Catalysis of Hydrogen–Deuterium Exchange Reactions by 4-Substituted Proline Derivatives

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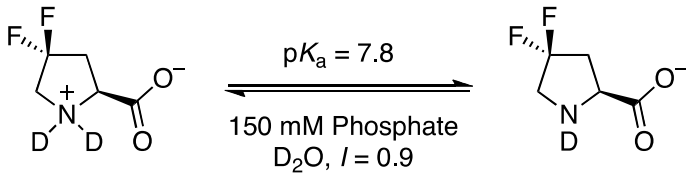
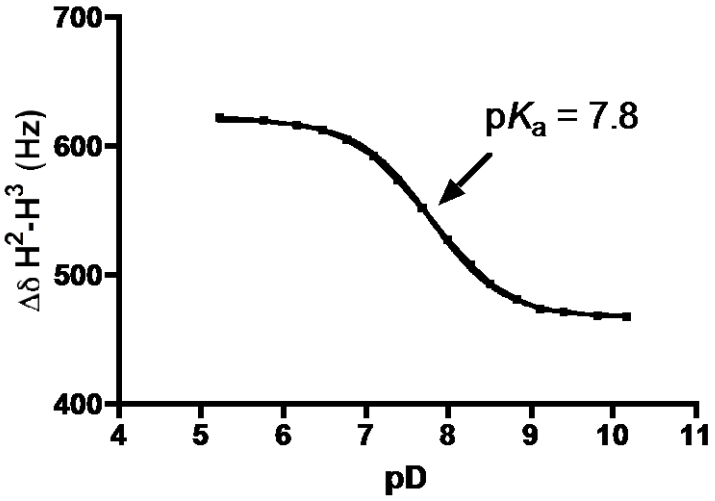
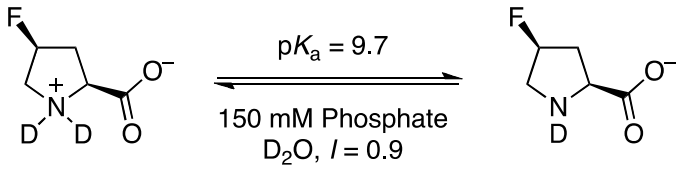
1 p*K*$_a$ Values

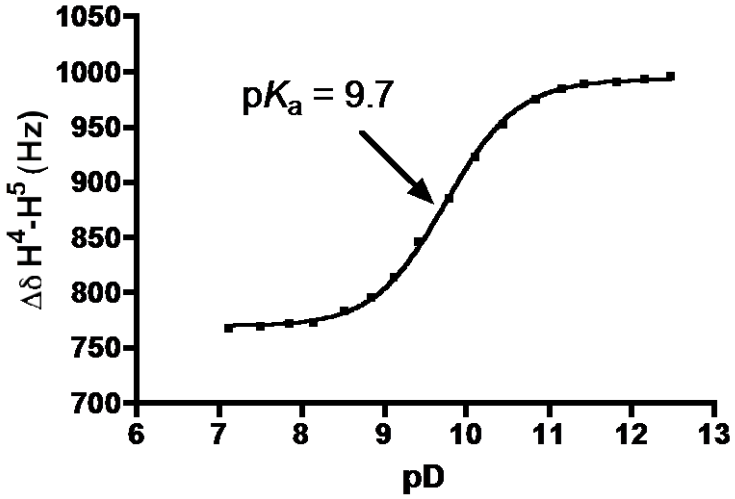
(2*S*)-4,4-Difluoroproline in D_2O (**1**)



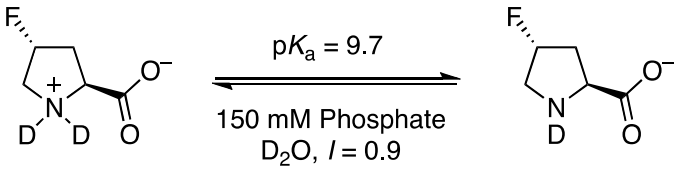


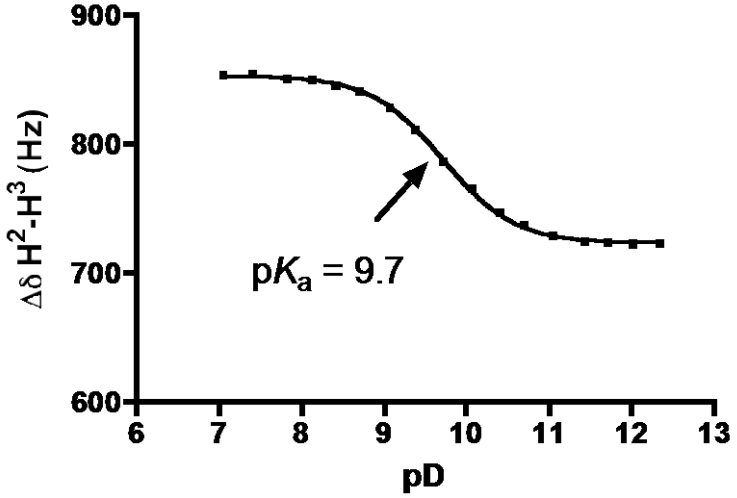
(2*S*,4*S*)-4-Fluoroproline (**2**)



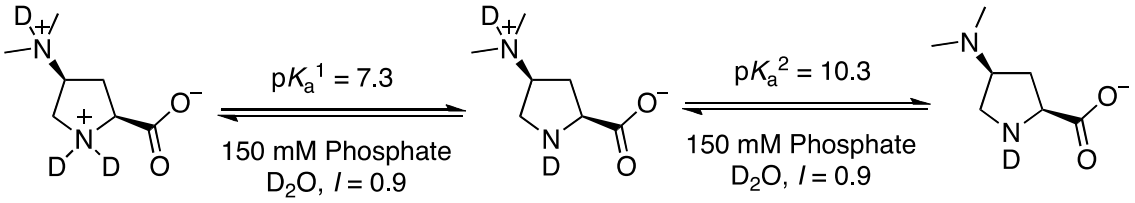


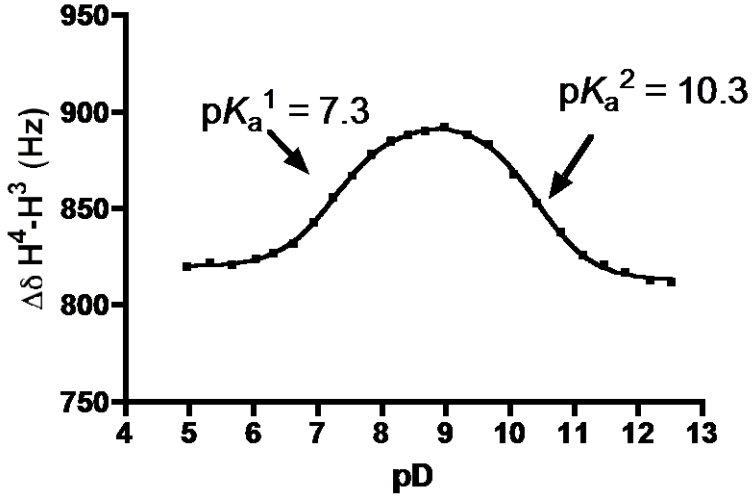
(2*S*,4*R*)-4-Fluoroproline (**3**)



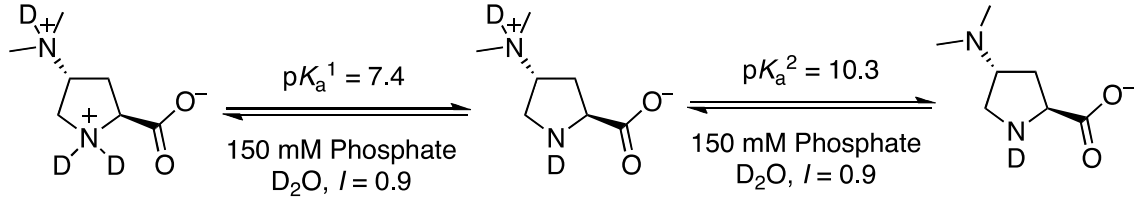


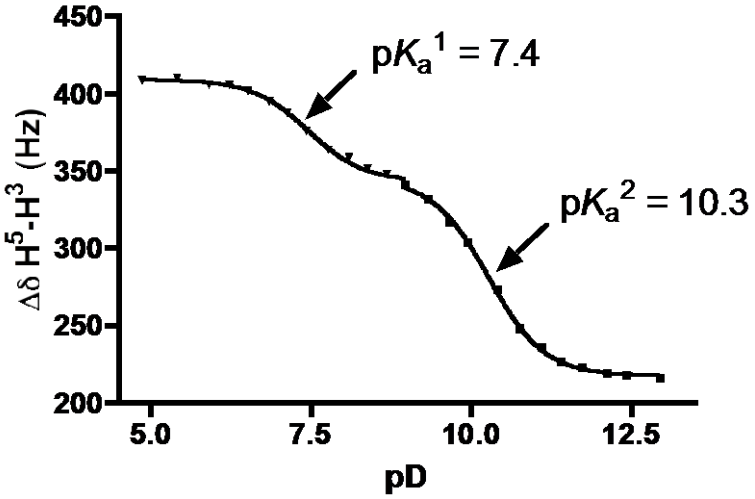
(2*S*,4*S*)-4-Dimethylaminoproline (**4**)



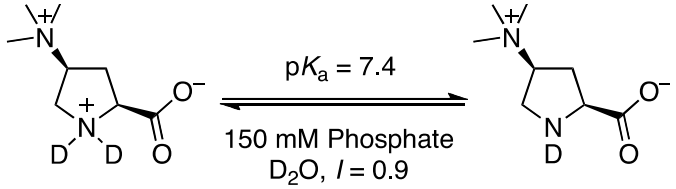


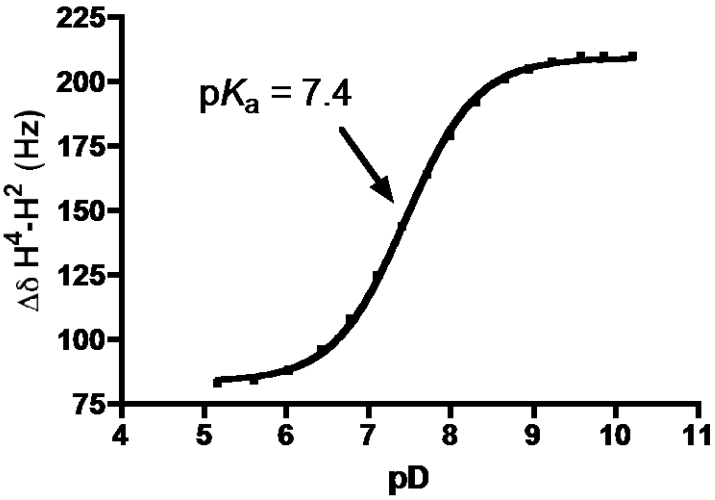
(2*S*,4*R*)-4-Dimethylaminoproline (**5**)



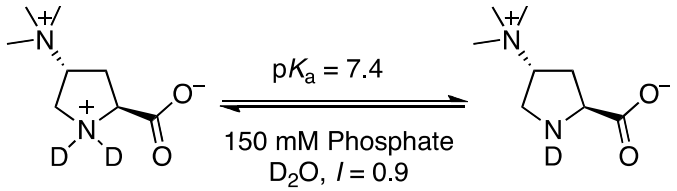


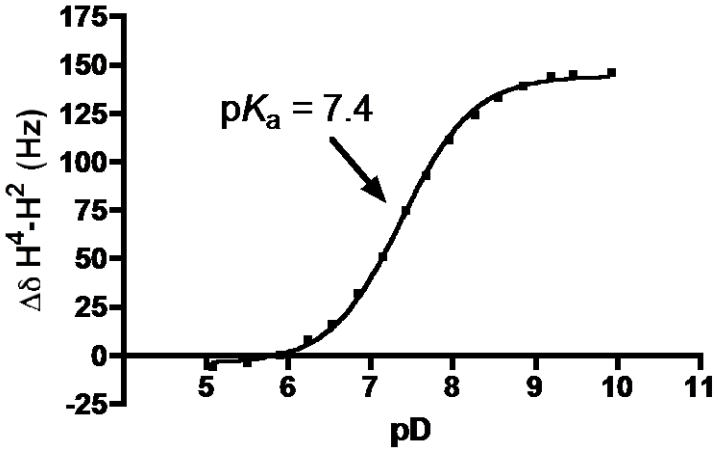
(2*S*,4*S*)-4-Trimethylaminoproline (**6**)



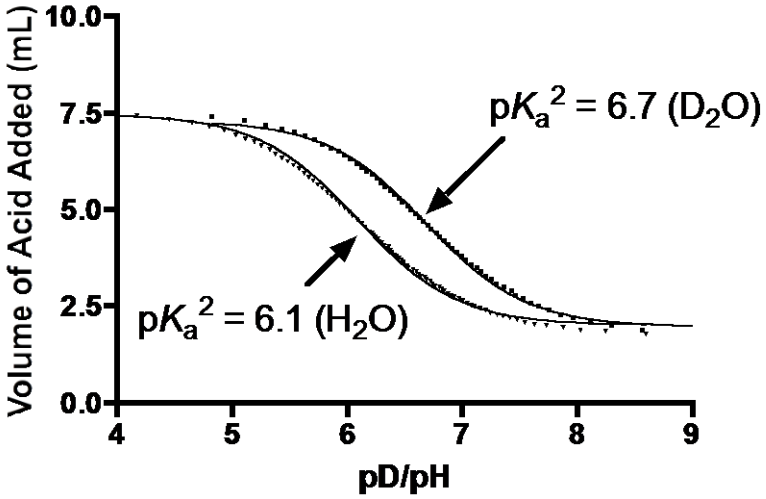


(2*S*,4*R*)-4-Trimethylaminoproline (**7**)



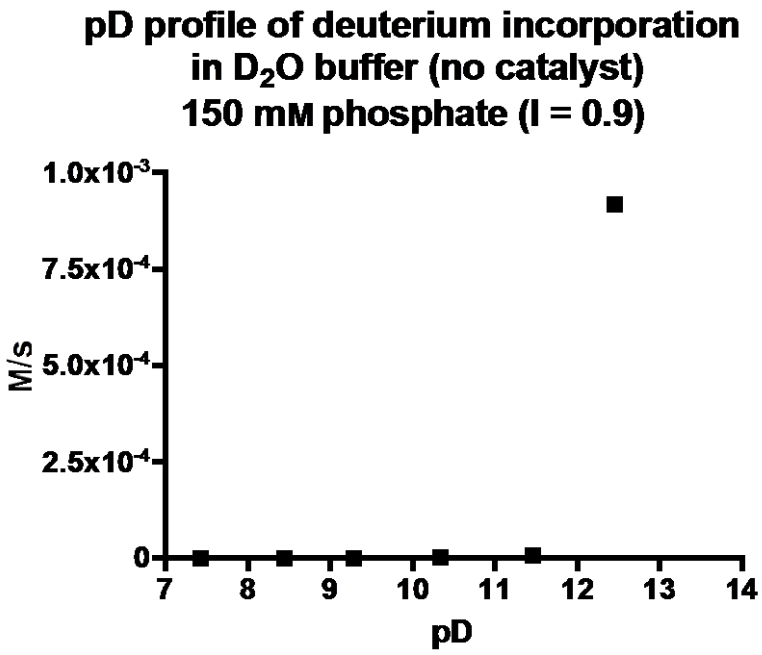


pK_a^2 Values for phosphate in D_2O and H_2O

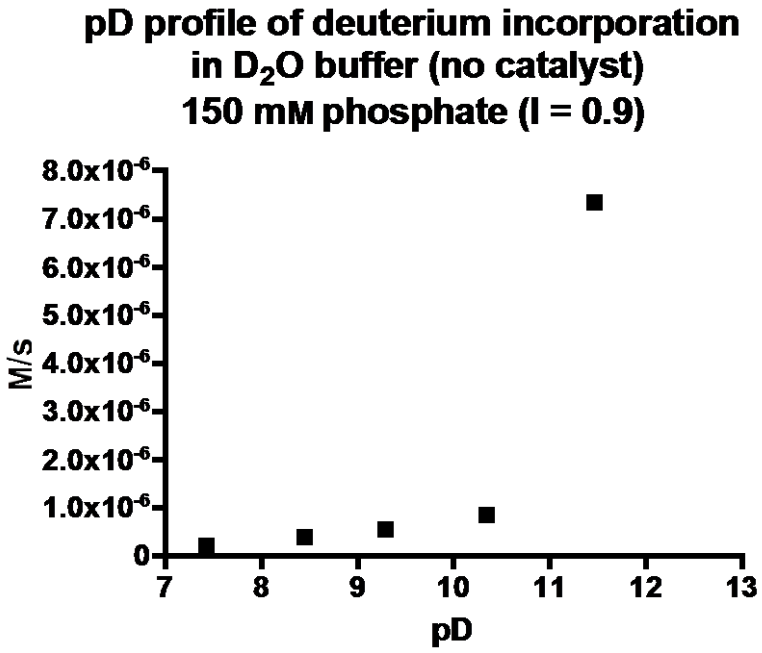


2 Rate versus pD/pH/pH-meter–reading profiles

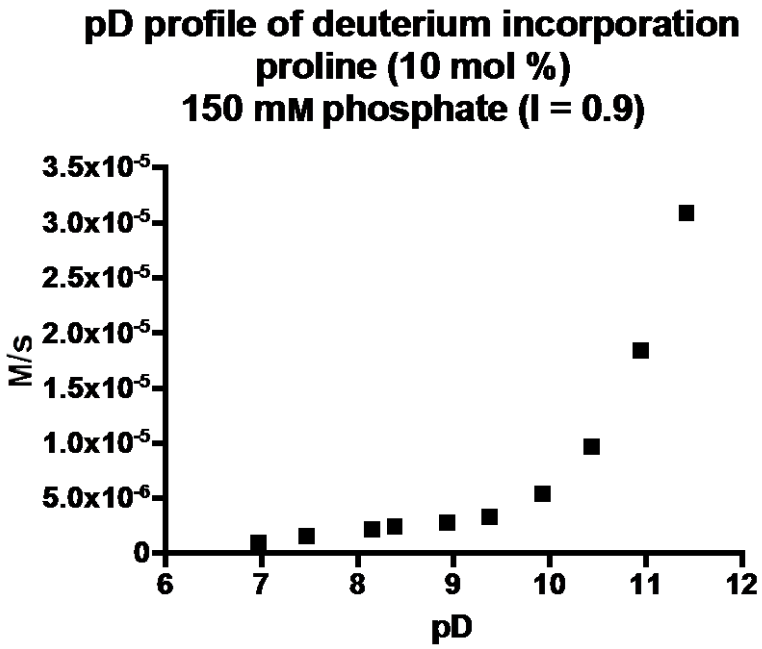
Background (no catalyst; all pD values)



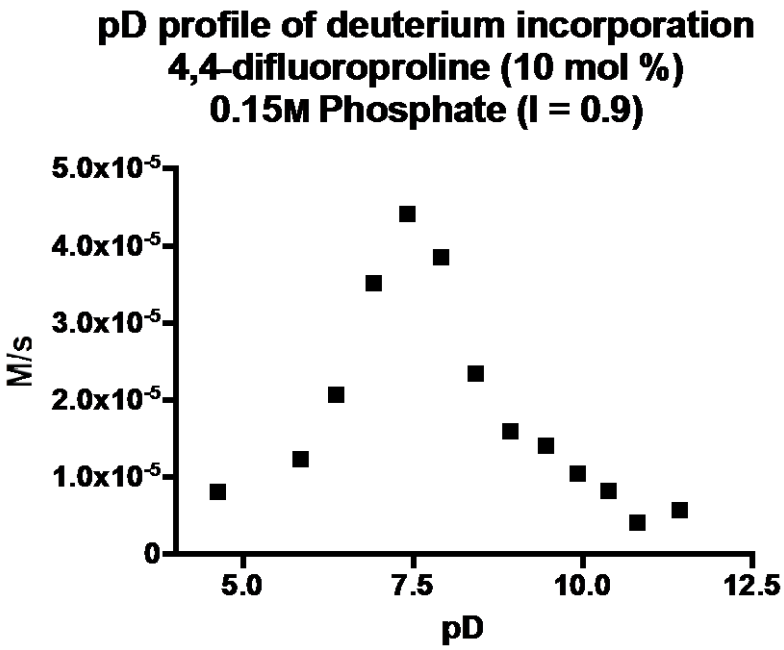
Background (no catalyst; to pD = 11.5)

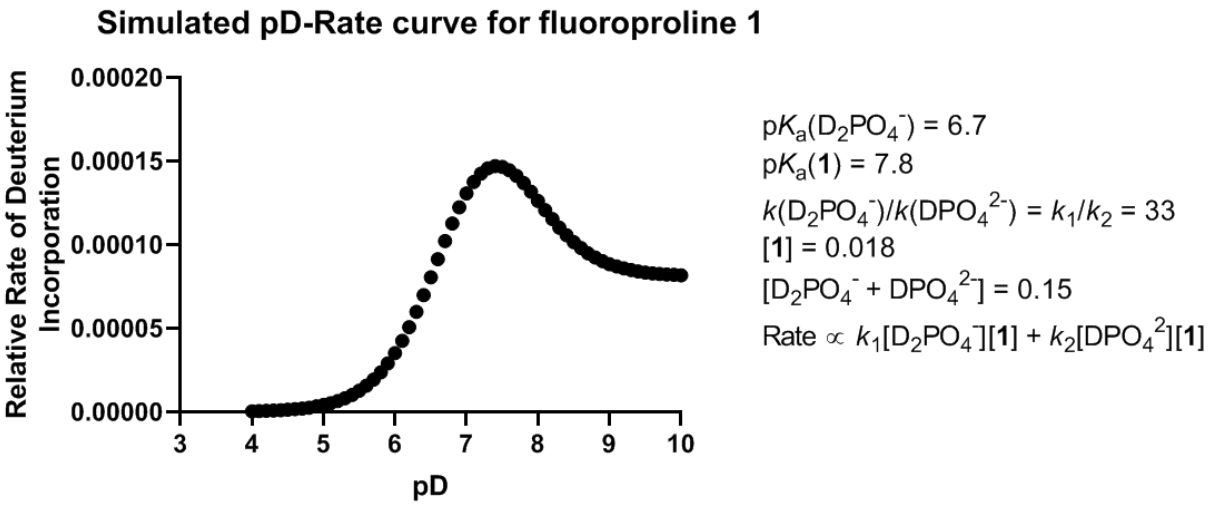


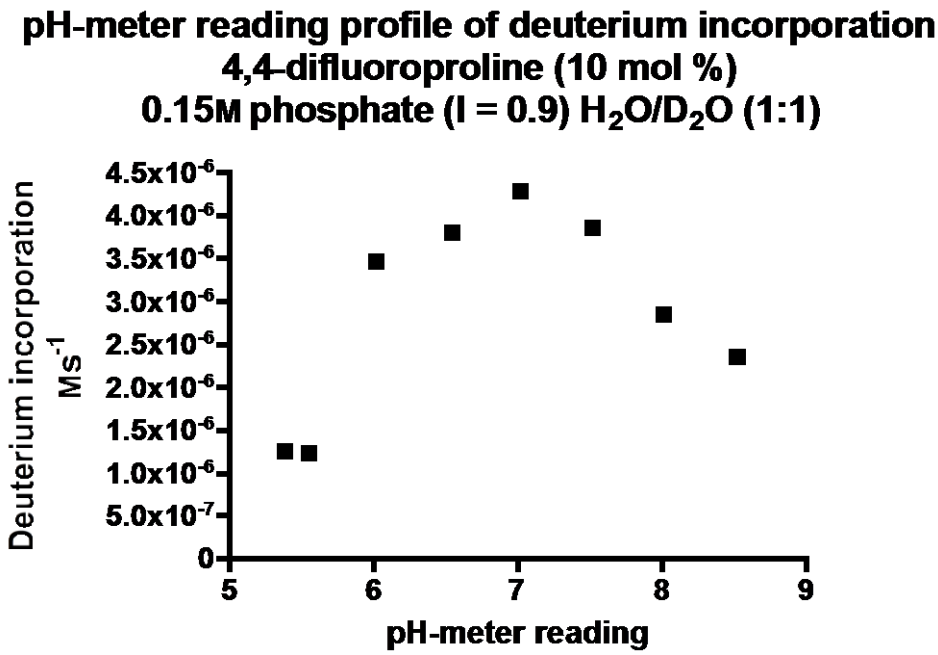
(2*S*)-Proline



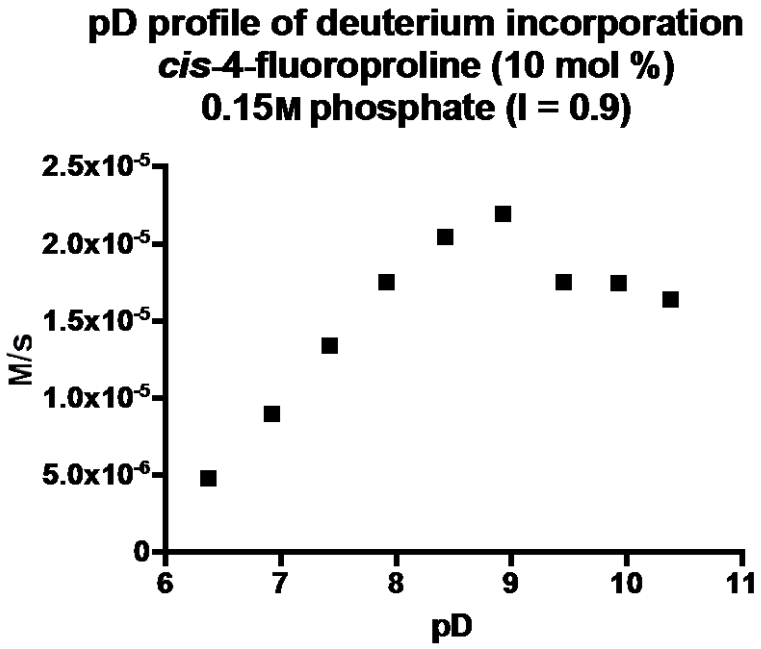
(2*S*)-4,4-Difluoroproline (**1**)

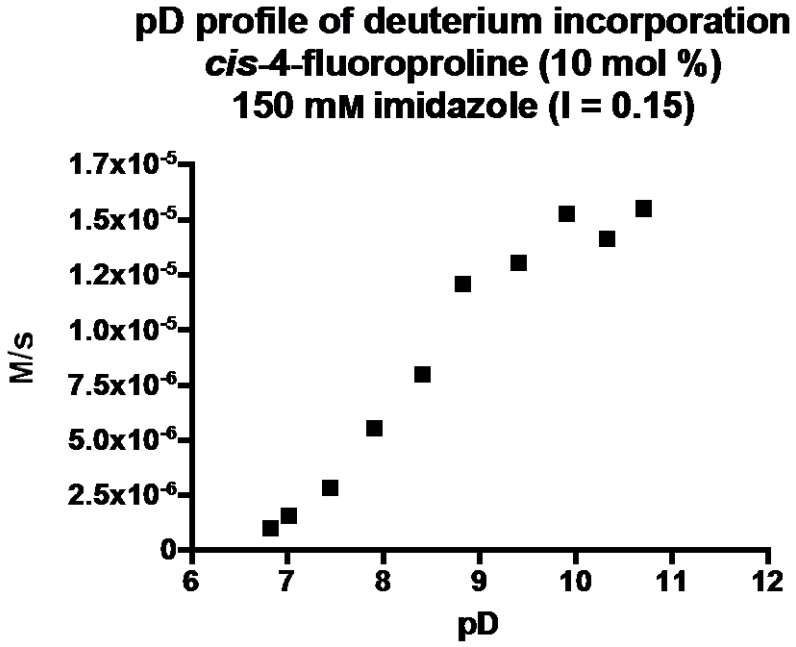



Simulated pD-Rate curve for fluoroproline 1
Relative Rate of Deuterium Incorporation
0.00020
0.00015
0.00010
0.00005
0.00000
3
4
5
6
7
8
9
10
pD
$pK_a(D_2PO_4^-) = 6.7$
$pK_a(\mathbf{1}) = 7.8$
$k(D_2PO_4^-)/k(DPO_4^{2-}) = k_1/k_2 = 33$
$[\mathbf{1}] = 0.018$
$[D_2PO_4^- + DPO_4^{2-}] = 0.15$
$\text{Rate} \propto k_1[D_2PO_4^-][\mathbf{1}] + k_2[DPO_4^{2}][\mathbf{1}]$

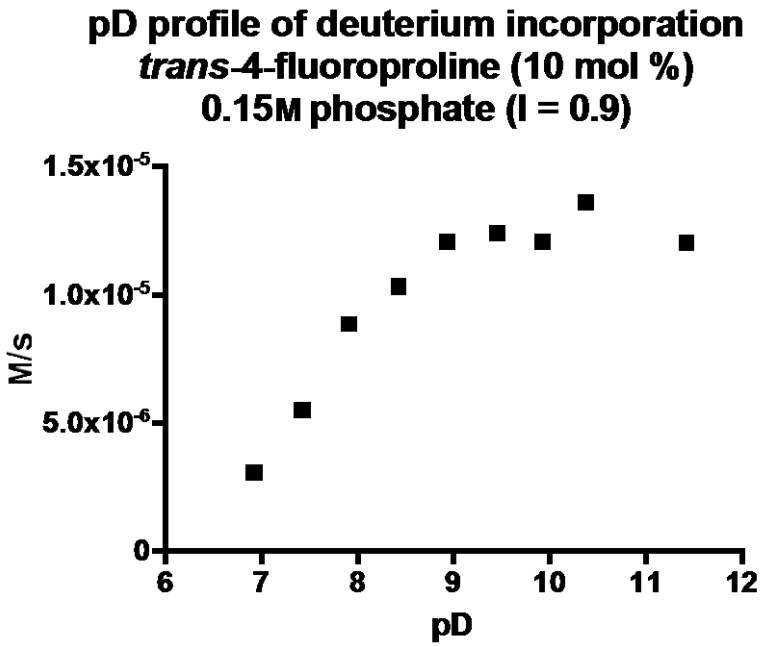

pH-meter reading profile of deuterium incorporation
4,4-difluoroproline (10 mol %)
0.15M phosphate (I = 0.9) H_2O/D_2O (1:1)
Deuterium incorporation Ms^{-1}
$4.5x10^{-6}$
$4.0x10^{-6}$
$3.5x10^{-6}$
$3.0x10^{-6}$
$2.5x10^{-6}$
$2.0x10^{-6}$
$1.5x10^{-6}$
$1.0x10^{-6}$
$5.0x10^{-7}$
0
5
6
7
8
9
pH-meter reading

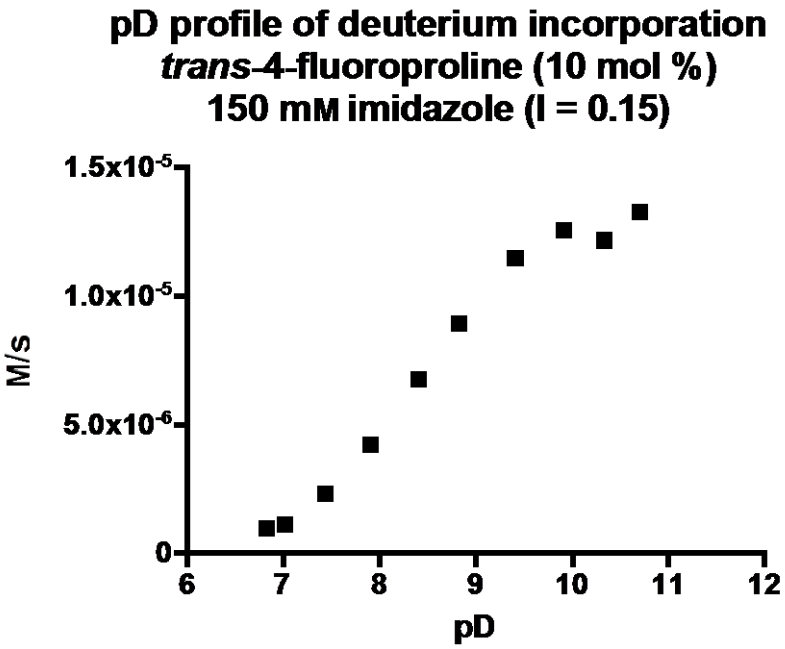
(2*S*,4*S*)-4-Fluoroproline (**2**)



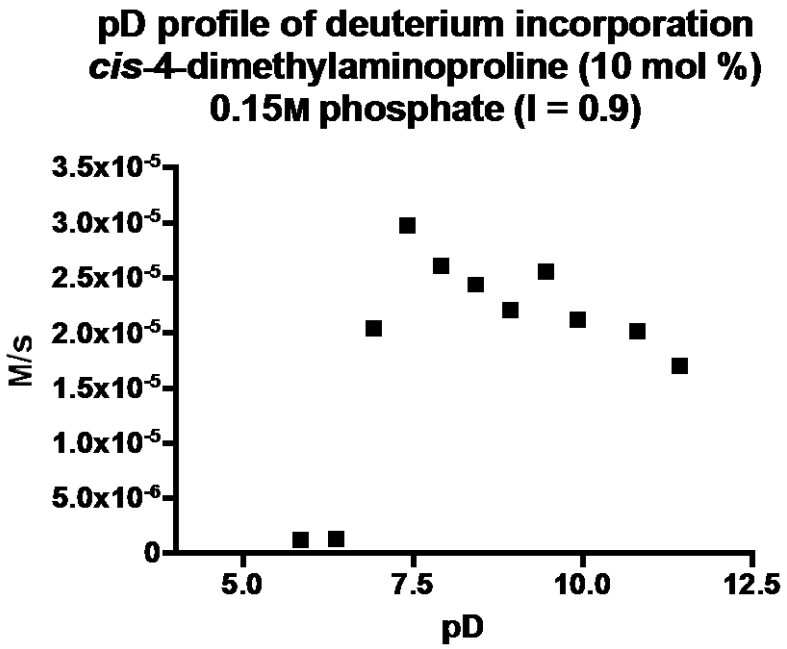


(2*S*,4*R*)-4-Fluoroproline (**3**)

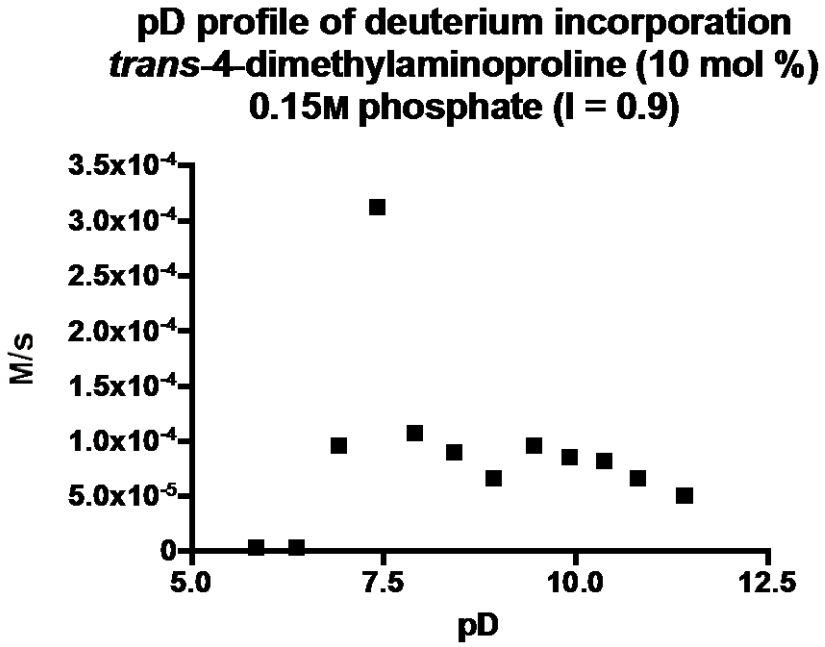




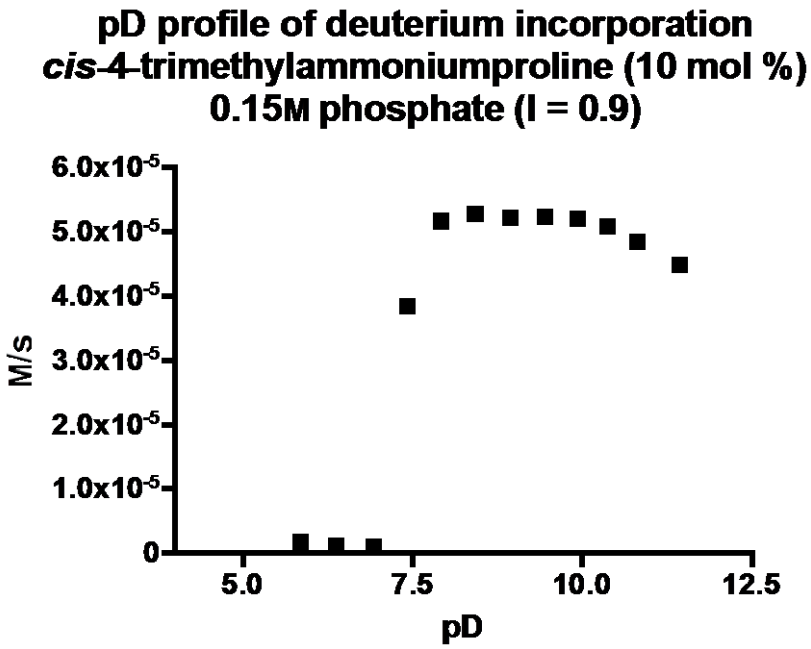
(2*S*,4*S*)-4-Dimethylaminoproline (**4**)

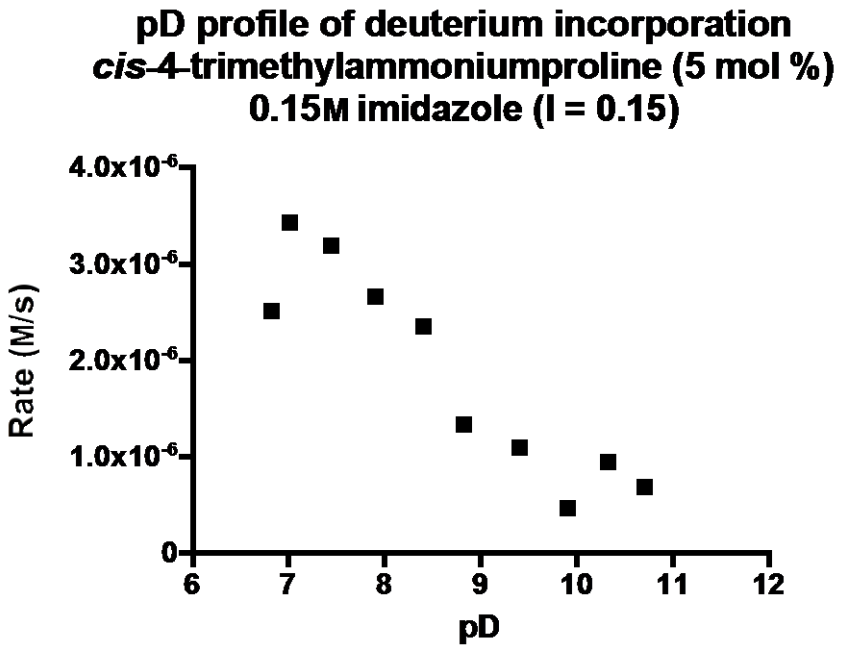


(2*S*,4*R*)-4-Dimethylaminoproline (**5**)

