Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information

Metallophyte wastes and polymetallic catalysis: a promising combination in green chemistry. The illustrative synthesis of 5'-capped RNA

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MATERIALS AND METHODS

Carbon tetrachloride was distilled under phosphorus pentoxide. Pyridine, acetonitrile, triethylamine, butylamine and tri-*n*-butylamine were distilled under calcium hydride. Solvents and other reagents were purchased from Aldrich and used without further purifications. Analytical and semi-preparative high performance liquid chromatographies were performed on a Dionex DX 600 HPLC system or a Dionex U 3000 HPLC system equipped with anion-exchange DNAPac PA 100 columns (4 x 250 mm for analysis or 9 x 250 mm for purification, Dionex). The following HPLC solvent systems were used: 5% CH_3CN in 25 mM Tris-HCl buffer, pH 8 (buffer A) and 5% CH_3CN containing 400 mM $NaClO_4$ in 25 mM Tris-HCl buffer, pH 8 (buffer B). Flow rates were 1.5 $mL.min^{-1}$ and 5 $mL.min^{-1}$ for analysis and semi-preparative purposes, respectively. MALDI-TOF mass spectra were recorded on a Voyager-DE spectrometer (Perseptive Biosystems, USA) using a 10:1 (m/m) mixture of 2,4,6-trihydroxyacetophenone/ammonium citrate as a saturated solution in acetonitrile/water (1:1, v/v) for the matrix. Analytical samples were mixed with the matrix in a 1:5 (v/v) ratio, crystallized on a 100-well stainless steel plate and analyzed. UV quantitation of RNAs was performed on a Varian Cary 300 Bio UV/Visible spectrometer by measuring absorbance at 260 nm.

The solid-supported 5'-*H*-phosphonate **2** and 5'-phosphoroimidazolide **3** derivatives, as well as GpppRNAs were synthetized as previously described (Y. Thillier and al. *RNA* **2012**, *18*, 856-868).

Synthesis of T_6 and RNA on solid-support

Oligonucleotides T_6 and RNA synthesis was performed on an ABI 394 synthesizer (Applied Biosystems) at 1 μmol scale from commercially available (Link Technologies) long chain alkylamine controlled-pore glass (LCAA-CPG) solid support with a pore size of 1000 Å derivatized through the succinyl linker with 5'-*O*-dimethoxytrityl-[thymidine, 2'-*O*-acetyl-N^6-phenoxyacetyl adenosine or 2'-*O*-acetyl-N^2-dimethylformamide guanosine]. T_6 and RNAs sequences were assembled on a 1 μmol scale in Twist oligonucleotide synthesis columns (Glen research) using 5'-*O*-DMTr-dT-3'-*O*-(*O*-cyanoethyl-*N,N*-diisopropyl-phosphoramidite) from Pierce technologies; the PivOM amidites (5'-*O*-DMTr-2'-*O*-PivOM-[U, C^{Ac}, A^{Pac} or G^{iPrPac}]-3'-*O*-(*O*-cyanoethyl-*N,N*-diisopropyl-phosphoramidite) and the 5'-*O*-DMTr-2'-*O*-Me-A^{Pac}-3'-*O*-(*O*-cyanoethyl-*N,N*-diisopropyl-phosphoramidite) were all purchased from Chemgenes. The PrOM amidites (5'-*O*-DMTr-2'-*O*-PrOM-[U, A^{Pac}]-3'-*O*-(*O*-cyanoethyl-*N,N*-diisopropyl-phosphoramidite) were prepared according to an unpublished procedure. Phosphoramidites were vacuum dried prior to their dissolution in extra dry acetonitrile

(Biosolve) at 0.1 M. For the coupling reaction, the activator was 5-benzylmercaptotetrazole (BMT, Chemgenes) used at 0.3 M concentration. Dichloroacetic acid (3% in CH_2Cl_2) (Glen research) was the detritylation reagent. The capping step was performed with a mixture of 5% phenoxyacetic anhydride (Pac_2O) in THF and 10% *N*-methylimidazole in THF (Link Technologies). The oxidizing solution was 0.1 M iodine in THF/pyridine/H_2O (78:20:2; v/v/v) (Link Technologies). After RNA assembly completion, the column was removed from the synthesizer and dried under a stream of argon.

Capping reaction with GDP

Guanosine-5'-diphosphate sodium salt (0.5 g, 1.2 mmol) was purchased from Jena Bioscience and before use it was converted into its tri-*n*-butylammonium salt by passing through a glass column filled with 20 mL of wet DOWEX-50W X 8 resin, H^+ form. It was collected in a 250 mL round flask containing absolute ethanol (12 mL) and tri-*n*-butylamine (0.72 mL), stirred at 0°C. The Dowex column was rinsed with water (80 mL) to reach pH 5 to 6. The solvents were evaporated from the collected solution and the residue was coevaporated four times with absolute ethanol then was lyophilized from water to afford a white hygroscopic powder (0.938 g, 1.15 mmol, 96%). The desired GDP tri-*n*-butylammonium salt was characterized by two doublets at -10.55 and -11.20 ppm (^{31}P-NMR, 121 MHz, D_2O). It can be stored as a solid for several weeks at -20°C.

In a dry 2 mL microcentifuge tube (Costar), bis (tri-*n*-butylammonium) guanosine diphosphate (103 mg, 0.14 mmol) and the freshly dehydrated catalytic extracts required for the coupling (0.2 mmol) whose the amount was estimated from the concentration of the metal (Zn or Ni) determined by ICP-MS (Table 1) were mixed in anhydrous DMF (0.5 mL) to get a final concentration of 0.4 M in the capping solution. The tube was closed and the mixture was vortexed for 5 minutes on a Top-Mix 1118 (Fischerbrand) and centrifuged in a tabletop centrifuge (Sigma 1-13) at 6000 min^{-1} for 30 seconds. This was repeated twice. Then the supernatant was taken using a glass syringe filled with 3 beads of 4Å molecular sieves. Using another syringe, the solution was applied to the column containing the solid-supported 5'-phosphoroimidazolidate oligonucleotide (1 μmol), and left to react for 18 h at 30°C. The solution was removed and the support was washed with water (2 x 2 mL), a 0.1 M aqueous solution of EDTA (pH 7, 2 x 2 mL), and dry CH_3CN (4 x 2 mL). Finally the column was dried by blowing argon through it during 1 min.

Deprotection and release of $GpppT_6$ (1-12) and GpppRNAs (13, 15, 16, 18, 19, 21)

The solid-supported $GpppT_6$ and GpppRNAs were deprotected and released from the support as follows: firstly, a 1 M solution of 1,8-diazadicyclo-[5,4,0]undec-7-ene (DBU) in anhydrous CH_3CN was applied to the column for 3 min. Then the solution was removed and the solid-support was washed with anhydrous CH_3CN. The support was dried by a 1 min flush with argon. Secondly, a 30 % aqueous ammonia solution was applied to the column in three batches (1.5 mL, 1mL, 0.5 mL) for 30 min each. The three ammonia fractions were collected in a 4 mL screw-capped glass vial and were left to react at room temperature for 1.5 h. The fully deprotected oligonucleotides were transferred to 50 mL round-bottomed flasks and isopropylamine (15% of total volume: 0.45 mL) was added only to the solutions of GpppRNAs. Then the mixtures were evaporated under reduced pressure with a bath at 30°C maximum until the volumes were reduced to 0.3 mL. The mixtures were coevaporated three times with 1 mL of water following the same protocol. The residues were redissolved in water (1.5 mL divided in three portions for flask rinse: 0.8 mL, 0.4 mL, 0.3 mL) and transferred to 2 mL Eppendorf-vials then lyophilized from water.

Analytical and purification of $GpppT_6$ (1-12) and GpppRNAs (15, 18, 21)

The crude $GpppT_6$ (**1-12**) and GpppRNAs 6-mers (**15**, **18**, **21**) were analyzed on a Dionex DX 600 HPLC system monitored at 260 nm with a 0%-30% linear gradient of buffer B in buffer A. Only GpppRNAs (**15**, **18**, **21**) were purified using a 0%-30% linear gradient of buffer B in buffer A. The fractions containing the pure 5'-capped oligonucleotides were pooled in a 100 mL round-bottomed flask and were concentrated to a volume of 0.5 mL under reduced pressure with a bath at 30°C. Then the pure GpppRNAs were ready for the desalting procedure.

Sephadex procedure for crude $GpppT_6$ (5, 9)

The crude $GpppT_6$ **5** and **9** were dissolved in 1 mL of water, and were loaded on a NAP-10 Column (Sephadex G-25 DNA grade, GE healthcare). Oligonucleotides were eluted in a 2 mL Eppendorf-vial with 1.5 mL of water then lyophilized.

Capping reaction with ^{7m}GDP

7-Methylguanosine-5'-diphosphate sodium salt (0.25 g, 0.5 mmol) was purchased from Jena Bioscience and before use, it was converted into its tri-*n*-butylammonium following the same procedure as above using 15 mL of wet DOWEX-50W X 8 resin, H^+ form ; 10 mL of absolute ethanol and tri-*n*-butylamine (0.3 mL, 1.26 mmol). The solvents were evaporated from the collected solution and the residue was coevaporated four times with absolute ethanol then

was lyophilized from water to afford a white hygroscopic powder (0.353 g, 0.47 mmol, 94 %). The desired ^{7m}GDP, tri-*n*-butylammonium salt was characterized by two doublets at -9.98 and -11.13 ppm (^{31}P-NMR, 121 MHz, D_2O). It can be stored as a solid for several days at -20°C.

The capping reaction with bis (tri-*n*-butylammonium)-^{7m}GDP was performed as previously described. A two-fold decrease in the quantities of reagents used was applied in this case. In a 2 mL Eppendorf vial, bis (tri-*n*-butylammonium)-^{7m}GDP (50 mg, 0.07 mmol) and the correct amount of freshly dehydrated catalytic extract were mixed in anhydrous DMF (0.25 mL). The tube was closed and the mixture was vortexed for 5 minutes and centrifuged in a tabletop centrifuge at 6000 min^{-1} for 30 seconds. This was repeated twice. Then the supernatant was taken using a 1 mL plastic syringe filled with a bead of 4Å molecular sieves. The solution was applied to the column containing the solid-supported 5'-phosphoroimidazolidate oligonucleotide and pushed back and forth for 5 minutes then left to react for 18 h at 30°C. The solution was removed and the support was washed with water (2 x 2 mL), a 0.1 M aqueous solution of EDTA (pH 7, 2 x 2 mL), and dry CH_3CN (4 x 2 mL). Finally the column was dried by blowing argon through it during 1 min.

Deprotection and release of ^{7m}GpppRNAs (14, 17, 20)

The solid-supported ^{7m}GpppRNAs were deprotected and released from the support as follows: it was first treated with a 1M solution of 1,8-diazadicyclo-[5,4,0]undec-7-ene (DBU) in anhydrous CH_3CN for 3 min. Then the solution was removed and the solid-support was washed with anhydrous CH_3CN. The support was reverse-flushed by a 1 min flush with argon and dried under vacuum over P_2O_5 for 2 hours.

In a dry 10 mL round-bottomed flask, 2.7 mL of dry butylamine was added in 1.3 mL of dry THF under argon. Then the Twist oligonucleotide synthesis column was opened and a half quantity of the solid-support was transferred in another column. 2 mL of the butylamine / THF (2:1) solution was applied to each column using two glass syringes filled of 4Å molecular sieves (5 beads each). The solution was pushed back and forth through the synthesis column for 2 min and left to react 4 hours at 30°C.

The deprotecting solution was removed by deconnecting the syringes from the column without moving the plunger. The column was rinsed with 1 mL of dry THF followed by a gently 1 min flush with argon. Both columns were eluted with 2 mL of 25 mM TEAB in the same 100 mL flask. Then the mixtures were evaporated under reduced pressure at 30°C

maximum until the volumes were reduced to 0.3 mL. The residues were redissolved in water (1.5 mL divided in three portions for flask rinse: 0.8 mL, 0.4 mL, 0.3 mL) and transferred to 2 mL Eppendorf-vials then lyophilized from water.

Analytical and purification of GpppRNAs (13, 16, 19) and ^{7m}GpppRNAs (14, 17, 20)

The crude GpppRNAs (**13, 14**, **16, 17**, **19, 20**) were analyzed and purified on a Dionex U 3000 HPLC system monitored at 260 nm and 300 nm with the columns kept at 50°C in a thermostated column compartment. Analytical IEX-HPLC was performed using a DNAPac PA-200 column (4 X 250 mm, Dionex). GpppRNAs (**13, 14**, **16, 17**, **19, 20**) were eluted using a 0%-50% or 0%-75% linear gradient of buffer B in buffer A, flow rates were 1.2 mL.min^{-1}. The crude mixtures were purified by semi-preparative HPLC with a 0%-30% or 0%-75% linear gradient of buffer B in buffer A. The fractions containing the pure GpppRNAs were pooled in a 100 mL round-bottomed flask and were concentrated to a volume of 0.5 mL under reduced pressure with a bath at 30°C. Then the pure GpppRNAs were ready for the desalting procedure.

Desalting procedure for pure GpppRNAs and ^{7m}GpppRNAs

Next to evaporation, the purified materials were redissolved in TEAB 100 mM (6 mL divided in three portions for flask rinse: 3 mL, 2 mL, 1mL) and were loaded on a Sep Pak C_{18} cartridge. Elution was performed with 10 mL of 100 mM TEAB, pH 7 then with 10 mL of 50% CH_3CN in 12.5 mM TEAB, pH 7. The second fraction containing the desired 5'-capped RNA was collected in a 100 mL round-bottomed flask and was freeze-dried. The residue was dissolved in 1.5 mL water (divided in 3 portions of 0.8 mL, 0.4 mL, 0.3 mL for flask rinse) and transferred to a 2 mL Eppendorf-vial and lyophilized from water. Lyophilized GpppRNAs and ^{7m}Gppp-RNAs were stored at -20°C for several months without any degradation.

