SUPPORTING INFORMATION

Modulation of the stability of i-motif structures using an acyclic threoninol cytidine derivative

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1. General information.

L-threoninol and other chemicals were purchased from Sigma-Aldrich orFluka and were used without further purification. Dry solvents were purchased as well from Sigma-Aldrich or Fluka and used as supplied. All the standard phosphoramidites and ancillary reagents used for oligonucleotide synthesis were purchased from Applied Biosystems or Link Technologies. The benzoyl (Bz) group was used for the protection of the amino group of C and A and the isobutyryl (iBu) group was used for the protection of G. All oligonucleotides were synthesized DMT-ON and were purified by cartridge (Glen-Pak^{TM} DNA purification cartridge) from Glen Research. The oligonucleotides were desalted with Sephadex G-25 (IllustraNAP-10 or NAP-5 columns), from GE Healthcare Life Sciences. Flash column chromatography was carried out on silica gel SDS 0.063–0.2 mm/70–230 mesh. ^{1}H, ^{13}C and ^{31}P spectra of compounds **1-4** were recorded at 25ºC on a Varian Mercury 400 MHz spectrometer (Agilent Technologies) using deuterated solvents. Tetramethylsilane (TMS) was used as an internal reference (0 ppm) for ^{1}H spectra recorded in $CDCl_3$ and the residual signal of the solvent (77.16 ppm) for ^{13}C and ^{31}P-spectra. For CD_3OD the residual signal of the solvent was used as a reference. Chemical shifts are reported in part per million (ppm) in the δ scale, coupling constants in Hz and multiplicity as follows: s (singlet), d (doublet), m (multiplet). HPLC analyses were performed using a Waters 2695 Separations Module equipped with a Waters 2998 Photodiode Array Detector. The HPLC solvent used where: A: 5% ACN in 100 mMtriethylammonium acetate (TEAA) (pH = 7) and solvent B: 70% ACN in 100 mM TEAA (pH = 7).The matrix assisted laser desorption ionization time-of-flight (MALDI-TOF/TOF) mass spectrometer used to characterize the oligonucleotides was an AutoFLEX III (BrukerDaltonics, Bremen, Germany) equipped with smart beam laser operated at 200 Hz. Mass spectra were recorded in negative mode (2,4,6-trihidroxyacetophenone matrix with ammonium citrate as an additive).High-resolution electrospray ionization mass spectra ((HR) ESI-MS) were recorded on an Agilent 1100 LC/MS-TOF instrument. UV analyses and melting experiments were performed using a Jasco V-650 instrument equipped with a thermoregulated cell holder. CD analyses were recorded on a Jasco J-810 spectropolarimeter equipped with a Julabo F-25/HD temperature control unit. NMR spectra of Lt_0, Lt_3 and Lt_1/2/3 were acquired in a BrukerAvancespectrometer operating at 600 MHz and equipped with a cryoprobe.

2. Melting experiments.

The melting experiments were performed in duplicate at 2.7 μM concentration of oligonucleotide. Samples of each oligonucleotide were prepared using a solution 150 mM NaCl and 20 mM sodium acetate buffer (pH=5.3). Concentrations of all oligonucleotides were estimated by UV-Vis absorption at 30ºC, in a pH 8.0 buffer, using the ε_{260} values calculated by the nearest-neighbour method for the DNA coil state. The samples were heated at 90ºC for 5 minutes, allowed to cool slowly to room temperature to induce *i*-motif formation and the kept overnight in a refrigerator (4ºC). The melting curves were recorded monitoring the absorbance at 295 nm. The samples were heated with a temperature controller from 10 to 70ºC at a constant rate of 0.5ºC/min using 1 cm quartz path-length cuvettes with a ground hole at the top to adapt a PTFE stopper to provide a suitable seal to avoid evaporation of the sample. During the experiment, when the temperature was below 25ºC, argon was flushed to prevent water condensation. At least three different samples were prepared for each melting experiment. Thermodynamic data are calculated from the absorbance trace at a characteristic wavelength following the next procedure. First, the appropriate baselines at low and high temperatures are drawn ($L0_T$ and $L1_T$, respectively). Second, the relative amounts of folded and unfolded DNA are calculated for each one of measured absorbance values (A_T) from these relations:

$$Fraction\ of\ folded\ DNA = \frac{L0_T - A_T}{L0_T - A_T} \qquad (Equation\ 1)$$

$$Fraction\ of\ unfolded\ DNA = 1 - fractions\ of\ folded\ DNA\ (Equation\ 2)$$

Third, the appropriate expression for the equilibrium constant is applied. In the case of an intramolecular unfolding, the equilibrium constant expression is:

$$K_{unfolding} = \frac{fraction\ of\ unfolded\ DNA}{fraction\ of\ folded\ DNA} \qquad (Equation\ 3)$$

Fourth, fitting the values of the equilibrium constant to the van't Hoff equation provides values for thermodynamic data (ΔH and ΔS) associated to the considered equilibrium:

$$\ln K_{unfolding} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad (Equation\ 4)$$

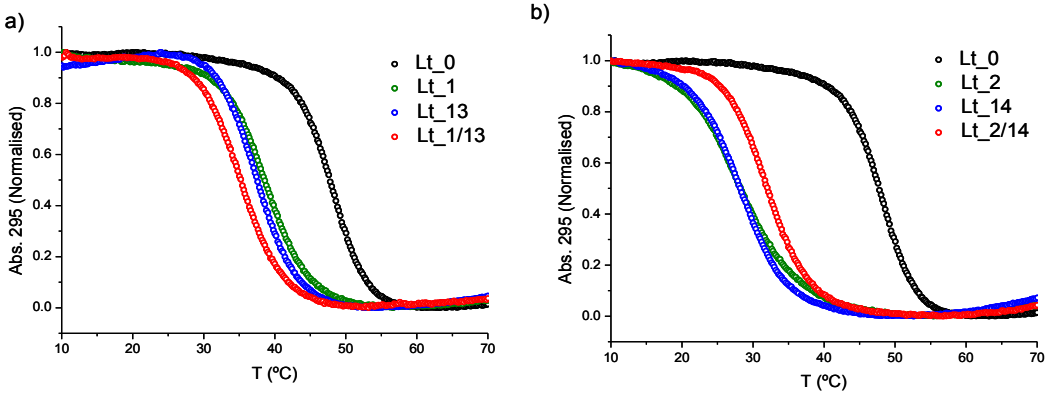
where R is the gas constant. This analysis assumes that the thermodynamic constants (ΔH and ΔS) will not change throughout the range of temperatures studied.

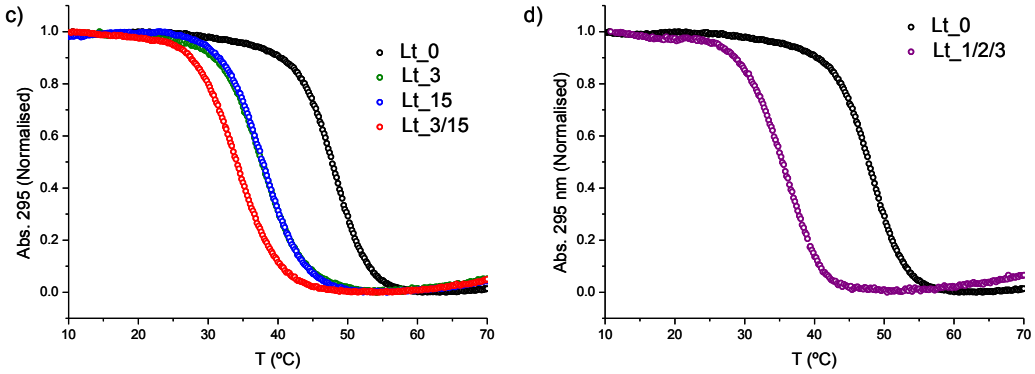
Table S1. Thermal stabilities and thermodynamic parameters with the corresponding uncertainty (Uncert.) of Lt_0 and modified oligonucleotides at pH 5.3.[a]

Name	T_m	Uncert. T_m	ΔG^o_{298}	Uncert. ΔG^o_{298}	ΔH^o	Uncert. ΔH^o	$T\Delta S^o_{298}$	Uncert. $T\Delta S^o_{298}$
Lt_0	47.81	0.17	-5.68	0.19	-79.35	1.95	-73.67	1.76
Lt_1	39.54	0.21	-2.87	0.04	-61.68	1.82	-58.80	1.94
Lt_2	29.12	0.60	-0.62	0.02	-45.60	2.46	-44.98	2.34
Lt_3	38.42	0.07	-2.59	0.10	-60.95	2.65	-58.36	2.55
Lt_13	37.44	0.16	-2.68	0.10	-67.45	3.55	-64.77	3.44
Lt_14	29.32	0.20	-0.83	0.01	-56.35	3.05	-55.51	3.04
Lt_15	37.93	0.15	-2.75	0.06	-65.13	2.15	-62.40	2.09
Lt_1/13	35.43	0.12	-2.26	0.01	-67.50	1.30	-65.23	1.31
Lt_2/14	32.40	0.15	-1.63	0.04	-66.41	3.76	-64.77	2.46
Lt_3/15	34.71	0.23	-2.06	0.07	-65.84	2.05	-63.68	1.98
Lt_1/2/3	35.64	0.59	-2.38	0.10	-69.35	1.45	-66.96	1.55

[a] T_m is expressed in ºC and ΔG^o_{298}, ΔH^o and $T\Delta S^o_{298}$ in kcal/mol.

Figure S1. Melting profiles of a) Lt_1, Lt_13 and Lt_1/13, b) Lt_2, Lt_14 and Lt_2/14, c) Lt_3, Lt_15 and Lt_3/15 and d) Lt_1/2/3. In all cases the melting profile of Lt_0 (in black) was represented for comparison purposes.





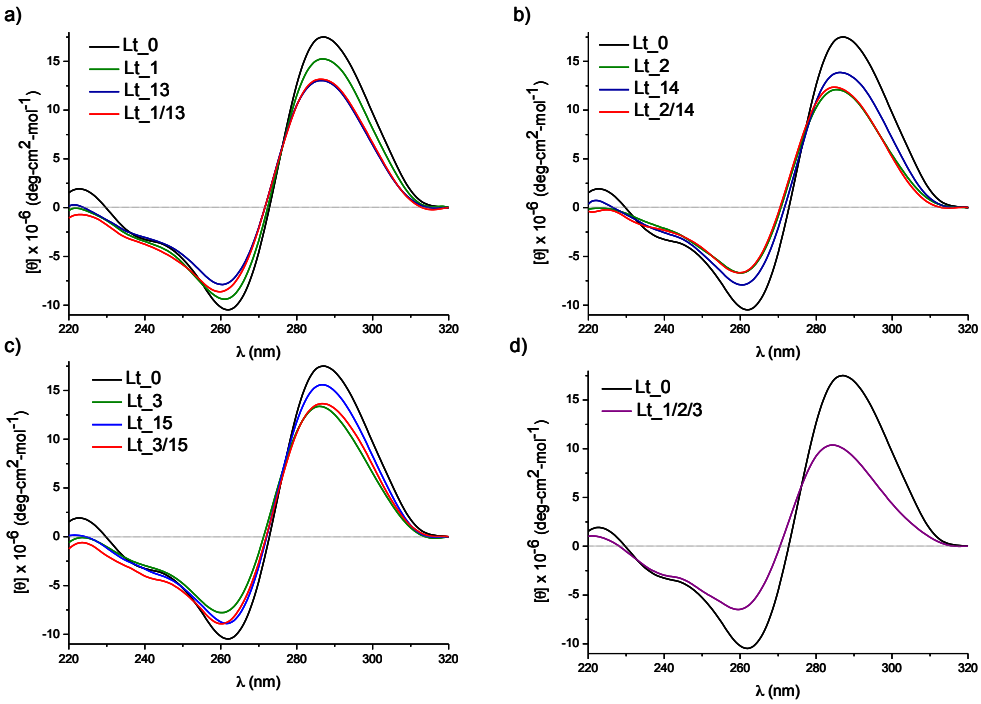
3. CD experiments.

Samples of each oligonucleotide were prepared at 2.7 μM concentration using a solution 150 mM NaCl and 20 mM sodium acetate buffer (pH=5.3). Concentrations of each oligonucleotide were estimated as described before. The samples were heated at 90ºC for 5 minutes, allowed to cool slowly to room temperature to induce *i*-motif formation and the kept overnight in a refrigerator (4ºC). The CD profiles were recorded at 15ºC from 320 to 220 nm at wavelength steps of 0.5 nm using 1 cm quartz path-length cuvettes.

Table S2. λ and molar ellipticity values of Lt_0 and modified oligonucleotides.

Name	λ_1 (nm)	$[\theta]_1 x10^{-6}$ (deg-cm^2-mol^{-1})	λ_2 (nm)	$[\theta]_2 x10^{-6}$ (deg-cm^2-mol^{-1})
Lt_0	287.0	17.5	262.0	-10.5
Lt_1	287.0	15.2	261.0	-9.4
Lt_13	286.5	13.0	260.5	-7.9
Lt_1/13	286.5	13.2	259.5	-8.6
Lt_3	286.0	13.4	260.5	-7.8
Lt_15	287.0	15.6	261.5	-8.9
Lt_3/15	286.5	13.6	260.0	-8.9
Lt_2	285.5	12.1	260.0	-6.7
Lt_14	286.5	13.8	260.5	-7.9
Lt_2/14	284.5	12.4	260.0	-6.7
Lt_1/2/3	284.5	10.4	259.5	-6.5

Figure S2. CD profiles of a) Lt_1, Lt_13 and Lt_1/13, b) Lt_2, Lt_14 and Lt_2/14, c) Lt_3, Lt_15 and Lt_3/15 and d) Lt_1/2/3. In all cases the CD profile of Lt_0 (in black) was included as a reference.