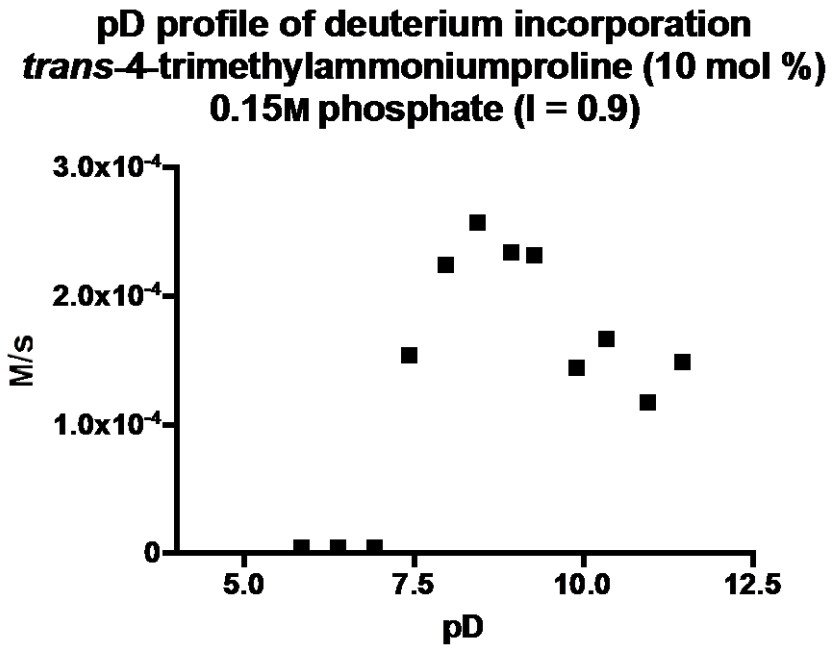


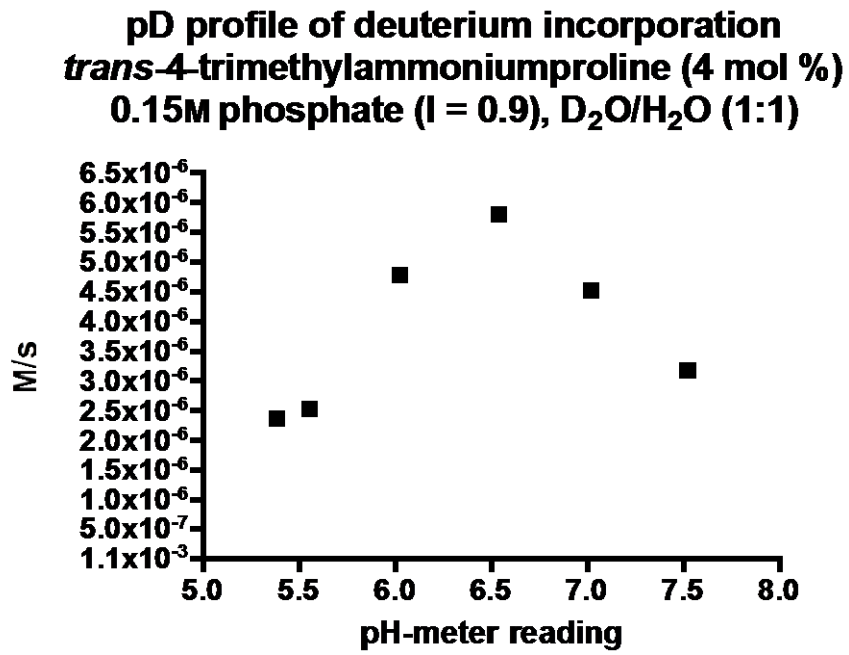
(2*S*,4*S*)-4-trimethylammoniumproline (**6**)

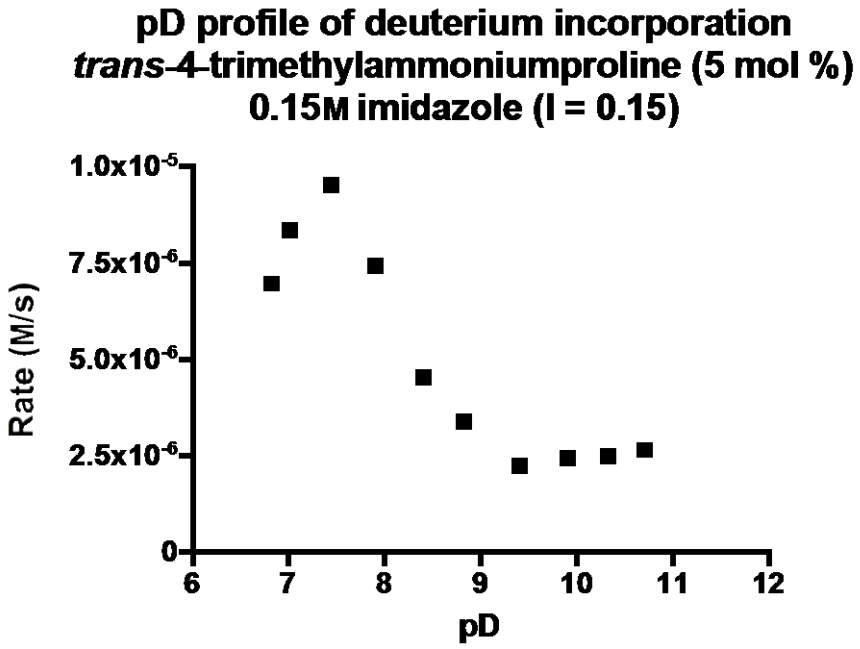




(2*S*,4*R*)-4-trimethylammoniumproline (**7**)

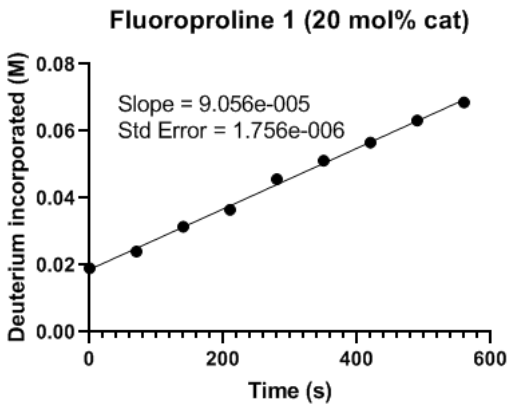


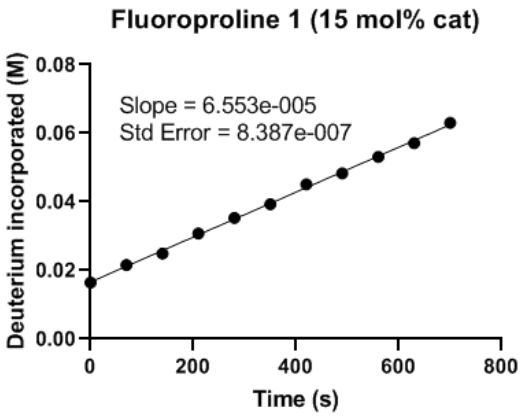


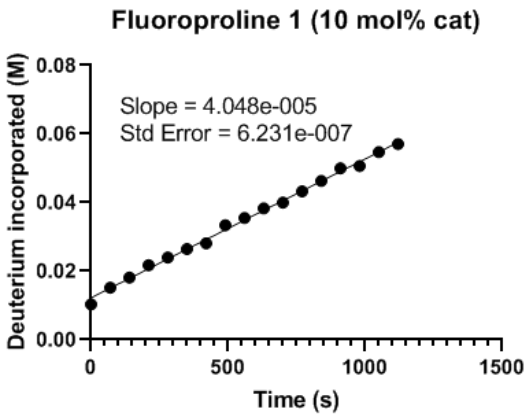

pD profile of deuterium incorporation
trans-4-trimethylammoniumproline (5 mol %)
0.15M imidazole (I = 0.15)
Rate (M/s)
1.0x10-5
7.5x10-6
5.0x10-6
2.5x10-6
0
6
7
8
9
10
11
12
pD

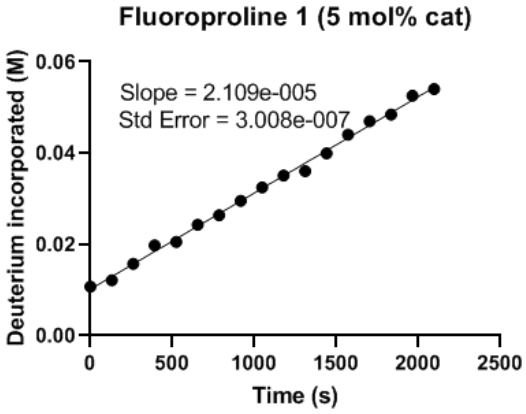
3 Concentration/Time and Log/Log Plots for Determination of Rate Laws

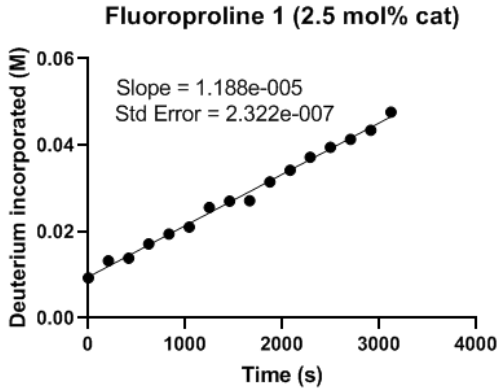
Fluoroproline 1: Variation of concentration of catalyst (0.15M Phosphate, pD 7.4, *I* = 0.9)

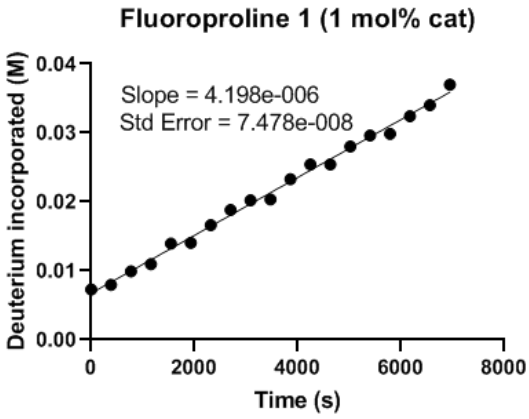


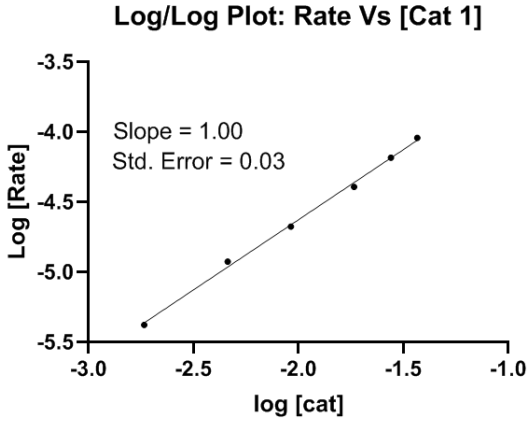




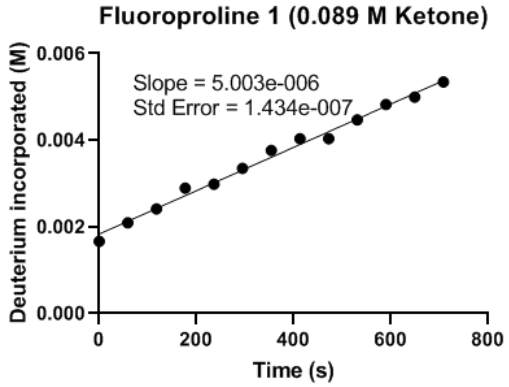


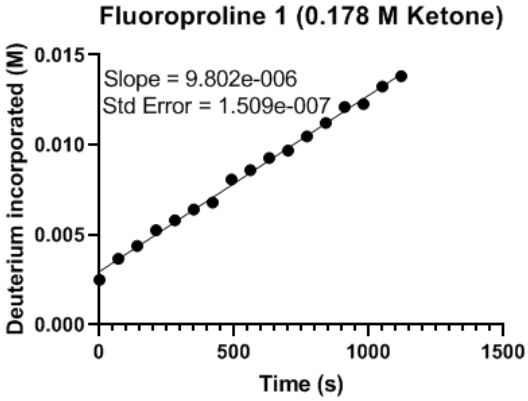


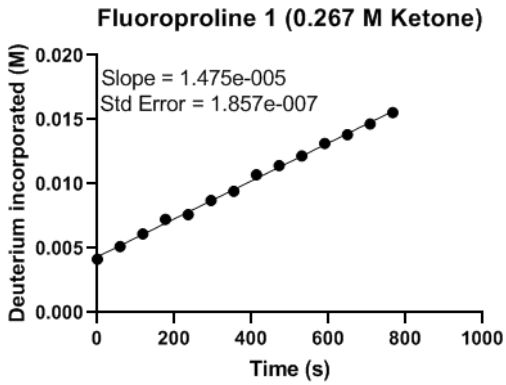


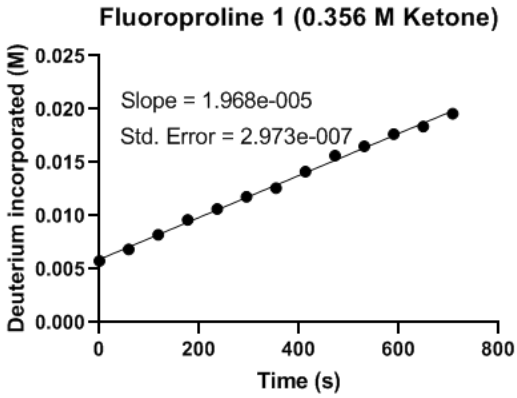


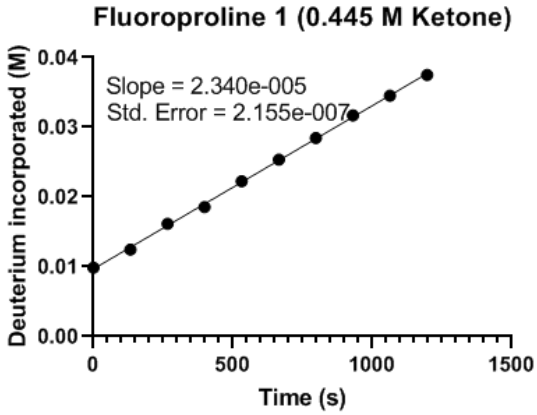
Fluoroproline 1: Variation of concentration of ketone (0.15M Phosphate, pD 7.4, *I* = 0.9)

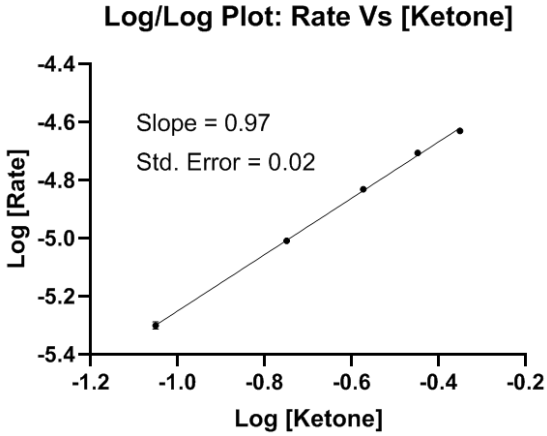




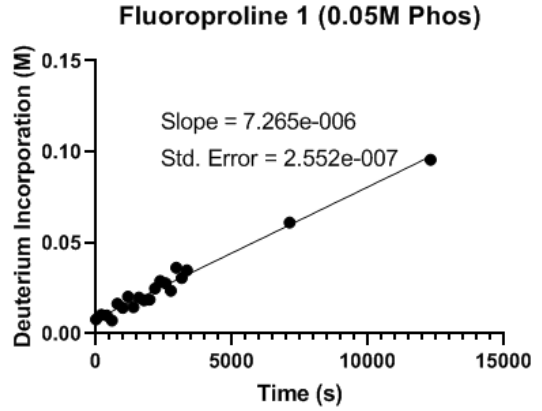


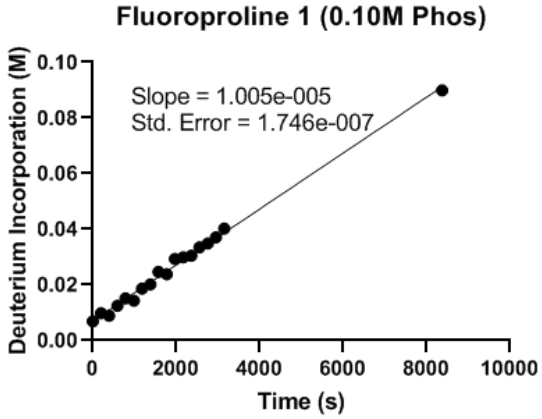


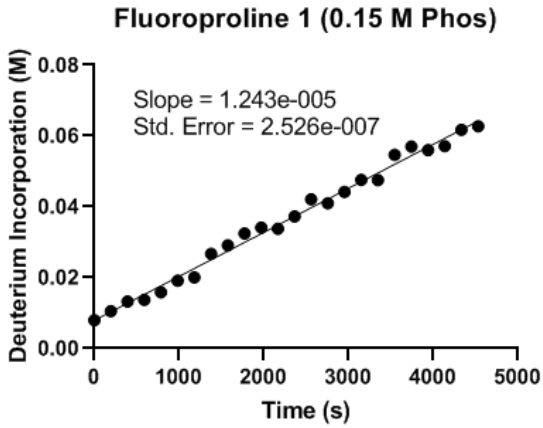


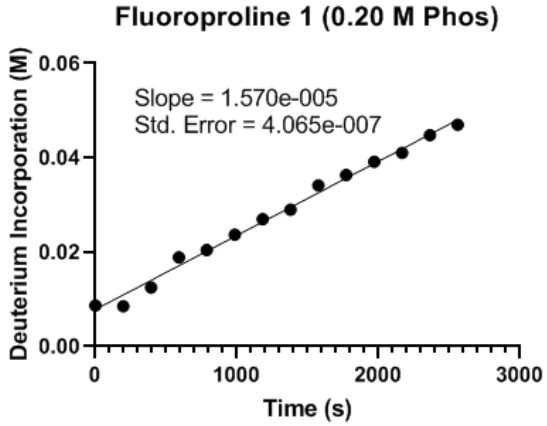


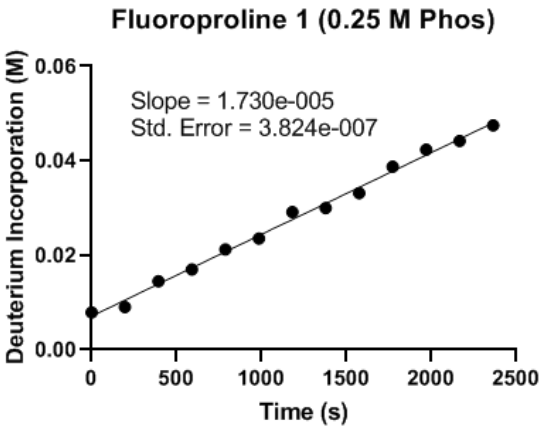
Fluoroproline 1: Variation of concentration of phosphate (pD = 8.0, *I* = 3)

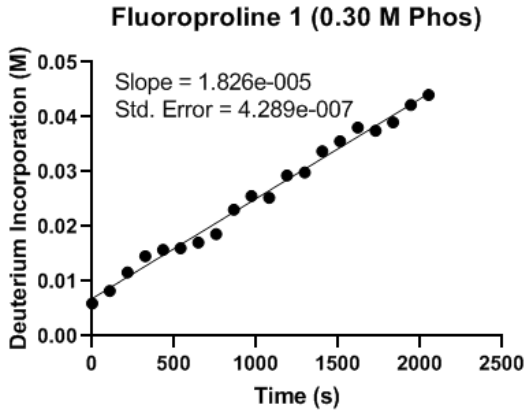


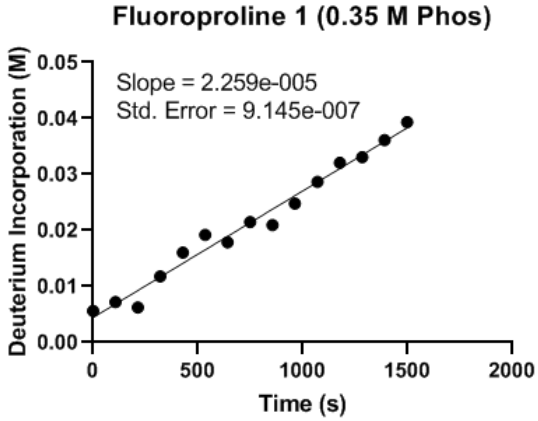


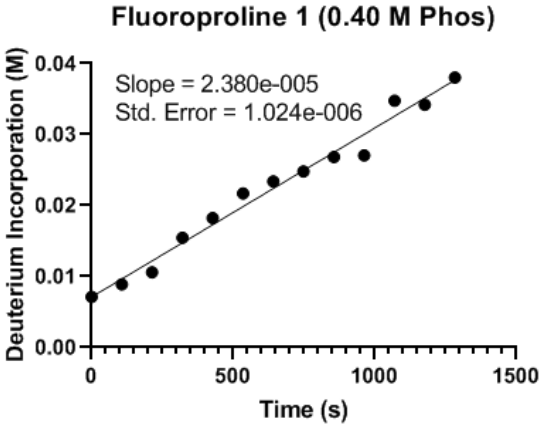


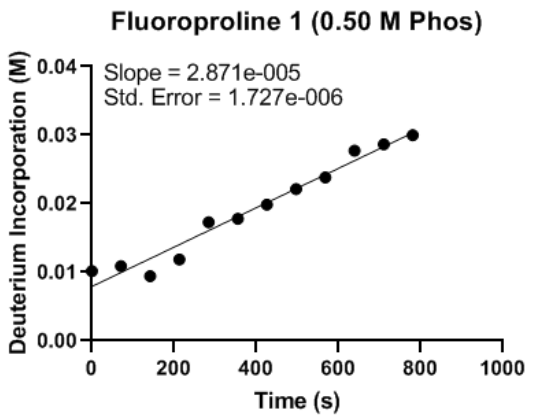


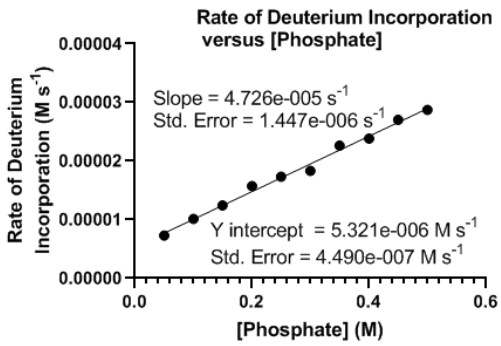


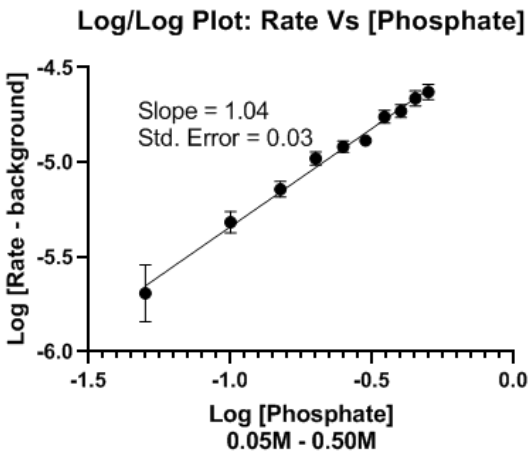






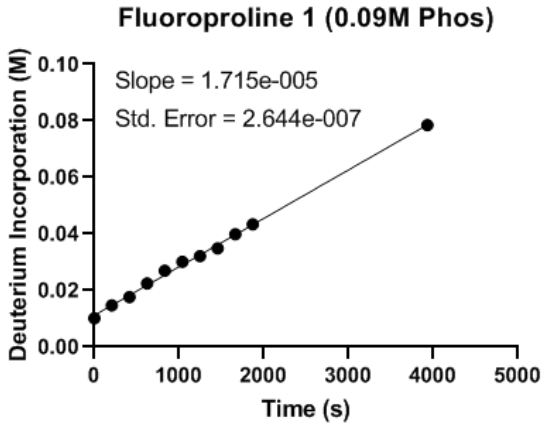


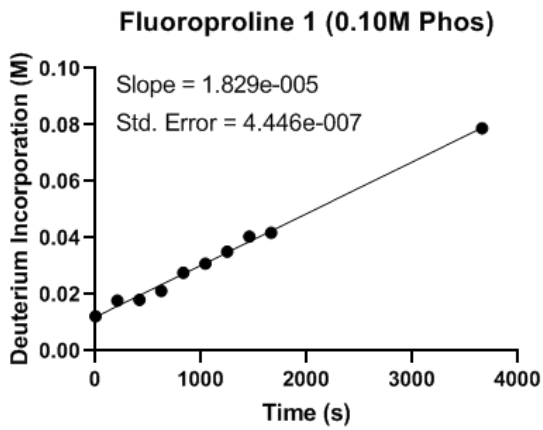


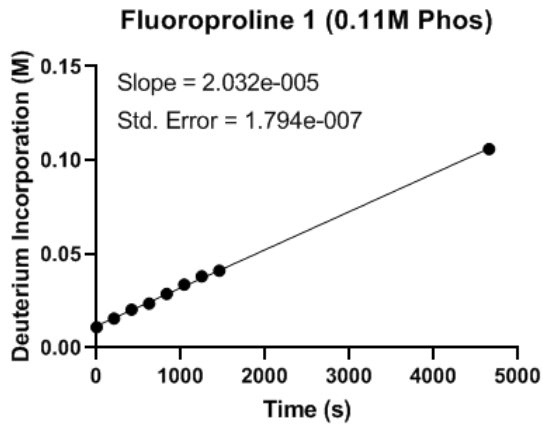


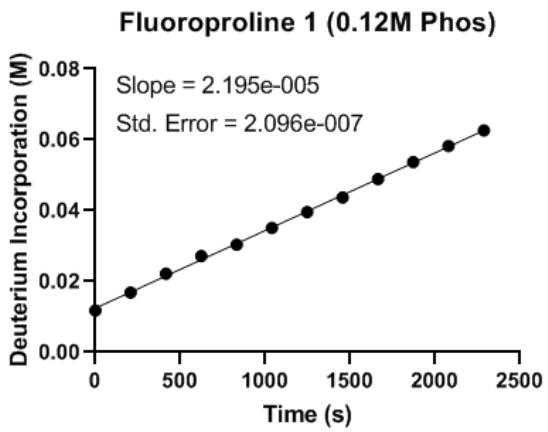
The Y Intercept of the above Rate versus [Phosphate] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of phosphate, a value close to unity is obtained (see below). The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [phosphate] plot.)

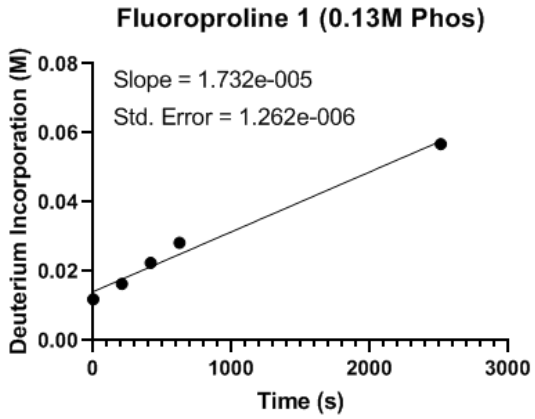
Fluoroproline 1: Variation of concentration of phosphate (pD = 6.5, *I* = 0.9)

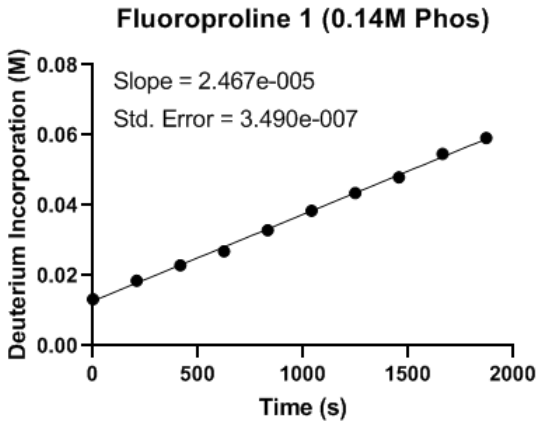


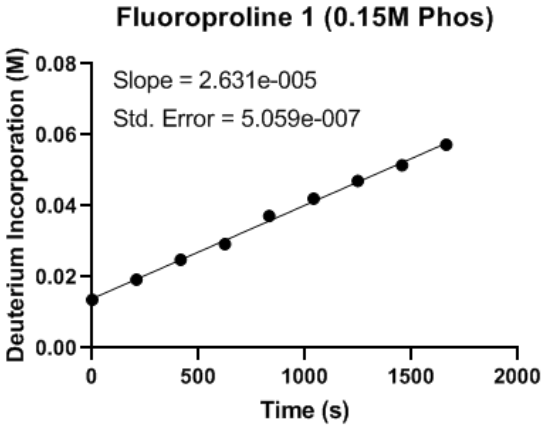


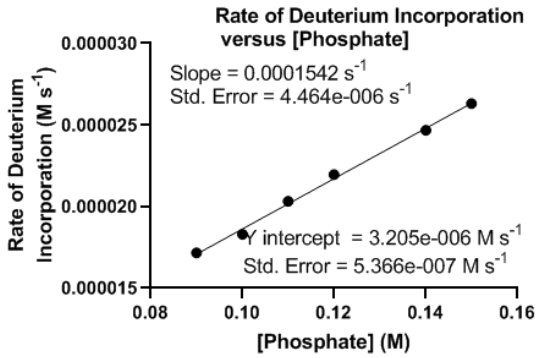






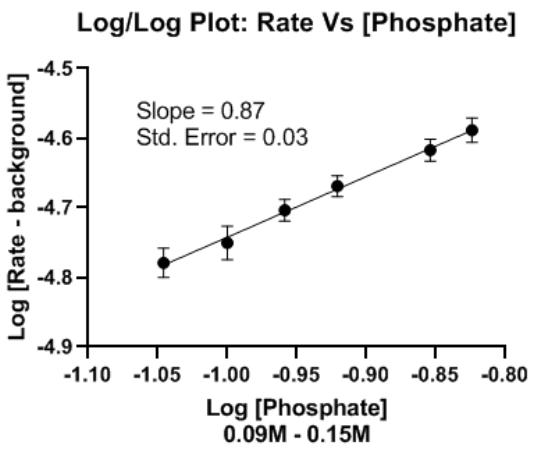




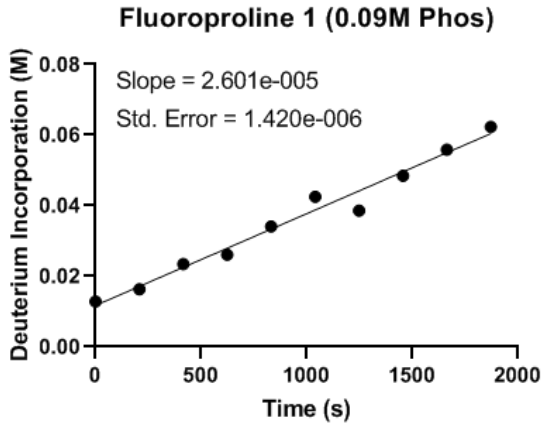


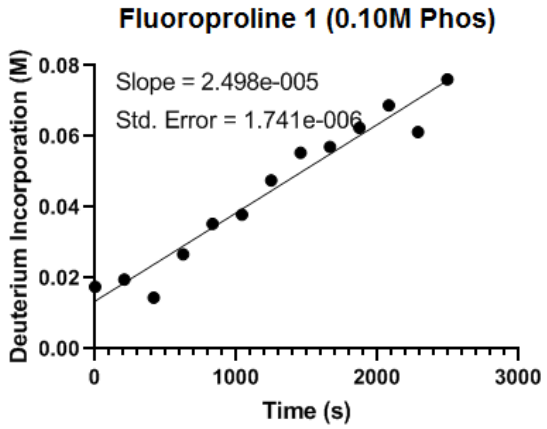
Value at 0.13M (0.00001732) excluded owing to being an extreme outlier

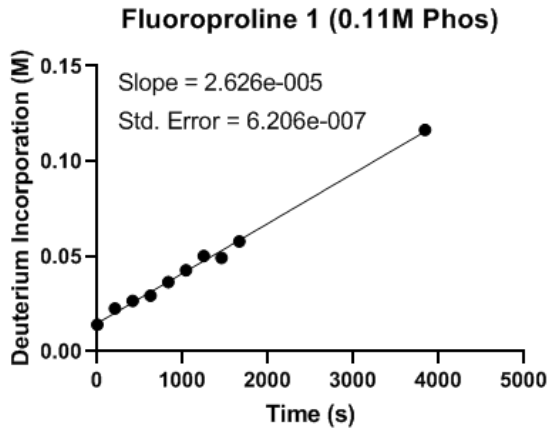
The Y Intercept of the above Rate versus [Phosphate] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of phosphate, a value of 0.87 is obtained (see below). The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [phosphate] plot.)

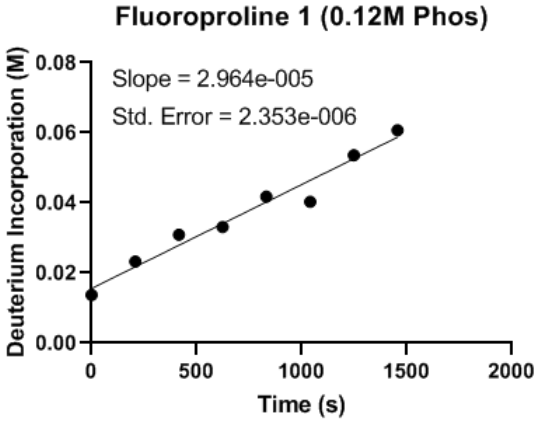


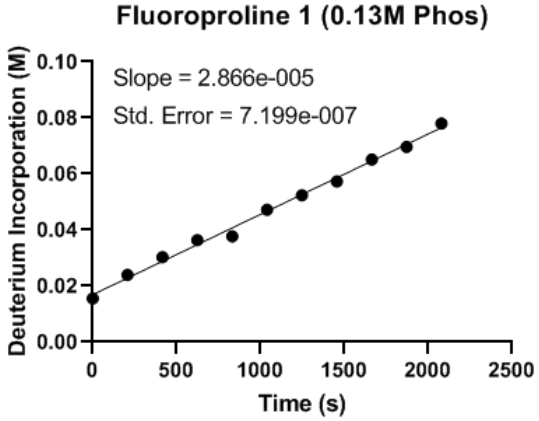
Fluoroproline 1: Variation of concentration of phosphate (pD = 8.5, *I* = 0.9)

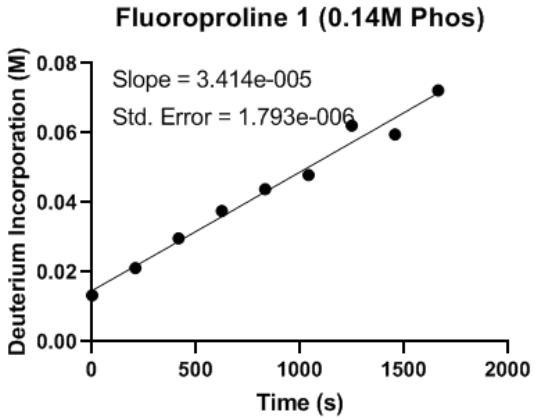


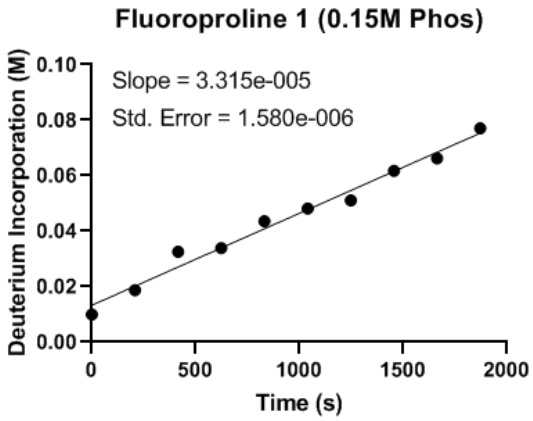


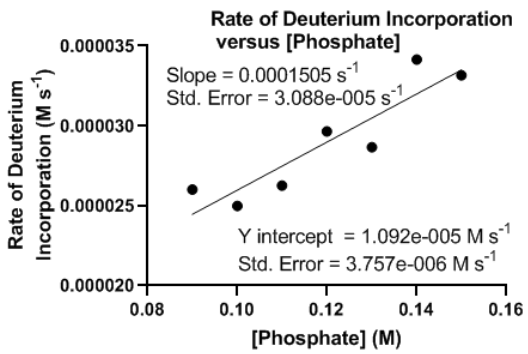




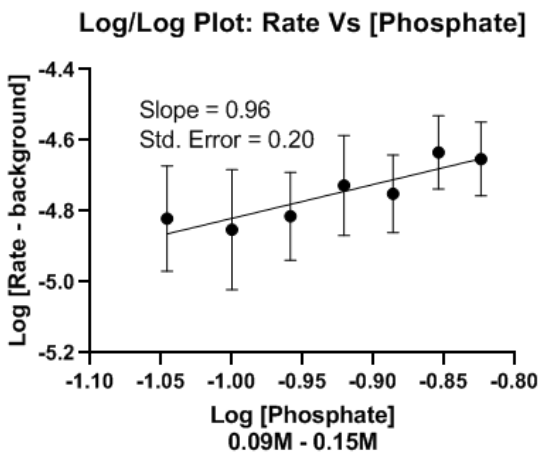




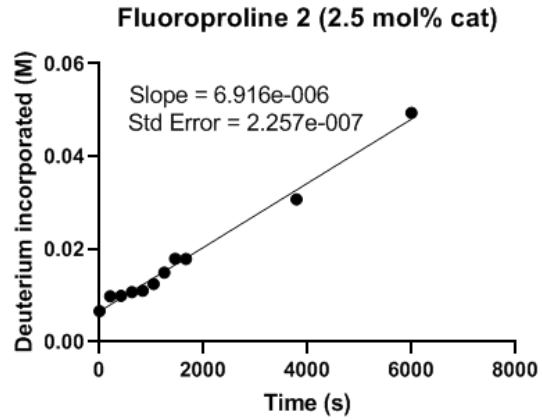


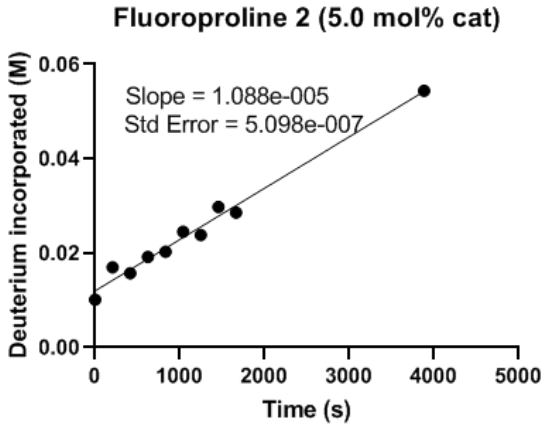


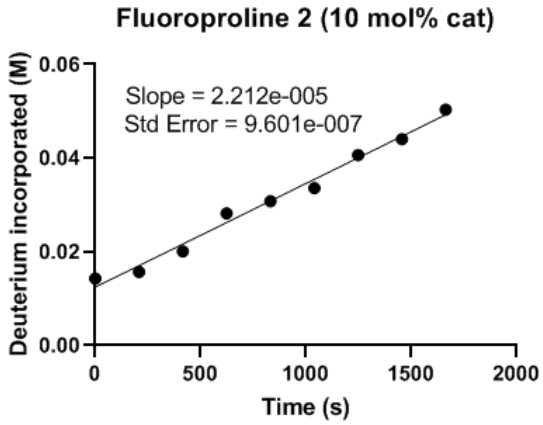
The Y Intercept of the above Rate versus [Phosphate] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of phosphate, a value of 0.96 is obtained (see below). The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [phosphate] plot.)

