Electronic supporting information for

A versatile solid-phase approach to the synthesis of oligonucleotide conjugates with biodegradable hydrazone linker†

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Content

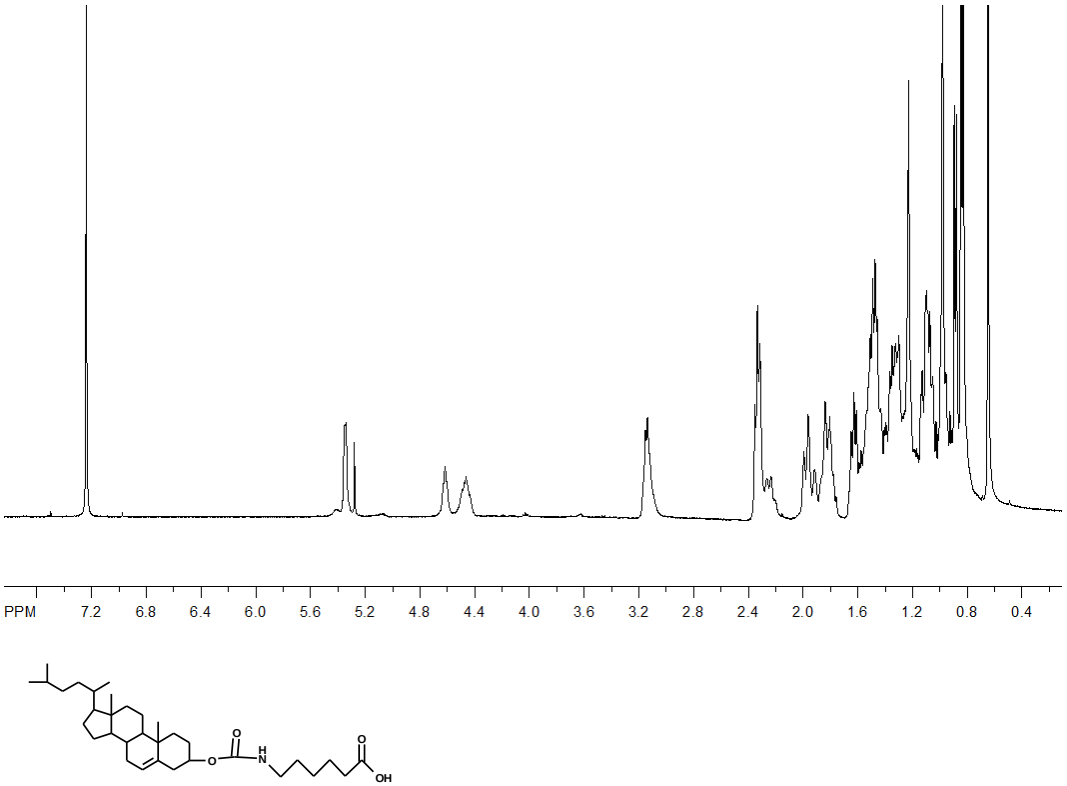


Figure S1. ^{1}H-NMR spectrum of 6-(cholesteryloxycarbonylamino)-hexanoic acid (**IV**). NMR spectrum was measured with $CDCl_3$ as a solvent using AVANCE III 400 NMR spectrometer. The assignment of peaks in the NMR spectrum is given in the experimental part.