4. pH Denaturation studies by UV-Vis.

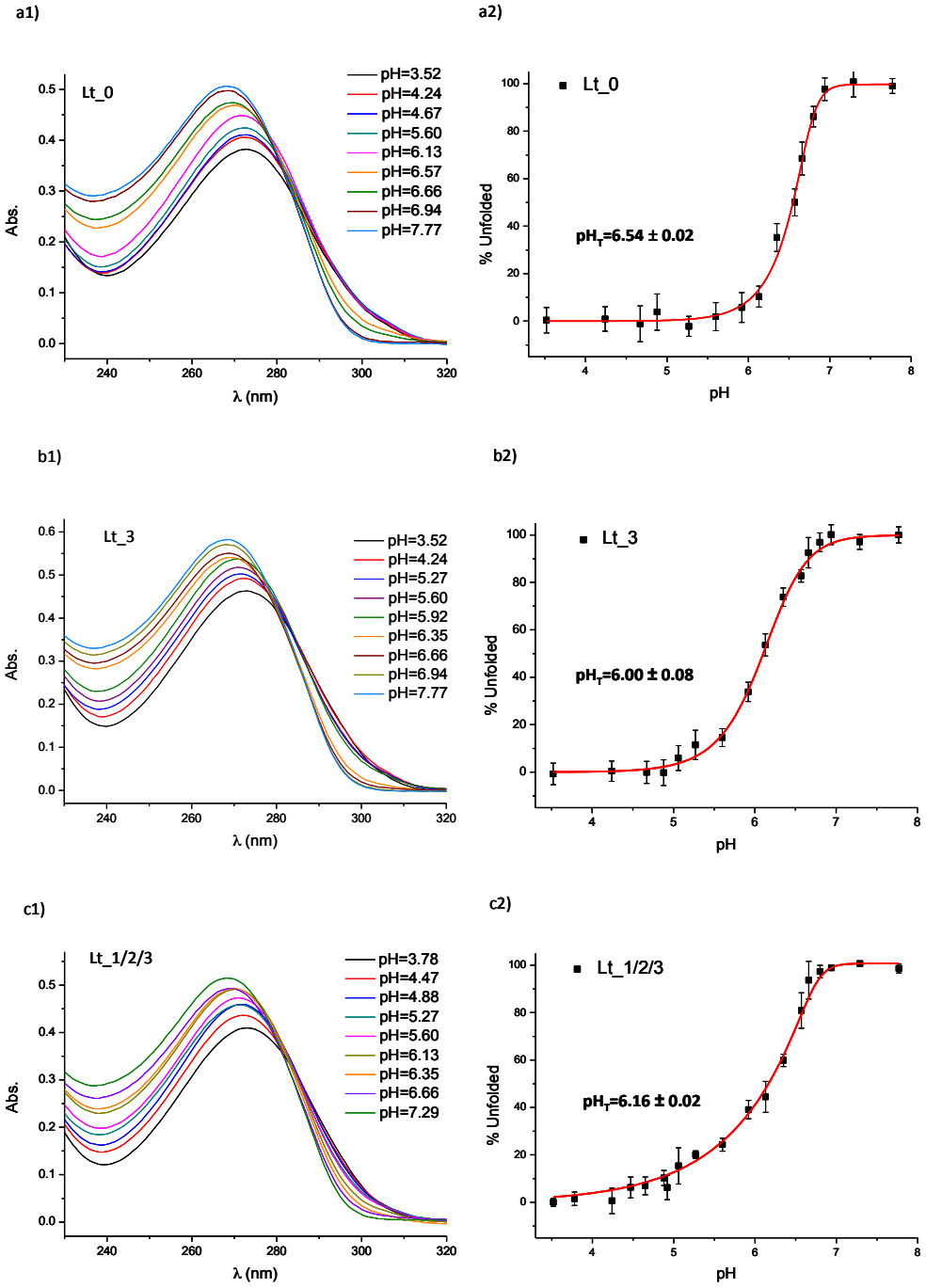
A typical sample (200 μL) was prepared at 2.7 μM concentration of oligonucleotide using the appropriate buffer (150 mM NaCl and 20 mM sodium acetate for pH=3.50-5.30 and 150 mM NaCl and 20 mM sodium phosphate for pH=5.60-7.70). Concentrations of each oligonucleotide were estimated as described before. The samples were heated at 90ºC for 5 minutes, allowed to cool slowly to room temperature to induce *i*-motif formation and the kept overnight in a refrigerator (4ºC). The UV-Vis profiles were recorded at 25ºC from 320 to 220 nm using low volume quartz cuvettes (200 μL) of 1 cm path-length. For each pH at least two different samples were prepared. The *i*-motif unfolding was monitored at 295 nm. The absorbance values were converted into % Unfolded:

$$\%\, Unfolded = \frac{FoldBS - Abs.}{FoldBS - UnfoldBS} \times 100 \quad (Equation\ 5)$$

Where "Abs." is the absorbance at 295 nm at a given pH and "FoldBS" and "UnfoldBS" correspond to the baseline values of the folded and unfolded species

respectively. The transition midpoint (pH_T) was obtained by plotting %Unfolded *.vs*. pH and fitting to a sigmoidal (SRichards2) using the Origin Pro 8 software.

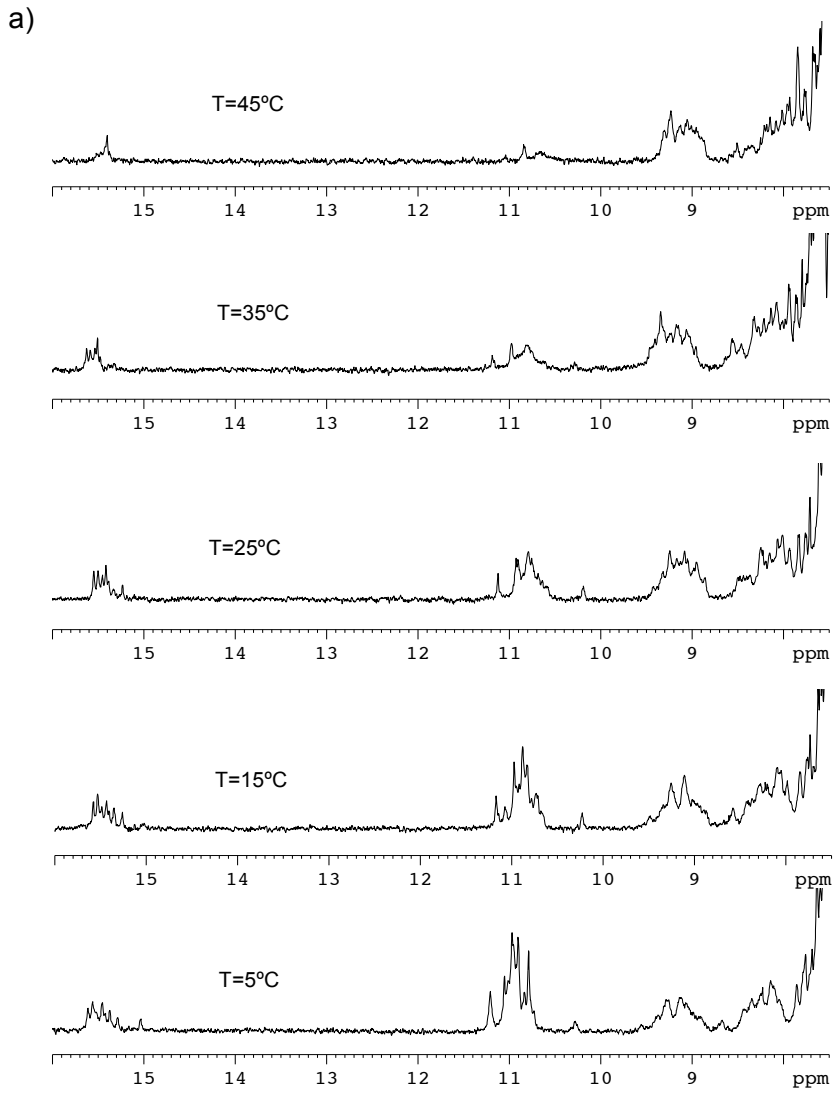
Figure S3. a1), b1) and c1) UV-Vis absorption spectra at different pH values of Lt_0, Lt_3 and Lt_1/2/3 respectively. a2), b2) and c2) % Unfolded of *i*-motif as a function of pH of Lt_0, Lt_3 and Lt_1/2/3 respectively.



5. NMR of Lt_0, Lt_3 and Lt_1/2/3.

Samples for NMR experiments were dissolved in 9:1 H_2O/D_2O. Experiments were performed either with no buffer added or in 25 mM sodium phosphate buffer, and 100 mM NaCl. The pHwas adjusted by adding aliquots of either concentrated solution of DCl or NaOD (pH = 4.2). Water suppression was achieved by including a WATERGATE module in the pulse sequence prior to acquisition

Fig S4. NMR spectra at different temperature of a) Lt_0, b) Lt_3 and c) Lt_1/2/3 at pH=4.2.



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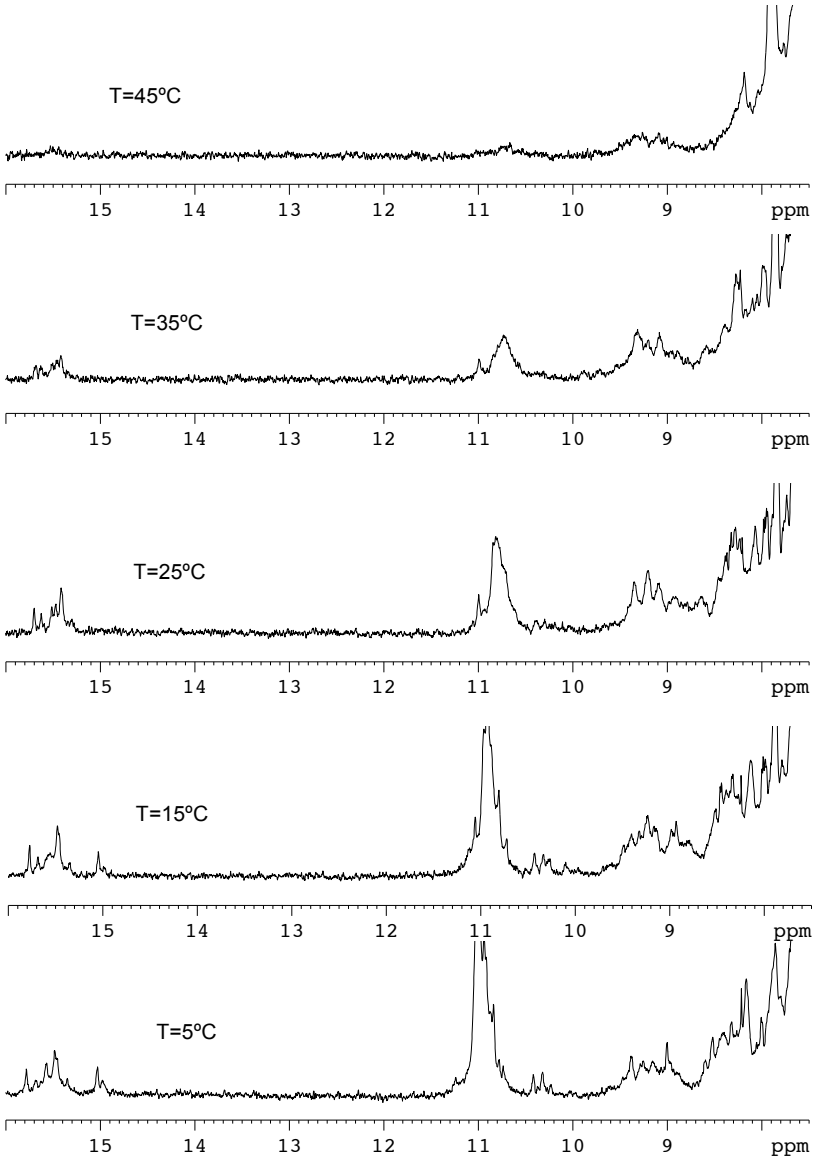
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T=35ºC
15 14 13 12 11 10 9 ppm
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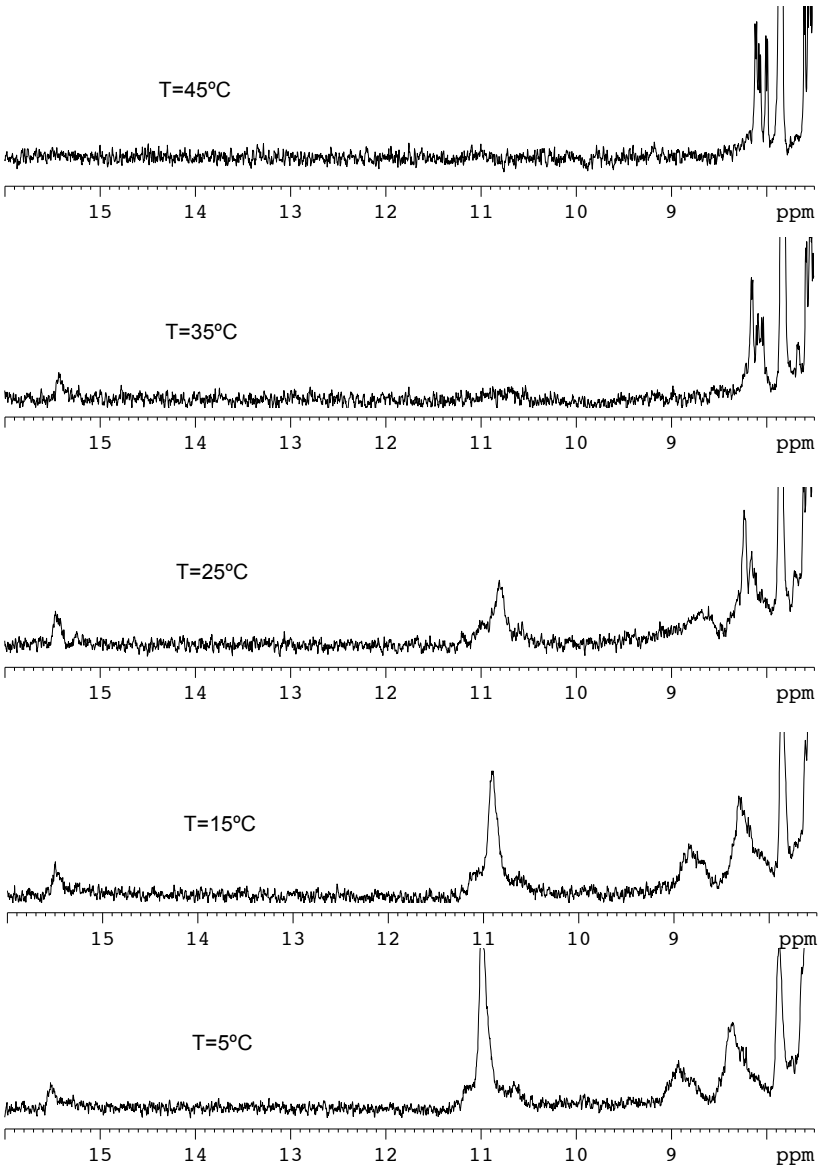
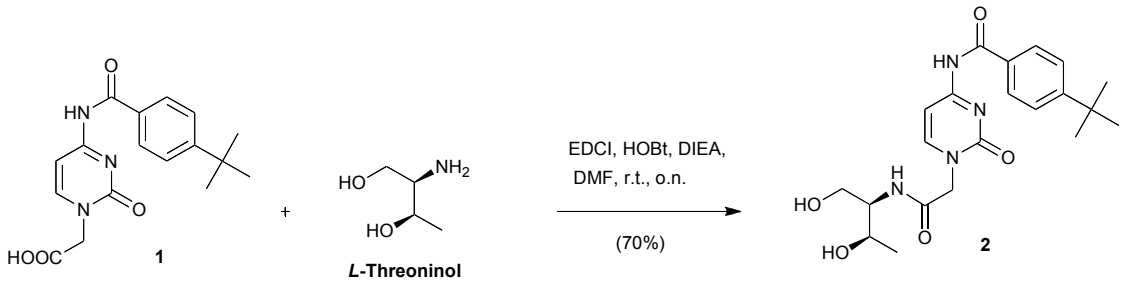
T=5ºC
15 14 13 12 11 10 9 ppm

