Supplementary Information

Relationship between the Physicochemical Properties of Lipid Nanoparticles and the Quality of siRNA Delivery to Liver Cells

Yusuke Sato, Hiroto Hatakeyama, Mamoru Hyodo, and Hideyoshi Harashima

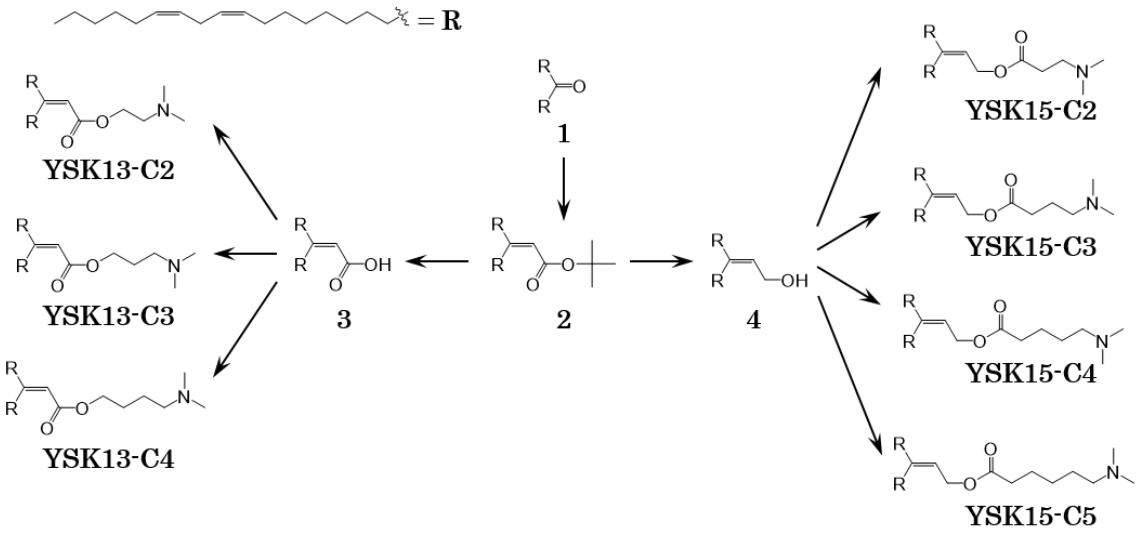
Laboratory for Molecular Design of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-Ku, Sapporo 060-0812, Japan.

General Information

^{1}H and ^{13}C NMR spectra were measured on a JEOL ECA500, ECX400P or ECS400 instrument, with tetramethylsilane as the internal standard (0 ppm). The following abbreviations were used to express the peaks: s = singlet; d = doublet; t = triplet; m = multiplet; br = broad. ^{1}H NMR cheimcal shifts are reported in ppm on the sigma scale downfield from tetramethylsilane. All reactions were monitored by thin-layer chromatography on pre-coated TLC plates (Millipore), visualization was achieved by using UV light (254 nm), phosphomolybdic acid stain or *p*-anisaldehyde stain. The products were purified by flash column chromatography on silica gel or an automated Teledyne ISCO combiflash Rf chromatography system. All ordinary chemicals were purchased and used without further purification. Compound **1** was prepared via a previously reported method.[1]

Methods

Synthesis of pH-sensitive cationic lipids.



Synthesis of tert-butyl (12Z,15Z)-3-((9Z,12Z)-octadeca-9,12-dien-1-yl)henicosa-2,12,15-trienoate (2). *tert*-Butyl diethylphosphonoacetate (2.14 mL, 9.11 mmol) and *t*-BuOK (937 mg, 8.35 mmol) were successively added to anhydrous *t*-BuOH (40 mL) and the resulting solution stirred at room temperature for 5 min, and the ketone **1** (4.00 g, 7.59 mmol) dissolved in anhydrous *t*-BuOH (10 mL) was then added. The mixture was refluxed for 3 h and then concentrated. The residue was dissolved in EtOAc (50 mL), and the organic layer was washed with a saturated NH_4Cl solution (2×50 mL). The organic

phase was dried over anhydrous Na_2SO_4. Evaporation of the solvent resulted in a yellowish oily residue. The residue was chromatographed on silica gel (SiO_2, hexane/EtOAc) to give 3.54 g (75%) of **2** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.52 (1H, s), 5.32-5.37 (8H, m), 2.76 (4H, t), 2.53 (2H, t), 1.97-2.09 (10H, m), 1.47 (9H, s), 1.20-1.45 (36H, m), 0.87 (6H, t). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 163.31, 162.78, 130.26, 128.01, 117.02, 79.41, 77.43, 77.10, 76.78, 38.40, 31.90, 31.62, 30.01, 29.86, 29.78, 29.61, 29.54, 29.44, 28.77, 28.36, 27.84, 28.29, 25.71, 22.67, 14.17. HRMS (*m/z*) Calcd. for $C_{43}H_{76}O_2Na$ $(M+Na)^+$ 647.5738; Found 647.5735.

Synthesis of (12Z,15Z)-3-((9Z,12Z)-octadeca-9,12-dien-1-yl)henicosa-2,12,15-trienoic acid (3). *tert*-Butyl ester **2** (453 mg, 0.725 mmol) was dissolved in dichloromethane (DCM), and trifluoroacetic acid (TFA, 10%) was added. The mixture was stirred at room temperature for 4 h. EtOAc (50 mL) was added to the mixture, which was then washed with a NaOH solution (1 M, 2×50 mL), a HCl solution (1 M, 50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4. Evaporation of the solvent gave crude **3** as a pale yellow oily residue, which was used in the following step without further purification.

Synthesis of 2-(dimethylamono)ethyl (12Z,15Z)-3-((9Z,12Z)-octadeca-9,12-dien-1-yl)henicosa-2,12,15-trienoate (YSK13-C2). Acid **3** (569 mg, 1.00 mmol) was dissolved in DCM (20 mL) and DMAP (122 mg, 1.00 mmol) and EDCI (959 mg, 5.00 mmol) were added to this mixture. After stirring for 1 h at ambient temperature, dimethylaminoethanol (503 μL, 5.00 mmol) was added and the reaction mixture was stirred at ambient temperature overnight. The reaction mixture was diluted with DCM (50 mL) and washed with a NaOH solution (1 M, 2×50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4 and the solvents were removed *in vacuo*. The crude product was chromatographed on silica gel (SiO_2, DCM/MeOH) to give 539 mg of **YSK13-C2** as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.66 (1H, s), 5.29-5.41 (8H, m), 4.17 (2H, t), 2.77 (4H, t), 2.58 (2H, t), 2.29 (6H, s), 1.97-2.13 (12H, m), 1.20-1.47 (36H, m), 0.88 (6H, t). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.62, 165.65, 130.27, 128.01, 114.86, 77.44, 77.12, 76.80, 61.25, 58.05, 45.82, 45.82, 38.52, 32.28, 31.62, 30.09, 29.78, 29.62, 29.54, 29.44, 28.77, 27.74, 27.29, 25.71, 22.67, 14.18. HRMS (*m/z*) Calcd. for $C_{43}H_{78}NO_2$ $(M+H)^+$ 640.6027; Found 640.6015.

Synthesis of 2-(dimethylamono)propyl (12Z,15Z)-3-((9Z,12Z)-octadeca-9,12-dien-1-yl)henicosa-2,12,15-trienoate (YSK13-C3). Acid **3** (3.00 g, 5.28 mmol) was dissolved in DCM (26 mL) and DMAP (323 mg, 2.64 mmol) and EDCI (2.53 g, 13.2 mmol) were added. After stirring for 1 h at ambient temperature, dimethylaminopropanol (1.54 mL, 13.2 mmol) was added and the reaction mixture was stirred at ambient temperature overnight. The reaction mixture was diluted with DCM (100 mL) and washed with a NaOH solution (1 M, 2×100 mL) and brine (100 mL). The organic phase was dried over Na_2SO_4 and the solvents were removed *in vacuo*. The crude product was chromatographed on silica gel (SiO_2, DCM/MeOH) to give 1.90 g of **YSK13-C3** as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.60 (1H, s), 5.29-5.40 (8H, m), 4.10 (2H, t), 2.76 (4H, t), 2.57 (2H, t), 2.33 (2H, t), 2.21 (6H, s), 2.12 (2H, t), 1.97-2.09 (8H, m), 1.81 (2H, m), 1.20-1.45 (36H, m), 0.88 (6H, t). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.66, 165.21, 130.27, 128.01, 114.96, 77.43, 77.11, 76.79, 61.99, 56.50, 45.60, 38.52, 32.26, 31.62, 30.09, 29.86, 29.78, 29.62, 29.55, 29.44, 28.82, 27.78, 27.29, 27.24, 25.71, 22.67, 14.18. HRMS (*m/z*) Calcd. for $C_{44}H_{80}NO_2$ $(M+H)^+$ 654.6184; Found 654.6173.

Synthesis of 2-(dimethylamono)butyl (12Z,15Z)-3-((9Z,12Z)-octadeca-9,12-dien-1-yl)henicosa-2,12,15-trienoate (YSK13-C4). Acid **3** (285 mg, 0.50 mmol) was dissolved in DCM (10 mL) and to it DMAP (61.1 mg, 0.50 mmol) and EDCI (479 mg, 2.5 mmol) were added. After stirring for 1 h at ambient temperature, dimethylaminopropanol (333 μL, 2.5 mmol) was added and the reaction mixture was stirred at ambient temperature overnight. The reaction mixture was diluted with DCM (50 mL) and washed with a NaOH solution (1 M, 2×50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4 and the solvents were removed *in vacuo*. The crude product was chromatographed on silica gel (SiO_2, DCM/MeOH) to give 228 mg of **YSK13-C4** as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.59 (1H, s), 5.27-5.40 (8H, m), 4.08 (2H, t), 2.76 (4H, t), 2.56 (2H, t), 2.28 (2H, t), 2.21 (6H, s), 2.12 (2H, t), 1.97-2.09 (8H, m), 1.65 (2H, m), 1.20-1.55 (38H, m), 0.88 (6H, t). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.73, 165.18, 130.27, 128.01, 114.99, 77.44, 77.12, 76.80, 63.45, 59.40, 63.45, 59.40, 45.54, 38.52, 31.62, 29.78, 29.61, 29.54, 29.44, 27.29, 25.71, 22.67, 14.18. HRMS (*m/z*) Calcd. for $C_{45}H_{82}NO_2$ $(M+H)^+$ 668.6340; Found 668.6321.

Synthesis of (12Z,15Z)-3-((9Z,12Z)-octadeca-9,12-dien-1-yl)henicosa-2,12,15-trien-1-ol (4).

