Supporting information

Modification of oligodeoxynucleotides by on-column Suzuki cross-coupling reactions

Maria Ejlersen,[a] Chenguang Lou,[a] Yogesh S. Sanghvi,[b] Yitzhak Tor[c] and Jesper Wengel[a]*

[a]Biomolecular Nanoscale Engineering Center, Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, DK-5230 Odense M, Denmark

[b]Rasayan Inc. 2802, Crystal Ridge Road, Encinitas, California, 92024-6615, United States

[c]Deparment of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093-0358, United States

*correspondence to: jwe@sdu.dk

Oligonucleotide (ON) synthesis, cleavage and purification

ONs were synthesized on an Expedite Nucleic Acid Synthesis system (PerSeptive Biosystems) in 0.2 μmol scale. Synthesis followed the standard DMT-OFF protocol employing CPG-supports (GE Healthcare) with the first nucleotide attached from the manufacturer. The 5IdU (2'-deoxy-5-iodouridine) phosphoramidite was manually coupled[1] for 15 min using 5-[3,5-bis(trifluoromethyl)phenyl]-1*H*-tetrazole (Activator 42®, 0.25M in MeCN) and acetonitrile as solvent. Coupling yields were >99% for unmodified phosphoramidites and >98% for 5IdU phosphoramidite. Following Suzuki cross-coupling reactions, the ONs were cleaved from solid support and deprotected using mild deprotection conditions (32% aqueous ammonia, RT, 24 h). RP-HPLC purification of **ON1**, **ON2**, **ON3**, **ON4**, **ON5**, **ON8** and **ON9** was performed on a Merck Hitachi 7000 system equipped with a Waters XBridge C18 column (2.5 μm/138 Å, 10×50 mm). Elution was performed at 60 °C with an isocratic hold of buffer A for 2 min followed by a linear gradient to 100% Buffer B over 68 min at a flowrate of 1 mL/min (Buffer A: 50 mM TEAA buffer, pH 7.4; Buffer B: 80% MeOH in buffer A). RP-HPLC purification of **ON6(b)** and **ON7** was performed on a Waters 600 system equipped with a Kinetex C8 column (5 μm/100 Å, 100×21.2 mm). Elution was performed at 50 °C with an isocratic hold of buffer A for 2 min followed by a linear gradient to 70% Buffer B over 17 min at a flow rate of 8 mL/min (Buffer A: 50 mM TEAA buffer, pH 7.4; Buffer B: 75% MeCN in buffer A). Collected fractions from RP-purification were analyzed by MALDI-MS and pure fractions showing the desired mass were pooled. Purification of **ON6(a)** was performed on anion-exchange HPLC (IE-HPLC) using the DIONEX Ultimate 3000 system equipped with a DNAPac PA100 semipreparative column (13 μm, 250×9 mm) heated to 60 °C. Elution was performed with an isocratic hold of buffer B (10%), Buffer A (2%) and Milli-Q water (solvent A, 88%) for 2 min, followed by a linear gradient to 30% buffer A in 20 min at a flow rate of 2.0 mL/min (buffer A: 1.0 M sodium perchlorate; buffer B: 0.25 M Tris-Cl, pH 8.0, solvent A: Milli-Q water). After IE-HPLC purification, **ON6**(a) was desalted in ethanol. Final purity of ONs was evaluated by RP-HPLC performed on a Merck Hitachi 7000 system equipped with a Waters XBridge C18 column (2.5 μm/138 Å, 10×50 mm). Elution was performed at 60 °C with an isocratic hold of buffer A for 2 min followed by a linear gradient to 70% Buffer B over 28 min at a flow rate of 1 mL/min (Buffer A: 50 mM TEAA buffer, pH 7.4; Buffer B: 75% MeCN in buffer A). MALDI-TOF was

carried out on an Ultraflex II TOF/TOF instrument from Bruker using a HPA matrix (10 mg 3-hydroxypicolinic acid, 50 mM ammonium citrate in 70% aqueous acetonitrile) to verify the composition of all ONs.

Reagents and conditions for Suzuki cross-coupling reactions (**ON1**-**ON5**, **ON7**-**ON9**): $Pd(OAc)_2$ [6.2 mM], TPPTS [28 mM] and boronic acid monomer [170 mM] were mixed in a mixture of Tris buffer (50 mM, pH 8.50) and MeCN (3:2 v/v, 1 mL) in a 1 mL syringe. Syringes, columns and solvents were carefully degassed with argon prior to mixing. The syringe with the coupling-reagents was attached to the column with the CPG-bound ON with an empty syringe at the other end. The reaction was carried out in an oven at 70 °C where the support was treated with the reagents every 30 min for a total of 4 h. The reagents were removed and the column containing CPG-bound ON was washed with a mixture of MQ water and MeCN (1:1 v/v, 4 mL) and MeCN (4 mL).

Reagents and conditions for Suzuki cross-coupling reactions (**ON6**): $Pd(OAc)_2$ [6.2 mM], TPPTS [14 mM] and pyrene-1-boronic acid [85 mM] were mixed in a mixture of Tris buffer (50 mM, pH 8.50) and DMF (1:3 v/v, 1 mL) in a 1 mL syringe. Syringes, columns and solvents were carefully degassed with argon prior to mixing. The syringe with the coupling-reagents was attached to the column with the CPG-bound ON with an empty syringe at the other end. The reaction was carried out in an oven at 70 °C where the support was treated with the reagents every 30 min for a total of 4 h. The reagents were removed and the column containing CPG-bound ON was washed with a mixture of MQ water and MeCN (1:1 v/v, 4 mL) and MeCN (4 mL).

Reagents and conditions for initial Suzuki cross-coupling reactions (test ONa and ONb): $Pd(OAc)_2$ [6.2 mM], TPPTS [28 mM] Na_2CO_3 [340 mM], 2-thienylboronic acid [170 mM] were mixed in a mixture of MQ H_2O and MeCN (3:2 v/v, 1 mL) in a 1 mL syringe. Syringes, columns and solvents were carefully degassed with argon prior to mixing. The syringe with the coupling-reagents was attached to the column with the CPG-bound ON with an empty syringe at the other end. The reaction was carried out in an oven at 70 °C where the support was treated with the reagents every 30 min for a total of 4 h. The reagents were removed and the column containing CPG-bound ON was washed with a mixture of MQ water and MeCN (1:1 v/v, 4 mL) and MeCN (4 mL). Method 1: Suzuki cross-coupling reaction was carried out after synthesis of the full-length ON followed by cleavage, deprotection and purification of the ON. 4 h reaction time was used for ONs with one modification while 8 h and double cross-couplings were used for ONs with multiple modifications.

Method 2: Suzuki cross-coupling reaction was carried out after incorporation of the 5IdU nucleotide. ON synthesis was continued after the Suzuki reaction to furnish the full-length ON, or continued until incorporation of the next 5IdU nucleotide which was then subjected to another Suzuki cross-coupling reaction, etc.

Yields of the ONs were calculated from the maximal yield obtained from a 0.2 μmol synthesis using the absorbance at 260 nm and the molar extinction coefficients of the obtained products. The yield was calculated from the absorbance at 260 nm utilizing the extinction coefficients (ε_{260} in H_2O) for the relevant nucleosides; dA 13700 $M^{-1}cm^{-1}$, dC 7300 $M^{-1}cm^{-1}$, dG 10800 $M^{-1}cm^{-1}$, dT/dU^{Ph} 8400 $M^{-1}cm^{-1}$,[2] dU^{Th}/dU^{MeTh} 10140 $M^{-1}cm^{-1}$,[3] dU^{Py} 18600 $M^{-1}cm^{-1}$,[4] and dU^{Dap} 12100 $M^{-1}cm^{1}$.

MALDI-MS spectra of synthesized ONs

Table S1 Summary of experimental and calculated masses of synthesized ONs

ODN	Sequence	Found m/z	Calculated m/z
ON1	5'-GTG AdU^{I}A TGC	2866.0	2865.7
ON2	5'-GdU^{I}G AdU^{I}A dU^{I}GC	3089.8	3089.4
ON3(a)	5'-GTG AdUThA TGC	2821.7	2821.9
ON3(b)	5'-GTG AdUThA TGC	2821.3	2821.9
ON4	5'-GTG AdU^{Ph}A TGC	2815.8	2815.9
ON5	5'-GTG AdU^{MeTh}A TGC	2836.3	2835.5
ON6(a)	5'-GTG AdU^{Py}A TGC	2940.4	2940.1
ON6(b)	5'-GTG AdU^{Py}A TGC	2940.4	2940.1
ON7(a)	5'-GTG AdU^{Dap}A TGC	3062.8	3062.9
ON7(b)	5'-GTG AdU^{Dap}A TGC	3063.0	3062.9
ON8(a)	5'-GdUThG AdUThA dUThGC	2958.7	2958.0
ON8(b)	5'-GdUThG AdUThA dUThGC	2958.4	2958.0
ON8(c)	5'-GdUThG AdUThA dUThGC	2958.7	2958.0
ON8(d)	5'-GdUThG AdUThA dUThGC	2958.7	2958.0
ON9	5'-GdU^{Ph}G AdU^{MeTh}A dUThGC	2966.1	2966.1

Th = 2'-thienyl, MeTh = 5-methyl-2-thienyl, Ph = phenyl, Py = pyrene, Dap = 3-(Dansylamino)phenyl

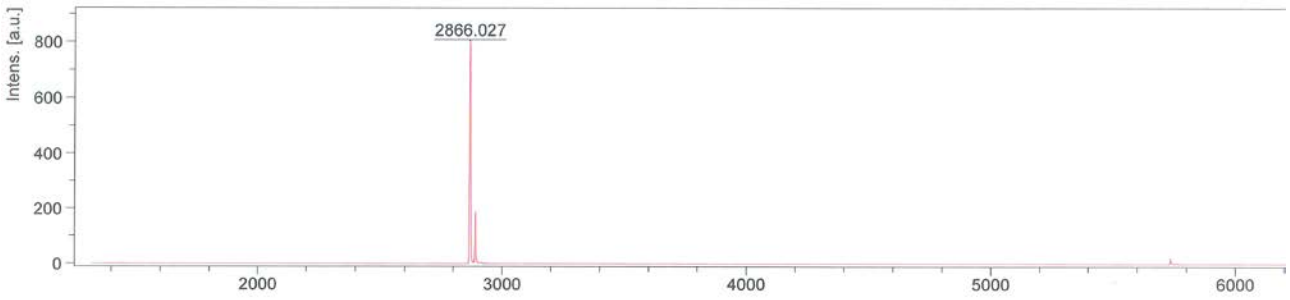

Figure S1. MALDI-MS spectrum for **ON1**.

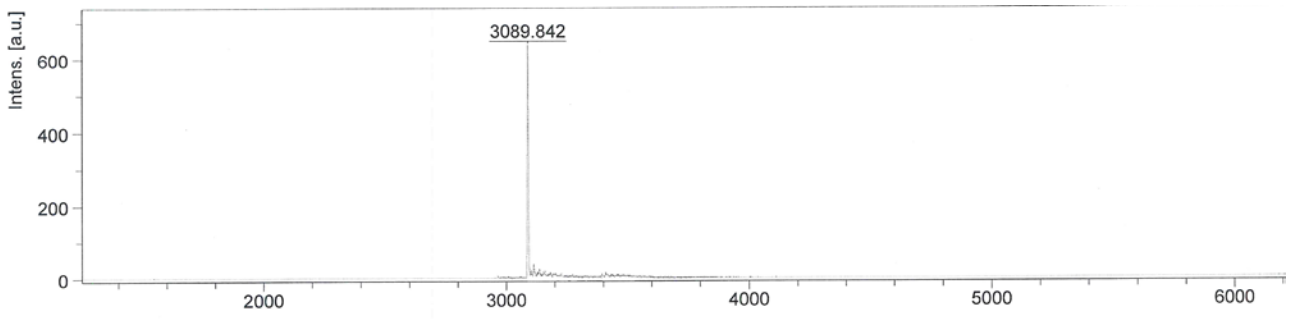

Figure S2. MALDI-MS spectrum for **ON2**.