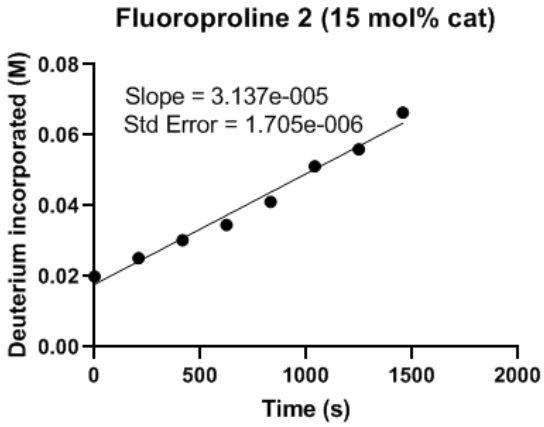


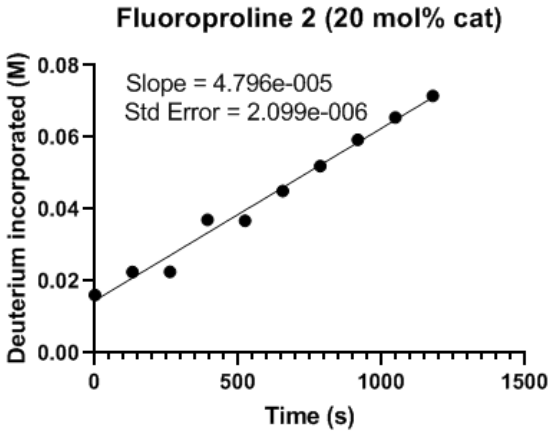
Fluoroproline 2: Variation of concentration of catalyst (0.25M Phosphate, pD 8.4, *I* = 1.5)

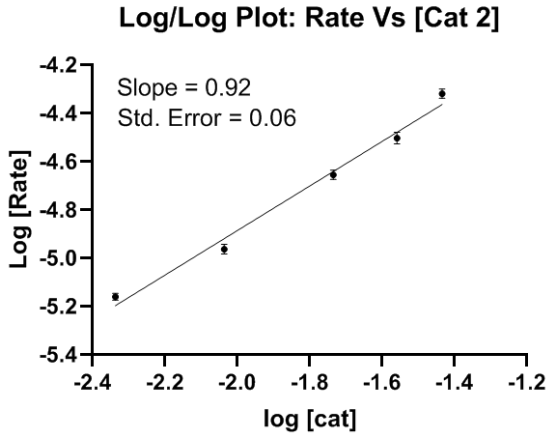




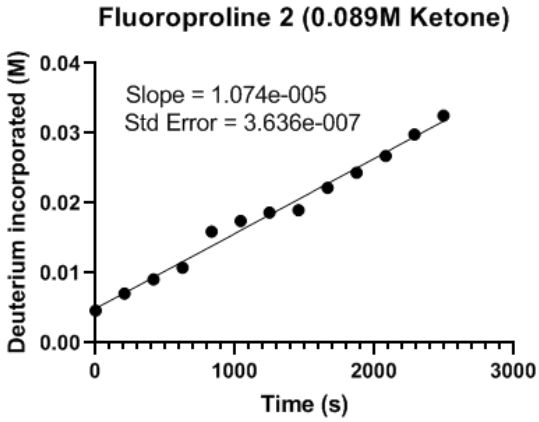


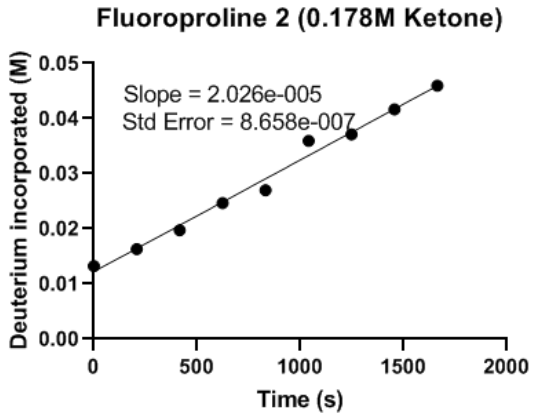


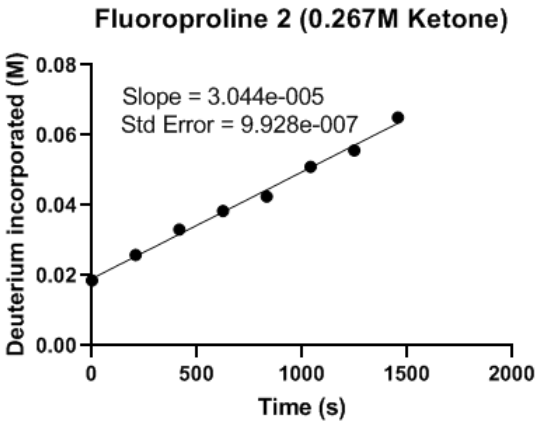


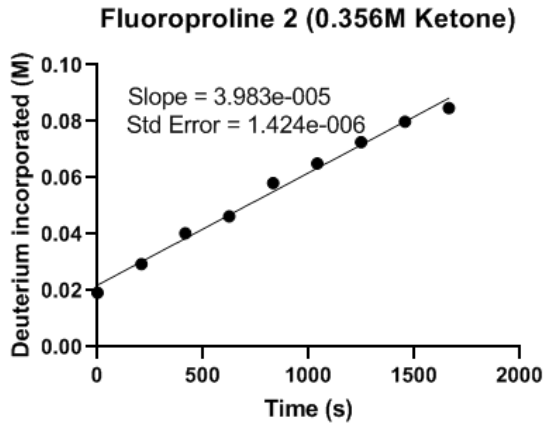


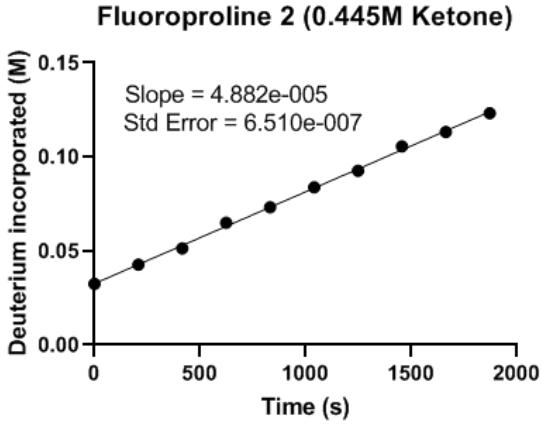
Fluoroproline 2: Variation of concentration of ketone (0.25M Phosphate, pD 8.4, *I* = 1.

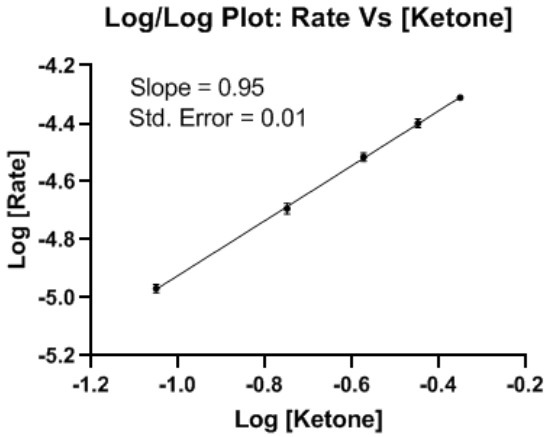




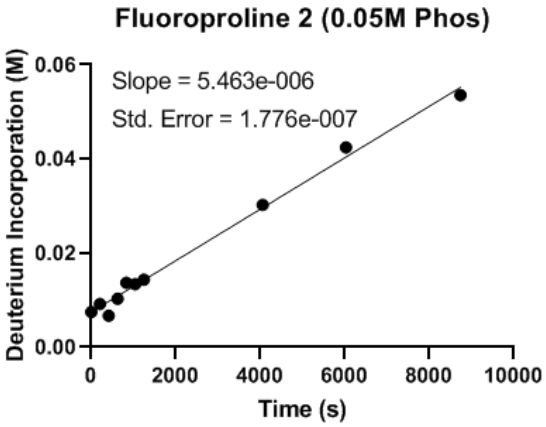


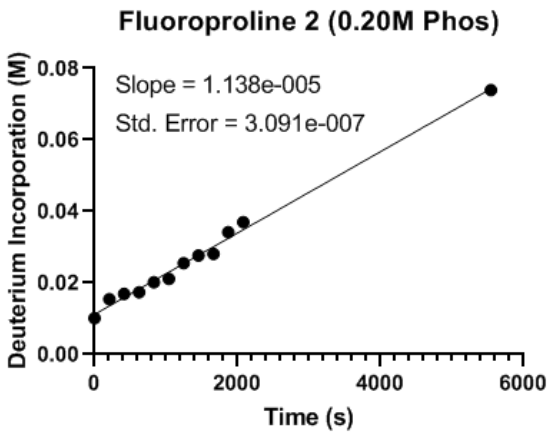


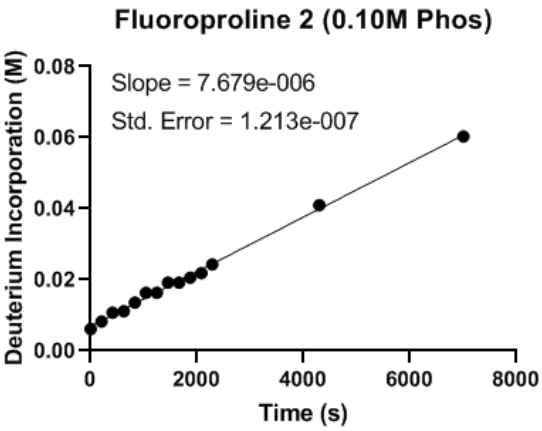


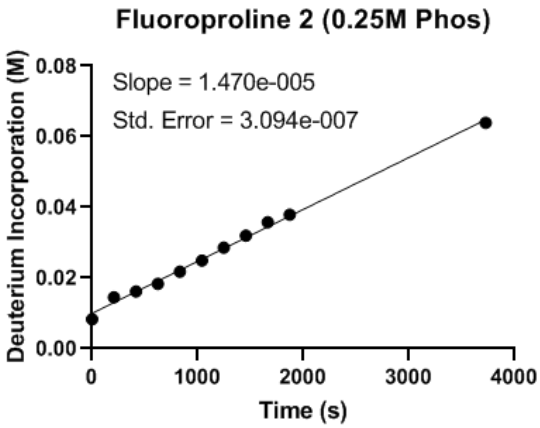


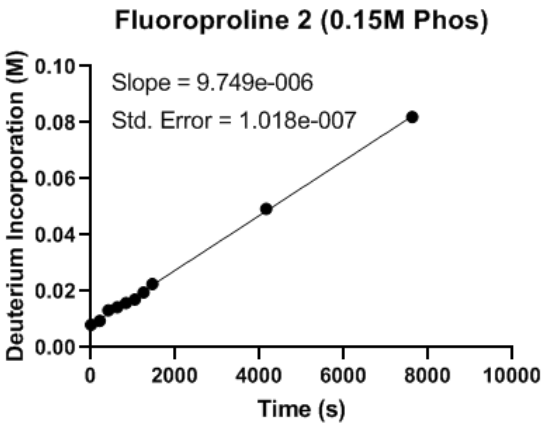
Fluoroproline 2: Variation of concentration of phosphate (pD = 8.4, *I* = 3)

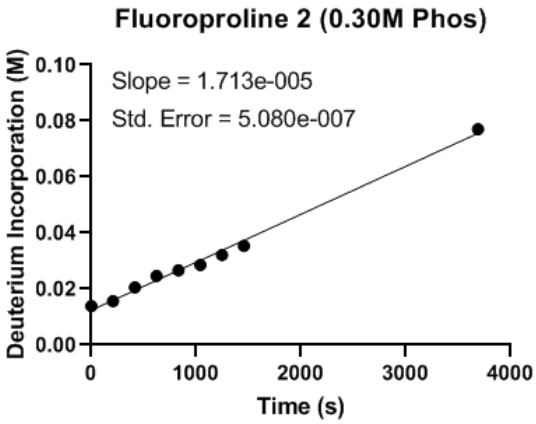


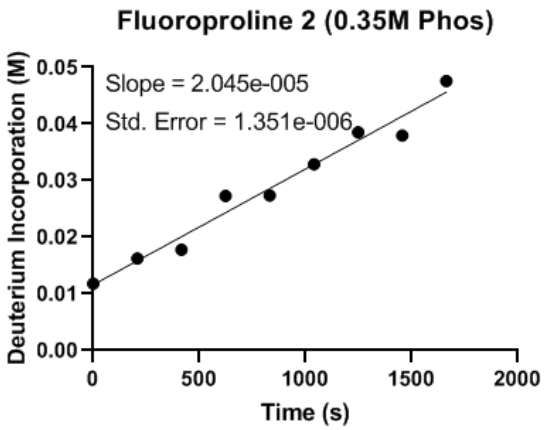


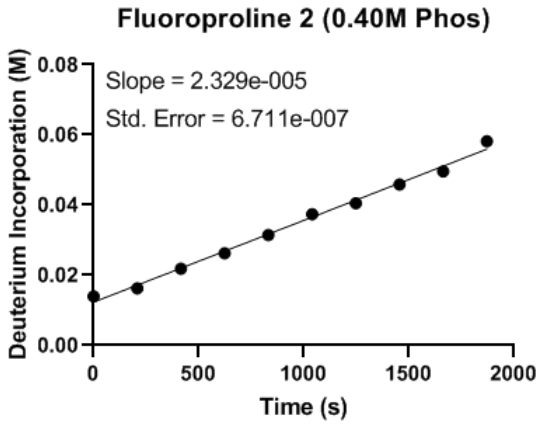


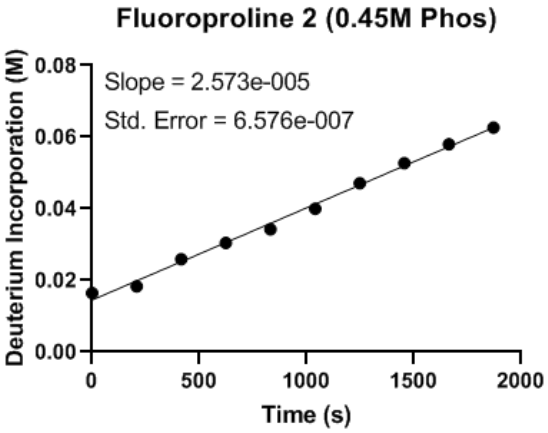


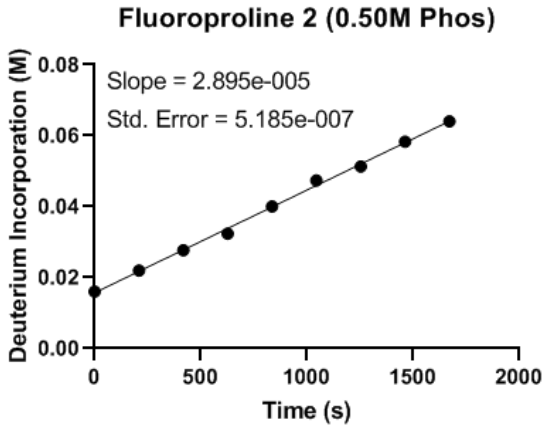


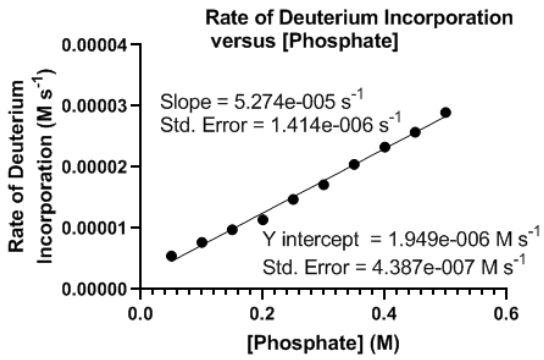




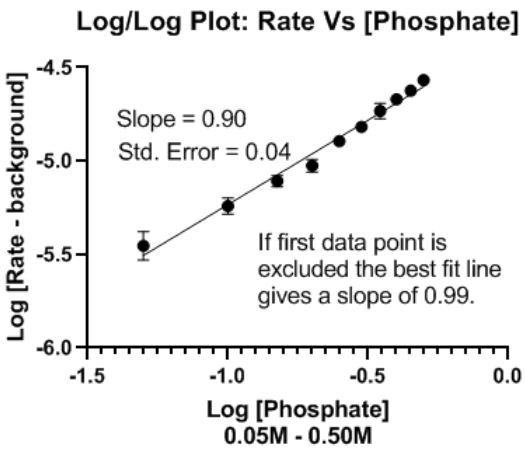




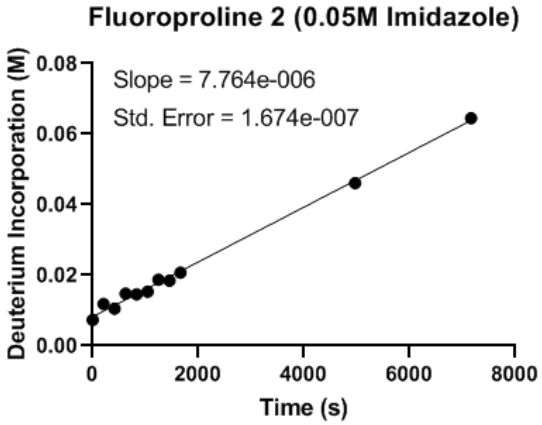


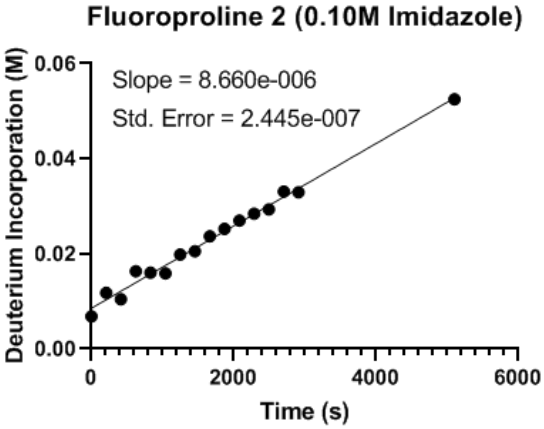


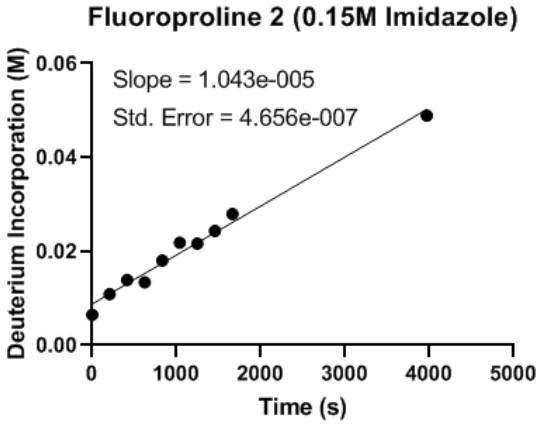
The Y Intercept of the above Rate versus [Phosphate] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of phosphate, a value of 0.90 is obtained (see below). If the first data point is excluded, the best-fit line gives a slope of 0.99. The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [phosphate] plot.)

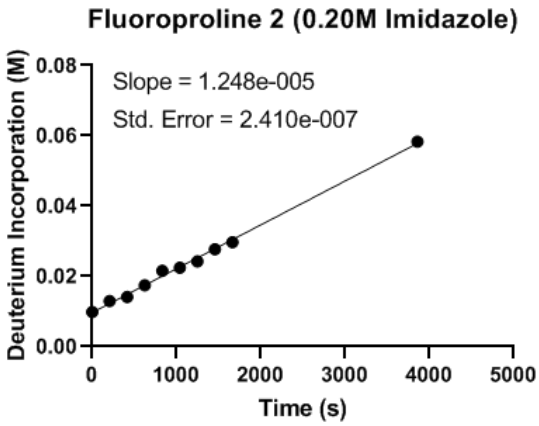


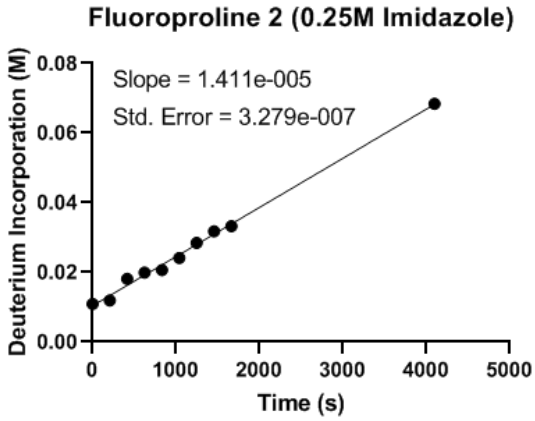
Fluoroproline 2: Variation of concentration of imidazole (pD = 8.4, *I* = 0.5)

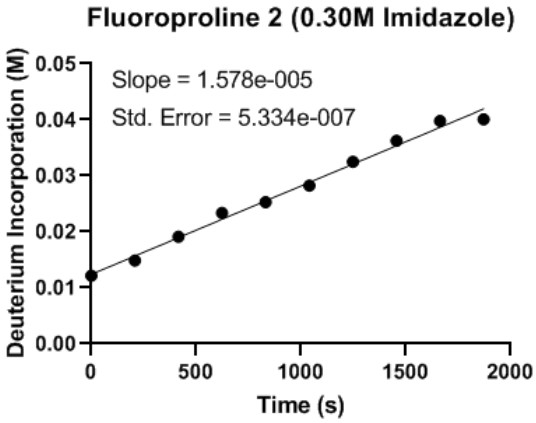


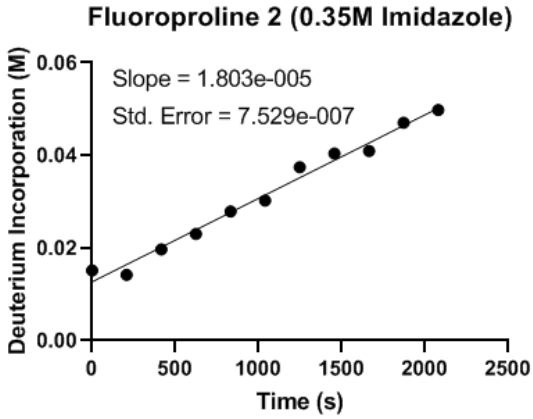


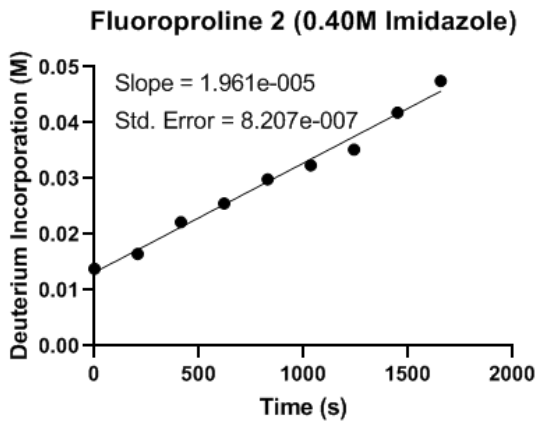


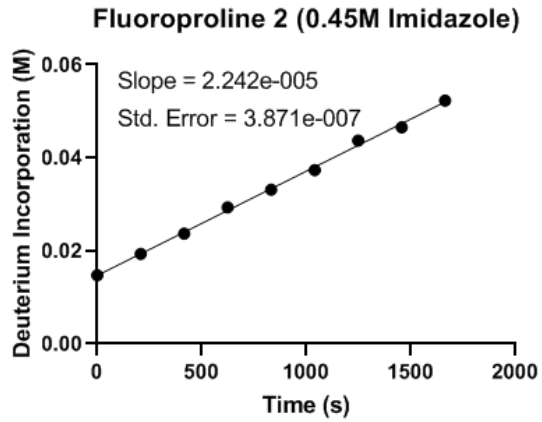


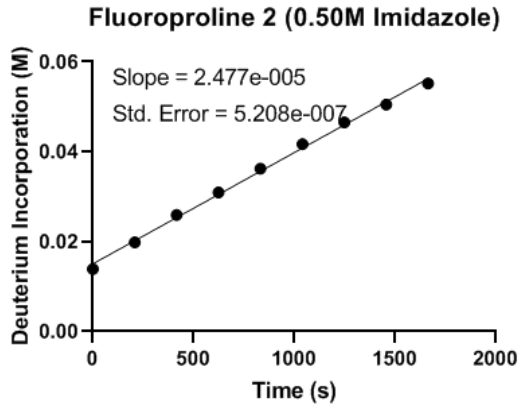


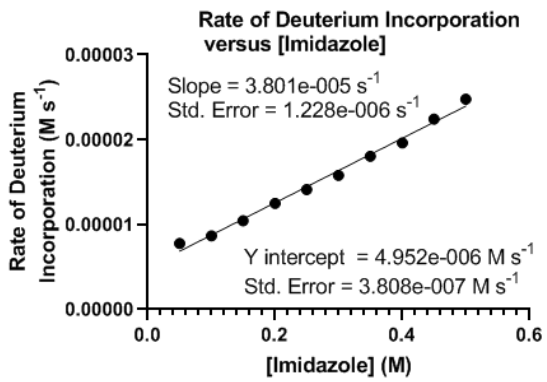




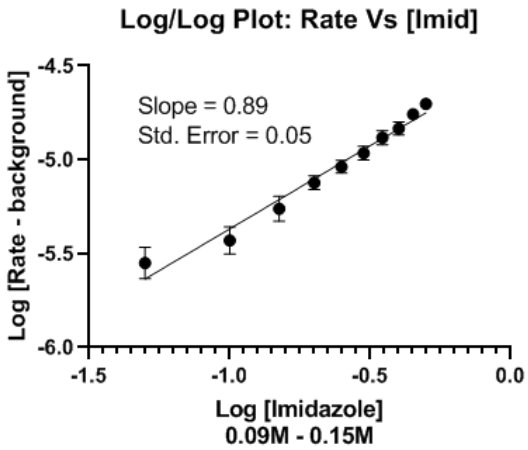




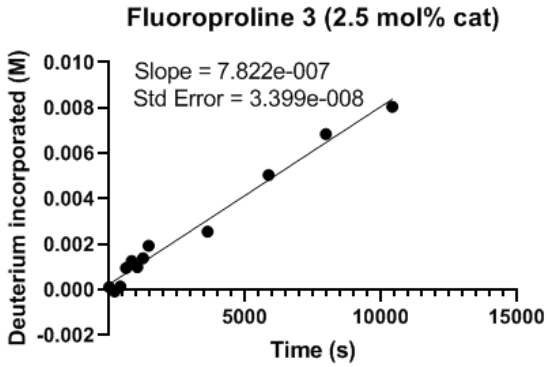


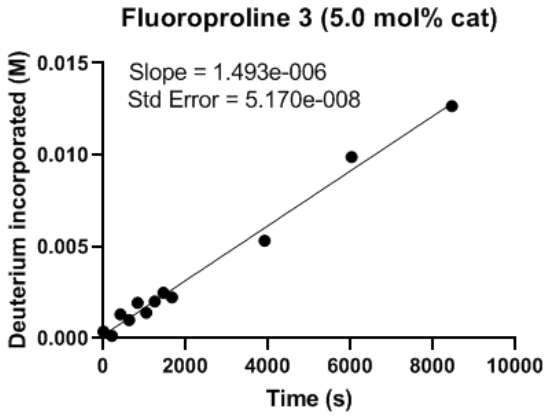


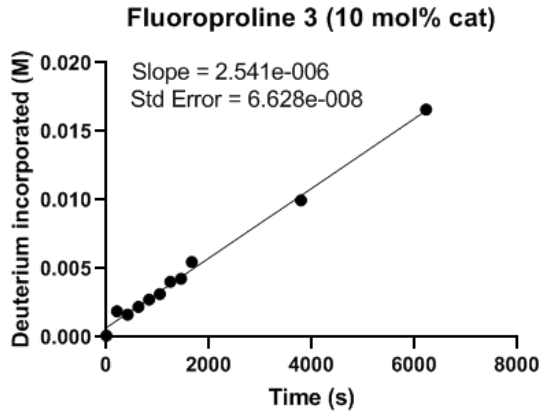
The Y Intercept of the above Rate versus [Imidazole] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of imidazole, a value of 0.89 is obtained (see below). If the first data point is excluded, the best-fit line gives a slope of 1.03. The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [imidazole] plot.)

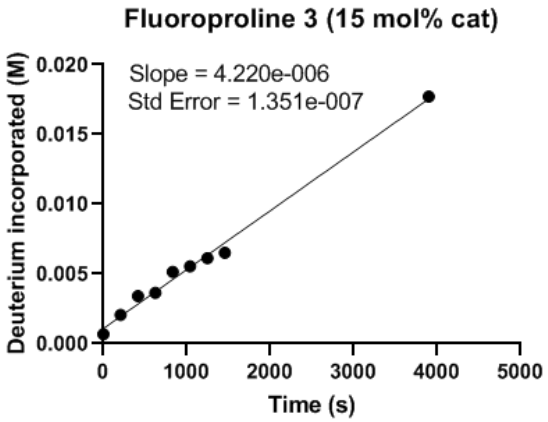


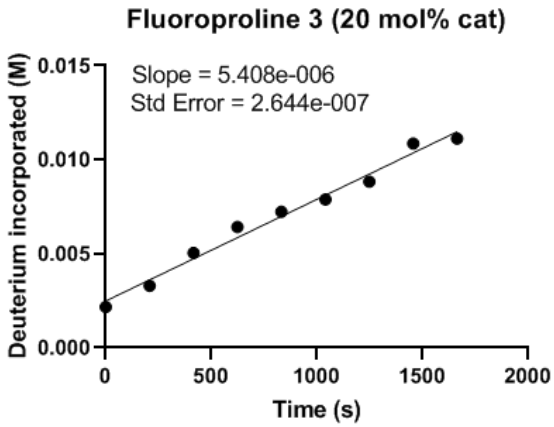
Fluoroproline 3: Variation of concentration of catalyst (0.25M Phosphate, pD 8.4, *I* = 1.5)

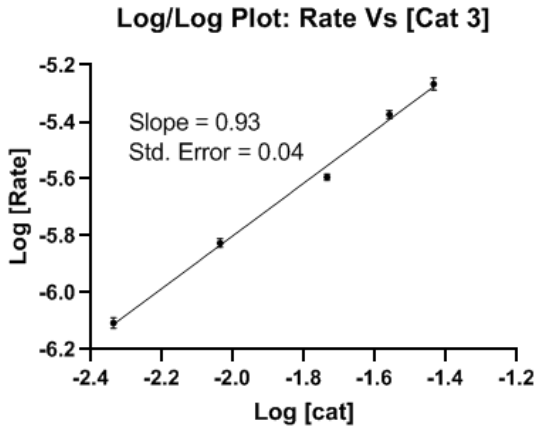




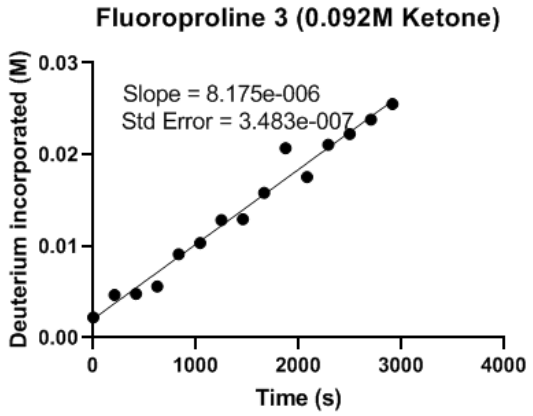


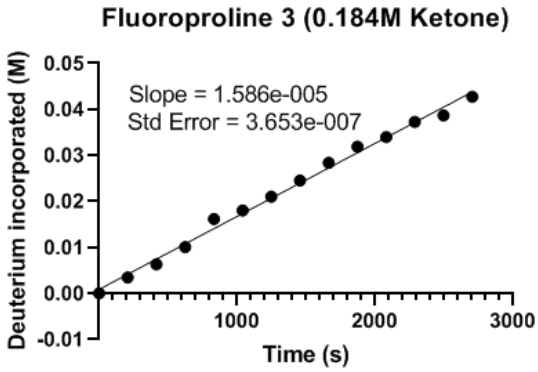


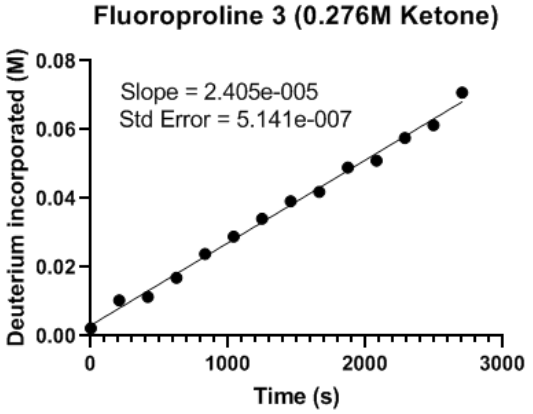


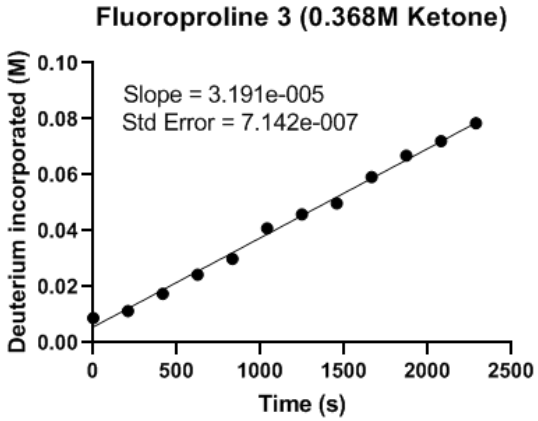


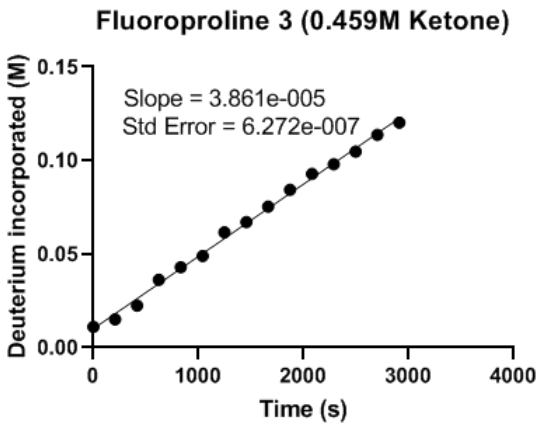
Fluoroproline 3: Variation of concentration of ketone (0.25M Phosphate, pD 8.4, *I* = 1.5)

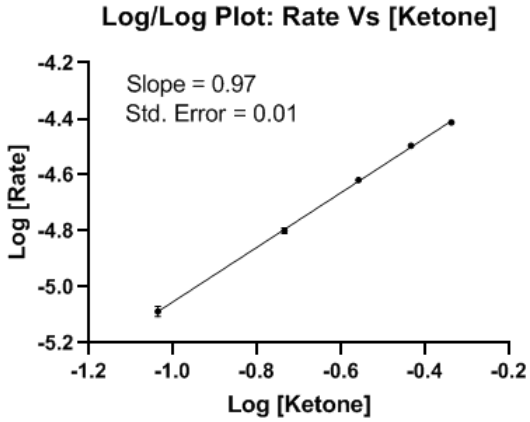




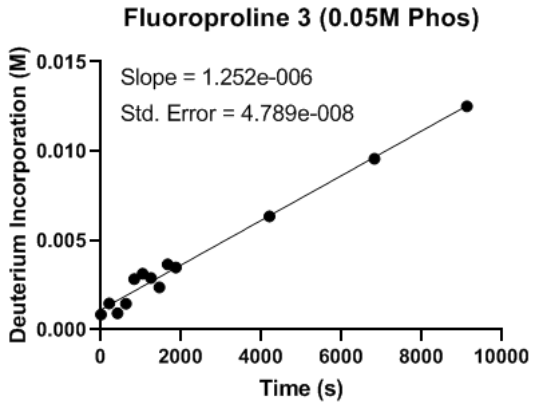


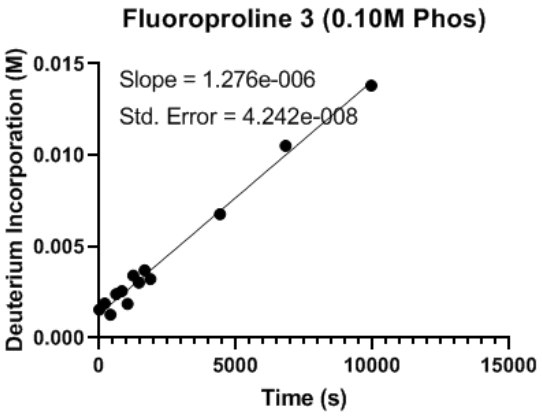


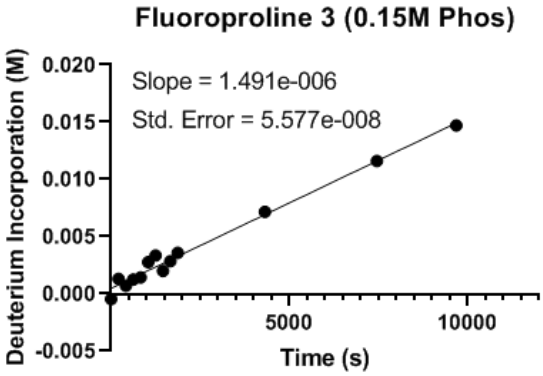


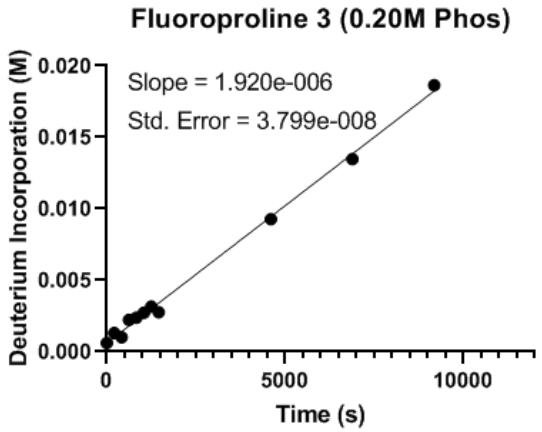


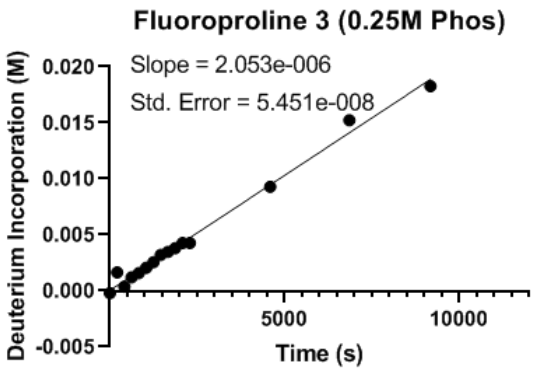
Fluoroproline 3: Variation of concentration of phosphate (pD = 8.4, *I* = 3)

