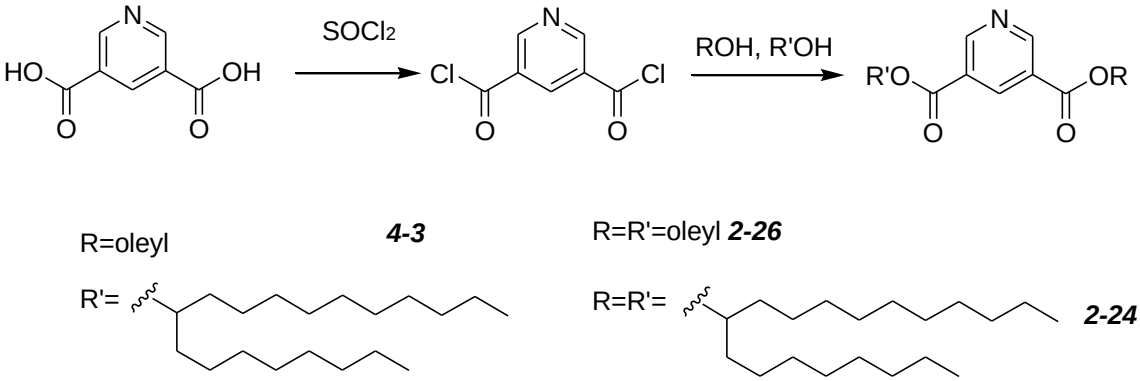
Synthesis of (C16:0)(C11:1)

Methylation conditions: To a solution of ***(C16:0)(C11:1) pyridine*** (200 mg, 0.367 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0543 g, 0.367 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave colorless oil in quantitative yield which was later determined via ESI mass spectroscopy method of methylated analogues to contain 22% of ***2-5***, 28% of ***2-29*** and 50% of ***4-2***. NMR ($CDCl_3$) ^{1}H δ: 9.39 (m, 3H), 5.80 (m, 2H), 4.94 (m, 4H), 4.66 (s, 3H), 4.42 (t, J=15 Hz, 4H), 2.00 (m, 4H), 1.79 (m, 4H), 1.27 (m, 38H), 0.88 (br t, 1.5H). ^{13}C δ: 164.4, 149.2, 139.1, 131.4, 114.1, 67.8, 50.0, 33.7, 31.9, 29.3, 28.8, 25.7, 22.6, 14.0. MS (+ve ESI; low res): calc'd for ***(2-5)*** $C_{40}H_{72}NO_4^+$ = 630.55 amu, obtained = 630.47 amu. calc'd for ***(2.29)*** $C_{30}H_{48}NO_4^+$ = 486.3 amu, obtained = 486.3 amu, calc'd for ***(4-2)*** $C_{35}H_{60}NO_4^+$ = 558.4 amu, obtained = 558.4 amu.

Synthesis of (C18:1)(brC20:0)

Methylation conditions: To a solution of ***(C18:1)(brC20:0) pyridine*** (200 mg, 0.286 mmol) in DCM, trimethyloxoniumtetrafluoroborate (0.0424 g, 0.286 mmol) was added and the mixture was stirred at room temperature overnight. Concentration in vacuum gave colorless oil in quantitative yield which was determined via ESI mass spectroscopy method to contain 28% of ***2-25***, 16% of ***2-27*** and 56% of ***4-4***. NMR ($CDCl_3$) ^{1}H δ: 9.35 (m, 3H), 5.35 (m, 2H), 4.68 (s, 3H), 4.42 (m, 4H), 3.49 (m, 0H, exp. 2H), 2.00 (m, 8H), 1.27 (m, 67H), 0.88 (br t, 10 H). ^{13}C δ: 160.5, 149.2, 144.1, 131.2, 129.9, 129.7, 126.3, 70.4, 58.5, 50.1, 37.2, 31.0, 29.3, 27.2, 26.7, 22.6, 14.0. MS (+ve ESI; low res): calc'd for ***(2-25)*** $C_{48}H_{88}NO_4^+$ = 742.6 amu, obtained = 742.6 amu, calc'd for ***(2.27)*** $C_{44}H_{76}NO_4^+$ = 682.5 amu, obtained = 682.4 amu, calc'd for ***(4-4)*** $C_{46}H_{82}NO_4^+$ = 712.6 amu, obtained = 712.3 amu.

Alternate Synthesis of (18:1)(brC20:0)



To 1 equivalent of 3,5-pyridinedicarboxylic acid (0.835 g, 5 mmol) (Scheme above), thionyl chloride (8.4 g, 70 mmol) was added. The solution was reflux for 24 h under N_2 to yield pyridine-3,5-dicarboyl dichloride. The residual thionyl chloride was removed under reduced pressure and then the remaining product was dissolved in DCM (20 mL). 1.2 equivalent of nonadecan-9-ol (1.791 g, 6 mmol) and 1.2 equivalents of oleyl alcohol (1.610 g, 6 mmol) were added and the reaction solution was refluxed for 3 h. The solvent was removed under reduced pressure and then the residue was dissolved in ether (50 mL) and washed with 4M NaOH, dried over sodium sulphate and the crude product was purified by silica gel chromatography, using 15% ether/hexanes as eluent, yields 3.099 g of colorless oil which was determined via ESI mass spectroscopy method of the methylated product to contain 28% of ***2-24***, 16% of ***2-26*** and 56% of ***4-3***. NMR ($CDCl_3$) ^{1}H δ: 9.34 (d, 2H, 3), 8.83 (t, J=3 Hz, 1H), 5.34 (m, 4H), 4.36 (t, J=18 Hz, 4H), 2.00 (m, 8H), 1.82 (m, 4H), 1.25 (m, 52H), 0.86 (br t, 9H). ^{13}C: δ 164.5, 154.0, 139.3, 137.9, 126.3, 114.1, 65.9, 33.7, 31.8, 29.6, 28.8, 28.6, 25.9, 22.6, 14.0. MS (+ve ESI): calc'd for (***2-24***) $C_{47}H_{86}NO_4^+$ = 728.65 amu, obtained = 728.53 amu, calc'd for (***2.26***) $C_{47}H_{74}NO_4^+$ = 668.55 amu, obtained = 668.42 amu, calc'd for **(*4-3*)** $C_{45}H_{80}NO_4^+$ = 698.6 amu, obtained = 698.7 amu.

Lipid fractionation by ESI-MS

This experiment investigates the potential fractionation of EPC/**dibrC20:0** binary mixtures during lipid hydration relative to EPC/**diC16:0** binary mixtures. A solution of DOPE/EPC/pyridinum lipid in a 2/1.5/1.5 mole ratio and total lipid concentration of 2mM in $CHCl_3$ was prepared as well as a reference solution of tetrabutyl ammonium tribromide ($CHCl_3$ 2mM). An aliquot of the lipid mixture and the reference (10 μL each) was diluted to 1 mL in MeOH. ESI-MS (ESI+) gave spectra that showed strong ions for the parent cationic lipids (EPC, m/z 706.5; **diC16:0**, m/z 630.5; **dibrC20:0**, m/z 742.5) and the standard (m/z 242.1). The ion intensity of each peak in the mixture was measured relative to the ion intensity of the tetrabutylammonium reference to give a relative response factor for each species. The stock solution was evaporated to form a thin layer of lipids and the layer was hydrated to a final concentration of 2 mM in lipid using water as occurs in the first stage of liposome formation. After sonication of this solution, a 10μL aliquot plus a known amount of reference was analysed as previously. Fractionation was observed as a change in the apparent response factor of the different species. The data is tabulated below using the convention that the amount of pyridinium lipid detected in the chloroform stock solution = 100.

Compound	**%EPC**	**%diC16:0**
diC16:0 stock CHCl3	64	100
diC16:0 water dispersion	49	14
	%EPC	**%dibrC20:0**
dibrC20:0 stock CHCl3	67	100
dibrC20:0 water dispersion	35	0.06

Biological Methods

Cationic lipids included the synthetic pyridinum lipids and commercial lipid 1,2-dimyristoyl-sn-glycero-3-ethylphophocholine (EPC). The co-lipids used for the formulation of liposomes and lipoplexes were 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and cholesterol. As neutral lipids, the concentrations of DOPE and cholesterol were not included in the final calculation of charge ratios when forming lipoplexes between cationic lipids and DNA.

Preparation of lipid ethanolic stock solutions

Stock solutions of pyridinium lipids, commercial cationic lipid EPC and co-lipids DOPE and cholesterol were made by dissolving a known amount of each lipid in dichloromethane in a round-bottom flask. The solutions were placed on a rotary evaporator for 1 h to obtain a film. The film was dissolved in a known amount of anhydrous EtOH in order to achieve a 1 mM stock, and subsequently stored at −80 °C.

Liposome formulations

An overall 3:2 molar ratio of total cationic lipid (synthetic lipid combined 1:1 with EPC, or control lipid EPC alone) to co-lipid, DOPE or cholesterol, in ethanolic solutions were prepared separately and evaporated under reduced pressure to generate thin films. The lipid films were hydrated with a known amount of sterile water to give 2 mM final hydrated stock solutions, which were stored overnight at 4 °C. Before use, the hydrated stocks were warmed to 37 °C and sonicated for 30 min.

Preparation of Lipoplexes (lipid/pDNA complexes)

Lipoplexes of concentrations 0.081 mM, 0.243 mM, 0.486 mM, 0.81 mM and 1.62 mM, corresponding to the N/P (+/-) molar charge ratios of 0.5:1, 1.5:1, 3:1, 5.0:1 and 10.0:1, respectively, were prepared from the 2 mM liposome stocks. OPTI-MEM buffer (57.6 µL) and *p*DNA (14.4 µL; 250 ng/µL) in Elution solution, were first combined, followed by the addition of an equal volume of corresponding liposome (72 µL) to this and mixed. These lipoplex formulations were incubated at rt for 30 min. 48 µL of lipoplex formulation was used for the gel assays and to each of the remaining lipoplex formulations, 204 µL of OPTI-MEM was added prior to use for transfection experiments. The Lipofectamine 2000/DNA control was prepared as per manufacturer's protocol.

Liposome and lipoplex sizing

The hydrodynamic diameter, d_H, of liposomes and lipoplexes was measured by dynamic light scattering (DLS) at 25 °C with a detection angle of 90°. All data are the mean ± standard deviation (SD) of three measurements.

Gel retardation assays of lipoplexes

To 20 µL of the lipoplexes, 2 µL of the gel loading dye (6X) was added and mixed by pipetting. Eighteen microliters of each sample was then loaded onto a 1% agarose gel impregnated with ethidium bromide and run at 105 V for 1 h in 1x TBE buffer. The migration of *p*DNA complexed with the cationic lipids was impeded in the electric field. The *p*DNA bands were observed using a Geliance transilluminator.

DNase I degradation assays of lipoplexes

Twenty microliters of the lipoplexes was incubated with DNase I (1 µL) at 37 °C for 1 h. After incubation, 5% SDS (4 µL) was added and incubated for a further 30 min, followed by 2 µL of gel loading dye (6x). Eighteen microliters of each sample was then loaded onto a 1% agarose gel impregnated with ethidium bromide and run at 105 V for 1 h in 1x TBE buffer. The *p*DNA bands were observed using a Geliance transilluminator.

Cell culture

CHO-K1 cells were grown in RPMI media supplemented with 10% fetal calf serum and 100 U/mL of penicillin/streptomycin and 0.25 µg/mL amphotericin B. Cells were seeded 48 h before transfection onto opaque and transparent 96-well plate at a density of 10^4 cells per well and incubated at 37 °C in presence of 5% CO_2 atmosphere. Cells were grown to 80% confluence before being washed with 1x PBS and incubated with 45 µL of each lipid-*p*DNA complex in triplicate for 4 h at 37 °C in the presence of 5% CO_2 atmosphere. Complexes were then removed and the cells washed with 1x PBS before adding 100 µL of complete RPMI media. Cells were left to incubate for an additional 44 h. Following the incubation, transfection and cytotoxicity assays were performed according to the below mentioned protocols.

β-galactosidase assay

Forty-eight hours after the application of lipoplexes, β-galactosidase activity was determined using a Beta-Glo® Assay System (Promega). Treated cells in the opaque 96-well plate were washed with 1x PBS, then 50 µL of DMEM (phenol red-free media) was added to each well. This was followed by the addition of 50 µL of Beta- Glo™ working solution, prepared according to the manufacturer's directions (Promega), to each well and thorough mixing by pipetting. After 1 h incubation at rt, luminescence was then read on a Victor Envision high throughput plate reader. β-Galactosidase activity was expressed as relative light units produced by the luminescence of luciferin, which was normalized for protein content.

Total protein (BCA) assay

Total protein content was measured using Pierce® BCA Protein Assay (Pierce Biotechnology, Rockford, IL). Forty-eight hours after the application of lipoplexes, treated cells in the transparent 96-well plate were washed with 1x PBS, and 10 µL of passive lysis buffer (Promega) was added to each well. Plates were incubated at rt for 30 min. BCA working reagent (200 µL), prepared according to the manufacturer's directions, was then added to each well, gently mixed by pipetting, and incubated at rt for 1 h prior to reading at 562 nm on a Victor Envision plate reader. A calibration curve obtained from a bovine serum albumin standard solution was used to determine cellular protein content per well.

Cytotoxicity assay

The cytotoxicity associated with the lipoplex formulations at N:P (+/-) molar charge ratios ranging from 0.5:1 to 10:1 was evaluated using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. Forty-eight hours after the application of lipoplexes, CHO-K1 cells in the transparent 96-well plates were washed with 1x PBS, 50 µL of DMEM (phenol red-free media) followed by 10 µL of CellTiter96® Aqueous One Solution Cell Proliferation Assay (Promega) was added to each well and mixed by gentle rocking. The plates were incubated further for 1 h at 37 °C. The absorbance of converted dye, which correlates with the number of viable cells, was measured at 492 nm using a Victor Envision high throughput plate reader. The percentage of viable cells was calculated as the absorbance ratio of treated to untreated cells.

Gel retardation assays

Assays related to experiments labelled Exp1 and Exp4 in Tables S1-S5 were previously reported: Parvizi, P.; Jubeli, E.; Raju, L.; Khalique, N. A.; Almeer, A.; Allam, H.; Manaa, M. A.; Larsen, H.; Nicholson, D.; Pungente, M. D.; Fyles, T. M. Aspects of nonviral gene therapy: Correlation of molecular parameters with lipoplex structure and transfection efficacy in pyridinium-based cationic lipids *Int. J. Pharm.* **2014**, *461*, 145. doi: http://dx.doi.org/10.1016/j.ijpharm.2013.11.045

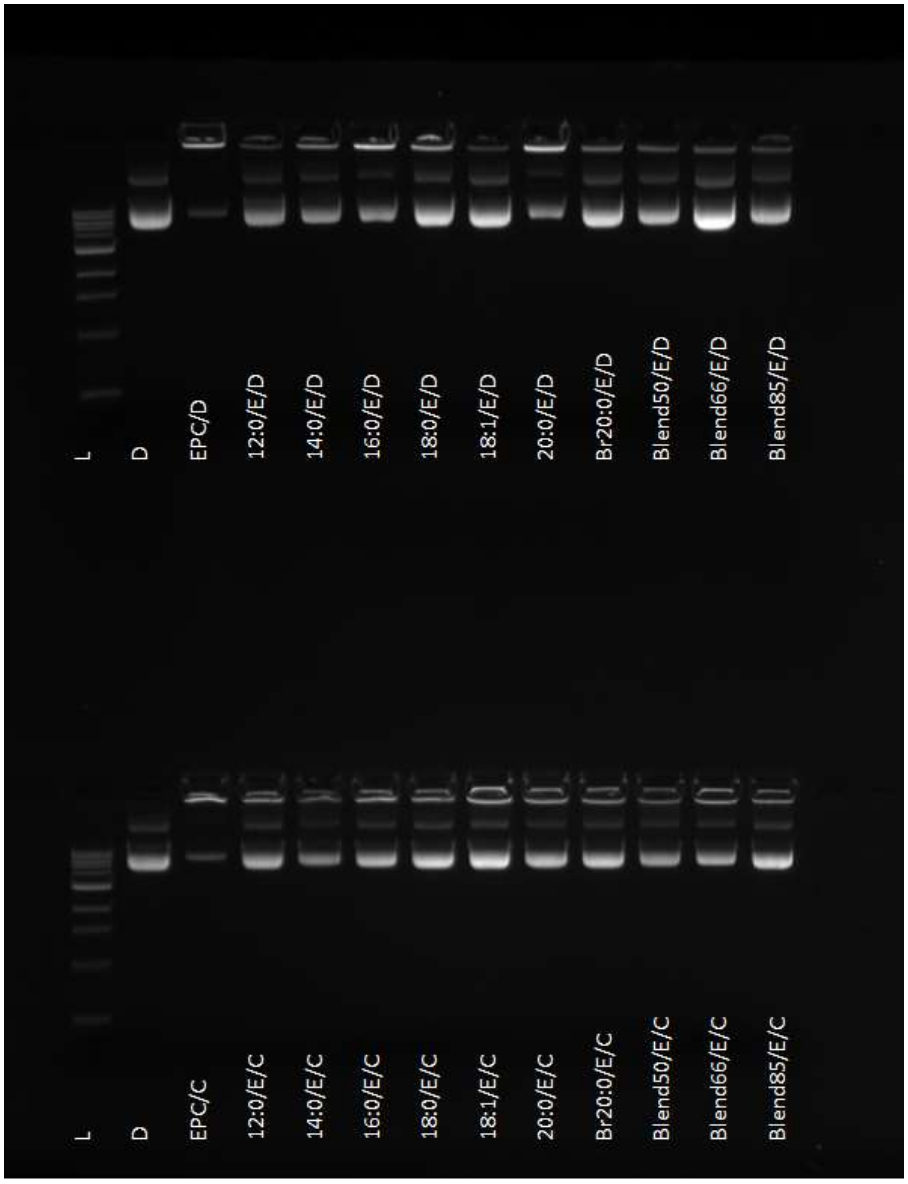


Figure S1 -Gel retardation assay of lipids diC12:0 to diC20:0 co-formulated with commercial lipid EPC (E) and neutral co-lipid DOPE (D) or cholesterol (C) at molar charge ratios 3, and run through a 1% agarose gel impregnated with the pDNA gel stain, ethidium bromide. Lanes λ and DNA denote the 1 kb DNA ladder and pDNA, respectively. Data from the experiment labelled Exp7 in Tables S1-S5.

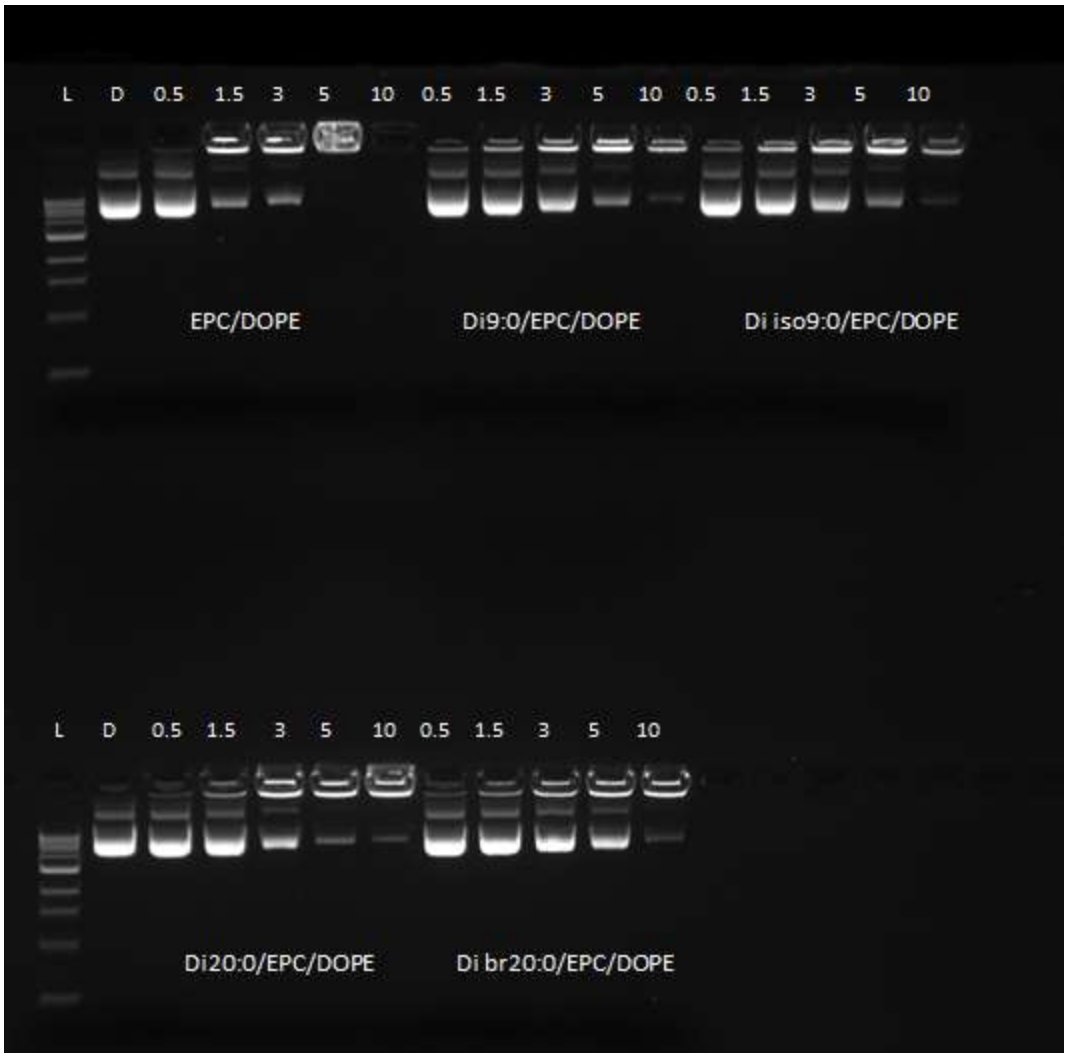


Figure S2 - Gel retardation assay of lipids diC9:0, diCisoC9:0, diC20:0 or dibrC20:0 co-formulated with commercial lipid EPC and neutral co-lipid DOPE at charge ratios 0.5, 1.5, 3, 5 and 10 and run through a 1% agarose gel impregnated with the pDNA gel stain, ethidium bromide. Lanes λ and DNA denote the 1 kb DNA ladder and pDNA, respectively. Data from the experiment labelled Exp8 in Tables S1-S5.

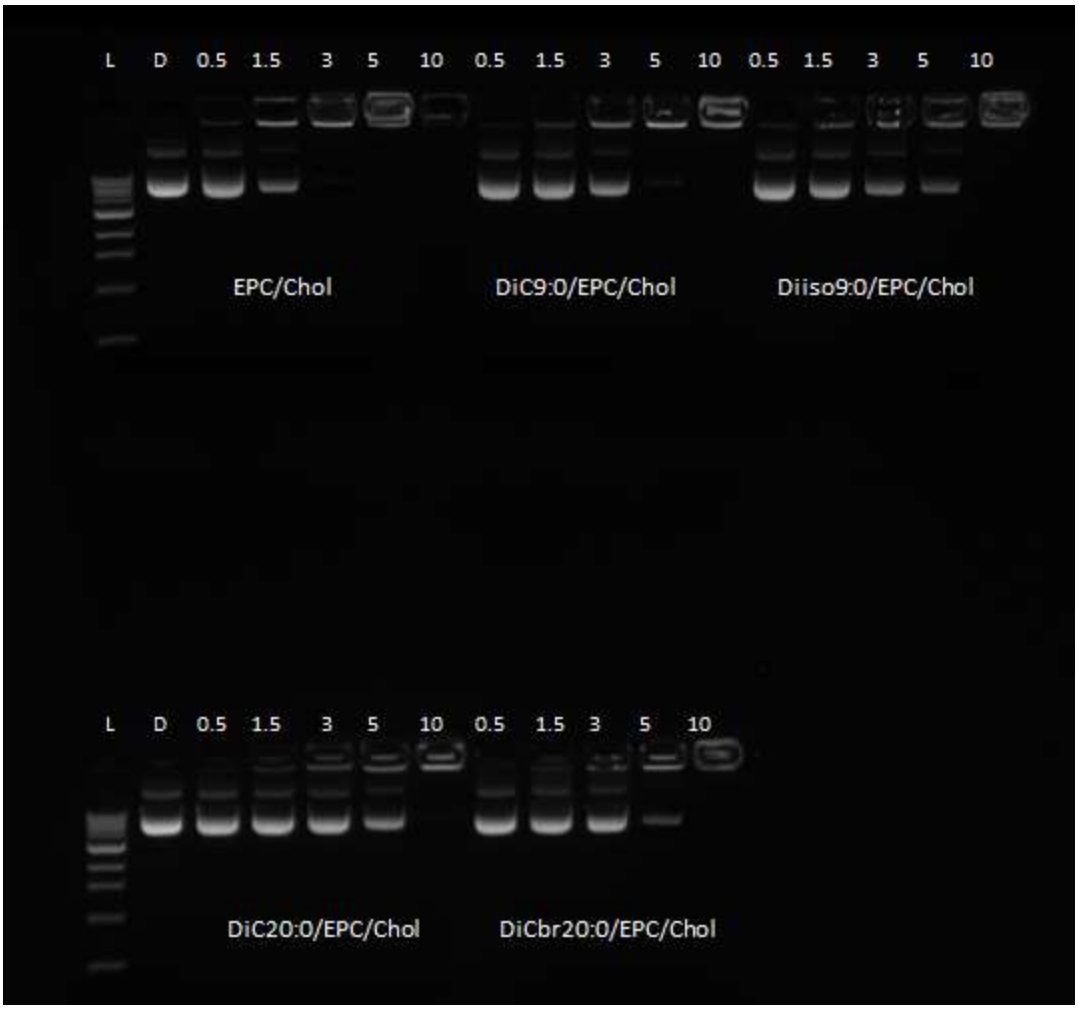


Figure S3 - Gel retardation assay of lipids diC9:0, diCisoC9:0, diC20:0 or dibrC20:0 co-formulated with commercial lipid EPC and neutral co-lipid cholesterol at charge ratios 0.5, 1.5, 3, 5 and 10 and run through a 1% agarose gel impregnated with the pDNA gel stain, ethidium bromide. Lanes λ and DNA denote the 1 kb DNA ladder and pDNA, respectively. Data from the experiment labelled Exp8a in Tables S1-S5.

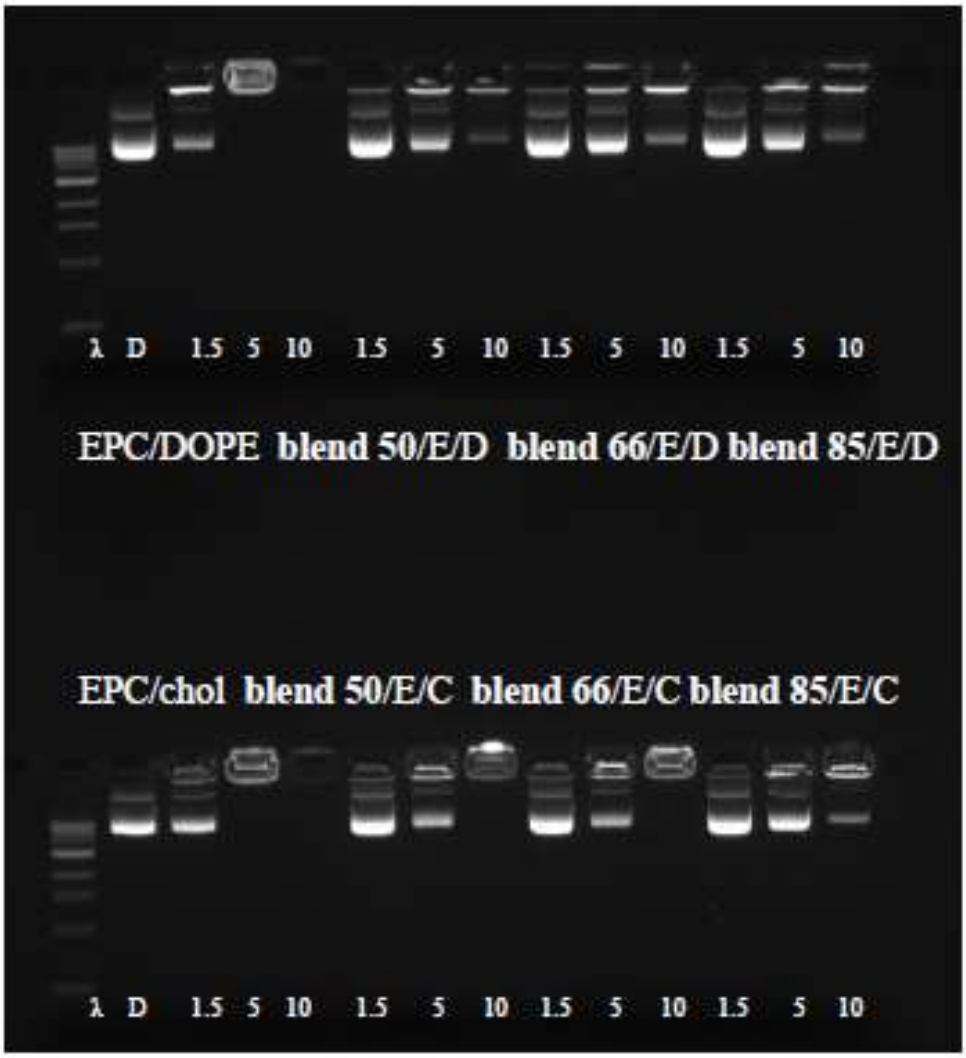


Figure S4 - Gel retardation assay of lipids for blend 50, blend 66 and blend 85 co-formulated with commercial lipid EPC (E) and neutral co-lipid DOPE (D) or cholesterol (C) at charge ratios 1.5, ,5 and 10 and run through a 1% agarose gel impregnated with the pDNA gel stain, ethidium bromide. Lanes λ and DNA denote the 1 kb DNA ladder and pDNA, respectively. Data from the experiment labelled Exp9 in Tables S1-S5.

Figure S5 -Gel retardation assay of lipids for synthesized ternary lipid mixture (New TF) and binary blend of pure lipids (blend) co-formulated with commercial lipid EPC and neutral co-lipid DOPE or cholesterol at charge ratios 0.5, 1.5, 3, 5 and 10 and run through a 1% agarose gel impregnated with the pDNA gel stain, ethidium bromide. Lanes λ and DNA denote the 1 kb DNA ladder and pDNA, respectively. Data from the experiment labelled Exp17 in Tables S1-S5.

DNase I degradation assays

Assays related to experiments labelled Exp1 and Exp4 in Tables S1-S5 were previously reported: Parvizi, P.; Jubeli, E.; Raju, L.; Khalique, N. A.; Almeer, A.; Allam, H.; Manaa, M. A.; Larsen, H.; Nicholson, D.; Pungente, M. D.; Fyles, T. M. Aspects of nonviral gene therapy: Correlation of molecular parameters with lipoplex structure and transfection efficacy in pyridinium-based cationic lipids *Int. J. Pharm.* **2014**, *461*, 145. doi: http://dx.doi.org/10.1016/j.ijpharm.2013.11.045

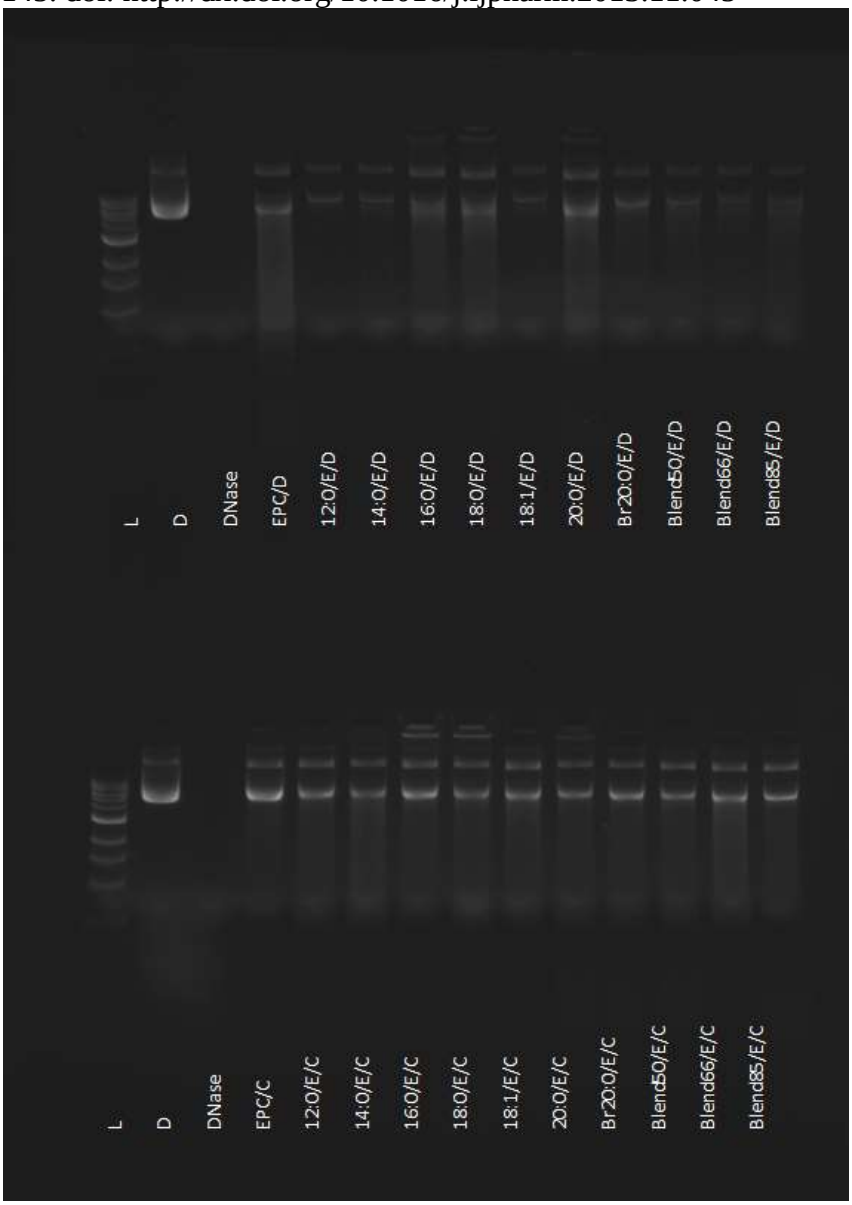


Figure S6 -DNase I degradation assay of lipids diC12:0 to diC20:0 co-formulated with commercial lipid EPC (E) and neutral co-lipid DOPE (D) or cholesterol (C) at molar charge ratios 3, and run through a 1% agarose gel impregnated with the pDNA gel stain, ethidium bromide. Lanes λ and DNA denote the 1 kb DNA ladder and pDNA, respectively. Data from the experiment labelled Exp7 in Tables S1-S5.

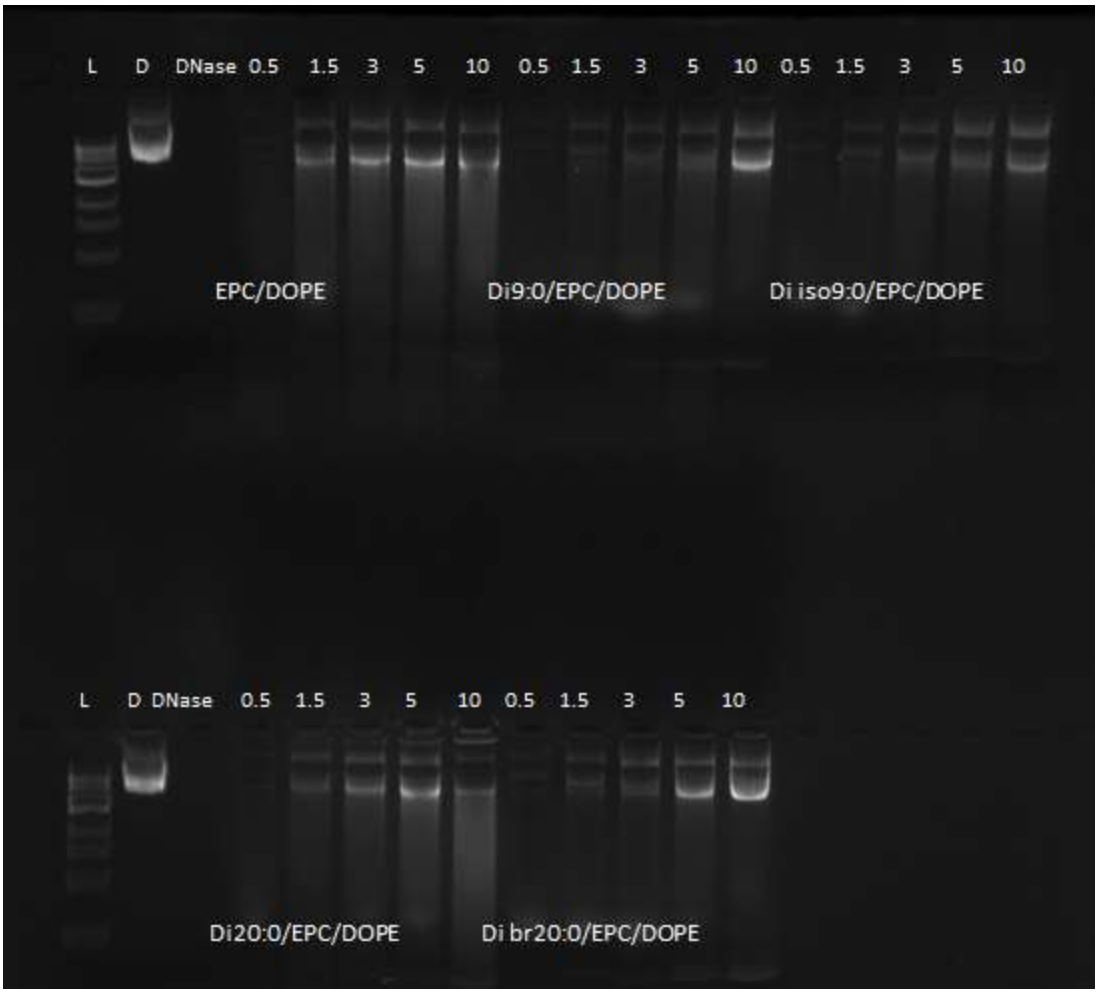


Figure S7 - DNase I degradation assay of lipids diC9:0, diCisoC9:0, diC20:0 or dibrC20:0 co-formulated with commercial lipid EPC and neutral co-lipid DOPE at charge ratios 0.5, 1.5, 3, 5 and 10 and run through a 1% agarose gel impregnated with the pDNA gel stain, ethidium bromide. Lanes λ and DNA denote the 1 kb DNA ladder and pDNA, respectively. Data from the experiment labelled Exp8 in Tables S1-S5.

