Electronic Supplementary Information (ESI)

Rapid synthesis of nucleotide pyrophosphate linkages in a ball mill

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General information

All substrates were purchased from Sigma and used as supplied: AMP-morpholidate (A1127); AMP.$H_{0.9}$.$Na_{1.1}$ (A1752); ADP.$H_{1.6}$.$Na_{1.4}$ (A2754); ATP.Na_2.H_2 (A6419); TMP.Na_2 (T7004), β-NMN (N3501) 5-ribose monophosphate (83870). Other reagents or solvents were purchased from commercial suppliers (Sigma, Aldrich or Fluka) and used without further purification. H_2O was purified to 18.2 MΩ by reverse osmosis (Barnstead NANOpure Diamond water purification system).

All ball mill reactions were performed using a Retsch Mixer Mill MM 400[1] and a 15mm stainless steel ball (13.67g) according to the conditions described below.

Stock solutions of triethylammonium bicarbonate (TEAB) in 18.2 MΩ H_2O were prepared by bubbling CO_2 through a sintered frit into a mixture of triethylamine and H_2O at 0°C overnight to give homogenous solutions and subsequently diluted to 1 M. This stock was stored at 4°C until required (up to 2 days) and then diluted as required to give: 100 mM TEAB (aq.), pH 7.8 (Buffer A); or 100 mM TEAB in 65:35 (v/v) MeCN:H_2O, pH 8.2 (Buffer B).

To the ball-milled reaction mixtures was added H_2O (1 mL) giving turbid solutions and treated as below:

Analytical HPLC

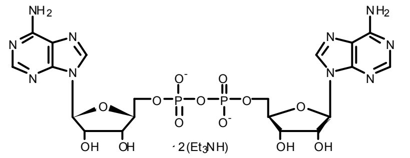
Analysis of crude reaction mixtures was performed following further dilution (10 µl in 1 mL H_2O) and injection of a 10 µl aliquot of this diluted solution onto HPLC (Merck Hitachi D7000 system). Elution from the C-18 reversed phase silica column (Phenomenex Clarity 5µm Oligo-RP - 150 x 4.60 mm) was performed using a gradient of: 0-25 min, 0-20% Buffer B; 25-30 min, isocratic elution at 20% B; flow rate, 1.0 mL min^{-1}. Chromatograms were recorded using a Merck Hitachi Diode Array Detector L-7455 monitoring at 260 nm. Compositional analysis was based upon molar extinction coefficients described in the literature[2-4]

Preparative HPLC

The stock solution was dilution following addition of a further aliquot of H_2O (0.5 mL) and this diluted solution purified by preparative HPLC using the same system as described above with the following changes: the material was injected in two aliquots (0.75 mL); a larger column was utilised (Phenomenex Clarity 5µm Oligo-RP - 250 x 21.2 mm); the gradient was modified: 0-7.5 min, isocratic elution at 0% B; 7.5-67.5 min, 0-20% B; flow rate, 7.0 mL min^{-1}; monitoring was performed at 280 nm

^{1}H, ^{13}C or ^{31}P NMR NMR spectra were recorded on a Bruker III-400 or DRX-500 at 20°C. All spectra were recorded at ambient temperature in D_2O. Mass spectra were recorded using a VG Quattro II Triple Quadrupole Mass Spectrometer (Electrospray). Mass spectrometry was performed by Analytical Services and Environmental Projects (ASEP) at Queen's University Belfast.

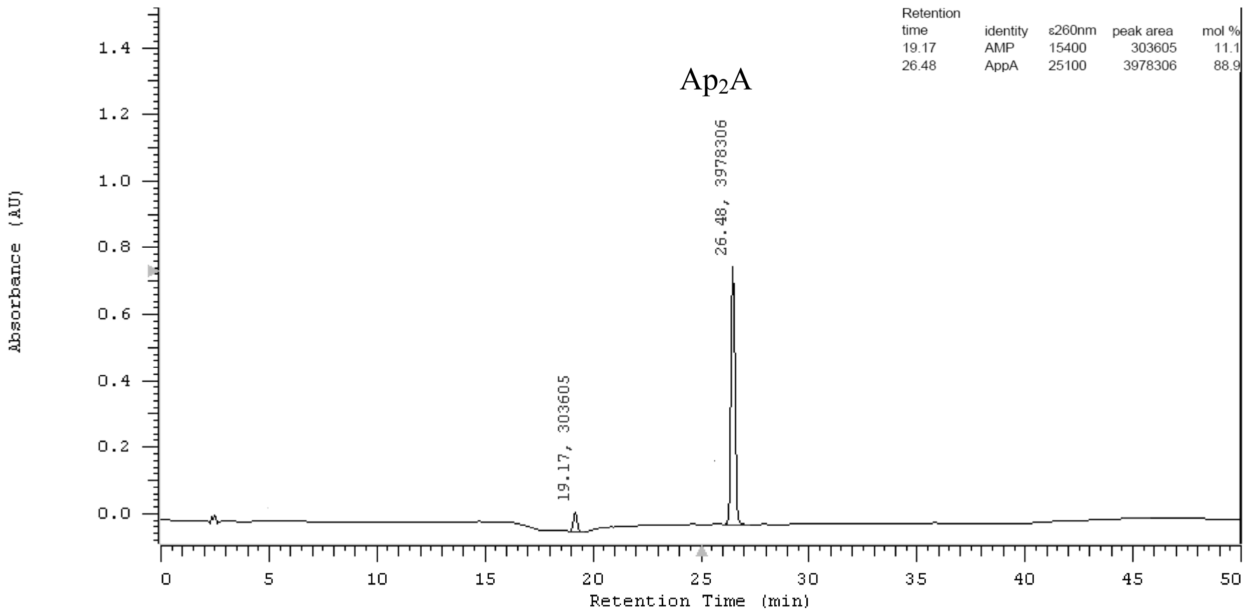
Experimental procedures and analytical data



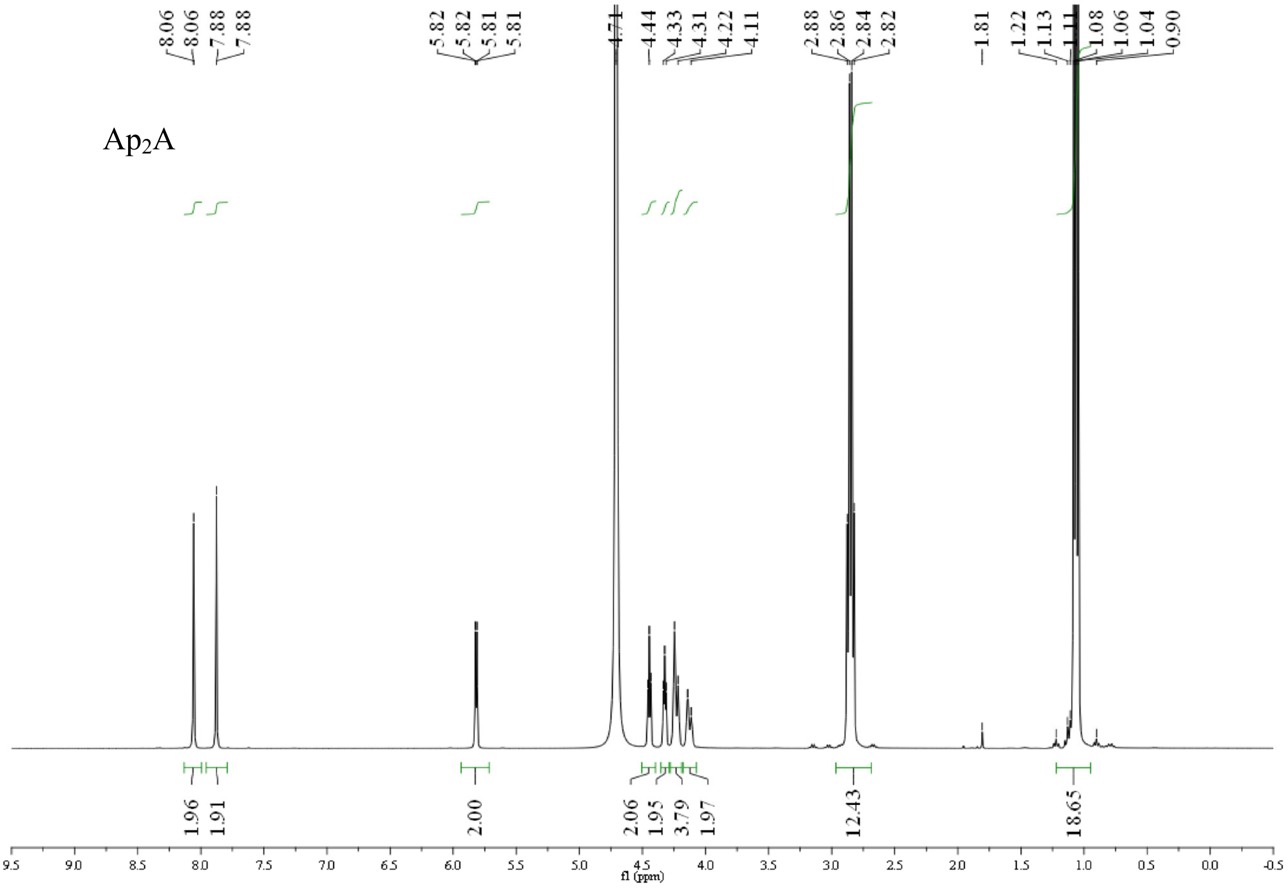
P^1,P^2-di(adenosin-5'-yl)diphosphate bis(triethylammonium) salt - AppA (**3a**).

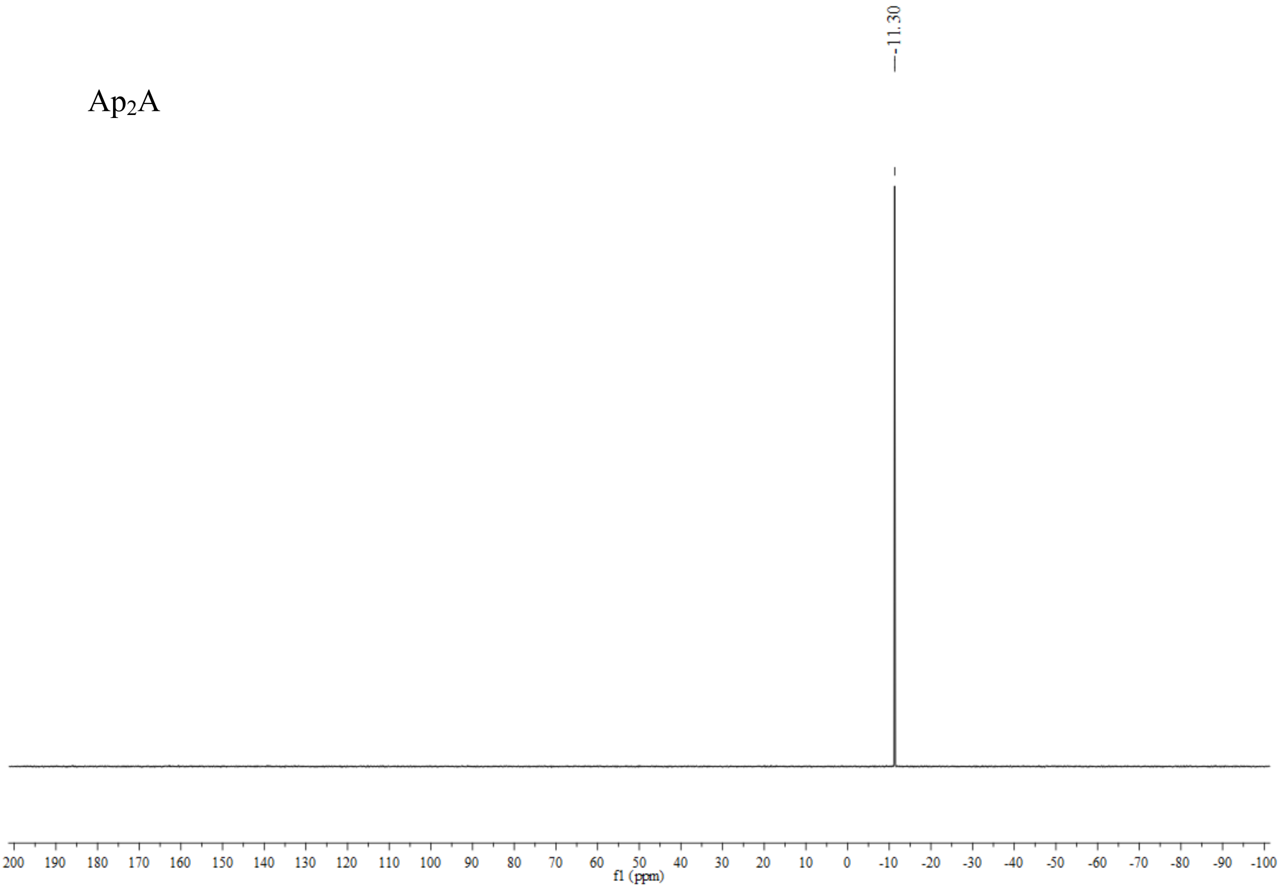
A stainless steel jar (25 mL) was charged with a mixture of adenosine-5′-phosphoromorpholidate 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt (**1**) (48 mg, 0.068 mmol), adenosine-5'-monophosphate (**2a**) (25 mg, 0.067 mmol, 1 eq.), $MgCl_2(H_2O)_6$ (21 mg, 0.10 mmol, 1.5 eq.), tetrazole (10 mg, 0.14 mmol, 2.1 eq.), H_2O (7.5 µL, 0.42 mmol, 6 eq.) and a 15.0 mm stainless steel ball. The vessel was vibrated at 30 Hz for 90 minutes and allowed to cool to room temperature. The reaction mixture was suspended in H_2O, analysed and then purified by C18 RP-HPLC – see general information. Appropriate fractions were lyophilised to yield pure **3a** (45 mg, 0.051 mmol, 75%). ^{1}H NMR (400 MHz, D_2O) δ = 8.06 (2H, s), 7.88 (2H, s), 5.82 (2H, d, J = 5.0 Hz), 4.44 (2H, t, J = 5.0 Hz), 4.32 (2H, t, J = 4.5 Hz), 4.23 (4H, d, J = 11.2 Hz), 4.13 (2H, d, J = 11.6 Hz), 2.85 (6H, q, J = 7.3 Hz), 1.06 (9H, t, J = 7.3 Hz); ^{13}C NMR (101 MHz, D_2O) δ = 154.66, 152.27, 148.02, 139.09, 117.68, 87.08, 83.30 (d, $^2J_{C,P}$ = 8.6 Hz), 74.67, 69.86, 65.09, 46.65, 8.20; ^{31}P (162 MHz, D_2O) δ = -11.30; ES+ MS ($C_{20}H_{27}N_{10}O_{13}P_2$) (M + H$^+$), calc. 677.1234, found 677.1215.

