

Supplementary Materials: Development of a Library of Disulfide Bond-containing Cationic Lipids for mRNA Delivery

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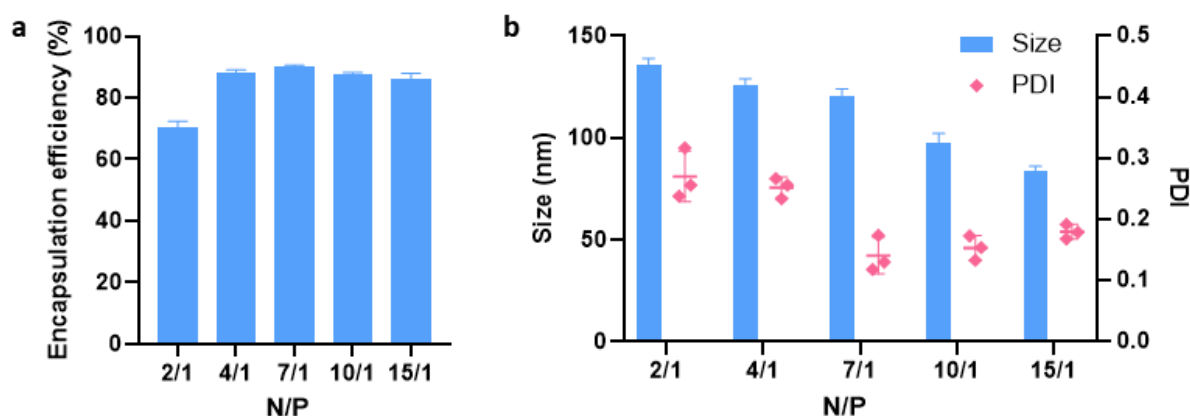
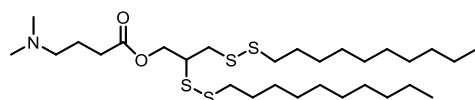


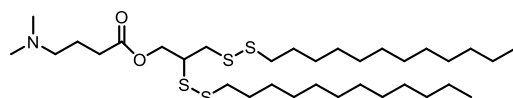
Figure S1. Characterization of LNP with different N/P ratio using C4S18A formulation. **a)** encapsulation efficiency of LNP with different N/P ratio. **b)** the variance of particle size and PDI over N/P ratio. Data presented as mean \pm SEM of three separate experiments.

Characterization of disulfide lipids



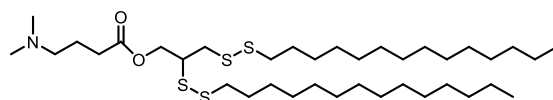
2,3-bis(decyldisulfanyl)propyl 4-(dimethylamino)butanoate (C4S10)

Synthesized by using the general procedure. ^1H NMR (500 MHz, CDCl_3) δ = 4.39 – 4.33 (m, 2H), 3.30 – 3.25 (m, 1H), 3.05 (dd, J = 13.8, 6.2 Hz, 1H), 2.92 (dd, J = 13.8, 8.1 Hz, 1H), 2.72 – 2.68 (m, 4H), 2.38 (t, J = 7.5 Hz, 2H), 2.30 (t, J = 7.1 Hz, 2H), 2.22 (s, 6H), 1.84 – 1.77 (m, 2H), 1.71 – 1.63 (m, 4H), 1.41 – 1.35 (m, 4H), 1.32 – 1.24 (m, 24H), 0.88 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ = 173.30, 64.14, 58.86, 49.26, 45.49, 40.19, 39.96, 39.03, 32.06, 32.04, 29.70, 29.67, 29.66, 29.46, 29.38, 29.38, 29.34, 29.31, 28.66, 28.64, 22.94, 22.82, 14.27. **HR-MS** (ESI) m/z calcd for $\text{C}_{29}\text{H}_{60}\text{O}_2\text{NS}_4$ $[\text{M}+\text{H}]^+$ 582.3501, found 582.3503.



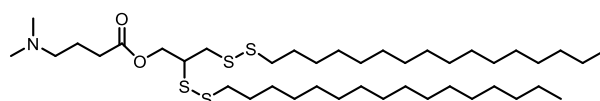
2,3-bis(dodecyldisulfanyl)propyl 4-(dimethylamino)butanoate (C4S12)

Synthesized by using the general procedure. ^1H NMR (400 MHz, CDCl_3) δ = 4.36 (d, J = 5.7 Hz, 2H), 3.28 (dq, J = 8.1, 5.8 Hz, 1H), 3.08 – 3.02 (m, 1H), 2.91 (dd, J = 13.8, 8.2 Hz, 1H), 2.74 – 2.67 (m, 4H), 2.41 (dd, J = 15.5, 8.1 Hz, 4H), 2.31 (s, 6H), 1.91 – 1.82 (m, 2H), 1.71 – 1.62 (m, 4H), 1.41 – 1.34 (m, 4H), 1.31 – 1.24 (m, 32H), 0.87 (t, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ = 173.07, 64.20, 58.58, 49.22, 45.08, 40.15, 39.98, 39.03, 32.06, 31.86, 29.81, 29.79, 29.76, 29.68, 29.67, 29.50, 29.39, 29.35, 29.32, 28.66, 28.64, 22.84, 22.41, 14.28. **HR-MS** (ESI) m/z calcd for $\text{C}_{33}\text{H}_{68}\text{O}_2\text{NS}_4$ $[\text{M}+\text{H}]^+$ 638.4127, found 638.4132.



