Supporting Information:

Mechanistic Insights into the Hydrolysis of Organophosphorus Compounds by Paraoxonase 1: Exploring the Limits of Selectivity of a Promiscuous Enzyme

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Table of Contents

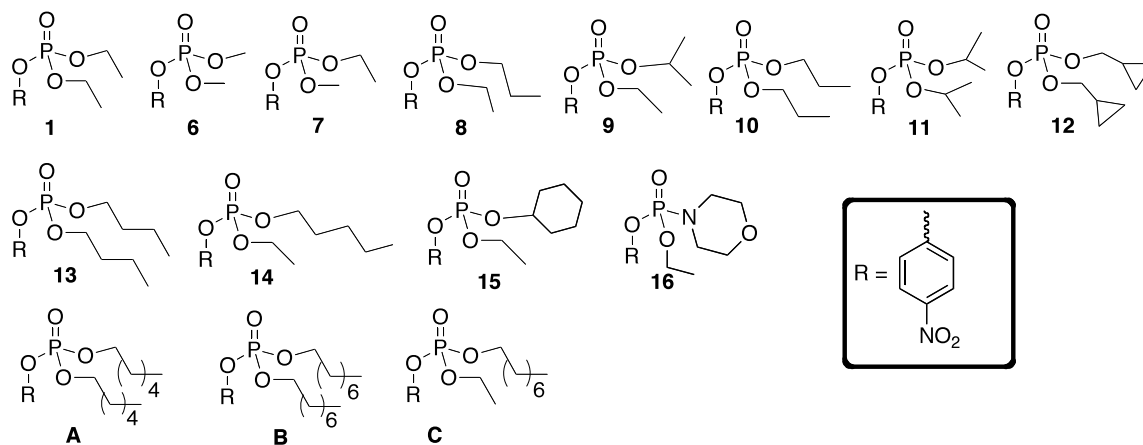## 1. General synthetic procedures for OP substrates and Inhibitors:

The general reaction for the preparation of the photoaffinity labels (PAL-Group ID) was the addition of a phosphoryl dichloridate (1 equivalent) to the corresponding alcohol (1 equivalent) in the presence of toluene at 0^0C. A solution of triethylamine (1 equivalent) in toluene was added dropwise, and the reaction was brought to room temperature and stirred overnight. The reaction mixture was filtered and the supernatant was evaporated. The solid residue was then re-suspended in dry acetone. Upon cooling the mixture to 0^0C, sodium azide (1 equivalent) was added and the reaction was stirred for 8 hours. The reaction mixture was filtered, and the solvent was evaporated. The crude product was purified by silica gel column chromatography (hexane:ethyl acetate 80:20 as the eluent). Compounds were obtained in 40–80% yield. The purified compounds were analyzed by IR, ^{1}H and ^{13}C NMR as well as HRMS.

The general reaction for the formation of the SAR substrates/inhibitors was the addition of a phosphoryl dichloridate (1 equivalent) to the corresponding alcohol (2 equivalents) in the presence of toluene at 0^0C. A solution of triethylamine (2 equivalents) in toluene was added dropwise, and the reaction was stirred at room temperature overnight. The reaction mixture was filtered, and the solvent was then evaporated. The crude product was purified by silica gel column chromatography (hexane:ethyl acetate 80:20 as the eluent). Compounds were isolated in 50–80% yield. The purified compounds were analyzed by IR, ^{1}H and ^{13}C NMR as well as HRMS.
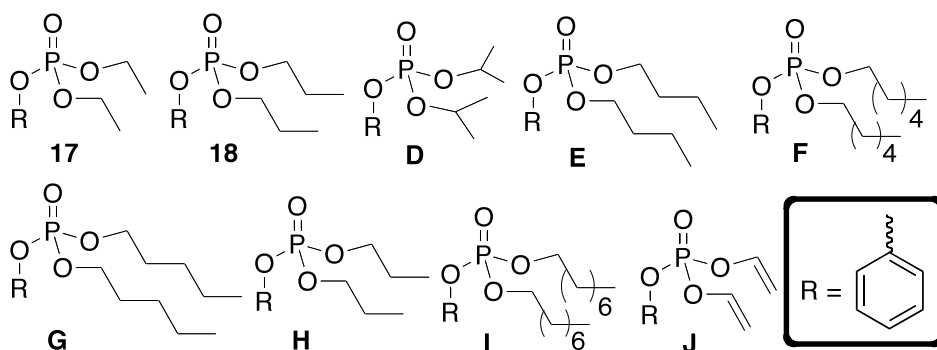
2. Complete Library of ligands studied

Compounds **A-P** were also synthesized and screened; since they had no hydrolytic activity against G2E6 PON1, these molecules were not discussed in the manuscript.
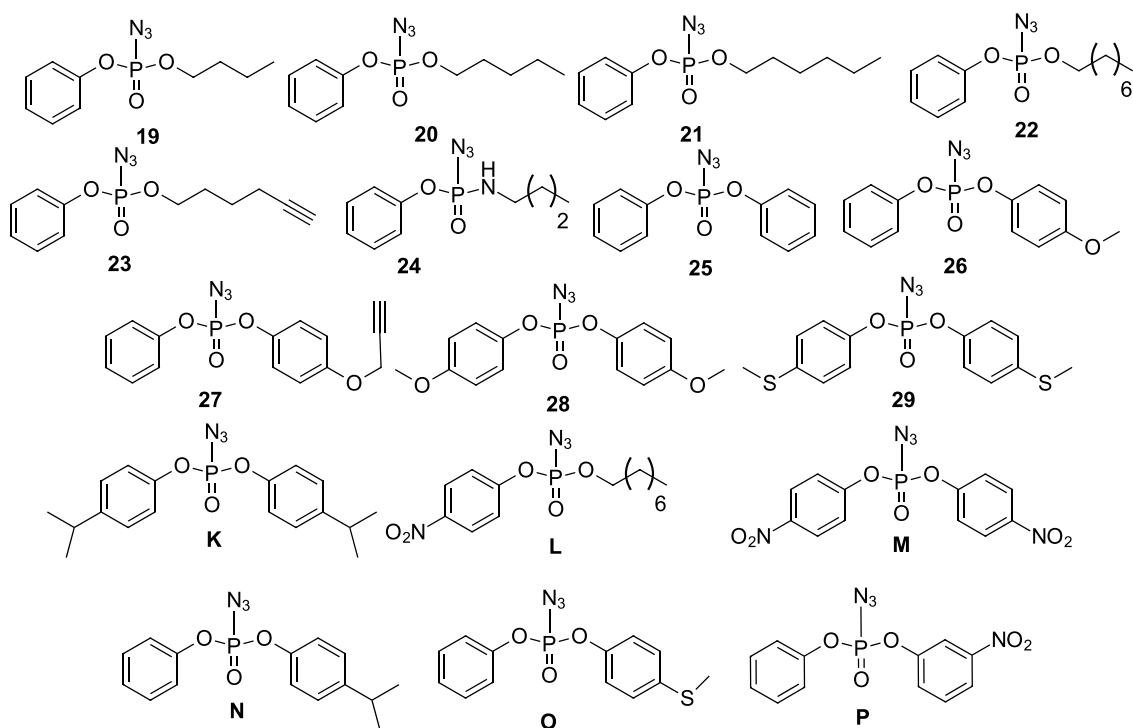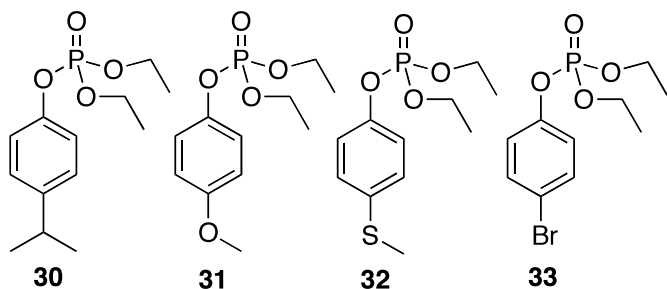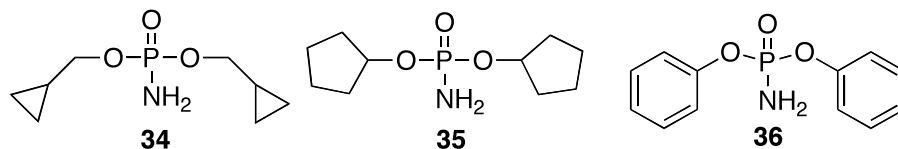
Group I

Group II

17 **18** **D** **E** **F**

G **H** **I** **J** R = (phenyl)

Group III

19 **20** **21** **22**

23 **24** **25** **26**

27 **28** **29**

K **L** **M**

N **O** **P**

Group IV

30   **31**   **32**   **33**
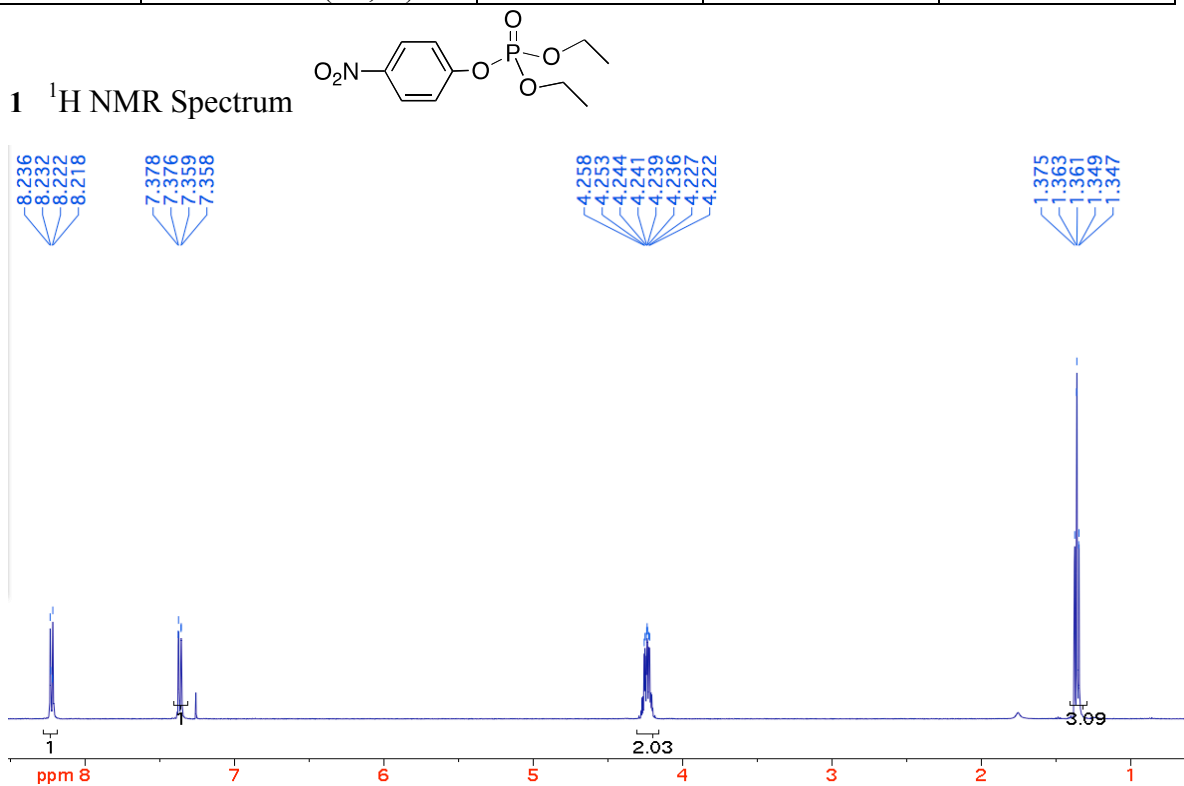
Group V

34   **35**   **36**

3. ^{1}H NMR, ^{13}C NMR, IR spectroscopic and Mass Spectrometric data of newly synthesized molecules.

Molecule	^{1}H NMR	^{13}C NMR	IR	MS
1	8.22 (2H, d, *J* 10 Hz), 7.36 (2H, d, *J* 10 Hz), 4.23 (4H, q, *J* 5Hz), 1.35 (6H, t, *J* 5 Hz)	155.8, 144.8, 125.9, 120.7, 65.4, 16.3	2988, 29840, 1590, 1345	Calc. (M+Na)$^+$ 298.0456

Expt 298.0449 |
| 6 | 8.25 (2H, d, *J* 10Hz), 7.38 (2H, d, *J* 10Hz), 3.91 (6H, d, *J* 10Hz) | 155.3, 144.8, 125.7, 120.5, 55.3 | 3017, 2914, 2860, 1252, 1045 | Calc (M+Na)$^+$ 270.0143

Expt 270.0141 |
| 7 | 8.25 (2H, d, *J* 10Hz), 7.38 (2H, d, *J* 10Hz), 4.28-4.25 (2H, m), 3.90 (6H, d, *J* 10Hz), 1.40-1.37 (3H, m), | 155.8, 145.1, 126.0, 120.9, 65.8, 55.5, 16.5 | 2928, 2846, 1048 | Calc (M+Na)$^+$ 284.0300

Expt 284.0301 |
| 8 | 8.20 (2H, d, *J* 10Hz), 7.35 (2H, d, *J* 10Hz), 4.24-4.22 (2H, m), 4.11-4.09 (2H, m), 1.72-1.68 (2H, m), 1.36-1.33 (3H, m), 0.94-0.92 (3H, m) | 155.6, 144.6, 125.6, 120.5, 70.6, 65.2, 23.5, 16.1, 9.9 | 2987, 1591, 1342, 1290, 1023, | Calc (M+Na)$^+$ 312.0613

Expt 312.0610 |
| 9 | 8.18 (2H, d, *J* 10Hz), 7.33 (2H, d, *J* 10Hz), 4.76-4.74 (1H, m), 4.20-4.17(2H, m), 1.29-1.34 (9H, m), | 155.7, 144.5, 125.5, 120.5, 74.6, 65.0, 50.4, 23.4, 16.0 | 2980, 1580, 1349, 1256, 1025 | Calc (M+Na)$^+$ 312.0613

Expt 312.0611 |
| 10 | 8.22 (2H, d, *J* 10Hz), | 155.9, 144.8, | 2960, 1590, | Calc (M+H)$^+$ |

