Novel bivalent spermine-based neutral neogalactolipids for modular gene delivery systems

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Supplementary Information

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8-amino-1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-3,6-dioxaoctane (4a) was prepared within 3 synthetic steps starting from 2,3,4,6-tetra-*O*-acetyl-*α*-D-galactopyranosyl bromide.

1) $HgCN_2$ (2.247 g, 8.896 mmol) and 4 Å crushed molecular sieves were added to a solution of 3,6-dioxa-8-chlorooctane-1-ol (1.00g, 5.931 mmol) in dry DCM (15 mL) at 20°C under stirring. After 15 min, the solution of 2,3,4,6-tetra-*O*-acetyl-*α*-D-galactopyranosyl bromide (3.658 g, 8.896 mmol) in dry DCM (15 mL) was added dropwise within 1 h. After 4 h at 40 °C, the reaction mixture was cooled to ambient temperature, filtered through Celite 545® pad, and washed with 20% aq. KI (4 × 50 mL), water (3 × 30 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by column chromatography on a silica gel (toluene – ethyl acetate, 2:1) to give 8-chloro-1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-3,6-dioxaoctane (2.078 g, 75%) as a yellowish oil. δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.91 (3H, s), 1.98 (3H, s), 2.99 (3H, s), 2.08 (3H, s, 4 OCOMe), 3.50-3.64 (8 H, m, C$\underline{H}_2$O(C$\underline{H}_2$)$_2$OC$\underline{H}_2$), 3.65-3.75 (3 H, m, C$\underline{H}_2$Cl, OC$\underline{H}_a$H), 3.81-3.93 (2 H, m, 5-H Gal, OC$\underline{H}_b$H), 4.06 (1 H, dd, *J* 6.5, 11.1, 6-H_a Gal), 4.07 (1 H, dd, *J* 6.5, 11.1, 6-H_b Gal), 4.52 (1 H, d, *J* 8.0, 1-H Gal), 4.95 (1 H, dd, *J* 3.4, 10.5, 3-H Gal), 5.14 (1 H, dd, *J* 8.0, 10.5, 2-H Gal), 5.32 (1 H, dd, *J* 1.0, 3.4, 4-H Gal). δ_c (75 MHz; $CDCl_3$): 20.56, 20.64, 20.66, 20.76, 42.78, 61.25, 67.01, 68.73, 69.02, 70.33, 70.55, 70.58, 70.61, 70.83, 101.25, 169.46, 170.12, 170.24, 170.36. *m/z*: 521.893 (M^++Na, 100%).

2) Sodium azide (0.542 g, 8.329 mmol) was added to a solution of 8-azido-1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-3,6-dioxaoctane (2.078 g, 4.164 mmol) in dry DMF (100 mL) and stirred for 40 h at 100º C. The solvent was removed under diminished pressure, the residue was dissolved in DCM (70 mL) and washed by 3% aq. HCl (4 × 20 mL) and water (3 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, the solvent was removed under diminished pressure. Column chromatography on silica gel (toluene – ethylacetate, 1:2) gave 8-azido-1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-3,6-dioxaoctane (6) (1.747 g, 83%) as a yellowish oil. δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.92 (3H, s), 1.98 (3H, s), 2.00 (3H, s), 2.08 (3H, s, 4 OCOMe), 3.35 (2 H, t, *J* 5.0, CH_2N_3), 3.40-3.64 (8 H, m, C$\underline{H}_2$O(C$\underline{H}_2$)$_2$OC$\underline{H}_2$), 3.65-3.75 (1 H, m, OC$\underline{H}_a$H), 3.81-3.93 (2 H, m, 5-H Gal, OC$\underline{H}_b$H), 4.06 (1 H, dd, *J* 6.6, 11.1, 6-H_a Gal), 4.11 (1 H, dd, *J* 6.5, 11.1, 6-H_b Gal), 4.51 (1 H, d, *J* 8.0, 1-H Gal), 4.95 (1 H, dd, *J* 3.4, 10.5, 3-H Gal), 5.15 (1 H, dd, *J* 8,0, 10.5, 2-H Gal), 5.32 (1 H, dd, *J* 1.0, 3.4, 4-H Gal). δ_c (75 MHz; $CDCl_3$): 20.72, 20.81, 20.90, 50.77, 61.39, 67.14, 68.89, 69.17, 70.12, 70.51, 70.73, 70.78, 70.82, 71.00, 101.45, 169.63, 170.31, 170.41. *m/z*: 528.031 (M^++Na, 100%).

3) A catalytic amount of 10% Pd/C was added to a solution of 8-azido-1-(2,3,4,6-tetra-*O*-cetyl-β-D-galactopyranosyloxy)-3,6-dioxaoctane (6) (0.721 g, 1.978 mmol) and ammonium formate (0.500 g, 7.913 mmol) in methanol (10 mL) heated to 60 °C. After 15 min the catalyst was filtered off and methanol was removed under diminished pressure. Column chromatography on a silica gel (DCM – methanol, 10:1) gave compound **4a** (0.308 g, 45%) as a colorless oil. δ_H (300 MHz; $CDCl_3$; Me_4Si): 1.98 (3H, s), 2.05 (3H, s), 2.08 (3H, s), 2.18 (3H, s, 4 OCOMe), 3.15-3.26 (2 H, m, C$\underline{H}_2$NH$_2$), 3.49-3.70 (8 H, m, C$\underline{H}_2$O(C$\underline{H}_2$)$_2$OC$\underline{H}_2$), 3.73-4.04 (3 H, m, OC$\underline{H}_2$, 5-H- Gal), 4.05-4.22 (2 H, m, 6-H Gal), 4.55 (1 H, d, *J* 7.9, 1-H Gal), 5.03 (1 H, dd, *J* 3.3, 10.5, 3-H Gal), 5.16 (1 H, dd, *J* 7.9, 10.5, 2-H Gal), 5.38 (1 H, dd, *J* 0.8, 3.3, 4-H Gal), 5.90-6.70 (2 H, m, N$\underline{H}_2$). δ_c (75 MHz; $CDCl_3$): 20.74, 20.84, 20.86, 21.03, 39.95, 61.33, 66.83, 67.11, 68.95, 69.19, 70.10, 70.21, 70.47, 70.81, 70.83, 101.38, 170.14, 170.30, 170.42, 170.63. *m/z*: 480.400 (M^+ + H, 100%)

(9-Aza-12-carboxy-3,6-dioxa-10-oxododecyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (4b). To a solution of compound **4a** (1.250 g, 2.607 mmol) in dry CH_2Cl_2 (10 mL) anhydrous Et_3N (0.27 mL, 3.84 mmol) and succinic anhydride (0.211 g, 2.112 mmol) were added. After keeping 40 min at 40 °C reaction mixture was cooled to 24 °C, washed with 3% aq. HCl (1 × 15 mL), and water to pH 7. The organic layer was dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by column chromatography ($CHCl_3$ – MeOH – 1% aq. AcOH, 18 : 1 : 0.01 → 10 : 1 : 0.01) to give compound **4b** as a colorless oil (1.044 g, 69%). $[\alpha]_D^{27}$ -9.3 (*c* 1.0, $CHCl_3$). MALDI-TOFMS: *m/z*: 602.320 $[M + Na]^+$; Calcd for $C_{24}H_{37}NNaO_{15}$: 602.206 $[M + Na]^+$. ^{1}H NMR (300 MHz): 1.94 (s, 3 H), 2.01 (s, 3 H), 2.02 (s, 3 H), 2.10 (s, 3 H, 4 $COCH_3$), 2.39-2.51 m, 2 H) and 2.53-2.65 (m, 2 H, C(O)CH_2CH_2C(O)), 3.34-3.43 (m, 2 H, CH_2N), 3.47-3.76 (m, 9 H, $CH_2O(CH_2)_2OCH_2$, OC$\underline{H}_a$H), 3.85-3.99 (m, 2 H, OC$\underline{H}_b$H, H-5 Gal), 4.01-4.19 (m, 2 H, H-6 Gal), 4.52 (d, 1 H, *J* 7.9, H-1 Gal), 4.99 (dd, 1

H, *J* 3.3, 10.5, H-3 Gal), 5.14 (dd, 1 H, *J* 7.9, 10.5, H-2 Gal), 5.34 (dd, 1 H, *J* 0.8, 3.3, H-4 Gal), 6.65-6.88 (m, 1 H, NH); ^{13}C NMR (75 MHz): 20.62, 20.69, 20.72, 20.82, 30.47, 31.06, 39.45, 61.32, 67.11, 68.91, 69.13, 69.66, 70.18, 70.64, 70.71, 70.88, 101.33, 169.73, 170.24, 170.32, 170.54, 172.94, 176.61.

4,9-Bis-[4-(benzyloxy)-4-oxobutanoyl]-1,12-bis-(trifluoroacetamido)-4,9-diazadodecane (7). Ethyl trifluoroacetate (1.26 mL, 10.626 mmol) and water (11 ml, 5.9323 mmol) were added dropwise to a solution of spermine (**5**) (0.500 g, 2.471 mmol) in acetonitrile (15 mL). Reaction mixture was refluxed within 6 h, after removing of organic solvent in a vacuum the compound **6** was yielded (0.965 g, 99% technical grade) as white amorphous solid. To a solution of bis-trifluoroacetamide **6** (0.200 g, 0.507 mmol) in dry CH_2Cl_2 (2 mL) anhydrous pyridine (1.4 mL, 13.133 mmol) and DMAP (9 mg, 0.076 mmol) were added. Reaction mixture was cooled to 0 °C, and the chloride of succinic acid monobenzyl ester (0.460 g, 2.029 mmol) was added dropwise within 15 min, and mixture was kept for 1 h. Reaction mixture was diluted by CH_2Cl_2 (20 mL), washed with 3% aq. HCl (2 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by column chromatography on silica gel (petroleum ether – ethyl acetate, 1 : 5) to give compound **7** (145 mg, 37%) as a colorless oil. MALDI-TOFMS: *m/z* 797.570 $[M + Na]^+$; Calcd for $C_{36}H_{44}F_6N_4NaO_8$: 797.296 $[M + Na]^+$. ^{1}H NMR (300 MHz): 1.40-1.68 (m, 8 H, $NCH_2(C\underline{H}_2)_2CH_2N$, $NCH_2C\underline{H}_2$), 2.45-2.73 (m, 8 H, $C(O)CH_2CH_2C(O)$), 3.05-3.42 (m, 12 H, 6 CH_2N), 5.03 (wide s, 4 H, 2 CH_2Ph), 7.20 – 7.35 (m, 10 H, 2 Ph).

4,9-Bis-[4-(carboxy)butanoyl]-1,12-bis-(trifluoroacetamido)-4,9-diazadodecane (8). A catalytic amount of 10% Pd/C was added to a solution of compound **7** (49 mg, 0.063 mmol) and ammonium formate (8 mg, 0.126 mmol) in methanol (10 mL), and a reaction mixture was heated to 60 °C. After 15 min the catalyst was filtered off and methanol was removed under diminished pressure. Column chromatography on a silica gel ($CHCl_3$ – MeOH, 1 : 1) gave compound **8** (36.6 mg, 97%) as a colorless oil. MALDI-TOFMS: *m/z* 594.213 $[M]^+$; Calcd for $C_{22}H_{32}F_6N_4O_8$: 594.212 $[M]^+$. ^{1}H NMR (300 MHz): 1.29-1.60 (m, 4 H, $NCH_2(C\underline{H}_2)_2CH_2N$), 1.61-1.89 (m, 4 H, 2 $NCH_2C\underline{H}_2$), 2.23-2.68 (m, 8 H, $C(O)CH_2CH_2C(O)$), 3.09-3.42 (m, 12 H, CH_2N).

1,28-Bis-{[*rac*-2,3-bis(tetradecyloxy)propyloxy]carbonylamino}-*N*^{12},*N*^{17}-bis-[3-(trifluoroacetamido)propyl]-7,12,17,22-tetraaza-8,11,18,21-tetraoxooctacosane (9a). A solution of compound **8** (75 mg 0.127 mmol) and DIEA (66 μL, 0.380 mmol) in anhydrous DMF (2 mL) was cooled to 0 °C and stirred within 10 min. A solution of compound **2a** (0.238 g, 0.380 mmol) and HBTU (144 mg, 0.380 mmol) in anhydrous DMF (3 mL) was added dropwise into the reaction mixture and stirred within 4 day at 24 °C. DMF and DIEA were evaporated; the residue was dissolved in $CHCl_3$, washed with 3% aq. HCl (2 × 10 mL), and water to pH 7. The organic layer was dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by column chromatography on silica gel (toluene - $CHCl_3$ – MeOH – acetone, 1 : 1 : 0.1 : 0.1) to give 0.175 g (72%) of compound **9a** as a colorless oil. MALDI-TOFMS: *m/z* 1835.768 $[M + Na]^+$; Calcd for $C_{98}H_{184}F_6N_8NaO_{14}$: 1834.373 $[M + Na]^+$. ^{1}H NMR (300 MHz): 0.81 (t, 12 H, *J* 6.7, 4 $CH_2C\underline{H}_3$), 1.09-1.32 (m, 96 H, 4 $(CH_2)_{11}$, 2 $NHCH_2CH_2(C\underline{H}_2)_2$), 1.32-1.73 (m, 24 H, 6 $NCH_2C\underline{H}_2$, 4 $OCH_2C\underline{H}_2$ $NCH_2(C\underline{H}_2)_2CH_2N$), 2.04-2.14 (m, 4 H) и 2.39-2.52 (m, 4 H, 2 $C(O)CH_2CH_2C(O)$), 3.01-3.63 (m, 36 H, 4 CH_2N, 6 $NHC\underline{H}_2$, 2 OCH_2CH, 4 $OC\underline{H}_2CH_2$), 4.01 (dd, 2 H, *J* 11.4, 4.0, 2 $CH_2OC(O)$), 4.11 (dd, 2 H, *J* 11.4, 5.4, 2 $CH_2OC(O)$), 4.71-4.90 (m, 2 H, 2 NH), 5.82-6.18 (m, 2 H, 2 NH). ^{13}C NMR (75 MHz): 14.21, 22.78, 24.92, 26.13, 26.18, 26.25, 26.28, 26.36, 26.93, 27.84, 28.20, 28.38, 29.45, 29.59, 29.75, 29.79, 29.89, 30.09, 31.06, 31.31, 31.46, 32.01, 36.00, 37.36, 39.47, 40.87, 42.45, 44. 22, 44.59, 47.53, 64.30, 65.26, 70.49, 70.69, 71.86, 76.95, 79.11, 116.17 (q, *J* 287.8, $C(O)CF_3$), 156.61, 157.08, 157.56, 157.98 (q, *J* 37.0, $C(O)CF_3$), 171.81, 172.09, 172.33, 172.67, 173.42.

