Supplementary Information

Enhancing mRNA Stability and Translational Potential Through Tailored Modifications at the 3' End

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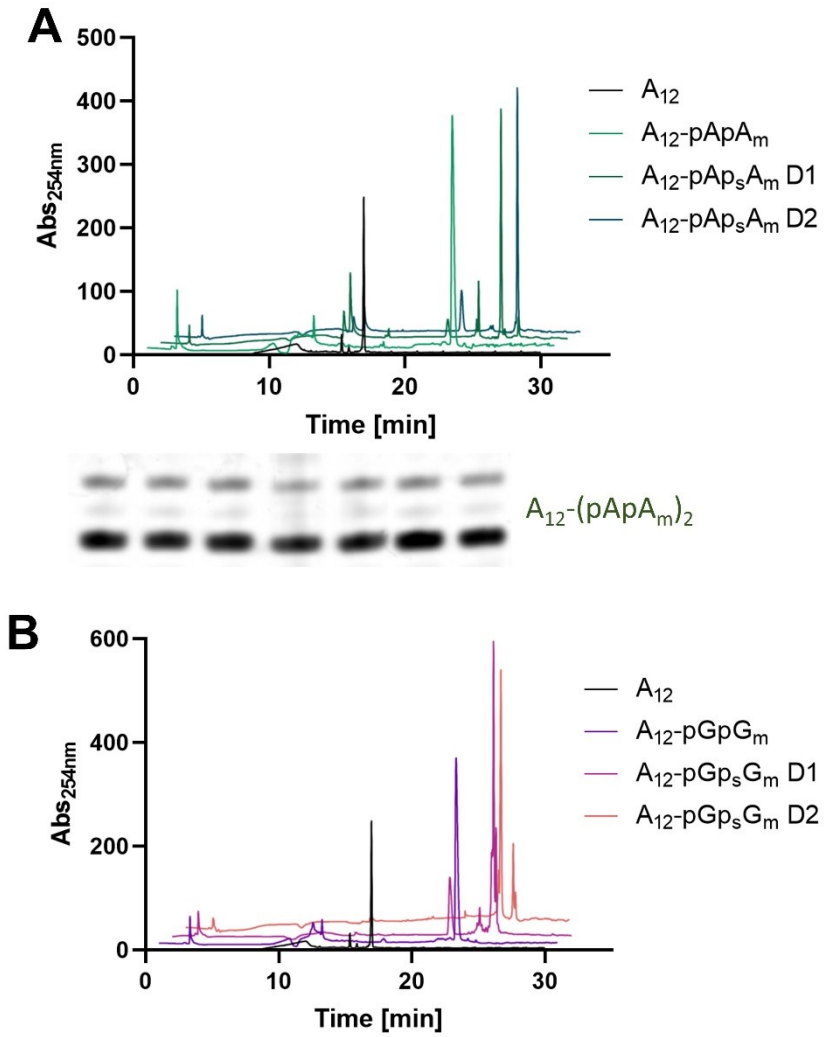


Figure S1. RP-HPLC analysis of short RNA after ligation with (A) adenosine and (B) guanosine dinucleotides. A_{12} RNA was used as reference. In (A) gel image of 16-mer product of double ligation with $pApA_m$ dinucleotide.

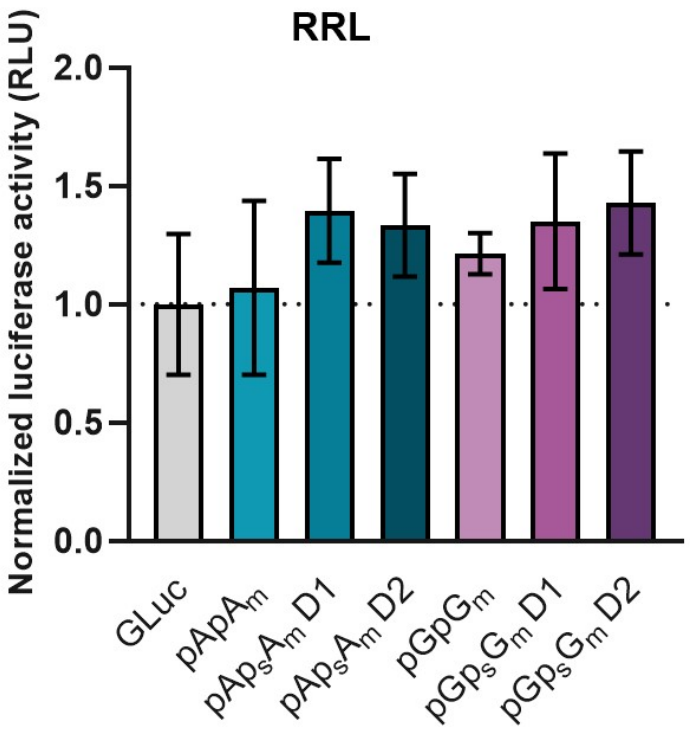


Figure S2. Translation efficiency experiment in Rabbit Reticulocyte Lysate (RRL) system.

