

Electronic Supplementary Information

Synthesis and evaluation of fluorescent cap analogues for mRNA labelling

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Data presented in **Table S1** illustrates effect of polarity of different solvents on the spectroscopic properties of AntOMe and MantOMe. In this study MeOH, EtOH and iPrOH were chosen as appropriate organic solvents since their protic properties and dipole moments provide a close resemblance to water. Despite that absorption maxima and absorption coefficients displays only a slightly variation upon a change of solvent in case of AntOMe a 9 nm shift on changing from PBS to organic solvents was observed. Interestingly, fluorescence of AntOMe and MAntOMe decreases in intensity and is red shifted with increasing solvent polarity. Quantum yields calculated for alcohol solutions are almost five times higher than in phosphate buffer solutions. Time-resolved experiments revealed a tendency to increase fluorescence lifetime if solvent polarity is decreased. The most significant changes were observed for AntOMe as its lifetime in aqueous medium (PBS, pH 5.175) was equal 1.81 ns, however, in ethanol it was prolonged to 8.25 ns (**Table S1**).

Table S1. Spectroscopic properties of methyl anthranilate and methyl *N*-methylantranilate in phosphate buffer and organic solvents.

compound	Solution	Absorption		Fluorescence		
		λ_{\max} (nm)	$\epsilon_{\lambda_{\max}}$ ($M^{-1}cm^{-1}$)	λ_{\max} (nm)	QY	lifetime
AntOMe	PBS pH = 5.18	327	4140	420	0.099	$\tau = 1.81$ ns $\chi_R^2 = 0.954$
	PBS pH = 8.96	327	4140	420	0.099	$\tau = 1.71$ ns $\chi_R^2 = 0.958$
	MeOH	336	5050	409	0.539	$\tau = 7.36$ ns $\chi_R^2 = 1.004$
	EtOH	337	5110	409	0.597	$\tau = 8.25$ ns $\chi_R^2 = 0.998$
	iPrOH	337.5	5200	407.5	0.577	$\tau = 7.84$ ns $\chi_R^2 = 0.977$
MantOMe	PBS pH = 5.18	349.5	4780	441.5	0.175	$\tau = 3.50$ ns $\chi_R^2 = 0.946$
	PBS pH = 8.96	349.5	4800	441.5	0.176	$\tau = 3.49$ ns $\chi_R^2 = 0.965$
	MeOH	352.5	6110	423	0.480	$\tau = 7.23$ ns $\chi_R^2 = 0.987$
	EtOH	353	6170	422.5	0.513	$\tau = 8.07$ ns $\chi_R^2 = 1.001$
	iPrOH	352	6230	419.5	0.493	$\tau = 7.61$ ns $\chi_R^2 = 0.971$

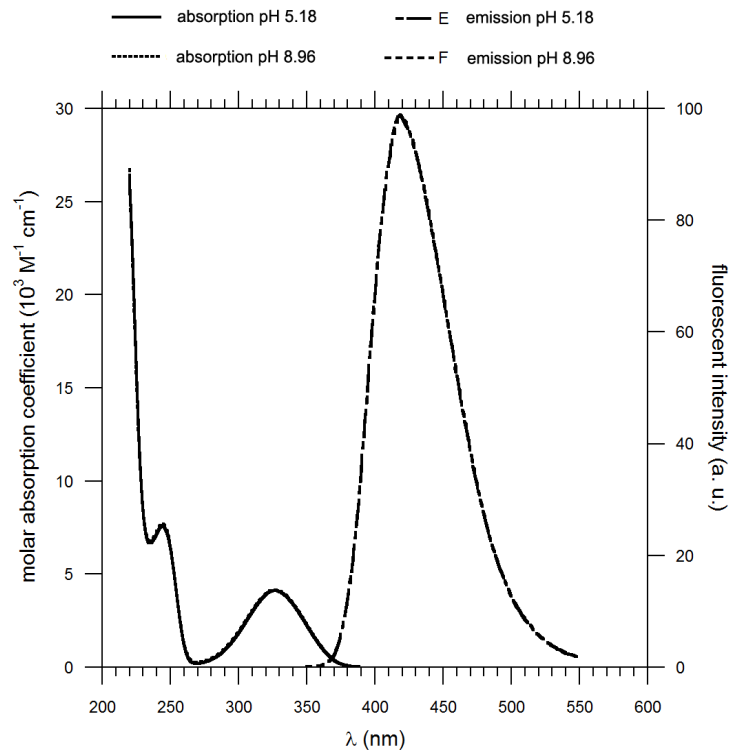


Fig. S1. Absorbance and emission spectra of AntOMe

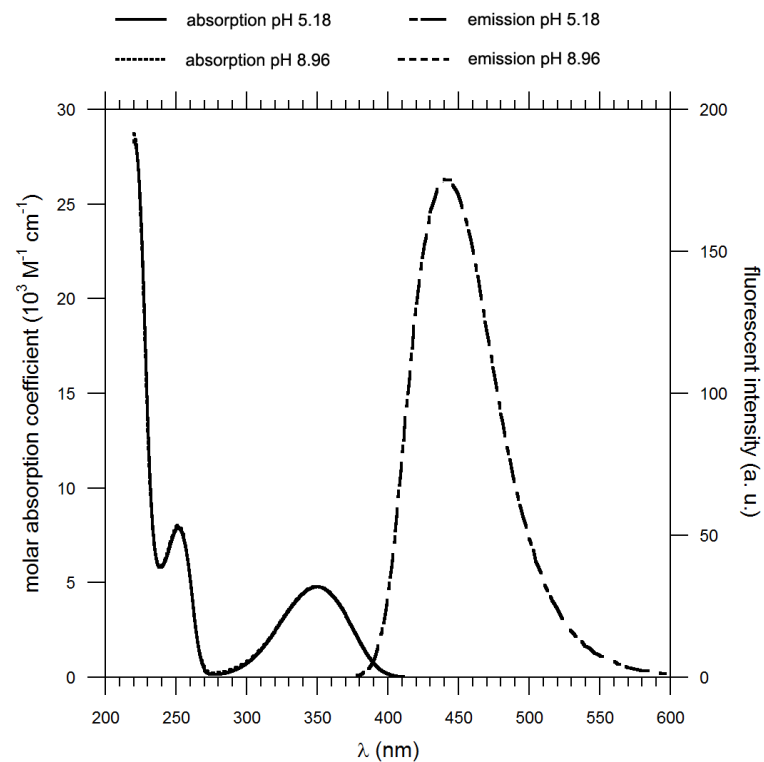


Fig. S2. Absorbance and emission spectra of MantOMe

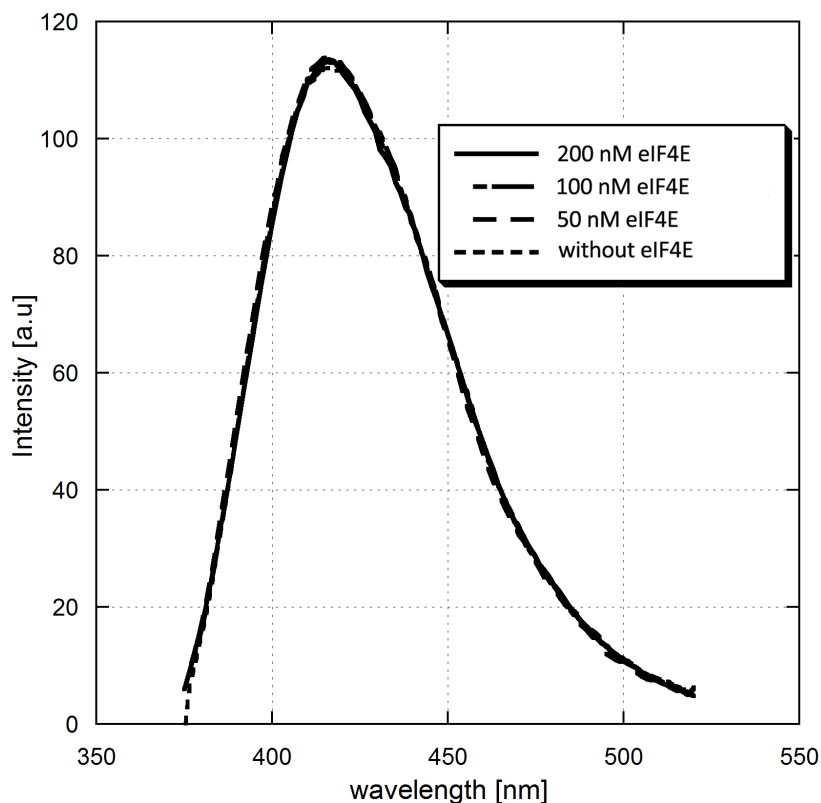


Fig. S3. MantOME fluorescence intensity observed upon addition of increasing amounts of eIF4E protein. The concentration of MantOME was 200 nM and the eIF4E concentrations were in range 50-1000 nM.

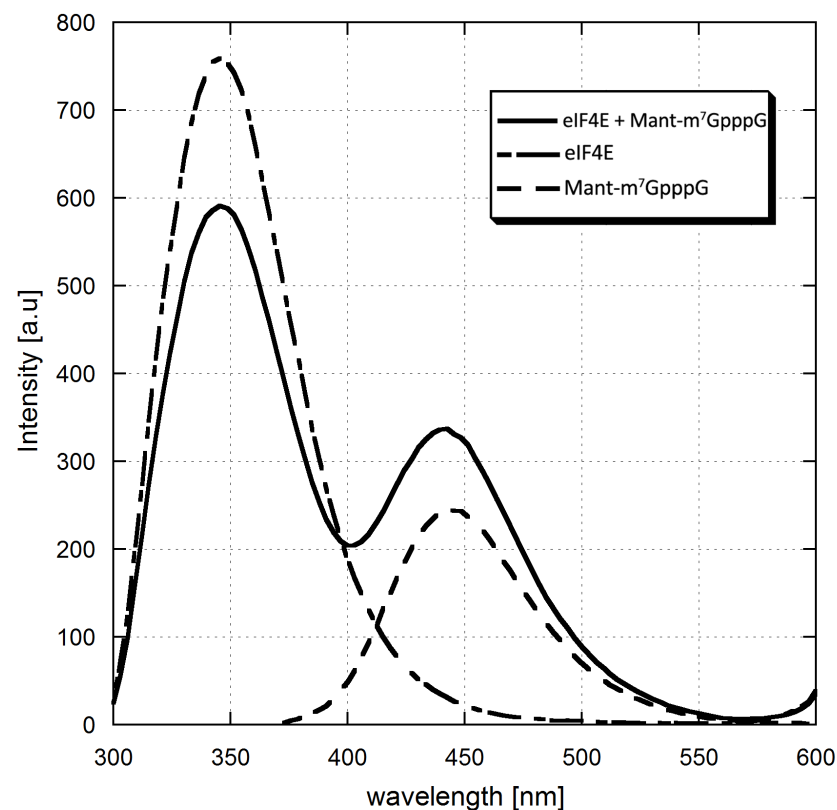


Fig. S4. Changes of fluorescence intensity of Mant-m⁷GpppG and Trp residues in eIF4E upon complex formation. The excitation wavelength used in the experiment was 280 nm (maximum absorption for Trp residues). eIF4E concentration was 100 nM and Mant-m⁷GpppG concentration was 200 nM.

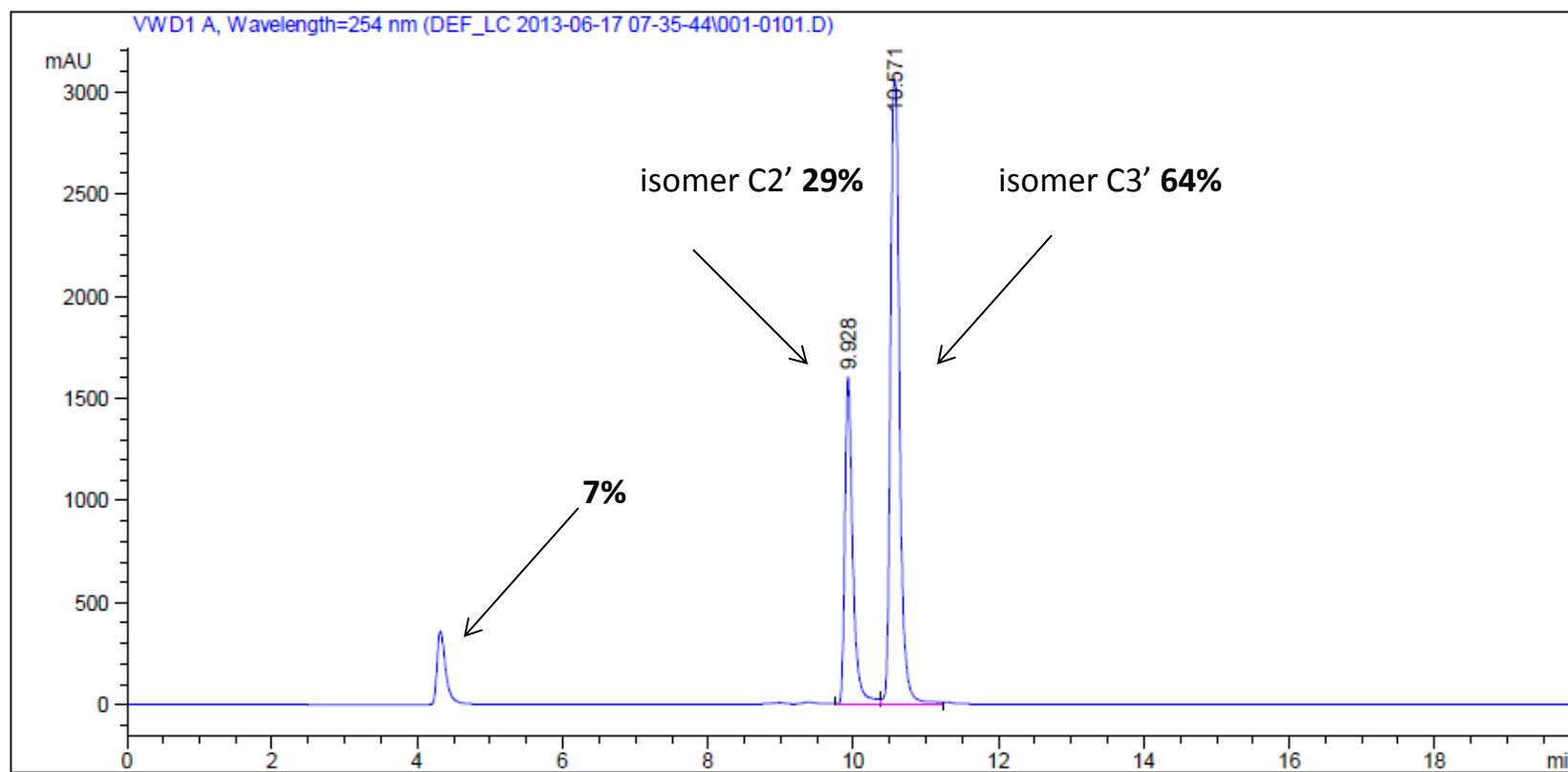
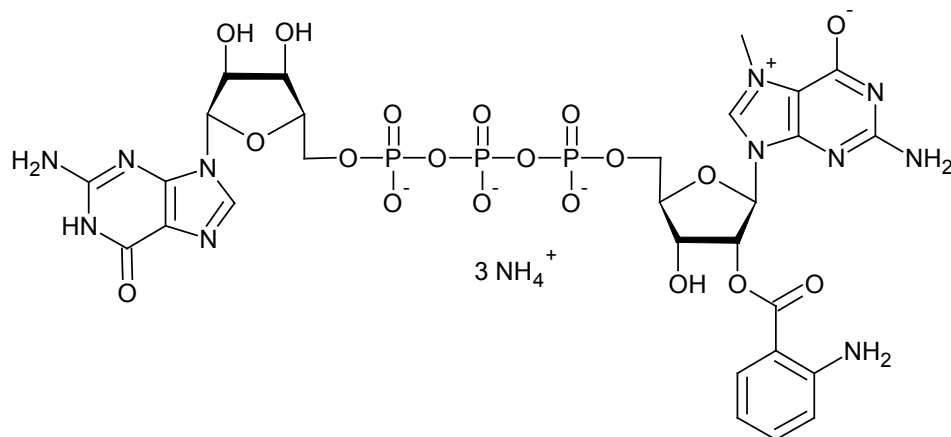
Table S2. ¹H NMR signals of 2' and 3' regioisomers of cap analogues **1-2**

Proton	Ant-m ⁷ GpppG (1)		Mant-m ⁷ GpppG (2)	
	2' isomer	3' isomer	2' isomer	3' isomer
N8	31.6	68.4	42.7	57.3
H1' (m ⁷ G)	32.1	67.9	42.9	57.1
H2'/H3' (m ⁷ G)	33.1	66.9	40.9	59.1
N-CH ₃	31.7	68.3	-	-
H3 (Ant)	32.6	67.4	-	-
H5 (Mant)	-	-	44.1	55.9
H6 (Mant)	-	-	43.1	56.9
N-CH ₃ (Mant)	-	-	41.1	58.9

Table S3. Conformational and regioisomeric equilibria for some (M)Ant labelled nucleotides

Compound	Coupling constants	regioisomer equilibrium	%N conformation	Amplitude of exponential component from fluorescence lifetimes measurements
Ant-m ₂ ^{7,2'-O} GpppG (5)	J _{H1-H2} = 5.5 J _{H2-H3} = 3.2 J _{H3-H4} = 3.2	3' isomer = 100%	41 % N conformation	α ₁ = 36.89% α ₂ = 63.11% (pH 5) α ₁ = 39.03% α ₂ = 60.97% (pH 9)
Ant-m ⁷ GpppG (1)	J _{H1-H2(3')} = 6.0 J _{H2-H3(3')} = 5.1 J _{H1-H2(2')} = 3.0 J _{H2-H3(2')} = 4.5	2' isomer = 32% 3' isomer = 68%	2' isomer: 62% N conformation 3' isomer: 35% N conformation total: 44% N conformation	α ₁ = 31.81% α ₂ = 68.19% (pH 5) α ₁ = 31.72% α ₂ = 68.28% (pH 9)
Mant-m ⁷ GpppG (2)	J _{H1-H2(3')} = 5.7 J _{H2-H3(3')} = 5.2 J _{H1-H2(2')} = 2.7 J _{H2-H3(2')} = 5.1 J _{H3-H342')} = 5.0	2' isomer = 42% 3' isomer = 58%	2' isomer: 71% N conformation 3' isomer: 39% N conformation total: 52% N conformation	α ₁ = 40.50% α ₂ = 59.50% (pH 5) α ₁ = 44.98% α ₂ = 55.02% (pH 9)
Ant-m ⁷ GDP (6)	J _{H1-H2(3')} = 5.6 J _{H1-H2(2')} = 1.8 J _{H2-H3(2')} = 5.9	2' isomer = 52% 3' isomer = 48%	2' isomer: 81% N conformation 3' isomer: 40% N conformation total: 61% N conformation	
Ant-m ₂ ^{7,2'-O} GMP (8)	J _{H1-H2} = 5.5 J _{H2-H3} = 2.7 J _{H3-H4} = 4.5	3' isomer = 100%	40% N conformation	
Mant-m ⁷ GMP (10)	J _{H1-H2(3')} = 5.6 J _{H2-H3(3')} = 4.8 J _{H3-H4(2')} = 3.0 J _{H1-H2(2')} = 4.2 J _{H2-H3(2')} = 5.0	2' isomer = 42% 3' isomer = 58%	2' isomer: 55% N conformation 3' isomer: 40% N conformation total: 46% N conformation	

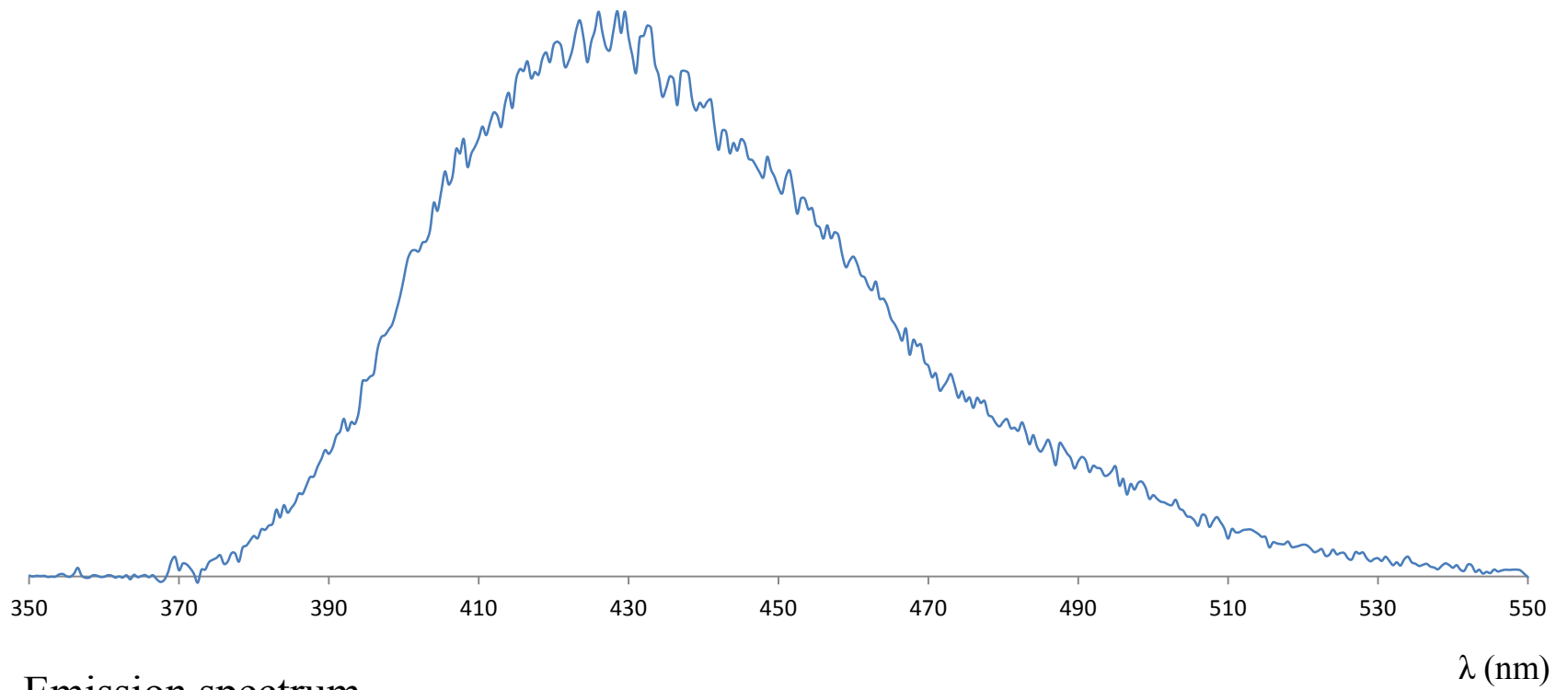
Ant-m⁷GpppG



HPLC profile

Ant-m⁷GpppG

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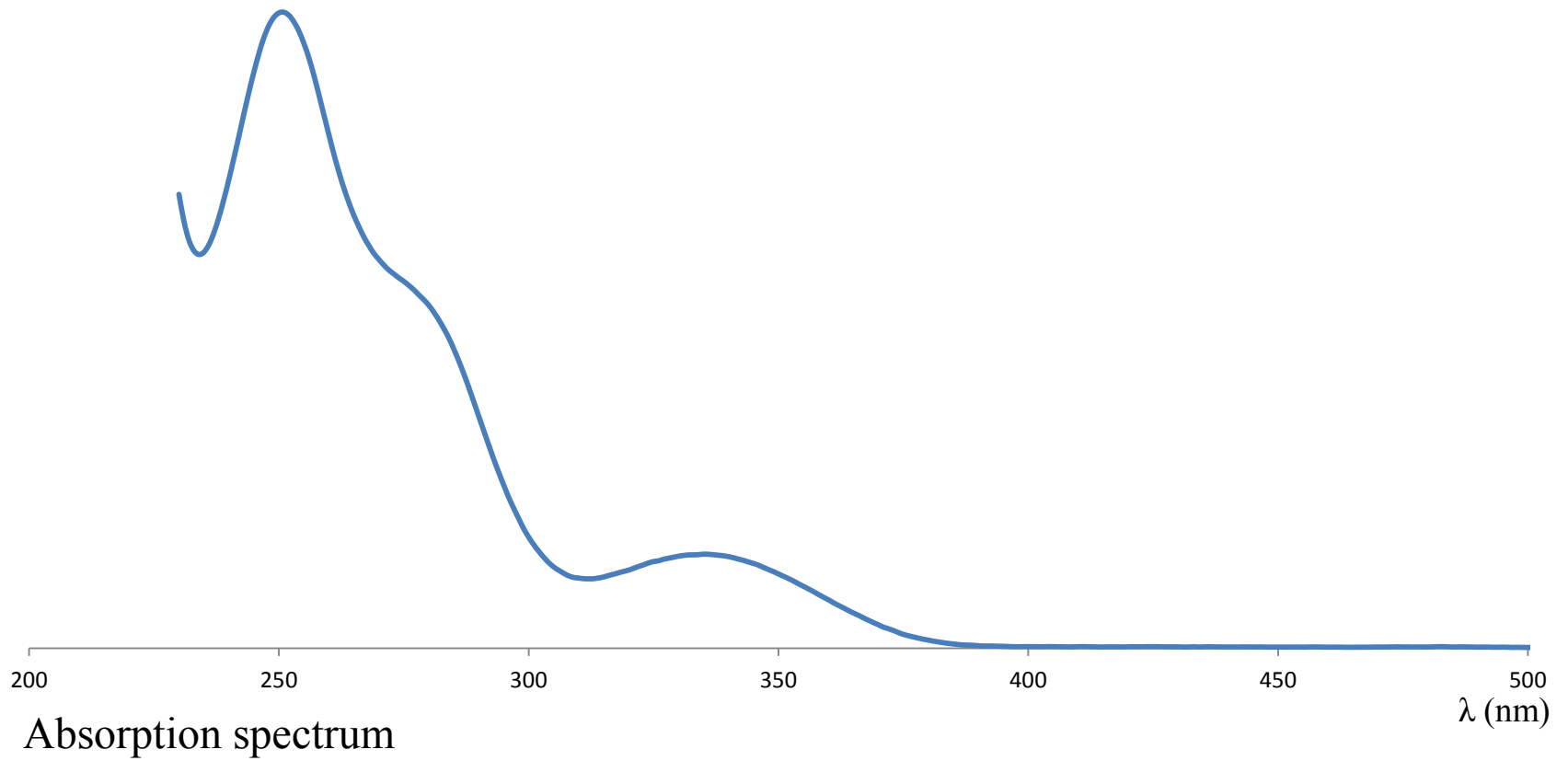


Emission spectrum

λ (nm)

