

Assessing Enzyme-Catalysed Phosphorylation of
Nucleosides to Aid Synthetic Approaches to Novel
Antiviral Nucleotides: **Appendices**

By

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A-I.1 Optimisation of Component Concentrations

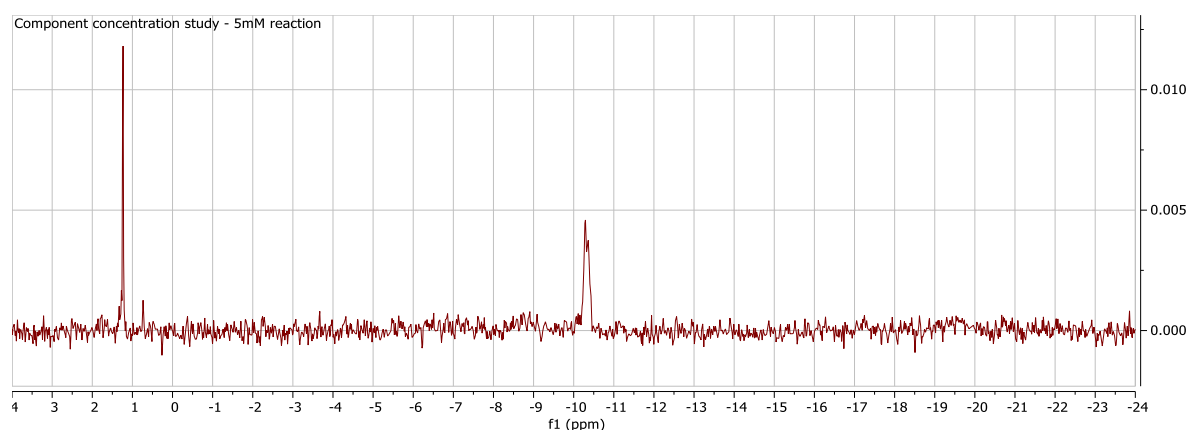


Figure A-I.1: ^{31}P NMR spectra of 5 mM reaction of component concentration study. Uridine 5 mM. ATP 5 mM. Process described in Section 4.4. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.2.

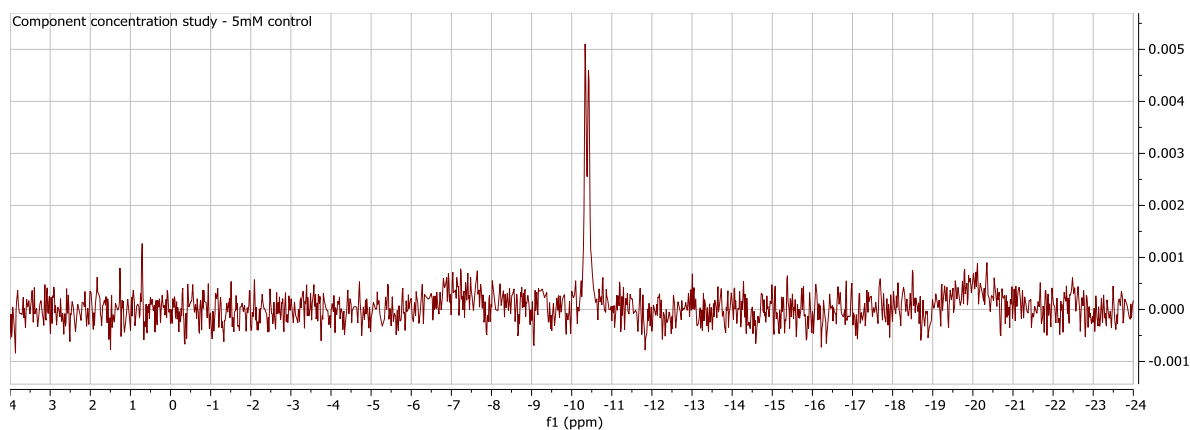


Figure A-I.2: ^{31}P NMR spectra of 5 mM control of component concentration study. Uridine 0 mM. ATP 5 mM. Process described in Section 4.4. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.2.

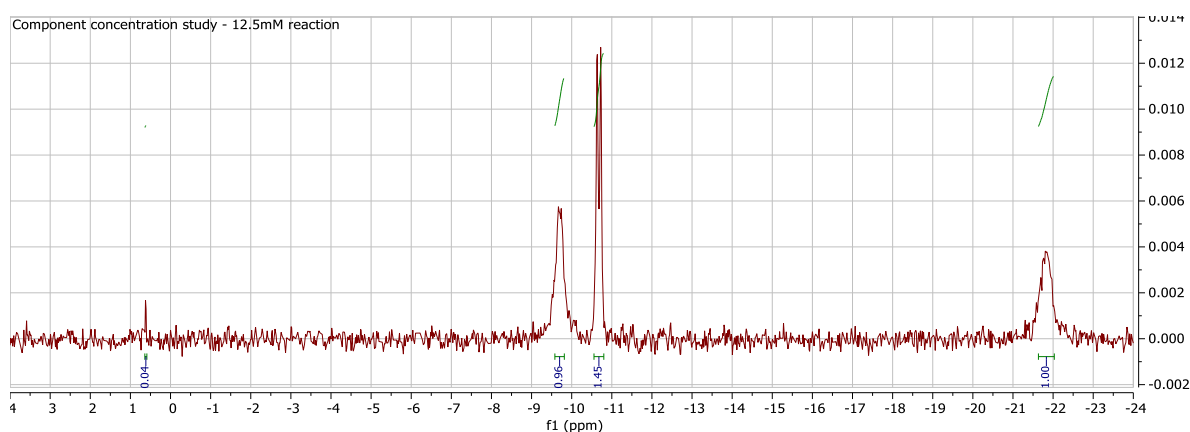


Figure A-I.3: ^{31}P NMR spectra of 12.5 mM reaction of component concentration study. Uridine 12.5 mM. ATP 12.5 mM. Process described in Section 4.4. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.2.

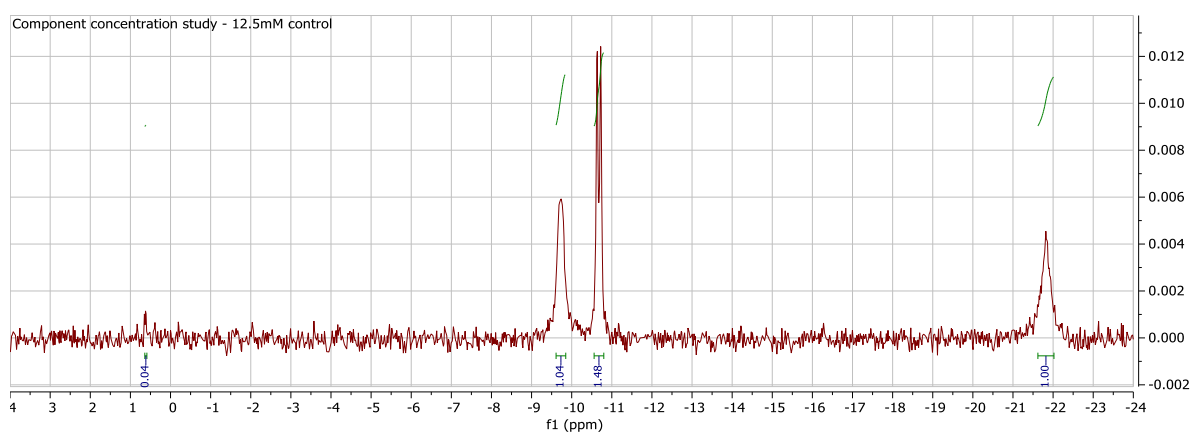


Figure A-I.4: ^{31}P NMR spectra of 12.5 mM control of component concentration study. Uridine 0 mM. ATP 12.5 mM. Process described in Section 4.4. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.2.

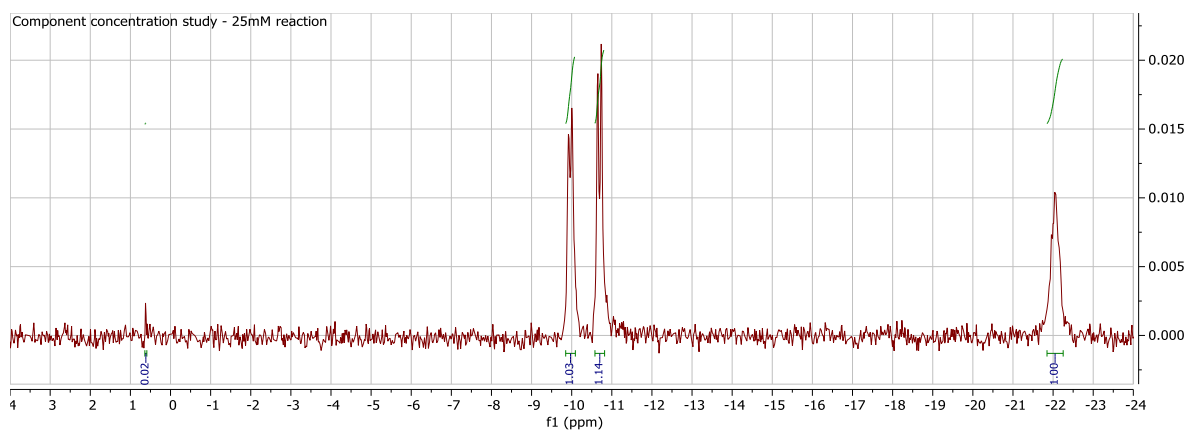


Figure A-I.5: ^{31}P NMR spectra of 25 mM reaction of component concentration study. Uridine 25 mM. ATP 25 mM. Process described in Section 4.4. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.2.

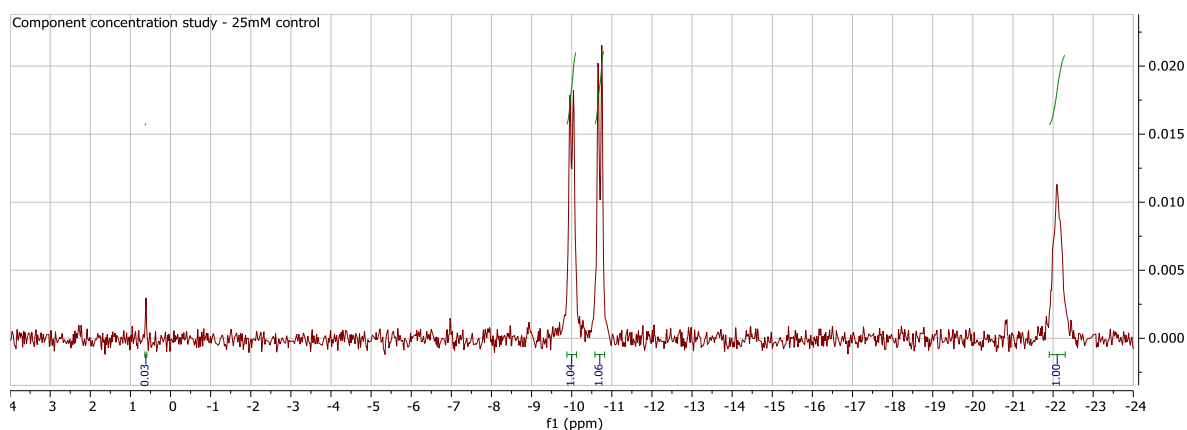


Figure A-I.6: ^{31}P NMR spectra of 25 mM control of component concentration study. Uridine 0 mM. ATP 25 mM. Process described in Section 4.4. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.2.

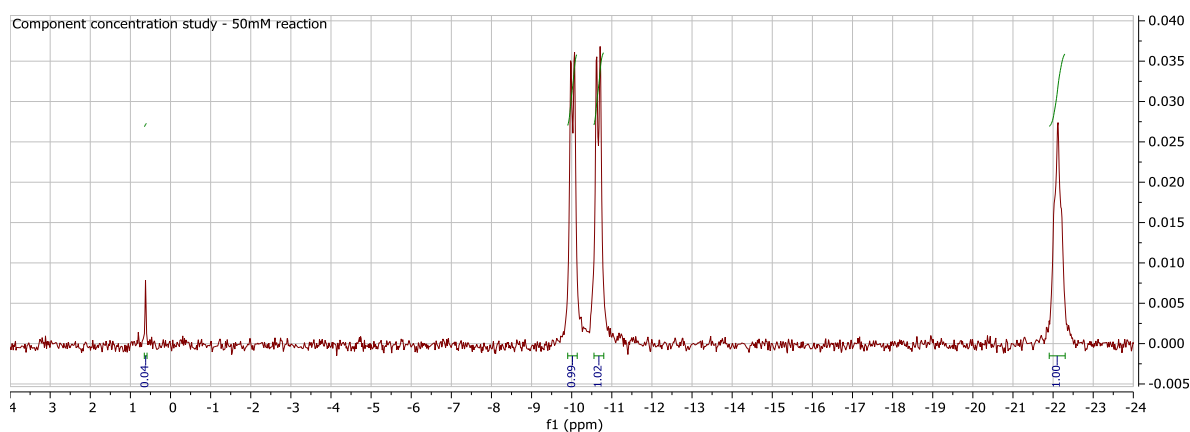


Figure A-I.7: ^{31}P NMR spectra of 50 mM reaction of component concentration study. Uridine 50 mM. ATP 50 mM. Process described in Section 4.4. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.2.

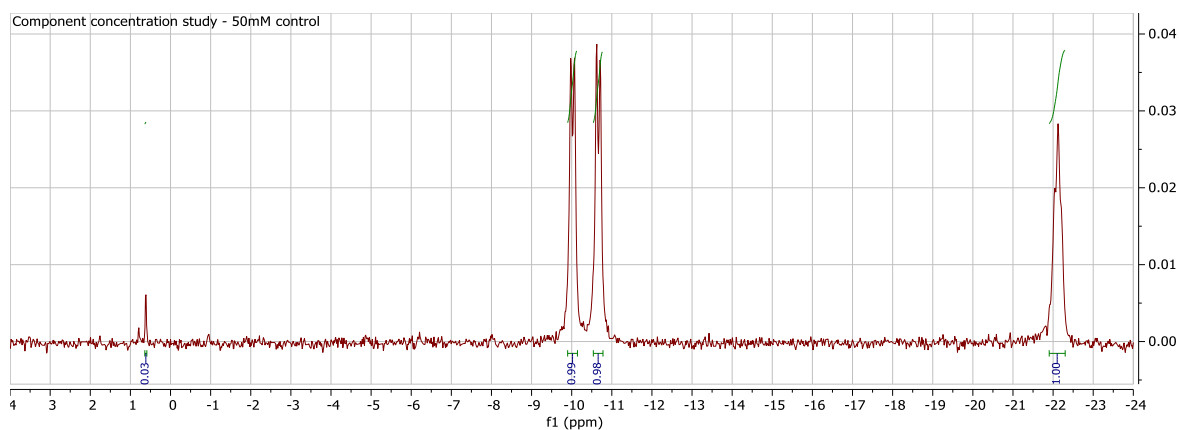


Figure A-I.8: ^{31}P NMR spectra of 50 mM reaction of component concentration study. Uridine 0 mM. ATP 50 mM. Process described in Section 4.4. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.2.

A-I.2 Reaction Duration Study

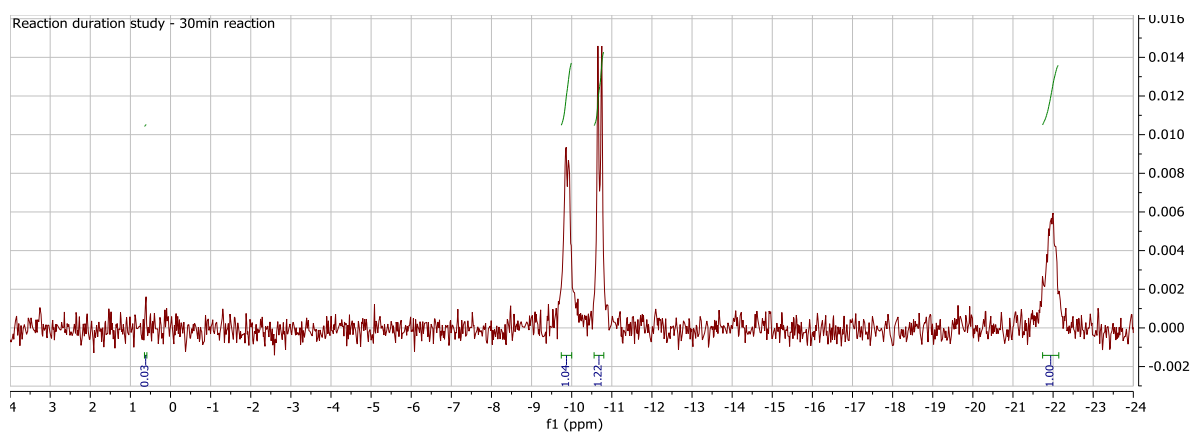


Figure A-I.9: ^{31}P NMR spectra of 30 min reaction of reaction duration study. 30-minute duration of reaction. Process described in Section 4.5. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.3.

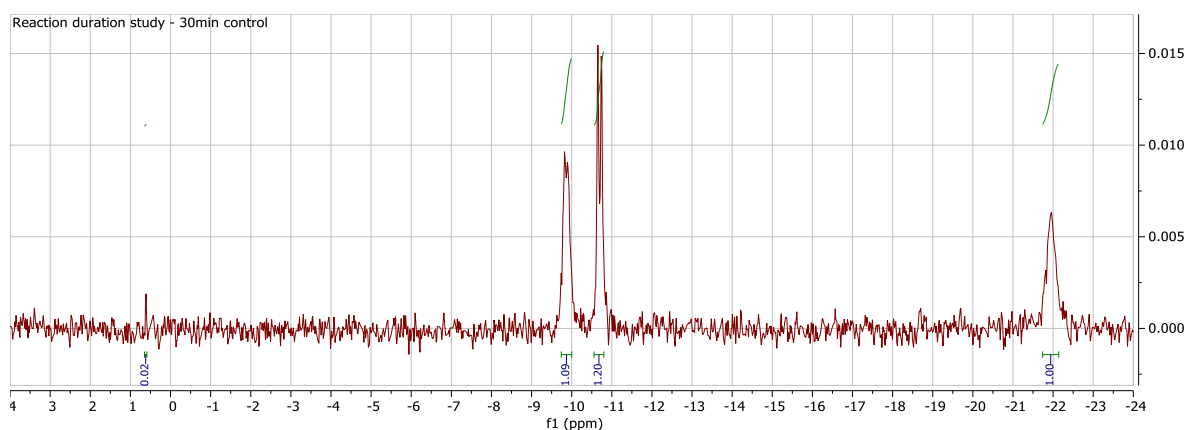


Figure A-I.10: ^{31}P NMR spectra of 30 min control of reaction duration study. 30-minute duration of reaction. Process described in Section 4.5. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.3.

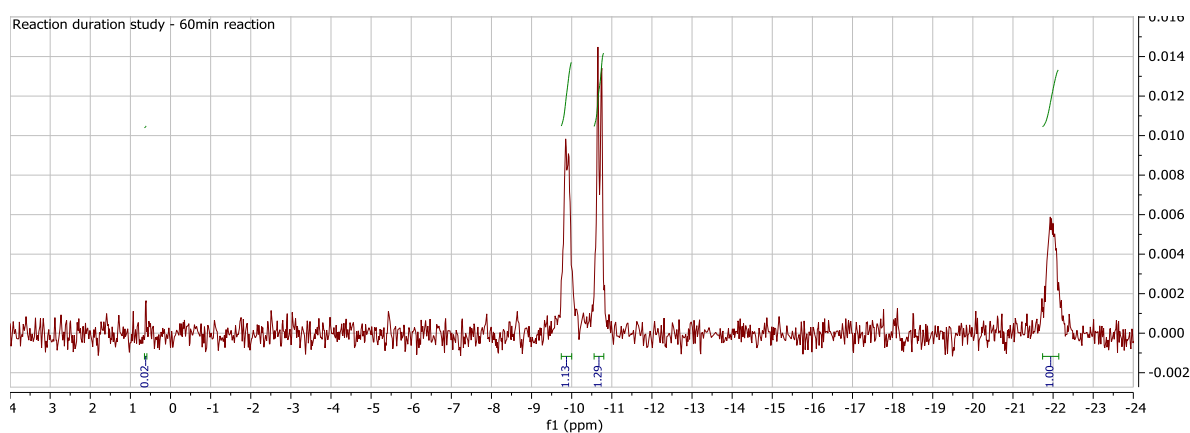


Figure A-I.11: ^{31}P NMR spectra of 60 min reaction of reaction duration study. 60-minute duration of reaction. Process described in Section 4.5. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.3.

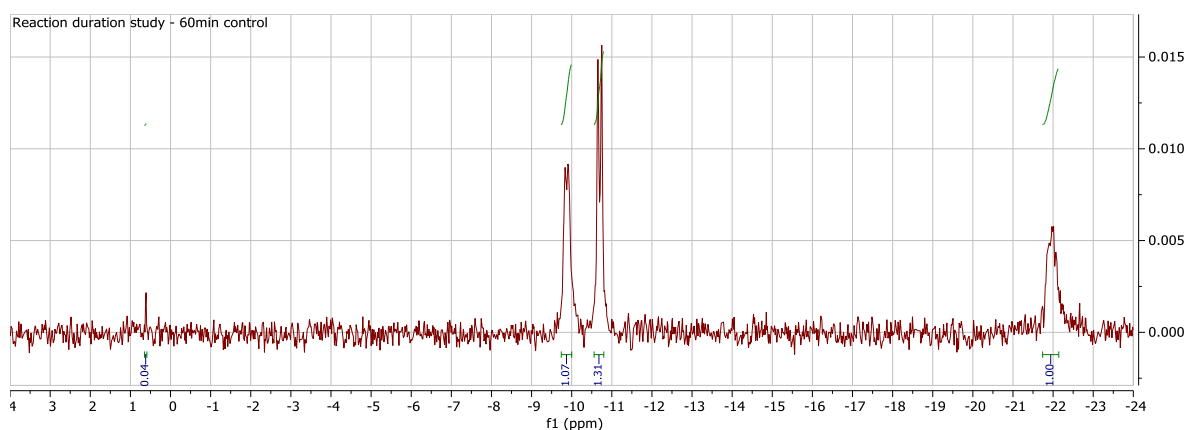


Figure A-I.12: ^{31}P NMR spectra of 60 min control of reaction duration study. 60-minute duration of reaction. Process described in Section 4.5. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.3.

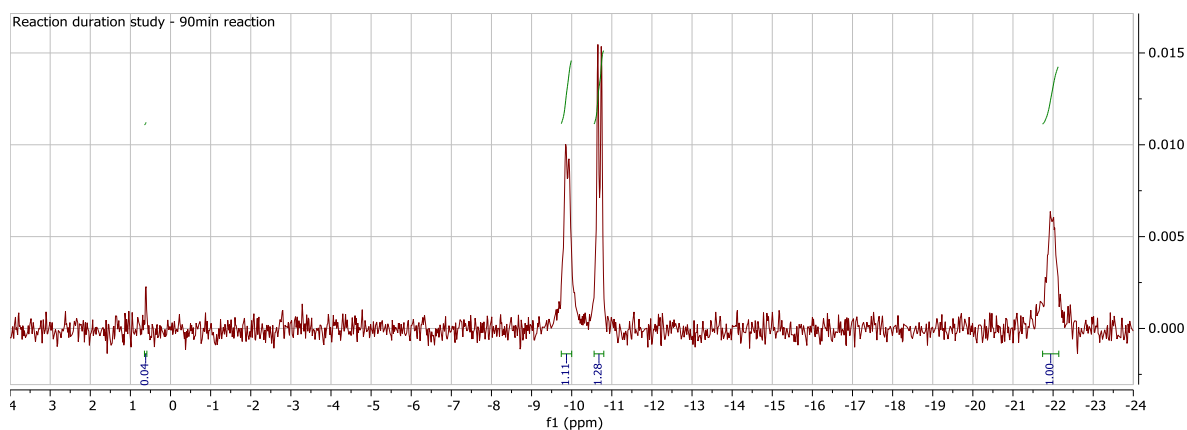


Figure A-I.13: ^{31}P NMR spectra of 90 min reaction of reaction duration study. 90-minute duration of reaction. Process described in Section 4.5. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.3.

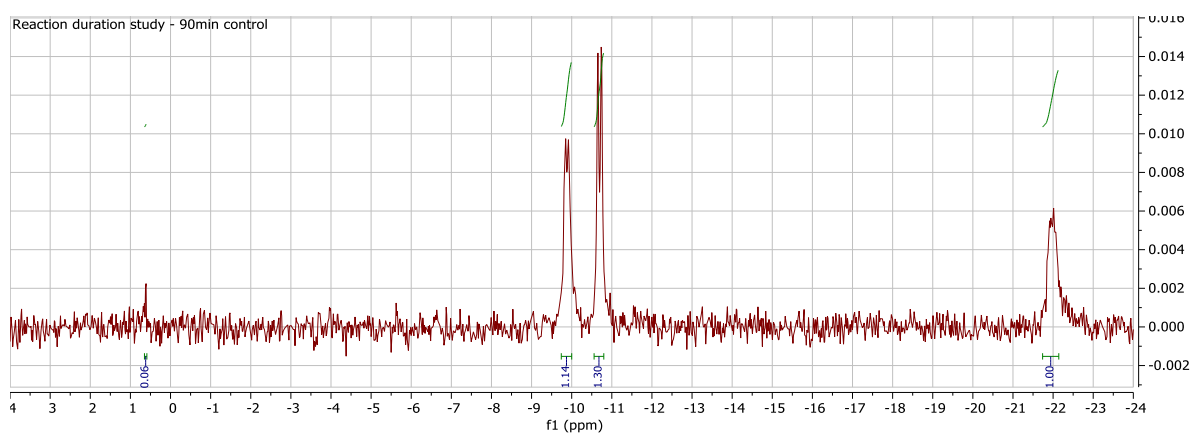


Figure A-I.14: ^{31}P NMR spectra of 90 min control of reaction duration study. 90-minute duration of reaction. Process described in Section 4.5. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.3.

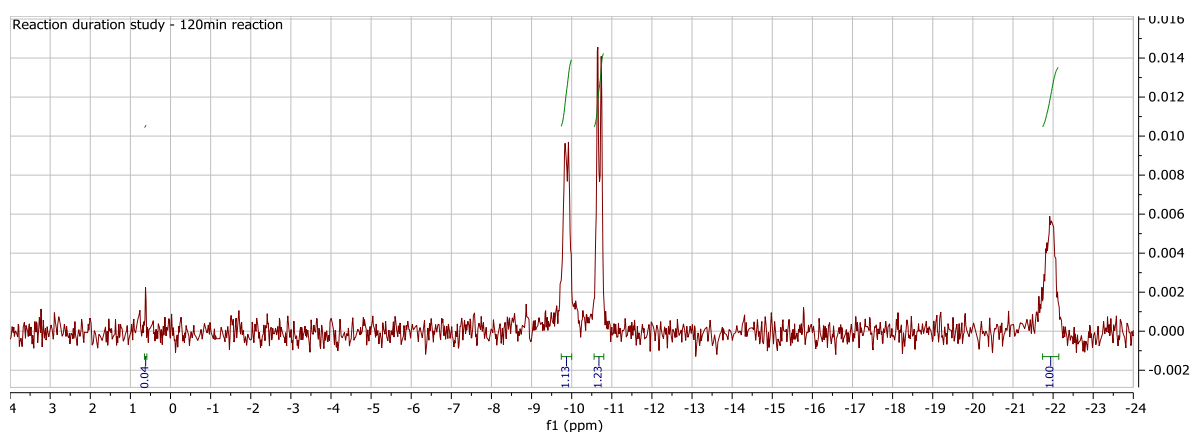


Figure A-I.15: ^{31}P NMR spectra of 120 min reaction of reaction duration study. 120-minute duration of reaction. Process described in Section 4.5. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.3.

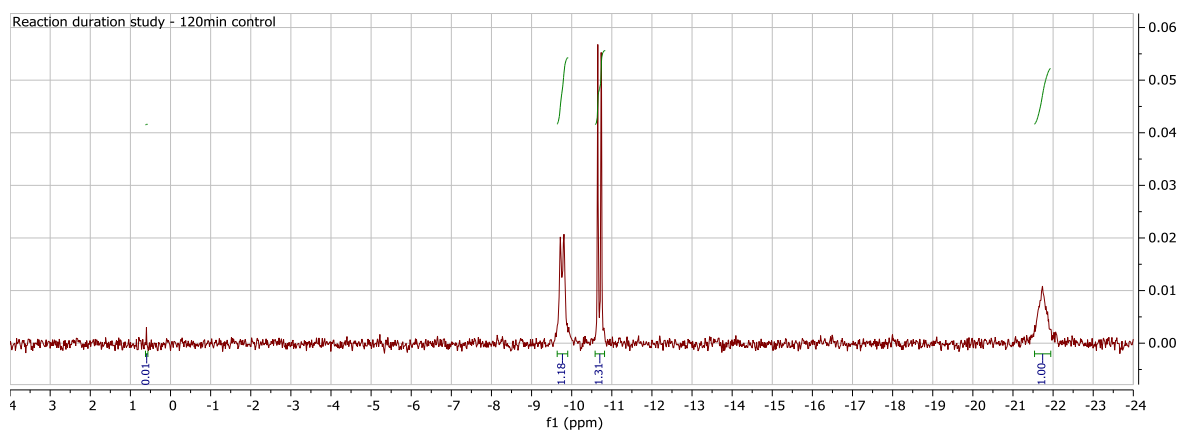


Figure A-I.16: ^{31}P NMR spectra of 120 min control of reaction duration study. 120-minute duration of reaction. Process described in Section 4.5. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.3.

A-I.3 Substrate Excess Study

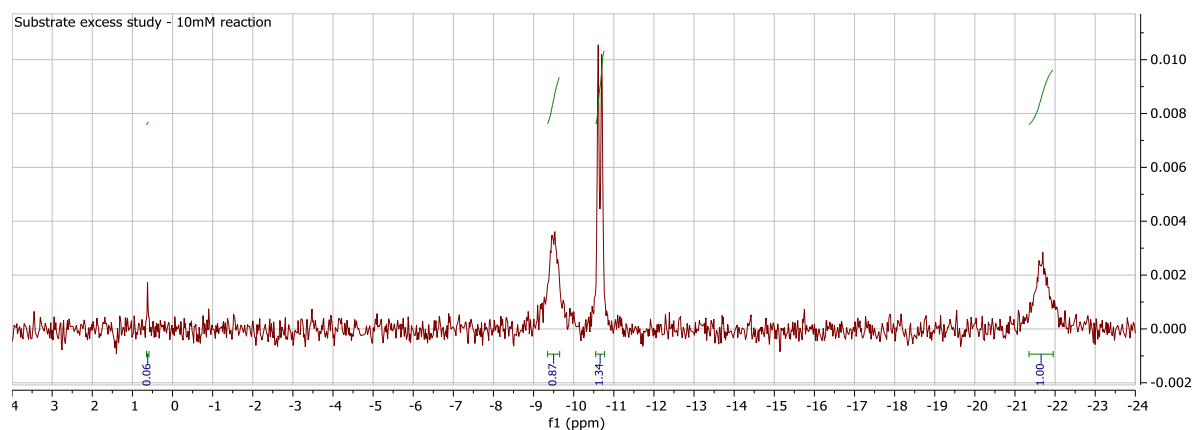


Figure A-I.17: ^{31}P NMR spectra of 10 mM reaction of substrate excess study. Uridine 25 mM. ATP 10 mM. Process described in Section 4.6. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.4.

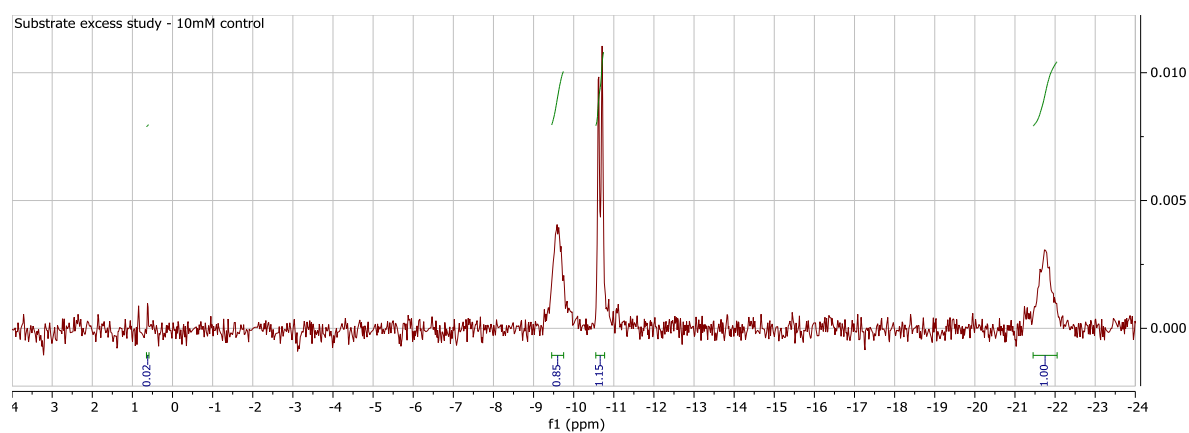


Figure A-I.18: ^{31}P NMR spectra of 10 mM control of substrate excess study. Uridine 0 mM. ATP 10 mM. Process described in Section 4.6. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.4.

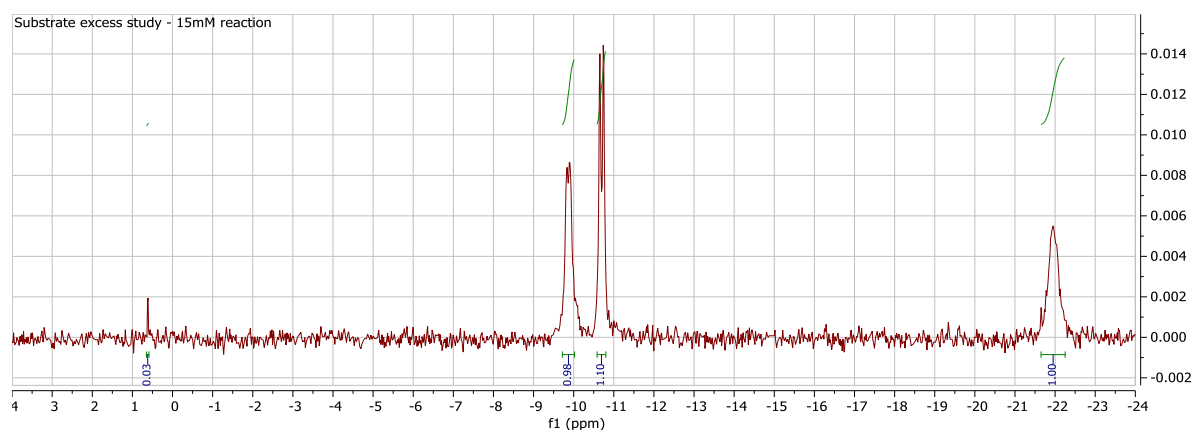


Figure A-I.19: ^{31}P NMR spectra of 15 mM reaction of substrate excess study. Uridine 25 mM. ATP 15 mM. Process described in Section 4.6. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.4.

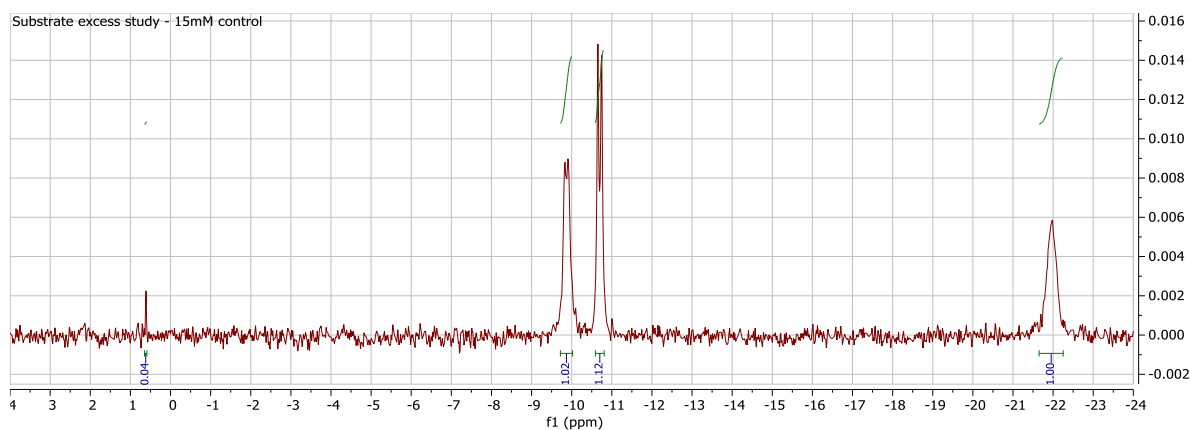


Figure A-I.20: ^{31}P NMR spectra of 15 mM control of substrate excess study. Uridine 0 mM. ATP 15 mM. Process described in Section 4.6. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.4.

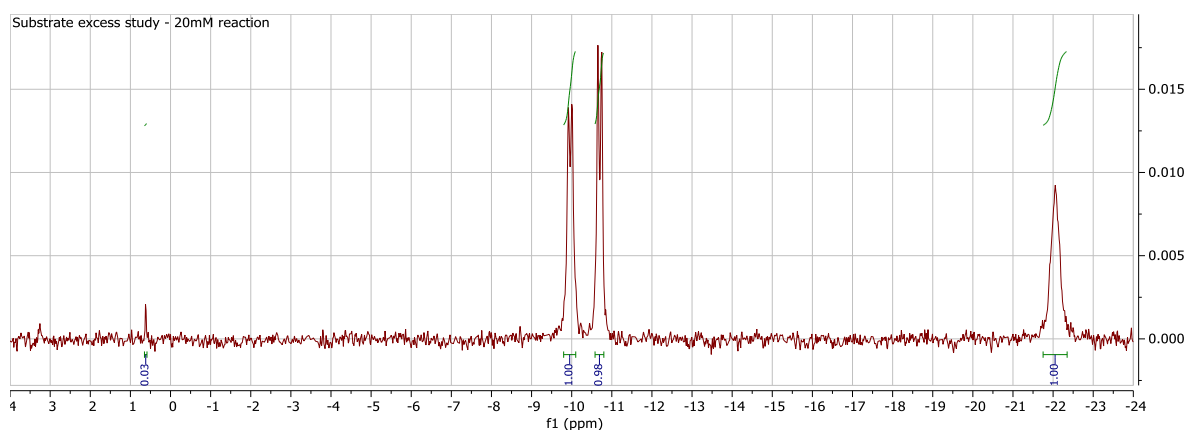


Figure A-I.21: ^{31}P NMR spectra of 20 mM reaction of substrate excess study. Uridine 25 mM. ATP 20 mM. Process described in Section 4.6. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.4.

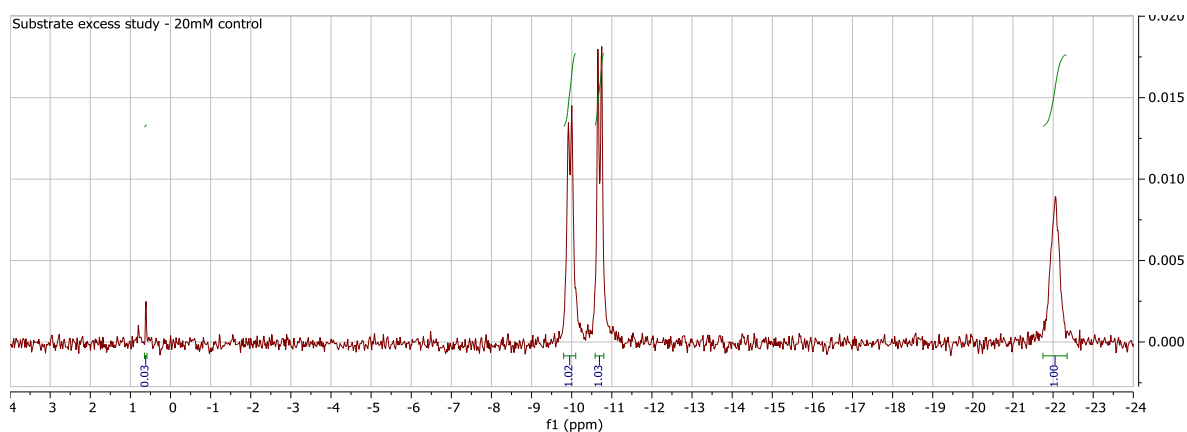


Figure A-I.22: ^{31}P NMR spectra of 20 mM control of substrate excess study. Uridine 0 mM. ATP 20 mM. Process described in Section 4.6. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.4.

A-I.4 Use of Pyruvate Kinase

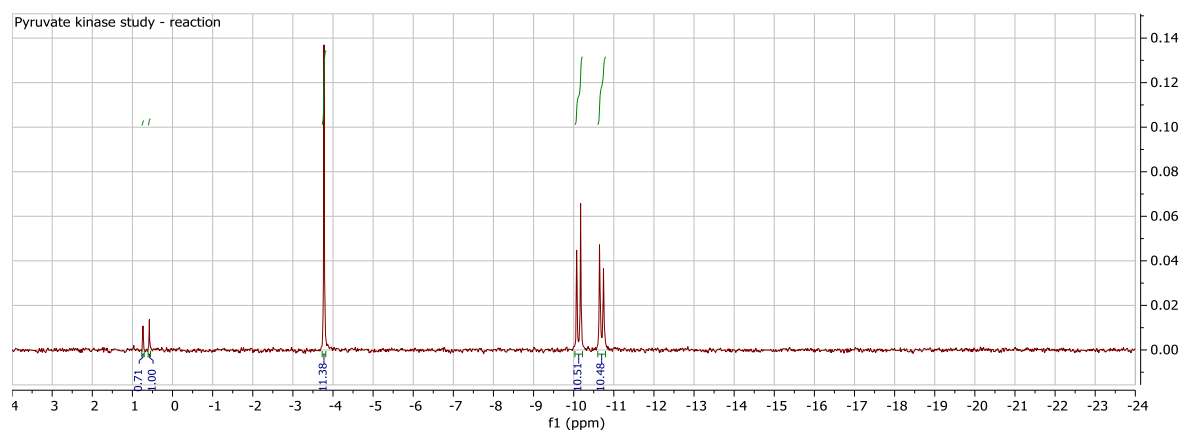


Figure A-I.23: ^{31}P NMR spectra of reaction sample of pyruvate kinase study. Process described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.5.

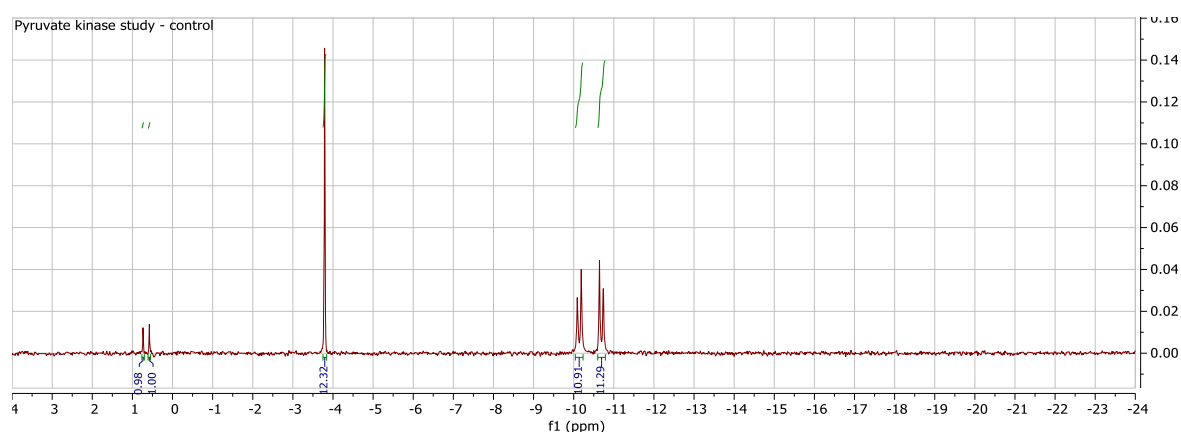


Figure A-I.24: ^{31}P NMR spectra of control sample of pyruvate kinase study. Process described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.5.

A-I.5 Confirmation of Enzymatic Activity

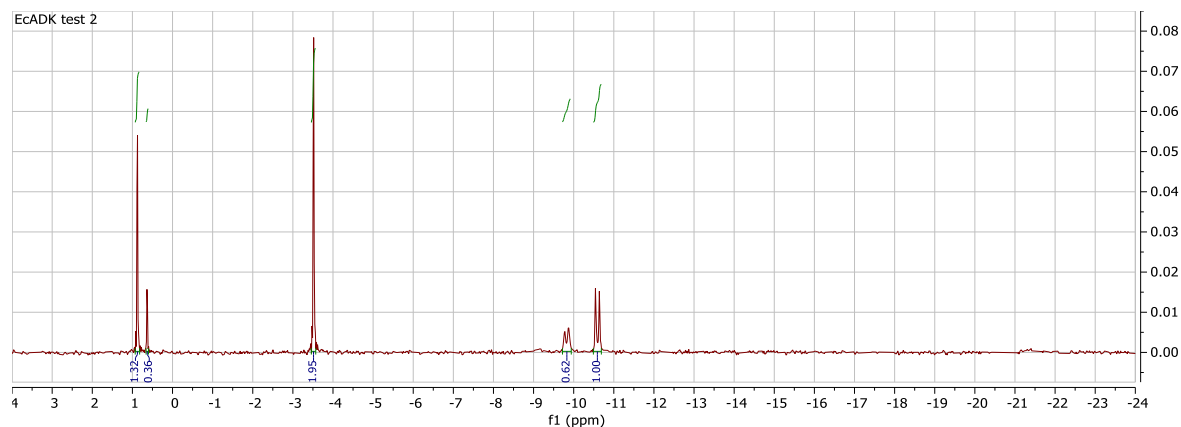


Figure A-I.25: ^{31}P NMR spectra of EcADK activity test reaction sample. Process described in Section 4.8.1. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.6.

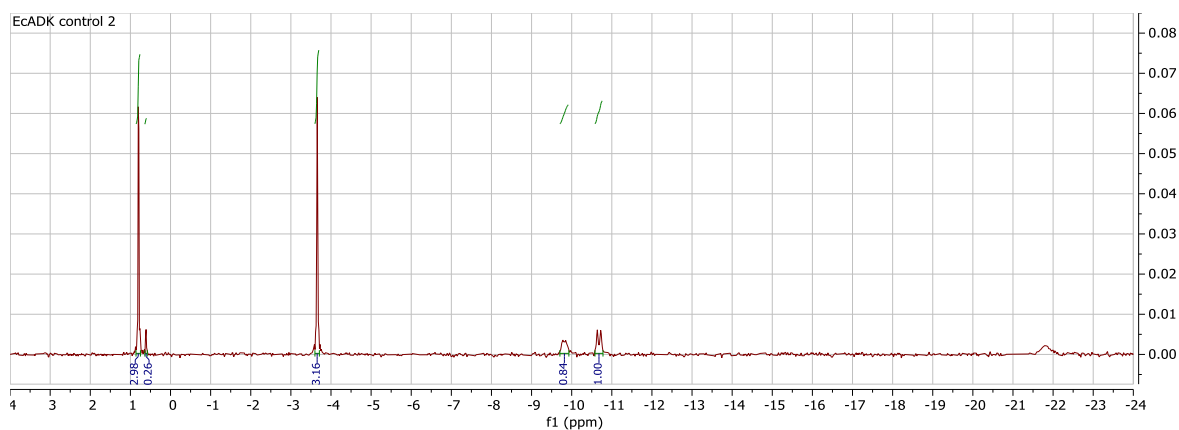


Figure A-I.26: ^{31}P NMR spectra of EcADK activity test negative control sample. Process described in Section 4.8.1. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.6.

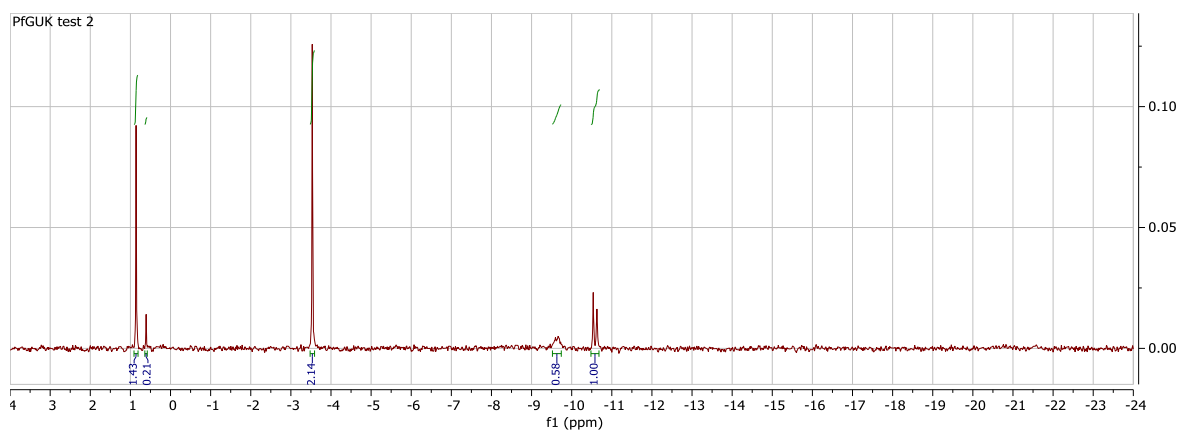


Figure A-I.27: ^{31}P NMR spectra of PfGUK activity test reaction sample. Process described in Section 4.8.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.7.

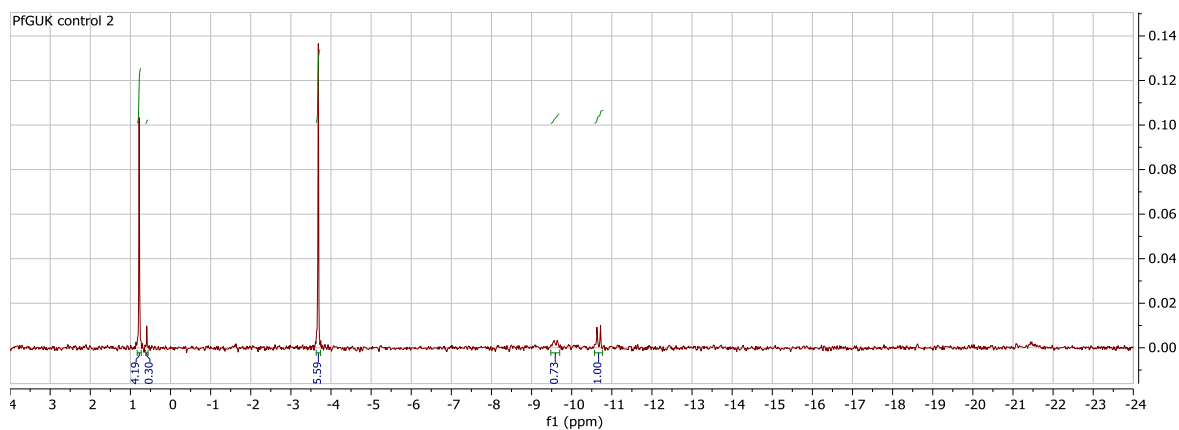


Figure A-I.28: ^{31}P NMR spectra of PfGUK activity test negative control sample. Process described in Section 4.8.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.7.

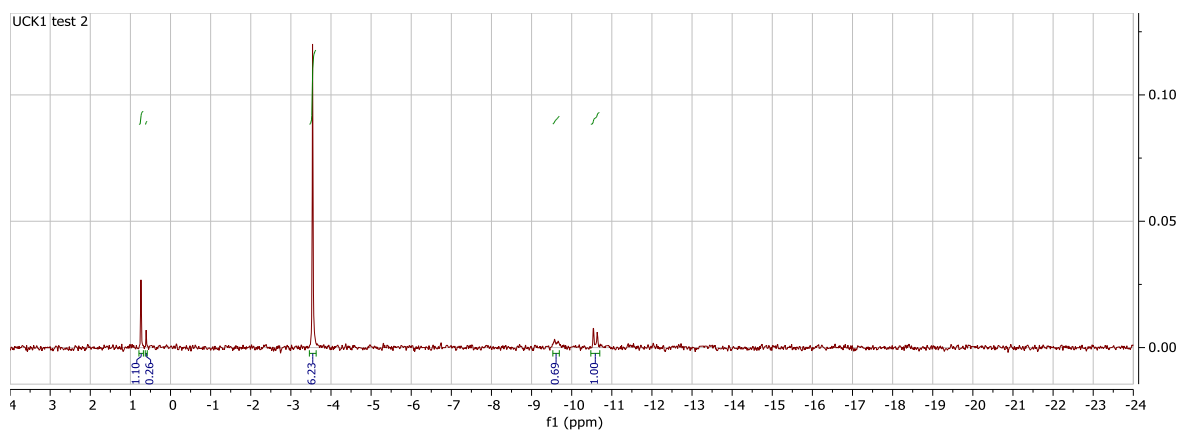


Figure A-I.29: ^{31}P NMR spectra of UCK1 activity test reaction sample. Process described in Section 4.8.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.8.

