Supporting Information

Solid phase synthesis, hybridizing ability, uptake and nucleases resistant profile of position-selective cationic and hydrophobic phosphotriester oligonucleotides

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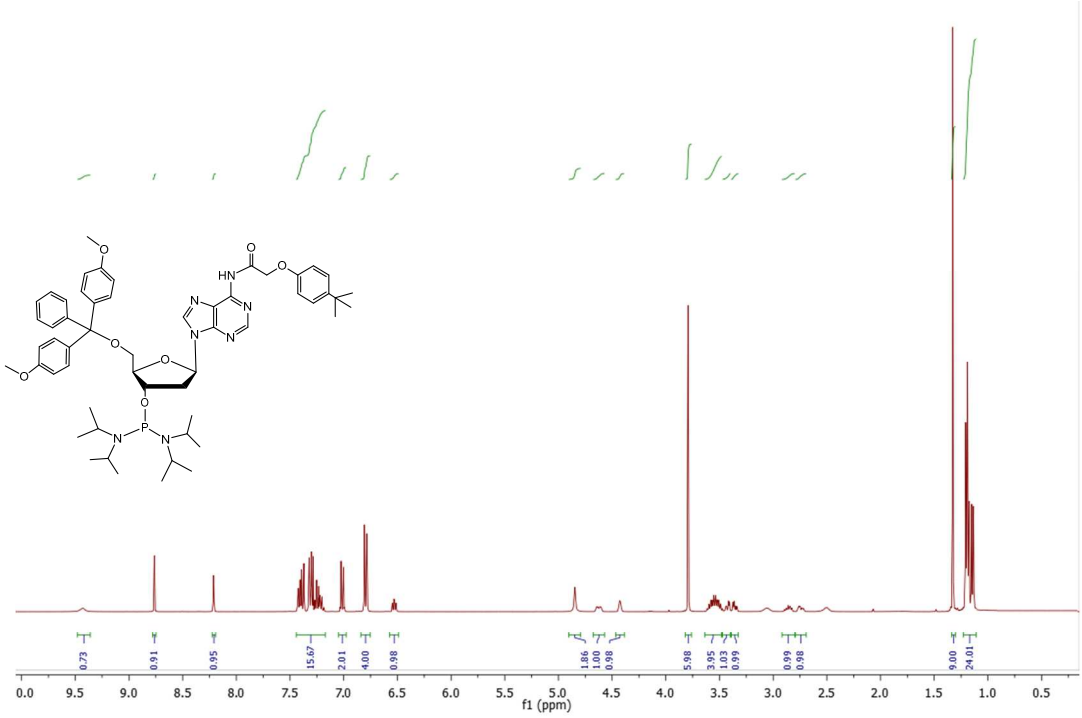
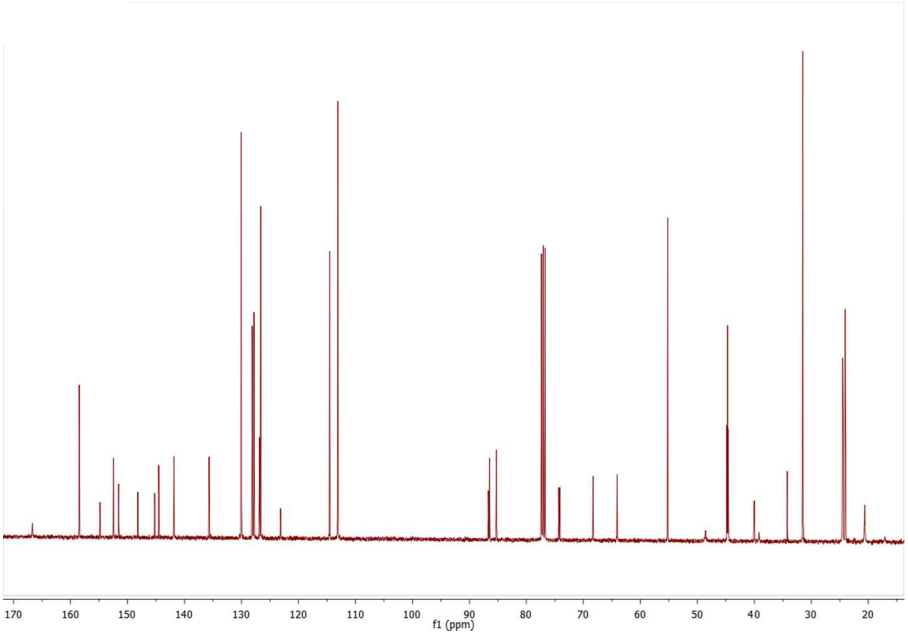
1) NMR spectra