Aluminum chloride (581 mg, 4.36 mmol) in dry ether (15 mL) was added dropwise to a stirred, ice-cooled suspension of lithium aluminium hydride (248 mg, 6.54 mmol) in anhydrous ether (15 mL). After stirring the hydride mixture at ice-bath temperature for 30 min, the *tert*-butyl ester **2** (1.36 g, 2.18 mmol) in anhydrous ether (10 mL) was added dropwise. The reaction mixture was stirred for an additional 1 h at ice-bath temperature. The reaction mixture was quenched with ice-cold water and then filtered through a celite pad. Evaporation of solvent gave the crude reduced alcohol. The crude product was chromatographed on silica gel (SiO_2, hexane/EtOAc) to give 689 mg of **4** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.27-5.41 (9H, m), 4.12 (2H, d), 2.76 (4H, t), 1.94-2.09 (12H, m), 1.20-1.45 (36H, m), 0.88 (6H, t). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 144.66, 130.28, 128.05, 123.27, 77.44, 77.12, 76.80, 59.32, 36.88, 31.63, 30.47, 29.80, 29.77, 29.61, 29.57, 29.45, 29.41, 28.99, 28.08, 27.30, 25.71, 22.67, 14.18. HRMS (*m/z*) Calcd. for $C_{39}H_{70}ONa$ $(M+Na)^+$ 577.5319; Found 577.5313.

Synthesis of (12Z,15Z)-3-((9Z,12Z)-octadeca-9,12-dien-1-yl)henicosa-2,12,15-trien-1-yl 3-(dimethylamino)propanoate (YSK15-C2). Alcohol **4** (331 mg, 0.60 mmol) was dissolved in DCM (5 mL) and DMAP (29.1 mg, 0.24 mmol), triethylamine (TEA) (249 μL, 1.79 mmol) and 3-*N*,*N*-dimethylamino propanoic acid hydrochloride (220 mg, 1.43 mmol) were added to the solution. After stirring for 5 min at ambient temperature, EDCI (343 mg, 1.79 mmol) was added and the reaction mixture was stirred at ambient temperature for 4 h. The reaction mixture was siluted with DCM (50 mL) and washed with NaOH solution (1 M, 2×50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4 and the solvents were removed *in vacuo*. The crude product was purified with fluch column chromatography (SiO_2, DCM/MeOH). This gave 376 mg of **YSK15-C2** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.28-5.41 (9H, m), 4.59 (2H, d), 2.77 (4H, t), 2.61 (2H, t), 2.47 (2H, t), 2.23 (6H, s), 1.94-2.07 (12H, m), 1.20-1.42 (36H, m), 0.88 (6H, t). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 172.63, 147.12, 130.27, 128.04, 118.04, 77.44, 77.12, 76.80, 61.35, 54.86, 45.39, 31.62, 29.77, 29.62, 29.44, 29.41, 27.33, 27.29, 25.71, 22.67, 14.18. HRMS (*m/z*) Calcd. for $C_{44}H_{80}NO_2$ $(M+H)^+$ 654.6184; Found 654.6176.

Synthesis of (12Z,15Z)-3-((9Z,12Z)-octadeca-9,12-dien-1-yl)henicosa-2,12,15-trien-1-yl 3-(dimethylamino)butanoate (YSK15-C3). Alcohol **4** (277.5 mg, 0.50 mmol) was dissolved in DCM (4 mL) and DMAP (24.4 mg, 0.20 mmol), TEA (209 μL, 1.50 mmol)

and 4-(dimethylamino)butyric acid hydrochloride (201 mg, 1.20 mmol) were then added. After stirring for 5 min at ambient temperature, EDCI (288 mg, 1.50 mmol) was added and the reaction mixture was stirred at ambient temperature overnight. The reaction mixture was diluted with DCM (50 mL) and washed with a NaOH solution (1 M, 2×50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4 and solvents were removed *in vacuo*. The crude product was chromatographed on silica gel (SiO_2, DCM/MeOH) to give 305 mg of **YSK15-C3** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.28-5.41 (9H, m), 4.58 (2H, d), 2.76 (4H, t), 2.32 (2H, t), 2.28 (2H, t), 2.20 (6H, s), 1.95-2.08 (12H, m), 1.78 (2H, m), 1.20-1.40 (36H, m), 0.88 (6H, t). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.72, 146.96, 130.27, 128.04, 118.15, 77.43, 77.11, 76.79, 61.21, 58.95, 45.49, 36.9, 32.35, 31.62, 30.68, 29.77, 29.62, 29.54, 29.44, 29.41, 28.87, 27.95, 27.33, 27.29, 25.71, 23.09, 22.67, 14.18. HRMS (*m/z*) Calcd. for $C_{45}H_{82}NO_2$ $(M+H)^+$ 668.6340; Found 668.6332.

Synthesis of (12Z,15Z)-3-((9Z,12Z)-octadeca-9,12-dien-1-yl)henicosa-2,12,15-trien-1-yl 3-(dimethylamino)pentanoate (YSK15-C4). Alcohol **4** (201 mg, 0.36 mmol) was dissolved in DCM (4 mL) and to it DMAP (17.7 mg, 0.15 mmol), TEA (252 μL, 1.81 mmol) and 5-(dimethylamino)pentanoic acid (90 mg, 0.63 mmol) were added. After stirring for 5 min at ambient temperature, EDCI (347 mg, 1.81 mmol) was added and the reaction mixture was stirred at ambient temperature overnight. The reaction mixture was siluted with DCM (50 mL) and washed with a NaOH solution (1 M, 2×50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4 and the solvents were removed *in vacuo*. The crude product was chromatographed on silica gel (SiO_2, DCM/MeOH) to give 211 mg of **YSK15-C4** as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.28-5.41 (9H, m), 4.58 (2H, d), 2.76 (4H, t), 2.32 (2H, t), 2.28 (2H, t), 2.20 (6H, s), 1.95-2.10 (12H, m), 1.64 (2H, m), 1.49 (2H, m), 1.20-1.40 (36H, m), 0.88 (6H, t). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.70, 146.99, 130.27, 128.04, 118.13, 77.43, 77.11, 76.79, 61.17, 59.38, 45.50, 36.91, 34.31, 31.62, 30.68, 29.77, 29.61, 29.44, 27.40, 28.87, 27.95, 27.32, 27.29, 27.20, 25.71, 22.94, 22.67, 14.18. HRMS (*m/z*) Calcd. for $C_{46}H_{84}NO_2$ $(M+H)^+$ 682.6497; Found 682.6488.

Synthesis of (12Z,15Z)-3-((9Z,12Z)-octadeca-9,12-dien-1-yl)henicosa-2,12,15-trien-1-yl 3-(dimethylamino)hexanoate (YSK15-C5). Alcohol **4** (201 mg, 0.36 mmol) was dissolved in DCM (8 mL) and to it DMAP (17.7 mg, 0.15 mmol), TEA (252 μL, 1.81

mmol) and 6-(dimethylamino)hexanoic acid (94 mg, 0.66 mmol) were added. After stirring for 5 min at ambient temperature, EDCI (347 mg, 1.81 mmol) was added and the reaction mixture was stirred at ambient temperature overnight. The reaction mixture was diluted with DCM (50 mL) and washed with a NaOH solution (1 M, 2×50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4 and the solvents were removed *in vacuo*. The crude product was chromatographed on silica gel (SiO_2, DCM/MeOH) to give 173 mg of **YSK15-C5** as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.28-5.41 (9H, m), 4.58 (2H, d), 2.35-2.57 (6H, br), 2.76 (4H, t), 2.31 (2H, t), 1.93-2.09 (12H, m), 1.63 (4H, m), 1.20-1.40 (40H, m), 0.88 (6H, t). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.83, 147.00, 130.27, 128.04, 118.14, 77.44, 77.12, 76.80, 61.15, 59.61, 45.42, 36.91, 34.38, 31.62, 30.68, 29.77, 29.61, 29.55, 29.44, 29.40, 28.86, 27.95, 27.32, 27.29, 27.07, 25.71, 25.00, 22.67, 14.17. HRMS (*m/z*) Calcd. for $C_{47}H_{87}NO_2$ $(M+H)^+$ 696.6653; Found 696.6646.

Table S1. Physiological parameters of MENDs.

lipid	size (nm)	polydispersity	ζ-potential (mV)	siRNA encapsulation (%)	apparent pKa	% charged lipid at pH7.4
YSK05	73.3±2.7	0.07±0.03	6.0±3.1	95.1±0.5	6.40	9.1
YSK13-C2	75.2±2.4	0.05±0.02	-2.6±1.9	89.9±2.2	5.70	2.0
YSK13-C3	74.3±3.3	0.08±0.02	2.5±1.6	93.9±1.1	6.45	10.1
YSK13-C4	73.8±1.4	0.08±0.02	2.2±2.6	93.5±1.4	6.80	20.1
YSK15-C2	75.4±5.4	0.10±0.03	-3.5±1.9	93.0±1.3	5.80	2.5
YSK15-C3	75.5±2.2	0.08±0.03	-0.2±1.6	94.2±0.9	6.65	15.1
YSK15-C4	76.5±5.2	0.11±0.01	6.4±1.2	95.5	7.10	33.4
YSK15-C5	80.0	0.09	-5.3	95.7	7.25	41.5

Data are represented as the mean±SD (n = 3) or the mean (n = 2).

Table S2. List of siRNA sequences used in this study.

siRNA	sense strand (5‘ - 3‘)	antisense strand (5‘ - 3‘)
siFVII	GGAucAucucAAGucuuACT*T	GuAAGAcuuGAGAuG AuccT*T
siPLK1	AGAuCACCCuCCUUAAAuAUU	UAUUUAAGGAGGGUGAuCUUU
siGL4	CCGUCGUCUUCGUGAGCAATT	UUGCUCACGAAUACGACGGTT
siCD31-1	GCACAGUGAUGCUGAACAATT	UUGUUCAGCAUCACUGUGCTT
siCD31-2	GUGCAUAGUUCAAGUGACATT	UGUCACUUGAACUAUGCACTT
siCD31-3	GCAAGAAGCAGGAAGGACATT	UGUCCUUCCUGCUUCUUGCTT
Cy5-siGL3	Cy5-GCGCUGCUGGUGCCAACCCTT	GGGUUGGCACCAGCAGCGCTT

2’-OMe modified nucleotides are in lower case letters, 2’-fluoro modified nucleotides are in bold lower case letters, and phosphorothioate linkages are represented by asterisks.

Table S3. List of PCR primers used in this study.