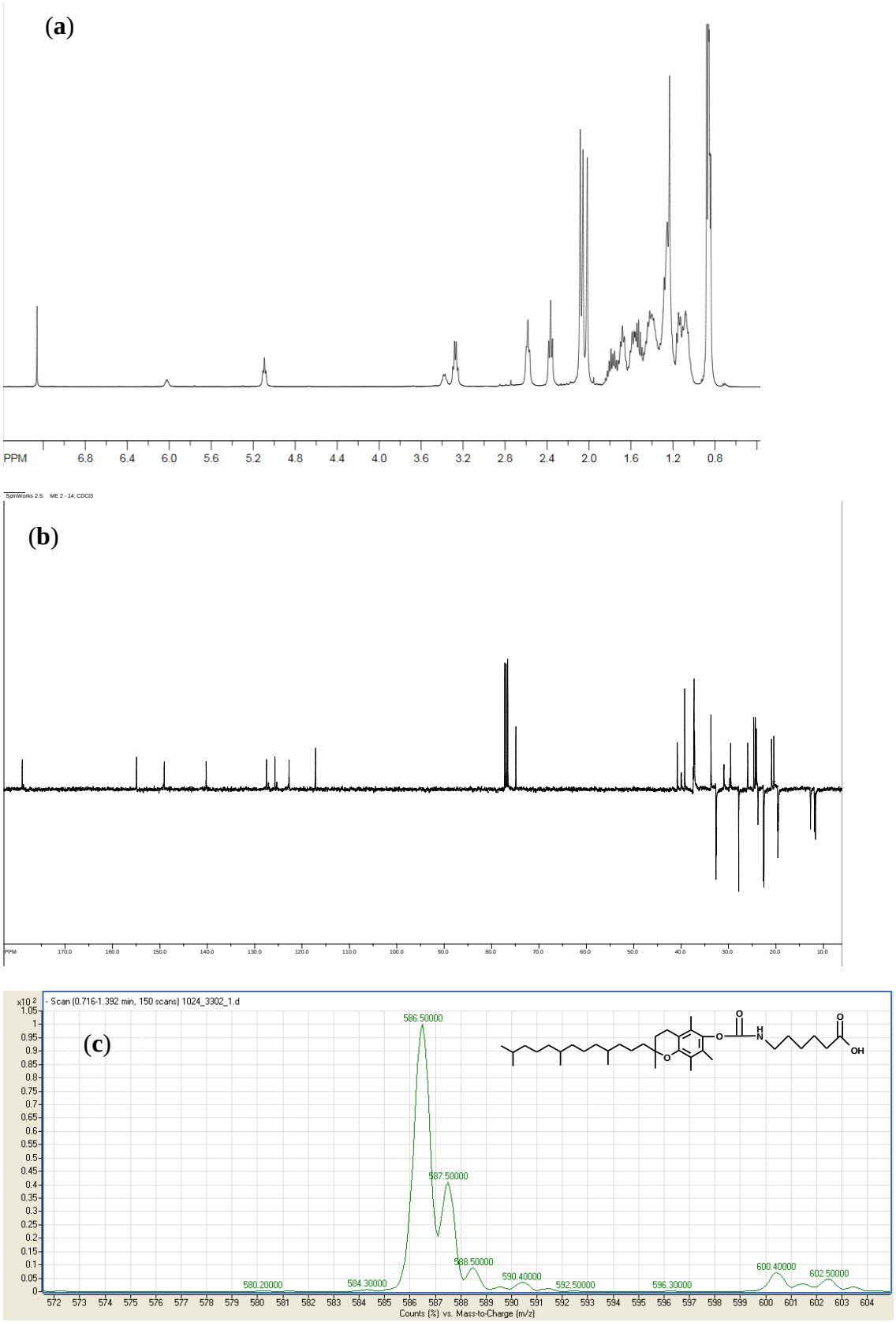


Figure S2. ^{1}H-NMR (**a**), ^{13}C-NMR (**b**) and ESI-MS (**c**) spectra of 6-[2,5,7,6-tetramethyl-2-(4',8',12'-trimethyltridecyl)-chroman-6-yloxycarbonyl]-hexanoic acid (**V**). NMR spectra were measured with $CDCl_3$ as a solvent using AVANCE III 400 NMR spectrometer. Mass spectrum was recorded by the ESI LC/MS XCT. The assignment of peaks in the NMR spectra is given in the experimental part.

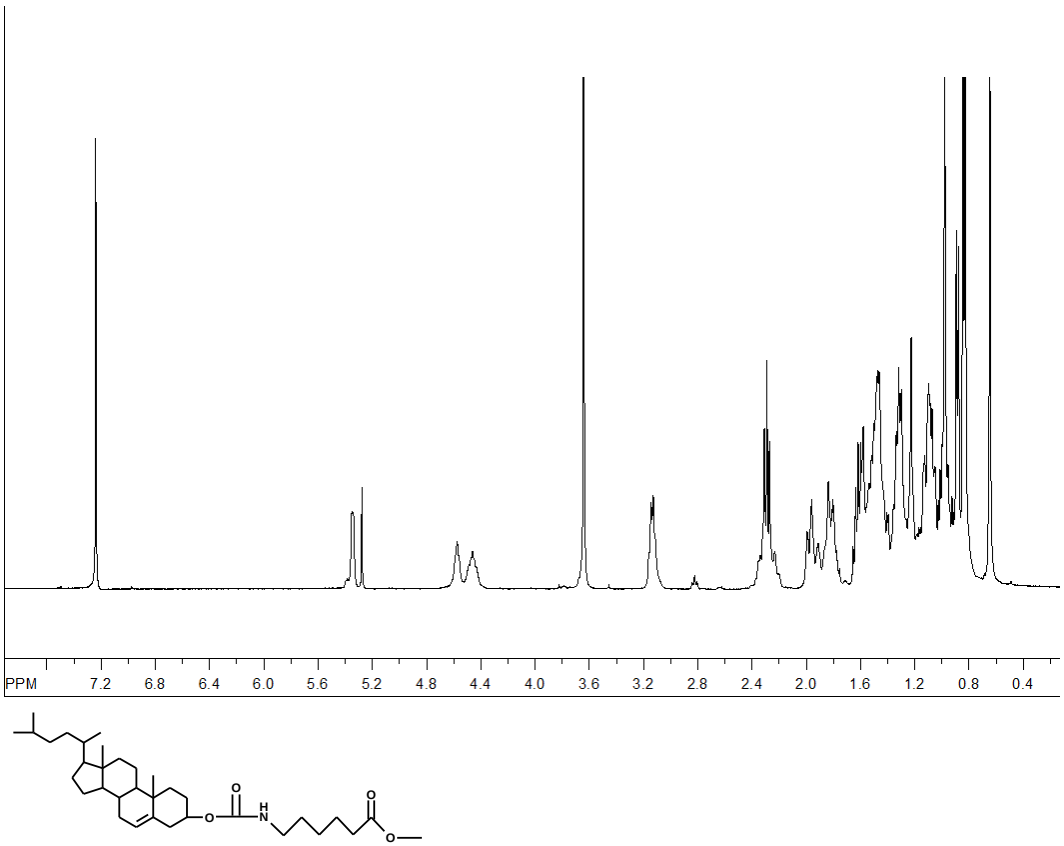


Figure S3. ^{1}H-NMR spectrum of methyl 6-(cholesteryloxycarbonylamino)-hexanoate (**VI**). NMR spectrum was measured with $CDCl_3$ as a solvent using AVANCE III 400 NMR spectrometer. The assignment of peaks in the NMR spectrum is given in the experimental part.

