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Synthesis of novel lipophilic polyamines with cytotoxic activity

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1. Experimental section

1.1. Chemistry

All column chromatography solvents were distilled before use. Thin layer chromatography was performed using pre-coated aluminum plates (Kieselgel 60 F_{254}, Merck), which were visualized with the phosphomolybdic acid–ceric sulfate reagent. Flash column chromatography (FC) was performed on Kieselgel 60 (40–63 μm, Merck). ^{1}H and ^{13}C NMR spectra were recorded at 30°C with Bruker DPX-300, DPX-500, DPX-400, and DPX-600 instruments in $CDCl_3$, MeOH-d_4, and D_2O as solvents. The signals of $SiMe_4$ (δ = 0.00 ppm) and $CDCl_3$ (δ = 77.16 ppm) were used as internal references. The *J* values are given in Hz. Mass spectra were recorded on a high-resolution Q-TOF G6550A spectrometer (Agilent) with a Dual-ESI-Jetstream ionisation source or on a Finnigan MAT 900XL-TRAP spectrometer with electrospray ionization (ESI).

*1.1.1. General procedure for synthesis of bromo derivatives **1a-e***

Triphenylphosphine (5.28 mmol) and tetrabromomethane (5.28 mmol) were added to a solution of diglycerides (2-*O*-ethyl-1-*O*-octadecyl-*rac*-glycerol, 2-*O*-ethyl-3-*O*-hexadecyl-*rac*-glycerol, 2-*O*-ethyl-3-*O*-tetradecyl-*rac*-glycerol, 3-*O*-dodecyl-2-*O*-ethyl-*rac*-glycerol, 2-*O*-ethyl-*rac*-glycerol) (3.52 mmol) in anhydrous dichloromethane (15 ml), and the reaction mixture was stirred for 3 h at 24 ºC, quenched with methanol (10 ml) and stirred for additional 15 min. The solvents were removed under reduced pressure, and the residue was chromatographed on silica gel using light petroleum – ethyl acetate mixture (30:1) to give compounds **1a-e**.

*1.1.1.1. 1-Bromo-1-deoxy-2-O-ethyl-3-O-octadecyl-rac-glycerol (**1a**)*

Yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (t, *J* = 6.9 Hz, 3 H, $(CH_2)_{15}C\underline{H}_3$), 1.25 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.30 (br. s, 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.54-1.64 (m, 2 H, $OCH_2C\underline{H}_2$), 3.39-3.47 (m, 3 H, $OC\underline{H}_2CH_2$, $CH\underline{H}_aBr$), 3.47-3.69 (m, 6 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $CH\underline{H}_bBr$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz): 14.23, 15.58, 22.81, 26.19, 29.49, 29.57, 29.70, 29.73, 29.80, 29.83, 32.05, 32.71, 65.84, 70.81, 71.86, 76.84. MS-ESI m/z: $[M]^+$ calcd for $C_{23}H_{47}BrO_2$ 434.28, found 434.93

*1.1.1.2. 1-Bromo-1-deoxy-2-O-ethyl-3-O-hexadecyl-rac-glycerol (**1b**)*

Yield 98 %**.** ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.5 Hz, 3 H, $(CH_2)_{13}C\underline{H}_3$), 1.20 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.25 (br.s, 26 H, $(C\underline{H}_2)_{13}CH_3$), 1.48 – 1.60 (m, 2 H, $OCH_2C\underline{H}_2$), 3.36 – 3.47 (m, 3 H, $OC\underline{H}_2CH_2$, $CH\underline{H}_aBr$), 3.45-3.70 (m, 6 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $CH\underline{H}_bBr$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 14.13, 15.54, 22.76, 26.20, 29.44, 29.54, 29.70, 29.73, 29.76, 29.79, 32.02, 32.61, 65.81, 70.97, 71.86, 77.95. HRMS-ESI m/z: $[M+K]^+$ calcd for $C_{21}H_{43}BrO_2$ 445.2090, found 445.2084.

*1.1.1.3. 1-Bromo-1-deoxy-2-O-ethyl-3-O-tetradecyl-rac-glycerol (**1c**)*

Yield 82 %**.** ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.7 Hz, 3 H, $(CH_2)_{11}C\underline{H}_3$), 1.20 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.24 (br.s, 22 H, $(C\underline{H}_2)_{11}CH_3$), 1.45 – 1.66 (m, 2 H, $OCH_2C\underline{H}_2$), 3.35 – 3.47 (m, 3 H, $OC\underline{H}_2CH_2$, $CH\underline{H}_aBr$), 3.47-3.70 (m, 6 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $CH\underline{H}_bBr$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 14.01, 15.38, 22.61, 26.01, 29.29, 29.37, 29.53, 29.59, 29.61,

31.86, 32.49, 65.64, 70.68, 71.67, 77.66. HRMS-ESI m/z: $[M+Na]^+$ calcd for $C_{19}H_{39}BrO_2$ 401.2031, found 401.2030.

*1.1.1.4. 1-Bromo-1-deoxy-3-O-dodecyl-2-O-ethyl-rac-glycerol (**1d**)*

Yield 94 %**.** ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, *J* = 6.7 Hz, 3 H, $(CH_2)_9C\underline{H}_3$), 1.14 – 1.50 (m, 21 H, $(C\underline{H}_2)_9CH_3$; $OCH_2C\underline{H}_3$), 1.50 – 1.62 (m, 2 H, $OCH_2C\underline{H}_2$), 3.39-3.49 (m, 3 H, $OC\underline{H}_2CH_2$, $CH\underline{H}_aBr$), 3.49-3.72 (m, 6 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $CH\underline{H}_bBr$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 14.09, 15.46, 22.67, 26.07, 29.34, 29.44, 29.59, 29.60, 29.63, 29.65, 31.91, 32.67, 65.75, 70.76, 71.78, 77.71. HRMS-ESI m/z: $[M+Na]^+$ calcd for $C_{17}H_{35}BrO_2$ 373.1718, found 373.1711.

*1.1.1.5. 1-Bromo-3-O-decyl-1-deoxy-2-O-ethyl-rac-glycerol (**1e**)*

Yield 90 %**.** ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, *J* = 6.7 Hz, 3 H, $(CH_2)_7C\underline{H}_3$), 1.15 – 1.49 (m, 17 H, $(C\underline{H}_2)_7CH_3$; $OCH_2C\underline{H}_3$), 1.48 – 1.66 (m, 2 H, $OCH_2C\underline{H}_2$), 3.39 – 3.49 (m, 3 H, $OC\underline{H}_2CH_2$, $CH\underline{H}_aBr$), 3.49-3.73 (m, 6 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $CH\underline{H}_bBr$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 14.08, 15.45, 22.66, 26.07, 29.30, 29.43, 29.55, 29.59, 31.89, 32.66, 65.75, 70.75, 71.78, 77.71. HRMS-ESI m/z: $[M+Na]^+$ calcd for $C_{15}H_{31}BrO_2$ 345.1405, found 345.1405.

*1.1.2. General procedure for synthesis of lipophilic polyamines **3a-g** by the Fukuyama reaction*

Regioselectively protected polyamines (**2a-c**) (0.344 mmol), Cs_2CO_3 (0.517 mmol) were added to a solution of compounds **1a-e** (0.344 mmol) in anhydrous DMF (5 ml), and the reaction mixture was stirred for 25 h at 80 ºC. After DMF was removed under reduced pressure, the residue was re-dissolved in CH_2Cl_2 (10 ml), and water (20 ml) was added. The organic layer was separated, and the water layer was extracted with CH_2Cl_2 (3 × 10 ml). The combined organic phase was washed with water (3 × 10 ml), then dried with Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The product was isolated using column chromatography on silica gel (toluene—acetone, 10:1) to give compounds **3a-g**.

*1.1.2.1. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-12-(2-nitrophenylsulfonylamino)-4,9-diazadodecane (**3a**)*

Yield 33%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, *J* = 6.8 Hz, 3 H, $(CH_2)_{15}C\underline{H}_3$), 1.07 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.25 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.43 (br.s, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.48-1.56 (m, 2 H, $OCH_2C\underline{H}_2$), 1.63-1.74 (m, 2 H) and 1.75-1.90 (m, 2 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.04-3.18 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.20-3.68 (m, 13 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 3 NCH_2), 6.35 (br.s, 1 H, $N\underline{H}CH_2$), 7.55-7.87 (m, 6 H) and 7.98-8.17 (m, 2 H, 2 C_6H_4). ^{13}C NMR (126 MHz, $CDCl_3$) 14.24, 15.66, 22.83, 26.23, 27.19, 28.55, 28.60, 28.86, 29.50, 29.64, 29.74, 29.80, 29.84, 32.07, 44.57, 47.01, 65.73, 70.55, 71.94, 77.55, 79.67, 80.02, 124.21, 131.22, 131.68, 132.65, 133.46, 133.89, 148.29, 155.58.

*1.1.2.2. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-hexadecyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-12-(2-nitrophenylsulfonylamino)-4,9-diazadodecane (**3b**)*

Yield 30 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, *J* = 6.7 Hz, 3 H, $(CH_2)_{13}C\underline{H}_3$), 1.06 (t, *J* = 7.0, 3 H, $OCH_2C\underline{H}_3$), 1.24 (br.s, 26 H, $(C\underline{H}_2)_{13}CH_3$), 1.42 (br.s, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.48-1.59 (m, 2 H, $OCH_2C\underline{H}_2$), 1.59-1.74 (m, 2 H) and 1.75-1.89 (m, 2 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.00-3.18 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.18-3.67 (m, 13 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 3 NCH_2), 6.37 (br.s, 1 H, $N\underline{H}CH_2$), 7.55-7.85 (m, 6 H) and 7.95-8.15 (m, 2 H, 2 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$) 14.23, 15.64, 22.61, 26.21, 27.16, 28.53, 28.58, 28.84, 29.48, 29.62, 29.78, 29.82, 32.05, 44.56, 46.86, 47.10, 47.35, 49.16, 65.71, 70.53, 71.92, 77.48, 79.61,

80.00, 124.08, 124.19, 131.17, 131.68, 132.64, 132.90, 133.46, 148.26, 148.32, 155.56. HRMS-ESI m/z: $[M+K]^+$ calcd for $C_{53}H_{90}N_6O_{14}S_2$ 1137.5593, found 1137.5600.

*1.1.2.3. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-tetradecyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-12-(2-nitrophenylsulfonylamino)-4,9-diazadodecane (**3c**)*

Yield 17 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, *J* = 6.8 Hz, 3 H, $(CH_2)_{11}C\underline{H}_3$), 1.06 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.24 (br.s, 22 H, $(C\underline{H}_2)_{11}CH_3$), 1.41 (br.s, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.48-1.58 (m, 2 H, $OCH_2C\underline{H}_2$), 1.60-1.73 (m, 2 H) and 1.73-1.90 (m, 2 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.00-3.19 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.20-3.68 (m, 13 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 3 $NC\underline{H}_2$), 7.55-7.87 (m, 6 H) and 7.92-8.14 (m, 2 H, 2 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$) 14.18, 15.60, 22.76, 26.00, 26.16, 27.12, 28.48, 28.53, 28.84, 29.48, 29.56, 29.72, 29.76, 32.00, 43.66, 44.52, 46.86, 47.02, 49.20, 65.65, 70.49, 71.87, 77.36, 79.54, 79.93, 124.15, 131.08, 131.66, 132.64, 133.46, 133.78, 148.21, 155.51. HRMS-ESI m/z: $[M+Na]^+$ calcd for $C_{51}H_{86}N_6O_{14}S_2$ 1093.5541, found 1093.5550.

*1.1.2.4. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-3-dodecyloxy-2-ethyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-12-(2-nitrophenylsulfonylamino)-4,9-diazadodecane (**3d**)*

Yield 18 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, *J* = 6.8 Hz, 3 H, $(CH_2)_9C\underline{H}_3$), 1.07 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.25 (br.s, 18 H, $(C\underline{H}_2)_9CH_3$), 1.43 (br.s, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.48-1.56 (m, 2 H, $OCH_2C\underline{H}_2$), 1.63-1.74 (m, 2 H) and 1.75-1.90 (m, 2 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.04-3.18 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.20-3.68 (m, 13 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 3 $NC\underline{H}_2$), 7.55-7.87 (m, 6 H), 7.98-8.17 (m, 2 H, 2 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.17, 15.58, 22.74, 26.15, 27.10, 28.47, 28.52, 28.80, 29.41, 29.55, 29.69, 29.74, 31.98, 43.59, 44.49, 46.84, 47.05, 49.20, 65.64, 70.48, 71.85, 77.45, 79.52, 79.92, 124.15, 131.07, 131.65, 132.60, 133.45, 133.75, 148.20, 155.51. HRMS-ESI m/z: $[M+Na]^+$ calcd for $C_{49}H_{82}N_6O_{14}S_2$ 1065.5228, found 1065.5239.

*1.1.2.5. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-3-decyloxy-2-ethyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-12-(2-nitrophenylsulfonylamino)-4,9-diazadodecane (**3e**)*

Yield 22 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, *J* = 6.8 Hz, 3 H, $(CH_2)_7C\underline{H}_3$), 1.07 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.25 (br.s, 14 H, $(C\underline{H}_2)_7CH_3$), 1.43 (br.s, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.48-1.56 (m, 2 H, $OCH_2C\underline{H}_2$), 1.63-1.74 (m, 2 H) and 1.75-1.90 (m, 2 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.04-3.18 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.20-3.68 (m, 13 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 3 $NC\underline{H}_2$), 6.35 (br.s, 1 H, $N\underline{H}CH_2$), 7.55-7.87 (m, 6 H), 7.98-8.17 (m, 2 H, 2 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): 14.13, 15.54, 22.69, 26.10, 27.06, 28.42, 28.47, 28.71, 29.33, 29.49, 29.60, 29.63, 31.91, 43.41, 44.46, 46.79, 46.99, 49.21, 65.59, 70.42, 71.80, 77.40, 79.47, 79.86, 124.11, 130.77, 130.99, 131.65, 132.64, 133.47, 133.68, 148.14, 155.46. HRMS-ESI m/z: $[M+Na]^+$ calcd for $C_{47}H_{78}N_6O_{14}S_2$ 1037.4915, found 1037.4927.

*1.1.2.6. 4,8-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-11-(2-nitrophenylsulfonylamino)-4,8-diazaundecane (**3f**)*

Yield 27 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.7 Hz, 3 H, $(CH_2)_{17}C\underline{H}_3$), 1.05 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.24 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.41 (s, 18 H, 2 $C(C\underline{H}_3)_3$), 1.46-1.58 (m, 2 H, $OCH_2C\underline{H}_2$), 1.58-1.74 (m, 4 H) and 1.74-1.88 (m, 2 H, 3 $NCH_2C\underline{H}_2CH_2N$), 2.97-3.20 (m, 8 H, 4 $C\underline{H}_2NHBoc$), 3.20-3.66 (m, 13 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 3 $NC\underline{H}_2$), 7.54-7.84 (m, 6 H), 7.94-8.05 (m, 1 H) and 8.05-8.15 (m, 1 H, 2 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$) 14.19, 15.60, 22.77, 26.17, 27.14, 28.49, 28.54, 28.77, 29.43, 29.58, 29.74, 29.78, 32.01, 43.57, 44.59, 44.91, 45.06, 46.86, 49.32, 65.65, 70.47, 71.87, 77.47, 79.70, 80.07, 124.16, 131.08, 131.66,

132.67, 133.47, 133.74, 148.21, 155.44. HRMS-ESI m/z: $[M+K]^+$ calcd for $C_{54}H_{92}N_6O_{14}S_2$ 1151.5750, found 1151.5765.

*1.1.2.7. 3,6-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-8-(2-nitrophenylsulfonylamino)-3,6-diazaoctane (**3g**)*

Yield 20 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3 H, $(CH_2)_{17}C\underline{H}_3$), 0.95-1.13 (m, 3 H, $OCH_2C\underline{H}_3$), 1.27 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.43 (br.s, 18 H, 2 $C(C\underline{H}_3)_3$), 1.49-1.57 (m, 2 H, $OCH_2C\underline{H}_2$), 3.10-3.24 (m, 8 H, 4 $C\underline{H}_2$NBoc), 3.24-3.63 (m, 13 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 3 NCH_2), 7.52-7.89 (m, 6 H) and 7.96-8.15 (m, 2 H, 2 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$) 14.24, 15.59, 22.83, 26.22, 28.50, 28.55, 29.50, 29.65, 29.80, 29.84, 32.07, 42.24, 42.88, 45.82, 49.87, 65.58, 70.51, 71.91, 77.36, 79.35, 124.14, 130.83, 131.08, 132.90, 133.55, 148.26, 155.93. HRMS-ESI m/z: $[M+K]^+$ calcd for $C_{51}H_{86}N_6O_{14}S_2$ 1109.5280, found 1109.5287.

*1.1.3. General procedure for synthesis modified lipophilic polyamines **5a-g***

Cesium carbonate (0.179 mmol) and bromoethane (0.597 mmol) were added to a solution of compounds **3a-g** (0.060 mmol) in anhydrous DMF (4 ml), and the reaction mixture was stirred for 5 h at 80 ºC. After DMF was removed under reduced pressure, the residue was re-dissolved in dichloromethane (10 ml), and water (20 ml) was added. The organic layer was separated, and the water layer was extracted with dichloromethane (3 × 10 ml). The combined organic phase was washed with water (3 × 10 ml), then dried with Na_2SO_4, filtered, and the solvent was evaporated to dryness under reduced pressure. The product was isolated by column chromatography on silica gel (toluene—acetone solvent system, 10:1) to obtain products **5a-g**.

*1.1.3.1. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-12-[N-ethyl-N-(2-nitrophenylsulfonyl)amino]-4,9-diazadodecane (**5a**)*

Yield 83%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3 H, *J* = 6.9 Hz, $(CH_2)_{15}C\underline{H}_3$), 1.07 (t, 3 H, *J* = 7.0 Hz, $OCH_2C\underline{H}_3$), 1.14 (t, 3 H, *J* = 7.1 Hz, $NCH_2C\underline{H}_3$), 1.26 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.41-1.48 (m, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.49-1.56 (m, 2 H, $OCH_2C\underline{H}_2$), 1.75-1.90 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.03-3.18 (m, 8H, 4 $C\underline{H}_2$NBoc), 3.26-3.66 (m, 15 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 4 NCH_2), 7.58-7.72 (m, 6 H) and 7.96-8.08 (m, 2 H, 2 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.25, 14.04, 15.47, 22.62, 26.02, 28.40, 29.29, 29.43, 29.54, 31.86, 65.56, 70.41, 71.72, 79.42, 124.07, 130.59, 131.55, 133.34, 133.59, 148.10, 155.35. HRMS-ESI m/z: $[M+K]^+$ calcd for $C_{57}H_{98}N_6O_{14}S_2$ 1193.6220, found 1193.6235.

*1.1.3.2. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-hexadecyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-12-[N-ethyl-N-(2-nitrophenylsulfonyl)amino]-4,9-diazadodecane (**5b**)*

Yield 90 %. ^{1}H NMR (300 MHz, $CDCl_3$): ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3 H, *J* = 6.9 Hz, $(CH_2)_{15}C\underline{H}_3$), 1.02-1.18 (m, $OCH_2C\underline{H}_3$, $NCH_2C\underline{H}_3$), 1.25 (br.s, 26 H, $(C\underline{H}_2)_{13}CH_3$), 1.38-1.49 (m, 24 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$, $OCH_2C\underline{H}_2$), 1.67-1.91 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.01-3.23 (m, 8H, 4 $C\underline{H}_2$NBoc), 3.24-3.70 (m, 15 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 4 NCH_2), 7.56-7.75 (m, 6 H) and 7.94-8.10 (m, 2 H, 2 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.76, 14.17, 15.65, 22.78, 26.22, 27.17, 27.69, 28.60, 29.45, 29.61, 29.76, 29.81, 32.04, 42.37, 44.61, 44.92, 46.94, 49.36, 65.73, 70.71, 71.92, 77.35, 79.53, 124.19, 124.24, 130.81, 131.16, 131.62, 131.67, 133.47, 133.88, 148.36, 155.55. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{55}H_{94}N_6O_{14}S_2$ 1127.6358, found 1127.6374.

*1.1.3.3. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-tetradecyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-12-[N-ethyl-N-(2-nitrophenylsulfonyl)amino]-4,9-diazadodecane (**5c**)*

Yield 91 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3 H, *J* = 6.9 Hz, $(CH_2)_{15}C\underline{H}_3$), 1.07 (t, 3 H, *J* = 7.0 Hz, $OCH_2C\underline{H}_3$), 1.12 (t, 3 H, *J* = 7.1 Hz, $NCH_2C\underline{H}_3$), 1.25 (br.s, 22 H, $(C\underline{H}_2)_{11}CH_3$), 1.39-1.47 (m, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.48-1.60 (m, 2 H, $OCH_2C\underline{H}_2$), 1.68-1.89 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.00-3.23 (m, 8H, 4 $C\underline{H}_2NBoc$), 3.23-3.68 (m, 15 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 4 NCH_2), 7.53-7.77 (m, 6 H) and 7.91-8.06 (m, 2 H, 2 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.57, 14.19, 15.62, 22.77, 26.22, 27.07, 27.58, 28.56, 29.44, 29.58, 29.74, 29.78, 32.01, 42.29, 44.51, 44.81, 46.80, 65.73, 70.57, 71.88, 77.58, 79.58, 124.19, 124.23, 130.75, 131.11, 131.66, 131.71, 133.47, 133.51, 133.76, 133.83, 148.21, 148.26, 155.54**.** HRMS-ESI m/z: $[M+K]^+$ calcd for $C_{53}H_{90}N_6O_{14}S_2$ 1137.5593, found 1137.5595.