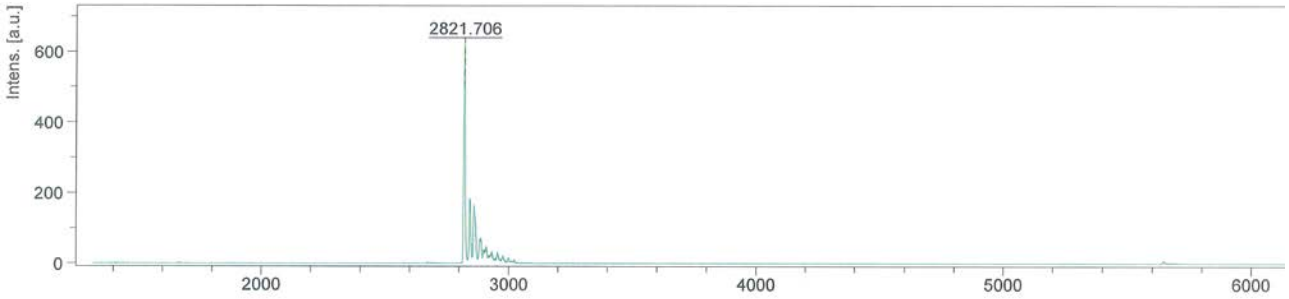

Figure S3. MALDI-MS spectrum for **ON3**(a).

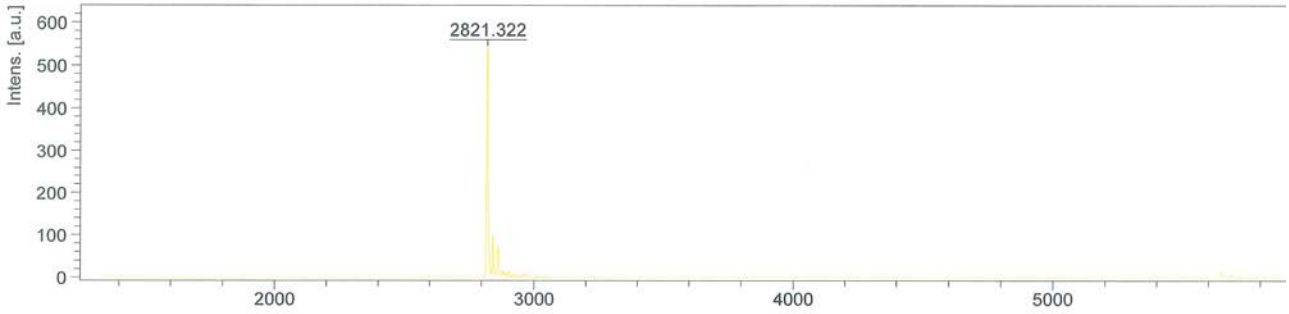

Figure S4. MALDI-MS spectrum for **ON3**(b).

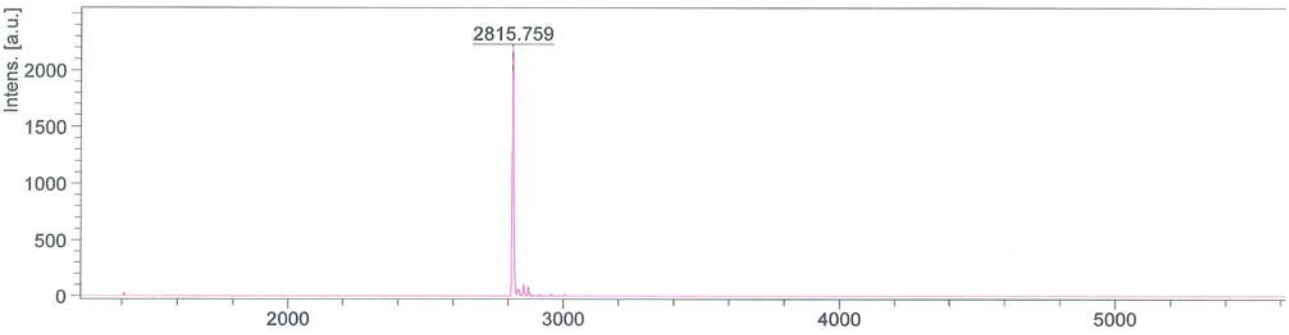

Figure S5. MALDI-MS spectrum for **ON4**.

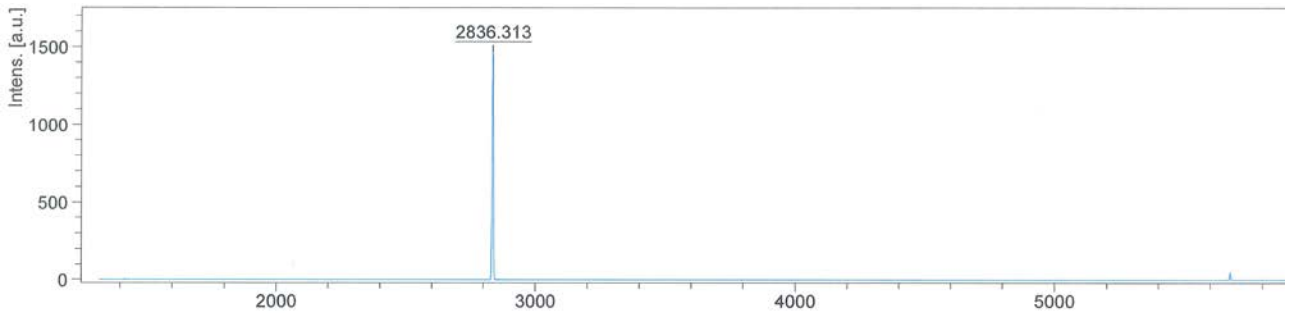

Figure S6. MALDI-MS spectrum for **ON5**.

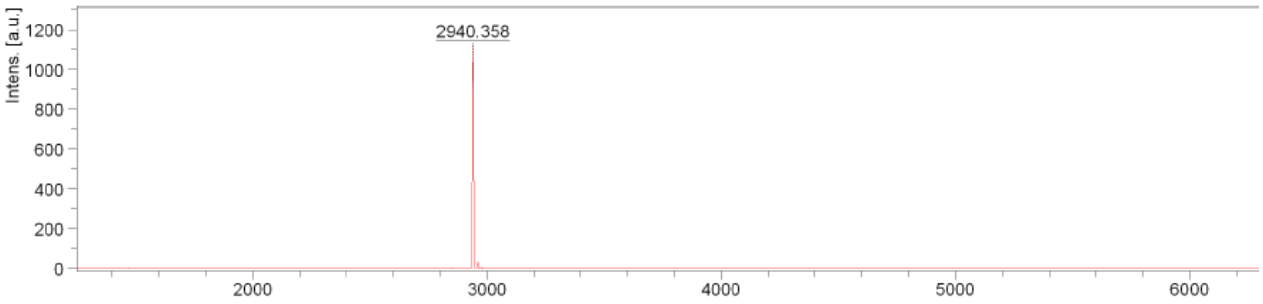

Figure S7. MALDI-MS spectrum for **ON6**(a).

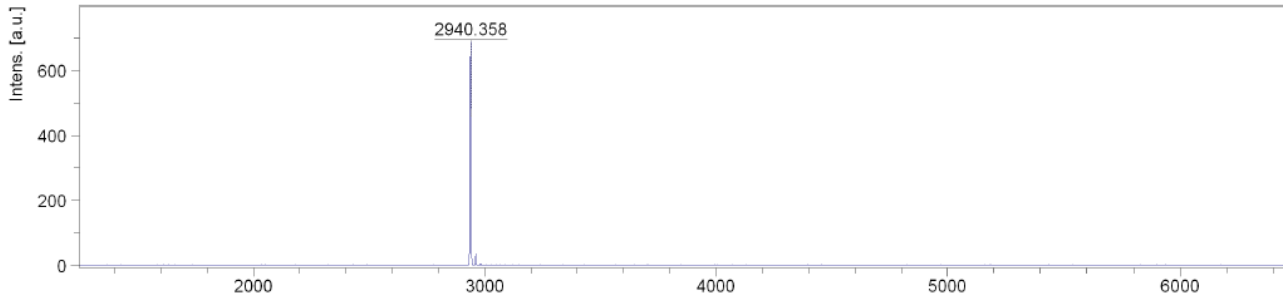

Figure S8. MALDI-MS spectrum for **ON6**(b).

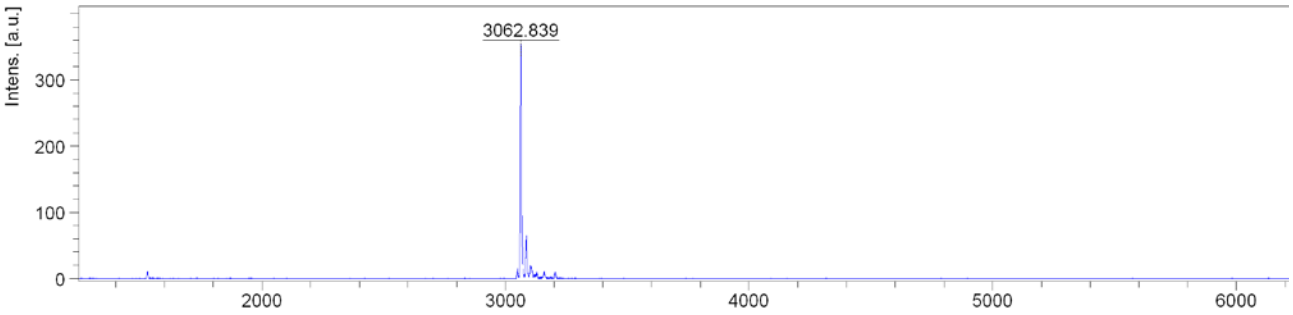

Figure S9. MALDI-MS spectrum for **ON7**(a).

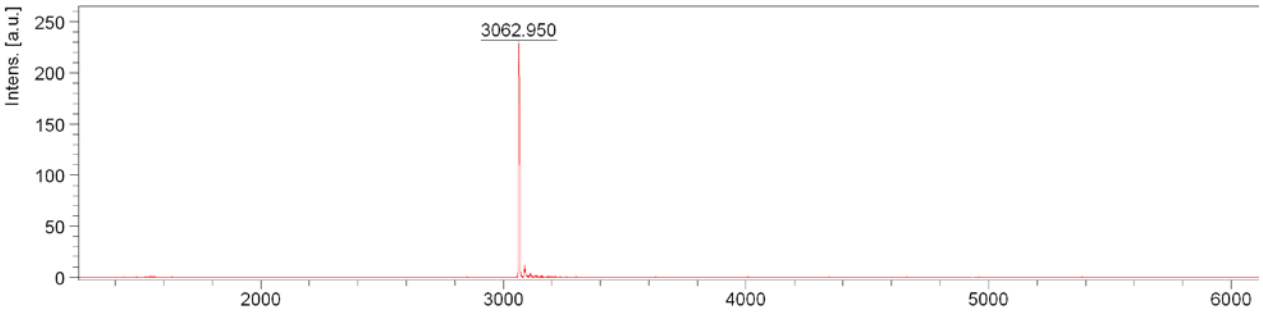

Figure S10. MALDI-MS spectrum for **ON7**(b).