1,28-Bis-[(cholest-5-en-3β-yloxy)carbonylamino]-*N*^{12},*N*^{17}-bis-[3-(trifluoroacetamido)propyl]-7,12,17,22-tetraaza-8,11,18,21-tetraoxooctacosane (9b) was synthesized as described for **9a** using compound **8** (36 mg, 0.061 mmol), HBTU (69 mg, 0.182 mmol), compound **2b** (96 mg, 0.182 mmol) and DIEA (32 μL, 0.182 mmol). Compound **9b** was obtained in 89% yield (87 mg). MALDI-TOFMS: *m/z* 1639.589 $[M + Na]^+$; Calcd for $C_{90}H_{148}F_6N_8NaO_{10}$: 1638.112 $[M + Na]^+$. ^{1}H NMR (300 MHz): 0.61 (s, 6 H, 2 C(13)Me), 0.78 (d, 6 H, *J* 6.6, 2 C(25)Me), 0.79 (d, 6 H, *J* 6.6, 2 C(25)Me), 0.84 (d, 6 H, *J*

6.4, 2 C(20)Me), 0.94 (s, 6 H, 2 C(10)Me), 0.90–2.00 (m, 75 H, Chol protons, 6 $NHCH_2C\underline{H}_2$, $NCH_2(C\underline{H}_2)_2CH_2N$, 2 $NHCH_2CH_2(C\underline{H}_2)_2$), 2.15-2.28 (m, 4 H, 2 $H_2C(4)$ Chol), 2.41-2.50 (m, 4 H) и 2.51-2.62 (m, 4 H, 2 $C(O)CH_2CH_2C(O)$), 3.00-3.41 (m, 20 H, 4 NCH_2, 6 $NHC\underline{H}_2$), 4.35-4.46 (m, 2 H, H(3) Chol), 4.65-4.76 (m, 2 H, 2 NH), 5.29 (br. s, 2 H, H(6) Chol), 5.84-6.20 (m, 2 H, 2 NH). ^{13}C NMR (75 MHz): 11.97, 18.83, 19.43, 21.16, 22.67, 22.92, 23.96, 24.40, 28.12, 28.30, 28.34, 32.01, 32.03, 35.91, 36.31, 36.69, 37.12, 39.64, 39.87, 42.44, 50.16, 56.29, 56.82, 74.37, 79.19, 116.21 (q, *J* 287.8, $C(O)CF_3$), 122.56, 139.99, 156.43, 157.25, 157.49, 171.89, 172.15, 172.40, 172.70, 173.43.

1,28-Bis-{[*rac*-2,3-bis(tetradecyloxy)propyloxy]carbonylamino}-*N*^{12},*N*^{17}-bis-[3-aminopropyl]-7,12,17,22-tetraaza-8,11,18,21-tetraoxooctacosane (10a). K_2CO_3 (52 mg, 0.379 mmol) was added to a solution of compound **9a** (0.122 g, 0.063 mmol) in MeOH (5 mL), and a reaction mixture was stirred 48 h at 50 °C. After cooling to 24 °C methanol was evaporated, the residue was dissolved in CH_2Cl_2 (30 mL), washed with water to pH 7. The organic layer was dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$ – MeOH – aq. NH_3, 4 : 1: 0.2) to give compound **9a** as a colorless oil (63 mg, 60%). MALDI-TOFMS: *m/z* 1642.934 $[M + Na]^+$; Calcd for $C_{94}H_{186}N_8NaO_{12}$: 1642.409 $[M + Na]^+$.

1,28-Bis-[(choles-5-en-3β-yloxy)carbonylamino]-*N*^{12},*N*^{17}-bis-[3-aminopropyl]-7,12,17,22-tetraaza-8,11,18,21-tetraoxooctacosane (10b). To a solution of compound **9b** (41 mg, 0.025 mmol) in MeOH (5 mL) 0.01 *M* solution of NaOH in MeOH (20 mL) was added and a mixture was stirred for 22 h at 24 °C. MeOH was removed in a vacuum; the residue was dissolved in CH_2Cl_2 (50 mL), washed with water, dried over Na_2SO_4, filtered and evaporated. Product was isolated by means of column chromatography ($CHCl_3$ – MeOH – aq. NH_3, 4 : 1: 0.2). Compound **10b** was obtained in 81% yield (30 mg) as a colorless oil. MALDI-TOFMS: *m/z* 1443.601 $[M + Na]^+$; Calcd for $C_{94}H_{186}N_8NaO_{12}$: 1444.132 $[M + Na]^+$.

1,28-Bis-{[*rac*-2,3-bis(tetradecyl)propyloxy]carbonylamino}-*N*^{12},*N*^{17}-bis-[17-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-4,9-diaza-12,15-dioxa-5,8-dioxoheptadecyl]-7,12,17,22-tetraaza-8,11,18,21-tetraoxooctacosane (11a) was synthesized as described for **9a** using compound **10a** (63 mg, 0.039 mmol), HBTU (44 mg, 0.116 mmol), compound **4b** (68 mg, 0.116 mmol) and DIEA (20 μL, 0.116 mmol). Column chromatography on a silica gel ($CHCl_3$ – MeOH, 40 : 1) gave compound **11a** (47 mg, 43%) as a colorless oil. MALDI-TOFMS: *m/z* 2766.153 $[M + Na + H]^+$; Calcd for $C_{142}H_{257}N_{10}NaO_{40}$: 2765.828 $[M + Na + H]^+$. ^{1}H NMR (300 MHz): 0.81 (t, 12 H, *J* 6.1, 4 $CH_2C\underline{H}_3$), 1.13-1.29 (m, 96 H, 4 $(CH_2)_{11}$, 2 $NHCH_2CH_2(C\underline{H}_2)_2$), 1.32-1.73 (m, 24 H, 6 $NCH_2C\underline{H}_2$, 4 $OCH_2C\underline{H}_2$ $NCH_2(C\underline{H}_2)_2CH_2N$), 1.92, 1.98, 1.99, 2.08 (4 s, 24 H, 8 $C(O)CH_3$), 2.39-2.51 (m, 16 H, 4 $C(O)CH_2CH_2C(O)$), 2.98-3.14 (m, 12 H, 6 $NHC\underline{H}_2$), 3.16-3.62 (m, 44 H, 4 CH_2N, 2 $C\underline{H}_2NH$, 2 $CH_2O(CH_2)_2OCH_2$, 2 $OC\underline{H}_aH$, 4 $OC\underline{H}_2CH_2$, 2 OCH_2CH), 3.80-4.15 (m, 12 H, 2 $OC\underline{H}_bH$, 2 H-5 Gal, 4 H-6 Gal, 2 $CH_2OC(O)$), 4.50 (d, 2 H, *J* 7.9, 2 H-1 Gal), 4.96 (dd, 2 H, *J* 3.4, 10.4, 2 H-3 Gal), 5.13 (dd, 2 H, *J* 7.9, 10.4, 2 H-2 Gal), 5.32 (d, *J* 3.4, 2 H, 2 H-4 Gal), 6.15-6.20 (m, 2 H, 2 NH), 6.53-6.75 (m, 2 H, 4 NH), 6.91-7.15 (m, 2 H, 2 NH). ^{13}C NMR (75 MHz): 14.20, 20.68, 20.75, 20.77, 20.88, 22.77, 26.13, 26.19, 26.41, 26.51, 29.44, 29.59, 29.74, 29.78, 29.90, 30.11, 31.71, 32.00, 39.38, 39.50, 40.99, 54.90, 61.36, 64.27, 67.16, 68.95, 69.23, 69.89, 70.25, 70.33, 70.56, 70.68, 70.79, 70.94, 71.86, 76.99, 101.43, 156.58, 169.71, 170.24, 170.35, 170.52, 172.46, 172.56, 172.61, 172.68, 172.94.

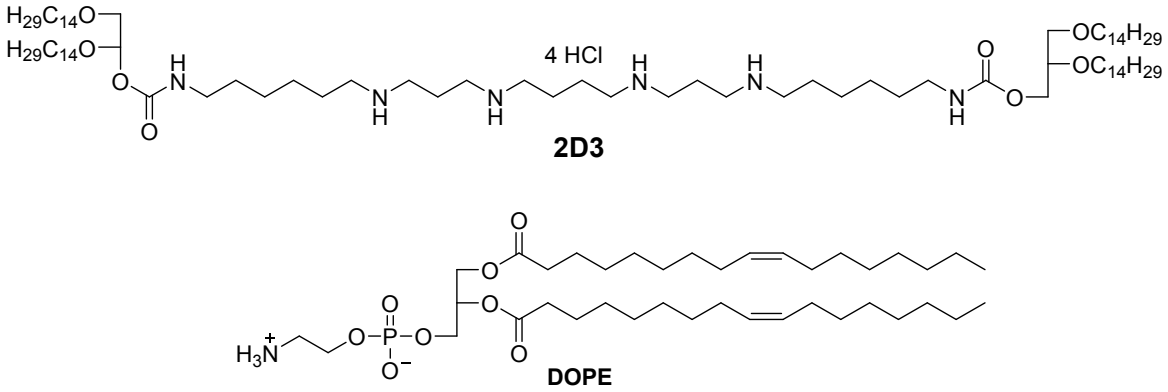
1,28-Bis-[(cholest-5-en-3β-yloxy)carbonylamino]-*N*^{12},*N*^{17}-bis-[17-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)-4,9-diaza-12,15-dioxa-5,8-dioxoheptadecyl]-7,12,17,22-tetraaza-8,11,18,21-tetraoxooctacosane (11b) was synthesized as described for **9a** using compound **10b** (30 mg, 0.021 mmol), HBTU (24 mg, 0.063 mmol), compound **4b** (50 mg, 0.084 mmol) and DIEA (10 μL, 0.084 mmol). Column chromatography on a silica gel (toluene - $CHCl_3$ – MeOH – acetone, 1 : 1 : 0.2 : 0.5) gave compound **11b** (32 mg, 47%) as a colorless oil. $[\alpha]_D^{26}$ -4.0 (*c* 0.5, $CHCl_3$ - CH_3OH, 1 : 1). MALDI-TOFMS: *m/z* 2570.471 $[M + Na + H]^+$; Calcd for $C_{134}H_{220}N_{10}NaO_{36}$: 2569.567 $[M + Na + H]^+$. ^{1}H NMR (300 MHz): 0.61 (s, 6 H, 2 C(13)Me), 0.78 (d, 6 H, *J* 6.6, 2 C(25)Me), 0.80 (d, 6 H, *J* 6.6, 2 C(25)Me), 0.85 (d, 6 H, *J* 6.4, 2 C(20)Me), 0.94 (s, 6 H, 2 C(10)Me), 0.90–1.60 (m, 72 H, Chol protons, 6 $NHCH_2C\underline{H}_2$, $NCH_2(C\underline{H}_2)_2CH_2N$, 2 $NHCH_2CH_2(C\underline{H}_2)_2$), 1.66-1.89 (m, 14 H, Chol protons), 1.92, 1.96, 1.99, 2.08 (4 s, 24 H, 8 $COCH_3$), 2.13–2.34 (m, 4 H, 2 $H_2C(4)$ Chol), 2.40-2.64 (m, 16 H, 4

C(O)CH_2CH_2C(O)), 3.00-3.49 (m, 24 H, 4 NCH_2, 8 NHC$\underline{H}_2$), 3.50-3.73 (m, 14H, 2 $CH_2O(CH_2)_2OCH_2$, 2 OC$\underline{H}_a$H), 3.82-3.97 (m, 4 H, 2 OC$\underline{H}_b$H, 2 H-5 Gal),4.06 (dd, 1 H, *J* 6.6, 11.2) and 4.11 (dd, 1 H, *J* 6.7, 11.2, 4 H-6 Gal), 4.35-4.46 (m, 2 H, H(3) Chol), 4.50 (d, 2 H, *J* 7.9, 2 H-1 Gal), 4.96 (dd, 2 H, *J* 3.4, 10.4, 2 H-3 Gal), 5.13 (dd, 2 H, *J* 7.9, 10.4, 2 H-2 Gal), 5.27-5.31 (m, 2 H, H(6) Chol), 5.32 (dd, *J* 1.1, 3.4, 2 H, 2 H-4 Gal). ^{13}C NMR (75 MHz): 11.99, 18.84, 19.48, 20.74, 20.81, 20.83, 20.94, 21.17, 22.69, 22.95, 23.97, 24.42, 26.44, 26.55, 27.71, 28.14, 28.36, 28.66, 29.54, 29.79, 29.83, 29.99, 30.15, 31.06, 31.71, 31.79, 32.01, 32.04, 35.93, 36.31, 36.70, 36.95, 37.14, 38.75, 39.44, 39.53, 39.64, 39.87, 40.88, 42.44, 43.01, 45.97, 50.14, 56.27, 56.82, 61.41, 67.20, 68.98, 69.29, 69.92, 70.33, 70.41, 70.77, 70.82, 71.01, 74.30, 101.48, 122.58, 140.01, 156.39, 169.69, 170.30, 170.40, 170.55, 172.19, 172.25, 172.34, 172.48, 172.55, 172.62, 172.95.