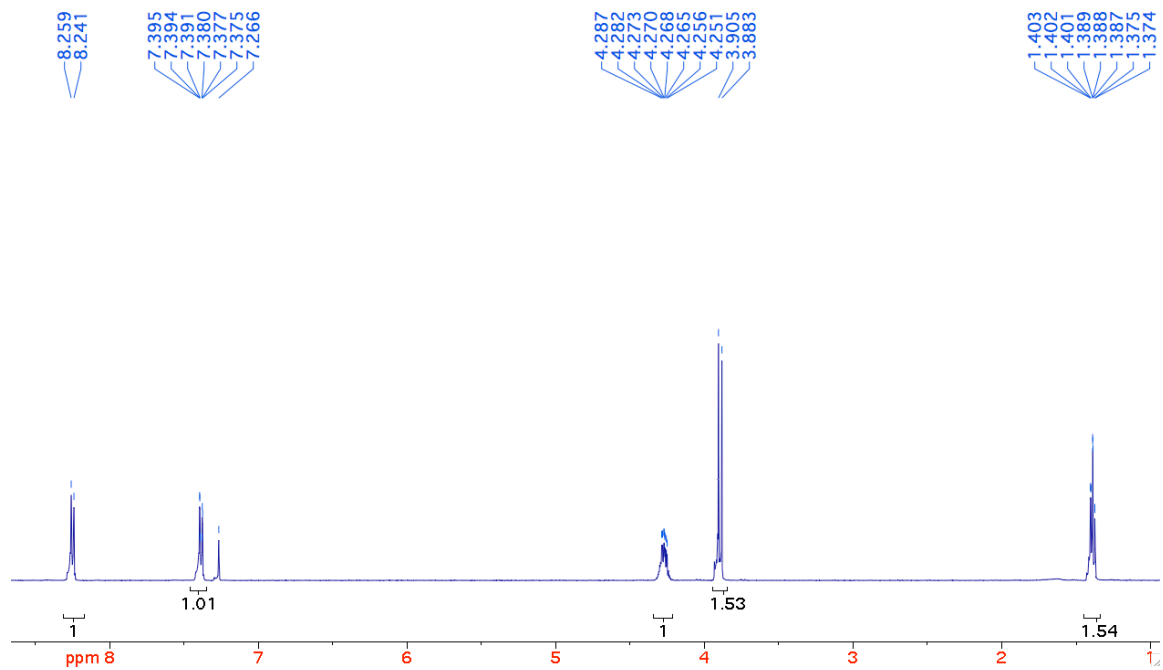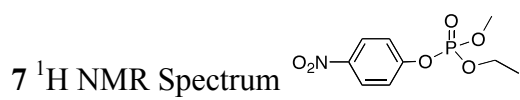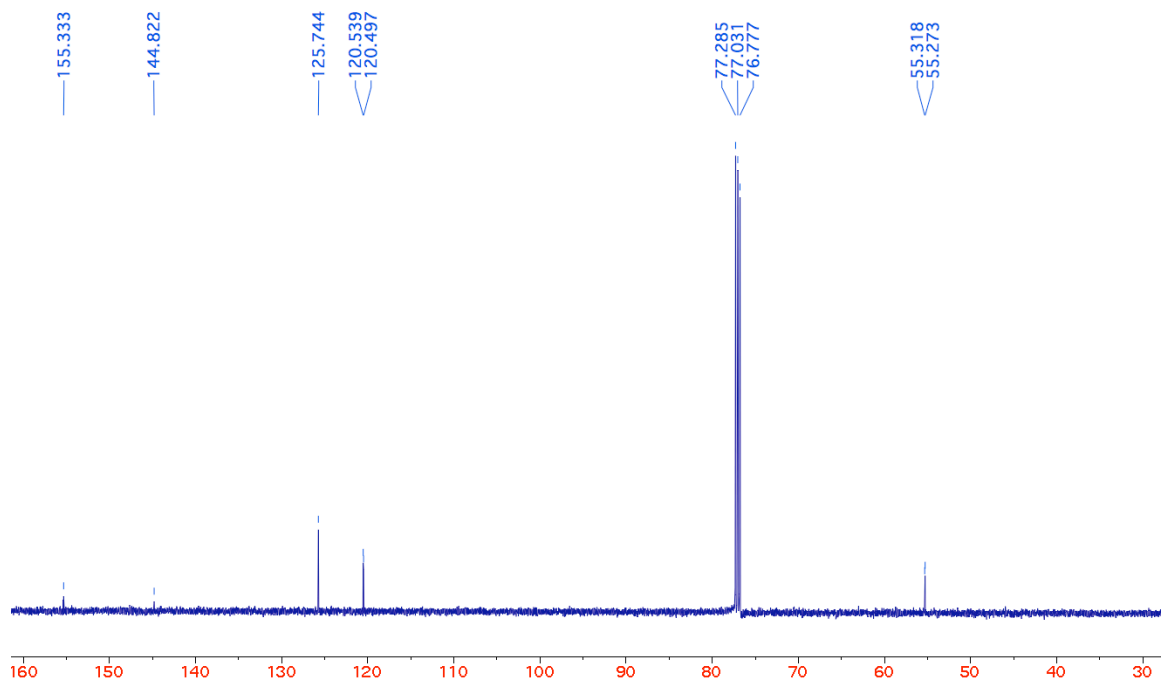|				
	7.37 (2H, d, *J* 10Hz), 4.13 (4H, q, *J* 5Hz), 1.74-1.71 (4H, m), 0.95 (6H, q, *J* 5Hz)	125.8, 120.7, 70.7, 23.8, 10.1	1339, 1236, 1015	304.0950 Expt 304.0965
11	8.20 (2H, d, *J* 10 Hz), 7.34 (2H, d, *J* 10 Hz), 4.74 (2H, q, *J* 5Hz), 1.32 (12H, d, *J* 5 Hz)	156.4, 144.6, 125.7, 120.6, 74.4, 14.3	2984, 2938, 1592, 1347, 1008	Calc. (M+Na)⁺ 326.0769 Expt 326.0739
12	8.23 (2H, d, *J* 10Hz), 7.38 (2H, d, *J* 10Hz), 4.04 (4H, q, *J* 10Hz), 1.19-1.21 (2H, m), 0.60 (4H, d, *J* 10Hz), 0.32 (4H, d, *J* 10Hz)	151.2, 130.0, 125.2, 120.4, 73.7, 11.5, 3.8	3114, 2894, 1522, 1347, 1036	Calc (M+Na)⁺ 350.0769 Expt 350.0786
13	8.25 (2H, d, *J* 10Hz), 7.38 (2H, d, *J* 10Hz), 4.18 (4H, q, *J* 5Hz), 1.72-1.68 (4H, m), 1.44-1.41 (4H, m), 0.94 (6H, t, *J* 5Hz)	155.8, 144.8, 125.8, 120.7, 69.0, 32.3, 18.8, 13.7	2956, 1591, 1523, 1346, 1233	Calc (M+Na)⁺ 354.1082 Expt 354.1078
14	8.23 (2H, d, *J* 10Hz), 7.35 (2H, d, *J* 10Hz), 4.25-4.24 (2H, m), 4.18-4.15 (2H, m), 1.71-1.70 (2H, m), 1.39-1.35 (7H, m), 0.89-0.88 (3H, m)	155.8, 144.8, 125.8, 120.6, 69.3, 65.3, 29.5, 27.6, 22.2, 16.2, 14.0	2950, 1601, 1388, 1295, 1101	Calc (M+Na)⁺ 340.0926 Expt 340.0923
15	8.25 (2H, d, *J* 10Hz), 7.39 (2H, d, *J* 10Hz), 4.54-4.52 (1H, m), 4.26-4.24 (2H, m), 1.96-1.93 (2H, m), 1.77-1.74 (2H, m), 1.39-1.30 (3H, m), 1.28 (3H, t, *J* 5Hz), 1.27-1.26 (3H, m)	155.8, 144.5, 125.6, 120.6, 79.2, 65.0, 33.2, 24.9, 23.4, 16.0	2921, 2866, 1259, 1045	Calc (M+Na)⁺ 352.1395 Expt 352.1390
16	8.20 (2H, d, *J* 10Hz), 7.34 (2H, d, *J* 10Hz), 4.19-4.17 (2H, m), 3.61-3.60 (4H, m), 3.21-3.19 (4H, m), 1.35 (3H, t, *J* 5Hz),	156.0, 144.4, 125.6, 120.6, 66.8, 63.7, 44.6, 16.1	3010, 2921, 2860, 1255, 1048	Calc (M+Na)⁺ 339.0722 Expt 339.0722
A	8.18 (2H, d, *J* 10Hz), 7.34 (2H, d, *J* 10Hz), 4.12 (4H, q, *J* 5Hz), 1.65 (4H, t, *J* 5Hz), 1.31 -1.24 (12H, m), 0.82 (6H, t, *J* 5Hz)	155.6, 144.6, 125.5, 120.5, 69.1, 31.1, 30.1, 24.9, 22.4, 13.8	2928, 1593, 1346, 1023, 936	Calc (M+Na)⁺ 410.1708 Expt 410.1724
B	8.15 (2H, d, *J* 10Hz), 7.31 (2H, d, *J* 10Hz), 4.10-4.09 (4H, m), 4.02-	155.6, 144.5, 125.5, 120.5, 69.0, 60.2, 31.6,	2957, 1593, 1346, 1295,	Calc (M+Na)⁺ 466.2334

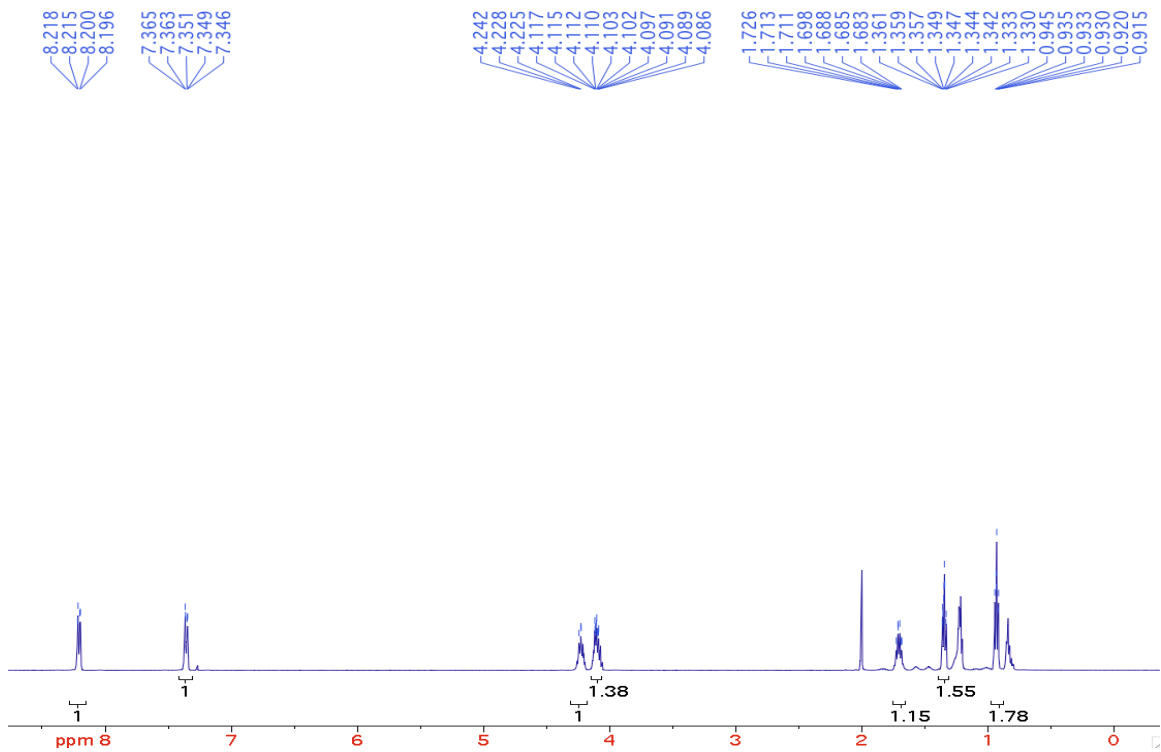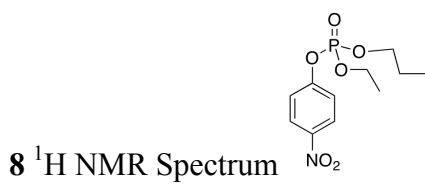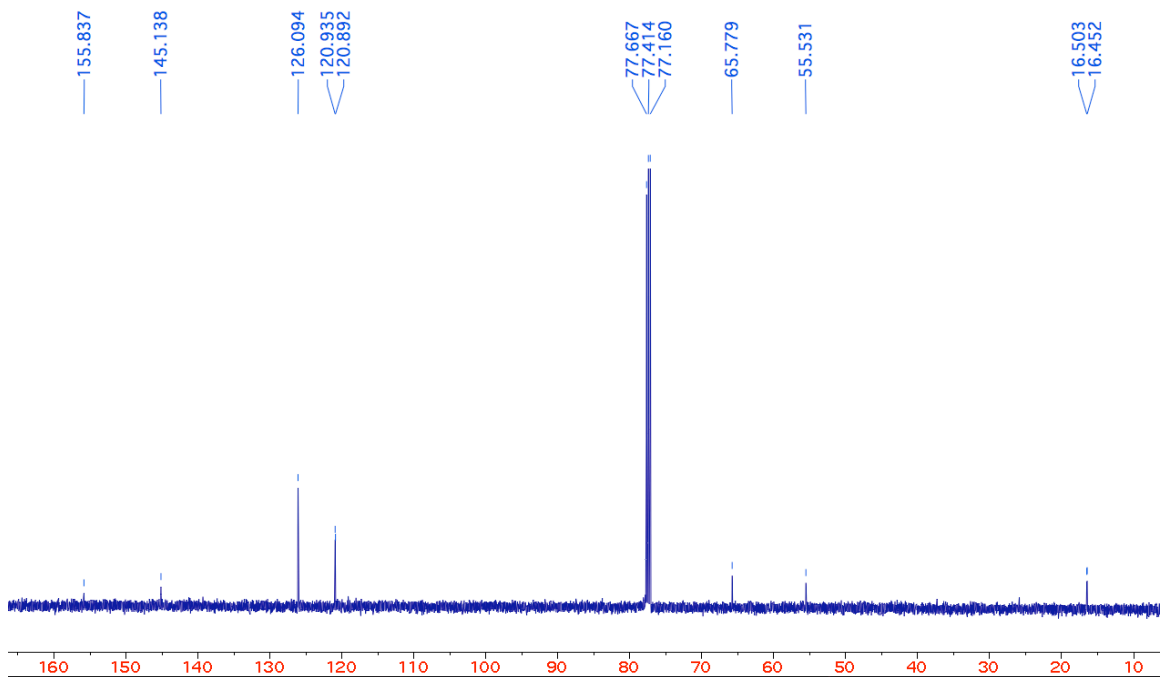|			
	4.01(4H,m), 1.63-1.61(4H, m), 1.27-1.14 (16H, m), 0.8-0.6 (6H, m)	30.2, 29.1, 25.3, 22.5, 13.9	1023,	Expt 466.2292
C	8.24 (2H, d, J 10Hz), 7.38 (2H, d, J 10Hz), 4.26-4.24 (2H, m), 4.18-4.17(2H, m), 1.71-1.70 (2H, m), 1.40-1.37 (3H, m), 1.28-1.25 (10H, m), 0.89-0.86 (3H, m)	155.8, 144.8, 125.8, 120.7, 69.4, 65.4, 31.9, 30.4, 29.3, 25.5, 22.8, 16.3, 14.2	2955, 1590, 1346, 1295, 1028,	Calc (M+Na)$^+$ 382.1395 Expt 382.1390

1 ^{1}H NMR Spectrum

^{13}C NMR Spectrum

160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

155.782
144.887
125.891
120.747
77.535
77.280
77.026
65.426
65.379
16.330
16.278

6 ^{1}H NMR Spectrum

8.259
8.257
8.240
7.391
7.390
7.389
7.262
3.914
3.891

0.997
1
3.02

ppm 8   7   6   5   4   3   2   1

^{13}C NMR Spectrum

S7

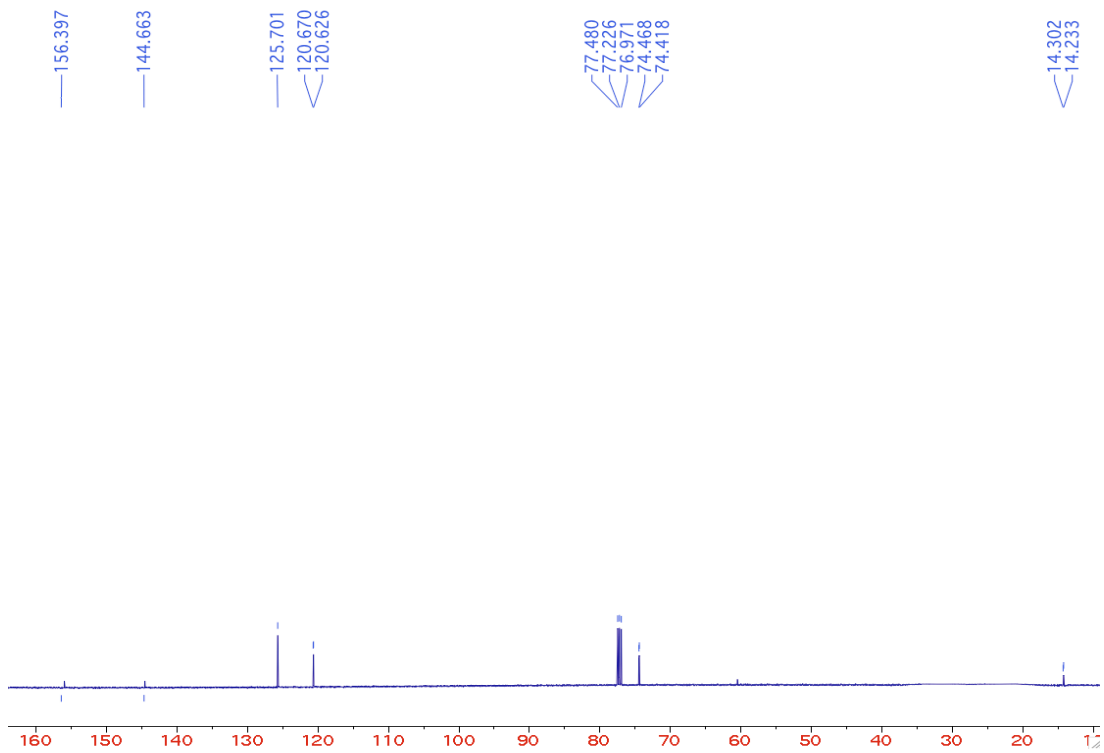**13C NMR Spectrum values (top):** 155.333, 144.822, 125.744, 120.539, 120.497, 77.285, 77.031, 76.777, 55.318, 55.273

7 ^{1}H NMR Spectrum

1H NMR values: 8.259, 8.241, 7.395, 7.394, 7.391, 7.380, 7.377, 7.375, 7.266, 4.287, 4.282, 4.273, 4.270, 4.268, 4.265, 4.256, 4.251, 3.905, 3.883, 1.403, 1.402, 1.401, 1.389, 1.388, 1.387, 1.375, 1.374

^{13}C NMR Spectrum

13C NMR peaks: 155.837, 145.138, 126.094, 120.935, 120.892, 77.667, 77.414, 77.160, 65.779, 55.531, 16.503, 16.452

8 ^{1}H NMR Spectrum

1H NMR peaks: 8.218, 8.215, 8.200, 8.196, 7.365, 7.363, 7.351, 7.349, 7.346, 4.242, 4.228, 4.225, 4.117, 4.115, 4.112, 4.110, 4.103, 4.102, 4.097, 4.091, 4.089, 4.086, 1.726, 1.713, 1.711, 1.698, 1.688, 1.685, 1.683, 1.361, 1.359, 1.357, 1.349, 1.347, 1.344, 1.342, 1.333, 1.330, 0.945, 0.935, 0.933, 0.930, 0.920, 0.915

Integrations: 1, 1, 1.38, 1, 1.15, 1.55, 1.78

^{13}C NMR Spectrum

S9

155.573
144.601
125.658
120.509
70.602
65.155
23.592
16.113
9.922

9 ^{1}H NMR Spectrum

8.192
8.174
7.344
7.343
7.326
7.324
4.767
4.755
4.754
4.742
4.741
4.729
4.207
4.202
4.192
4.190
4.188
4.185
4.176
4.171
1.343
1.330
1.318
1.316
1.304
1.302
1.291

1.01
1.06
1
0.455
4.67

ppm 8   7   6   5   4   3   2   1

^{13}C NMR Spectrum

13C NMR

155.661
155.610
144.527
125.556
120.538
120.495
77.408
77.153
76.898
74.571
74.521
65.003
64.953
50.361
23.409
16.003
15.947

pm 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

10 ^{1}H NMR Spectrum

8.235
8.234
8.221
8.220
8.216
7.384
7.382
7.381
7.368
7.366
7.364
7.269
4.143
4.140
4.137
4.134
4.132
4.130
1.747
1.738
1.733
1.732
1.723
1.720
1.719
0.966
0.958
0.956
0.953
0.951

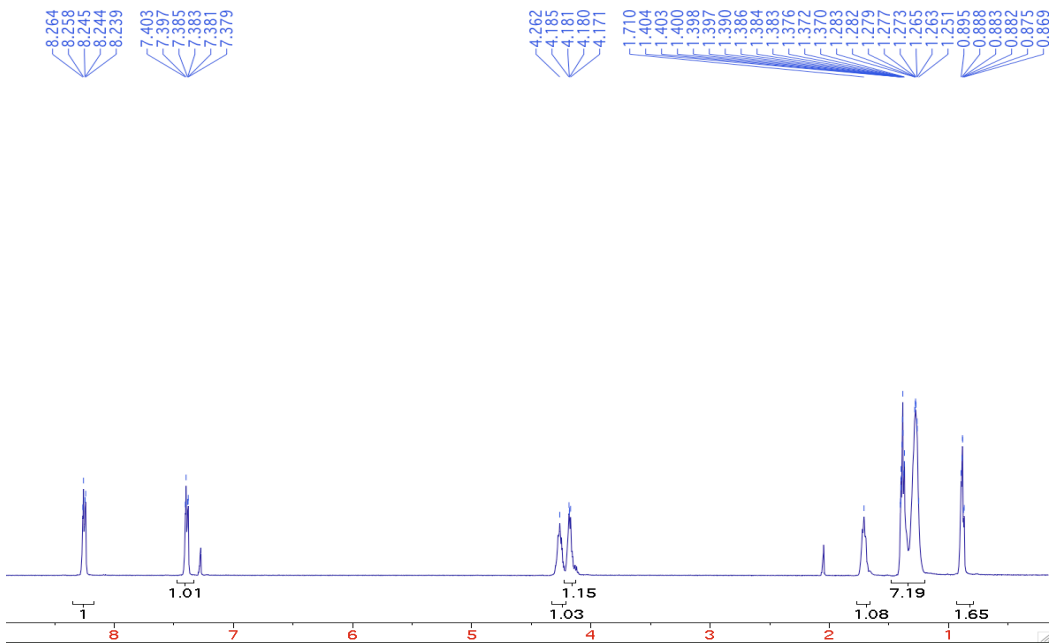1
1.01
2.24
2.14
3.22

ppm 8 7 6 5 4 3 2 1 0

^{13}C NMR Spectrum

11 ^{1}H NMR Spectrum

^{13}C NMR Spectrum

¹³C NMR peaks:
156.397, 144.663, 125.701, 120.670, 120.626, 77.480, 77.226, 76.971, 74.468, 74.418, 14.302, 14.233

160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 1?

