N1-propargylguanosine modified mRNA cap analogs: synthesis, reactivity, and applications to the study of cap-binding proteins

Michal Kopcial[1,2,3], Blazej A. Wojtczak[2], Renata Kasprzyk[1,2,3], Joanna Kowalska[3] and Jacek Jemielity[2*]

[1] College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw
[2] Centre of New Technologies, University of Warsaw
[3] Faculty of Physics, University of Warsaw
* Correspondence: j.jemielity@cent.uw.edu.pl; Tel.: +48 22 55 43 774, Centre of New Technologies, University of Warsaw, S. Banacha 2c, 02-097, Warsaw

Supporting Information (SI2)

Tables

Table S1. Murine eIF4E binding affinity assay IC_{50} and K_D values.

Compound	IC_{50} (μM)		
	20°C	30°C	37°C
1	2.92 ± 0.19	3.57 ± 0.25	3.68 ± 0.70
2	6.57 ± 0.49	6.37 ± 0.73	8.20 ± 0.79
3	3.06 ± 0.16	3.48 ± 0.22	3.75 ± 0.89
4	5.51 ± 0.33	6.04 ± 0.46	8.10 ± 0.61
5	1.85 ± 0.15	2.16 ± 0.20	3.21 ± 0.33
m^{7}Gp$_3$G	3.04 ± 0.26	3.46 ± 0.30	3.31 ± 0.87
Compound	K_D (μM)		
	20°C	30°C	37°C
1	0.268 ± 0.040	0.305 ± 0.045	0.371 ± 0.082
2	0.610 ± 0.094	0.548 ± 0.095	0.836 ± 0.121
3	0.281 ± 0.041	0.297 ± 0.043	0.379 ± 0.100
4	0.511 ± 0.075	0.520 ± 0.078	0.826 ± 0.109
5	0.168 ± 0.026	0.182 ± 0.029	0.324 ± 0.048
m^{7}Gp$_3$G	0.279 ± 0.045	0.294 ± 0.046	0.334 ± 0.096

Table S2. Half-life for m^{7}Gp$_3$G and compound **4**

Compound	m^{7}Gp$_3$G	**1**
Half-life [min]	5.703	15.75

Figures

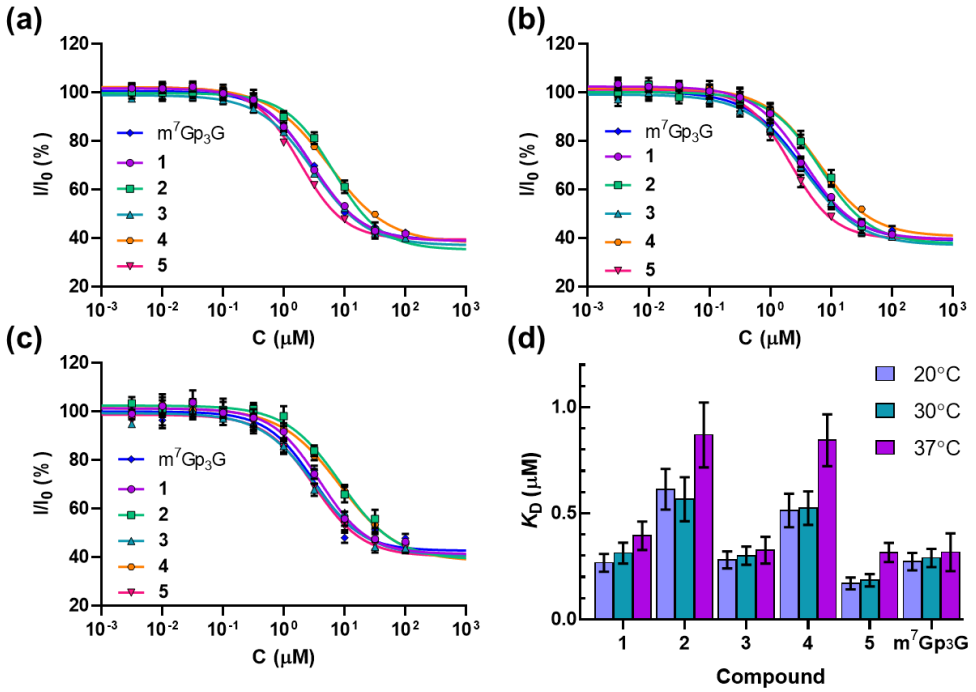


Figure S1. Murine eIF4E binding affinity assay

Competitive binding of five eIF4E ligands **1-5** and m^7Gp_3G as a control at three different temperatures (**(a)** 20°C, **(b)** 30°C and **(c)** 37°C), using previously described pyrene fluorescence intensity binding assay [1]; **(d)** comparison of calculated K_D values. Data shown are average values ± SD of 3 independent experiments.

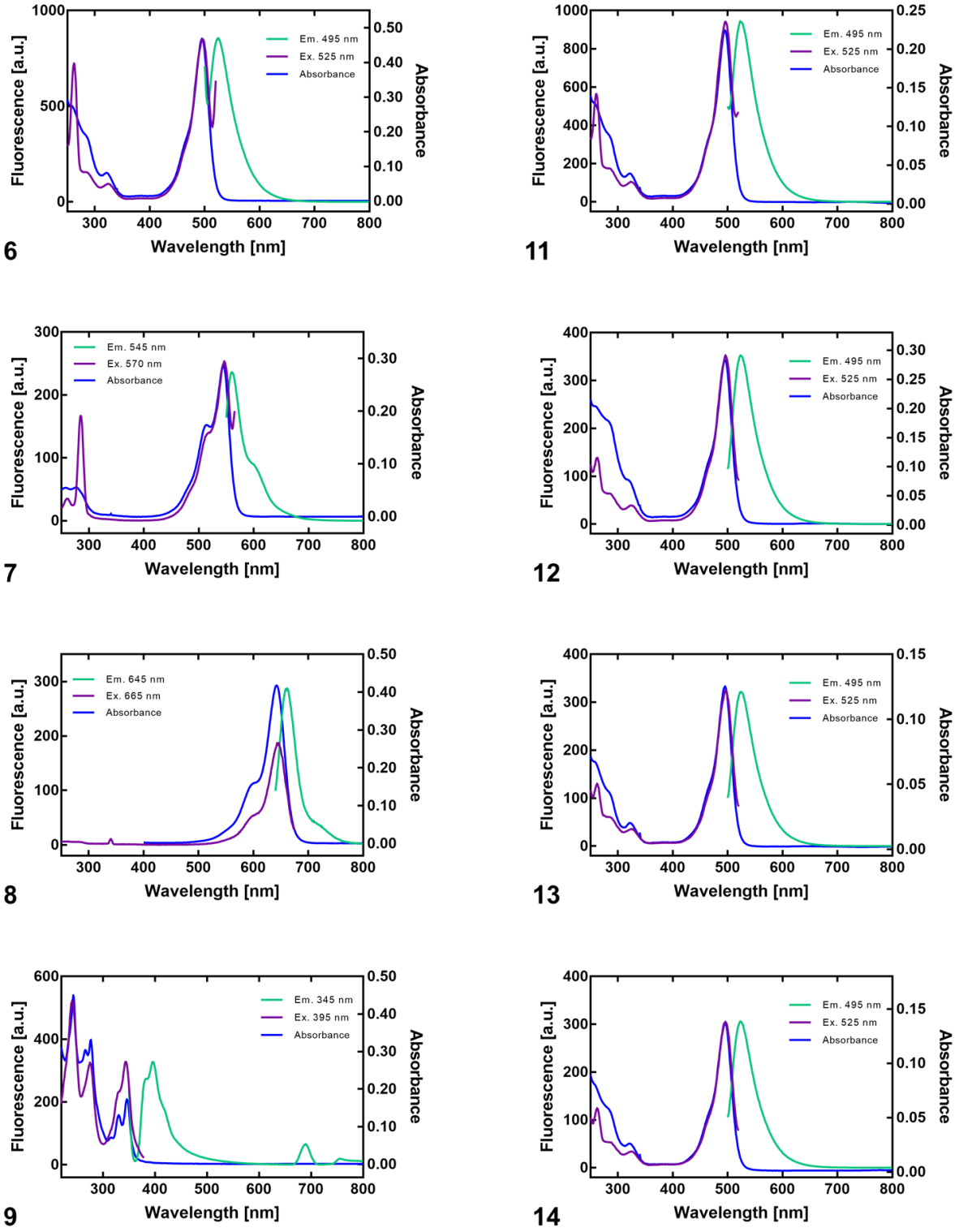


Figure S2. Absorption, emission and excitation spectra of probes 16a-d, 17a-20a.

Absorption spectra were recorded in 0.1 M NaOH for probes containing fluorescein (**16a-20a**) or 50 mM Tris/HCl, 200 mM KCl, 0.5 mM EDTA, pH=7.6 for all others (**16b-d**). Emission and excitation

spectra were recorded in 50 mM Tris/HCl, 200 mM KCl, 0.5 mM EDTA, pH=7.6 for 100 nM compound (except 200 nM for **16d**).

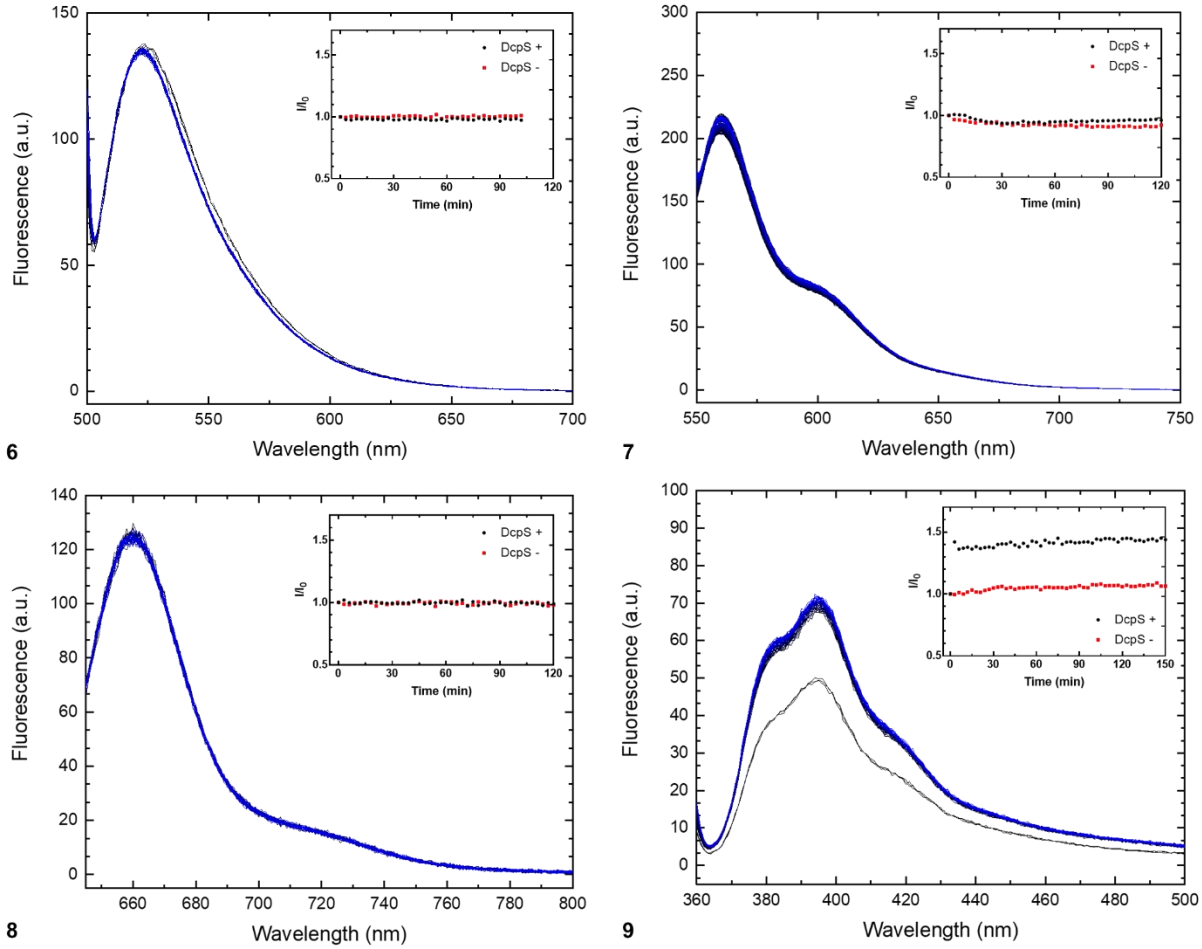


Figure S3. Probe **16a-d** hydrolysis by hDcpS enzyme monitored by emission spectroscopy

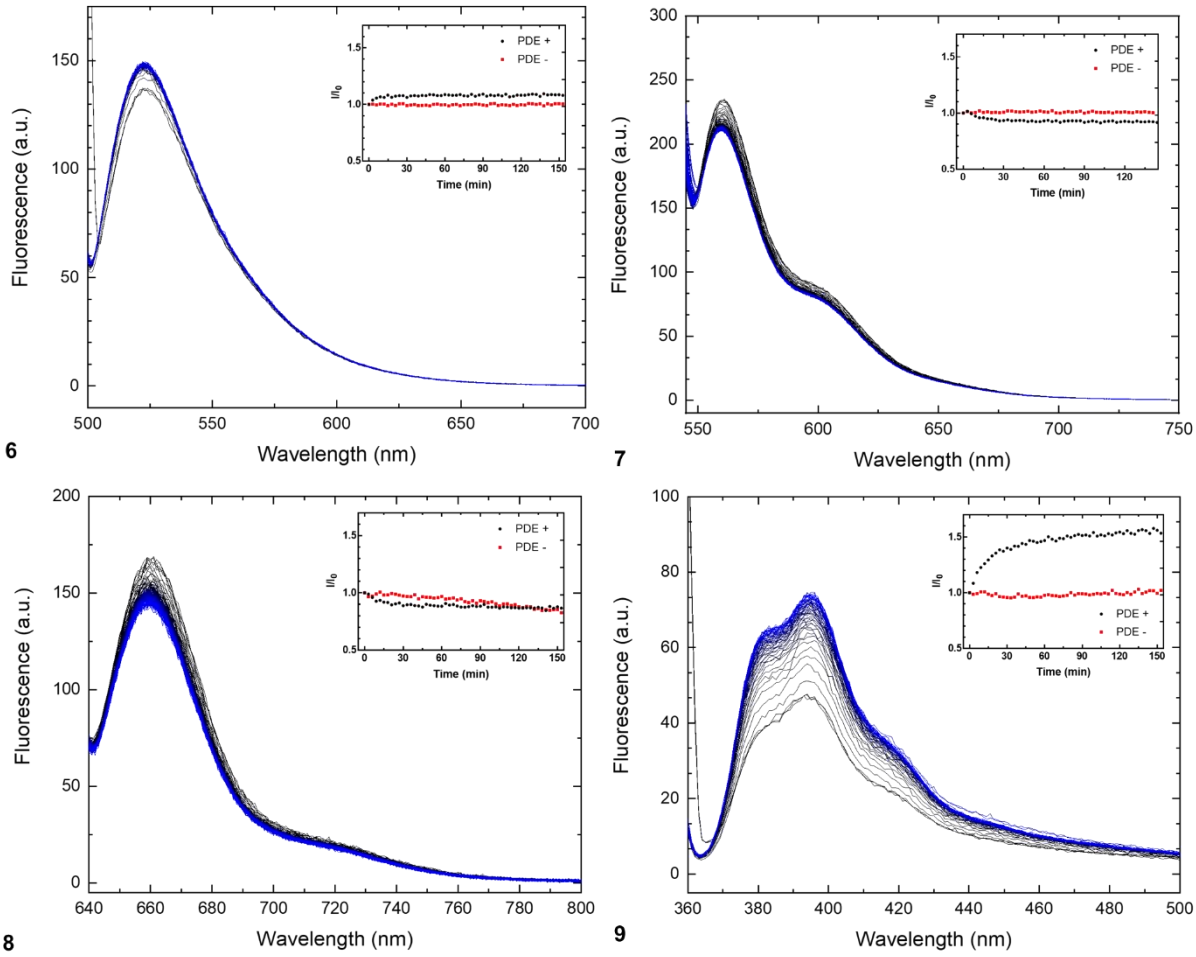


Figure S4. Probe **16a-d** hydrolysis by PDE-I enzyme monitored by emission spectroscopy

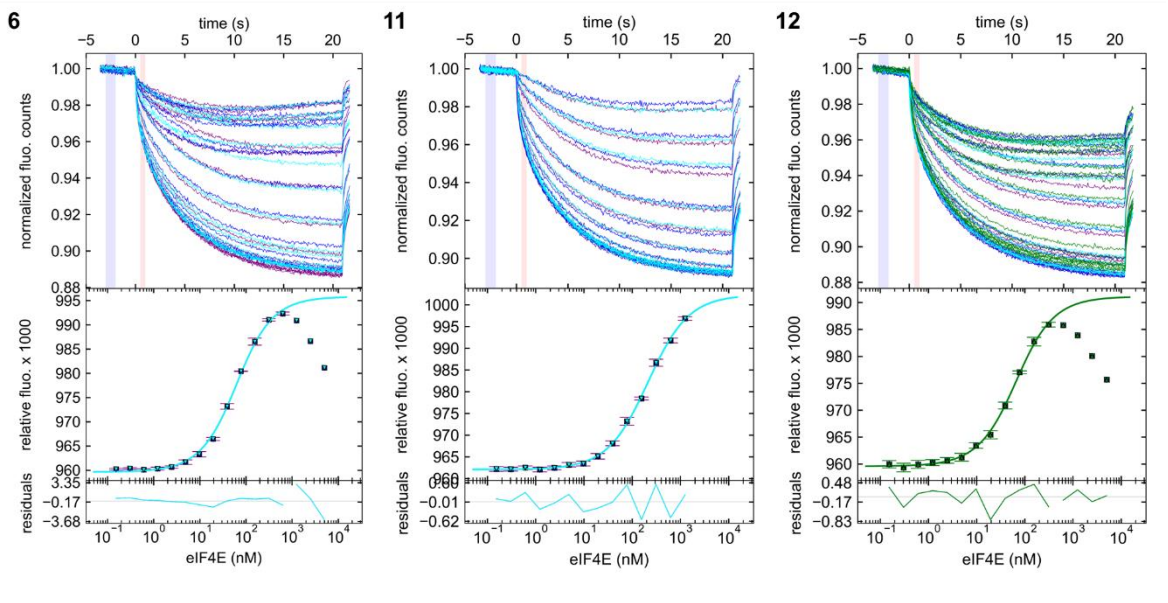


Figure S5. Probe binding affinity for meIF4E measured by microscale thermophoresis

Binding affinities of compounds **6, 11** and **12** for meIF4E determined by MST measurements. Three independent measurements were conducted (except for **12**, n=4), error bars represent standard deviation.