*1.1.3.4. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-3-dodecyloxy-2-ethoxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-12-[N-ethyl-N-(2-nitrophenylsulfonyl)-amino]-4,9-diazadodecane (**5d**)*

Yield 73 %**.** ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 3 H, *J* = 6.9 Hz, $(CH_2)_{15}C\underline{H}_3$), 1.07 (t, 3 H, *J* = 7.0 Hz, $OCH_2C\underline{H}_3$), 1.14 (t, 3 H, *J* = 7.1 Hz, $NCH_2C\underline{H}_3$), 1.26 (br.s, 18 H, $(C\underline{H}_2)_9CH_3$), 1.40-1.49 (m, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.49-1.56 (m, 2 H, $OCH_2C\underline{H}_2$), 1.75-1.90 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.03-3.18 (m, 8H, 4 $C\underline{H}_2NBoc$), 3.26-3.66 (m, 15 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 4 NCH_2), 7.58-7.72 (m, 6 H) and 7.96-8.08 (m, 2 H, 2 C_6H_4). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.72, 14.22, 15.62, 22.78, 26.16, 27.02, 27.51, 28.54, 29.45, 29.58, 29.58, 29.67, 29.73, 32.01, 42.37, 44.74, 48.95, 65.72, 70.50, 71.87, 77.53, 79.57, 124.23, 130.75, 131.02, 131.11, 133.50, 133.70, 133.79, 148.15, 155.52. HRMS-ESI m/z: $[M+K]^+$ calcd for $C_{51}H_{86}N_6O_{14}S_2$ 1109.5280, found 1109.5293.

*1.1.3.5. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-3-decyloxy-2-ethyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-12-[N-ethyl-N-(2-nitrophenylsulfonyl)-amino]-4,9-diazadodecane (**5e**)*

Yield 69 %. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, 3 H, *J* = 6.9 Hz, $(CH_2)_{15}C\underline{H}_3$), 1.07 (t, 3 H, *J* = 7.0 Hz, $OCH_2C\underline{H}_3$), 1.14 (t, 3 H, *J* = 7.1 Hz, $NCH_2C\underline{H}_3$), 1.26 (br.s, 14 H, $(C\underline{H}_2)_7CH_3$), 1.38-1.48 (m, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.48-1.56 (m, 2 H, $OCH_2C\underline{H}_2$), 1.75-1.90 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.03-3.18 (m, 8H, 4 $C\underline{H}_2NBoc$), 3.20-3.68 (m, 15 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 4 NCH_2), 7.58-7.72 (m, 6 H) and 7.96-8.08 (m, 2 H, 2 C_6H_4). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.74, 14.22, 15.63, 22.78, 26.17, 27.04, 28.55, 29.43, 29.58, 29.68, 29.72, 32.00, 42.35, 44.74, 44.89, 65.72, 70.49, 71.87, 77.53, 79.56, 124.19, 124.23, 130.75, 131.03, 131.11, 131.69, 131.74, 133.50, 133.71, 133.79, 148.15, 148.21, 155.44, 155.58. HRMS-ESI m/z: $[M+Na]^+$ calcd for $C_{49}H_{82}N_6O_{14}S_2$ 1065.5228, found 1065.5230.

*1.1.3.6. 4,8-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-11-[N-ethyl-N-(2-nitrophenylsulfonyl)amino]-4,8-diazaundecane (**5f**)*

Yield 89 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3 H, *J* = 6.7, $(CH_2)_{15}C\underline{H}_3$), 1.07 (t, 3 H, *J* = 7.0, $OCH_2C\underline{H}_3$), 1.12 (t, 3 H, *J* = 7.3, $NCH_2C\underline{H}_3$) 1.24 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.42 (s, 18 H, 2 $C(C\underline{H}_3)_3$), 1.47-1.59 (m, 2 H, $OCH_2C\underline{H}_2$), 1.59-1.72 (m, 2 H) and 1.72-1.89 (m, 4 H, 3 $NCH_2C\underline{H}_2CH_2N$), 2.98-3.21 (m, 8 H, 4 $C\underline{H}_2NHBoc$), 3.21-3.68 (m, 15 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 4 NCH_2), 7.53-7.73 (m, 6 H) and 7.91-8.08 (m, 2 H, 2 C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.76, 14.17, 15.64, 22.78, 26.22, 27.15, 27.64, 28.60, 29.45, 29.61, 29.76, 29.81, 32.04, 42.36, 44.62, 44.82, 44.90, 45.24, 46.93, 49.35, 65.73, 70.71, 71.92, 77.58, 79.73, 124.19, 124.25, 130.82, 131.15, 131.63, 131.69, 133.43, 133.47, 133.87, 133.97, 148.32, 148.38, 155.49, 155.52**.** HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{56}H_{96}N_6O_{14}S_2$ 1141.6504, found 1141.6507.

*1.1.3.7. 3,6-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)-N-(2-nitrophenylsulfonyl)amino]-8-[N-ethyl-N-(2-nitrophenylsulfonyl)amino]-3,6-diazaoctane (**5g**)*

Yield 66 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3 H, *J* = 6.9, $(CH_2)_{15}C\underline{H}_3$), 0.98-1.10 (m, 3 H, $OCH_2C\underline{H}_3$), 1.14 (t, 3 H, *J* = 7.1, $NCH_2C\underline{H}_3$), 1.25 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.36-1.61 (m, 20 H, 2 $C(C\underline{H}_3)_3$, $OCH_2C\underline{H}_2$), 3.19-3.73 (m, 23 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$, 8 NCH_2), 7.54-7.74 (m, 6 H) and 7.95-8.11 (m, 2 H, 2 C_6H_4). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.80, 14.22, 15.62, 22.80, 26.21, 28.53, 29.47, 29.63, 29.78, 29.82, 32.05, 43.14, 43.52, 45.53, 65.68, 70.60, 71.87, 77.33, 80.30, 124.16, 124.23, 130.80, 131.23, 131.36, 131.82, 133.50, 133.61, 148.27, 155.46. HRMS-ESI m/z: $[M+K]^+$ calcd for $C_{53}H_{90}N_6O_{14}S_2$ 1137.5593, found 1137.5602.

1.1.4. General procedure for removal of 2-nitrophenylsulfonyl groups

Thiophenol (0.988 mmol) and potassium carbonate (0.988 mmol) were added to a solution of compounds **3a-g, 5a-g** (0.049 mmol) in anhydrous DMF (2 ml). The reaction mixture was stirred for 40 min at 23 ºC and then was filtered through Celite®. The organic solvent was removed under reduced pressure, and the residue was chromatographed on silica (CH_2Cl_2 – MeOH – 25% aq. ammonia, 8:1:0.1) to yield the products**.**

1.1.4.1. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-12-ethylamino-4,9-diazadodecane

Yield 97%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3 H, *J* = 6.8, $(CH_2)_{15}C\underline{H}_3$), 1.19 (t, 3 H, *J* = 7.0, $OCH_2C\underline{H}_3$), 1.24 (br.s, 33 H, $(C\underline{H}_2)_{15}CH_3$, $NCH_2C\underline{H}_3$), 1.38-1.49 (m, 24 H, $NCH_2(C\underline{H}_2)_2CH_2N$, 2 $C(C\underline{H}_3)_3$, $OCH_2C\underline{H}_2$), 1.58-2.05 (m, 2 H, 2 $NCH_2C\underline{H}_2CH_2N$), 2.52-2.92 (m, 8 H, 4 $NHC\underline{H}_2$), 3.05-3.36 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.36-3.78 (m, 7 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.14, 15.65, 22.79, 25.89, 26.20, 28.58, 28.61, 29.47, 29.57, 29.66, 29.79, 32.05, 43.62, 45.21, 47.18, 51.32, 65.73, 71.86, 79.51, 155.82. MS-ESI m/z: $[M+H]^+$ calcd for $C_{45}H_{92}N_4O_6$ 784.701, found 785.725.

1.1.4.2. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-hexadecyloxyprop-1-yl)amino]-12-ethylamino-4,9-diazadodecane

Yield 92 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ0 .87 (t, 3 H, *J* = 6.8, $(CH_2)_{15}C\underline{H}_3$), 1.20 (t, 3 H, *J* = 7.0, $OCH_2C\underline{H}_3$), 1.21-1.29 (m, 29 H, $(C\underline{H}_2)_{13}CH_3$, $NCH_2C\underline{H}_3$), 1.39-1.48 (m, 24 H, $OCH_2C\underline{H}_2$, $NCH_2(C\underline{H}_2)_2CH_2N$, 2 $C(C\underline{H}_3)_3$), 1.66-1.98 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 2.50-2.97 (m, 8 H, 4 $NHC\underline{H}_2$), 3.10-3.33 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.37-3.78 (m, 7 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.09, 15.53, 22.77, 25.90, 26.17, 28.40, 28.44, 29.45, 29.57, 29.67, 29.72, 29.79, 32.03, 43.46, 45.16, 47.18, 51.02, 65.73, 71.96, 79.32, 155.78. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{43}H_{88}N_4O_6$ 757.6782, found 757.6788.

1.1.4.3. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-tetradecyloxyprop-1-yl)amino]-12-ethylamino-4,9-diazadodecane

Yield 62 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, 3 H, *J* = 6.8, $(CH_2)_{15}C\underline{H}_3$), 1.13-1.22 (m, 6 H, $OCH_2C\underline{H}_3$, $NCH_2C\underline{H}_3$), 1.26 (br.s, 22 H, $(C\underline{H}_2)_{11}CH_3$), 1.40-1.49 (m, 24 H, $OCH_2C\underline{H}_2$, $NCH_2(C\underline{H}_2)_2CH_2N$, 2 $C(C\underline{H}_3)_3$), 1.61-1.90 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 2.55-2.81 (m, 8 H, 4 $NHC\underline{H}_2$), 3.03-3.35 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.36-3.77 (m, 7 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.18, 15.81, 22.80, 26.07, 26.27, 28.61, 28.64, 29.47, 29.62, 29.75, 29.78, 29.81, 32.05, 44.04, 45.19, 47.02, 47.54, 51.59, 65.67, 71.86, 72.06, 77.70, 79.42, 155.80. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{41}H_{84}N_4O_6$ 729.6470, found 729.6481.

1.1.4.4. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-3-dodecyloxy-2-ethyloxyprop-1-yl)amino]-12-ethylamino-4,9-diazadodecane

Yield 81 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, 3 H, *J* = 6.8, $(CH_2)_{15}C\underline{H}_3$), 1.12-1.21 (m, 6 H, $OCH_2C\underline{H}_3$, $NCH_2C\underline{H}_3$), 1.25 (br.s, 18 H, $(C\underline{H}_2)_9CH_3$), 1.37-1.47 (m, 24 H, $OCH_2C\underline{H}_2$, $NCH_2(C\underline{H}_2)_2CH_2N$, 2 $C(C\underline{H}_3)_3$), 1.61-1.97 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 2.50-2.91 (m, 8 H, 4 $NHC\underline{H}_2$), 3.02-3.34 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.34-3.76 (m, 7 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.24, 15.75, 22.79, 25.90, 26.18, 28.49, 28.55, 29.45, 29.57,

29.71, 29.77, 32.01, 43.76, 45.27, 47.13, 51.57, 65.66, 71.80, 79.54, 155.70. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{39}H_{80}N_4O_6$ 701.6156, found 701.6160.

1.1.4.5. 4,9-Bis(tert-butyloxycarbonyl)-1-[N-(rac-3-decyloxy-2-ethyloxyprop-1-yl)amino]-12-ethylamino-4,9-diazadodecane

Yield 73 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3 H, *J* = 6.8, $(CH_2)_{15}C\underline{H}_3$), 1.17 (t, 3 H, *J* = 7.0, $OCH_2C\underline{H}_3$), 1.19-1.27 (m, 17 H, $(C\underline{H}_2)_7CH_3$, $NCH_2C\underline{H}_3$), 1.45-1.54 (m, 24 H, $OCH_2C\underline{H}_2$, $NCH_2(C\underline{H}_2)_2CH_2N$, 2 $C(C\underline{H}_3)_3$), 1.58-1.97 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 2.53-2.89 (m, 8 H, 4 $NHC\underline{H}_2$), 3.05-3.34 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.34-3.78 (m, 7 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.23, 15.75, 22.77, 25.63, 26.18, 28.50, 28.55, 29.42, 29.56, 29.66, 29.70, 31.99, 43.80, 46.58, 47.14, 51.60, 65.65, 71.79, 79.52, 155.88. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{37}H_{88}N_4O_6$ 673.5843, found 673.5854.

1.1.4.6. 4,8-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-11-ethylamino-4,8-diazaundecane

Yield 84 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3 H, *J* = 7.0, $(CH_2)_{13}C\underline{H}_3$), 1.11-1.22 (m, 6 H, $NCH_2C\underline{H}_3$, $OCH_2C\underline{H}_3$), 1.24 (br.s., 30H, $(C\underline{H}_2)_{15}CH_3$), 1.43 (br.s, 18 H, 2 $C(C\underline{H}_3)_3$), 1.47-1.61 (m, 2 H, $OCH_2C\underline{H}_2$), 1.62-1.84 (m, 6 H, 3 $NCH_2C\underline{H}_2CH_2N$), 2.53-2.79 (m, 8 H, 4 $NHC\underline{H}_2$), 3.08-3.33 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.36-3.76 (m, 7 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.21, 15.80, 22.80, 26.24, 28.59, 28.61, 29.47, 29.61, 29.75, 29.77, 29.81, 32.04, 44.11, 45.06, 47.60, 51.61, 65.66, 71.82, 72.00, 77.71, 79.51, 155.65. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{44}H_{90}N_4O_6$ 771.6939, found 771.6949.

1.1.4.7. 3,6-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-8-ethylamino-3,6-diazaoctane

Yield 84 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3 H, J = 6.8, $(CH_2)_{15}C\underline{H}_3$), 1.20 (t, 3 H, J = 7.0, $OCH_2C\underline{H}_3$), 1.28 (br.s, 33 H, $(C\underline{H}_2)_{15}CH_3$, $NCH_2C\underline{H}_3$), 1.47 (br.s, 18 H, 2 $C(C\underline{H}_3)_3$), 1.52-1.60 (m, 2 H, $OCH_2C\underline{H}_2$), 2.53-3.03 (m, 8 H, 4 $NHC\underline{H}_2$), 3.20-3.77 (m, 15 H, 4 $C\underline{H}_2NBoc$, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.22, 15.81, 22.81, 26.25, 28.61, 29.48, 29.62, 29.77, 29.82, 32.05, 46.19, 47.78, 47.86, 48.37, 51.45, 65.68, 71.84, 77.82, 80.09, 155.66. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{41}H_{84}N_4O_6$ 729.6469, found 729.6471.

1.1.4.8. 4,9-Bis(tert-butyloxycarbonyl)-1-(rac-2-ethoxy-3-octadecyloxyprop-1-yl)amino-12-amino-4,9-diazadodecane

Yield 72%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, *J* = 6.8 Hz, 3 H, $(CH_2)_{15}C\underline{H}_3$), 1.19 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.25 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.39-1.47 (m, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.48-1.56 (m, 2 H, $OCH_2C\underline{H}_2$), 1.63-1.74 (m, 2 H) and 1.75-1.90 (m, 2 H, 2 $NCH_2C\underline{H}_2CH_2N$), 2.52-2.93 (m, 6 H, $C\underline{H}_2NHC\underline{H}_2$, $C\underline{H}_2NH_2$), 3.04-3.37 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.37-3.78 (m, 7 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.23, 15.78, 22.81, 25.81, 26.24, 28.60, 29.48, 29.62, 29.81, 32.05, 45.33, 46.93, 51.64, 65.70, 71.88, 79.82, 155.81. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{43}H_{88}N_4O_6$ 757.6782, found 757.6781.

1.1.4.9. 4,9-Bis(tert-butyloxycarbonyl)-1-[(rac-2-ethyloxy-3-hexadecyloxyprop-1-yl)amino]-12-amino-4,9-diazadodecane

Yield 71 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.7 Hz, 3 H, $(CH_2)_{13}C\underline{H}_3$), 1.19 (t, *J* = 7.0, 3 H, $OCH_2C\underline{H}_3$), 1.28 (br.s, 26 H, $(C\underline{H}_2)_{13}CH_3$), 1.41-1.48 (m, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.50-1.59 (m, 2 H, $OCH_2C\underline{H}_2$), 1.59-1.78 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 2.51-2.63 (m, 2H) and 2.63-2.79 (m, 4 H, $C\underline{H}_2NHC\underline{H}_2$, $C\underline{H}_2NH_2$), 3.04-3.33 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.35-3.77 (m, 7 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.25, 15.80, 22.82, 25.80, 26.24, 28.60, 29.48, 29.61, 29.78, 29.82, 32.05, 49.89, 46.74, 51.59, 65.69, 71.83, 71.94, 79.49, 155.72. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{41}H_{84}N_4O_6$ 729.6469, found 729.6471.

1.1.4.10. 4,8-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-11-amino-4,8-diazaundecane

Yield 63 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.8 Hz, 3 H, $(CH_2)_{15}C\underline{H}_3$), 1.14-1.235 (m, 30 H, $(C\underline{H}_2)_{15}CH_3$, $OCH_2C\underline{H}_3$), 1.40-1.49 (m, 22 H, 2 $C(C\underline{H}_3)_3$, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.63-1.94 (m, 2 H, $OCH_2C\underline{H}_2$), 1.63-1.94 (m, 6 H, 3 $NCH_2C\underline{H}_2CH_2N$), 2.49-2.92 (m, 6 H, $C\underline{H}_2NHC\underline{H}_2$, $C\underline{H}_2NH_2$), 3.02-3.35 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.36-3.49 (m, 4 H), 3.49-3.62 (m, 1 H), 3.63-3.77 (m, 2 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.23, 15.76, 22.81, 26.23, 28.32, 28.60, 29.48, 29.62, 29.77, 29.83, 32.05, 45.11, 65.71, 71.91, 79.78, 155.68.

1.1.4.11. 3,6-Bis(tert-butyloxycarbonyl)-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-8-amino-3,6-diazaoctane

Yield 84 %. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3 H, *J* = 6.7 Hz, $(CH_2)_{15}C\underline{H}_3$), 1.19 (t, 3 H, *J* = 7.0 Hz, $OCH_2C\underline{H}_3$), 1.25 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.46 (br.s, 18 H, 2 $C(C\underline{H}_3)_3$), 1.51-1.60 (m, 2 H, $OCH_2C\underline{H}_2$), 2.63-2.92 (m, 6 H, $C\underline{H}_2NHC\underline{H}_2$, $C\underline{H}_2NH_2$), 3.17-3.38 (m, 8 H, 4 $C\underline{H}_2NBoc$), 3.38-3.75 (m, 7 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.98, 15.62, 22.64, 25.87, 26.03, 28.28, 28.54, 29.20, 29.31, 29.44, 29.53, 29.60, 29.66, 31.89, 42.02, 45.86, 47.80, 54.89, 65.58, 77.37, 79.01, 155.50. MS-ESI m/z: $[M+H]^+$ calcd for $C_{39}H_{80}N_4O_6$ 701.62, found 701.97.

1.1.5. General procedure for Boc-deprotection

A solution of 3 *N* HCl in anhydrous dioxane (1 ml) was added to a pre-cooled (4 °C) solution of Boc-derivatives (0.048 mmol) in anhydrous dichloromethane (2 ml). After the reaction mixture was stirred for 2 h at 23 ºC, the volatiles were removed under reduced pressure, and the residue was re-crystallized from diethyl ether (4 ml) to afford final products as beige or white flakes.

*1.1.5.1. 12-Ethylamino-1-[(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**6a**)*

Yield (97 %). ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD): δ 0.86 (t, 3 H, *J* = 6.8, $(CH_2)_{15}C\underline{H}_3$), 1.24-1.31 (m, 36 H, $(C\underline{H}_2)_{15}CH_3$, $OCH_2C\underline{H}_3$, $NCH_2C\underline{H}_3$), 1.51-1.61 (m, 2 H, $OCH_2C\underline{H}_2$), 1.78-1.87 (m, 4 H, $NCH_2(C\underline{H}_2)_2CH_2N$), 2.08-2.24 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.00-3.23 (m, 16 H, 8 $C\underline{H}_2NH$), 3.40-3.54 (m, 2 H, $OC\underline{H}_2CH_2$) and (1 H, $OCH\underline{H}_bCH$), 3.54-3.64 (m, 1 H, $OCH\underline{H}_bCH_3$) and (1 H, $OCH\underline{H}_aCH$), 3.70-3.79 (m, 1 H, $OCH\underline{H}_bCH_3$), 3.80-3.88 (m, 1 H $C\underline{H}OCH_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.43, 14.65, 15.72, 23.49, 23.65, 23.70, 23.82, 26.78, 30.14, 30.24, 30.33, 30.55, 30.61, 32.81, 44.06, 44.90, 45.58, 45.64, 46.01, 48.06, 49.96, 66.64, 70.82, 72.63, 74.78. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{35}H_{76}N_4O_2$ 585.6047, found 585.6044.

*1.1.5.2. 12-Ethylamino-1-[(rac-2-ethyloxy-3-hexadecyloxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**6b**)*

Yield 72 %. ^{1}H NMR (600 MHz, CD_3OD-D_2O): δ 0.88 (t, 3 H, *J* = 6.8, $(CH_2)_{13}C\underline{H}_3$), 1.22 (t, 3 H, *J* = 7.0, $NCH_2C\underline{H}_3$) 1.25-1.33 (m, 29 H, $(C\underline{H}_2)_{13}CH_3$, $OCH_2C\underline{H}_3$), 1.50-1.61 (m, 2 H, $OCH_2C\underline{H}_2$), 1.77-1.89 (m, 4 H, $NCH_2(C\underline{H}_2)_2CH_2N$), 2.08-2.25 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.06-3.27 (m, 16 H, 8 $C\underline{H}_2NH$), 3.44-3.54 (m, 2 H, $OC\underline{H}_2CH_2$) and (1 H, $OCH\underline{H}_bCH$), 3.57-3.69 (m, 1 H, $OCH\underline{H}_bCH_3$) and (1 H, $OCH\underline{H}_aCH$), 3.72-3.80 (m, 1 H, $OCH\underline{H}_bCH_3$), 3.83-3.91 (m, 1 H $C\underline{H}OCH_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.51, 14.54, 15.69, 23.46, 23.69, 23.84, 23.88, 26.75, 30.11, 30.14, 30.23, 30.43, 30.45, 32.75, 44.12, 45.00, 45.65, 45.96, 48.08, 50.00, 66.61, 70.52, 72.72, 74.76. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{33}H_{73}N_4O_2$ 557.5734, found 557.5728.