1,28-Bis-{[*rac*-2,3-bis(tetradecyloxy)propyloxy]carbonylamino}-*N*^{12},*N*^{17}-bis-[17-(β-D-galactopyranosyloxy)-4,9-diaza-12,15-dioxa-5,8-dioxoheptadecyl]-7,12,17,22-tetraaza-8,11,18,21-tetraoxooctacosane (1a). To a solution of compound **11a** (46 mg, 0.017 mmol) in $CHCl_3$ (4 mL) 0.04 *M* MeONa in MeOH (1 mL) was added. A reaction mixture was stirred for 1 h at 24 °C, and solvents were removed in a vacuum. Column chromatography on a silica gel ($CHCl_3$ – MeOH – 1% aq. AcOH, 5 : 1 : 0.08) gave compound **1a** (29 mg, 72%) as a white amorphous solid. MALDI-TOFMS: *m/z* 2429.970 $[M + Na]^+$. Calcd for $C_{126}H_{240}N_{10}NaO_{32}$: 2428.736 $[M + Na]^+$. ^{1}H NMR (600 MHz, CD_3Cl-CD_3OD, 1:1): 0.80 (t, 12 H, *J* 6.1, 4 $CH_2C\underline{H}_3$), 1.04-1.31 (m, 96 H, 4 $(CH_2)_{11}$, 2 $NHCH_2CH_2(C\underline{H}_2)_2$), 1.34-1.75 (m, 24 H, 6 $NCH_2C\underline{H}_2$, 4 $OCH_2C\underline{H}_2$ $NCH_2(C\underline{H}_2)_2CH_2N$), 2.36-2.48 (m, 16 H, 4 C(O)CH_2CH_2C(O)), 2.98-3.14 (м, 12 H, 6 NHC$\underline{H}_2$), 3.19-3.76 (m, 54 H, 4 CH_2N, 2 C$\underline{H}_2$NH, 2 $CH_2O(CH_2)_2OCH_2$, 2 OC$\underline{H}_a$H, 4 OC$\underline{H}_2CH_2$, 2 OCH_2CH, 2 H-5 Gal, 4 H-6 Gal, 2 H-3 Gal, 2 H-2 Gal), 3.80-3.83 (m, 2 H, 2 H-4 Gal), 3.97-4.04 (m, 4 H, 2 OCH$\underline{H}_b$, 2 CH$\underline{H}_a$OC(O)), 4.07 (dd, 2 H, *J* 4.3, 11.7, 2 CH$\underline{H}_b$OC(O)), 4.22 (d, 2 H, *J* 7.9, 2 H-1 Gal). ^{13}C NMR (150 MHz): 14.20, 20.68, 20.75, 20.77, 20.88, 22.77, 26.13, 26.19, 26.41, 26.51, 29.44, 29.59, 29.74, 29.78, 29.90, 30.11, 31.71, 32.00, 39.38, 39.50, 40.99, 54.90, 61.36, 64.27, 67.16, 68.95, 69.23, 69.89, 70.25, 70.33, 70.56, 70.68, 70.79, 70.94, 71.86, 76.99, 101.43, 156.58, 170.35, 170.52, 172.46, 172.56, 172.61, 172.68, 172.94.

1,28-Bis-[(cholest-5-en-3β-yloxy)carbonylamino]-*N*^{12},*N*^{17}-bis-[17-(β-D-galactopyranosyloxy)-4,9-diaza-12,15-dioxa-5,8-dioxoheptadecyl]-7,12,17,22-tetraaza-8,11,18,21-tetraoxooctacosane (1b) was synthesized as described for **1a** starting from compound **11b** (17 мг, 0.0067 ммоль) and 0.04 *M* MeONa in MeOH (0.5 mL). Column chromatography on a silica gel ($CHCl_3$ – MeOH – 1% aq. AcOH, 5 : 1 : 0.08) gave compound **1b** (13 mg, 89%) as a white amorphous solid. $[\alpha]_D^{28}$ -6.7 (*c* 0.5, $CHCl_3$ - CH_3OH, 1 : 1). MALDI-TOFMS: *m/z* 2233.500 $[M + Na]^+$. Calcd for $C_{118}H_{204}N_{10}NaO_{28}$: 2232.474 $[M + Na]^+$. ^{1}H NMR (600 MHz, CD_3Cl-CD_3OD, 1:1): 0.61 (s, 6 H, 2 C(13)Me), 0.77 (d, 6 H, *J* 6.6, 2 C(25)Me), 0.79 (d, 6 H, *J* 6.6, 2 C(25)Me), 0.84 (d, 6 H, *J* 6.5, 2 C(20)Me), 0.94 (c, 6 H, 2 C(10)Me), 0.90 – 1.60 (m, 72 H, Chol protons, 6 $NHCH_2C\underline{H}_2$, $NCH_2(C\underline{H}_2)_2CH_2N$, 2 $NHCH_2CH_2(C\underline{H}_2)_2$), 1.64-1.95 (м, 14 H, Chol protons), 2.15–2.29 (m, 4 H, 2 H_2C(4) Chol), 2.36-2.60 (m, 16 H, 4 C(O)CH_2CH_2C(O)), 3.01 (t, 4 H, *J* 7.0), 3.07 (t, 6 H, *J* 7.0) and 3.14 (t, 2 H, *J* 6.5, 6 CH_2NH), 3.19-3.74 (m, 40 H, 4 NCH_2, 2 $CH_2CH_2O(CH_2)_2OCH_2CH_2$, 4 H-6 Gal, 2 H-3 Gal, 2 H-2 Gal), 3.82 (br. d, *J* 3.3, 2 H, 2 H-4 Gal), 3.98 (ddd, *J* 2.3, 5.0, 10.4, 2 H-5 Gal), 4.12-4.20 (m, 2 H, H(3) Chol), 4.21 (m, 2 H, *J* 7.7, 2 H-1 Gal), 5.23-5.32 (m, 2 H, H(6) Chol). ^{13}C NMR ^{13}C (150 MHz): 11.29, 18.14, 18.73, 20.67, 21.88, 22.13, 22.53, 23.42, 23.85, 25.99, 26.13, 27.58, 27.81, 28.06, 28.78, 28.92, 29.23, 29.30, 30.86, 30.99, 31.52, 31.56, 35.44, 35.81, 36.21, 36.38, 36.42, 36.68, 38.22, 38.83, 39.01, 39.14, 39.43, 40.16, 41.96, 49.81, 55.85, 56.42, 60.93, 67.84, 68.64, 69.47, 69.66, 69.71, 69.75, 69.77, 70.89, 70.91, 73.13, 74.02, 74.85, 76.95, 77.16, 77.16, 77.37, 103.00, 122.05, 139.55, 156.84, 172.15, 172.39, 172.70, 172.85, 173.15, 173.21.

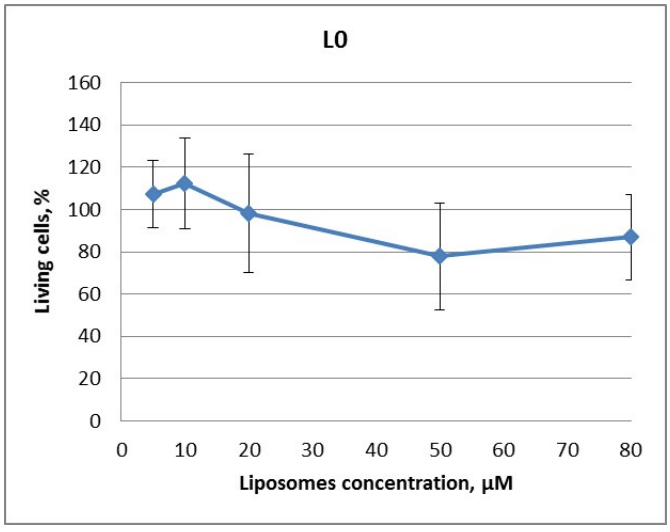
Structure of polycationic lipid 2D3 and zwitter-ionic lipid DOPE

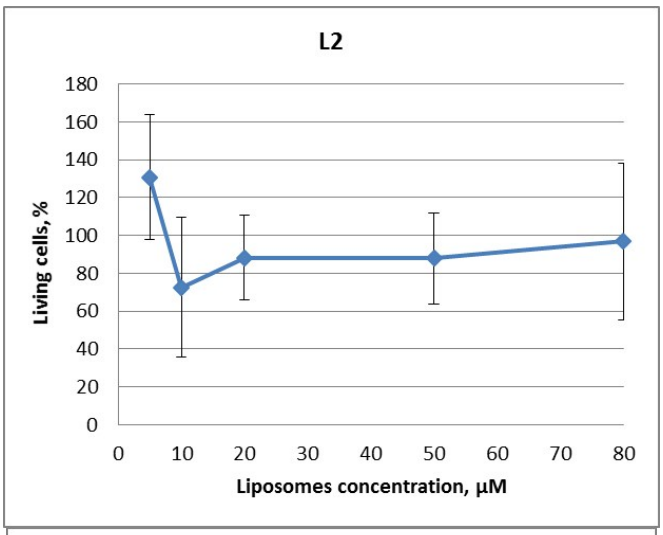


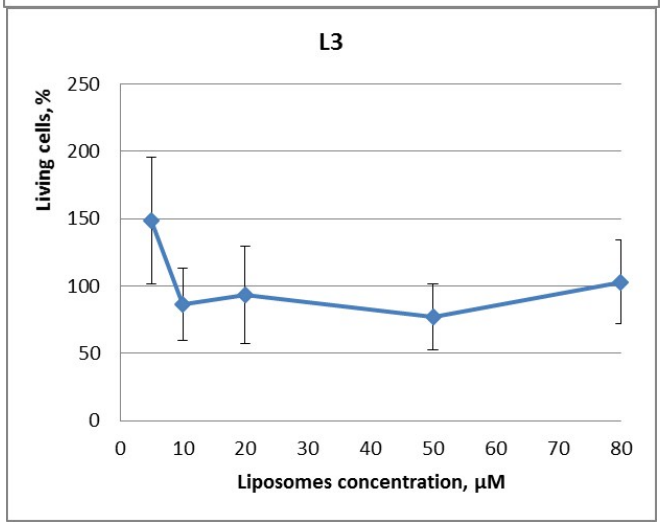
Preparation of liposomes

The non-targeted liposomes 2D3-DOPE (**L0**) were composed of lipid 2D3 and DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, Avanti Polar Lipids, USA) at a 1 : 1 molar ratio. To prepare the targeted liposomes, 2.5, 5 or 10% mol. of galactose-containing lipids **1a** were added to the core liposomes 2D3-DOPE. All liposomal formulations were made by the hydration of lipid thin-film followed by sonication. Briefly, cationic lipid 2D3, DOPE and lipid **1a** were dissolved in chloroform-methanol (1 : 1 v/v) mixture and gently agitated. The solvents were carefully evaporated and the residue was dried under a vacuum (0.01 Torr) for 2-3 h. The obtained lipid film was hydrated in deionised water (MilliQ) for 4 h at 4 °C, and then the lipid suspensions were sonicated for 15 min at 65–70 °C in a bath-type sonicator (Bandelin Sonorex Digitec DT 52H, Germany). In the resulting dispersion, the cationic lipid 2D3 concentration was 1 mM.