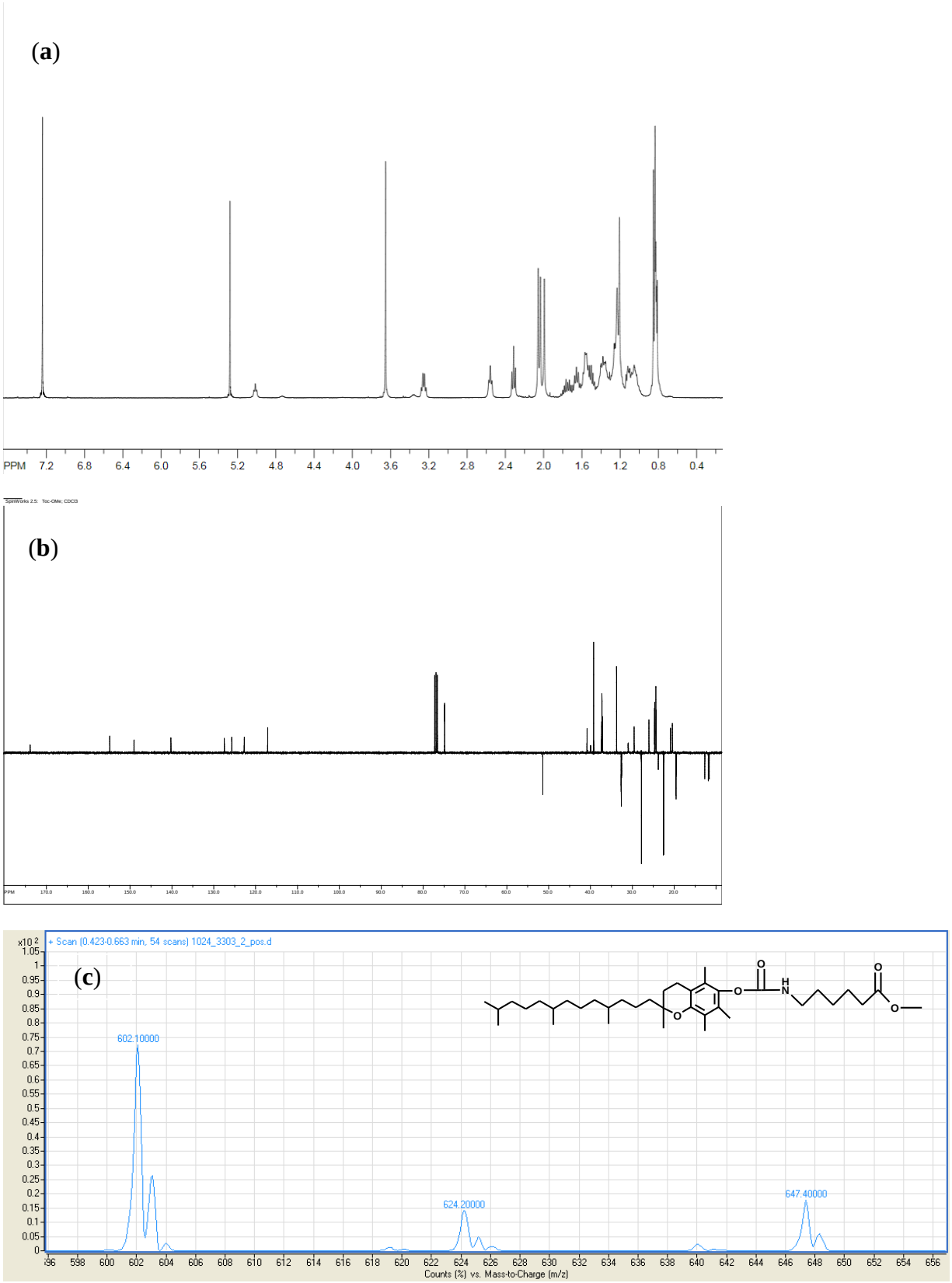


Figure S4. ^{1}H-NMR (**a**), ^{13}C-NMR (**b**) and ESI-MS (**c**) spectra of methyl 6-[2,5,7,6-tetramethyl-2-(4',8',12'-trimethyltridecyl)-chroman-6-yloxycarbonyl]-hexanoate (**VII**). NMR spectra were measured with $CDCl_3$ as a solvent using AVANCE III 400 NMR spectrometer. Mass spectrum was recorded by the ESI LC/MS XCT. The assignment of peaks in the NMR spectra is given in the experimental part.

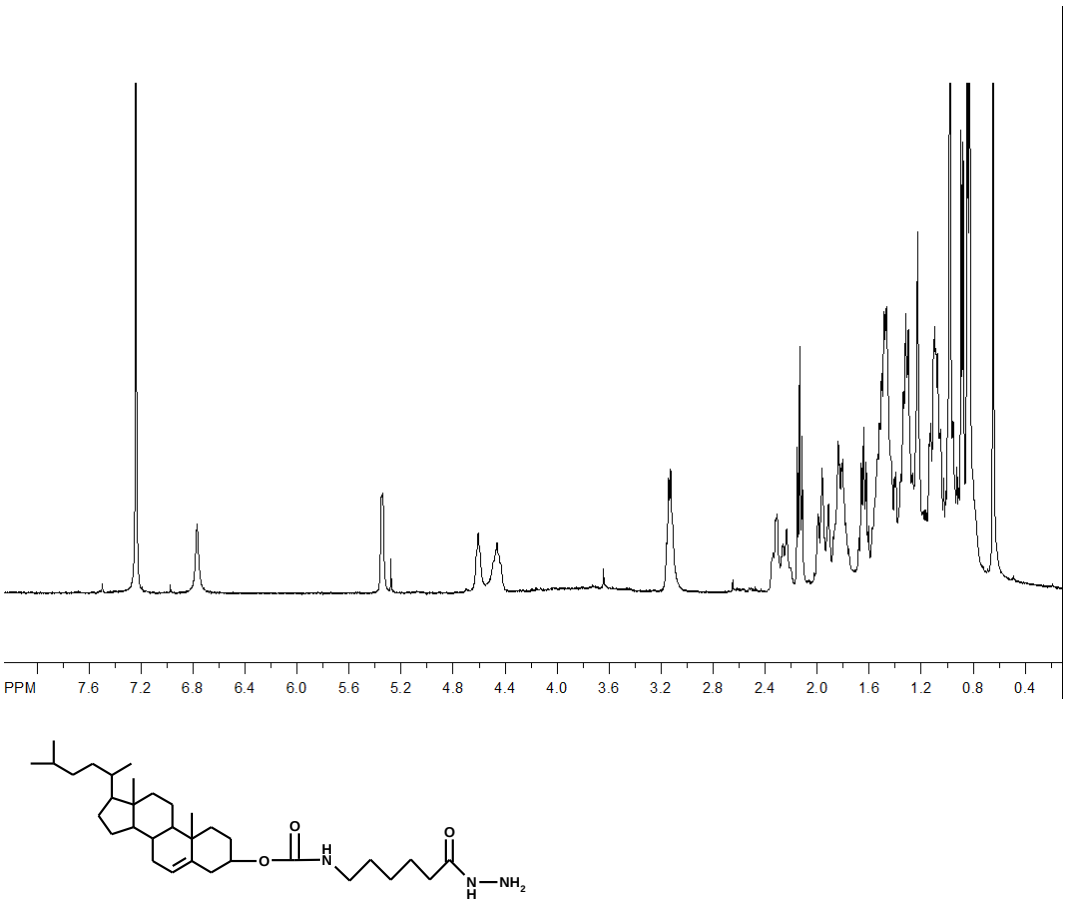


Figure S5. ^{1}H-NMR spectrum of hydrazide 6-(cholesteryloxycarbonylamino)-hexanoate (**VIII**). NMR spectrum was measured with $CDCl_3$ as a solvent using AVANCE III 400 NMR spectrometer. The assignment of peaks in the NMR spectrum is given in the experimental part.

