Supplementary Information

Novel convenient approach to the solid-phase synthesis of oligonucleotide conjugates

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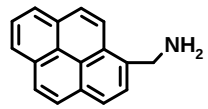
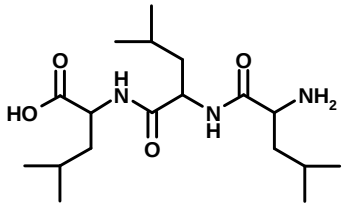
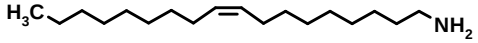
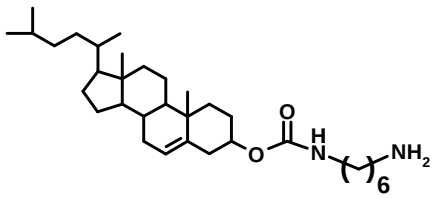
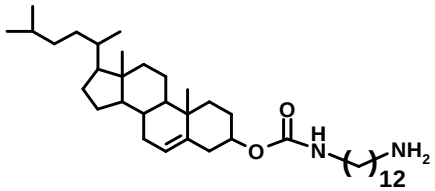
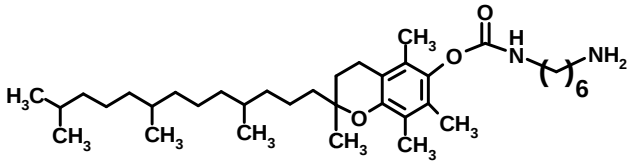
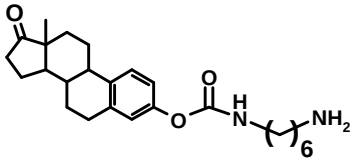
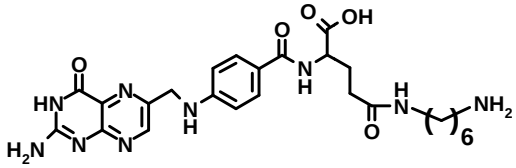
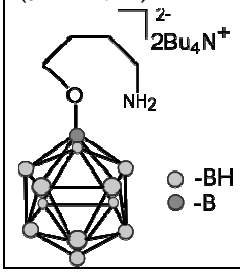
Amino ligands for solid-phase attachment to oligonucleotides using DSC activation and selected optimal solvents for this reaction

Table S1. The amino ligands used for solid-phase oligonucleotide conjugates synthesis.

Structure	**Solvent**	**Molecular weight**	**References**
Pyrenemethylamine (*Pyr*-NH_2) 	DMSO	231,29	Sigma-Aldrich
Hexamethylenediamine (*NH_2**L**$_6$*-NH_2, where ***L**$_6$*: -$(CH_2)_6$-**)**	THF	116,20	Sigma-Aldrich
Dodecamethylenediamine (*NH_2**L**$_{12}$*-NH_2, where ***L**$_{12}$*: -$(CH_2)_{12}$-**)**	THF	200,36	Sigma-Aldrich
Aminopropanol (***L**$_3$*-NH_2, where ***L**$_3$*: *HO*-$(CH_2)_3$-**)**	DMSO	75,11	Sigma-Aldrich
12-Amino-1-dodecanol (***L**$_{12}$*-NH_2, where ***L**$_{12}$*: *HO*-$(CH_2)_{12}$-**)**	DMSO	201,35	TCI Chemicals
Propargylamine $HC{\equiv}CCH_2NH_2$	THF	55,08	Sigma-Aldrich
Trileucine (*(Leu)$_3$*-NH_2**)** 	CH_3CN	357,49	Sigma-Aldrich
Oleylamine (*Oleyl*-NH_2**)** 	THF	267,49	Sigma-Aldrich
Cholesteryl-6-aminohexylcarbamate (I) (*Chol**L**$_6$*-NH_2, where ***L**$_6$*: -C(O)NH$(CH_2)_6$-**)**	THF	528,85	[1]

			
Cholesteryl-12-aminododecanylcarbamate (II) (*Chol**L**12*-NH_2, where ***L**12:* -C(O)NH$(CH_2)_{12}$-) 	THF	613,01	by analogy with [1]
α-Tocopheryl-6-aminohexylcarbamate (III) (*Toc**L**6*-NH_2, where ***L**6:* -C(O)NH$(CH_2)_6$-) 	THF	572,90	by analogy with [2,3]
Estronyl-6-aminohexylcarbamate (IV) (*Est**L**6*-NH_2, where ***L**12:* -C(O)NH$(CH_2)_{12}$-) 	THF	412,56	by analogy with [2,3]
Folate-γ-(6-aminohexylcarbamate) (V) (*Fol**L**6*-NH_2, where ***L**6:* -NH$(CH_2)_6$-) 	DMSO	583,60	by [4] with some modifications
Bis-tetrabutylammonium-(4-aminobuthoxy)-undecahydro-*closo*-dodecaborate (VI) (*c-B12*-NH_2) 	DMSO	289	[5,6]