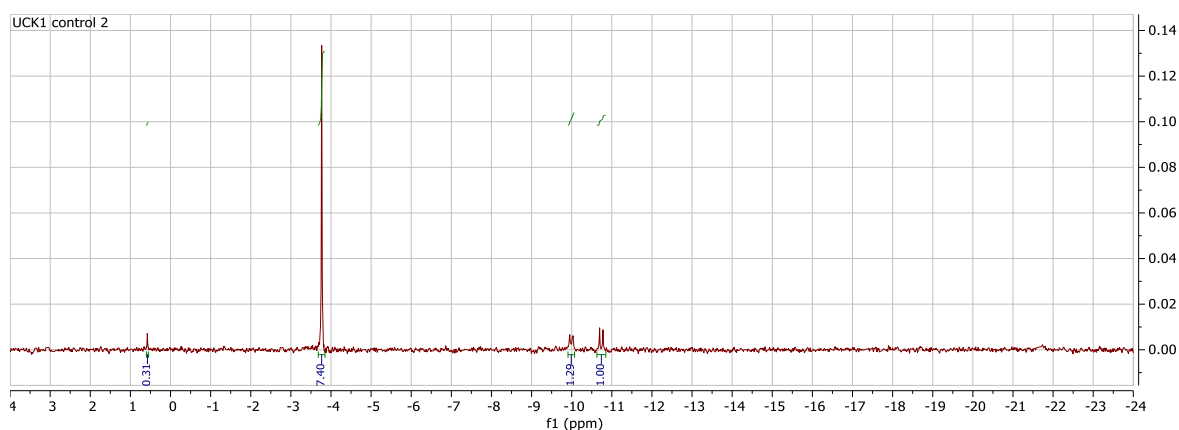


Figure A-I.30: ^{31}P NMR spectra of UCK1 activity test negative control sample. Process described in Section 4.8.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.8.

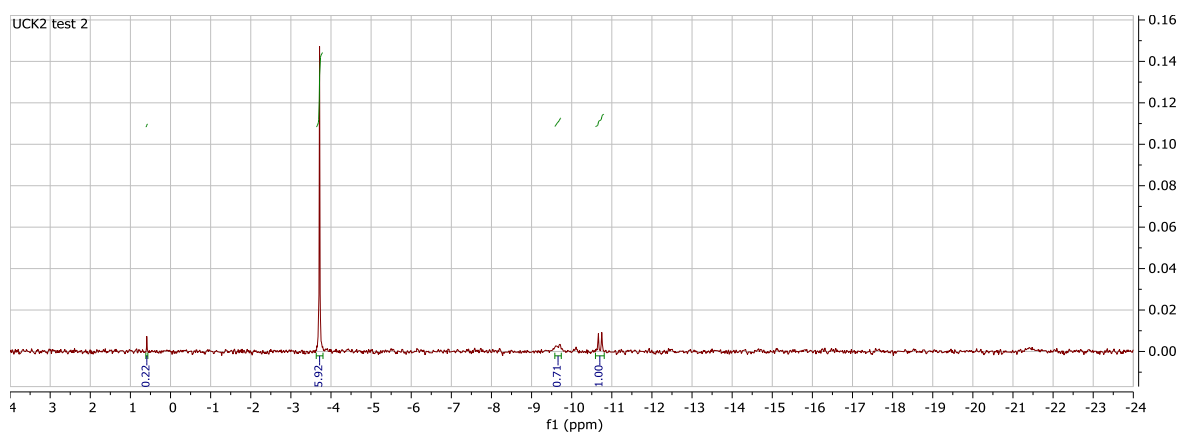


Figure A-I.31: ^{31}P NMR spectra of UCK2 activity test reaction sample. Process described in Section 4.8.4. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table 4.9.

Note: Figure A-I.30 was used as negative control for UCK2 activity test.

Note: ^{31}P NMR spectra for CMPK activity tests can be found in *Section 4.8.5*.

A-I.6 Quantification Model (12.5mM)

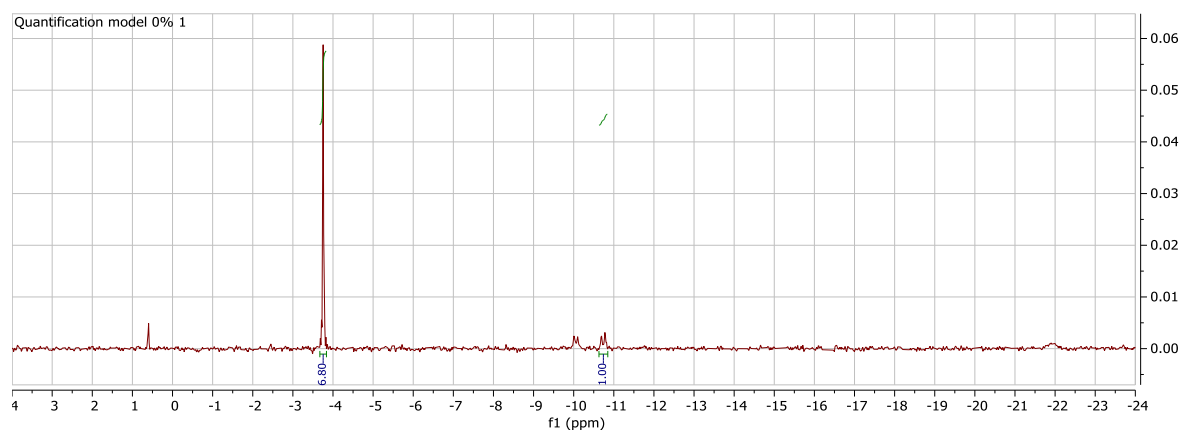


Figure A-I.32: ^{31}P NMR spectra from quantification model 0% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

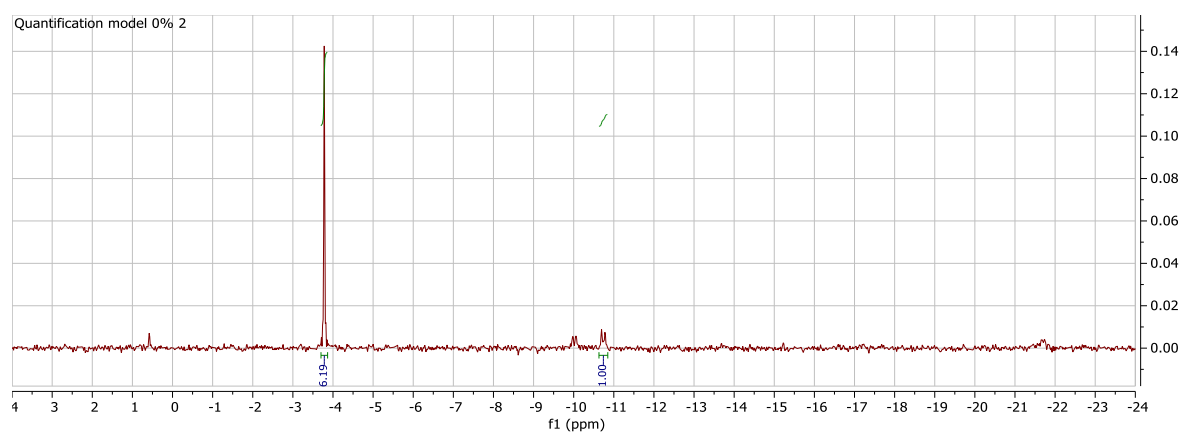


Figure A-I.33: ^{31}P NMR spectra from quantification model 0% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

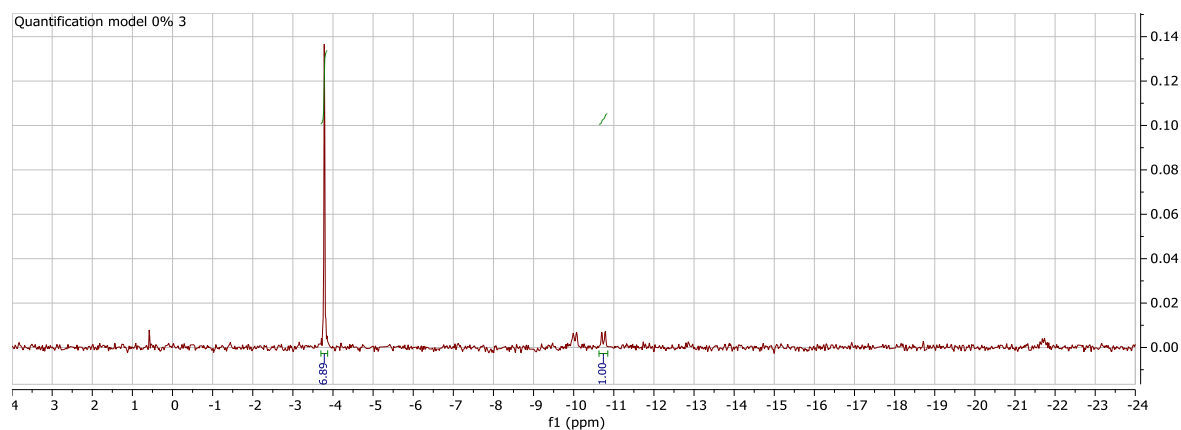


Figure A-I.34: ^{31}P NMR spectra from quantification model 0% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

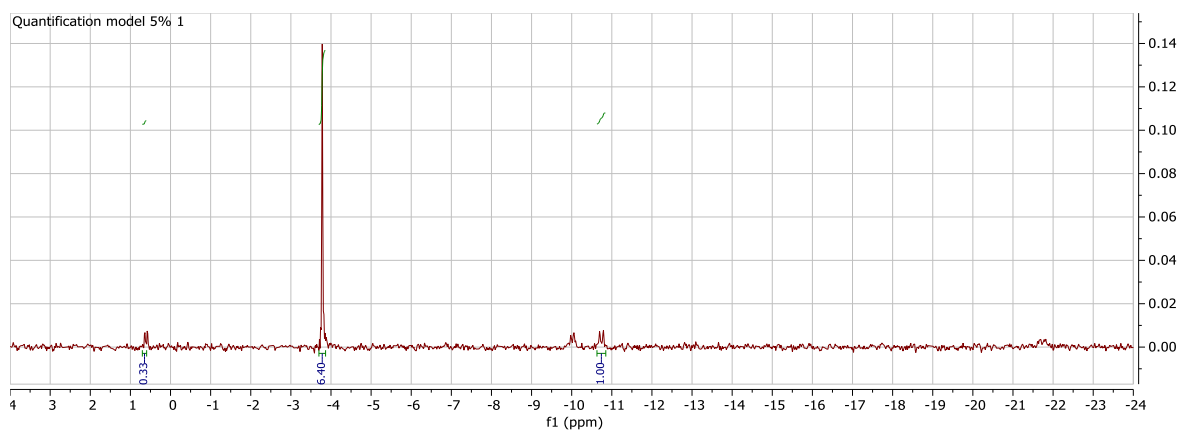


Figure A-I.35: ^{31}P NMR spectra from quantification model 5% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

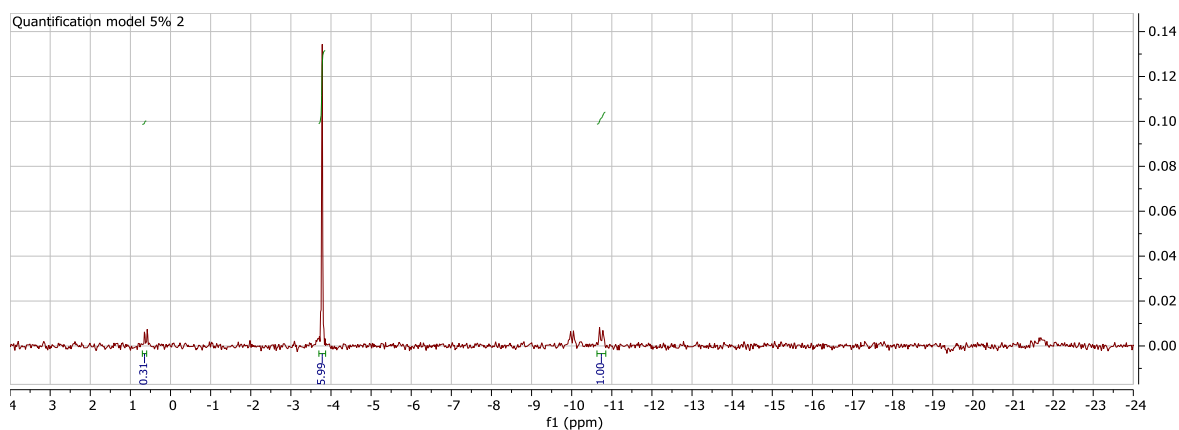


Figure A-I.36: ^{31}P NMR spectra from quantification model 5% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

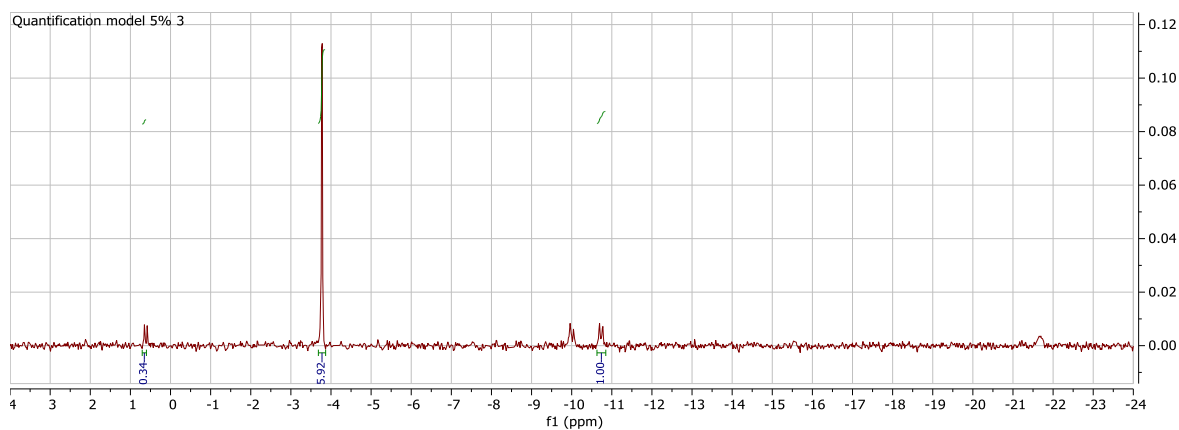


Figure A-I.37: ^{31}P NMR spectra from quantification model 5% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

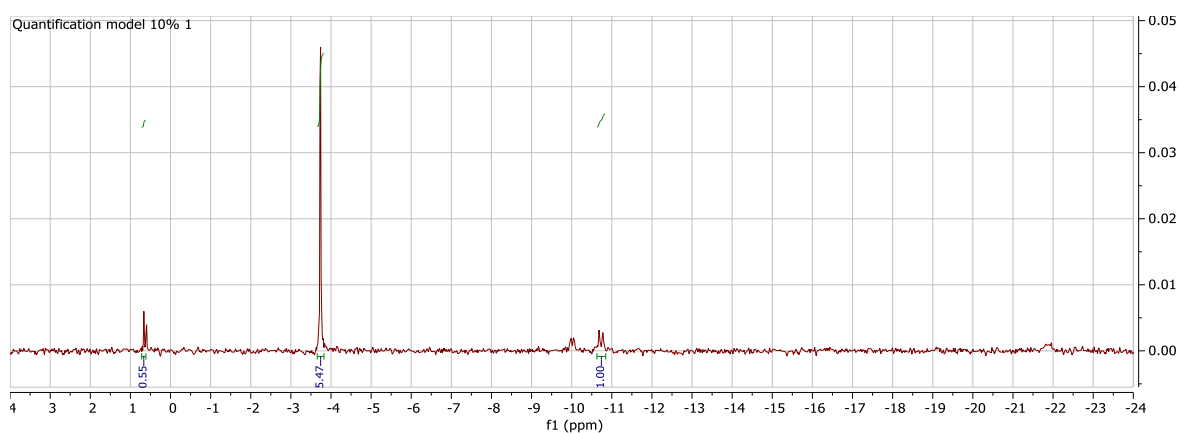


Figure A-I.38: ^{31}P NMR spectra from quantification model 10% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

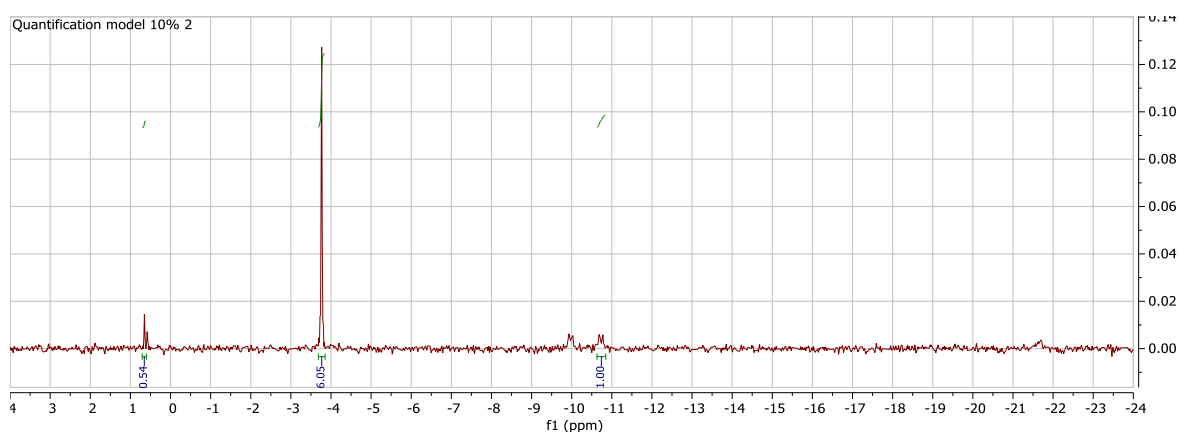


Figure A-I.39: ^{31}P NMR spectra from quantification model 10% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

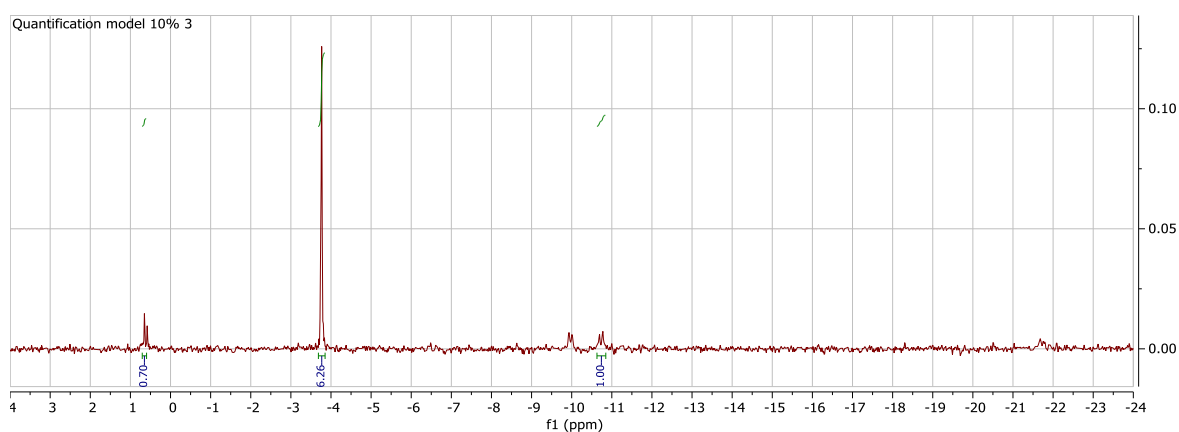


Figure A-I.40: ^{31}P NMR spectra from quantification model 10% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

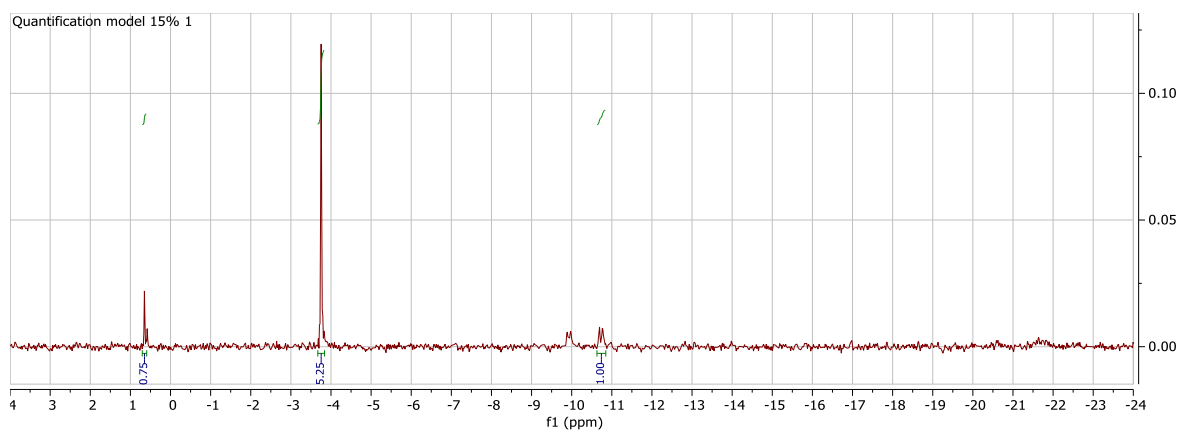


Figure A-I.41: ^{31}P NMR spectra from quantification model 15% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

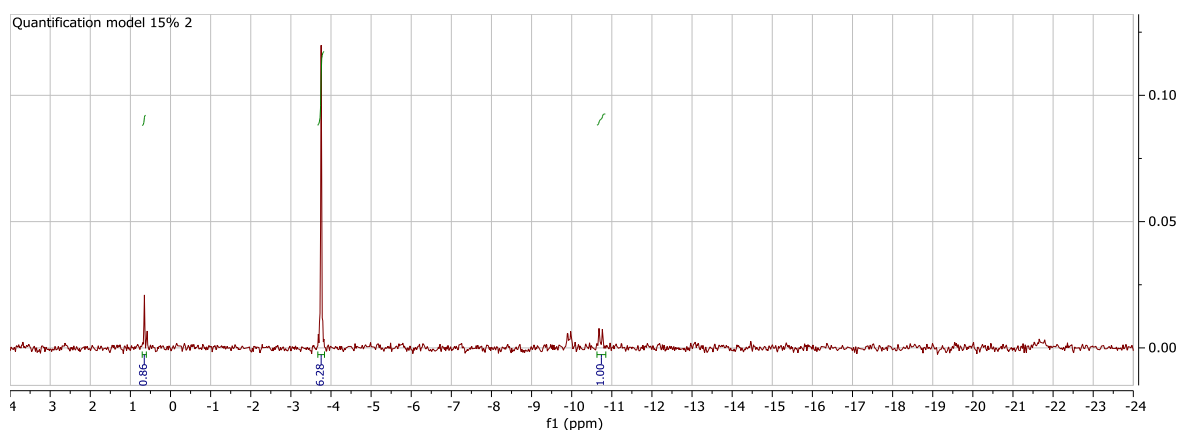


Figure A-I.42: ^{31}P NMR spectra from quantification model 15% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

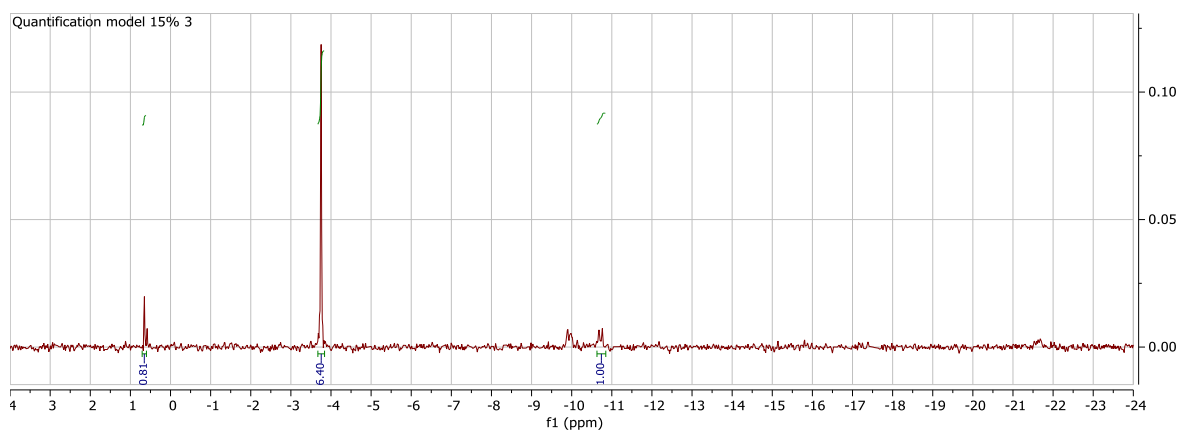


Figure A-I.43: ^{31}P NMR spectra from quantification model 15% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

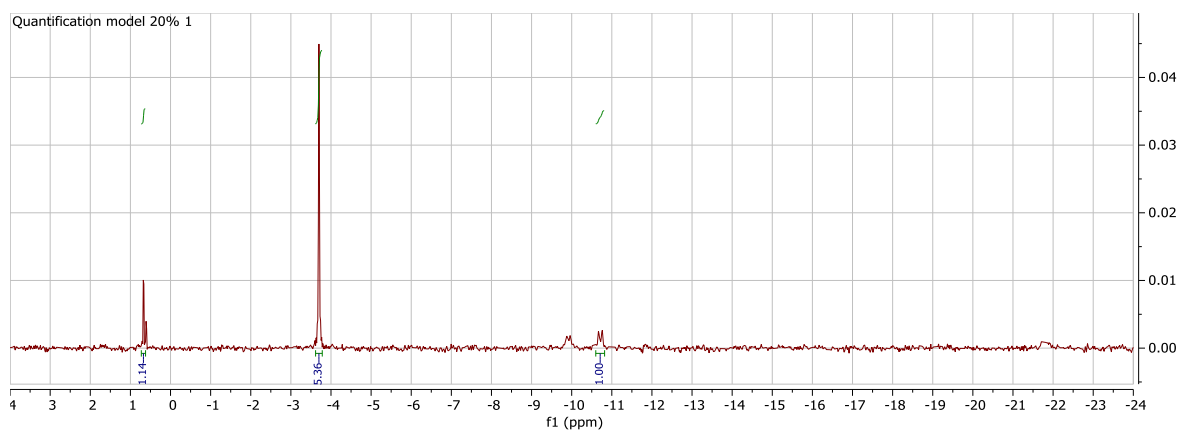


Figure A-I.44: ^{31}P NMR spectra from quantification model 20% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

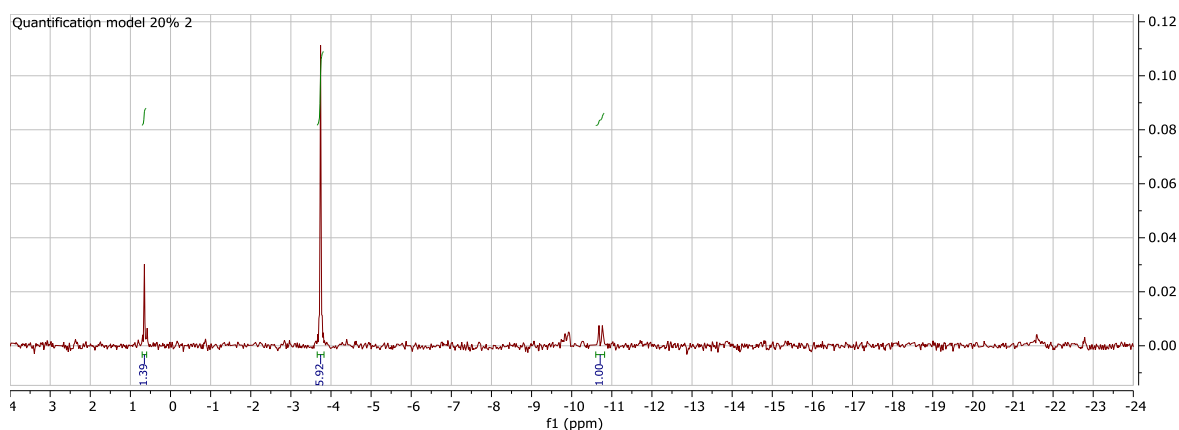


Figure A-I.45: ^{31}P NMR spectra from quantification model 20% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

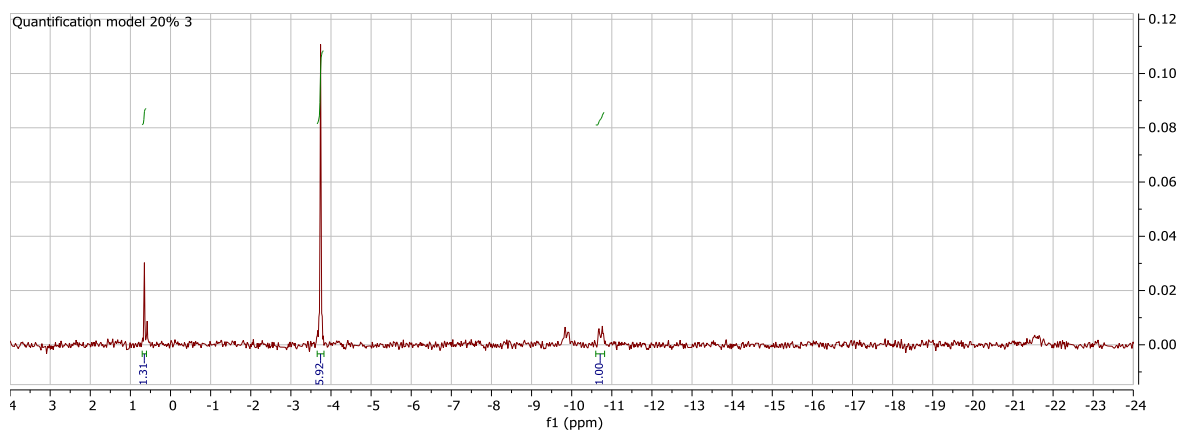


Figure A-I.46: ^{31}P NMR spectra from quantification model 20% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

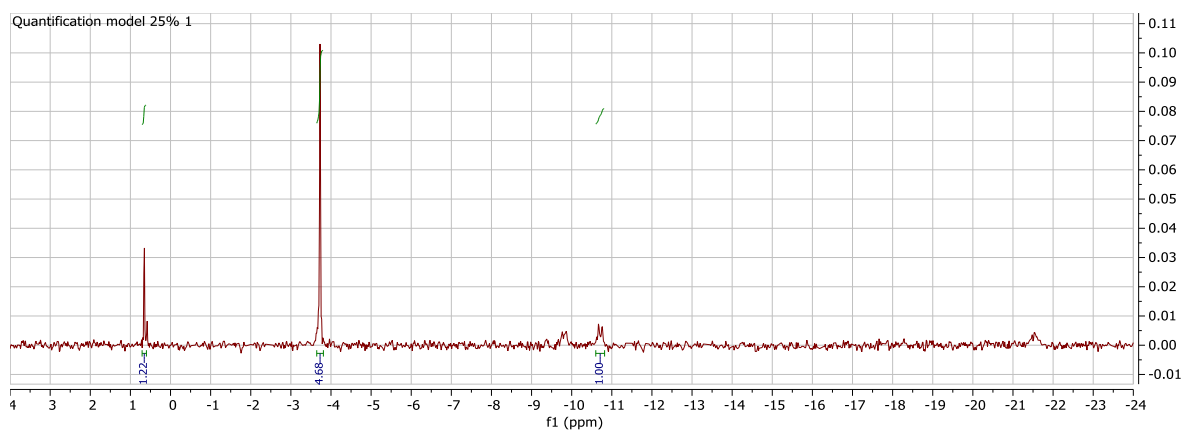


Figure A-I.47: ^{31}P NMR spectra from quantification model 25% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

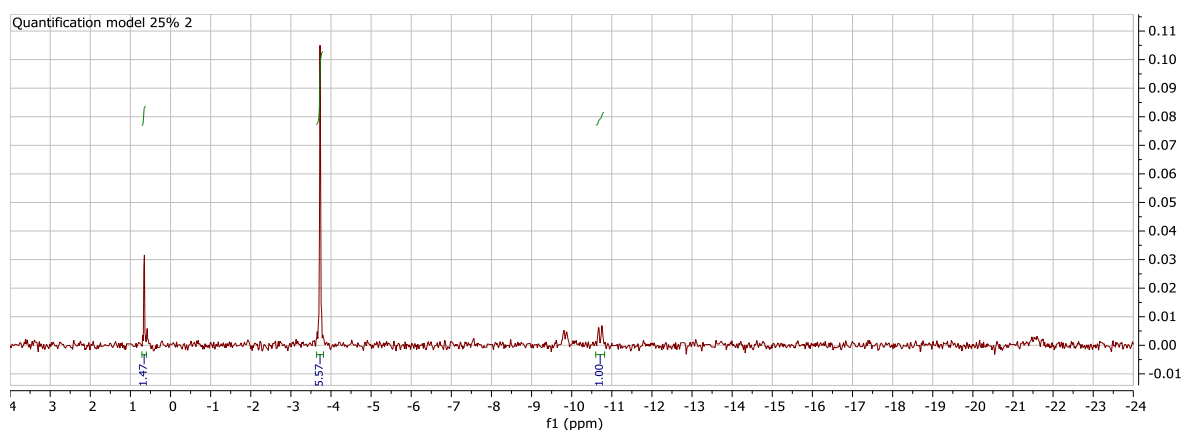


Figure A-I.48: ^{31}P NMR spectra from quantification model 25% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

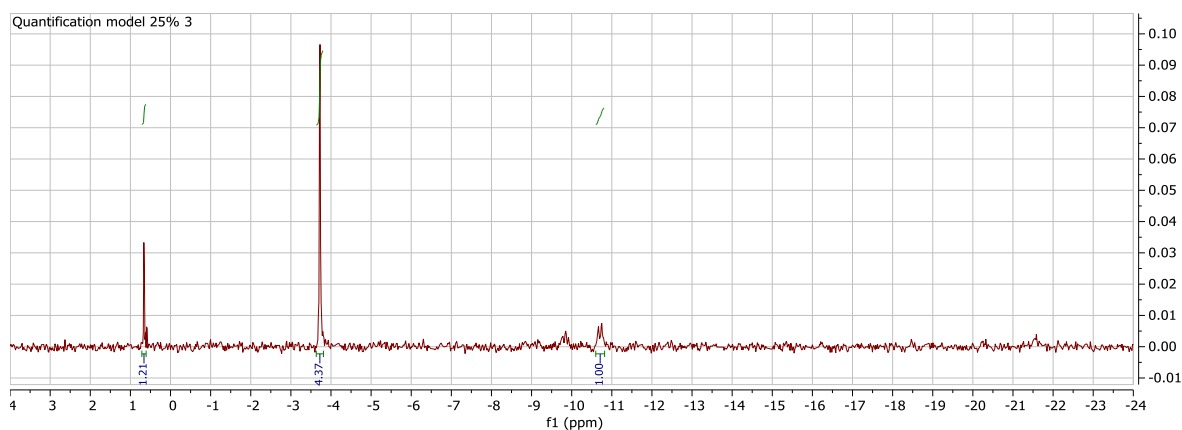


Figure A-I.49: ^{31}P NMR spectra from quantification model 25% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

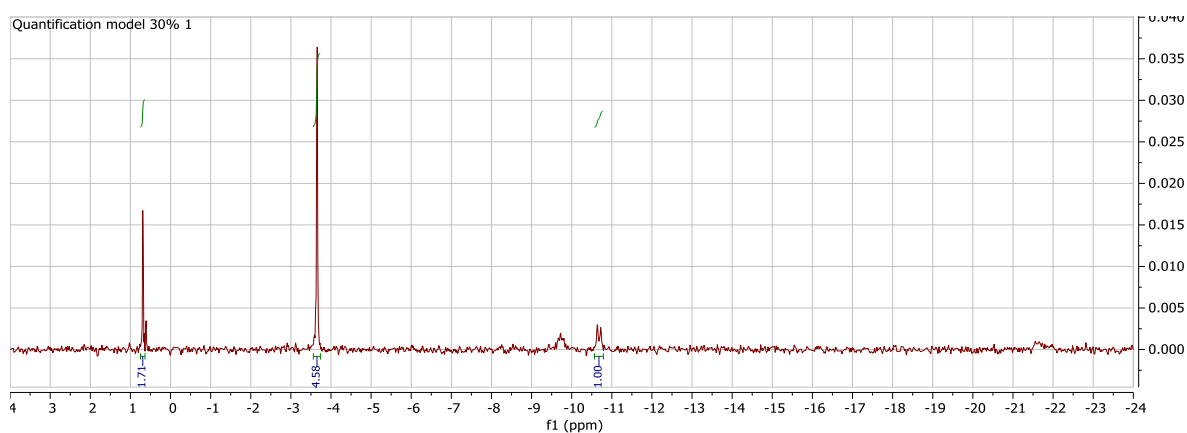


Figure A-I.50: ^{31}P NMR spectra from quantification model 30% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

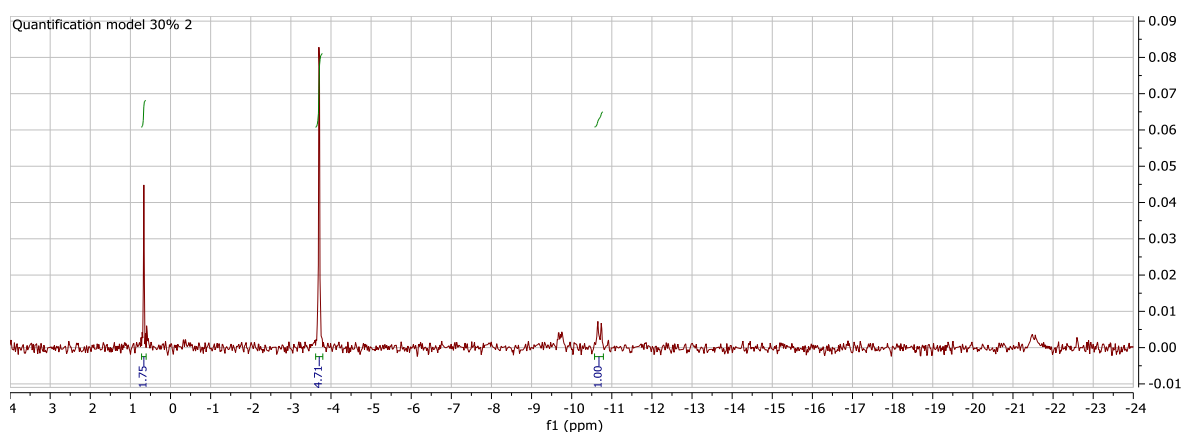


Figure A-I.51: ^{31}P NMR spectra from quantification model 30% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

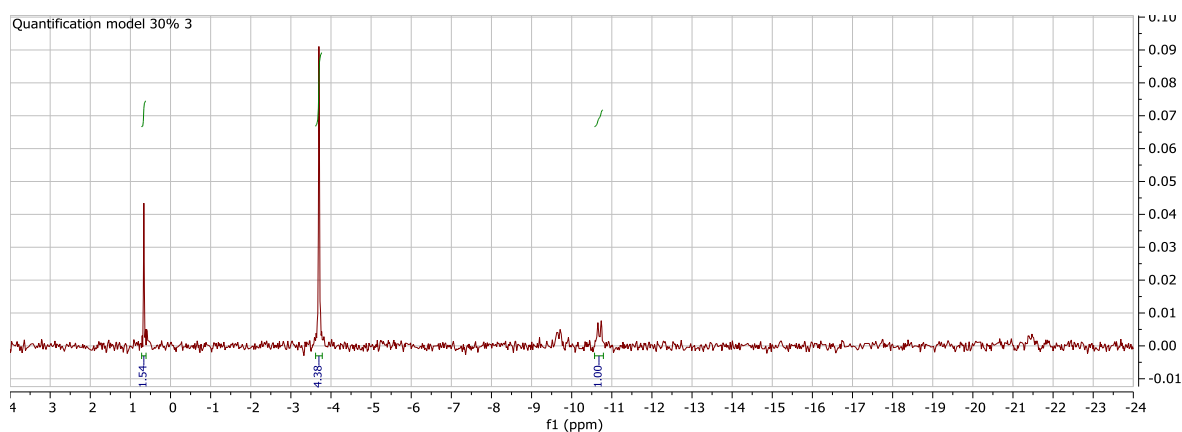


Figure A-I.52: ^{31}P NMR spectra from quantification model 30% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

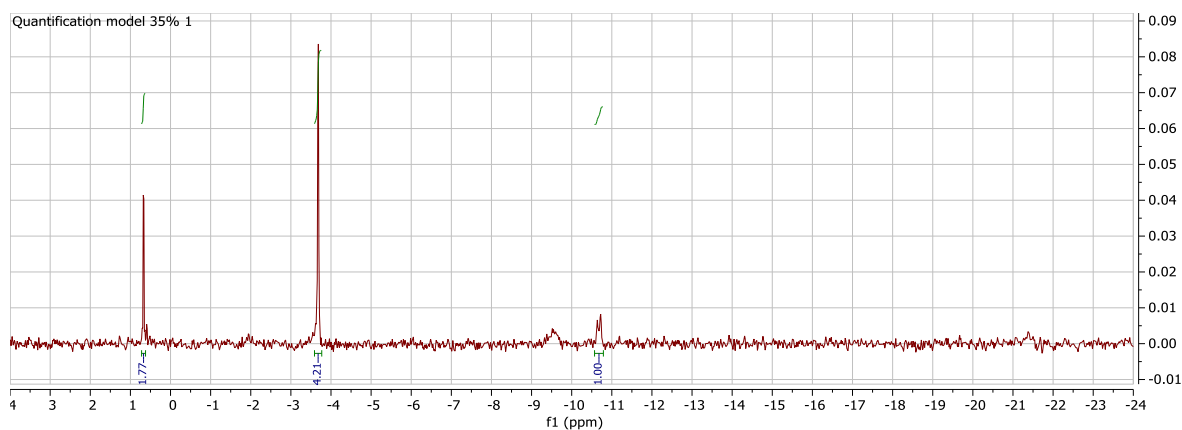


Figure A-I.53: ^{31}P NMR spectra from quantification model 35% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

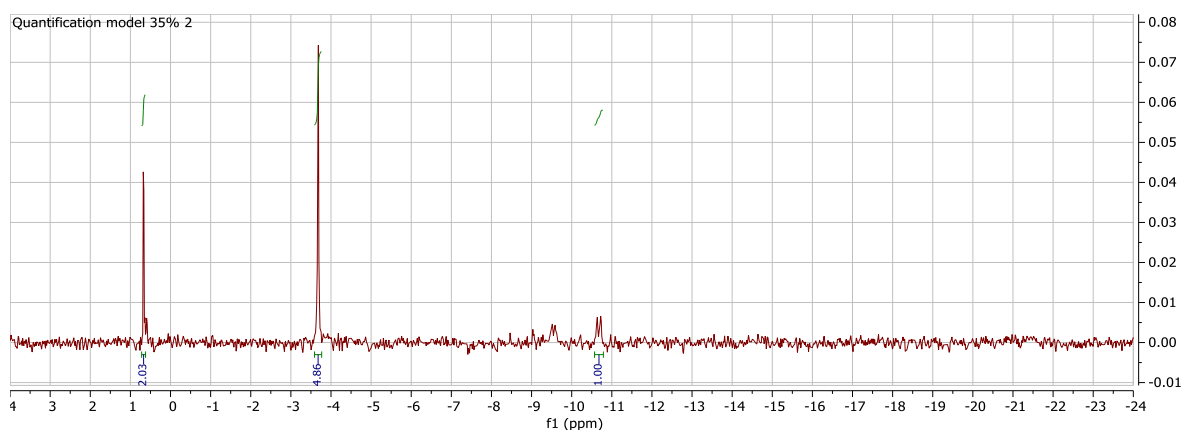


Figure A-I.54: ^{31}P NMR spectra from quantification model 35% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

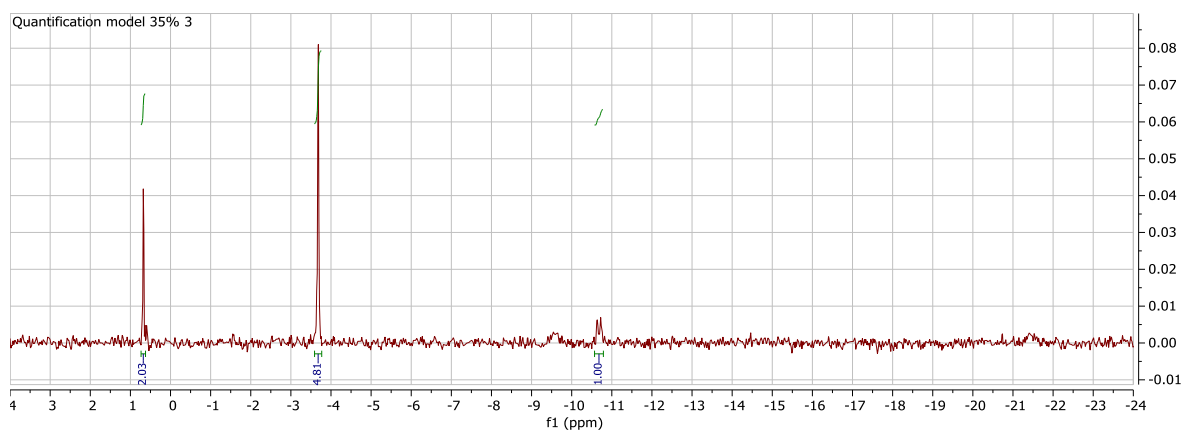


Figure A-I.55: ^{31}P NMR spectra from quantification model 35% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

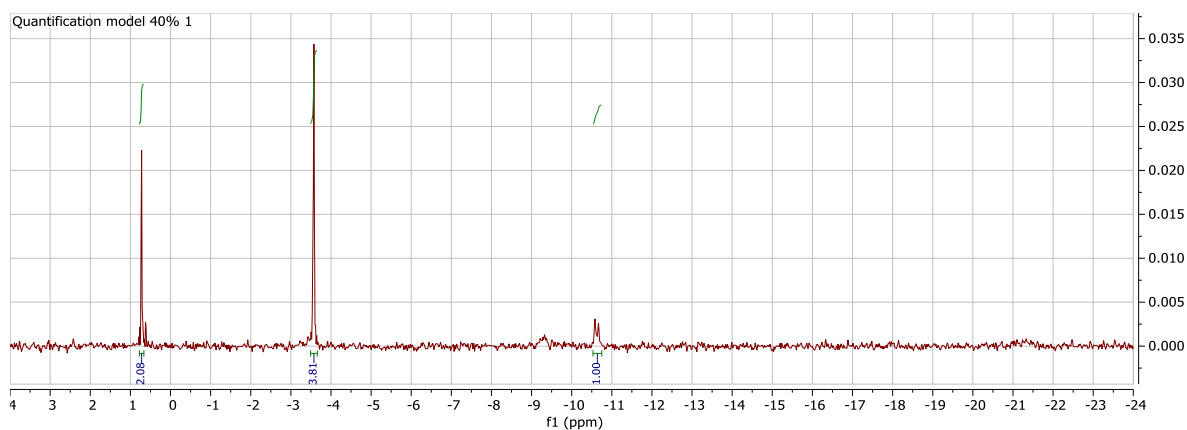


Figure A-I.56: ^{31}P NMR spectra from quantification model 40% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

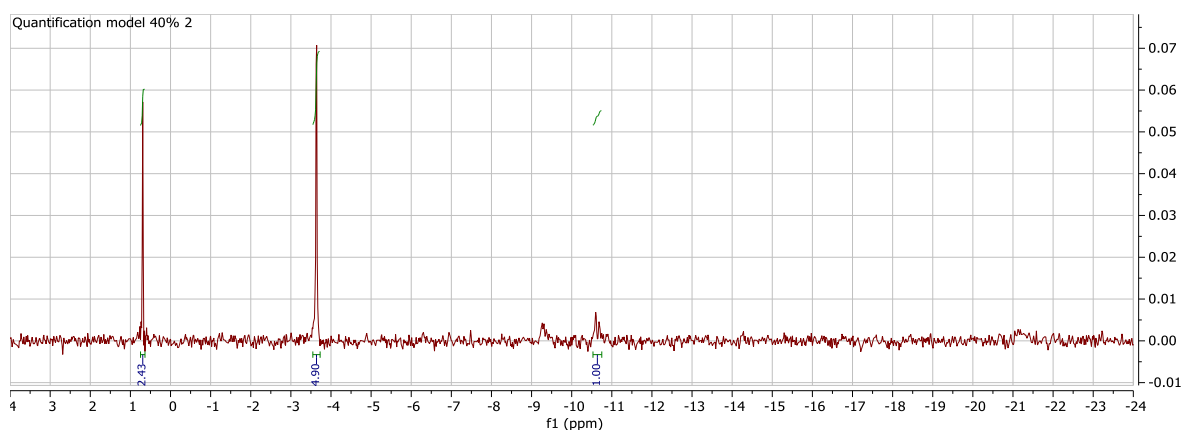


Figure A-I.57: ^{31}P NMR spectra from quantification model 40% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

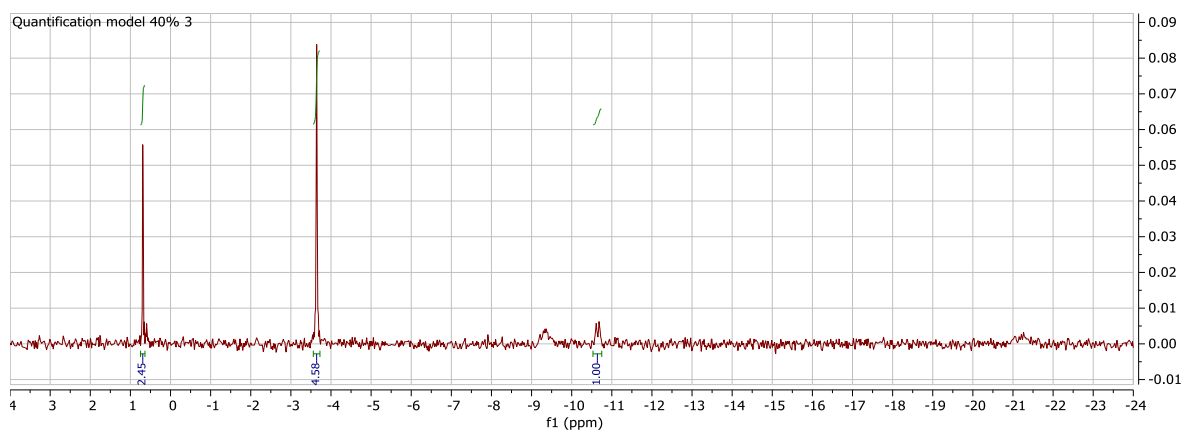


Figure A-I.58: ^{31}P NMR spectra from quantification model 40% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

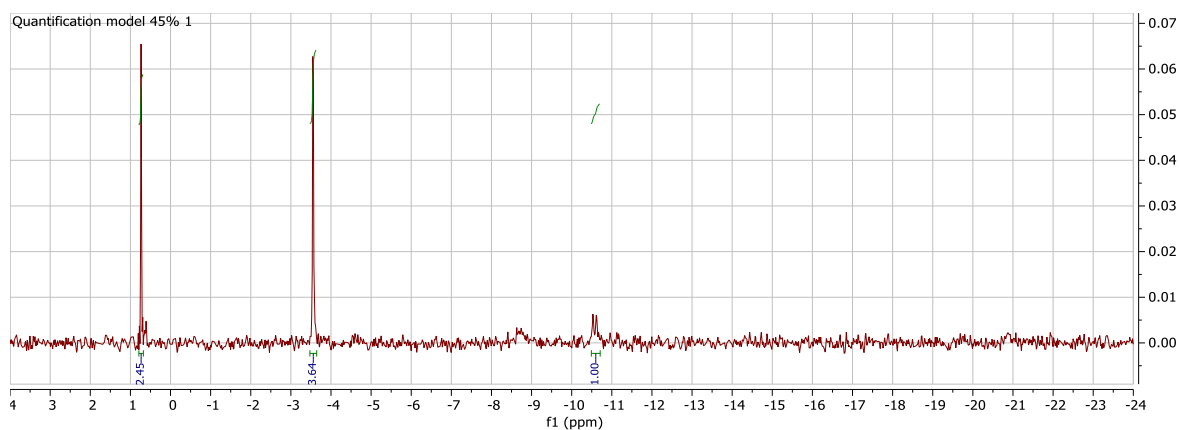


Figure A-I.59: ^{31}P NMR spectra from quantification model 45% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

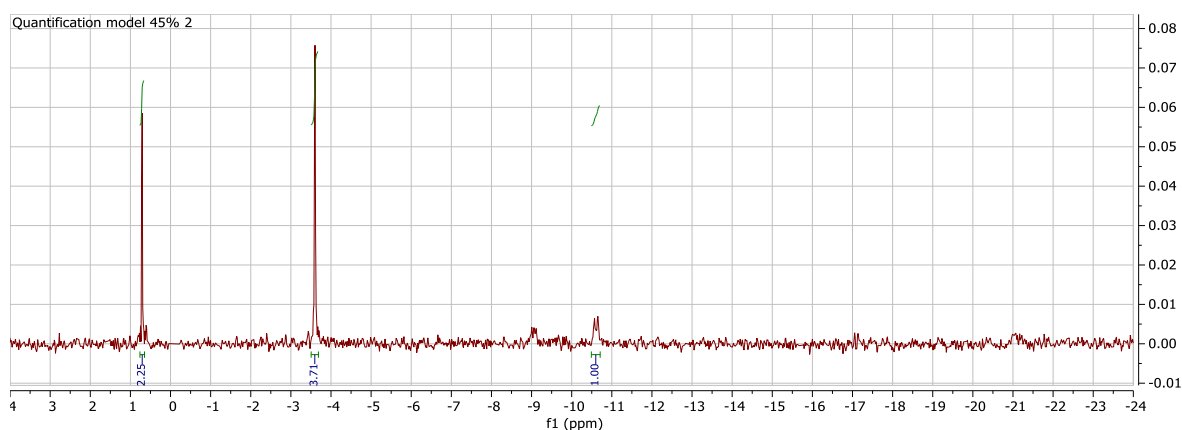


Figure A-I.60: ^{31}P NMR spectra from quantification model 45% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