12 ¹H NMR Spectrum

8.245, 8.239, 8.227, 7.401, 7.384, 7.260, 5.294, 4.027, 4.010, 3.995, 1.208, 0.612, 0.596, 0.331, 0.327, 0.325

1   1   1.89   1.22   1.89   1.89

ppm 8   7   6   5   4   3   2   1   0

¹³C NMR Spectrum

S13

13 ^{1}H NMR Spectrum

^{13}C NMR Spectrum

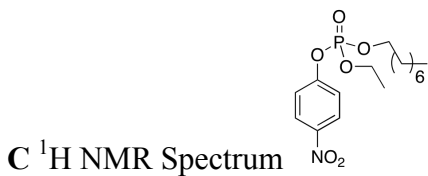**14** ^{1}H NMR Spectrum

^{13}C NMR Spectrum

13C NMR Spectrum (top spectrum)

155.774
155.725
144.779
125.776
120.675
120.632
77.414
77.160
76.906
69.289
69.238
65.300
65.251
30.004
29.950
27.586
22.243
16.244
16.191
14.008

160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

15 ¹H NMR Spectrum   SAR129

8.259
8.258
8.255
8.253
8.240
8.239
7.402
7.400
7.398
7.396
7.383
7.382
4.543
4.537
4.530
4.525
4.268
4.264
4.253
4.251
4.249
4.247
4.244
4.237
4.233
1.969
1.936
1.935
1.777
1.769
1.751
1.745
1.560
1.553
1.540
1.534
1.397
1.396
1.393
1.302
1.299
1.294
1.279
1.274
1.267
1.265
1.260

0.985   0.974   1.05 3.21
1   0.478   1.03 1.57

ppm 8   7   6   5   4   3   2   1

¹³C NMR Spectrum

S16

155.767
155.718
144.540
125.634
120.576
120.533
79.201
79.150
77.323
77.069
76.814
65.005
64.956
33.265
33.231
33.179
33.142
24.952
23.404
16.072

150 140 130 120 110 100 90 80 70 60 50 40 30 20

16 ^{1}H NMR Spectrum

8.215
8.215
8.200
8.197
7.361
7.359
7.344
7.342
7.260
4.192
4.190
4.178
4.176
4.173
4.162
3.617
3.609
3.600
3.212
3.206
3.202
3.198
3.191
1.364
1.363
1.358
1.350
1.349
1.336
1.334

1   0.998   1   2.08   2.06   1.61

ppm 8   7   6   5   4   3   2   1

^{13}C NMR Spectrum

S17

156.058
156.005
144.436
125.628
120.604
120.561
77.345
77.091
76.836
66.826
66.783
63.795
63.750
44.650
16.144
16.092

160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

A ^{1}H NMR Spectrum

8.194
8.190
8.179
8.176
7.352
7.351
7.334
7.332
4.146
4.139
4.133
4.124
4.118
4.111
1.670
1.656
1.655
1.641
1.318
1.247
1.240
1.233
1.226
1.219
0.835
0.821
0.807

1
1
1.98
1.93
6.37
3.26

ppm 8   7   6   5   4   3   2   1

^{13}C NMR Spectrum

B ^{1}H NMR Spectrum

^{13}C NMR Spectrum

C ^{1}H NMR Spectrum

^{13}C NMR Spectrum

155.859, 155.809, 144.862, 125.857, 120.754, 120.711, 77.490, 77.236, 76.981, 69.395, 69.344, 65.377, 65.331, 31.929, 30.408, 30.355, 29.325, 29.209, 25.553, 22.812, 16.332, 16.280, 14.259

Group II

17 **18** **D** **E** **F**

G **H** **I** **J** R =

Molecules **17, 18, D-H** are known and they were synthesized and characterized following reported procedure.[1]

Molecule	¹H NMR	¹³C NMR	IR	MS
I	7.31-7.29 (2H, m), 7.20-7.18 (2H, m), 7.12-7.14 (1H, m), 4.09-4.11 (4H, m), 1.62-1.66 (4H, m), 1.26-1.24 (20H, m), 0.85 (6H, t, *J* 5Hz)	151.0, 129.8, 125.1, 120.2, 68.8, 31.9, 30.5, 29.3, 25.6, 22.8, 14.2	2927, 1491, 1213, 1024, 945	Calc (M+Na)⁺ 398.2586 Expt 398.2582
J	7.33-7.30 (2H, m), 7.21-7.14 (3H, m),	150.7, 132.2, 129.7, 125.1,	3079, 2880, 1594, 1486,	Calc (M+Na)⁺

	5.94-5.90 (2H, m), 5.37.5.34 (2H, m), 5.25-5.23 (2H, m), 4.63-4.62 (4H, m)	120.1, 118.6, 68.8	1282, 1207, 1017	277.0606 Expt 277.0607

SAR70 ^{1}H NMR Spectrum

^{13}C NMR Spectrum

<superscript>13</superscript>C NMR labels:

151.040

129.818
125.068
120.196
120.154

77.570
77.315
77.061
68.763
68.712

31.950
30.463
30.406
29.345
29.252
25.595
22.825
14.254

ppm 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

J ^{1}H NMR Spectrum

7.339
7.325
7.307
7.222
7.219
7.217
7.206
7.204
7.202
7.176
7.161
7.160
7.147

5.944
5.935
5.933
5.931
5.921
5.910
5.900

5.378
5.375
5.372
5.344
5.341
5.257
5.236

4.637
4.631
4.621

0.501 0.728   0.473   0.256 0.492   1
                        0.227

7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0

^{13}C NMR Spectrum
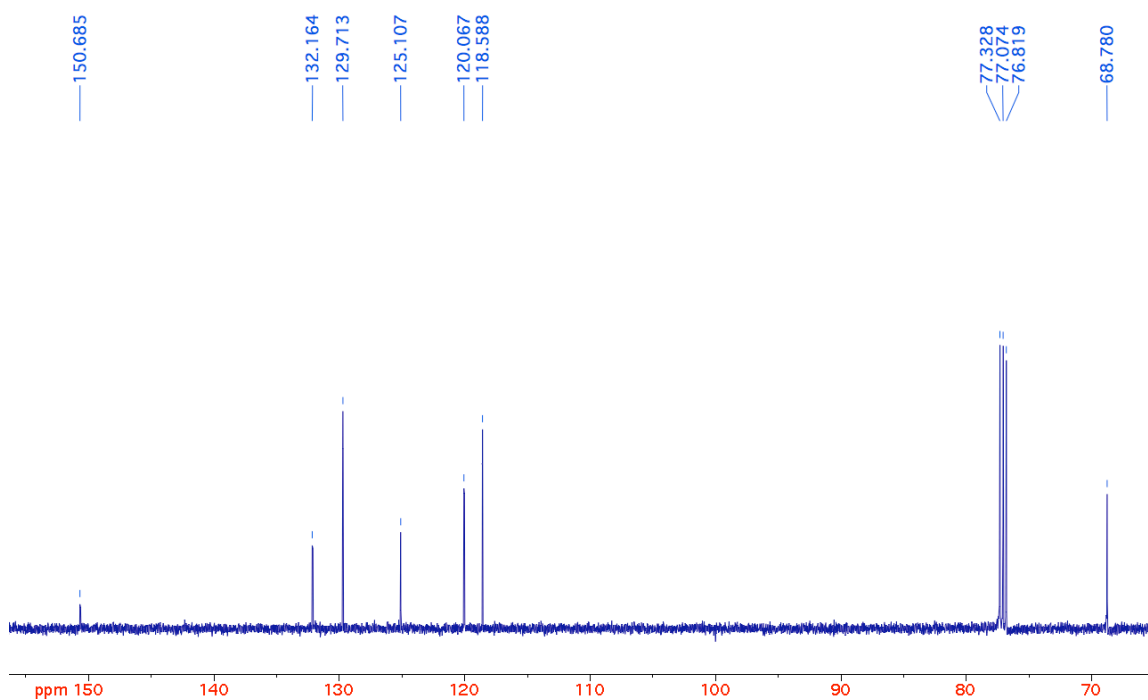
Group III
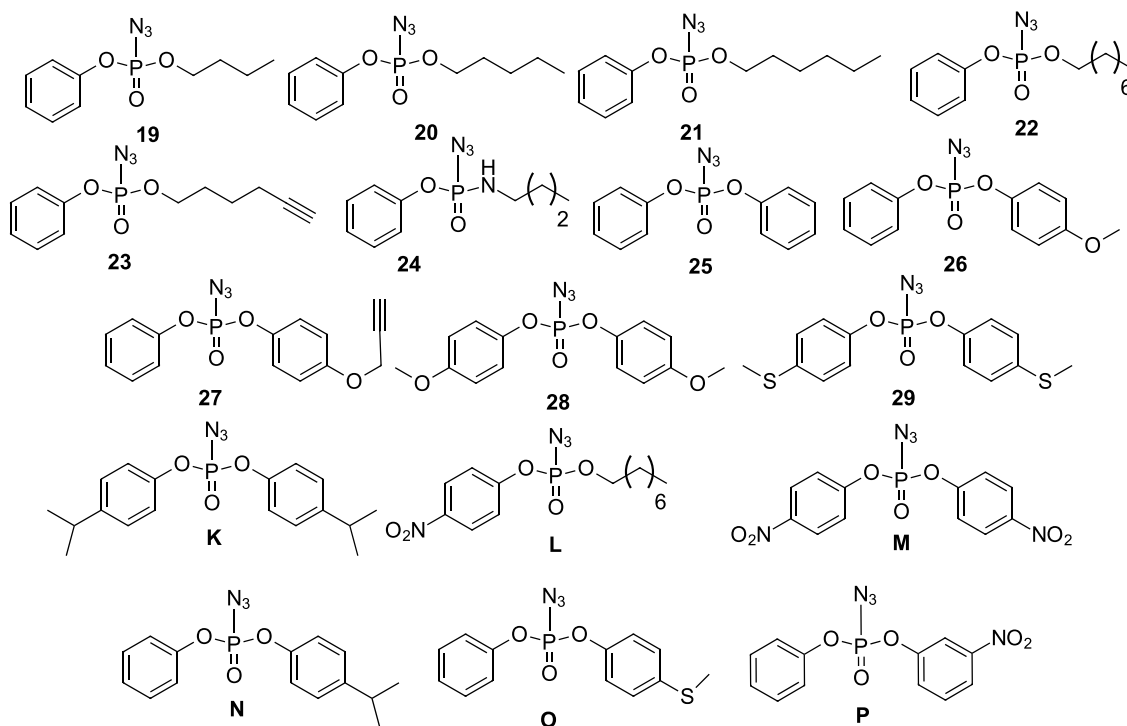
Molecules **M** and **25** are commercially available.

Molecule	1H NMR	13C NMR	IR	MS
19	7.40-7.37 (2H, m), 7.28-7.24 (3H, m), 4.28-4.24 (2H, m), 1.77-1.71 (2H, m), 1.46-1.42 (4H, m), 0.96 (3H, t, J 5Hz)	150.2, 130.2, 126.1, 120.4, 69.7, 32.3, 18.8, 13.7	2970, 2164, 1511, 1209	Calc (M+Na)$^+$ 278.0670 Expt 278.0673
20	7.41-7.38 (2H, m), 7.28-7.25 (3H, m), 4.28-4.23 (2H, m), 1.78-1.75 (2H, m), 1.39-1.35 (4H, m), 0.93 (3H, t, J 5Hz)	150.0, 130.0, 125.8, 120.2, 69.8, 31.2, 30.0, 24.9, 22.5, 14.0	2962, 2171, 1589, 1216	Calc (M+Na)$^+$ 292.0827 Expt 292.0829
21	7.37-7.34 (2H, m), 7.23-7.21 (3H, m), 4.24-4.19 (2H, m), 1.73-1.69 (2H, m), 1.38-1.27 (6H, m), 0.87 (3H, t, J 5Hz)	150.2, 130.2, 126.0, 120.4, 69.9, 31.4, 30.2, 25.2, 22.7, 14.2	2929, 2171, 1593, 1217	Calc (M+Na)$^+$ 306.2532 Expt 306.2530
22	7.37-7.34 (2H, m), 7.23-7.21 (3H, m), 4.24-4.19 (2H, m), 1.73-1.70 (2H, m), 1.38-1.25 (10H, m), 0.87 (3H, t, J 5Hz)	150.0, 129.5, 125.8, 120.2, 69.8, 31.7, 30.1, 30.0, 29.1, 25.3, 22.6, 14.1	2969, 2156, 1588, 1291	Calc (M+Na)$^+$ 334.1296 Expt 334.1291
23	7.38-7.35 (2H, m), 7.24-7.21 (3H, m), 4.28-4.24 (2H, m), 2.24-2.22 (2H, m), 1.98 (1H, s), 1.88-1.85 (2H, m), 1.65-1.60 (2H, m),	149.9, 130.0, 125.9, 120.2, 83.4, 69.1, 60.4, 29.1, 24.2, 21.0, 17.9	3303, 3252, 2949, 2167, 1490, 1271	Calc (M+H)$^+$ 280.0846 Expt 280.0842
24	7.37-7.34 (2H, m), 7.24-7.19 (2H, m), 3.14 (1H, bs), 3.07-3.03 (2H, m), 1.52-1.49(2H, m), 1.37-1.33 (2H, m), 0.92 (3H, t, J 5Hz)	150.4, 130.2, 125.8, 120.6, 41.7, 33.7, 19.9, 13.9	3219, 2962, 2143, 1595, 1256	Calc (M+Na)$^+$ 277.0830 Expt 277.0825
26	7.39-7.38 (3H, m), 7.19-7.17 (4H, m), 6.89-6.88 (2H, m), 3.80 (3H, s)	157.6, 150.0, 143.5, 130.2, 126.2, 121.2, 120.4, 115.0, 55.8	3111, 2947, 1589, 1339, 1216	Calc (M+H)$^+$ 306.0644 Expt 306.0647
27	7.41-7.38 (2H, m), 7.26-7.22 (3H, m), 6.98	155.6, 150.0, 144.2, 130.2,	3294, 3024, 2919, 2171,	Calc (M+H)$^+$ 330.0644

