

# Towards combining backbone and sugar constraint in 3'-3' bis-phosphonate tethered 2'-4' bridged LNA oligonucleotide trimers

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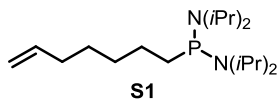
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## I. Synthesis of phosphordiamine S1



To a suspension of Mg (1.1 g, 45.2 mmol) and a catalytic amount of I<sub>2</sub> in THF (23 ml), 7-Bromo-1-heptene (3.98 g, 22.5 mmol) was added at r.t for about 30 min. In a separate oven dried flask, bis(diisopropylamino)chlorophosphine (5.0 g, 18.7 mmol) was suspended in Et<sub>2</sub>O (100 mL) under Ar and cooled to 0 °C with constant stirring. The above freshly prepared solution of hept-6-en-1-ylmagnesium bromide (22.5 mL, 22.4 mmol, 1M solution in THF) was cannulated into the stirred solution of chlorophosphine suspension dropwise and the reaction mixture was allowed to stir at 0 °C for 1h. The progress of the reaction was monitored by <sup>31</sup>P NMR. <sup>31</sup>P NMR showed major peaks at 48.6 ppm (product) and 20.6 ppm (minor). The reaction mixture was filtered through a plug of Celite and the solids were rinsed with Et<sub>2</sub>O. The solvent was evaporated in vacuo. The residue was suspended in dry MeCN, transferred to a separatory funnel and extracted with hexanes (2x100 mL). The hexanes layer was washed with MeCN (50 mL). The hexanes layer was collected and passed through a cotton plug. The filtrate was concentrated at 30 °C, dried under high vacuum, and used immediately in the synthesis of phosphoramidite **7** without any further purification.

**Yield and physical aspect:** 5.1 g, 85%; colorless oil

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 5.92-5.76 (m, 1H), 5.09 – 4.87 (m, 2H), 3.51 – 3.29 (m, 4H), 2.14-1.99 (m, 2H), 1.72 – 1.54 (m, 2H), 1.52 – 1.36 (m, 6H), 1.36 – 1.26 (m, 4H), 1.21 (d, J = 6.7 Hz, 10H), 1.16 – 1.02 (d, J = 6.7 Hz, 11H)

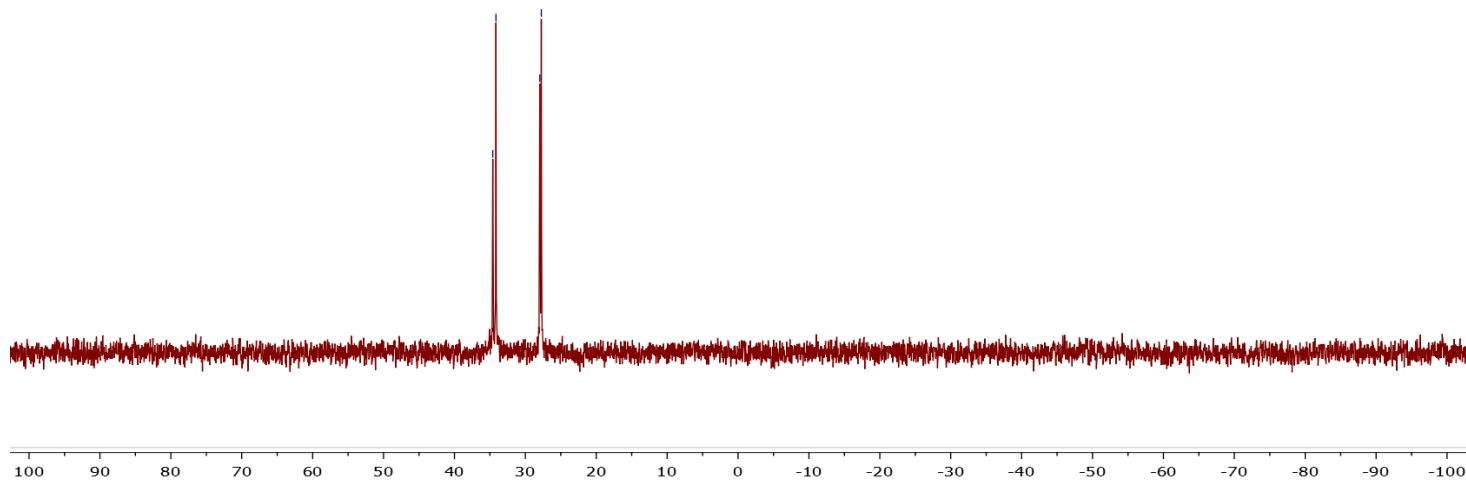
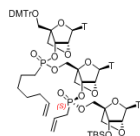
**<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>) δ 139.4, 114.2, 46.54, 46.45, 34.0, 31.8, 31.3, 31.2, 29.1, 28.84, 28.80, 25.9, 25.7, 24.64, 24.57, 24.3, 24.2, 22.8, 14.3

**<sup>31</sup>P NMR** (160 MHz, CDCl<sub>3</sub>) δ 47.6

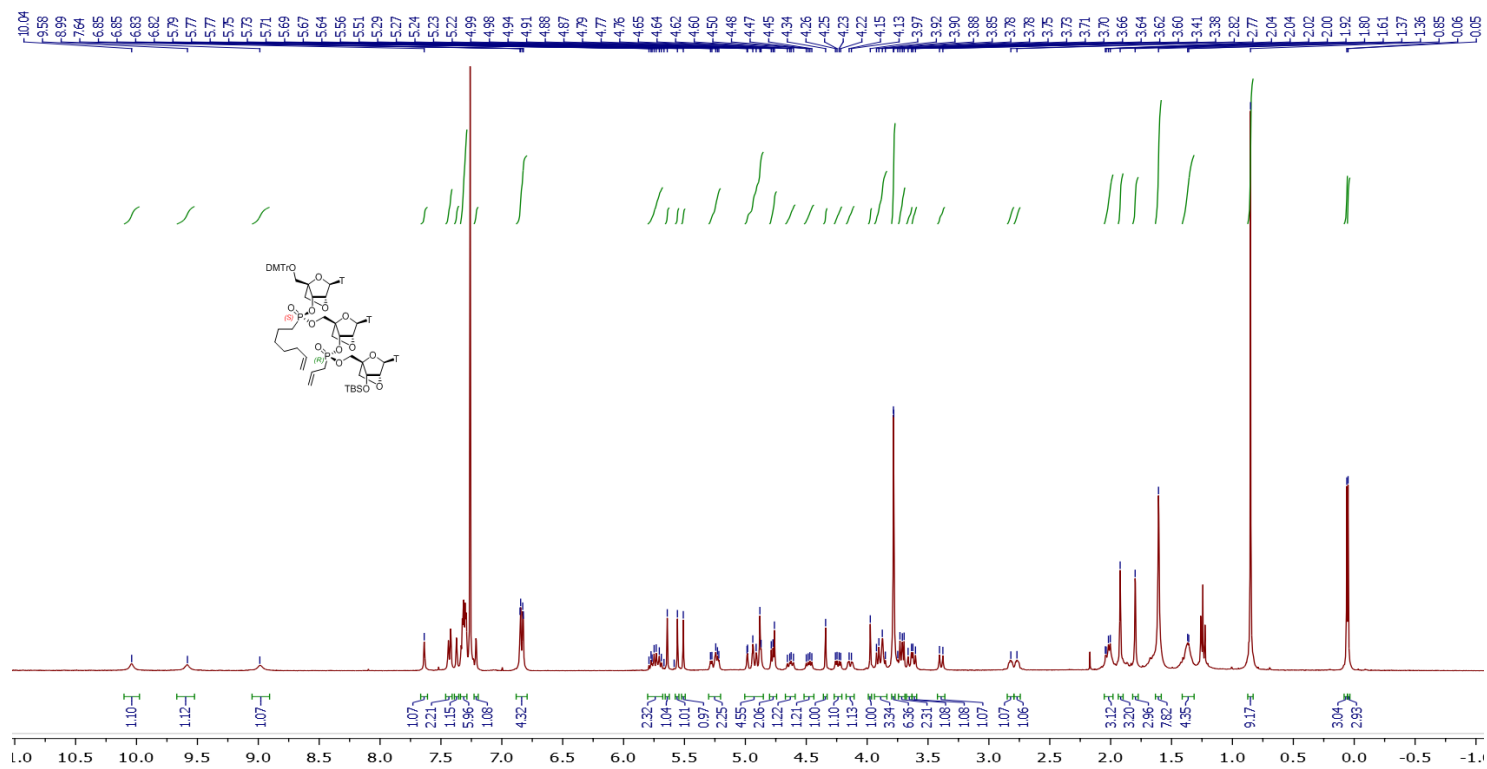
## II. $^1\text{H}$ , $^{31}\text{P}$ and $^{13}\text{C}$ NMR spectra

8a/8b:  $^{31}\text{P}$ , 200 MHz,  $\text{CDCl}_3$

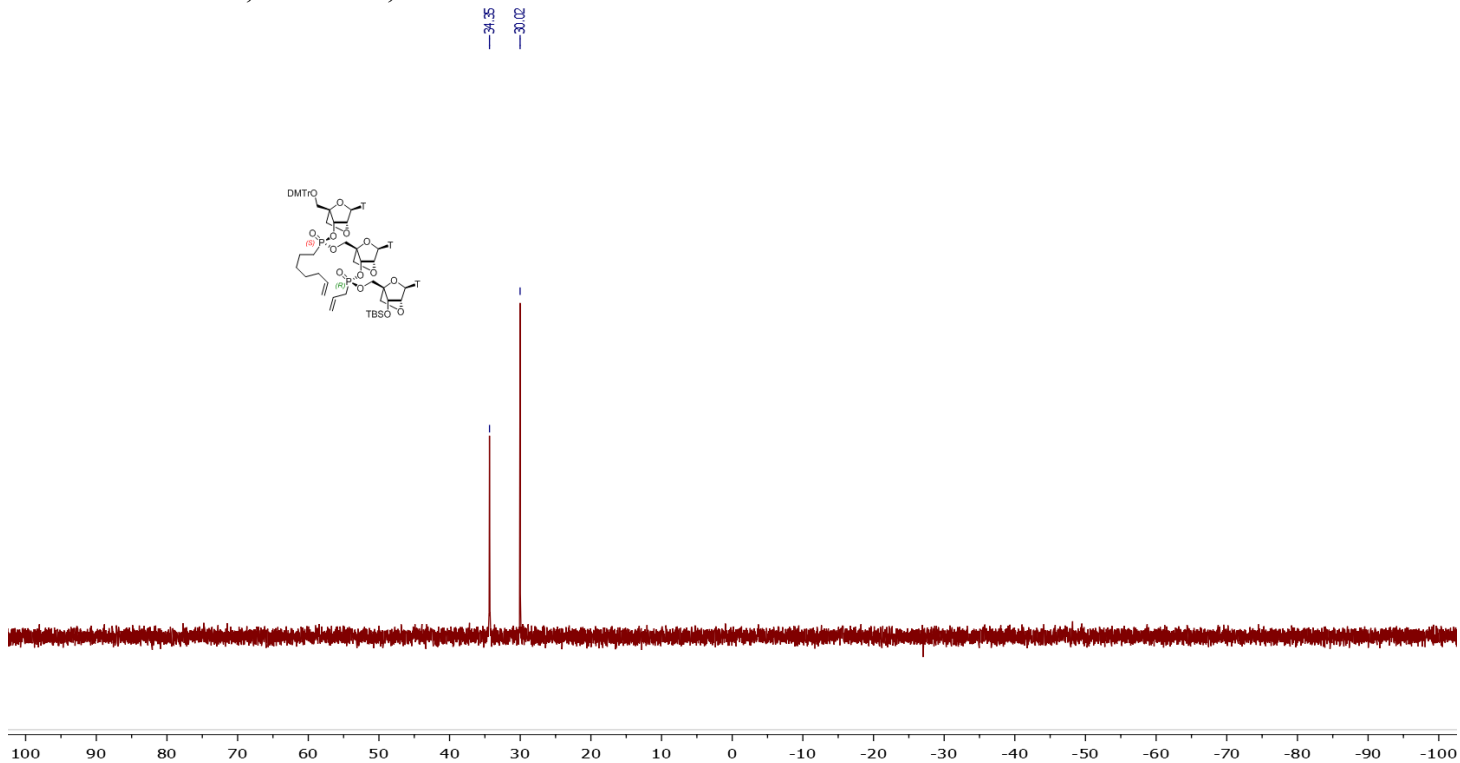
34.59  
34.14  
27.96  
27.74



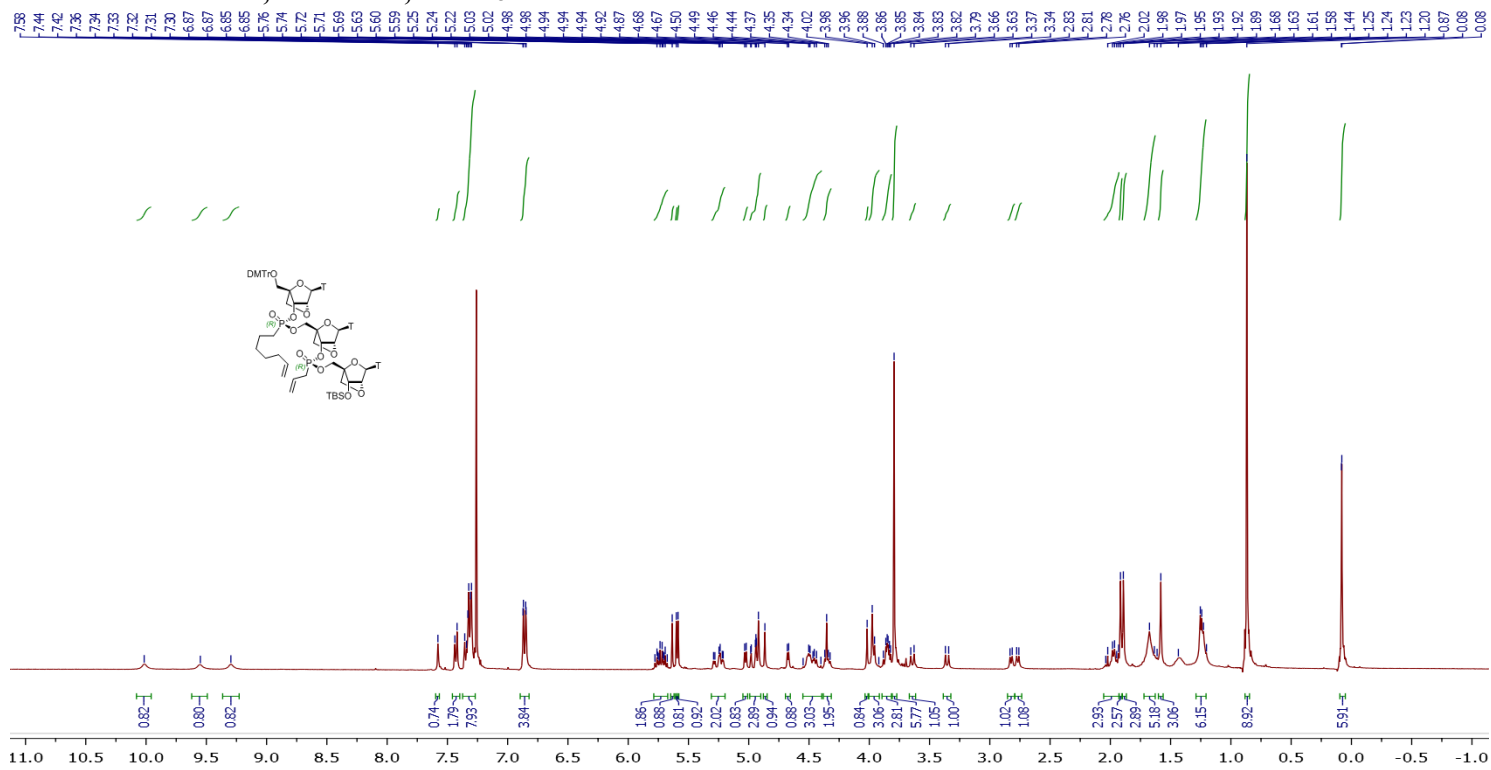
**8c:  $^1\text{H}$ , 400 MHz,  $\text{CDCl}_3$**