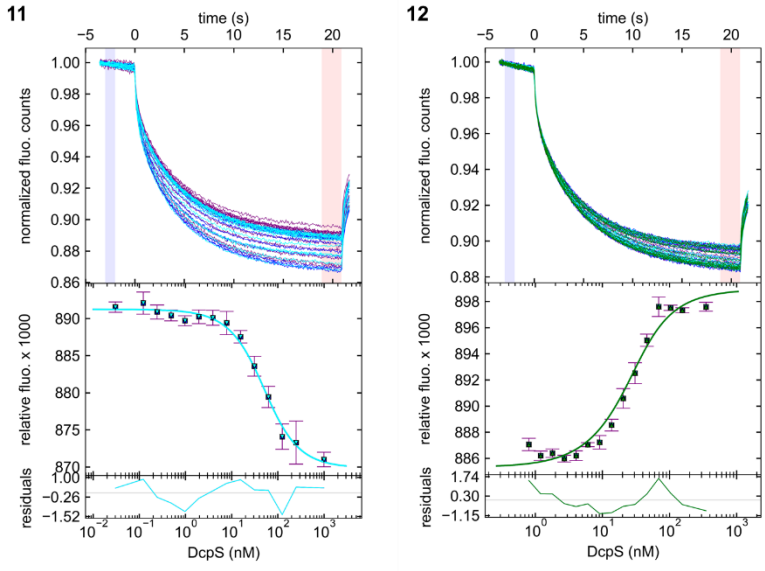
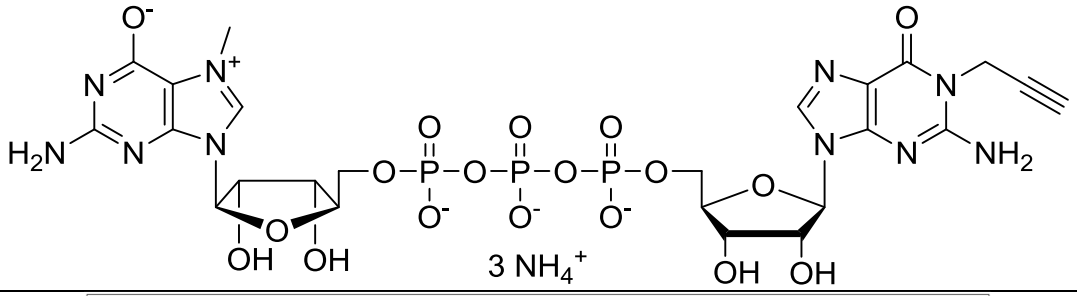
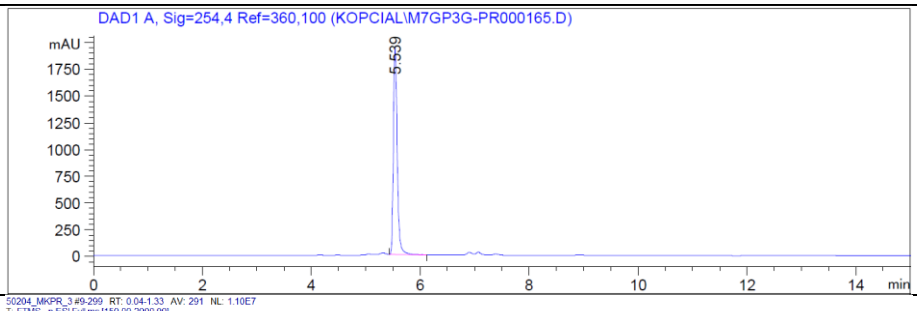
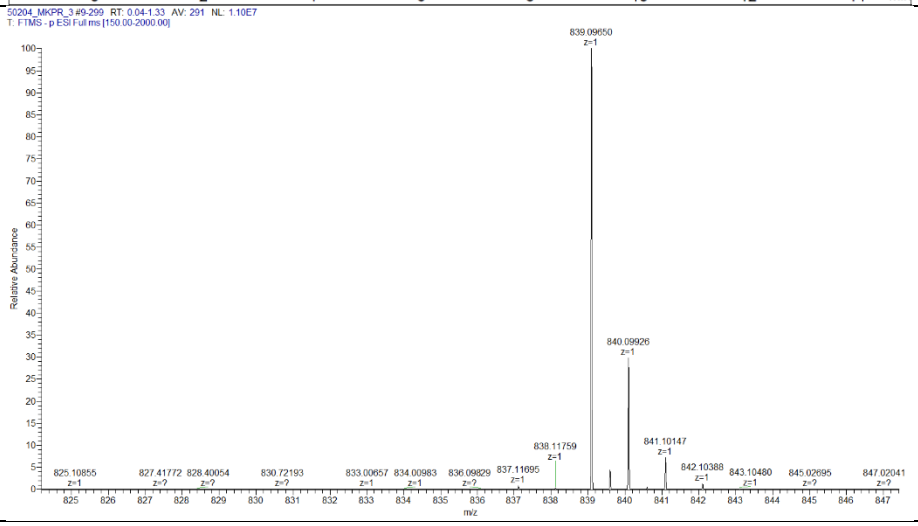
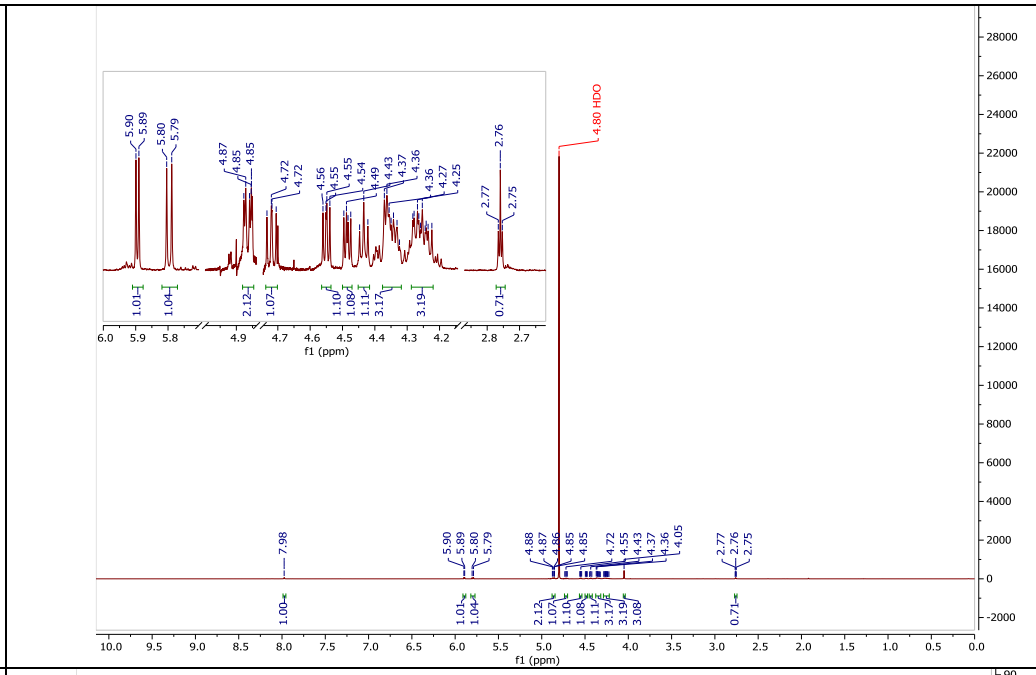
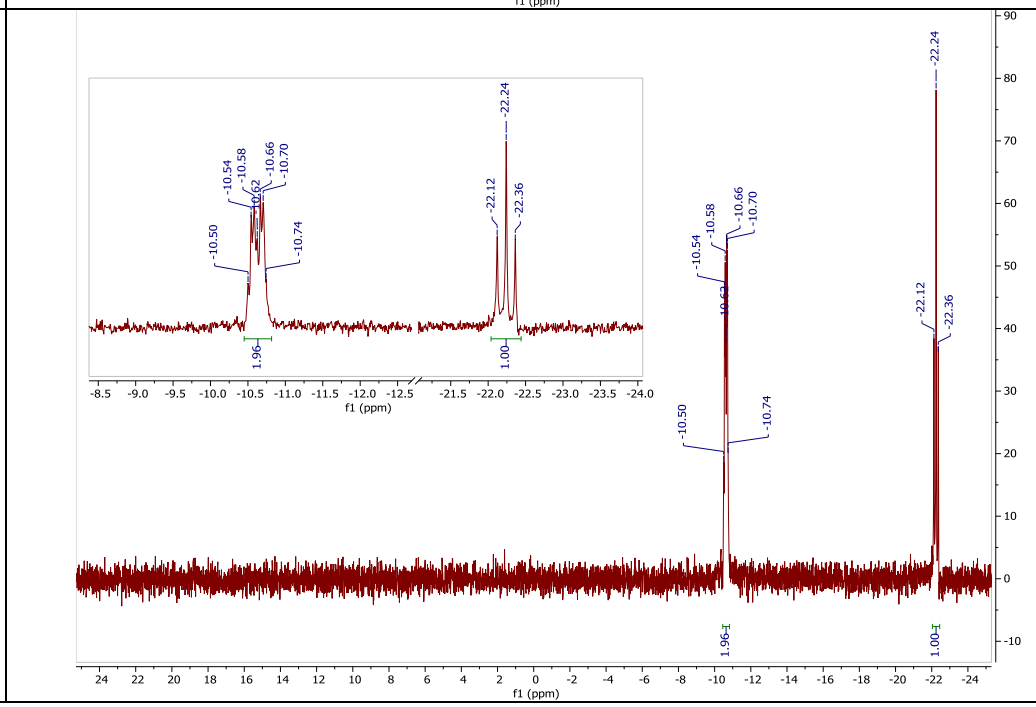


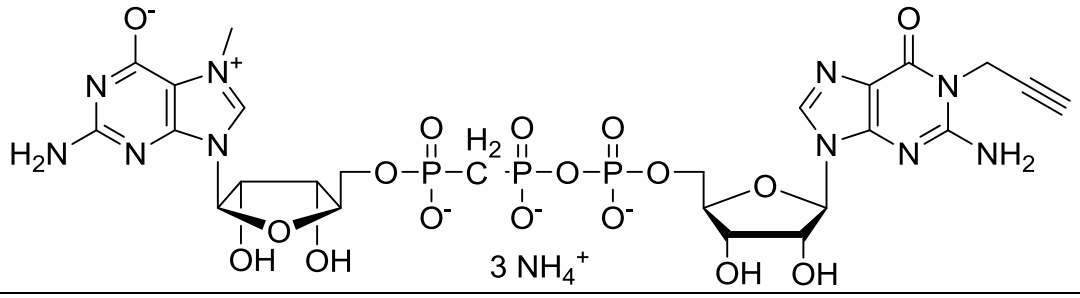
Figure S6. Probes affinity binding with hDcpS measured by microscale thermophoresis

Binding affinity of compounds **11** and **12** for hDcpS determined by MST measurements. Three independent measurements were conducted (except four for **12**, n=4), error bars represent standard deviation.

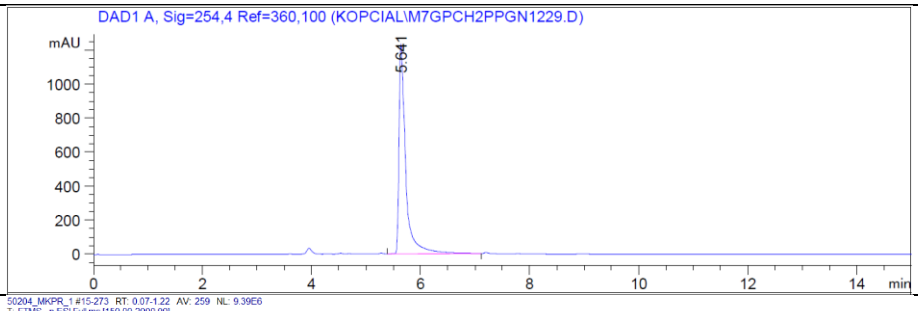
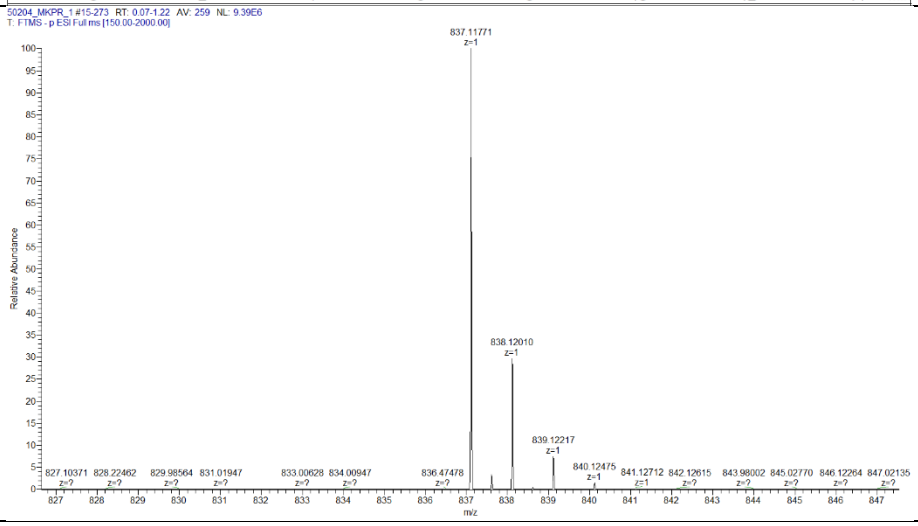
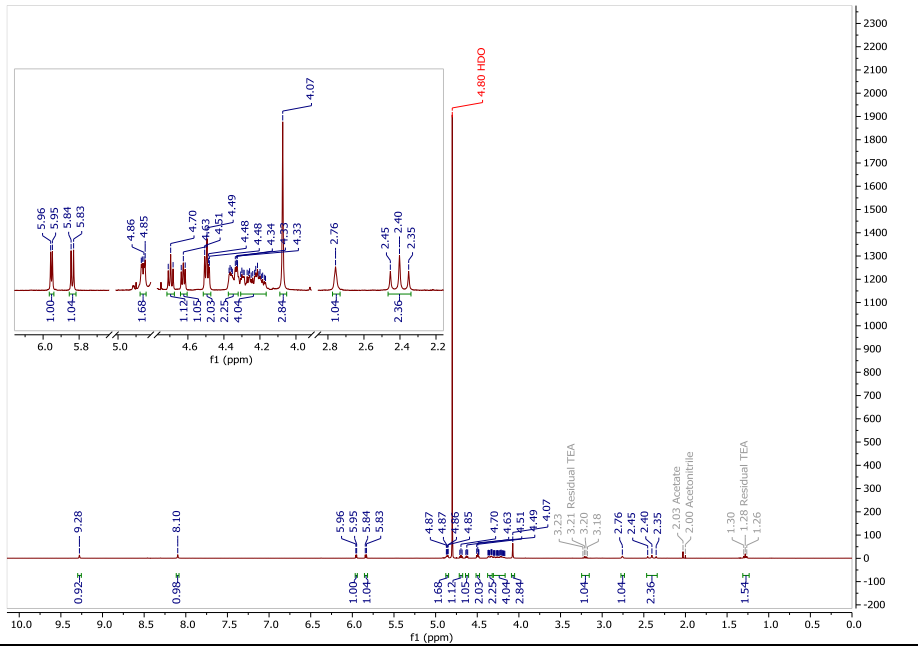
Compound characterization

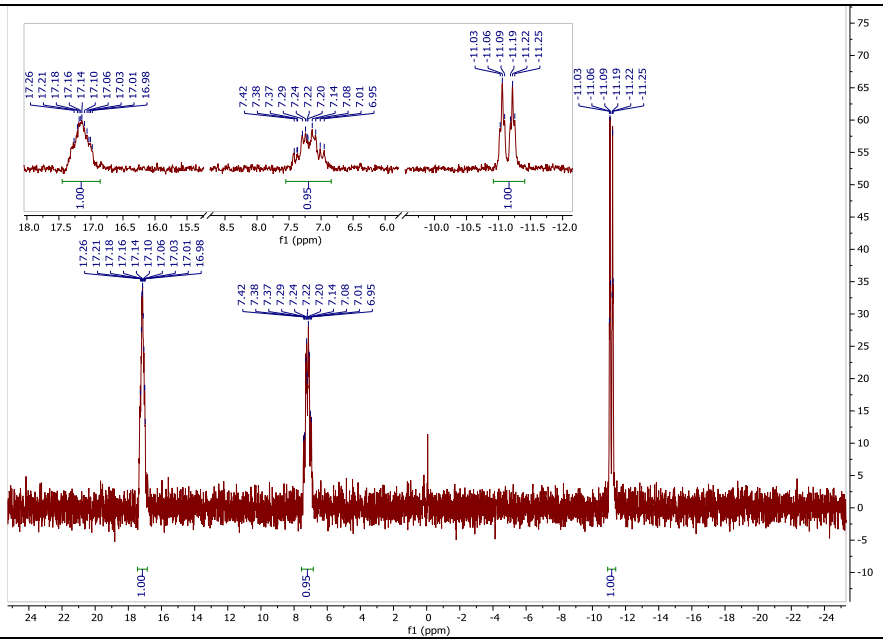
m^7Gp_3G-N1-propargyl **(1)**	
Structure	
HPLC of purified product (method A)	
HRMS	

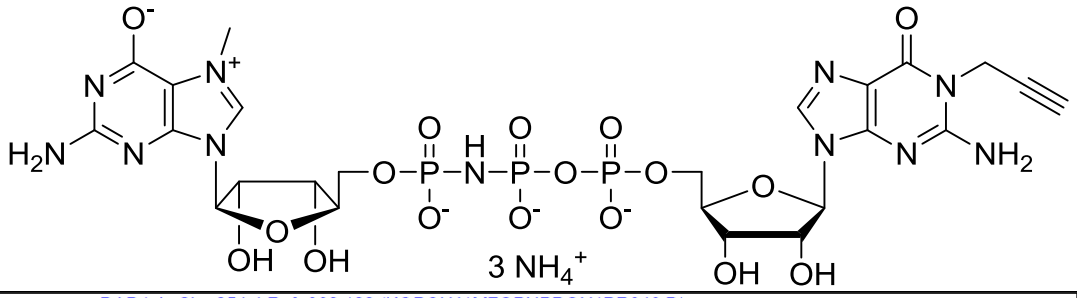
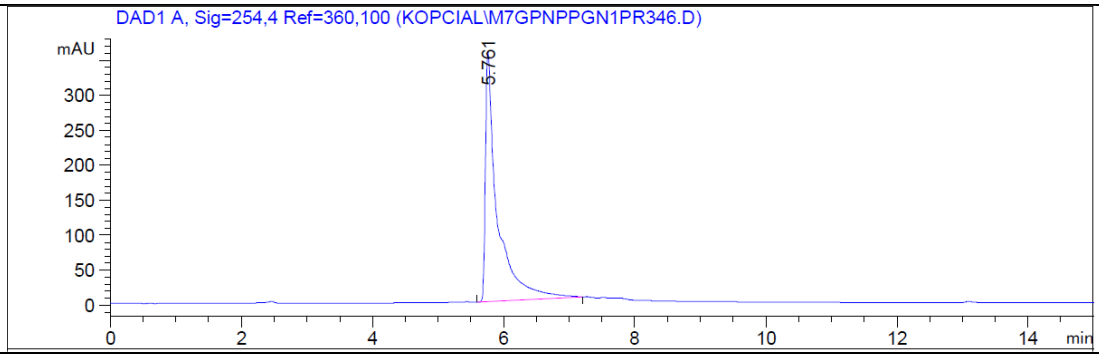
^{1}H NMR (400 MHz, D_2O)	
^{31}P NMR (162 MHz, D_2O)	

Structure	

m^7GpCH_2ppG-N1-pr **(2)**

HPLC of purified product (method A)	
HRMS	
^{1}H NMR (400 MHz, D_2O)	

^{31}P NMR (162 MHz, D_2O)	

m^{7}GpNHppG-N1-propargyl **(3)**	
Structure	
HPLC of purified product (method A)	