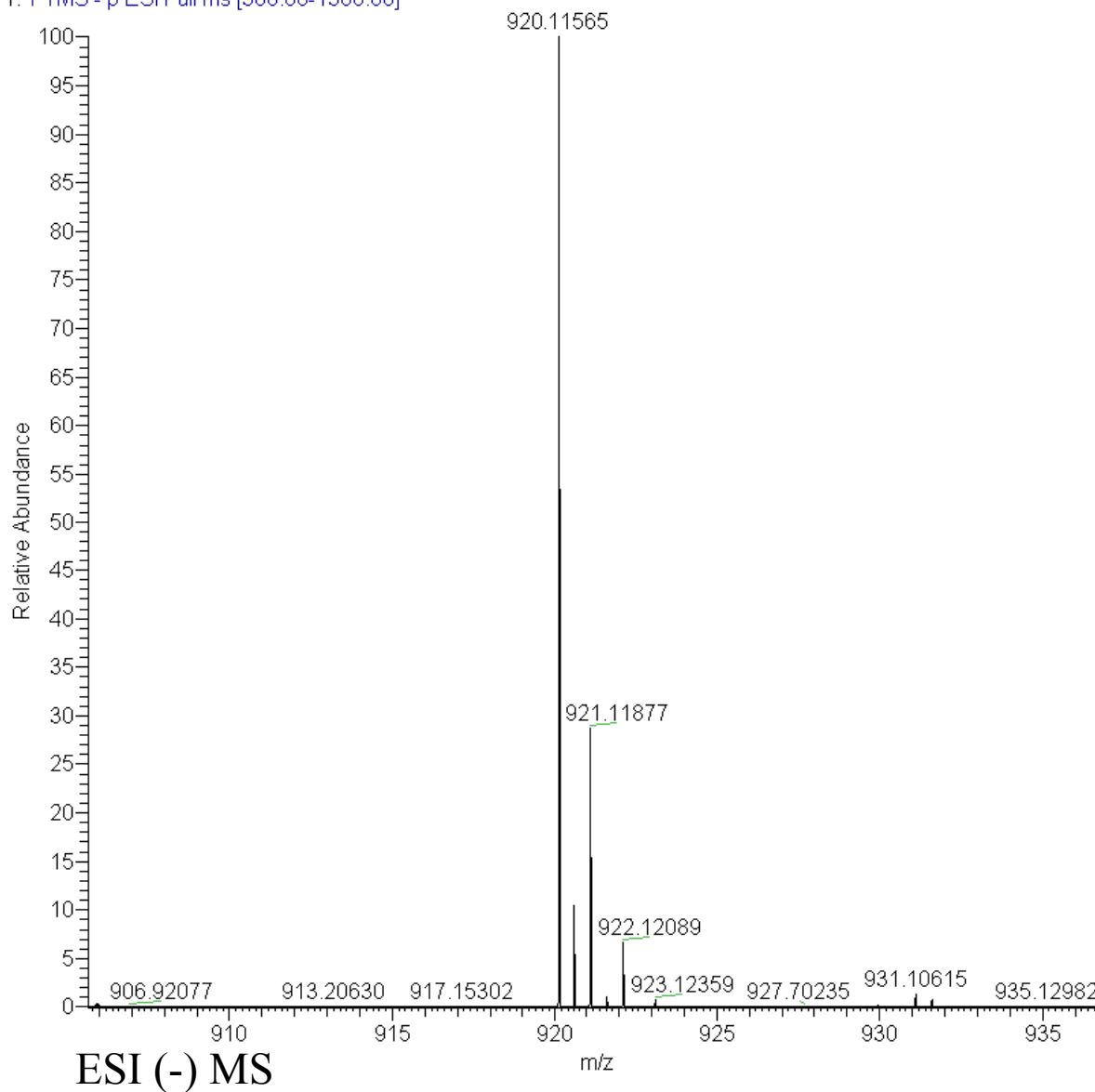
Ant-m⁷GpppG

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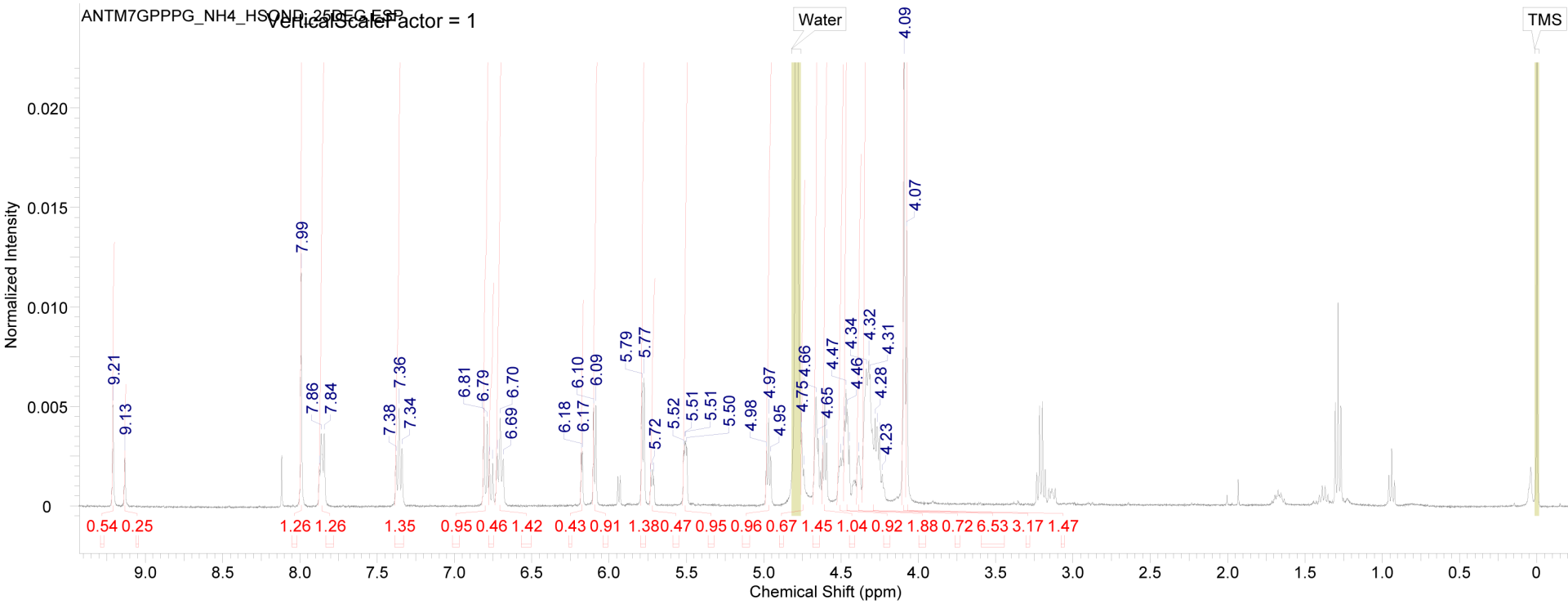


Ant-m⁷GpppG

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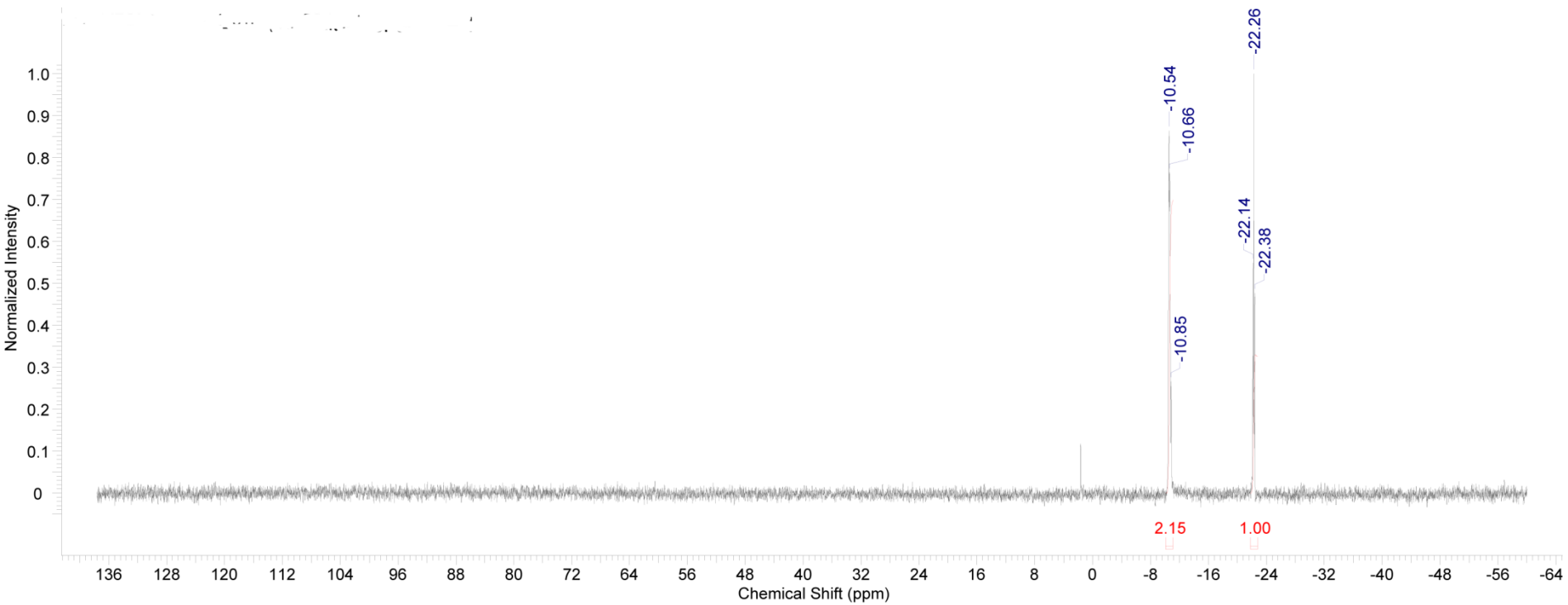


Ant-m⁷GpppG



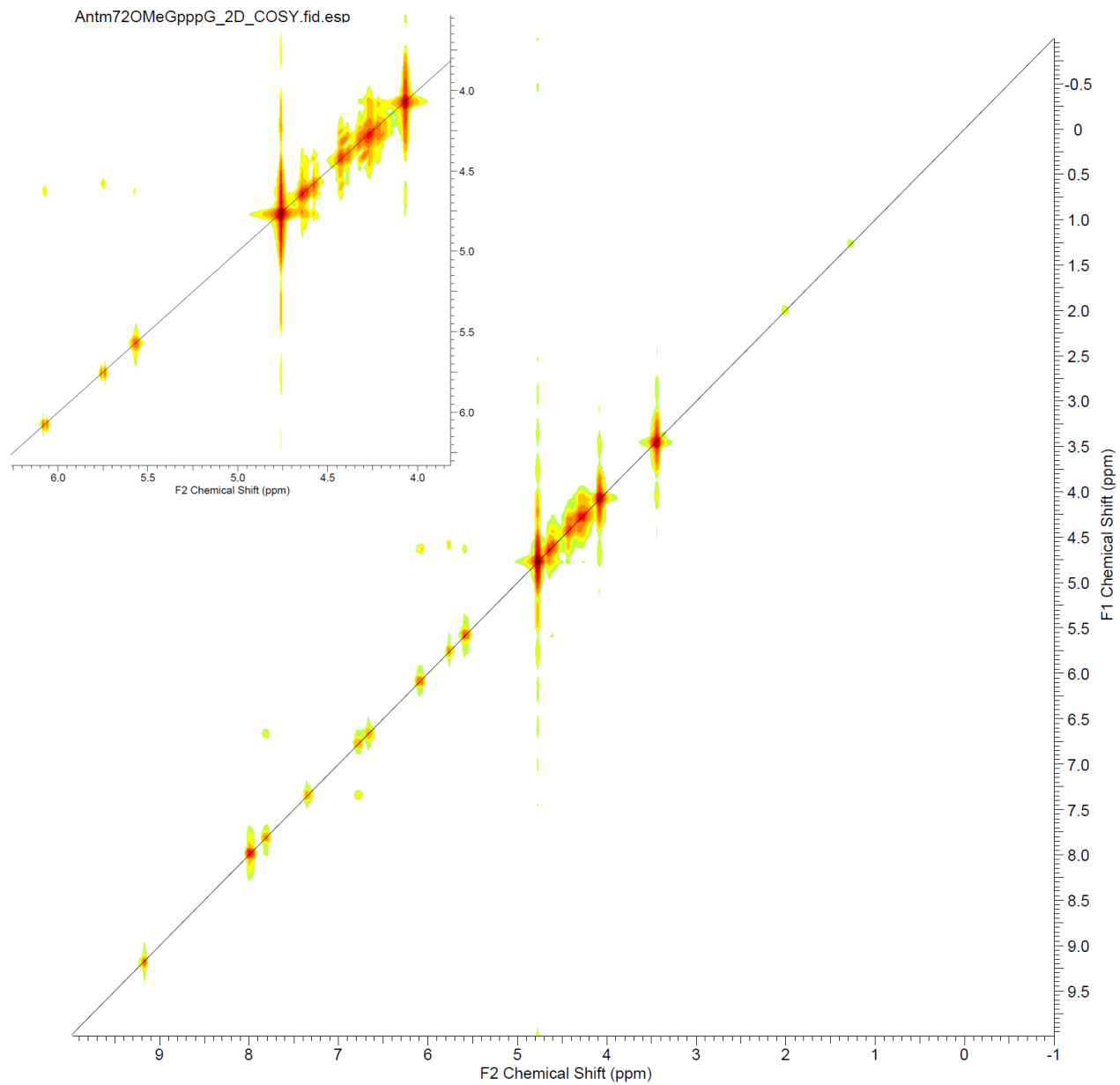
¹H NMR

Ant-m⁷GpppG

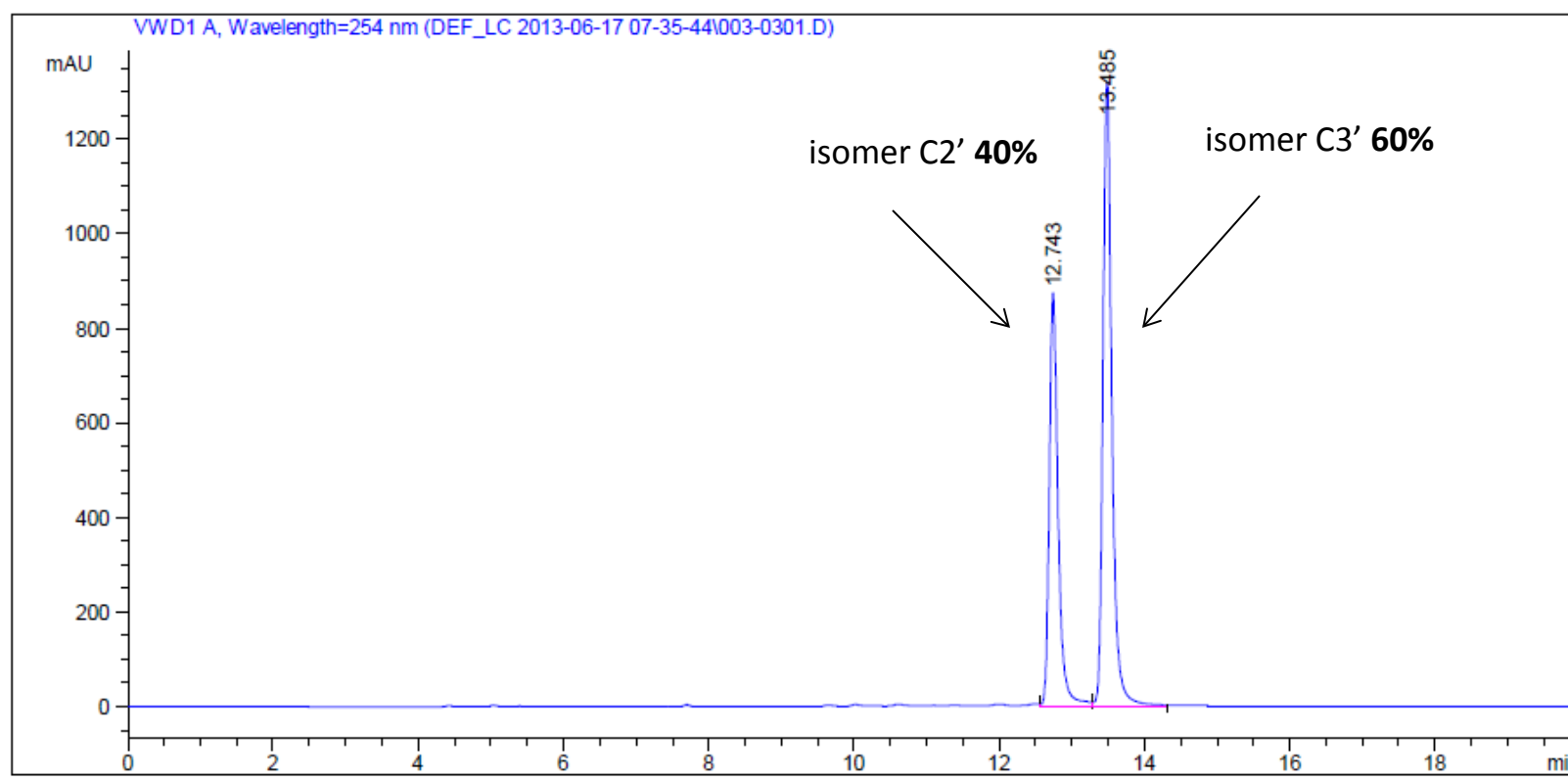
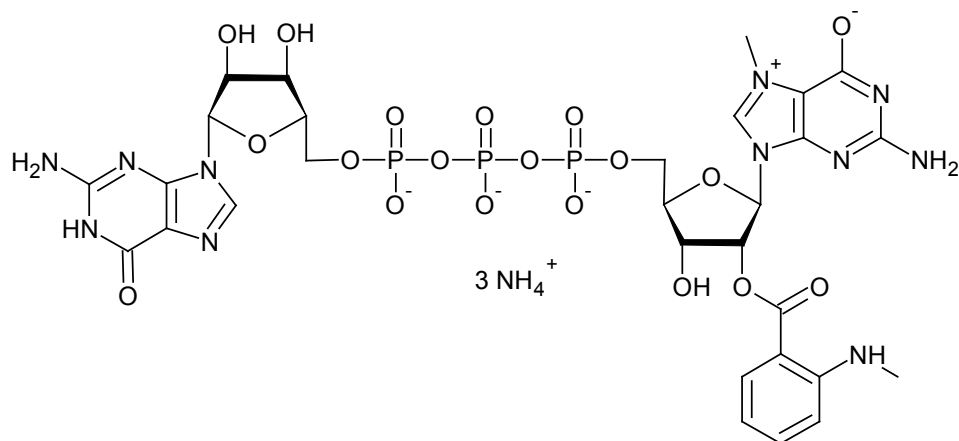


³¹P NMR

Ant-m⁷GpppG

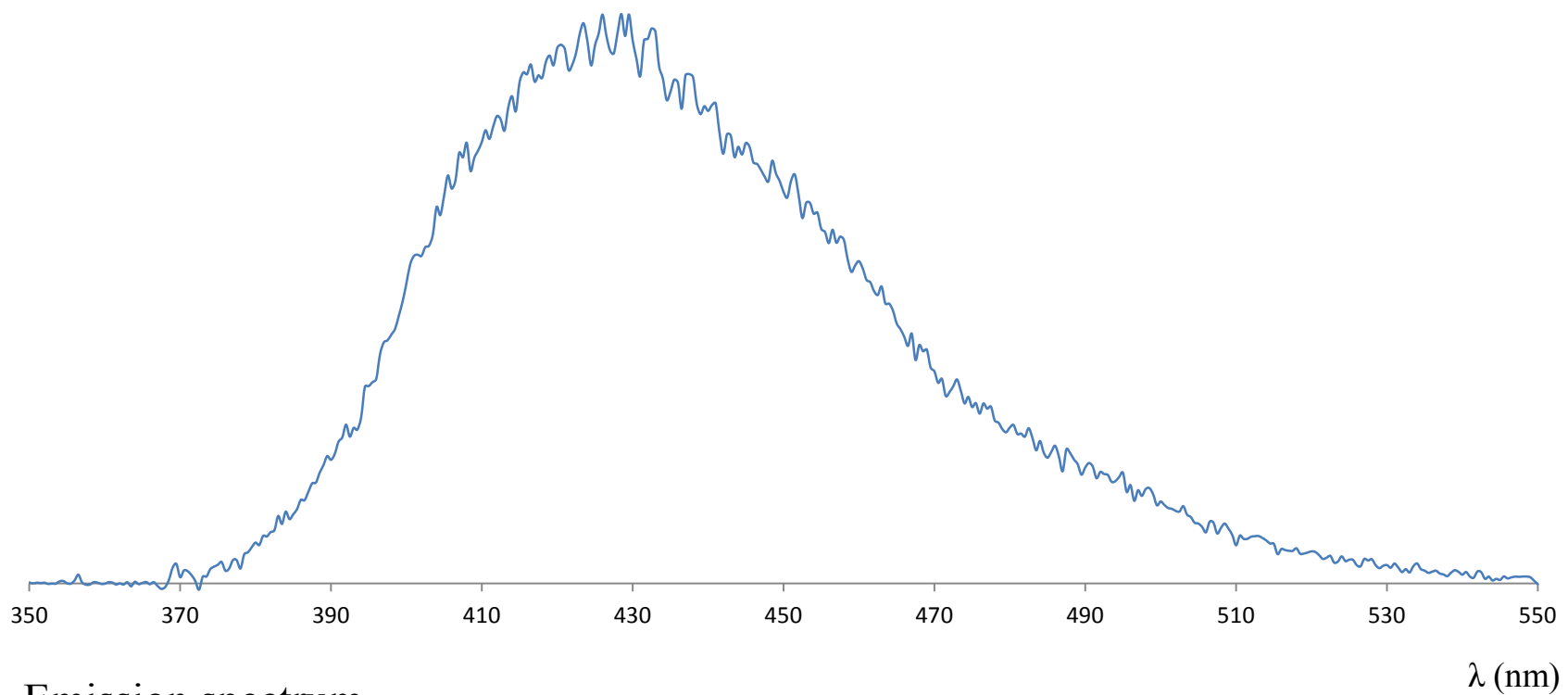


Mant-m⁷GpppG



HPLC profile

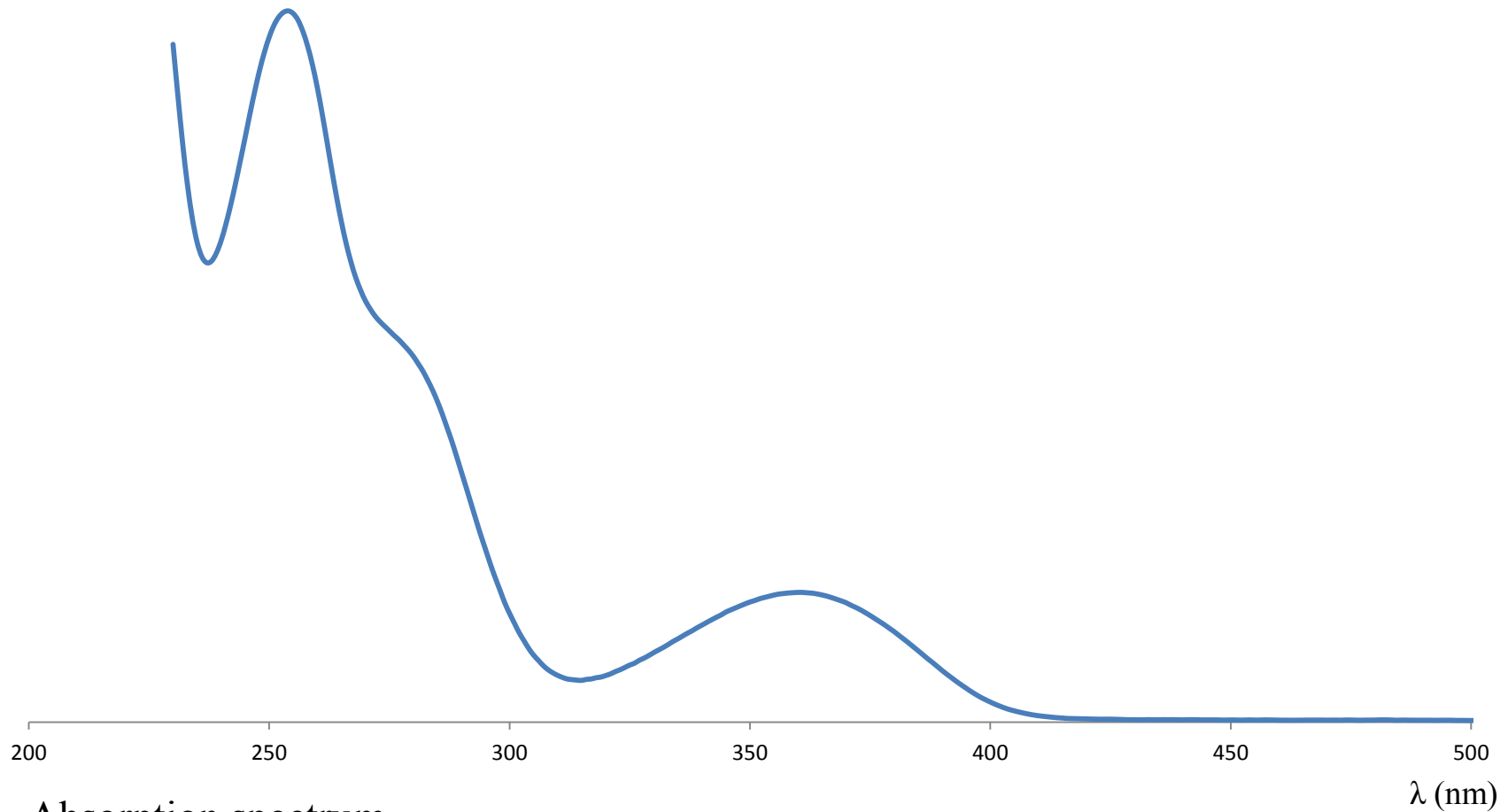
Mant-m⁷GpppG



Emission spectrum

λ (nm)