Gene	forward (5‘ - 3‘)	reverse (5‘ - 3‘)
Gapdh	AGCAAGGACACTGAGCAAG	TAGGCCCCTCCTGTTATTATG
Actb	AGAGGGAAATCGTGCGTGAC	CAATAGTGATGACCTGGCCGT
Cd31	TACAGTGGACACTACACCTG	GACTGGAGGAGAACTCTAAC
VE-cad	GCCAGTAAACCCAAAGTTC	TAAACTGCCCATACTTGACC

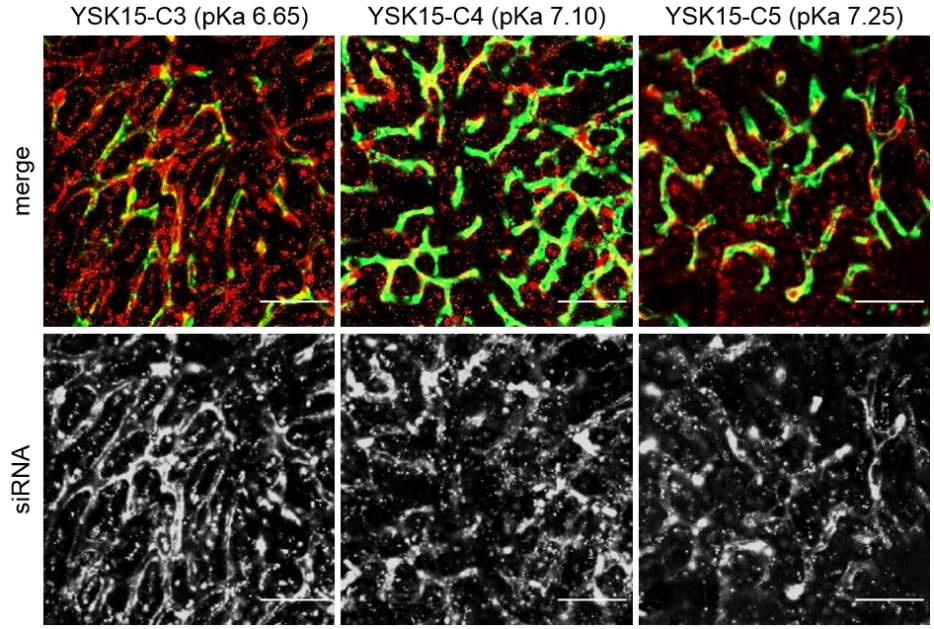


Figure S1. Intrahepatic localization of siRNAs formulated in 3 kinds of YSK15-MENDs (pKa 6.65 - 7.25). Mice were intravenously injected with MENDs encapsulating Cy5-labeled siRNAs. Liver tissues were collected, and blood vessels were stained with FITC-conjugated Isolectin B4. Blood vessels and siRNAs are visualized as green and red, respectively. Bars represent 50 μm.

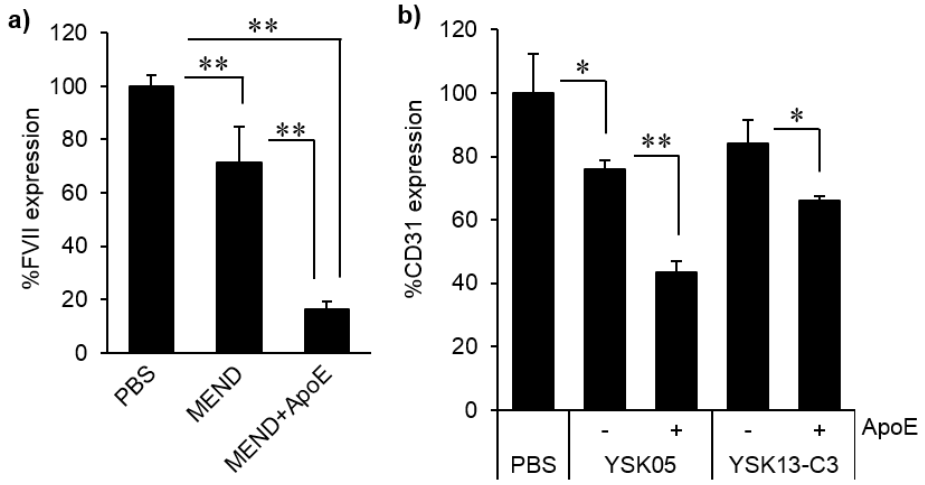


Figure S2. ApoE-dependency of gene silenicing in hepatocytes and LSECs. (a) ApoE-deficient mice were injected with YSK13-C3-MEND at 0.1 mg kg^{-1}. (b) ApoE-deficient mice were injected with YSK05-MEND or YSK13-C3-MEND at 0.3 mg kg^{-1}. ApoE was added to the MEND solution prior to injection and the dose of ApoE was fixed at 0.1 mg kg^{-1}. The data are represented as the mean ± SD (n = 3). *P < 0.05, **P < 0.01 (by one-way nrANOVA, followed by SNK test).

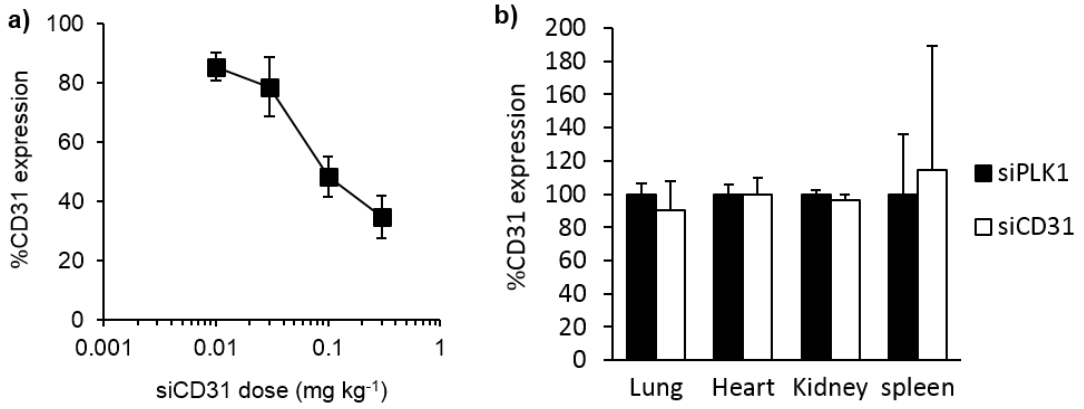


Figure S3. Efficient and liver specific CD31 gene silencing of YSK15-C4-MEND. (a) Dose-dependency of hepatic CD31 gene silencing. (b) CD31 expression in lung, heart, kidneys and spleen after the injection of YSK15-C4-MEND at 0.3 mg kg^{-1}. siRNA against polo-like kinase 1 (siPLK1) was used as a control siRNA. The data are represented as the mean ± SD (n = 3).

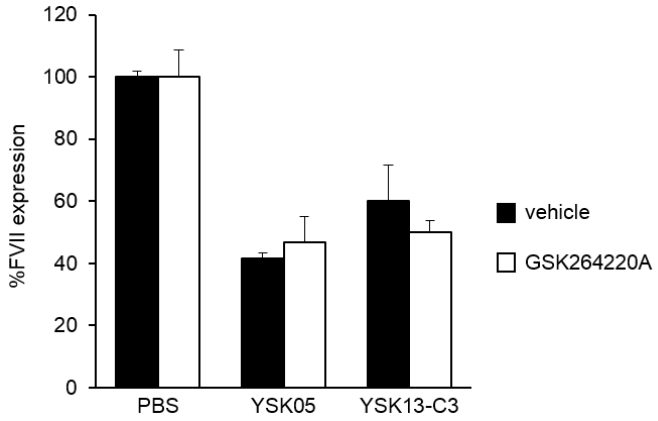
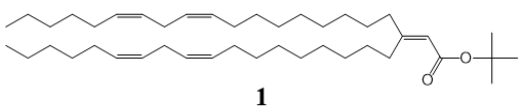


Figure S4. Effect of GSK264220A on FVII gene silencing activity in liver. Mice were intraperitoneally injected with GSK264220A (30 mg kg^{-1}) or vehicle and intravenously injected with siFVII formulated in the YSK05-MEND at 0.1 mg kg^{-1} or the YSK13-C3-MEND at 0.01 mg kg^{-1}. Plasma FVII activity was measured in 24 hours. Data are represented as the mean±SD (n = 3).

References

1. Jayaraman, M.; Ansell, S. M.; Mui, B. L.; Tam, Y. K.; Chen, J.; Du, X.; Butler, D.; Eltepu, L.; Matsuda, S.; Narayanannair, J. K.; Rajeev, K. G.; Hafez, I. M.; Akinc, A.; Maier, M. A.; Tracy, M. A.; Cullis, P. R.; Madden, T. D.; Manoharan, M.; Hope, M. J., Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew Chem Int Ed Engl* **2012,** *51* (34), 8529-33.



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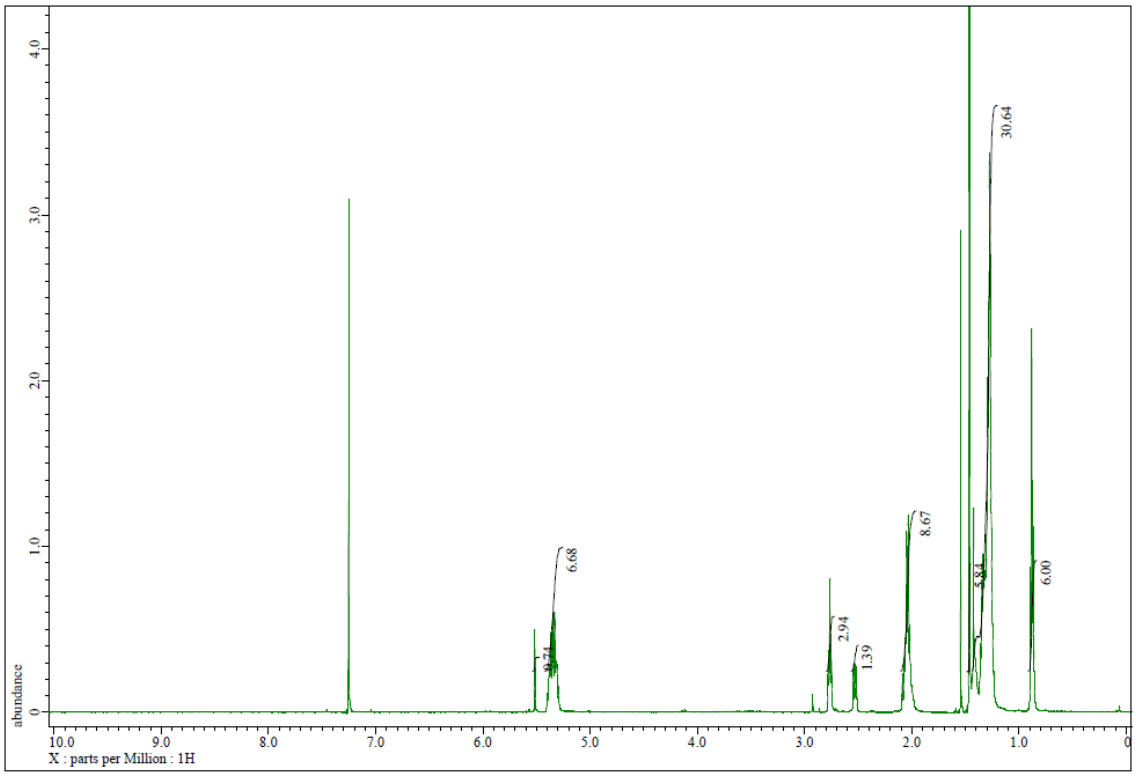
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2.0
1.0
0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
X : parts per Million : 1H
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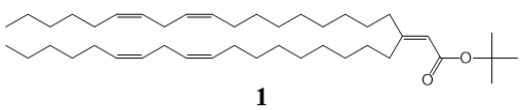
2.94
1.39
8.67
30.64
6.00