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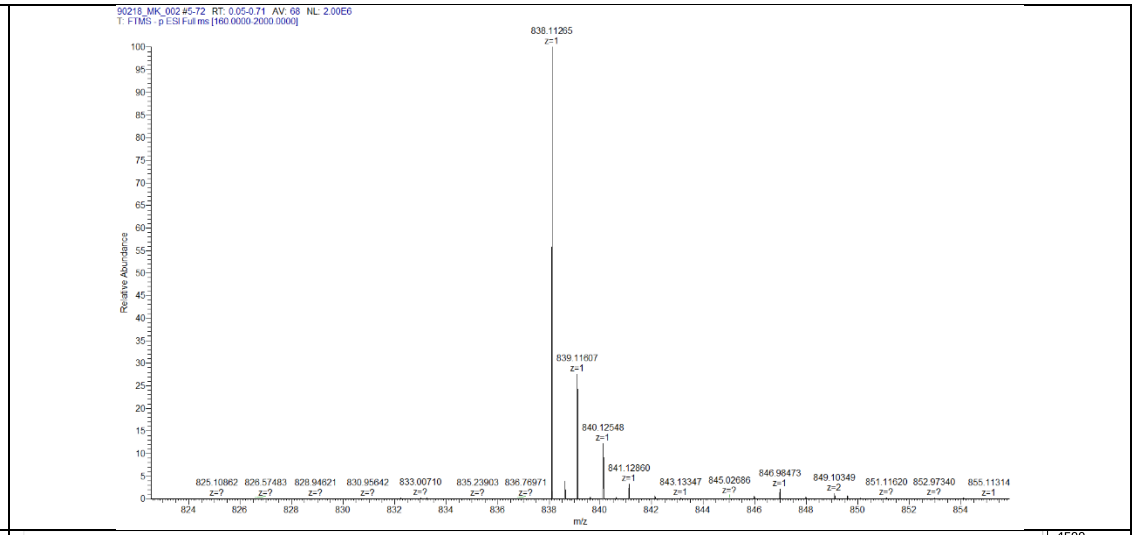
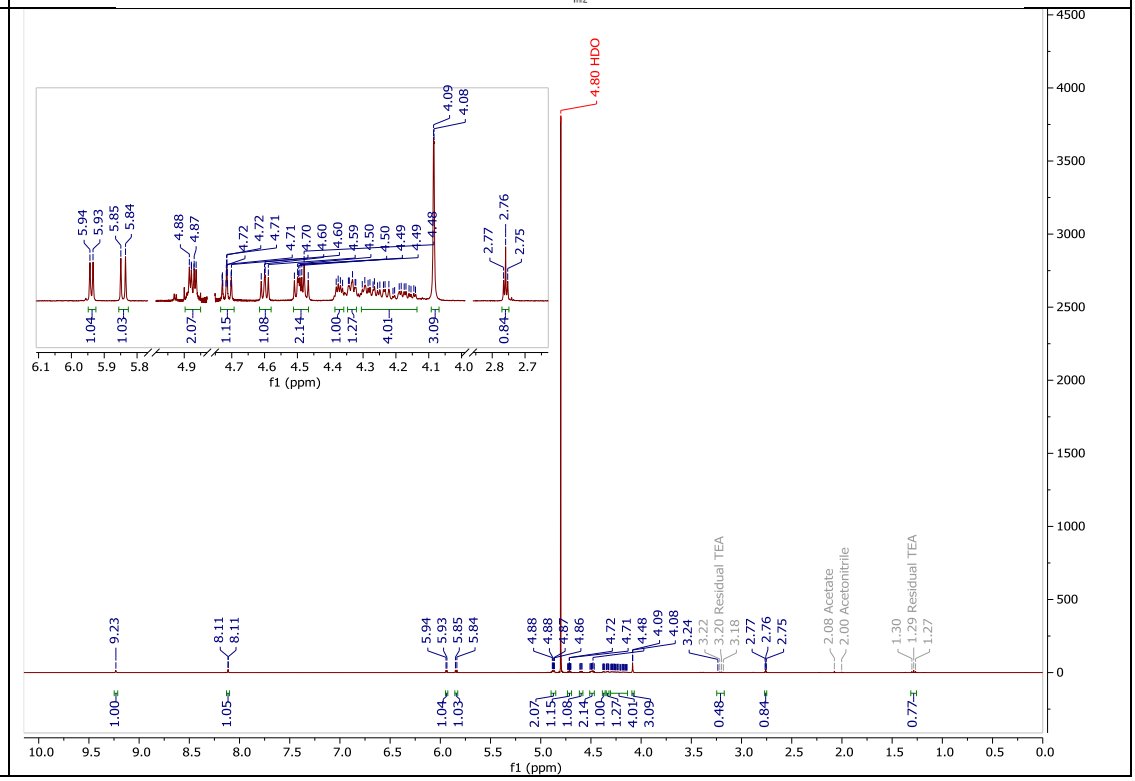
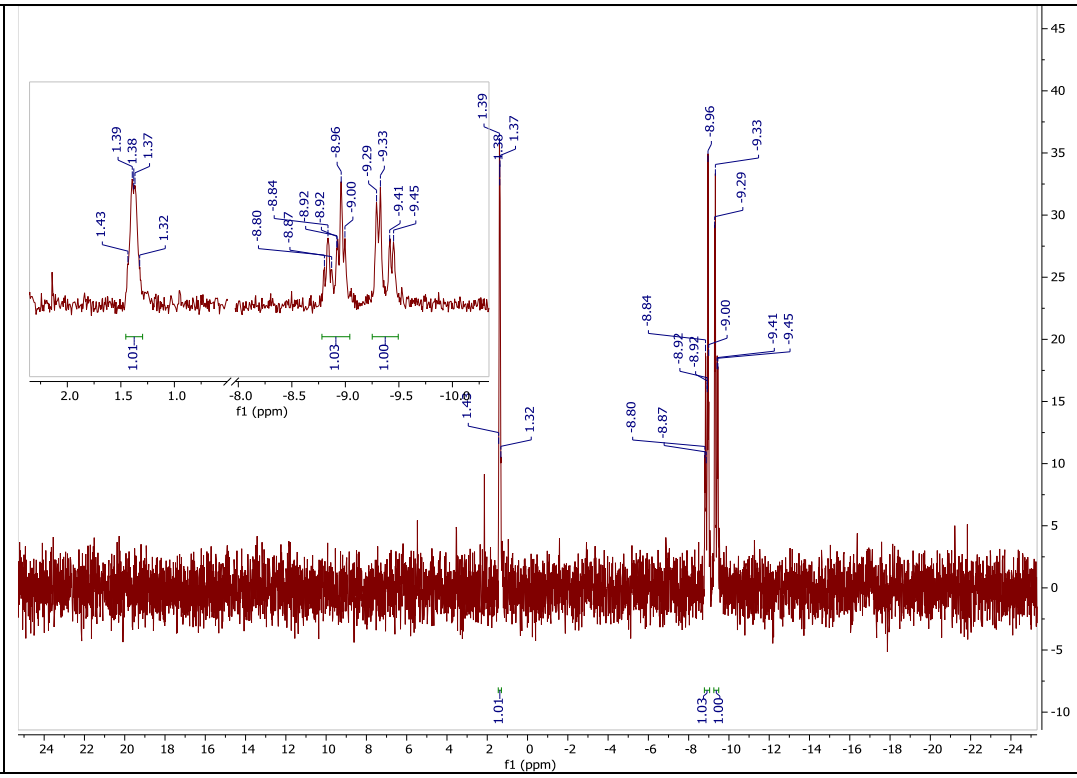
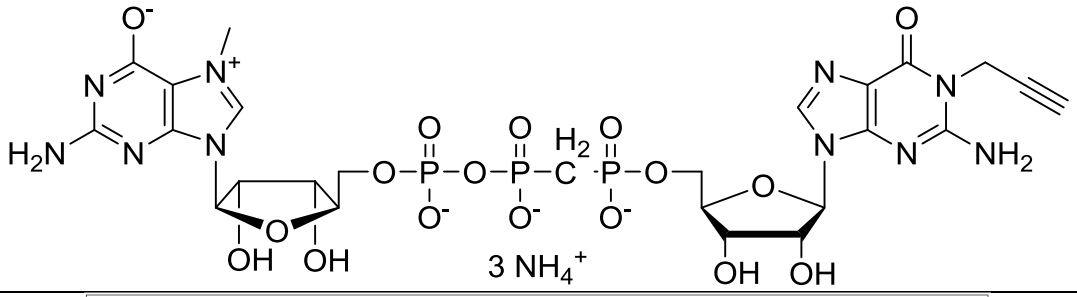
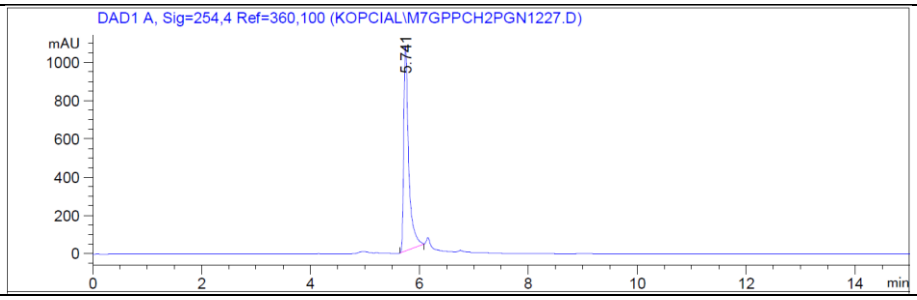
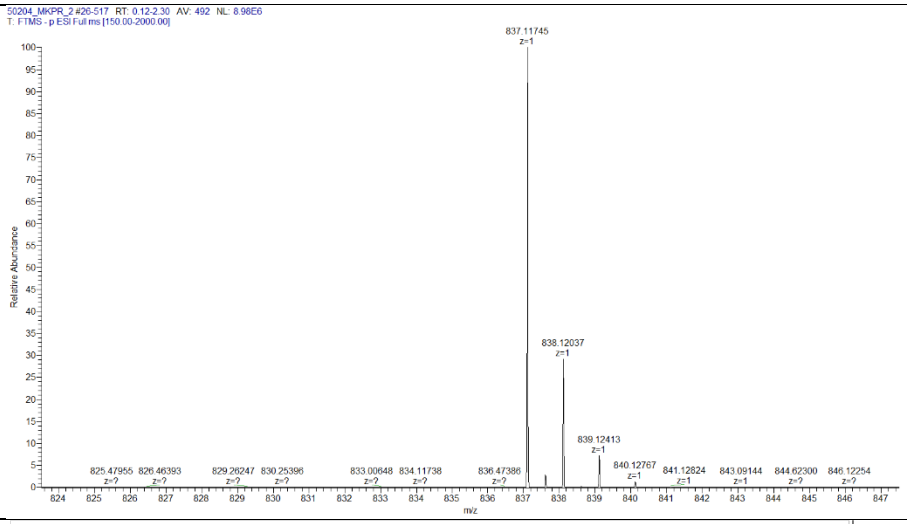
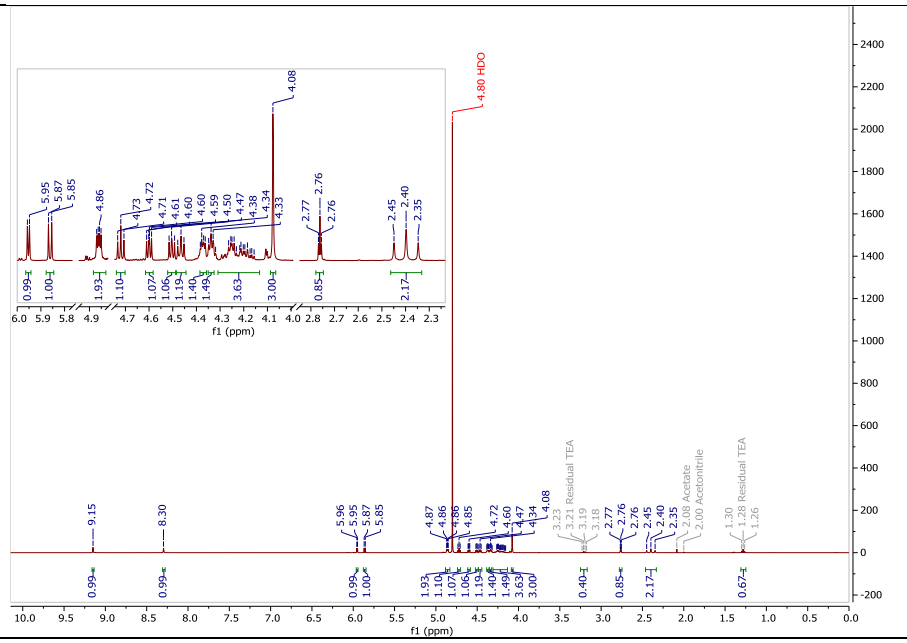


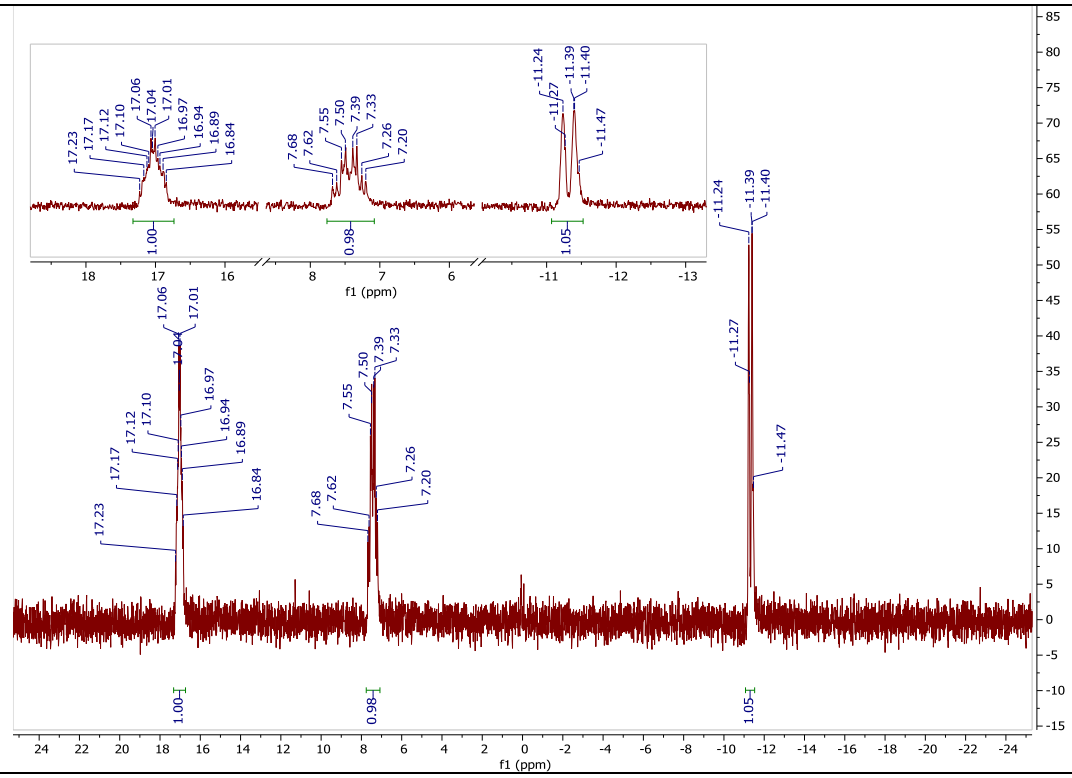
^{1}H NMR (400 MHz, D_2O)

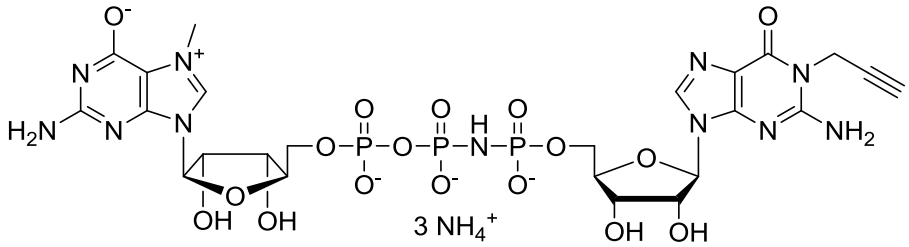
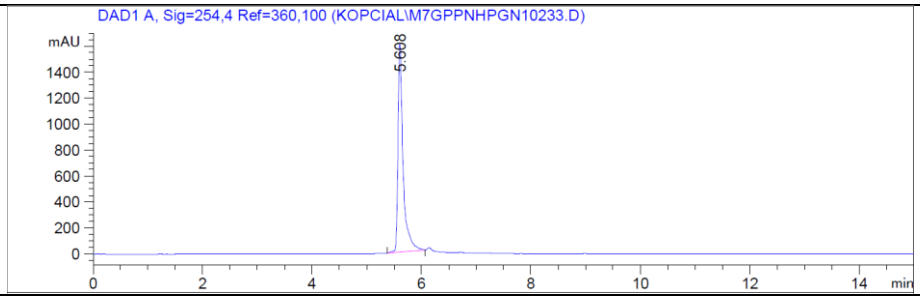


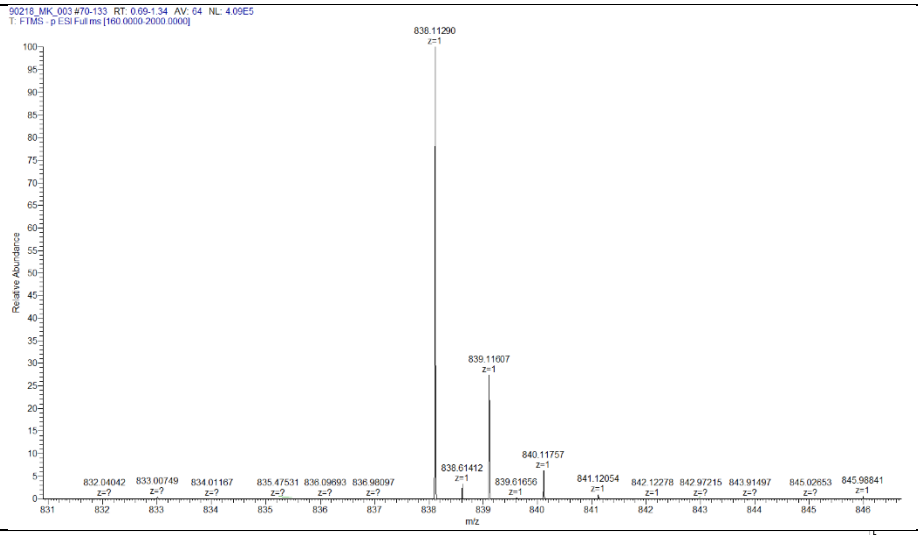
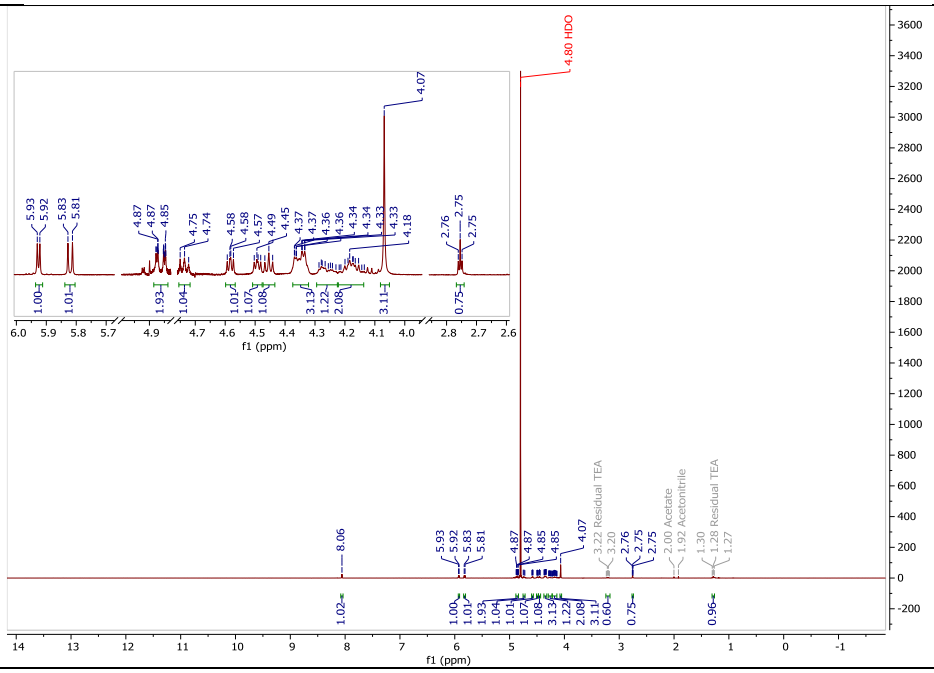
^{31}P NMR (162 MHz, D_2O)	

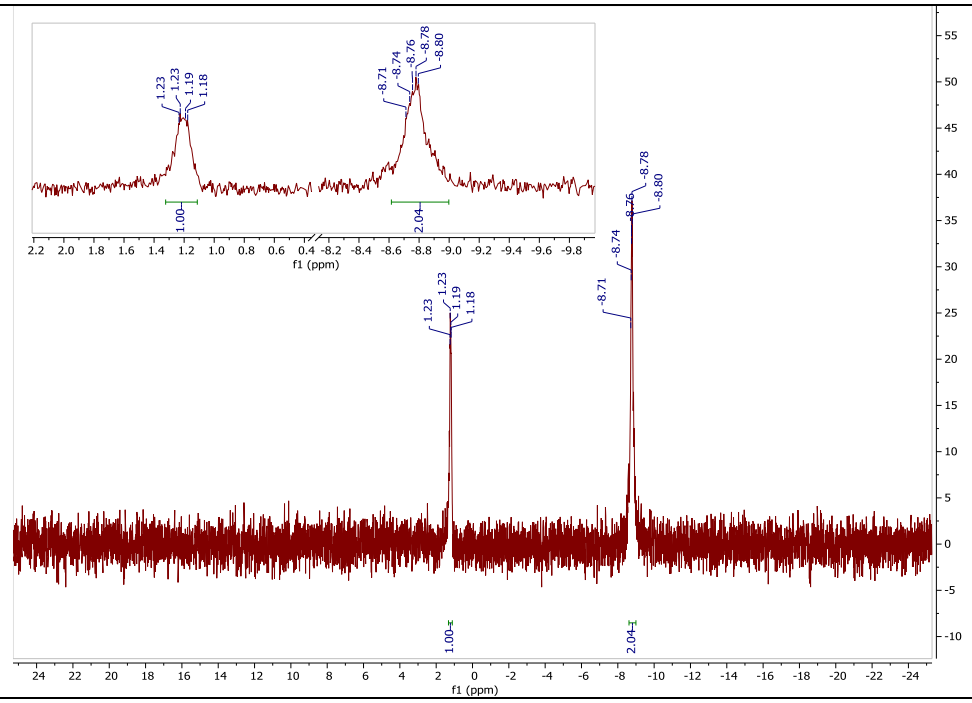
m^{7}GppCH$_2$pG-N1-propargyl **(4)**	
Structure	
HPLC of purified product (method A)	