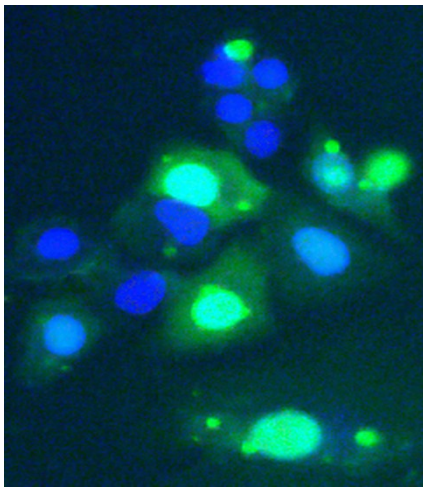
Cytotoxicity of liposomes estimated by MTT test

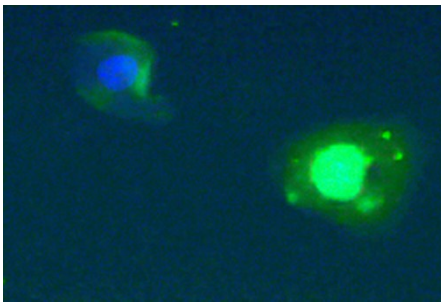






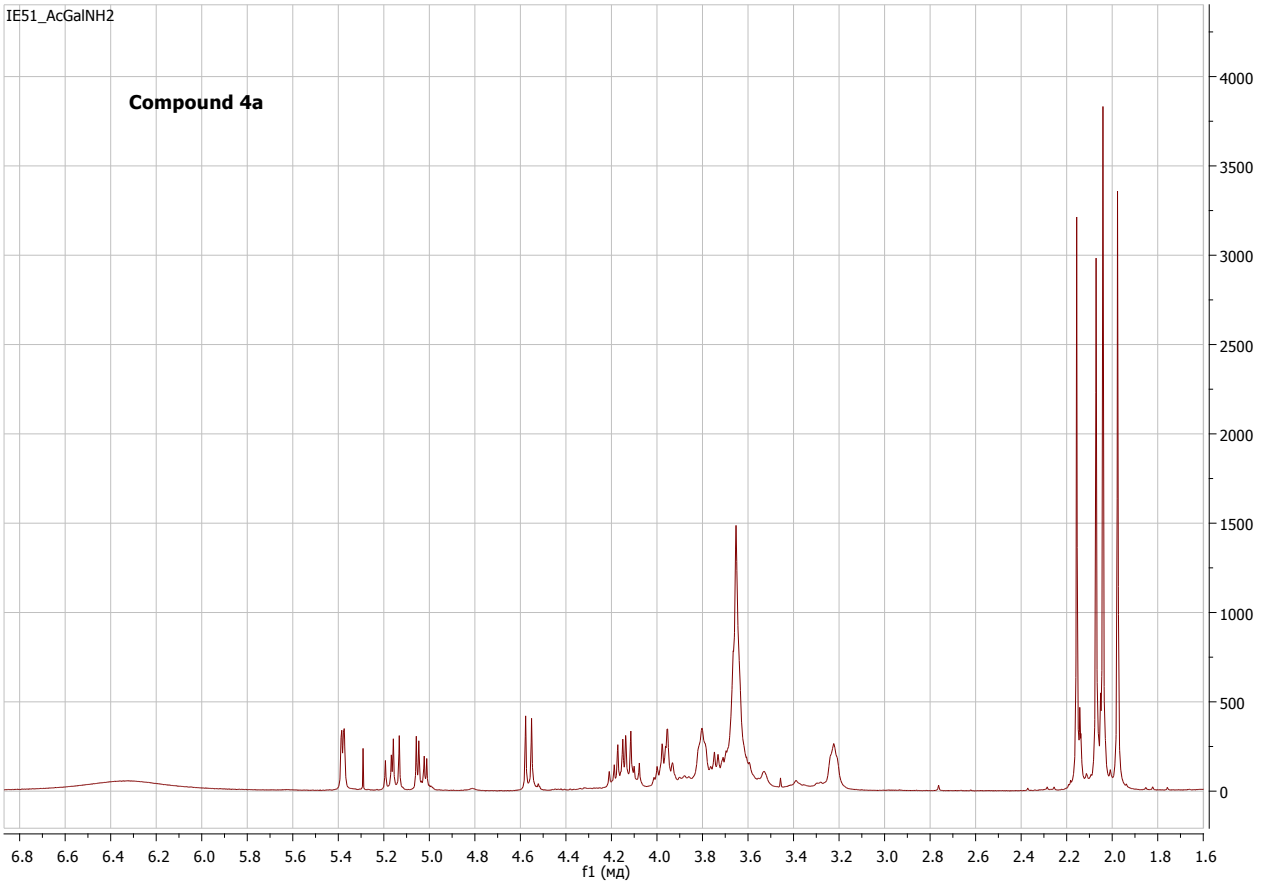
Localization of FITC-labeled oligonucleotide

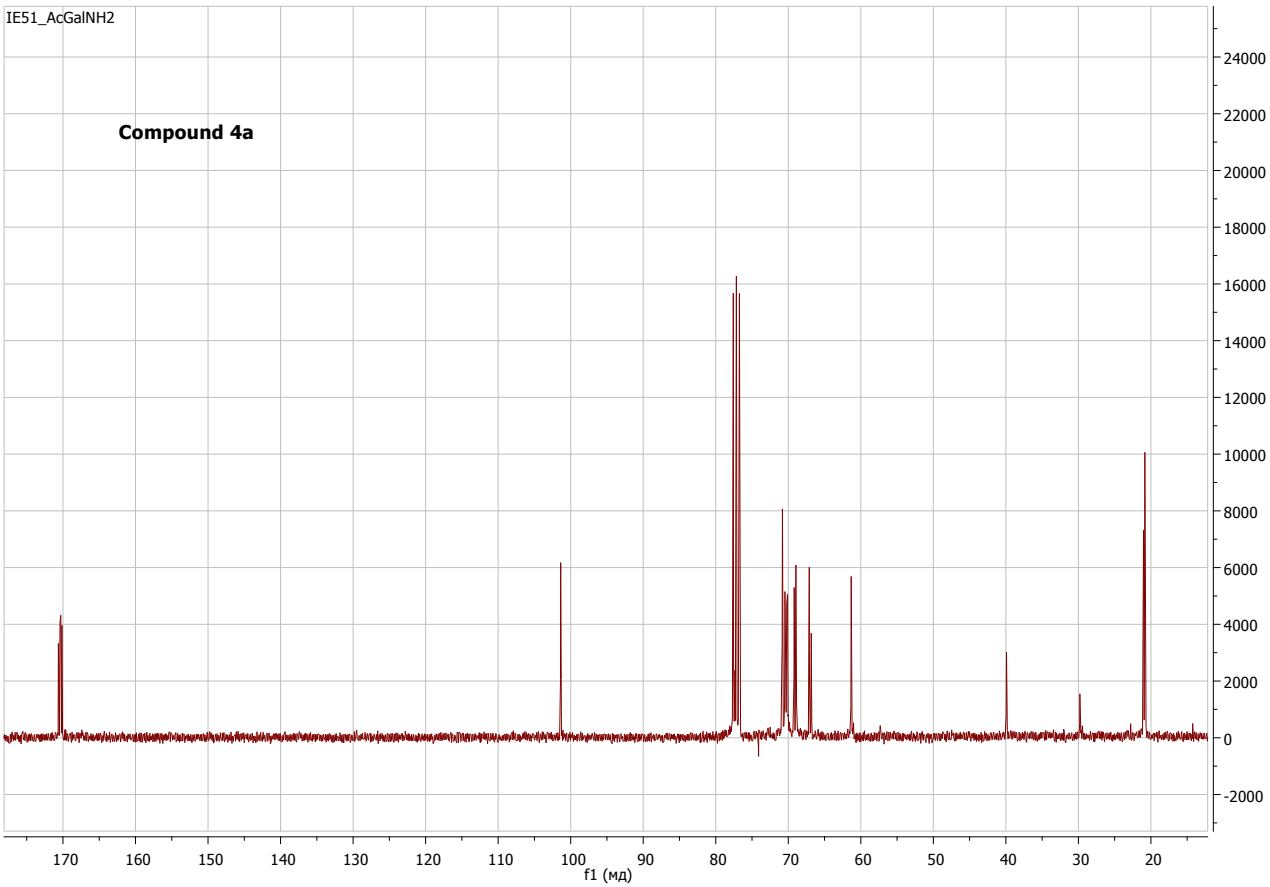




Localization of FITC-labeled oligonucleotide in Hep G2 cells after 4 h incubation with FITC-ODN/L2 lipoplexes using InCell Analyser. Green signal corresponds to the FITC-labeled oligonucleotide; blue signal, to the nuclei stained with DAPI.

NMR spectra of compounds synthesized





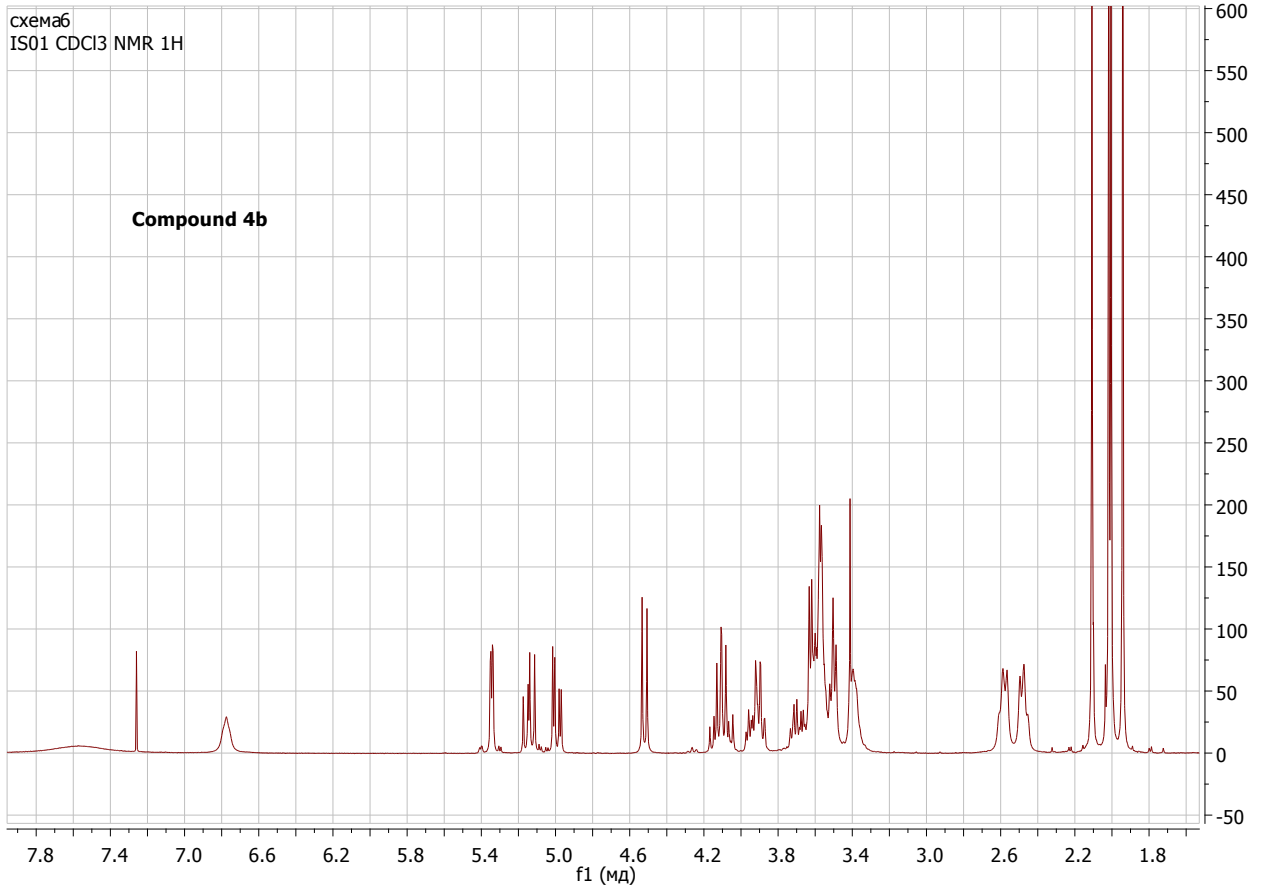


схема6
IS01 CDCl3 NMR 1H
Compound 4b
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
7.8
7.4
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
f1 (мд)

