Four phosphates at one blow: access to pentaphosphorylated magic spot nucleotides and their analysis by capillary electrophoresis

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Supporting Information:

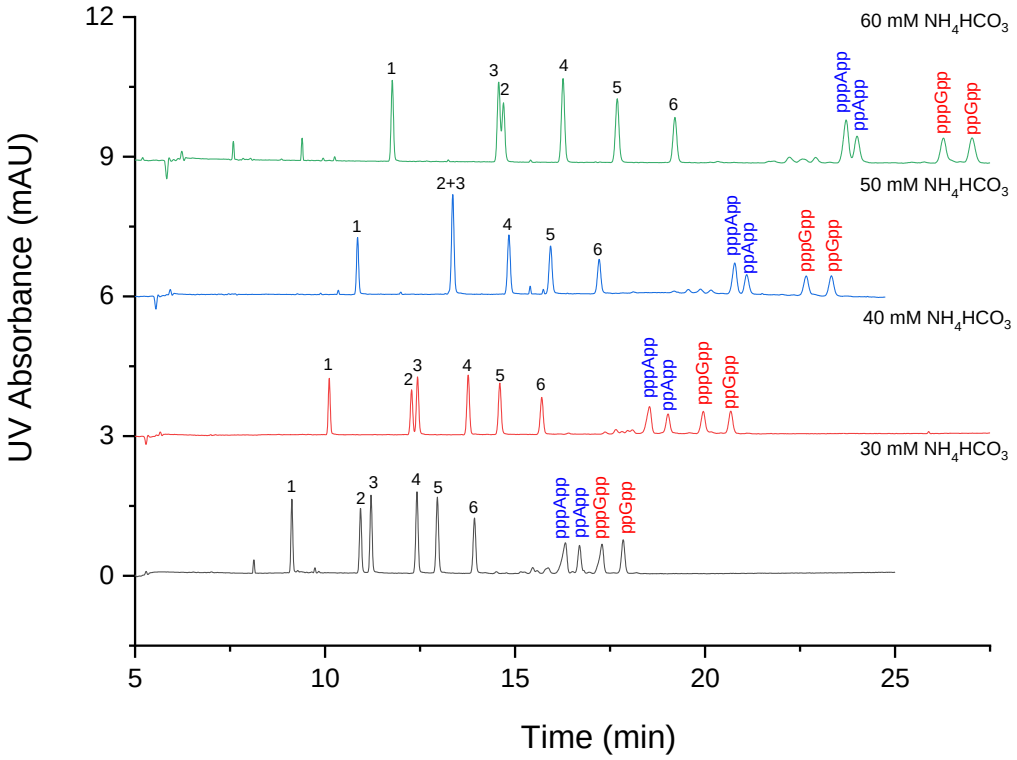


Fig. S1 Influence of the salt concentration on the separation of ten nucleotide standards: AMP (1), GMP (2), ADP (3), ATP (4), GDP (5), GTP (6). Composition of the running buffer: 30-60 mM ammonium bicarbonate titrated by ammonium hydroxide solution to pH 10.1. Other conditions as in Fig.3.

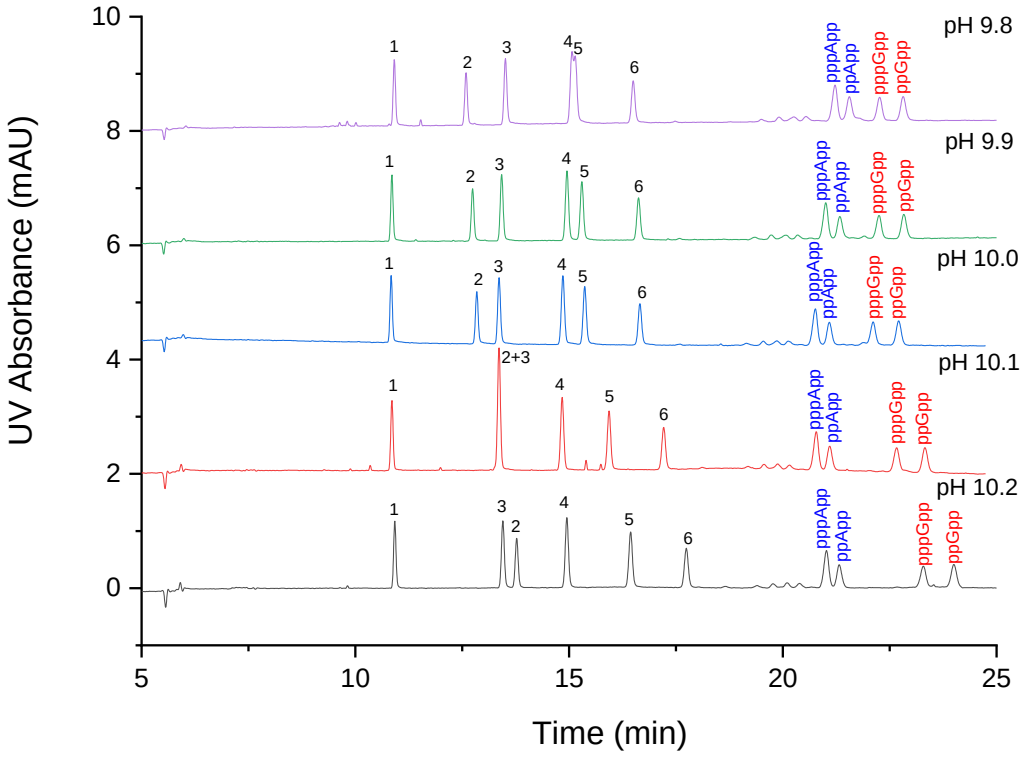


Fig. S2 Influence of pH on the separation of ten nucleotide standards: AMP (1), GMP (2), ADP (3), ATP (4), GDP (5), GTP (6). Composition of the running buffer: 50 mM ammonium bicarbonate titrated by ammonium hydroxide solution to pH 9.8-10.2. Other conditions as in Fig.3.

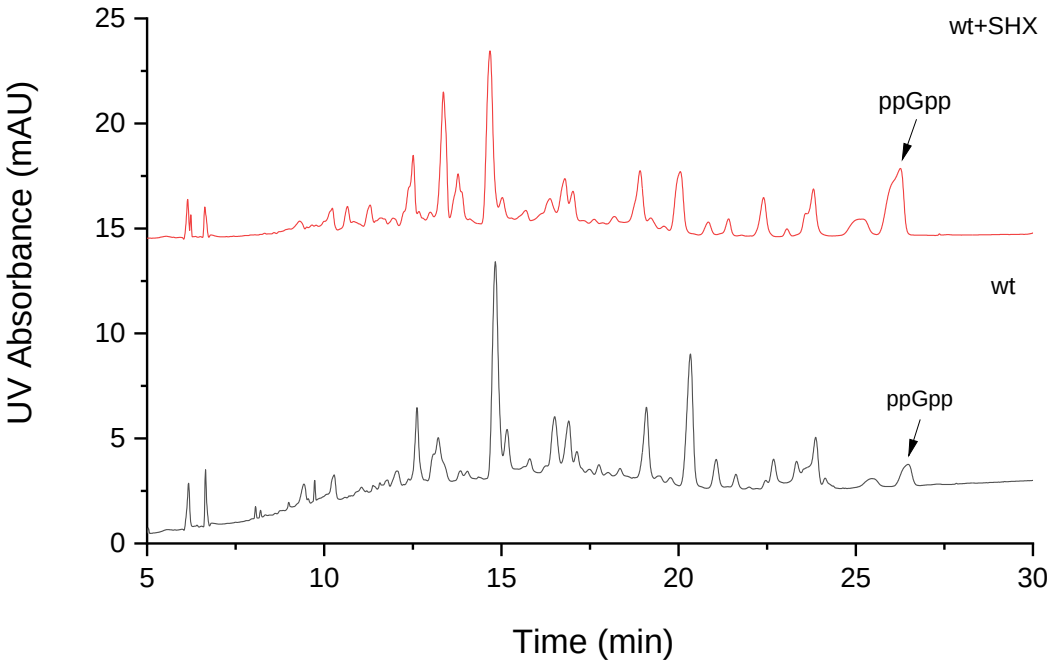
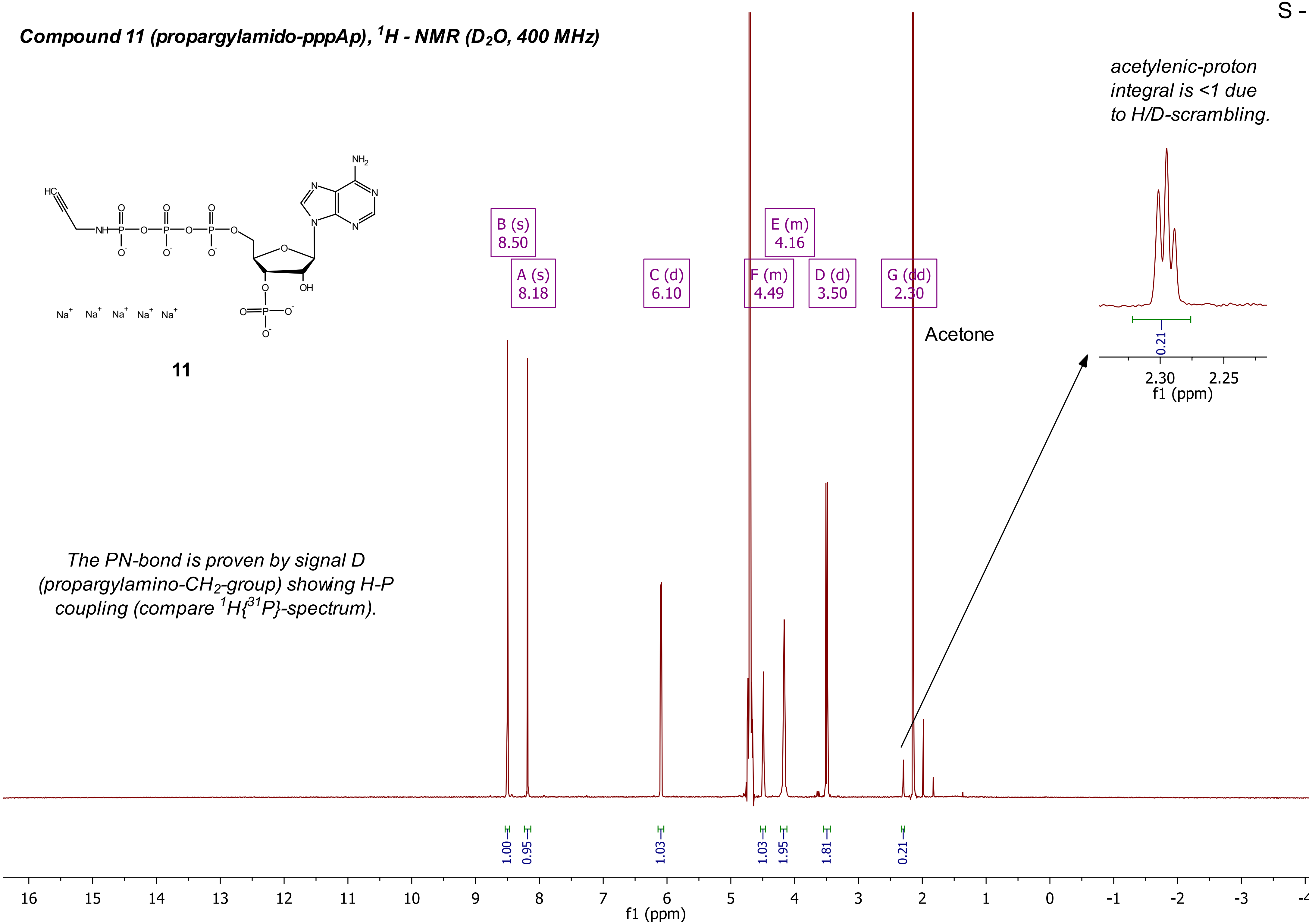
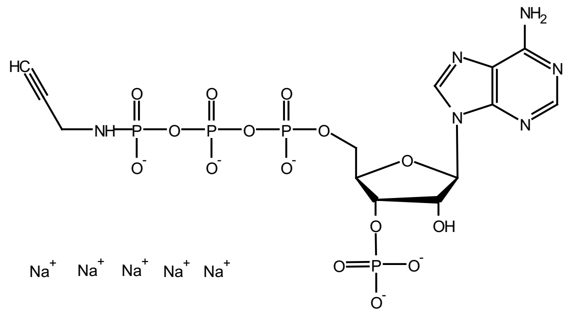


Fig.S3 Electropherogram of *E. Coli* cell extracts before (black) and 1h after adding SHX (red). Composition of the running buffer: 50 mM ammonium bicarbonate titrated by ammonium hydroxide solution to pH 10.0; Separation voltage: 18 kV; UV detection at 250 nm; Temperature: 25 °C; Injection: 100 mbar, 5s.

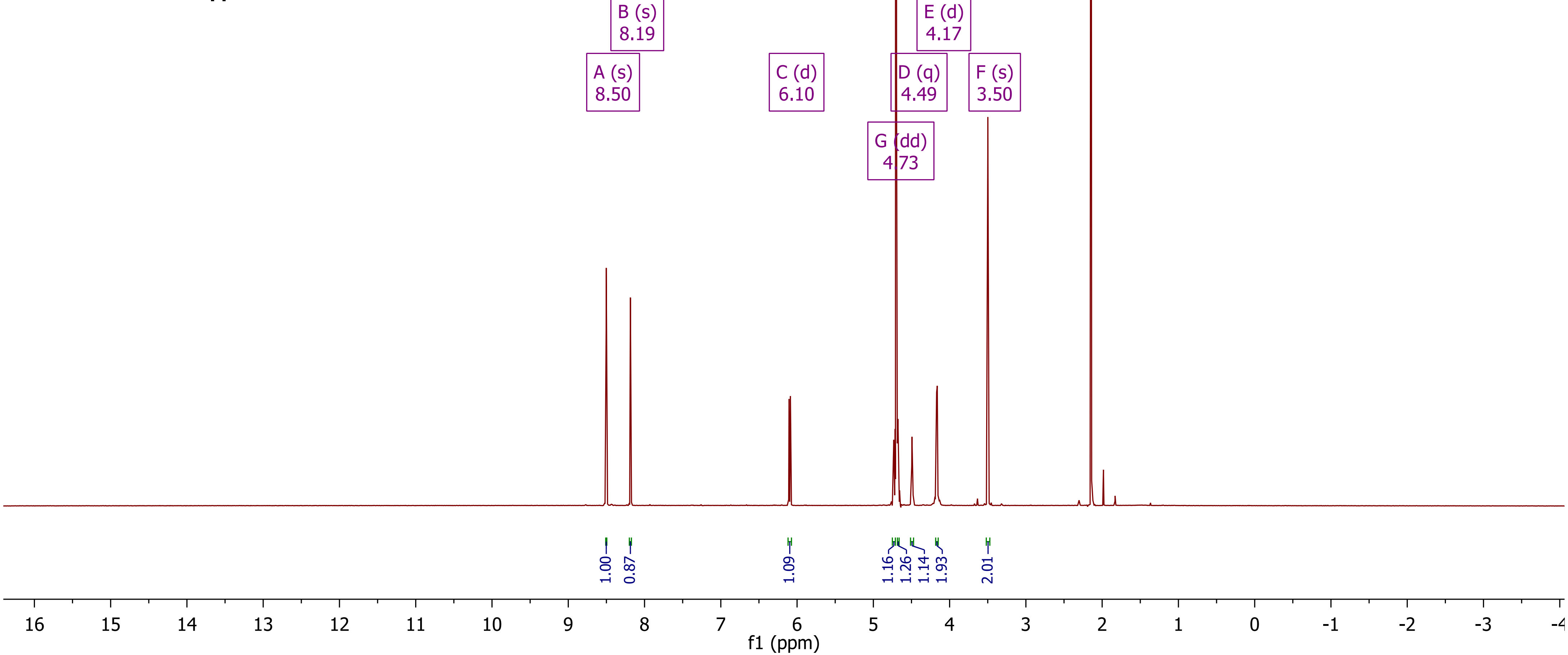
Compound 11 (propargylamido-pppAp), ^{1}H - NMR (D_2O, 400 MHz)



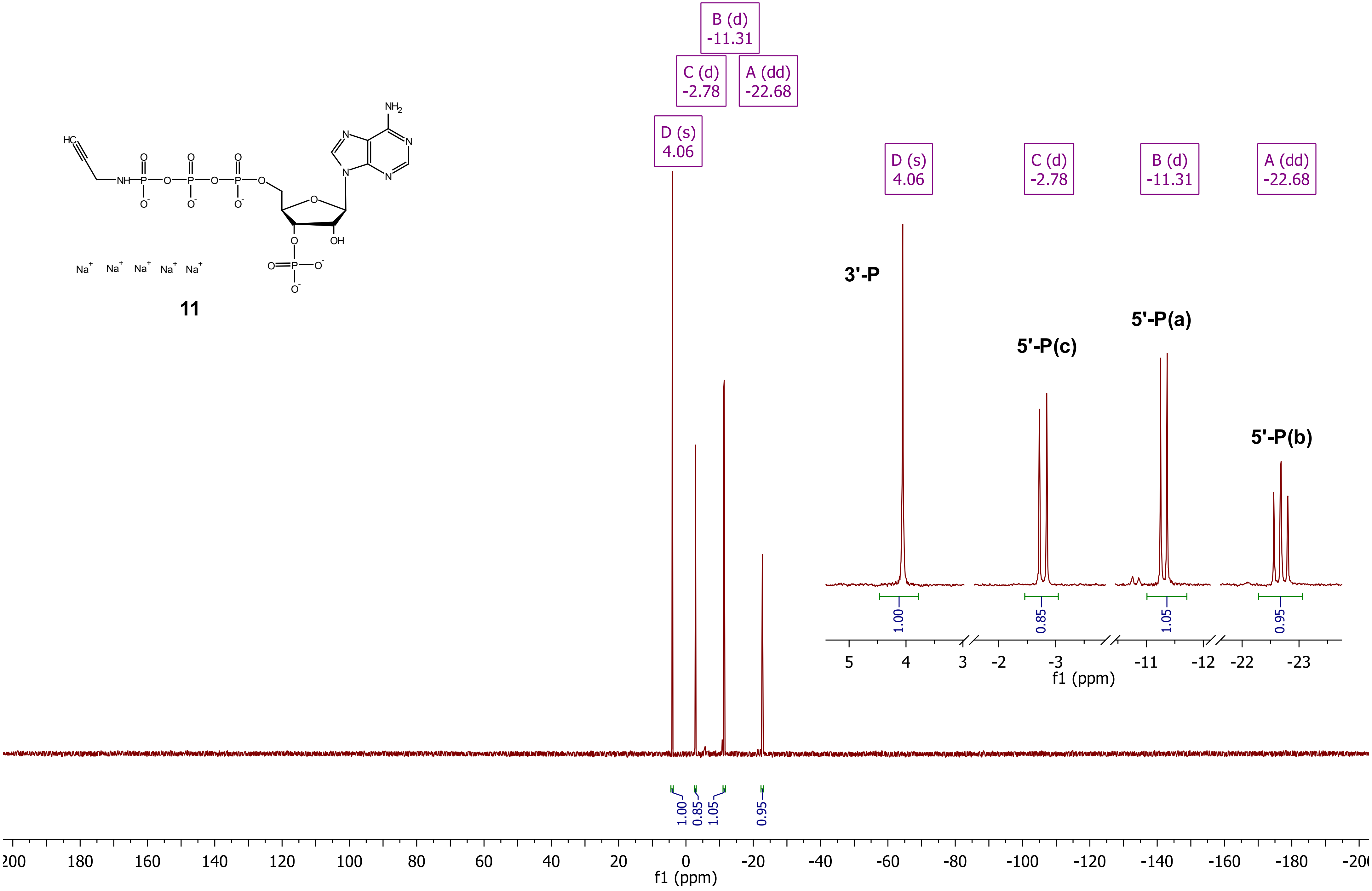
Compound 11 (propargylamido-pppAp), ^{1}H [^{31}P] - NMR (D_2O, 400 MHz)



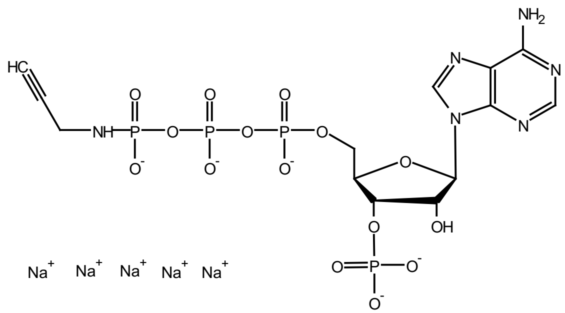
11



Compound 11 (propargylamido-pppAp), ^{31}P [^{1}H] - NMR (D_2O, 162 MHz)



Compound 11 (propargylamido-pppAp), ^{31}P - NMR (non-decoupled, D_2O, 162 MHz)



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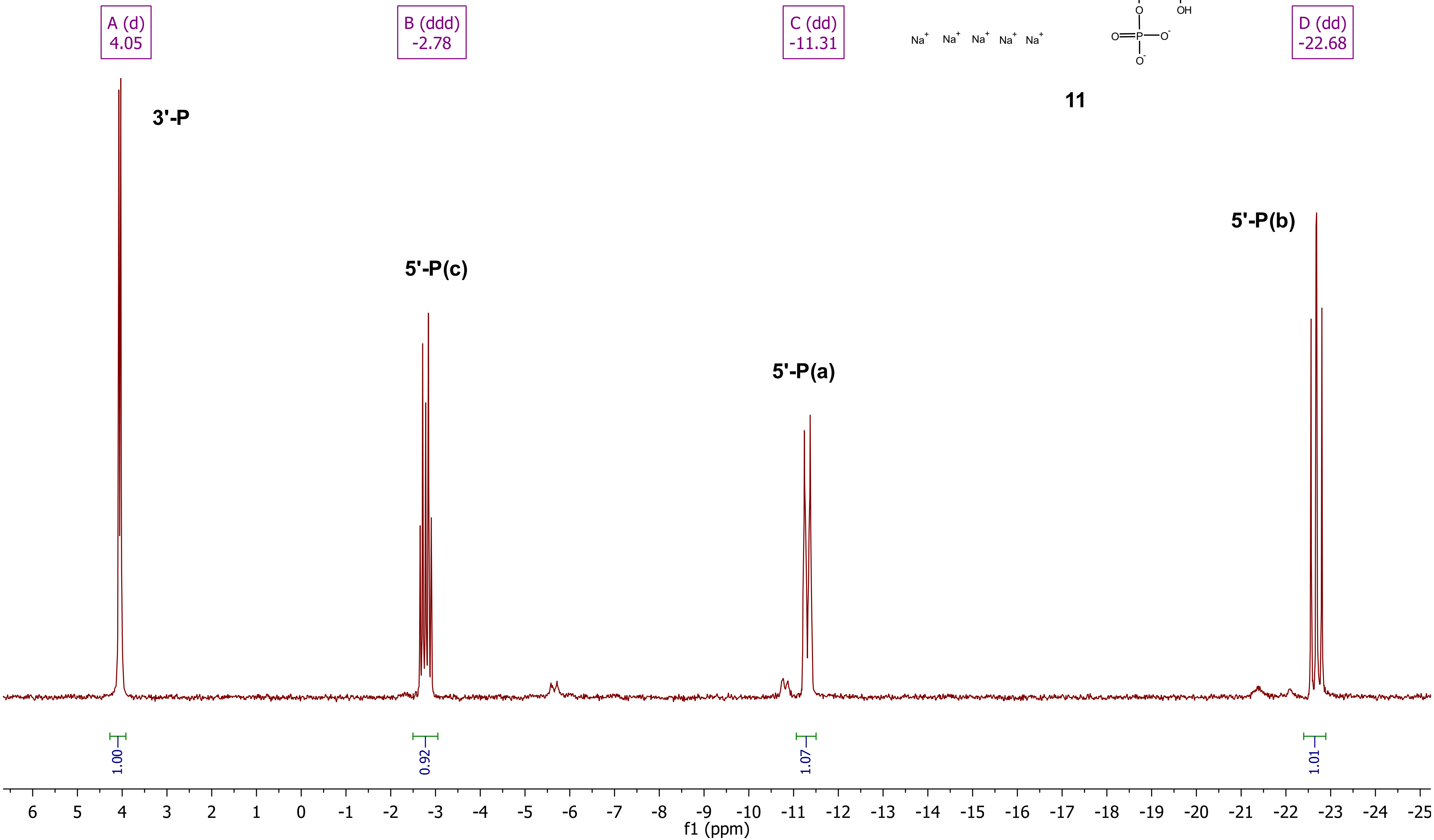
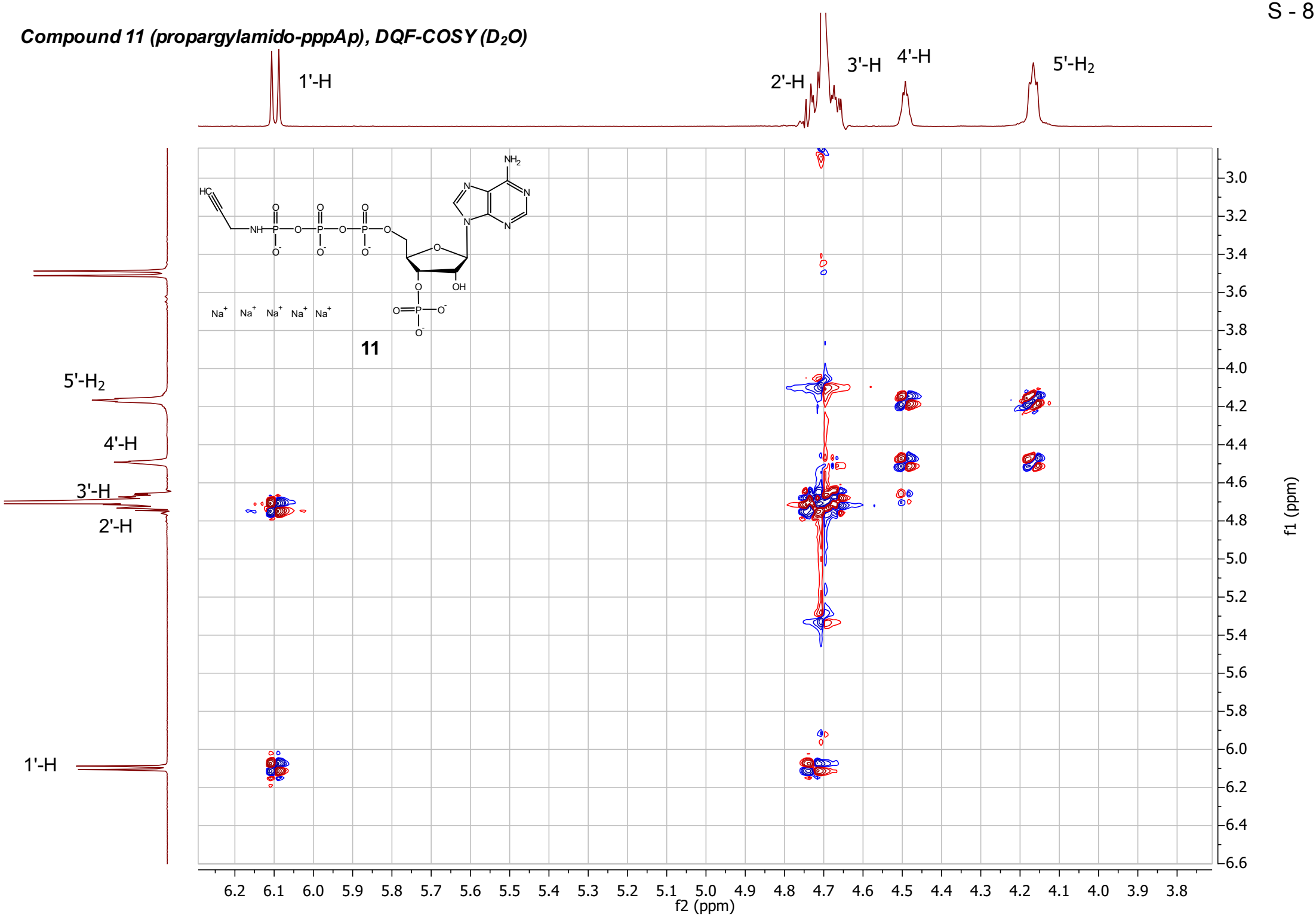
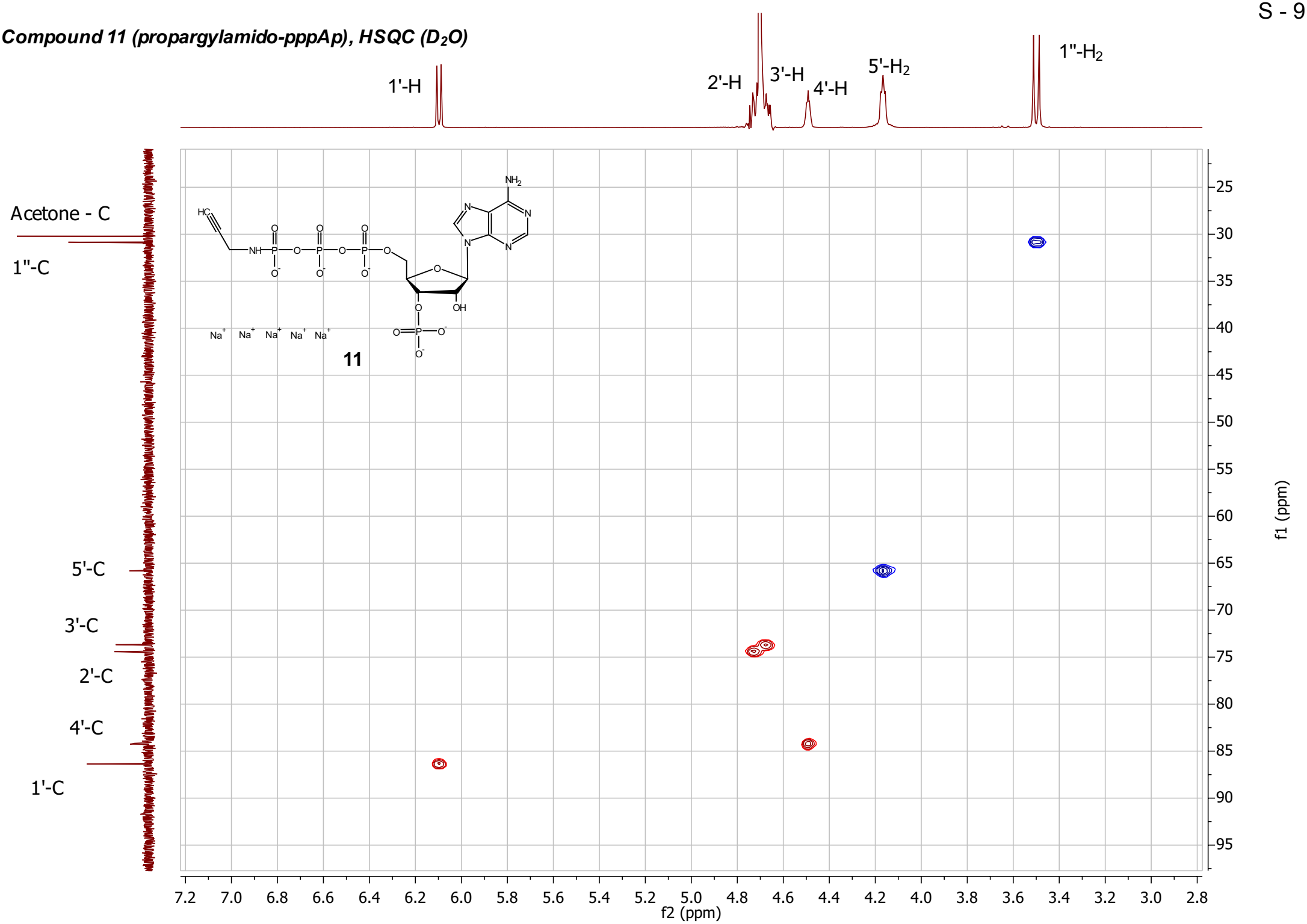


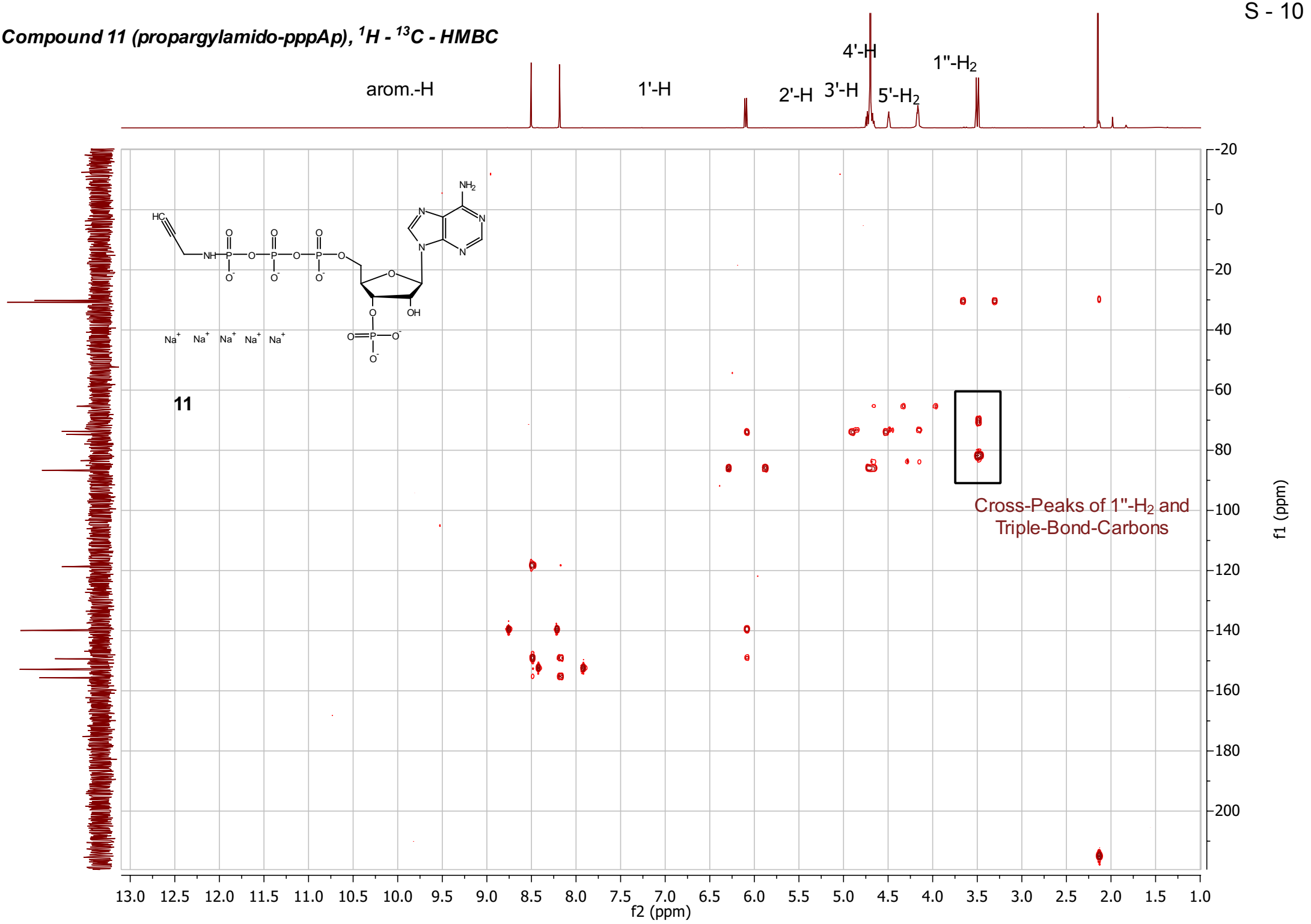
Compound 11 (propargylamido-pppAp), DQF-COSY (D_2O)



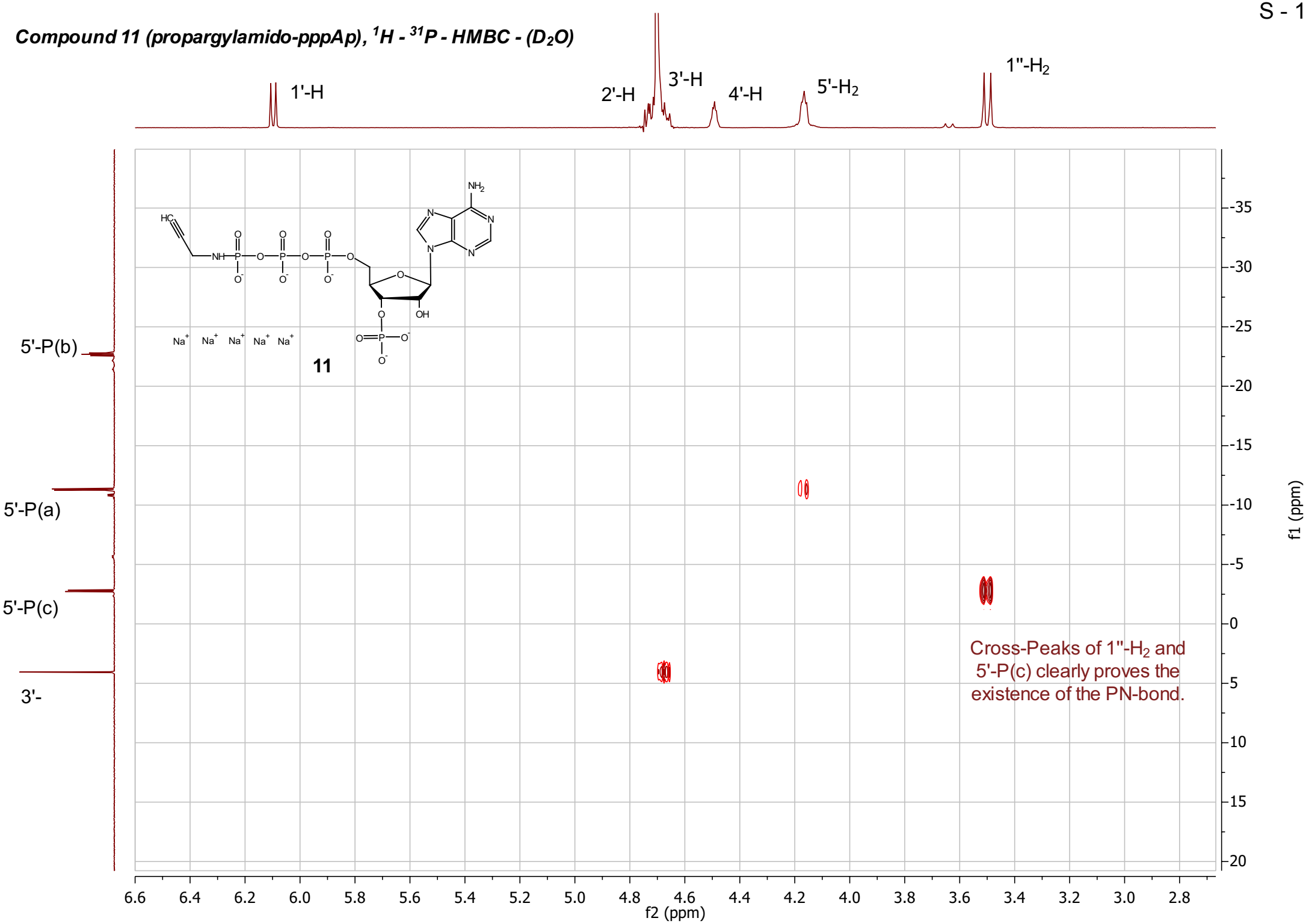
Compound 11 (propargylamido-pppAp), HSQC (D_2O)