Mant-m⁷GpppG

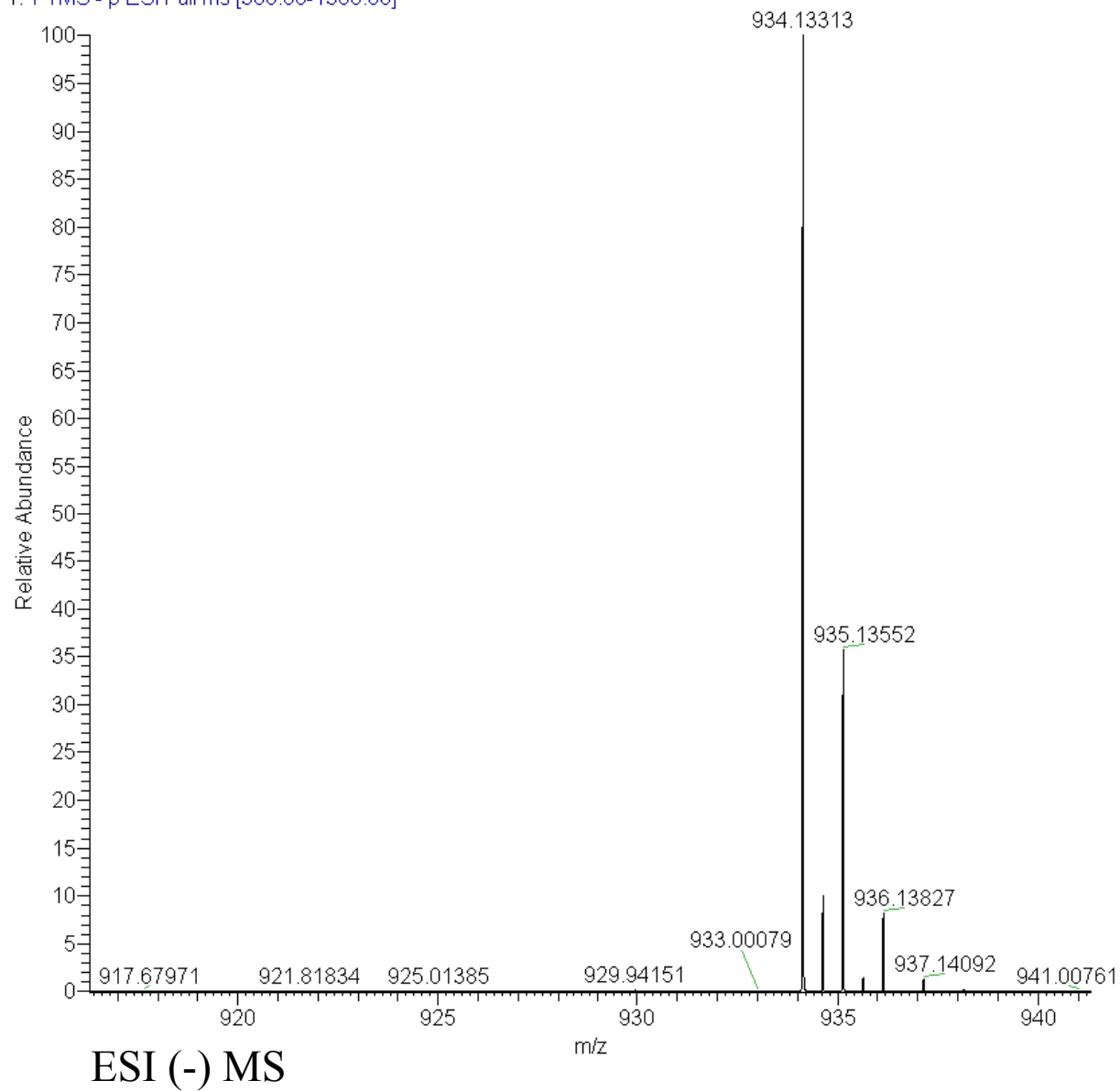


Absorption spectrum

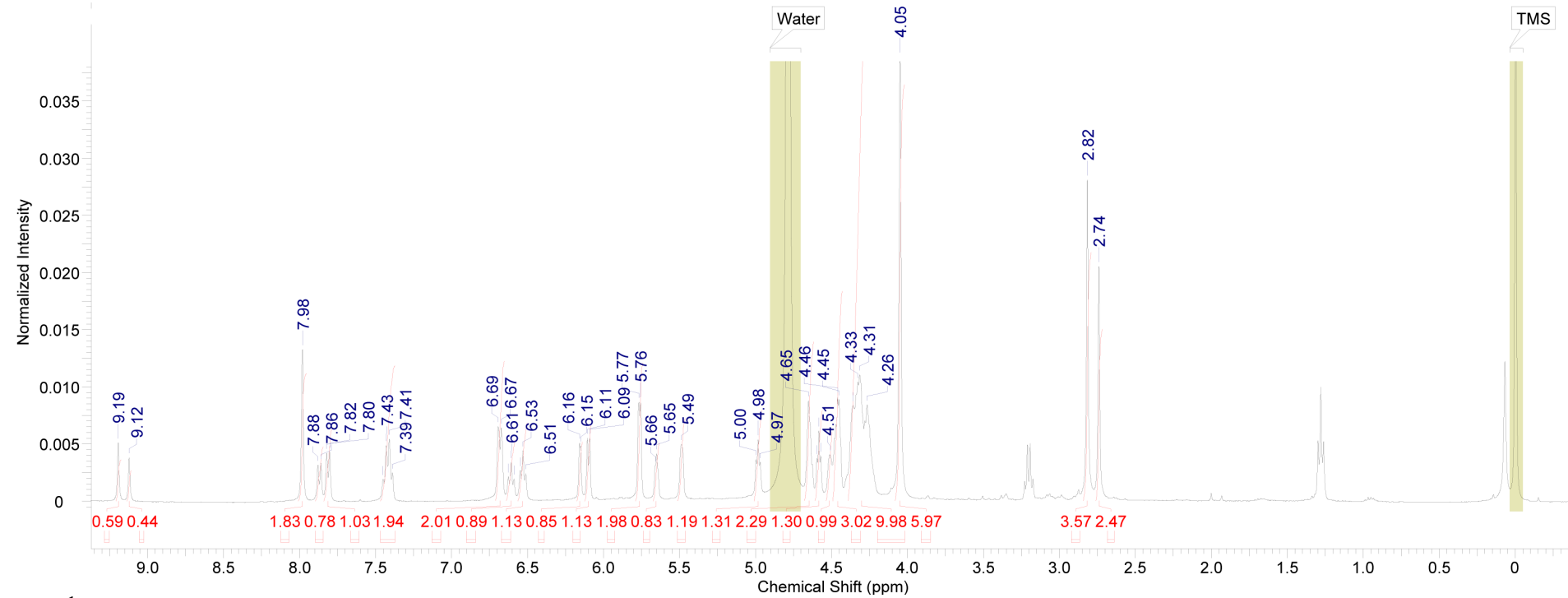
λ (nm)

Mant-m⁷GpppG

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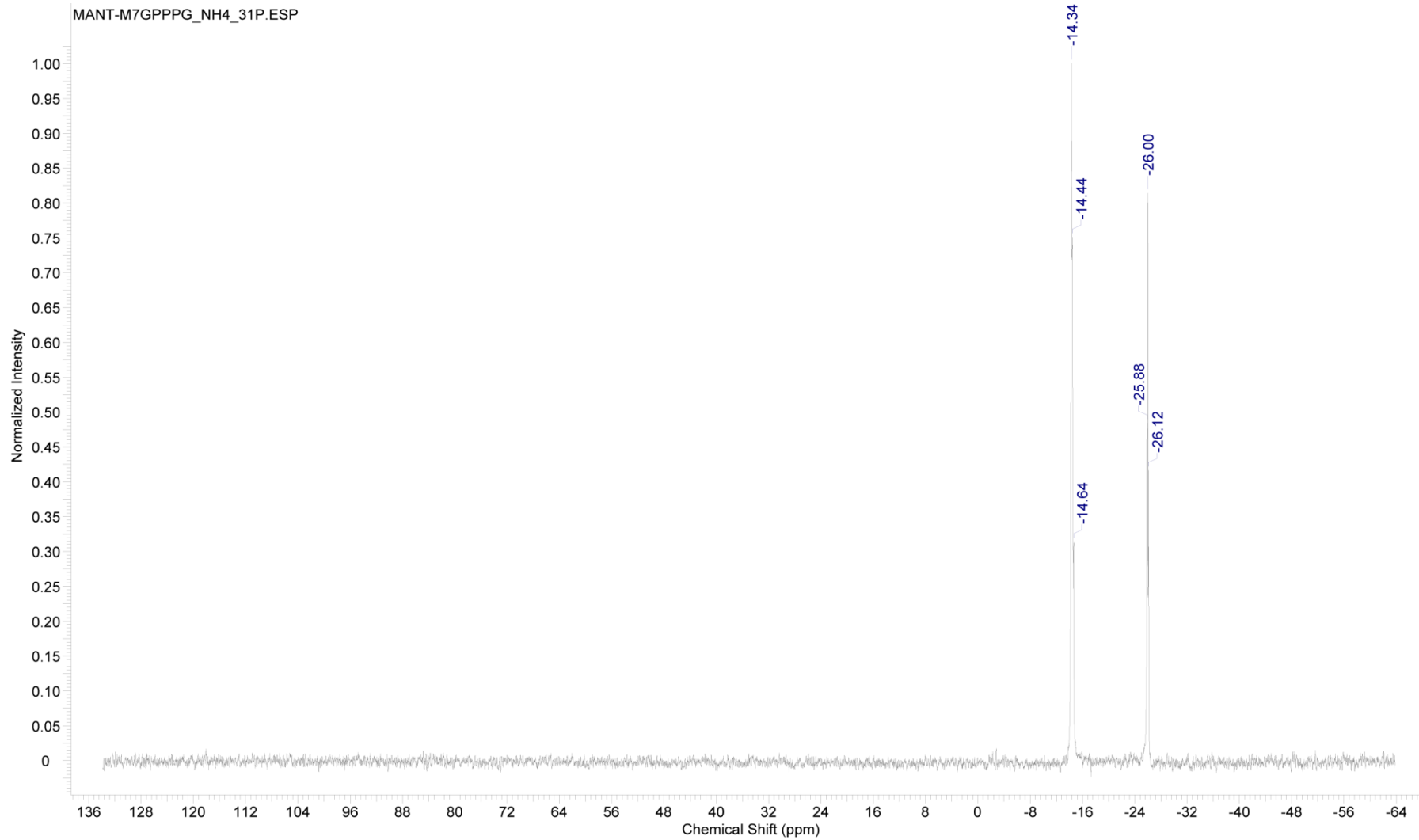


Mant-m⁷GpppG



¹H NMR

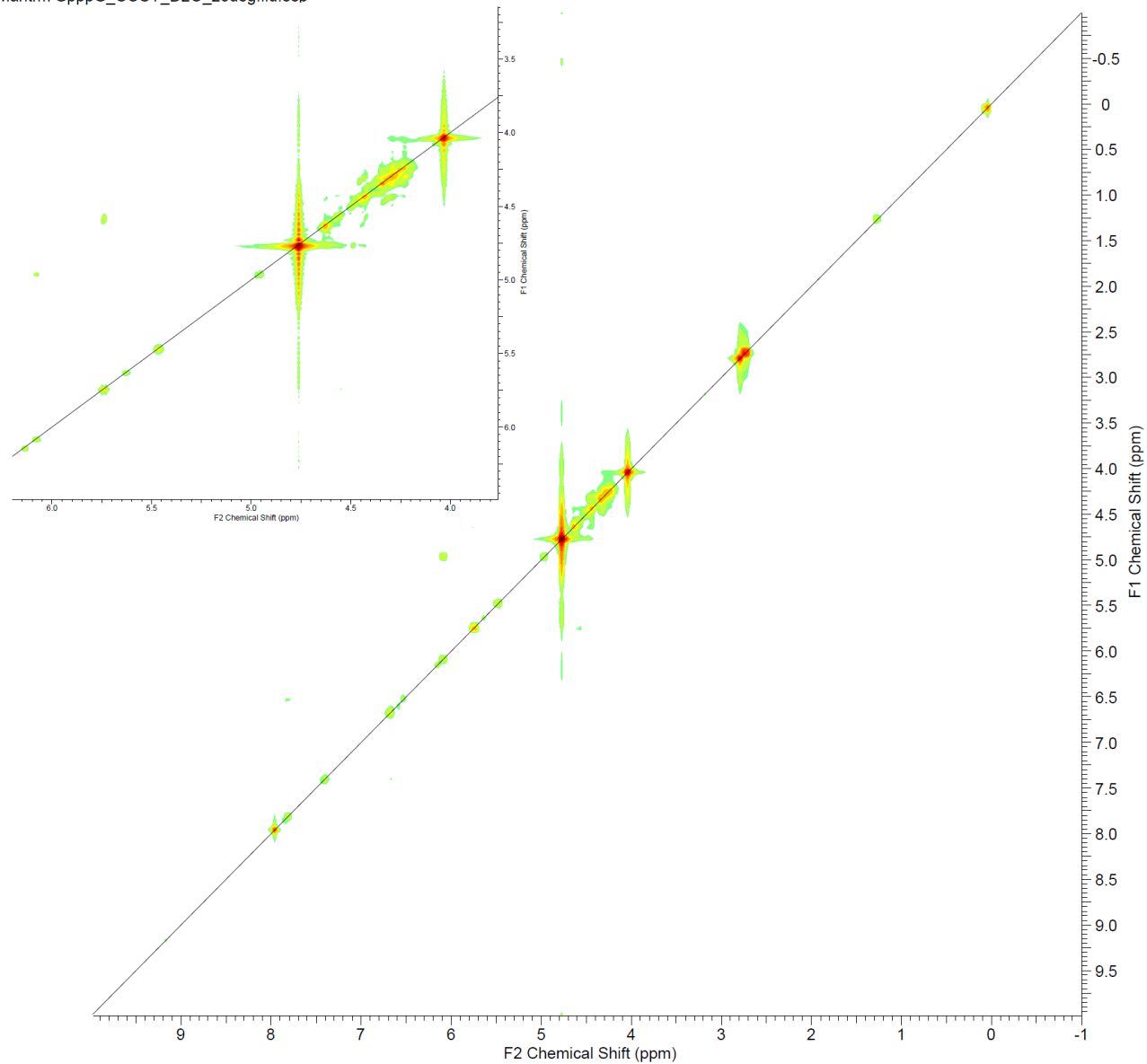
Mant-m⁷GpppG



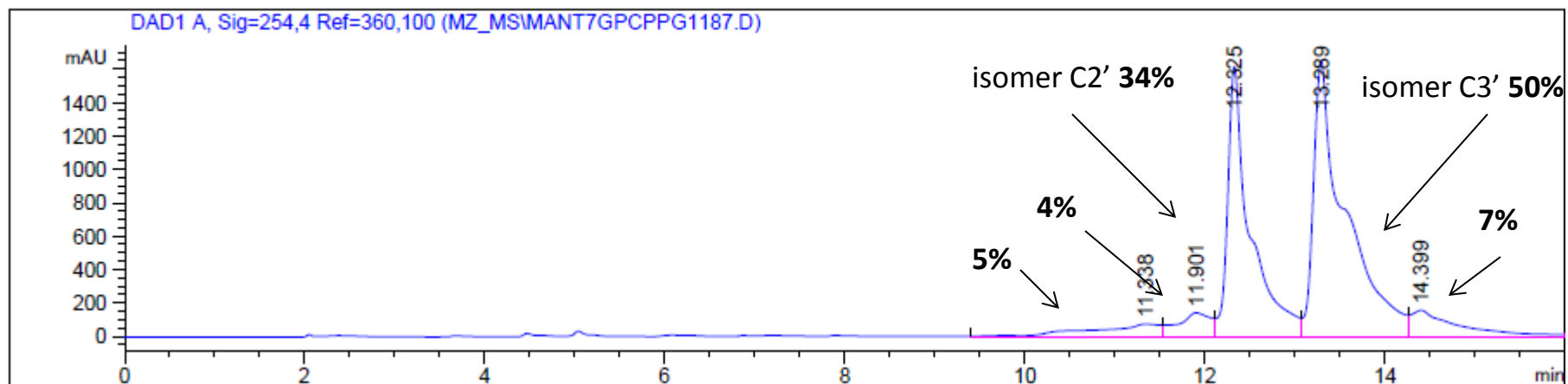
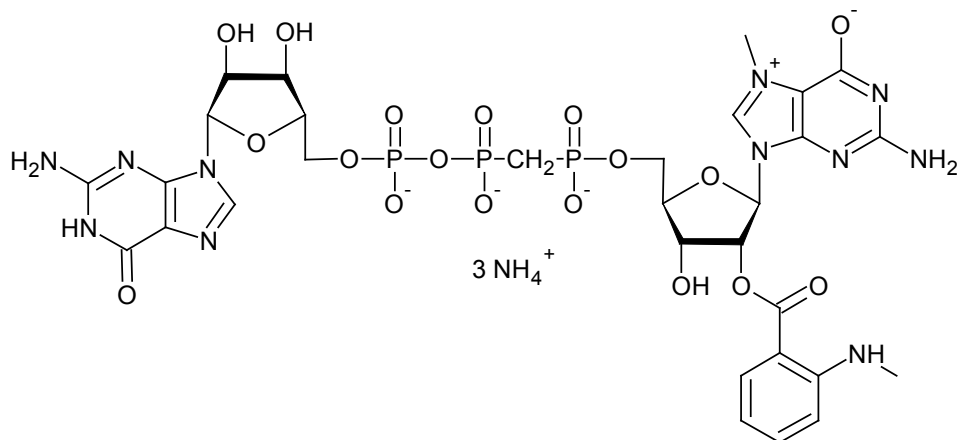
³¹P NMR

Mant-m⁷GpppG

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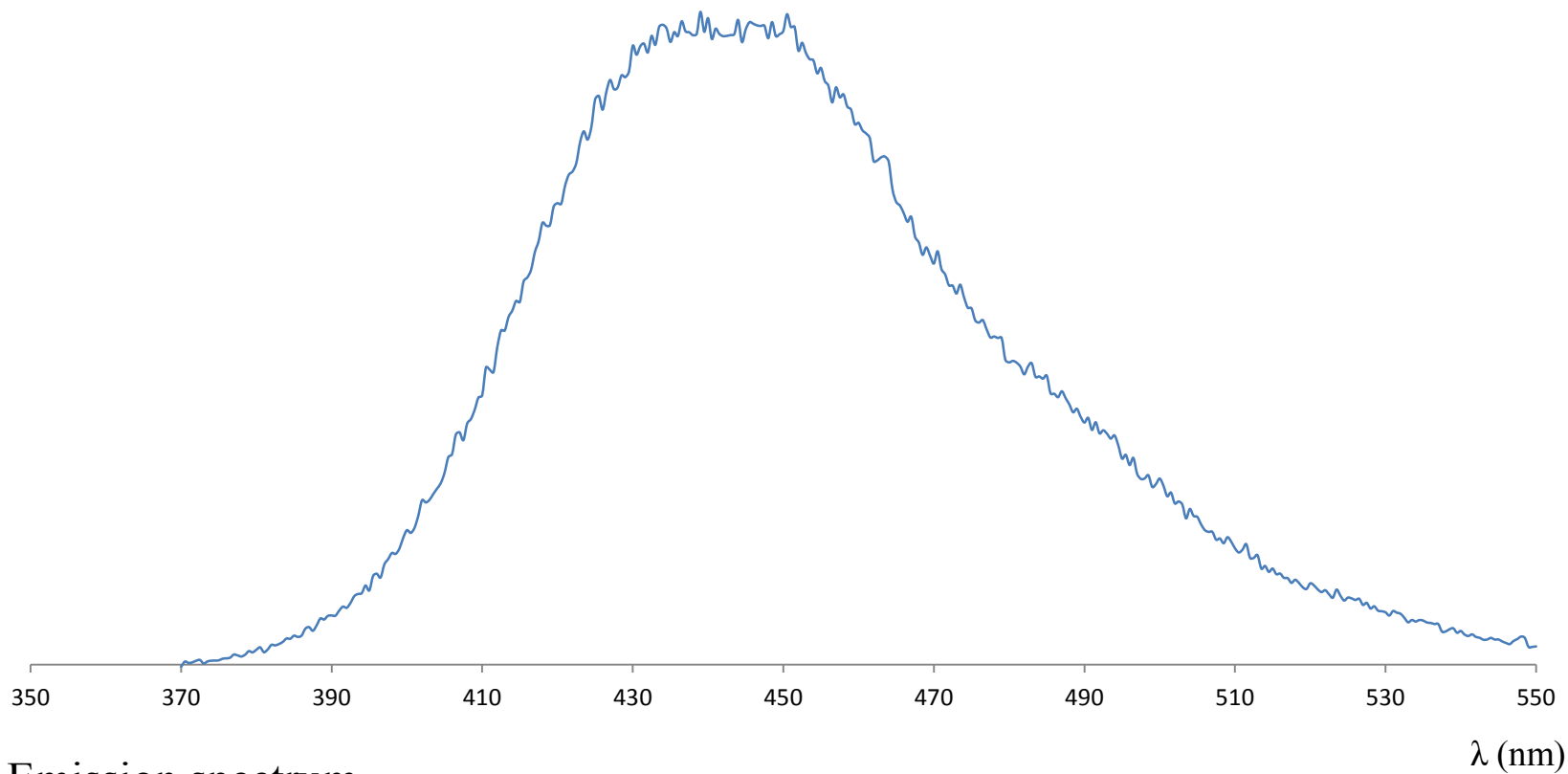
Mant-m⁷GpCH₂ppG



HPLC profile

Mant-m⁷GpCH₂ppG

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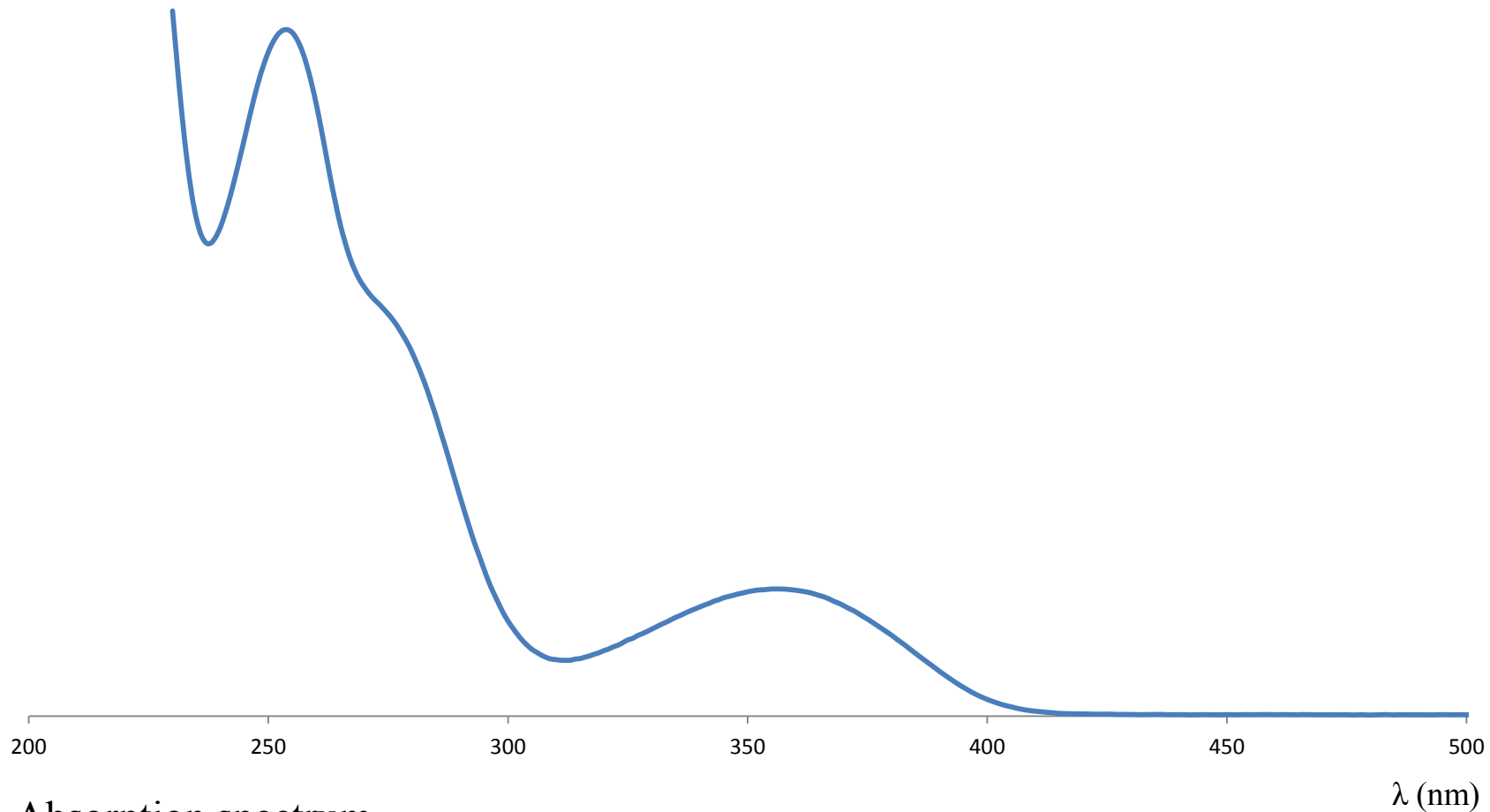


Emission spectrum

λ (nm)

Mant-m⁷GpCH₂ppG

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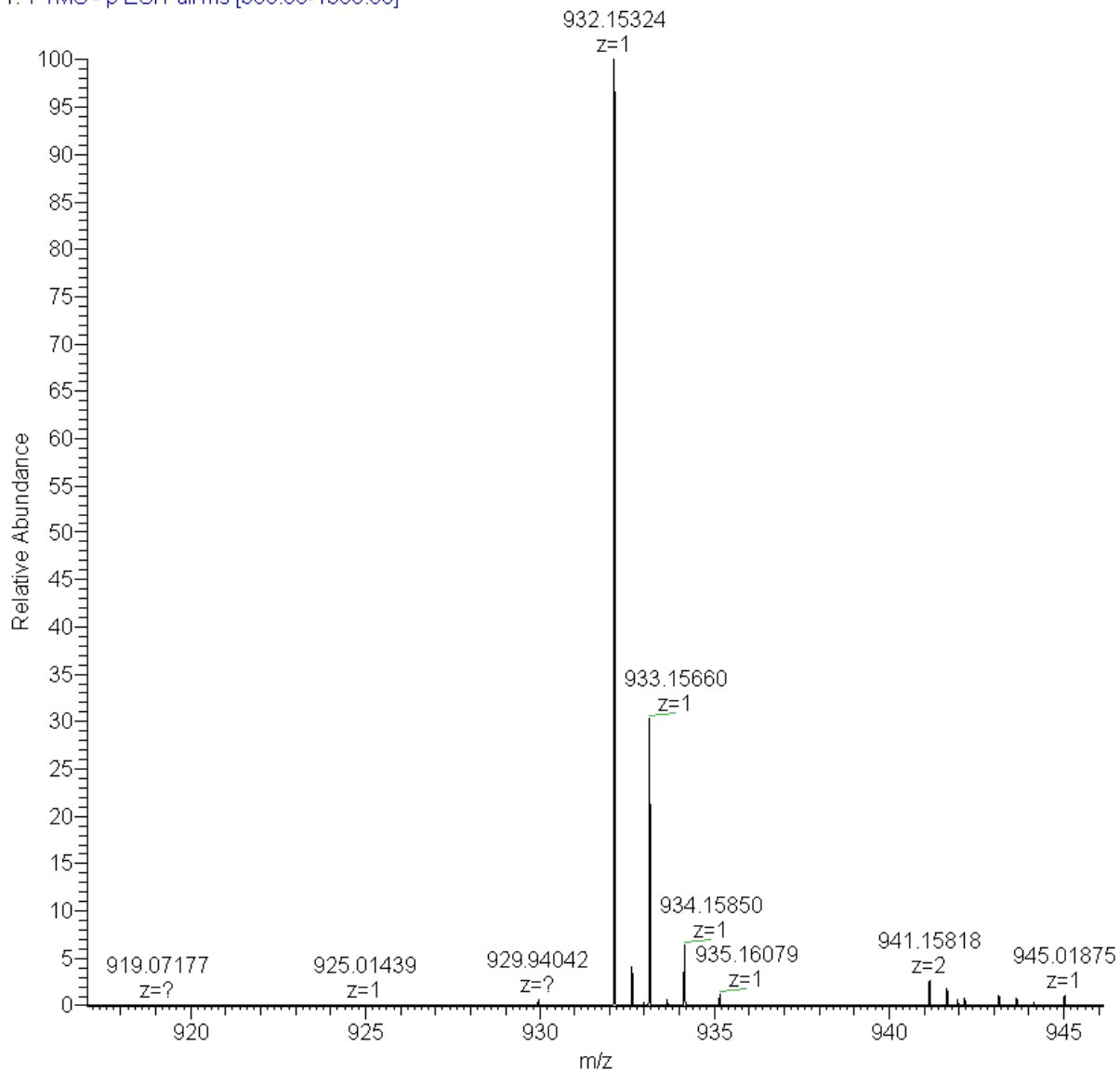