*1.1.5.3. 12-Ethylamino-1-[(rac-2-ethyloxy-3-tetradecyloxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**6c**)*

Yield 92 %. ^{1}H NMR (600 MHz, CD_3OD-D_2O): δ 0.88 (t, 3 H, *J* = 6.8, $(CH_2)_{11}C\underline{H}_3$), 1.25 (t, 3 H, *J* = 7.0, $NCH_2C\underline{H}_3$) 1.29 (br.s, 22 H, $(C\underline{H}_2)_{11}CH_3$) , 1.36 (t, 3 H, *J* = 7.3, $OCH_2C\underline{H}_3$), 1.56-

1.64 (m, 2 H, $OCH_2C\underline{H}_2$), 1.83-1.94 (m, 4 H, $NCH_2(C\underline{H}_2)_2CH_2N$), 2.14-2.28 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.07-3.30 (m, 16 H, 8 $C\underline{H}_2NH$), 3.47-3.59 (m, 2 H, $OC\underline{H}_2CH_2$) and (1 H, $OCH\underline{H}_bCH$), 3.60-3.69 (m, 1 H, $OCH\underline{H}_bCH_3$) and (1 H, $OCH\underline{H}_aCH$), 3.76-3.83 (m, 1 H, $OCH\underline{H}_bCH_3$), 3.85-3.90 (m, 1 H $C\underline{H}OCH_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.50, 14.32, 15.67, 23.45, 23.79, 24.00, 24.07, 26.88, 30.10, 30.25, 30.30, 30.39, 30.41, 32.76, 44.23, 45.23, 45.82, 46.16, 48.20, 50.36, 66.56, 70.57, 72.89, 74.84. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{31}H_{69}N_4O_2$ 529.5421, found 529.5415.

*1.1.5.4. 1-[(rac-3-Dodecyloxy-2-ethoxyprop-1-yl)amino]-12-ethylamino-4,9-diazadodecane tetrahydrochloride (**6d**)*

Yield 94 %. ^{1}H NMR (300 MHz, CD_3OD-D_2O): δ 0.86 (t, 3 H, *J* = 6.8, $(CH_2)_9C\underline{H}_3$), 1.15-1.38 (m, 24 H, $NCH_2C\underline{H}_3$, $(C\underline{H}_2)_9CH_3$, $OCH_2C\underline{H}_3$), 1.49-1.63 (m, 2 H, $OCH_2C\underline{H}_2$), 1.76-1.91 (m, 4 H, $NCH_2(C\underline{H}_2)_2CH_2N$), 2.06-2.28 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.02-3.28 (m, 16 H, 8 $C\underline{H}_2NH$), 3.44-3.57 (m, 2 H, $OC\underline{H}_2CH_2$) and (1 H, $OCH\underline{H}_bCH$), 3.57-3.79 (m, 3 H, $OCH\underline{H}_bCH_3$, $OCH\underline{H}_aCH$, $OCH\underline{H}_bCH_3$), 3.79-3.90 (m, 1 H $C\underline{H}OCH_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.36, 14.21, 15.51, 23.11, 23.51, 23.69, 23.78, 26.44, 29.68, 29.80, 29.98, 32.37, 44.06, 44.93, 45.60, 45.93, 48.04, 50.02, 66.50, 70.26, 72.73, 74.52. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{29}H_{65}N_4O_2$ 501.5108, found 501.5102.

*1.1.5.5. 1-[(rac-3-Decyloxy-2-ethoxyprop-1-yl)amino]-12-ethylamino-4,9-diazadodecane tetrahydrochloride (**6e**)*

Yield 65 %**.** ^{1}H NMR (300 MHz, CD_3OD-D_2O): δ 0.86 (t, 3 H, *J* = 6.8, $(CH_2)_7C\underline{H}_3$), 1.13-1.36 (m, 24 H, $NCH_2C\underline{H}_3$, $(C\underline{H}_2)_7CH_3$, $OCH_2C\underline{H}_3$), 1.50-1.63 (m, 2 H, $OCH_2C\underline{H}_2$), 1.77-1.91 (m, 4 H, $NCH_2(C\underline{H}_2)_2CH_2N$), 2.08-2.27 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.05-3.29 (m, 16 H, 8 $C\underline{H}_2NH$), 3.46-3.58 (m, 2 H, $OC\underline{H}_2CH_2$) and (1 H, $OCH\underline{H}_bCH$), 3.58-3.70 (m, 1 H, $OCH\underline{H}_bCH_3$) and (1 H, $OCH\underline{H}_aCH$), 3.70-3.82 (m, 1 H, $OCH\underline{H}_bCH_3$), 3.82-3.90 (m, 1 H $C\underline{H}OCH_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.42, 14.24, 15.56, 23.20, 23.61, 23.80, 23.90, 26.57, 29.80, 29.92, 29.94, 30.06, 30.10, 32.47, 44.12, 45.03, 45.68, 45.99, 48.11, 50.12, 66.53, 70.32, 72.80, 74.62. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{27}H_{61}N_4O_2$ 473.4795, found 473.4789.

*1.1.5.6. 11-Ethylamino-1-[(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-4,8-diazaundecane tetrahydrochloride (**6f**)*

Yield 95 %. ^{1}H NMR (600 MHz, CD_3OD-D_2O): δ 0.88 (t, 3 H, *J* = 6.8, $(CH_2)_{15}C\underline{H}_3$), 1.22 (t, 3 H, *J* = 7.0, $NCH_2C\underline{H}_3$) 1.26 (br.s., 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.32 (t, 3 H, *J* = 7.3, $OCH_2C\underline{H}_3$), 1.52-1.60 (m, 2 H, $OCH_2C\underline{H}_2$), 2.10-2.24 (m, 6 H, 3 $NCH_2C\underline{H}_2CH_2N$), 3.09-3.27 (m, 16 H, 8 $C\underline{H}_2NH$), 3.47-3.56 (m, 2 H, $OC\underline{H}_2CH_2$) and (1 H, $OCH\underline{H}_bCH$), 3.57-3.69 (m, 1 H, $OCH\underline{H}_bCH_3$) and (1 H, $OCH\underline{H}_aCH$), 3.71-3.80 (m, 1 H, $OCH\underline{H}_bCH_3$), 3.82-3.90 (m, 1 H $C\underline{H}OCH_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.37, 14.31, 15.56, 23.23, 23.56, 23.70, 23.87, 26.56, 29.84, 29.92, 29.97, 30.08, 30.11, 32.50, 44.07, 44.89, 45.71, 45.78, 45.92, 50.03, 66.54, 70.40, 72.75, 74.63. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{34}H_{75}N_4O_2$ 571.5890, found 571.5878.

*1.1.5.7. 8-Ethylamino-1-[(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-3,6-diazaoctane tetrahydrochloride (**6g**)*

Yield 92 %. ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD): δ 0.86 (t, 3 H, *J* = 6.8, $(CH_2)_{15}C\underline{H}_3$), 1.25 (br.s, 33 H, $(C\underline{H}_2)_{15}CH_3$, $NCH_2C\underline{H}_3$), 1.37 (t, 3 H, *J* = 7.1, $OCH_2C\underline{H}_3$), 1.52-1.65 (m, 2 H, $OCH_2C\underline{H}_2$), 3.06-3.25 (m, 16 H, 8 $C\underline{H}_2NH$), 3.37-3.68 (m, 4 H, $OC\underline{H}_2CH_2$, $OC\underline{H}_2CH$, $OCH\underline{H}_aCH_3$), 3.71-3.82 (m, 1 H, $OCH\underline{H}_bCH_3$), 3.86-3.93 (m, 1 H $C\underline{H}OCH_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.43, 14.57, 15.67, 23.38, 26.66, 30.00, 30.08, 30.16, 30.38, 32.68, 44.39, 44.63, 44.72, 45.57, 45.84, 45.98, 50.29, 66.65, 70.50, 72.69, 74.68. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{31}H_{69}N_4O_2$ 529.5421, found 529.5415.

*1.1.5.8. 12-Amino-1-[(rac-2-Ethoxy-3-octadecyloxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**7a**)*

Yield (99 %). ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD): 0.88 (t, *J* = 6.9 Hz, 3 H, $(CH_2)_{15}C\underline{H}_3$), 1.24 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.28 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.51-1.63 (m, 2 H, $OCH_2C\underline{H}_2$), 1.78-1.96 (m, 4 H, $NCH_2(C\underline{H}_2)_2CH_2N$), 2.05-2.15 (m, 2 H) and 2.15-2.25 (m, 2 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.01-3.27 (m, 14 H, 7 $C\underline{H}_2NH$), 3.52-3.71 (m, 4 H) and 3.71-3.86 (m, 3 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.36, 15.54, 23.19, 23.61, 23.81, 23.76, 24.79, 26.44, 29.74, 29.78, 29.83, 29.91, 29.99, 32.42, 37.60, 39.90, 45.58, 45.87, 49.96, 66.53, 70.21, 72.72, 74.63. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{33}H_{73}N_4O_2$ 557.5734, found 557.5728.

*1.1.5.9. 12-Amino-1-[(rac-2-ethoxy-3-hexadecyloxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**7b**)*

Yield 98 %. ^{1}H NMR (300 MHz, $CDCl_3$-CD_3OD): 0.87 (t, *J* = 6.7, 3 H, $(CH_2)_{13}C\underline{H}_3$), 1.28 (br.s, 29 H, $OCH_2C\underline{H}_3$, $(C\underline{H}_2)_{15}CH_3$), 1.49-1.63 (m, 2 H, $OCH_2C\underline{H}_2$), 1.74-1.92 (m, 4 H, $NCH_2(C\underline{H}_2)_2CH_2N$), 2.04-2.27 (m, 4 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.02-3.29 (m, 14 H, 7 $C\underline{H}_2NH$), 3.47-3.80 (m, 7 H $C\underline{H}_2OC\underline{H}_2CH_2$, $CHOC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.27, 15.48, 23.06, 23.53, 23.80, 24.79, 26.31, 29.54, 29.63, 29.73, 29.79, 32.27, 37.59, 39.90, 45.56, 45.80, 48.01, 49.94, 66.48, 69.97, 72.75, 74.50. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{31}H_{69}N_4O_2$ 529.5421, found 529.5419.

*1.1.5.10. 11-Amino-1-[N-(rac-2-ethoxy-3-octadecyloxyprop-1-yl)amino]-4,8-diazaundecane tetrahydrochloride (**7c**)*

Yield 89 %. ^{1}H NMR (300 MHz, CD_3OD-D_2O): 0.88 (t, *J* = 6.9 Hz, 3 H, $(CH_2)_{15}C\underline{H}_3$), 1.22 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\underline{H}_3$), 1.27 (br.s, 30 H, $(C\underline{H}_2)_{15}CH_3$), 1.52-1.62 (m, 2 H, $OCH_2C\underline{H}_2$), 2.06-2.26 (m, 6 H, 2 $NCH_2C\underline{H}_2CH_2N$), 3.07-3.27 (m, 14 H, 7 $C\underline{H}_2NH$), 3.45-3.55 (m, 3 H), 3.57-3.70 (m, 2 H), 3.72-3.80 (m, 1H) and 3.84-3.90 (m, 1 H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.68, 15.72, 23.51, 23.67, 23.76, 24.82, 26.76, 30.10, 30.24, 30.30, 30.57, 32.81, 37.59, 45.66, 45.76, 45.91, 49.95, 66.65, 70.62, 72.67, 74.77. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{32}H_{71}N_4O_2$ 543.5577, found 543.5577.

*1.1.5.11. 12-Amino-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-3,6-diazaoctane tetrahydrochloride (**7d**)*

Yield 74 %. ^{1}H NMR (300 MHz, $CDCl_3$-CD_3OD): 0.87 (t, 3 H, *J* = 6.8 Hz, $(CH_2)_{15}C\underline{H}_3$), 1.19-1.27 (m, 33 H, $(C\underline{H}_2)_{15}CH_3$, $OCH_2C\underline{H}_3$), 1.51-1.62 (m, 2 H, $OCH_2C\underline{H}_2$), 3.09-3.52 (m, 14 H, 7 $C\underline{H}_2NH$), 3.58-3.94 (m, 7H, $C\underline{H}_2OC\underline{H}_2CH_2$, $C\underline{H}OC\underline{H}_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.57, 15.80, 23.55, 23.65, 26.82, 30.18, 30.29, 30.36, 30.45, 30.63, 30.86, 32.85, 37.31, 39.86, 44.71, 45.73, 45.76, 45.82, 50.28, 66.73, 70.67, 72.69, 74.77. HRMS-ESI m/z: $[M+H]^+$ calcd for $C_{29}H_{65}N_4O_2$ 501.5108, found 501.5099.

1.2. Biology

1.2.1. Cell lines and culture conditions

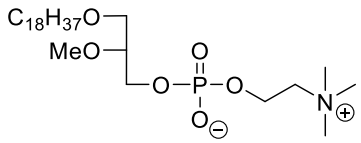
All reagents were from Sigma-Aldrich unless specified otherwise. The HCT116 (colon carcinoma), K562 (chronic myelogenous leukemia), and MCF7 (breast adenocarcinoma) cell lines were from American Type Culture Collection (Manassas, VA). Cells were propagated at 37°C, 5% CO_2 in Dulbecco modified Eagle's medium (HCT116 and MCF7) or RPMI-1640 (K562) media supplemented with 5% fetal bovine serum (HyClone, Logan, UT), penicillin (100 U ml^{-1}), streptomycin (100 μg ml^{-1}) and 2 mM *L*-glutamine.

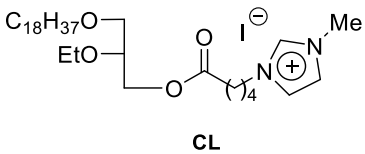
1.2.2. MTT-assay

The MTT-assay was used to evaluate the cytotoxicity of our compounds. For this purpose, HCT-116, K562, and MCF7 sells were seeded in 96-well plates at a density of $5x10^3$ cells per well and incubated for 24 h. The cells were then treated with aliquots of lipophilic polyamines solutions in water to reach final concentrations of 0.1; 0.2; 0.4; 0.8; 1.6; 3.2; 6.4; 12.5; 25; 50 μM. After 72 h of incubation at 37°C and 5% CO_2, 20 μl of MTT-solutions [3-(4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 5 mg ml^{-1} in distilled water) were added per well, and the plates were incubated for an additional 2 h. The supernatant was removed, DMSO (100 μl) was added to each well, and the plates were mixed to dissolve the formazan crystals. The optical density was measured at 571 nm on Multiscan FC (Thermo Scientific, USA). Finally, the results were processed using GraphPad Prism software version 6. The cytotoxicity data are expressed in IC_{50} values (concentration that inhibits 50% of cell growth). For each compound, the assay was repeated at least in triplicate.

1.2.3. Hemolysis

To study hemolysis, erythrocytes were obtained from peripheral blood of healthy persons. The blood was collected in test-tubes containing 1% sodium citrate, stirred, and incubated for 1 h at 4 °C. The suspension of erythrocytes was washed with buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4), the cells were suspended in 50 μl of the same buffer. Tested compounds were added to the final concentrations given in Table S2. The volume of the added solutions of tested glycerolipids did not exceed 10% of a total volume of the mixture. In the control test-tube, distilled water (50 μl) was added to the suspension of erythrocytes. The samples were incubated for 1 h at 37 °C with gentle stirring, then centrifuged at 2000 rpm. Optical density of the supernatant was measured on a LKB spectrophotometer (Sweden) at absorption wavelength λ = 545 nm. The obtained values of absorption were compared with the hemolysis induced by water (the absorption was taken as 100%).





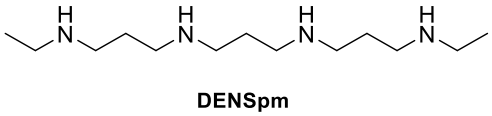


Table S1. Cytotoxicity of modified polyamines for human tumour cell lines.

Compound	IC_{50}[a], μmol dm^{-3}		
	K562	HCT116	MCF7
6a	1.30 ±0.40	6.20±1.90	5.80±0.93
6b	1.89±0.05	3.29±0.03	1.82±0.41
6c	2.07±1.12	3.21±0.22	1.94±0.12
6d	2.01±0.13	2.97±0.19	1.93±0.48
6e	2.25±0.25	3.83±0.07	1.86±0.31
6f	1.85±0.34	1.64±0.02	1.28±0.24
6g	1.80±0.10	2.70±0.50	n.d.[b]
7a	1.90±0.20	8.50±0.70	2.70±0.93
7b	2.06±0.11	4.23±0.12	1.99±0.36
7c	2.18±0.30	3.33±0.14	2.02±0.44
7d	2.40±0.10	6.80±1.10	n.d.
Edelfosine	32.0±2.0	1.0±0.5	6.5±0.5
CL	17.0±0.60	10.8±0.30	20.4±0.60
DENSpm	>50	>50	>50

[a]The IC_{50} value represents the concentration that induced 50% cell death after a 72 h exposure;

[b]n.d. – no data.

Table S2. Hemolytic effect of lipophilic polyamines.

Compound	Hemolysis[a] (%)
6b	8.8
6c	8.1
6d	6.8
6e	2.9
7b	8.3
6f	4.7
7c	6.3
Edelfosine[b,c]	18.5
CL[c]	2.8
DENSpm	2.9

[a]Hemolysis caused by resuspension of erythrocytes in pure water was taken as 100%;

[b]Edelfosine concentration was 8 μmol dm^{-3}

[c]E. V. Shmendel, K. A. Perevoshchikova, D. K. Shishova, T. S. Kubasova, L. L. Tyutyunnik, M. A. Maslov, A. A. Shtil, *Russ. Chem. Bull.*, 2015, **64**, 1648.

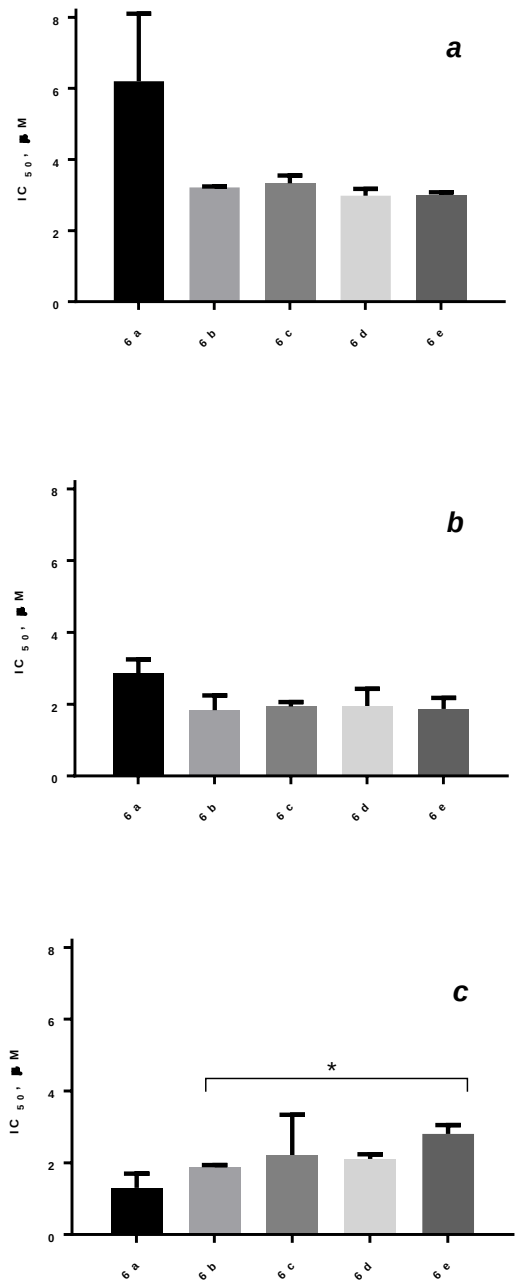


Figure S1. The impact of the length of the *O*-alkyl chain at the C1 position of the glycerol backbone on polyamines cytotoxicity against (***a***) colon cancer (HCT116), (***b***) breast adenocarcinoma (MCF-7), and (***c***) myelogenous leukaemia (K562) cells. The data are presented as mean values ± SEM for n = 3 experiments. No significant difference in the row C10-C18 was observed by the paired t-test, with the exception of pair **6b** and **6e** against the K562 cells, *P < 0.1.