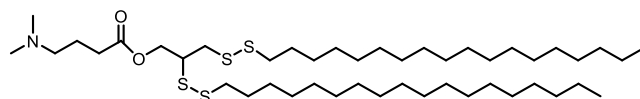
2,3-bis(tetradecyldisulfanyl)propyl 4-(dimethylamino)butanoate (C4S14)

Synthesized by using the general procedure. ^1H NMR (400 MHz, CDCl_3) δ = 4.39 – 4.33 (m, 2H), 3.28 (dq, J = 8.0, 5.8 Hz, 1H), 3.05 (dd, J = 13.7, 6.2 Hz, 1H), 2.95 – 2.88 (m, 1H), 2.70 (td, J = 7.7, 2.3 Hz, 4H), 2.38 (t, J = 7.5 Hz, 2H), 2.30 (d, J = 7.5 Hz, 2H), 2.23 (s, 6H), 1.85 – 1.76 (m, 2H), 1.67 (dq, J = 14.4, 7.1 Hz, 4H), 1.43 – 1.34 (m, 4H), 1.31 – 1.22 (m, 40H), 0.88 (t, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ = 173.30, 64.15, 58.85, 49.26, 45.46, 40.19, 39.97, 39.04, 32.07, 29.85, 29.84, 29.81, 29.77, 29.68, 29.68, 29.52, 29.40, 29.39, 29.35, 29.32, 28.67, 28.65, 22.91, 22.85, 14.28. **HR-MS** (ESI) m/z calcd for $\text{C}_{37}\text{H}_{76}\text{O}_2\text{NS}_4$ $[\text{M}+\text{H}]^+$ 694.4759, found 694.4763.



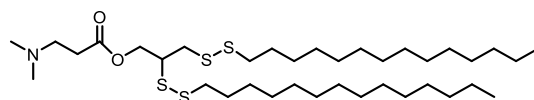
2,3-bis(hexadecyldisulfanyl)propyl 4-(dimethylamino)butanoate (C4S16)

Synthesized by using the general procedure. ^1H NMR (400 MHz, CDCl_3) δ = 4.35 (dd, J = 9.2, 3.8 Hz, 2H), 3.27 (dq, J = 8.0, 5.8 Hz, 1H), 3.03 (dd, J = 12.4, 6.2 Hz, 1H), 2.91 (dd, J = 13.7, 8.1 Hz, 1H), 2.70 (td, J = 7.8, 2.1 Hz, 4H), 2.42 – 2.32 (m, 4H), 2.26 (s, 6H), 1.82 – 1.77 (m, 2H), 1.71 – 1.62 (m, 4H), 1.39 – 1.22 (m, 52H), 0.87 (t, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ = 173.19, 64.16, 58.75, 49.24, 45.32, 40.17, 39.96, 39.03, 32.07, 31.97, 29.84, 29.81, 29.76, 29.68, 29.67, 29.51, 29.39, 29.39, 29.34, 29.31, 28.66, 28.64, 22.84, 22.72, 14.27. **HR-MS** (ESI) m/z calcd for $\text{C}_{41}\text{H}_{84}\text{O}_2\text{NS}_4$ $[\text{M}+\text{H}]^+$ 750.5379, found 750.5388.



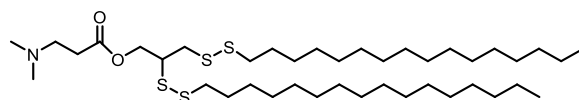
2,3-bis(octadecyldisulfanyl)propyl 4-(dimethylamino)butanoate (C4S18)

Synthesized by using the general procedure. ^1H NMR (400 MHz, CDCl_3) δ = 4.35 (dd, J = 5.6, 1.2 Hz, 2H), 3.27 (dq, J = 8.0, 5.8 Hz, 1H), 3.04 (dd, J = 13.7, 6.2 Hz, 1H), 2.91 (dd, J = 13.8, 8.1 Hz, 1H), 2.69 (td, J = 7.7, 2.1 Hz, 4H), 2.36 (dt, J = 15.1, 7.5 Hz, 4H), 2.24 (s, 6H), 1.85 – 1.77 (m, 2H), 1.66 (dq, J = 14.4, 7.1 Hz, 4H), 1.39 – 1.20 (m, 60H), 0.86 (t, J = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ = 173.17, 64.14, 58.68, 49.22, 45.25, 40.15, 39.94, 39.00, 32.05, 31.96, 29.82, 29.79, 29.74, 29.65, 29.65, 29.49, 29.37, 29.36, 29.32, 29.29, 28.64, 28.62, 22.81, 22.68, 14.25. **HR-MS** (ESI) m/z calcd for $\text{C}_{45}\text{H}_{92}\text{O}_2\text{NS}_4$ $[\text{M}+\text{H}]^+$ 806.6005, found 806.6009.



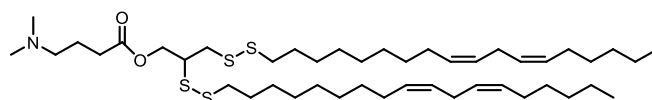
2,3-bis(tetradecyldisulfanyl)propyl 3-(dimethylamino)propanoate (C3S14)

Synthesized by using the general procedure. ^1H NMR (500 MHz, CDCl_3) δ = 4.42 – 4.34 (m, 2H), 3.28 (dq, J = 7.8, 5.9 Hz, 1H), 3.04 (dd, J = 13.8, 6.3 Hz, 1H), 2.93 (dd, J = 13.6, 8.3 Hz, 1H), 2.73 – 2.68 (m, 4H), 2.65 – 2.60 (m, 2H), 2.52 (dd, J = 8.8, 5.3 Hz, 2H), 2.25 (s, 6H), 1.71 – 1.63 (m, 4H), 1.37 (d, J = 4.8 Hz, 4H), 1.30 – 1.24 (m, 40H), 0.87 (t, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ = 172.19, 64.31, 54.75, 49.25, 45.34, 40.17, 39.97, 39.03, 32.88, 32.07, 29.84, 29.83, 29.81, 29.76, 29.68, 29.67, 29.51, 29.40, 29.39, 29.35, 29.32, 28.67, 28.64, 22.84, 14.28. **HR-MS** (ESI) m/z calcd for $\text{C}_{36}\text{H}_{74}\text{O}_2\text{NS}_4$ $[\text{M}+\text{H}]^+$ 680.4597, found 680.4601.