Absorption spectrum

λ (nm)

Mant-m⁷GpCH₂ppG

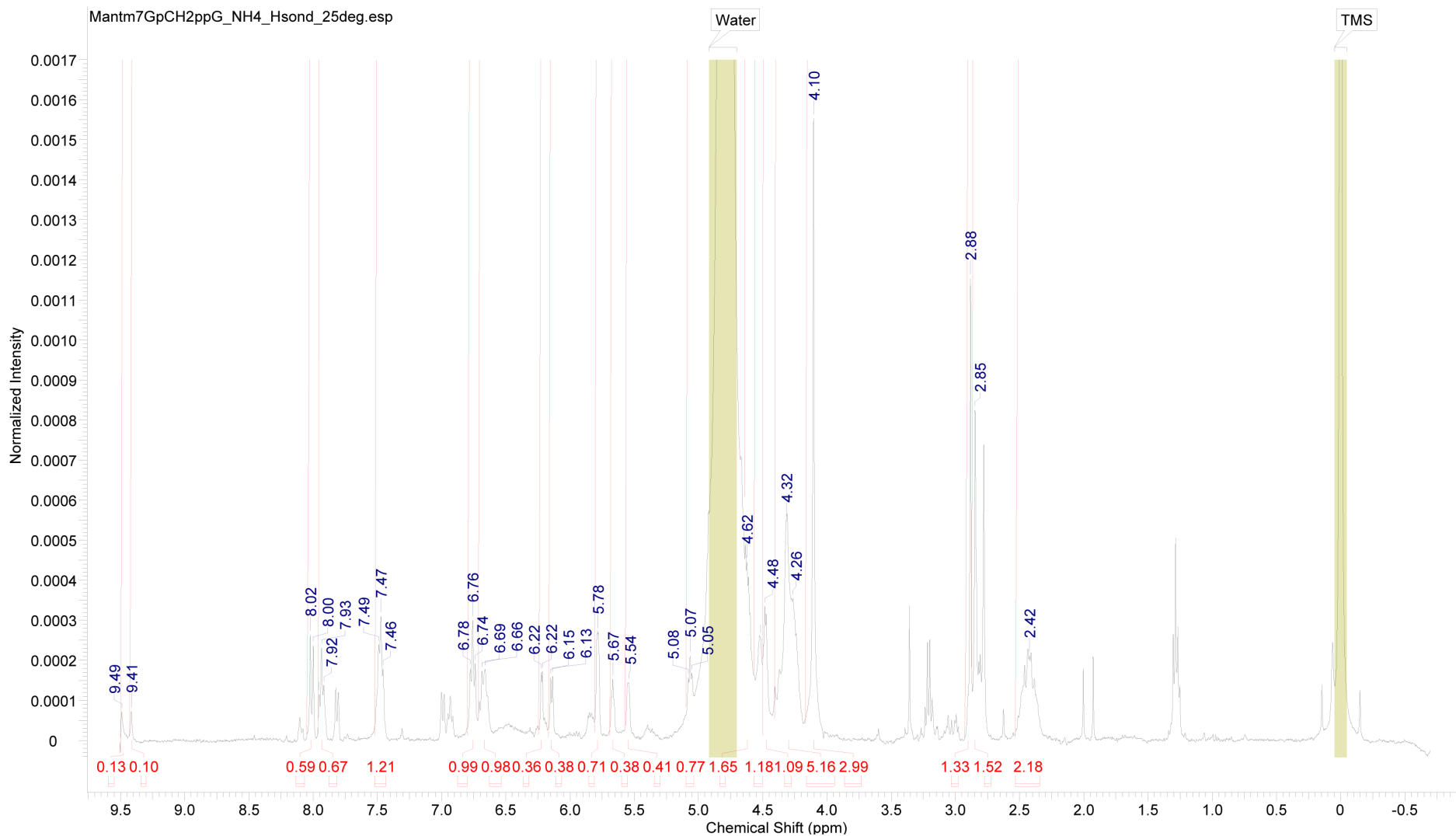
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ESI (-) MS

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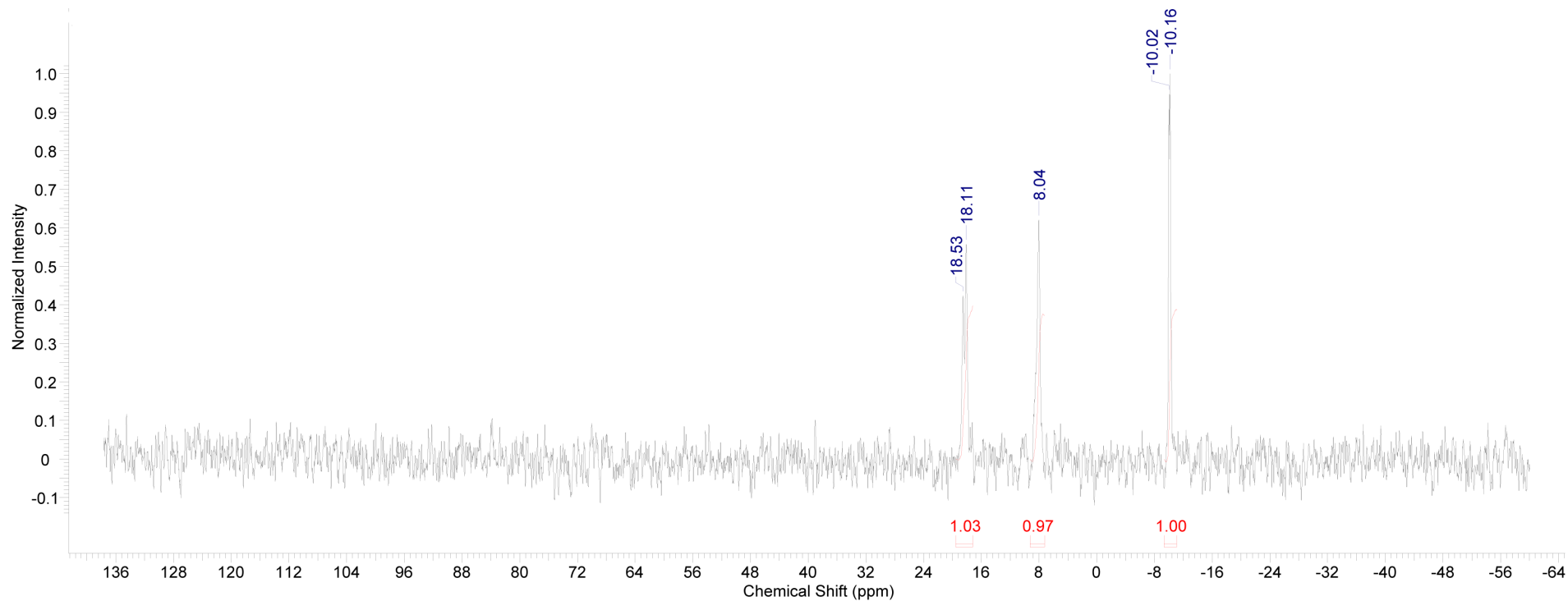
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¹H NMR

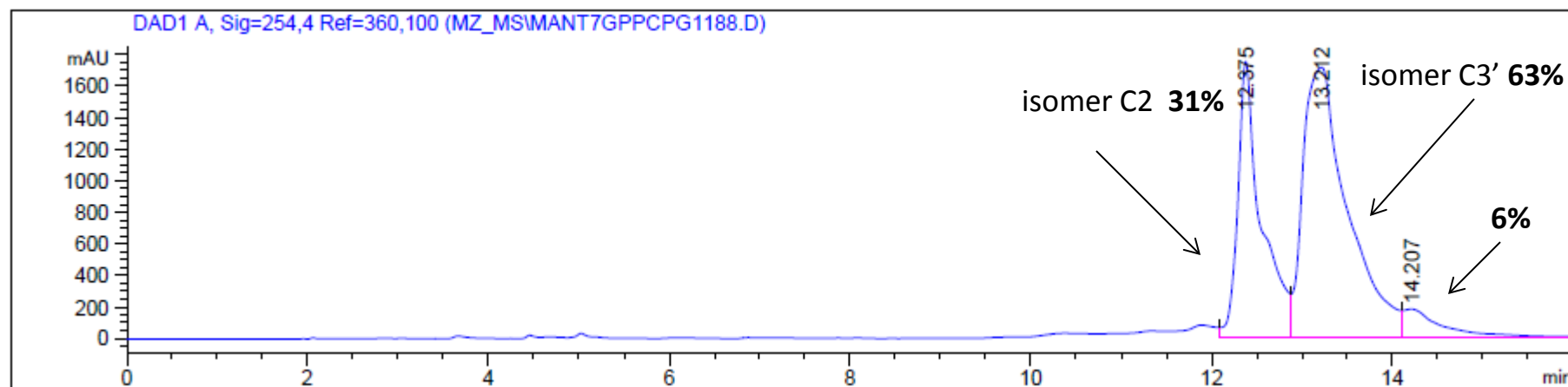
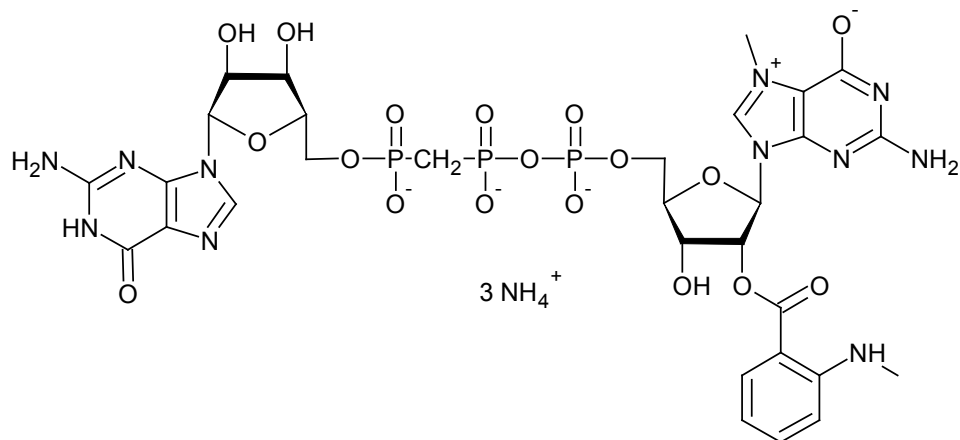
Mant-m⁷GpCH₂ppG

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³¹P NMR

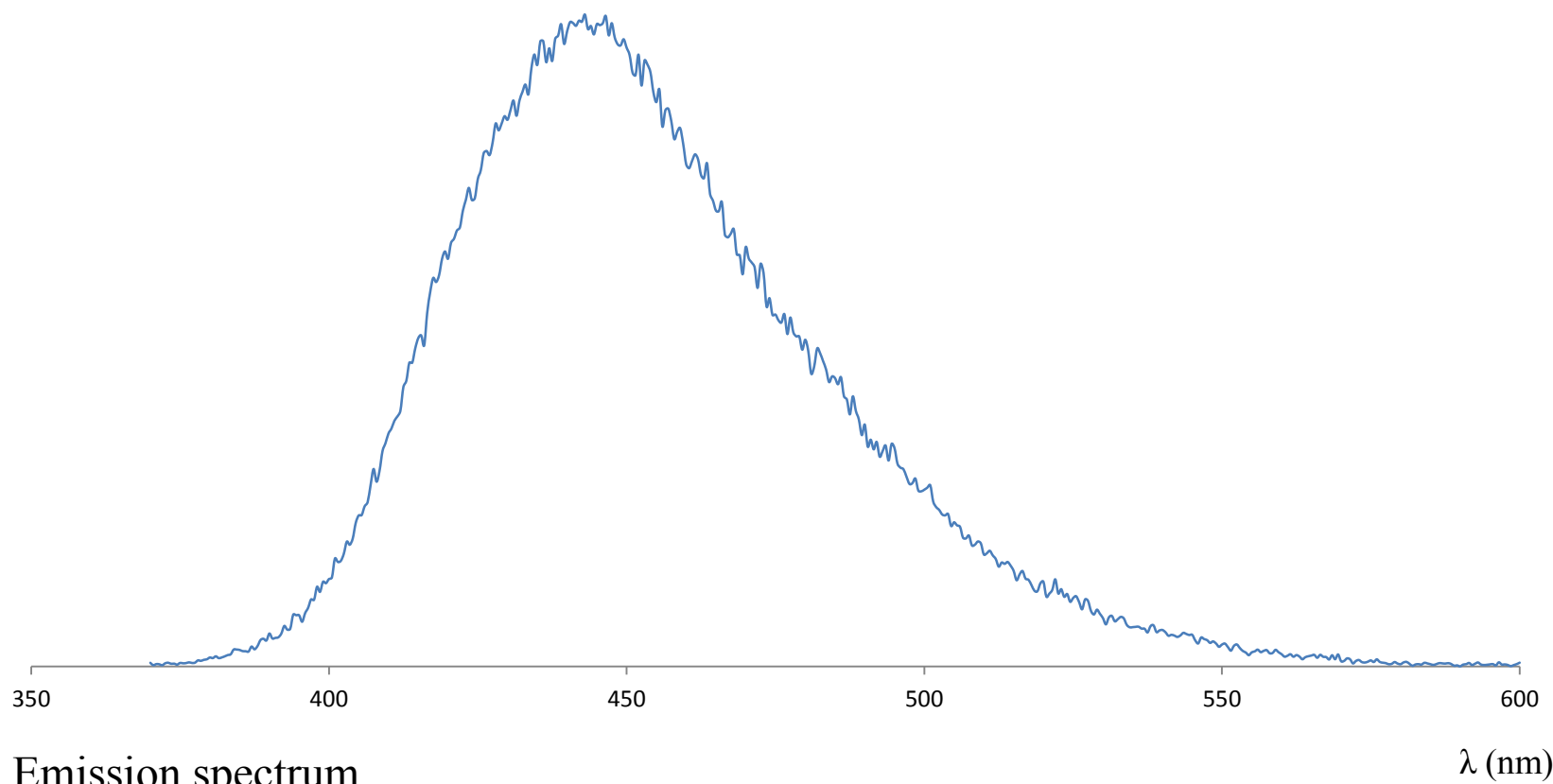
Mant-m⁷GppCH₂pG



HPLC profile

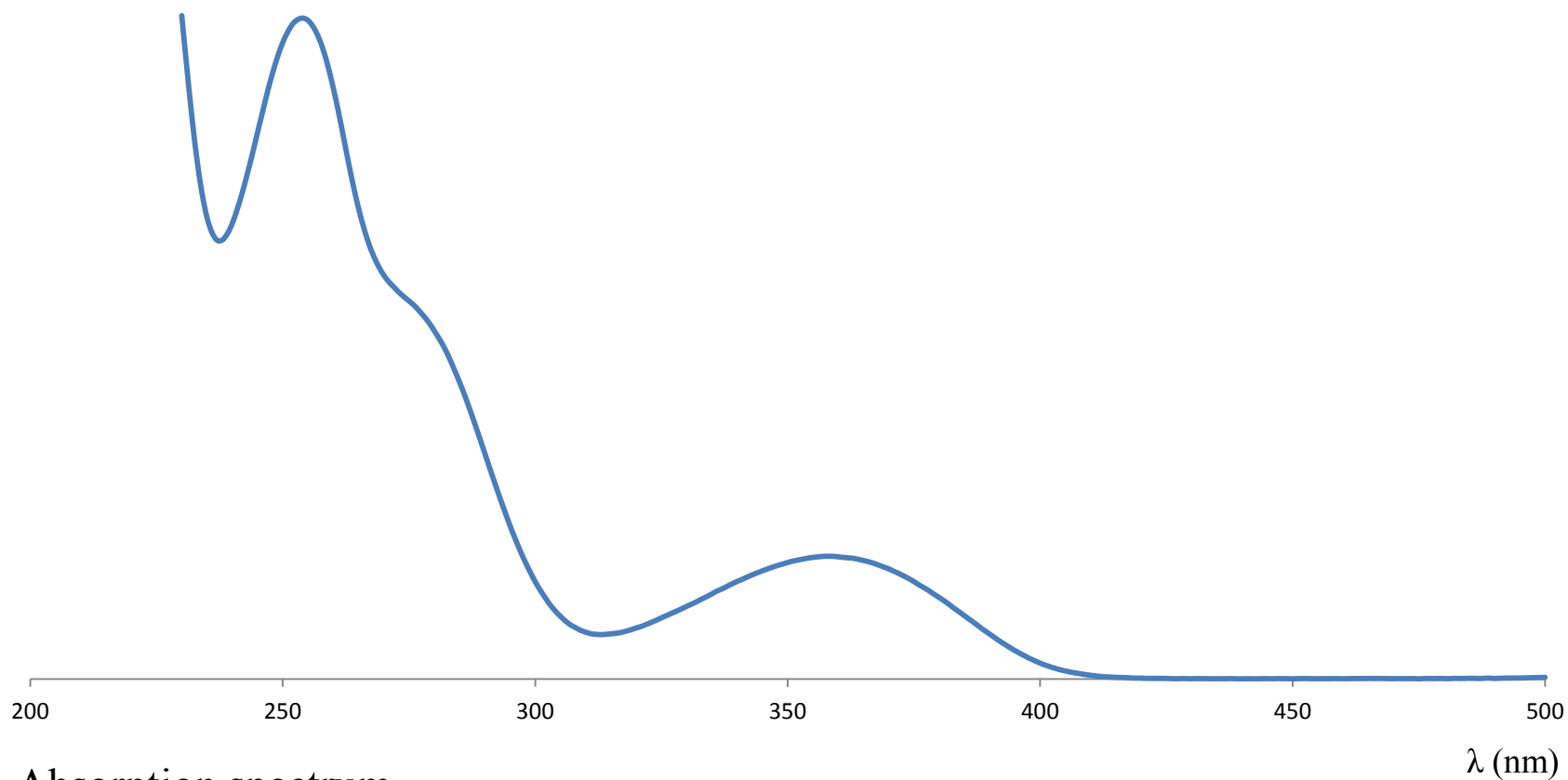
Mant-m⁷GppCH₂pG

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Mant-m⁷GppCH₂pG

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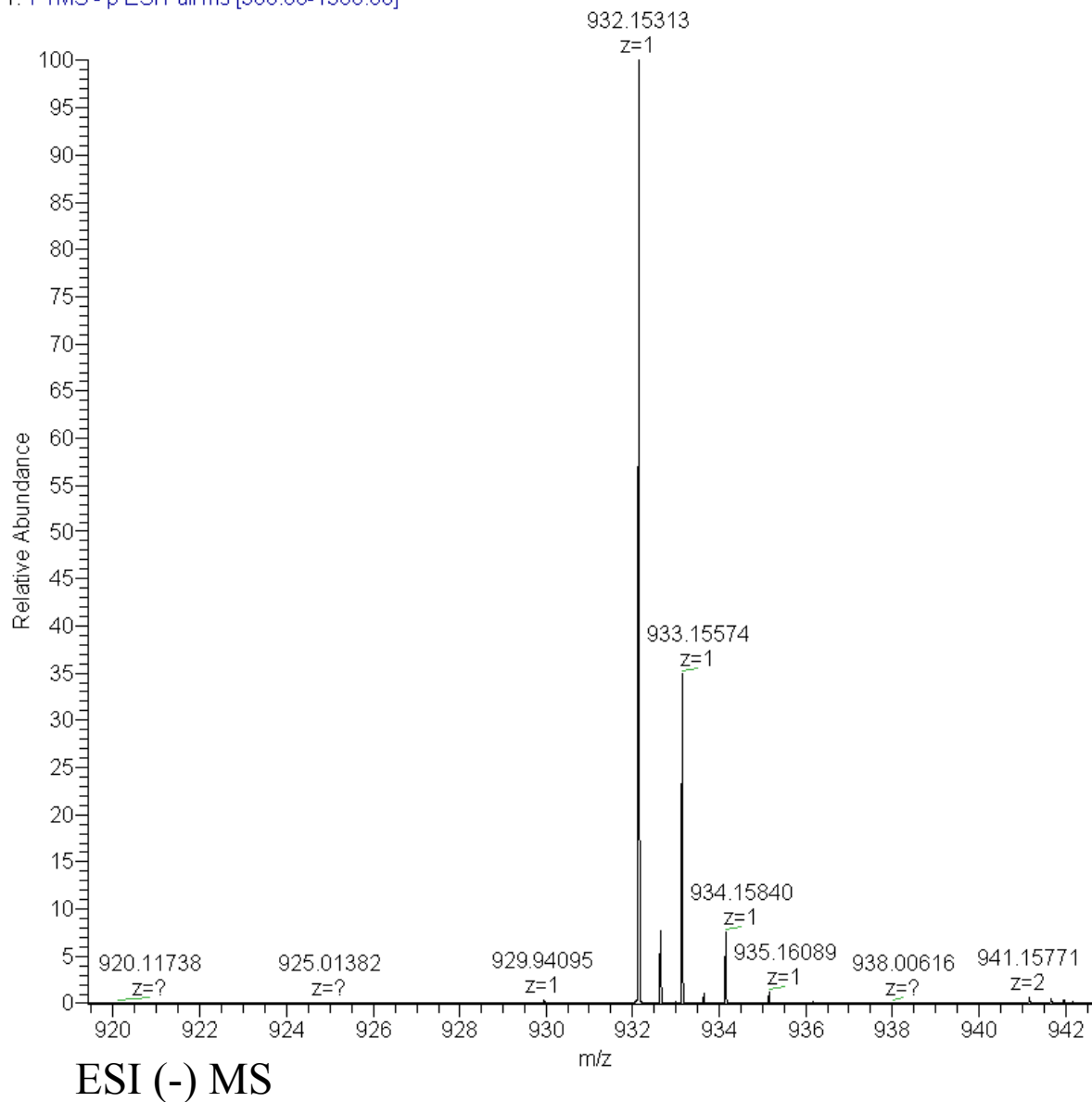


Absorption spectrum

λ (nm)

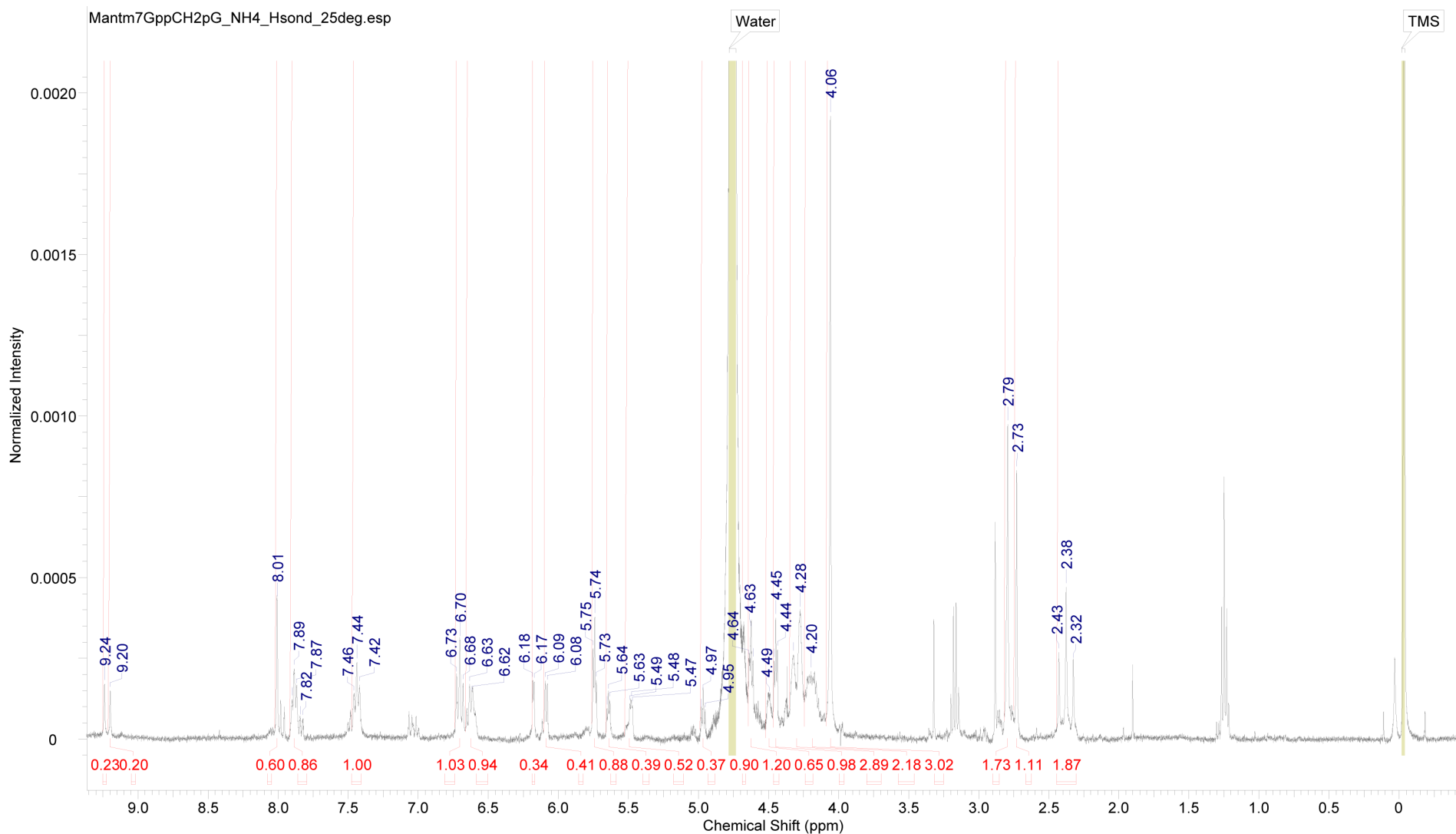
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Mant-m⁷GppCH₂pG