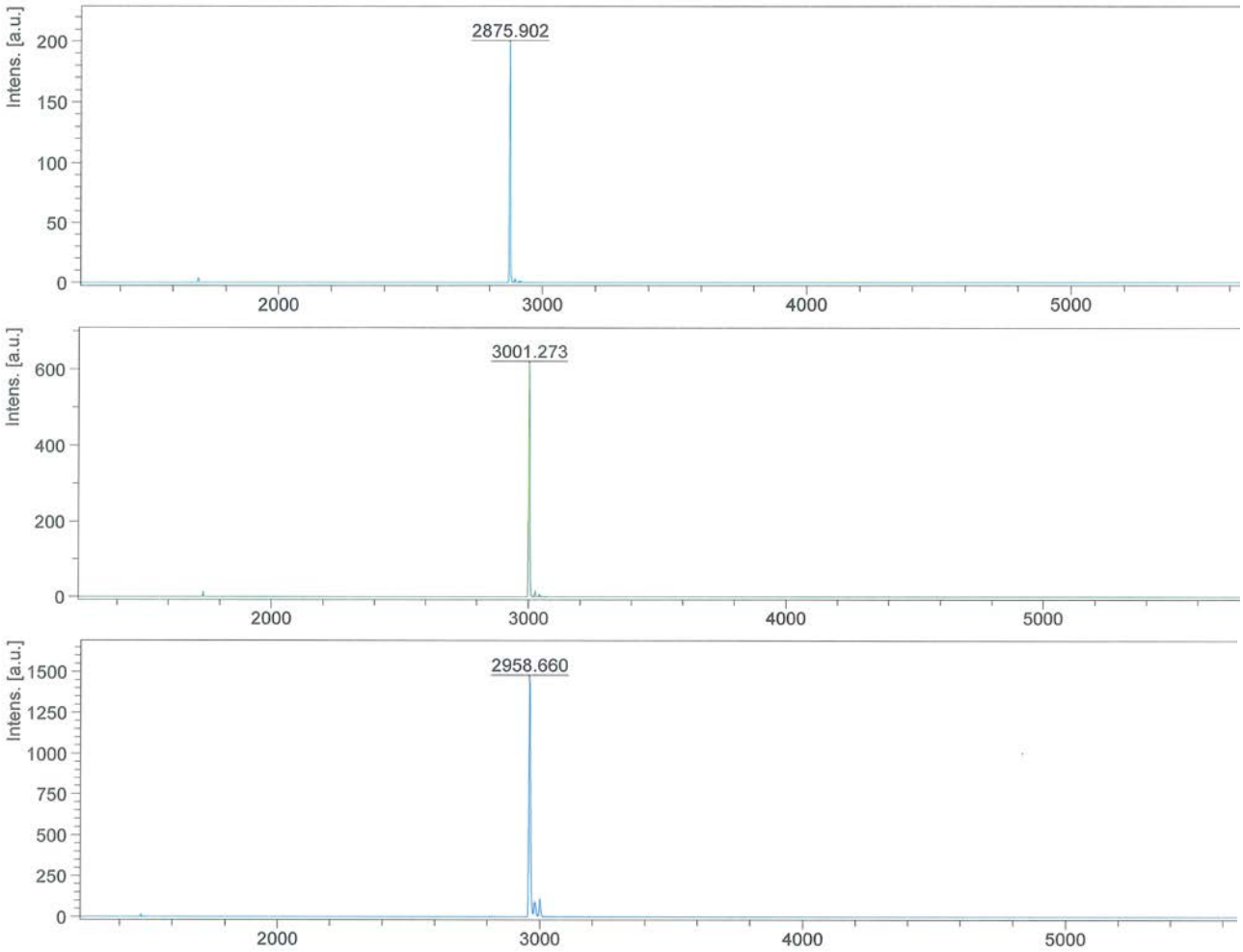

Figure S11. MALDI-MS spectra for **ON8**(a). (Top) Dehalogenation in third position (calculated mass: 2875.0); (middle) no reaction in third position (calculated mass: 3001.9); (bottom) three substitutions (calculated mass: 2958.0).

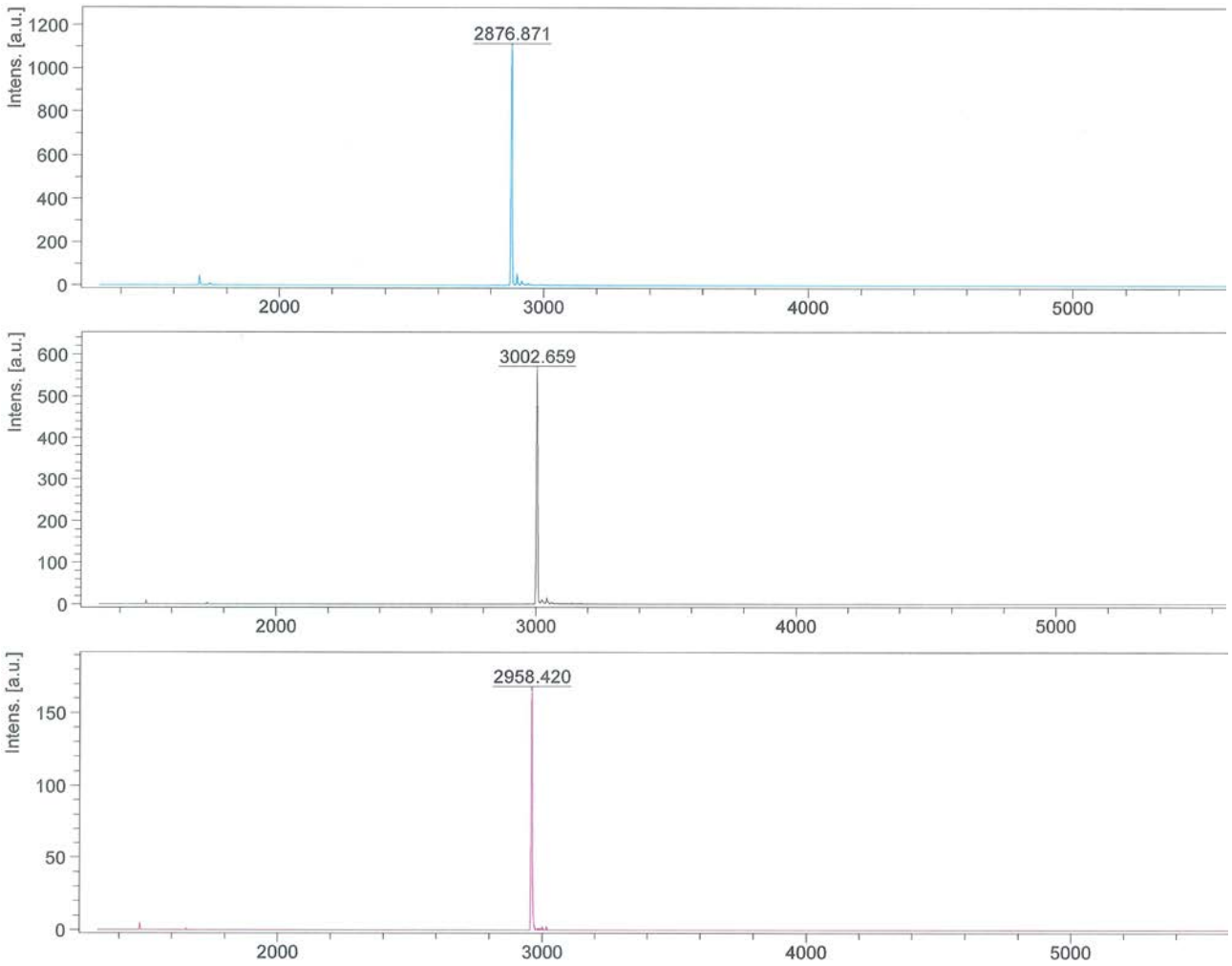

Figure S12. MALDI-MS spectra for **ON8**(b). (Top) Dehalogenation in third position (calculated mass: 2875.0); (middle) no reaction in third position (calculated mass: 3001.9); (bottom) three substitutions (calculated mass: 2958.0).

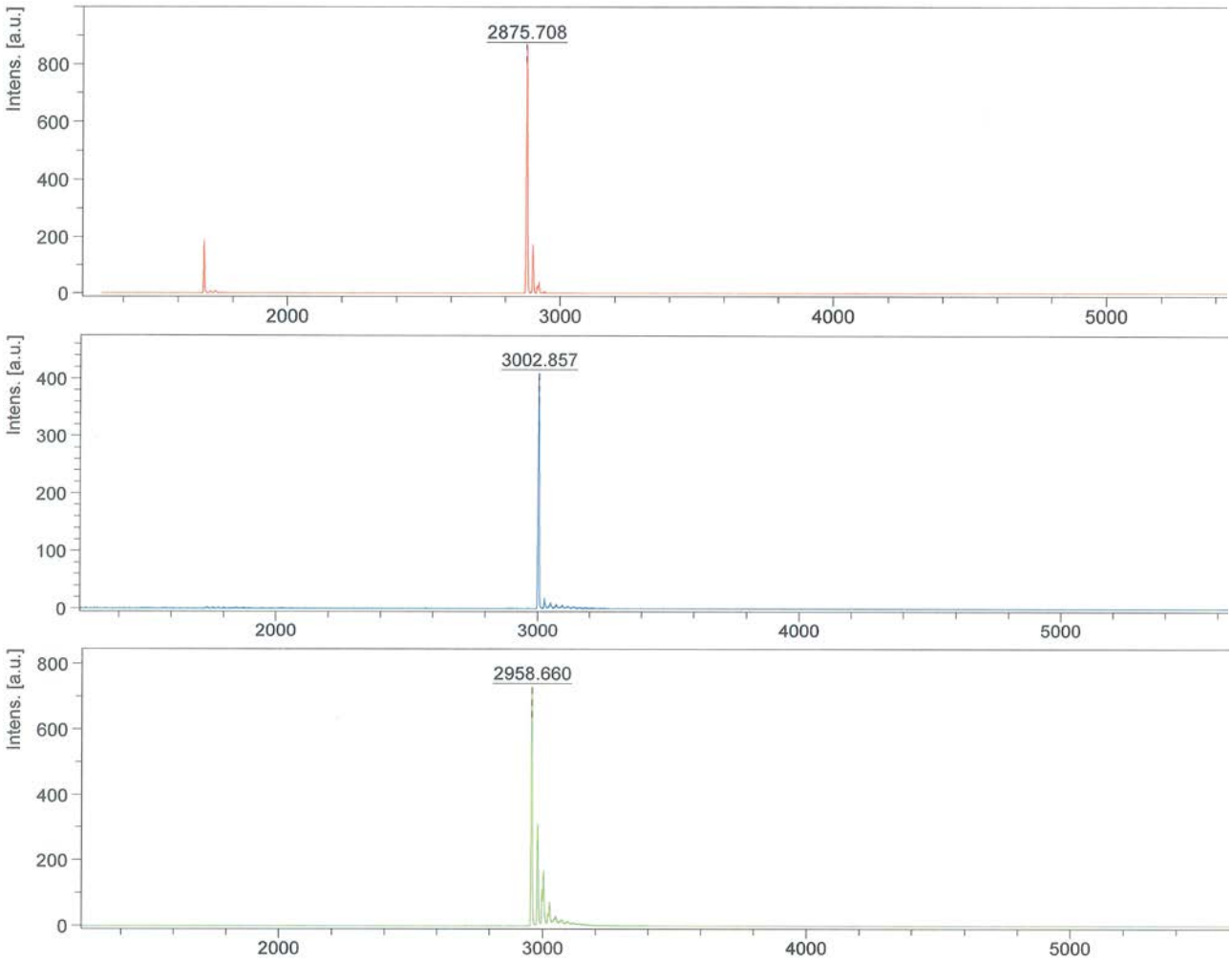

Figure S13. MALDI-MS spectra for **ON8**(c). (Top) Dehalogenation in third position (calculated mass: 2875.0); (middle) no reaction in third position (calculated mass: 3001.9); (bottom) three substitutions (calculated mass: 2958.0).

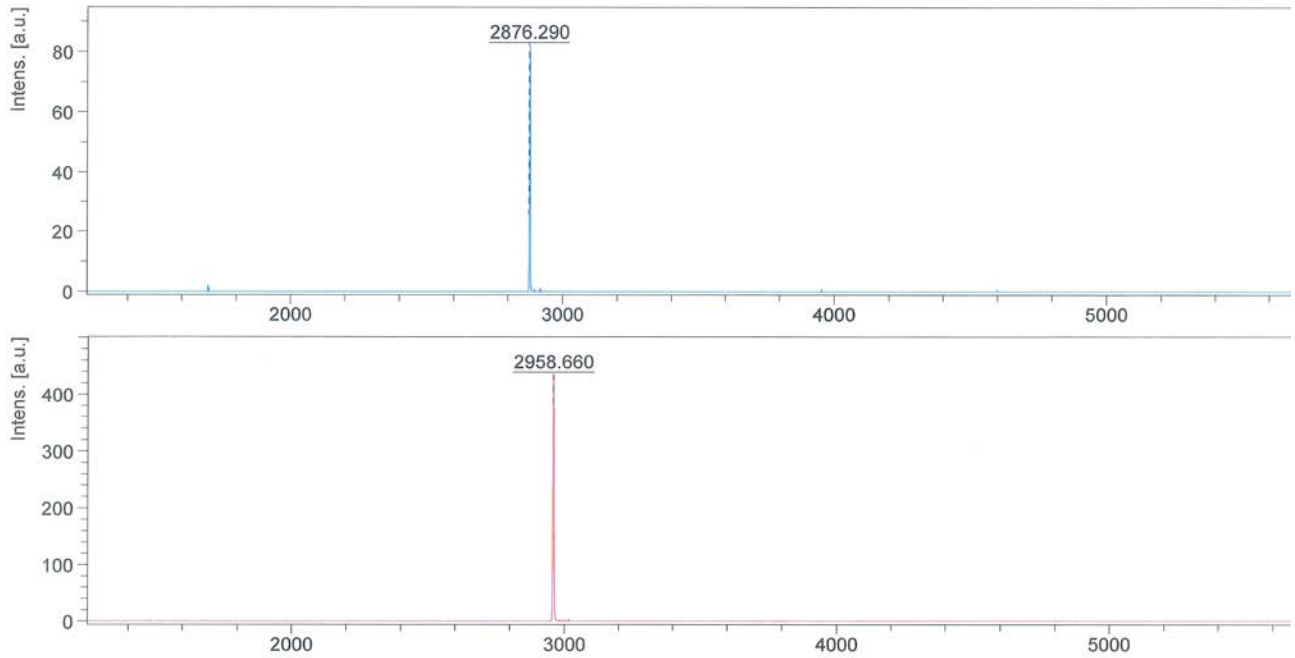

Figure S14. MALDI-MS spectra for **ON8**(d). (Top) Dehalogenation in third position (calculated mass: 2875.0); (bottom) three substitutions (calculated mass: 2958.0).

