

# 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*-methylanthranilate in phosphate buffer and organic solvents.

compound	Solution	Absorption		Fluorescence		
		$\lambda_{\max}$ (nm)	$\epsilon_{\lambda_{\max}}$ ( $M^{-1}cm^{-1}$ )	$\lambda_{\max}$ (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 $\chi_R^2 = 0.971$

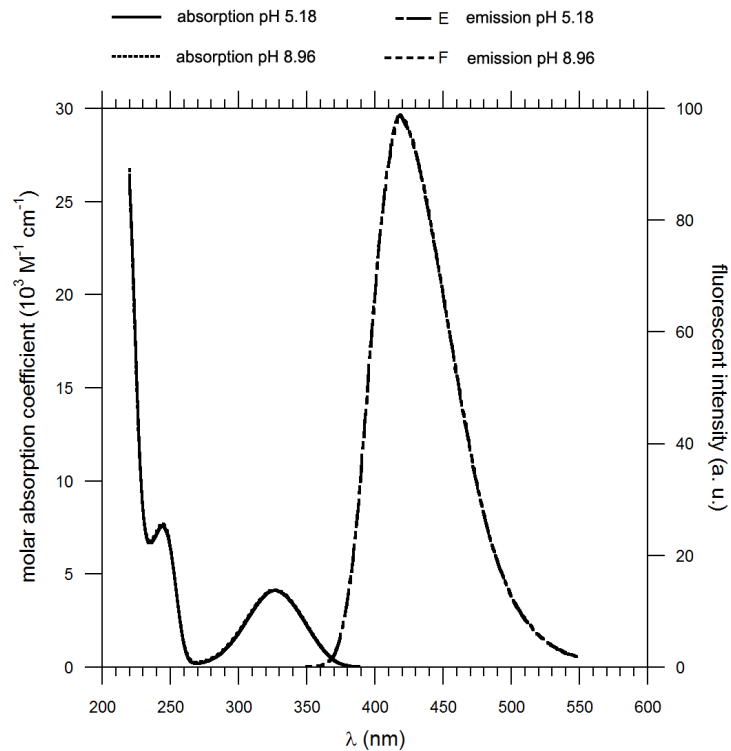


Fig. S1. Absorbance and emission spectra of AntOMe

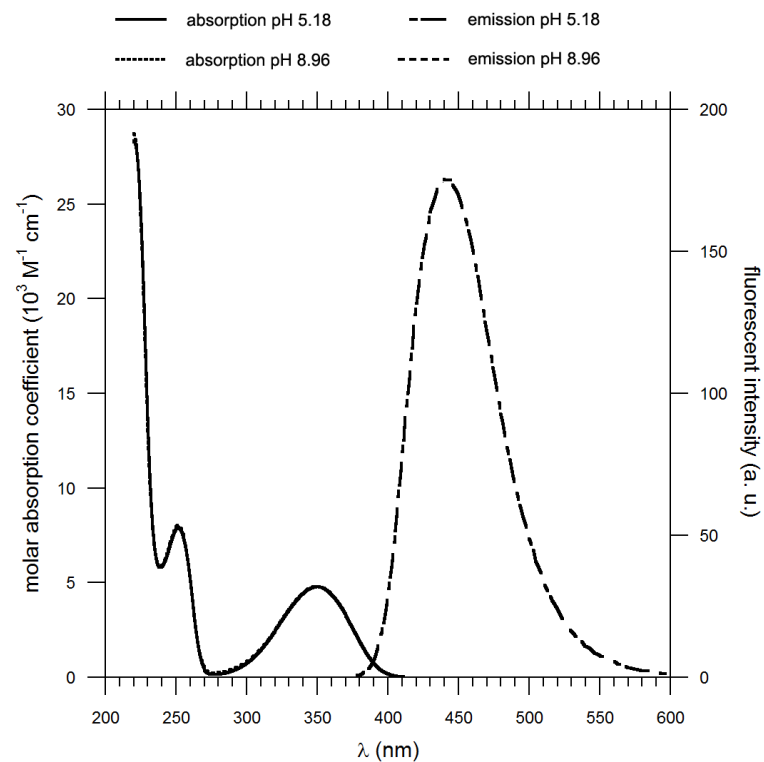


Fig. S2. Absorbance and emission spectra of MantOMe

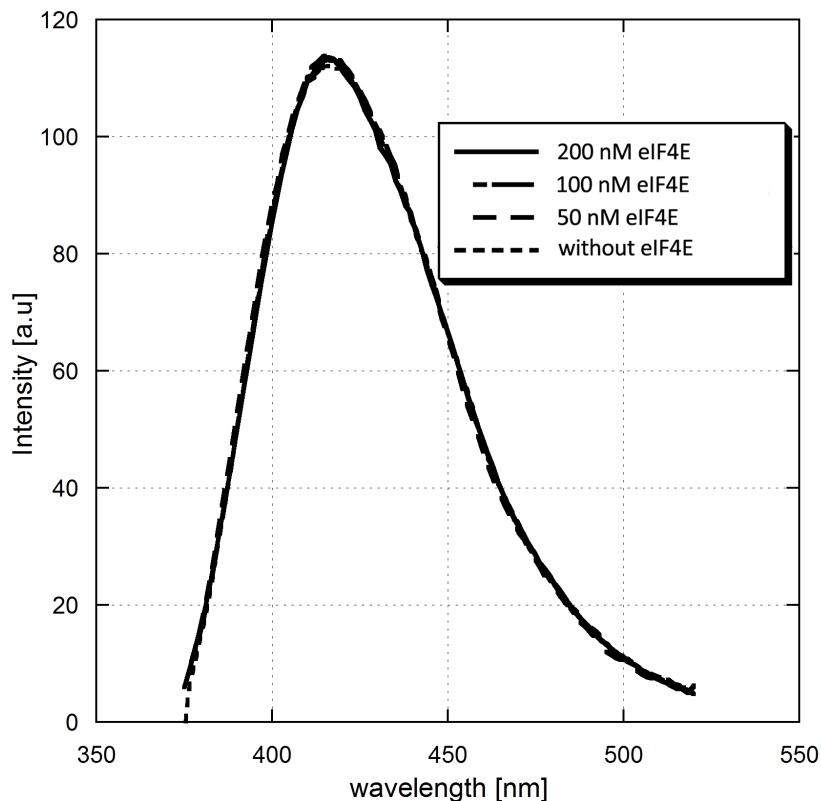


Fig. S3. MantOME fluorescence intensity observed upon addition of increasing amounts of eIF4E protein. The concentration of MantOME was 200 nM and the eIF4E concentrations were in range 50-1000 nM.

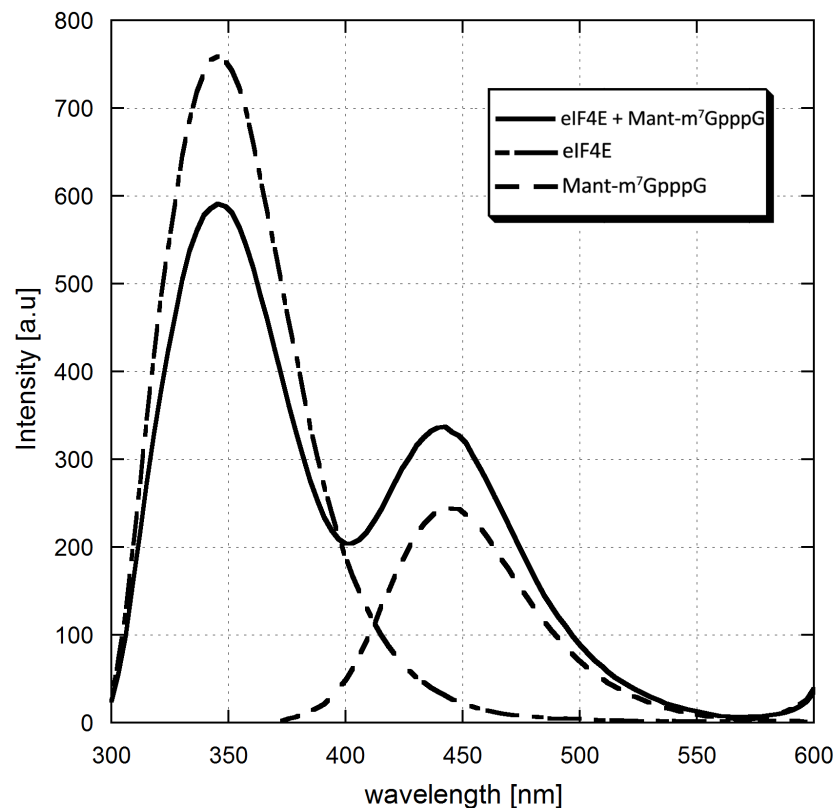


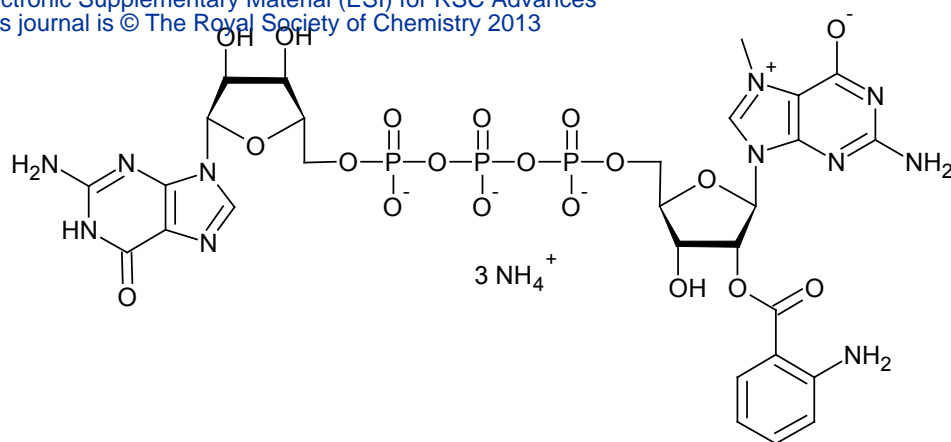
Fig. S4. Changes of fluorescence intensity of Mant-m<sup>7</sup>GpppG and Trp residues in eIF4E upon complex formation. The excitation wavelength used in the experiment was 280 nm (maximum absorption for Trp residues). eIF4E concentration was 100 nM and Mant-m<sup>7</sup>GpppG concentration was 200 nM.

**Table S2.**  $^1\text{H}$  NMR signals of 2' and 3' regioisomers of cap analogues **1-2**

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

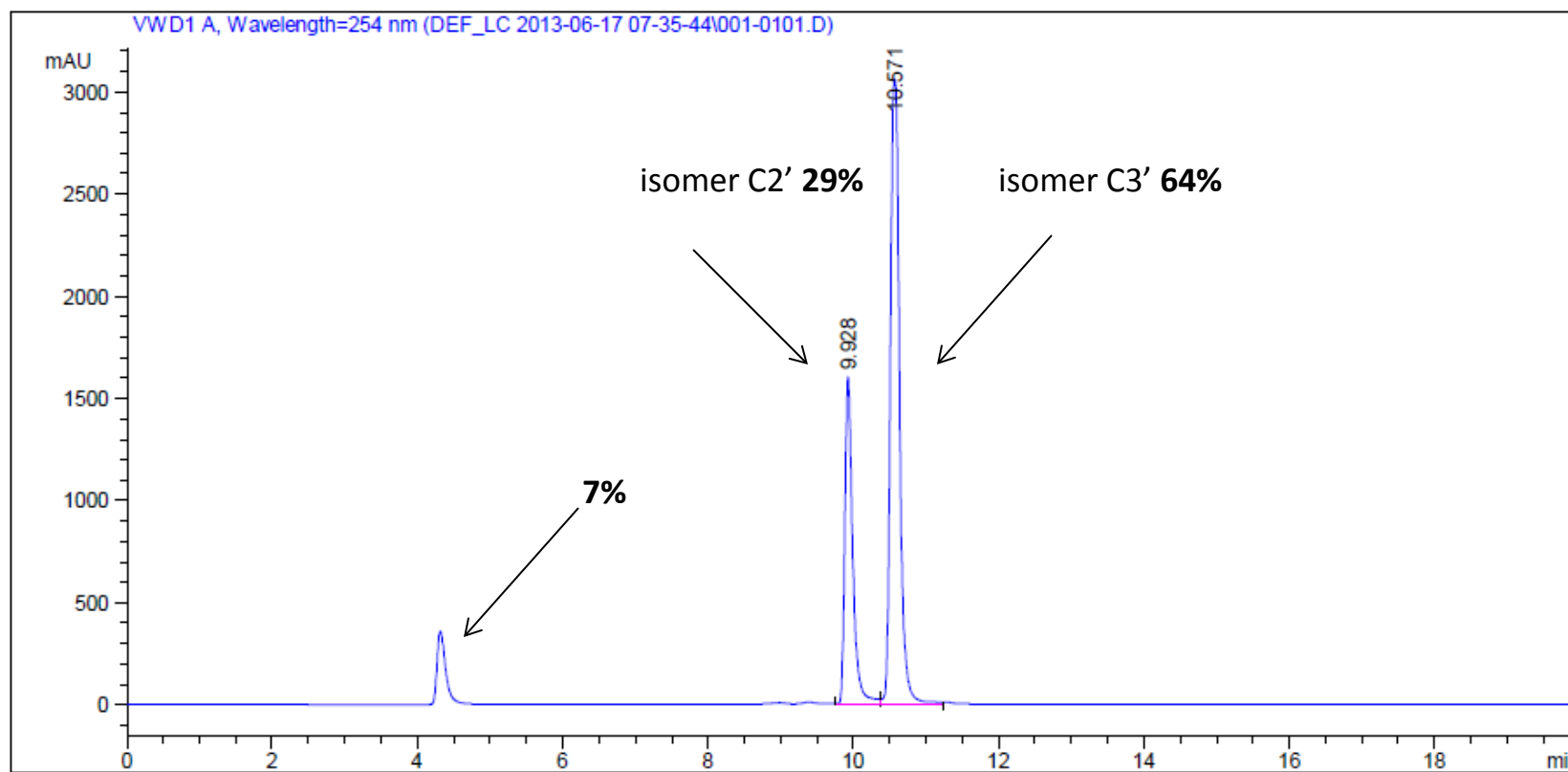
# Table S3 Conformational and regioisomeric equilibria for some (M)Ant labelled nucleotides

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



# Ant-m<sup>7</sup>GpppG

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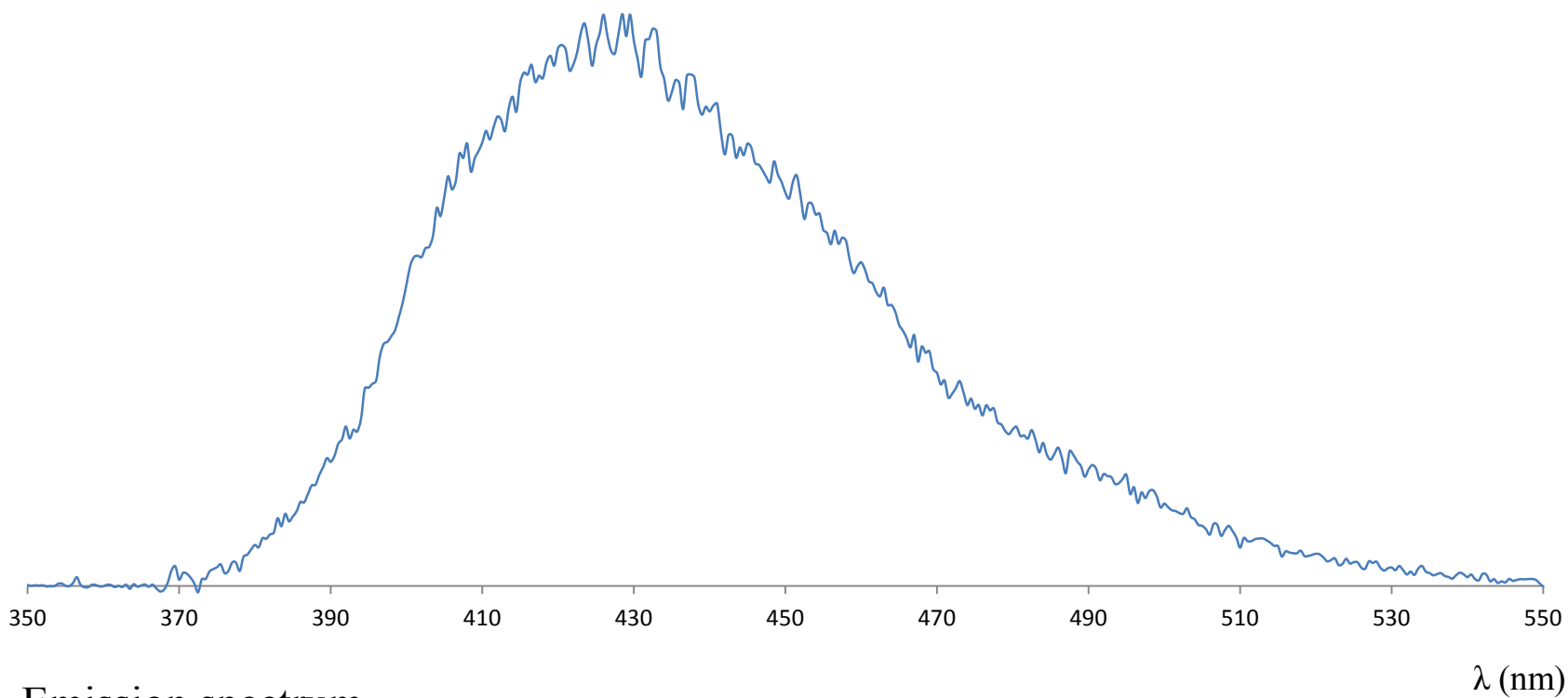


HPLC profile



# Ant-m<sup>7</sup>GpppG

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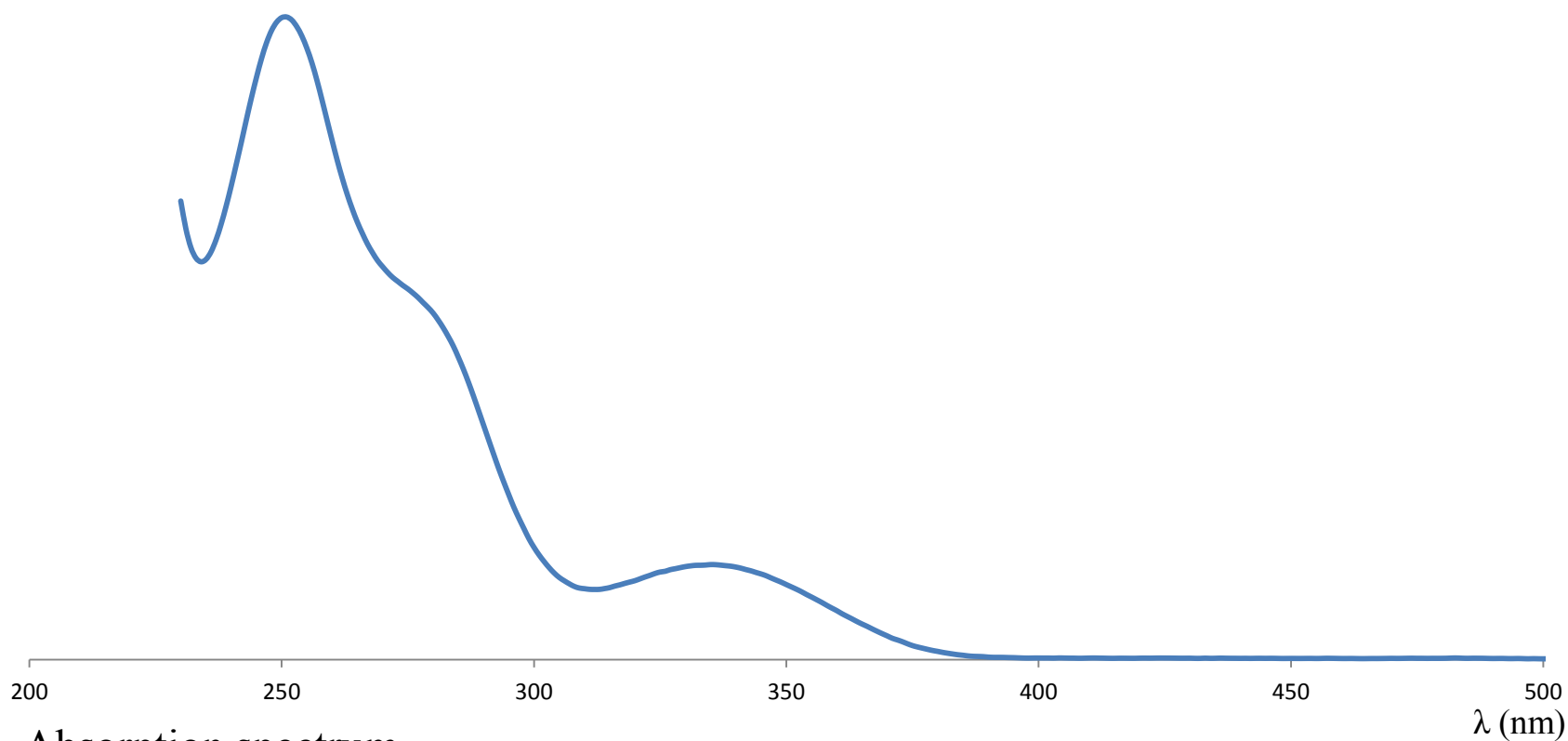


Emission spectrum

λ (nm)

# Ant-m<sup>7</sup>GpppG

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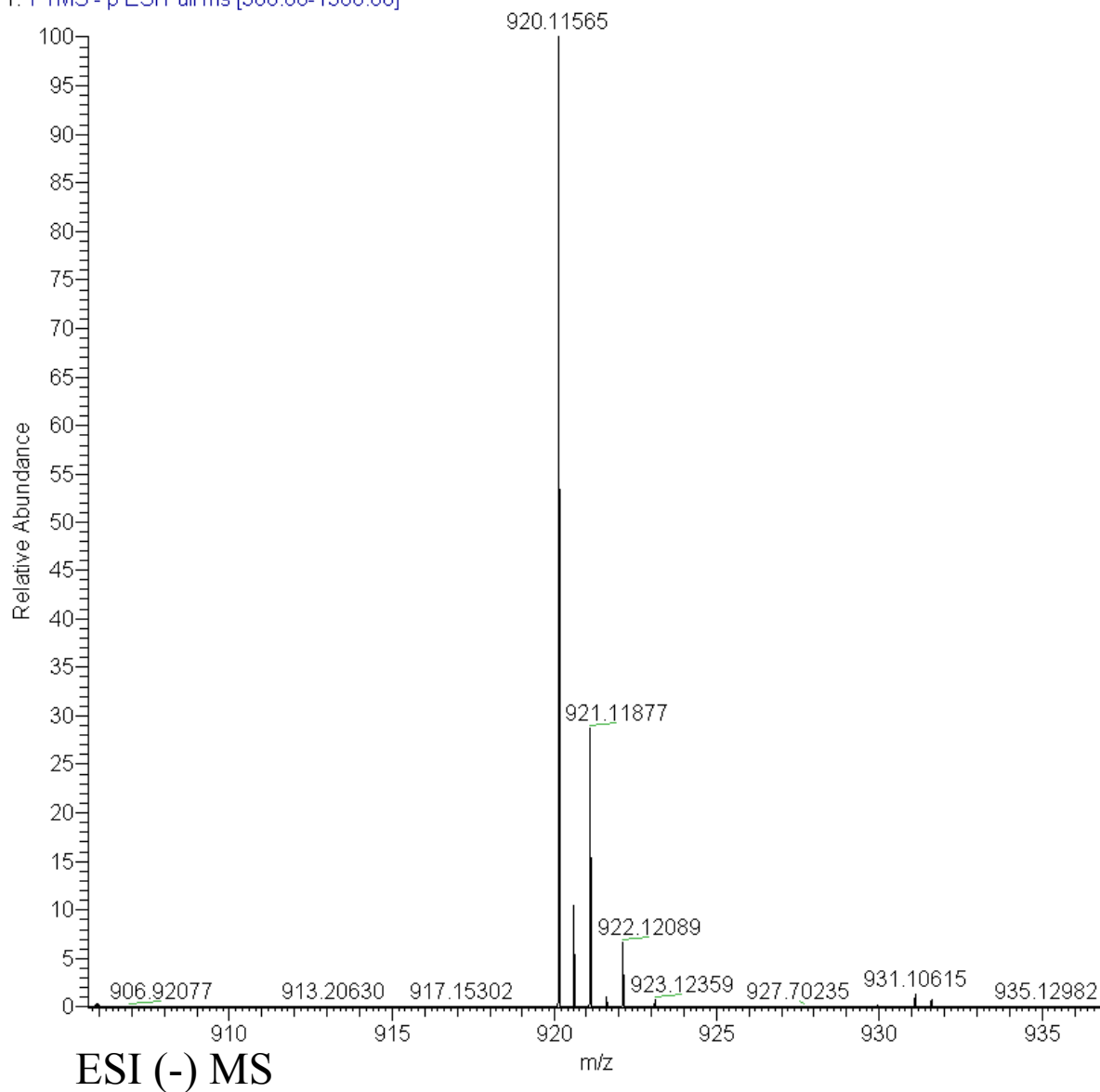