Figure S8 - DNase I degradation assay of lipids diC9:0, diCisoC9:0, diC20:0 or dibrC20:0 co-formulated with commercial lipid EPC and neutral co-lipid cholesterol at charge ratios 0.5, 1.5, 3, 5 and 10 and run through a 1% agarose gel impregnated with the pDNA gel stain, ethidium bromide. Lanes λ and DNA denote the 1 kb DNA ladder and pDNA, respectively. Data from the experiment labelled Exp8a in Tables S1-S5.

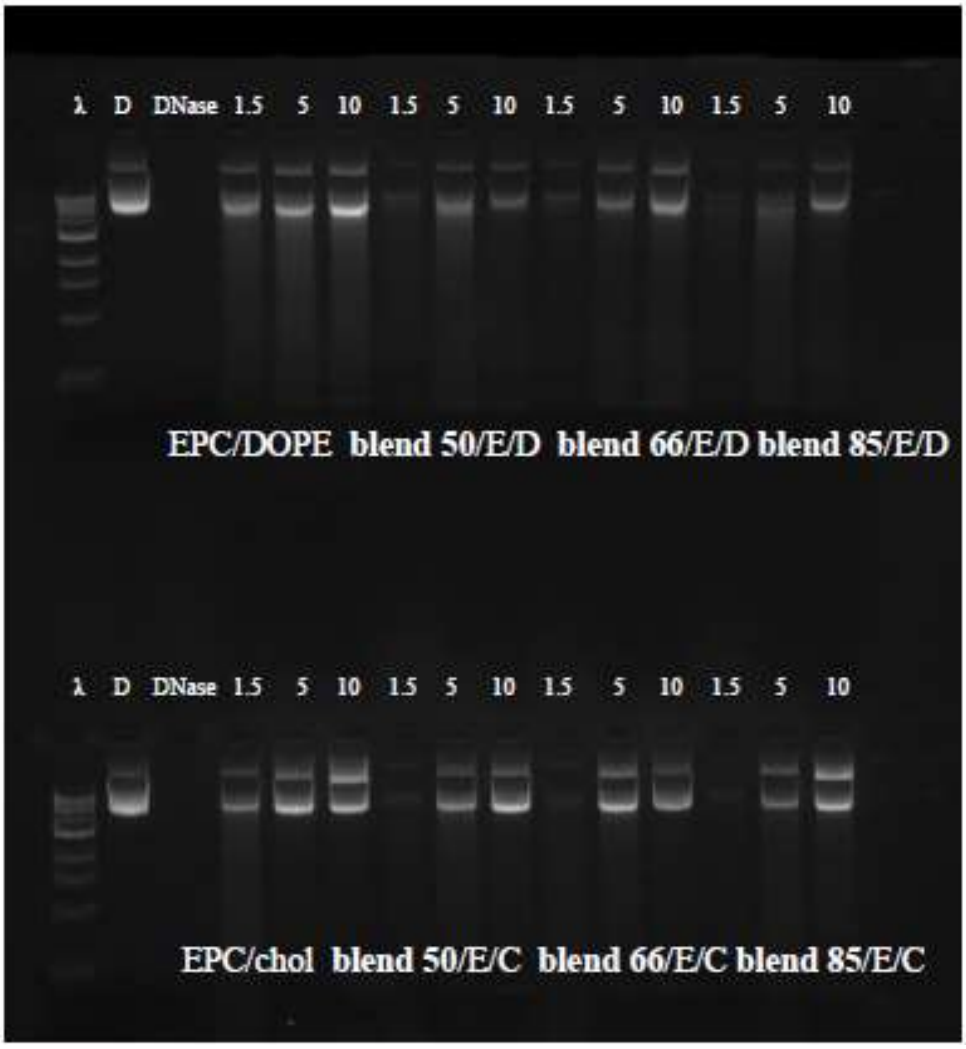


Figure S9 - DNase I degradation assay of blend 50, blend 66 and blend 85 co-formulated with commercial lipid EPC (E) and neutral co-lipid DOPE (D) or cholesterol (C)at charge ratios 1.5, 5 and 10 and run through a 1% agarose gel impregnated with the pDNA gel stain, ethidium bromide. Lanes λ and DNA denote the 1 kb DNA ladder and pDNA, respectively. Data from the experiment labelled Exp9 in Tables S1-S5.

Figure S10 - DNase I degradation assay of lipids synthesized ternary lipids (New TF) and binary blend of pure lipids (blend) co-formulated with commercial lipid EPCand neutral co-lipid DOPE or cholesterol at charge ratios 0.5, 1.5, 3, 5 and 10 and run through a 1% agarose gel impregnated with the pDNA gel stain, ethidium bromide. Lanes λ and DNA denote the 1 kb DNA ladder and pDNA, respectively. Data from the experiment labelled Exp17 in Tables S1-S5.

Figure S11 - DNase I degradation assay of lipids synthesized ternary lipids (New TF) and binary blend of pure lipids (blend) co-formulated with commercial lipid EPCand neutral co-lipid DOPE or cholesterol at charge ratios 0.5, 1.5, 3, 5 and 10 and run through a 1% agarose gel impregnated with the pDNA gel stain, ethidium bromide. Lanes λ and DNA denote the 1 kb DNA ladder and pDNA, respectively. Data from the experiment labelled Exp17 in Tables S1-S5.

Transfection and cell viability bar charts

Charts related to experiments labelled Exp1 and Exp4 in Table S3 were previously reported: Parvizi, P.; Jubeli, E.; Raju, L.; Khalique, N. A.; Almeer, A.; Allam, H.; Manaa, M. A.; Larsen, H.; Nicholson, D.; Pungente, M. D.; Fyles, T. M. Aspects of nonviral gene therapy: Correlation of molecular parameters with lipoplex structure and transfection efficacy in pyridinium-based cationic lipids *Int. J. Pharm.* **2014**, *461*, 145. doi: http://dx.doi.org/10.1016/j.ijpharm.2013.11.045

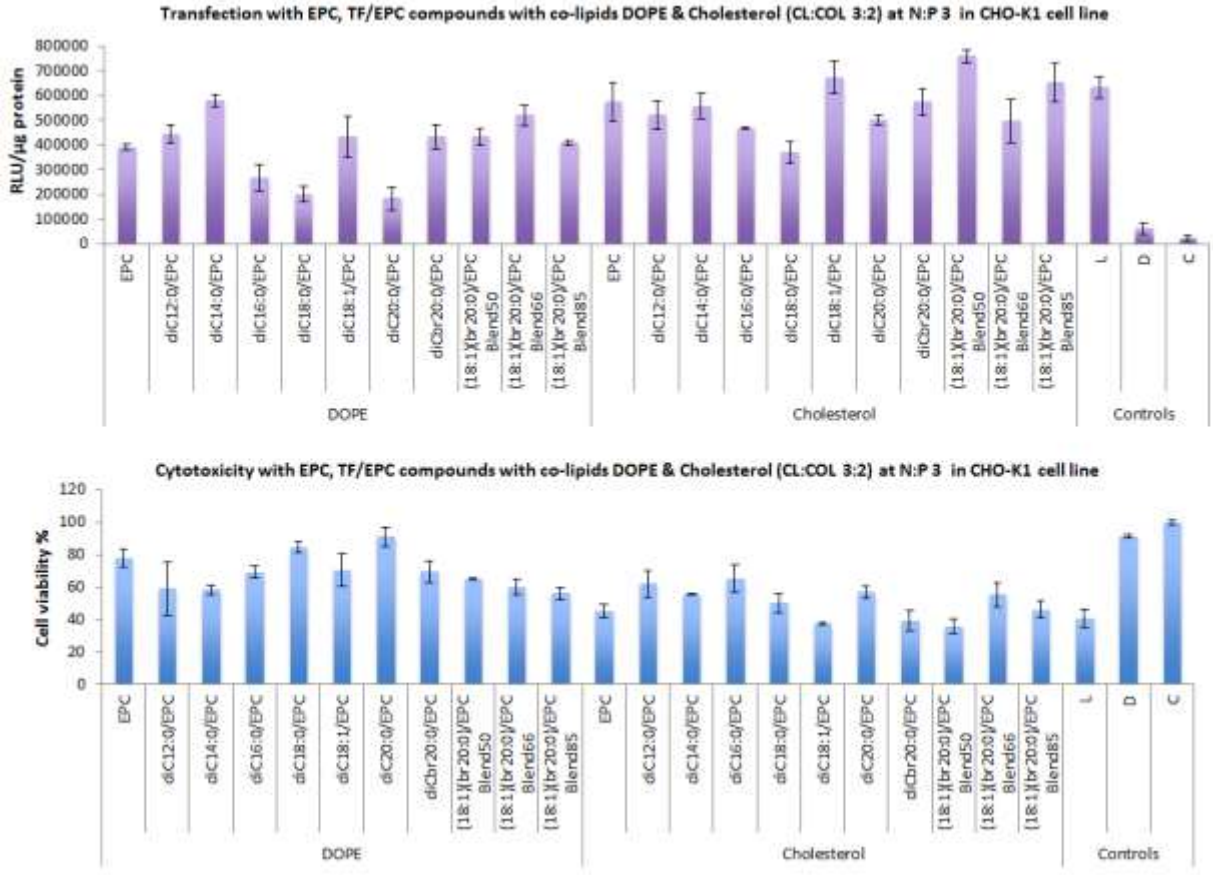


Figure S12 -Transfection efficiency as luminescence readings of β-galactosidase (left) and cytotoxicity (right) (after 48 h) of synthetic lipid diC12:0 to diC20:0/co-lipid/DNA lipoplexes compared to EPC/co-lipid/DNA at molar charge ratio of 3 and Lipofectamine 2000™ (Lipo) (n = 9; mean ± SD) as positive controls, and plasmid DNA alone and CHO-K1 cells alone as negative controls. Numerical from this experiment is labelled Exp7 in Table S3.

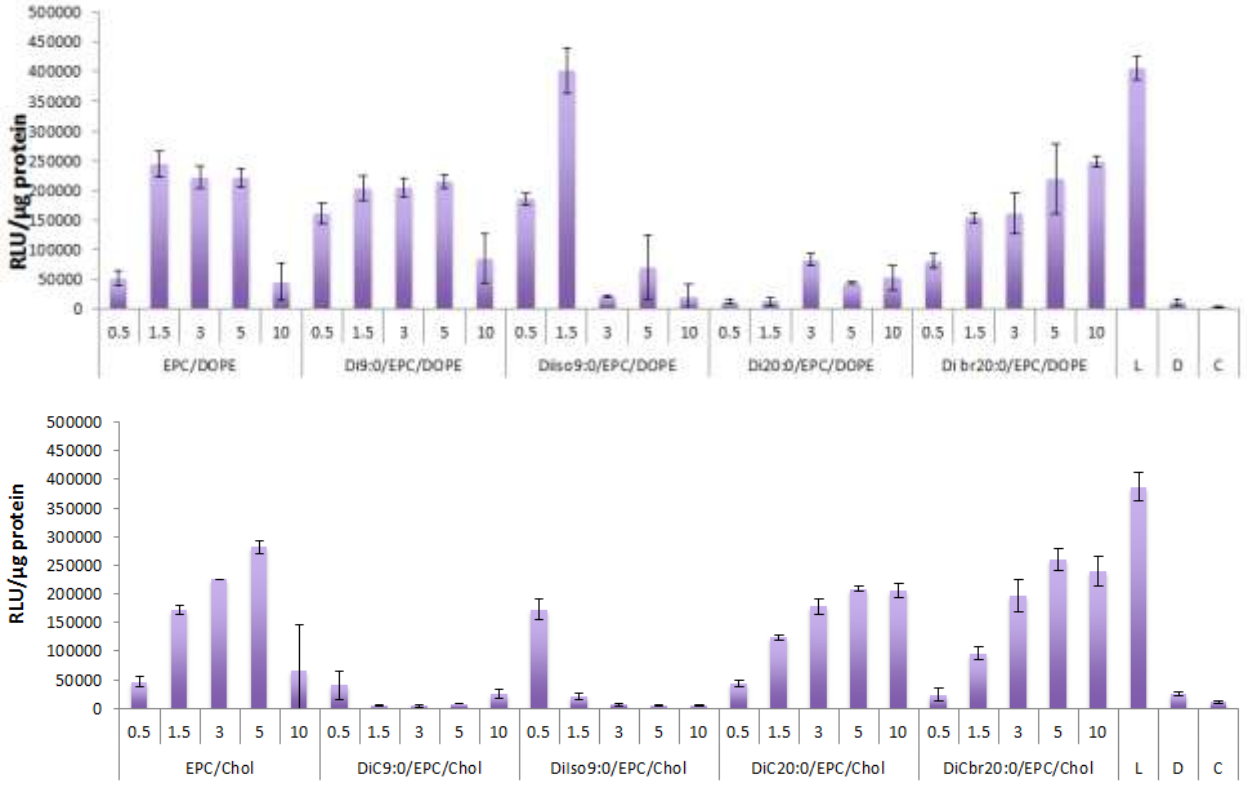


Figure S13 -Transfection efficiency as luminescence readings of β-galactosidase (after 48 h) of diC9:0, diCisoC9:0, diC20:0 or dibrC20:0 co-formulated with commercial lipid EPC and neutral co-lipid DOPE (top) or cholesterol (bottom) at molar charge ratio of 0.5 to 10 and Lipofectamine 2000^{TM} (Lipo) (n = 9; mean ± SD) as positive controls, and plasmid DNA alone and CHO-K1 cells alone as negative controls. Numerical data from this experiment is labelled Exp8 (top) or Exp8a(bottom) in Table S3.

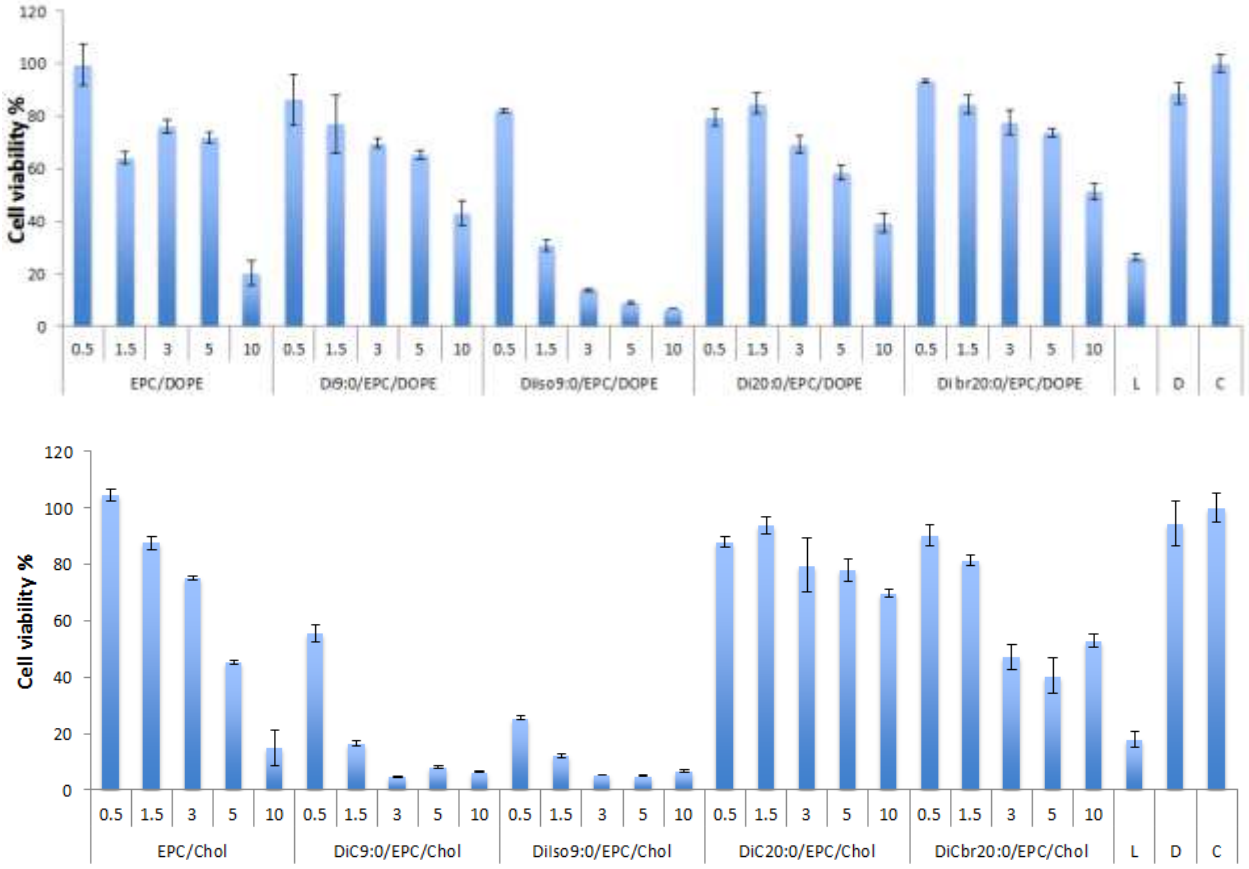


Figure S14 -Cytotoxicity (after 48 h) of diC9:0, diCisoC9:0, diC20:0 or dibrC20:0 co-formulated with commercial lipid EPC and neutral co-lipid DOPE (top) or cholesterol (bottom) at molar charge ratio of 0.5 to 10 and Lipofectamine 2000^{TM} (Lipo) (n = 9; mean ± SD) as positive controls, and plasmid DNA alone and CHO-K1 cells alone as negative controls. Numerical data from this experiment is labelled Exp8 (top) or Exp8a(bottom) in Table S3.

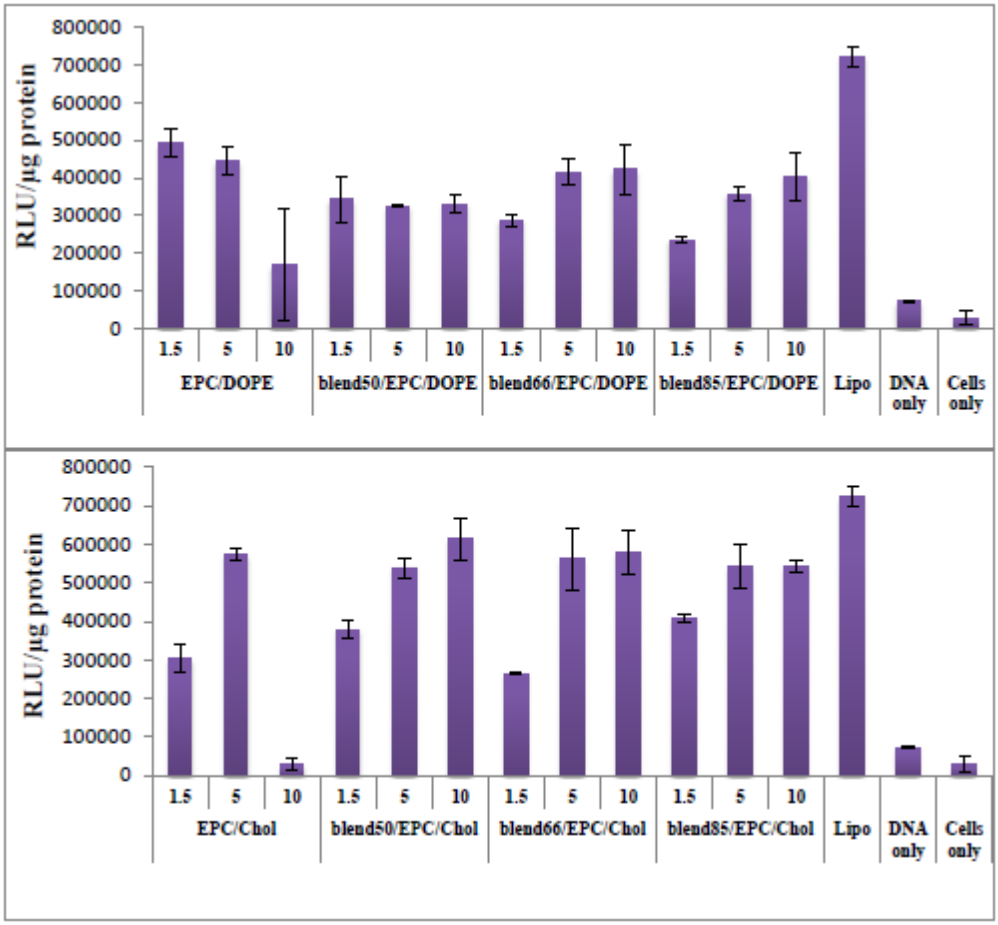


Figure S15 - Transfection efficiency as luminescence readings of β-galactosidase (after 48 h) of blend 50, blend 66 and blend 85/co-lipid/DNA lipoplexes compared to EPC/co-lipid/DNA at molar charge ratios of 1.5, 5 and 10 and Lipofectamine 2000^{TM} (Lipo) (n = 9; mean ± SD) as positive controls, and plasmid DNA alone and CHO-K1 cells alone as negative controls. Numerical data from this experiment is labelled Exp9 in Table S3.

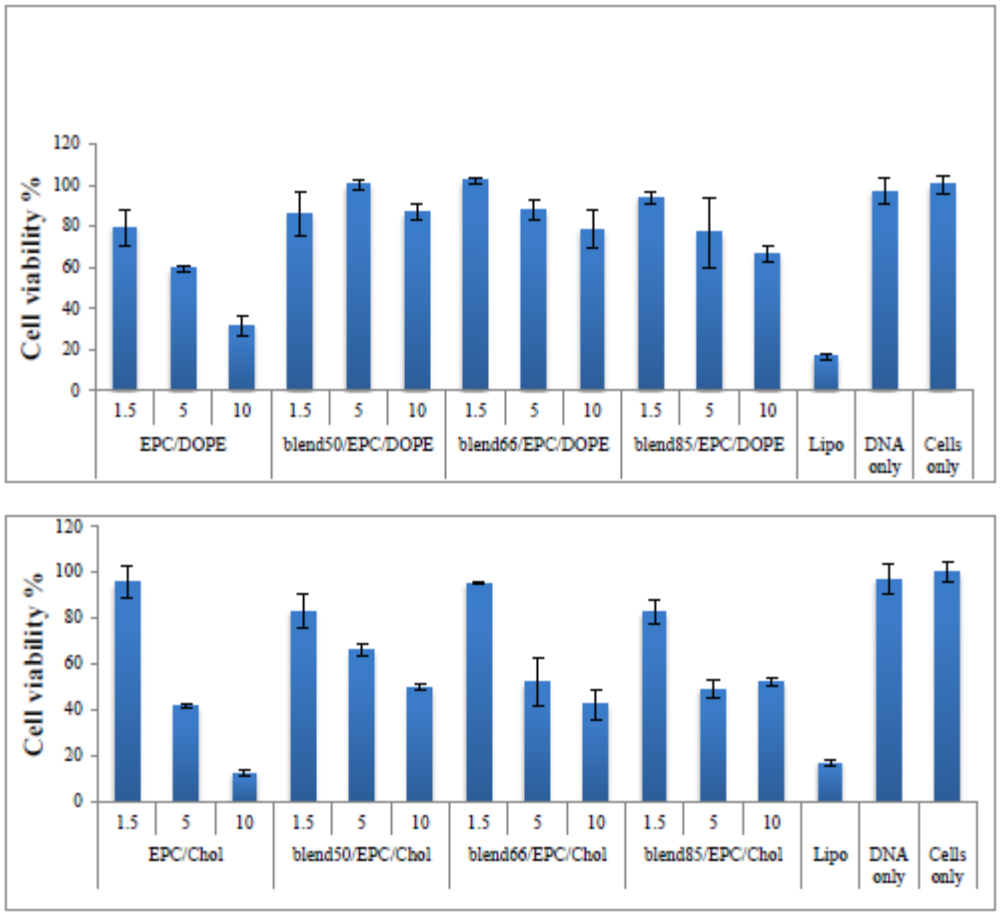


Figure S16 - Cytotoxicity (after 48 h) of blend 50, blend 66 and blend 85/co-lipid/DNA lipoplexes compared to EPC/co-lipid/DNA at molar charge ratios of 1.5, 5 and 10 and Lipofectamine 2000™ (Lipo) (n = 9; mean ± SD) as positive controls, and plasmid DNA alone and CHO-K1 cells alone as negative controls. Numerical data from this experiment is labelled Exp9 in Table S3.

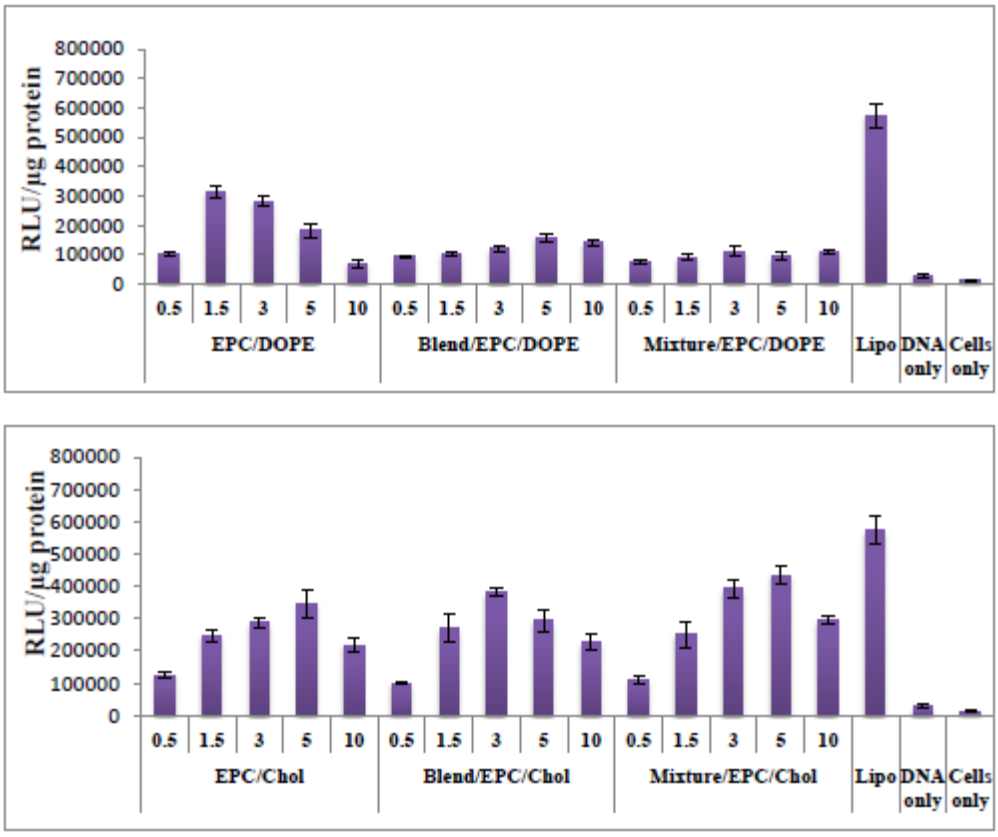


Figure S17 -Transfection efficiency as luminescence readings of β-galactosidase (after 48 h) of synthesized ternary lipids (mixture) and binary blend of pure lipids (blend)/co-lipid/DNA lipoplexes compared to EPC/co-lipid/DNA at molar charge ratio of 0.5 to 10 and Lipofectamine 2000^{TM} (Lipo) (n = 9; mean ± SD) as positive controls, and plasmid DNA alone and CHO-K1 cells alone as negative controls. Numerical data from this experiment is labelled Exp17 in Table S3.

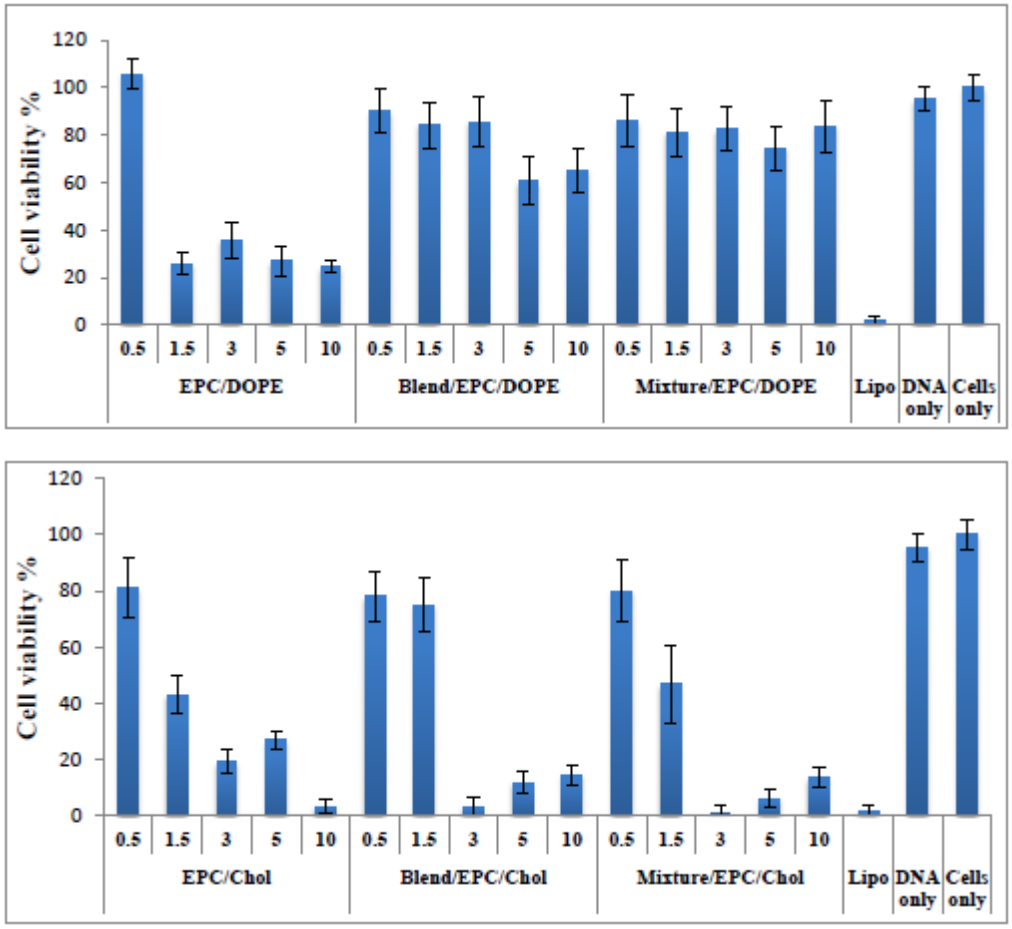


Figure S18 - Cytotoxicity (after 48 h) of synthesized ternary lipids (mixture) and binary blend of pure lipids (blend)/co-lipid/DNA lipoplexes compared to EPC/co-lipid/DNA at molar charge ratio of 0.5 to 10 and Lipofectamine 2000™ (Lipo) (n = 9; mean ± SD) as positive controls, and plasmid DNA alone and CHO-K1 cells alone as negative controls. Numerical data from this experiment is labelled Exp17 in Table S3.

Table S1: Particle sizing data

The column headings are: Experiment label; Formulation label used as the experiments were conducted;; Identity of the pyridinium lipid if present (Cat A); Identity of the other cationic lipid (Cat B); Identity of the co-lipid (Co-lipid); three columns giving the molar ratio of Cat A: CatB:Co-lipid; Charge ration of the experiment (CR) as the ratio of N/P cationic lipid:DNA phosphate; Average liposome diameter (Å); Polydispersity index (PDI) of the liposome; Average lipoplex diameter (Å); PDI of the lipoplex

Label	Formulation labels	Cat A	Cat B	Co-lipid	mol Cat A	mol Cat B	mol Co	CR	Zavg dia liposome	PDI liposome /A	Zavg dia lipoplex	PDI lipoplex
Exp1_1	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	0.5	671	0.5	890	0.3
Exp1_3	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	3.0	671	0.5	1375	0.4
Exp1_4	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	5.0	671	0.5	1205	0.4
Exp1_5	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	10.0	671	0.5	1160	0.4
Exp1_6	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	0.5	288	0.4	1142	0.3
Exp1_7	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	1.5	288	0.4	1334	0.3
Exp1_8	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	3.0	288	0.4	1568	0.4
Exp1_9	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	5.0	288	0.4	2380	0.6
Exp1_10	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	10.0	288	0.4	1118	0.3
Exp1_16	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	0.5	501	0.5	876	0.3
Exp1_17	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	1.5	501	0.5	952	0.3
Exp1_18	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	3.0	501	0.5	1335	0.4
Exp1_19	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	5.0	501	0.5	1203	0.4
Exp1_2	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	1.5	671	0.5	981	0.4
Exp1_20	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	10.0	501	0.5	1244	0.5
Exp1_21	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	0.5	256	0.3	497	0.3
Exp1_22	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	1.5	256	0.3	436	0.3
Exp1_23	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	3.0	256	0.3	1230	0.4
Exp1_24	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	5.0	256	0.3	9493	0.5
Exp1_25	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	10.0	256	0.3	2102	0.7
Exp4_6	(16:0)(11:1)EPC/DOPE	(16:0)(11:1)mix	EPC	DOPE	1.5	1.5	2	0.5	260	0.3	1472	0.4
Exp4_7	(16:0)(11:1)EPC/DOPE	(16:0)(11:1)mix	EPC	DOPE	1.5	1.5	2	1.5	260	0.3	1733	0.3
Exp4_8	(16:0)(11:1)EPC/DOPE	(16:0)(11:1)mix	EPC	DOPE	1.5	1.5	2	3.0	260	0.3	1611	0.5
Exp4_9	(16:0)(11:1)EPC/DOPE	(16:0)(11:1)mix	EPC	DOPE	1.5	1.5	2	5.0	260	0.3	4130	0.5
Exp4_10	(16:0)(11:1)EPC/DOPE	(16:0)(11:1)mix	EPC	DOPE	1.5	1.5	2	10.0	260	0.3	5200	0.3
Exp7_2	diC12:0/EPC/DOPE	diC12:0	EPC	DOPE	1.5	1.5	2	3.0	1891	0.5	2964	0.6
Exp7_3	diC14:0/EPC/DOPE	diC14:0	EPC	DOPE	1.5	1.5	2	3.0	908	0.5	2532	0.3
Exp7_4	diC16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	3.0	209	0.2	3621	0.4
Exp7_5	diC18:0/EPC/DOPE	diC18:0	EPC	DOPE	1.5	1.5	2	3.0	357	0.3	649	0.3
Exp7_6	diC18:1/EPC/DOPE	diC18:1	EPC	DOPE	1.5	1.5	2	3.0	723	0.8	4932	0.4
Exp7_7	diC20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	3.0	392	0.3	4219	0.4
Exp7_8	diCbr20:0/EPC/DOPE	diCbr20:0	EPC	DOPE	1.5	1.5	2	3.0	542	0.9	661	0.3
Exp7_9	(18:1)(br20:0)EPC Blend50/DOPE	(18:1)(br20:0)blend50	EPC	DOPE	1.5	1.5	2	3.0	481	0.5	864	0.5
Exp7_10	(18:1)(br20:0)EPC Blend66/DOPE	(18:1)(br20:0)blend66	EPC	DOPE	1.5	1.5	2	3.0	1923	0.7	807	0.4
Exp7_11	(18:1)(br20:0)EPC Blend85/DOPE	(18:1)(br20:0)blend85	EPC	DOPE	1.5	1.5	2	3.0	324	0.4	470	0.3
Exp7_13	diC12:0/EPC/chol	diC12:0	EPC	chol	1.5	1.5	2	3.0	1367	0.5	1096	0.4
Exp7_14	diC14:0/EPC/chol	diC14:0	EPC	chol	1.5	1.5	2	3.0	330	0.9	1227	0.5

Label	Formulation labels	Cat A	Cat B	Co-lipid	mol Cat A	mol Cat B	mol Co	CR	liposome /A	PDI liposome /A	lipoplex /A	lipoplex /A
Exp7_15	diC16: 0/EPC/chol	diC16:0	EPC	chol	1.5	1.5	2	3.0	842	0.5	2451	0.5
Exp7_16	diC18: 0/EPC/chol	diC18:0	EPC	chol	1.5	1.5	2	3.0	372	0.3	1589	0.4
Exp7_17	diC18:1/EPC/chol	diC18:1	EPC	chol	1.5	1.5	2	3.0	338	0.4	344	0.2
Exp7_18	diC20: 0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	3.0	668	0.5	658	0.4
Exp7_19	diCbr20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	3.0	392	0.4	497	0.3
Exp7_20	(18:1)(br20:0)/EPC Blend50/chol	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	3.0	274	0.3	2074	0.6
Exp7_21	(18:1)(br20:0)/EPC Blend66/chol	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	3.0	181	0.3	3367	0.6
Exp7_22	(18:1)(br20:0)/EPC Blend85/chol	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	3.0	414	0.5	511	0.4
Exp8_6	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	0.5	197	0.4	1103	0.2
Exp8_7	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	1.5	197	0.4	1066	0.3
Exp8_8	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	3.0	197	0.4	834	0.3
Exp8_9	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	5.0	197	0.4	3782	0.3
Exp8_10	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	10.0	197	0.4	4756	0.6
Exp8_11	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	0.5	396	0.4	1394	0.3
Exp8_12	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	1.5	396	0.4	1677	0.3
Exp8_13	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	3.0	396	0.4	1526	0.4
Exp8_14	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	5.0	396	0.4	2468	0.3
Exp8_15	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	10.0	396	0.4	4150	0.6
Exp8_16	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	0.5	369	0.4	483	0.3
Exp8_17	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	1.5	369	0.4	541	0.3
Exp8_18	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	3.0	369	0.4	4388	0.5
Exp8_19	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	5.0	369	0.4	5339	0.4
Exp8_20	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	10.0	369	0.4	1989	0.4
Exp8_21	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	0.5	473	0.5	542	0.2
Exp8_22	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	1.5	473	0.5	502	0.2
Exp8_23	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	3.0	473	0.5	505	0.2
Exp8_24	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	5.0	473	0.5	569	0.3
Exp8_25	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	10.0	473	0.5	4135	0.8
Exp8a_6	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	0.5	287	0.2	353	0.3
Exp8a_7	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	1.5	287	0.2	377	0.3
Exp8a_8	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	3.0	287	0.2	1498	0.4
Exp8a_9	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	5.0	287	0.2	5896	0.4
Exp8a_10	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	10.0	287	0.2	429	0.2
Exp8a_11	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	0.5	1186	0.5	678	0.3
Exp8a_12	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	1.5	1186	0.5	620	0.3
Exp8a_13	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	3.0	1186	0.5	2016	0.6