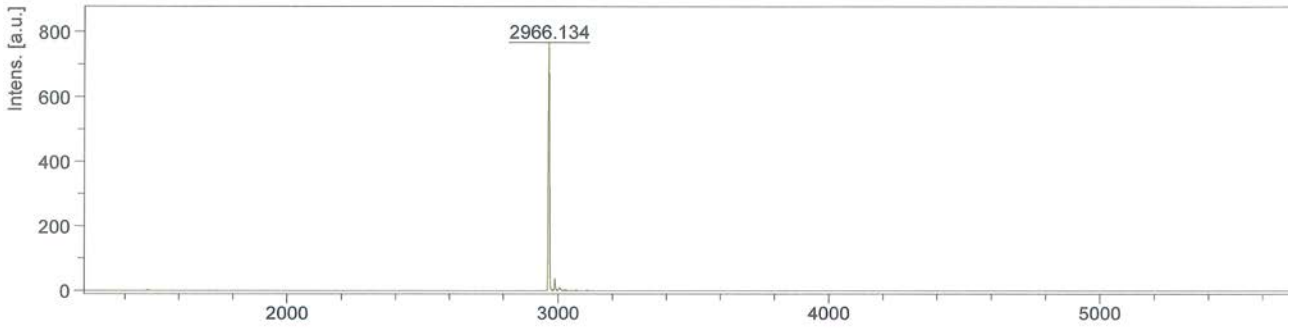

Figure S15. MALDI-MS spectrum for **ON9.**

RP-HPLC profiles

ONa 5'- dU^{I}TA CG (crude)

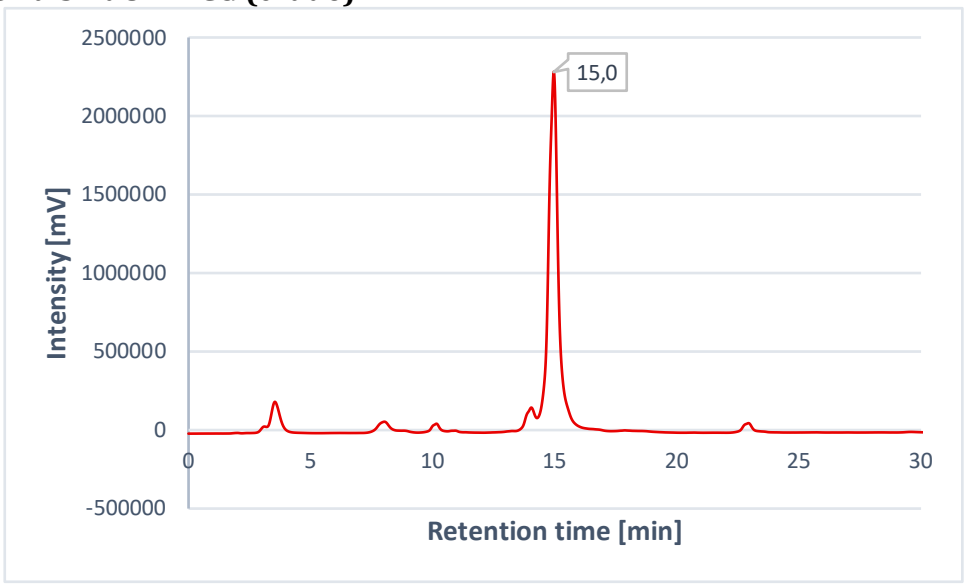

Figure S16. Crude **ONa** before cross-coupling reaction, (eluent: MeOH/50 mM TEAA buffer pH 7.0)

ONa 5'- dUThTA CG (crude)

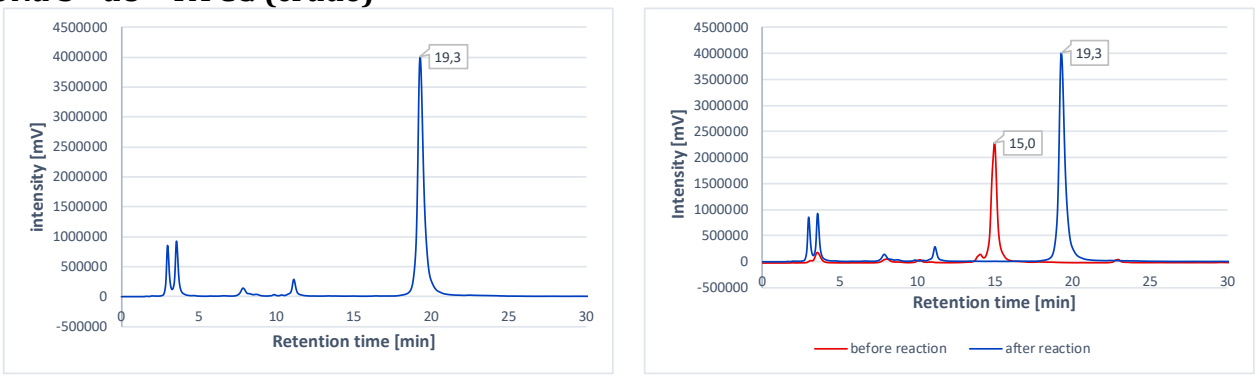

Figure S17. (left) crude **ONa** after reaction; (right) overlay of spectra from crude **ONa** before and after reaction, (eluent: MeOH/50 mM TEAA buffer pH 7.0)

ONb 5'- TAdU^{I} CG (crude)

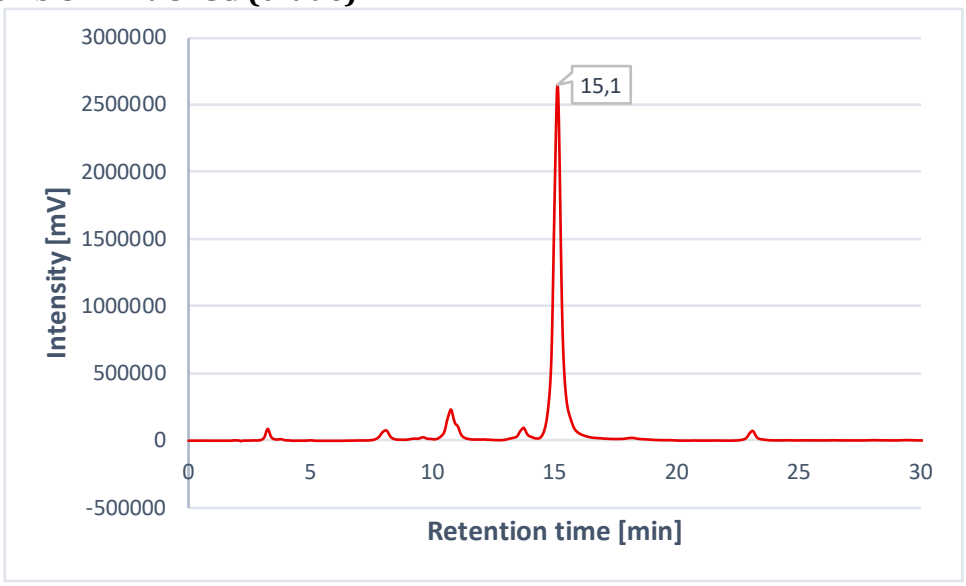

Figure S18. Crude **ONb** before cross-coupling reaction, (eluent: MeOH/50 mM TEAA buffer pH 7.0)

ONb 5'- TAdUTh CG (crude)

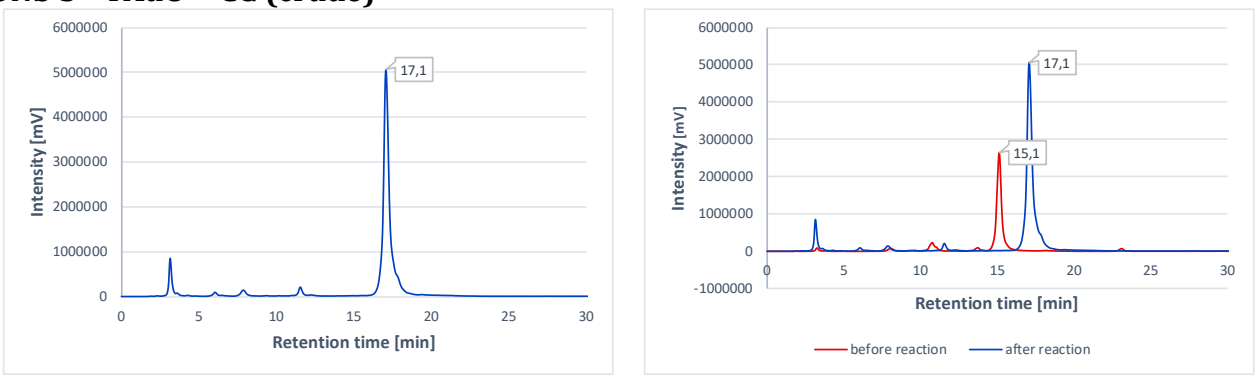

Figure S19. (left) crude **ONb** after reaction; (right) overlay of spectra from crude **ONb** before and after reaction, (eluent: MeOH/50 mM TEAA buffer pH 7.0)

ON1 5'-GTG AdU^{I}A TGC

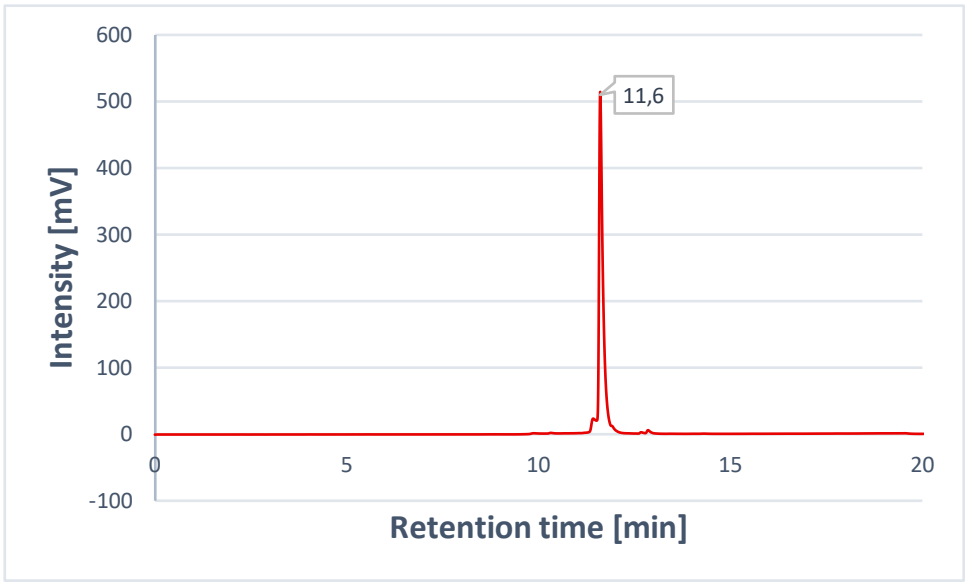

Figure S20. Purified **ON1** (eluent: MeCN/50 mM TEAA buffer pH 7.4)