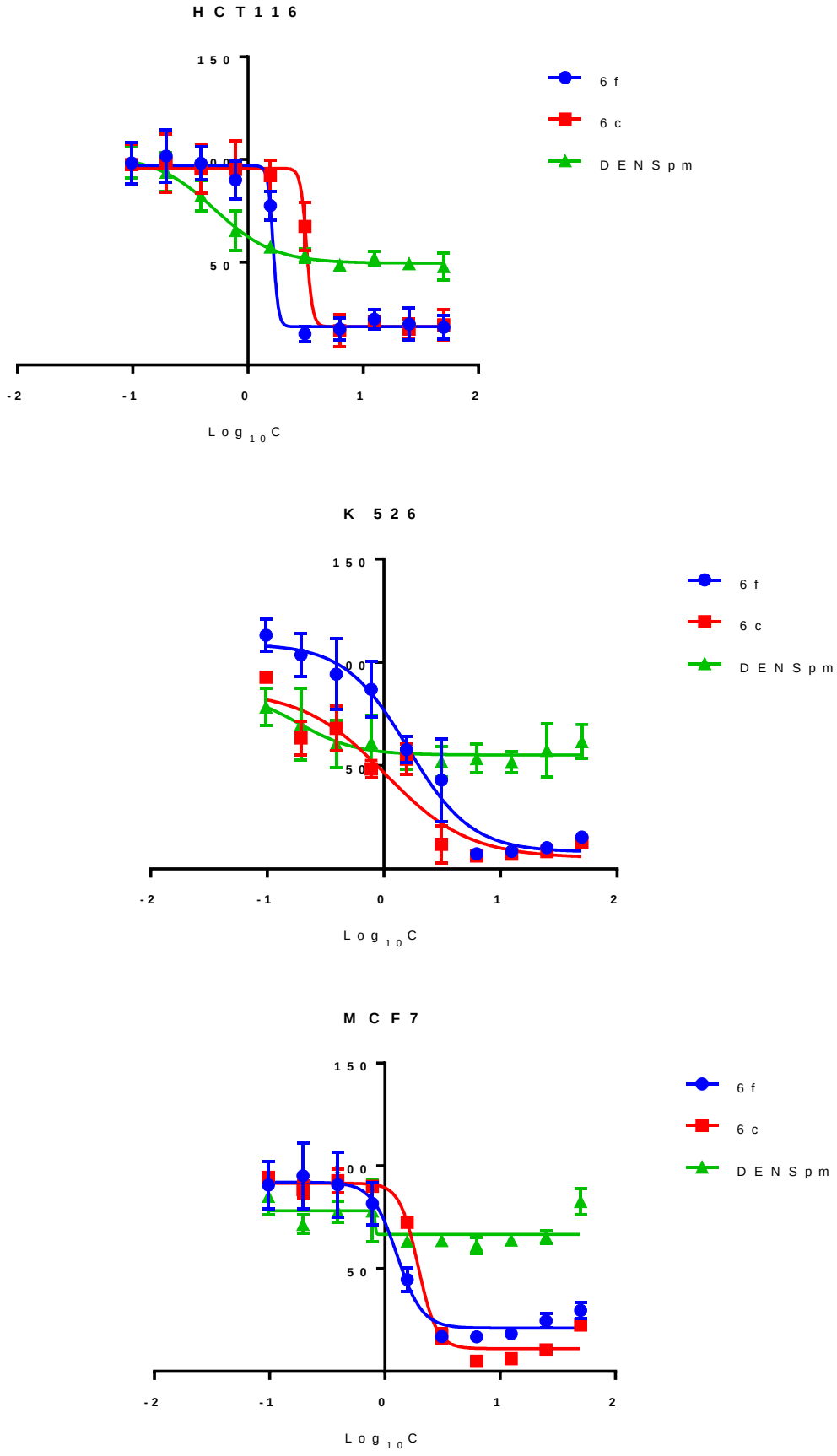
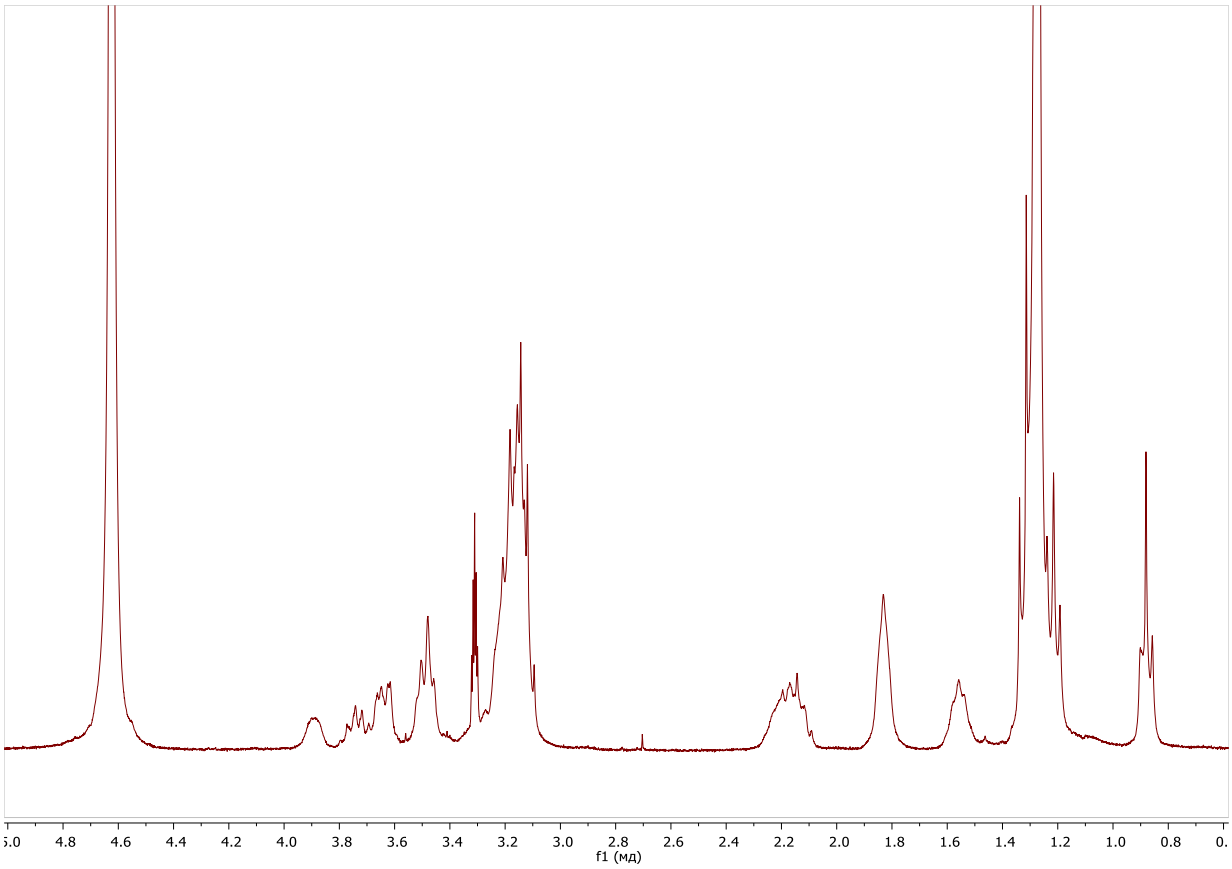
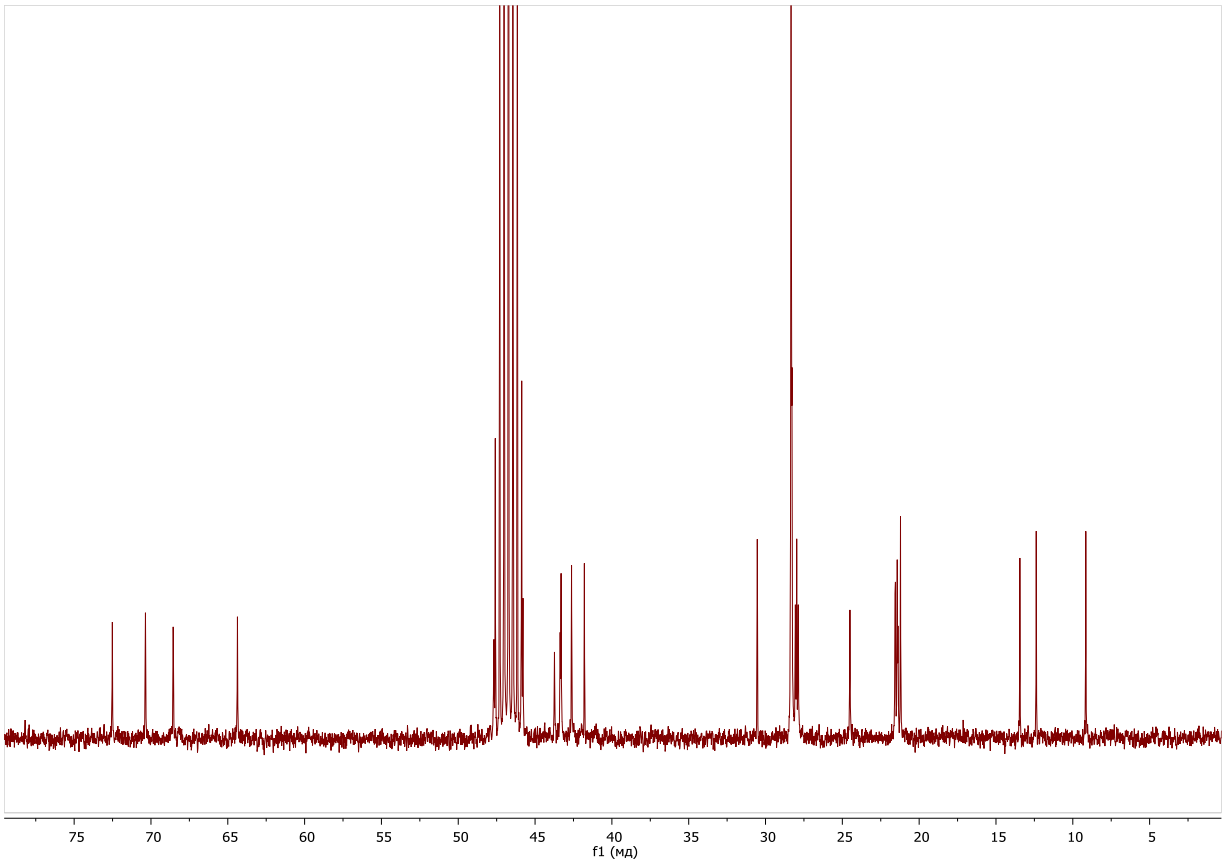
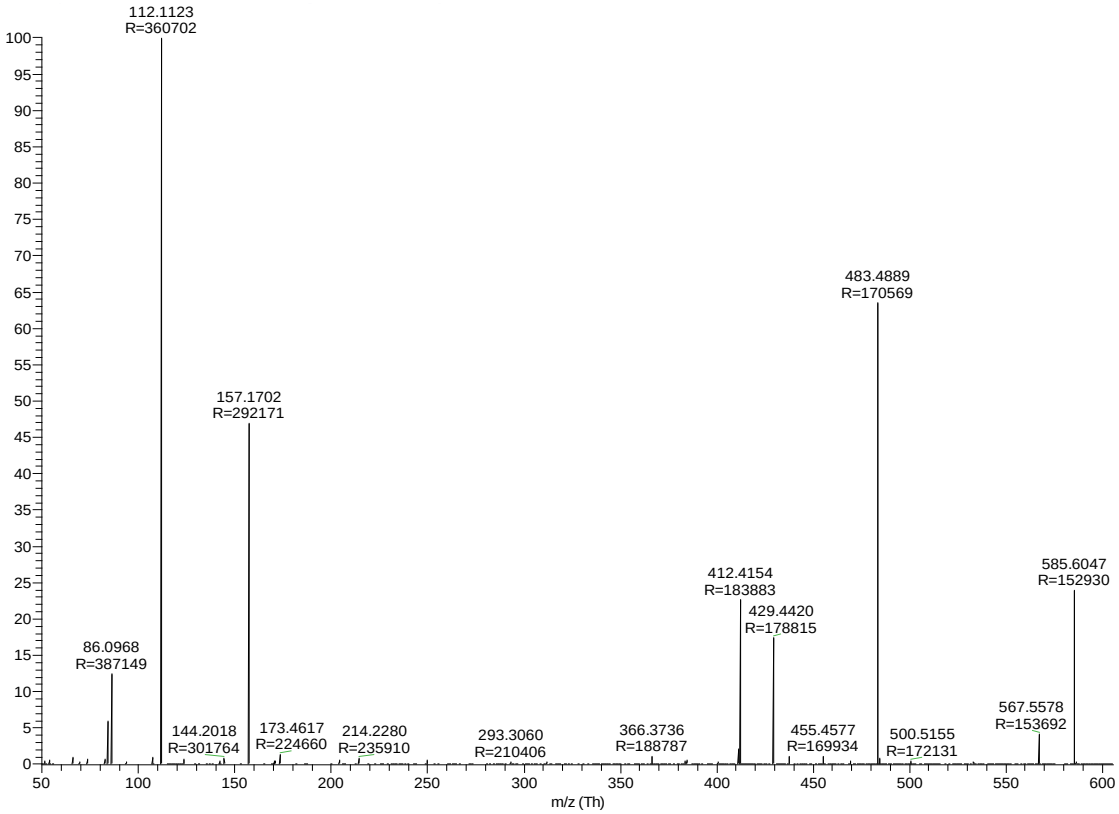


Figure S2. Cytotoxic profiles of **6c**, **6f** and **DENSpm** against HCT 116, K562 and MCF7 cells.

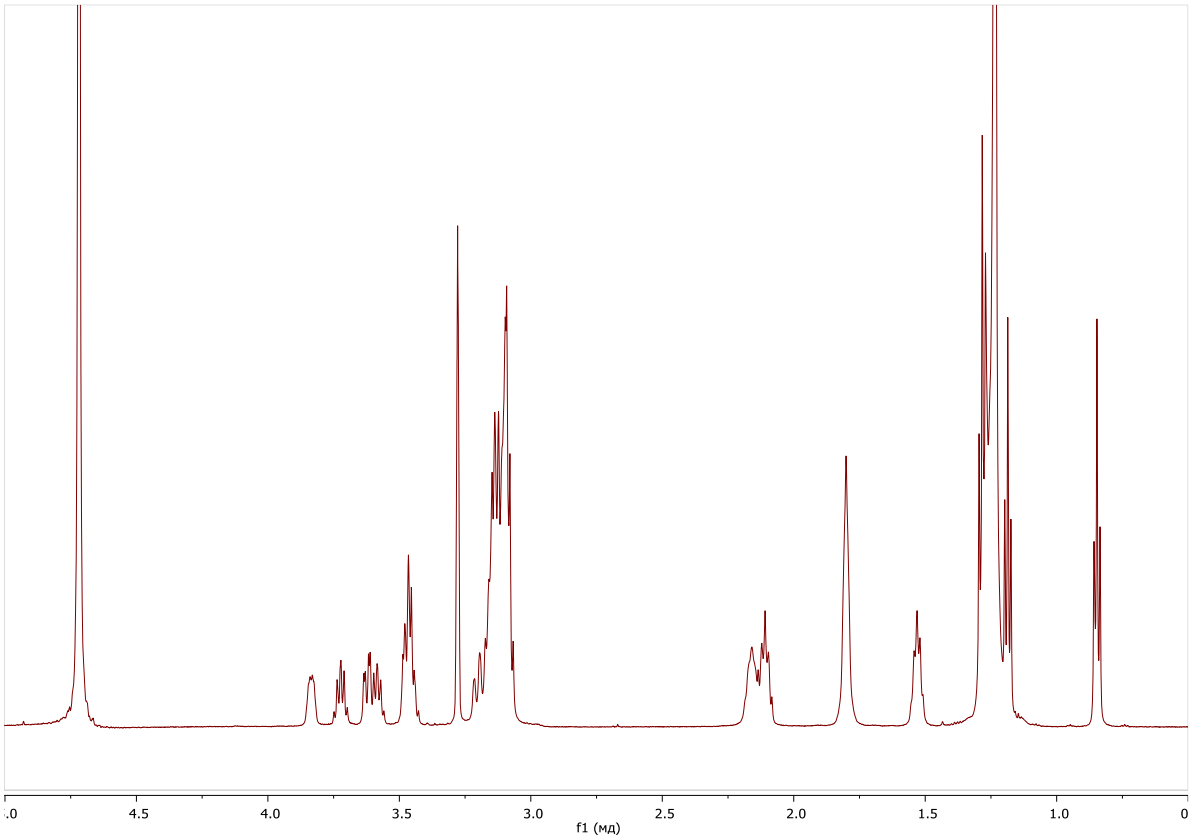
*12-Ethylamino-1-[(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**6a**)*

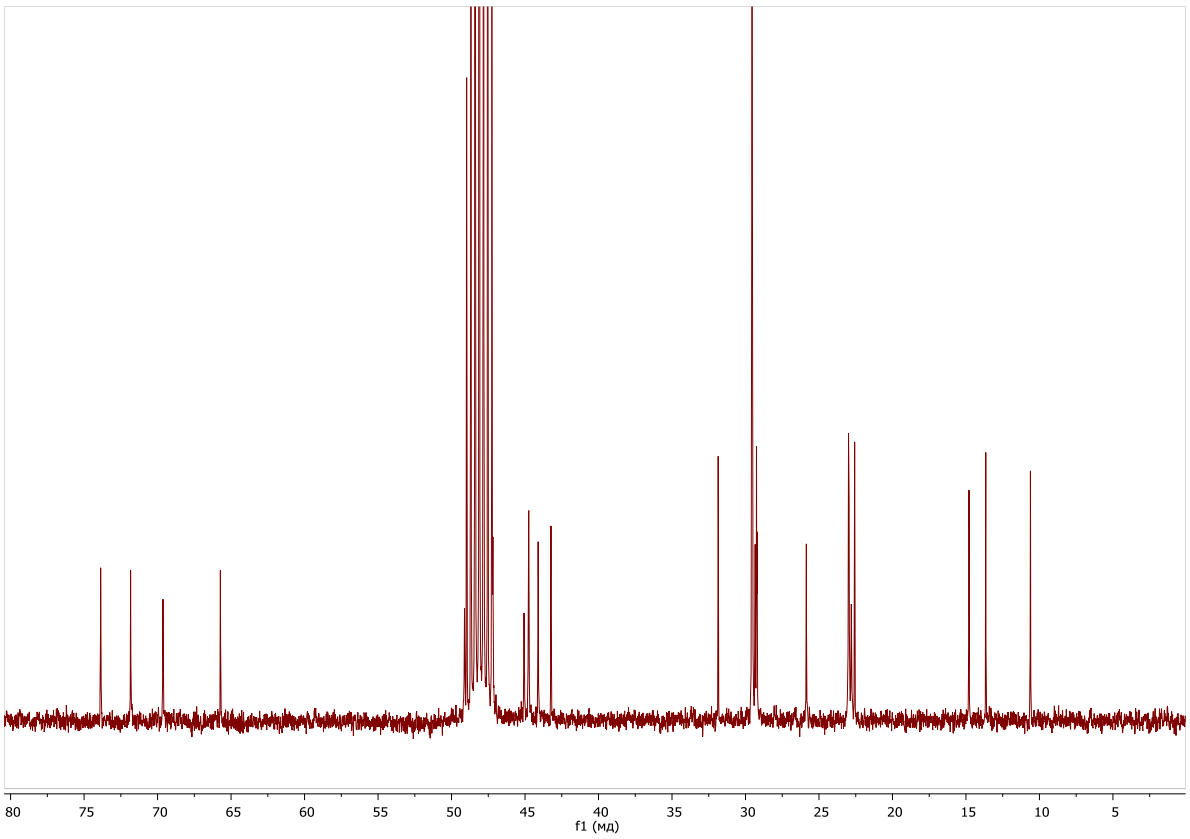






*12-Ethylamino-1-[(rac-2-ethyloxy-3-hexadecyloxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**6b**)*