c)



6. Synthesis of compounds 1-4.

N- [N^4-(4-*tert*-Butylbenzoyl)-cytosin-1-yl)acetyl]-*L*-threoninol, (compound 2).

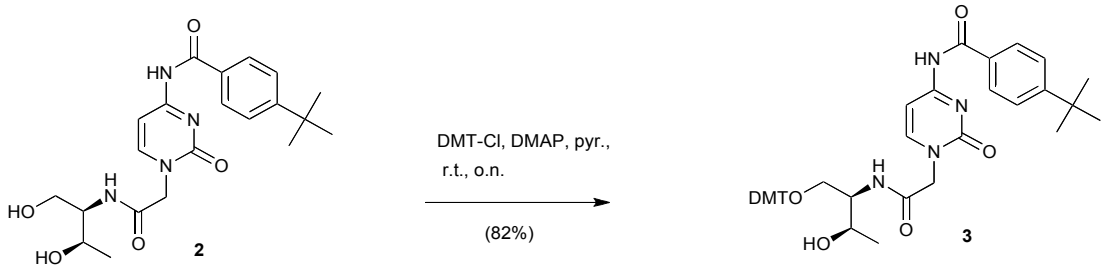


N^4-(4-*tert*-Butylbenzoyl)cytosine (compound **1**) was synthesized as described in the bibliography[1]. A solution of the carboxylic acid **1** (800 mg; 2.4 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (558 mg; 3.6 mmol), hydroxybenzotriazole (HOBt) (486 mg; 3.6 mmol) and diisopropylethylamine (DIEA) (0.63 mL; 3.6 mmol) in anhydrous dimethylformamide (DMF) (45 mL) was prepared under argon. After stirring the mixture for 5 minutes, *L*-threoninol (273 mg; 2.6 mmol) was added and the reaction mixture was stirred at room temperature overnight. Then, the solvent was evaporated under reduced pressure. The residue was dissolved in toluene and concentrated to dryness under reduces pressure (3x) and dissolved in dichloromethane (DCM) (150 mL). The organic phase was washed with 10% aqueous $NaHCO_3$ (2x 50 mL) and with saturated aqueous NaCl (50 mL), dried over anhydrous Na_2SO_4 and evaporated to dryness. Finally, the crude was purified by flash column chromatography on silica gel with a gradient of methanol from 3 to 15% in DCM. The desired compound was obtained as a white solid in 70% yield (711 mg; 1.7 mmol). TLC (DCM/Methanol 10:1) R_f=0.31. ^{1}H NMR (400 MHz, CD_3OD)δ_H: 8.00 (d, J = 7.2 Hz, 1H, cytosine H-6), 7.90 (d, J= 8.4 Hz, 2H, tBuBz), 7.59 - 7.54 (m, 3H, tBuBz and cytosine H-5), 4.71 – 4.60 (AB system, 2H, J_{AB} = 16.0 Hz, N-C$\underline{H}_2$-CO), 4.05-3.96 (m, 1H, CH_3-C$\underline{H}$-CH-NH), 3.85-3.82 (m, 1H, CH_3-CH-C$\underline{H}$-NH), 3.70 – 3.58 (ABX system, 2H, J_{AB}= 11.2 Hz, J_{AX} =5.6 Hz, J_{BX} = 6.4 Hz, OH-C$\underline{H}_2$-CH), 1.34 (s, 9H, tBu), 1.17 (d, 3H, J = 6.4 Hz, C$\underline{H}_3$-CH).^{13}C NMR (100 MHz, CD_3OD)δ_c: 167.92 (CO), 163.86 (CO), 156.62 (C), 150.79 (CO), 130.31 (CH, cytosine), 128.96 (C), 127.68 (CH, tBuBz), 125.36 (CH, tBuBz), 125.10 (C), 96.93 (CH, cytosine), 65.76 (CH, CH_3-C$\underline{H}$-CH-NH), 61.38 (CH_2, OH-C$\underline{H}_2$-

[1] D. W. Will, G. Breipohl, D. Langner, J. Knolle, E. Uhlmann, *Tetrahedron*, 1995, **51**, 44, 12069-12082

CH), 56.59 (CH, CH_3-CH-C<u>H</u>-NH), 52.12 (CH_2, N-C<u>H</u>$_2$-CO), 34.55 (C, tBu), 30.04 (CH_3, tBu), 18.93 (CH_3, C<u>H</u>$_3$-CH). HRMS m/z: Calc for $C_{21}H_{29}N_4O_5$ $(M+H)^+$ 417.2132, found 417.2121.

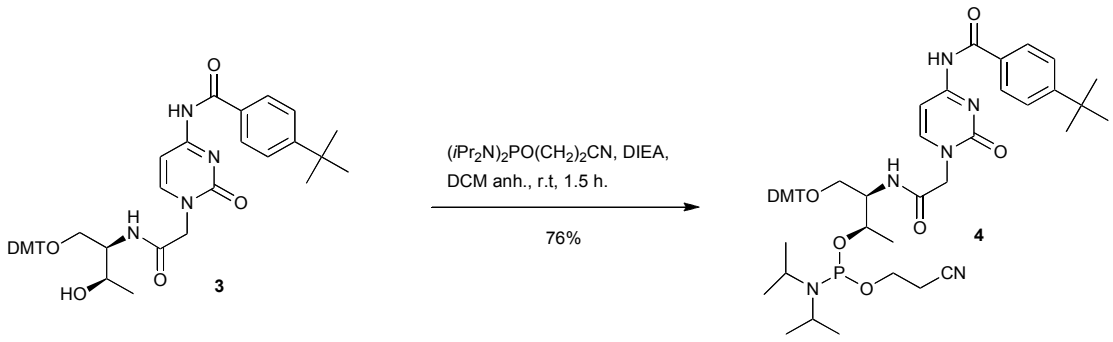
O^1-(4,4'-Dimethoxytriphenylmethyl)-*N*- [N^4-(4-*tert*-Butylbenzoyl)-cytosin-1-yl)acetyl]-*L*-threoninol, (compound 3).



Compound **2** (400 mg; 0.96 mmol) was dried by evaporation with anhydrous acetonitrile (ACN) under reduced pressure and left in a desiccator for 30 min. Next the compound was dissolved in anhydrous pyridine (30 mL) under argon at 0ºC and dimethylaminopyridine (catalytic amount) and 4,4'-dimethoxytriphenylmethyl chloride (390 mg; 1.15 mmol) were added with exclusion of moisture. After 15 minutes the mixture was allowed to reach room temperature and left to react under these conditions overnight. Afterward the reaction was complete as judged by TLC and quenched with methanol. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (EtOAc) (80 mL). The solution was washed with 10% aqueous $NaHCO_3$ (2x 30 mL) and with saturated aqueous sodium chloride (30 mL). After drying the organic phase with Na_2SO_4, the solvent was evaporated under reduced pressure and the product was purified by flash column chromatography. The product was eluted with a gradient of methanol from 3 to 5% in EtOAc always with a 1% of triethylamine (TEA). Compound **3**was isolated as a white solid in 82% yield (564 mg; 0.78 mmol). TLC (EtOAc/Methanol 10:0.3) R_f=0.32. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 7.79 (d, *J*= 8.4 Hz, 2H, tBuBz), 7.63 (d, *J* = 7.2 Hz, 1H, cytosine H-6), 7.45 (d, *J*= 8.4 Hz, 2H, tBuBz), 7.39-7.36 (m, 3H, cytosine H-5 and arom. DMT), 7.26-6.75 (m, 9H, arom. DMT), 4.62 – 4.42 (AB system, 2H, J_{AB} = 14.8 Hz, N-C<u>H</u>$_2$-CO), 4.10-4.07 (m, 1H, CH_3-C<u>H</u>-CH-NH), 3.97-3.94 (m, 1H, CH_3-CH-C<u>H</u>-NH), 3.72 (s, 6H, O-C<u>H</u>$_3$), 3.36 – 3.17 (ABX system, 2H, J_{AB}= 9.6 Hz, J_{AX} = 4.4 Hz, J_{BX} = 5.2 Hz, OH-C<u>H</u>$_2$-CH), 1.31 (s, 9H, tBu), 1.08 (d, 3H, *J* = 6.4 Hz, C<u>H</u>$_3$-CH).^{13}C NMR (100 MHz, $CDCl_3$) δ_c: 166.68

(CO), 162.84 (CO), 158.47 (C, DMT), 157.00 (C), 149.61 (CO), 144.50 (C, DMT), 135.67 (C, arom.), 135.47 (C, arom.), 130.00 (CH, DMT), 129.93 (CH, DMT), 129.10 (CH, cytosine), 127.98 (CH, arom.), 127.87 (CH, arom.), 127.59 (CH, DMT), 126.85 (C, tBuBz), 125.90 (CH, tBuBz), 113.17 (CH, DMT), 113.12 (CH, cytosine), 86.40 (C, C-O DMT), 67.59 (CH, CH_3-C<u>H</u>-CH-NH), 63.97 (CH_2, O-C<u>H_2</u>-CH), 55.16 (CH_3, CH_3-O), 54.70 (CH, CH_3-CH-C<u>H</u>-NH), 52.79 (CH_2, N-C<u>H_2</u>-CO), 35.11 (C, tBu), 31.03 (CH_3, tBu), 20.02 (CH_3, C<u>H_3</u>-CH).HRMS m/z: Calc for $C_{42}H_{47}N_4O_7$ $(M+H)^+$ 719.3439, found 719.3430.

O^1-(4,4'-Dimethoxytriphenylmethyl)-*N*- [N^4-(4-*tert*-Butylbenzoyl)-cytosin-1-yl)acetyl]-O^3-(2-cyanoethyl-*N,N'*-diisopropylaminophosphinyl)- *L*-threoninol, (compound 4).