ON2 5'-GdU^{I}G AdU^{I}A dU^{I}GC

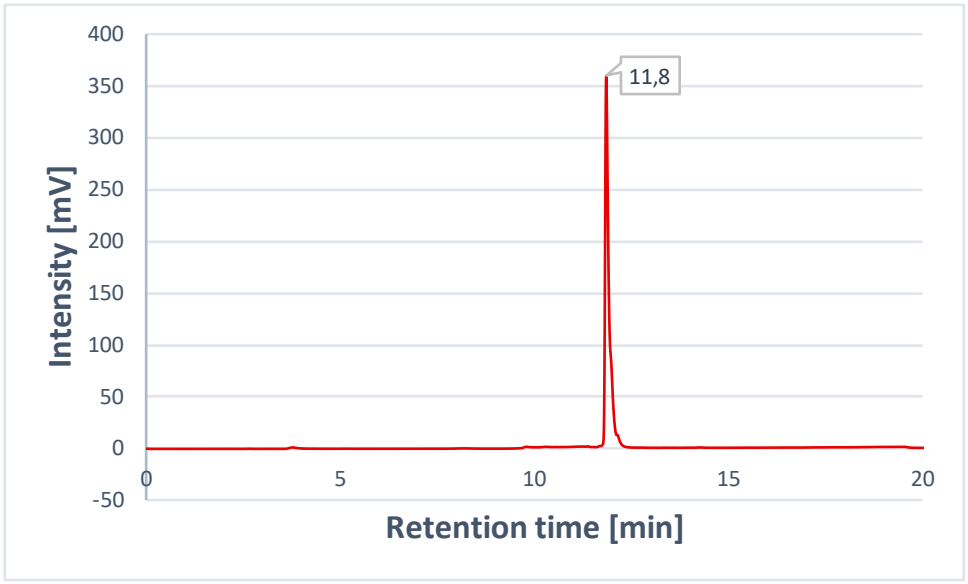

Figure S21. Purified **ON2** (eluent: MeCN/50 mM TEAA buffer pH 7.4)

ON3(a) 5'-GTG AdUThA TGC

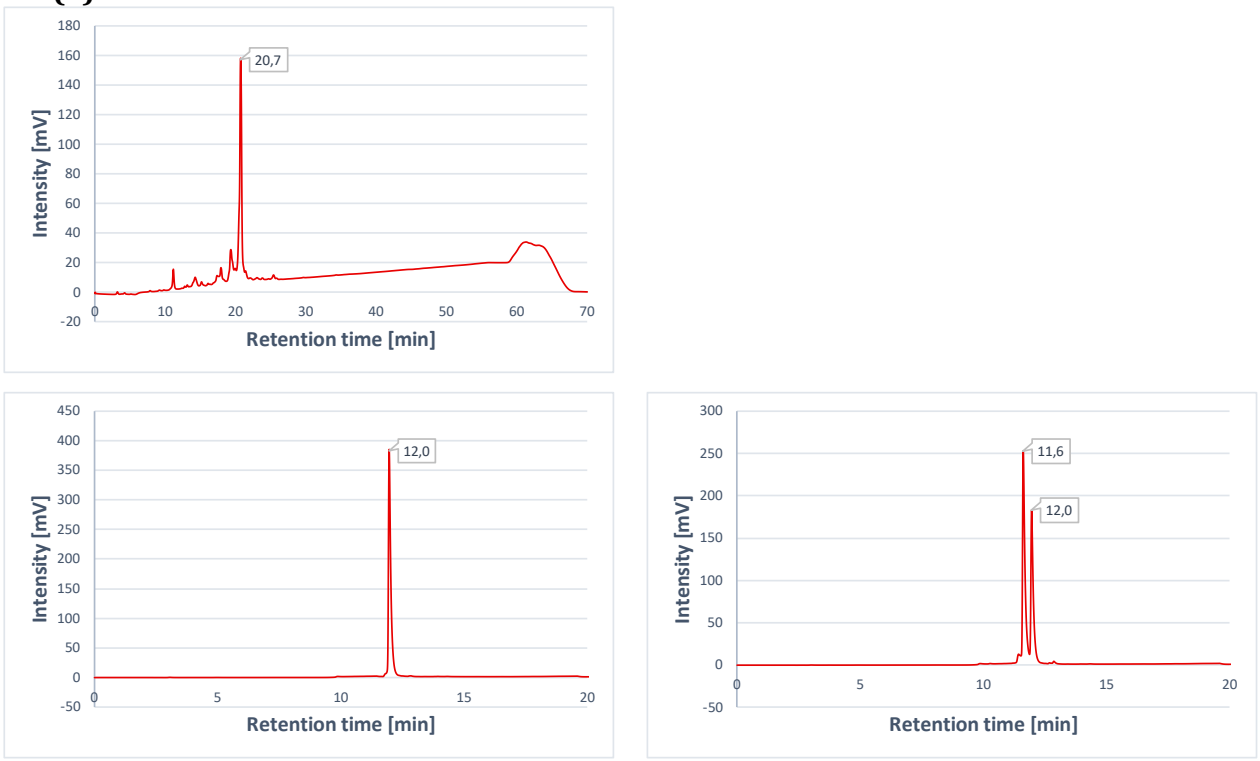

Figure S22. RP-HPLC spectra for **ON3**(a). (top left) Crude **ON3**(a), (eluent: MeOH/50 mM TEAA buffer pH 7.4); (bottom left) purified **ON3**(a); (bottom right) co-injection of **ON3**(a) and **ON1**, (eluent: MeCN/50 mM TEAA buffer pH 7.4)

ON3(b) 5'-GTG AdUThA TGC

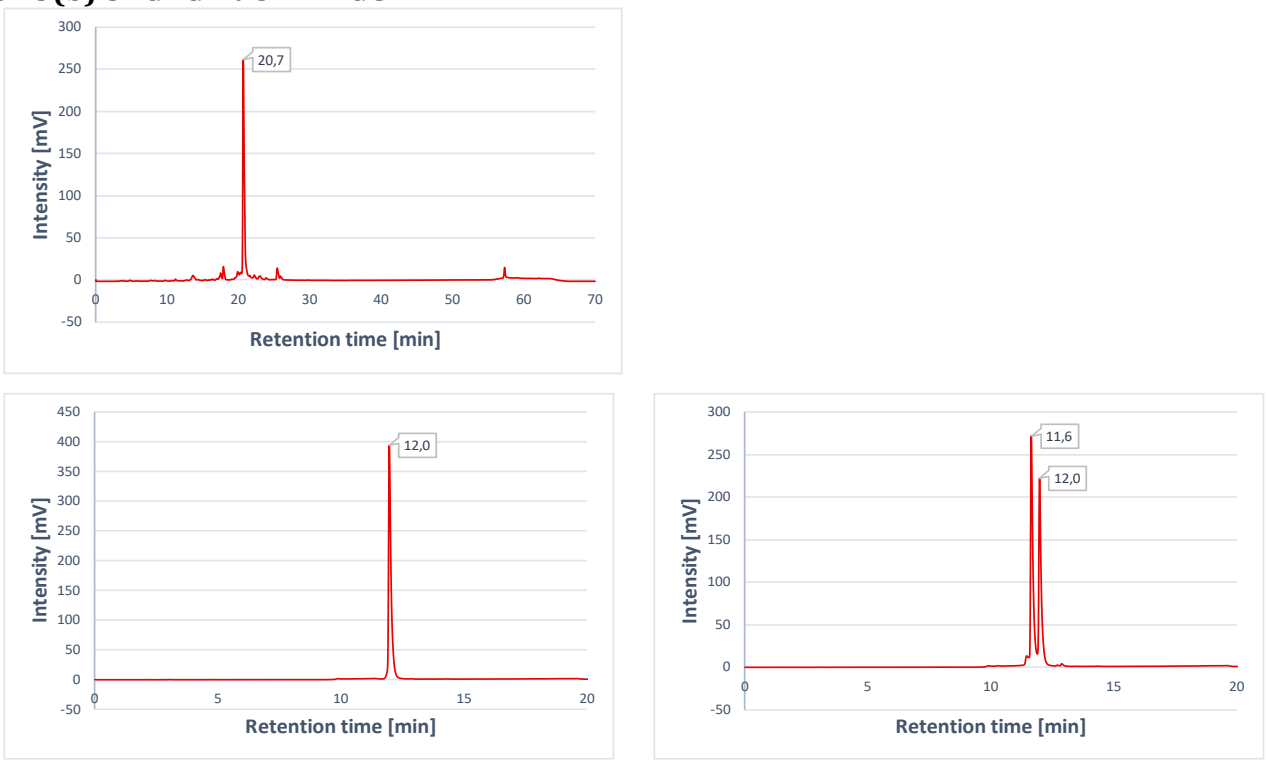

Figure S23. RP-HPLC spectra for **ON3**(b). (top left) Crude **ON3**(b), (eluent: MeOH/50 mM TEAA buffer pH 7.4); (bottom left) purified **ON3**(b); (bottom right) co-injection of **ON3**(b) and **ON1**, (eluent: MeCN/50 mM TEAA buffer pH 7.4)

ON4 5'-GTG AdU^{MeTh}A TGC

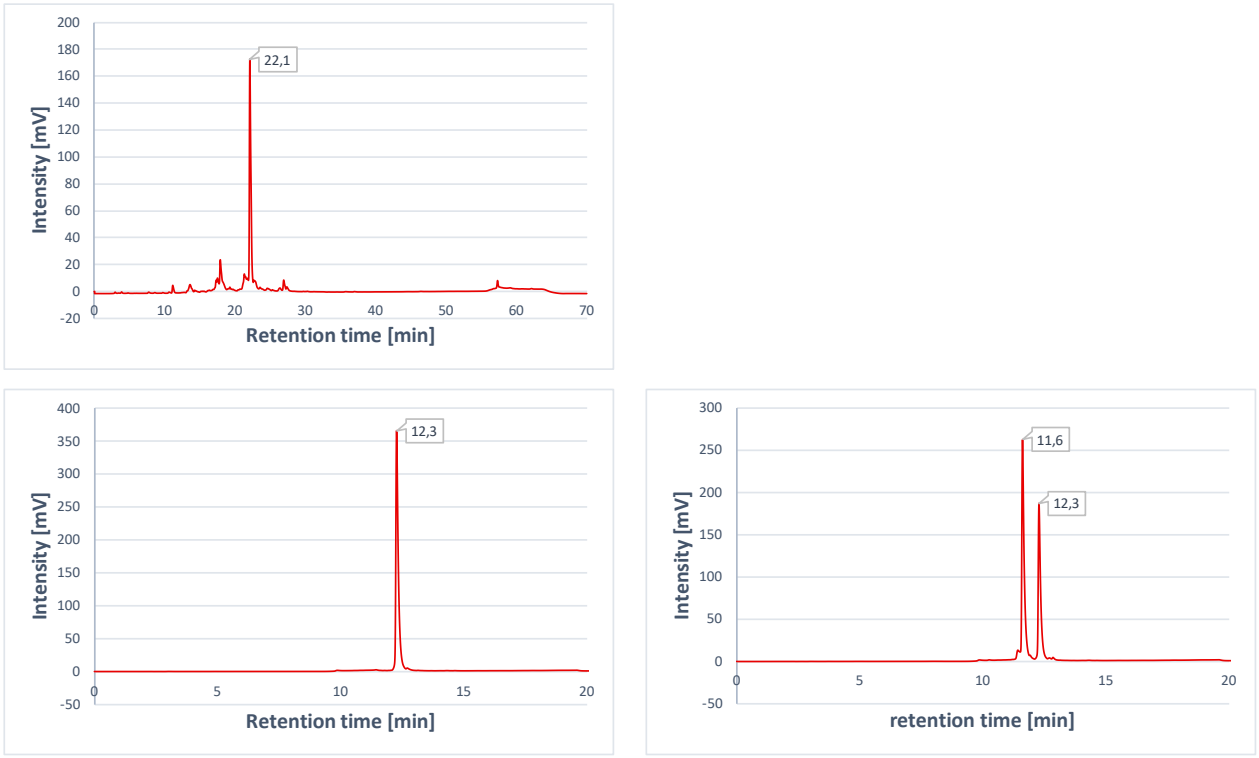

Figure S24. RP-HPLC spectra for **ON4**. (top left) Crude **ON4**, (eluent: MeOH/50 mM TEAA buffer pH 7.4); (bottom left) purified **ON4**; (bottom right) co-injection of **ON4** and **ON1**, (eluent: MeCN/50 mM TEAA buffer pH 7.4)