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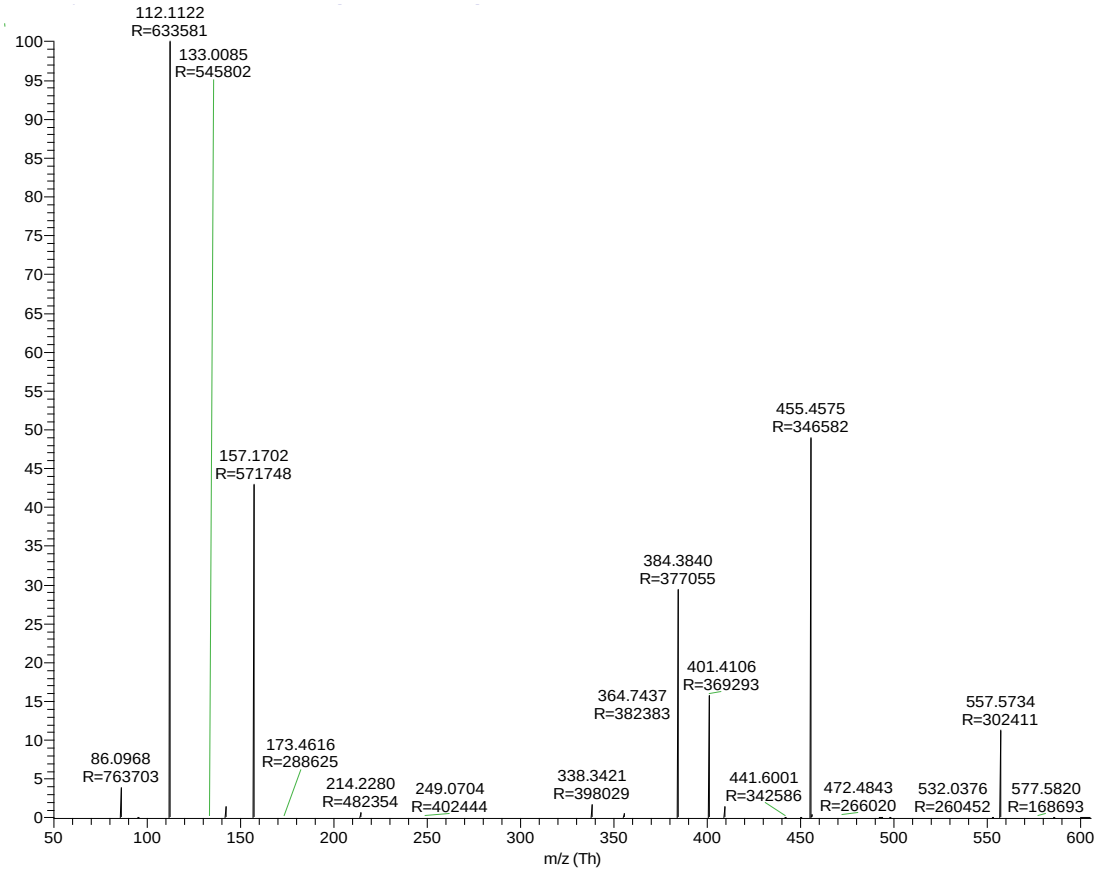
f1 (мд)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532.0376
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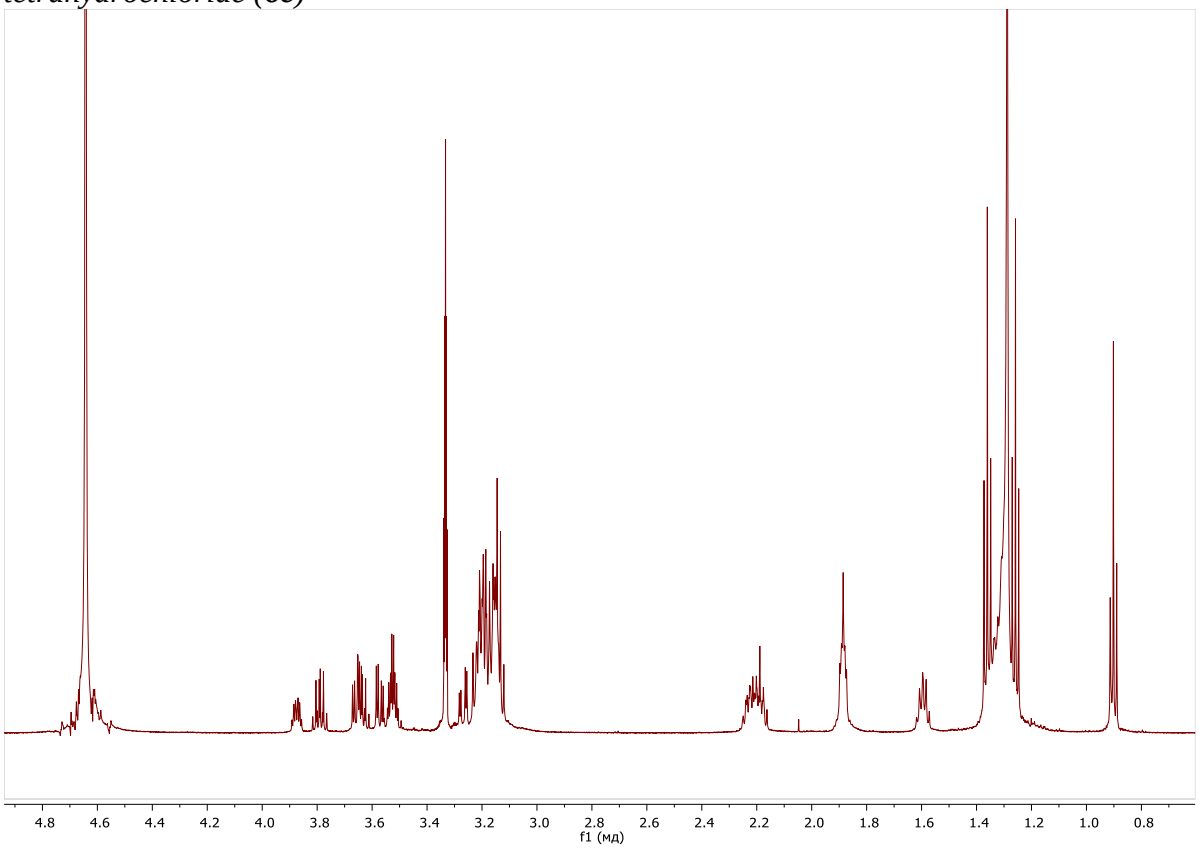
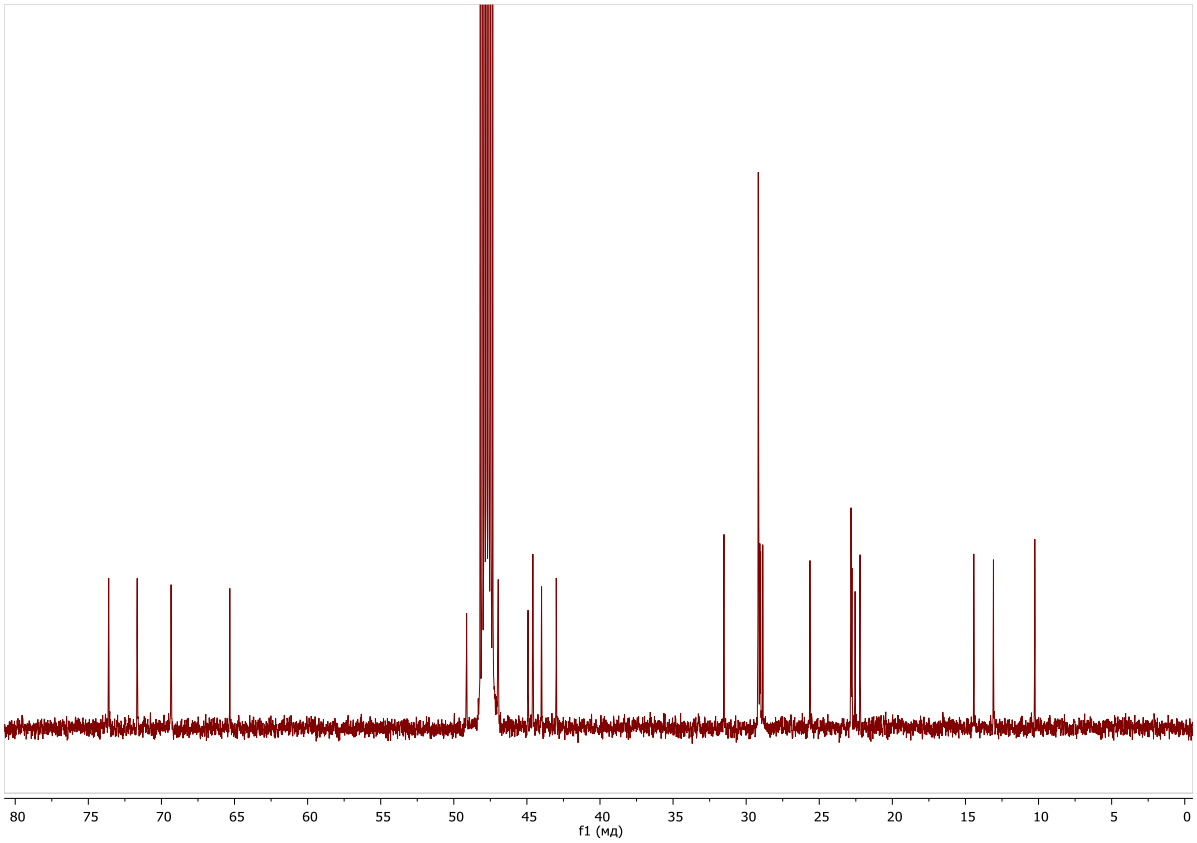
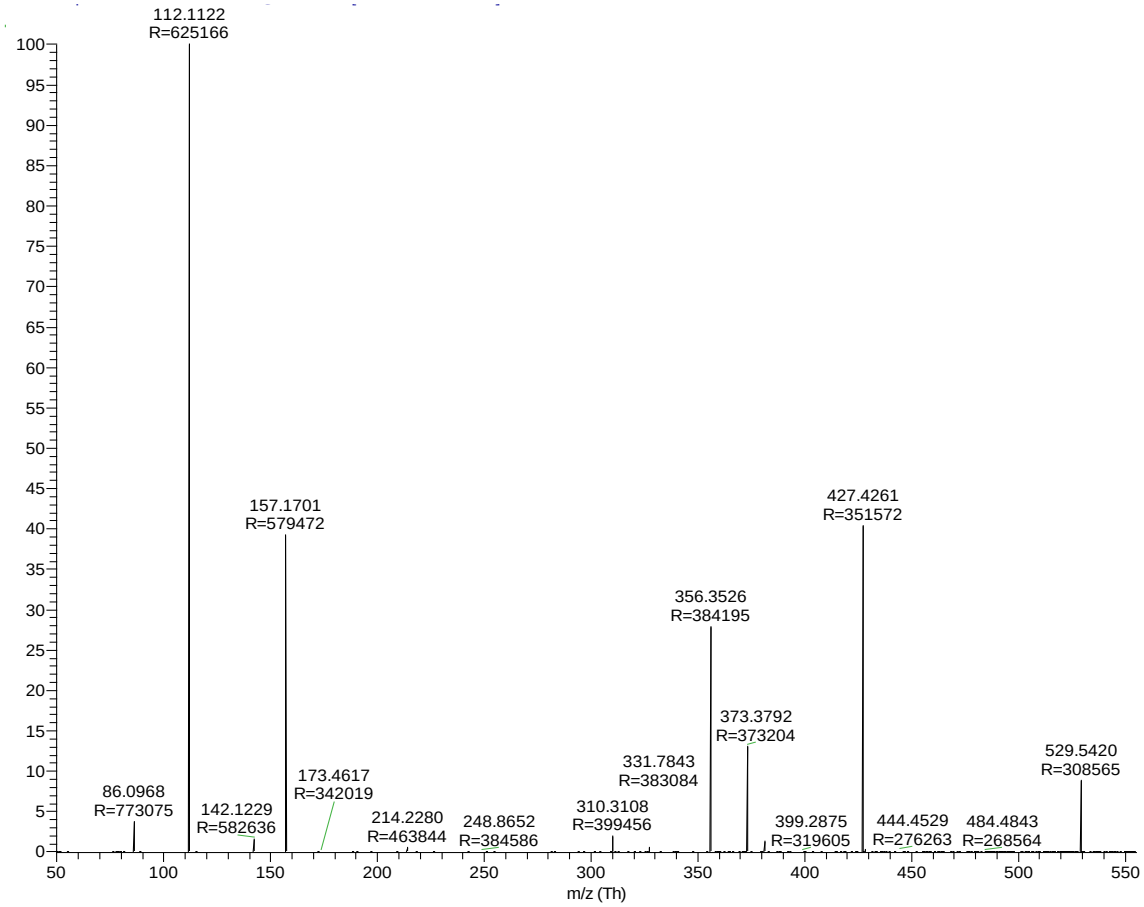
m/z (Th)

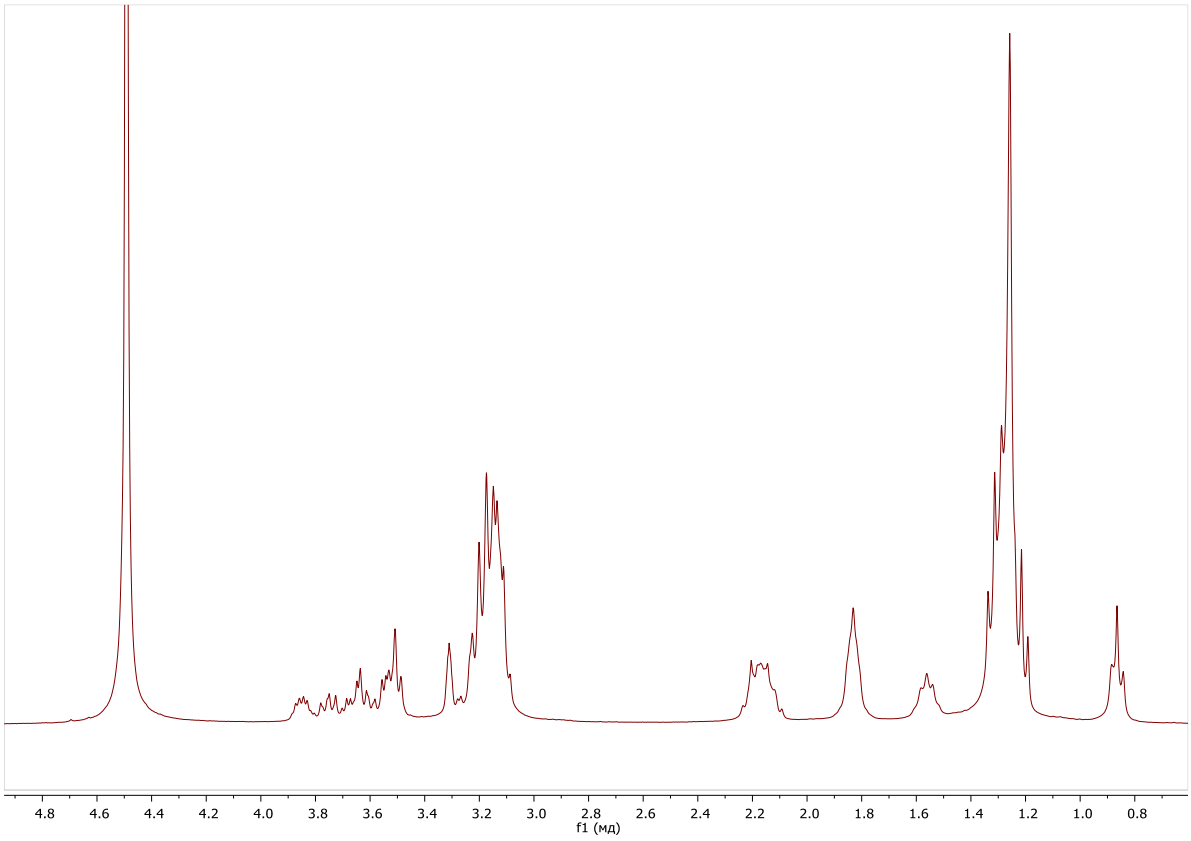
*12-Ethylamino-1-[(rac-2-ethyloxy-3-tetradecyloxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**6c**)*

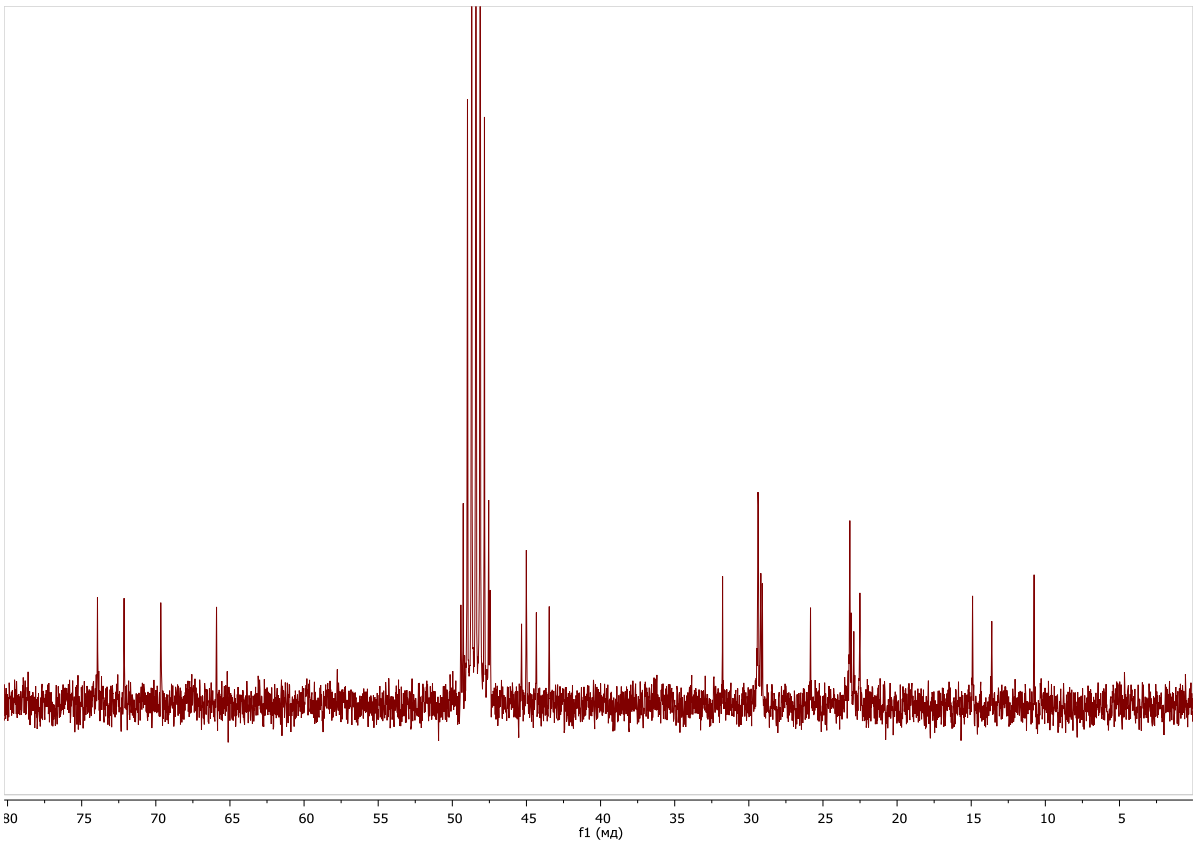






*12-Ethylamino-1-[(rac-3-dodecyloxy-2-ethoxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**6d**)*

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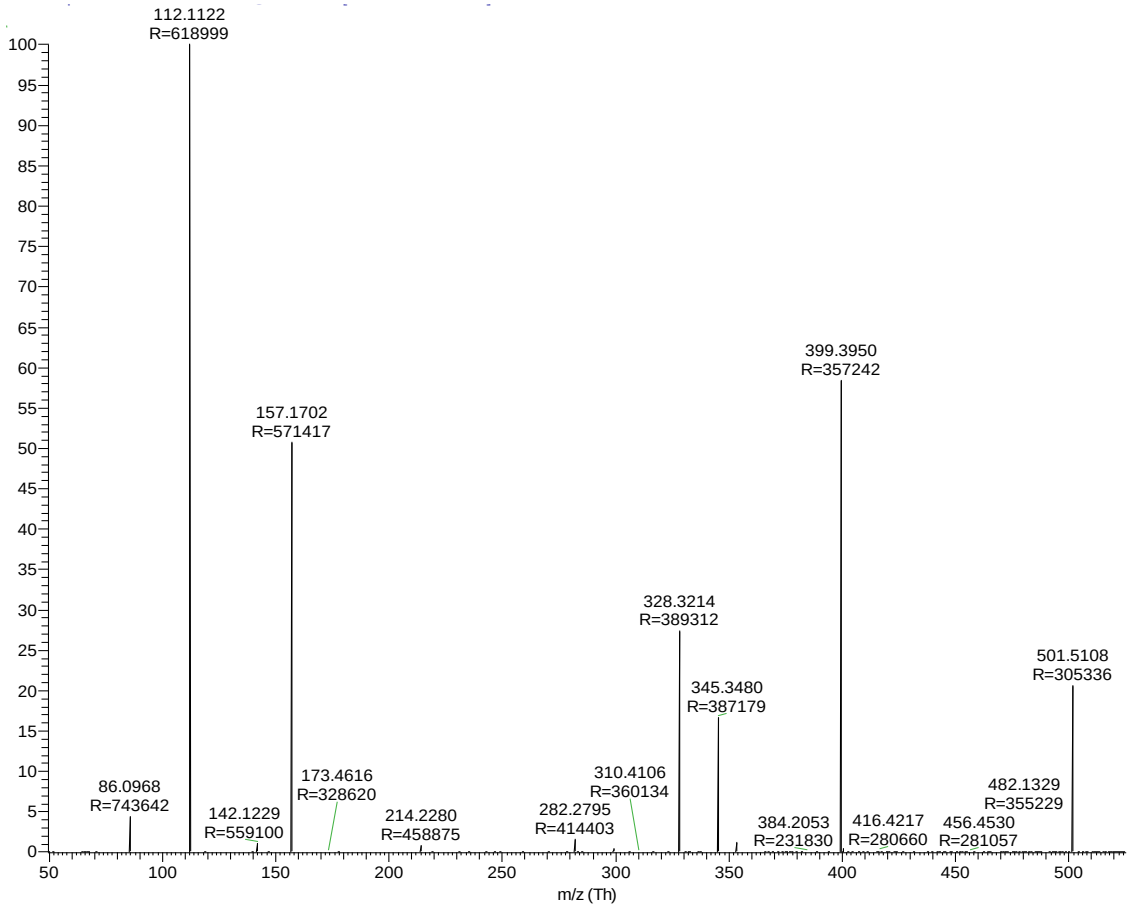
f1 (мд)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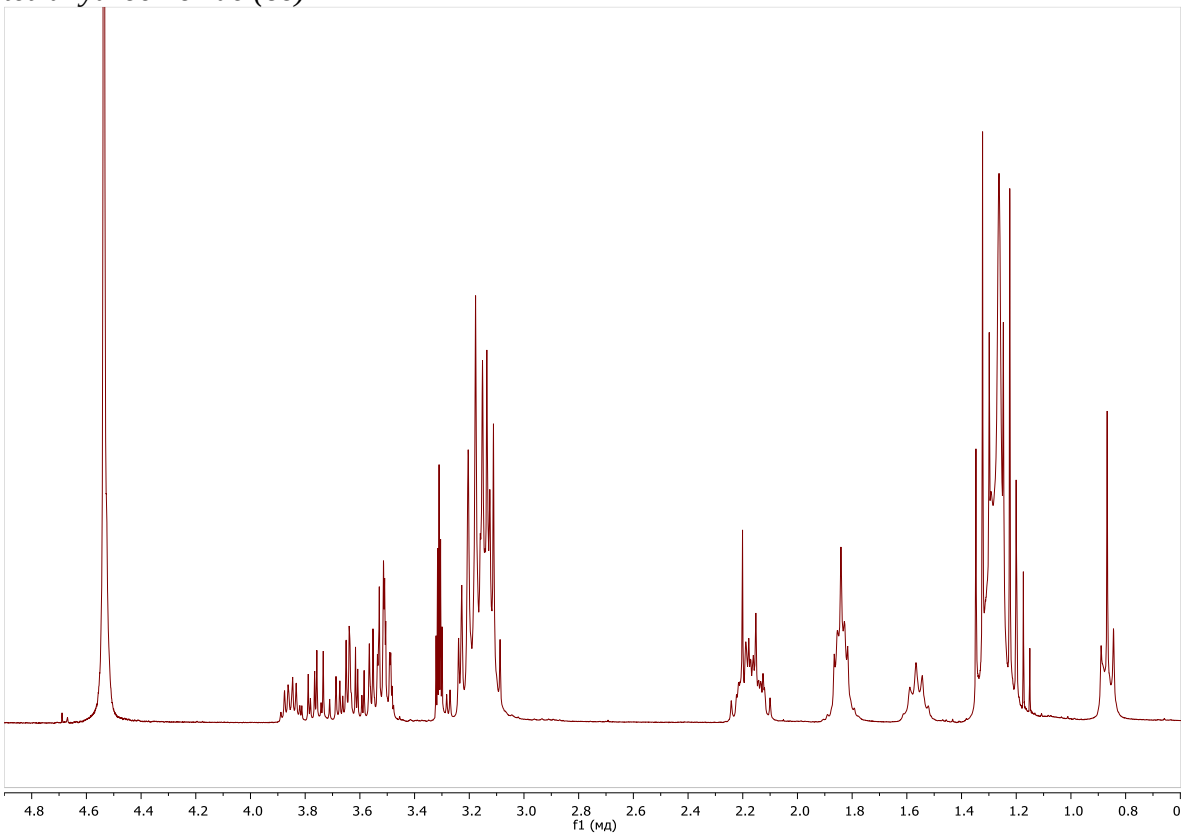
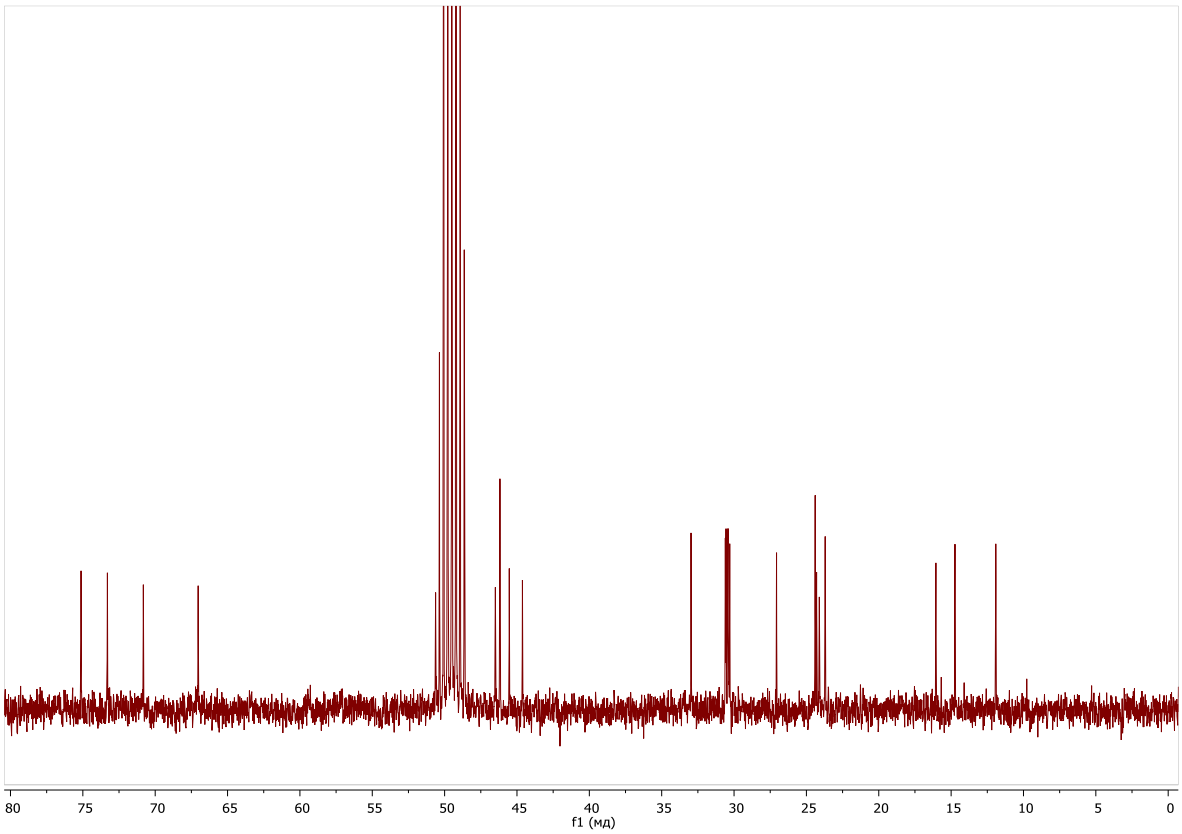
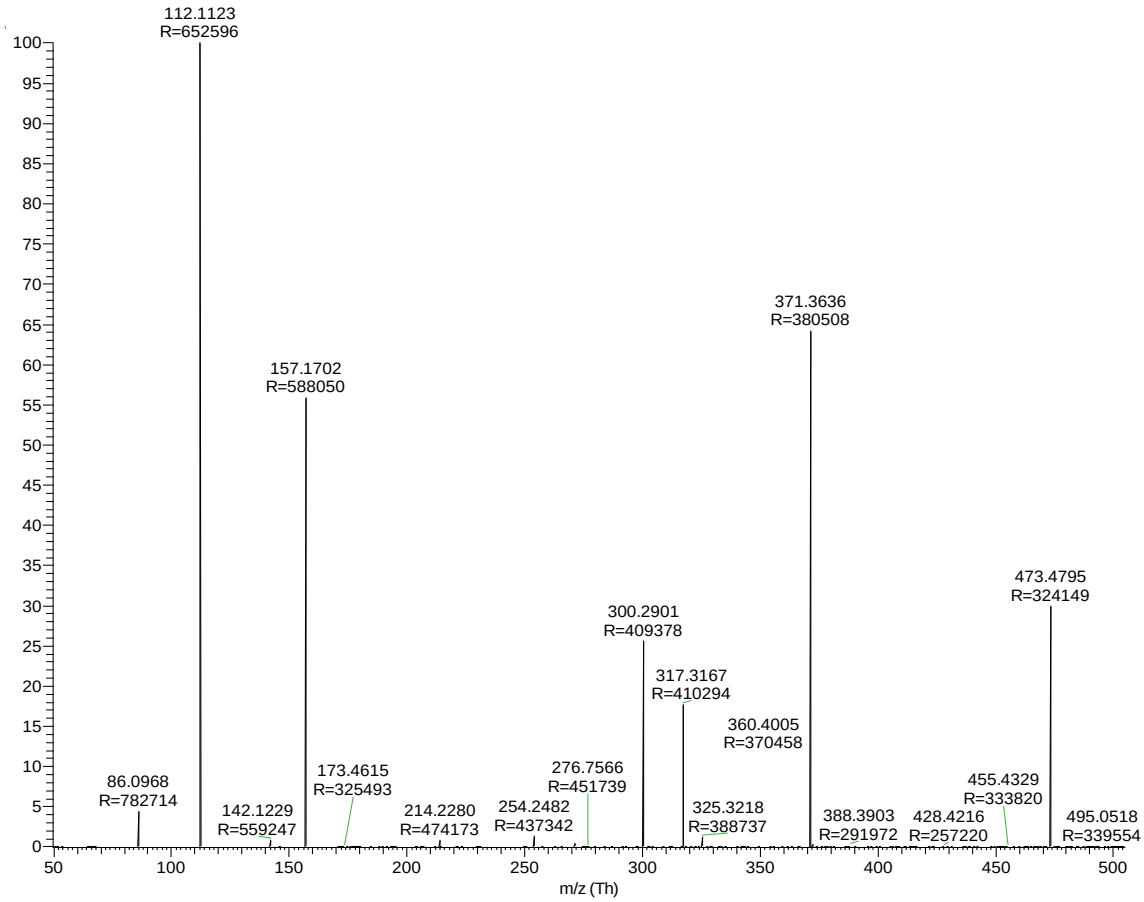
m/z (Th)