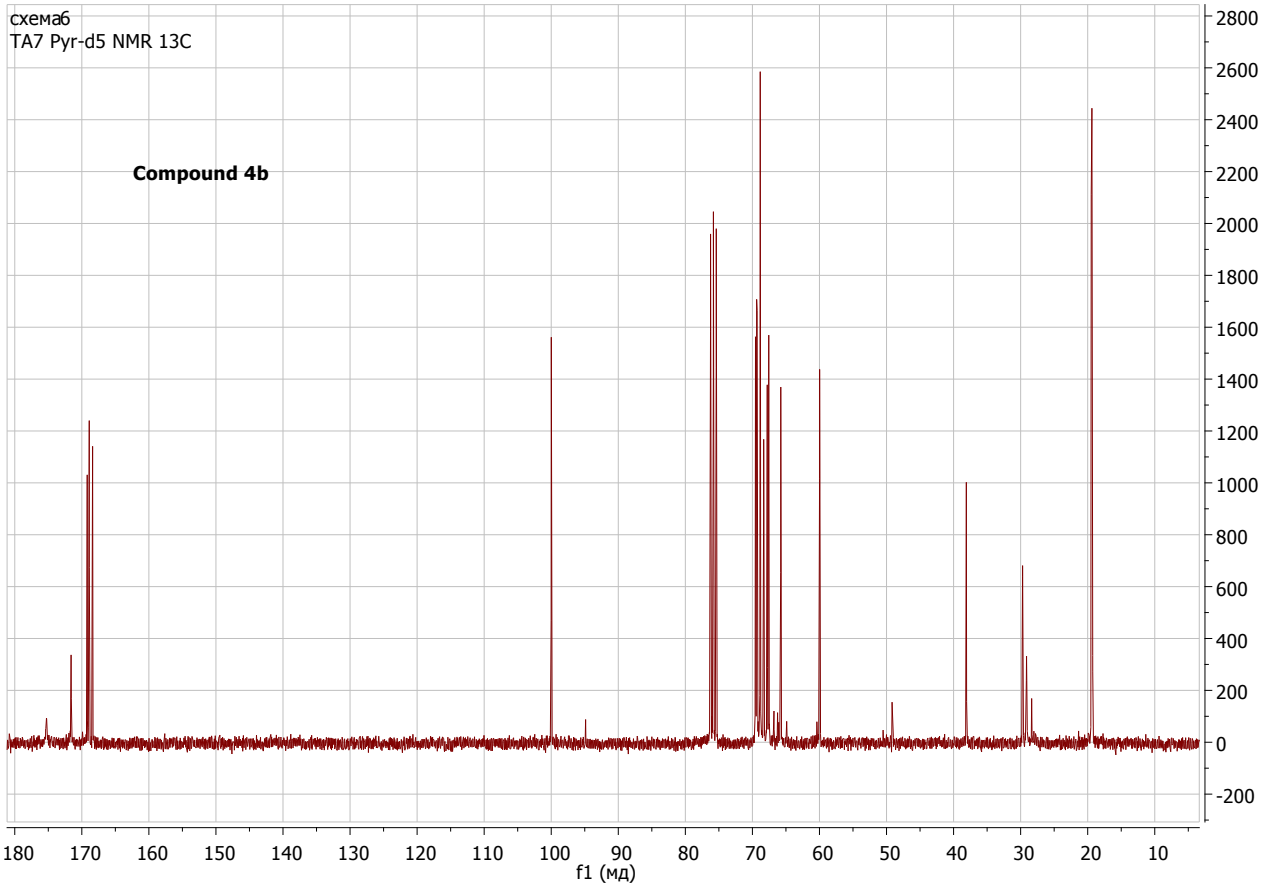
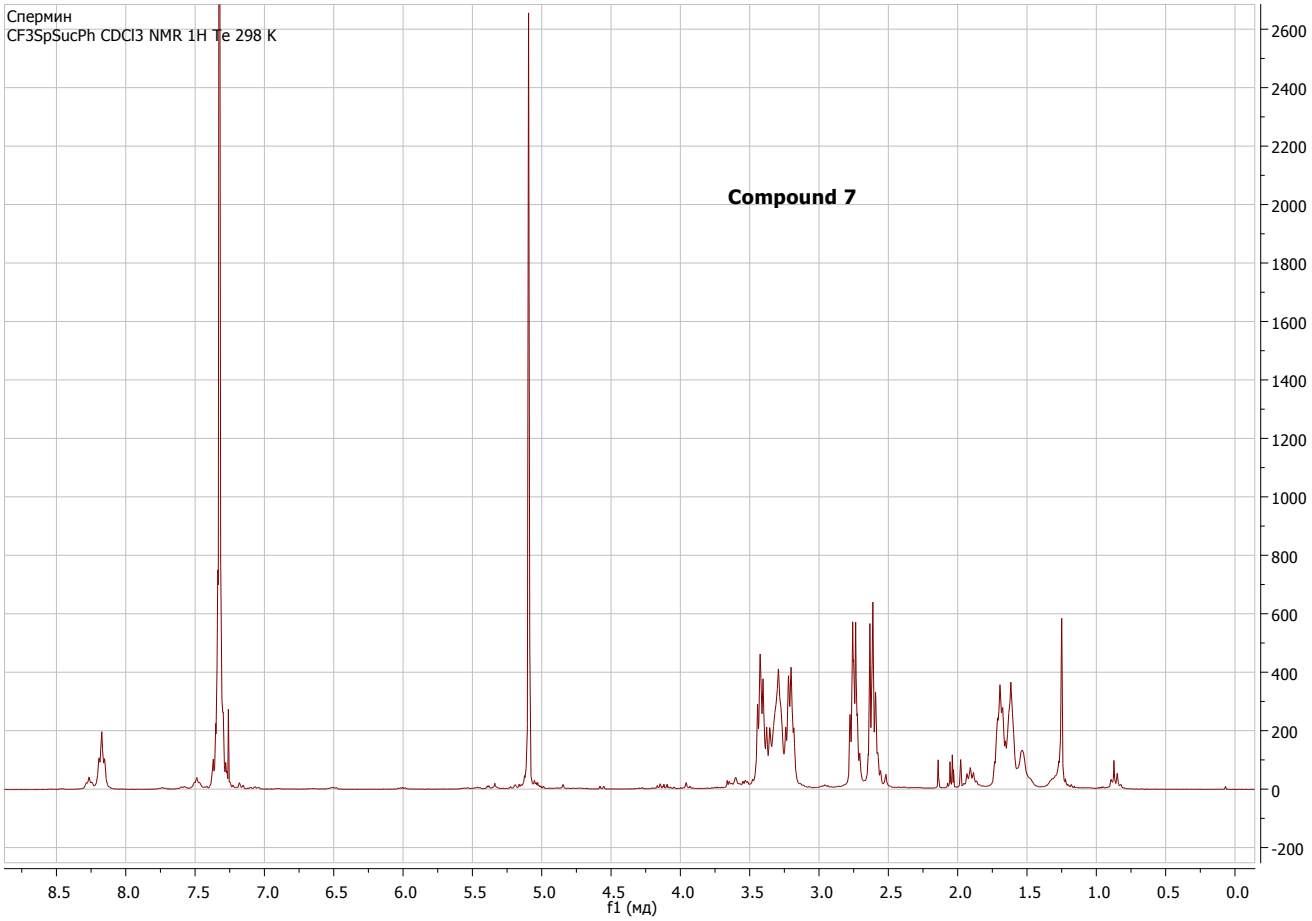
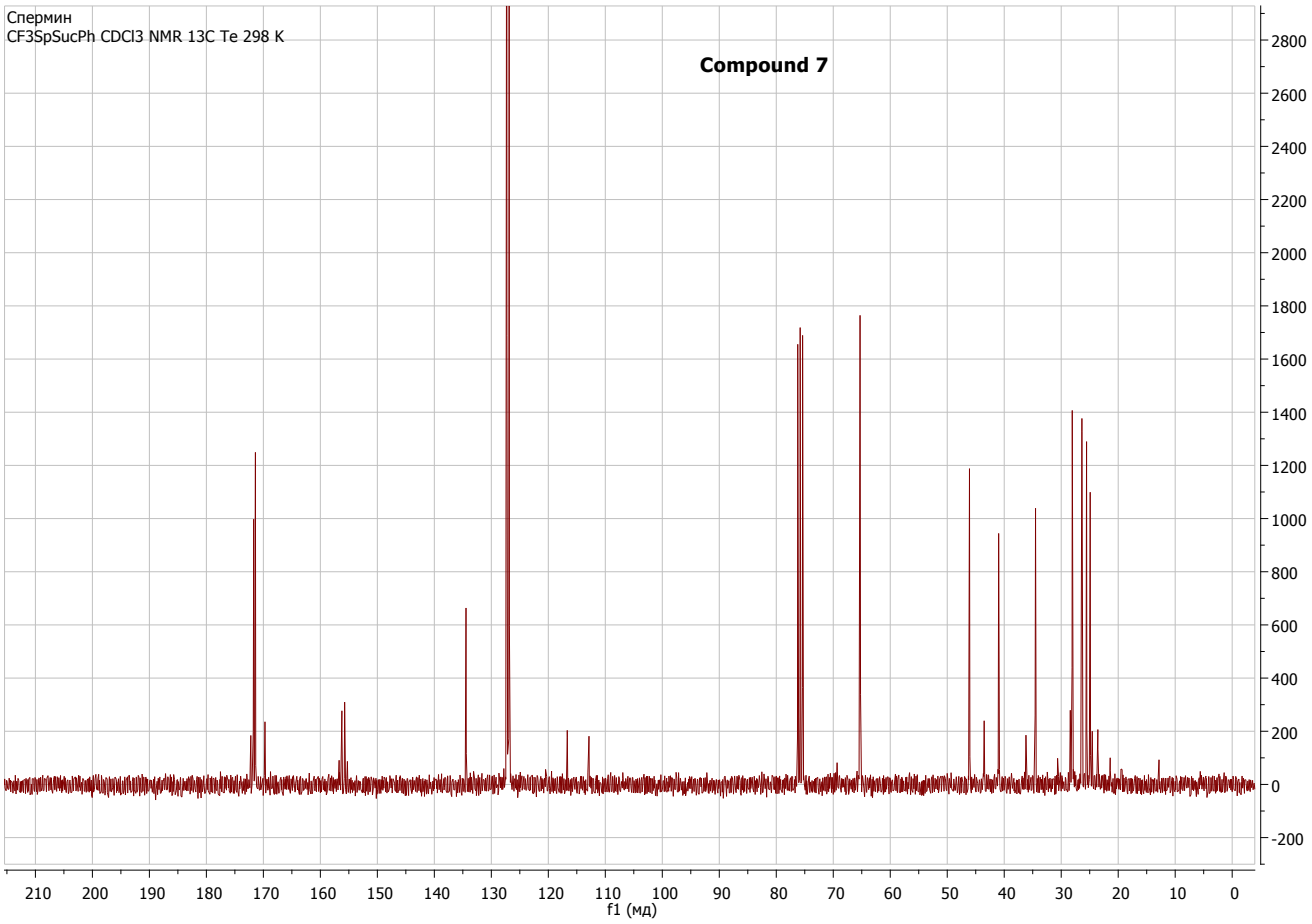
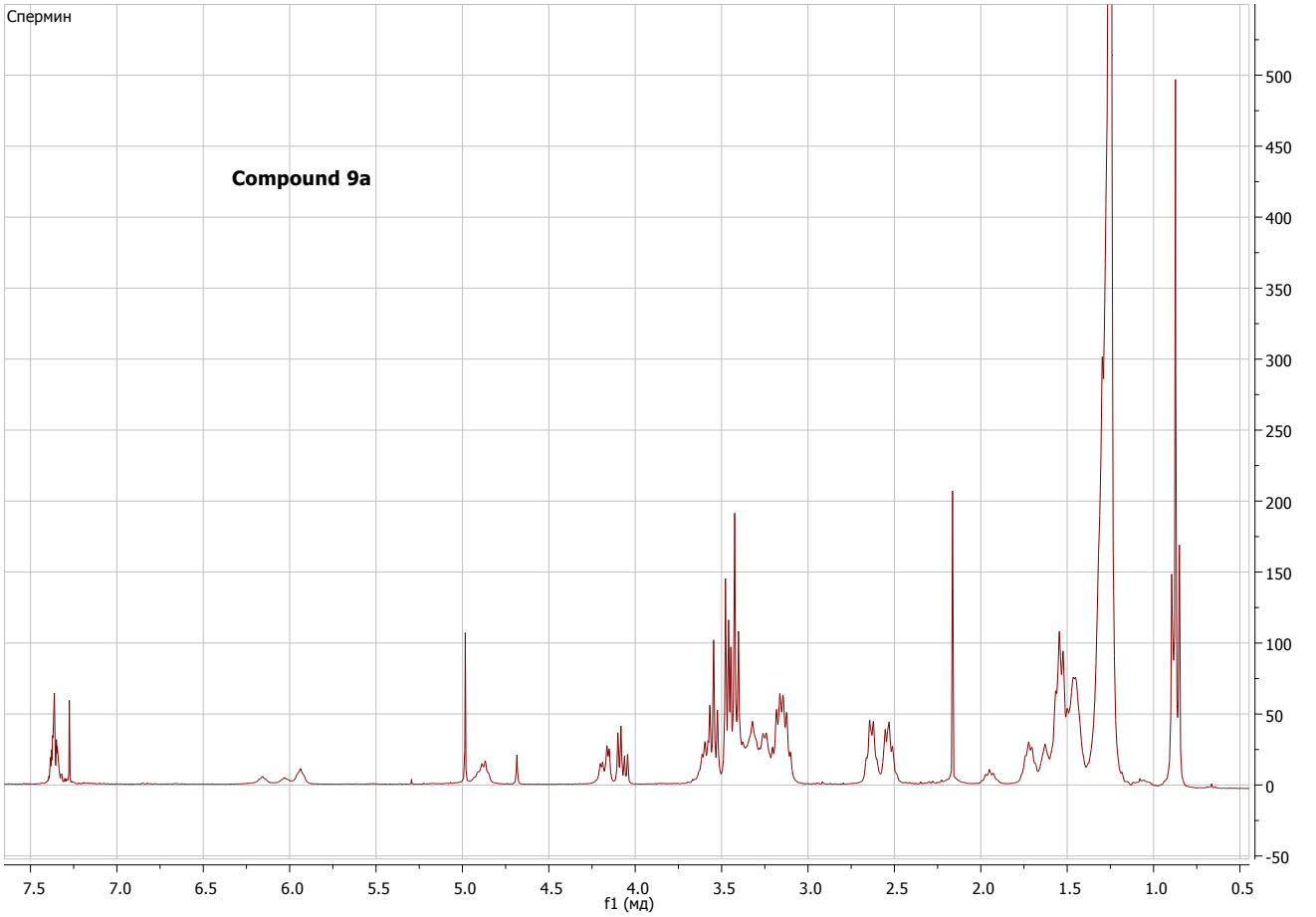
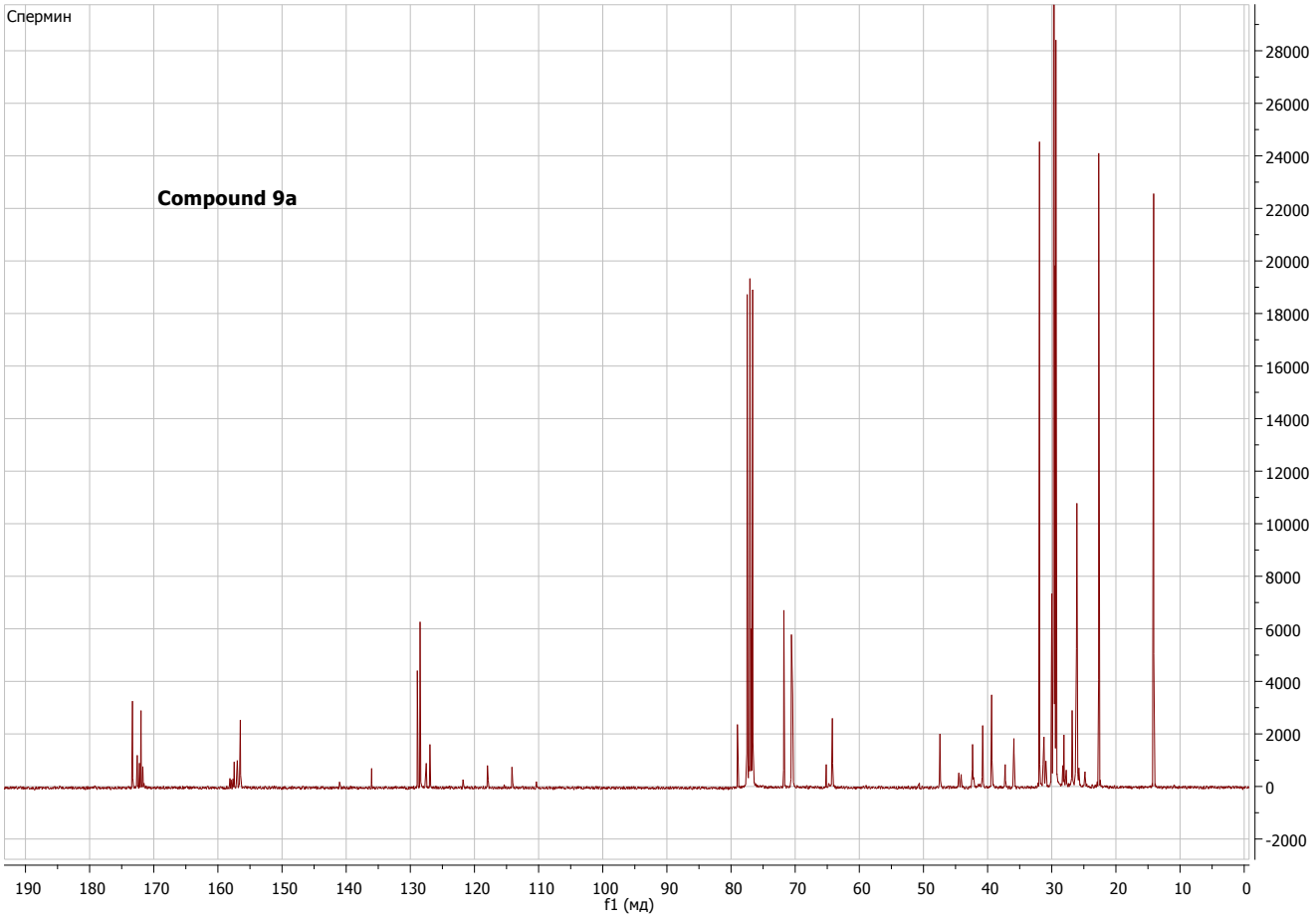