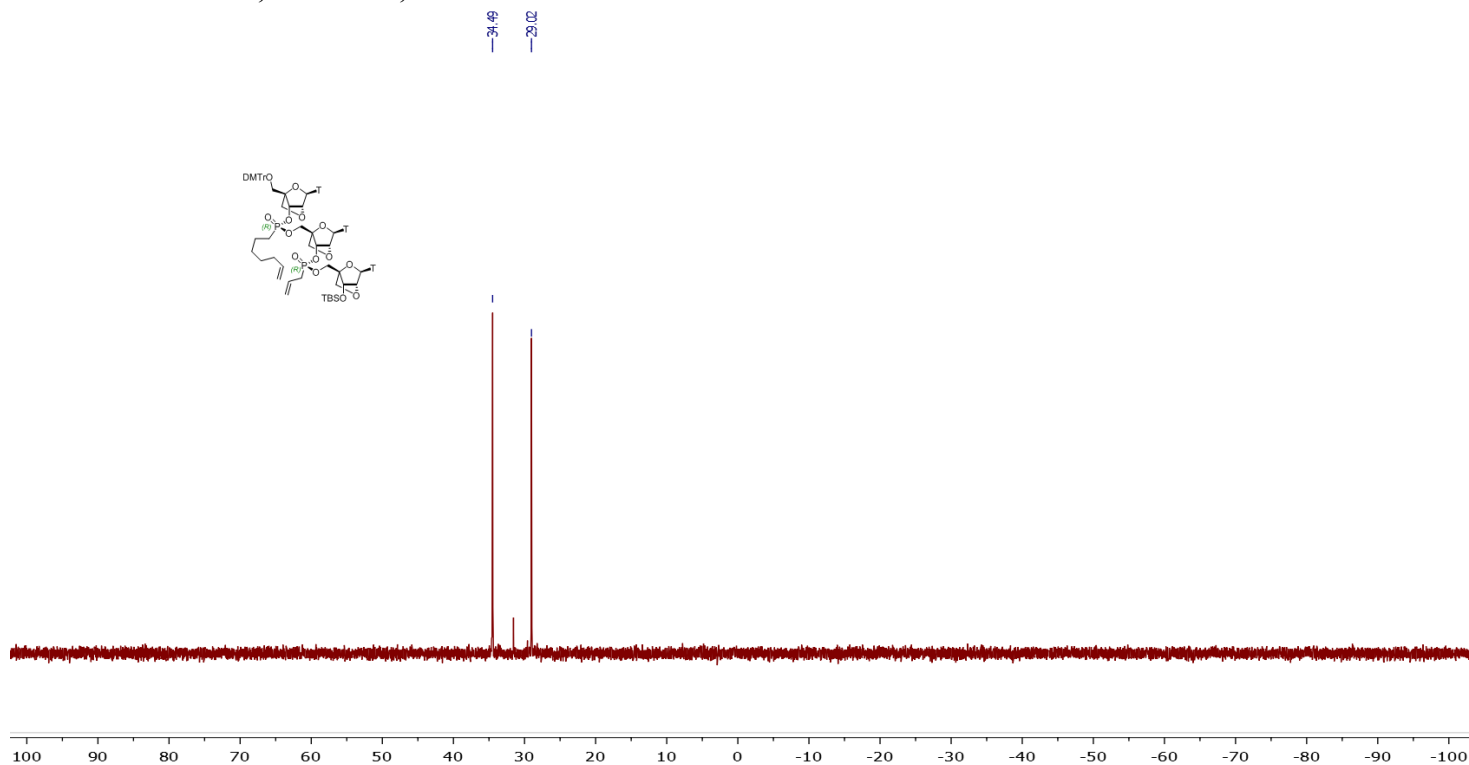
**8c:  $^{31}\text{P}$ , 160 MHz,  $\text{CDCl}_3$**



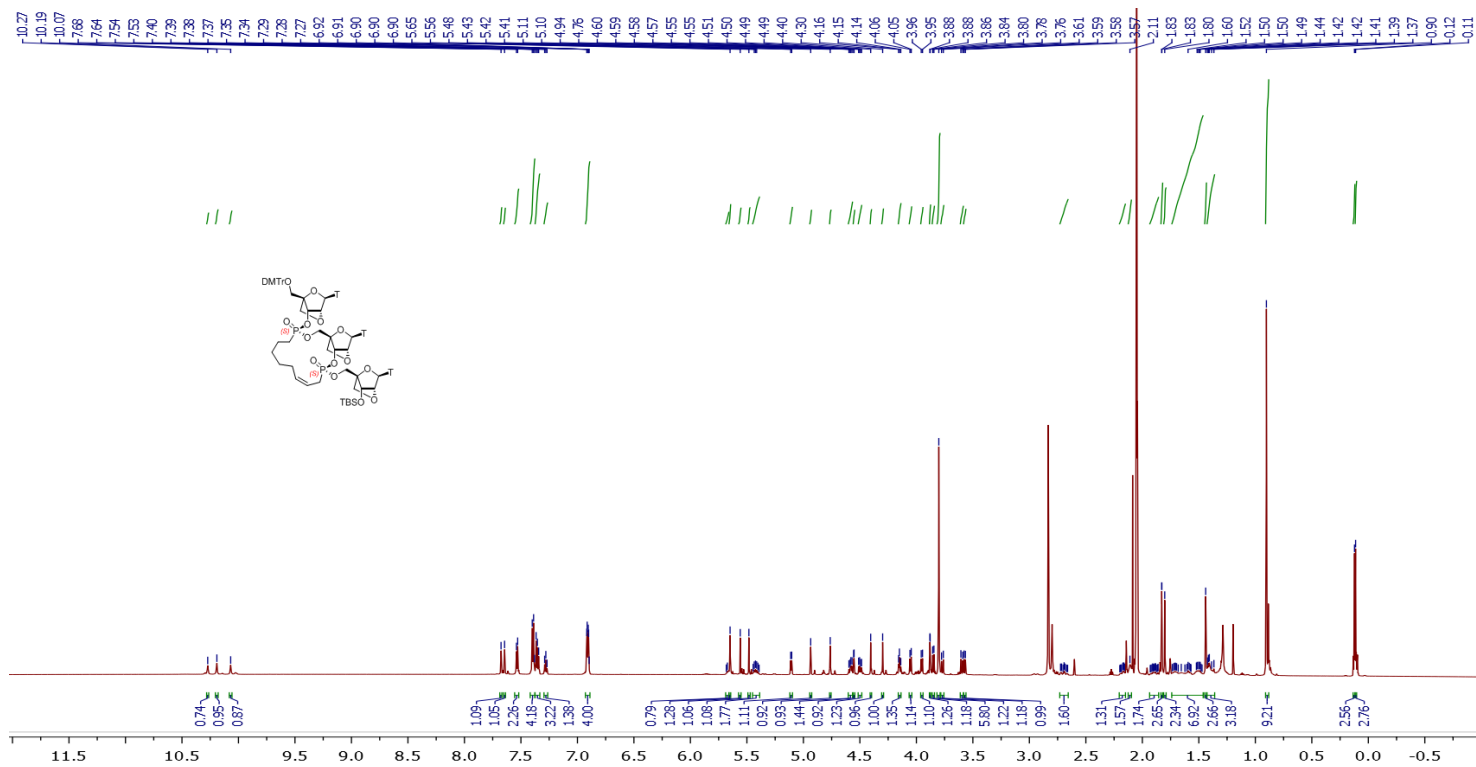
**8d: <sup>1</sup>H, 400 MHz, CDCl<sub>3</sub>**



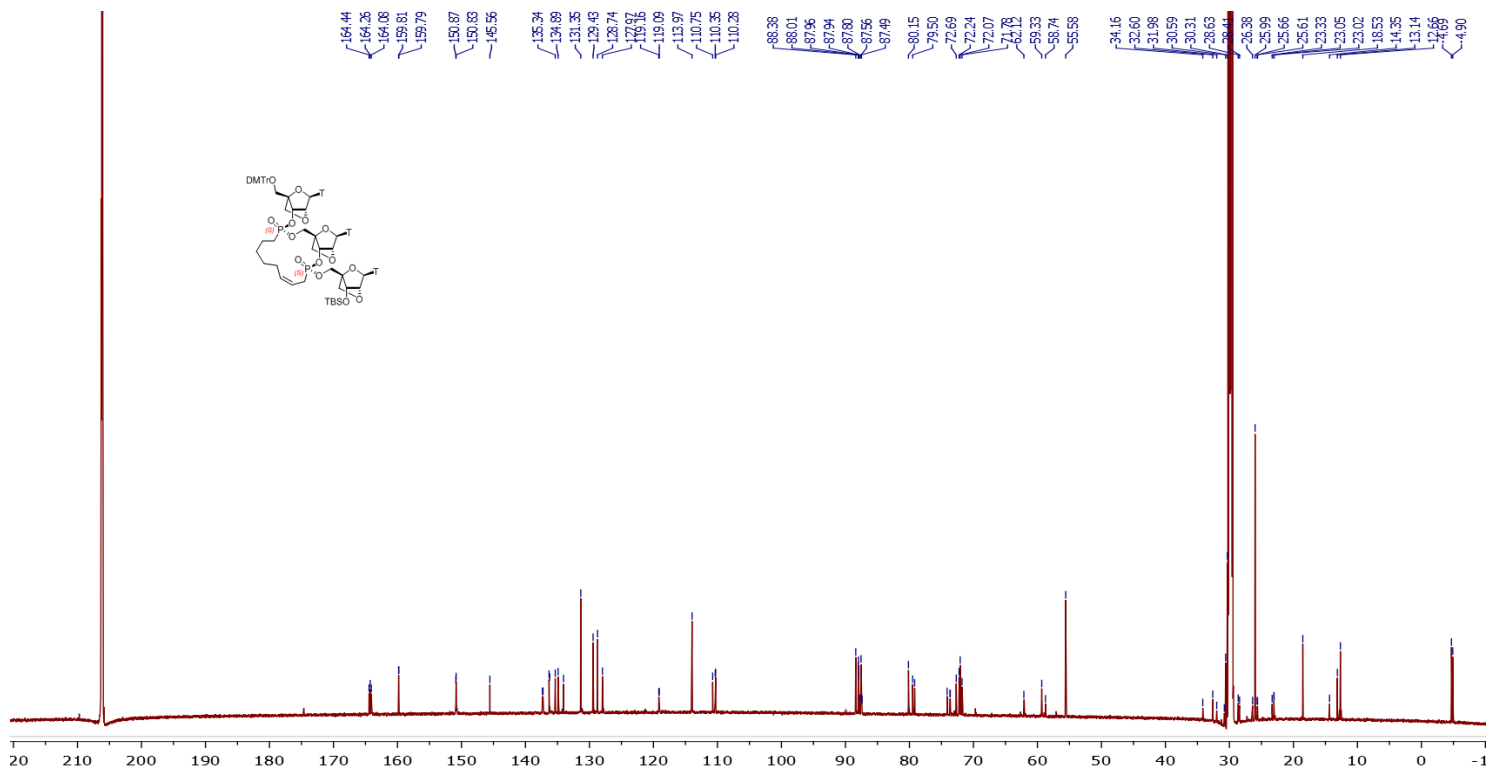
**8d: <sup>31</sup>P, 160 MHz, CDCl<sub>3</sub>**



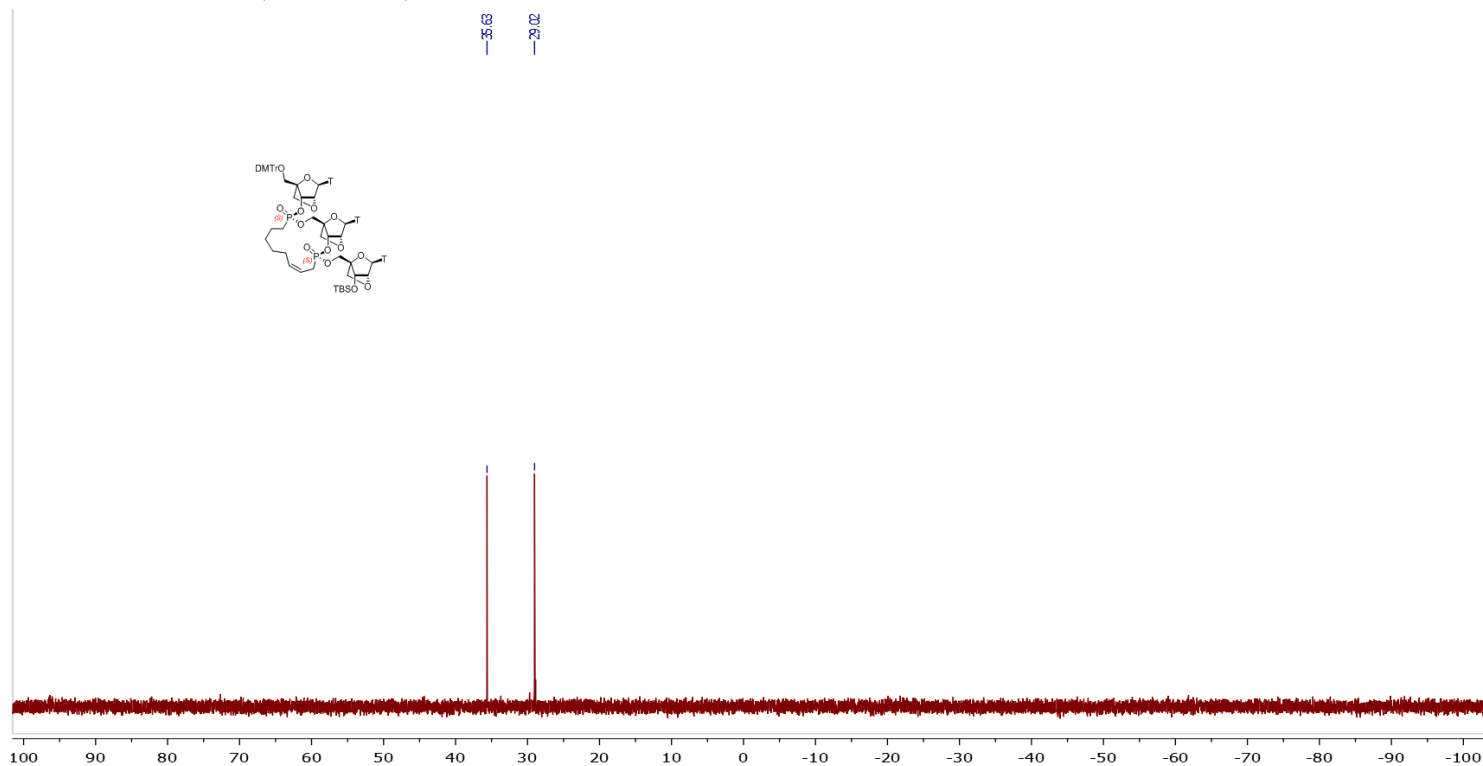
9a: <sup>1</sup>H, 700 MHz, acetone-d<sub>6</sub>