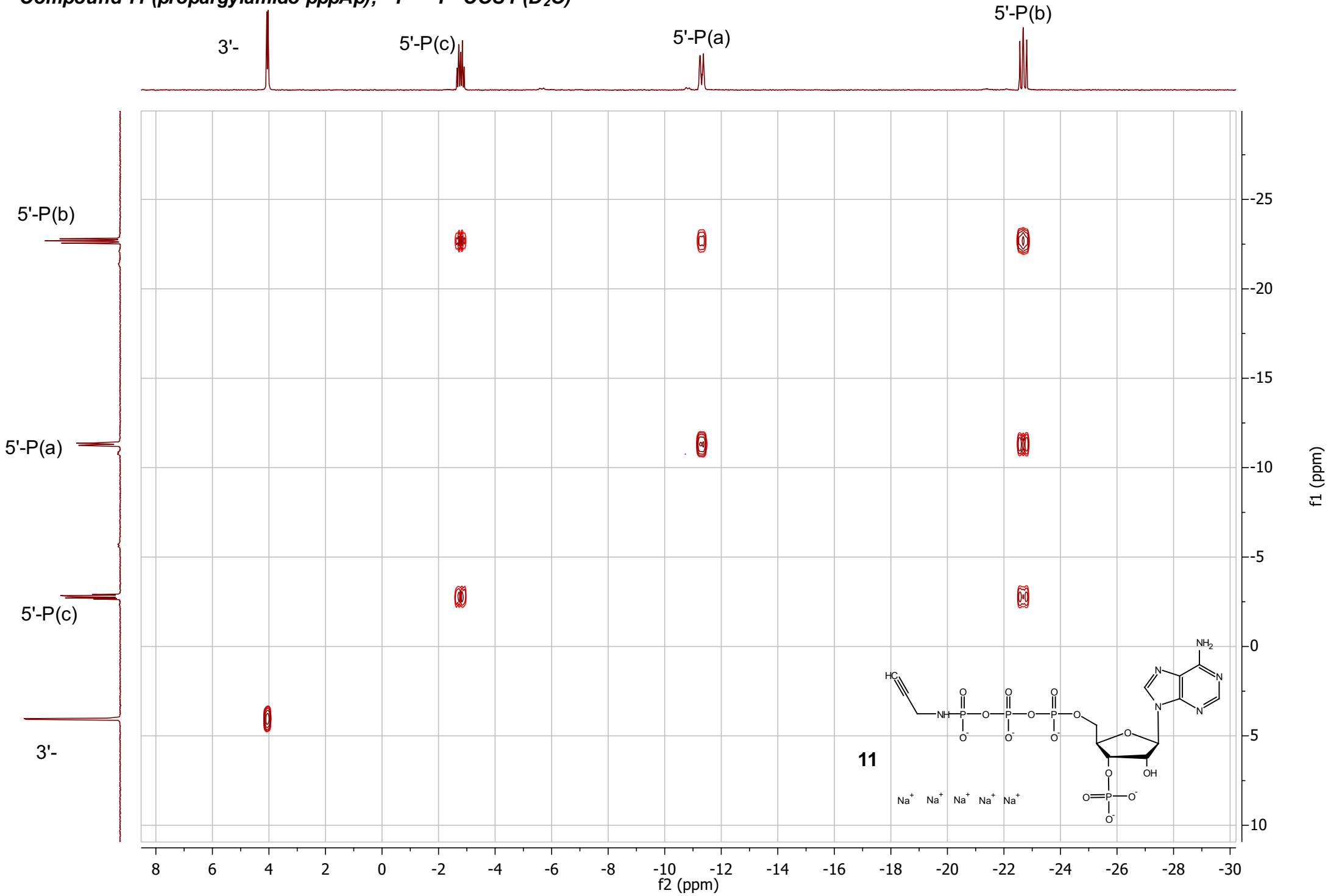
Compound 11 (propargylamido-pppAp), ^{1}H - ^{13}C - HMBC



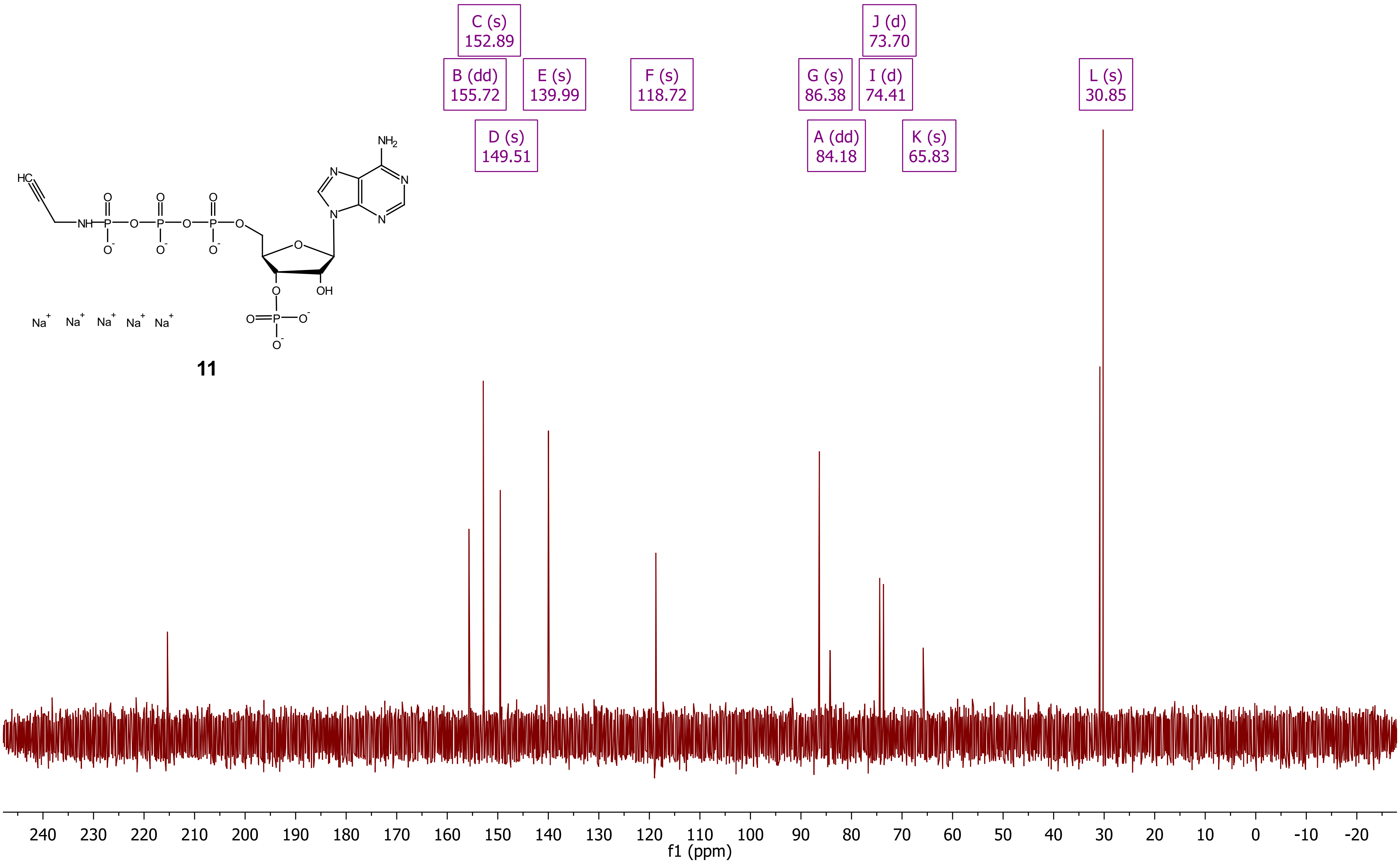
Compound 11 (propargylamido-pppAp), ^{1}H - ^{31}P - HMBC - (D_2O)



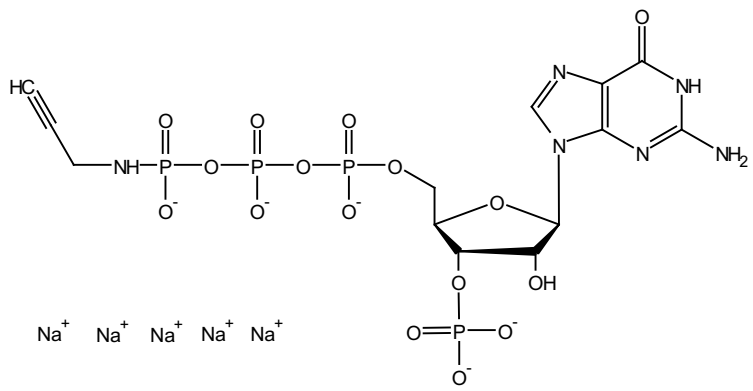
Compound 11 (propargylamido-pppAp), ^{31}P - ^{31}P- COSY (D_2O)



Compound 11 (propargylamido-pppAp), ^{13}C [^{1}H] - NMR (D_2O, 400 MHz)

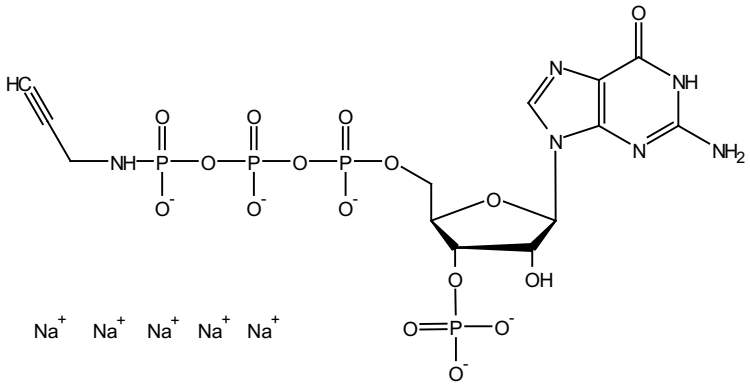


Compound 12 (propargylamido-pppGp), ^{1}H - NMR (D_2O, 400 MHz)



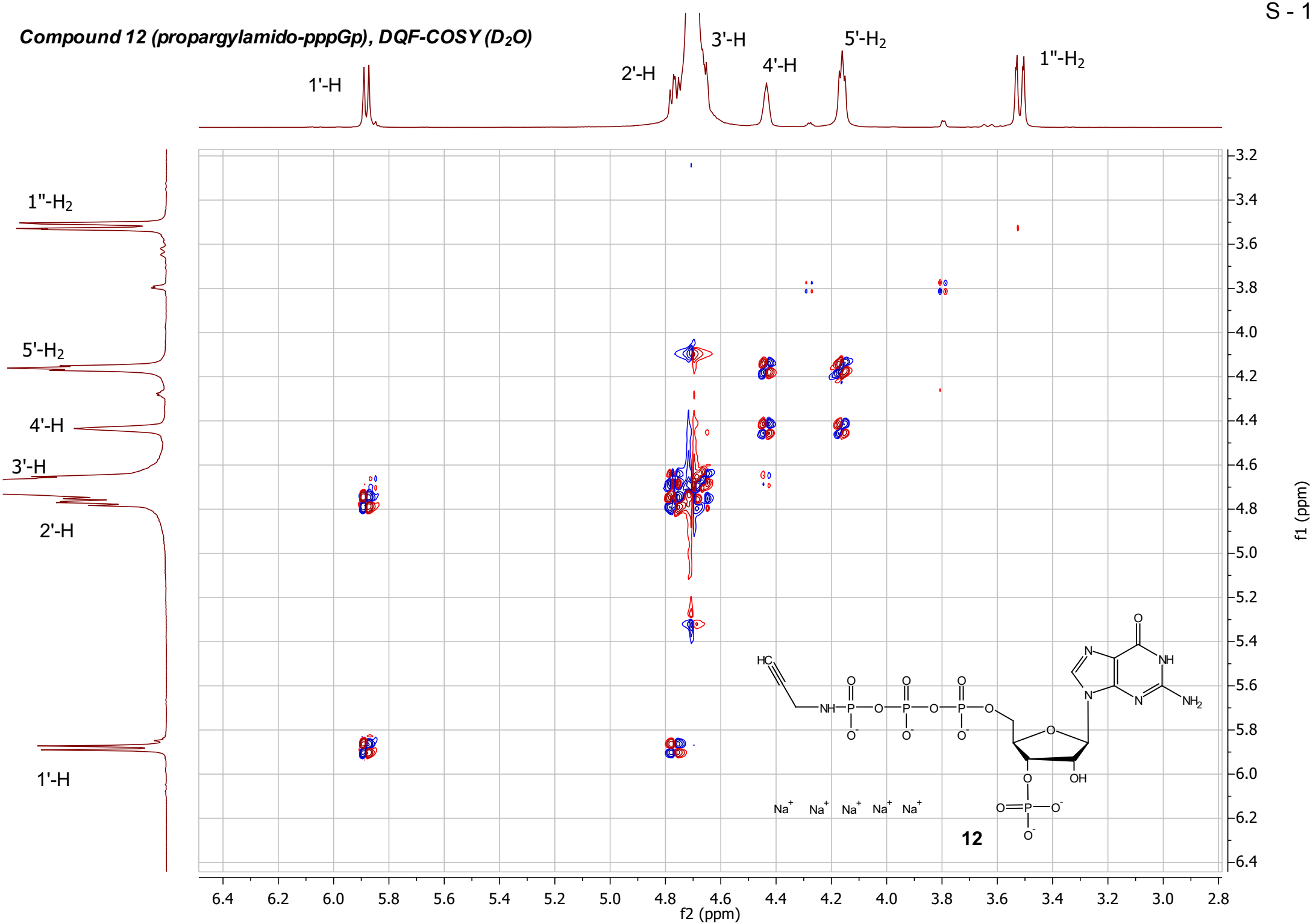
12

Compound 12 (propargylamido-pppGp), $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)

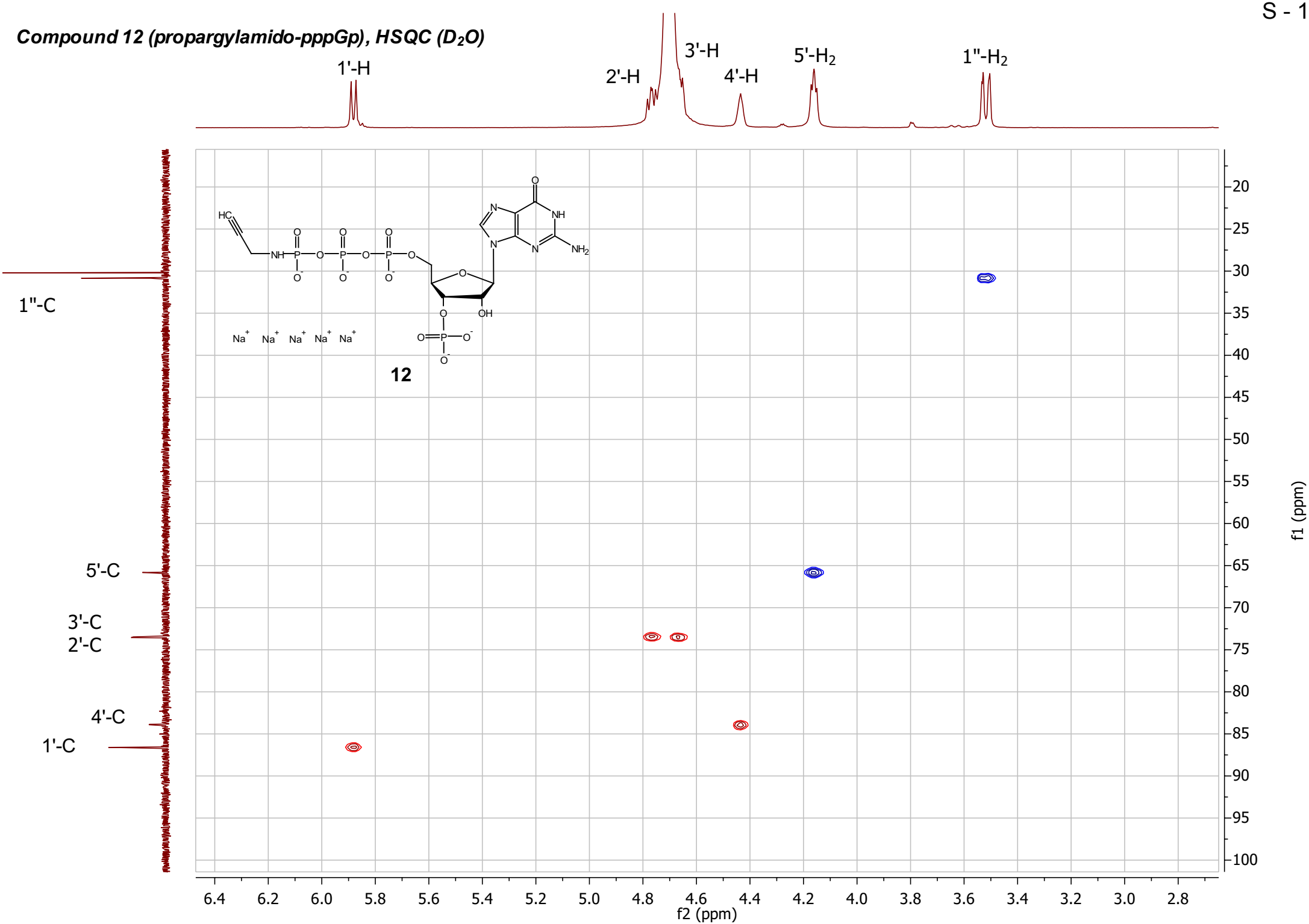


12

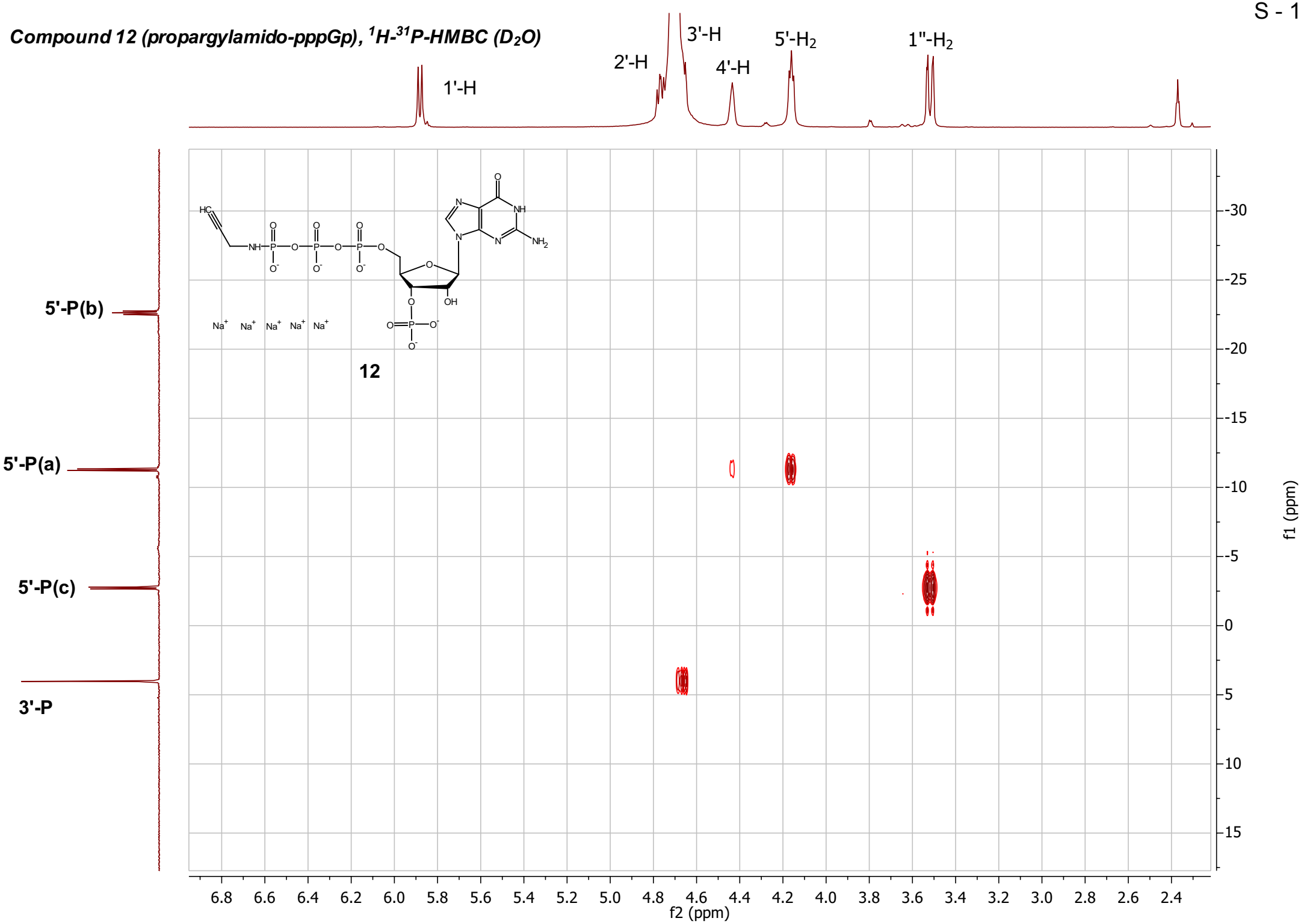
Compound 12 (propargylamido-pppGp), DQF-COSY (D_2O)



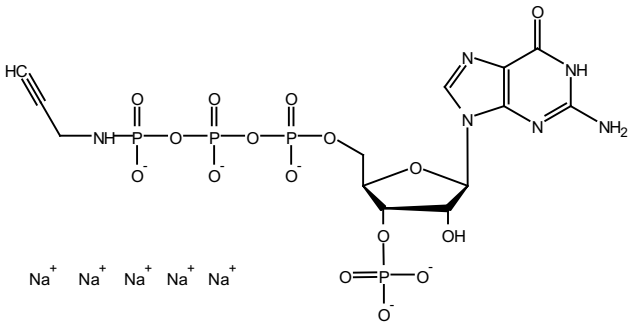
Compound 12 (propargylamido-pppGp), HSQC (D_2O)



Compound 12 (propargylamido-pppGp), ^{1}H-^{31}P-HMBC (D_2O)

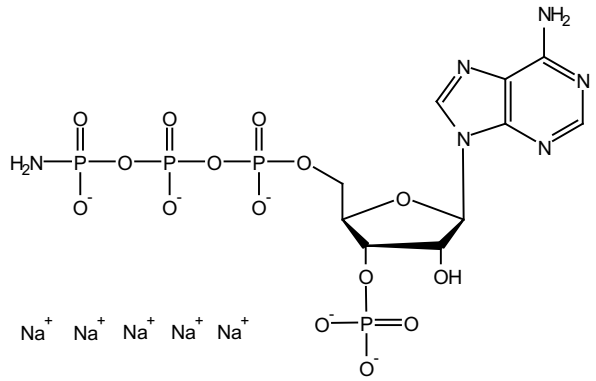


Compound 12 (propargylamido-pppGp), $^{13}C\{^{1}H\}$ - NMR (D_2O, 101 MHz)



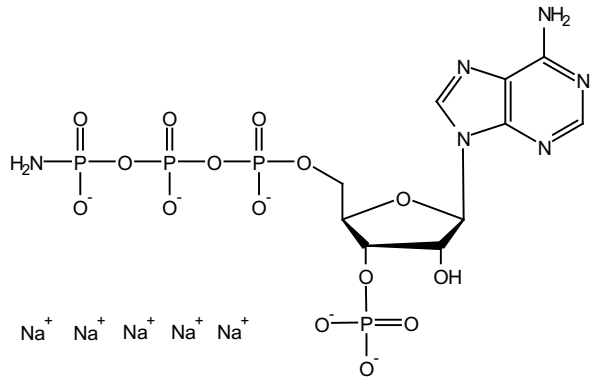
12

Compound 13 (amido-pppAp), ^{1}H - NMR (D_2O, 400 MHz)



13

Compound 13 (amido-pppAp), ^{1}H {^{31}P} - NMR (D_2O, 400 MHz)



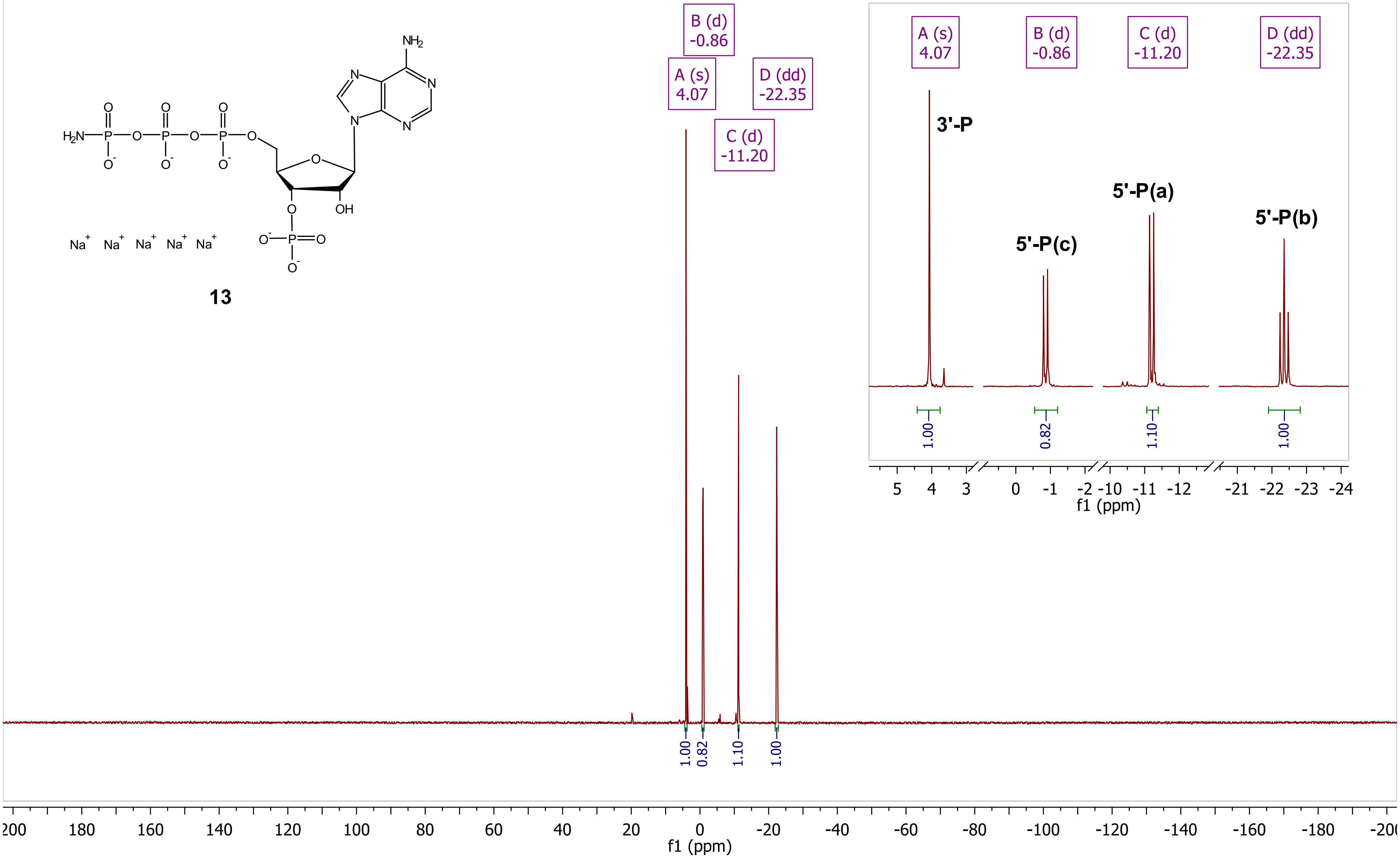
13

C (s) 8.48
B (s) 8.18
A (d) 6.10
G (m) 4.73
E (m) 4.51
D (m) 4.18
F (dd) 4.68
acetone

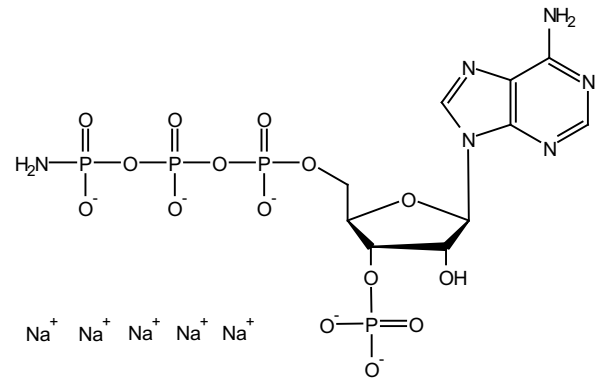
1.00 0.96 1.00 0.75 1.12 1.03 2.26

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)

Compound 13 (amido-pppAp), $^{31}P\{^1H\}$ - NMR (D_2O, 162 MHz)



Compound 13 (amido-pppAp), ^{31}P - NMR (D_2O, 162 MHz)



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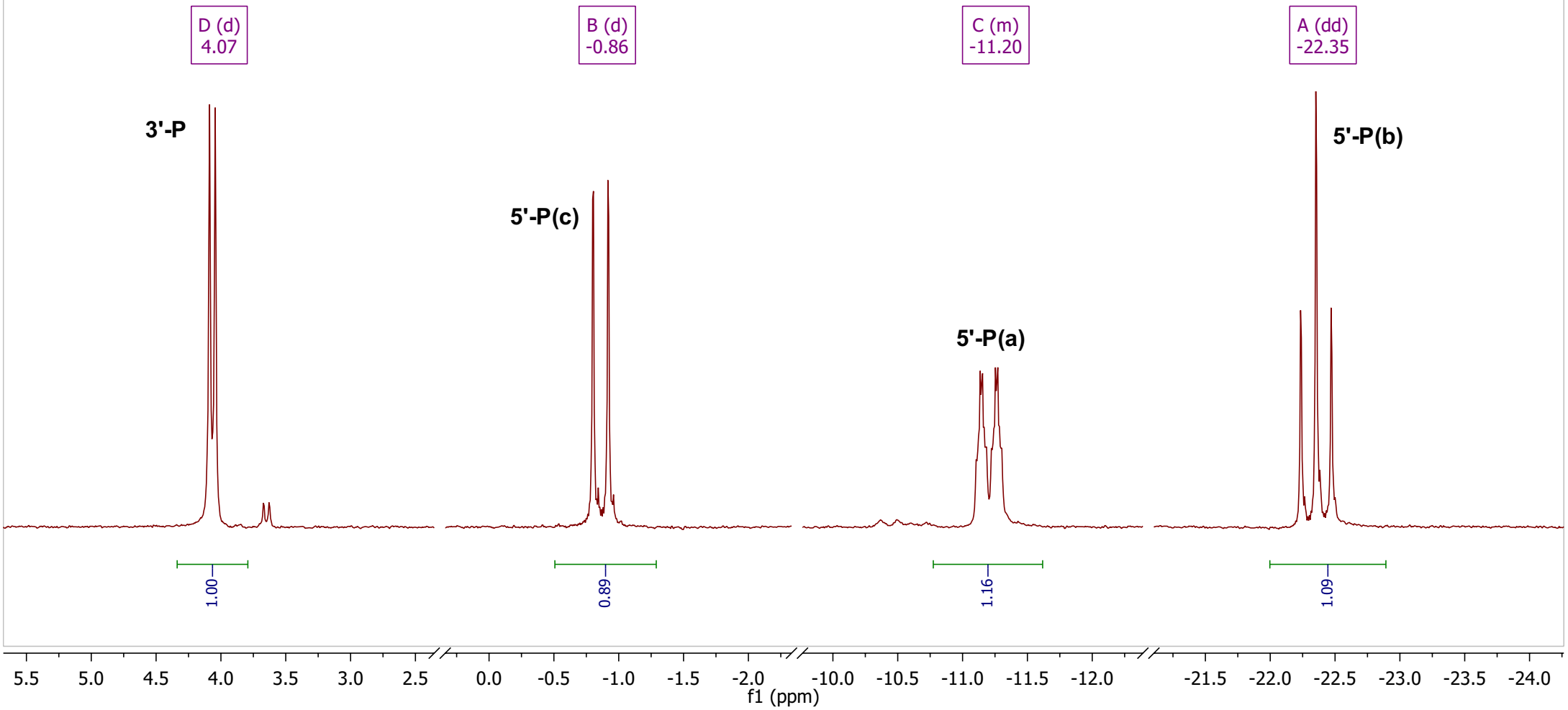
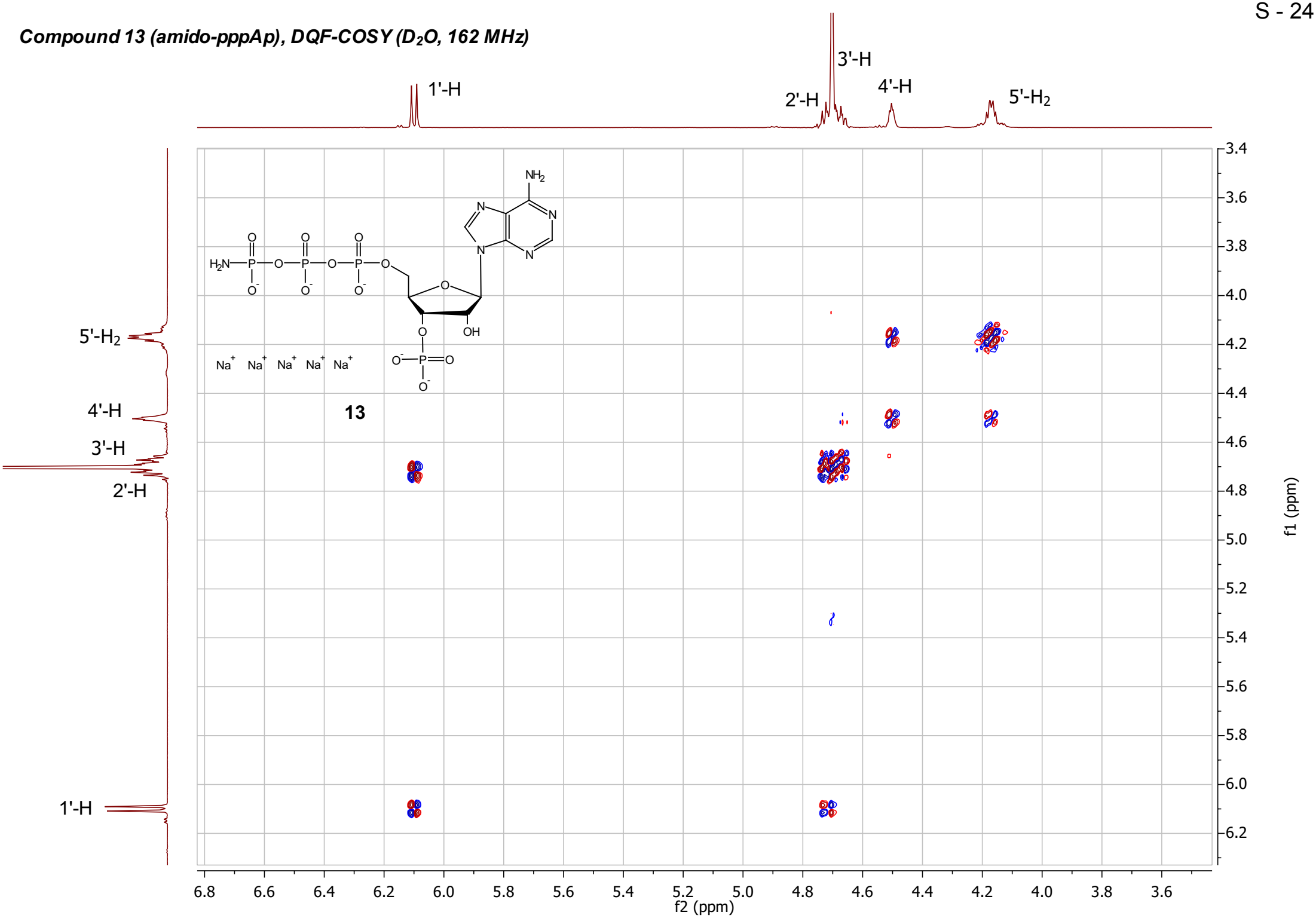
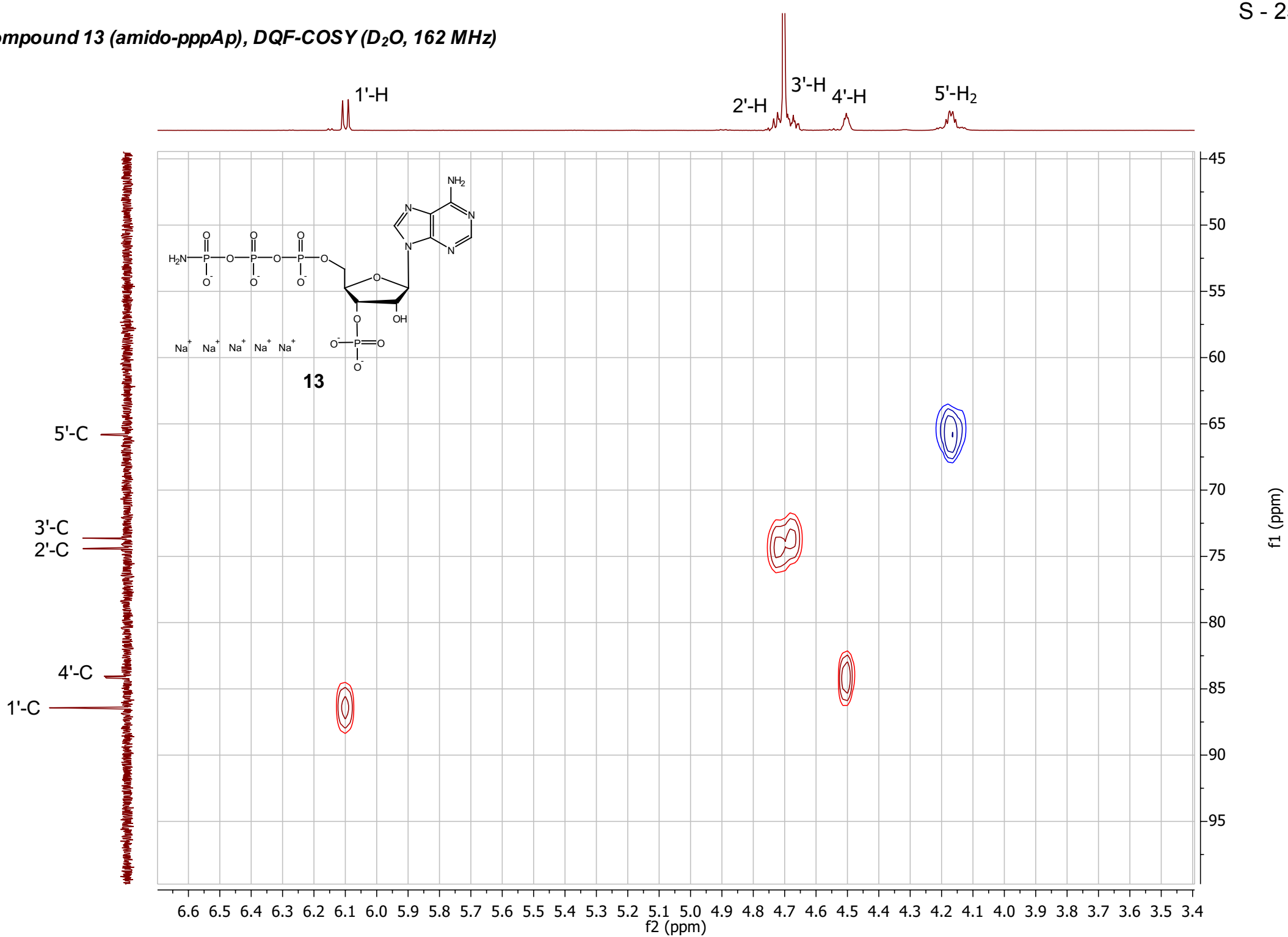


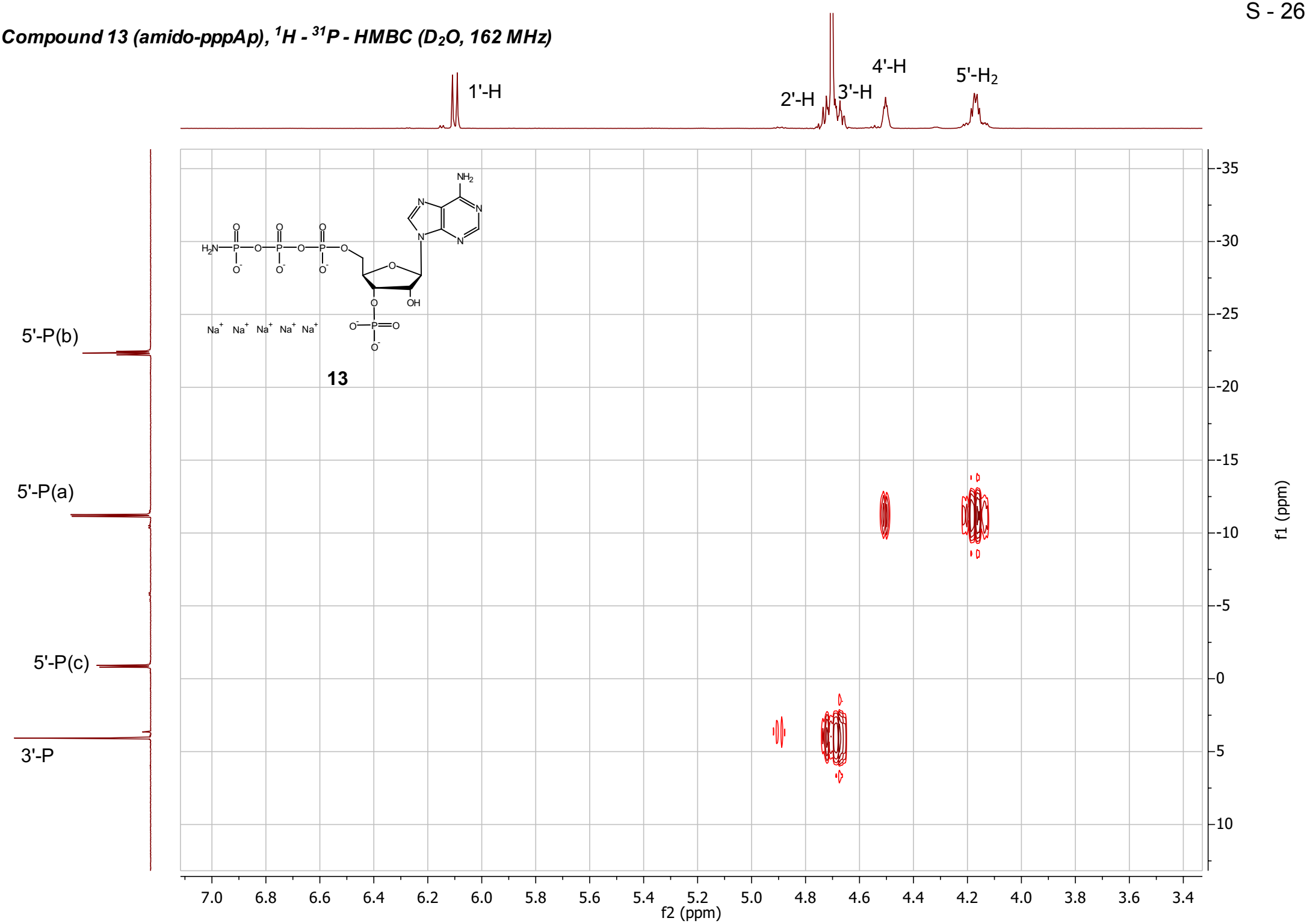
Compound 13 (amido-pppAp), DQF-COSY (D_2O, 162 MHz)