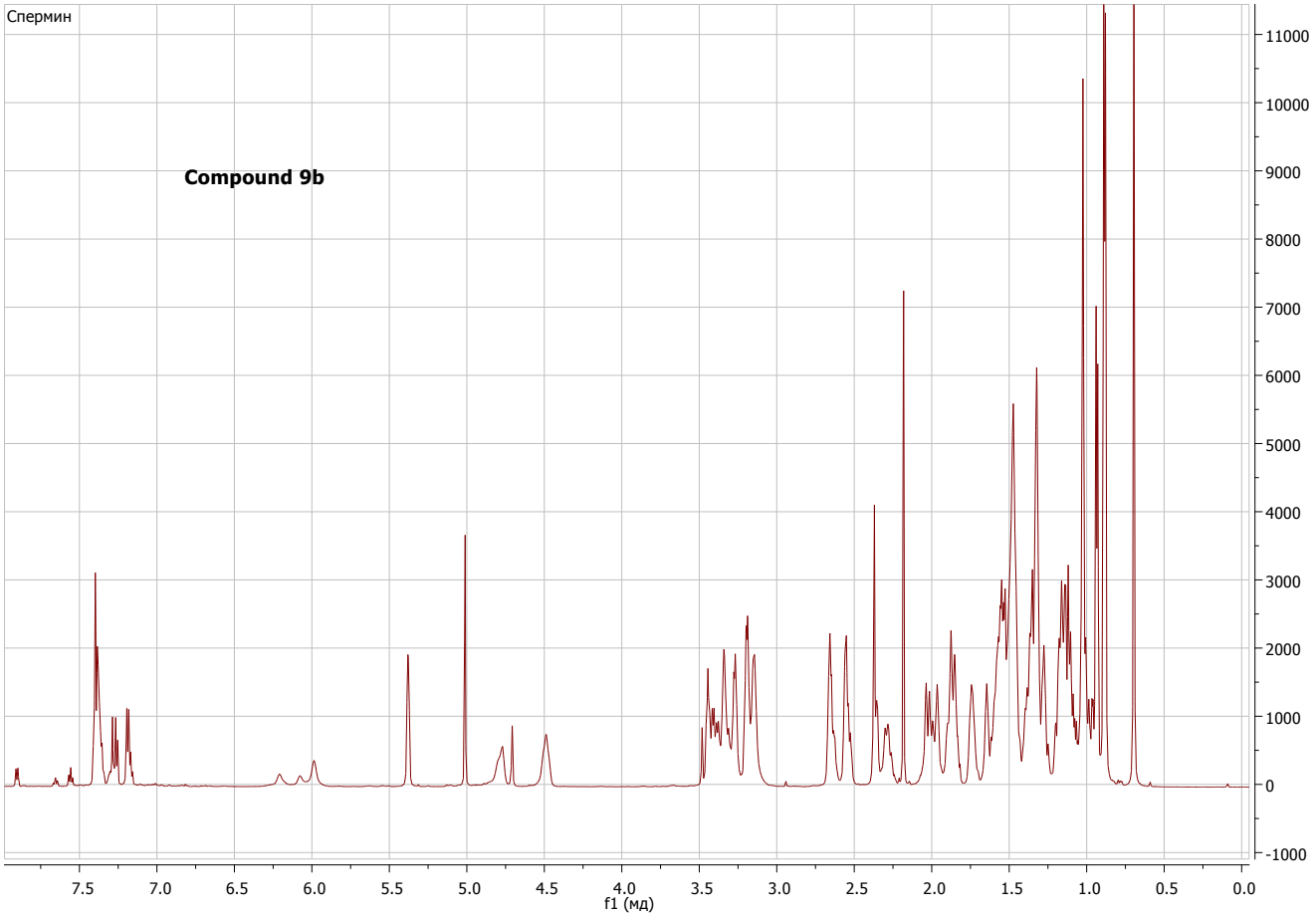
схема6
TA7 Pyr-d5 NMR 13C
Compound 4b
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (мд)

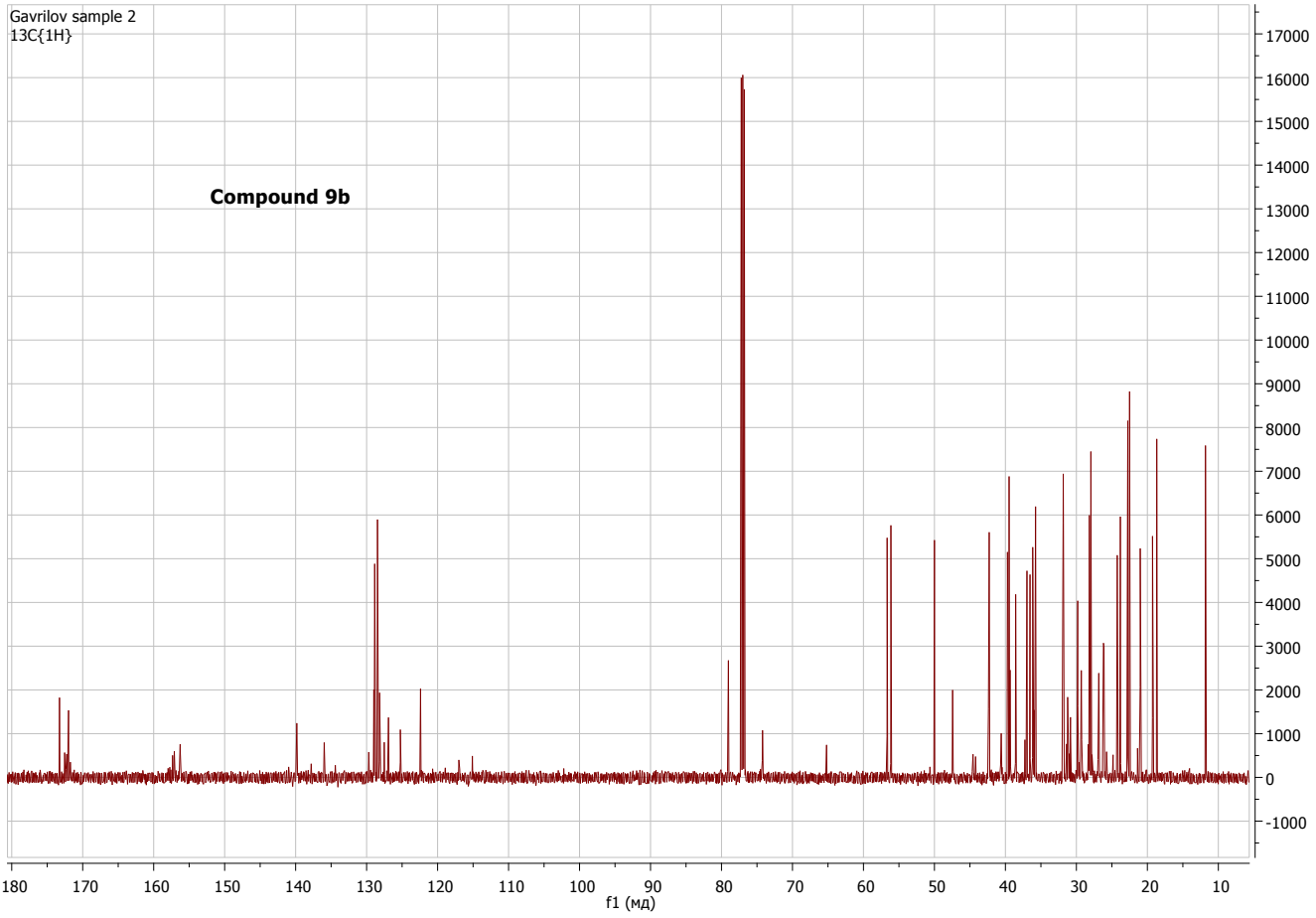

Спермин
CF3SpSucPh CDCl3 NMR 1H Te 298 K
Compound 7
f1 (мд)

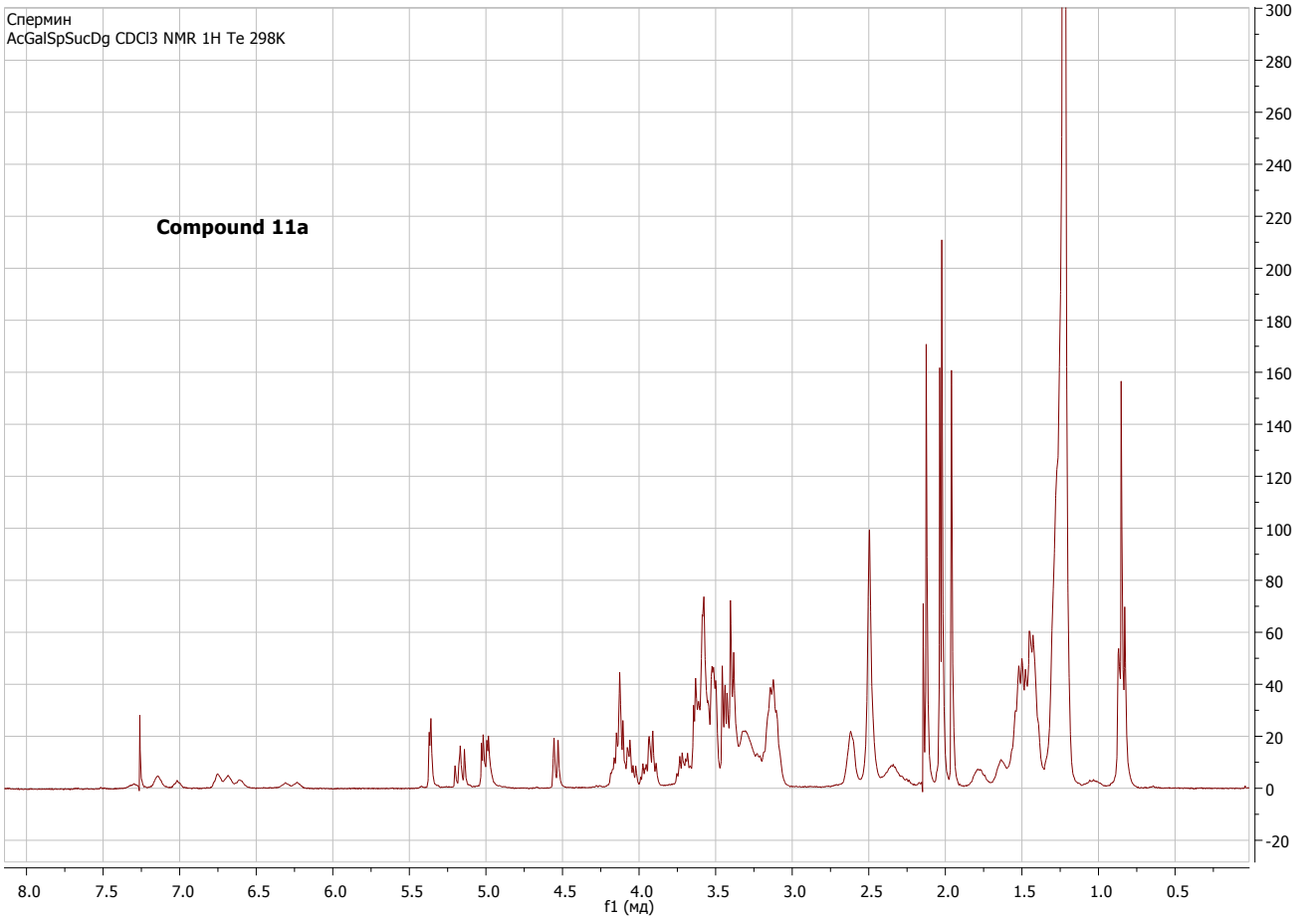

Спермин
CF3SpSucPh CDCl3 NMR 13C Te 298 K
Compound 7
f1 (мд)