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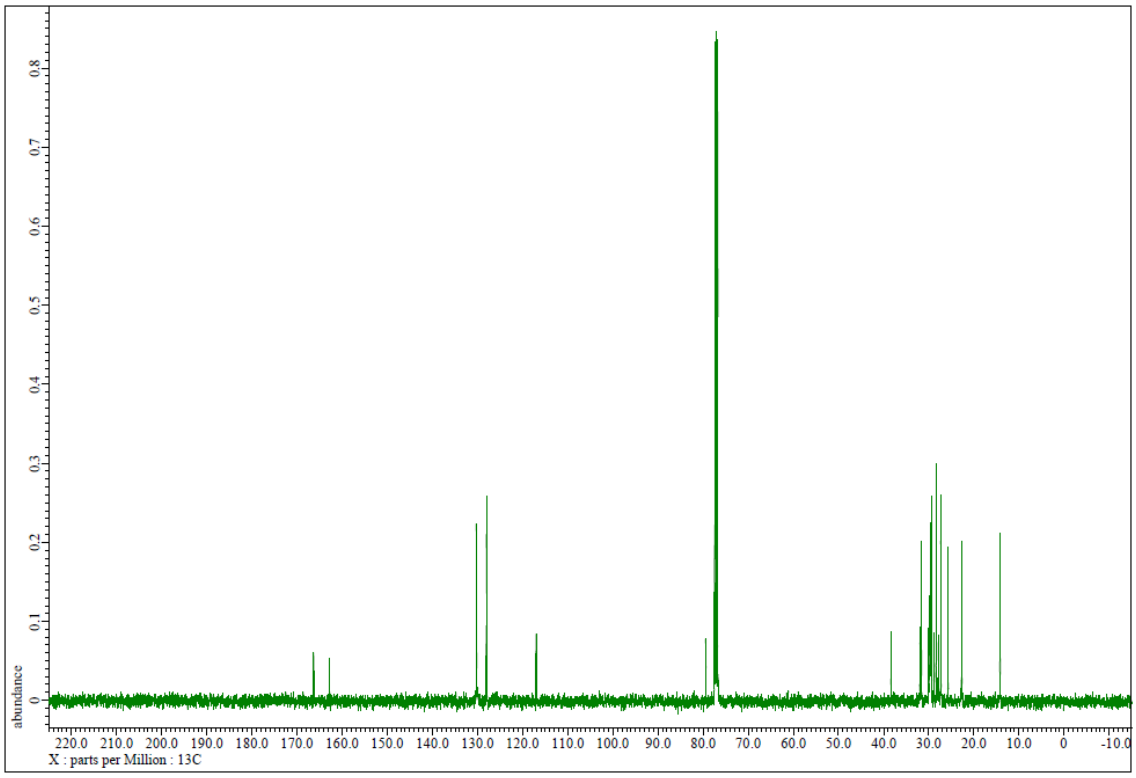
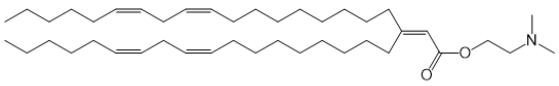

abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0
X : parts per Million : 13C



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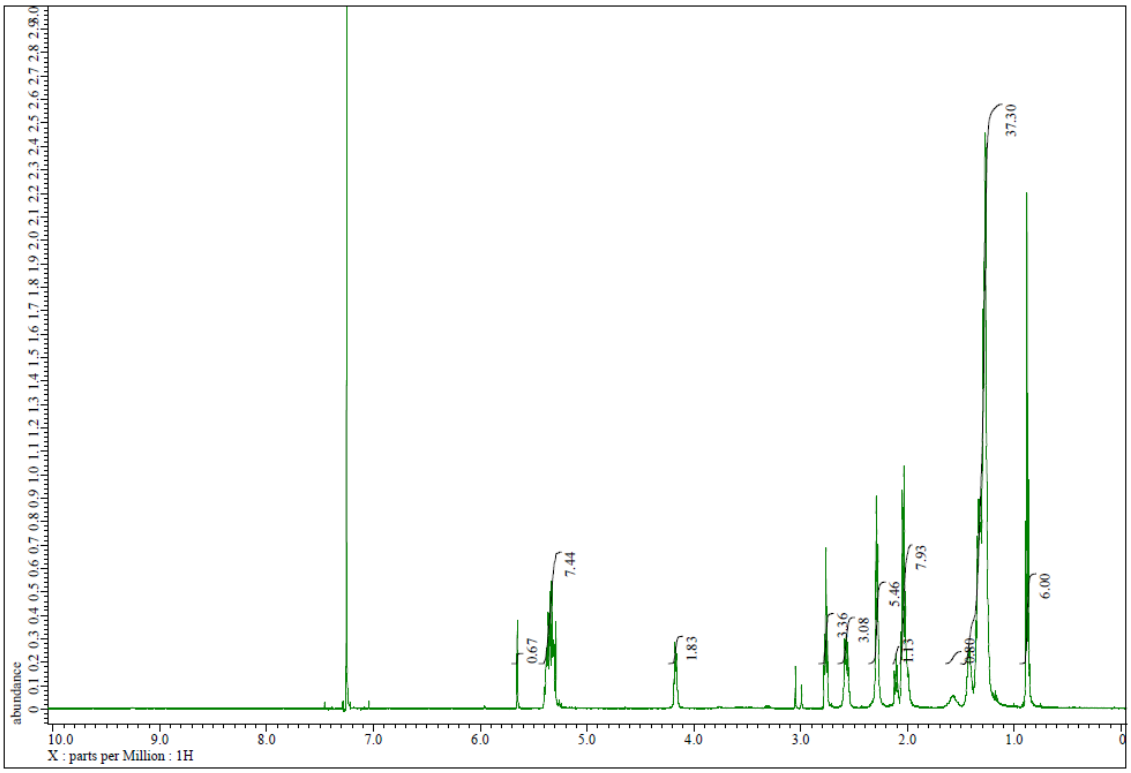
abundance
X : parts per Million : 1H
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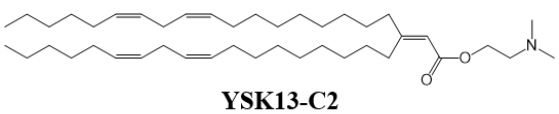
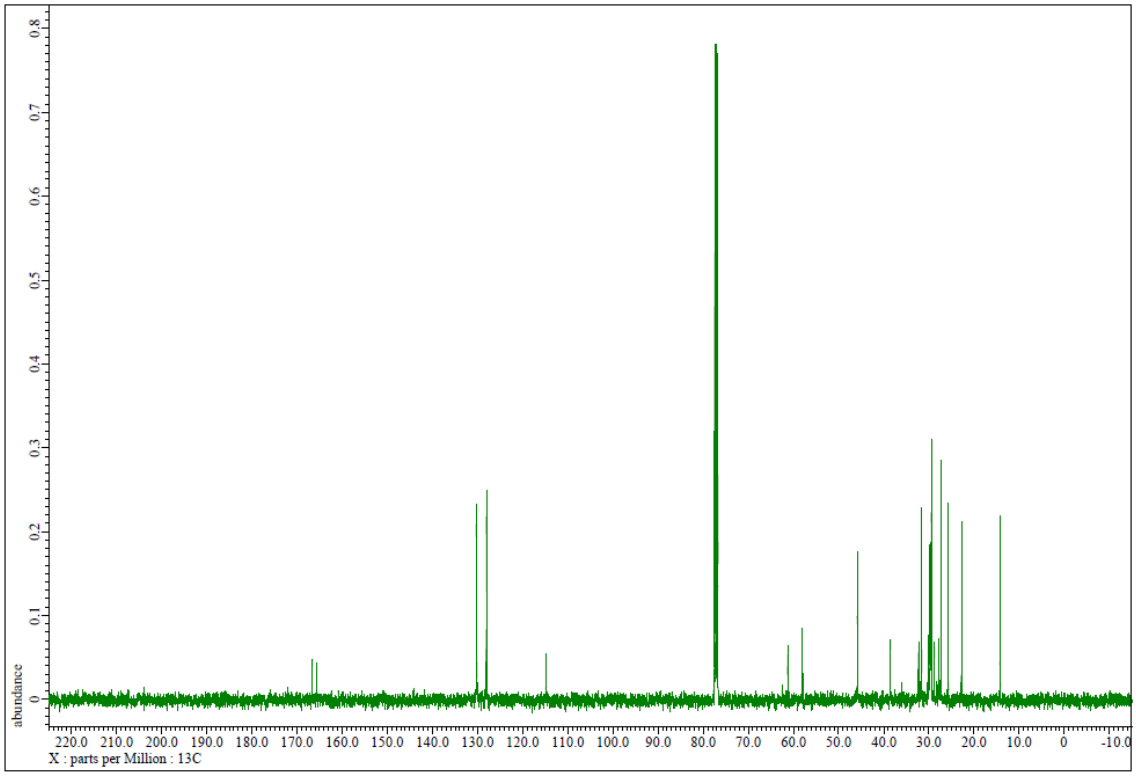
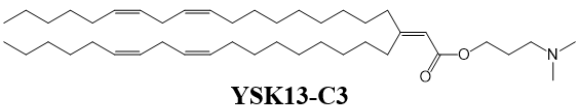
7.44
1.83
3.36
3.08
5.46
7.93
37.30
6.00