Absorption spectrum

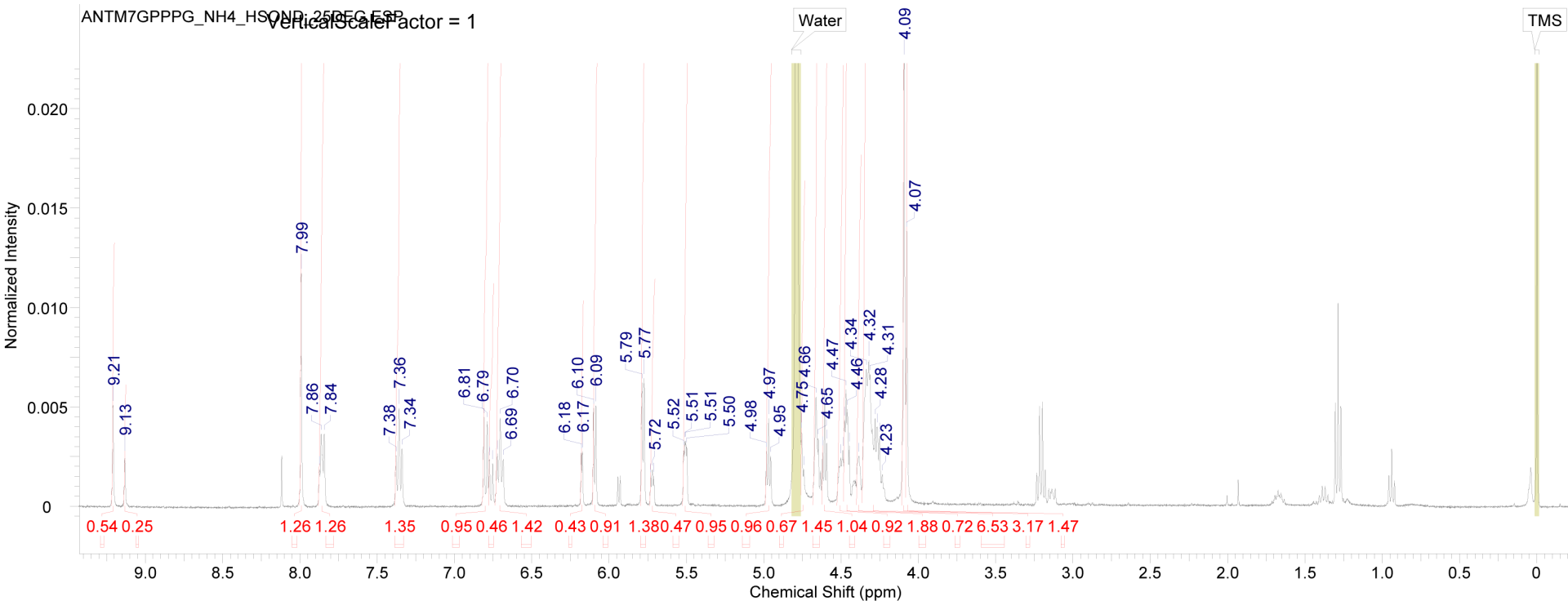
λ (nm)

# Ant-m<sup>7</sup>GpppG

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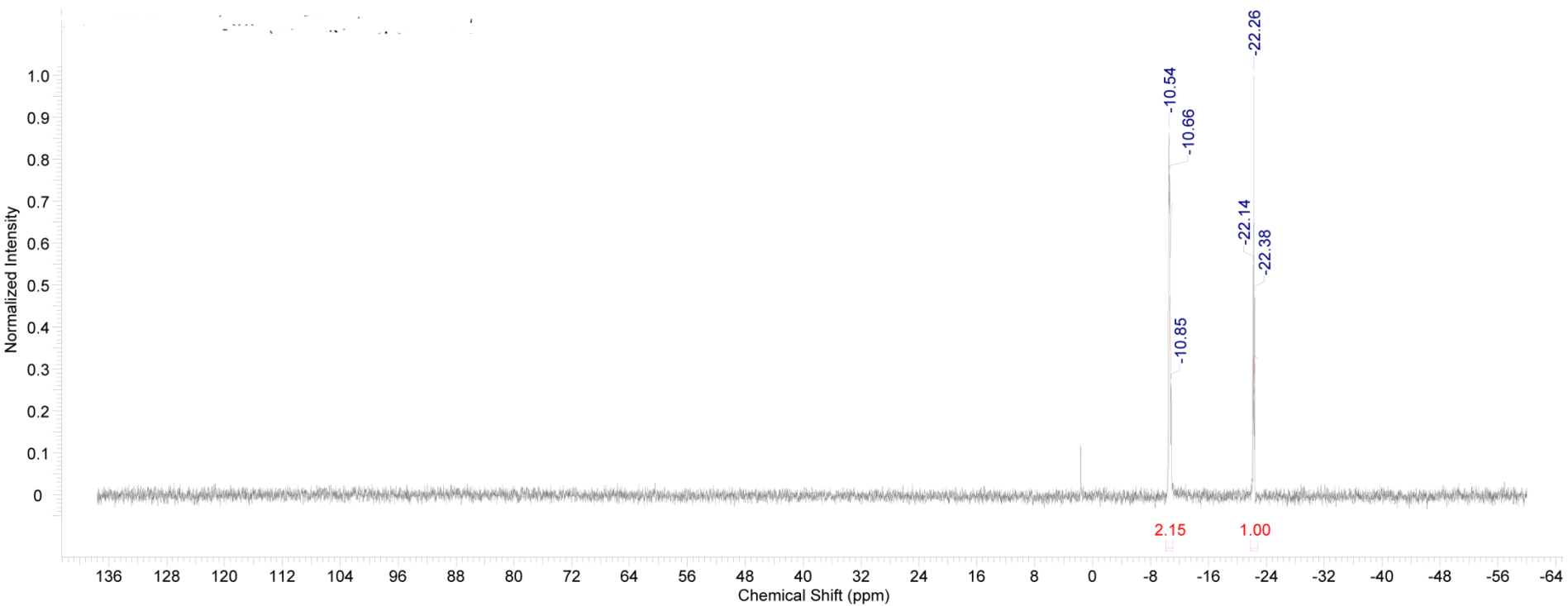
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<sup>1</sup>H NMR

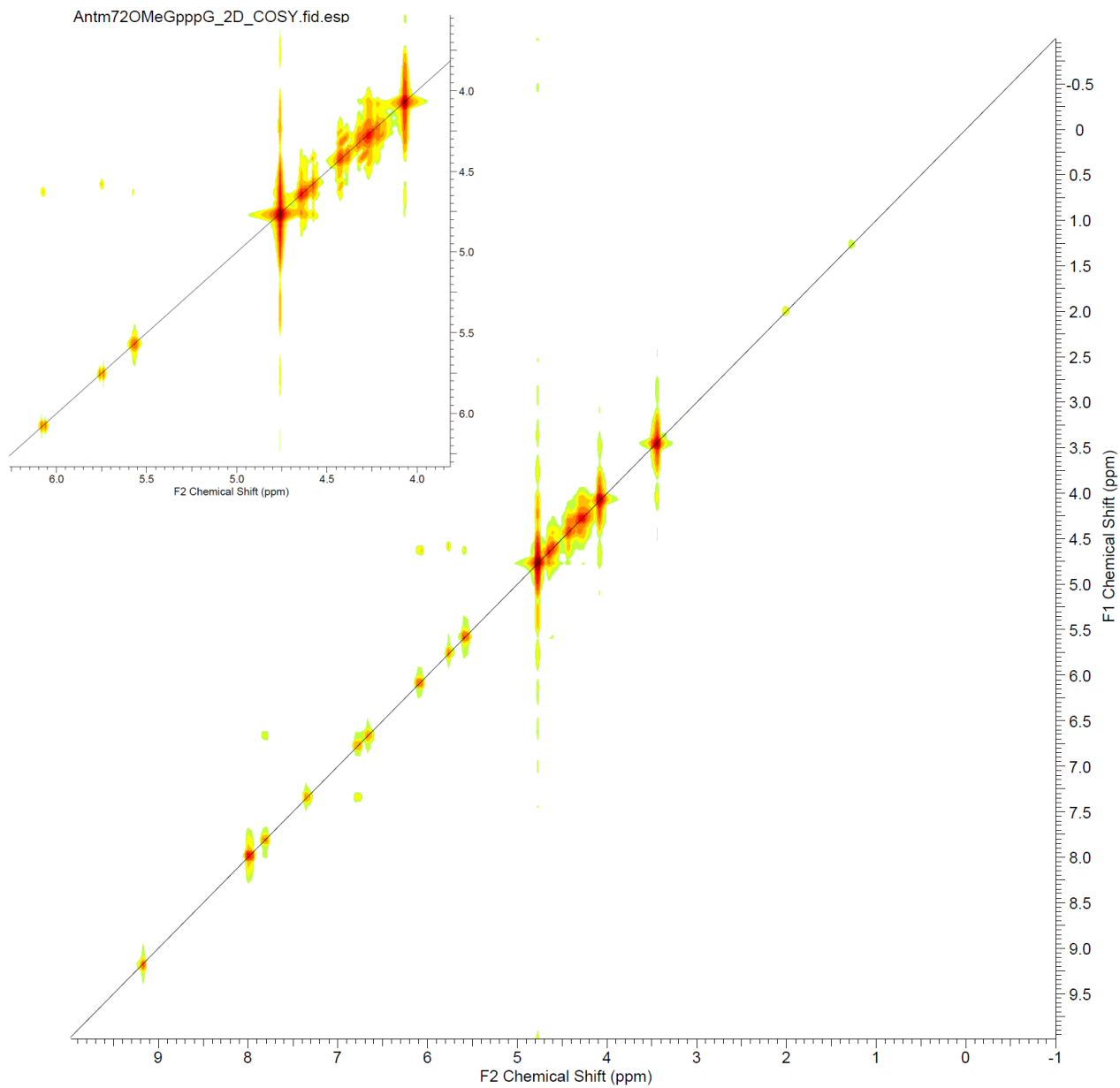
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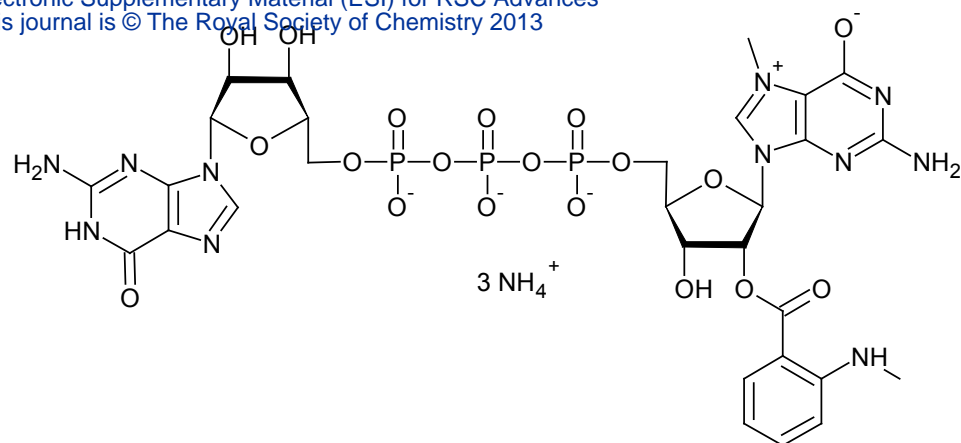
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<sup>31</sup>P NMR

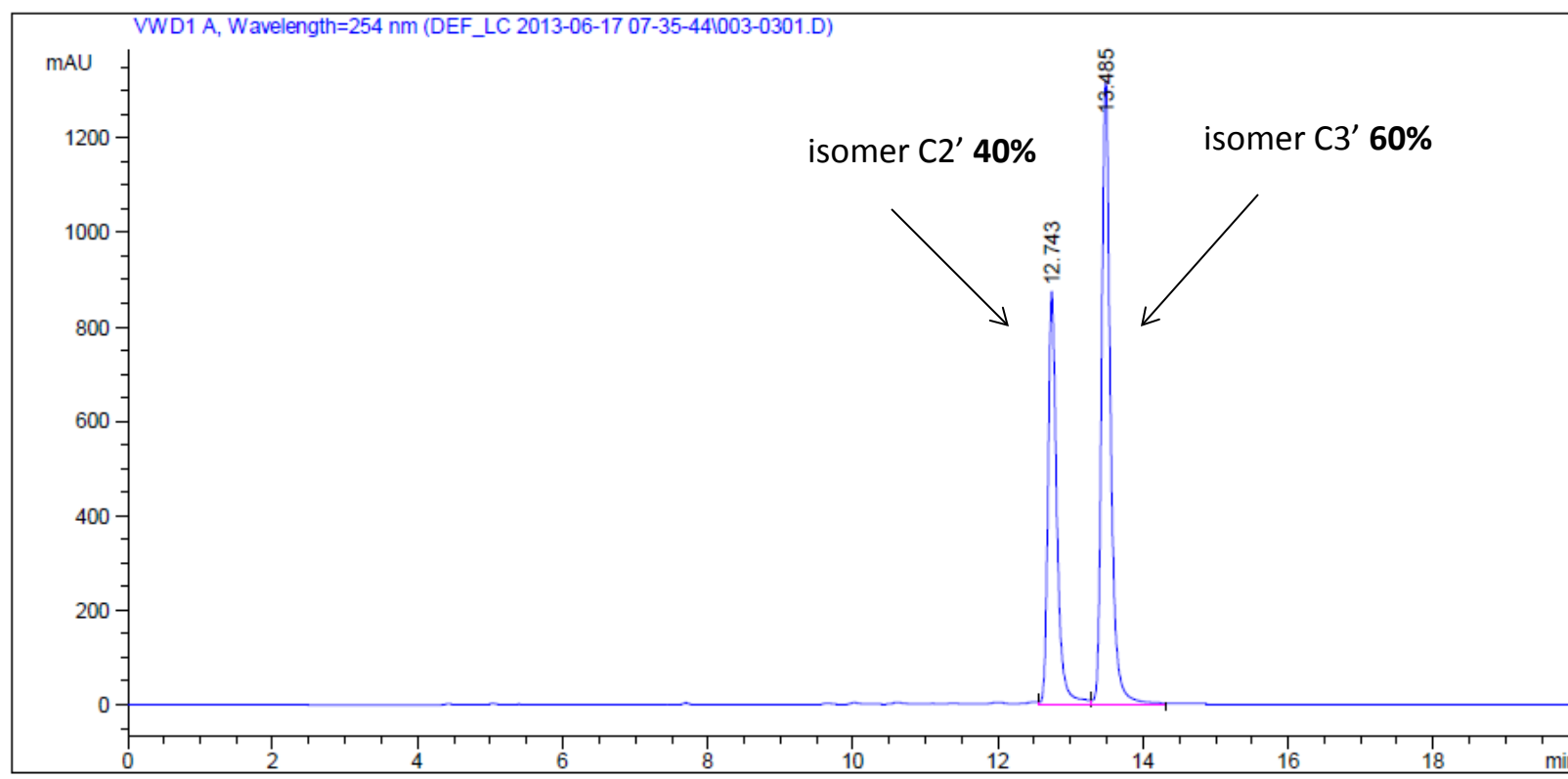
# Ant-m<sup>7</sup>GpppG





# Mant-m<sup>7</sup>GpppG

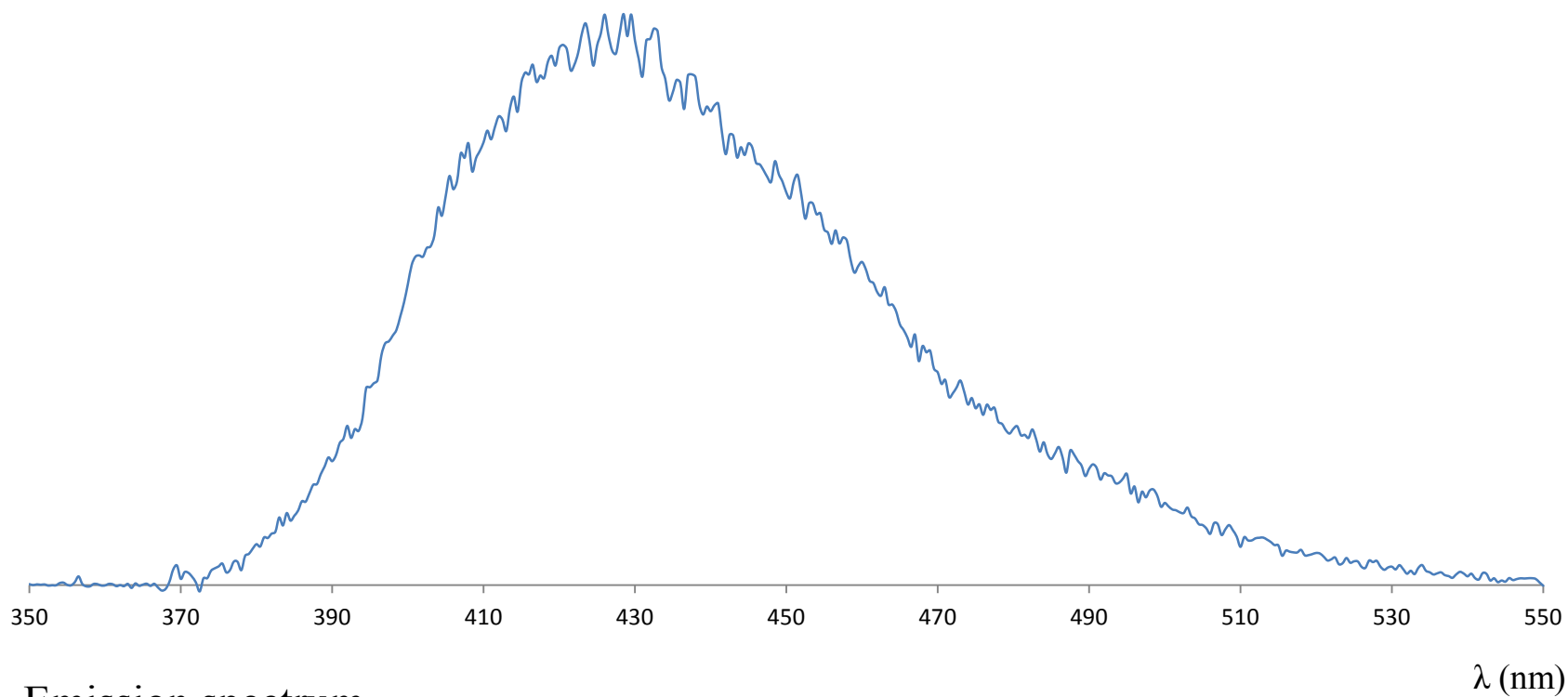
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HPLC profile

# Mant-m<sup>7</sup>GpppG

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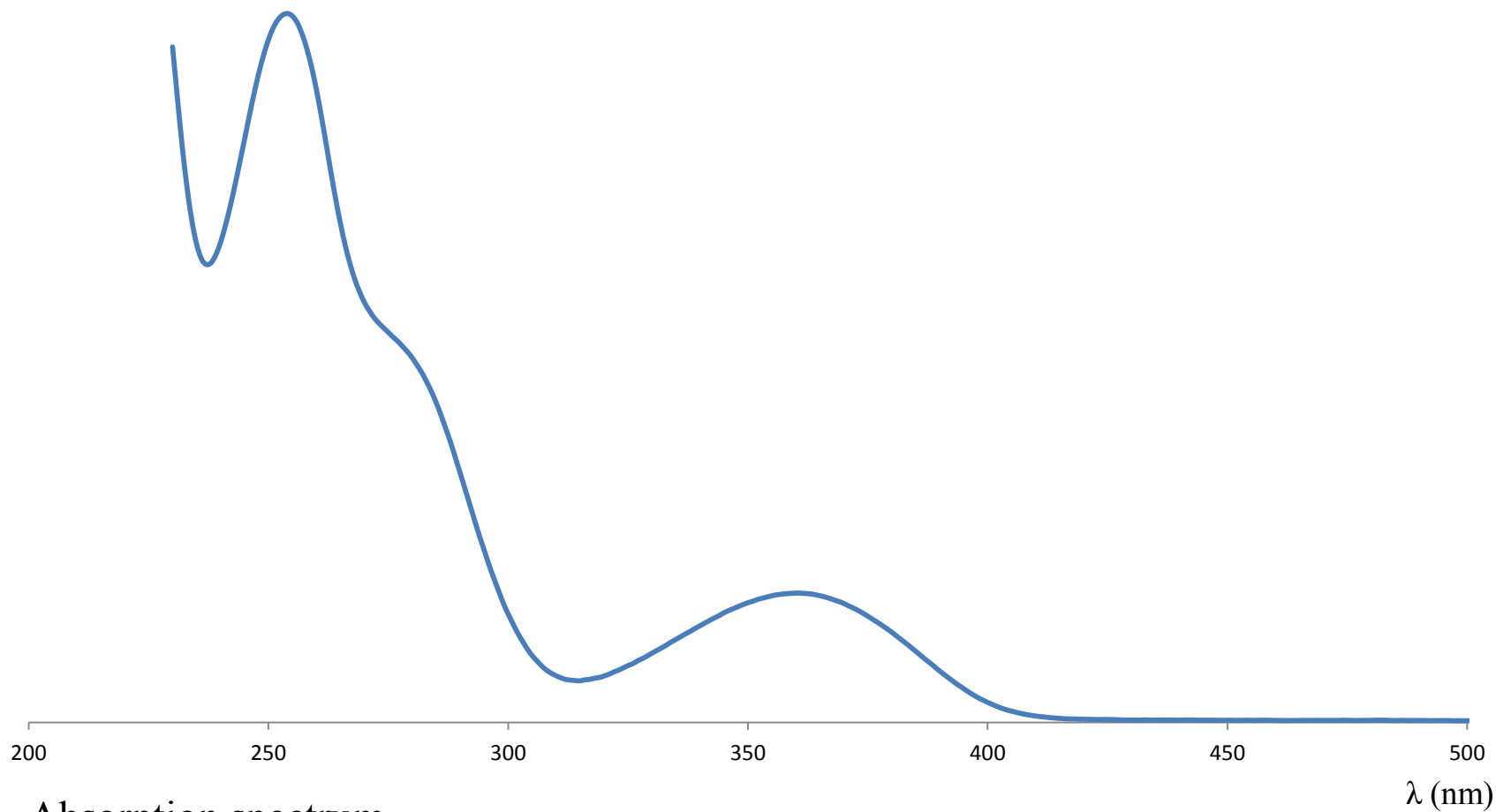
Emission spectrum

λ (nm)



# Mant-m<sup>7</sup>GpppG

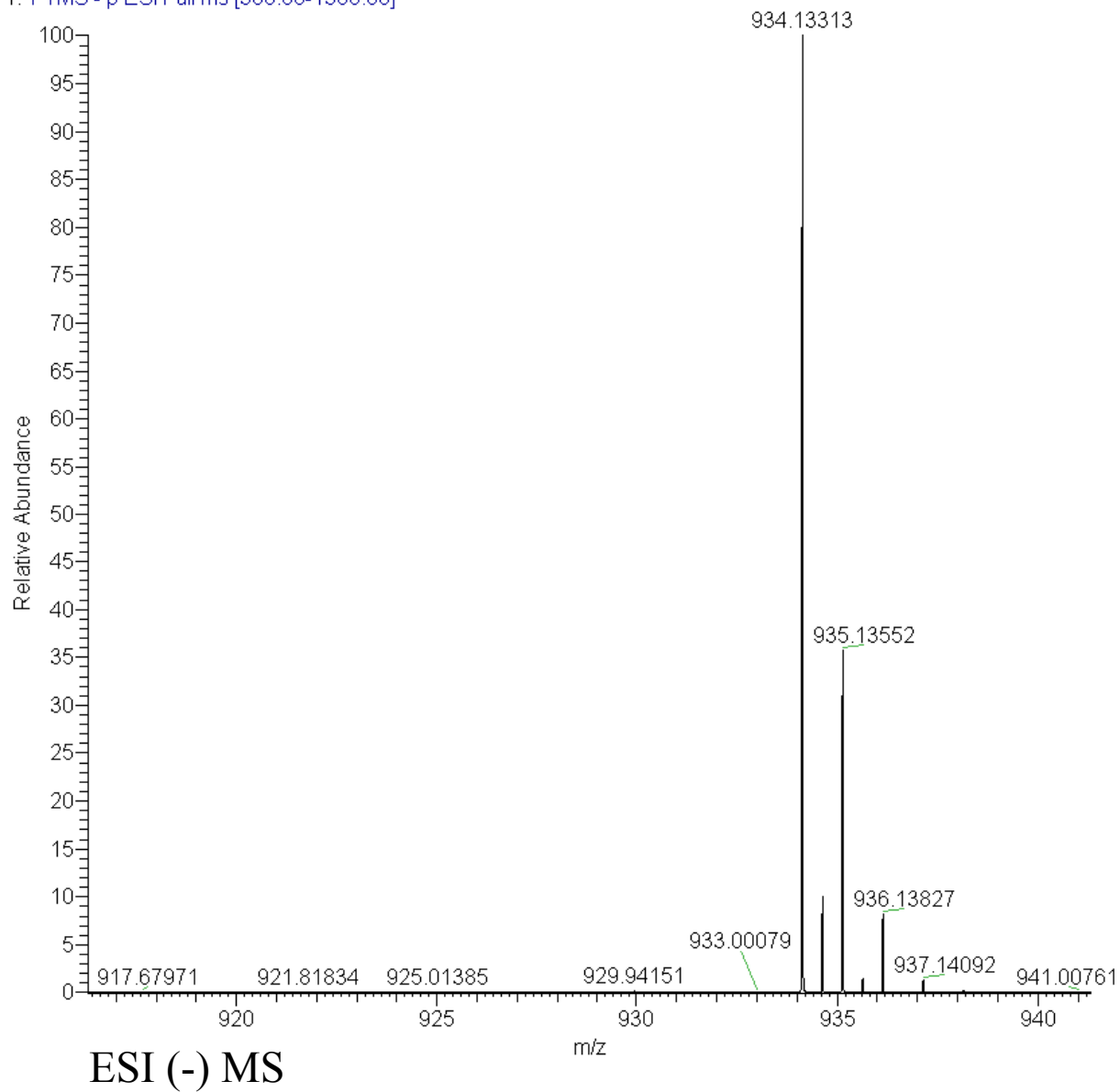
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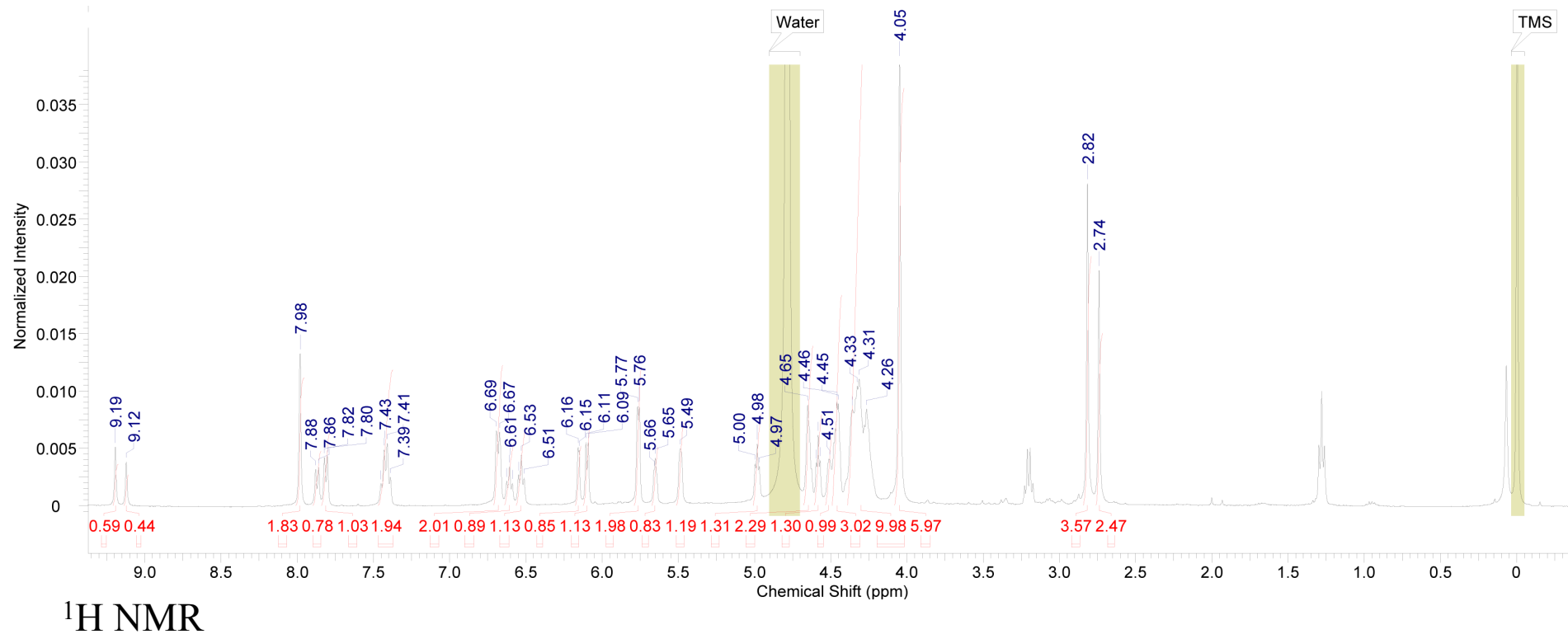
Absorption spectrum

