Supplementary Material (ESI) for New Journal of Chemistry

Towards mRNA with superior translational activity: synthesis and properties of ARCA tetraphosphates with single phosphorothioate modifications

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Supplementary information

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Table S1. Chemical shift differences of selected signals in ^{1}H and ^{31}P NMR between D1 and D2 diastereomers of compounds **1**–**6**

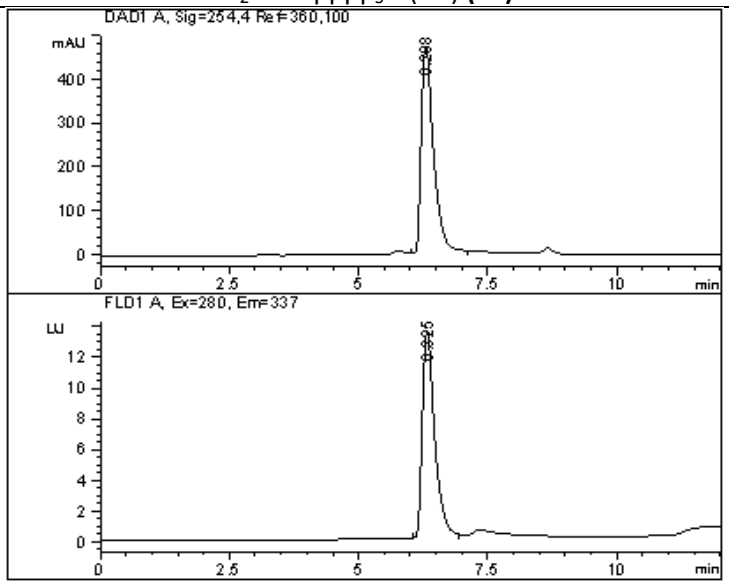
	$\Delta\delta_H$ (ppm) (δ D1 – δ D2)[a,b]			
Compound	H8 Guo	H8 $m_2^{7,2'\text{-O}}$Guo	H1' G	H1' $m_2^{7,2'\text{-O}}$Guo
1	0.02	0.00	0.00	0.00
2	–0.01	0.00	–0.01	0.00
3	0.00	–0.02	–0.02	–0.04
4	0.00	0.02	0.00	0.03
5	0.07	—	–0.005	—
6	—	0.03	—	0.01

	$\Delta\delta_P$ (ppm) (δ D1 – δ D2)[a,b]			
Compound	Pα	Pβ	Pγ	Pδ
1	0.25	–0.02	0.00	0.00
2	0.00	–0.14	0.02	0.00
3	0.00	0.00	0.00	0.00
4	0.00	0.00	–0.01	0.34
5	0.19	–0.03	0.00	—
6	0.24	–0.04	0.00	—
GTPαS (δ_{Sp}-δ_{Rp})[b]	0.20	–0.28	0.00	—

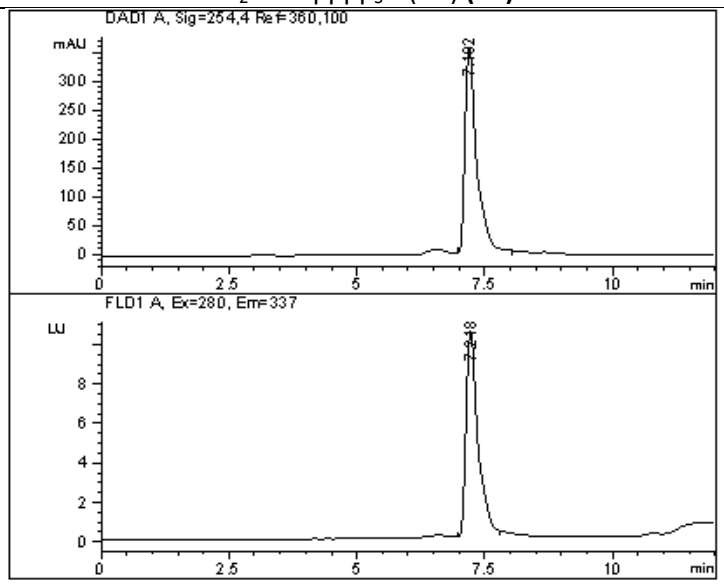
[a] D1 refers to the diastereomer with shorter retention on RP HPLC column [b]the Δδ values are calculated from NMR spectra of mixtures containing unequal amounts of the D1 and D2 diastereomers [b]data from J. Ludwig and F. Eckstein, *J. Org. Chem.*, 1989**, 54**, 631-635.

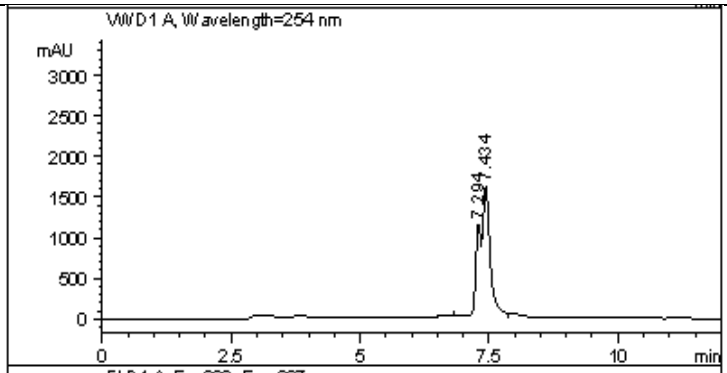
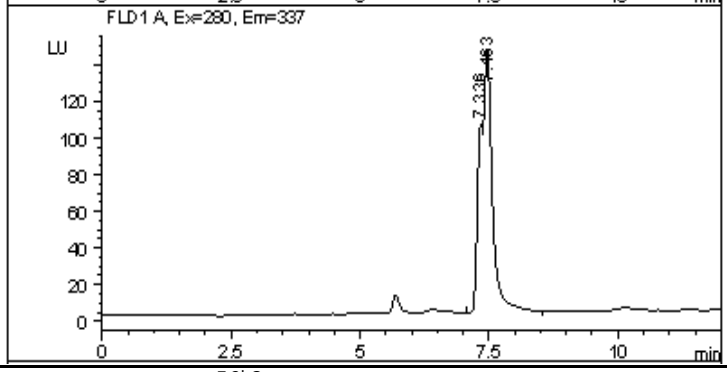
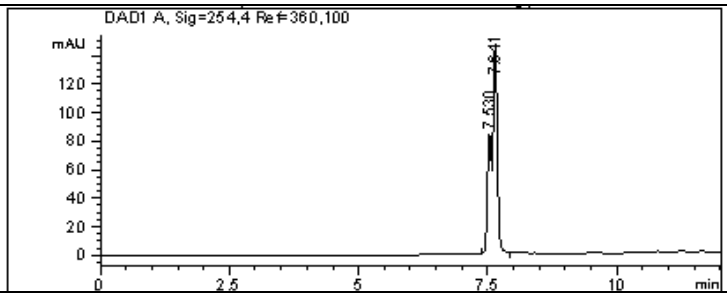
RP HPLC profiles of compounds **1**-**4**

$m_2^{7,2'\text{-}O}Gppp_SpG$ (D1) **(1a)**

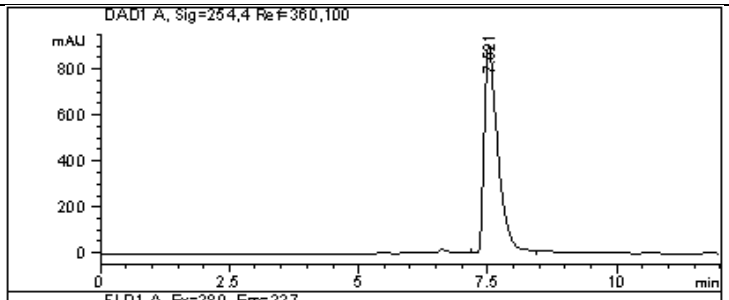
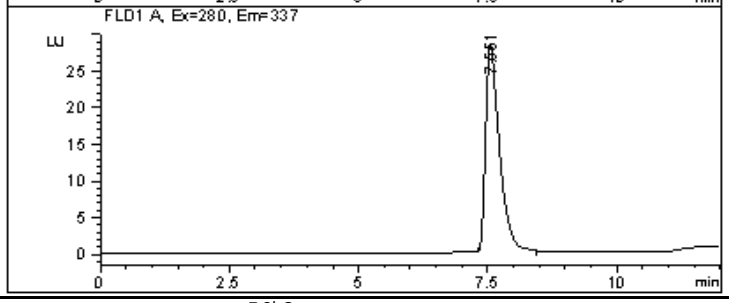
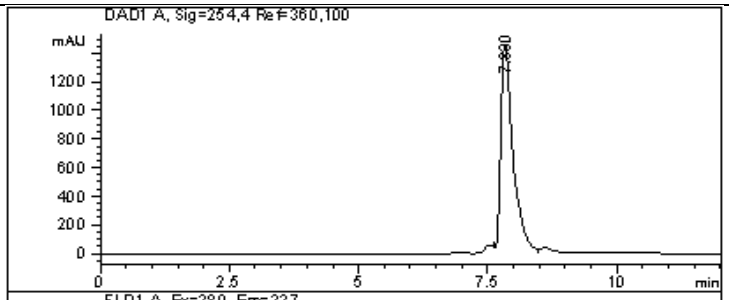
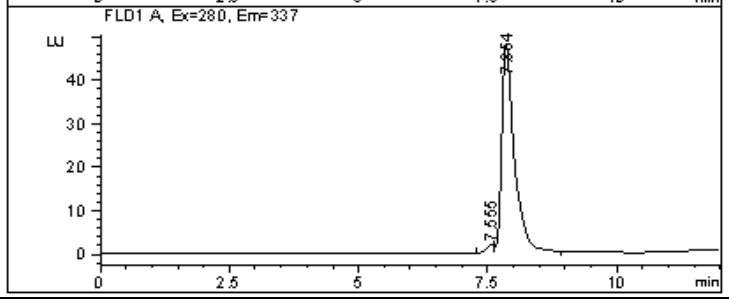