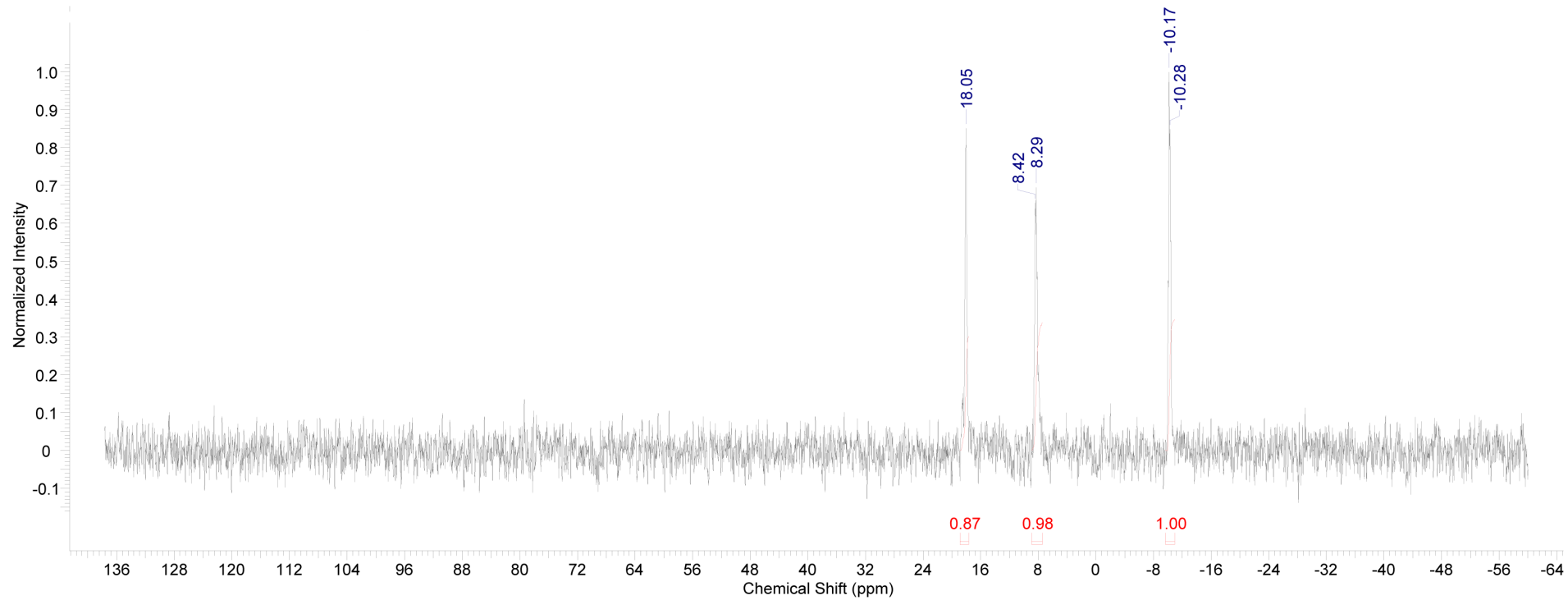
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¹H NMR

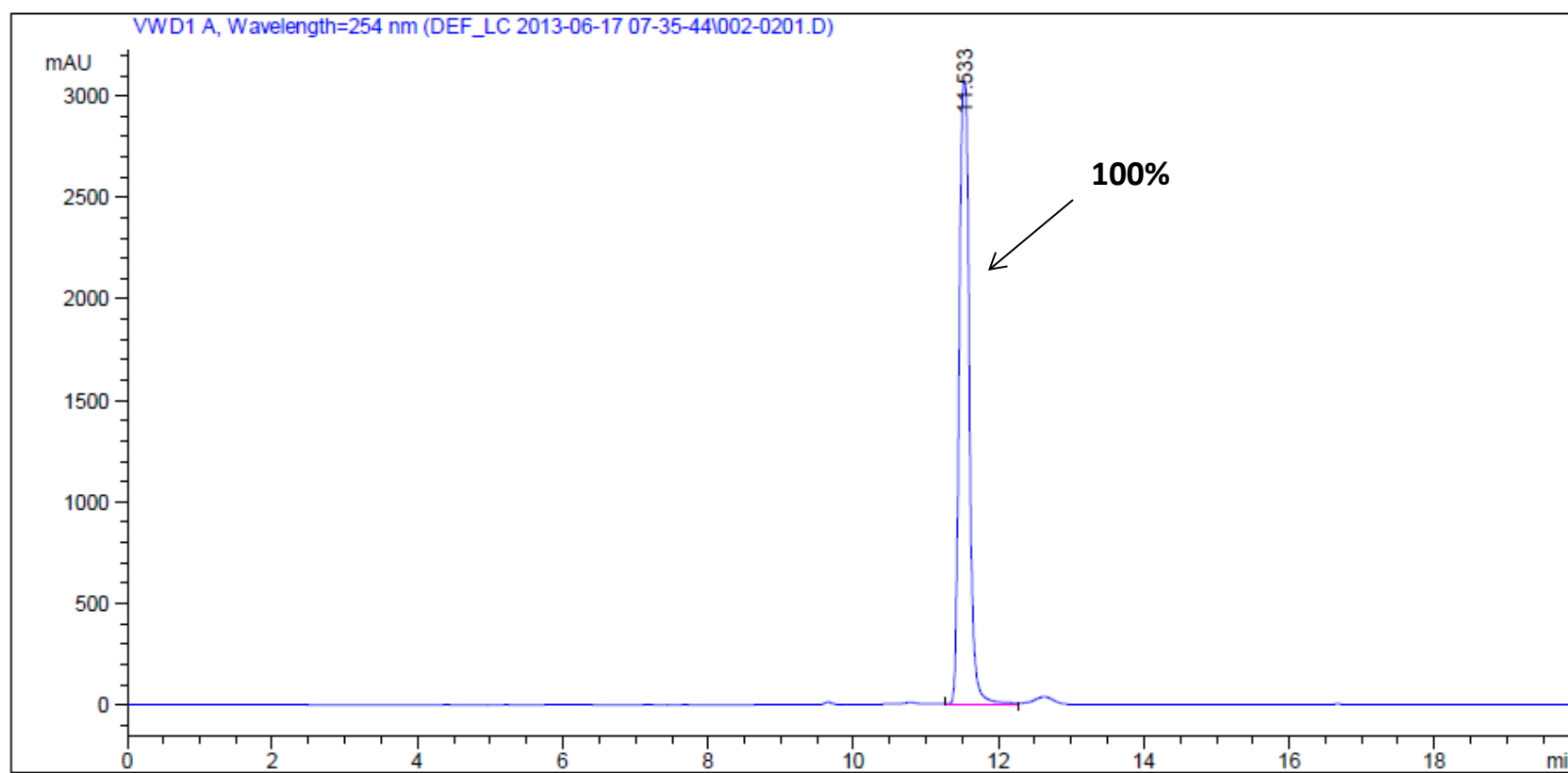
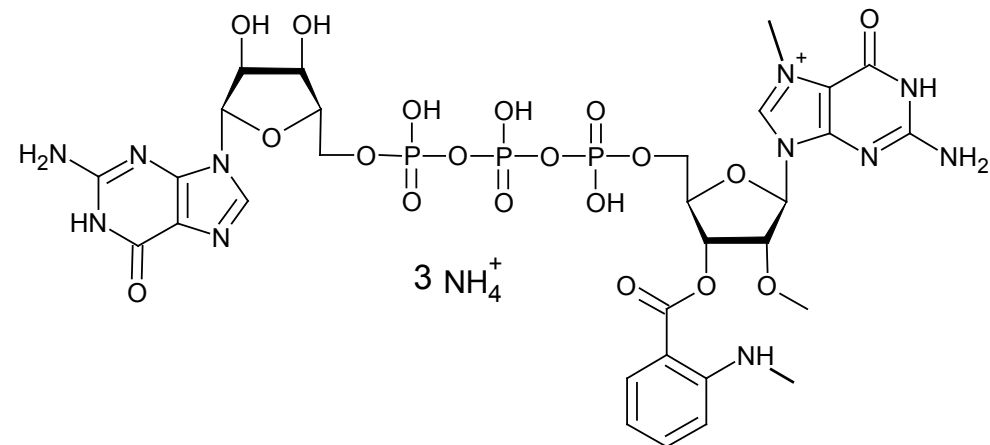
Mant-m⁷GppCH₂pG

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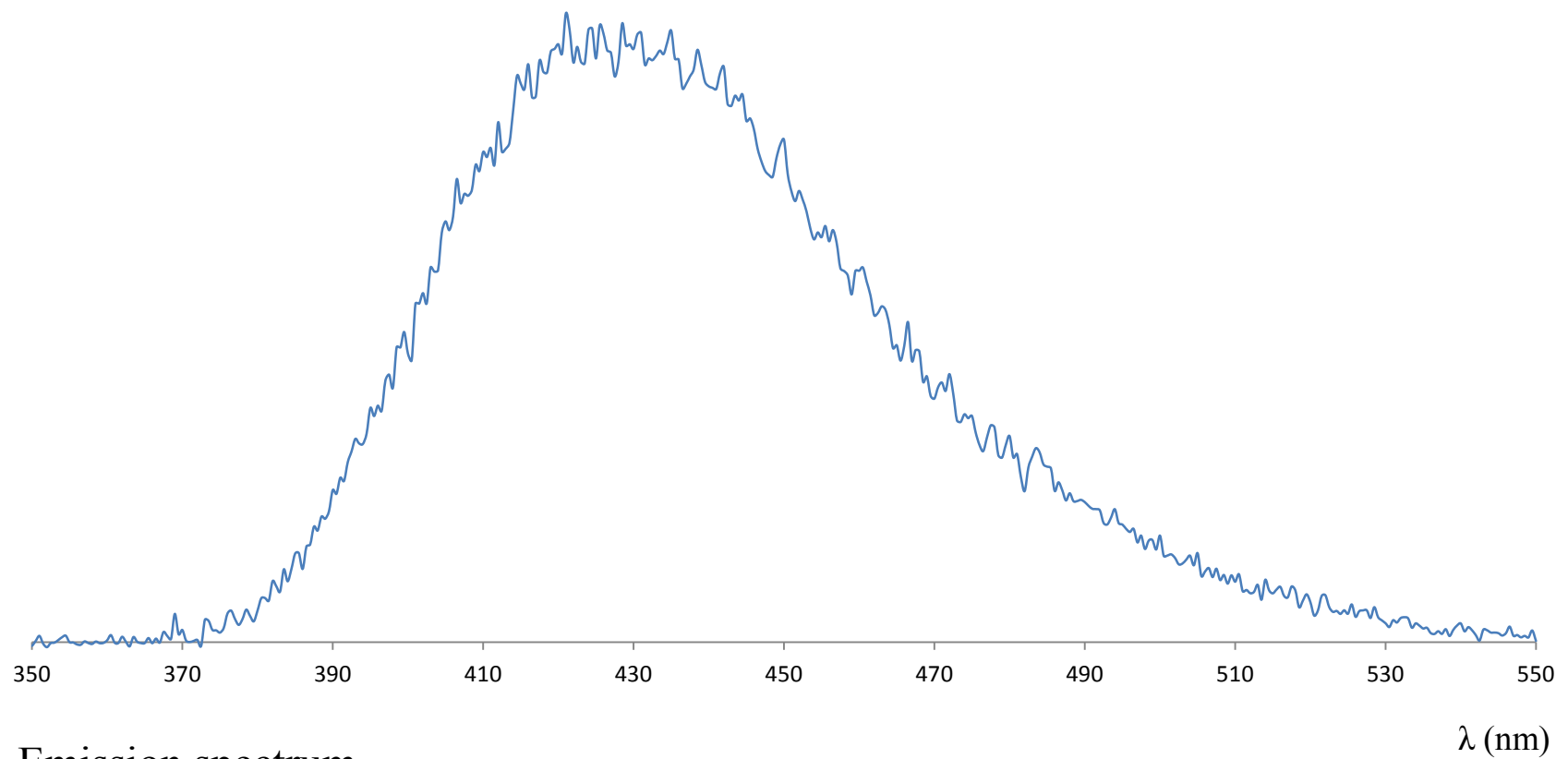
³¹P NMR

Ant-m₂^{7,2'-O}GpppG



HPLC profile

Ant-m₂^{7,2'-O}GpppG

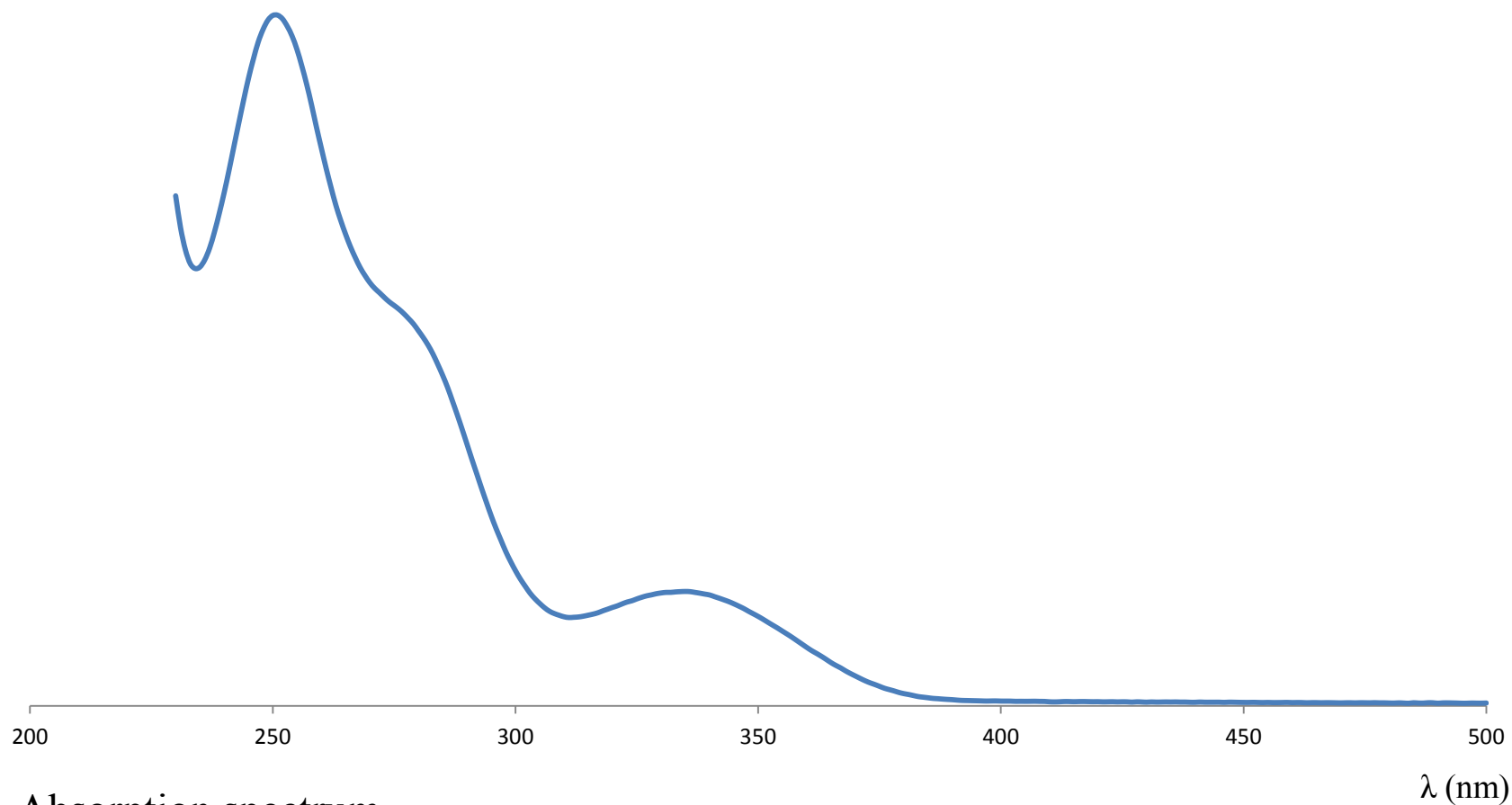


Emission spectrum

λ (nm)

Ant-m₂^{7,2'-O}GpppG

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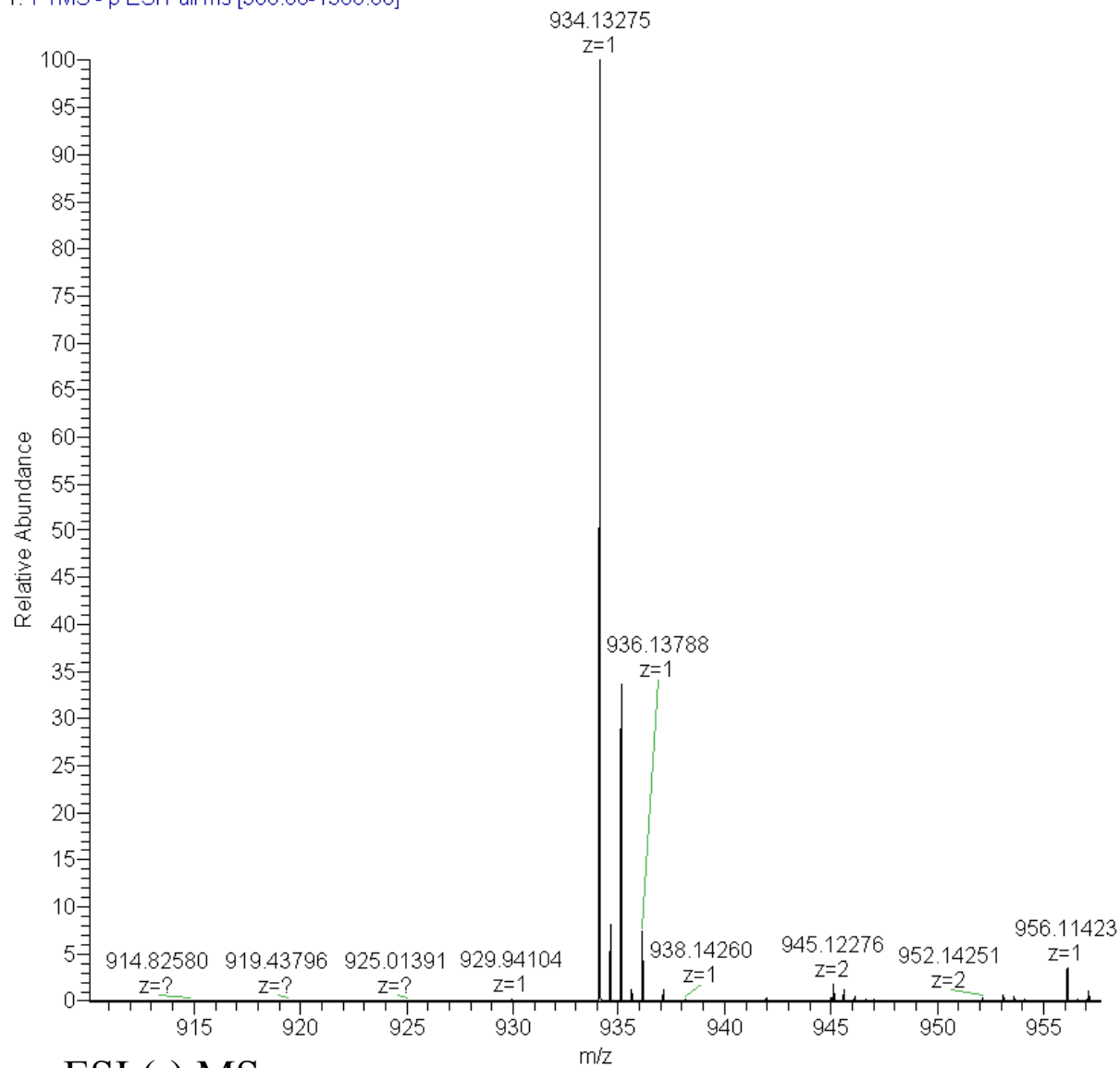


Absorption spectrum

λ (nm)

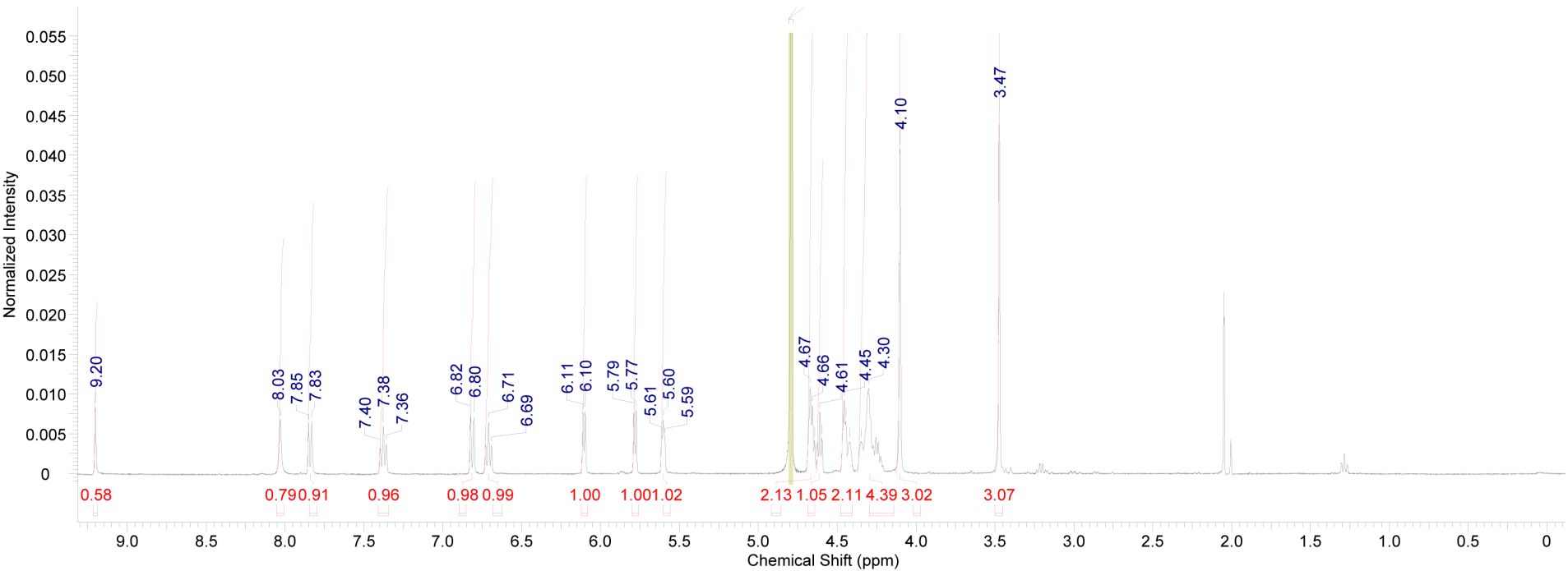
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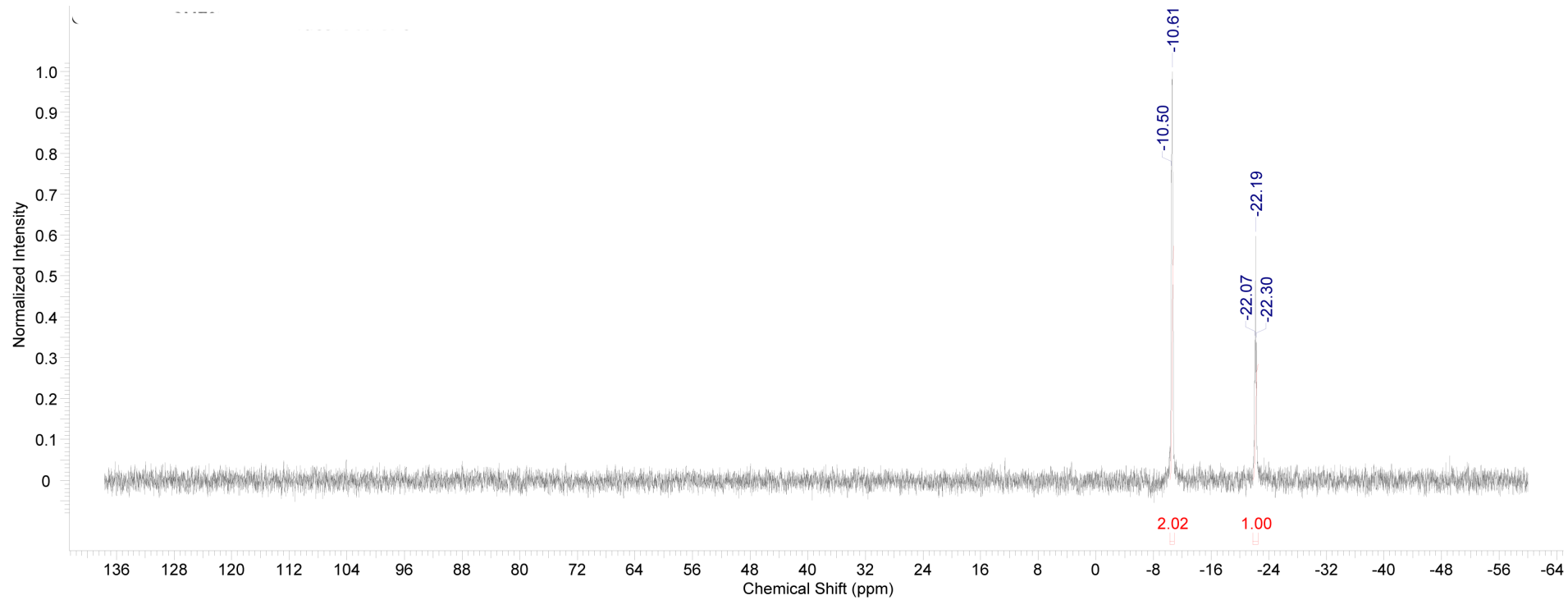
ESI (-) MS