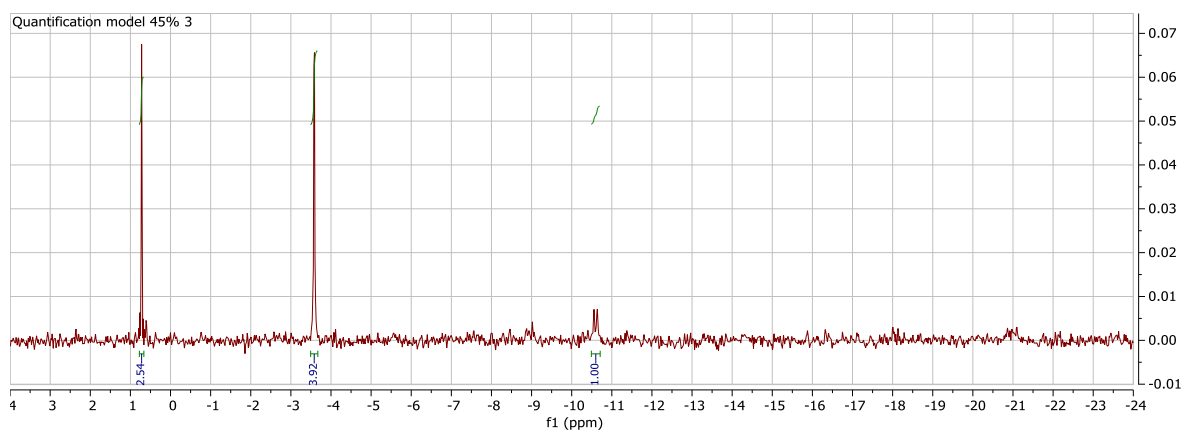


Figure A-I.61: ^{31}P NMR spectra from quantification model 45% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

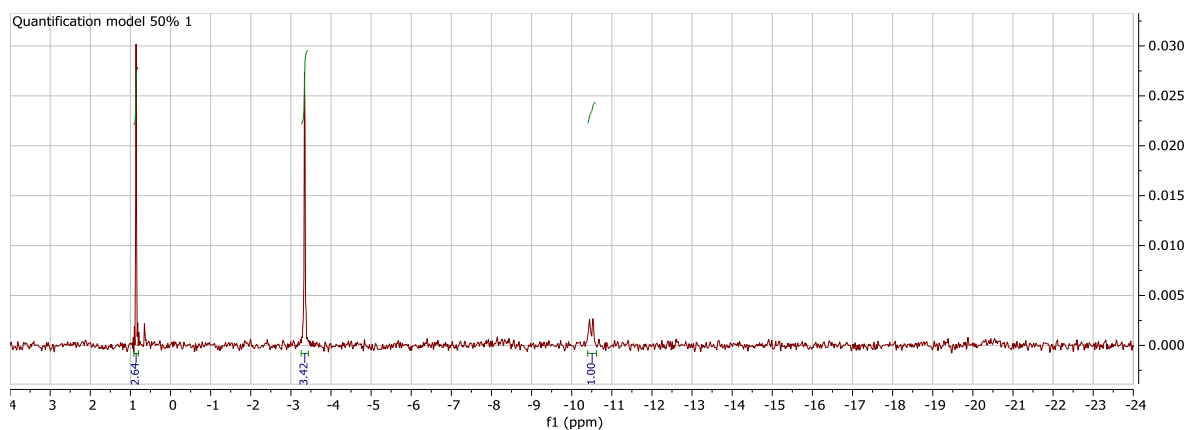


Figure A-I.62: ^{31}P NMR spectra from quantification model 50% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

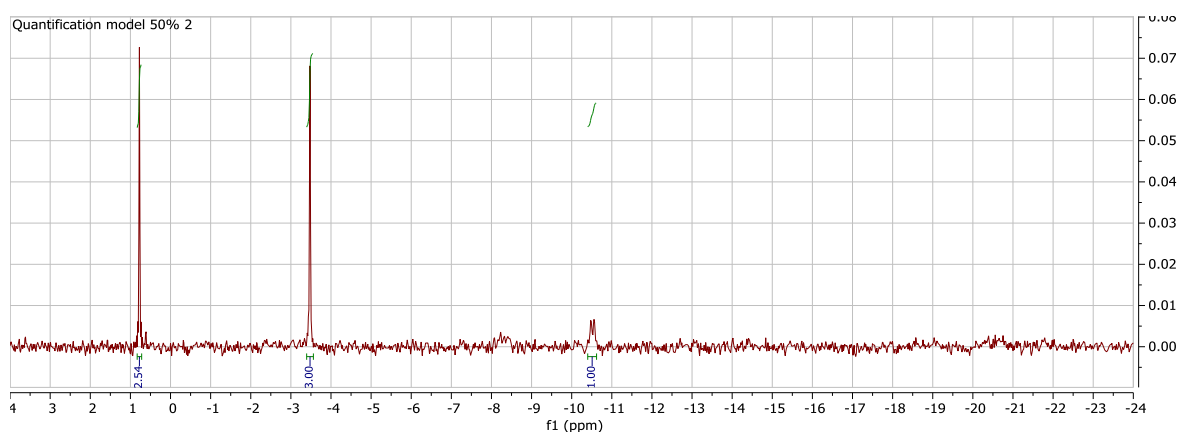


Figure A-I.63: ^{31}P NMR spectra from quantification model 50% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

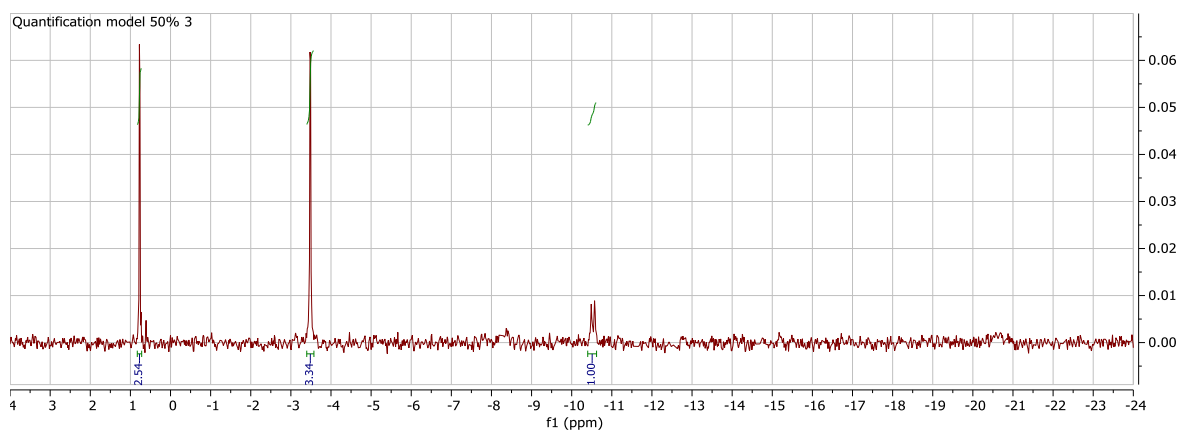


Figure A-I.64: ^{31}P NMR spectra from quantification model 50% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

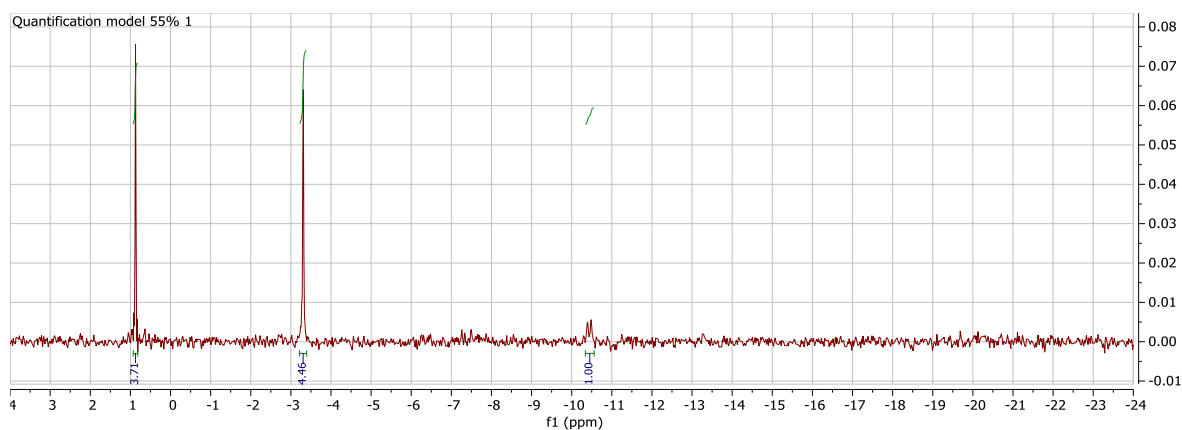


Figure A-I.65: ^{31}P NMR spectra from quantification model 55% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

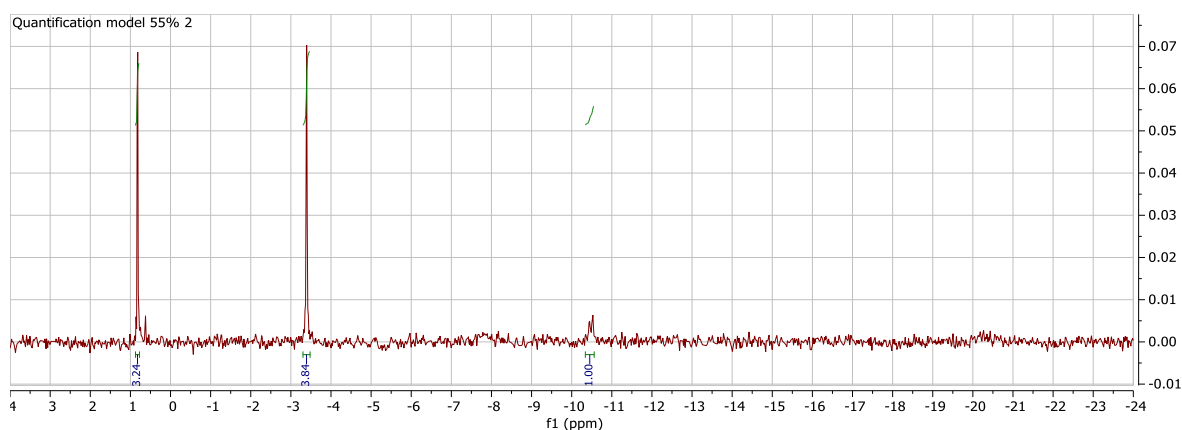


Figure A-I.66: ^{31}P NMR spectra from quantification model 55% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

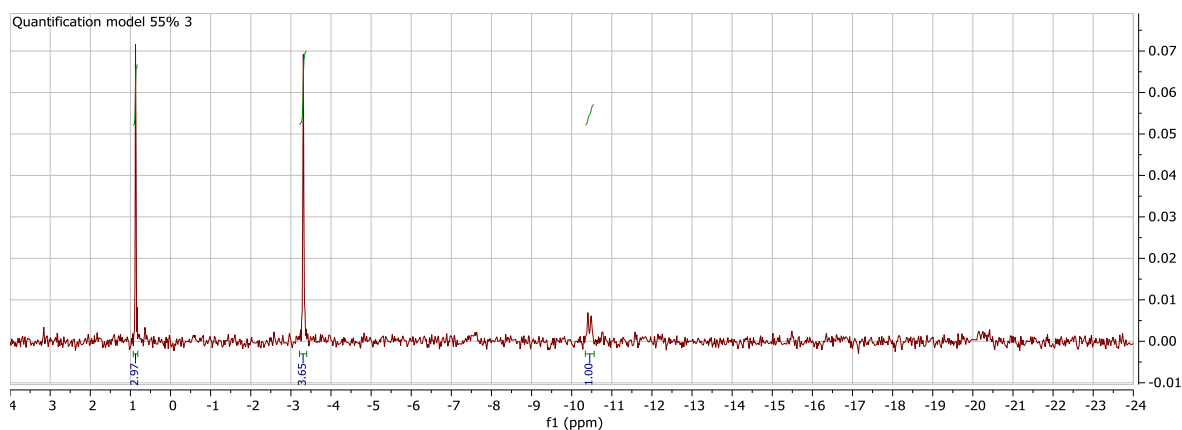


Figure A-I.67: ^{31}P NMR spectra from quantification model 55% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

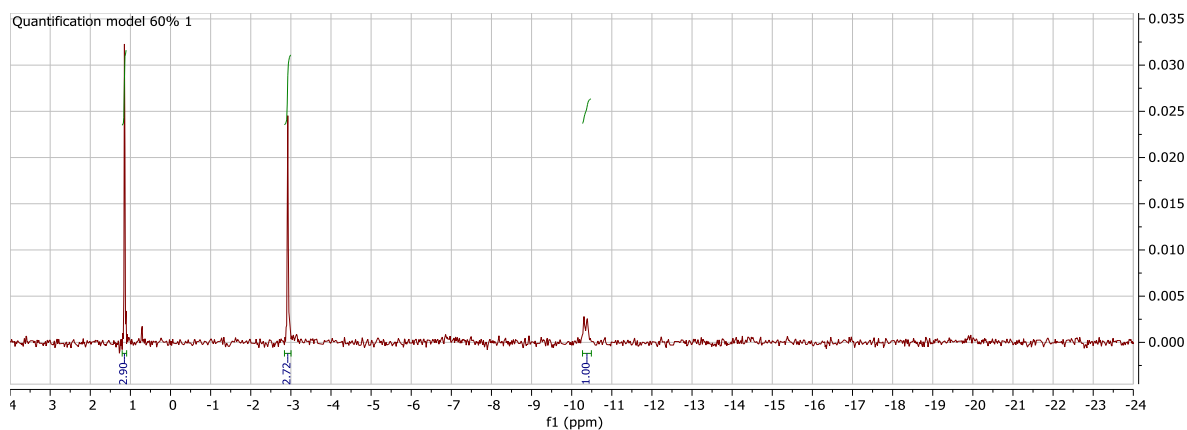


Figure A-I.68: ^{31}P NMR spectra from quantification model 60% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

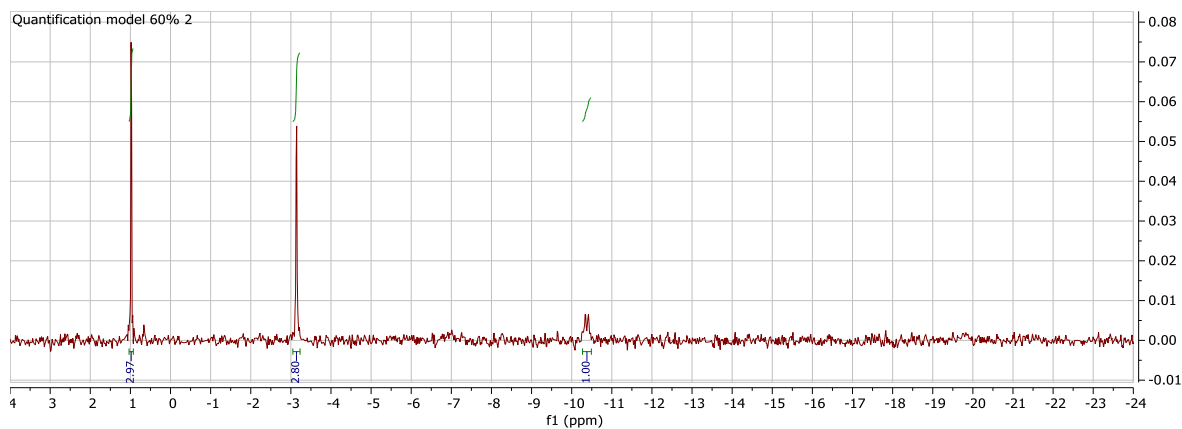


Figure A-I.69: ^{31}P NMR spectra from quantification model 60% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

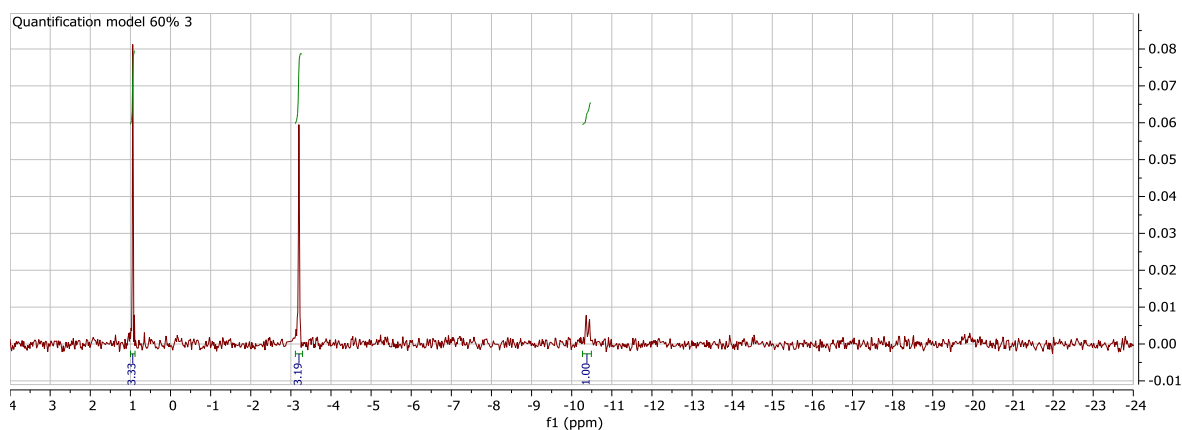


Figure A-I.70: ^{31}P NMR spectra from quantification model 60% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

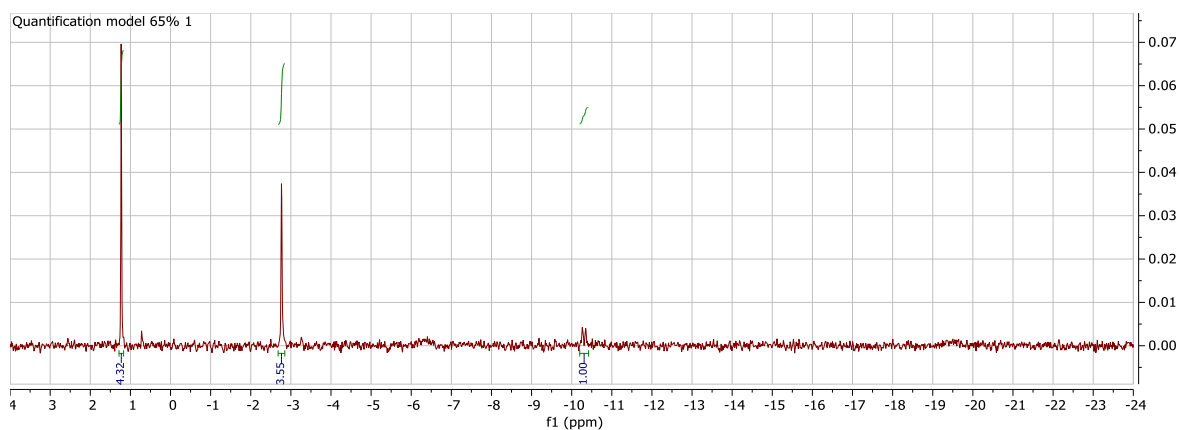


Figure A-I.71: ^{31}P NMR spectra from quantification model 65% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

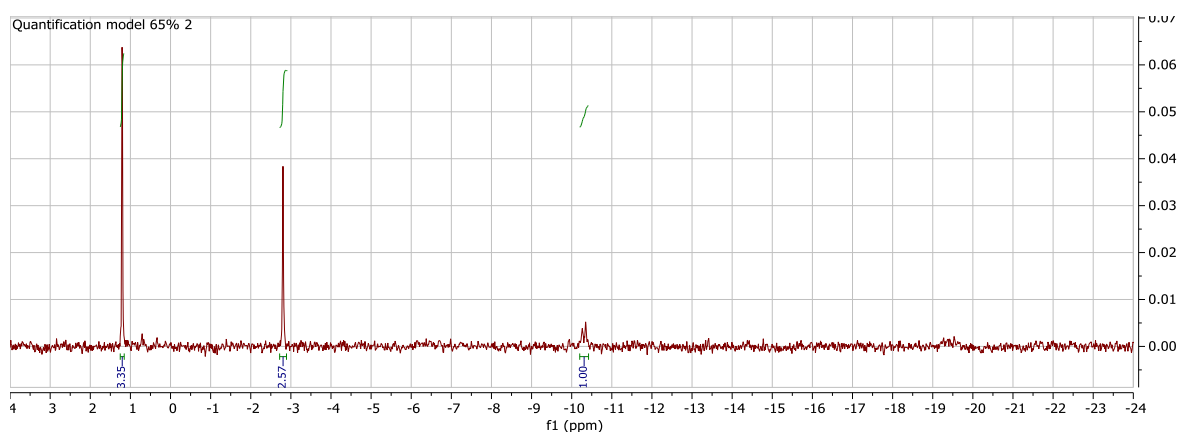


Figure A-I.72: ^{31}P NMR spectra from quantification model 65% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

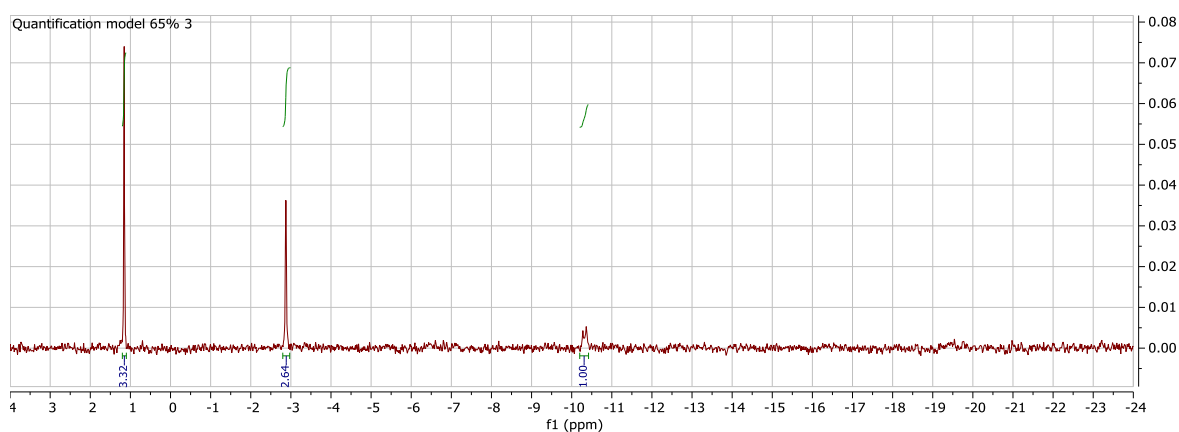


Figure A-I.73: ^{31}P NMR spectra from quantification model 65% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

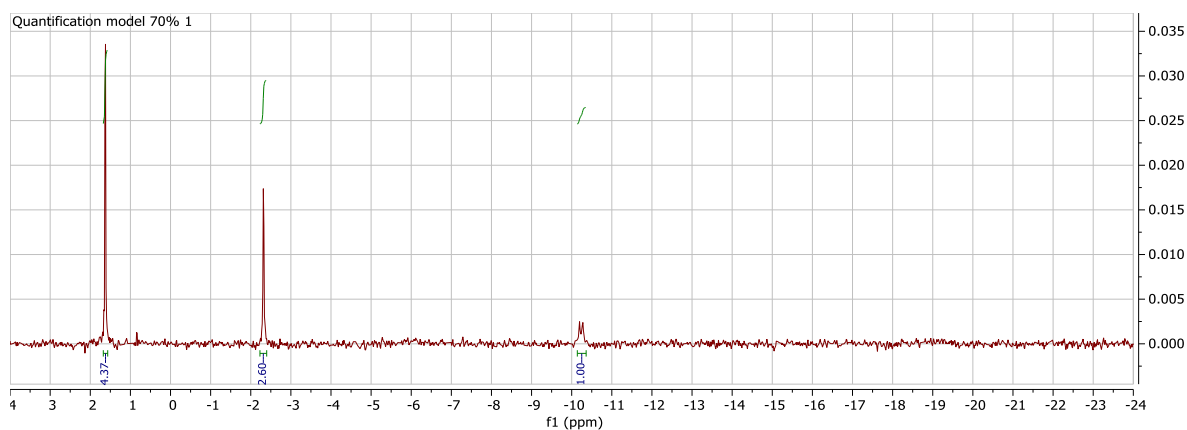


Figure A-I.74: ^{31}P NMR spectra from quantification model 70% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

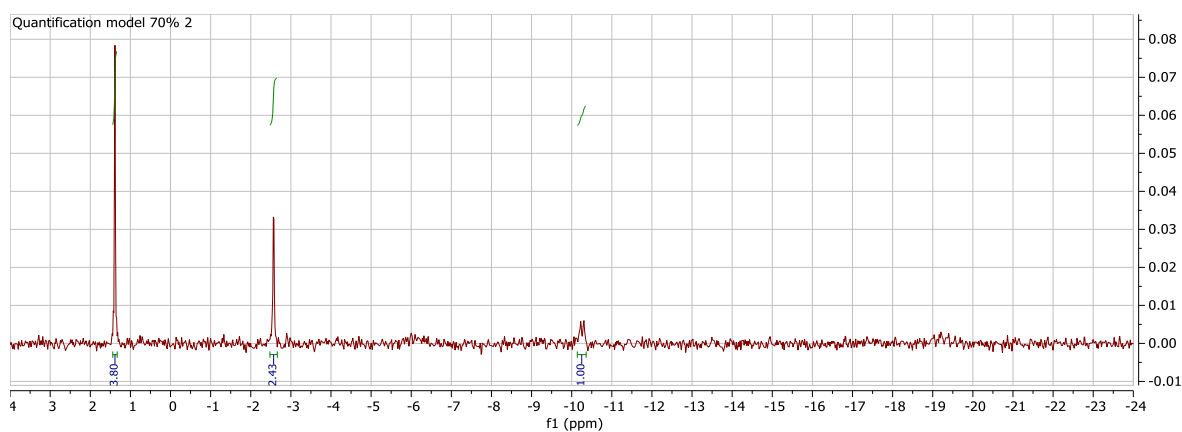


Figure A-I.75: ^{31}P NMR spectra from quantification model 70% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

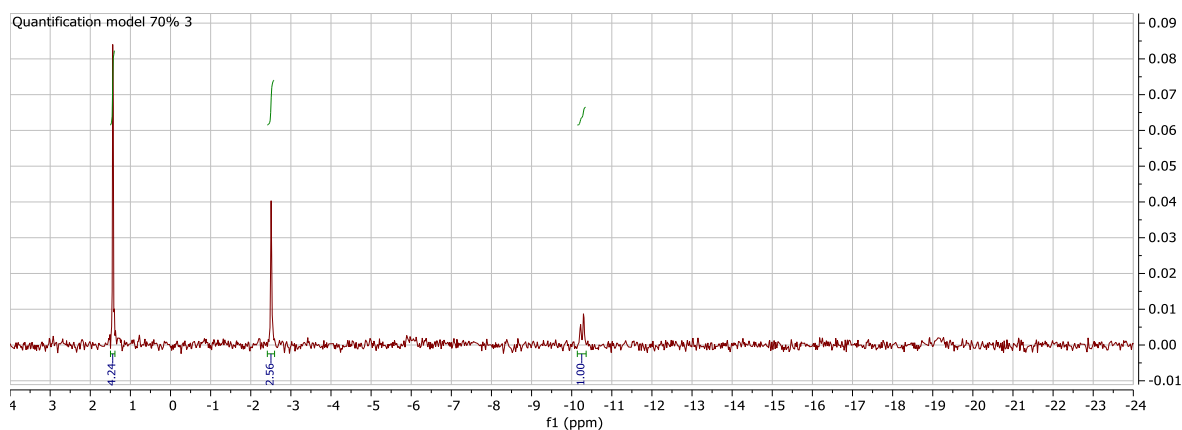


Figure A-I.76: ^{31}P NMR spectra from quantification model 70% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

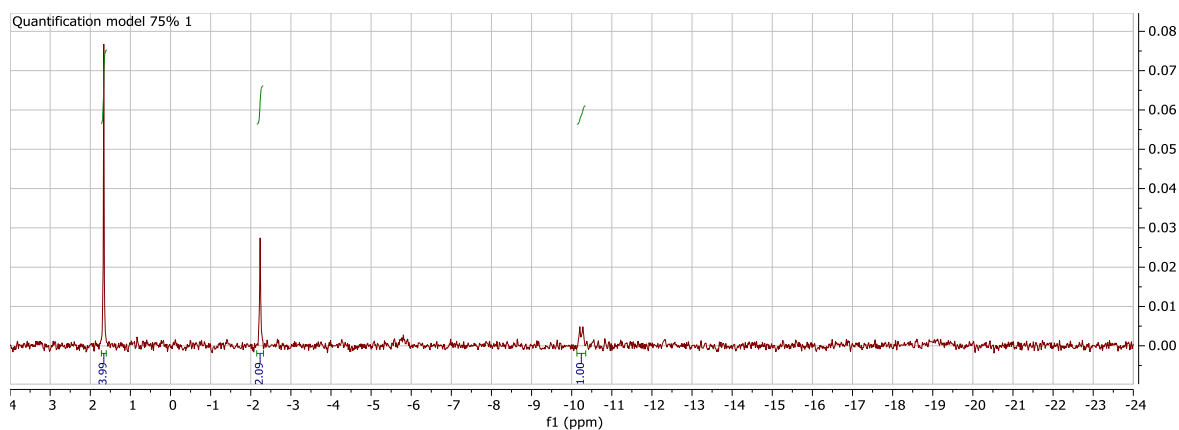


Figure A-I.77: ^{31}P NMR spectra from quantification model 75% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

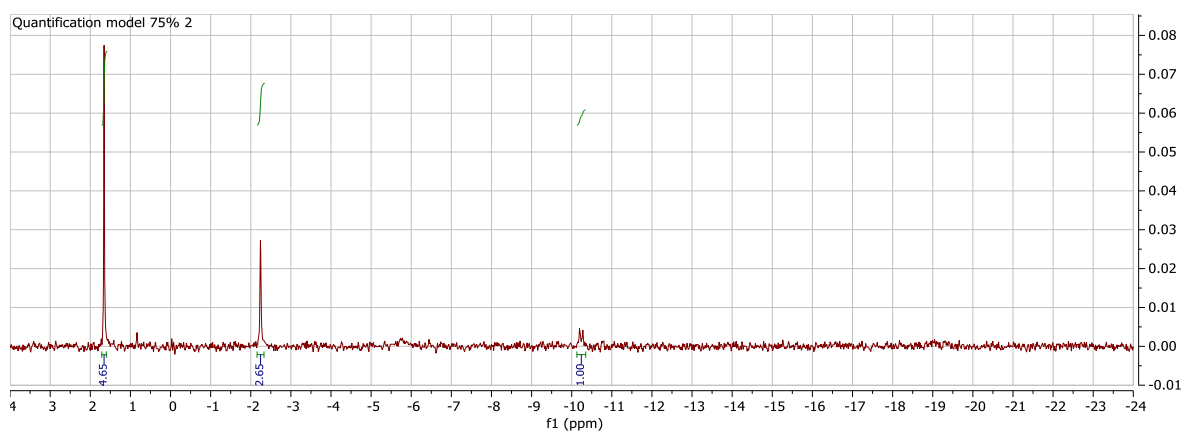


Figure A-I.78: ^{31}P NMR spectra from quantification model 75% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

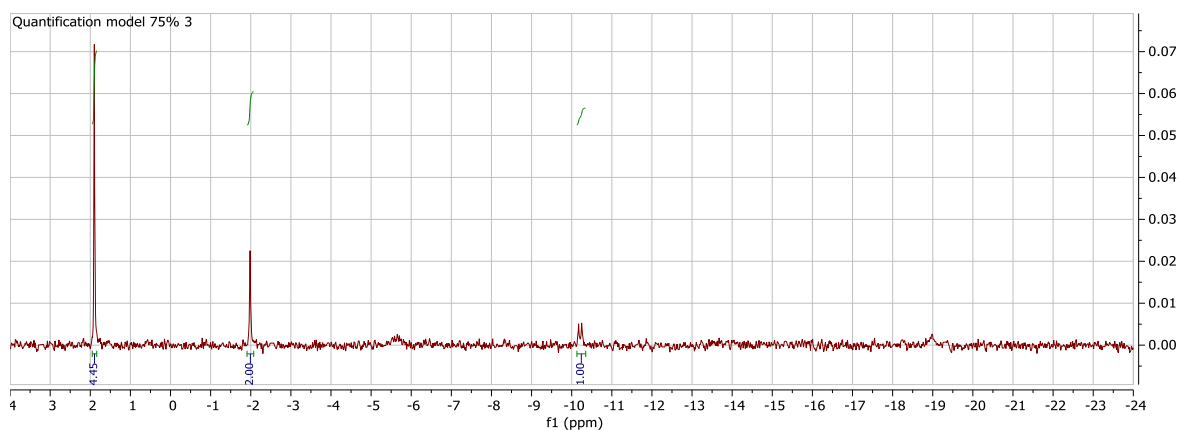


Figure A-I.79: ^{31}P NMR spectra from quantification model 75% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

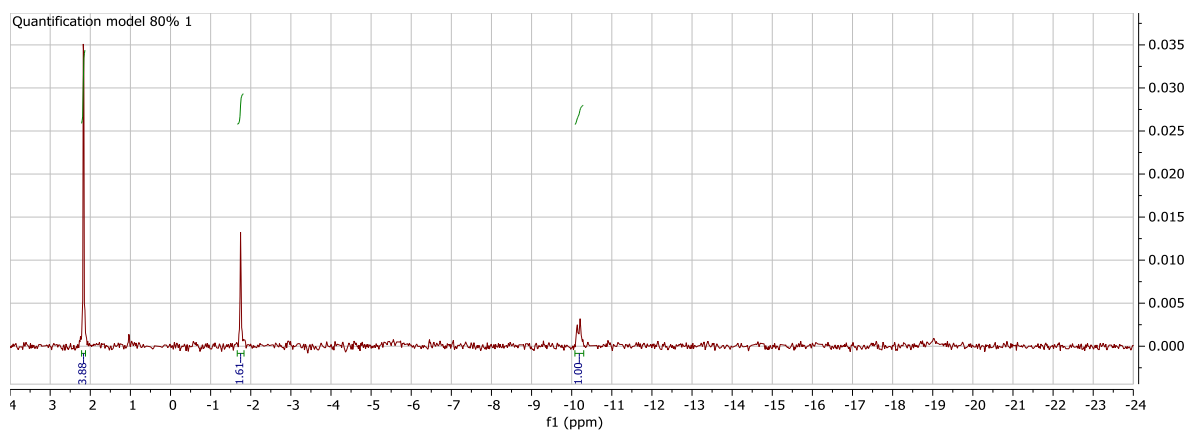


Figure A-I.80: ^{31}P NMR spectra from quantification model 80% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

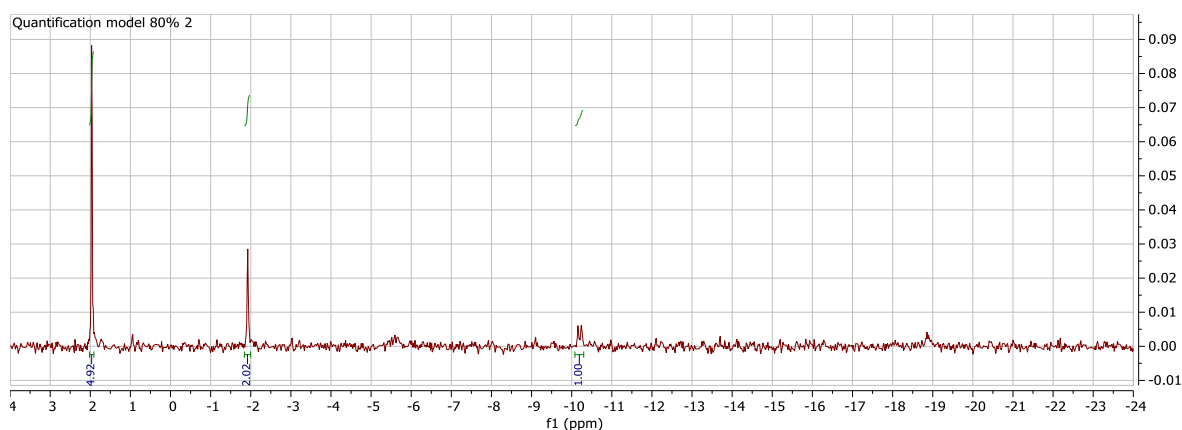


Figure A-I.81: ^{31}P NMR spectra from quantification model 80% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

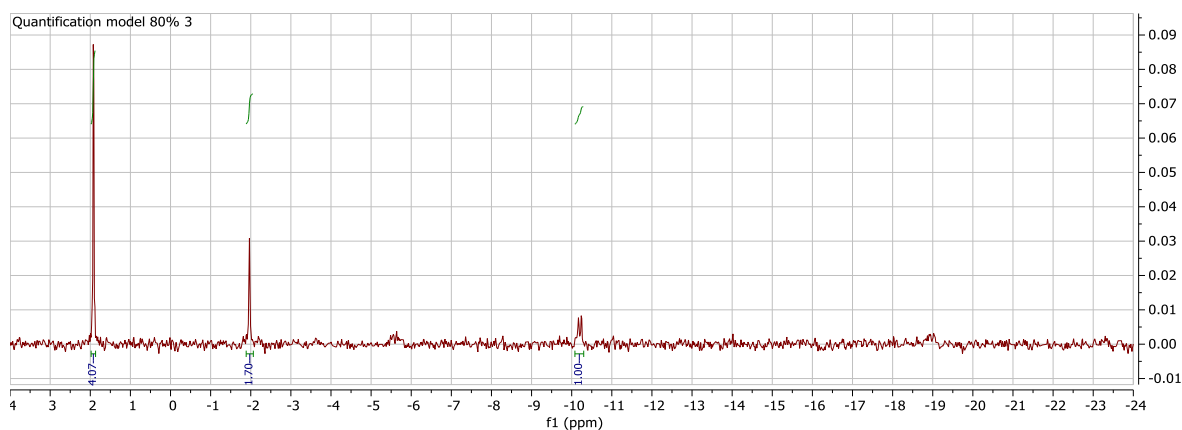


Figure A-I.82: ^{31}P NMR spectra from quantification model 80% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

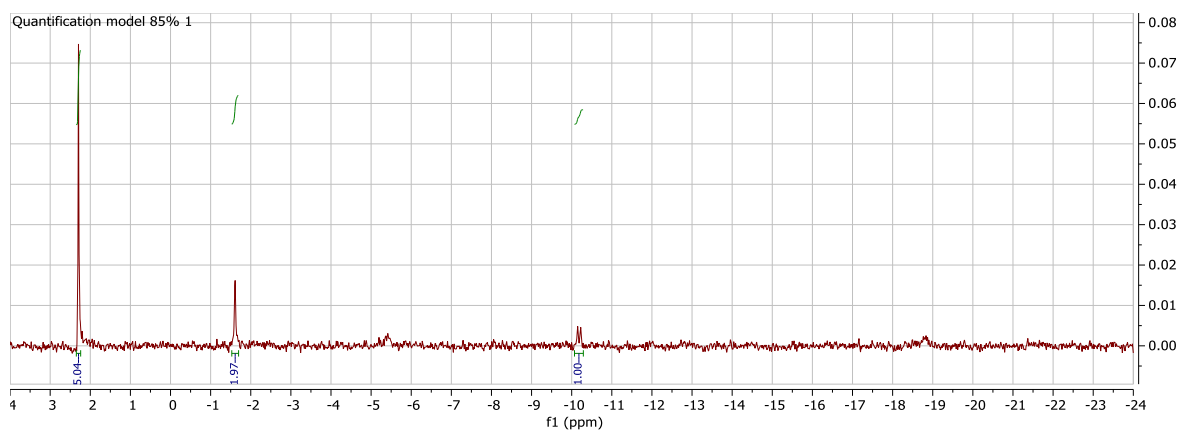


Figure A-I.83: ^{31}P NMR spectra from quantification model 85% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

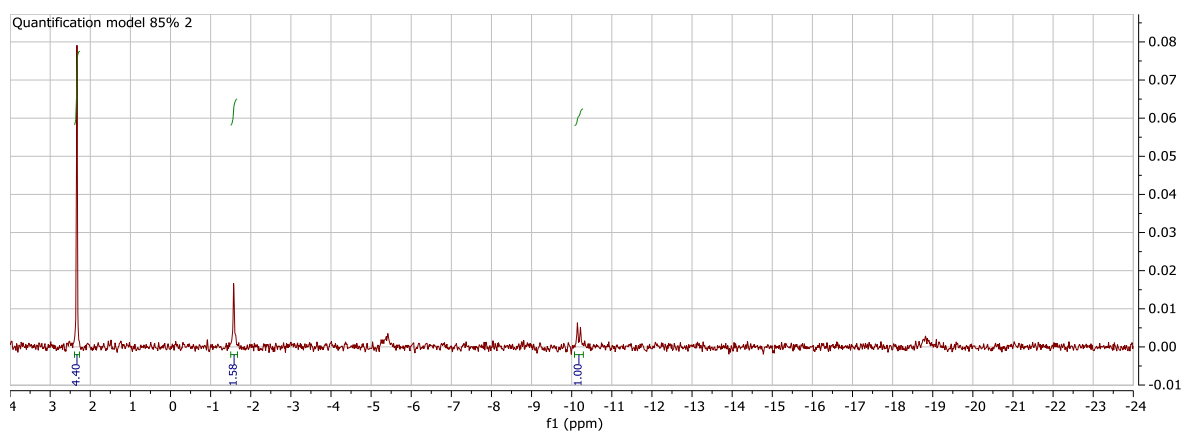


Figure A-I.84: ^{31}P NMR spectra from quantification model 85% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

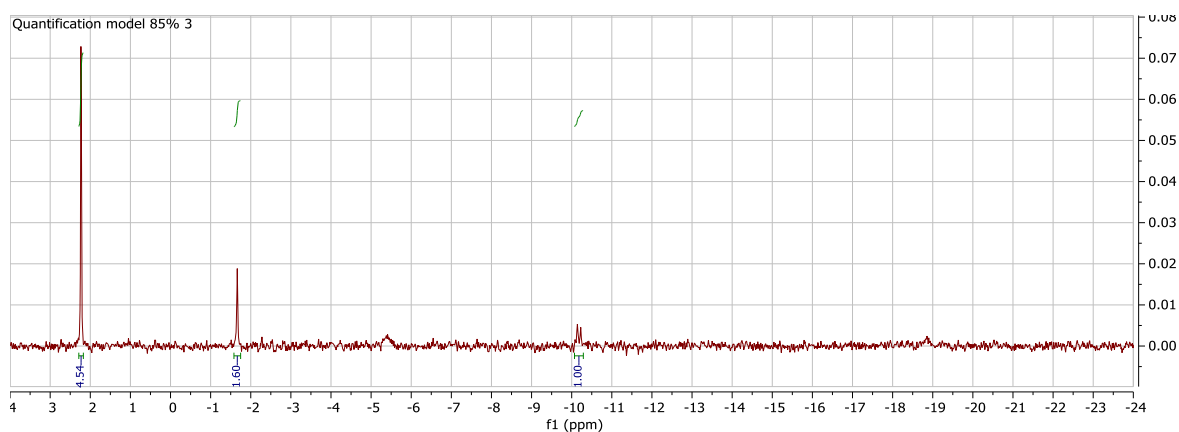


Figure A-I.85: ^{31}P NMR spectra from quantification model 85% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

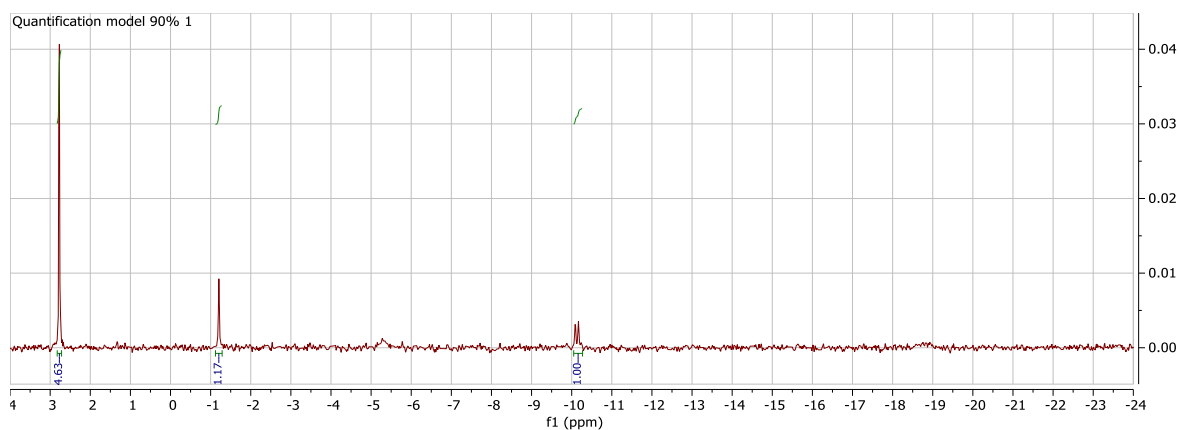


Figure A-I.86: ^{31}P NMR spectra from quantification model 90% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

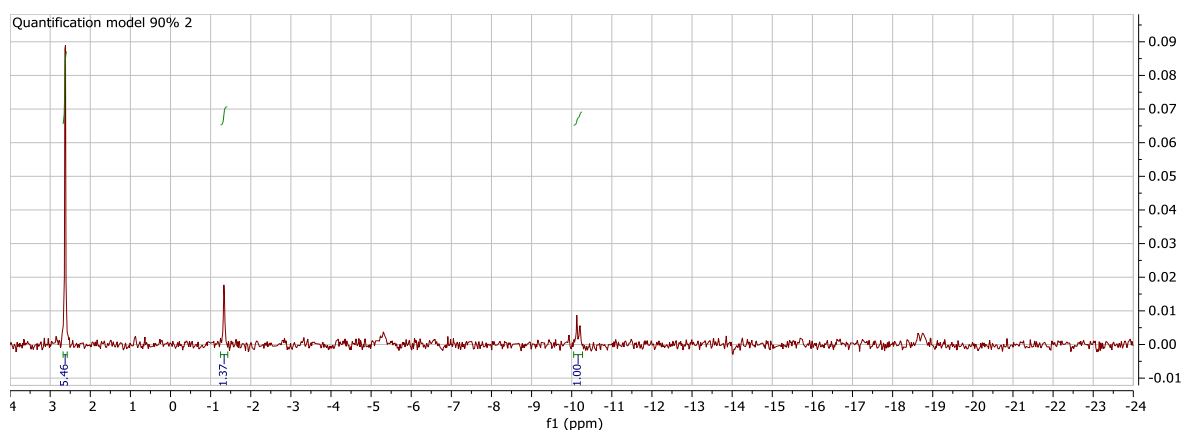


Figure A-I.87: ^{31}P NMR spectra from quantification model 90% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

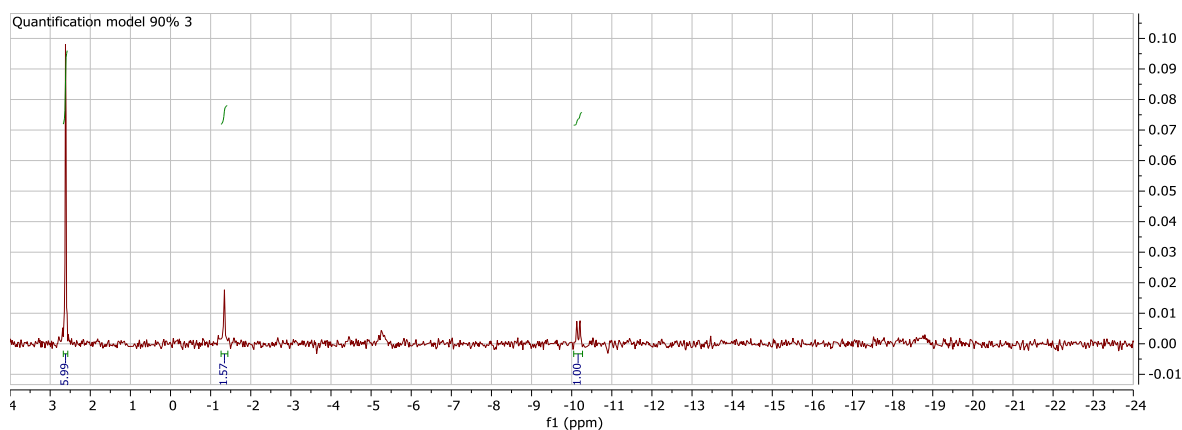


Figure A-I.88: ^{31}P NMR spectra from quantification model 90% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

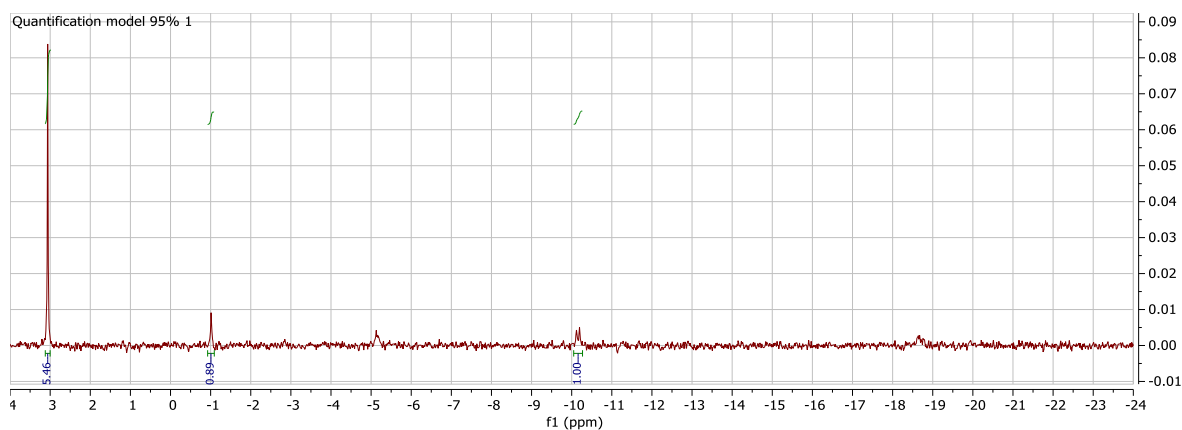


Figure A-I.89: ^{31}P NMR spectra from quantification model 95% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

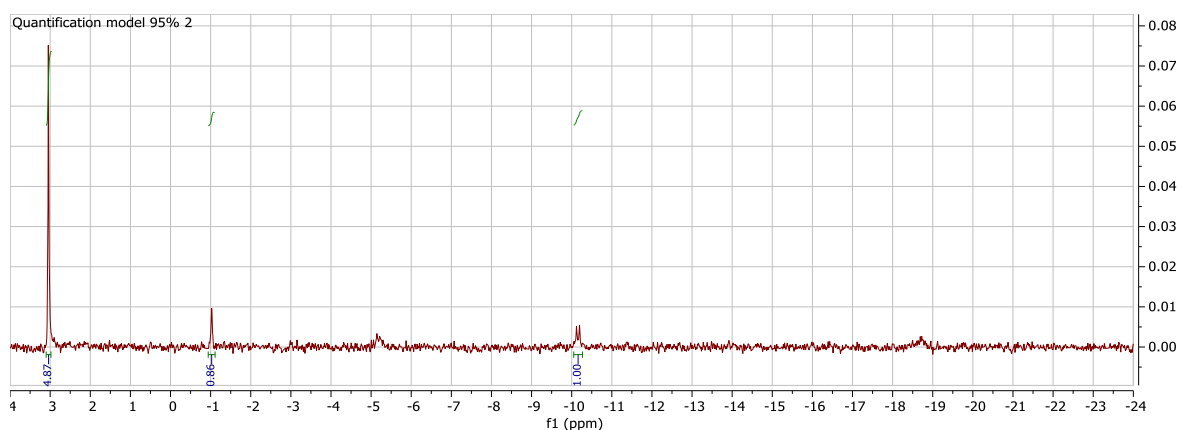


Figure A-I.90: ^{31}P NMR spectra from quantification model 95% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

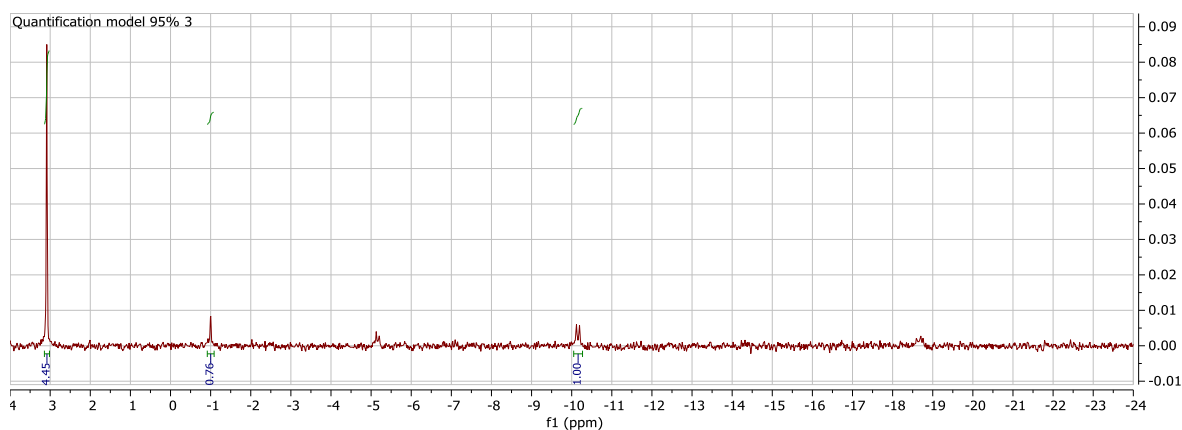


Figure A-I.91: ^{31}P NMR spectra from quantification model 95% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

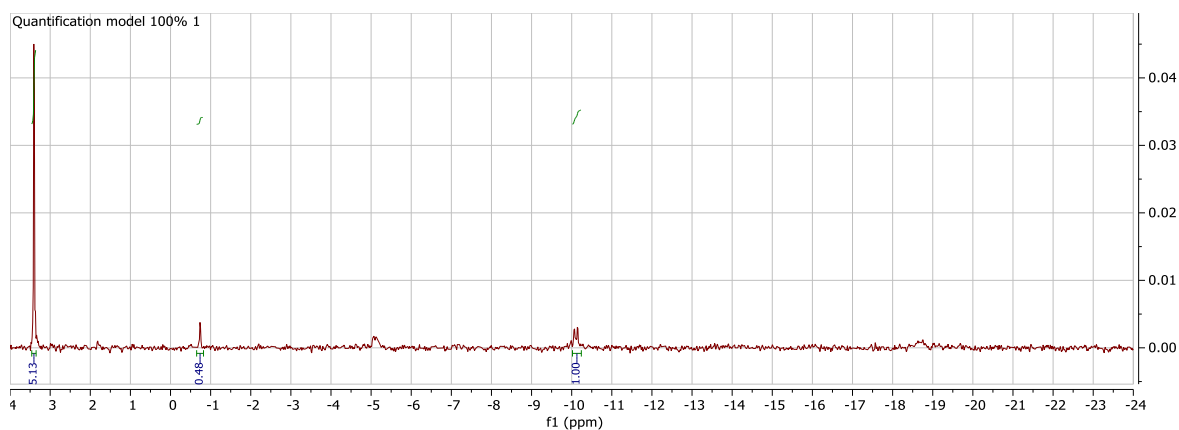


Figure A-I.92: ^{31}P NMR spectra from quantification model 100% conversion sample 1. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

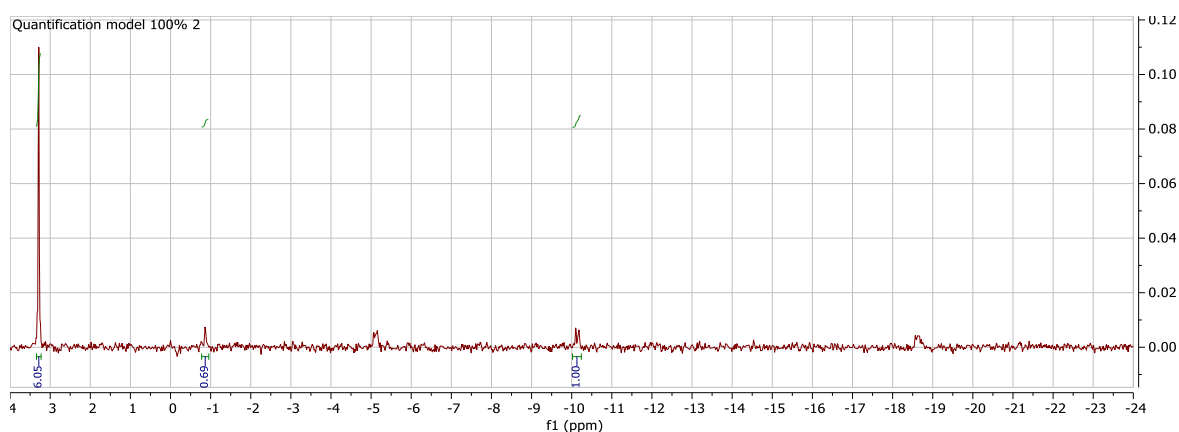


Figure A-I.93: ^{31}P NMR spectra from quantification model 100% conversion sample 2. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

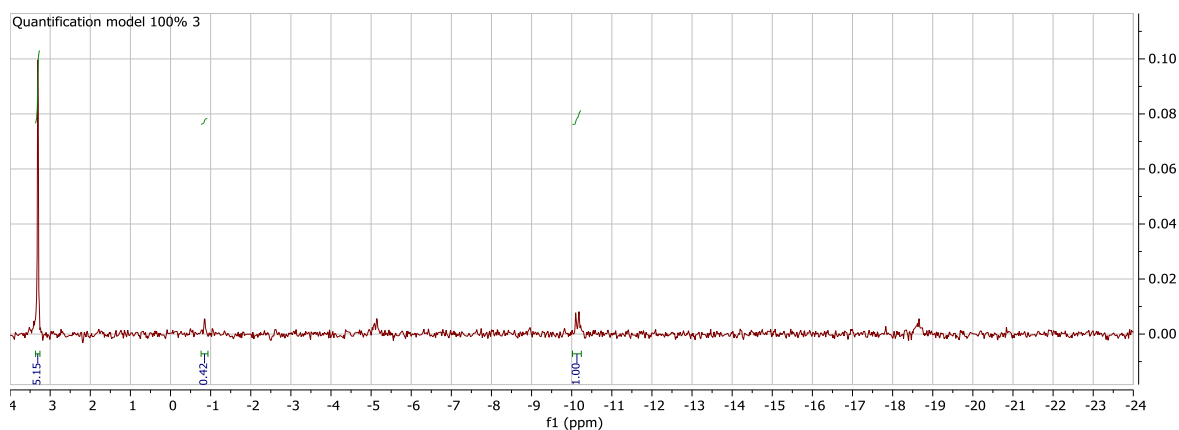


Figure A-I.94: ^{31}P NMR spectra from quantification model 100% conversion sample 3. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.1.