# Mant-m<sup>7</sup>GpppG

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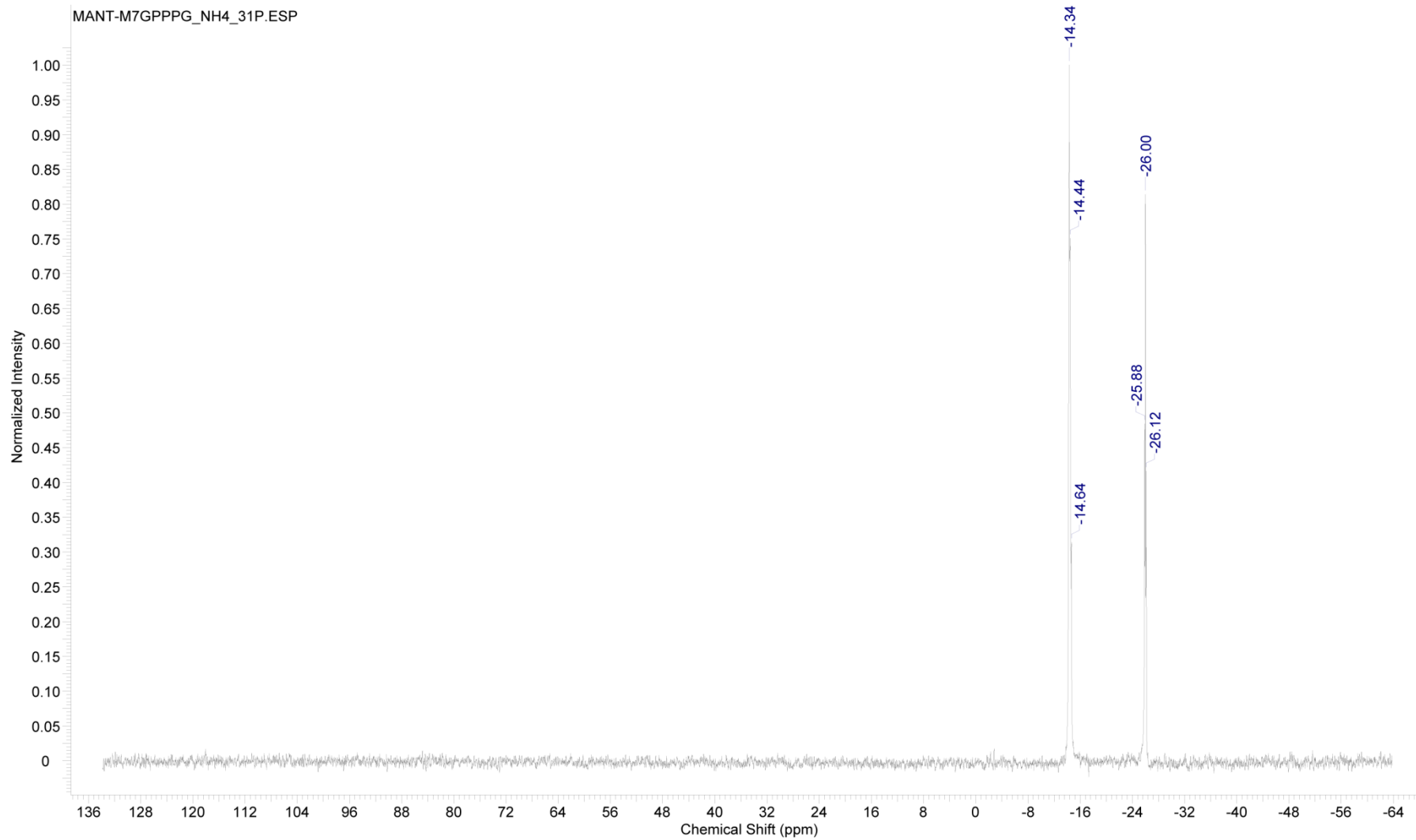


# Mant-m<sup>7</sup>GpppG



# Mant-m<sup>7</sup>GpppG

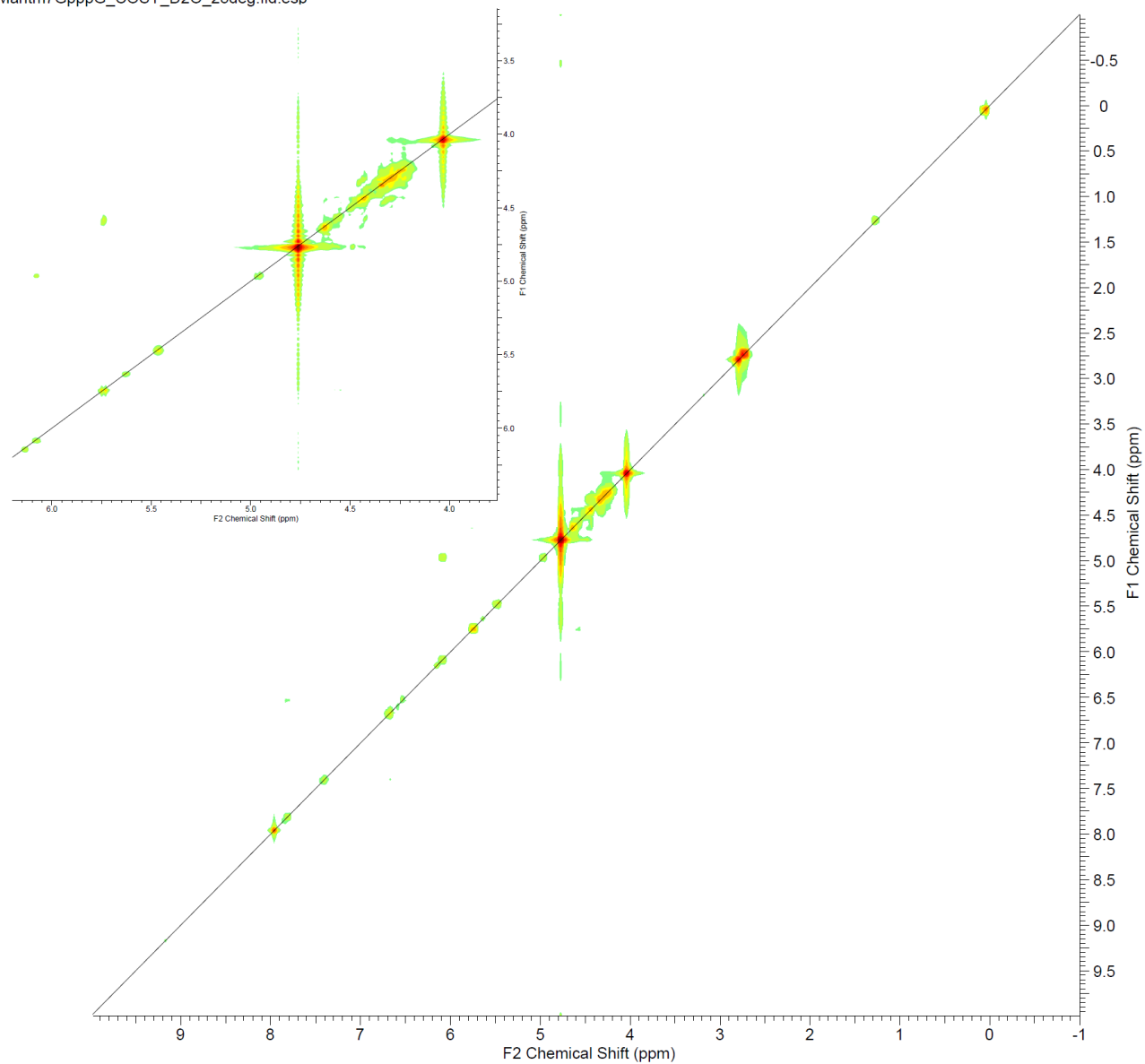
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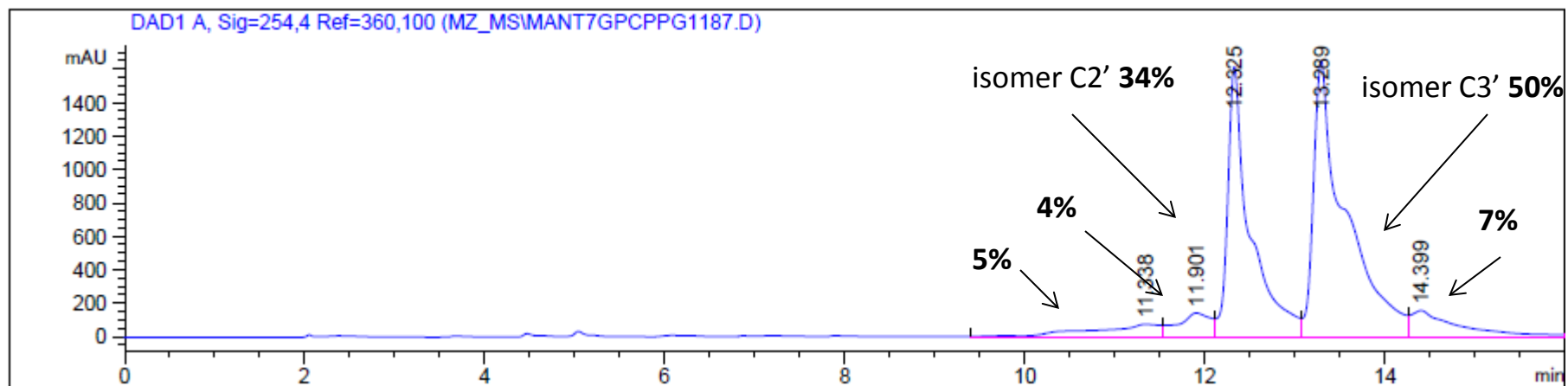
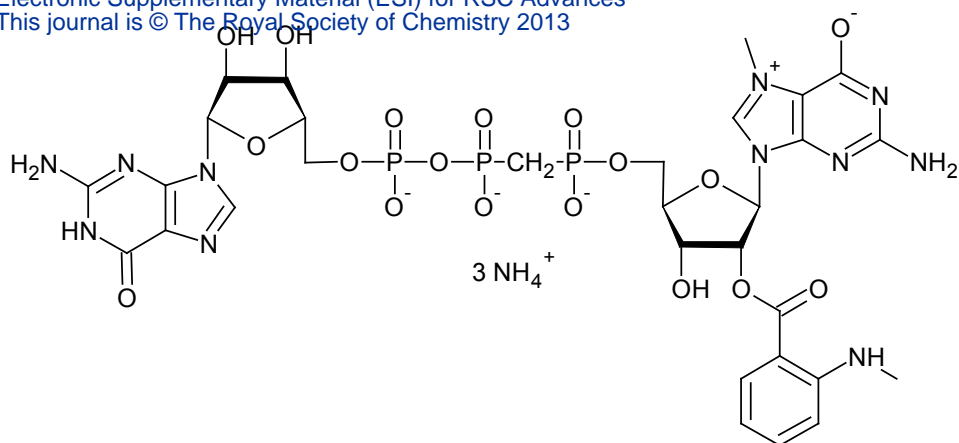
<sup>31</sup>P NMR

# Mant-m<sup>7</sup>GpppG

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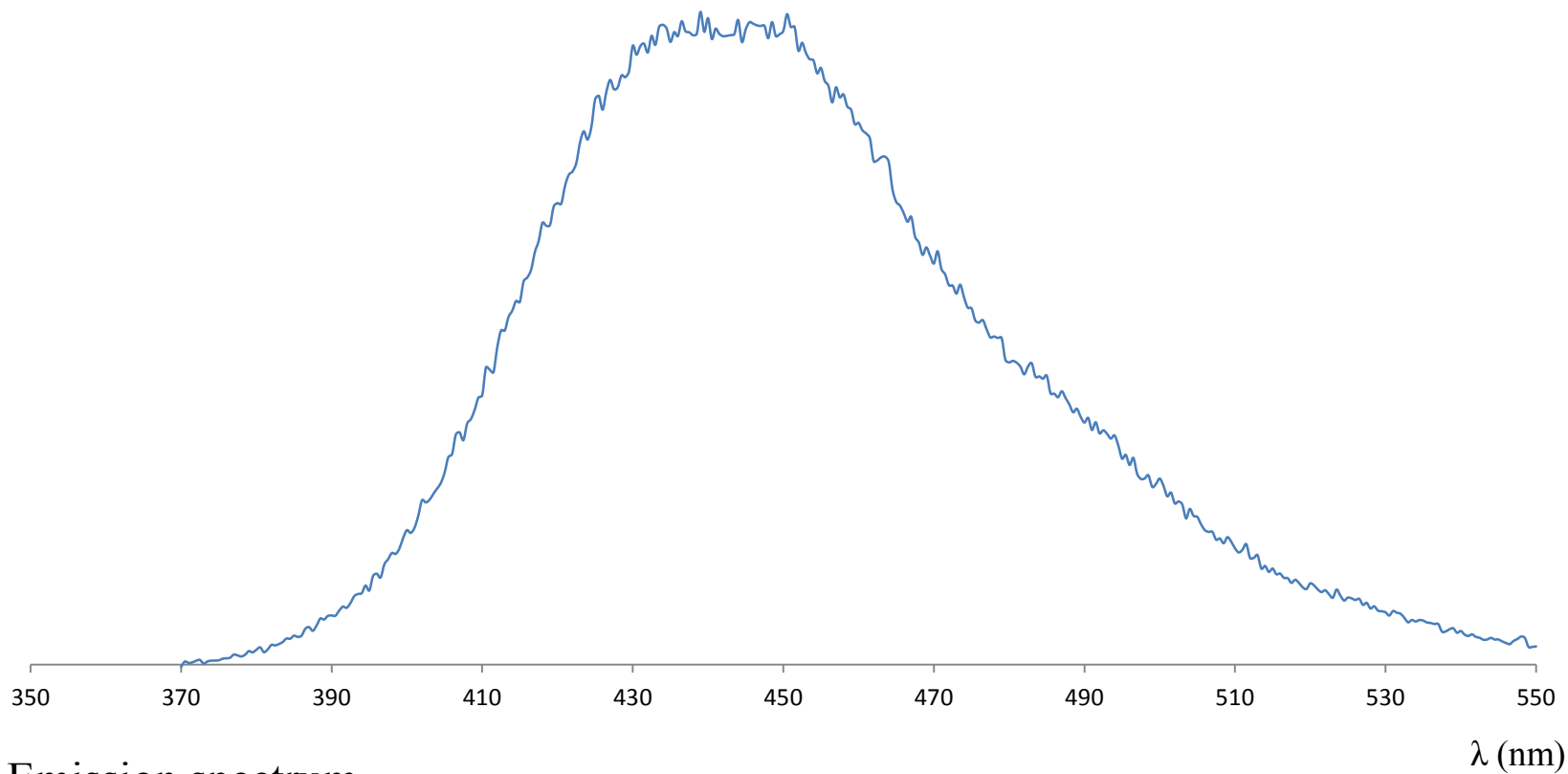
# Mant-m<sup>7</sup>GpCH<sub>2</sub>ppG



HPLC profile

# Mant-m<sup>7</sup>GpCH<sub>2</sub>ppG

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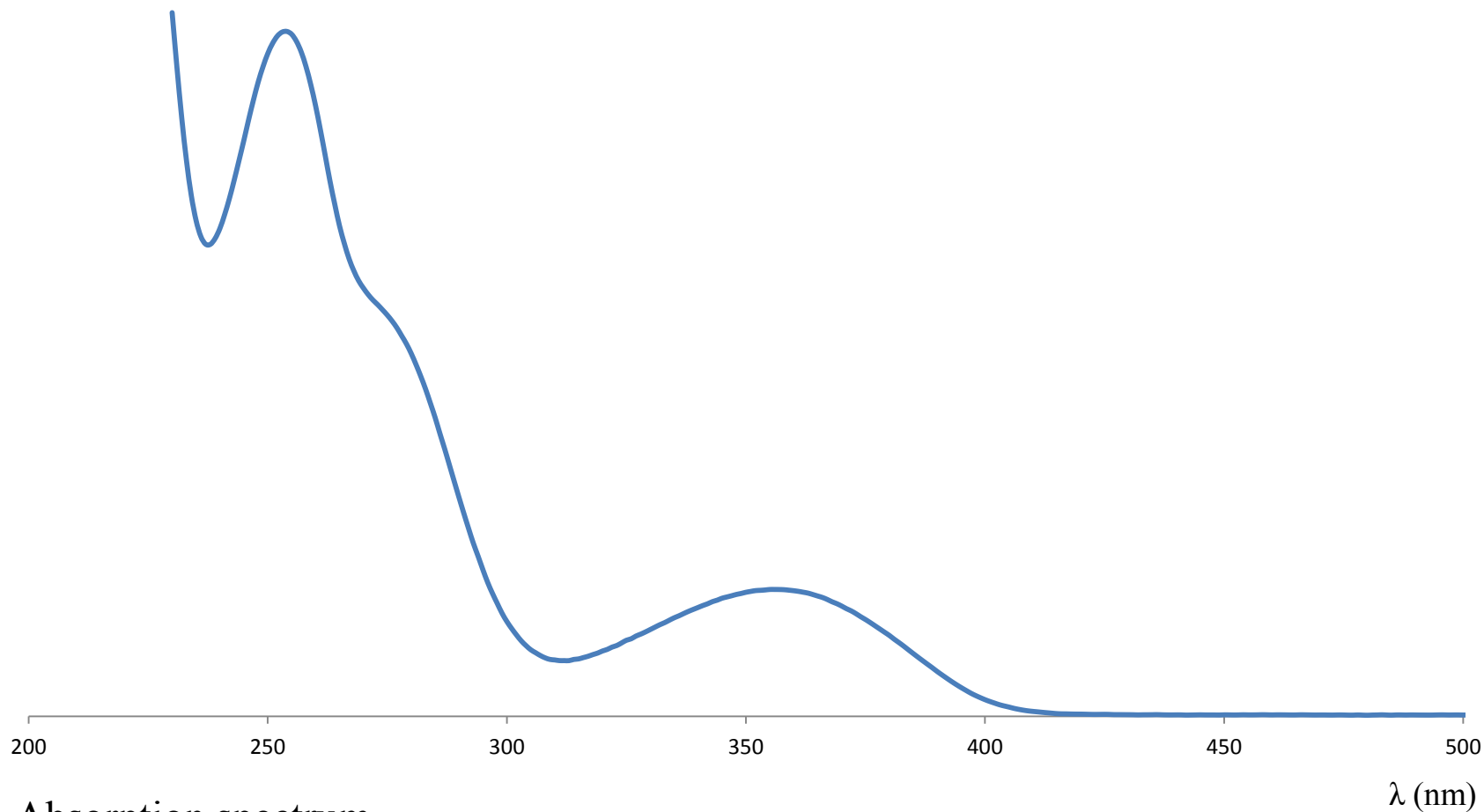


Emission spectrum

λ (nm)

# Mant-m<sup>7</sup>GpCH<sub>2</sub>ppG

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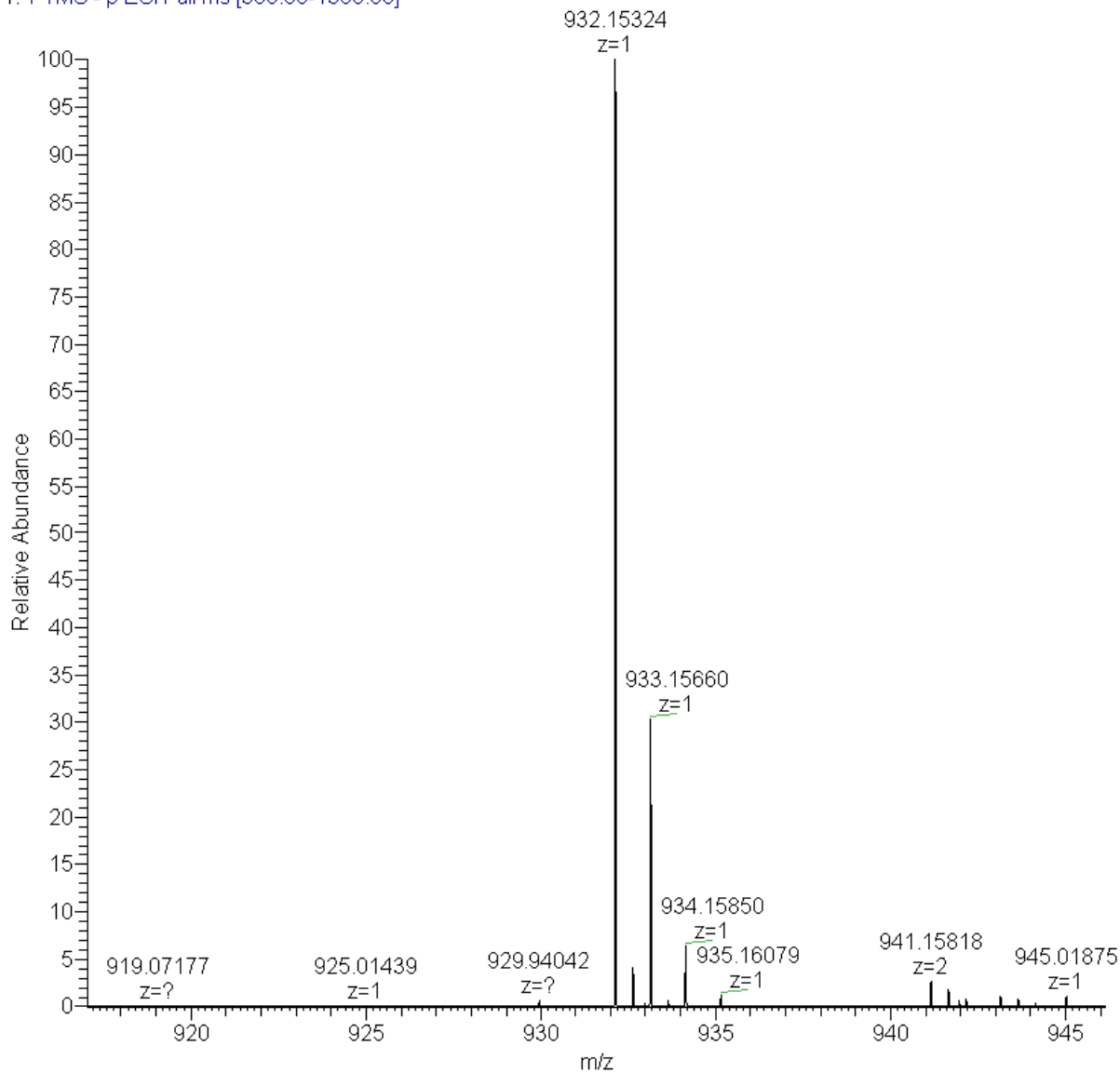
Absorption spectrum

λ (nm)