	(2H, d, J 10Hz), 4.69 (2H, s), 2.54 (4H, s)	126.3, 121.4, 120.4, 116.3, 116.3, 78.3, 76.1, 54.5	1508, 1297, 1161	Expt 330.0641
28	7.18(4H, d, J 10 Hz), 6.88(4H, d, J 10Hz), 3.77(6H, s)	157.0, 144.2, 121.1, 114.7, 55.6	3073, 2837, 2157, 1501, 1300, 958	Calc (M+Na)$^+$ 358.0569 Expt 358.0565
29	7.26(2H, d, J 10Hz), 7.16(2H, d, J 10Hz), 2.42(6H, s)	157.0, 144.2, 121.1, 114.7, 55.6	2170, 1487, 1191, 967	Calc (M+Na)$^+$ 390.0079 Expt 390.0078
K	7.24-7.20 (4H, m), 2.92 (2H, m), 3.77(6H, d, J 5Hz)	147.9, 146.7, 127.9. 120.0, 33.6, 23.9	2962, 2100, 1506, 974	Calc (M+Na)$^+$ 382.1296 Expt 382.1283
L	8.27 (2H, d, J 10Hz), 7.40 (2H, d, J 10Hz), 4.30-4.25 (2H, m), 1.77-1.72 (2H, m), 1.28-1.26(10H, m), 0.88 (3H, t, J 5Hz)	154.5, 145.3, 125.8, 120.9, 70.4, 31.7, 30.0, 29.1, 28.9, 25.2, 22.6, 14.0	2929, 2170, 1526, 1348, 1025	Calc (M+Na)$^+$ 379.1147 Expt 379.1149
N	7.42-7.19 (9H, m), 2.93-2.92 (1H, m), 1.27 (6H, d, J 5Hz)	146.8, 130.0, 127.9, 126.1, 120.3, 120.0, 33.6, 24.0	3097, 2917, 2158, 1589, 1190	Calc (M+Na)$^+$ 340.0827 Expt 340.0824
O	7.42-7.19 (5H, m), 7.22-7.21 (4H, m), 2.50 (3H, s)	149.9, 147.5, 136.4, 130.1, 128.4, 126.1, 120.8, 16.4	3014, 2962, 2171, 16112, 1112	Calc (M+H)$^+$ 322.0415 Expt 322.0416
P	8.28-8.26 (3H, m), 7.43-7.39 (3H, m), 7.25-7.24 (3H, m)	154.5, 149.8, 145.5, 130.3, 126.0, 120.8, 119.9, 115.6	3067, 2955, 2156, 1589, 1196	Calc 329.0389 Expt 329.0385
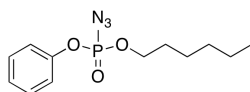
19 ^{1}H NMR Spectrum   SAR132
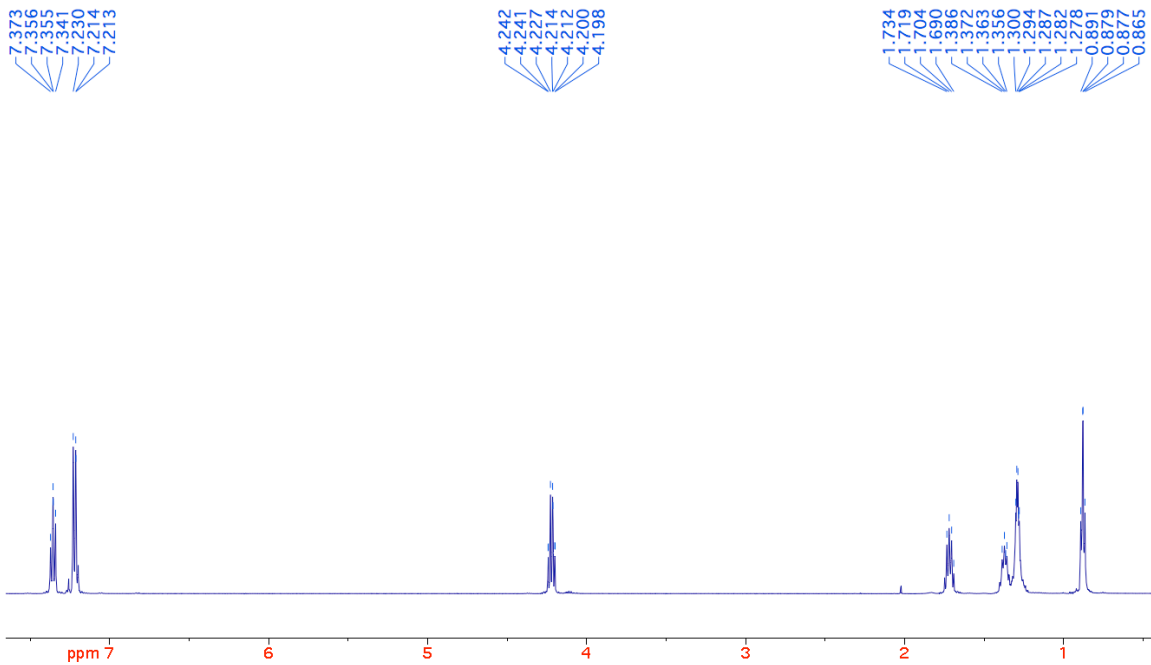
^{13}C NMR Spectrum
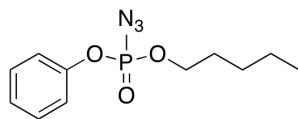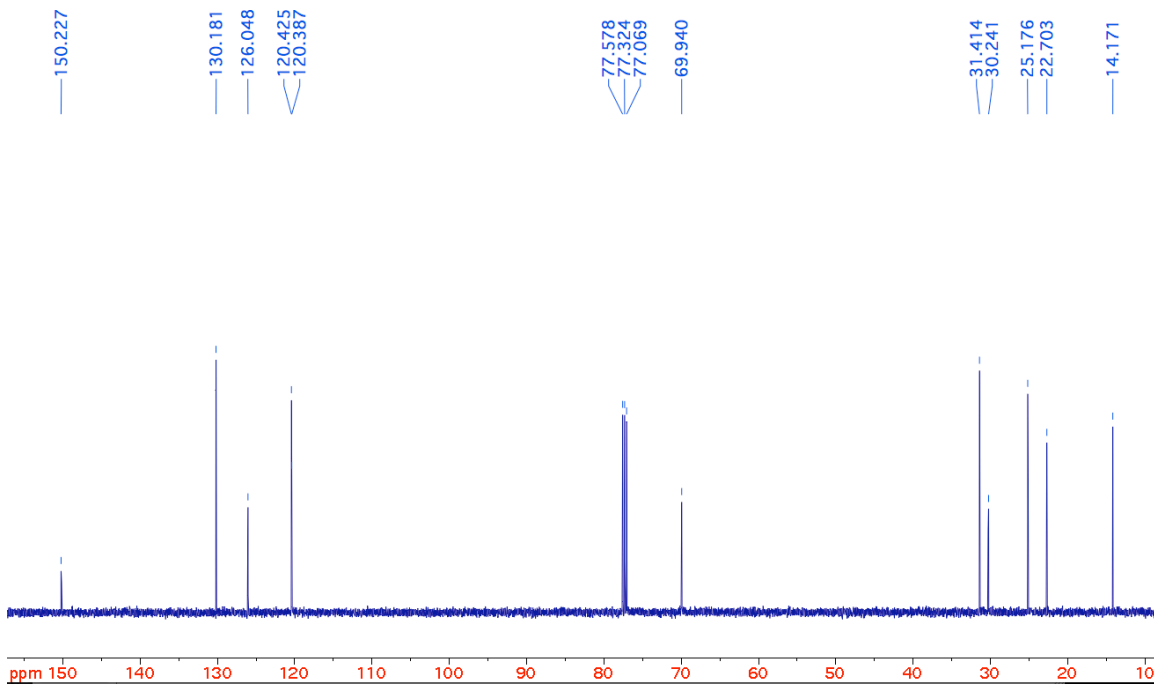
20 ^{1}H NMR Spectrum SAR134

^{13}C NMR Spectrum

21 ^{1}H NMR Spectrum   SAR132

7.373
7.356
7.355
7.341
7.230
7.214
7.213

4.242
4.241
4.227
4.214
4.212
4.200
4.198

1.734
1.719
1.704
1.690
1.386
1.372
1.363
1.356
1.300
1.294
1.287
1.282
1.278
0.891
0.879
0.877
0.865

ppm 7   6   5   4   3   2   1

^{13}C NMR Spectrum

150.227

130.181
126.048
120.425
120.387

77.578
77.324
77.069

69.940

31.414
30.241
25.176
22.703

14.171

ppm 150   140   130   120   110   100   90   80   70   60   50   40   30   20   10

22 ^{1}H NMR Spectrum   SAR133

S29

^{13}C NMR Spectrum

23 ^{1}H NMR Spectrum   SAR131

^{13}C NMR Spectrum

24 ^{1}H NMR Spectrum

^{13}C NMR Spectrum

26 ^{1}H NMR Spectrum

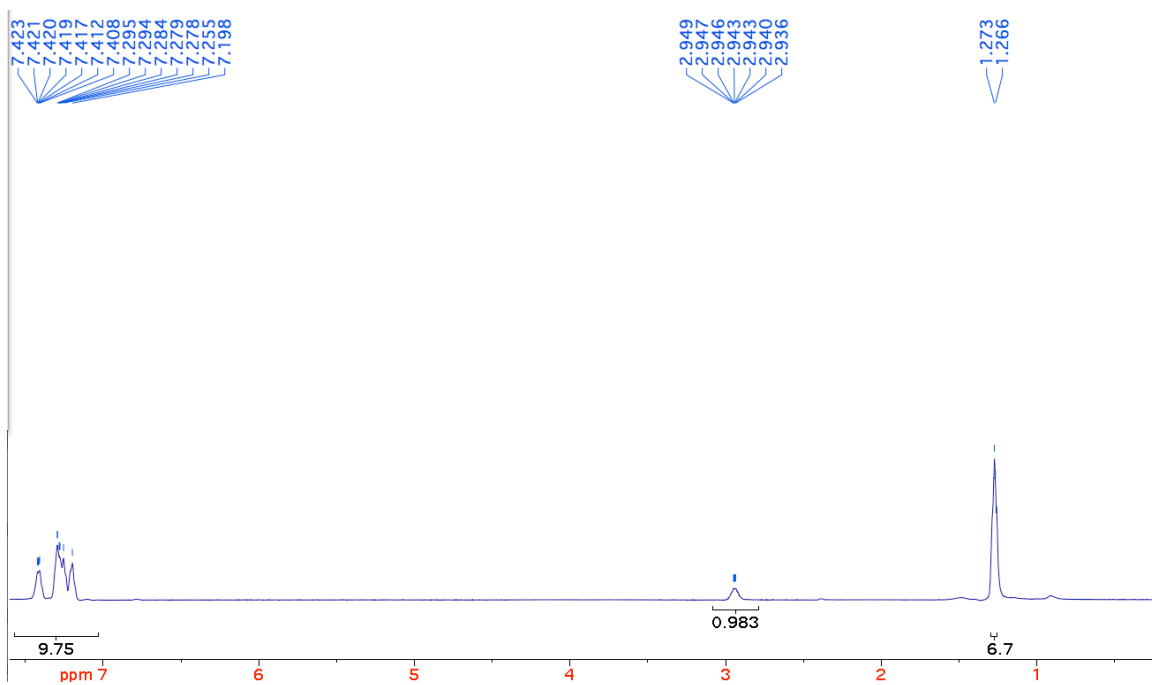## ¹³C NMR Spectrum

27 ¹H NMR Spectrum

^{13}C NMR Spectrum

28 ^{1}H NMR Spectrum

7.190
7.167
6.889
6.867

3.777

1.03

1

1.52

ppm 8   7   6   5   4   3   2   1   0

^{13}C NMR Spectrum

157.046
144.180
121.064
114.746
77.356
77.039
76.721
55.645

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

29 ^{1}H NMR Spectrum

^{13}C NMR Spectrum

7.244
7.242
7.224
7.181
7.160

2.429

0.568
0.802

1

ppm 7   6   5   4   3   2   1   0

157.046
144.180
121.064
114.740
77.356
77.039
76.721
55.645

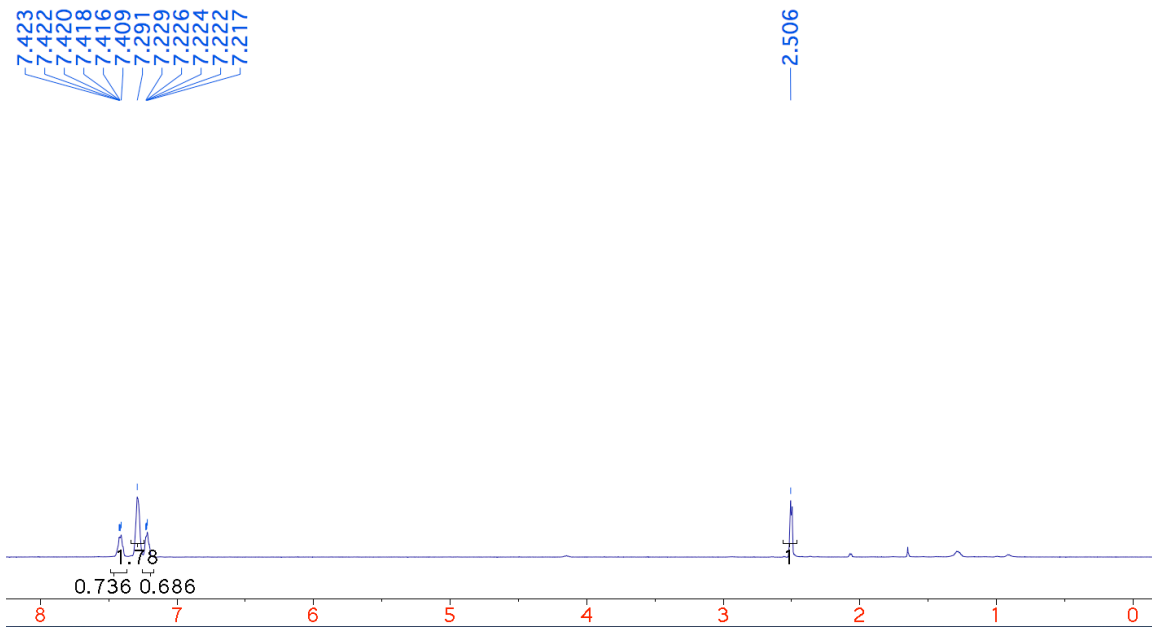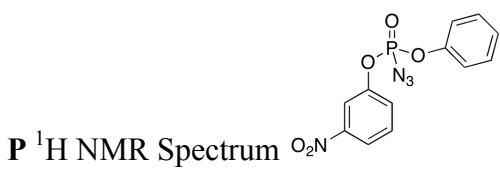ppm 200   180   160   140   120   100   80   60   40   20   0

K ^{1}H NMR Spectrum

^{13}C NMR Spectrum

L ^{1}H NMR Spectrum

¹³C NMR Spectrum

N ¹H NMR Spectrum

S38

7.423, 7.421, 7.420, 7.419, 7.417, 7.412, 7.408, 7.295, 7.294, 7.284, 7.279, 7.278, 7.255, 7.198
2.949, 2.947, 2.946, 2.943, 2.943, 2.940, 2.936
1.273, 1.266

9.75   0.983   6.7

ppm 7   6   5   4   3   2   1

^{13}C NMR Spectrum

146.829
130.040, 127.914, 126.074, 120.278, 120.242, 119.974, 119.935
77.297, 77.043, 76.789
33.590
24.002

160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

O ^{1}H NMR Spectrum

S39

^{13}C NMR Spectrum

P ^{1}H NMR Spectrum

^{13}C NMR Spectrum

Group IV

30   **31**   **32**   **33**

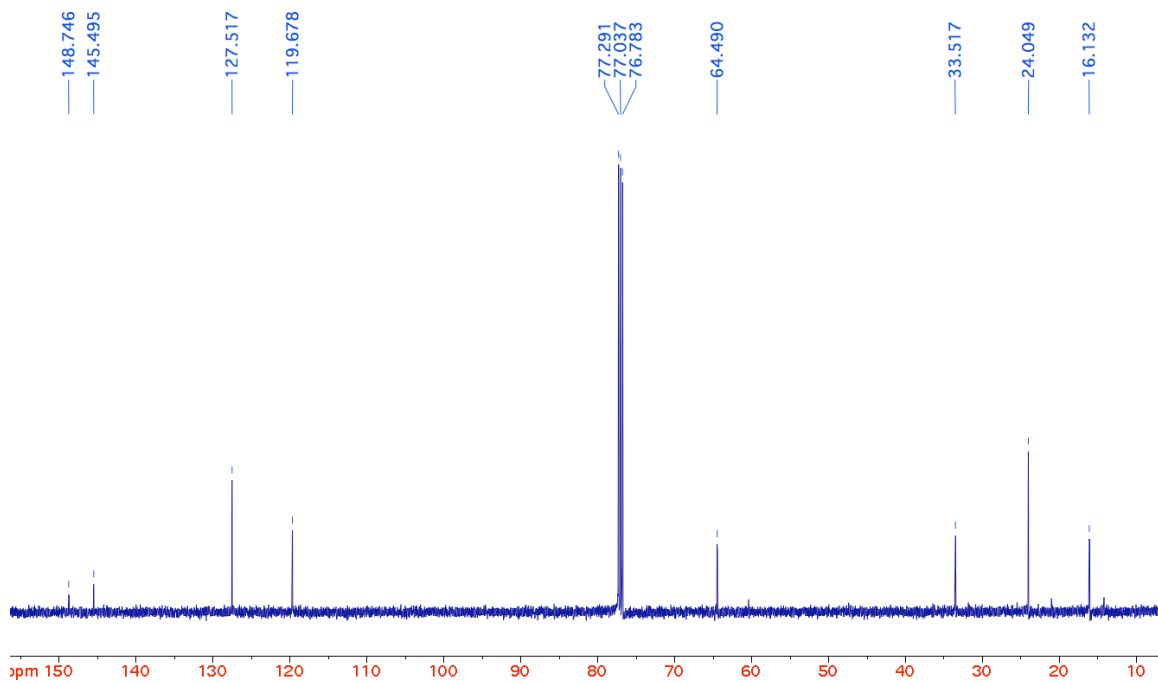**Group IV**

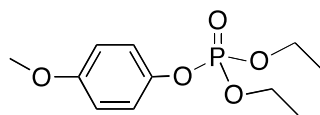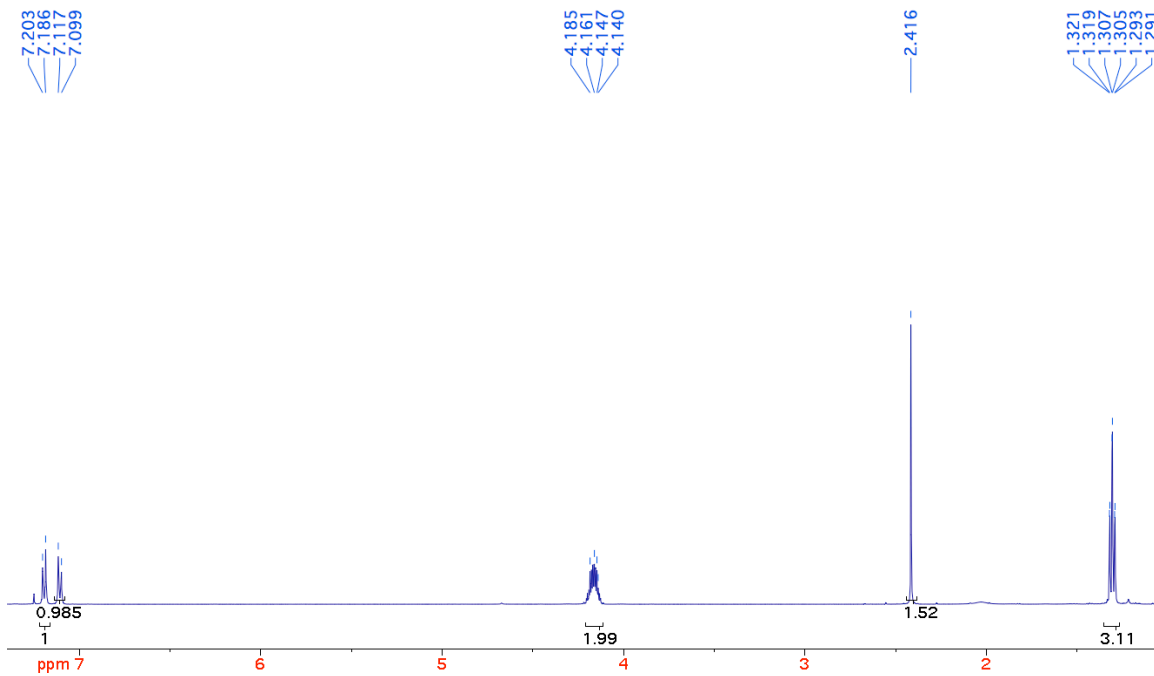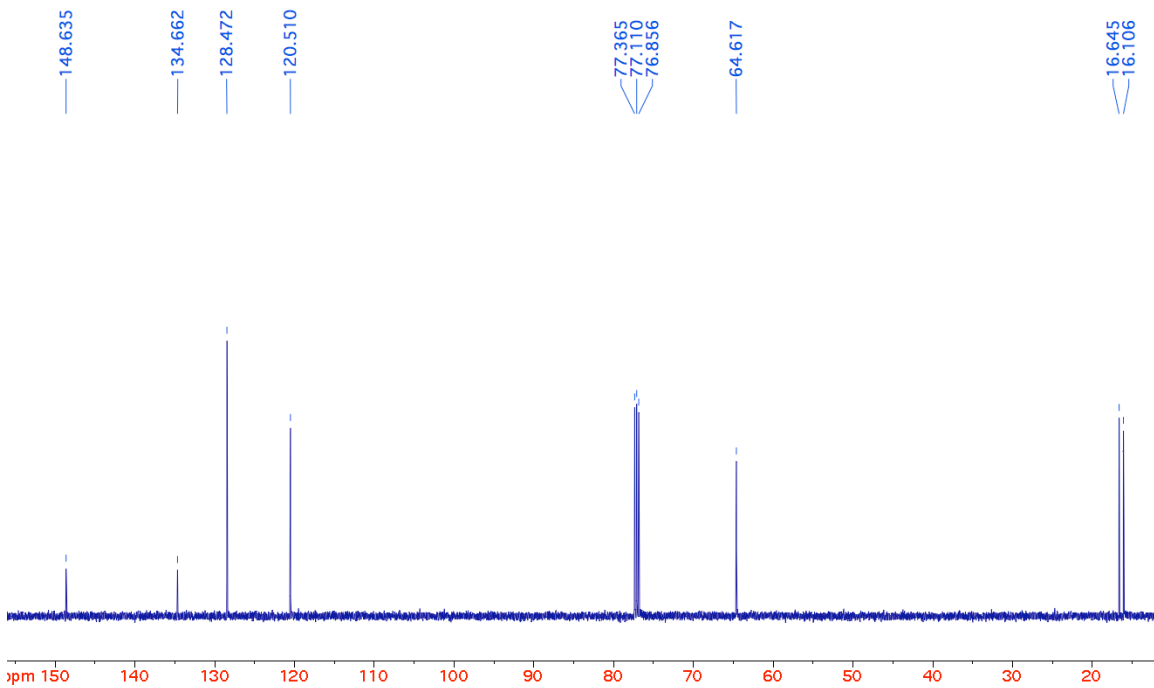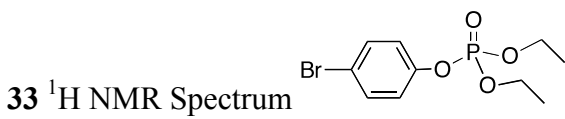| Molecule	^{1}H NMR	^{13}C NMR	IR	MS
30	7.16 (2H, d, *J* 10Hz), 7.12 (2H, q, *J* 10Hz), 4.21-4.20 (4H, m), 2.89-2.88 (1H, m), 1.35 (6H, t, *J* 5Hz), 1.22 (6H, d, *J* 5Hz)	148.7, 145.5, 127.5, 119.7, 64.5, 33.5, 24.1, 16.1	2956, 1602, 1507, 1282, 1216	Calc (M+Na)$^+$ 256.1075 Expt 256.1071
31	7.20 (2H, d, *J* 10Hz), 7.11 (2H, d, *J* 10Hz), 4.16-4.14 (4H, m), 2.41 (3H, s), 1.32-1.29 (6H, m)	148.6, 134.7, 128.5, 120.5, 64.6, 16.6, 16.1	2981, 1490, 1218, 1030, 958, 772	Calc (M+Na)$^+$ 283.0711 Expt 283.0715
32	7.16 (2H, d, *J* 10Hz), 7.07 (2H, q, *J* 10Hz), 4.12 (4H, q, *J* 5Hz), 2.36 (3H, s), 1.28 (6H, t, *J* 5Hz)	148.5, 134.6, 128.3, 120.4, 64.5, 16.5, 16.0	2984, 1491, 1279, 1031, 883, 550	Calc (M+Na)$^+$ 299.0483 Expt 299.0488
33	7.43 (2H, d, *J* 10Hz), 7.10 (2H, q, *J* 10Hz), 4.21-4.17 (4H, m), 1.33 (6H, t, *J* 5Hz)	150.1, 132.9, 122.0, 118.0, 64.9, 16.3	2988, 1485, 1218, 1030, 958, 834	Calc (M+Na)$^+$ 330.9711 Expt 330.9705