Analytical C18 RP-HPLC of crude reaction mixture

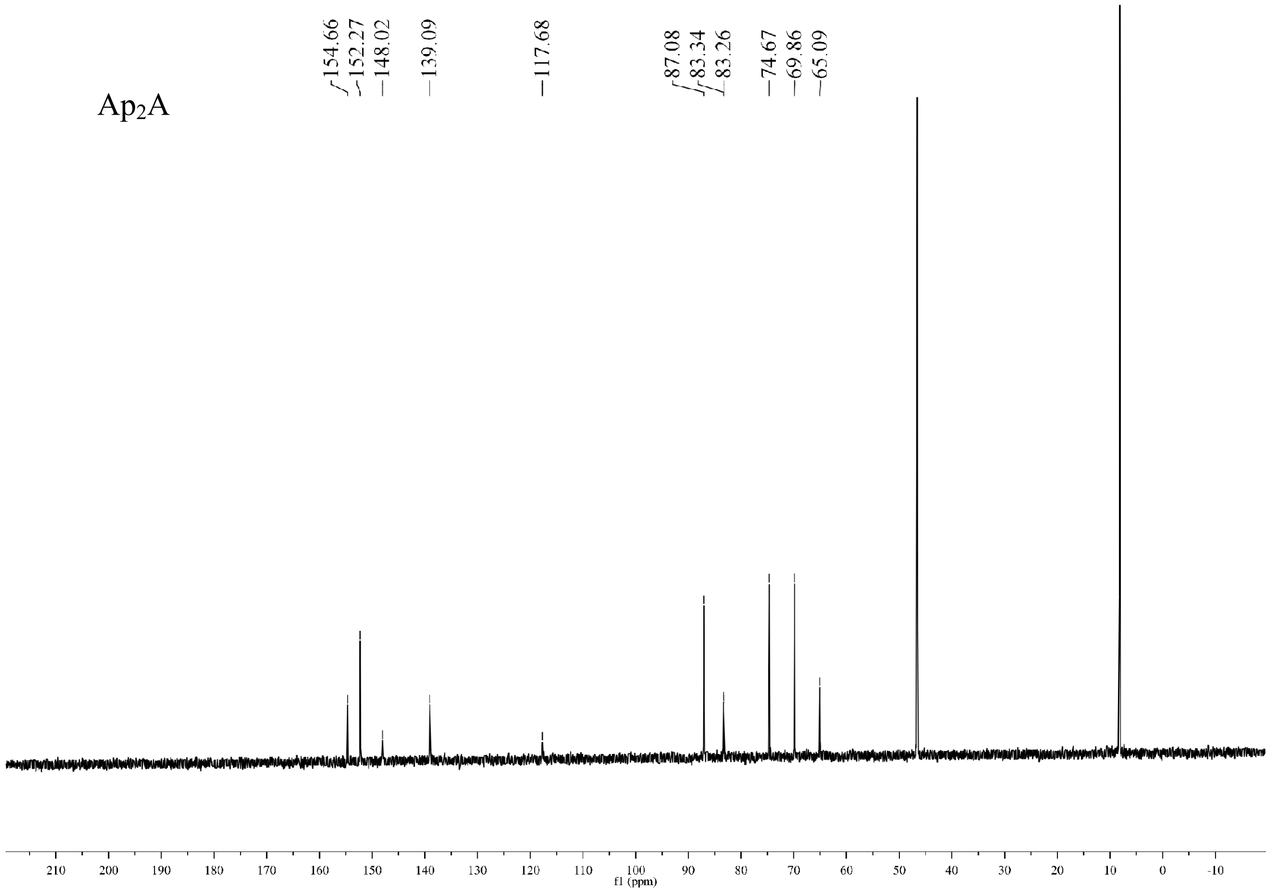


Retention time	identity	ε260nm	peak area	mol %
19.17	AMP	15400	303605	11.1
26.48	AppA	25100	3978306	88.9

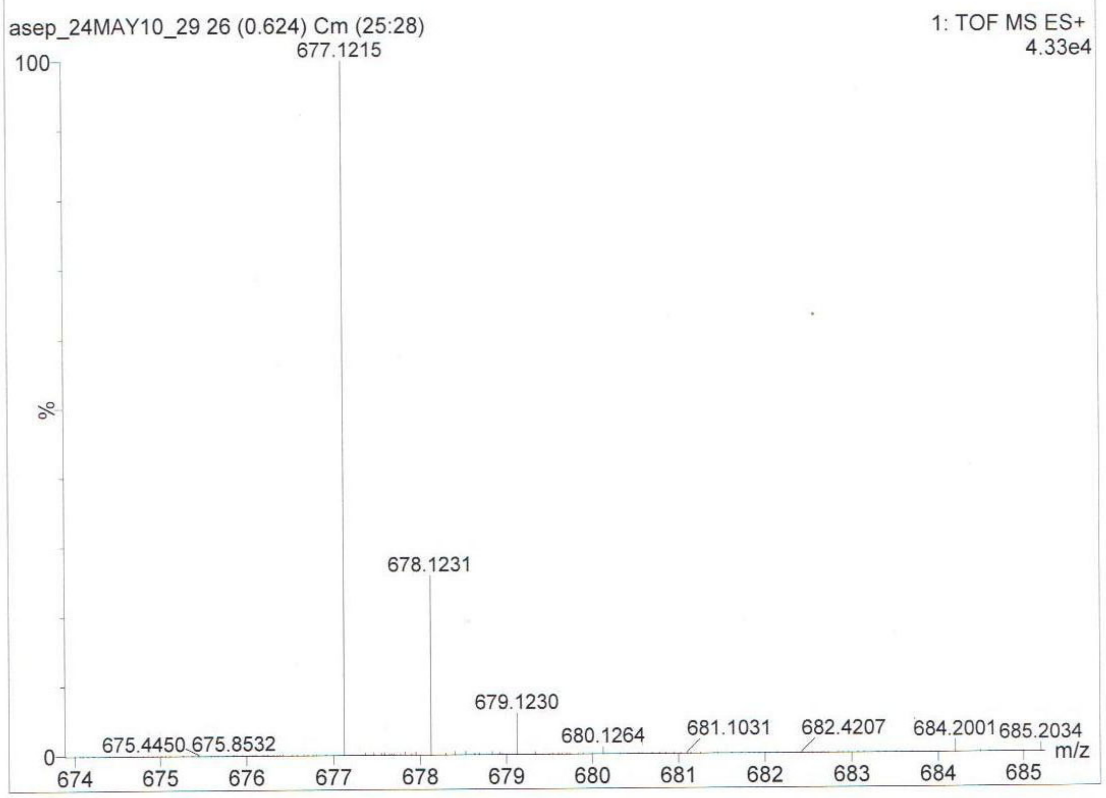

8.06
8.06
7.88
7.88
5.82
5.82
5.81
5.81
4.71
4.44
4.33
4.31
4.22
4.11
2.88
2.86
2.84
2.82
1.81
1.22
1.13
1.11
1.08
1.06
1.04
0.90
Ap2A
1.96
1.91
2.00
2.06
1.95
3.79
1.97
12.43
18.65
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


-11.30
Ap2A
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100
f1 (ppm)

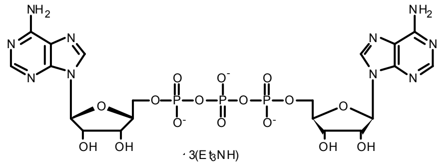
Ap$_2$A


154.66
152.27
148.02
139.09
117.68
87.08
83.34
83.26
74.67
69.86
65.09
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

Ap$_2$A

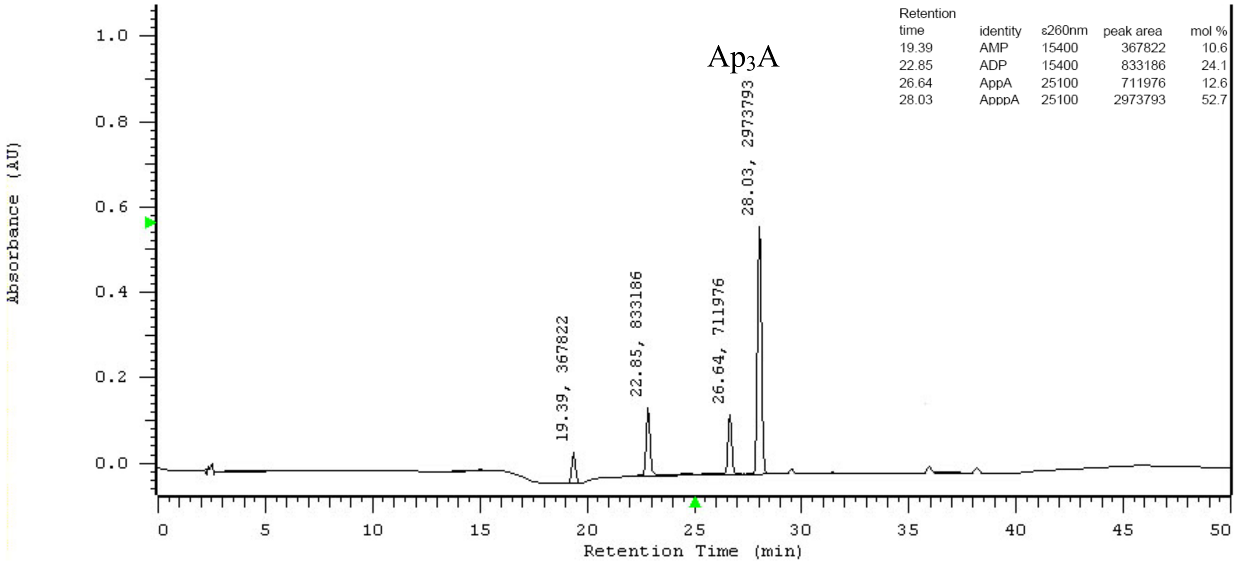

asep_24MAY10_29 26 (0.624) Cm (25:28)
1: TOF MS ES+
4.33e4
100
%
0
677.1215
678.1231
679.1230
680.1264
681.1031
682.4207
684.2001
685.2034
675.4450
675.8532
674
675
676
677
678
679
680
681
682
683
684
685
m/z

P^1,P^3-di(adenosin-5'-yl)triphosphate tris(triethylammonium) salt – AppppA (**3b**)