# Mant-m<sup>7</sup>GpCH<sub>2</sub>ppG

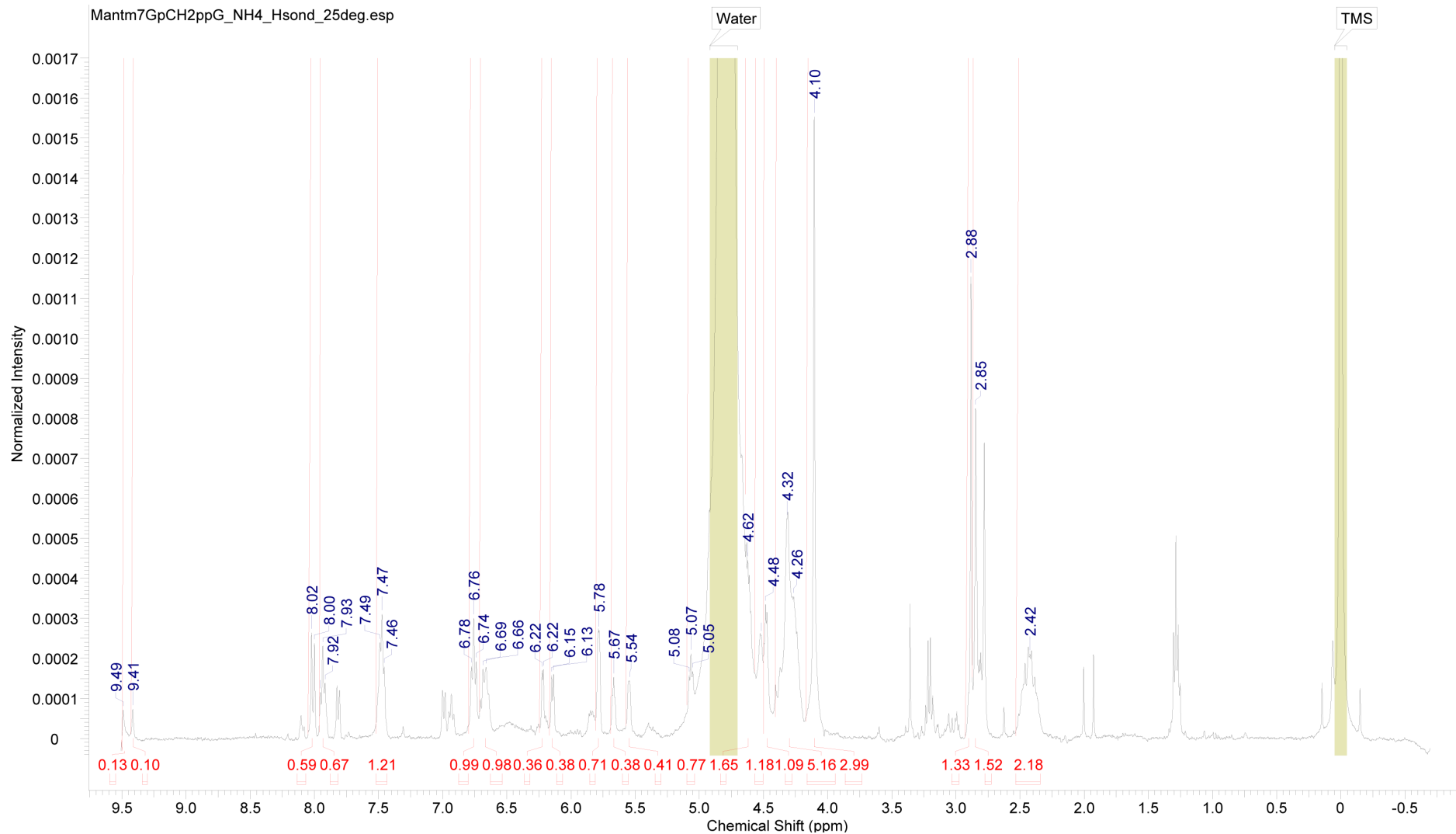
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ESI (-) MS

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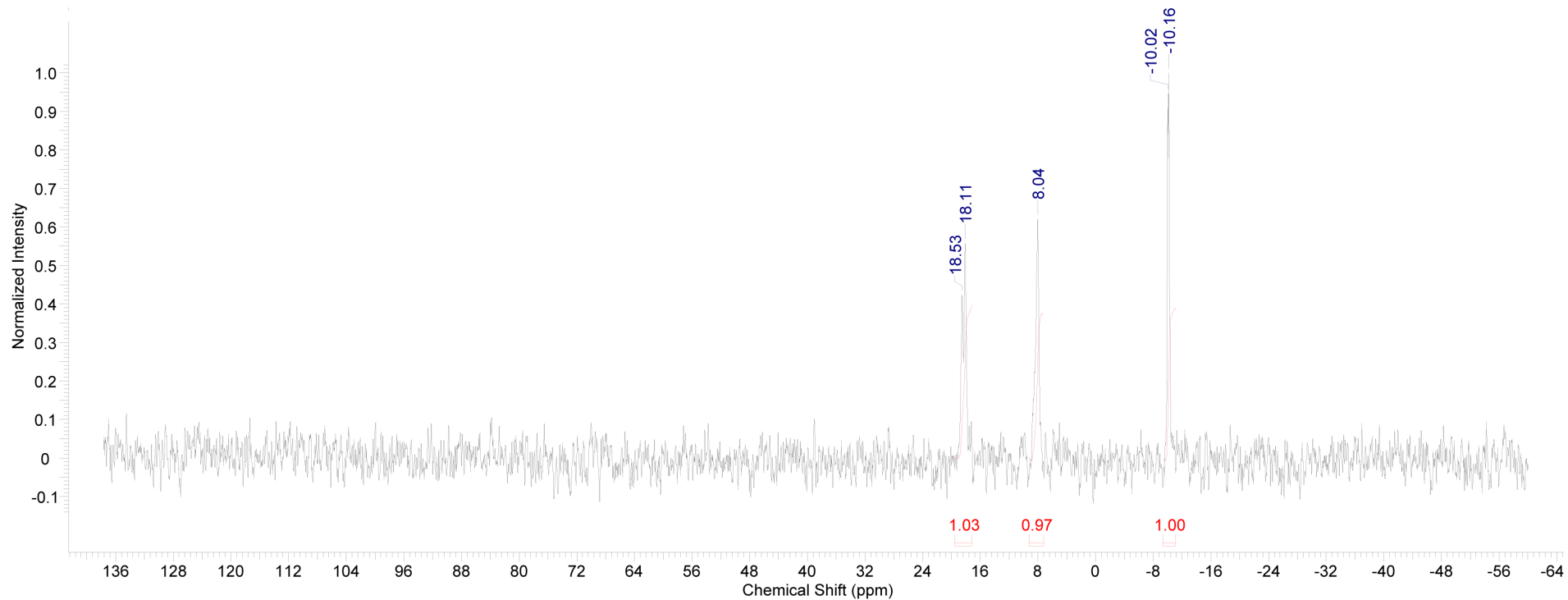
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<sup>1</sup>H NMR

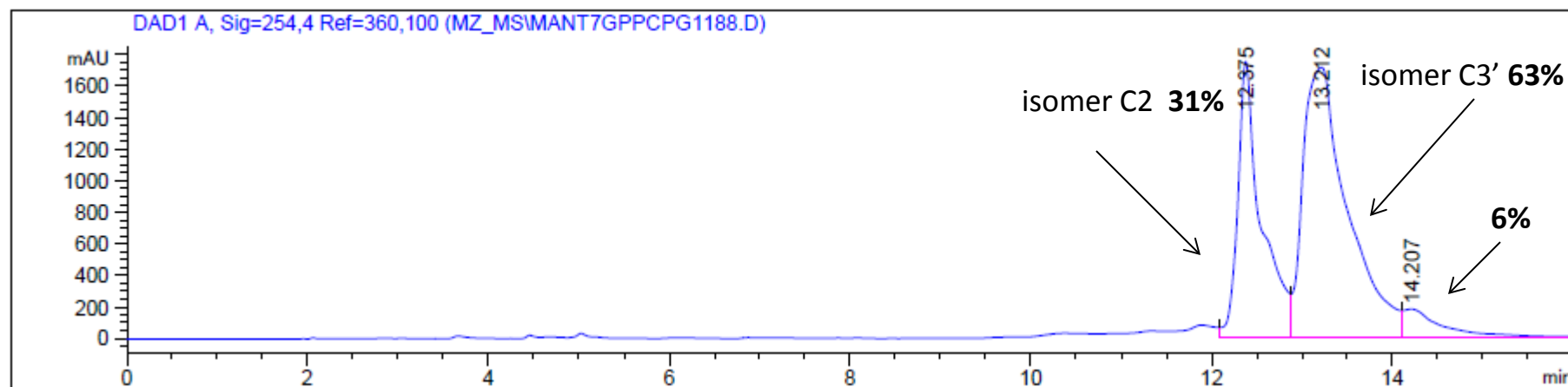
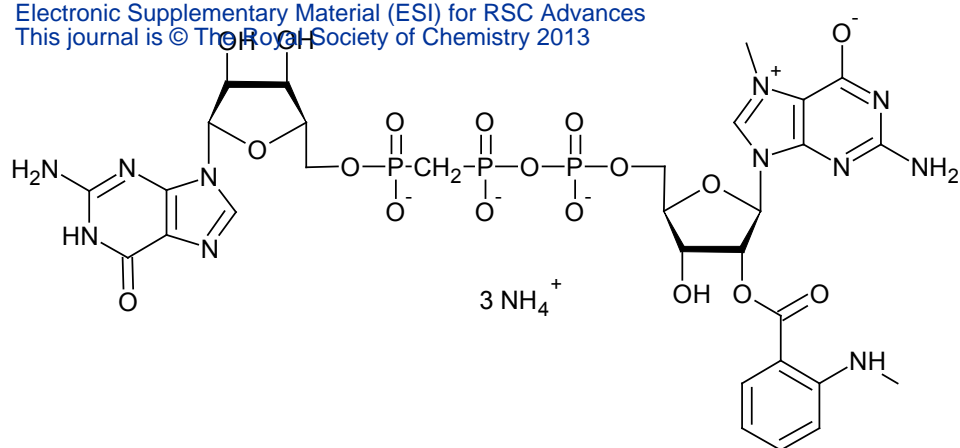
# Mant-m<sup>7</sup>GpCH<sub>2</sub>ppG

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<sup>31</sup>P NMR

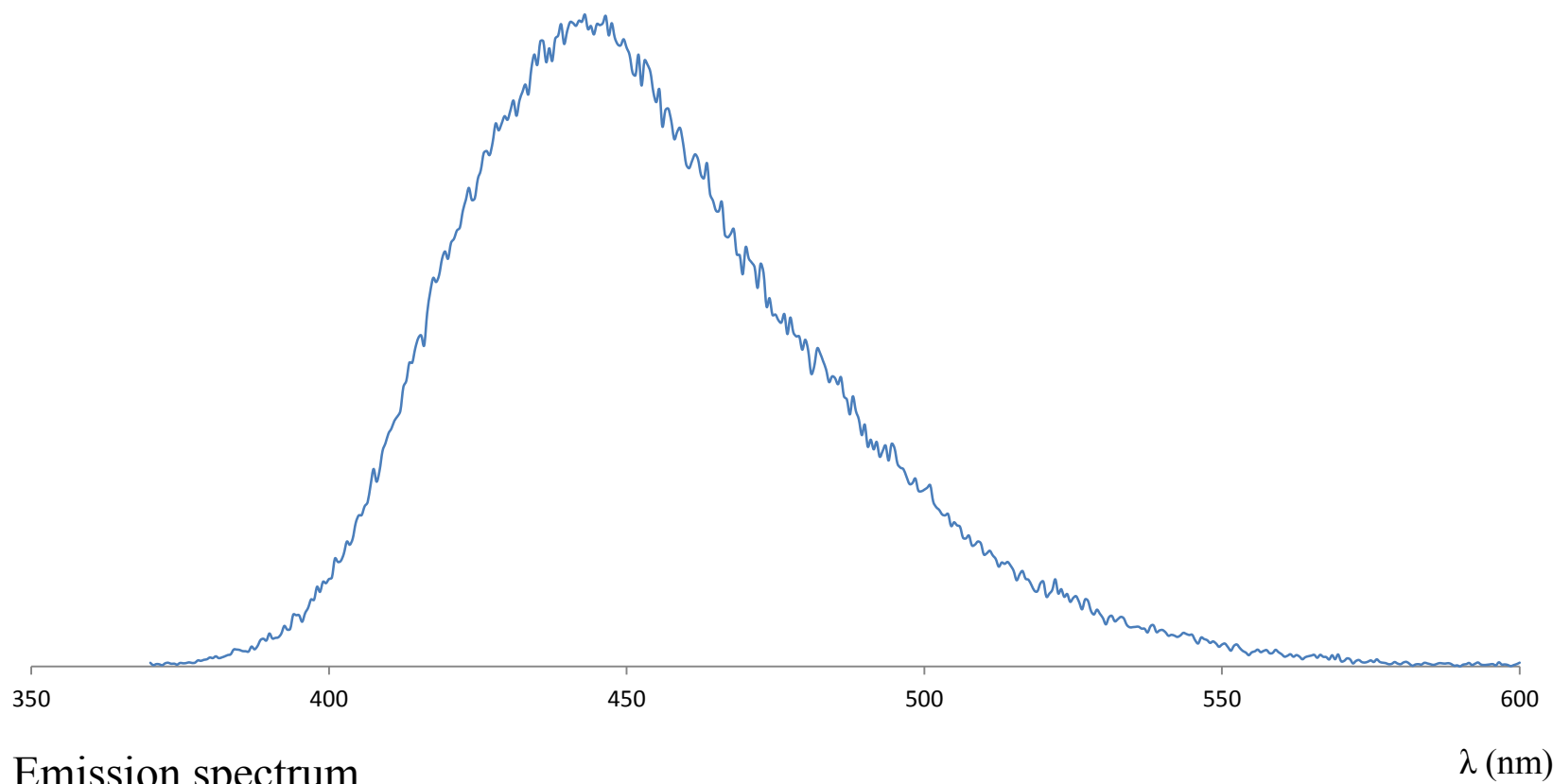
# Mant-m<sup>7</sup>GppCH<sub>2</sub>pG



HPLC profile

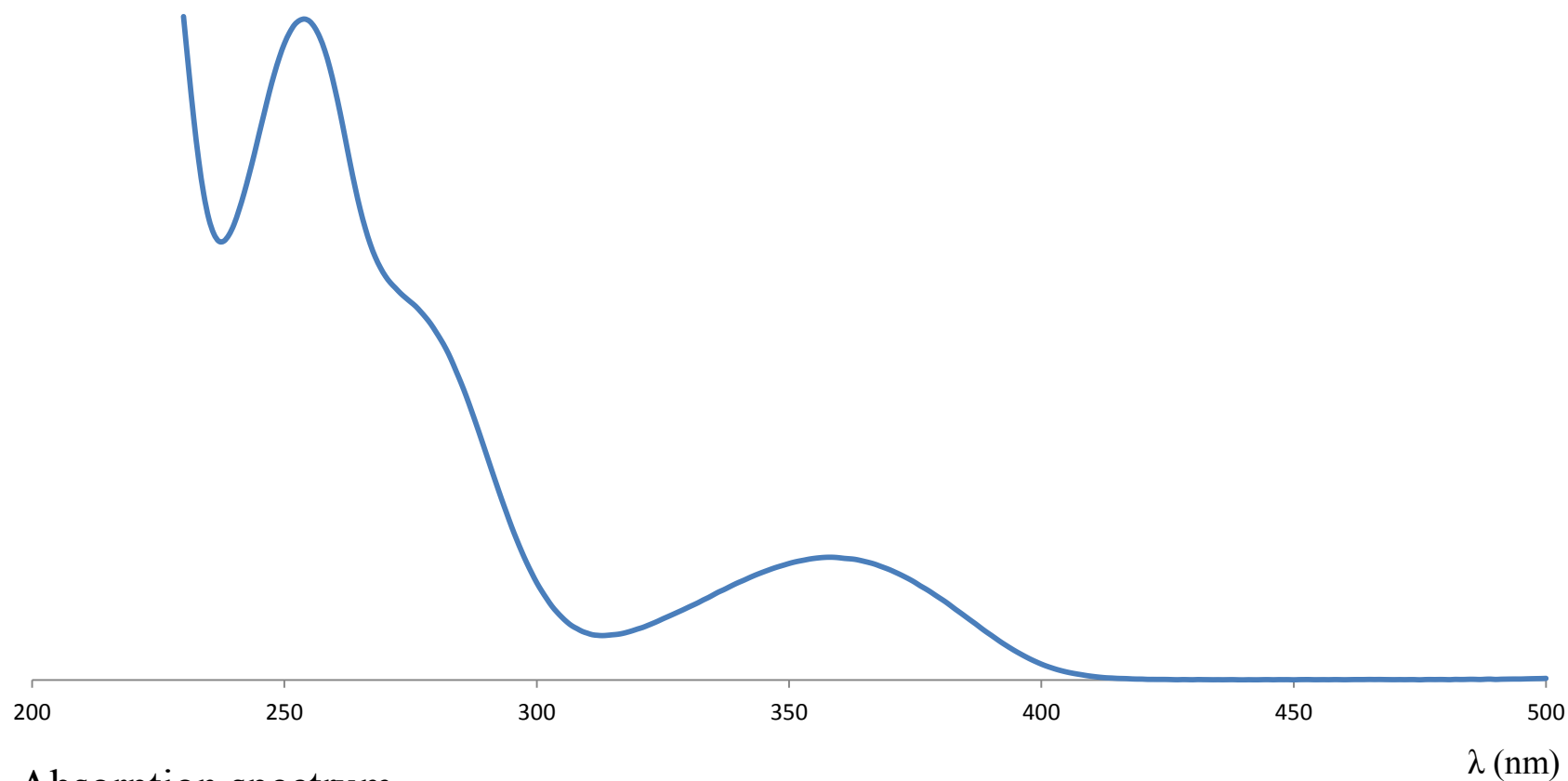
# Mant-m<sup>7</sup>GppCH<sub>2</sub>pG

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# Mant-m<sup>7</sup>GppCH<sub>2</sub>pG

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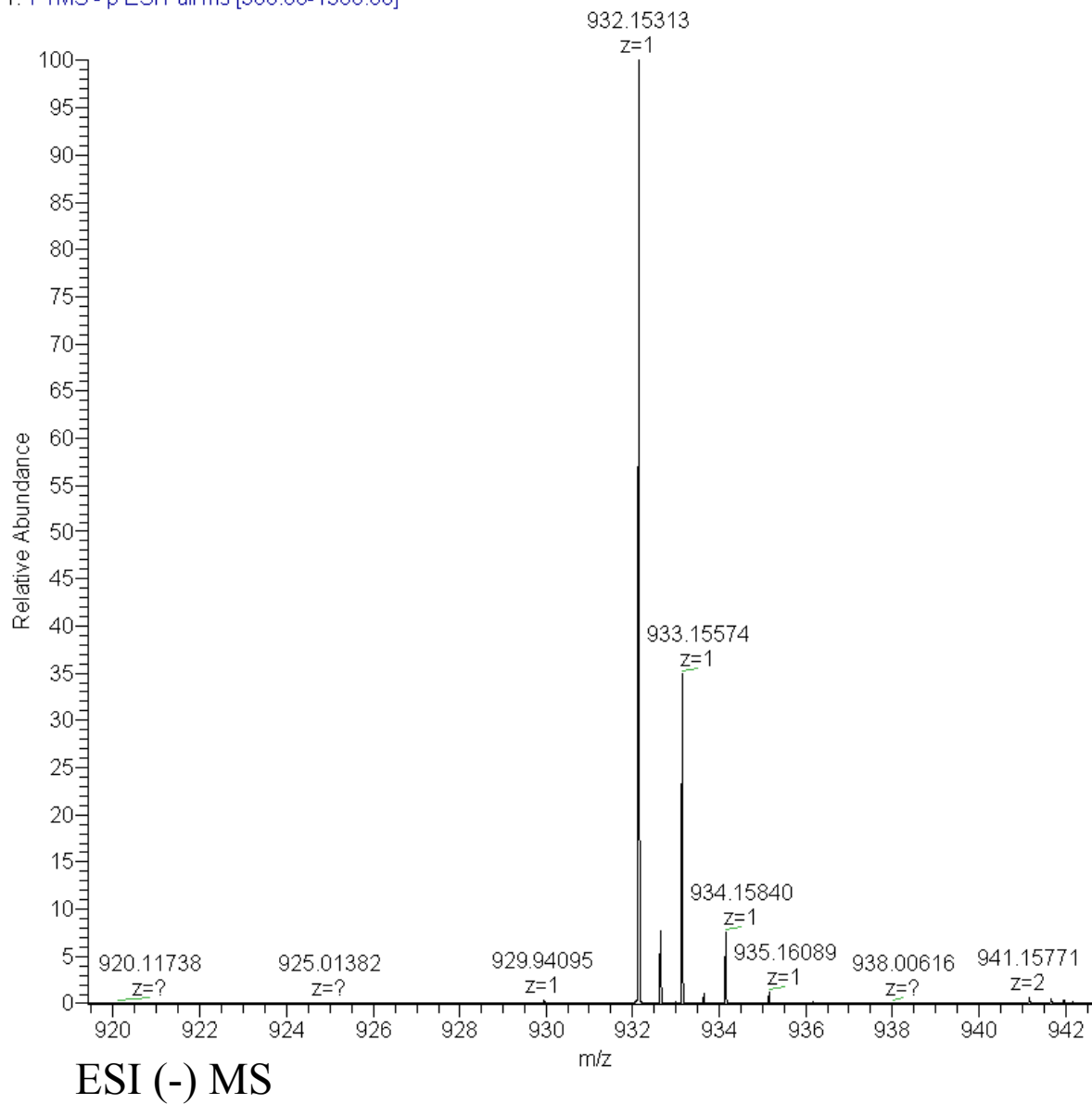


Absorption spectrum

λ (nm)