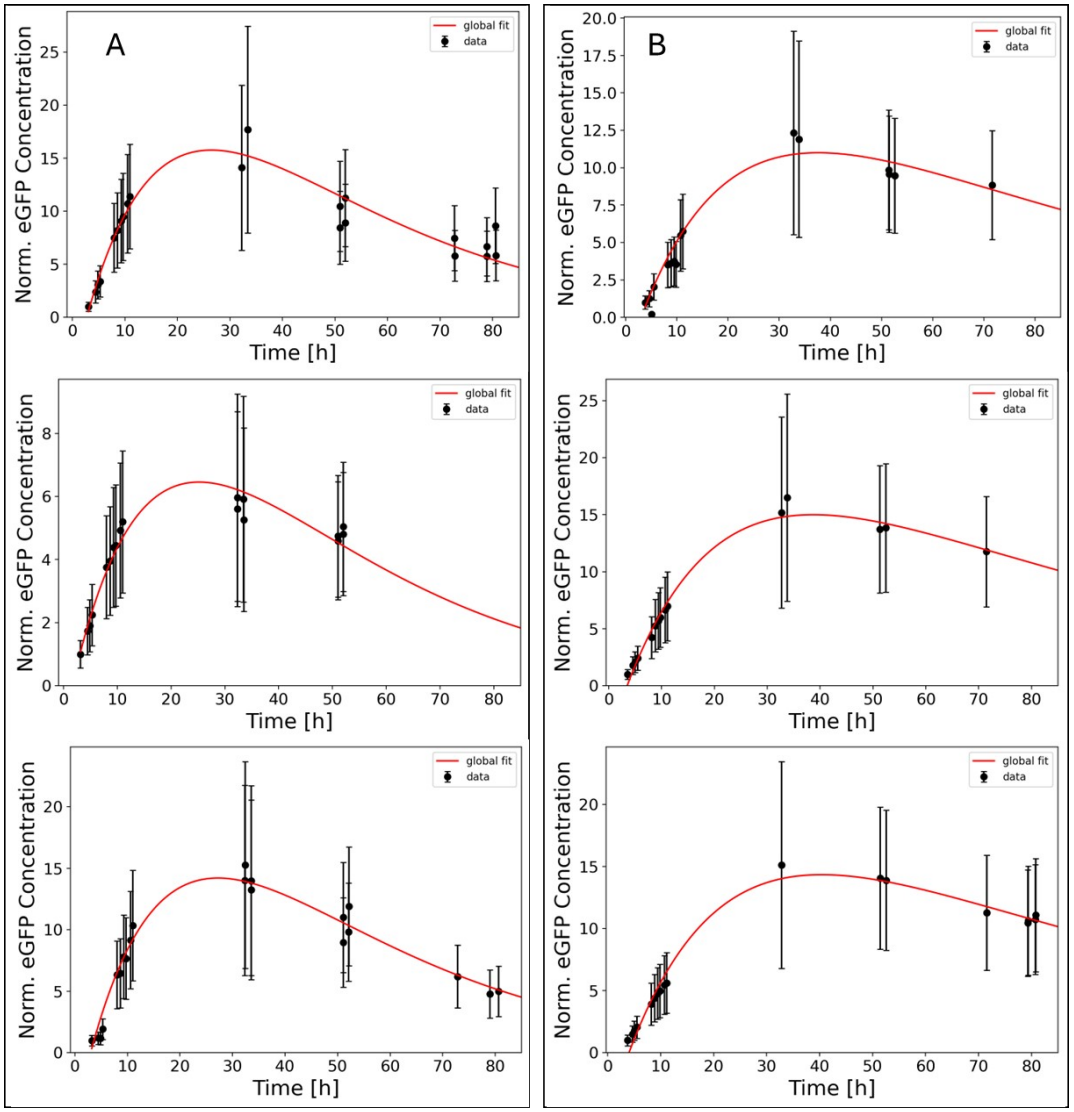
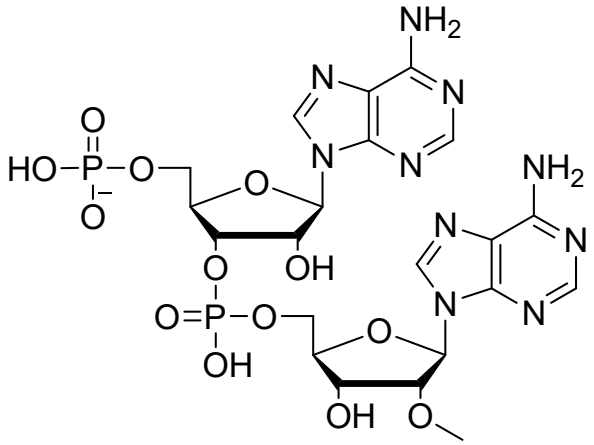
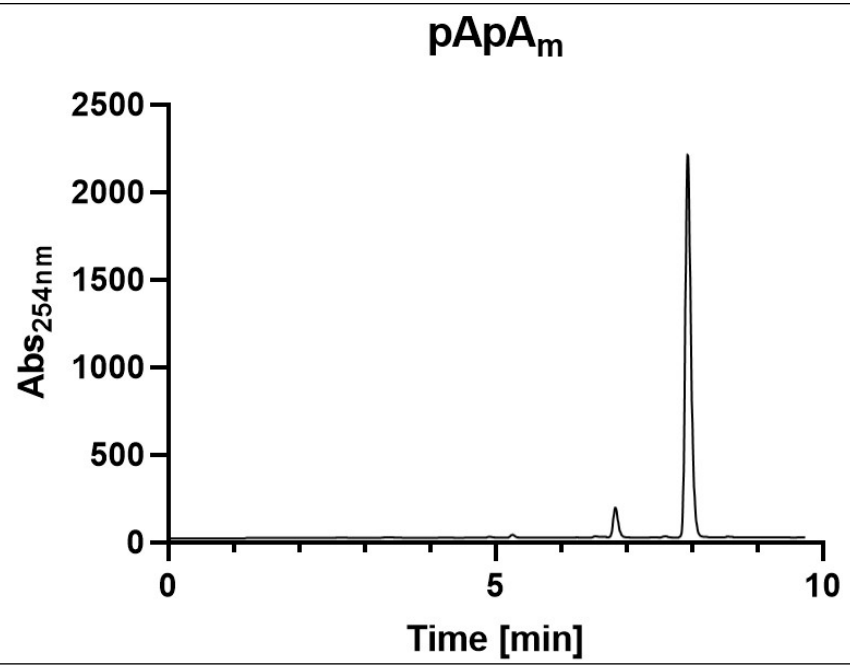


Figure S3. Simple kinetic model (red lines, Eq. 3) fitted to the eGFP concentration data registered from single mRNA-injected cells. Two distinct mRNA variants were tested: (A) unmodified eGFP and (B) eGFP-pAp$_s$A$_m$ D1.