ON5 5'-GTG AdU^{Ph}A TGC

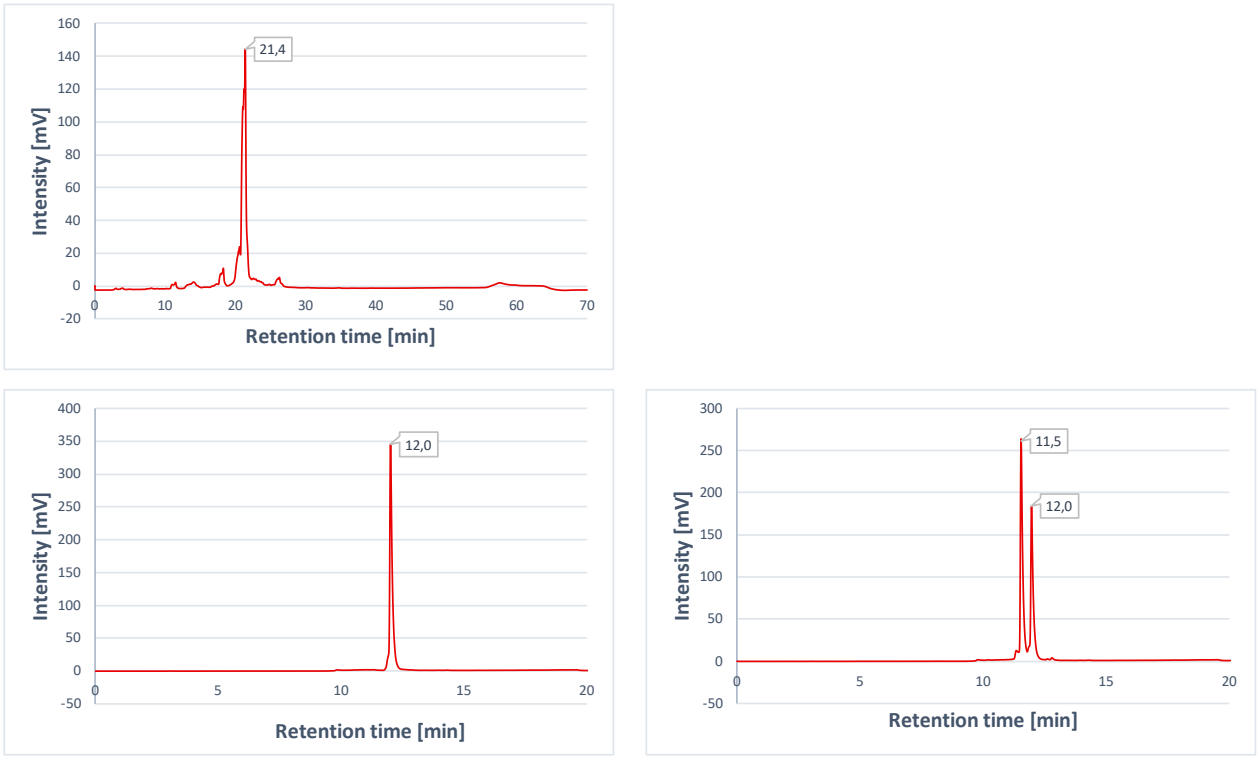

Figure S25. RP-HPLC spectra for **ON5**. (top left) Crude **ON5**, (eluent: MeOH/50 mM TEAA buffer pH 7.4); (bottom left) purified **ON5**; (bottom right) co-injection of **ON5** and **ON1**, (eluent: MeCN/50 mM TEAA buffer pH 7.4)

ON6(a) 5'-GTG AdU^{Py}A TGC

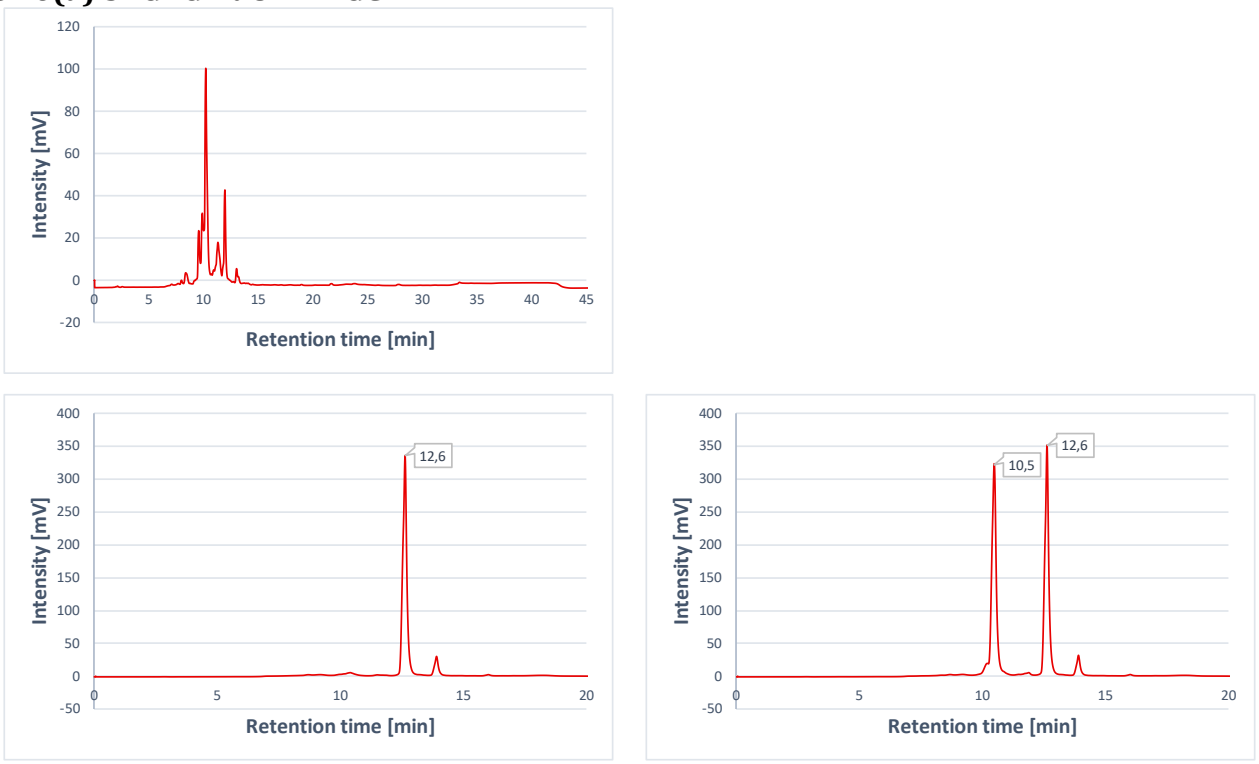

Figure S26. RP-HPLC spectra for **ON6**(a). (top left) Crude **ON6**(a); (bottom left) purified **ON6**(a); (bottom right) co-injection of **ON6**(a) and **ON1**, (eluent: MeCN/50 mM TEAA buffer pH 7.4)

ON6(b) 5'-GTG AdU^{Py}A TGC

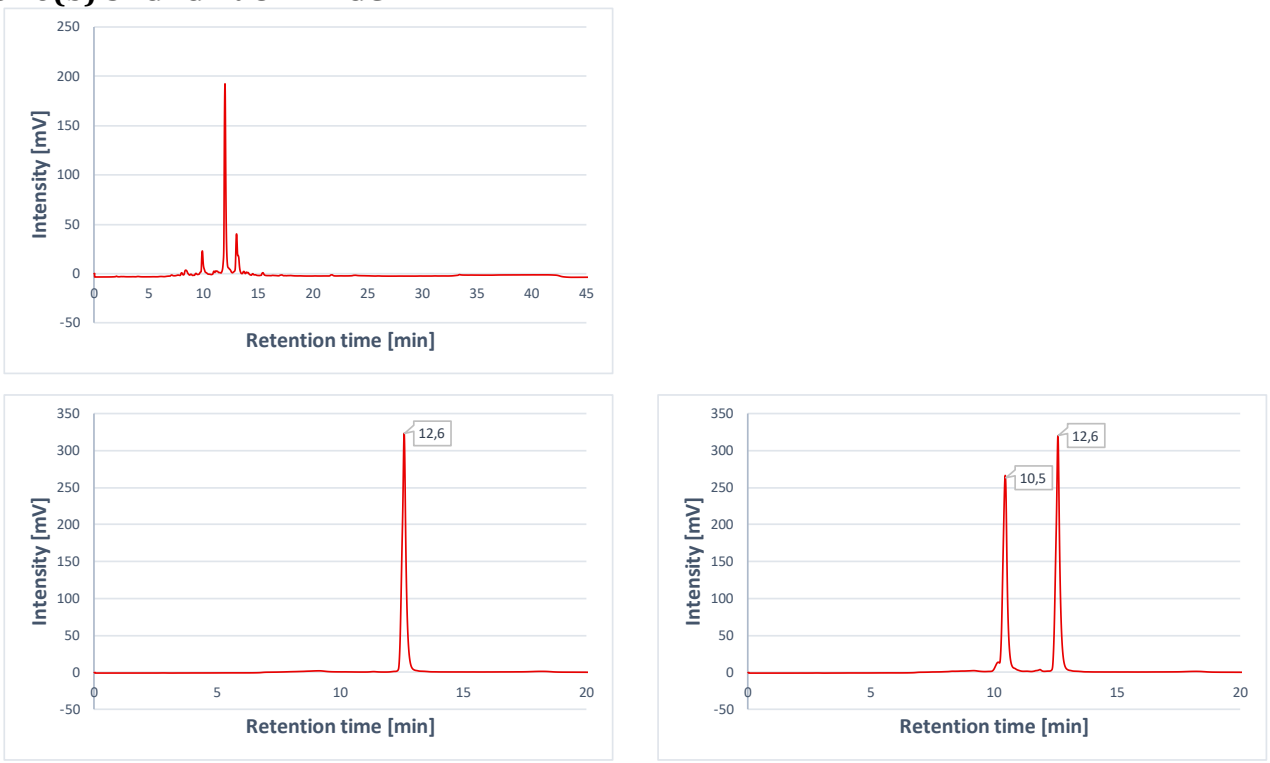

Figure S27. RP-HPLC spectra for **ON6**(b). (top left) Crude **ON6**(b); (bottom left) purified **ON6**(b); (bottom right) co-injection of **ON6**(b) and **ON1**, (eluent: MeCN/50 mM TEAA buffer pH 7.4)

ON7(a) 5'-GTG AdU^{Dap}A TGC

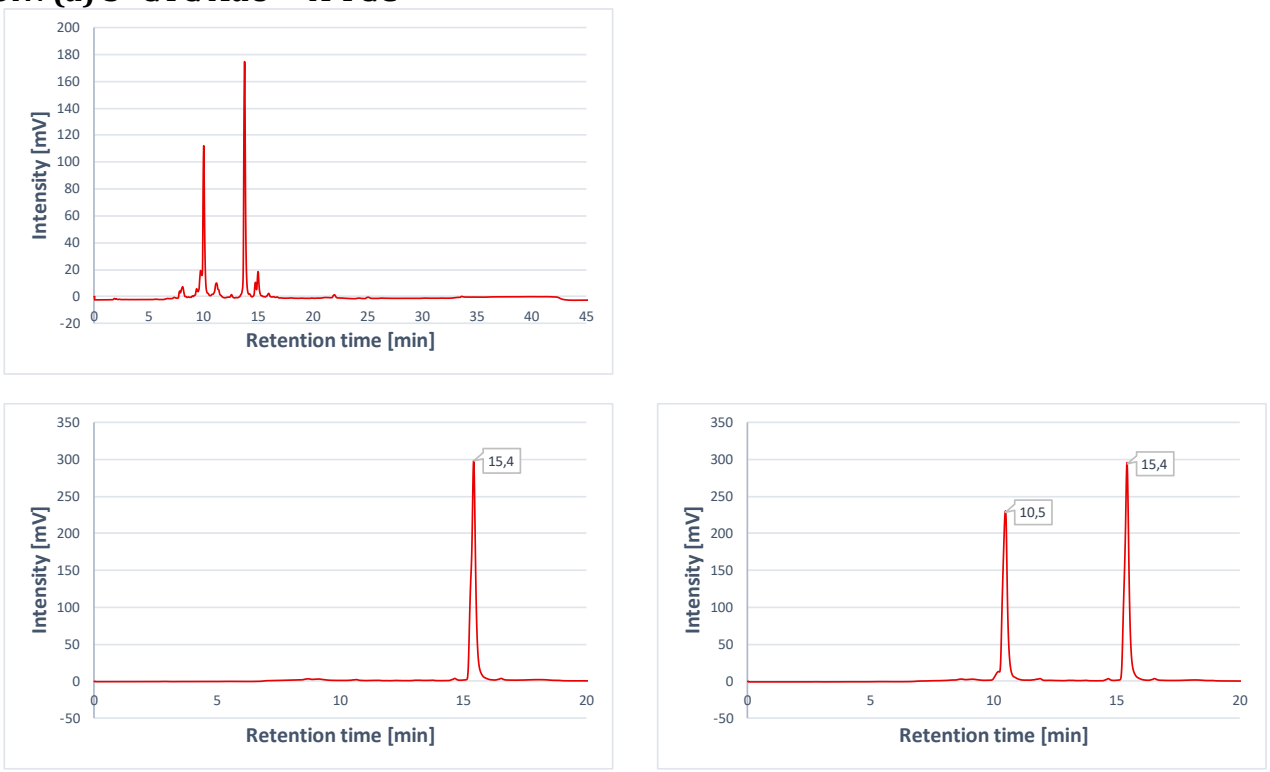

Figure S28. RP-HPLC spectra for **ON7**(a). (top left) Crude **ON7**(a); (bottom left) purified **ON7**(a); (bottom right) co-injection of **ON7**(a) and **ON1**, (eluent: MeCN/50 mM TEAA buffer pH 7.4)