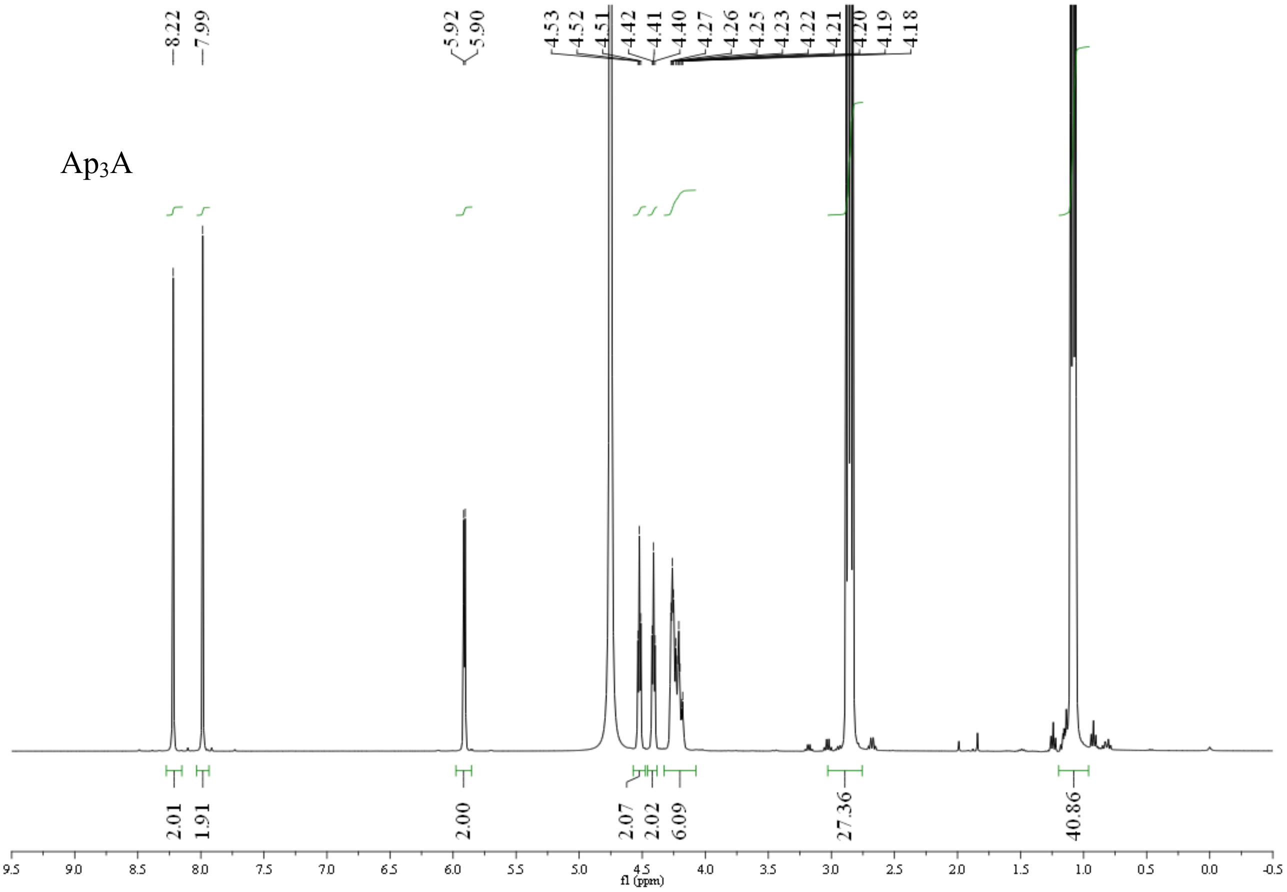


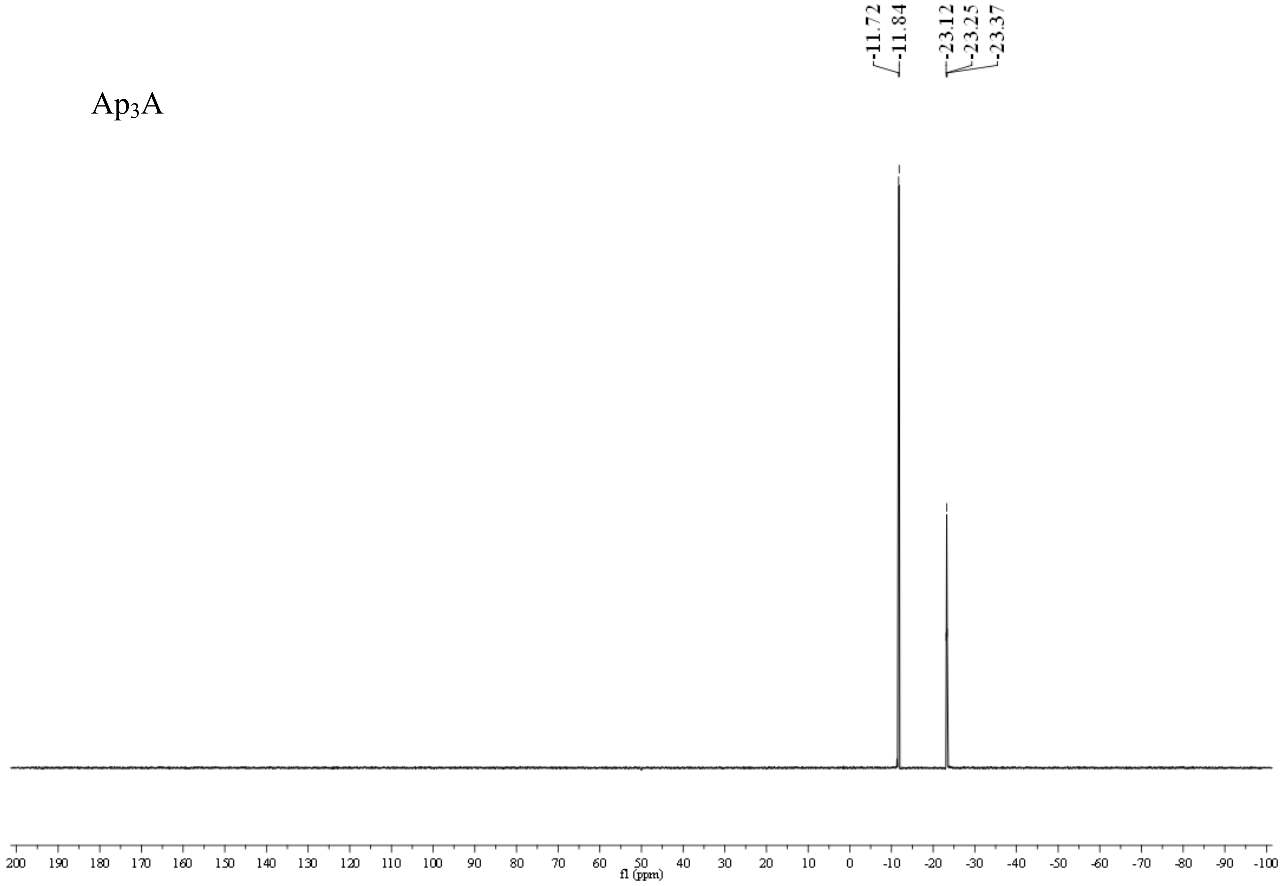
A stainless steel jar (25 mL) was charged with a mixture of adenosine-5'-phosphoromorpholidate 4-morpholine-*N*,*N'*-dicyclohexylcarboxamidine salt (**1**) (50 mg, 0.070 mmol), adenosine-5'-diphosphate (**2b**) (30 mg, 0.065 mmol, 0.9 eq.), $MgCl_2(H_2O)_6$ (22 mg, 0.11 mmol, 1.5 eq.), tetrazole (10 mg, 0.14 mmol, 2 eq.), H_2O (7.5 µL, 0.42 mmol, 6 eq.) and a 15.0 mm stainless steel ball. The vessel was vibrated at 30 Hz for 90 minutes and allowed to cool to room temperature. The reaction mixture was suspended in H_2O, analysed and then purified by C18 RP-HPLC – see general information. Appropriate fractions were lyophilised to yield **3b·(TEAB)$_{1.5}$** (39 mg, 0.032 mmol, 49%). ^{1}H NMR(400 MHz, D_2O) δ = 8.17 (2H, s), 7.91 (2H, s), 5.85 (2H, d, J = 3.8 Hz), 4.47 (2H, t, J = 4.6 Hz), 4.37 (2H, t, J = 4.6 Hz), 4.30 – 4.10 (6H, m), 2.86 (27H, q, J = 7.3 Hz), 1.08 (40.3H, t, J = 7.3 Hz); ^{13}C NMR (101 MHz, D_2O) δ = 154.84, 152.55, 148.11, 139.04, 117.75, 87.15, 83.04 (d, $^2J_{C,P}$ = 9.4), 74.78, 69.57, 64.58 (d, $^3J_{C,P}$ = 5.4 Hz), 46.11, 8.78; ^{31}P NMR (162 MHz, D_2O) δ = -11.78 (2 P$_\alpha$, d, J = 19.7 Hz), -23.25 (1 P$_\beta$, t, J = 19.7); ES+ MS, ($C_{20}H_{28}N_{10}O_{16}P_2$) (M + H$^+$), calc. 757.0898, found 757.0906.

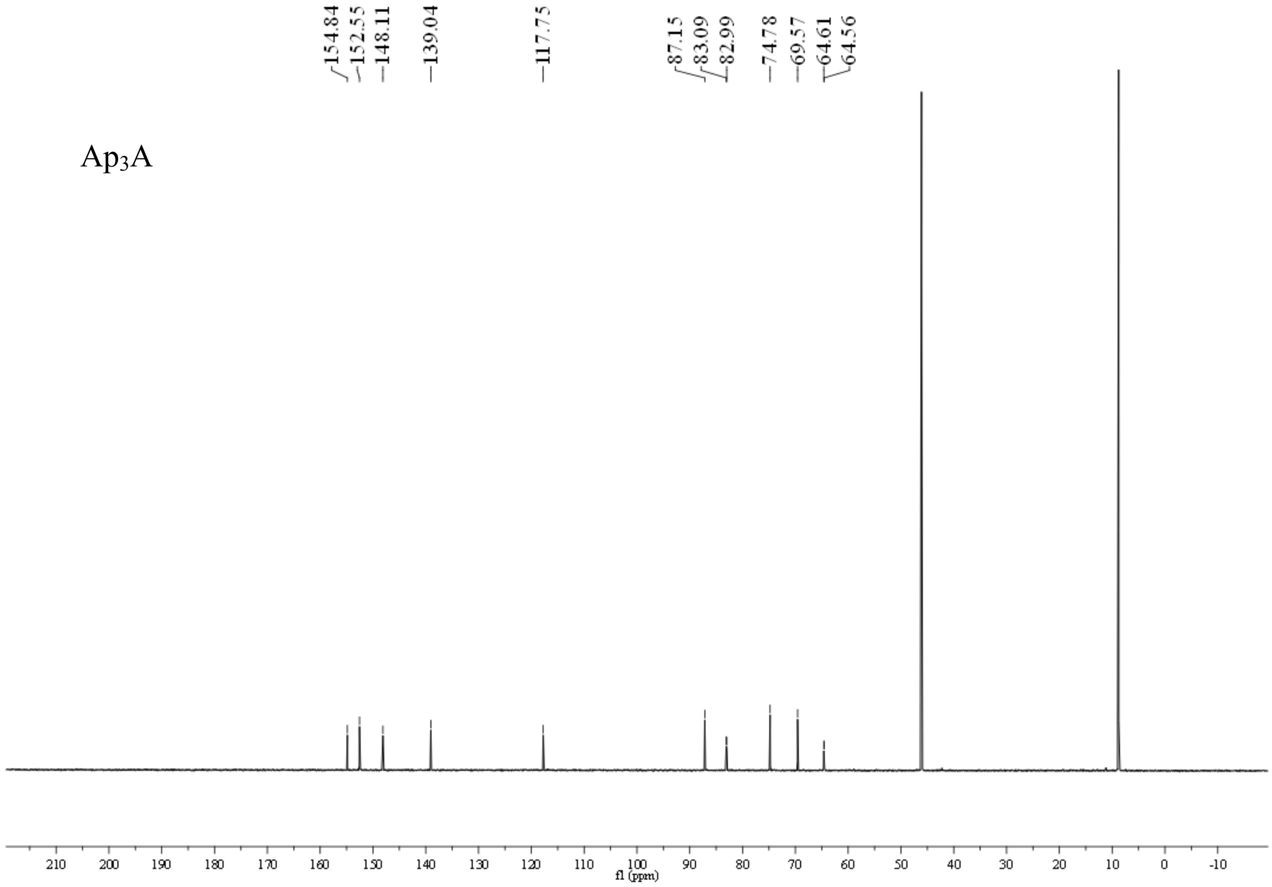
Analytical C18 RP-HPLC of crude reaction mixture

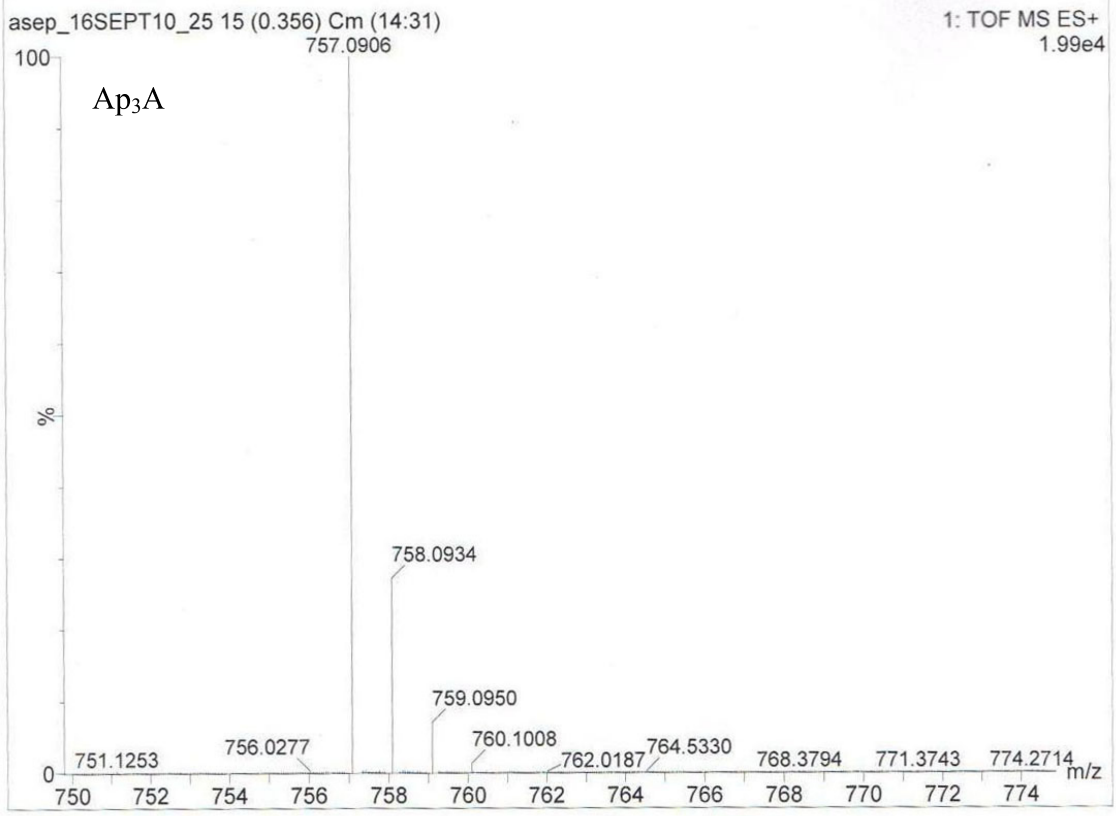


Retention time	identity	ε260nm	peak area	mol %
19.39	AMP	15400	367822	10.6
22.85	ADP	15400	833186	24.1
26.64	AppA	25100	711976	12.6
28.03	ApppA	25100	2973793	52.7

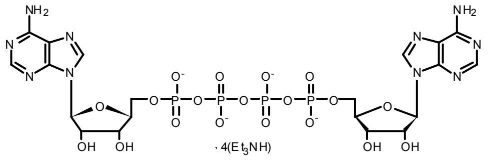

Ap3A
8.22
7.99
5.92
5.90
4.53
4.52
4.51
4.42
4.41
4.40
4.27
4.26
4.25
4.23
4.22
4.21
4.20
4.19
4.18
2.01
1.91
2.00
2.07
2.02
6.09
27.36
40.86
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


Ap3A
-11.72
-11.84
-23.12
-23.25
-23.37
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100
f1 (ppm)


Ap$_3$A
154.84
152.55
148.11
139.04
117.75
87.15
83.09
82.99
74.78
69.57
64.61
64.56
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

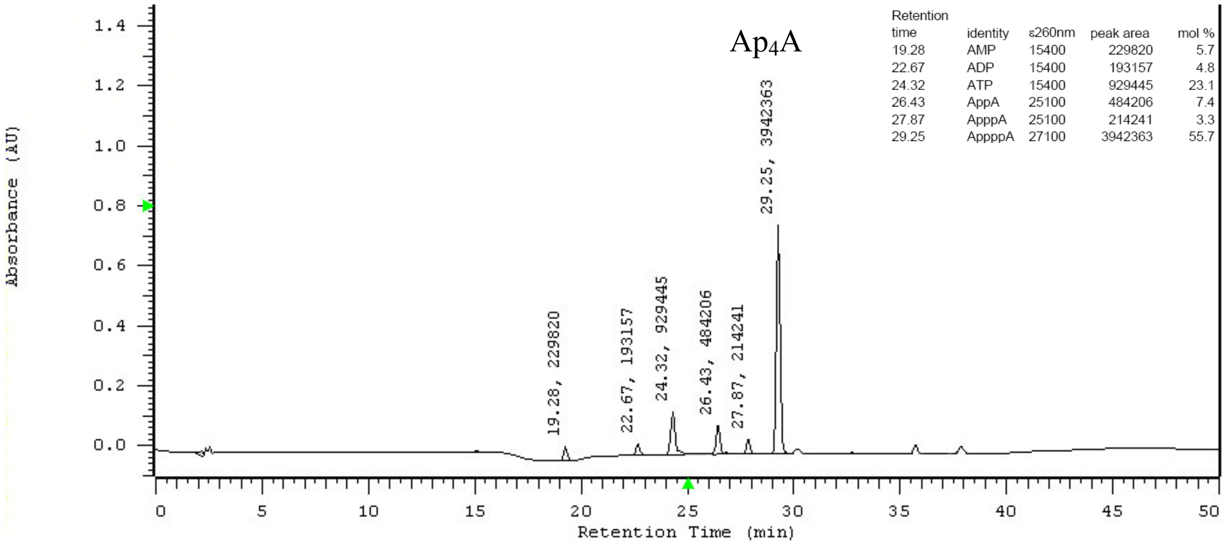

asep_16SEPT10_25 15 (0.356) Cm (14:31)
1: TOF MS ES+
1.99e4
Ap$_3$A
100
%
0
757.0906
758.0934
759.0950
760.1008
751.1253
756.0277
762.0187
764.5330
768.3794
771.3743
774.2714
m/z
750
752
754
756
758
760
762
764
766
768
770
772
774

P^1,P^4-di(adenosin-5'-yl)tetraphosphate tetrakis(triethylammonium) salt - ApppppA (**3c**)