Ant-m₂^{7,2'-O}GpppG

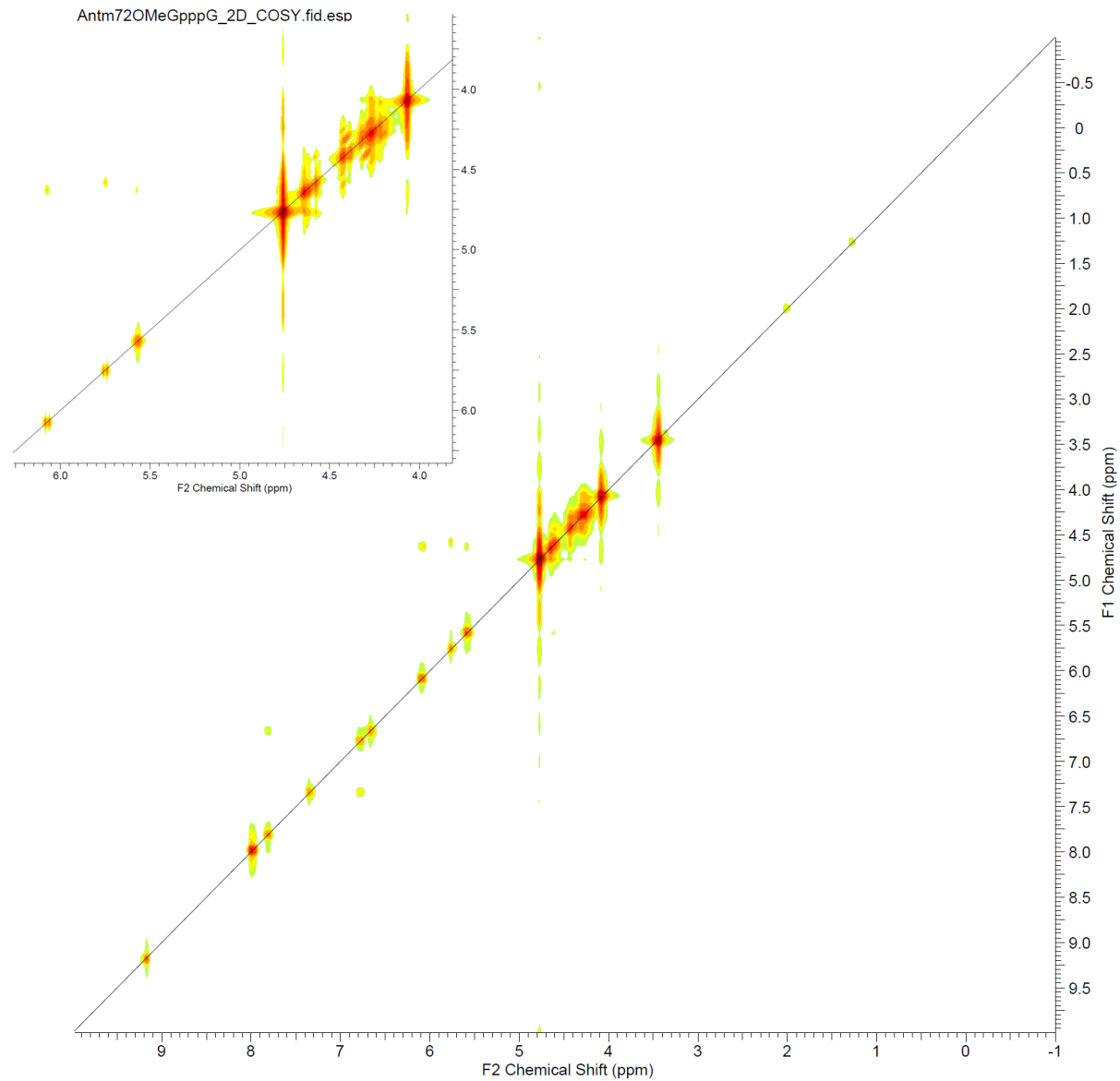


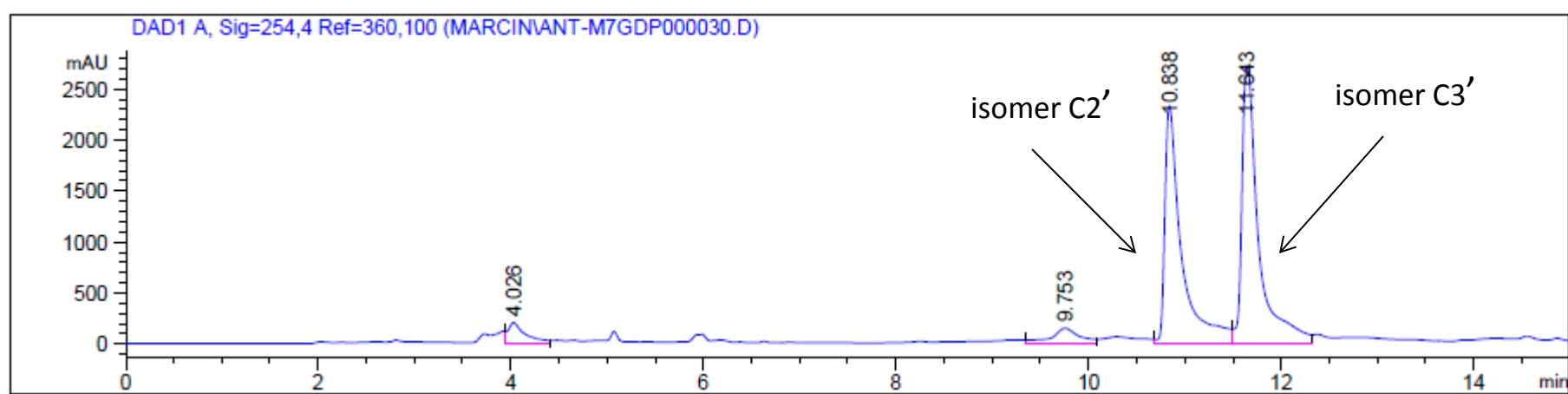
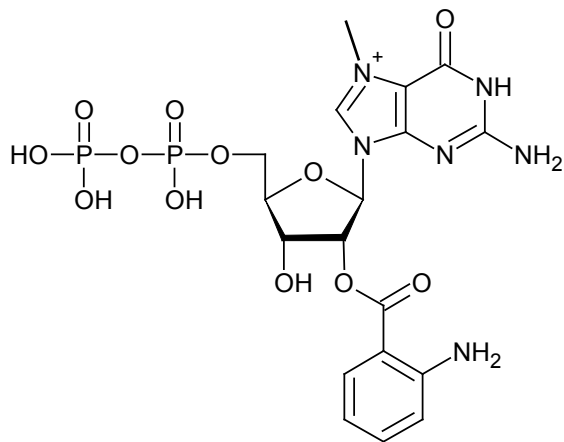
¹H NMR

Ant-m₂^{7,2'-O}GpppG



³¹P NMR

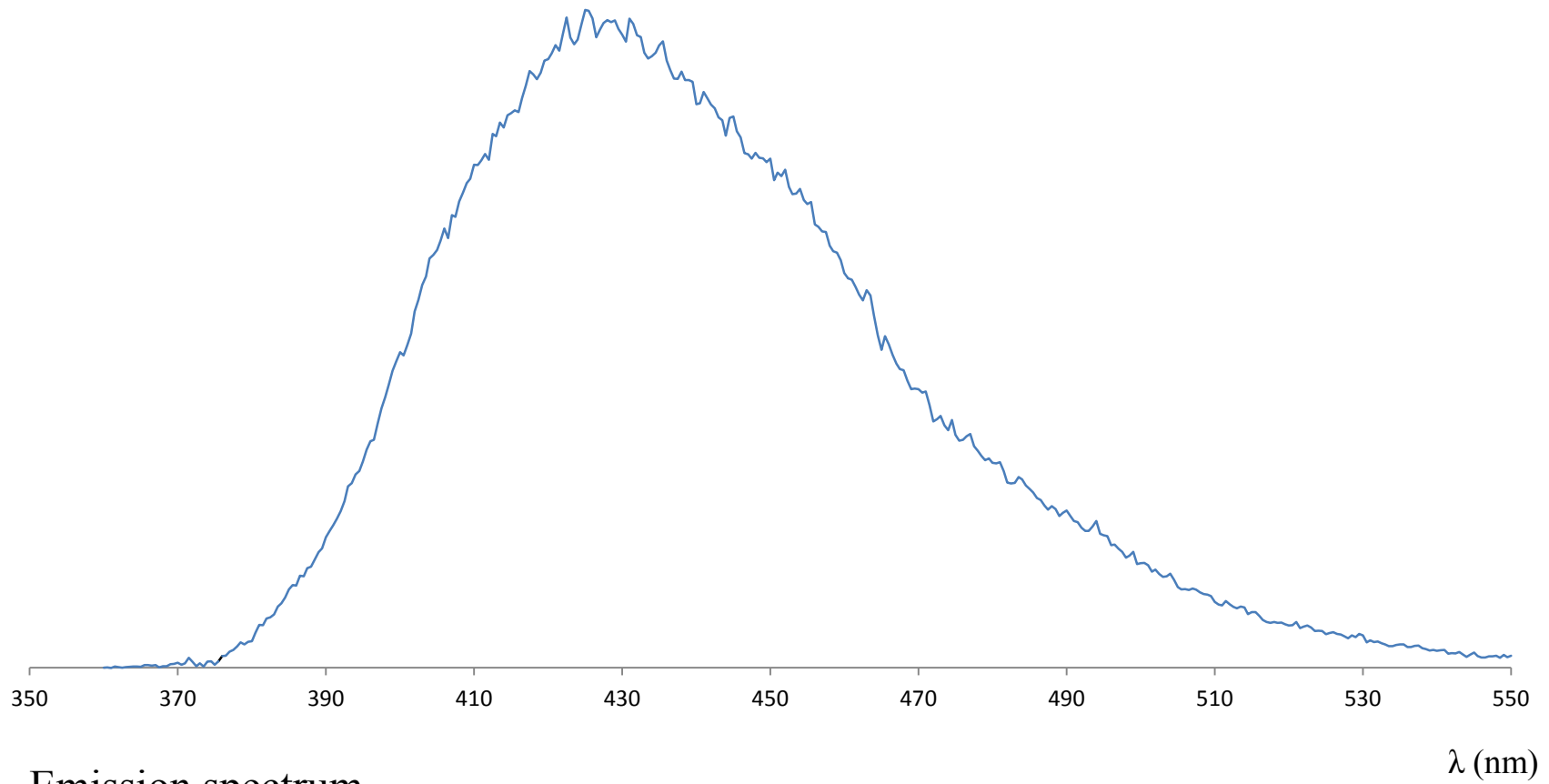
Ant-m₂^{7,2'-O}GpppG

Ant-m⁷GDP

HPLC profile

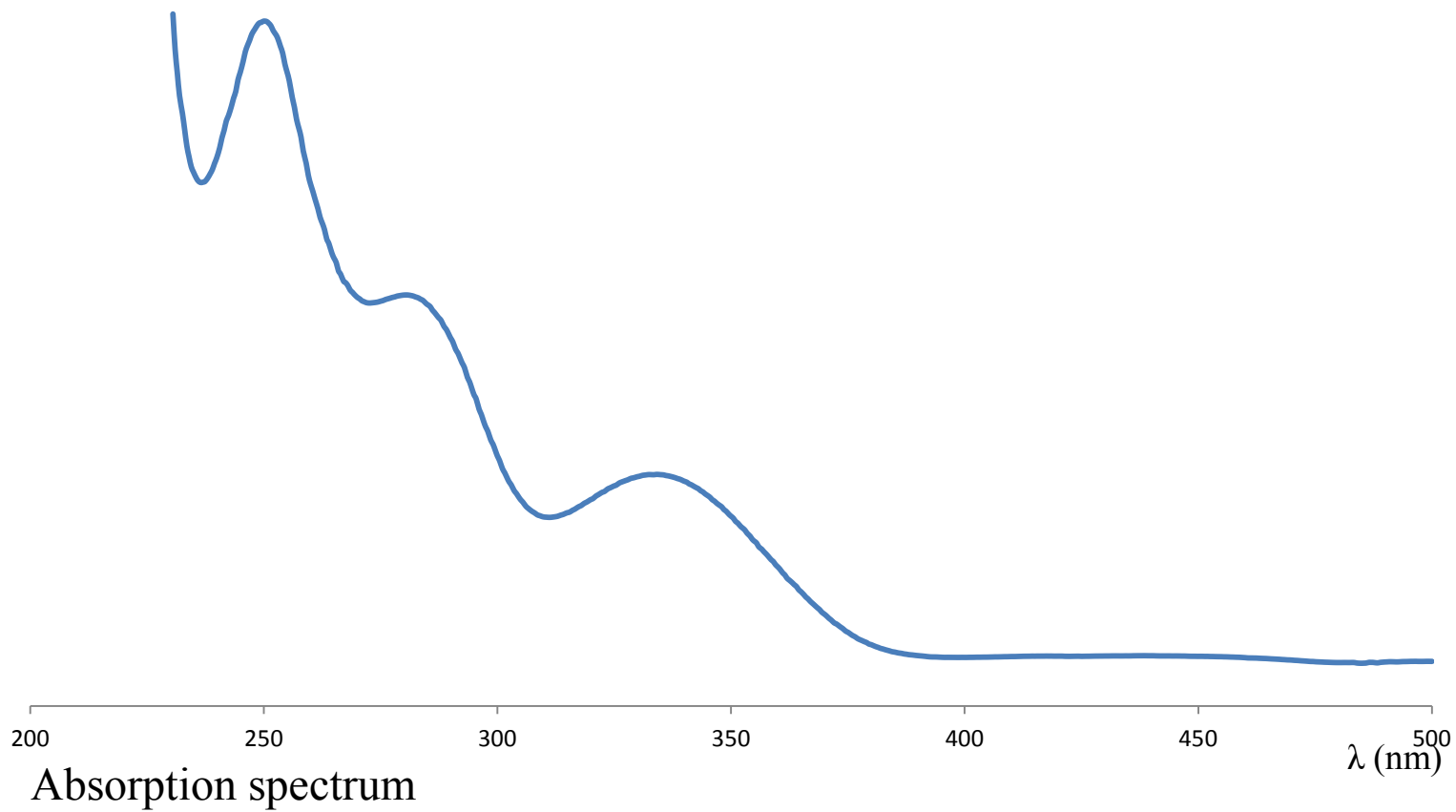
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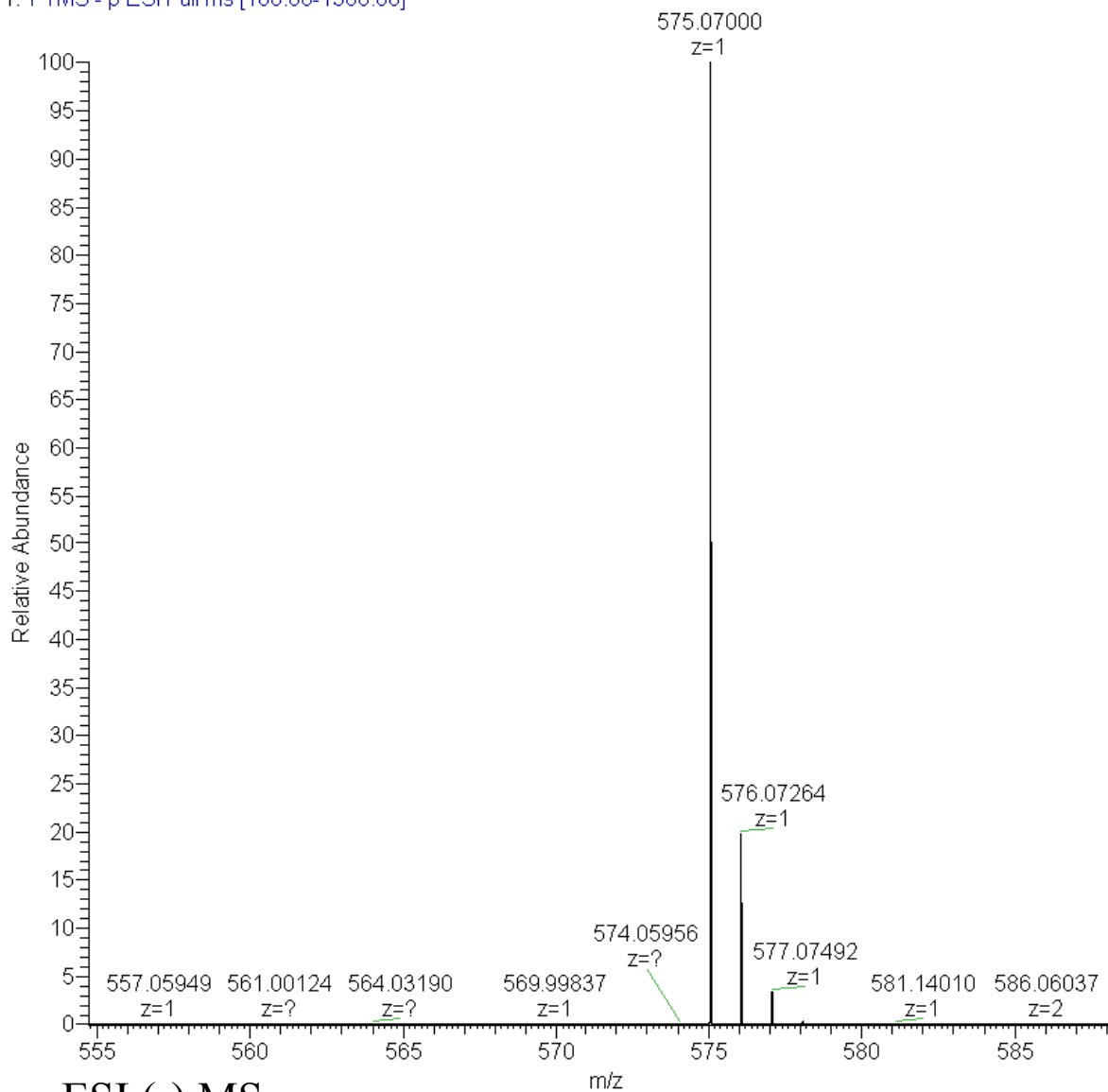
Ant-m⁷GDP

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Ant-m⁷GDP

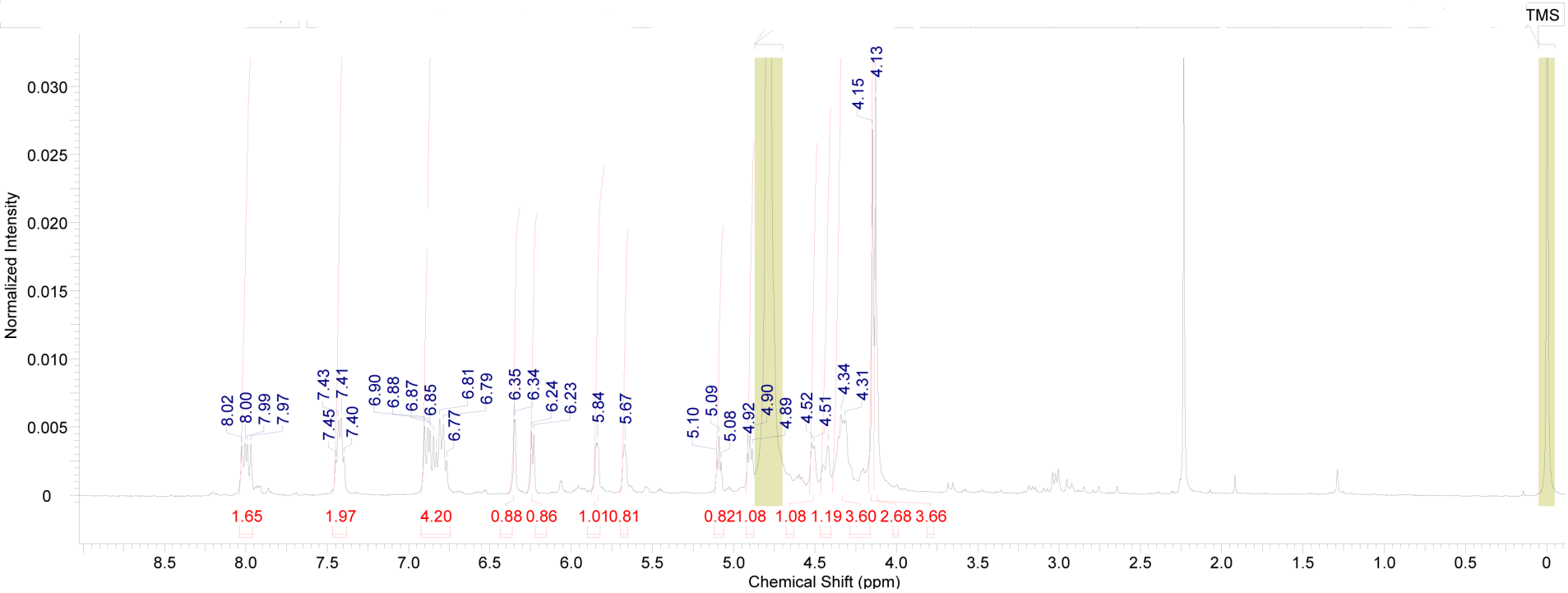
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ESI (-) MS

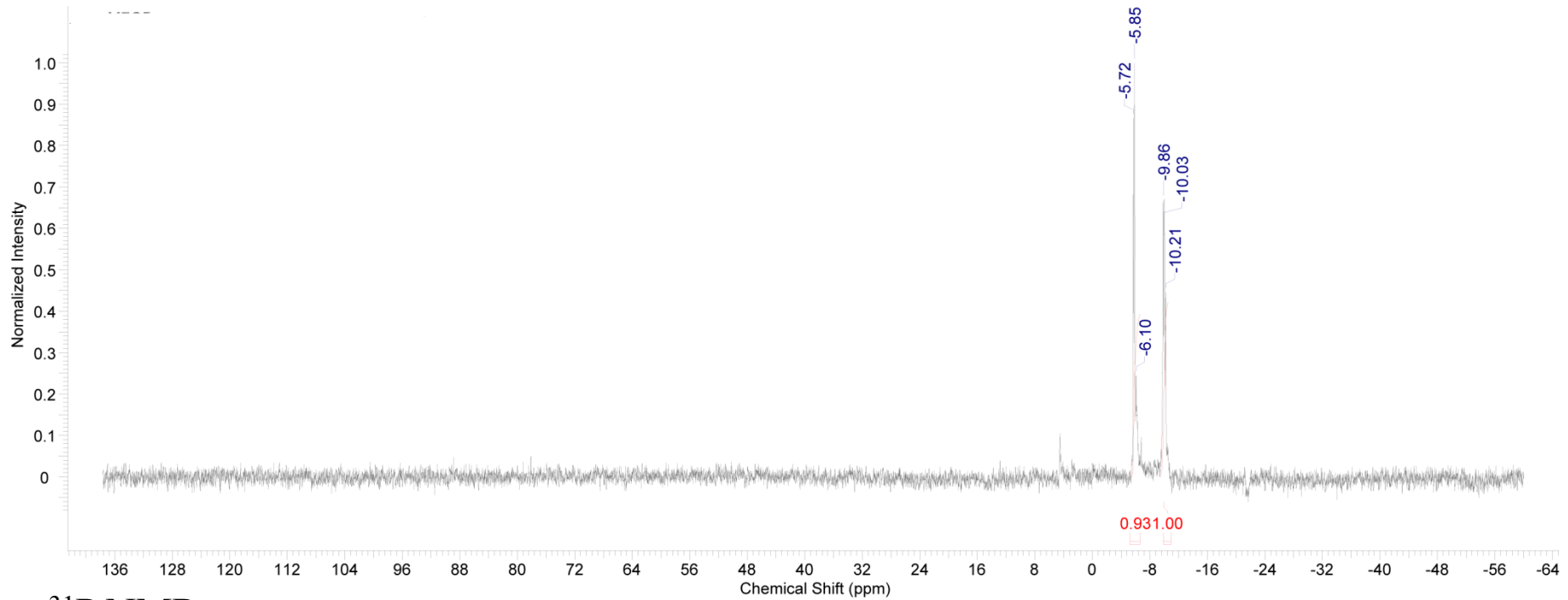
Ant-m⁷GDP

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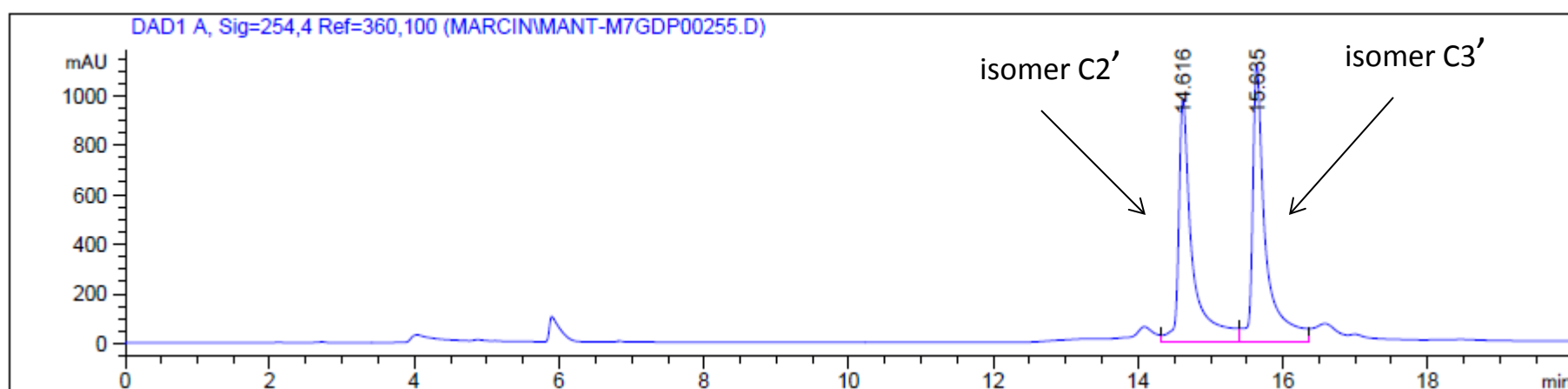
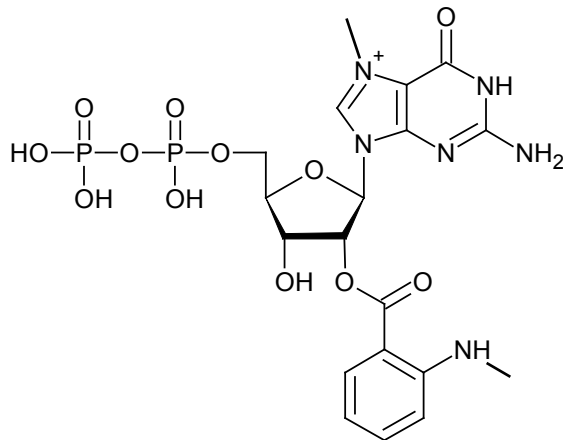
Ant-m⁷GDP