HRMS	
^{1}H NMR (400 MHz, D_2O)	

^{31}P NMR (162 MHz, D_2O)	

m^{7}GppNHpG-N1-propargyl **(5)**	
Structure	
HPLC of purified product (method A)	

HRMS	
^{1}H NMR (400 MHz, D_2O)	

^{31}P NMR (162 MHz, D_2O)	

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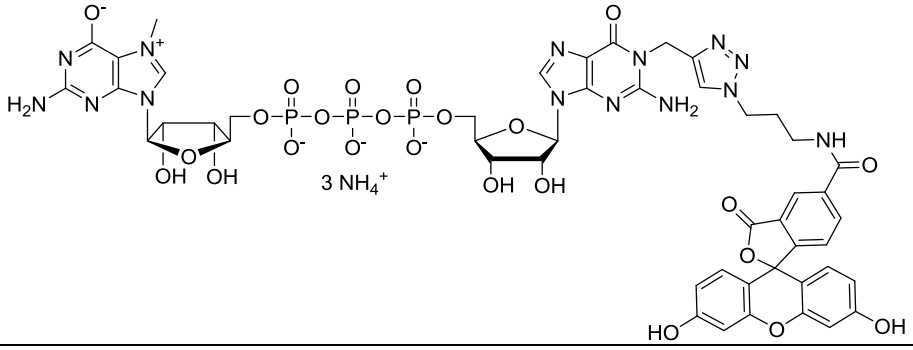
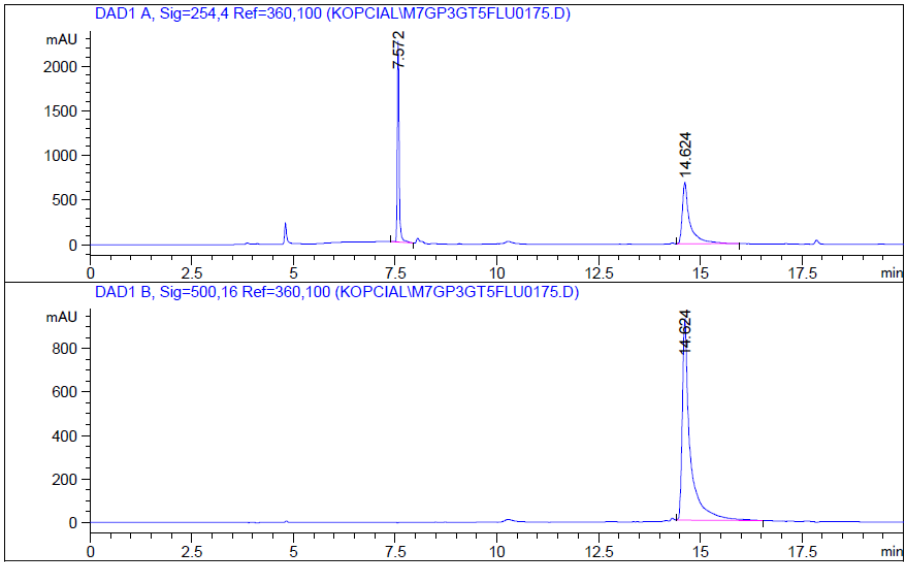
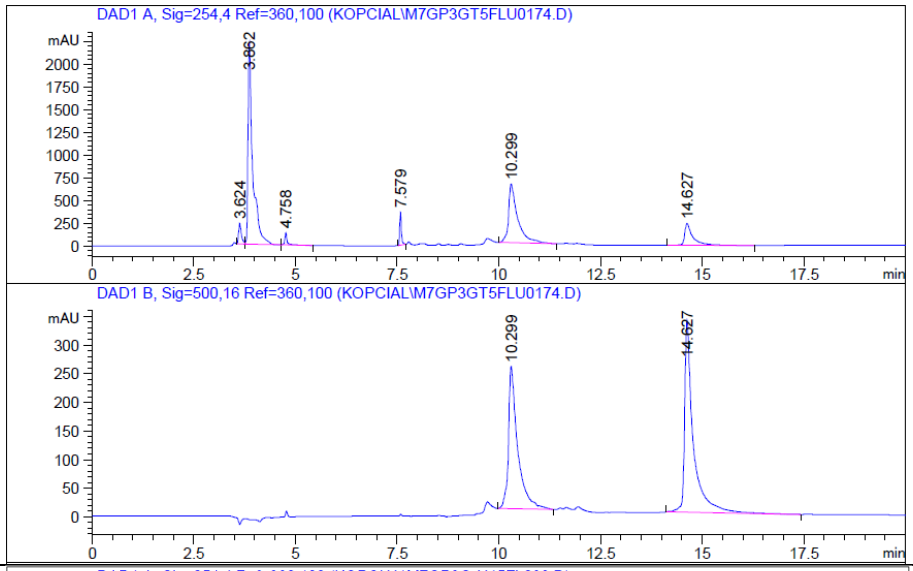
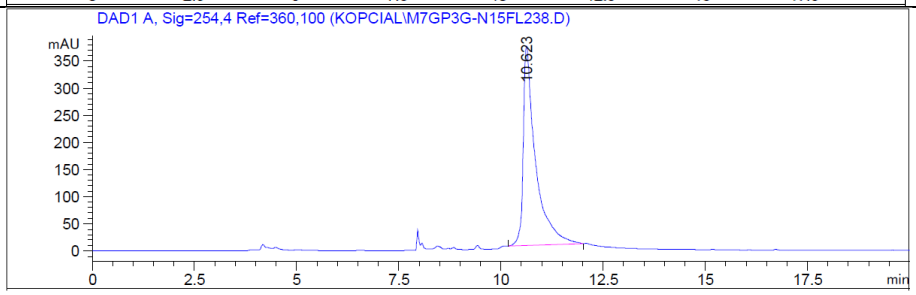
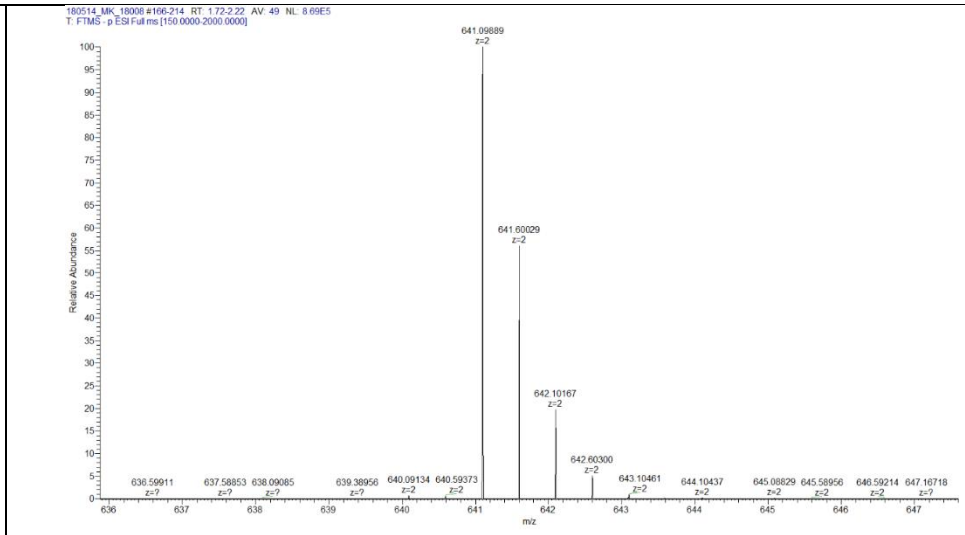
Structure	

Reaction HPLC (Method C)	t = 0  t = 1 h 
HPLC of purified product (method C)	

HR MS	

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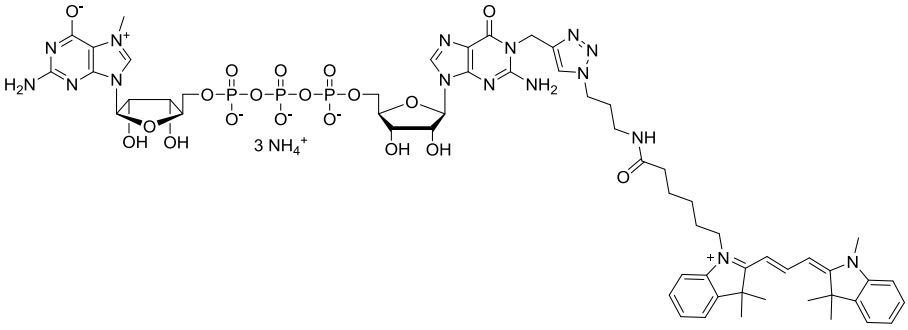
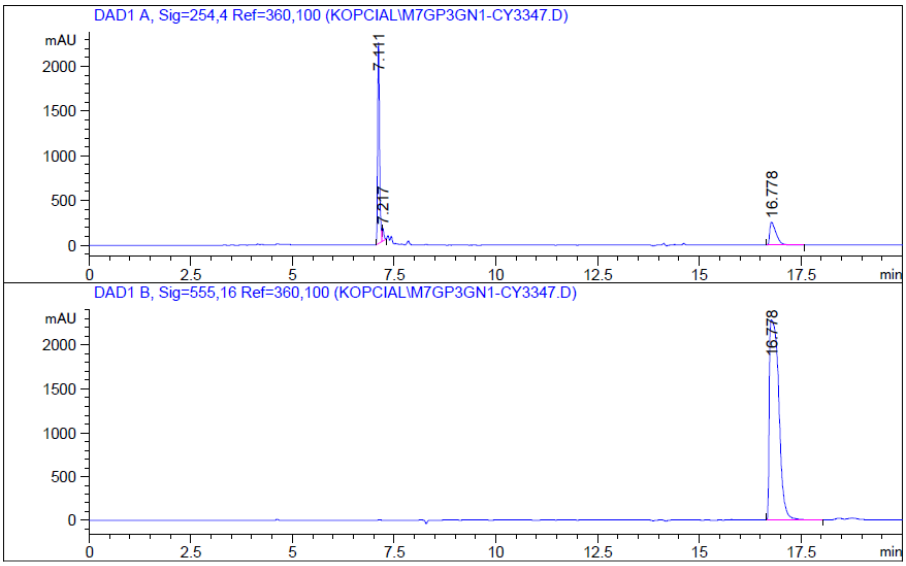
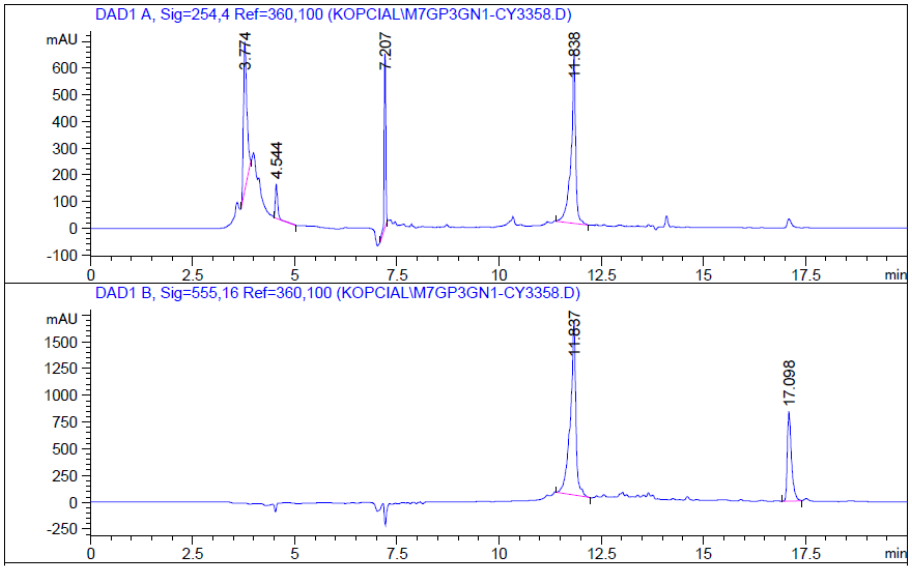
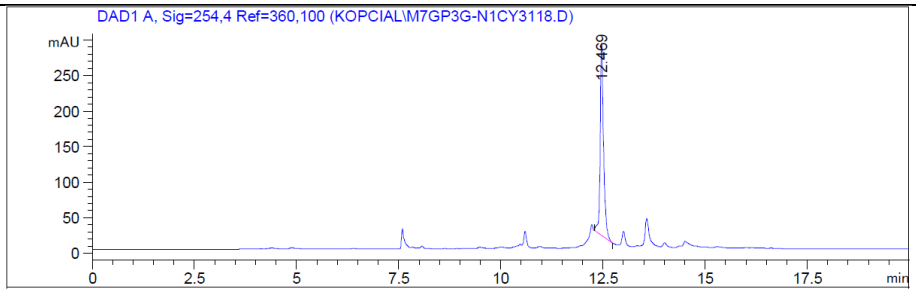
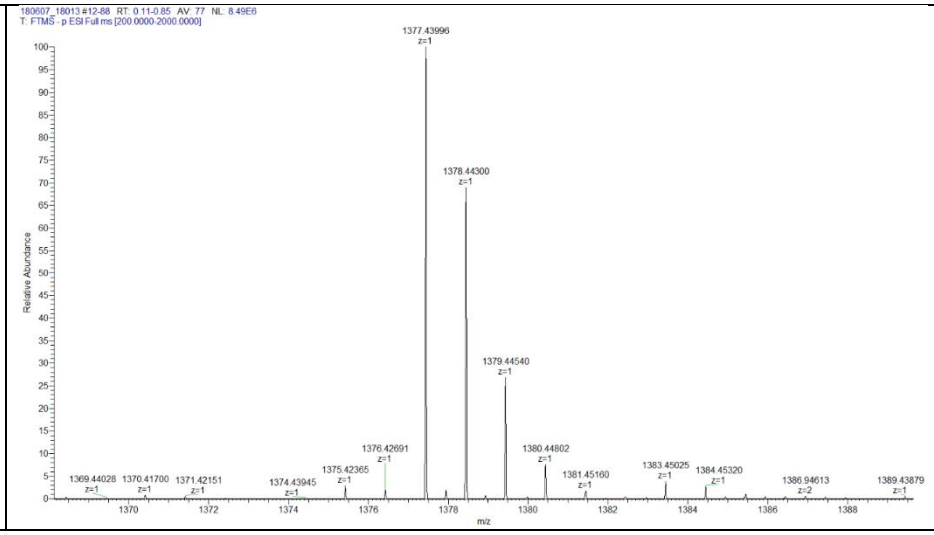
Structure	

Reaction HPLC (Method C)	t = 0  t = 1 h 
HPLC of purified product (method C)	

HR MS	

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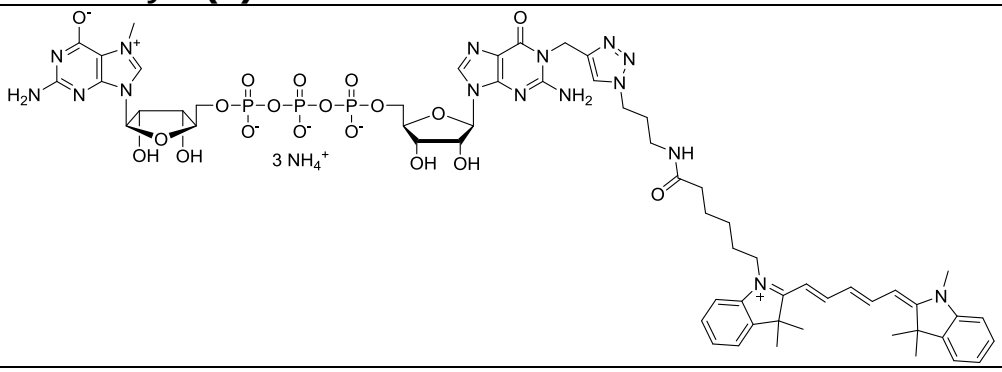
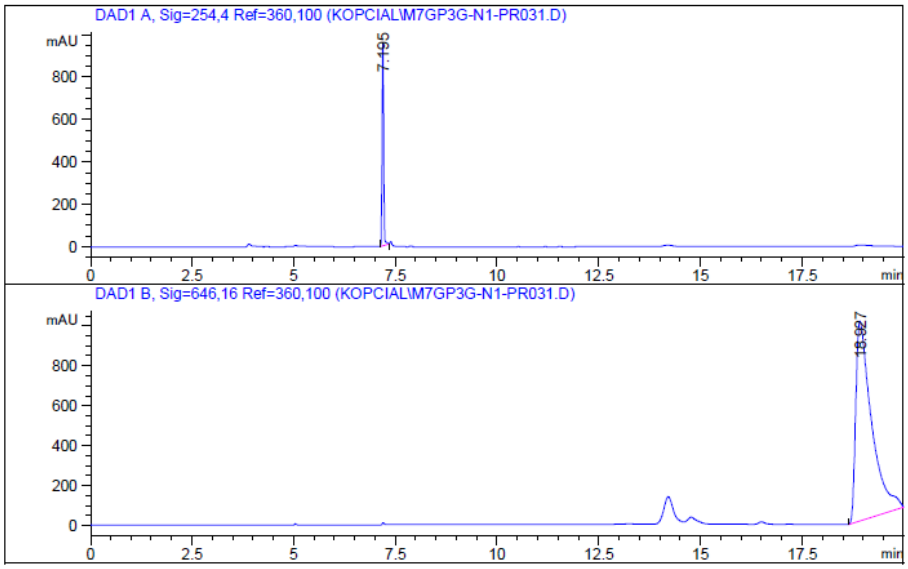
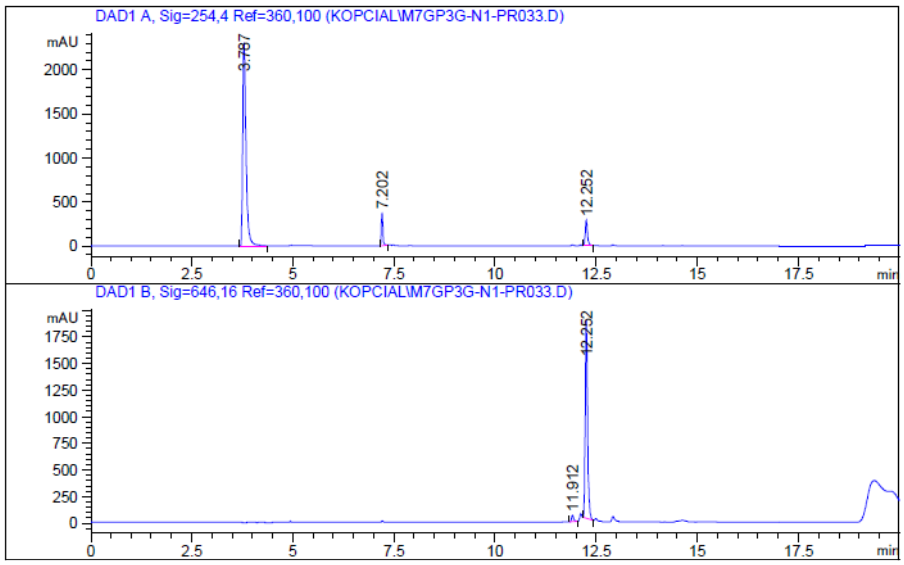
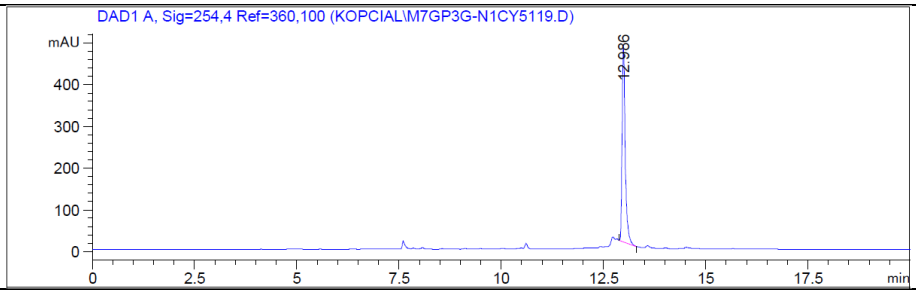
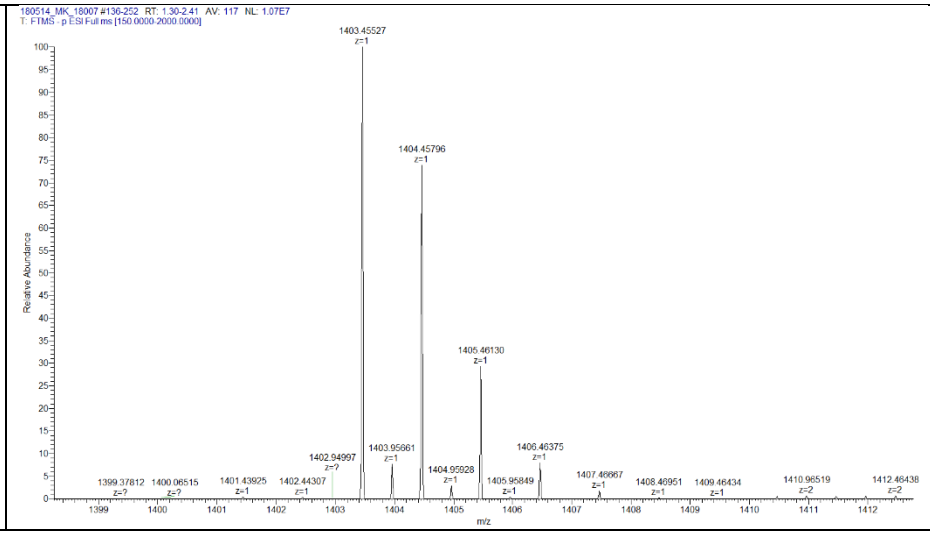
Structure	