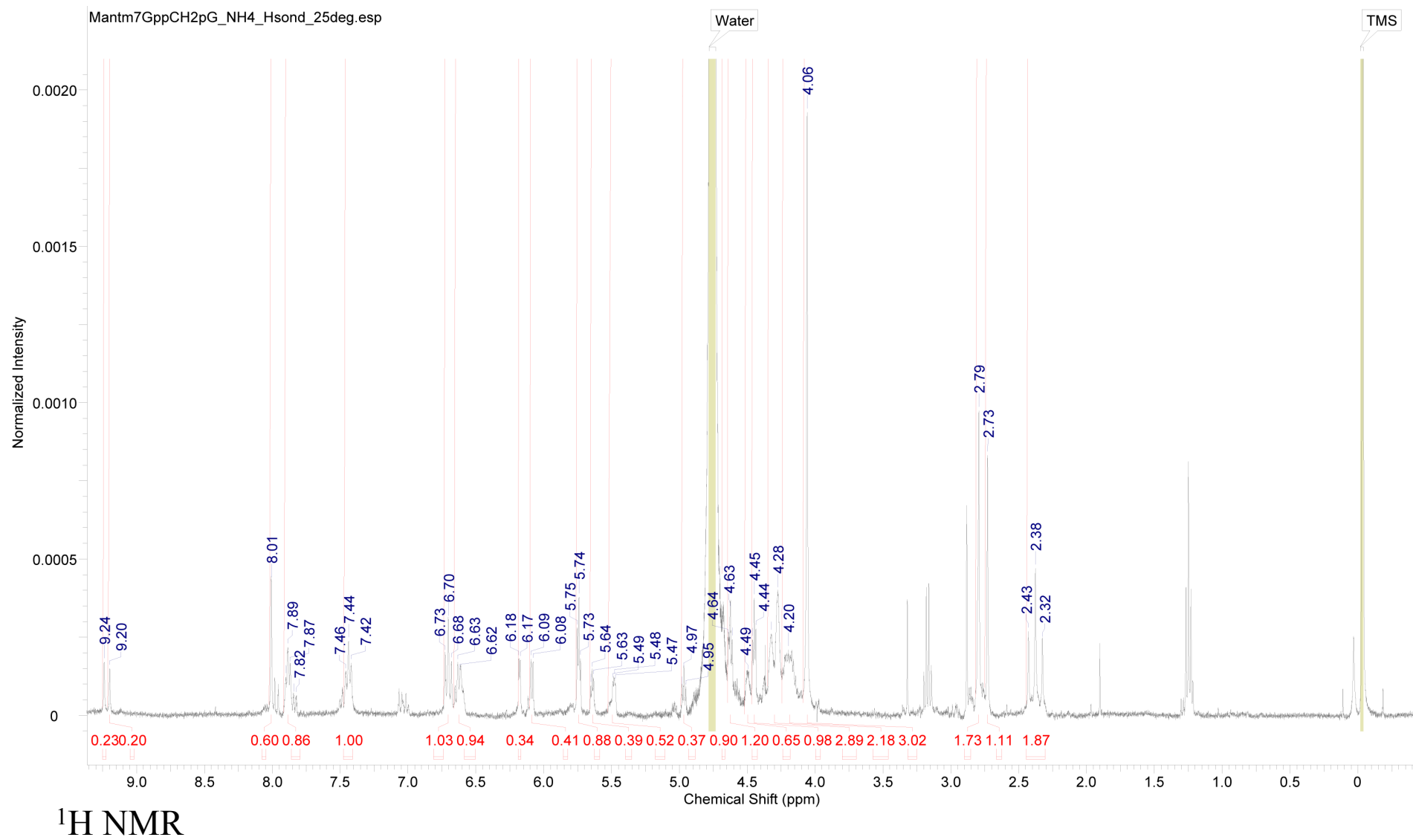
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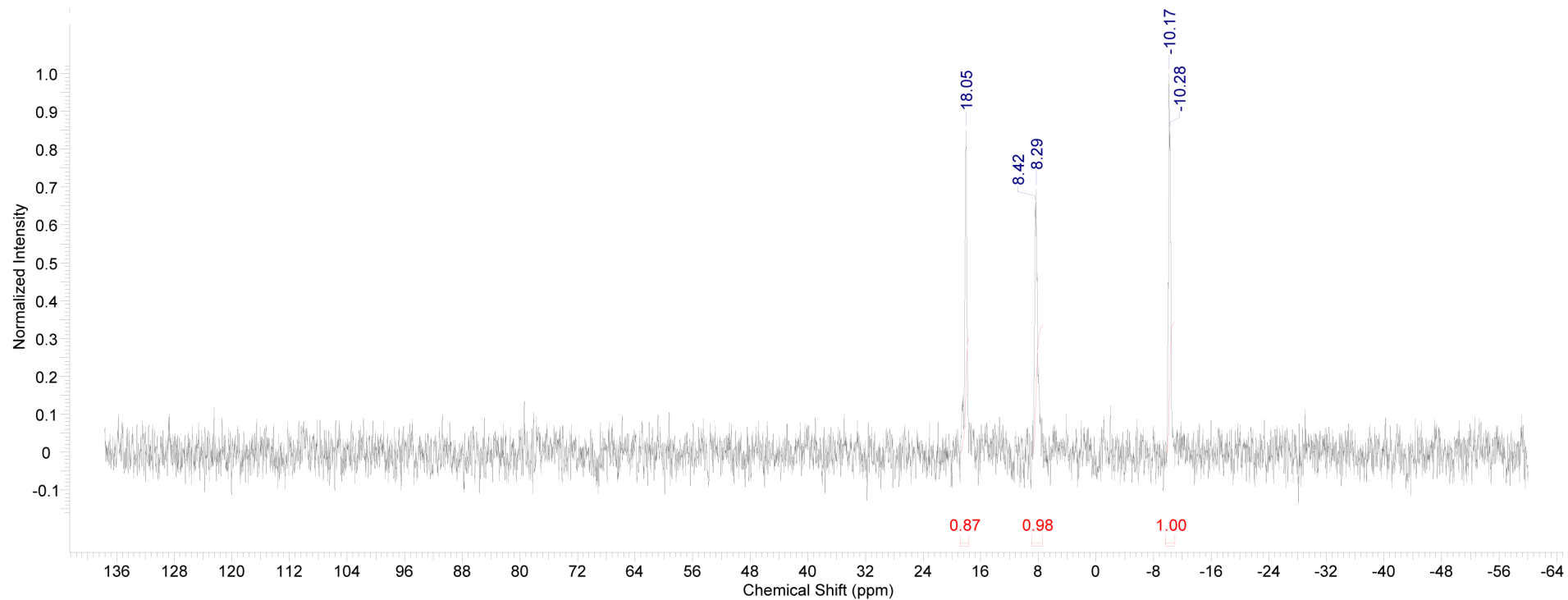
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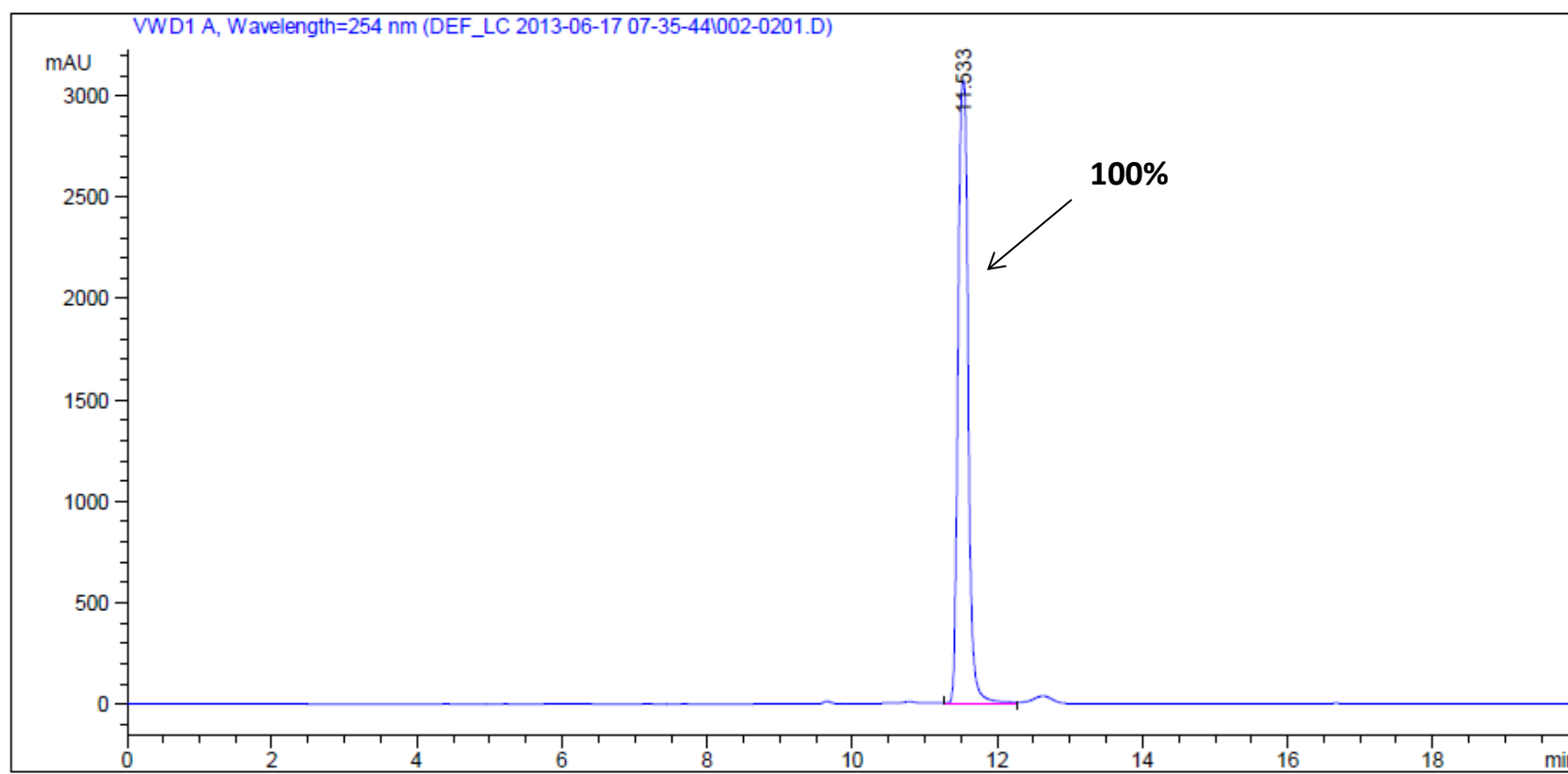
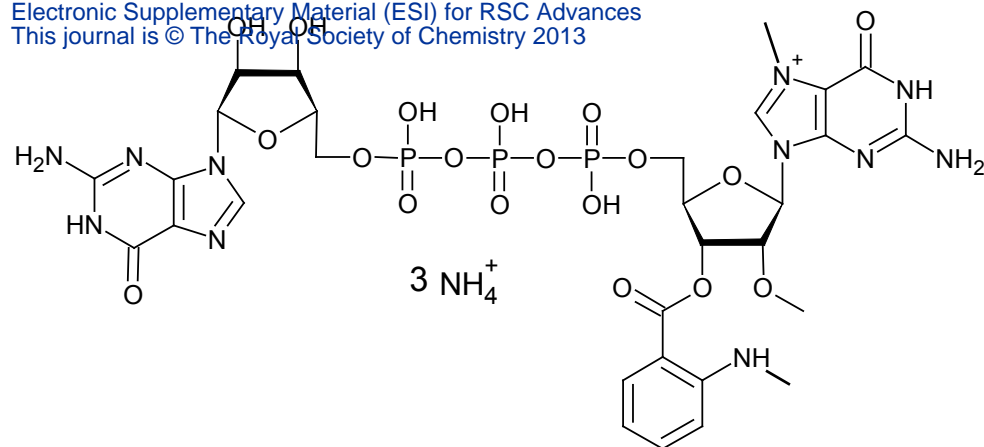
# Mant-m<sup>7</sup>GppCH<sub>2</sub>pG

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<sup>31</sup>P NMR

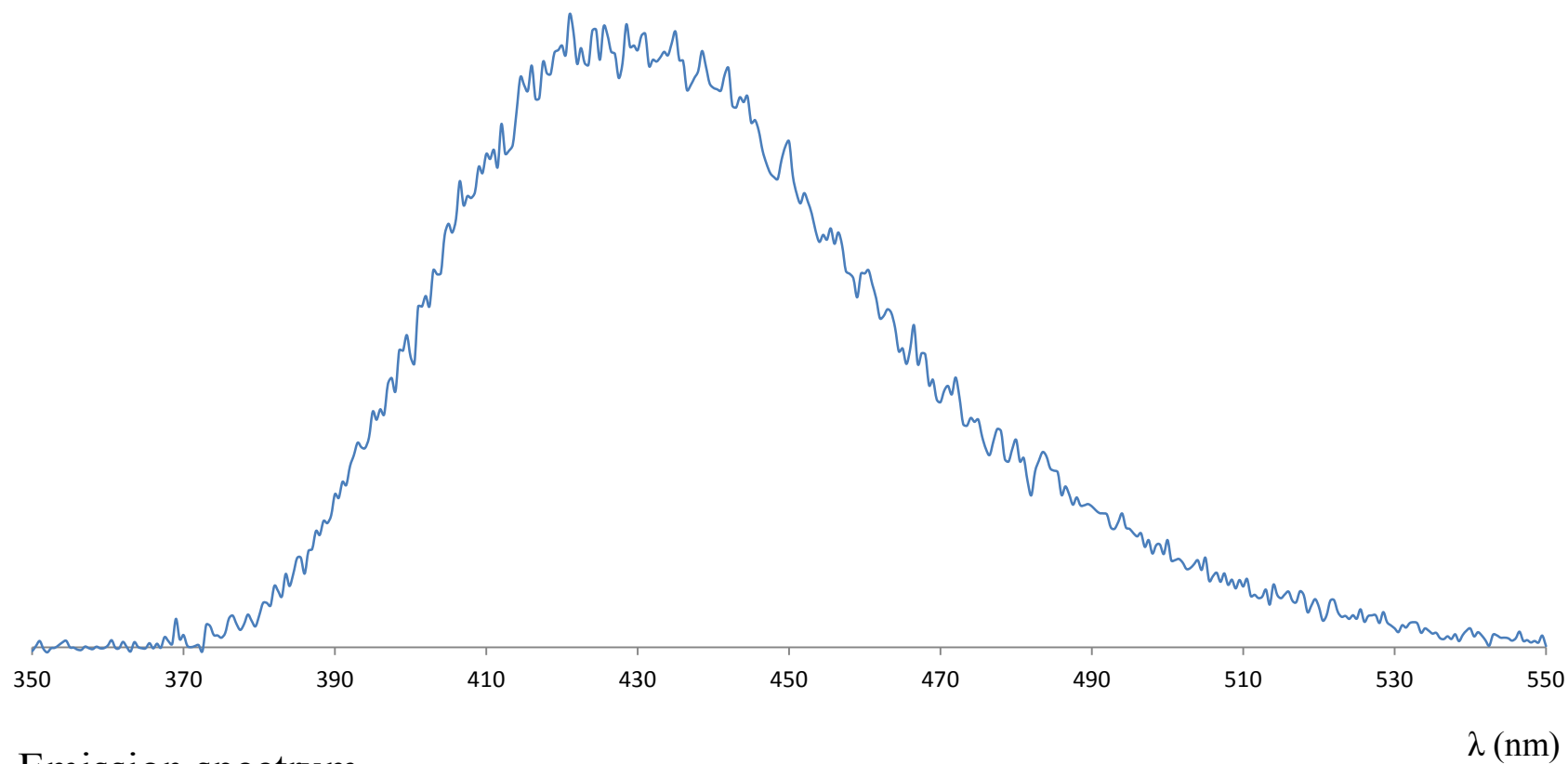
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HPLC profile

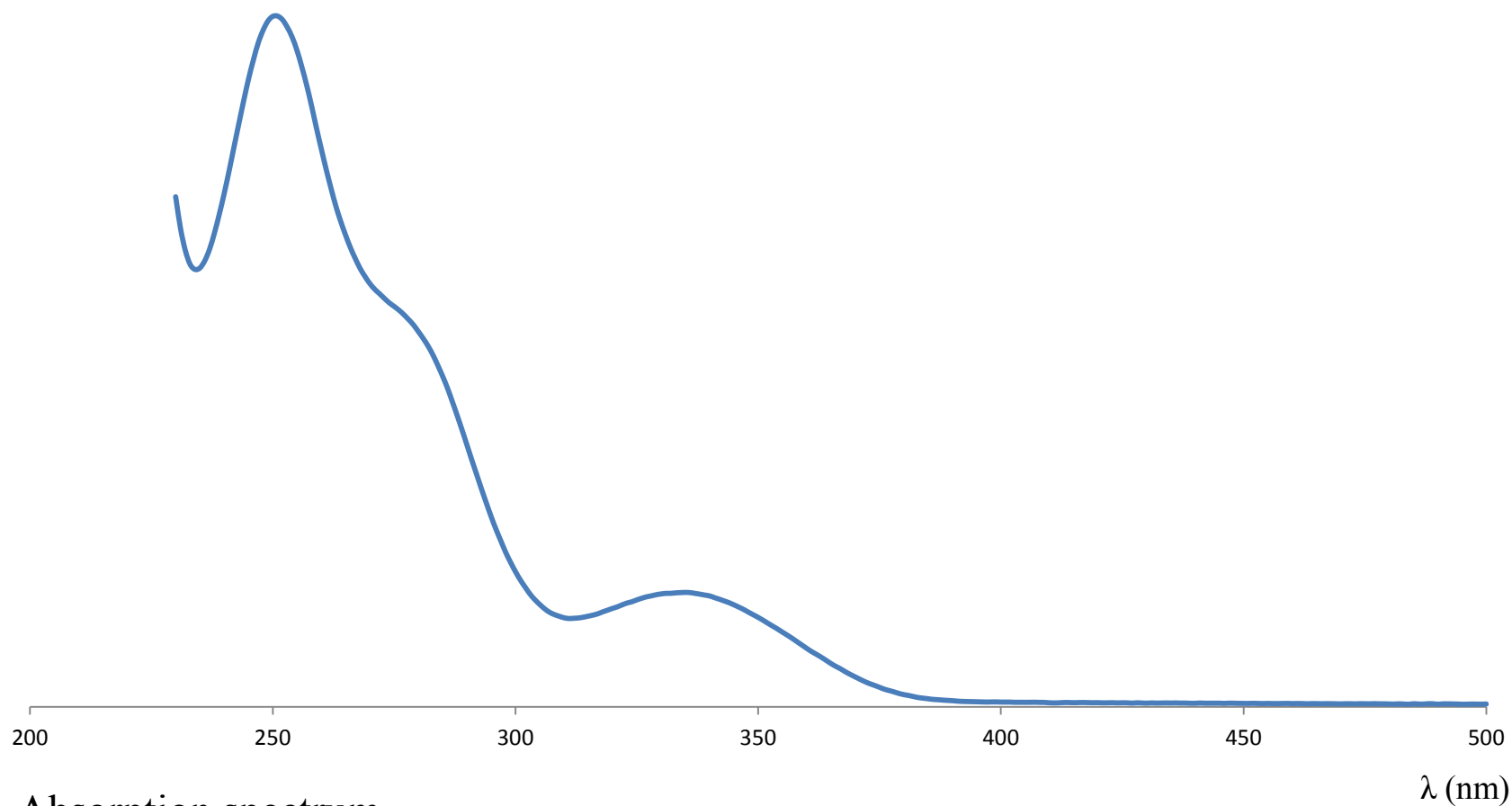
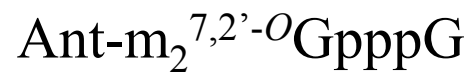
# Ant-m<sub>2</sub><sup>7,2'-O</sup>GpppG

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Emission spectrum

λ (nm)

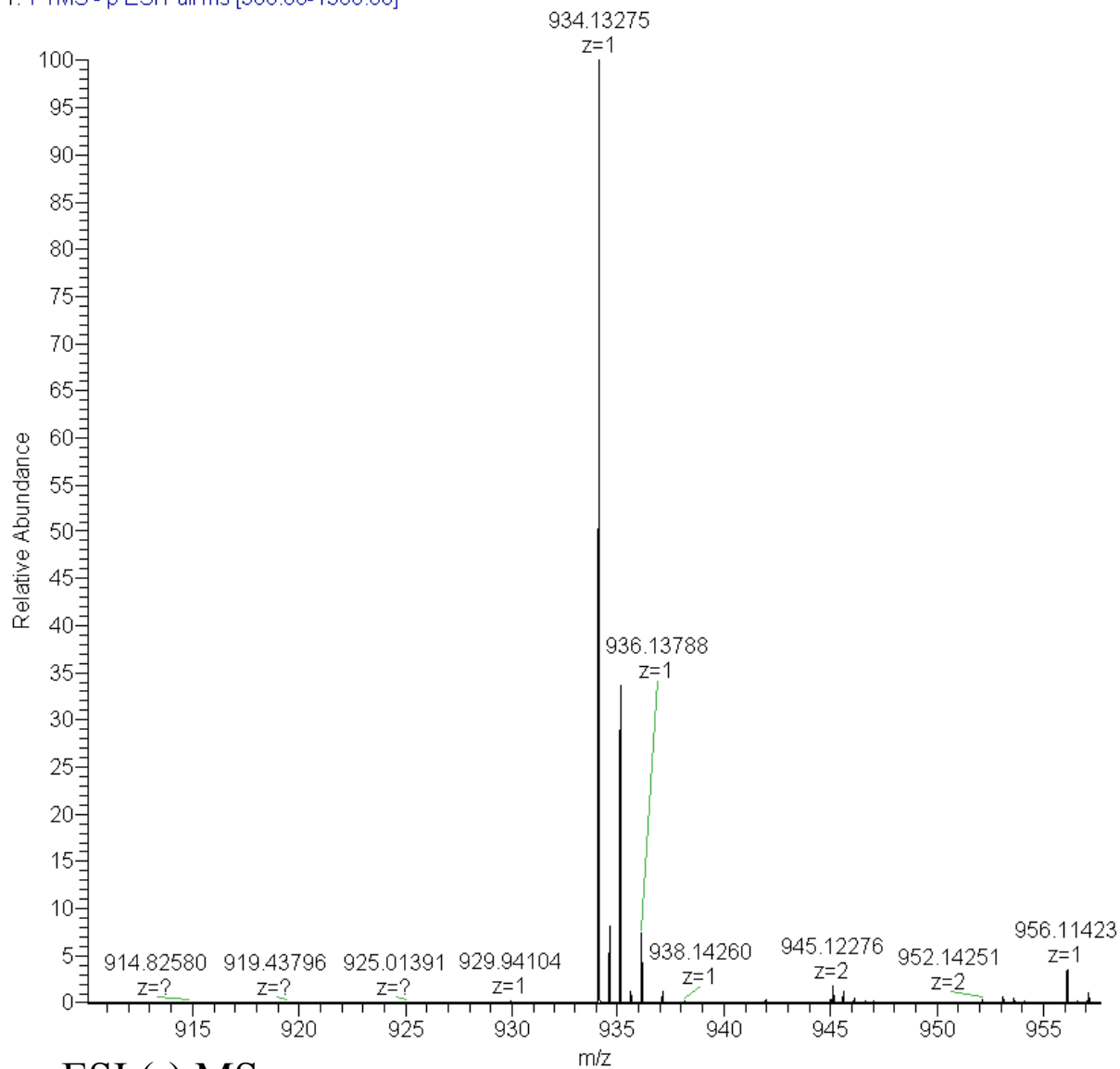


Absorption spectrum

λ (nm)

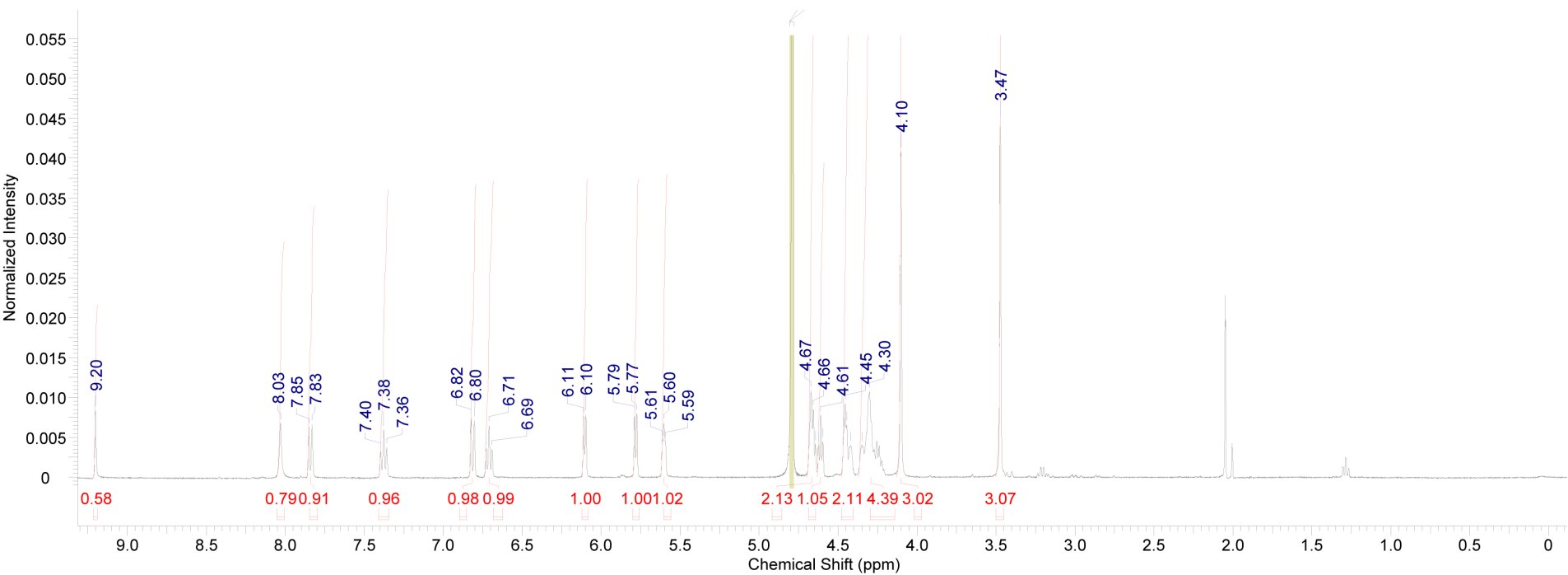
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ESI (-) MS

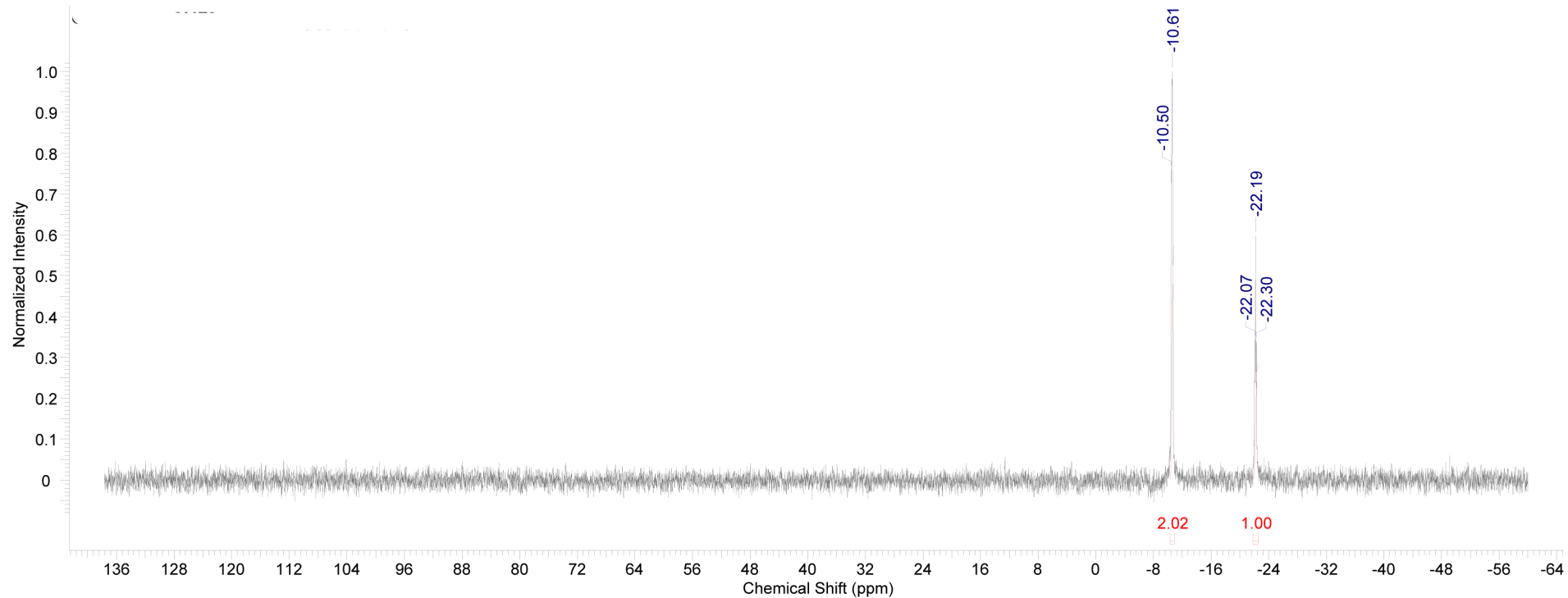
# Ant-m<sub>2</sub><sup>7,2'-O</sup>GpppG