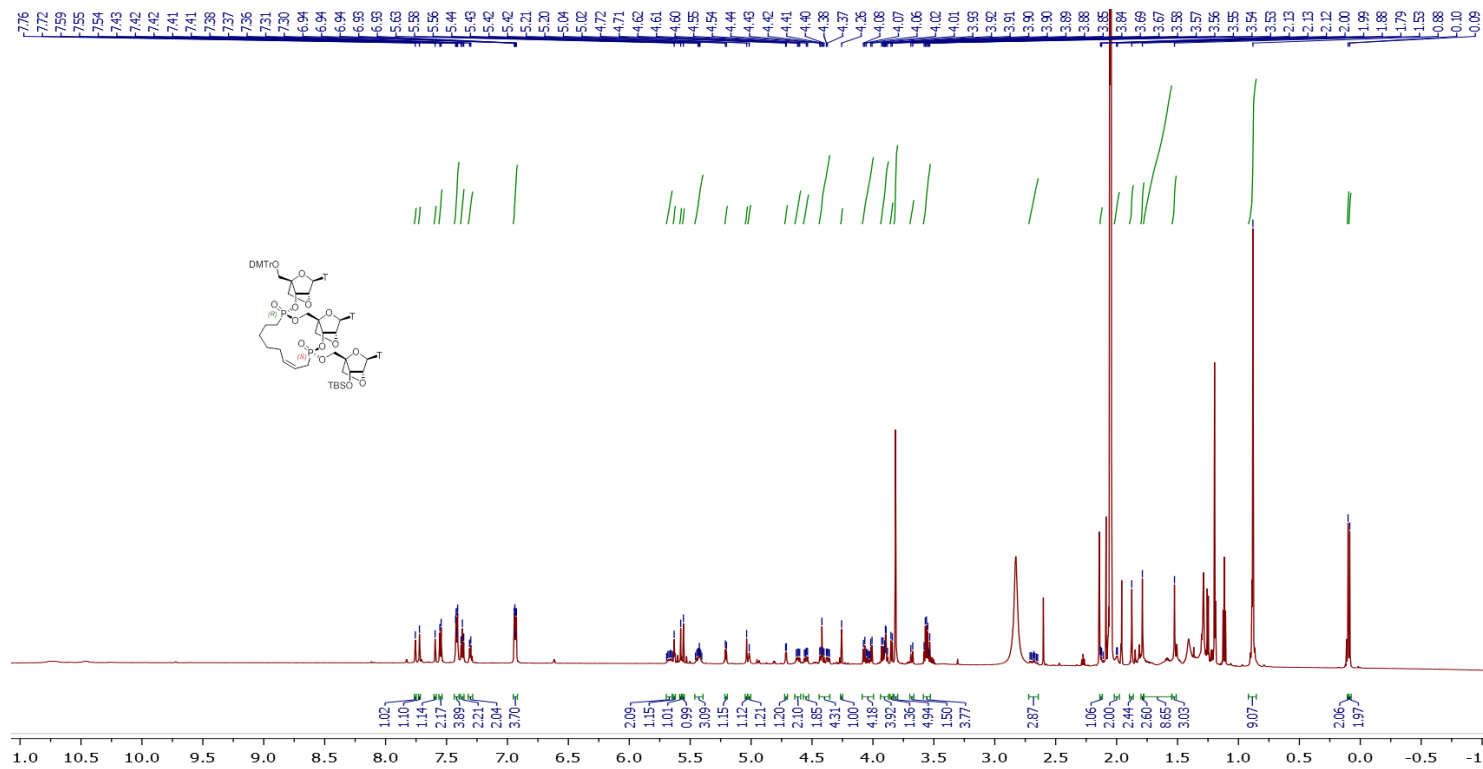
9a: <sup>13</sup>C, 175 MHz, acetone-d<sub>6</sub>



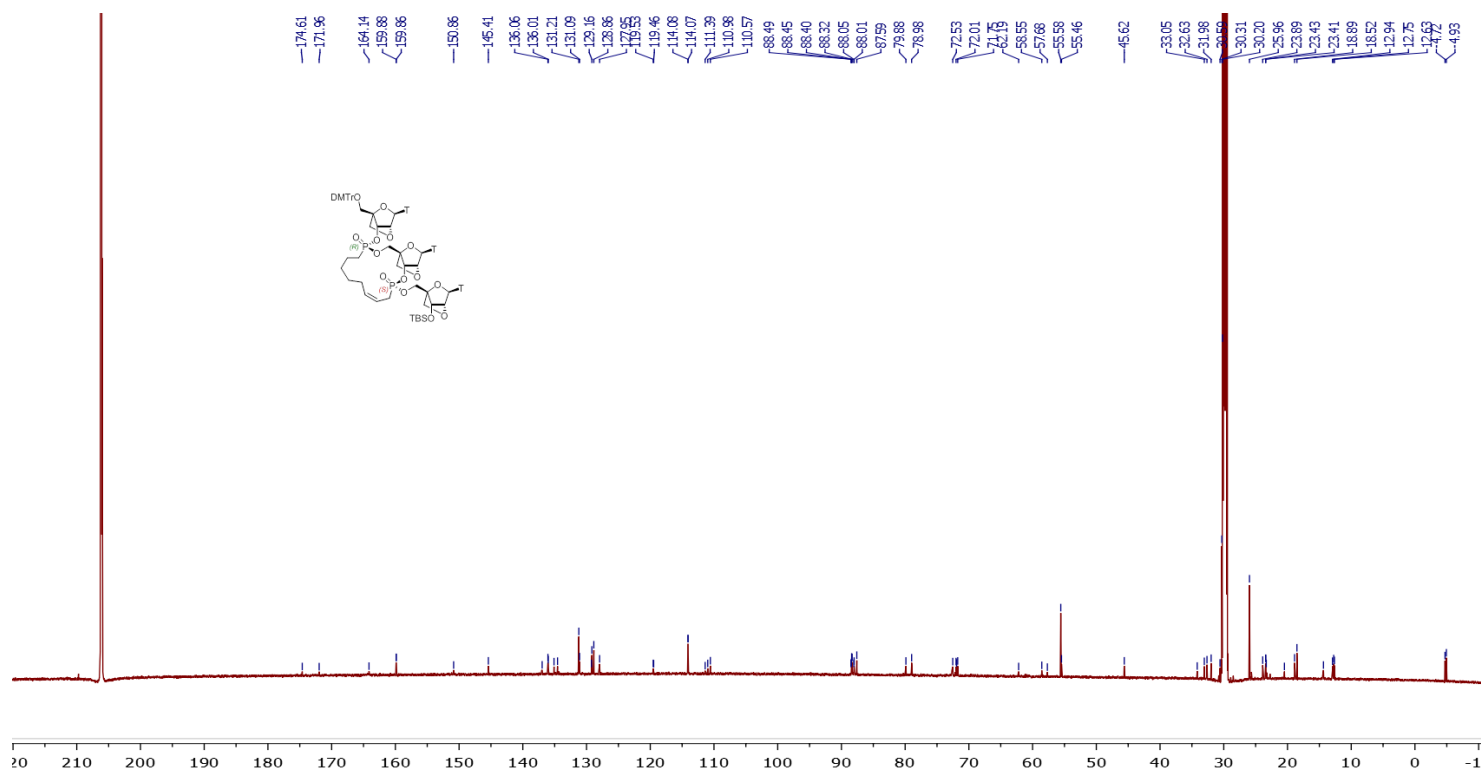
9a:  $^{31}\text{P}$ , 700 MHz, acetone- $d_6$



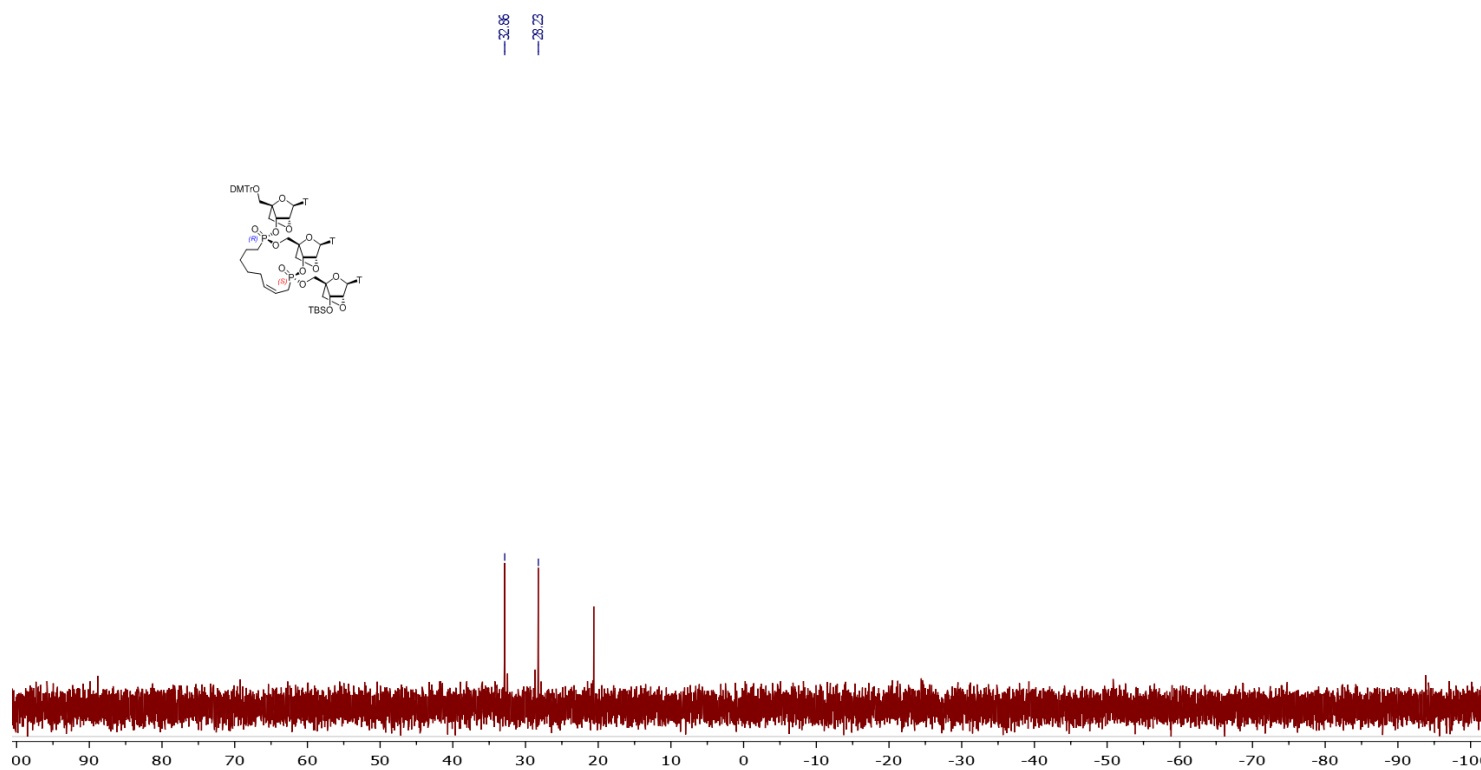
9b:  $^1\text{H}$ , 700 MHz, acetone- $d_6$



9b:  $^{13}\text{C}$ , 175 MHz, acetone- $d_6$



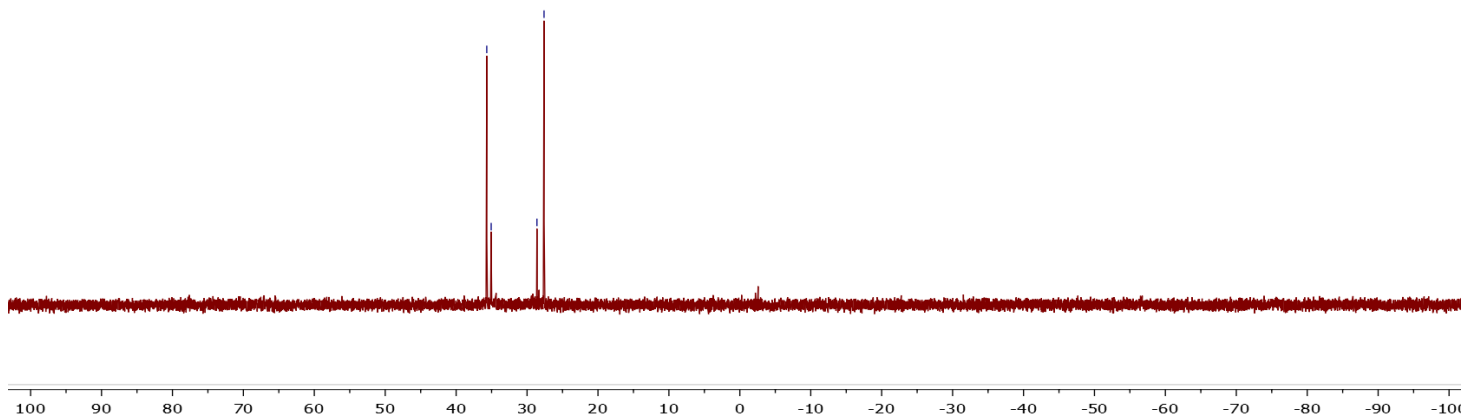
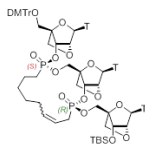
9b:  $^{31}\text{P}$ , 160 MHz, acetone- $d_6$





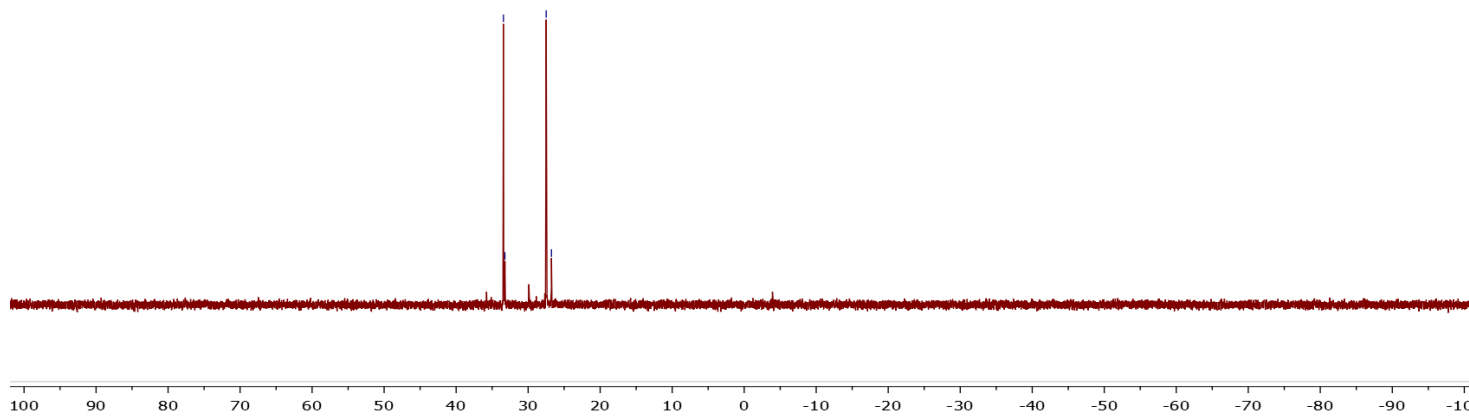
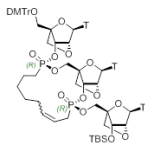
9c:  $^{31}\text{P}$ , 101 MHz, acetone- $d_6$