$m_2^{7,2'\text{-}O}Gppp_SpG$ (D2) **(1b)**

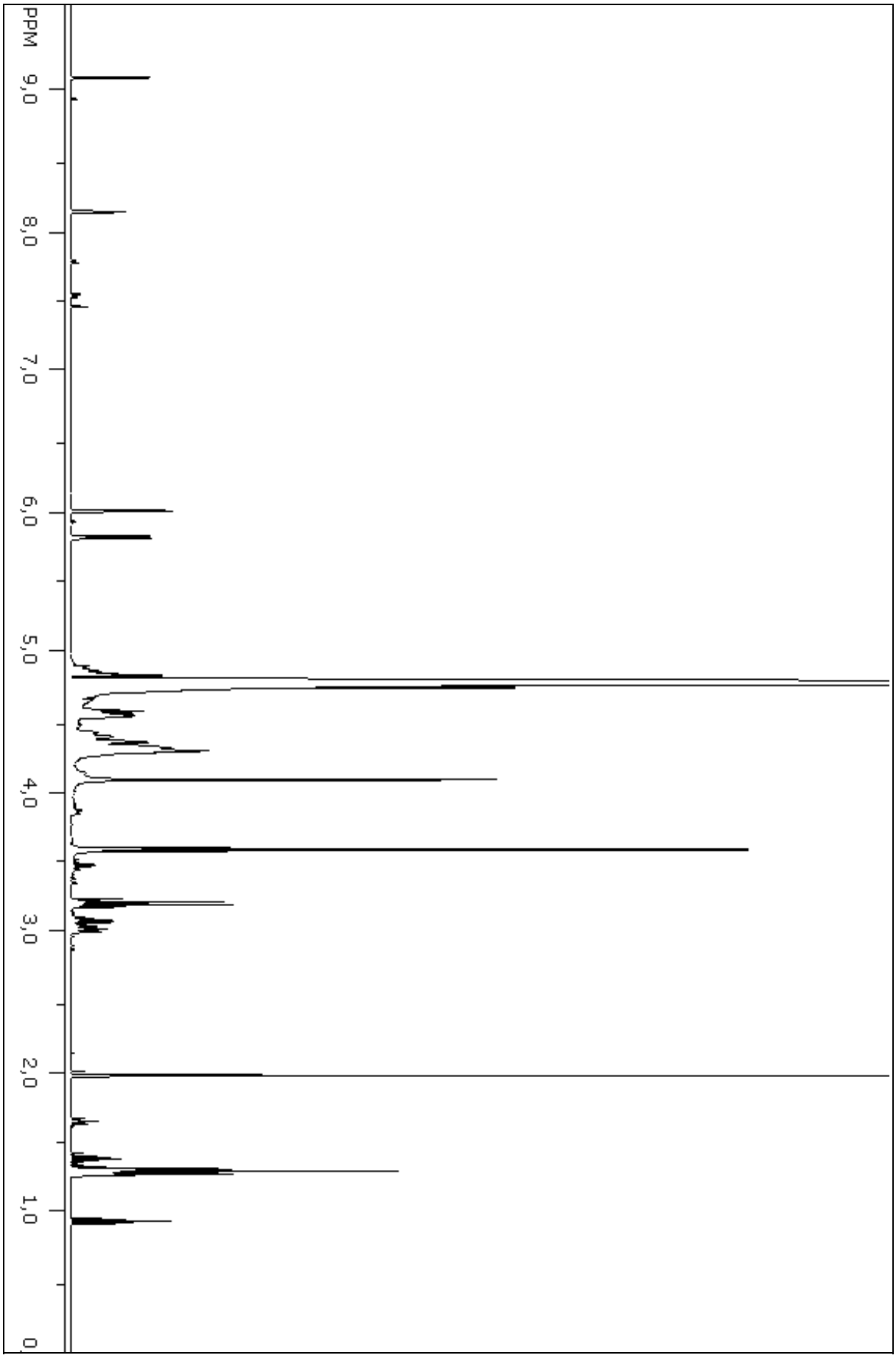


	$m_2^{7,2'\text{-}O}Gppp_SpG$ (D1:D2) **(2a,b)**
Absorbance	
Fluorescence	
	$m_2^{7,2'\text{-}O}Gpp_SppG$ (D1:D2) **(3a,b)**
Absorbance	

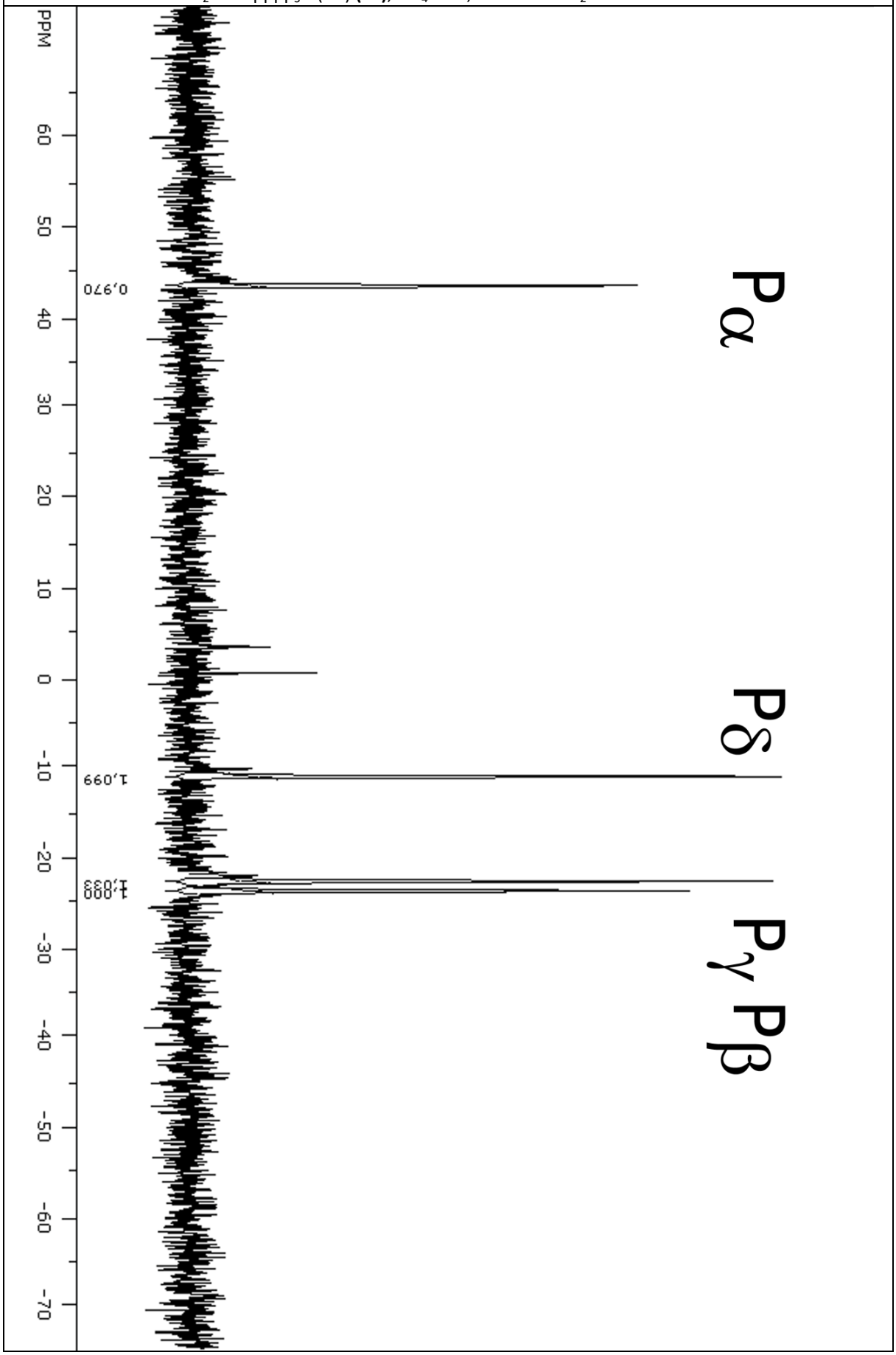
Fl	

	$m_2^{7,2'\text{-}O}Gp_SpppG$ (D1) **(4a)**
Absorbance	
Fluorescence	
	$m_2^{7,2'\text{-}O}Gp_SpppG$ (D2) **(4b)**
Absorbance	
Fluorescence	