Label	Formulation labels	Cat A	Cat B	Co-lipid	mol Cat A	mol Cat B	mol Co	CR	liposome /A	PDI liposome /A	lipoplex /A	lipoplex /A
Exp8a_14	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	5.0	1186	0.5	5765	0.4
Exp8a_15	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	10.0	1186	0.5	2923	0.5
Exp8a_16	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	0.5	632	0.7	465	0.4
Exp8a_17	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	1.5	632	0.7	467	0.3
Exp8a_18	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	3.0	632	0.7	559	0.4
Exp8a_19	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	5.0	632	0.7	4421	0.7
Exp8a_20	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	10.0	632	0.7	3651	0.6
Exp8a_21	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	0.5	278	0.3	304	0.3
Exp8a_22	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	1.5	278	0.3	350	0.2
Exp8a_23	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	3.0	278	0.3	3278	0.7
Exp8a_24	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	5.0	278	0.3	5839	0.4
Exp8a_25	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	10.0	278	0.3	301	0.2
Exp9_4	(18:1)(br20:0)blend50/EPC/DOPE	(18:1)(br20:0)blend50	EPC	DOPE	1.5	1.5	2	1.5	481	0.5	515	0.3
Exp9_5	(18:1)(br20:0)blend50/EPC/DOPE	(18:1)(br20:0)blend50	EPC	DOPE	1.5	1.5	2	5.0	481	0.5	641	0.3
Exp9_6	(18:1)(br20:0)blend50/EPC/DOPE	(18:1)(br20:0)blend50	EPC	DOPE	1.5	1.5	2	10.0	481	0.5	7320	0.5
Exp9_7	(18:1)(br20:0)blend66/EPC/DOPE	(18:1)(br20:0)blend66	EPC	DOPE	1.5	1.5	2	1.5	1923	0.7	618	0.3
Exp9_8	(18:1)(br20:0)blend66/EPC/DOPE	(18:1)(br20:0)blend66	EPC	DOPE	1.5	1.5	2	5.0	1923	0.7	607	0.3
Exp9_9	(18:1)(br20:0)blend66/EPC/DOPE	(18:1)(br20:0)blend66	EPC	DOPE	1.5	1.5	2	10.0	1923	0.7	3056	0.7
Exp9_10	(18:1)(br20:0)blend85/EPC/DOPE	(18:1)(br20:0)blend85	EPC	DOPE	1.5	1.5	2	1.5	324	0.4	395	0.3
Exp9_11	(18:1)(br20:0)blend85/EPC/DOPE	(18:1)(br20:0)blend85	EPC	DOPE	1.5	1.5	2	5.0	324	0.4	522	0.3
Exp9_12	(18:1)(br20:0)blend85/EPC/DOPE	(18:1)(br20:0)blend85	EPC	DOPE	1.5	1.5	2	10.0	324	0.4	4961	0.5
Exp9_16	(18:1)(br20:0)blend50/EPC/Chol	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	1.5	274	0.3	301	0.2
Exp9_17	(18:1)(br20:0)blend50/EPC/Chol	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	5.0	274	0.3	10153	0.4
Exp9_18	(18:1)(br20:0)blend50/EPC/Chol	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	10.0	274	0.3	385	0.2
Exp9_19	(18:1)(br20:0)blend66/EPC/Chol	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	1.5	181	0.3	252	0.2
Exp9_20	(18:1)(br20:0)blend66/EPC/Chol	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	5.0	181	0.3	13990	0.6
Exp9_21	(18:1)(br20:0)blend66/EPC/Chol	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	10.0	181	0.3	280	0.1
Exp9_22	(18:1)(br20:0)blend85/EPC/Chol	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	1.5	414	0.5	409	0.3
Exp9_23	(18:1)(br20:0)blend85/EPC/Chol	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	5.0	414	0.5	2745	0.6
Exp9_24	(18:1)(br20:0)blend85/EPC/Chol	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	10.0	414	0.5	5680	0.8
Exp17_11	blend(18:1)(br20:0) 32/EPC/Chol	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	0.5	445	0.3	689	0.3
Exp17_12	blend(18:1)(br20:0) 32/EPC/Chol	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	1.5	445	0.3	586	0.3
Exp17_13	blend(18:1)(br20:0) 32/EPC/Chol	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	3.0	445	0.3	827	0.3
Exp17_14	blend(18:1)(br20:0) 32/EPC/Chol	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	5.0	445	0.3	5750	0.5
Exp17_15	blend(18:1)(br20:0) 32/EPC/Chol	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	10.0	445	0.3	6887	0.4
Exp17_16	blend(18:1)(br20:0) 32/EPC/Chol	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	0.5	379	0.4	277	0.4

Label	Formulation labels	Cat A	Cat B	Co-lipid	mol Cat A	mol Cat B	mol Co	CR	liposome /A	PDI liposome /A	lipoplex /A	lipoplex /A
Exp17_17	blend(18:1)(br20:0) 32/EPC/Chol	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	1.5	379	0.4	324	0.3
Exp17_18	blend(18:1)(br20:0) 32/EPC/Chol	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	3.0	379	0.4	7738	0.3
Exp17_19	blend(18:1)(br20:0) 32/EPC/Chol	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	5.0	379	0.4	3625	0.7
Exp17_20	blend(18:1)(br20:0) 32/EPC/Chol	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	10.0	379	0.4	294	0.2
Exp17_21	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	0.5	462	0.4	737	0.4
Exp17_22	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	1.5	462	0.4	530	0.3
Exp17_23	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	3.0	462	0.4	468	0.3
Exp17_24	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	5.0	462	0.4	798	0.3
Exp17_25	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	10.0	462	0.4	5409	0.4
Exp17_26	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	0.5	319	0.4	251	0.3
Exp17_27	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	1.5	319	0.4	301	0.2
Exp17_28	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	3.0	319	0.4	8004	0.3
Exp17_29	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	5.0	319	0.4	1745	0.4
Exp17_30	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	10.0	319	0.4	264	0.2

Table S2: SAXD data

The column headings are: Experiment label; Formulation label used as the experiments were conducted; Identity of the pyridinium lipid if present (Cat A); Identity of the other cationic lipid (Cat B); Identity of the co-lipid (Co-lipid); three columns giving the molar ratio of Cat A: CatB:Co-lipid; Charge ration of the experiment (CR) as the ratio of N/P cationic lipid:DNA phosphate; SAXD phase observed; lattice parameter of the phase (Å)

Label	Formulation labels	Cat A	Cat B	Co-lipid	mol Cat A	mol Cat B	mol Co	CR	SAXS phase	lattice parameter
Exp1_1	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	0.5	hexagonal	70
Exp1_2	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	1.5	hexagonal	70
Exp1_3	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	3.0	hexagonal	70
Exp1_4	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	5.0	hexagonal	70
Exp1_5	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	10.0	hexagonal	70
Exp1_6	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	0.5	hexagonal	65
Exp1_7	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	1.5	hexagonal	65
Exp1_8	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	3.0	hexagonal	65
Exp1_9	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	5.0	hexagonal	65
Exp1_10	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	10.0	hexagonal	65
Exp1_16	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	0.5	ND	ND
Exp1_17	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	1.5	ND	ND
Exp1_18	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	3.0	ND	ND
Exp1_19	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	5.0	ND	ND
Exp1_20	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	10.0	ND	ND
Exp1_21	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	0.5	hexagonal	71
Exp1_22	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	1.5	hexagonal	71
Exp1_23	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	3.0	hexagonal	71
Exp1_24	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	5.0	hexagonal	71
Exp1_25	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	10.0	hexagonal	71
Exp7_2	diC12:0/EPC/DOPE	diC12:0	EPC	DOPE	1.5	1.5	2	3.0	hexagonal	60-62
Exp7_2x	diC12:0/EPC/DOPE	diC12:0	EPC	DOPE	1.5	1.5	2	1.5	hexagonal?	63
Exp7_3	diC14:0/EPC/DOPE	diC14:0	EPC	DOPE	1.5	1.5	2	3.0	hexagonal?	64
Exp7_3x	diC14:0/EPC/DOPE	diC14:0	EPC	DOPE	1.5	1.5	2	1.5	hexagonal?	69
Exp7_4	diC16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	3.0	indeterminate	na
Exp7_4x	diC16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	1.5	hexagonal	68
Exp7_5	diC18:0/EPC/DOPE	diC18:0	EPC	DOPE	1.5	1.5	2	3.0	hexagonal?	73
Exp7_5x	diC18:0/EPC/DOPE	diC18:0	EPC	DOPE	1.5	1.5	2	1.5	hexagonal	76-84
Exp7_6	diC18:1/EPC/DOPE	diC18:1	EPC	DOPE	1.5	1.5	2	3.0	no XRD	
Exp7_6x	diC18:1/EPC/DOPE	diC18:1	EPC	DOPE	1.5	1.5	2	1.5	hexagonal	68
Exp7_7	diC20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	3.0	no XRD	
Exp7_7x	diC20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	1.5	no XRD	
Exp7_13	diC12:0/EPC/chol	diC12:0	EPC	chol	1.5	1.5	2	3.0	hexagonal?	84
Exp7_13x	diC12:0/EPC/chol	diC12:0	EPC	chol	1.5	1.5	2	1.5	hexagonal?	80
Exp7_14	diC14:0/EPC/chol	diC14:0	EPC	chol	1.5	1.5	2	3.0	indeterminate	na
Exp7_14x	diC14:0/EPC/chol	diC14:0	EPC	chol	1.5	1.5	2	1.5	hexagonal	84
Exp7_15	diC16:0/EPC/chol	diC16:0	EPC	chol	1.5	1.5	2	3.0	hexagonal	82
Exp7_15x	diC16:0/EPC/chol	diC16:0	EPC	chol	1.5	1.5	2	1.5	hexagonal?	75-90
Exp7_16	diC18:0/EPC/chol	diC18:0	EPC	chol	1.5	1.5	2	3.0	lamellar	70
Exp7_16x	diC18:0/EPC/chol	diC18:0	EPC	chol	1.5	1.5	2	1.5	indeterminate	na
Exp7_17	diC18:1/EPC/chol	diC18:1	EPC	chol	1.5	1.5	2	3.0	indeterminate	na
Exp7_17x	diC18:1/EPC/chol	diC18:1	EPC	chol	1.5	1.5	2	1.5	hexagonal	82
Exp7_18	diC20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	3.0	indeterminate	na
Exp7_18x	diC20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	1.5	indeterminate	na
Exp8_7	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	1.5	lamellar	63
Exp8_12	Diiso3:0/EPC/DOPE	diisoC3:0	EPC	DOPE	1.5	1.5	2	1.5	hexagonal	68
Exp8_18	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	3.0	hexagonal	64
Exp8_23	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	3.0	hexagonal	68
Exp8a_7	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	1.5	lamellar	68
Exp8a_12	Diiso3:0/EPC/chol	diisoC3:0	EPC	chol	1.5	1.5	2	1.5	lamellar	66-85
Exp8a_18	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	3.0	lamellar	71
Exp8a_23	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	3.0	lamellar	70

Table S3: Transfection and cell viability data

The column headings are: Experiment label; Formulation label used as the experiments were conducted; Identity of the pyridinium lipid if present (Cat A); Identity of the other cationic lipid (Cat B); Identity of the co-lipid (Co-lipid); three columns giving the molar ratio of Cat A: CatB:Co-lipid; Charge ration of the experiment (CR) as the ratio of N/P cationic lipid:DNA phosphate; Average transfection; Standaed error in Transfection; Normalized transfection efficiency based on the efficieincy of Lipofetamine = 100 and cells-alone = 0; Average cell viability; Standard deviation in cell viability.

Label	Formulation labels	Cat A	Cat B	Co-lipid	mol Cat A	mol Cat B	mol Co	CR	T Avg	T SE	TE norm	T SE Norm	Cyt avg	Cyt SD
Exp1_1	DiC16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	0.5	58924	9136	0.152	0.030	113.9	20.8
Exp1_2	DiC16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	1.5	68418	8538	0.189	0.031	107.7	20.4
Exp1_3	DiC16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	3.0	88966	8925	0.267	0.036	106.3	9.8
Exp1_4	DiC16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	5.0	121390	13363	0.392	0.051	82.8	15.8
Exp1_5	DiC16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	10.0	86290	12856	0.257	0.045	75.7	17.6
Exp1_6	DiC16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	0.5	39649	5175	0.078	0.018	139.4	11.6
Exp1_7	DiC16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	1.5	51208	6679	0.122	0.024	119.5	18.1
Exp1_8	DiC16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	3.0	58884	11996	0.152	0.036	117.8	17.6
Exp1_9	DiC16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	5.0	64384	8681	0.173	0.031	109.1	13.2
Exp1_10	DiC16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	10.0	54714	2915	0.136	0.020	105.1	16.6
Exp1_16	DiC16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	0.5	50132	5370	0.118	0.022	108.2	18.1
Exp1_17	DiC16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	1.5	91697	7976	0.278	0.034	108.2	9.9
Exp1_18	DiC16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	3.0	187306	18388	0.645	0.071	67.7	15.2
Exp1_19	DiC16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	5.0	188444	38650	0.650	0.137	51.0	39.4
Exp1_20	DiC16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	10.0	87038	18508	0.260	0.060	49.3	37.3
Exp1_21	DiC16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	0.5	73271	5632	0.207	0.027	114.9	12.6
Exp1_22	DiC16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	1.5	119358	10218	0.384	0.042	104.8	19.3
Exp1_23	DiC16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	3.0	227503	19568	0.800	0.078	60.3	16.3
Exp1_24	DiC16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	5.0	171634	14831	0.585	0.060	84.1	12.0
Exp1_25	DiC16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	10.0	143416	13847	0.477	0.054	74.8	10.7
Exp1_31	Lipofectamine								279549	5866	1.000	0.047	14.8	0.3
Exp1_32	DNA alone								14453	4675	-0.019		100.7	44.6
Exp1_33	Cells alone								19371	7224	0.000		100.0	48.6
Exp4_6	(16:0)[(1t:1)EPC/DOPE	(16:0)[(1t:1)mix	EPC	DOPE	1.5	1.5	2	0.5	59611	8912	0.120	0.028	102.7	5.2
Exp4_7	(16:0)[(1t:1)EPC/DOPE	(16:0)[(1t:1)mix	EPC	DOPE	1.5	1.5	2	1.5	66451	8148	0.134	0.027	106.4	10.3
Exp4_8	(16:0)[(1t:1)EPC/DOPE	(16:0)[(1t:1)mix	EPC	DOPE	1.5	1.5	2	3.0	96917	5913	0.196	0.025	85.9	4.2
Exp4_9	(16:0)[(1t:1)EPC/DOPE	(16:0)[(1t:1)mix	EPC	DOPE	1.5	1.5	2	5.0	75372	20704	0.152	0.047	78.9	1.1
Exp4_10	(16:0)[(1t:1)EPC/DOPE	(16:0)[(1t:1)mix	EPC	DOPE	1.5	1.5	2	10.0	57663	9746	0.117	0.029	70.3	1.9
Exp4_11	Lipofectamine								494825	19997	1.000	0.064	10.9	0.8
Exp4_12	DNA alone								18174	3328	0.037		96.7	1.0
Exp4_13	Cells alone								8565	9945	0.017		100.0	2.0
Exp7_2	diC12:0/EPC/DOPE	diC12:0	EPC	DOPE	1.5	1.5	2	3.0	445353	36644	0.693	0.078	59.1	16.2
Exp7_3	diC14:0/EPC/DOPE	diC14:0	EPC	DOPE	1.5	1.5	2	3.0	578507	25100	0.912	0.079	58.4	2.9
Exp7_4	diC16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	3.0	268641	52171	0.402	0.085	68.9	3.7
Exp7_5	diC18:0/EPC/DOPE	diC18:0	EPC	DOPE	1.5	1.5	2	3.0	201873	23951	0.293	0.051	84.5	3.6
Exp7_6	diC18:1/EPC/DOPE	diC18:1	EPC	DOPE	1.5	1.5	2	3.0	433876	82735	0.674	0.139	70.4	10.2
Exp7_7	diC20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	3.0	183658	46359	0.263	0.071	90.7	5.9
Exp7_8	diCbr20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	3.0	434017	48869	0.674	0.092	63.2	6.7
Exp7_9	(18:1)[br20:0)/EPC Blend50/DO	(18:1)[br20:0)blend50	EPC	DOPE	1.5	1.5	2	3.0	432533	33231	0.672	0.073	64.9	0.8
Exp7_10	(18:1)[br20:0)/EPC Blend66/DO	(18:1)[br20:0)blend66	EPC	DOPE	1.5	1.5	2	3.0	519408	39770	0.814	0.088	60.1	4.7
Exp7_11	(18:1)[br20:0)/EPC Blend85/DO	(18:1)[br20:0)blend85	EPC	DOPE	1.5	1.5	2	3.0	409881	7975	0.634	0.050	56.0	4.0
Exp7_13	diC12:0/EPC/chol	diC12:0	EPC	chol	1.5	1.5	2	3.0	520807	58483	0.817	0.111	62.1	8.2
Exp7_14	diC14:0/EPC/chol	diC14:0	EPC	chol	1.5	1.5	2	3.0	556771	50858	0.876	0.104	55.3	0.3
Exp7_15	diC16:0/EPC/chol	diC16:0	EPC	chol	1.5	1.5	2	3.0	467554	3256	0.729	0.056	65.0	8.5
Exp7_16	diC18:0/EPC/chol	diC18:0	EPC	chol	1.5	1.5	2	3.0	369694	44352	0.568	0.081	50.0	6.0
Exp7_17	diC18:1/EPC/chol	diC18:1	EPC	chol	1.5	1.5	2	3.0	674285	64639	1.069	0.130	37.4	1.1
Exp7_18	diC20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	3.0	499328	19661	0.782	0.067	56.8	3.9
Exp7_23	Lipo+DNA								632277	44732	1.000	0.103	40.8	5.7
Exp7_24	DNA alone								61595	22682	0.062		91.1	1.0
Exp7_25	Cells alone								23842	10593	0.000		100.0	1.5
Exp8_6	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	0.5	161269	6833	0.392	0.018	86.4	9.6
Exp8_7	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	1.5	203491	8343	0.497	0.023	76.9	10.8

Label	Formulation labels	Cat A	Cat B	Co-lipid	mol Cat A	mol Cat B	mol Co	CR	T Avg	T SE	TE norm	T SE Norm	Cyt avg	Cyt SD
Exp7_19	diCbr20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	3.0	573175	53795	0.903	0.108	39.3	6.5
Exp7_20	(18:1)(br20:0)/EPC Blend50/chc	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	3.0	759474	26750	1.209	0.099	35.7	4.5
Exp7_21	(18:1)(br20:0)/EPC Blend66/chc	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	3.0	497548	86312	0.779	0.147	55.2	7.5
Exp7_22	(18:1)(br20:0)/EPC Blend85/chc	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	3.0	653813	76992	1.035	0.144	46.1	5.2
Exp8_8	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	3.0	204182	6320	0.499	0.018	69.7	1.9
Exp8_9	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	5.0	214562	4687	0.525	0.016	65.4	1.6
Exp8_10	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	10.0	84854	16850	0.202	0.040	43.1	4.8
Exp8_11	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	0.5	185653	4153	0.453	0.014	82.0	0.8
Exp8_12	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	1.5	401669	15478	0.990	0.043	30.8	2.0
Exp8_13	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	3.0	22191	816	0.046	0.002	13.8	0.5
Exp8_14	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	5.0	70734	21743	0.167	0.051	8.8	0.5
Exp8_15	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	10.0	20538	9035	0.042	0.018	6.7	0.2
Exp8_16	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	0.5	12421	1847	0.022	0.003	79.4	3.2
Exp8_17	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	1.5	13362	2772	0.024	0.005	84.7	4.0
Exp8_18	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	3.0	83495	4318	0.199	0.011	69.2	3.3
Exp8_19	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	5.0	44028	968	0.100	0.003	58.5	2.9
Exp8_20	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	10.0	52854	8504	0.122	0.020	39.5	3.8
Exp8_21	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	0.5	81336	5402	0.193	0.013	93.3	0.7
Exp8_22	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	1.5	154651	3329	0.376	0.011	84.4	3.4
Exp8_23	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	3.0	161748	14114	0.393	0.035	77.6	4.8
Exp8_24	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	5.0	219192	24047	0.536	0.060	73.6	1.5
Exp8_25	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	10.0	248256	3935	0.608	0.016	51.3	3.2
Exp8_26	Lipo+DNA								405616	8128	1.000	0.028	26.3	1.5
Exp8_27	DNA alone								11350	2003	0.019		88.7	4.0
Exp8_28	Cells alone								3700	456	0.000		100.0	3.4
Exp8a_6	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	0.5	39945	10322	0.094	0.024	55.5	2.8
Exp8a_7	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	1.5	4186	511	0.000	0.000	16.4	1.1
Exp8a_8	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	3.0	4304	836	0.001	0.000	4.7	0.1
Exp8a_9	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	5.0	7975	128	0.010	0.002	8.1	0.4
Exp8a_10	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	10.0	25706	2906	0.056	0.007	6.3	0.3
Exp8a_11	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	0.5	172545	7173	0.440	0.022	25.5	0.8
Exp8a_12	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	1.5	20768	2250	0.044	0.005	12.1	0.8
Exp8a_13	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	3.0	6127	672	0.005	0.001	5.3	0.1
Exp8a_14	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	5.0	4818	133	0.002	0.000	4.8	0.3
Exp8a_15	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	10.0	4604	667	0.001	0.000	6.7	0.4
Exp8a_16	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	0.5	43583	2690	0.103	0.007	88.1	1.8
Exp8a_17	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	1.5	123850	1939	0.313	0.010	93.9	3.2
Exp8a_18	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	3.0	178468	5482	0.455	0.019	79.7	9.4
Exp8a_19	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	5.0	208523	1930	0.534	0.015	78.0	4.0
Exp8a_20	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	10.0	206353	5286	0.528	0.020	69.6	1.4
Exp8a_21	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	0.5	23809	4672	0.051	0.011	90.3	3.9
Exp8a_22	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	1.5	95793	4465	0.239	0.013	81.4	2.0
Exp8a_23	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	3.0	196281	11385	0.502	0.032	47.2	4.3
Exp8a_24	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	5.0	259709	8120	0.667	0.028	40.5	6.3
Exp8a_25	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	10.0	239287	10672	0.614	0.032	52.9	2.3
Exp8a_26	Lipo+DNA								387139	10186	1.000	0.037	17.8	2.9
Exp8a_27	DNA alone								25224	1319	0.055		94.4	8.1
Exp8a_28	Cells alone								10983	1187	0.018		100.0	5.3
Exp9_4	(18:1)(br20:0)blend50/EPC/DOF	(18:1)(br20:0)blend50	EPC	DOPE	1.5	1.5	2	1.5	344403	61299	0.455	0.088	85.6	10.9
Exp9_5	(18:1)(br20:0)blend50/EPC/DOF	(18:1)(br20:0)blend50	EPC	DOPE	1.5	1.5	2	5.0	325691	3488	0.428	0.034	100.1	2.5
Exp9_6	(18:1)(br20:0)blend50/EPC/DOF	(18:1)(br20:0)blend50	EPC	DOPE	1.5	1.5	2	10.0	329937	25080	0.434	0.047	86.6	4.0
Exp9_7	(18:1)(br20:0)blend66/EPC/DOF	(18:1)(br20:0)blend66	EPC	DOPE	1.5	1.5	2	1.5	286877	13958	0.372	0.036	102.0	1.4
Exp9_8	(18:1)(br20:0)blend66/EPC/DOF	(18:1)(br20:0)blend66	EPC	DOPE	1.5	1.5	2	5.0	415016	34365	0.557	0.060	87.7	4.7

Label	Formulation labels	Cat A	Cat B	Co-lipid	mol Cat A	mol Cat B	mol Co	CR	T Avg	T SE	TE norm	T SE Norm	Cyt avg	Cyt SD
Exp9_9	(18:1)(br20:0)blend66/EPC/DOF	(18:1)(br20:0)blend66	EPC	DOPE	1.5	1.5	2	10.0	422258	66717	0.567	0.097	78.6	8.9
Exp9_10	(18:1)(br20:0)blend85/EPC/DOF	(18:1)(br20:0)blend85	EPC	DOPE	1.5	1.5	2	1.5	235546	10033	0.299	0.032	93.7	2.8
Exp9_11	(18:1)(br20:0)blend85/EPC/DOF	(18:1)(br20:0)blend85	EPC	DOPE	1.5	1.5	2	5.0	357239	17864	0.474	0.042	76.8	16.8
Exp9_12	(18:1)(br20:0)blend85/EPC/DOF	(18:1)(br20:0)blend85	EPC	DOPE	1.5	1.5	2	10.0	403305	61858	0.540	0.091	66.4	3.5
Exp9_16	(18:1)(br20:0)blend50/EPC/Cho	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	1.5	379126	21140	0.505	0.046	82.9	7.5
Exp9_17	(18:1)(br20:0)blend50/EPC/Cho	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	5.0	539314	25734	0.736	0.056	66.0	2.4
Exp9_18	(18:1)(br20:0)blend50/EPC/Cho	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	10.0	613986	54256	0.843	0.089	49.7	1.2
Exp9_19	(18:1)(br20:0)blend66/EPC/Cho	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	1.5	264227	1275	0.340	0.031	94.9	0.6
Exp9_20	(18:1)(br20:0)blend66/EPC/Cho	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	5.0	560840	78330	0.767	0.116	51.9	10.6
Exp9_21	(18:1)(br20:0)blend66/EPC/Cho	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	10.0	579727	57516	0.794	0.091	42.1	6.5
Exp9_22	(18:1)(br20:0)blend85/EPC/Cho	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	1.5	407372	10155	0.546	0.040	82.7	5.2
Exp9_23	(18:1)(br20:0)blend85/EPC/Cho	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	5.0	541839	57169	0.740	0.090	48.8	3.7
Exp9_24	(18:1)(br20:0)blend85/EPC/Cho	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	10.0	541066	14889	0.738	0.049	52.0	1.4
Exp9_25	Lipofectamine								722757	27378	1.000	0.067	16.5	1.6
Exp9_26	DNA alone								72649	3832	0.064		96.9	6.2
Exp9_27	Cells alone								28223	20175	0.000		100.0	4.6
Exp17_16	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	0.5	99819	4171	0.156	0.013	90.1	27.4
Exp17_17	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	1.5	270250	44092	0.460	0.082	84.4	29.2
Exp17_18	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	3.0	381234	12131	0.658	0.052	85.6	31.9
Exp17_19	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	5.0	294124	34942	0.502	0.070	60.6	30.8
Exp17_20	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	10.0	226004	24238	0.381	0.049	65.2	28.2
Exp17_26	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	0.5	109394	13614	0.173	0.025	78.2	26.5
Exp17_27	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	1.5	250544	39980	0.424	0.074	75.0	29.1
Exp17_28	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	3.0	393463	28063	0.679	0.069	3.0	10.7
Exp17_29	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	5.0	432762	28254	0.750	0.073	11.8	12.1
Exp17_30	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	10.0	294897	10545	0.504	0.041	14.6	10.8
Exp17_11	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	0.5	94530	5107	0.146	0.014	86.0	33.2
Exp17_12	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	1.5	105110	5584	0.165	0.015	81.0	31.3
Exp17_13	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	3.0	122005	8362	0.195	0.020	82.5	28.1
Exp17_14	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	5.0	159308	14571	0.262	0.031	74.4	28.0
Exp17_15	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	10.0	141009	12379	0.229	0.026	83.6	32.1
Exp17_21	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	0.5	73424	6318	0.108	0.012	79.8	33.6
Exp17_22	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	1.5	92533	8429	0.143	0.017	46.7	41.9
Exp17_23	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	3.0	112667	15523	0.178	0.028	0.7	8.9
Exp17_24	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	5.0	94910	12318	0.147	0.022	6.1	9.6
Exp17_25	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	10.0	111950	6688	0.177	0.017	13.6	10.9
Exp17_31	Lipofectamine								573168	41492	1.000	0.102	1.8	4.9
Exp17_32	DNA alone								28890	5731	0.029	0.199	95.2	15.6
Exp17_33	Cells alone								12618	1813	0.000	0.145	100.0	15.8

Table S4: calculated molecular parameters

Compound name	lc Å	a head Å^2	V tails Å^3	S	clogP	ltot Å	Vtot Å^2	nC
chol	17.4	27.5	545	1.14	9.9	23.3	654	14
EPC	17.9	54.5	876	0.90	9.8	26.2	1179	12
DOPE	18.7	45.4	967	1.14	14.8	26.3	1198	8
diC9:0	10.3	42.0	506	1.17	5.1	17.6	712	8
diisoC9:0	7.2	42.0	506	1.67	4.0	14.5	712	8
diC11:1	12.0	41.9	593	1.18	6.3	19.3	798	8
diC11:0	12.3	41.9	614	1.19	7.3	19.6	819	9
diC12:0	13.3	41.9	668	1.20	8.3	20.6	872	10
diC14:0	15.3	41.8	775	1.21	10.5	22.6	979	12
diC16:0	17.4	41.7	883	1.22	12.6	24.6	1086	14
diC16:1	17.4	41.7	862	1.19	11.6	24.6	1066	13
diC18:0	19.4	41.7	990	1.23	14.7	26.7	1193	16
diC18:1	18.7	41.7	969	1.25	13.7	26.0	1173	8
(C16:1)(C11:0)	14.7	41.8	728	1.19	9.4	22.0	932	11
diC20:0	21.4	41.6	1097	1.23	16.8	28.7	1300	18
dibrC20:0	12.3	41.6	1108	2.16	16.5	19.6	1311	10
(C16:0)(C11:1)	14.7	41.8	738	1.20	9.4	22.0	942	11.5
(C18:1)(brC20:0)	15.5	41.7	1033	1.60	15.1	22.8	1236	9
(16:0)(11:1)mix	14.5	41.8	722.9	1.2	9.2	21.8	927.1	10.8
diC16:1 mix	17.1	41.7	849	1.19	11.4	24.4	1052	12.8
(C18:1)(brC20:0)mix	15.1	41.7	1044	1.70	14.9	22.4	1247	8.84
(18:1)(br20:0)blend0.32/0.68	14.3	41.6	1063	1.87	15.6	21.6	1266	9.36
(18:1)(br20:0)blend50	15.5	41.7	1039	1.70	15.1	22.8	1242	9
(18:1)(br20:0)blend66	14.5	41.6	1061	1.85	15.5	21.7	1264	9.32
(18:1)(br20:0)blend85	13.3	41.6	1087	2.03	16.1	20.5	1290	9.7

Table S5: TI_{PVM} for experimental mixtures

The column headings are: Experiment label; Formulation label used as the experiments were conducted; Identity of the pyridinium lipid if present (Cat A); Identity of the other cationic lipid (Cat B); Identity of the co-lipid (Co-lipid); three columns giving the molar ratio of Cat A: CatB:Co-lipid; Charge ration of the experiment (CR) as the ratio of N/P cationic lipid:DNA phosphate;; calculated logP of the mixed lipids; calculated S of the mixed lipids; calculated l_c of the mixed lipids (Å); calculated volume of the mixed lipids (Å^3); calculated TI_{PVM}; calculated partition term; calculated volume filling term; calculated melting term.

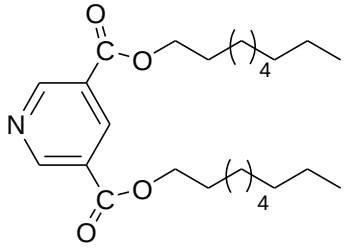
Label	Formulation labels	Cat A	Cat B	Co-lipid	mol Cat A	mol Cat B	mol Co	CR	T Avg	T SE	TE norm	T SE Norm	Cpt avg	Cpt SD
Exp1_1	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	0.5	58924	9136	0.152	0.030	113.9	20.8
Exp1_2	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	1.5	68418	8538	0.189	0.031	107.7	20.4
Exp1_3	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	3.0	88966	8925	0.267	0.036	106.3	9.8
Exp1_4	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	5.0	121390	13363	0.392	0.051	82.8	15.8
Exp1_5	Di16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	10.0	86290	12856	0.257	0.045	75.7	17.6
Exp1_6	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	0.5	39649	5175	0.078	0.018	139.4	11.6
Exp1_7	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	1.5	51208	6679	0.122	0.024	119.5	18.1
Exp1_8	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	3.0	58884	11996	0.152	0.036	117.8	17.6
Exp1_9	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	5.0	64384	9681	0.173	0.031	109.1	13.2
Exp1_10	Di16:1/EPC/DOPE	diC16:1	EPC	DOPE	1.5	1.5	2	10.0	54714	2915	0.136	0.020	105.1	16.6
Exp1_16	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	0.5	50132	5370	0.118	0.022	108.2	18.1
Exp1_17	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	1.5	91697	7976	0.278	0.034	108.2	9.9
Exp1_18	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	3.0	187306	18388	0.645	0.071	67.7	15.2
Exp1_19	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	5.0	188444	38650	0.650	0.137	51.0	39.4
Exp1_20	Di16:0/EPC/Chol	diC16:0	EPC	chol	1.5	1.5	2	10.0	87038	18508	0.260	0.060	43.3	37.3
Exp1_21	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	0.5	73271	5632	0.207	0.027	114.9	12.6
Exp1_22	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	1.5	119358	10218	0.384	0.042	104.8	19.3
Exp1_23	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	3.0	227503	19568	0.800	0.078	60.3	16.3
Exp1_24	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	5.0	171634	14831	0.585	0.060	84.1	12.0
Exp1_25	Di16:1/EPC/Chol	diC16:1	EPC	chol	1.5	1.5	2	10.0	143416	13847	0.477	0.054	74.8	10.7
Exp1_31	Lipofectamine								279549	5866	1.000	0.047	14.8	0.3
Exp1_32	DNA alone								14453	4675	-0.019		100.7	44.6
Exp1_33	Cells alone								19371	7224	0.000		100.0	48.6
Exp4_6	(16:0)(11:1)EPC/DOPE	(16:0)(11:1)mix	EPC	DOPE	1.5	1.5	2	0.5	59611	8912	0.120	0.028	102.7	5.2
Exp4_7	(16:0)(11:1)EPC/DOPE	(16:0)(11:1)mix	EPC	DOPE	1.5	1.5	2	1.5	66451	8148	0.134	0.027	106.4	10.3
Exp4_8	(16:0)(11:1)EPC/DOPE	(16:0)(11:1)mix	EPC	DOPE	1.5	1.5	2	3.0	96917	5913	0.196	0.025	85.9	4.2
Exp4_9	(16:0)(11:1)EPC/DOPE	(16:0)(11:1)mix	EPC	DOPE	1.5	1.5	2	5.0	75372	20704	0.152	0.047	78.9	1.1
Exp4_10	(16:0)(11:1)EPC/DOPE	(16:0)(11:1)mix	EPC	DOPE	1.5	1.5	2	10.0	57663	9746	0.117	0.029	70.3	1.9
Exp4_11	Lipofectamine								494825	19997	1.000	0.064	10.9	0.8
Exp4_12	DNA alone								18174	3328	0.037		96.7	1.0
Exp4_13	Cells alone								8565	9945	0.017		100.0	2.0
Exp7_2	diC12:0/EPC/DOPE	diC12:0	EPC	DOPE	1.5	1.5	2	3.0	445353	36644	0.693	0.078	59.1	16.2
Exp7_3	diC14:0/EPC/DOPE	diC14:0	EPC	DOPE	1.5	1.5	2	3.0	578507	25100	0.912	0.079	58.4	2.9
Exp7_4	diC16:0/EPC/DOPE	diC16:0	EPC	DOPE	1.5	1.5	2	3.0	268641	52171	0.402	0.085	68.3	3.7
Exp7_5	diC18:0/EPC/DOPE	diC18:0	EPC	DOPE	1.5	1.5	2	3.0	201873	23951	0.293	0.051	84.5	3.6
Exp7_6	diC18:1/EPC/DOPE	diC18:1	EPC	DOPE	1.5	1.5	2	3.0	433876	82735	0.674	0.139	70.4	10.2
Exp7_7	diC20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	3.0	183658	46359	0.263	0.071	90.7	5.9
Exp7_8	diCbr20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	3.0	434017	48869	0.674	0.092	63.2	6.7
Exp7_9	(18:1)[br20:0]/EPC Blend50/DO	(18:1)[br20:0]blend50	EPC	DOPE	1.5	1.5	2	3.0	432533	33231	0.672	0.073	64.9	0.8
Exp7_10	(18:1)[br20:0]/EPC Blend66/DO	(18:1)[br20:0]blend66	EPC	DOPE	1.5	1.5	2	3.0	519408	39770	0.814	0.088	60.1	4.7
Exp7_11	(18:1)[br20:0]/EPC Blend85/DO	(18:1)[br20:0]blend85	EPC	DOPE	1.5	1.5	2	3.0	409881	7975	0.634	0.050	56.0	4.0
Exp7_13	diC12:0/EPC/chol	diC12:0	EPC	chol	1.5	1.5	2	3.0	520807	58483	0.817	0.111	62.1	8.2
Exp7_14	diC14:0/EPC/chol	diC14:0	EPC	chol	1.5	1.5	2	3.0	556771	50858	0.876	0.104	55.3	0.3
Exp7_15	diC16:0/EPC/chol	diC16:0	EPC	chol	1.5	1.5	2	3.0	467554	3256	0.729	0.056	65.0	8.5
Exp7_16	diC18:0/EPC/chol	diC18:0	EPC	chol	1.5	1.5	2	3.0	369694	44352	0.568	0.081	50.0	6.0
Exp7_17	diC18:1/EPC/chol	diC18:1	EPC	chol	1.5	1.5	2	3.0	674285	64639	1.069	0.130	37.4	1.1
Exp7_18	diC20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	3.0	499928	19661	0.782	0.067	56.8	3.9
Exp7_23	Lipo-DNA								632277	44732	1.000	0.103	40.8	5.7
Exp7_24	DNA alone								61595	22682	0.062		91.1	1.0
Exp7_25	Cells alone								23842	10593	0.000		100.0	1.5
Exp8_6	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	0.5	161269	6833	0.392	0.018	86.4	9.6
Exp8_7	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	1.5	203491	8343	0.497	0.023	76.9	10.8

