

Supplementary Information

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

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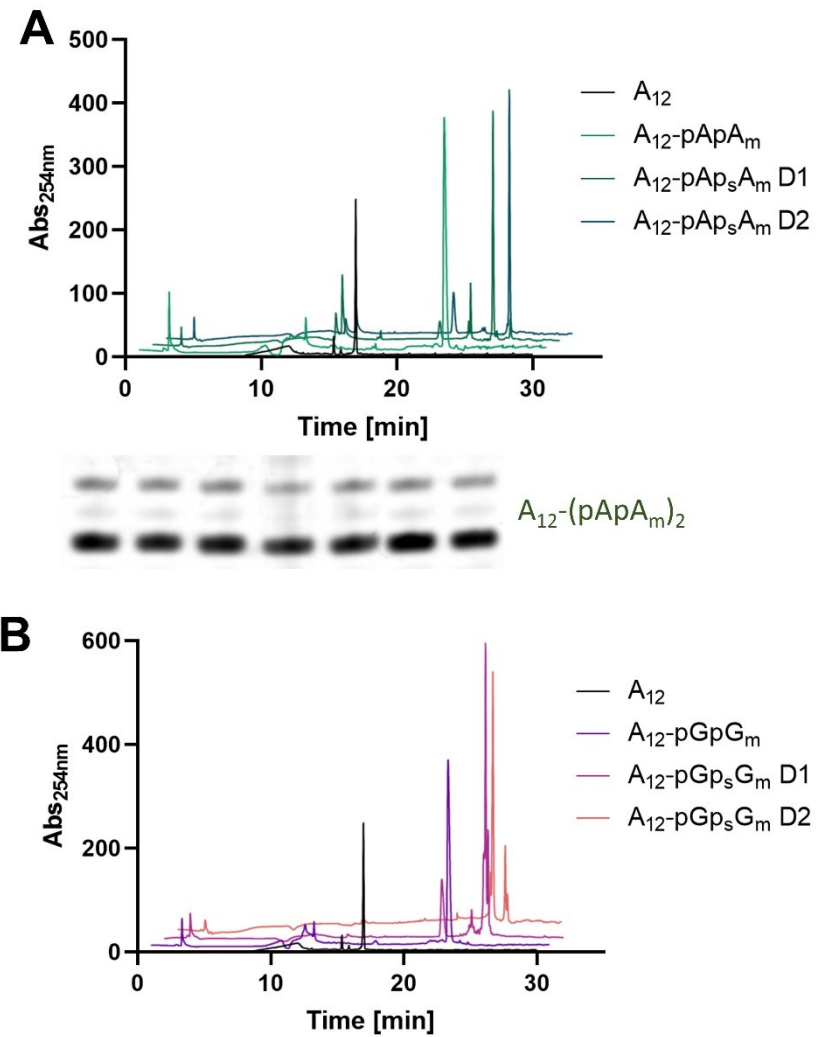


Figure S1. RP-HPLC analysis of short RNA after ligation with (A) adenosine and (B) guanosine dinucleotides. A_{12} RNA was used as reference. In (A) gel image of 16-mer product of double ligation with pApA_m dinucleotide.

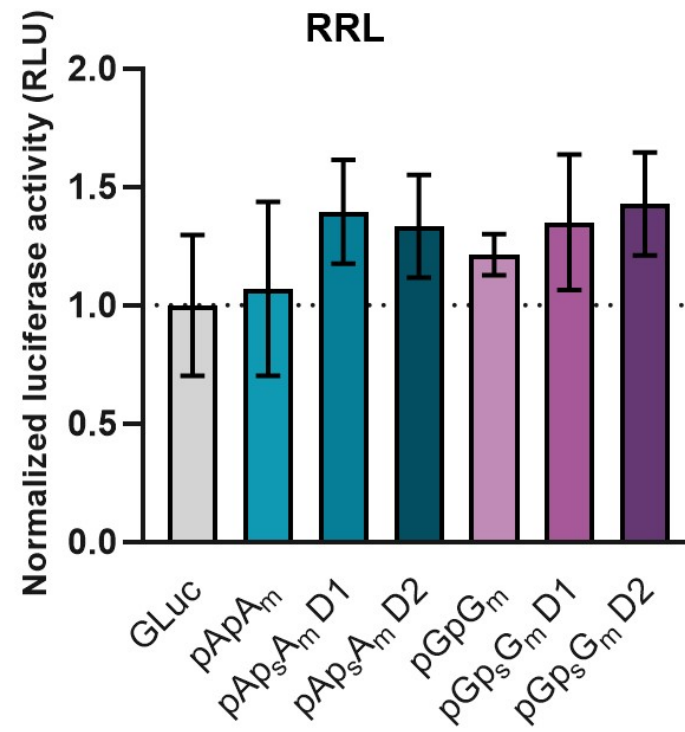


Figure S2. Translation efficiency experiment in Rabbit Reticulocyte Lysate (RRL) system.