<sup>1</sup>H NMR

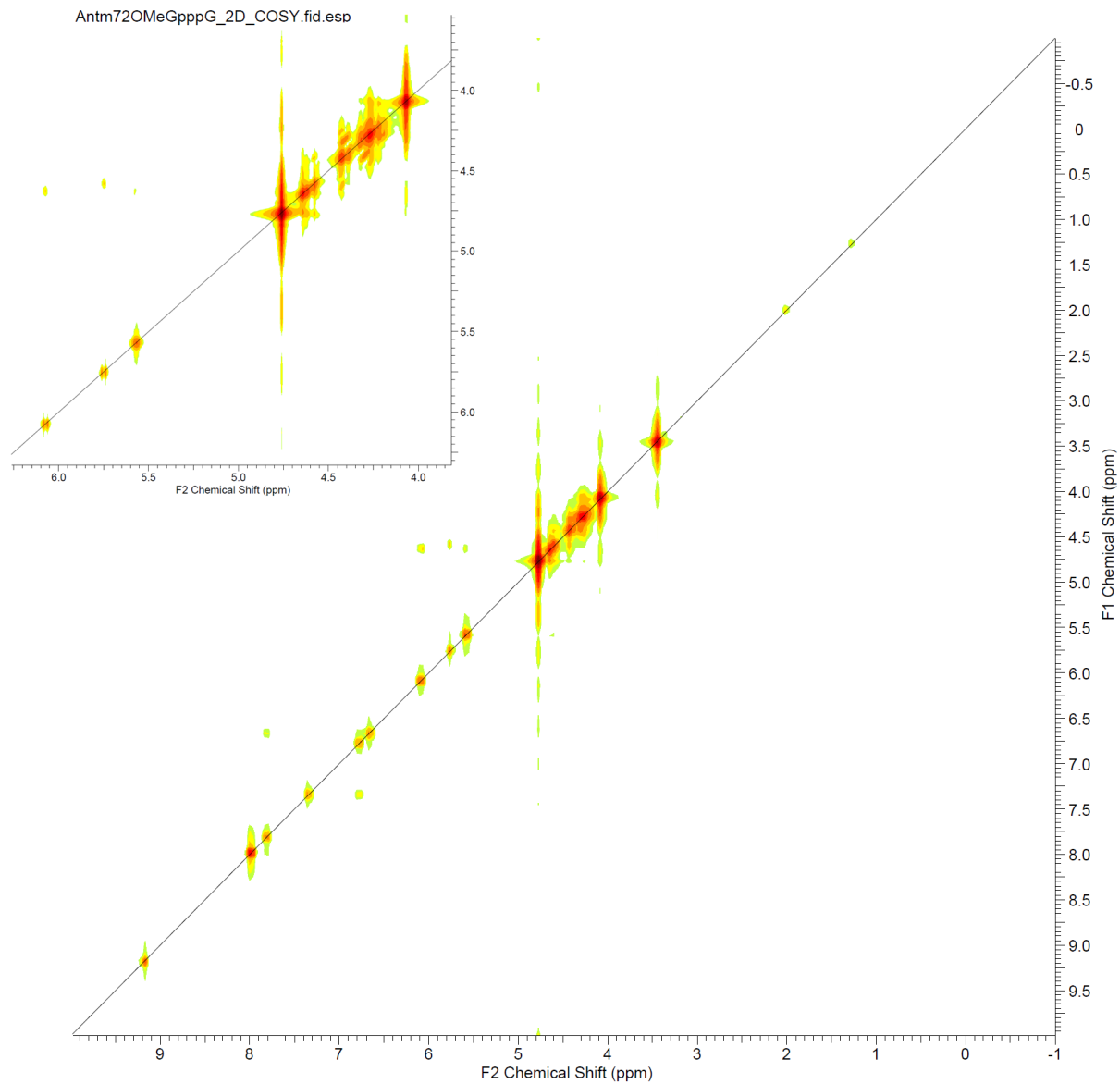
# Ant-m<sub>2</sub><sup>7,2'-O</sup>GpppG

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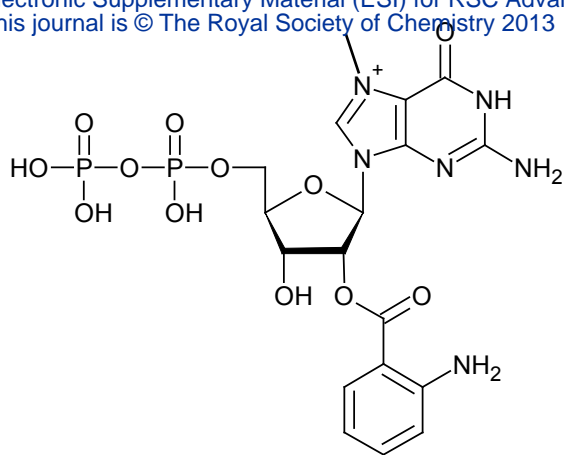


<sup>31</sup>P NMR

# Ant-m<sub>2</sub><sup>7,2'-O</sup>GpppG

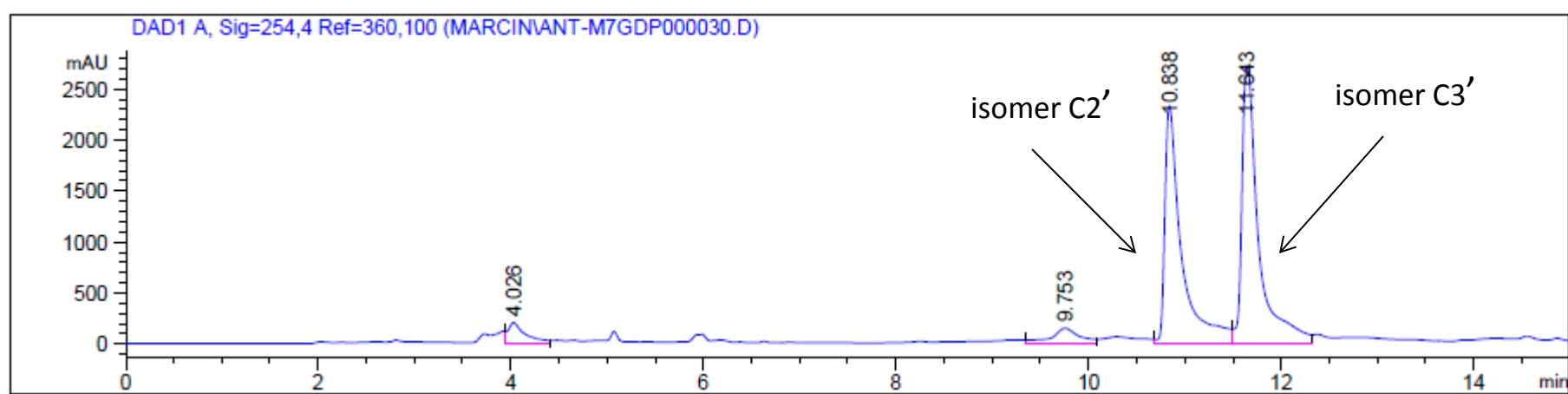






# Ant-m<sup>7</sup>GDP

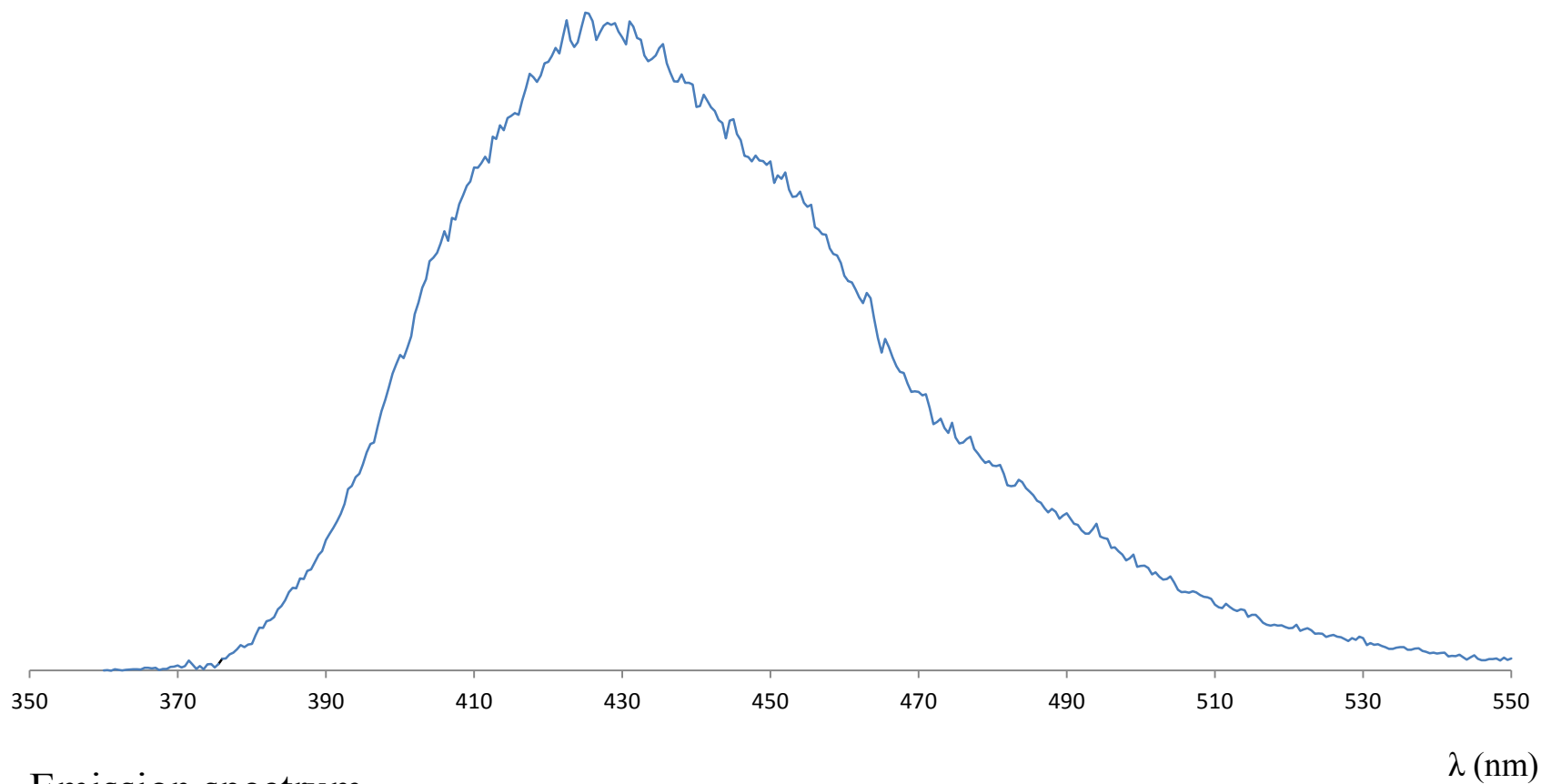
**Ziemiak *et al.***  
Synthesis and evaluation of fluorescent  
cap analogues for mRNA labelling  
**supplementary information**



HPLC profile

# Ant-m<sup>7</sup>GDP

**Ziemniak *et al.***  
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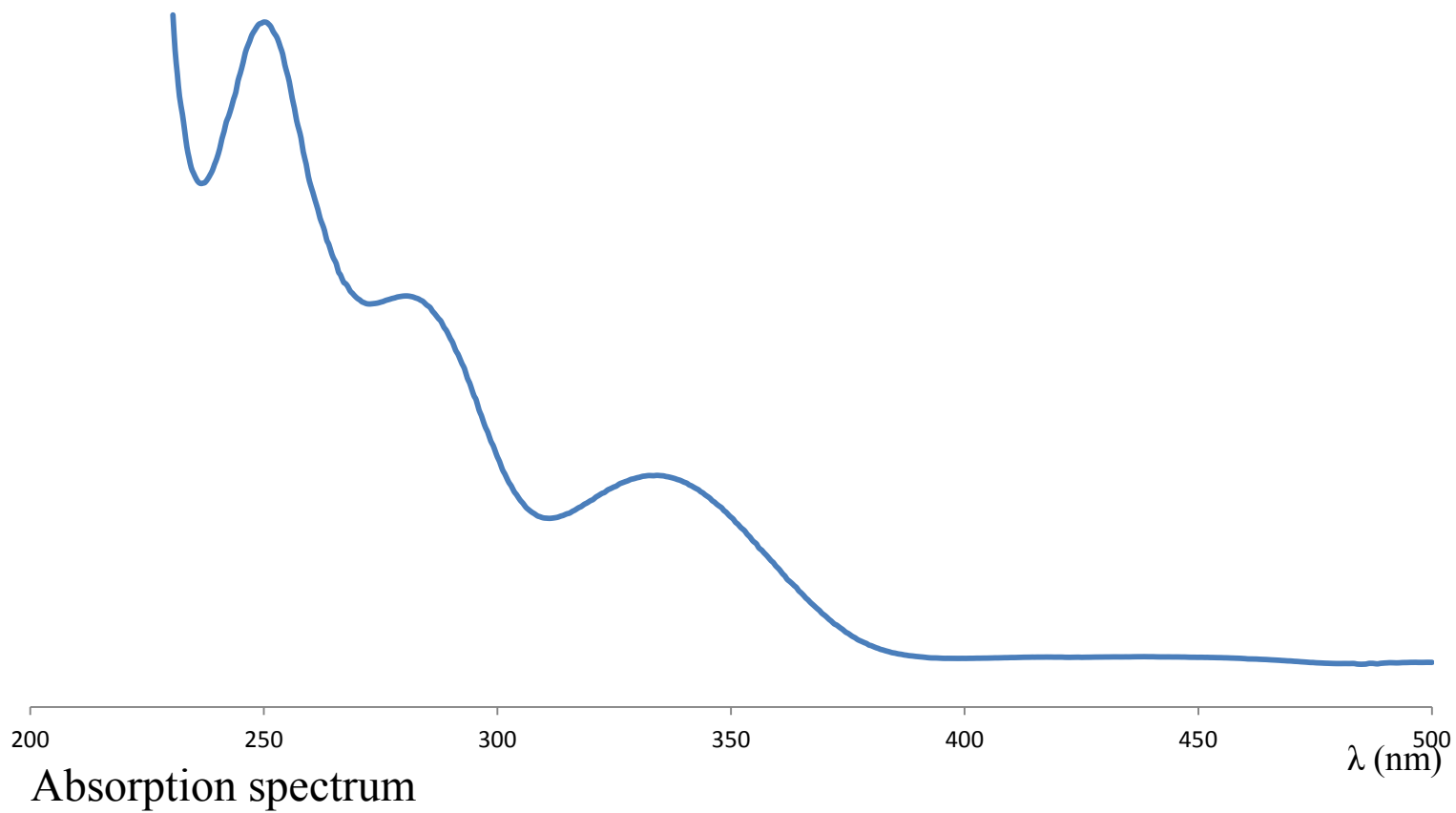


Emission spectrum

λ (nm)

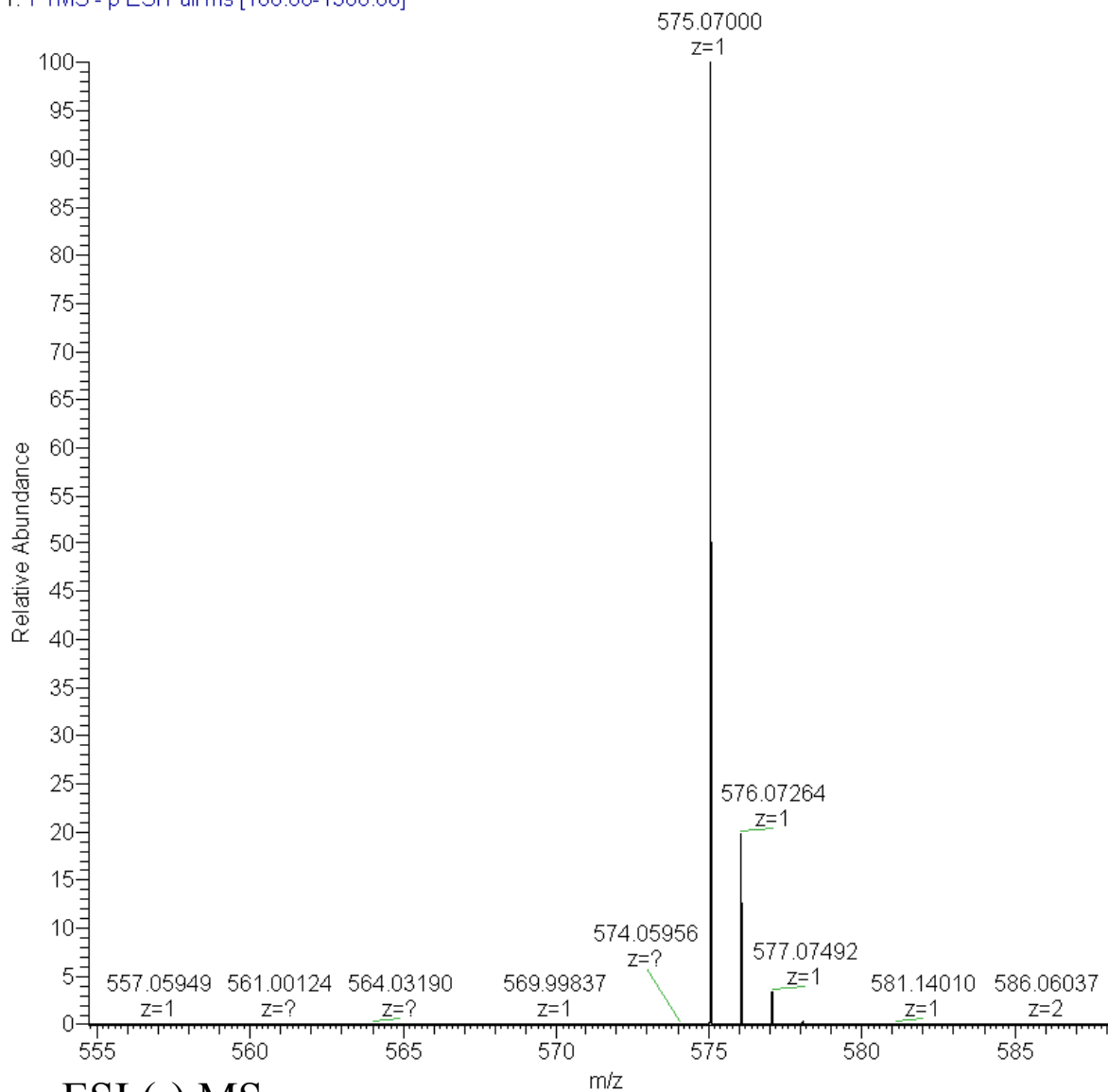
# Ant-m<sup>7</sup>GDP

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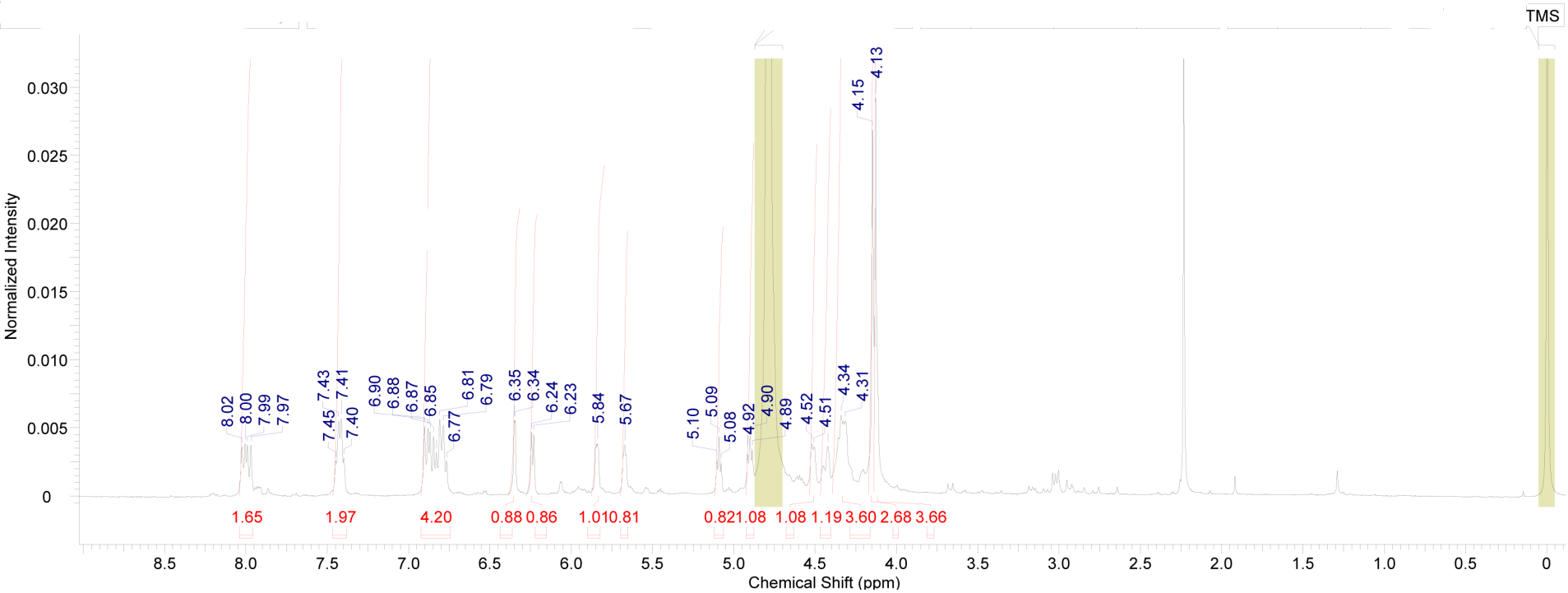
# Ant-m<sup>7</sup>GDP

11229ant\_m7gdp #9-80 RT: 0.16-1.43 AV: 72 NL: 2.61E5  
T: FTMS - p ESI Full ms [100.00-1300.00]



ESI (-) MS

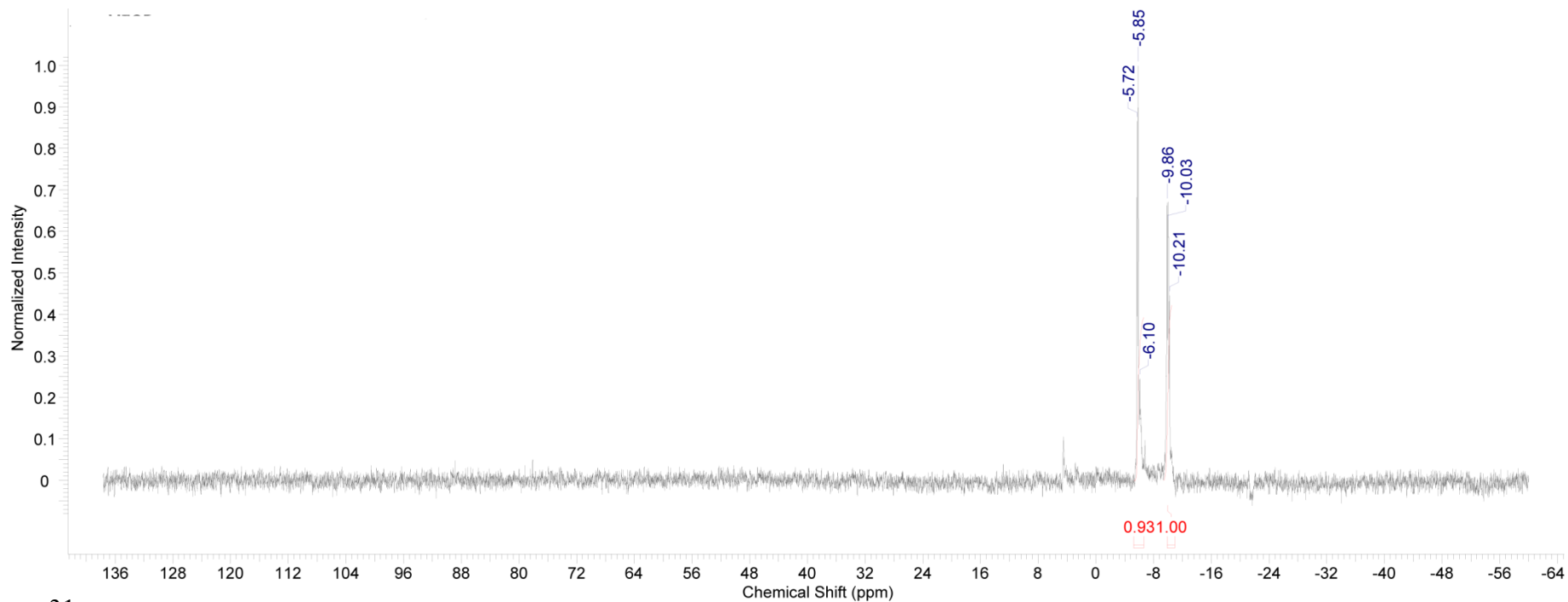
# Ant-m<sup>7</sup>GDP



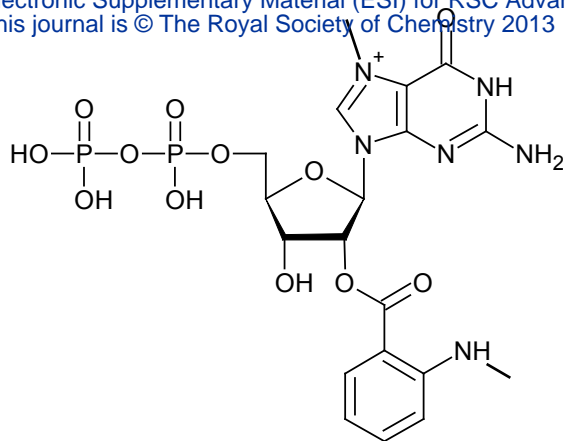
**<sup>1</sup>H NMR**

# Ant-m<sup>7</sup>GDP

**Ziemiak *et al.***  
Synthesis and evaluation of fluorescent  
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**supplementary information**