35.68  
35.06  
28.61  
27.61

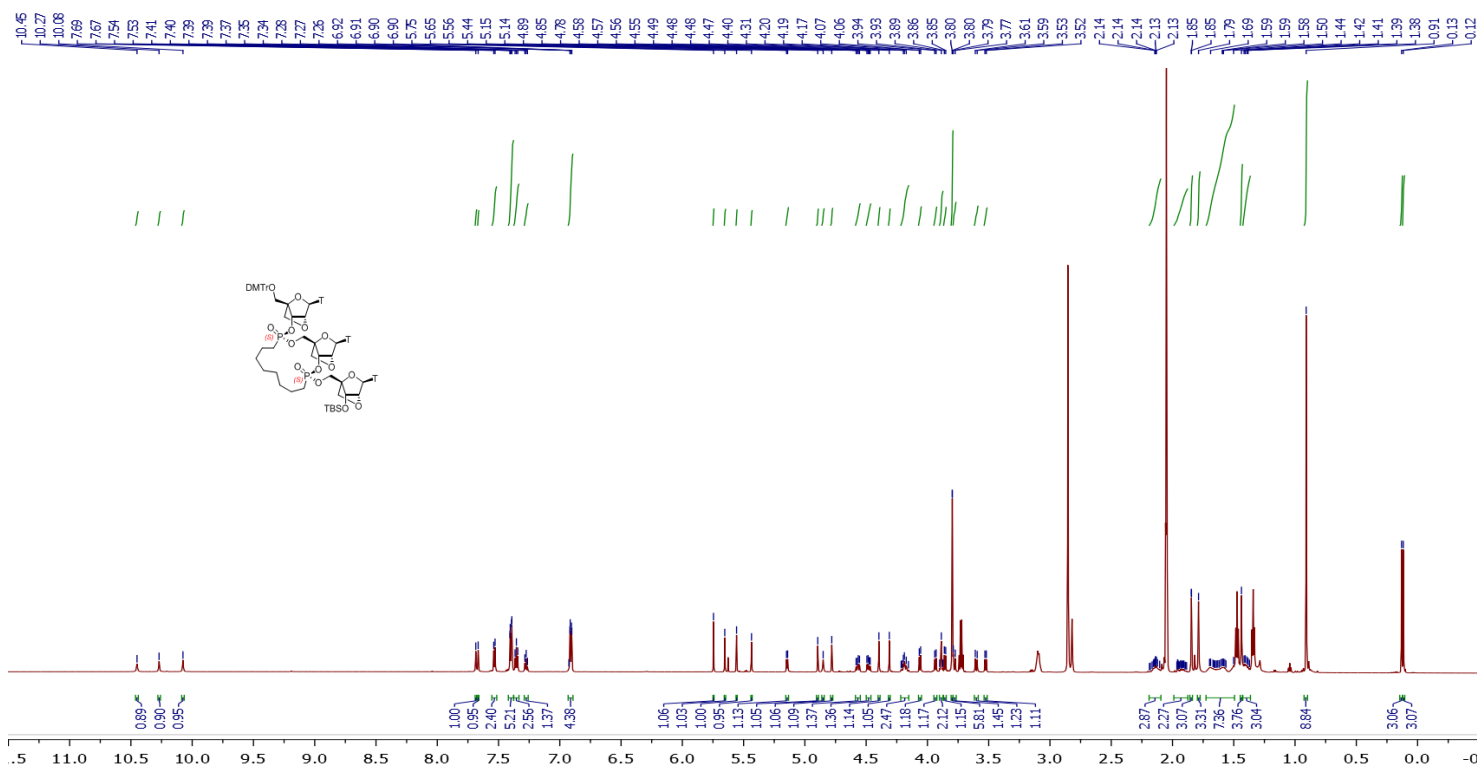


9d:  $^{31}\text{P}$ , 101 MHz, acetone- $d_6$

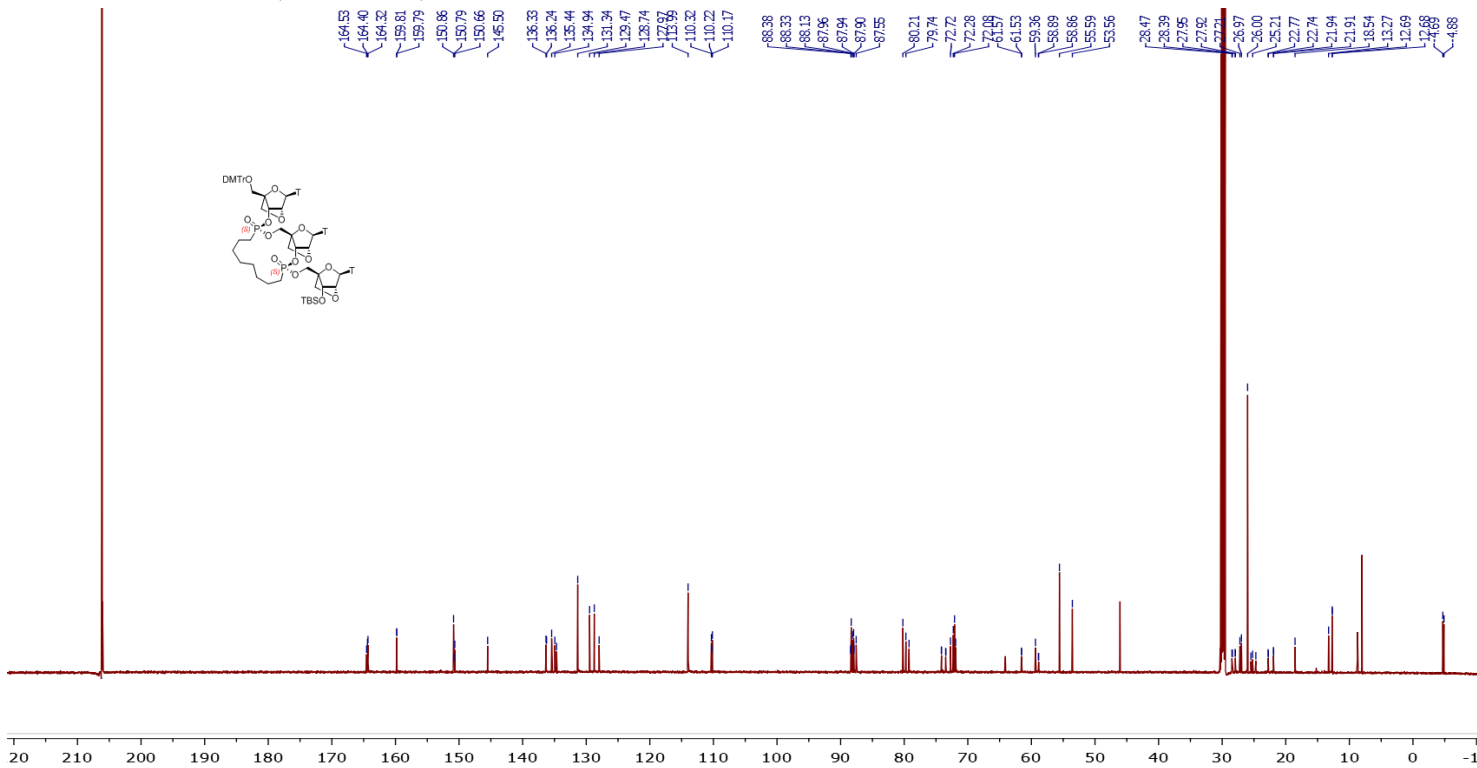
33.45  
32.23  
27.49  
26.76



10a: <sup>1</sup>H, 700 MHz, acetone-d<sub>6</sub>

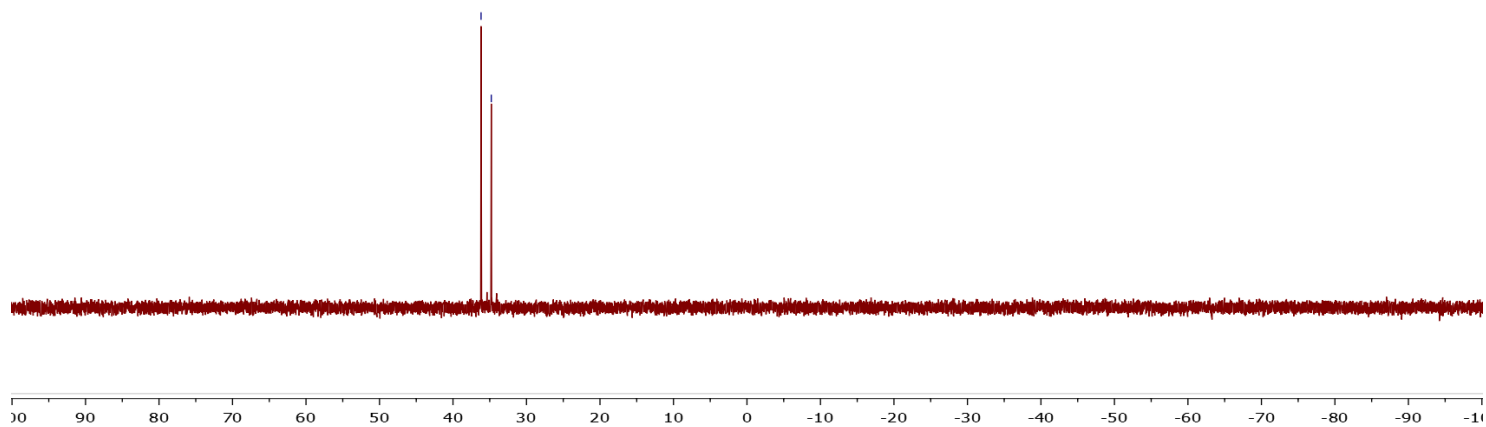
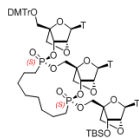


10a: <sup>13</sup>C, 175 MHz, acetone-d<sub>6</sub>



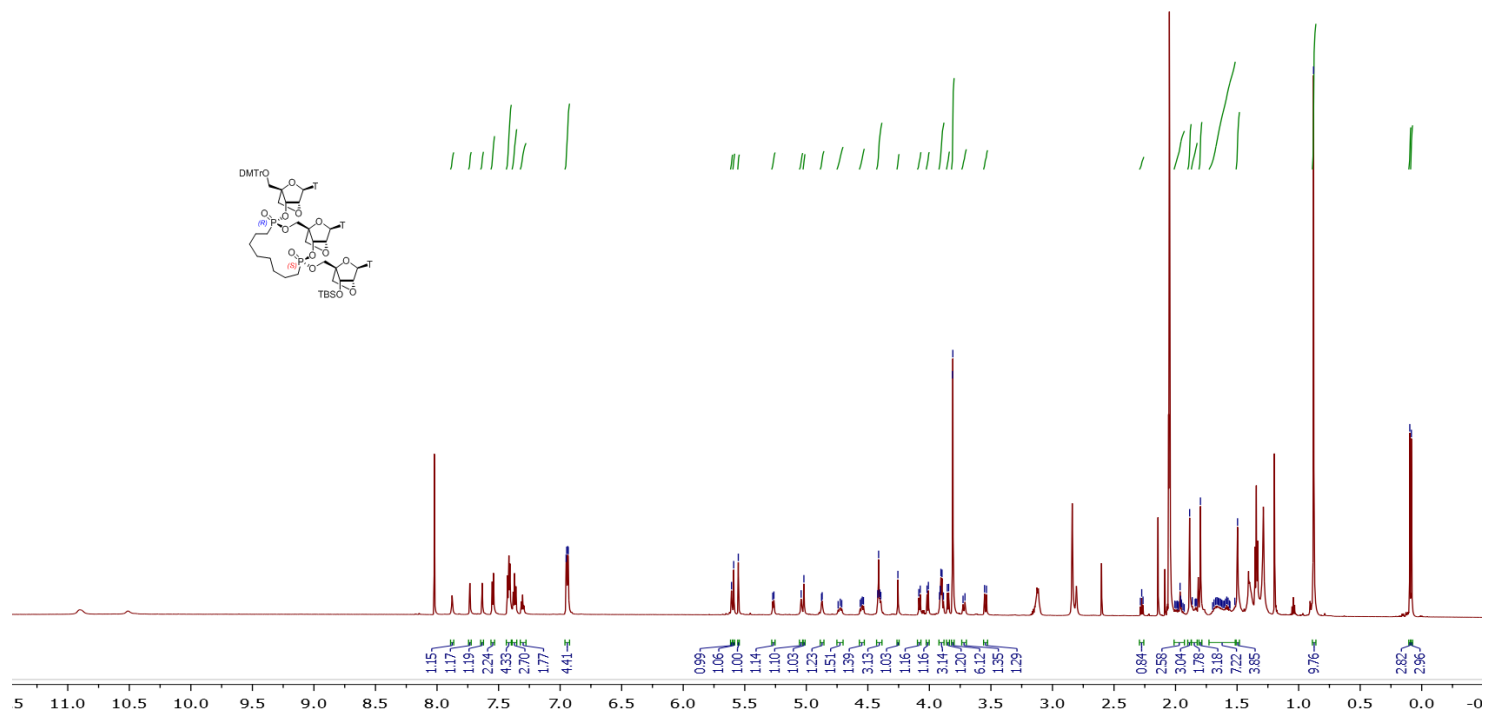
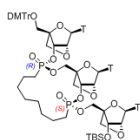
10a:  $^{31}\text{P}$ , 101 MHz, acetone- $d_6$