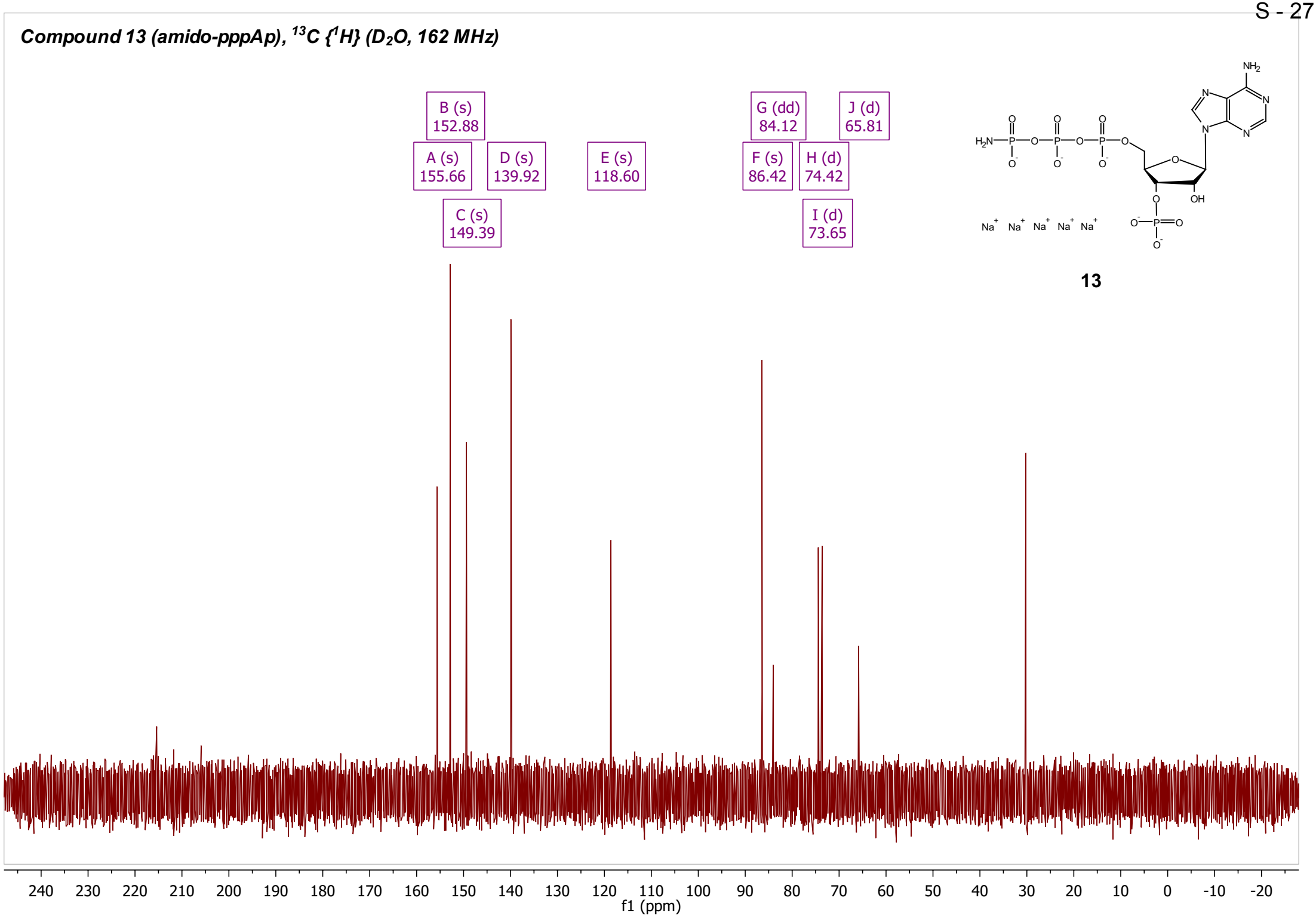
Compound 13 (amido-pppAp), DQF-COSY (D_2O, 162 MHz)



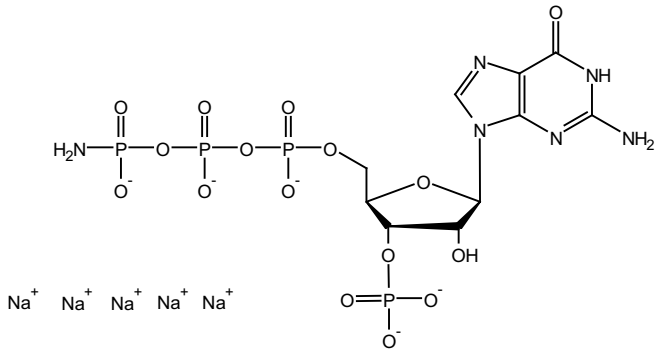
Compound 13 (amido-pppAp), ^{1}H - ^{31}P - HMBC (D_2O, 162 MHz)



Compound 13 (amido-pppAp), ^{13}C {^{1}H} (D_2O, 162 MHz)

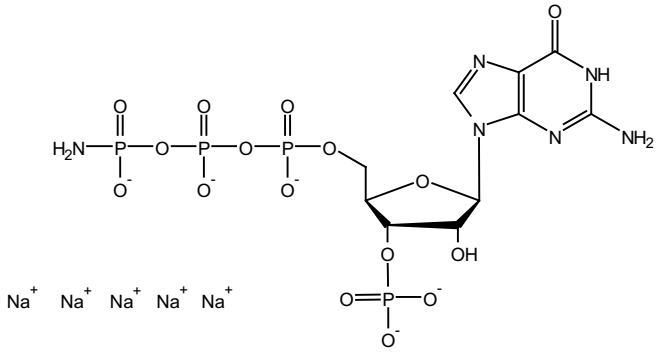


Compound 14 (amido-pppGp), ^{1}H - NMR (D_2O, 162 MHz)



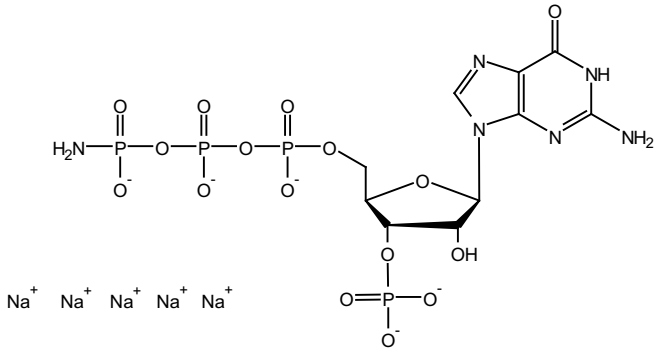
14

Compound 14 (amido-pppGp), ^{1}H {^{31}P} - NMR (D_2O, 400 MHz)



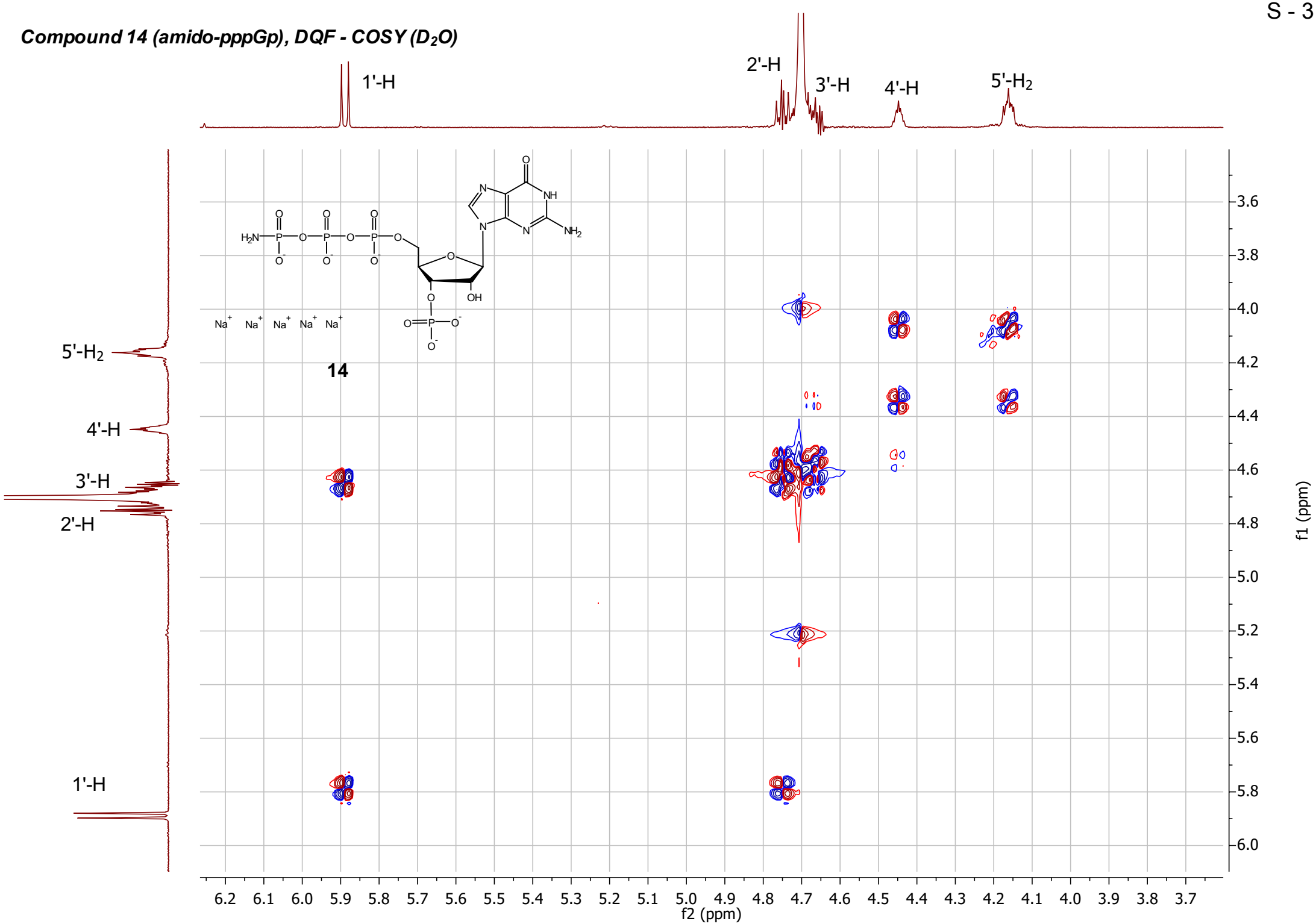
14

Compound 14 (amido-pppGp), $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)

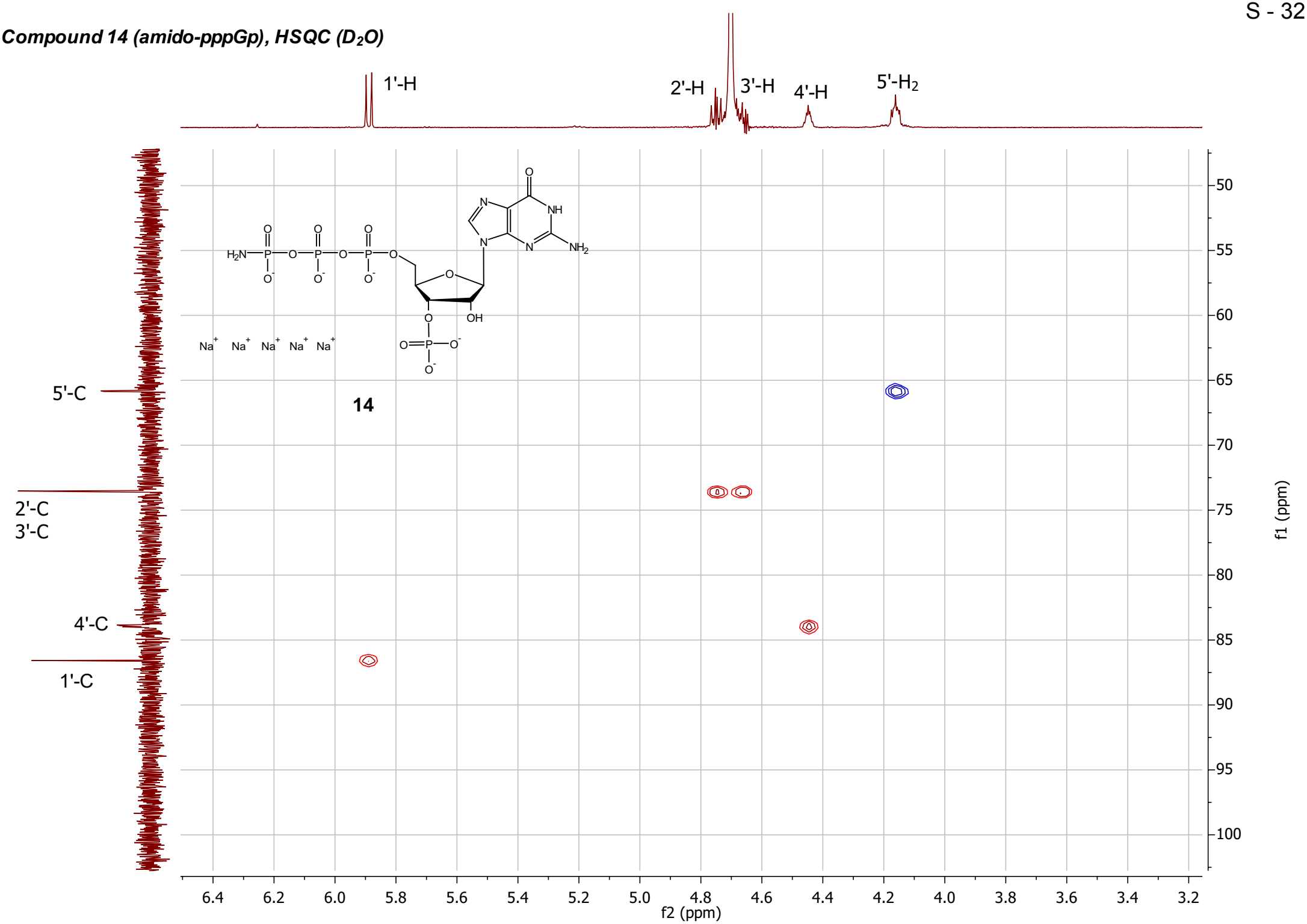


14

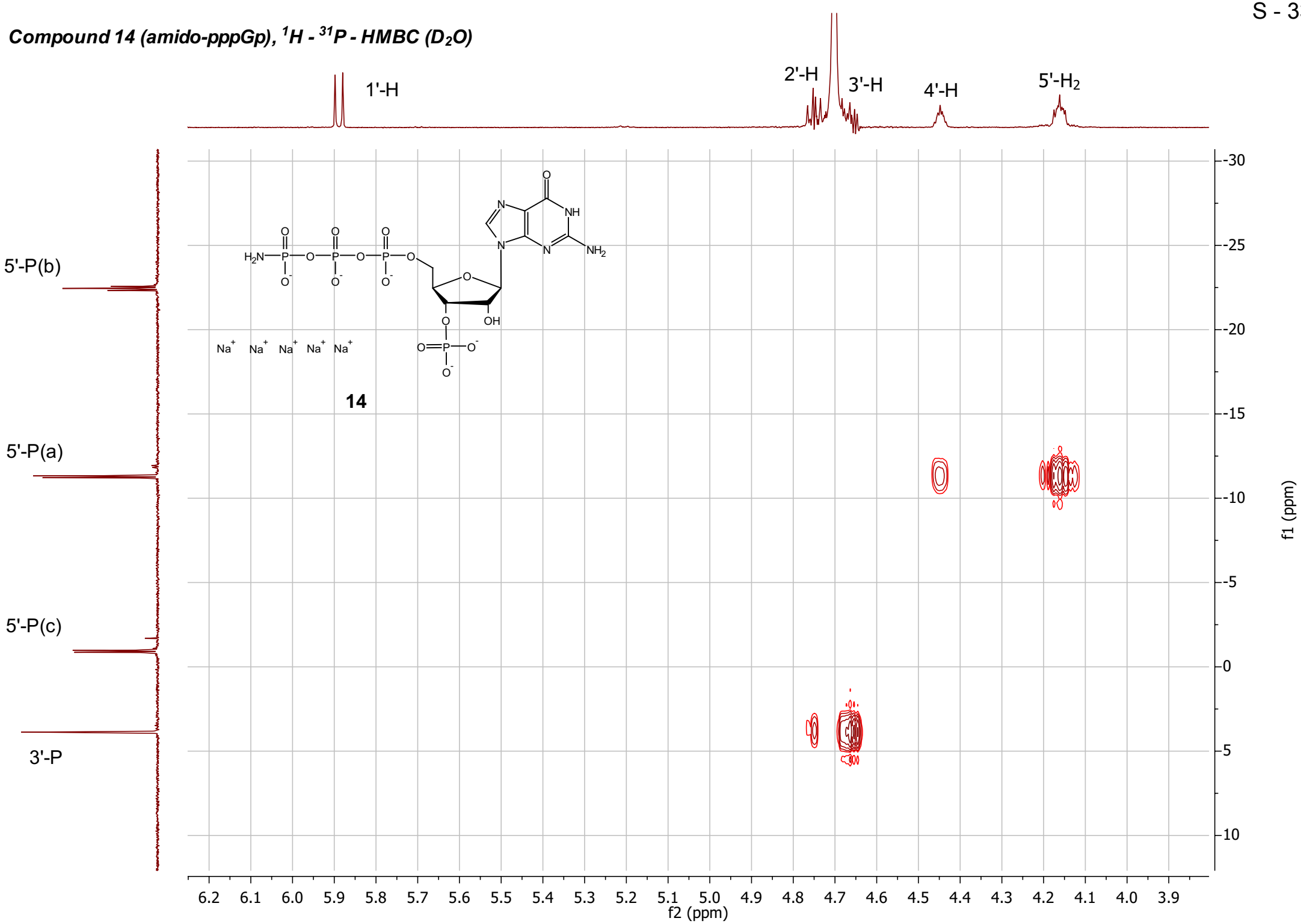
Compound 14 (amido-pppGp), DQF - COSY (D_2O)



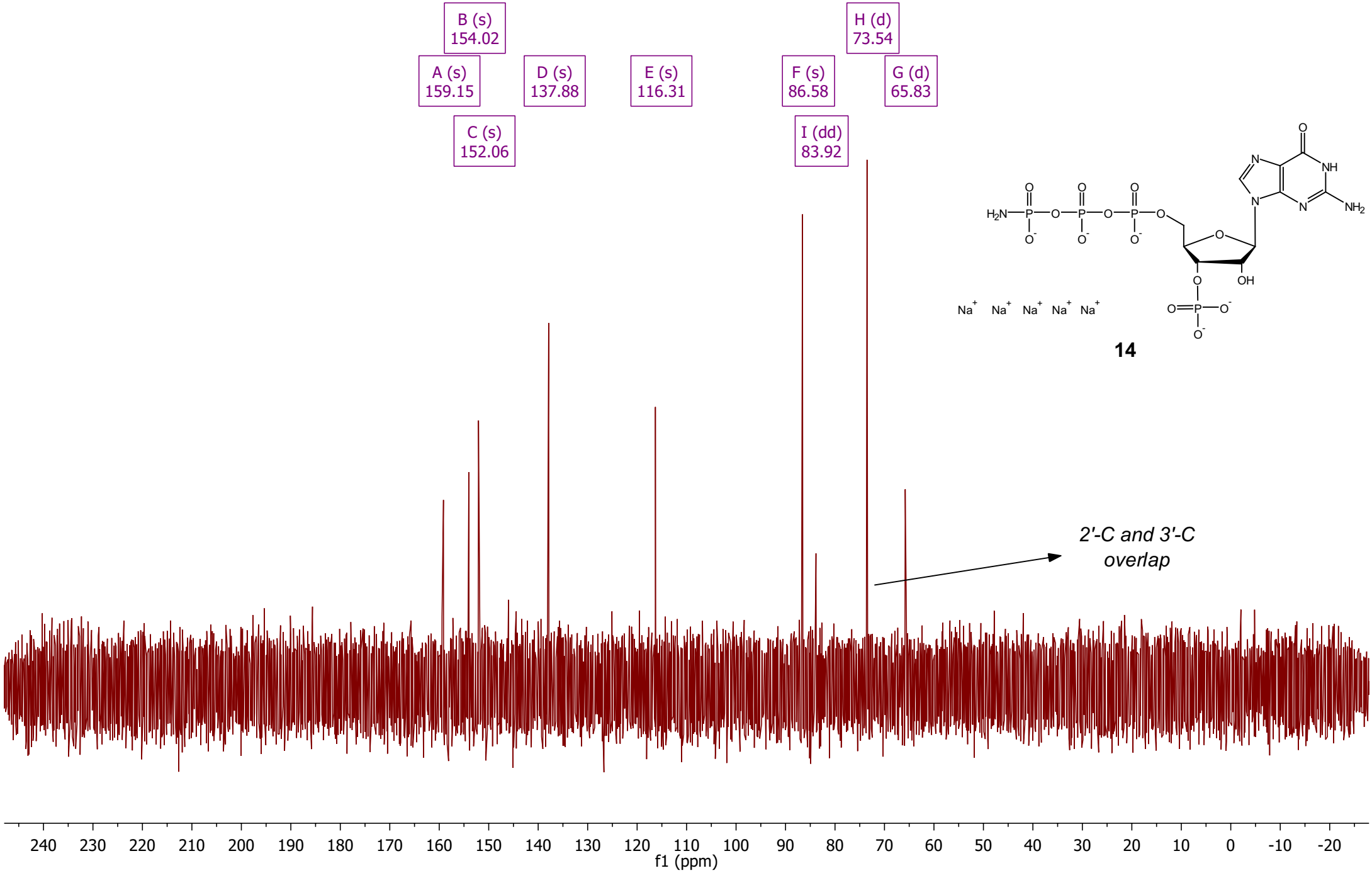
Compound 14 (amido-pppGp), HSQC (D_2O)



Compound 14 (amido-pppGp), ^{1}H - ^{31}P - HMBC (D_2O)

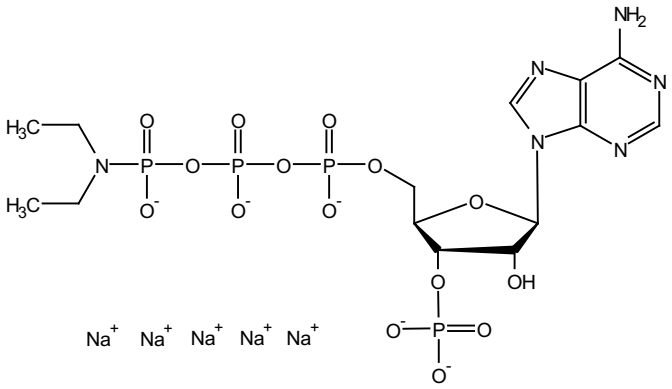


Compound 14 (amido-pppGp), ^{13}C {^{1}H} (D_2O, 162 MHz)



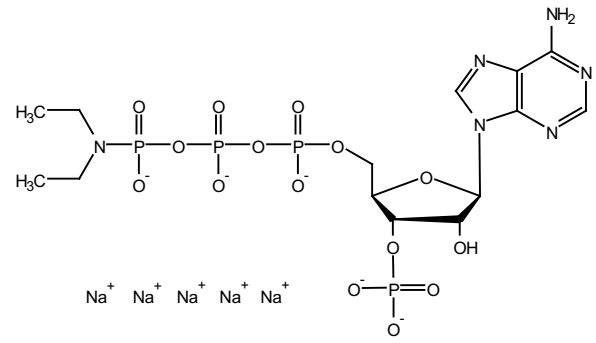
14

Compound 15 (diethylamido-pppAp), ^{1}H - NMR (D_2O, 400 MHz)



15

Compound 15 (diethylamido-pppAp), ^{1}H {^{31}P} - NMR (D_2O, 400 MHz)



15

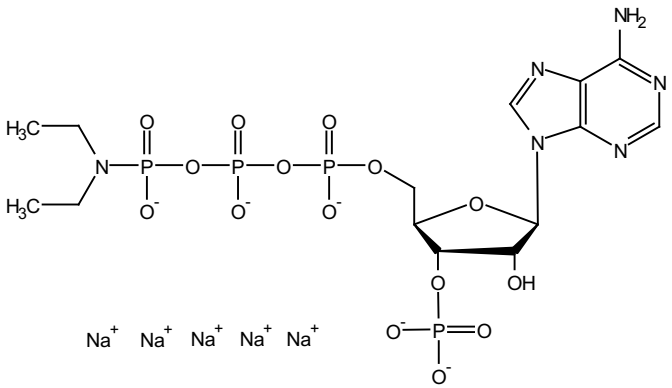
A (s) 8.49
B (s) 8.18
C (d) 6.09
G (d) 4.68
H (m) 4.47
F (m) 4.76
E (d) 4.14
D (q) 2.84
I (t) 0.86

1.00 0.89 1.00 0.97 1.14 1.09 2.23 4.02 6.03

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4

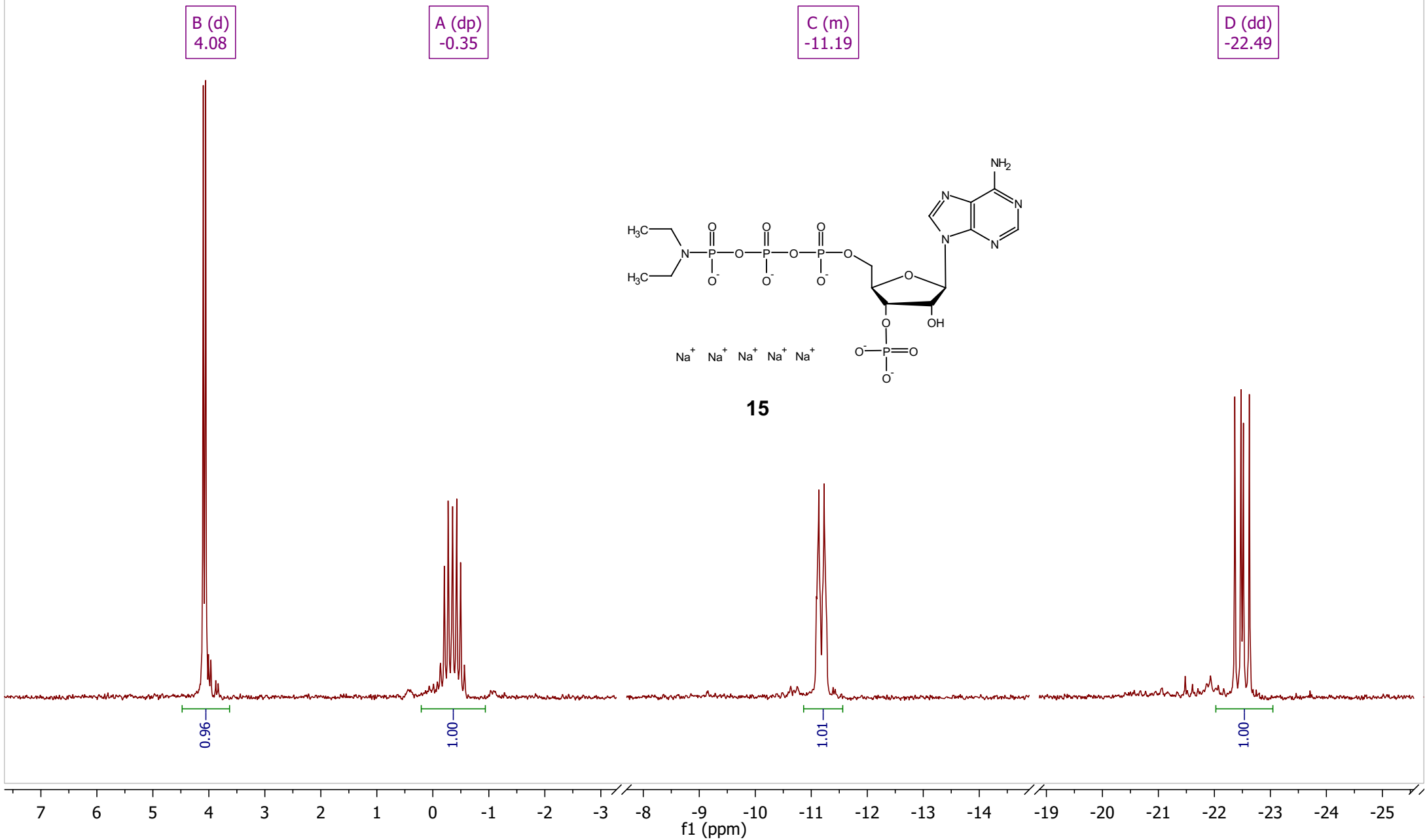
f1 (ppm)

Compound 15 (diethylamido-pppAp), $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)



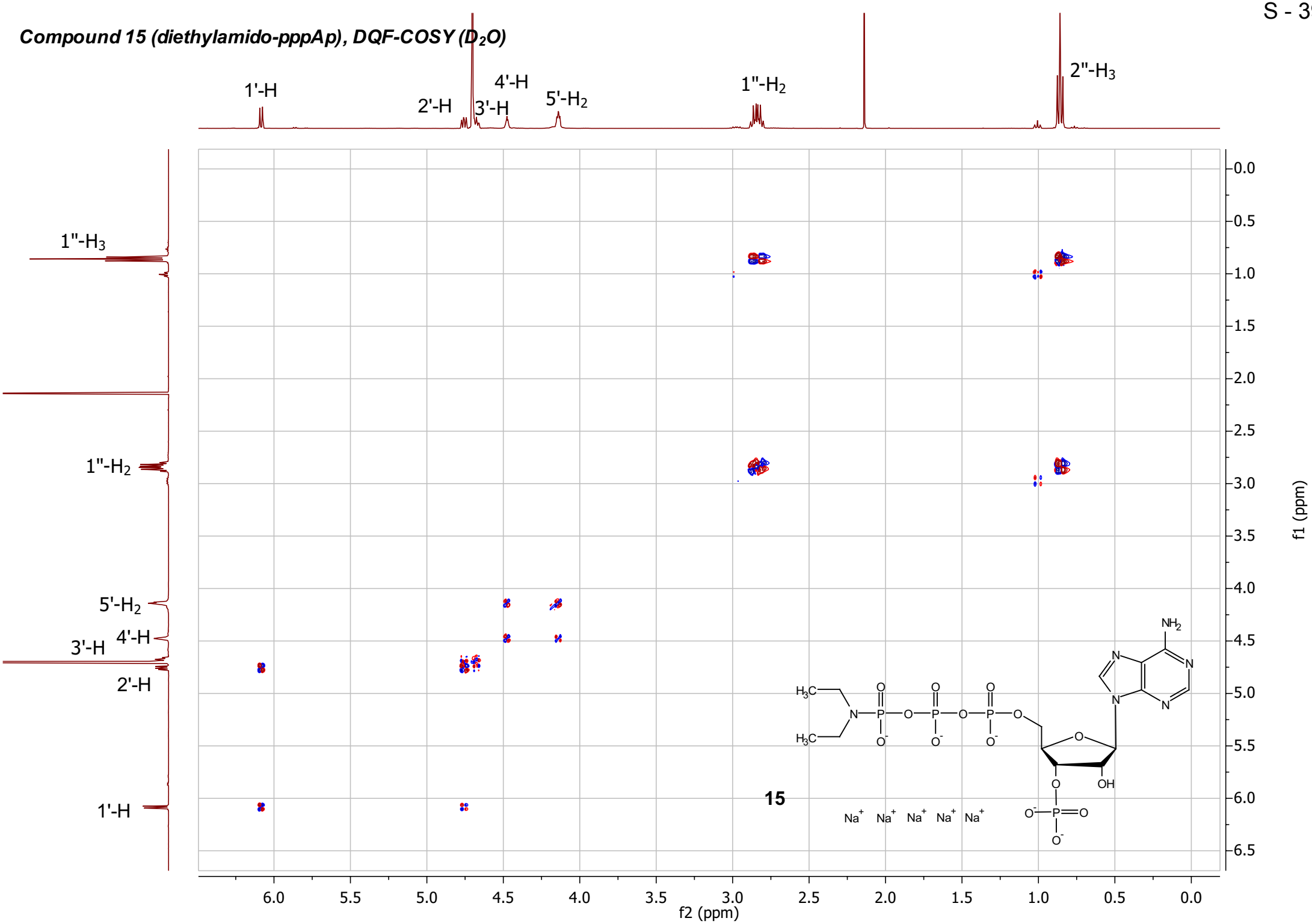
15

Compound 15 (diethylamido-pppAp), ^{31}P - NMR (D_2O, 162 MHz)

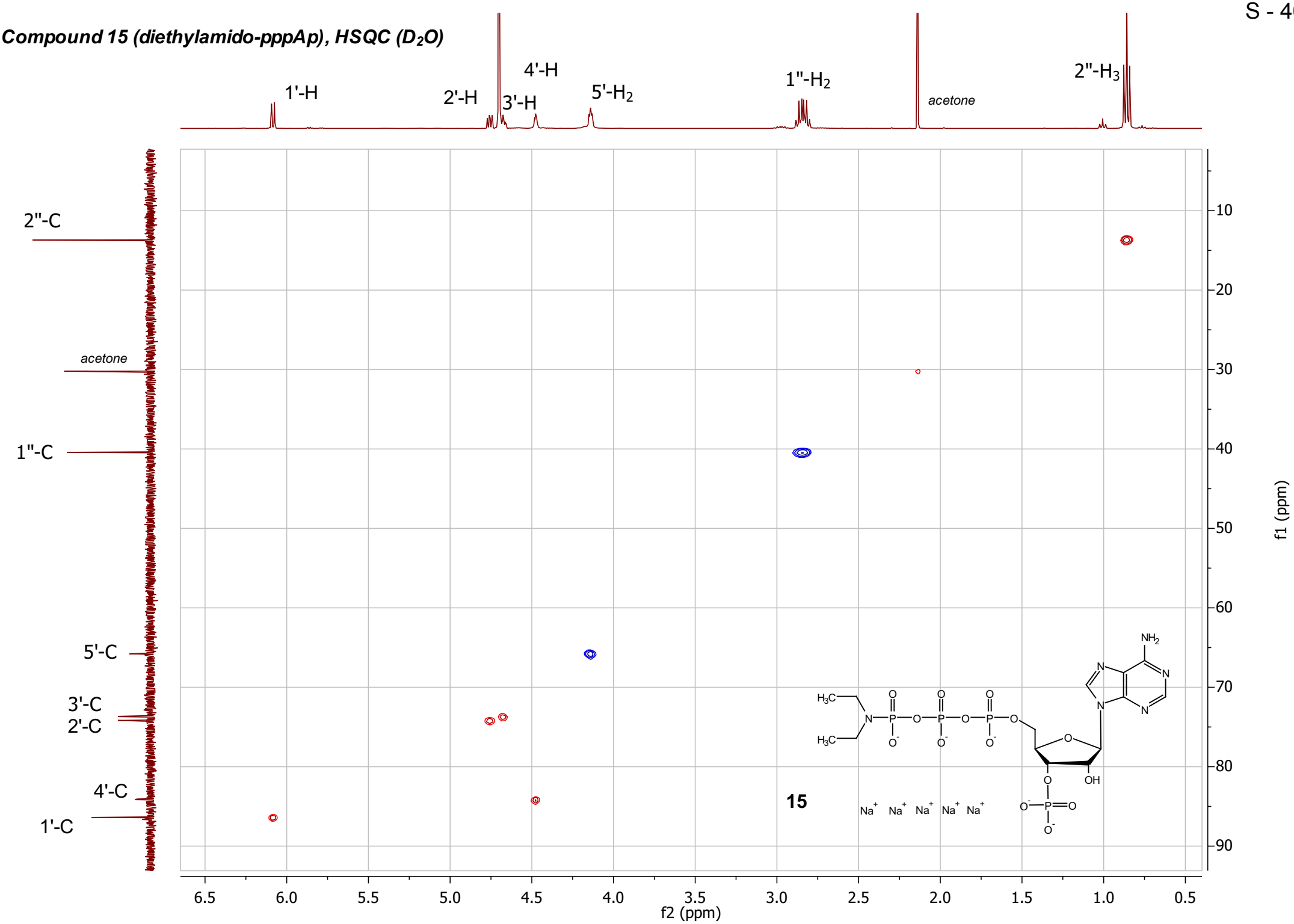


15

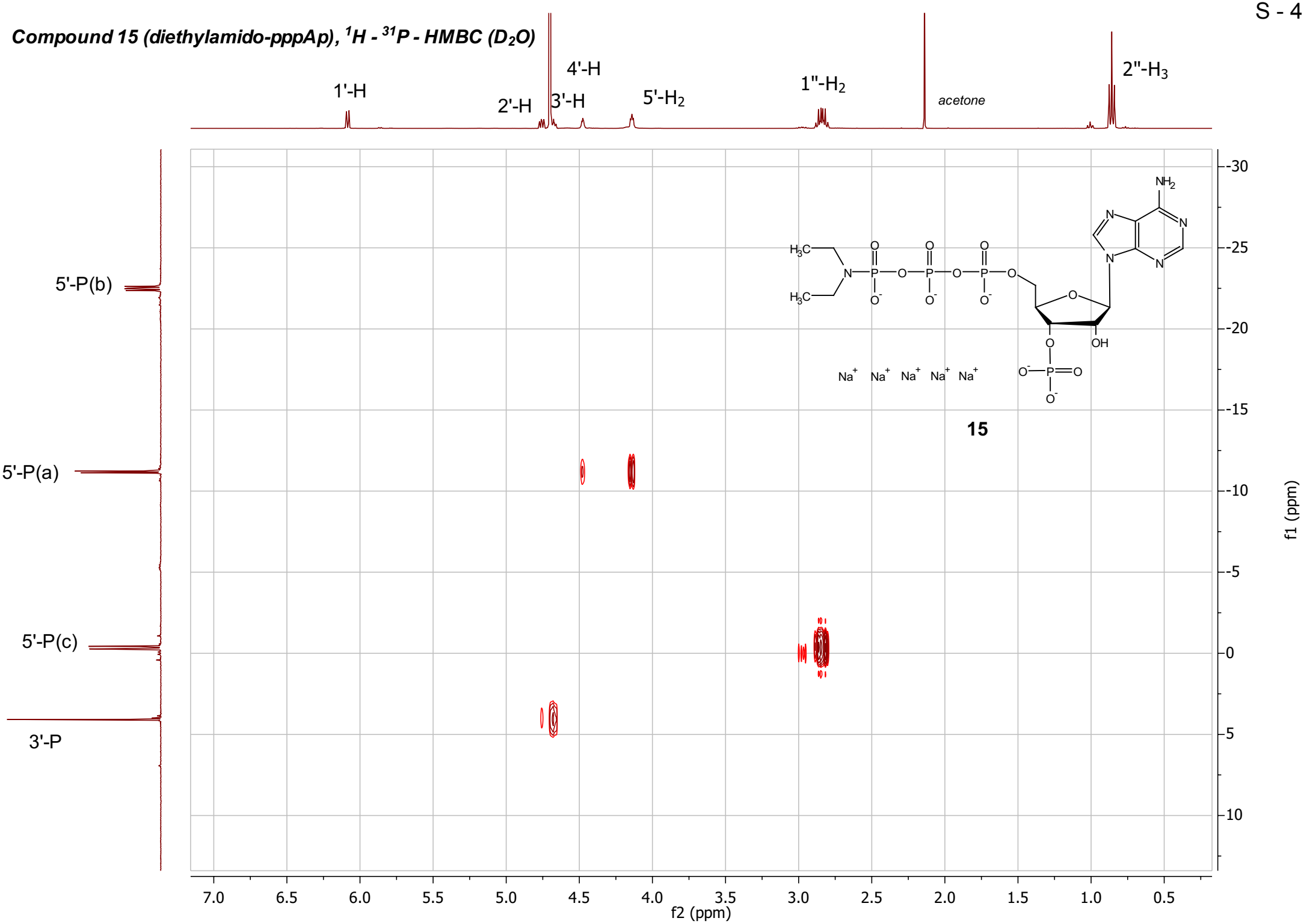
Compound 15 (diethylamido-pppAp), DQF-COSY (D_2O)