Conversion (%)	Sample	UMP Integral	PEP Integral	X
0	1	0.000	6.803	0.000
	2	0.000	6.193	0.000
	3	0.000	6.889	0.000
5	1	0.329	6.402	0.049
	2	0.310	5.990	0.049
	3	0.336	5.925	0.054
10	1	0.550	5.470	0.091
	2	0.536	6.050	0.081
	3	0.700	6.256	0.101
15	1	0.751	5.247	0.125
	2	0.862	6.281	0.121
	3	0.815	6.405	0.113
20	1	1.139	5.358	0.175
	2	1.395	5.922	0.191
	3	1.309	5.922	0.181
25	1	1.223	4.678	0.207
	2	1.465	5.568	0.208
	3	1.210	4.371	0.217
30	1	1.711	4.584	0.272
	2	1.745	4.711	0.270
	3	1.537	4.381	0.260
35	1	1.767	4.212	0.296
	2	2.025	4.865	0.294
	3	2.028	4.813	0.296
40	1	2.081	3.808	0.353
	2	2.429	4.905	0.331
	3	2.452	4.585	0.348
45	1	2.455	3.644	0.403
	2	2.252	3.709	0.378
	3	2.540	3.922	0.393
50	1	2.643	3.417	0.436
	2	2.541	2.996	0.459
	3	2.544	3.338	0.433
55	1	3.707	4.460	0.454
	2	3.241	3.843	0.458
	3	2.972	2.652	0.528
60	1	2.901	2.717	0.516
	2	2.970	2.797	0.515
	3	3.332	3.192	0.511
65	1	4.355	3.579	0.549
	2	3.295	2.530	0.566
	3	3.336	2.655	0.557
70	1	4.372	2.596	0.627
	2	3.800	2.430	0.610
	3	3.241	2.555	0.559
75	1	3.990	2.091	0.656
	2	4.648	2.648	0.637
	3	4.454	2.000	0.690
80	1	3.883	1.607	0.707

	2	4.918	2.023	0.709
	3	4.068	1.702	0.705
85	1	5.109	2.001	0.719
	2	4.432	1.592	0.736
	3	4.581	1.620	0.739
90	1	4.704	1.166	0.801
	2	5.480	1.371	0.800
	3	5.757	1.566	0.786
95	1	5.462	0.892	0.860
	2	4.867	0.860	0.850
	3	4.450	0.761	0.854
100	1	5.134	0.478	0.915
	2	6.046	0.690	0.898
	3	5.152	0.422	0.924

Table A-I.1: Peak data from ^{31}P NMR analysis of samples used in uridine substrate conversion quantification model. Spectra can be found in Section A-I.6. Model described in Section 4.7. NMR analysis conducted as described in Section 2.7.1.2. Peak integrals normalised to peak relating to α -phosphate of ATP. $X = \text{UMP peak} / (\text{UMP peak} + \text{PEP peak})$.

A-I.7 Quantification Model Test (25mM)

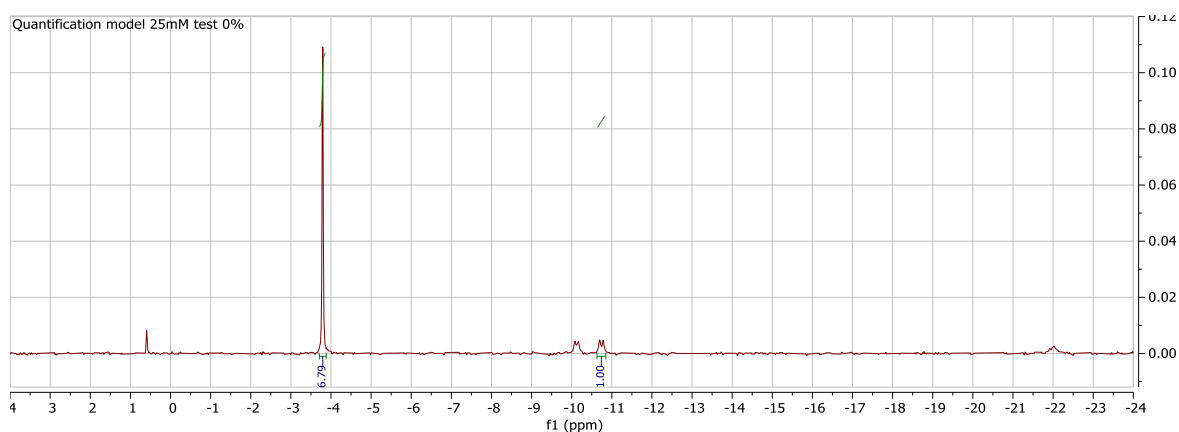


Figure A-I.95: ^{31}P NMR spectra from quantification model 25mM test 0% conversion sample. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.2.

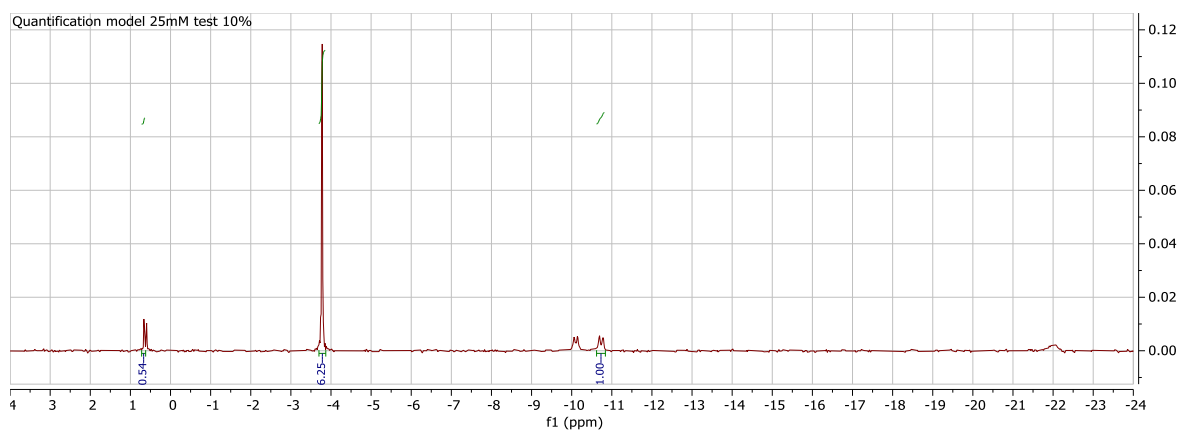


Figure A-I.96: ^{31}P NMR spectra from quantification model 25mM test 10% conversion sample. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.2.

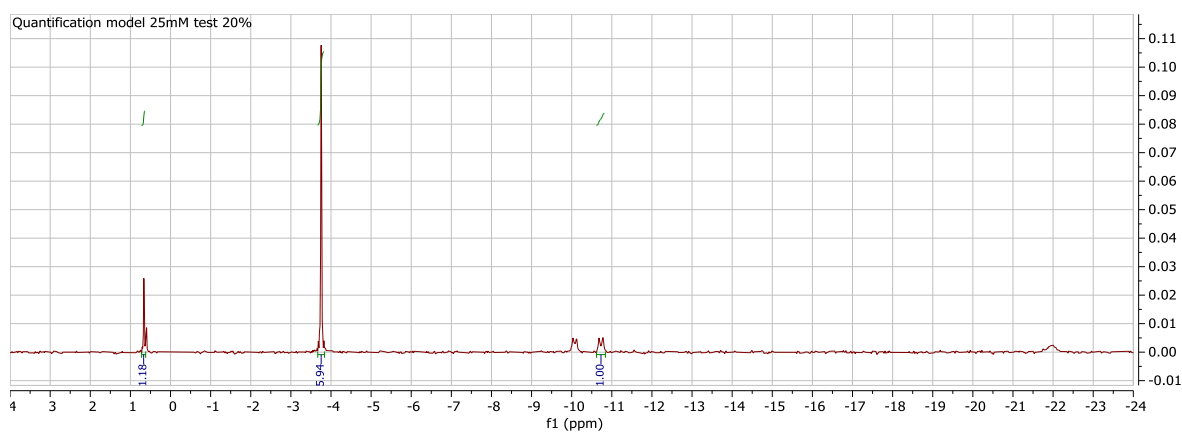


Figure A-I.97: ^{31}P NMR spectra from quantification model 25mM test 20% conversion sample. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.2.

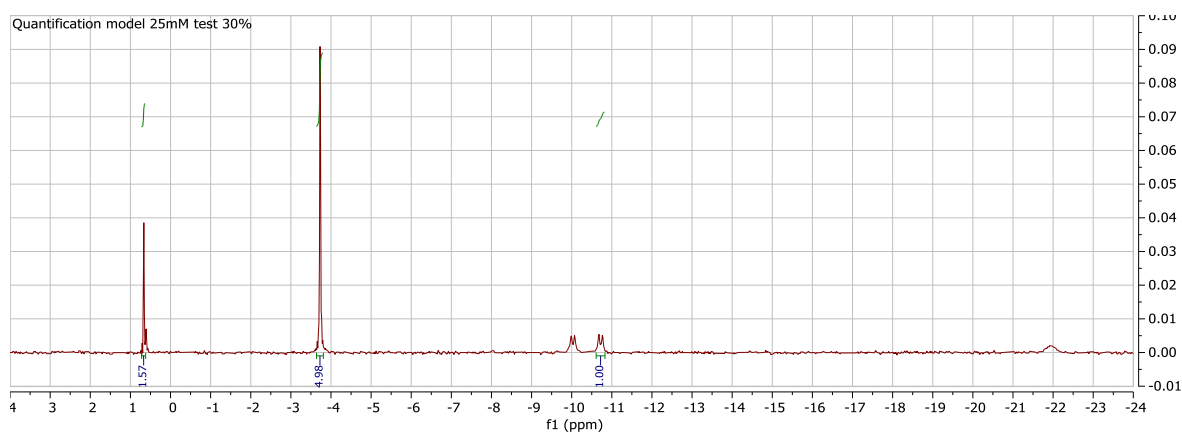


Figure A-I.98: ^{31}P NMR spectra from quantification model 25mM test 30% conversion sample. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.2.

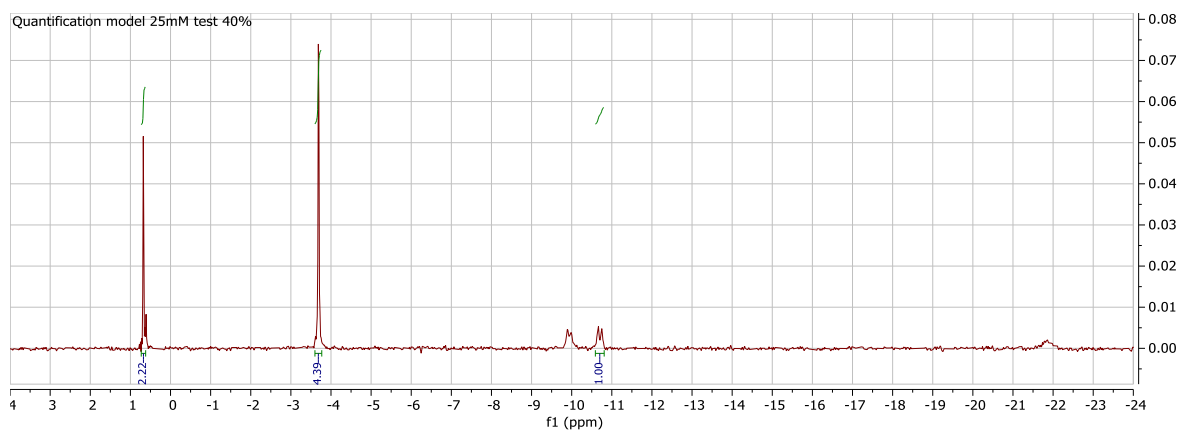


Figure A-I.99: ^{31}P NMR spectra from quantification model 25mM test 40% conversion sample. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.2.

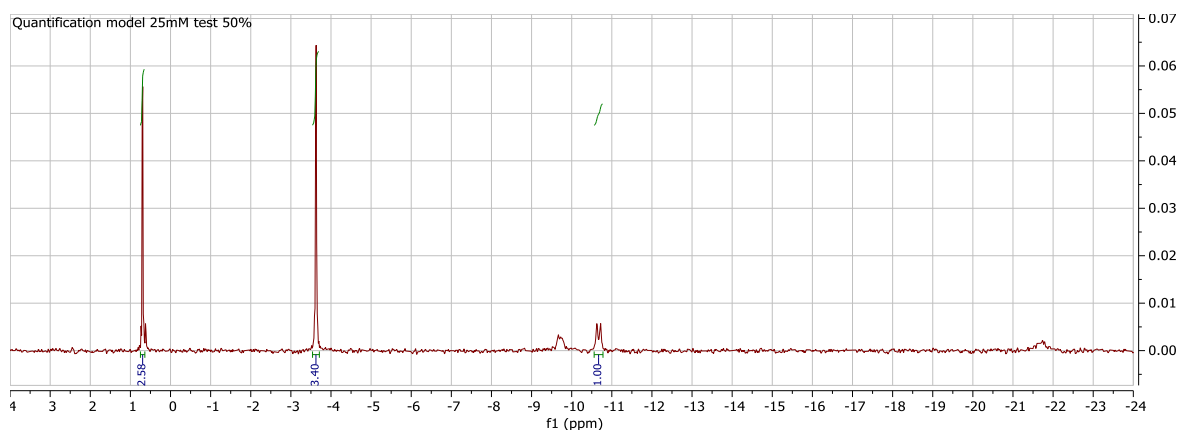


Figure A-I.100: ^{31}P NMR spectra from quantification model 25mM test 50% conversion sample. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.2.

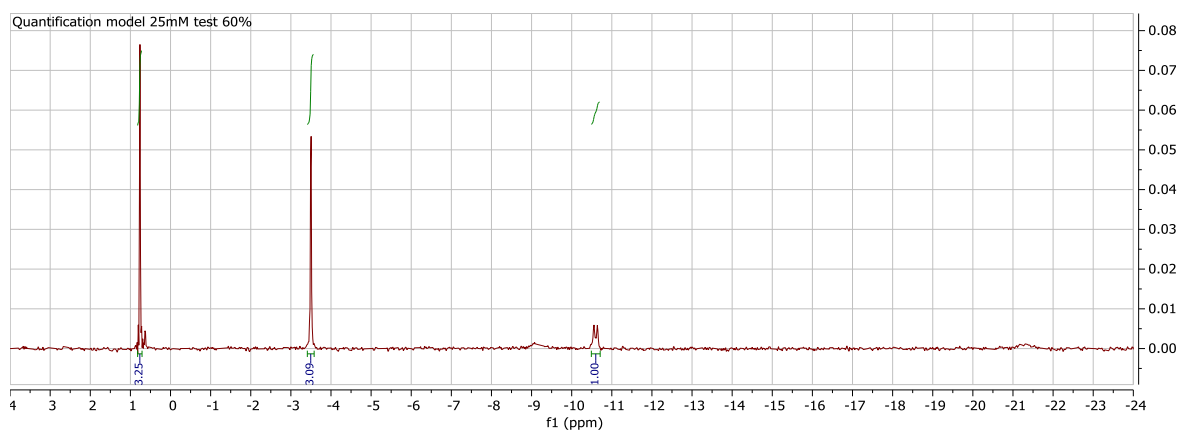


Figure A-I.101: ^{31}P NMR spectra from quantification model 25mM test 60% conversion sample. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.2.

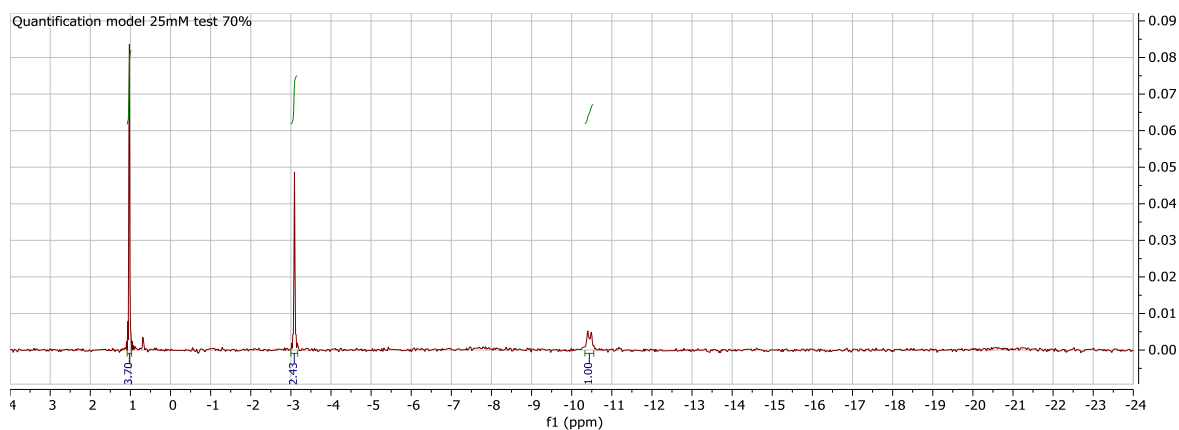


Figure A-I.102: ^{31}P NMR spectra from quantification model 25mM test 70% conversion sample. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.2.

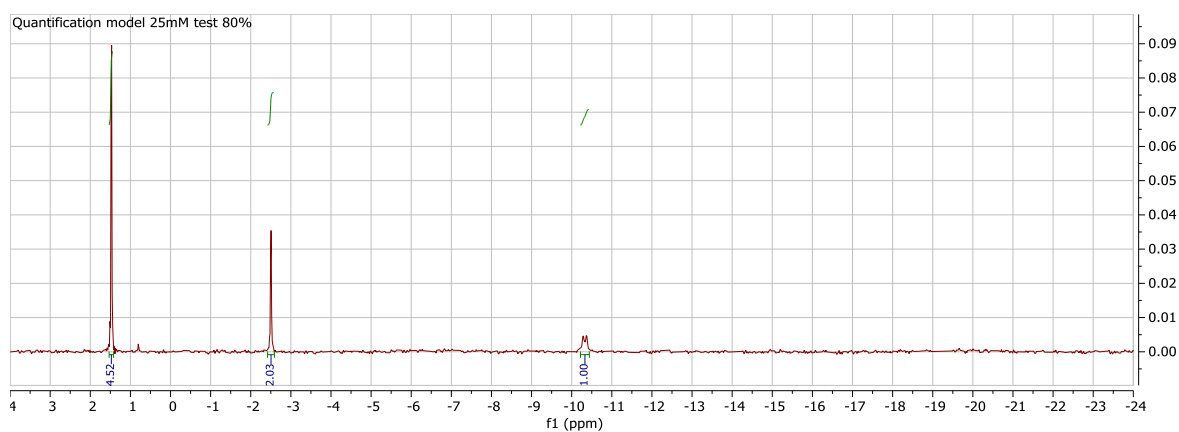


Figure A-I.103: ^{31}P NMR spectra from quantification model 25mM test 80% conversion sample. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.2.

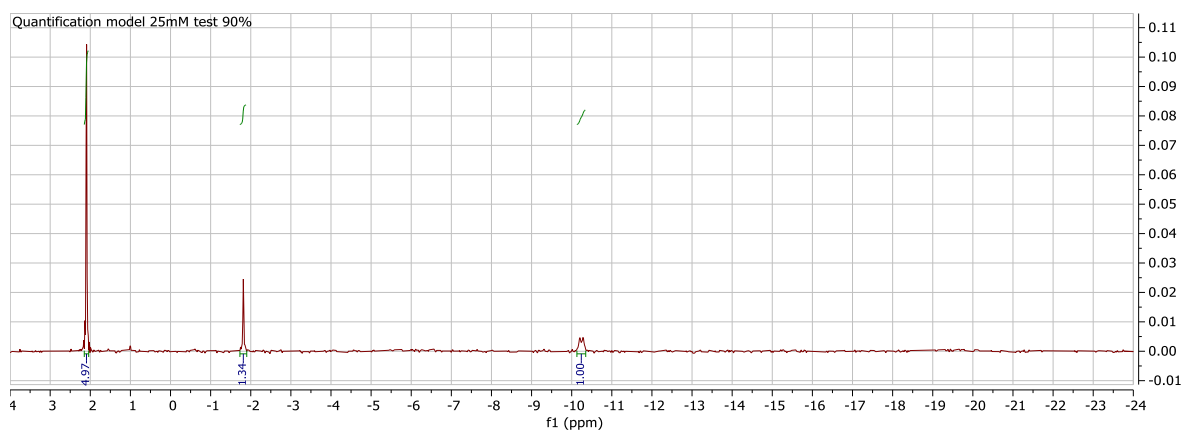


Figure A-I.104: ^{31}P NMR spectra from quantification model 25mM test 90% conversion sample. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.2.

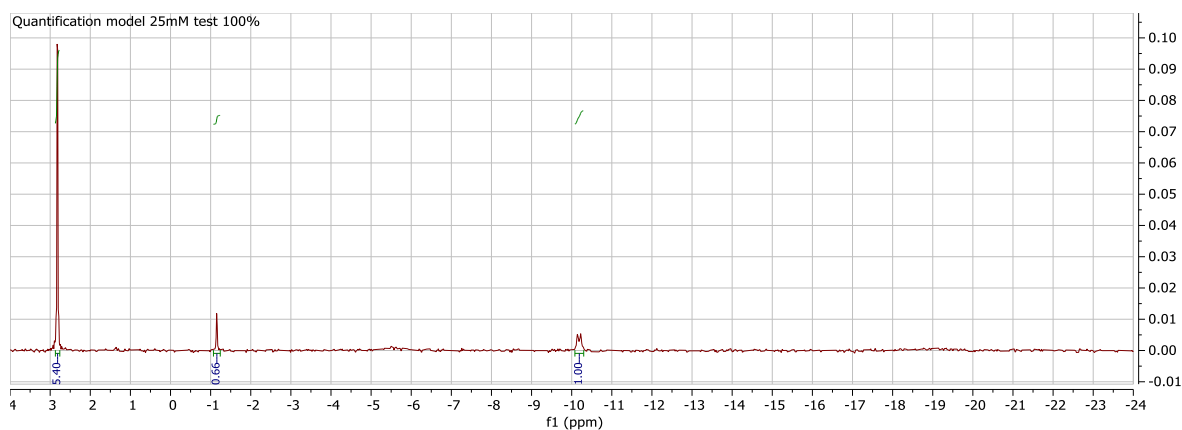


Figure A-I.105: ^{31}P NMR spectra from quantification model 25mM test 100% conversion sample. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.2.

Conversion (%)	UMP Integral	PEP Integral	X	Calculated Conversion
0	0.000	6.803	0.000	0.91
10	0.000	6.193	0.000	9.86
20	0.000	6.889	0.000	19.49
30	0.329	6.402	0.049	27.66
40	0.310	5.990	0.049	38.51
50	0.336	5.925	0.054	49.15
60	0.550	5.470	0.091	58.32
70	0.536	6.050	0.081	68.51
80	0.700	6.256	0.101	78.13
90	0.751	5.247	0.125	89.1
100	0.862	6.281	0.121	100.74

Table A-I.2: Peak data from ^{31}P NMR analysis of samples used in 25mM uridine substrate conversion quantification model test. Spectra can be found in Section A-I.7. Model test described in Section 4.7.3. NMR analysis conducted as described in Section 2.7.1.2. Peak integrals normalised to peak relating to α -phosphate of ATP. $X = \text{UMP peak} / (\text{UMP peak} + \text{PEP peak})$. Substrate conversion calculated using model described in Section 4.7.

A-I.8 UCK1 Concentration Study

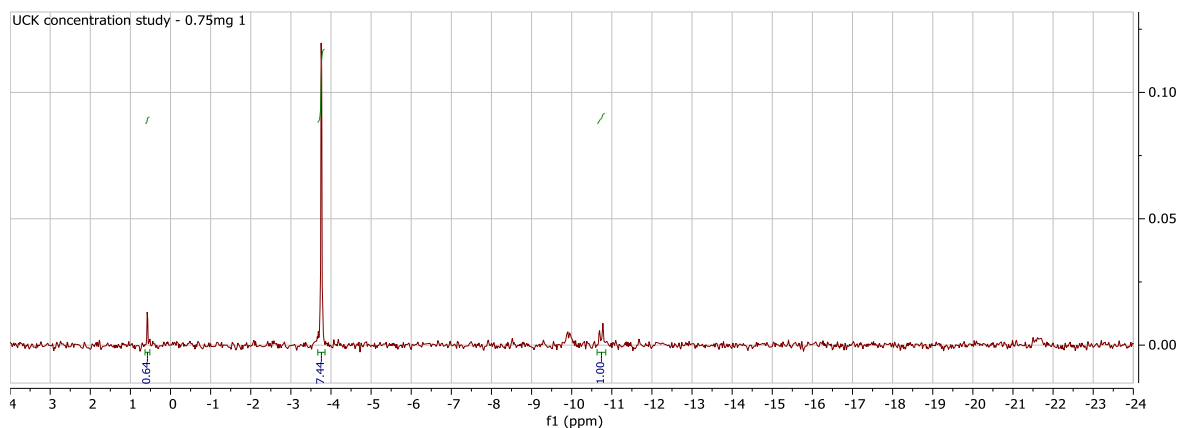


Figure A-I.106: ^{31}P NMR spectra of UCK1 concentration study 0.75 mg reaction sample 1. 0.75 mg UCK, 0.75 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

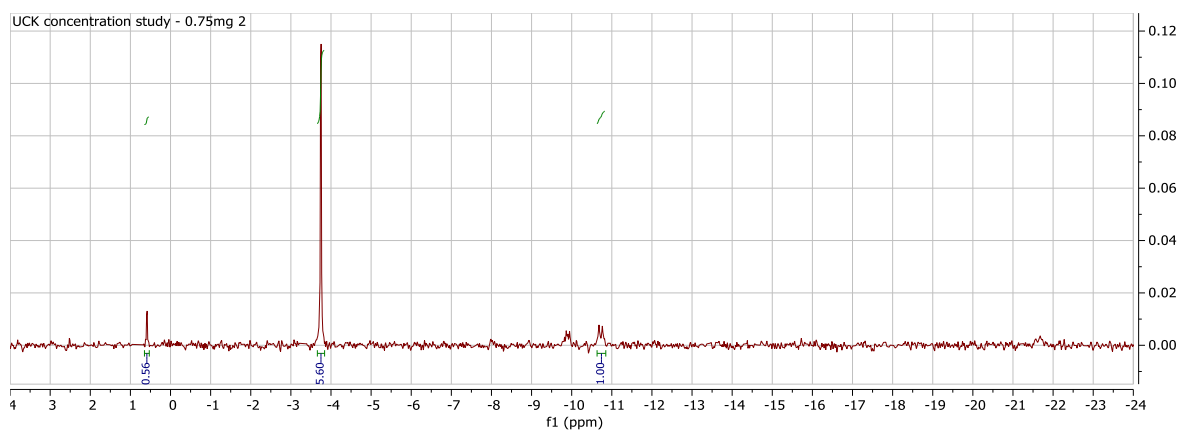


Figure A-I.107: ^{31}P NMR spectra of UCK concentration study 0.75 mg reaction sample 2. 0.75 mg UCK, 0.75 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

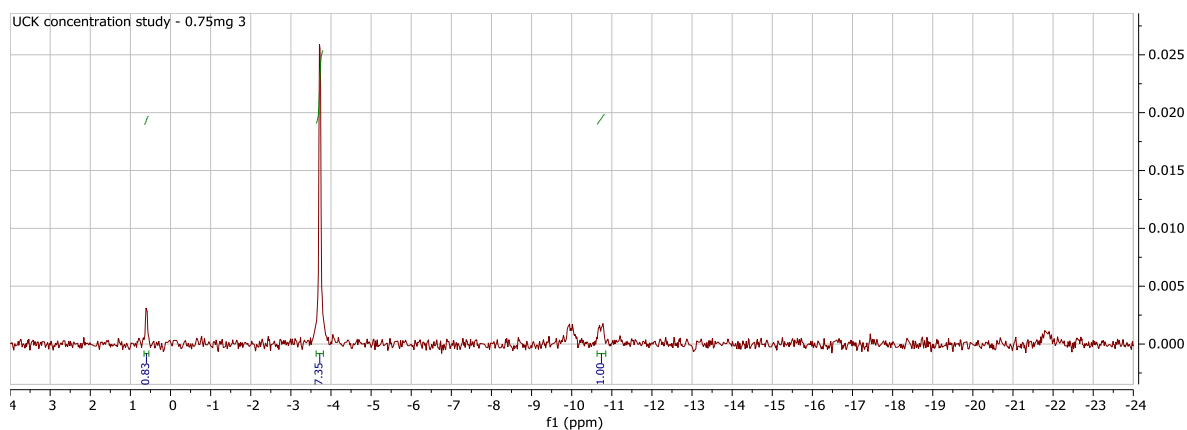


Figure A-I.108: ^{31}P NMR spectra of UCK concentration study 0.75 mg reaction sample 3. 0.75 mg UCK, 0.75 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

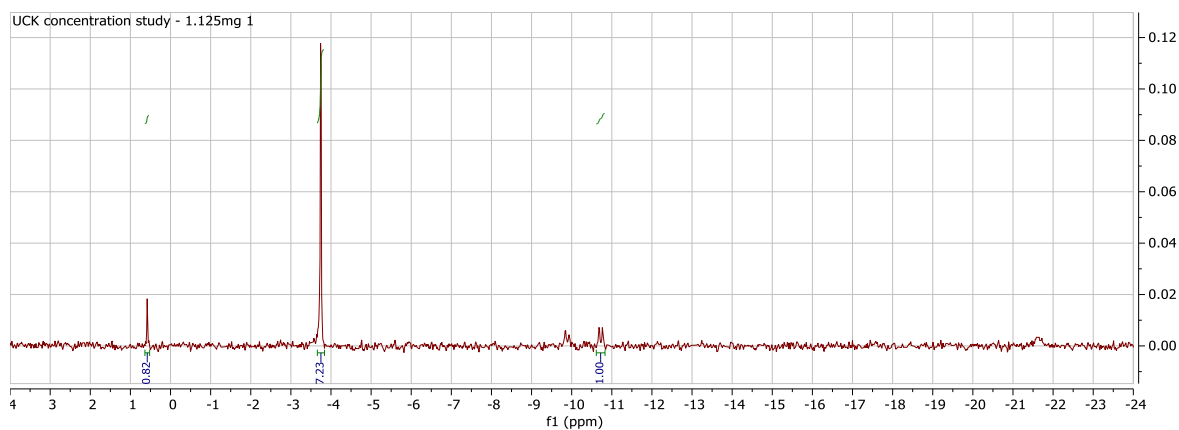


Figure A-I.109: ^{31}P NMR spectra of UCK concentration study 1.125 mg reaction sample 1. 1.125 mg UCK, 1.125 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

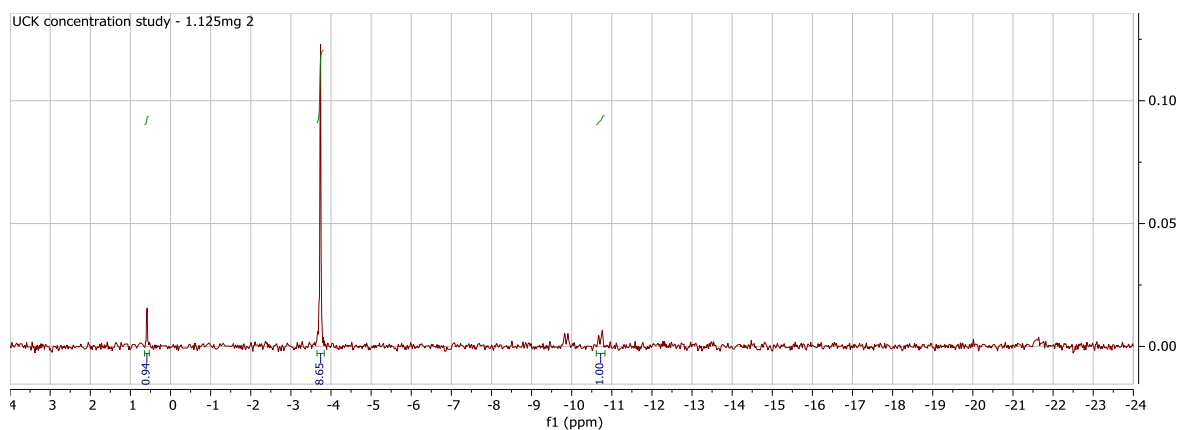


Figure A-I.110: ^{31}P NMR spectra of UCK concentration study 1.125 mg reaction sample 2. 1.125 mg UCK, 1.125 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

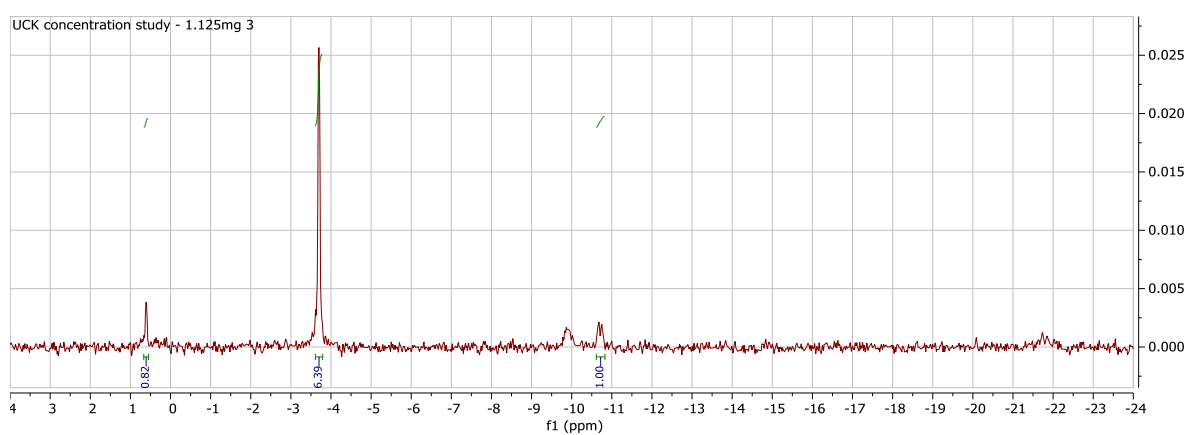


Figure A-I.111: ^{31}P NMR spectra of UCK concentration study 1.125 mg reaction sample 3. 1.125 mg UCK, 1.125 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

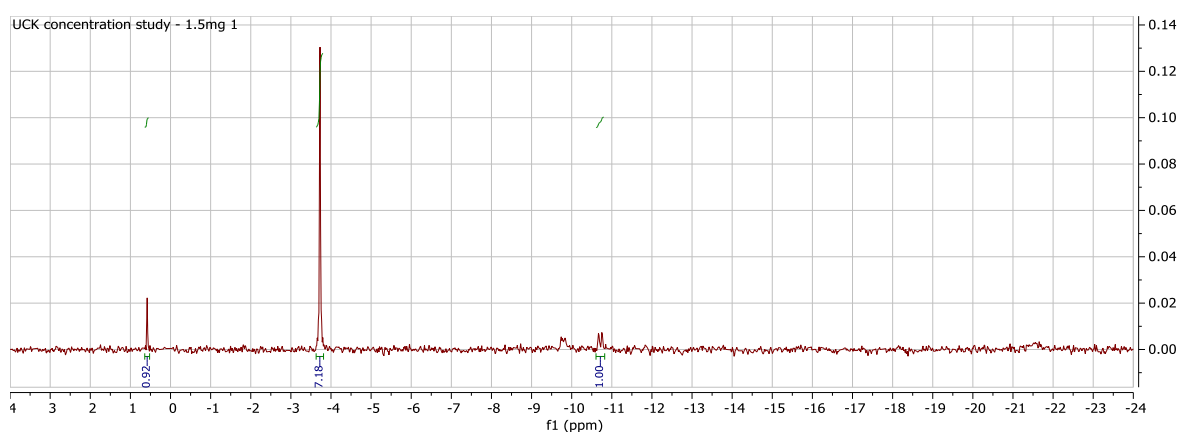


Figure A-I.112: ^{31}P NMR spectra of UCK concentration study 1.5 mg reaction sample 1. 1.5 mg UCK, 1.5 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

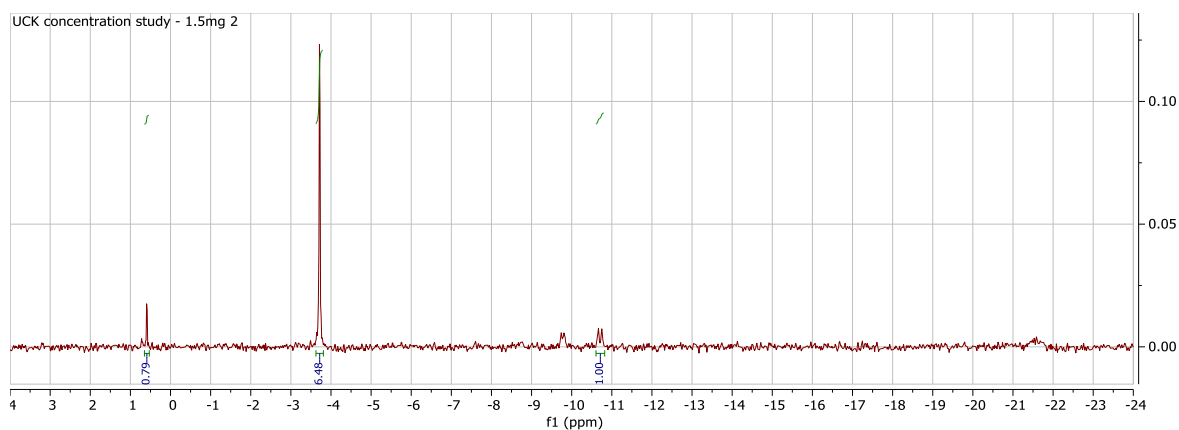


Figure A-I.113: ^{31}P NMR spectra of UCK concentration study 1.5 mg reaction sample 2. 1.5 mg UCK, 1.5 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

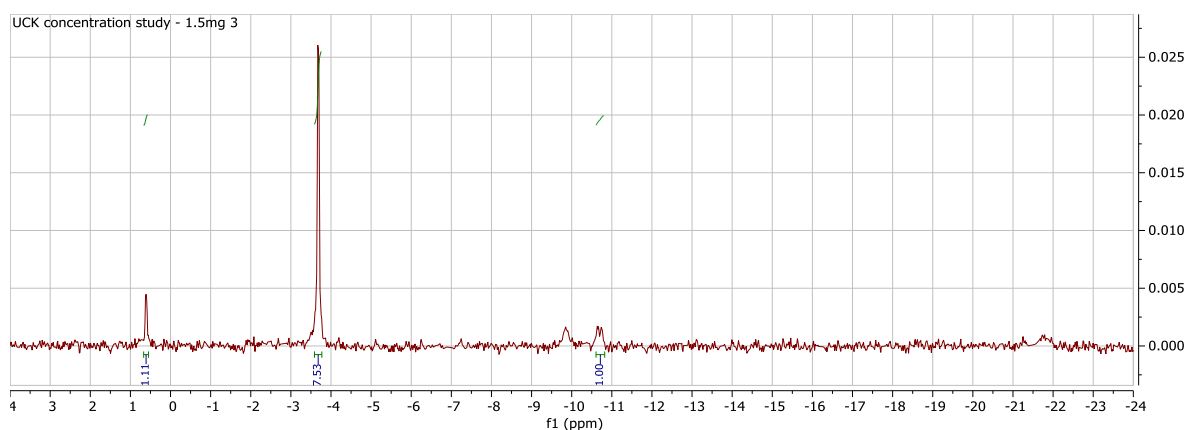


Figure A-I.114: ^{31}P NMR spectra of UCK concentration study 1.5 mg reaction sample 3. 1.5 mg UCK, 1.5 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

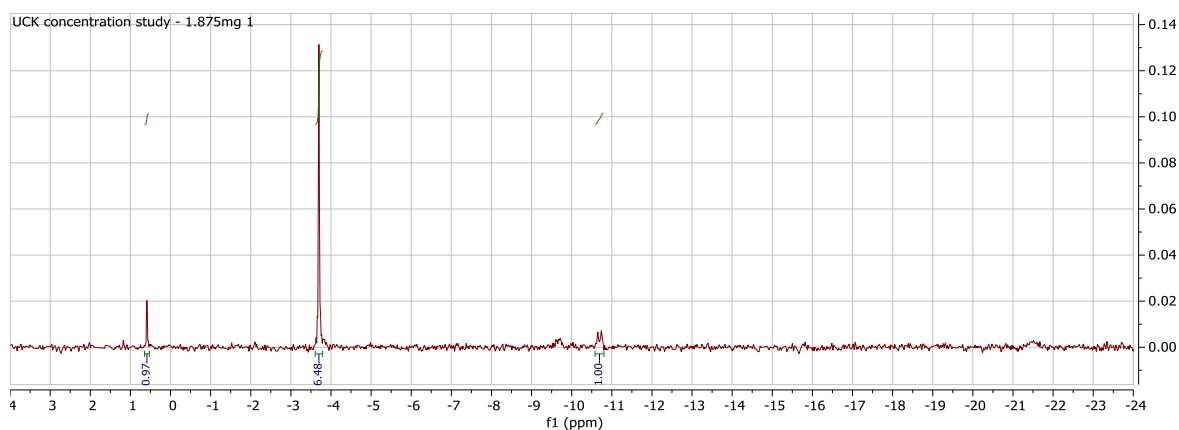


Figure A-I.115: ^{31}P NMR spectra of UCK concentration study 1.875 mg reaction sample 1. 1.875 mg UCK, 1.875 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

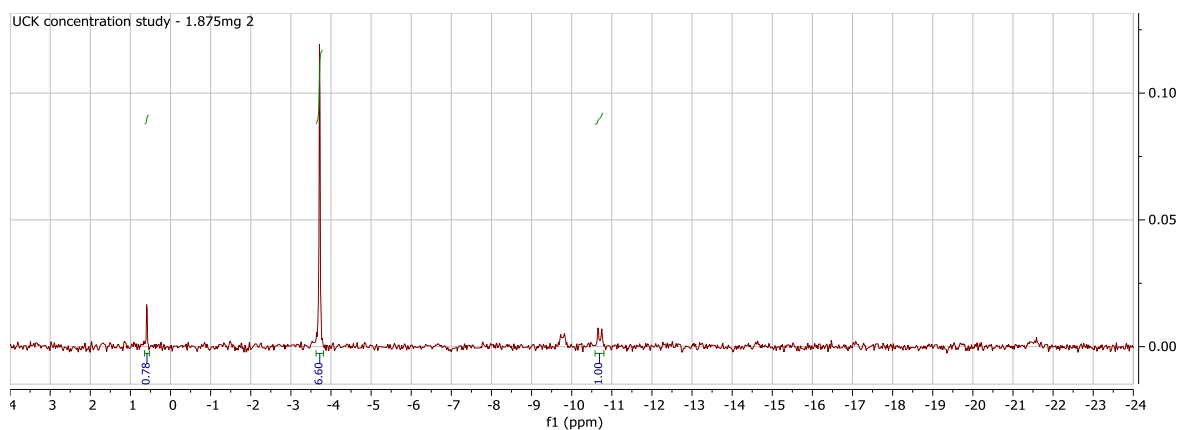


Figure A-I.116: ^{31}P NMR spectra of UCK concentration study 1.875 mg reaction sample 2. 1.875 mg UCK, 1.875 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

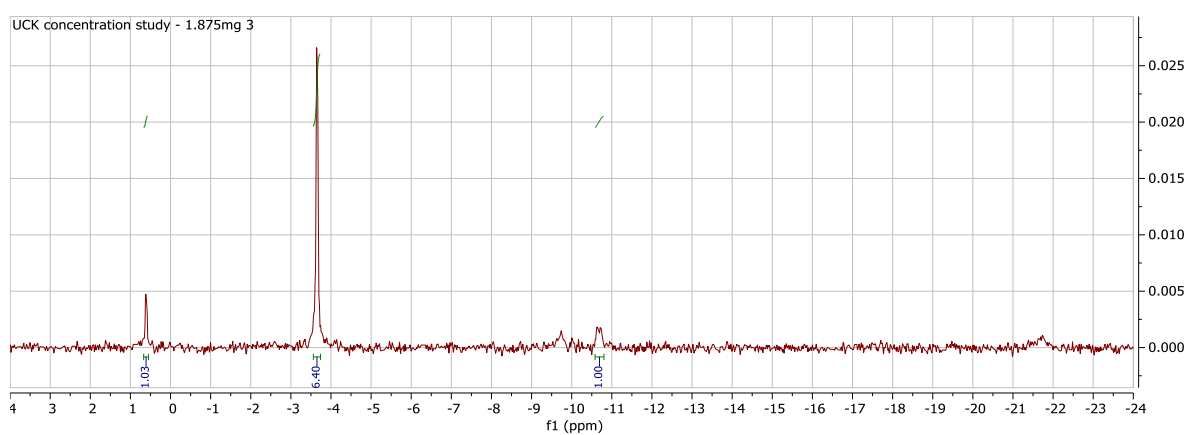


Figure A-I.117: ^{31}P NMR spectra of UCK concentration study 1.875 mg reaction sample 3. 1.875 mg UCK, 1.875 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

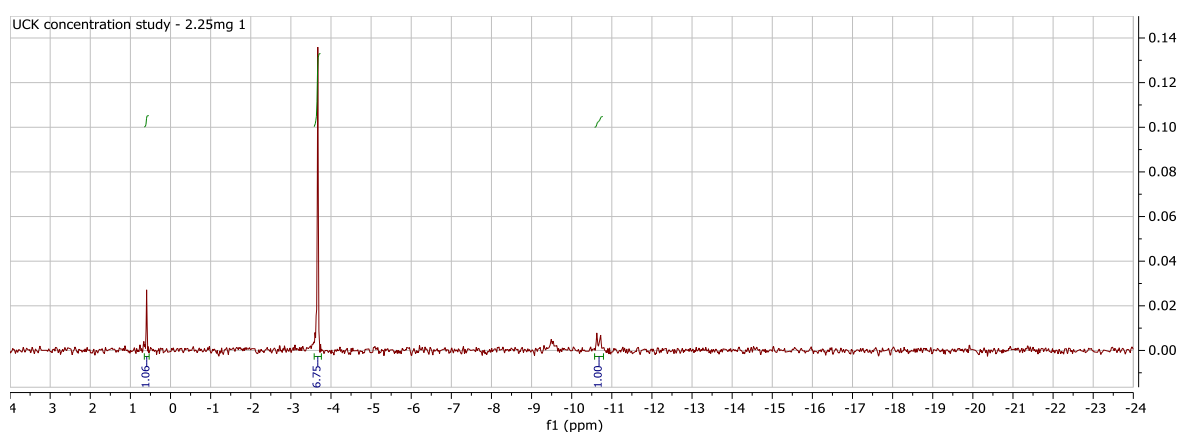


Figure A-I.118: ^{31}P NMR spectra of UCK concentration study 2.25 mg reaction sample 1. 2.25 mg UCK, 2.25 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

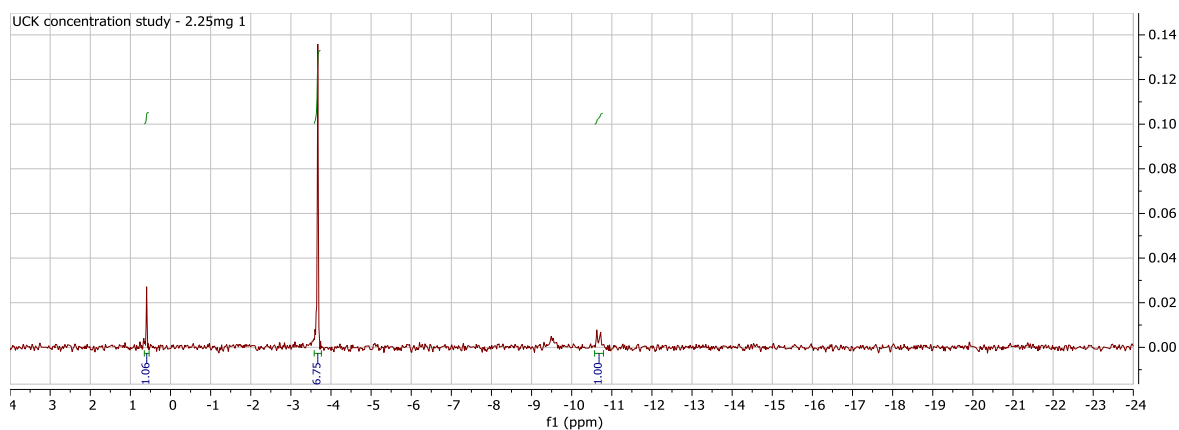


Figure A-I.119: ^{31}P NMR spectra of UCK concentration study 2.25 mg reaction sample 2. 2.25 mg UCK, 2.25 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

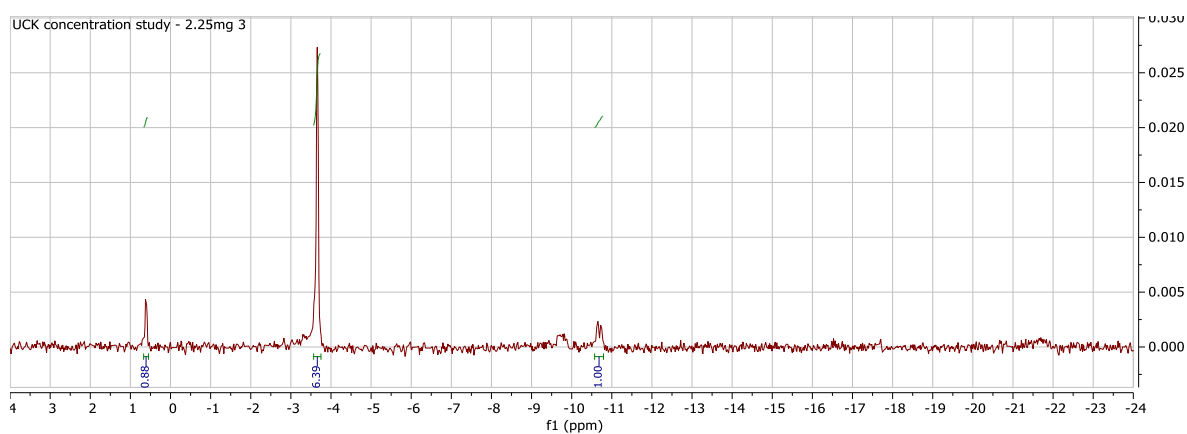


Figure A-I.120: ^{31}P NMR spectra of UCK concentration study 2.25 mg reaction sample 3. 2.25 mg UCK, 2.25 mg PK. Process described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.3.