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³¹P NMR

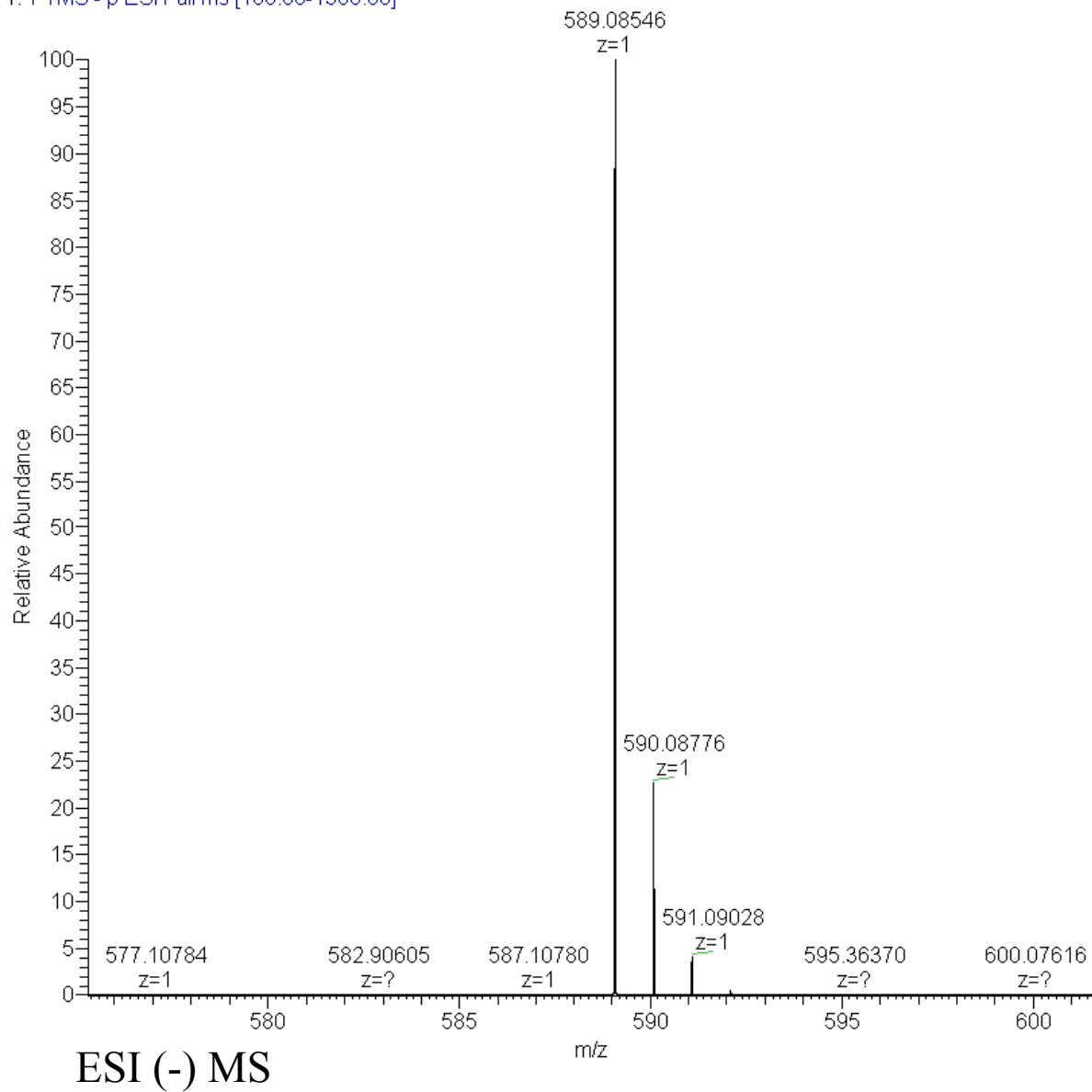
Mant-m⁷GDP



HPLC profile

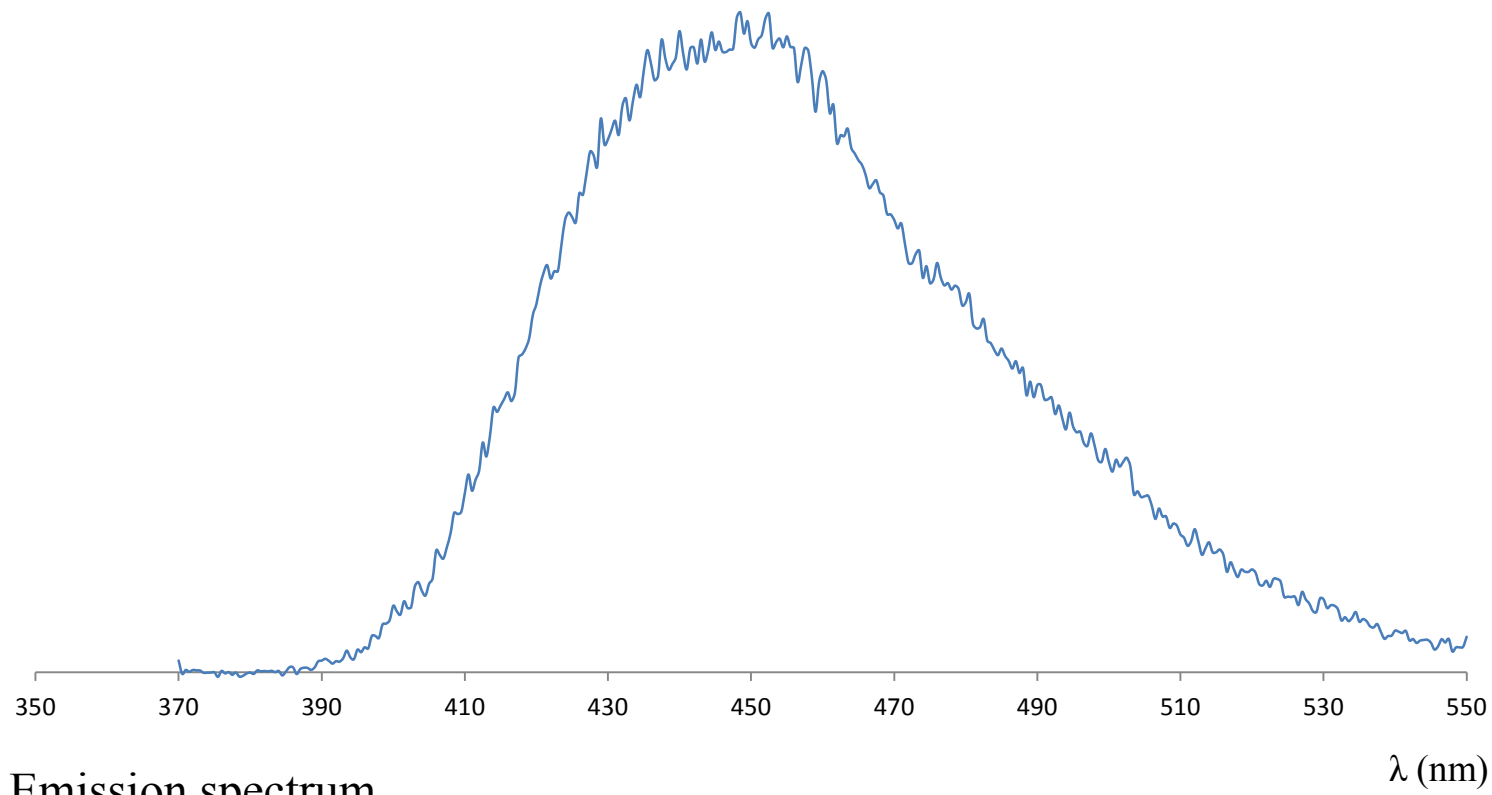
Mant-m⁷GDP

11229mant_m7gdp #16-63 RT: 0.28-1.13 AV: 48 NL: 3.98E5
T: FTMS - p ESI Full ms [100.00-1300.00]



Mant-m⁷GDP

Ziemniak et al.
Synthesis and evaluation of fluorescent
cap analogues for mRNA labelling
supplementary information

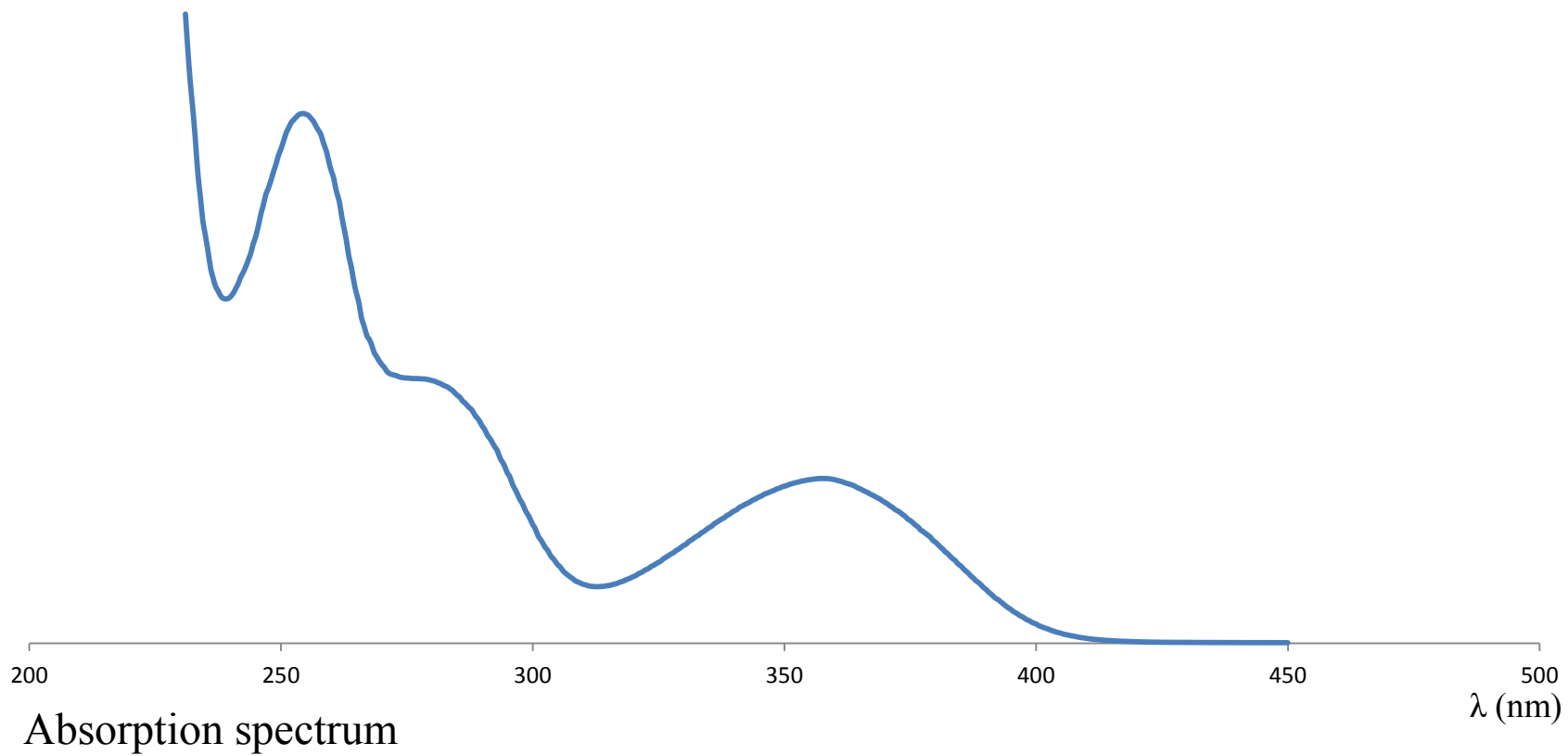


Emission spectrum

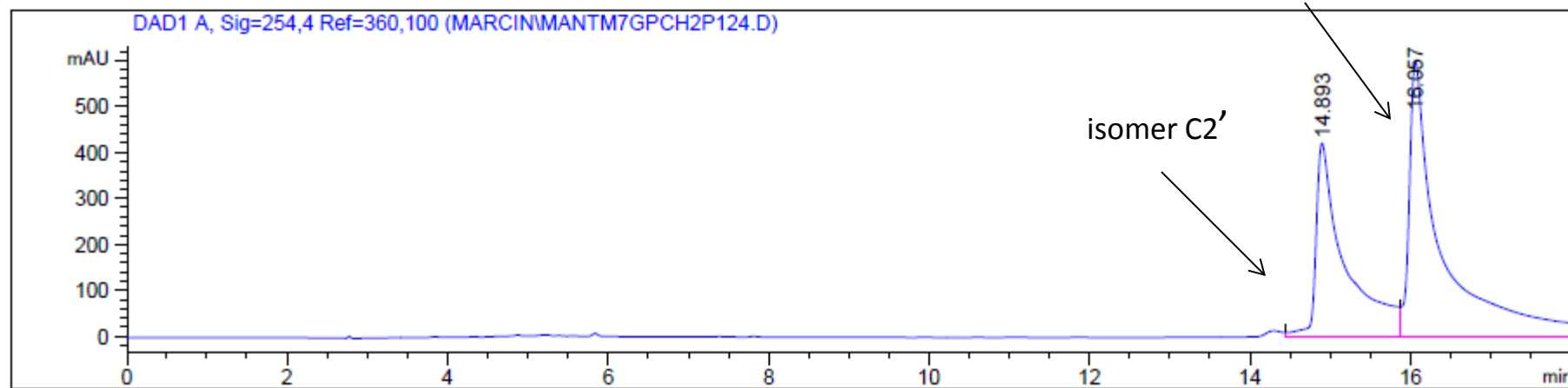
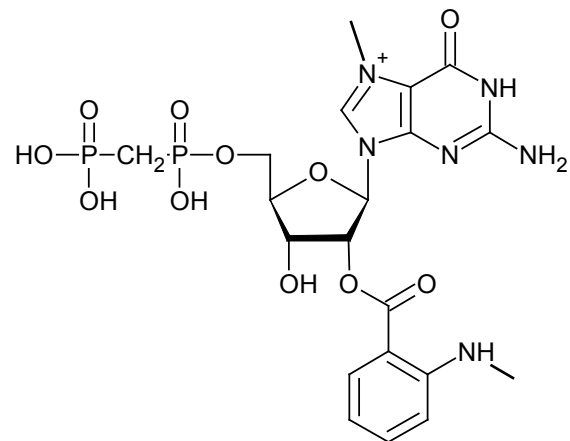
λ (nm)

Mant-m⁷GDP

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supplementary information



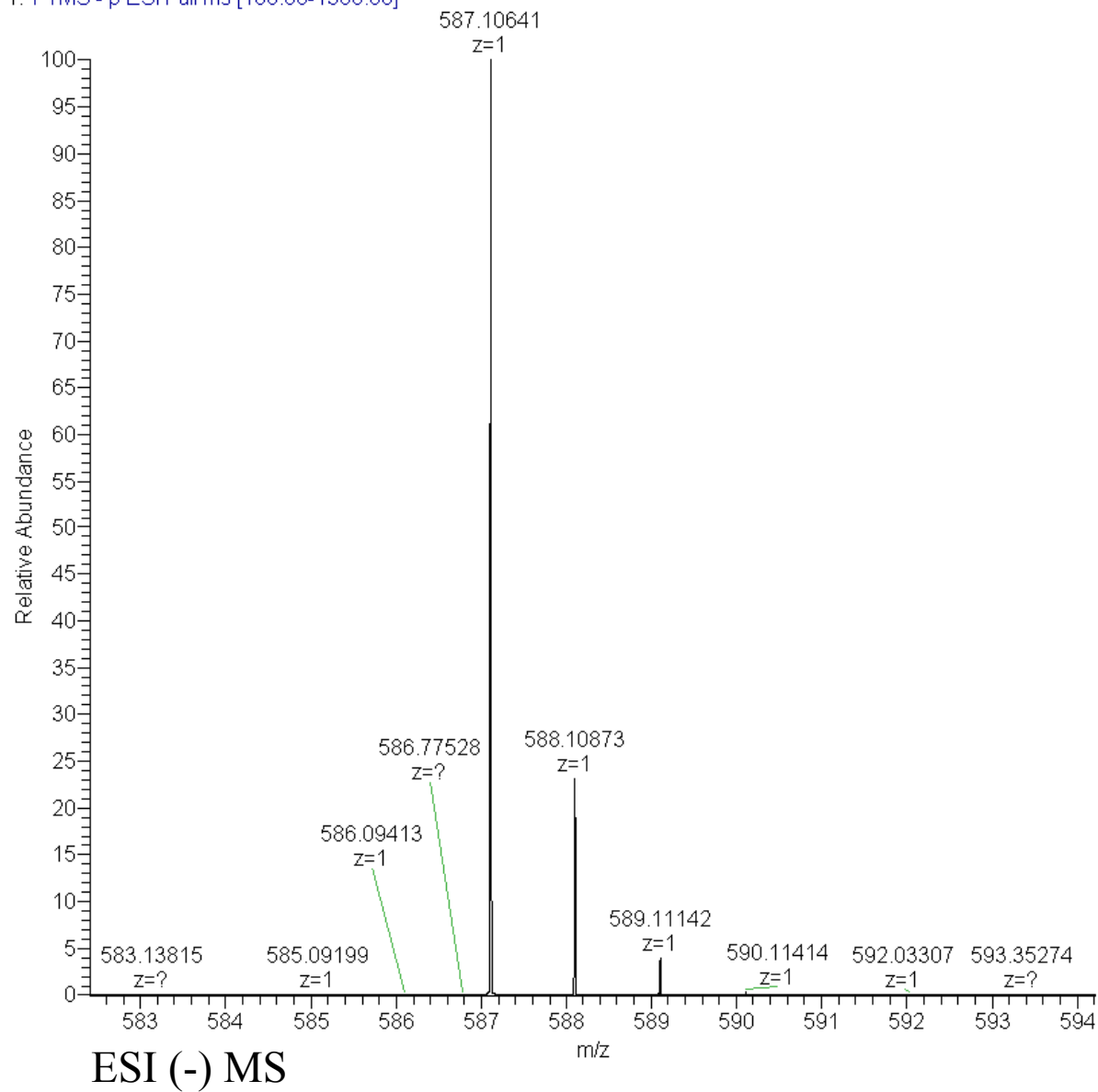
Mant-m⁷GpCH₂p



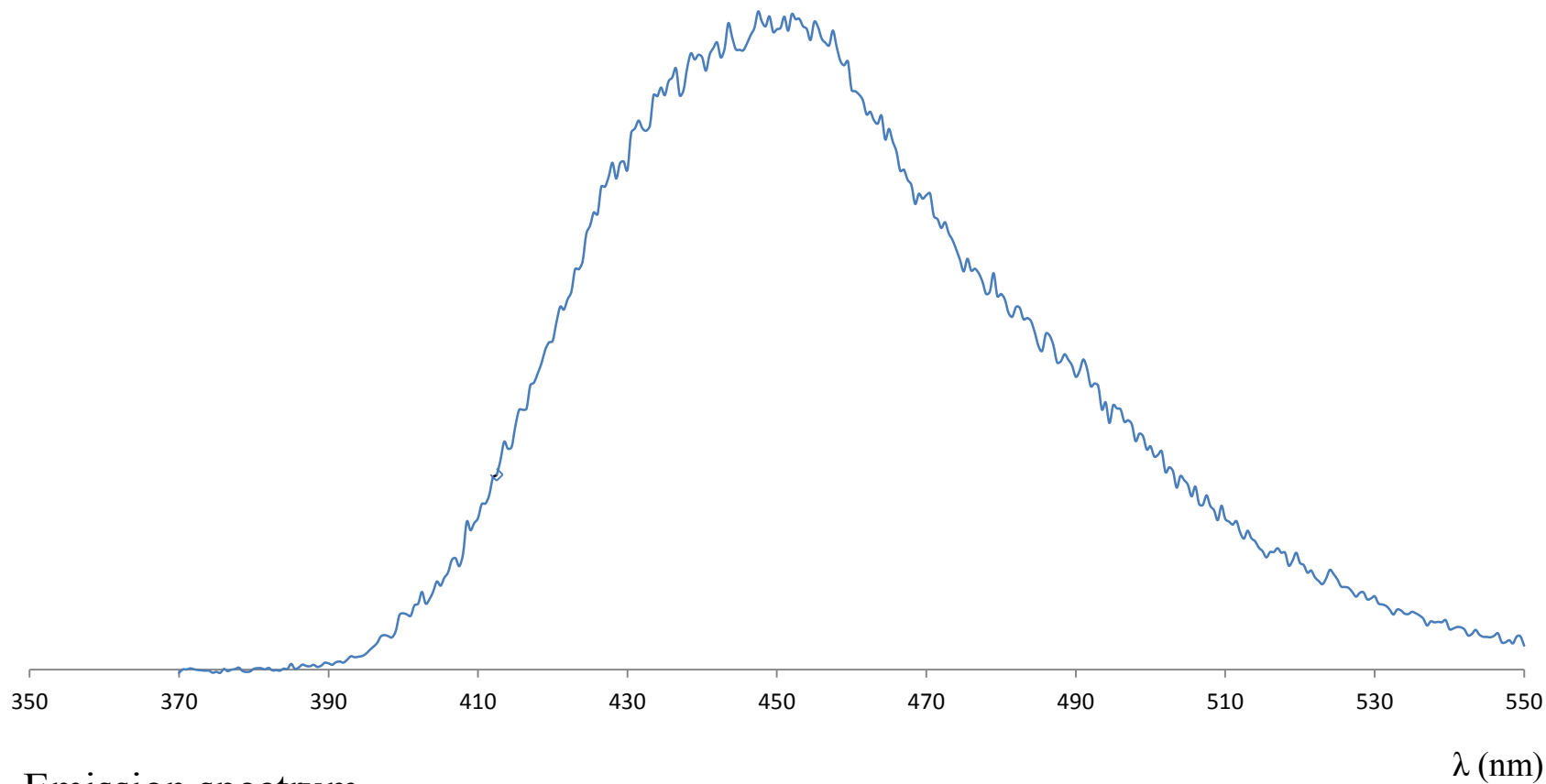
HPLC profile

Mant- m^7 GpCH₂p

11229mant_m7gpch2p #32-96 RT: 0.57-1.72 AV: 65 NL: 3.56E5
T: FTMS - p ESI Full ms [100.00-1300.00]



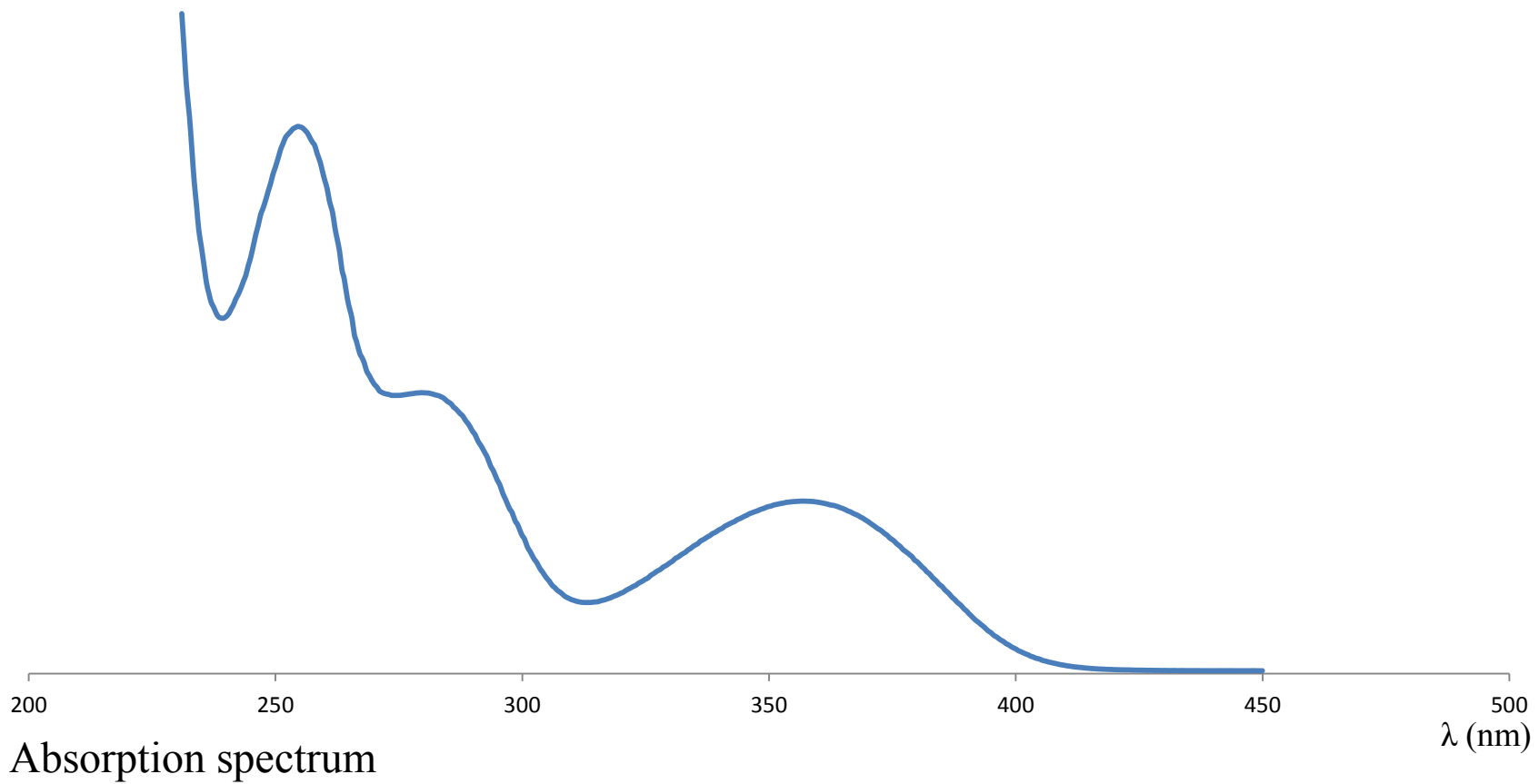
Mant-m⁷GpCH₂p

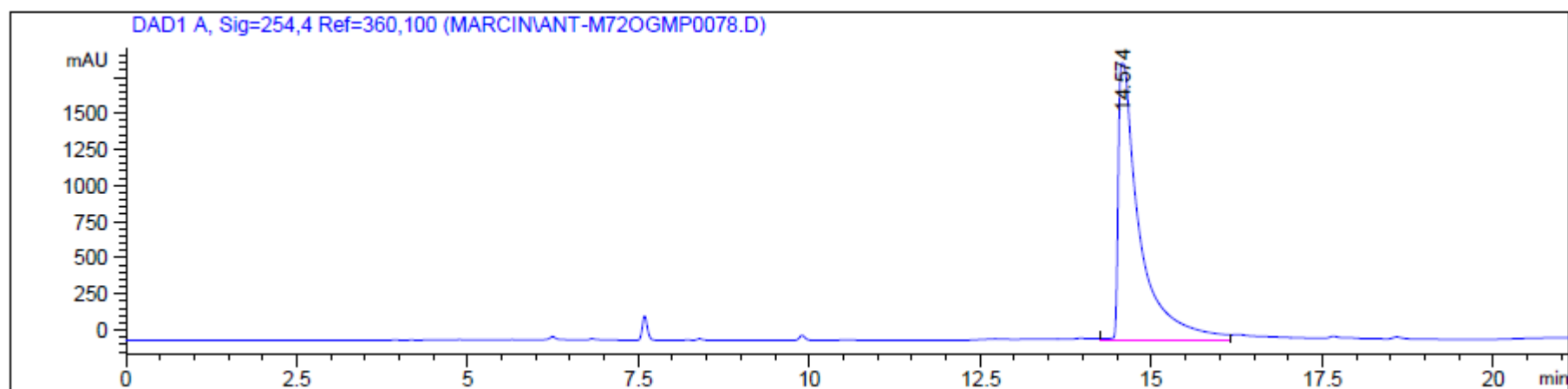
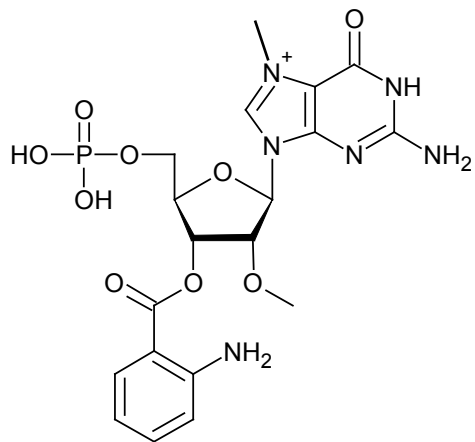