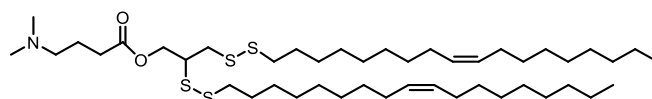
2,3-bis(hexadecyldisulfanyl)propyl 3-(dimethylamino)propanoate (C3S16)

Synthesized by using the general procedure. ^1H NMR (500 MHz, CDCl_3) δ = 4.42 – 4.34 (m, 2H), 3.28 (dq, J = 7.8, 5.8 Hz, 1H), 3.05 (dd, J = 13.7, 6.3 Hz, 1H), 2.98 – 2.90 (m, 1H), 2.71 (t, J = 6.8 Hz, 4H), 2.63 (dd, J = 10.9, 3.9 Hz, 2H), 2.51 (dd, J = 8.8, 5.3 Hz, 2H), 2.24 (s, 6H), 1.71 – 1.63 (m, 4H), 1.40 – 1.35 (m, 4H), 1.30 – 1.23 (m, 48H), 0.88 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ = 172.21, 64.31, 54.79, 49.27, 45.38, 40.18, 39.98, 39.04, 32.94, 32.07, 29.85, 29.81, 29.77, 29.68, 29.52, 29.40, 29.36, 29.32, 28.67, 28.65, 22.84, 14.28. **HR-MS** (ESI) m/z calcd for $\text{C}_{40}\text{H}_{82}\text{O}_2\text{NS}_4$ $[\text{M}+\text{H}]^+$ 736.5223, found 736.5231.



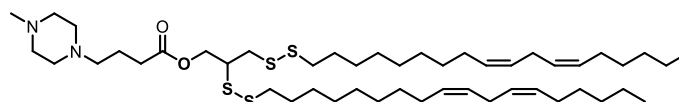
2,3-bis(((9Z,12Z)-octadeca-9,12-dien-1-yl)disulfanyl)propyl 4-(dimethylamino) butanoate (C4S18A)

Synthesized by using the general procedure. ^1H NMR (400 MHz, CDCl_3) δ = 5.42 – 5.29 (m, 8H), 4.36 (d, J = 5.6 Hz, 2H), 3.28 (dq, J = 11.8, 5.8 Hz, 1H), 3.05 (dd, J = 13.7, 6.2 Hz, 1H), 2.92 (dt, J = 12.6, 3.7 Hz, 1H), 2.77 (t, J = 6.4 Hz, 4H), 2.70 (t, J = 6.6 Hz, 4H), 2.39 (t, J = 7.4 Hz, 2H), 2.34 (t, J = 7.4 Hz, 2H), 2.25 (s, 6H), 2.05 (dd, J = 13.7, 6.8 Hz, 8H), 1.86 – 1.77 (m, 2H), 1.72 – 1.62 (m, 4H), 1.42 – 1.24 (m, 32H), 0.89 (t, J = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ = 173.24, 130.36, 130.21, 128.17, 128.05, 64.17, 58.79, 49.27, 45.37, 40.20, 39.97, 39.00, 32.01, 31.67, 29.80, 29.57, 29.56, 29.49, 29.39, 29.36, 29.36, 29.33, 29.30, 28.65, 28.62, 27.36, 27.35, 25.77, 22.80, 22.72, 14.23. **HR-MS** (ESI) m/z calcd for $\text{C}_{45}\text{H}_{84}\text{O}_2\text{NS}_4$ $[\text{M}+\text{H}]^+$ 798.5379, found 798.5364.



2,3-bis(((Z)-octadec-9-en-1-yl)disulfanyl)propyl 4-(dimethylamino)butanoate (C4S18B)

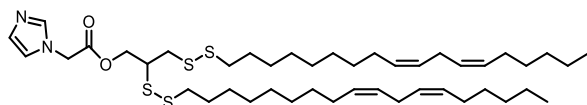
Synthesized by using the general procedure. ^1H NMR (500 MHz, CDCl_3) δ = 5.37 – 5.30 (m, 4H), 4.36 (dd, J = 5.6, 2.0 Hz, 2H), 3.27 (dq, J = 7.8, 5.8 Hz, 1H), 3.04 (dd, J = 13.7, 6.2 Hz, 1H), 2.91 (dd, J = 13.8, 8.1 Hz, 1H), 2.70 (td, J = 7.7, 2.1 Hz, 4H), 2.36 (dd, J = 15.8, 7.6 Hz, 4H), 2.26 (s, 6H), 2.03 – 1.96 (m, 8H), 1.70 – 1.63 (m, 2H), 1.70 – 1.62 (m, 4H), 1.38 – 1.23 (m, 44H), 0.87 (t, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ = 173.17, 130.10, 129.88, 64.15, 58.69, 49.21, 45.25, 40.14, 39.93, 38.97, 32.03, 31.94, 29.89, 29.87, 29.83, 29.79, 29.74, 29.65, 29.55, 29.45, 29.37, 29.35, 29.34, 29.31, 29.28, 28.63, 28.61, 27.34, 27.31, 22.82, 22.66, 14.26. **HR-MS** (ESI) m/z calcd for $\text{C}_{45}\text{H}_{88}\text{O}_2\text{NS}_4$ $[\text{M}+\text{H}]^+$ 802.5692, found 802.5699.