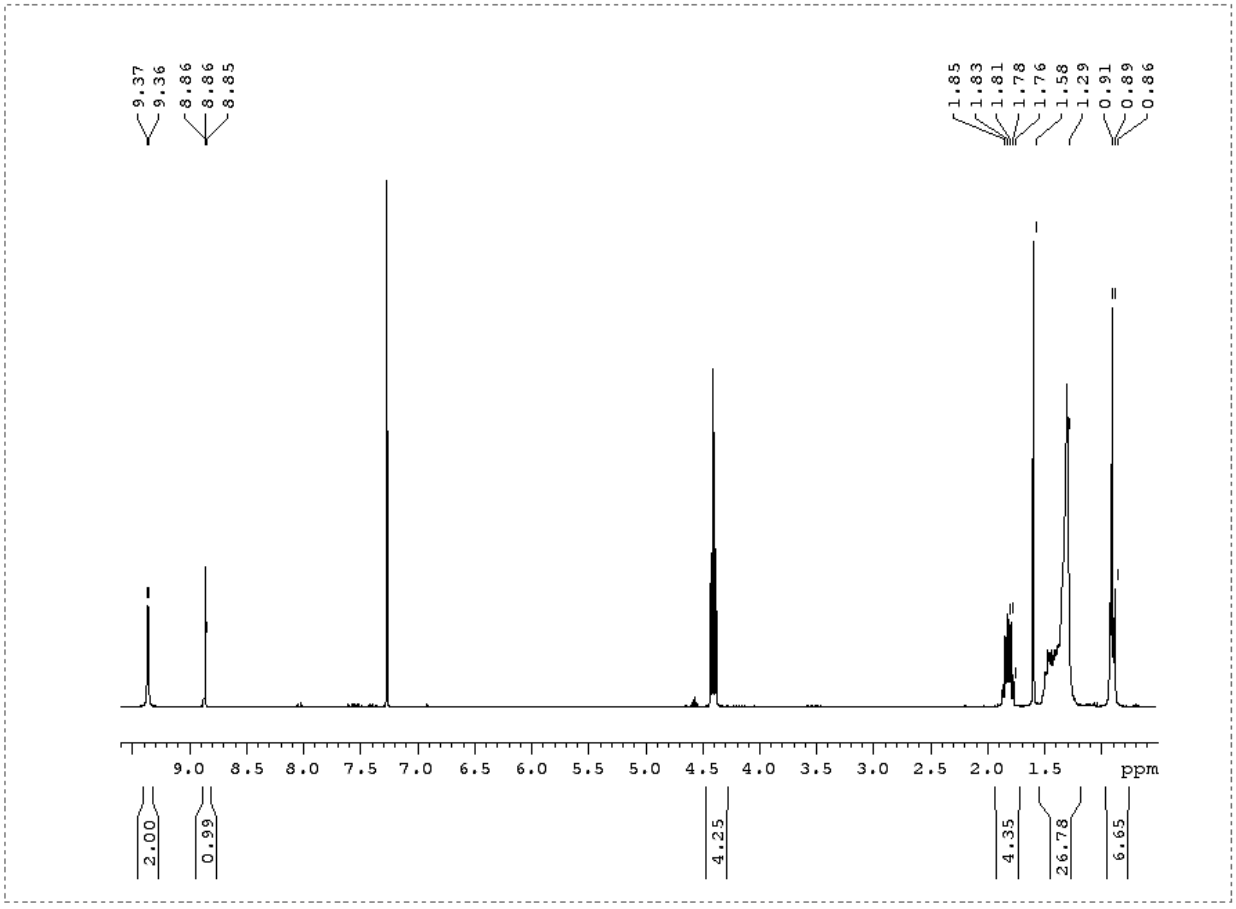
Label	Formulation labels	Cat A	Cat B	Co-lipid	mol Cat A	mol Cat B	mol Co	CR	T Avg	T SE	TE norm	T SE Norm	Cyt avg	Cyt SD
Exp7_19	diCbr20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	3.0	573175	53795	0.903	0.108	39.3	6.5
Exp7_20	(18:1)(br20:0)/EPC Blend50/chc	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	3.0	759474	26750	1.209	0.099	35.7	4.5
Exp7_21	(18:1)(br20:0)/EPC Blend66/chc	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	3.0	497548	86312	0.779	0.147	55.2	7.5
Exp7_22	(18:1)(br20:0)/EPC Blend85/chc	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	3.0	653813	76992	1.035	0.144	46.1	5.2
Exp8_8	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	3.0	204182	6320	0.499	0.018	69.7	1.9
Exp8_9	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	5.0	214562	4687	0.525	0.016	65.4	1.6
Exp8_10	Di9:0/EPC/DOPE	diC9:0	EPC	DOPE	1.5	1.5	2	10.0	84854	16850	0.202	0.040	43.1	4.8
Exp8_11	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	0.5	185653	4153	0.453	0.014	82.0	0.8
Exp8_12	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	1.5	401669	15478	0.990	0.043	30.8	2.0
Exp8_13	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	3.0	22191	816	0.046	0.002	13.8	0.5
Exp8_14	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	5.0	70734	21743	0.167	0.051	8.8	0.5
Exp8_15	Diiso9:0/EPC/DOPE	diisoC9:0	EPC	DOPE	1.5	1.5	2	10.0	20538	9035	0.042	0.018	6.7	0.2
Exp8_16	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	0.5	12421	1847	0.022	0.003	79.4	3.2
Exp8_17	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	1.5	13362	2772	0.024	0.005	84.7	4.0
Exp8_18	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	3.0	83495	4318	0.199	0.011	69.2	3.3
Exp8_19	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	5.0	44028	968	0.100	0.003	58.5	2.9
Exp8_20	Di20:0/EPC/DOPE	diC20:0	EPC	DOPE	1.5	1.5	2	10.0	52854	8504	0.122	0.020	39.5	3.8
Exp8_21	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	0.5	81336	5402	0.193	0.013	93.3	0.7
Exp8_22	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	1.5	154651	3329	0.376	0.011	84.4	3.4
Exp8_23	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	3.0	161748	14114	0.393	0.035	77.6	4.8
Exp8_24	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	5.0	219192	24047	0.536	0.060	73.6	1.5
Exp8_25	Di br20:0/EPC/DOPE	dibrC20:0	EPC	DOPE	1.5	1.5	2	10.0	248256	3935	0.608	0.016	51.3	3.2
Exp8_26	Lipo+DNA								405616	8128	1.000	0.028	26.3	1.5
Exp8_27	DNA alone								11350	2003	0.019		88.7	4.0
Exp8_28	Cells alone								3700	456	0.000		100.0	3.4
Exp8a_6	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	0.5	39945	10322	0.094	0.024	55.5	2.8
Exp8a_7	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	1.5	4186	511	0.000	0.000	16.4	1.1
Exp8a_8	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	3.0	4304	836	0.001	0.000	4.7	0.1
Exp8a_9	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	5.0	7975	128	0.010	0.002	8.1	0.4
Exp8a_10	Di9:0/EPC/chol	diC9:0	EPC	chol	1.5	1.5	2	10.0	25706	2906	0.056	0.007	6.3	0.3
Exp8a_11	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	0.5	172545	7173	0.440	0.022	25.5	0.8
Exp8a_12	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	1.5	20768	2250	0.044	0.005	12.1	0.8
Exp8a_13	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	3.0	6127	672	0.005	0.001	5.3	0.1
Exp8a_14	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	5.0	4818	133	0.002	0.000	4.8	0.3
Exp8a_15	Diiso9:0/EPC/chol	diisoC9:0	EPC	chol	1.5	1.5	2	10.0	4604	667	0.001	0.000	6.7	0.4
Exp8a_16	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	0.5	43583	2690	0.103	0.007	88.1	1.8
Exp8a_17	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	1.5	123850	1939	0.313	0.010	93.9	3.2
Exp8a_18	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	3.0	178468	5482	0.455	0.019	79.7	9.4
Exp8a_19	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	5.0	208523	1930	0.534	0.015	78.0	4.0
Exp8a_20	Di20:0/EPC/chol	diC20:0	EPC	chol	1.5	1.5	2	10.0	206353	5286	0.528	0.020	69.6	1.4
Exp8a_21	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	0.5	23809	4672	0.051	0.011	90.3	3.9
Exp8a_22	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	1.5	95793	4465	0.239	0.013	81.4	2.0
Exp8a_23	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	3.0	196281	11385	0.502	0.032	47.2	4.3
Exp8a_24	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	5.0	259709	8120	0.667	0.028	40.5	6.3
Exp8a_25	Di br20:0/EPC/chol	dibrC20:0	EPC	chol	1.5	1.5	2	10.0	239287	10672	0.614	0.032	52.9	2.3
Exp8a_26	Lipo+DNA								387139	10186	1.000	0.037	17.8	2.9
Exp8a_27	DNA alone								25224	1319	0.055		94.4	8.1
Exp8a_28	Cells alone								10983	1187	0.018		100.0	5.3
Exp9_4	(18:1)(br20:0)blend50/EPC/DOF	(18:1)(br20:0)blend50	EPC	DOPE	1.5	1.5	2	1.5	344403	61299	0.455	0.088	85.6	10.9
Exp9_5	(18:1)(br20:0)blend50/EPC/DOF	(18:1)(br20:0)blend50	EPC	DOPE	1.5	1.5	2	5.0	325691	3488	0.428	0.034	100.1	2.5
Exp9_6	(18:1)(br20:0)blend50/EPC/DOF	(18:1)(br20:0)blend50	EPC	DOPE	1.5	1.5	2	10.0	329937	25080	0.434	0.047	86.6	4.0
Exp9_7	(18:1)(br20:0)blend66/EPC/DOF	(18:1)(br20:0)blend66	EPC	DOPE	1.5	1.5	2	1.5	286877	13958	0.372	0.036	102.0	1.4
Exp9_8	(18:1)(br20:0)blend66/EPC/DOF	(18:1)(br20:0)blend66	EPC	DOPE	1.5	1.5	2	5.0	415016	34365	0.557	0.060	87.7	4.7

Label	Formulation labels	Cat A	Cat B	Co-lipid	mol Cat A	mol Cat B	mol Co	CR	T Avg	T SE	TE norm	T SE Norm	Cyt avg	Cyt SD
Exp9_9	(18:1)(br20:0)blend66/EPC/DOF	(18:1)(br20:0)blend66	EPC	DOPE	1.5	1.5	2	10.0	422258	66717	0.567	0.097	78.6	8.9
Exp9_10	(18:1)(br20:0)blend85/EPC/DOF	(18:1)(br20:0)blend85	EPC	DOPE	1.5	1.5	2	1.5	235546	10033	0.299	0.032	93.7	2.8
Exp9_11	(18:1)(br20:0)blend85/EPC/DOF	(18:1)(br20:0)blend85	EPC	DOPE	1.5	1.5	2	5.0	357239	17864	0.474	0.042	76.8	16.8
Exp9_12	(18:1)(br20:0)blend85/EPC/DOF	(18:1)(br20:0)blend85	EPC	DOPE	1.5	1.5	2	10.0	403305	61858	0.540	0.091	66.4	3.5
Exp9_16	(18:1)(br20:0)blend50/EPC/Cho	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	1.5	379126	21140	0.505	0.046	82.9	7.5
Exp9_17	(18:1)(br20:0)blend50/EPC/Cho	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	5.0	539314	25734	0.736	0.056	66.0	2.4
Exp9_18	(18:1)(br20:0)blend50/EPC/Cho	(18:1)(br20:0)blend50	EPC	chol	1.5	1.5	2	10.0	613986	54256	0.843	0.089	49.7	1.2
Exp9_19	(18:1)(br20:0)blend66/EPC/Cho	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	1.5	264227	1275	0.340	0.031	94.9	0.6
Exp9_20	(18:1)(br20:0)blend66/EPC/Cho	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	5.0	560840	78330	0.767	0.116	51.9	10.6
Exp9_21	(18:1)(br20:0)blend66/EPC/Cho	(18:1)(br20:0)blend66	EPC	chol	1.5	1.5	2	10.0	579727	57516	0.794	0.091	42.1	6.5
Exp9_22	(18:1)(br20:0)blend85/EPC/Cho	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	1.5	407372	10155	0.546	0.040	82.7	5.2
Exp9_23	(18:1)(br20:0)blend85/EPC/Cho	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	5.0	541839	57169	0.740	0.090	48.8	3.7
Exp9_24	(18:1)(br20:0)blend85/EPC/Cho	(18:1)(br20:0)blend85	EPC	chol	1.5	1.5	2	10.0	541066	14889	0.738	0.049	52.0	1.4
Exp9_25	Lipofectamine								722757	27378	1.000	0.067	16.5	1.6
Exp9_26	DNA alone								72649	3832	0.064		96.9	6.2
Exp9_27	Cells alone								28223	20175	0.000		100.0	4.6
Exp17_16	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	0.5	99819	4171	0.156	0.013	90.1	27.4
Exp17_17	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	1.5	270250	44092	0.460	0.082	84.4	29.2
Exp17_18	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	3.0	381234	12131	0.658	0.052	85.6	31.9
Exp17_19	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	5.0	294124	34942	0.502	0.070	60.6	30.8
Exp17_20	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	chol	1.5	1.5	2	10.0	226004	24238	0.381	0.049	65.2	28.2
Exp17_26	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	0.5	109394	13614	0.173	0.025	78.2	26.5
Exp17_27	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	1.5	250544	39980	0.424	0.074	75.0	29.1
Exp17_28	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	3.0	393463	28063	0.679	0.069	3.0	10.7
Exp17_29	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	5.0	432762	28254	0.750	0.073	11.8	12.1
Exp17_30	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	chol	1.5	1.5	2	10.0	294897	10545	0.504	0.041	14.6	10.8
Exp17_11	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	0.5	94530	5107	0.146	0.014	86.0	33.2
Exp17_12	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	1.5	105110	5584	0.165	0.015	81.0	31.3
Exp17_13	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	3.0	122005	8362	0.195	0.020	82.5	28.1
Exp17_14	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	5.0	159308	14571	0.262	0.031	74.4	28.0
Exp17_15	blend(18:1)(br20:0) 32/EPC/Chc	(18:1)(br20:0)blend0.32/0.68	EPC	DOPE	1.5	1.5	2	10.0	141009	12379	0.229	0.026	83.6	32.1
Exp17_21	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	0.5	73424	6318	0.108	0.012	79.8	33.6
Exp17_22	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	1.5	92533	8429	0.143	0.017	46.7	41.9
Exp17_23	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	3.0	112667	15523	0.178	0.028	0.7	8.9
Exp17_24	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	5.0	94910	12318	0.147	0.022	6.1	9.6
Exp17_25	mix (br20:0)(18:1)/EPC/Chol	(C18:1)(brC20:0)mix	EPC	DOPE	1.5	1.5	2	10.0	111950	6688	0.177	0.017	13.6	10.9
Exp17_31	Lipofectamine								573168	41492	1.000	0.102	1.8	4.9
Exp17_32	DNA alone								28890	5731	0.029	0.199	95.2	15.6
Exp17_33	Cells alone								12618	1813	0.000	0.145	100.0	15.8

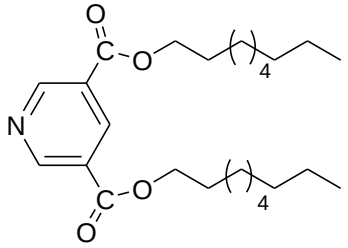
Proton and Carbon NMR spectra

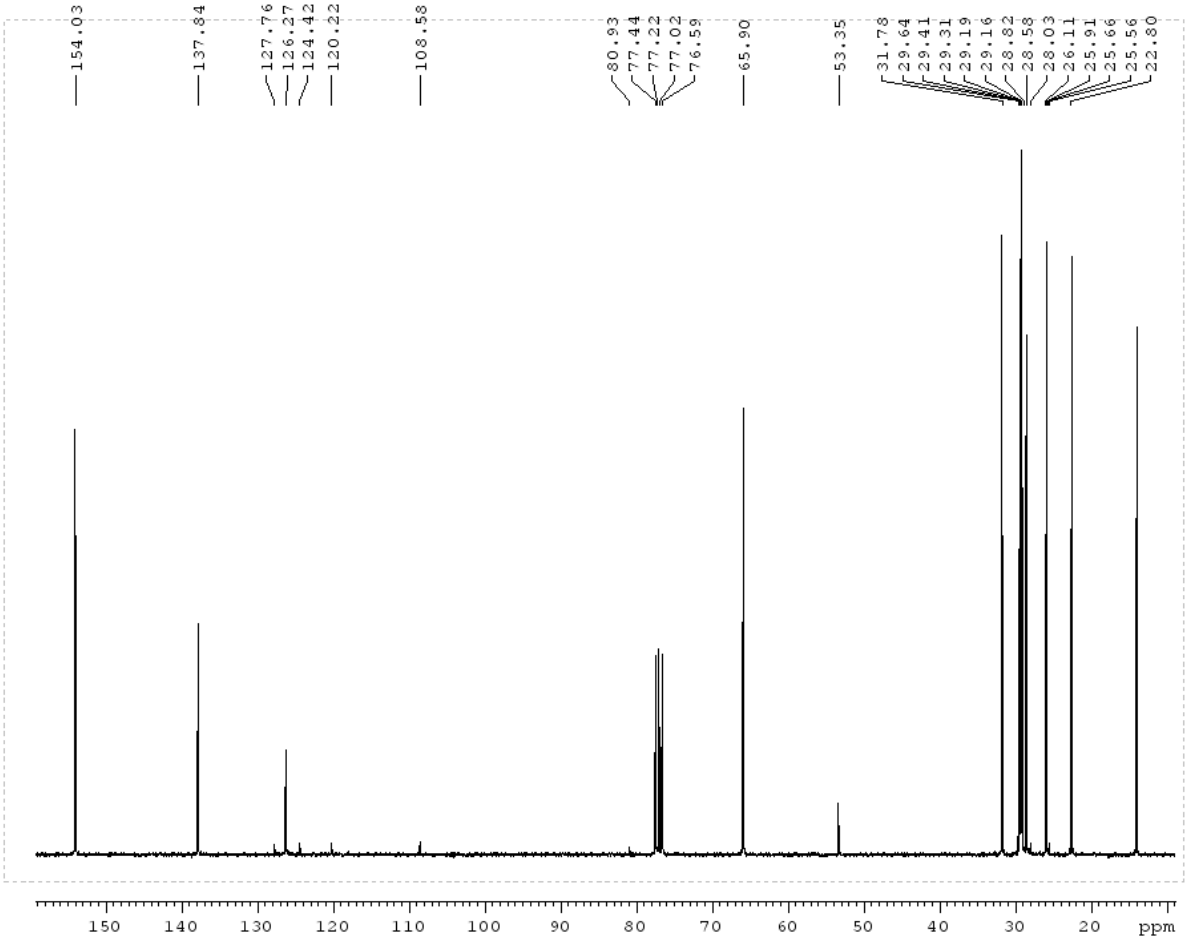
^{1}HNMR for compound **diC9:0 pyridine**



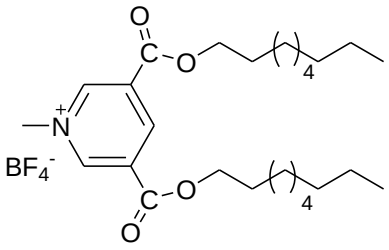


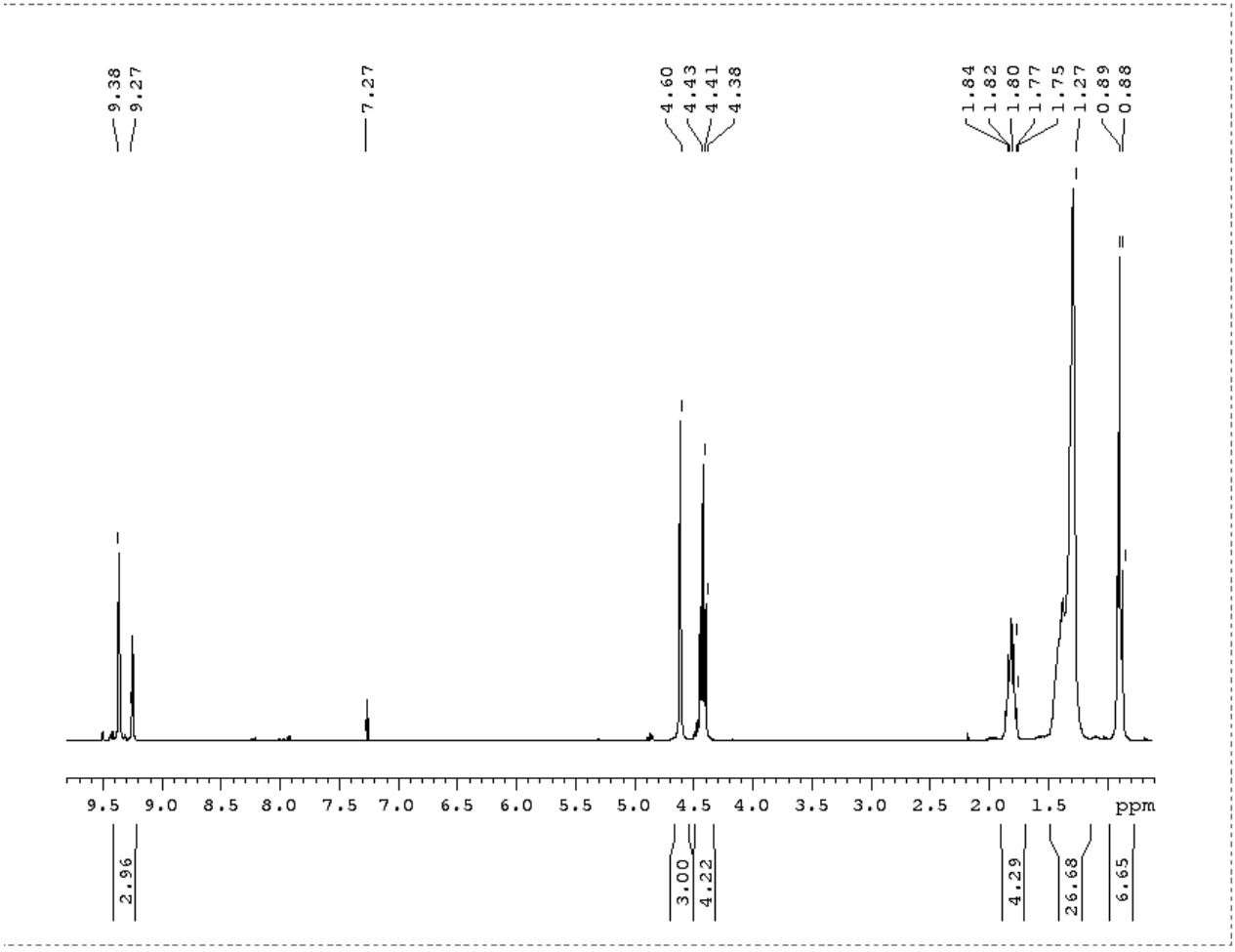
^{13}CNMR for compound **diC9:0 pyridine**



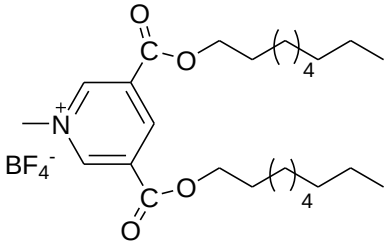


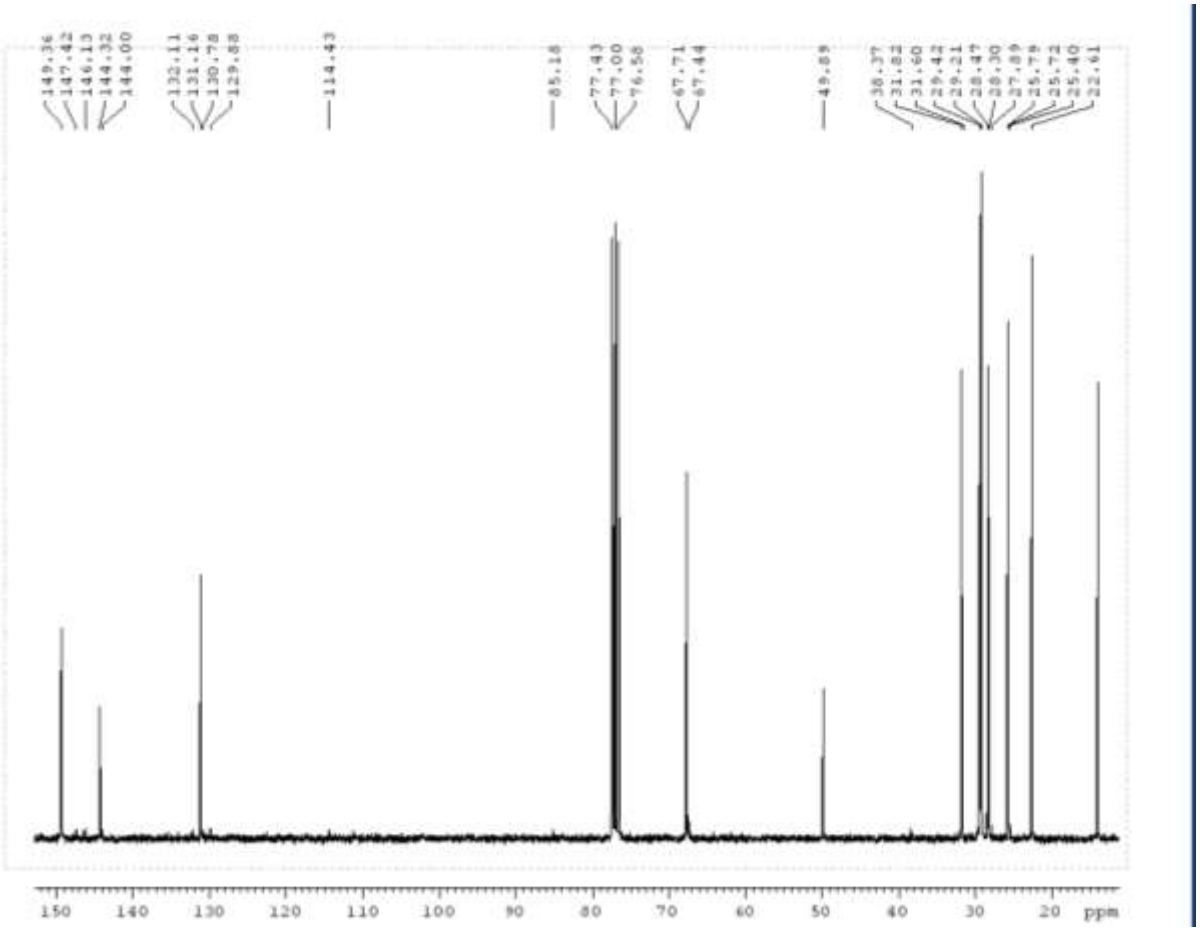
[1]HNMR for compound **diC9:0**



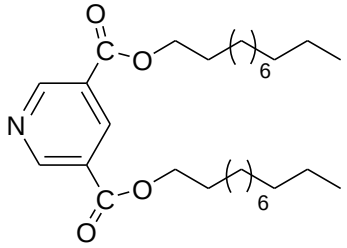


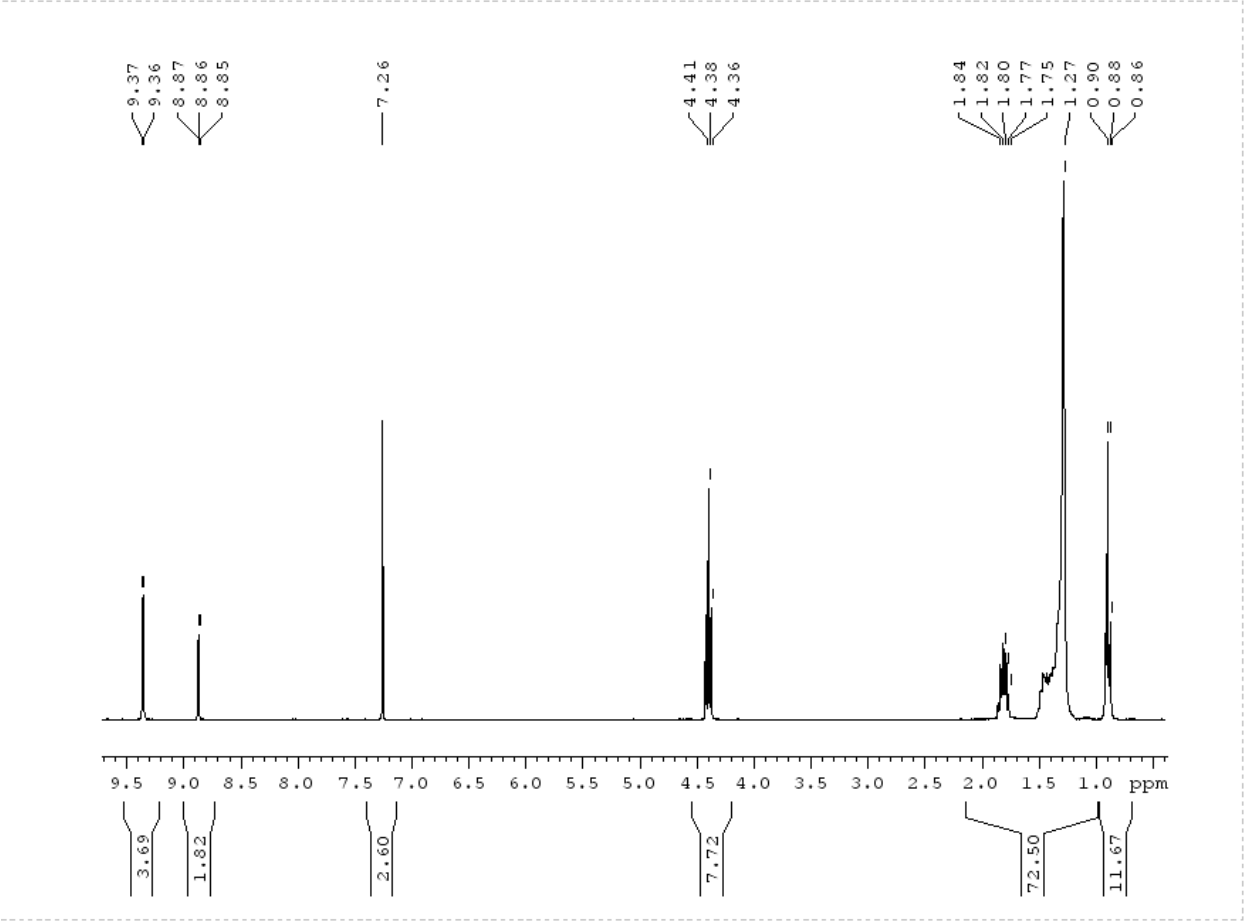
^{13}CNMR for compound **diC9:0**



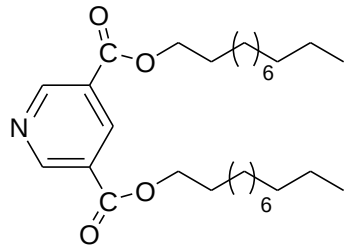


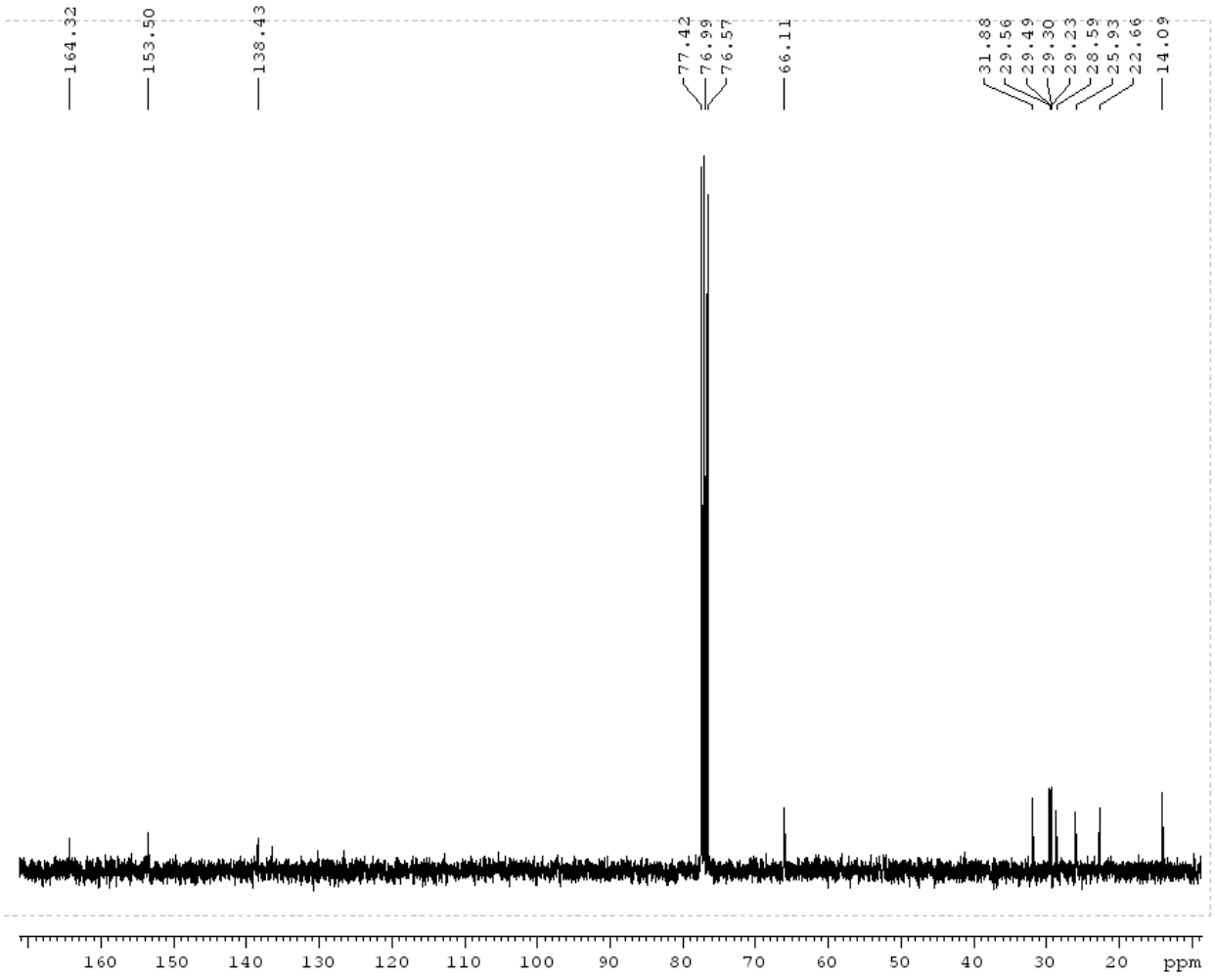
[1]HNMR for compound **diC11:0 pyridine**



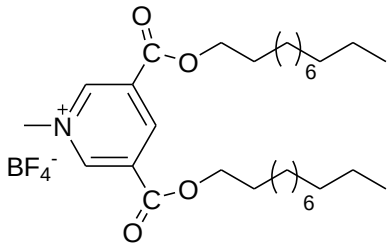


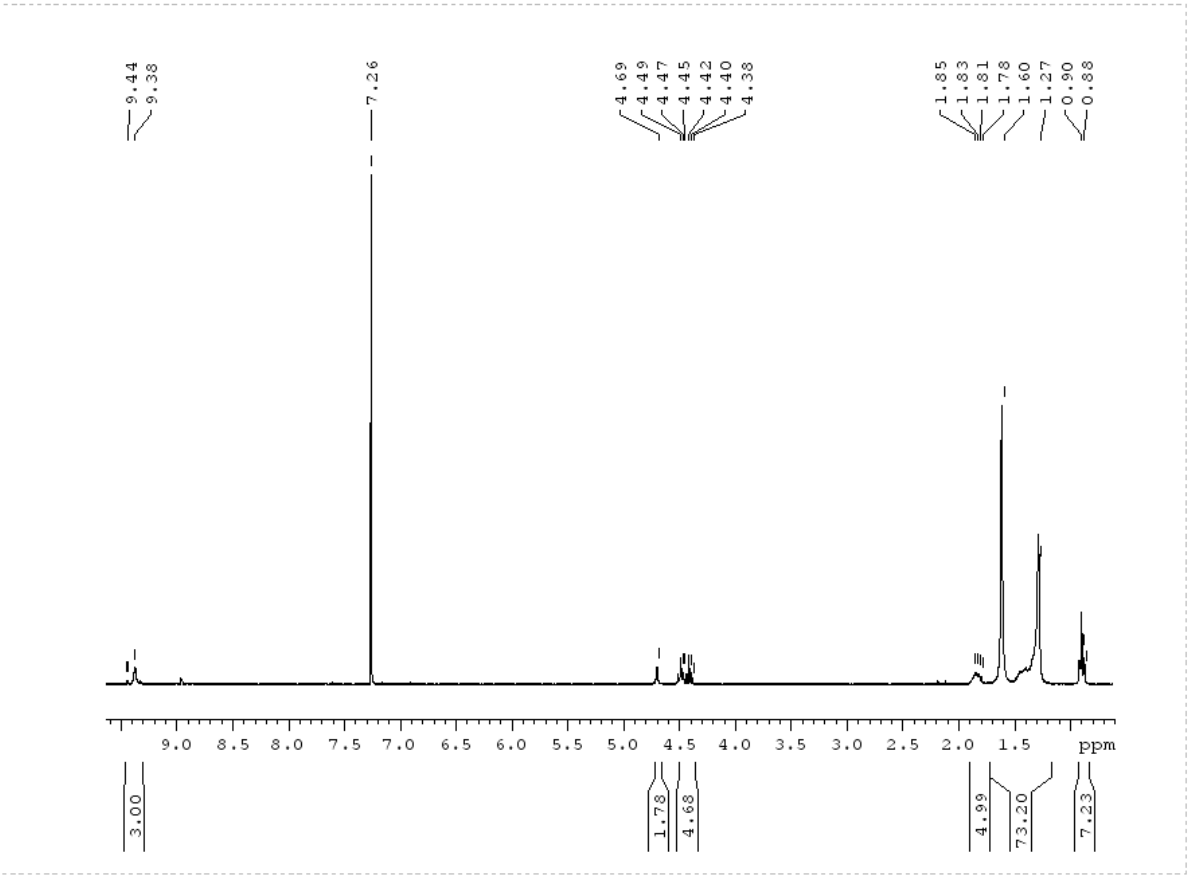
^{13}CNMR for compound **diC11:0 pyridine**