ON7(b) 5'-GTG AdU^{Dap}A TGC

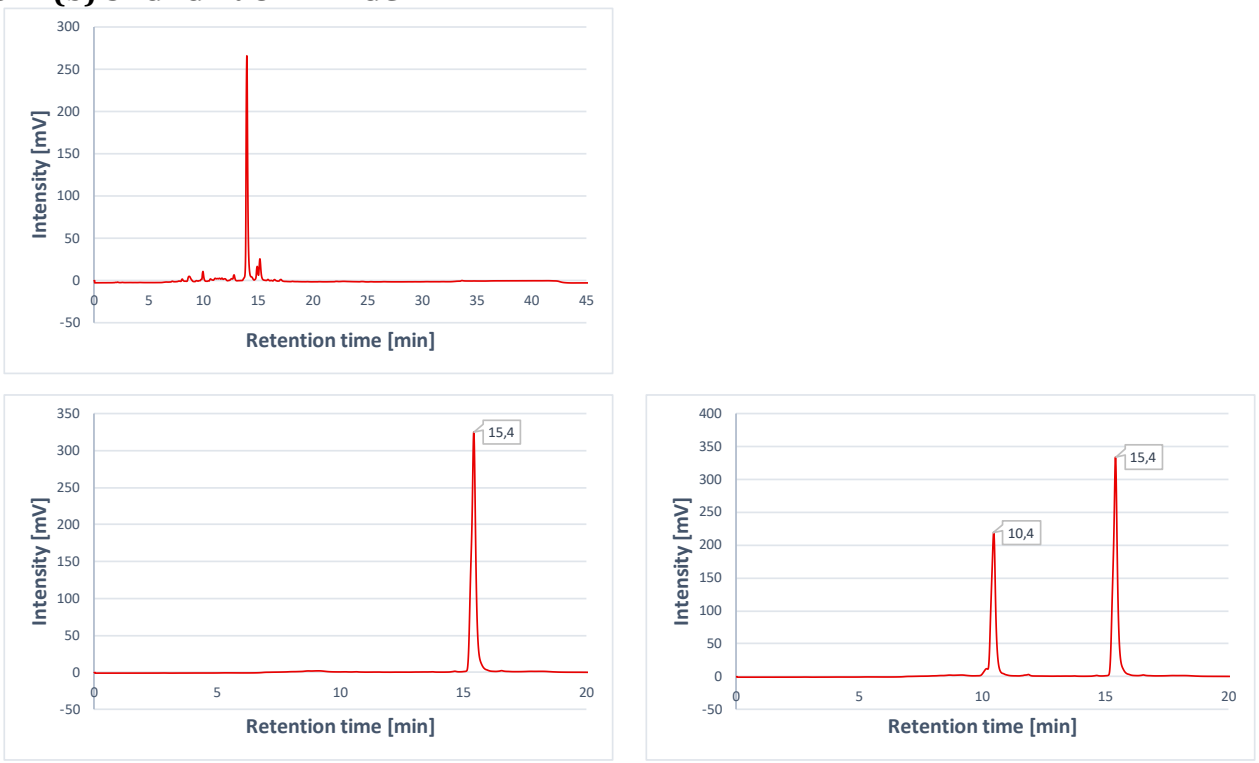

Figure S29. RP-HPLC spectra for **ON7**(b). (top left) Crude **ON7**(b); (bottom left) purified **ON7**(b); (bottom right) co-injection of **ON7**(b) and **ON1**, (eluent: MeCN/50 mM TEAA buffer pH 7.4)

ON8(a) 5'-GdUThG AdUThA dUThGC

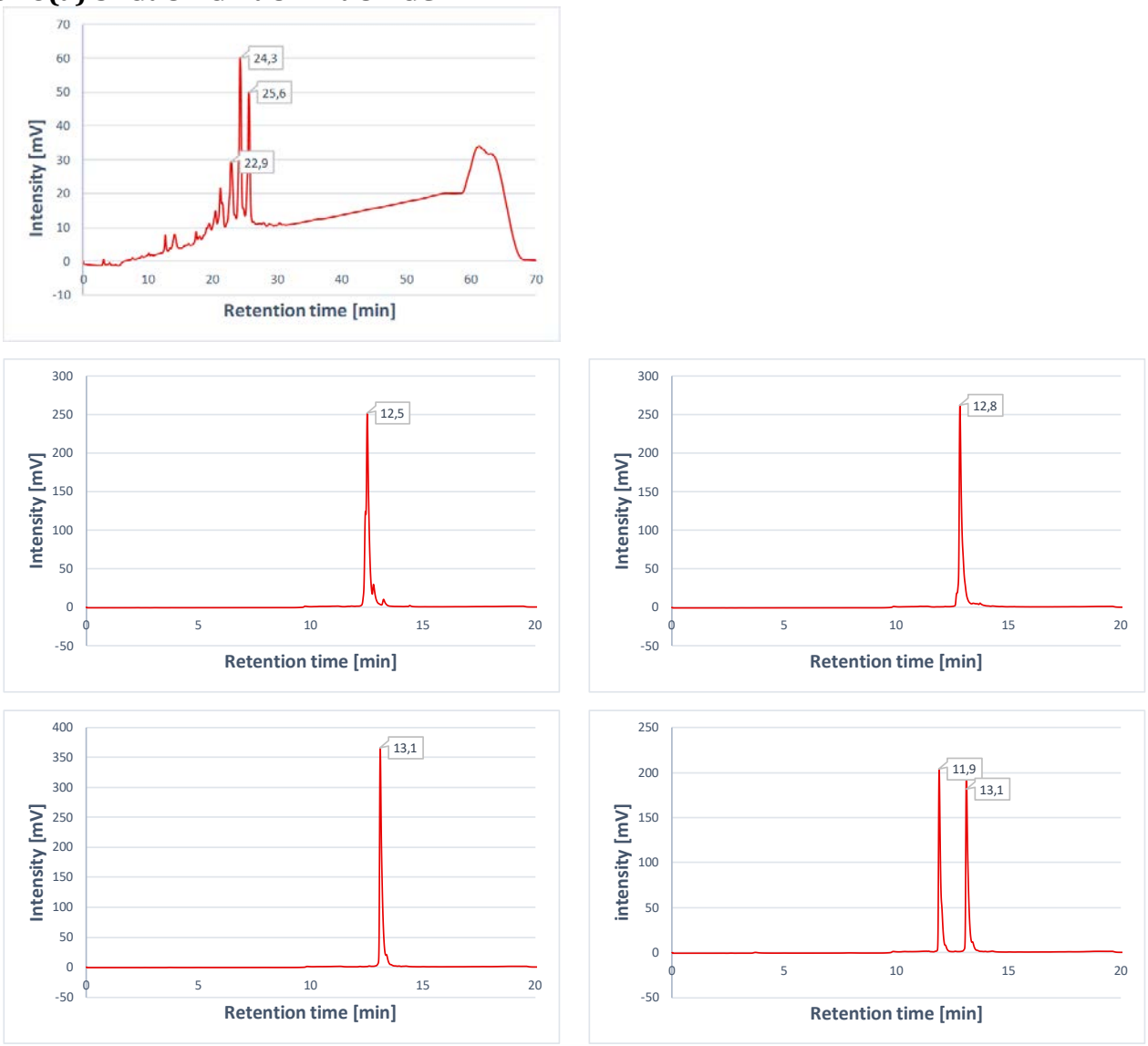

Figure S30. RP-HPLC spectra for **ON8**(a). (top left) Crude **ON8**(a) (eluent: MeOH/50 mM TEAA buffer pH 7.4); (middle left) dehalogenation in third position; (middle right) no reaction in third position; (bottom left) purified **ON8**(a); (bottom right) co-injection of **ON8**(a) and **ON2**, (eluent: MeCN/50 mM TEAA buffer pH 7.4).

ON8(b) 5’-GdUThG AdUThA dUThGC

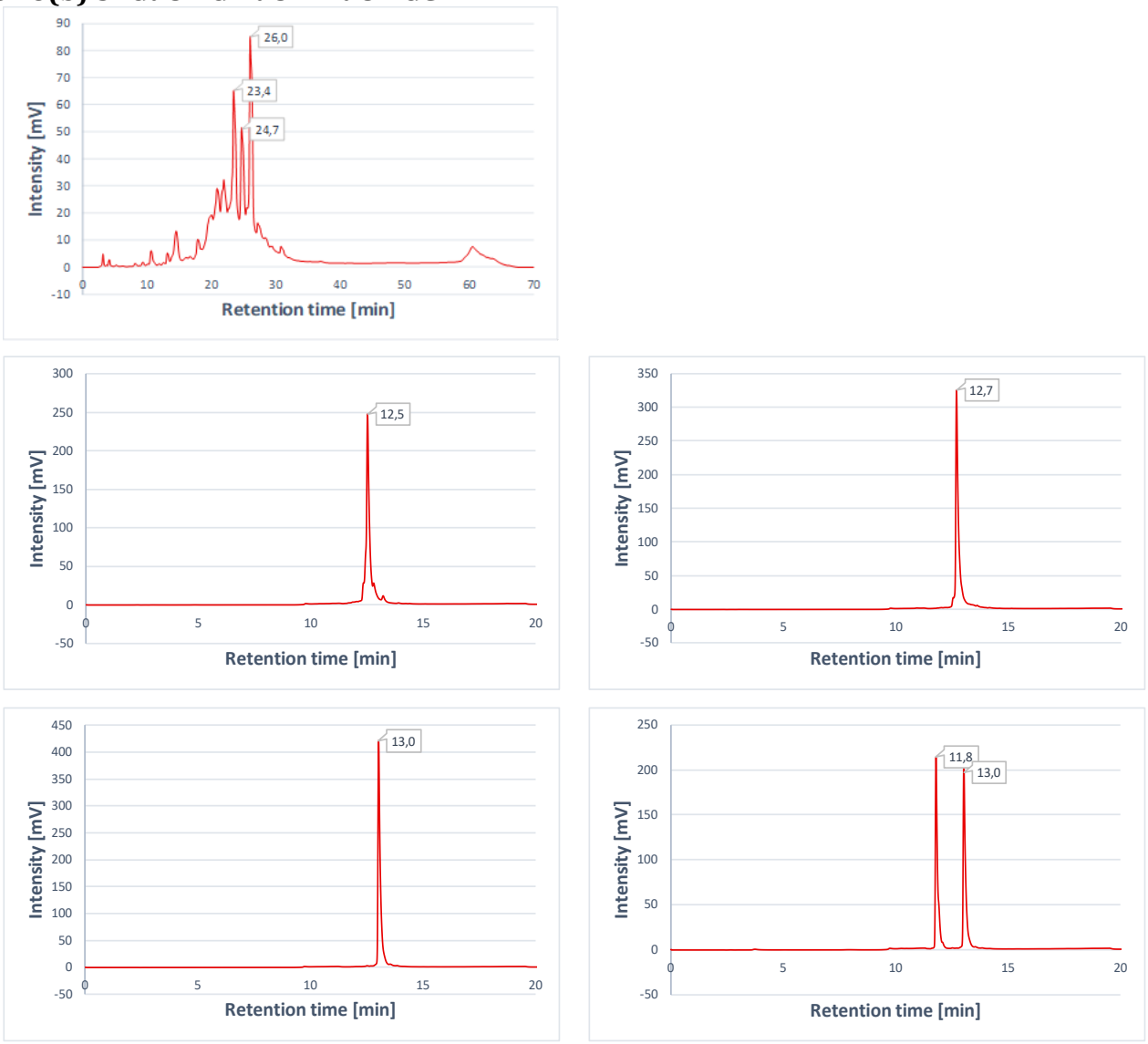

Figure S31. RP-HPLC spectra for **ON8**(b). (top left) Crude **ON8**(b) (eluent: MeOH/50 mM TEAA buffer pH 7.4); (middle left) dehalogenation in third position; (middle right) no reaction in third position; (bottom left) purified **ON8**(b); (bottom right) co-injection of **ON8**(b) and **ON2**, (eluent: MeCN/50 mM TEAA buffer pH 7.4).