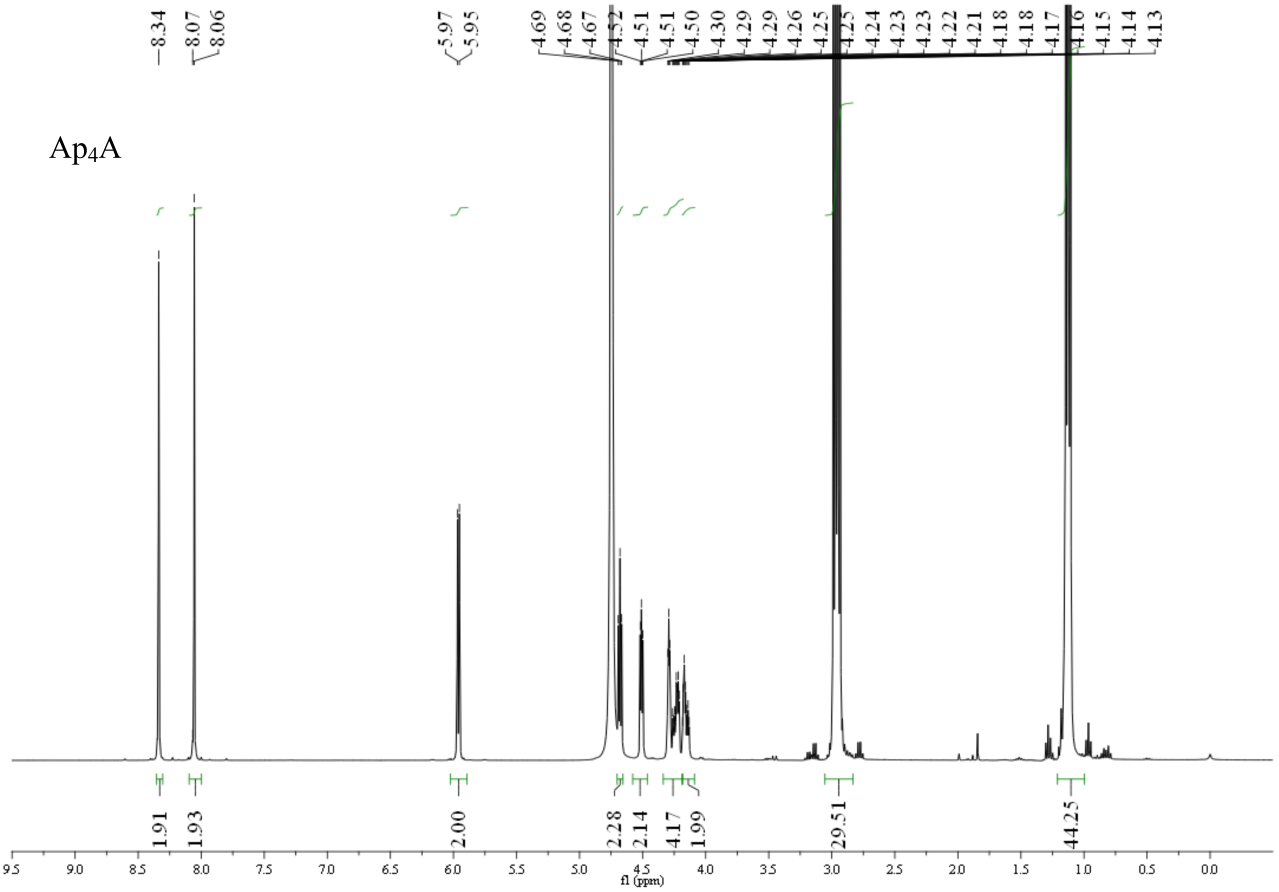


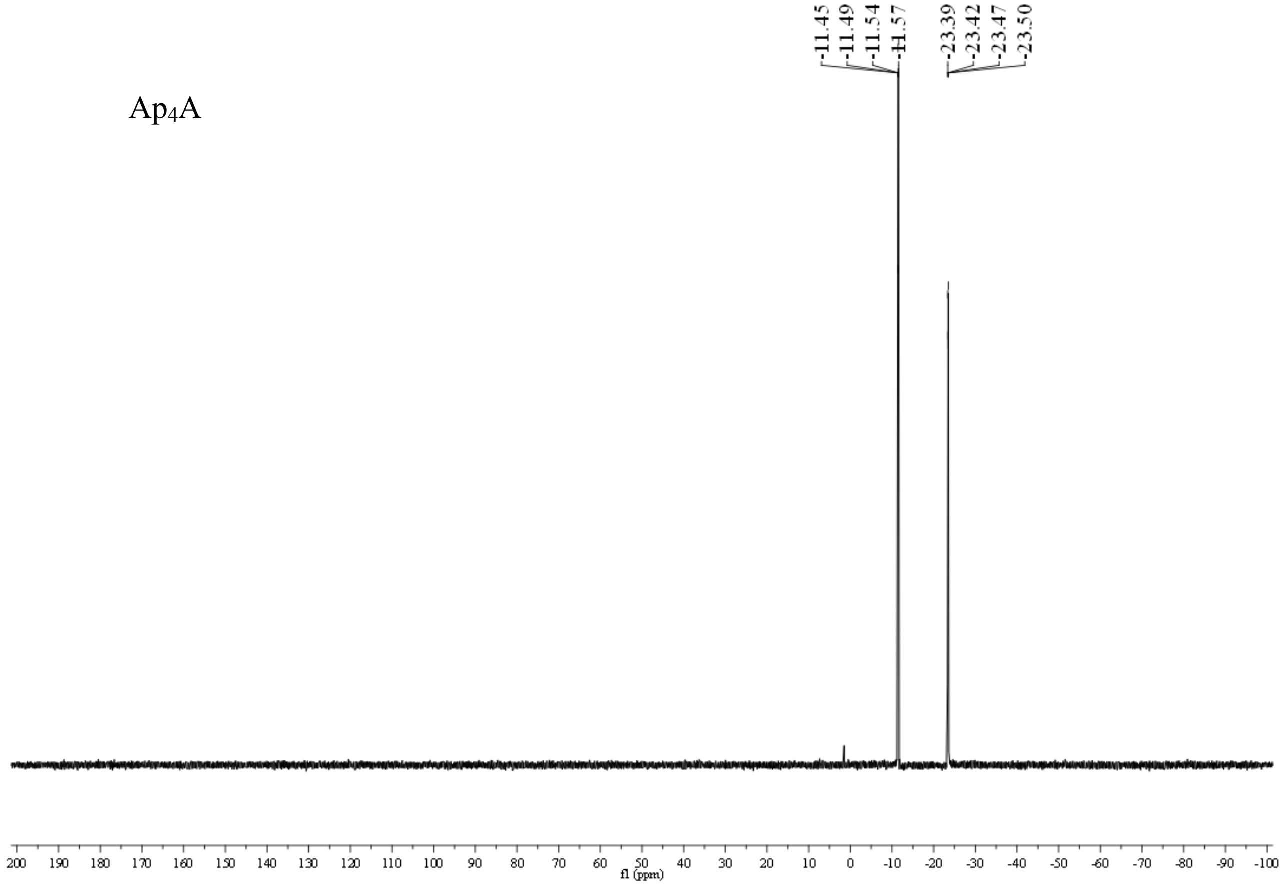
A stainless steel jar (25 mL) was charged with a mixture of adenosine-5'-phosphoromorpholidate 4-morpholine-*N*,*N*'-dicyclohexylcarboxamidine salt (**1**) (51 mg, 0.072 mmol), adenosine-5'-triphosphate (**2c**) (40 mg, 0.073 mmol, 1 eq.), $MgCl_2(H_2O)_6$ (22 mg, 0.11 mmol, 1.5 eq.), tetrazole (10 mg, 0.14 mmol, 2 eq.), H_2O (7.5 µL, 0.42 mmol, 6 eq.) and a 15.0 mm stainless steel ball. The vessel was vibrated at 30 Hz for 90 minutes and allowed to cool to room temperature. The reaction mixture was suspended in H_2O, analysed and then purified by C18 RP-HPLC – see general information. Appropriate fractions were lyophilised to yield pure **3c·(TEAB)$_{0.6}$** (50 mg, 0.037 mmol, 52 %). ^{1}H NMR(400 MHz, D_2O) δ = 8.34 (2H, s), 8.06 (2 H, s), 5.96 (2H, d, J = 5.9), 4.68 (2H, t, J = 4.5), 4.51 (2H, t, J = 4.5), 4.34 – 4.27 (2H, m), 4.27 – 4.20 (2H, m), 4.20 – 4.11 (2H, m), 2.96 (27.6H, q, J = 7.3 Hz), 1.13 (41.3H, t, J = 7.3 Hz); ^{13}C NMR (101 MHz, D_2O) δ = 155.10, 152.63, 148.69, 139.58, 118.00, 86.55, 83.89 (d, $^2J_{C,P}$ = 9.3), 74.36, 70.31, 65.14 (d, $^3J_{C,P}$ = 5.5), 46.29, 8.51; ^{31}P NMR (162 MHz, D_2O) δ = -11.51 (2 P_α, AA'XX'), -23.44 (2 P_β, AA'XX'); ES+ MS , ($C_{20}H_{29}N_{10}O_{19}P_4$) (M + H$^+$)calc. 837.0561, found 837.0557.

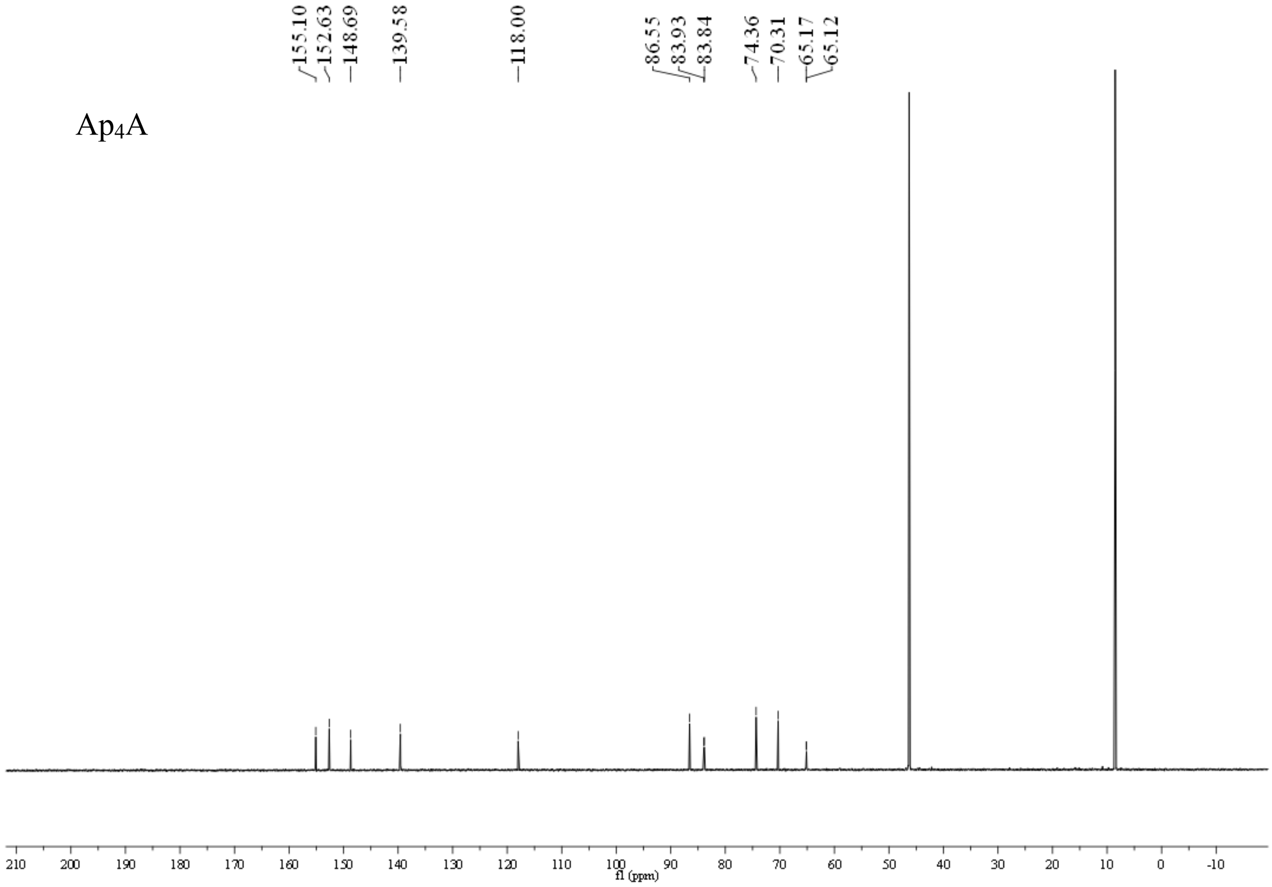
Analytical C18 RP-HPLC of crude reaction mixture

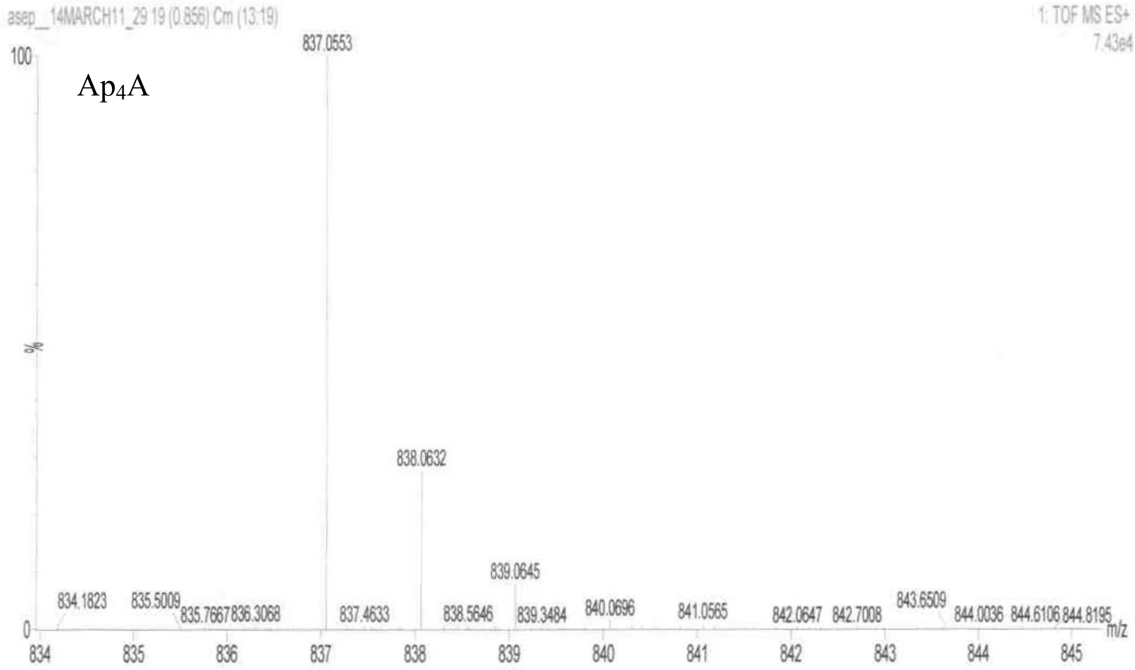


Retention time	identity	ε260nm	peak area	mol %
19.28	AMP	15400	229820	5.7
22.67	ADP	15400	193157	4.8
24.32	ATP	15400	929445	23.1
26.43	AppA	25100	484206	7.4
27.87	ApppA	25100	214241	3.3
29.25	ApppppA	27100	3942363	55.7

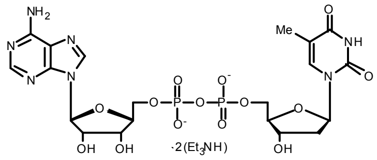

Ap4A
8.34
8.07
8.06
5.97
5.95
4.69
4.68
4.67
4.52
4.51
4.51
4.50
4.30
4.29
4.29
4.26
4.25
4.25
4.24
4.23
4.23
4.22
4.21
4.18
4.18
4.17
4.16
4.15
4.14
4.13
1.91
1.93
2.00
2.28
2.14
4.17
1.99
29.51
44.25
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Ap4A
-11.45
-11.49
-11.54
-11.57
-23.39
-23.42
-23.47
-23.50
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
f1 (ppm)


Ap$_4$A
155.10
152.63
148.69
139.58
118.00
86.55
83.93
83.84
74.36
70.31
65.17
65.12
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

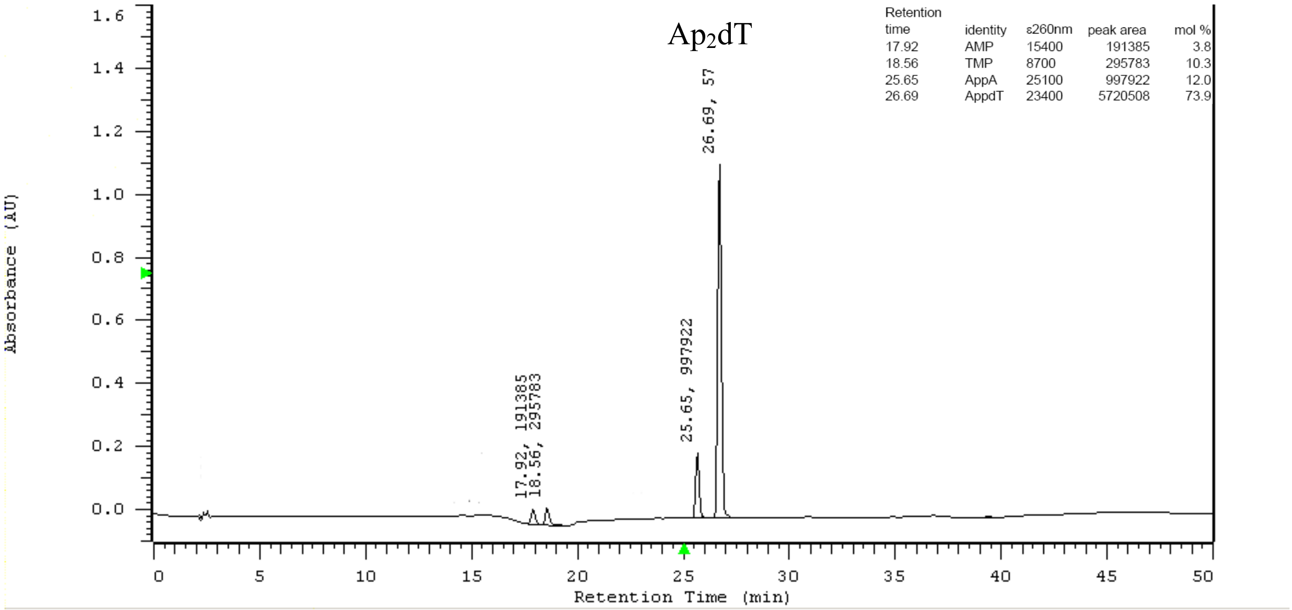

Ap$_4$A
asep__14MARCH11_29 19 (0.856) Cm (13:19)
1: TOF MS ES+
7.43e4
100
%
0
837.0553
838.0632
839.0645
834.1823
835.5009
835.7667
836.3068
837.4633
838.5646
839.3484
840.0696
841.0565
842.0647
842.7008
843.6509
844.0036
844.6106
844.8195
m/z
834
835
836
837
838
839
840
841
842
843
844
845