Table: Synthesized 5'-GpppT_6 and 5'-GpppRNAs

ON	Catalysts[a]	5'-sequence-3'	Molecular Formula	Calc. m/z[b]	Found m/z[b]
1	**$ZnCl_2$**	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	2267.29
2	**$NiCl_2$**	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	2267.61
	***N. caerulescens* extract**				
3	crude	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	2267.34
4	purified (f1)	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	-
5	purified (f3) [c]	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	2267.25
6	purified (f3)	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	2267.65
	***N. caerulescens* + *A. vulneraria* extract**				
7	crude	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	2267.57
	***P. douarrei* extract**				
8	crude	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	2267.36
9	purified[c]	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	2267.80
	***G. pruinosa* extract**				
10	crude	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	2267.34
11	purified	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	2267.29
	***P. accuminata* extract**				
12	crude	GpppT$_6$	$C_{70}H_{93}N_{17}O_{57}P_8$	2267.35	2267.19
13	**$ZnCl_2$**	GpppAGUUGUUAGUCUUACUGG	$C_{151}H_{225}N_{70}O_{141}P_{20}$	6254.57	6254.86
14	**$ZnCl_2$**	GpppAUAUUA	$C_{67}H_{83}N_{26}O_{53}P_8$	2348.31	2348.55
15	**$ZnCl_2$**	^{7m}GpppAUAUUA	$C_{68}H_{85}N_{26}O_{53}P_8$	2362.34	2362.67
	***N. caerulescens* extract**				
16	purified (f3)	GpppA$_{OMe}$GUUGUUAGUCUUACUGGA	$C_{192}H_{239}N_{75}O_{145}P_{21}$	6597.80	6597.45
17	purified (f3)	GpppAUAUUA	$C_{67}H_{83}N_{26}O_{53}P_8$	2348.31	2348.15
18	purified (f3) [d]	^{7m}GpppAUAUUA	$C_{68}H_{85}N_{26}O_{53}P_8$	2362.34	2362.59
	***P. douarrei* extract**				
19	purified	GpppA$_{OMe}$GUUGUUAGUCUUACUGGA	$C_{192}H_{239}N_{75}O_{145}P_{21}$	6597.80	6597.03
20	purified	^{7m}GpppAUAUUA	$C_{68}H_{85}N_{26}O_{53}P_8$	2362.34	2362.36
21	purified	GpppAUAUUA	$C_{67}H_{83}N_{26}O_{53}P_8$	2348.31	2348.32

[a] **[Zn]=[Ni]= 0.4µm**
[b] MALDI-TOF characterization in negative mode
[c] The catalyst-GDP solution was not centrifuged, but final mixture was eluted on Sephadex
[d] The catalyst was treated by the system Zn/dioxan to eliminate traces of HCl and to prevent depurination

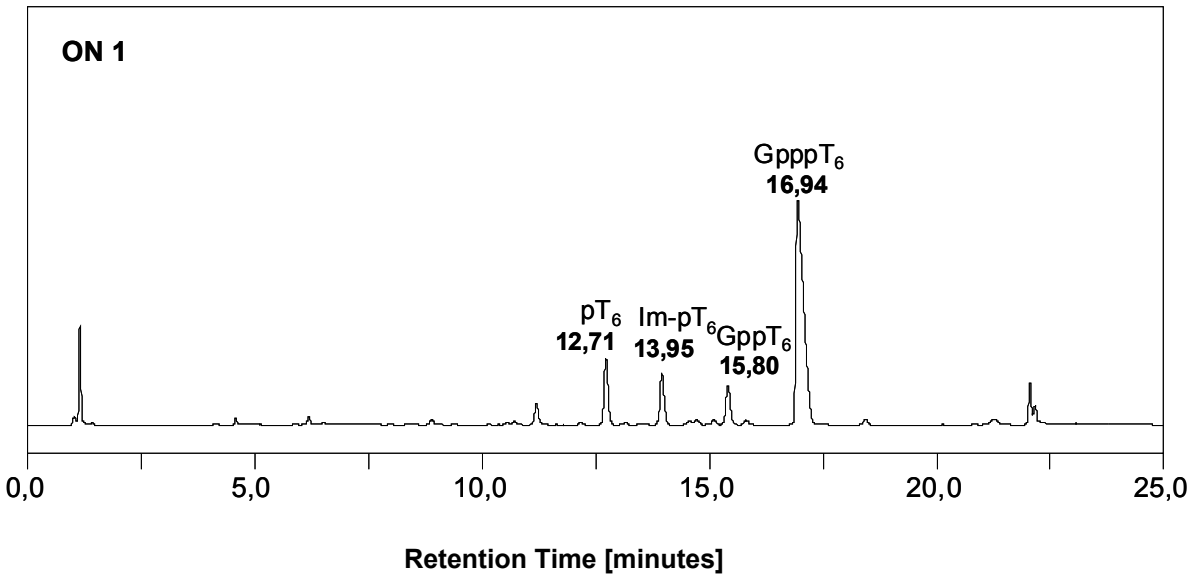

Figure S1: IEX-HPLC analysis of **crude ON 1**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 $mL.min^{-1}$, λ 260 nm.

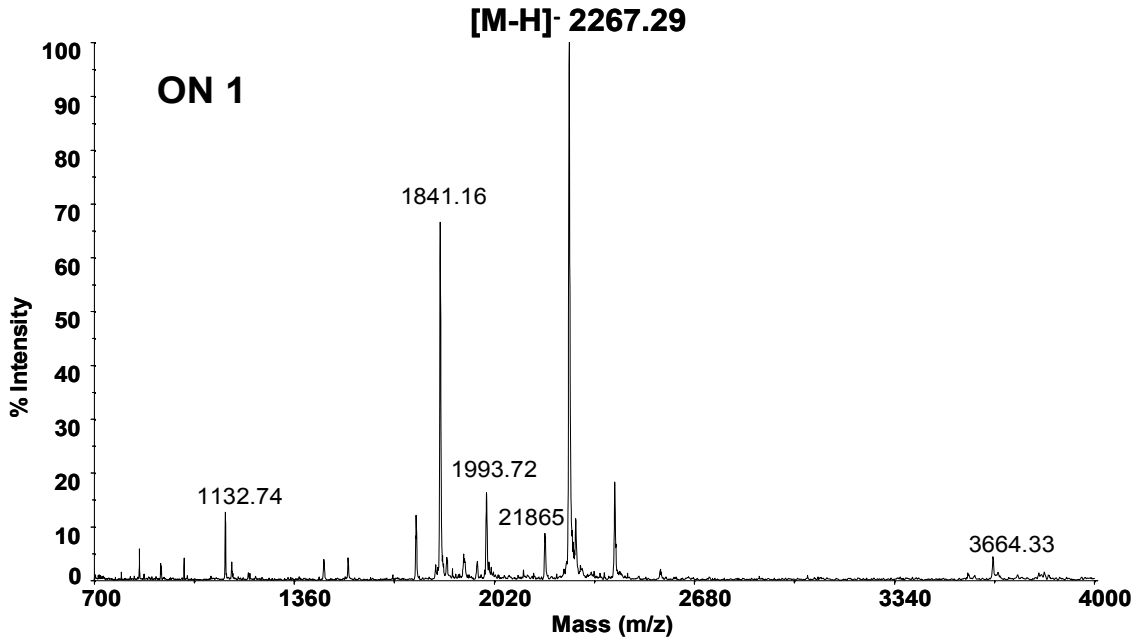

Figure S2: MALDI-TOF MS analysis of **crude ON 1**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

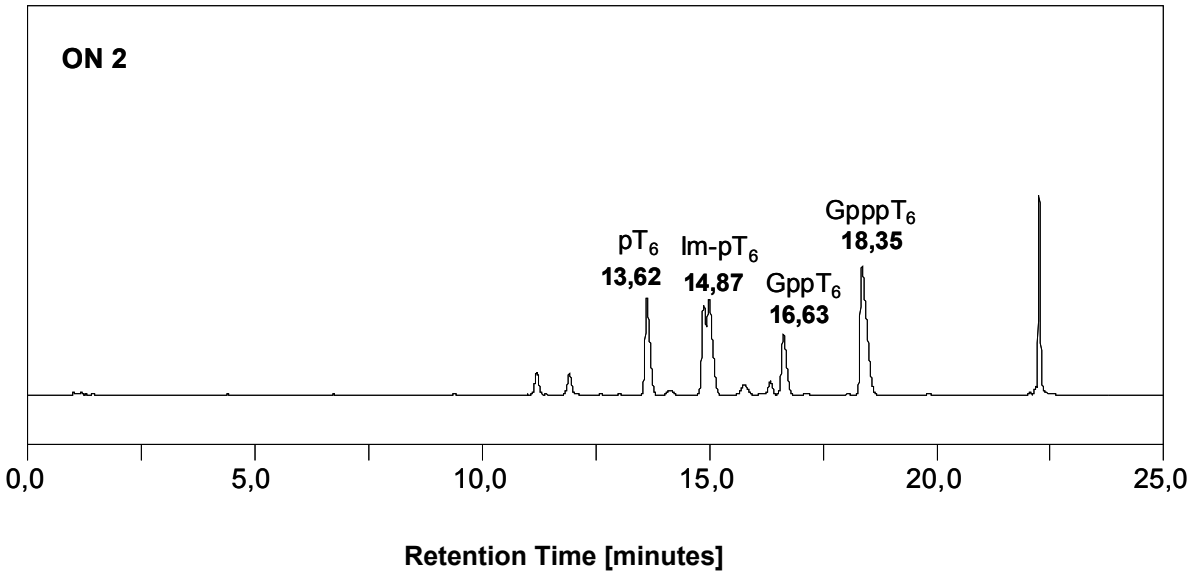

Figure S3: IEX-HPLC analysis of **crude ON 2**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 $mL.min^{-1}$, λ 260 nm.

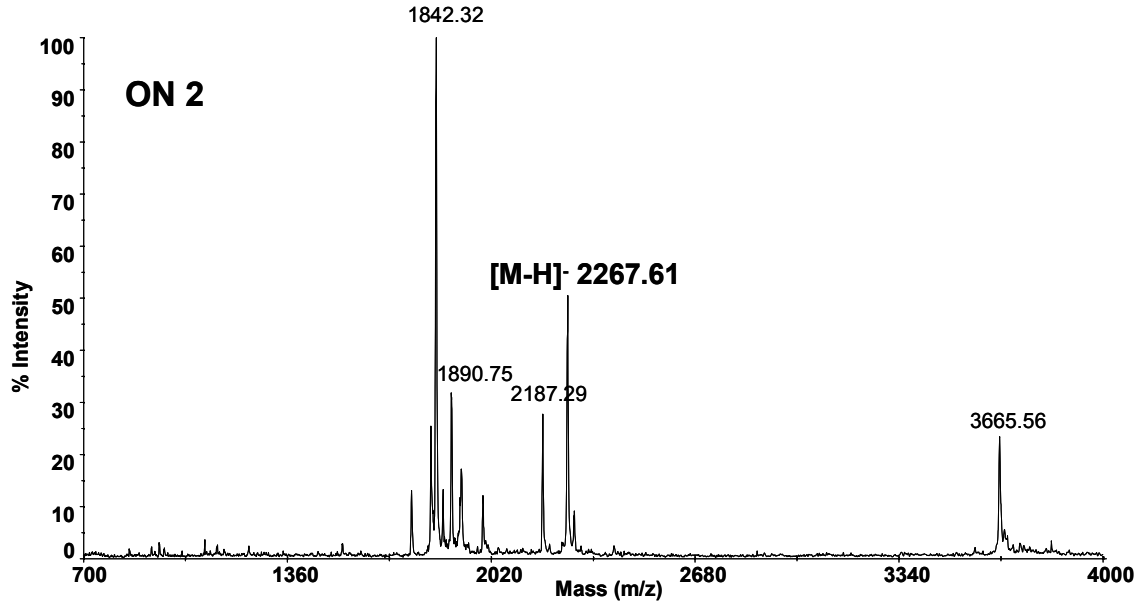

Figure S4: MALDI-TOF MS analysis of **crude ON 2**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

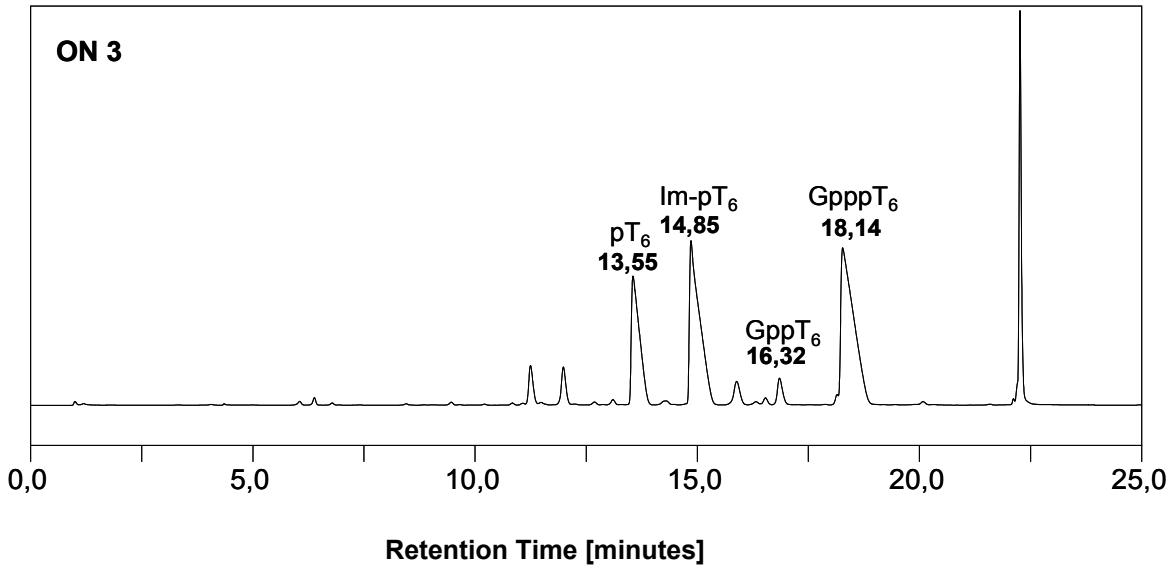

Figure S5: IEX-HPLC analysis of **crude ON 3**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 mL.min^{-1}, λ 260 nm.

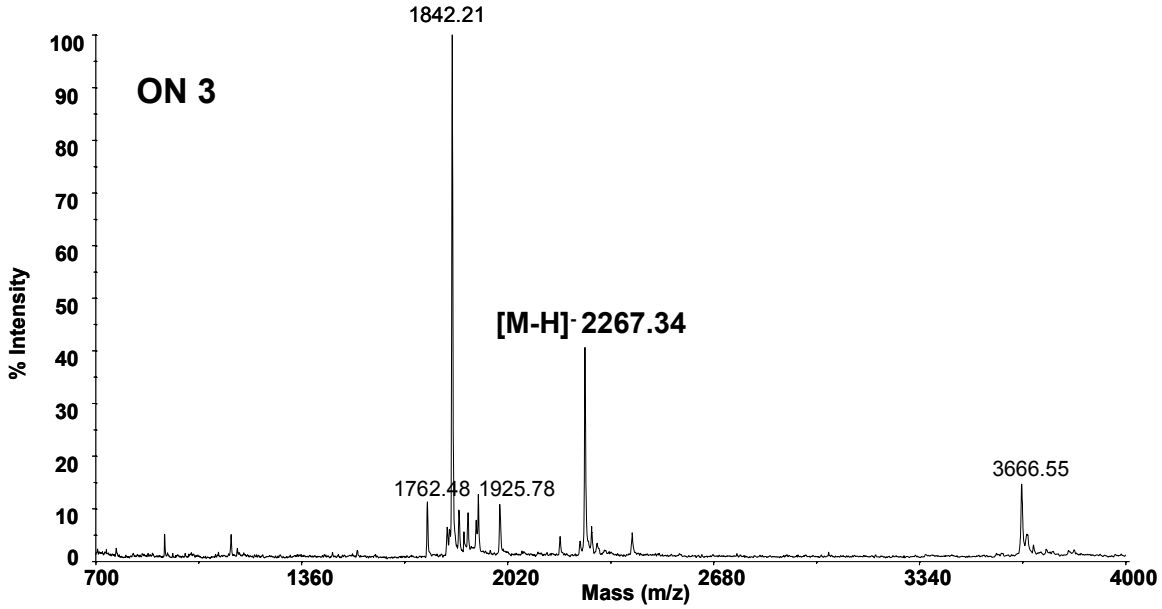

Figure S6: MALDI-TOF MS analysis of **crude ON 3**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

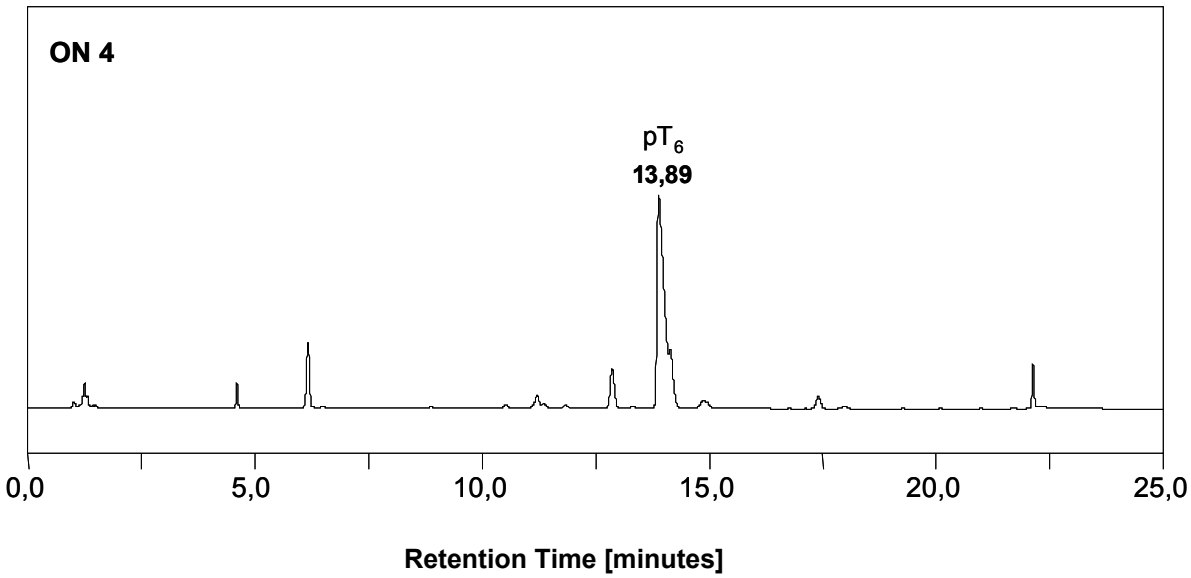

Figure S7: IEX-HPLC analysis of **crude ON 4**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 $mL.min^{-1}$, λ 260 nm.