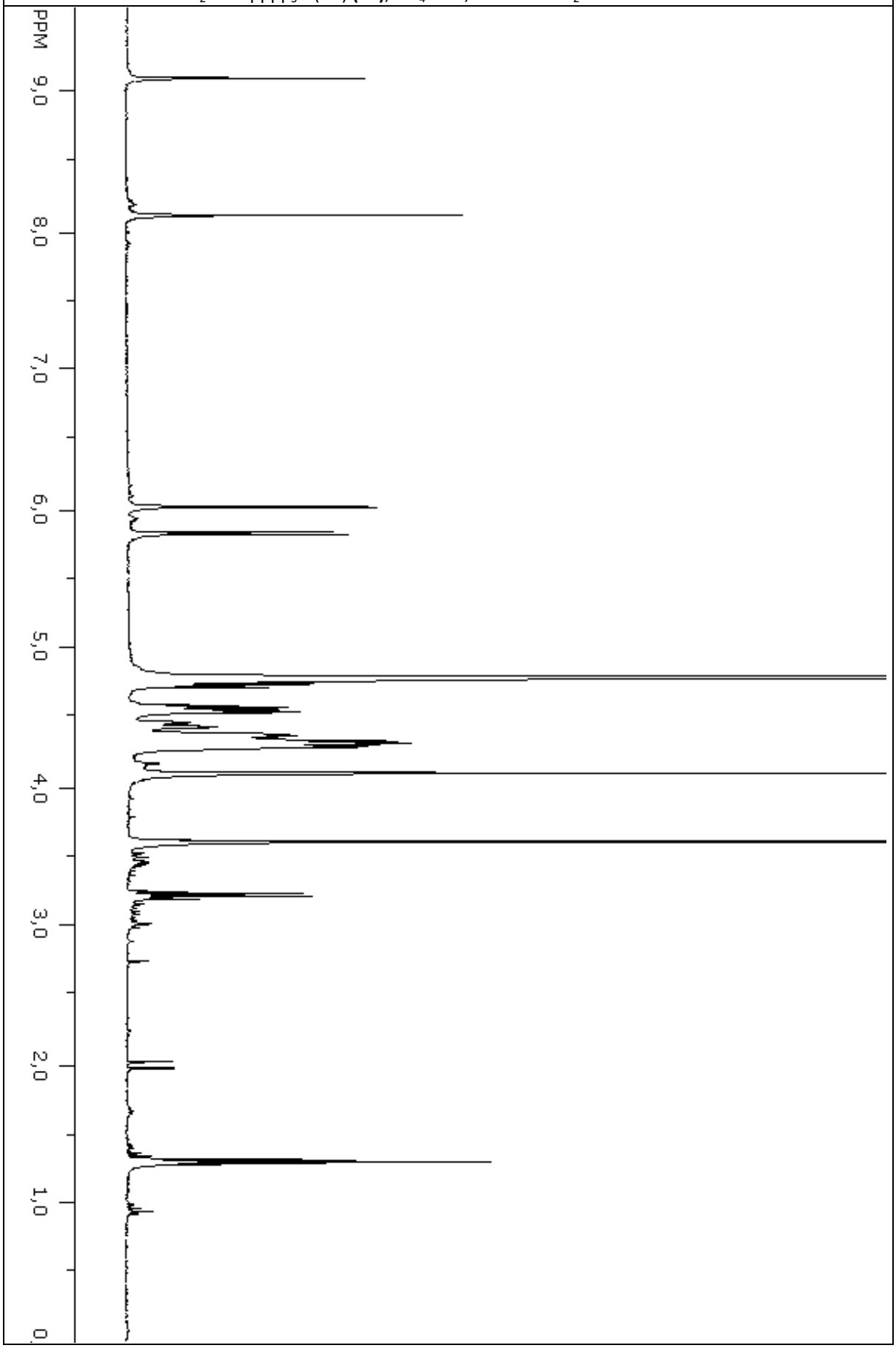
$m_2^{7,2'\text{-}O}Gpppp_SG$ (D1) **(1a)**, NH_4^+ salt, ^1H NMR in D_2O at 399.94 MHz



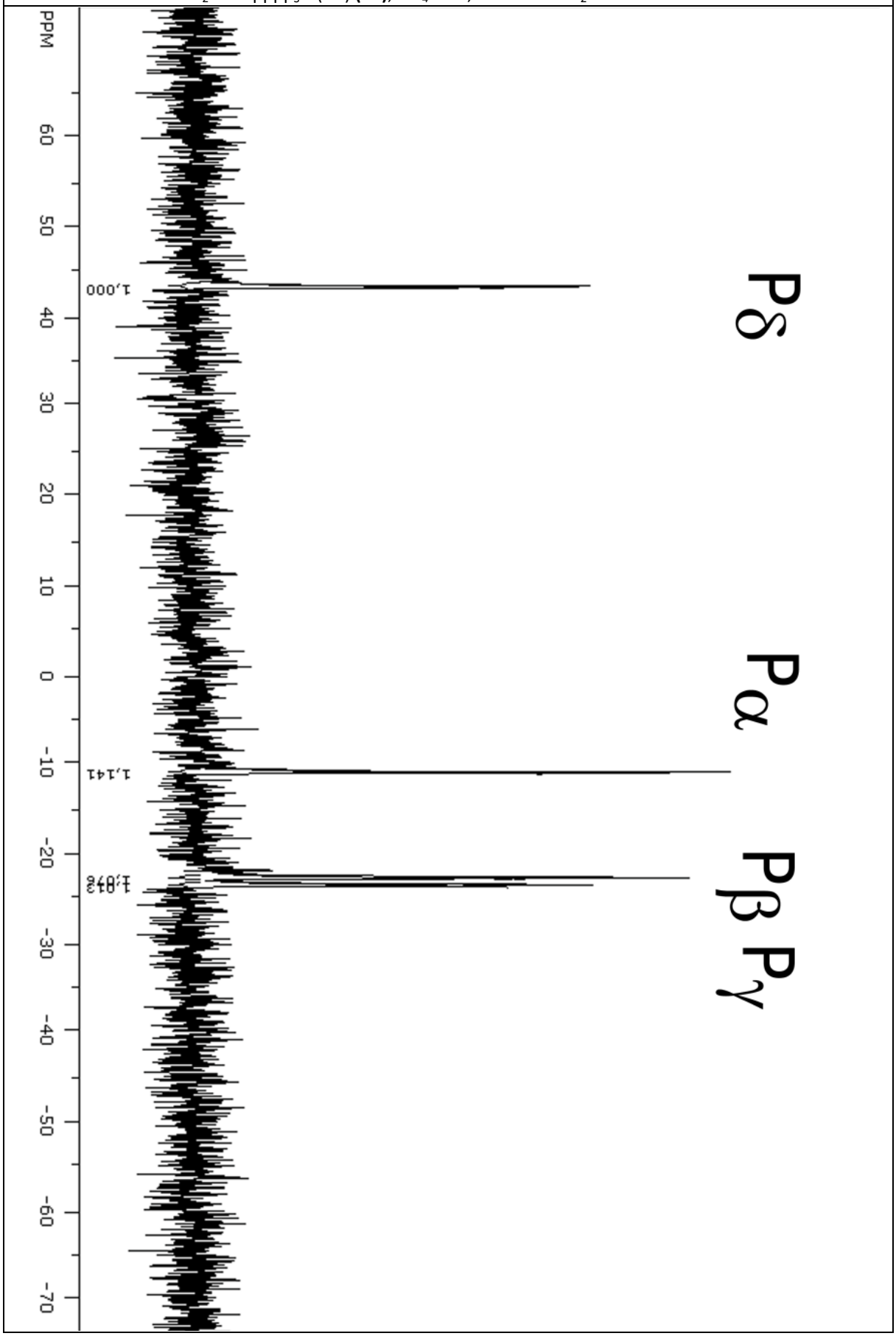
$m_2^{7,2'\text{-}O}Gpppp_SG$ (D1) **(1a)**, NH_4^+ salt, ^{31}P NMR in D_2O at 161.9 MHz



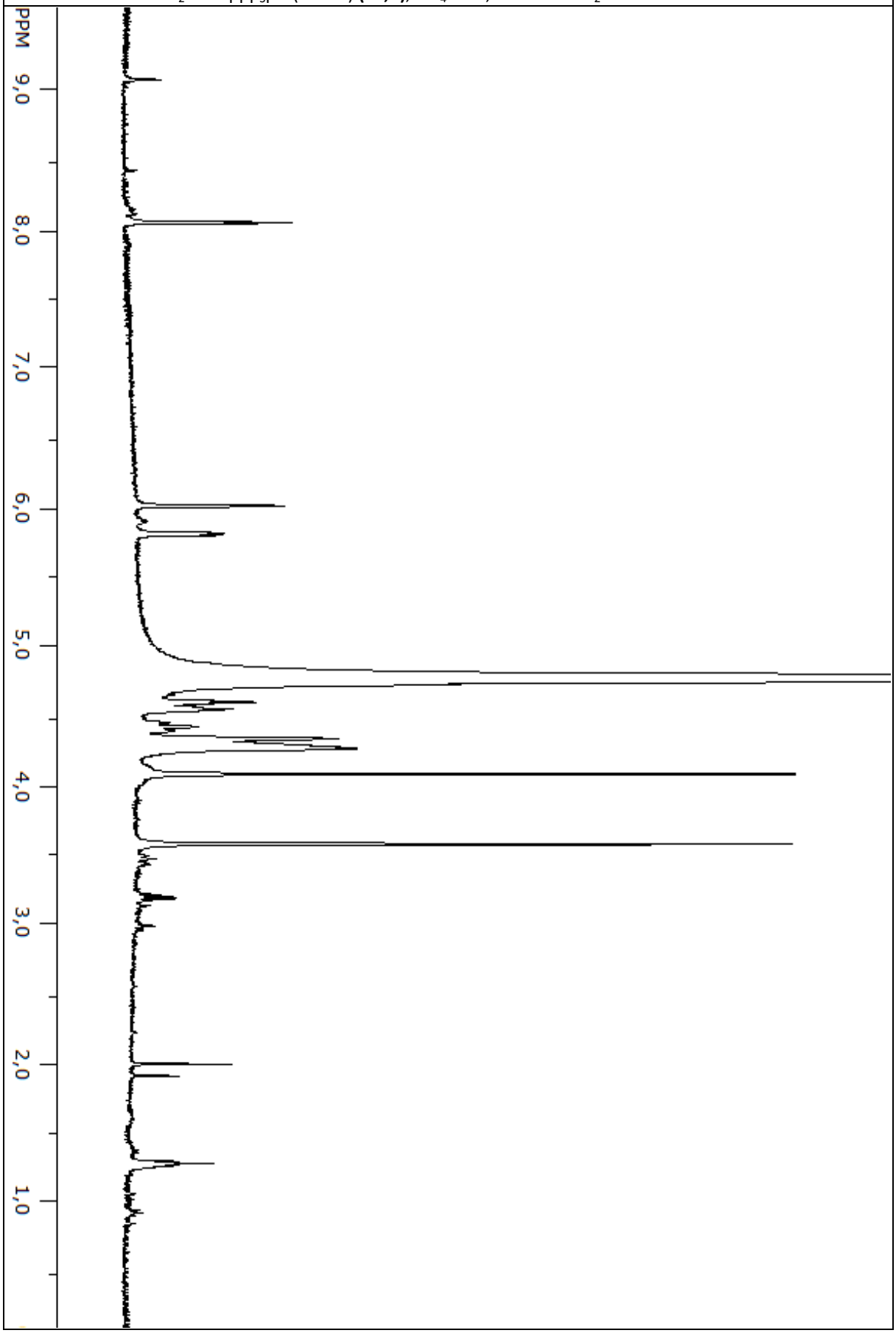
$m_2^{7,2'\text{-}O}Gpppp_SG$ (D2) **(1b)**, NH_4^+ salt, ^1H NMR in D_2O at 399.94 MHz