30 ^{1}H NMR Spectrum
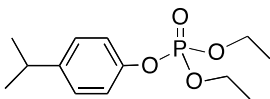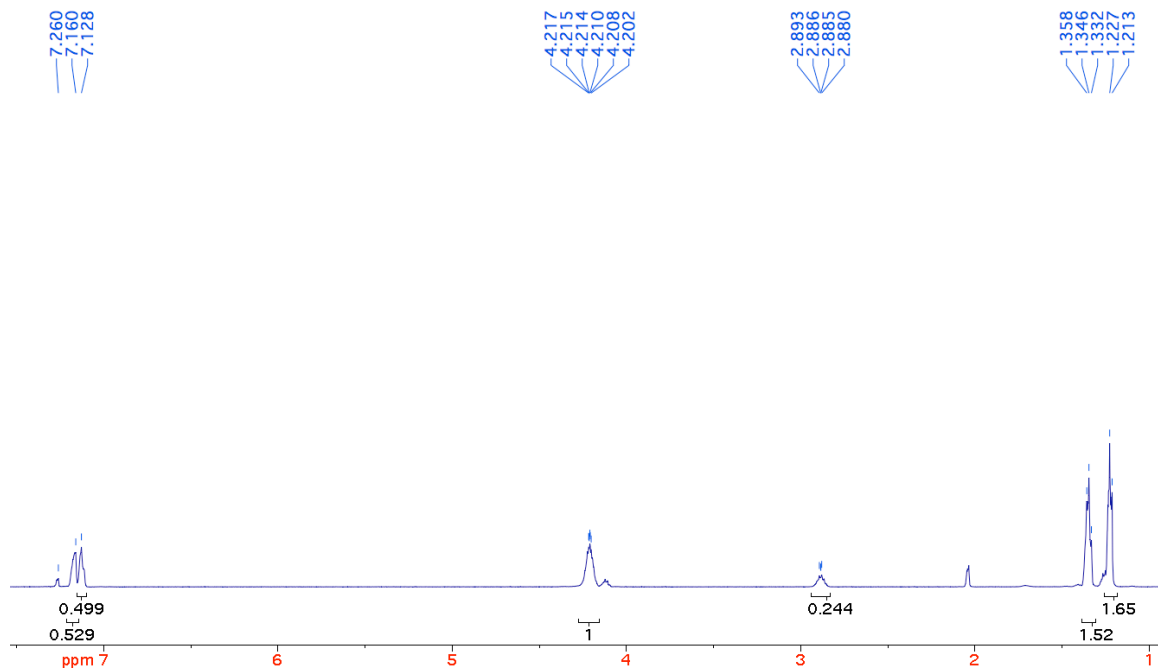
^{13}C NMR Spectrum

31 ^{1}H NMR Spectrum

7.203
7.186
7.117
7.099

4.185
4.161
4.147
4.140

2.416

1.321
1.319
1.307
1.305
1.293
1.291

0.985
1

1.99

1.52

3.11

ppm 7   6   5   4   3   2

^{13}C NMR Spectrum

148.635

134.662
128.472

120.510

77.365
77.110
76.856

64.617

16.645
16.106

ppm 150   140   130   120   110   100   90   80   70   60   50   40   30   20

32 ^{1}H NMR Spectrum

^{13}C NMR Spectrum

33 ^{1}H NMR Spectrum

1.01

1

2.02

3.05

ppm 7   6   5   4   3   2

^{13}C NMR Spectrum

150.072

132.897

122.040
118.091

77.516
77.262
77.007

64.957

16.270

150 140 130 120 110 100 90 80 70 60 50 40 30 20
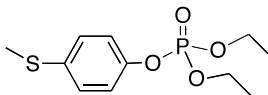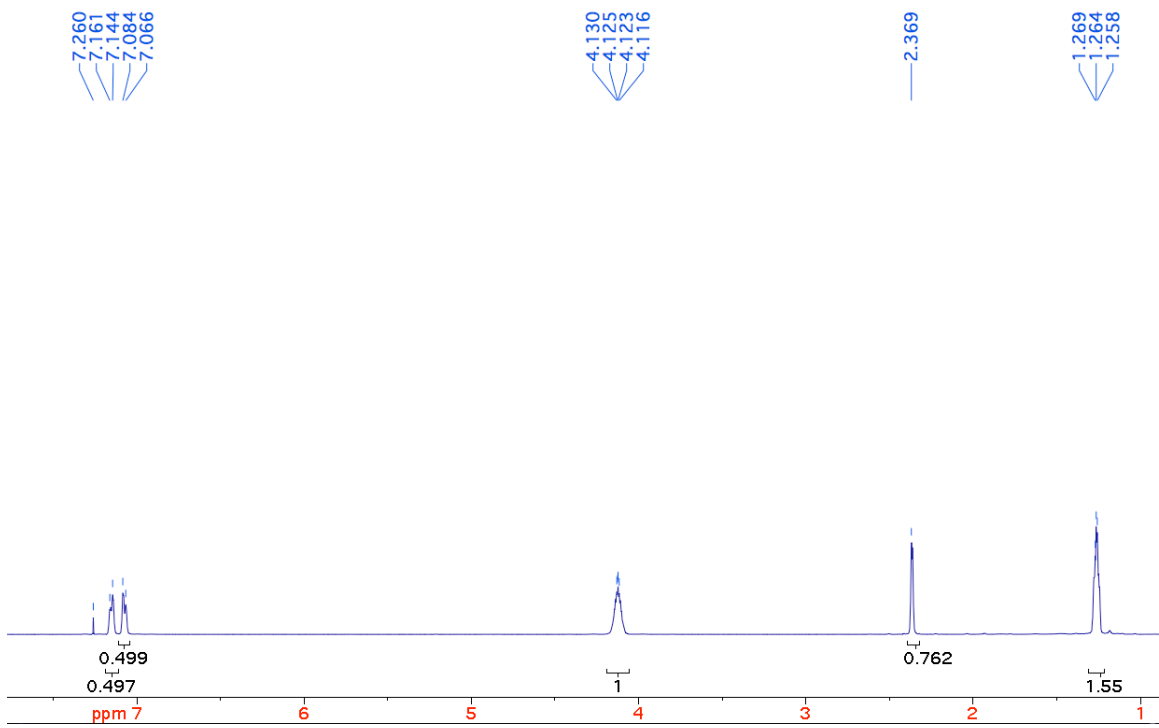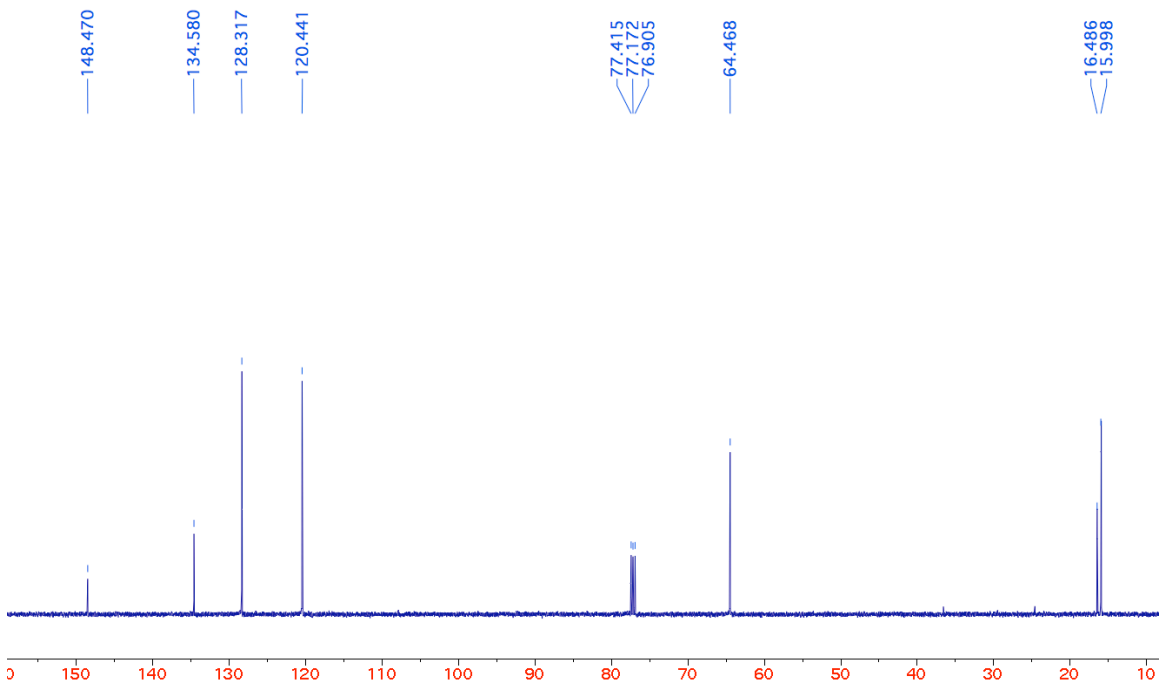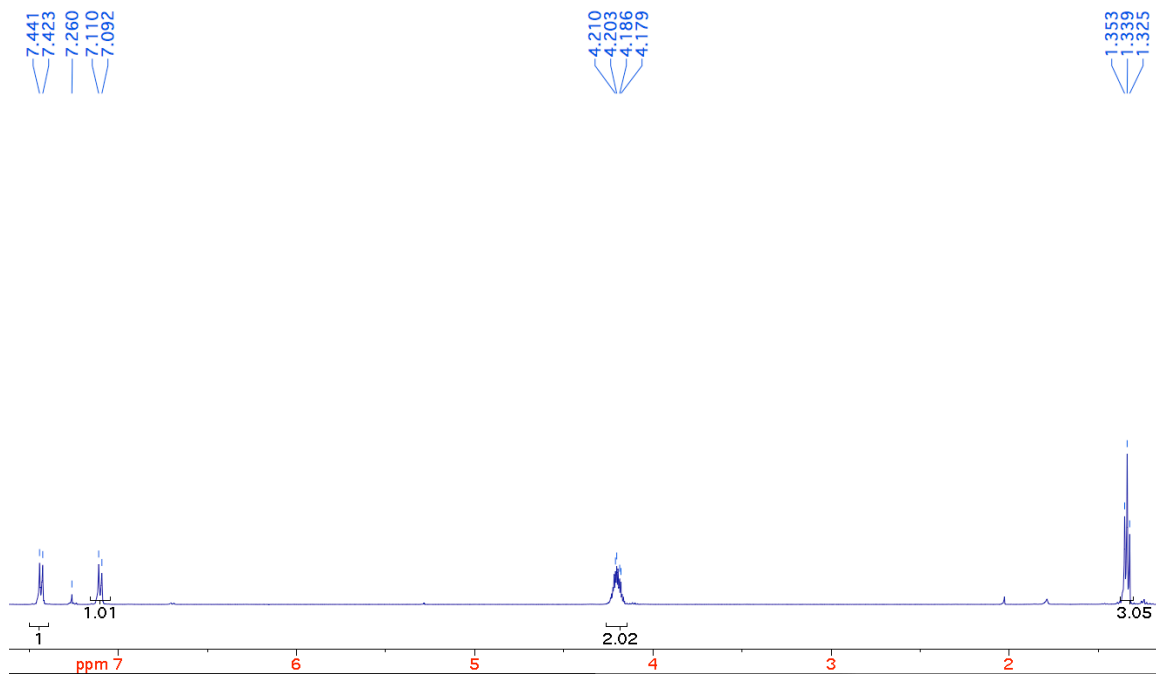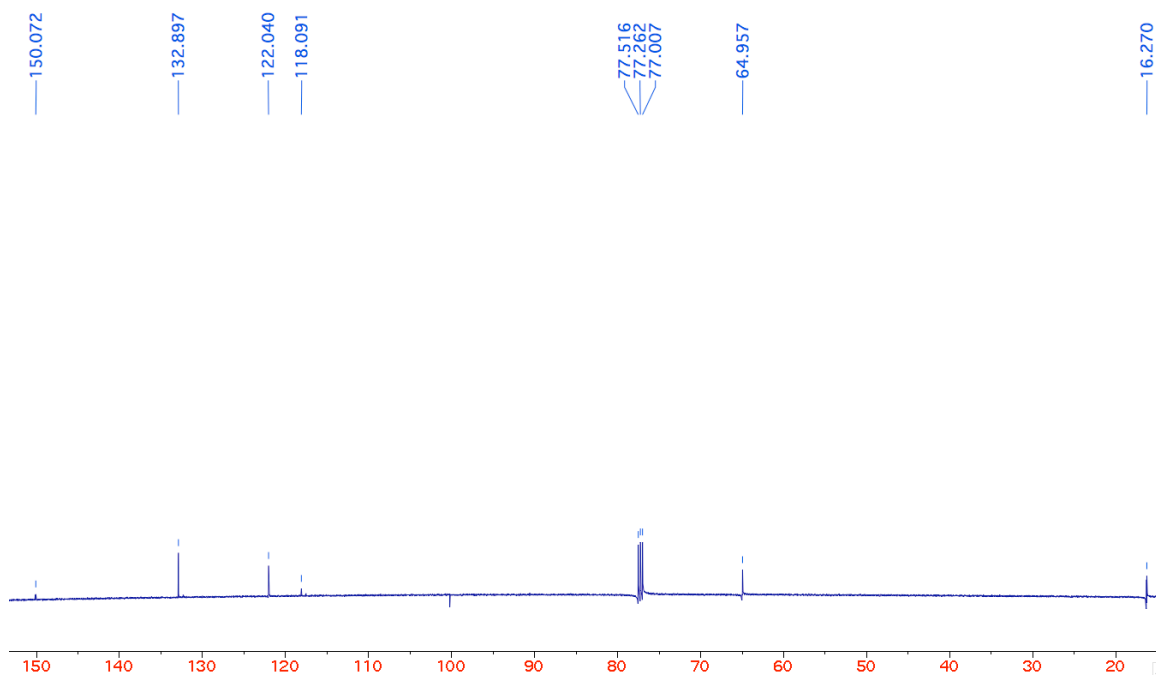
Group V
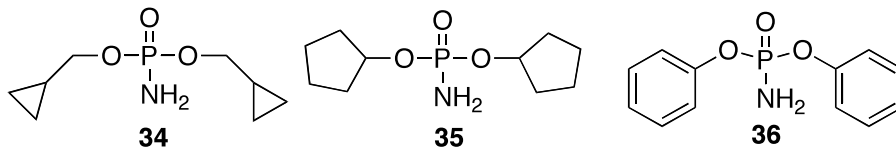
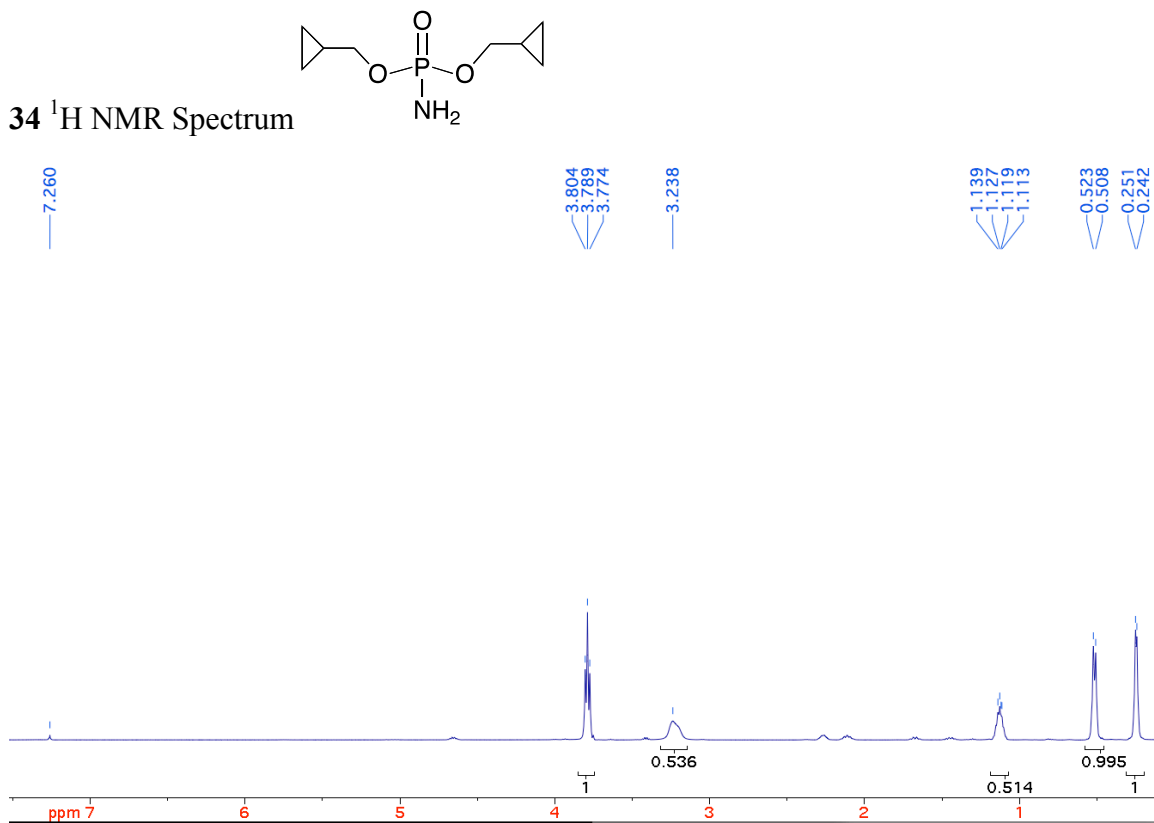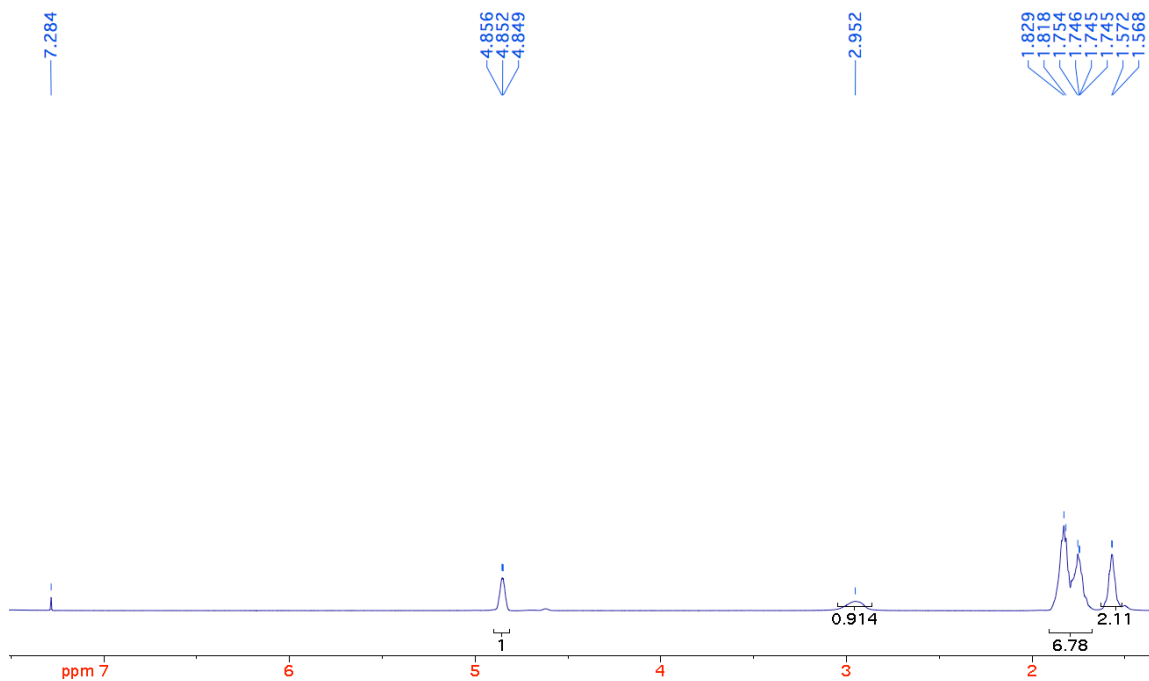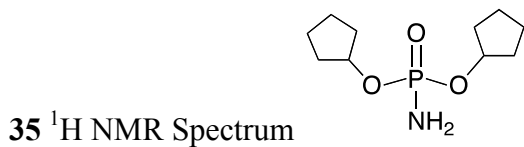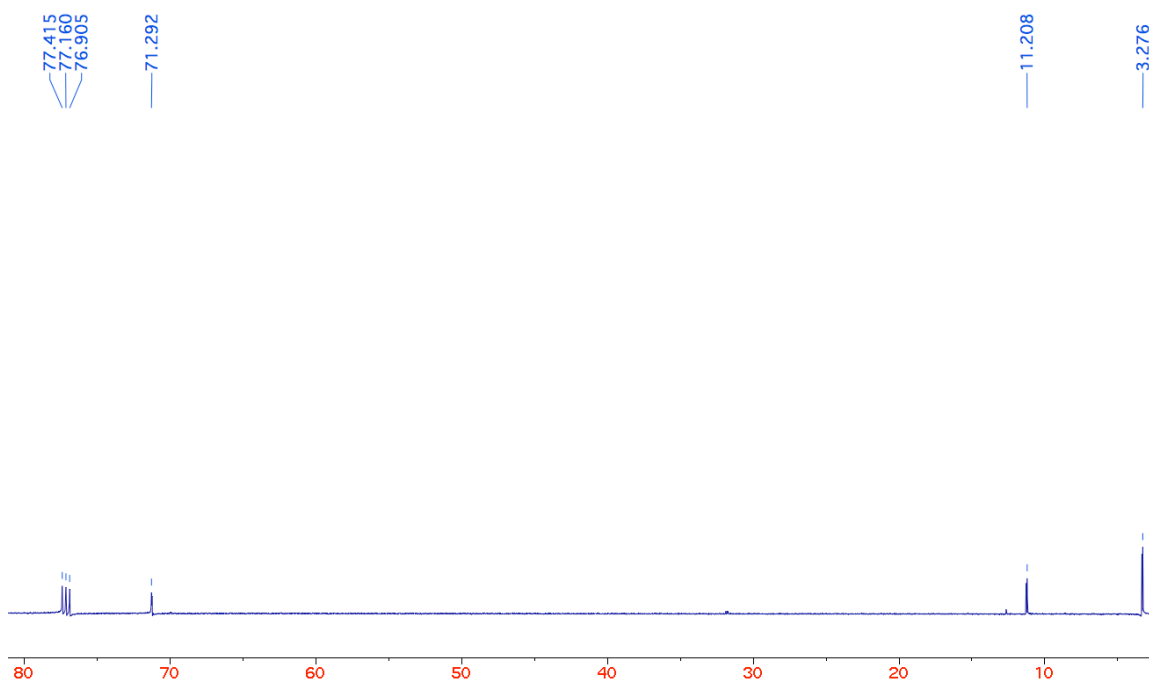**34** and **35** were synthesized following a reported procedure and the two molecules are known DFPase inhibitors.[3] **36** is commercially available
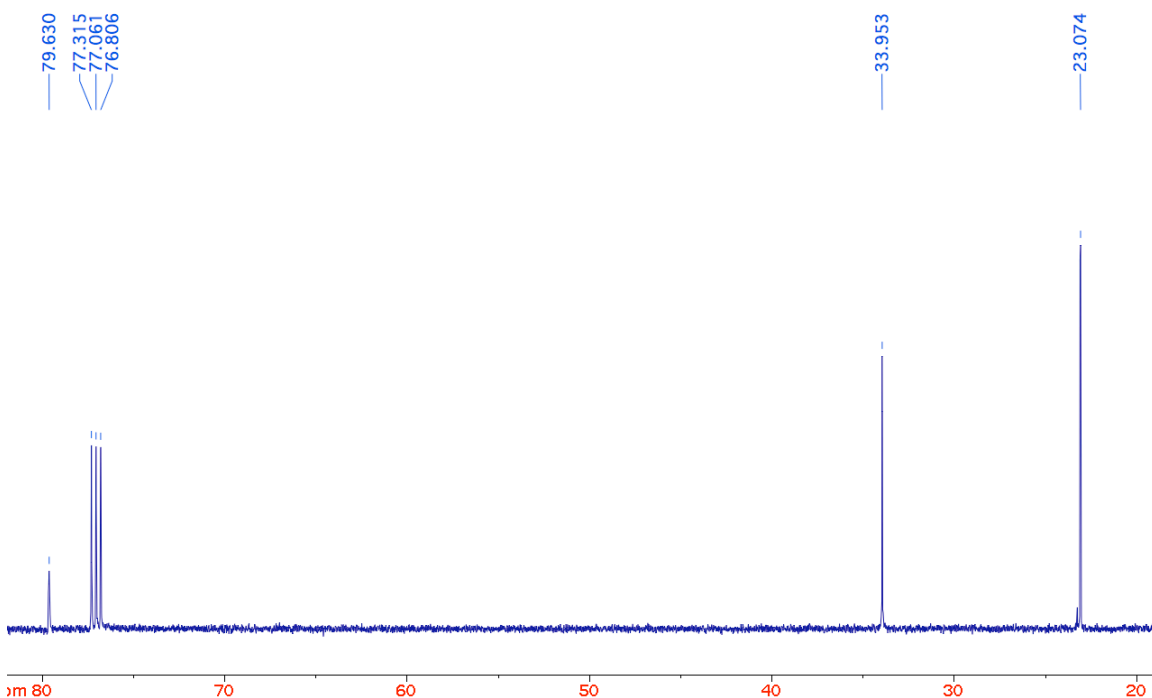
Molecule	¹H NMR	¹³C NMR	IR	MS
34	3.78 (3H, d, *J* 10Hz), 3.23(2H, s), 1.13-1.11 (2H, m), 0.52-0.50 (4H, d, *J* 5Hz), 0.25 (4H, d, *J* 5Hz)	71.3, 11.2, 3.3	3343, 1567, 1226, 1039, 730	Calc (M+Na)$^+$ 228.0766 Expt 228.0767
35	4.85-4.84(2H, m), 2.95(2H, bs), 1.82-1.74 (10H, m), 1.57-1.56 (4H, m)	79.6, 33.9, 23.1	3328, 2955, 1570, 1446, 990	Calc (M+Na)$^+$ 256.1078 Expt 256.1077