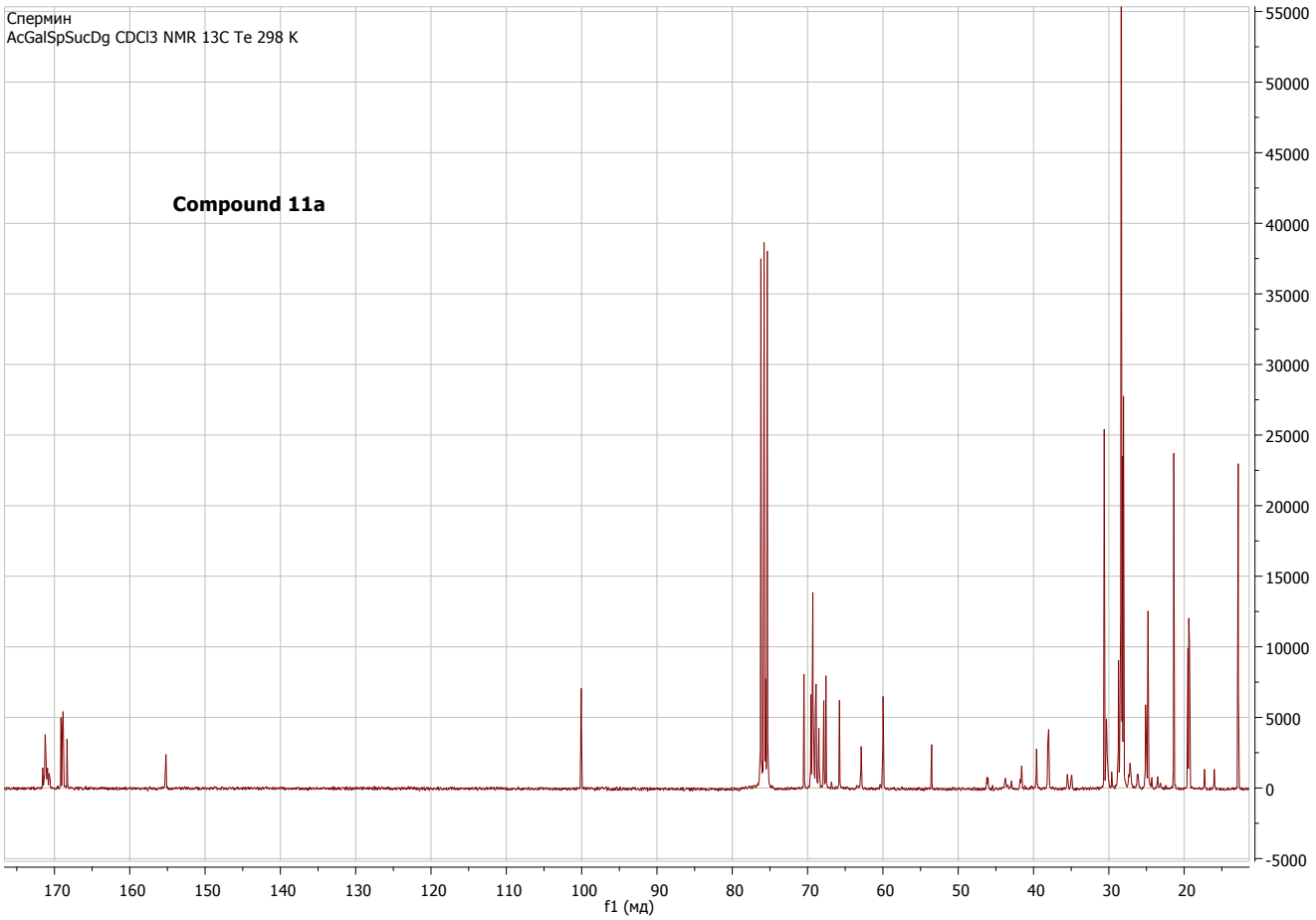

Спермин
Compound 9a
500
450
400
350
300
250
200
150
100
50
0
-50
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (мд)

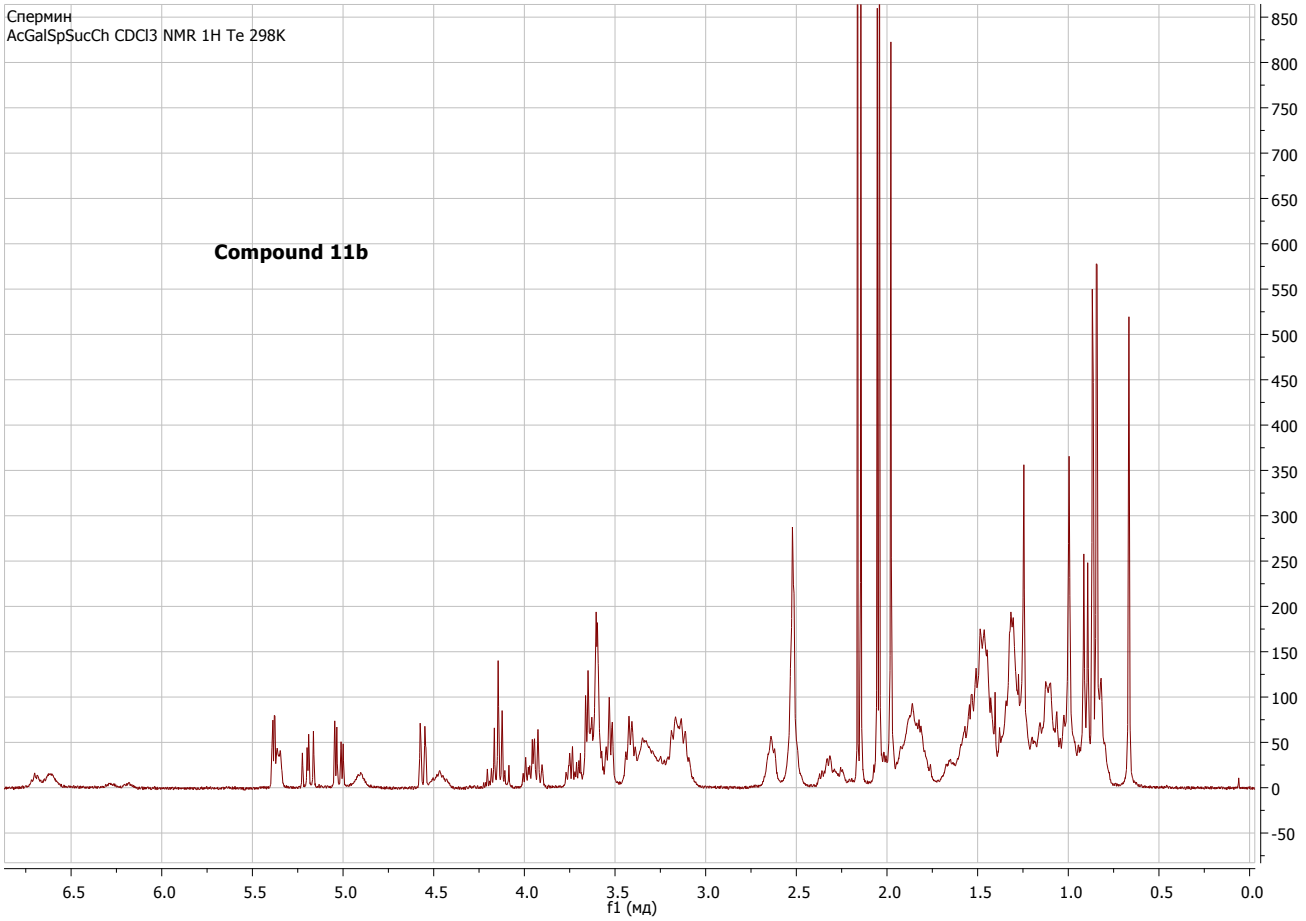

Спермин
Compound 9a
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (мд)

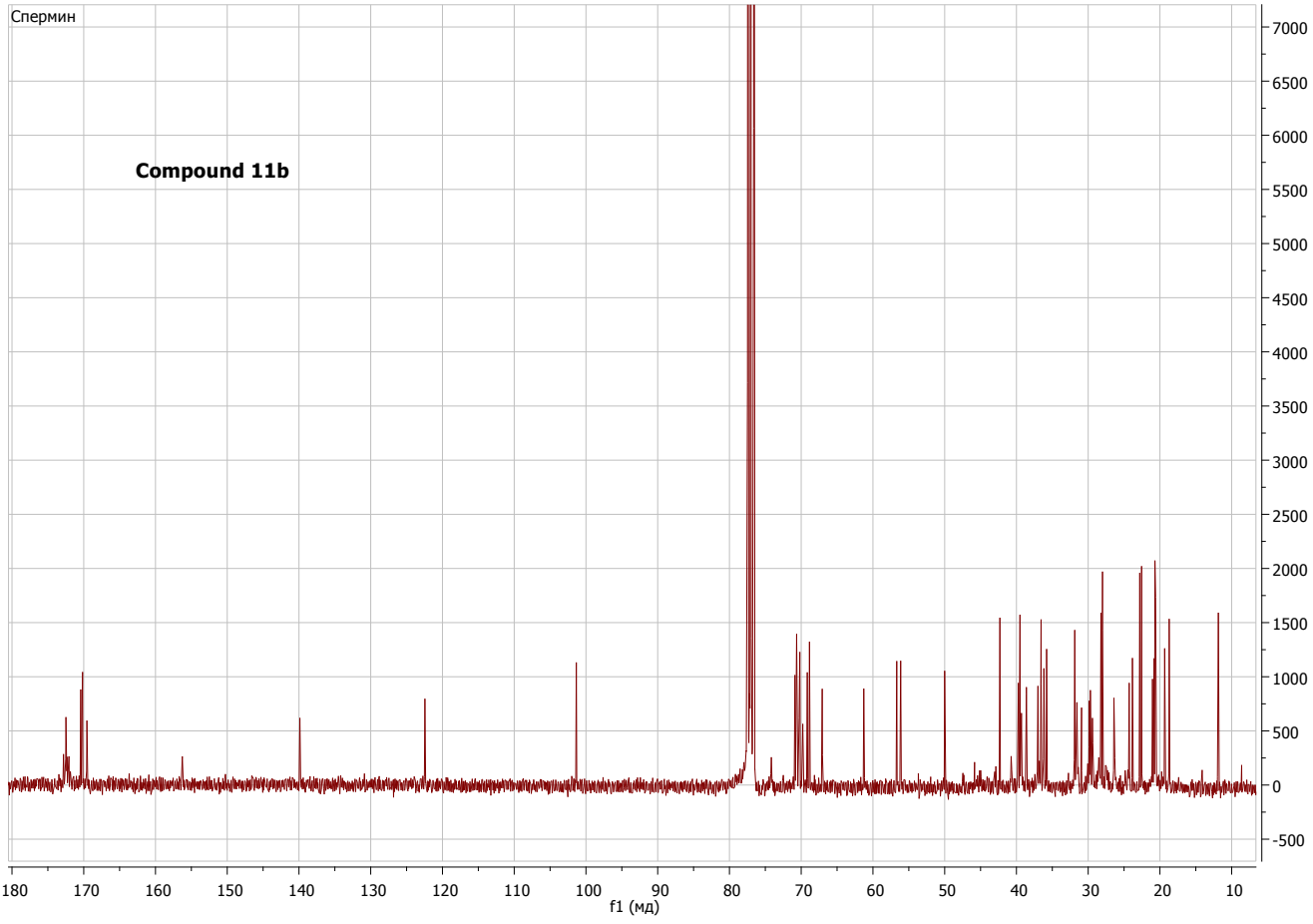

Спермин
Compound 9b
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (мд)