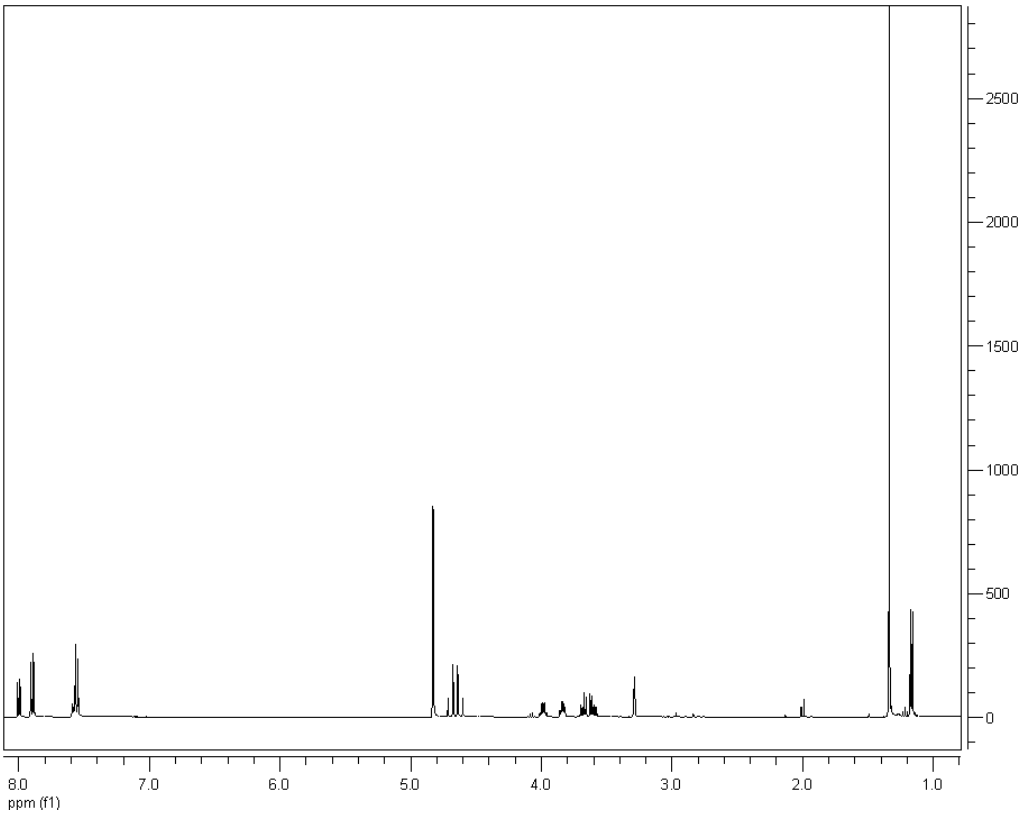


Compound **3** (200 mg; 0.28 mmol) was dried by evaporation with anhydrous ACN) under reduced pressure and left in a desiccator for 30 min. Next the product was dissolved in anhydrous DCM (10 mL) under argon and DIEA (196 µL; 1.12mmol)was added with exclusion of moisture. The solution was cooled and 2-cyanoethoxy-*N,N'*-diisopropylaminochlorophosphine (99 µL; 0.42mmol) was added dropwise with a syringe.Afterward, the solution was stirred at room temperature for 1.5 h. Then 15 mL of DCM were added to the reaction mixture and the organic phase was washed with saturated aqueous sodium chloride (15 mL). After drying the organic phase with Na_2SO_4, the solvent was evaporated under reduced pressure and the product was purified by flash column chromatography on silica gel. The column was packed with silica gel using a 10% TEA solution in EtOAc/hexane 90:10 and the gradient used was EtOAc/hexane 90:10 →EtOAc. The pure compound was obtained as white solid (196 mg, 76%). TLC (EtOAc) R_f=0.55 and 0.45. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: Most of signals are duplicated due to the presence of diastereoisomers, 7.81-7.49 (m, 5H, cytosine H-6 and aromatic protons of tBuBz), 7.38-6.77 (m, 14H, cytosine H-5 and

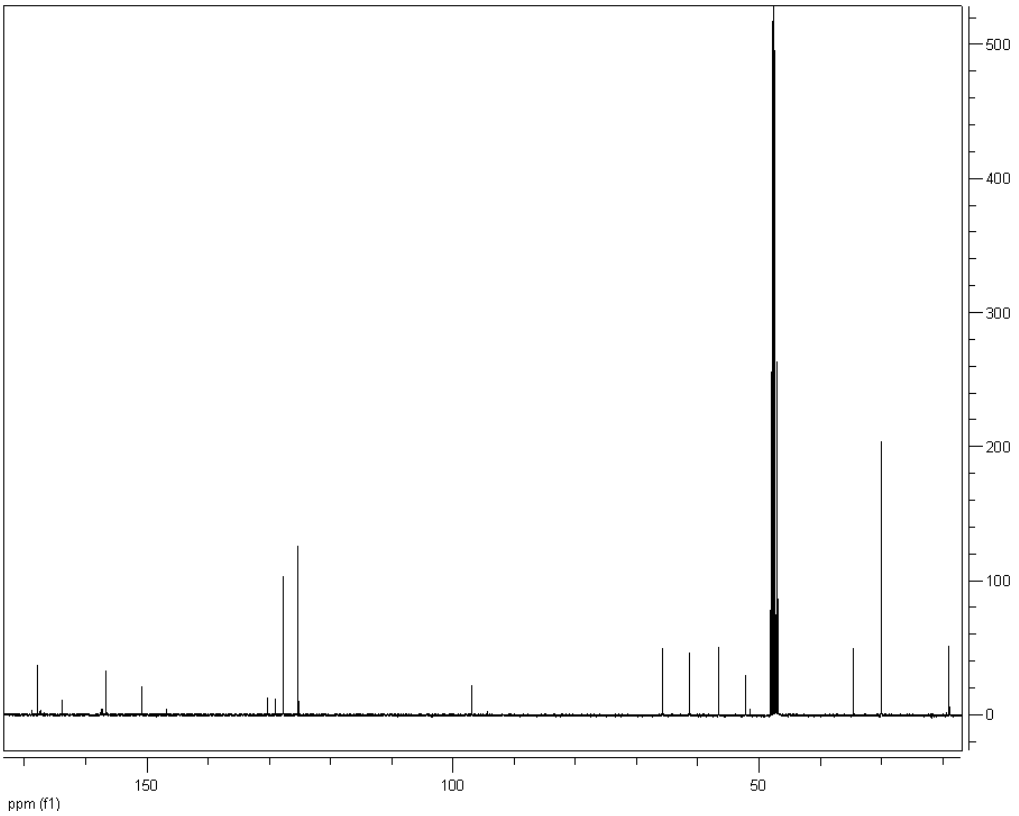
aromatic protons of DMT), 6.66-6.61 (m, 1H, NH, -OCH_2-CH-N<u>H</u>-CO), 4.60 – 4.44 (2H, two diastereisomers, two AB systems, J_{AB} = 14.8 Hz, N-C<u>H</u>$_2$-CO), 4.32-4.24 (m, 1H, CH_3-C<u>H</u>-CH-NH), 4.17-4.07 (m, 1H, CH_3-CH-C<u>H</u>-NH), 3.76 and 3.75 (6H, 2s, two diastereoisomers, O-C<u>H</u>$_3$), 3.52-3.16 (m, 6H, -OC<u>H</u>$_2$$CH_2$CN, CH *i*Pr and OH-C<u>H</u>$_2$-CH), 2.65-2.43 (m, 2H, -OCH_2C<u>H</u>$_2$CN), 1.33 (s, 9H, tBu), 1.17-1.08 (m, 12H, CH_3*i*Pr), 0.95 (d, 3H, J = 6.8 Hz, C<u>H</u>$_3$-CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ_c: Most of signals are duplicated due to the presence of diastereoisomers 166.32 and 166.24 (CO), 158.41 and 158.38 (C, DMT), 157.11 (C), 151.12 (CO), 144.67 (C, DMT), 135.98-135.82, two diastereoisomers, two (C, arom.), 130.00-129.98 (two diastereisomers, two CH, DMT), 128.15-127.41, (2CH DMT and 1CH tBuBz), 126.74 and 126.70 (C, tBuBz), 126.00 (CH, tBuBz), 118.20 (C, CN), 113.05 (CH, DMT), 102.48 (CH, cytosine), 86.10 (C, C-O DMT), 68.36-68.20 (CH, CH_3-C<u>H</u>-CH-NH), 62.98-62.46 (CH_2, O-C<u>H</u>$_2$-CH), 58.38-58.05 (CH_2, O-<u>C</u>H_2-CH_2-CN), 55.18 and 55.15 (CH_3, CH_3-O), 54.83-54.45 (CH, CH_3-CH-C<u>H</u>-NH), 43.15-42.96 (CH, *i*Pr), 35.14 (C, tBu), 31.03 (CH_3, tBu), 24.69-24.26 (CH3, *i*Pr), 20.51-20.27 (CH_2, O-CH_2-<u>C</u>H_2-CN), 19.61-19.46 (CH_3, C<u>H</u>$_3$-CH).^{31}P NMR (162 MHz, $CDCl_3$) δ_P: 148.00 and 146.65. HRMS m/z: Calc for $C_{51}H_{62}N_6O_8P$ $(M-H)^-$ 917.4372, found 917.4353.