UCK Amount (mg)	Sample	UMP Integral	PEP Integral	X	Calculated Conversion
0.75	1	0.640	7.438	0.079	8.95
	2	0.564	5.603	0.091	10.31
	3	0.564	5.603	0.091	10.31
1.125	1	0.816	7.228	0.101	11.44
	2	0.944	8.649	0.098	11.10
	3	0.820	6.391	0.114	12.92
1.5	1	0.921	7.175	0.114	12.92
	2	0.787	6.478	0.108	12.24
	3	1.107	7.531	0.128	14.50
1.875	1	0.971	6.477	0.130	14.73
	2	0.775	6.597	0.105	11.90
	3	1.030	6.399	0.139	15.75
2.25	1	1.063	6.748	0.136	15.41
	2	1.005	6.739	0.130	14.73
	3	0.884	6.393	0.121	13.71

Table A-I.3: Peak data from ^{31}P NMR analysis of samples used in UCK concentration study. Spectra can be found in Section A-I.8. Study described in Section 4.8. NMR analysis conducted as described in Section 2.7.1.2. Peak integrals normalised to peak relating to α -phosphate of ATP. $X = \text{UMP peak} / (\text{UMP peak} + \text{PEP peak})$. Substrate conversion calculated using model described in Section 4.7.

A-I.9 PK Concentration Study

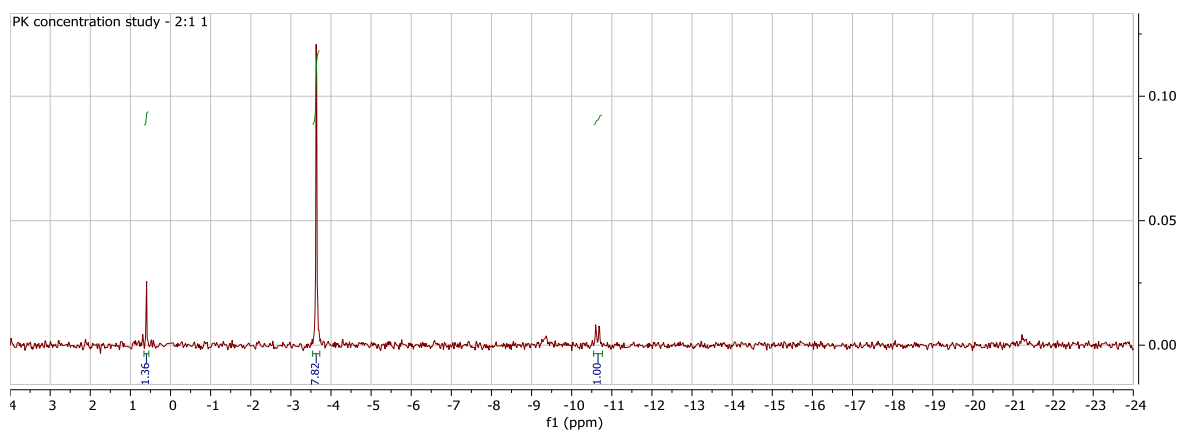


Figure A-I.121: ^{31}P NMR spectra of PK concentration study 2:1 (PK:UCK) reaction sample 1. 1.5 mg UCK, 3 mg PK. Process described in Section 4.9. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.4.

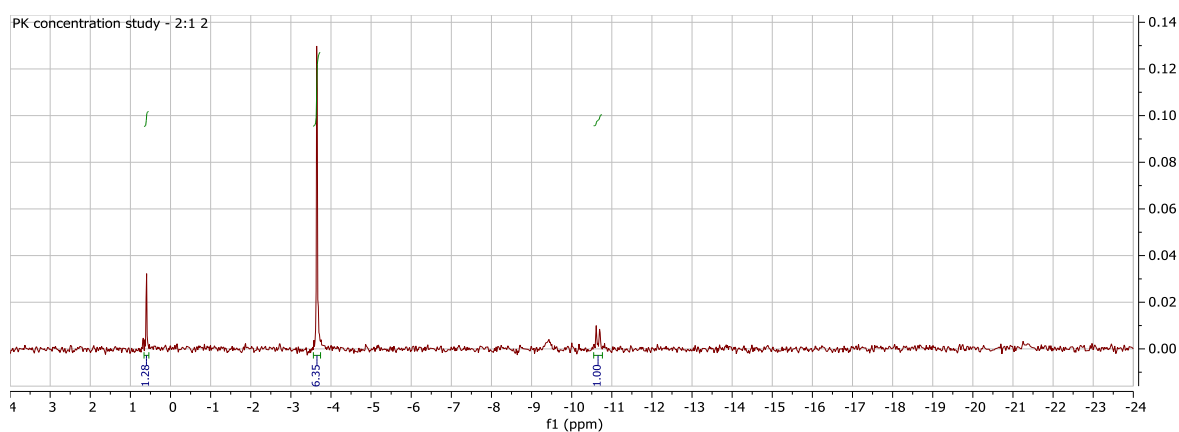


Figure A-I.122: ^{31}P NMR spectra of PK concentration study 2:1 (PK:UCK) reaction sample 2. 1.5 mg UCK, 3 mg PK. Process described in Section 4.9. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.4.

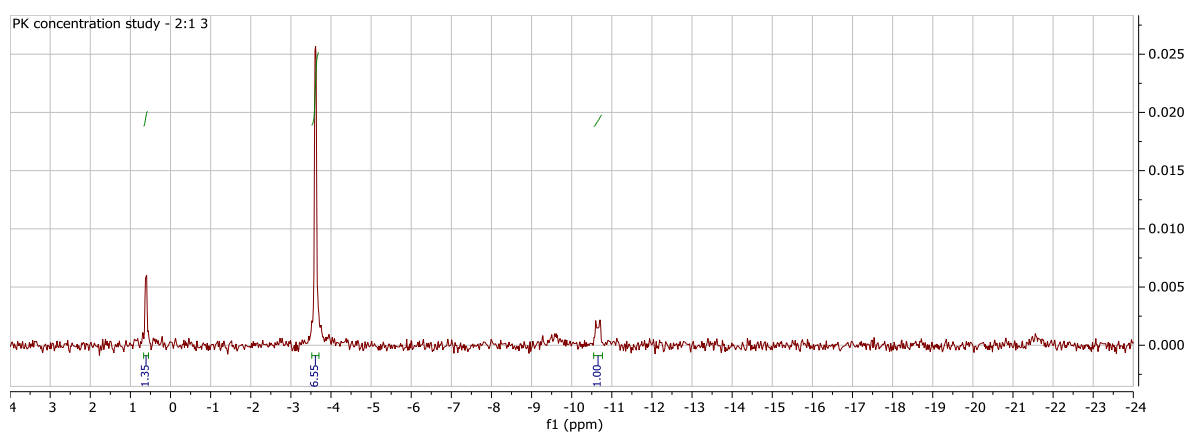


Figure A-I.123: ^{31}P NMR spectra of PK concentration study 2:1 (PK:UCK) reaction sample 3. 1.5 mg UCK, 3 mg PK. Process described in Section 4.9. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.4.

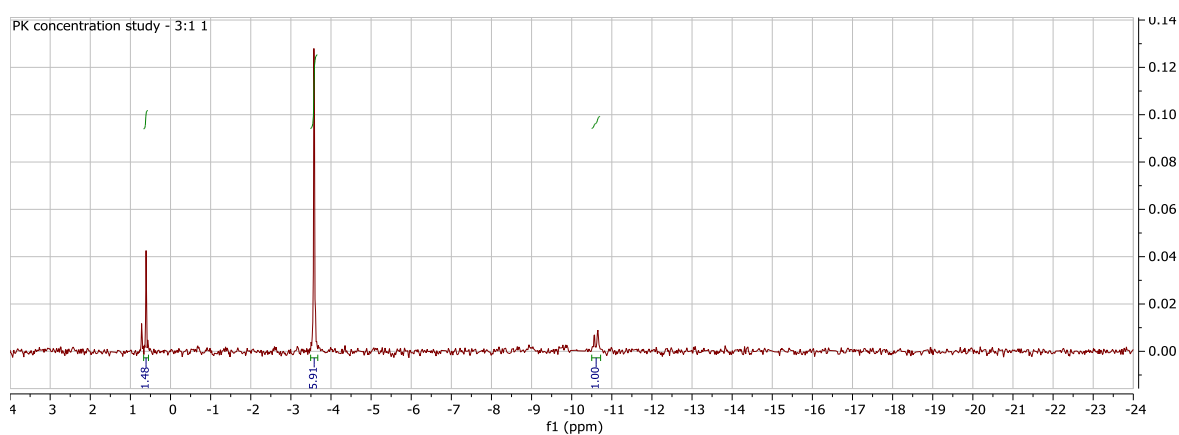


Figure A-I.124: ^{31}P NMR spectra of PK concentration study 3:1 (PK:UCK) reaction sample 1. 1.5 mg UCK, 4.5 mg PK. Process described in Section 4.9. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.4.

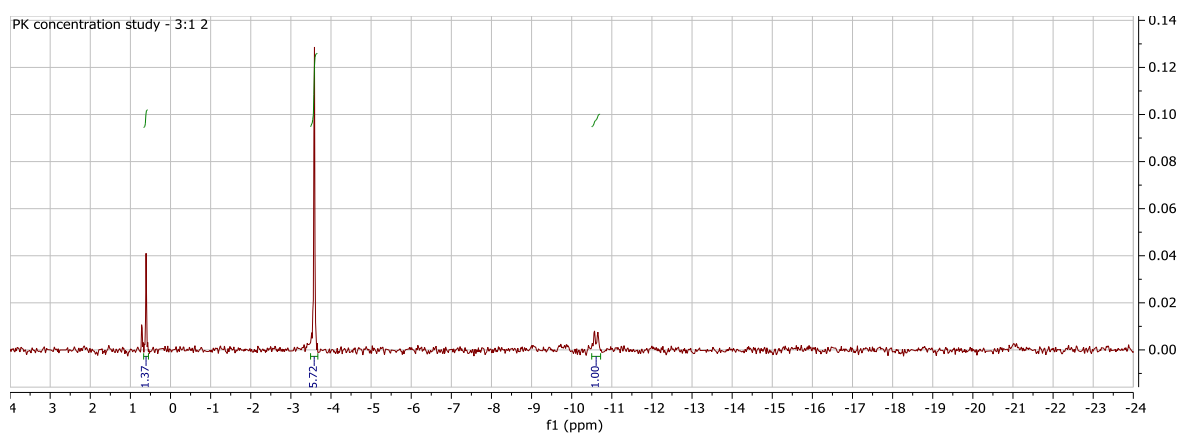


Figure A-I.125: ^{31}P NMR spectra of PK concentration study 3:1 (PK:UCK) reaction sample 2. 1.5 mg UCK, 4.5 mg PK. Process described in Section 4.9. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.4.

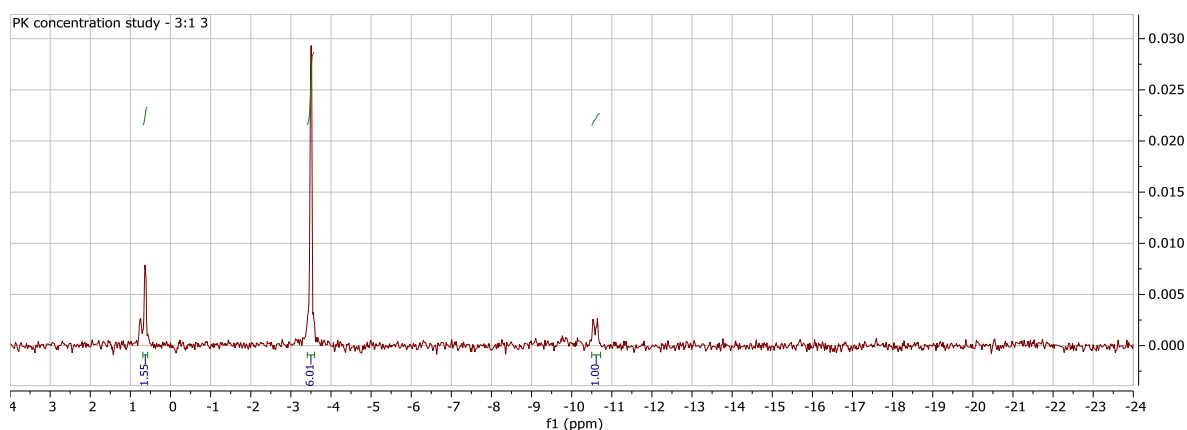


Figure A-I.126: ^{31}P NMR spectra of PK concentration study 3:1 (PK:UCK) reaction sample 3. 1.5 mg UCK, 4.5 mg PK. Process described in Section 4.9. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.4.

PK:UCK Ratio	Sample	UMP Integral	PEP Integral	X	Calculated Conversion
1:1	1	0.921	7.175	0.114	12.92
	2	0.787	6.478	0.108	12.24
	3	1.107	7.531	0.128	14.50
2:1	1	1.365	7.816	0.149	16.88
	2	1.279	6.350	0.168	19.04
	3	1.345	6.546	0.170	19.27
3:1	1	1.483	5.912	0.201	22.78
	2	1.367	5.720	0.193	21.87
	3	1.548	6.008	0.205	23.23

Table A-I.4: Peak data from ^{31}P NMR analysis of samples used in PK concentration study. Spectra can be found in Section A-I.9. Study described in Section 4.9. NMR analysis conducted as described in Section 2.7.1.2. Peak integrals normalised to peak relating to α -phosphate of ATP. $X = \text{UMP peak} / (\text{UMP peak} + \text{PEP peak})$. 1:1 PK:UCK ratio data is derived from 1.5mg samples of UCK concentration study (Section A-I.8). Substrate conversion calculated using model described in Section 4.7.

Note: ^{31}P NMR spectra for 1:1 PK:UCK ratio are *Figure A-I.112*, *Figure A-I.113*, and *Figure A-I.114*.

A-I.10 Addition Strategy Study

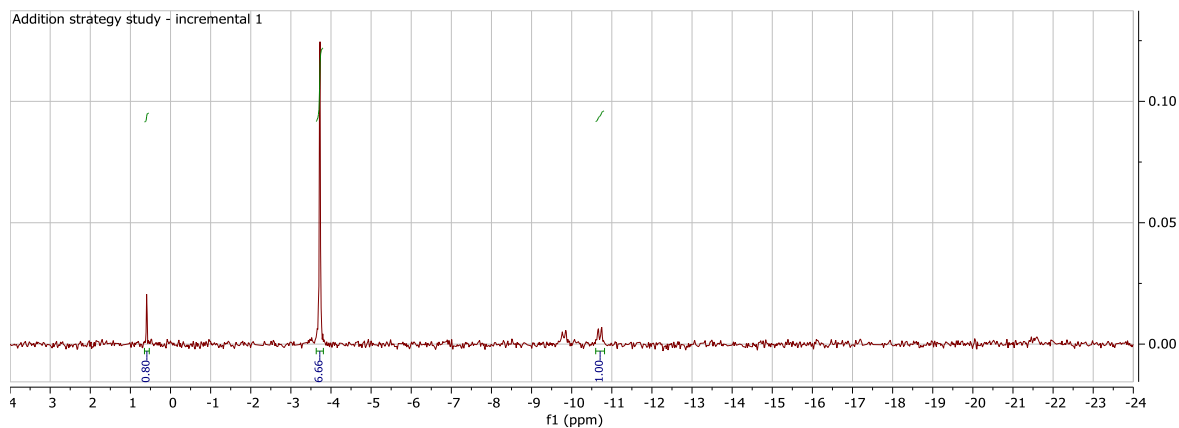


Figure A-I.127: ^{31}P NMR spectra of UCK addition strategy study incremental addition reaction sample 1. 1.5 mg UCK (added to reaction mixture in three 0.5 mg aliquots at 10-minute intervals), 1.5 mg PK. Process described in Section 4.10. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.5.

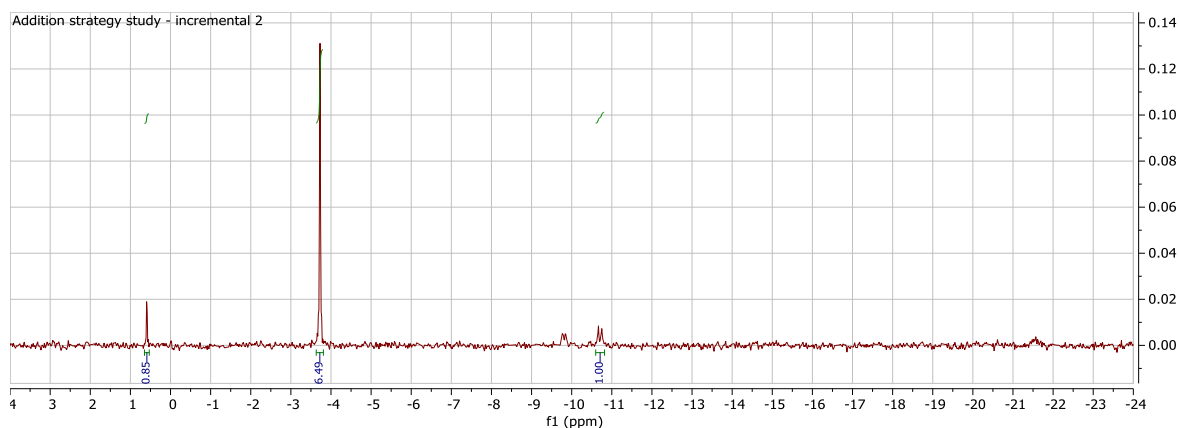


Figure A-I.128: ^{31}P NMR spectra of UCK addition strategy study incremental addition reaction sample 2. 1.5 mg UCK (added to reaction mixture in three 0.5 mg aliquots at 10-minute intervals), 1.5 mg PK. Process described in Section 4.10. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.5.

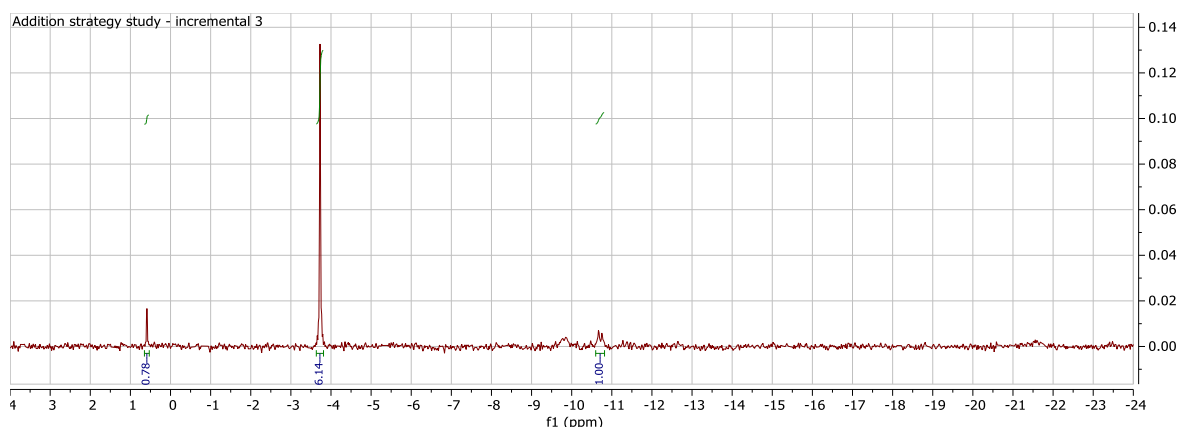


Figure A-I.129: ^{31}P NMR spectra of UCK addition strategy study incremental addition reaction sample 3. 1.5 mg UCK (added to reaction mixture in three 0.5 mg aliquots at 10-minute intervals), 1.5 mg PK. Process described in Section 4.10. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.5.

UCK Addition Strategy	Sample	UMP Integral	PEP Integral	X	Calculated Conversion
Fixed	1	0.921	7.175	0.114	12.92
	2	0.787	6.478	0.108	12.24
	3	1.107	7.531	0.128	14.50
Incremental	1	0.801	6.659	0.107	12.12
	2	0.847	6.487	0.115	13.03
	3	0.780	6.143	0.113	12.80

Table A-I.5: Peak data from ^{31}P NMR analysis of samples used in UCK addition strategy study. Spectra can be found in Section A-I.10. Study described in Section 4.10. NMR analysis conducted as described in Section 2.7.1.2. Peak integrals normalised to peak relating to α -phosphate of ATP. $X = \text{UMP peak} / (\text{UMP peak} + \text{PEP peak})$. Fixed UCK addition strategy data is derived from 1.5mg samples of UCK concentration study (Section A-I.8). Substrate conversion calculated using model described in Section 4.7.

Note: ^{31}P NMR spectra for fixed UCK addition strategy are Figure A-I.112, Figure A-I.113, and Figure A-I.114.

A-I.11 Activity Against Natural Substrates

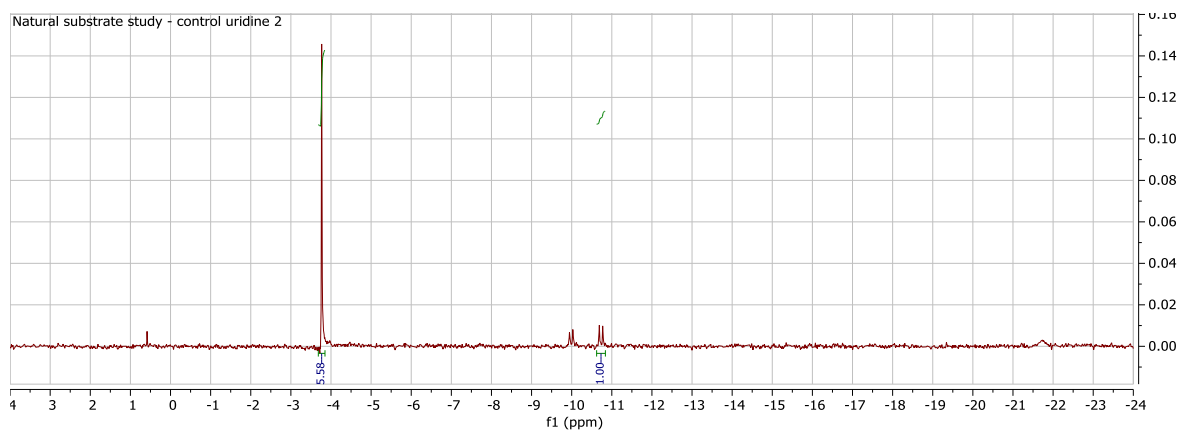


Figure A-I.130: ^{31}P NMR spectra of natural substrate study uridine control sample 2. 0 mg UCK, 0 mg PK, 12.5 mM uridine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

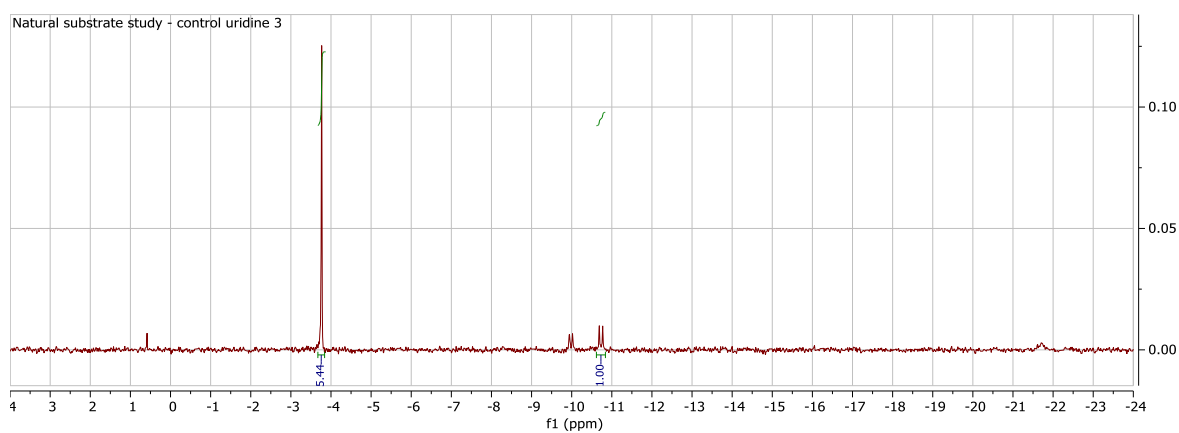


Figure A-I.131: ^{31}P NMR spectra of natural substrate study uridine control sample 3. 0 mg UCK, 0 mg PK, 12.5 mM uridine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

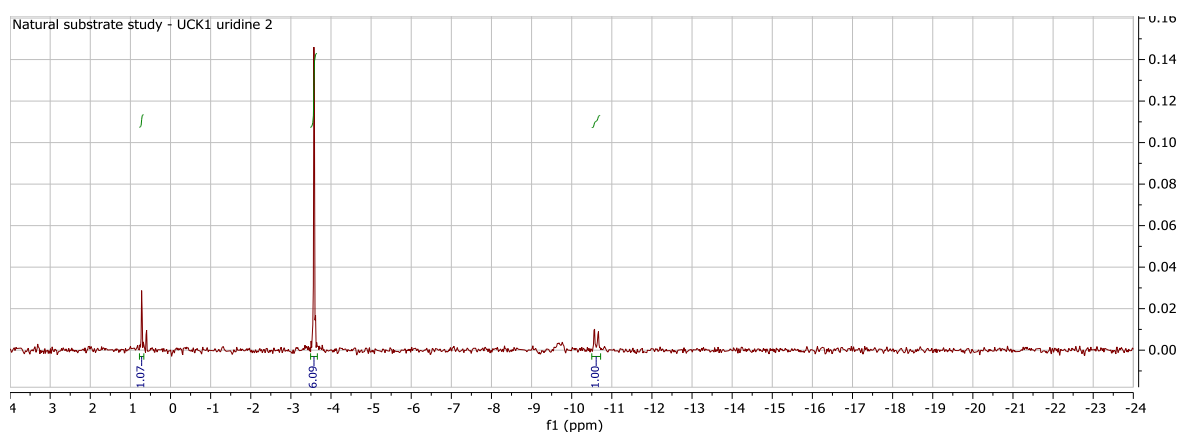


Figure A-I.132: ^{31}P NMR spectra of natural substrate study UCK1-uridine reaction sample 2. 1.5 mg UCK1, 3 mg PK, 12.5 mM uridine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

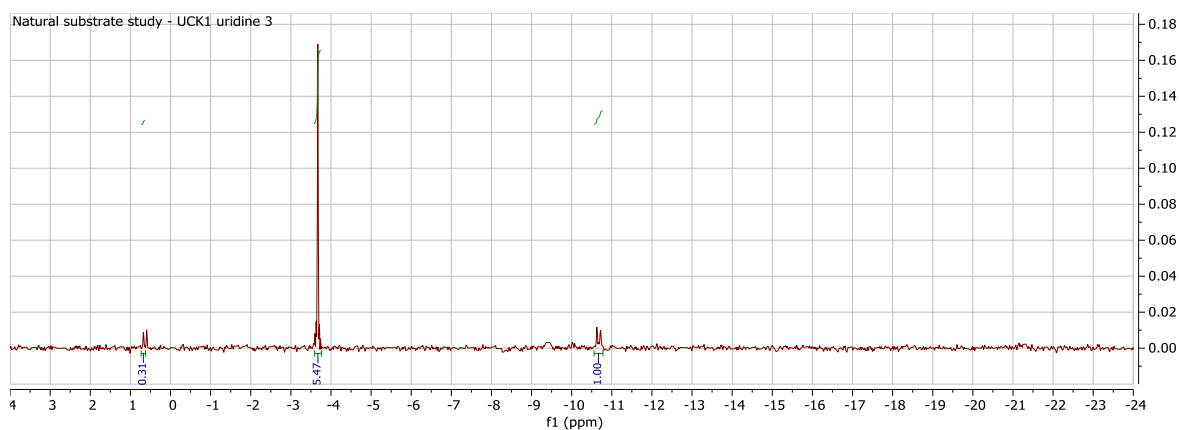


Figure A-I.133: ^{31}P NMR spectra of natural substrate study UCK1-uridine reaction sample 3. 1.5 mg UCK1, 3 mg PK, 12.5 mM uridine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

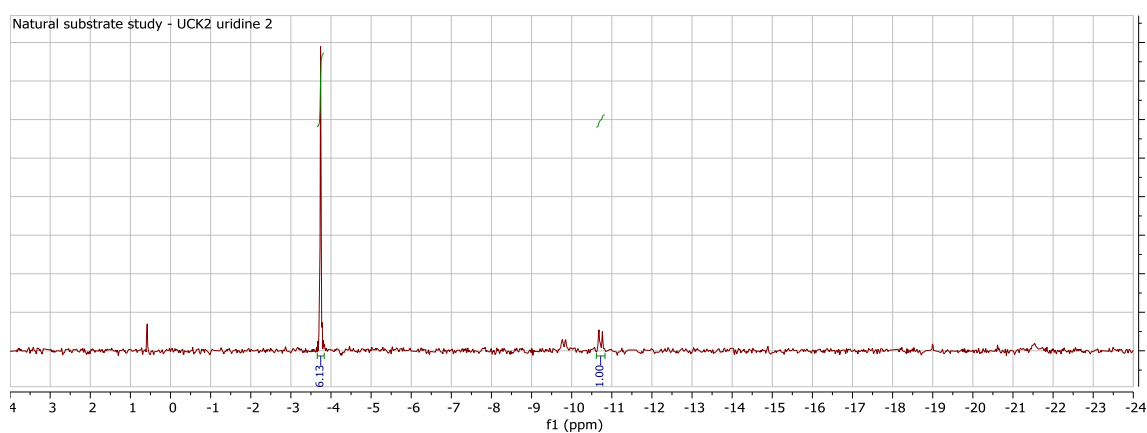


Figure A-I.134: ^{31}P NMR spectra of natural substrate study UCK2-uridine reaction sample 2. 1.5 mg UCK2, 3 mg PK, 12.5 mM uridine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

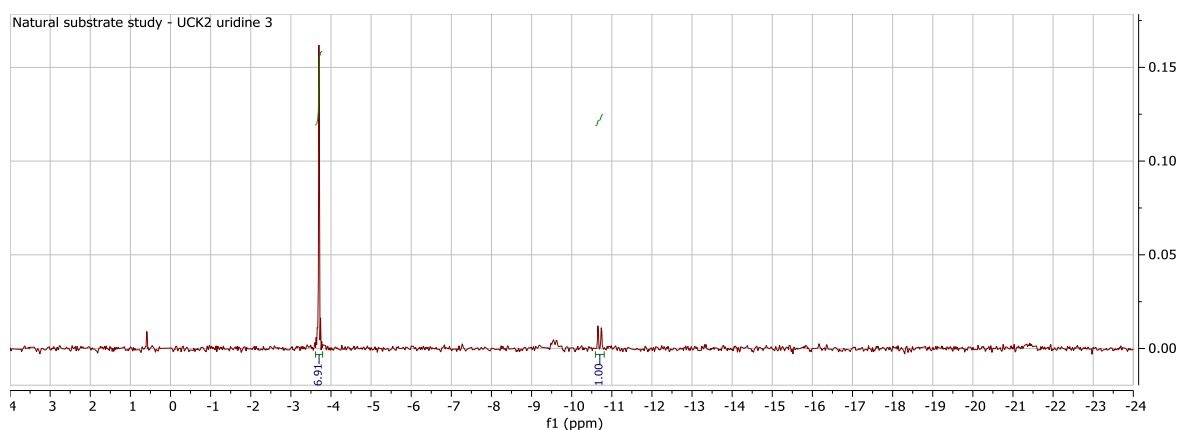


Figure A-I.135: ^{31}P NMR spectra of natural substrate study UCK2-uridine reaction sample 3. 1.5 mg UCK2, 3 mg PK, 12.5 mM uridine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

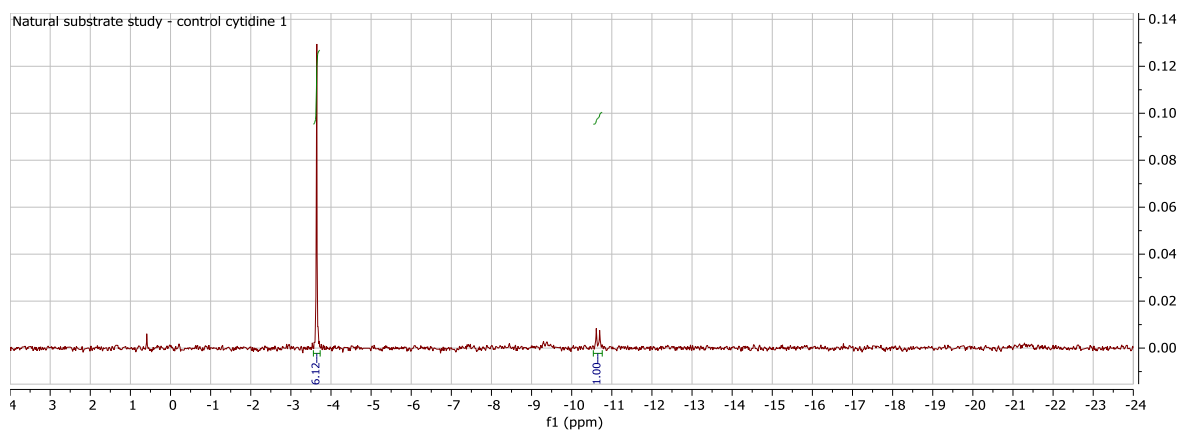


Figure A-I.136: ^{31}P NMR spectra of natural substrate study cytidine control sample 1. 0 mg UCK, 0 mg PK, 12.5 mM cytidine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

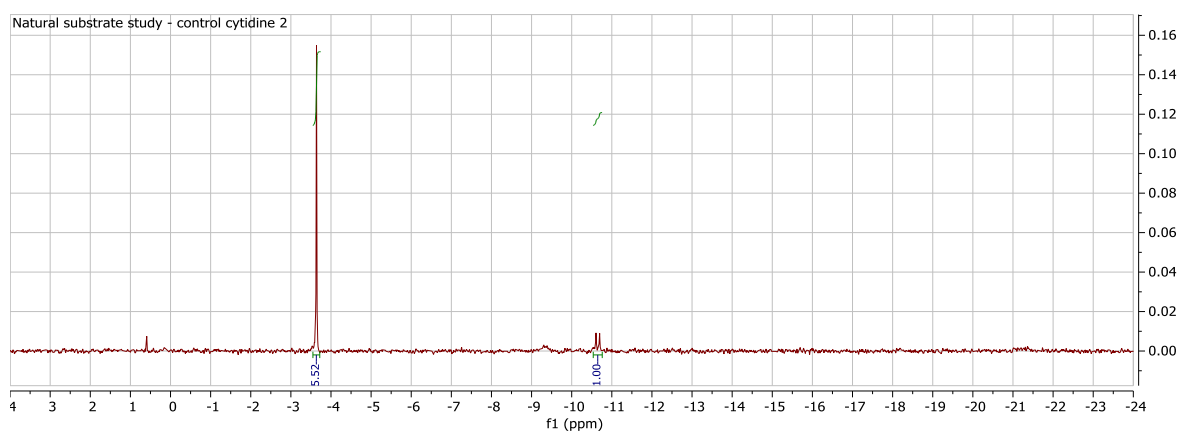


Figure A-I.137: ^{31}P NMR spectra of natural substrate study cytidine control sample 2. 0 mg UCK, 0 mg PK, 12.5 mM cytidine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

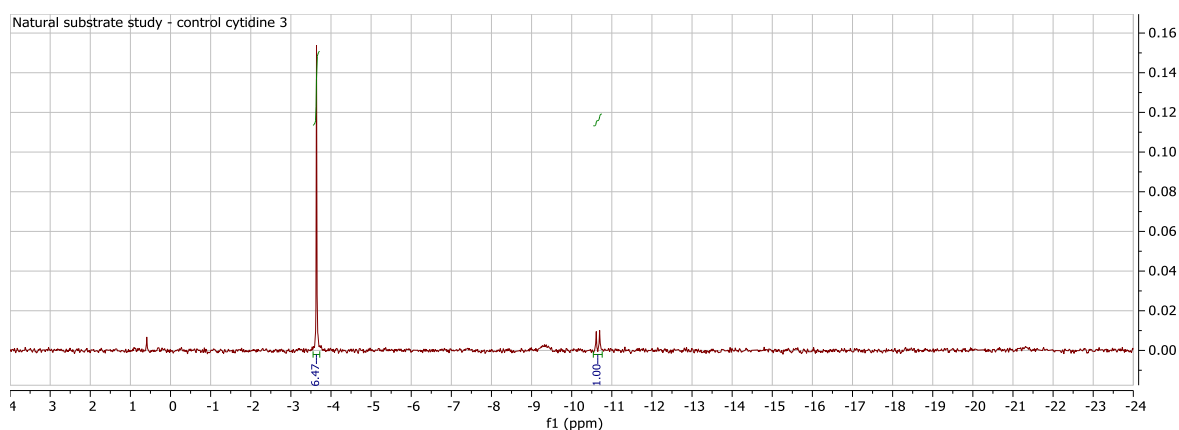


Figure A-I.138: ^{31}P NMR spectra of natural substrate study cytidine control sample 3. 0 mg UCK, 0 mg PK, 12.5 mM cytidine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

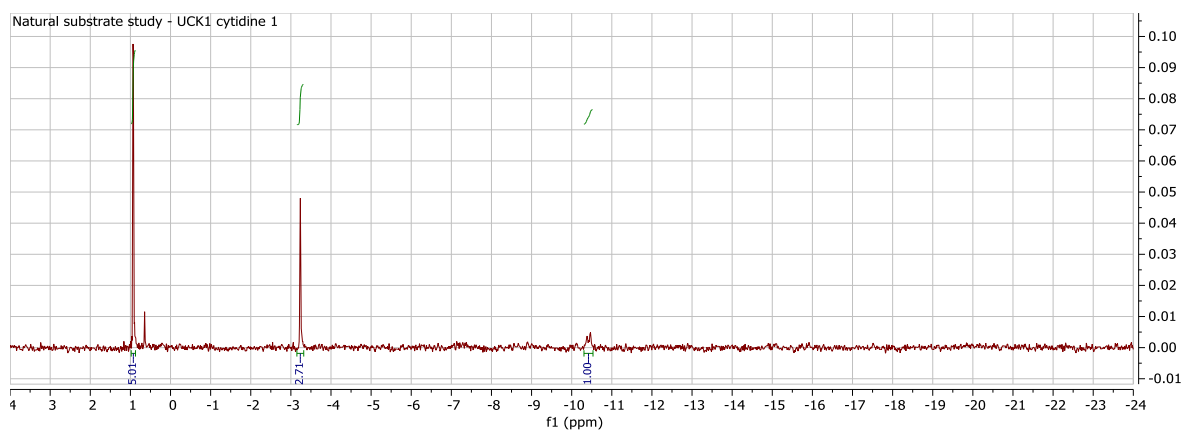


Figure A-I.139: ^{31}P NMR spectra of natural substrate study UCK1-cytidine reaction sample 1. 1.5 mg UCK1, 3 mg PK, 12.5 mM cytidine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

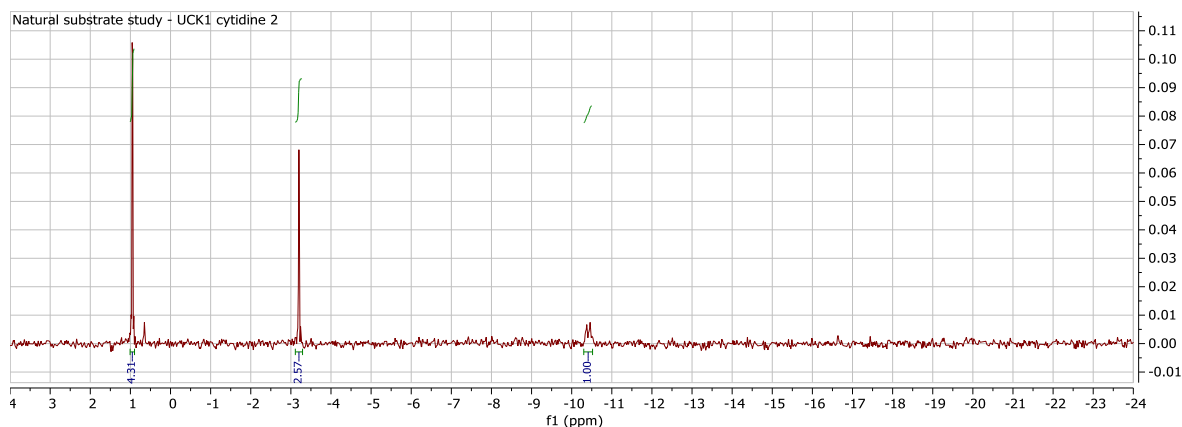


Figure A-I.140: ^{31}P NMR spectra of natural substrate study UCK1-cytidine reaction sample 2. 1.5 mg UCK1, 3 mg PK, 12.5 mM cytidine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

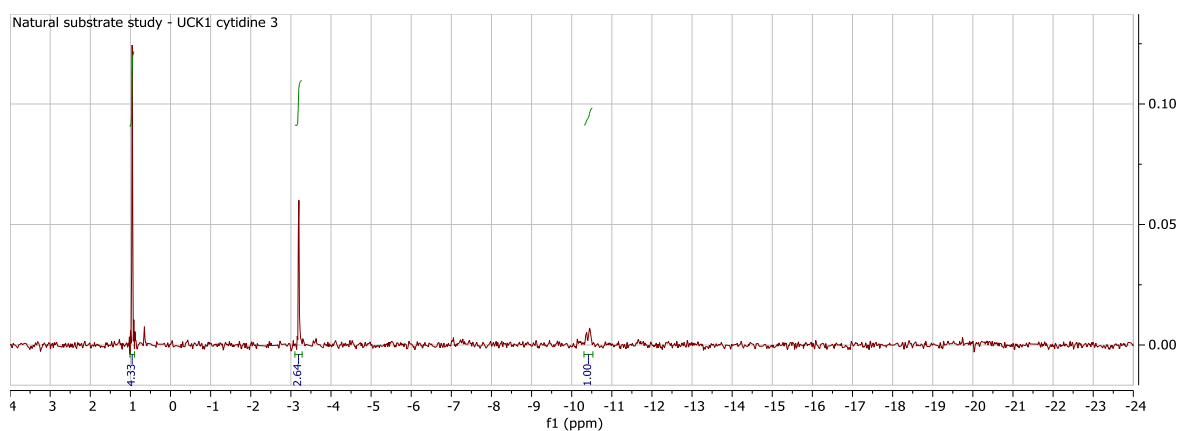


Figure A-I.141: ^{31}P NMR spectra of natural substrate study UCK1-cytidine reaction sample 3. 1.5 mg UCK1, 3 mg PK, 12.5 mM cytidine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

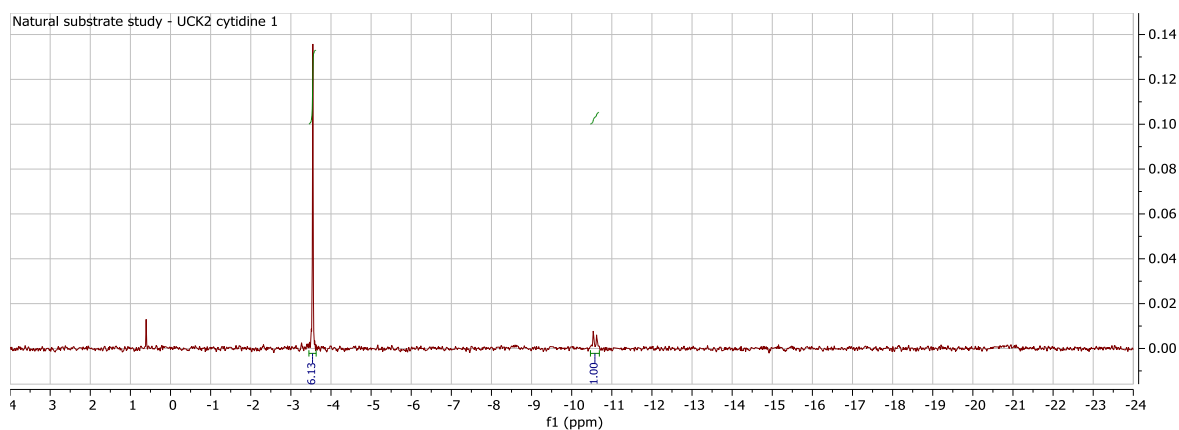


Figure A-I.142: ^{31}P NMR spectra of natural substrate study UCK2-cytidine reaction sample 1. 1.5 mg UCK2, 3 mg PK, 12.5 mM cytidine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

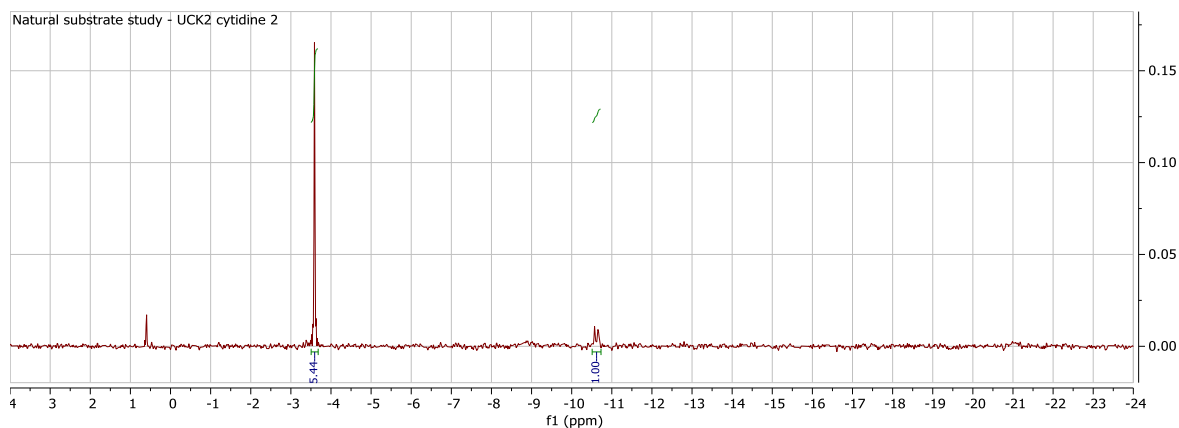


Figure A-I.143: ^{31}P NMR spectra of natural substrate study UCK2-cytidine reaction sample 2. 1.5 mg UCK2, 3 mg PK, 12.5 mM cytidine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

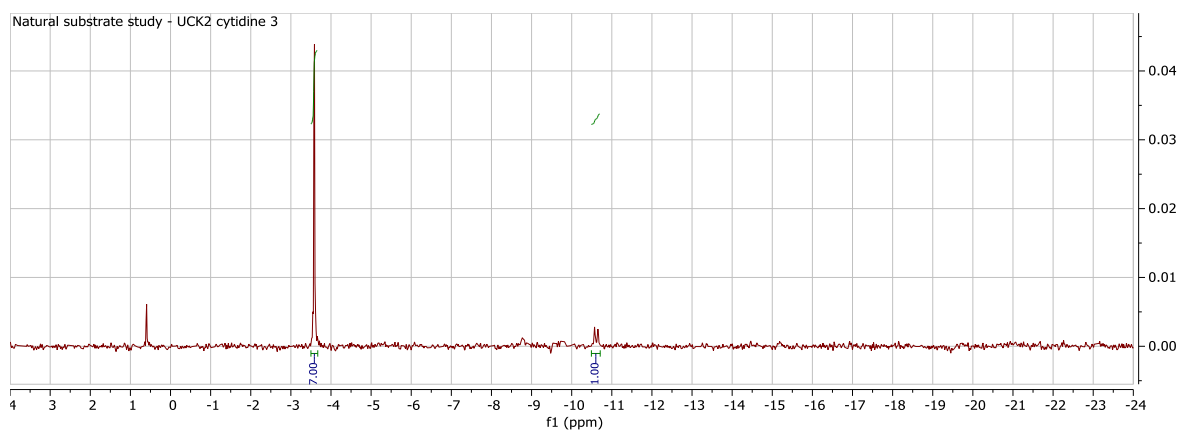


Figure A-I.144: ^{31}P NMR spectra of natural substrate study UCK2-cytidine reaction sample 3. 1.5 mg UCK2, 3 mg PK, 12.5 mM cytidine. Process described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.6.

Substrate	Enzyme	Sample	UMP Integral	PEP Integral	X	Calculated Conversion
Uridine	Control	1	0.000	7.401	0.000	0.91
		2	0.000	5.579	0.000	0.91
		3	0.000	5.437	0.000	0.91
	UCK1	1	1.097	6.232	0.150	17.70
		2	1.071	6.086	0.150	17.70
		3	0.313	5.466	0.054	6.95
	UCK2	1	0.000	5.923	0.000	0.91
		2	0.000	6.128	0.000	0.91
		3	0.000	6.913	0.000	0.91
Cytidine	Control	1	0.000	6.118	0.000	0.91
		2	0.000	5.524	0.000	0.91
		3	0.000	6.469	0.000	0.91
	UCK1	1	5.010	2.709	0.649	73.54
		2	4.308	2.567	0.627	71.08
		3	4.328	2.641	0.621	70.41
	UCK2	1	0.000	6.126	0.000	0.91
		2	0.000	5.441	0.000	0.91
		3	0.000	7.001	0.000	0.91

Table A-I.6: Peak data from ^{31}P NMR analysis of samples used in natural substrate study. Spectra can be found in Section A-I.11. Study described in Section 5.2. NMR analysis conducted as described in Section 2.7.1.2. Peak integrals normalised to peak relating to α -phosphate of ATP. $X = \text{UMP peak} / (\text{UMP peak} + \text{PEP peak})$. Sample 1 data for each of uridine control, UCK1-uridine, and UCK2-uridine reactions is derived from UCK1 and UCK2 activity tests (Section 4.8). Substrate conversion calculated using model described in Section 4.7.

Note: ^{31}P NMR spectra for sample 1 of uridine control, UCK1-uridine reaction, and UCK2-uridine reaction are *Figure A-I.112*, *Figure A-I.113*, and *Figure A-I.114* respectively.