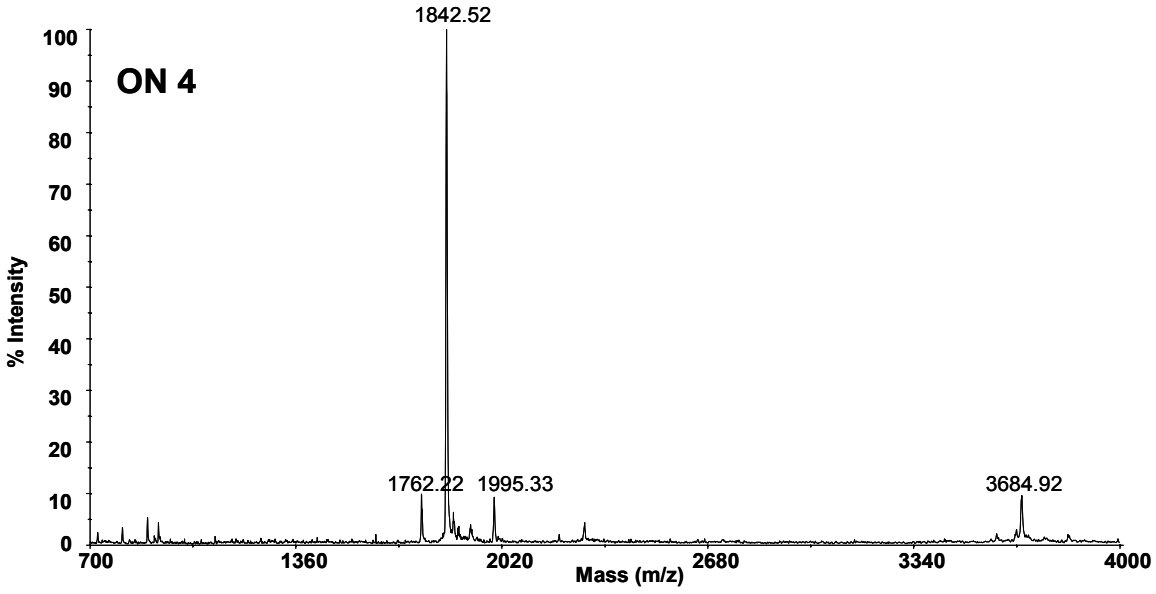

Figure S8: MALDI-TOF MS analysis of **crude ON 4**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

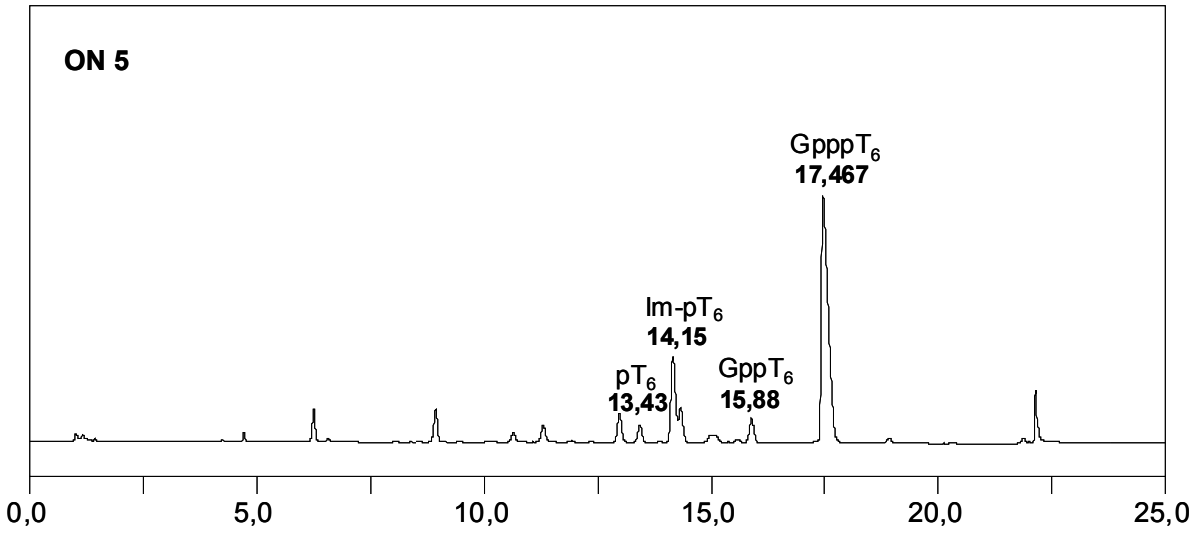

Figure S9: IEX-HPLC analysis of **crude ON 5**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 mL.min^{-1}, λ 260 nm.

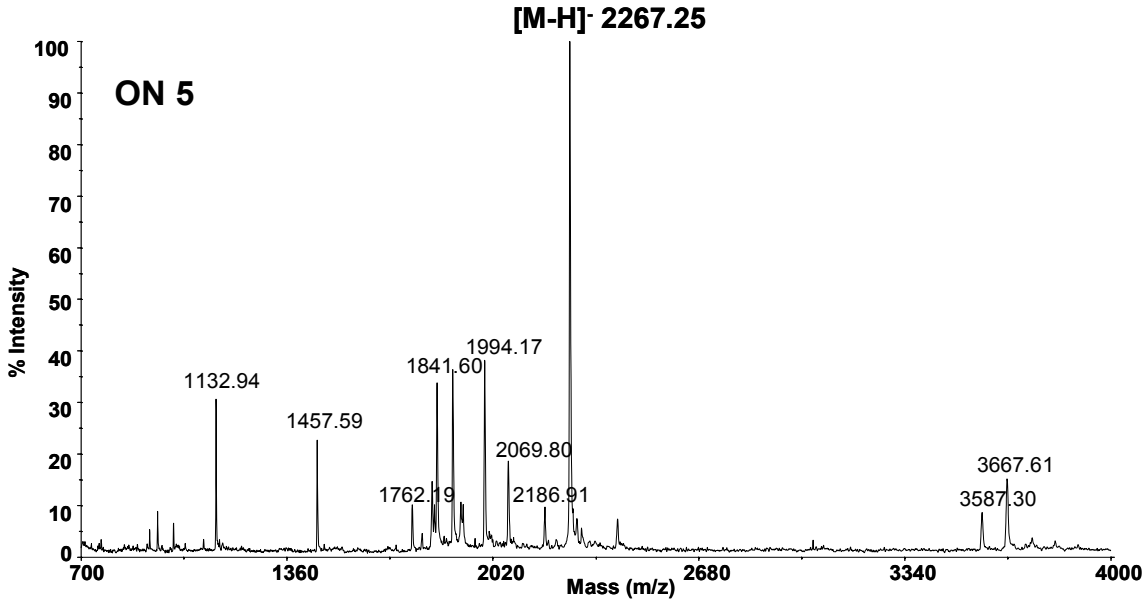

Figure S10: MALDI-TOF MS analysis of **crude ON 5**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

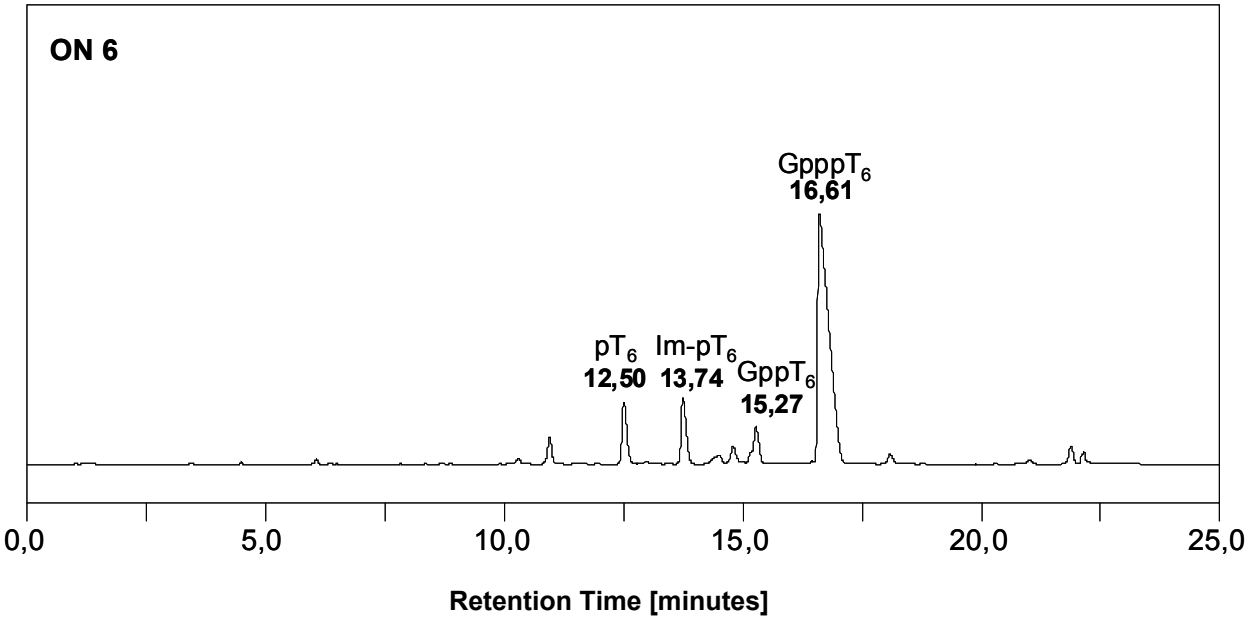

Figure S11: IEX-HPLC analysis of **crude ON 6**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 mL.min^{-1}, λ 260 nm.

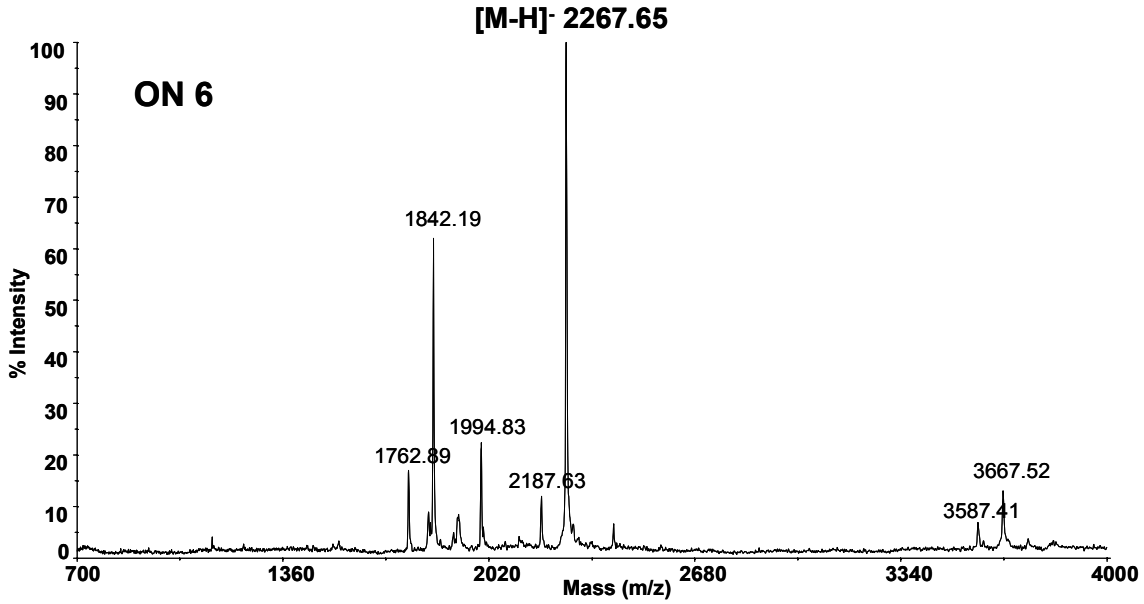

Figure S12: MALDI-TOF MS analysis of **crude ON 6**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

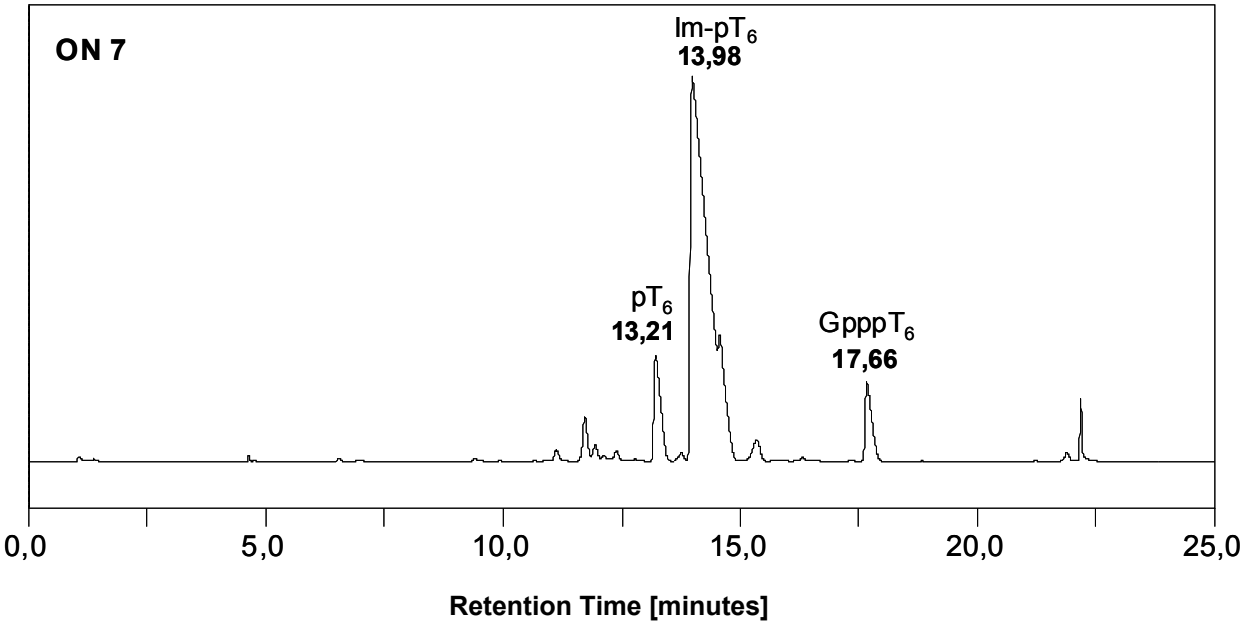

Figure S13: IEX-HPLC analysis of **crude ON 7**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 $mL.min^{-1}$, λ 260 nm.