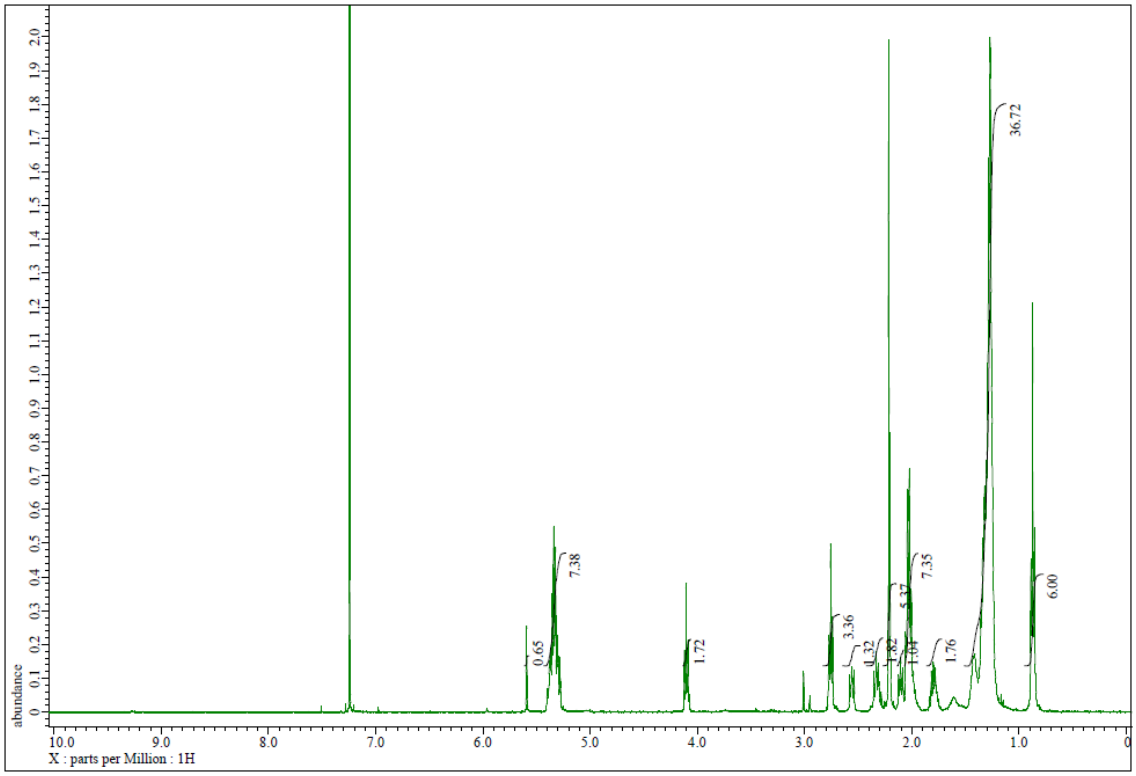

O
O
N

YSK13-C2


abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0
X : parts per Million : 13C



YSK13-C3


abundance
X : parts per Million : 1H
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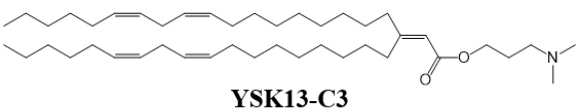
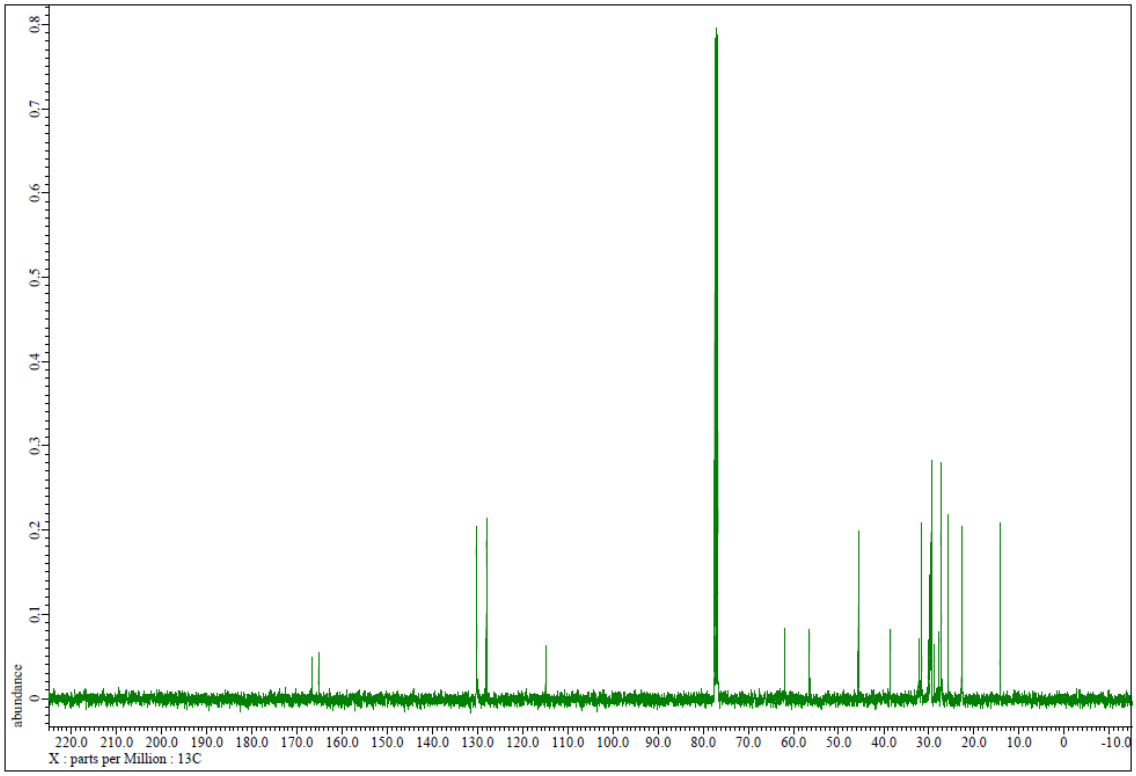
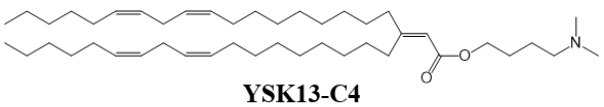
7.38
1.72
3.36
1.32
5.37
7.35
1.76
36.72
6.00


O
O
N

YSK13-C3


abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0
X : parts per Million : 13C



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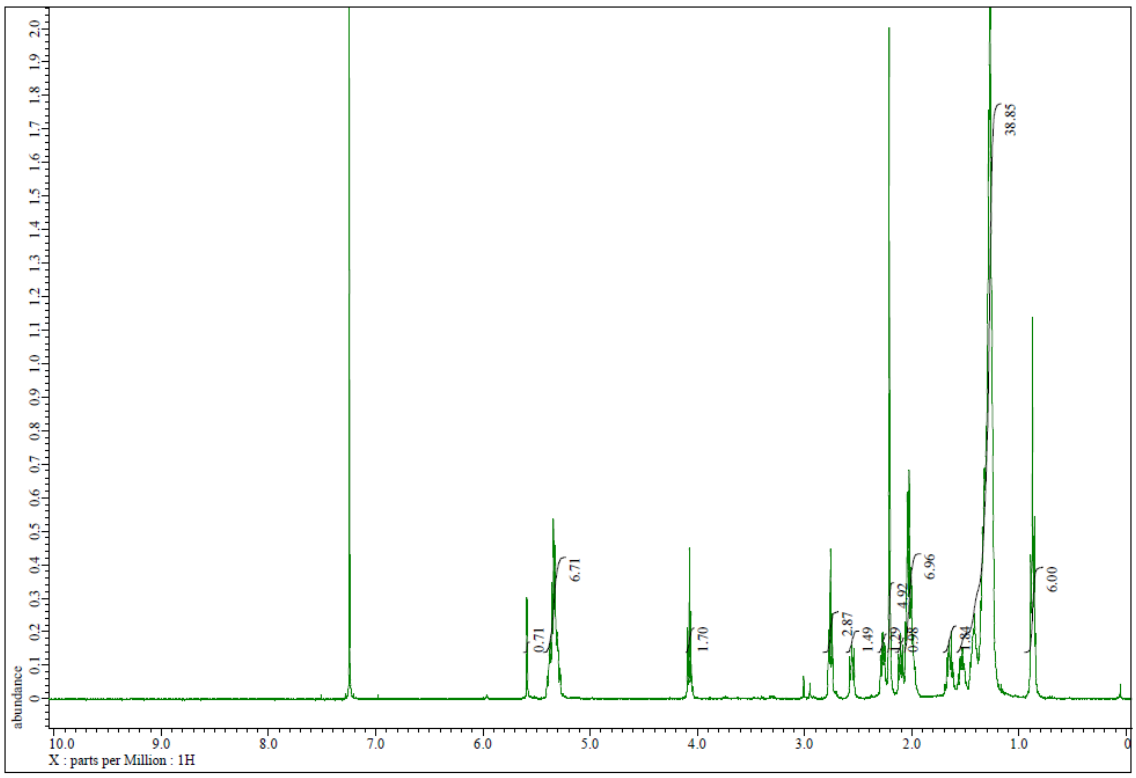
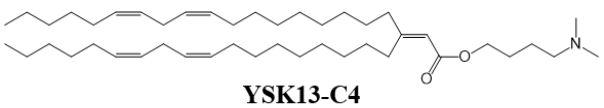
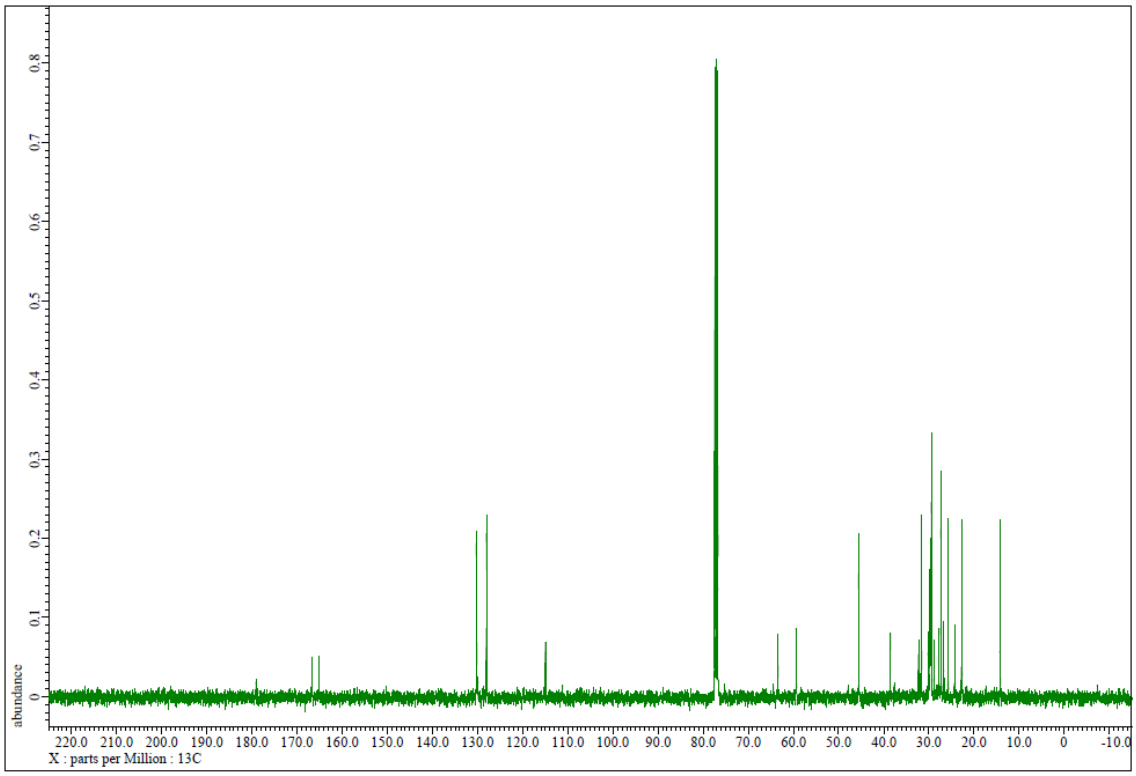
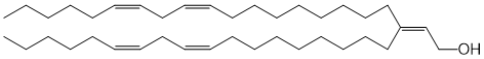

abundance
X : parts per Million : 1H
0.71
6.71
1.70
2.87
1.49
4.92
6.96
38.85
6.00