Electrophoretic analysis of reaction mixtures upon solid-phase conjugation

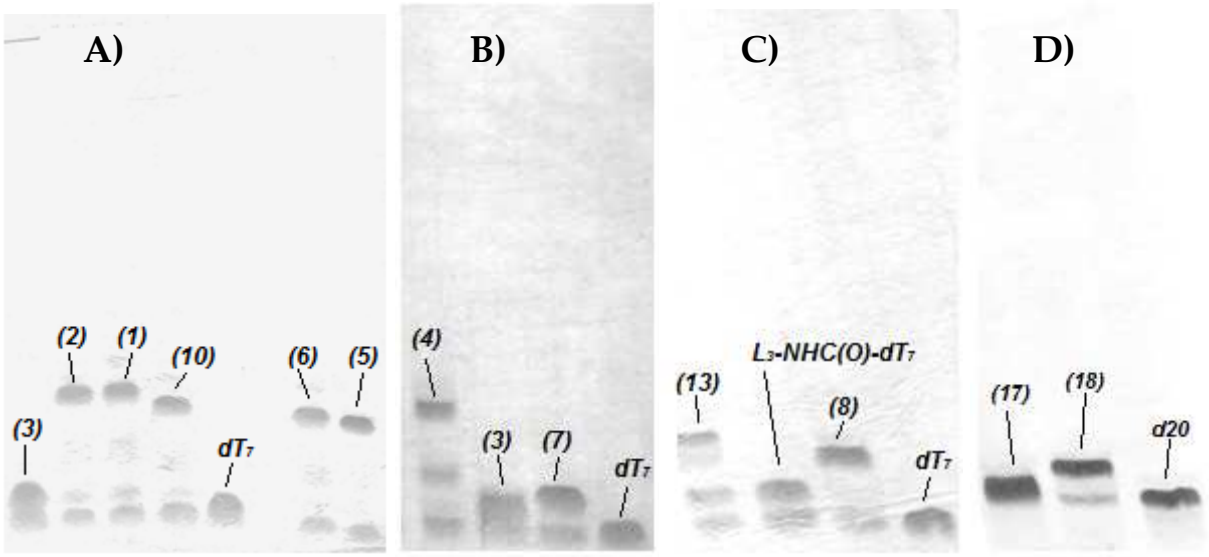


Figure S1. Analysis of reaction mixtures by PAGE: **A)** (***1***) – *Chol***L**$_6$-NHC(O)-dT$_7$, (***2***) – *Chol***L**$_{12}$-NHC(O)-dT$_7$, (***3***) – *Oleyl*-NHC(O)-dT$_7$, (***5***) – *Toc***L**$_6$-NHC(O)-dT$_7$, ***(6)*** – *Fol***L**$_6$-NHC(O)-dT$_7$, ***(10)*** – *NH*$_2$***L***$_6$-NHC(O)-dT$_7$; **B)** ***(3)*** – *Oleyl*-NHC(O)-dT$_7$, ***(4)*** – *Est***L**$_6$-NHC(O)-dT$_7$, ***(7)*** – *(Leu)*$_3$-NHC(O)-dT$_7$; **C)** ***(8)*** – *Pyr*-NHC(O)-dT$_7$, ***(13)*** – *Pyr*-NHC(O)-***L***$_3$-NHC(O)-dT$_7$; **D)** ***(17)*** – *c-B*$_{12}$-NHC(O)-d20, ***(18)*** – *Oleyl*-NHC(O)-d20. dT$_7$ = 5'-d(TTTTTTT); d20 = 5'-d(ATACGTTAACGATCCTTCAC); ***L***$_3$ = HO-(CH$_2$)$_3$-. Conditions: 15% denaturating PAAG (7M urea, acrylamide/*N*,*N*'-methylene bis-acrylamide 19:1) in TBE buffer. Gel stained with "Stains-all".

Functionalization of the 5′-diamino-modified oligonucleotides (10, 11) with N-(2-hydroxyethyl)phenazinium chloride