7. NMR of compounds 1-4

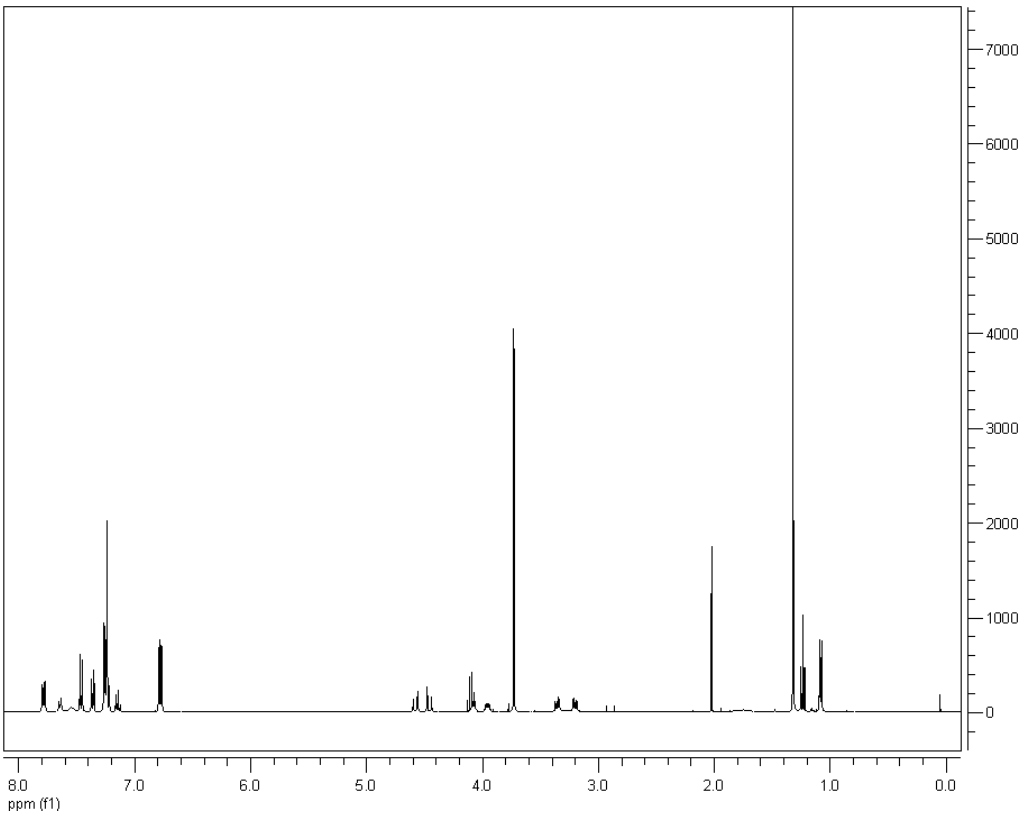
^{1}H NMR of Compound 2



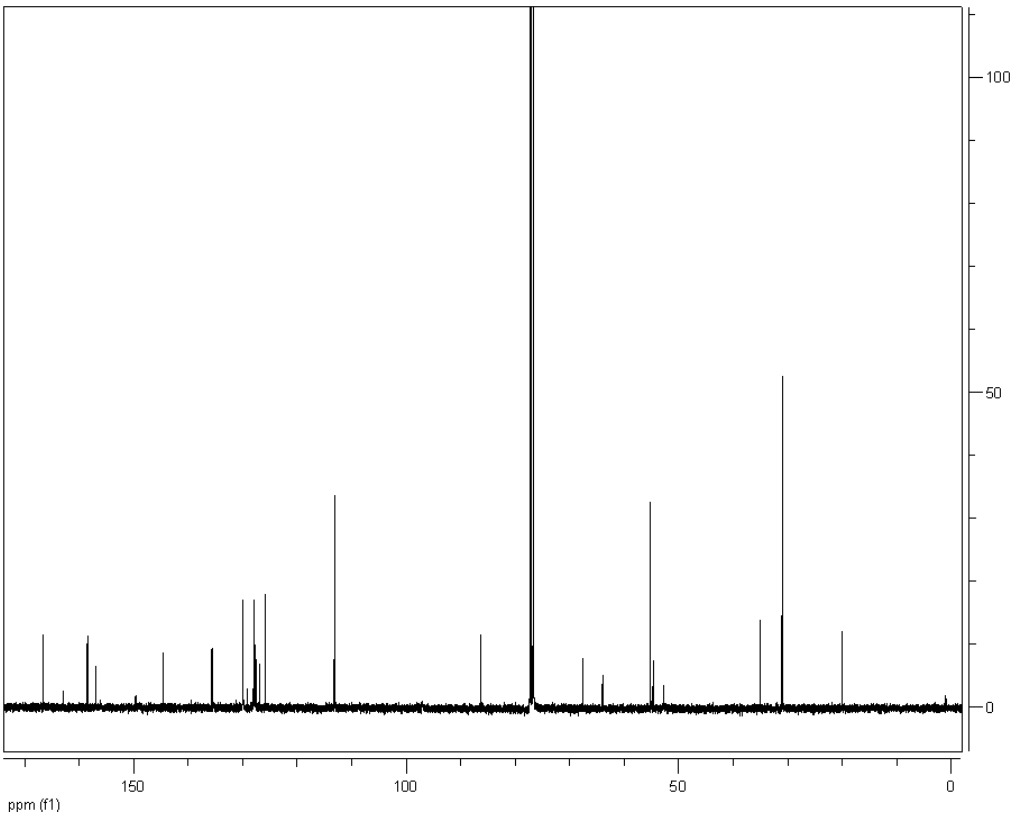
^{13}C NMR of Compound 2



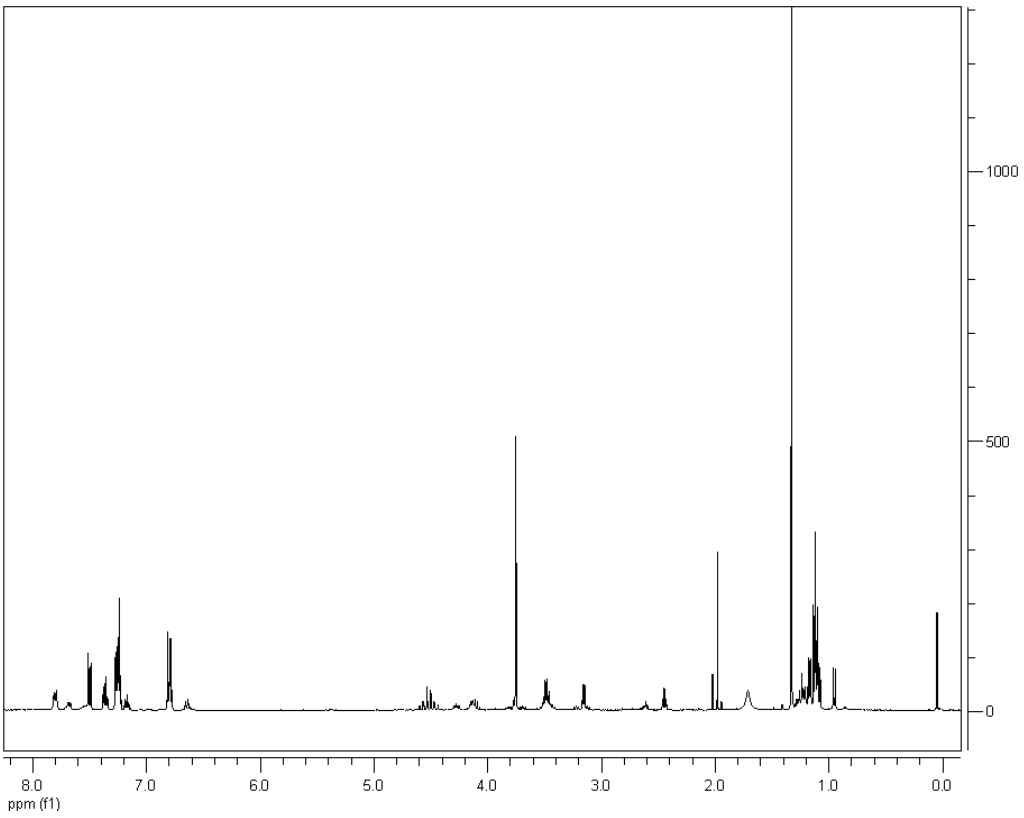
^{1}H NMR of Compound 3



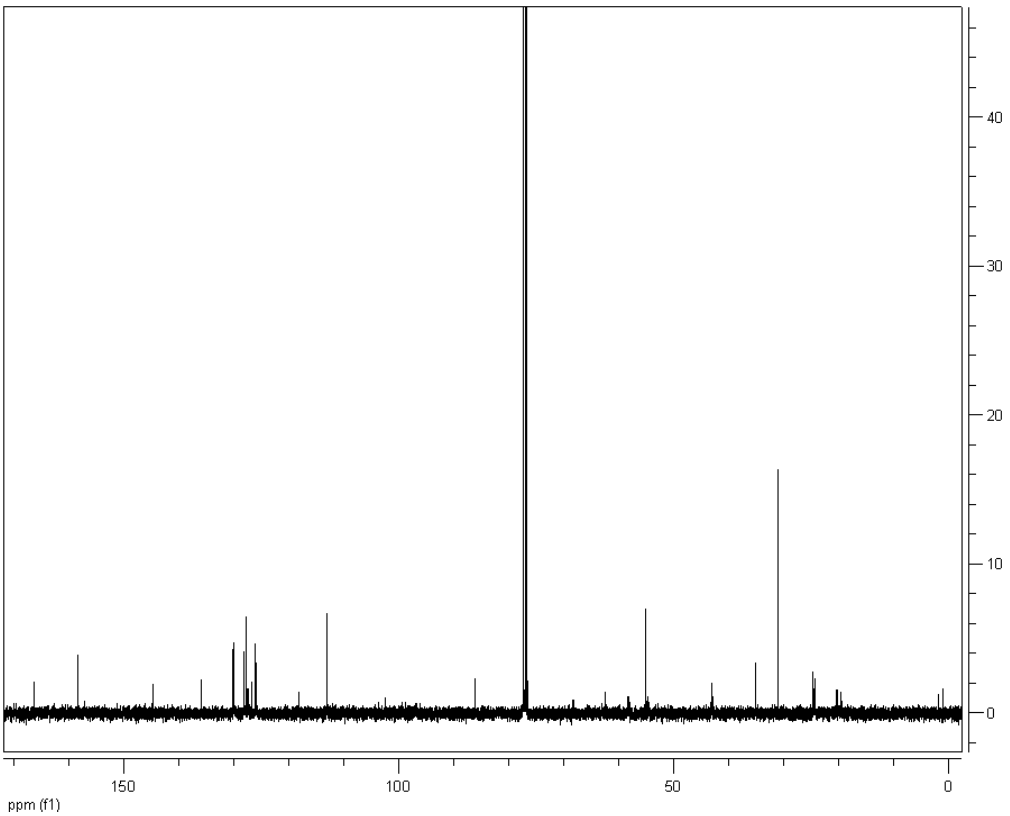
^{13}C NMR of Compound 3



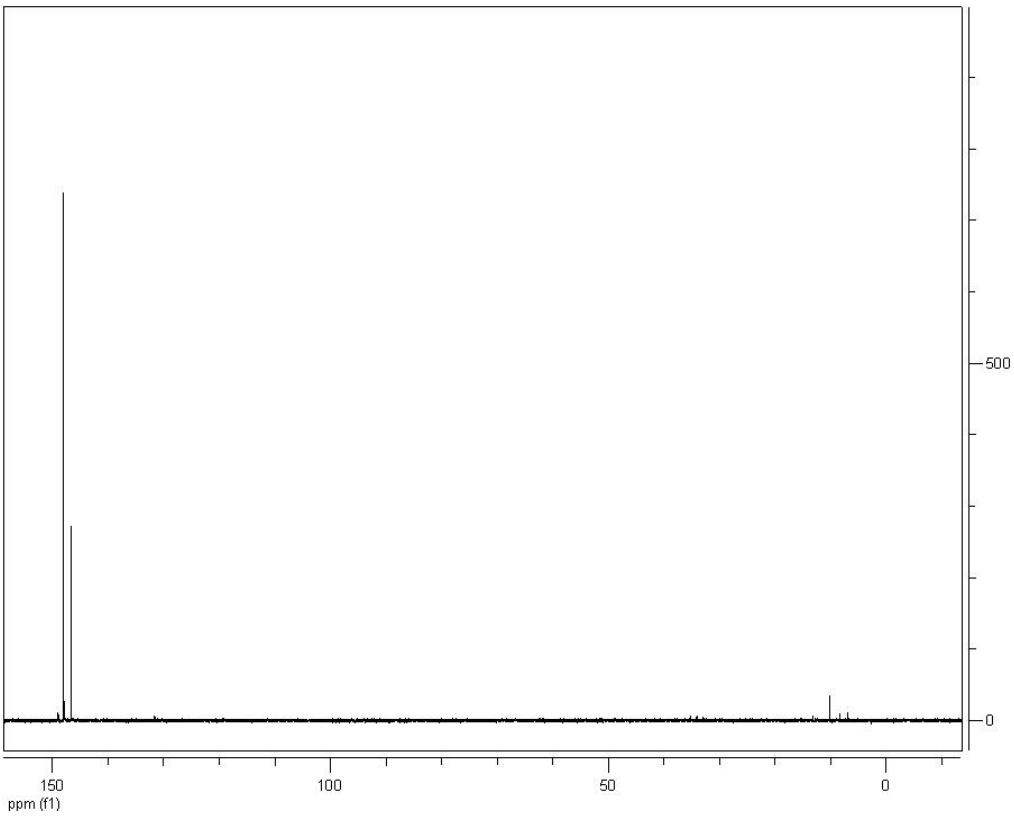
^{1}H NMR of Compound 4



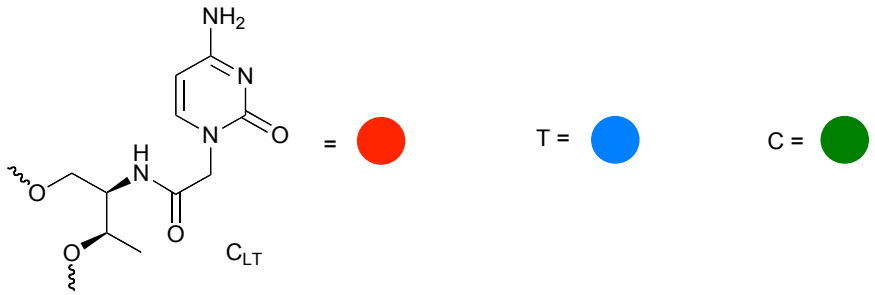
^{13}C NMR of Compound 4



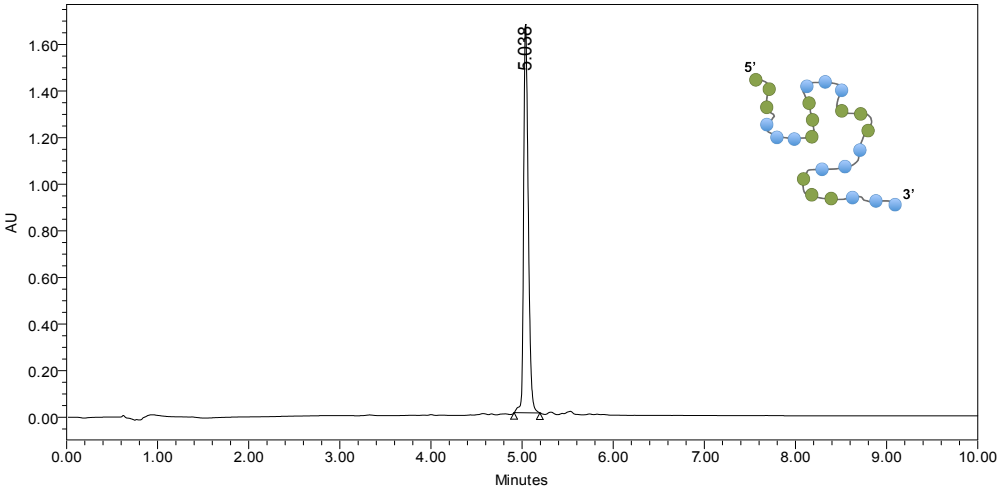
^{31}P NMR of Compound 4

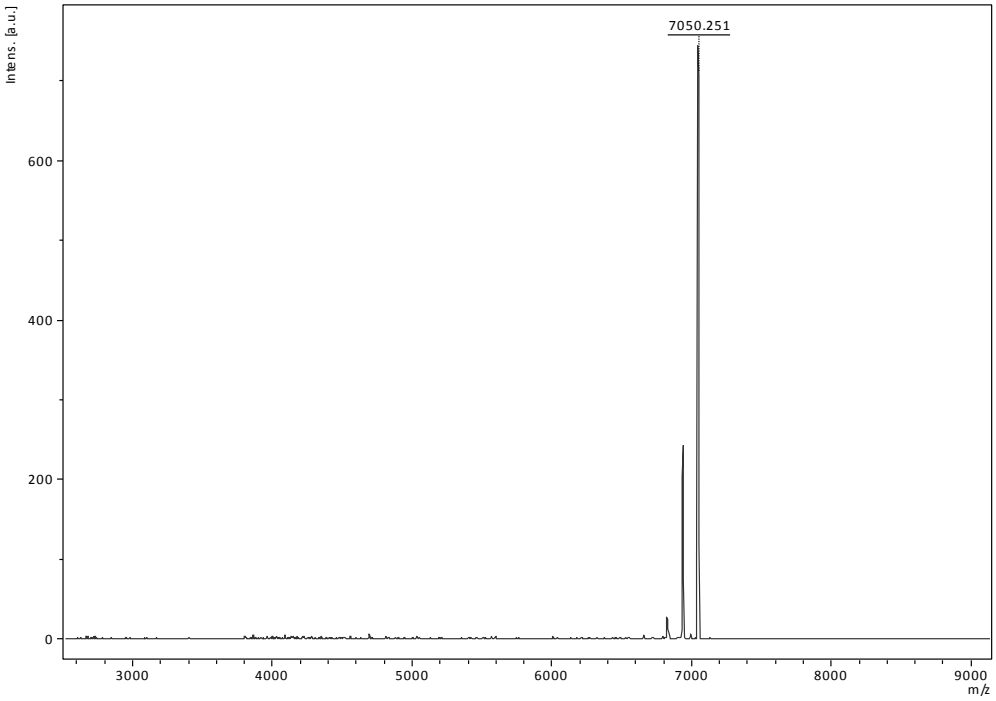


8. HPLC and mass spectrometry analysis of oligonucleotides Lt_0 and modified oligonucleotides.

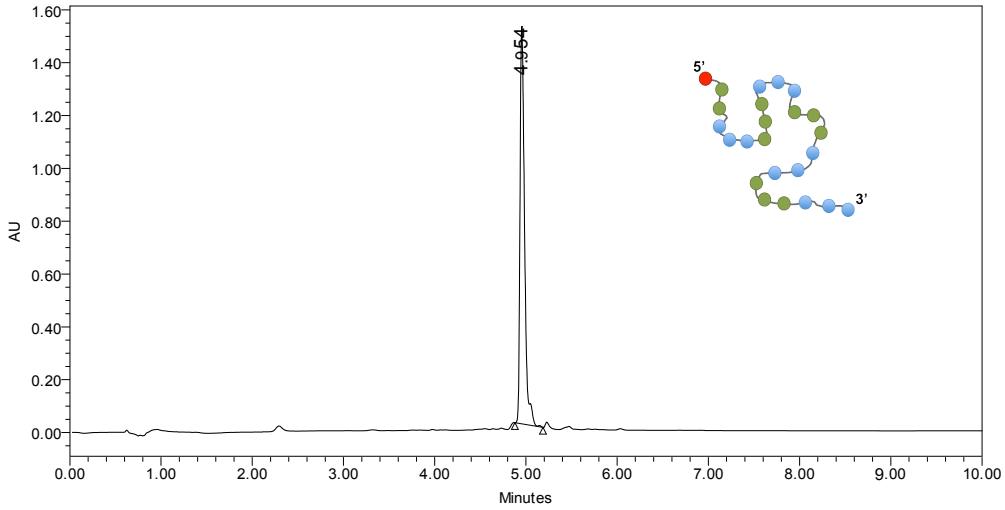


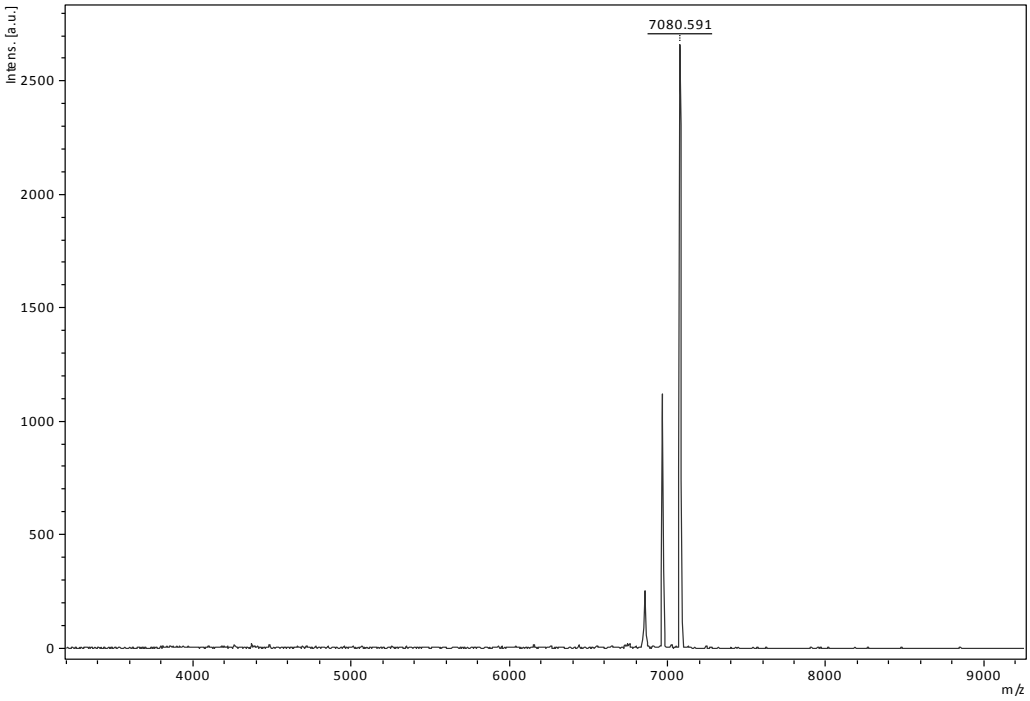
Lt_0