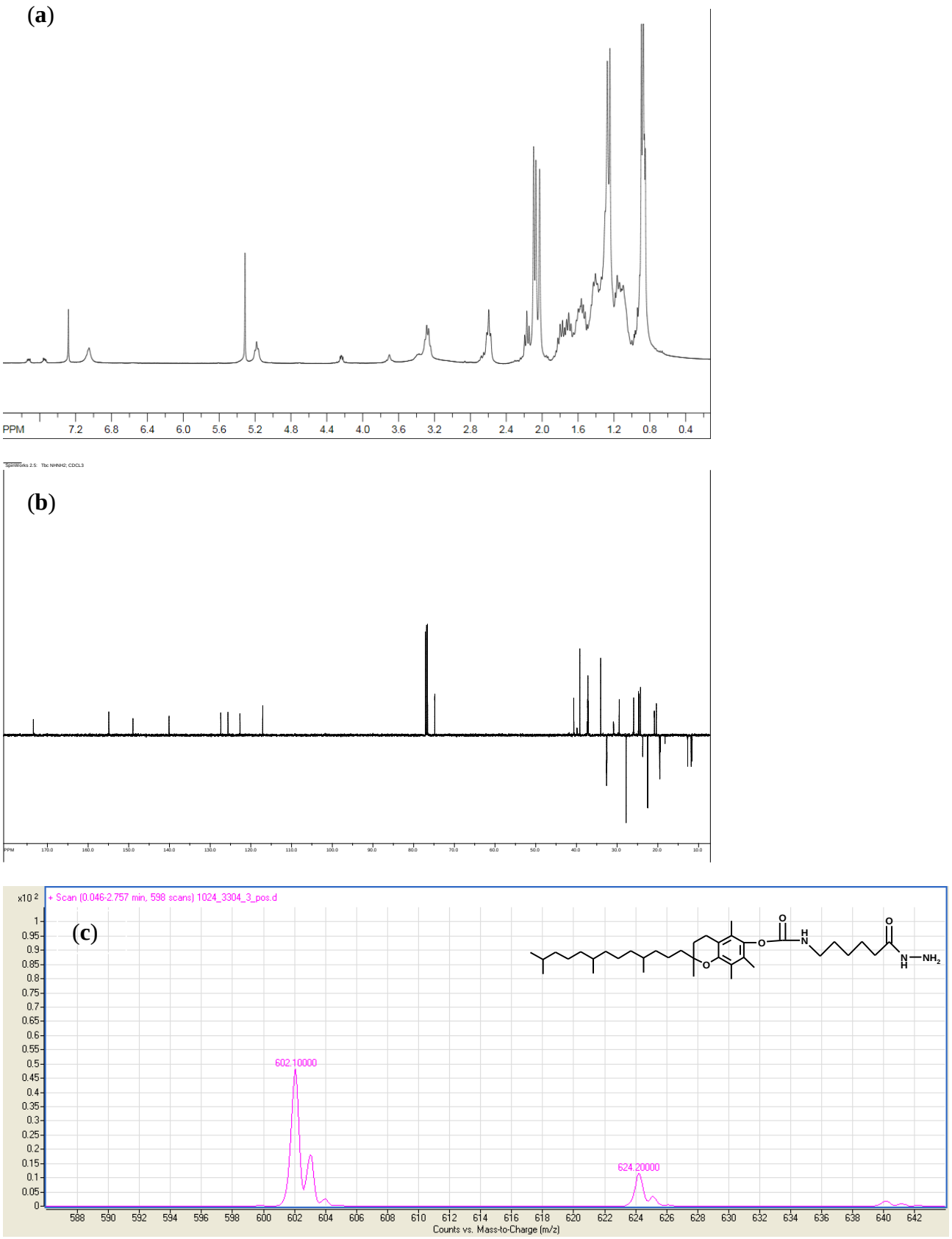


Figure S6. ^{1}H-NMR (**a**), ^{13}C-NMR (**b**) and ESI-MS (**c**) spectra of hydrazide 6-[2,5,7,6-tetramethyl-2-(4',8',12'-trimethyltridecyl)-chroman-6-yloxycarbonyl])-hexanoate (**IX**). NMR spectra were measured with $CDCl_3$ as a solvent using AVANCE III 400 and 500 NMR spectrometers. Mass spectrum was recorded by the ESI LC/MS XCT. The assignment of peaks in the NMR spectra is given in the experimental part.