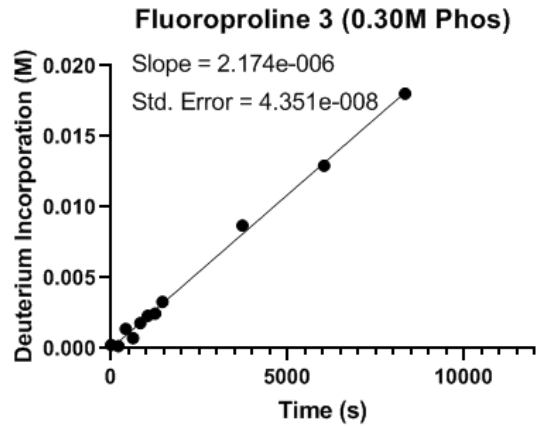


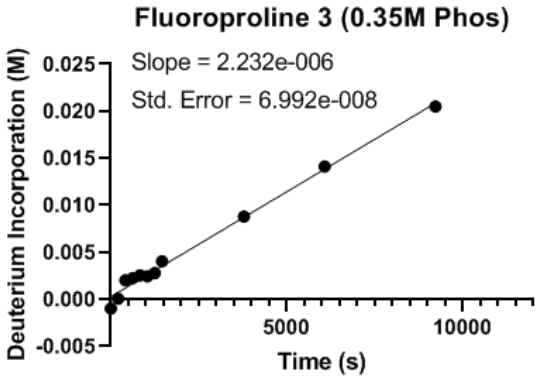


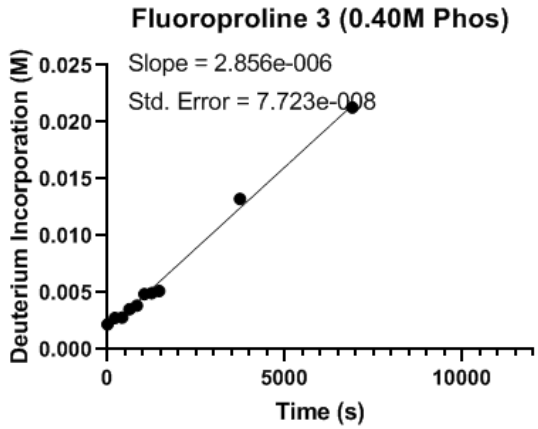


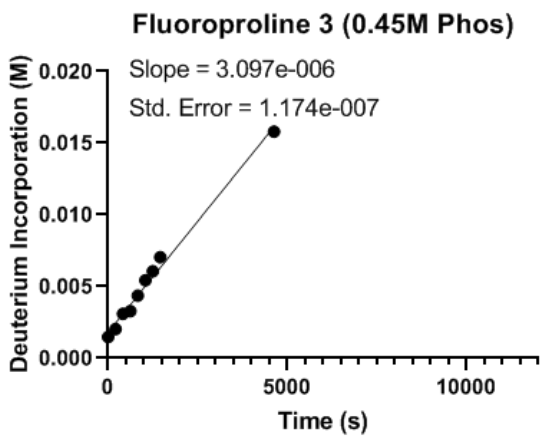


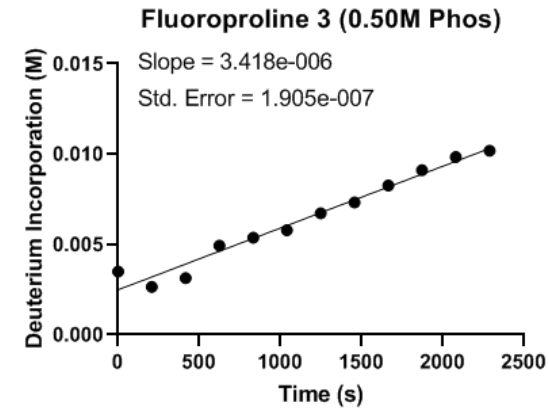


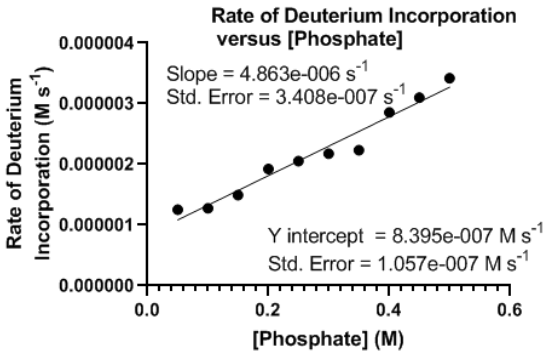




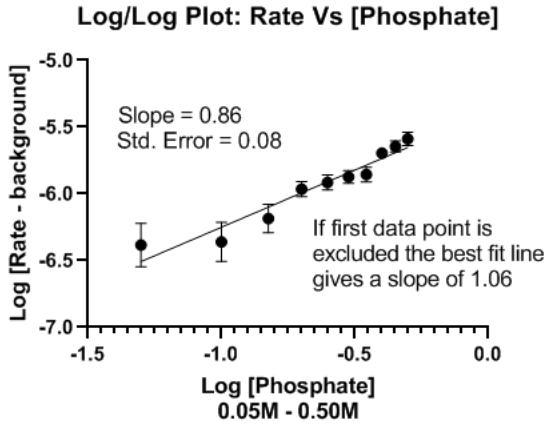




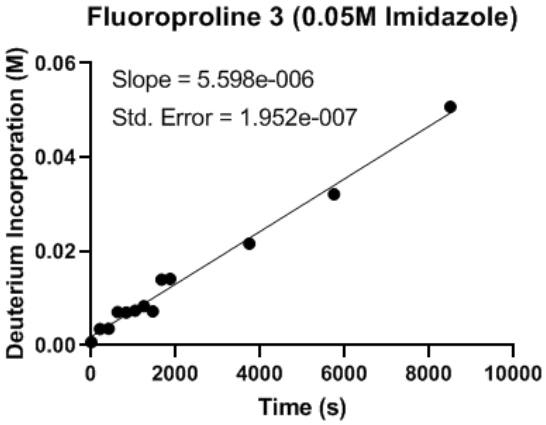


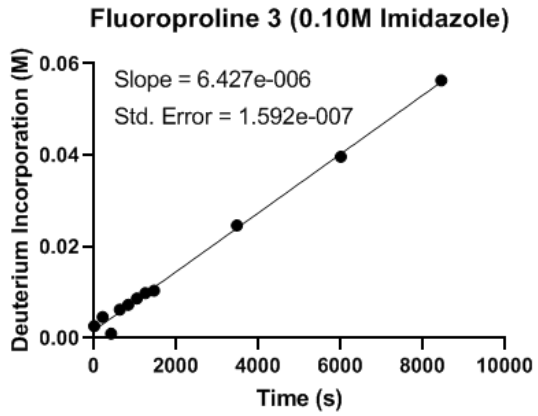


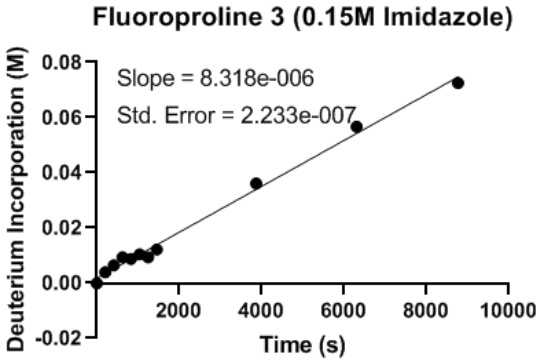
The Y Intercept of the above Rate versus [Phosphate] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of phosphate, a value of 0.86 is obtained (see below). If the first data point is excluded, the best-fit line gives a slope of 1.06. The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [phosphate] plot.)

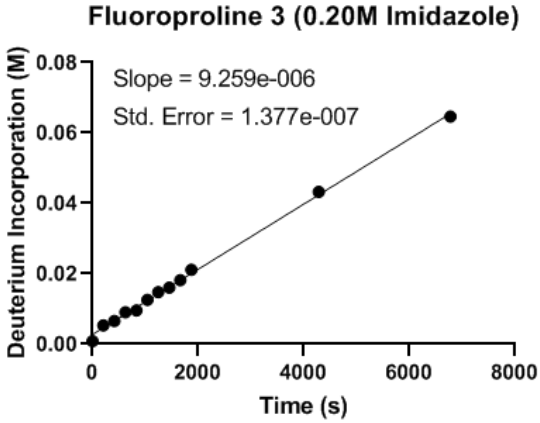


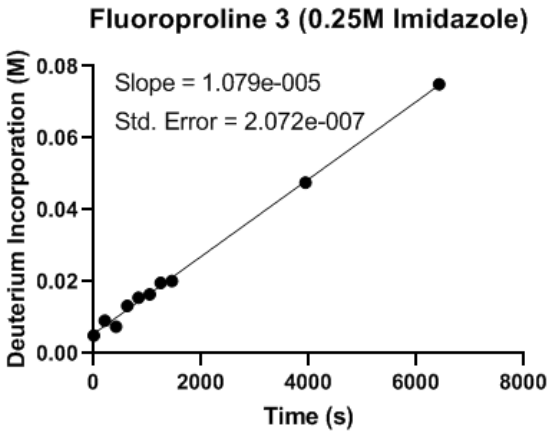
Fluoroproline 3: Variation of concentration of imidazole (pD = 8.4, *I* = 0.5)

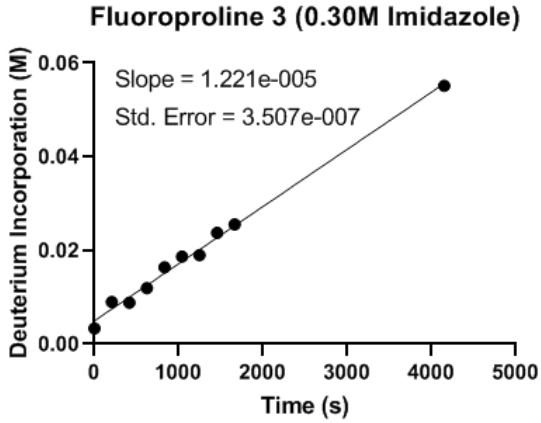


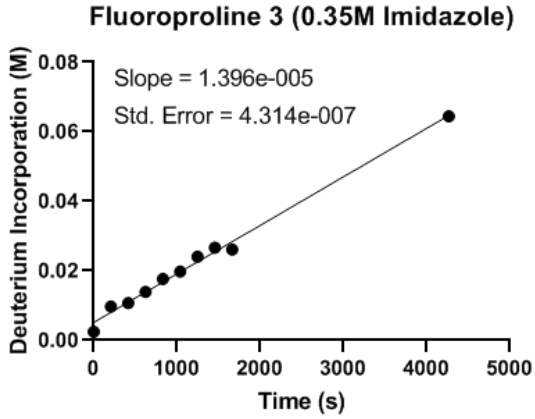


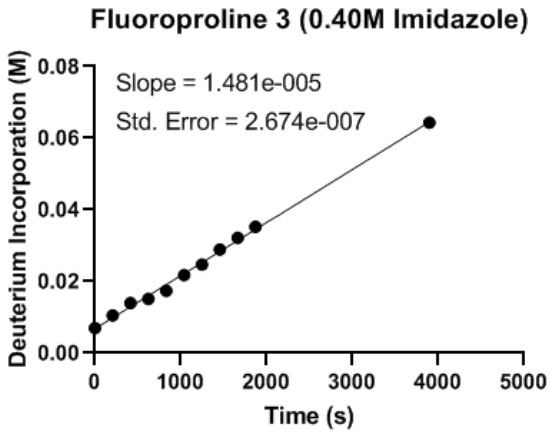


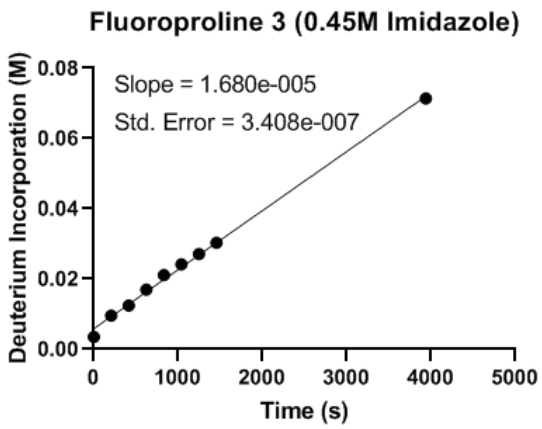


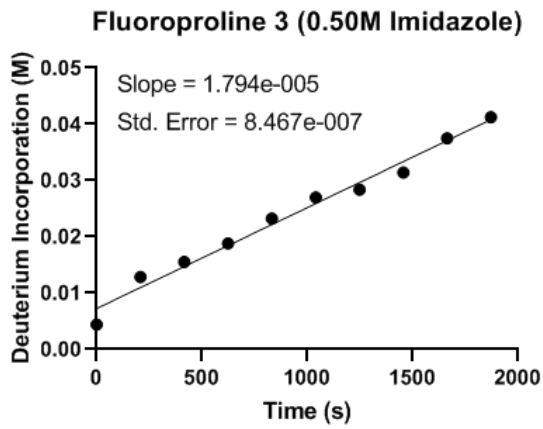


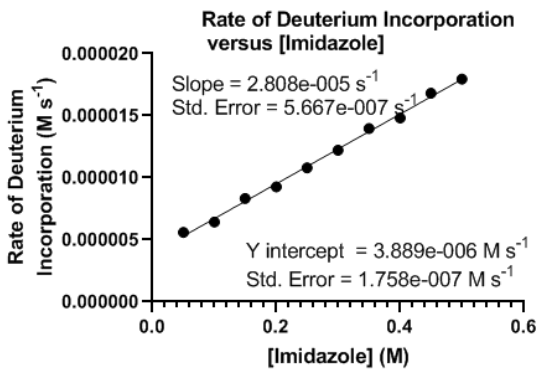




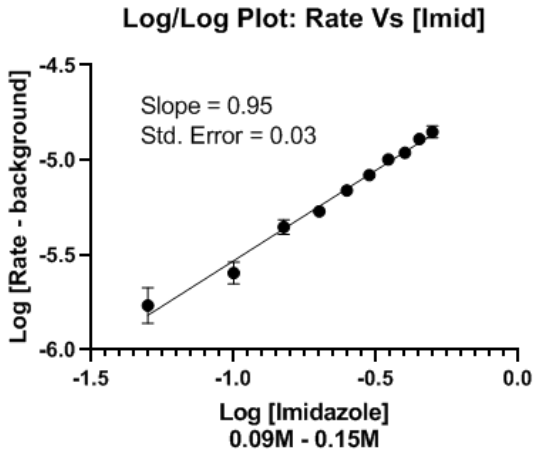




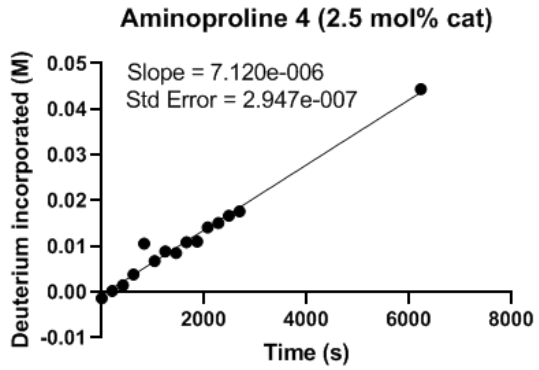


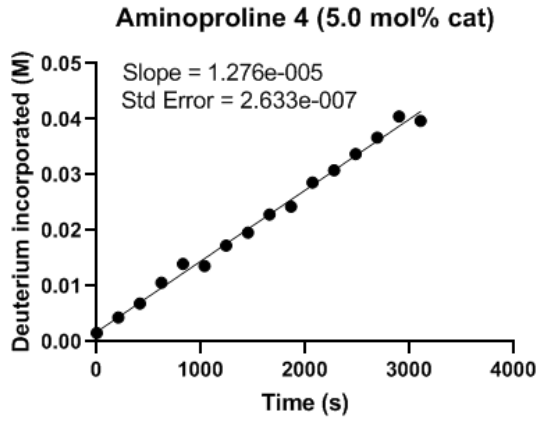


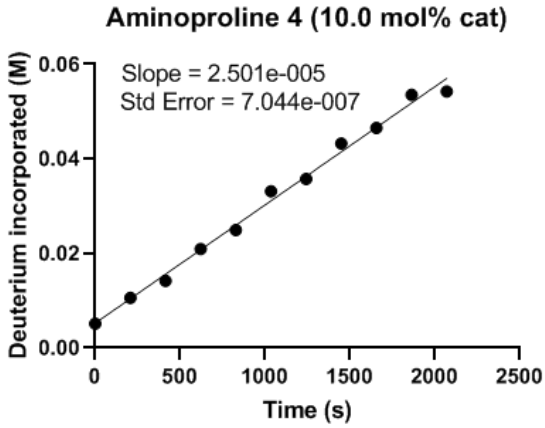
The Y Intercept of the above Rate versus [Imidazole] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of imidazole, a value of 0.95 is obtained (see below). excluded, the best-fit The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [imidazole] plot.)

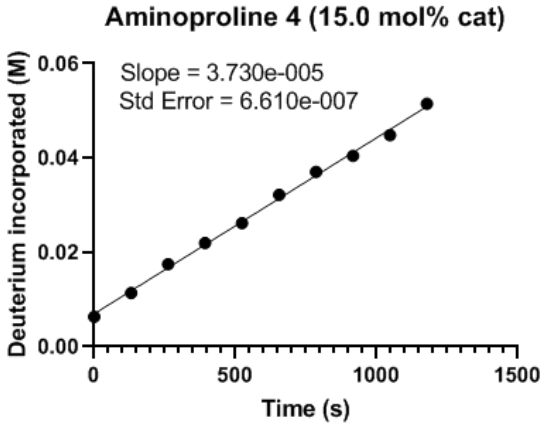


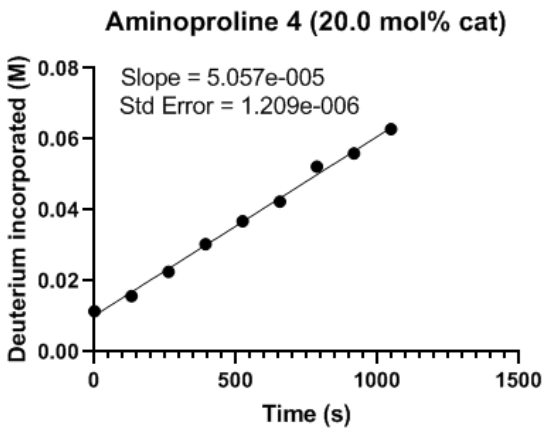
Aminoproline 4: Variation of concentration of catalyst (0.15M Phosphate, pD 7.4, *I* = 0.9)

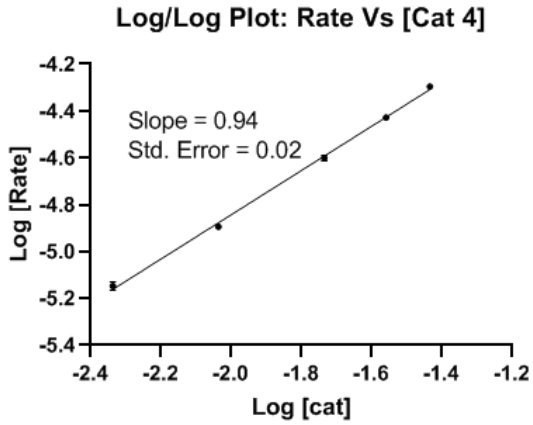




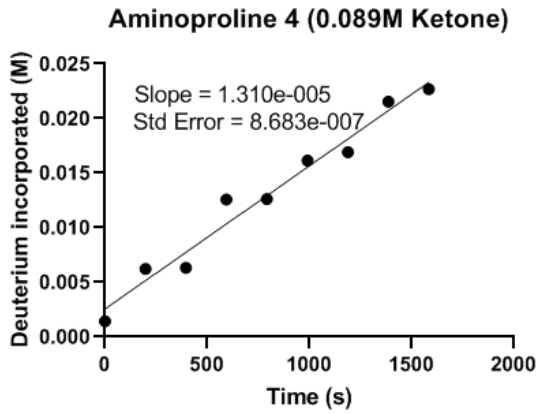


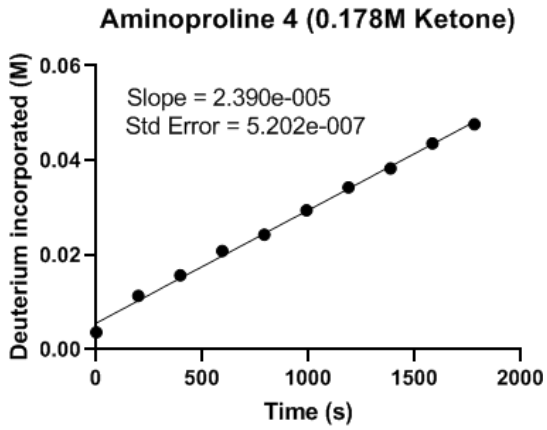


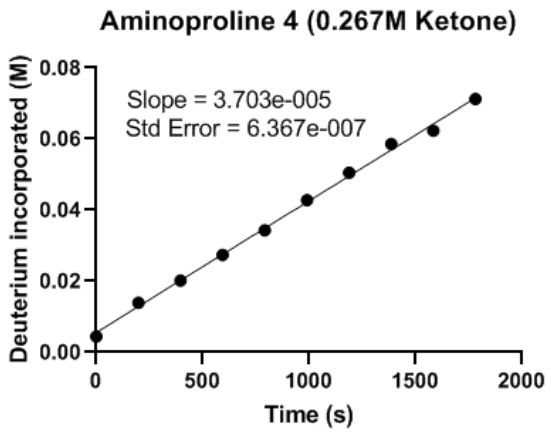


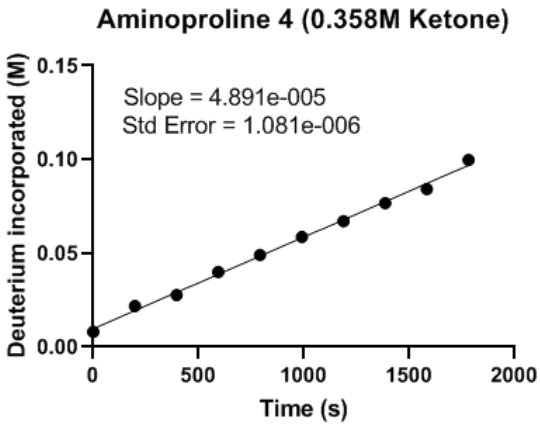


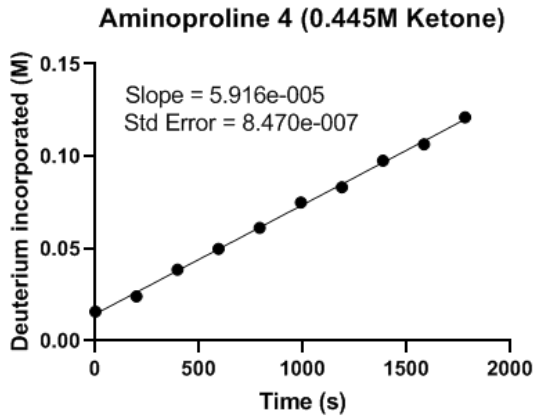
Aminoproline 4: Variation of concentration of ketone (0.15M Phosphate, pD 7.4, *I* = 0.9)

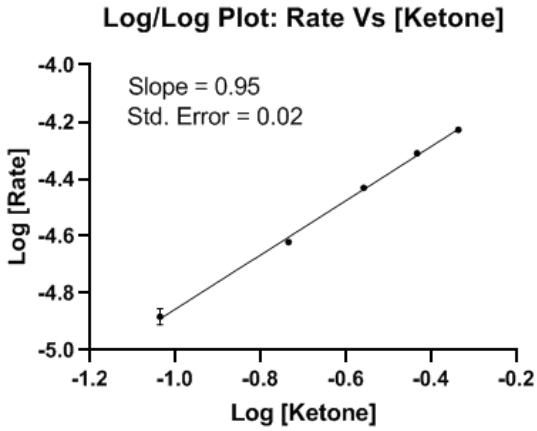




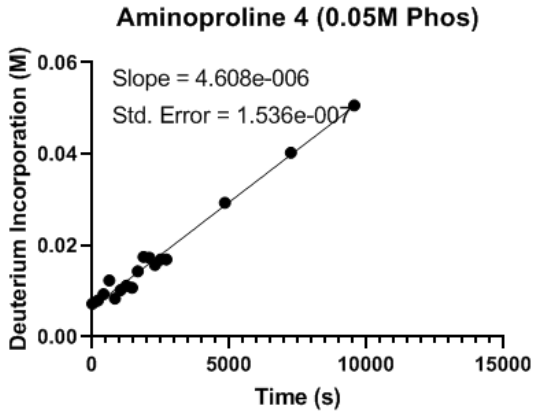


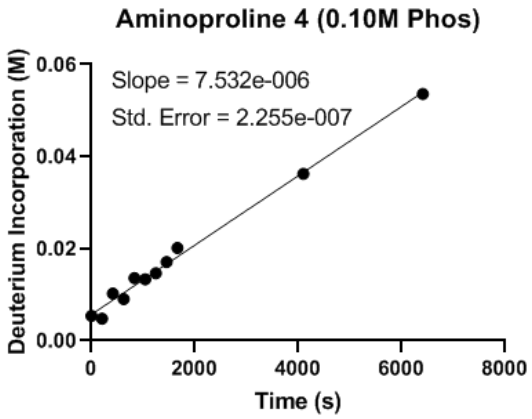


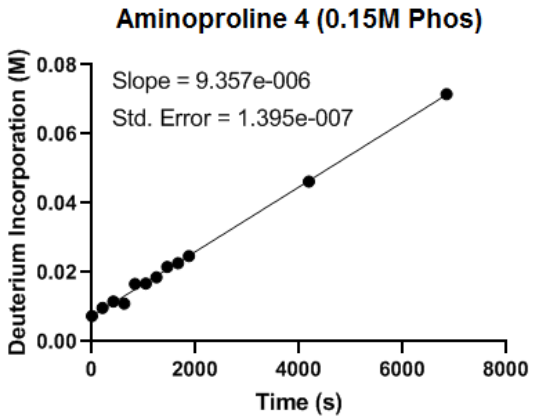


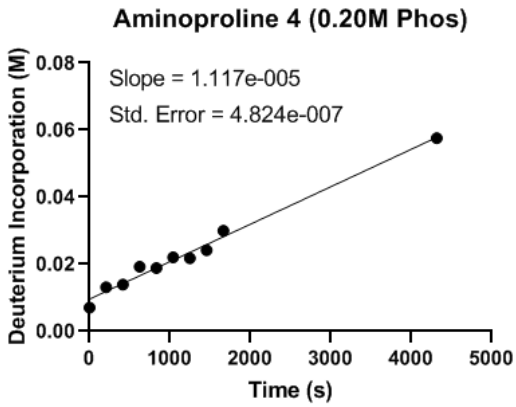


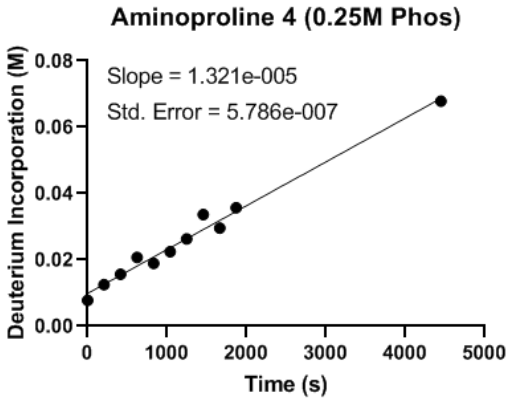
Aminoproline 4: Variation of concentration of phosphate (pD = 7.4, *I* = 3)

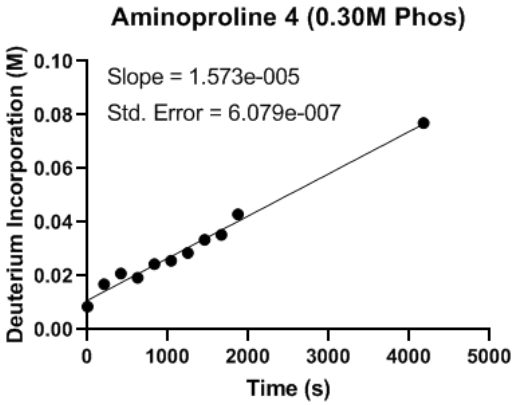


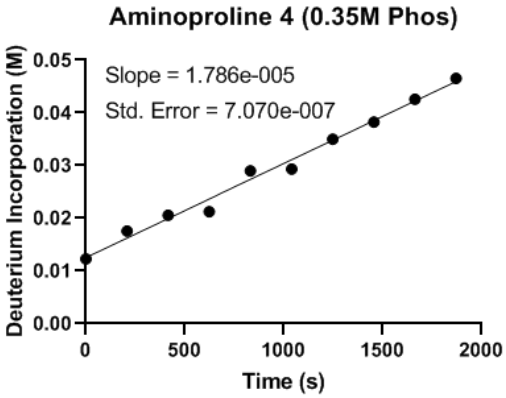


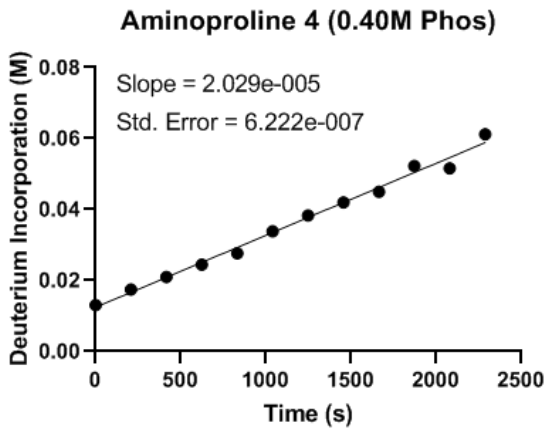


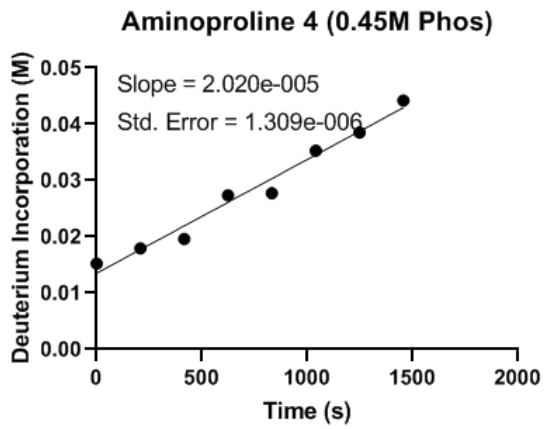


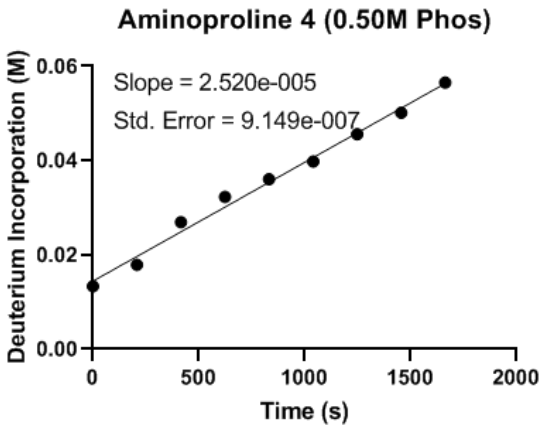


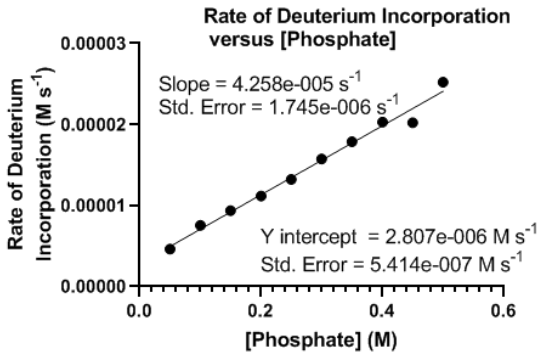




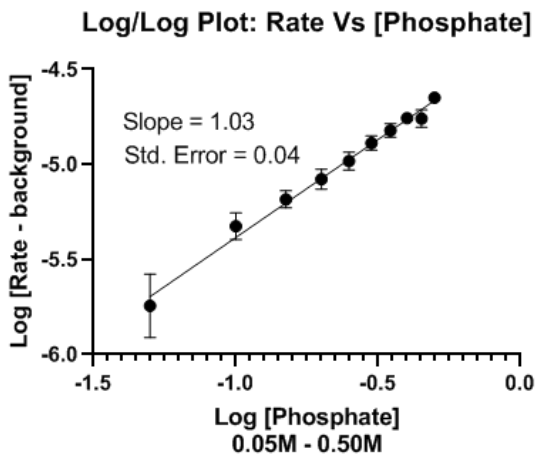




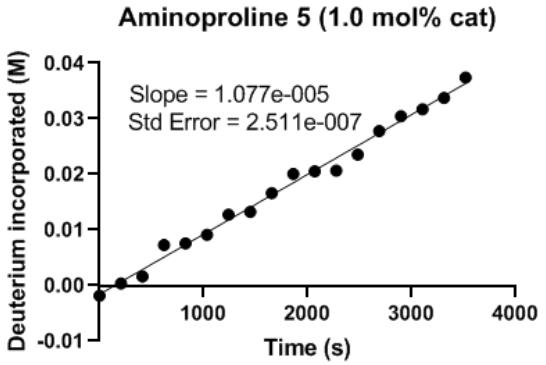


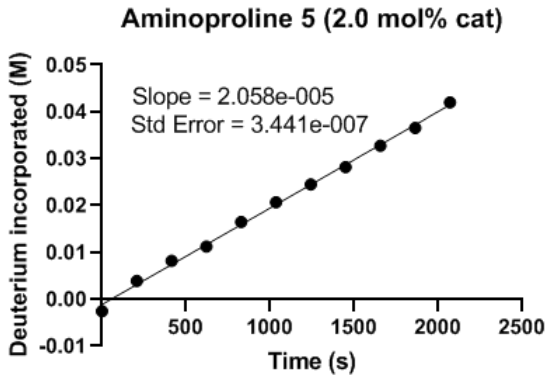


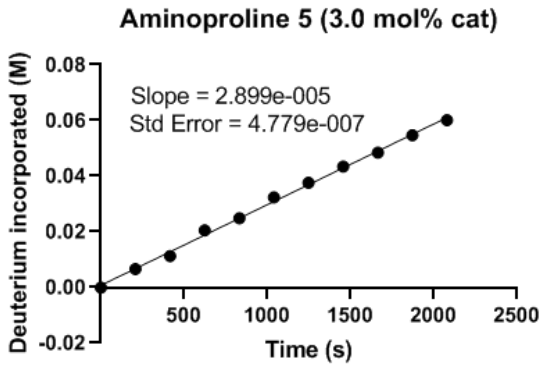
The Y Intercept of the above Rate versus [Phosphate] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of phosphate, a value of 1.03 is obtained (see below). The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [phosphate] plot.)