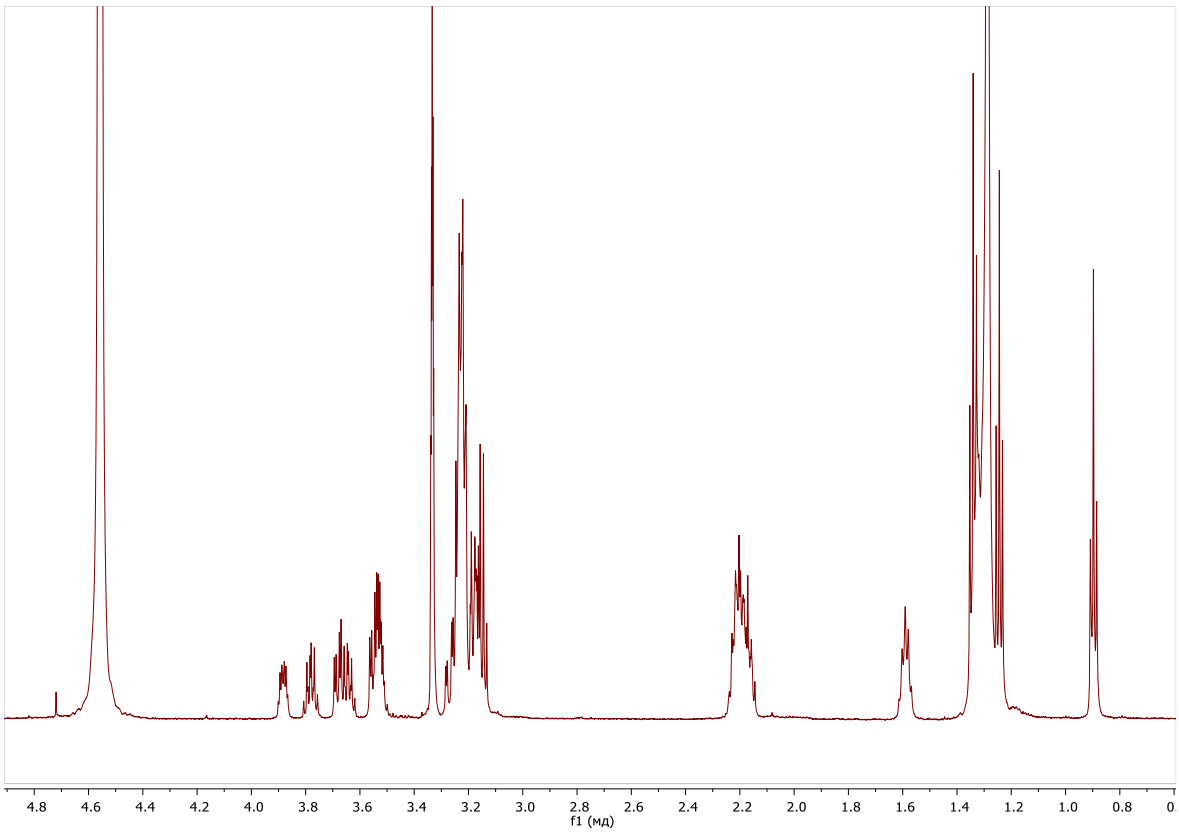
*12-Ethylamino-1-[(rac-3-decyloxy-2-ethoxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**6e**)*

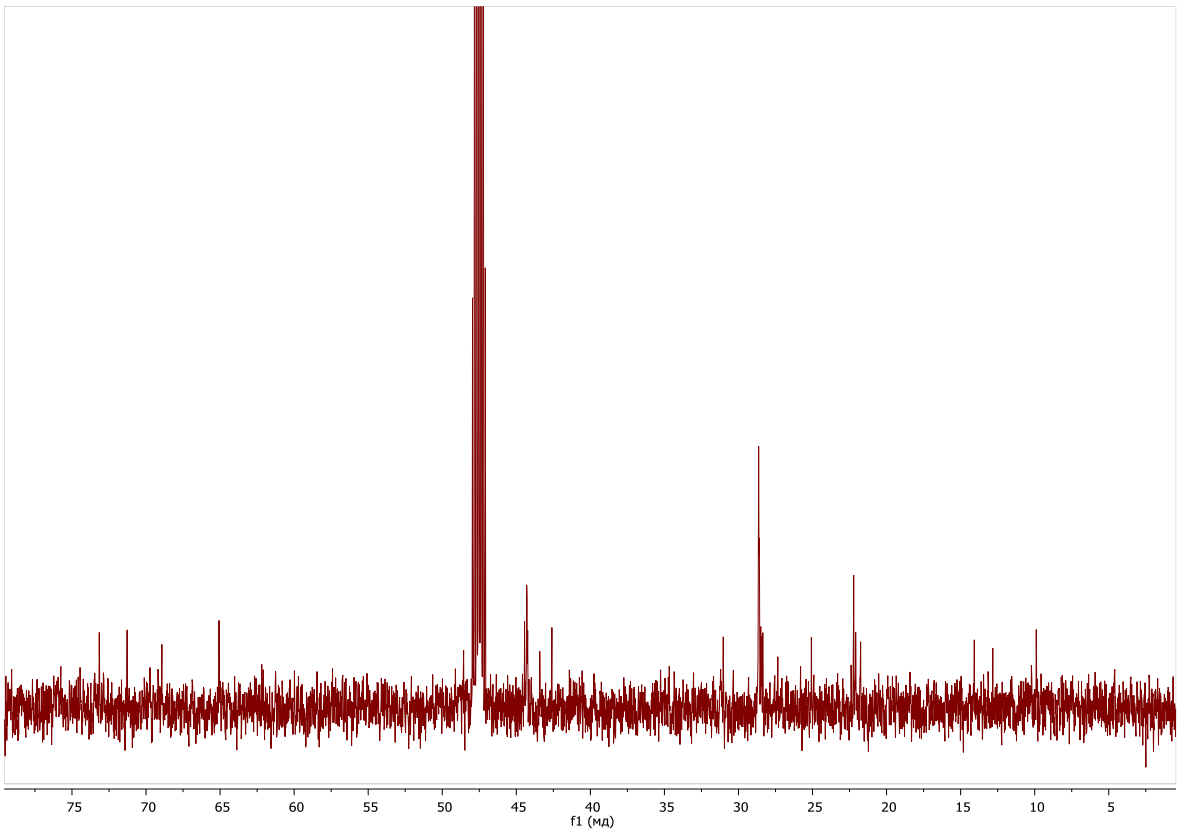






*11-Ethylamino-1-[(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-4,8-diazaundecane tetrahydrochloride (**6f**)*

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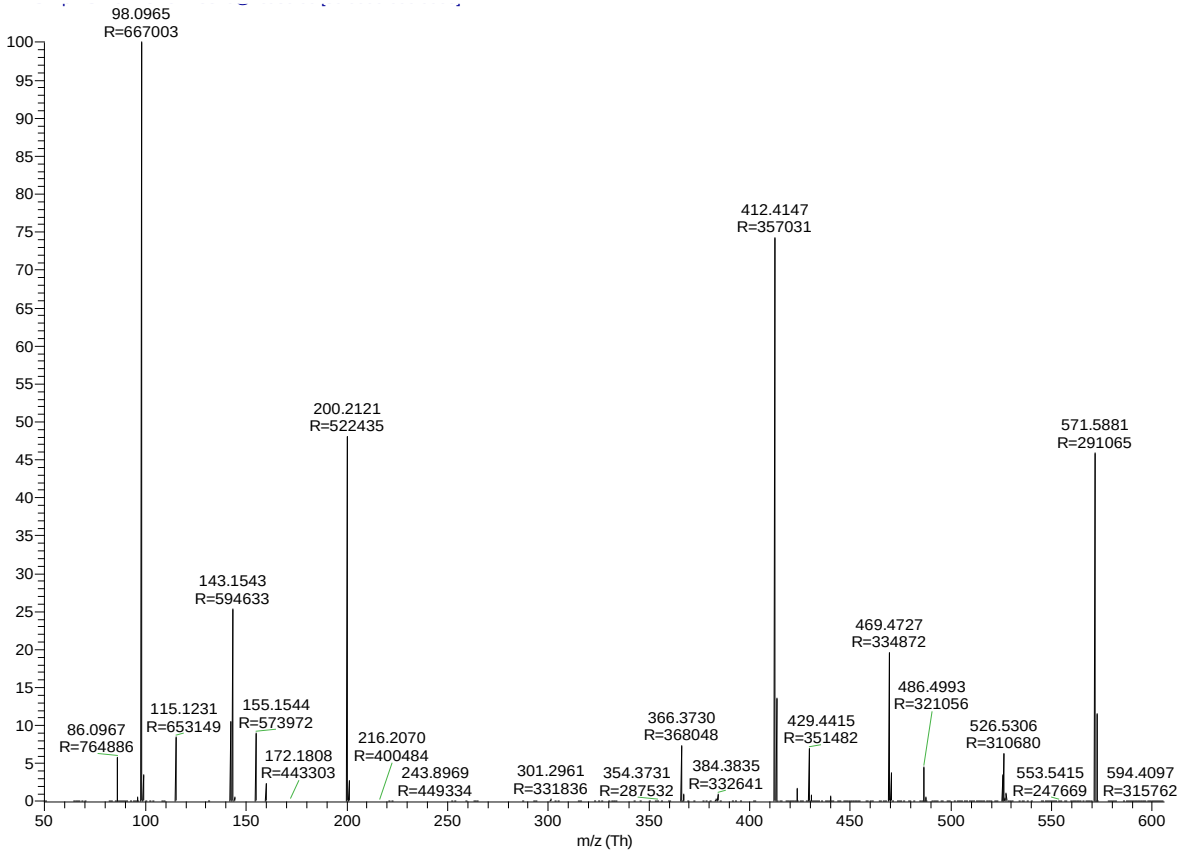
f1 (мд)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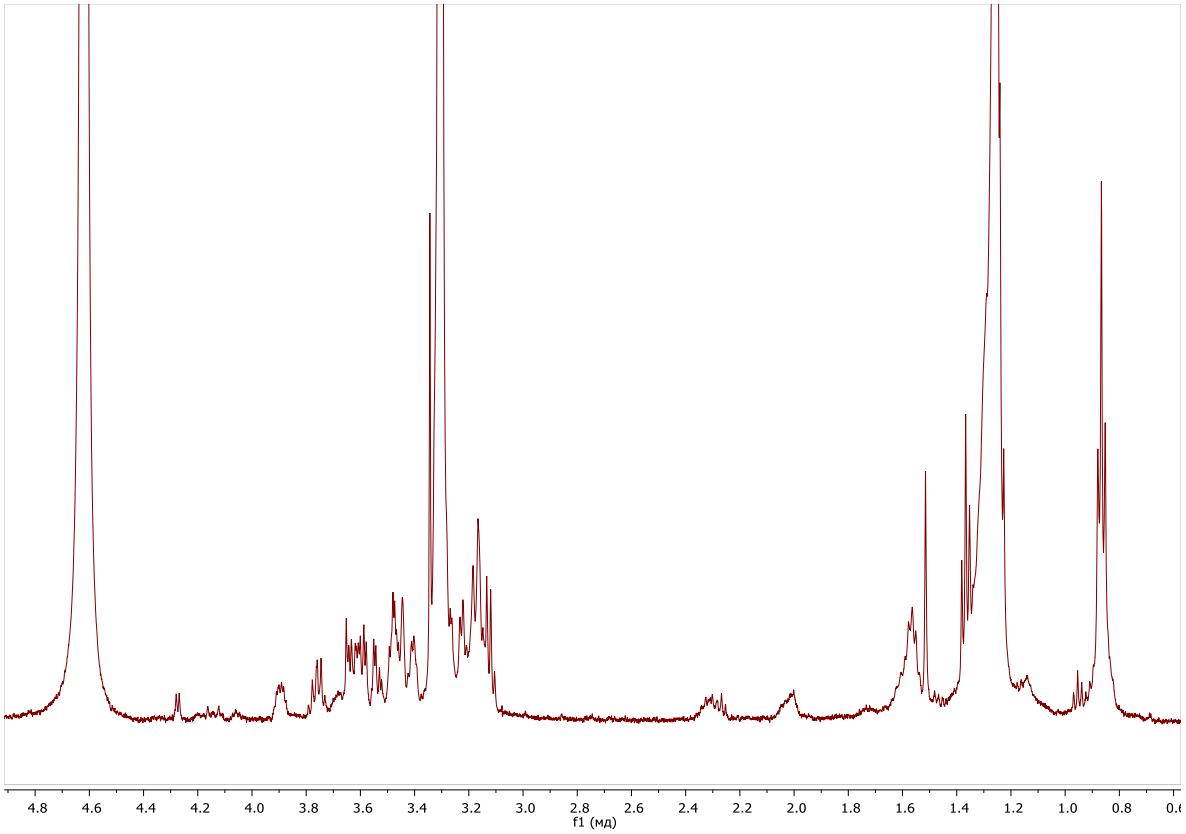
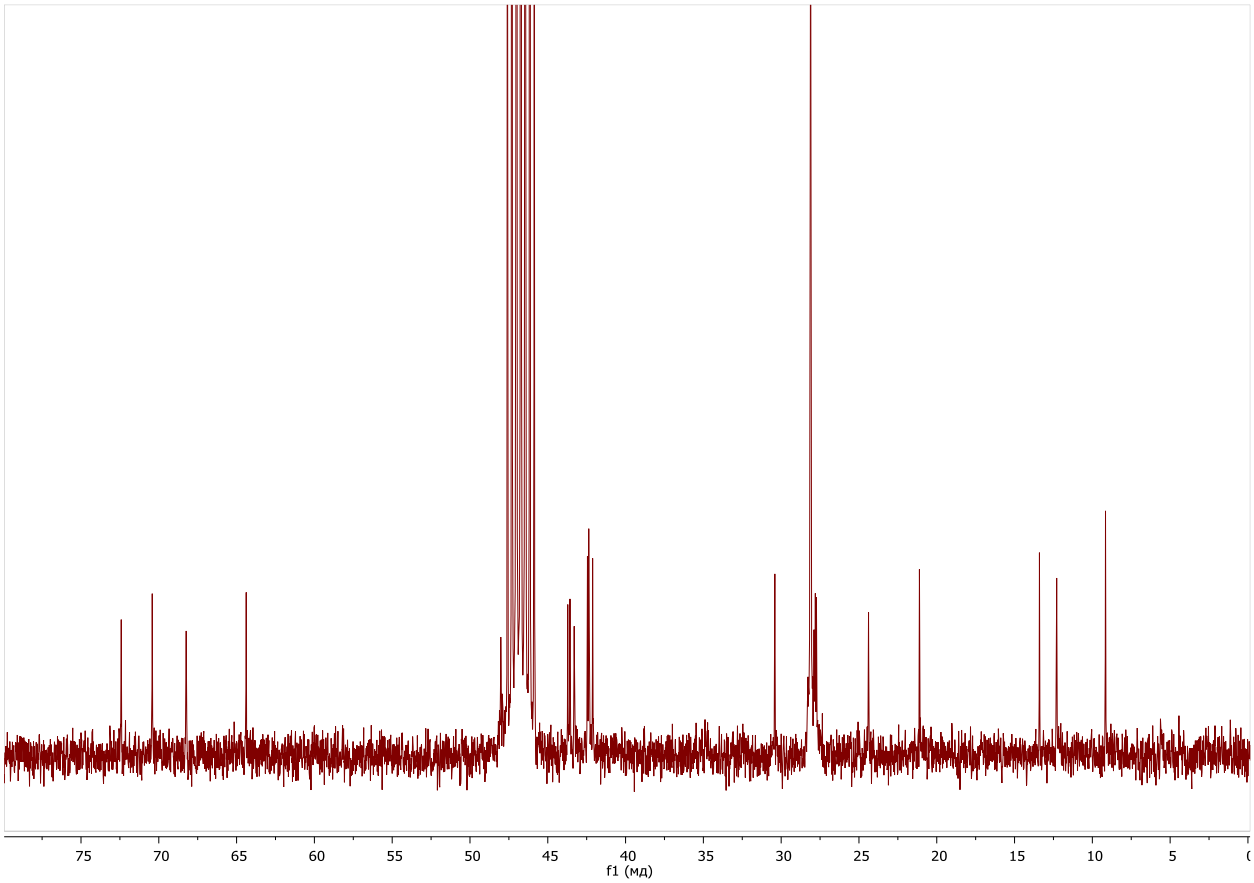
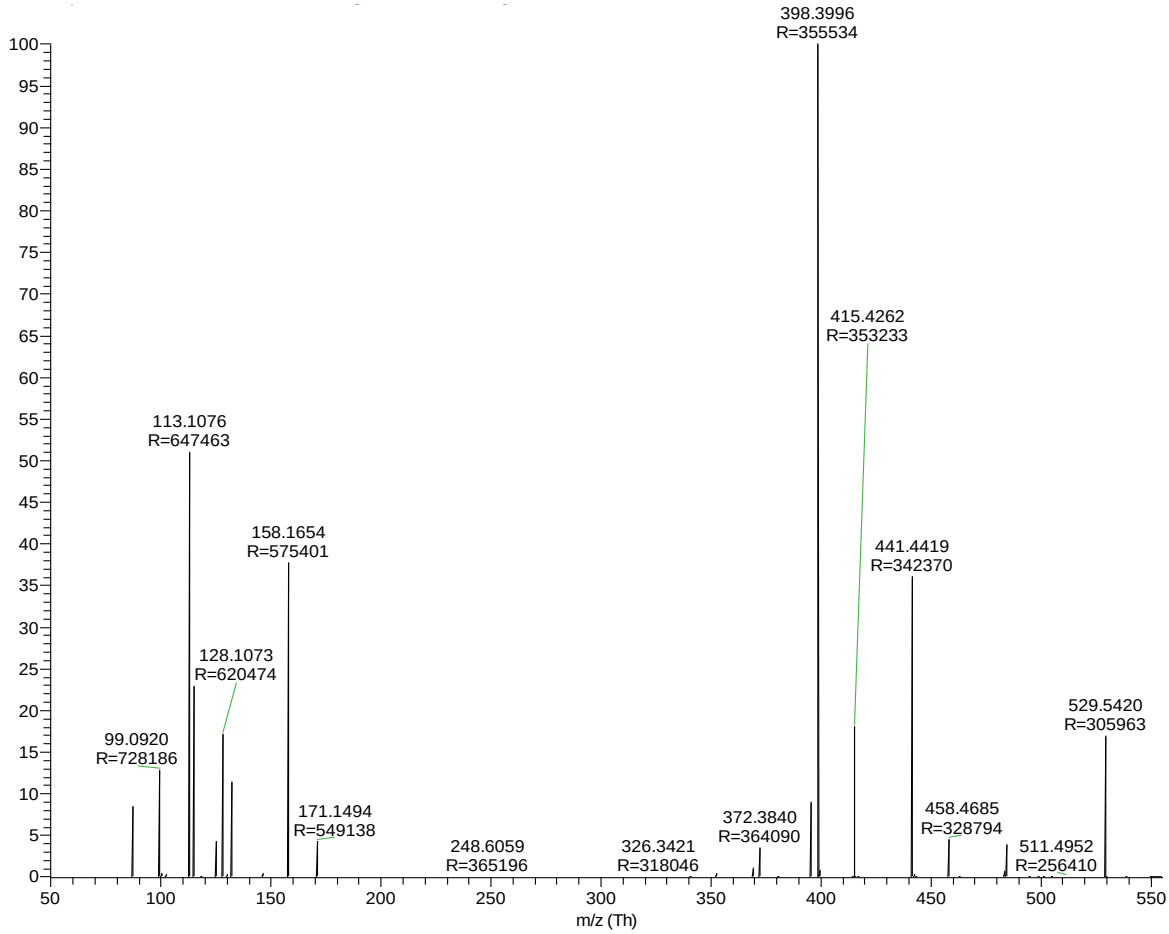
m/z (Th)