Emission spectrum

λ (nm)

Mant-m⁷GpCH₂p

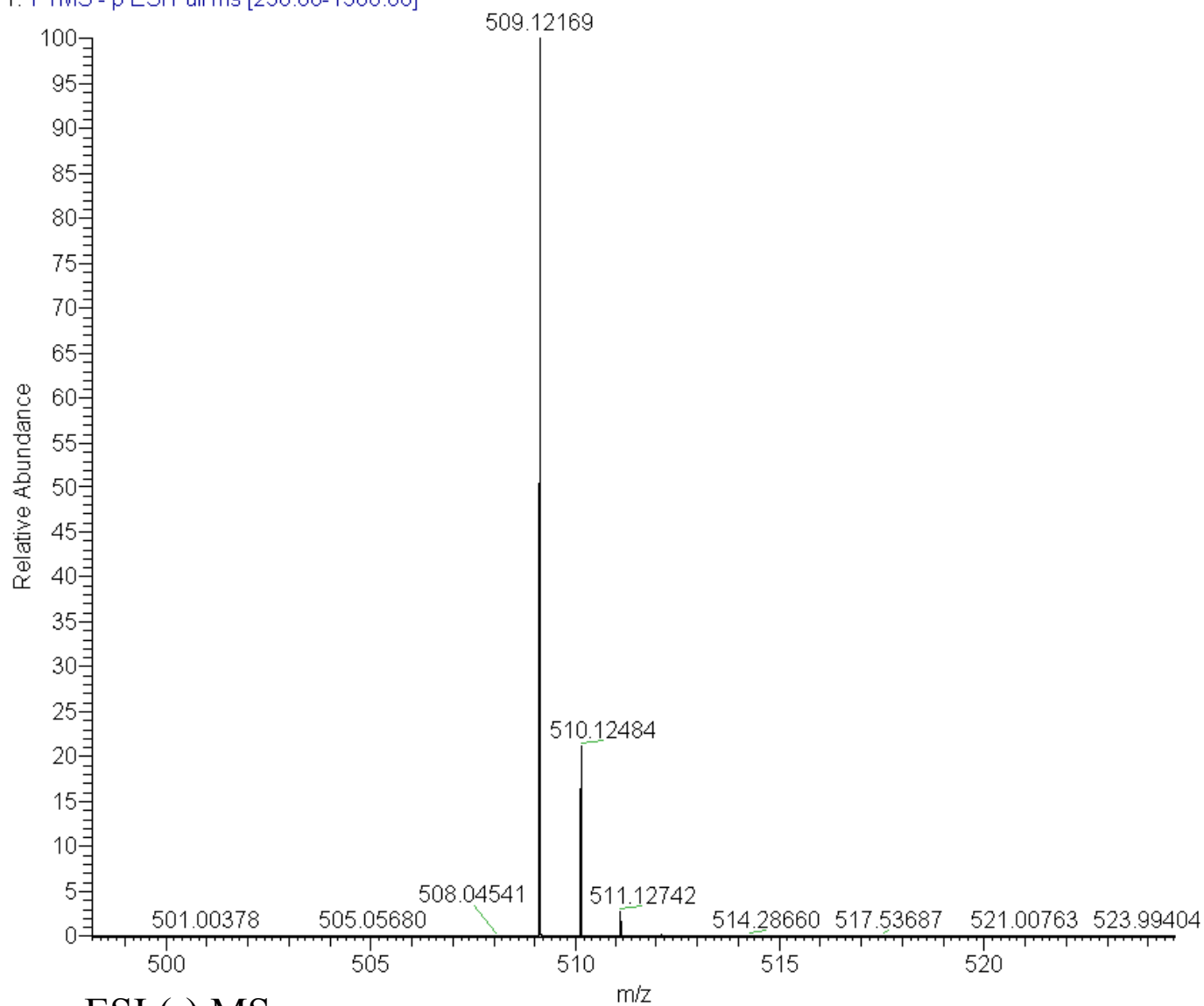


Ant-m₂^{7,2'-O}GMP

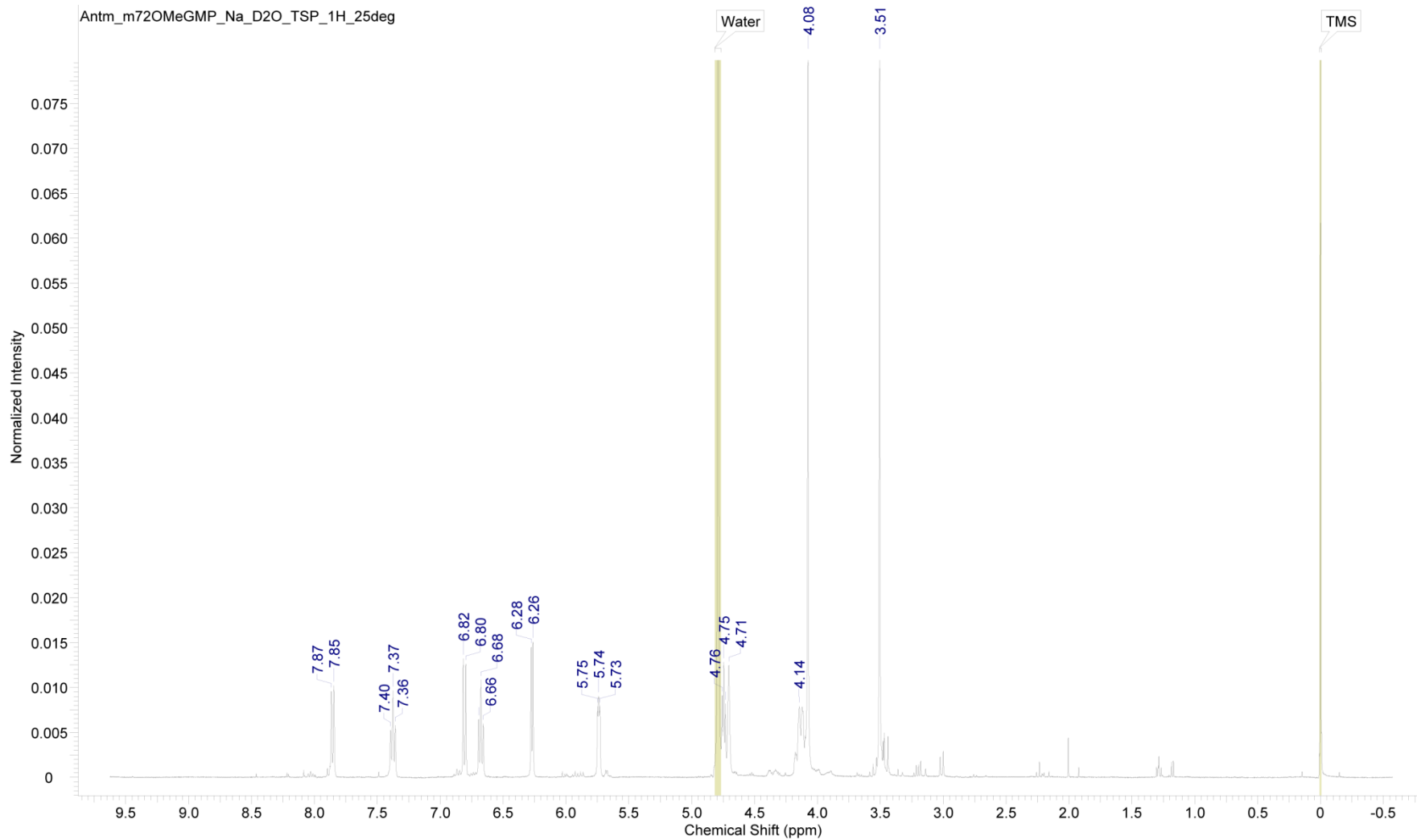
HPLC profile

Ant-m₂^{7,2'-O}GMP

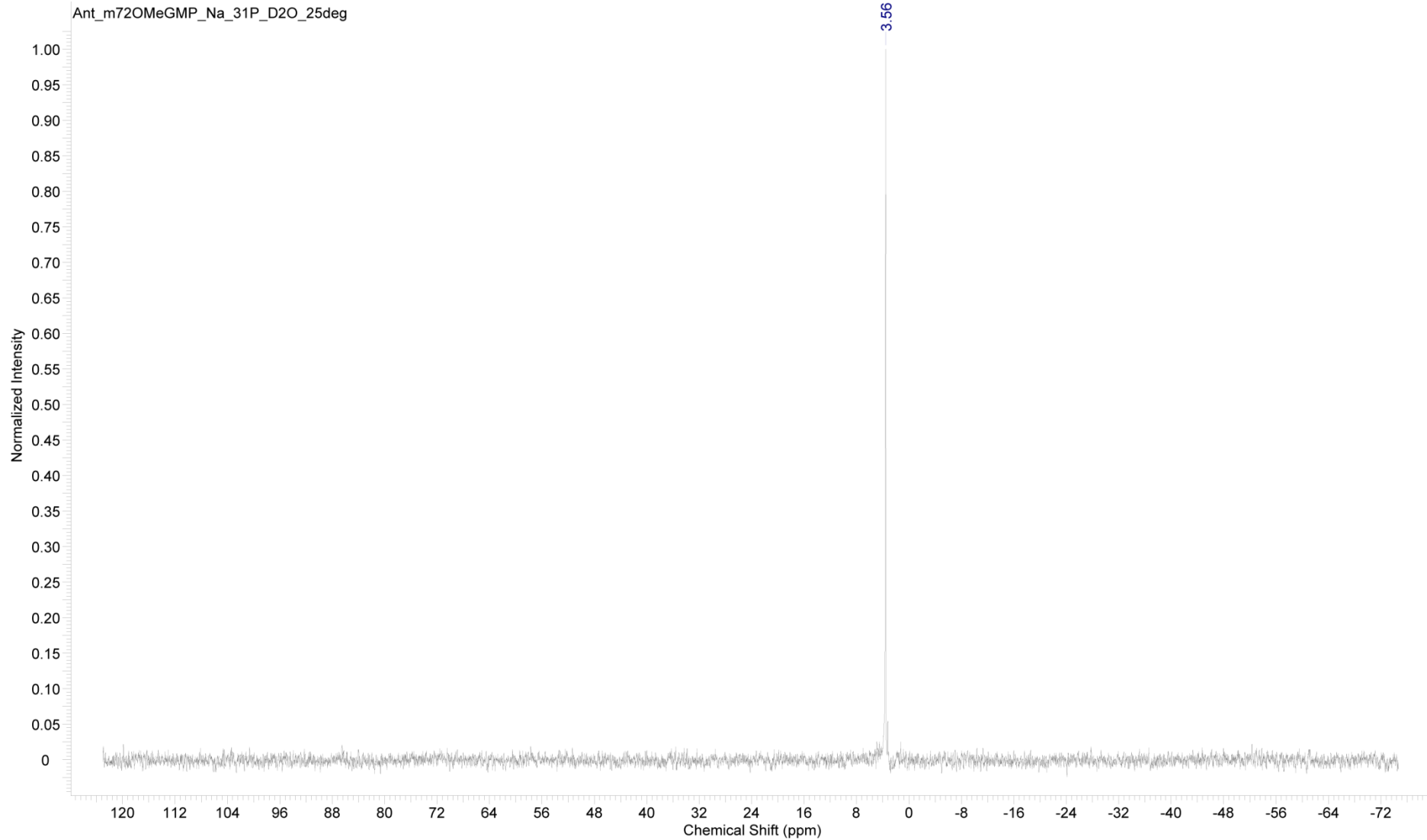
130312_mz_001 #24-45 RT: 0.37-0.70 AV: 22 NL: 2.51E6
T: FTMS - p ESI Full ms [250.00-1500.00]



ESI (-) MS

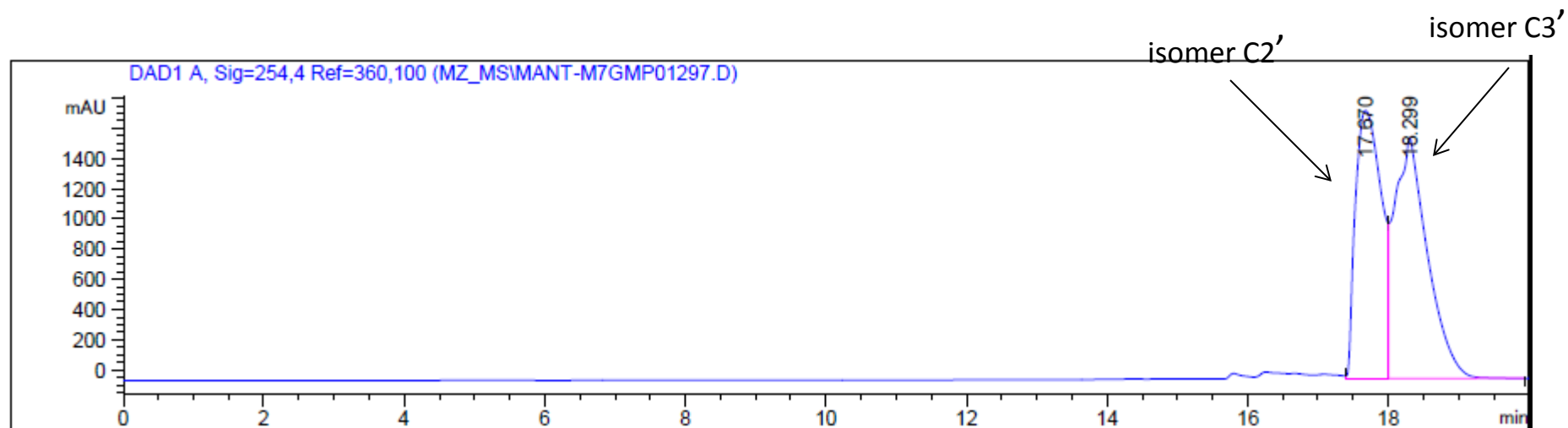
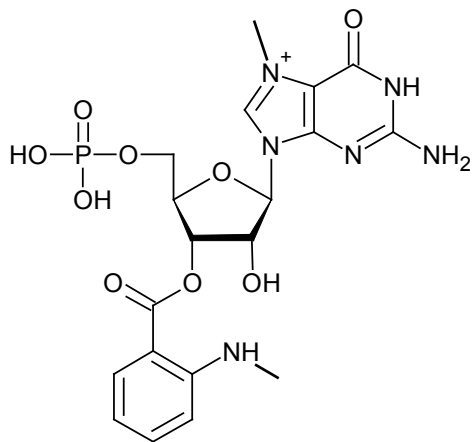
Ant-m₂^{7,2'-O}GMP¹H NMR

Ant-m₂^{7,2'-O}GMP



³¹P NMR

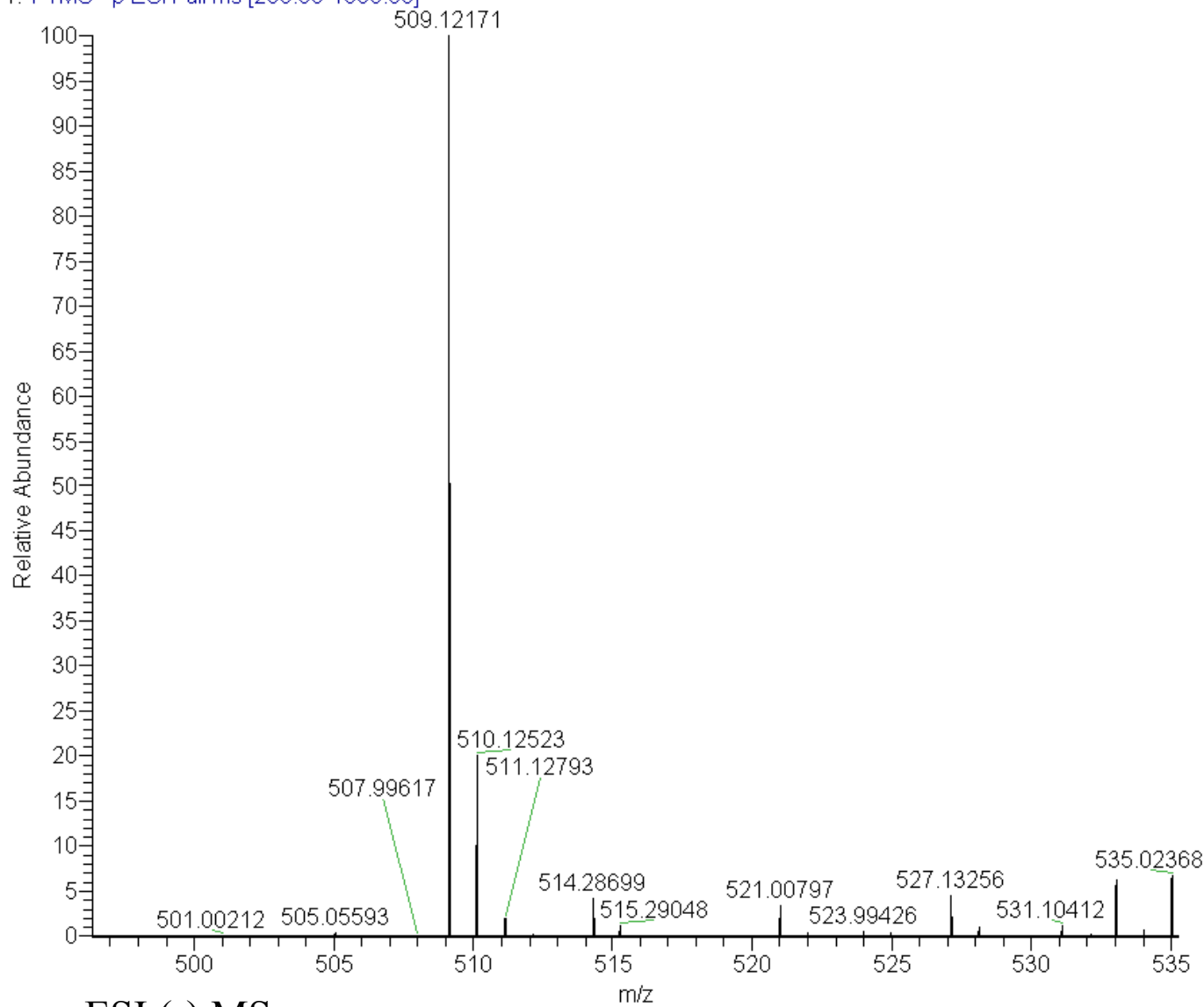
Mant-m⁷GMP



HPLC profile

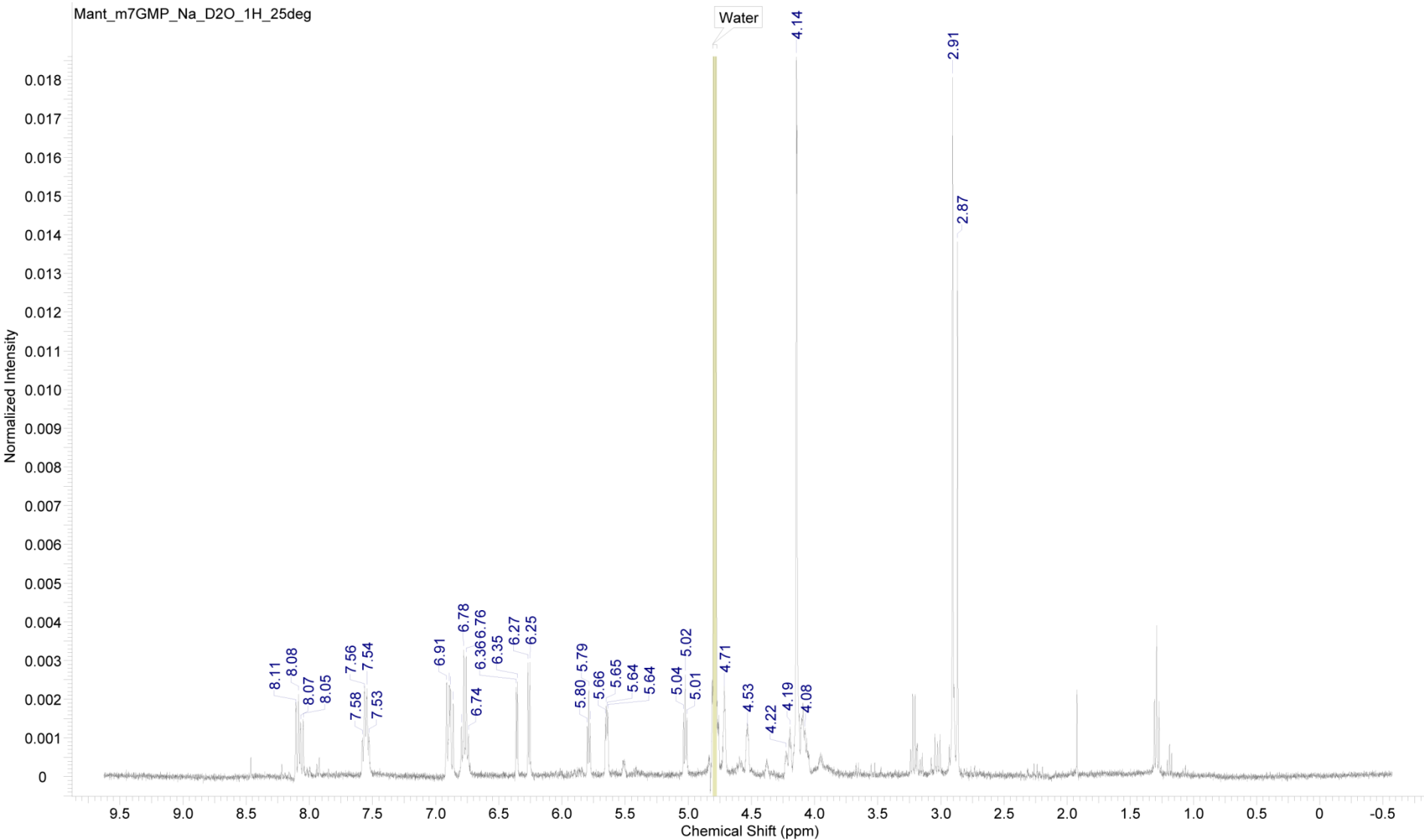
Mant-m⁷GMP

130312_mz_002 #2-26 RT: 0.02-0.38 AV: 25 NL: 5.84E5
T: FTMS - p ESI Full ms [250.00-1500.00]



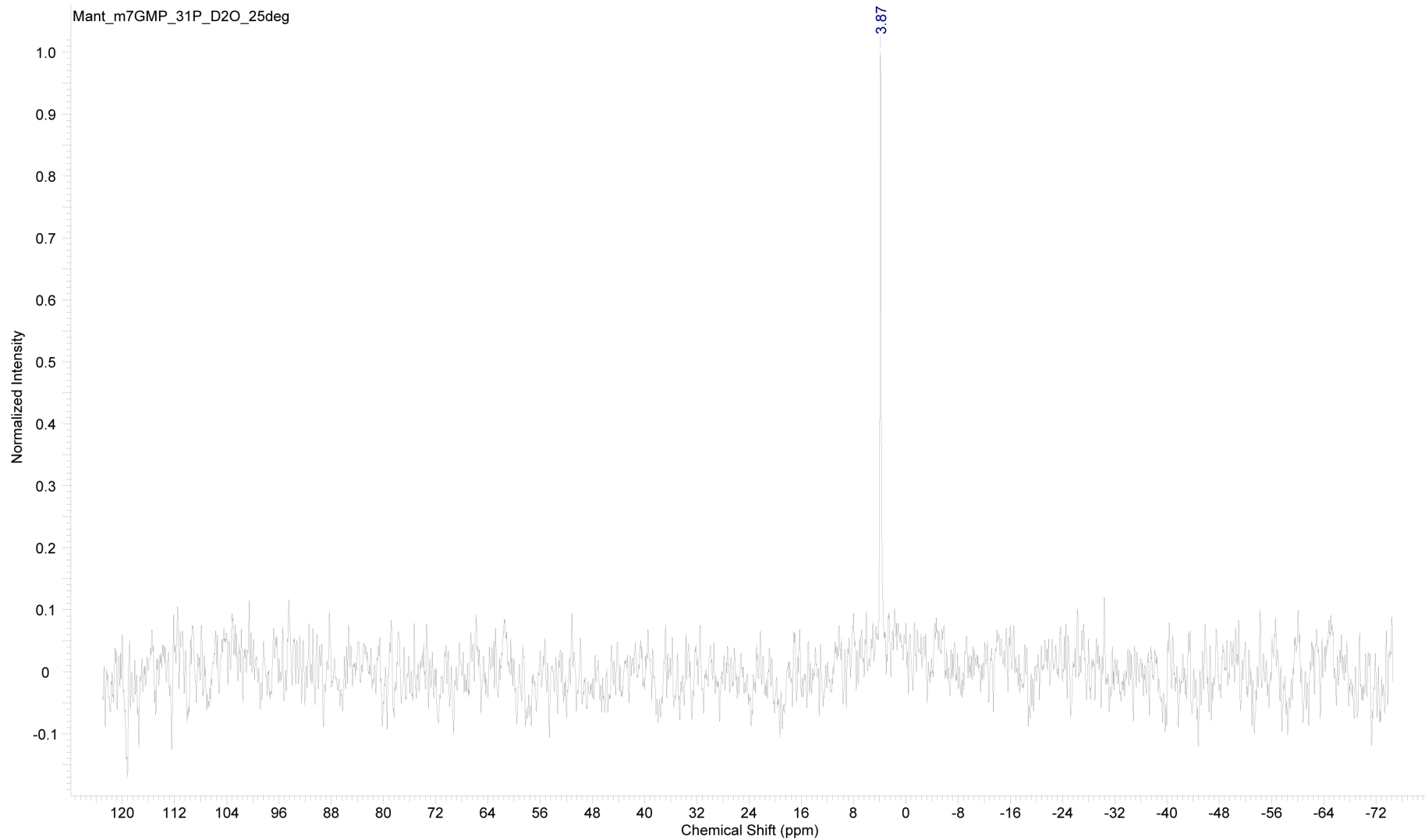
ESI (-) MS

Mant-m⁷GMP



¹H NMR

Mant-m⁷GMP



³¹P NMR