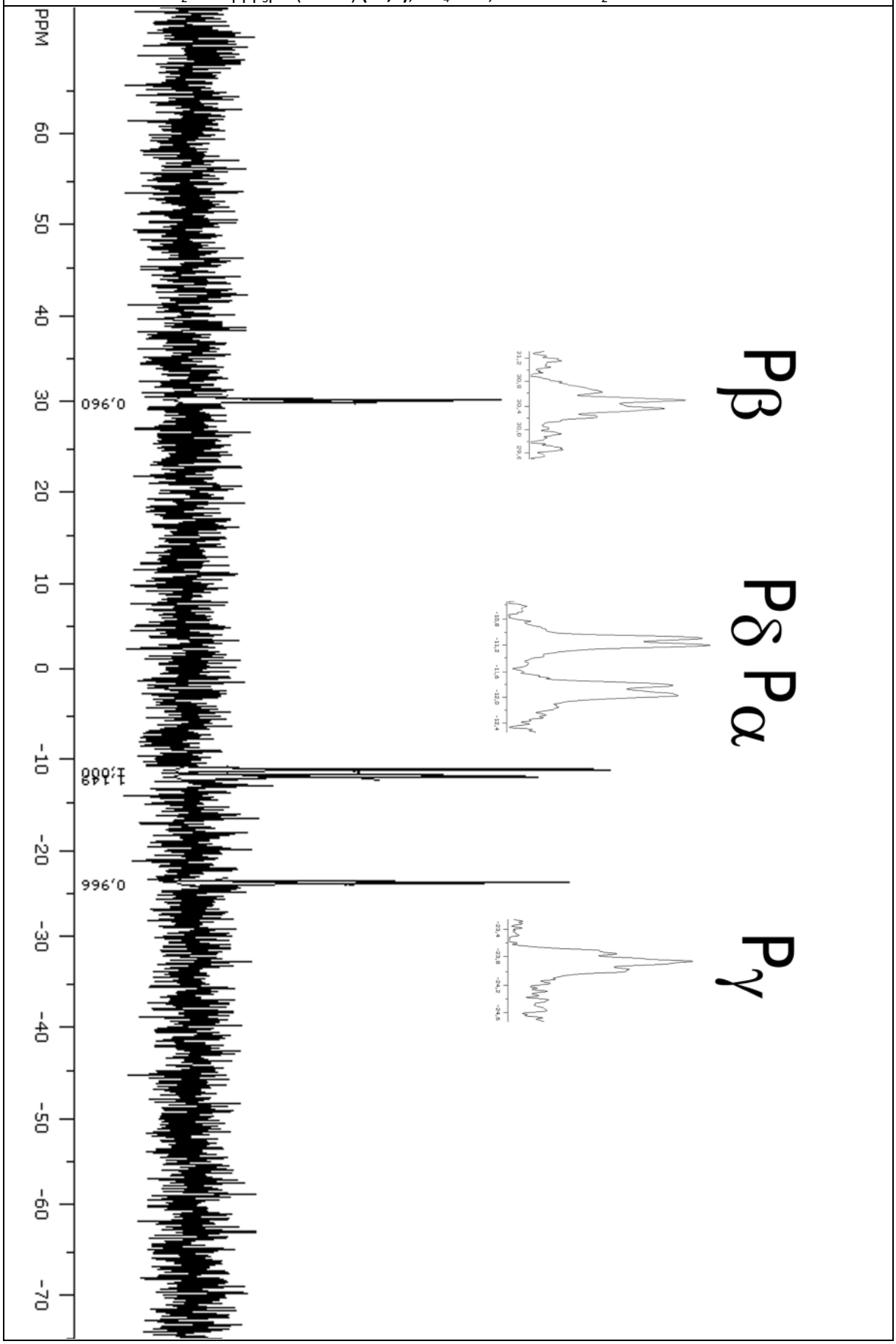
$m_2^{7,2'\text{-}O}Gpppp_SG$ (D2) **(1b)**, NH_4^+ salt, ^{31}P NMR in D_2O at 161.9 MHz



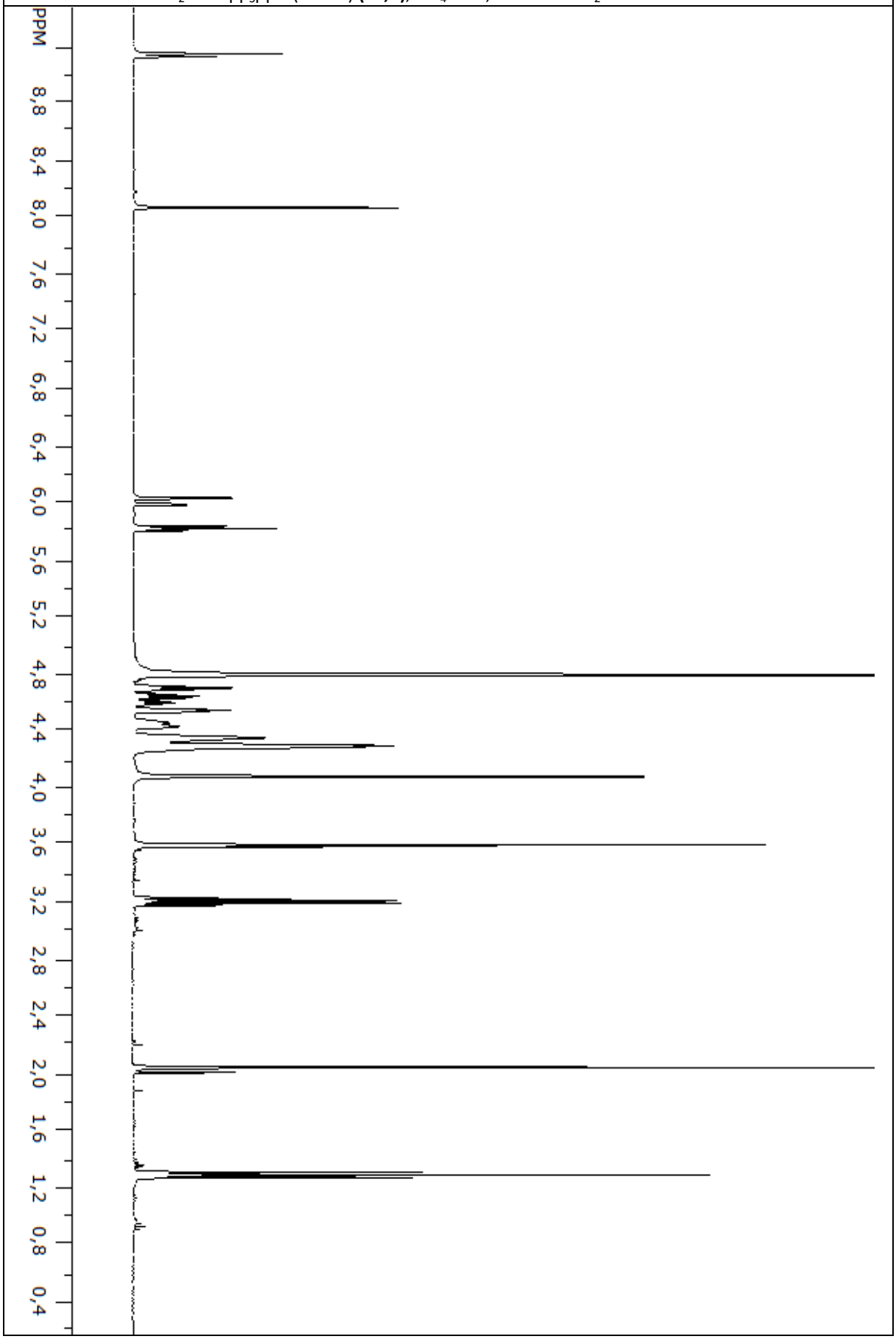
$m_2^{7,2'\text{-}O}Gppp_SpG$ (D1:D2) **(2a,b)**, NH_4^+ salt, ^1H NMR in D_2O at 399.94 MHz