P^1-(adenosin-5'-yl)-P^2-(thymidin-5'''-yl) bis(triethylammonium) salt - AppdT (3d)

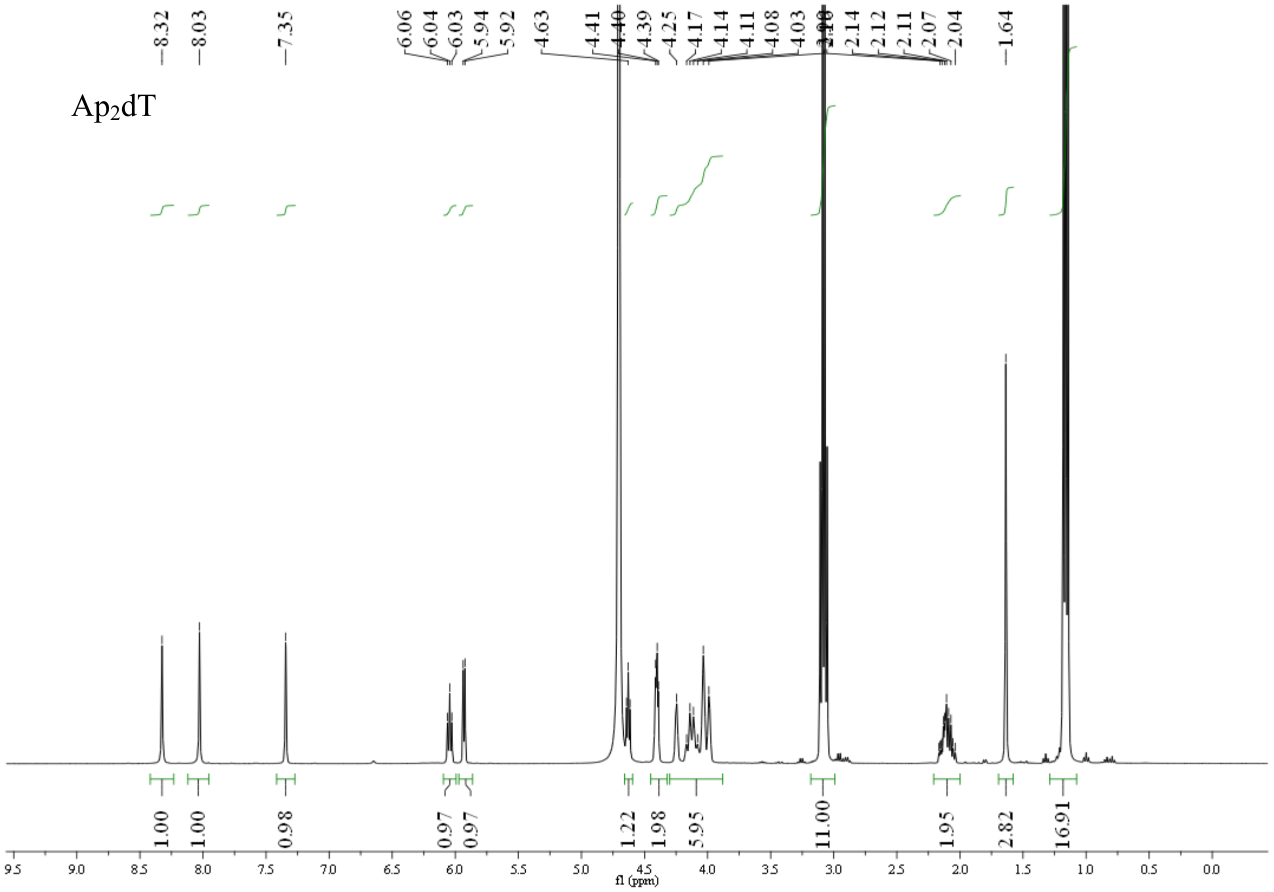


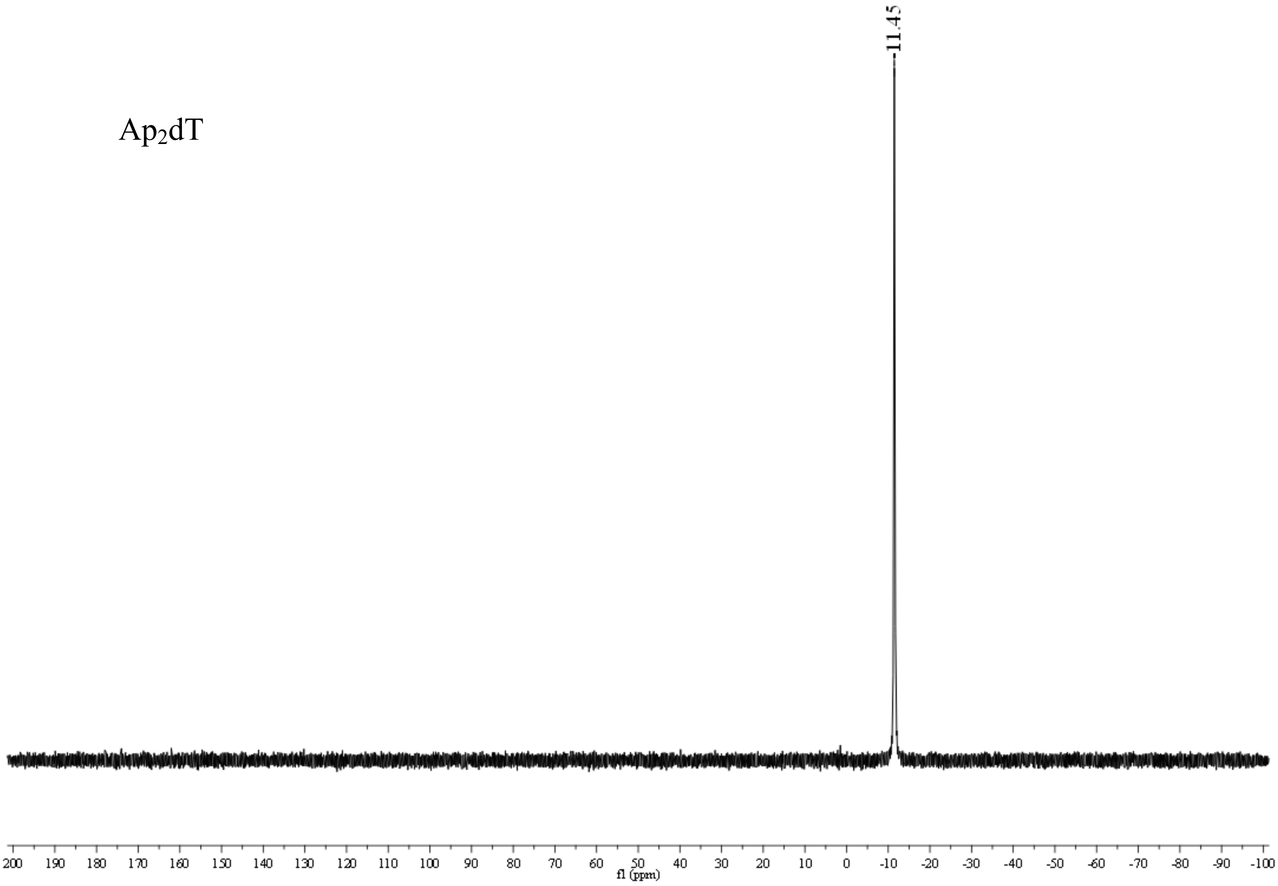
A stainless steel jar (25 mL) was charged with a mixture of adenosine-5'-phosphoromorpholidate 4-morpholine- *N*,*N'*- dicyclohexylcarboxamidine salt (**1**) (100 mg, 0.14 mmol), thymidine-5'-monophosphate (**2d**) (52 mg, 0.14 mmol, 1 eq.), $MgCl_2(H_2O)_6$ (43 mg, 0.21 mmol, 1.5 eq.), tetrazole (20 mg, 0.28 mmol, 2 eq.), H_2O (15 µL, 0.83 mmol, 6 eq.) and a 15.0 mm stainless steel ball. The vessel was vibrated at 30 Hz for 90 minutes and allowed to cool to room temperature. The reaction mixture was suspended in H_2O, analysed and then purified by C18 RP-HPLC – see general information. Appropriate fractions were lyophilised to yield pure **3d** (86 mg, 0.10 mmol, 71 %). ^{1}H NMR (400 MHz, D_2O) δ = 8.32 (1H, s), 8.03 (1H, s), 7.35 (1H, s), 6.04 (1H, t, J = 6.9), 5.93 (1H, d, J = 5.8), 4.63 (1H, t, J = 5.5), 4.45 – 4.32 (2H, m), 4.30 – 3.88 (6H, m), 2.85 (12H, q, J = 7.3Hz), 2.21 – 2.00 (2H, m), 1.64 (3H, s), 1.06 (18H, t, J = 7.3 Hz); ^{13}C NMR (101 MHz, D_2O) δ = 166.02, 155.30, 152.65, 151.25,148.84, 139.66, 136.95, 118.31, 111.22, 86.69, 85.22 (d, $^2J_{C,P}$ = 8.9), 84.65, 83.67 (d, $^2J_{C,P}$ = 9.1), 74.14, 70.84, 70.24, 65.38 (d, $^3J_{C,P}$ = 4.5), 65.26 (d, $^3J_{C,P}$ = 4.6), 38.65, 11.59; ^{31}P NMR (162 MHz, D_2O) δ = -11.45; ES+ MS ($C_{32}H_{57}N_9O_{14}P_2$) (M + 2Et$_3$NH$^+$), calc. 855.3657, found 855.3649, ($C_{26}H_{43}N_8O_{14}P_2$) (M + Et$_3$NH$^+$), calc. 753.2374, found 753.2375.

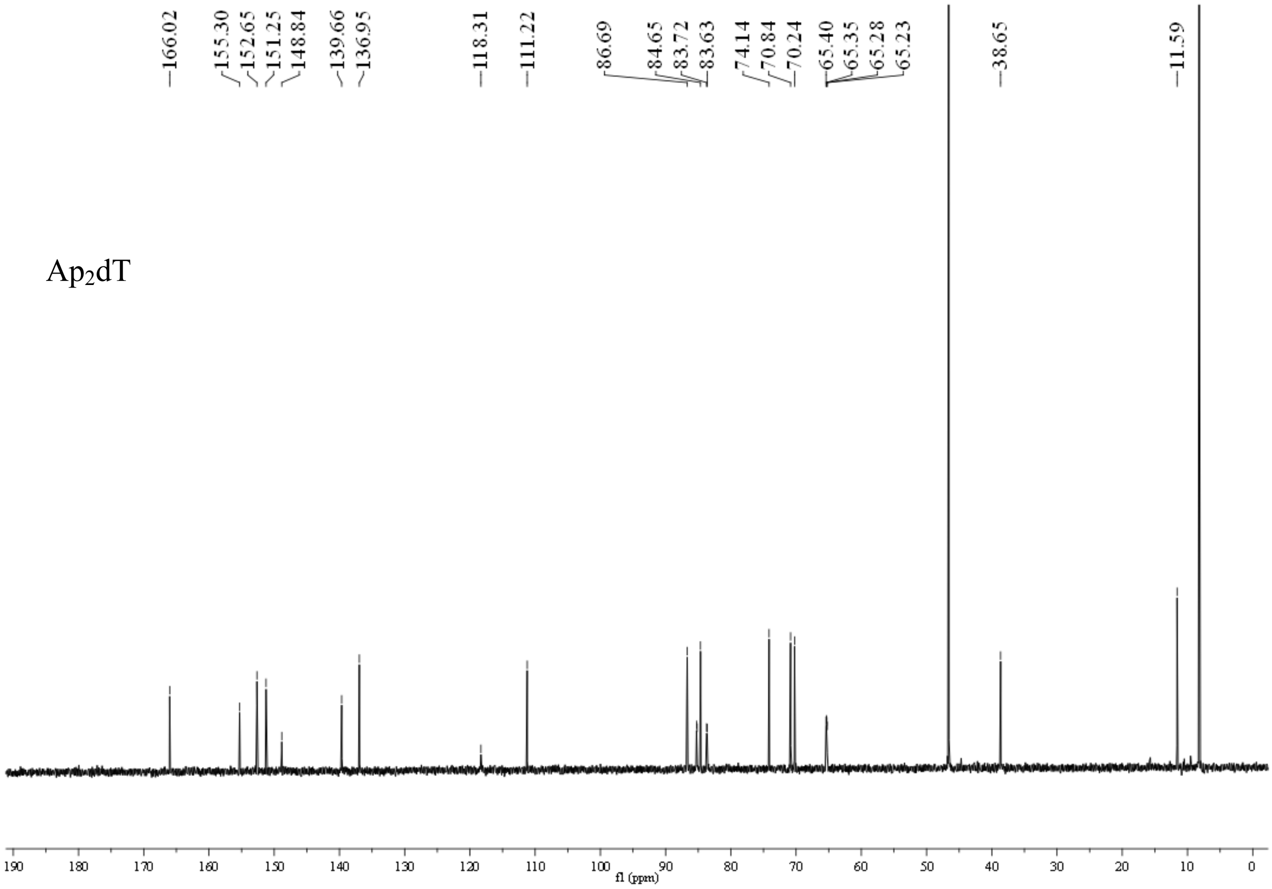
Analytical C18 RP-HPLC of crude reaction mixture