2,3-bis(((9Z,12Z)-octadeca-9,12-dien-1-yl)disulfanyl)propyl 4-(4-methylpiperazin-1-yl)butanoate (C4AS18A)

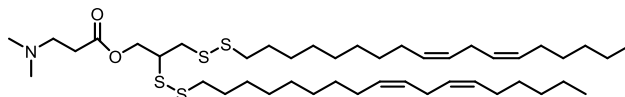
^1H NMR (400 MHz, CDCl_3) δ 5.39 – 5.30 (m, 8H), 4.35 (d, J = 5.6 Hz, 2H), 3.27 (dq, J = 8.3, 5.8 Hz, 1H), 3.04 (dd, J = 13.7, 6.2 Hz, 1H), 2.95 – 2.88 (m, 1H), 2.76 (t, J = 6.5 Hz, 4H), 2.69 (dt, J = 7.2, 3.7 Hz, 4H), 2.36 (t, J = 7.3 Hz, 6H), 2.28 (s, 3H), 2.03 (q, J = 6.9 Hz, 8H), 1.82 (q, J = 7.3 Hz, 2H), 1.66 (q, J = 7.7 Hz, 4H), 1.39 – 1.14 (m, 34H), 0.87 (t, J = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.24, 130.31, 130.16, 128.12, 127.99, 64.10, 57.60, 55.16, 53.05, 49.19, 46.06, 40.13, 39.89, 38.93, 32.18, 31.64, 29.76, 29.54, 29.46, 29.36, 29.33, 29.30, 29.26,

28.62, 28.59, 27.32, 27.32, 27.31, 25.74, 22.70, 22.17, 14.23. **HR-MS** (ESI) m/z calcd for $C_{48}H_{89}N_2O_2S_4$ $[M+H]^+$ 853.5807, found 853.5812.



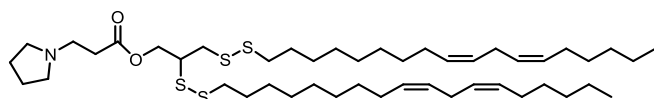
2,3-bis(((9Z,12Z)-octadeca-9,12-dien-1-yl)disulfanyl)propyl 2-(1H-imidazol-1-yl)acetate (C2S18A)

Synthesized by using the general procedure. 1H NMR (500 MHz, $CDCl_3$) δ = 7.51 (s, 1H), 7.10 (s, 1H), 6.96 (s, 1H), 5.42 – 5.29 (m, 8H), 4.74 (s, 2H), 4.49 (qd, J = 11.6, 5.6 Hz, 2H), 3.29 (dq, J = 8.6, 5.7 Hz, 1H), 3.02 (dd, J = 13.8, 5.8 Hz, 1H), 2.83 – 2.75 (m, 5H), 2.70 (td, J = 7.5, 2.6 Hz, 4H), 2.05 (q, J = 6.9 Hz, 8H), 1.72 – 1.62 (m, 4H), 1.40 – 1.25 (m, 32H), 0.89 (t, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 167.18, 138.07, 130.36, 130.18, 129.97, 128.17, 128.02, 120.11, 65.50, 48.74, 48.09, 40.00, 39.78, 38.92, 31.67, 29.78, 29.56, 29.55, 29.49, 29.38, 29.35, 29.34, 29.29, 28.62, 28.59, 27.34, 25.77, 22.72, 14.24. **HR-MS** (ESI) m/z calcd for $C_{44}H_{77}O_2N_2S_4$ $[M+H]^+$ 793.4862, found 793.4862.



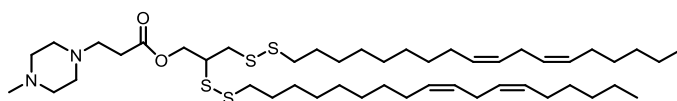
2,3-bis(((9Z,12Z)-octadeca-9,12-dien-1-yl)disulfanyl)propyl 3-(dimethylamino)propanoate (C3S18A)

Synthesized by using the general procedure. 1H NMR (400 MHz, $CDCl_3$) δ = 5.47 – 5.26 (m, 8H), 4.45 – 4.32 (m, 2H), 3.33 – 3.24 (m, 1H), 3.04 (dd, J = 13.2, 6.8 Hz, 1H), 2.94 (dd, J = 13.6, 8.0 Hz, 1H), 2.77 (t, J = 6.3 Hz, 4H), 2.70 (t, J = 7.3 Hz, 4H), 2.62 (t, J = 7.0 Hz, 2H), 2.51 (t, J = 7.2 Hz, 2H), 2.24 (s, 3H), 2.04 (dd, J = 13.5, 6.7 Hz, 8H), 1.73 – 1.61 (m, 8H), 1.41 – 1.24 (m, 32H), 0.89 (t, J = 6.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.22, 130.36, 130.22, 128.17, 128.05, 64.32, 54.83, 49.29, 45.41, 40.21, 39.97, 39.01, 32.98, 31.68, 29.81, 29.58, 29.50, 29.40, 29.37, 29.35, 29.31, 28.67, 28.63, 27.41, 27.37, 27.36, 25.78, 22.74, 14.25. **HR-MS** (ESI) m/z calcd for $C_{44}H_{82}O_2NS_4$ $[M+H]^+$ 784.5223, found 784.5222.