Table S1. Kinetic parameters (protein half-life, t_{dp} and mRNA half-life t_{dm}) of fits plotted in Figure S3.

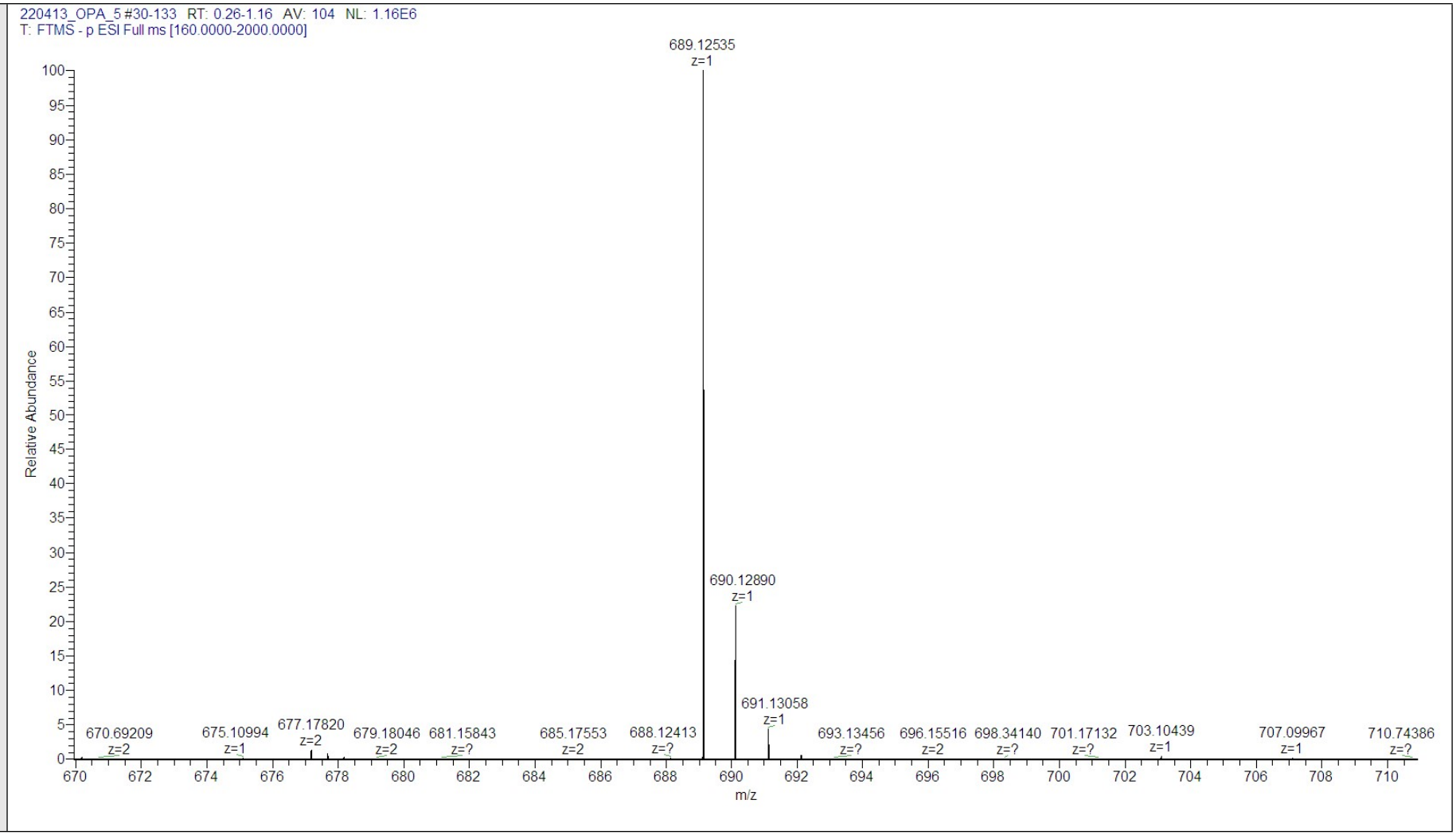
mRNA variant	t_{dp} [h]	t_{dm} [h]
eGFP-mRNA	21 ± 5	27 ± 6
eGFP-mRNA	21 ± 5	27 ± 9
eGFP-mRNA	21 ± 5	28 ± 7
eGFP-pAp_sA_m D1-mRNA	21 ± 5	66 ± 24
eGFP-pAp_sA_m D1-mRNA	21 ± 5	71 ± 21
eGFP-pAp_sA_m D1-mRNA	21 ± 5	63 ± 24