Note: The observed 0.91% substrate conversion results can be considered the result of model error as all samples with nucleoside monophosphate peak integrals of zero are calculated as having 0.91% substrate conversion.

A-I.12 Activity Against Unnatural Substrates

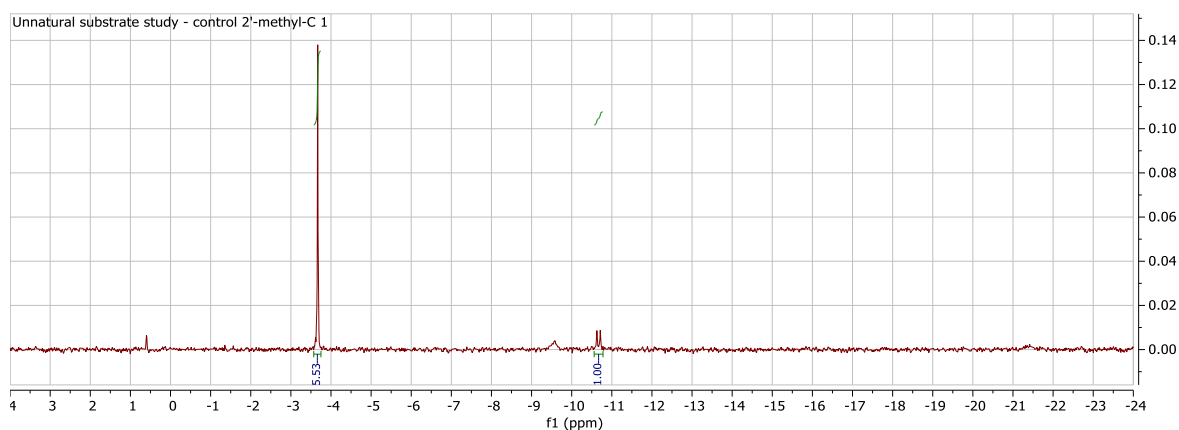


Figure A-I.145: ³¹P NMR spectra of unnatural substrate study 2'-methyl-C control sample. 0 mg UCK1, 0 mg PK, 12.5 mM 2'-methyl-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

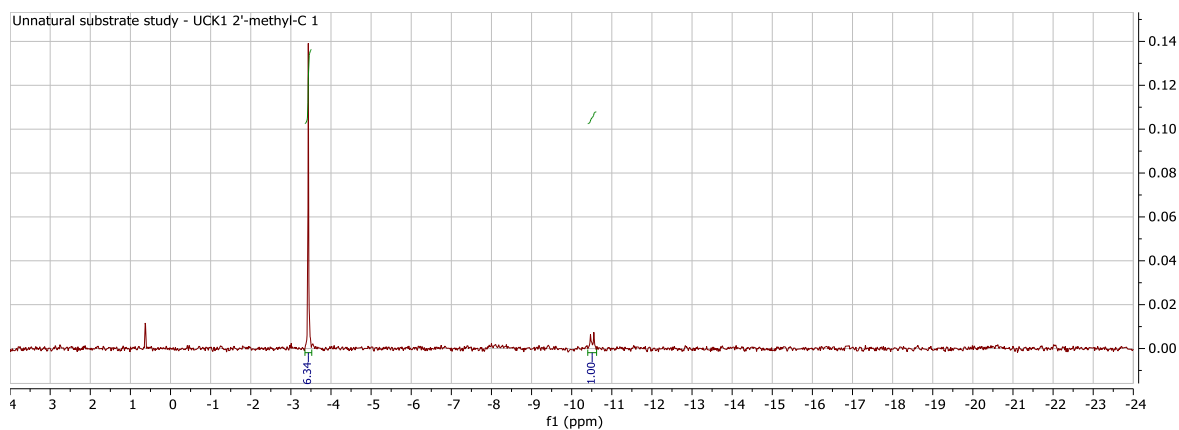


Figure A-I.146: ³¹P NMR spectra of unnatural substrate study 2'-methyl-C reaction sample 1. 1.5 mg UCK1, 3 mg PK, 12.5 mM 2'-methyl-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

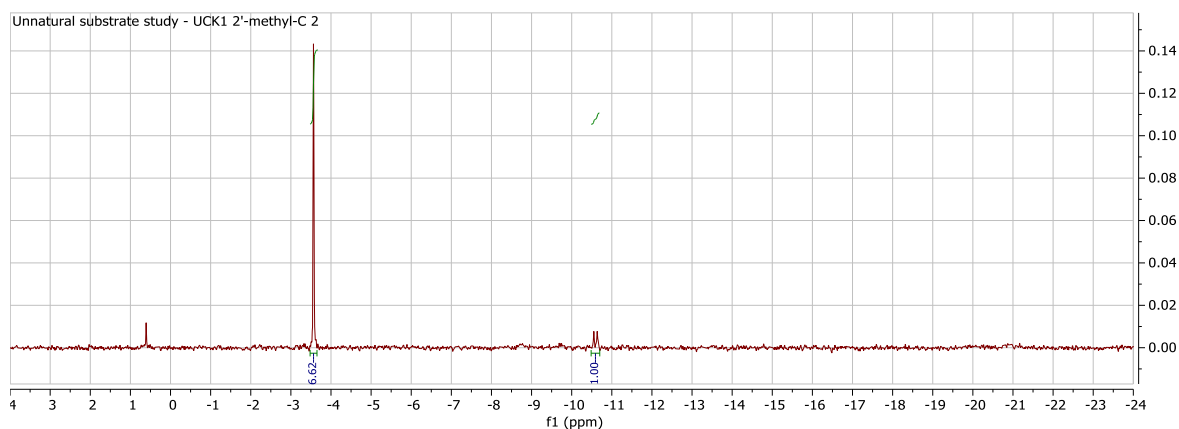


Figure A-I.147: ³¹P NMR spectra of unnatural substrate study 2'-methyl-C reaction sample 2. 1.5 mg UCK1, 3 mg PK, 12.5 mM 2'-methyl-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

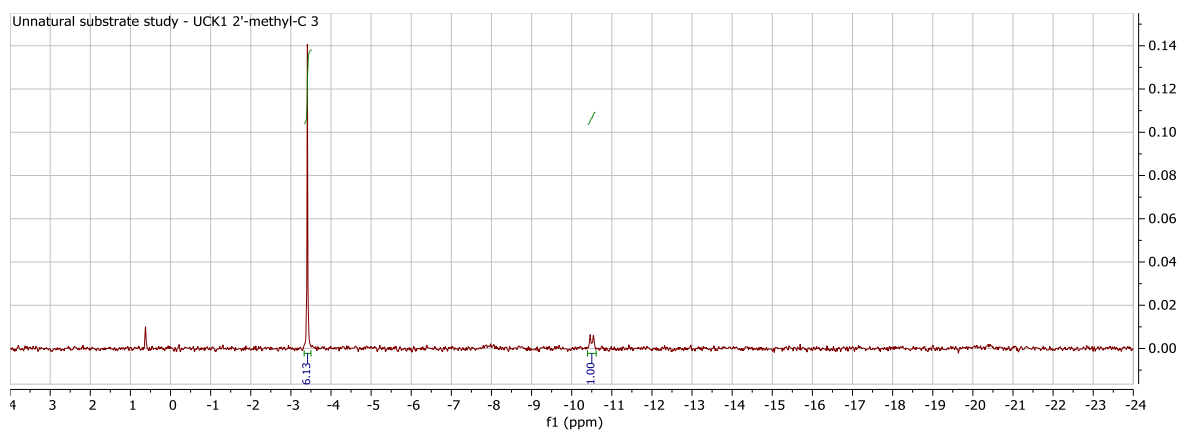


Figure A-I.148: ^{31}P NMR spectra of unnatural substrate study 2'-methyl-C reaction sample 3. 1.5 mg UCK1, 3 mg PK, 12.5 mM 2'-methyl-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

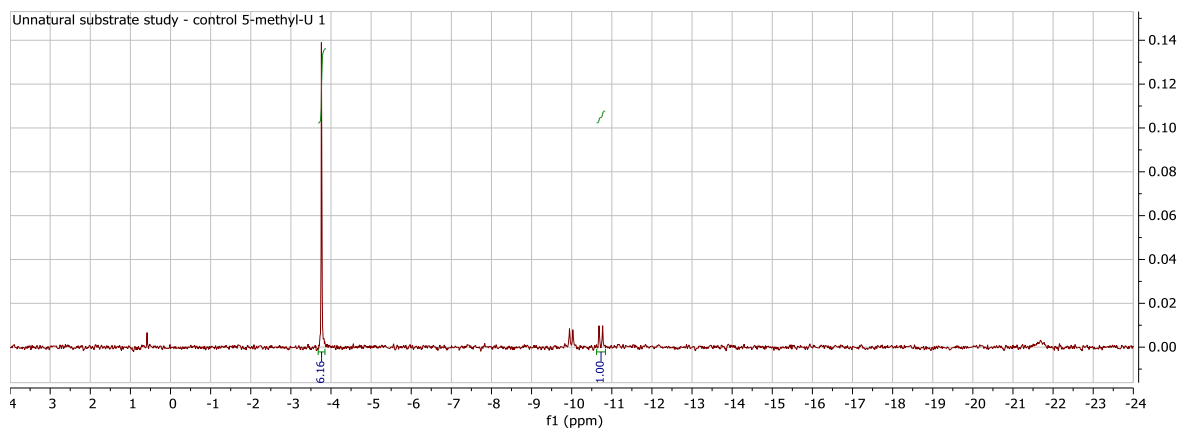


Figure A-I.149: ^{31}P NMR spectra of unnatural substrate study 5-methyl-U control sample. 0 mg UCK1, 0 mg PK, 12.5 mM 5-methyl-U. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

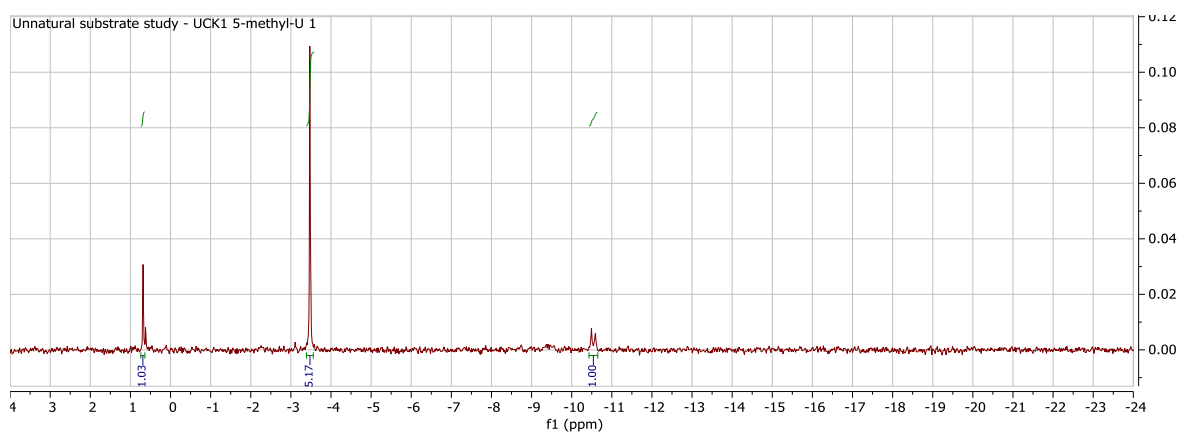


Figure A-I.150: ^{31}P NMR spectra of unnatural substrate study 5-methyl-U reaction sample 1. 1.5 mg UCK1, 3 mg PK, 12.5 mM 5-methyl-U. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

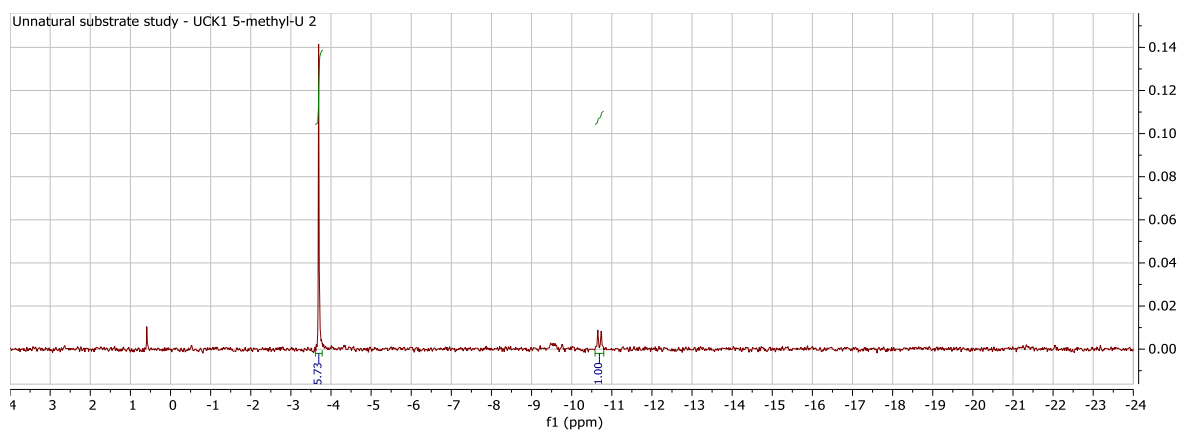


Figure A-I.151: ^{31}P NMR spectra of unnatural substrate study 5-methyl-U reaction sample 2. 1.5 mg UCK1, 3 mg PK, 12.5 mM 5-methyl-U. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

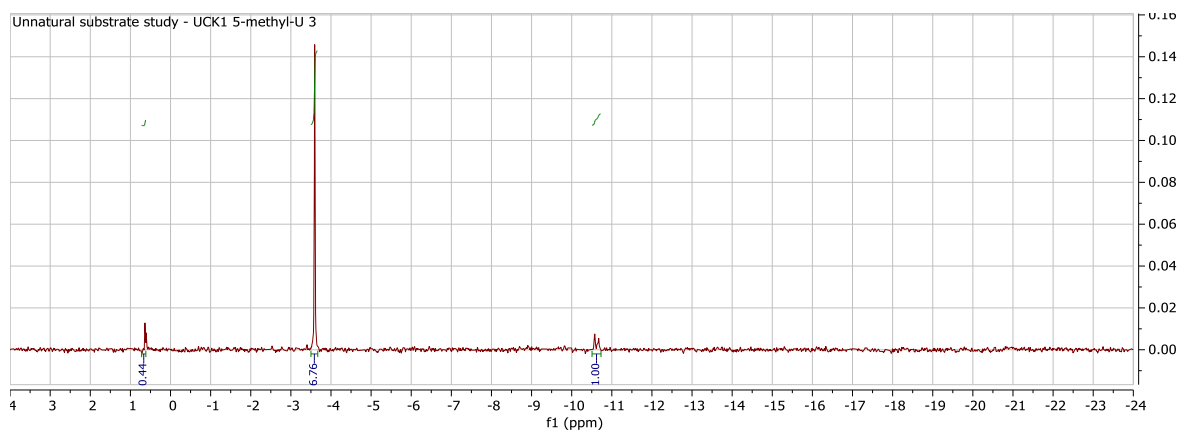


Figure A-I.152: ^{31}P NMR spectra of unnatural substrate study 5-methyl-U reaction sample 3. 1.5 mg UCK1, 3 mg PK, 12.5 mM 5-methyl-U. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

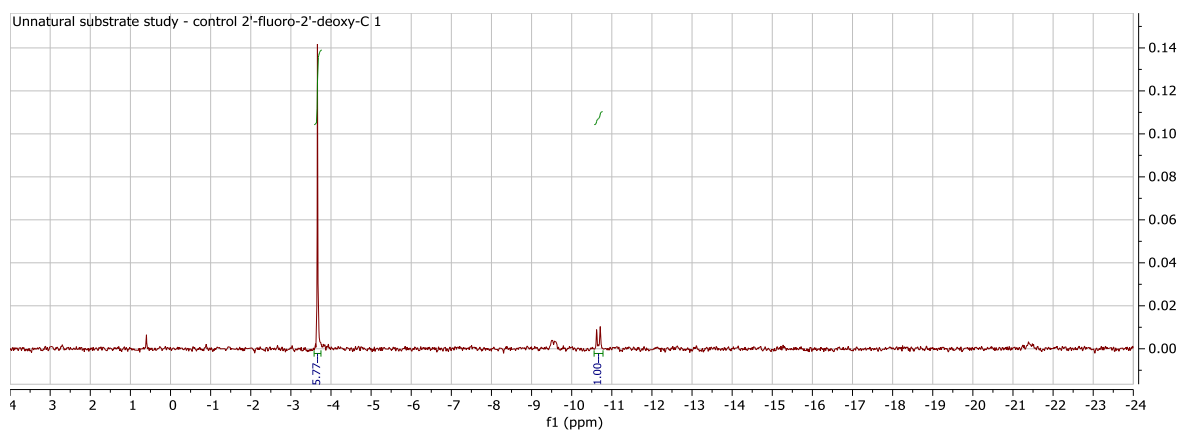


Figure A-I.153: ^{31}P NMR spectra of unnatural substrate study 2'-fluoro-2'-deoxy-C control sample. 0 mg UCK1, 0 mg PK, 12.5 mM 2'-fluoro-2'-deoxy-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

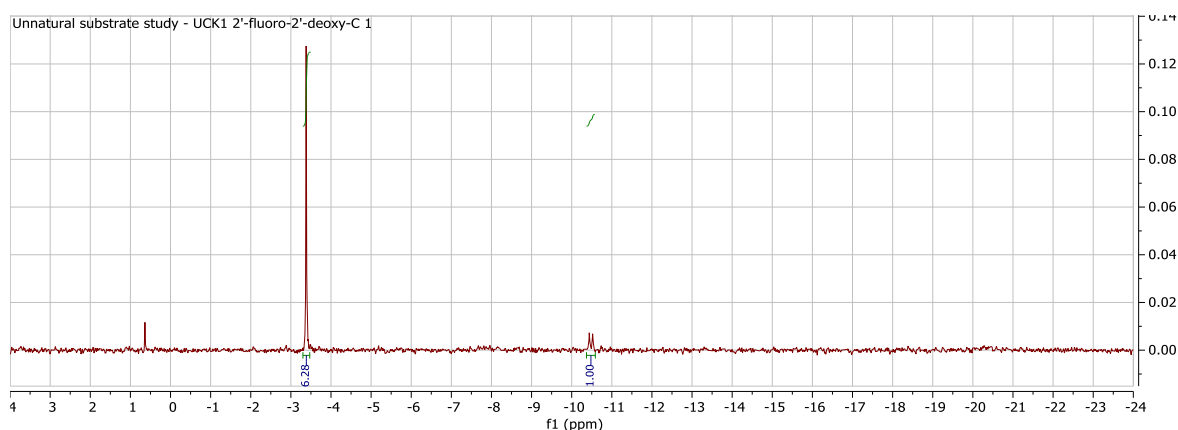


Figure A-I.154: ^{31}P NMR spectra of unnatural substrate study 2'-fluoro-2'-deoxy-C reaction sample 1. 1.5 mg UCK1, 3 mg PK, 12.5 mM 2'-fluoro-2'-deoxy-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

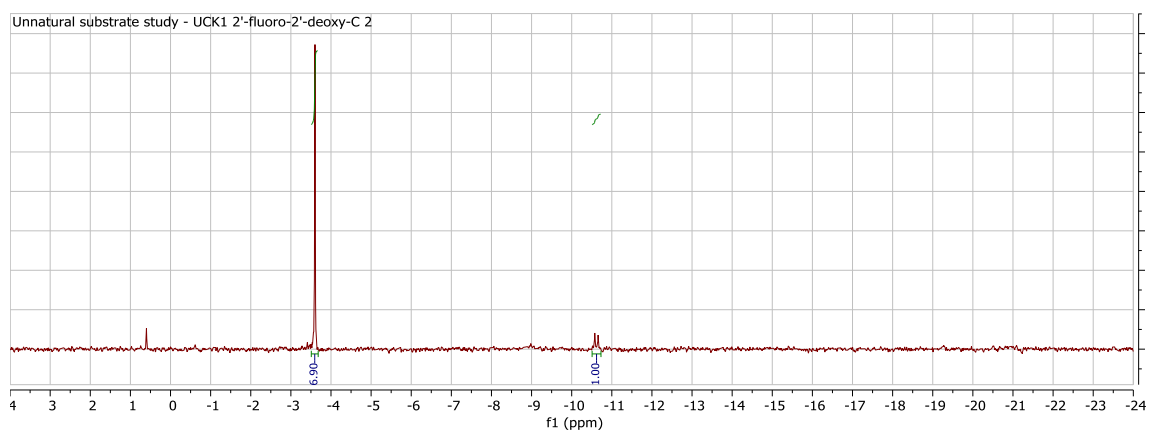


Figure A-I.155: ^{31}P NMR spectra of unnatural substrate study 2'-fluoro-2'-deoxy-C reaction sample 2. 1.5 mg UCK1, 3 mg PK, 12.5 mM 2'-fluoro-2'-deoxy-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

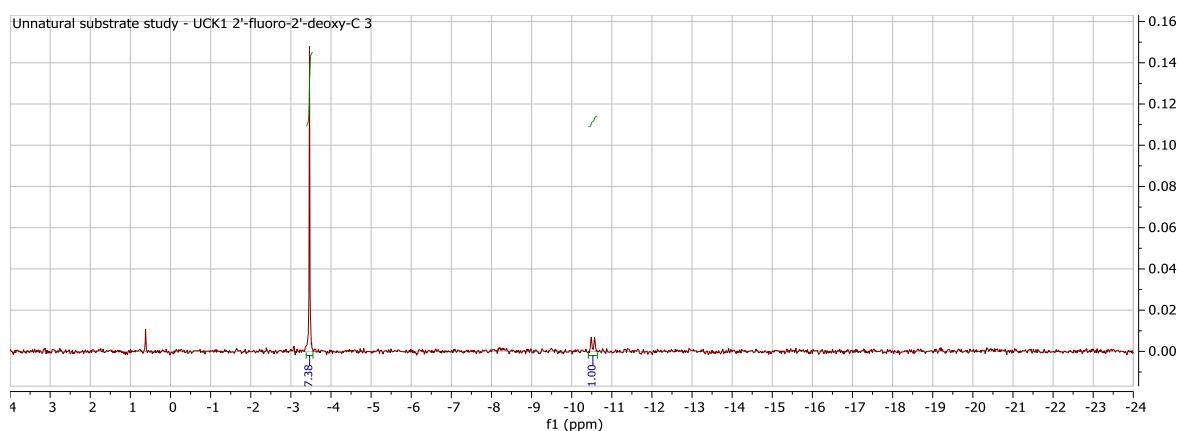


Figure A-I.156: ^{31}P NMR spectra of unnatural substrate study 2'-fluoro-2'-deoxy-C reaction sample 3. 1.5 mg UCK1, 3 mg PK, 12.5 mM 2'-fluoro-2'-deoxy-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

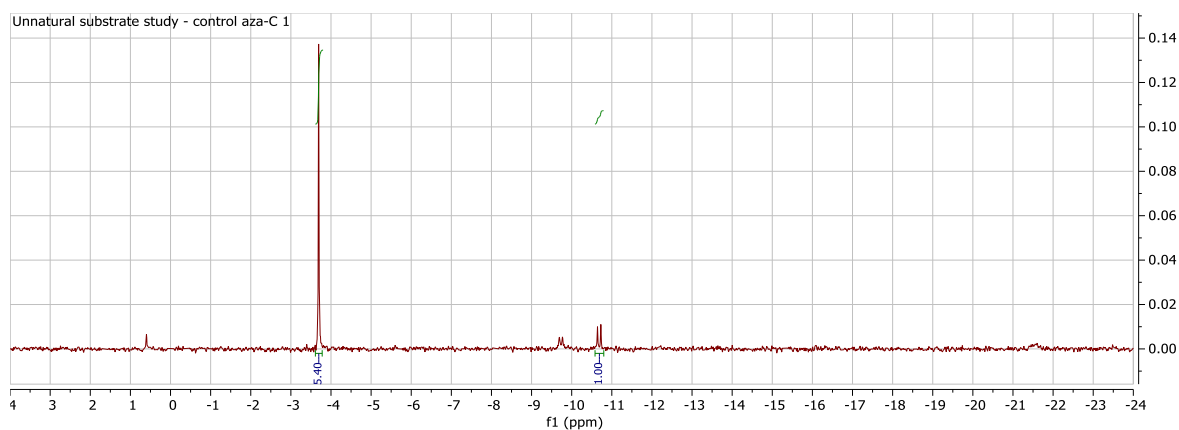


Figure A-I.157: ^{31}P NMR spectra of unnatural substrate study aza-C control sample. 0 mg UCK1, 0 mg PK, 12.5 mM aza-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

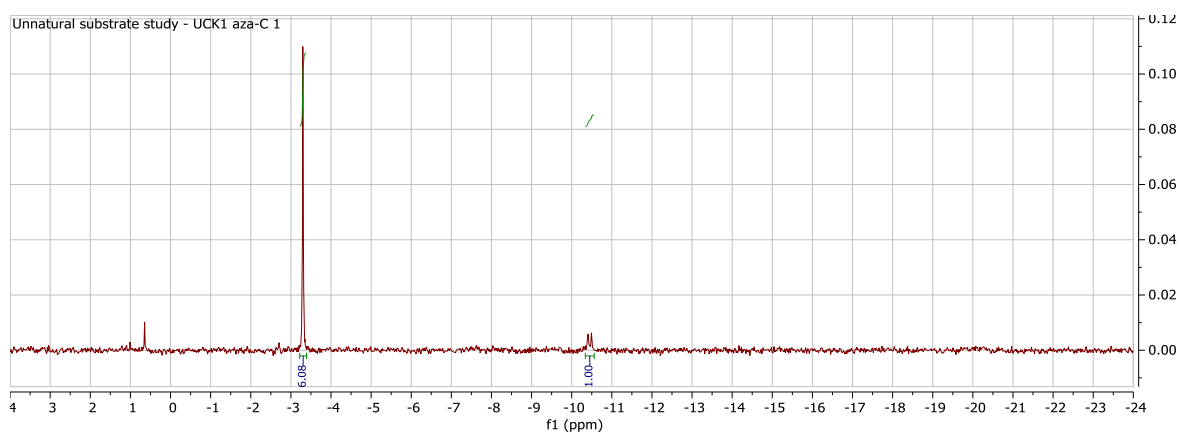


Figure A-I.158: ^{31}P NMR spectra of unnatural substrate study aza-C reaction sample 1. 1.5 mg UCK1, 3 mg PK, 12.5 mM aza-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

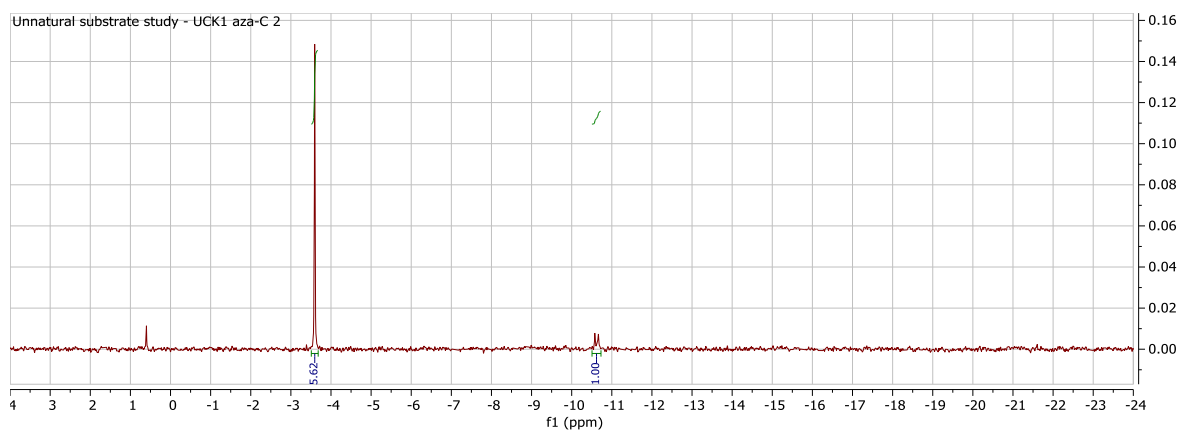


Figure A-I.159: ^{31}P NMR spectra of unnatural substrate study aza-C reaction sample 2. 1.5 mg UCK1, 3 mg PK, 12.5 mM aza-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

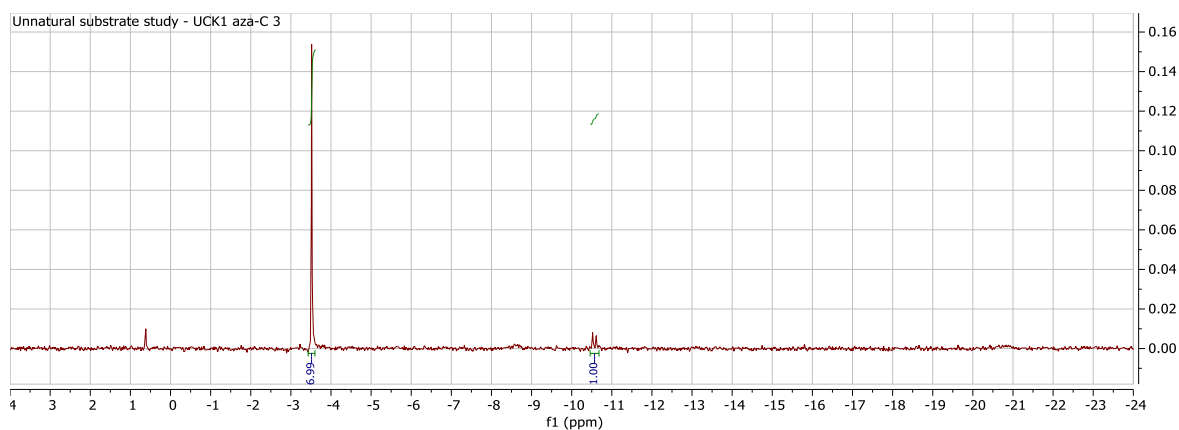


Figure A-I.160: ^{31}P NMR spectra of unnatural substrate study aza-C reaction sample 3. 1.5 mg UCK1, 3 mg PK, 12.5 mM aza-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

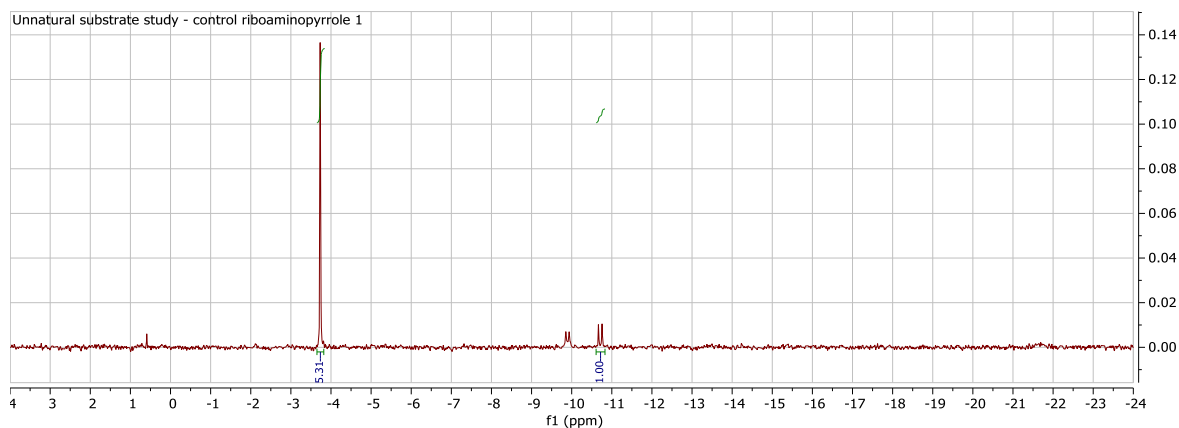


Figure A-I.161: ^{31}P NMR spectra of unnatural substrate study riboaminopyrrole control sample. 0 mg UCK1, 0 mg PK, 12.5 mM riboaminopyrrole. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

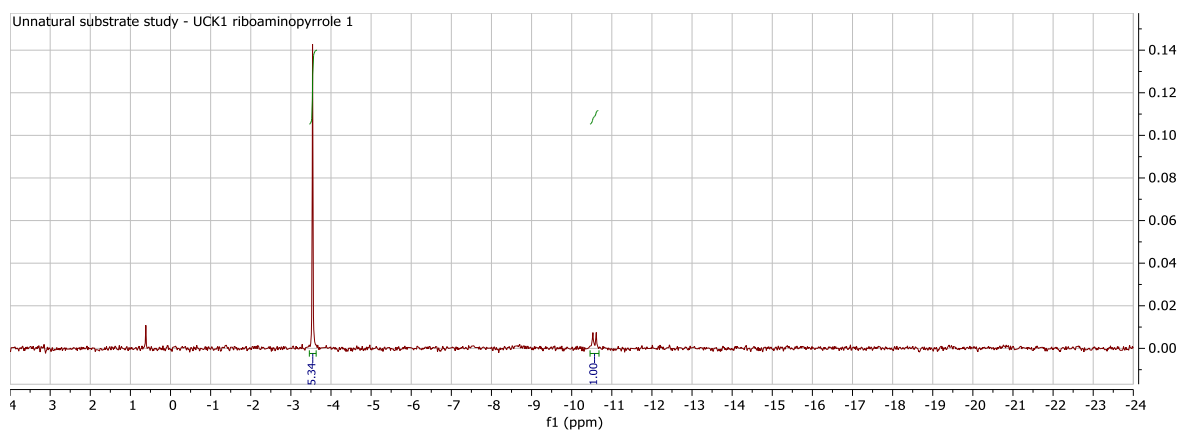


Figure A-I.162: ^{31}P NMR spectra of unnatural substrate study riboaminopyrrole reaction sample 1. 1.5 mg UCK1, 3 mg PK, 12.5 mM riboaminopyrrole. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

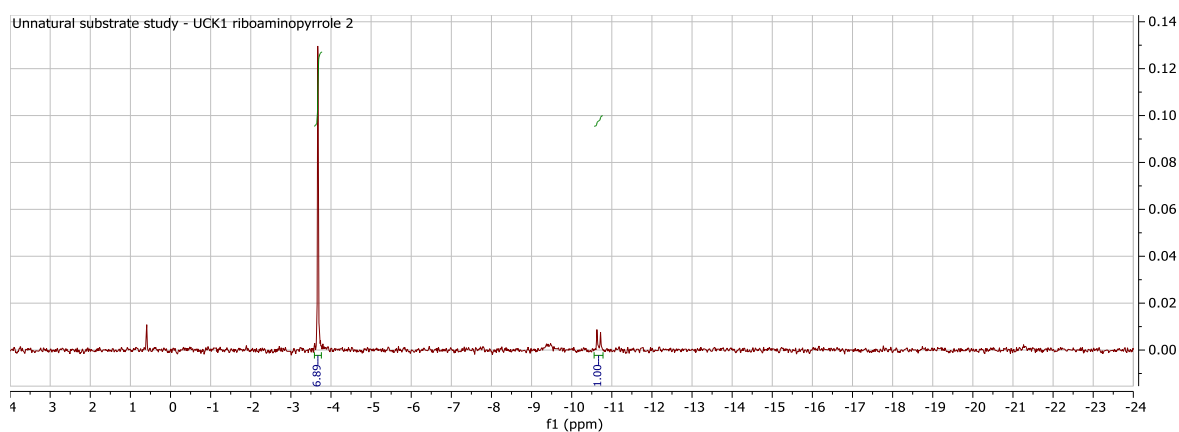


Figure A-I.163: ^{31}P NMR spectra of unnatural substrate study riboaminopyrrole reaction sample 2. 1.5 mg UCK1, 3 mg PK, 12.5 mM riboaminopyrrole. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

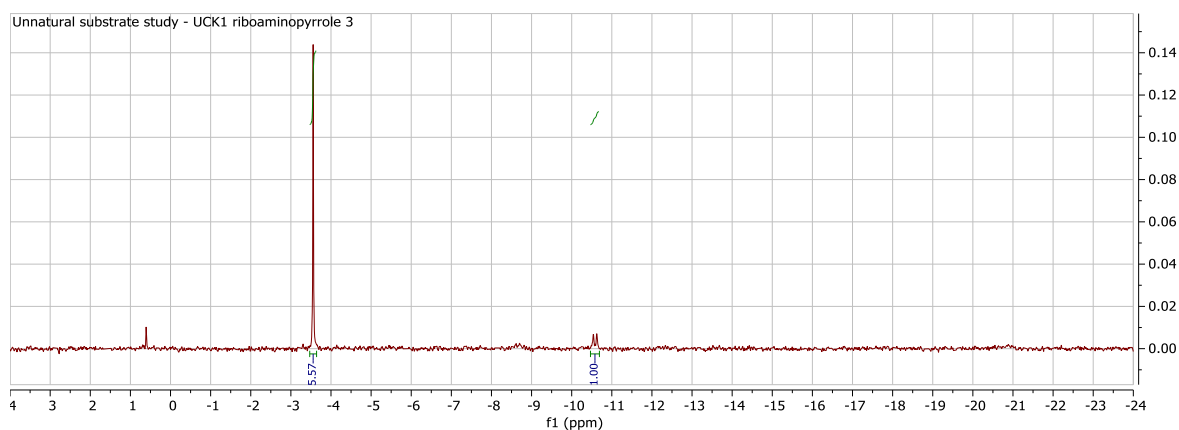


Figure A-I.164: ^{31}P NMR spectra of unnatural substrate study riboaminopyrrole reaction sample 3. 1.5 mg UCK1, 3 mg PK, 12.5 mM riboaminopyrrole. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

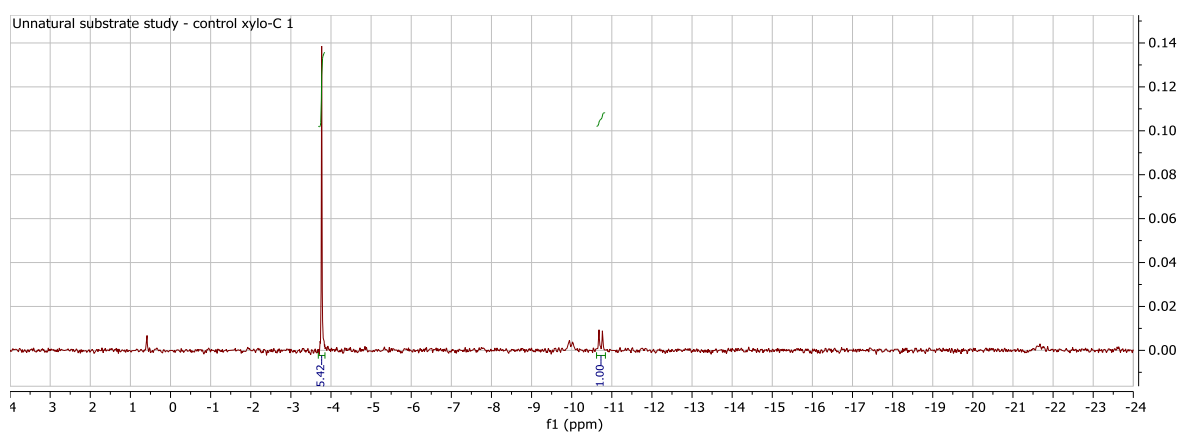


Figure A-I.165: ^{31}P NMR spectra of unnatural substrate study xylo-C control sample. 0 mg UCK1, 0 mg PK, 12.5 mM xylo-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

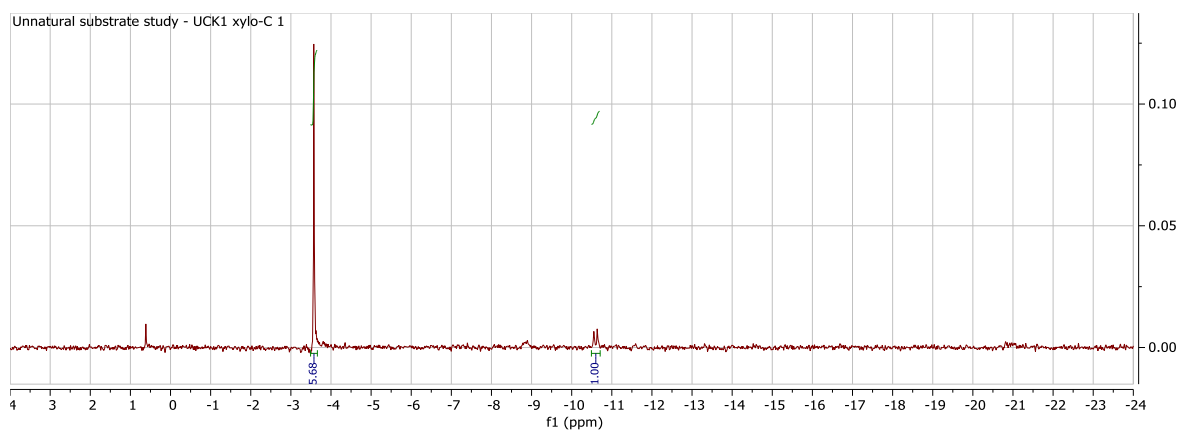


Figure A-I.166: ^{31}P NMR spectra of unnatural substrate study xylo-C reaction sample 1. 1.5 mg UCK1, 3 mg PK, 12.5 mM xylo-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

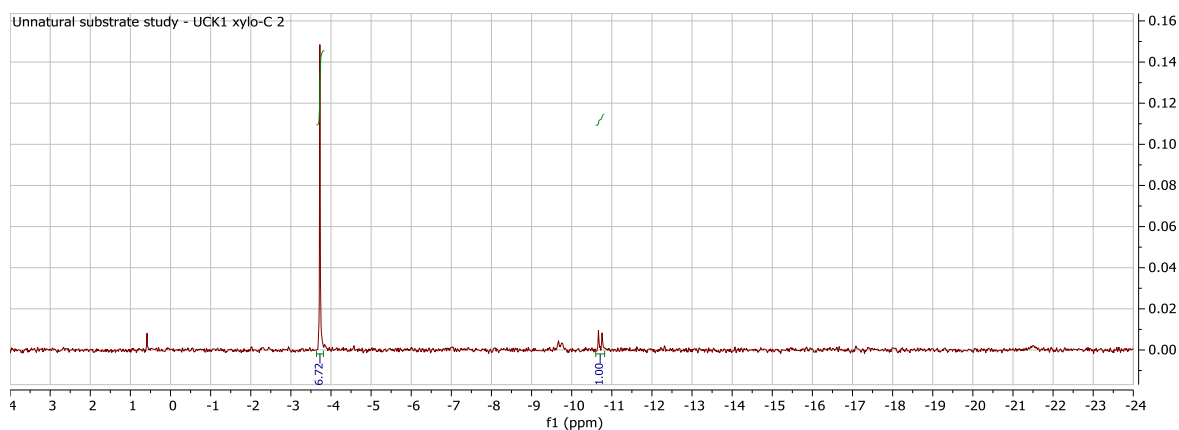


Figure A-I.167: ^{31}P NMR spectra of unnatural substrate study xylo-C reaction sample 2. 1.5 mg UCK1, 3 mg PK, 12.5 mM xylo-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

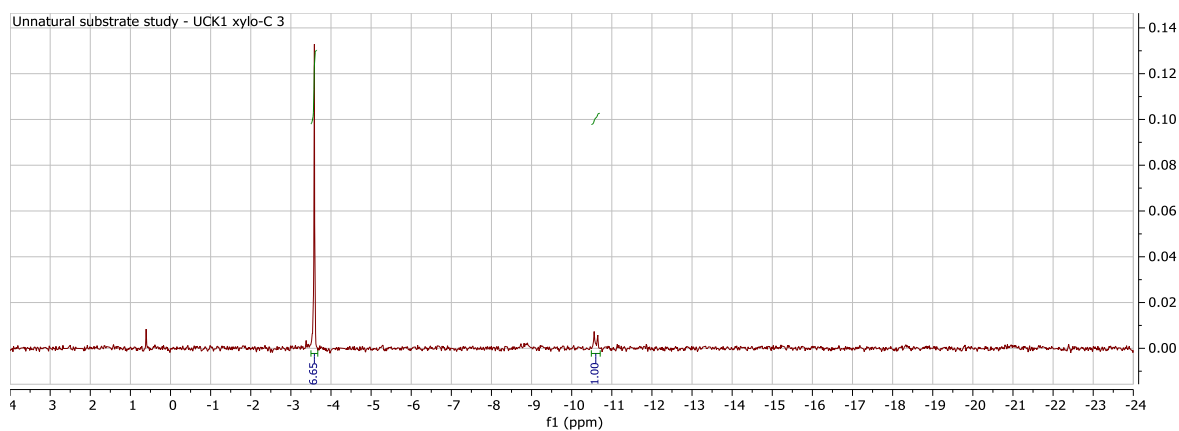


Figure A-I.168: ^{31}P NMR spectra of unnatural substrate study xylo-C reaction sample 3. 1.5 mg UCK1, 3 mg PK, 12.5 mM xylo-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

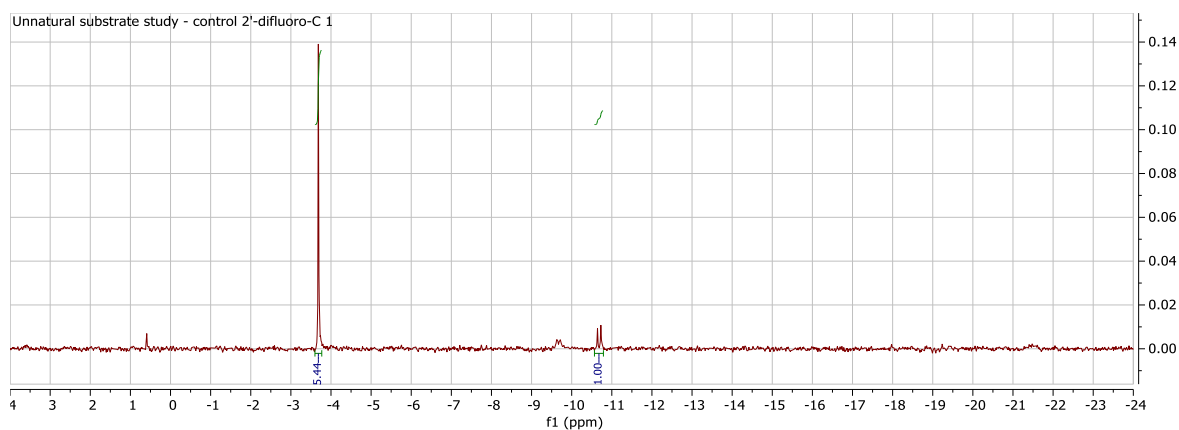


Figure A-I.169: ^{31}P NMR spectra of unnatural substrate study 2'-difluoro-C reaction sample 3. 0 mg UCK1, 0 mg PK, 12.5 mM 2'-difluoro-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

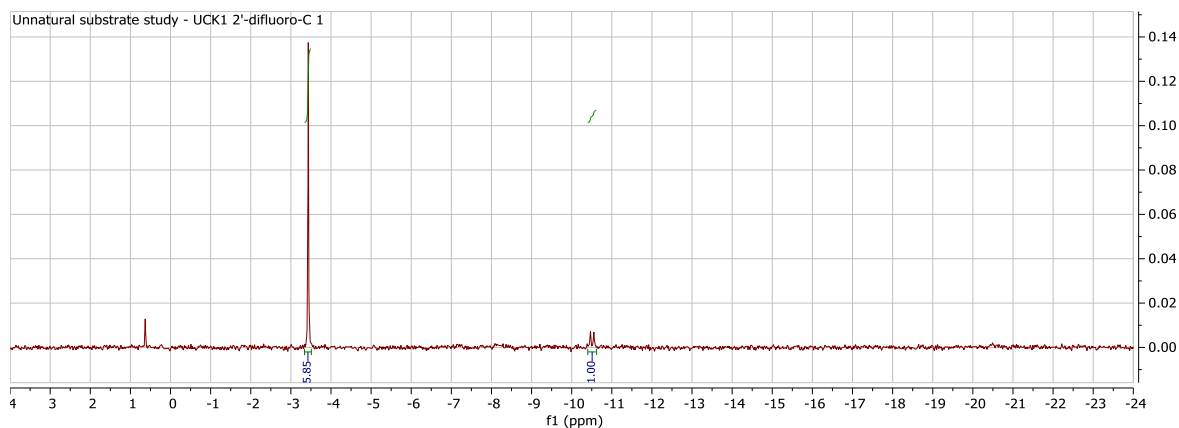


Figure A-I.170: ^{31}P NMR spectra of unnatural substrate study 2'-difluoro-C reaction sample 1. 1.5 mg UCK1, 3 mg PK, 12.5 mM 2'-difluoro-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

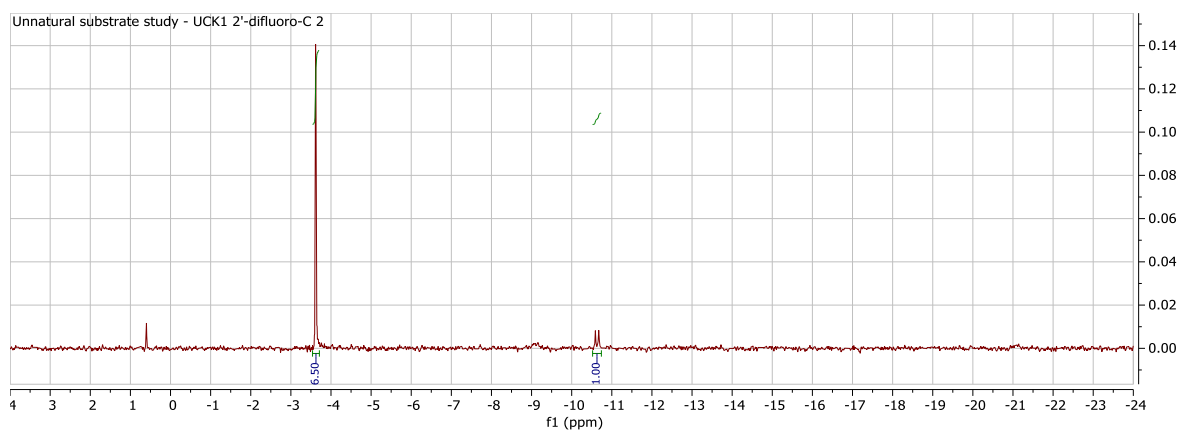


Figure A-I.171: ^{31}P NMR spectra of unnatural substrate study 2'-difluoro-C reaction sample 2. 1.5 mg UCK1, 3 mg PK, 12.5 mM 2'-difluoro-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

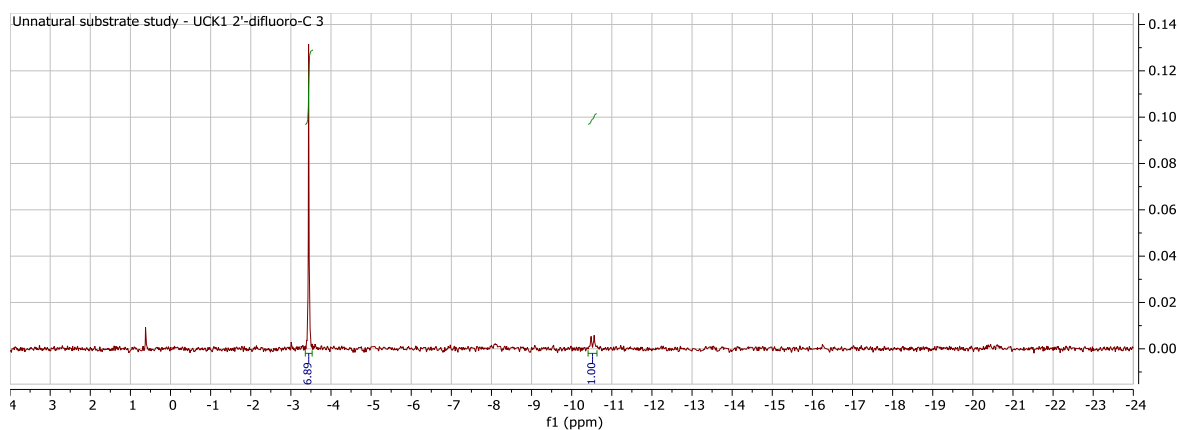


Figure A-I.172: ^{31}P NMR spectra of unnatural substrate study 2'-difluoro-C reaction sample 3. 1.5 mg UCK1, 3 mg PK, 12.5 mM 2'-difluoro-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

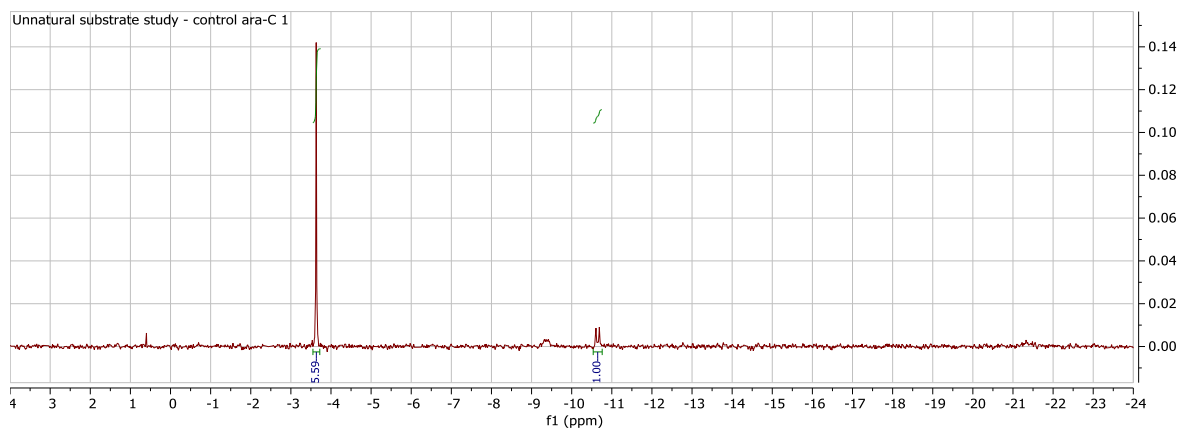


Figure A-I.173: ^{31}P NMR spectra of unnatural substrate study ara-C control sample. 0 mg UCK1, 0 mg PK, 12.5 mM ara-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

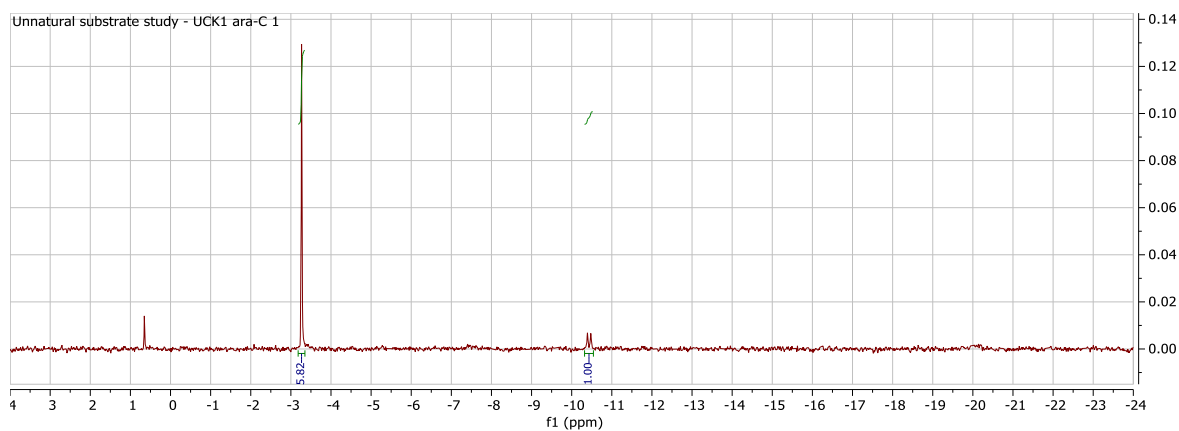


Figure A-I.174: ^{31}P NMR spectra of unnatural substrate study ara-C reaction sample 1. 1.5 mg UCK1, 3 mg PK, 12.5 mM ara-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

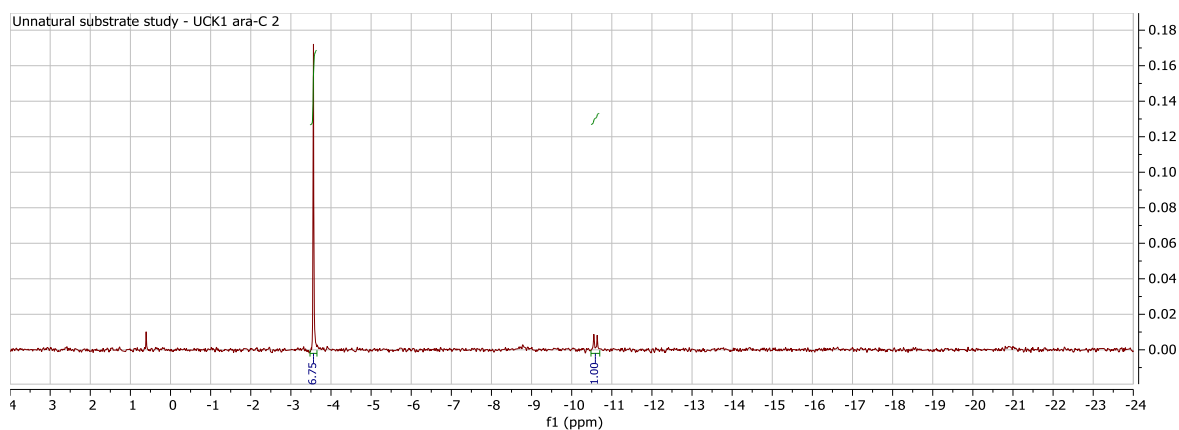


Figure A-I.175: ^{31}P NMR spectra of unnatural substrate study ara-C reaction sample 2. 1.5 mg UCK1, 3 mg PK, 12.5 mM ara-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

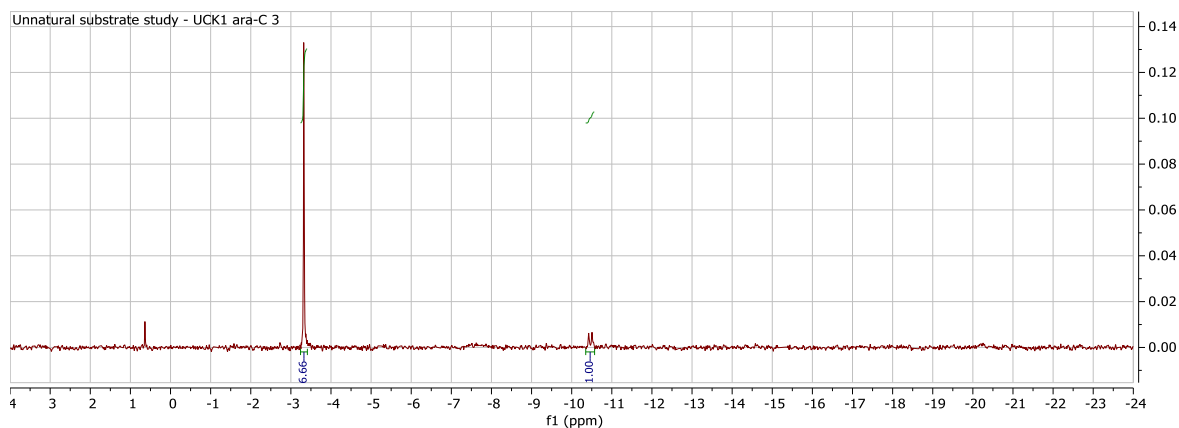


Figure A-I.176: ^{31}P NMR spectra of unnatural substrate study ara-C reaction sample 3. 1.5 mg UCK1, 3 mg PK, 12.5 mM ara-C. Process described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak data presented in Table A-I.7.