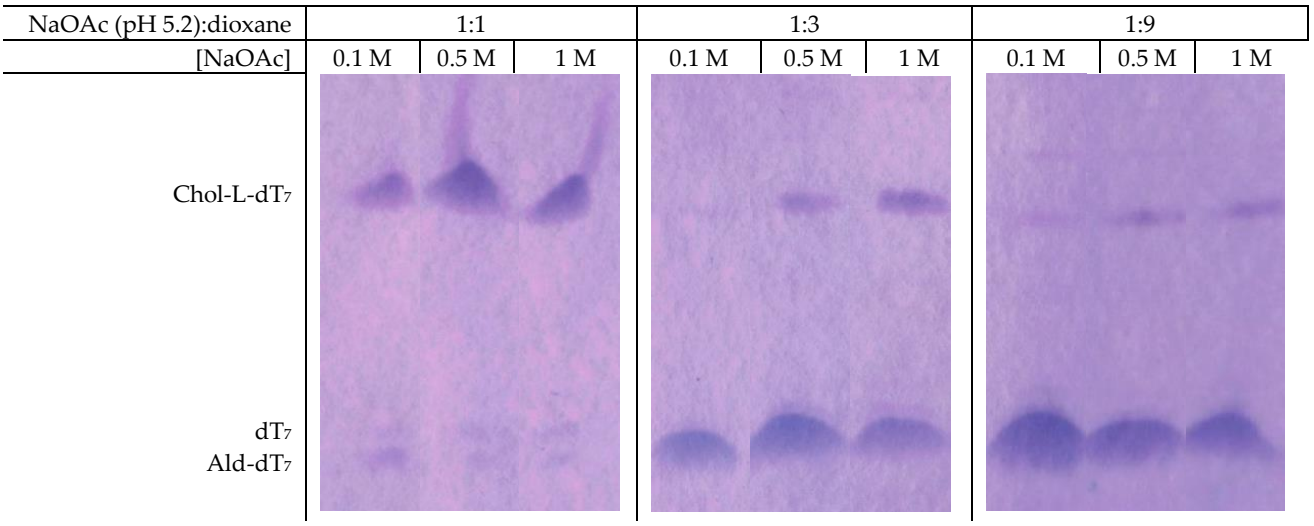


Figure S7. Optimization of conditions of the solid-phase synthesis of 5'-Chol-L-dT_7 (**1**). Chol: cholesterol residue; L: -$OC(O)NH(CH_2)_5C(O)NH$-N=CH-$C_6H_4O(CH_2)_2OP(O)(OH)$-; Ald: $HC(O)C_6H_4O(CH_2)_2OP(O)(OH)$-. Products were analyzed by gel electrophoresis in 20% PAAG under denaturing conditions (AA/bisAA 30:1, 7 M urea, TBE) and stained with Stains-all.

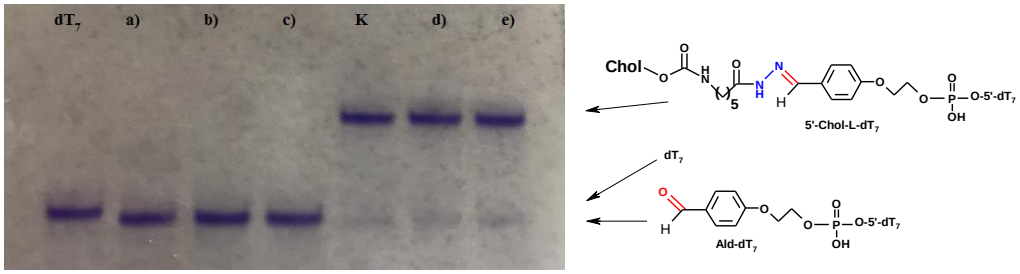


Figure S8. Stability of hydrazone bond of 5'-Chol-L-dT_7 (**1**) under treatment in different deblocking conditions: a) 28% NH_3 aq., 55 ^{O}C, 16 h; b) AMA-solution, 65 ^{O}C, 15 min; c) AMA-solution, RT, 16 h; d) 0.05M K_2CO_3 in methanol, RT, 16 h; e) NMP/TEA•3HF/TEA (150/100/75), 65 ^{O}C, 1.5 h. K: model 5'-Chol-L-dT_7; L: -OC(O)NH$(CH_2)_5$C(O)NH-N=CH-C_6H_4O$(CH_2)_2$OP(O)(OH)-; Chol: cholesterol residue. Products were analyzed by gel electrophoresis in 20% PAAG under denaturing conditions (AA/bisAA 30:1, 7 M urea, TBE) and stained with Stains-all.

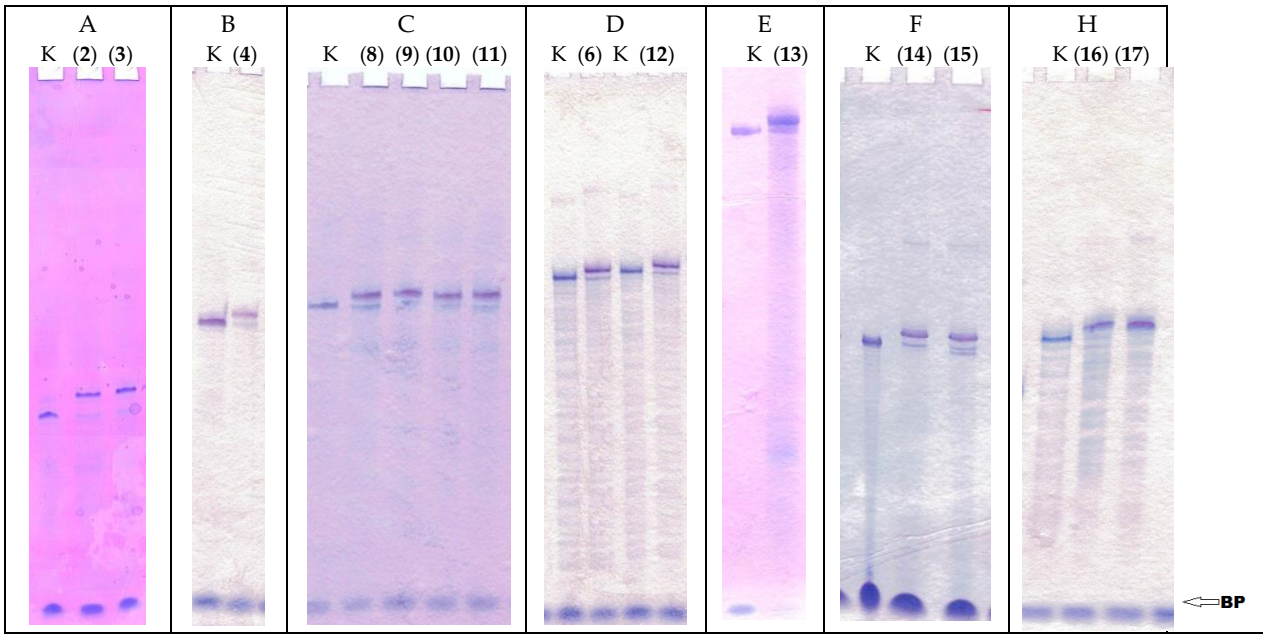
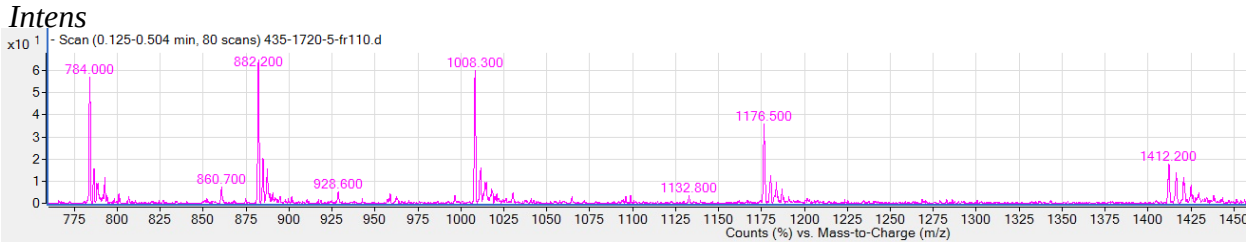
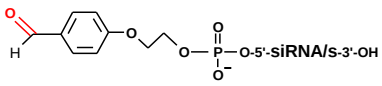