2,3-bis(((9Z,12Z)-octadeca-9,12-dien-1-yl)disulfanyl)propyl 3-(pyrrolidin-1-yl)propanoate (C3AS18A)

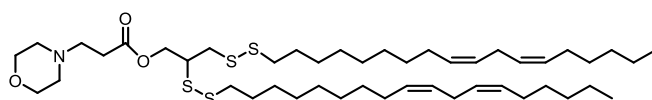
Synthesized by using the general procedure. 1H NMR (400 MHz, $CDCl_3$) δ = 5.42 – 5.29 (m, 8H), 4.38 (qd, J = 11.6, 5.6 Hz, 2H), 3.28 (dq, J = 11.9, 5.8 Hz, 1H), 3.04 (dd, J = 13.8, 6.3 Hz, 1H), 2.94 (dd, J = 13.8, 8.0 Hz, 1H), 2.83 – 2.75 (m, 6H), 2.70 (t, J = 7.3 Hz, 4H), 2.57 (t, J = 7.5 Hz, 2H), 2.53 (dd, J = 9.2, 3.9 Hz, 4H), 2.05 (q, J = 6.8 Hz, 8H), 1.81 – 1.75 (m, 4H), 1.72 – 1.62 (m, 4H), 1.44 – 1.22 (m, 32H), 0.89 (t, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.22, 130.35, 130.21, 128.16, 128.04, 64.24, 54.14, 51.46, 49.32, 40.16, 39.96, 39.00, 34.23, 31.68, 29.80, 29.57, 29.50, 29.40, 29.37, 29.34, 29.31, 28.66, 28.63, 27.37, 27.35, 25.78, 23.63, 22.73, 14.24. **HR-MS** (ESI) m/z calcd for $C_{46}H_{84}O_2NS_4$ $[M+H]^+$ 810.5379, found 810.5373.



2,3-bis(((9Z,12Z)-octadeca-9,12-dien-1-yl)disulfanyl)propyl 3-(4-methylpiperazin-1-yl)propanoate (C3BS18A)

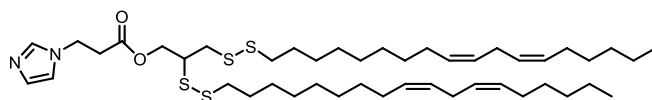
Synthesized by using the general procedure. 1H NMR (500 MHz, $CDCl_3$) δ = 5.41 – 5.30 (m, 8H), 4.41 (dd, J = 11.6, 5.7 Hz, 1H), 4.35 (dd, J = 11.6, 5.4 Hz, 1H), 3.28 (dq, J = 7.9,

5.7 Hz, 1H), 3.04 (dd, $J = 13.7, 6.3$ Hz, 1H), 2.94 (dd, $J = 13.8, 8.1$ Hz, 1H), 2.77 (t, $J = 6.7$ Hz, 4H), 2.73 – 2.65 (m, 7H), 2.57 – 2.41 (m, 6H), 2.28 (s, 3H), 2.08 – 2.01 (m, 8H), 1.80 – 1.71 (m, 3H), 1.66 (dt, $J = 14.6, 7.3$ Hz, 4H), 1.41 – 1.24 (m, 32H), 0.89 (t, $J = 6.9$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) $\delta = 172.19, 130.36, 130.20, 128.16, 128.03, 64.18, 55.13, 53.58, 52.94, 49.29, 46.10, 40.11, 39.95, 38.98, 32.43, 31.67, 29.80, 29.57, 29.49, 29.40, 29.37, 29.36, 29.33, 29.31, 28.66, 28.63, 27.36, 27.35, 25.77, 22.73, 14.24$. **HR-MS** (ESI) m/z calcd for $\text{C}_{47}\text{H}_{87}\text{O}_2\text{N}_2\text{S}_4$ $[\text{M}+\text{H}]^+$ 839.5645, found 839.5651.



2,3-bis(((9Z,12Z)-octadeca-9,12-dien-1-yl)disulfanyl)propyl 3-morpholino-propanoate (C3CS18A)

Synthesized by using the general procedure. ^1H NMR (400 MHz, CDCl_3) $\delta = 5.44 - 5.27$ (m, 8H), 4.39 (qd, $J = 11.6, 5.5$ Hz, 2H), 3.71 – 3.68 (m, 4H), 3.28 (dq, $J = 8.1, 5.7$ Hz, 1H), 3.05 (dd, $J = 13.7, 6.1$ Hz, 1H), 2.93 (dd, $J = 13.7, 8.2$ Hz, 1H), 2.77 (t, $J = 6.5$ Hz, 4H), 2.70 (dd, $J = 14.8, 7.3$ Hz, 6H), 2.53 (t, $J = 7.1$ Hz, 2H), 2.49 – 2.46 (m, 2H), 2.05 (dd, $J = 13.7, 6.8$ Hz, 8H), 1.67 (ddd, $J = 12.9, 10.6, 5.6$ Hz, 6H), 1.32 (td, $J = 13.3, 8.5$ Hz, 32H), 0.89 (t, $J = 6.8$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) $\delta = 171.96, 130.23, 130.07, 128.04, 127.91, 66.93, 64.05, 53.92, 53.40, 49.16, 39.99, 39.84, 38.88, 32.12, 31.55, 29.67, 29.44, 29.37, 29.27, 29.23, 29.21, 29.19, 28.53, 28.50, 27.22, 25.65, 22.60, 14.11$. **HR-MS** (ESI) m/z calcd for $\text{C}_{46}\text{H}_{84}\text{O}_3\text{N}_4\text{S}_4$ $[\text{M}+\text{H}]^+$ 826.5329, found 826.5330.