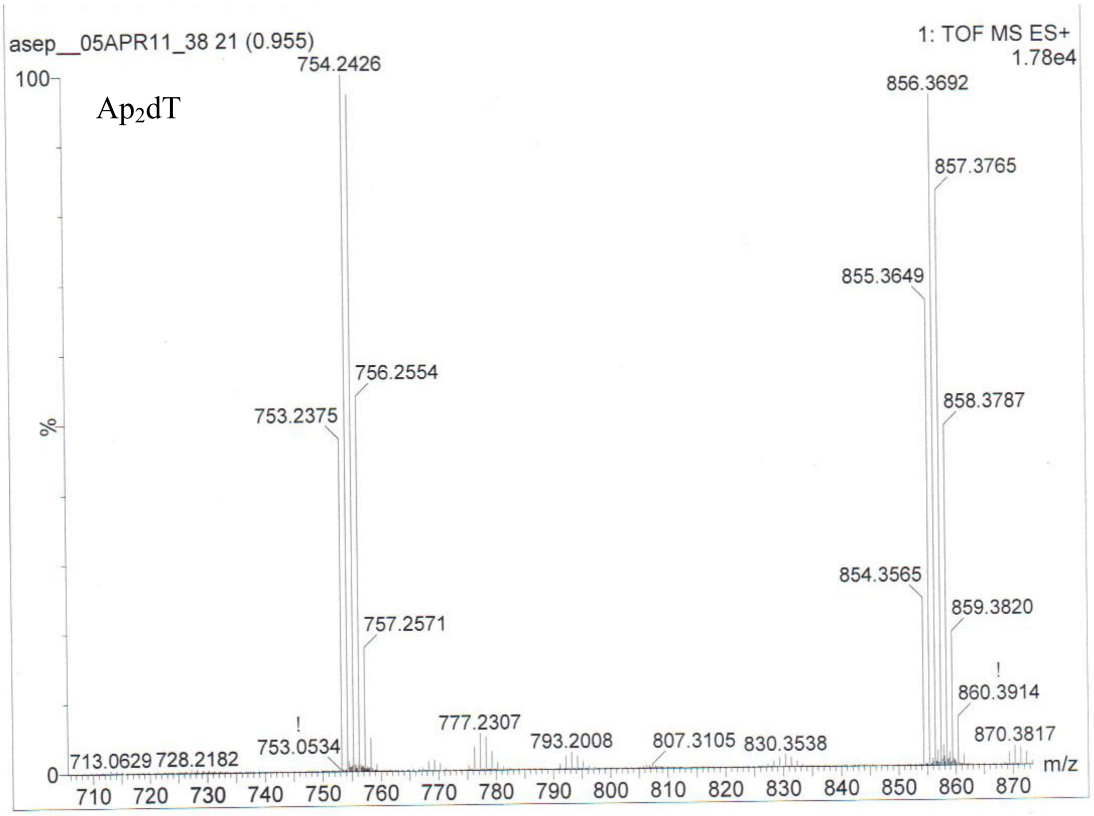


Retention time	identity	ε260nm	peak area	mol %
17.92	AMP	15400	191385	3.8
18.56	TMP	8700	295783	10.3
25.65	AppA	25100	997922	12.0
26.69	AppdT	23400	5720508	73.9

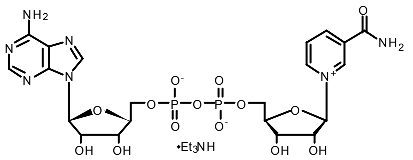

Ap$_2$dT
8.32
8.03
7.35
6.06
6.04
6.03
5.94
5.92
4.63
4.41
4.40
4.39
4.25
4.17
4.14
4.11
4.08
4.03
2.14
2.12
2.11
2.07
2.04
1.64
1.00
1.00
0.98
0.97
0.97
1.22
1.98
5.95
11.00
1.95
2.82
16.91
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Ap$_2$dT
-11.45
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
f1 (ppm)


166.02
155.30
152.65
151.25
148.84
139.66
136.95
118.31
111.22
86.69
84.65
83.72
83.63
74.14
70.84
70.24
65.40
65.35
65.28
65.23
38.65
11.59
Ap2dT
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

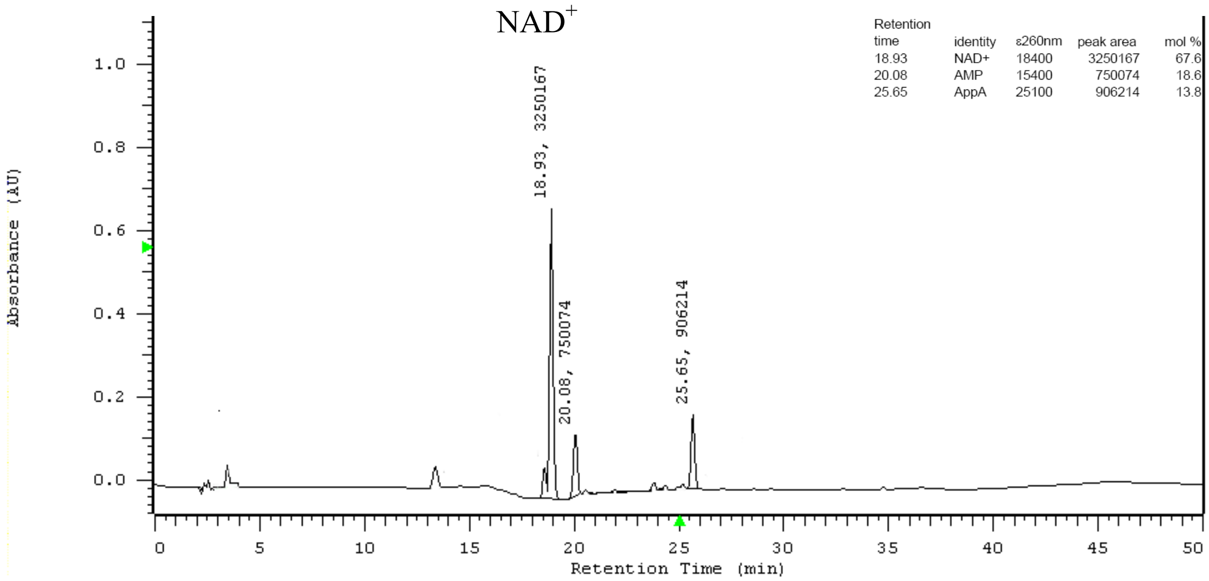

asep__05APR11_38 21 (0.955)
1: TOF MS ES+
1.78e4
Ap2dT
100
%
0
754.2426
753.2375
756.2554
757.2571
753.0534
713.0629
728.2182
777.2307
793.2008
807.3105
830.3538
856.3692
857.3765
855.3649
858.3787
854.3565
859.3820
860.3914
870.3817
m/z
710
720
730
740
750
760
770
780
790
800
810
820
830
840
850
860
870

β-Nicotinamide adenine dinucleotide triethylammonium salt - β-NAD$^+$ (3e)

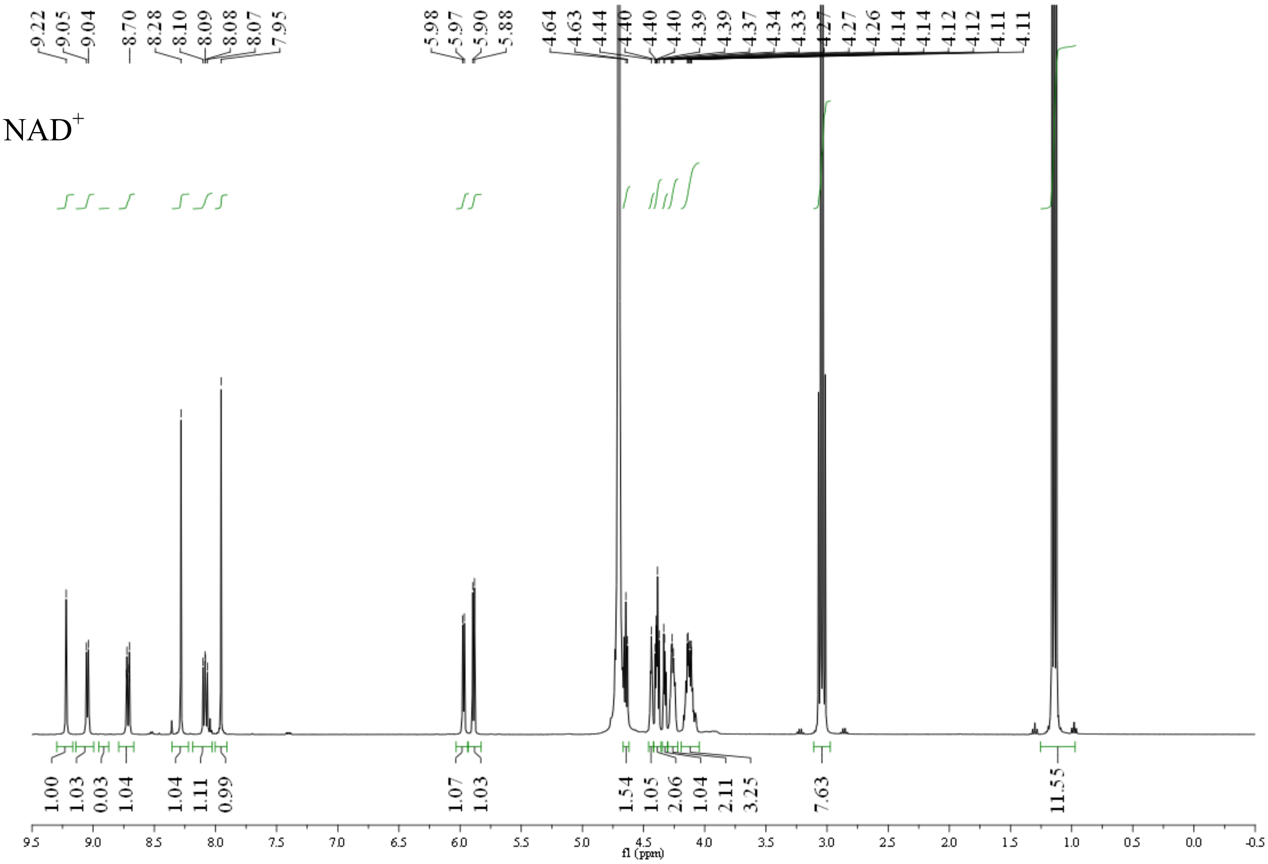


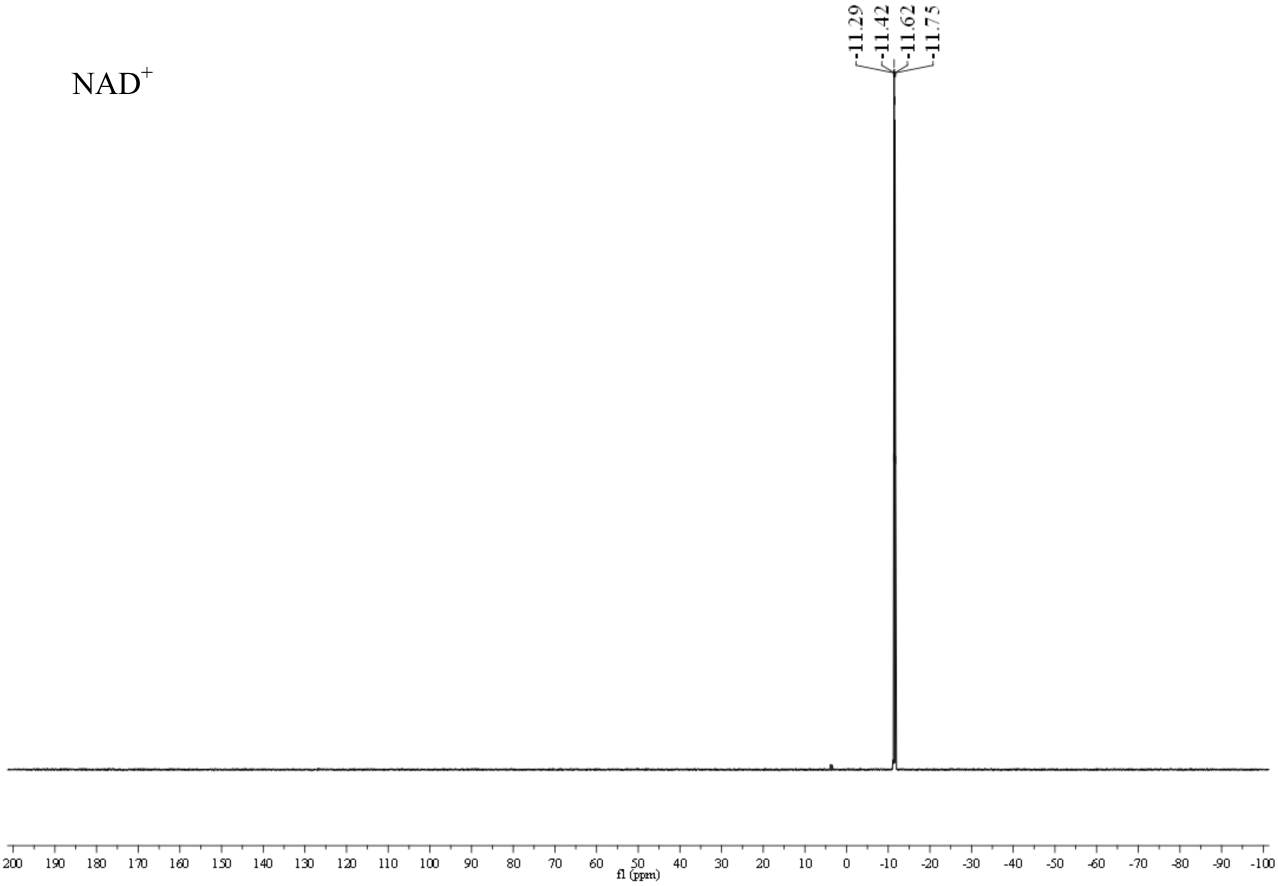
A stainless steel jar (25 mL) was charged with a mixture of adenosine-5'-phosphoromorpholidate 4-morpholine-*N*,*N'*-dicyclohexylcarboxamidine salt (**1**) (99 mg, 0.14 mmol), β-nicotinamide-5'-monophosphate (**2e**) (47 mg, 0.14 mmol, 1 eq.), $MgCl_2(H_2O)_6$ (43 mg, 0.21 mmol, 1.5 eq.), tetrazole (20 mg, 0.29 mmol, 2 eq.), H_2O (15 µL, 0.83 mmol, 6 eq.) and a 15.0 mm stainless steel ball. The vessel was vibrated at 30 Hz for 90 minutes and allowed to cool to room temperature. The reaction mixture was suspended in H_2O, analysed and then purified by C18 RP-HPLC – see general information. Appropriate fractions were lyophilised to yield **3e·(TEAB)$_{0.2}$** (65 mg, 0.081 mmol, 58 %). ^{1}H NMR (400 MHz, D_2O) δ = 9.22 (1H, s), 9.05 (1H, d, J = 6.3), 8.71 (1 H, d, J = 8.2), 8.28 (1 H, s), 8.17 – 8.00 (1H, m), 7.96 (1H, d, J = 5.7), 5.97 (1H, d, J = 5.4), 5.89 (1H, d, J = 6.0), 4.67 – 4.62 (2H, m), 4.46 – 4.42 (1H, m), 4.42 – 4.36 (2H, m), 4.35 – 4.31 (1H, m), 4.30 – 4.22 (2H, m), 4.19 – 4.05 (3H, m), 3.04 (7.3H, q, J = 7.3Hz), 1.14 (11H, t, J = 7.3Hz); ^{13}C NMR (101 MHz, D_2O) δ = 165.10, 155.16, 152.66, 148.77, 145.69, 142.33, 139.83, 139.62, 133.57, 128.56, 118.17, 99.98, 86.99 (d, $^2J_{C,P}$ = 8.7), 86.60, 83.79 (d, $^2J_{C,P}$ = 8.9), 77.57, 73.92, 70.59, 70.34, 65.35 (d, $^2J_{C,P}$ = 5.2), 64.86 (d, $^3J_{C,P}$ = 5.1); ^{31}P NMR (162 MHz, D_2O) δ = -11.56, -11.69 (2 P, ABq, J = 20.8). ES+ MS ($C_{21}H_{28}N_7O_{14}P_2$), (M$^+$), calc. 664.1170, found 664.1141.