Substrate	Sample	UMP Peak	PEP Peak	X	Calculated Conversion
2'-methyl-C	Control	0.000	5.526	0.000	0.91
	Reaction 1	0.000	6.337	0.000	0.91
	Reaction 2	0.000	6.624	0.000	0.91
	Reaction 3	0.000	6.127	0.000	0.91
5-methyl-U	Control	0.000	6.156	0.000	0.91
	Reaction 1	1.033	5.165	0.167	19.6
	Reaction 2	0.000	5.728	0.000	0.91
	Reaction 3	0.440	6.759	0.061	7.74
2'-fluoro-2'-deoxy-C	Control	0.000	5.775	0.000	0.91
	Reaction 1	0.000	6.278	0.000	0.91
	Reaction 2	0.000	6.898	0.000	0.91
	Reaction 3	0.000	7.382	0.000	0.91
aza-C	Control	0.000	5.398	0.000	0.91
	Reaction 1	0.000	6.075	0.000	0.91
	Reaction 2	0.000	5.616	0.000	0.91
	Reaction 3	0.000	6.993	0.000	0.91
riboaminopyrrole	Control	0.000	5.315	0.000	0.91
	Reaction 1	0.000	5.343	0.000	0.91
	Reaction 2	0.000	6.887	0.000	0.91
	Reaction 3	0.000	5.571	0.000	0.91
xylo-C	Control	0.000	5.416	0.000	0.91
	Reaction 1	0.000	5.682	0.000	0.91
	Reaction 2	0.000	6.723	0.000	0.91
	Reaction 3	0.000	6.652	0.000	0.91
2'-difluoro-C	Control	0.000	5.440	0.000	0.91
	Reaction 1	0.000	5.846	0.000	0.91
	Reaction 2	0.000	6.503	0.000	0.91
	Reaction 3	0.000	6.889	0.000	0.91
ara-C	Control	0.000	5.593	0.000	0.91
	Reaction 1	0.000	5.823	0.000	0.91
	Reaction 2	0.000	6.748	0.000	0.91
	Reaction 3	0.000	6.655	0.000	0.91

Table A-I.7: Peak data from ³¹P NMR analysis of samples used in unnatural substrate study. Spectra can be found in Section A-I.12. Study described in Section 5.3. NMR analysis conducted as described in Section 2.7.1.2. Peak integrals normalised to peak relating to α-phosphate of ATP. $X = \text{UMP peak} / (\text{UMP peak} + \text{PEP peak})$. Substrate conversion calculated using model described in Section 4.7.

Note: The observed 0.91% substrate conversion results can be considered the result of model error as all samples with nucleoside monophosphate peak integrals of zero are calculated as having 0.91% substrate conversion.

Appendix II: LCMS Data

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A-II.1 Representative MS Spectra for Target Compounds

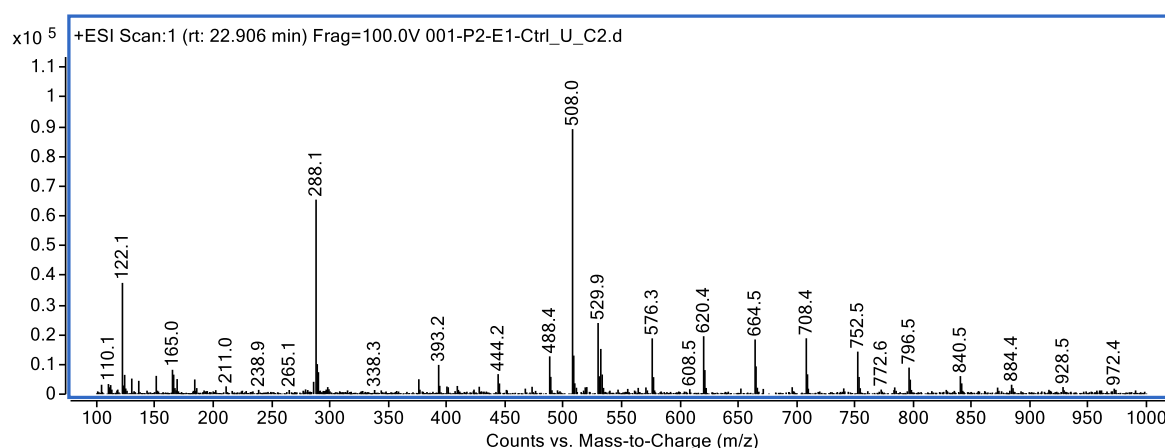


Figure A-II.1: Representative MS spectrum depicting the presence of ATP in sample. Spectrum taken from TIC of LCMS analysis of natural substrates study uridine control sample 1, at 22.906 min retention time. Peak at $m/z = 508.0$ identified as ATP. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

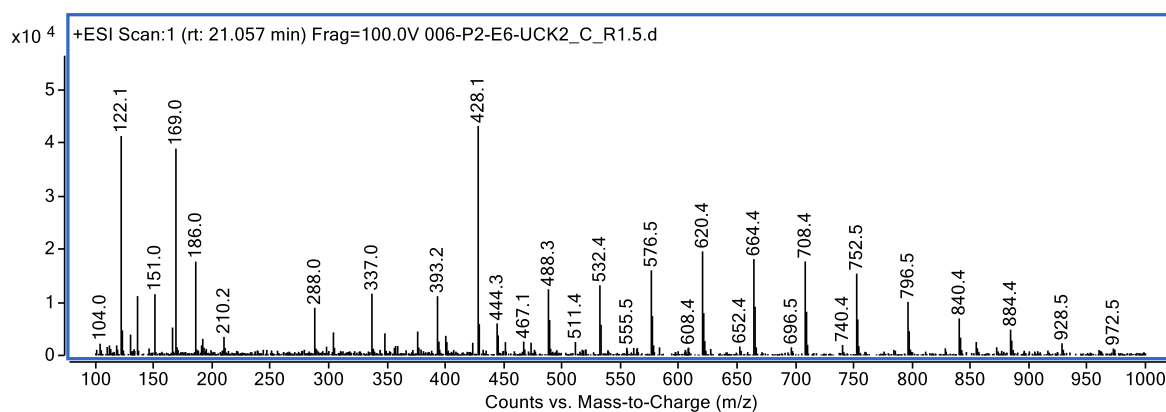


Figure A-II.2: Representative MS spectrum depicting the presence of ADP in sample. Spectrum taken from TIC of LCMS analysis of natural substrates study UCK2-cytidine reaction sample 1, at 21.057 min retention time. Peak at $m/z = 428.1$ identified as ADP. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

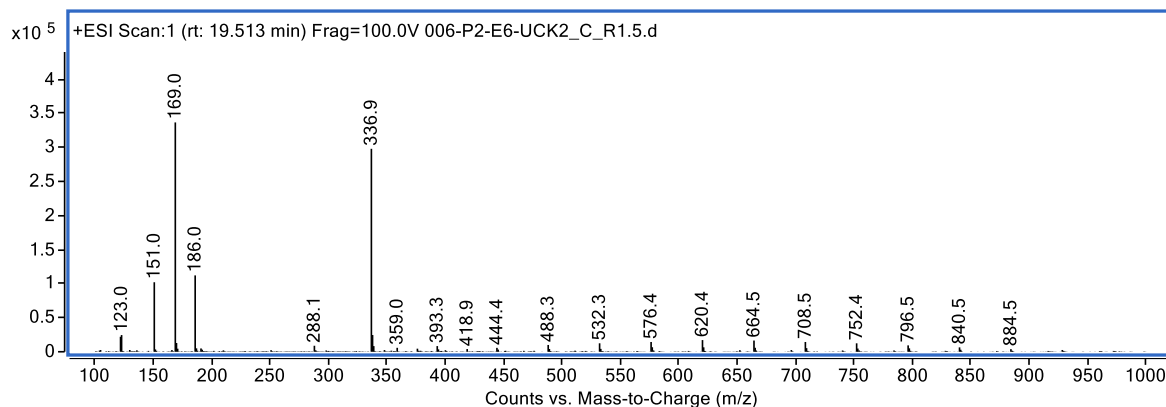


Figure A-II.3: Representative MS spectrum depicting the presence of PEP in sample. Spectrum taken from TIC of LCMS analysis of natural substrates study UCK2-cytidine reaction sample 1, at 19.513 min retention time. Peak at $m/z = 169.0$ identified as PEP. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

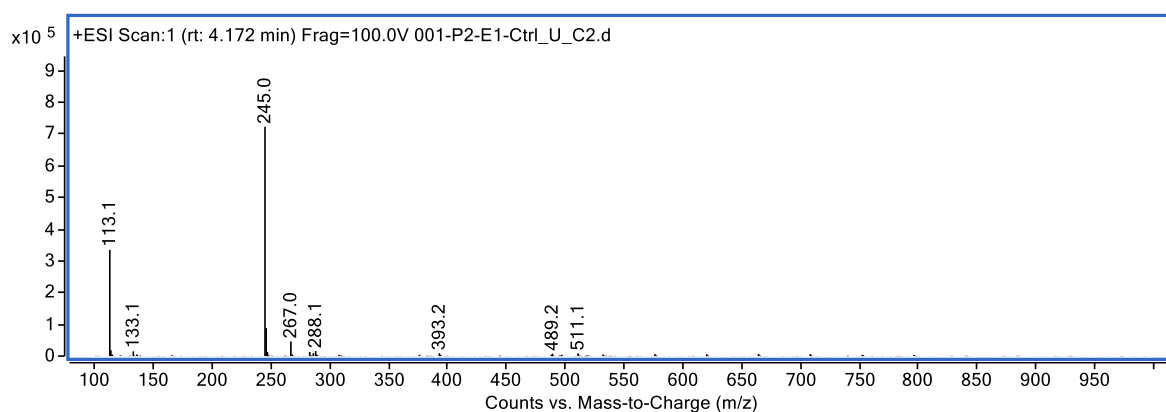


Figure A-II.4: Representative MS spectrum depicting the presence of uridine in sample. Spectrum taken from TIC of LCMS analysis of natural substrates study uridine control sample 1, at 4.172 min retention time. Peak at $m/z = 245.0$ identified as uridine. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

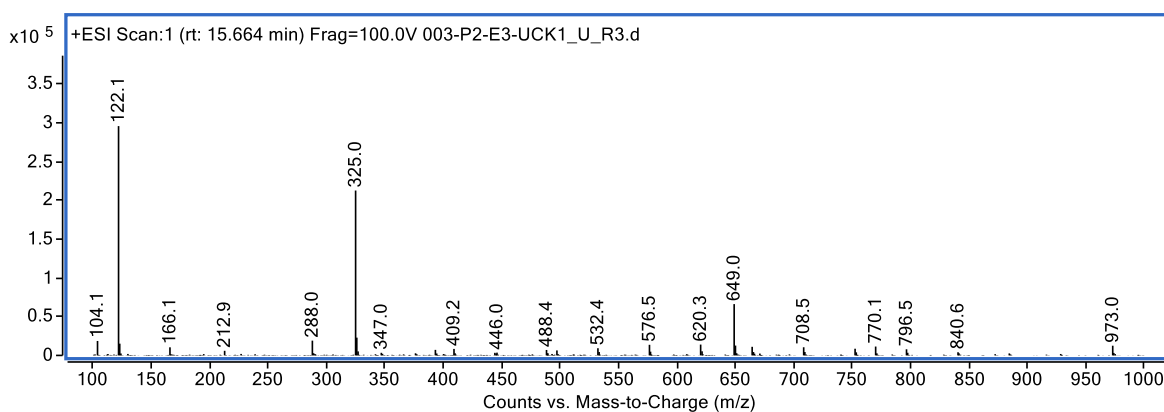


Figure A-II.5: Representative MS spectrum depicting the presence of UMP in sample. Spectrum taken from TIC of LCMS analysis of natural substrates study UCK1-uridine reaction sample 1, at 15.664 min retention time. Peak at $m/z = 325.0$ identified as UMP. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

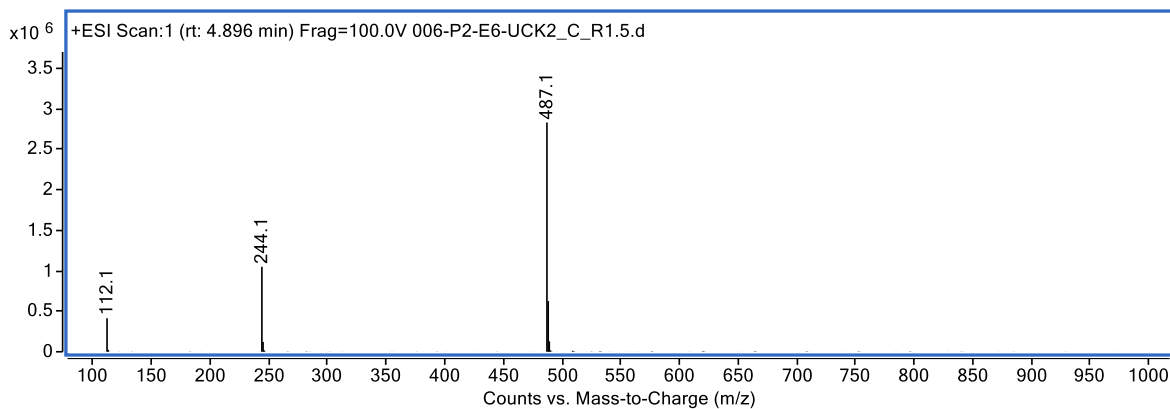


Figure A-II.6: Representative MS spectrum depicting the presence of cytidine in sample. Spectrum taken from TIC of LCMS analysis of natural substrates study UCK2-cytidine reaction sample 1, at 4.896 min retention time. Peak at $m/z = 244.1$ identified as cytidine. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

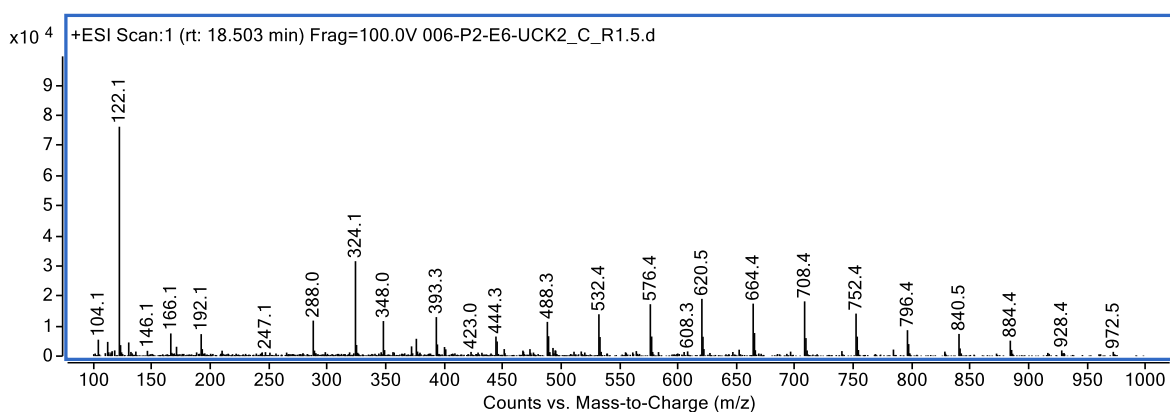


Figure A-II.7: Representative MS spectrum depicting the presence of CMP in sample. Spectrum taken from TIC of LCMS analysis of natural substrates study UCK2-cytidine reaction sample 1, at 18.503 min retention time. Peak at $m/z = 324.1$ identified as CMP. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

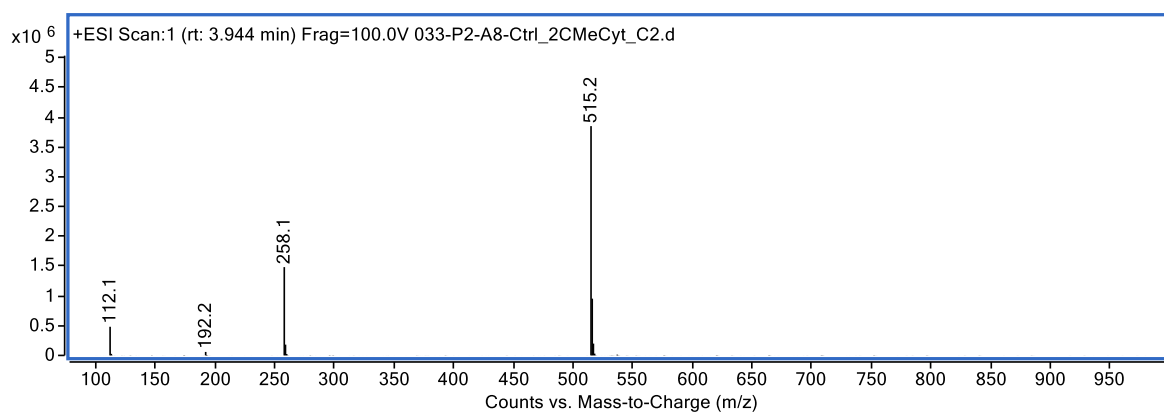


Figure A-II.8: Representative MS spectrum depicting the presence of 2'-methyl-C in sample. Spectrum taken from TIC of LCMS analysis of unnatural substrates study 2'-methyl-C control sample 1, at 3.944 min retention time. Peak at $m/z = 258.1$ identified as 2'-methyl-C. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

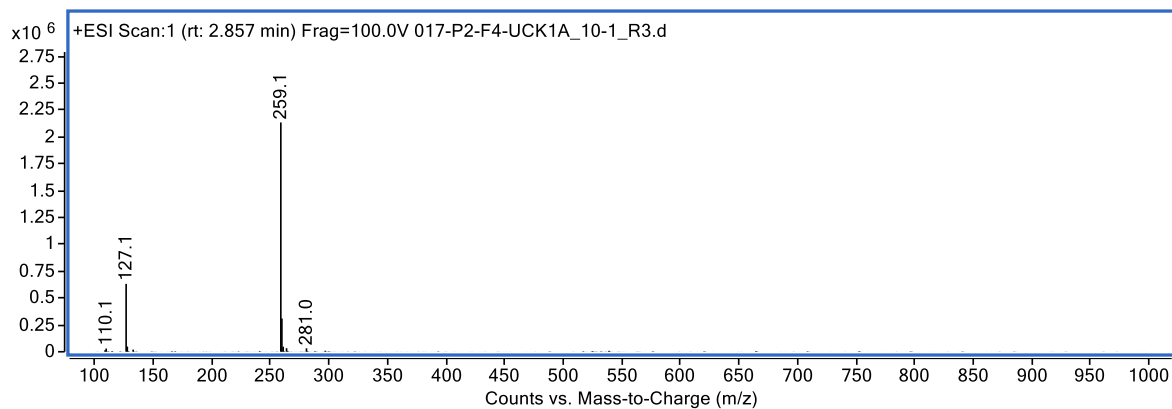


Figure A-II.9: Representative MS spectrum depicting the presence of 5-methyl-U in sample. Spectrum taken from TIC of LCMS analysis of unnatural substrates study UCK1-5-methyl-U reaction sample 1, at 2.857 min retention time. Peak at $m/z = 259.1$ identified as 5-methyl-U. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

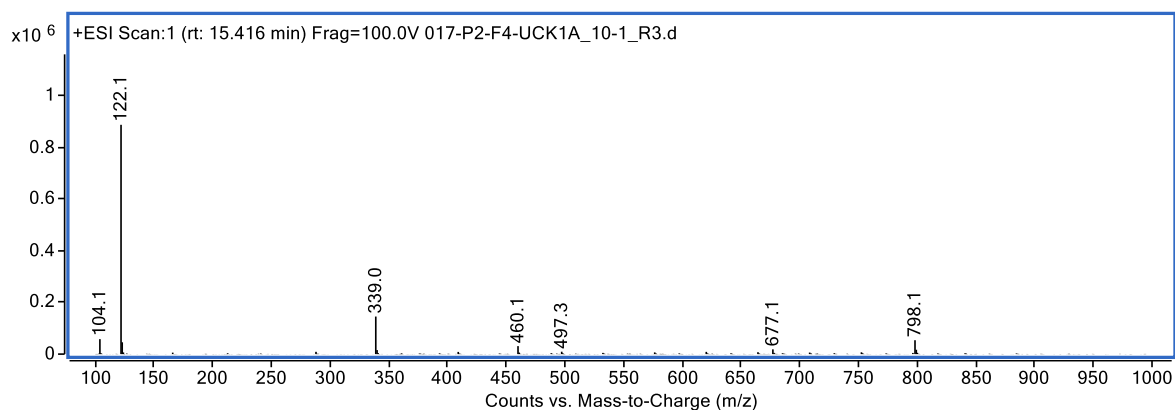


Figure A-II.10: Representative MS spectrum depicting the presence of 5-methyl-U monophosphate in sample. Spectrum taken from TIC of LCMS analysis of unnatural substrates study UCK1-5-methyl-U reaction sample 1, at 15.416 min retention time. Peak at $m/z = 339.0$ identified as 5-methyl-U monophosphate. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

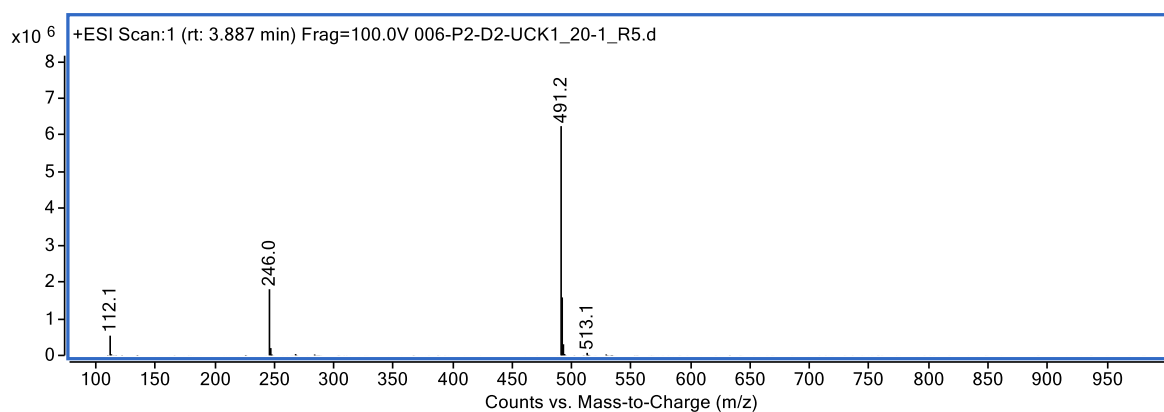


Figure A-II.11: Representative MS spectrum depicting the presence of 2'-fluoro-2'-deoxy-C in sample. Spectrum taken from TIC of LCMS analysis of unnatural substrates study UCK1-2'-fluoro-2'-deoxy-C reaction sample 3, at 3.887 min retention time. Peak at $m/z = 246.0$ identified as 2'-fluoro-2'-deoxy-C. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

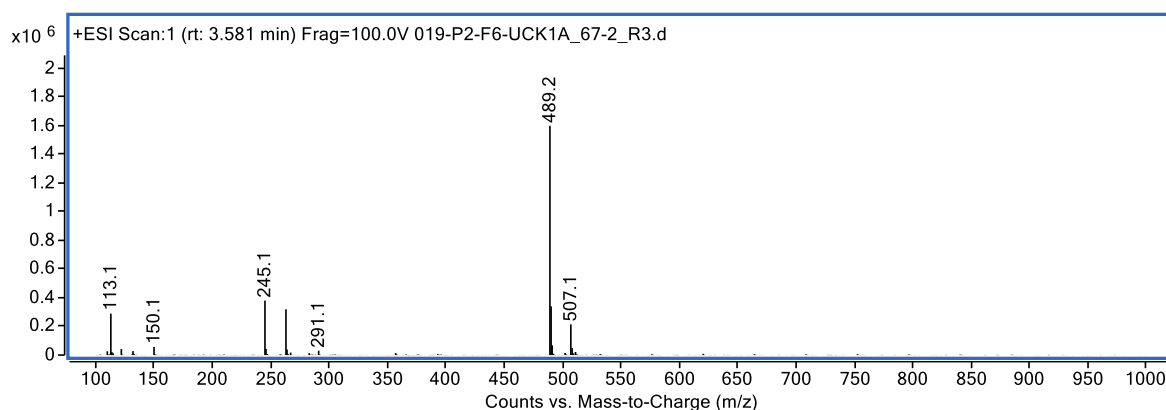


Figure A-II.12: Representative MS spectrum depicting the presence of aza-C in sample. Spectrum taken from TIC of LCMS analysis of unnatural substrates study UCK1-aza-C reaction sample 1, at 3.581 min retention time. Peak at $m/z = 245.1$ identified as aza-C. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

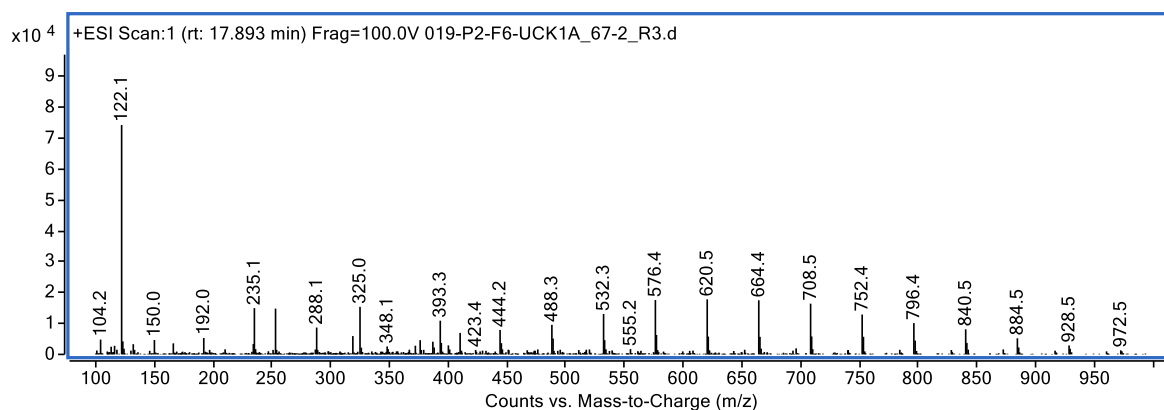


Figure A-II.13: Representative MS spectrum depicting the presence of aza-C monophosphate in sample. Spectrum taken from TIC of LCMS analysis of unnatural substrates study UCK1-aza-C reaction sample 1, at 17.893 min retention time. Peak at $m/z = 325.0$ identified as aza-C monophosphate. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

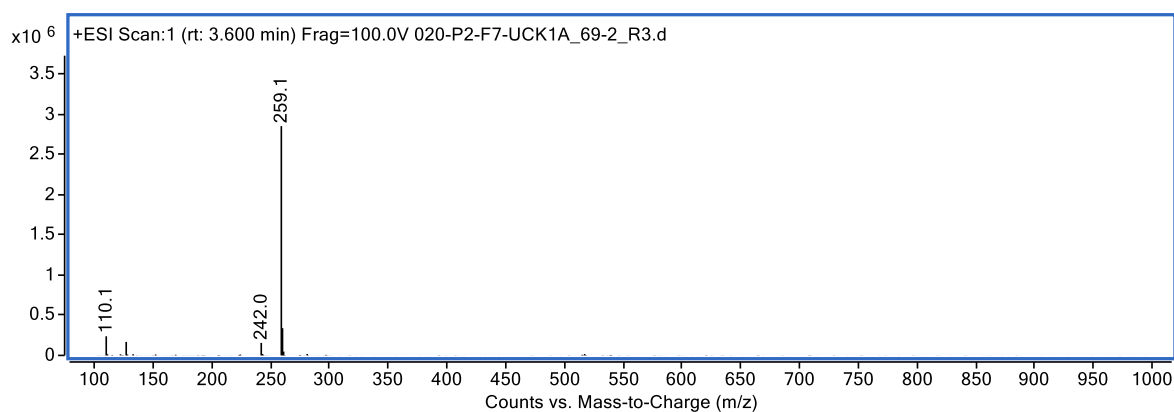


Figure A-II.14: Representative MS spectrum depicting the presence of riboaminopyrrole in sample. Spectrum taken from TIC of LCMS analysis of unnatural substrates study UCK1-riboaminopyrrole reaction sample 1, at 3.600 min retention time. Peak at $m/z = 259.1$ identified as riboaminopyrrole. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

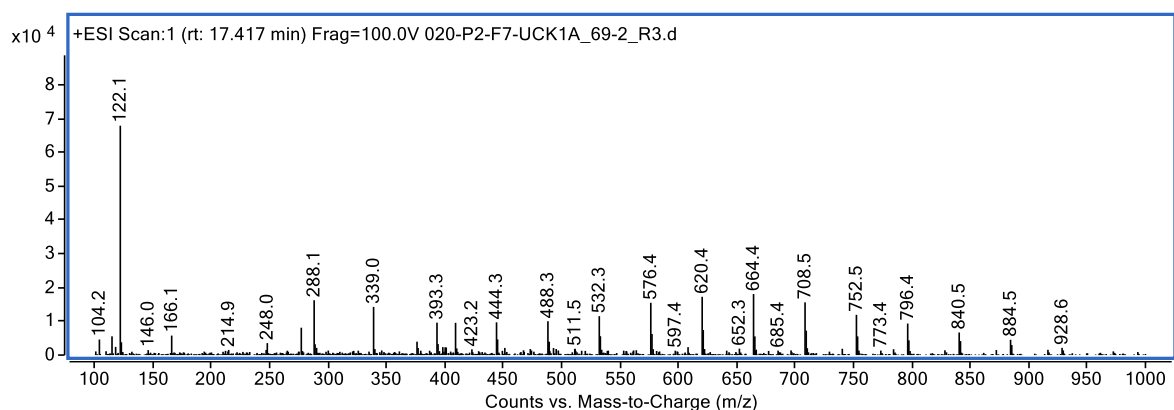


Figure A-II.15: Representative MS spectrum depicting the presence of riboaminopyrrole monophosphate in sample. Spectrum taken from TIC of LCMS analysis of unnatural substrates study UCK1-riboaminopyrrole reaction sample 1, at 17.417 min retention time. Peak at $m/z = 339.0$ identified as riboaminopyrrole monophosphate. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

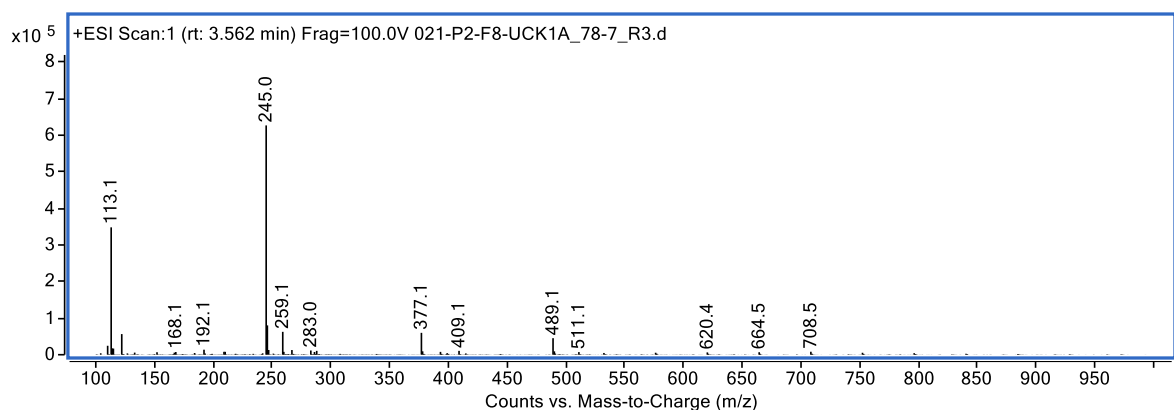


Figure A-II.16: Representative MS spectrum depicting the presence of xylo-C in sample. Spectrum taken from TIC of LCMS analysis of unnatural substrates study UCK1-xylo-C reaction sample 1, at 3.562 min retention time. Peak at $m/z = 245.0$ identified as xylo-C. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

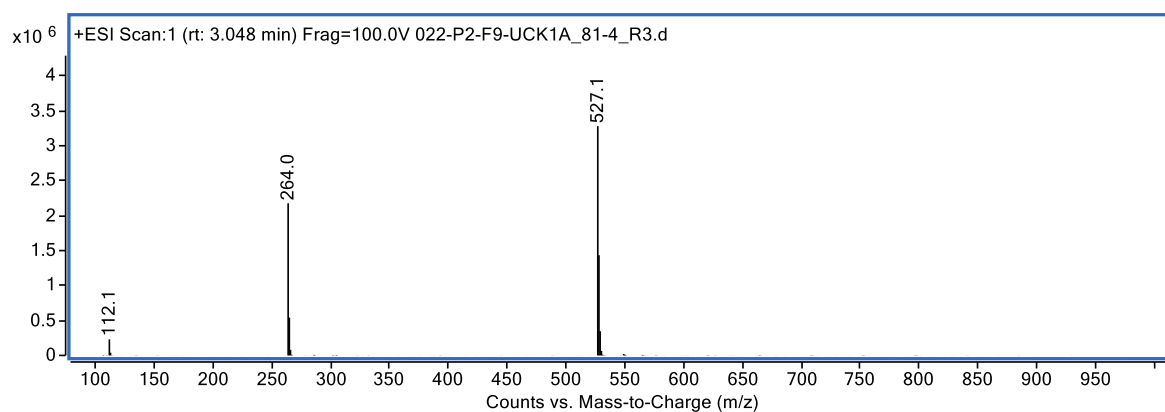


Figure A-II.17: Representative MS spectrum depicting the presence of 2'-difluoro-C in sample. Spectrum taken from TIC of LCMS analysis of unnatural substrates study UCK1-2'-difluoro-C reaction sample 1, at 3.048 min retention time. Peak at m/z = 264.0 identified as 2'-difluoro-C. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

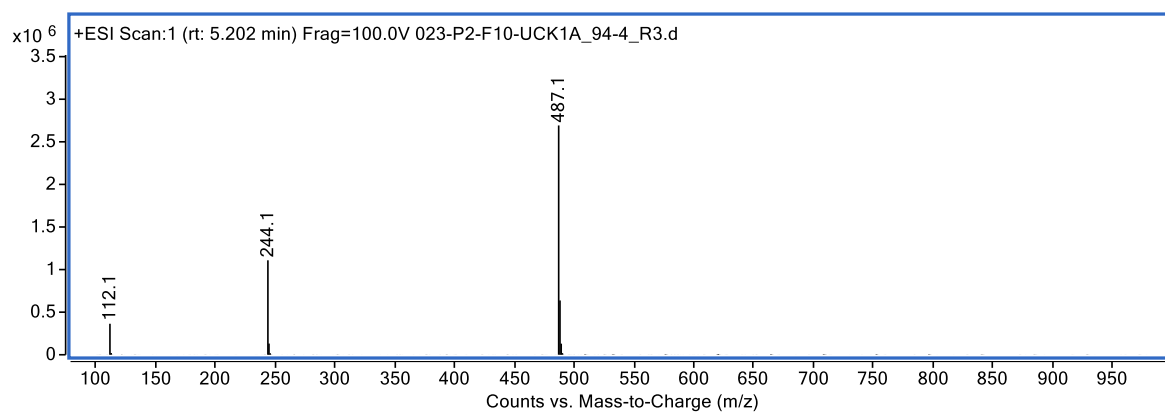


Figure A-II.18: Representative MS spectrum depicting the presence of ara-C in sample. Spectrum taken from TIC of LCMS analysis of unnatural substrates study UCK1-ara-C reaction sample 1, at 5.202 min retention time. Peak at m/z = 244.1 identified as ara-C. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

Note: Unnatural substrate monophosphates with no representative spectrum herein were not identified in any reaction mixture by EIC analysis of LCMS data.

A-II.2 Activity Against Natural Substrates

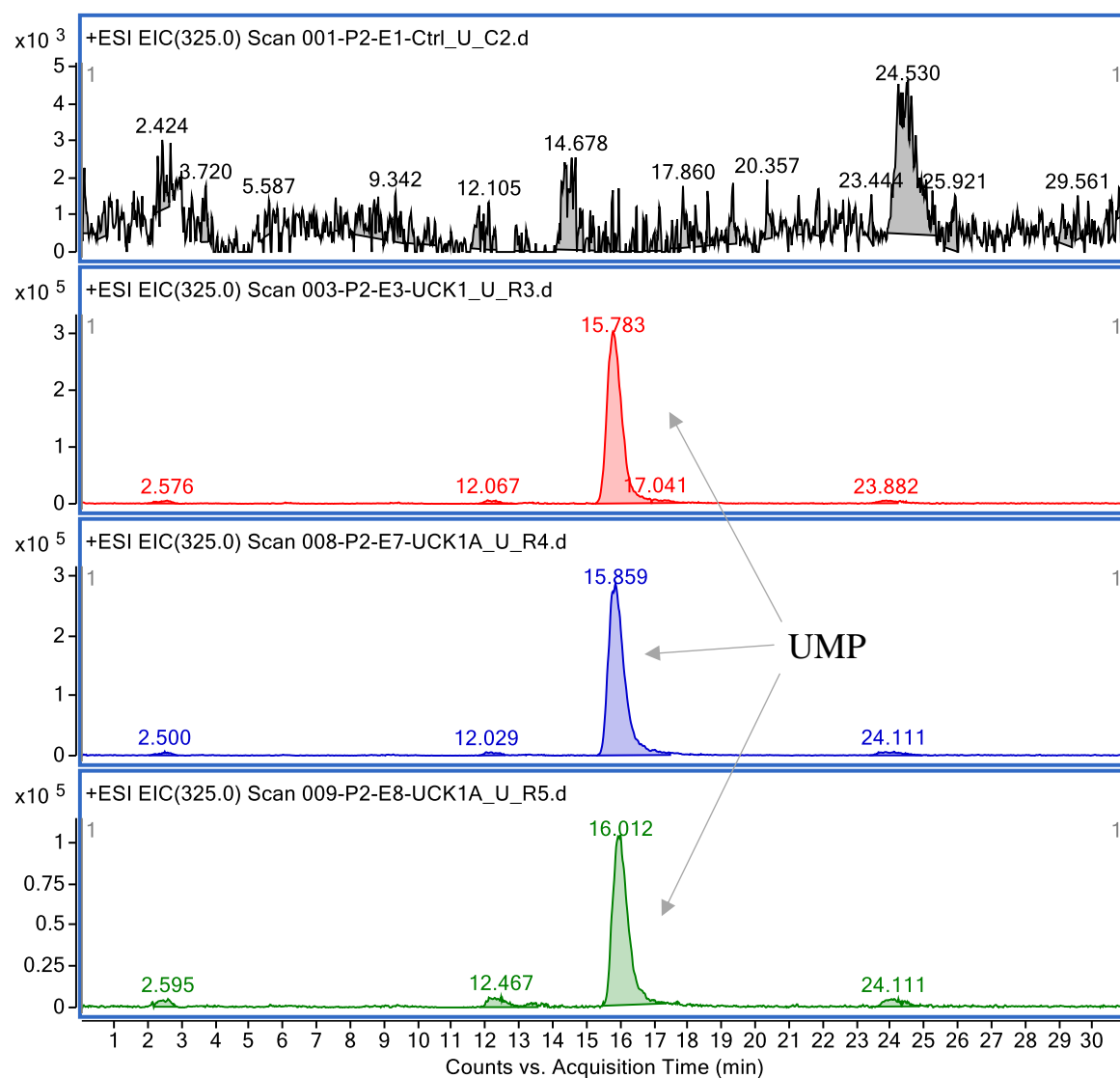


Figure A-II.19: Comparison of UMP-targeting EIC spectra of UCK1-uridine reactions and uridine control sample. Spectra taken from EIC of LCMS data of natural substrates study samples. All EIC extraction done targeting m/z value of 325.0 ± 0.5 . Black – uridine control sample 1. Red – UCK1-uridine reaction sample 1. Blue – UCK1-uridine reaction sample 2. Green – UCK1-uridine reaction sample 3. Peaks at approx. 16 min retention time include ion fragment at $m/z = 325.0$ that was identified as UMP. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

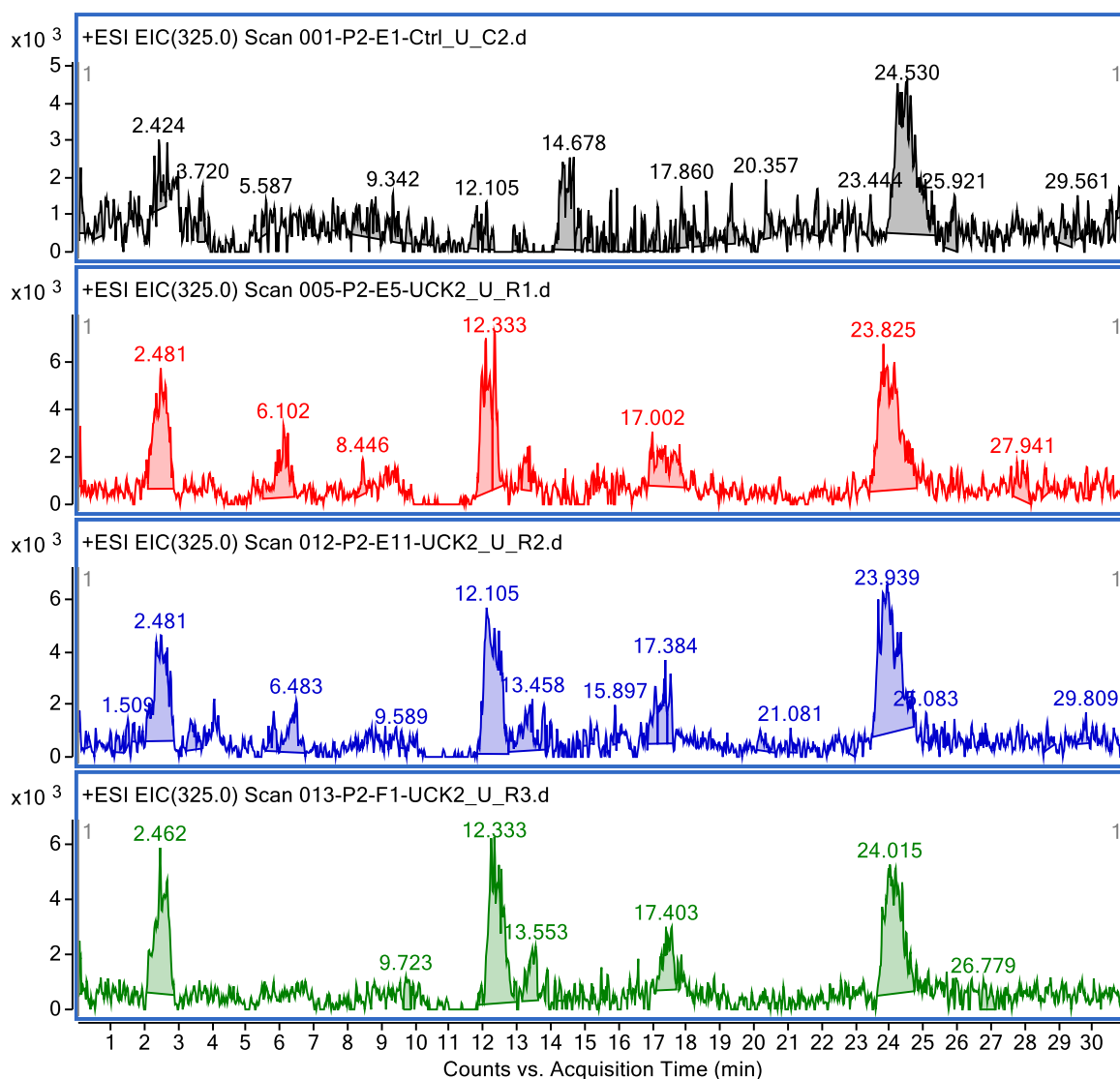


Figure A-II.20: Comparison of UMP-targeting EIC spectra of UCK2-uridine reactions and uridine control sample. Spectra taken from EIC of LCMS data of natural substrates study samples. All EIC extraction done targeting m/z value of 325.0 ± 0.5 . Black – uridine control sample 1. Red – UCK2-uridine reaction sample 1. Blue – UCK2-uridine reaction sample 2. Green – UCK2-uridine reaction sample 3. No peaks containing UMP could be distinguished. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

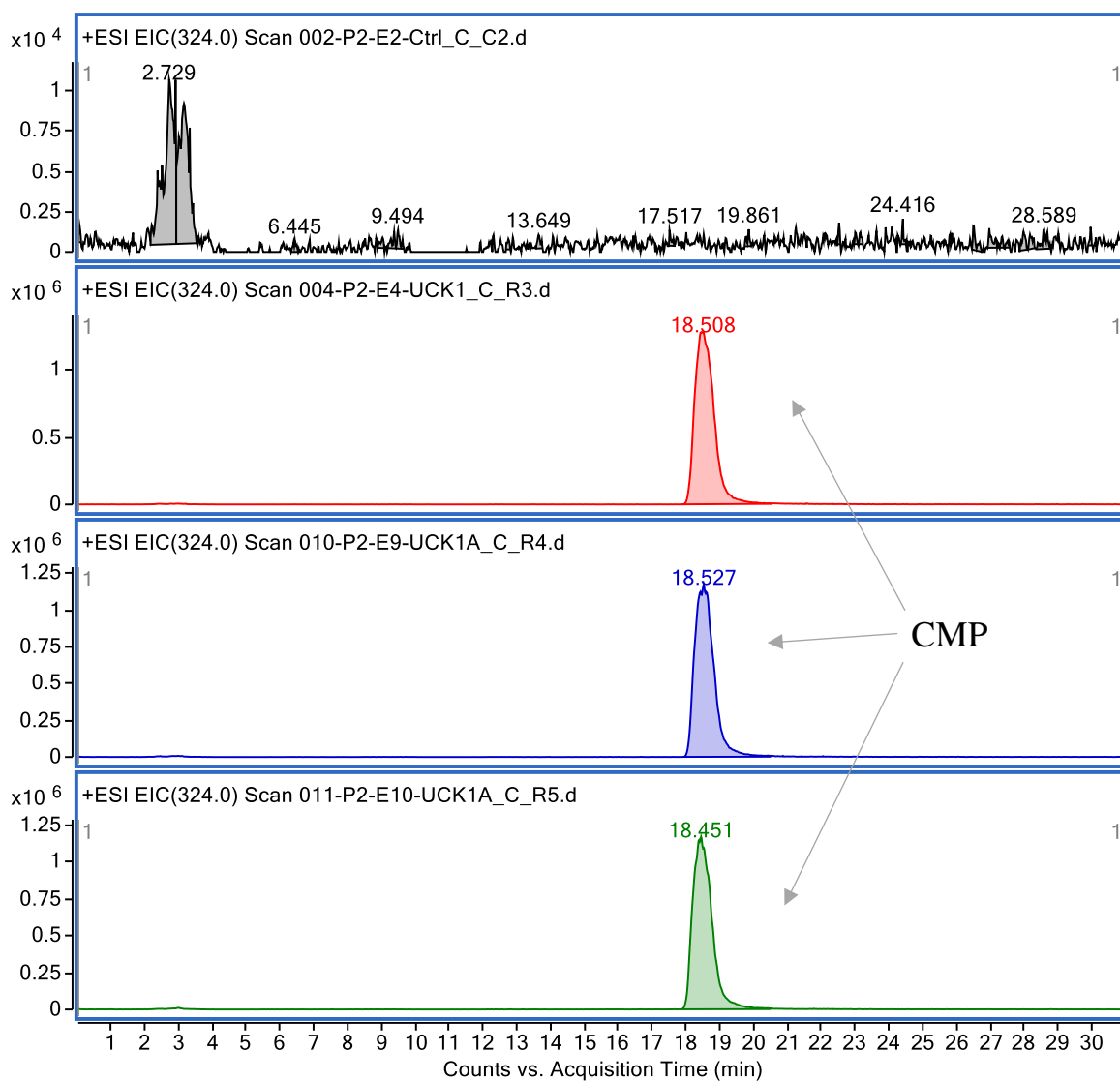


Figure A-II.21: Comparison of CMP-targeting EIC spectra of UCK1-cytidine reactions and cytidine control sample. Spectra taken from EIC of LCMS data of natural substrates study samples. All EIC extraction done targeting m/z value of 324.0 ± 0.5 . Black – cytidine control sample 1. Red – UCK1-cytidine reaction sample 1. Blue – UCK1-cytidine reaction sample 2. Green – UCK1-cytidine reaction sample 3. Peaks at approx. 18.5 min retention time include ion fragment at $m/z = 324.1$ that was identified as CMP. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