Reaction HPLC (Method C)	t = 0  t = 50 min 
HPLC of purified product (method C)	

HR MS	

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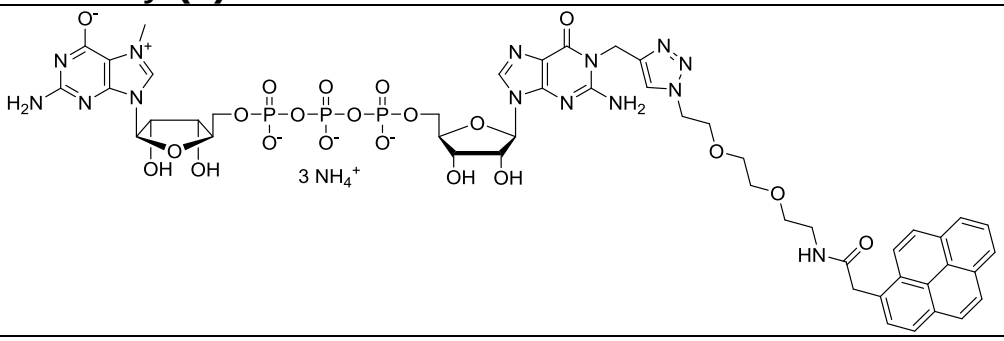
Structure	

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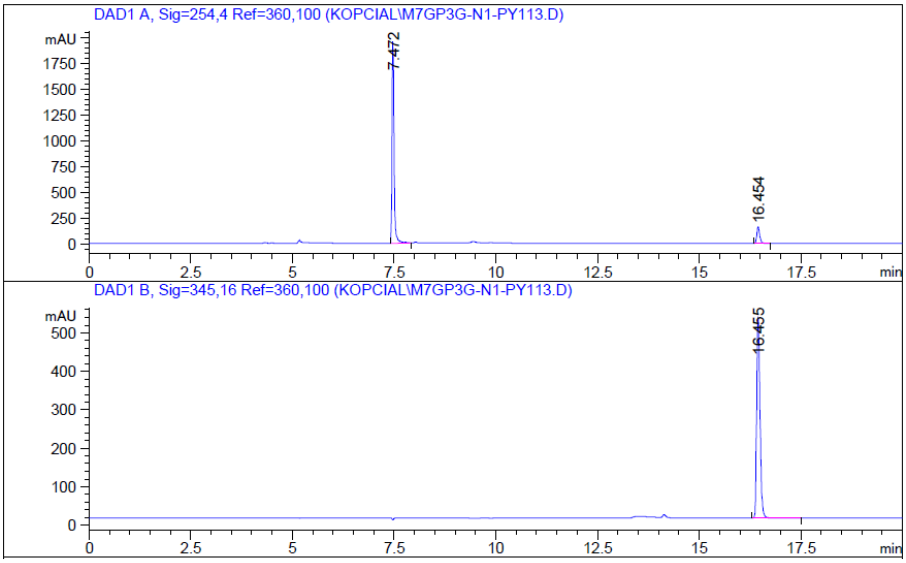

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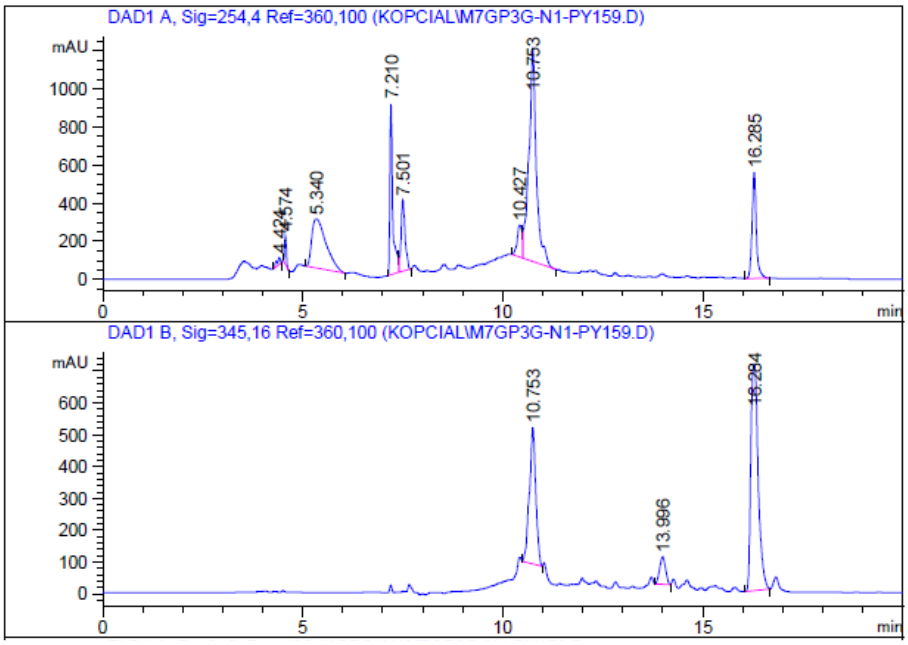
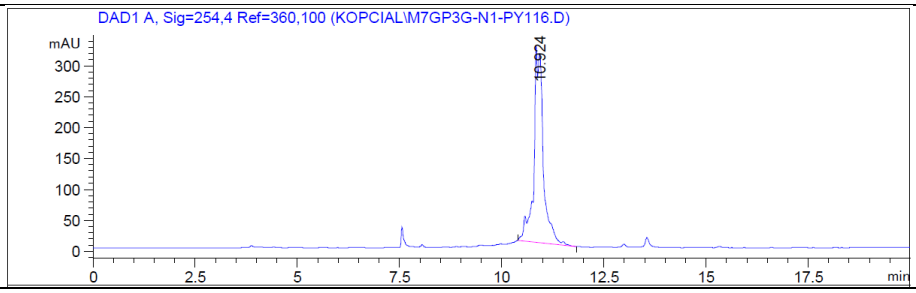
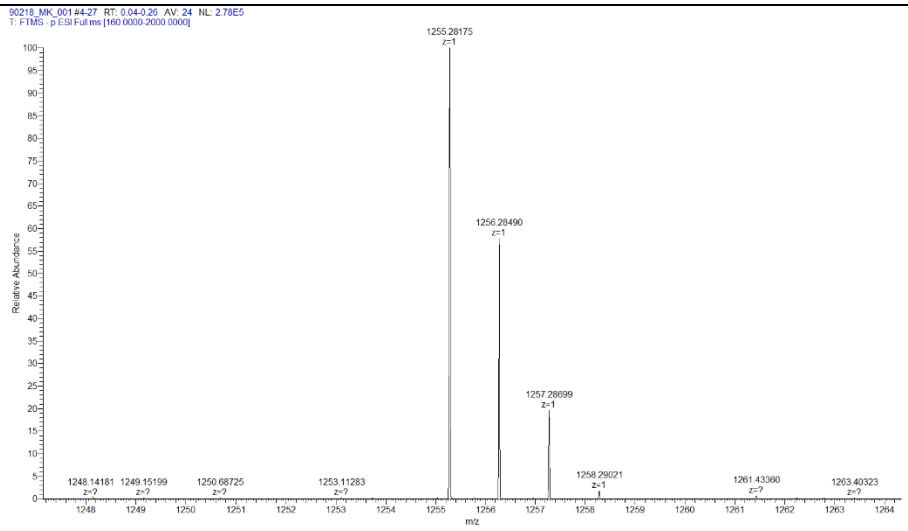
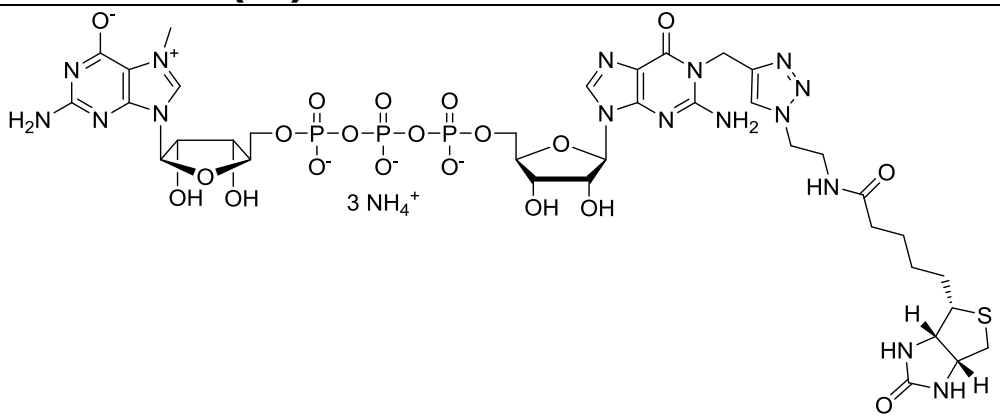
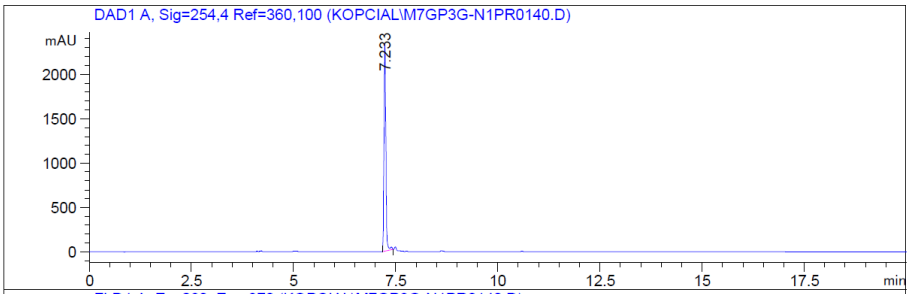
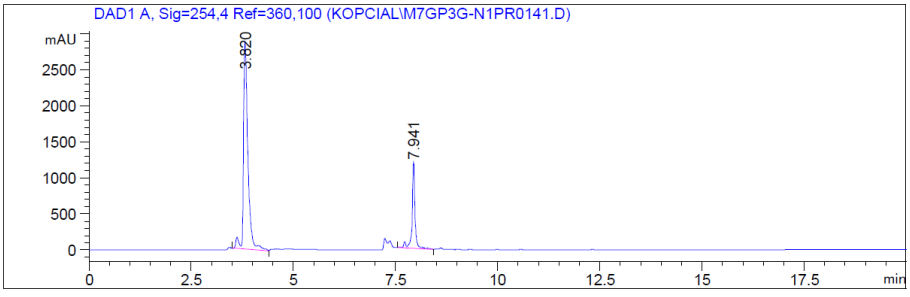
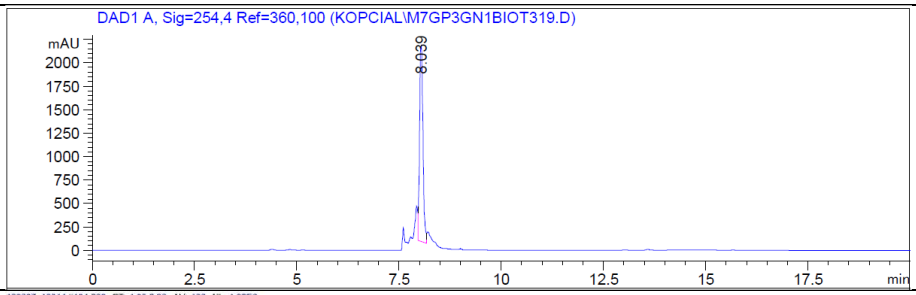
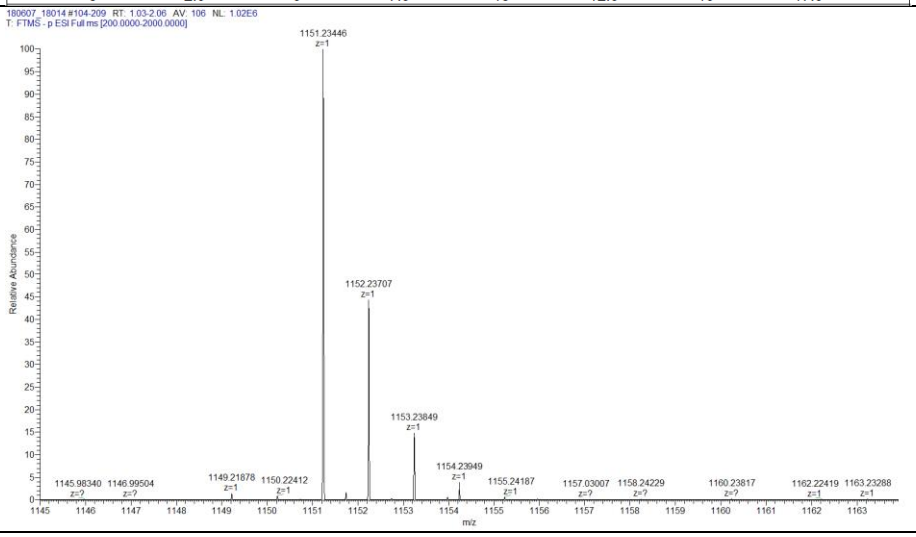


HPLC of purified product (method C)



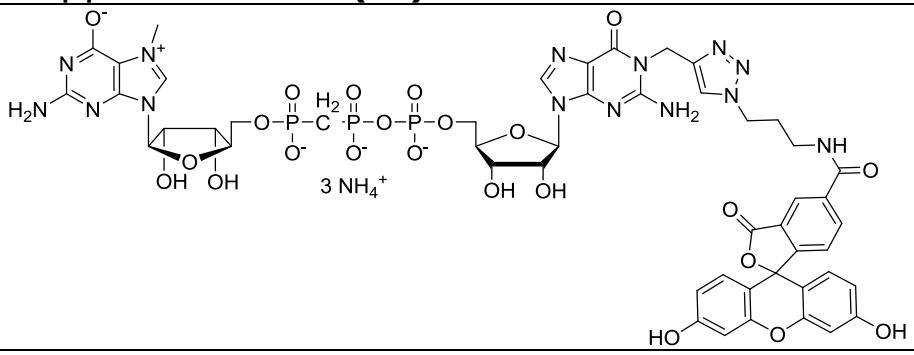
HR MS	

m^7Gp_3G-N1-biotin **(10)**	
Structure	
Reaction HPLC (Method C)	t = 0  t = 1 h 

HPLC of purified product (method C)	
HR MS	

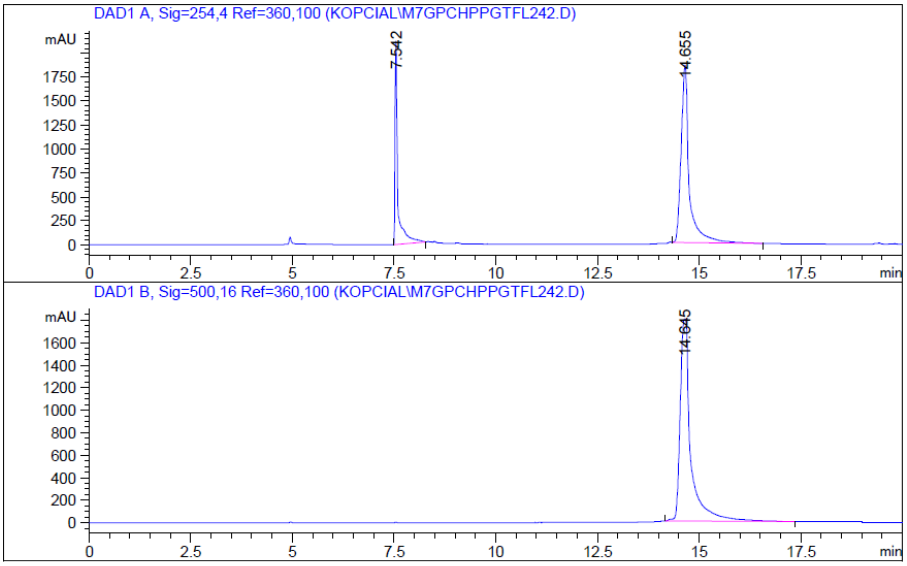
m^7GpCH_2ppG-N1-5FAM **(11)**

Structure

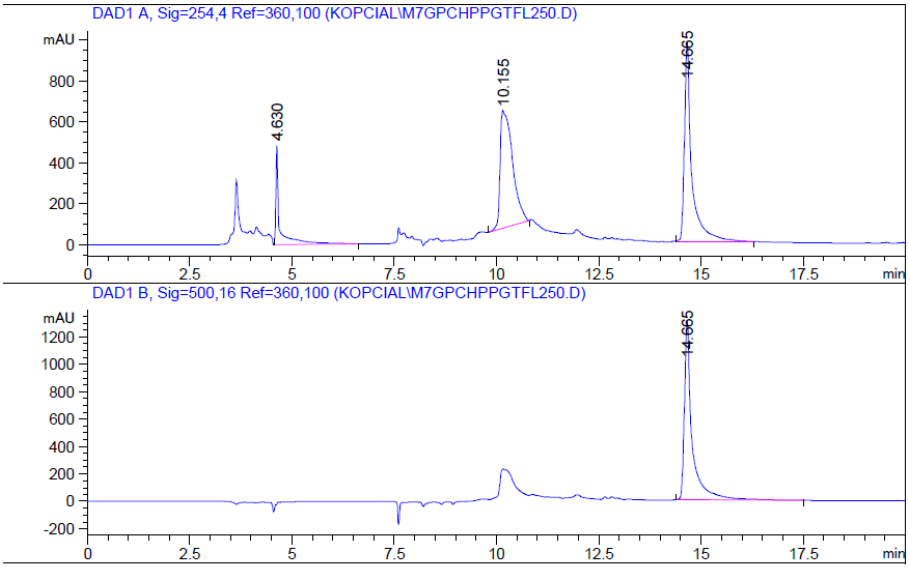


Reaction HPLC (Method D)