36.18  
34.79

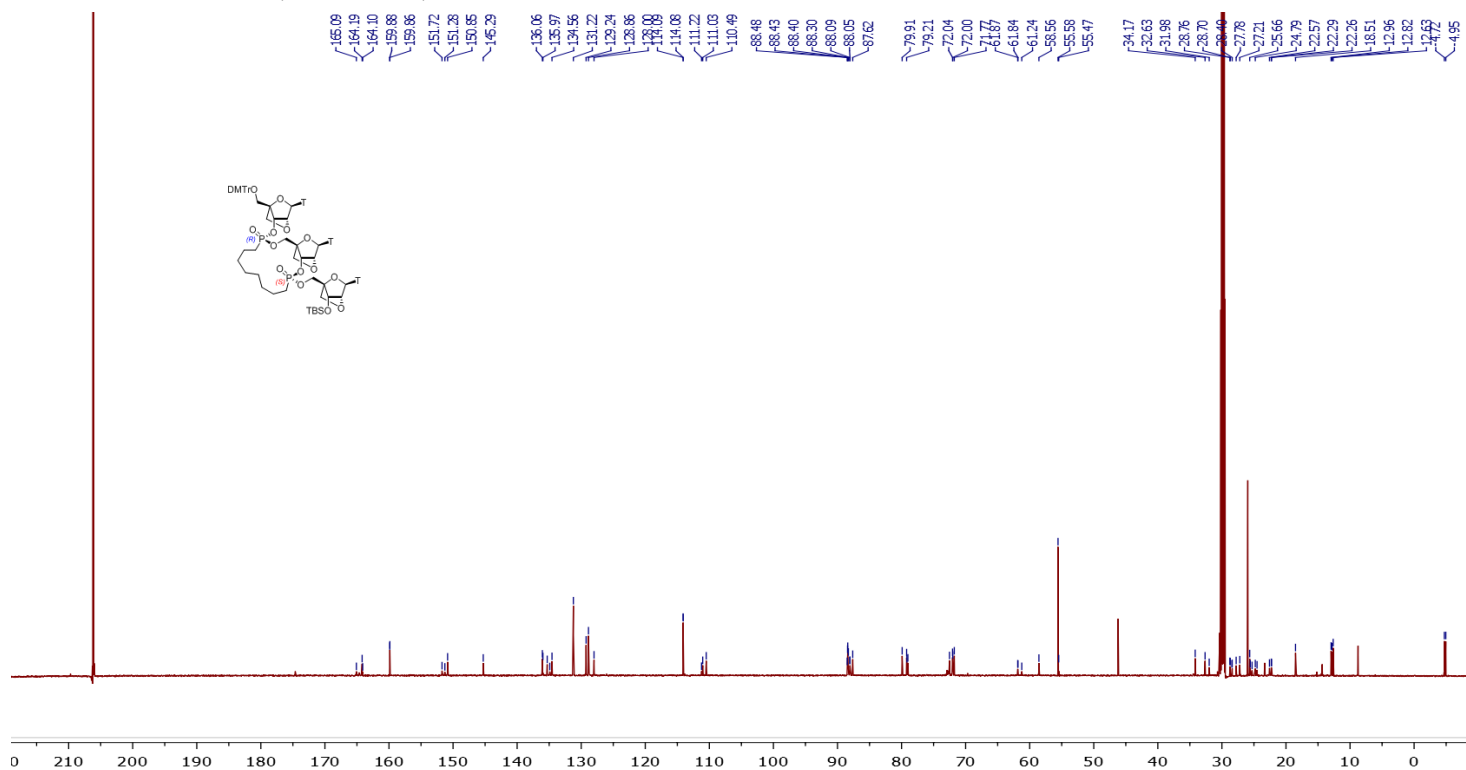


10b:  $^1\text{H}$ , 700 MHz, acetone- $d_6$

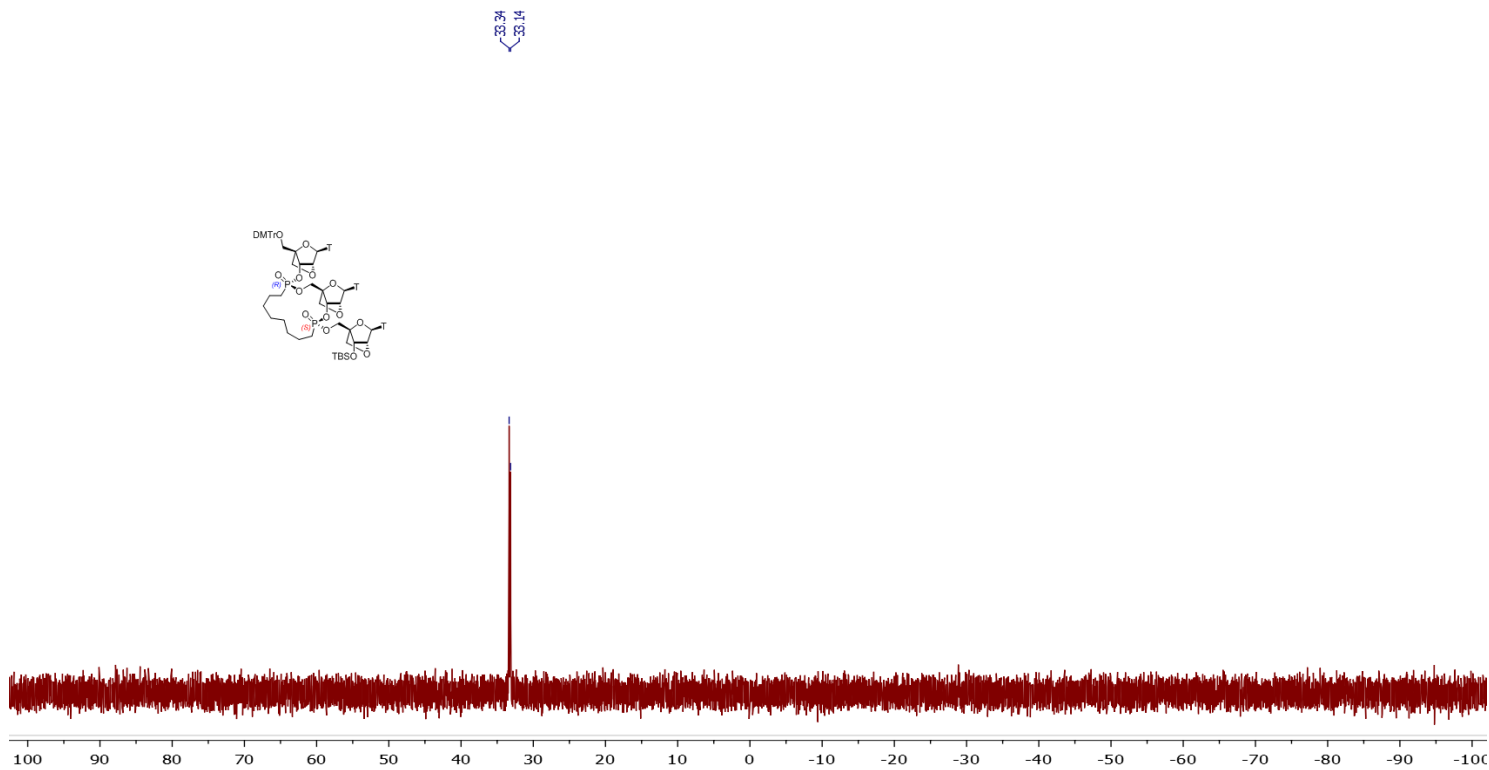
6.95  
6.96  
6.98  
6.99  
5.61  
5.59  
5.55  
5.27  
5.26  
5.04  
5.02  
4.88  
4.87  
4.74  
4.72  
4.71  
4.56  
4.55  
4.54  
4.54  
4.42  
4.41  
4.40  
4.39  
4.36  
4.09  
4.07  
4.02  
4.01  
3.92  
3.91  
3.91  
3.90  
3.89  
3.88  
3.84  
3.81  
3.81  
3.72  
3.71  
3.55  
3.54  
3.29  
2.29  
2.27  
2.26  
2.01  
2.00  
1.99  
1.98  
1.96  
1.96  
1.86  
1.85  
1.89  
1.89  
1.88  
1.84  
1.84  
1.83  
1.80  
1.70  
1.69  
1.67  
1.66  
1.65  
1.64  
1.63  
1.62  
1.61  
1.60  
1.59  
1.58  
1.57  
1.56  
1.56  
1.52  
1.50  
0.88  
0.10  
0.08



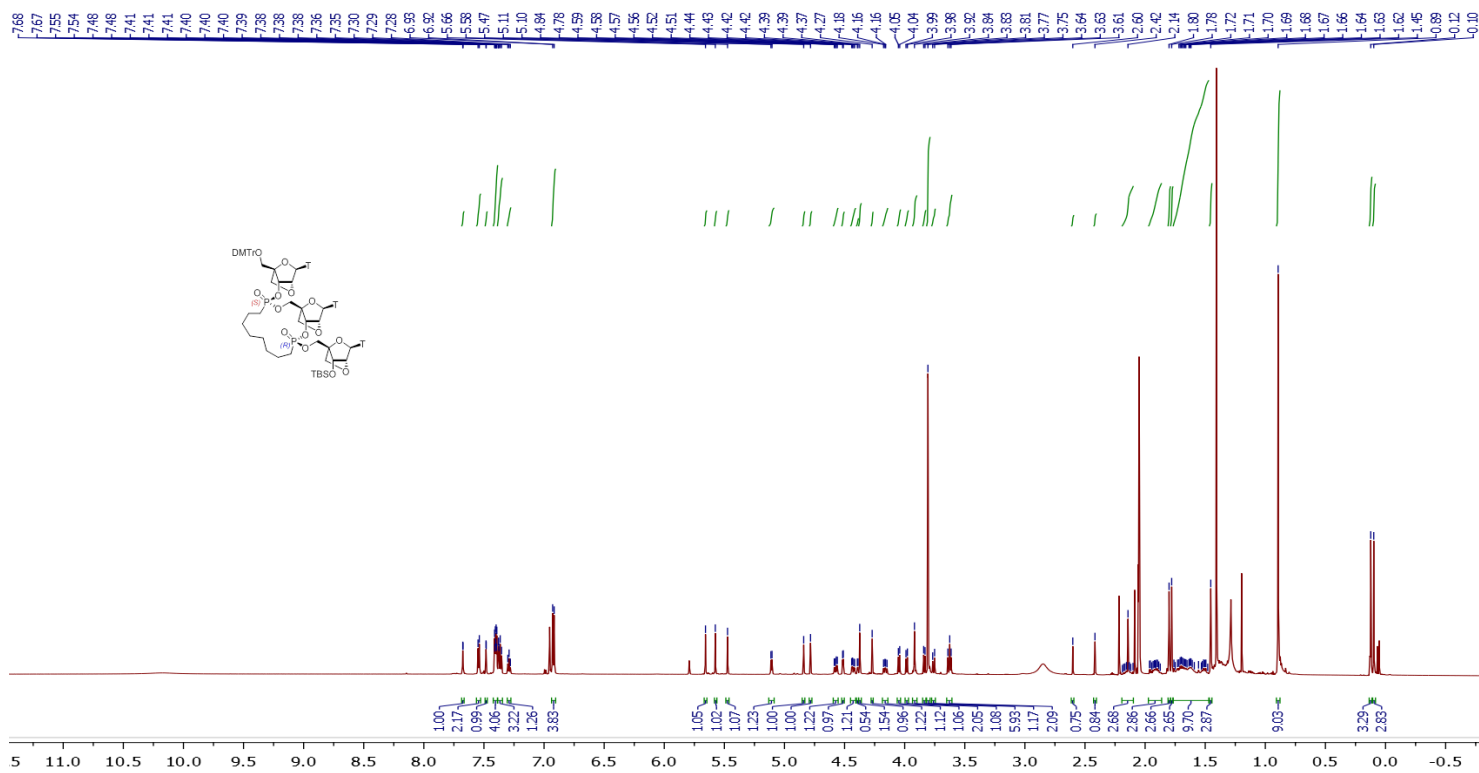
10b:  $^{13}\text{C}$ , 175 MHz, acetone- $d_6$



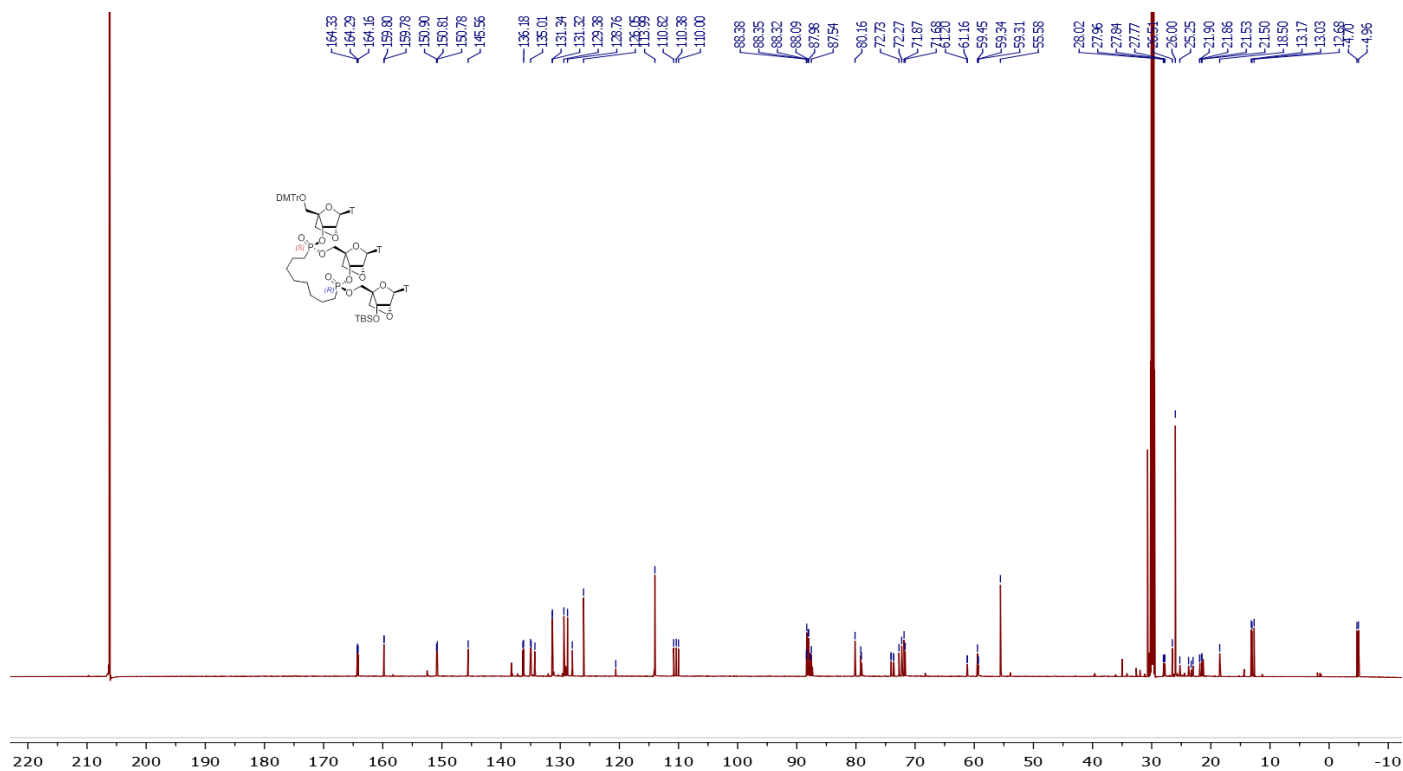
10b:  $^{31}\text{P}$ , 101 MHz, acetone- $d_6$



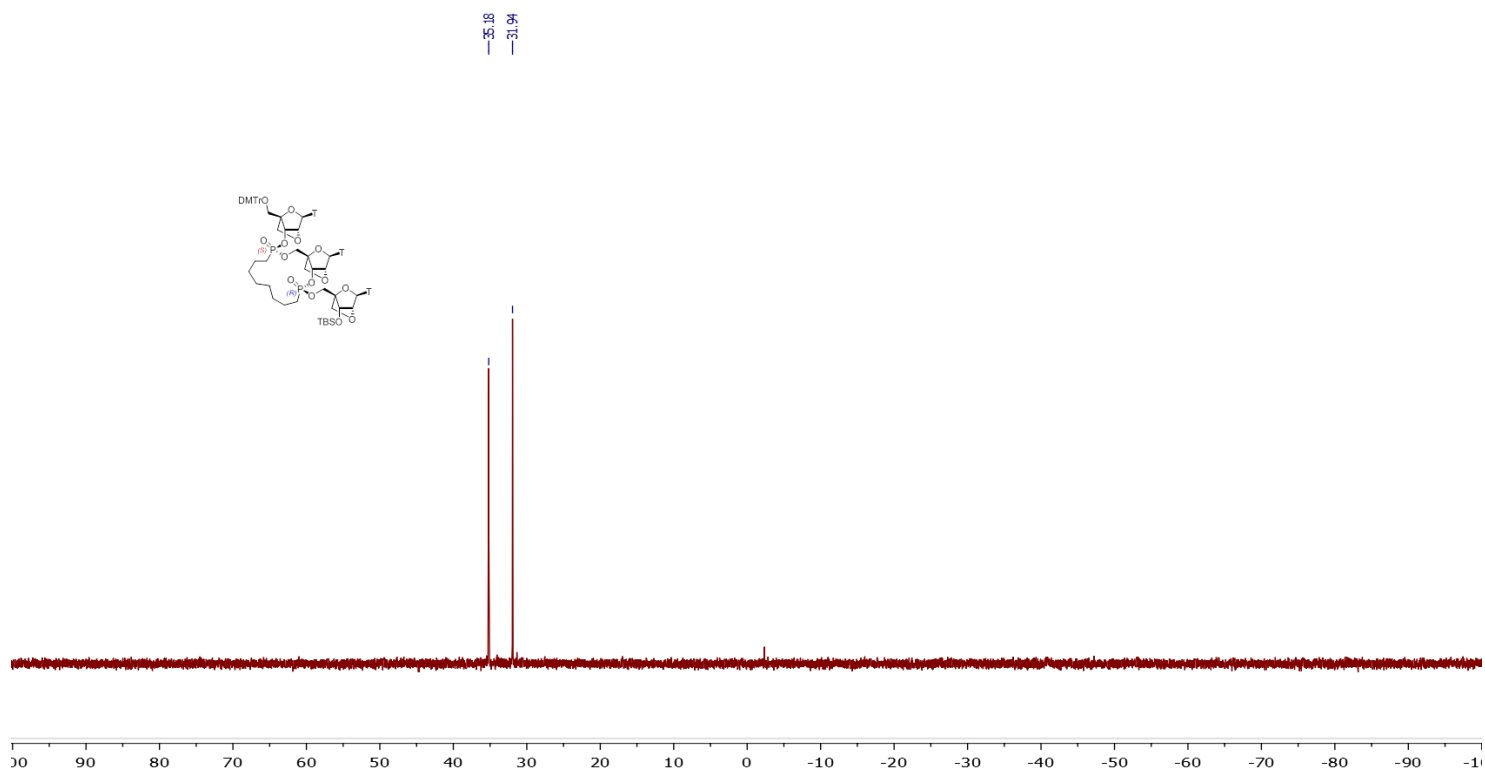
**10c: <sup>1</sup>H, 700 MHz, acetone-*d*<sub>6</sub>**