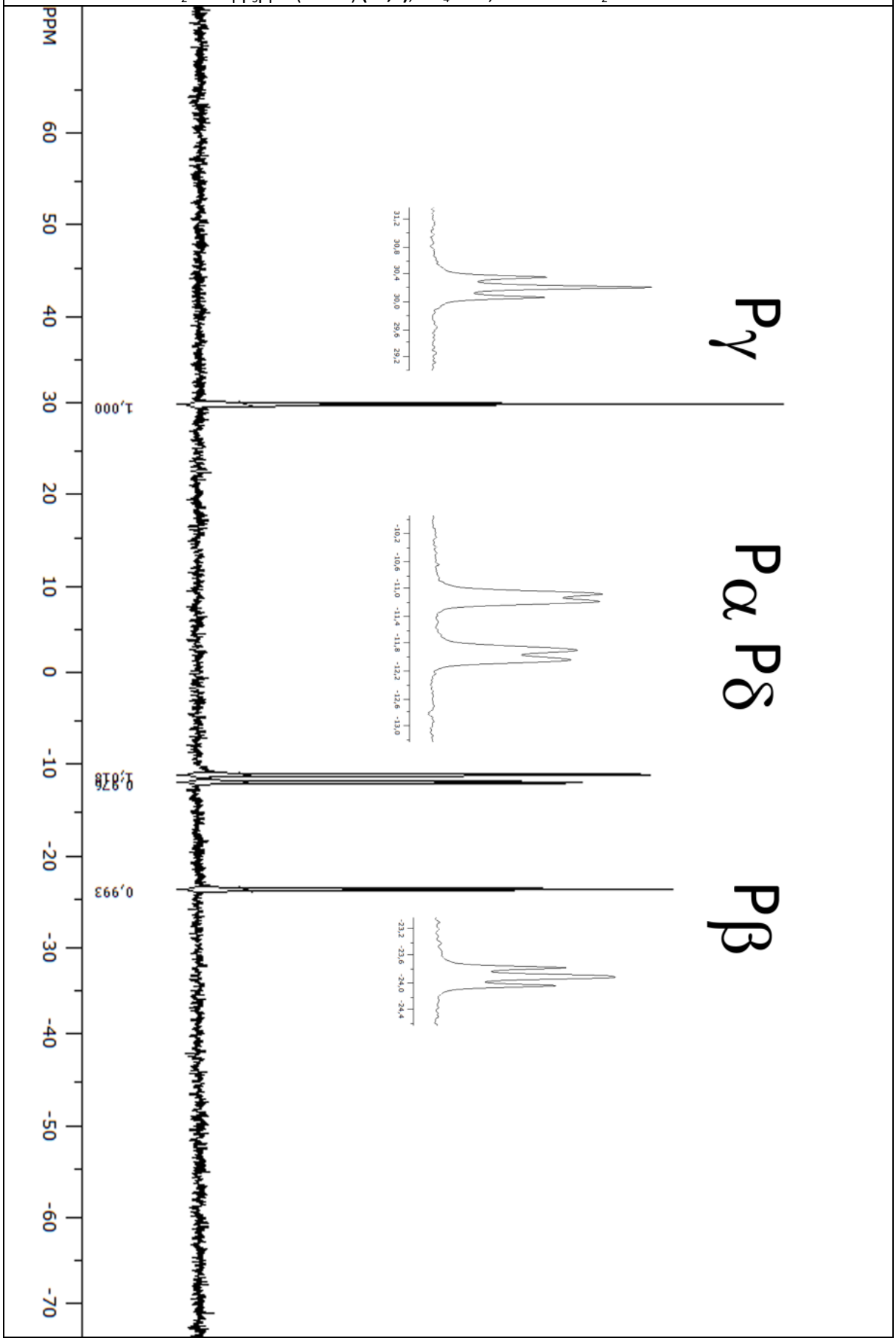
$m_2^{7,2'\text{-}O}Gppp_SpG$ (D1:D2) **(2a,b)**, NH_4^+ salt, ^{31}P NMR in D_2O at 161.9 MHz



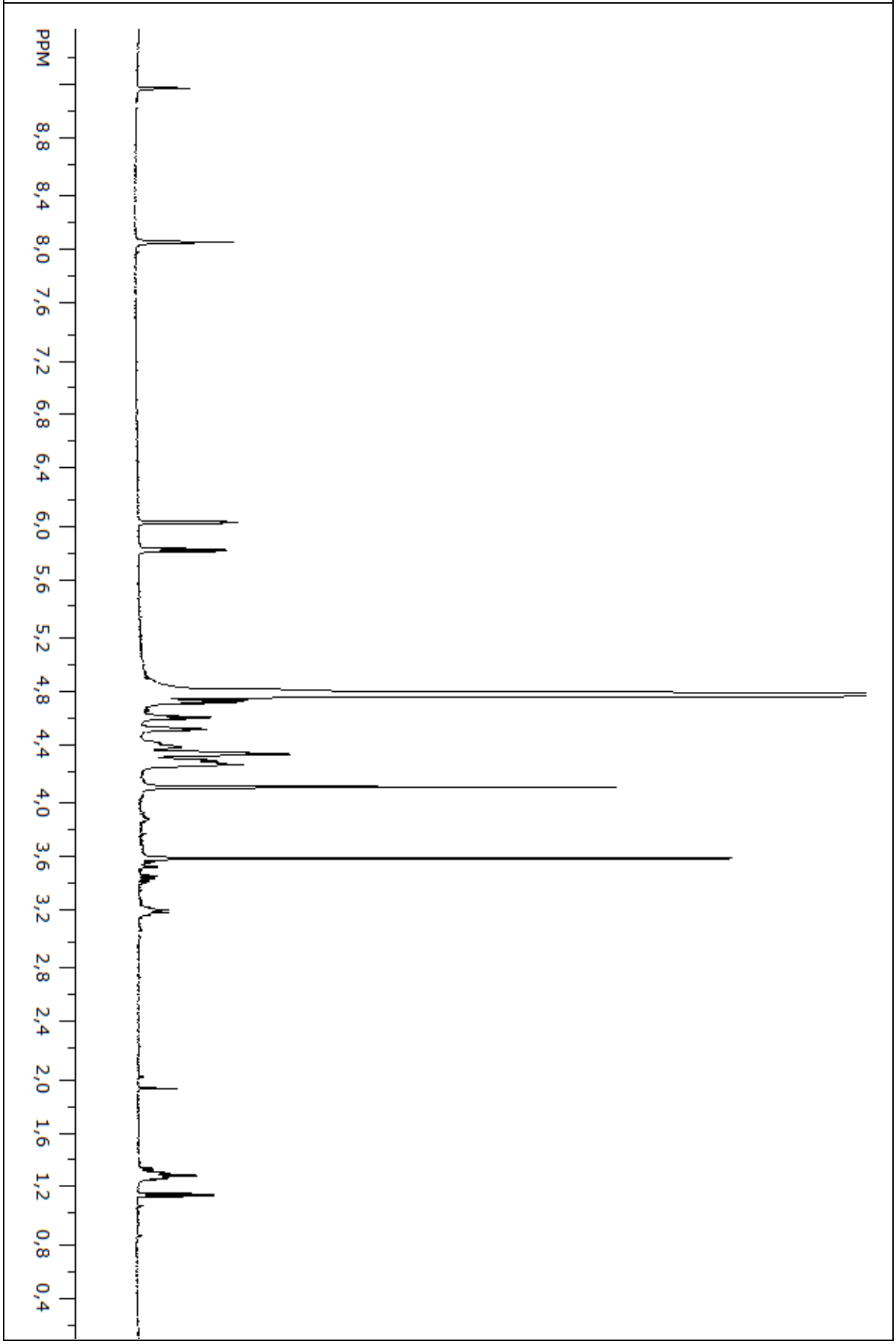
$m_2^{7,2'\text{-}O}Gpp_SppG$ (D1:D2) **(3a,b)**, NH_4^+ salt, ^1H NMR in D_2O at 399.94 MHz



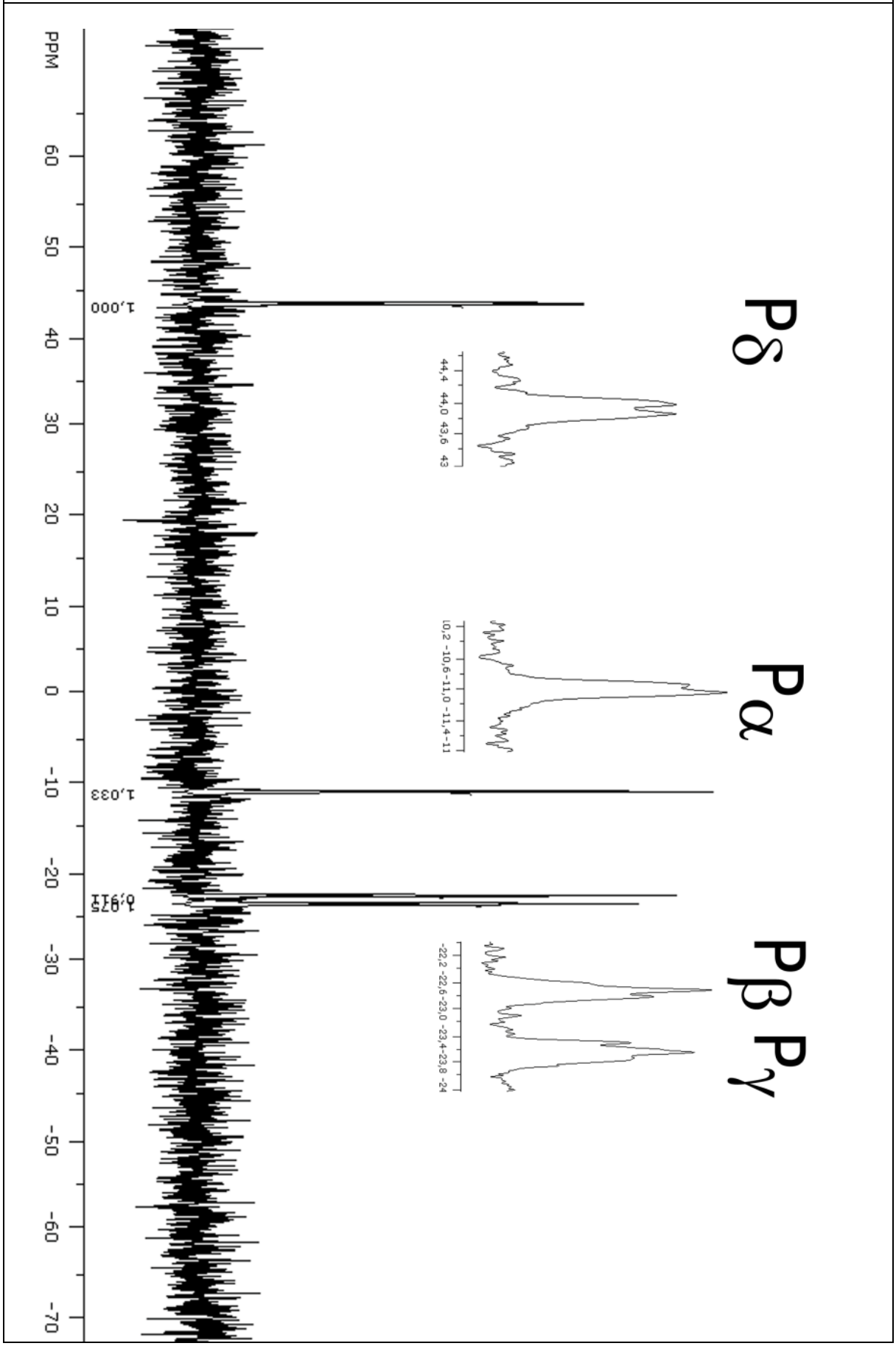
$m_2^{7,2'\text{-}O}Gpp_SppG$ (D1:D2) **(3a,b)**, NH_4^+ salt, ^{31}P NMR in D_2O at 161.9 MHz