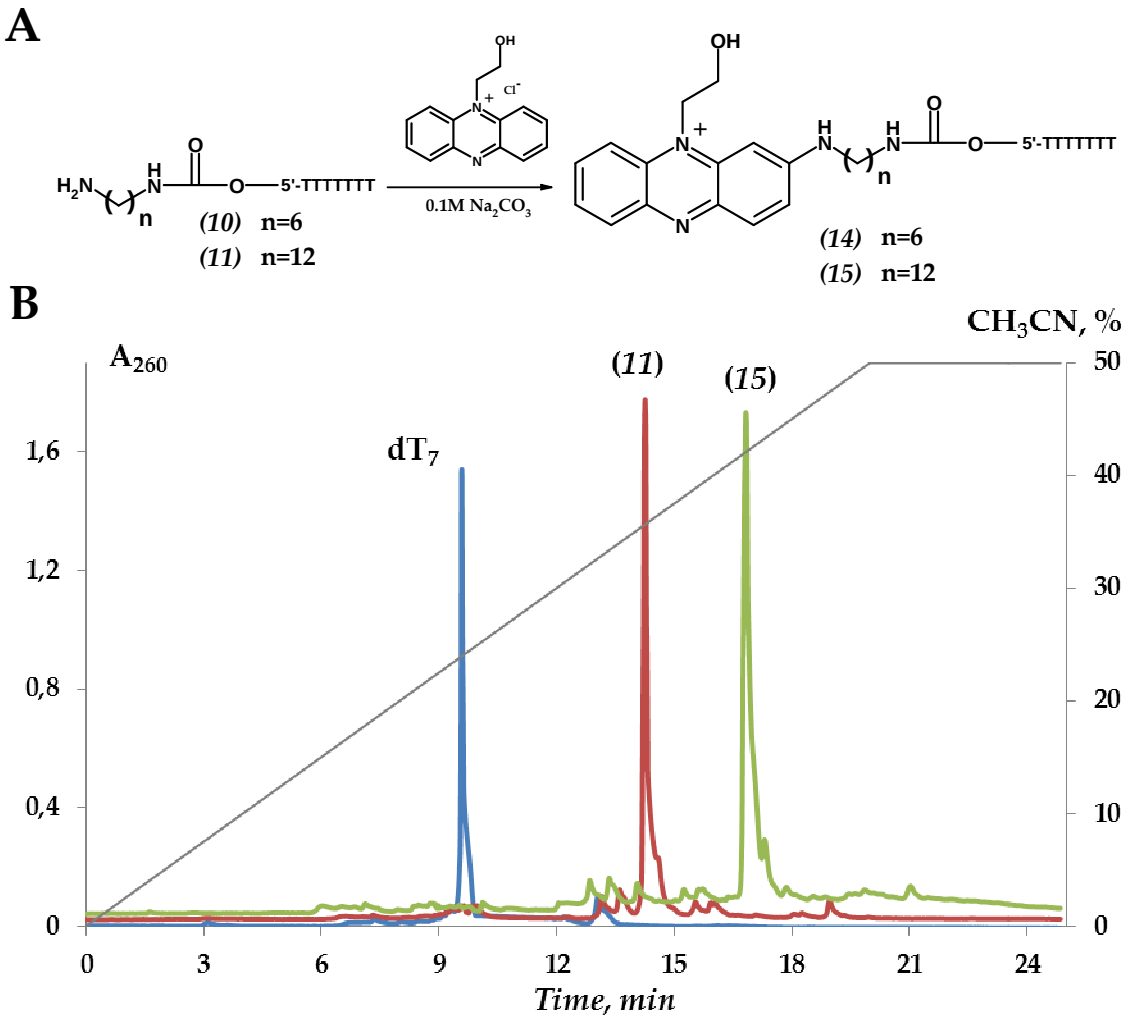


Figure S2. (**A**) Scheme of synthesis of 5'-*N*-(2-hydroxyethyl)phenazinium conjugates of dT7 (***14, 15***) using the specific oxidation reaction of quaternary phenazinium salts of amine attachment to second position of phenazinium dye in alkaline media [7]. (**B**) RP HPLC analysis of initial oligonucleotide (dT7) and reaction mixtures after solid-phase attachment of diamine to the activated with DSC dT7 (*NH2**L**12*-NHC(O)-dT7 (***11***)) and *N*-(2-hydroxyethyl)phenazinium modification of diamine derivative of dT7 in solution (*Phn-NH**L**12*-NHC(O)-dT7 (***15***)).

Reverse phase-HPLC (RP-HPLC) analysis of the oligonucleotides and their conjugates was performed on Alphachrom A-02 high performance liquid chromatograph (EcoNova, Russia) with the use of ProntoSil-120-5-C18 AQ (75×2.0 mm, 5.0 μm) column, applying a gradient elution from 0 to 50% (20 min) of acetonitrile in 0.02 M triethylammonium acetate buffer, pH 7.0 at a flow rate 100 μL per min, and detection at 260 nm.

Attachment of Cy3-fluorophore to the 5′-alkyne-modified oligonucleotide (14) using "click"-chemistry reaction