34 ¹H NMR Spectrum

¹³C NMR Spectrum

^{13}C NMR Spectrum

77.415
77.160
76.905
71.292
11.208
3.276

35 ^{1}H NMR Spectrum

7.284
4.856
4.852
4.849
2.952
1.829
1.818
1.754
1.746
1.745
1.745
1.572
1.568

0.914
2.11
6.78
1

ppm 7   6   5   4   3   2

^{13}C NMR Spectrum

4. Details on Enzyme Prepration and determination of Enzyme Kinetics

Expression and Purification of G2E6: Briefly, plasmid encoding Trx-G2E6 fusion gene was expressed in Origami B(DE3) cells (Novagen, Madison, WI). The expression was carried out in 2YT culture media. The culture was grown at 37°C until OD$_{600}$ was reached at 0.8 when it was induced with 0.1 mM IPTG and expressed further for 3 hours at 30°C. The cells were then harvested by centrifugation and stored at −80°C until further use. The harvested cells were resuspended in lysis buffer [50 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM CaCl$_2$, 0.1 mM DTT, and 10% glycerol] and were passed through a syringe needle. The crude lysate was then sonicated and recovered further by incubation with 0.1% tergitol NP-10 (Sigma-Aldrich, St. Louis, MO) with shaking at 4°C for 2.5 hours. After centrifugation, Ni-NTA resin (Qiagen, Valencia, CA), pre-equilibrated with lysis buffer containing 0.1% tergitol NP-10, was added to the supernatant, and the mixture was shaken for 3 hours. The resin was then washed with activity buffer [50 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM CaCl$_2$, 0.1% tergitol NP-10, 10% glycerol] containing increasing concentrations of imidazole (5 mM, 10 mM, and 25 mM, respectively). The fusion protein was eluted with activity buffer containing 125 mM imidazole. The final elution was then dialyzed (10000 MW cutoff; slide-a-lyzer, Pierce, Rockford, IL) in activity buffer to remove imidazole and monitored on SDS-PAGE gel for homogeneity. The protein concentration was determined using a Bradford assay (Bio-Rad Laboratories, Hercules, CA).

Determination of kinetic parameters: The kinetic parameters (k_{cat}, K_M) were determined using a Michaelis–Menten plot as demonstrated for a reference substrate. Briefly, to

assay buffer [50 mM Tris-HCl pH 7.4, 1mM $CaCl_2$], varying concentrations of substrates (paraoxon, 0.26 mM to 2.6 mM) and known concentration of enzyme (rePON1 G2E6) were added, and hydrolysis was monitored by UV-vis spectroscopy at 405 nm for 10 minutes. The rate of product (*p*-nitrophenol) formation was then plotted against substrate concentration and fitted to the Michaelis–Menten equation using KaleidaGraph. A typical curve is shown below.
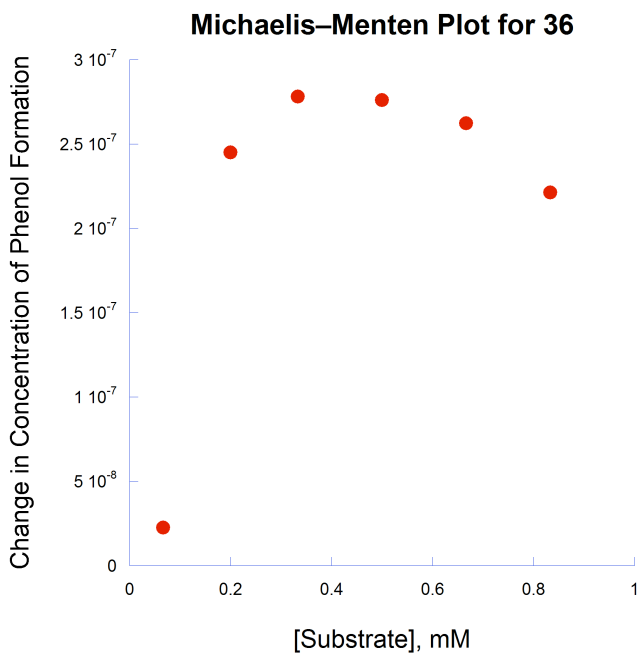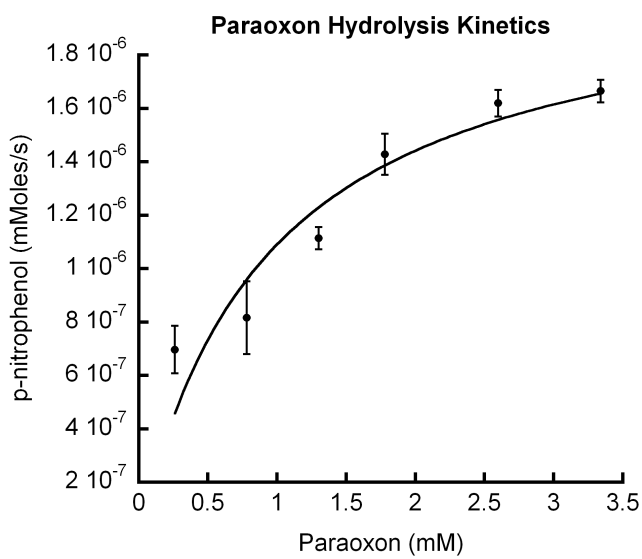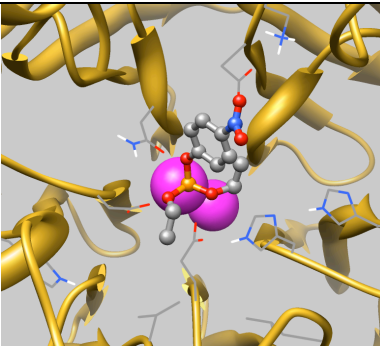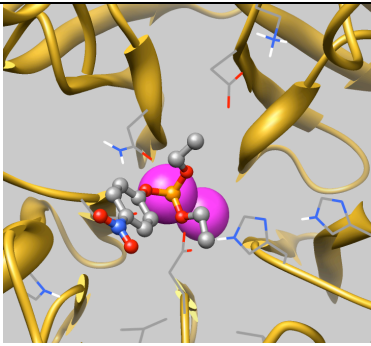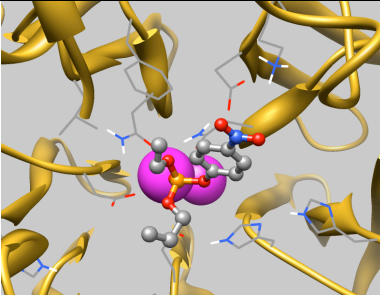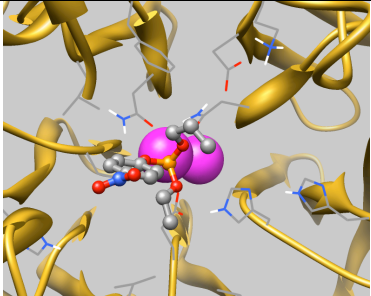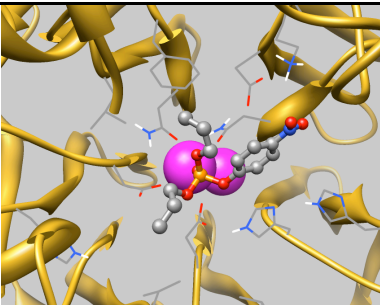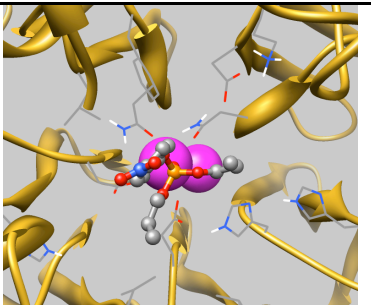