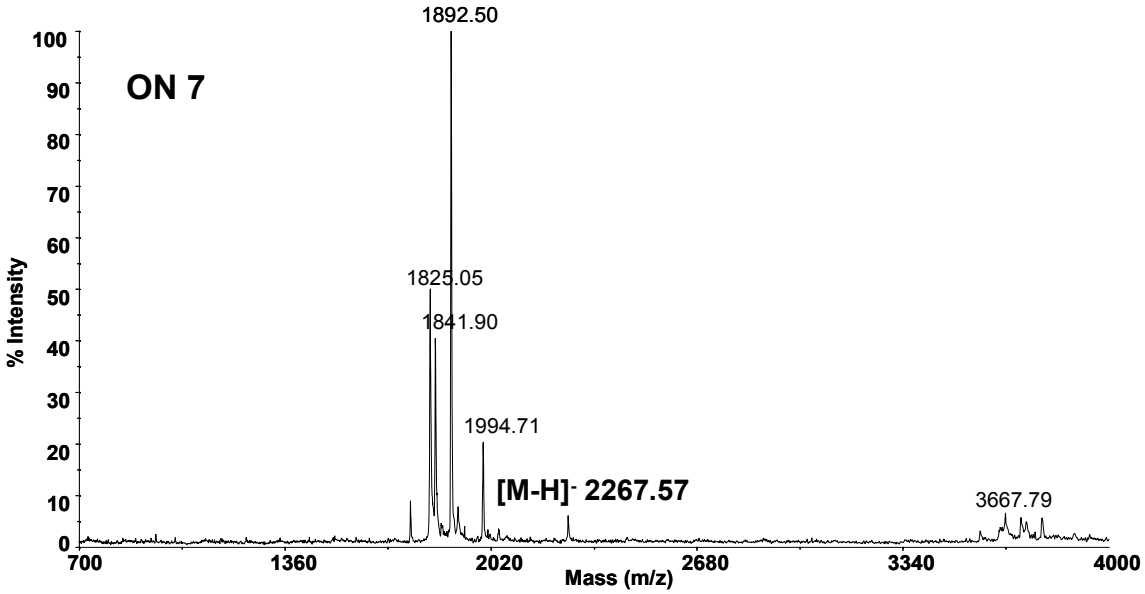

Figure S14: MALDI-TOF MS analysis of **crude ON 7**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

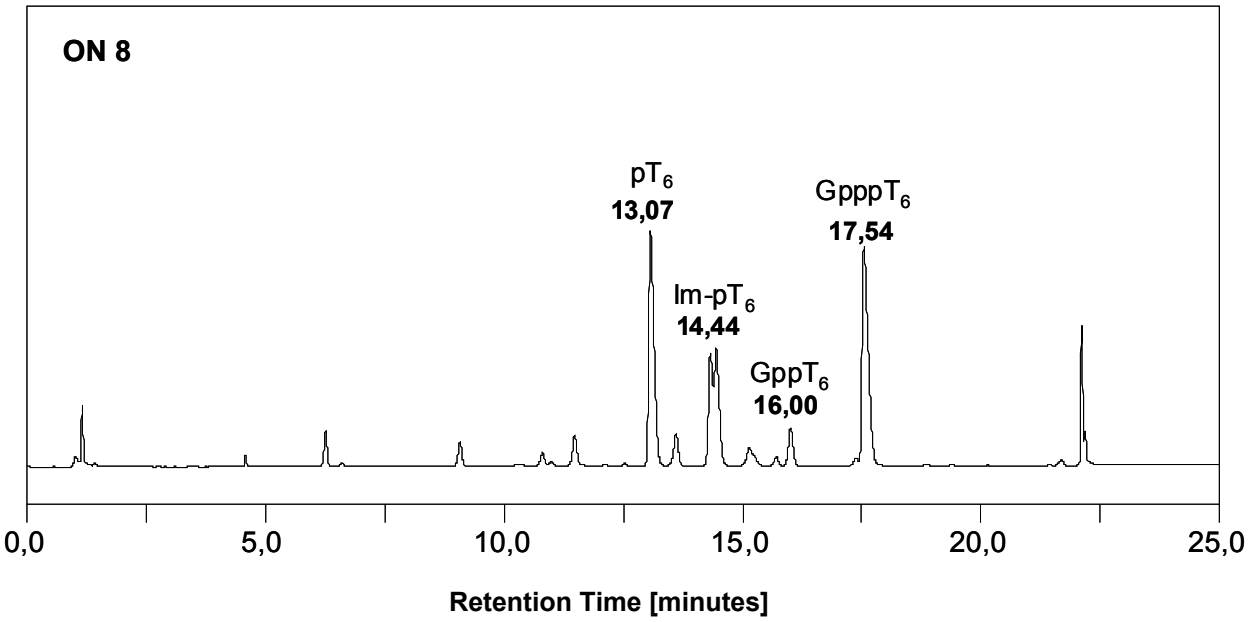

Figure S15: IEX-HPLC analysis of **crude ON 8**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 mL.min^{-1}, λ 260 nm.

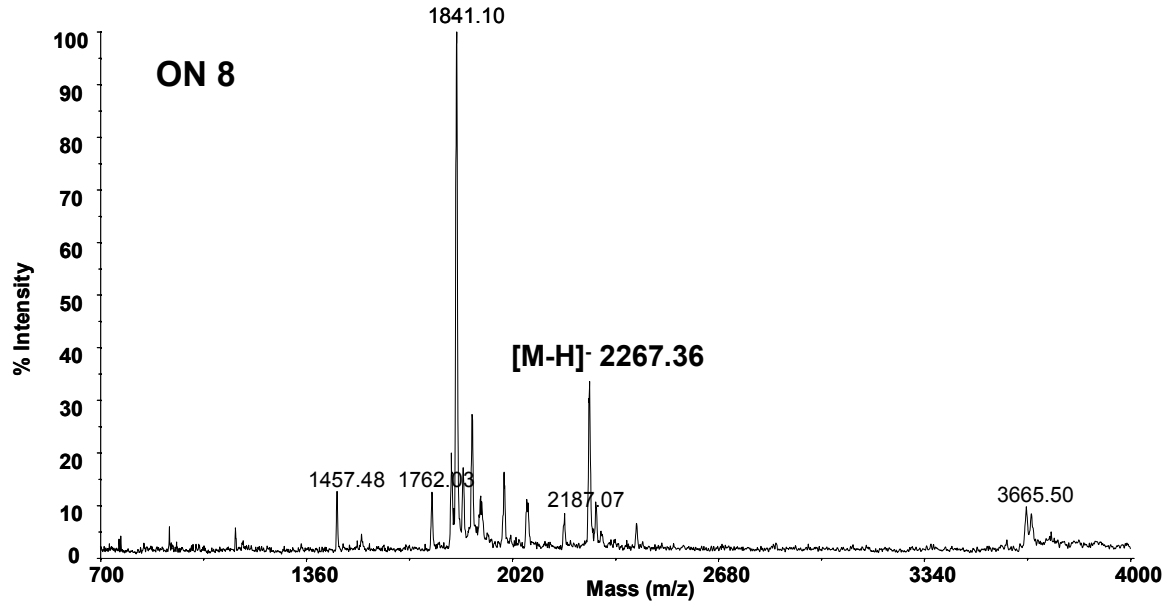

Figure S16: MALDI-TOF MS analysis of **crude ON 8**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

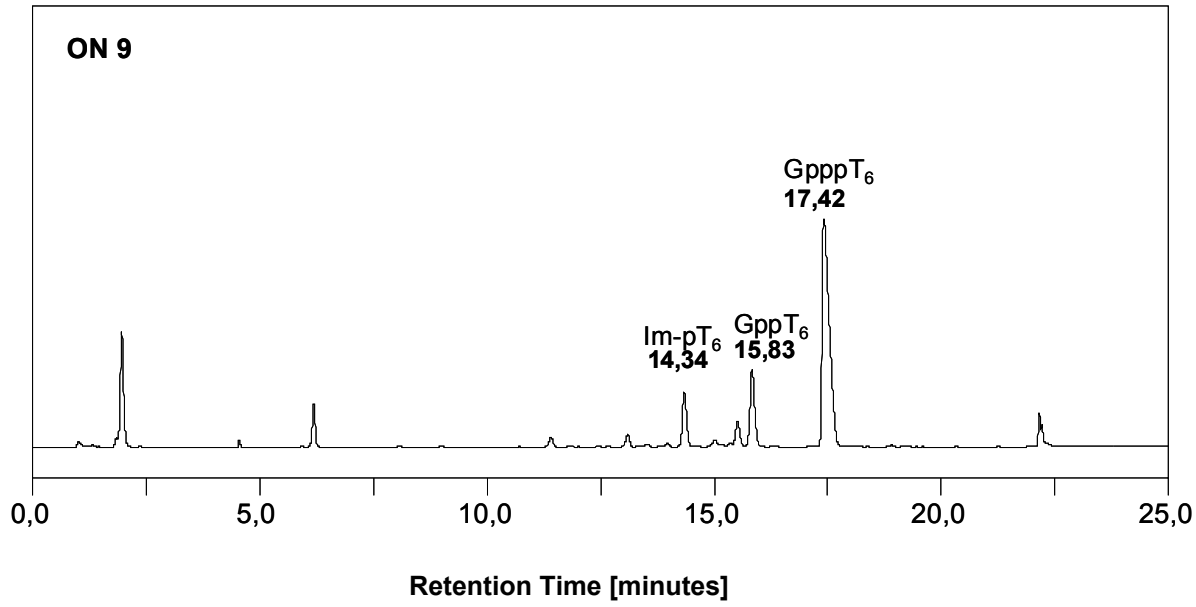

Figure S17: IEX-HPLC analysis of **crude ON 9**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 mL.min^{-1}, λ 260 nm.

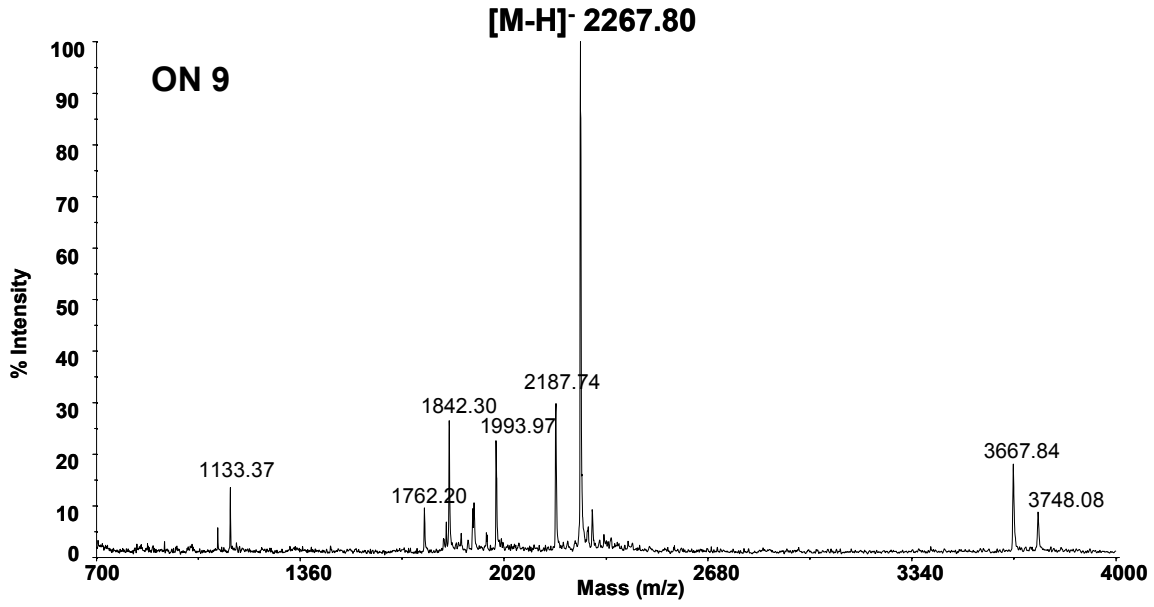

Figure S18: MALDI-TOF MS analysis of **crude ON 9**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

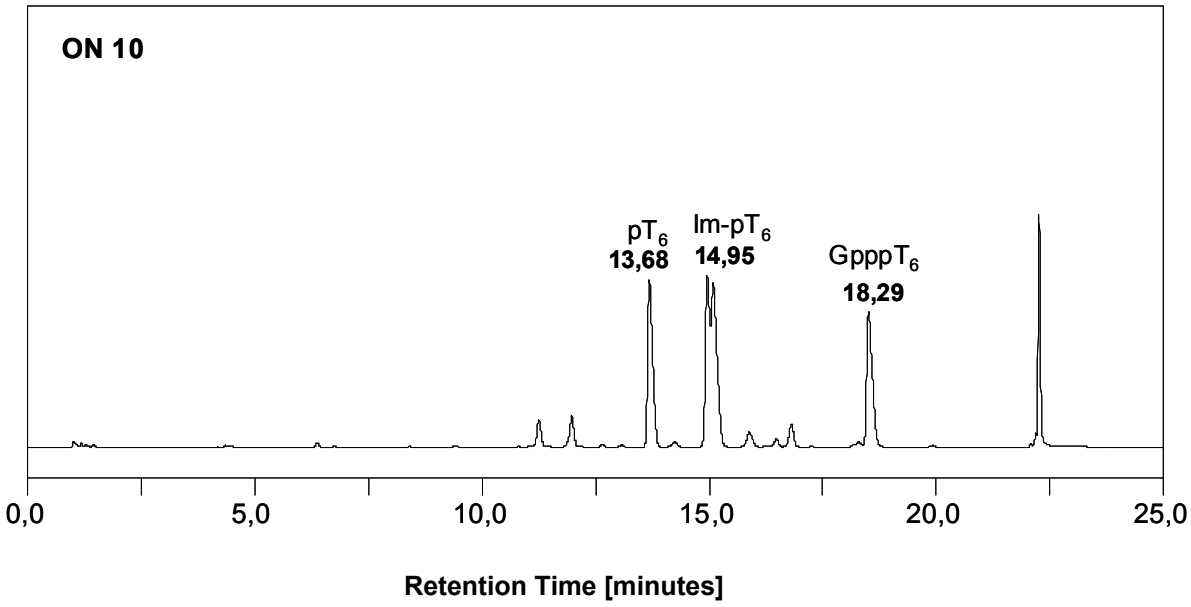

Figure S19: IEX-HPLC analysis of **crude ON 10**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 $mL.min^{-1}$, λ 260 nm.

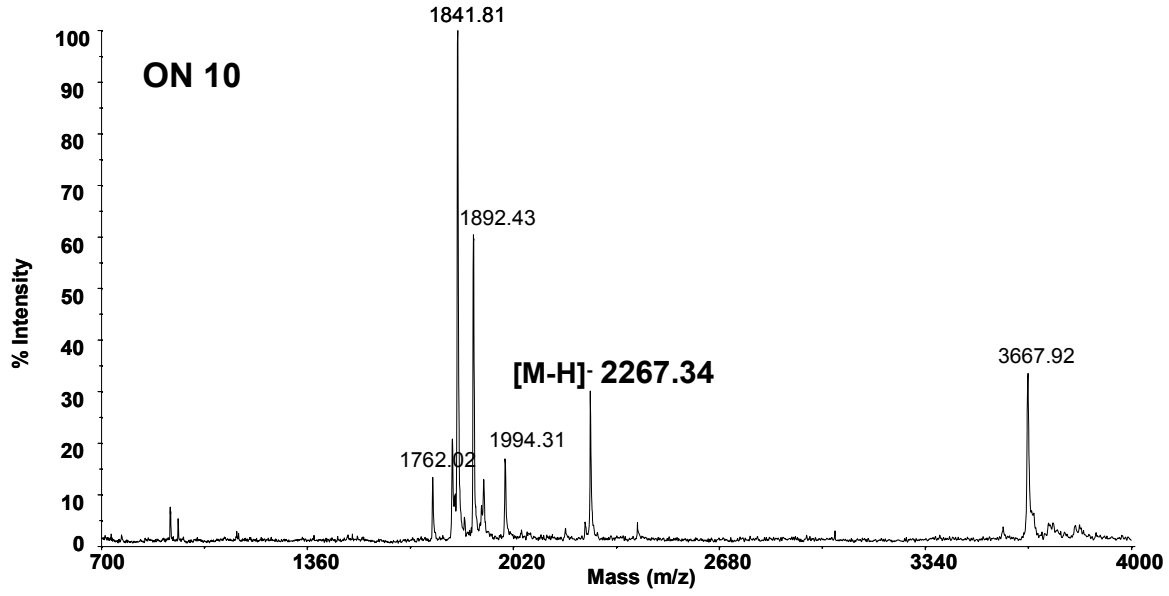

Figure S20: MALDI-TOF MS analysis of **crude ON 10**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

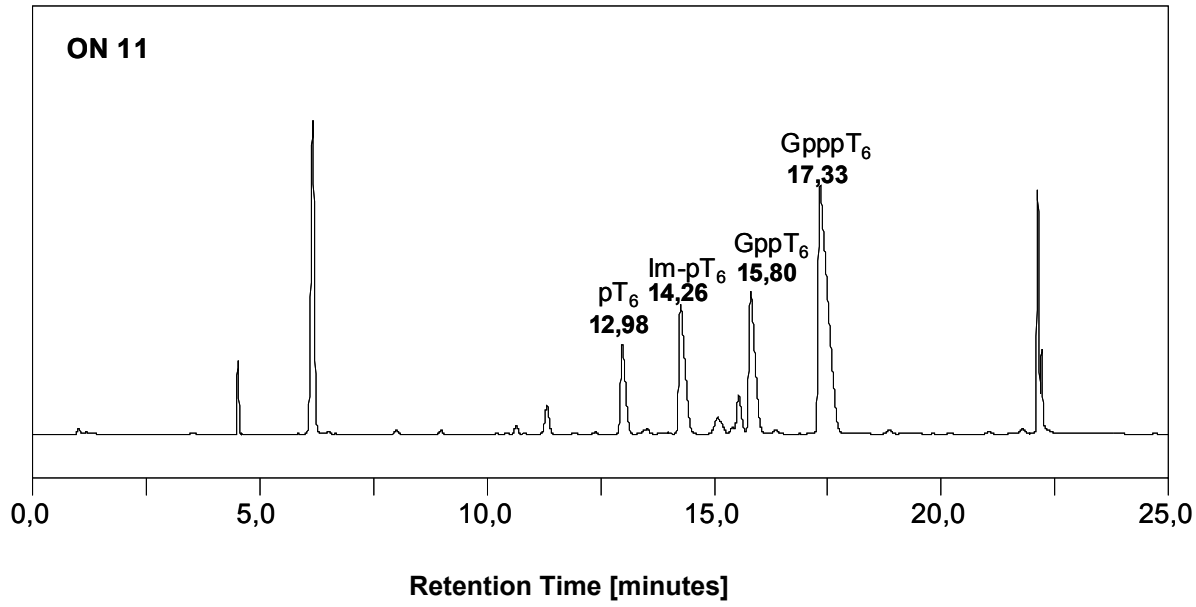

Figure S21: IEX-HPLC analysis of **crude ON 11**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 mL.min^{-1}, λ 260 nm.