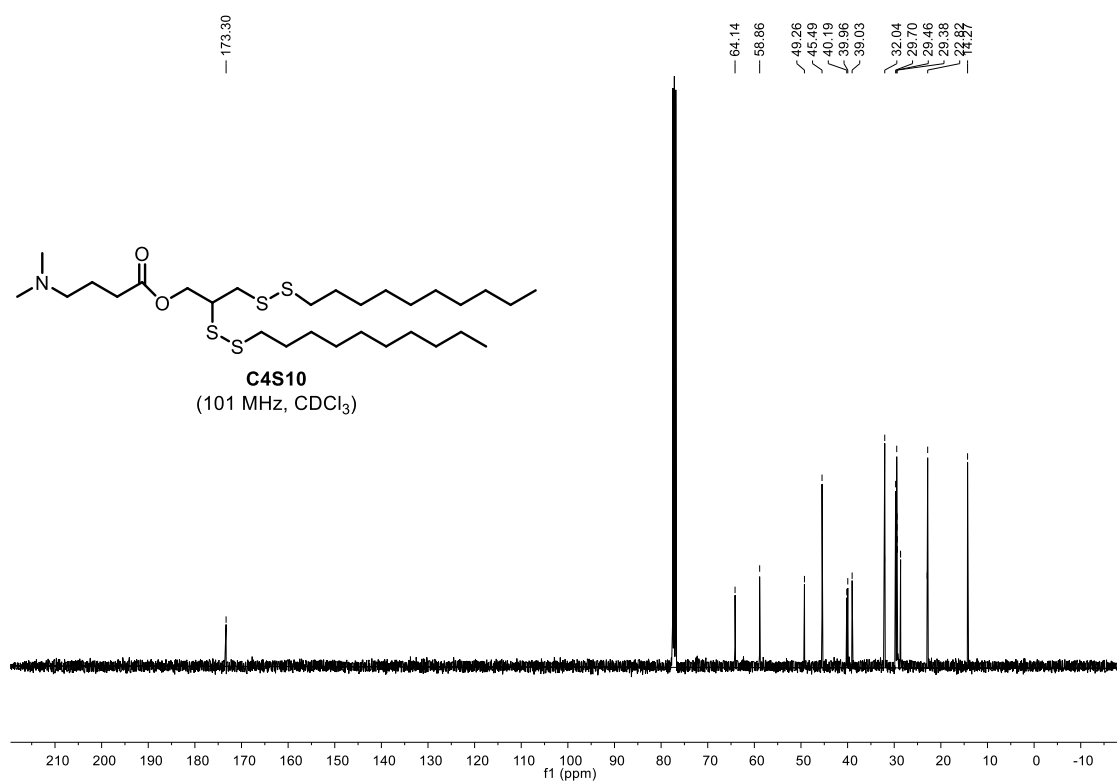
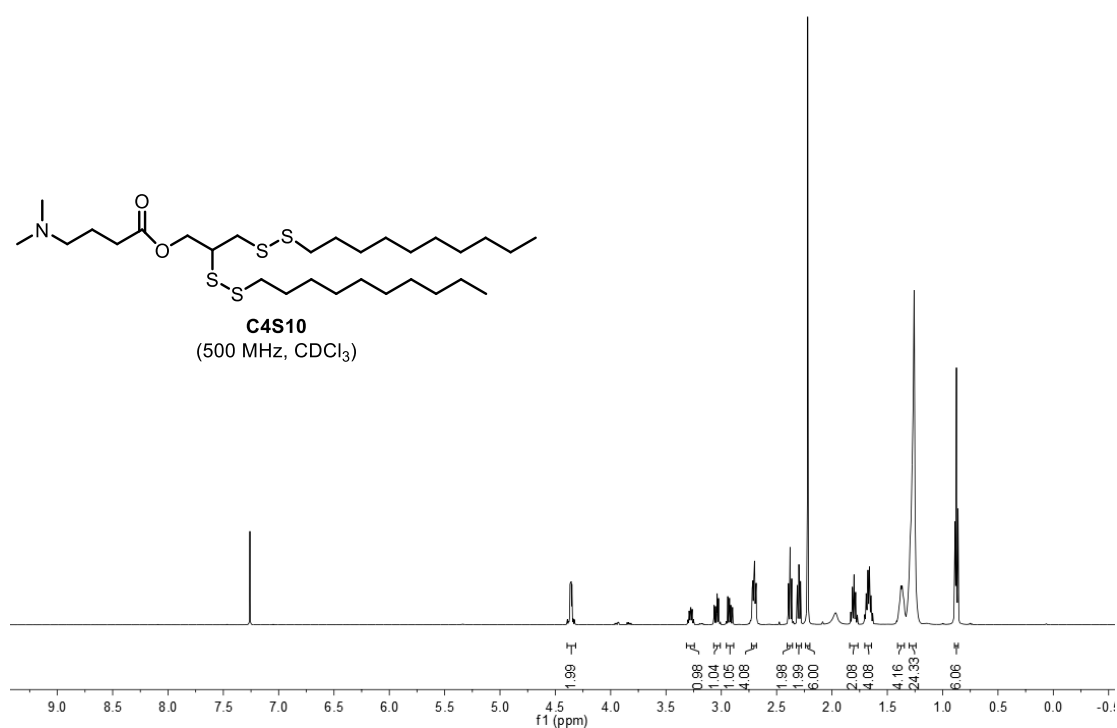
2,3-bis(((9Z,12Z)-octadeca-9,12-dien-1-yl)disulfanyl)propyl 3-(1H-imidazol-1-yl)propanoate (C3DS18A)