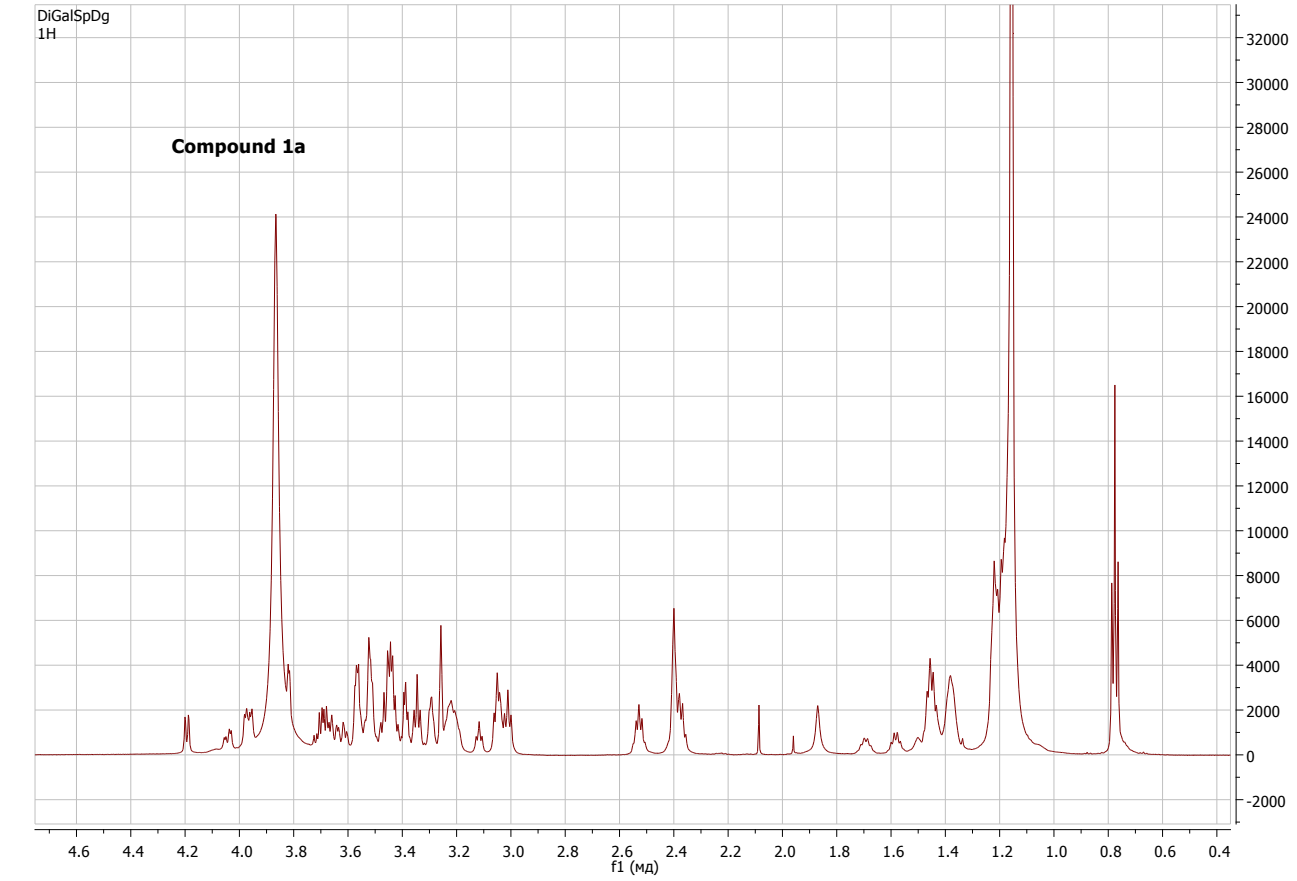

Gavrilov sample 2
13C{1H}
Compound 9b
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (мд)

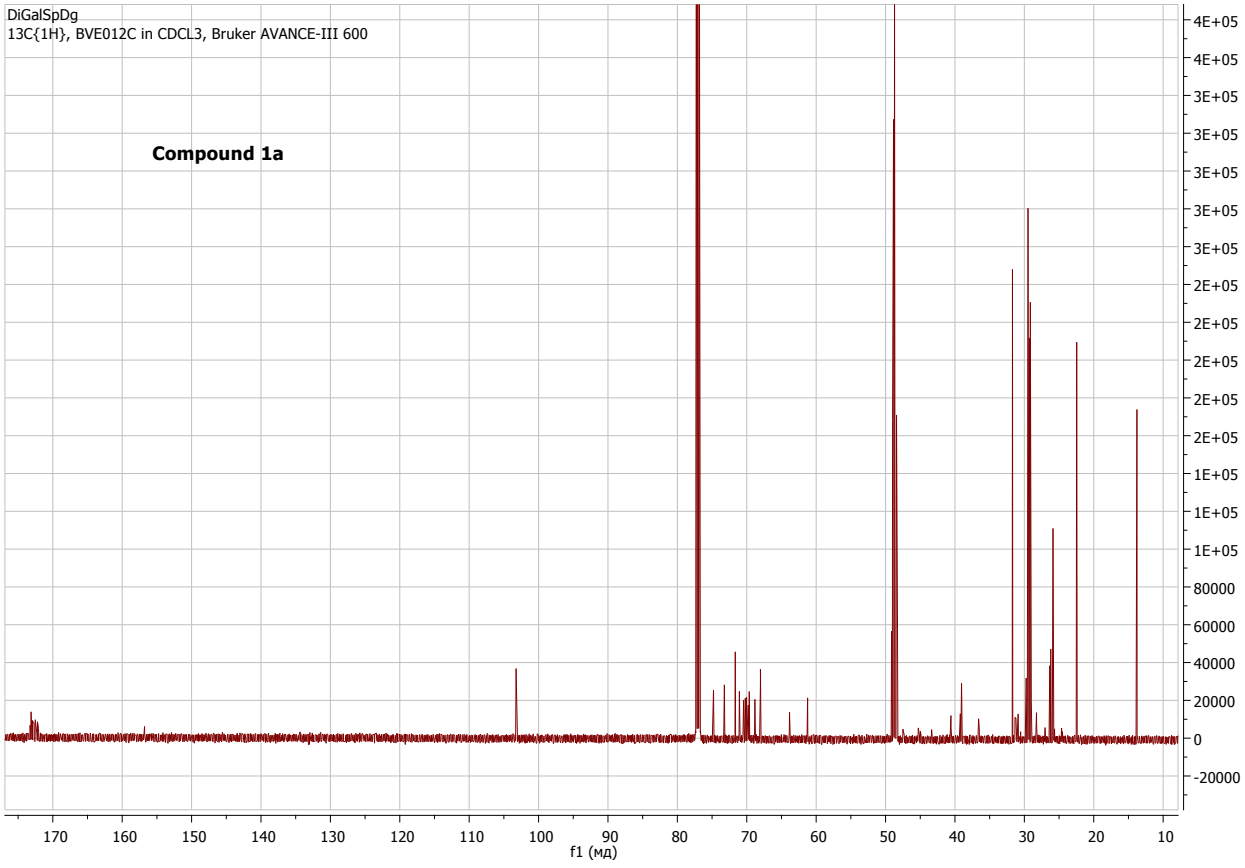

Спермин
AcGalSpSucDg CDCl3 NMR 1H Te 298K
Compound 11a
f1 (мд)

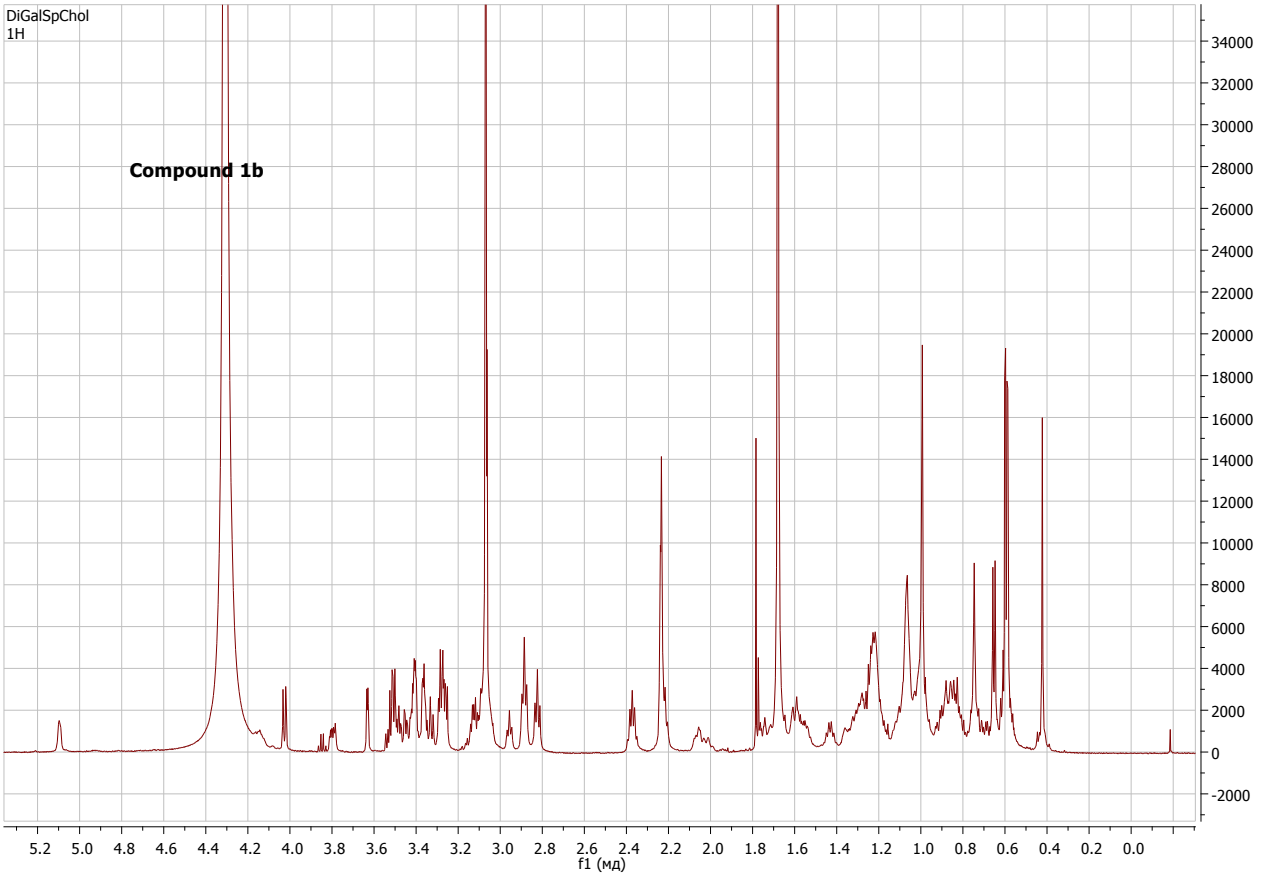

Спермин
AcGalSpSucDg CDCl3 NMR 13C Te 298 K
Compound 11a
f1 (мд)

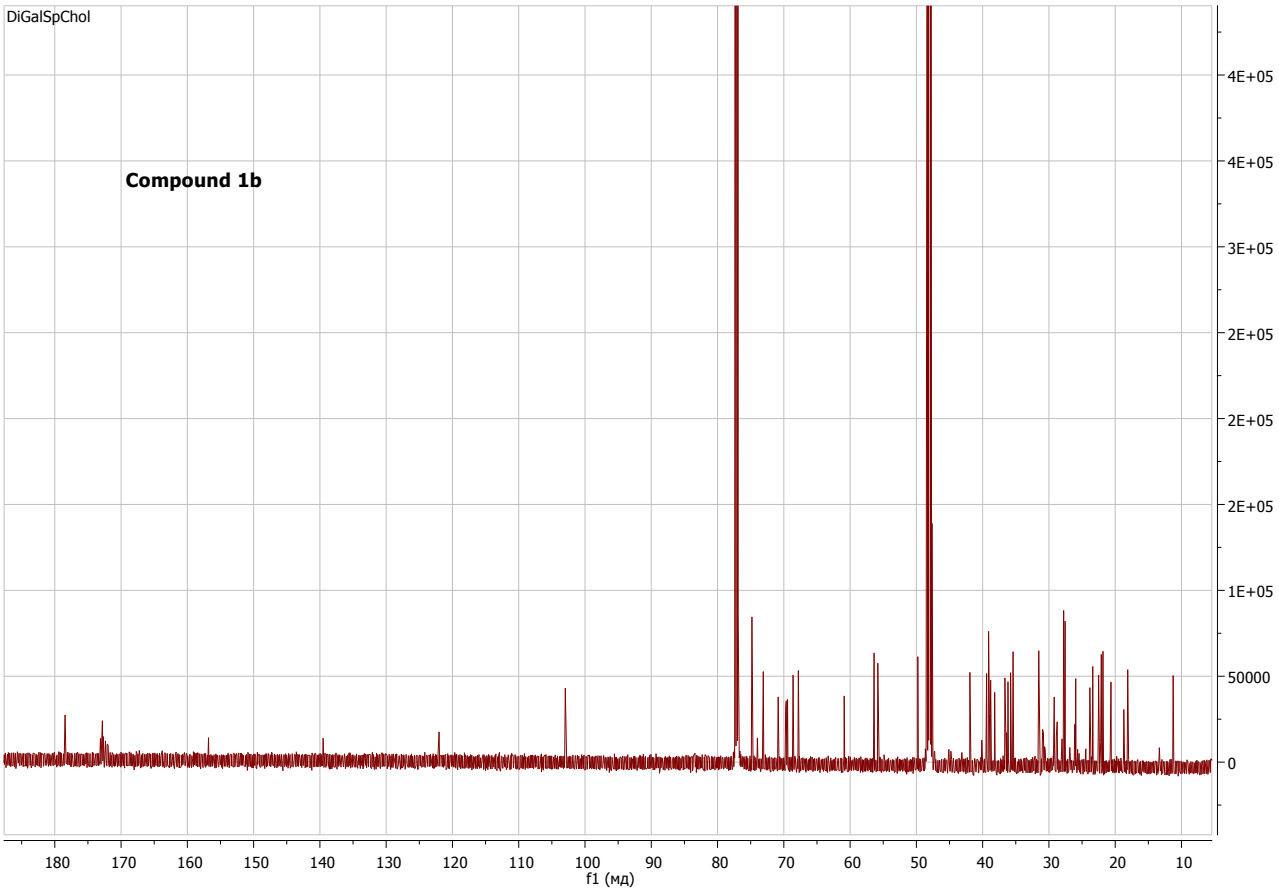

Спермин
AcGalSpSucCh CDCl3 NMR 1H Te 298K
Compound 11b
f1 (мд)


Спермин
Compound 11b
f1 (мд)


DiGalSpDg
1H
Compound 1a
f1 (мд)


DiGalSpDg
13C{1H}, BVE012C in CDCL3, Bruker AVANCE-III 600
Compound 1a
f1 (мд)


DiGalSpChol
1H
Compound 1b
34000
32000
30000
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 0.0
f1 (мд)


DiGalSpChol
Compound 1b
4E+05
4E+05
3E+05
2E+05
2E+05
2E+05
1E+05
50000
0
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (мд)