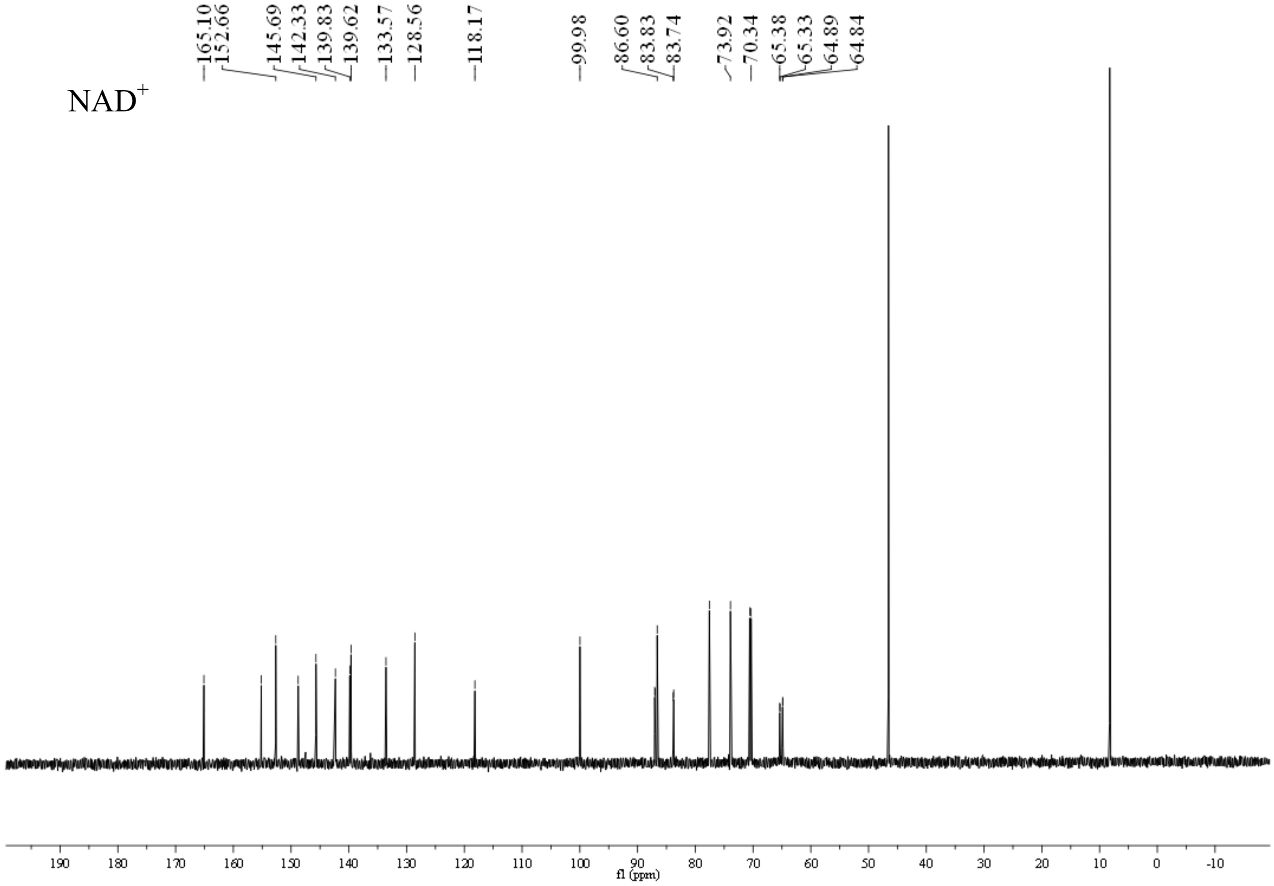
Analytical C18 RP-HPLC of crude reaction mixture

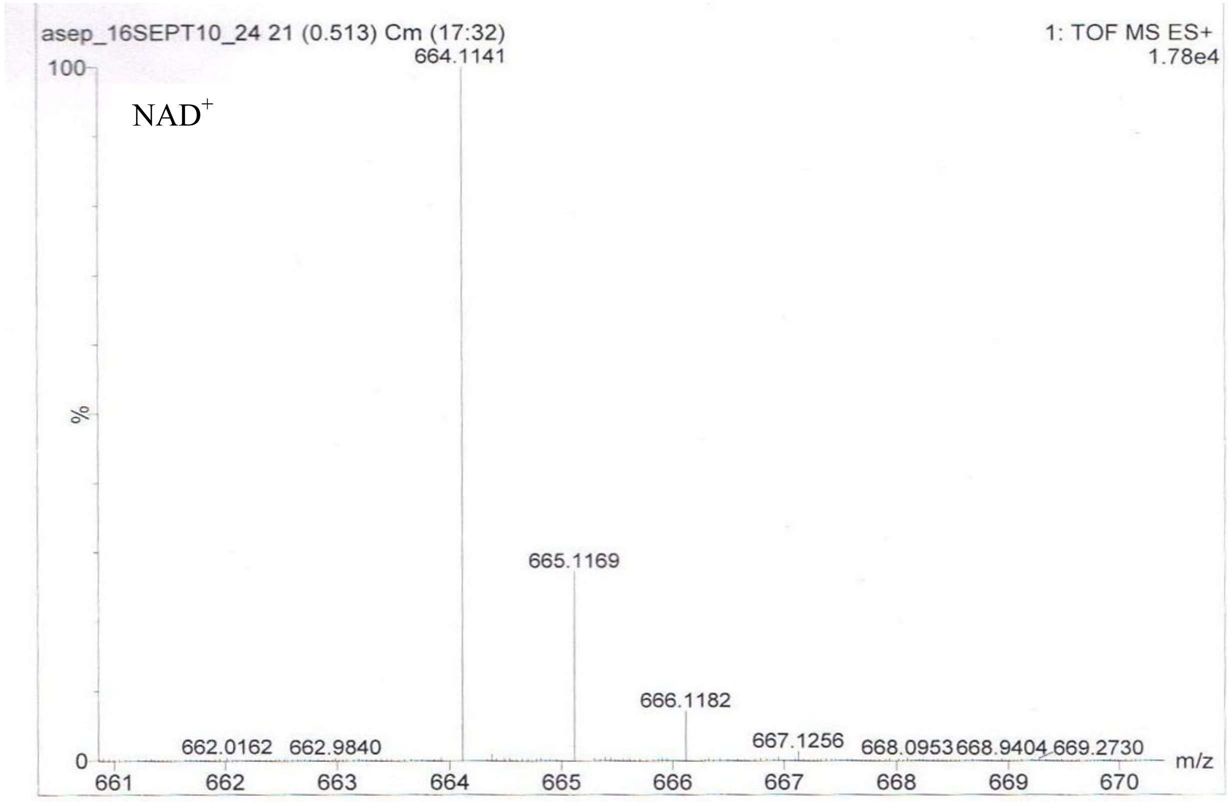


Retention time	identity	ε260nm	peak area	mol %
18.93	NAD+	18400	3250167	67.6
20.08	AMP	15400	750074	18.6
25.65	AppA	25100	906214	13.8

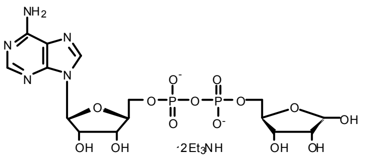

NAD+
9.22
9.05
9.04
8.70
8.28
8.10
8.09
8.08
8.07
7.95
5.98
5.97
5.90
5.88
4.64
4.63
4.44
4.40
4.40
4.40
4.39
4.39
4.37
4.34
4.33
4.27
4.27
4.26
4.14
4.14
4.12
4.12
4.11
4.11
1.00
1.03
0.03
1.04
1.04
1.11
0.99
1.07
1.03
1.54
1.05
2.06
1.04
2.11
3.25
7.63
11.55
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


NAD+
-11.29
-11.42
-11.62
-11.75
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
f1 (ppm)


NAD$^+$
165.10
152.66
145.69
142.33
139.83
139.62
133.57
128.56
118.17
99.98
86.60
83.83
83.74
73.92
70.34
65.38
65.33
64.89
64.84
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

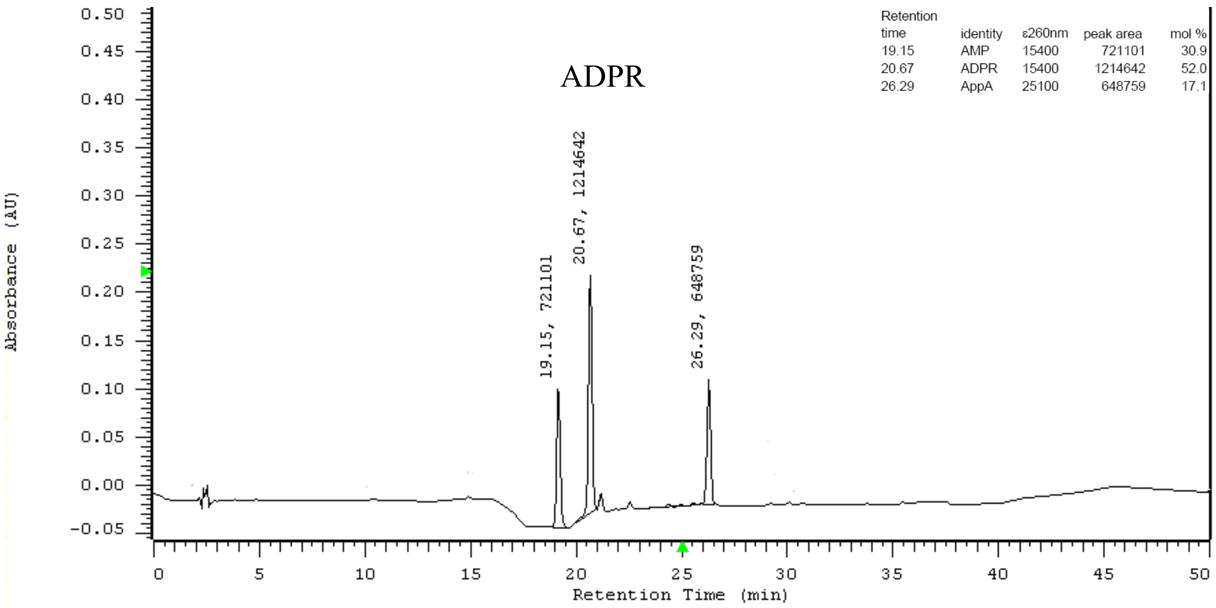

asep_16SEPT10_24 21 (0.513) Cm (17:32)
1: TOF MS ES+
1.78e4
NAD$^+$
100
%
0
664.1141
665.1169
666.1182
662.0162
662.9840
667.1256
668.0953
668.9404
669.2730
m/z
661
662
663
664
665
666
667
668
669
670

P^1-(adenosin-5'-yl)-P^2-(ribos-5''-yl) bis(triethylammonium) salt - ADPR (3f)



A stainless steel jar (25 mL) was charged with a mixture of adenosine-5'-phosphoromorpholidate 4-morpholine-*N*,*N'*-dicyclohexylcarboxamidine salt (**1**) (52 mg, 0.073 mmol), ribose-5-monophosphate (**2f**) (35 mg, 0.074 mmol, 1 eq.), $MgCl_2(H_2O)_6$ (23 mg, 1.1 mmol, 1.5 eq.), tetrazole (10 mg, 0.14 mmol, 2 eq.), H_2O (7.5 µL, 0.42 mmol, 6 eq.) and a 15.0 mm stainless steel ball. The vessel was vibrated at 30 Hz for 90 minutes and allowed to cool to room temperature. The reaction mixture was suspended in H_2O, analysed and then purified by C18 RP-HPLC – see general information. Appropriate fractions were lyophilised to yield pure **6c** (24 mg, 0.030 mmol, 43 %). ^{1}H NMR (500 MHz, D_2O) δ = 8.40 (1 H, s), 8.12 (1 H, s), 6.02 (1H, dd, *J* = 5.9, 1.7), 5.20 (0.37H, d, *J* = 4.1), 5.09 (0.63H, d, *J* =2.2), 4.67 – 4.63 (1H, m), 4.49 – 4.36 (1 H, m), 4.27 (1 H, brs), 4.21 – 4.15 (0.37 H, m), 4.11 (2 H, s), 4.07 – 4.03 (0.63 H, m), 4.03 – 3.81 (4 H, m); ^{13}C NMR (101 MHz, D_2O) δ = 155.51, 152.27, 139.82, 101.12, 96.33, 86.76, 83.93 (d, $^2J_{C,P}$ = 9.0), 81.07 (d, $^2J_{C,P}$ = 8.9) 75.12, 74.24, 70.66, 70.40, 69.98, 66.29 (d, $^3J_{C,P}$ = 4.7), 65.20; ^{31}P NMR (162 MHz, D_2O) δ = -11.34; ES+ MS ($C_{15}H_{24}N_5O_{14}P_2$) (M + H$^+$), calc. 560.0795, found 560.0796, ($C_{21}H_{39}N_6O_{14}P_2$) (M + TEA$^+$), calc. 661.2000, found 661.1995.