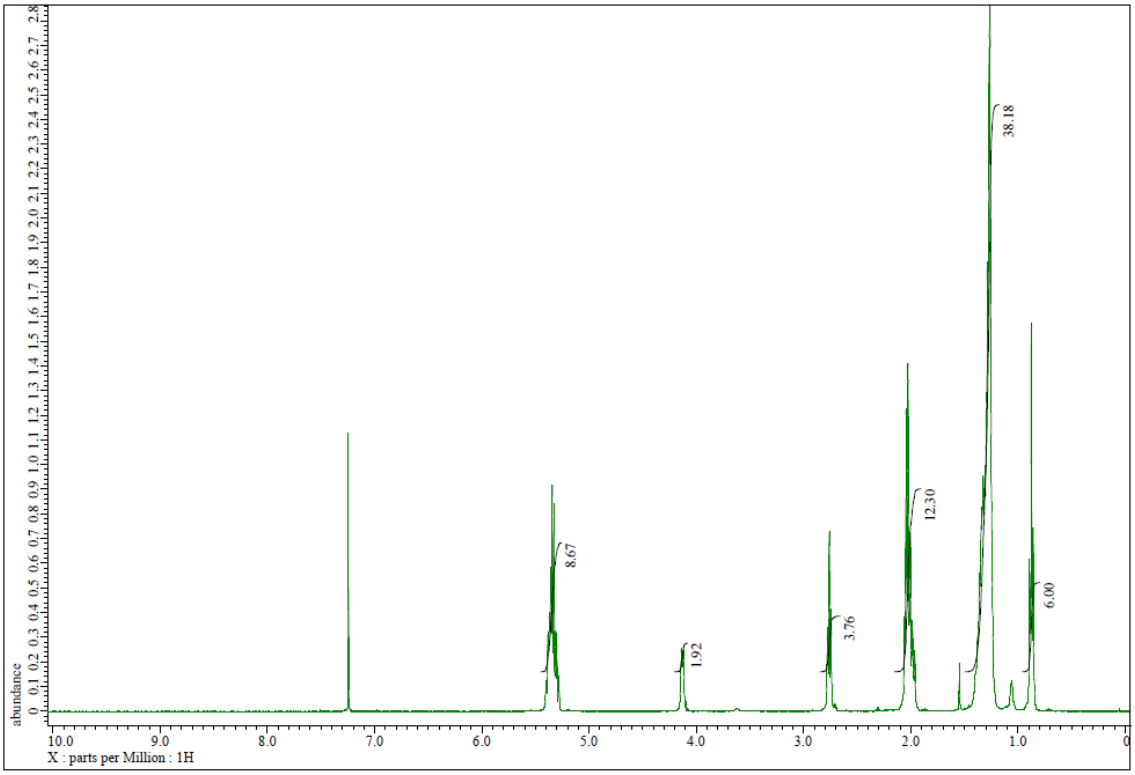

O
O
N

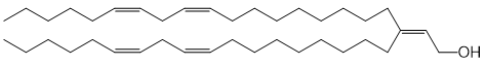
YSK13-C4


abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0
X : parts per Million : 13C

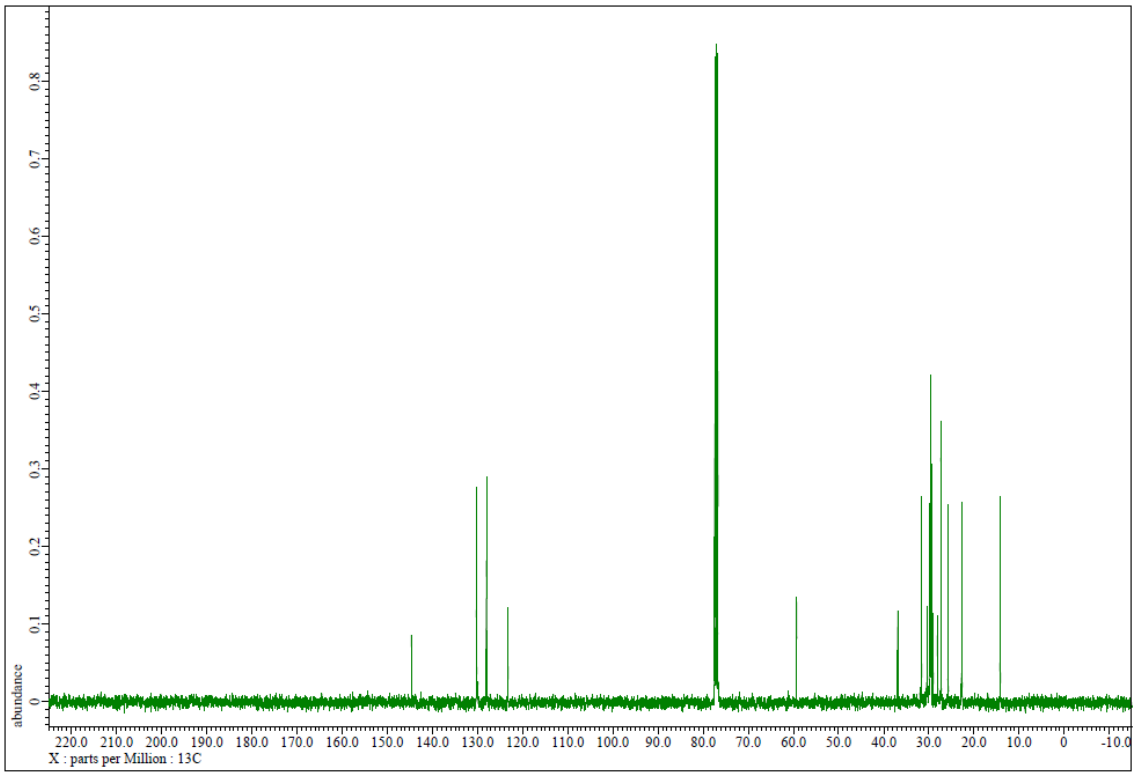


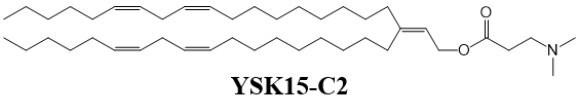
4




OH

4


abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0
X : parts per Million : 13C



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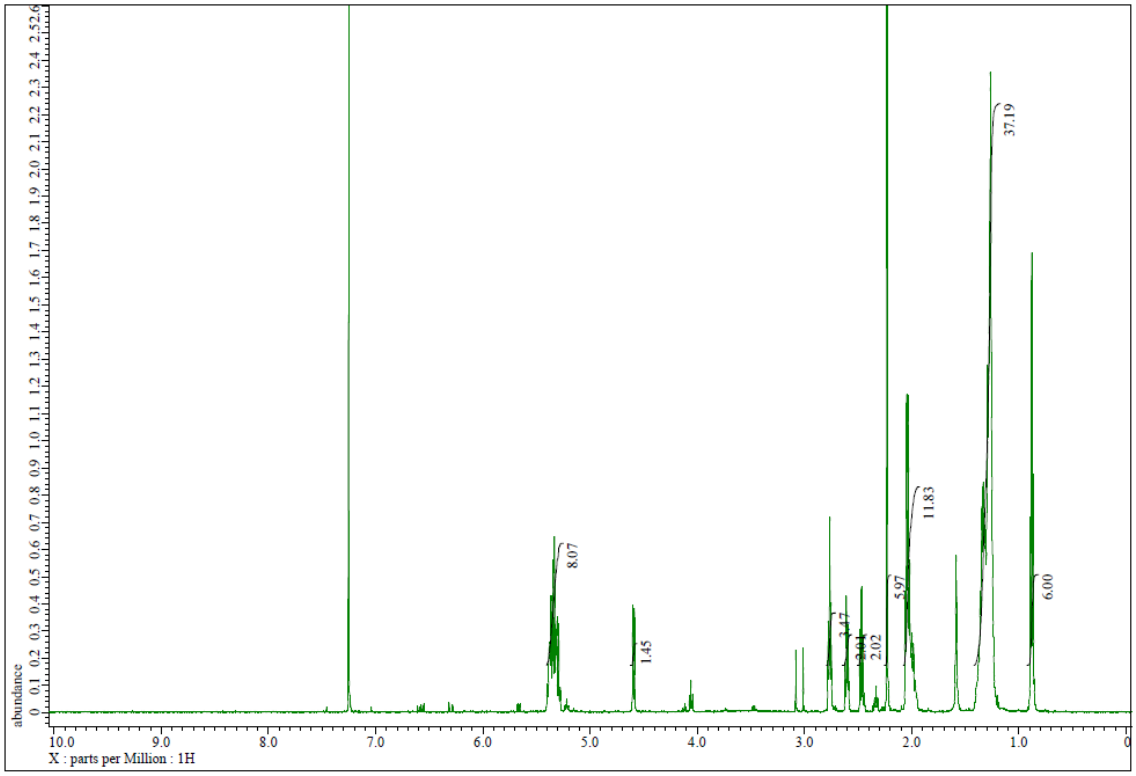
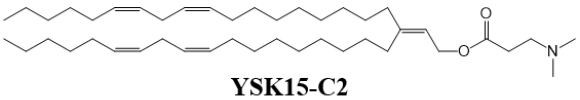
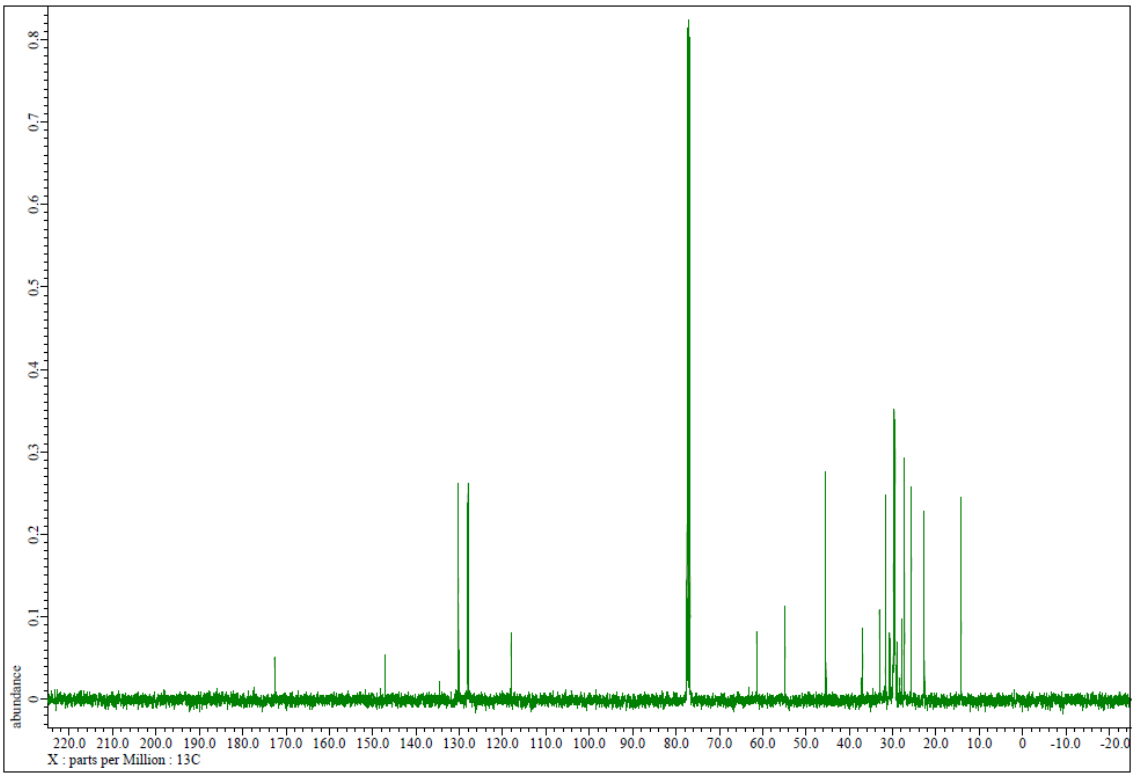
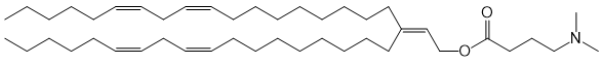