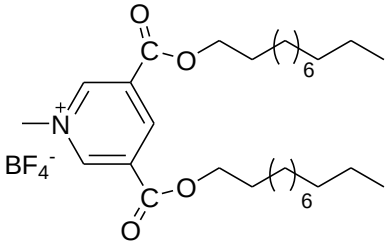


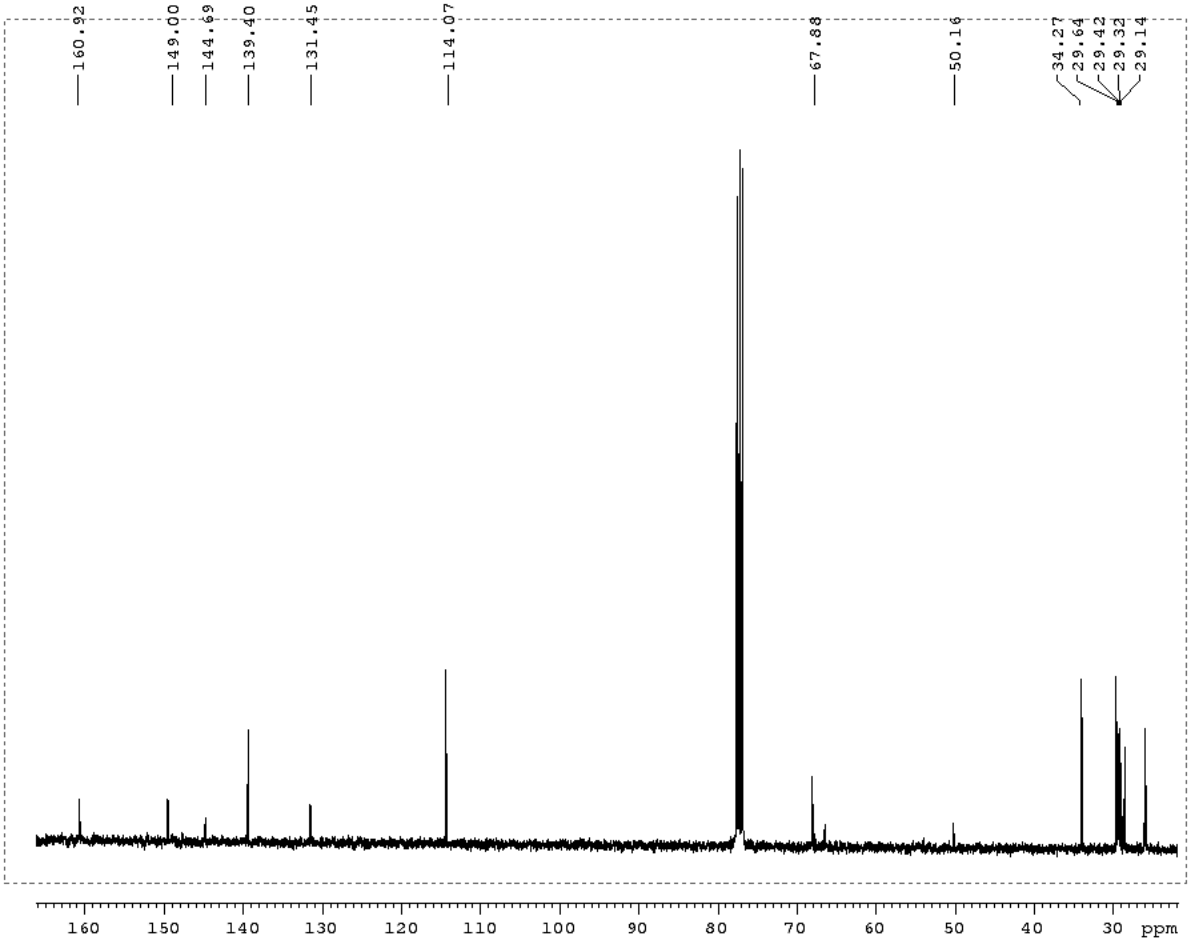
[1]HNMR for compound **diC11:0**



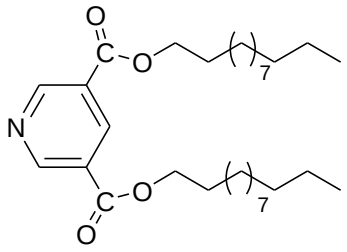


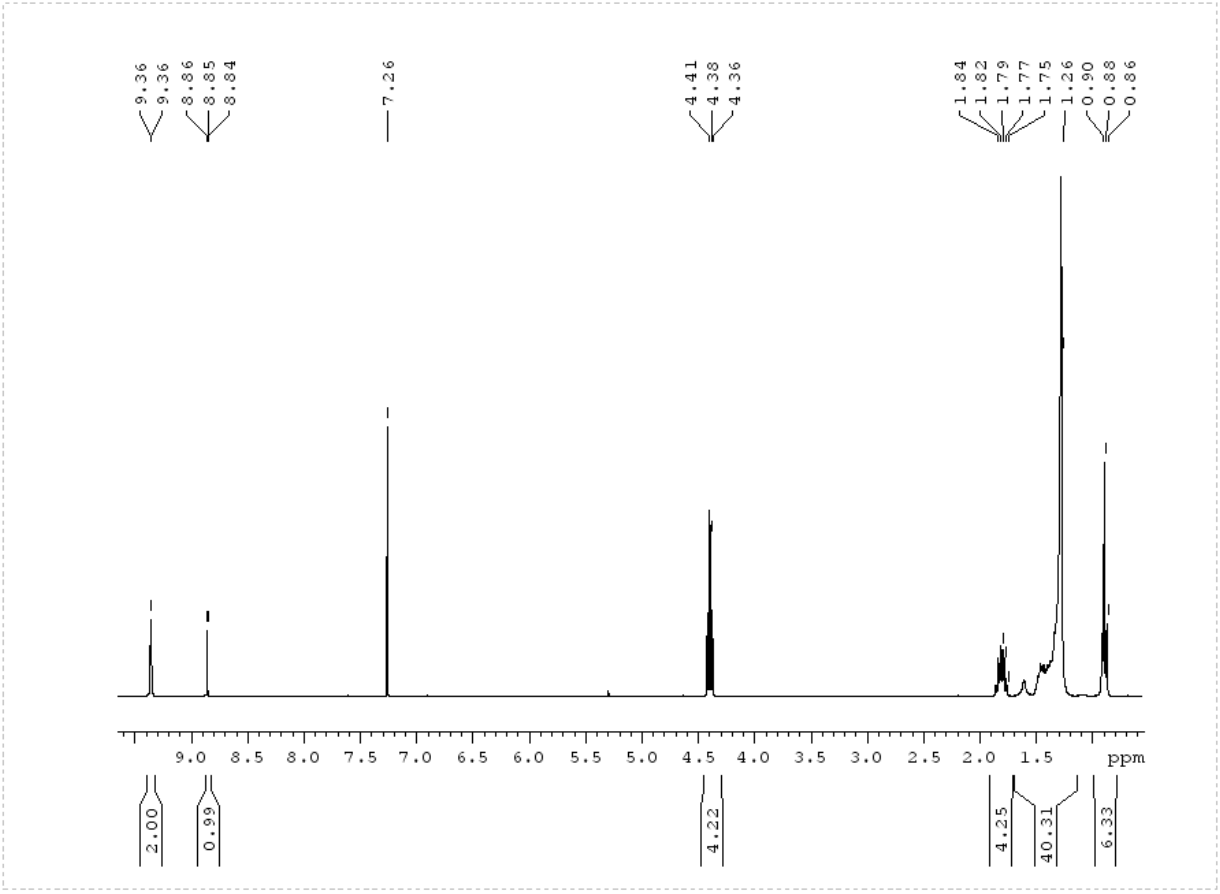
^{13}CNMR for compound **diC11:0**



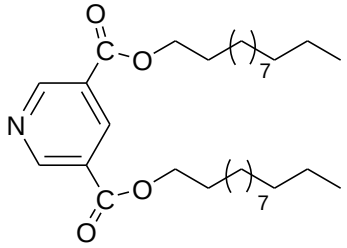


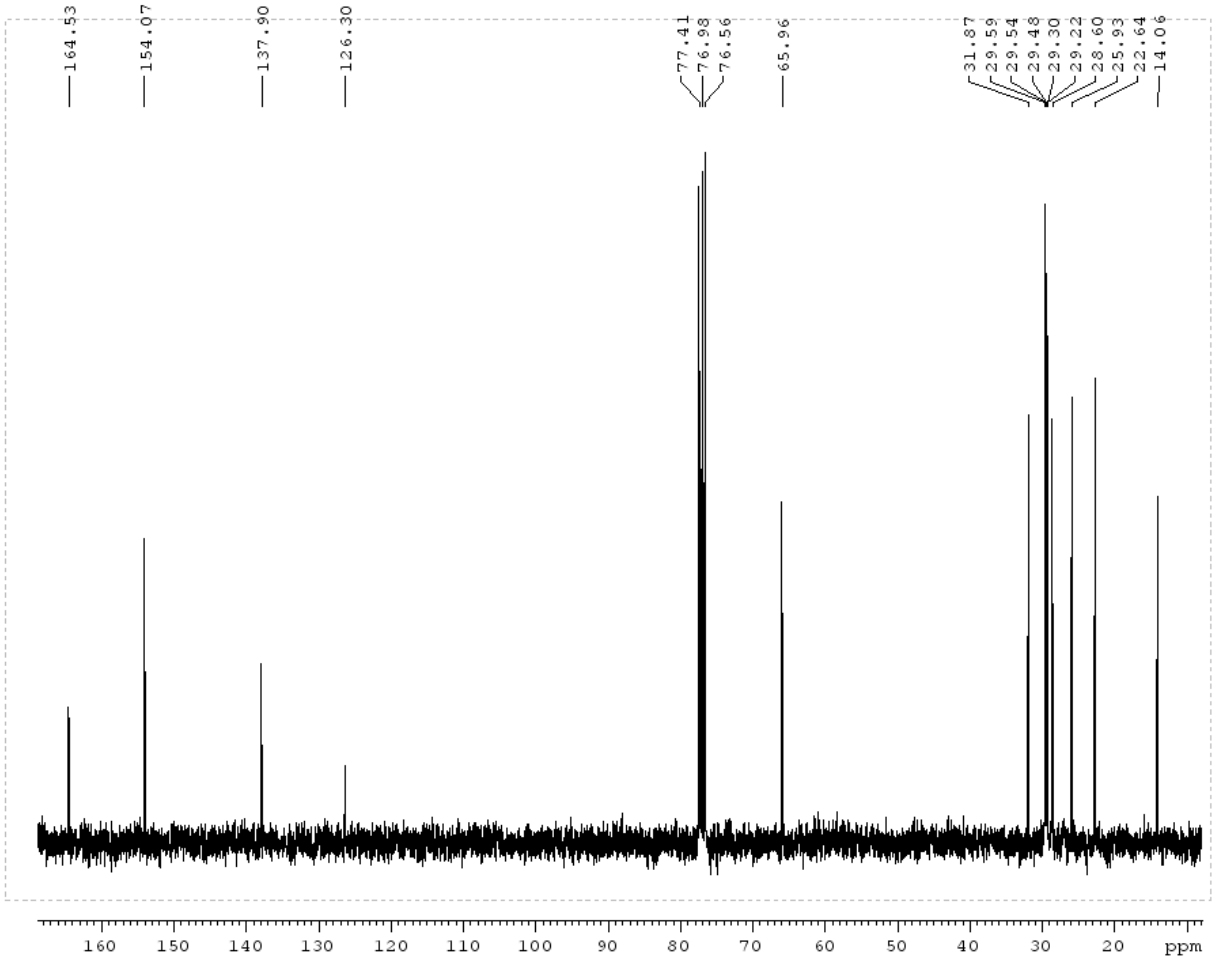
[1]HNMR for compound **diC12:0 Pyridine**



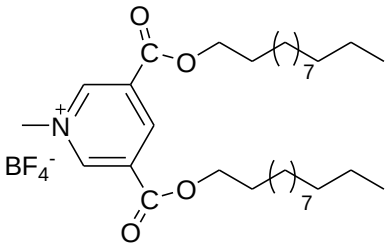


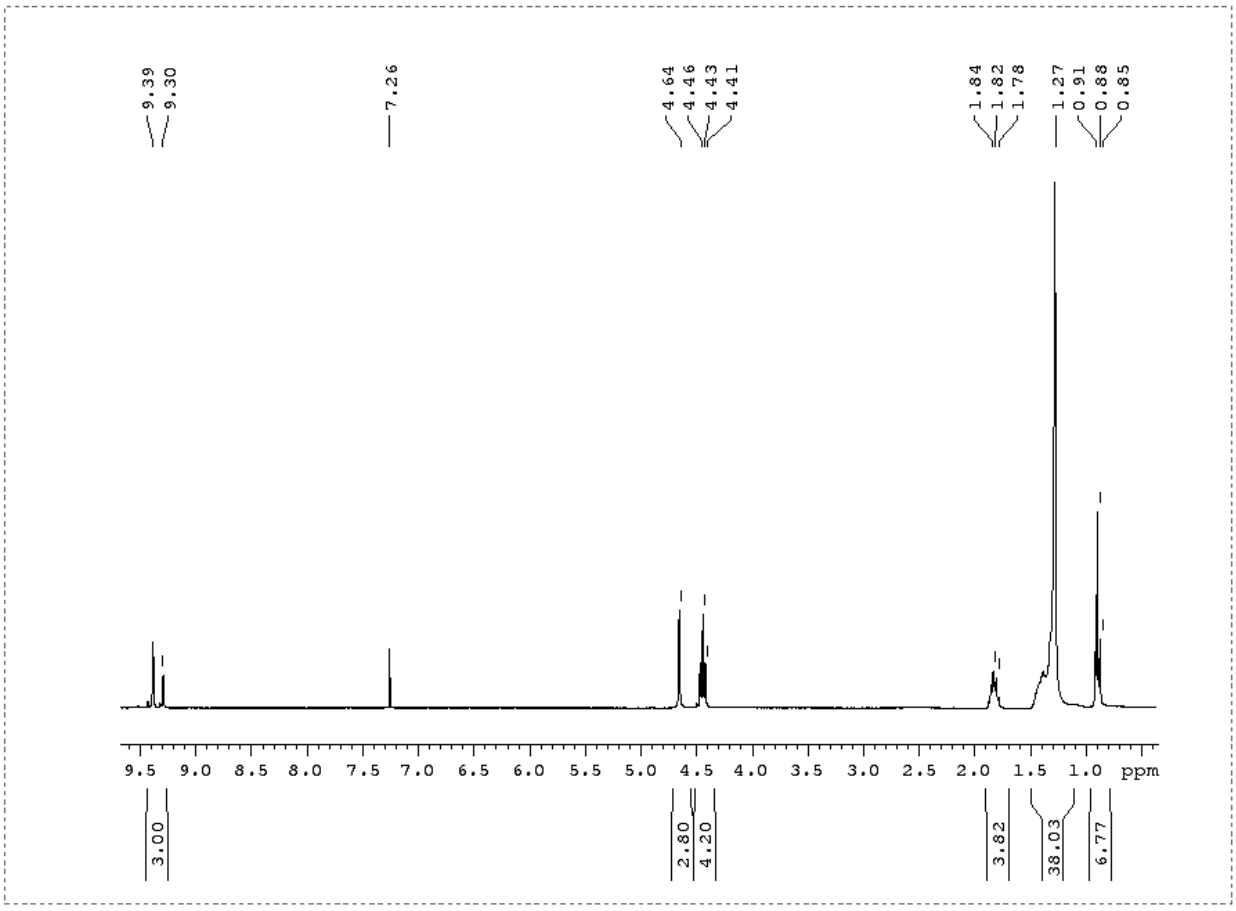
^{13}CNMR for compound **diC12:0 Pyridine**



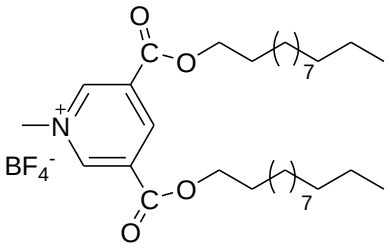


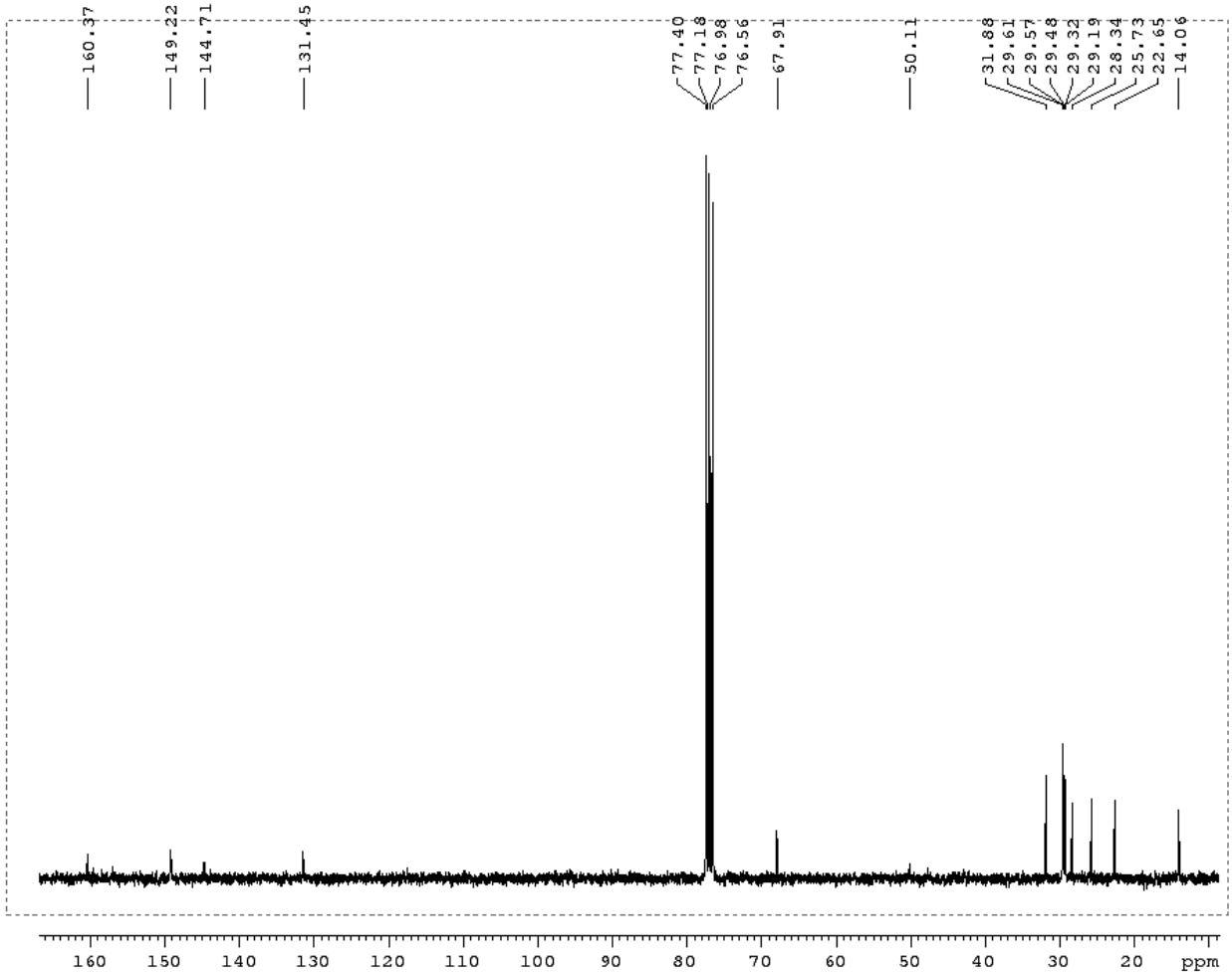
[1]HNMR for compound **diC12:0**



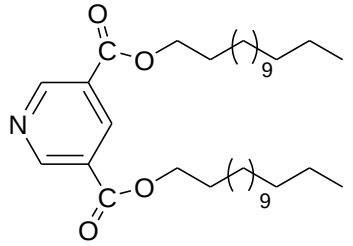


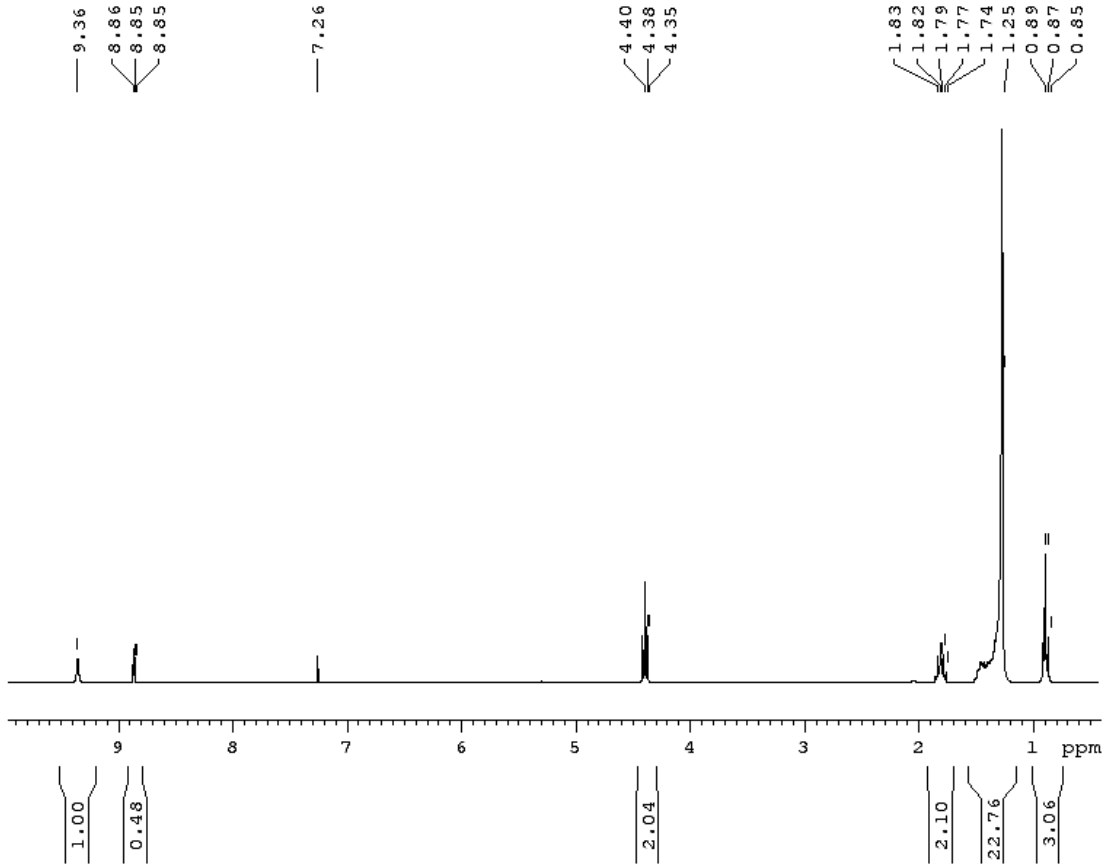
^{13}CNMR for compound **diC12:0**



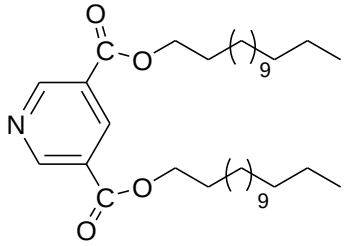


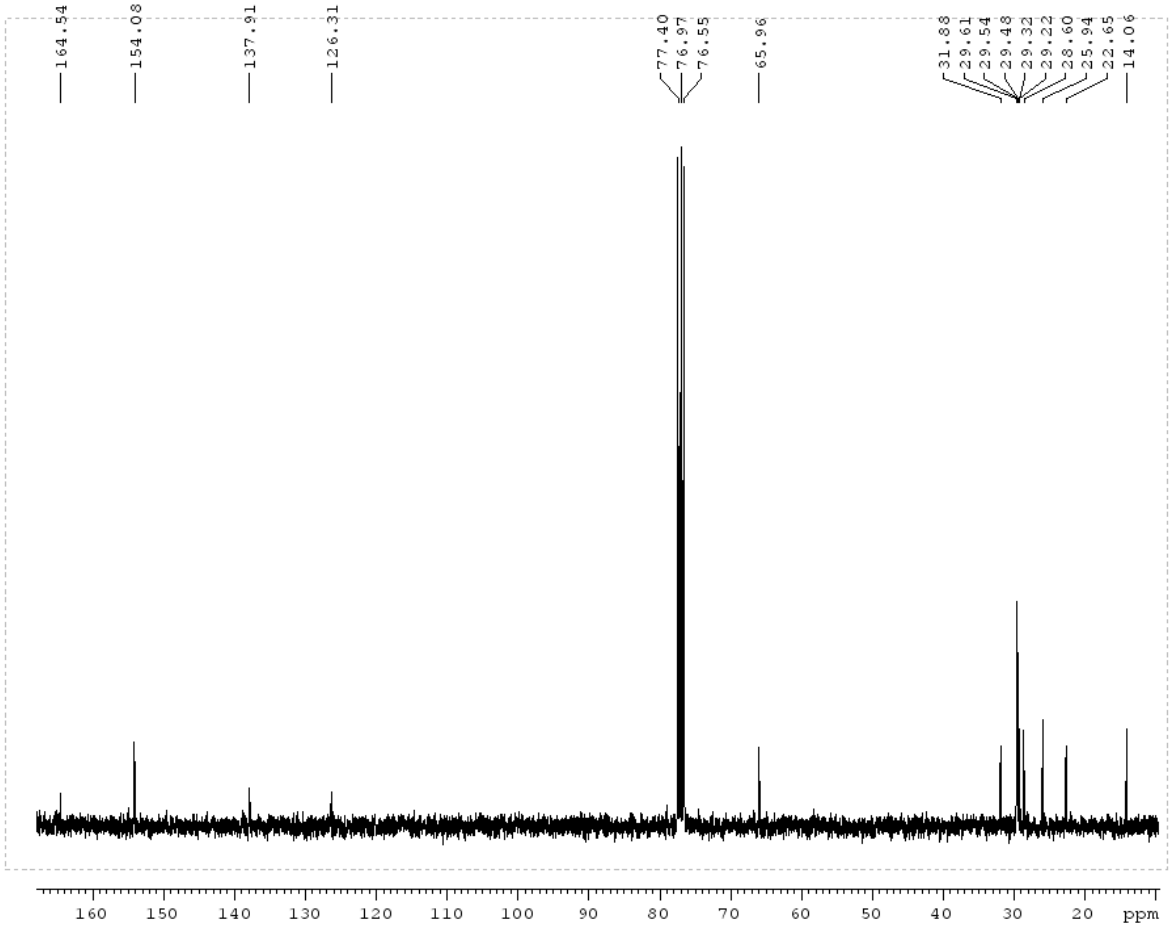
[1]HNMR for compound **diC14:0 pyridine**



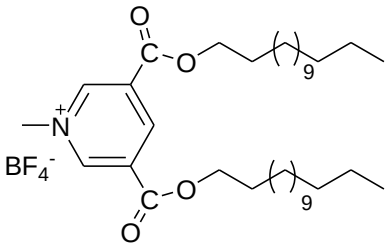


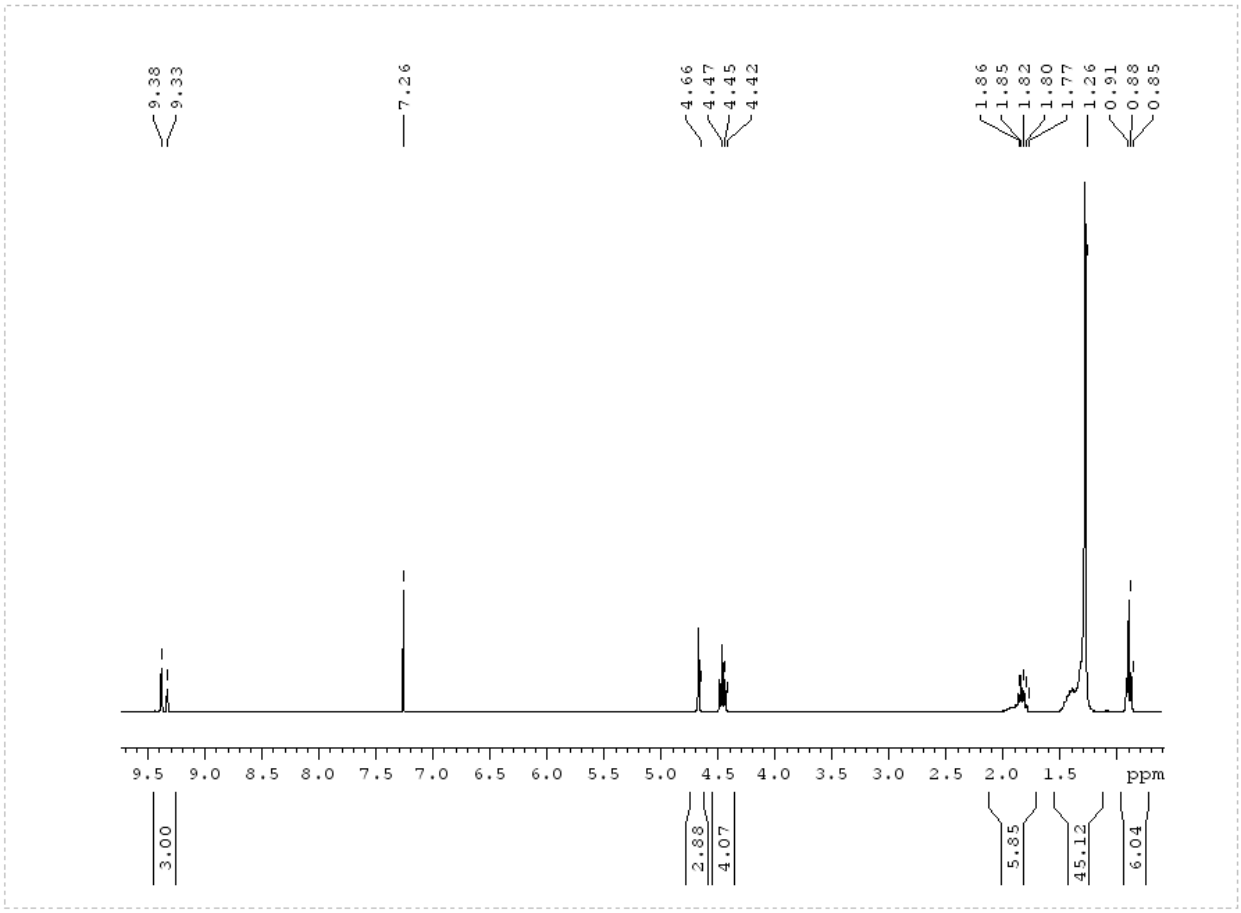
^{13}CNMR for compound **diC14:0 pyridine**



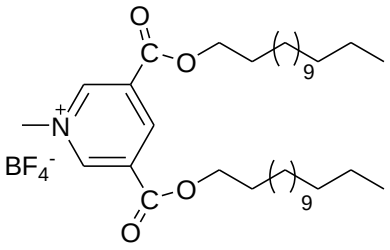


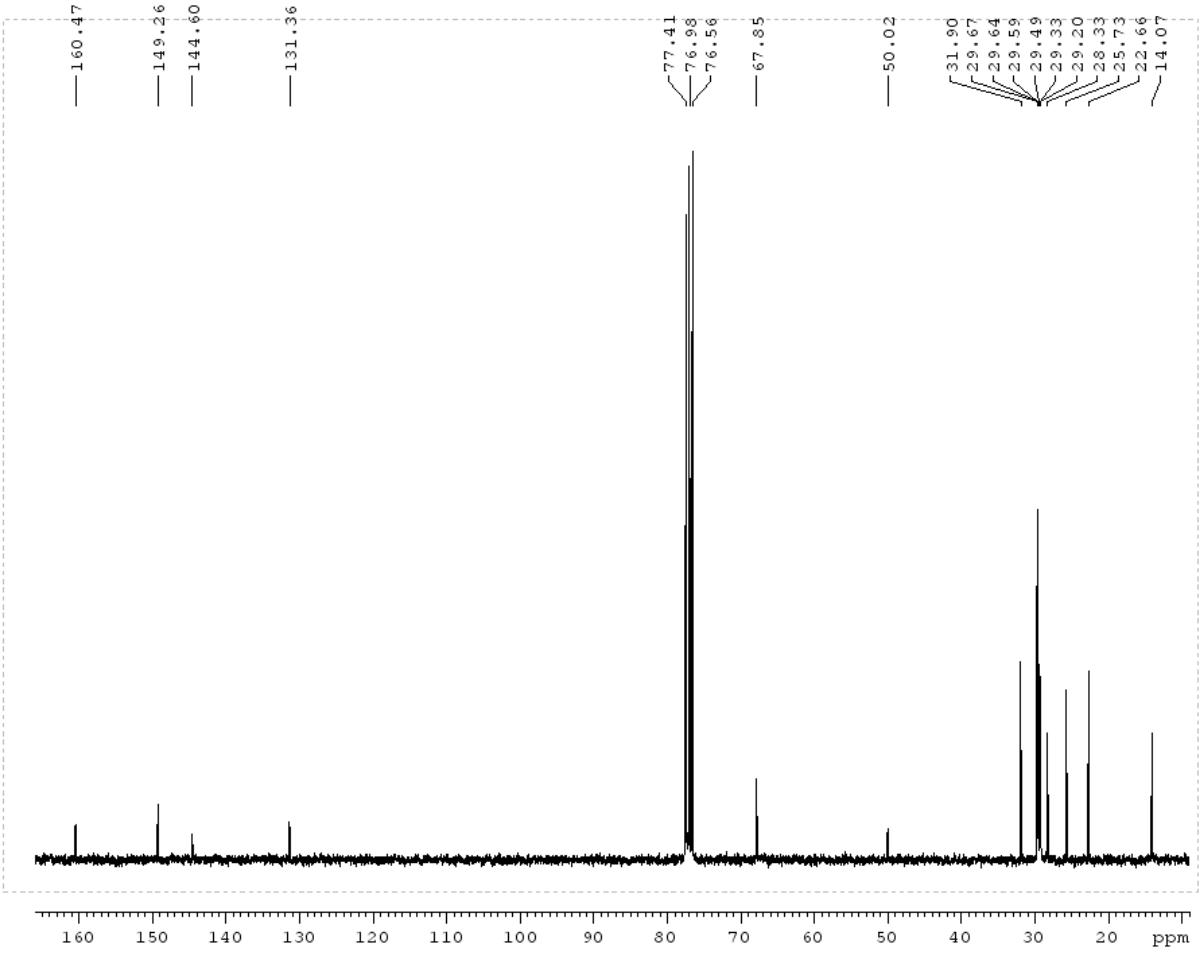
[1]HNMR for compound **diC14:0**



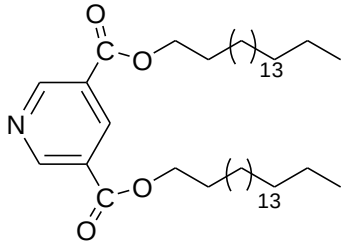


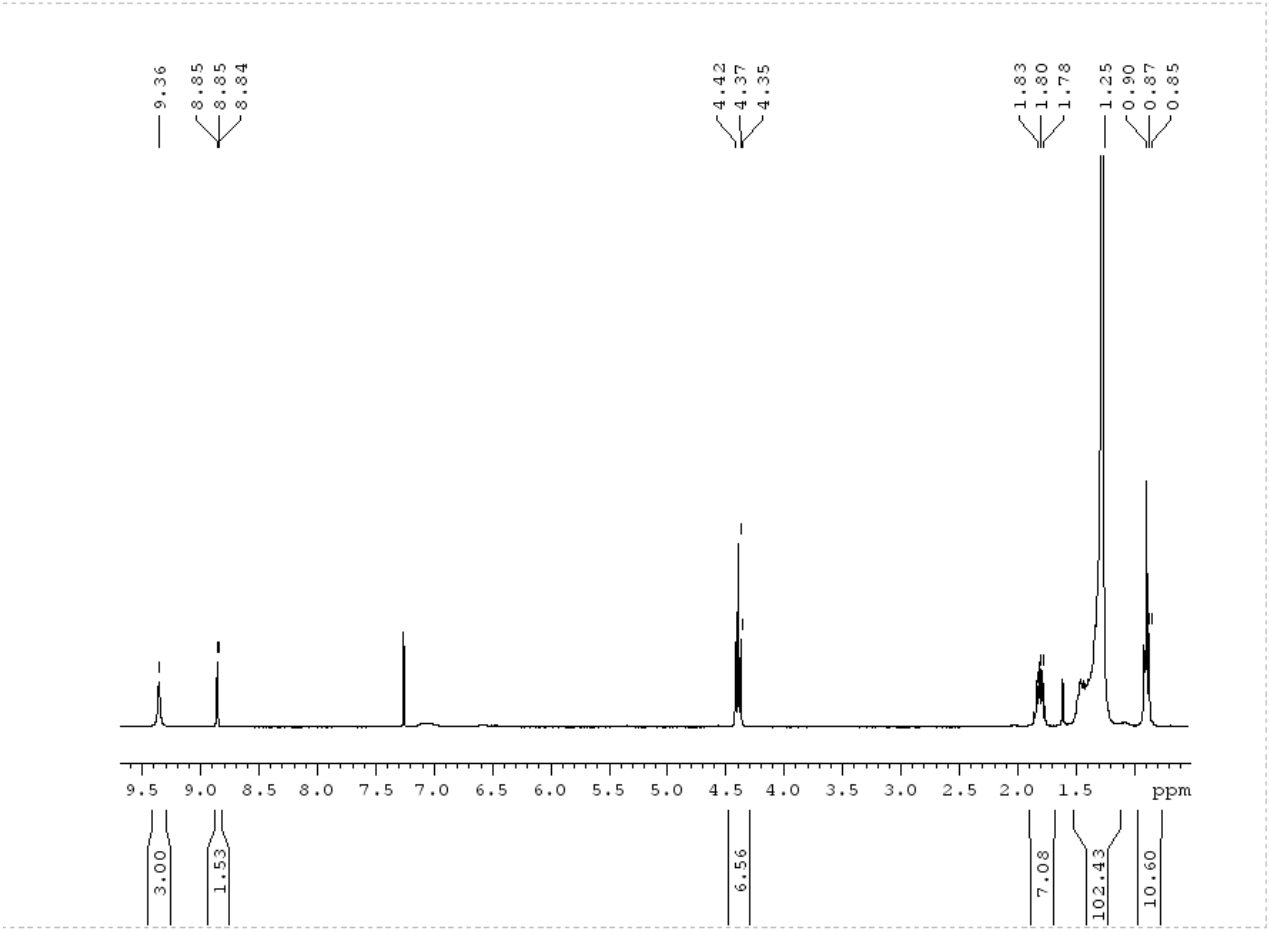
^{13}CNMR for compound **diC14:0**



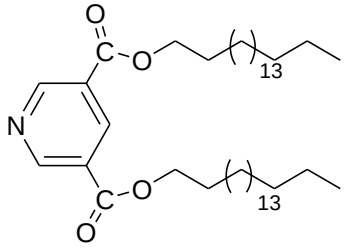


[1]HNMR for compound **diC18:0 pyridine**

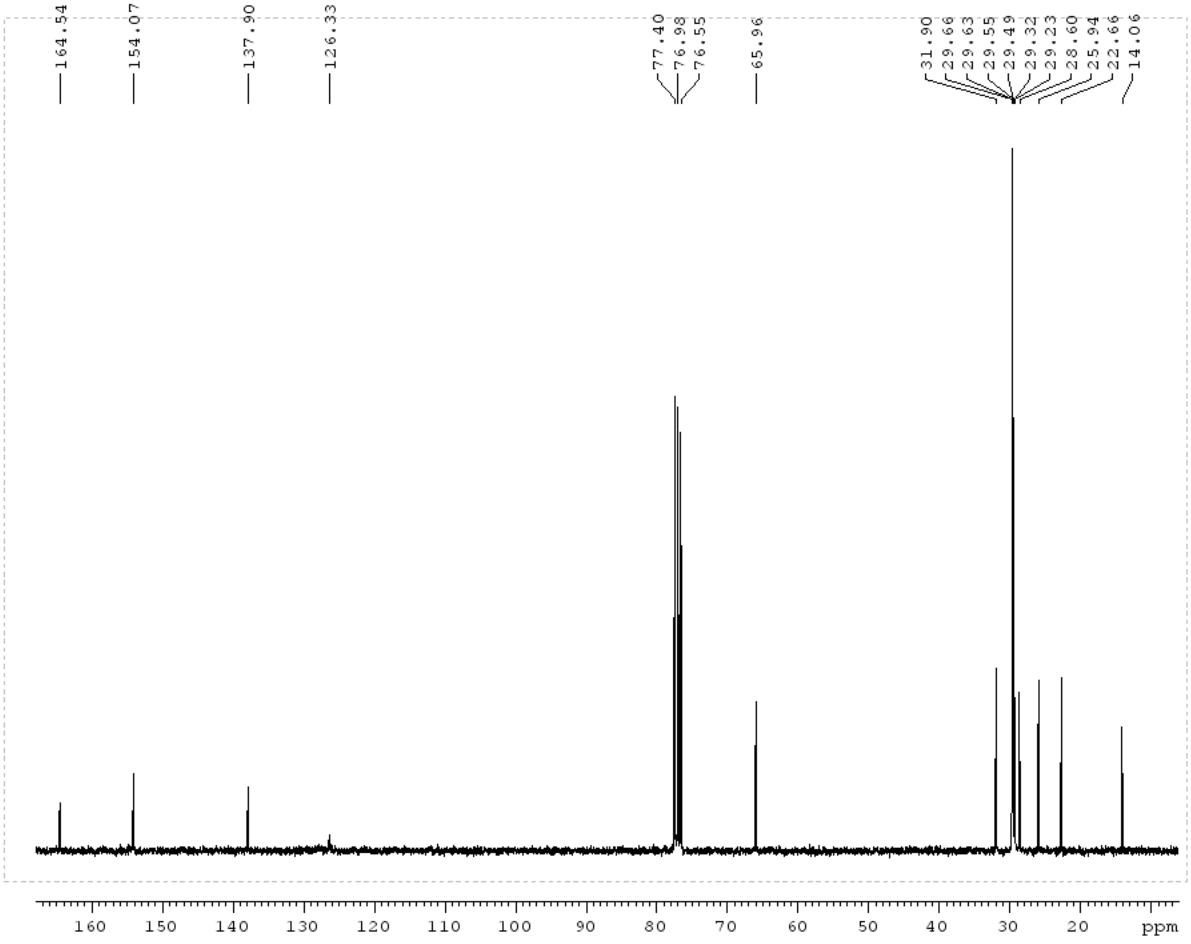




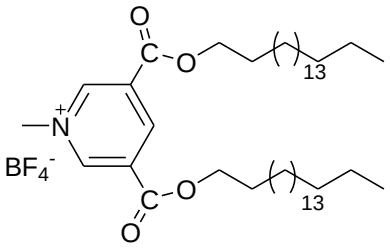
^{13}CNMR for compound **diC18:0 pyridine**