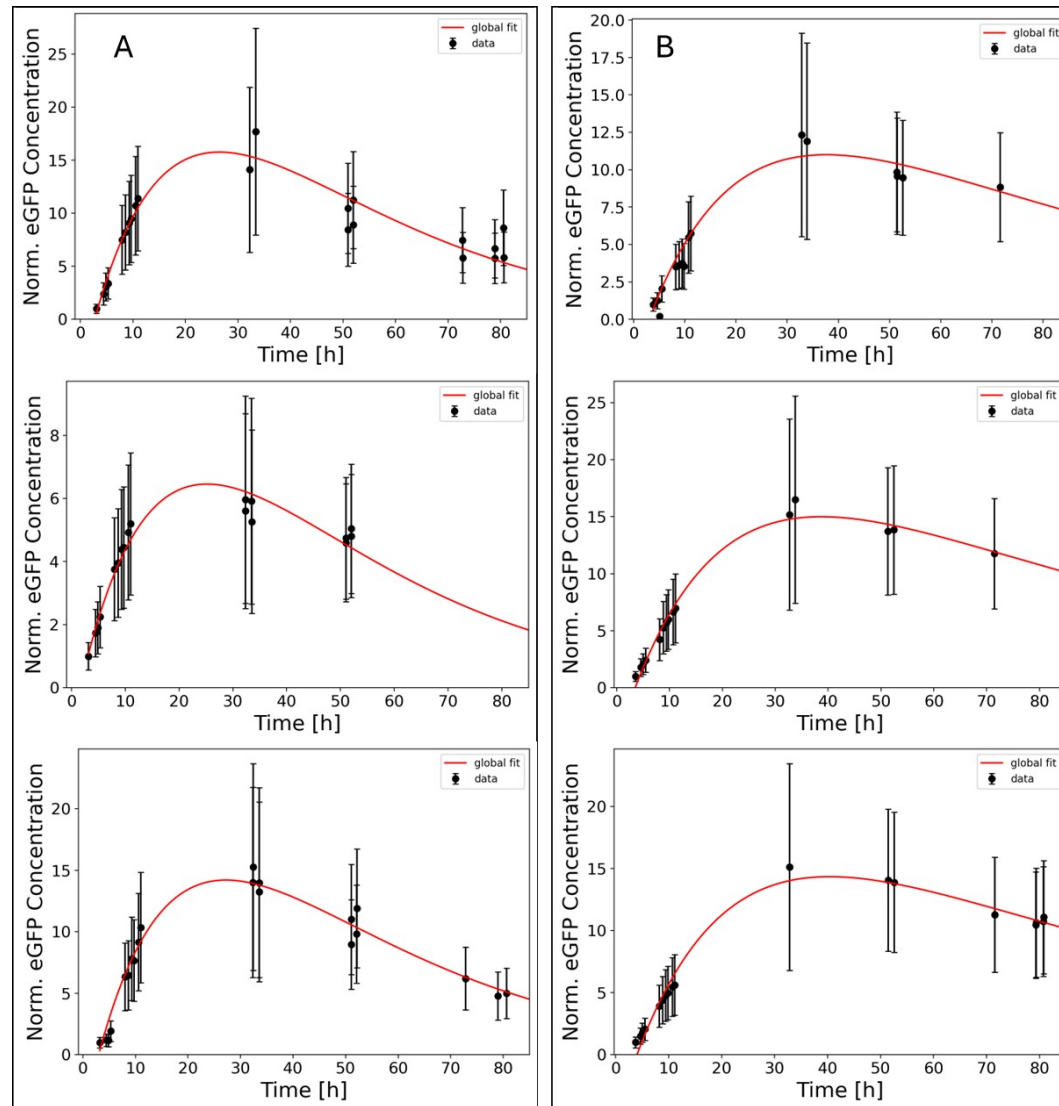


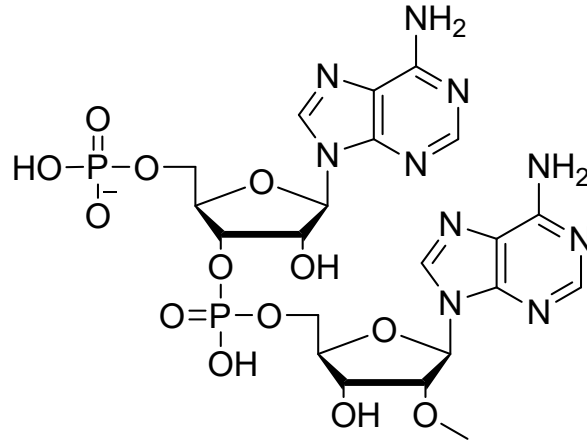
Figure S3. Simple kinetic model (red lines, Eq. 3) fitted to the eGFP concentration data registered from single mRNA-injected cells. Two distinct mRNA variants were tested: (A) unmodified eGFP and (B) eGFP-pAp_sA_mD1.

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 _s A _m D1-mRNA	21 ± 5	66 ± 24
eGFP-pAp _s A _m D1-mRNA	21 ± 5	71 ± 21
eGFP-pAp _s A _m D1-mRNA	21 ± 5	63 ± 24