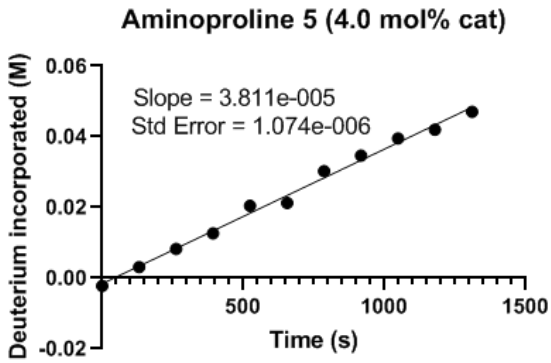


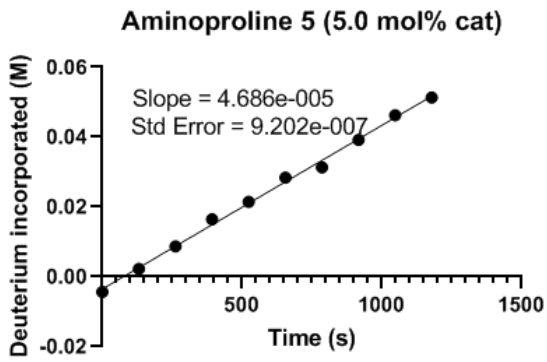
Aminoproline 5: Variation of concentration of catalyst (0.15M Phosphate, pD 7.4, *I* = 0.9)

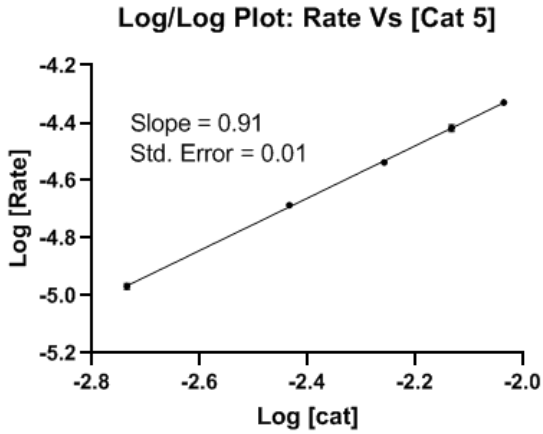




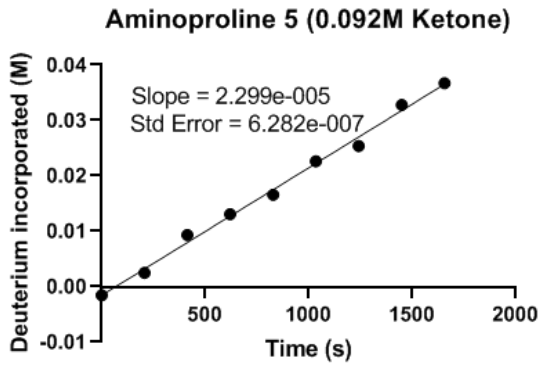


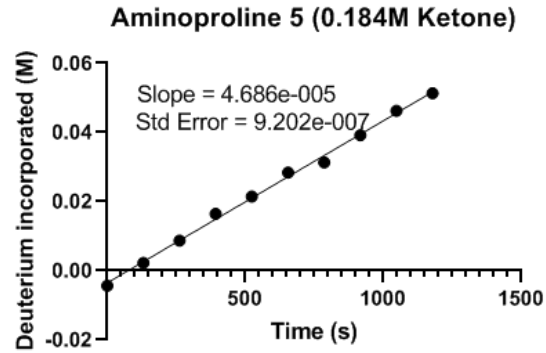


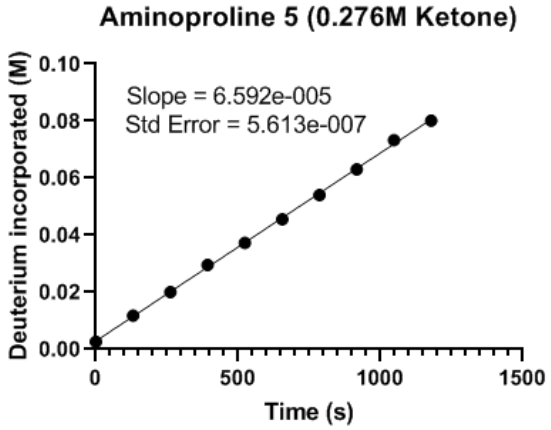


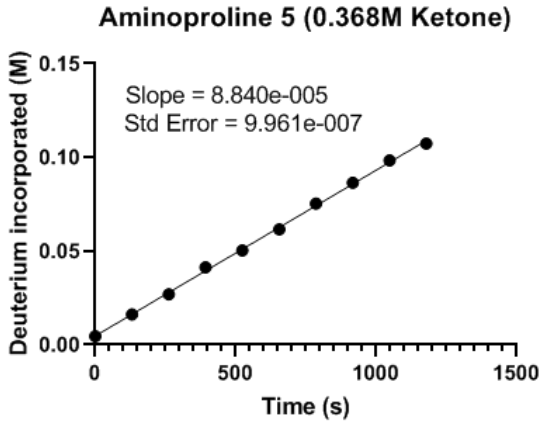


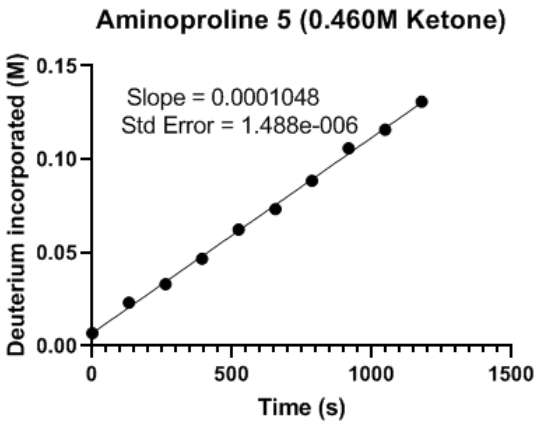
Aminoproline 5: Variation of concentration of ketone (0.15M Phosphate, pD 7.4, *I* = 0.9)

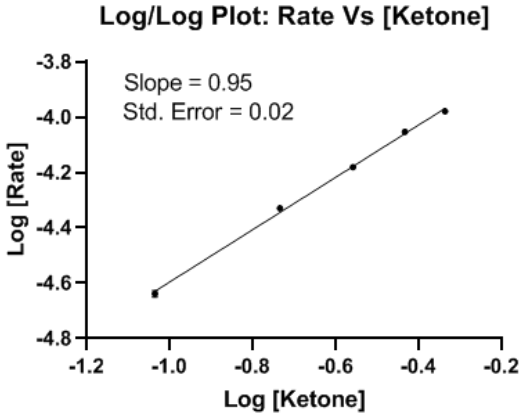




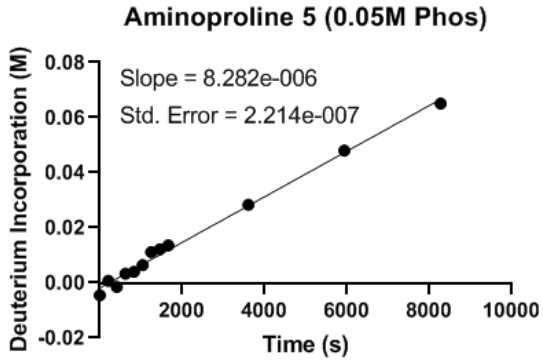


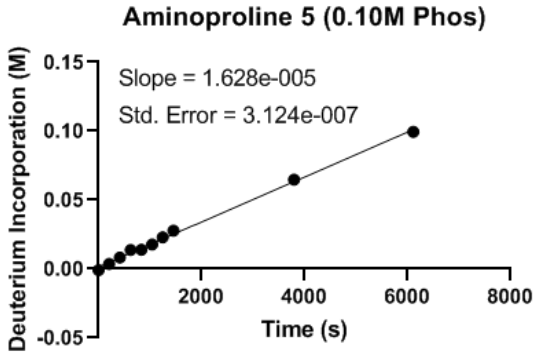


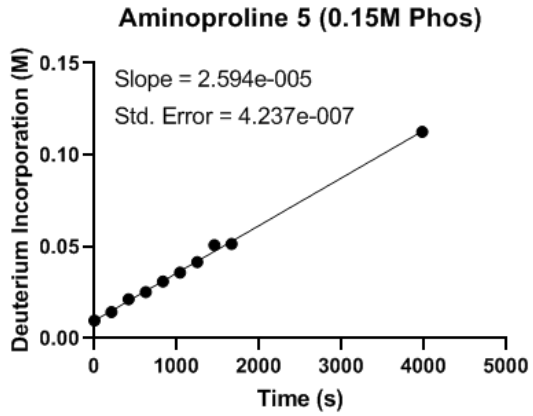


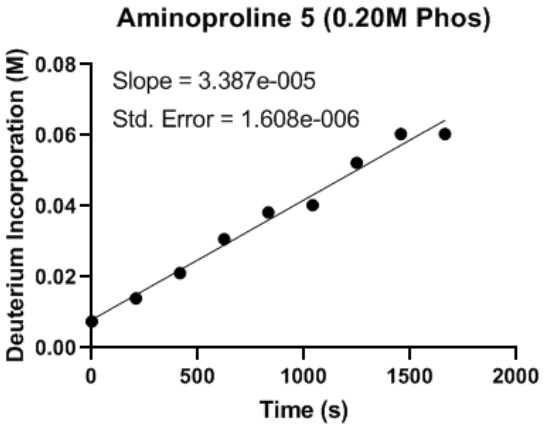


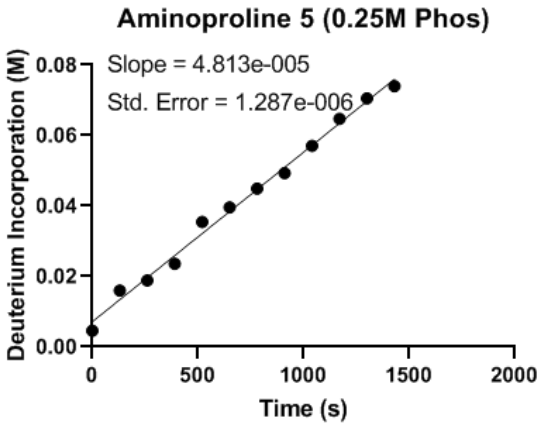
Aminoproline 5: Variation of concentration of phosphate (pD = 7.4, *I* = 3)

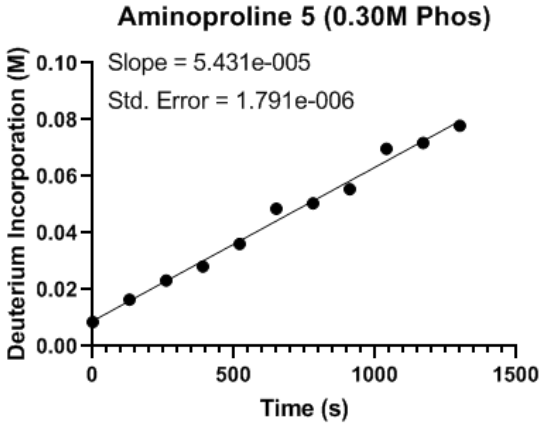


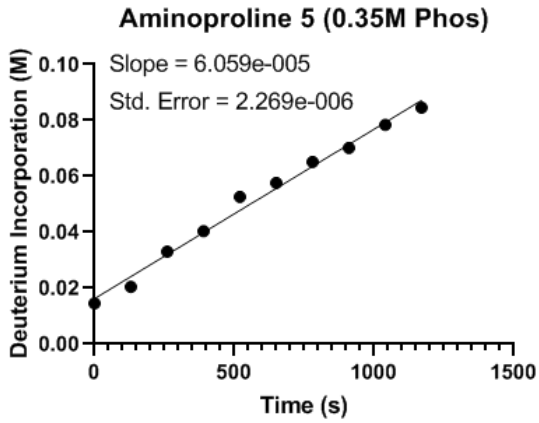


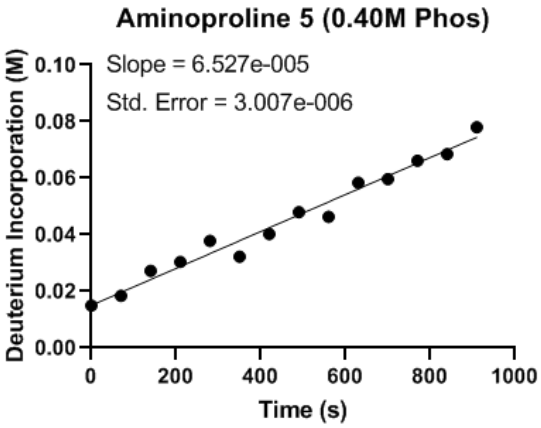


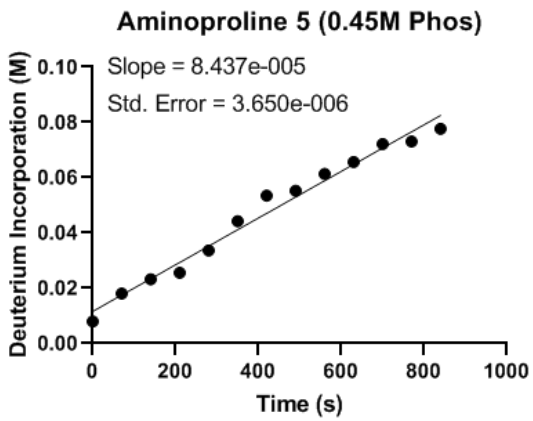


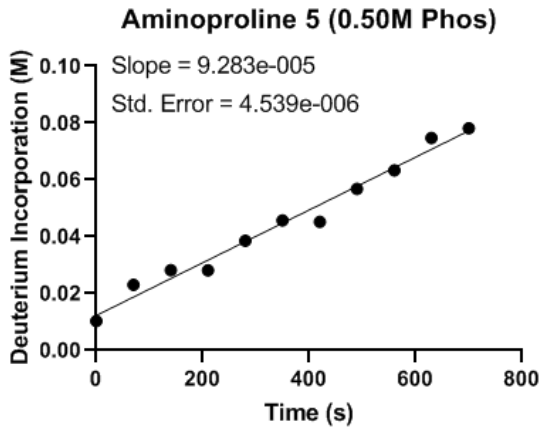


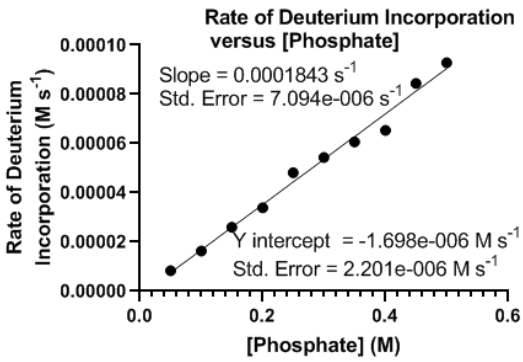




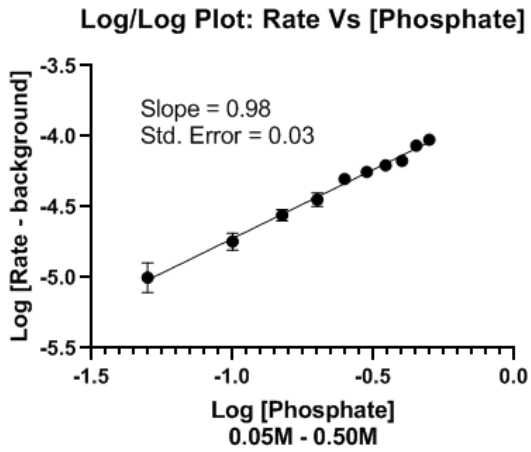




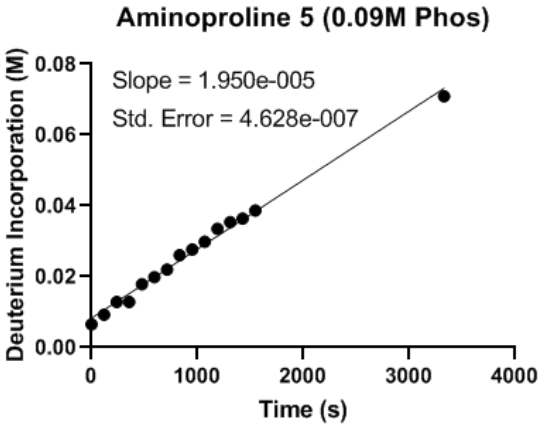


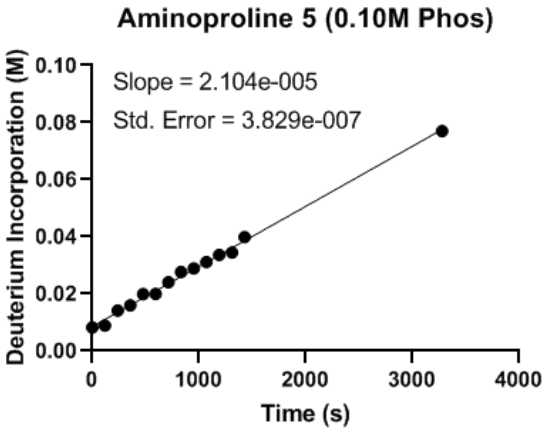


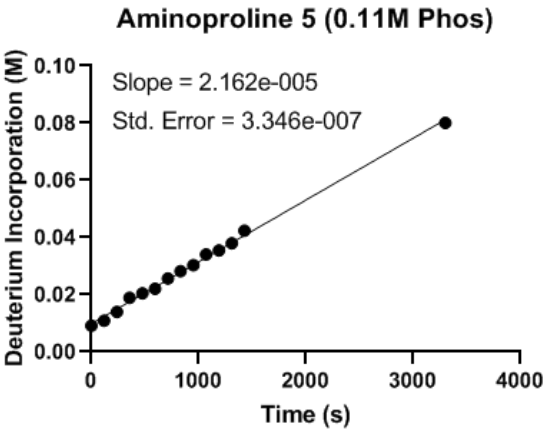
The Y Intercept of the above Rate versus [Phosphate] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of phosphate, a value of 0.98 is obtained (see below). The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [phosphate] plot.)

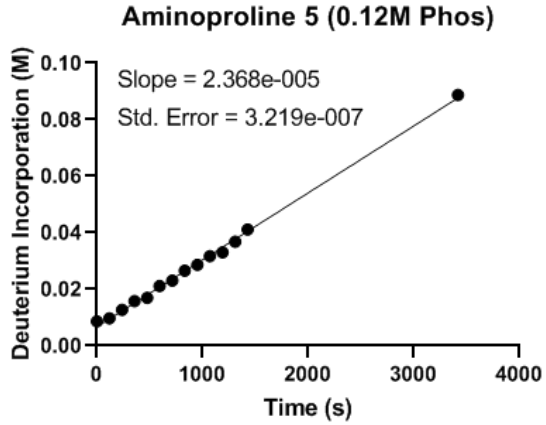


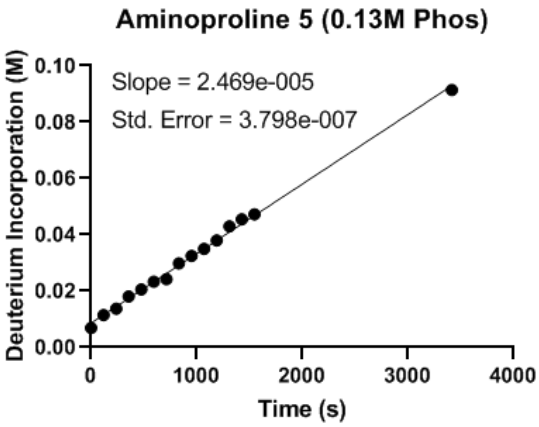
Aminoproline 5: Variation of concentration of phosphate (pD = 6.5, *I* = 0.9)

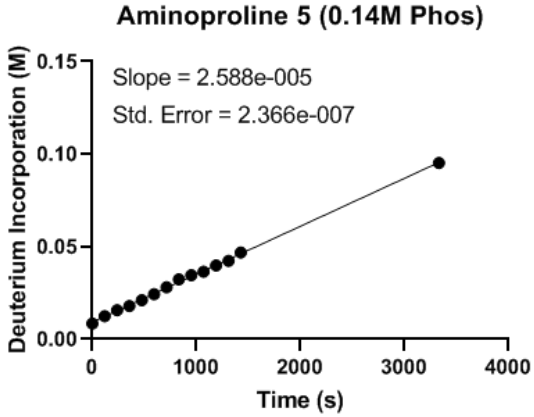


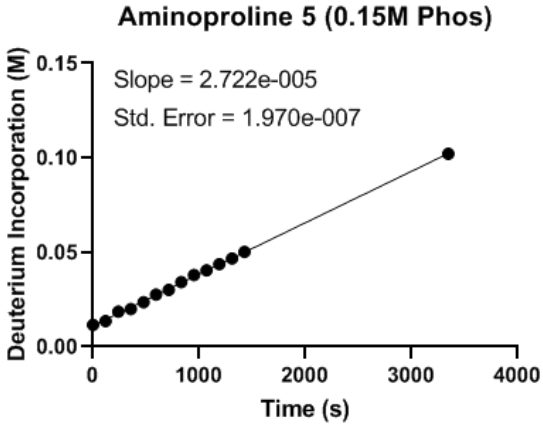


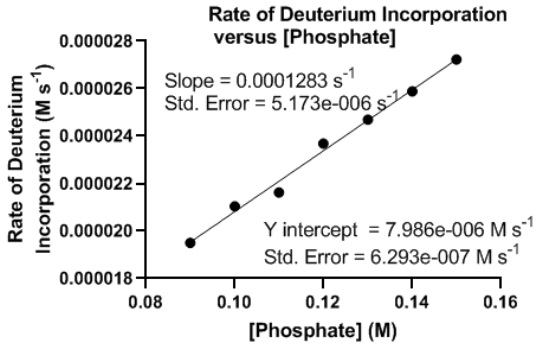






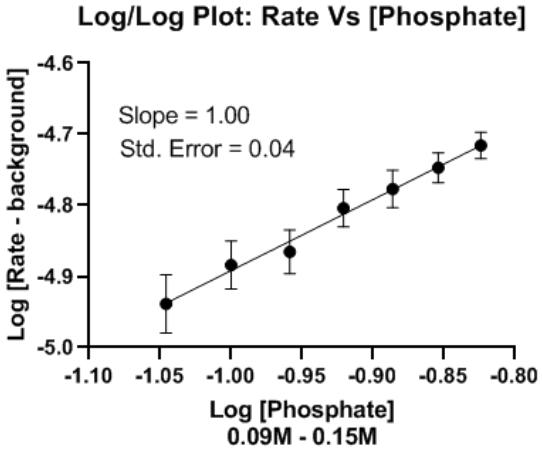




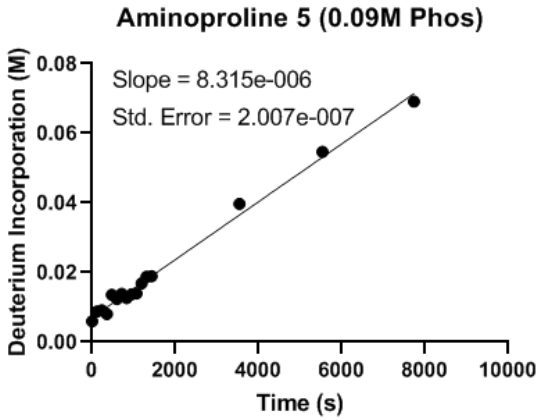


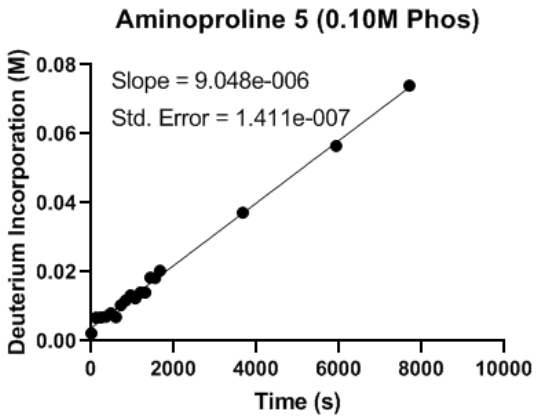
The Y Intercept of the above Rate versus [Phosphate] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent

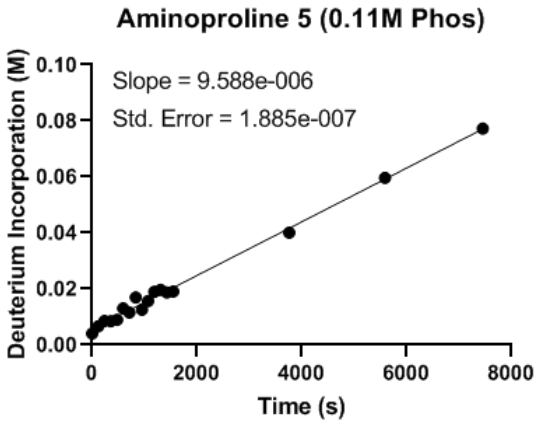
catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of phosphate, a value of 1.00 is obtained (see below). The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [phosphate] plot.)

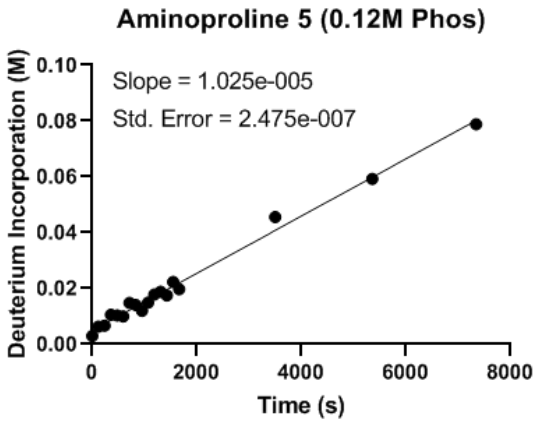


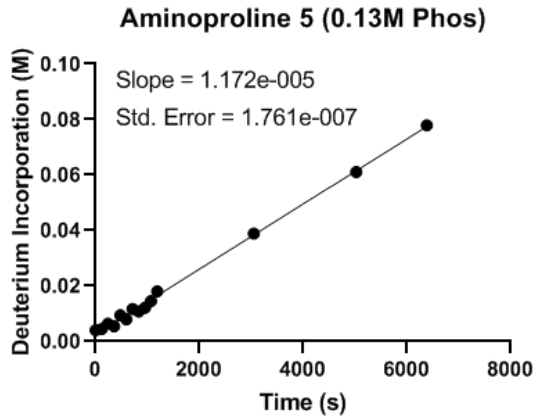
Aminoproline 5: Variation of concentration of phosphate (pD = 8.5, *I* = 0.9)

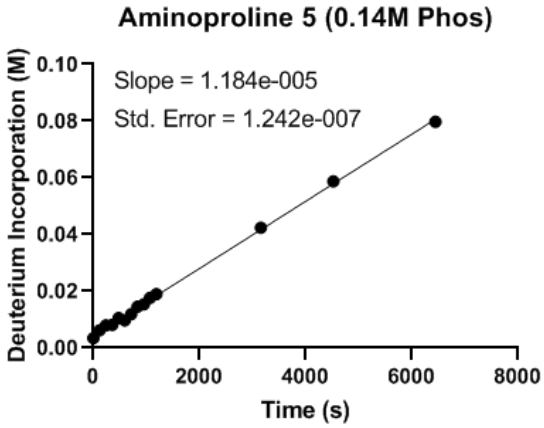


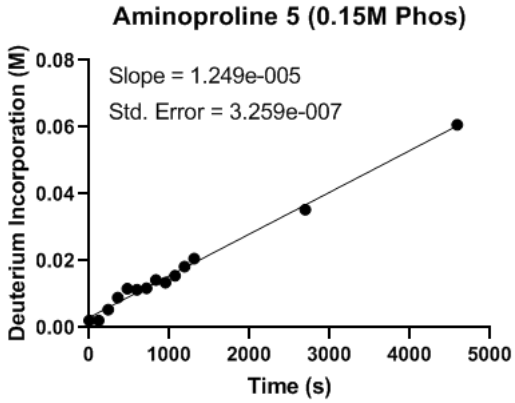


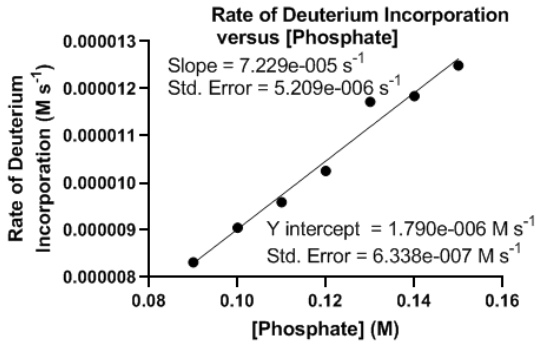




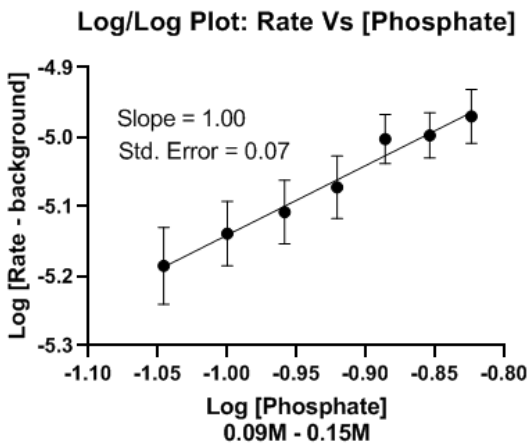




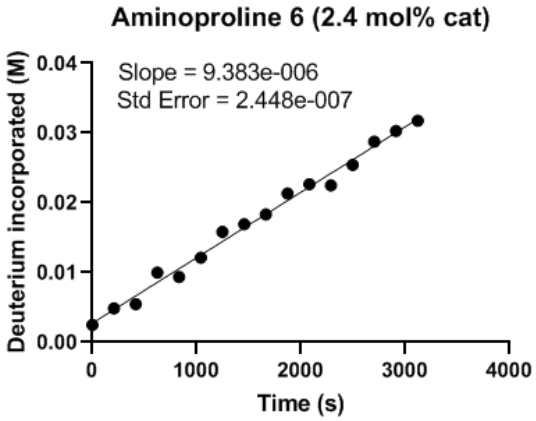


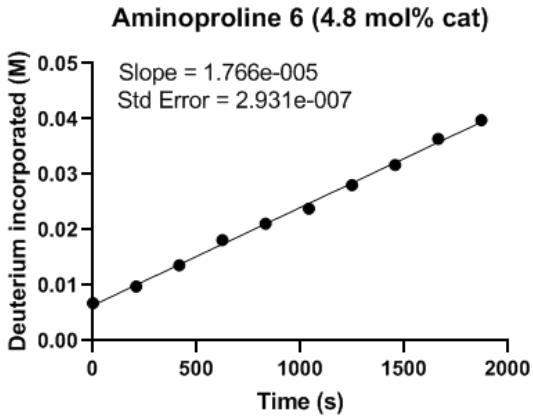


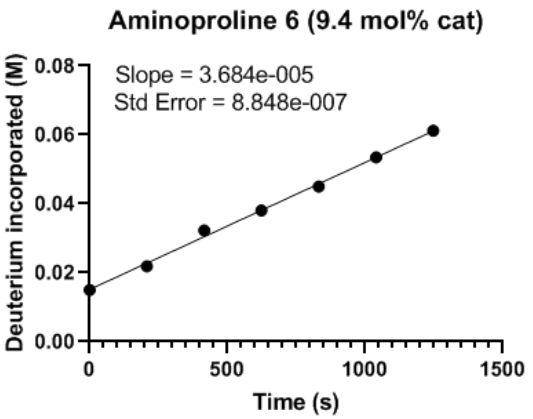
The Y Intercept of the above Rate versus [Phosphate] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of phosphate, a value of 1.00 is obtained (see below). The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [phosphate] plot.)

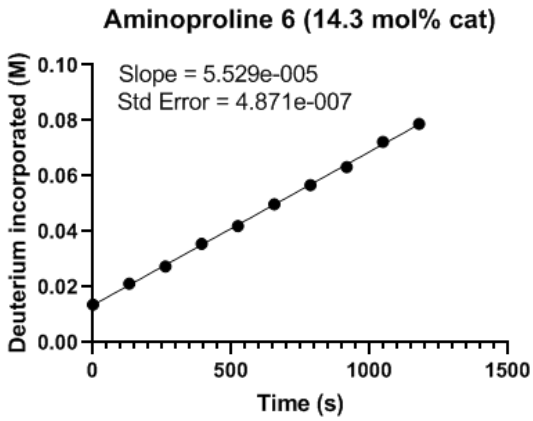


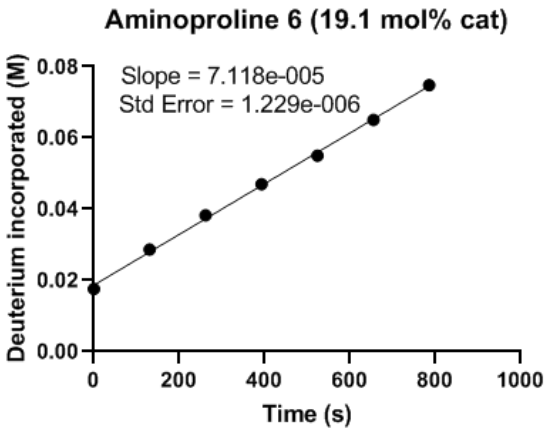
Aminoproline 6: Variation of concentration of catalyst (0.15M Phosphate, pD 7.4, *I* = 0.9)

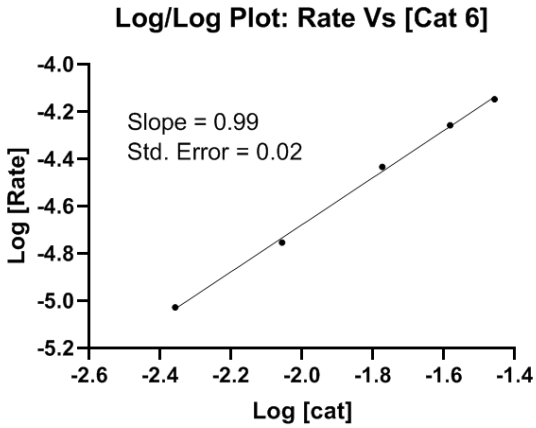




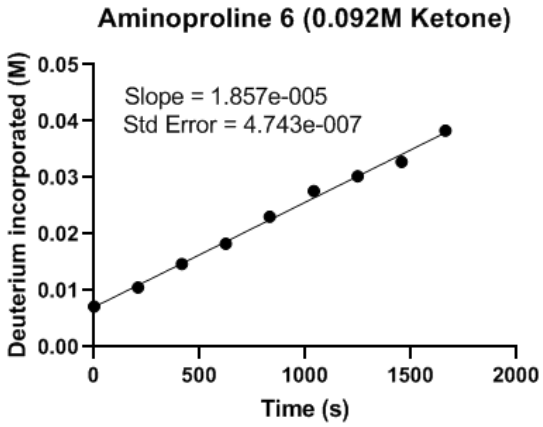


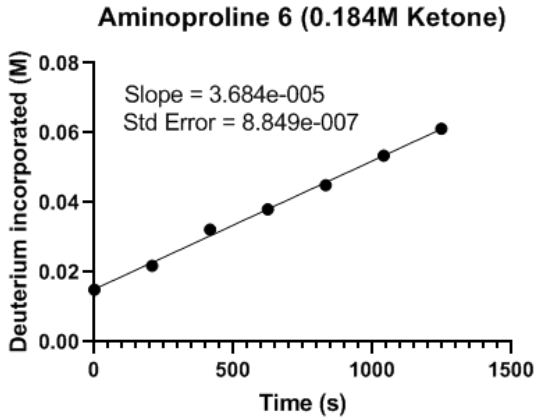


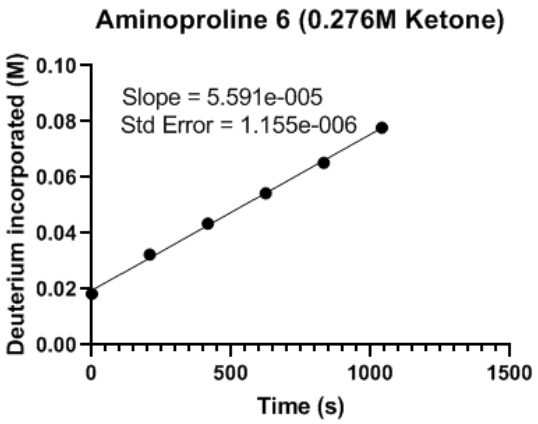


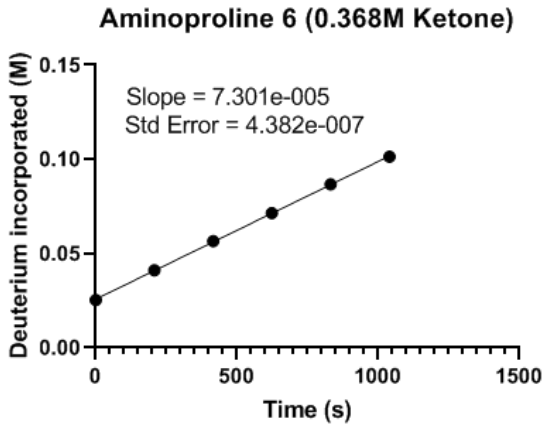


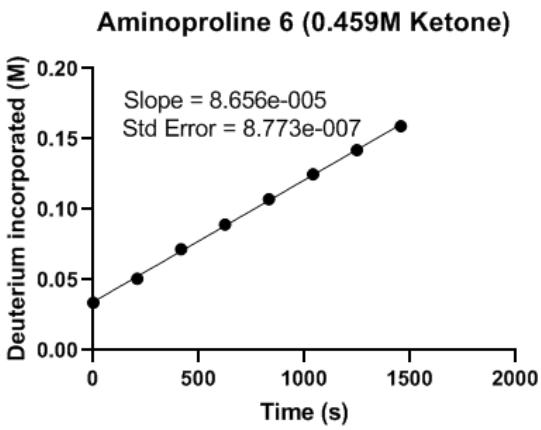
Aminoproline 6: Variation of concentration of ketone (0.15M Phosphate, pD 7.4, *I* = 0.9)

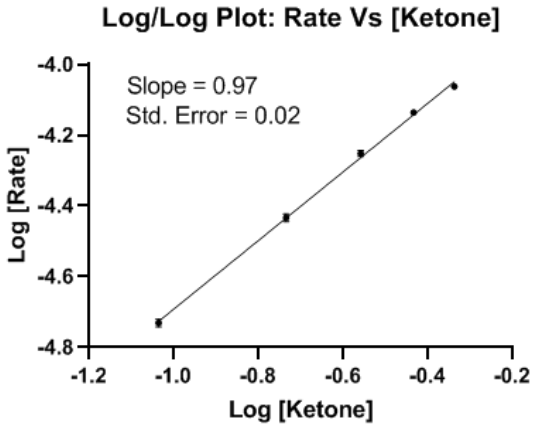




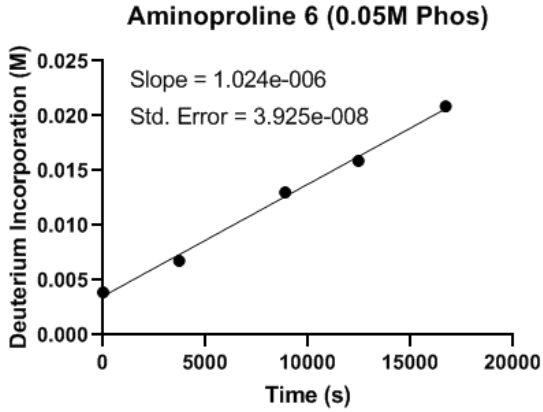


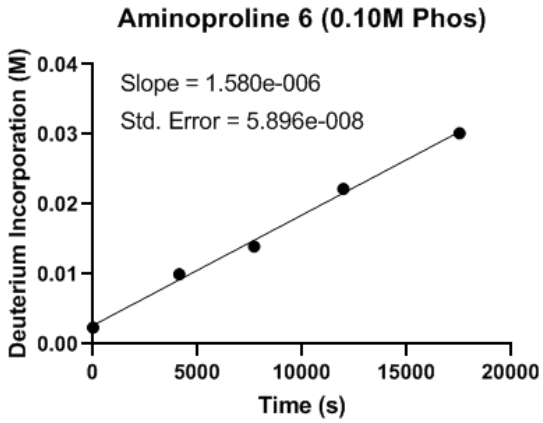


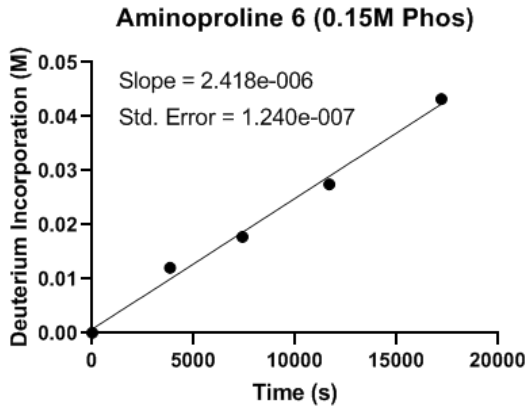


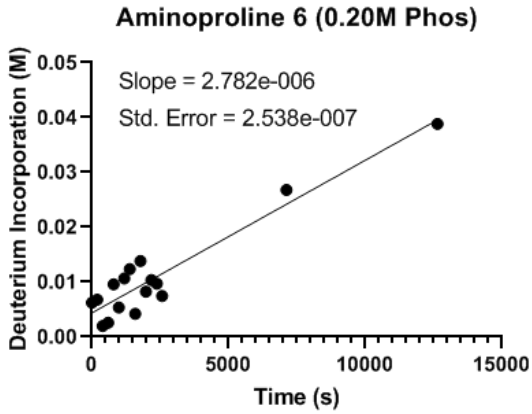


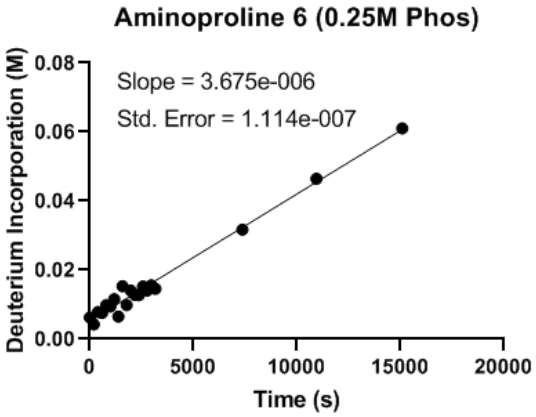
Aminoproline 6: Variation of concentration of phosphate (pD = 8.0, *I* = 3)