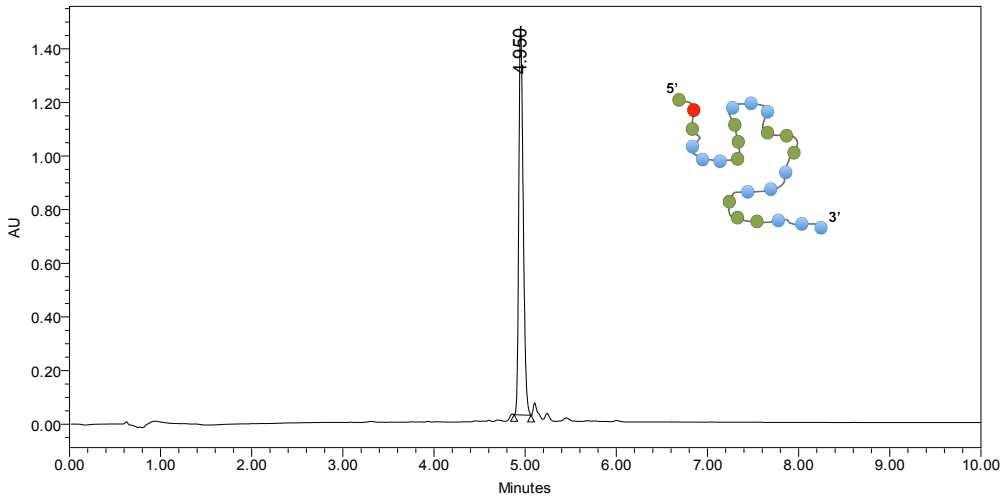


Lt_1





Lt_2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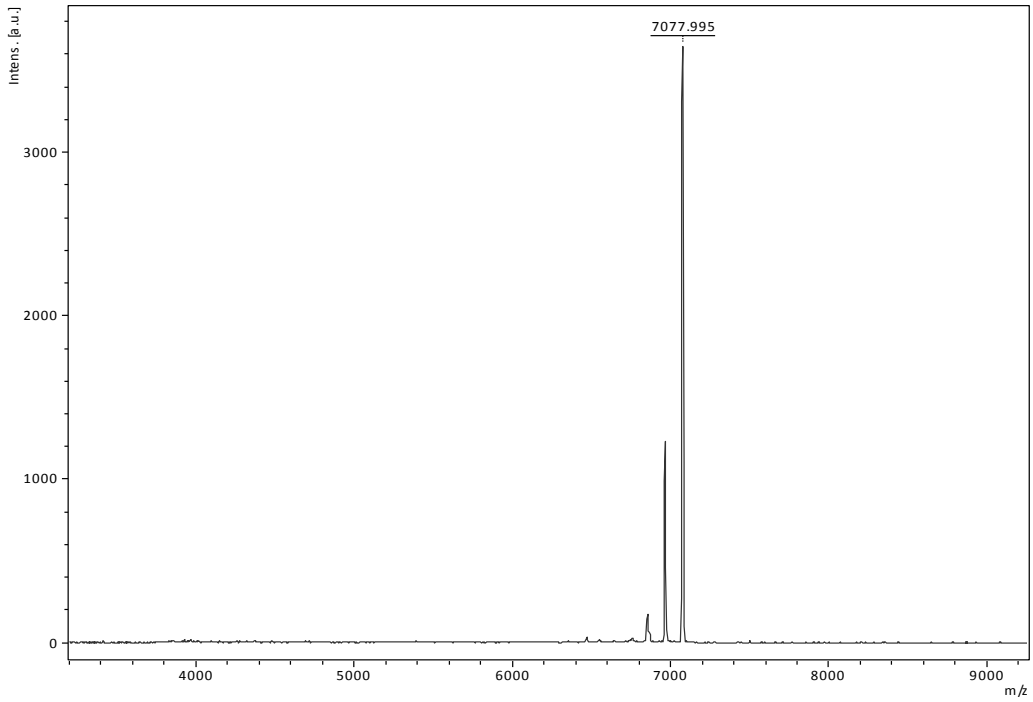
5'
3'
AU
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
Minutes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0
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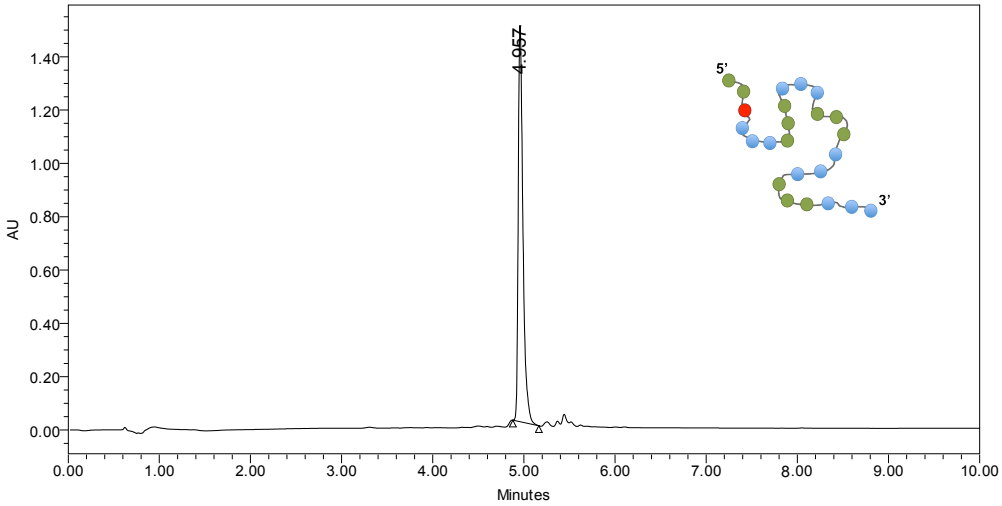
6000
7000
8000
9000
m/z

Lt_3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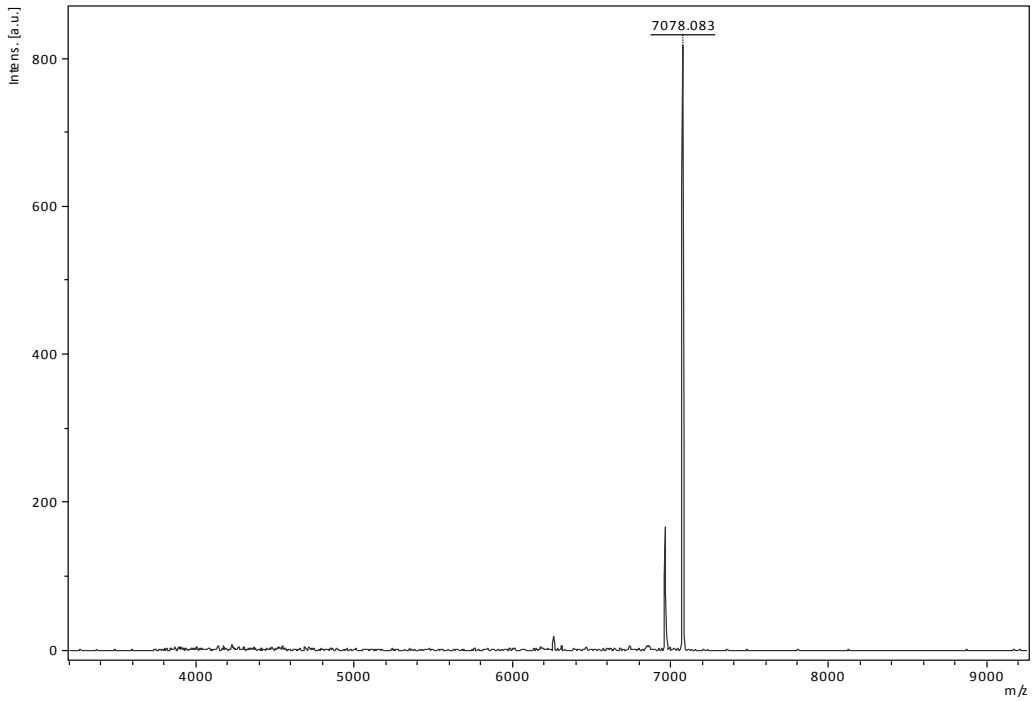
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
Minutes


7078.083
Intens. [a.u.]
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5000
6000
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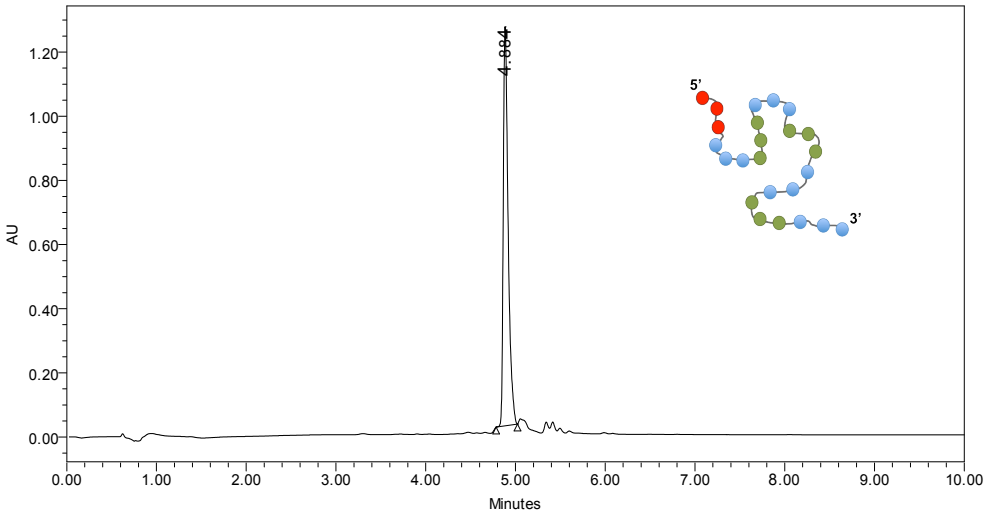
8000
9000
m/z