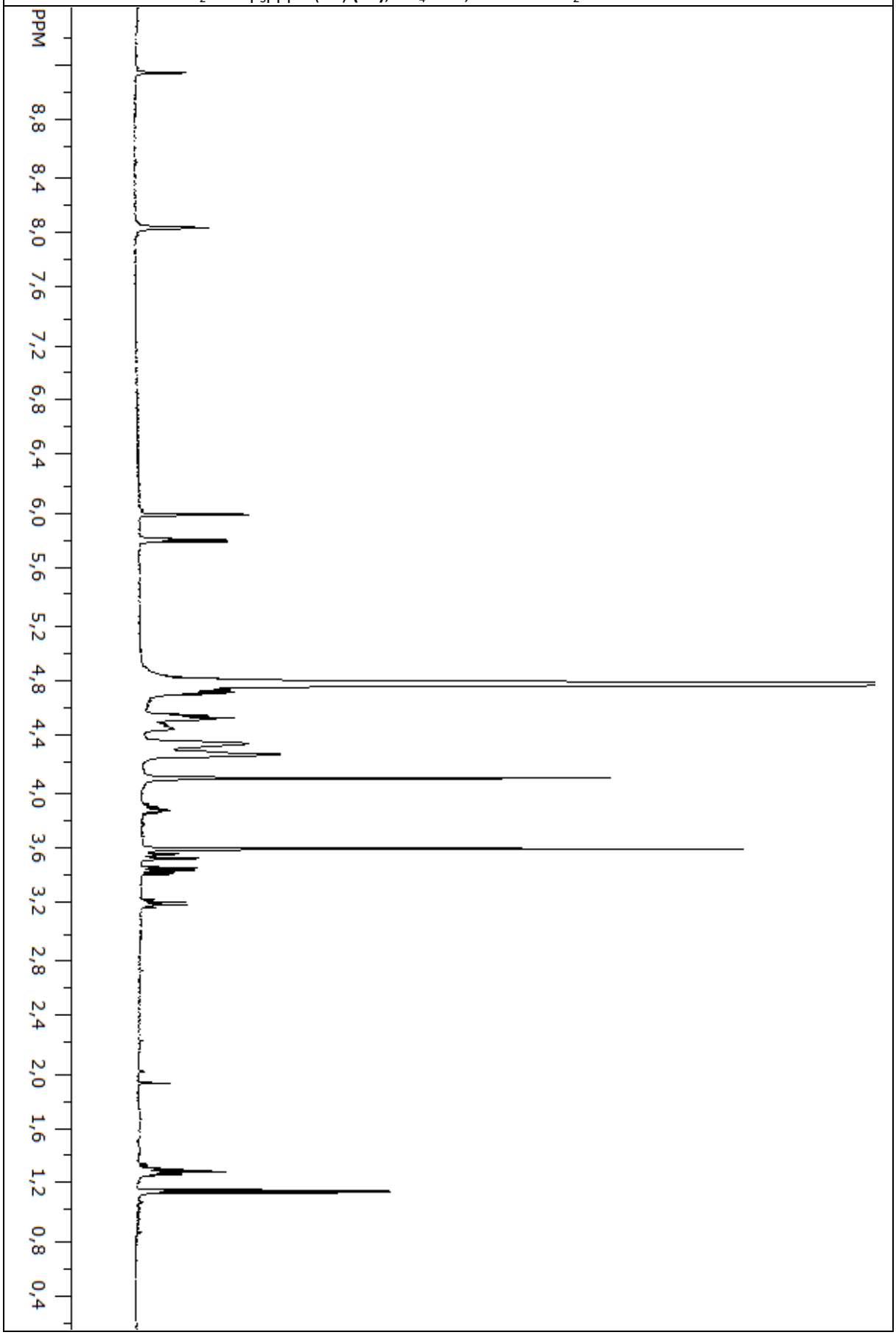
$m_2^{7,2'\text{-}O}Gp_SpppG$ (D1) **(4a)**, NH_4^+ salt, ^1H NMR in D_2O at 399.94 MHz



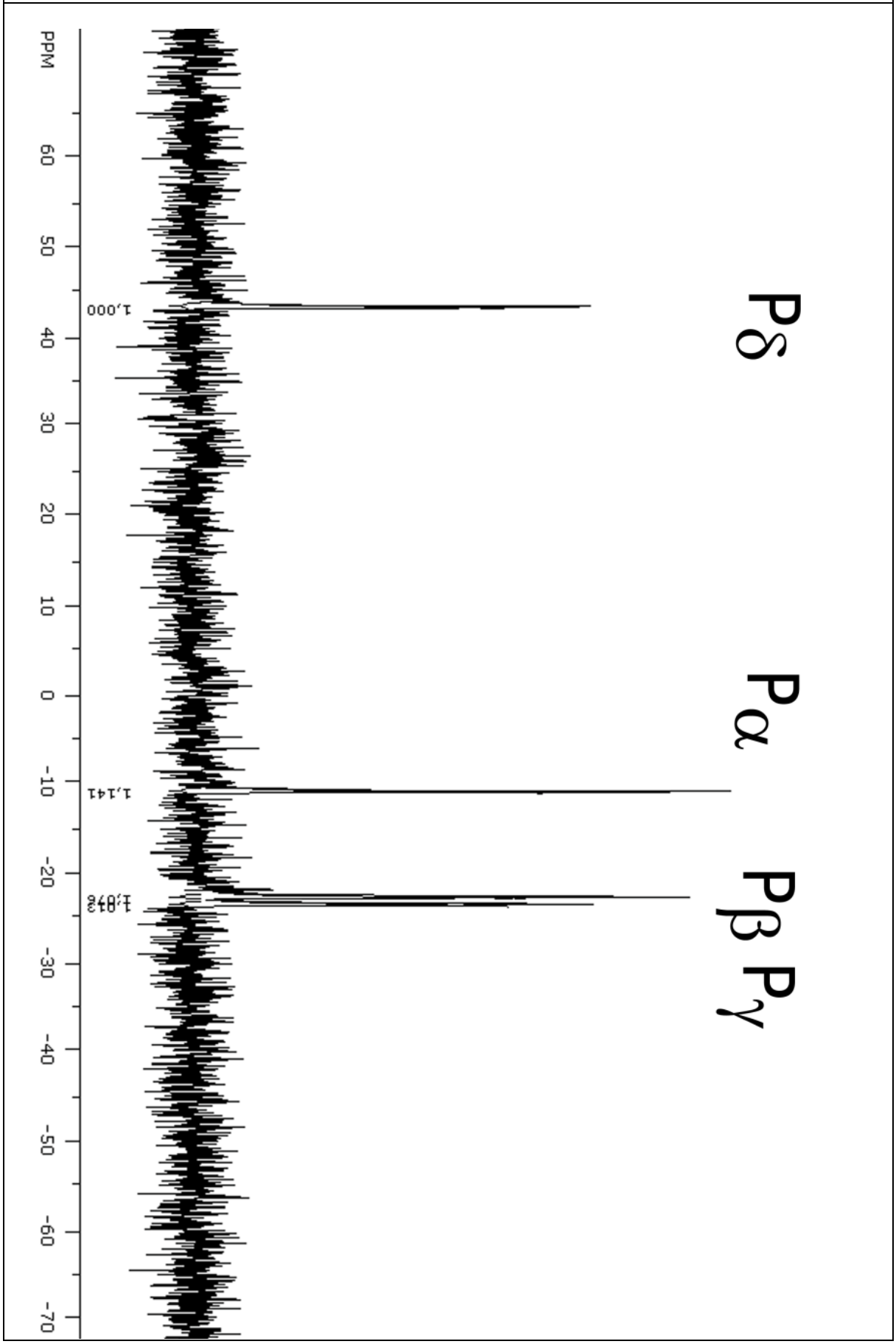
$m_2^{7,2'\text{-}O}Gp_SpppG$ (D1) **(4a),** NH_4^+ salt, ^{31}P NMR in D_2O at 161.9 MHz