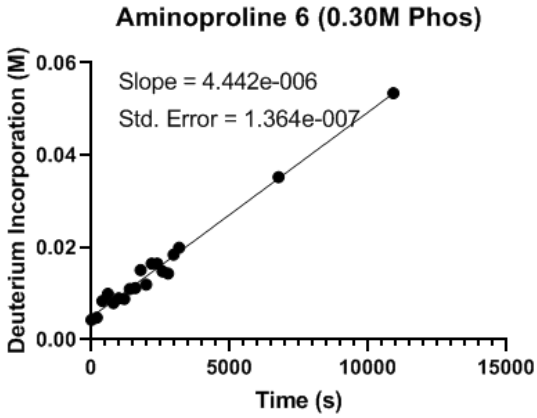


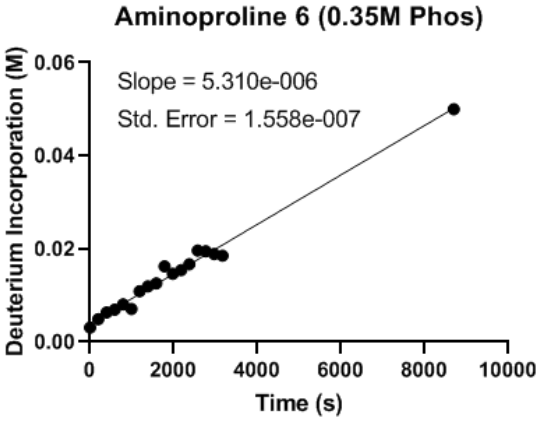


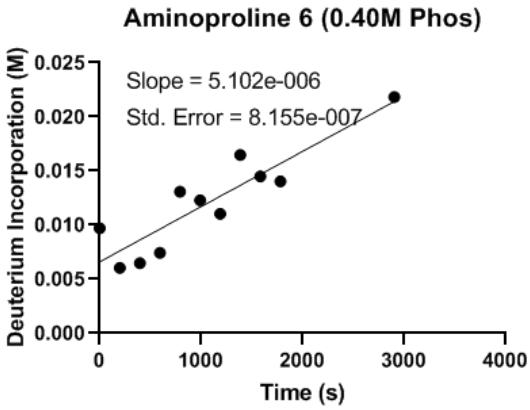


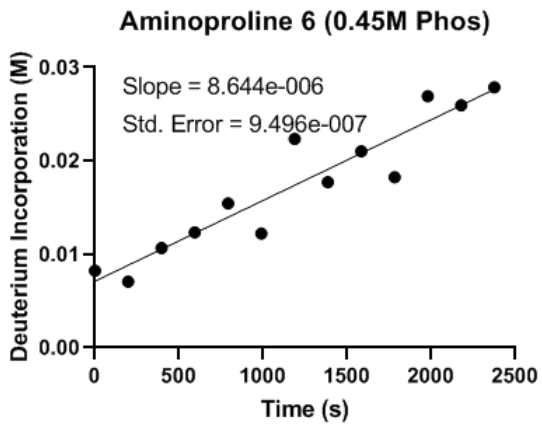


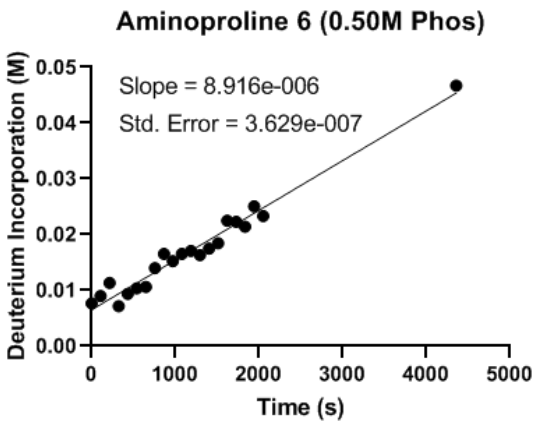


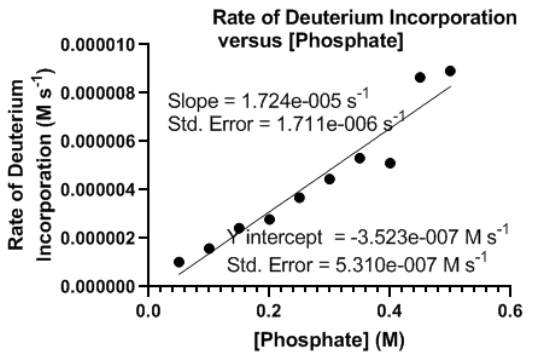




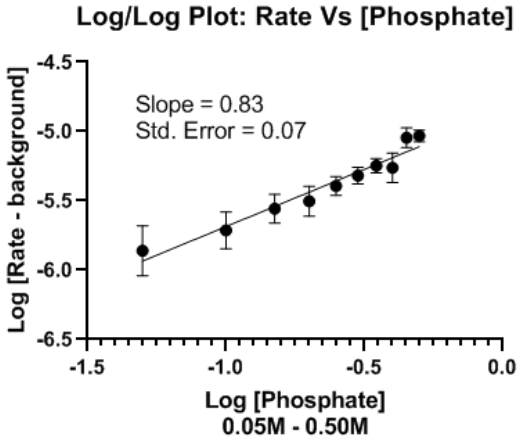




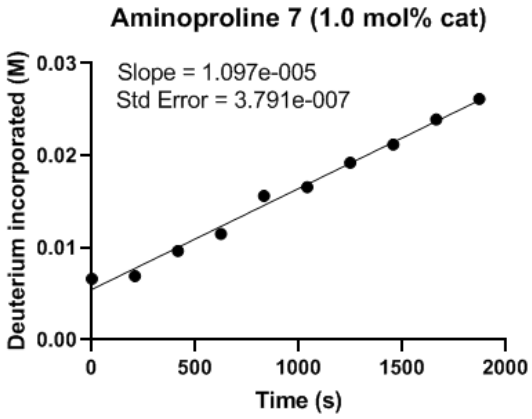


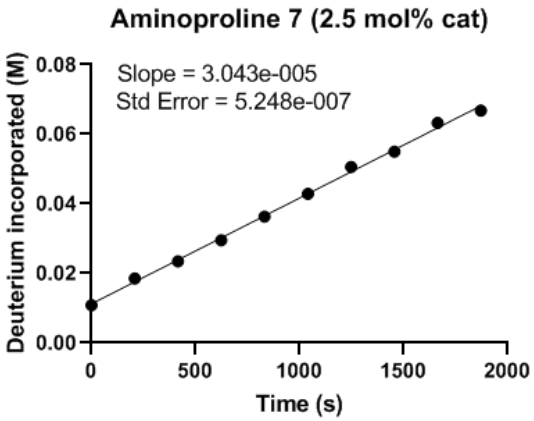


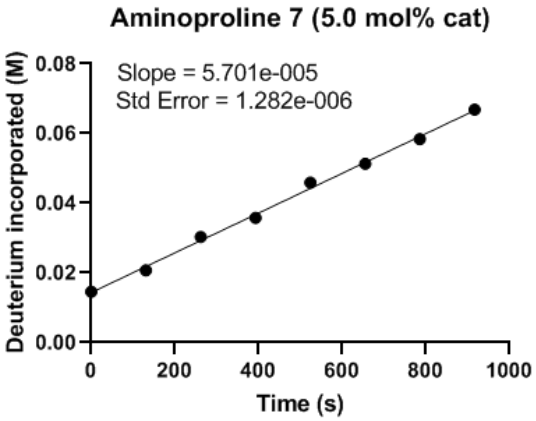
The Y Intercept of the above Rate versus [Phosphate] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of phosphate, a value of 0.83 is obtained (see below). If the first data point is excluded, the best-fit line has a slope of 0.95. The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [phosphate] plot.)

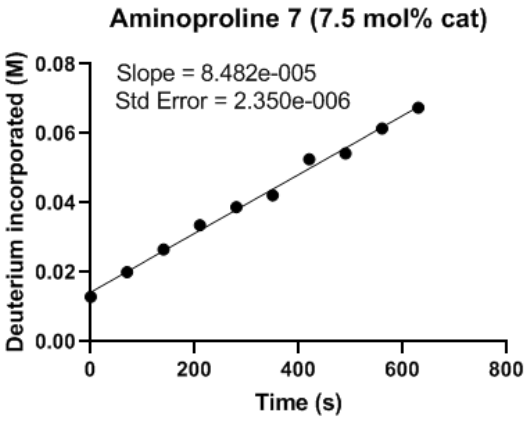


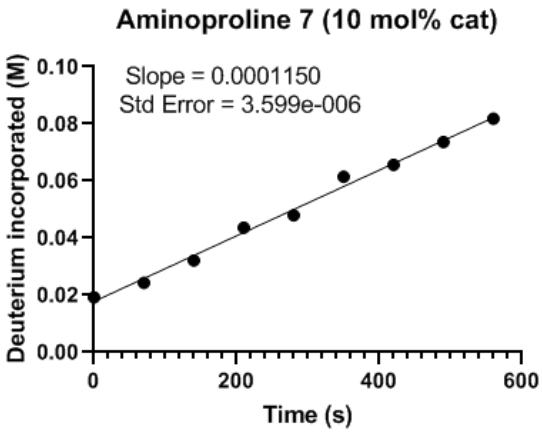
Aminoproline 7: Variation of concentration of catalyst (0.15M Phosphate, pD 7.4, *I* = 0.9)

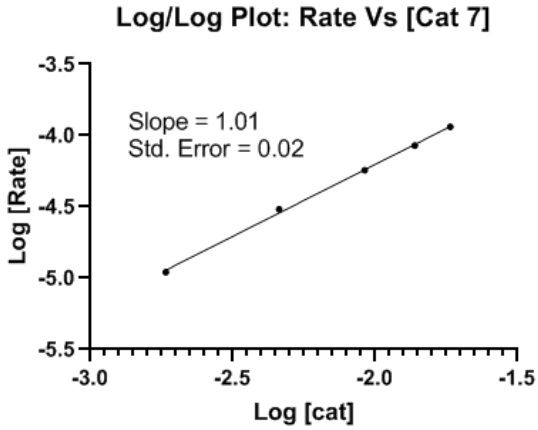




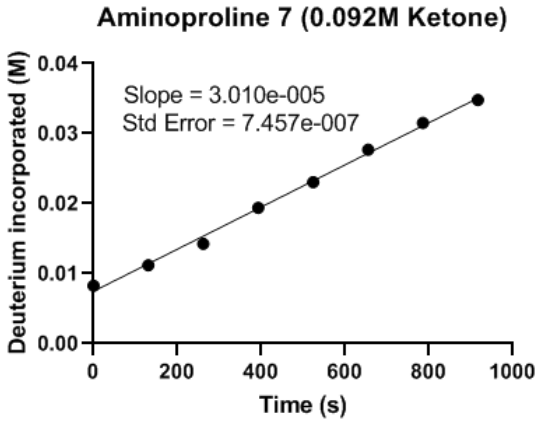


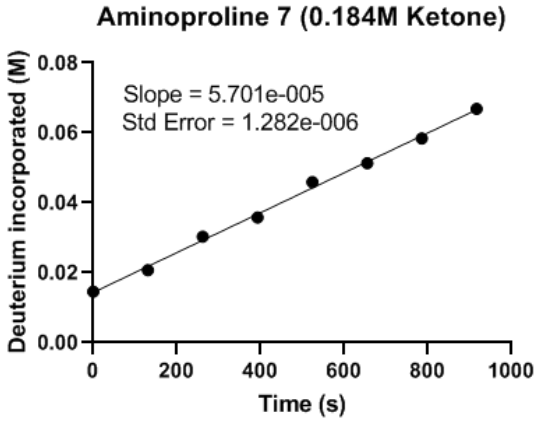


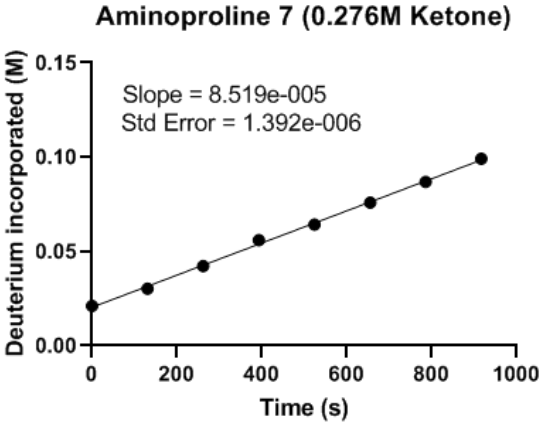


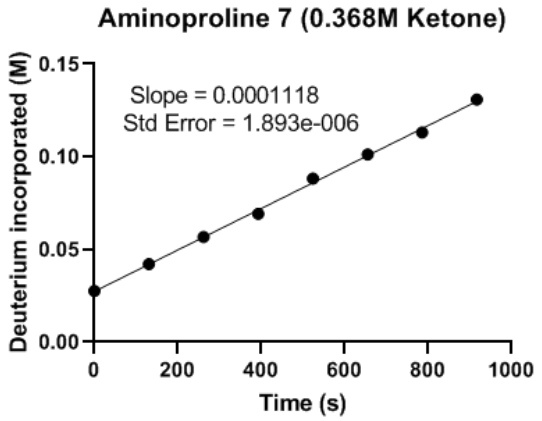


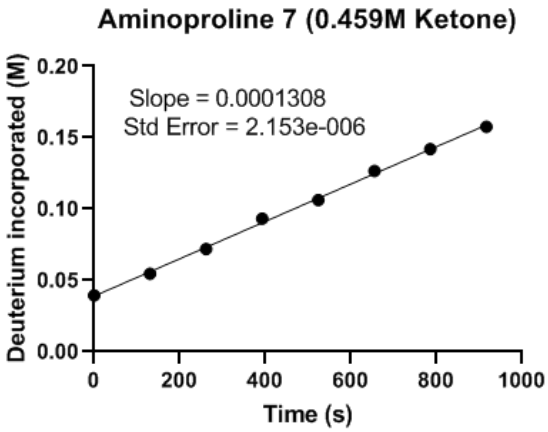
Aminoproline 7: Variation of concentration of ketone (0.15M Phosphate, pD 7.4, *I* = 0.9)

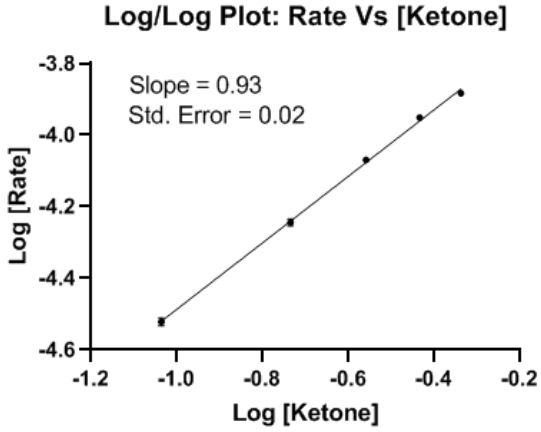




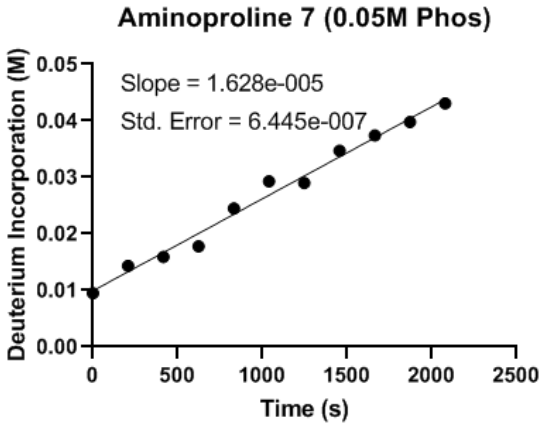


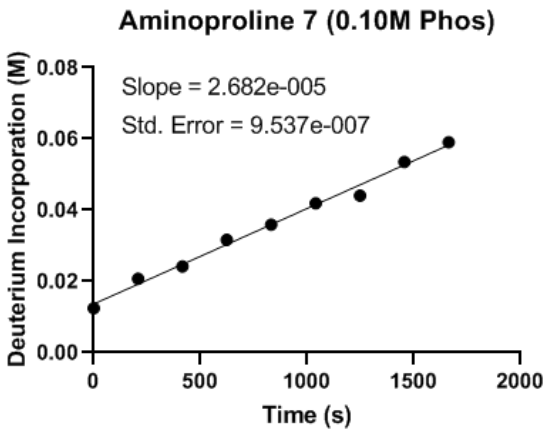


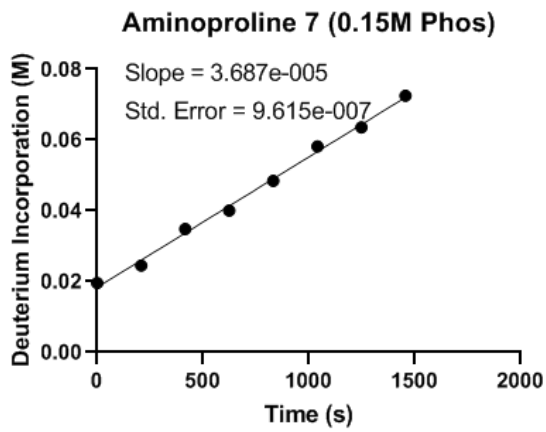


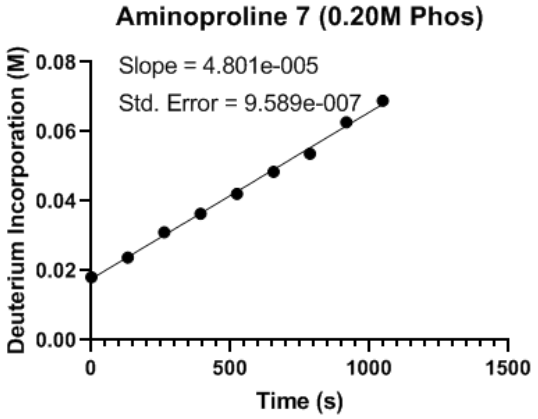


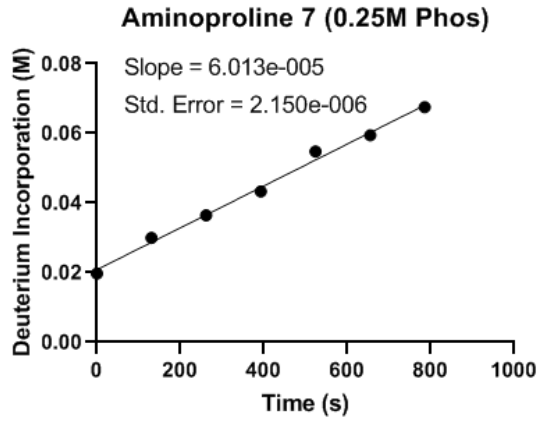
Aminoproline 7: Variation of concentration of phosphate (pD = 7.4, *I* = 3)

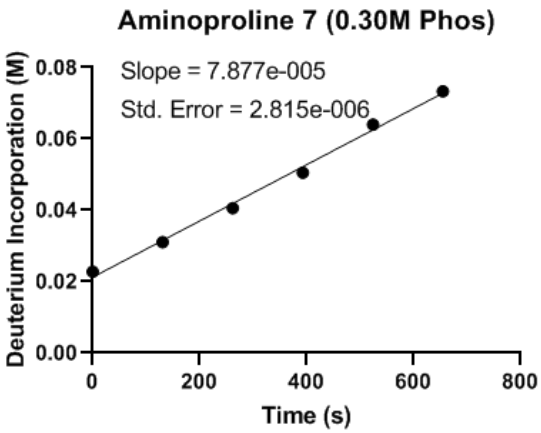


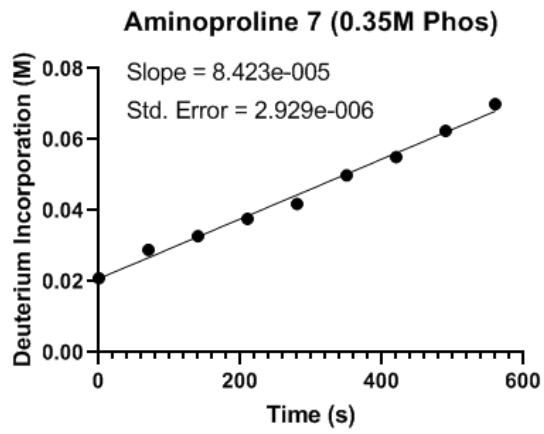


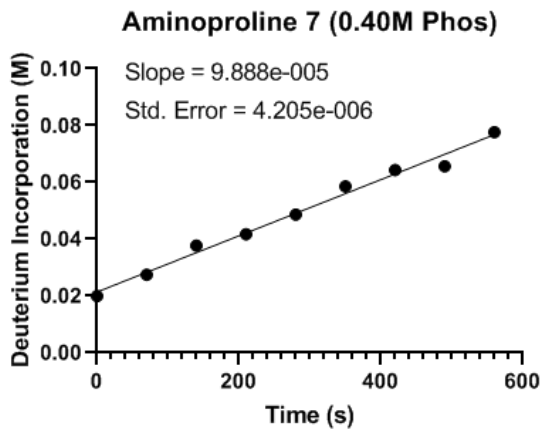


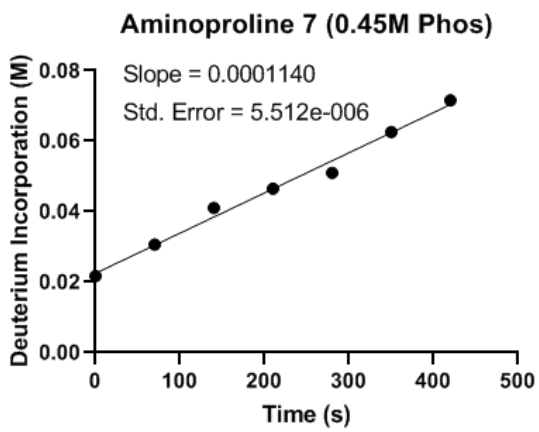


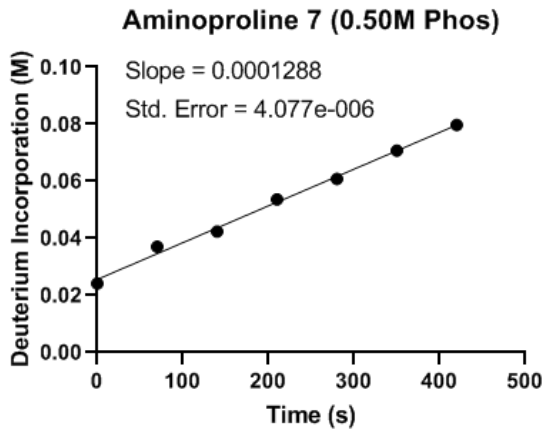


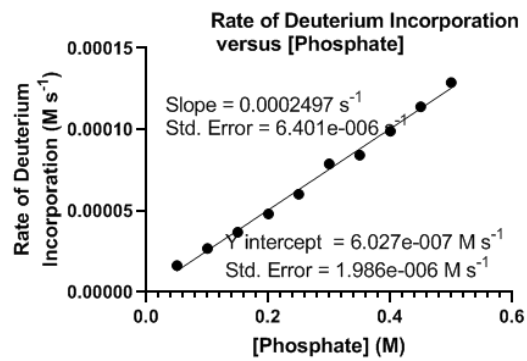




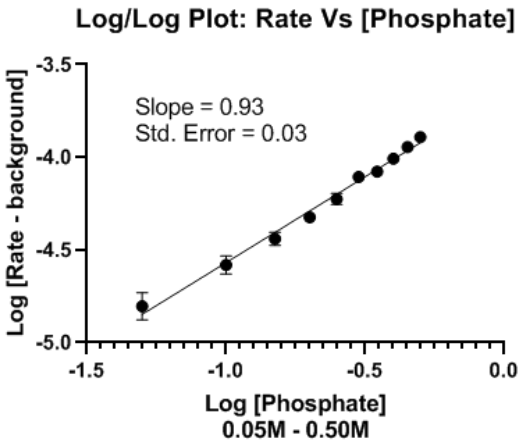








The Y Intercept of the above Rate versus [Phosphate] plot represents catalysis where the proline derivative acts as both the putative iminium/enamine covalent catalyst and the general acid/base (background). When the value corresponding to the Y intercept is subtracted from the values of the rate of deuterium incorporation at each concentration value and the log of the resulting values plotted against the log of the concentration of phosphate, a value of 0.93 is obtained (see below). If the first data point is excluded, the best-fit line has a slope of 1.00. The error bars in the log/log plot are 0.434*dy/y (y = rate of deuterium incorporation; dy = std. error obtained from plots of [deuterium] versus time + std. error of intercept in rate versus [phosphate] plot.)


Log/Log Plot: Rate Vs [Phosphate]
Slope = 0.93
Std. Error = 0.03
Log [Rate - background]
-3.5
-4.0
-4.5
-5.0
-1.5
-1.0
-0.5
0.0
Log [Phosphate]
0.05M - 0.50M

4 Substrate Isotope Effect Data

GC/MS data: **4,4-difluoroproline** (10 mol%), phosphate (150 mM, I = 0.9), D_2O/H_2O (1:1, pH-meter reading 6.0), Substrate = **[D_0]cyclohexanone**

Mass Ion	Integrations 0 min	20 min	40 min	60 min	80 min	100 min	120 min
97	14283	4994	7633	3992	1971	3901	2003
98	219875	76379	121170	64662	32642	63432	32864
99	15307	6276	12001	7225	4118	8972	5178
100	908	442	863	566	337	697	459
101	0	0	16	0	0	17	165
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0
M=97 species and higher MW isotopes of natural abundance							
97	14283	4994	7633	3992	1971	3901	2003
98	948	332	507	265	131	259	133
99	56	19	30	16	8	15	8
Subtraction of M=97 species and higher MW isotopes of natural abundance							
97	0	0	0	0	0	0	0
98	218927	76047	120663	64397	32511	63173	32731
99	15251	6257	11971	7209	4110	8957	5170
100	908	442	863	566	337	697	459
101	0	0	16	0	0	17	165
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0
M=98 species ([D_0]cyclohexanone) and higher MW isotopes of natural abundance							
97	0	0	0	0	0	0	0
98	**218927**	**76047**	**120663**	**64397**	**32511**	**63173**	**32731**
99	14537	5050	8012	4276	2159	4195	2173
100	854	297	471	251	127	246	128
101	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0
Subtraction M=98 species ([D_0]cyclohexanone) and higher MW isotopes of natural abundance							
97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	715	1207	3959	2933	1952	4762	2997
100	54	145	392	315	210	451	331

101	0	0	16	0	0	17	165
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

M=99 species ([D_1]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	**715**	**1207**	**3959**	**2933**	**1952**	**4762**	**2997**
100	47	80	263	195	130	316	199
101	0	0	1	1	1	1	1
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

Subtraction of M=99 species ([D_1]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0
100	**7**	**65**	**130**	**120**	**81**	**134**	**132**
101	0	0	15	-1	-1	16	164
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

Addition of the numbers in bold ([D_0]-, [D_1]-, and [D_2]cyclohexanone)

	219648	**77320**	**124752**	**67450**	**34543**	**68069**	**35860**

([D_0]-, [D_1]-, and [D_2]cyclohexanone) as a percentage of total

Species							
[D_0]	99.67161	98.35456	96.72252	95.47291	94.11696	92.80658	91.27387
[D_1]	0.32532	1.56102	3.17366	4.34908	5.64964	6.99594	8.35703
[D_2]	0.00307	0.08442	0.10382	0.17801	0.23339	0.19748	0.36910

Above values converted into moles of deuterium incorporated per liter

[D_0]	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
[D_1]	0.00060	0.00287	0.00584	0.00800	0.01040	0.01287	0.01538
[D_2]	0.00001	0.00031	0.00038	0.00066	0.00086	0.00073	0.00136
[D_1]+ [D_2]	0.00061	0.00318	0.00622	0.00866	0.01125	0.01360	0.01674

GC/MS data: **4,4-difluoroproline** (10 mol%), phosphate (150 mM, I = 0.9), D_2O/H_2O (1:1, pH-meter reading 6.0), Substrate = **[D_4]cyclohexanone**

Mass Ion	Integrations 0 min	20 min	40 min	60 min	80 min	100 min	120 min
97	0	0	0	0	0	0	0
98	346	0	0	0	0	0	0
99	298	0	21	0	10	0	130
100	2959	671	757	260	632	558	1304
101	23865	5031	6001	2156	4781	4069	8906
102	150423	28532	31588	10619	21422	17067	35359
103	10357	1902	2057	684	1385	1136	2337
104	1017	117	141	16	85	59	171

M=99 species and higher MW isotopes of natural abundance

99	298	0	21	0	10	0	130
100	20	0	1	0	1	0	9
101	1	0	0	0	0	0	1

Subtraction of M=98 integrations, and M=99 species with its higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0
100	2939	671	756	260	631	558	1295
101	23864	5031	6001	2156	4781	4069	8905
102	150423	28532	31588	10619	21422	17067	35359
103	10357	1902	2057	684	1385	1136	2337
104	1017	117	141	16	85	59	171

M=100 species ([D_2]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							
99							
100	**2939**	**671**	**756**	**260**	**631**	**558**	**1295**
101	195	45	50	17	42	37	86
102	11	3	3	1	2	2	5
103							
104							

Subtraction M=100 species ([D_2]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0
101	23669	4986	5951	2139	4739	4032	8819
102	150412	28529	31585	10618	21420	17065	35354
103	10357	1902	2057	684	1385	1136	2337

104	1017	117	141	16	85	59	171

M=101 species ([D_3]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							
99							
100							
101	**23669**	**4986**	**5951**	**2139**	**4739**	**4032**	**8819**
102	1572	331	395	142	315	268	586
103	92	19	23	8	18	16	34
104							

Subtraction of M=101 species ([D_3]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0
102	**148840**	**28198**	**31190**	**10476**	**21105**	**16797**	**34768**
103	10265	1883	2034	676	1367	1120	2303
104	1017	117	141	16	85	59	171

Addition of the numbers in bold ([D_4]-, [D_3]-, and [D_2]cyclohexanone)

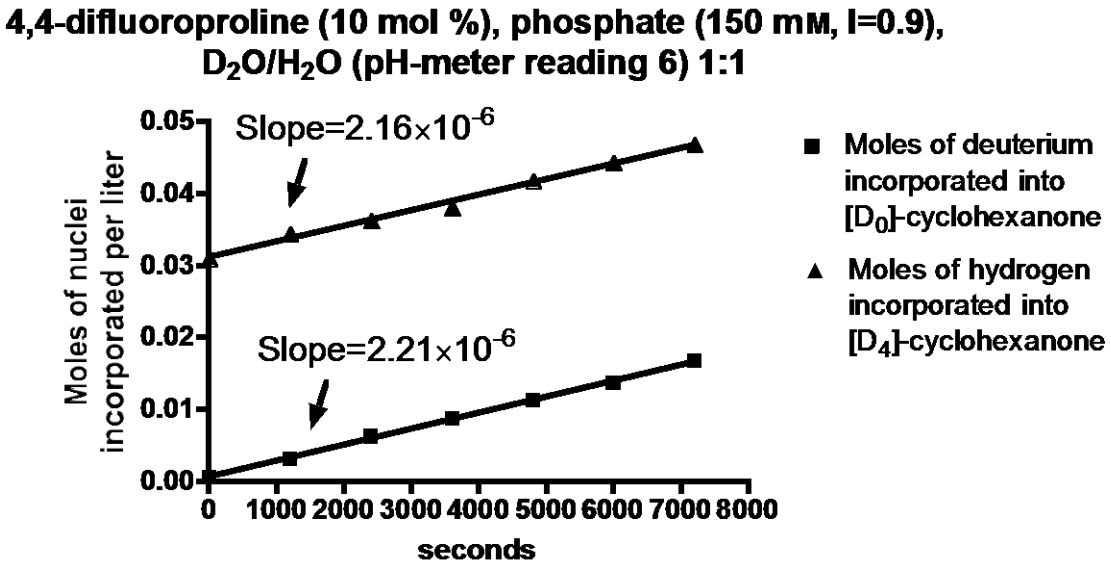
	175448	**33856**	**37896**	**12875**	**26475**	**21387**	**44883**

([D_4]-, [D_3]-, and [D_2]cyclohexanone) as a percentage of total

Species							
[D_4]	84.83430	83.28955	82.30340	81.36862	79.71548	78.53866	77.46406
[D_3]	13.49043	14.72851	15.70272	16.61192	17.89989	18.85229	19.64986
[D_2]	1.67526	1.98194	1.99388	2.01946	2.38463	2.60906	2.88609

Above values converted into moles of hydrogen incorporated per liter

[D_4]	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
[D_3]	0.02482	0.02710	0.02889	0.03057	0.03294	0.03469	0.03616
[D_2]	0.00616	0.00729	0.00734	0.00743	0.00878	0.00960	0.01062
[D_3]+ [D_2]	0.03099	0.03439	0.03623	0.03800	0.04171	0.04429	0.04678



GC/MS data: **4,4-difluoroproline** (10 mol%), phosphate (150 mM, I = 0.9), D_2O/H_2O (1:1, pH-meter reading 8.0), Substrate = **[D_0]cyclohexanone**

Mass Ion	Integrations 0 min	20 min	40 min	60 min	80 min	100 min	120 min
97	14283	4073	3538	2479	150	2578	2552
98	219875	65510	57742	40937	2476	42377	41249
99	15307	5802	6239	5122	338	7023	7725
100	908	400	476	409	0	641	750
101	0	0	0	0	0	12	52
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0
M=97 species and higher MW isotopes of natural abundance							
97	14283	4073	3538	2479	150	2578	2552
98	948	270	235	165	10	171	169
99	56	16	14	10	1	10	10
Subtraction of M=97 species and higher MW isotopes of natural abundance							
97	0	0	0	0	0	0	0
98	218927	65240	57507	40772	2466	42206	41080
99	15251	5786	6225	5112	337	7013	7715
100	908	400	476	409	0	641	750
101	0	0	0	0	0	12	52
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

M=98 species ([D_0]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	**218927**	**65240**	**57507**	**40772**	**2466**	**42206**	**41080**
99	14537	4332	3818	2707	164	2802	2728
100	854	254	224	159	10	165	160
101	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

Subtraction M=98 species ([D_0]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	715	1454	2407	2405	174	4210	4987
100	54	146	252	250	-10	476	590
101	0	0	0	0	0	12	52
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

M=99 species ([D_1]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	**715**	**1454**	**2407**	**2405**	**174**	**4210**	**4987**
100	47	97	160	160	12	280	331
101	0	0	1	1	0	1	1
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

Subtraction of M=99 species ([D_1]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0
100	**7**	**49**	**92**	**90**	**-21**	**197**	**259**
101	0	0	-1	-1	0	11	51
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

Addition of the numbers in bold ([D_0]-, [D_1]-, and [D_2]cyclohexanone)

219648	**66743**	**60006**	**43268**	**2619**	**46613**	**46326**

([D_0]-, [D_1]-, and [D_2]cyclohexanone) as a percentage of total

Species

[D_0]	99.67161	97.74775	95.83599	94.23280	94.17540	90.54494	88.67581
[D_1]	0.32532	2.17883	4.01084	5.55852	6.63227	9.03282	10.76591
[D_2]	0.00307	0.07343	0.15318	0.20868	-0.80767	0.42224	0.55829

Above values converted into moles of deuterium incorporated per liter

[D_0]	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
[D_1]	0.00060	0.00401	0.00738	0.01023	0.01220	0.01662	0.01981
[D_2]	0.00001	0.00027	0.00056	0.00077	-0.00297	0.00155	0.00205
[D_1]+ [D_2]	0.00061	0.00428	0.00794	0.01100	0.00923	0.01817	0.02186

GC/MS data: **4,4-difluoroproline** (10 mol%), phosphate (150 mM, I = 0.9), D_2O/H_2O (1:1, pH-meter reading 8.0), Substrate = **[D_4]cyclohexanone**

Mass Ion	Integrations 0 min	20 min	40 min	60 min	80 min	100 min	120 min
97	0	0	0	0	0	0	0
98	346	25	0	10	0	0	0
99	298	93	135	153	185	242	53
100	2959	1281	1507	1447	1767	2272	760
101	23865	10157	11658	10641	12556	15852	5097
102	150423	56779	58336	47362	51739	58915	17544
103	10357	3842	3937	3190	3463	4004	1151
104	1017	312	326	281	270	333	86

M=99 species and higher MW isotopes of natural abundance

99	298	93	135	153	185	242	53
100	20	6	9	10	12	16	4
101	1	0	1	1	1	1	0

Subtraction of M=98 integrations, and M=99 species with its higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	10	0	0	0
99	0	0	0	0	0	0	0
100	2939	1275	1498	1437	1755	2256	756
101	23864	10157	11657	10640	12555	15851	5097
102	150423	56779	58336	47362	51739	58915	17544
103	10357	3842	3937	3190	3463	4004	1151
104	1017	312	326	281	270	333	86

M=100 species ([D_2]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							
99							
100	**2939**	**1275**	**1498**	**1437**	**1755**	**2256**	**756**
101	195	85	99	95	117	150	50

102	11	5	6	6	7	9	3
103							
104							

Subtraction M=100 species ([D_2]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	10	0	0	0
99	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0
101	23669	10072	11558	10545	12439	15701	5047
102	150412	56774	58330	47356	51732	58906	17541
103	10357	3842	3937	3190	3463	4004	1151
104	1017	312	326	281	270	333	86

M=101 species ([D_3]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							
99							
100							
101	**23669**	**10072**	**11558**	**10545**	**12439**	**15701**	**5047**
102	1572	669	767	700	826	1043	335
103	92	39	45	41	49	61	20
104							

Subtraction of M=101 species ([D_3]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	10	0	0	0
99	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0
102	**148840**	**56105**	**57563**	**46656**	**50906**	**57864**	**17206**
103	10265	3803	3892	3149	3414	3943	1131
104	1017	312	326	281	270	333	86