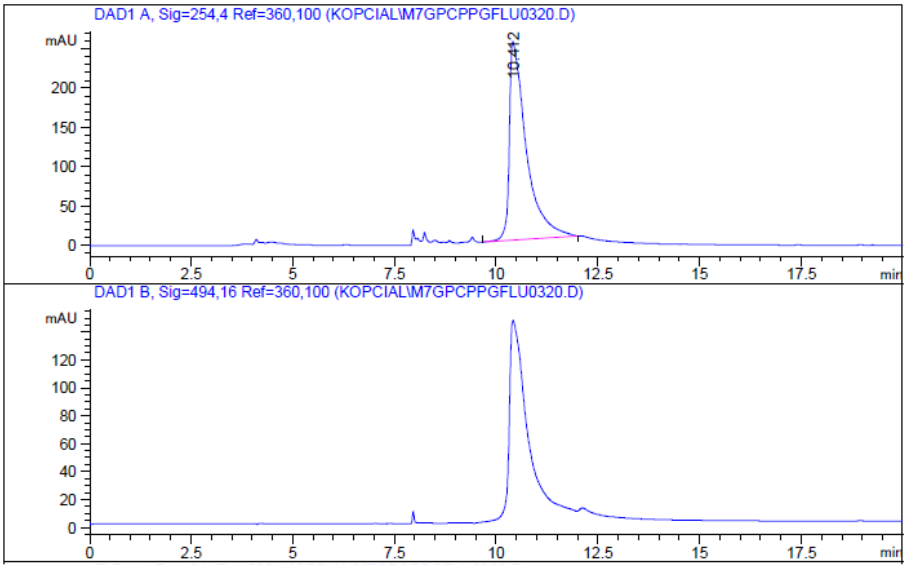
t = 0

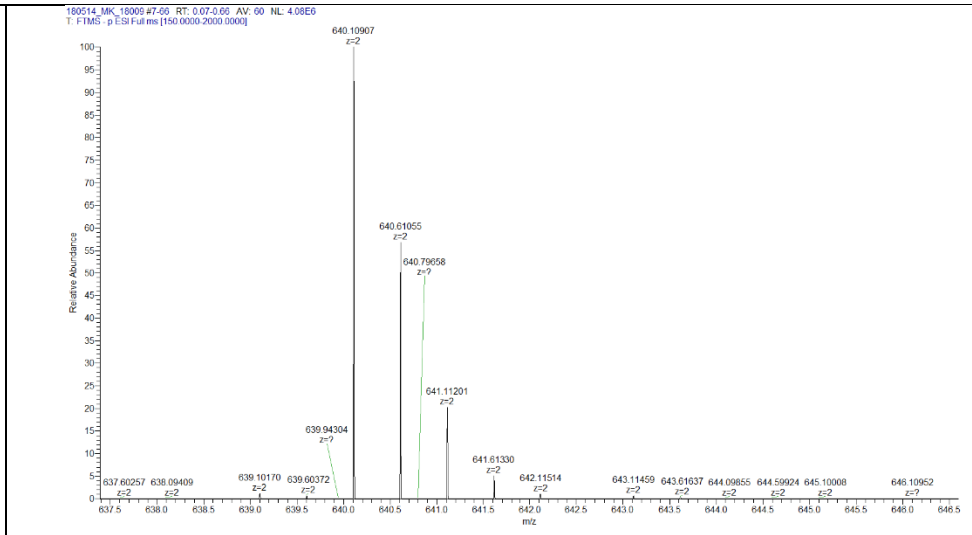


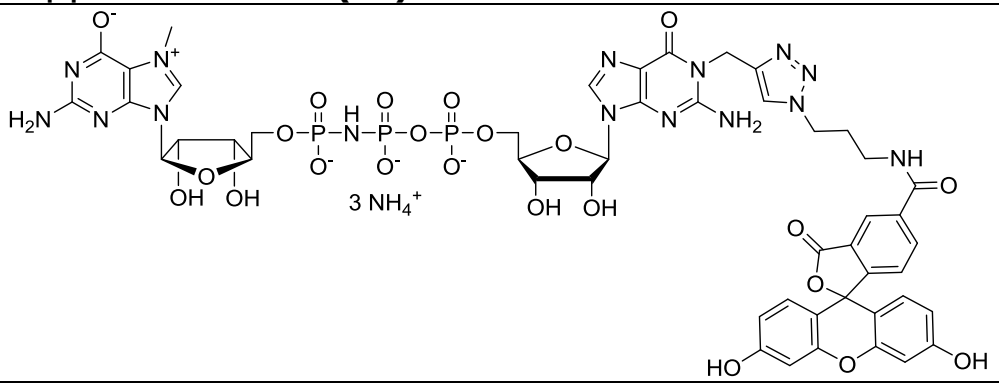
t = 1 h

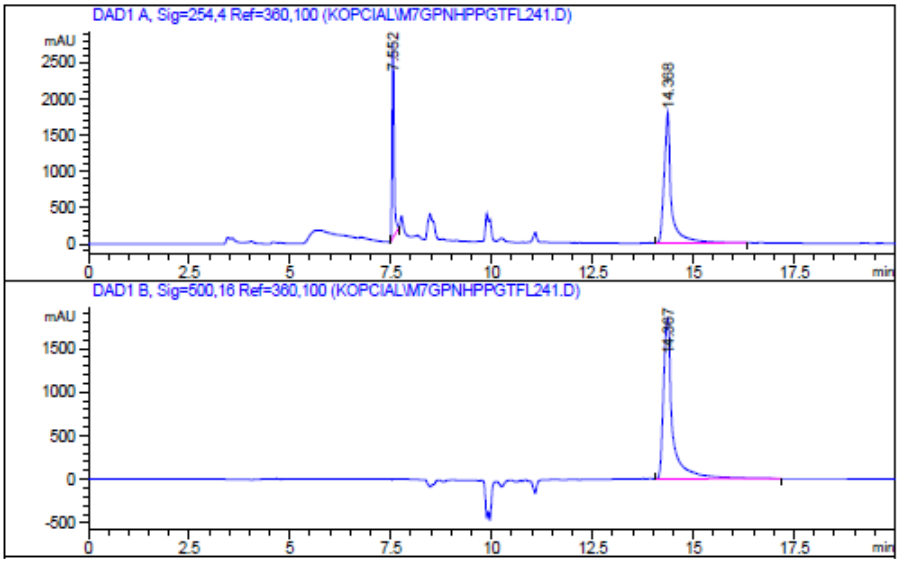
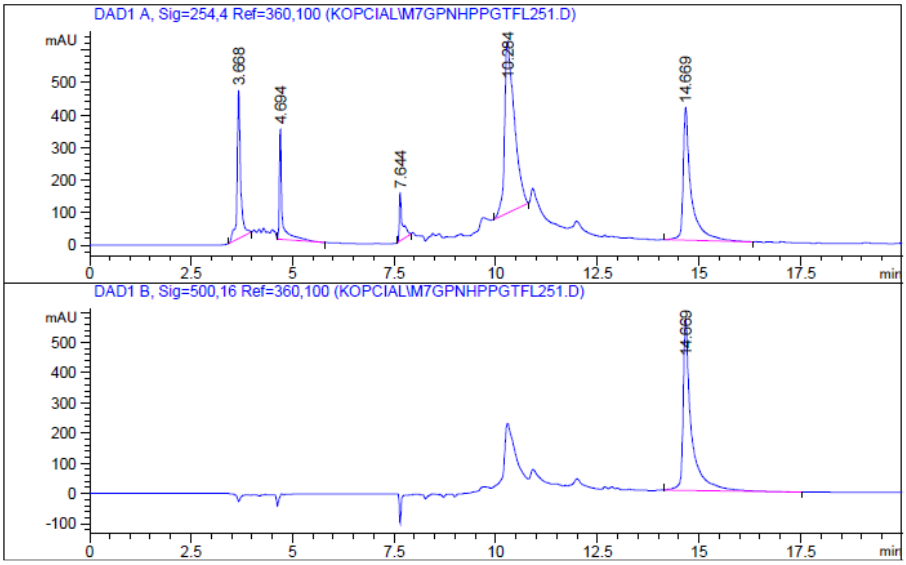
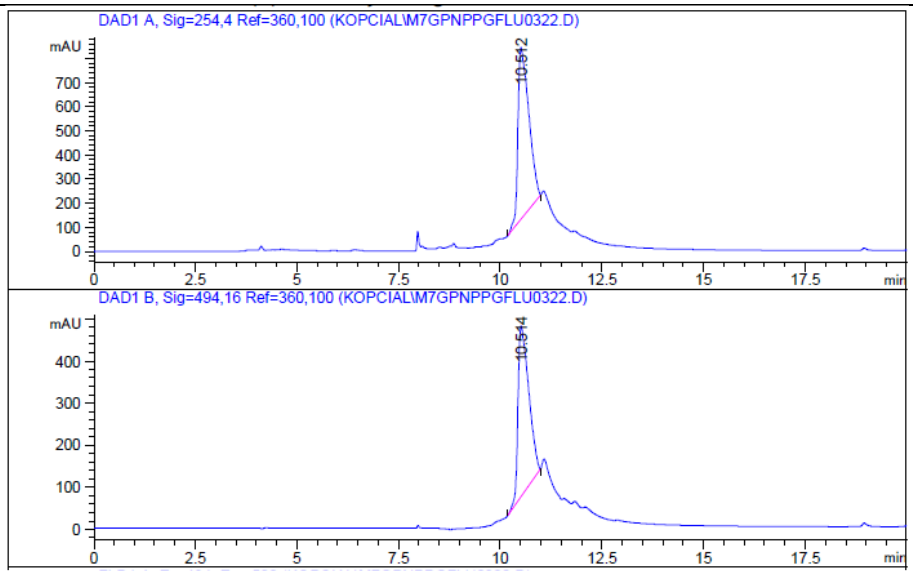


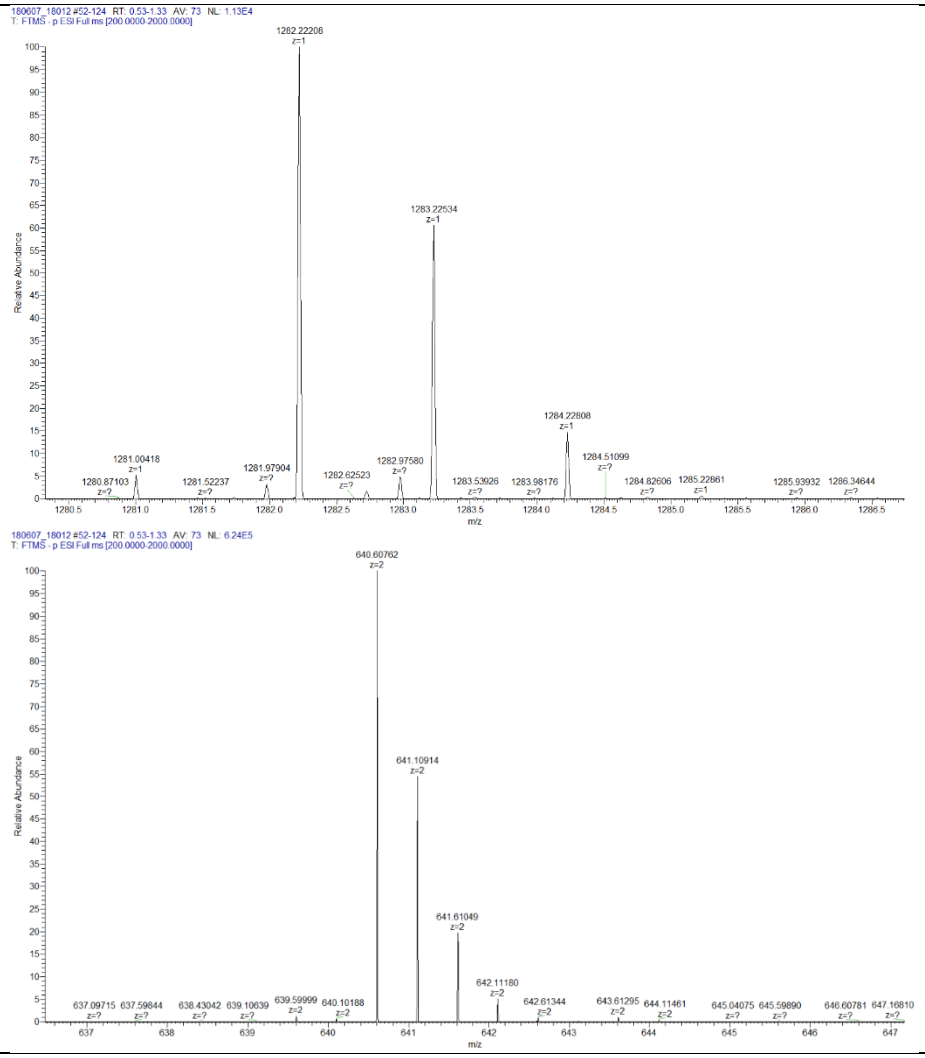
HPLC of purified product (method C)

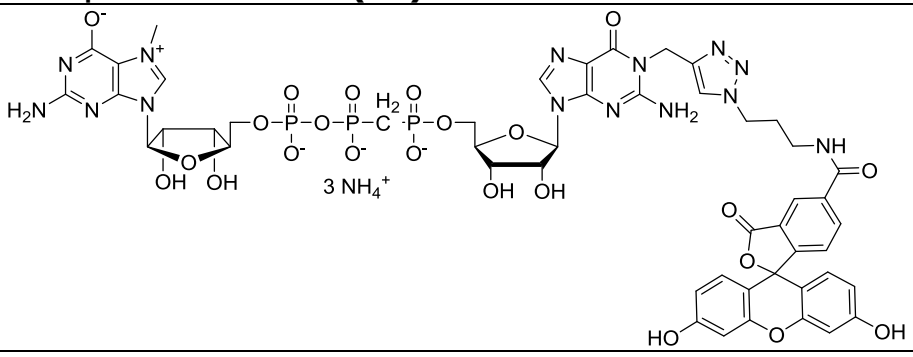


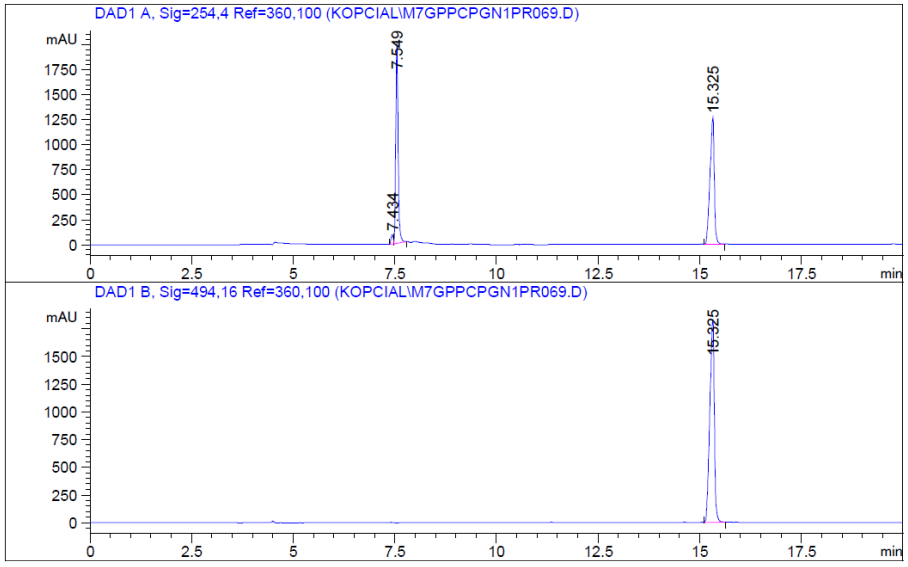
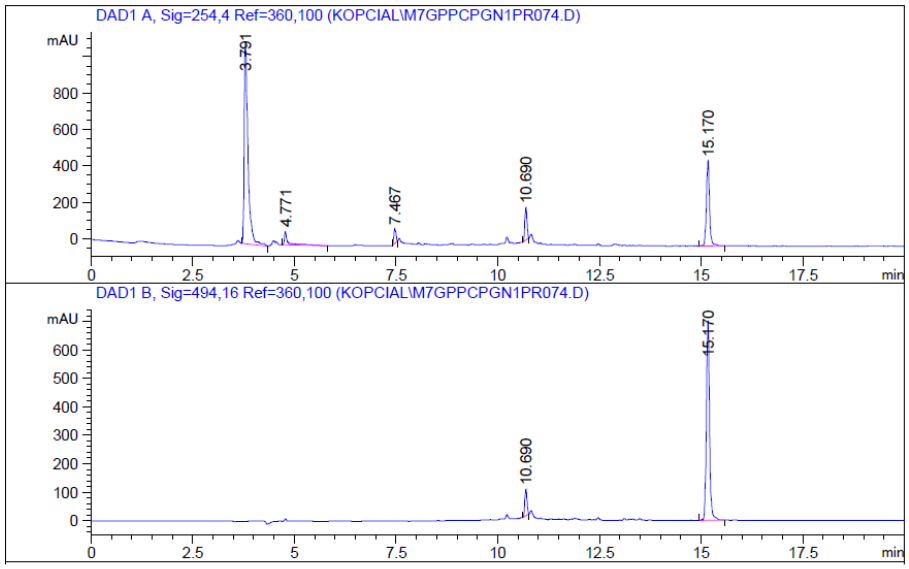
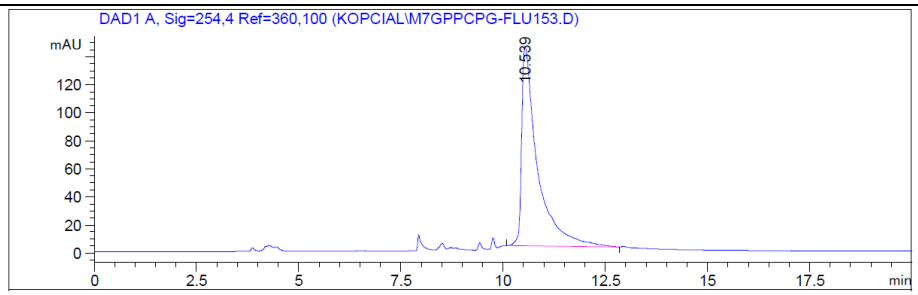
HR MS	

m^{7}GpNHppG-N1-5FAM **(12)**	
Structure	

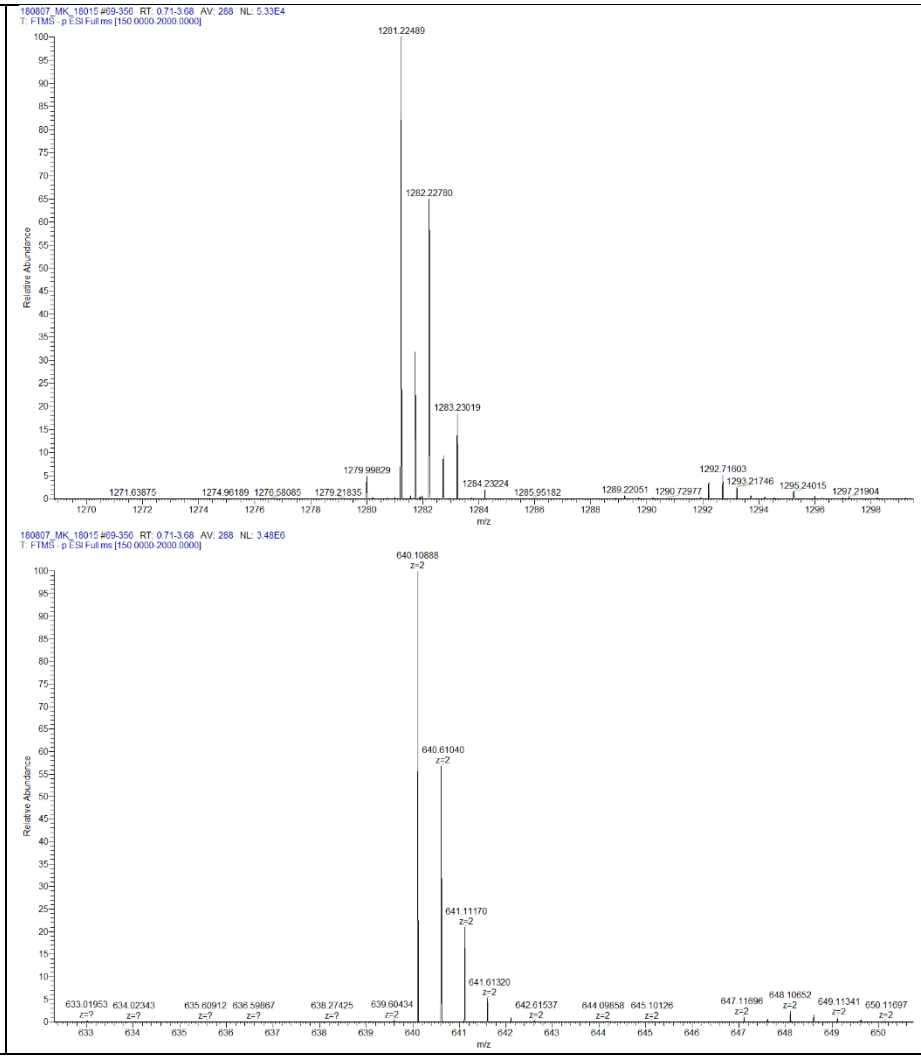
Reaction HPLC (Method D)	t = 0  t = 1 h 
HPLC of purified product (method C)	