5. Selected Poses from docking of ligands in G2E6

In general, the docking poses obtained from Autodock revealed an adequate job of sampling ligand orientations in the active site. Both the leaving groups as well as the

other phosphoryl substituents were found to orient themselves into the various pockets comprising the active site region. However, a significant percentage of poses were found to result in the substrate not coordinating to the calcium ion owing to the vertical extension of the active site box. These were discarded from further refinement due to the lack of catalytic relevance, based on the assumption that calcium coordination to the phosphoryl oxygen is critical for catalytic activity.
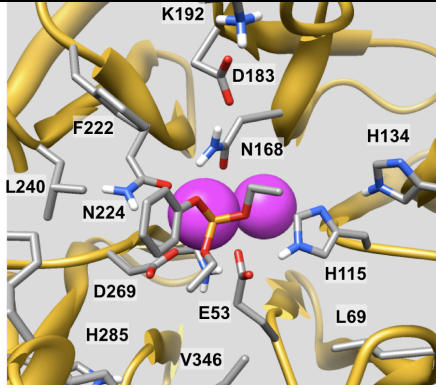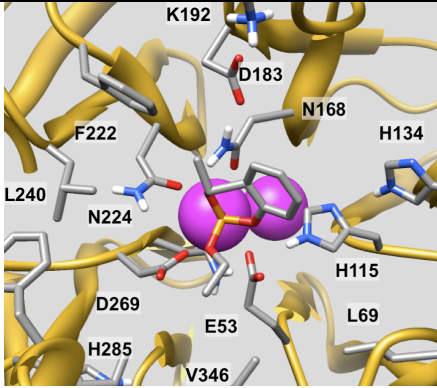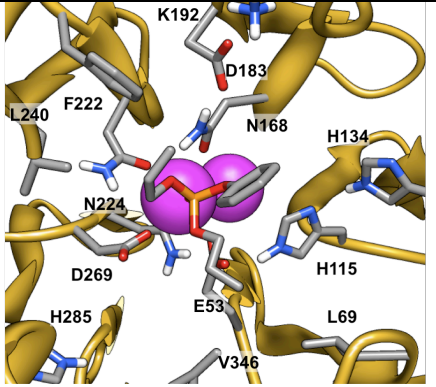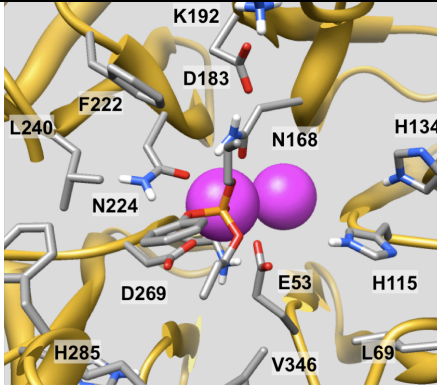
The energy scores from the docking simulations were not a useful metric for determining the quality of the pose. Similar energy scores were obtained for poses that placed the phosphoryl oxygen in proximity to calcium and those that placed the ligand into the HDL binding domain of the protein. Moreover, treatment of electrostatic interactions, particularly on the leaving group, was not very reliable. In many cases, the nitro group was placed in highly nonpolar pockets without a significant energetic penalty relative to those that provided suitable hydrogen bond donors, for instance. The principal utility of the docking simulations, then, was on giving a series of poses for subsequent MD simulations. Representative poses from docking are shown below:

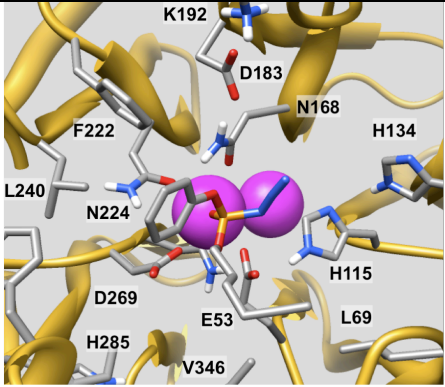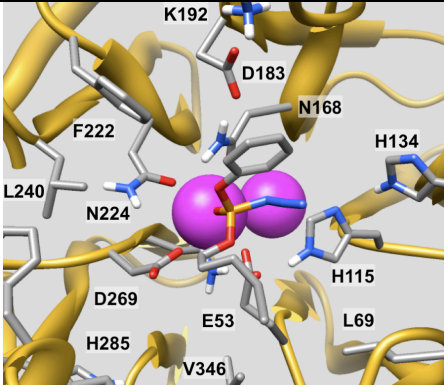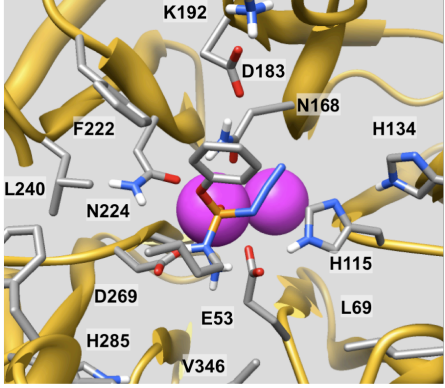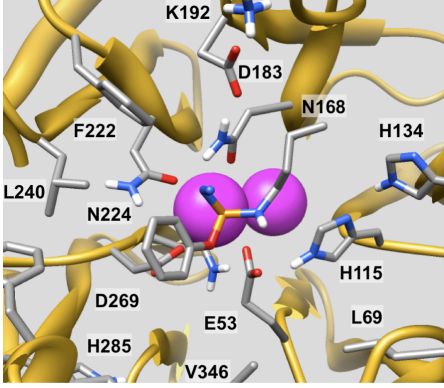**Representative Docking Poses from Group I Molecules. All scores are in kcal/mol**
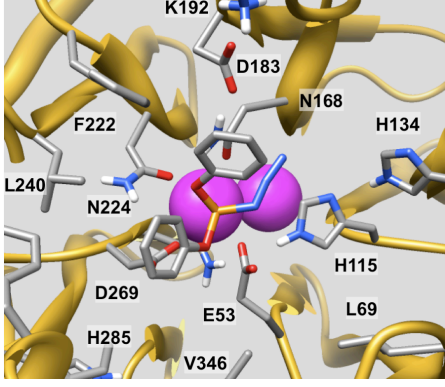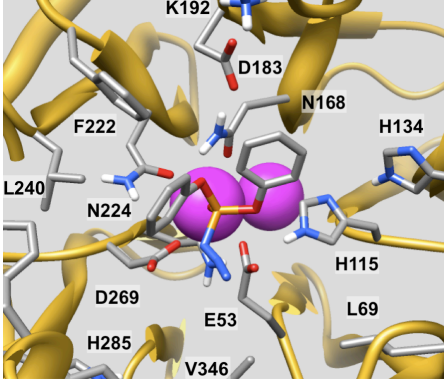
Molecule	Pose 1	Pose 2
Paraoxon (**1**)	Docking Score: -9.1	Docking Score: -8.8
8	Docking Score: -8.6	Docking Score: -7.5
10		

| | Docking Score: -9.2 | Docking Score: -8.4 |

Representative Docking Poses from Group II Molecules
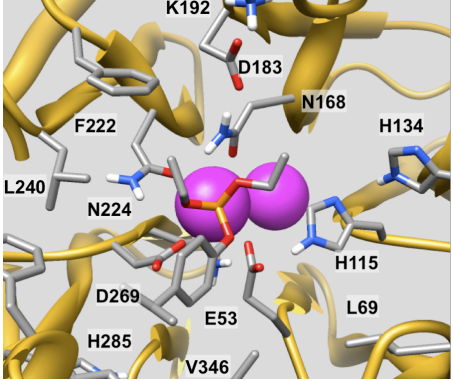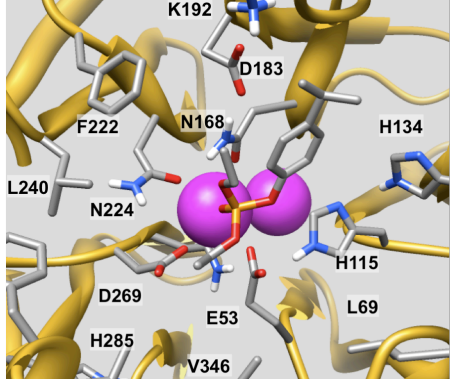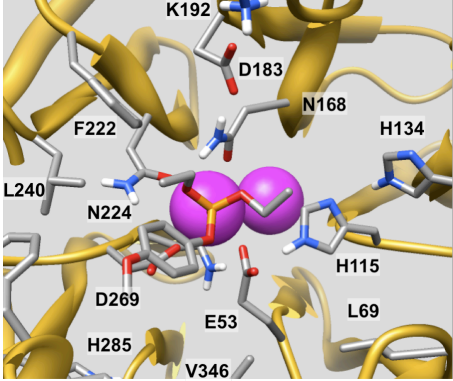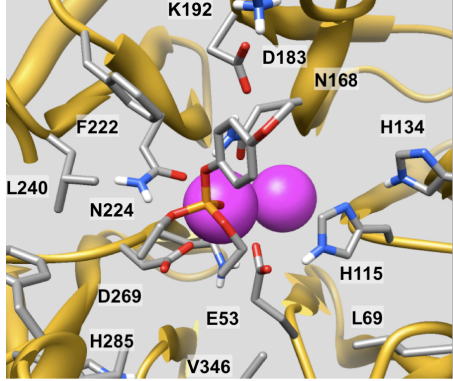
Molecule	Pose 1	Pose 2
17 (65)	 Docking Score: -9.7	 Docking Score: -9.4
18 (120)	 Docking Score: -9.1	 Docking Score: -7.5

Representative Docking Poses from Group III Molecules
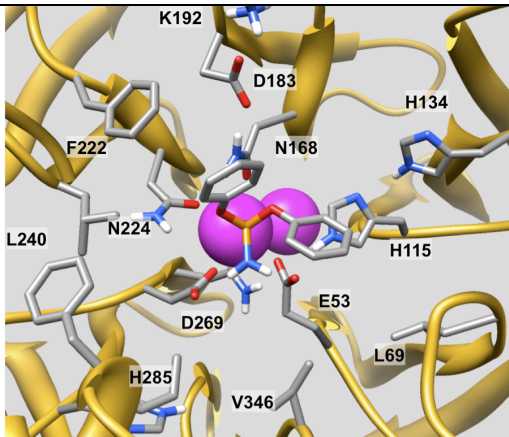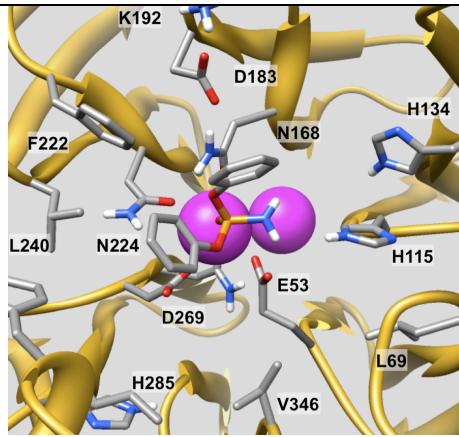
Molecule	Pose 1	Pose 2
23 (131)	Docking Score: -8.5	Docking Score: -8.0
24 (118)	Docking Score: -9.6	Docking Score: -9.5
25 (71)	Docking Score: -10.0	Docking Score: -9.1

Representative Docking Poses from Group IV Molecules

Molecule	Pose 1	Pose 2

30 (79)	Docking Score: -10.5	Docking Score: -9.4
31 (75)	Docking Score: -9.2	Docking Score: -7.1

Representative Docking Poses from Group V Molecules

Molecule	Pose 1	Pose 2
36	Docking Score: -11.2	Docking Score: -8.7

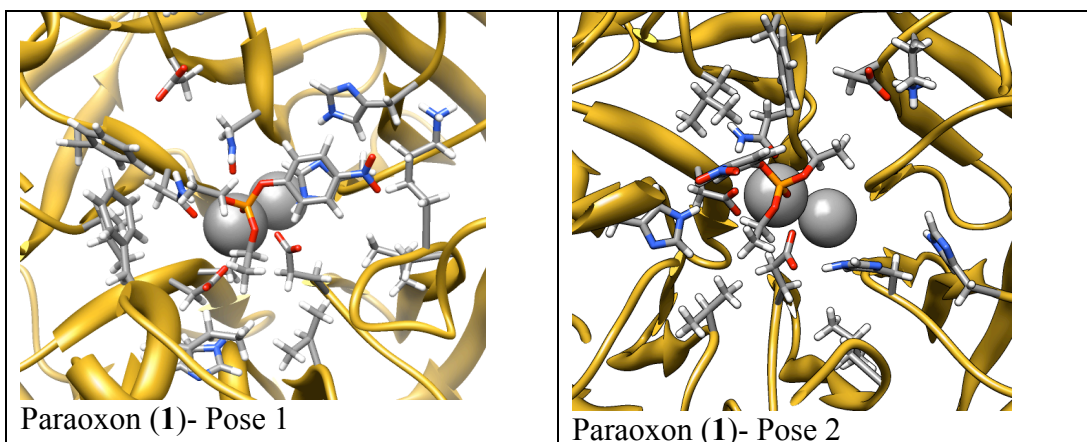**6. Selected Poses from MD simulations of ligand-G2E6 complex**

After analysis of the various docking poses for different OP ligands bound into the active-site snapshots, subsequent MD simulations were performed on select calcium-bound receptor-ligand complexes obtained from the docking simulations. A similar minimization protocol was utilized as described for the initial G2E6 model; the only difference was the inclusion of a moderate, flattened parabolic restraint on the calcium-phosphoryl oxygen bond coordinate, from 2.5 to 4.0 Å, to allow for enhanced relaxation of the ligand-receptor contacts prior to the possible dissociation of substrate. This was found to improve on the sub-optimal treatment of receptor flexibility in the docking protocol, and resulted in a reduction in the number of dissociative poses. A total of 4 ns of unrestrained MD simulations were performed on each ligand-receptor complex. A series of coordinate snapshots was extracted from the production MD trajectory from the terminal 1.5 ns of simulations, at 10 ps intervals, from which individual trajectories were generated for the unbound ligand, free receptor, and complex. Poisson-Boltzmann (MM-PBSA)[3] and Generalized Born (MM-GBSA)[4] simulations were performed on these snapshots, using the *sander* module to calculate individual components of the free energy for each component as in eq 1. From the individual results, the overall free energy of binding was calculated using eq 2.
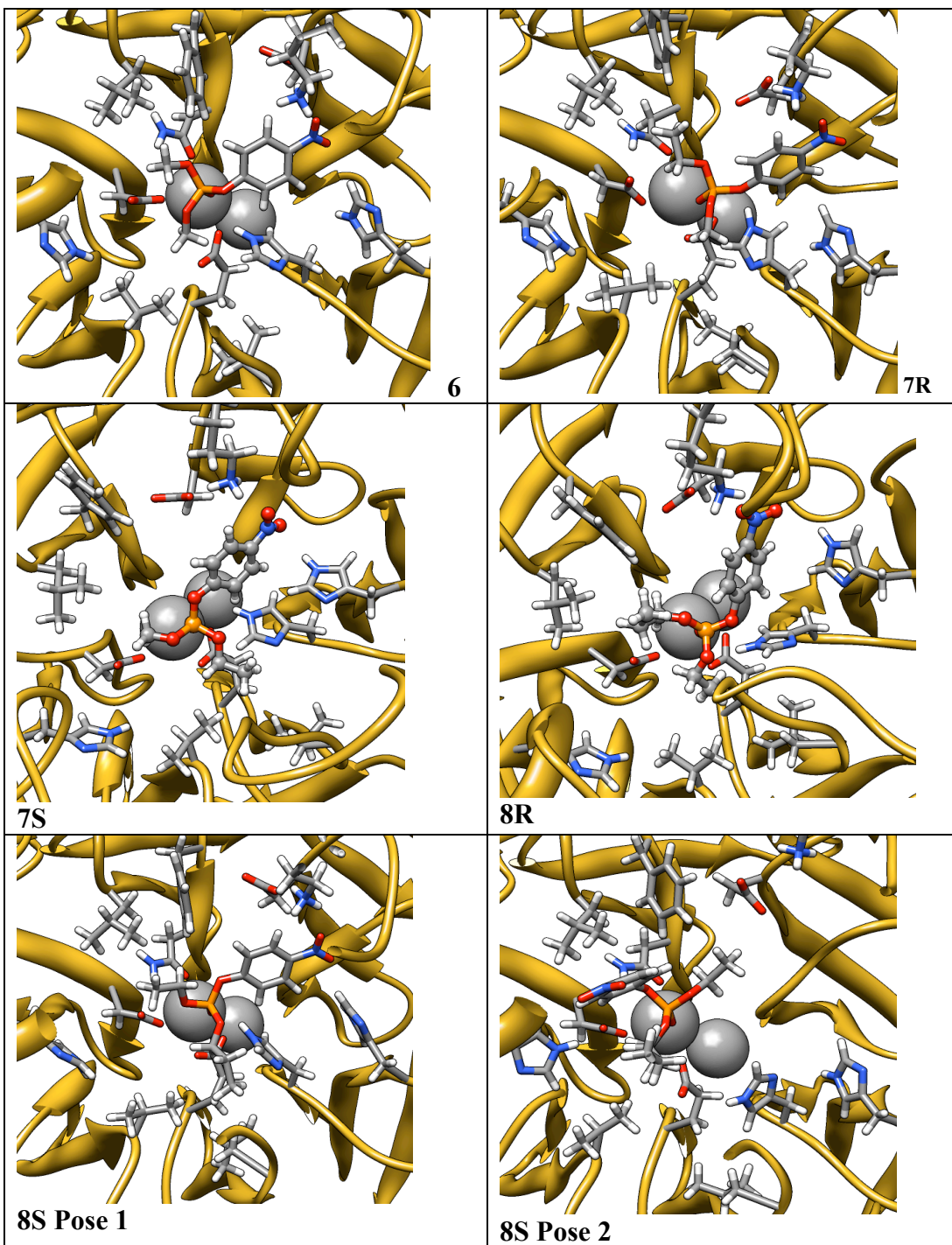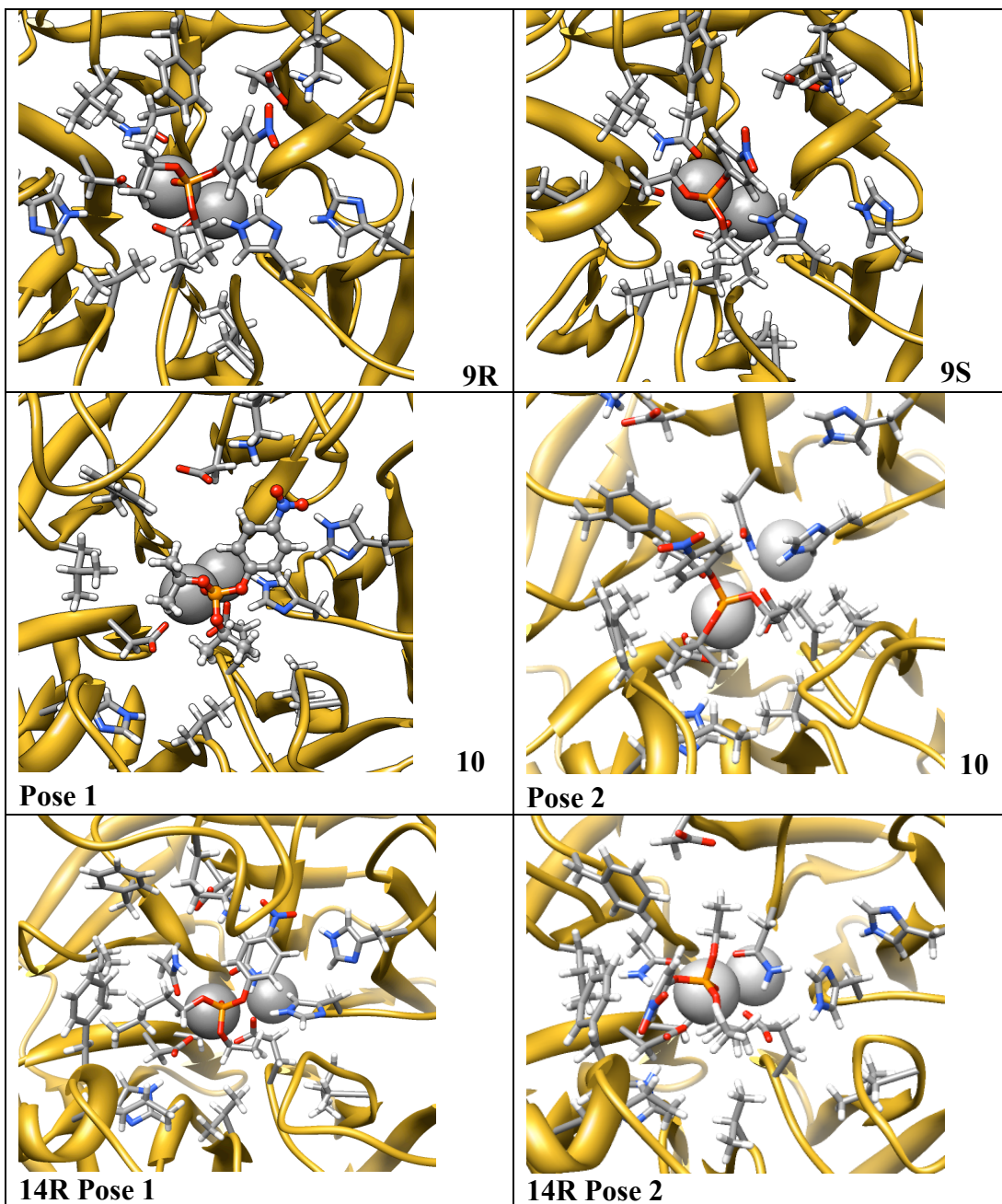
$$G = G_{hyd} + E_{MM} - TS_{solute} \tag{1}$$