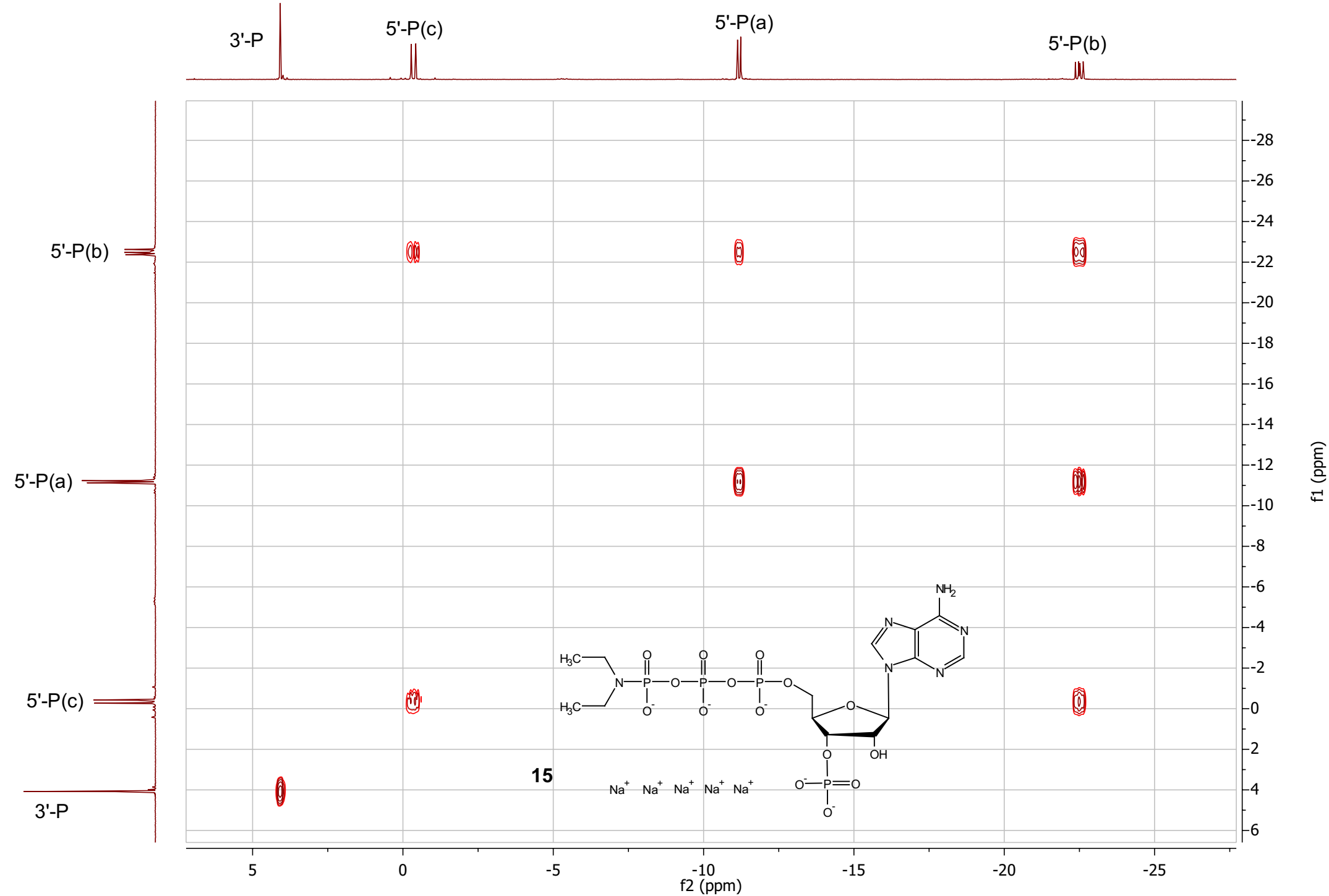
Compound 15 (diethylamido-pppAp), HSQC (D_2O)



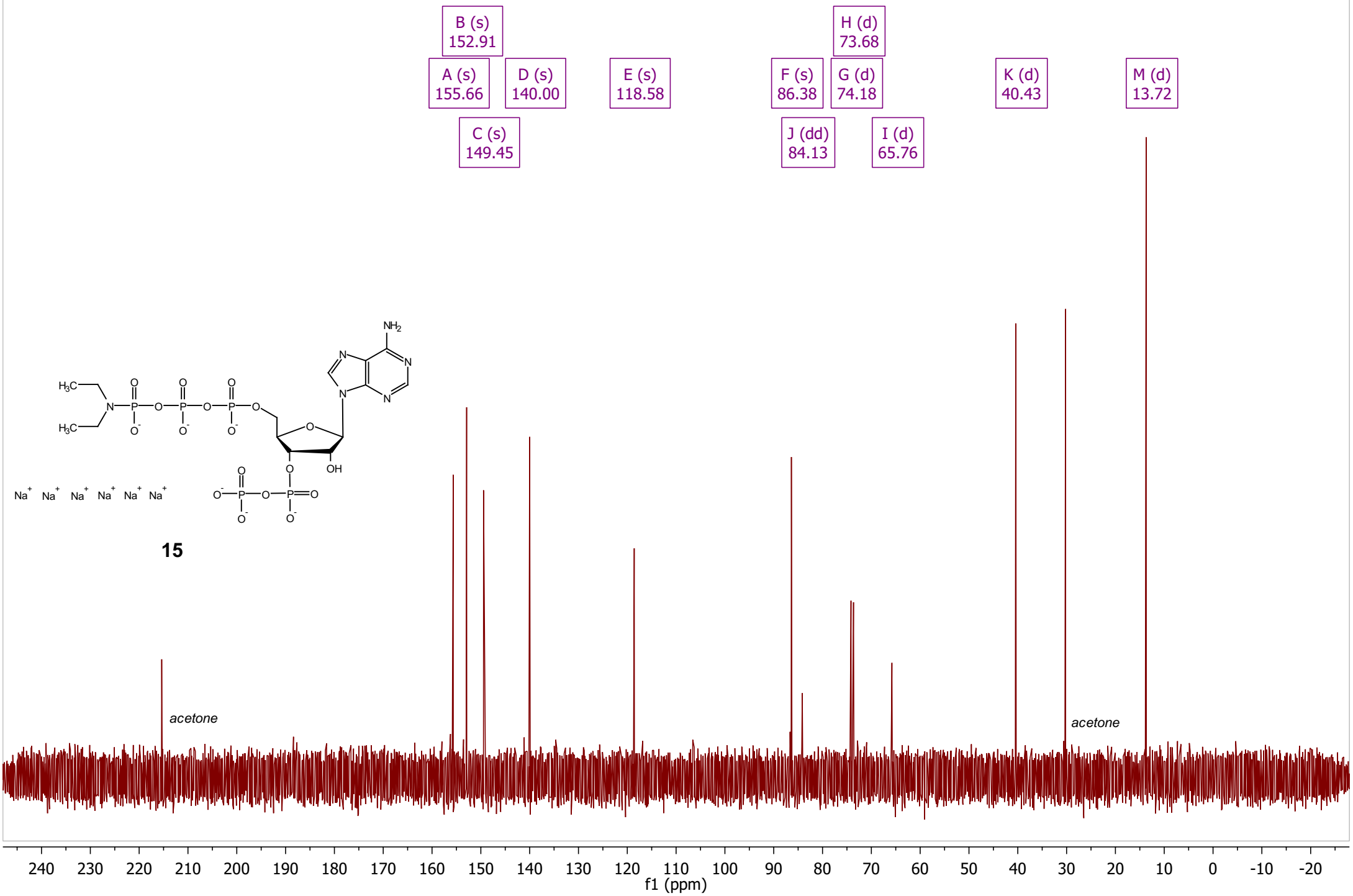
Compound 15 (diethylamido-pppAp), ^{1}H - ^{31}P - HMBC (D_2O)



Compound 15 (diethylamido-pppAp), PP-COSY (D_2O)

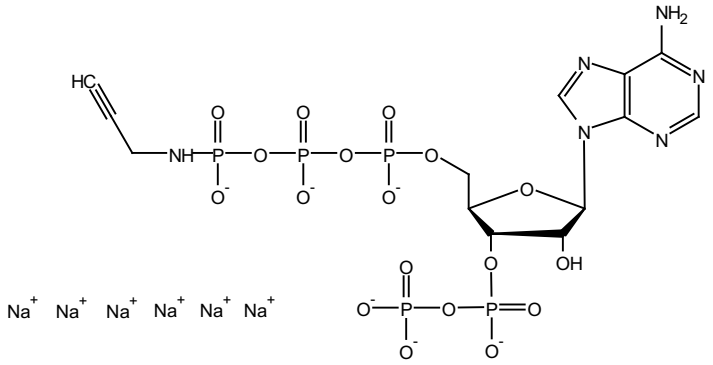


Compound 15 (diethylamido-pppAp), $^{13}C\{^{1}H\}$ - NMR (D_2O, 101 MHz)



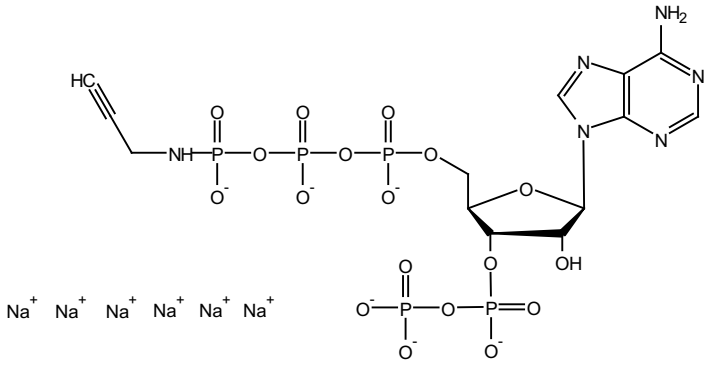
15

Compound 18 (propargylamido-pppApp), ^{1}H - NMR (D_2O, 400 MHz)



18

Compound 18 (propargylamido-pppApp), $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)



18

E (d) -10.75
B (d) -5.54
A (d) -2.66
C (dd) -22.42
D (d) -11.23

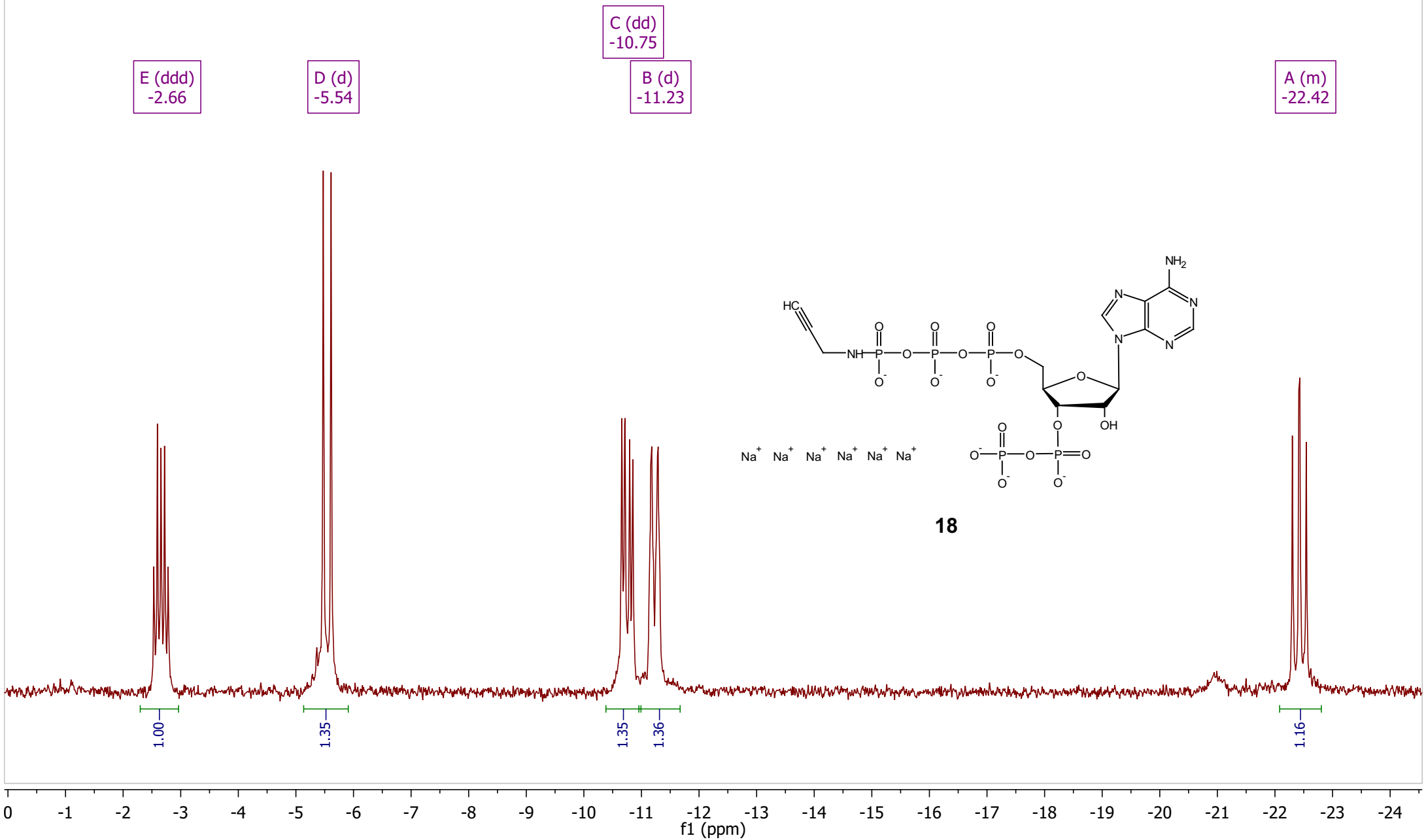
A (d) -2.66
B (d) -5.54
E (d) -10.75
D (d) -11.23
C (dd) -22.42

5'-P(c)
3'-P(b)
3'-P(a)
5'-P(a)
5'-P(b)

0.74 1.00 1.00 1.00 0.84

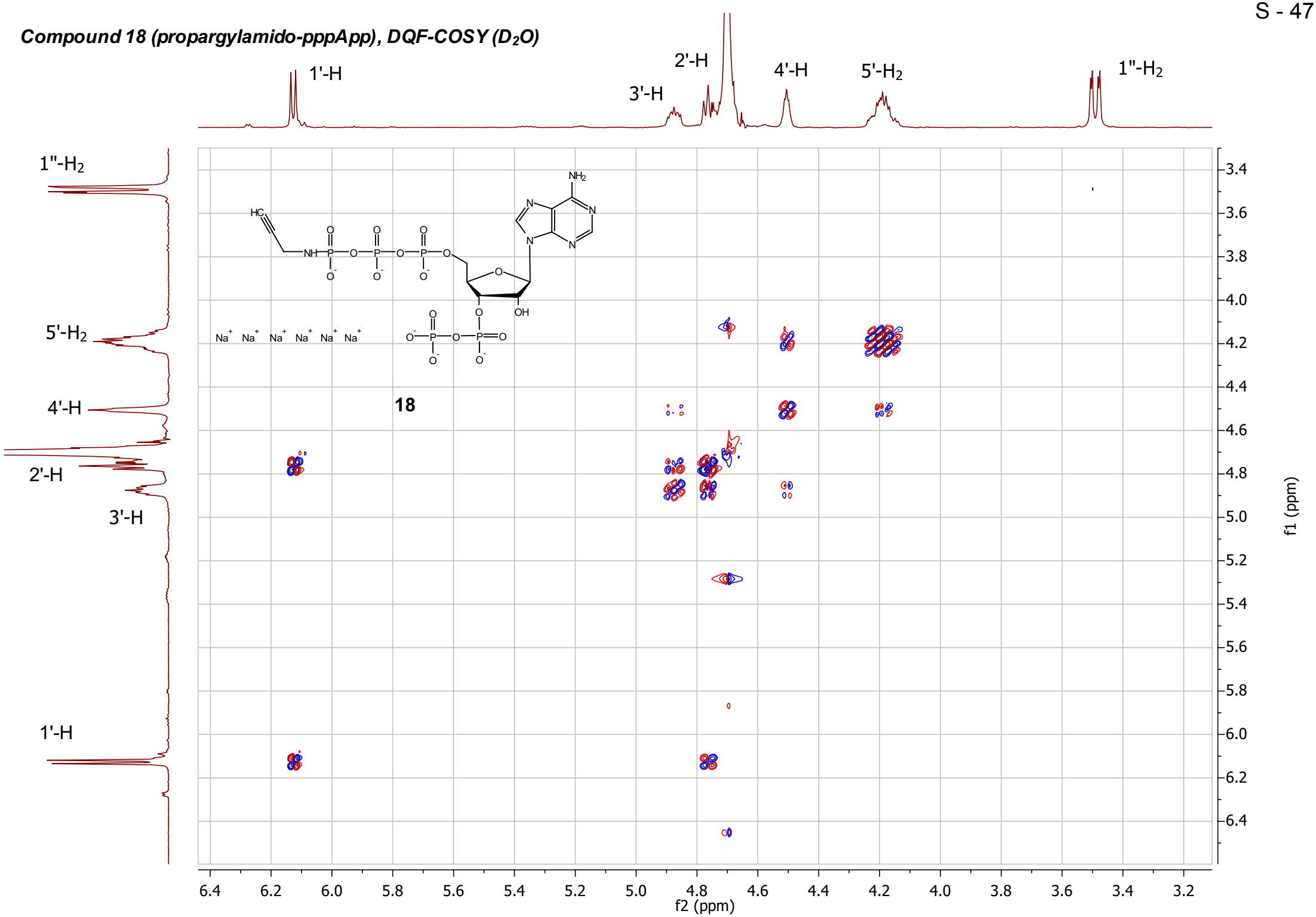
f1 (ppm)

Compound 18 (propargylamido-pppApp), ^{31}P - NMR (D_2O, 162 MHz)

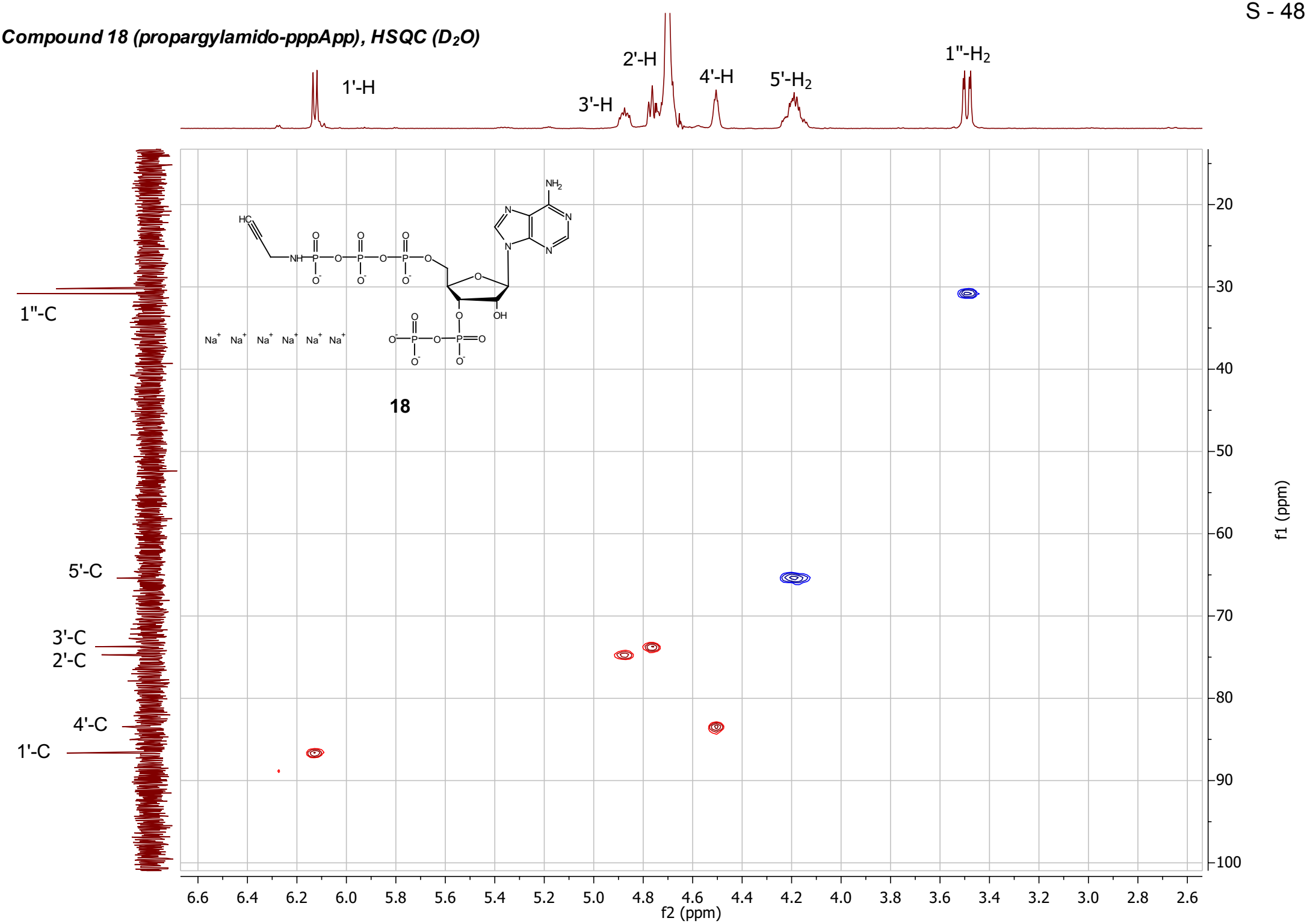


18

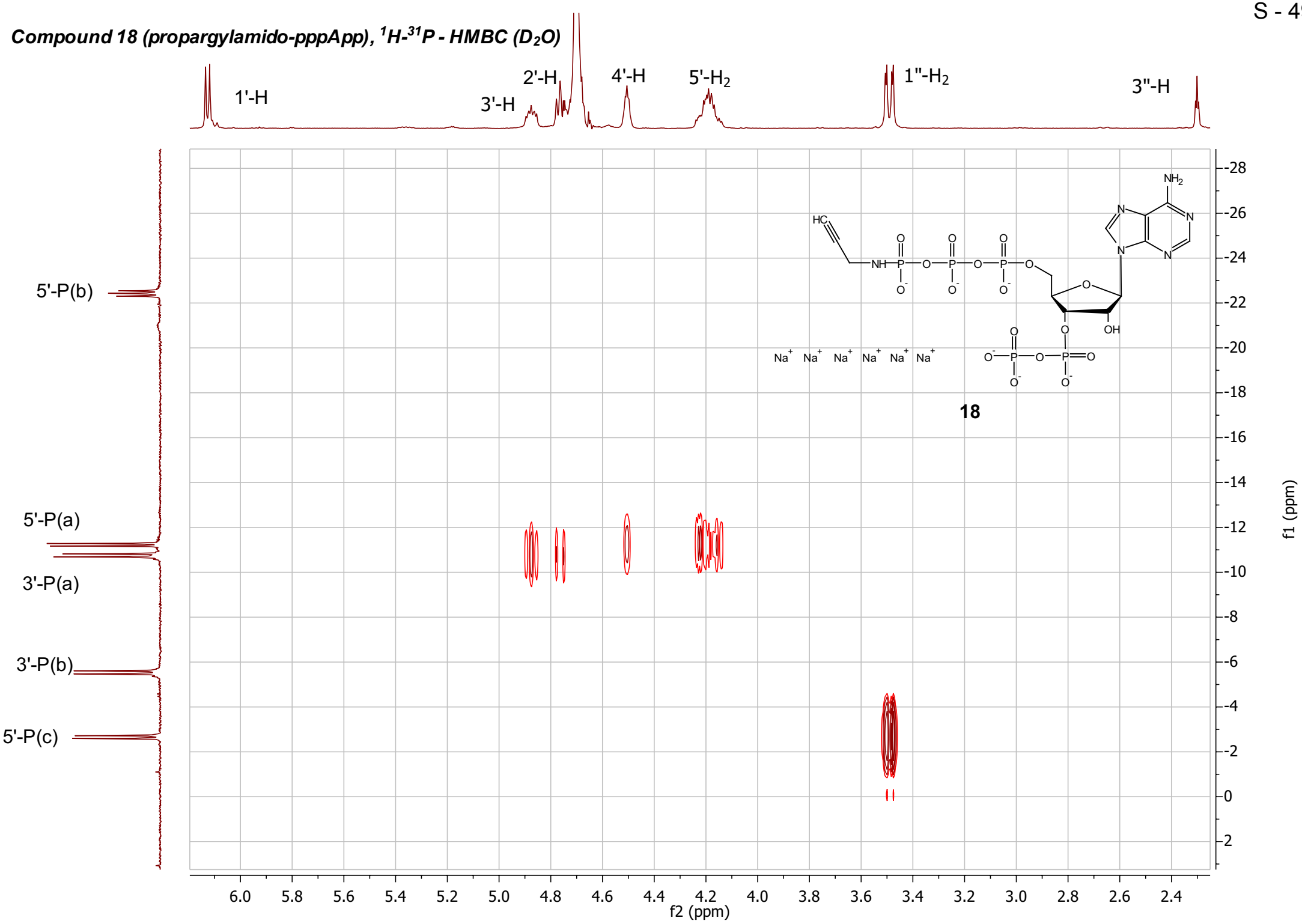
Compound 18 (propargylamido-pppApp), DQF-COSY (D_2O)



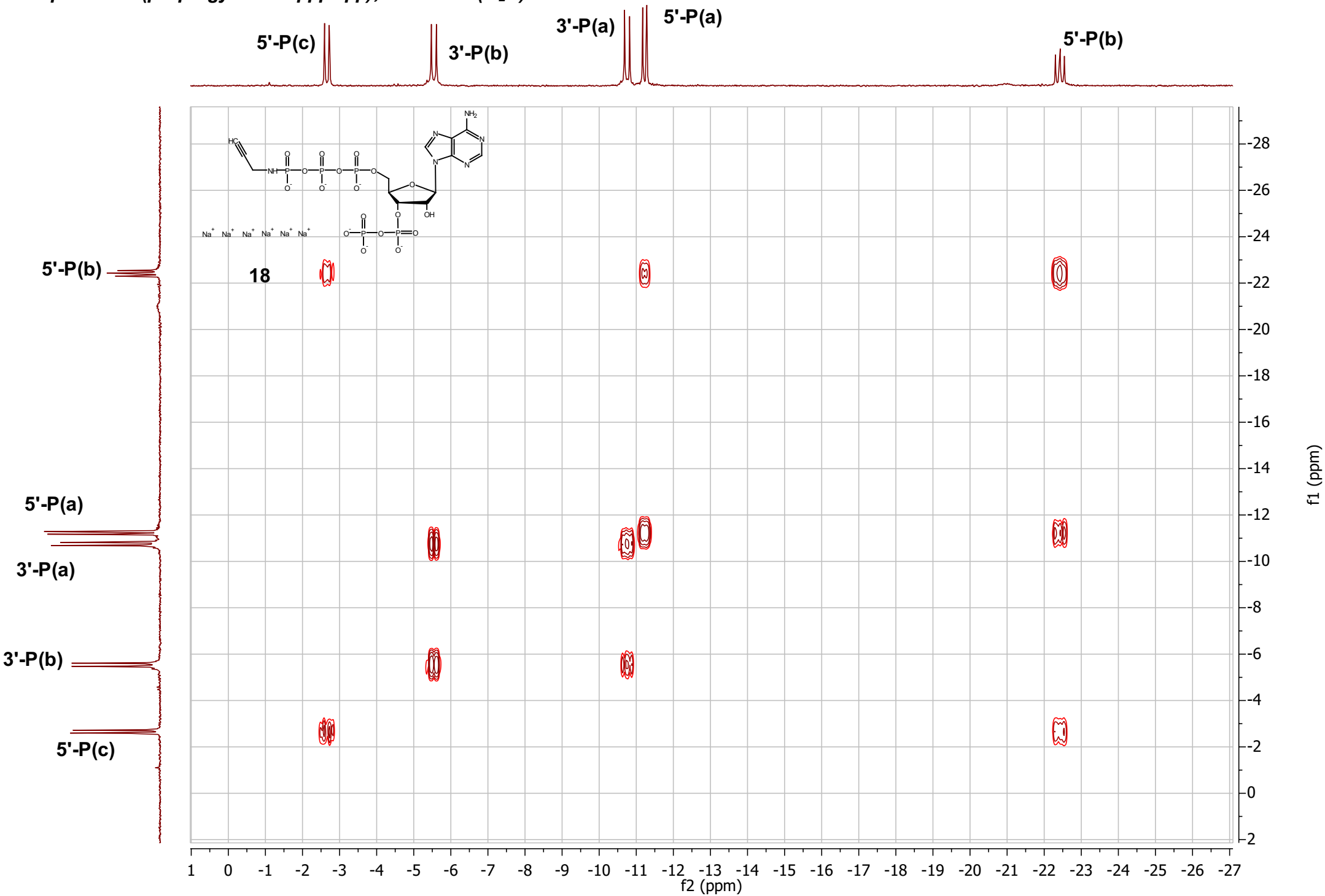
Compound 18 (propargylamido-pppApp), HSQC (D_2O)



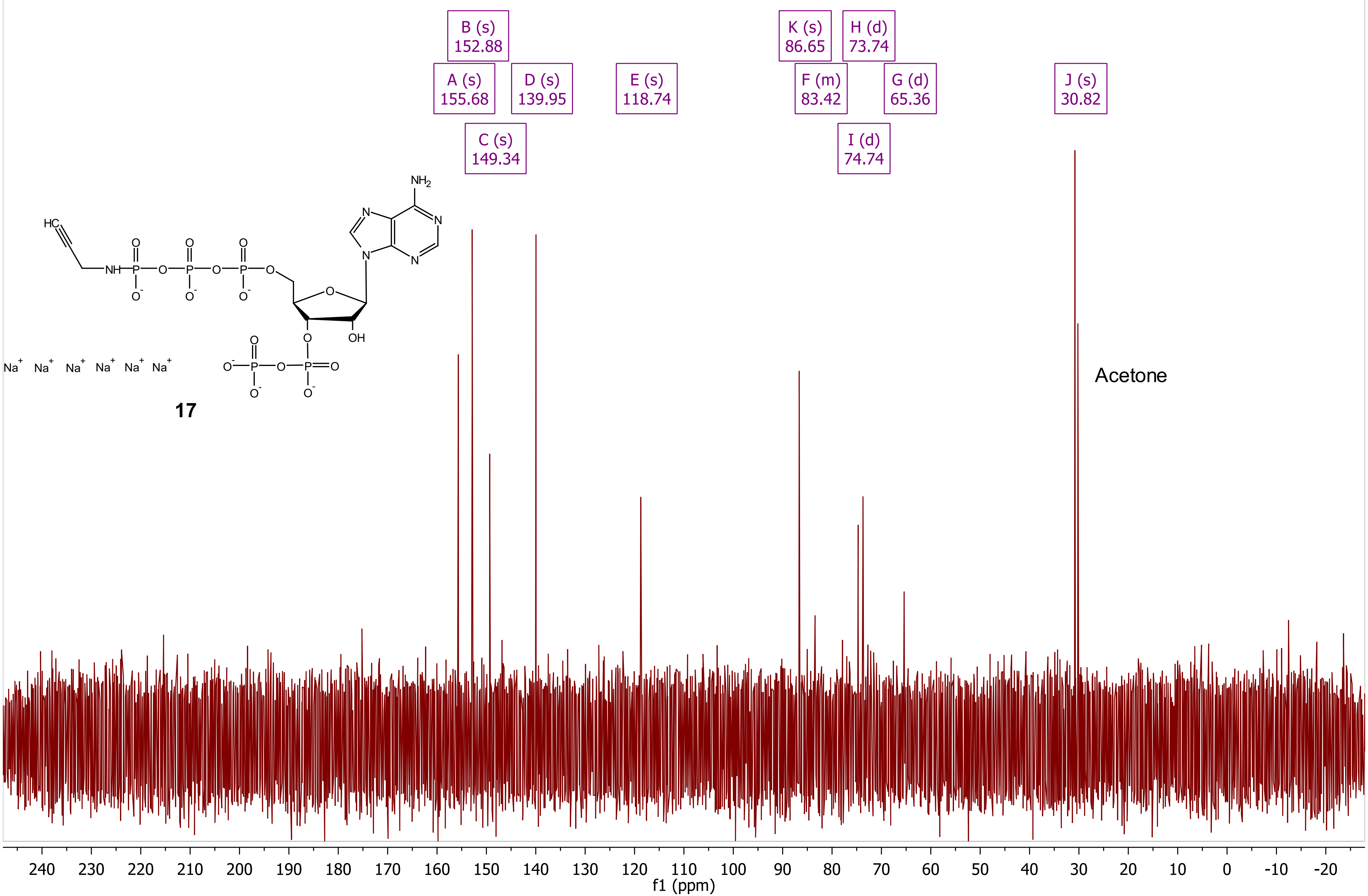
Compound 18 (propargylamido-pppApp), ^{1}H-^{31}P - HMBC (D_2O)



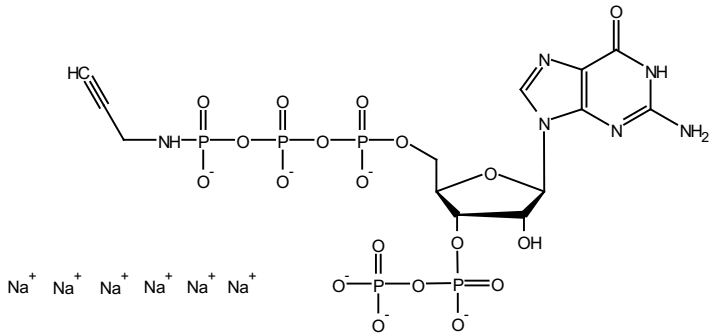
Compound 18 (propargylamido-pppApp), PP-COSY (D_2O)



Compound 18 (propargylamido-pppApp), $^{13}C\ \{^{1}H\}$ - NMR (D_2O, 101 MHz)

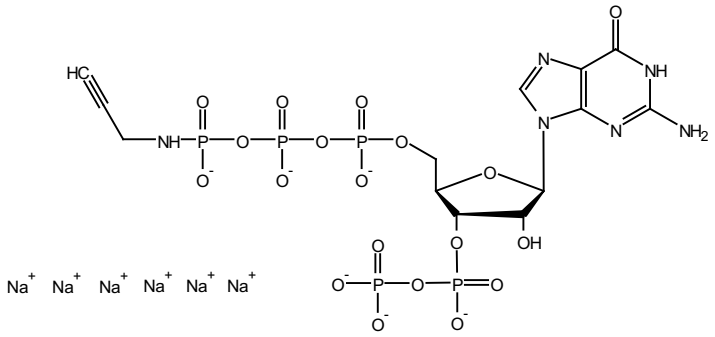


Compound 19 (propargylamido-pppGpp), ^{1}H - NMR (D_2O, 400 MHz)



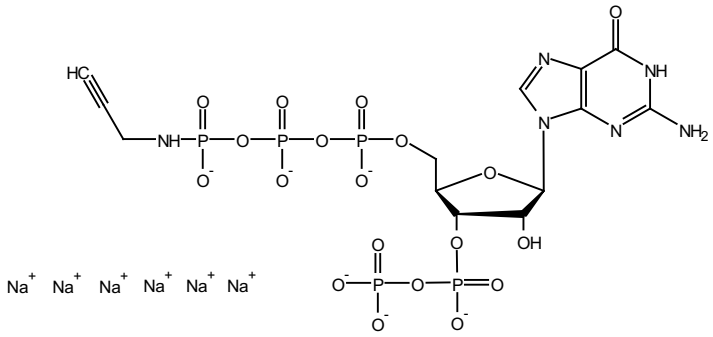
19

Compound 19 (propargylamido-pppGpp), ^{1}H {^{31}P} - NMR (D_2O, 400 MHz)



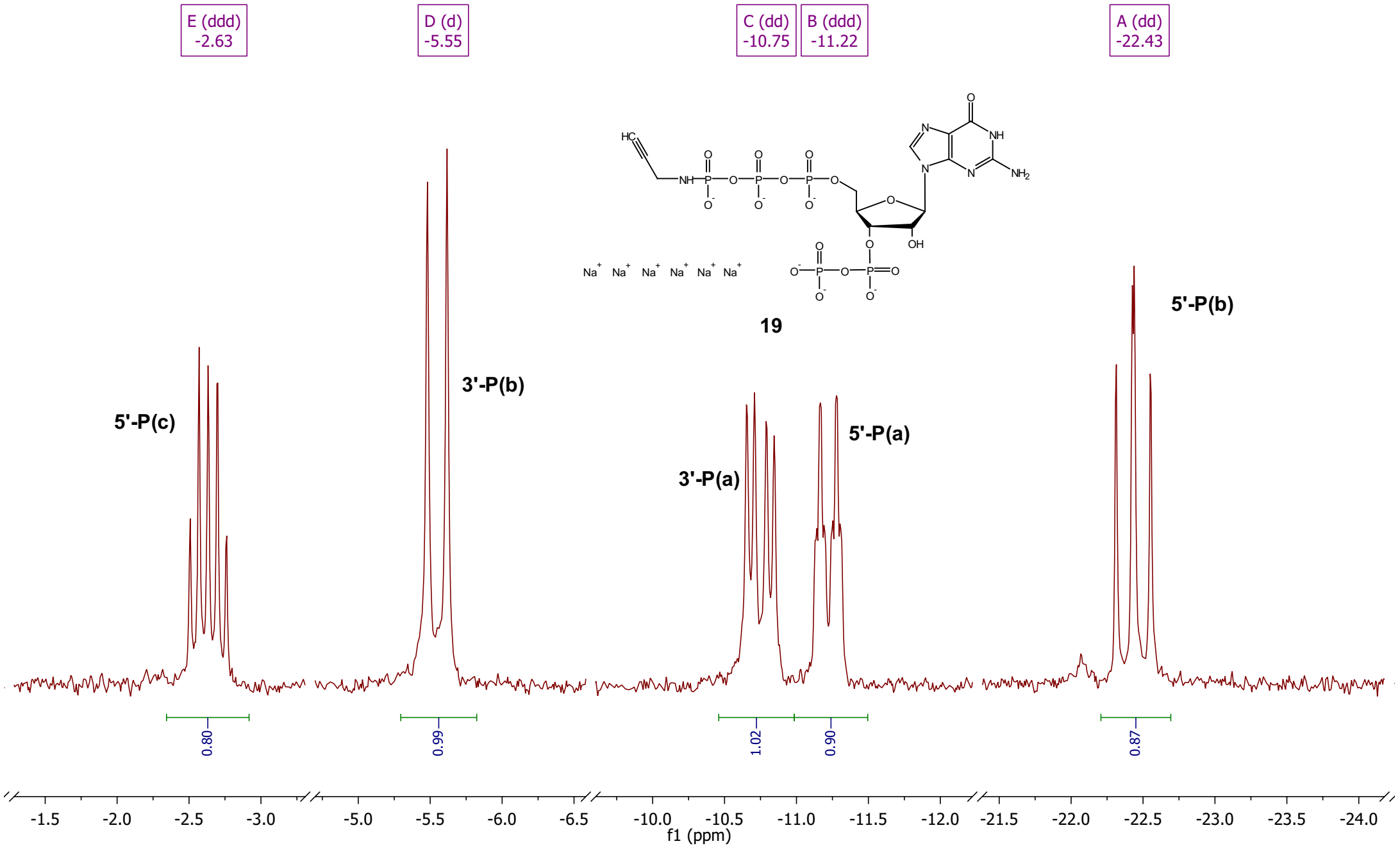
19

Compound 19 (propargylamido-pppGpp), $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)