Figure S9. PAGE-analysis of reaction mixtures after the synthesis of conjugates (**2-4,6,8-17**) with hydrazone bond. (**A**) 5'-Lipophilic conjugates of sense strand of siRNA (**2,3**); (**B**) 5'-Lipophilic conjugate of mitochondrial antireplicative RNA (**4**); (**C**) 5'- Lipophilic conjugates of mitochondrial antireplicative RNAs (**8-11**); (**D**) 5'- Lipophilic conjugates of mitochondrial antireplicative RNA (**6**) and guide RNA (**12**); (**E**) 5'- Lipophilic conjugate of mitochondrial guide RNA (**13**); (**F**) 5'-Lipophilic conjugates of crRNAs (**14,15**); (**H**) 5'-Lipophilic conjugates of crRNAs (**16,17**). K – initial oligonucleotide. Structures of conjugates are given in Table 1. Products were analyzed by gel electrophoresis in 15% or 20% PAAG under denaturing conditions (AA/bisAA 30:1, 7 M urea, TBE) and stained with Stains-all. BP - bromophenol blue.



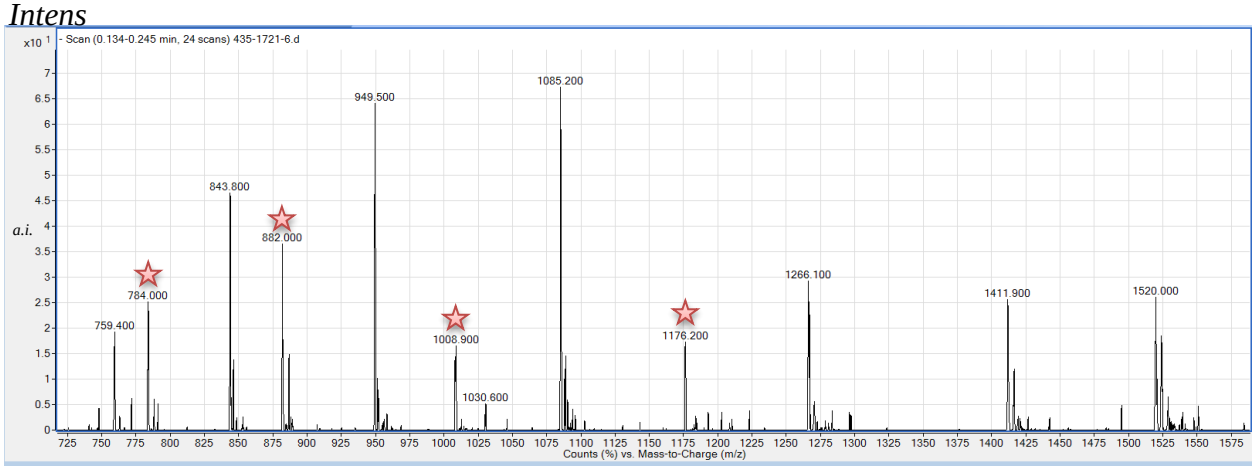


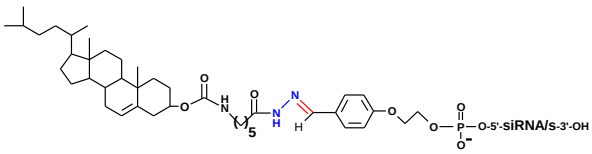
M_{calcd} 7066.34

[H9] / 784.00	M 7065.00
[H8] / 882.20	M 7065.60
[H7] / 1008.30	M 7065.10
[H6] / 1176.50	M 7065.00
[H5] / 1412.20	M 7066.00

M_{found} 7065.34

Figure S10. ESI-MS spectrum of Ald-siRNA/s. Ald: HC(O)$C_6H_4O(CH_2)_2OP(O)(OH)$-.





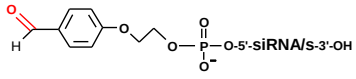
M_{calcd} 7606.65

[H10] / 759.40	M 7604.00
[H9] / 843.80	M 7603.20
[H8] / 949.50	M 7604.00
[H7] / 1085.20	M 7603.40
[H6] / 1266.10	M 7602.60
[H5] / 1520.00	M 7605.00

M_{found} 7603.70

Figure S11. ESI-MS spectra of Chol-L-siRNA/s and product (☆) of its destruction. Chol: cholesterol residue; L: - $OC(O)NH(CH_2)_5C(O)NH\text{-}N{=}CHC_6H_4O(CH_2)_2OP(O)(OH)$-.

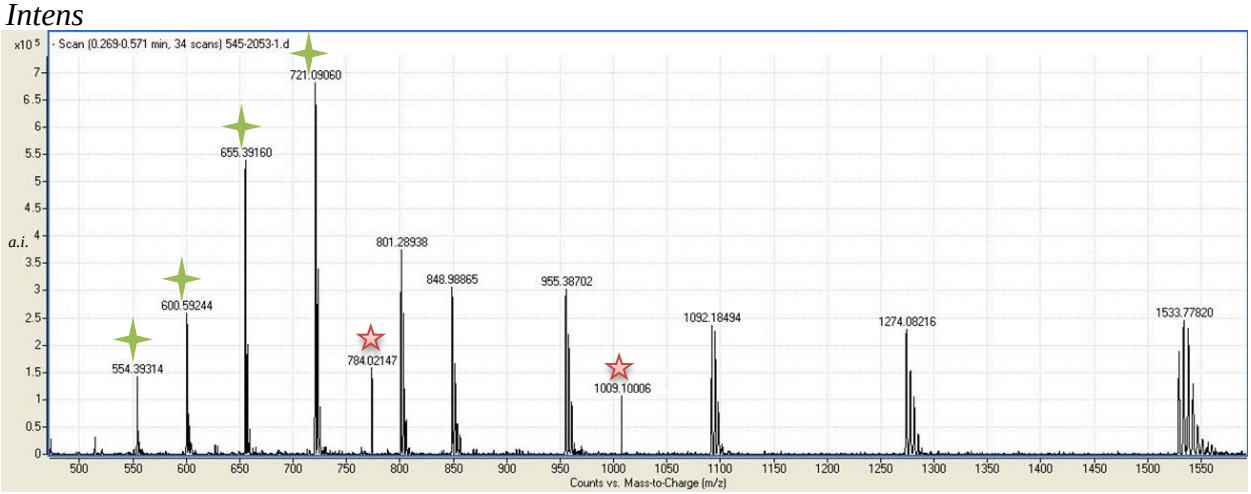
☆- Aldehyde-containing oligonucleotide Ald-siRNA/s (see Figure S10).