Addition of the numbers in bold ([D_4]-, [D_3]-, and [D_2]cyclohexanone)

175448	**67452**	**70619**	**58638**	**65100**	**75821**	**23009**

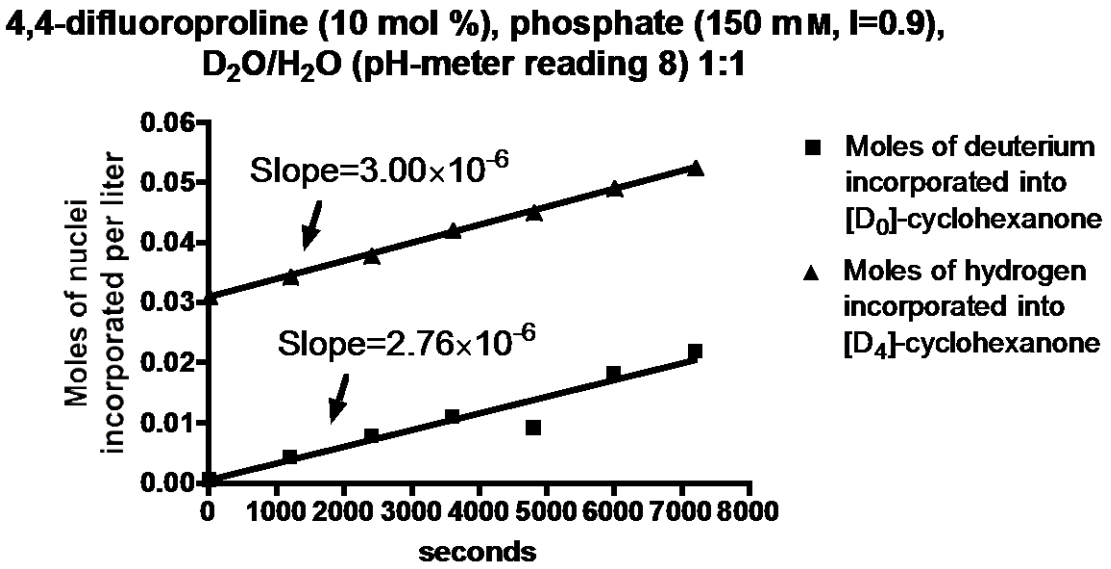
([D_4]-, [D_3]-, and [D_2]cyclohexanone) as a percentage of total

Species							
[D_4]	84.83430	83.17796	81.51193	79.56644	78.19732	76.31628	74.77925
[D_3]	13.49043	14.93207	16.36676	17.98320	19.10725	20.70838	21.93299
[D_2]	1.67526	1.88997	2.12130	2.45036	2.69543	2.97534	3.28776

Above values converted into moles of hydrogen incorporated per liter

[D_4]	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
[D_3]	0.02482	0.02748	0.03011	0.03309	0.03516	0.03810	0.04036
[D_2]	0.00616	0.00696	0.00781	0.00902	0.00992	0.01095	0.01210

$[D_3]+[D_2]$	0.03099	0.03443	0.03792	0.04211	0.04508	0.04905	0.05246



GC/MS data: ***trans*-4-trimethylammoniumproline** (5 mol%), phosphate (150 mM, I = 0.9), D_2O/H_2O (1:1, pH-meter reading 6.0), Substrate = **$[D_0]$cyclohexanone**

Mass Ion	Integrations 0 min	20 min	40 min	60 min	80 min	100 min	120 min
97	14283	5412	7105	7703	5918	5242	5811
98	219875	82334	110703	122411	93428	84424	92934
99	15307	7974	13682	18714	16995	17775	22249
100	908	570	1100	1602	1612	1866	2535
101	0	33	68	92	106	124	217
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0
M=97 species and higher MW isotopes of natural abundance							
97	14283	5412	7105	7703	5918	5242	5811
98	948	359	472	511	393	348	386
99	56	21	28	30	23	20	23
Subtraction of M=97 species and higher MW isotopes of natural abundance							
97	0	0	0	0	0	0	0
98	218927	81975	110231	121900	93035	84076	92548
99	15251	7953	13654	18684	16972	17755	22226
100	908	570	1100	1602	1612	1866	2535
101	0	33	68	92	106	124	217
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0

104	0	0	0	0	0	0	0

M=98 species ([D_0]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	**218927**	**81975**	**110231**	**121900**	**93035**	**84076**	**92548**
99	14537	5443	7319	8094	6178	5583	6145
100	854	320	430	475	363	328	361
101	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

Subtraction M=98 species ([D_0]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	715	2510	6335	10590	10794	12172	16081
100	54	250	670	1127	1249	1538	2174
101	0	33	68	92	106	124	217
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

M=99 species ([D_1]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	**715**	**2510**	**6335**	**10590**	**10794**	**12172**	**16081**
100	47	167	421	703	717	808	1068
101	0	1	2	3	3	3	4
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

Subtraction of M=99 species ([D_1]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0
100	**7**	**84**	**249**	**423**	**532**	**730**	**1106**
101	0	32	66	89	103	121	213
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

Addition of the numbers in bold ([D_0]-, [D_1]-, and [D_2]cyclohexanone)

219648	**84568**	**116816**	**132913**	**104362**	**96978**	**109736**

([D_0]-, [D_1]-, and [D_2]cyclohexanone) as a percentage of total

Species							
[D_0]	99.67161	96.93333	94.36343	91.71392	89.14660	86.69612	84.33743
[D_1]	0.32532	2.96776	5.42302	7.96750	10.34324	12.55125	14.65445
[D_2]	0.00307	0.09891	0.21355	0.31858	0.51016	0.75264	1.00813

Above values converted into moles of deuterium incorporated per liter

[D_0]	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
[D_1]	0.00060	0.00546	0.00998	0.01466	0.01903	0.02309	0.02696
[D_2]	0.00001	0.00036	0.00079	0.00117	0.00188	0.00277	0.00371
[D_1]+ [D_2]	0.00061	0.00582	0.01076	0.01583	0.02091	0.02586	0.03067

GC/MS data: ***trans*-4-trimethylammoniumproline** (5 mol%), phosphate (150 mM, I = 0.9), D_2O/H_2O (1:1, pH-meter reading 6.0), Substrate = **[D_4]cyclohexanone**

Mass Ion	Integrations 0 min	20 min	40 min	60 min	80 min	100 min	120 min
97	0	16	0	0	0	0	0
98	346	51	12	14	13	0	16
99	298	199	123	280	233	358	284
100	2959	2076	1514	2743	2476	3325	2697
101	23865	15998	11085	19229	16484	20758	16363
102	150423	85321	50764	78418	59169	67980	48743
103	10357	5781	3379	5252	4048	4565	3269
104	1017	437	264	451	315	371	227

M=99 species and higher MW isotopes of natural abundance

99	298	199	123	280	233	358	284
100	20	13	8	19	15	24	19
101	1	1	0	1	1	1	1

Subtraction of M=98 integrations, and M=99 species with its higher MW isotopes of natural abundance

97							
98							
99							
100	2939	2063	1506	2724	2461	3301	2678
101	23864	15997	11085	19228	16483	20757	16362
102	150423	85321	50764	78418	59169	67980	48743
103	10357	5781	3379	5252	4048	4565	3269
104	1017	437	264	451	315	371	227

M=100 species ([D_2]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							
99							
100	**2939**	**2063**	**1506**	**2724**	**2461**	**3301**	**2678**

101	195	137	100	181	163	219	178
102	11	8	6	11	10	13	10
103							
104							

Subtraction M=100 species ([D_2]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							
99							
100							
101	23669	15860	10985	19047	16320	20537	16184
102	150412	85313	50758	78407	59159	67967	48733
103	10357	5781	3379	5252	4048	4565	3269
104	1017	437	264	451	315	371	227

M=101 species ([D_3]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							
99							
100							
101	**23669**	**15860**	**10985**	**19047**	**16320**	**20537**	**16184**
102	1572	1053	729	1265	1084	1364	1075
103	92	62	43	74	64	80	63
104							

Subtraction of M=101 species ([D_3]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							
99							
100							
101							
102	**148840**	**84260**	**50029**	**77143**	**58076**	**66603**	**47658**
103	10265	5719	3336	5178	3984	4485	3206
104	1017	437	264	451	315	371	227

Addition of the numbers in bold ([D_4]-, [D_3]-, and [D_2]cyclohexanone)

	175448	**102183**	**62519**	**98914**	**76856**	**90442**	**66520**

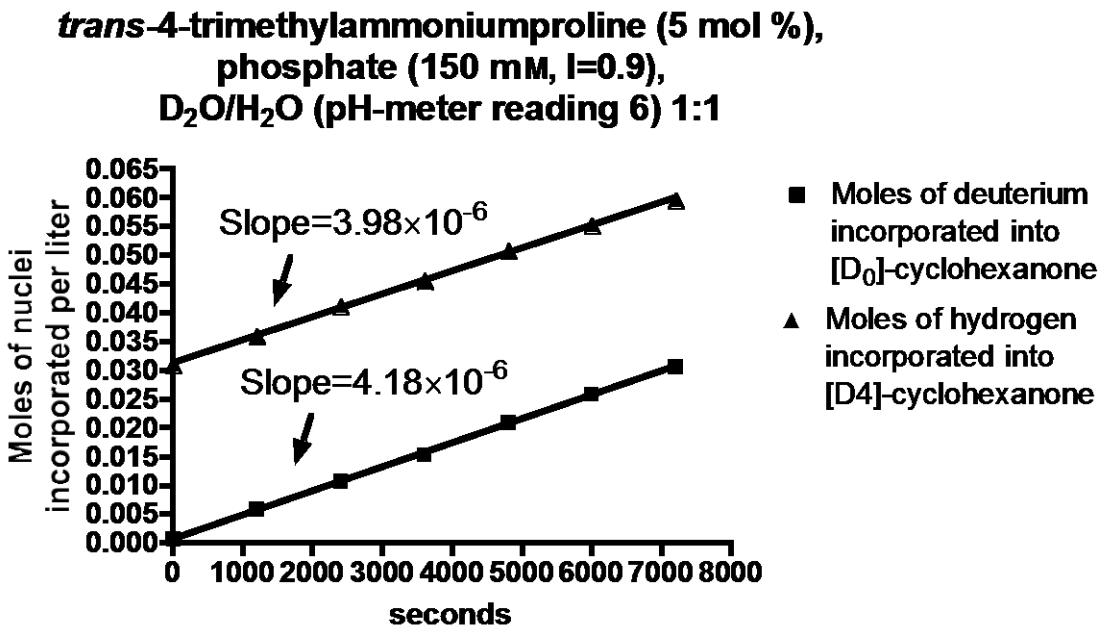
([D_4]-, [D_3]-, and [D_2]cyclohexanone) as a percentage of total

Species							
[D_4]	84.83430	82.45984	80.02153	77.98957	75.56438	73.64210	71.64437
[D_3]	13.49043	15.52144	17.56988	19.25612	21.23414	22.70780	24.32957
[D_2]	1.67526	2.01872	2.40860	2.75432	3.20148	3.65010	4.02606

Above values converted into moles of hydrogen incorporated per liter

[D_4]	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
[D_3]	0.02482	0.02856	0.03233	0.03543	0.03907	0.04178	0.04477

$[D_2]$	0.00616	0.00743	0.00886	0.01014	0.01178	0.01343	0.01482
$[D_3]+[D_2]$	0.03099	0.03599	0.04119	0.04557	0.05085	0.05521	0.05958



GC/MS data: ***trans*-4-trimethylammoniumproline** (5 mol%), phosphate (150 mM, I = 0.9), D_2O/H_2O (1:1, pH-meter reading 7.0), Substrate = **[D_0]cyclohexanone**

Mass Ion	0 min	20 min	40 min	Integrations 60 min	80 min	100 min	120 min
97	14283	6514	5678	6901	5324	6102	7703
98	219875	105687	91257	112926	87659	99292	124947
99	15307	10745	12309	18925	17417	23001	33187
100	908	791	986	1796	1789	2639	4150
101	0	17	17	107	135	200	327
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0
M=97 species and higher MW isotopes of natural abundance							
97	14283	6514	5678	6901	5324	6102	7703
98	948	433	377	458	354	405	511
99	56	25	22	27	21	24	30
Subtraction of M=97 species and higher MW isotopes of natural abundance							
97	0	0	0	0	0	0	0
98	218927	105254	90880	112468	87305	98887	124436
99	15251	10720	12287	18898	17396	22977	33157
100	908	791	986	1796	1789	2639	4150

101	0	17	17	107	135	200	327
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

M=98 species ([D_0]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	**218927**	**105254**	**90880**	**112468**	**87305**	**98887**	**124436**
99	14537	6989	6034	7468	5797	6566	8263
100	854	410	354	439	340	386	485
101	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

Subtraction M=98 species ([D_0]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	715	3731	6252	11430	11599	16411	24894
100	54	381	632	1357	1449	2253	3665
101	0	17	17	107	135	200	327
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

M=99 species ([D_1]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	**715**	**3731**	**6252**	**11430**	**11599**	**16411**	**24894**
100	47	248	415	759	770	1090	1653
101	0	1	2	3	3	4	6
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

Subtraction of M=99 species ([D_1]cyclohexanone) and higher MW isotopes of natural abundance

97	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0
100	**7**	**133**	**216**	**598**	**678**	**1164**	**2012**
101	0	16	15	104	132	196	321
102	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0

Addition of the numbers in bold ([D_0]-, [D_1]-, and [D_2]cyclohexanone)

219648	**109118**	**97349**	**124496**	**99583**	**116462**	**151342**

([D_0]-, [D_1]-, and [D_2]cyclohexanone) as a percentage of total

Species							
[D_0]	99.67161	96.45935	93.35500	90.33817	87.67111	84.90939	82.22158
[D_1]	0.32532	3.41896	6.42270	9.18117	11.64773	14.09144	16.44916
[D_2]	0.00307	0.12169	0.22230	0.48066	0.68117	0.99916	1.32925

Above values converted into moles of deuterium incorporated per liter

[D_0]	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
[D_1]	0.00060	0.00629	0.01182	0.01689	0.02143	0.02593	0.03027
[D_2]	0.00001	0.00045	0.00082	0.00177	0.00251	0.00368	0.00489
[D_1]+ [D_2]	0.00061	0.00674	0.01264	0.01866	0.02394	0.02961	0.03516

GC/MS data: ***trans*-4-trimethylammoniumproline** (5 mol%), phosphate (150 mM, I = 0.9), D_2O/H_2O (1:1, pH-meter reading 7.0), Substrate = **[D_4]cyclohexanone**

Mass Ion	Integrations 0 min	20 min	40 min	60 min	80 min	100 min	120 min
97	0	0	0	0	0	0	0
98	346	69	17	14	0	0	0
99	298	246	317	243	254	579	619
100	2959	2355	3178	2420	2521	5230	5484
101	23865	18236	23166	16361	16099	31147	30999
102	150423	94354	102420	61857	54399	93216	83300
103	10357	6345	6872	4173	3636	6382	5677
104	1017	515	587	331	286	537	470

M=99 species and higher MW isotopes of natural abundance

99	298	246	317	243	254	579	619
100	20	16	21	16	17	38	41
101	1	1	1	1	1	2	2

Subtraction of M=98 integrations, and M=99 species with its higher MW isotopes of natural abundance

97							
98							
99							
100	2939	2339	3157	2404	2504	5192	5443
101	23864	18235	23165	16360	16098	31145	30997
102	150423	94354	102420	61857	54399	93216	83300
103	10357	6345	6872	4173	3636	6382	5677
104	1017	515	587	331	286	537	470

M=100 species ([D_2]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							

99							
100	**2939**	**2339**	**3157**	**2404**	**2504**	**5192**	**5443**
101	195	155	210	160	166	345	361
102	11	9	12	9	10	20	21
103							
104							

Subtraction M=100 species ([D_2]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							
99							
100							
101	23669	18080	22955	16200	15932	30800	30635
102	150412	94345	102408	61848	54389	93196	83279
103	10357	6345	6872	4173	3636	6382	5677
104	1017	515	587	331	286	537	470

M=101 species ([D_3]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							
99							
100							
101	**23669**	**18080**	**22955**	**16200**	**15932**	**30800**	**30635**
102	1572	1200	1524	1076	1058	2045	2034
103	92	71	90	63	62	120	119
104							

Subtraction of M=101 species ([D_3]cyclohexanone) and higher MW isotopes of natural abundance

97							
98							
99							
100							
101							
102	**148840**	**93144**	**100883**	**60772**	**53331**	**91151**	**81245**
103	10265	6274	6782	4110	3574	6262	5558
104	1017	515	587	331	286	537	470

Addition of the numbers in bold ([D_4]-, [D_3]-, and [D_2]cyclohexanone)

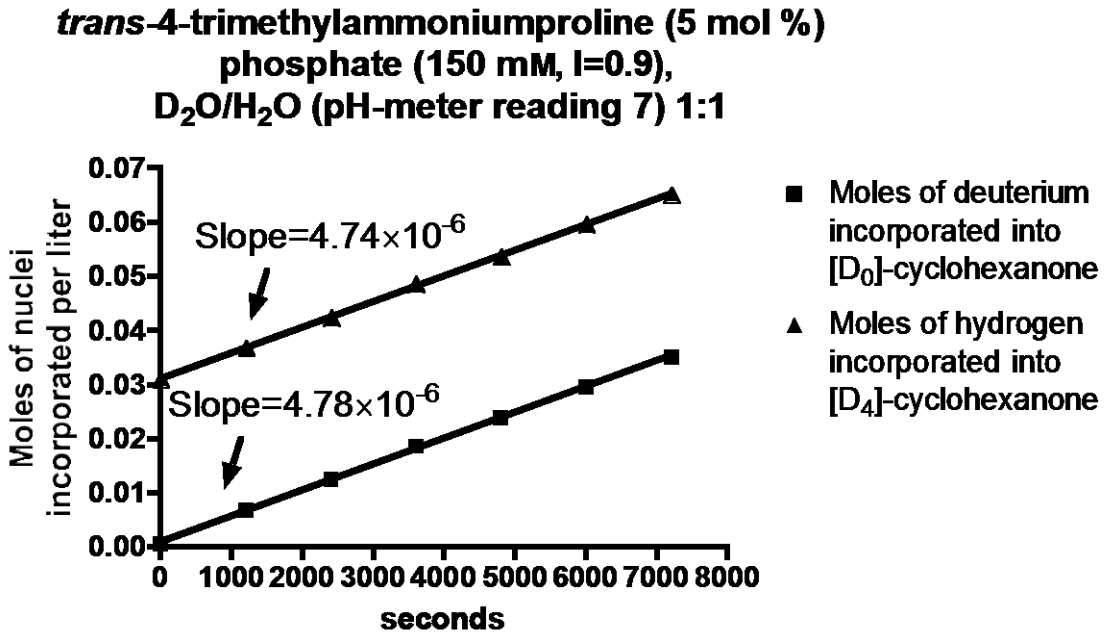
	175448	**113563**	**126996**	**79376**	**71767**	**127142**	**117323**

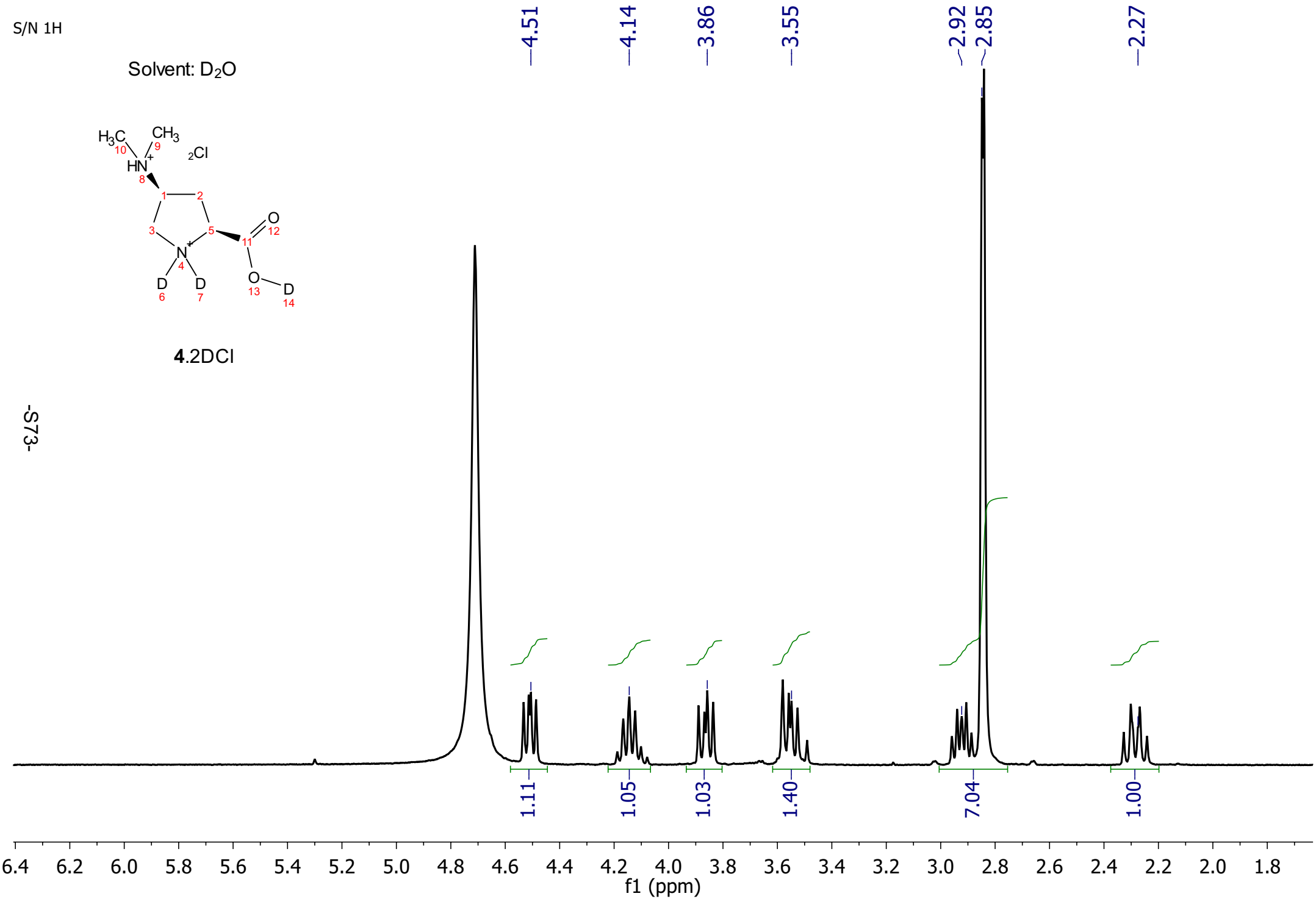
([D_4]-, [D_3]-, and [D_2]cyclohexanone) as a percentage of total

Species							
[D_4]	84.83430	82.02015	79.43858	76.56187	74.31158	71.69187	69.24885
[D_3]	13.49043	15.92049	18.07555	20.40968	22.19918	24.22486	26.11190
[D_2]	1.67526	2.05936	2.48588	3.02844	3.48924	4.08327	4.63926

Above values converted into moles of hydrogen incorporated per liter

$[D_4]$	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
$[D_3]$	0.02482	0.02929	0.03326	0.03755	0.04085	0.04457	0.04805
$[D_2]$	0.00616	0.00758	0.00915	0.01114	0.01284	0.01503	0.01707
$[D_3]+[D_2]$	0.03099	0.03687	0.04241	0.04870	0.05369	0.05960	0.06512



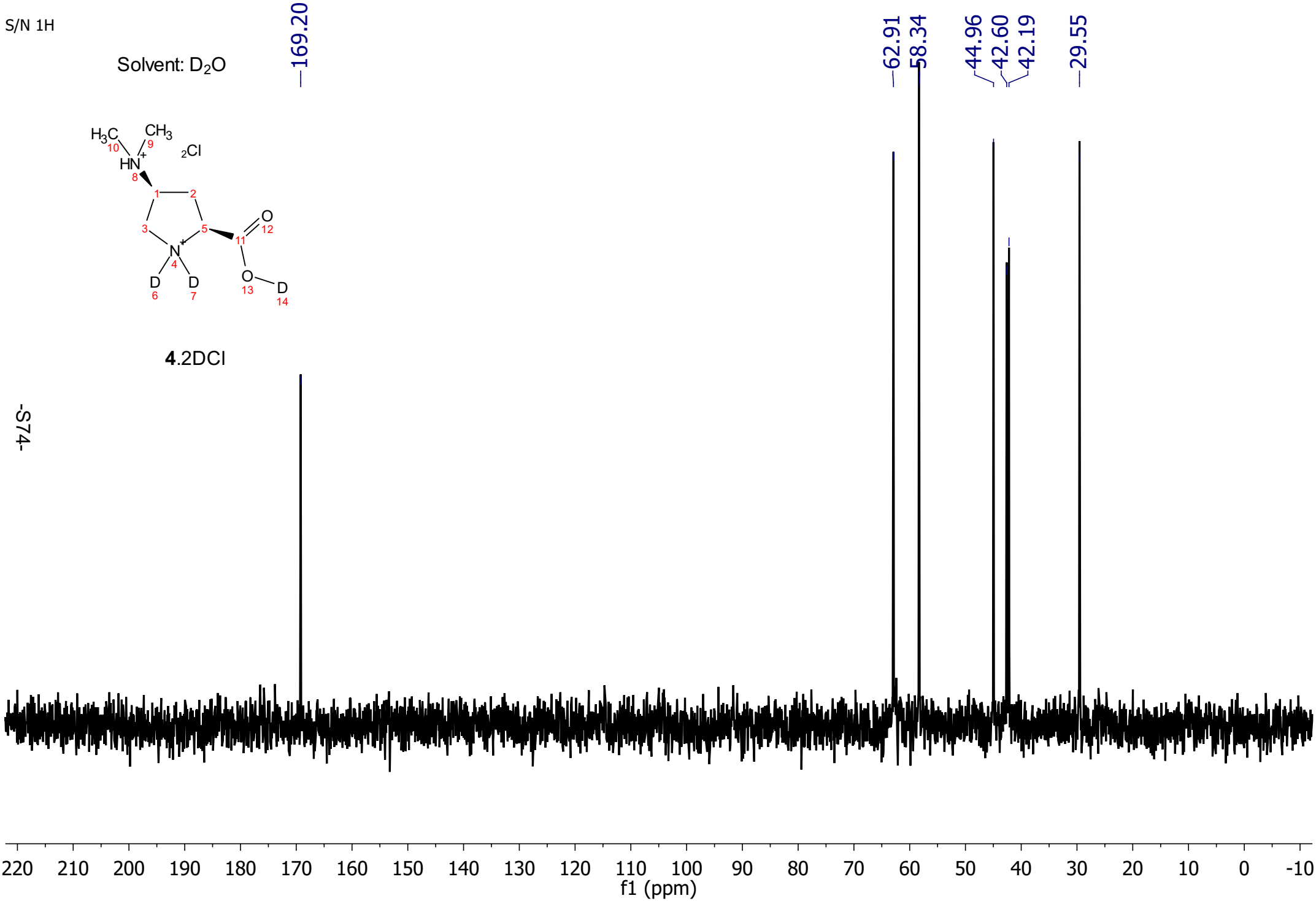

S/N 1H
Solvent: D2O
4.51
4.14
3.86
3.55
2.92
2.85
2.27
1.11
1.05
1.03
1.40
7.04
1.00
6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)