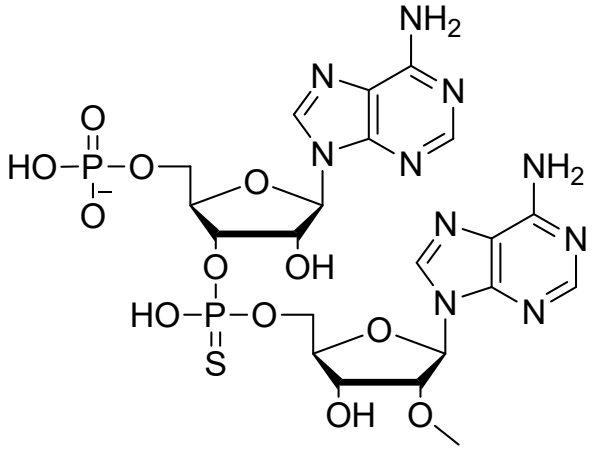
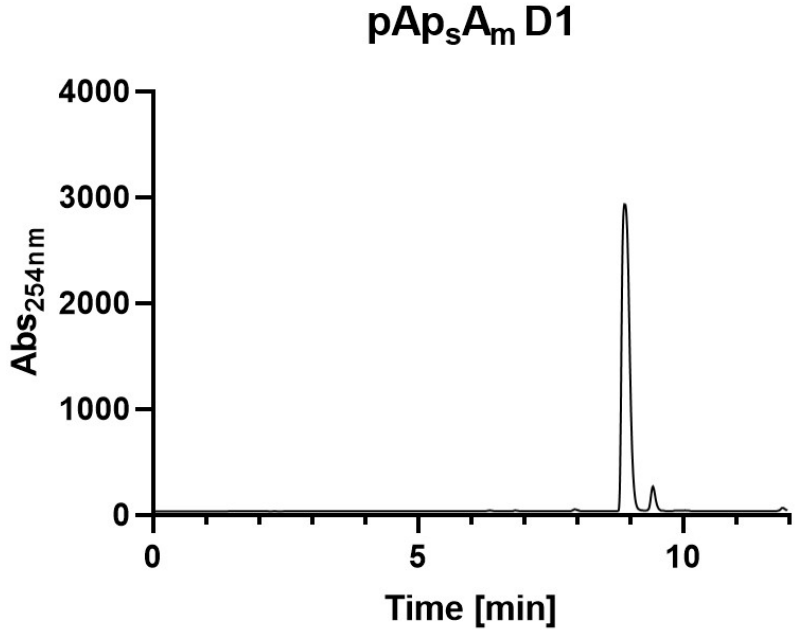
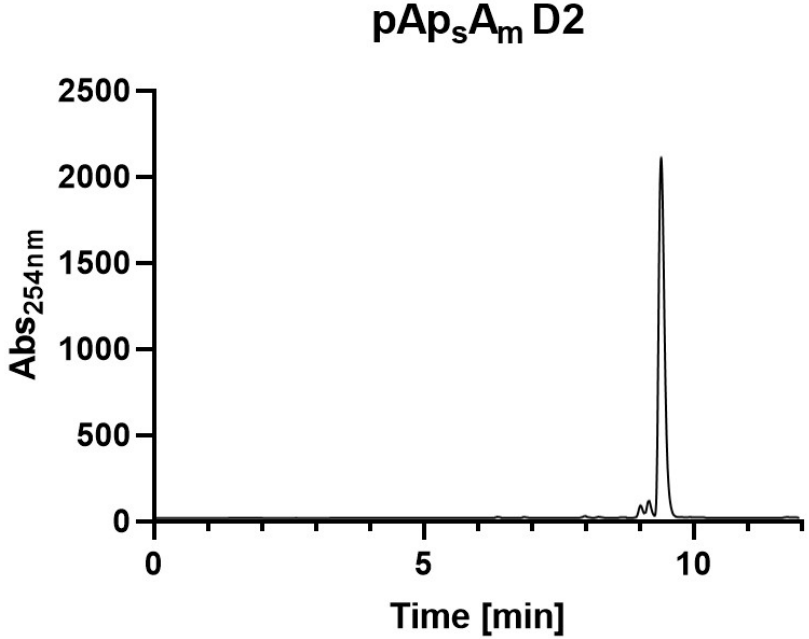
pApA_m	
Chemical structure	