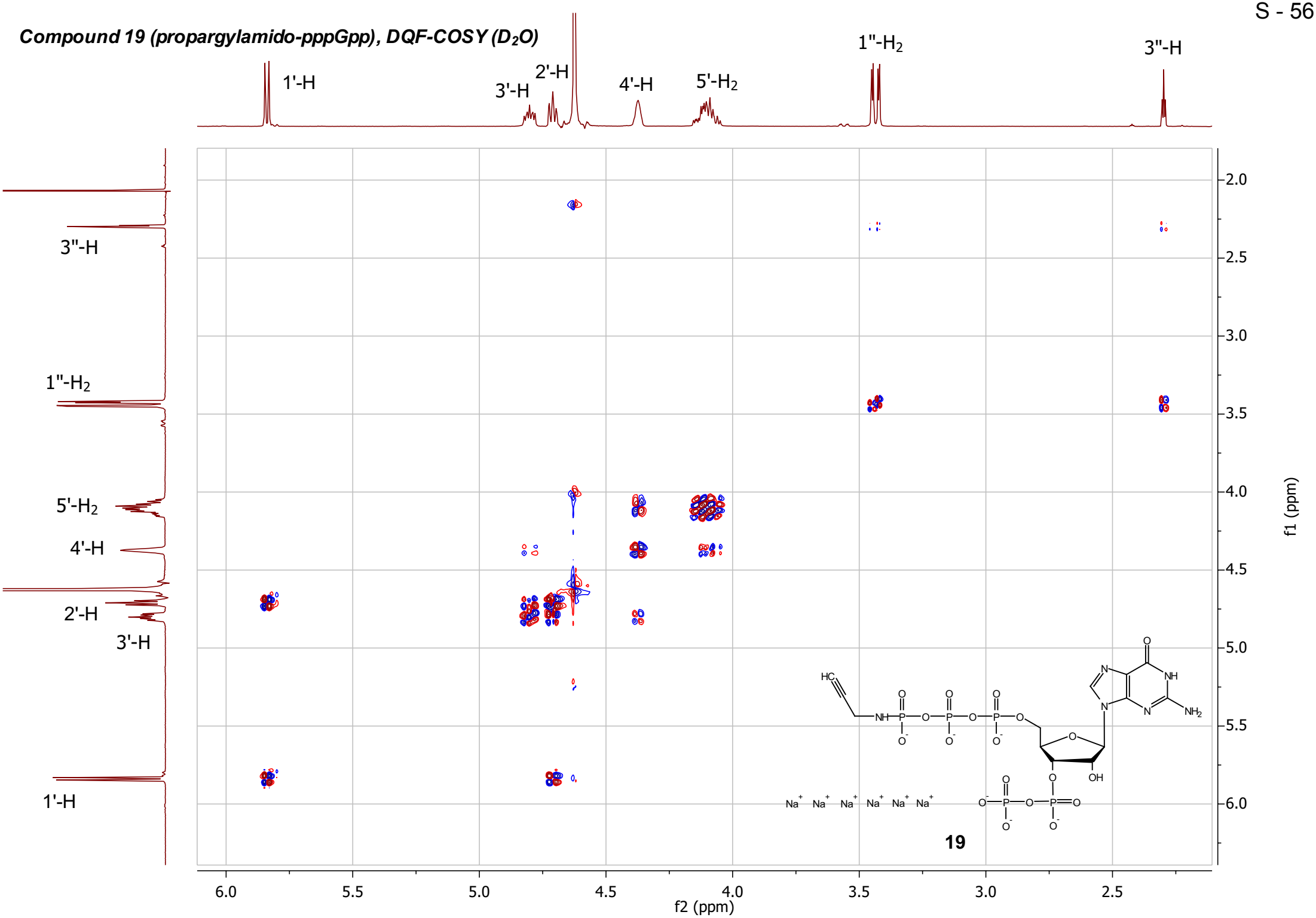
19

Compound 19 (propargylamido-pppGpp), ^{31}P - NMR (D_2O, 162 MHz)

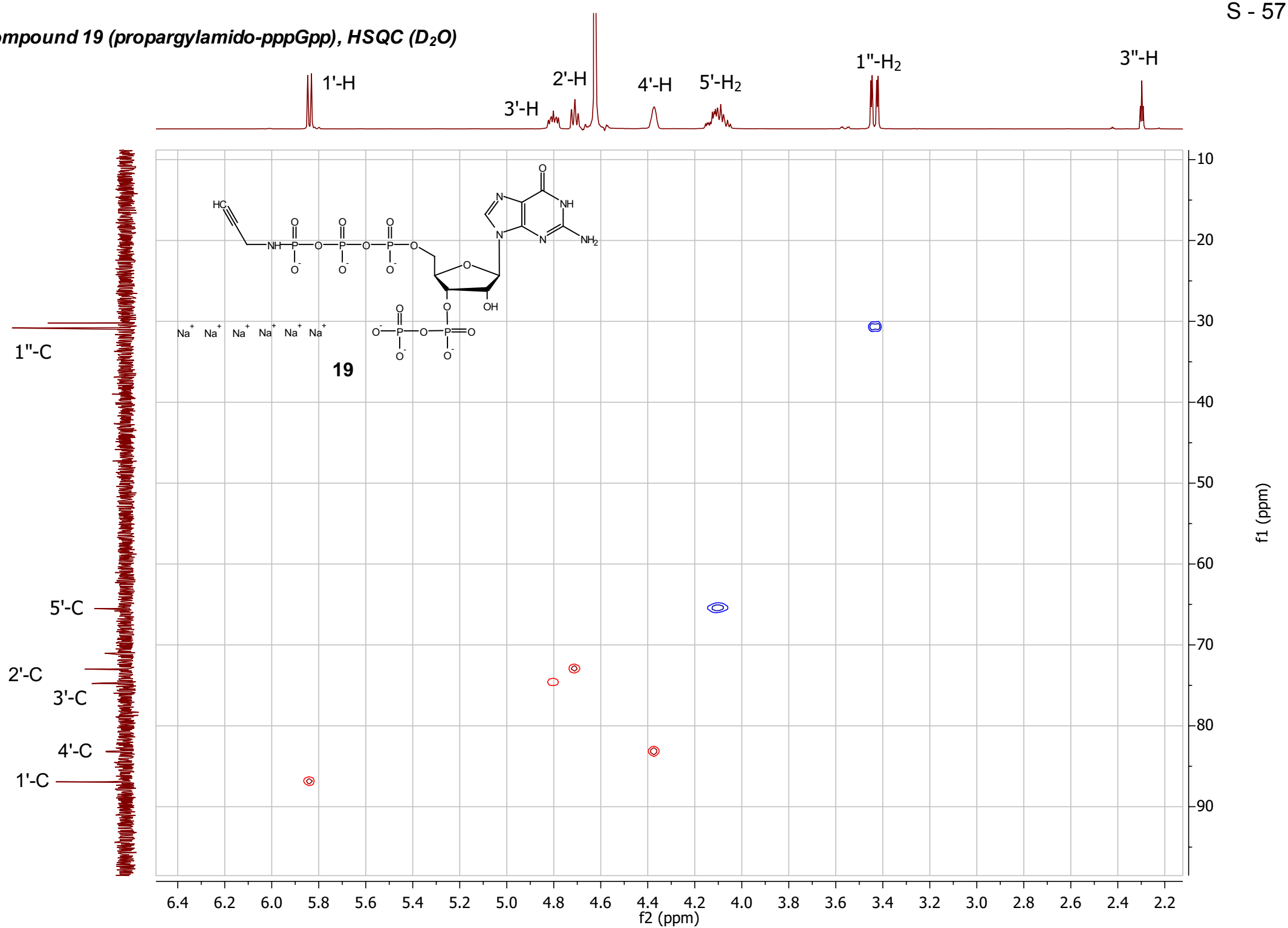


19

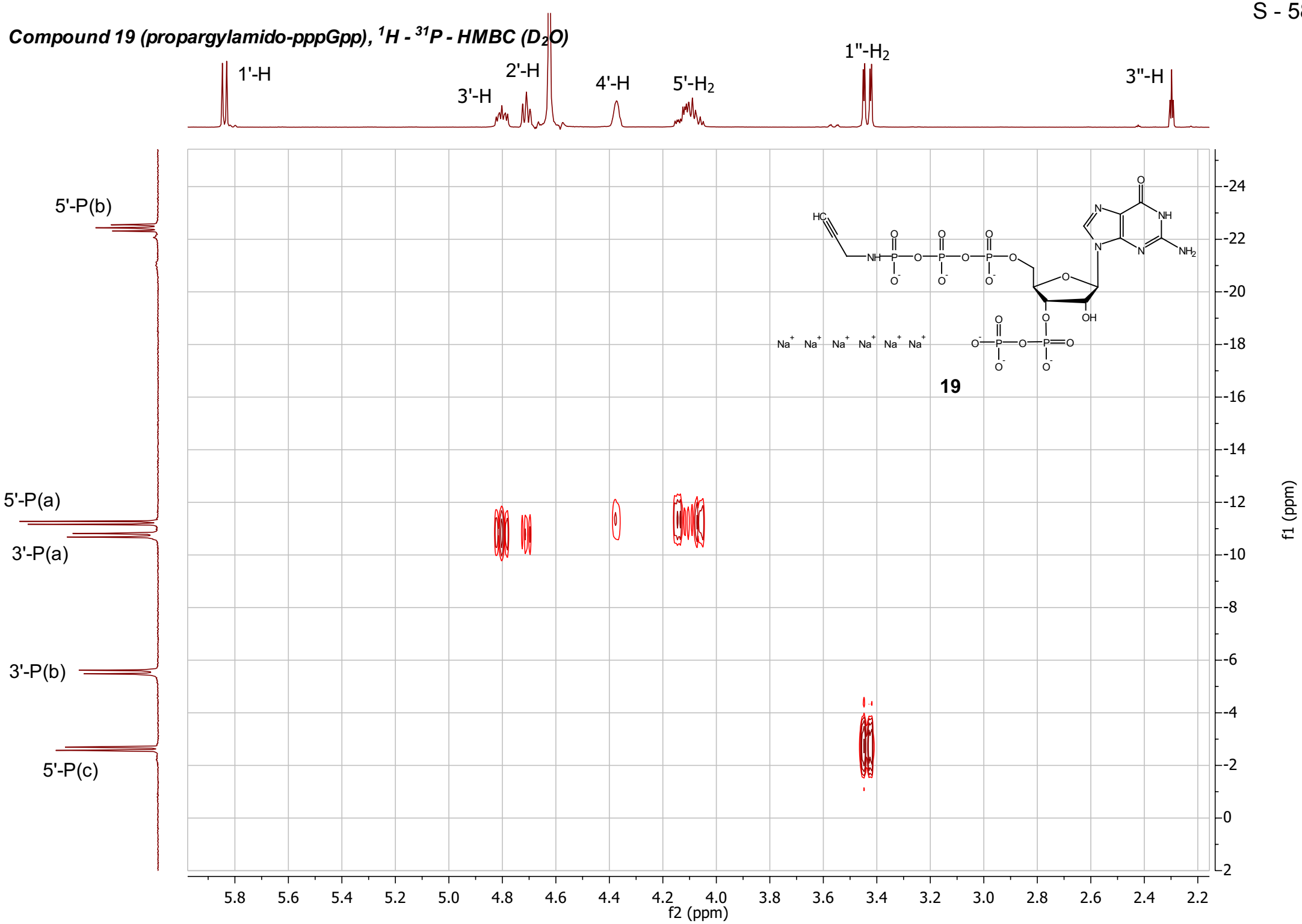
Compound 19 (propargylamido-pppGpp), DQF-COSY (D_2O)



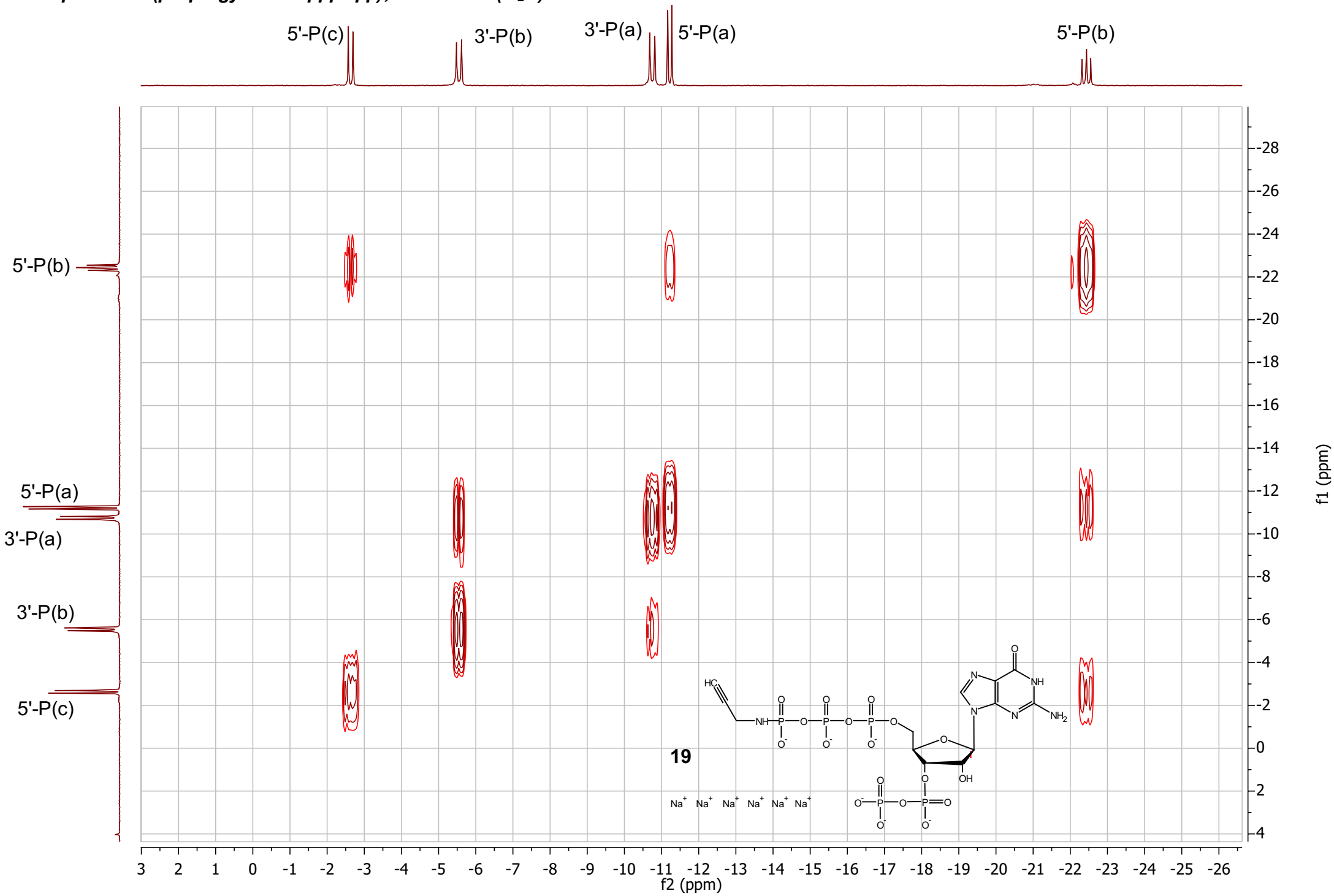
Compound 19 (propargylamido-pppGpp), HSQC (D_2O)



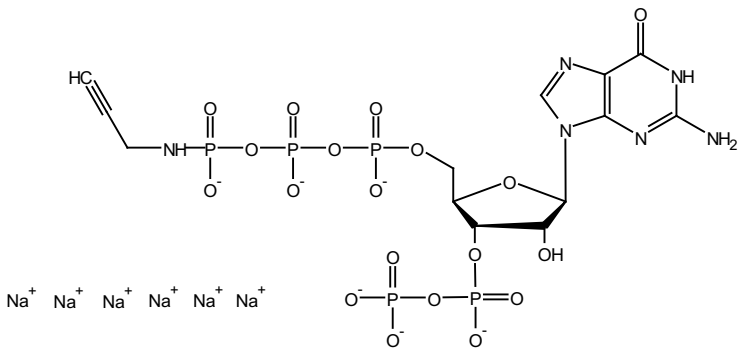
Compound 19 (propargylamido-pppGpp), ^{1}H - ^{31}P - HMBC (D_2O)



Compound 19 (propargylamido-pppGpp), PP - COSY (D_2O)

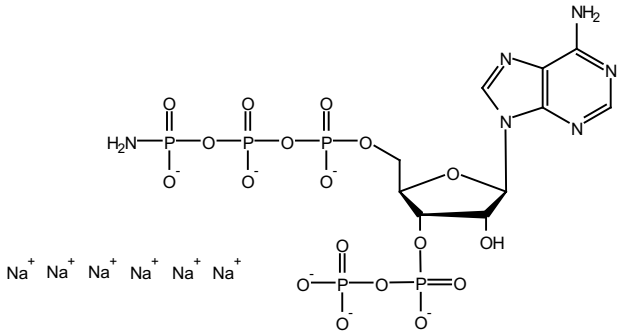


Compound 19 (propargylamido-pppGpp), ^{13}C {^{1}H} - NMR (D_2O, 400 MHz)



19

Compound 20 (amido-pppApp), ^{1}H - NMR (D_2O, 400 MHz)



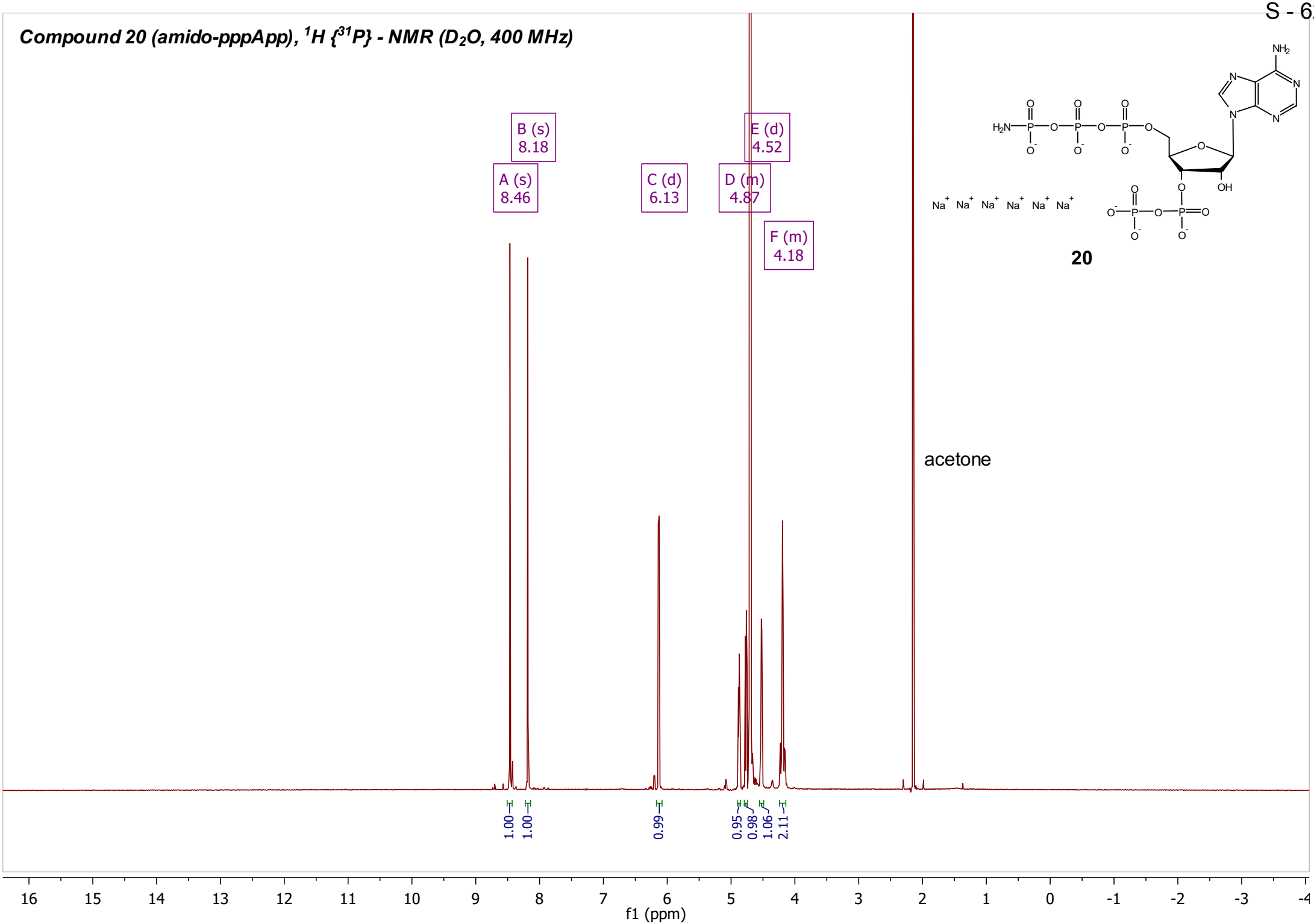
20

E (s) 8.18
D (s) 8.46
F (d) 6.13
G (m) 4.19
B (ddd) 4.87
A (m) 4.76
C (m) 4.52
acetone

1.00 1.00 1.01 1.00 1.13 1.04 2.15

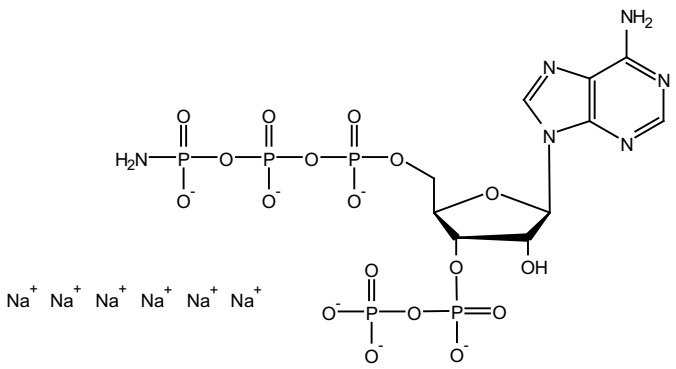
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)

Compound 20 (amido-pppApp), ^{1}H {^{31}P} - NMR (D_2O, 400 MHz)



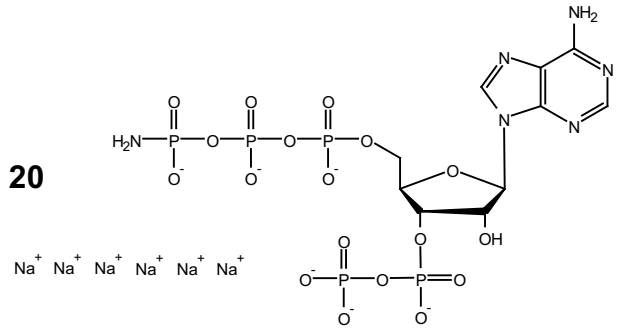
20

Compound 20 (amido-pppApp), $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)



20

Compound 20 (amido-pppApp), ^{31}P - NMR (D_2O, 162 MHz)



A (d)
-0.81

B (d)
-5.46

D (d)
-11.20

C (dd)
-10.67

E (dd)
-22.26

5'-P(c)

3'-P(b)

3'-P(a)

5'-P(a)

5'-P(b)

0.84

1.00

0.99

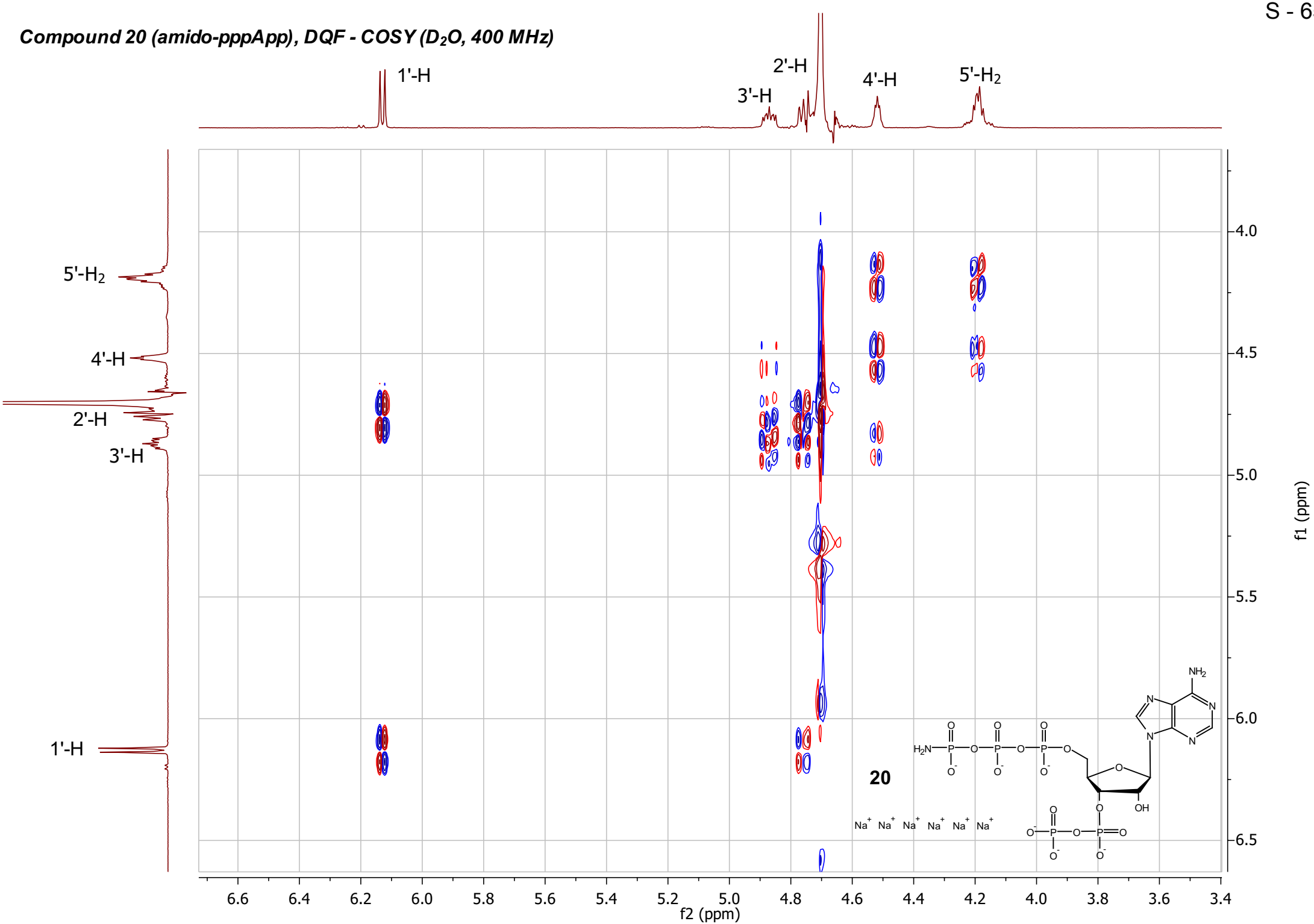
1.04

0.95

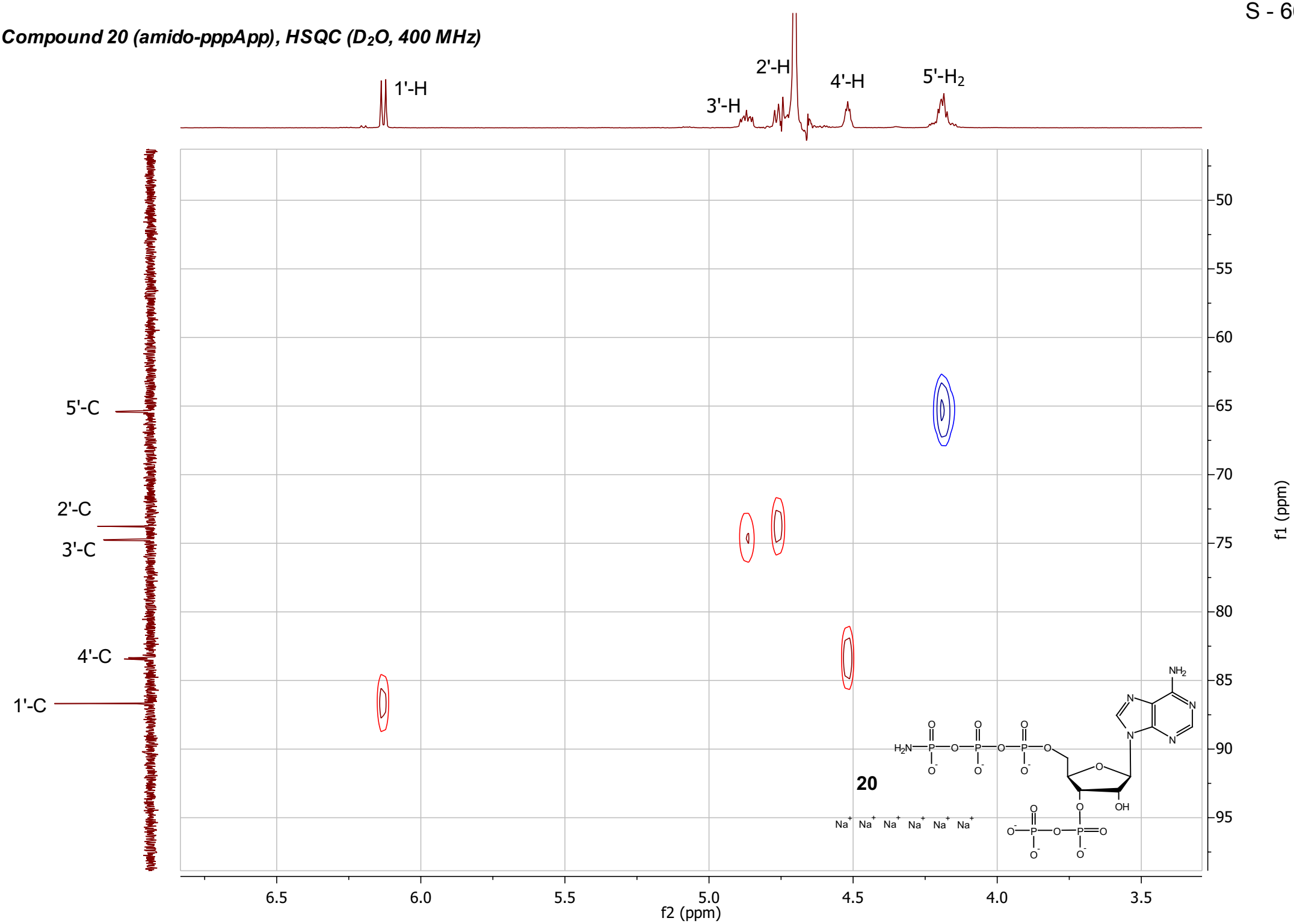
3 2 1 0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23 -24 -25 -26 -27

f1 (ppm)

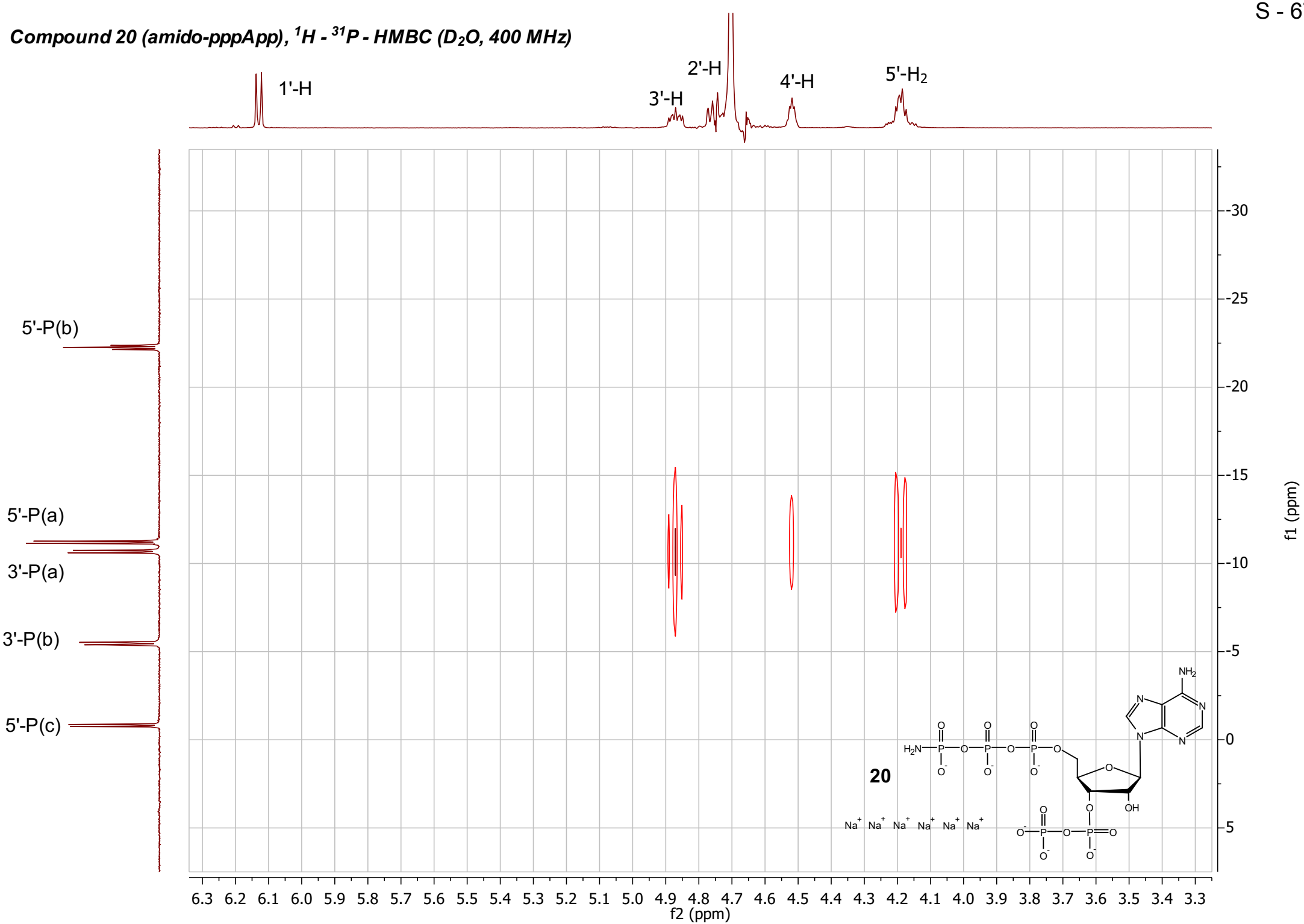
Compound 20 (amido-pppApp), DQF - COSY (D_2O, 400 MHz)



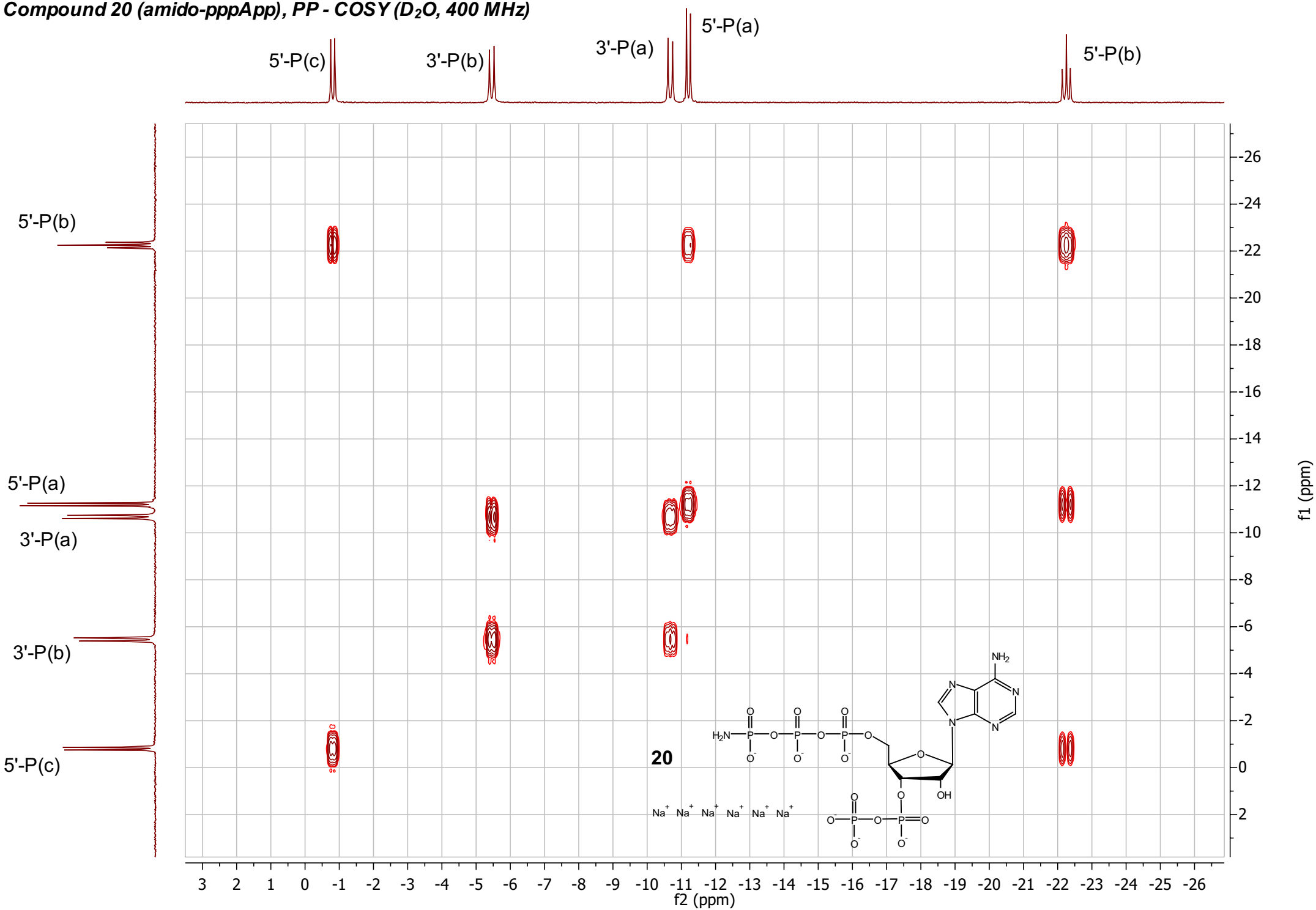
Compound 20 (amido-pppApp), HSQC (D_2O, 400 MHz)



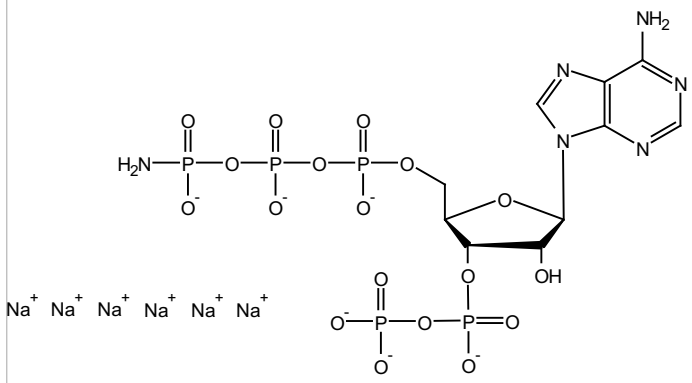
Compound 20 (amido-pppApp), ^{1}H - ^{31}P - HMBC (D_2O, 400 MHz)



Compound 20 (amido-pppApp), PP - COSY (D_2O, 400 MHz)



Compound 19 (amido-pppApp), ^{13}C {^{1}H} - NMR (D_2O, 101 MHz)



20

B (s) 152.89
A (s) 155.64
D (s) 139.92
E (s) 118.65
G (dd) 83.41
J (d) 65.41
F (s) 86.68
H (d) 74.76
C (s) 149.26
I (d) 73.78