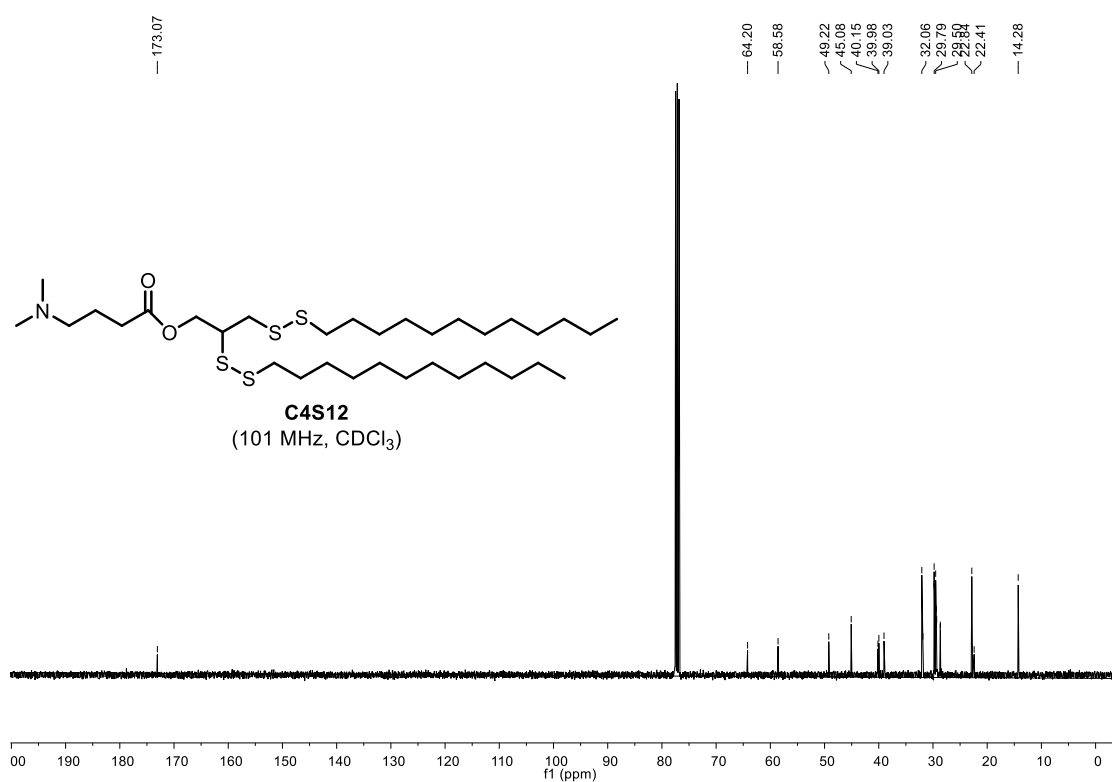
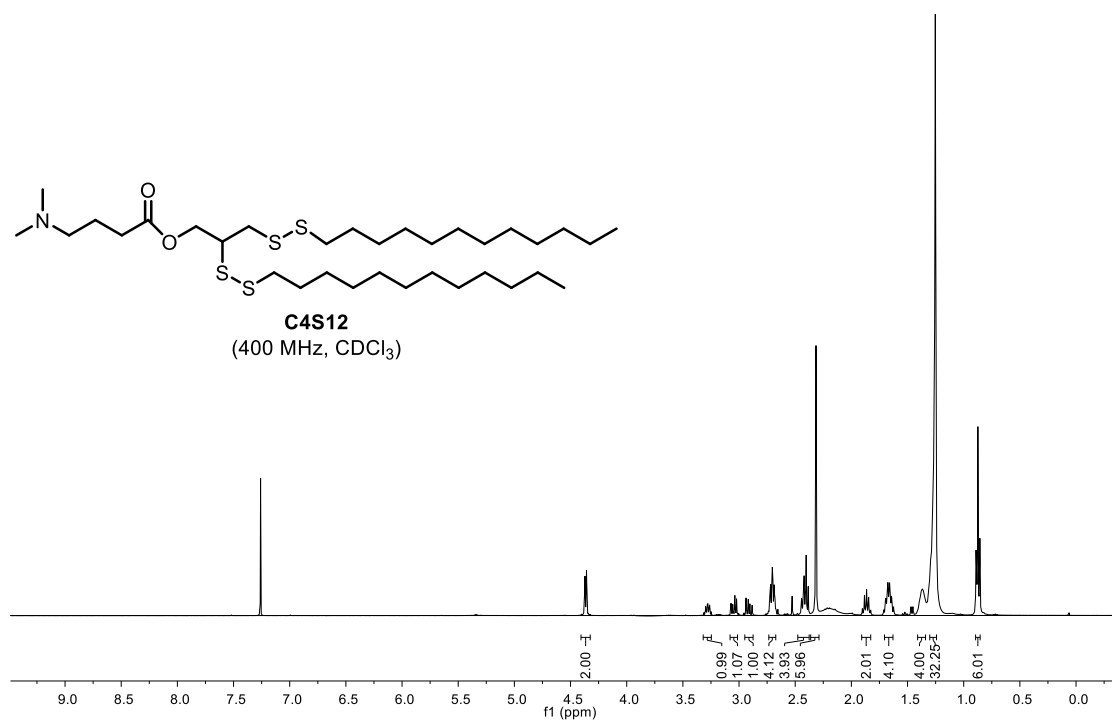
Synthesized by using the general procedure. ^1H NMR (400 MHz, CDCl_3) $\delta = 7.52$ (s, 1H), 7.05 (s, 1H), 6.94 (s, 1H), 5.43 – 5.28 (m, 8H), 4.40 (d, $J = 5.6$ Hz, 2H), 4.27 (t, $J = 6.6$ Hz, 2H), 3.26 (dq, $J = 8.3, 5.7$ Hz, 1H), 3.02 (dd, $J = 13.8, 6.0$ Hz, 1H), 2.85 – 2.75 (m, 6H), 2.69 (td, $J = 7.5, 2.8$ Hz, 3H), 2.09 – 1.99 (m, 8H), 1.65 (dt, $J = 14.6, 7.3$ Hz, 4H), 1.41 – 1.24 (m, 34H), 0.89 (t, $J = 6.8$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) $\delta = 170.29, 137.39, 130.36, 130.19, 129.92, 128.18, 128.03, 118.93, 64.81, 48.97, 42.28, 40.00, 38.97, 36.00, 31.67, 29.79, 29.56, 29.49, 29.39, 29.35, 29.34, 29.29, 28.64, 28.60, 27.35, 25.77, 22.72, 14.24$. **HR-MS** (ESI) m/z calcd for $\text{C}_{45}\text{H}_{79}\text{O}_2\text{N}_2\text{S}_4$ $[\text{M}+\text{H}]^+$ 807.5019, found 807.5018.