RP HPLC	

MS (-) ESI

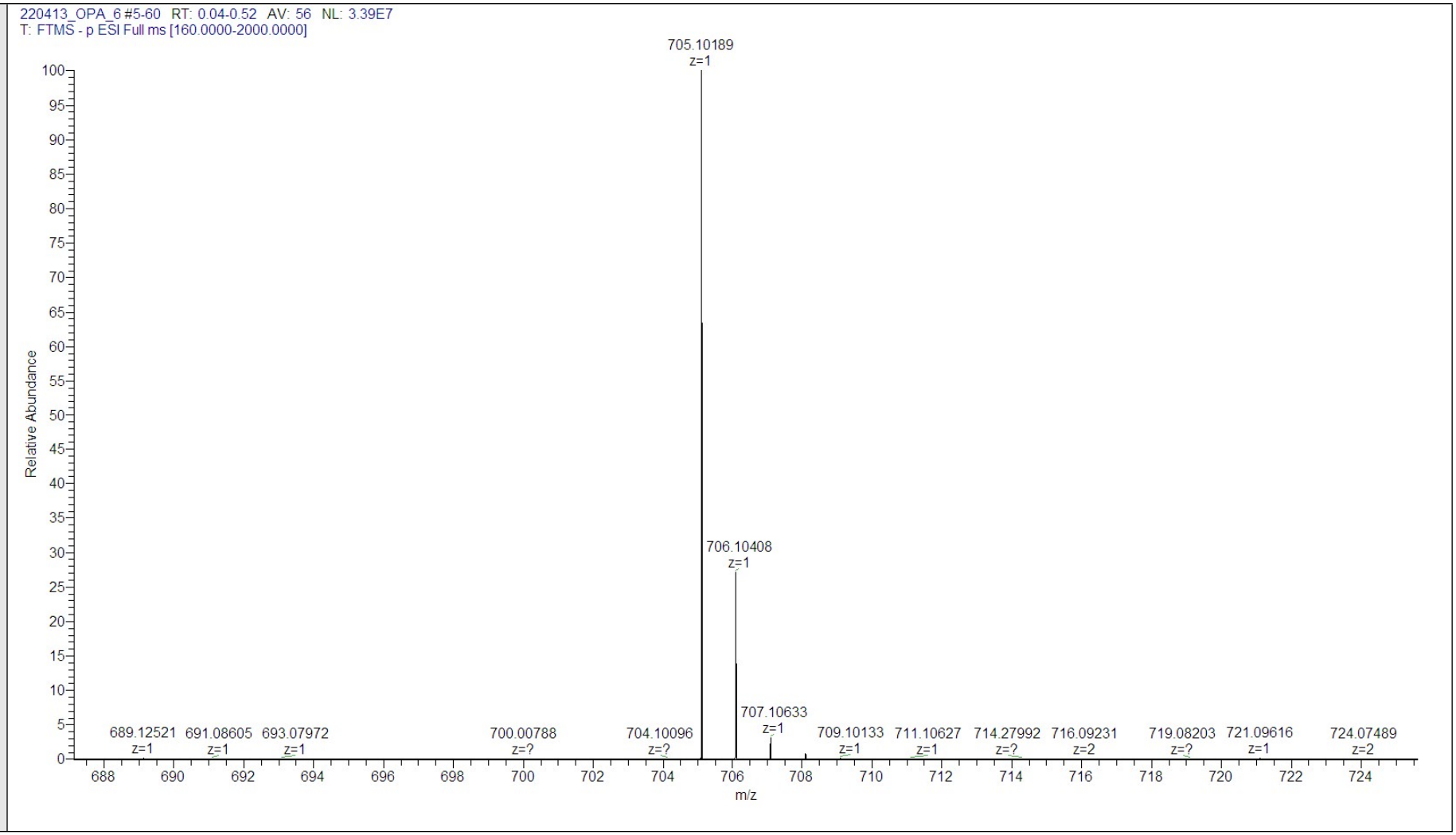
(Calc. $[M-H]^-$ $C_{21}H_{27}N_{10}O_{13}P_2^-$ 689.1240)