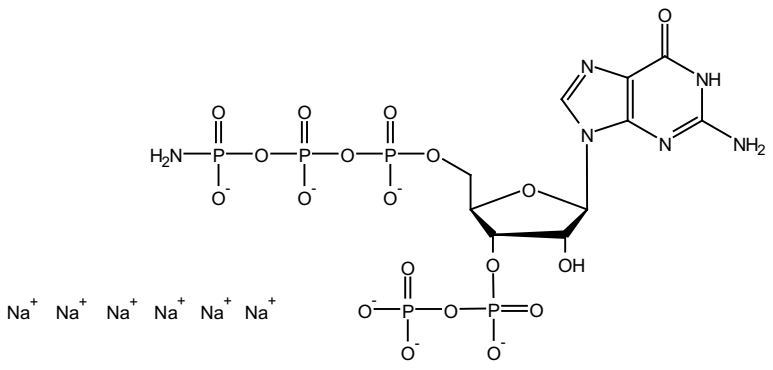
acetone

acetone

240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20

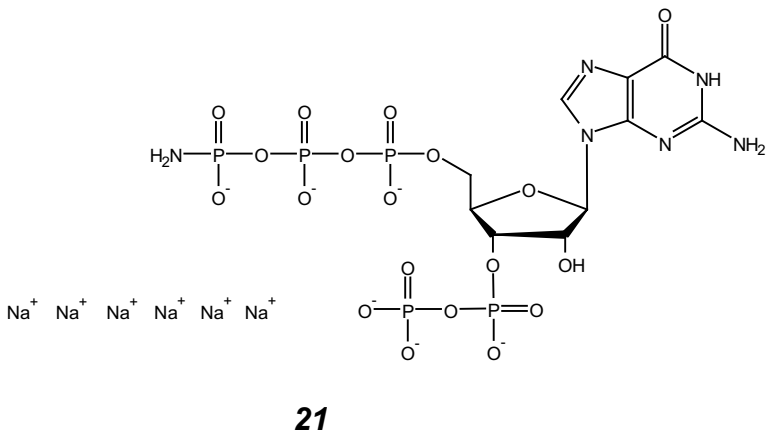
f1 (ppm)

Compound 21 (amino-pppGpp), ^{1}H - NMR (D_2O, 400 MHz)



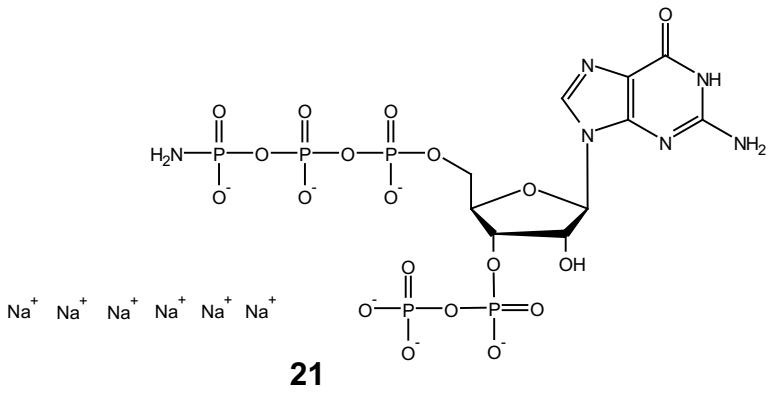
21

Compound 21 (amino-pppGpp), ^{1}H {^{31}P} - NMR (D_2O, 400 MHz)



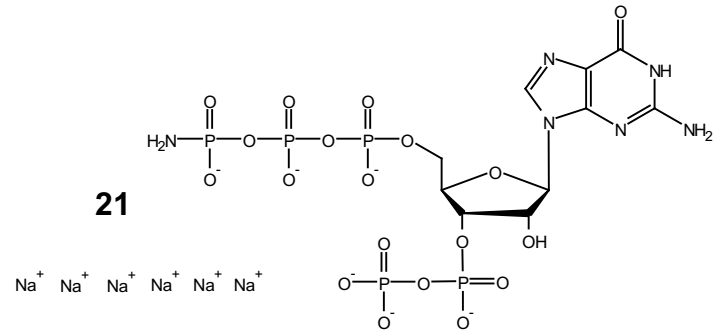
21

Compound 21 (amino-pppGpp), $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)

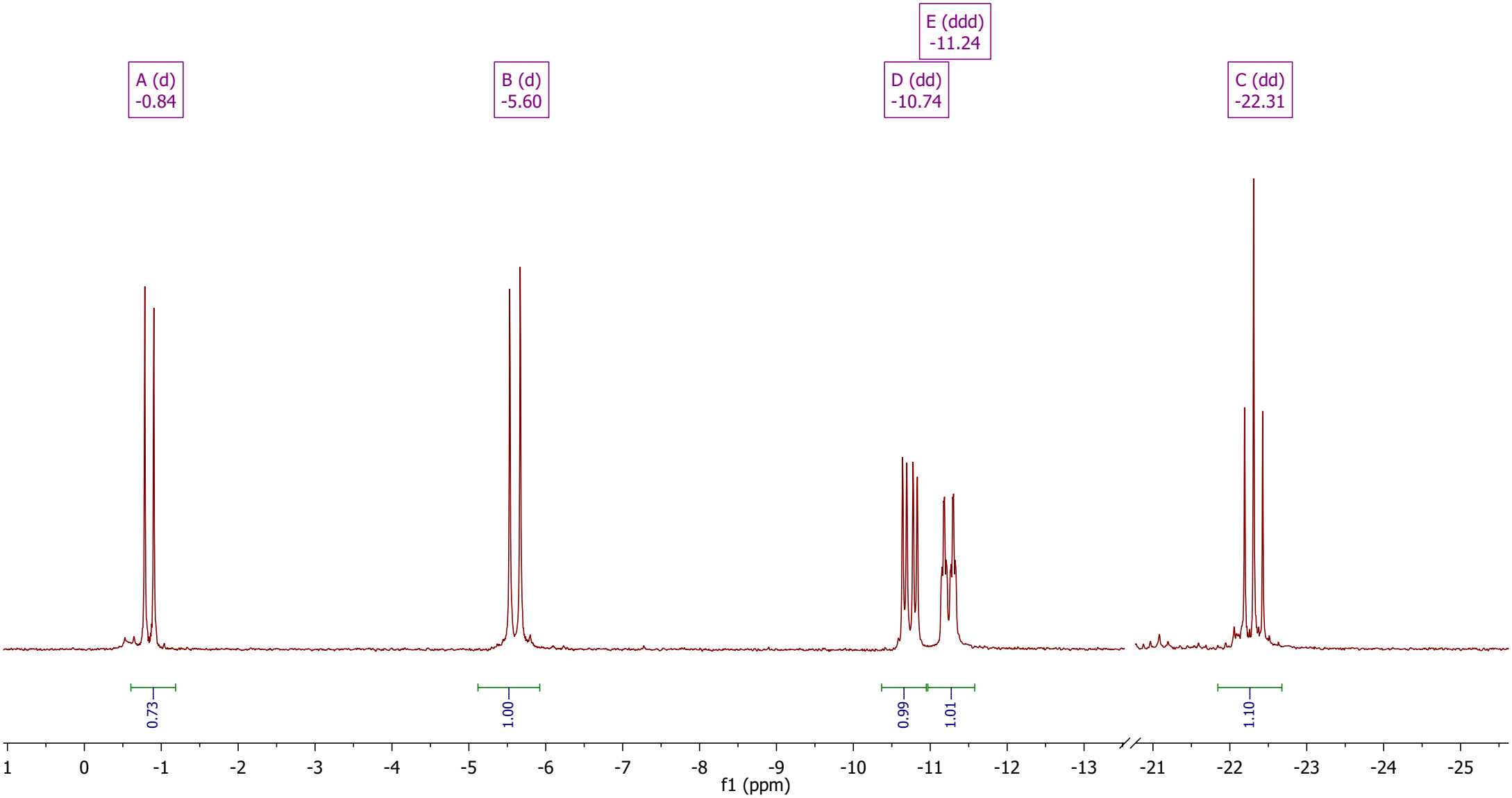


21

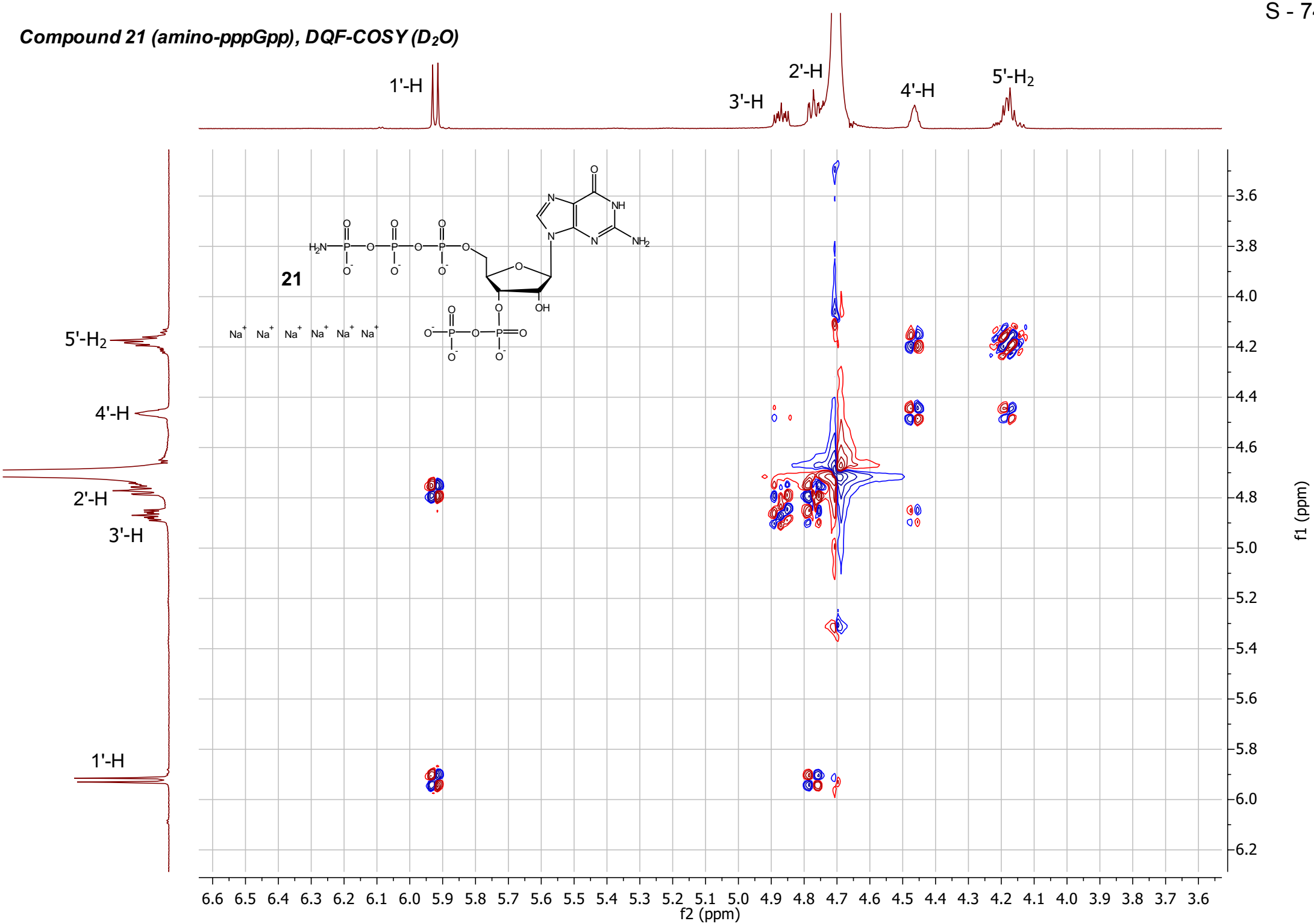
Compound 21 (amino-pppGpp), ^{31}P - NMR (D_2O, 162 MHz)



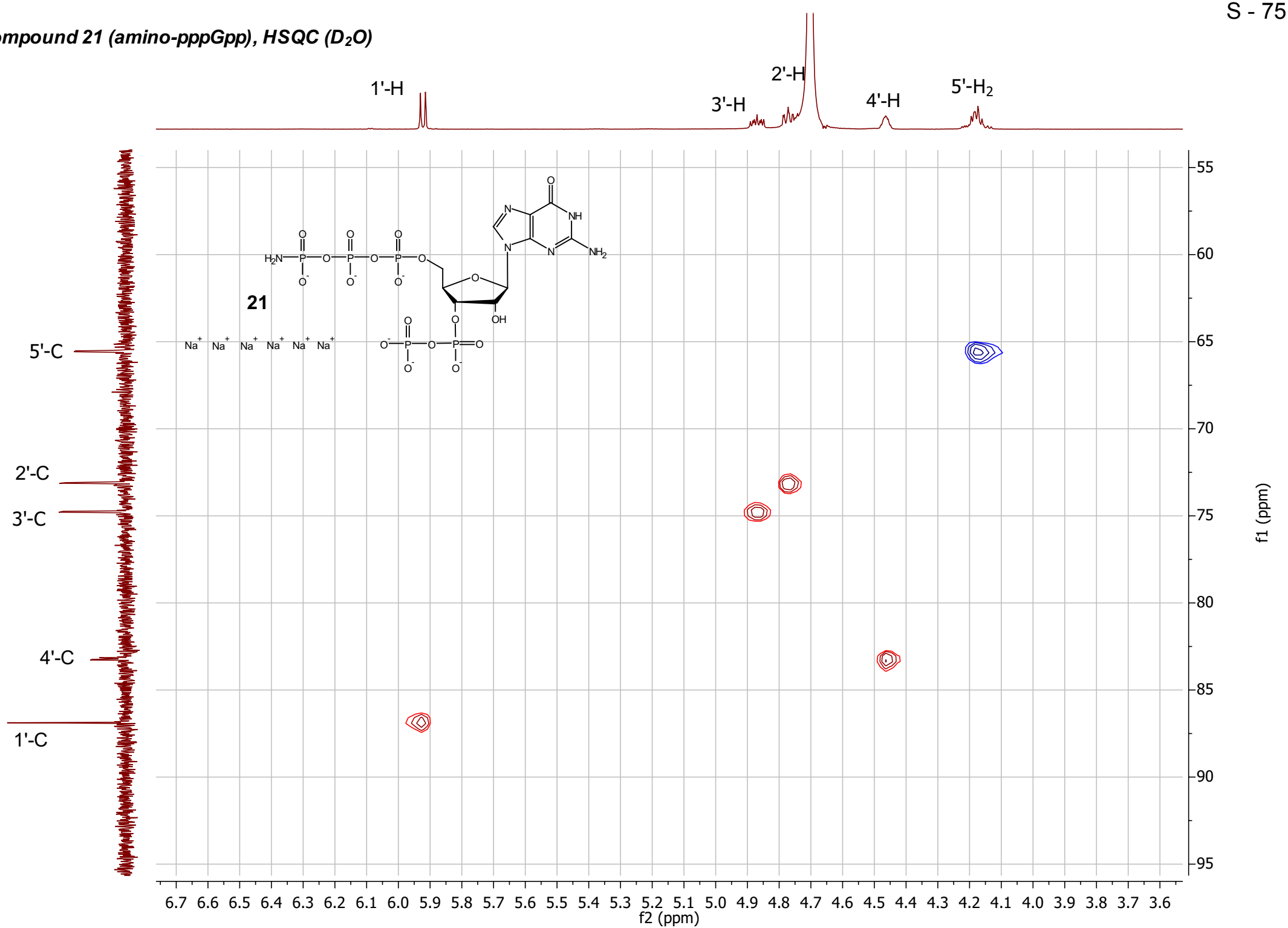
21



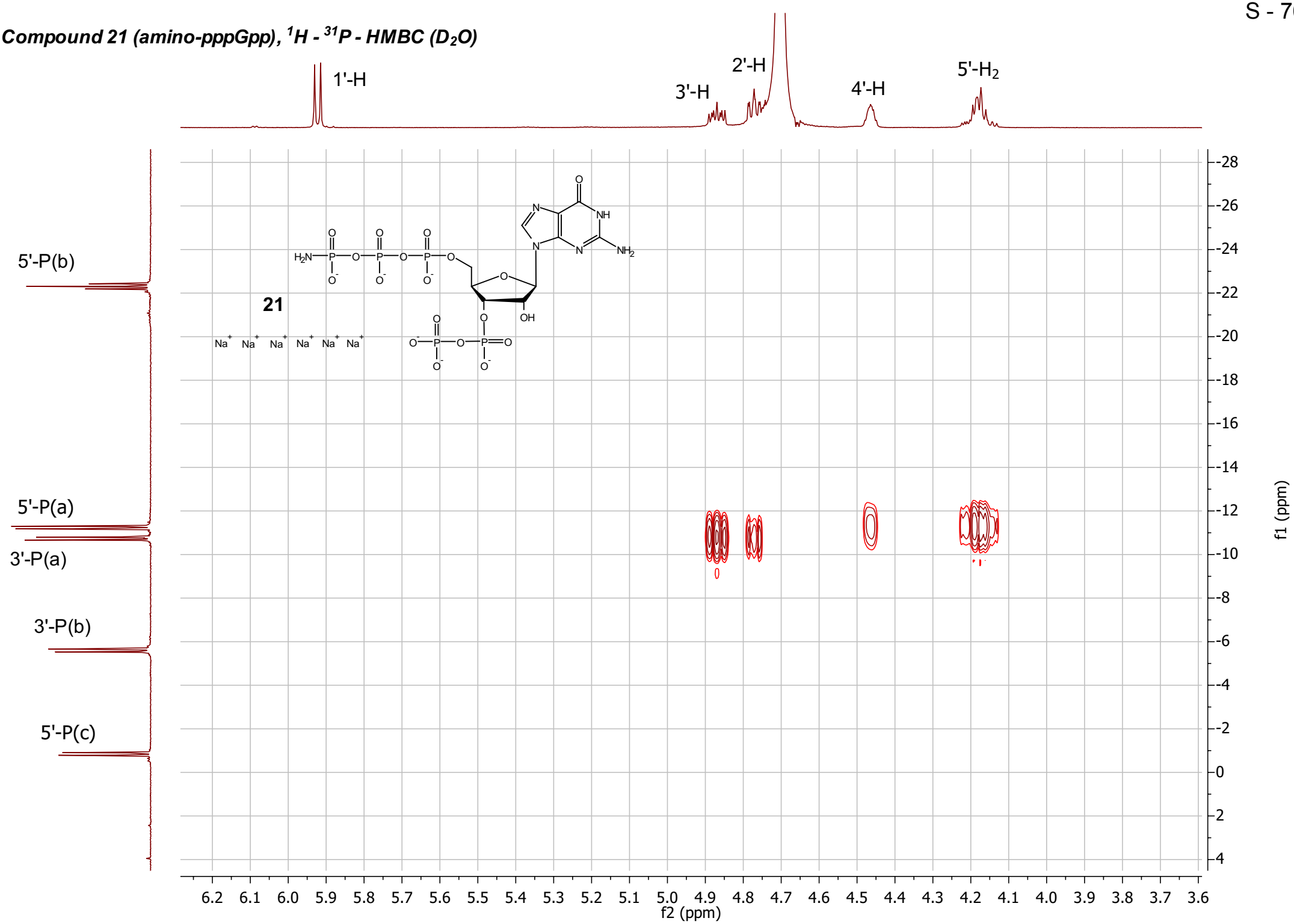
Compound 21 (amino-pppGpp), DQF-COSY (D_2O)



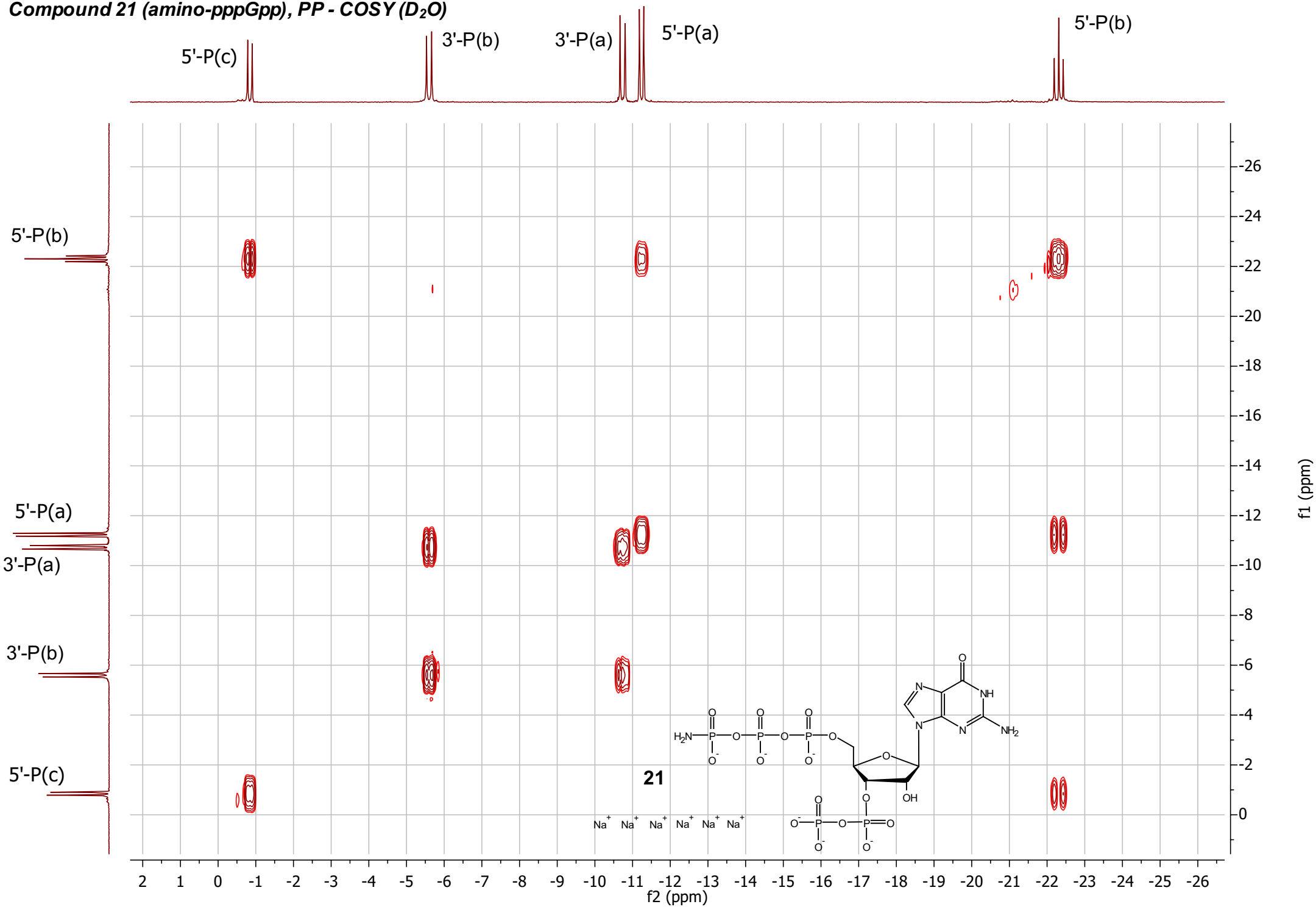
Compound 21 (amino-pppGpp), HSQC (D_2O)



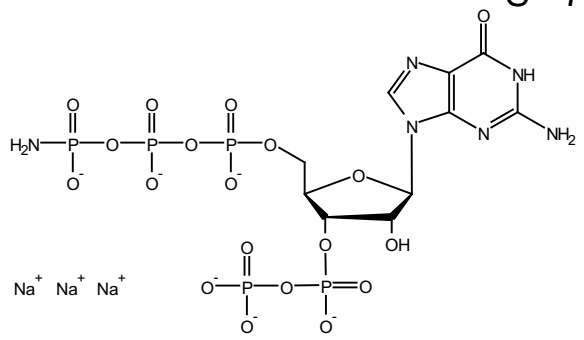
Compound 21 (amino-pppGpp), ^{1}H - ^{31}P - HMBC (D_2O)



Compound 21 (amino-pppGpp), PP - COSY (D_2O)



Compound 21 (amido-pppGpp), ^{13}C {^{1}H} - NMR (D_2O, 101 MHz)



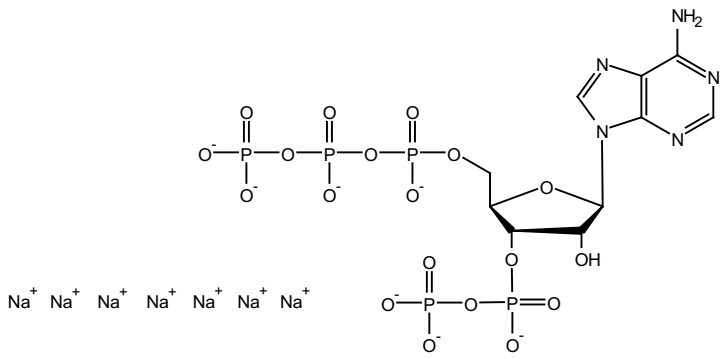
21

B (s) 154.00
A (s) 159.16
C (s) 151.93
K (s) 137.89
E (s) 116.32
G (dd) 83.21
F (s) 86.88
H (d) 74.77
I (d) 73.11
J (d) 65.55

240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20

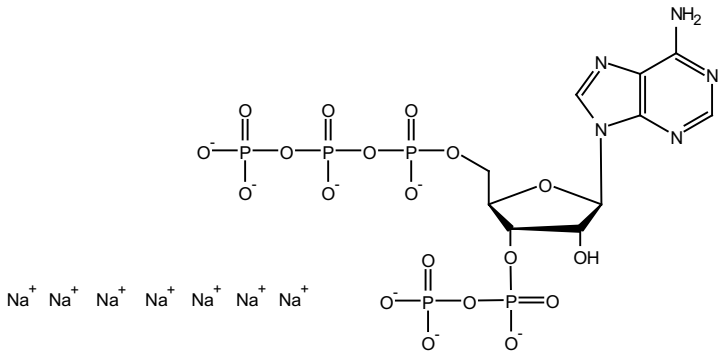
f1 (ppm)

Compound 4 (pppApp), ^{1}H - NMR (D_2O, 400 MHz)



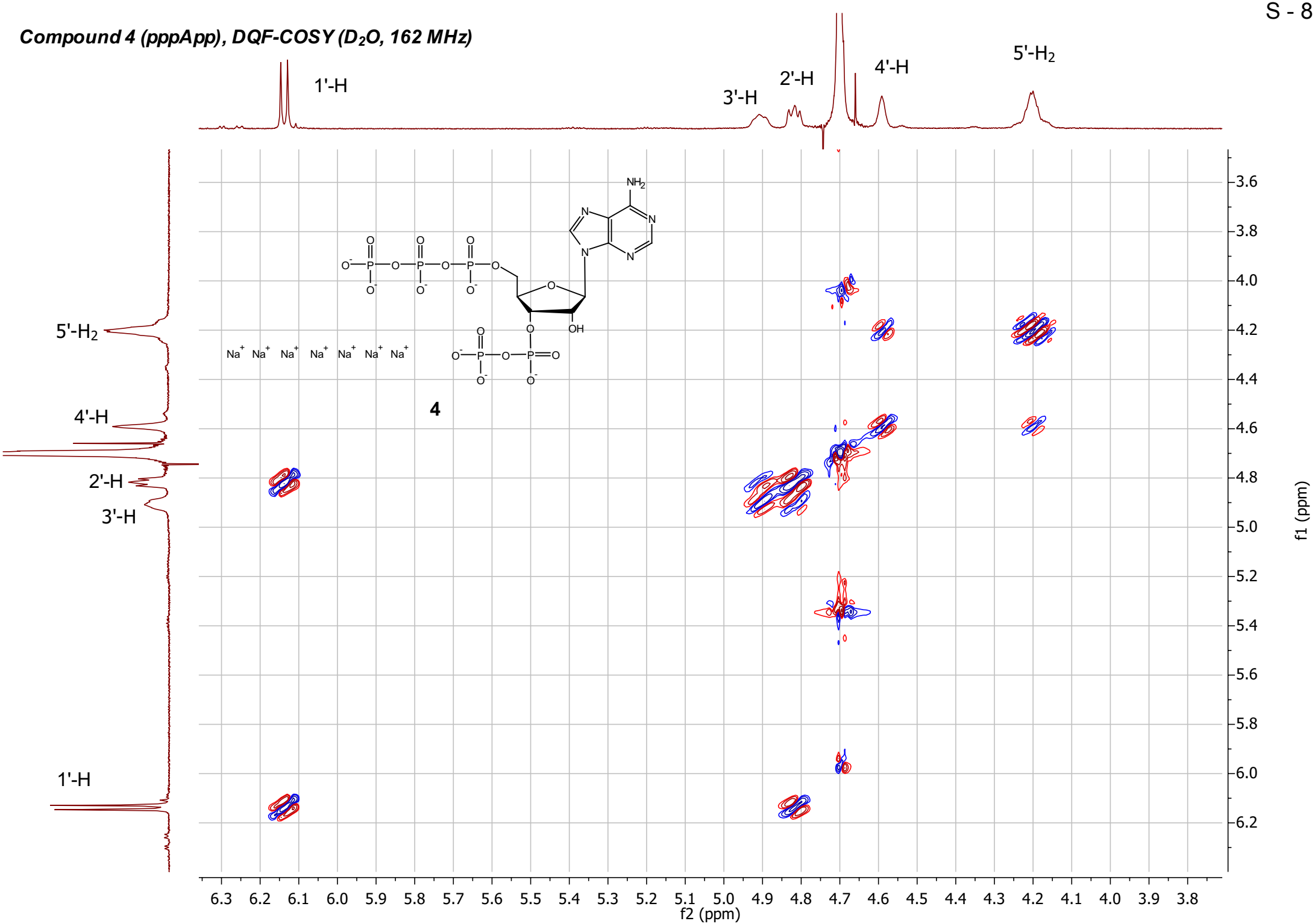
4

Compound 4 (pppApp), $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)

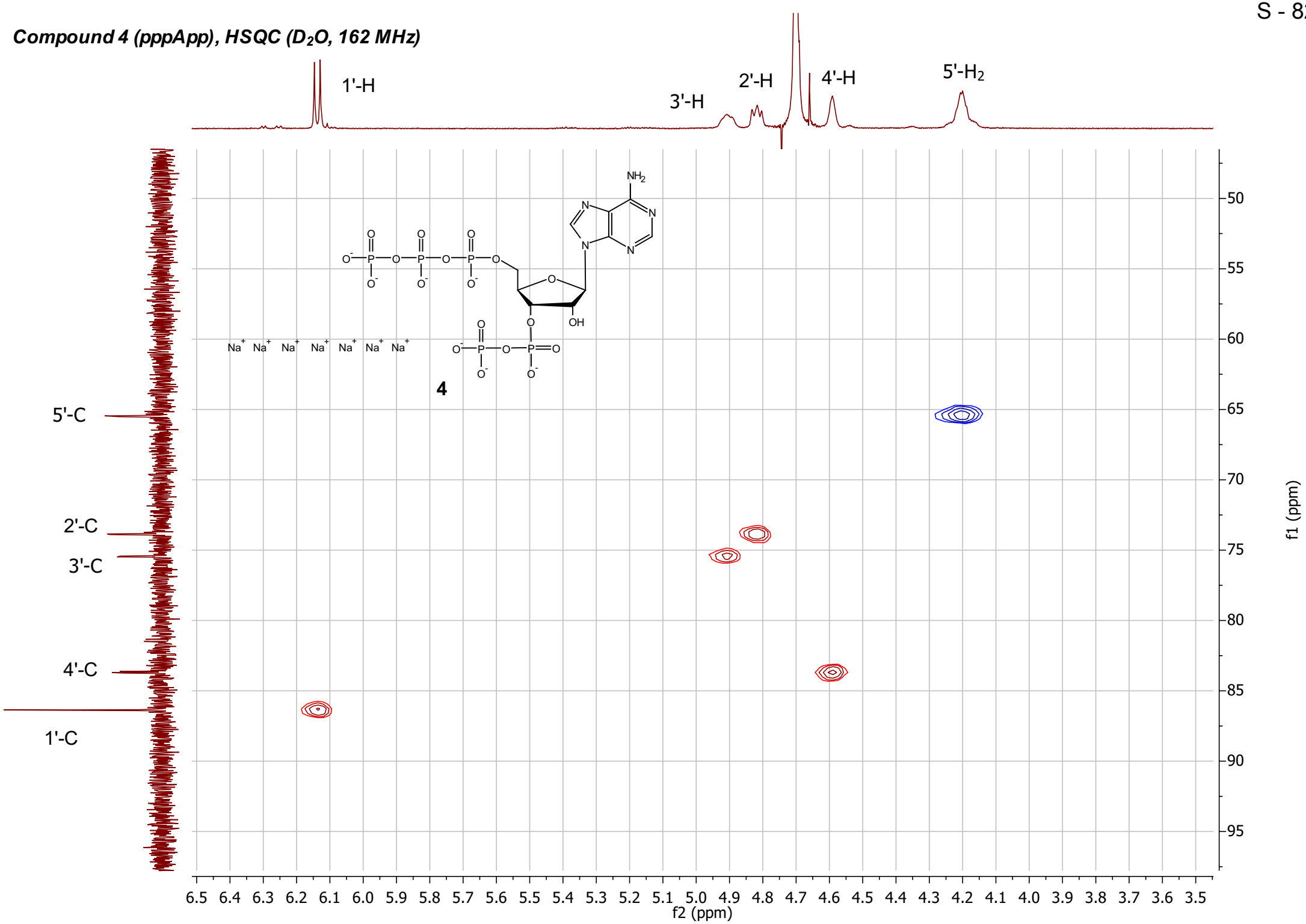


4

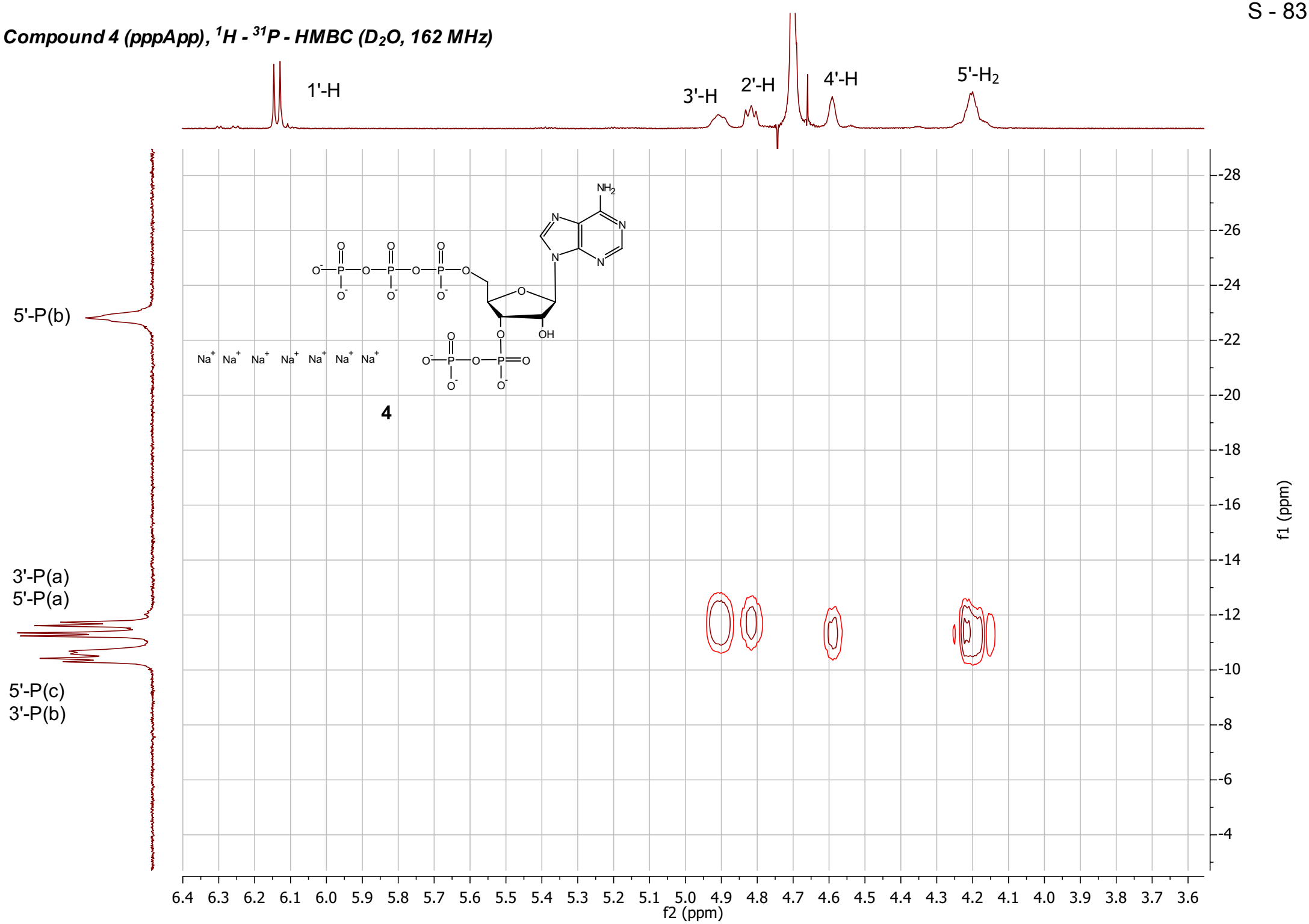
Compound 4 (pppApp), DQF-COSY (D_2O, 162 MHz)



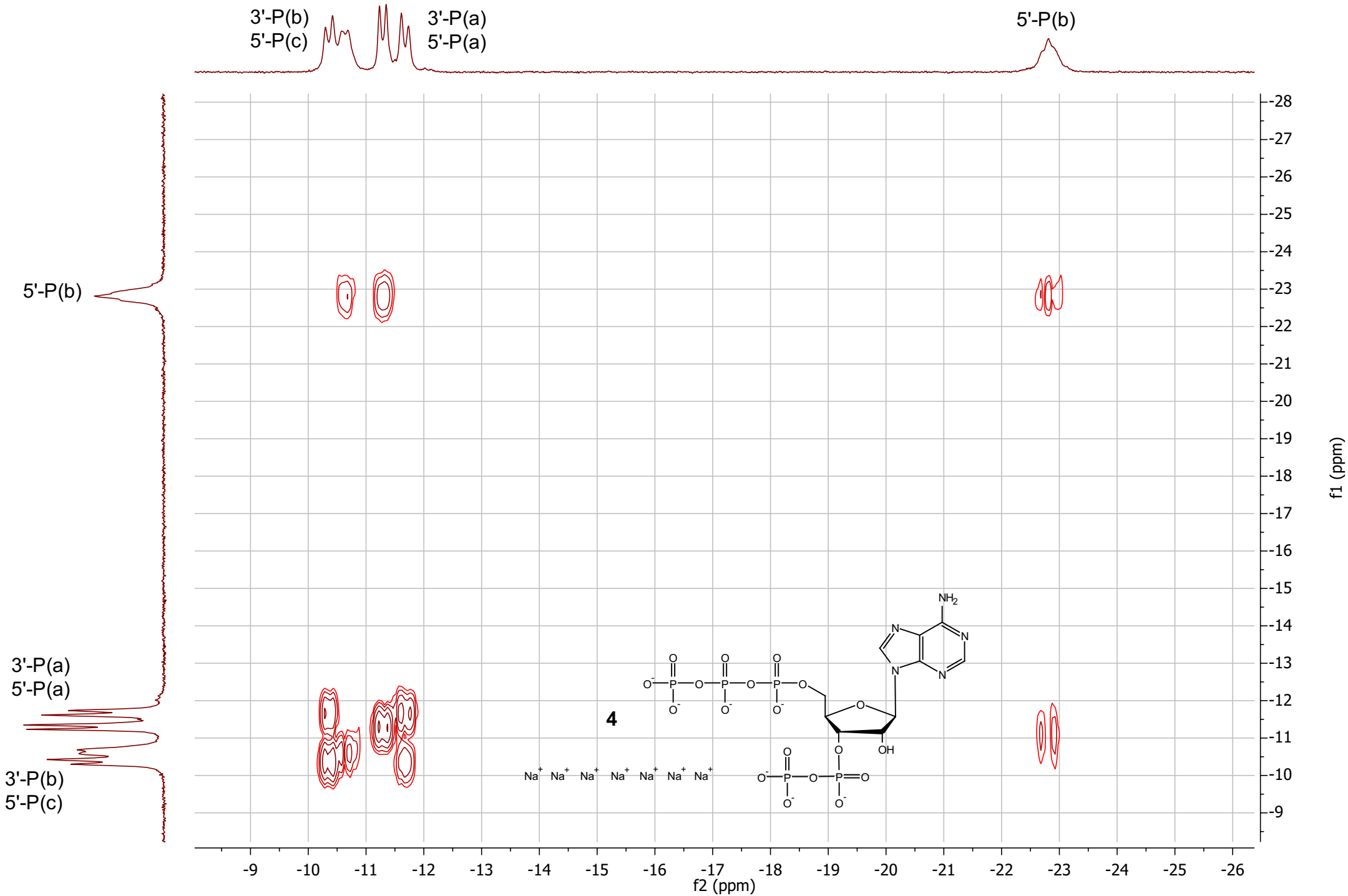
Compound 4 (pppApp), HSQC (D_2O, 162 MHz)