Analytical C18 RP-HPLC of crude reaction mixture



Retention time	identity	ε260nm	peak area	mol %
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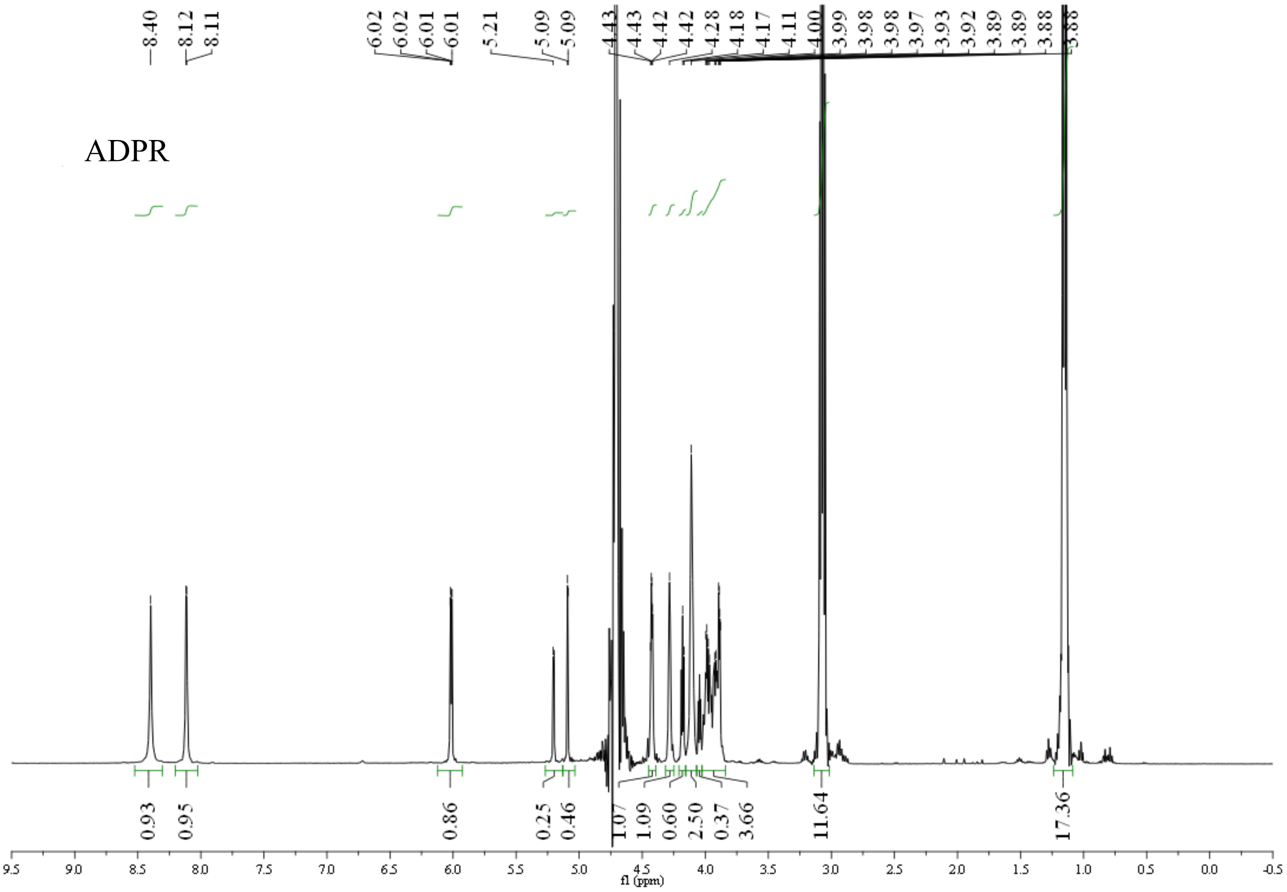
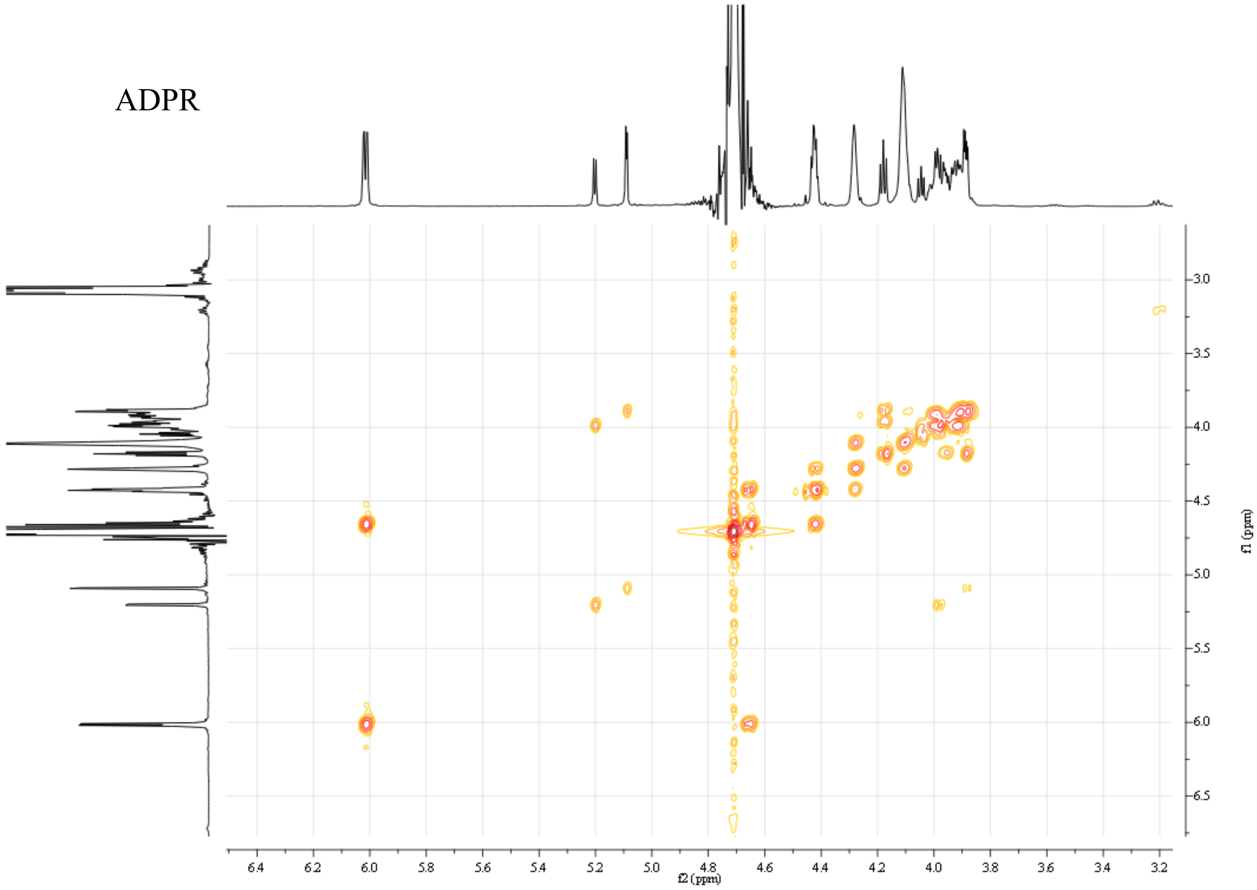
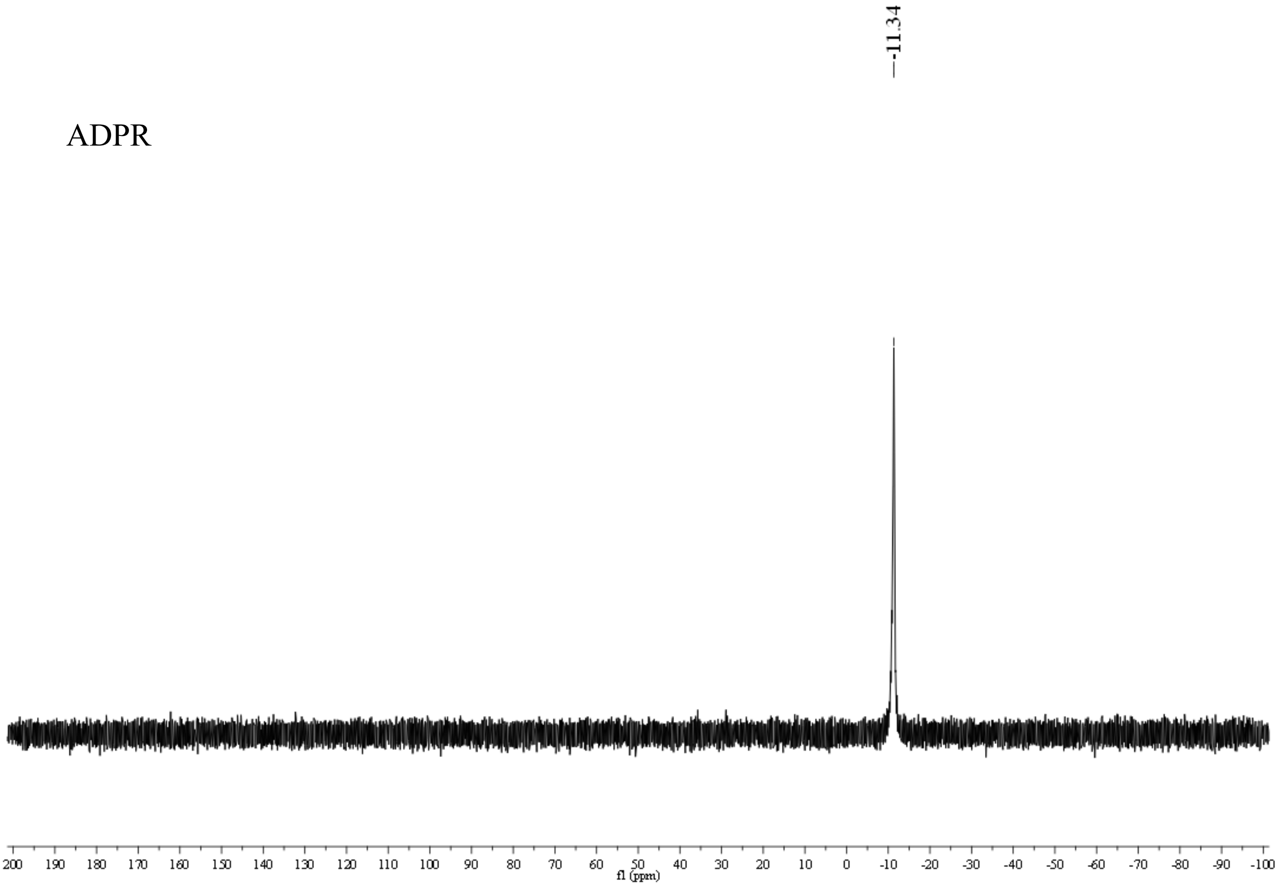
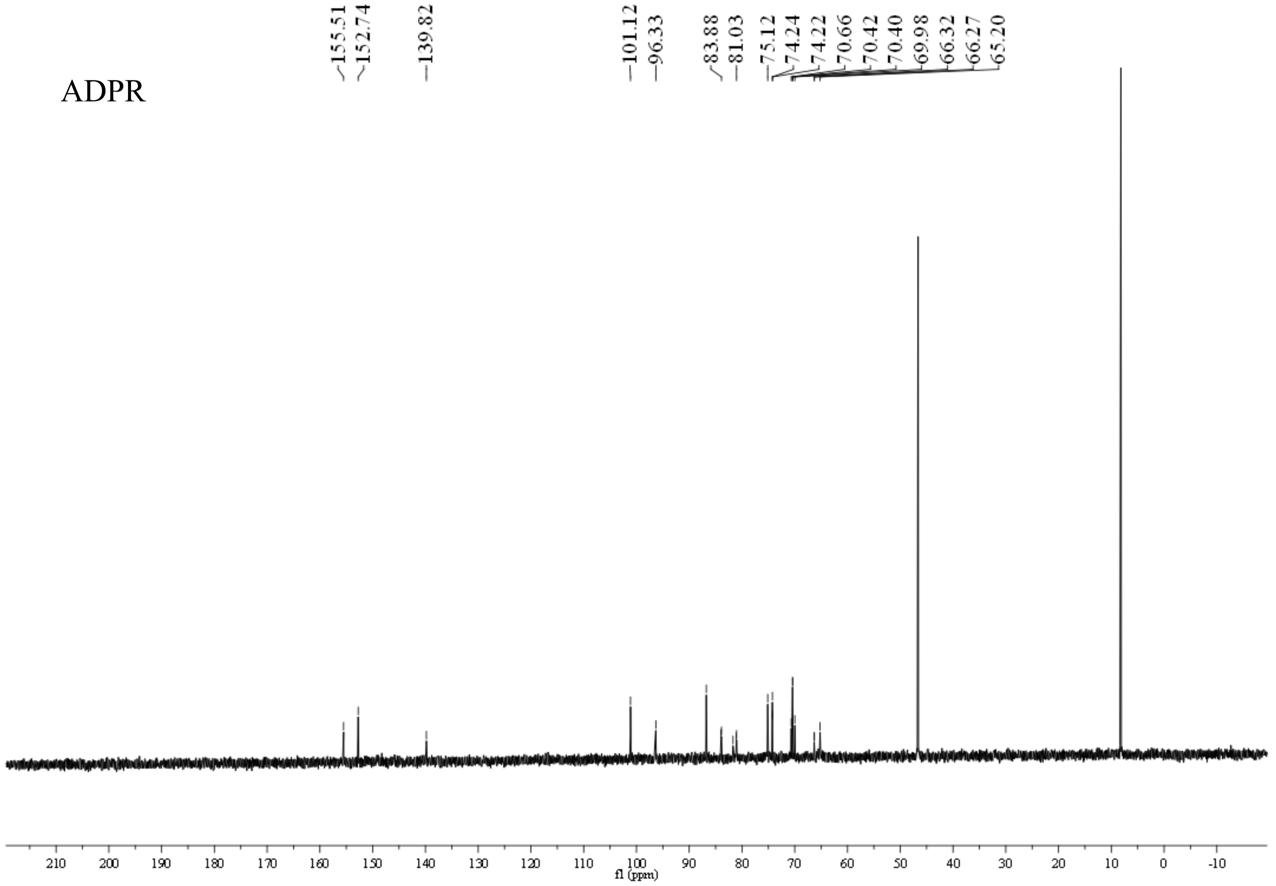
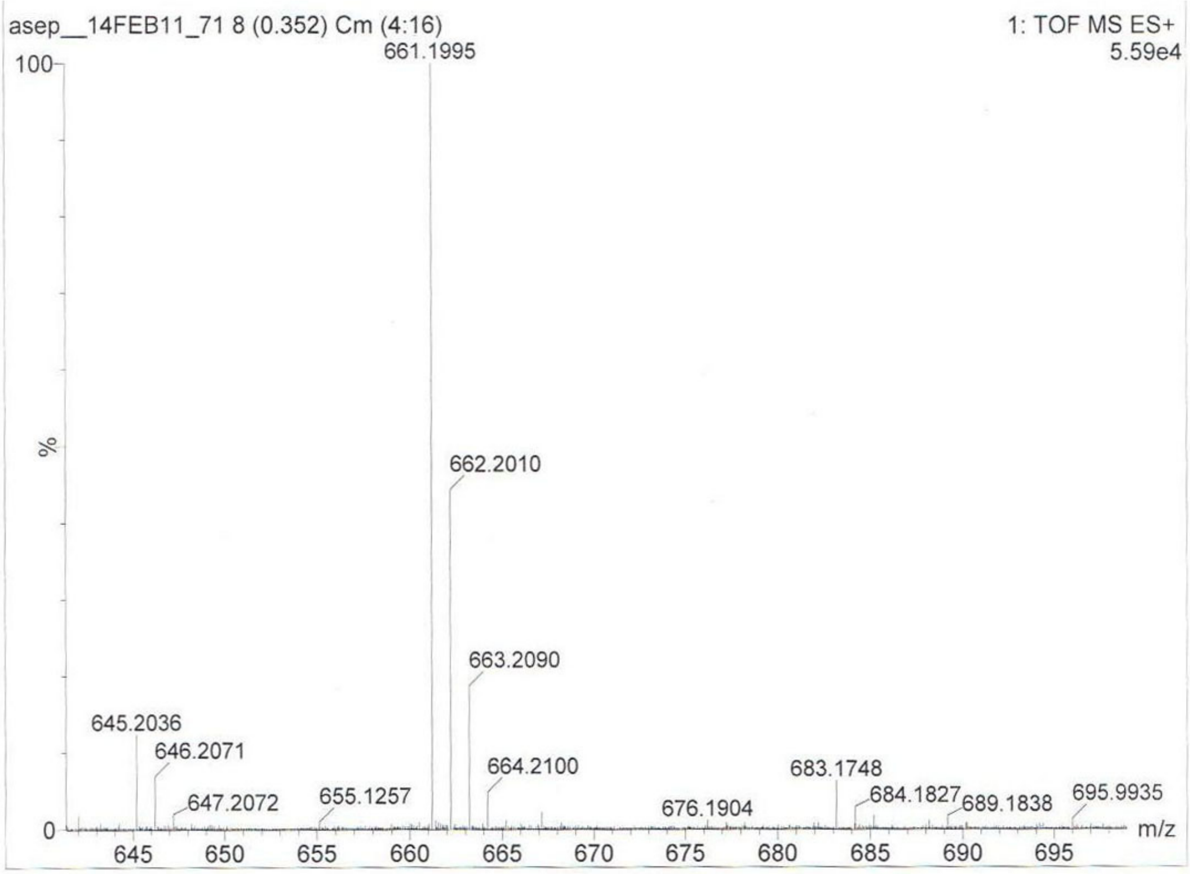
20.67	ADPR	15400	1214642	52.0
26.29	AppA	25100	648759	17.1


ADPR
8.40
8.12
8.11
6.02
6.02
6.01
6.01
5.21
5.09
5.09
4.43
4.43
4.42
4.42
4.28
4.18
4.17
4.11
4.00
3.99
3.98
3.98
3.97
3.93
3.92
3.89
3.89
3.88
3.88
0.93
0.95
0.86
0.25
0.46
1.07
1.09
0.60
2.50
0.37
3.66
11.64
17.36
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


ADPR
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
f2 (ppm)
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
f1 (ppm)


-11.34
ADPR
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
f1 (ppm)


155.51
152.74
139.82
101.12
96.33
83.88
81.03
75.12
74.24
74.22
70.66
70.42
70.40
69.98
66.32
66.27
65.20
ADPR
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)



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2. N. Russo and R. Shapiro, *J. Biol. Chem.*, 1999, **274**, 14902-14908.
3. E. Holler, B. Holmquist, B. L. Vallee, K. Taneja and P. Zamecnik, *Biochemistry*, 1983, **22**, 4924-4933.
4. J. C. Austin, C. W. Wharton and R. E. Hester, *Biochemistry*, 1989, **28**, 1533-1538.