ON8(c) 5’-GdUThG AdUThA dUThGC

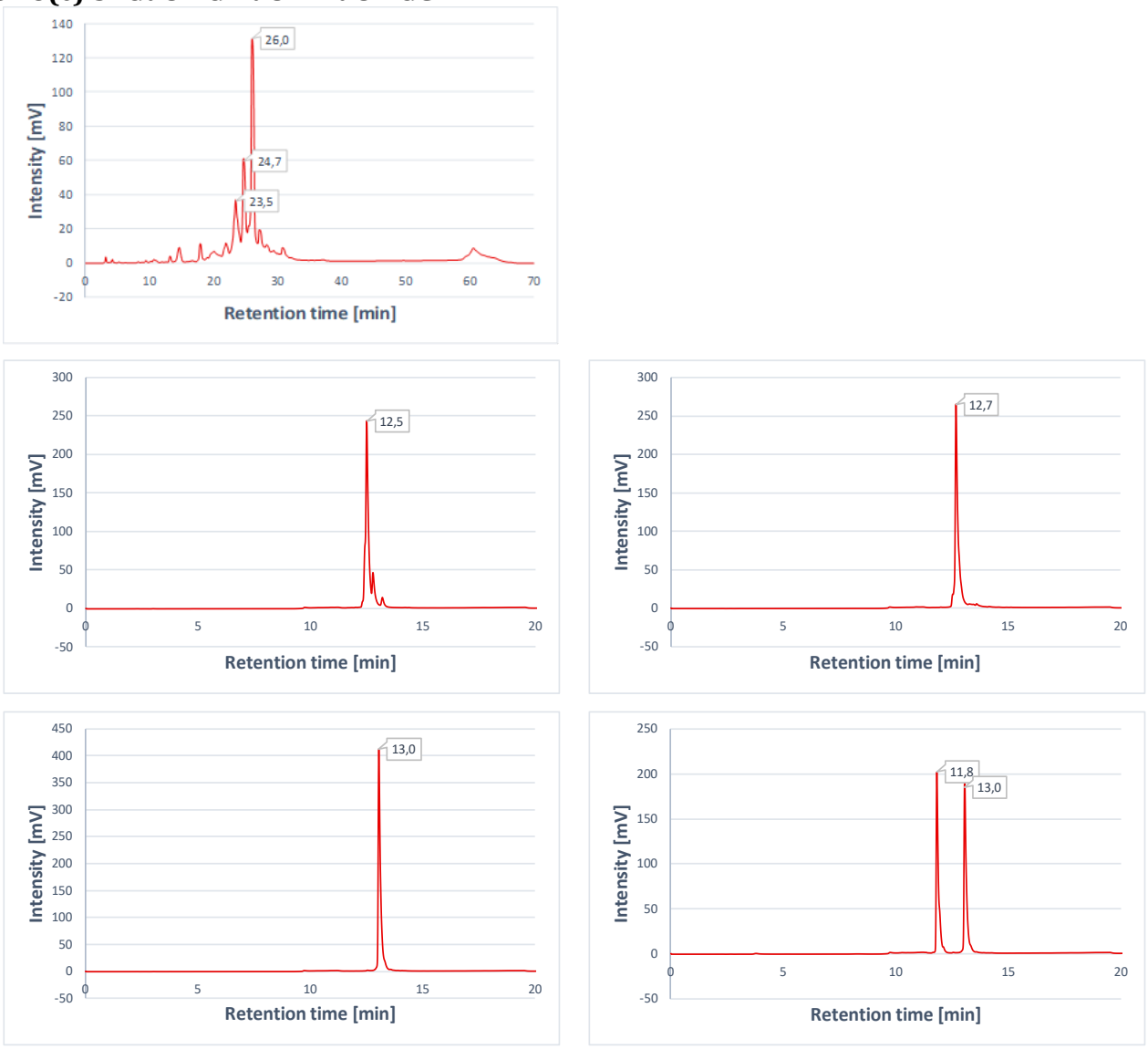

Figure S32. RP-HPLC spectra for **ON8**(c). (top left) Crude **ON8**(c) (eluent: MeOH/50 mM TEAA buffer pH 7.4); (middle left) dehalogenation in third position; (middle right) no reaction in third position; (bottom left) purified **ON8**(c); (bottom right) co-injection of **ON8**(c) and **ON2**, (eluent: MeCN/50 mM TEAA buffer pH 7.4).

ON8(d) 5'-GdUThG AdUThA dUThGC

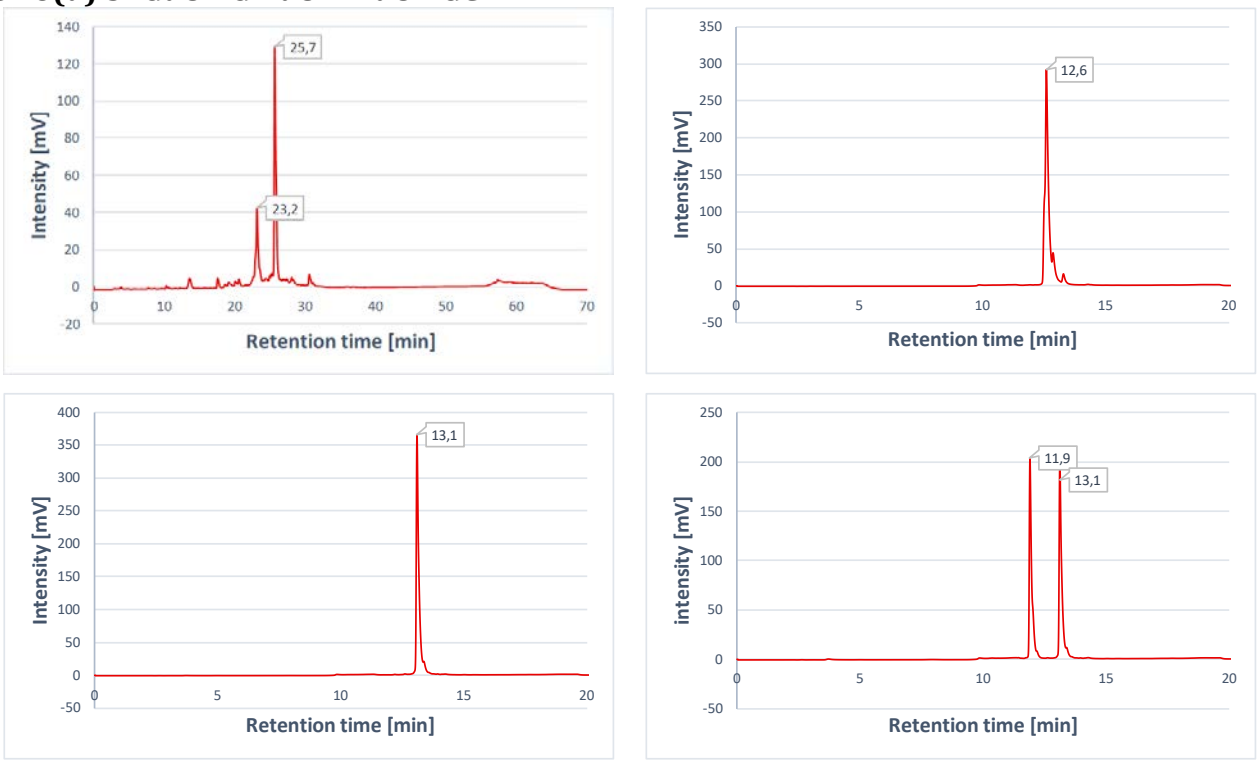

Figure S33. RP-HPLC spectra for purified **ON8**(d). (top left) Crude **ON8**(d), (eluent: MeOH/50 mM TEAA buffer pH 7.4); (top right) dehalogenation in third position; (bottom left) **ON8**(d); (bottom right) co-injection of **ON8**(d) and **ON2**, (eluent: MeCN/50 mM TEAA buffer pH 7.4).

ON9 5'-GdU^{Ph}G AdU^{MeTh}A dUThGC

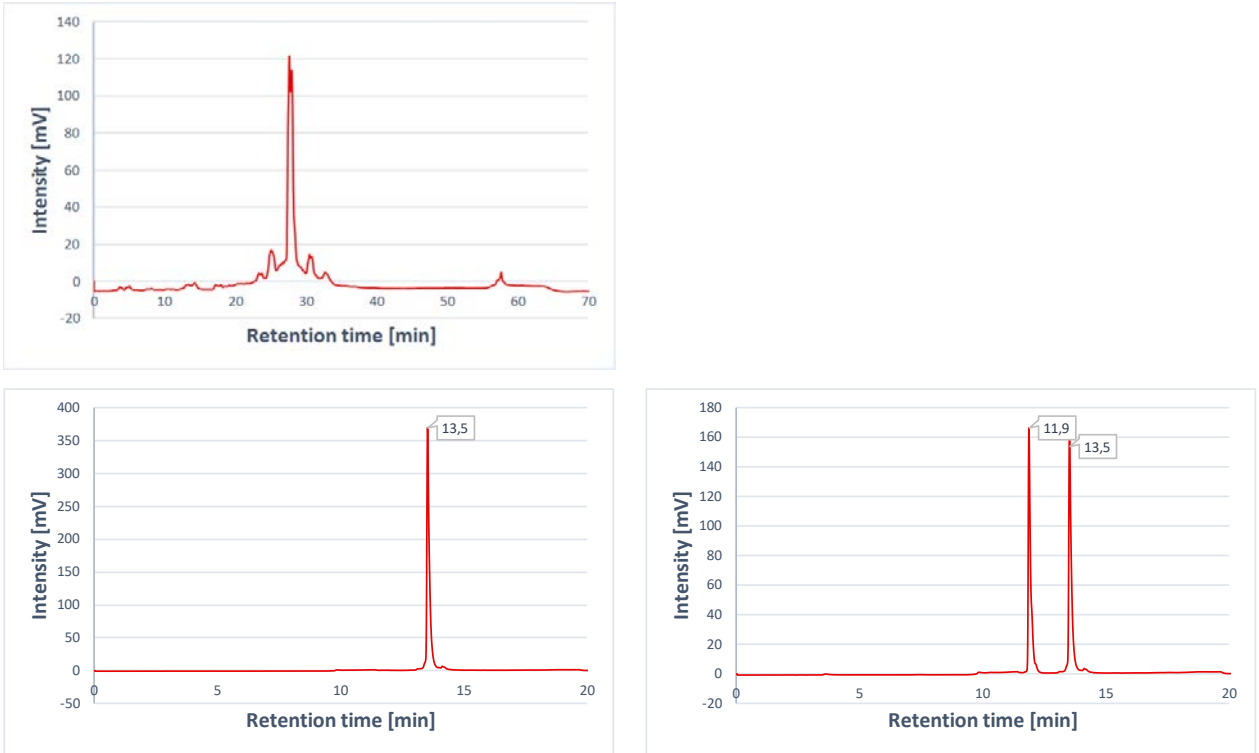

Figure S34. RP-HPLC spectra for **ON9**. (top left) Crude **ON9**, (eluent: MeOH/50 mM TEAA buffer pH 7.4); (bottom left) purified **ON9**; (bottom right) co-injection of **ON9** and **ON2**, (eluent: MeCN/50 mM TEAA buffer pH 7.4)

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