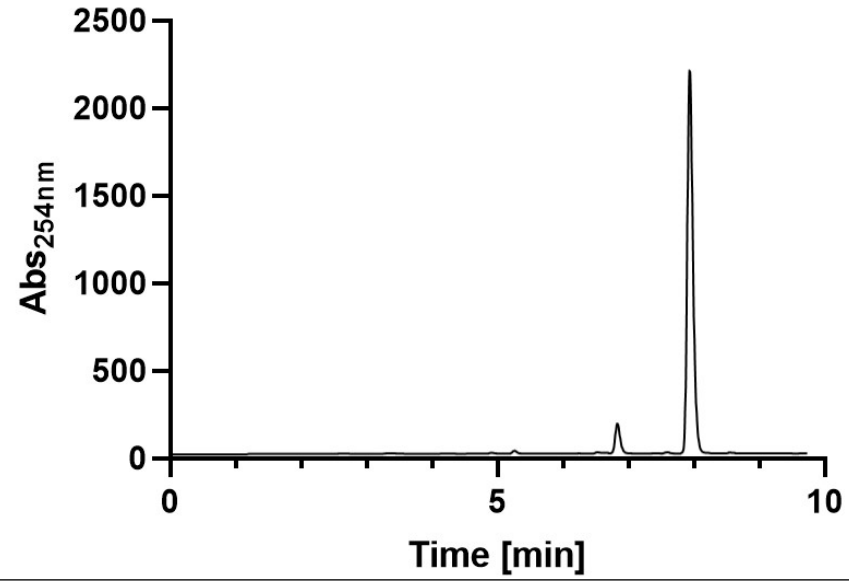
pApA_m

Chemical structure



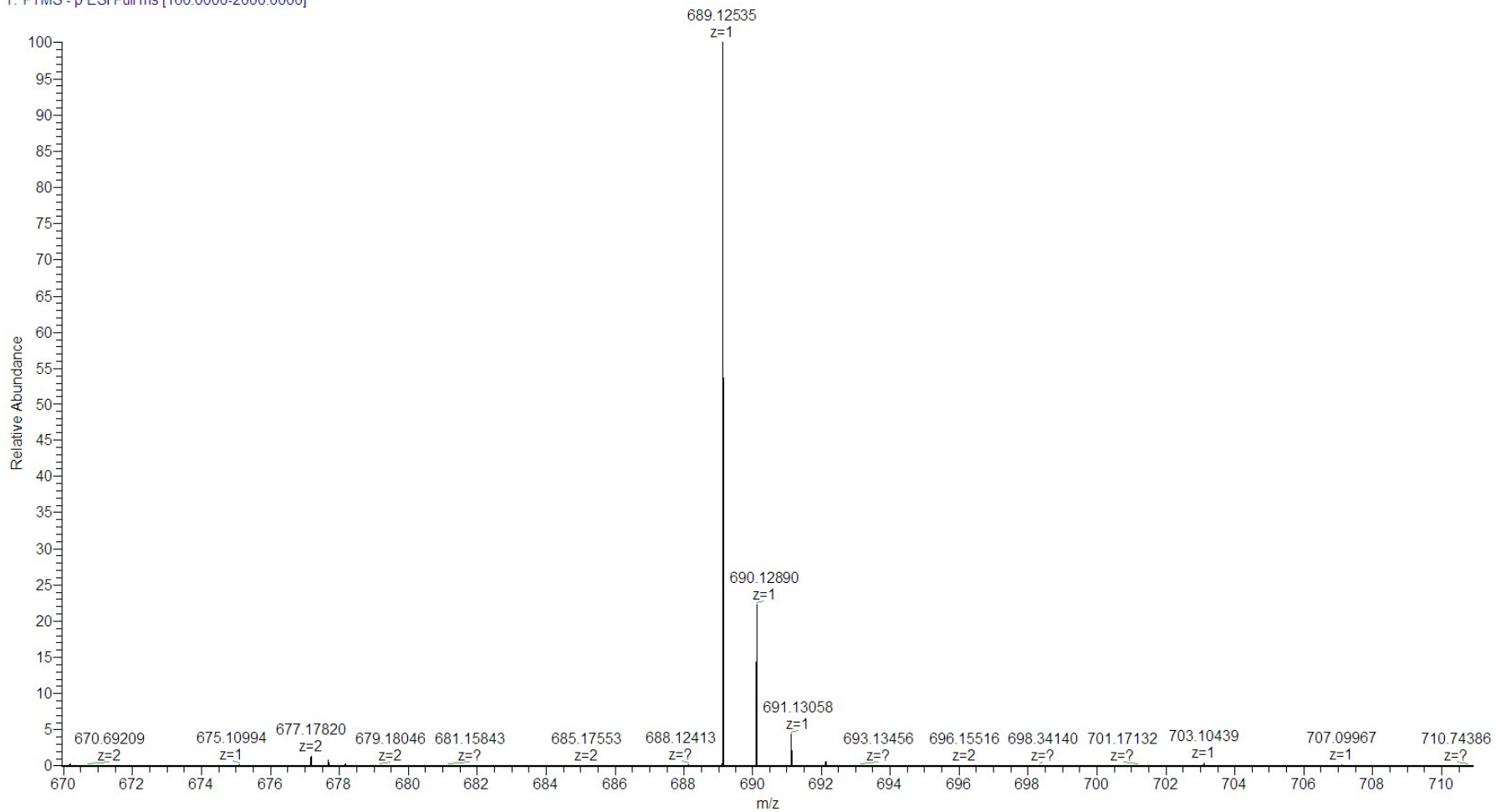
RP HPLC

pApA_m



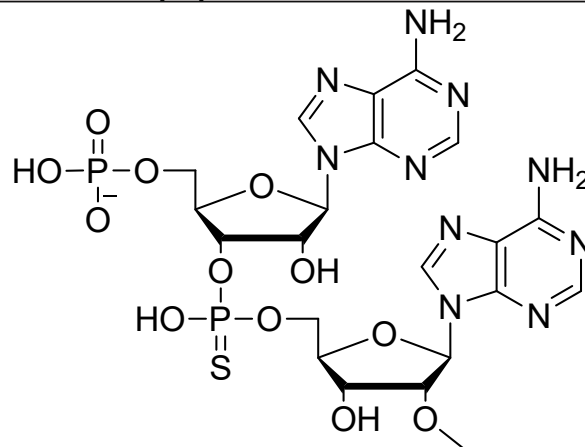
MS (-) ESI
(Calc. [M-H]⁻ C₂₁H₂₇N₁₀O₁₃P₂ 689.1240)