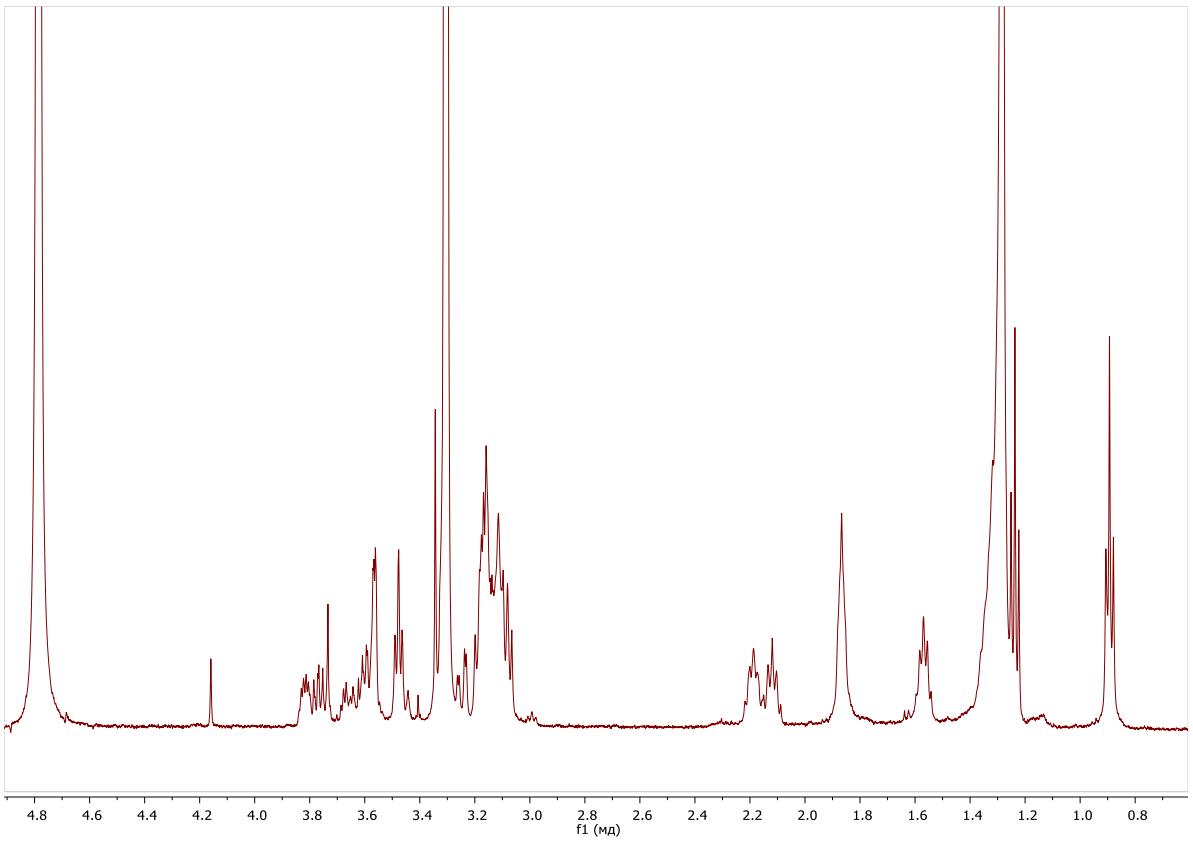
*8-Ethylamino-1-[(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-3,6-diazaoctane tetrahydrochloride (**6g**)*

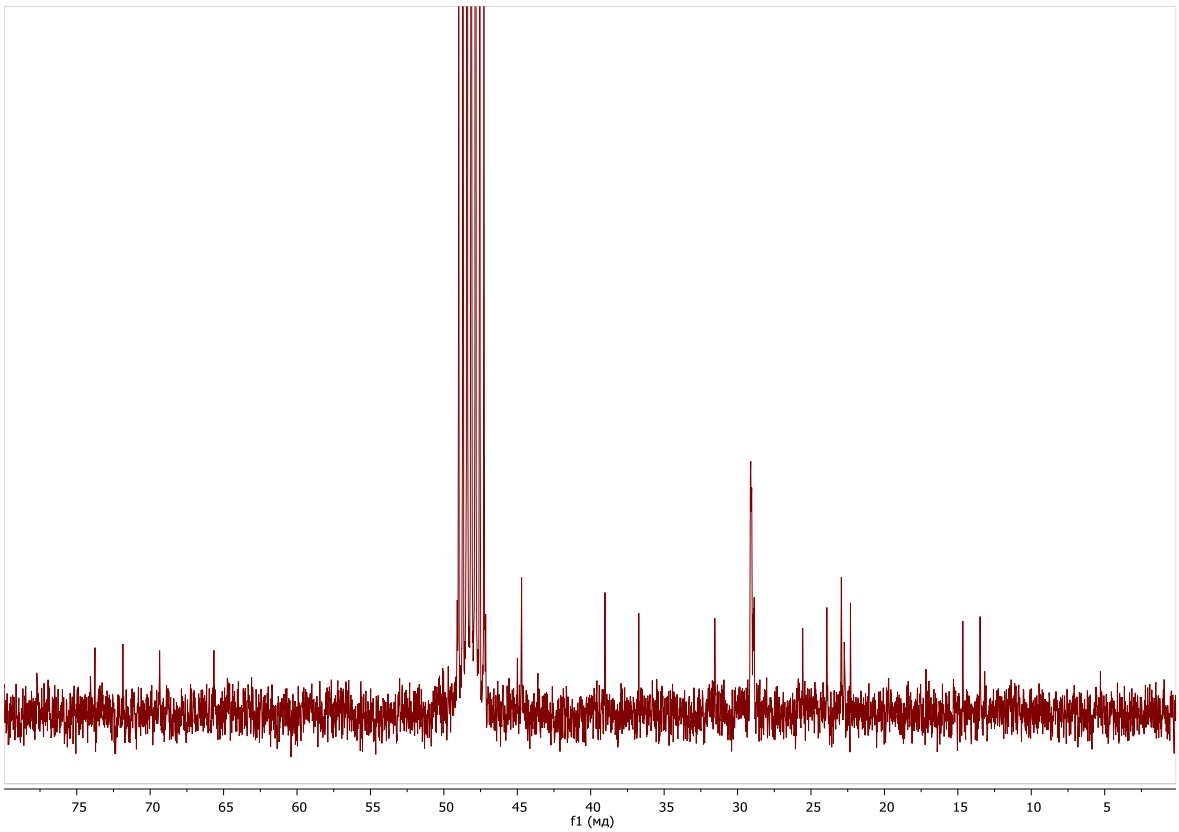






*12-Amino-1-[(rac-2-ethoxy-3-octadecyloxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**7a**)*

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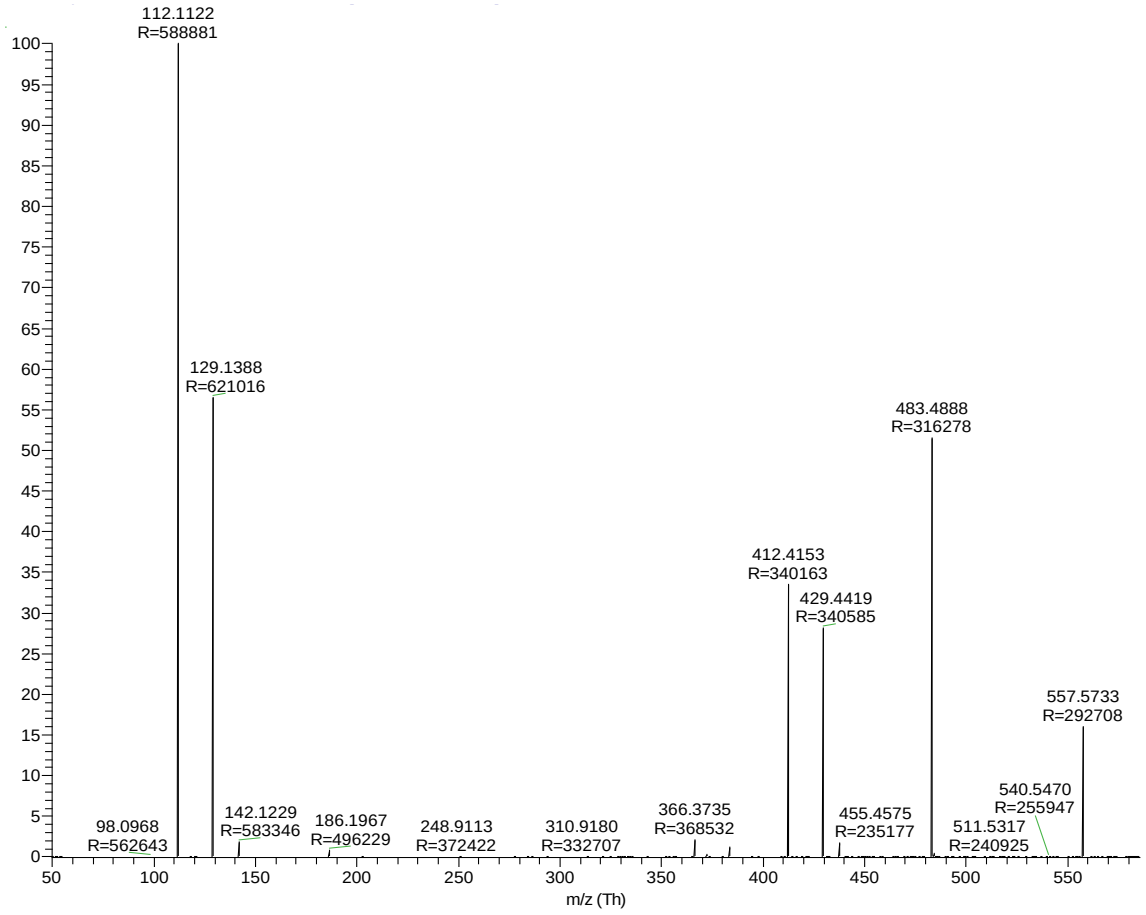
f1 (мд)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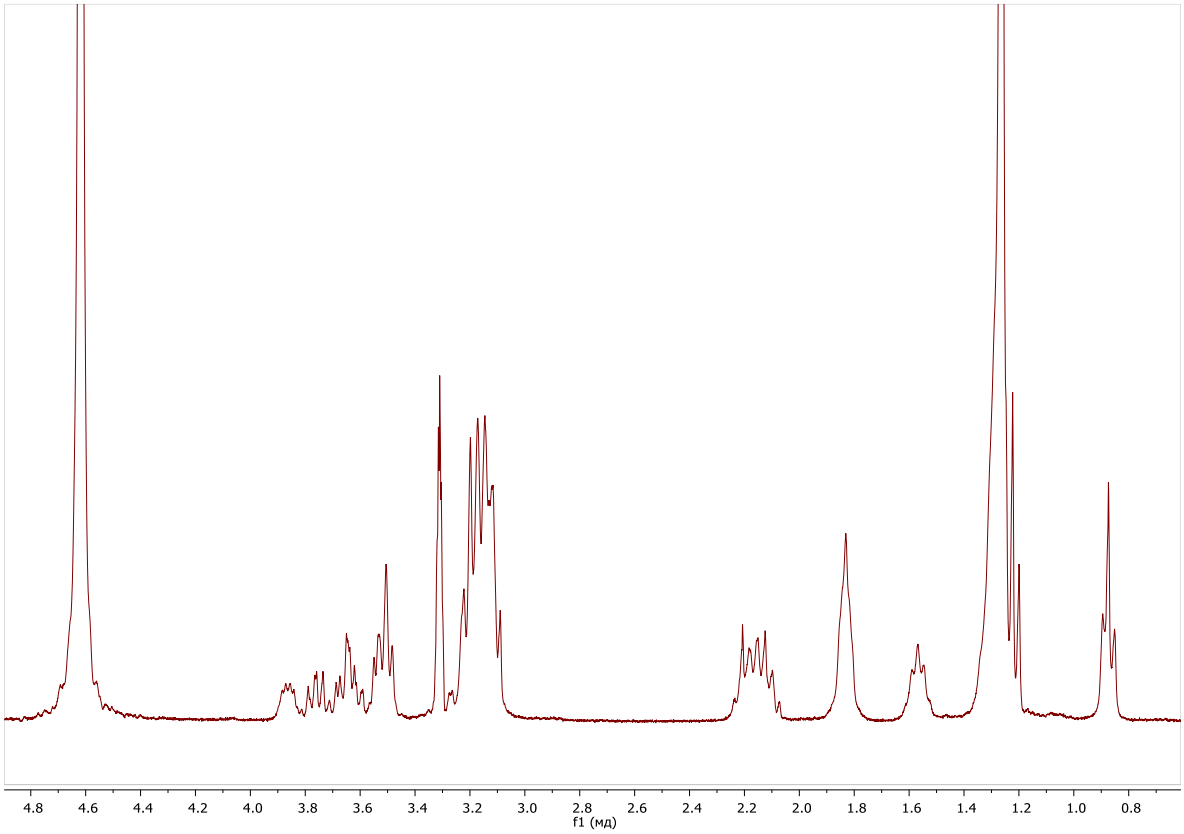
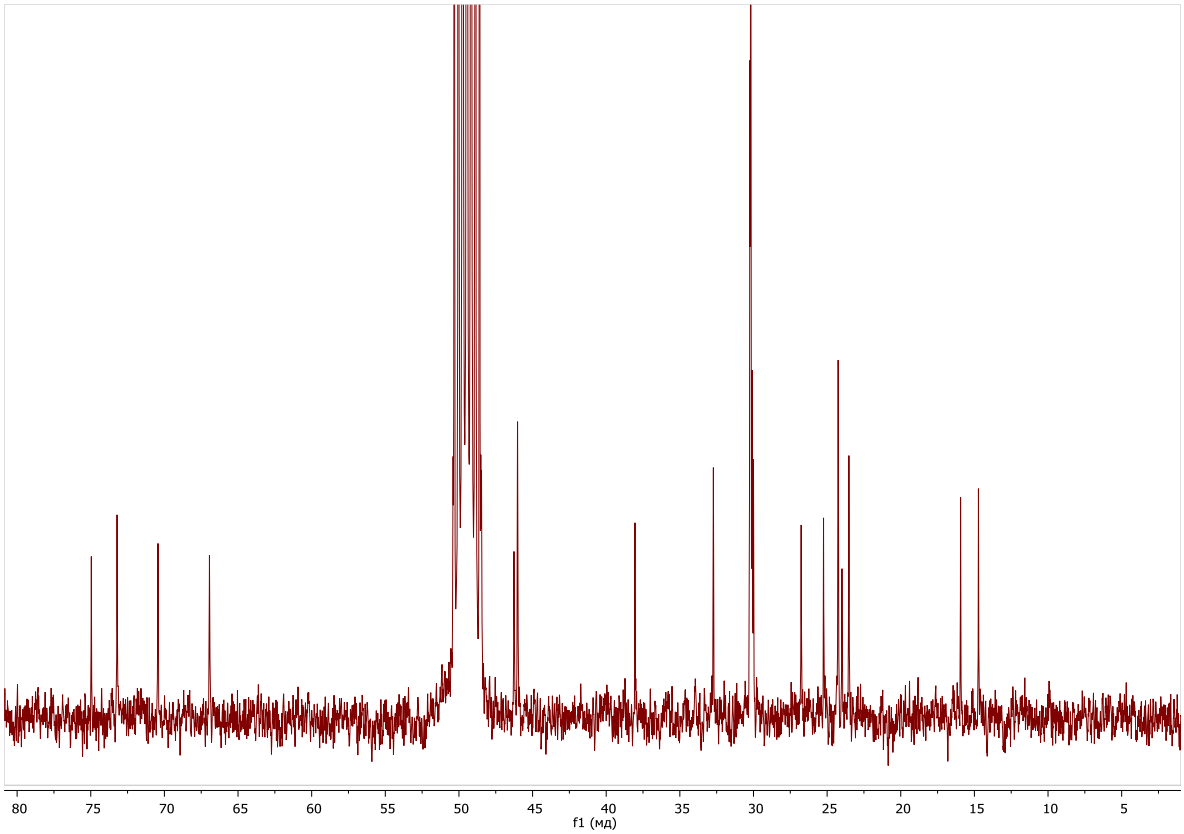
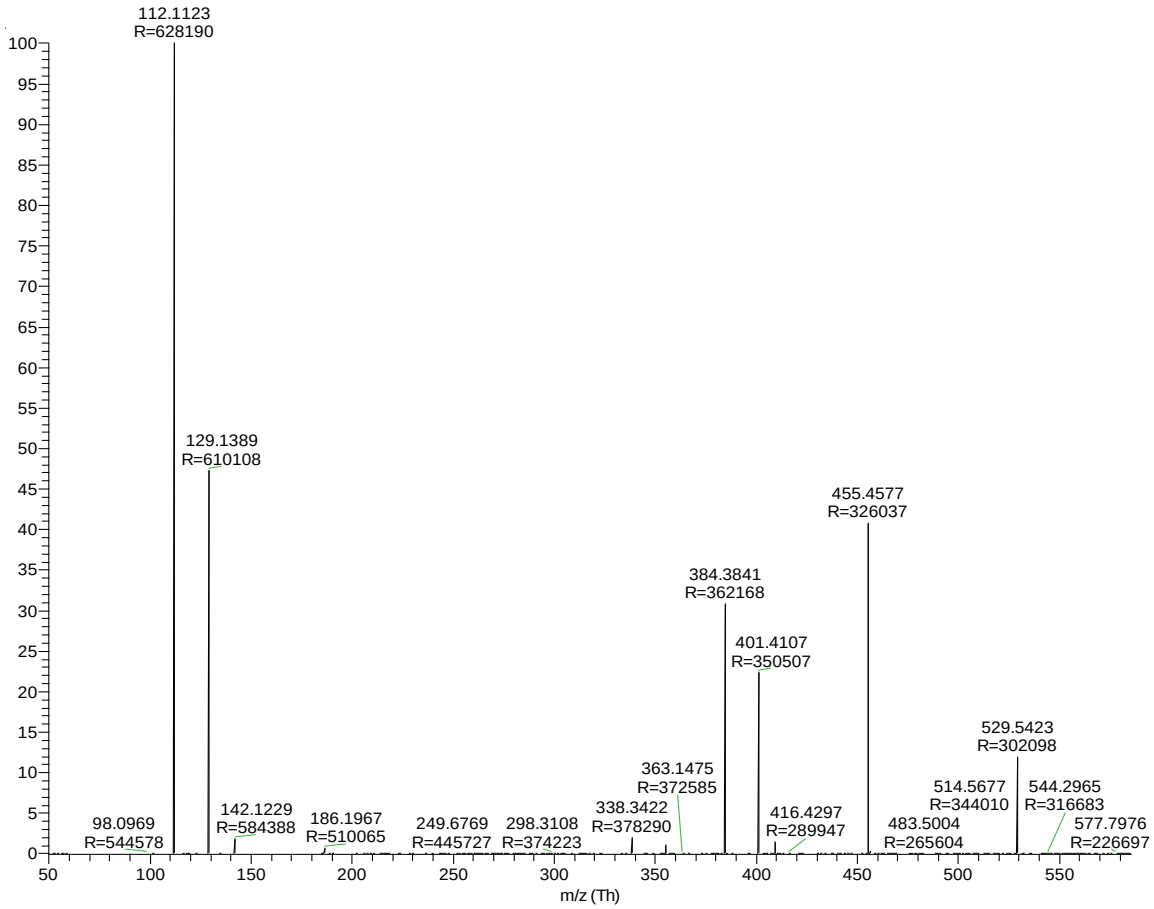
m/z (Th)