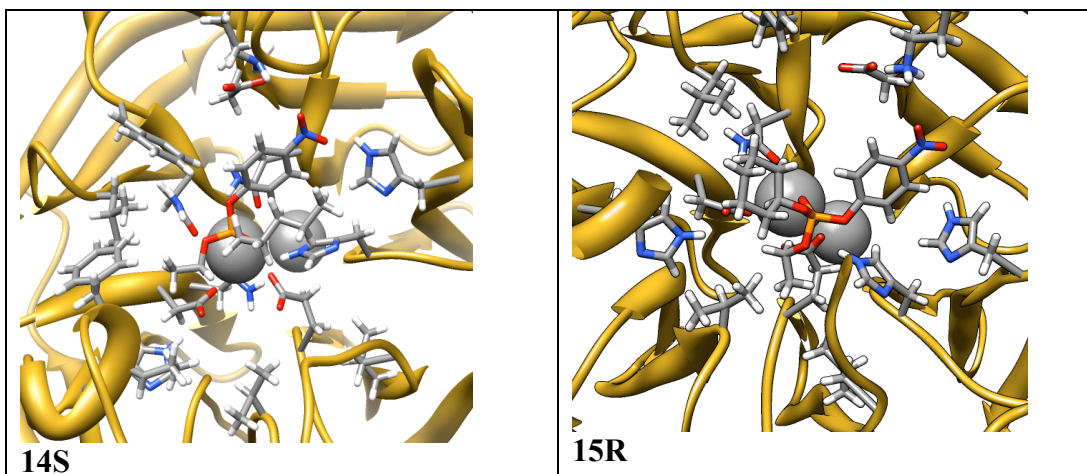$$\Delta G_{bind} = G_{complex} - (G_{receptor} + G_{ligand}) \tag{2}$$

The non-polar (SA) terms were estimated using the MSMS algorithm[5] using the equation $G_{SA} = \gamma\ SASA + \beta$, with γ and β set to 0.00542 kcal/(mol Å^{-2}) and 0.92 kcal/mol, respectively, and using a probe radius of 1.4 Å for estimating the solvent accessible surface area. For the polar (G_{polar}) energy terms, the Generalized Born and Poisson-Boltzmann methods were both utilized as implemented in the AMBER software package. In the GB calculations, dielectric constants of 1 and 78.5 were utilized with AMBER mbondi2 radii. The TS_{solute} term represents temperature and solute entropy, and in these calculations this term was omitted. As the binding energies were only compared within ligand families, the effect of entrotpy changes was estimated to be minimal. Such methods have been employed in estimating binding energies for organic molecules with good agreement with experimental data.[6,7]

Group I

Paraoxon (**1**)- Pose 1   Paraoxon (**1**)- Pose 2

6

7R

7S

8R

8S Pose 1

8S Pose 2

9R

9S

10 Pose 1

10 Pose 2

14R Pose 1

14R Pose 2

14S

15R

The energies obtained for poses of the group I molecules are shown in Table S1. The energy trend is consistently favorable for the orientation with the leaving group pointing toward the H115/H134/K192 region of the active site pocket.

Table S1. Energies determined for bound poses of the group I molecules bound in the G2E6 model of PON1, for the two primary orientations obtained. (MM-PBSA/MM-GBSA, respectively, in kcal/mol)

Molecule:	Leaving Group Orientation:	
	H115/K192 Pocket	H285 Pocket
Paraoxon (**1**)	-17.5/-20.5	-13.4/-17.9
6	-13.4/-17.7	a
7R	-16.2/-20.8	a
7S	-14.6/-28.0	a
8R	-20.4/-23.1	a
8S	-22.8/-28.4	-13.1/-21.1
9R	-15.7/-19.7	a
9S	-20.1/-28.8	a
10	-19.1/-25.7	-18.7/-20.3
14R	-20.3/-33.4	-15.1/-22.0
14S	-18.6/-28.8	a
15R	-28.3/-31.5	a

a - No pose determined in this pocket

Group II

| **17 Pose 1** | PBSA: -7.3 |
| | GBSA: -9.8 |

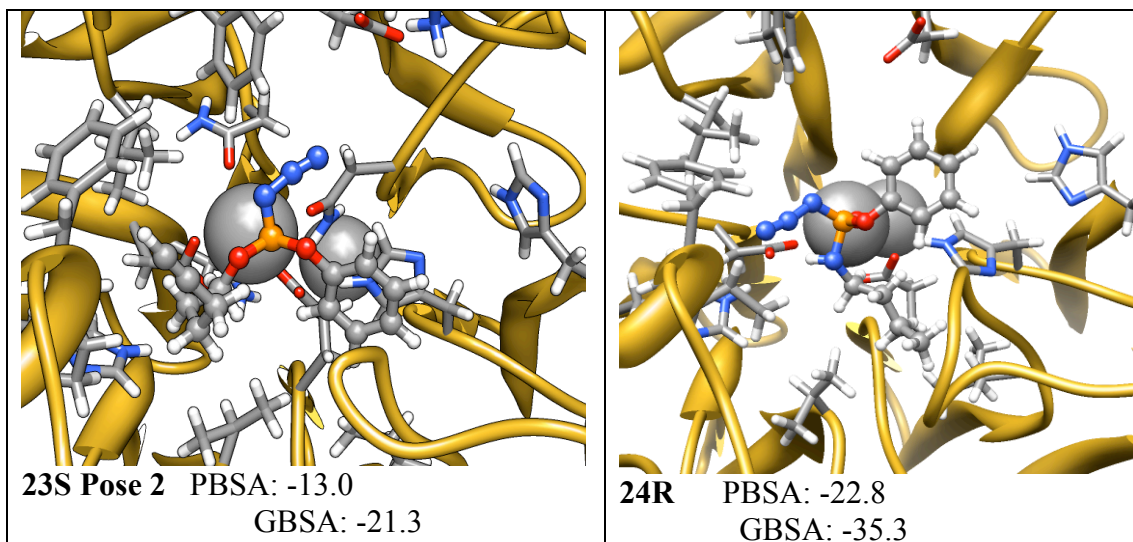| **17 Pose 2** | PBSA: -8.4 |
| | GBSA: -15.2 |
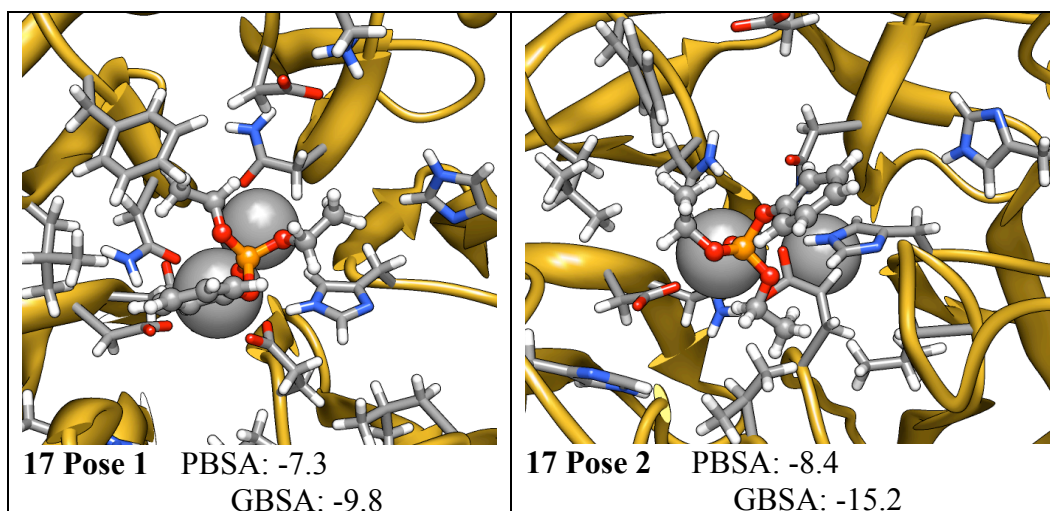
For the group II molecules, the lack of any electrostatic interactions with either pocket reduced the overall binding energies compared with paraoxon, but the poses do appear to be generally similar to those for the group I molecules.
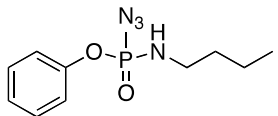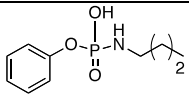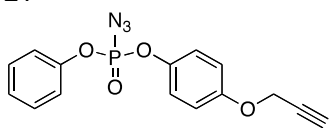
Group III

| **23R** | PBSA: -24.0 |
| | GBSA: -25.8 |

| **23S Pose 1** | PBSA: -22.1 |
| | GBSA: -22.2 |

23S Pose 2 PBSA: -13.0
GBSA: -21.3

24R PBSA: -22.8
GBSA: -35.3
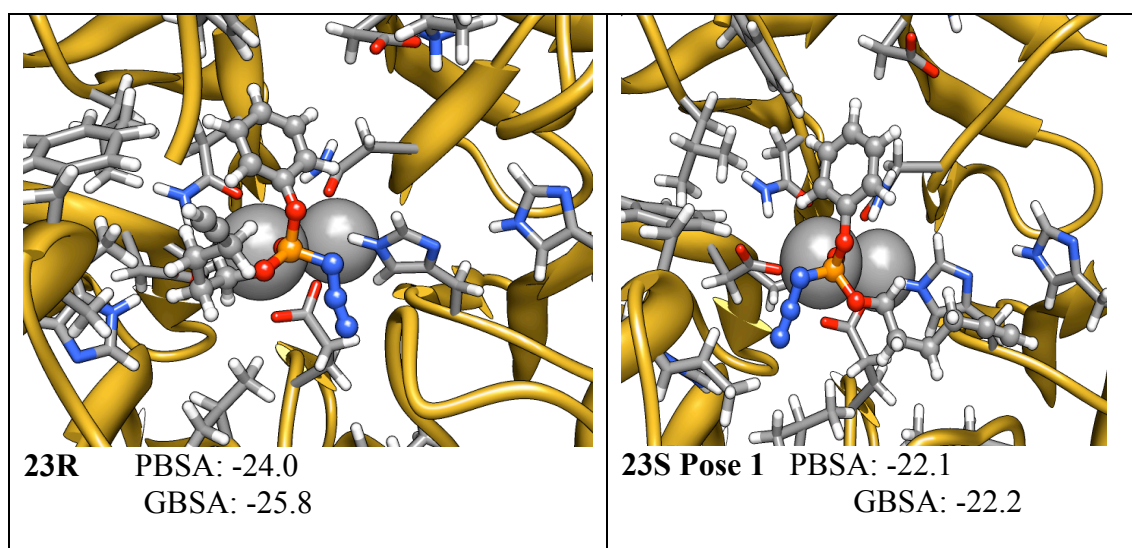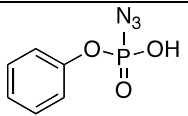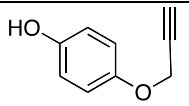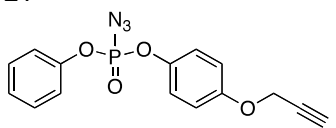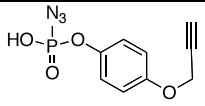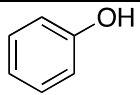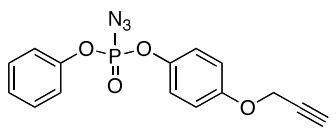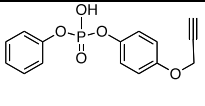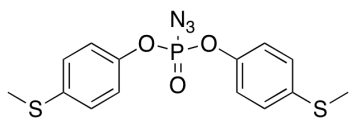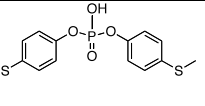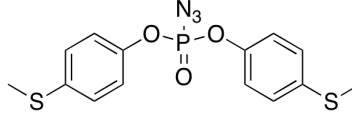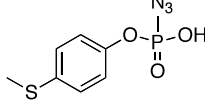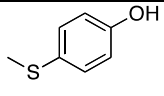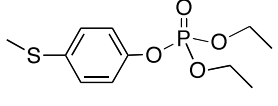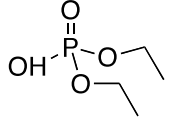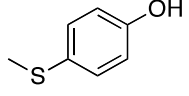
For the group III molecules the energetic preference was again similar, with electrostatic interactions observed between the azide and active site residues, specifically the aspartates and histidine 115. The leaving group is again positioned for hydrolysis by bases in proximity to D269 or E53.

Group V

36 Pose 1 PBSA: -7.4
GBSA: -13.6

36 Pose 2 PBSA: -19.5
GBSA: -21.5
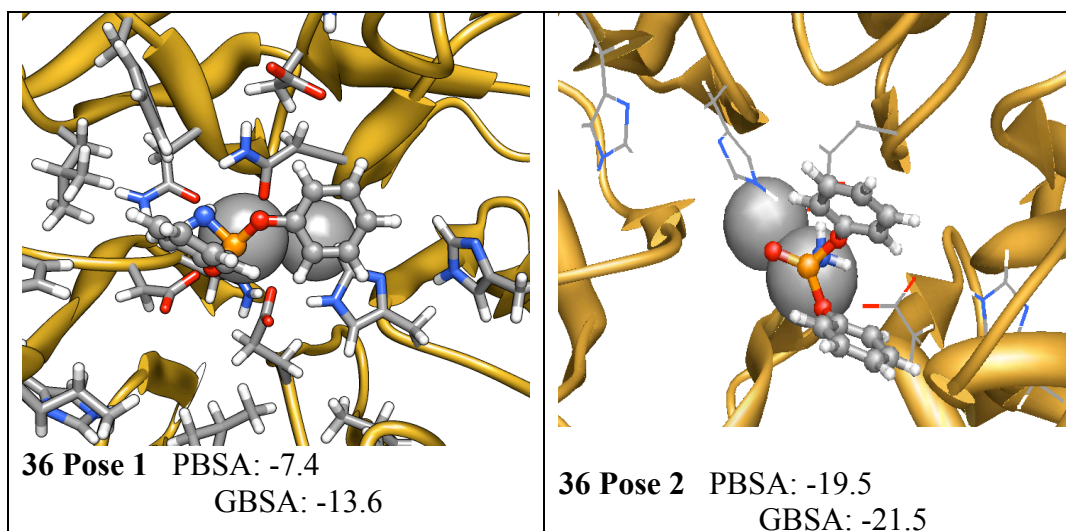
7. The $\Delta H_{298,hydrol}$ for the hydrolysis of representative ligands from each group of molecules by water was computed using density functional theory (DFT – B3LYP/6-31G*) using the Gaussian03 suite of programs.[8,9]

Molecule	Hydrolysis Products		ΔH_{hydrol} kcal/mol	Comment
1			-6.9	Favorable
10			-6.8	Favorable
8			-6.8	Favorable
9			-6.6	Favorable
23			-4.9	Favorable
23	HN$_3$		+11.4	Unfavorable
24			-3.9	Favorable

#				Value	
24		HN₃		+12.6	Unfavorable
27			HO— (4-propargyloxyphenol)	-4.5	Favorable
27			(phenol, OH)	-5.4	Favorable
27			HN3	+12.1	Unfavorable
29			HN3	+11.6	Unfavorable
29			(4-methylthiophenol, OH)	-5.4	Favorable
32			(4-methylthiophenol, OH)	-4.6	Favorable

8. References

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