abundance
X : parts per Million : 1H
8.07
1.45
3.47
2.02
5.97
11.83
37.19
6.00


O
O
N

YSK15-C2


abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0 -20.0
X : parts per Million : 13C



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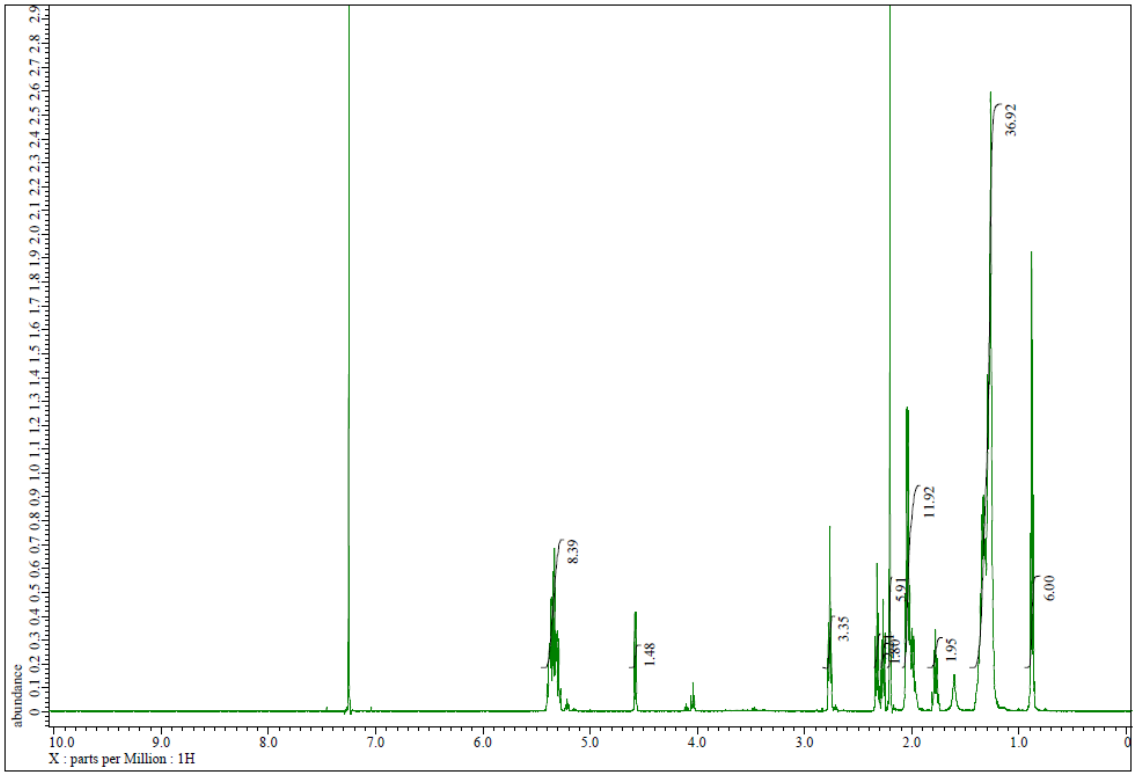
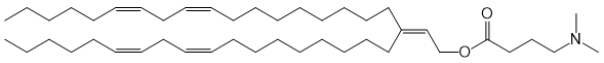
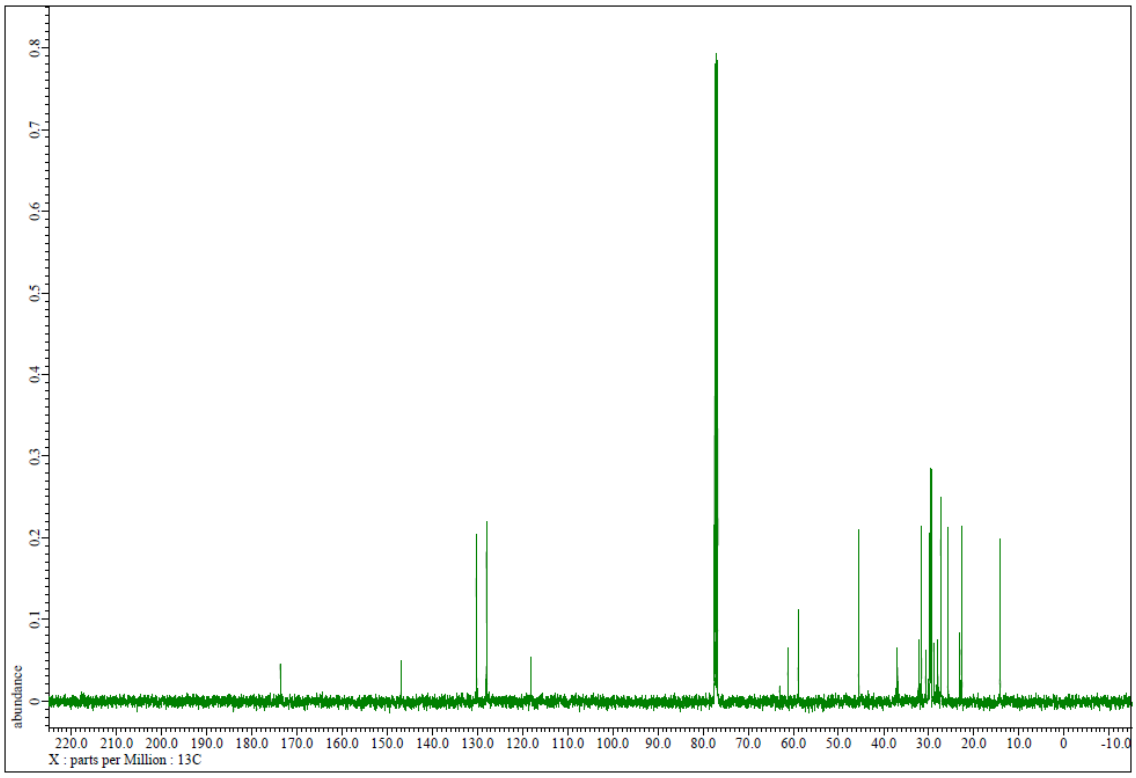
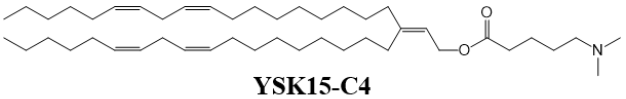

abundance
X : parts per Million : 1H
8.39
1.48
3.35
5.91
11.92
1.95
36.92
6.00

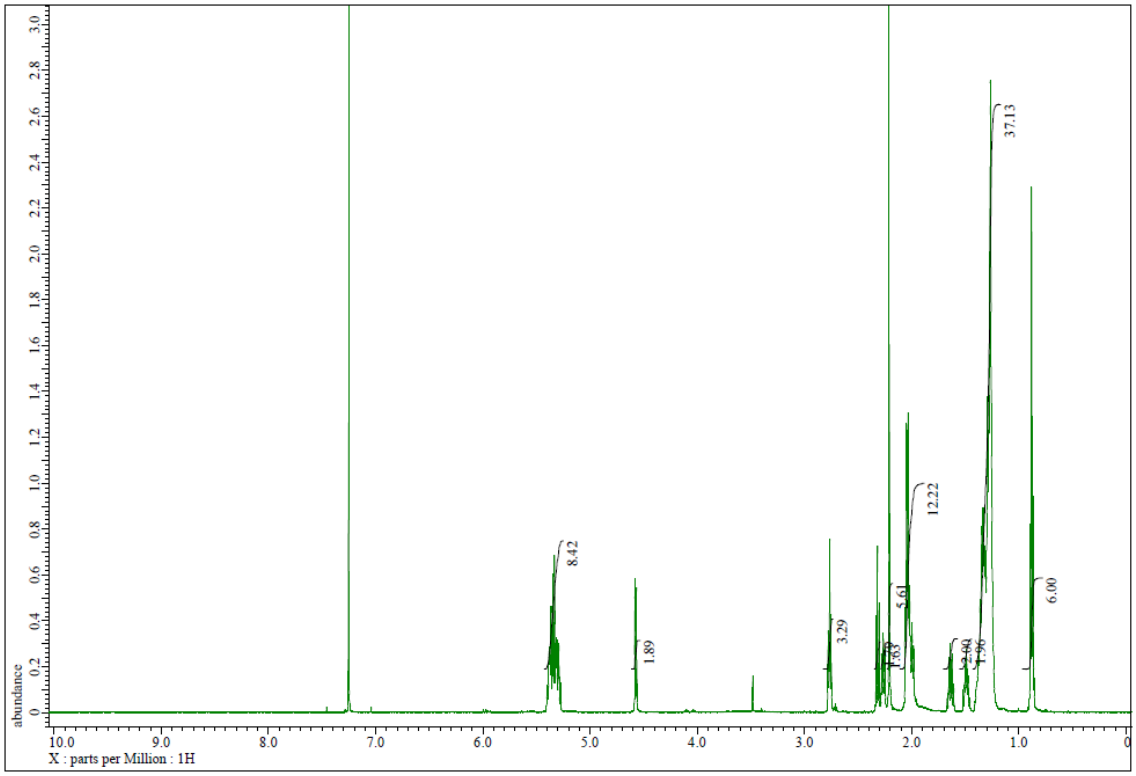

O
O
N

YSK15-C3


abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0
X : parts per Million : 13C