pAp$_s$A$_m$ D1/D2	
Chemical structure	
RP HPLC	 

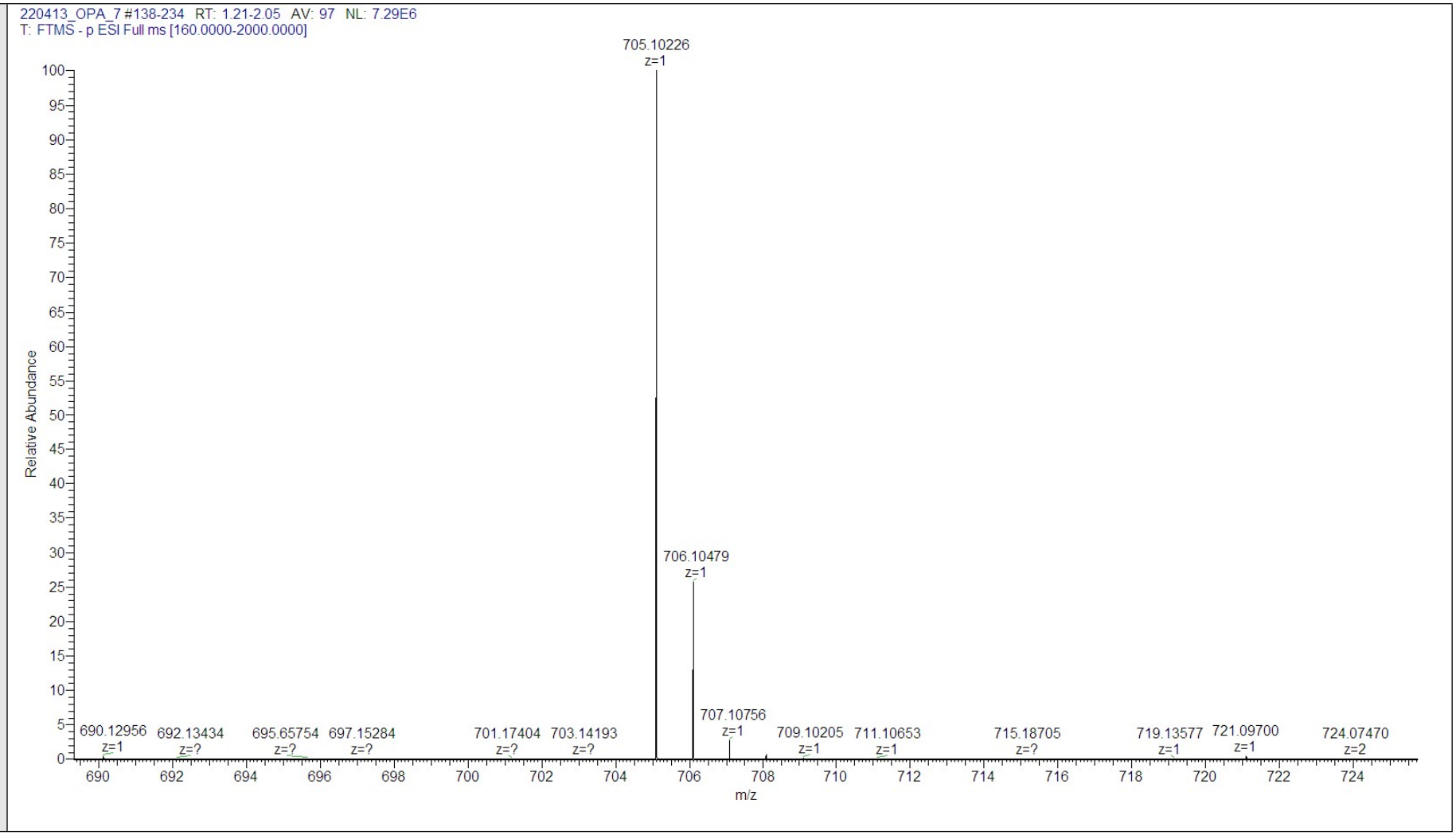
MS (-) ESI

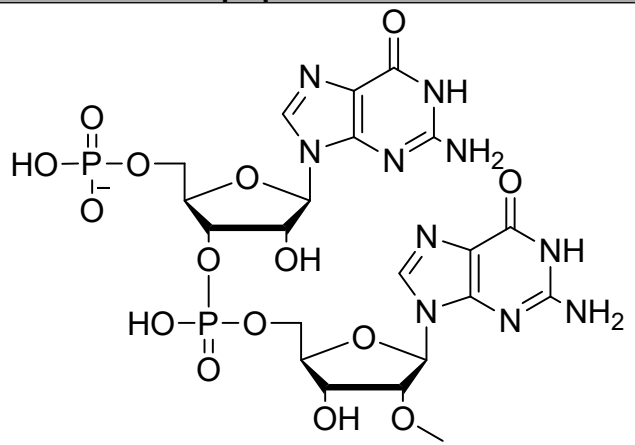
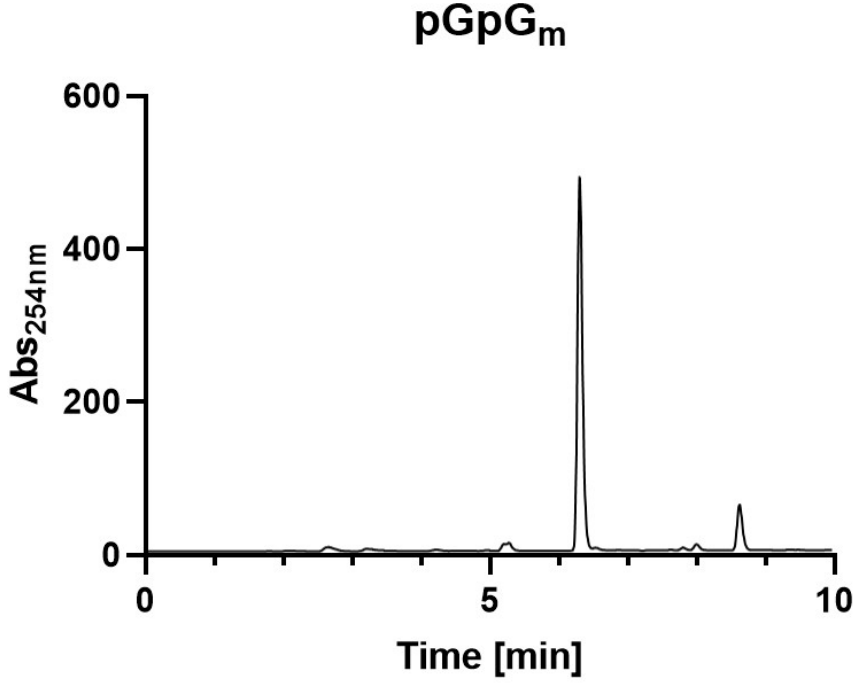
D1 (Calc. $[M-H]^-$ $C_{21}H_{27}N_{10}O_{12}P_2S^-$ 705.1011)



MS (-) ESI

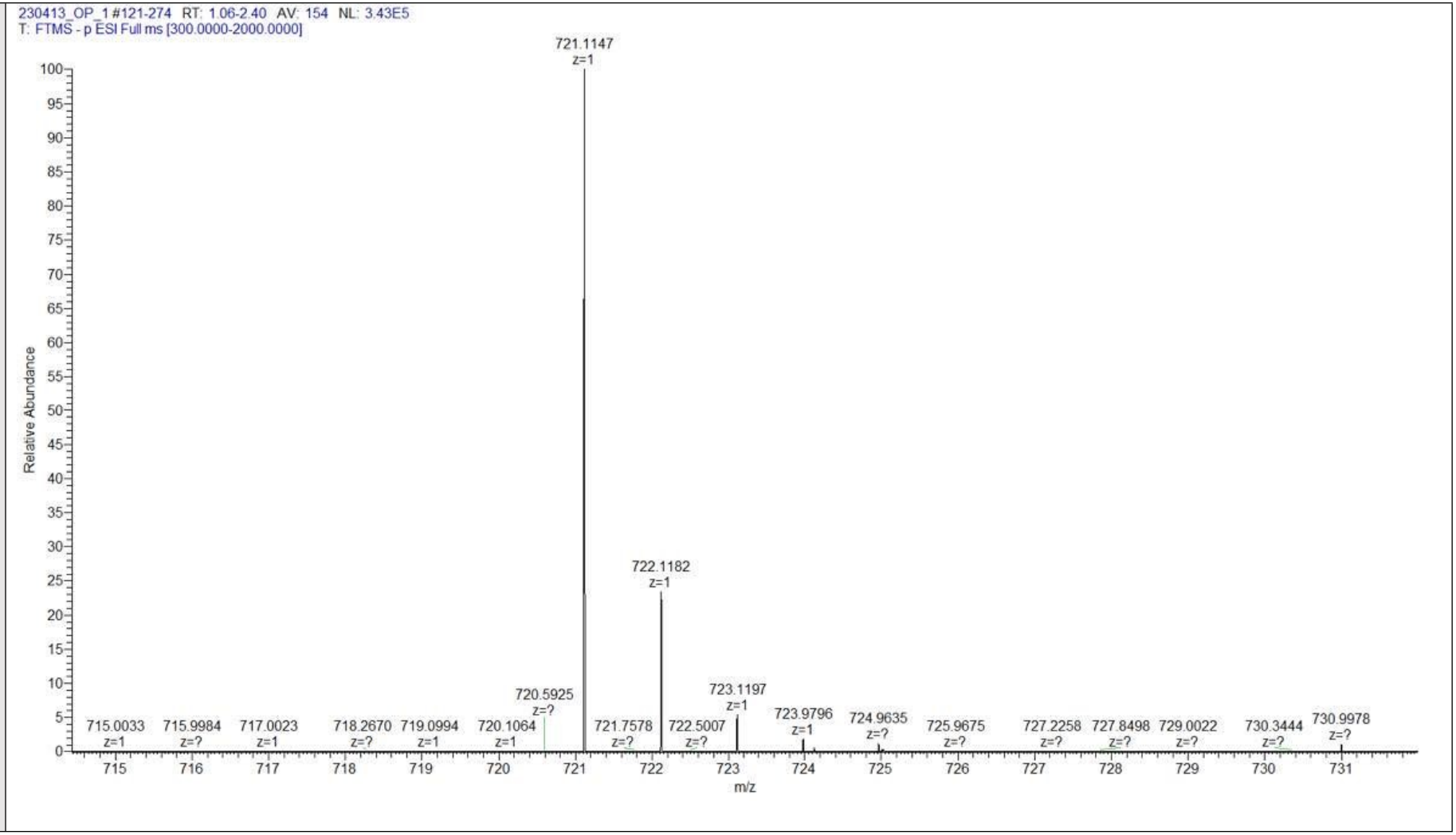
D2 (Calc. $[M-H]^-$ $C_{21}H_{27}N_{10}O_{12}P_2S^-$ 705.1011)