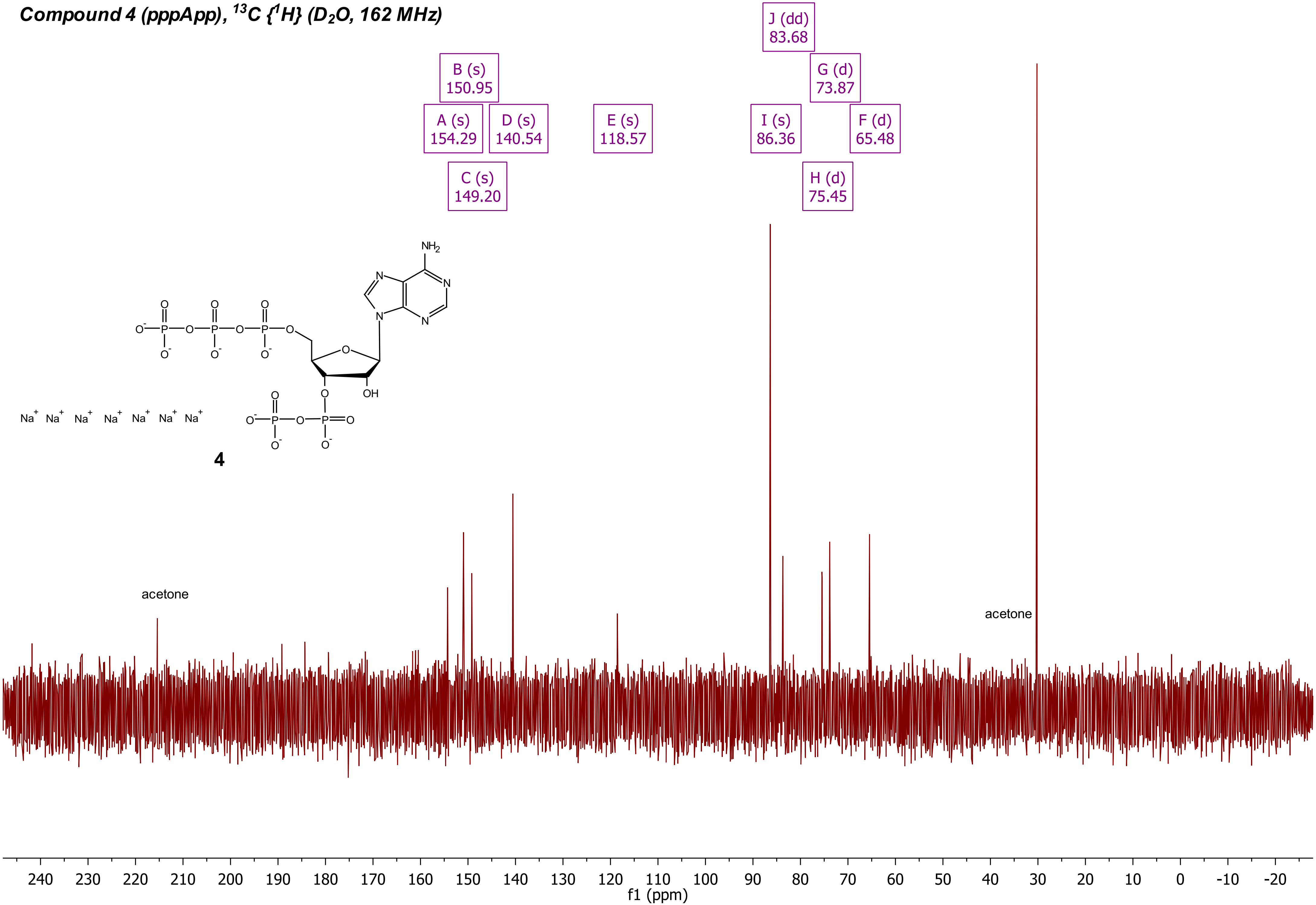
Compound 4 (pppApp), ^{1}H - ^{31}P - HMBC (D_2O, 162 MHz)



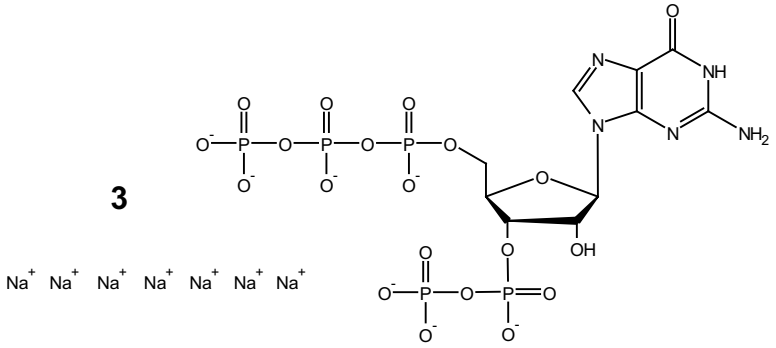
Compound 4 (pppApp), PP - COSY (D_2O, 162 MHz)



Compound 4 (pppApp), ^{13}C {^{1}H} (D_2O, 162 MHz)



Compound 3 (pppGpp), ^{1}H - NMR (D_2O, 400 MHz)



3

Na^+ Na^+ Na^+ Na^+ Na^+ Na^+ Na^+

A (s) 8.06

B (d) 5.91

C (br.s) 4.89

D (dd) 4.82

E (br.s) 4.51

F (br.s) 4.18

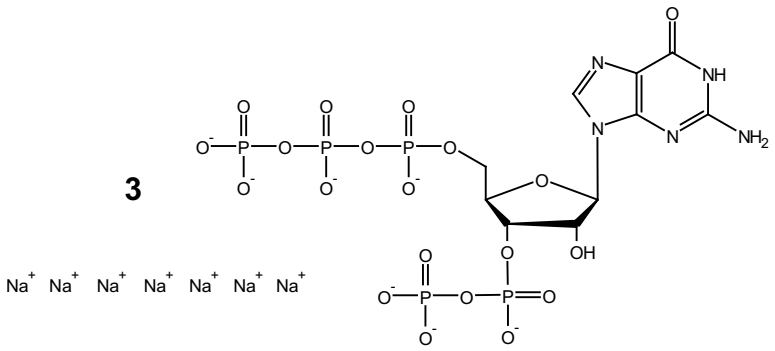
acetone

1.00 1.01 0.77 1.17 1.13 1.98

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4

f1 (ppm)

Compound 3 (pppGpp), ^{1}H {^{31}P} - NMR (D_2O, 400 MHz)

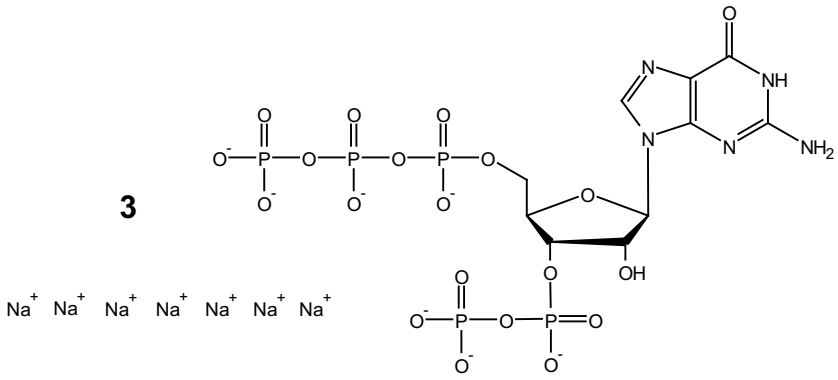


3

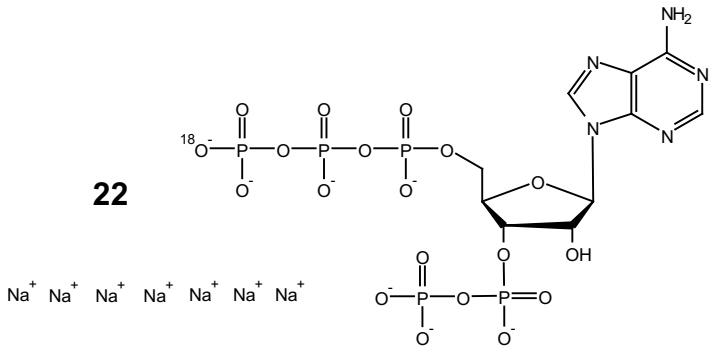
Na^+ Na^+ Na^+ Na^+ Na^+ Na^+ Na^+

D (m) 4.19
B (dd) 4.82
F (s) 8.06
E (d) 5.92
A (br.s) 4.89
C (br.s) 4.51
acetone
1.00
1.00
0.81
1.15
1.09
2.00
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)

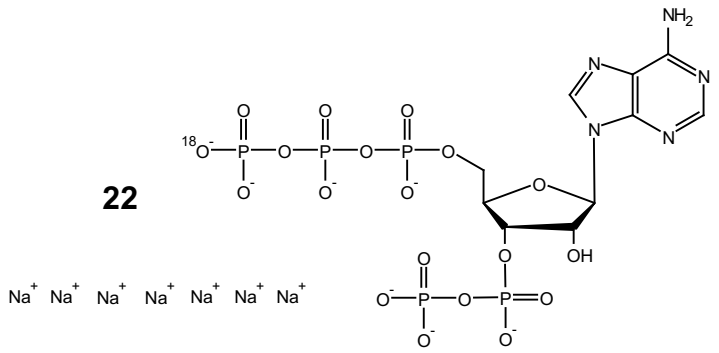
Compound 3 (pppGpp), $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)



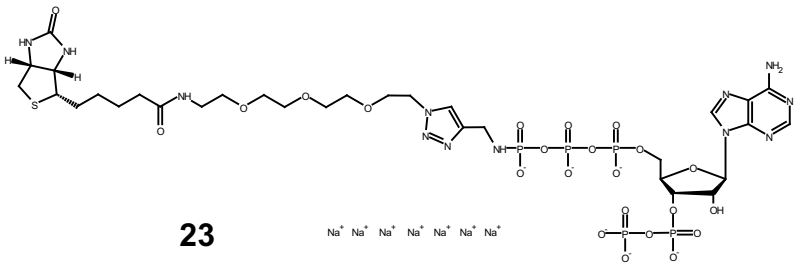
Compound 22 (^{18}O-pppApp), ^{1}H - NMR (D_2O, 400 MHz)



Compound 22 (^{18}O-pppApp), ^{31}P {^{1}H} - NMR (D_2O, 162 MHz)

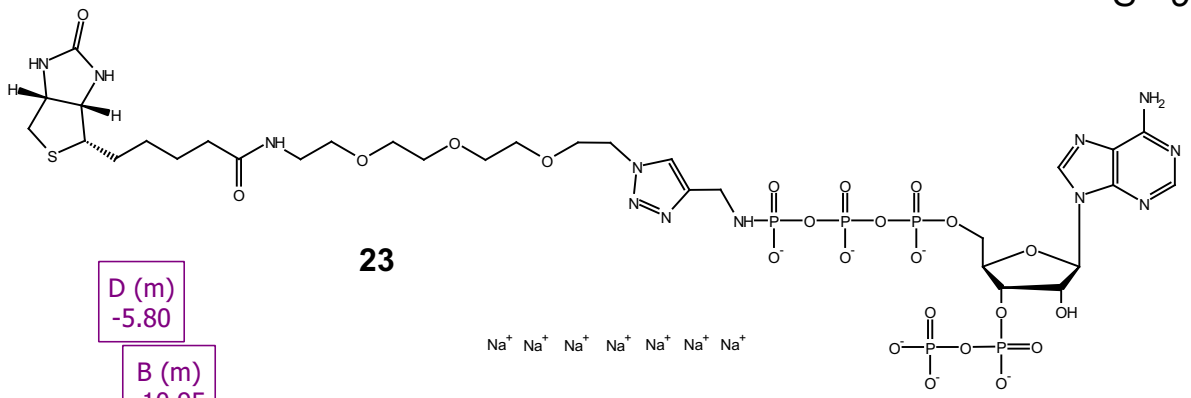


Compound 23 (biotin - pppApp), ^{1}H - NMR (D_2O, 400 MHz)

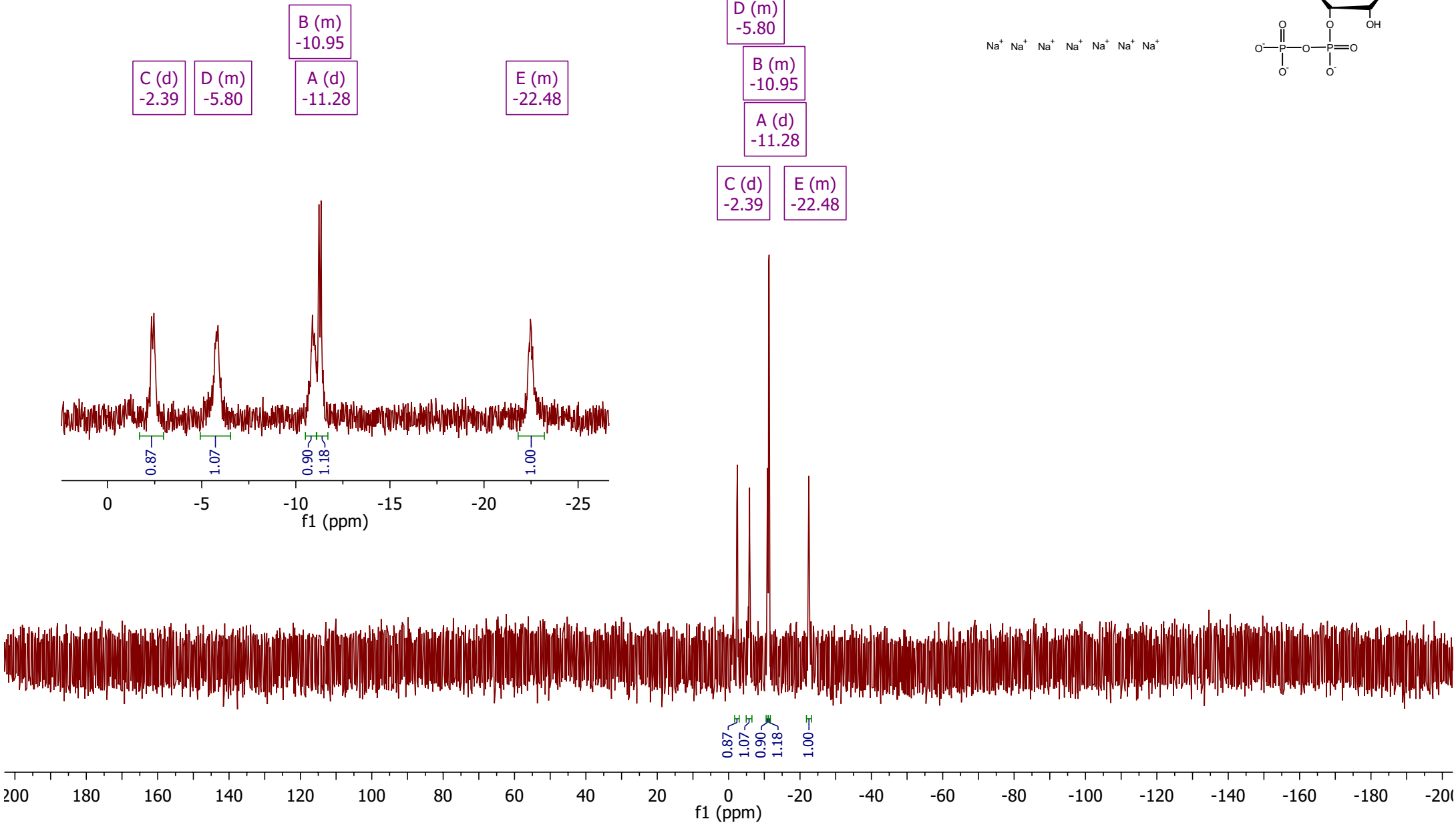


23

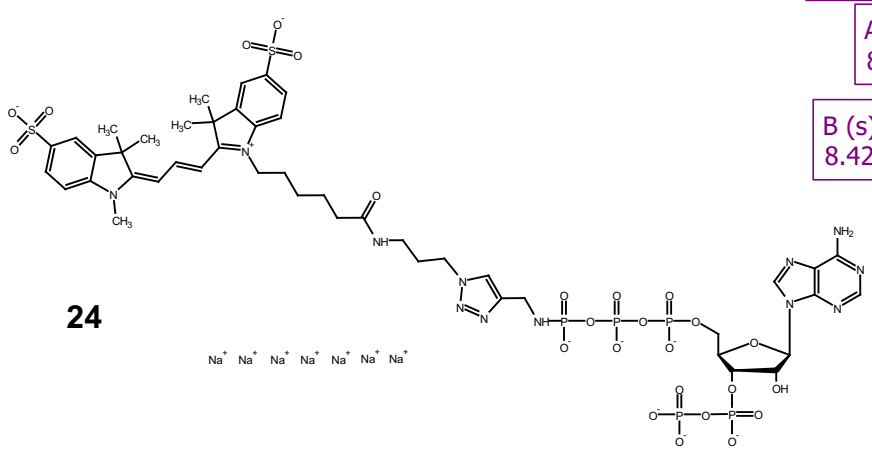
Compound 23 (biotin - pppApp), $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)



23



Compound 24 (fluorophor - pppApp), ^{1}H - NMR (D_2O, 400 MHz)



24

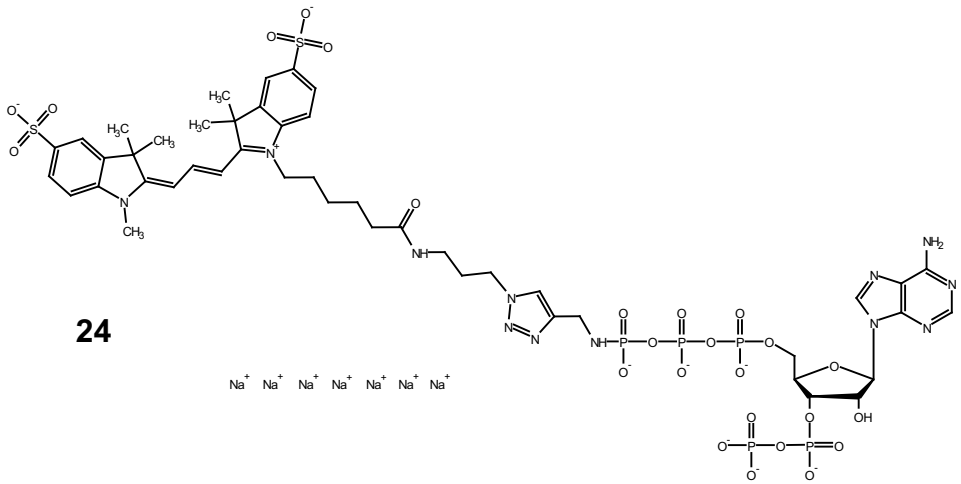
Z (d) 7.82
H (s) 7.74
E (dd) 7.79
C (dd) 8.41
G (m) 7.30
A (s) 8.02
B (s) 8.42
F (dd) 7.77
D (d) 7.81
X (d) 6.18
W (d) 6.03
Y (d) 6.24
P (m) 4.21
N (s) 4.50
M (s) 4.70
O (m) 3.98
Q (dd) 2.99
I (s) 3.49
V (dd) 1.75
T (m) 1.92
R (m) 1.51
K (s) 1.66
J (s) 1.68
L (s) 1.66
S (m) 1.29
U (dd) 2.09

2.03 1.00 1.05 1.08 1.13 1.19 0.71 2.11 1.01 1.06 0.98 0.60 0.93 4.05 3.94 3.01 1.97 2.13 1.91 1.92 6.15 3.07 3.18 2.23 2.09

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4

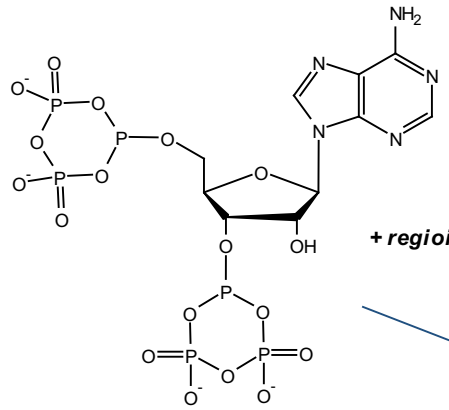
f1 (ppm)

Compound 24 (fluorophor - pppApp), $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)

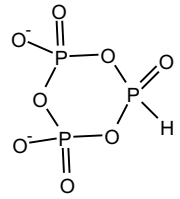


24

Exemplary P(III) - intermediate, $^{31}P\{^{1}H\}$ - NMR (DMF-d^{7}, 162 MHz)



+ regioisomer



H-Phosphonate
(hydrolysed P-Amidite)

P(III) - signals

pyrophosphat

P(V) - signals

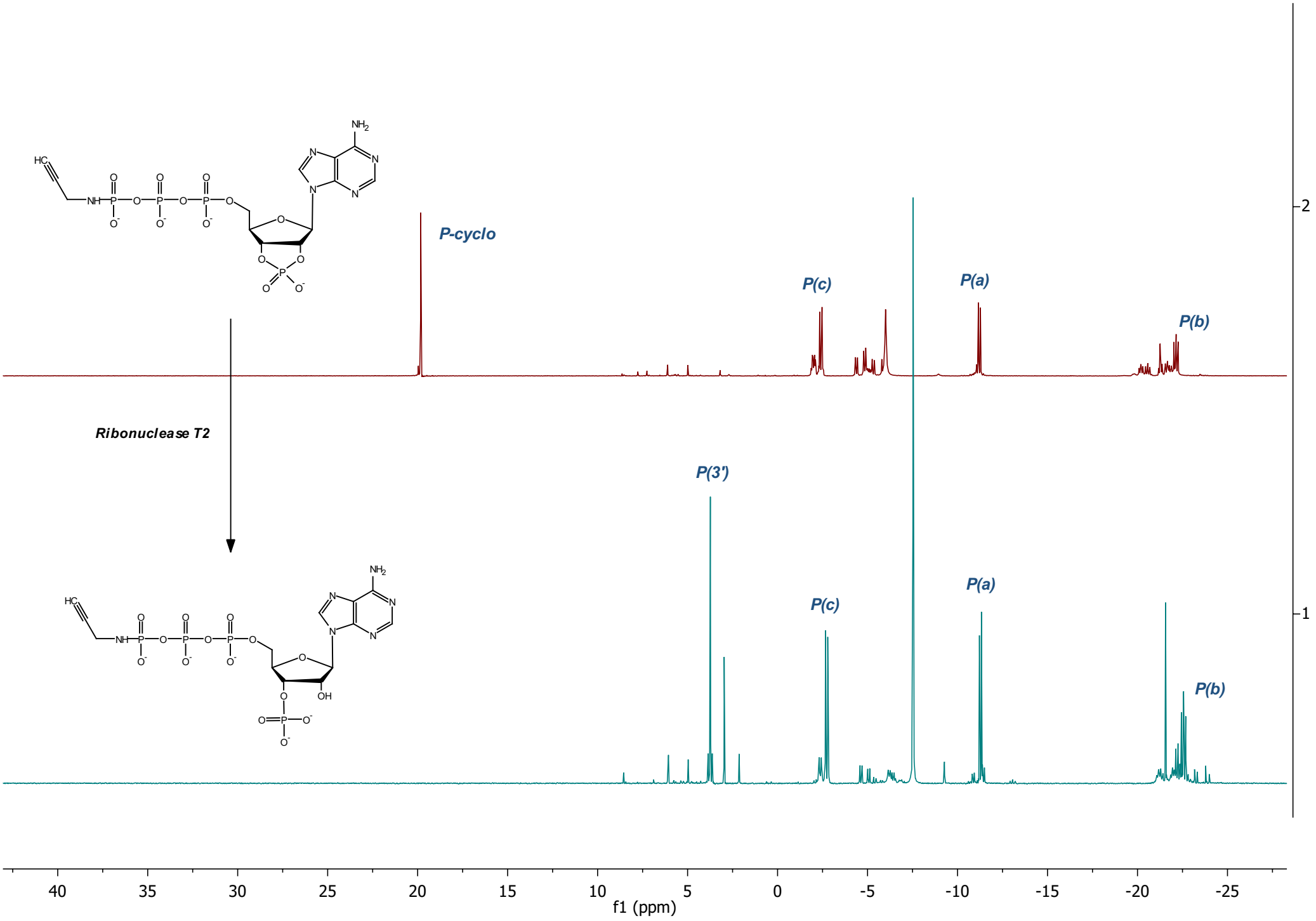
0.75

2.00

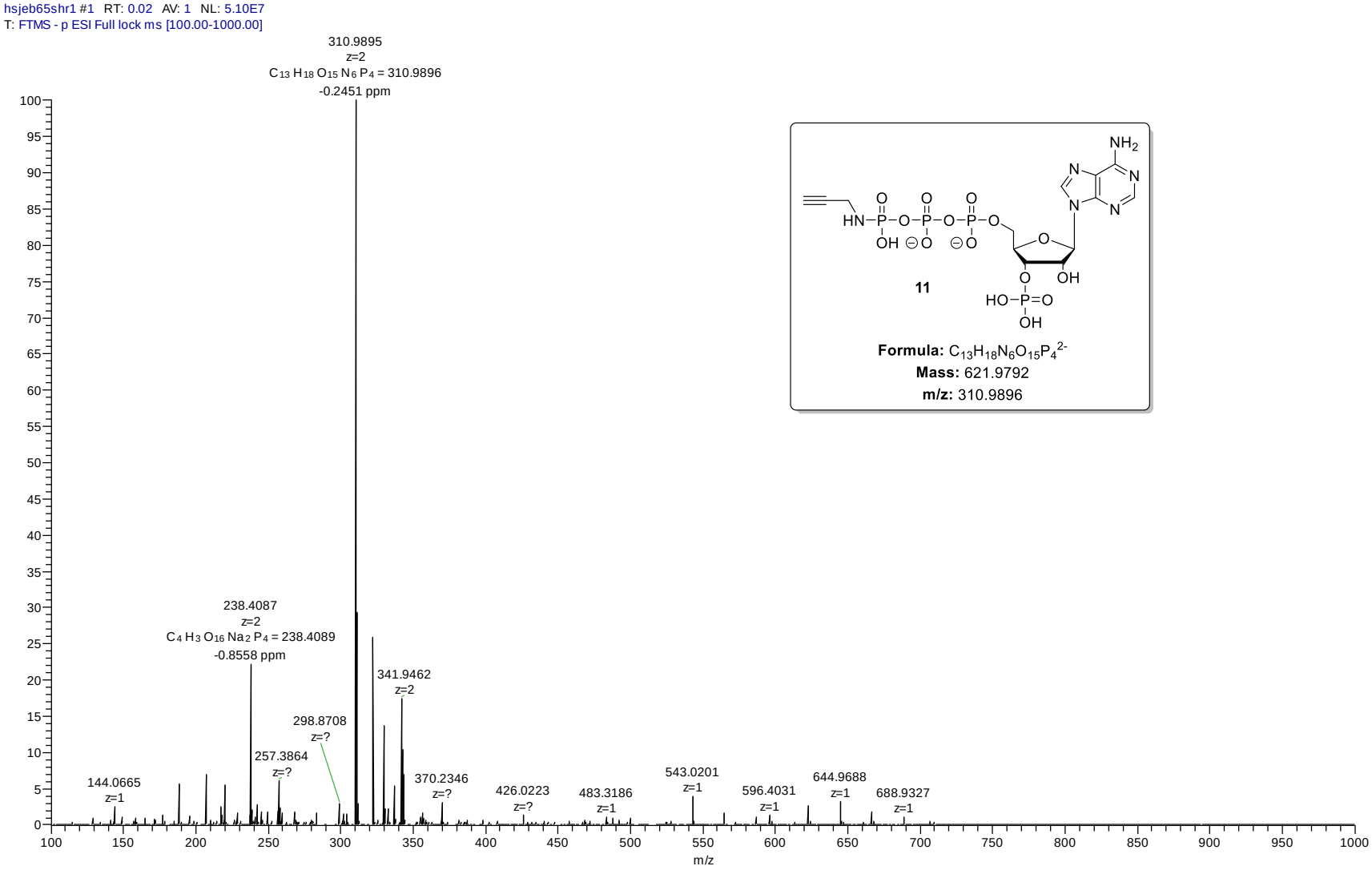
109 108 107 106 105 104 103 102 101 100 99 98 97 -4 -5 -6 -7 -8 -9 -10 -11 -12 -13 -14 -15 -16 -17 -18 -19 -20 -21 -22 -23 -24 -25 -26 -27 -28 -29 -30 -3

f1 (ppm)

Exemplary 2',3'-cyclophosphate intermediate 10 and crude product 11, $^{31}P\{^{1}H\}$ - NMR (D_2O, 162 MHz)

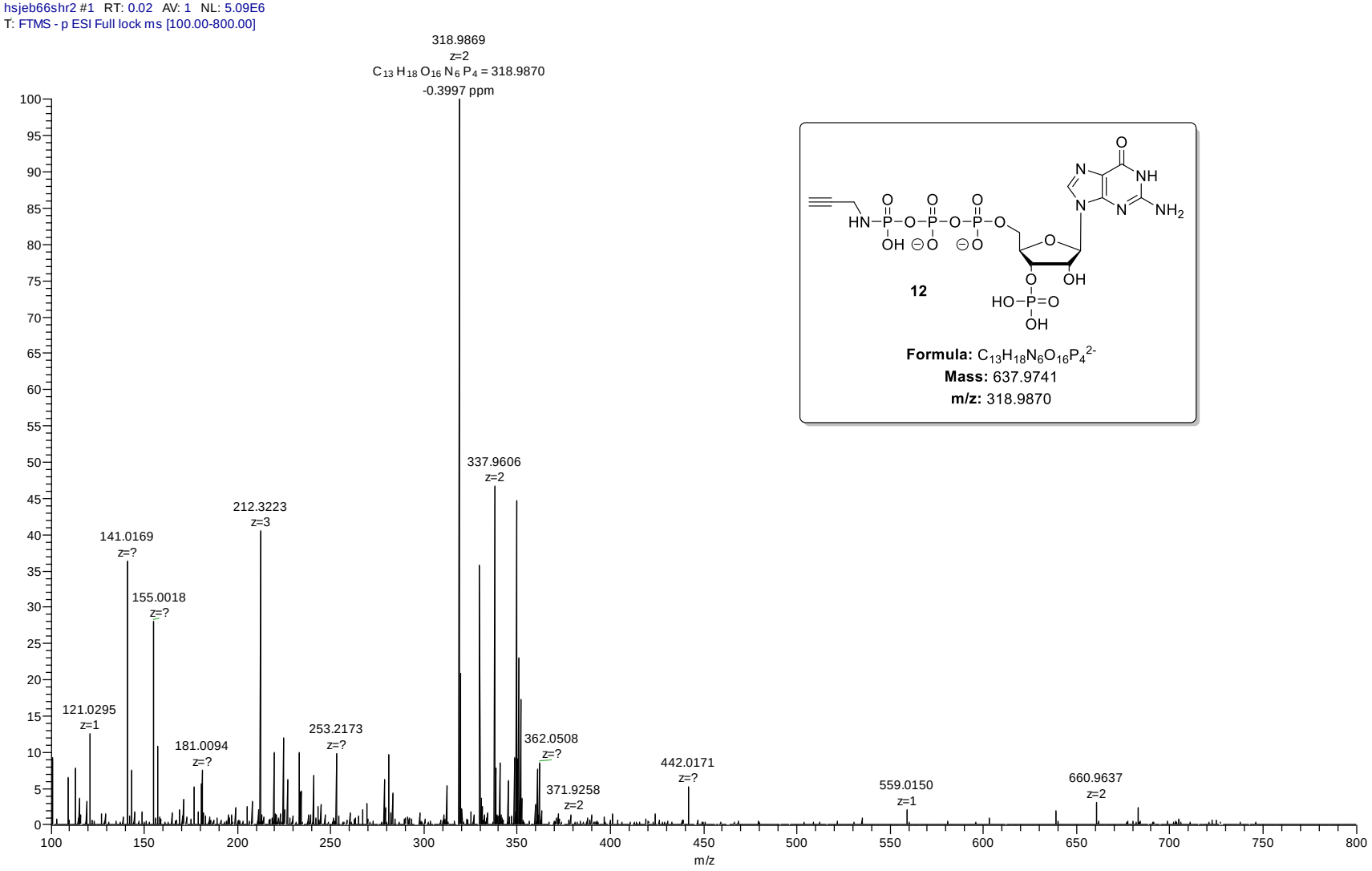


HRMS (ESI) Analysis of compound 11 (propargylamido-pppAp)

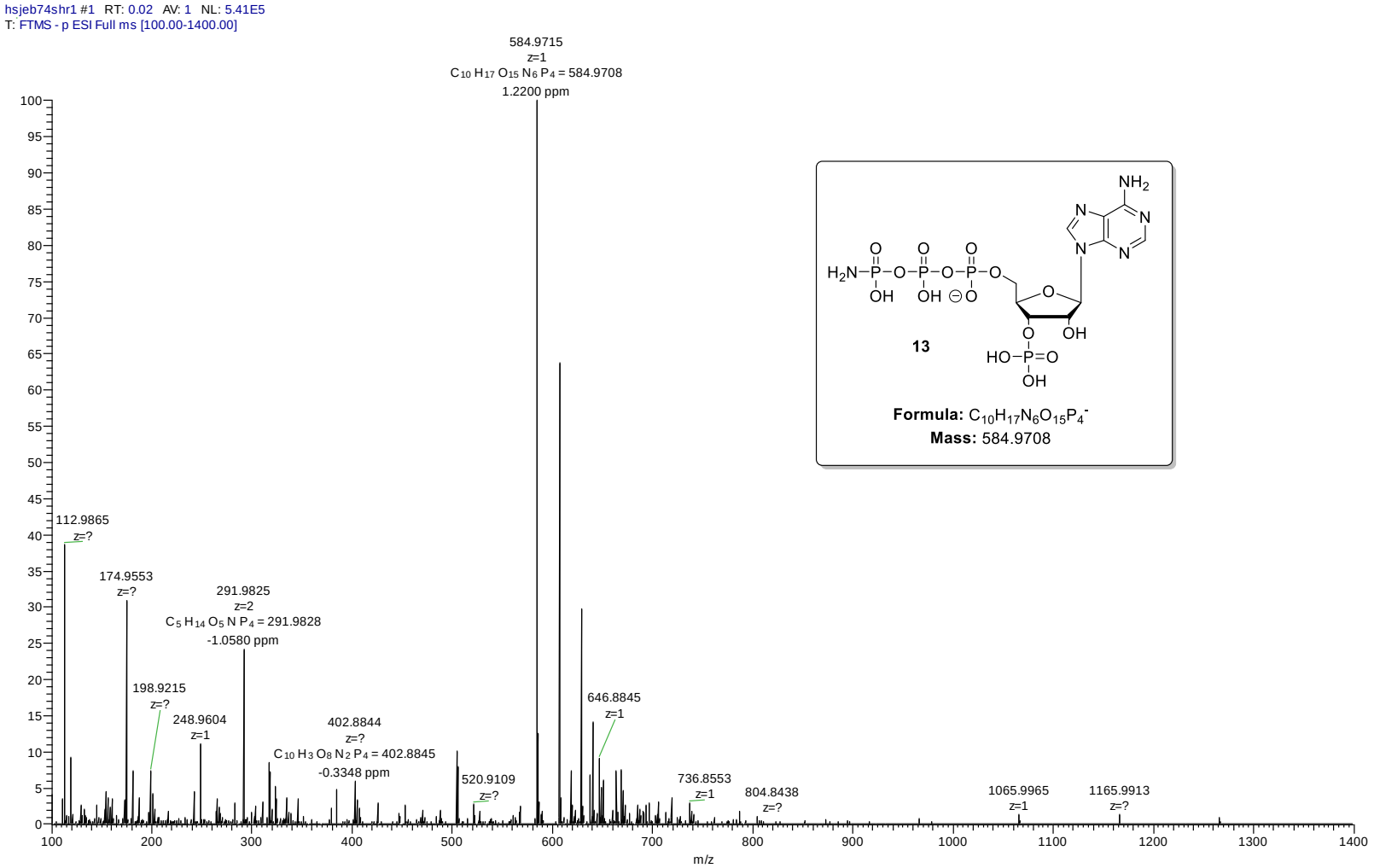


Formula: $C_{13}H_{18}N_6O_{15}P_4^{2-}$
Mass: 621.9792
m/z: 310.9896

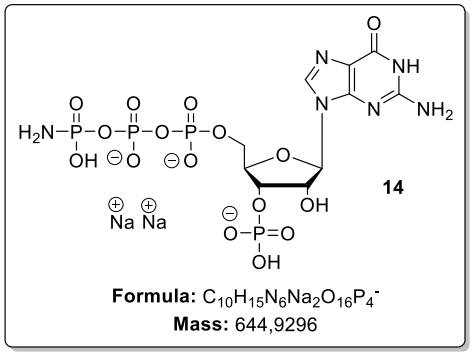
HRMS (ESI) Analysis of compound 12 (propargylamido-pppGp)



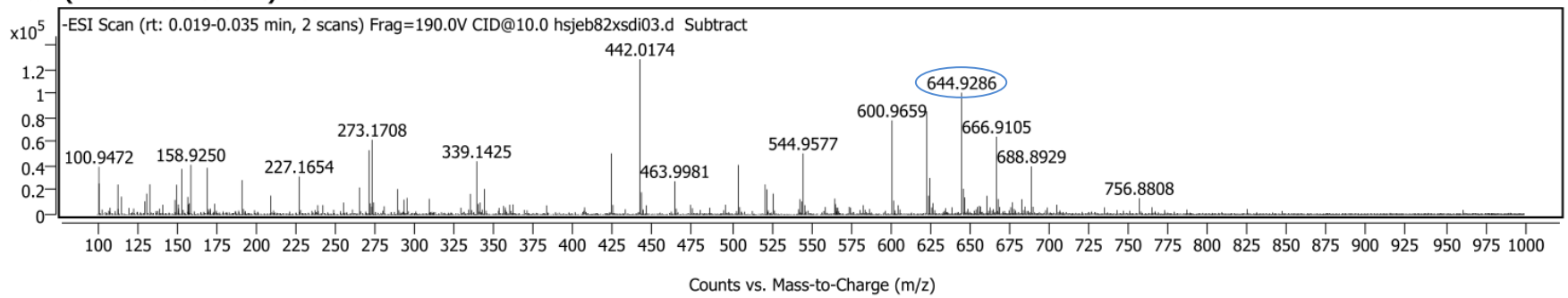
HRMS (ESI) Analysis of compound 13 (amido-pppAp)



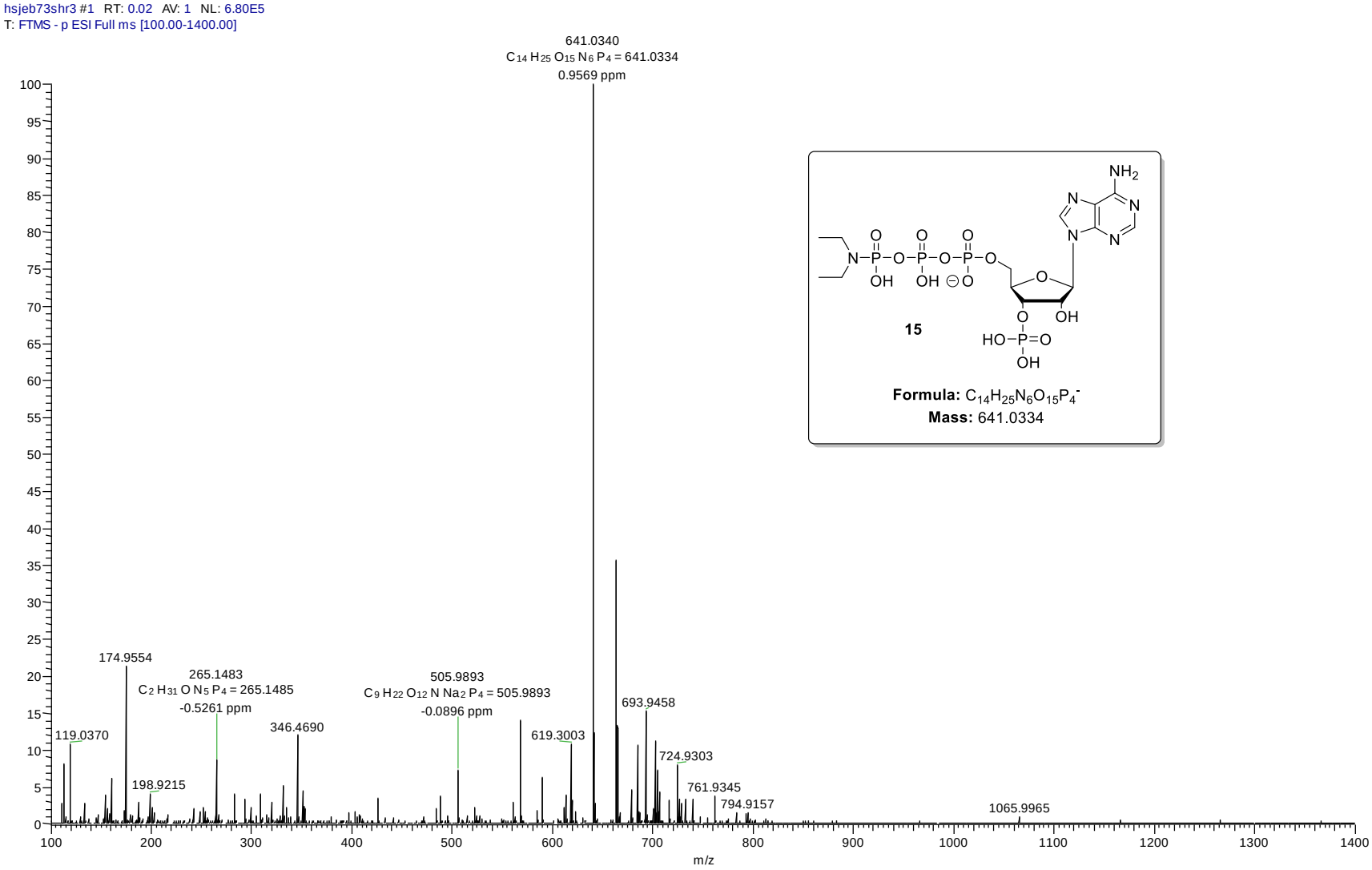
HRMS (ESI) Analysis of compound 14 (amido-pppGp)