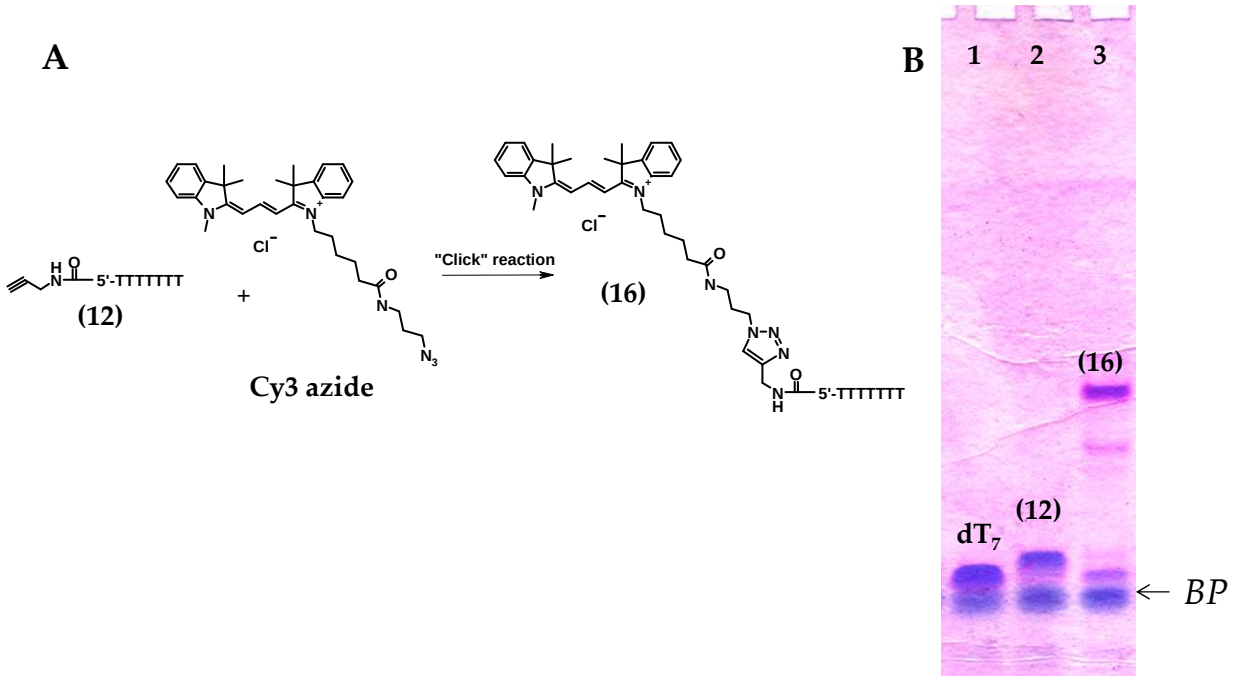
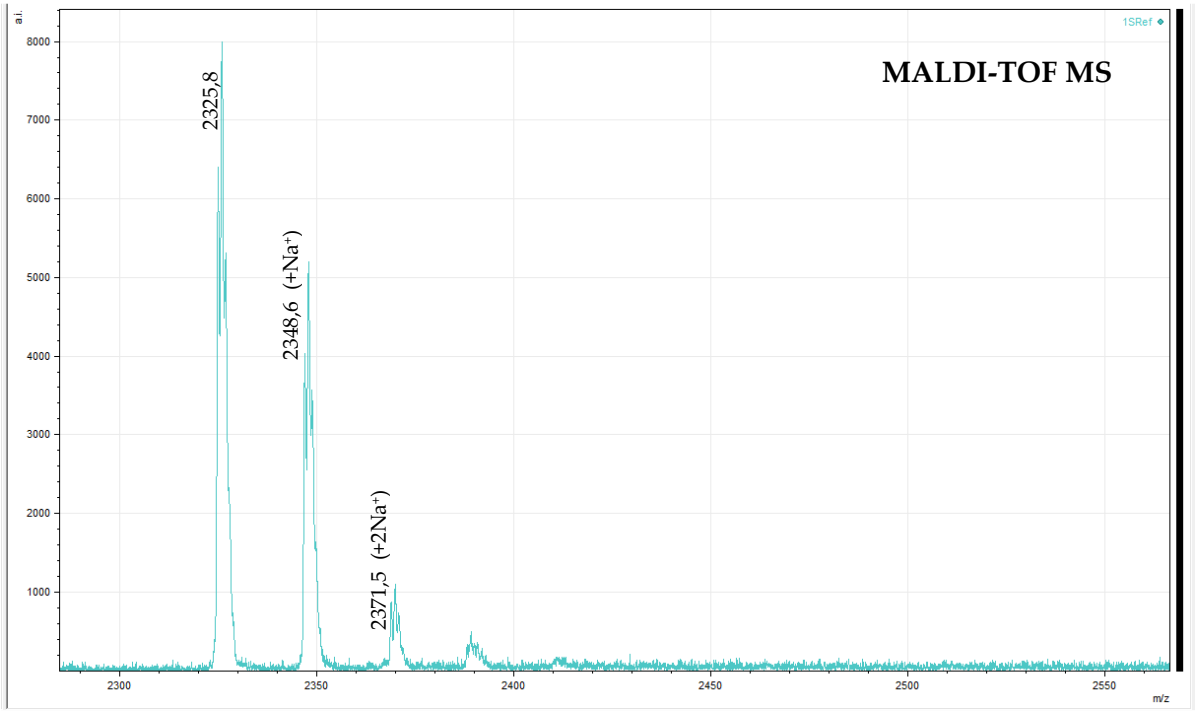