220413_OPA_5 #30-133 RT: 0.26-1.16 AV: 104 NL: 1.16E6
T: FTMS - p ESI Full ms [160.0000-2000.0000]



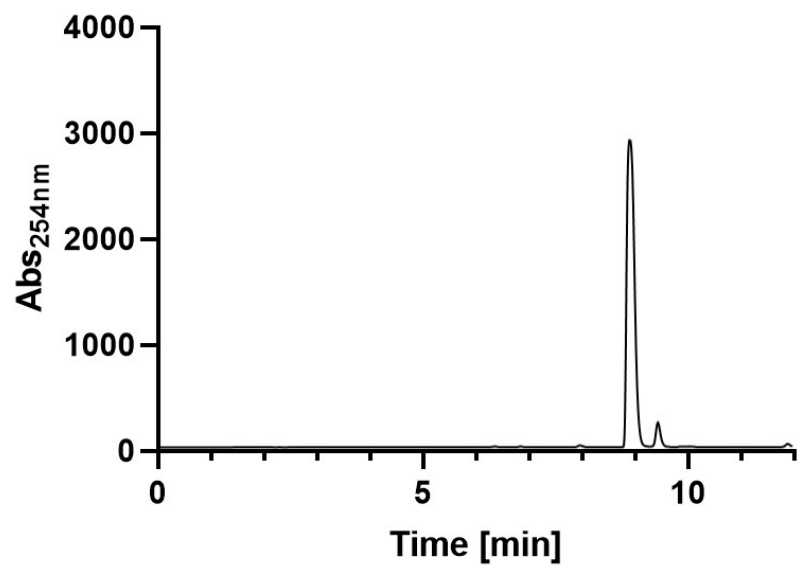
pAp_sA_m D1/D2

Chemical structure