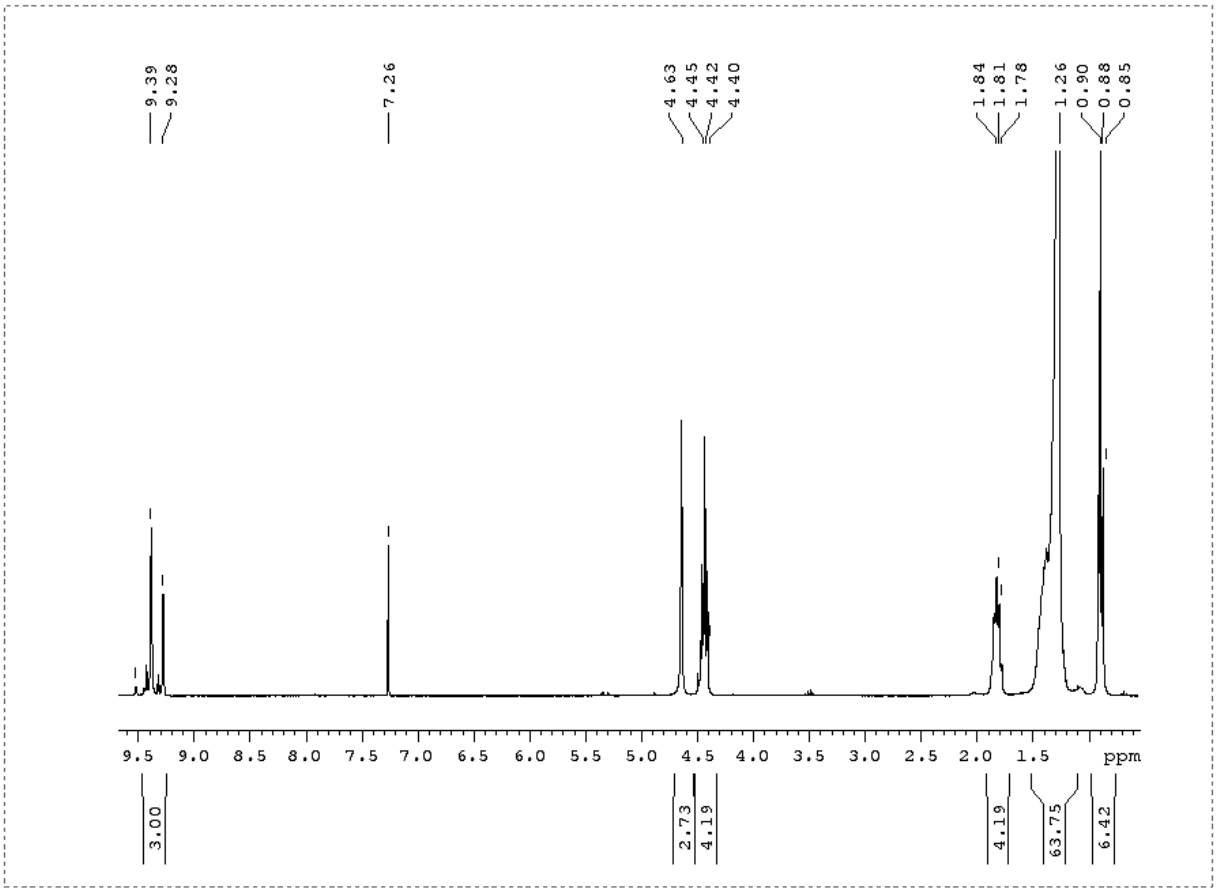


2-18

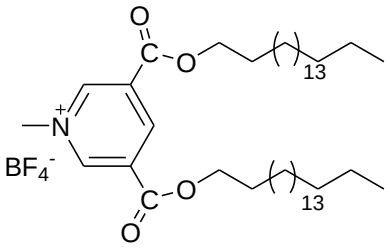


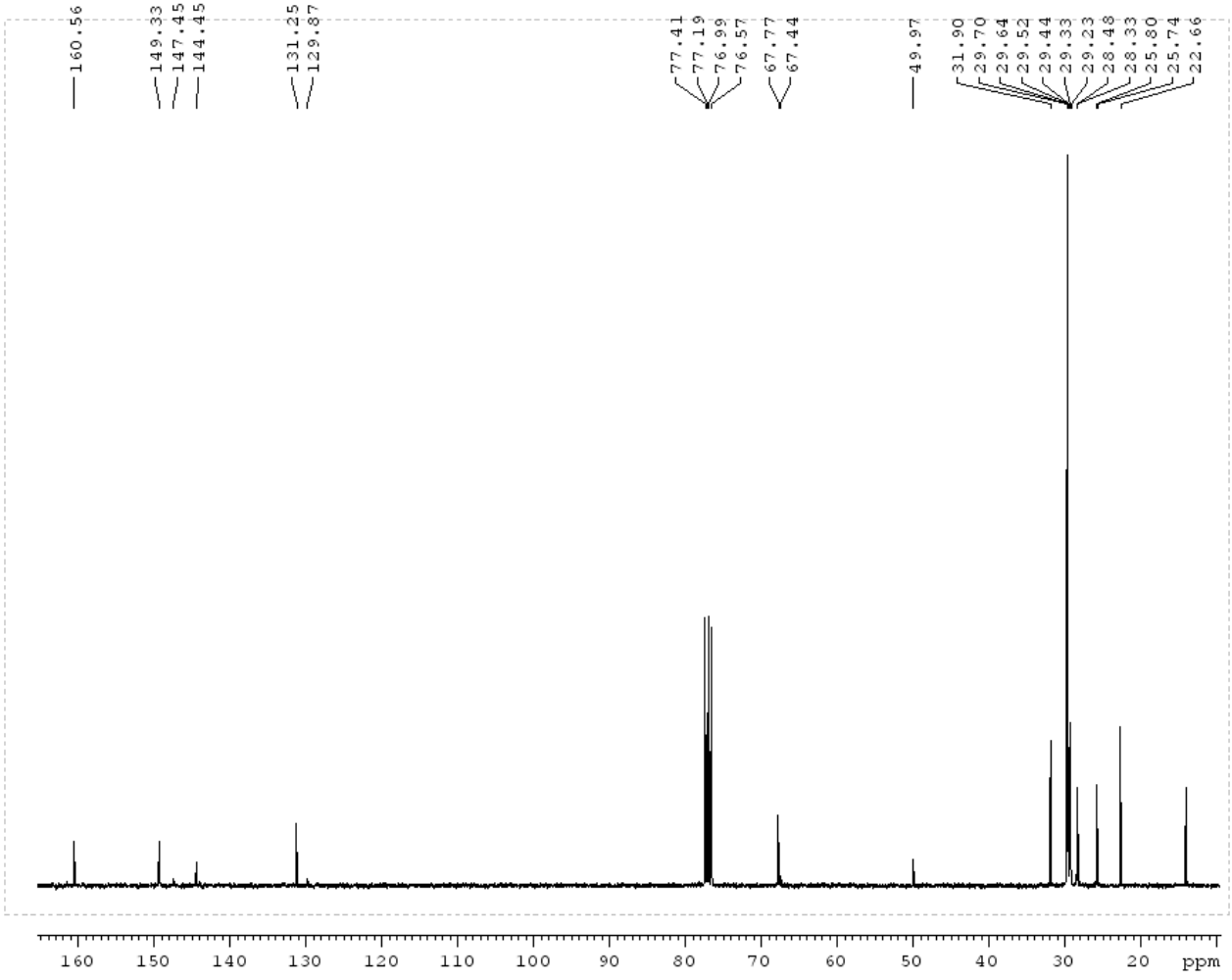
^{1}HNMR for compound **diC18:0**



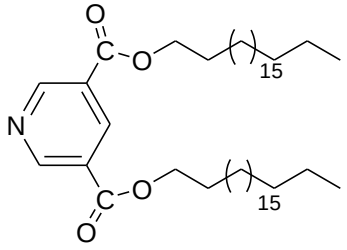


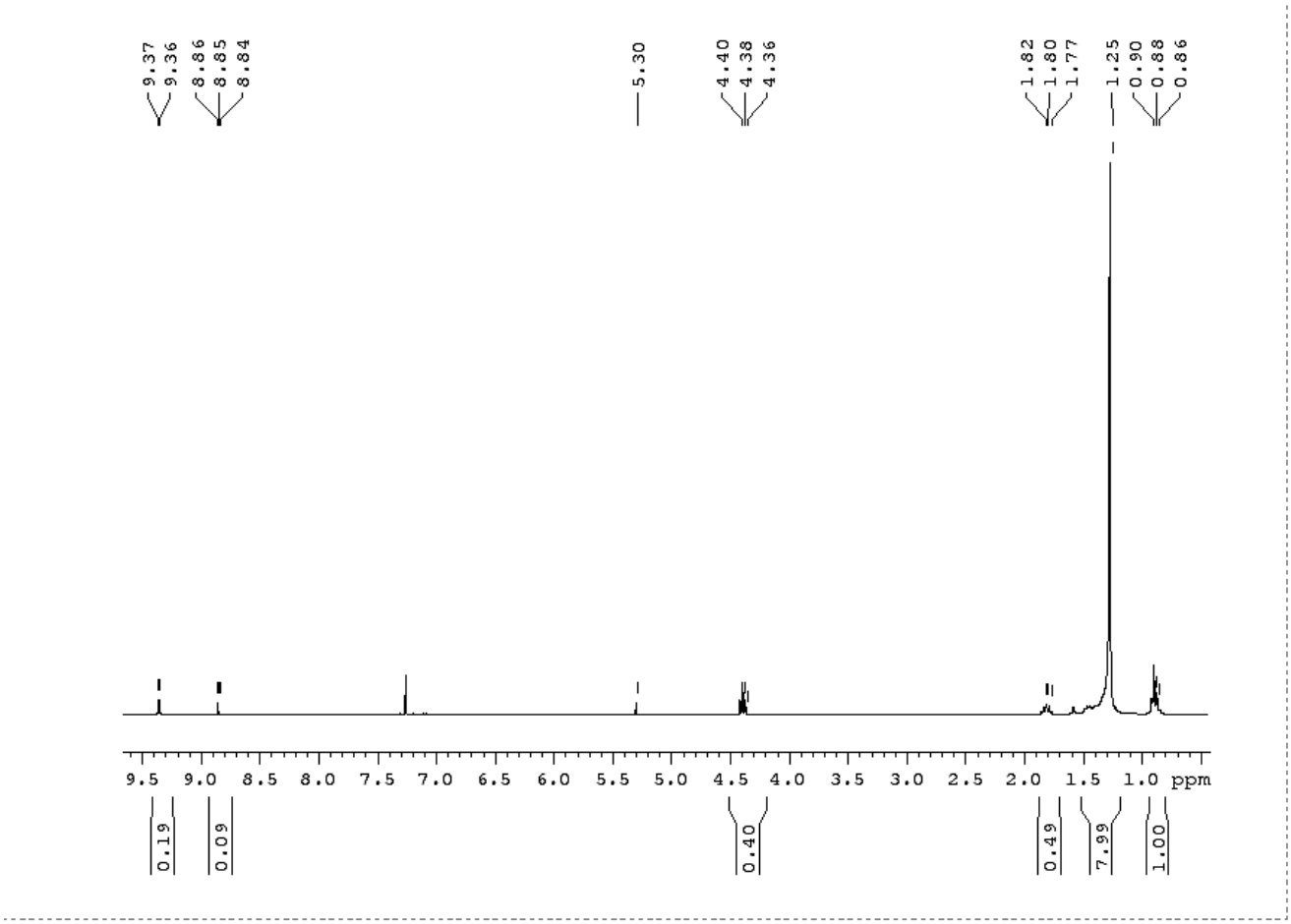
^{13}CNMR for compound **diC18:0**



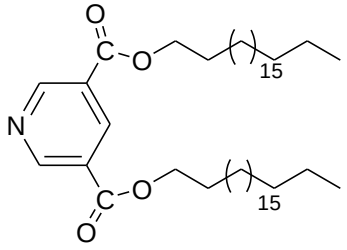


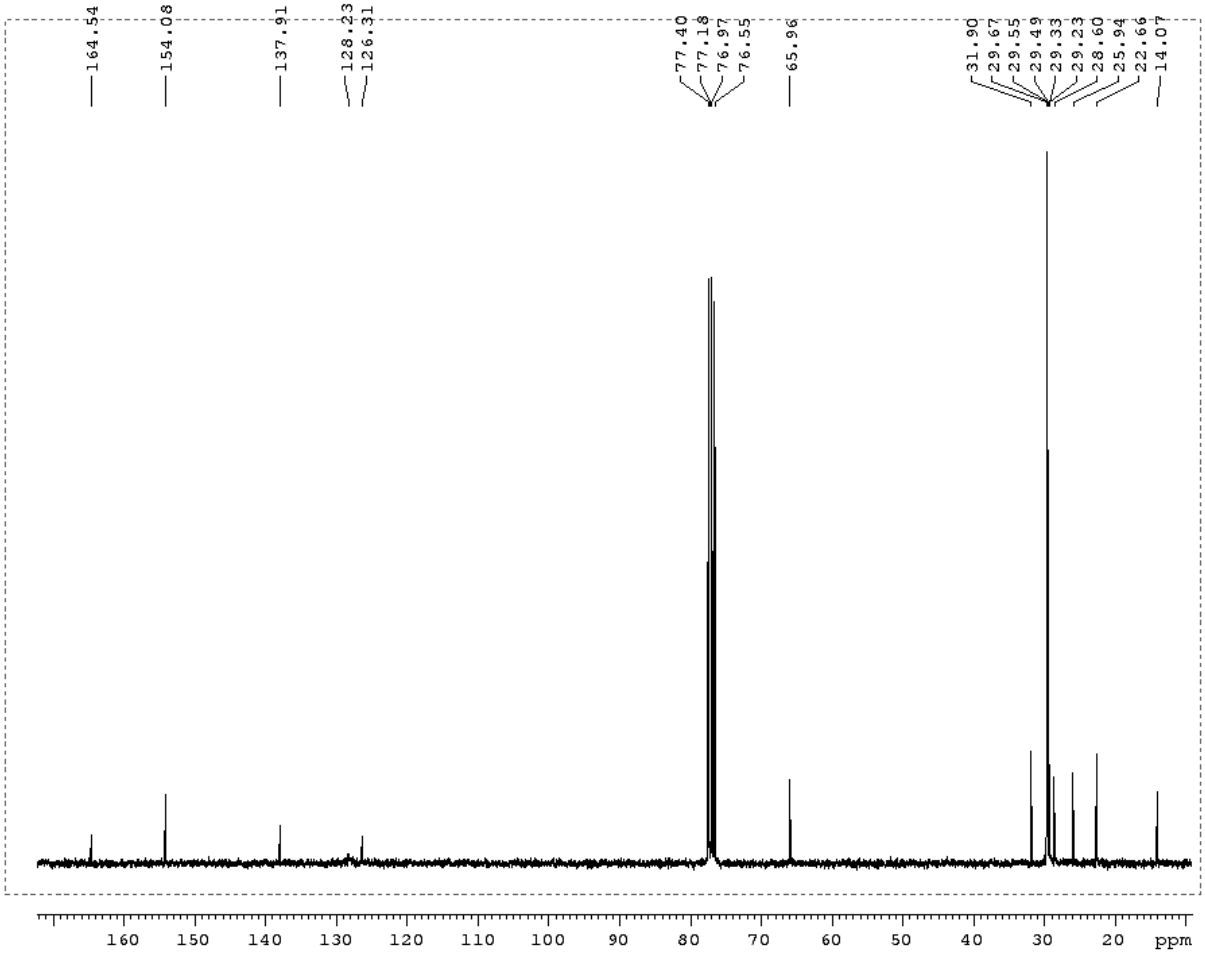
^{1}HNMR for compound **diC20:0 pyridine**



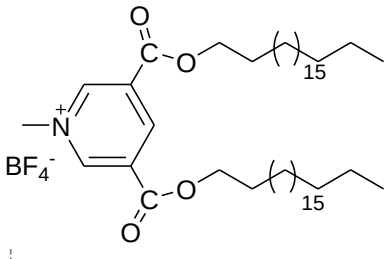


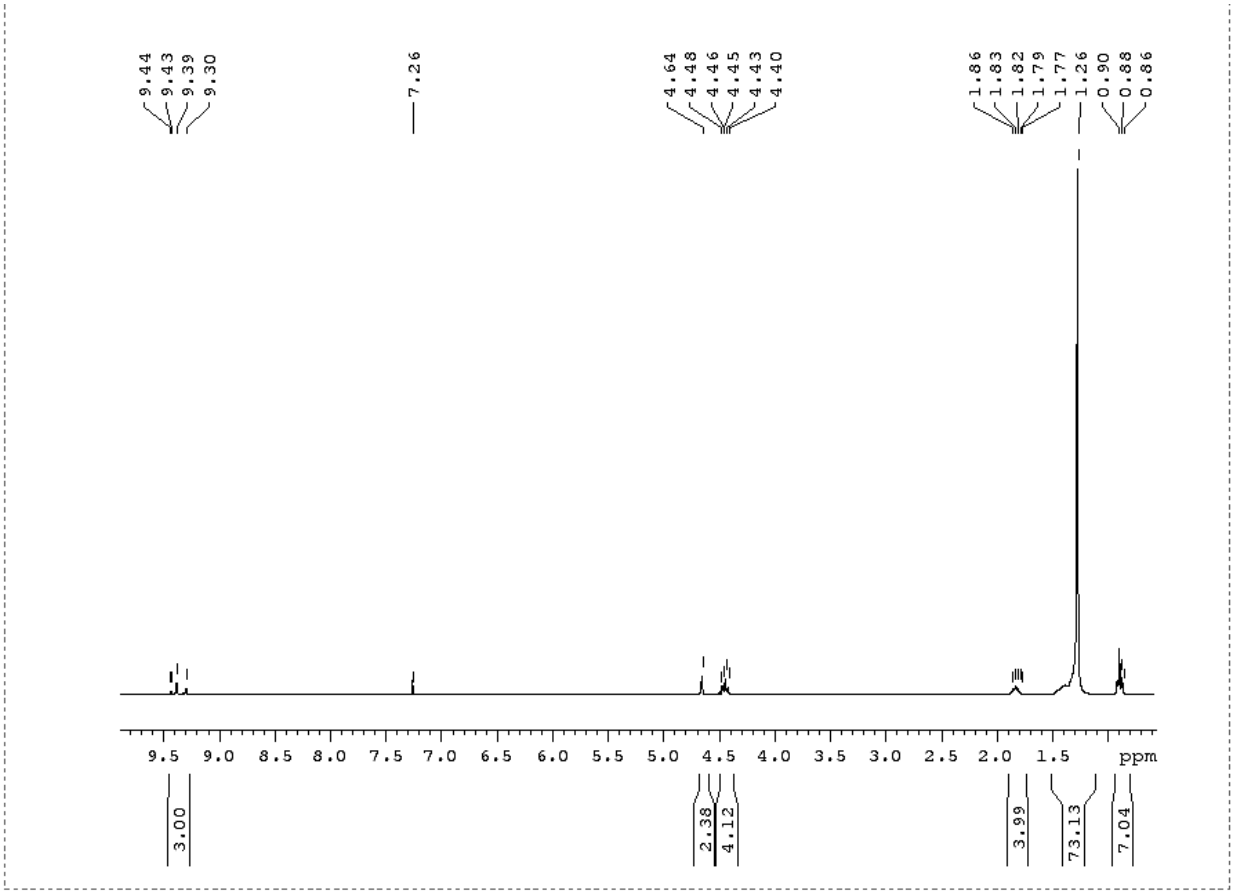
^{13}CNMR for compound **diC20:0 pyridine**



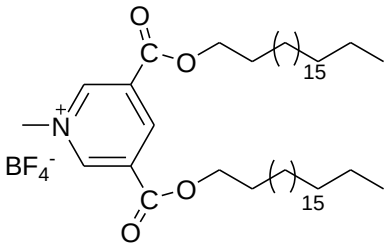


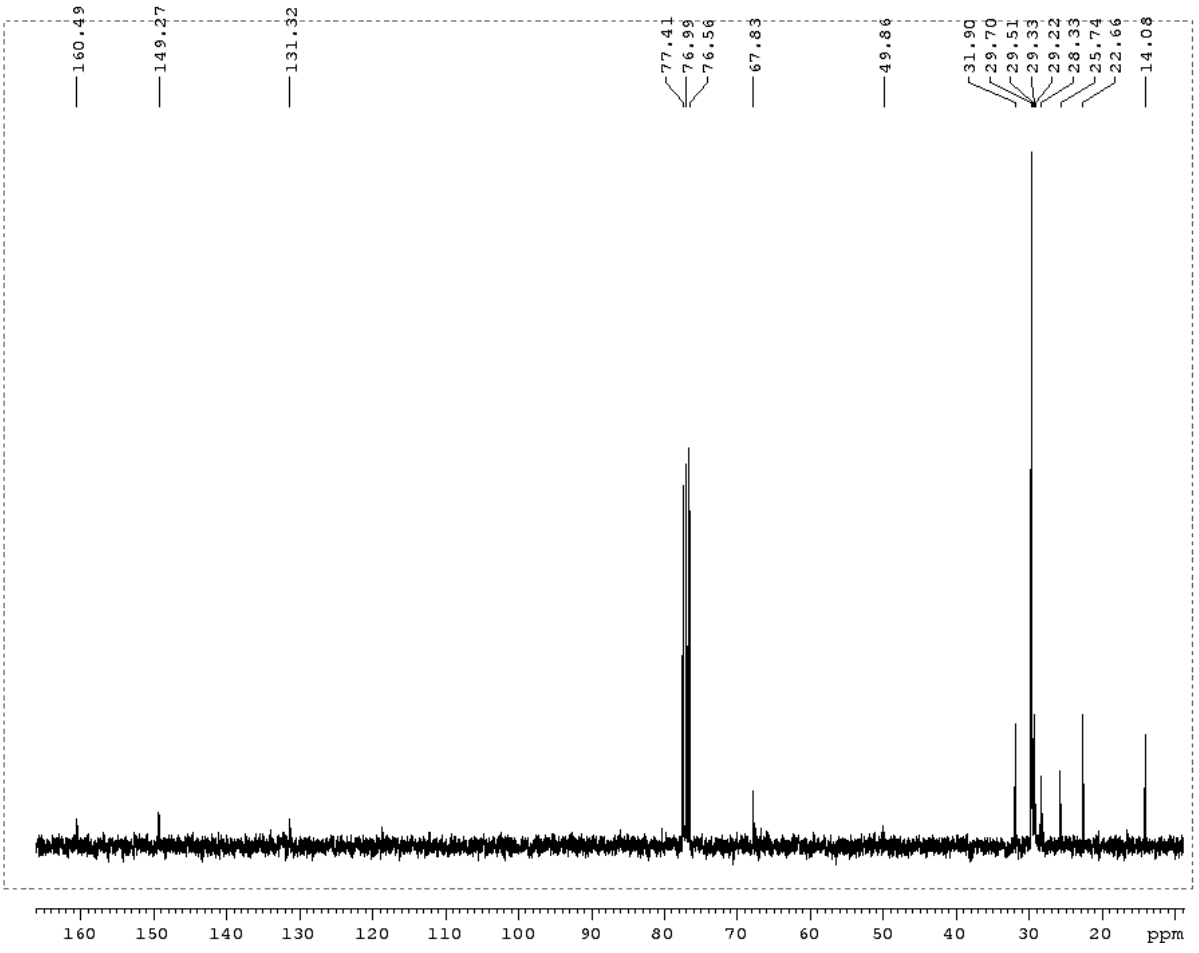
[1]HNMR for compound **diC20:0**



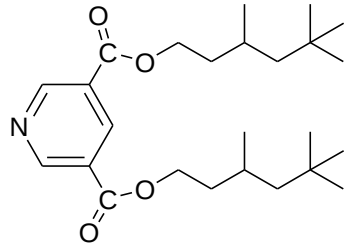


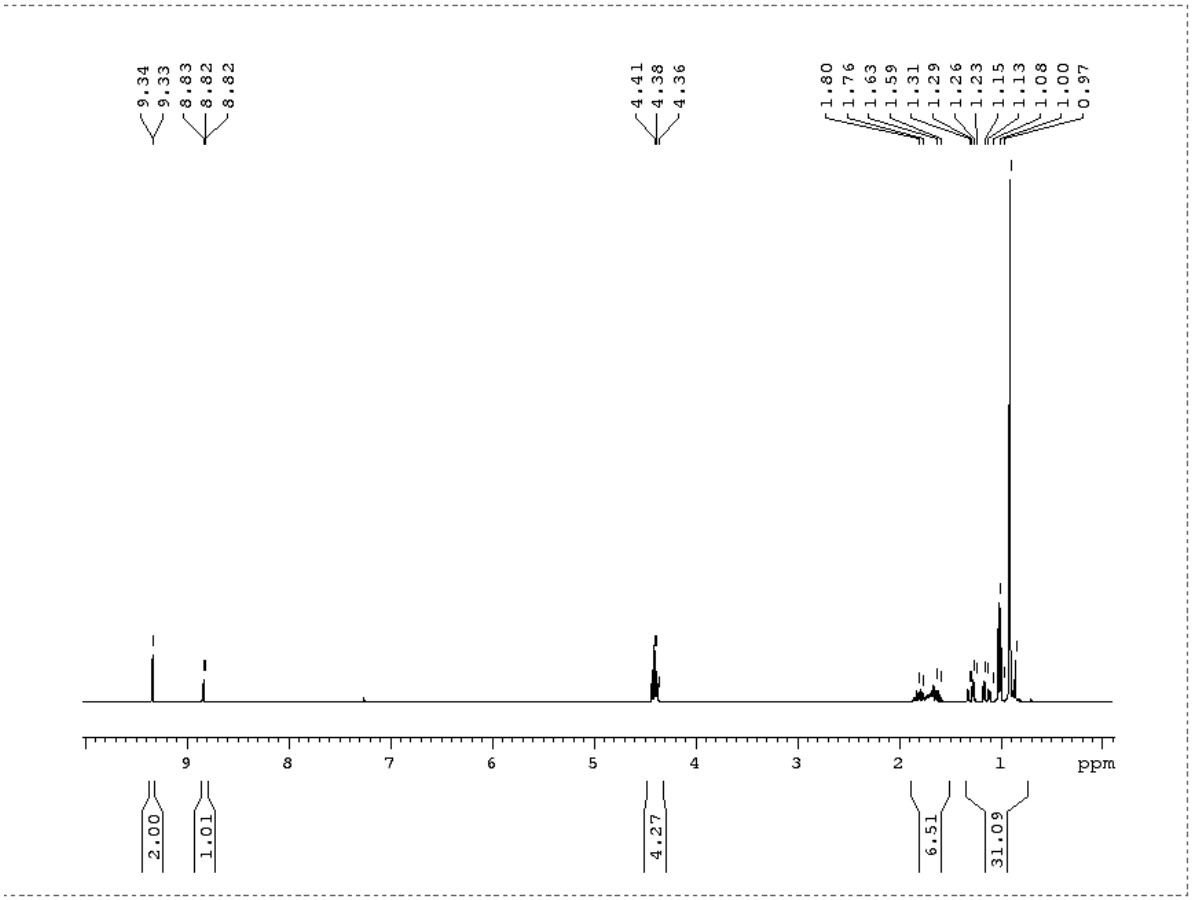
^{13}CNMR for compound **diC20:0**



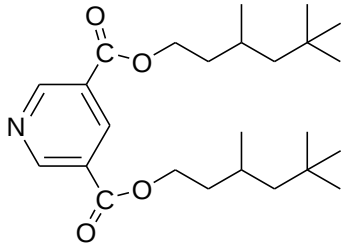


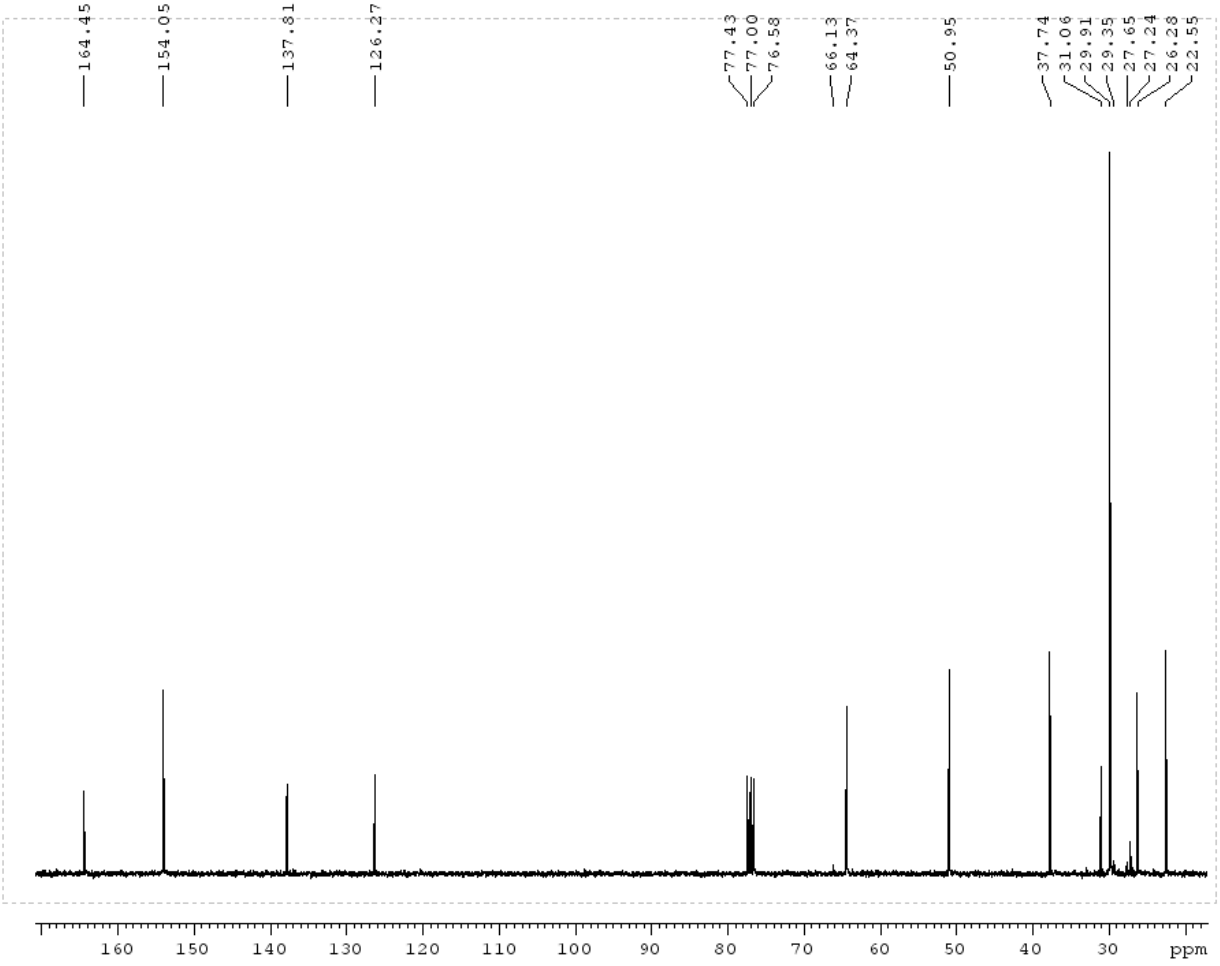
[1]HNMR for compound **diisoC9:0 pyridine**





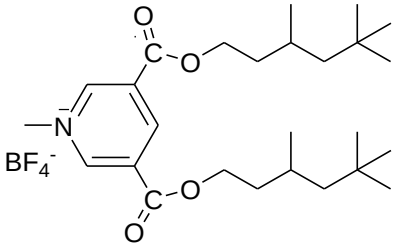
^{13}CNMR for compound **diisoC9:0 pyridine**