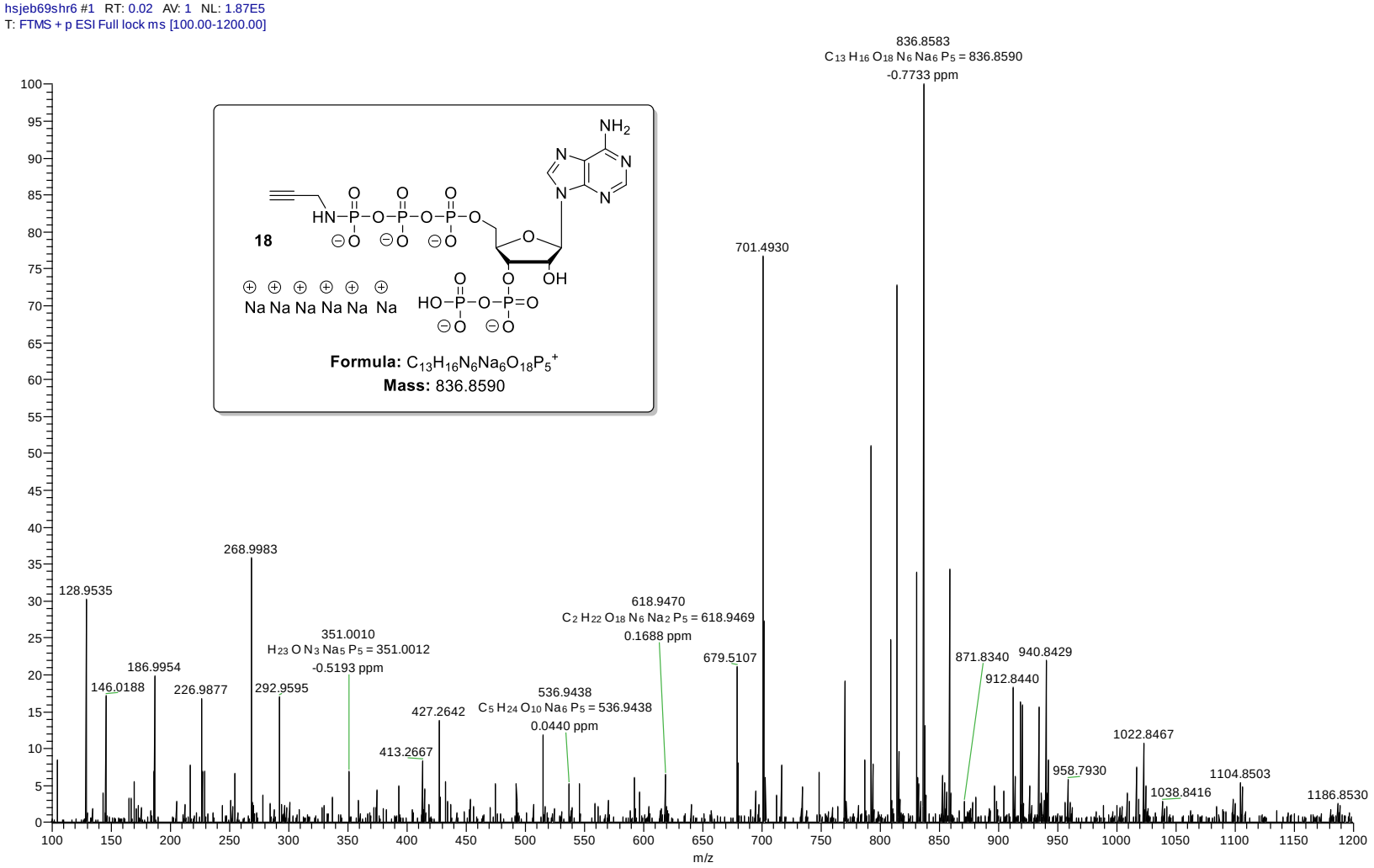
- Scan (rt: 0.019-0.035 min) Sub



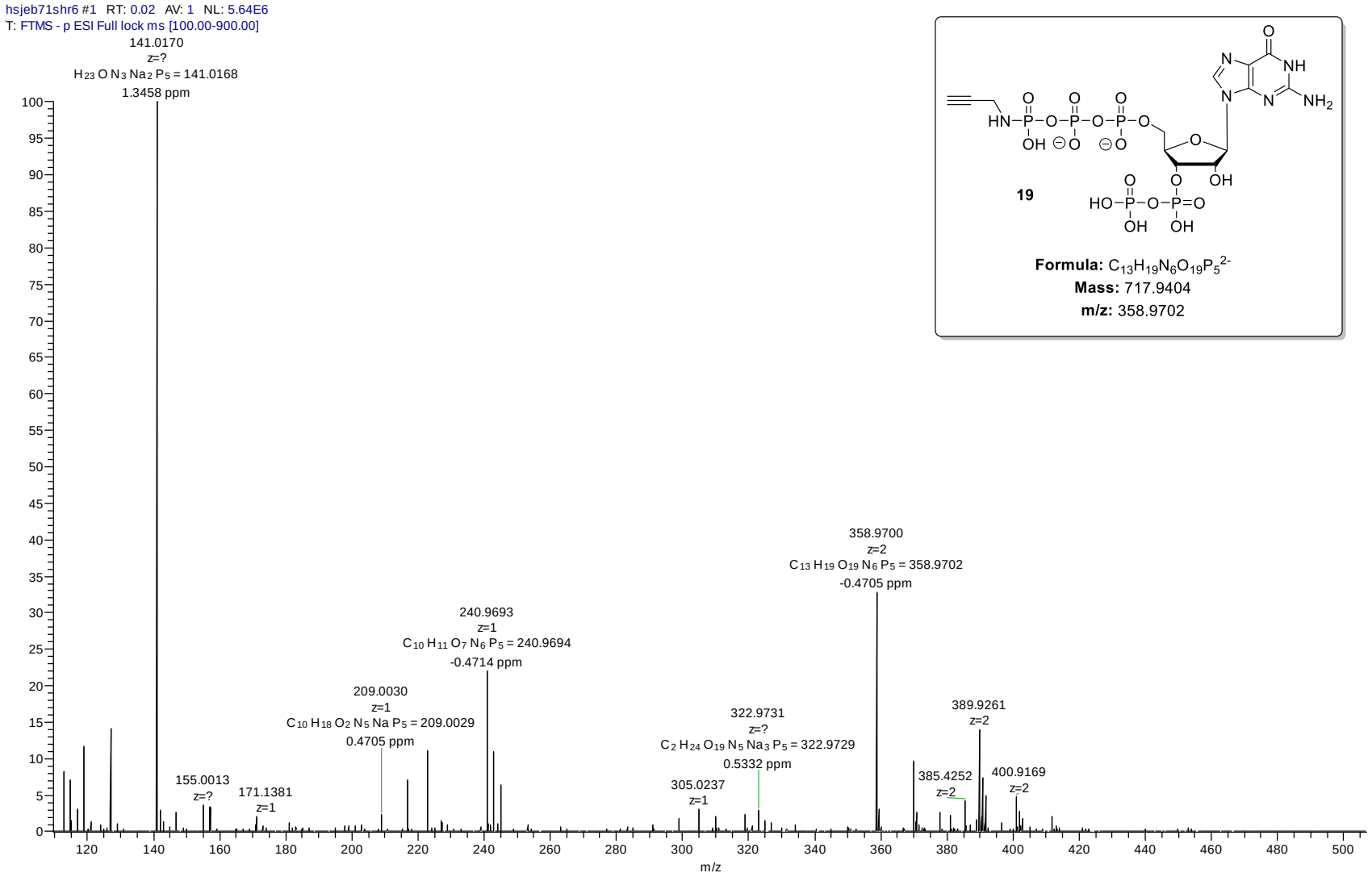
HRMS (ESI) Analysis of compound 15 (diethylamido-pppAp)



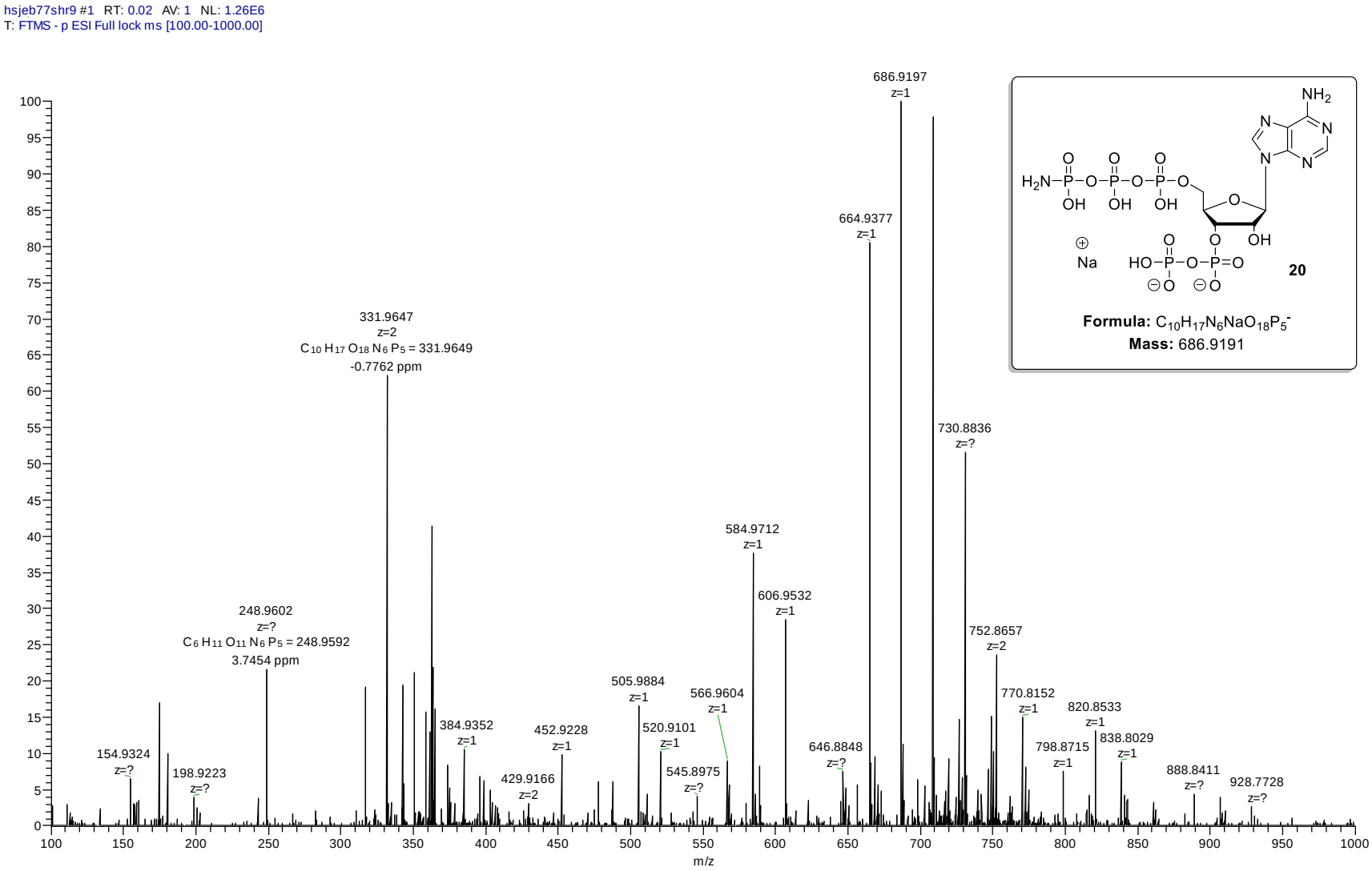
HRMS (ESI) Analysis of compound 18 (propargylamido-pppApp)



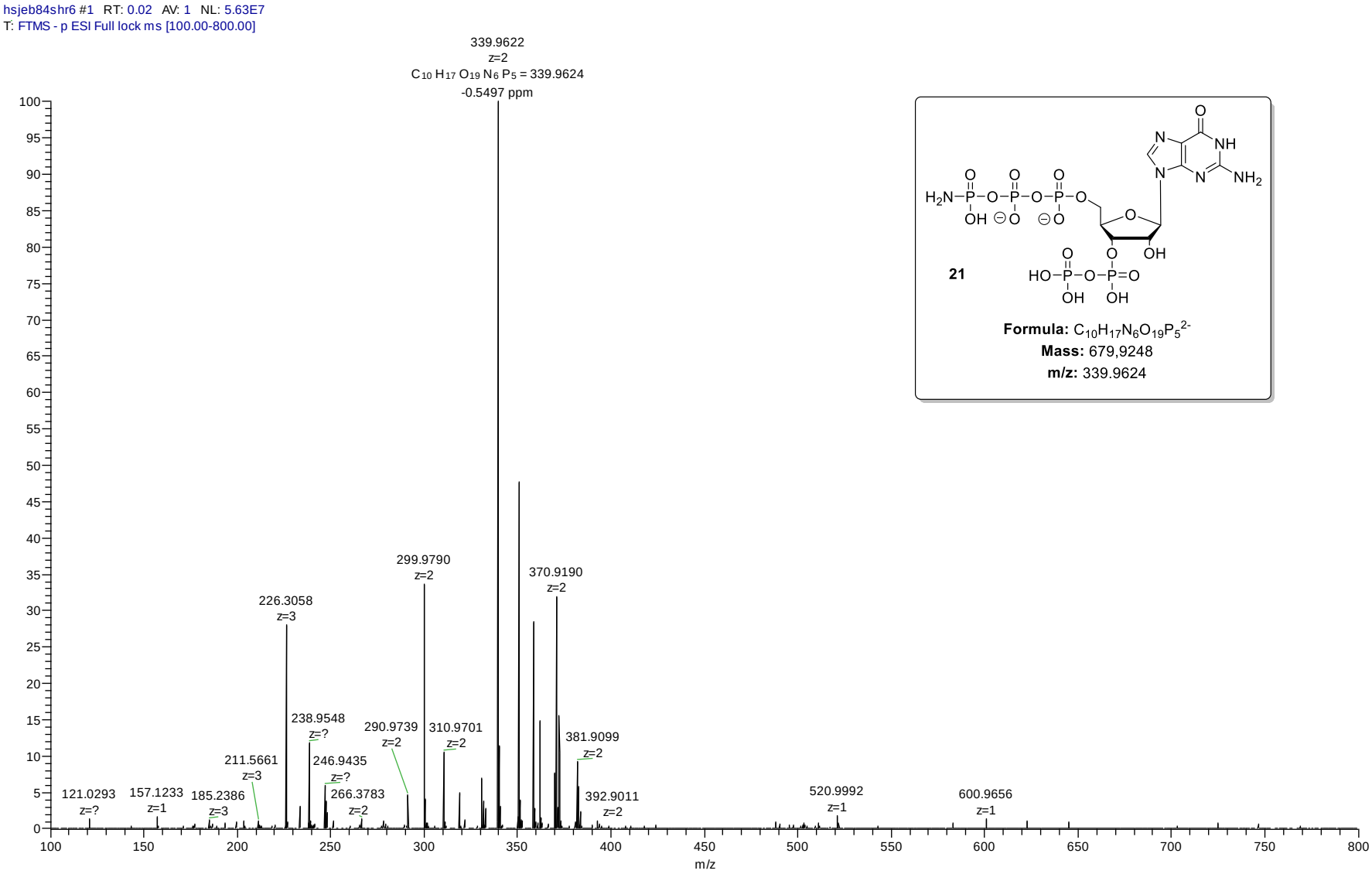
HRMS (ESI) Analysis of compound 19 (propargylamido-pppGpp)



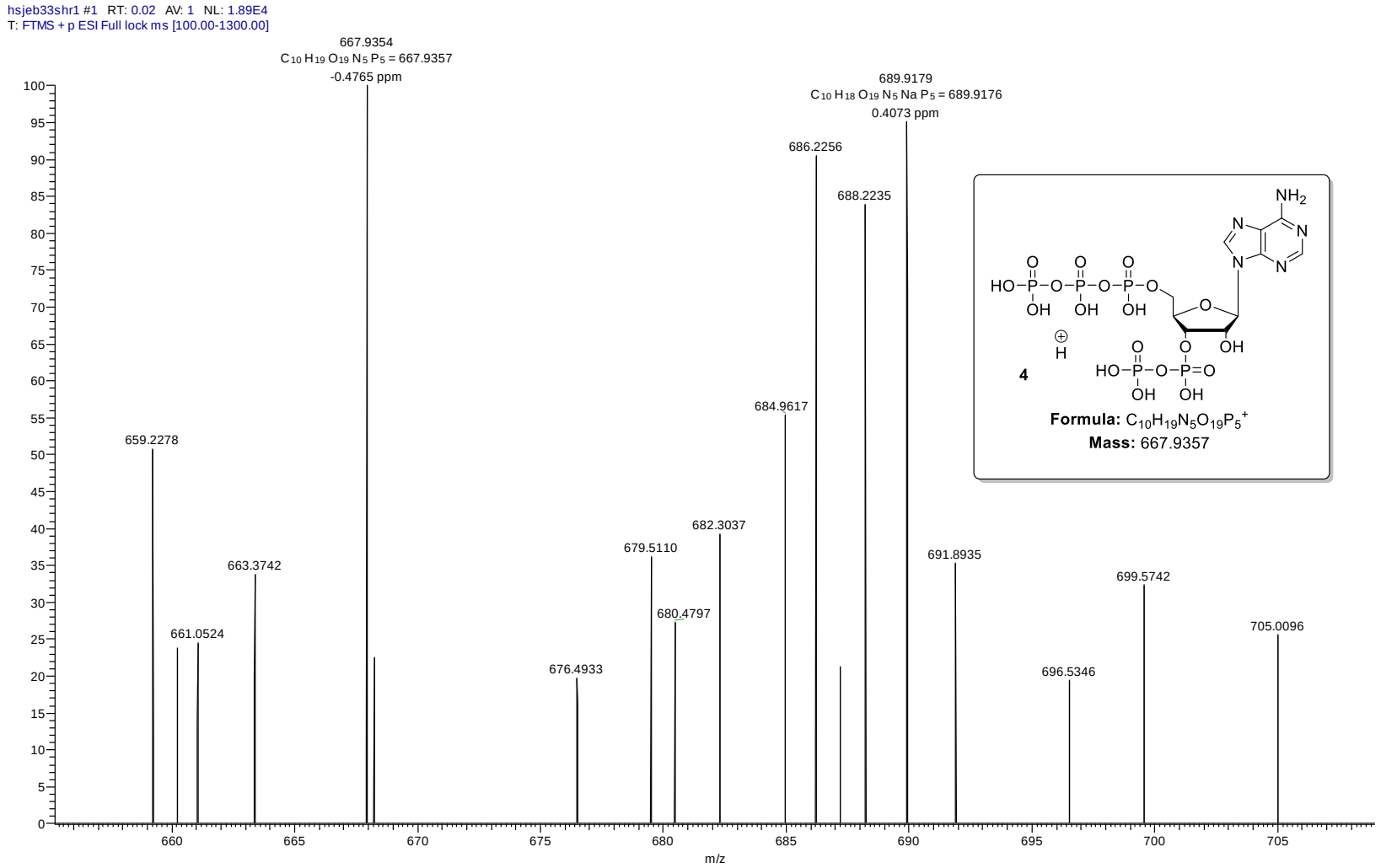
HRMS (ESI) Analysis of compound 20 (amido-pppApp)



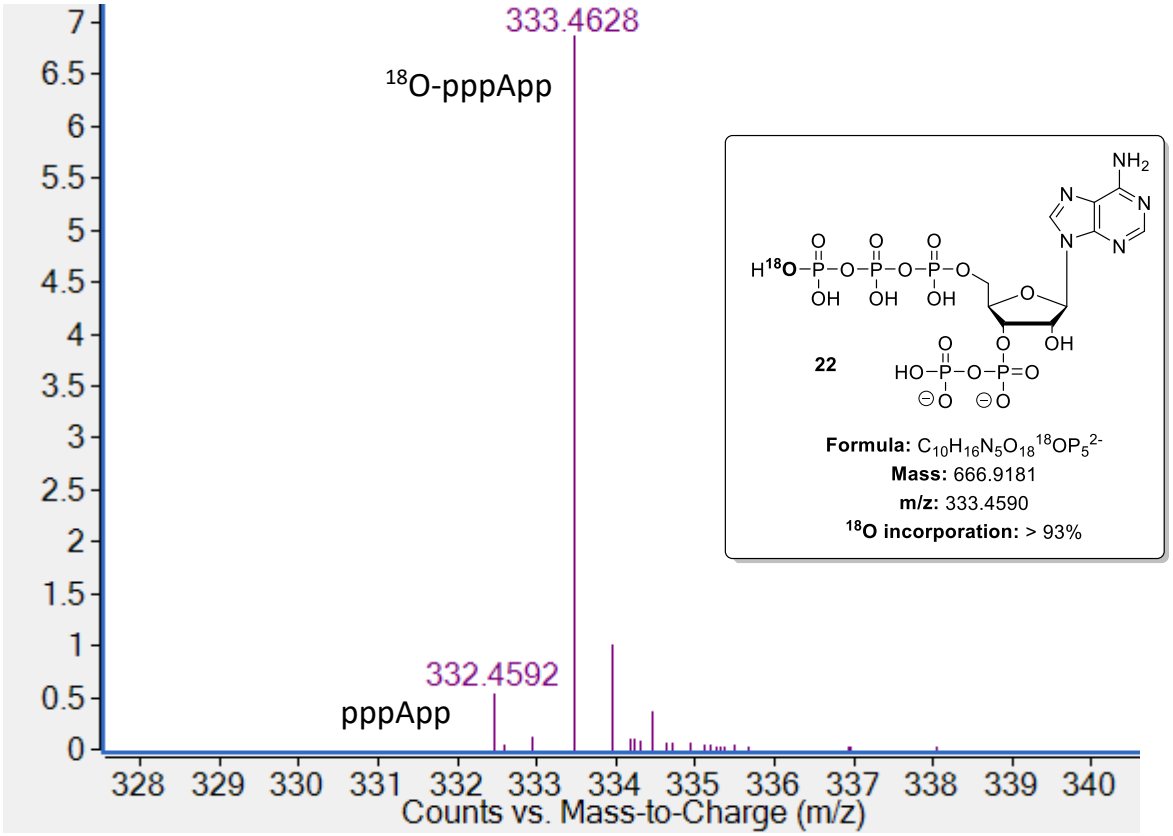
HRMS (ESI) Analysis of compound 21 (amido-pppGpp)



HRMS (ESI) Analysis of compound 4 (pppApp)

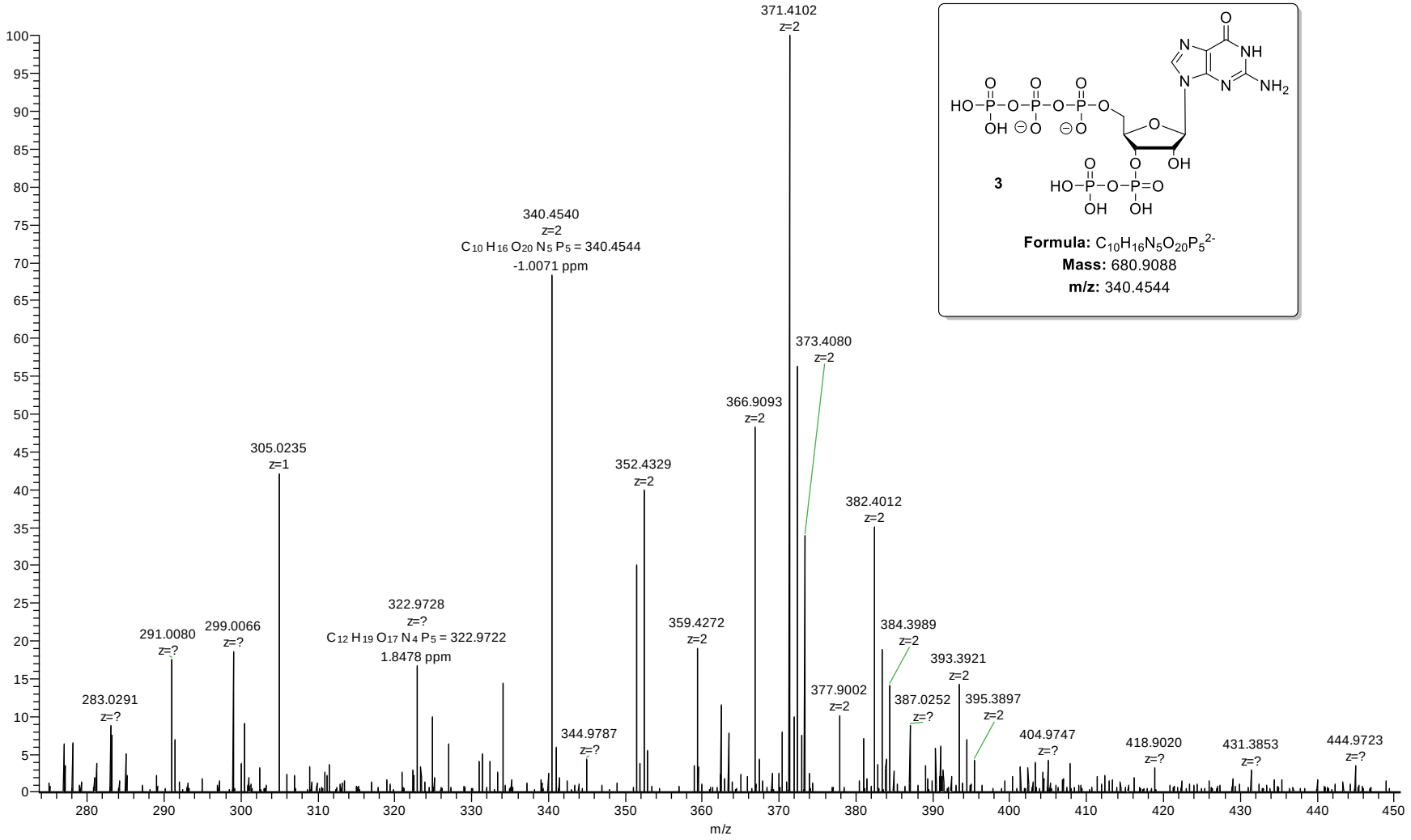


HRMS (ESI) Analysis of compound 22 (^{18}O-pppApp)

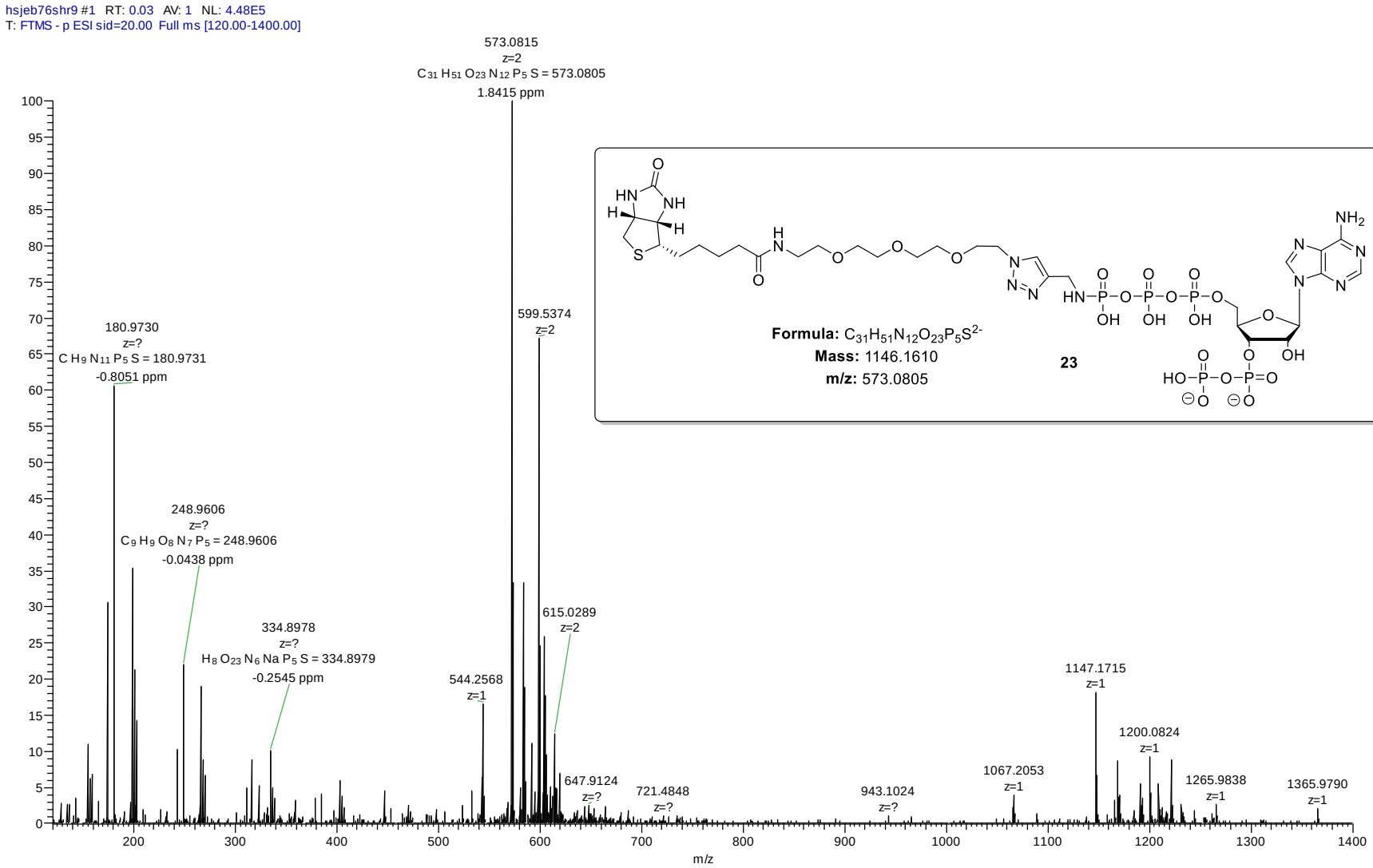


HRMS (ESI) Analysis of compound 3 (pppGpp)

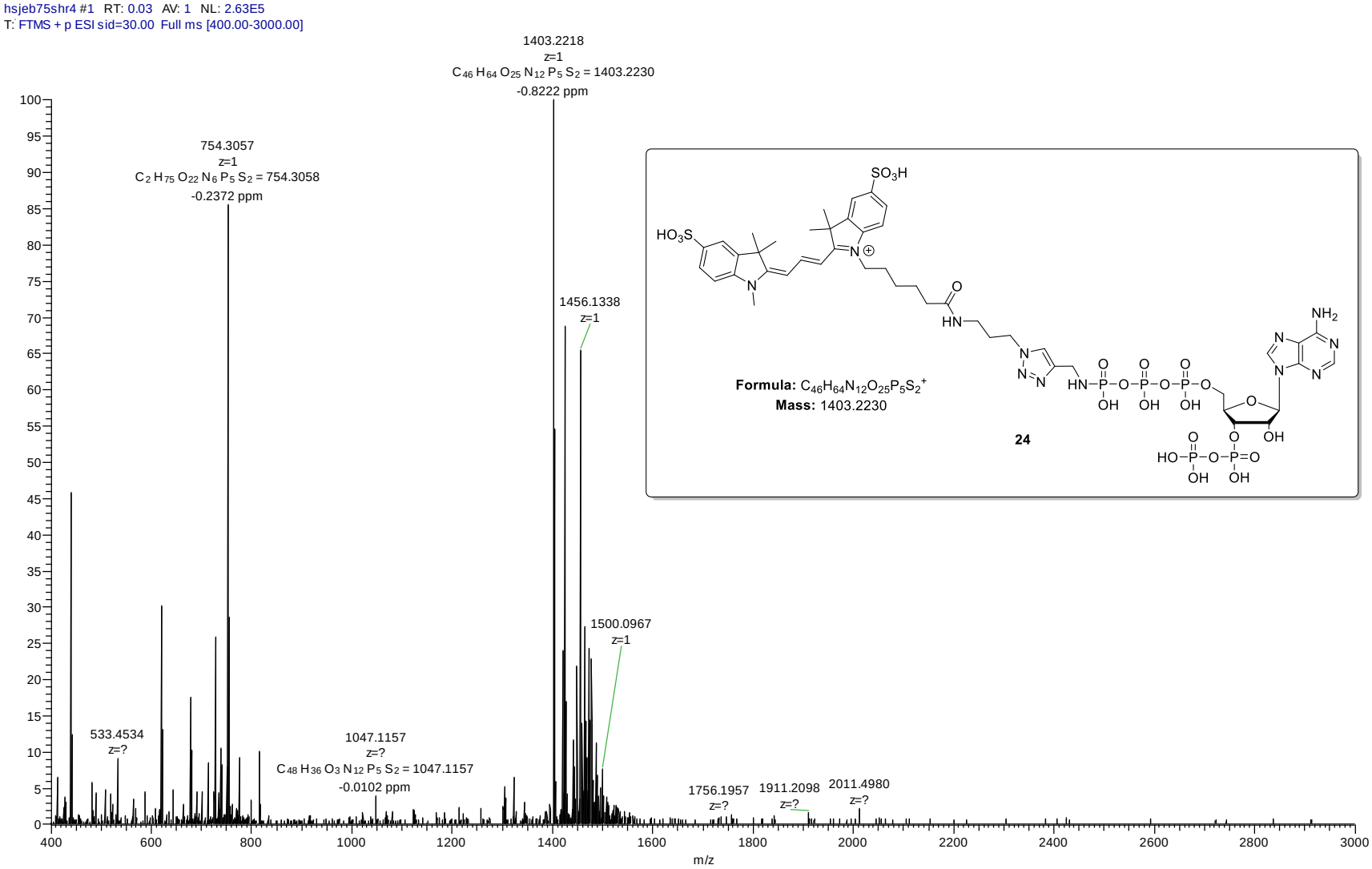
hsjeb72shr3 #1 RT: 0.03 AV: 1 NL: 2.23E5
T: FTMS - p ESI Full lock ms [150.00-700.00]



HRMS (ESI) Analysis of compound 23 (biotin - pppApp)



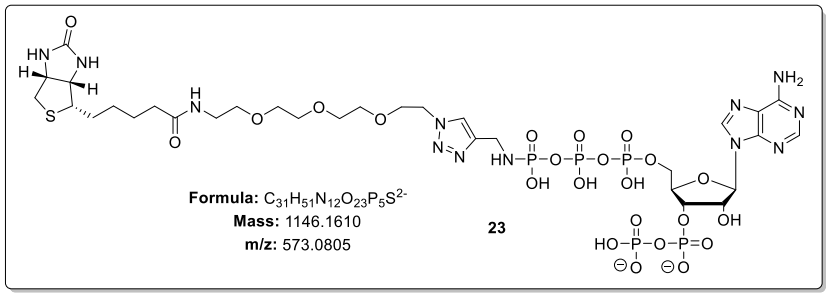
HRMS (ESI) Analysis of compound 24 (fluorophor - pppApp)

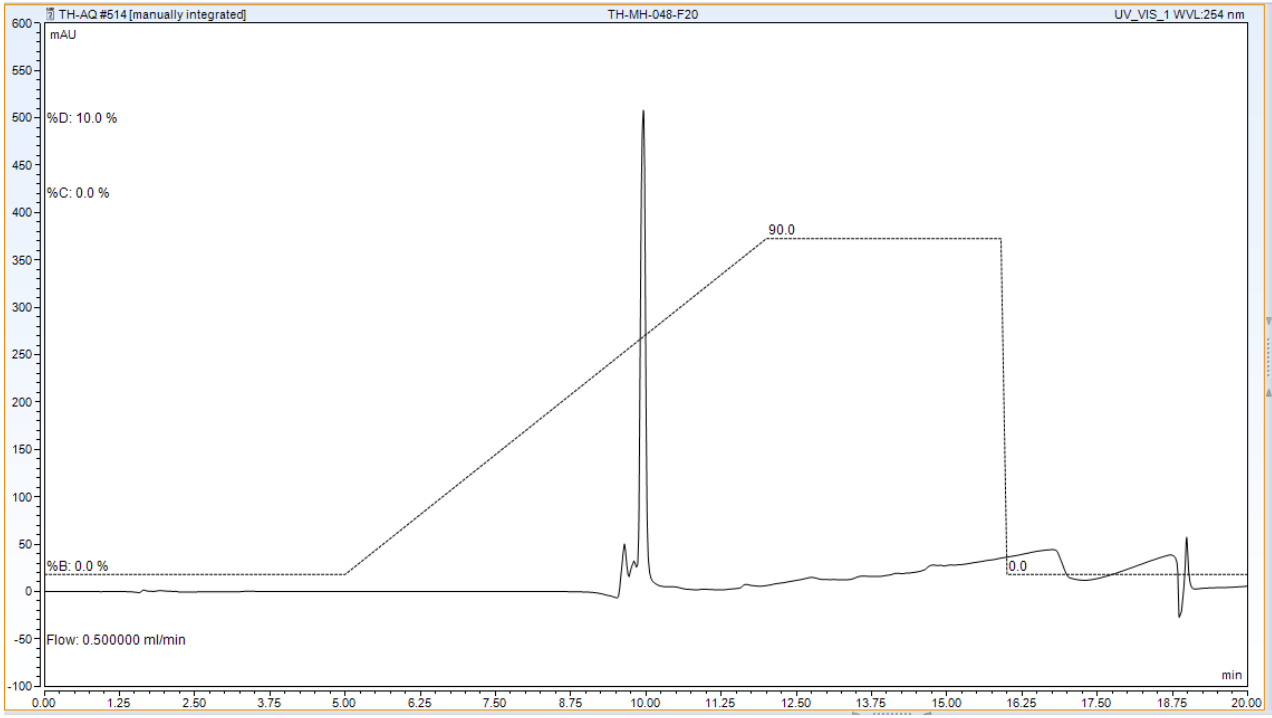


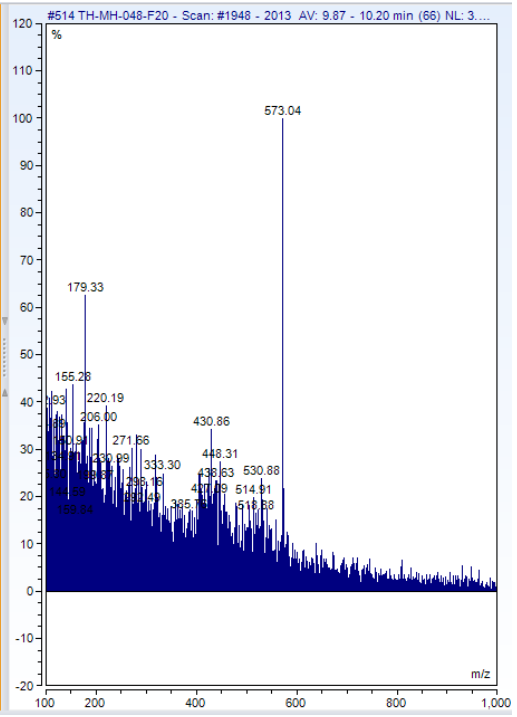
HPLC-MS Analysis of compound 23 (biotin-PEG$_3$-triazolmethanamino-pppApp)

HPLC-MS-System: *Dionex Ultimate 3000 – MSQ Plus, C18-AQ-column, WVL: 254 nm*

Gradient: *A (H_2O) / B (MeCN) / C (TEAA, 100 mM)*







HPLC-MS Analysis of compound 24 (Sulfo-cyanine3-triazolmethanamino-pppApp)

HPLC-MS-System: *Dionex Ultimate 3000 – MSQ Plus, C18-AQ-column, WVL: 254 nm*

Gradient: *A (H_2O) / B (MeCN) / C (TEAA, 100 mM)*