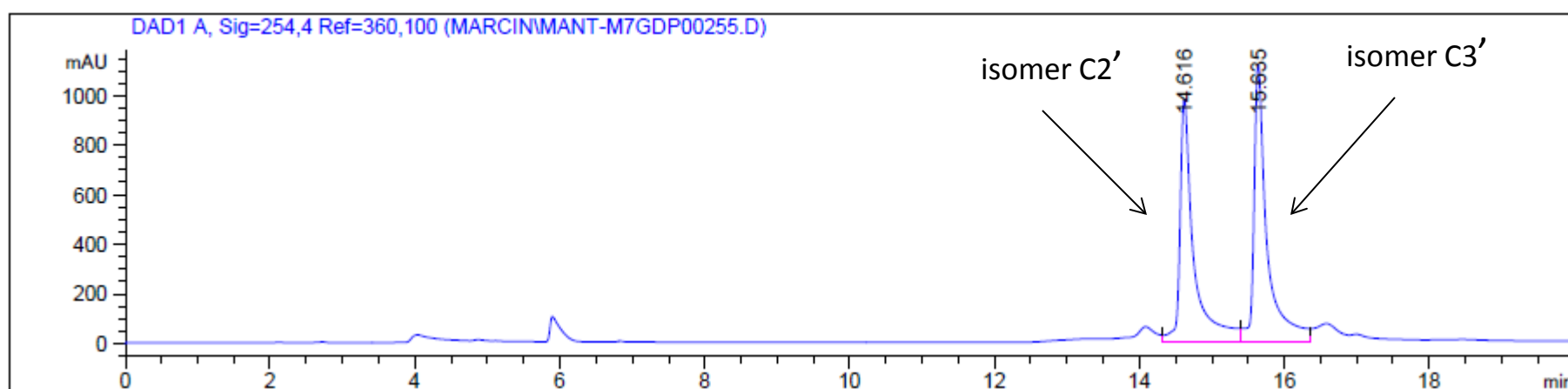


<sup>31</sup>P NMR



# Mant-m<sup>7</sup>GDP

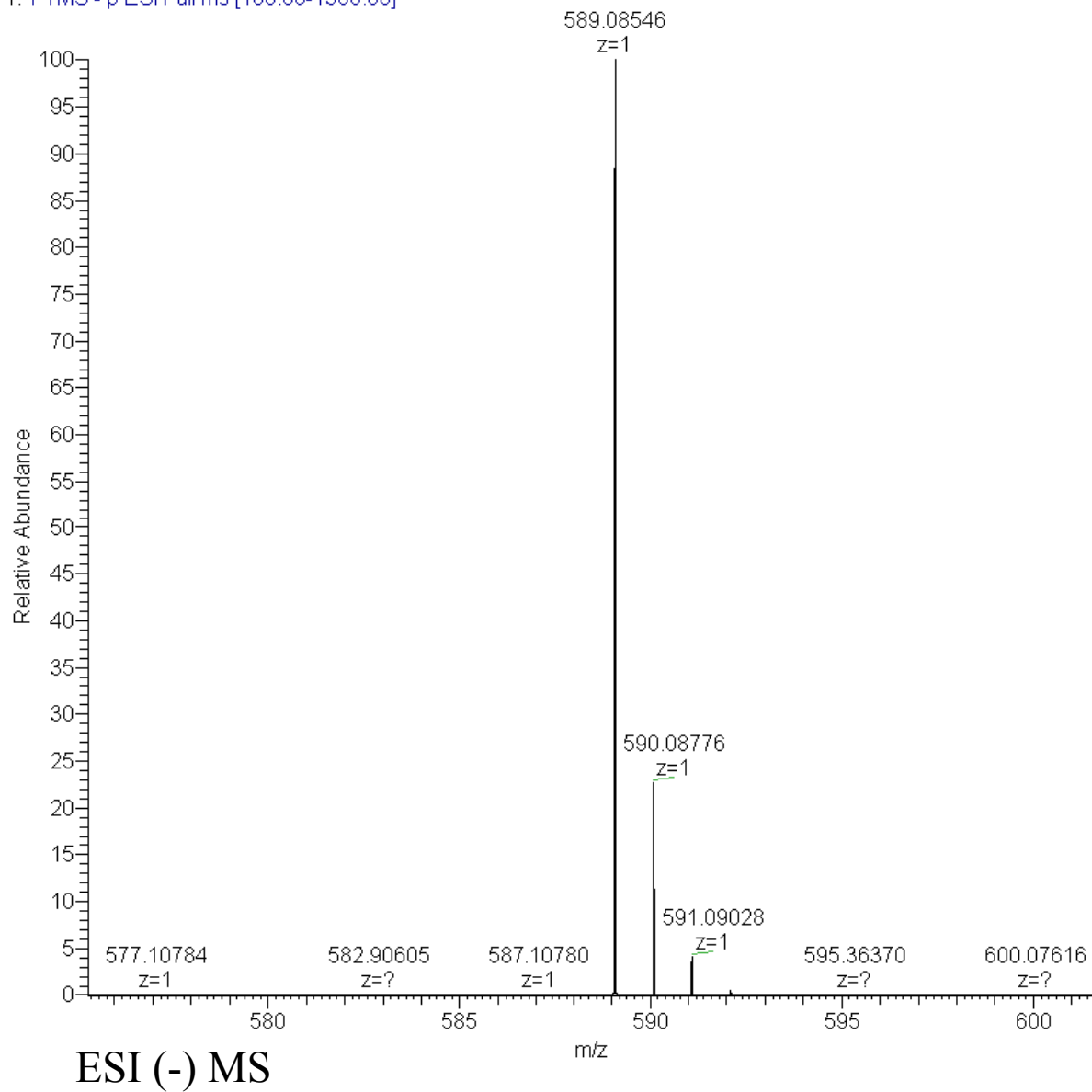
**Ziemiak *et al.***  
Synthesis and evaluation of fluorescent  
cap analogues for mRNA labelling  
**supplementary information**



HPLC profile

# Mant-m<sup>7</sup>GDP

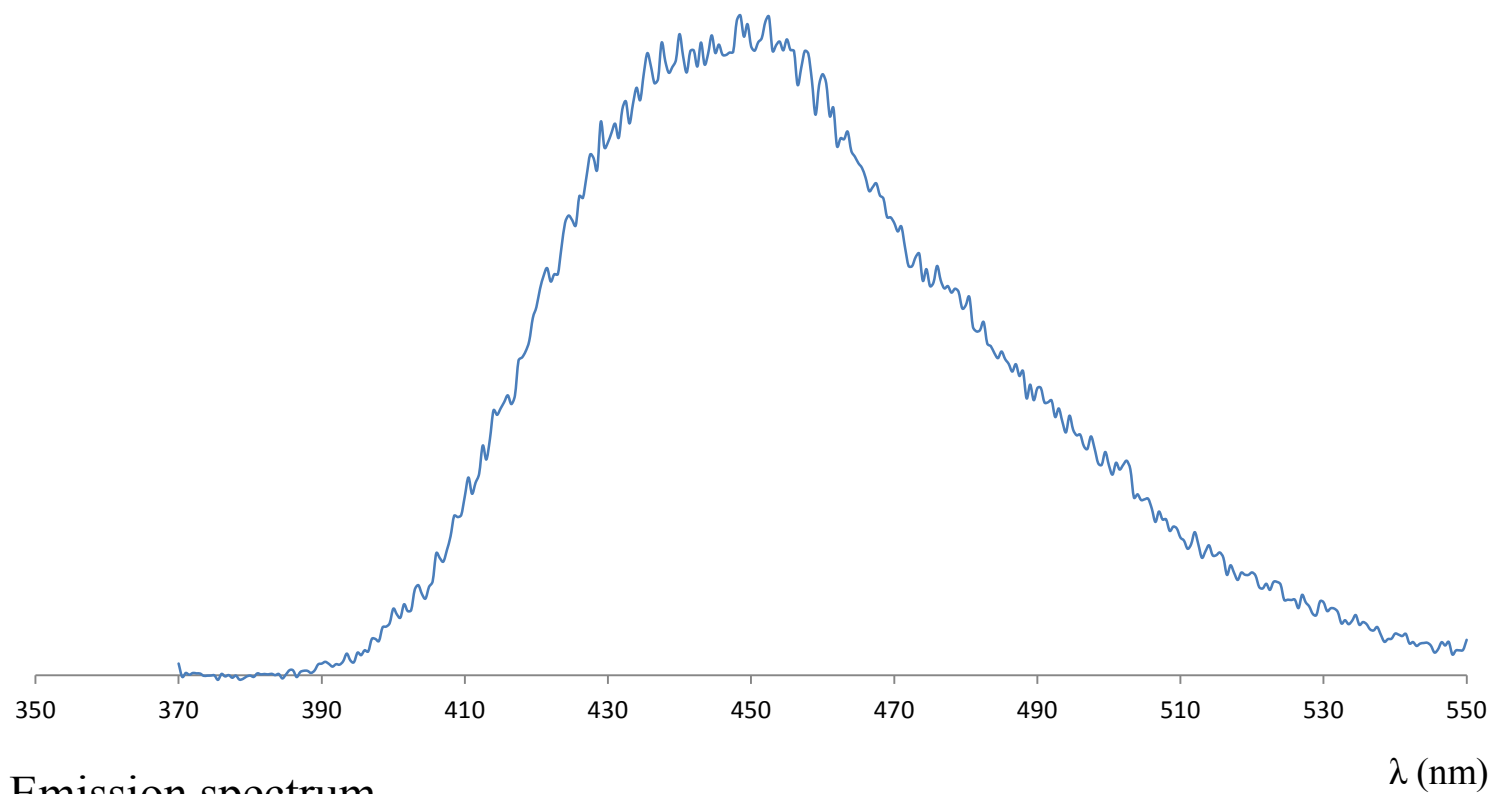
11229mant\_m7gdp #16-63 RT: 0.28-1.13 AV: 48 NL: 3.98E5  
T: FTMS - p ESI Full ms [100.00-1300.00]





# Mant-m<sup>7</sup>GDP

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

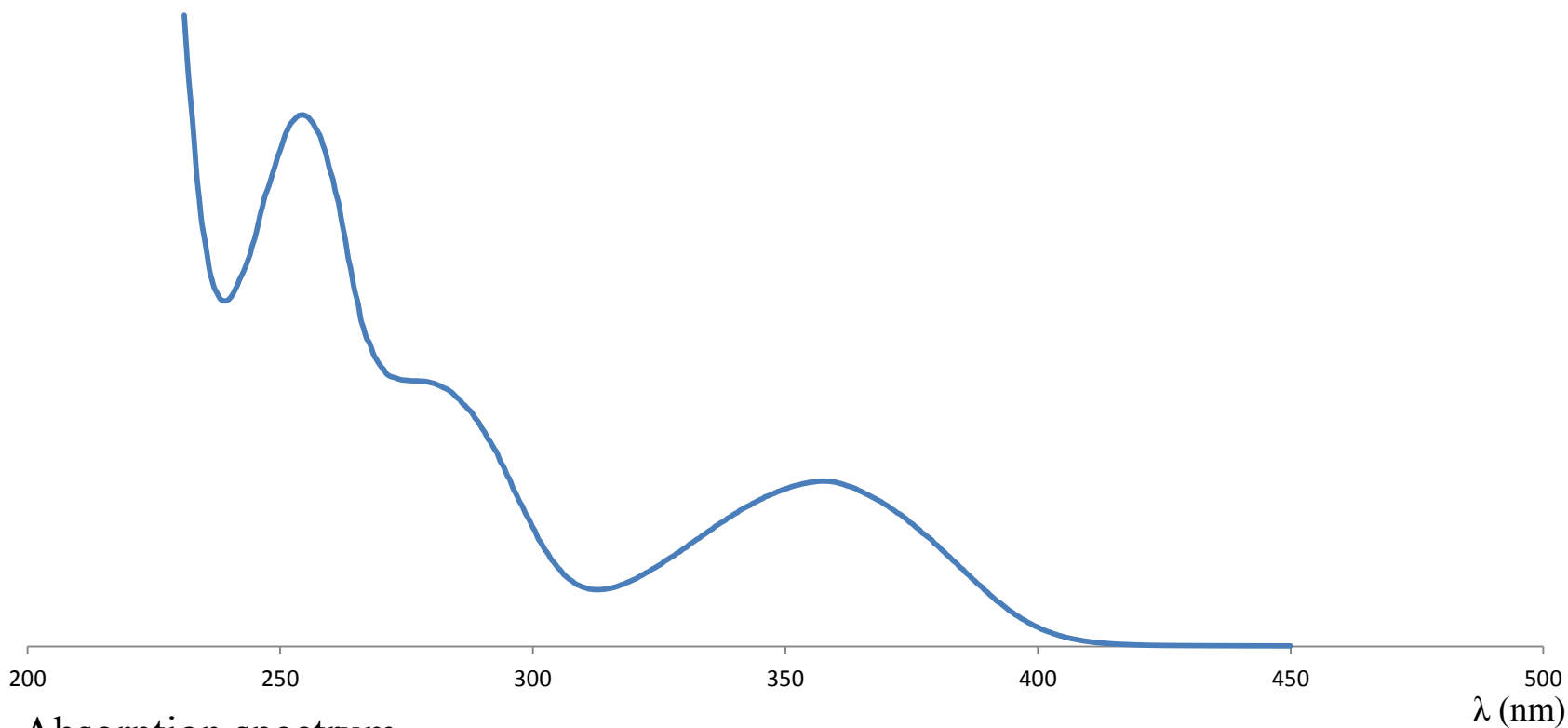


Emission spectrum

λ (nm)

# Mant-m<sup>7</sup>GDP

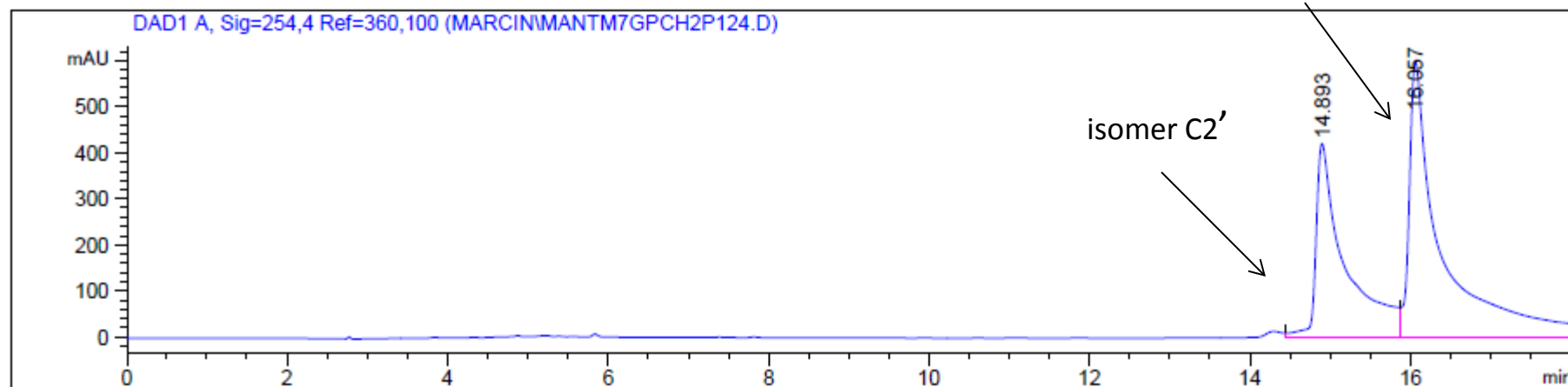
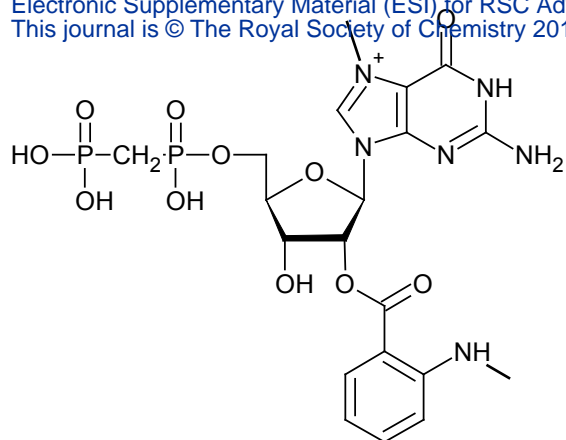
**Ziemniak *et al.***  
Synthesis and evaluation of fluorescent  
cap analogues for mRNA labelling  
**supplementary information**



Absorption spectrum

λ (nm)

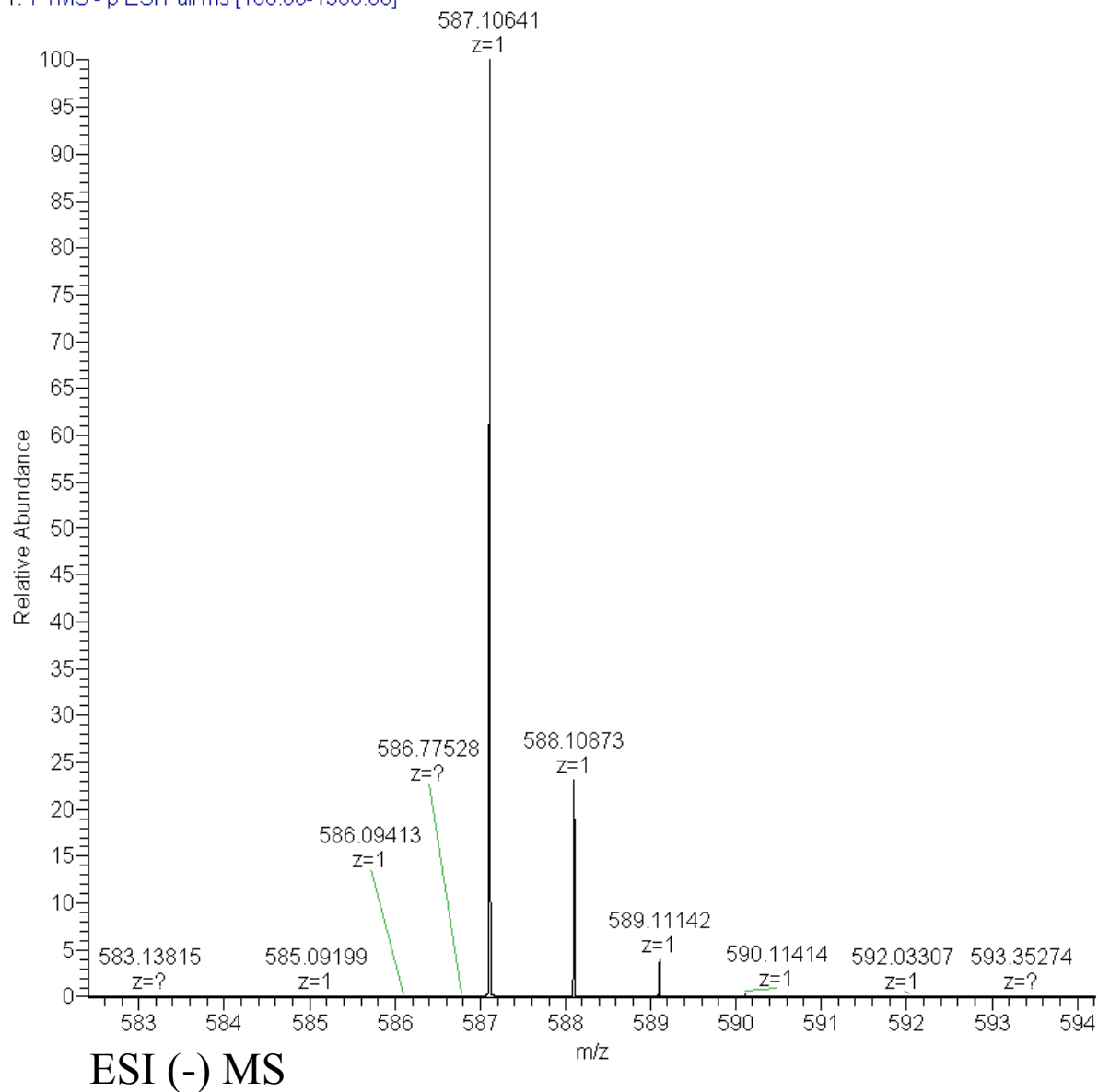
# Mant-m<sup>7</sup>GpCH<sub>2</sub>p



HPLC profile

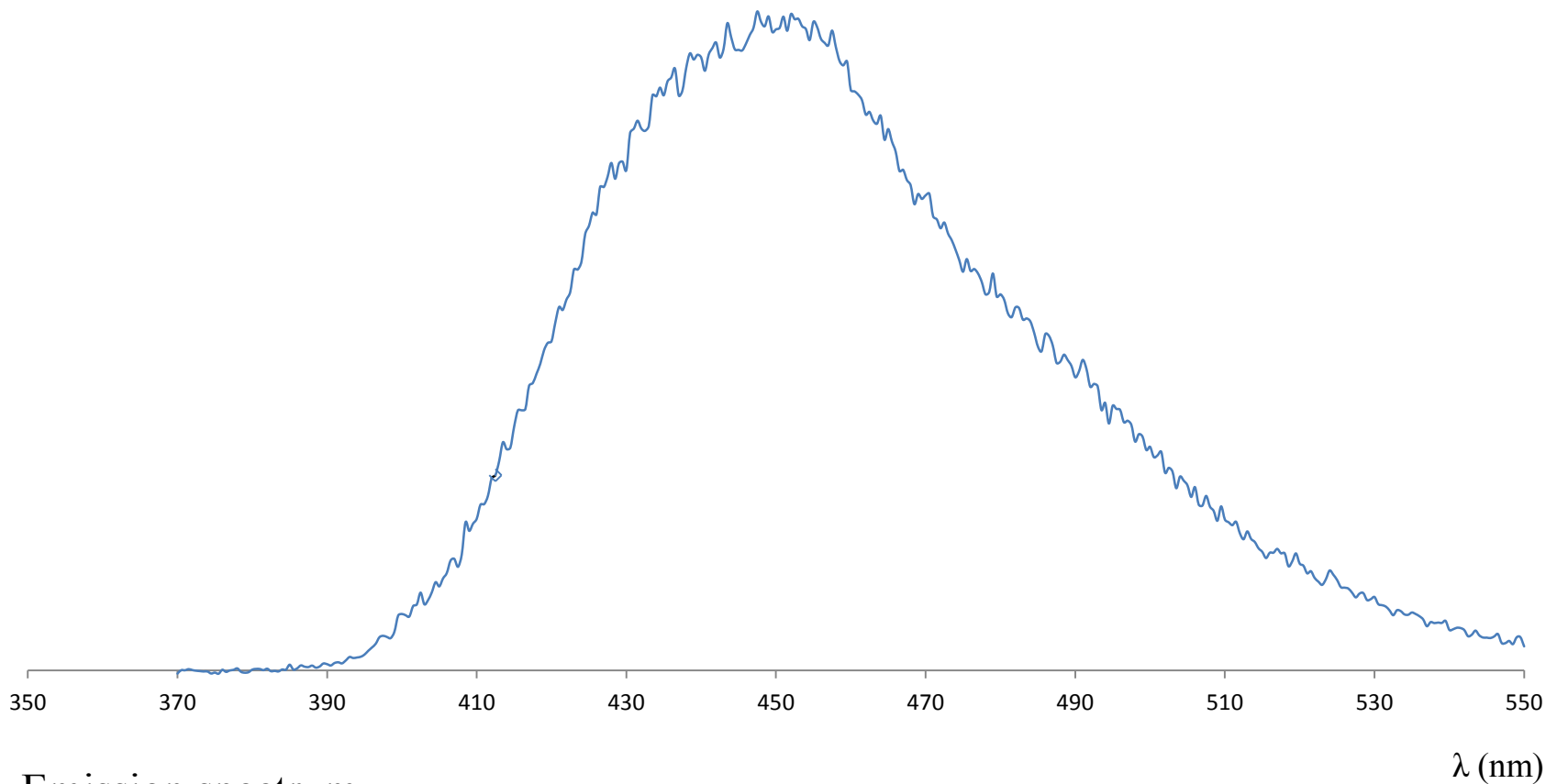
# Mant-m<sup>7</sup>GpCH<sub>2</sub>p

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-m<sup>7</sup>GpCH<sub>2</sub>p

**Ziemniak *et al.***  
Synthesis and evaluation of fluorescent  
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**supplementary information**

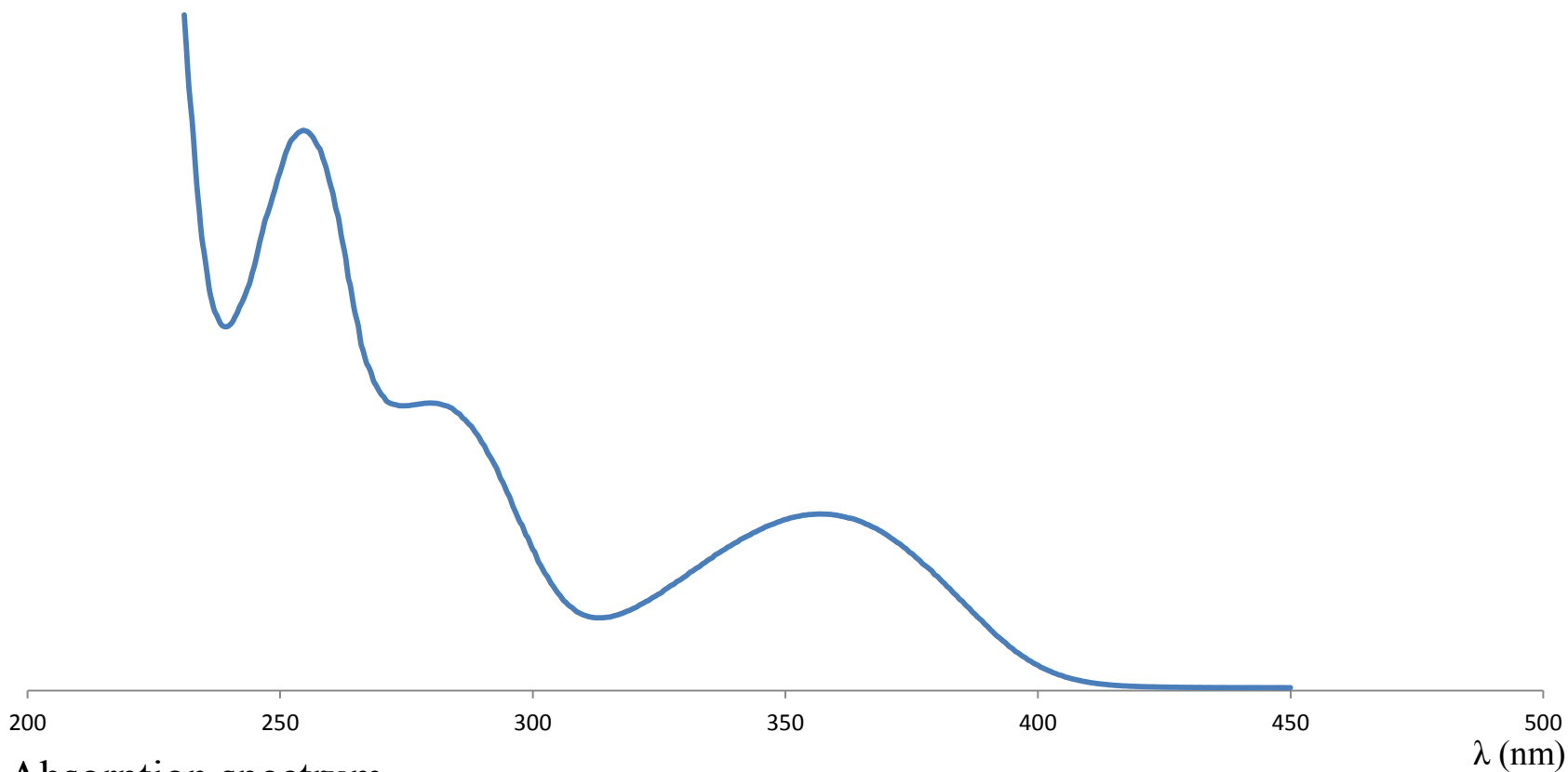


Emission spectrum

λ (nm)