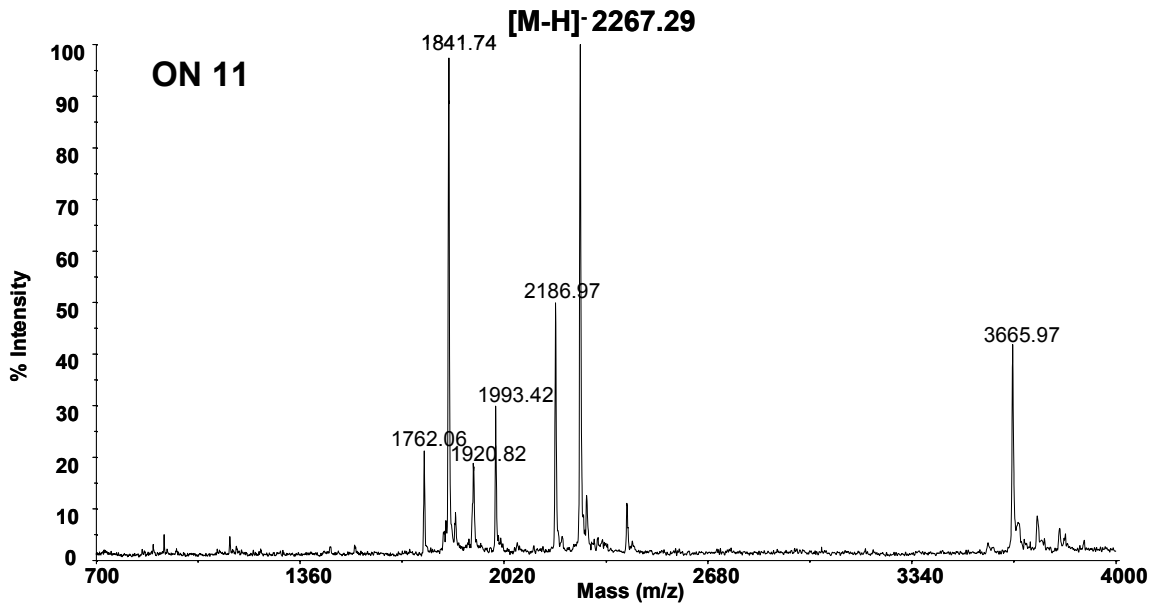

Figure S22: MALDI-TOF MS analysis of **crude ON 11**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

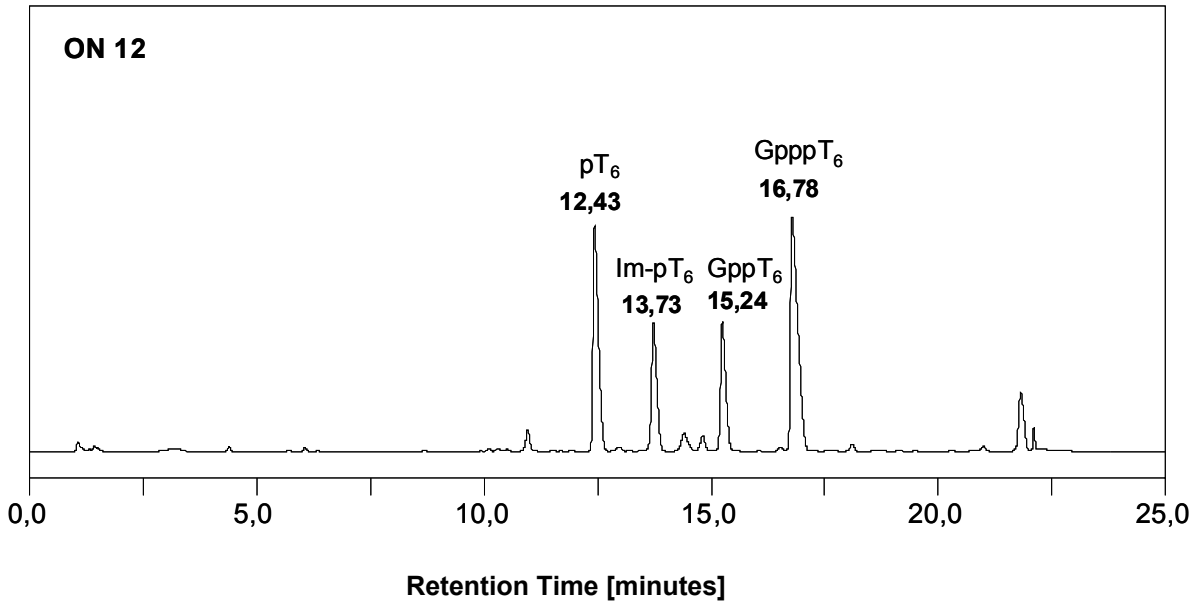

Figure S23: IEX-HPLC analysis of **crude ON 12**: $GpppT_6$

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 mL.min^{-1}, λ 260 nm.

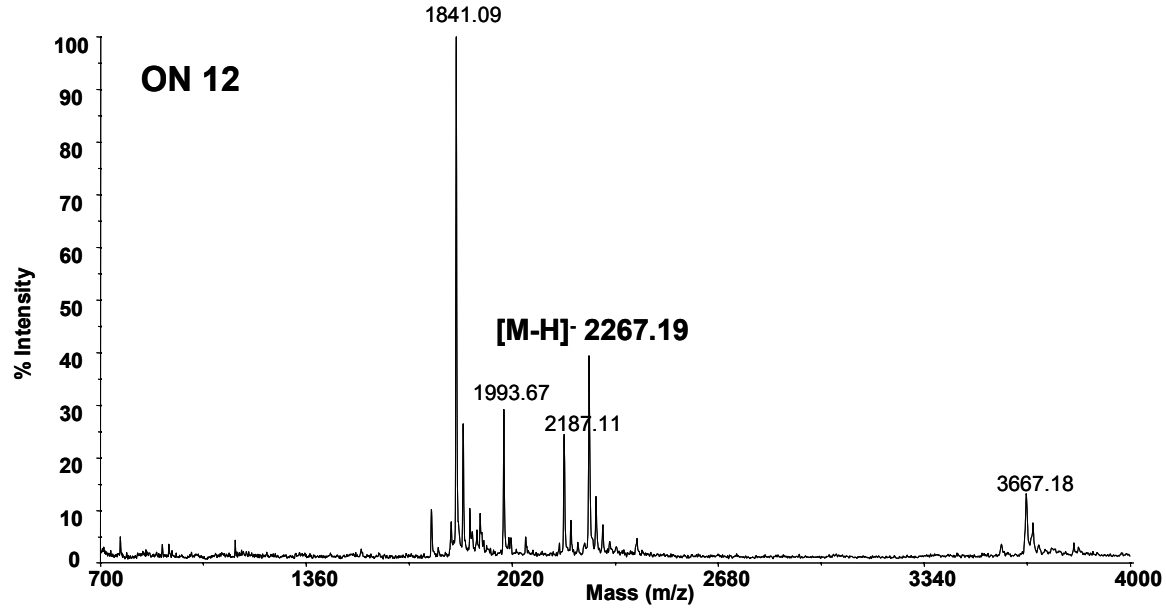

Figure S24: MALDI-TOF MS analysis of **crude ON 12**: $GpppT_6$

Calc. For $[M-H]^-$ 2267.35

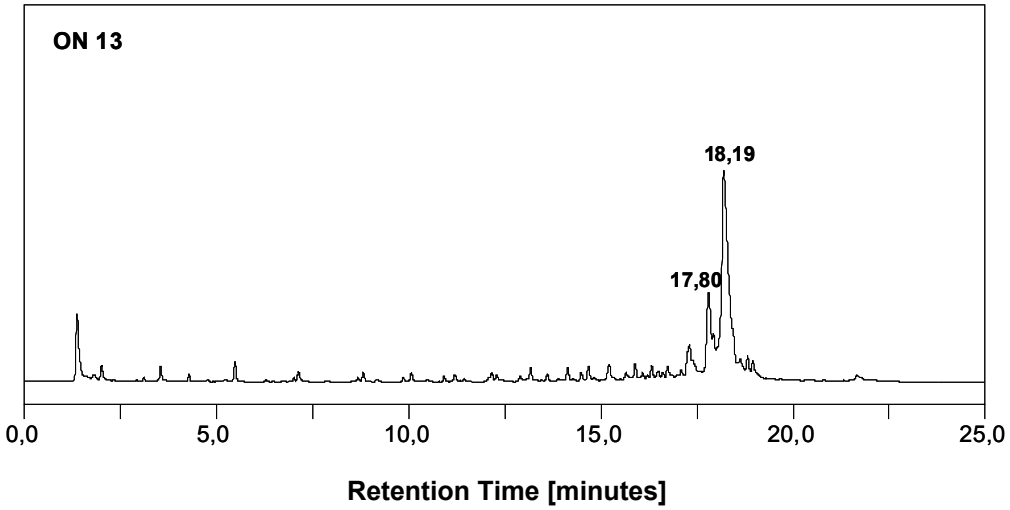

Figure S25: IEX-HPLC analysis of **crude ON 13**: GpppAGU UGU UAG UCU UAC GUG

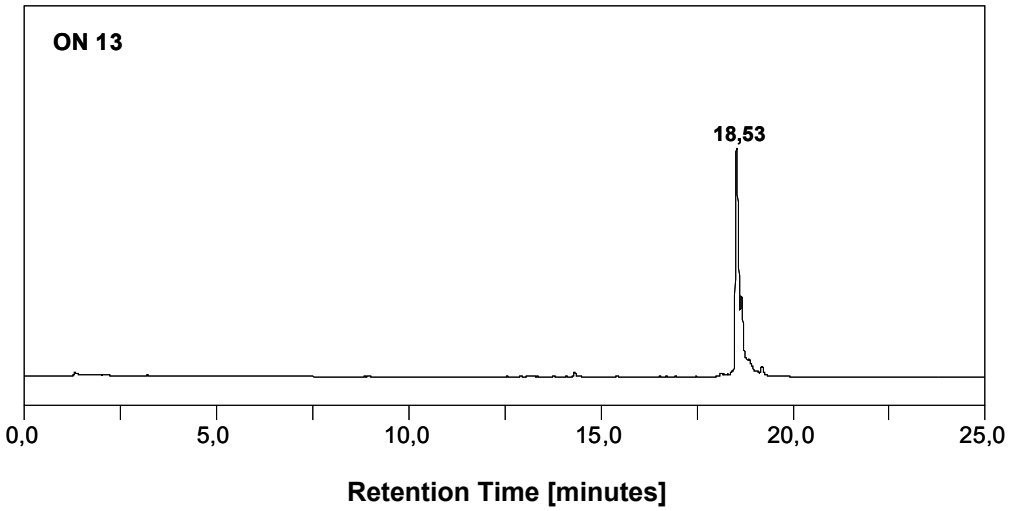

Figure S26: IEX-HPLC analysis of **pure ON 13**: GpppAGU UGU UAG UCU UAC GUG

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-200 (4 x 250 mm), elution with a linear gradient of 0% to 75% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, 50°C, Flow rate 1.2 mL.min^{-1}, λ 260 nm.

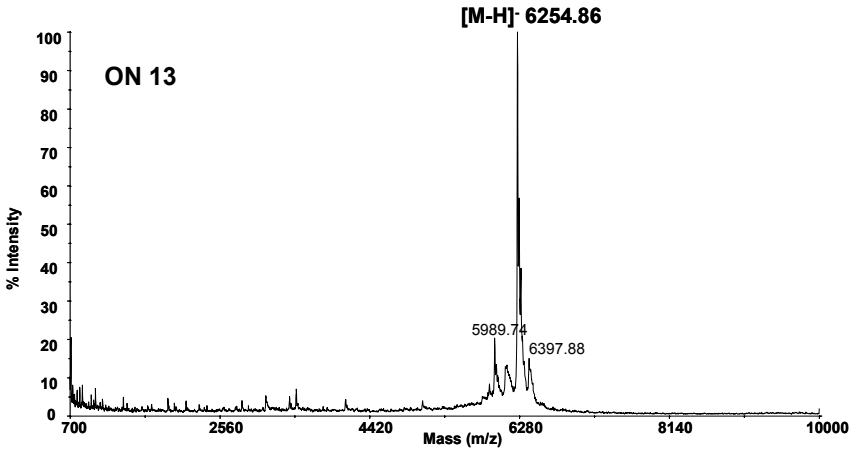

Figure S27: MALDI-TOF MS analysis of **pure ON 13**: GpppAGU UGU UAG UCU UAC GUG

Calc. For $[M-H]^-$ 6254.57

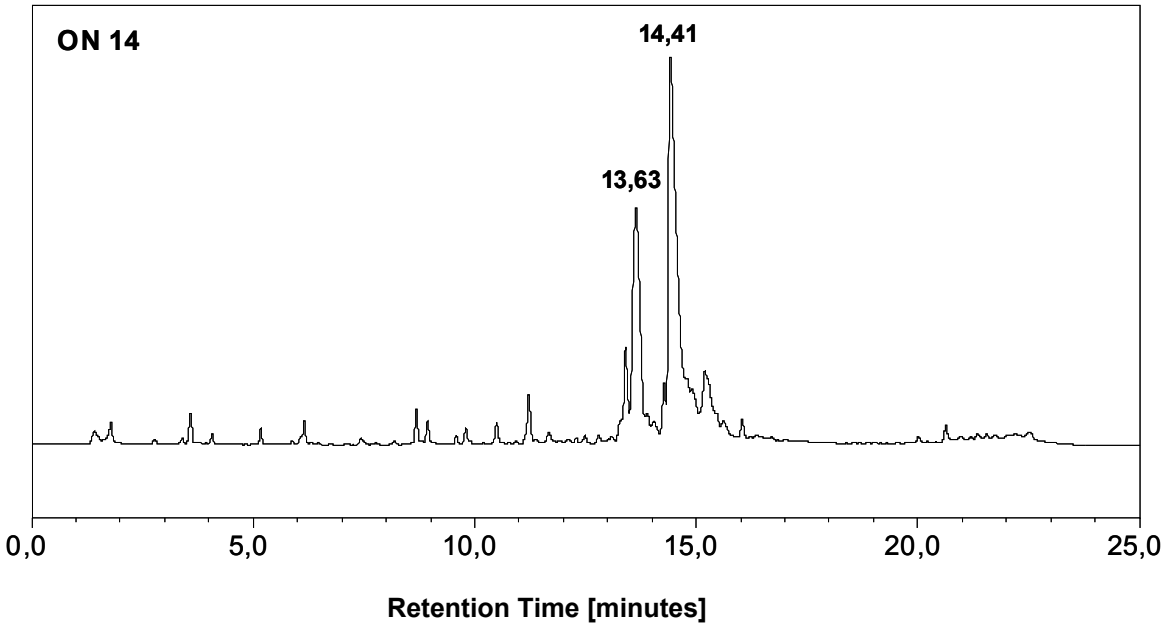

Figure S28: IEX-HPLC analysis of **crude ON 14**: ^{7m}GpppAUA UUA at λ =**260 nm**

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-200 (4 x 250 mm), elution with a linear gradient of 0% to 75% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, 50°C, Flow rate 1.2 mL.min^{-1}, λ 260 nm.