HR MS	

m^{7}GppCH$_2$pG-N1-5FAM **(13)**	
Structure	

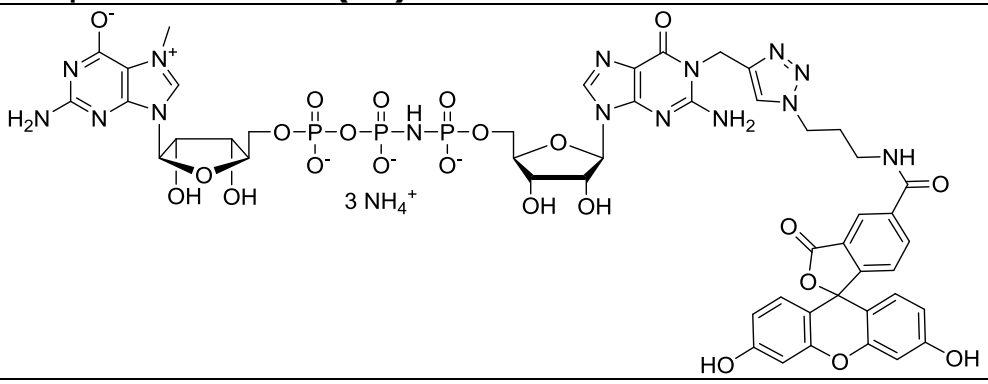
Reaction HPLC (Method C)	t = 0  t = 30 min 
HPLC of purified product (method C)	

HR MS



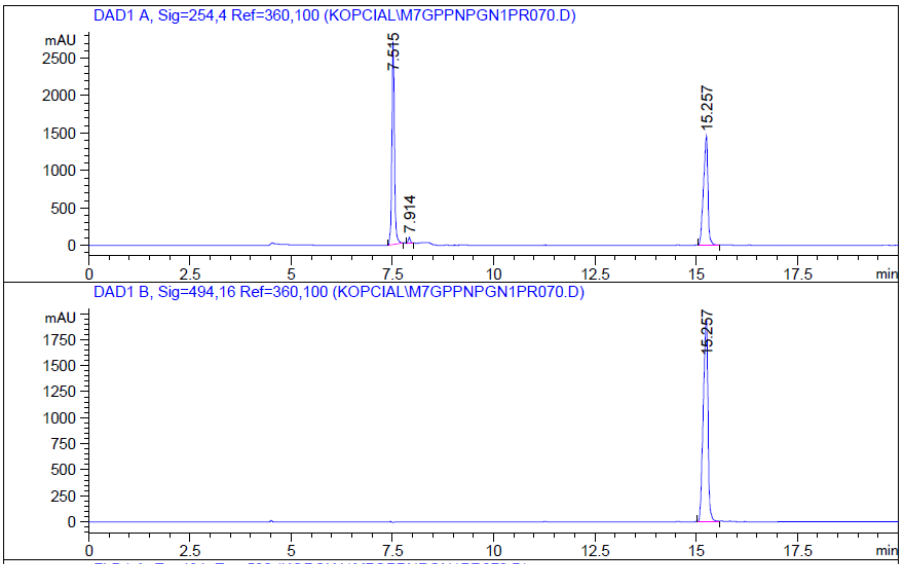
m^{7}GppNHpG-N1-5FAM **(14)**

Structure

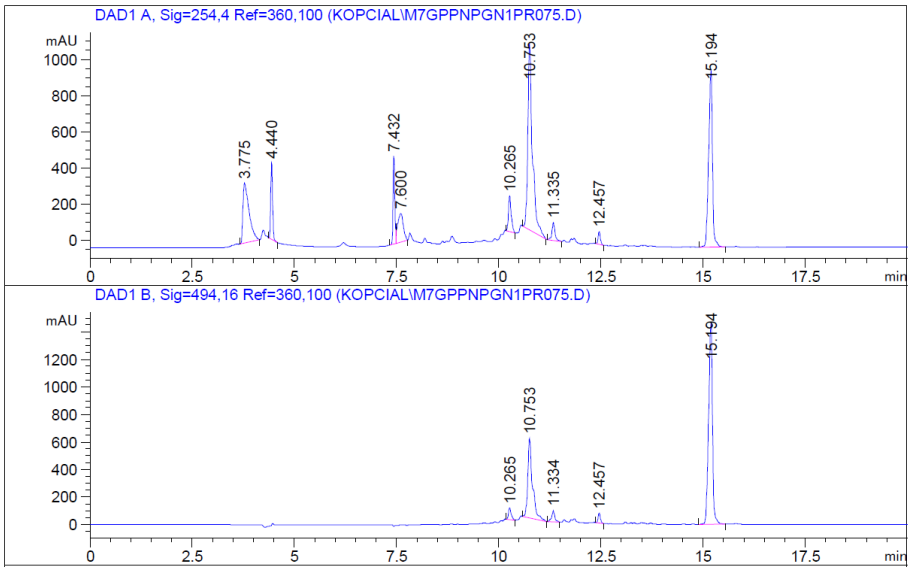


Reaction HPLC (Method D)

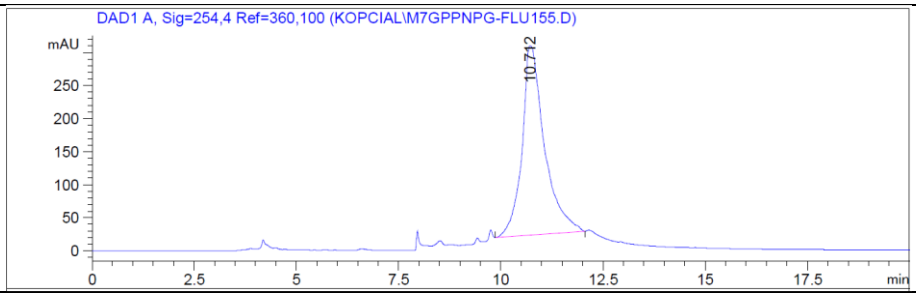
t = 0

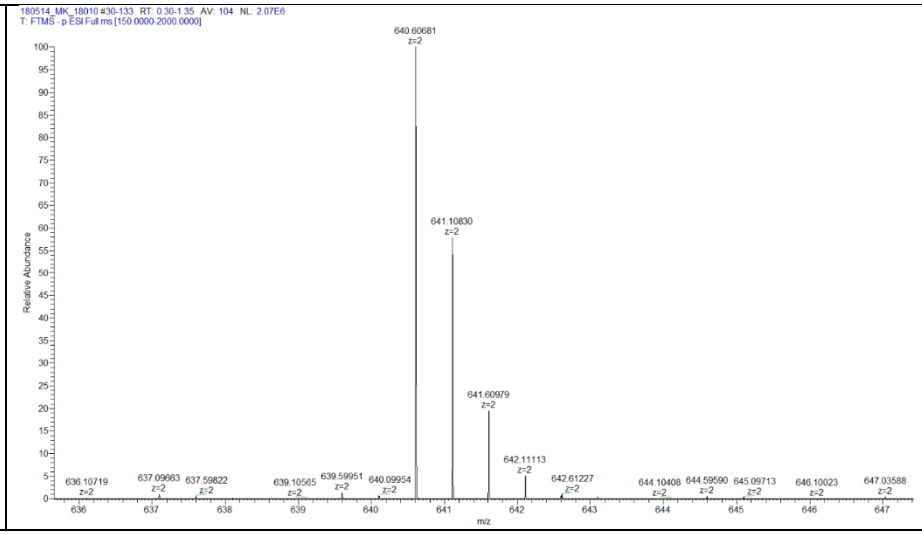


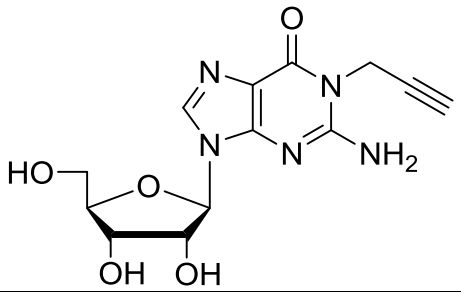
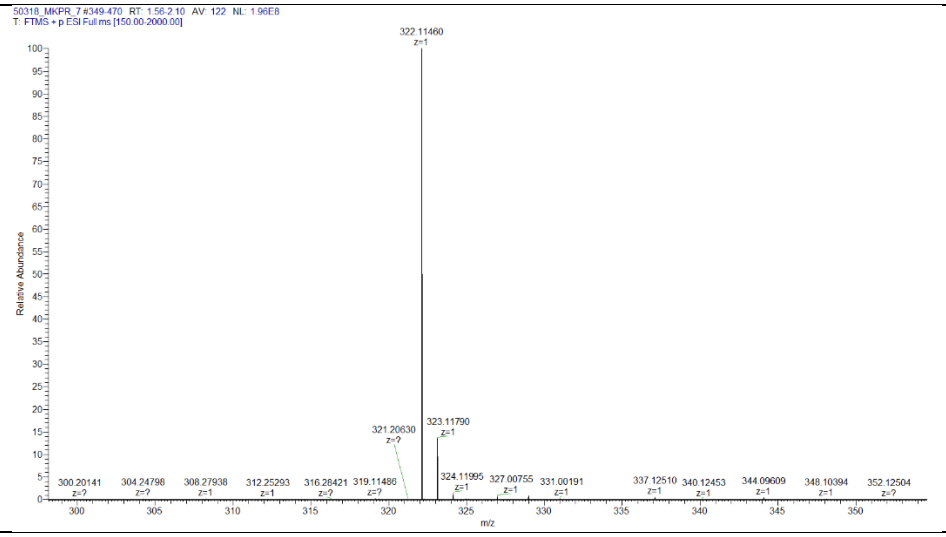
t = 1 h

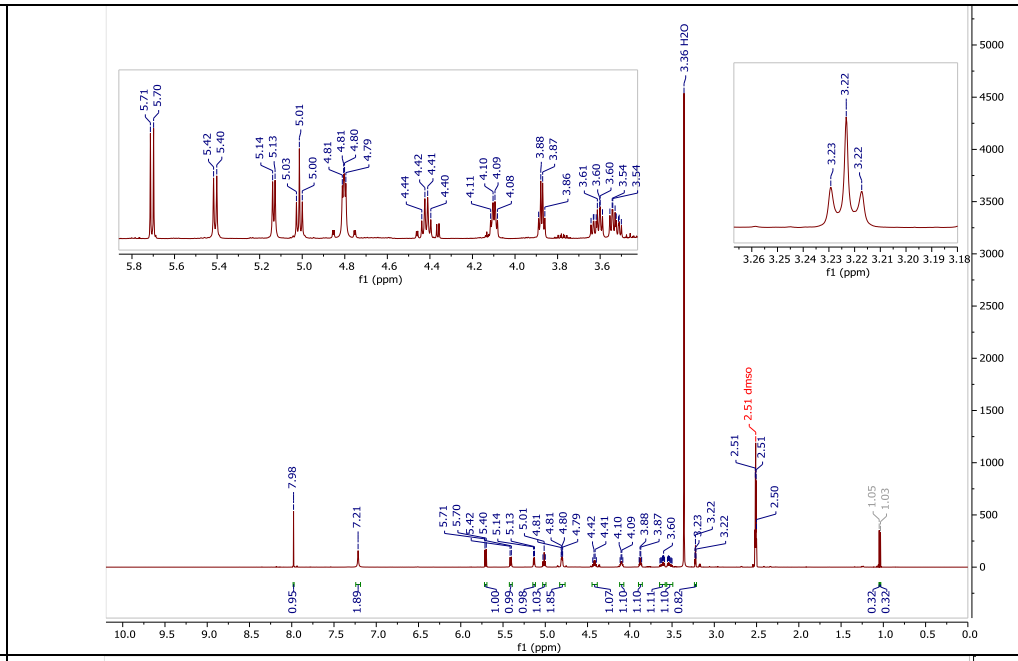
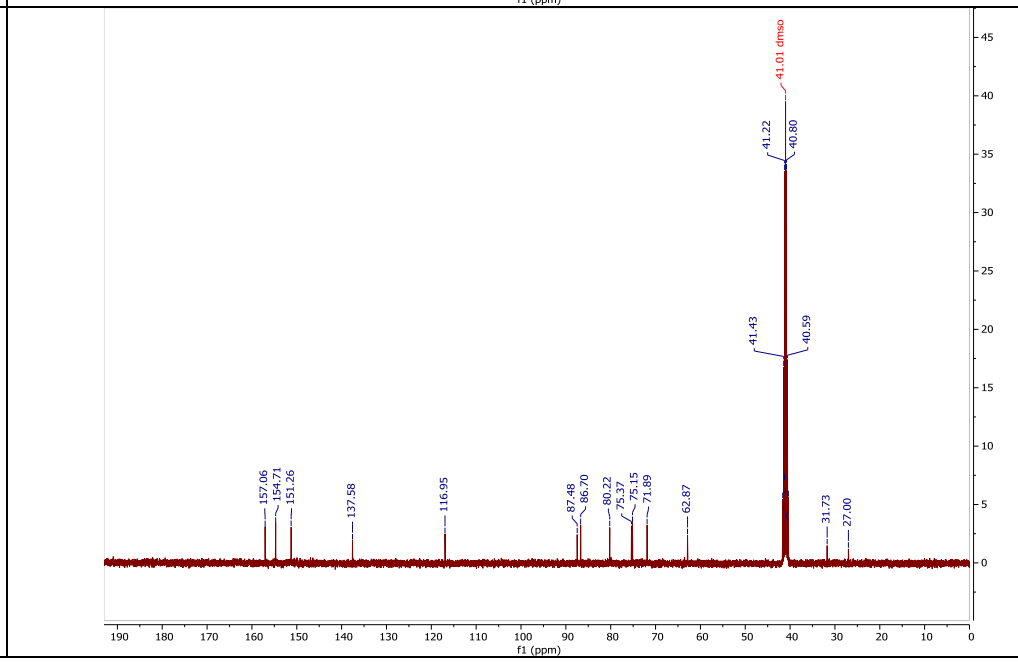


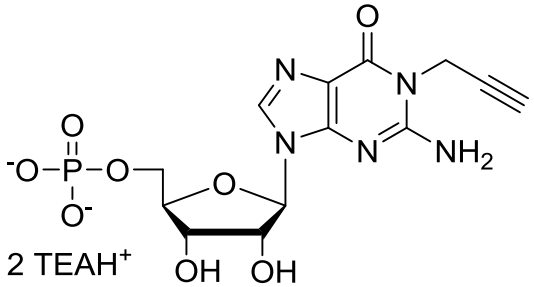
HPLC of purified product (method D)

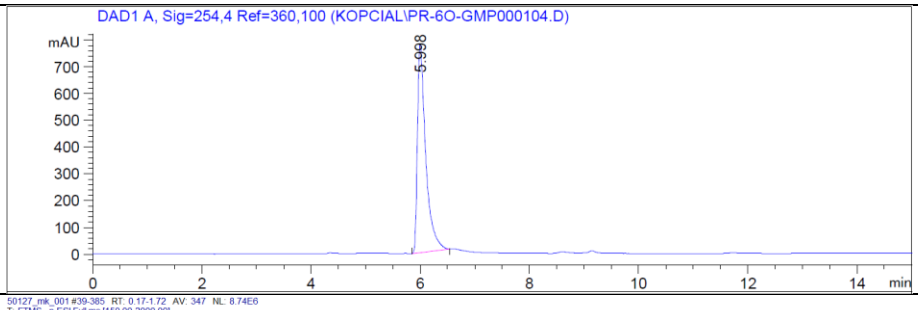
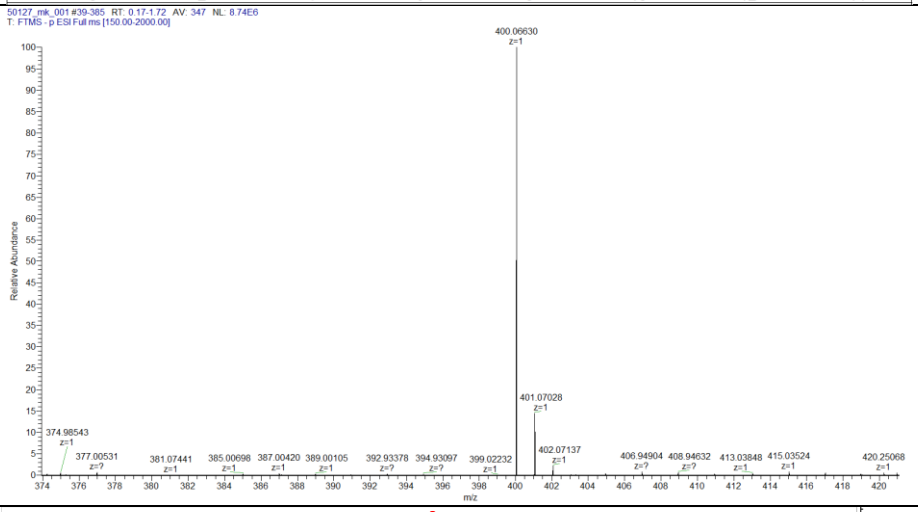
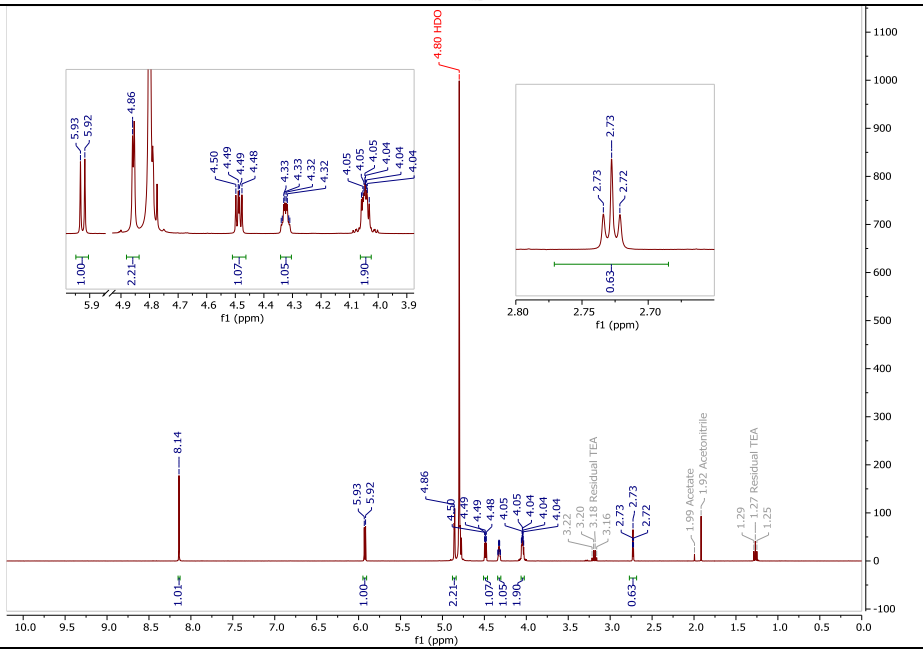


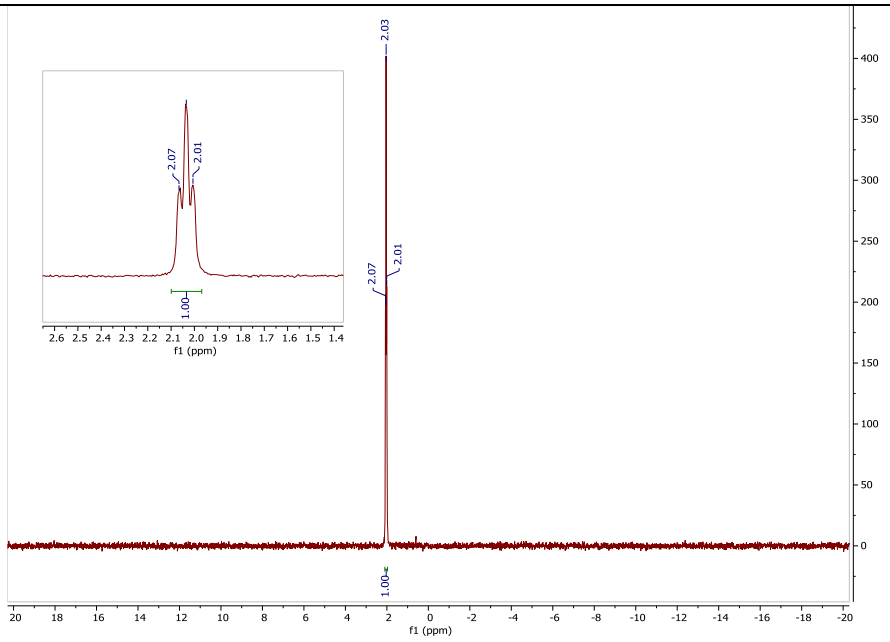
HR MS	

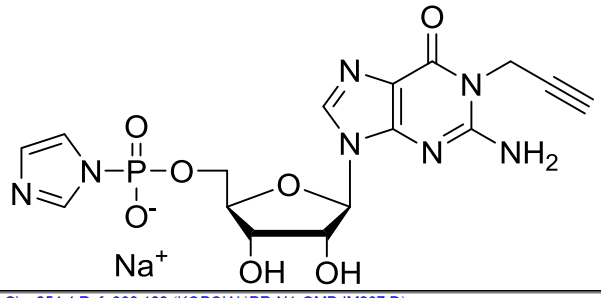
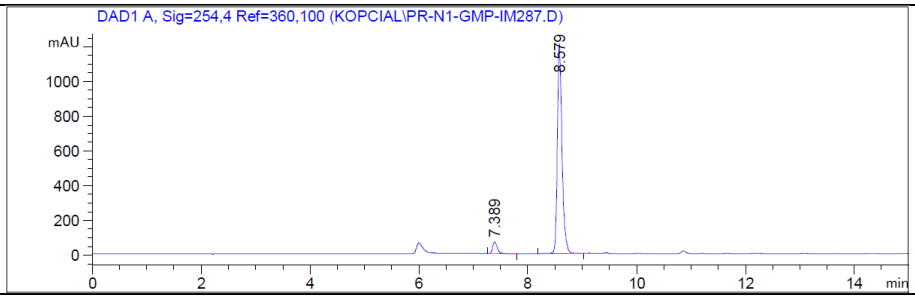
propargyl-N1-Guo **(16)**	
Structure	
HRMS	