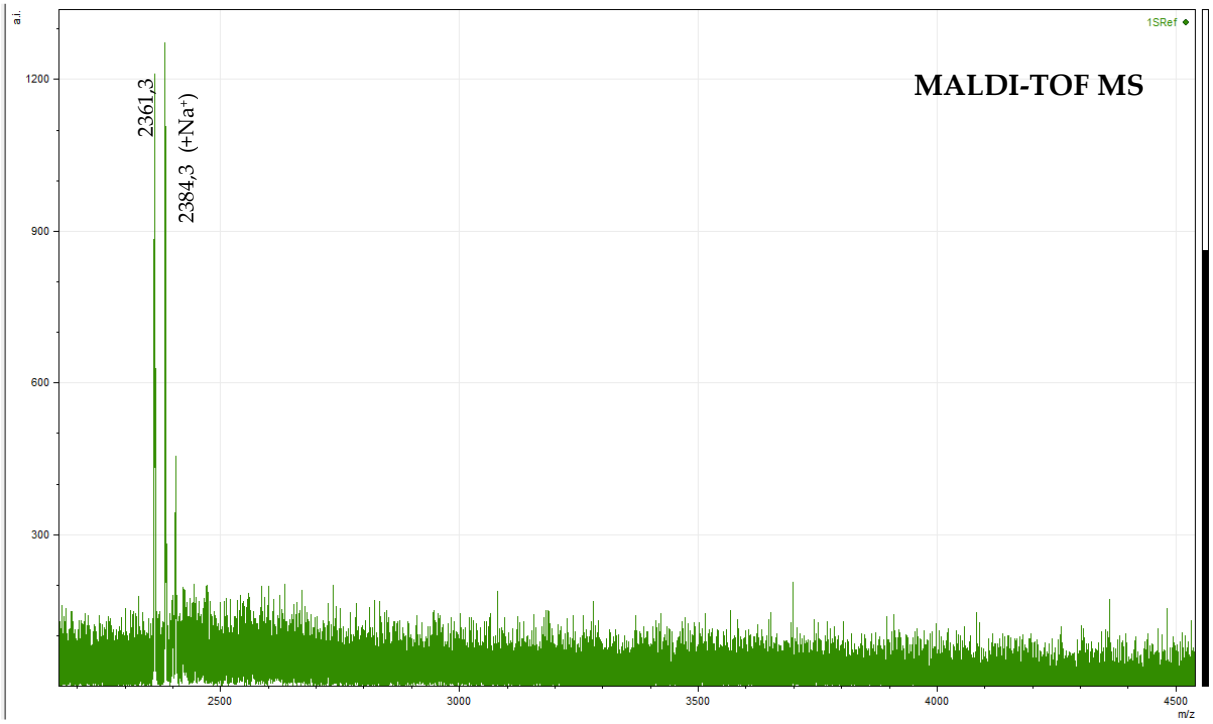
Figure S3. (**A**) Scheme of synthesis of conjugate (***16***) using "click"-reaction of Cy3 azide with 5'-propargylamine-modified dT_7 (***12***). (**B**) Analysis of reaction mixtures of dT_7 and conjugates (***12***) and (***16***) by PAGE: line **1 -** deblocked reaction mixtures of initial dT_7 oligonucleotide; line **2** – deblocked reaction mixture after solid-phase attachment of propargylamine to the activated with DSC dT_7 to obtain derivative (***12***); line **3 -** reaction mixture after Cy3 azide attachment to derivative (***12***) *via* "click"-chemistry in solution to obtain conjugate (***16***). Conditions: 15% denaturating PAAG (7M urea, acrylamide/*N*,*N*'-methylene bis-acrylamide 19:1) in TBE buffer. Gel stained with "Stains-all". BP – bromophenol blue.

Figure S4. *Representative mass spectra of the 5′-conjugates of oligonucleotides*

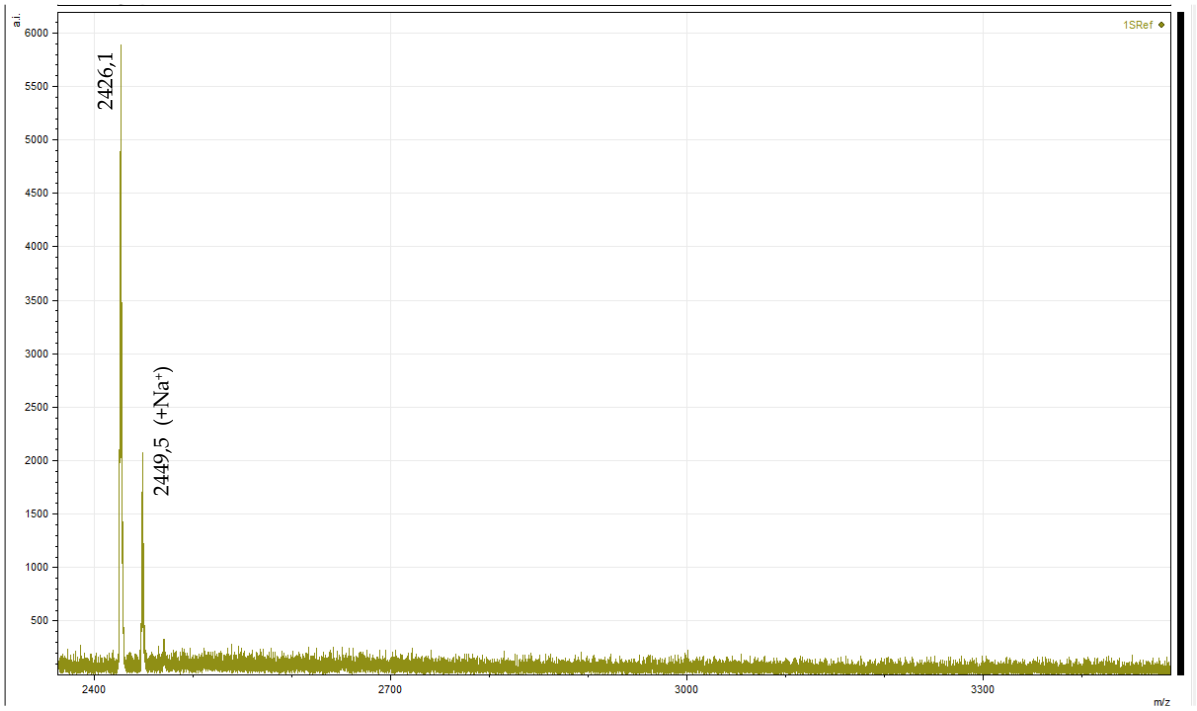
8	*Pyr*-NH-C(O)-d(TTTTTTT)