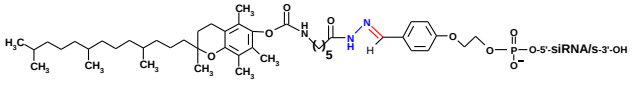


M_{calcd} 7066.34

[H9] / 784.00	M 7065.00
[H8] / 882.00	M 7064.00
[H7] / 1008.90	M 7069.30
[H6] / 1176.20	M 7063.20

M_{found} 7065.38





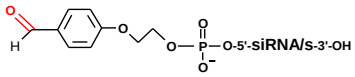
M_{calcd} 7650.7

[H9] / 848.99	M 7649.9
[H8] / 955.39	M 7651.1
[H7] / 1092.18	M 7652.29
[H6] / 1274.08	M 7650.49
[H5] / 1533.78	M 7673.89 – 23(M_{Na+}) + 1(M_{H+}) = M 7651.89

M_{found} 7651.13

Figure S12. ESI-MS spectra of Toc-L-siRNA/s and products (☆,✦) of its destruction. Toc: α-tocopherol residue; L: -OC(O)NH(CH_2)$_5$C(O)NH-N=C$HC_6H_4O(CH_2)_2OP(O)(OH)$-.

☆ - Aldehyde-containing oligonucleotide Ald-siRNA/s (see Figure S10).

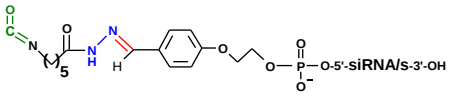


M_{calcd} 7066.34

[H9] / 784.02	M 7065.18
[H7] / 1009.10	M 7070.70

M_{found} 7067.94

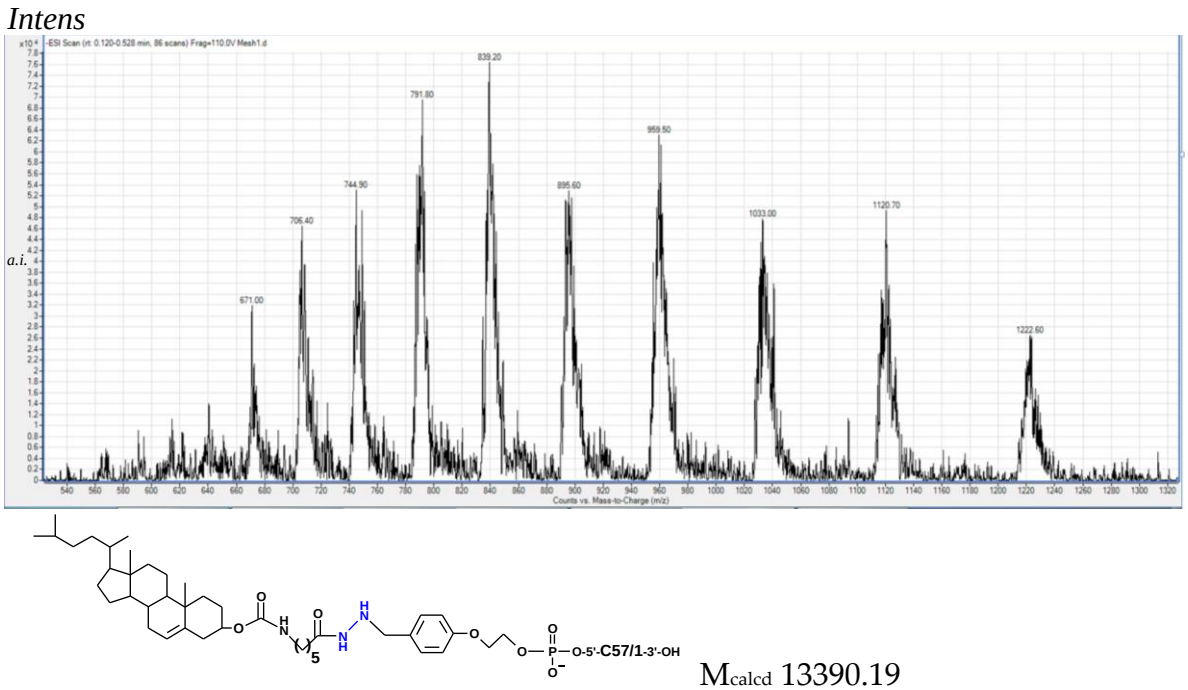
✦ - Isocyanate derivative of oligonucleotide siRNA/s.



M_{calcd} 7221.00

[H13] / 554.39	M 7220.07
[H12] / 600.59	M 7219.08
[H11] / 655.39	M 7220.29
[H10] / 721.09	M 7220.9
[H9] / 801.29	M 7220.61