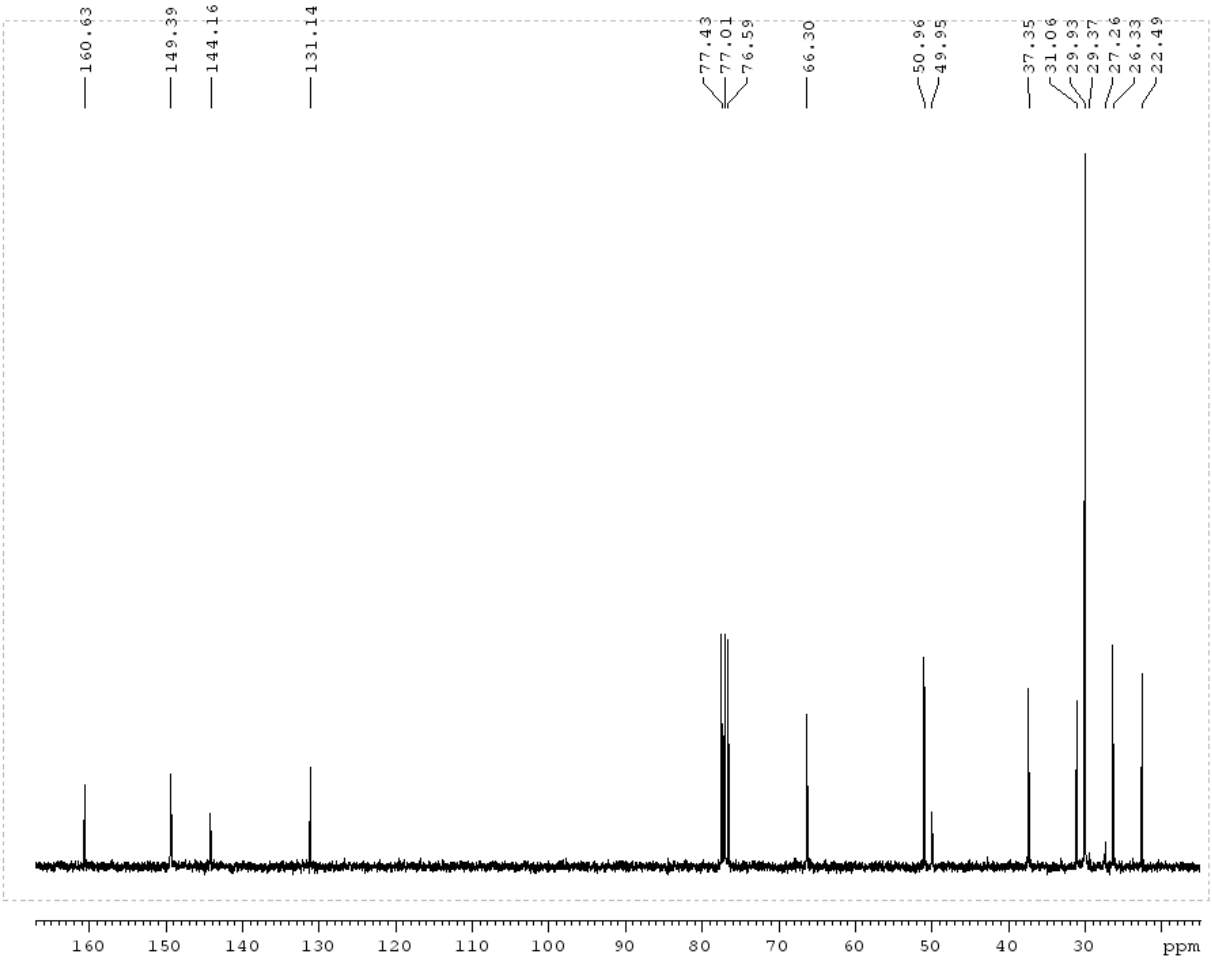




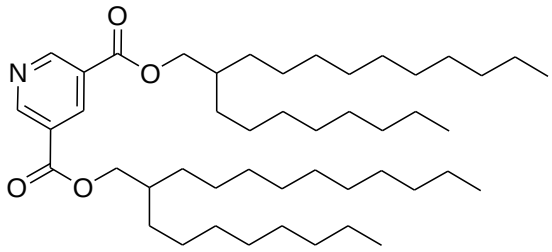
^{1}HNMR for compound **diisoC9:0**

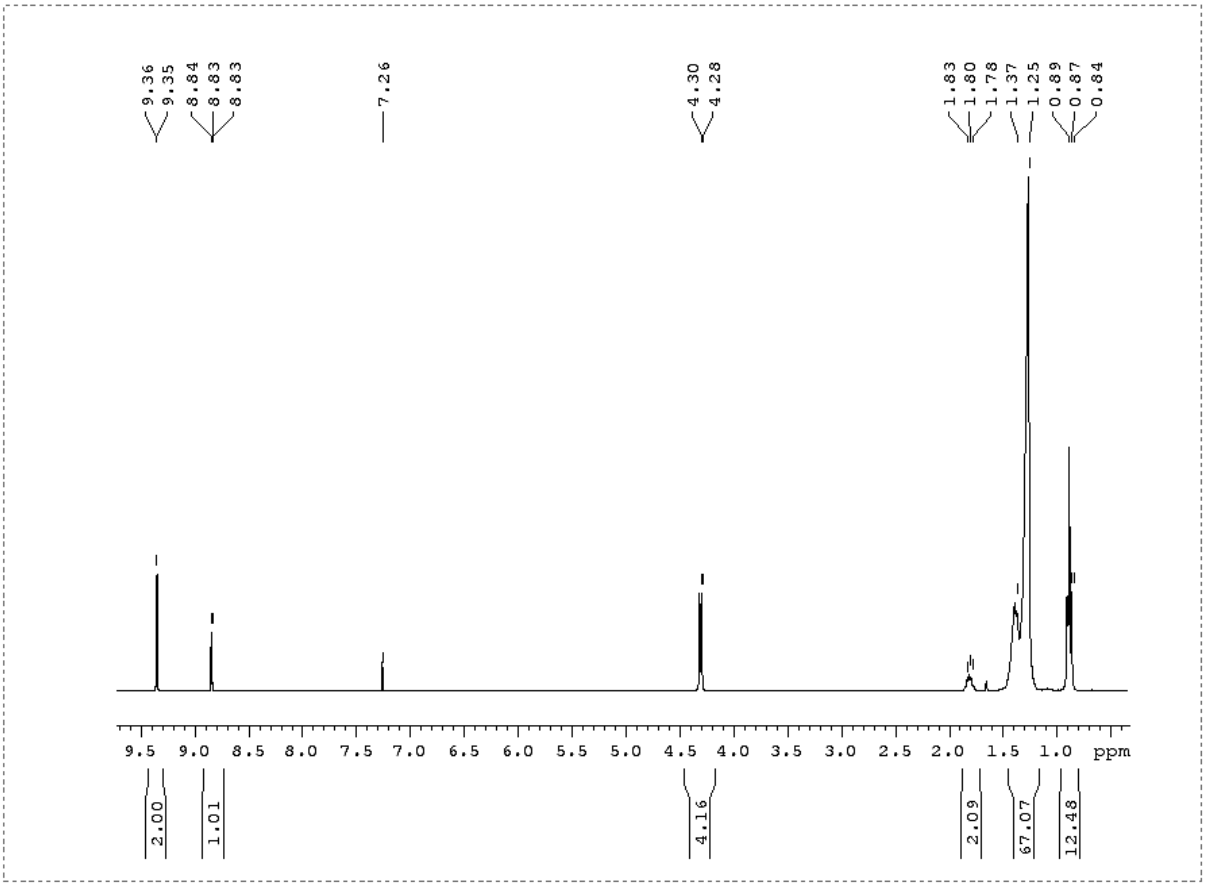
^{13}CNMR for compound **diisoC9:0**



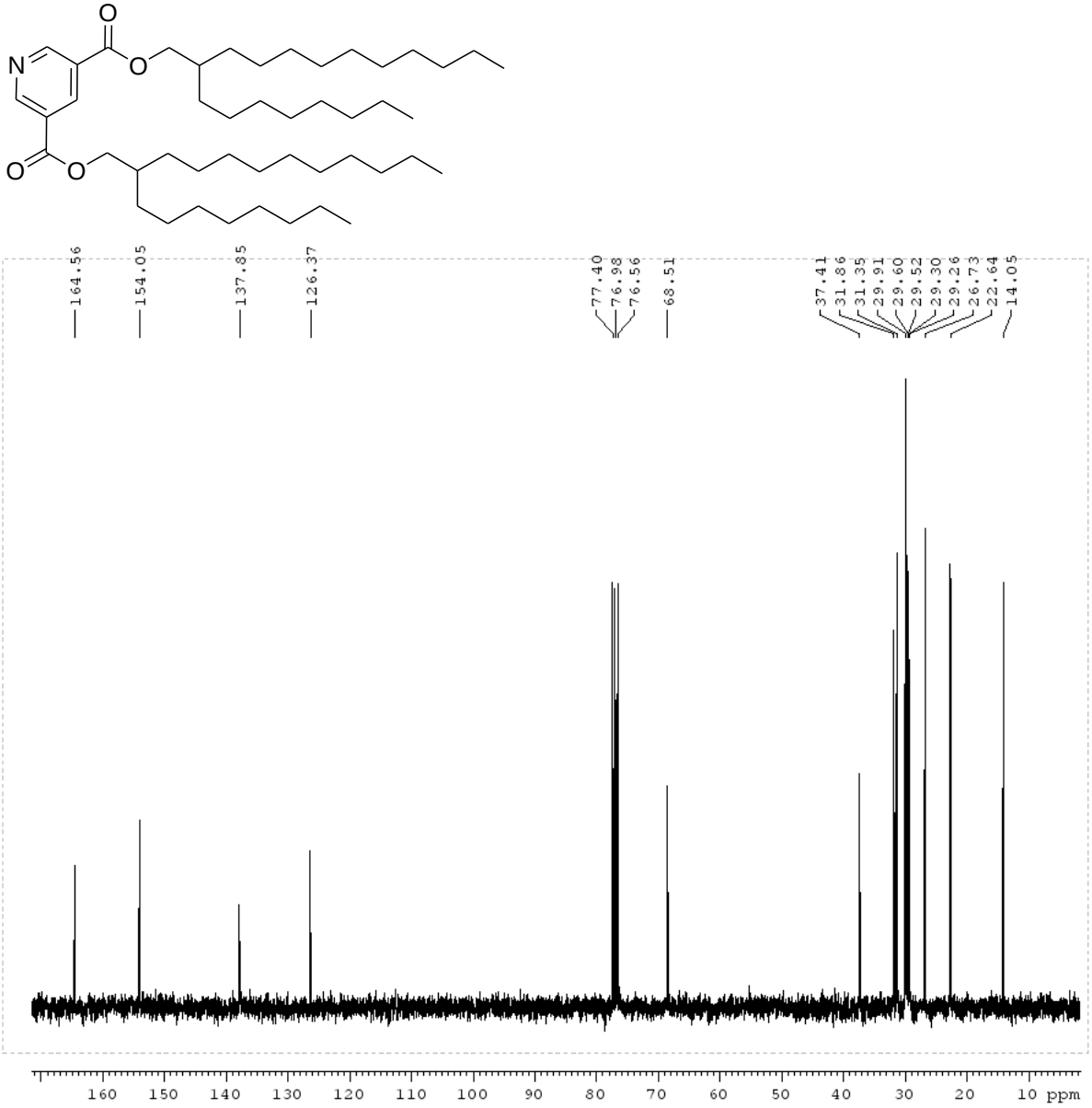


[1]HNMR for compound **dibrC20:0 pyridine**

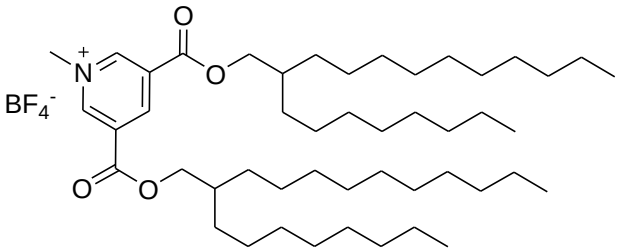


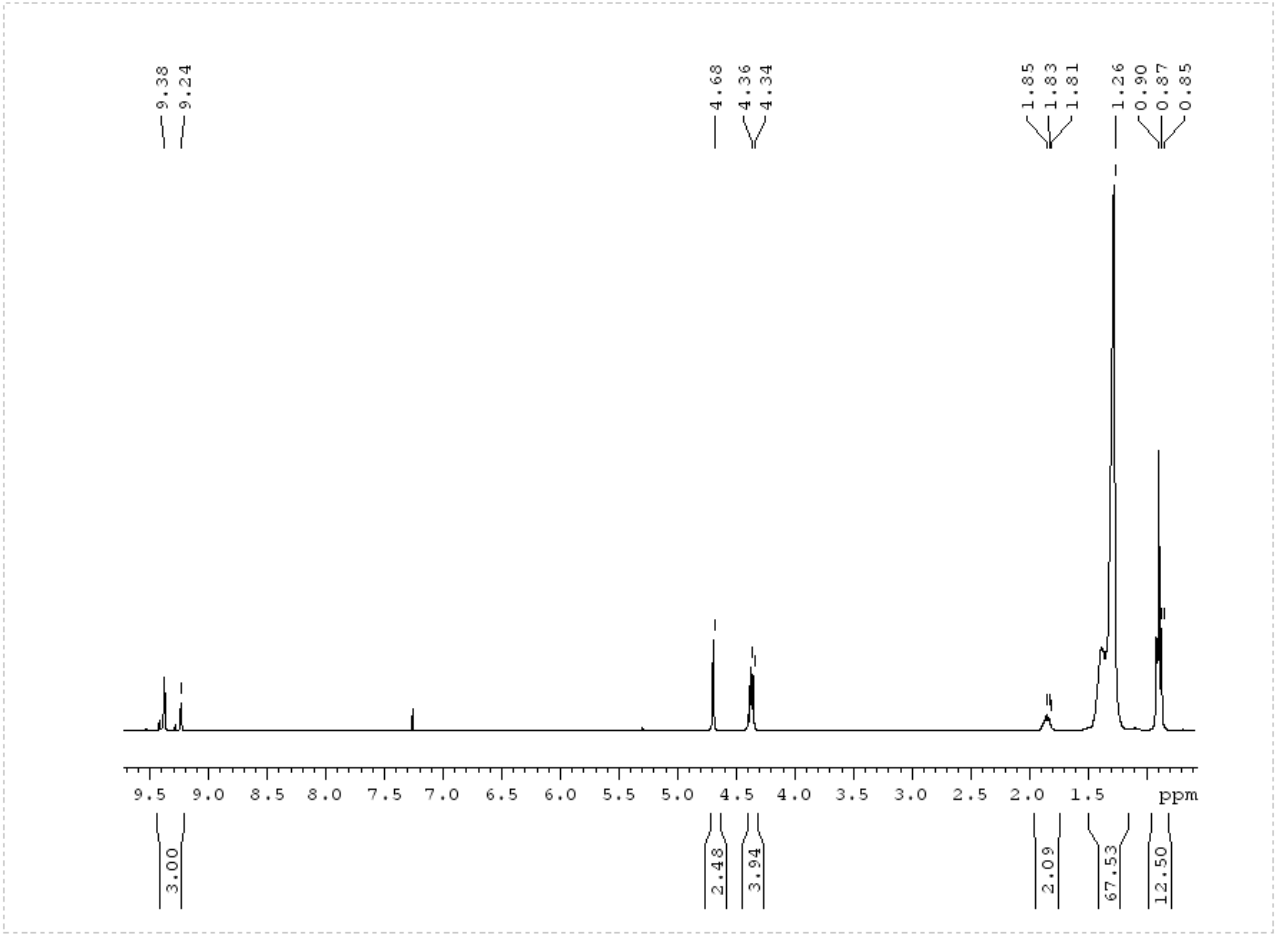


^{13}CNMR for compound **dibrC20:0 pyridine**

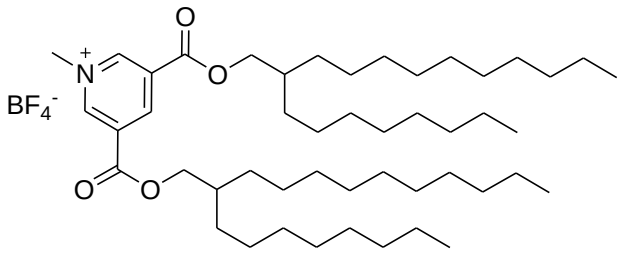


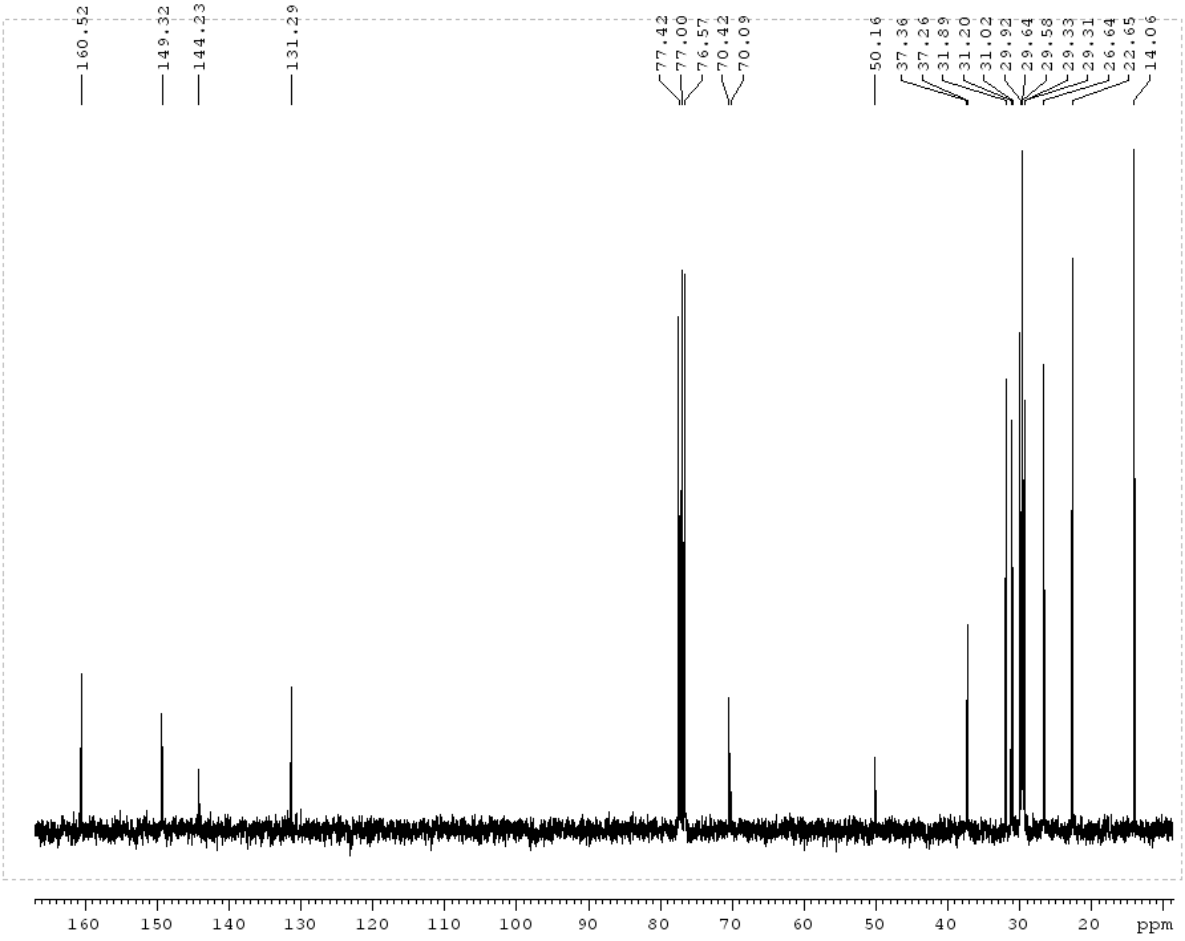
^{1}HNMR for compound **dibrC20:0**



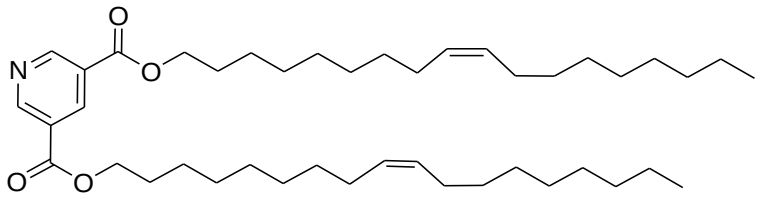


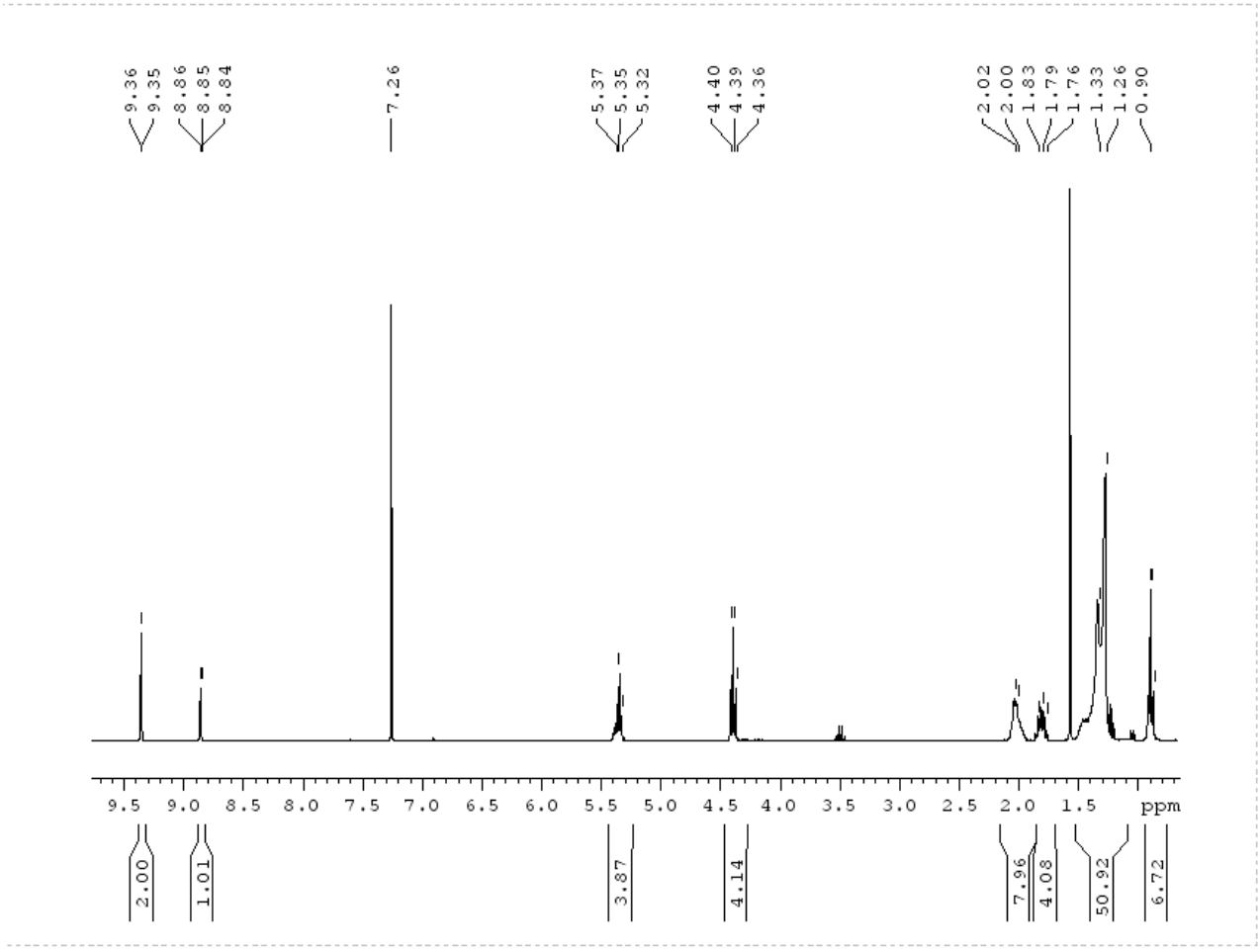
^{13}CNMR for compound **dibrC20:0**



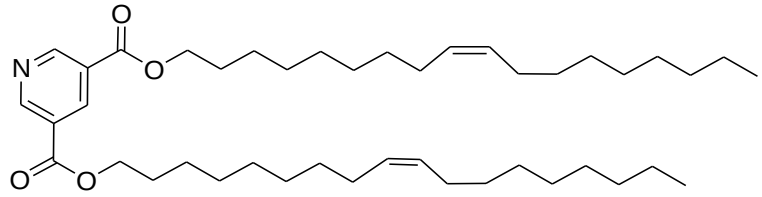


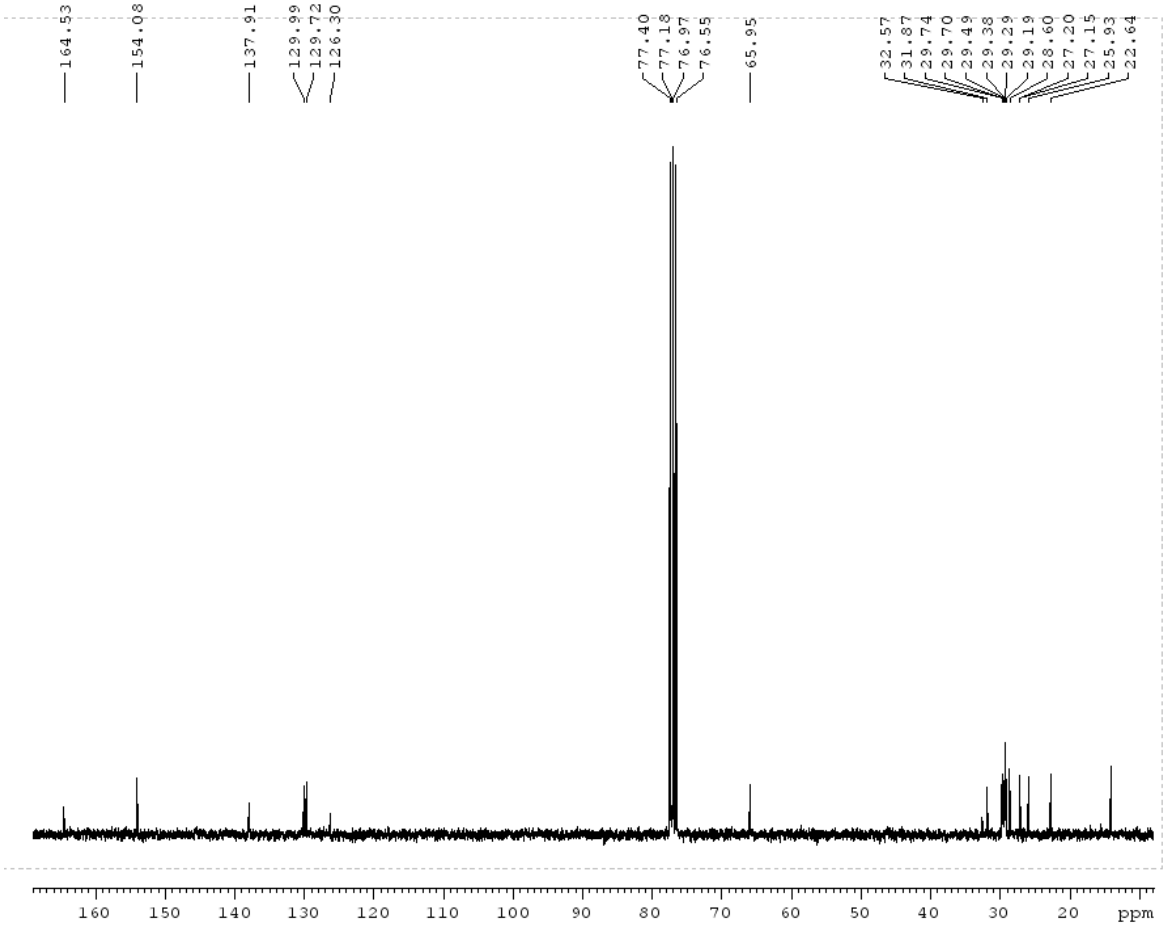
[1]HNMR for compound **diC18:1 pyridine**



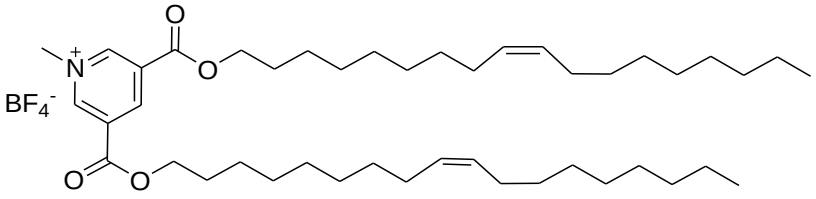


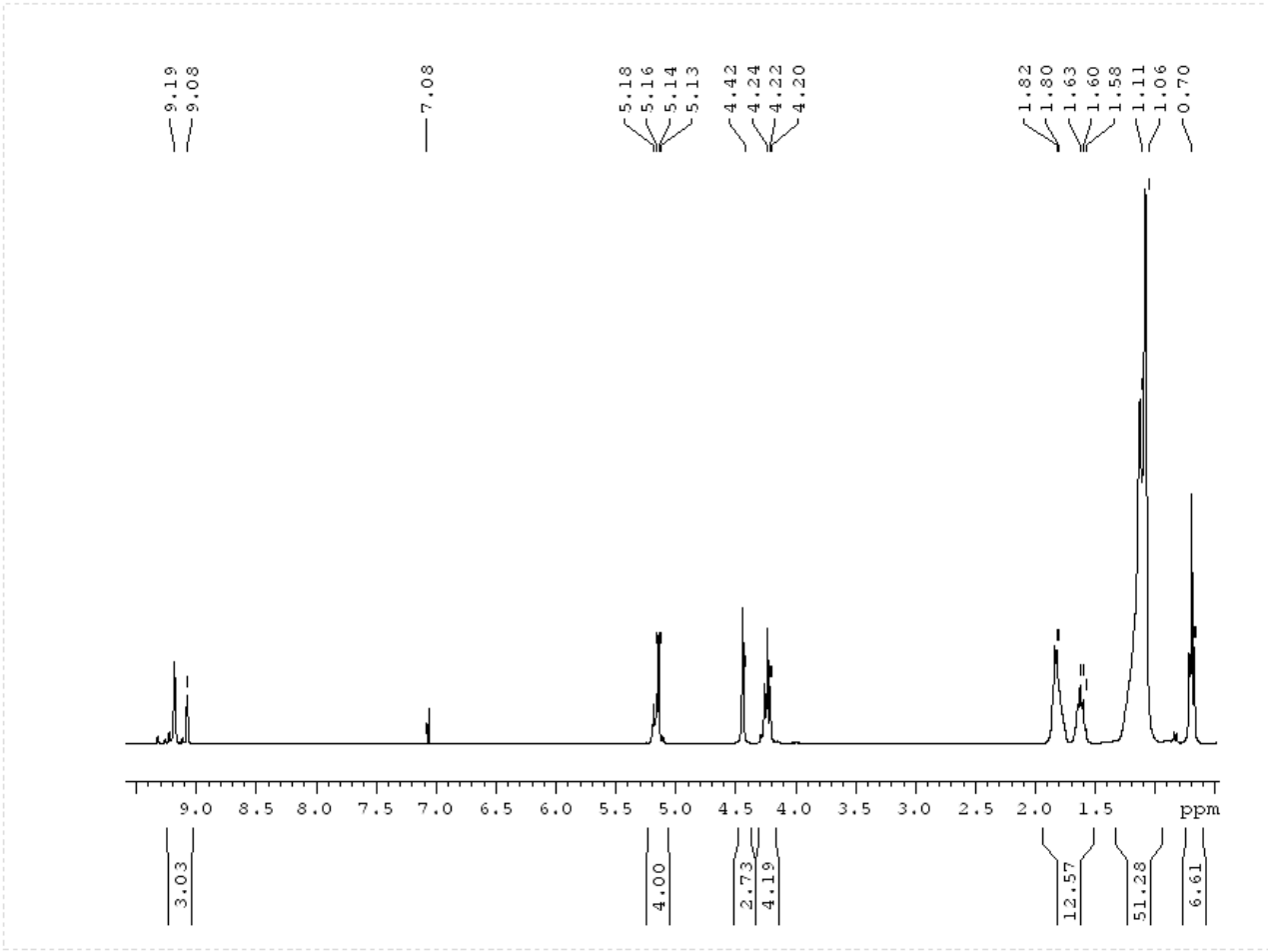
^{13}CNMR for compound **diC18:1 pyridine**



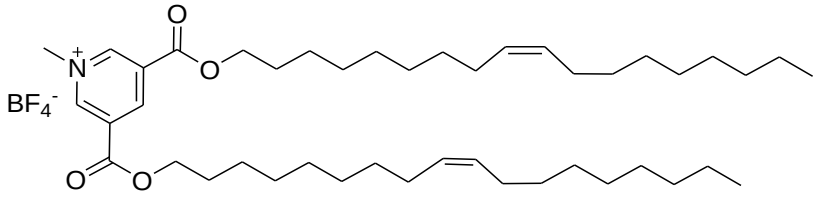


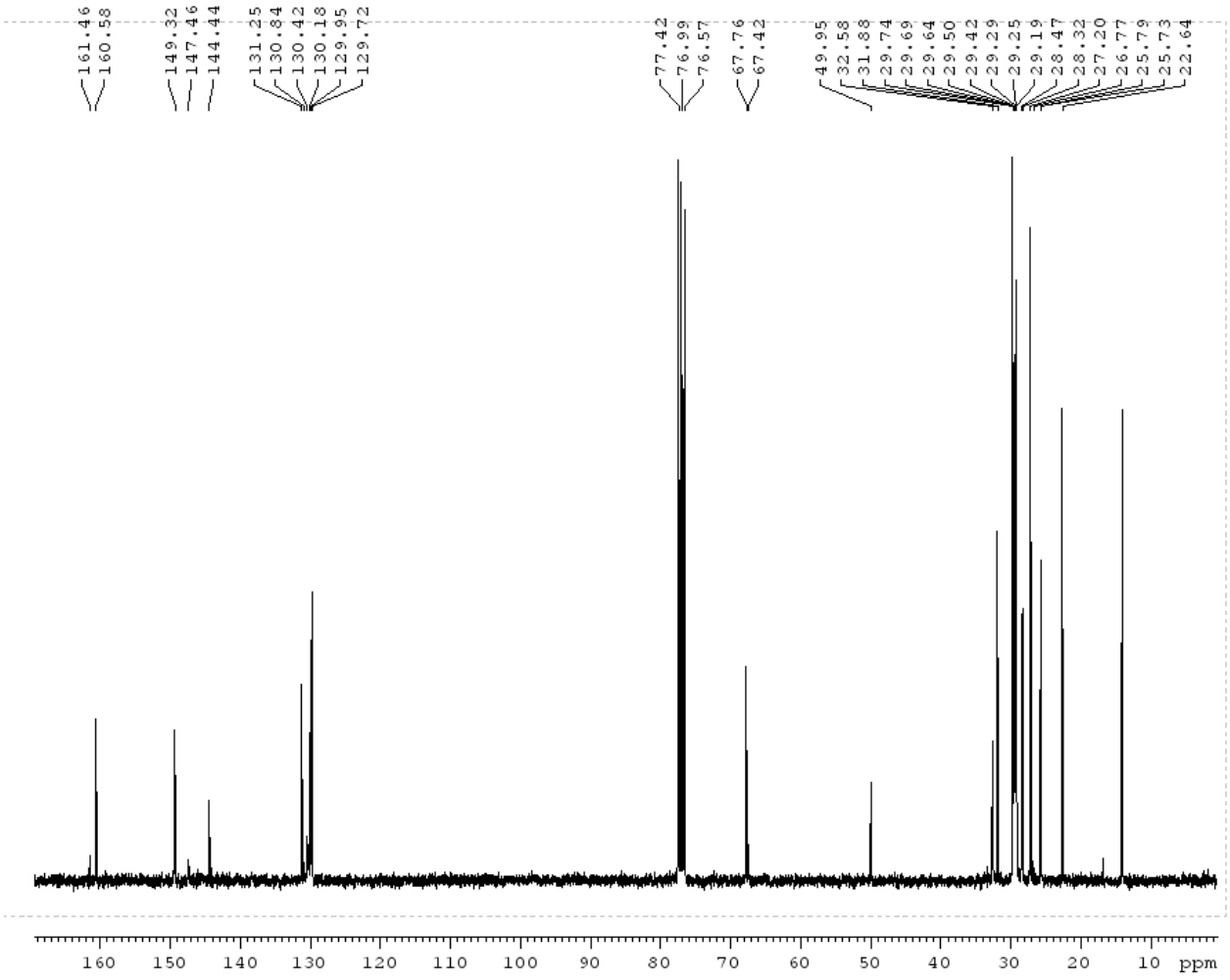
^{1}HNMR for compound **diC18:1**



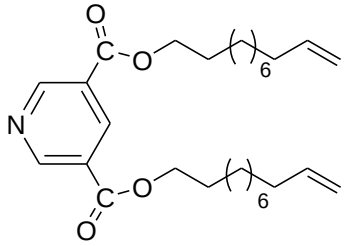


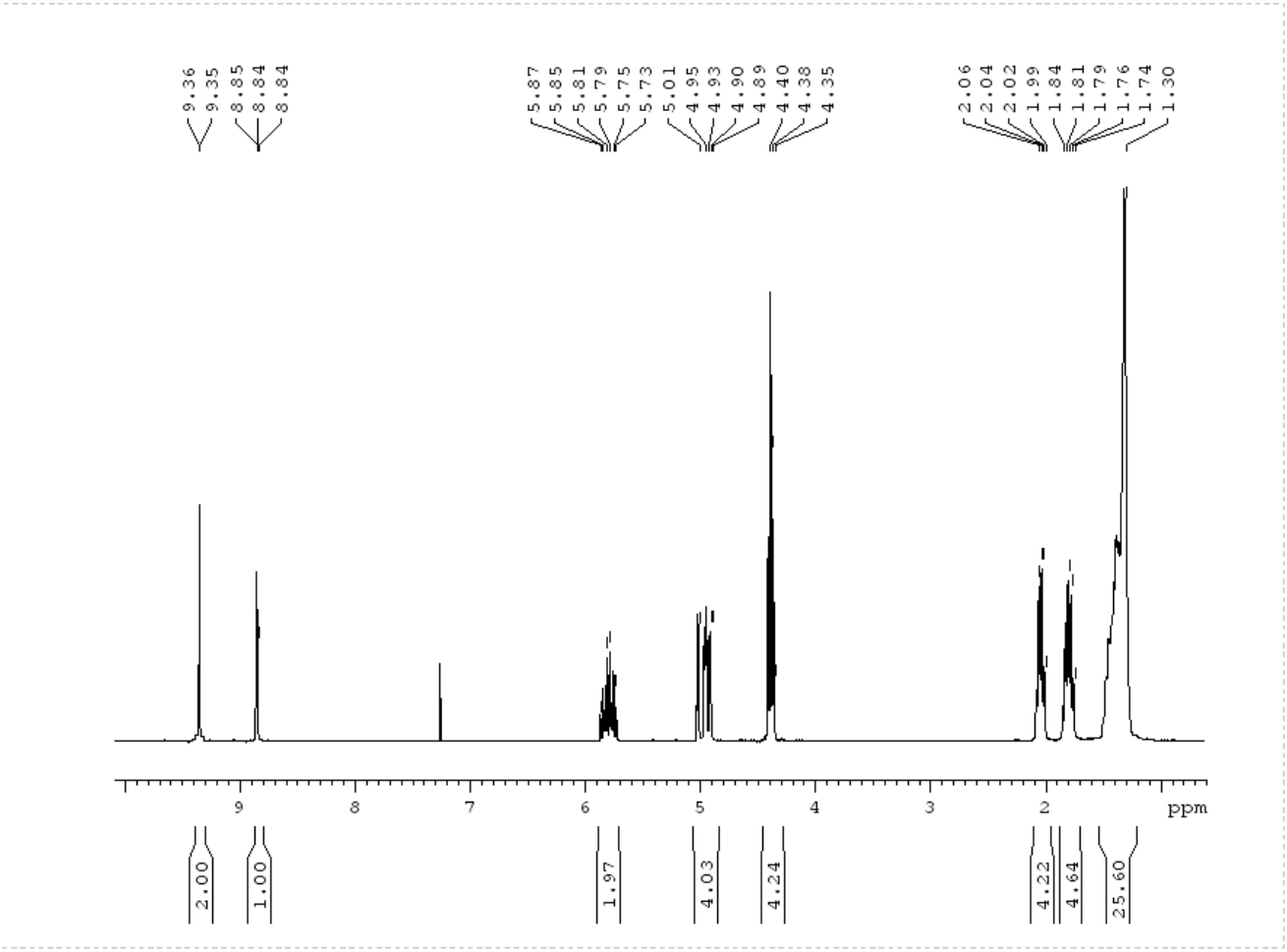
^{13}CNMR for compound **diC18:1**



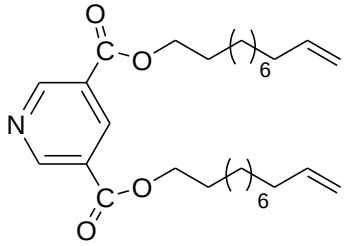


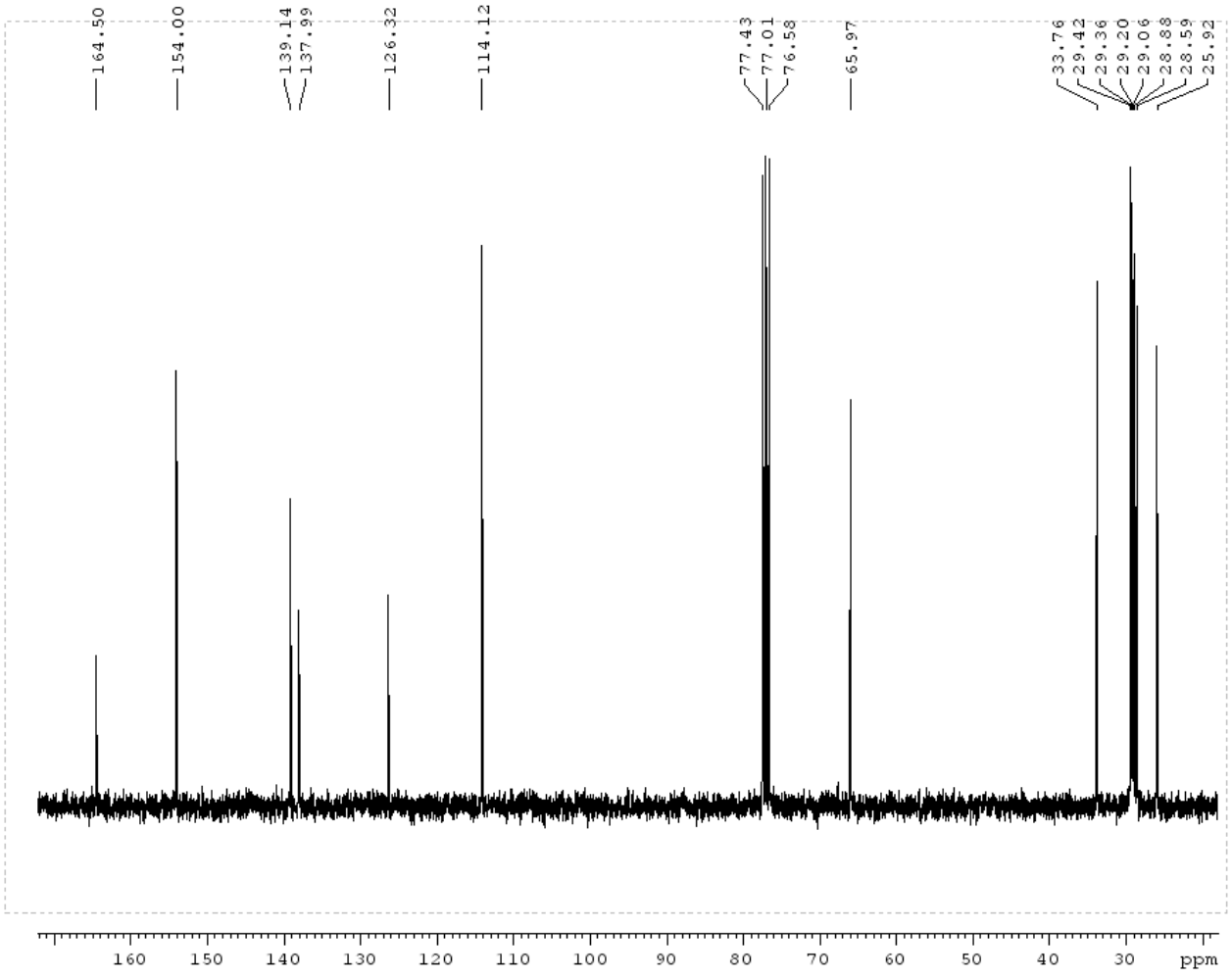
[1]HNMR for compound **diC11:1 pyridine**