# Mant-m<sup>7</sup>GpCH<sub>2</sub>p

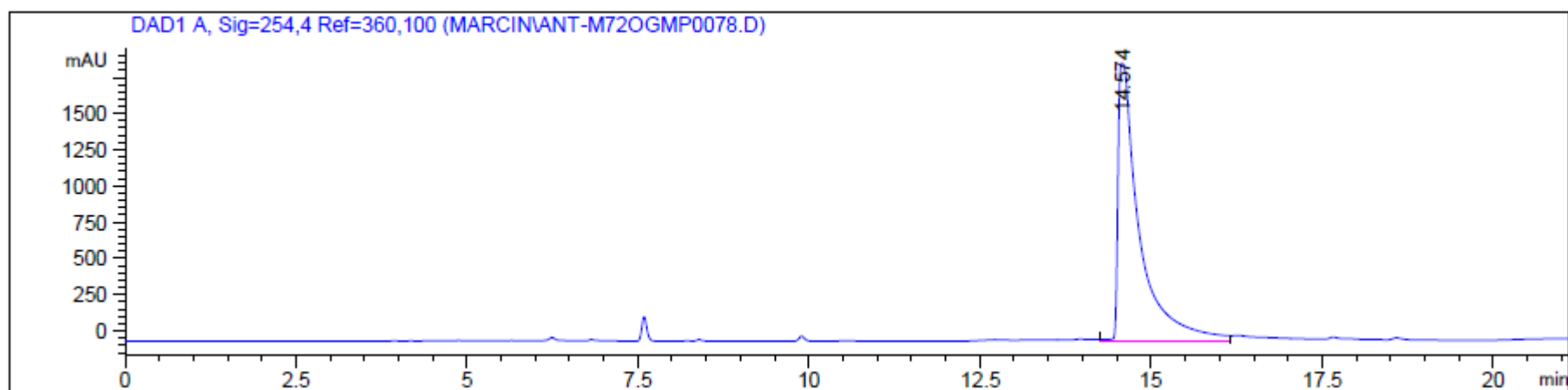
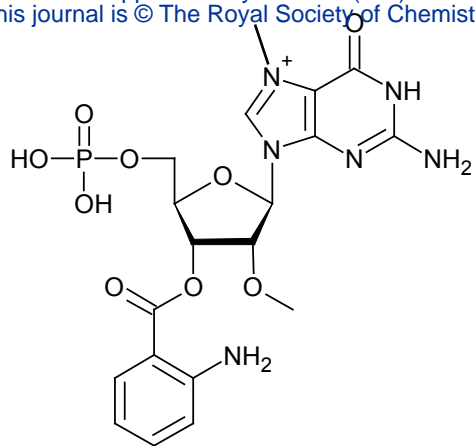
**Ziemniak *et al.***  
Synthesis and evaluation of fluorescent  
cap analogues for mRNA labelling  
**supplementary information**



Absorption spectrum

λ (nm)

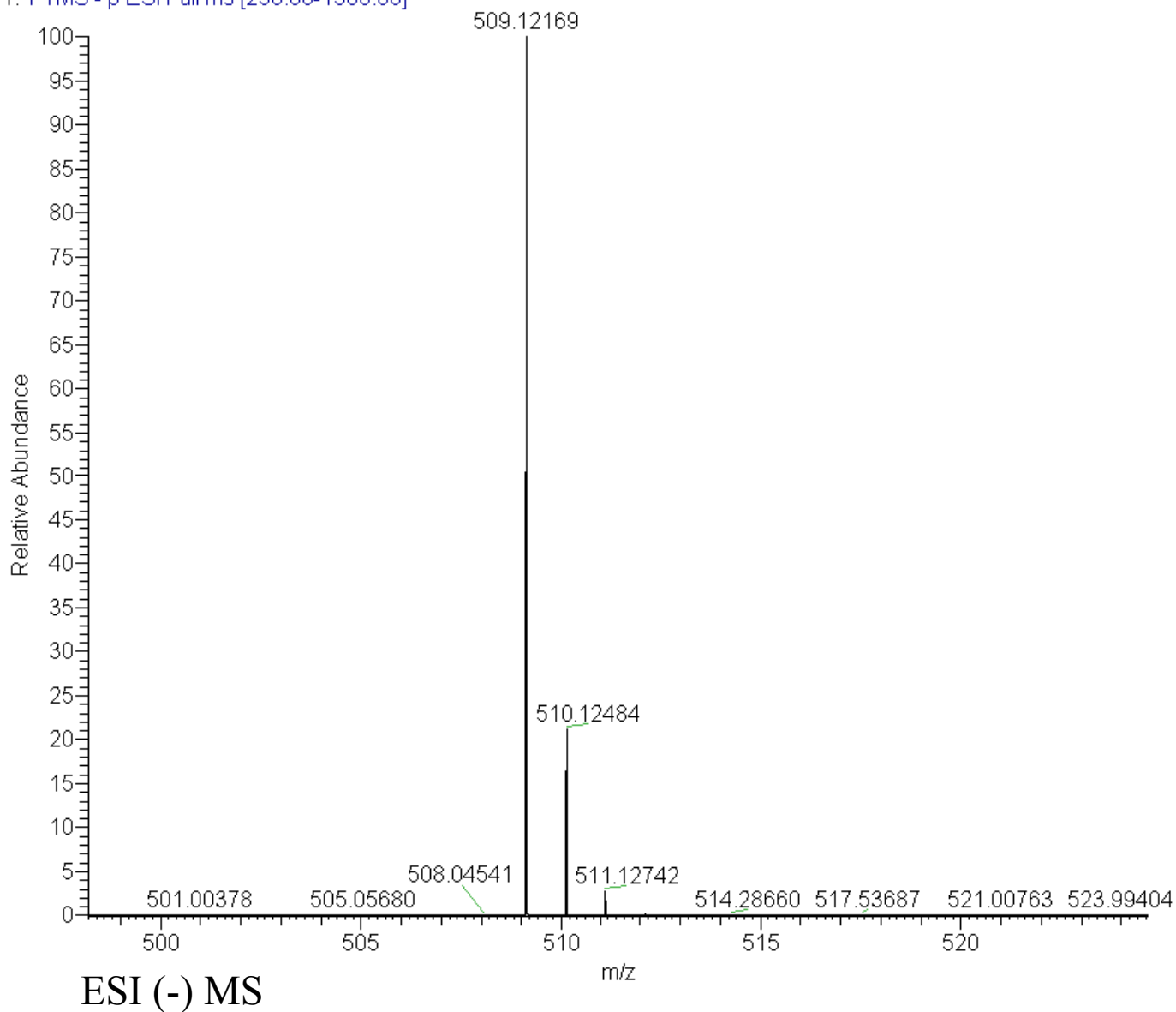
# Ant-m<sub>2</sub><sup>7,2'-O</sup>GMP



HPLC profile

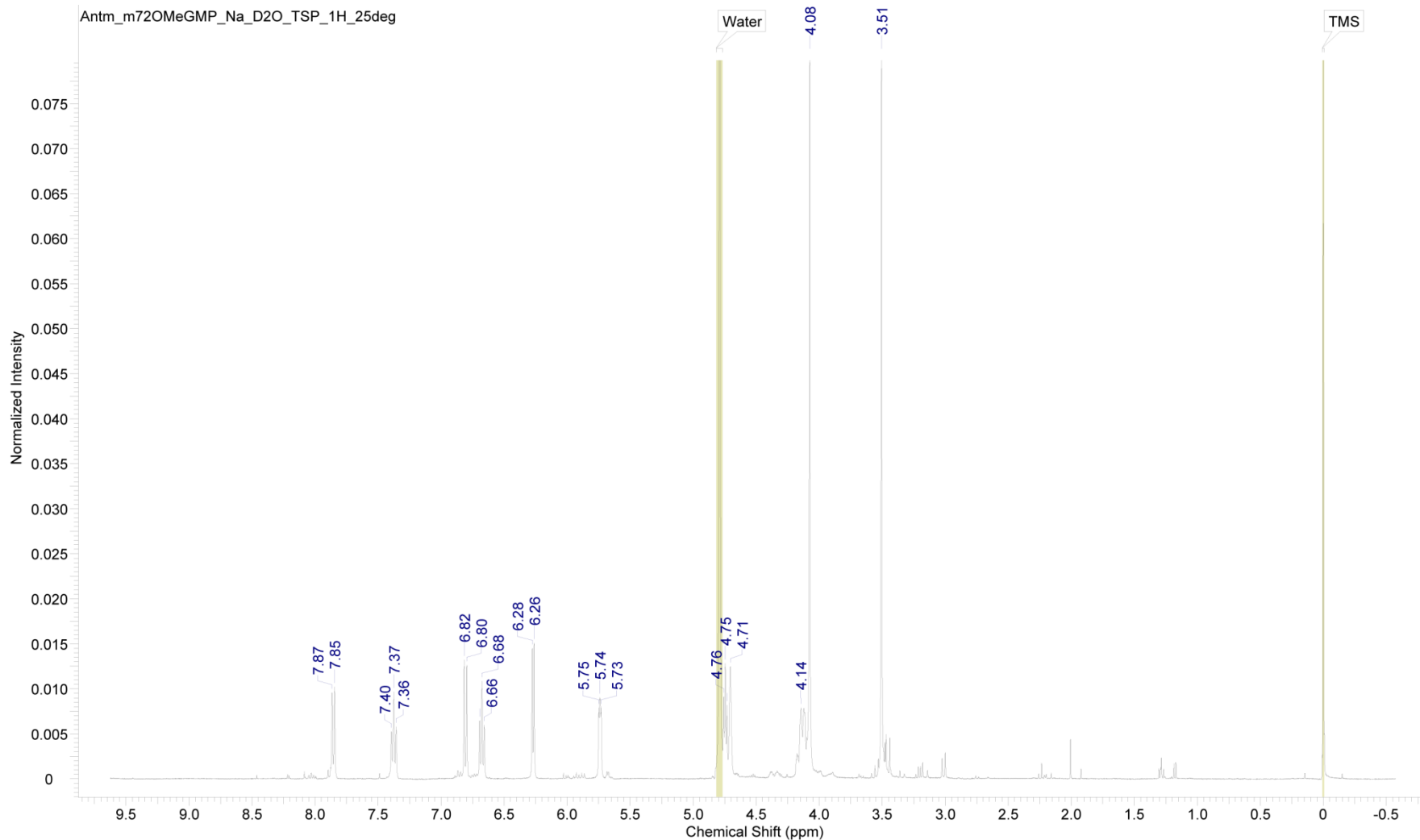
# Ant-m<sub>2</sub><sup>7,2'-O</sup>GMP

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]





# Ant-m<sub>2</sub><sup>7,2'-O</sup>GMP

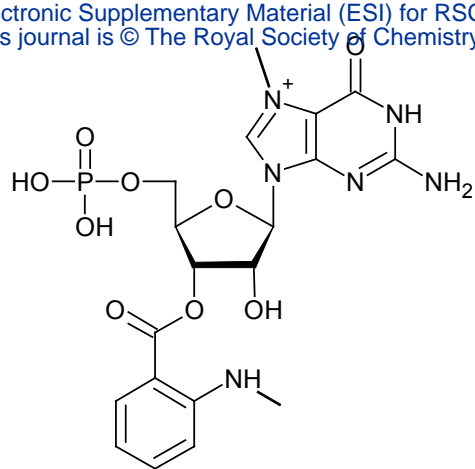


<sup>1</sup>H NMR

# Ant-m<sub>2</sub><sup>7,2'-O</sup>GMP

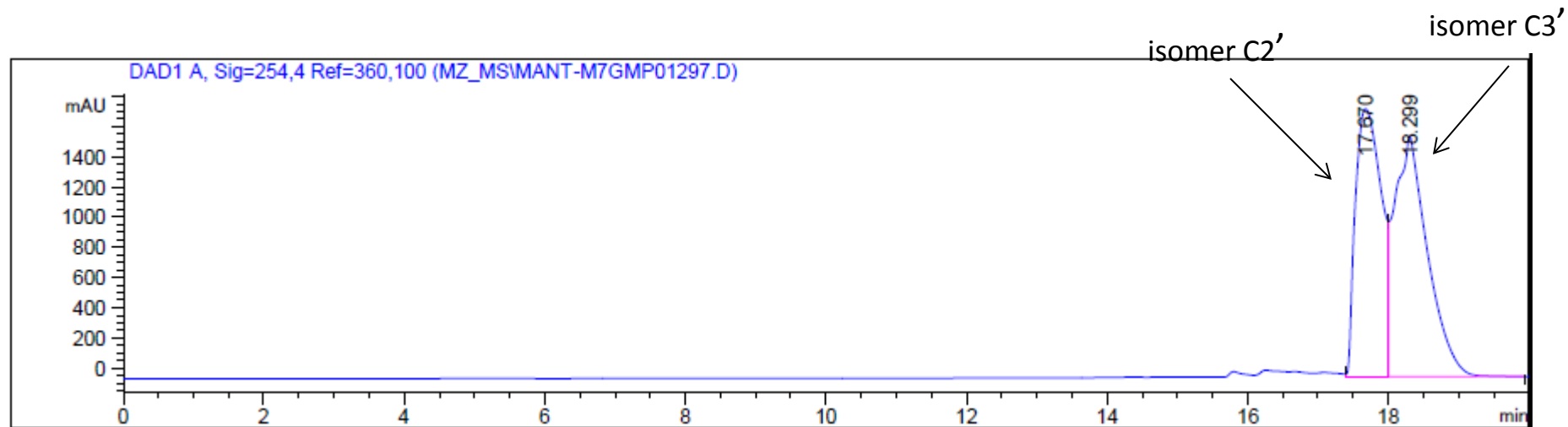


<sup>31</sup>P NMR



# Mant-m<sup>7</sup>GMP

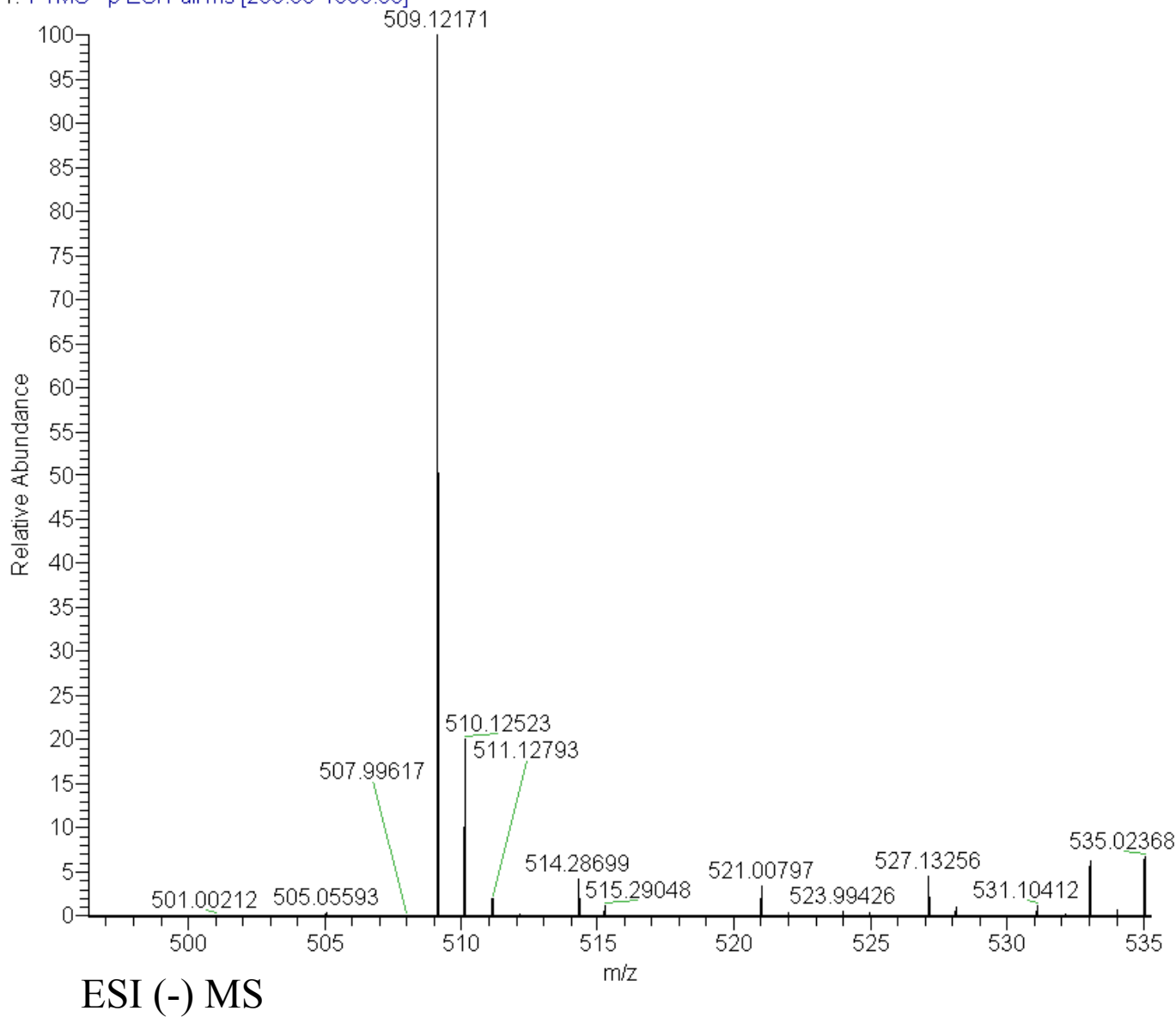
**Ziemiak *et al.***  
Synthesis and evaluation of fluorescent  
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**supplementary information**



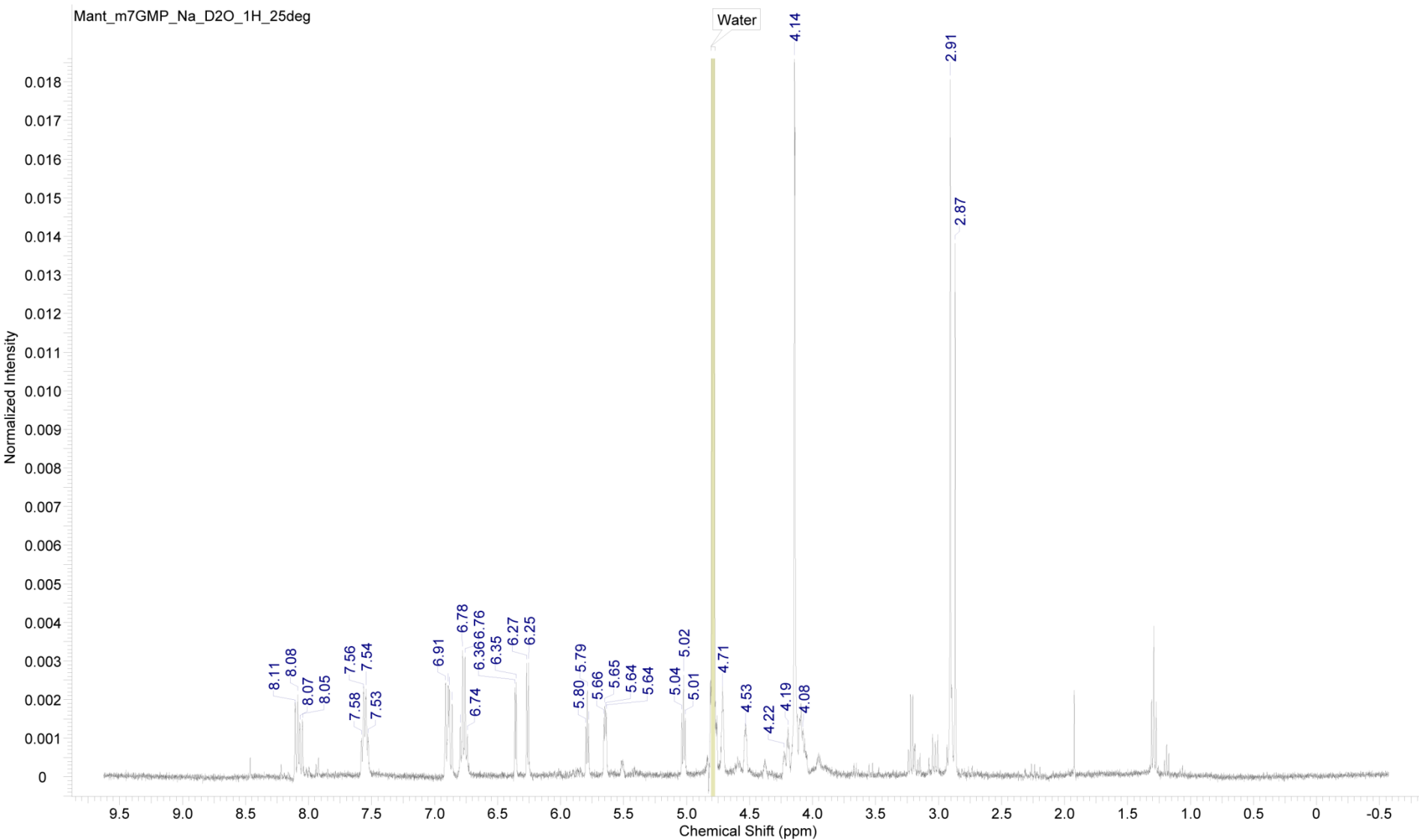
HPLC profile

# Mant-m<sup>7</sup>GMP

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]

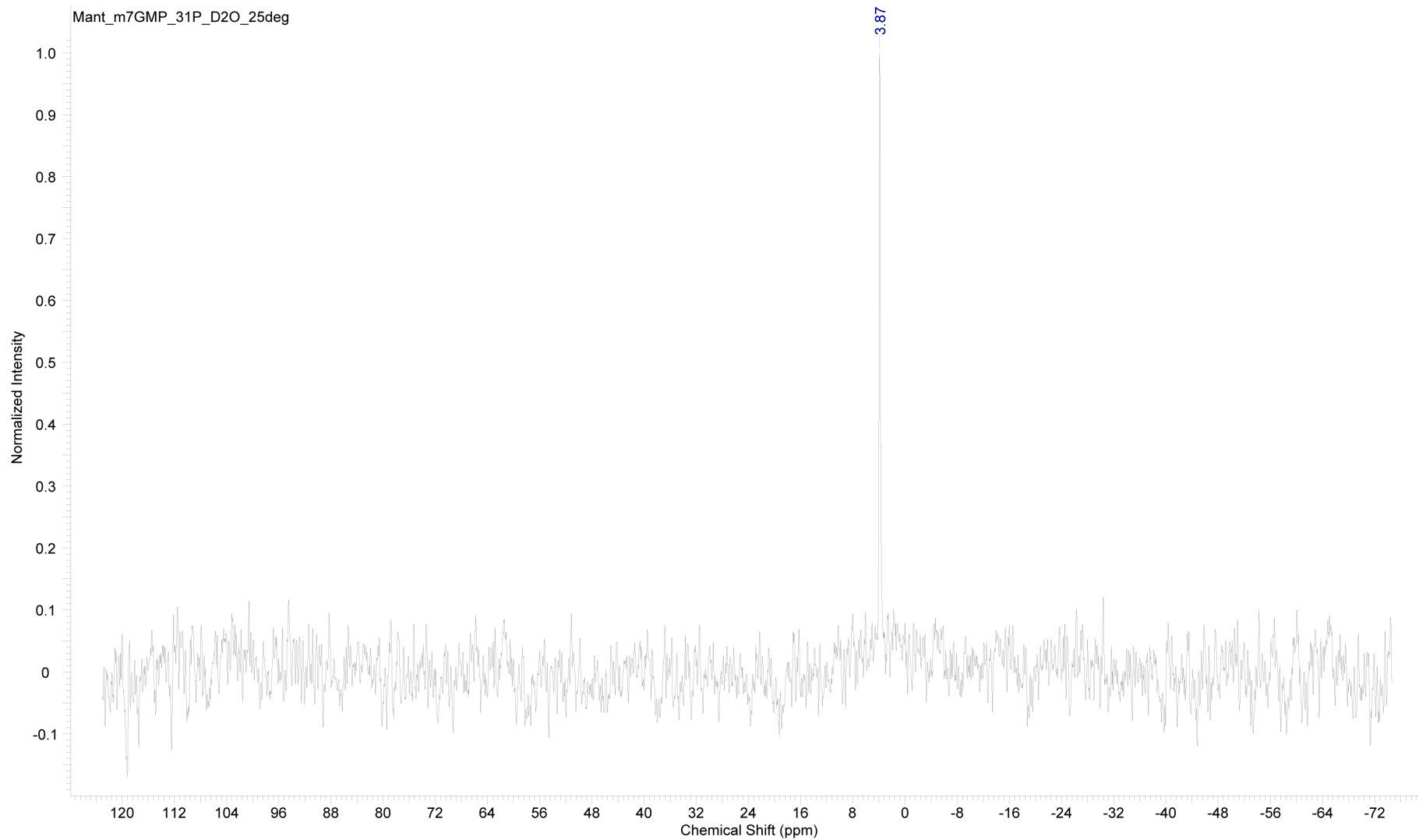


# Mant-m<sup>7</sup>GMP



<sup>1</sup>H NMR

# Mant-m<sup>7</sup>GMP



<sup>31</sup>P NMR