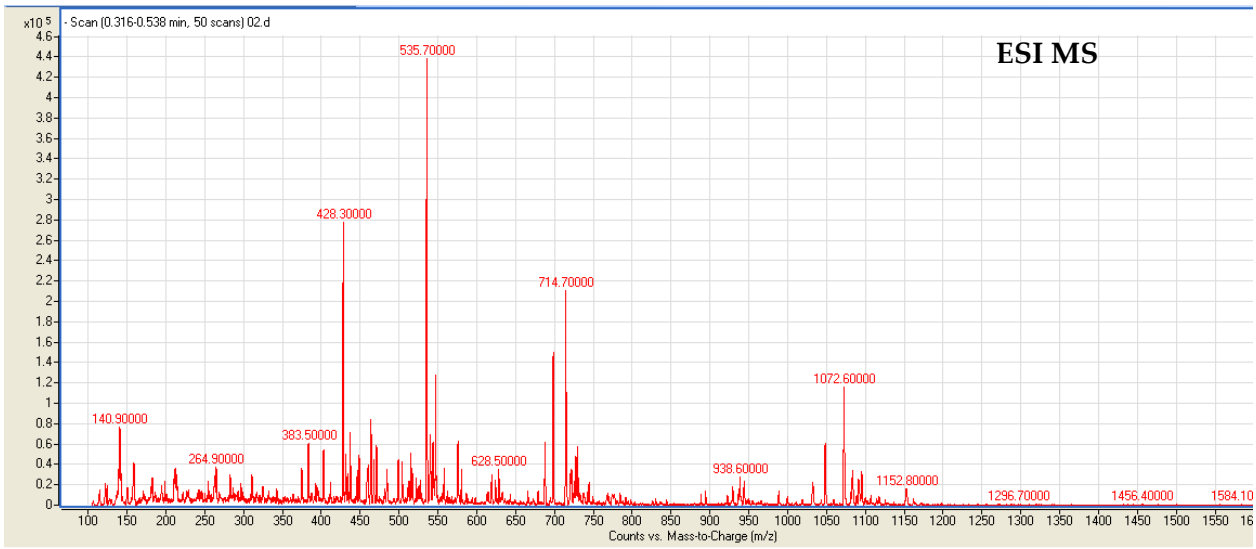
3	*Oleyl*-NH-C(O)-d(TTTTTTT)



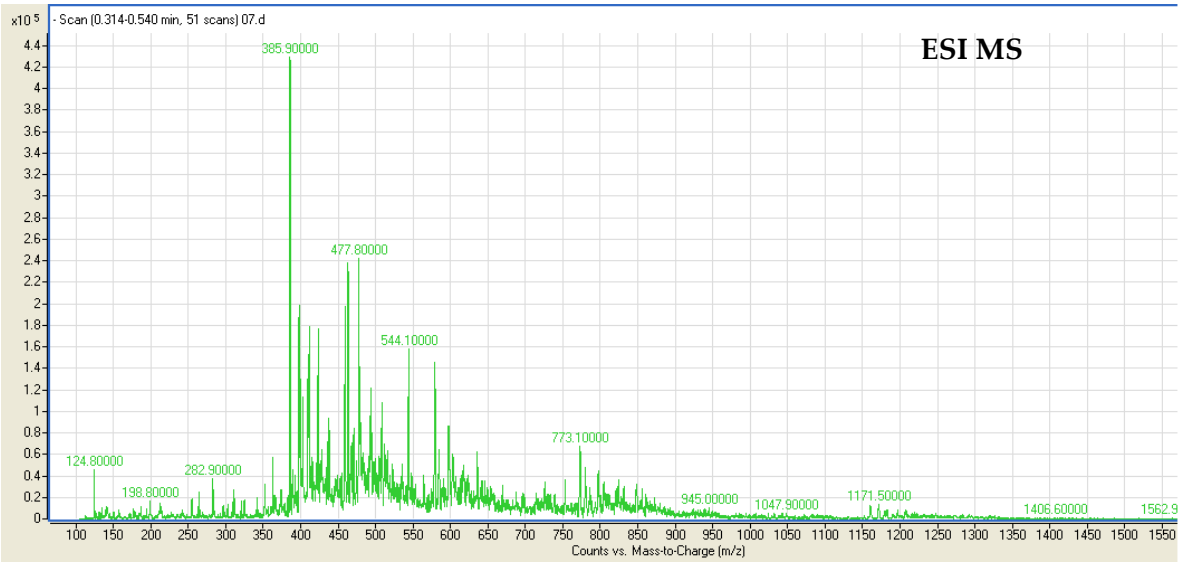
13	*Pyr*-NH-C(O)-***L***$_3$-NH-C(O)-d(TTTTTTT)