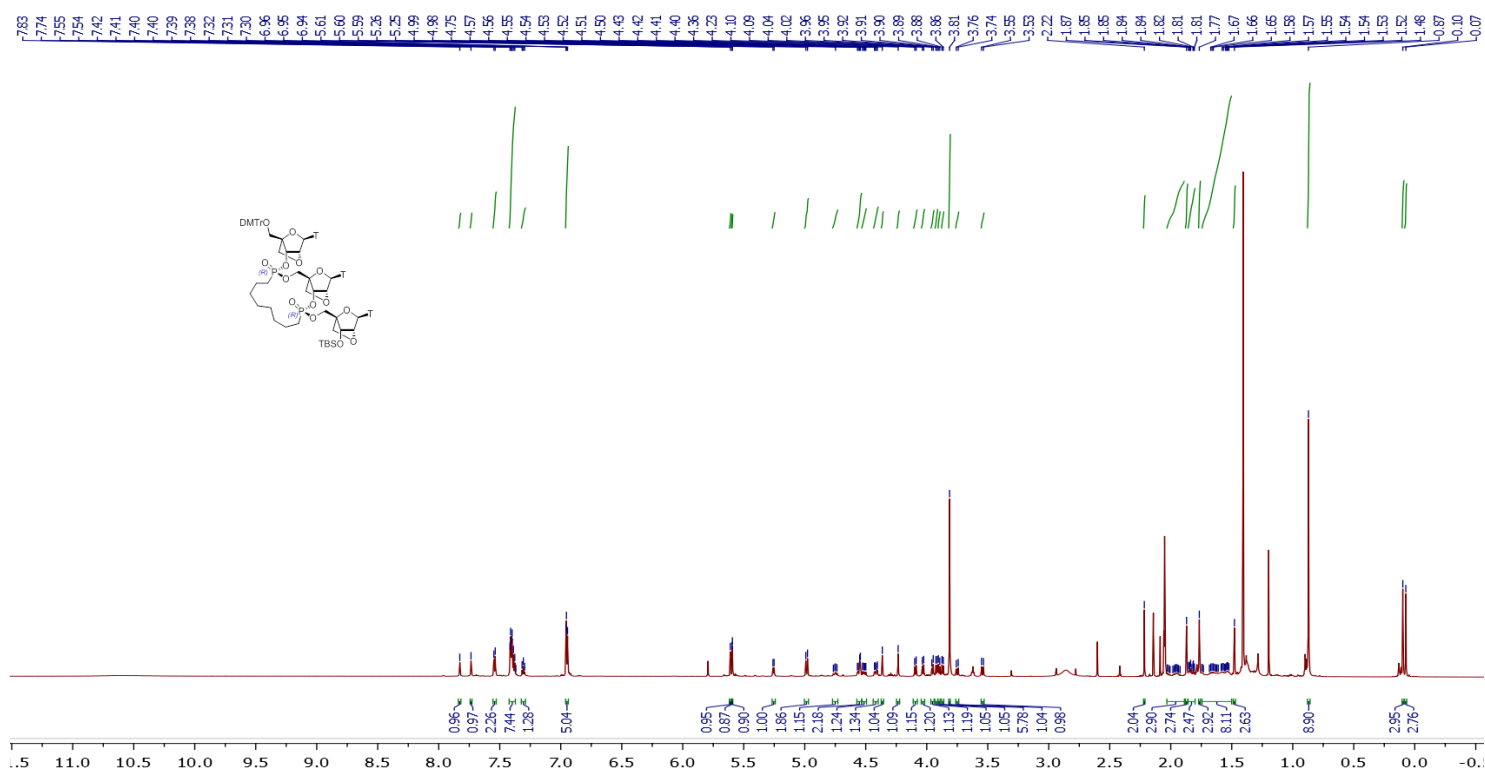
**10c: <sup>13</sup>C, 175 MHz, acetone-*d*<sub>6</sub>**



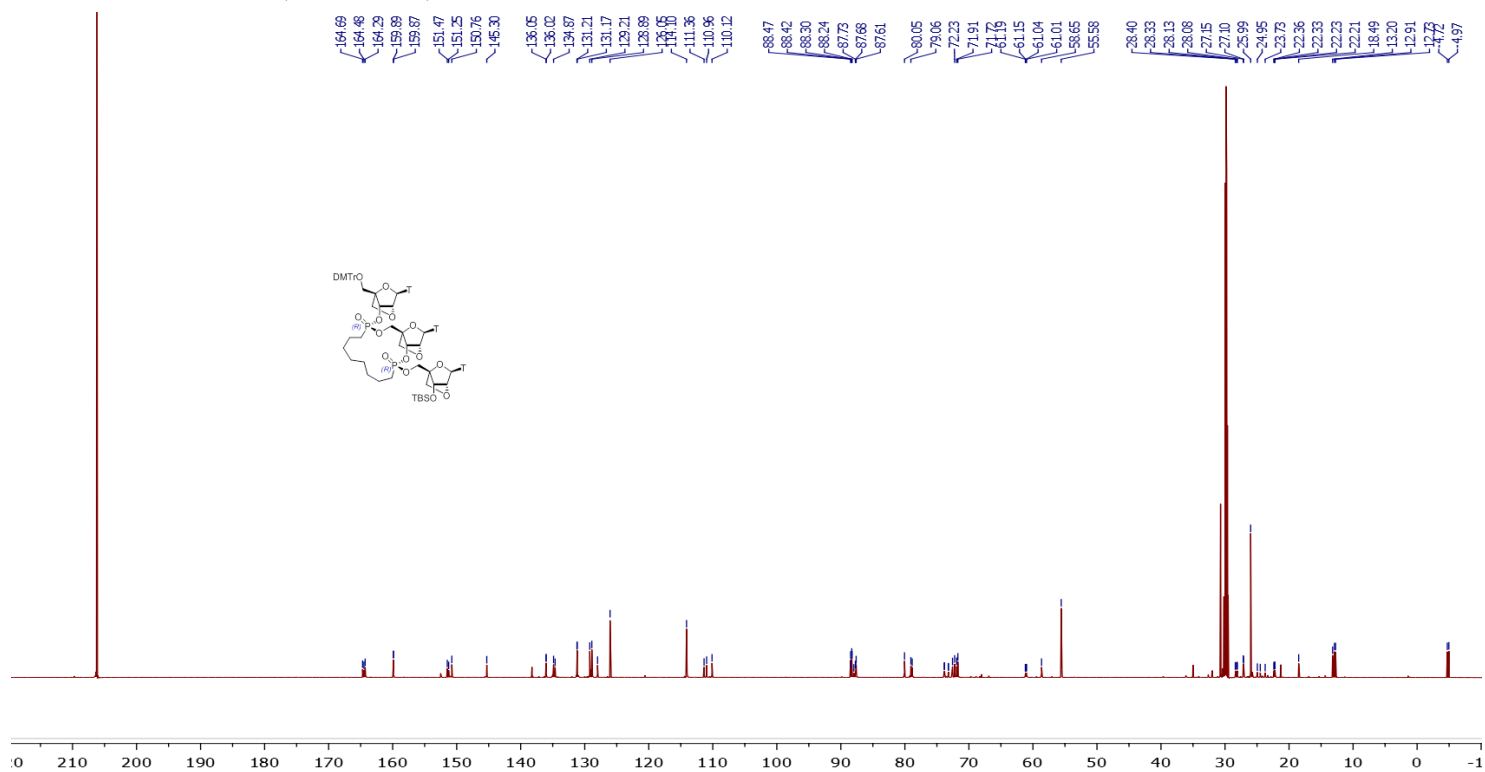
10c:  $^{31}\text{P}$ , 101 MHz, acetone- $d_6$



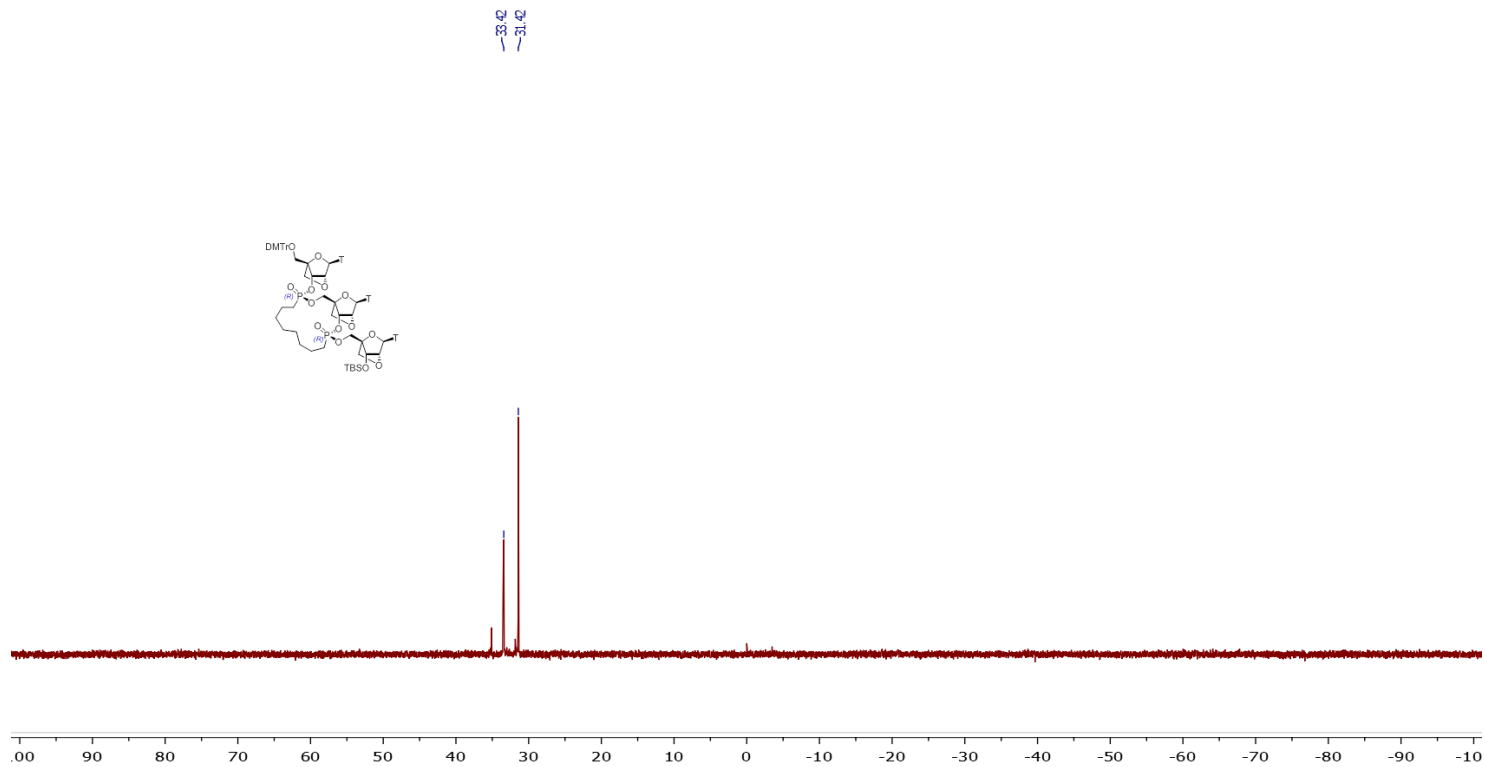
10d:  $^1\text{H}$ , 700 MHz, acetone- $d_6$



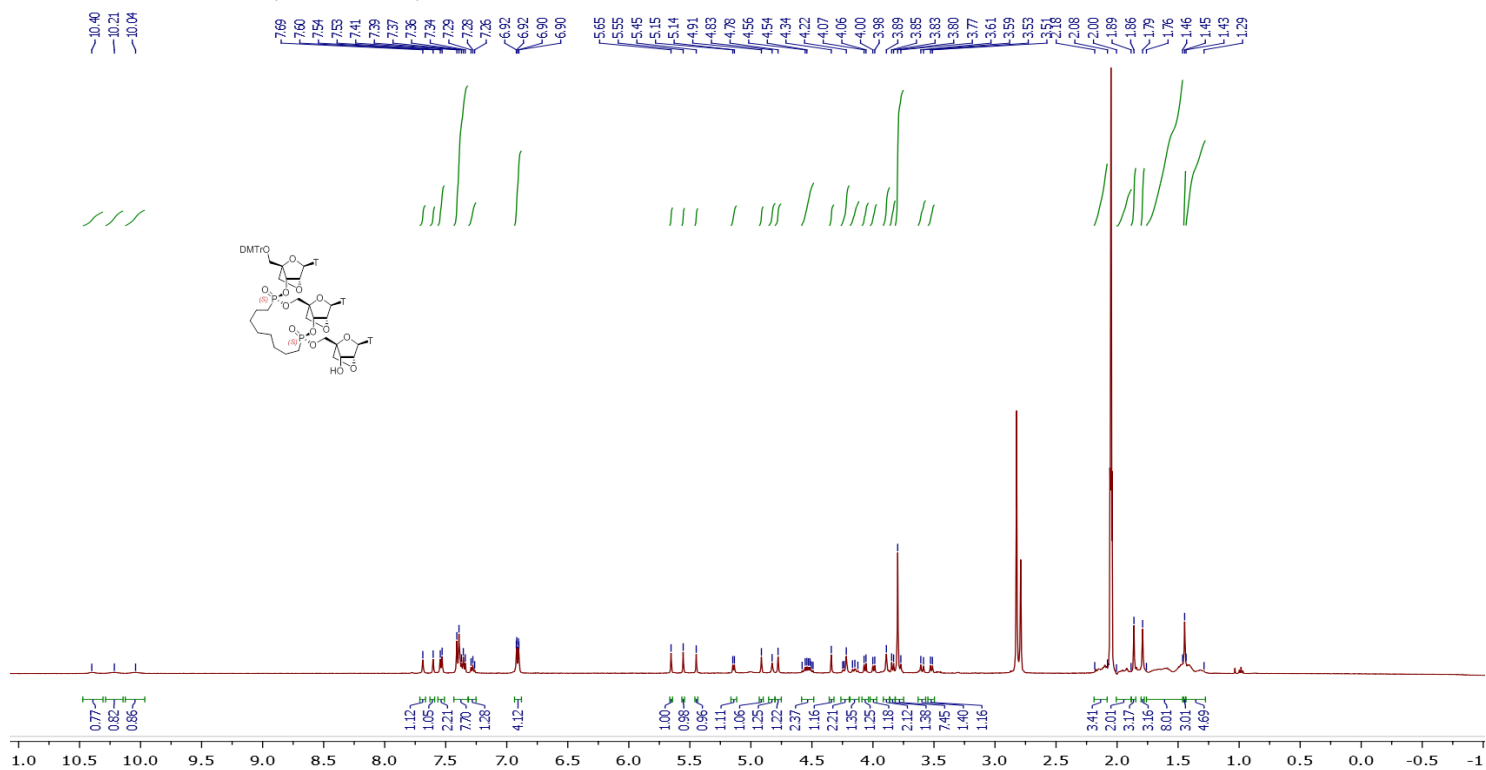
10d:  $^{13}\text{C}$ , 175 MHz, acetone- $d_6$