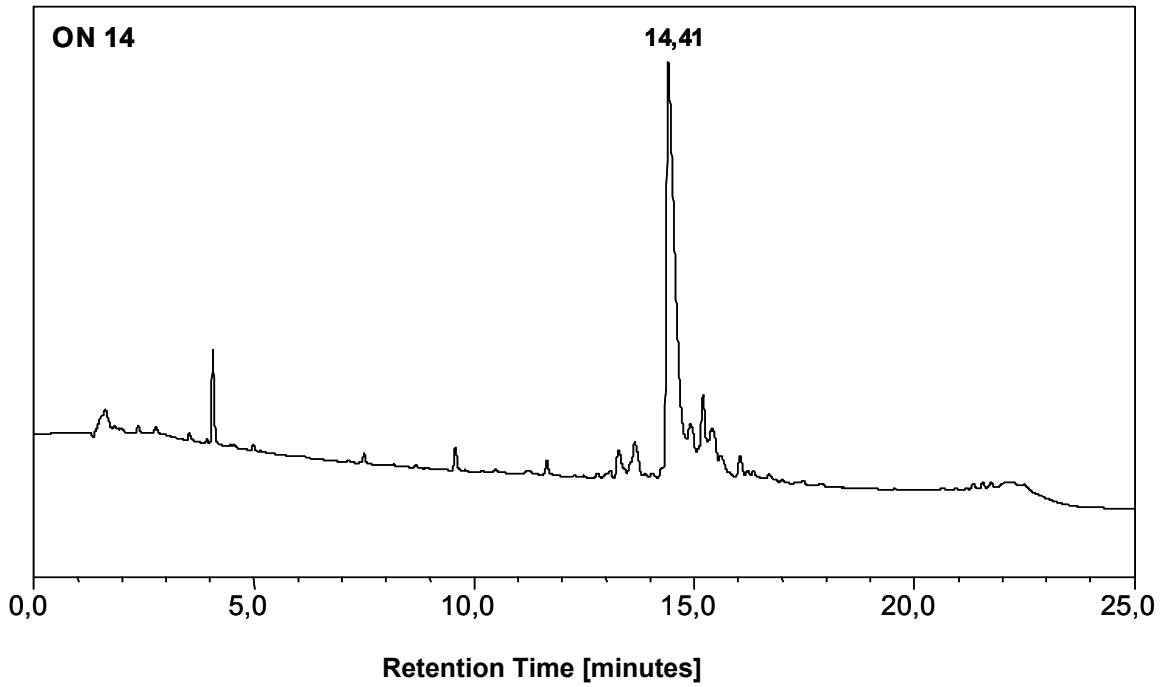

Figure S29: IEX-HPLC analysis of **crude ON 14**: ^{7m}GpppAUA UUA at λ =**300 nm**

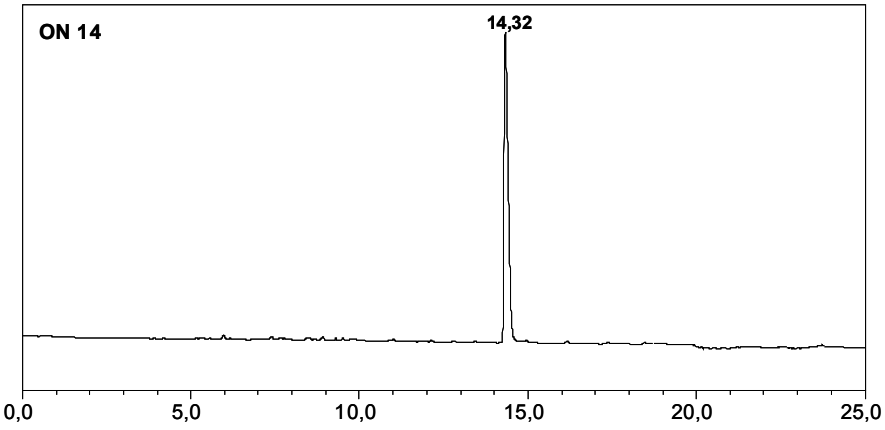

Figure S30: IEX-HPLC analysis of **pure ON 14**: ^{7m}GpppAUA UUA at **λ 260 nm**

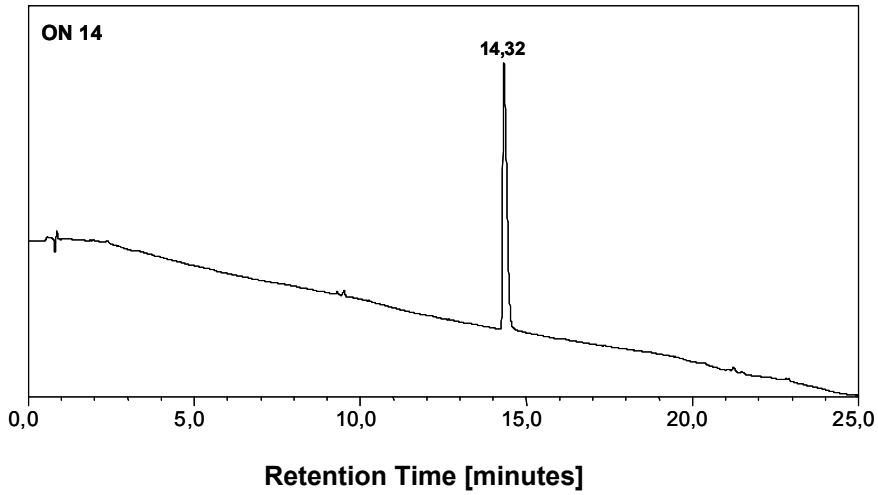

Figure S31: IEX-HPLC analysis of **pure ON 14**: ^{7m}GpppAUA UUA at **λ 300 nm**

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-200 (4 x 250 mm), elution with a linear gradient of 0% to 75% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, 50°C, Flow rate 1.2 mL.min^{-1}.

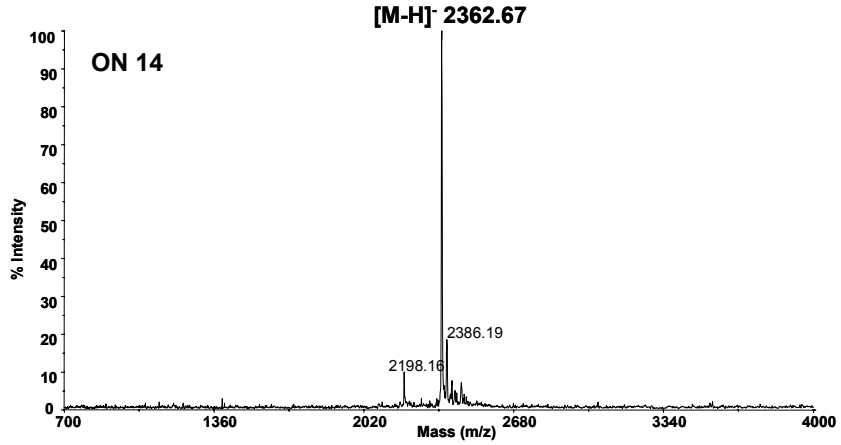

Figure S32: MALDI-TOF MS analysis of **pure ON 14**: ^{7m}GpppAUA UUA

Calc. For $[M-H]^-$ 2362.34

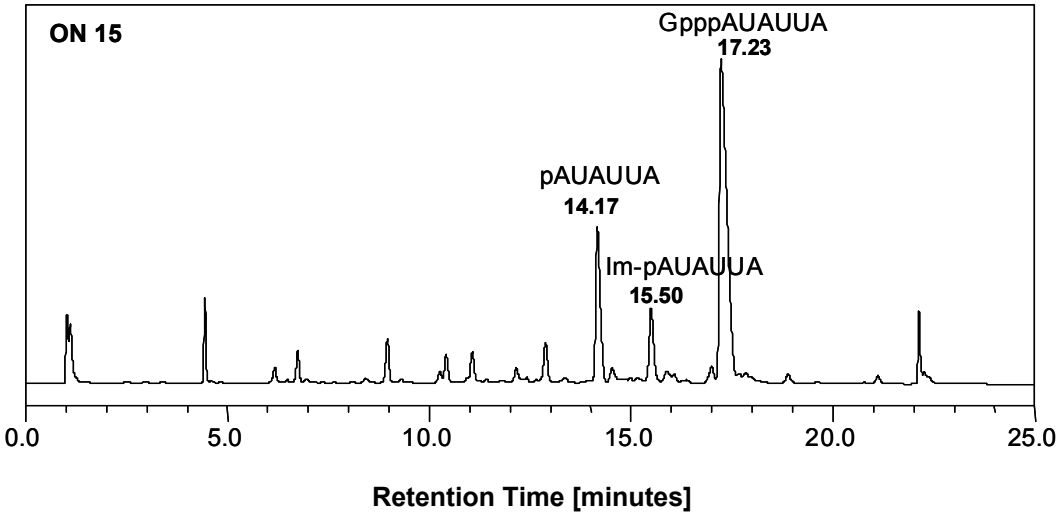

Figure S33: IEX-HPLC analysis of **crude ON 15**: GpppAUA UUA

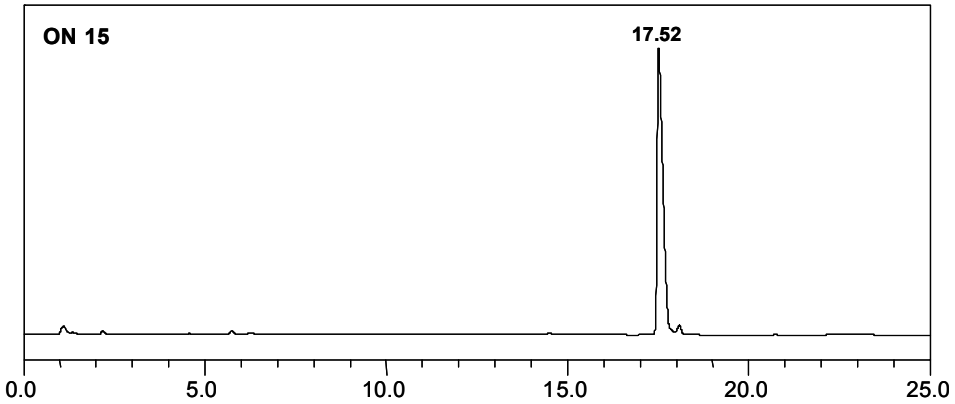

Figure S34: IEX-HPLC analysis of **pure ON 15**: GpppAUA UUA

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 $mL.min^{-1}$, λ 260 nm.

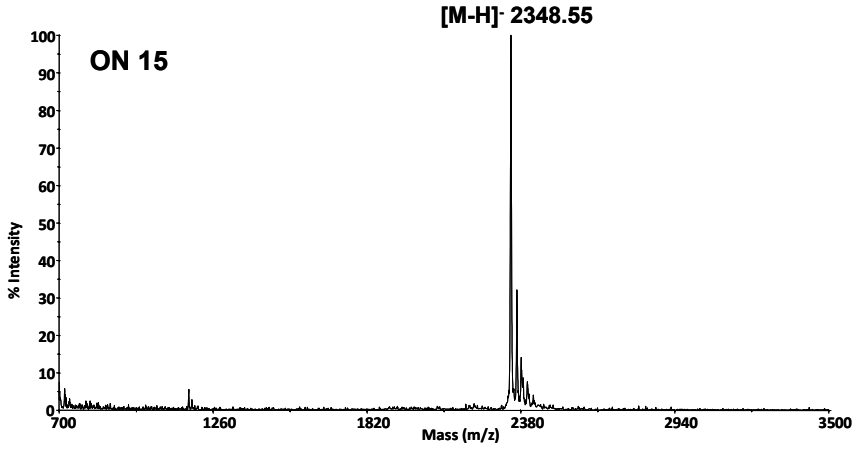

Figure S35: MALDI-TOF MS analysis of **pure ON 15**: GpppAUA UUA

Calc. For $[M-H]^-$ 2348.31

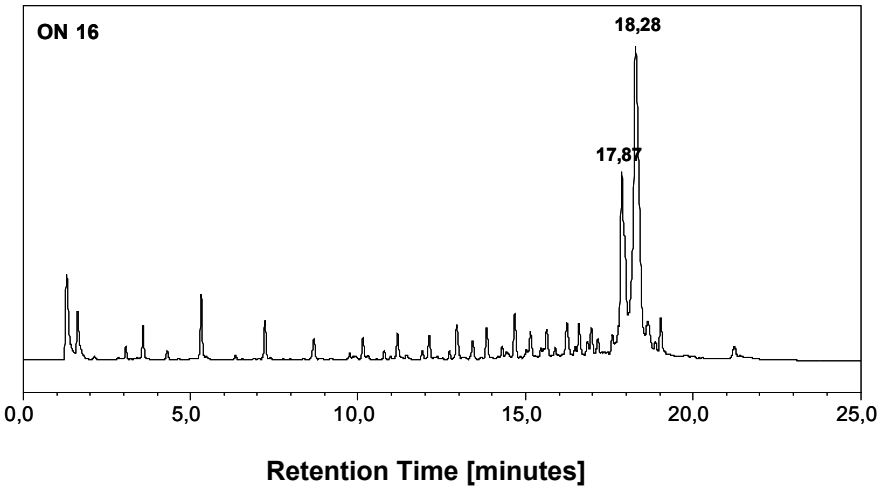

Figure S36: IEX-HPLC analysis of **crude ON 16**: GpppA$_{OMe}$GU UGU UAG UCU UAC GUG A

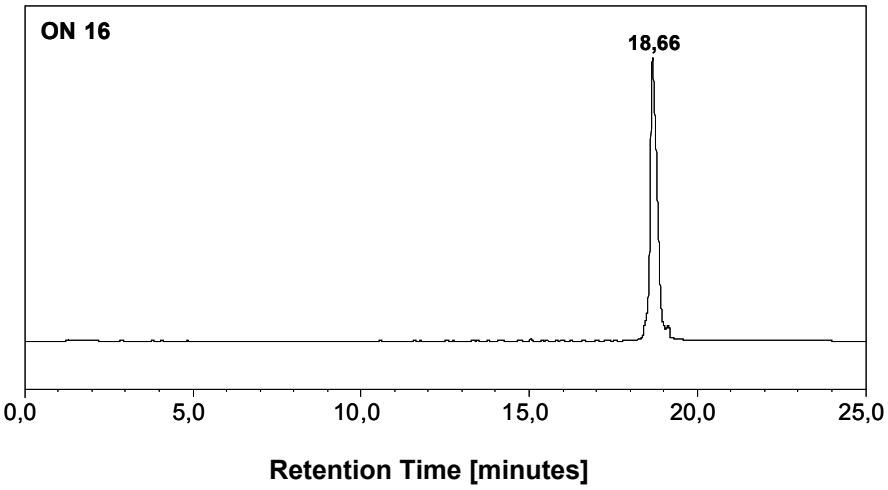

Figure S37: IEX-HPLC analysis of **pure ON 16**: GpppA$_{OMe}$GU UGU UAG UCU UAC GUG A

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-200 (4 x 250 mm), elution with a linear gradient of 0% to 75% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, 50°C, Flow rate 1.2 mL.min^{-1}, λ 260 nm