M_{found} 7220.19

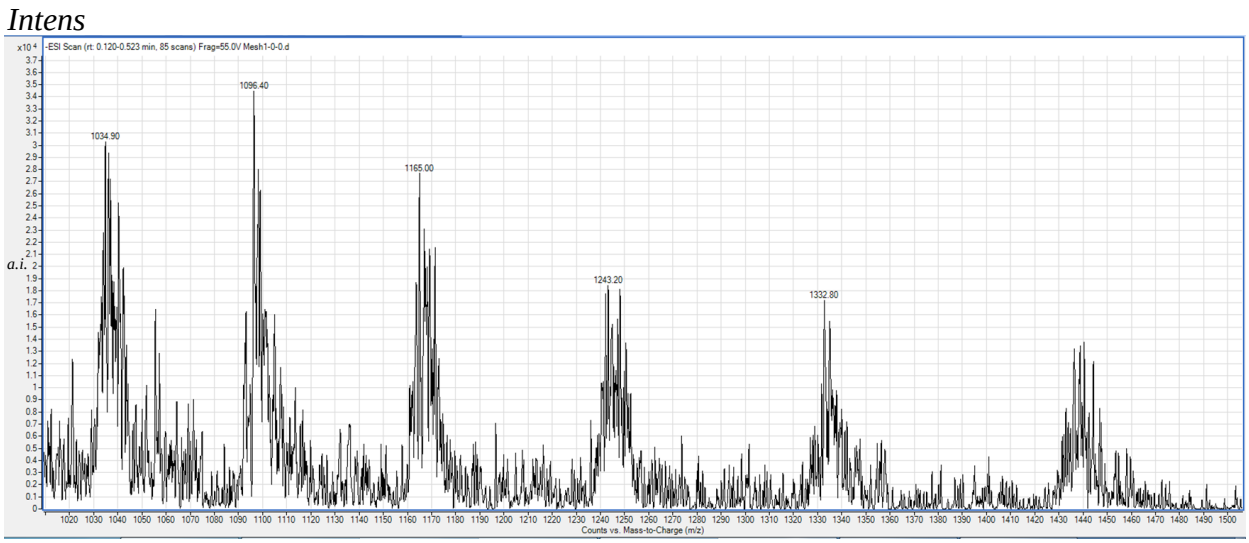


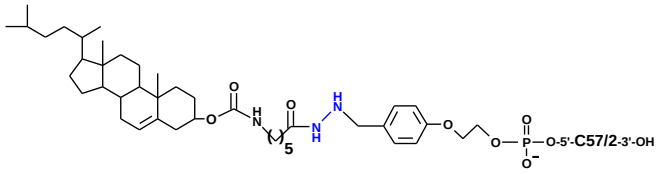
M_{calcd} 13390.19

[H20] / 671.00	M 13440.60 – 2*23(M_{Na+}) + 2*1(M_{H+}) = M 13396.00
[H19] / 706.40	M 13440.60 – 2*23(M_{Na+}) + 2*1(M_{H+}) = M 13396.60
[H18] / 744.90	M 13426.20 – 2*23(M_{Na+}) + 2*1(M_{H+}) = M 13382.20
[H17] / 791.80	M 13477.60 – 4*23(M_{Na+}) + 4*1(M_{H+}) = M 13389.60
[H16] / 839.20	M 13443.20 – 2*23(M_{Na+}) + 2*1(M_{H+}) = M 13399.20
[H15] / 895.60	M 13449.00 – 3*23(M_{Na+}) + 3*1(M_{H+}) = M 13383.00
[H14] / 959.50	M 13447.00 – 3*23(M_{Na+}) + 3*1(M_{H+}) = M 13381.00
[H13] / 1033.00	M 13442.00 – 2*23(M_{Na+}) + 2*1(M_{H+}) = M 13398.00
[H12] / 1120.70	M 13460.40 – 3*23(M_{Na+}) + 3*1(M_{H+}) = M 13394.40
[H11] / 1222.60	M 13459.60 – 3*23(M_{Na+}) + 3*1(M_{H+}) = M 13393.60

M_{found} 13391.16

Figure S13. ESI-MS spectrum of Chol-L_1-C57/1. Chol: cholesterol residue; L_1: -$OC(O)NH(CH_2)_5C(O)$NH-NH-$CH_2C_6H_4O(CH_2)_2OP(O)(OH)$-.



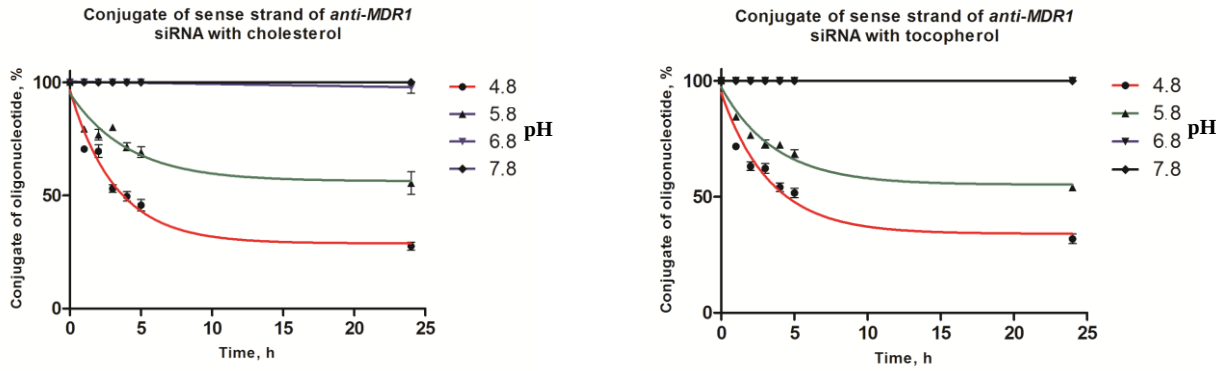


M_{calcd} 18587.28

[H18] / 1034.90	M 18646.20 – 3*23(M_{Na+}) + 3*1(M_{H+}) = M 18580.20
[H17] / 1096.40	M 18655.80 – 3*23(M_{Na+}) + 3*1(M_{H+}) = M 18589.80
[H16] / 1165.00	M 18656.00 – 3*23(M_{Na+}) + 3*1(M_{H+}) = M 18590.00
[H15] / 1243.20	M 18660.00 – 3*23(M_{Na+}) + 3*1(M_{H+}) = M 18594.00
[H14] / 1332.80	M 18673.20 – 4*23(M_{Na+}) + 4*1(M_{H+}) = M 18585.20

M_{found} 18587.86

Figure S14. ESI-MS spectrum of Chol-L_1-C57/2. Chol: cholesterol residue; L_1: -OC(O)NH(CH_2)$_5$C(O)NH-NH-$CH_2C_6H_4O(CH_2)_2OP(O)(OH)$-.



(**a**) (**b**)

Figure S15. Kinetic curves of hydrazone bond cleavage in lipophilic conjugates Chol-L-siRNA/s (**a**) and Toc-L-siRNA/s (**b**) at different pH. Quantification of the full size conjugate (%, axis Y) depending on pH and the time of incubation. The results are mean value (±SD) from three independent experiments.