^{1}H NMR (400 MHz, DMSO-d_6)	
^{13}C NMR (162 MHz, D_2O)	

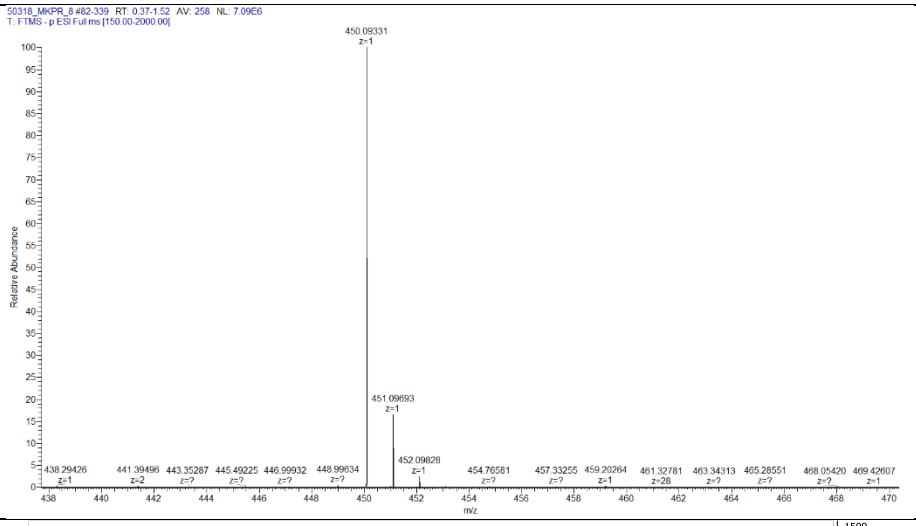
propargyl-N1-GMP **(17)**	
Structure	

HPLC of purified product (method A)	
HRMS	
^{1}H NMR (400 MHz, D_2O)	

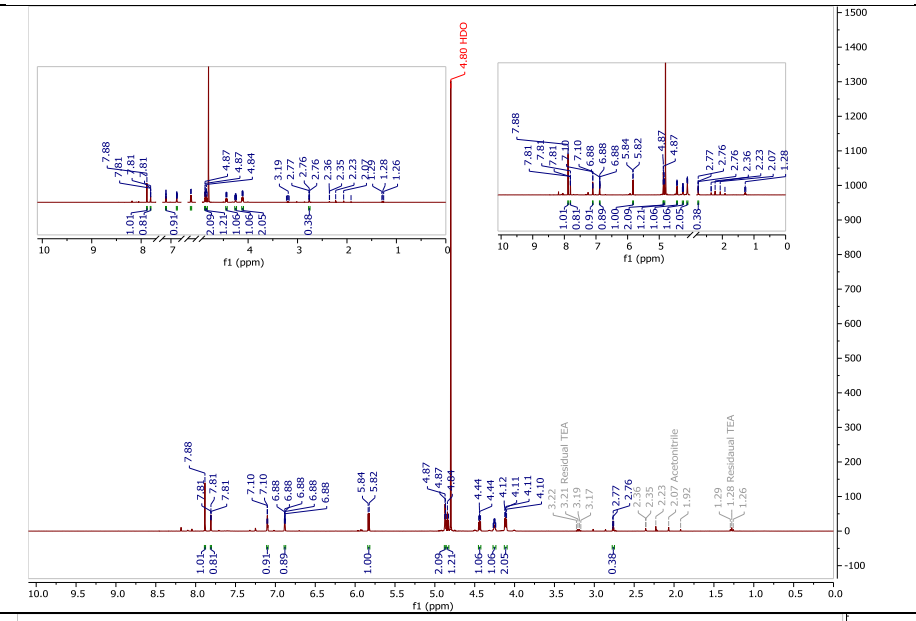
^{31}P NMR (162 MHz, D_2O)	

propargyl-N1-GMP-Im **(18)**	
Structure	
HPLC of purified product (method A)	

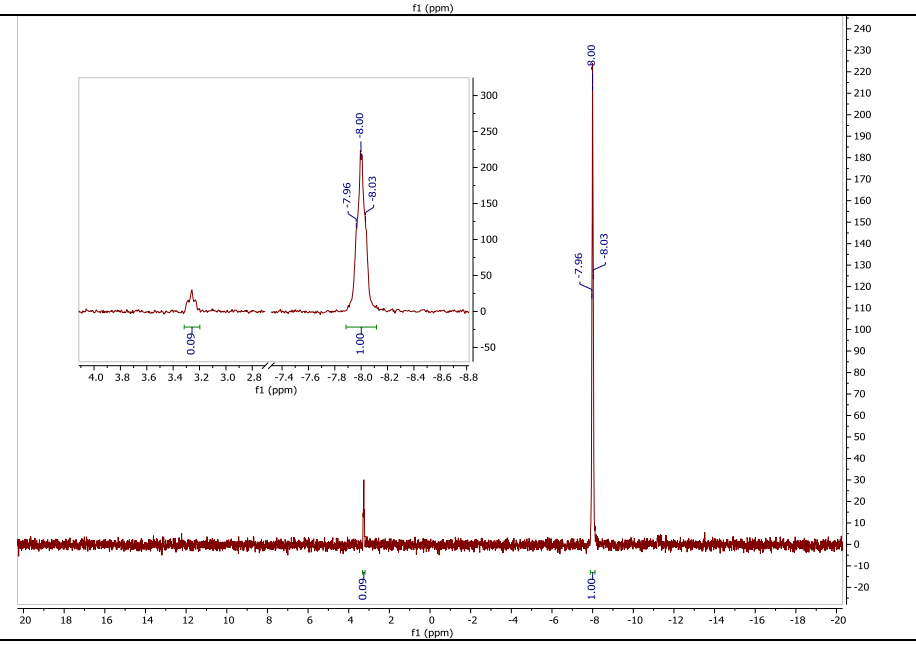
HRMS



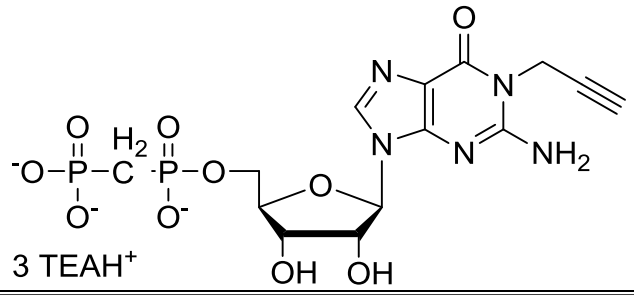
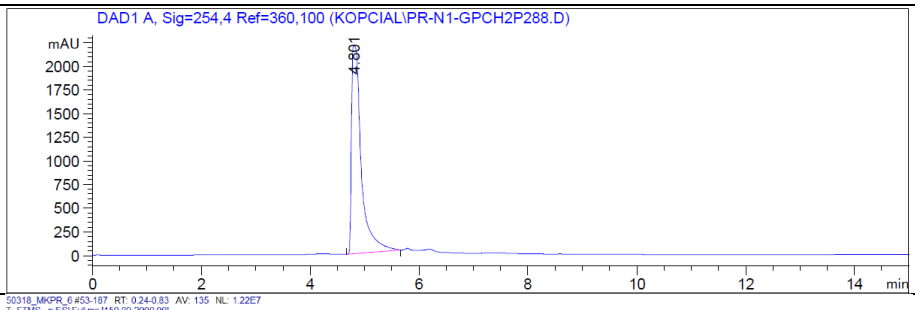
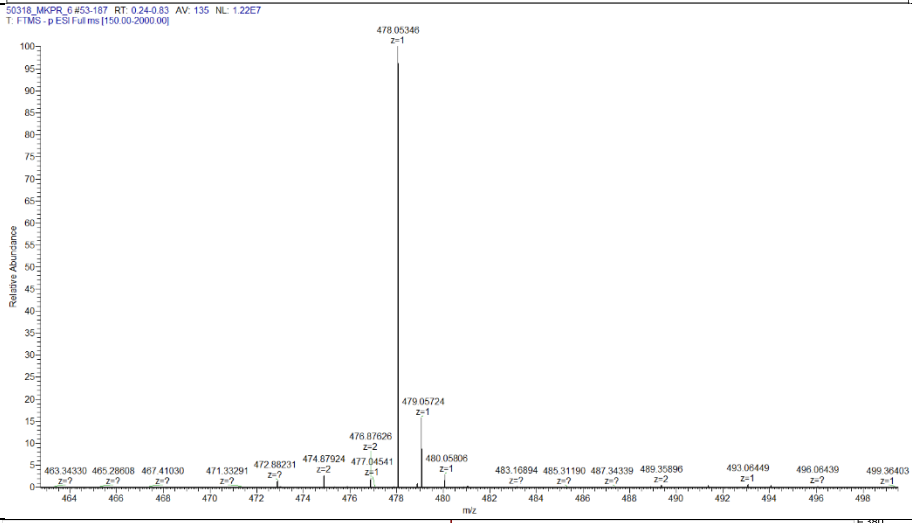
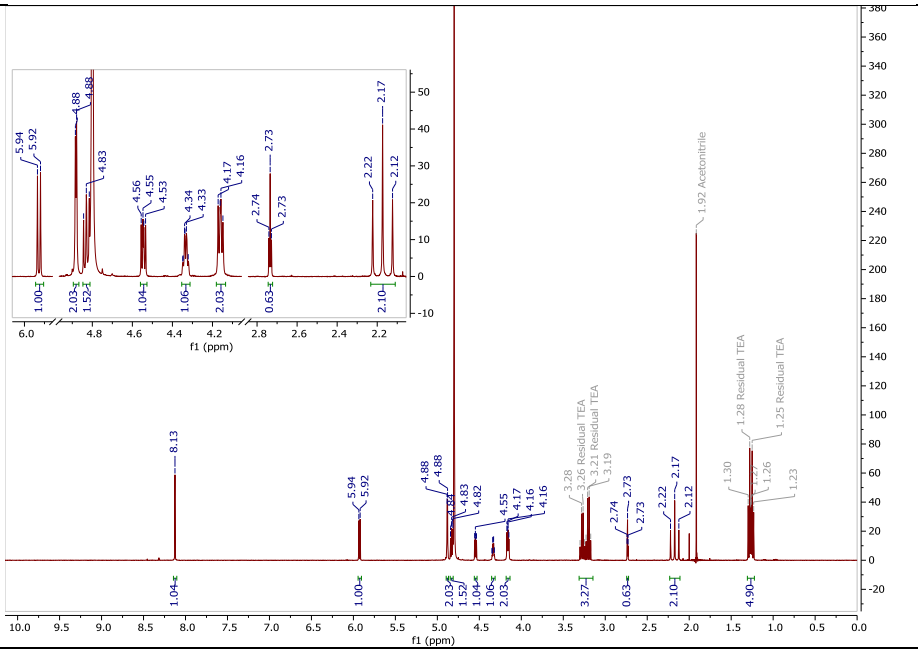
^{1}H NMR (400 MHz, D_2O)

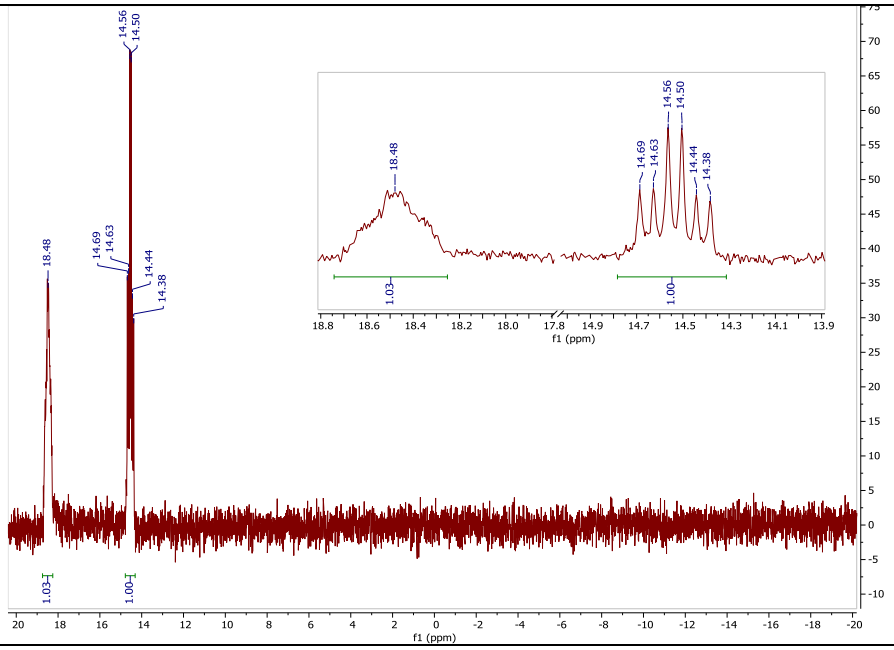


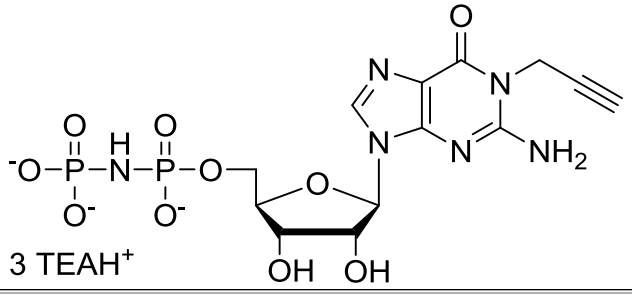
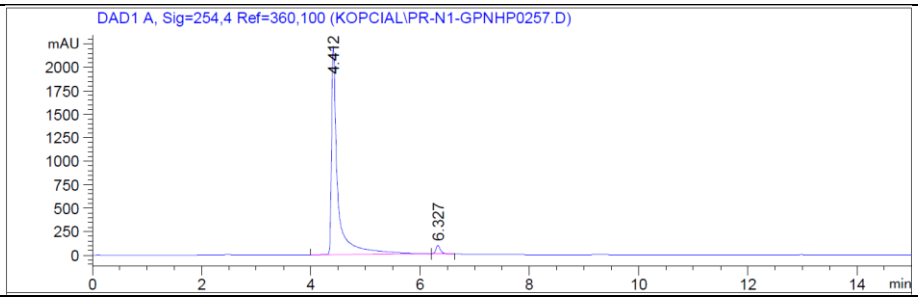
^{31}P NMR (162 MHz, D_2O)



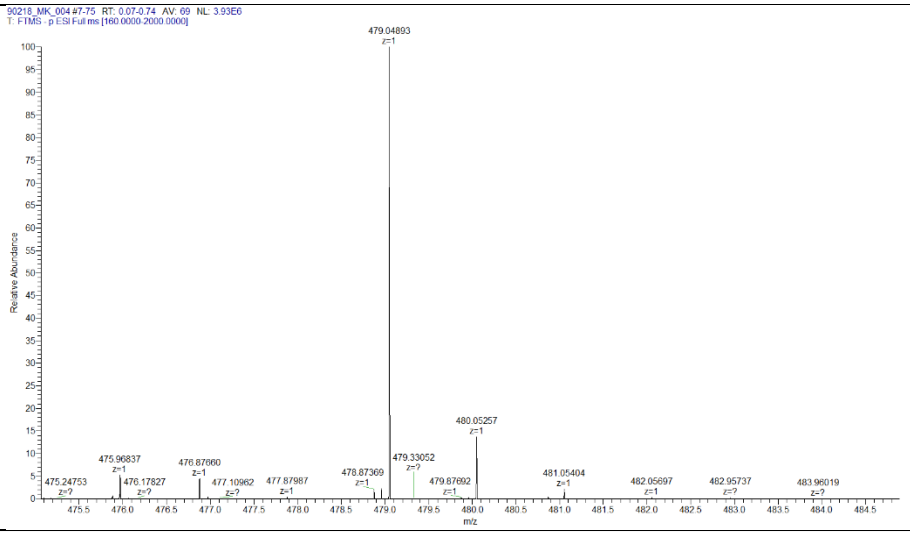
propargyl-N1-GpCH$_2$p **(22)**

Structure	
HPLC of purified product (method A)	
HRMS	
^{1}H NMR (400 MHz, D$_2$O)	

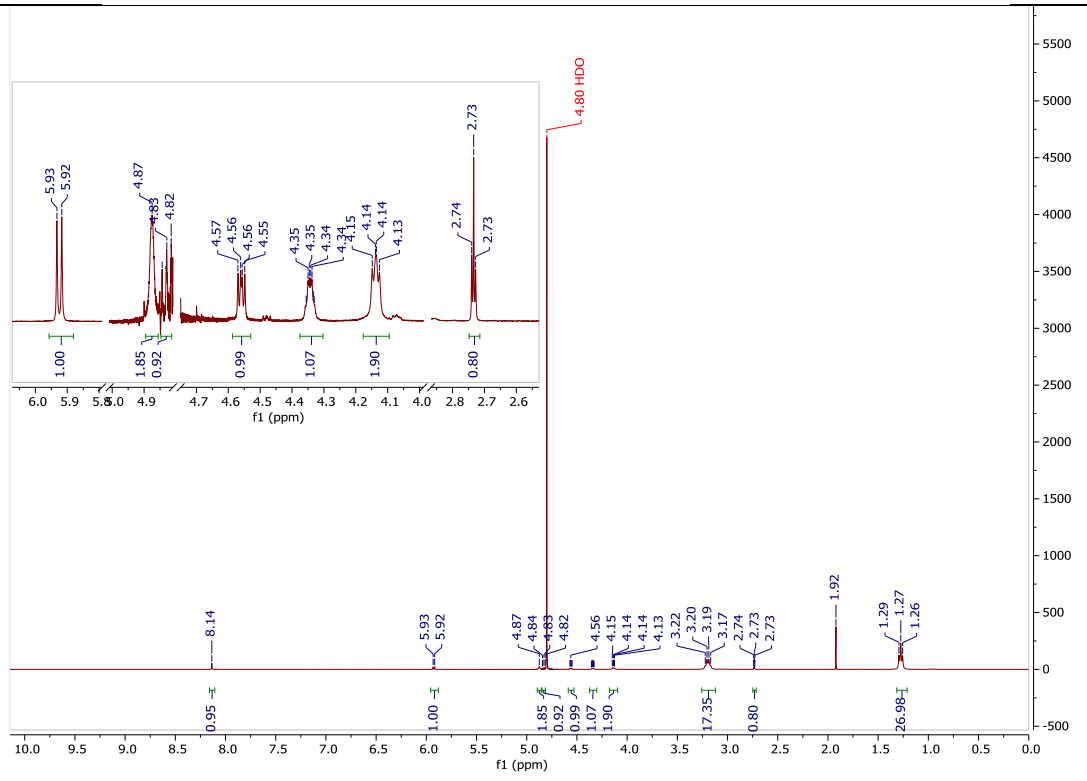
^{31}P NMR (162 MHz, D_2O)	

propargyl-N1-GpNHp **(23)**	
Structure	
HPLC of purified product (method A)	

HRMS



^{1}H NMR (400 MHz, D_2O)



^{31}P NMR (162 MHz, D_2O)