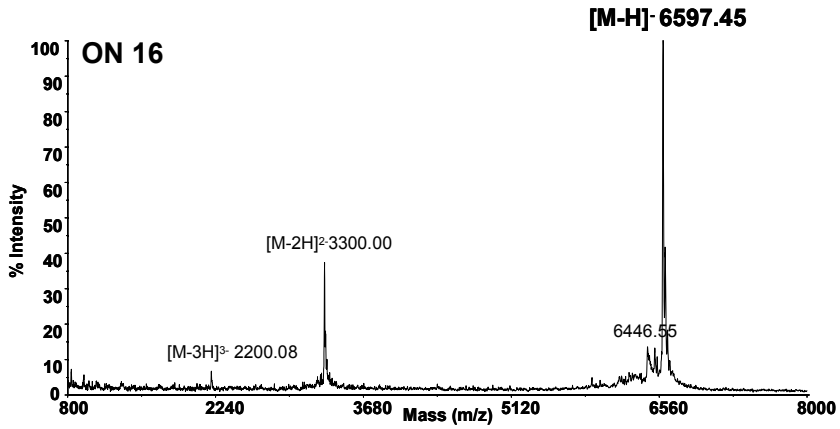

Figure S38: MALDI-TOF MS analysis of **pure ON 16**: GpppA$_{OMe}$GU UGU UAG UCU UAC GUG A

Calc. For [M-H]$^-$ 6597.80

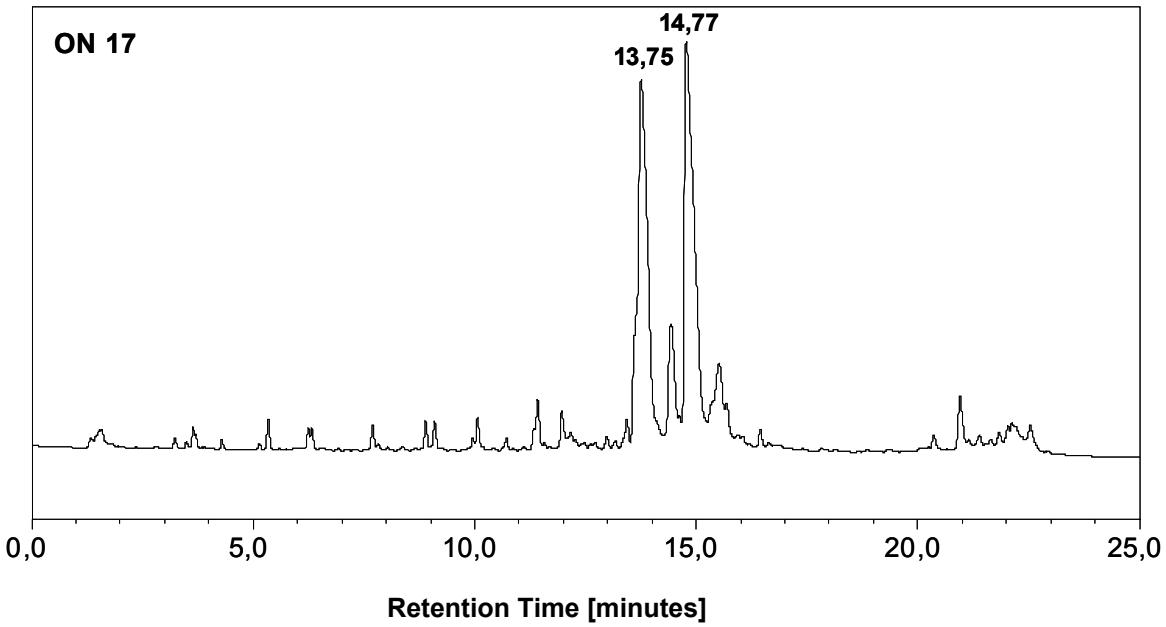

Figure S39: IEX-HPLC analysis of **crude ON 17**: ^{7m}GpppAUA UUA at **λ 260 nm**

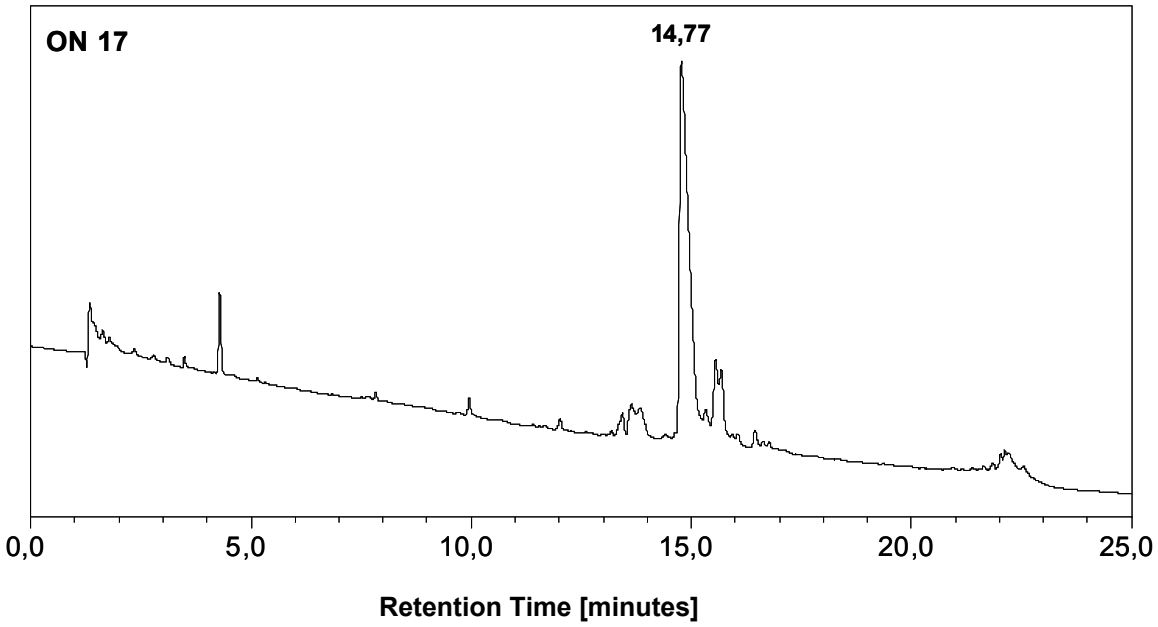

Figure S40: IEX-HPLC analysis of **crude ON 17**: ^{7m}GpppAUA UUA at **λ 300 nm**

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-200 (4 x 250 mm), elution with a linear gradient of 0% to 75% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, 50°C, Flow rate 1.2 mL.min^{-1}.

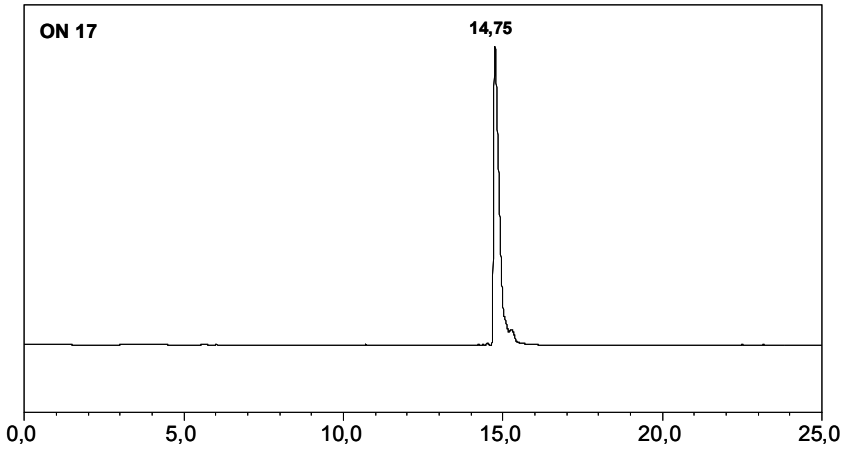

Figure S41: IEX-HPLC analysis of **pure ON 17**: ^{7m}GpppAUA UUA at **λ 260 nm**

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-200 (4 x 250 mm), elution with a linear gradient of 0% to 75% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, 50°C, Flow rate 1.2 mL.min^{-1}.

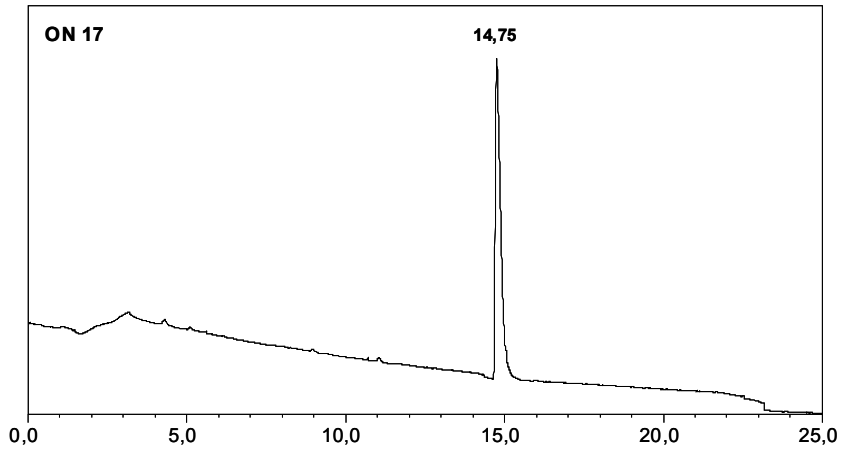

Figure S42: IEX-HPLC analysis of **pure ON 17**: ^{7m}GpppAUA UUA at **λ 300 nm**.

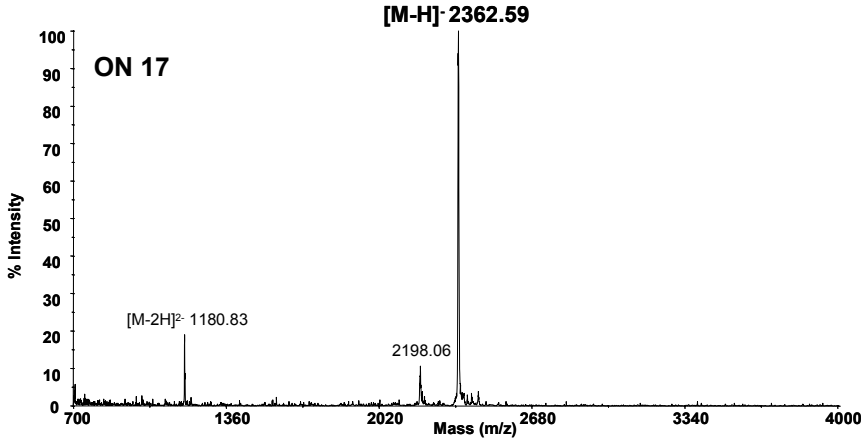

Figure S43: MALDI-TOF MS analysis of **pure ON 17**: ^{7m}GpppAUA UUA.

Calc. For $[M-H]^-$ 2362.34

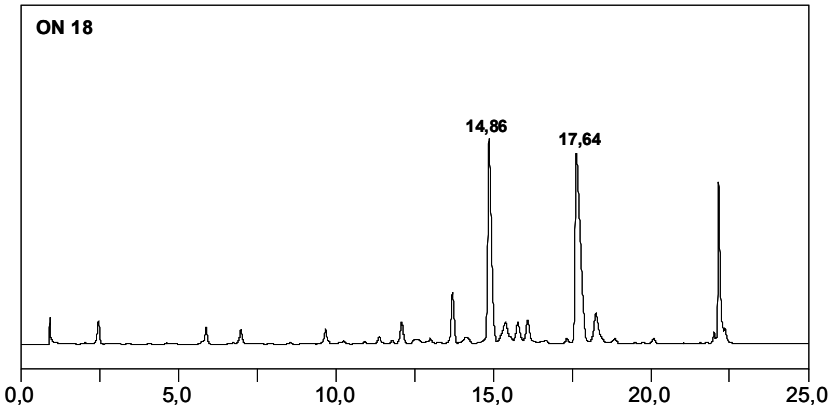

Figure S44: IEX-HPLC analysis of **crude ON 18**: GpppAUA UUA

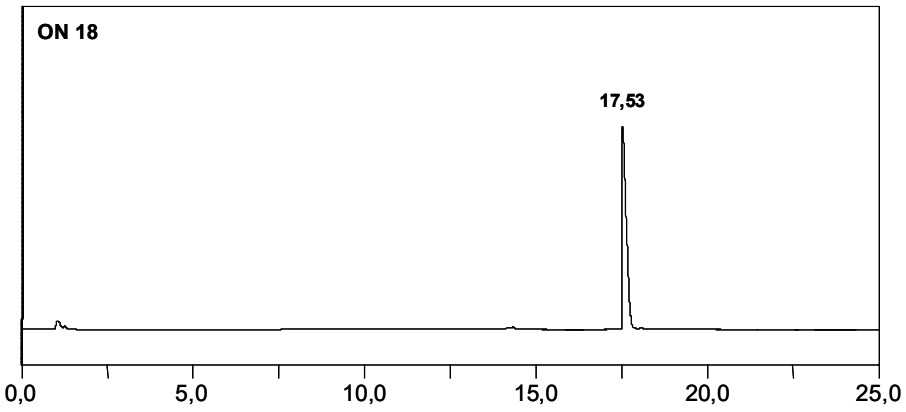

Figure S45: IEX-HPLC analysis of **pure ON 18**: GpppAUA UUA

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 mL.min^{-1}, λ 260 nm.

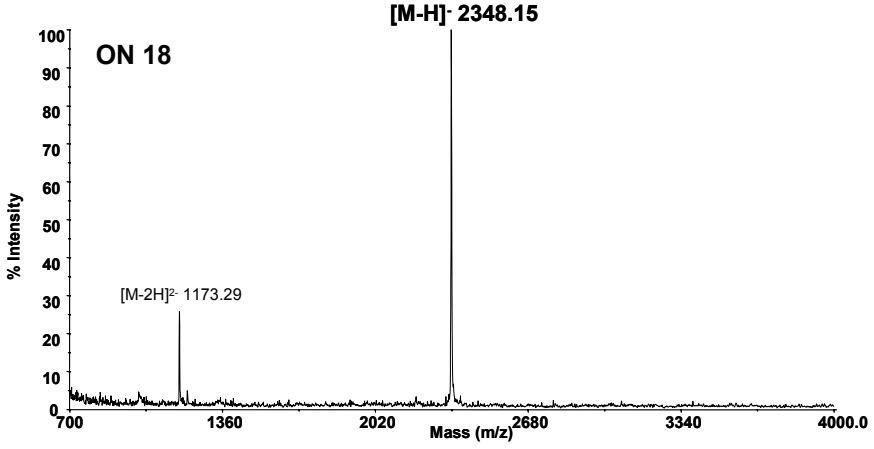

Figure S46: MALDI-TOF MS analysis of **pure ON 18**: GpppAUA UUA

Calc. For $[M-H]^-$ 2348.31

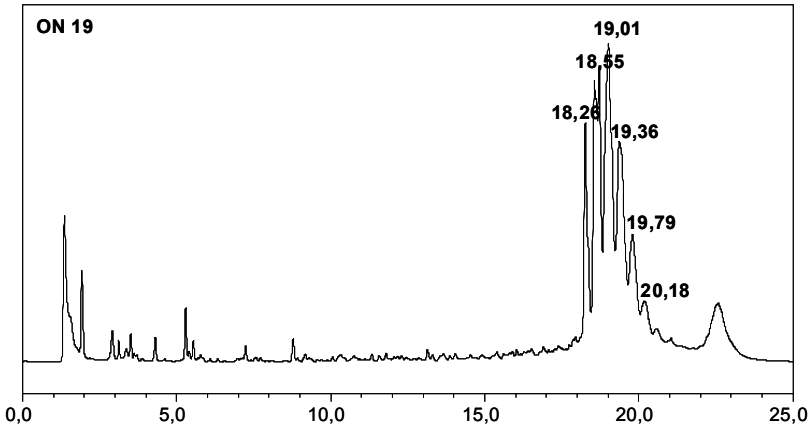

Figure S47: IEX-HPLC analysis of **1h30 NH_4OH ON 19**: GpppA_{OMe}GU UGU UAG UCU UAC GUG A