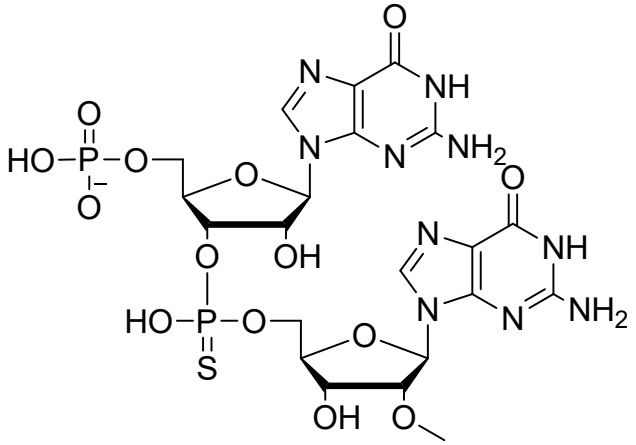
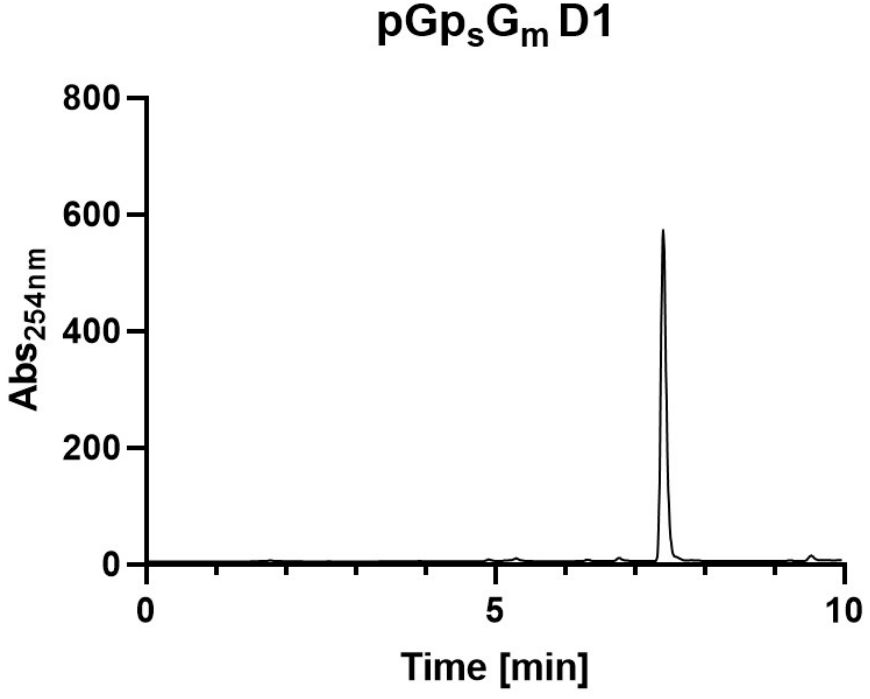
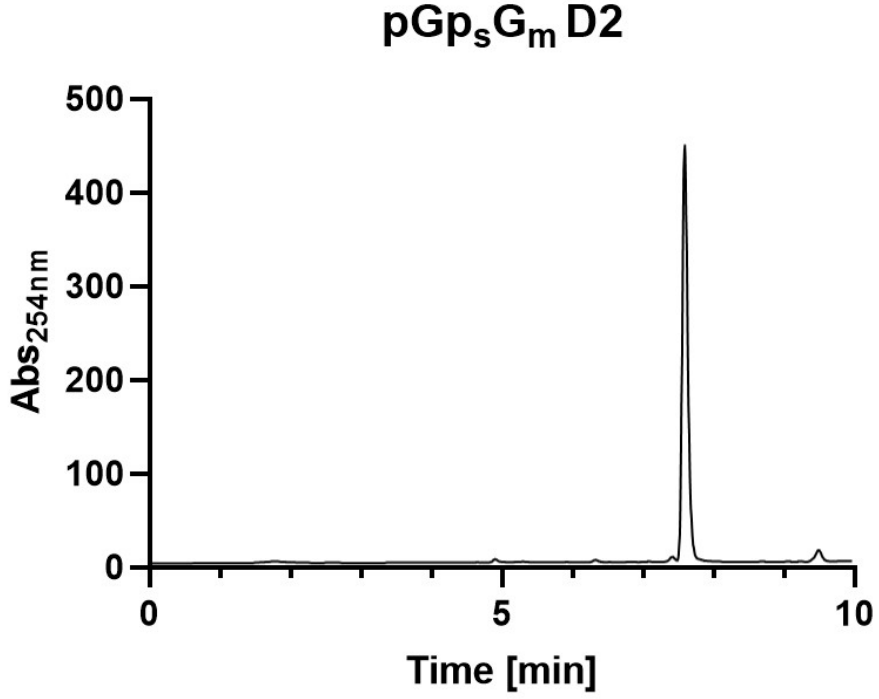