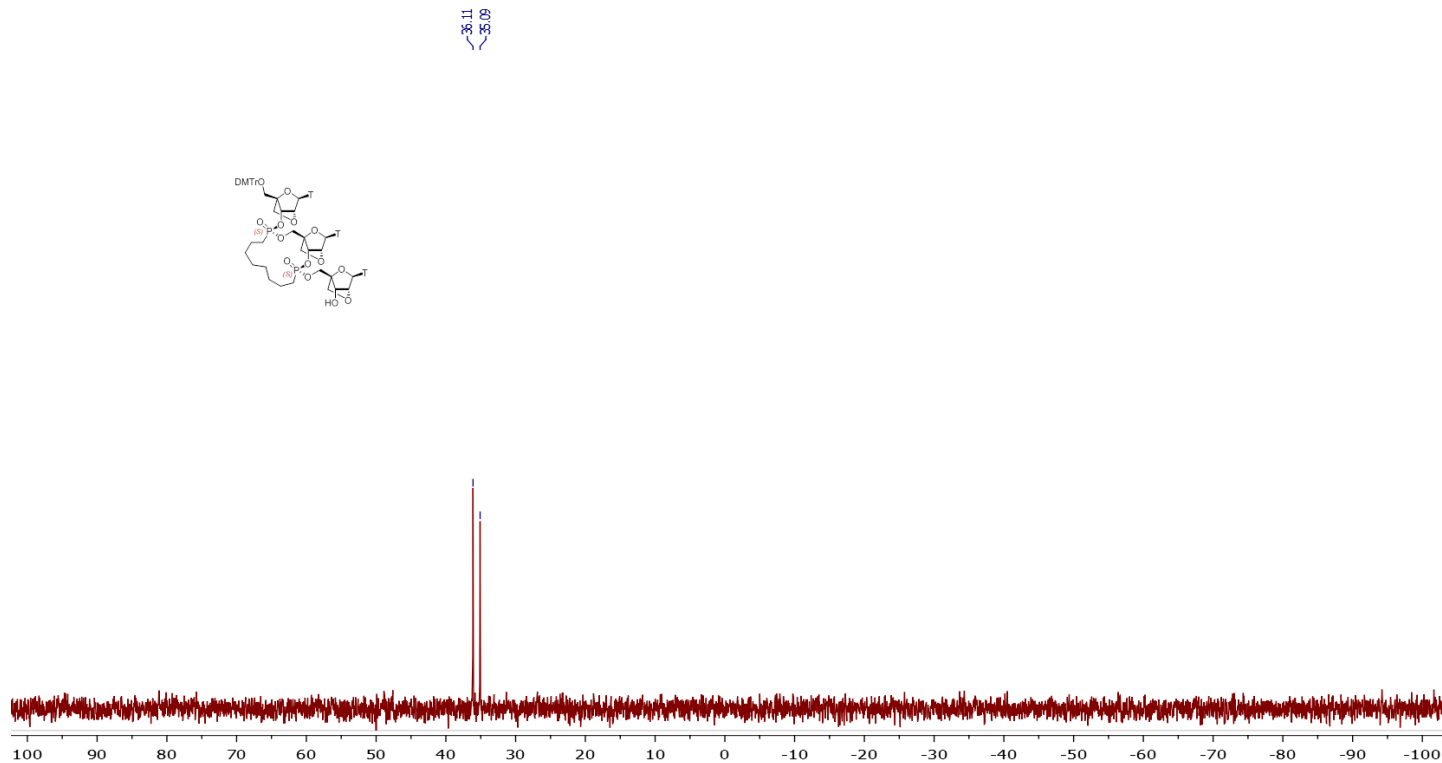
10d:  $^{31}\text{P}$ , 101 MHz, acetone- $d_6$



11a:  $^1\text{H}$ , 500 MHz, acetone- $d_6$

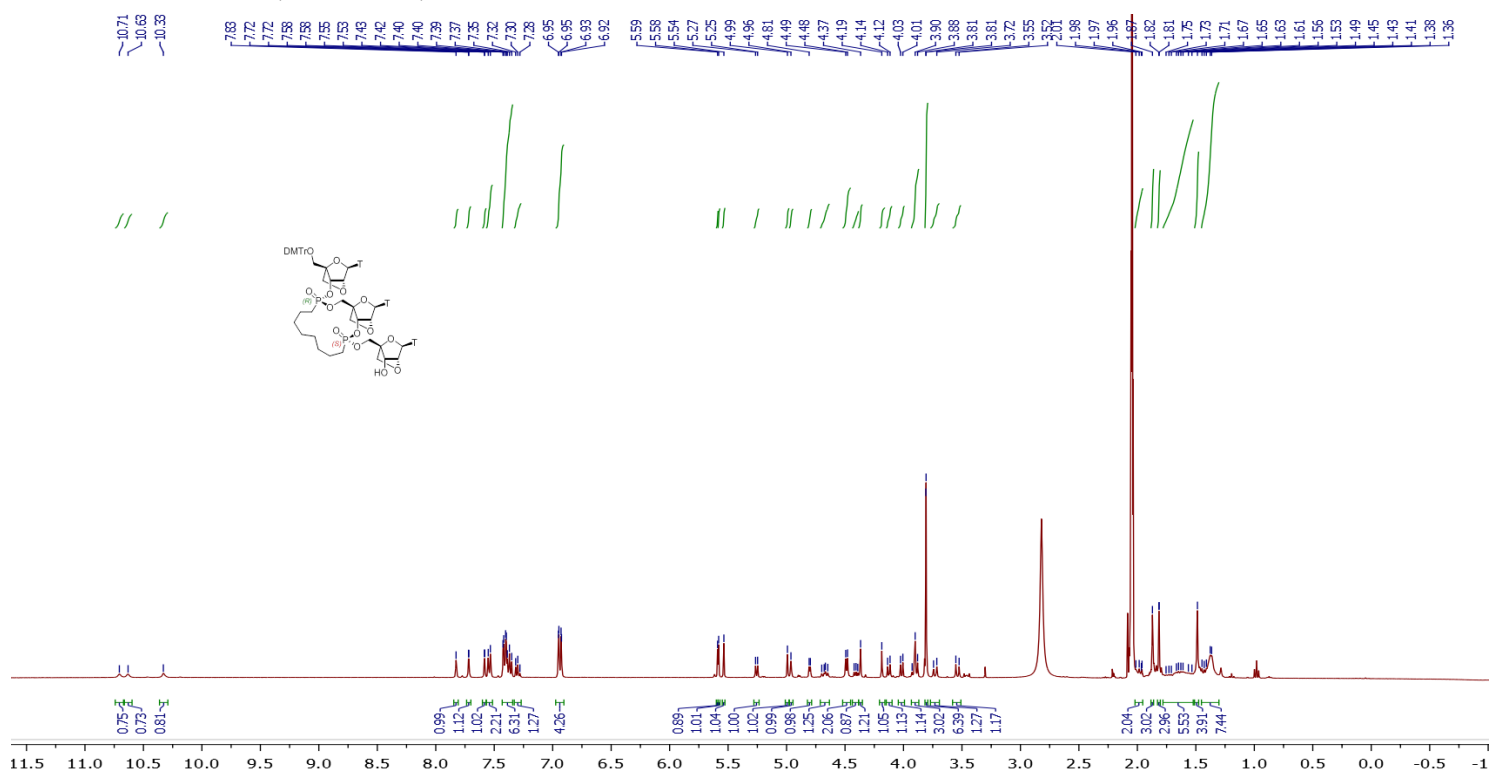


11a:  $^{31}\text{P}$ , 202 MHz, acetone- $d_6$

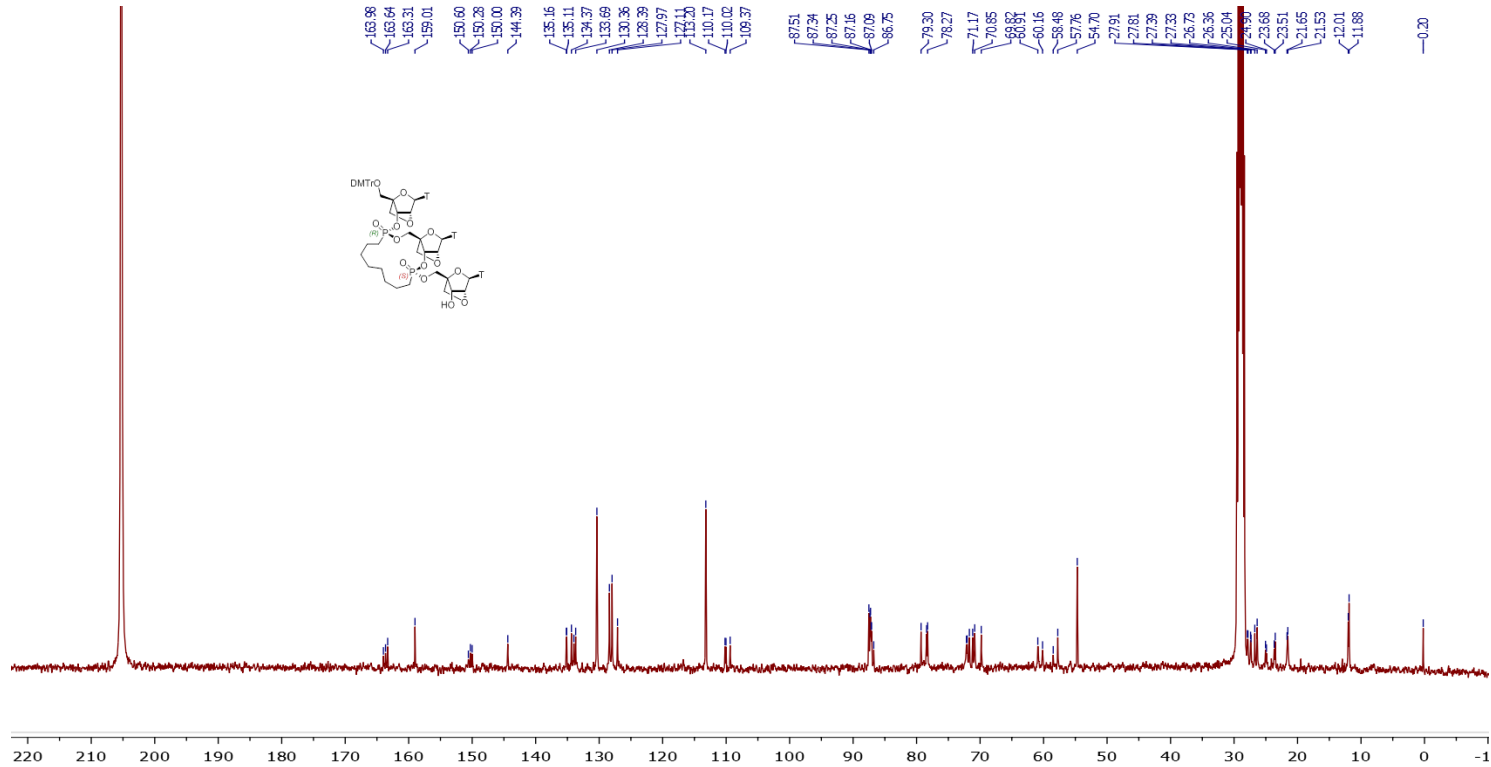




11b: <sup>1</sup>H, 500 MHz, acetone-d<sub>6</sub>

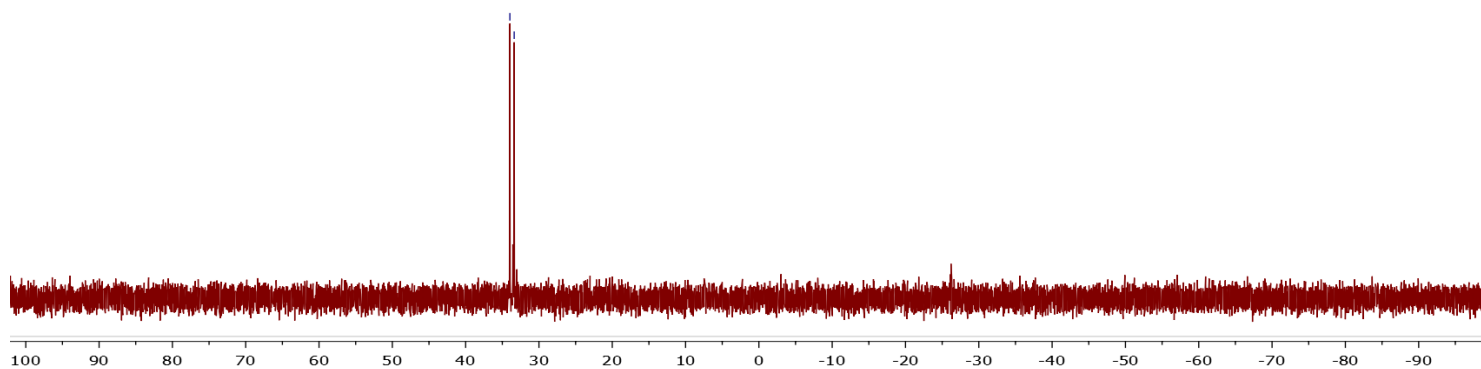
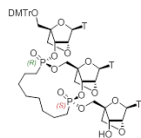


11b: <sup>13</sup>C, 100 MHz, acetone-d<sub>6</sub>



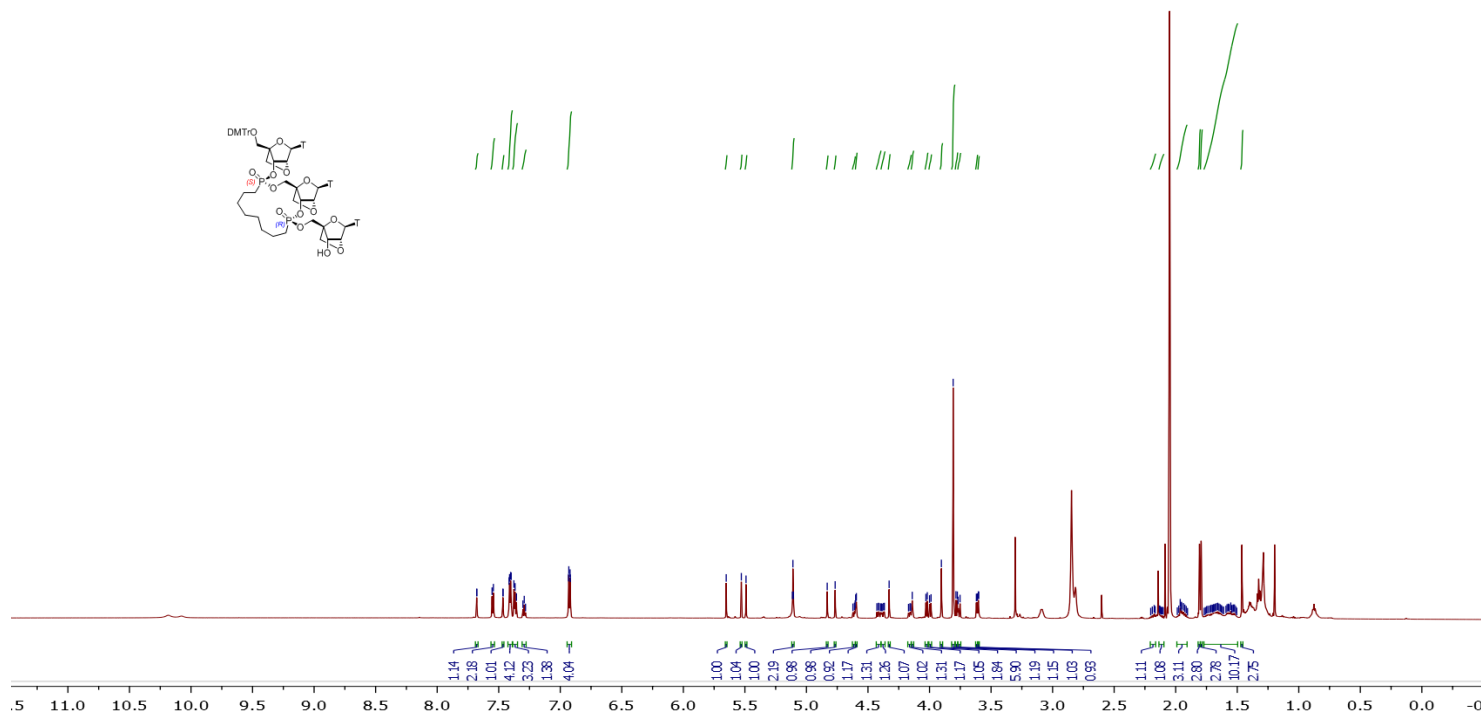
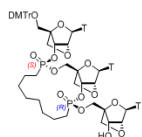
11b: <sup>31</sup>P, 202 MHz, acetone-*d*<sub>6</sub>