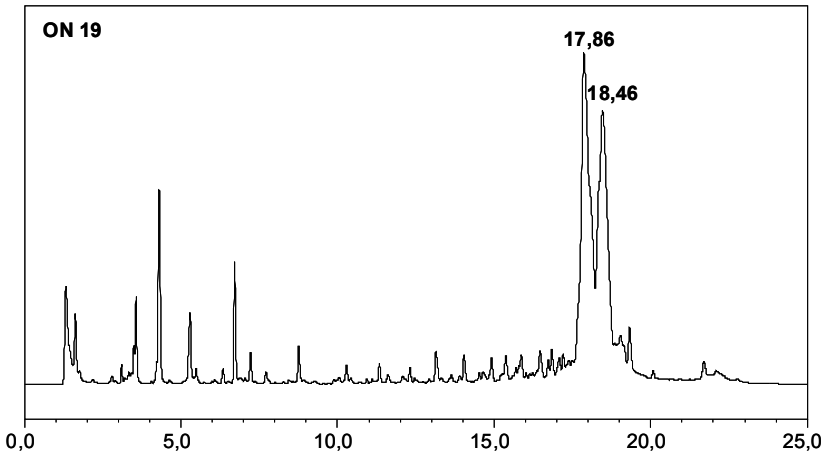

Figure S48: IEX-HPLC analysis of **crude ON 19**: GpppA_{OMe}GU UGU UAG UCU UAC GUG A

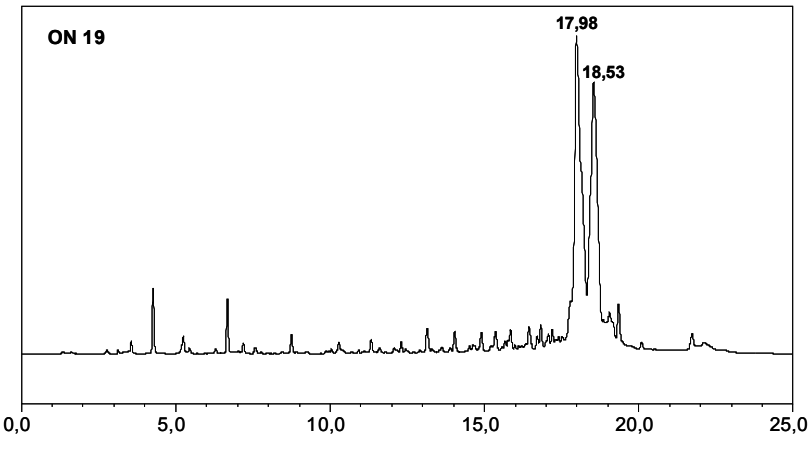

Figure S49: IEX-HPLC analysis of **crude ON 19 (after NAP-10 G-25 Sephadex)** :

GpppA_{OMe}GU UGU UAG UCU UAC GUG A

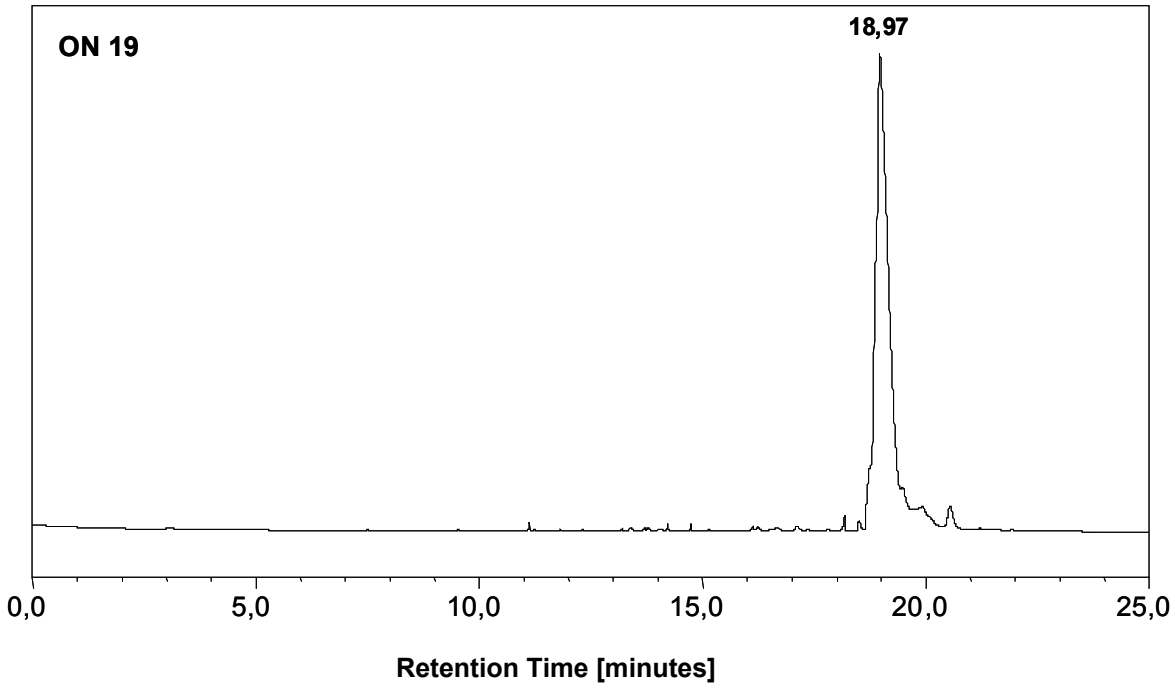

Figure S50: IEX-HPLC analysis of **pure ON 19**: GpppA$_{OMe}$GU UGU UAG UCU UAC GUG A

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-200 (4 x 250 mm), elution with a linear gradient of 0% to 75% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, 50°C, Flow rate 1.2 mL.min^{-1}, λ 260 nm

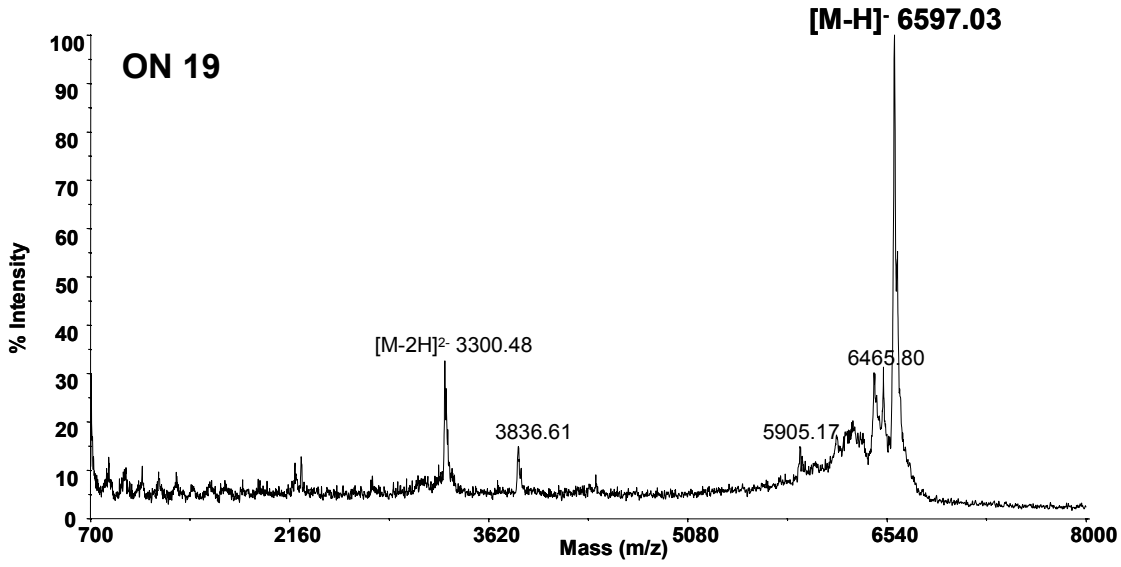

Figure S51: MALDI-TOF MS analysis of **pure ON 19**: GpppA$_{OMe}$GU UGU UAG UCU UAC GUG A

Calc. For [M-H]$^-$ 6597.80

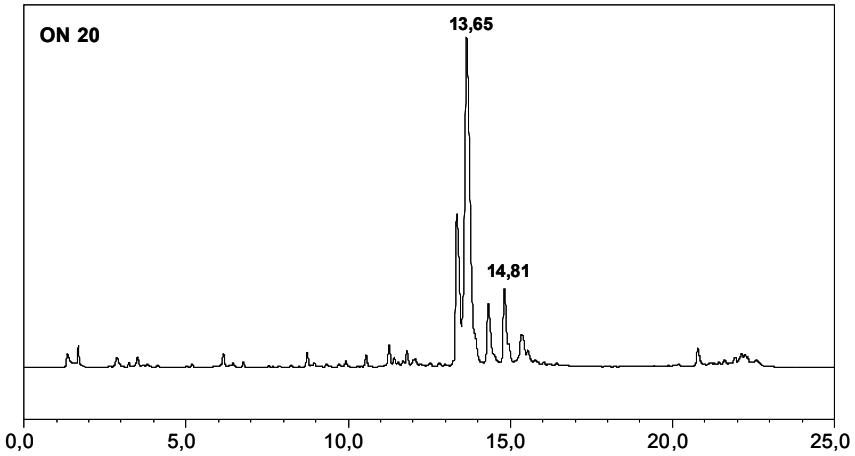

Figure S52: IEX-HPLC analysis of **crude ON 20**: ^{7m}GpppAUA UUA at **λ 260 nm**

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-200 (4 x 250 mm), elution with a linear gradient of 0% to 75% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, 50°C, Flow rate 1.2 mL.min^{-1}.

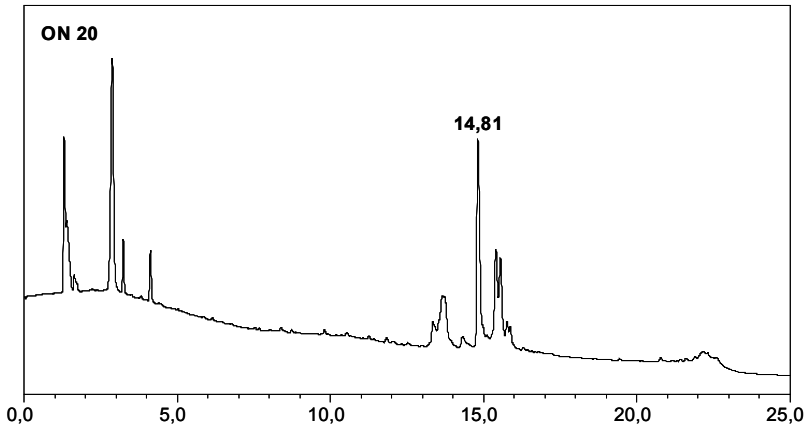

Figure S53: IEX-HPLC analysis of **crude ON 20**: ^{7m}GpppAUA UUA at **λ 300 nm**

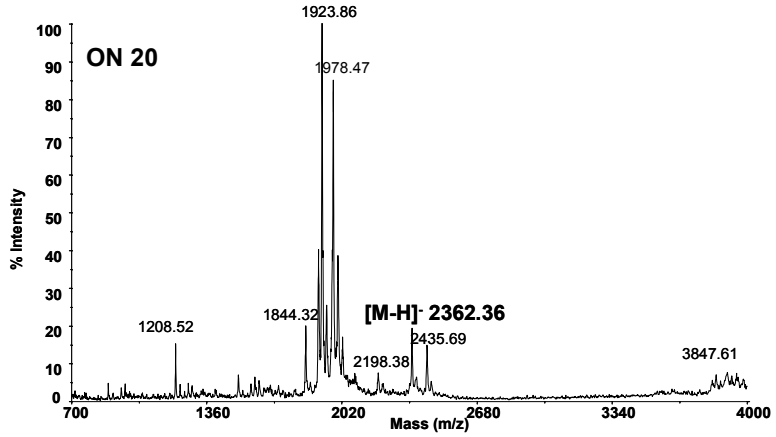

Figure S54: MALDI-TOF MS analysis of **crude ON 20**: ^{7m}GpppAUA UUA.

Calc. For $[M-H]^-$ 2362.34

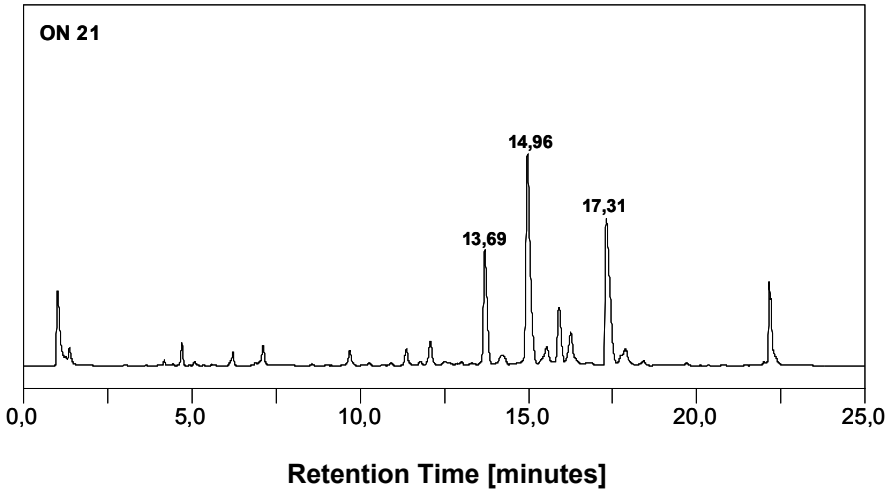

Figure S55: IEX-HPLC analysis of **crude ON 21**: GpppAUA UUA

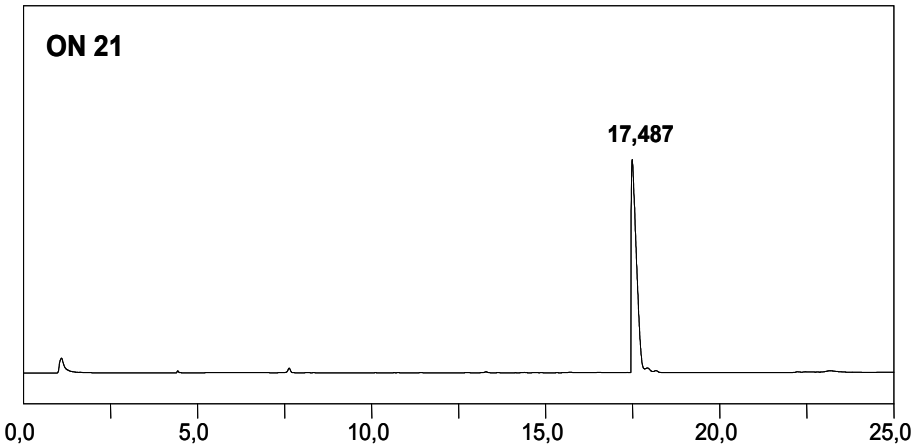

Figure S56: IEX-HPLC analysis of **pure ON 21**: GpppAUA UUA

IEX-HPLC analysis conditions: Column Dionex DNA PAC PA-100 (4 x 250 mm), elution with a linear gradient of 0% to 30% 400 mM $NaClO_4$ in Tris-HCl 25 mM pH 8 / ACN 95/5, in 20 min, Flow rate 1.5 mL.min^{-1}, λ 260 nm.

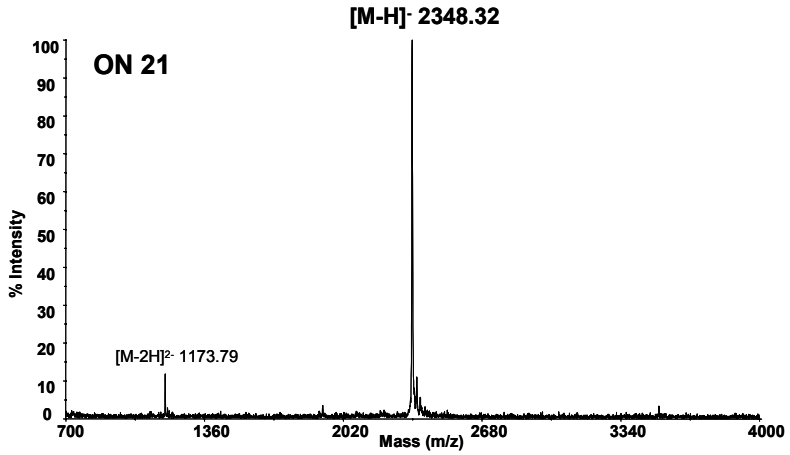

Figure S57: MALDI-TOF MS analysis of **pure ON 21**: GpppAUA UUA

Calc. For $[M-H]^-$ 2348.31