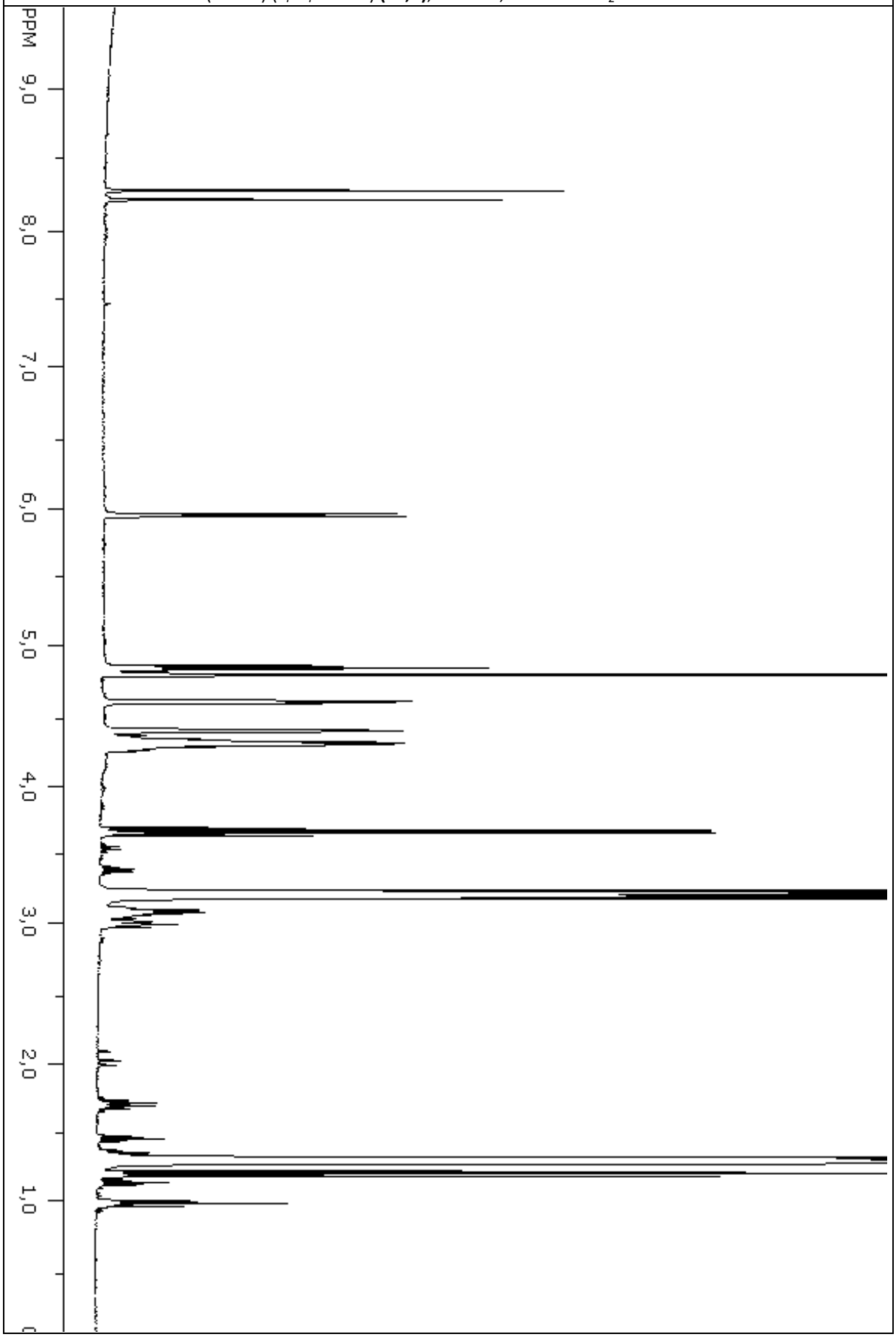
$m_2^{7,2'\text{-}O}Gp_SpppG$ (D2) **(4b)**, NH_4^+ salt, ^1H NMR in D_2O at 399.94 MHz



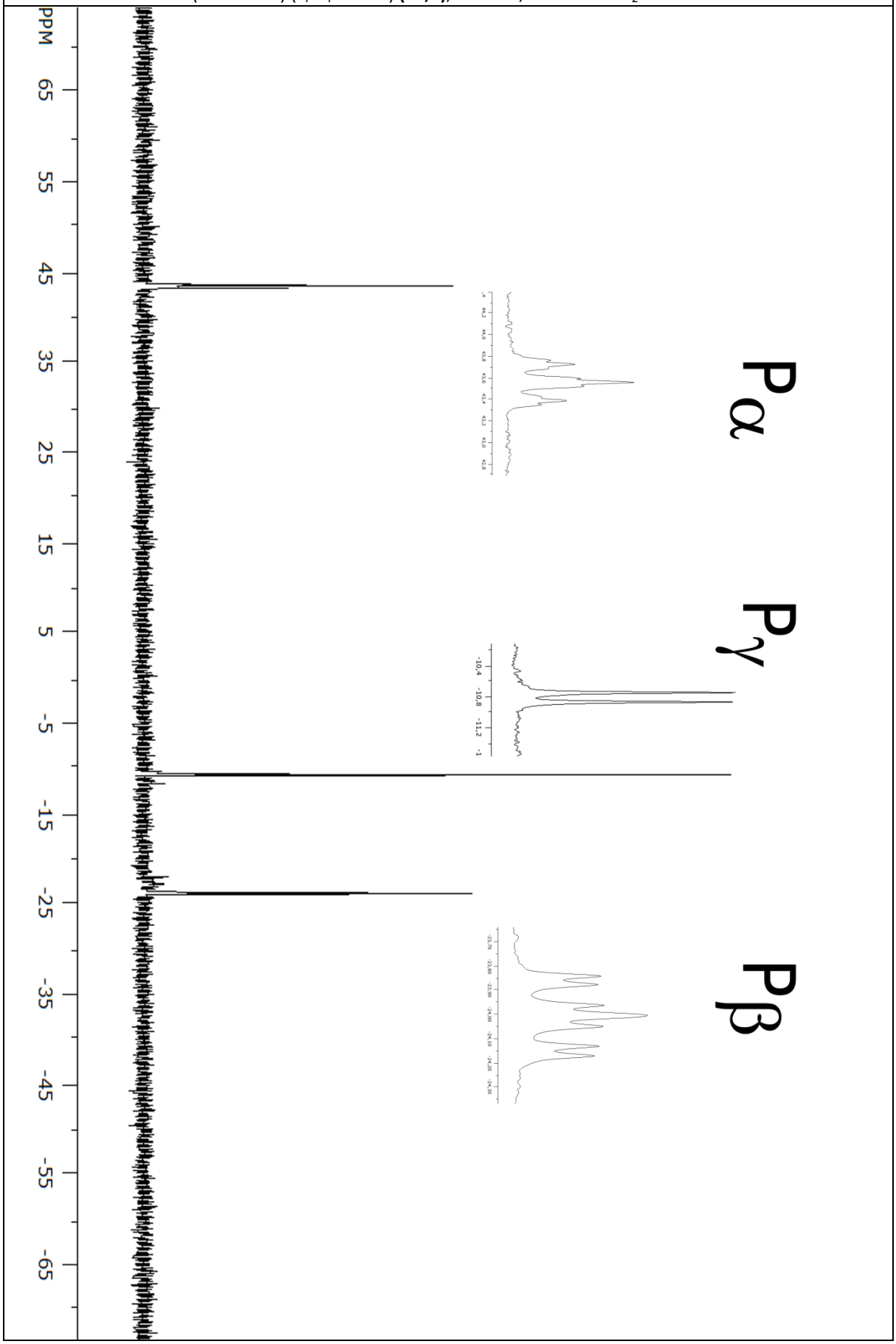
$m_2^{7,2'\text{-}O}Gp_SpppG$ (D2) **(4b)**, NH_4^+ salt, ^{31}P NMR in D_2O at 161.9 MHz



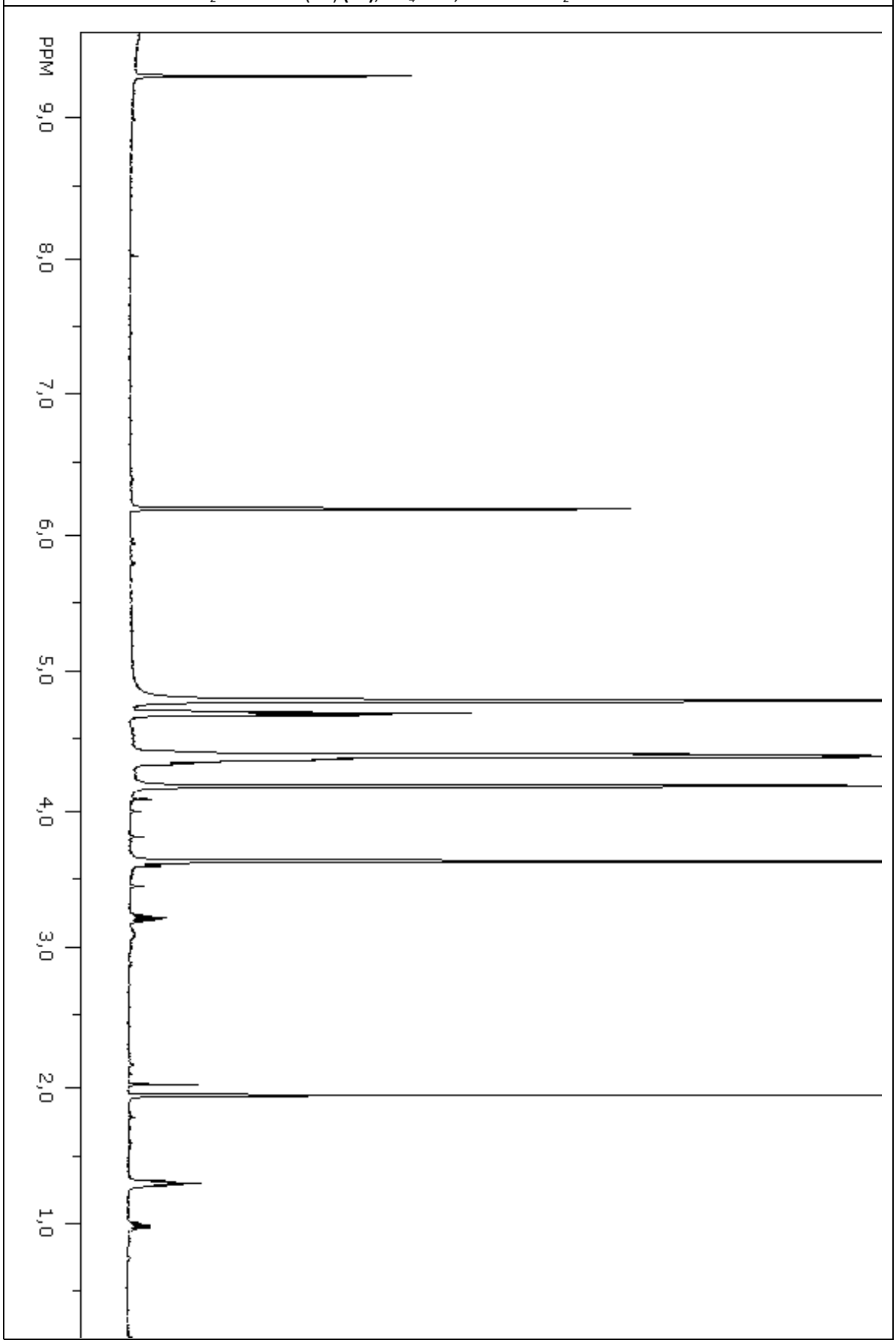
GTPαS (D1:D2) (S_P:R_P ~ 10:9) **(5a,b)**, TEA salt, ^{1}H NMR in D_2O at 399.94 MHz