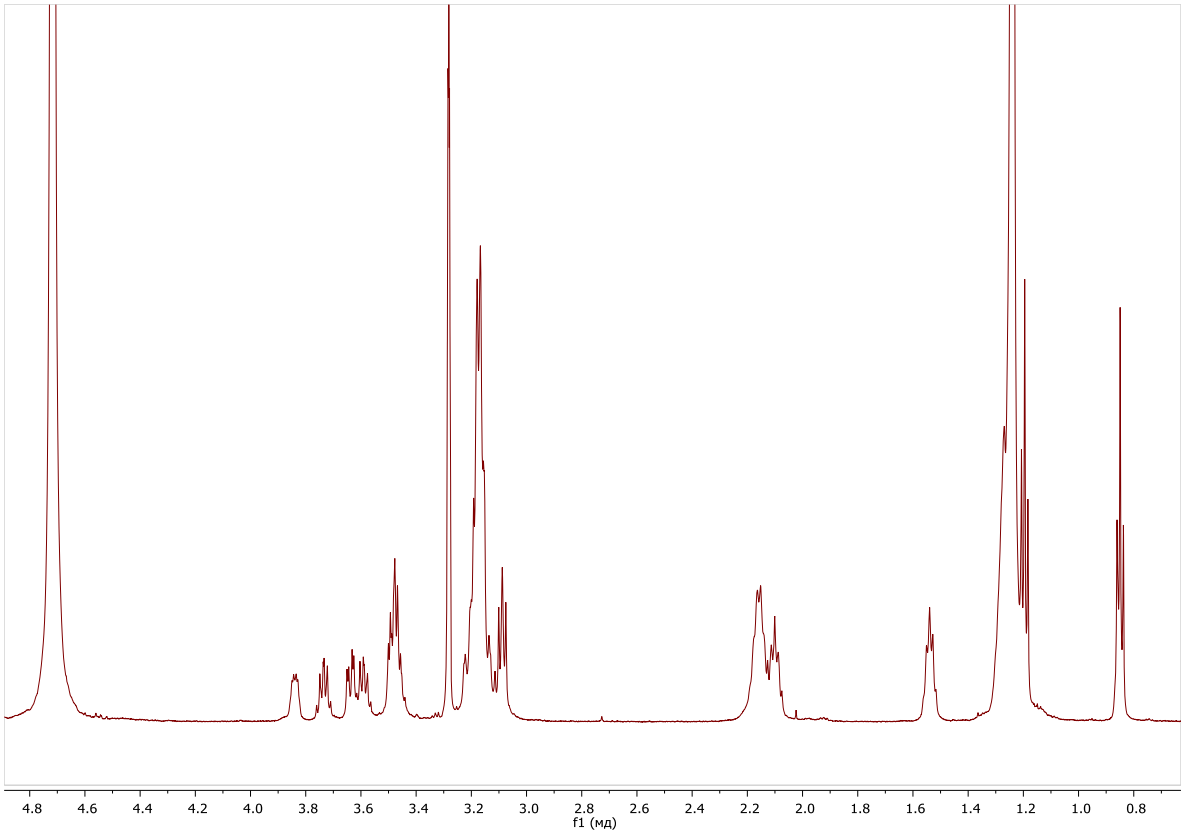
*12-Amino-1-[(rac-2-ethoxy-3-hexadecyloxyprop-1-yl)amino]-4,9-diazadodecane tetrahydrochloride (**7b**)*

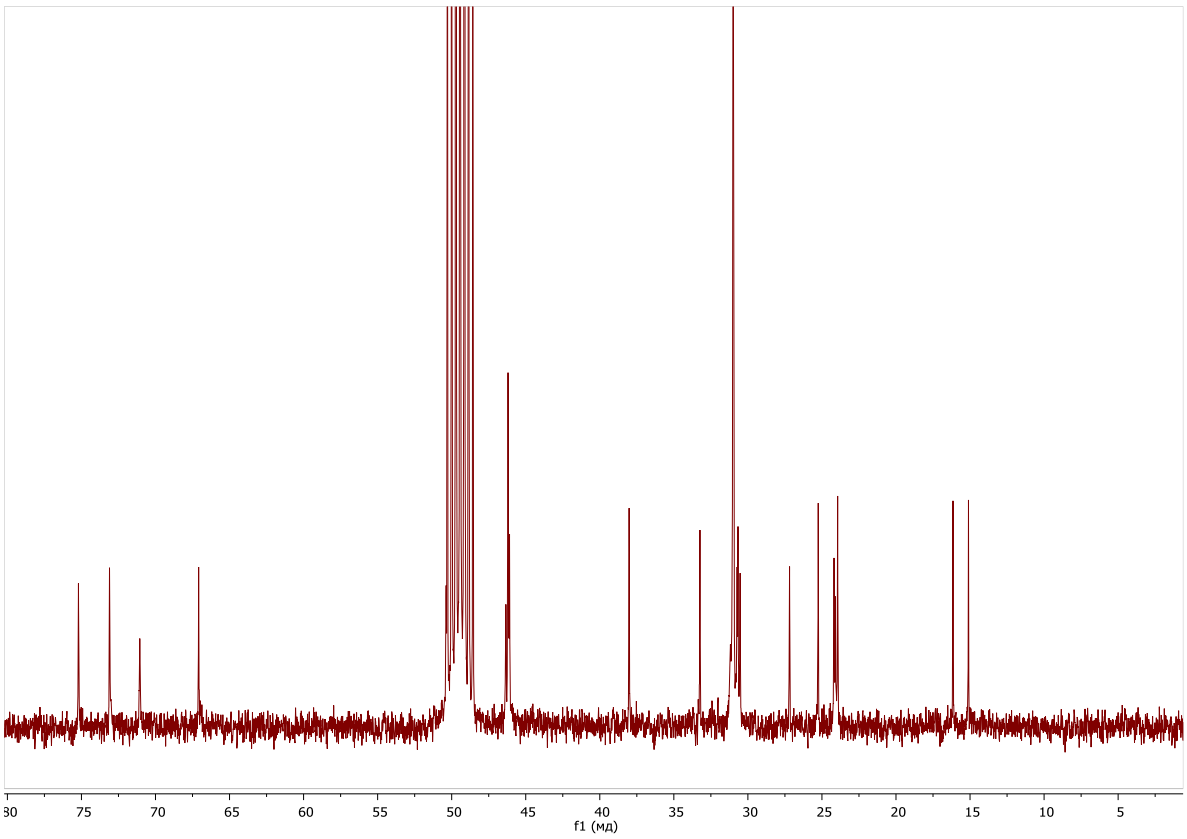


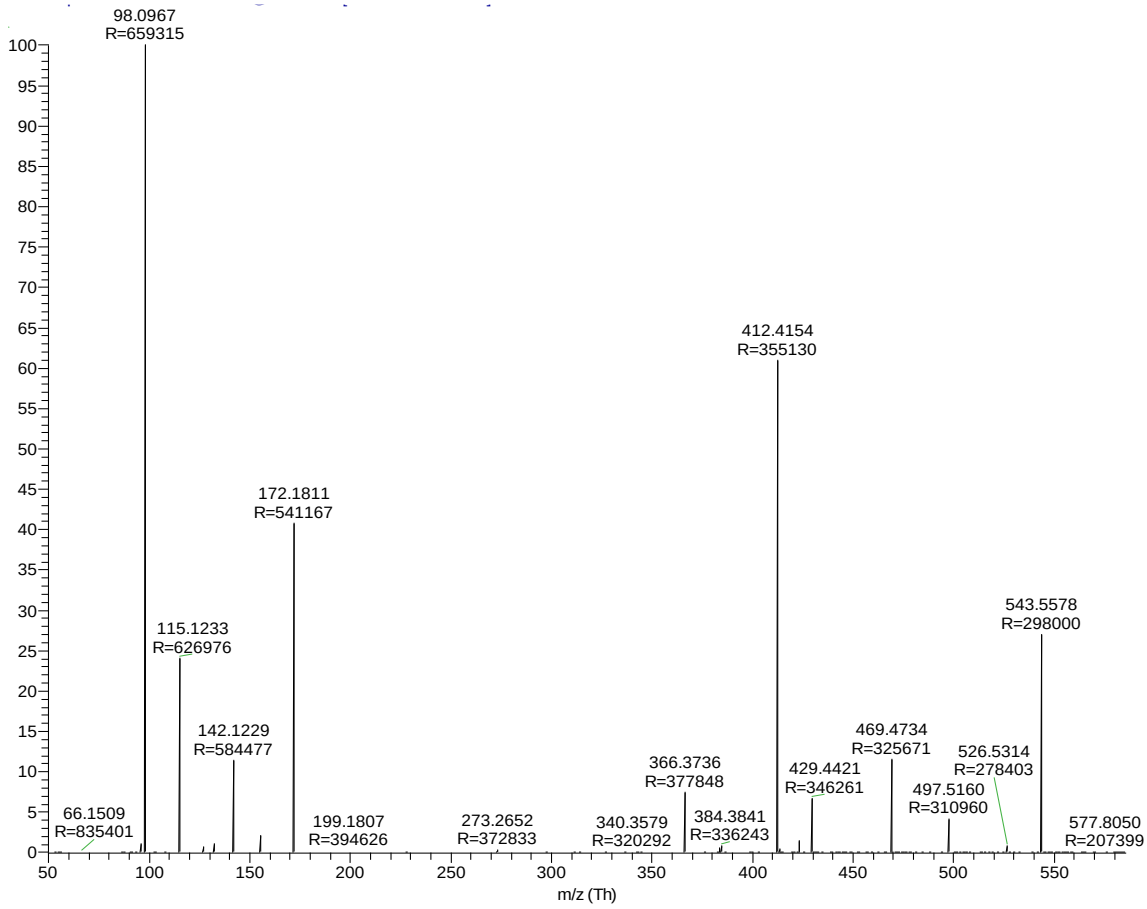




*11-Amino-1-[N-(rac-2-ethoxy-3-octadecyloxyprop-1-yl)amino]-4,8-diazaundecane tetrahydrochloride (**7c**)*




80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
f1 (мд)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R=659315
172.1811
R=541167
115.1233
R=626976
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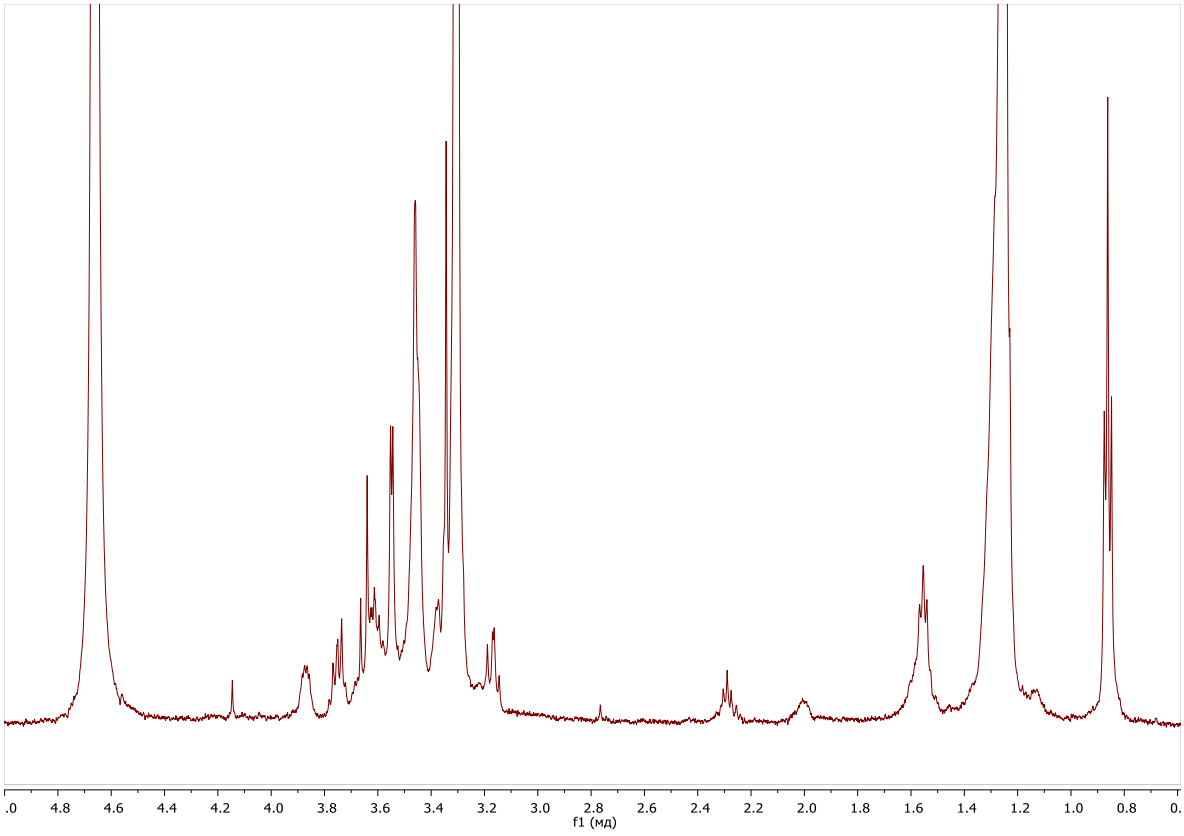
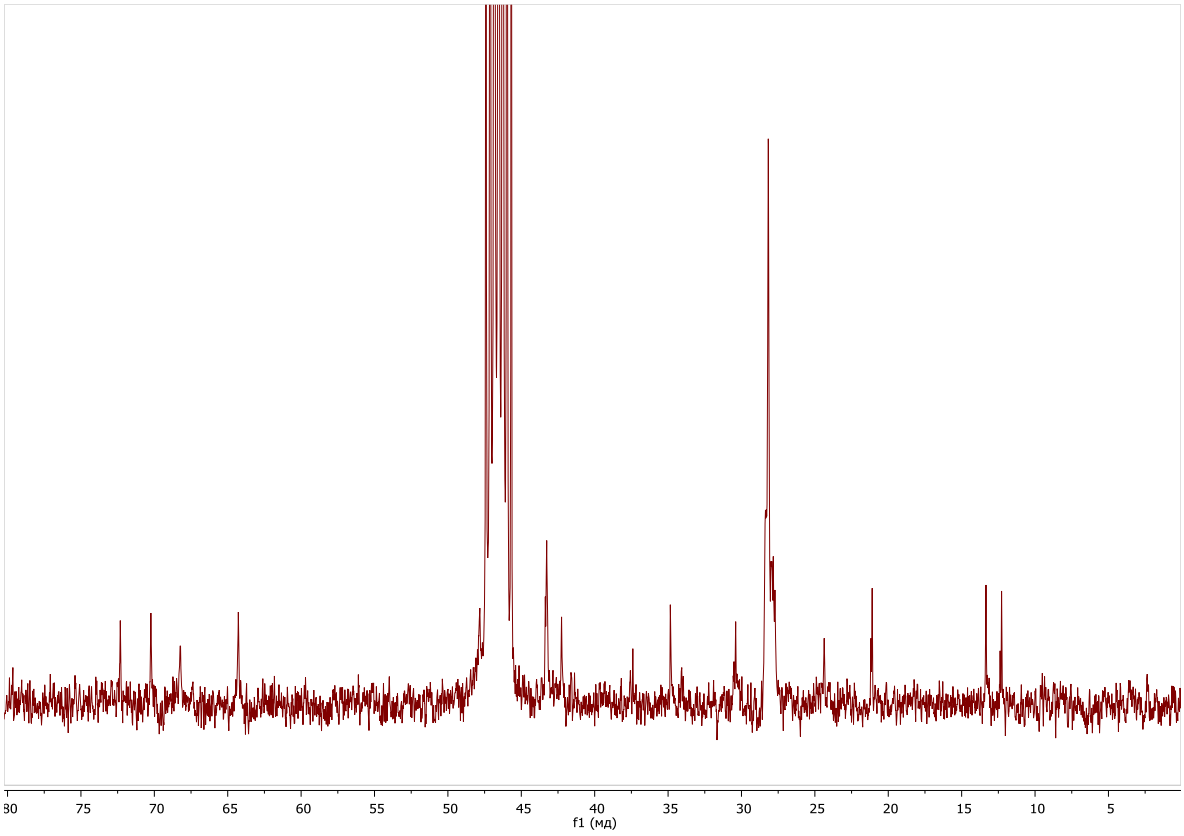
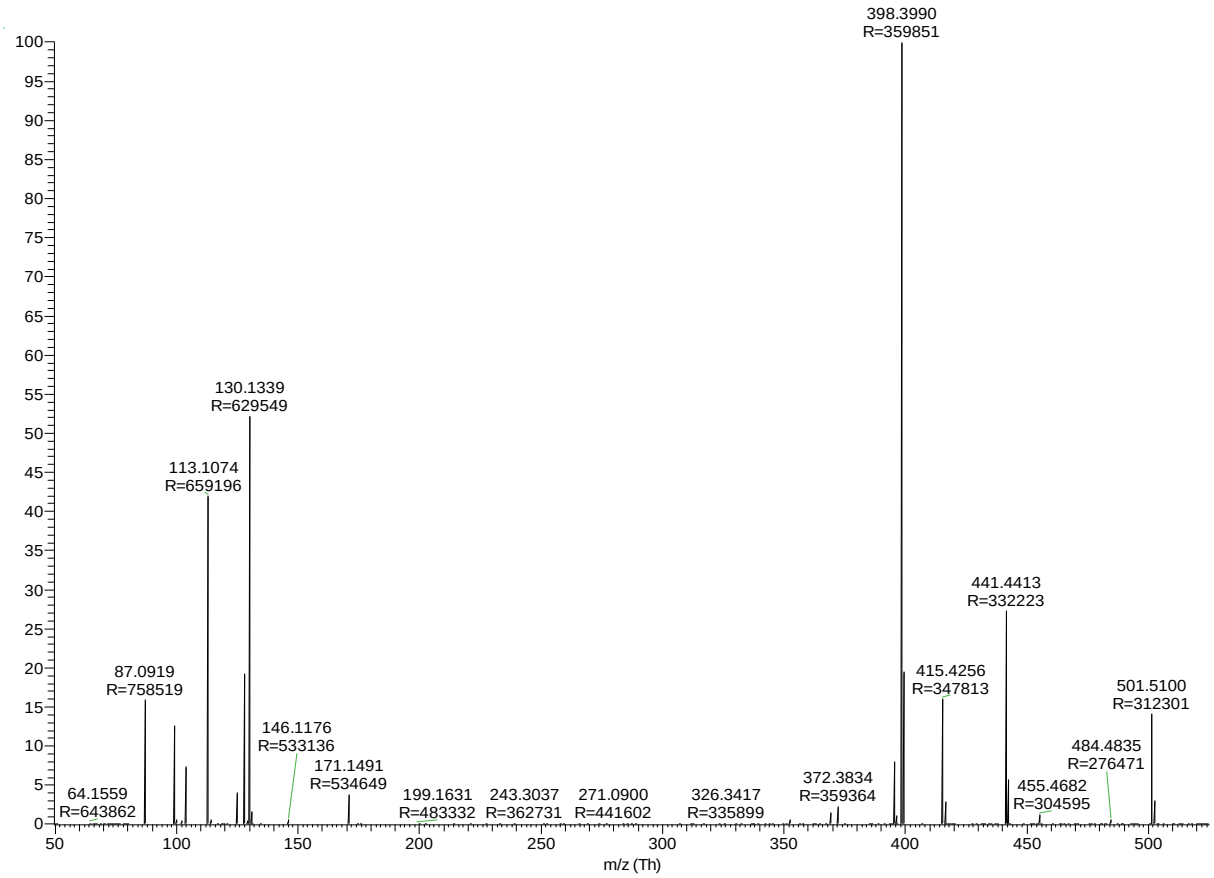
66.1509
R=835401
199.1807
R=394626
273.2652
R=372833
340.3579
R=320292
366.3736
R=377848
384.3841
R=336243
412.4154
R=355130
429.4421
R=346261
469.4734
R=325671
497.5160
R=310960
526.5314
R=278403
543.5578
R=298000
577.8050
R=207399
m/z (Th)

*8-Amino-1-[N-(rac-2-ethyloxy-3-octadecyloxyprop-1-yl)amino]-3,6-diazaoctane tetrahydrochloride (**7d**)*






398.3990
R=359851
130.1339
R=629549
113.1074
R=659196
441.4413
R=332223
87.0919
R=758519
415.4256
R=347813
501.5100
R=312301
146.1176
R=533136
484.4835
R=276471
171.1491
R=534649
372.3834
R=359364
455.4682
R=304595
64.1559
R=643862
199.1631
R=483332
243.3037
R=362731
271.0900
R=441602
326.3417
R=335899
m/z (Th)