4.2DCl

S/N 1H

Solvent: D_2O

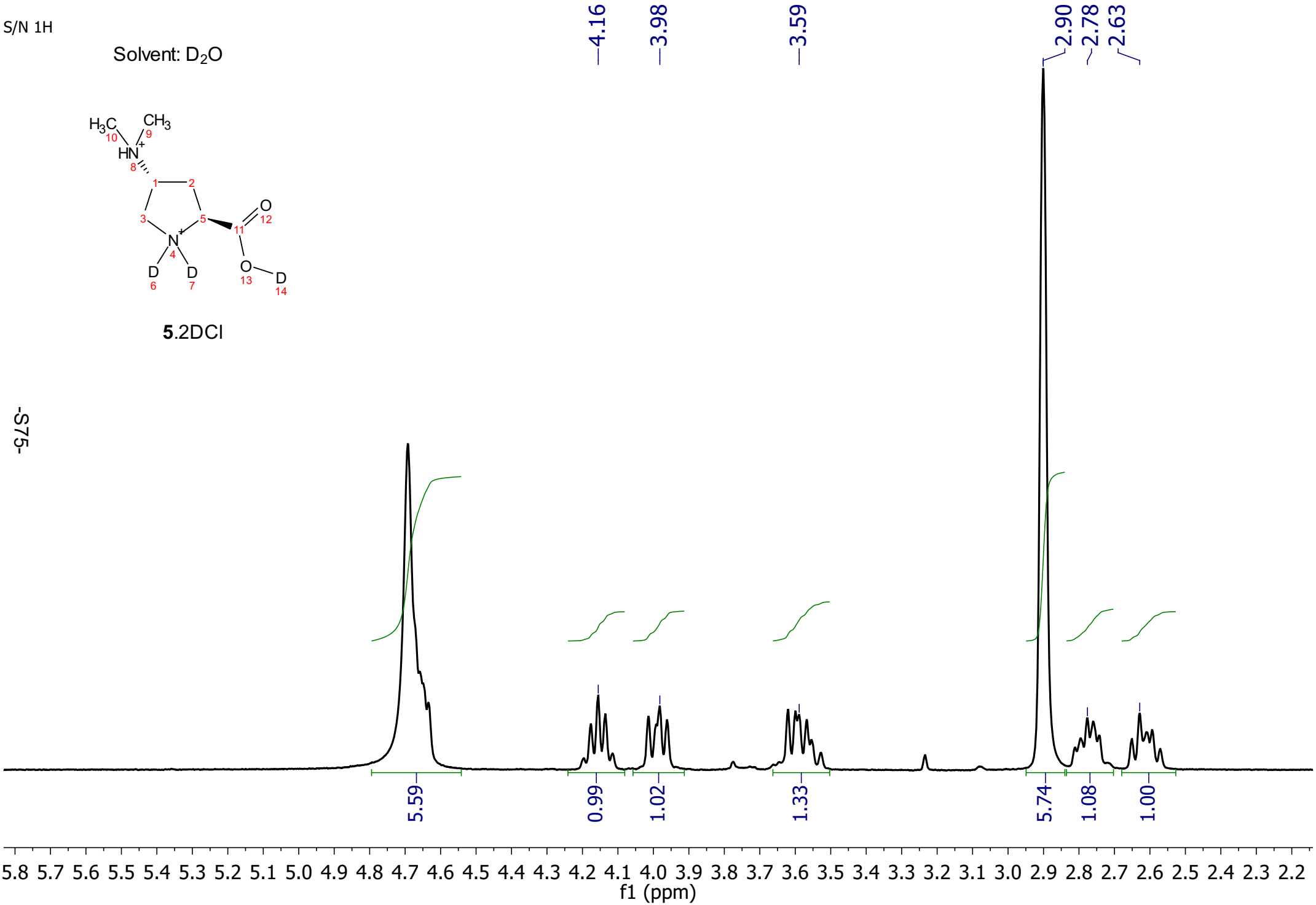
4.2DCl

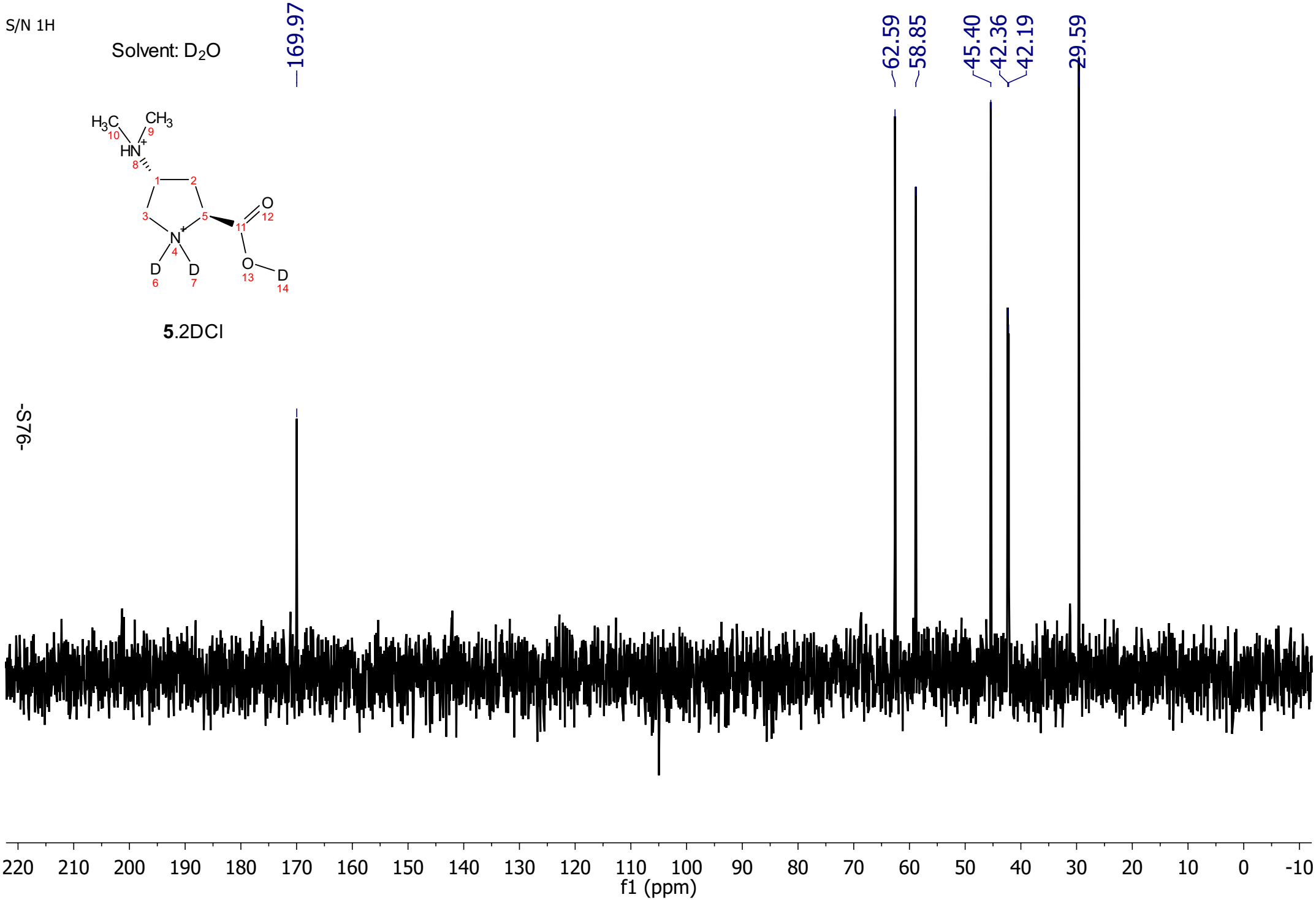


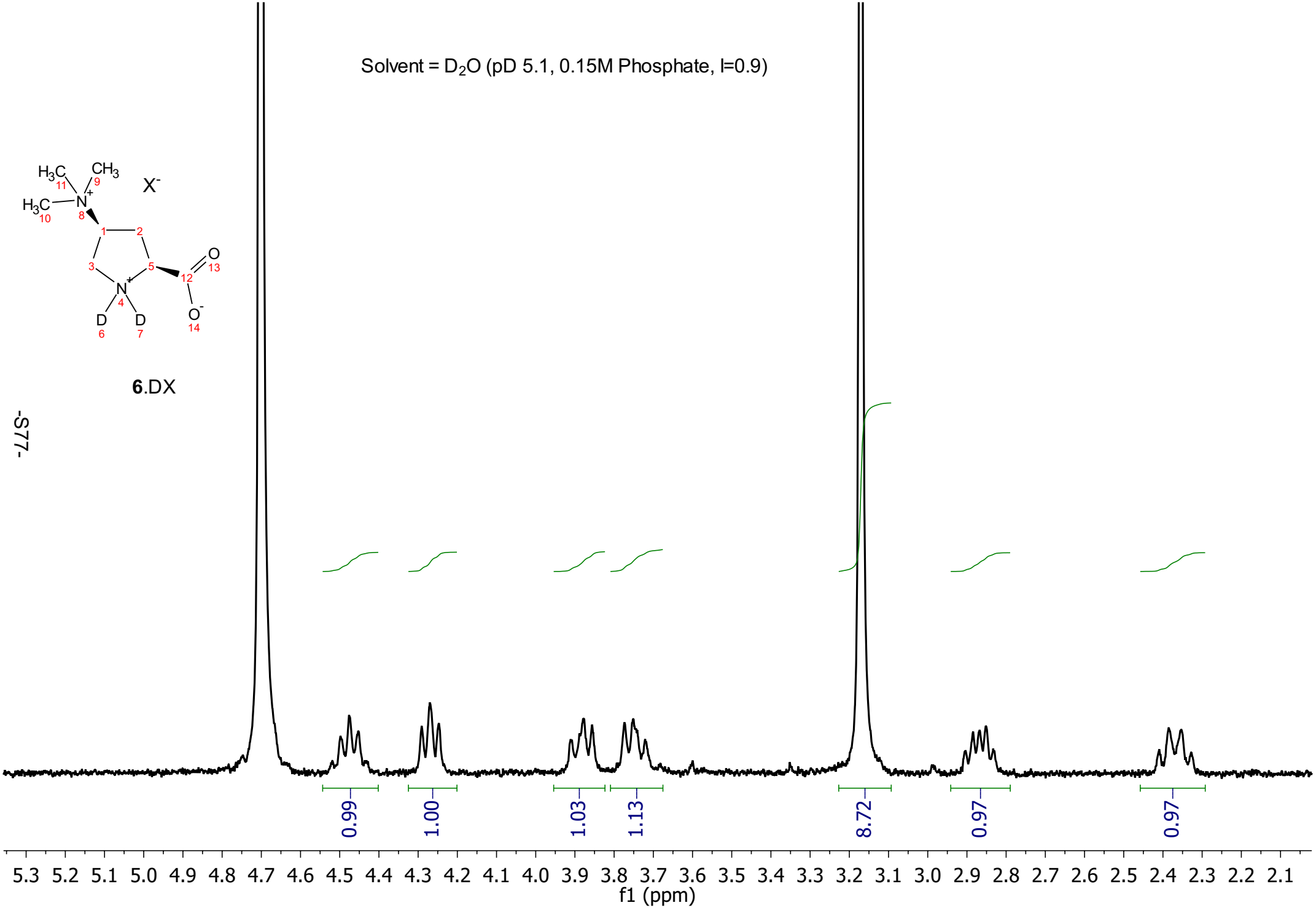
S/N 1H

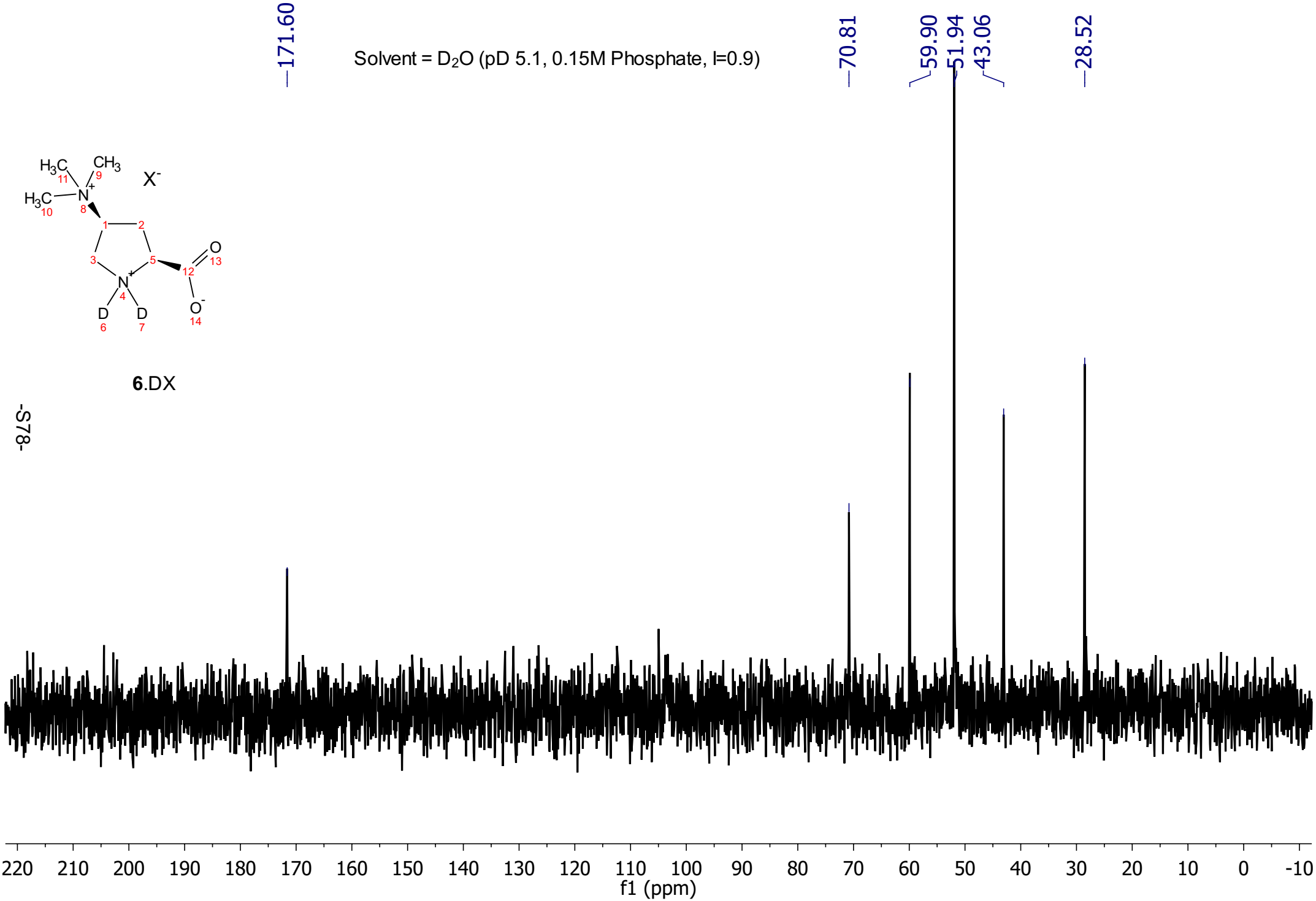
Solvent: D_2O

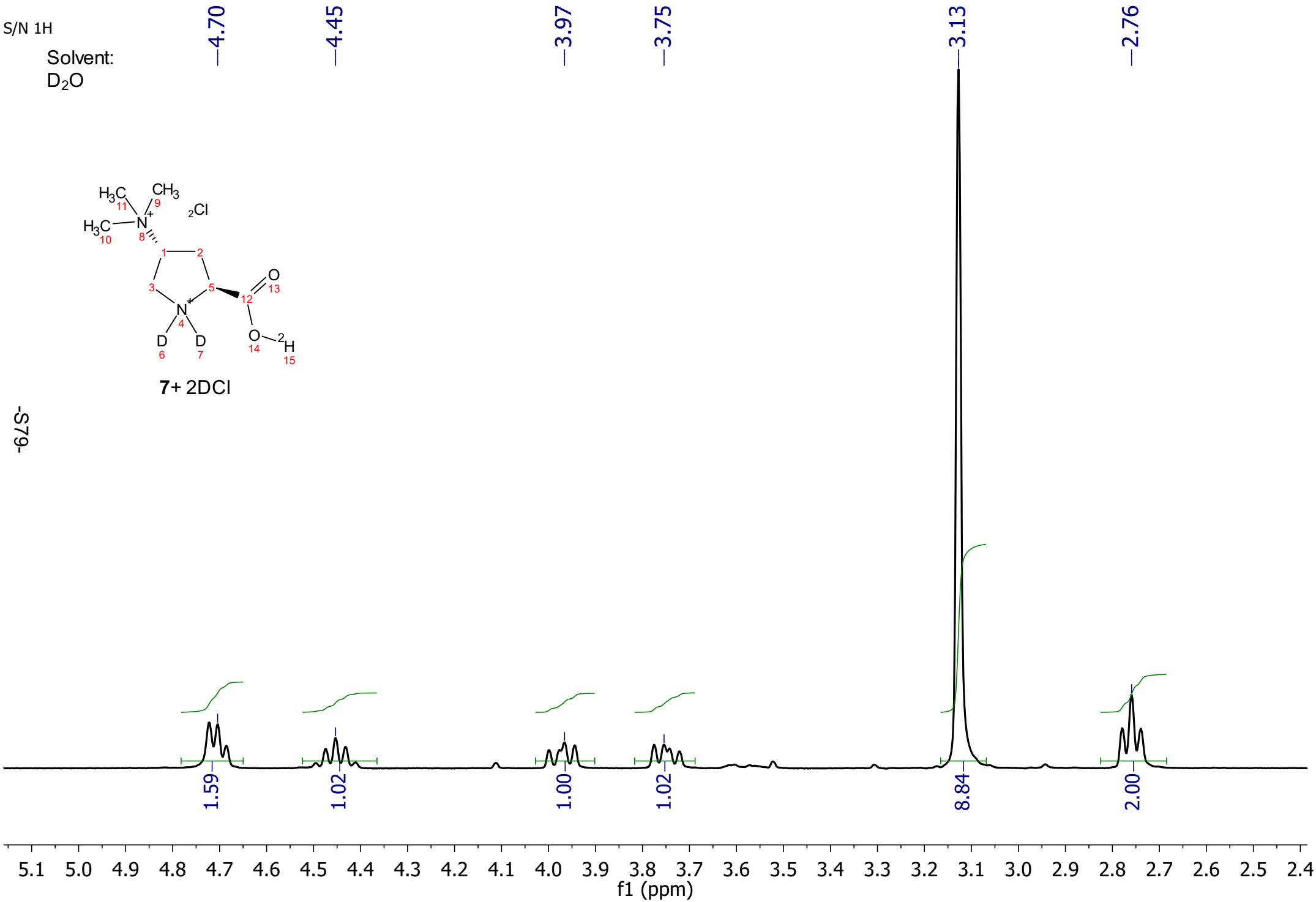
5.2DCl




S/N 1H
Solvent: D2O
169.97
62.59
58.85
45.40
42.36
42.19
29.59
H3C
CH3
HN
N
O
D
D
O
D
5.2DCl
f1 (ppm)


Solvent = D2O (pD 5.1, 0.15M Phosphate, I=0.9)
X-
6.DX
0.99
1.00
1.03
1.13
8.72
0.97
0.97
5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1
f1 (ppm)

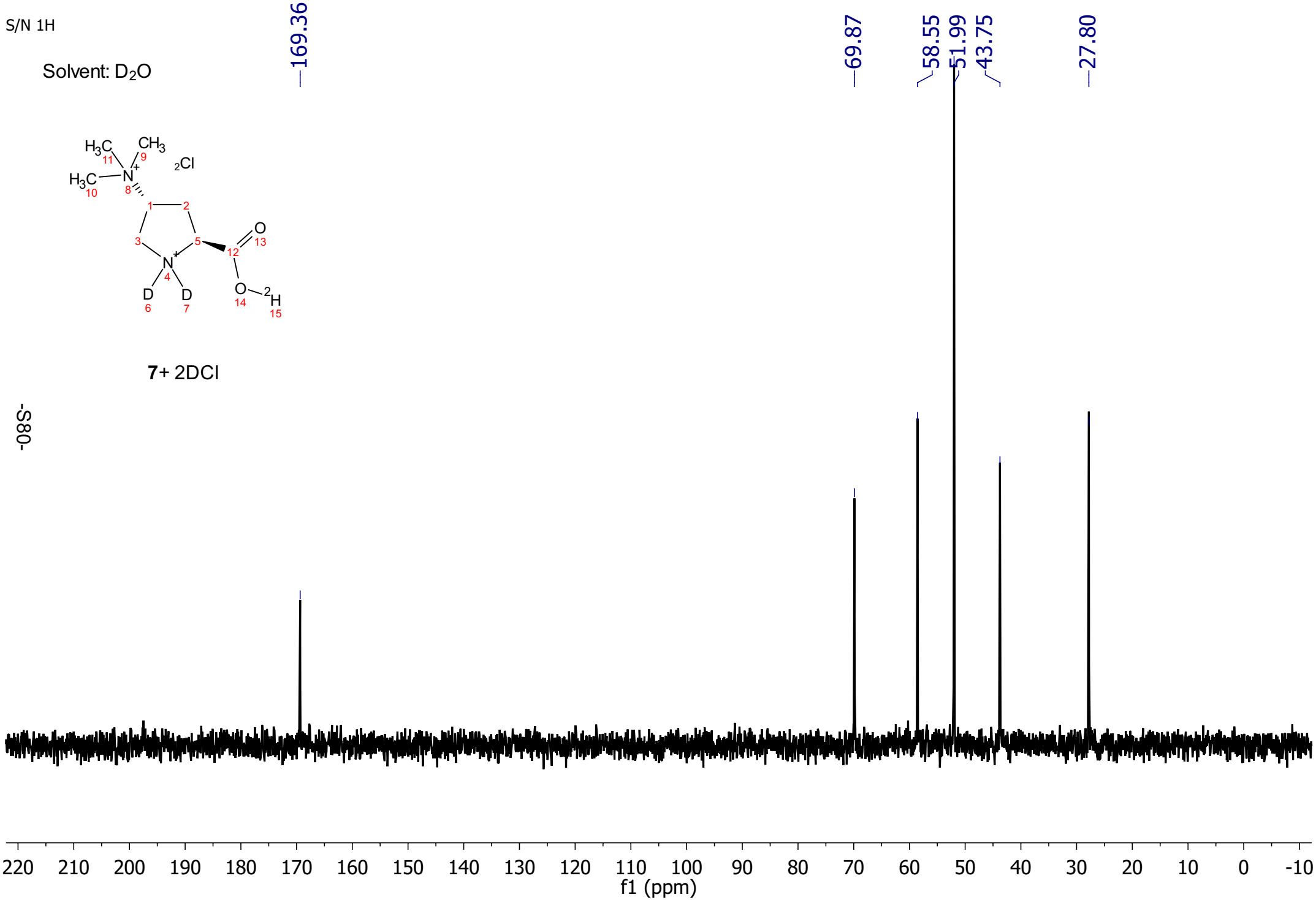

171.60
Solvent = D2O (pD 5.1, 0.15M Phosphate, I=0.9)
70.81
59.90
51.94
43.06
28.52
X-
6.DX
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

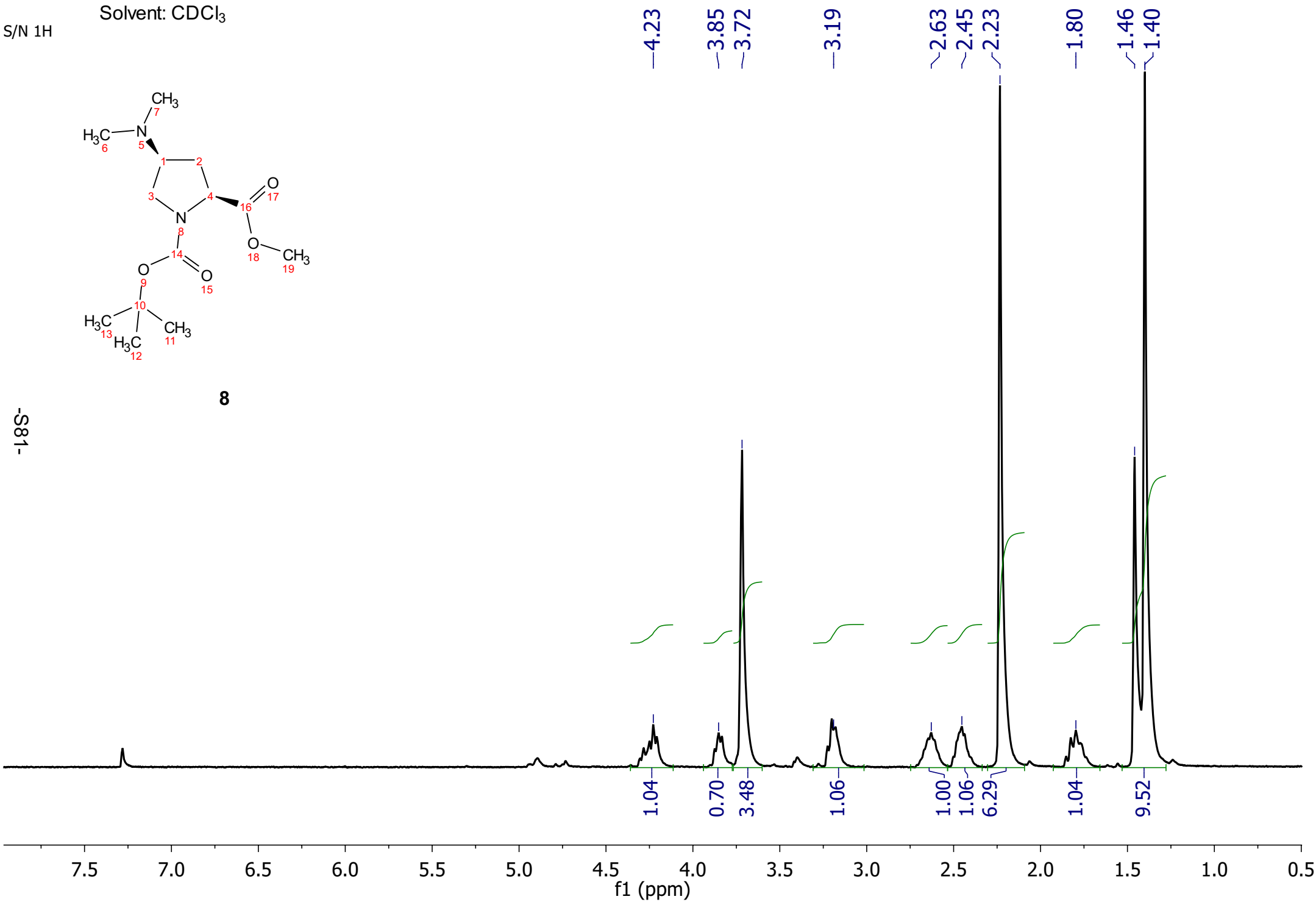

S/N 1H
Solvent:
D2O
4.70
4.45
3.97
3.75
3.13
2.76
7+ 2DCl
1.59
1.02
1.00
1.02
8.84
2.00
5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4
f1 (ppm)

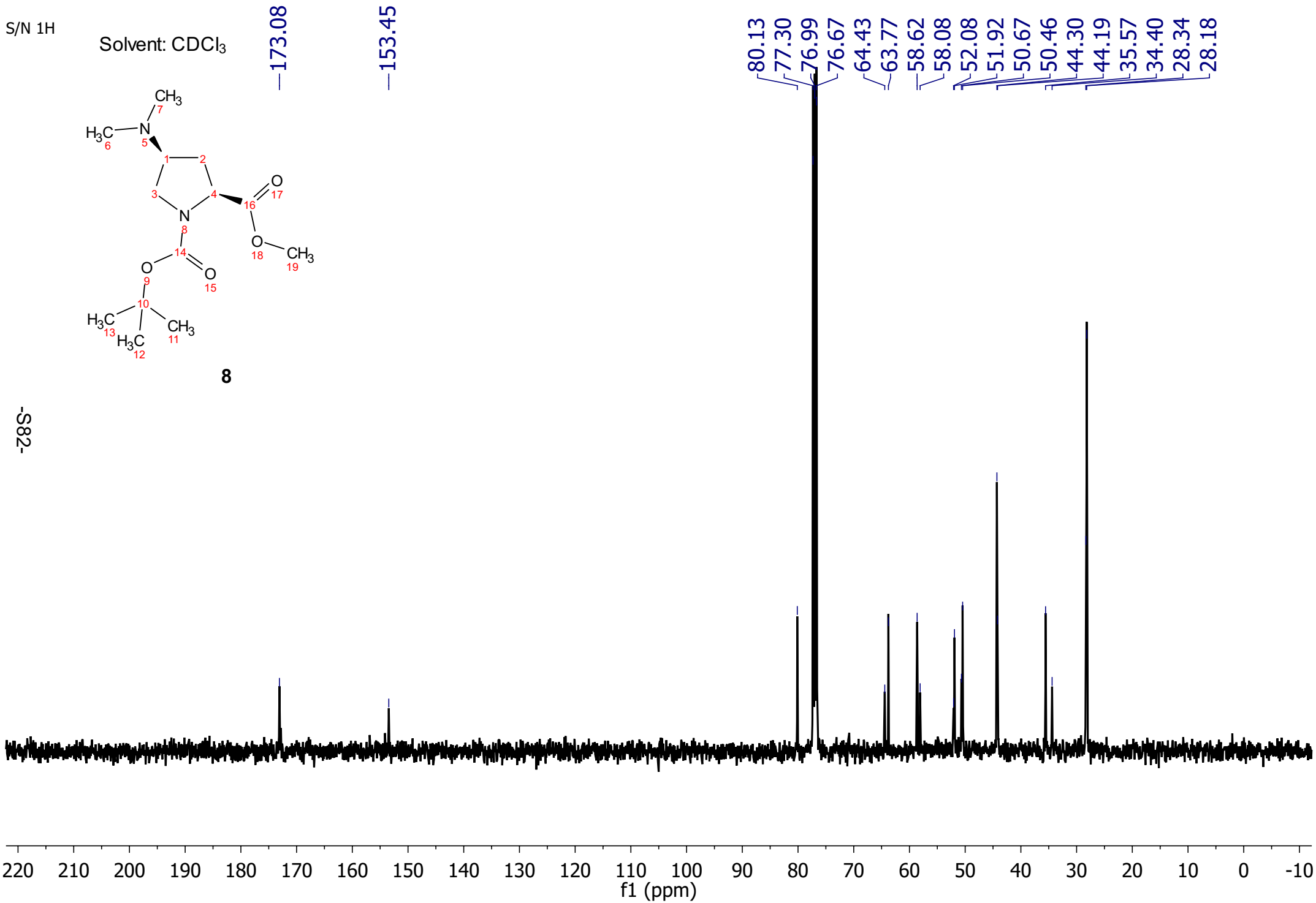
S/N 1H

Solvent: D_2O

7+ 2DCl



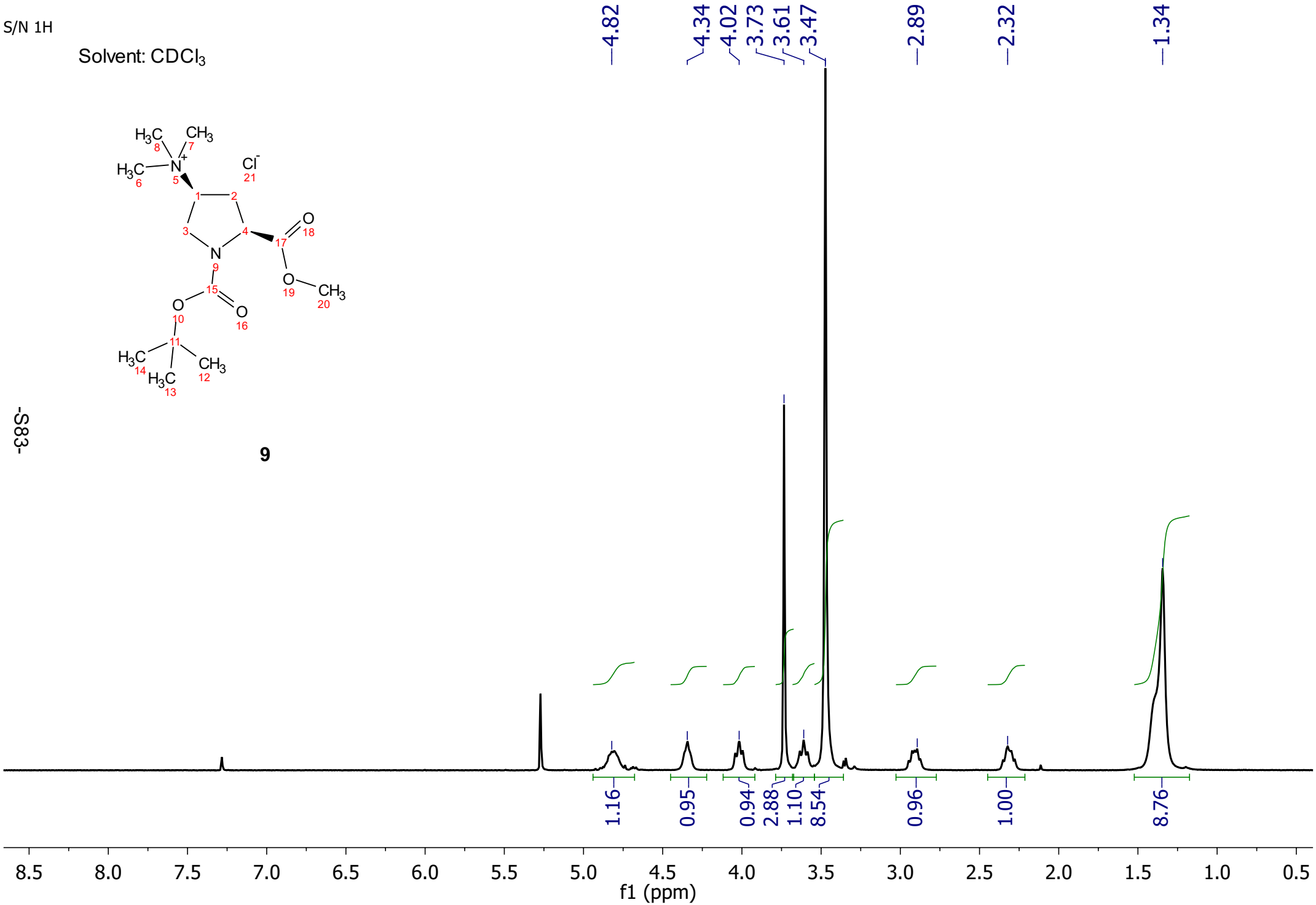

Solvent: CDCl3
S/N 1H
8
4.23
3.85
3.72
3.19
2.63
2.45
2.23
1.80
1.46
1.40
1.04
0.70
3.48
1.06
1.00
1.06
6.29
1.04
9.52
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


S/N 1H
Solvent: CDCl3
173.08
153.45
80.13
77.30
76.99
76.67
64.43
63.77
58.62
58.08
52.08
51.92
50.67
50.46
44.30
44.19
35.57
34.40
28.34
28.18
8
f1 (ppm)

S/N 1H

Solvent: $CDCl_3$

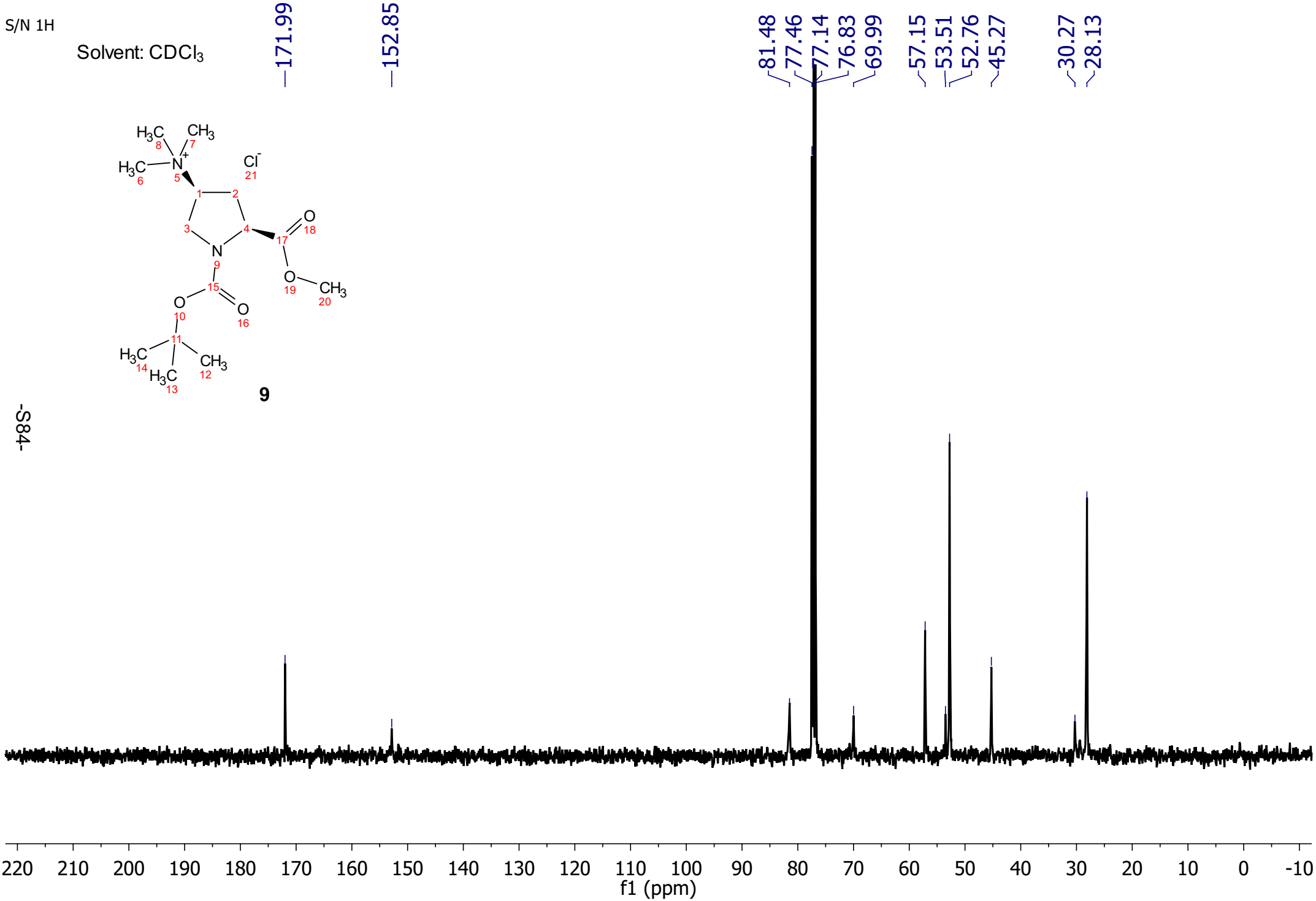
9



S/N 1H

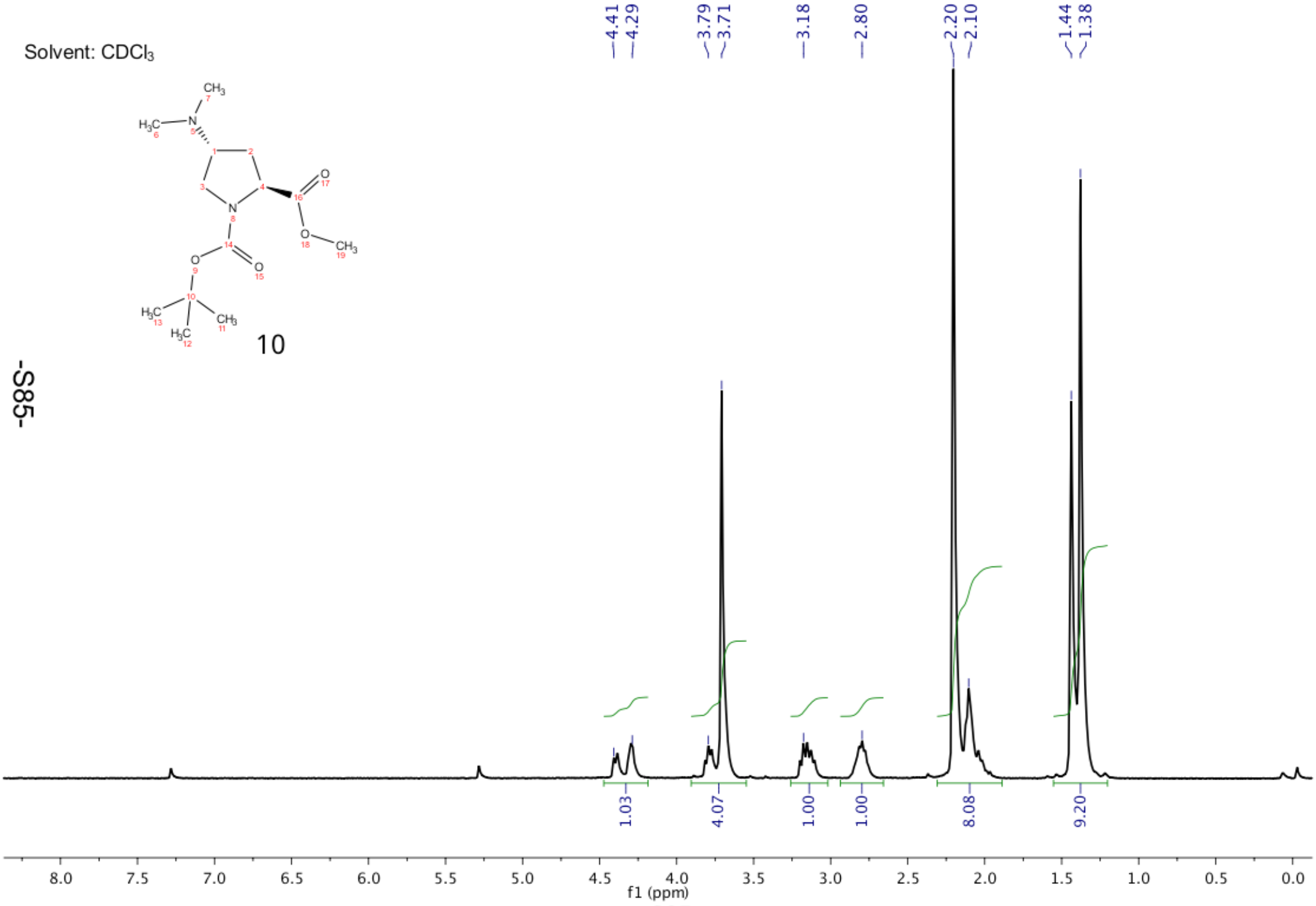
Solvent: $CDCl_3$

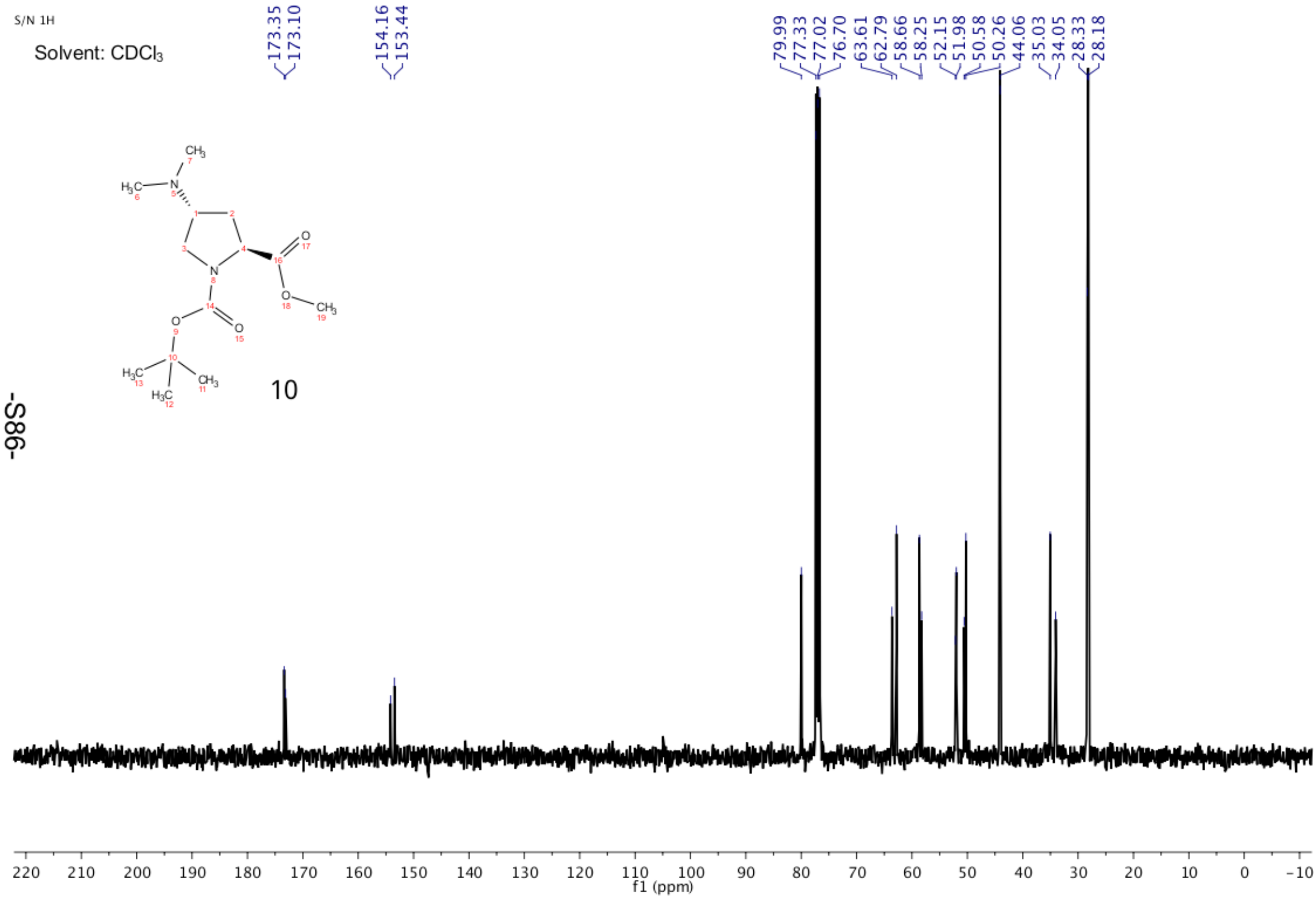
9



Solvent: $CDCl_3$

10

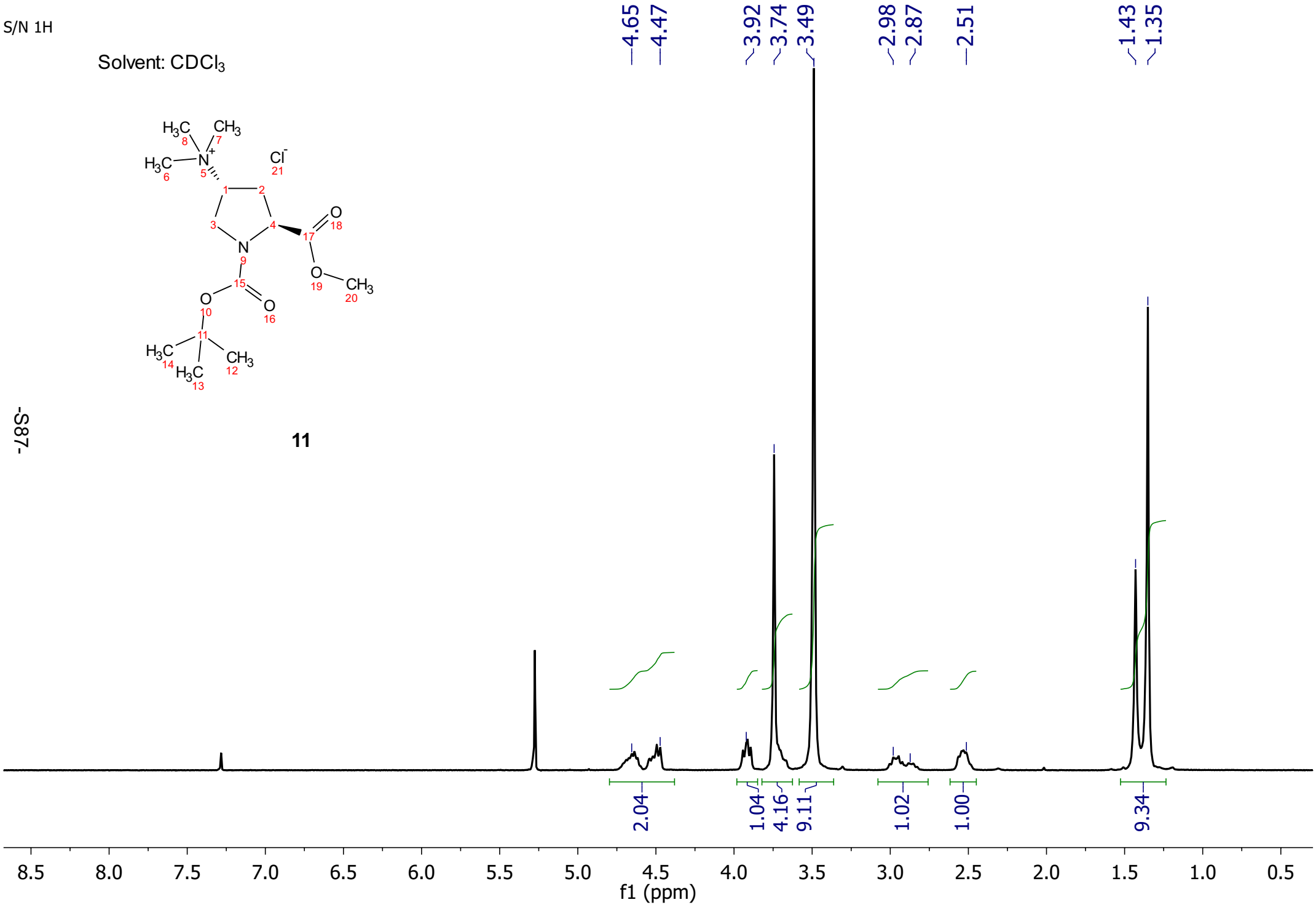



S/N 1H
Solvent: CDCl3
173.35
173.10
154.16
153.44
79.99
77.33
77.02
76.70
63.61
62.79
58.66
58.25
52.15
51.98
50.58
50.26
44.06
35.03
34.05
28.33
28.18
10
f1 (ppm)

S/N 1H

Solvent: $CDCl_3$

11



S/N 1H

Solvent: $CDCl_3$

11