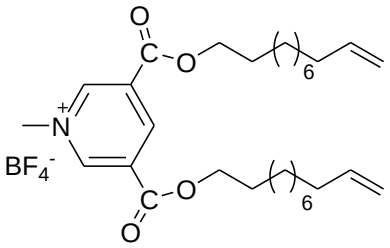


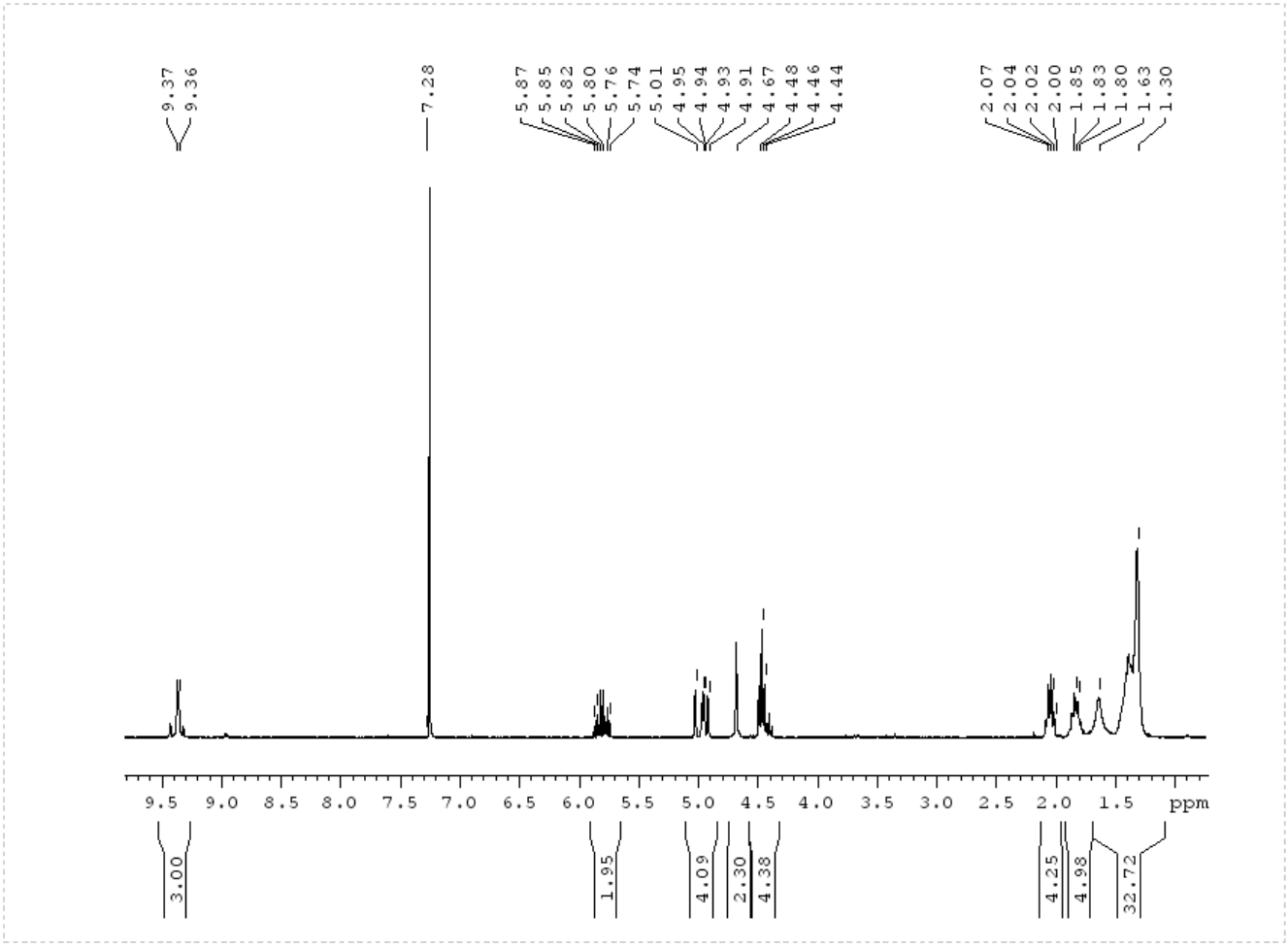
^{13}CNMR for compound **diC11:1 pyridine**



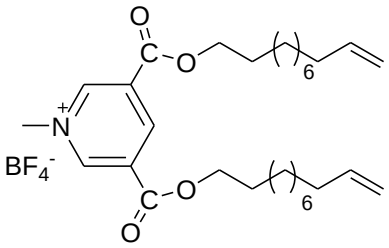


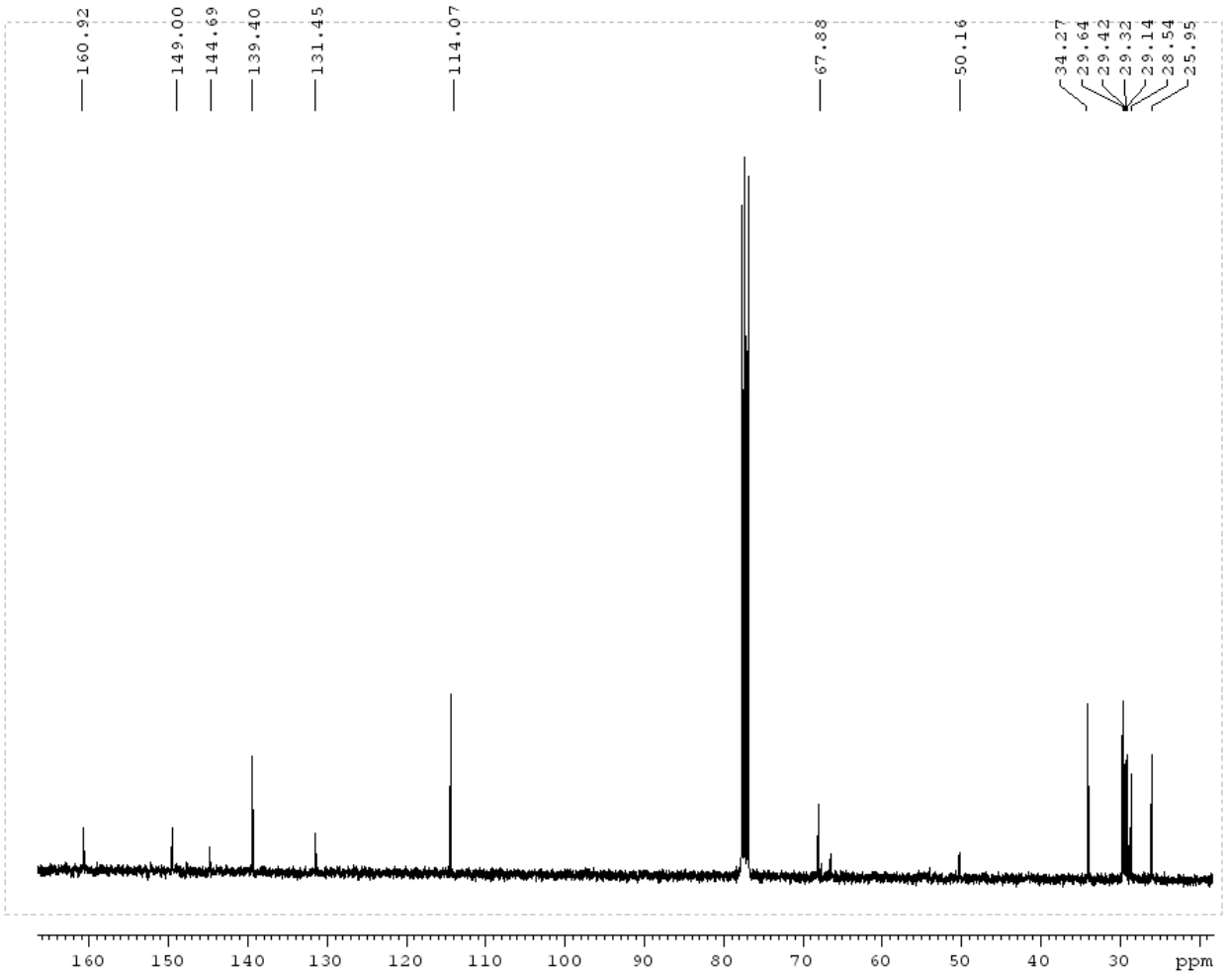
^{1}HNMR for compound **diC11:1**



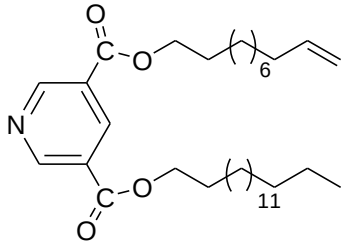


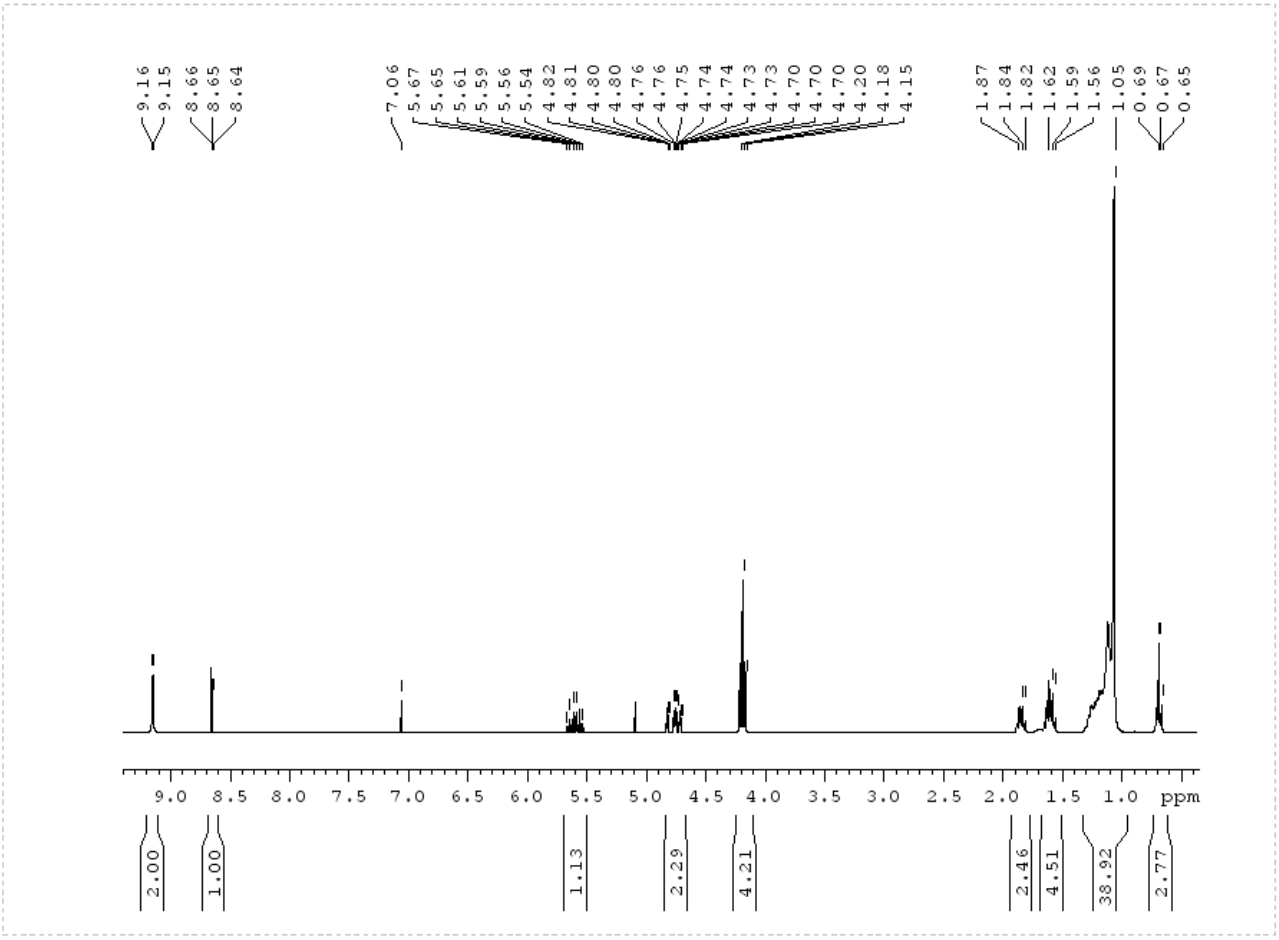
^{13}CNMR for compound **diC11:1**



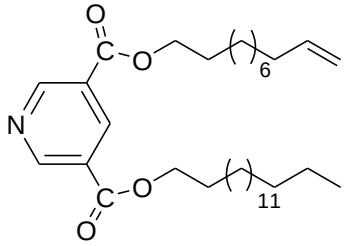


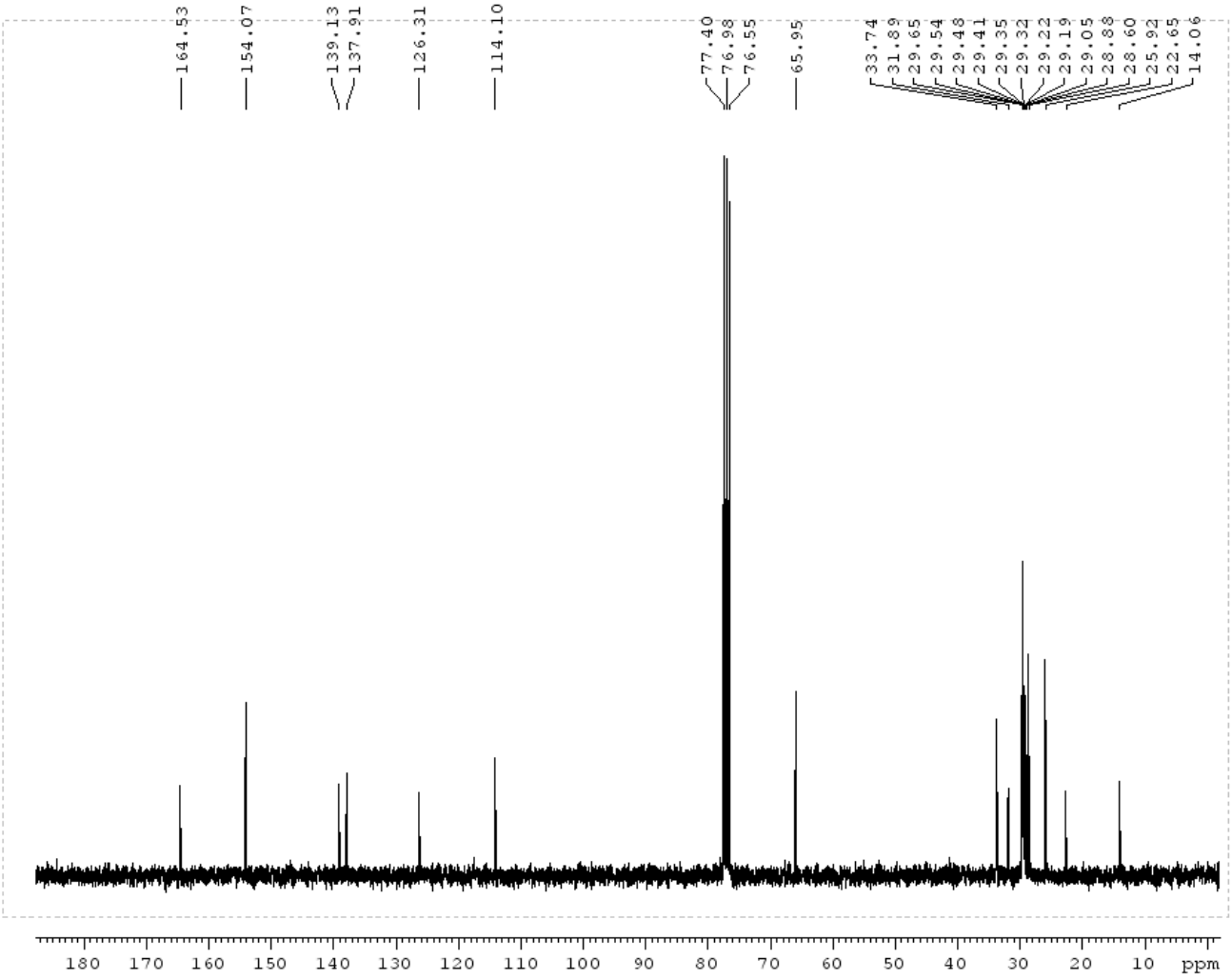
[1]HNMR for compound **(C16:0)(C11:1) pyridine**



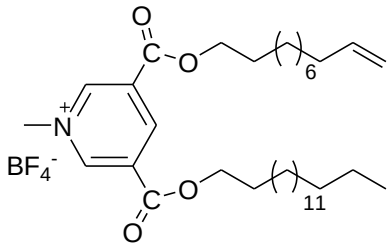


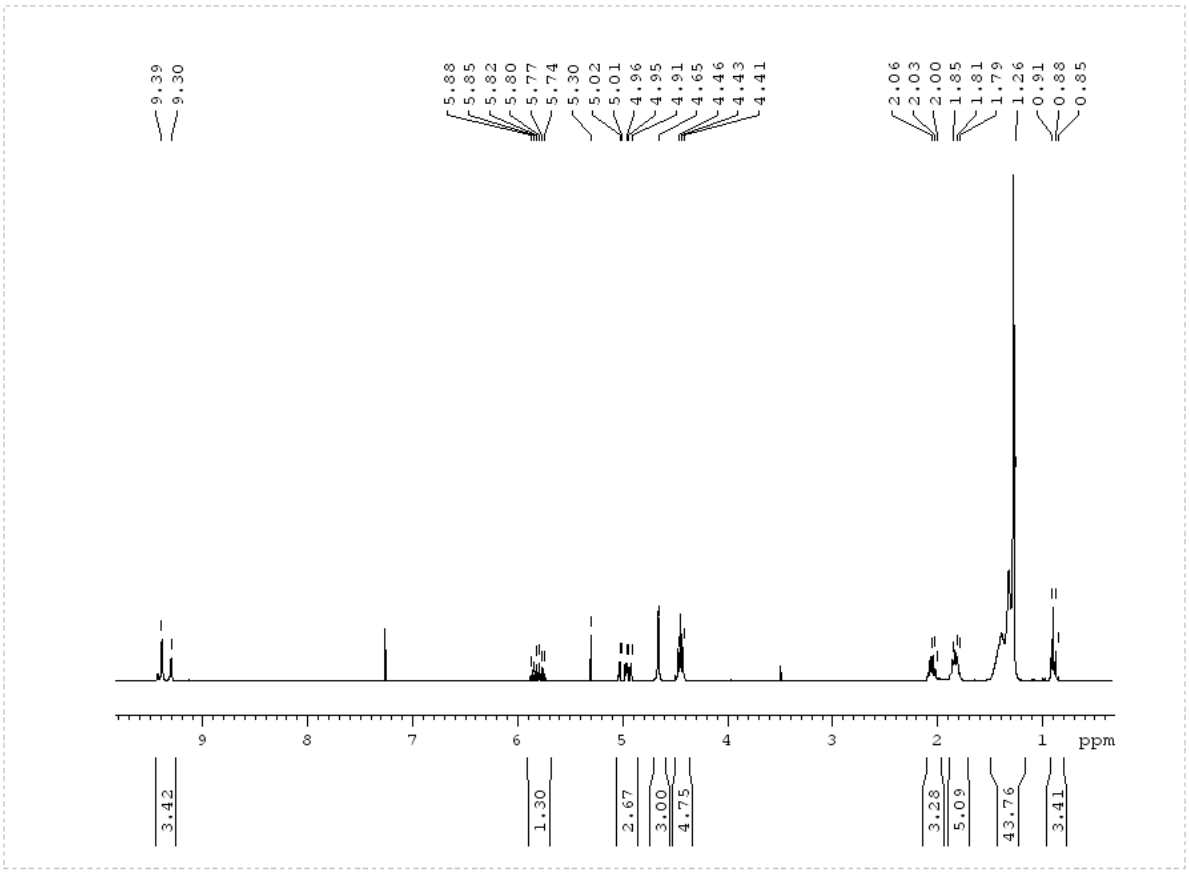
^{13}CNMR for compound **(C16:0)(C11:1) pyridine**



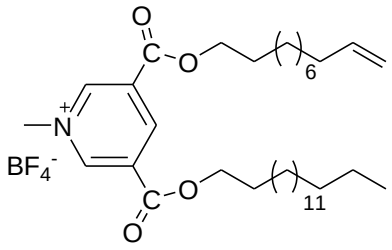


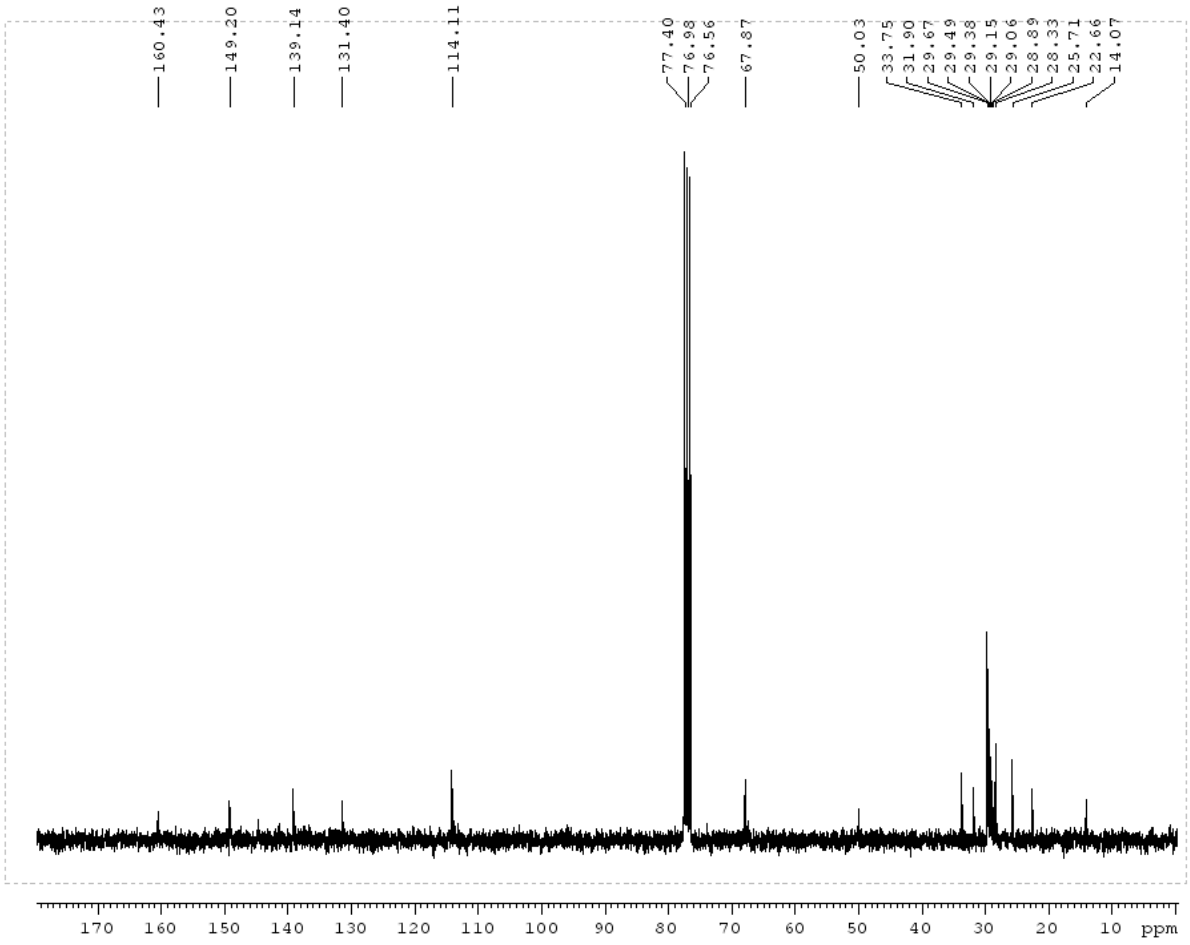
^{1}HNMR for compound **(C16:0)(C11:1)**



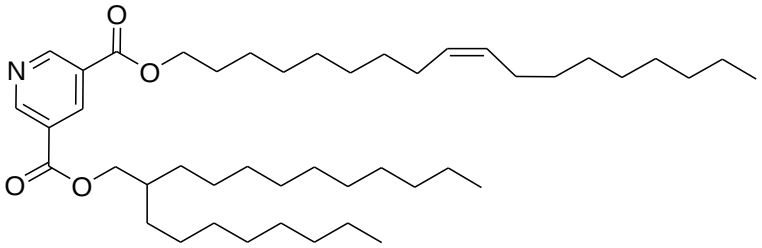


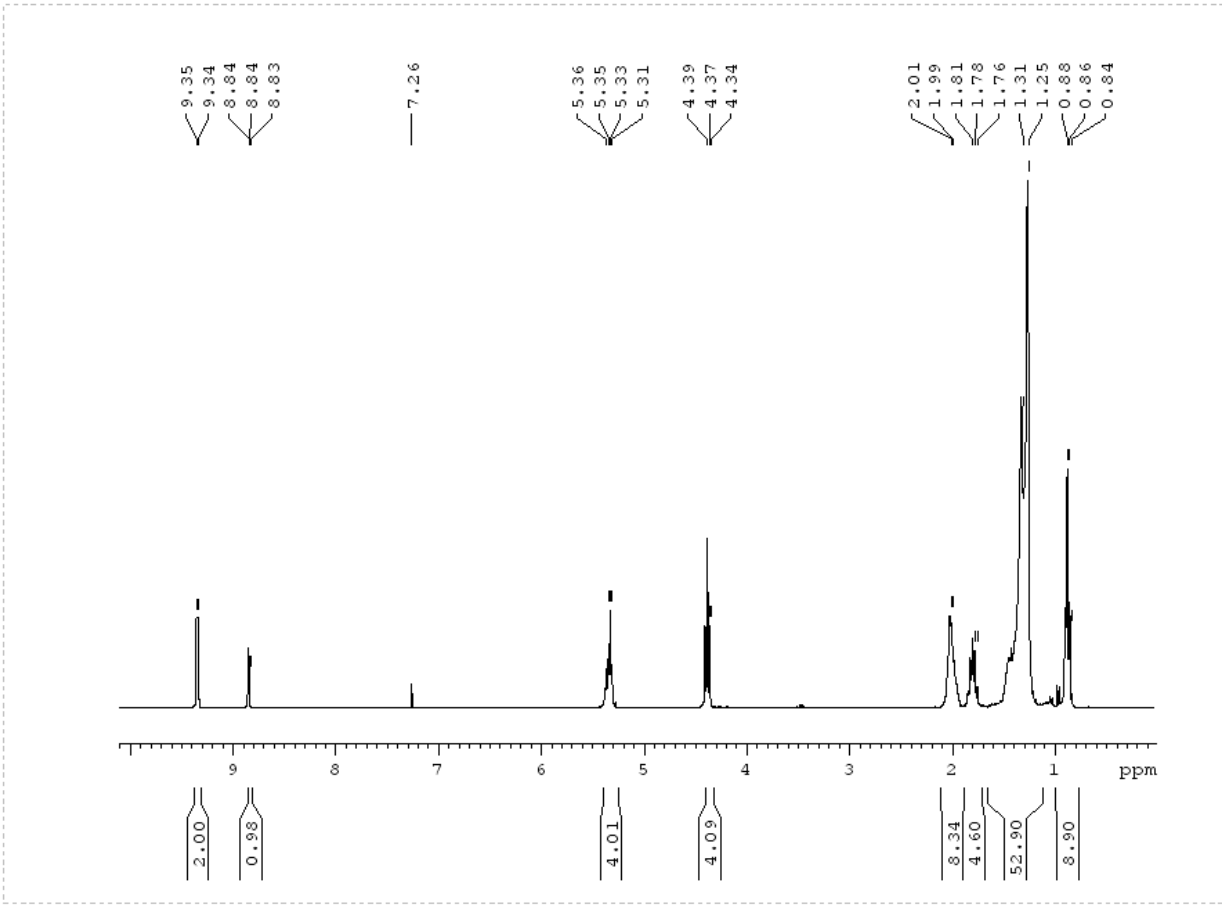
^{13}CNMR for compound **(C16:0)(C11:1)**



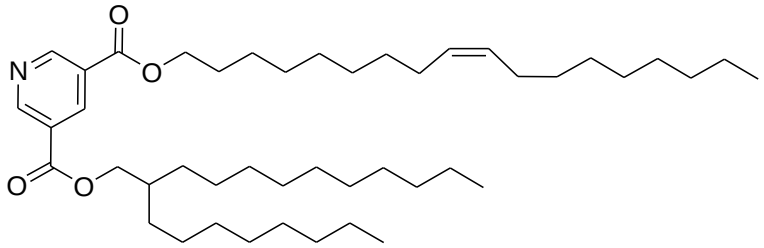


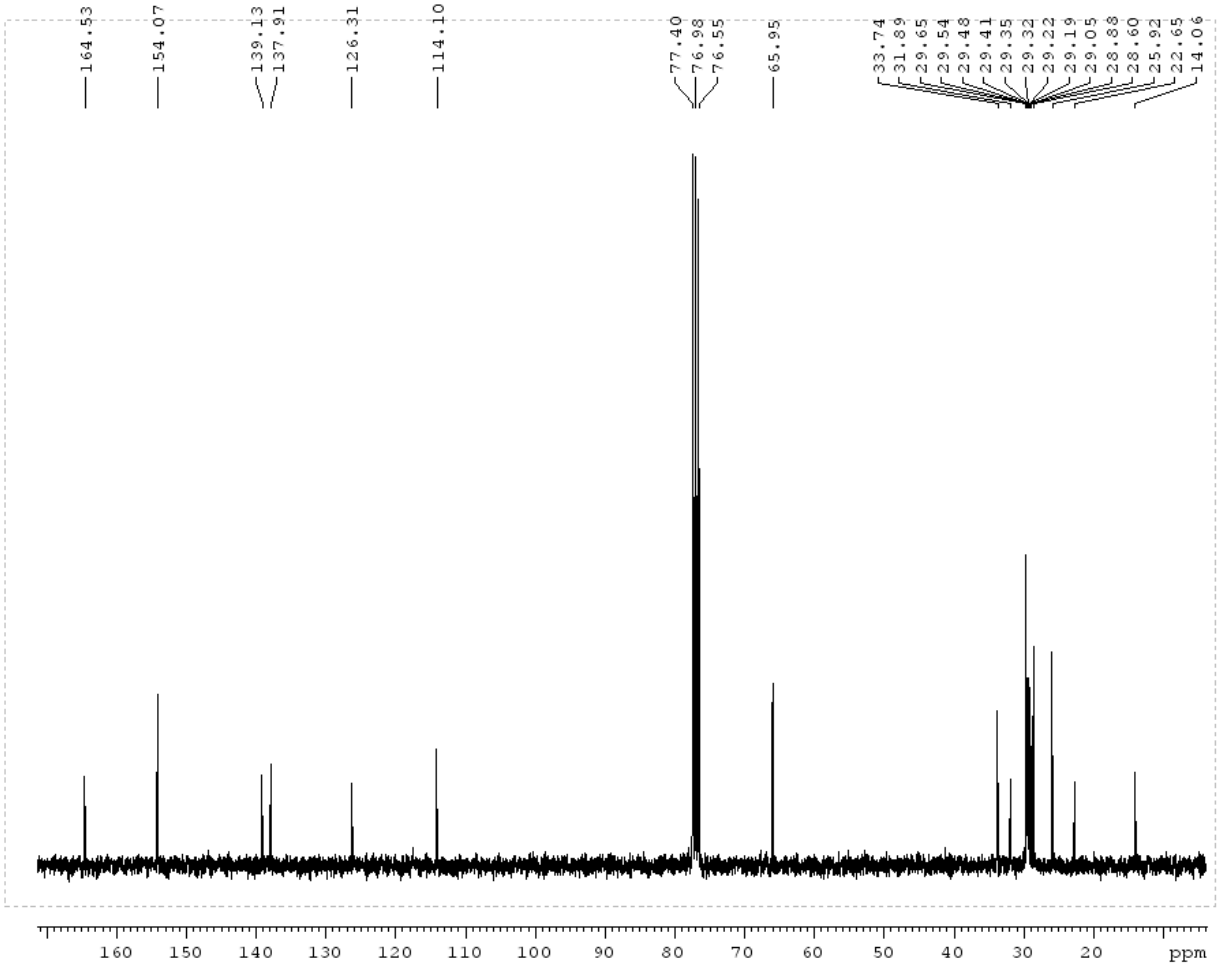
[1]HNMR for compound **(C18:1)(brC20:0) pyridine**



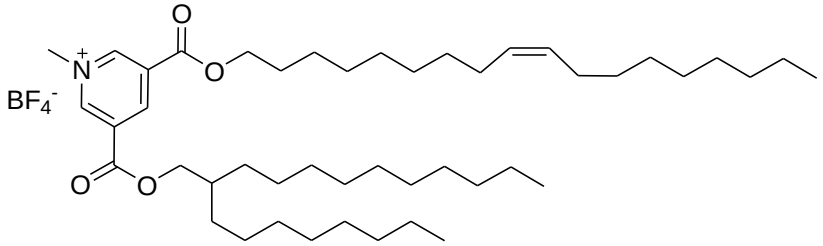


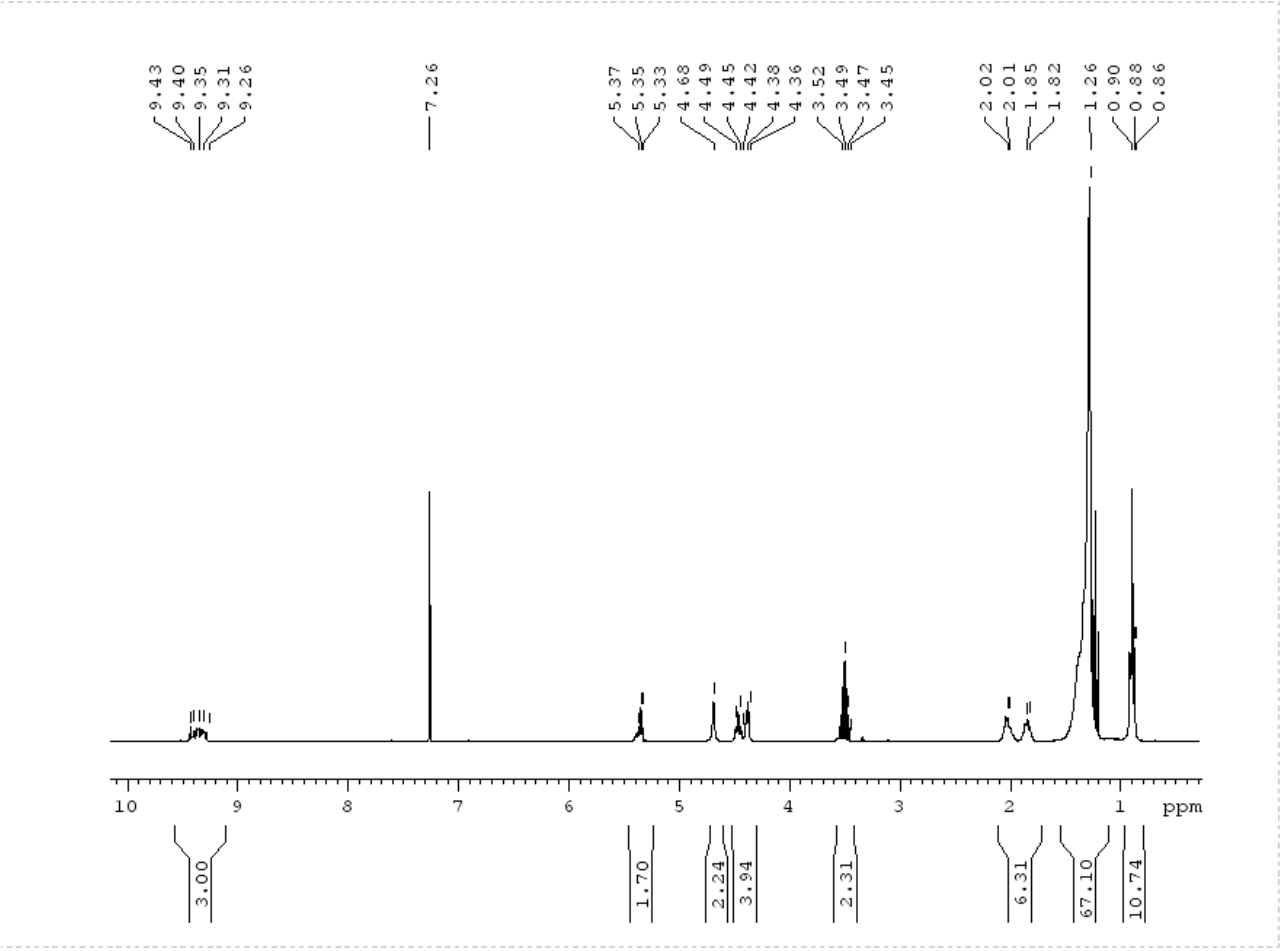
^{13}CNMR for compound **(C18:1)(brC20:0) pyridine**





^{1}HNMR for compound **(C18:1)(brC20:0)**





^{13}CNMR for compound **(C18:1)(brC20:0)**