Lt_1/2/3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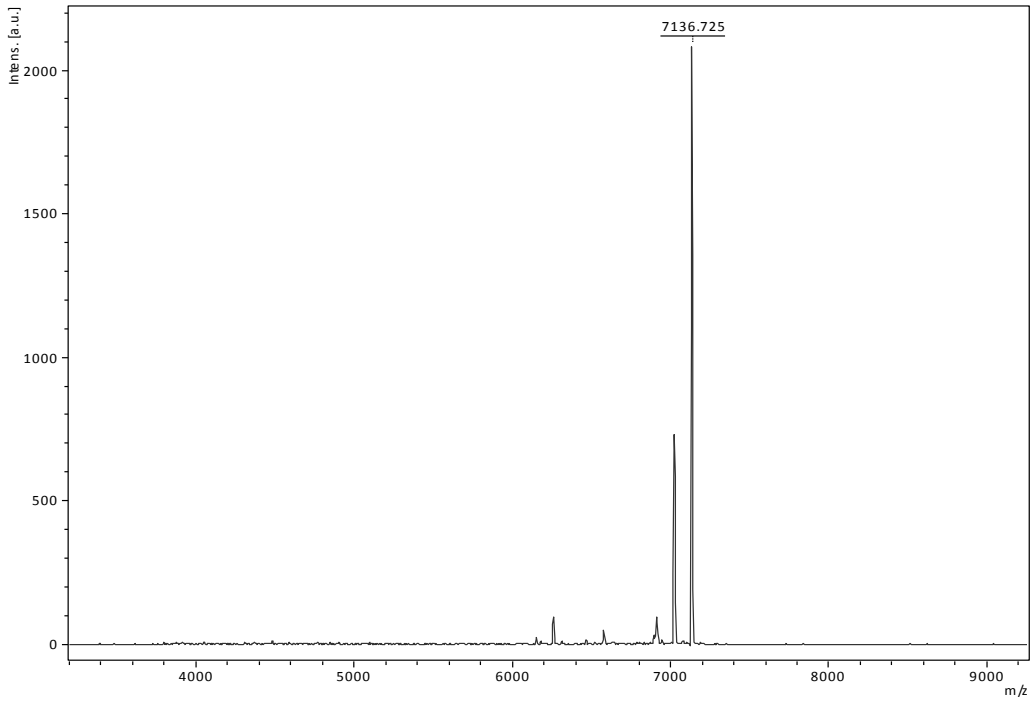
4.00
5.00
6.00
7.00
8.00
9.00
10.00
Minutes


7136.725
Intens. [a.u.]
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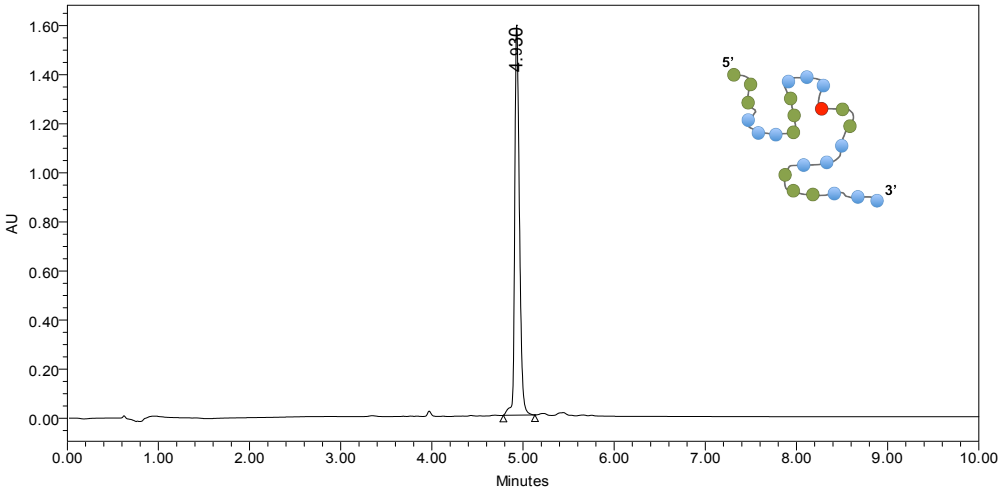
8000
9000
m/z

Lt_13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4.00
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6.00
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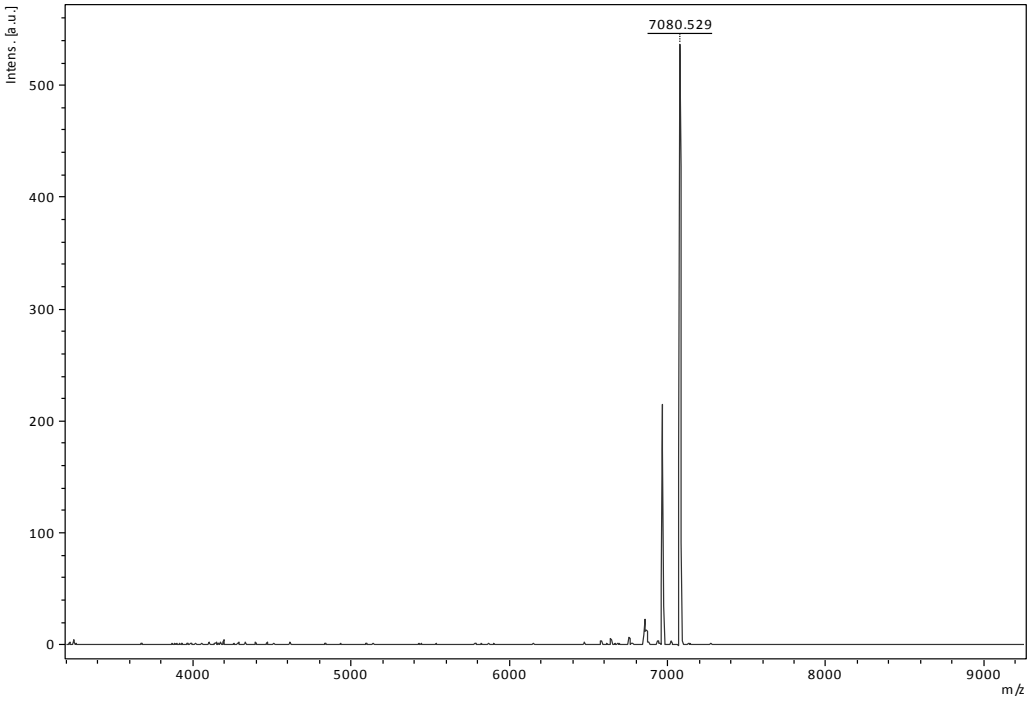
9.00
10.00


7080.529
Intens. [a.u.]
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200
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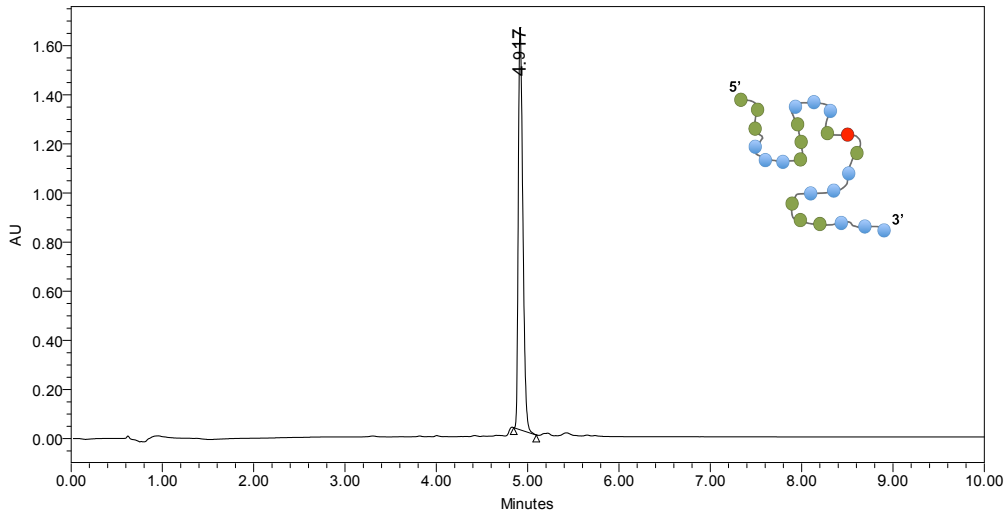
5000
6000
7000
8000
9000
m/z

Lt_14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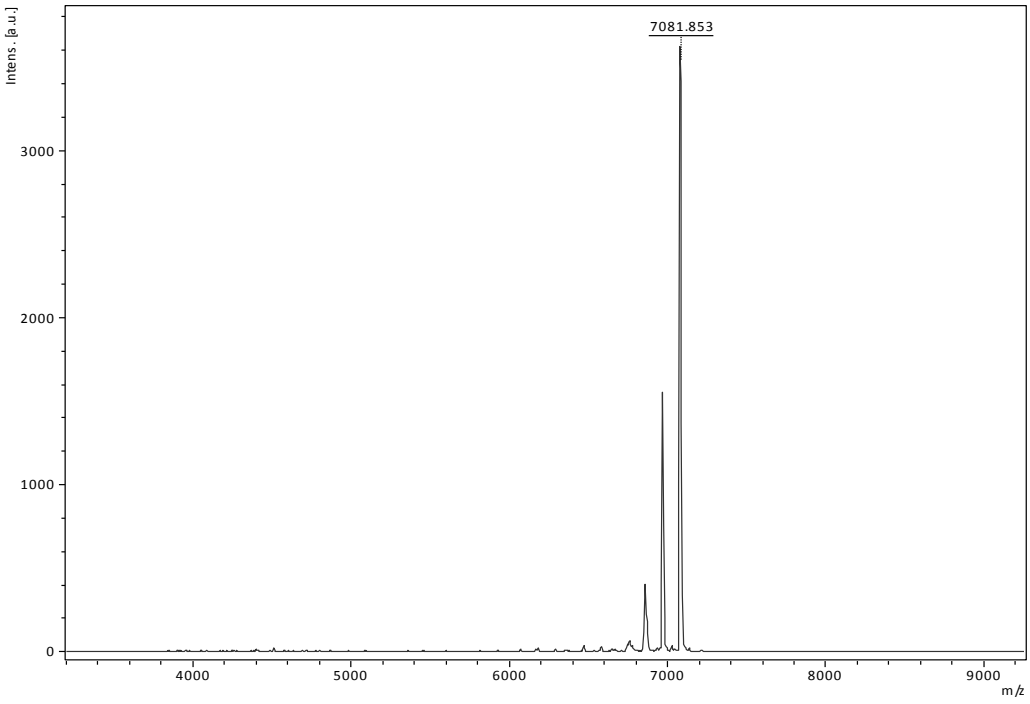
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
Minutes


7081.853
Intens. [a.u.]
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0
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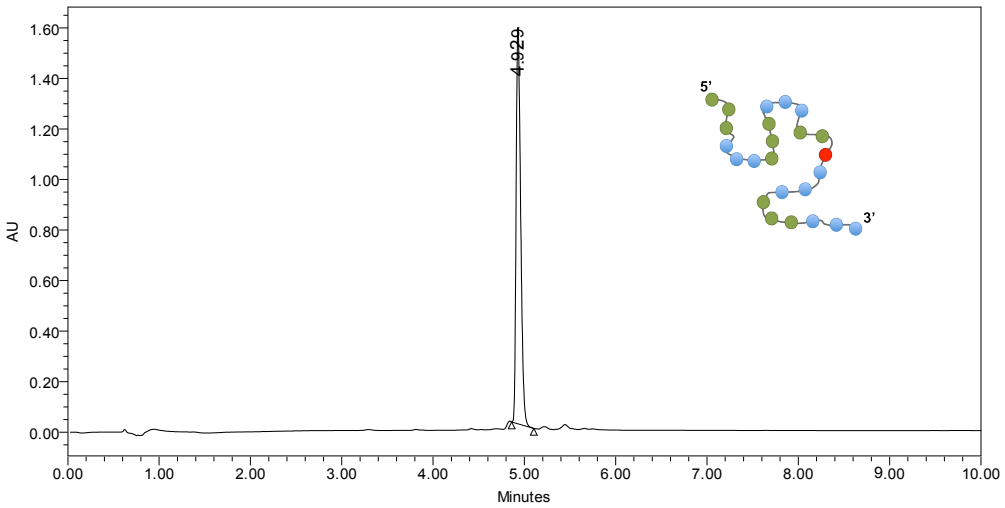
7000
8000
9000
m/z

Lt_15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2.00
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6.00
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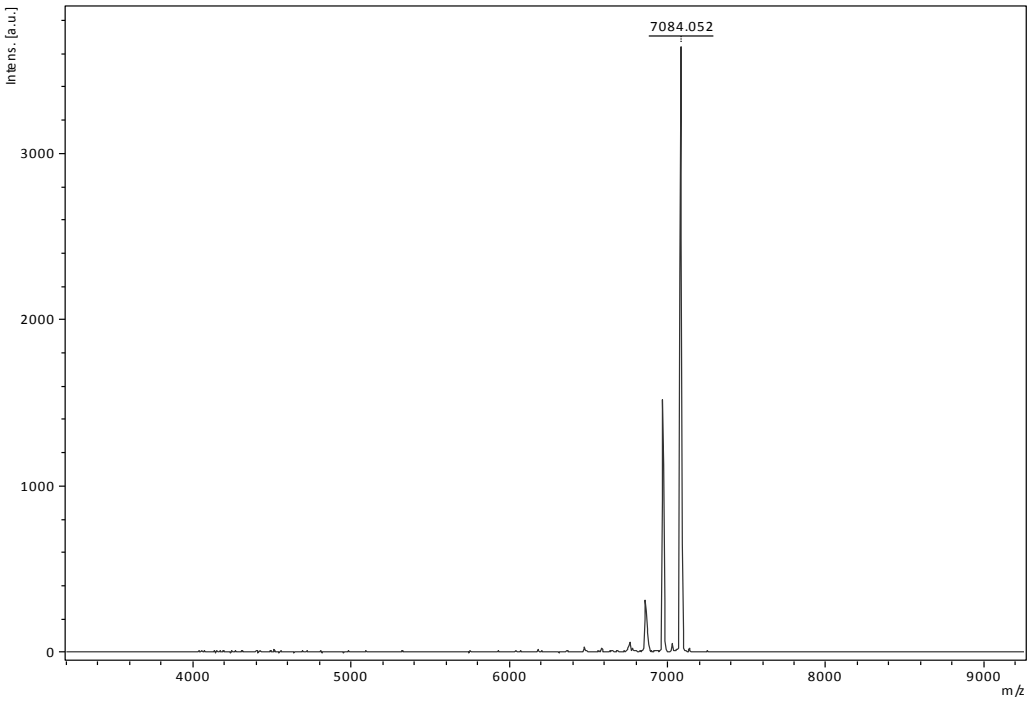
9.00
10.00