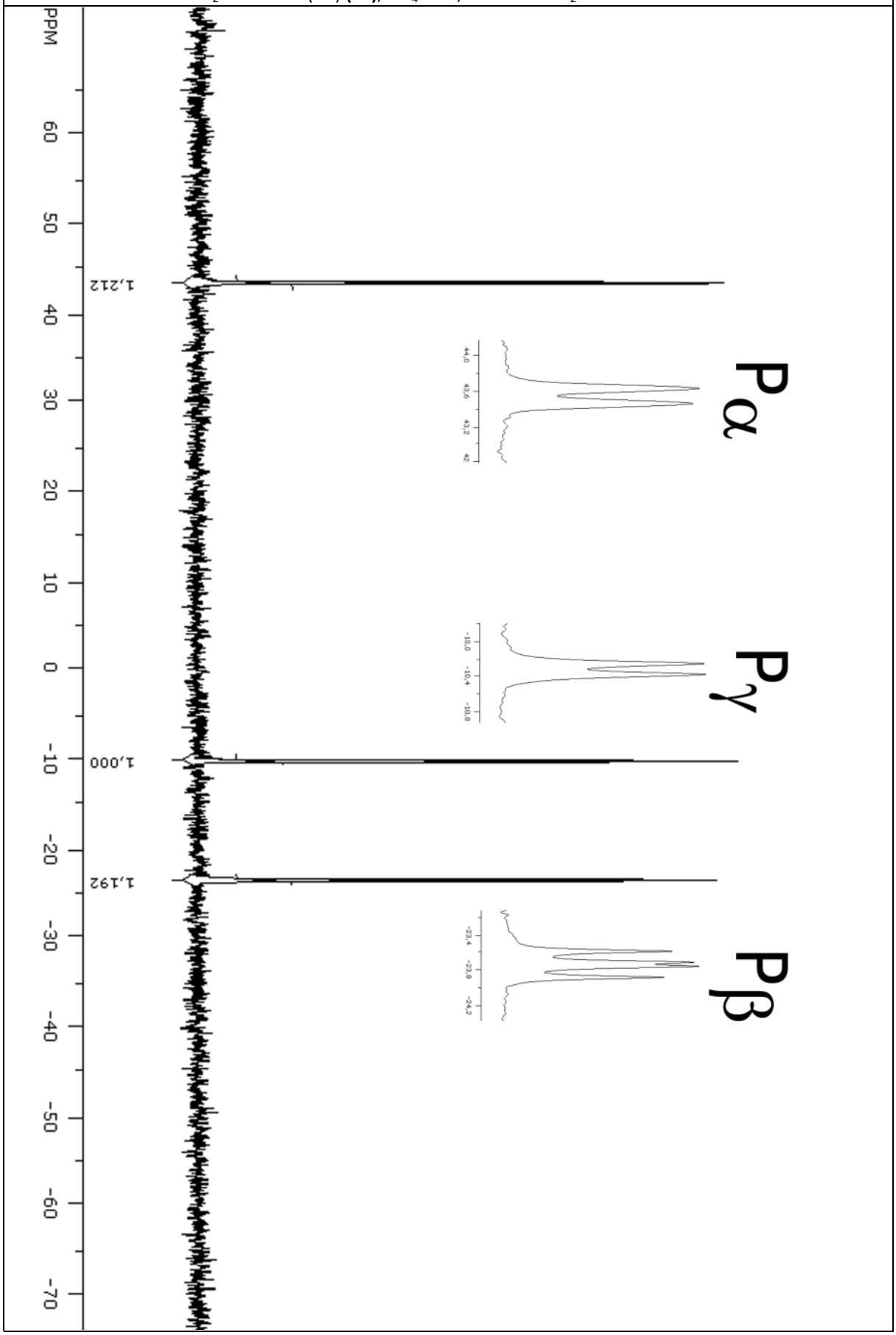
GTPαS (D1:D2 mix) (S_P:R_P ~ 10:9) **(5a,b)**, TEA salt, ^{31}P NMR in D_2O at 161.9 MHz



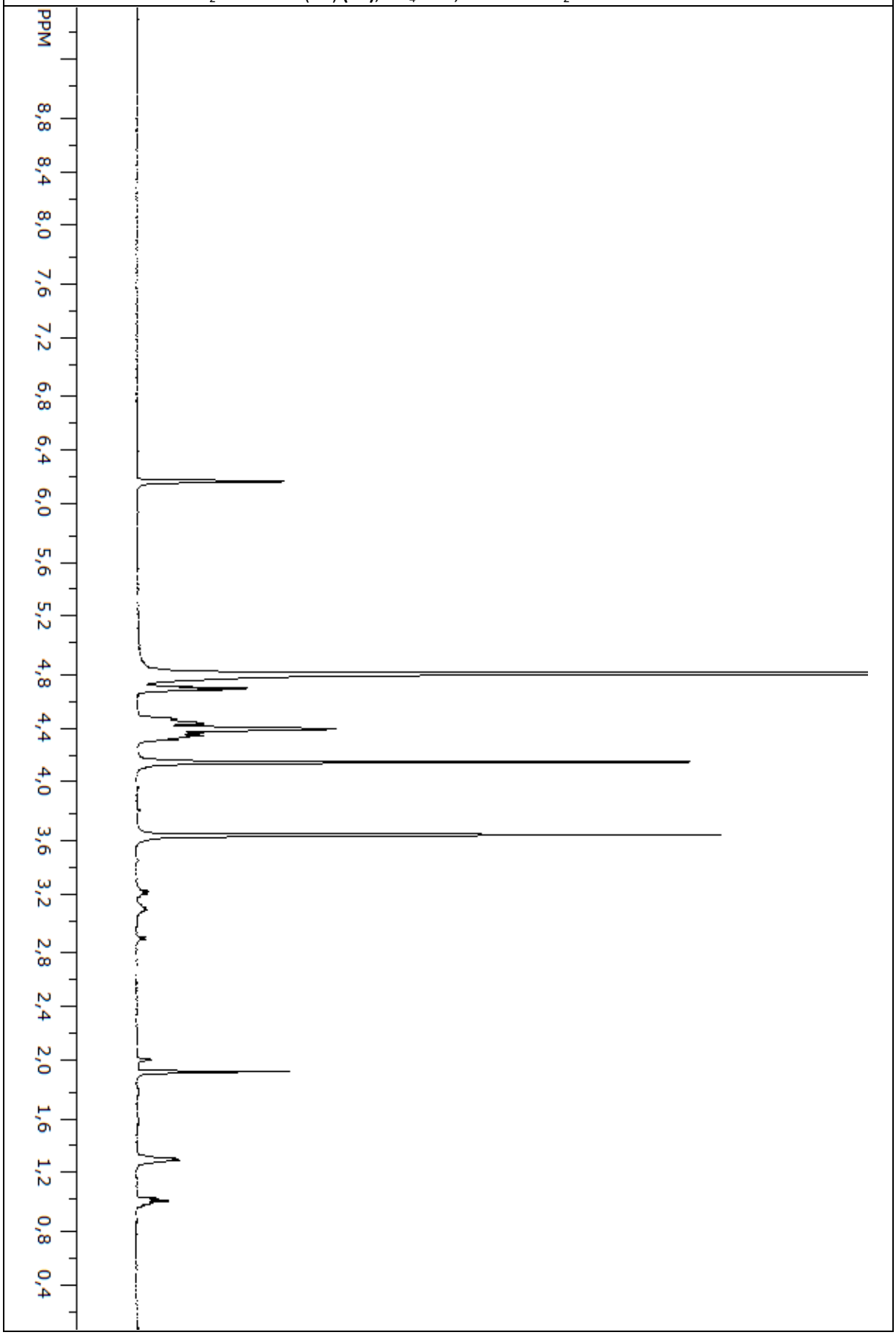
$m_2^{7,2'\text{-}O}$GTPαS (D1) **(6a)**, NH_4^+ salt, ^{1}H NMR in D_2O at 399.94 MHz



$m_2^{7,2'\text{-}O}$GTPαS (D1) **(6a)**, NH_4^+ salt, ^{31}P NMR in D_2O at 161.9 MHz



$m_2^{7,2'\text{-}O}$GTPαS (D2) **(6b)**, NH_4^+ salt, ^{1}H NMR in D_2O at 399.94 MHz



$m_2^{7,2'\text{-}O}$GTPαS (D2) **(6b)**, NH_4^+ salt, ^{31}P NMR in D_2O at 161.9 MHz