33.97  
33.88

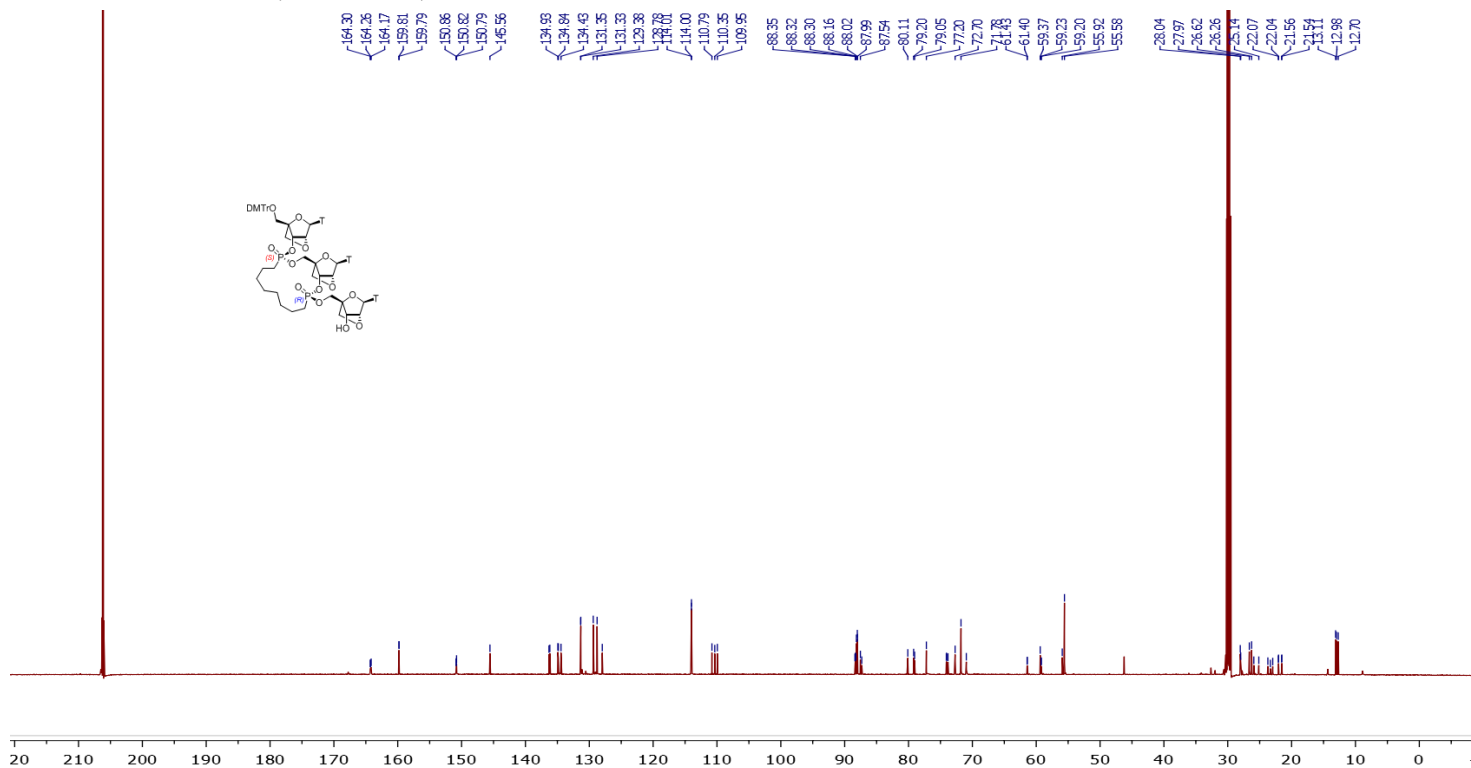


11c: <sup>1</sup>H, 700 MHz, acetone-*d*<sub>6</sub>

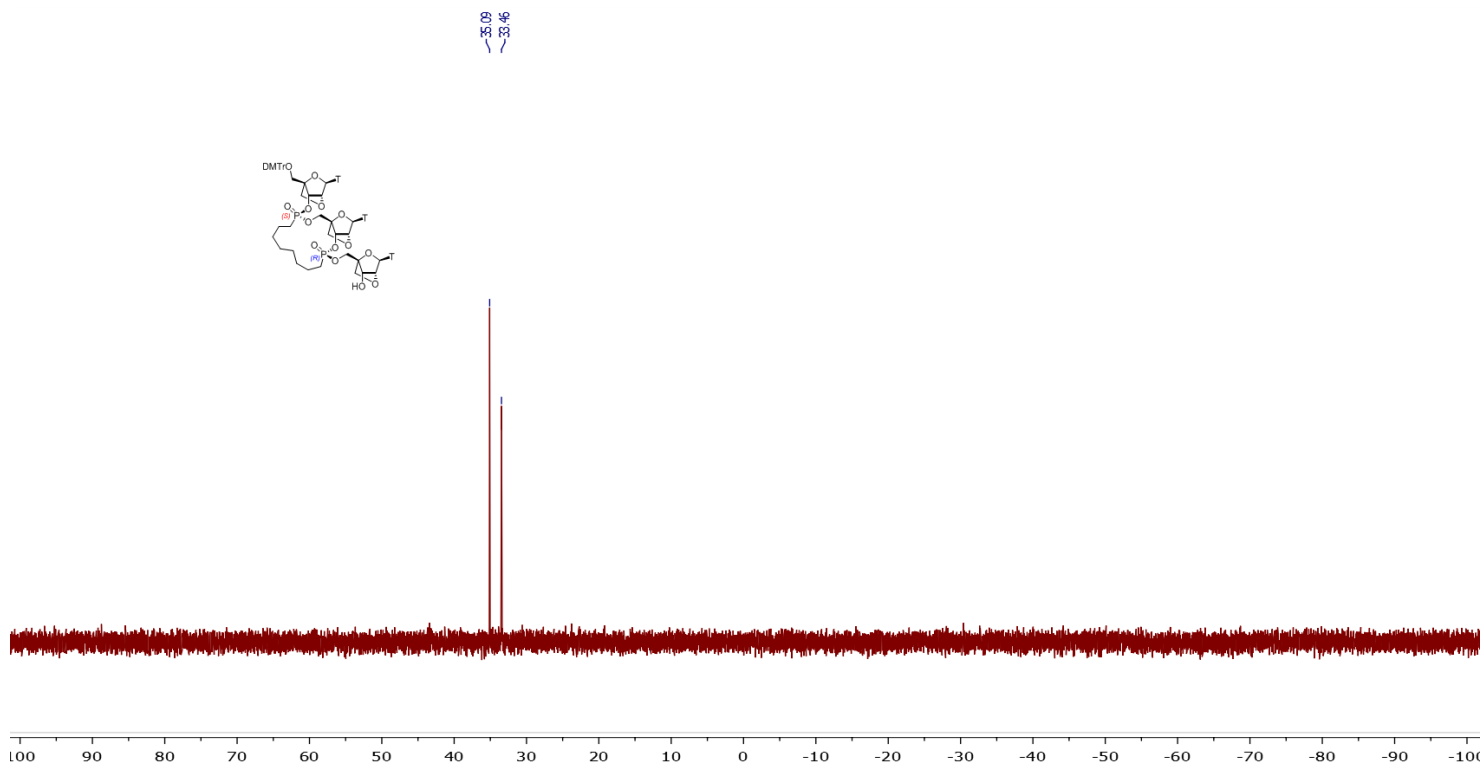
7.68 7.68 7.55 7.54 7.47 7.46 7.42 7.41 7.40 7.40 7.38 7.37 7.36 7.30 7.29 7.28 6.93 6.92 6.92 5.65 5.53 5.49 5.11 5.11 5.11 4.88 4.77 4.62 4.61 4.60 4.60 4.59 4.43 4.41 4.41 4.40 4.39 4.38 4.37 4.37 4.33 4.33 4.16 4.15 4.14 4.03 4.02 4.00 4.00 3.99 3.99 3.84 3.79 3.77 3.77 3.75 3.62 3.61 3.60 3.60 1.96 1.96 1.96 1.95 1.94 1.93 1.69 1.69 1.68 1.67 1.67 1.66 1.65 1.64 1.64 1.57 1.56 1.55 1.53 1.52



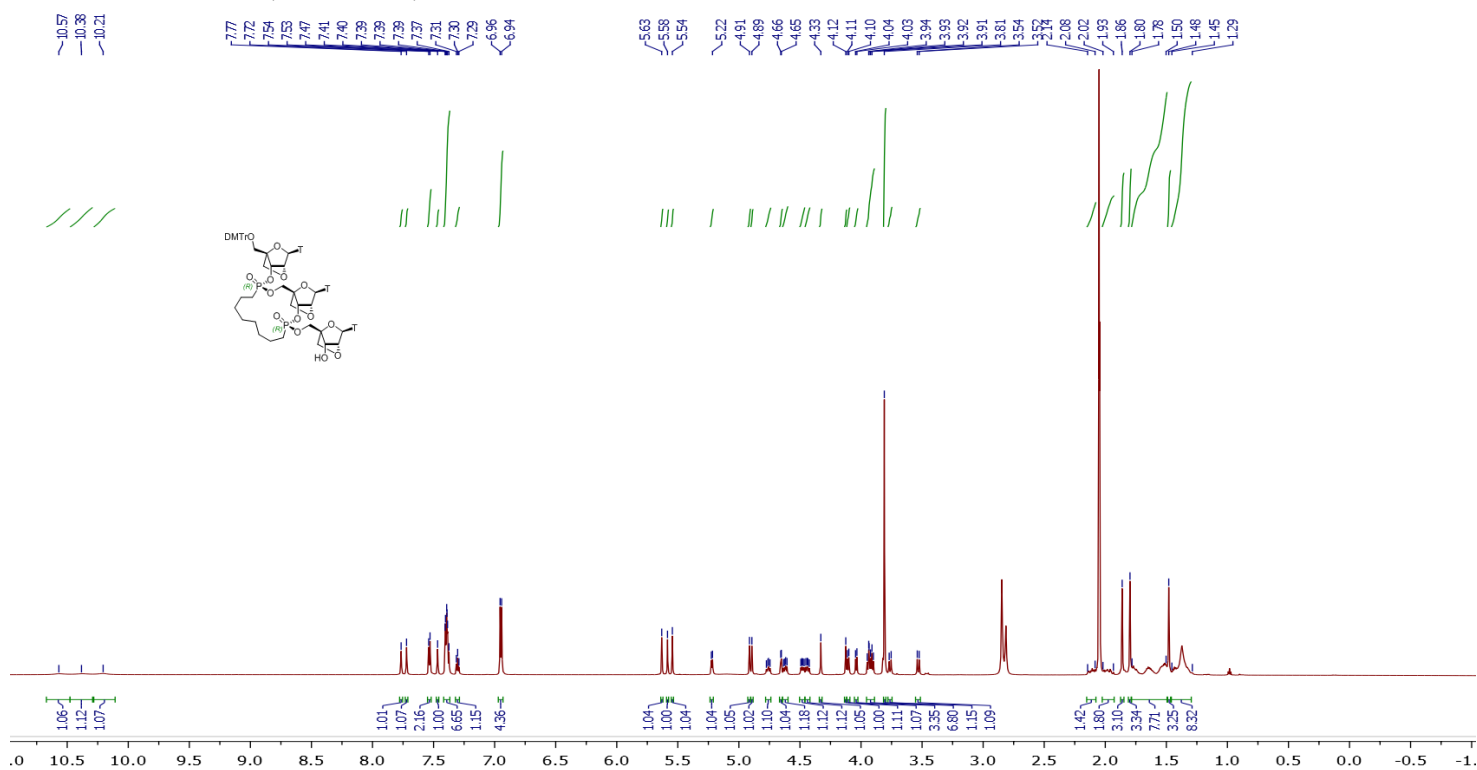
11c:  $^{13}\text{C}$ , 175 MHz, acetone- $d_6$



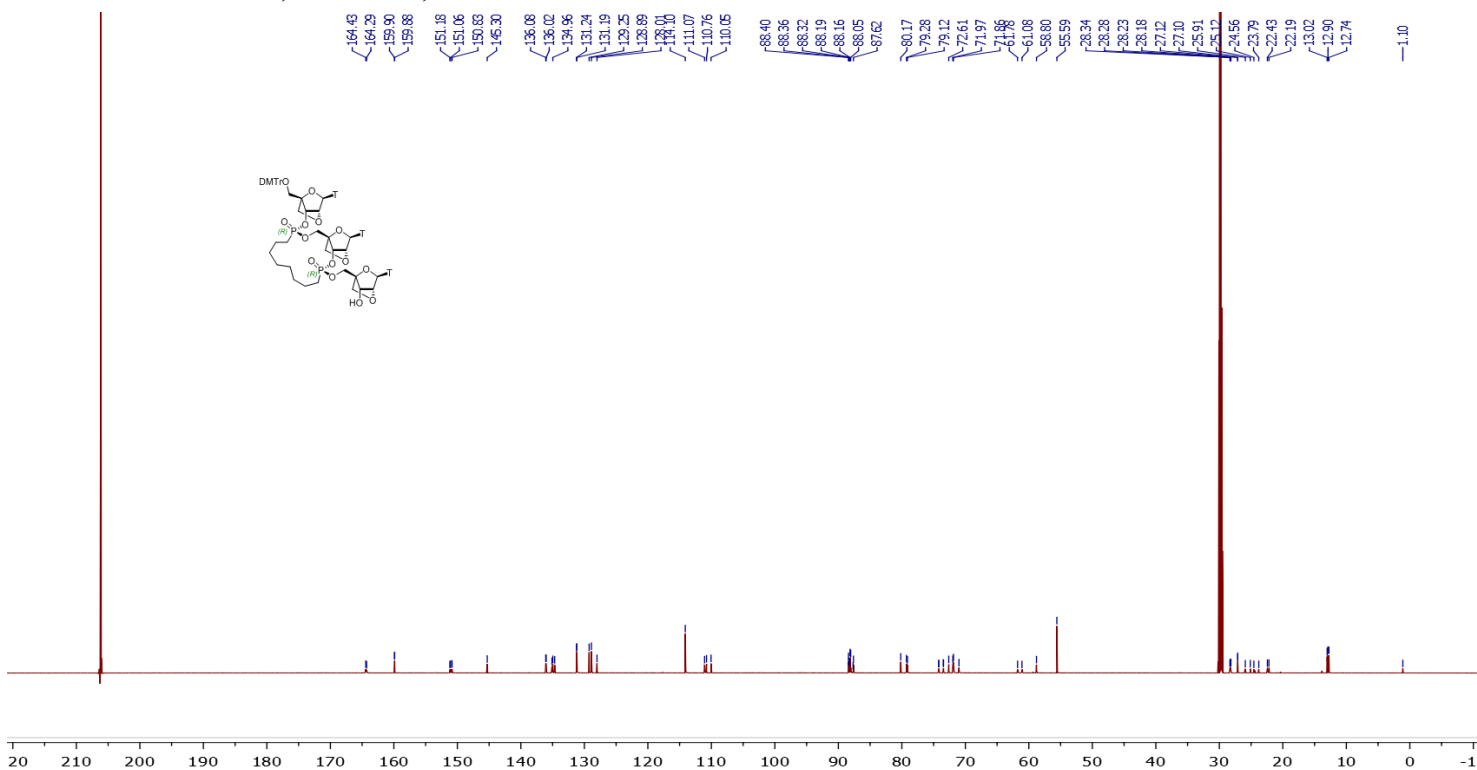
11c:  $^{31}\text{P}$ , 101 MHz, acetone- $d_6$



**11d: <sup>1</sup>H, 700 MHz, acetone-d<sub>6</sub>**



**11d: <sup>13</sup>C, 175 MHz, acetone-d<sub>6</sub>**



11d:  $^{31}\text{P}$ , 101 MHz, acetone- $d_6$

33.72  
33.08