7084.052
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m/z
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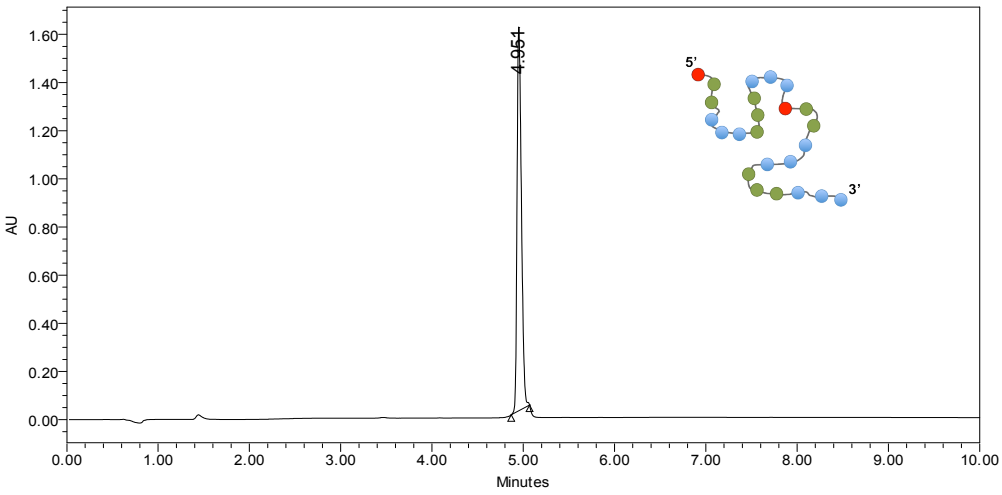
7000
8000
9000

Lt_1/13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3'
AU
Minutes
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2.00
3.00
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6.00
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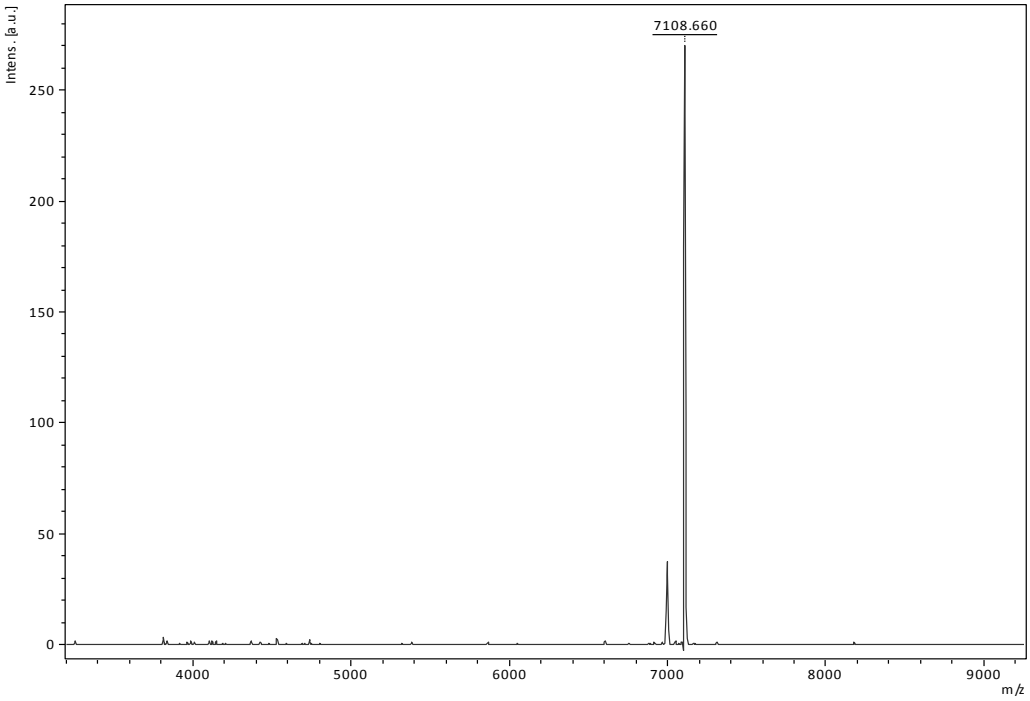
9.00
10.00


7108.660
Intens. [a.u.]
m/z
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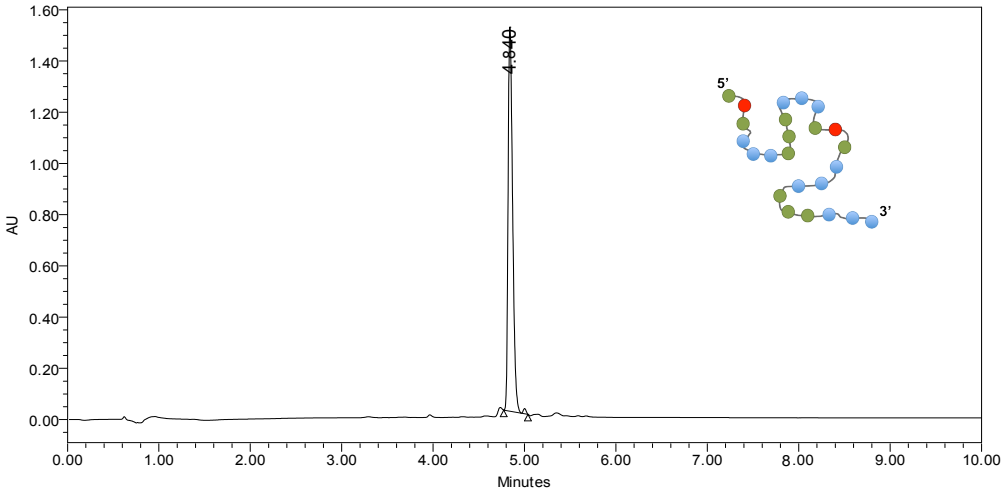
9000

Lt_2/14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3'
AU
Minutes
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0.40
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0.00
1.00
2.00
3.00
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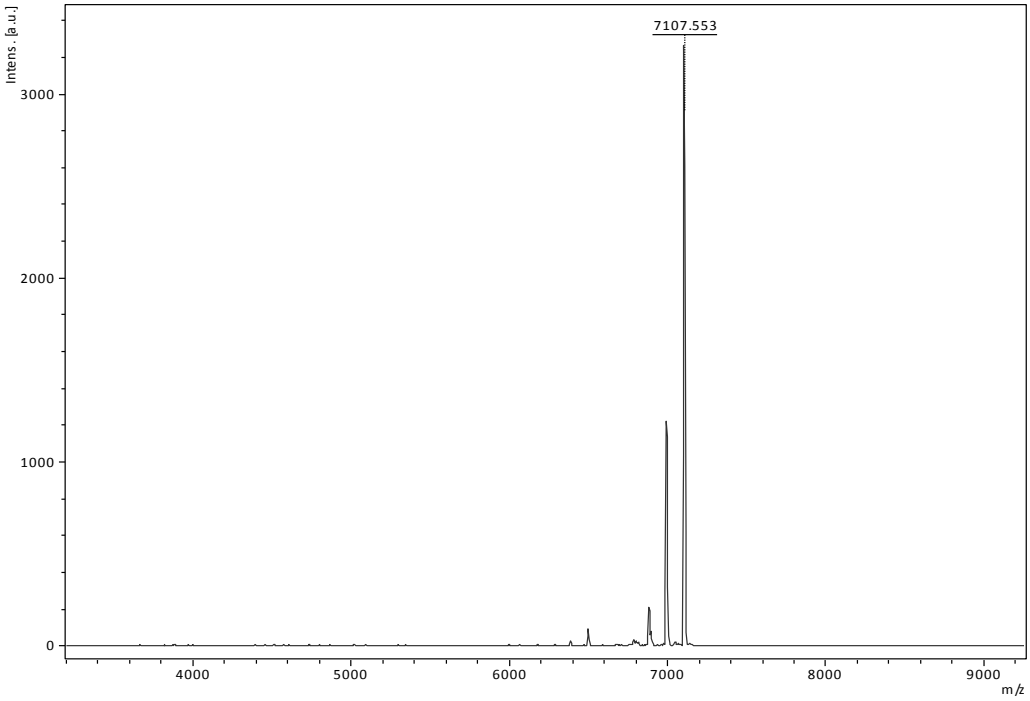
7107.553
Intens. [a.u.]
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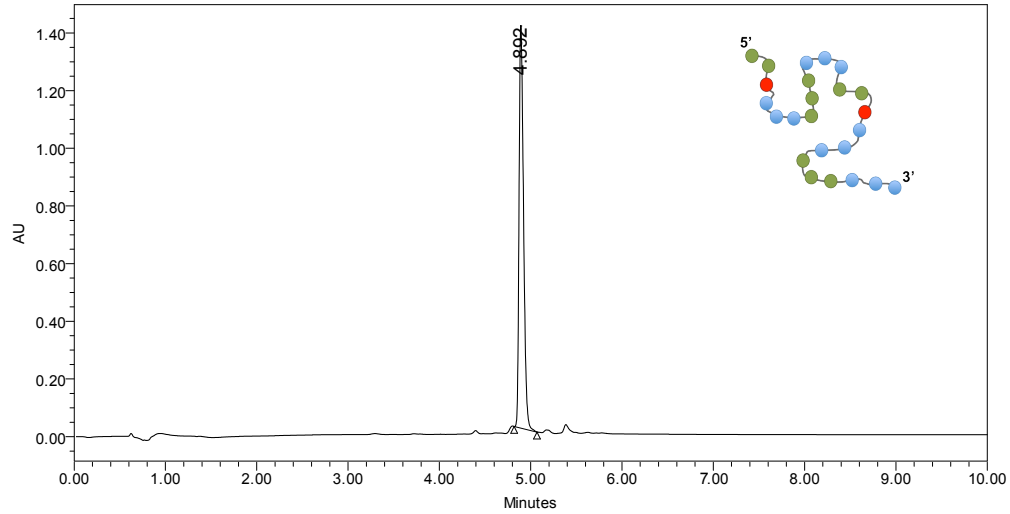
7000
8000
9000
m/z

Lt_3/15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AU
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2.00
3.00
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6.00
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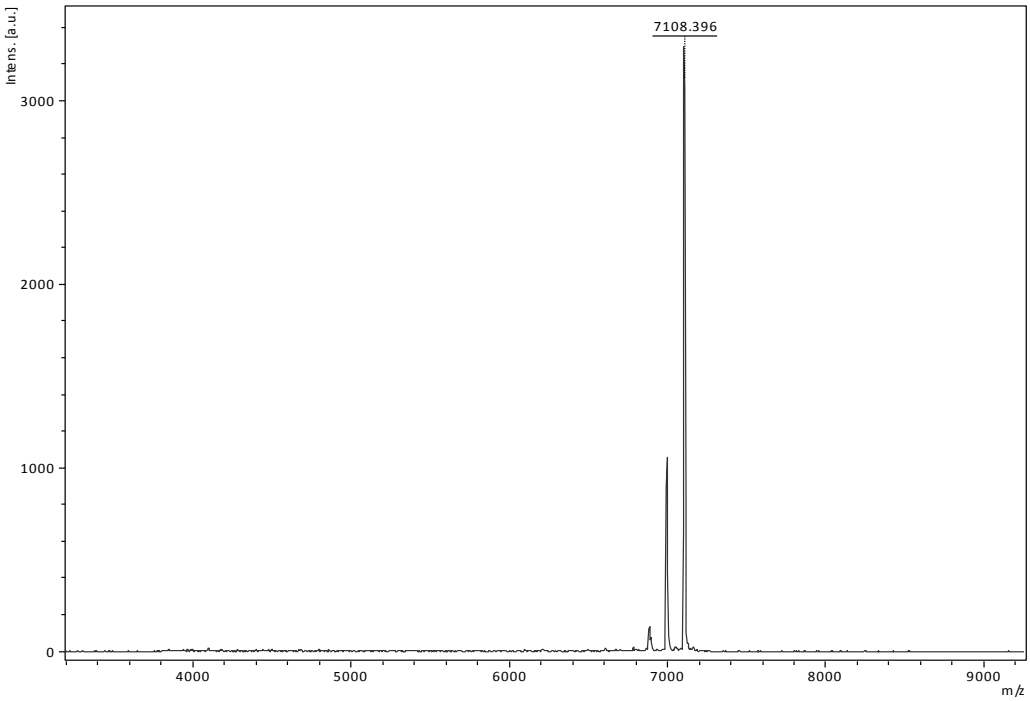
10.00


7108.396
Intens. [a.u.]
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2000
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0
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5000
6000
7000
8000
9000
m/z

Table S3. HPLC and mass spectrometry analysis (MALDI-TOF) of purified oligonucleotides.

Oligonucleotide	t_R (min.)	Mw (calcd)	Mw (found)
Lt_0	5.04	7053.8	7050.2
Lt_1	4.95	7082.8	7080.6
Lt_2	4.95	7082.8	7078.0
Lt_3	4.96	7082.8	7078.1
Lt_1/2/3	4.88	7140.8	7136.7
Lt_13	4.93	7082.8	7080.5
Lt_14	4.92	7082.8	7081.1
Lt_15	4.93	7082.8	7084.0
Lt_1/13	4.95	7111.8	7108.7
Lt_2/14	4.84	7111.8	7107.5
Lt_3/15	4.89	7111.8	7108.4