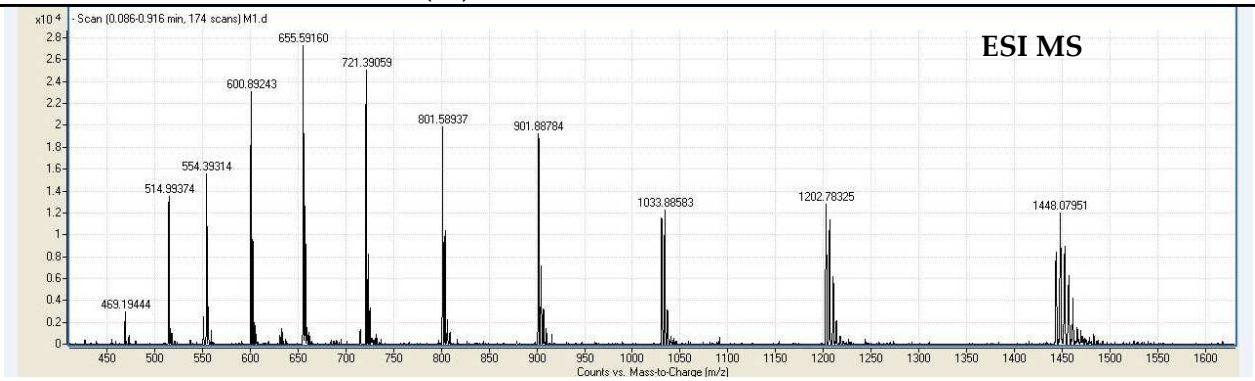
9	*c*-*B*$_{12}$-NH-C(O)-d(TTTTTTT)



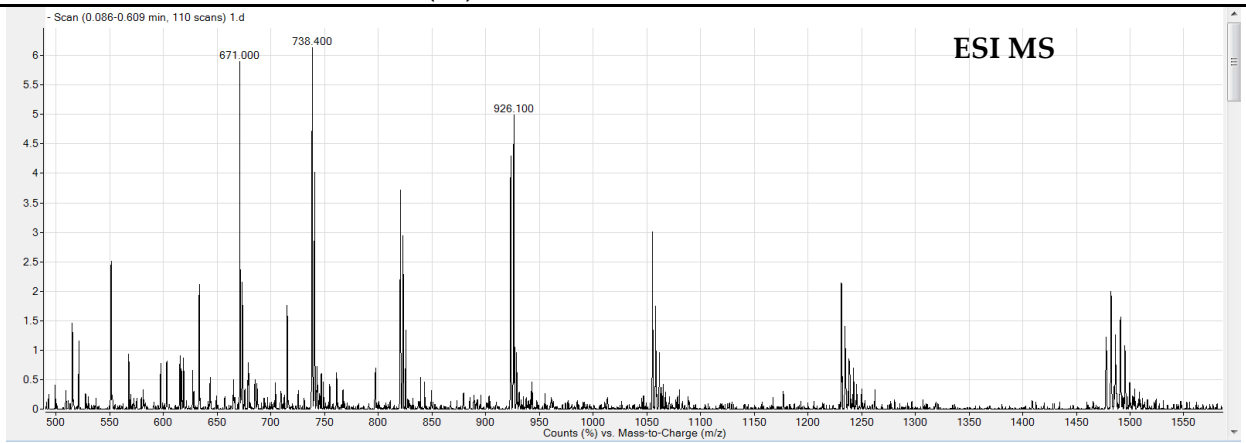
17	*c*-B_{12}-NH-C(O)-d(ATACGTTAACGATCCTTCAC)



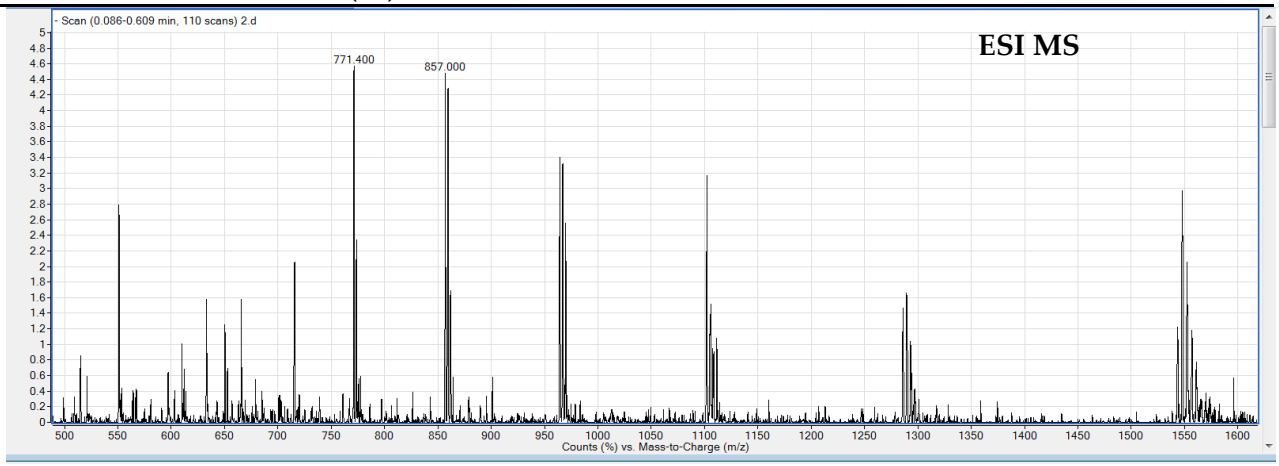
19	*(Leu)*$_3$-NH-C(O)-GGCUU^{m}GAC^{m}AAGUU^{m}GU^{m}AU^{m}AU^{m}GG