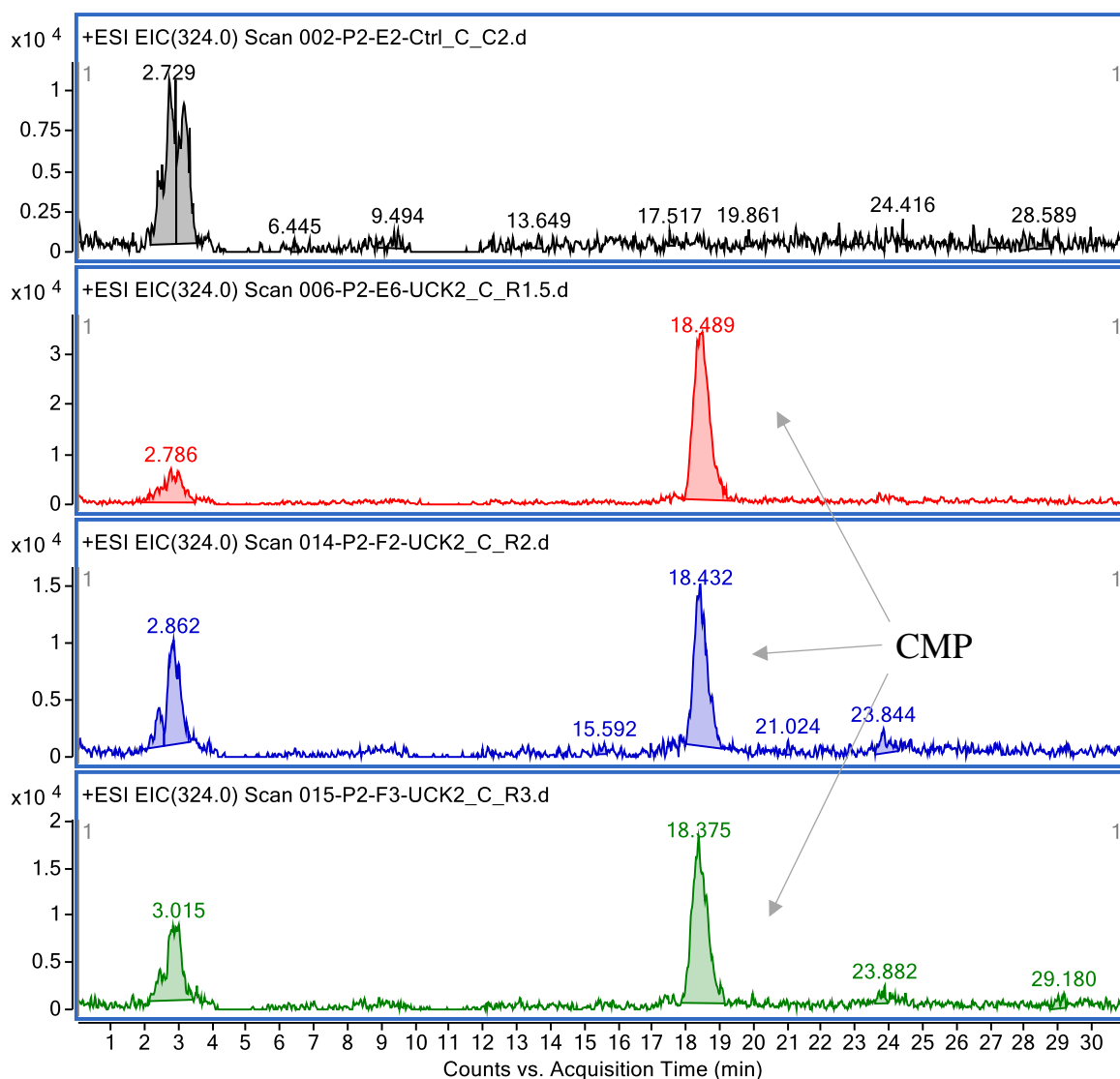


Figure A-II.22: Comparison of CMP-targeting EIC spectra of UCK2-cytidine reactions and cytidine control sample. Spectra taken from EIC of LCMS data of natural substrates study samples. All EIC extraction done targeting m/z value of 324.0 ± 0.5 . Black – cytidine control sample 1. Red – UCK2-cytidine reaction sample 1. Blue – UCK2-cytidine reaction sample 2. Green – UCK2-cytidine reaction sample 3. Peaks at approx. 18.5 min retention time include ion fragment at $m/z = 324.1$ that was identified as CMP. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

A-II.3 Activity Against Unnatural Substrates

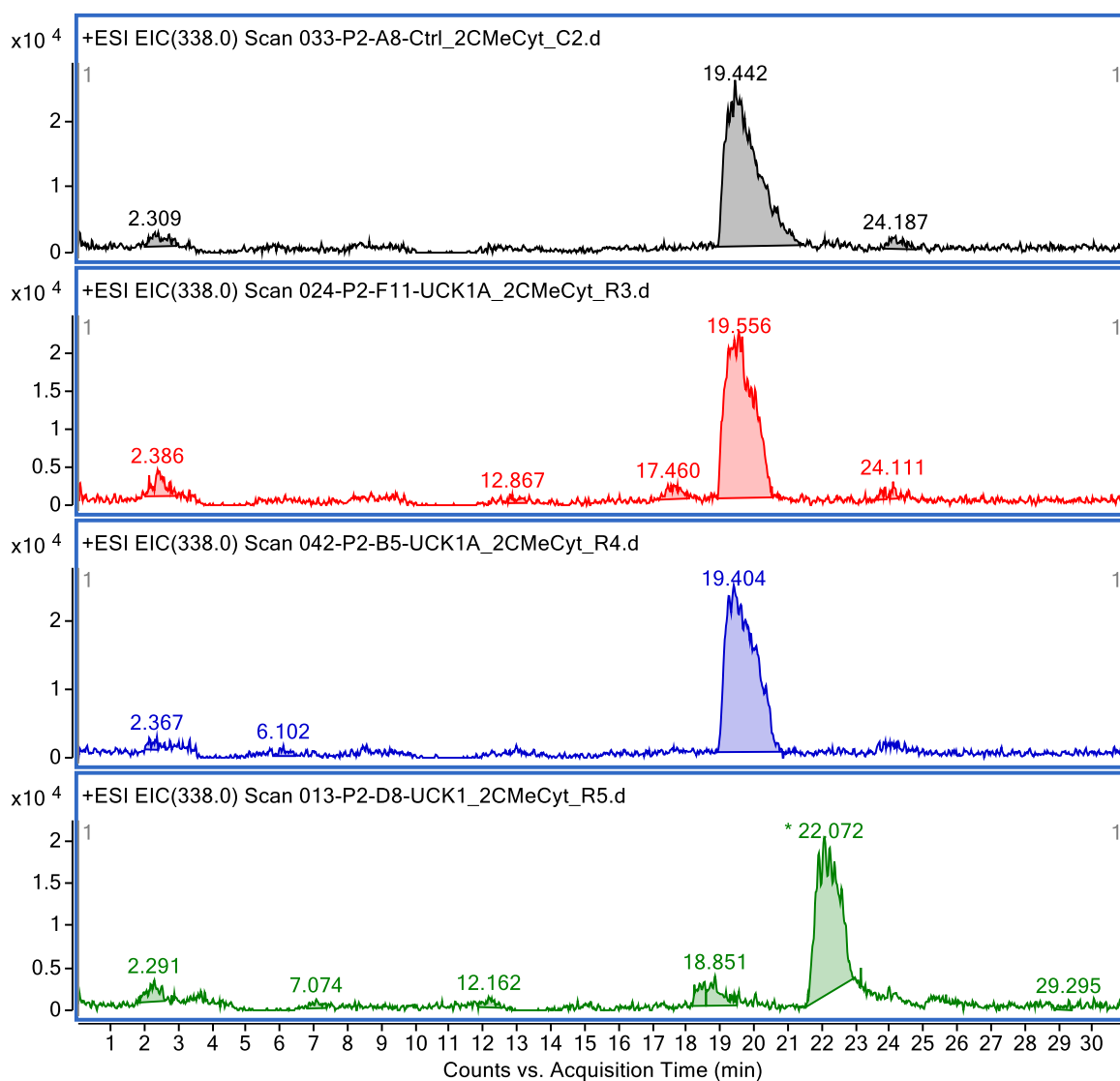


Figure A-II.23: Comparison of 2'-methyl-C-monophosphate-targeting EIC spectra of UCK1-2'-methyl-C reactions and 2'-methyl-C control sample. Spectra taken from EIC of LCMS data of unnatural substrates study samples. All EIC extraction done targeting m/z value of 338.0 ± 0.5 . Black – 2'-methyl-C control sample 1. Red – UCK1-2'-methyl-C reaction sample 1. Blue – UCK1-2'-methyl-C reaction sample 2. Green – UCK1-2'-methyl-C reaction sample 3. No peaks containing 2'-methyl-C monophosphate could be distinguished. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

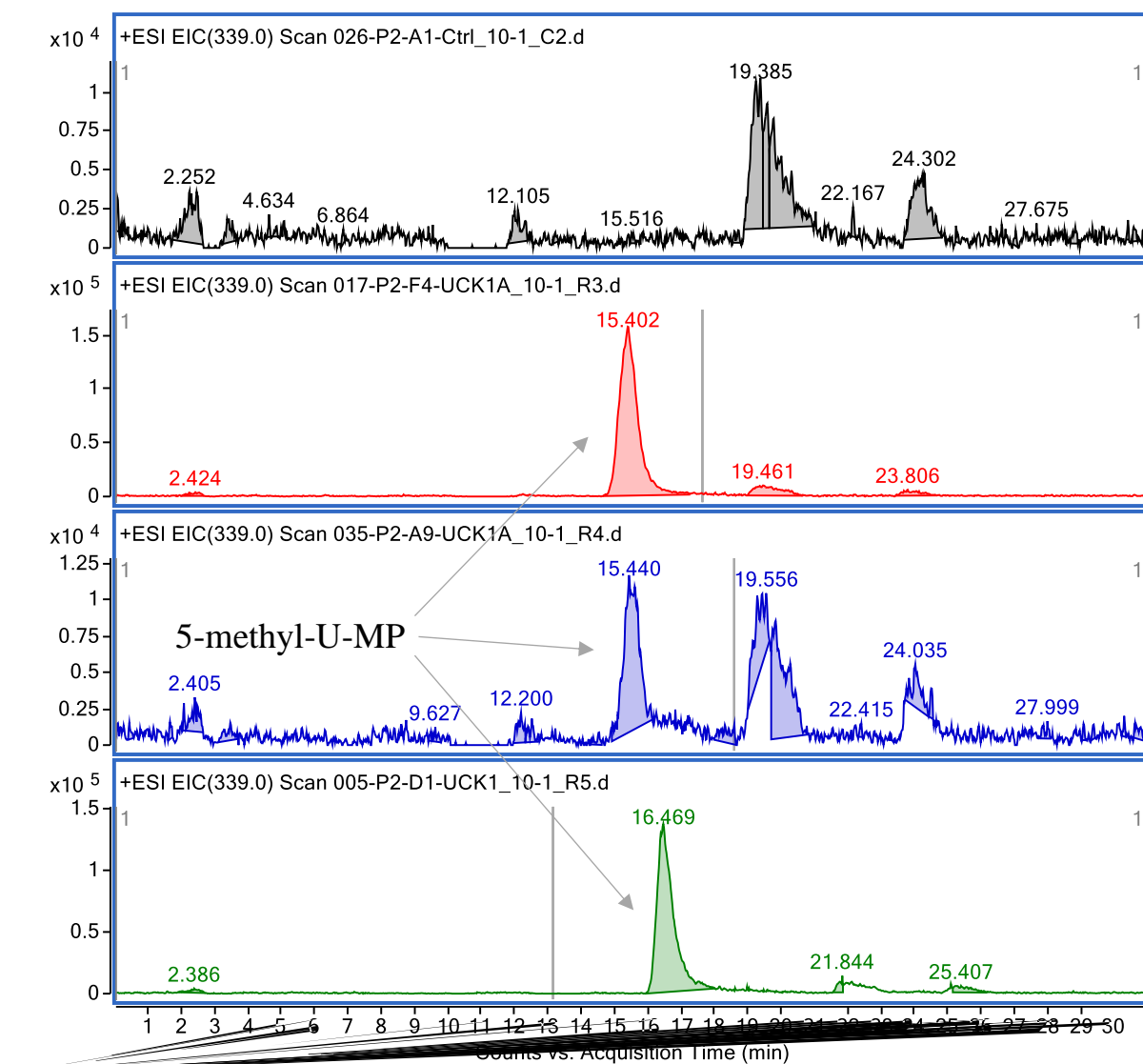


Figure A-II.24: Comparison of 5-methyl-U-monophosphate-targeting EIC spectra of UCK1-5-methyl-U reactions and 5-methyl-U control sample. Spectra taken from EIC of LCMS data of unnatural substrates study samples. All EIC extraction done targeting m/z value of 339.0 ± 0.5 . Black – 5-methyl-U control sample 1. Red – UCK1-5-methyl-U reaction sample 1. Blue – UCK1-5-methyl-U reaction sample 2. Green – UCK1-5-methyl-U reaction sample 3. Peaks at approx. 16 min retention time include ion fragment at $m/z = 339.0$ that was identified as 5-methyl-U monophosphate. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

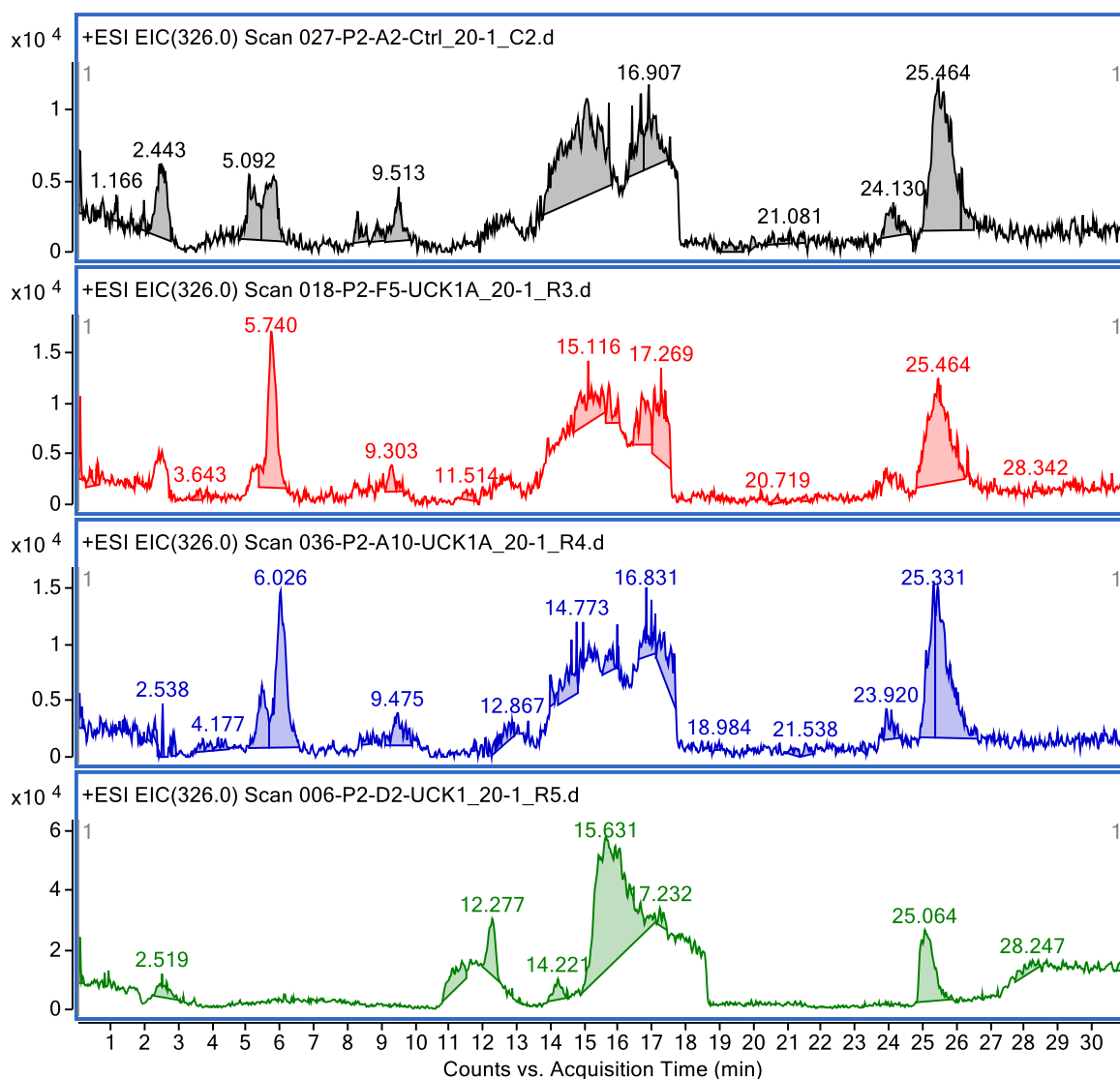


Figure A-II.25: Comparison of 2'-fluoro-2'-deoxy-C-monophosphate-targeting EIC spectra of UCK1-2'- fluoro-2'-deoxy-C reactions and 2'-fluoro-2'-deoxy-C control sample. Spectra taken from EIC of LCMS data of unnatural substrates study samples. All EIC extraction done targeting m/z value of 326.0 ± 0.5 . Black – 2'-fluoro-2'-deoxy-C control sample 1. Red – UCK1-2'-fluoro-2'-deoxy-C reaction sample 1. Blue – UCK1-2'-fluoro-2'-deoxy-C reaction sample 2. Green – UCK1-2'-fluoro-2'-deoxy-C reaction sample 3. No peaks containing 2'-fluoro-2'-deoxy-C monophosphate could be distinguished. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

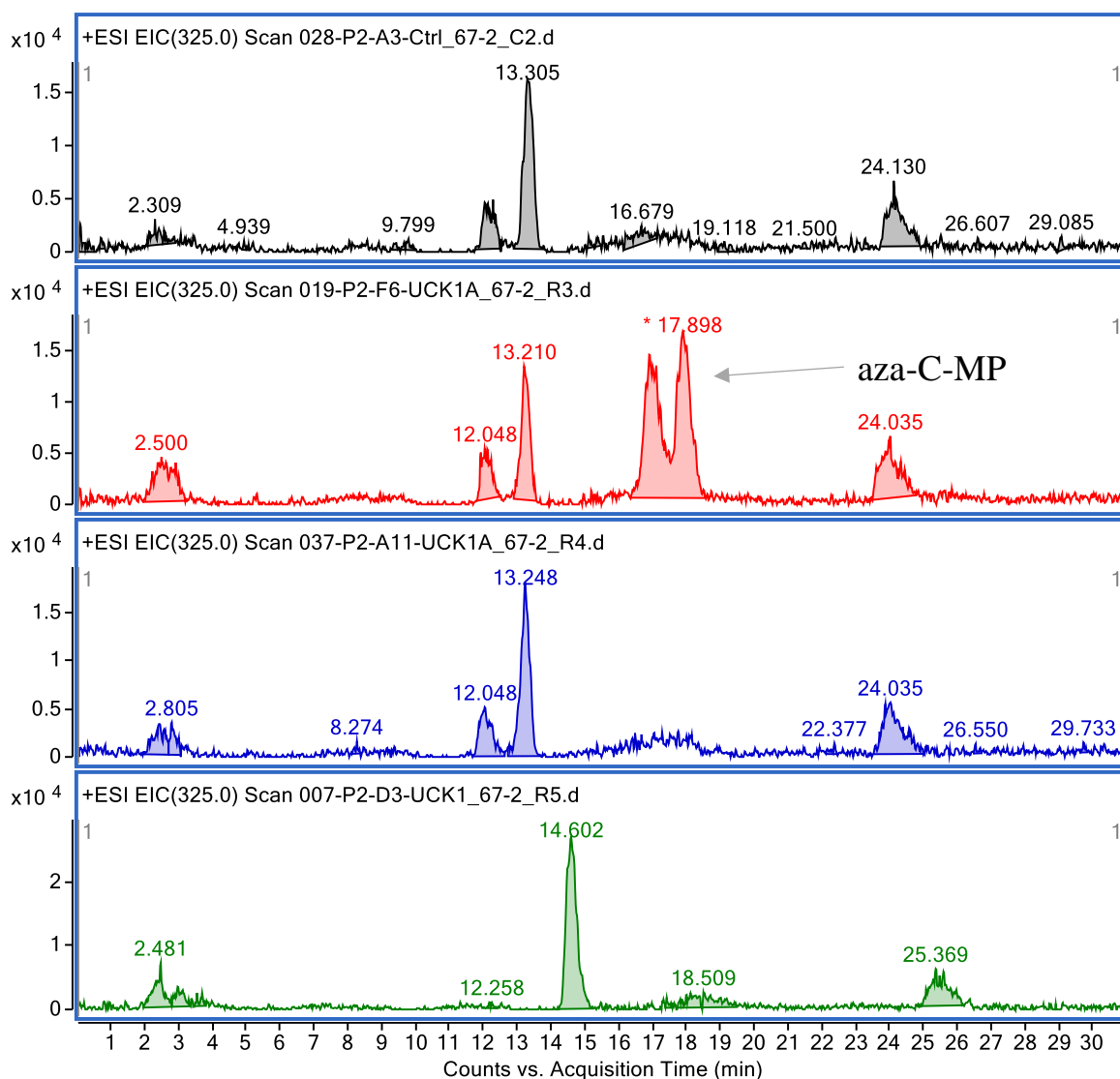


Figure A-II.26: Comparison of aza-C-monophosphate-targeting EIC spectra of UCK1-aza-C reactions and aza-C control sample. Spectra taken from EIC of LCMS data of unnatural substrates study samples. All EIC extraction done targeting m/z value of 325.0 ± 0.5 . Black – aza-C control sample 1. Red – UCK1-aza-C reaction sample 1. Blue – UCK1-aza-C reaction sample 2. Green – UCK1-aza-C reaction sample 3. Peak at approx. 18 min retention time (red) includes ion fragment at $m/z = 325.0$ that was identified as aza-C monophosphate. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

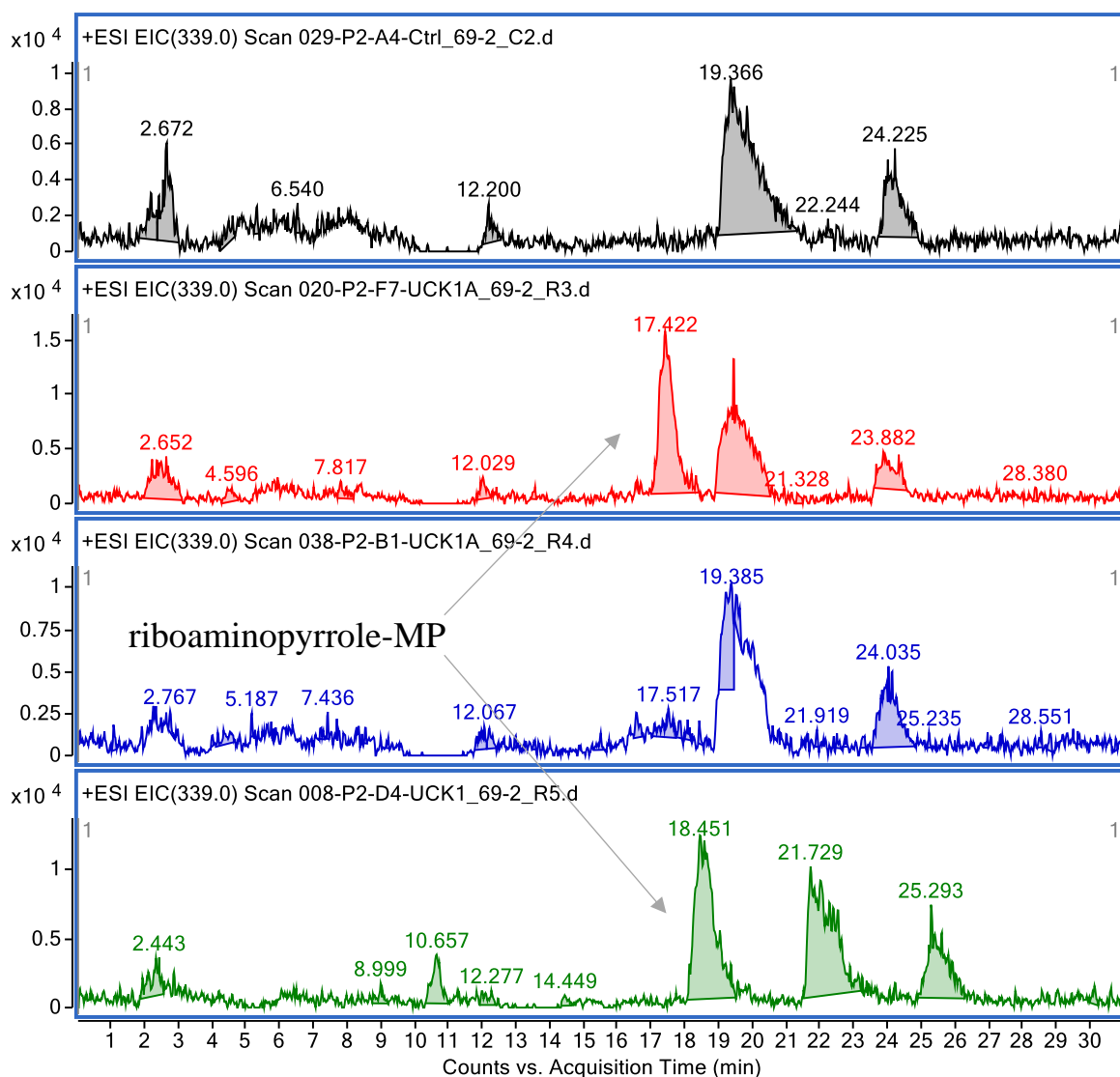


Figure A-II.27: Comparison of riboaminopyrrole-monophosphate-targeting EIC spectra of UCK1-riboaminopyrrole reactions and riboaminopyrrole control sample. Spectra taken from EIC of LCMS data of unnatural substrates study samples. All EIC extraction done targeting m/z value of 339.0 ± 0.5 . Black – riboaminopyrrole control sample 1. Red – UCK1-riboaminopyrrole reaction sample 1. Blue – UCK1-riboaminopyrrole reaction sample 2. Green – UCK1-riboaminopyrrole reaction sample 3. Peaks at approx. 17.5 (red) and 18.5 (green) min retention time include ion fragment at $m/z = 339.0$ that was identified as riboaminopyrrole monophosphate. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.2.

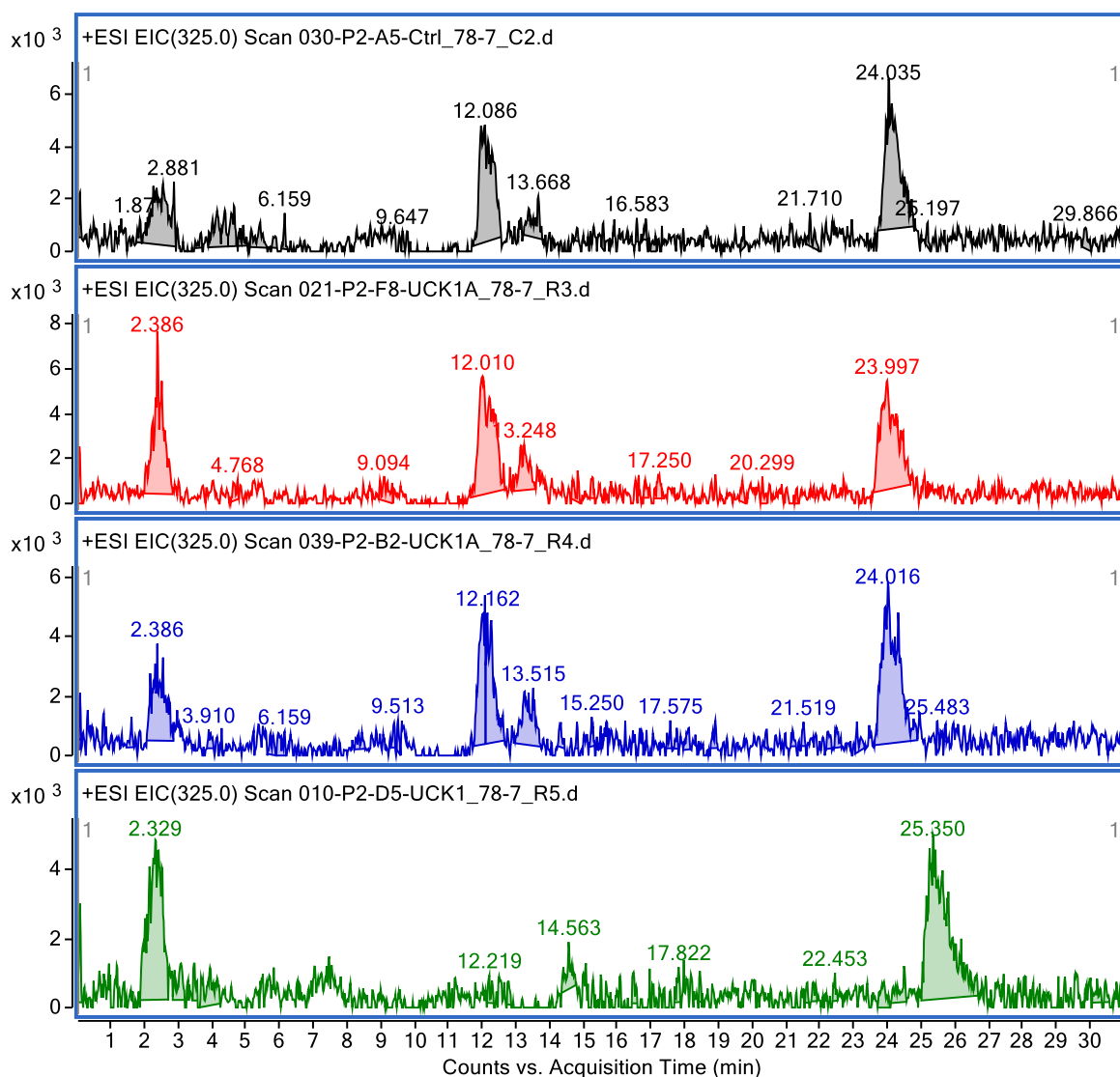


Figure A-II.28: Comparison of xylo-C-monophosphate-targeting EIC spectra of UCK1-xylo-C reactions and xylo-C control sample. Spectra taken from EIC of LCMS data of unnatural substrates study samples. All EIC extraction done targeting m/z value of 325.0 ± 0.5 . Black – xylo-C control sample 1. Red – UCK1-xylo-C reaction sample 1. Blue – UCK1-xylo-C reaction sample 2. Green – UCK1-xylo-C reaction sample 3. No peaks containing xylo-C monophosphate could be distinguished. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

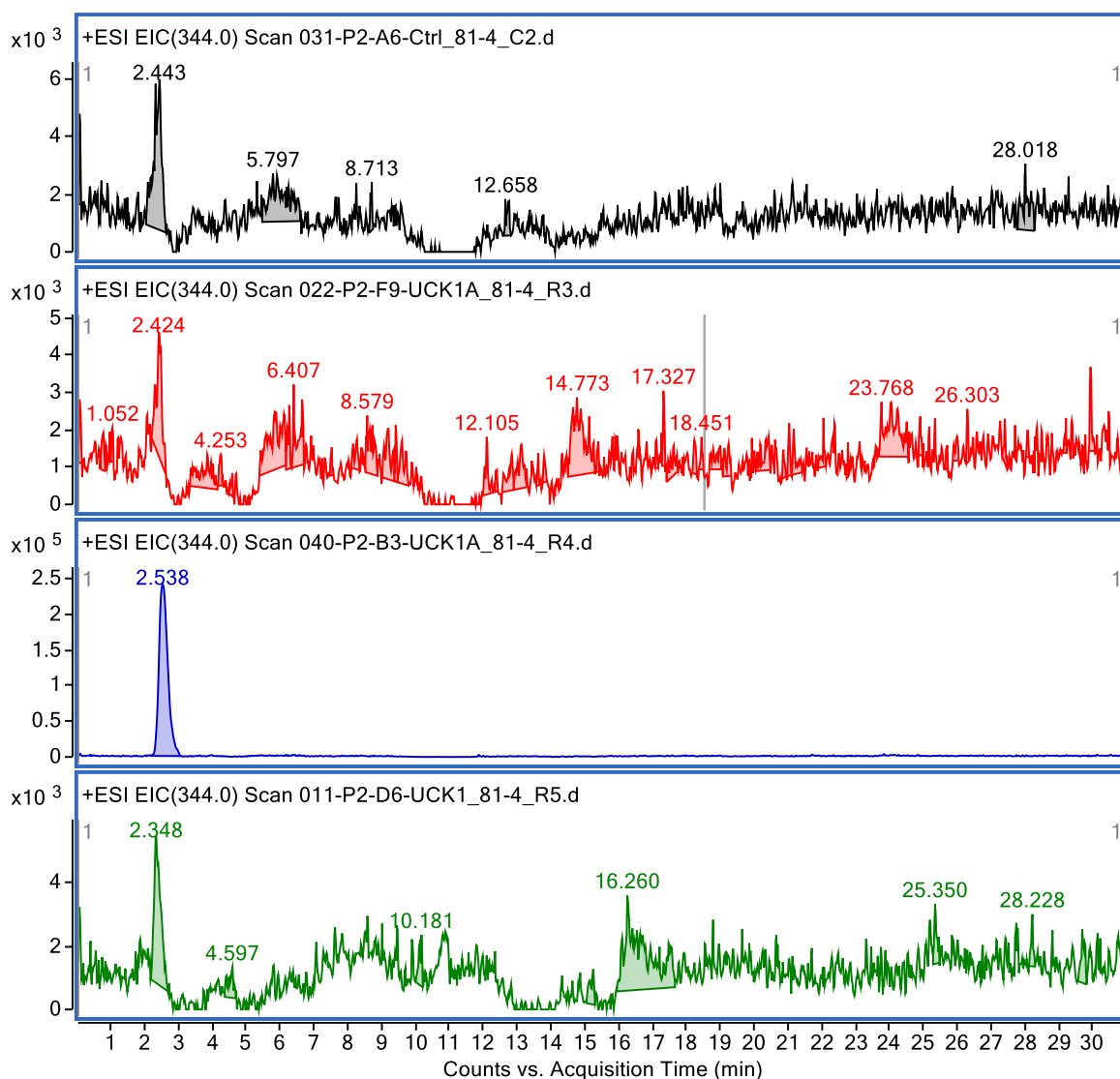


Figure A-II.29: Comparison of 2'-difluoro-C-monophosphate-targeting EIC spectra of UCK1-2'-difluoro-C reactions and 2'-difluoro-C control sample. Spectra taken from EIC of LCMS data of unnatural substrates study samples. All EIC extraction done targeting m/z value of 344.0 ± 0.5 . Black – 2'-difluoro-C control sample 1. Red – UCK1-2'-difluoro-C reaction sample 1. Blue – UCK1-2'-difluoro-C reaction sample 2. Green – UCK1-2'-difluoro-C reaction sample 3. No peaks containing 2'-difluoro-C monophosphate could be distinguished. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

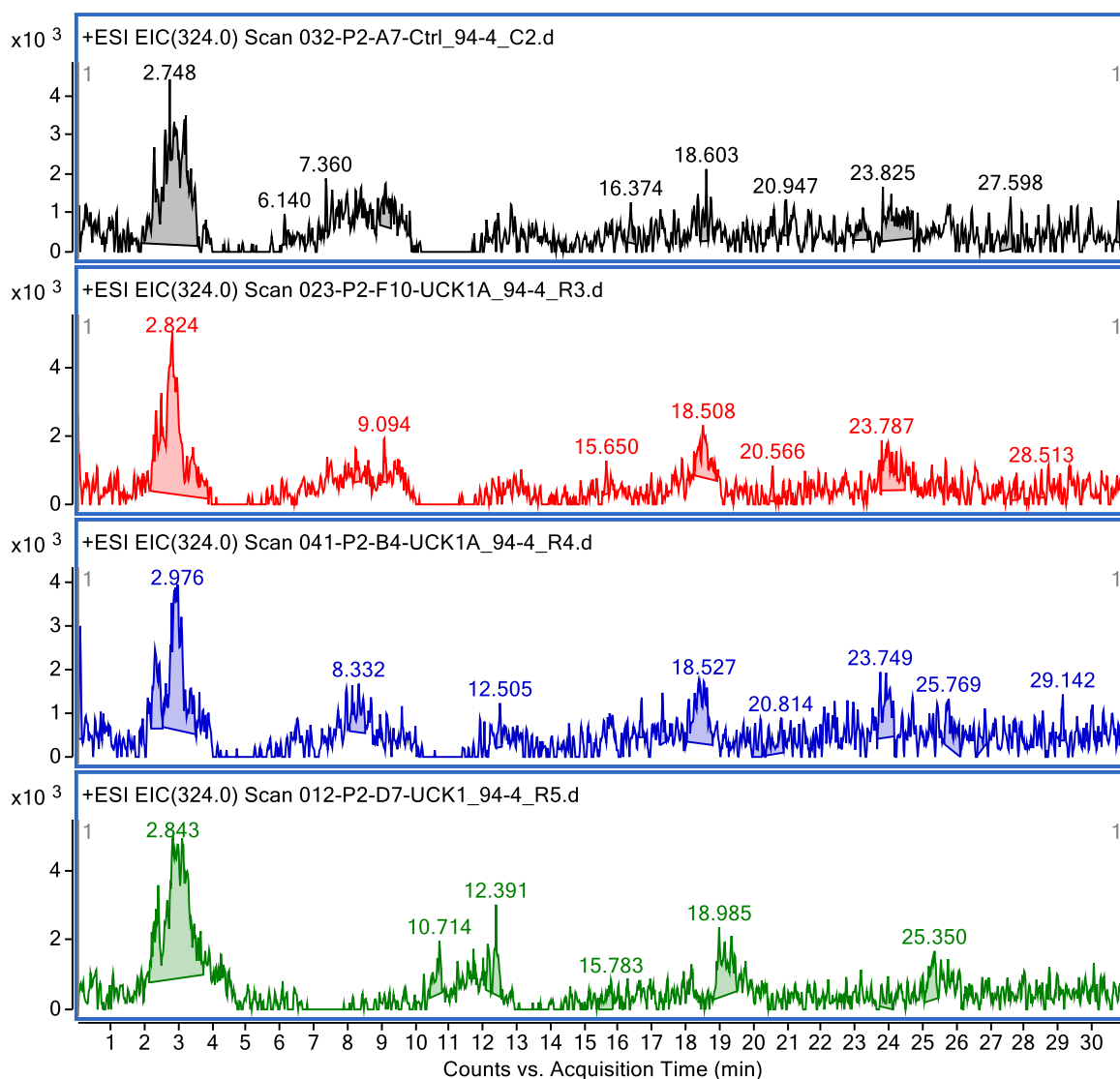


Figure A-II.30: Comparison of ara-C-monophosphate-targeting EIC spectra of UCK1-ara-C reactions and ara-C control sample. Spectra taken from EIC of LCMS data of unnatural substrates study samples. All EIC extraction done targeting m/z value of 324.0 ± 0.5 . Black – ara-C control sample 1. Red – UCK1-ara-C reaction sample 1. Blue – UCK1-ara-C reaction sample 2. Green – UCK1-ara-C reaction sample 3. No peaks containing ara-C monophosphate could be distinguished. LCMS analysis conducted as described in Section 2.7.1.1. Study described in Section 5.3.

Appendix III: Statistical Analysis Data

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A-III.1 UCK1 Concentration Study

UCK Amount	N	Mean	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
.750	3	10.9458	.87248	7.1918	14.6998	9.42	12.44
1.125	3	12.4753	.50188	10.3159	14.6347	11.77	13.45
1.500	3	14.0048	.69890	10.9976	17.0119	13.00	15.35
1.875	3	14.9001	1.10222	10.1576	19.6425	12.77	16.47
2.250	3	15.4596	.50465	13.2883	17.6309	14.45	16.02
Total	15	13.5571	.52854	12.4235	14.6907	9.42	16.47

Table A-III.1: Descriptive statistics from one-way ANOVA of UCK1 concentration study data. Dependent variable is calculated substrate conversion (%). Factor is UCK1 concentration (mg/mL). Study described in Section 4.8. Statistical analysis performed in SPSS statistics.

		Levene Statistic	df1	df2	Sig.
Calculated Substrate Conversion	Based on Mean	.806	4	10	.549

Table A-III.2: Results of Levene's test of homogeneity of variances as part of one-way ANOVA of UCK1 concentration study data. Dependent variable is calculated substrate conversion (%). Factor is UCK1 concentration (mg/mL). Study described in Section 4.8. Statistical analysis performed in SPSS statistics.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	40.839	4	10.210	5.727	.012
Within Groups	17.827	10	1.783		
Total	58.665	14			

Table A-III.3: Results of one-way ANOVA of UCK1 concentration study data. Dependent variable is calculated substrate conversion (%). Factor is UCK1 concentration (mg/mL). Study described in Section 4.8. Statistical analysis performed in SPSS statistics.

(I) UCK1 Conc. (mg/mL)	(J) UCK1 Conc. (mg/mL)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.750	1.125	-1.52948	1.09016	.639	-5.1173	2.0583
	1.500	-3.05897	1.09016	.105	-6.6468	.5288
	1.875	-3.95427*	1.09016	.030	-7.5421	-.3665
	2.250	-4.51384*	1.09016	.013	-8.1016	-.9260
1.125	.750	1.52948	1.09016	.639	-2.0583	5.1173
	1.500	-1.52948	1.09016	.639	-5.1173	2.0583
	1.875	-2.42479	1.09016	.246	-6.0126	1.1630
	2.250	-2.98436	1.09016	.117	-6.5722	.6034
1.500	.750	3.05897	1.09016	.105	-.5288	6.6468
	1.125	1.52948	1.09016	.639	-2.0583	5.1173
	1.875	-.89531	1.09016	.918	-4.4831	2.6925
	2.250	-1.45487	1.09016	.678	-5.0427	2.1329
1.875	.750	3.95427*	1.09016	.030	.3665	7.5421
	1.125	2.42479	1.09016	.246	-1.1630	6.0126
	1.500	.89531	1.09016	.918	-2.6925	4.4831
	2.250	-.55957	1.09016	.984	-4.1474	3.0282
2.250	.750	4.51384*	1.09016	.013	.9260	8.1016
	1.125	2.98436	1.09016	.117	-.6034	6.5722
	1.500	1.45487	1.09016	.678	-2.1329	5.0427
	1.875	.55957	1.09016	.984	-3.0282	4.1474

Table A-III.4: Results of Tukey's HSD post-hoc test following one-way ANOVA analysis of UCK1 concentration study data. Dependent variable is calculated substrate conversion (%). Factor is UCK1 concentration (mg/mL). Study described in Section 4.8. Statistical analysis performed in SPSS statistics.

A-III.2 PK Concentration Study

PK:UCK1 ratio (w/w)	N	Mean	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
1:1	3	14.0048	.69890	10.9976	17.0119	13.00	15.35
2:1	3	19.0409	.79398	15.6246	22.4571	17.47	20.05
3:1	3	23.2190	.38947	21.5432	24.8947	22.51	23.85
Total	9	18.7549	1.37105	15.5932	21.9165	13.00	23.85

Table A-III.5: Descriptive statistics from one-way ANOVA of PK concentration study data. Dependent variable is calculated substrate conversion (%). Factor is PK:UCK1 ratio (w/w). Study described in Section 4.9. Statistical analysis performed in SPSS statistics.

		Levene Statistic	df1	df2	Sig.
Calculated	Based on Mean	1.242	2	6	.354
Substrate					
Conversion					

Table A-III.6: Results of Levene's test of homogeneity of variances as part of one-way ANOVA of PK concentration study data. Dependent variable is calculated substrate conversion (%). Factor is PK:UCK1 ratio (w/w). Study described in Section 4.9. Statistical analysis performed in SPSS statistics.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	127.720	2	63.860	50.262	.000
Within Groups	7.623	6	1.271		
Total	135.344	8			

Table A-III.7: Results of one-way ANOVA of PK concentration study data. Dependent variable is calculated substrate conversion (%). Factor is PK:UCK1 ratio (w/w). Study described in Section 4.9. Statistical analysis performed in SPSS statistics.

(I) PK:UCK1 ratio (w/w)	(J) PK:UCK1 ratio (w/w)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-5.03610*	.92034	.004	-7.8600	-2.2122
	3.00	-9.21420*	.92034	.000	-12.0381	-6.3903
2.00	1.00	5.03610*	.92034	.004	2.2122	7.8600
	3.00	-4.17810*	.92034	.009	-7.0020	-1.3542
3.00	1.00	9.21420*	.92034	.000	6.3903	12.0381
	2.00	4.17810*	.92034	.009	1.3542	7.0020

Table A-III.8: Results of Tukey's HSD post-hoc test following one-way ANOVA analysis of UCK1 concentration study data. Dependent variable is calculated substrate conversion (%). Factor is PK:UCK1 ratio (w/w). Study described in Section 4.9. Statistical analysis performed in SPSS statistics.

A-III.3 Addition Strategy Study

		Levene Statistic	df1	df2	Sig.
Calculated Substrate Conversion	Based on Mean	3.011	1	4	.158

Table A-III.9: Results of Levene's test of homogeneity of variances as part of independent samples t-test analysis of PK concentration study data. Dependent variable is calculated substrate conversion (%). Factor is method of UCK1 addition. Study described in Section 4.10. Statistical analysis performed in SPSS statistics.

		Addition Strategy	N	Mean	Std. Deviation	Std. Error Mean
Calculated Substrate Conversion	Fixed		3	14.0048	1.21053	.69890
	Incremental		3	13.4079	.46593	.26901

Table A-III.10: Group statistics calculated as part of independent samples t-test analysis of PK concentration study data. Dependent variable is calculated substrate conversion (%). Factor is method of UCK1 addition. Study described in Section 4.10. Statistical analysis performed in SPSS statistics.

		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Calculated Substrate Conversion	Equal variances assumed	.797	4	.470	.59687	.74888	-1.48236	2.67610
	Equal variances not assumed	.797	2.580	.492	.59687	.74888	-2.02175	3.21549

Table A-III.11: Results of independent samples t-test analysis of PK concentration study data. Dependent variable is calculated substrate conversion (%). Factor is method of UCK1 addition. Study described in Section 4.10. Statistical analysis performed in SPSS statistics.

		Standardizer ^a	Point Estimate	95% Confidence Interval	
				Lower	Upper
Calculated	Cohen's d	.91719	.651	-1.044	2.273
Substrate	Hedges' correction	1.14953	.519	-.833	1.814
Conversion	Glass's delta	.46593	1.281	-.781	3.176

Table A-III.12: Independent samples effect sizes calculated as part of independent samples t-test analysis of PK concentration study data. Dependent variable is calculated substrate conversion (%). Factor is method of UCK1 addition. ^a = denominator used in estimating effect sizes. Cohen's d uses the pooled standard deviation. Hedge's correction uses the pooled standard deviation plus a correction factor. Glass's delta uses the sample standard deviation of the control group. Study described in Section 4.10. Statistical analysis performed in SPSS statistics.

A-III.4 Activity Against Natural Substrates

		Levene Statistic	df1	df2	Sig.
Calculated	Based on Mean	8.010	1	4	.047
Substrate					
Conversion					

Table A-III.13: Results of Levene's test of homogeneity of variances as part of independent samples t-test analysis of natural substrate study ³¹P NMR data relating to UCK1. Dependent variable is calculated substrate conversion (%). Factor is substrate. Study described in Section 5.2. Statistical analysis performed in SPSS statistics.

	Substrate	N	Mean	Std. Deviation	Std. Error Mean
Calculated Substrate	Uridine	3	14.1167	6.20652	3.58333
Conversion	Cytidine	3	71.6767	1.64810	.95153

Table A-III.14: Group statistics calculated as part of independent samples t-test analysis of natural substrate study ³¹P NMR data relating to UCK1. Dependent variable is calculated substrate conversion (%). Factor is substrate. Study described in Section 5.2. Statistical analysis performed in SPSS statistics.

		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Calculated	Equal variances assumed	-15.525	4	.000	-57.56000	3.70752	-67.85372	-47.26628
Substrate								
Conversion	Equal variances not assumed	-15.525	2.281	.002	-57.56000	3.70752	-71.77106	-43.34894

Table A-III.15: Results of independent samples t-test analysis of natural substrate study ³¹P NMR data relating to UCK1. Dependent variable is calculated substrate conversion (%). Factor is substrate. Study described in Section 5.2. Statistical analysis performed in SPSS statistics.

		Standardizer ^a	Point Estimate	95% Confidence Interval	
				Lower	Upper
Calculated	Cohen's d	4.54076	-12.676	-21.285	-4.223
Substrate	Hedges' correction	5.69100	-10.114	-16.983	-3.370
Conversion	Glass's delta	1.64810	-34.925	-67.110	-5.500

Table A-III.16: Independent samples effect sizes calculated as part of independent samples t-test analysis of natural substrate study ³¹P NMR data relating to UCK1. Dependent variable is calculated substrate conversion (%). Factor is substrate. ^a = denominator used in estimating effect sizes. Cohen's d uses the pooled standard deviation. Hedge's correction uses the pooled standard deviation plus a correction factor. Glass's delta uses the sample standard deviation of the control group. Study described in Section 5.2. Statistical analysis performed in SPSS statistics.

	N	Mean	Std. Error	95% Confidence Interval for		Minimum	Maximum
				Mean			
				Lower Bound	Upper Bound		
UCK1-U	3	7449312.11	2069136.045	-1453461.74	16352085.97	3.31E+6	9.63E+6
UCK1-C	3	47732252.75	1515908.189	41209826.25	54254679.26	4.60E+7	5.08E+7
UCK2-C	3	678492.55	220545.754	-270439.24	1627424.34	382340.30	1.11E+6
Total	9	18620019.14	7380890.591	1599654.92	35640383.36	382340.30	5.08E+7

Table A-III.17: Descriptive statistics from Welch's ANOVA of natural substrate study HPLCMS data. Dependent variable is integrated nucleoside monophosphate peak size. Factor is reaction type. UCK2-U reaction data excluded due to lack of variance. Study described in Section 5.2. Statistical analysis performed in SPSS statistics.

		Levene Statistic	df1	df2	Sig.
Peak Area	Based on Mean	6.392	2	6	.033

Table A-III.18: Results of Levene's test of homogeneity of variances as part of Welch's ANOVA of natural substrate study HPLCMS data. Dependent variable is integrated nucleoside monophosphate peak size. Factor is reaction type. UCK2-U reaction data excluded due to lack of variance. Study described in Section 5.2. Statistical analysis performed in SPSS statistics.

	Statistic ^a	df1	df2	Sig.
Welch	382.861	2	2.751	.000

Table A-III.19: Results of Welch's ANOVA of natural substrate study HPLCMS data. Dependent variable is integrated nucleoside monophosphate peak size. Factor is reaction type. UCK2-U reaction data excluded due to lack of variance. ^a = asymptotically F distributed. Study described in Section 5.2. Statistical analysis performed in SPSS statistics.

(I) Reaction Type	(J) Reaction Type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
UCK1-U	UCK1-C	-40282940.64000*	2565014.93367	.000	-49821335.8765	-30744545.4035
	UCK2-C	6770819.56333	2080856.65129	.144	-5198344.2273	18739983.3540
UCK1-C	UCK1-U	40282940.64000*	2565014.93367	.000	30744545.4035	49821335.8765
	UCK2-C	47053760.20333*	1531867.50920	.002	38413624.5148	55693895.8919
UCK2-C	UCK1-U	-6770819.56333	2080856.65129	.144	-18739983.3540	5198344.2273
	UCK1-C	-47053760.20333*	1531867.50920	.002	-55693895.8919	-38413624.5148

Table A-III.20: Results of Games-Howell post-hoc test following Welch's ANOVA of natural substrate study HPLCMS data.

Dependent variable is integrated nucleoside monophosphate peak size. Factor is reaction type. UCK2-U reaction data excluded due to lack of variance. Study described in Section 5.2. Statistical analysis performed in SPSS statistics.