pGpG$_m$	
Chemical structure	
RP HPLC	

MS (-) ESI

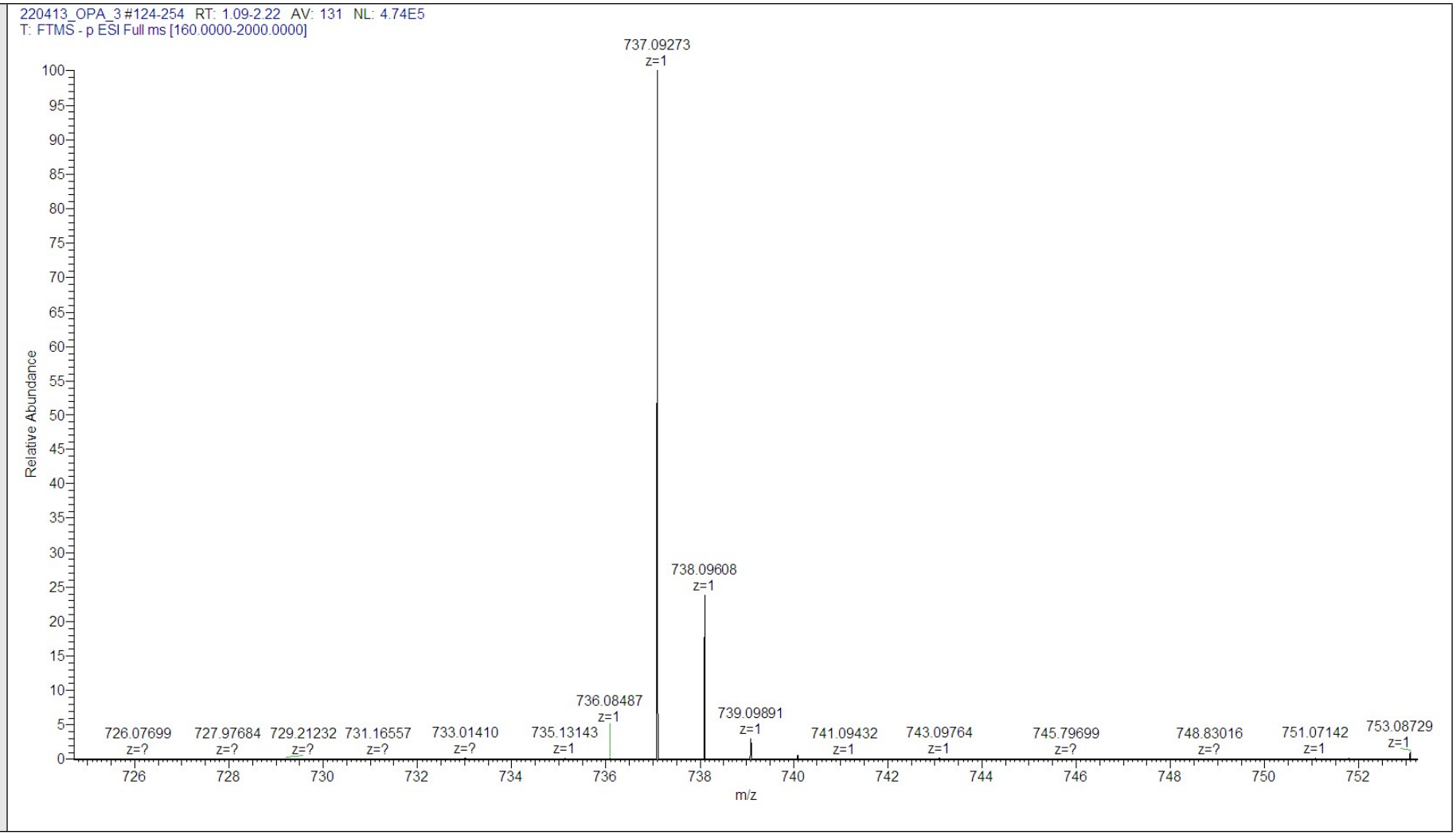
(Calc. $[M-H]^-$ $C_{21}H_{27}N_{10}O_{15}P_2^-$ 721.1138)



pGp$_s$Gm D1/D2	
Chemical structure	
RP HPLC	 

MS (-) ESI

D1 (Calc. $[M-H]^-$ $C_{21}H_{27}N_{10}O_{12}P_2S^-$ 737.0909)



MS (-) ESI

D2 (Calc. $[M-H]^-$ $C_{21}H_{27}N_{10}O_{12}P_2S^-$ 737.0909)