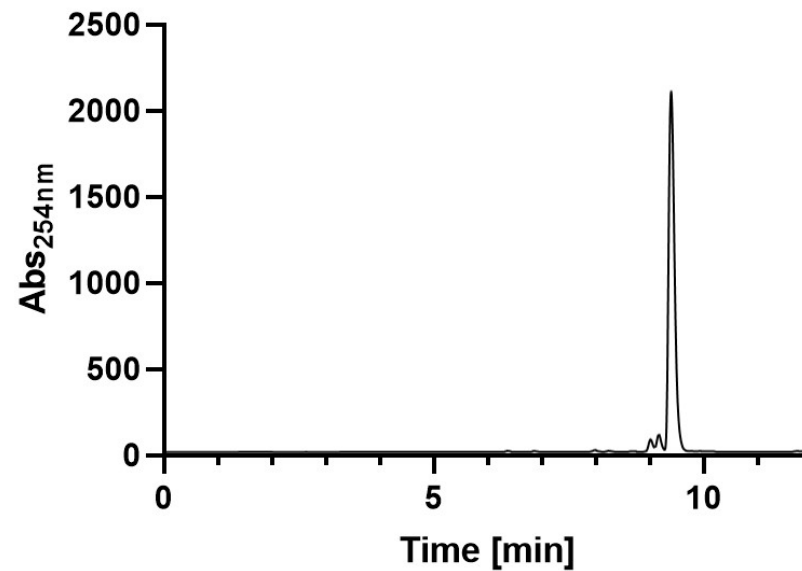


RP HPLC

pAp_sA_m D1

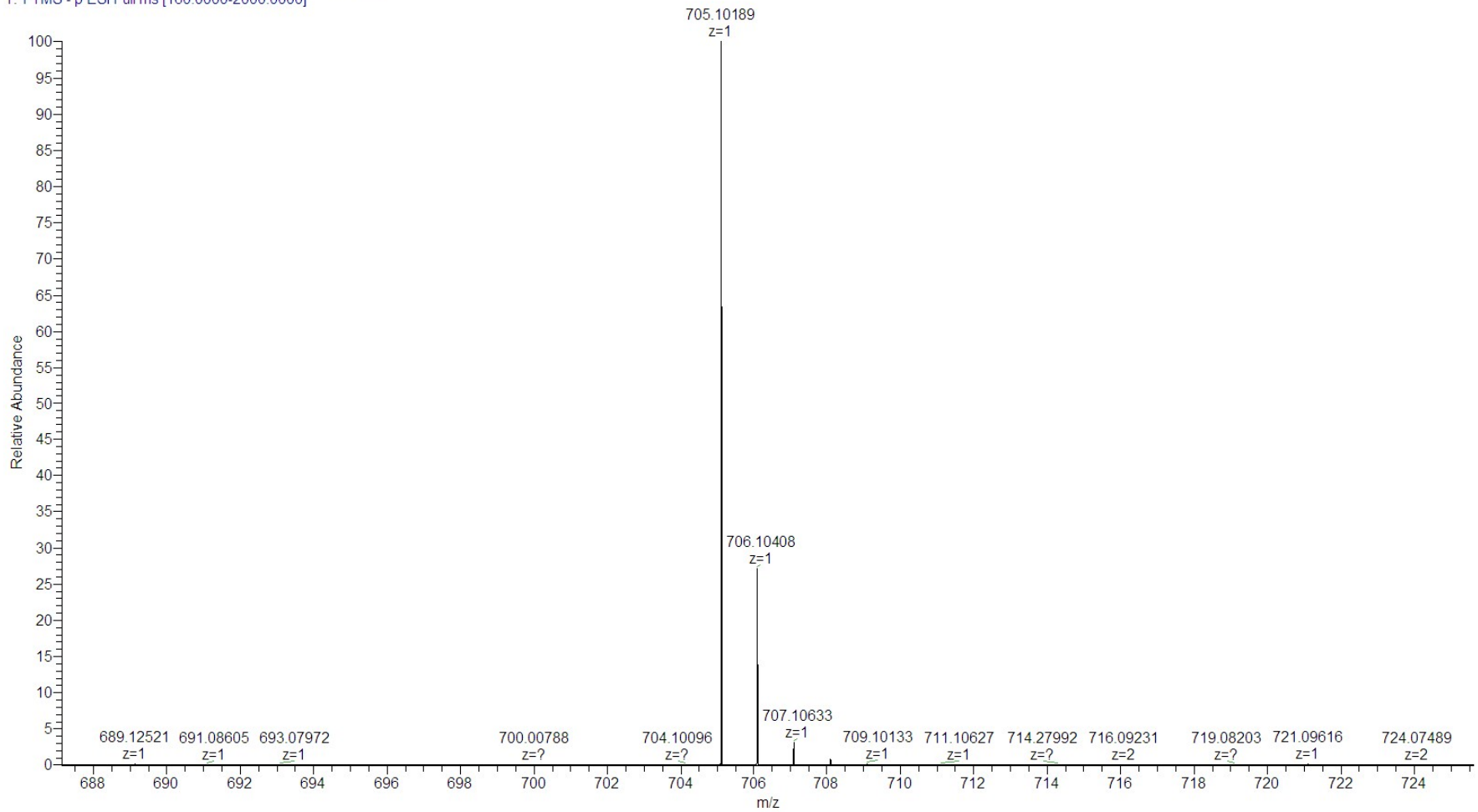


pAp_sA_m D2



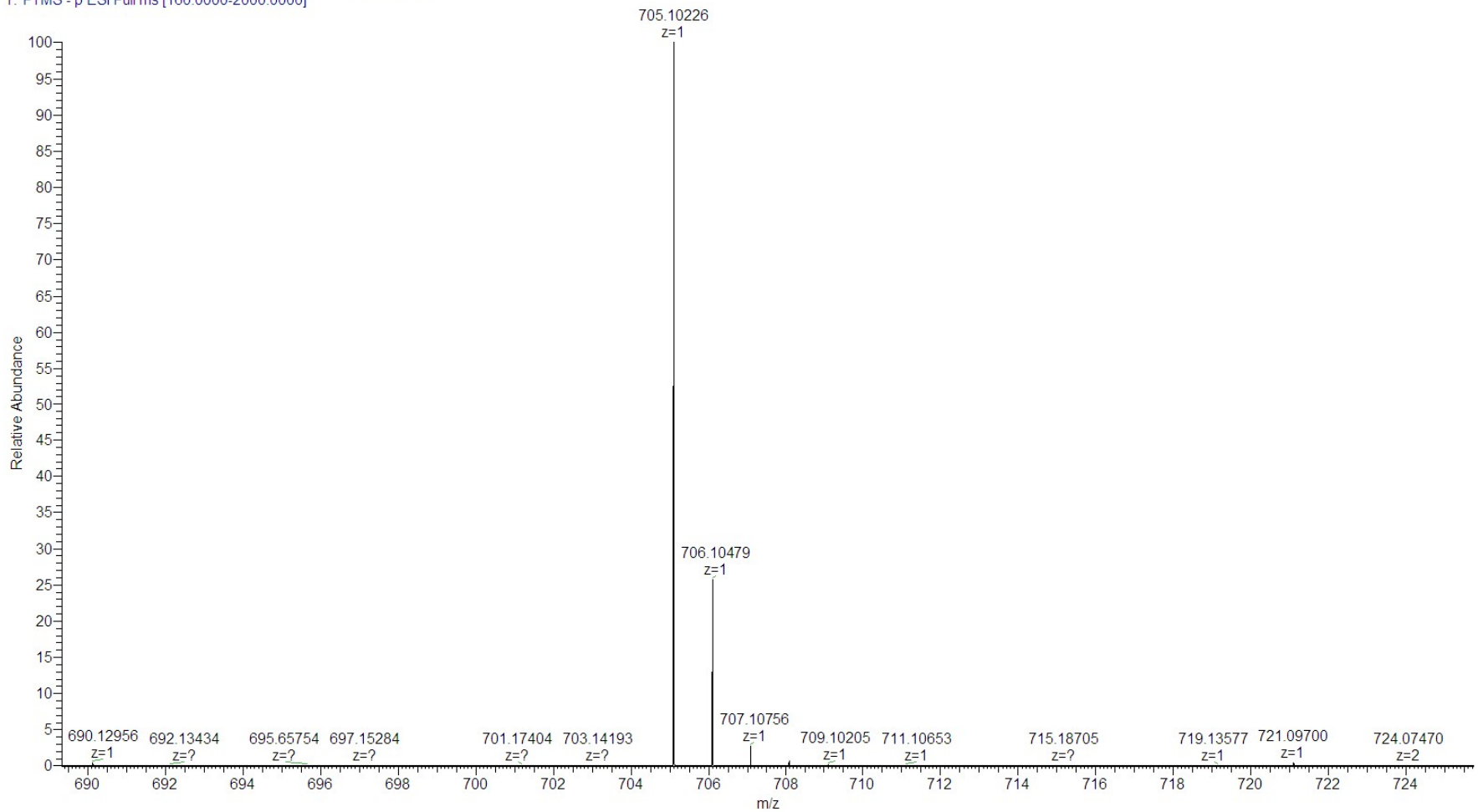
MS (-) ESI
D1 (Calc. [M-H]⁻ C₂₁H₂₇N₁₀O₁₂P₂S⁻ 705.1011)