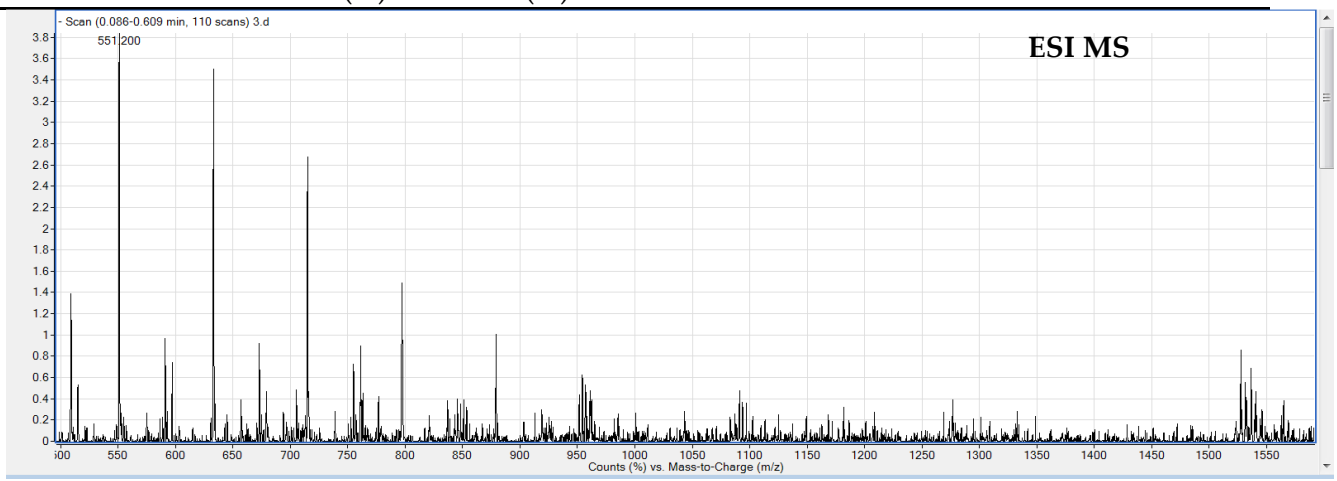
20	*Chol***L**$_6$-NH-C(O)-GGCUU^{m}GAC^{m}AAGUU^{m}GU^{m}AU^{m}AU^{m}GG



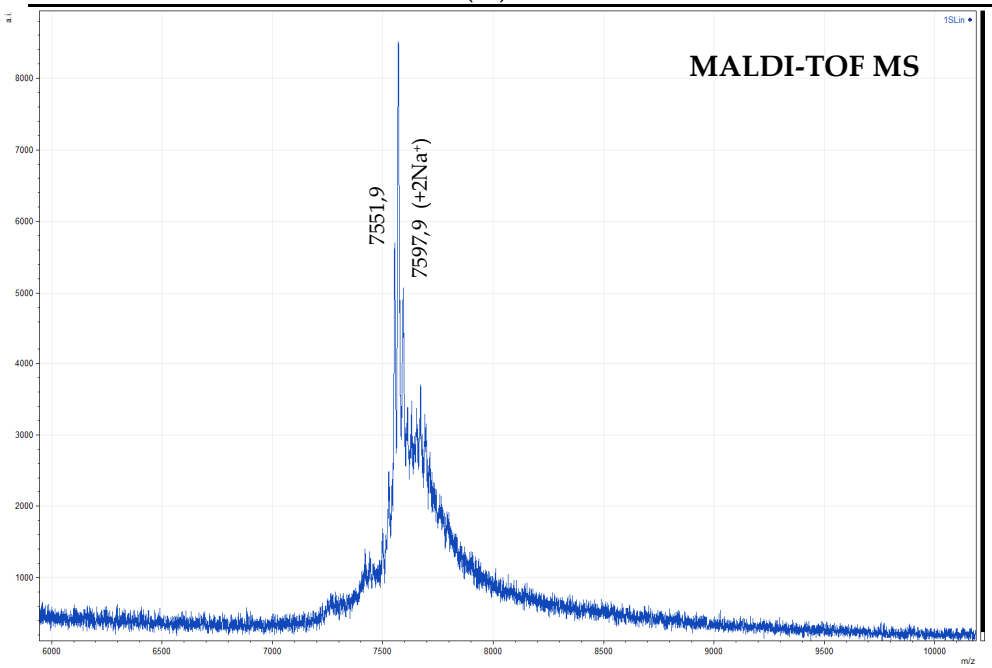
22 *Chol***L**$_6$-NH-C(O)-***LSSL***-GGCUU^{m}GAC^{m}AAGUU^{m}GU^{m}AU^{m}AU^{m}GG



23 *Chol***L**$_6$-NH-C(O)-***L***$_{12}$-NHC(O)-GGCUU^{m}GAC^{m}AAGUU^{m}GU^{m}AU^{m}AU^{m}GG



21 *(Leu)*$_3$-NH-C(O)-***LSSL***-GGCUU^{m}GAC^{m}AAGUU^{m}GU^{m}AU^{m}AU^{m}GG



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