YSK15-C4


abundance
X : parts per Million : 1H
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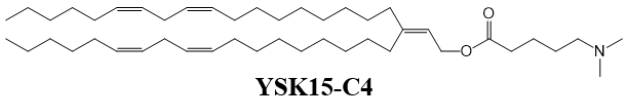
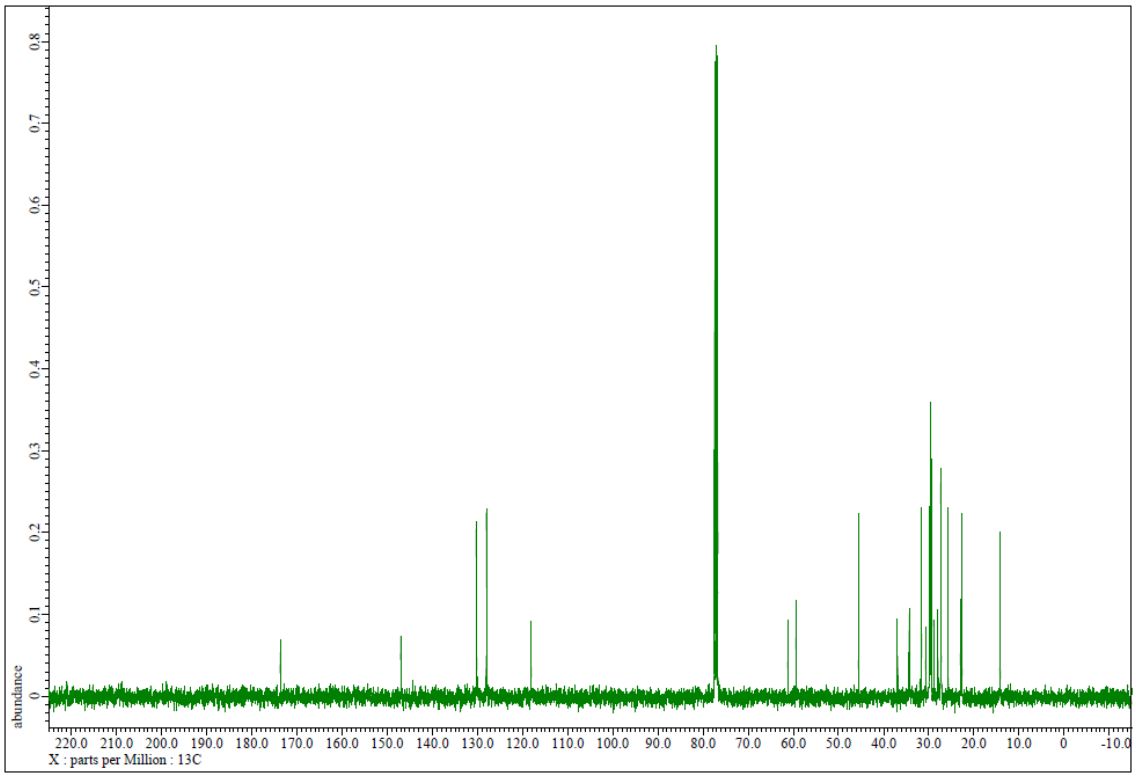
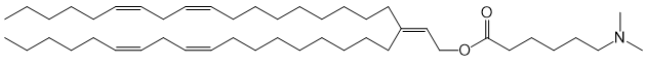
1.89
3.29
1.63
5.61
12.22
2.68
1.96
37.13
6.00

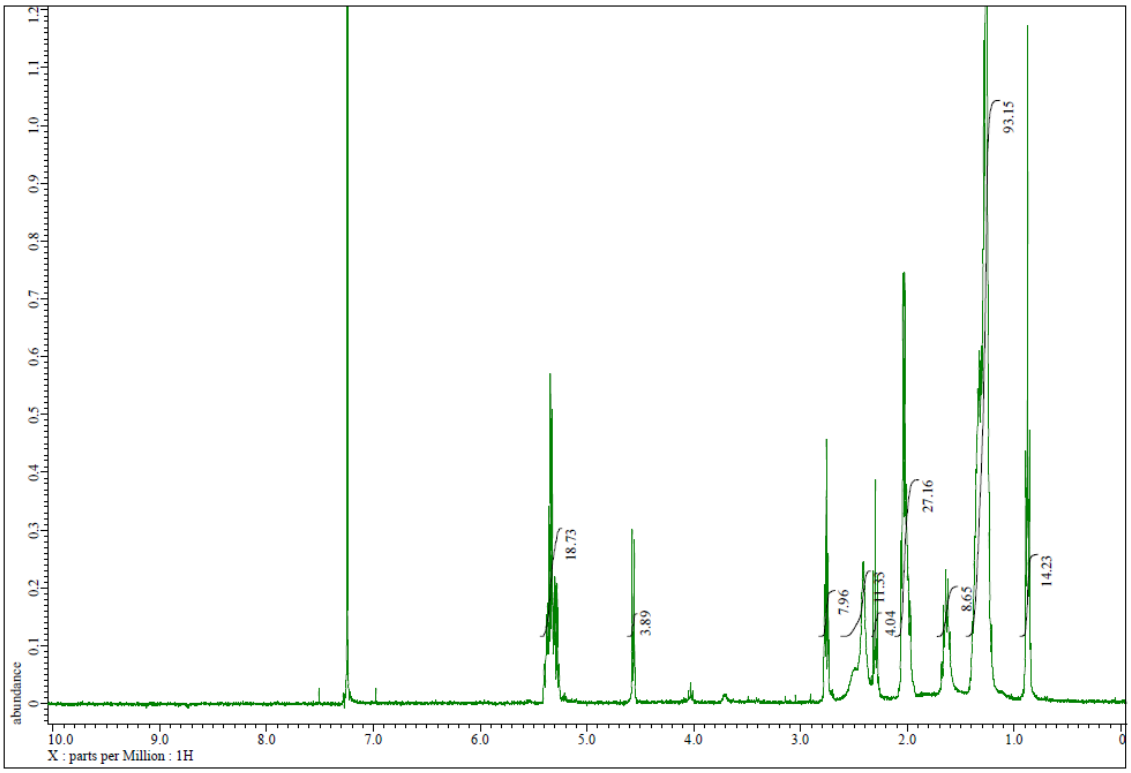

O
O
N

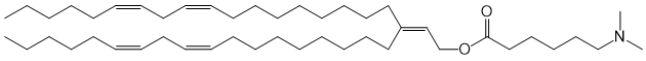
YSK15-C4


abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0
X : parts per Million : 13C

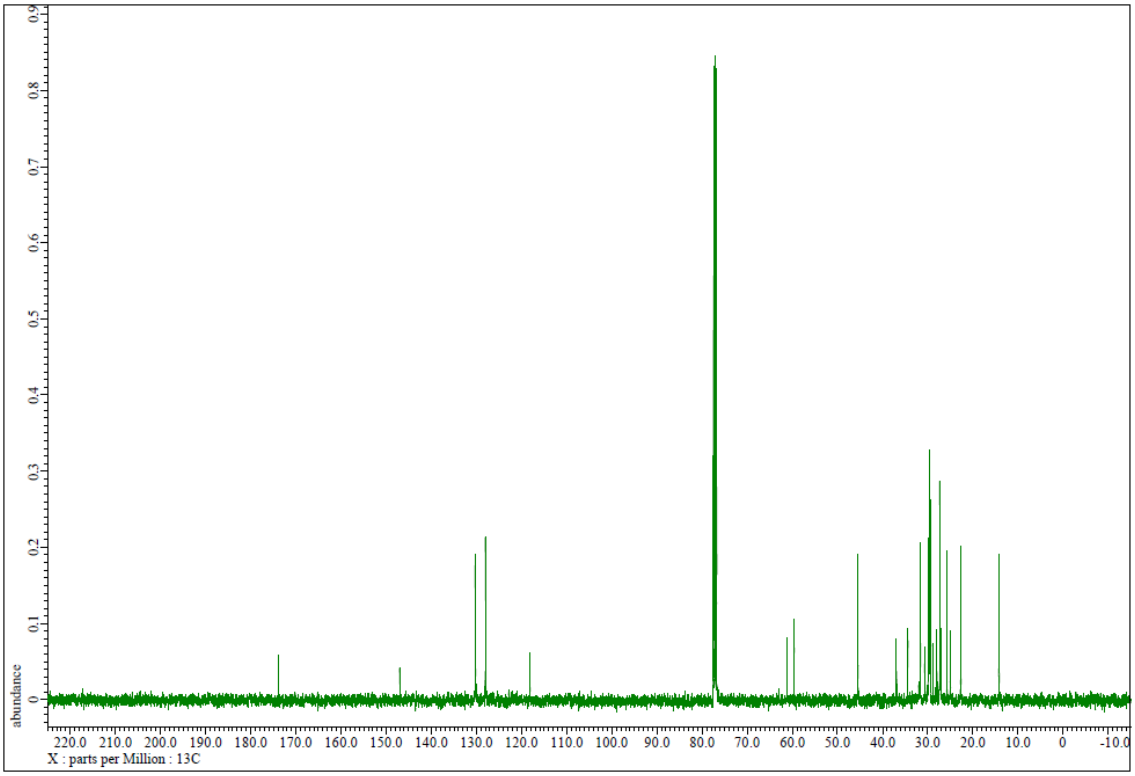


YSK15-C5


abundance
X : parts per Million : 1H
18.73
3.89
7.96
11.35
4.04
27.16
8.65
93.15
14.23


O
O
N

YSK15-C5


abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0
X : parts per Million : 13C