220413 OPA_6 #5-60 RT: 0.04-0.52 AV: 56 NL: 3.39E7
T: FTMS - p ESI Full ms [160.0000-2000.0000]

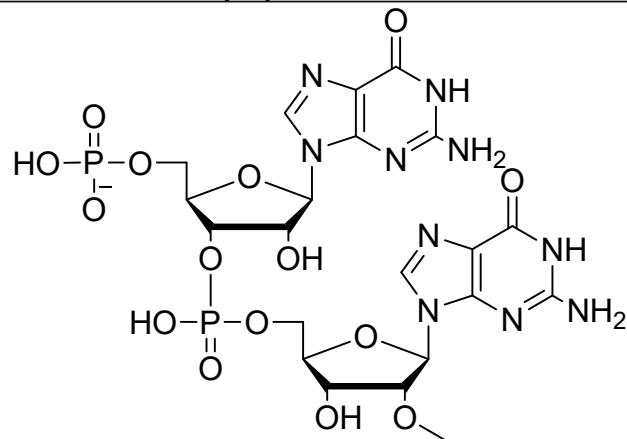


MS (-) ESI
D2 (Calc. [M-H]⁻ C₂₁H₂₇N₁₀O₁₂P₂S⁻ 705.1011)

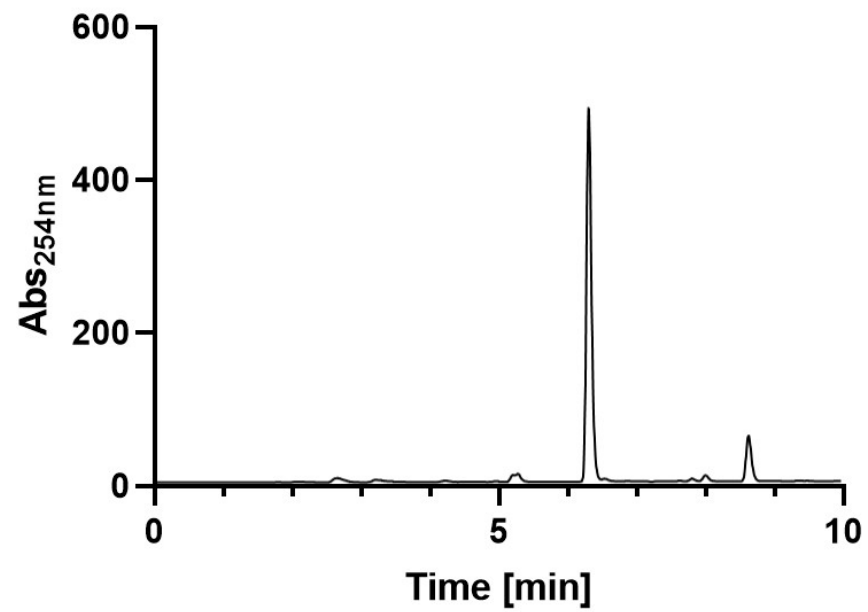
220413_OPA_7 #138-234 RT: 1.21-2.05 AV: 97 NL: 7.29E6
T: FTMS - p ESI Full ms [160.0000-2000.0000]