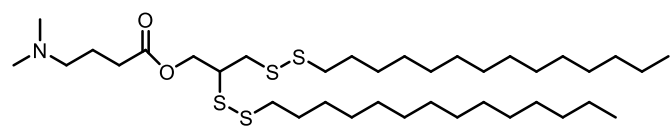
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- [1] a) Jayaraman, M.; Ansell, S. M.; Mui, B. L.; Tam, Y. K.; Chen, J.; Du, X.; Butler, D.; Eltepu, L.; Matsuda, S.; Narayanannair, J. K.; Rajeev, K. G.; Hafez, I. M.; Akinc, A.; Maier, M. A.; Tracy, M. A.; Cullis, P. R.; Madden, T. D.; Manoharan, M.; Hope, M. J. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew. Chem. Int. Ed.* **2012**, *51* (34), 8529; b) Sabnis, S.; Kumarasinghe, E. S.; Salerno, T.; Mihai, C.; Ketova, T.; Senn, J. J.; Lynn, A.; Bulychev, A.; McFadyen, I.; Chan, J.; Almarsson, O.; Stanton, M. G.; Benenato, K. E. A Novel amino lipid series for mRNA delivery: improved endosomal escape and sustained pharmacology and safety in non-human primates. *Mol. Ther.* **2018**, *26* (6), 1509.

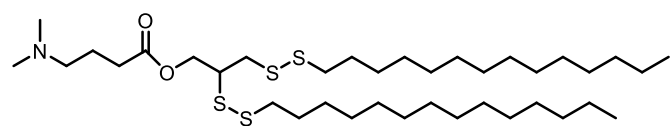
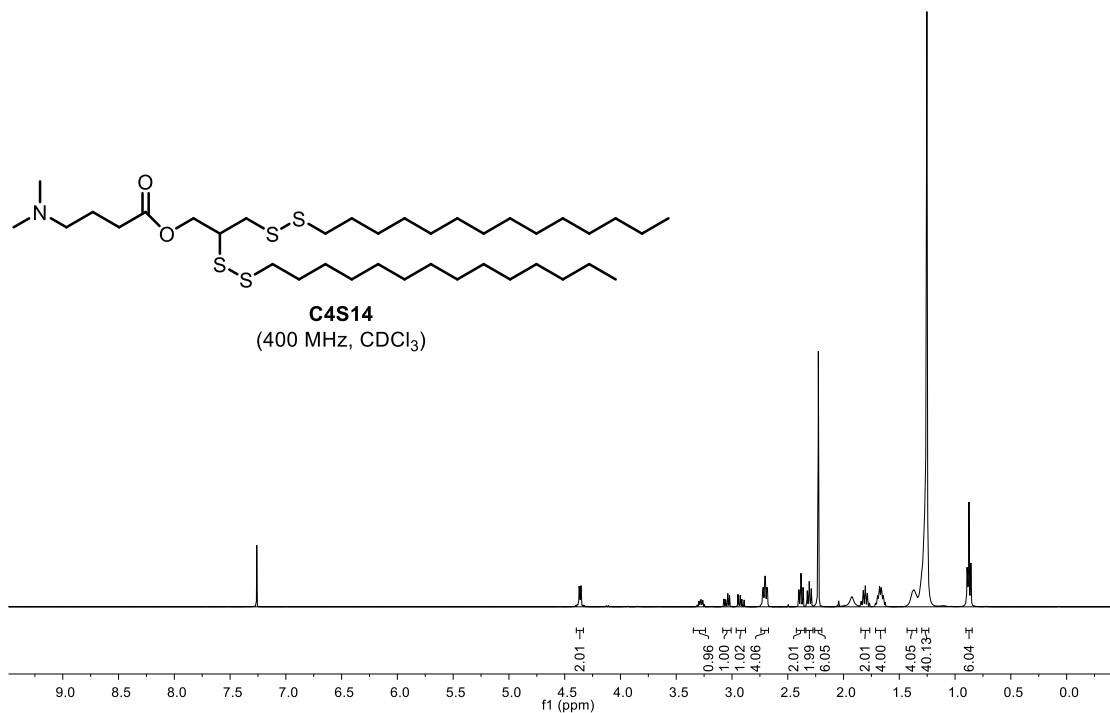
NMR Spectrum







C4S14
(400 MHz, CDCl₃)



C4S14
(101 MHz, CDCl₃)