pGpG_m



pGpG_m

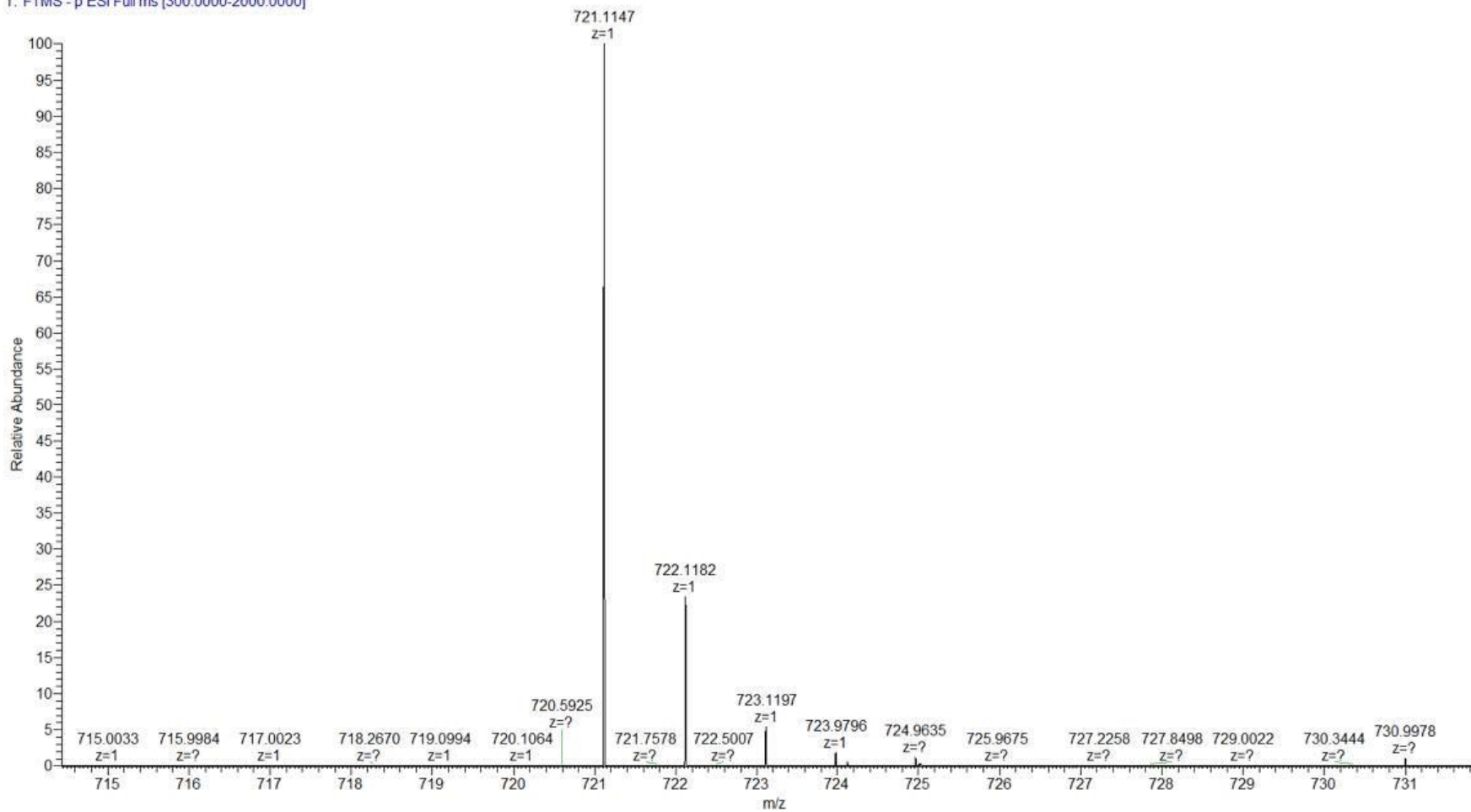


Chemical structure

RP HPLC

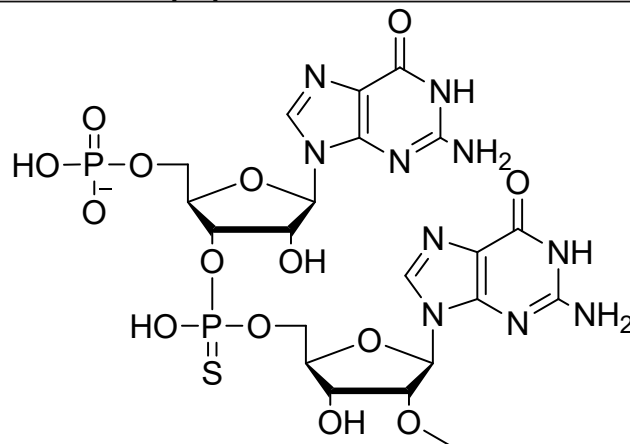
MS (-) ESI
(Calc. $[M-H]^-$ $C_{21}H_{27}N_{10}O_{15}P_2^-$ 721.1138)

230413 OP_1#121-274 RT: 1.06-2.40 AV: 154 NL: 3.43E5
T: FTMS - p ESI Full ms [300.0000-2000.0000]



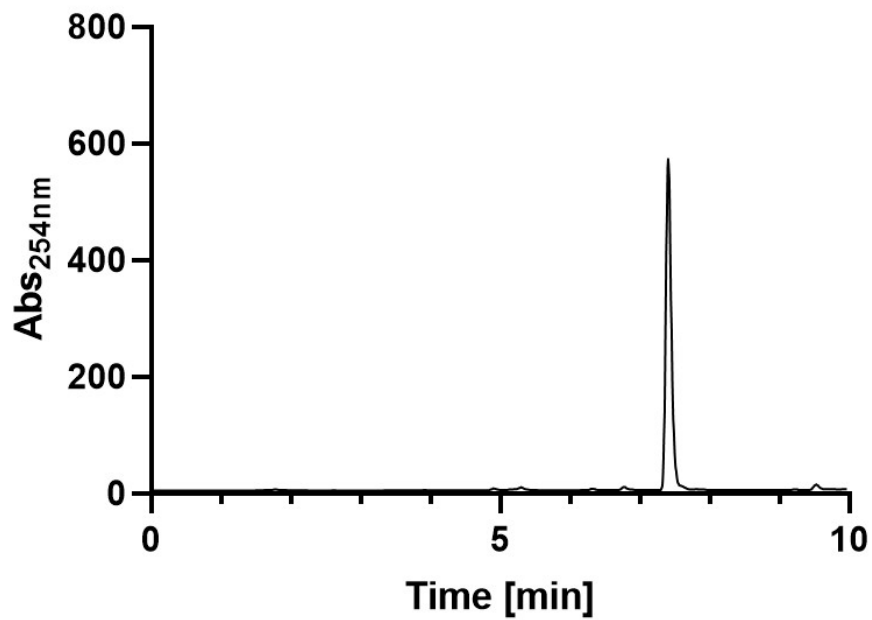
pGp_sG_m D1/D2

Chemical structure

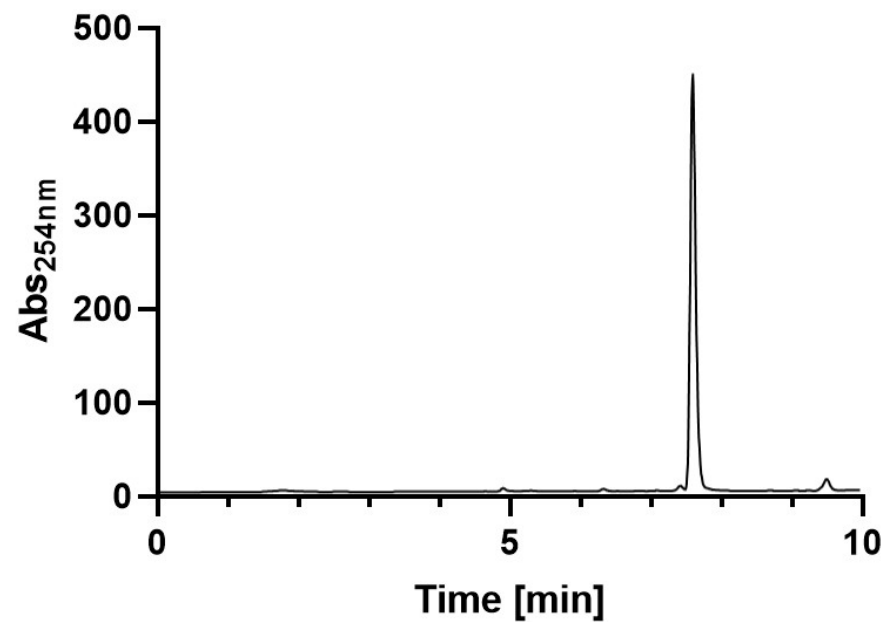


RP HPLC

pGp_sG_m D1

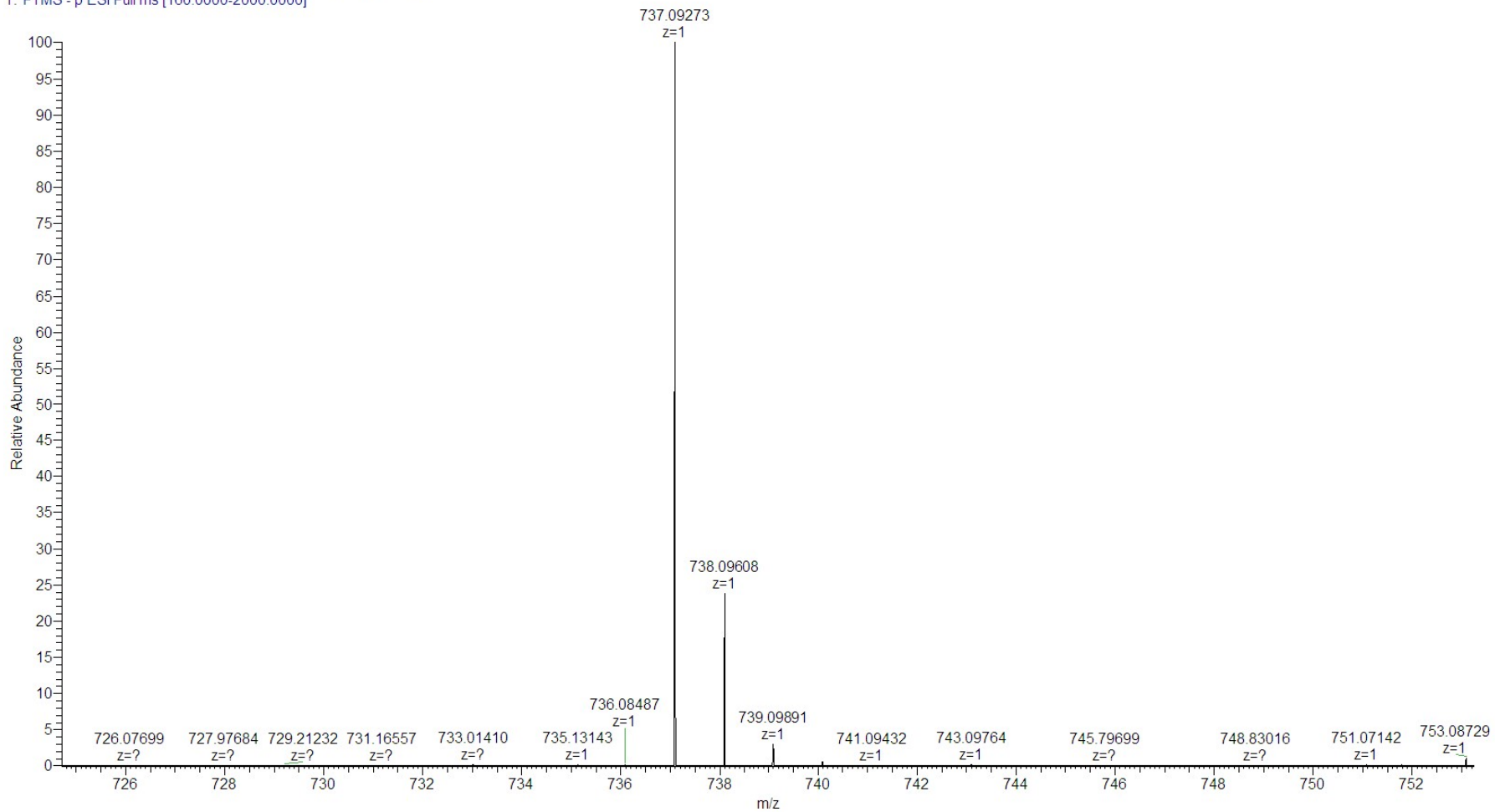


pGp_sG_m D2



MS (-) ESI
D1 (Calc. [M-H]⁻ C₂₁H₂₇N₁₀O₁₂P₂S⁻ 737.0909)

220413_OPA_3#124-254 RT: 1.09-2.22 AV: 131 NL: 4.74E5
T: FTMS - p ESI Full ms [160.0000-2000.0000]



MS (-) ESI
D2 (Calc. [M-H]⁻ C₂₁H₂₇N₁₀O₁₂P₂S⁻ 737.0909)

220413_OPA_4 #7-108 RT: 0.06-0.94 AV: 102 NL: 4.75E5
T: FTMS - p ESI Full ms [160.0000-2000.0000]