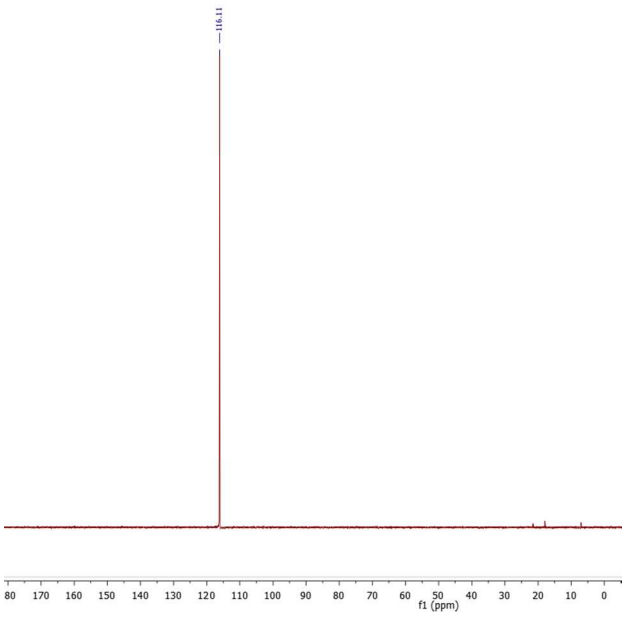
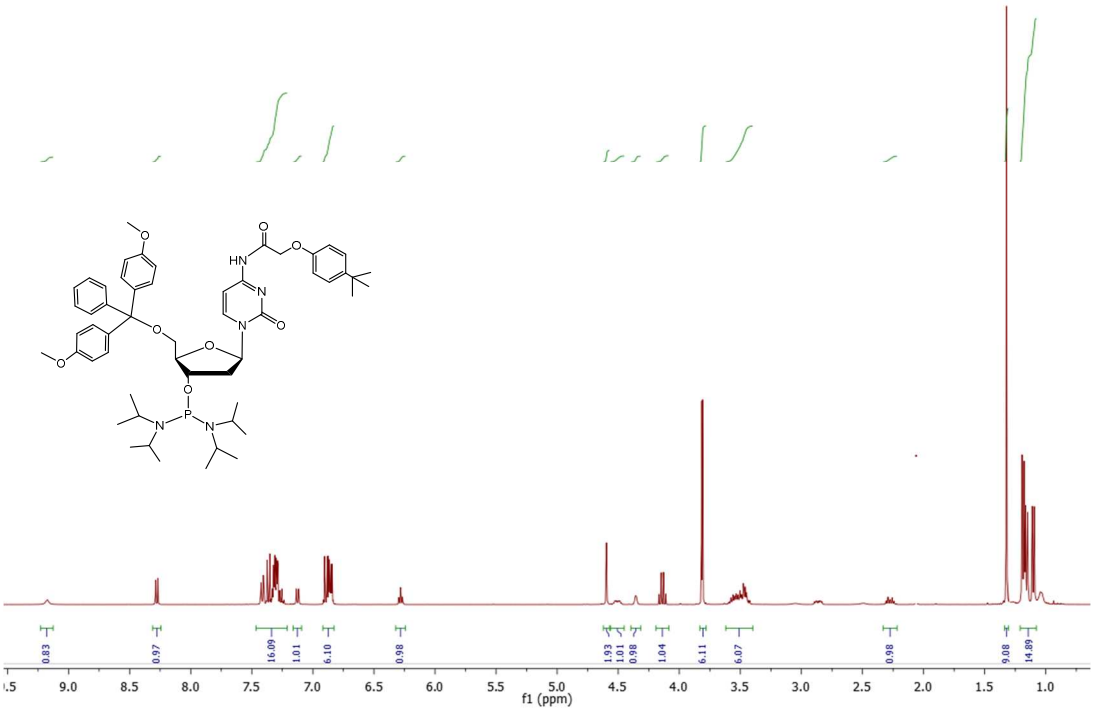


Figure S1. ^{1}H, ^{13}C and ^{31}P NMR of compound 5.



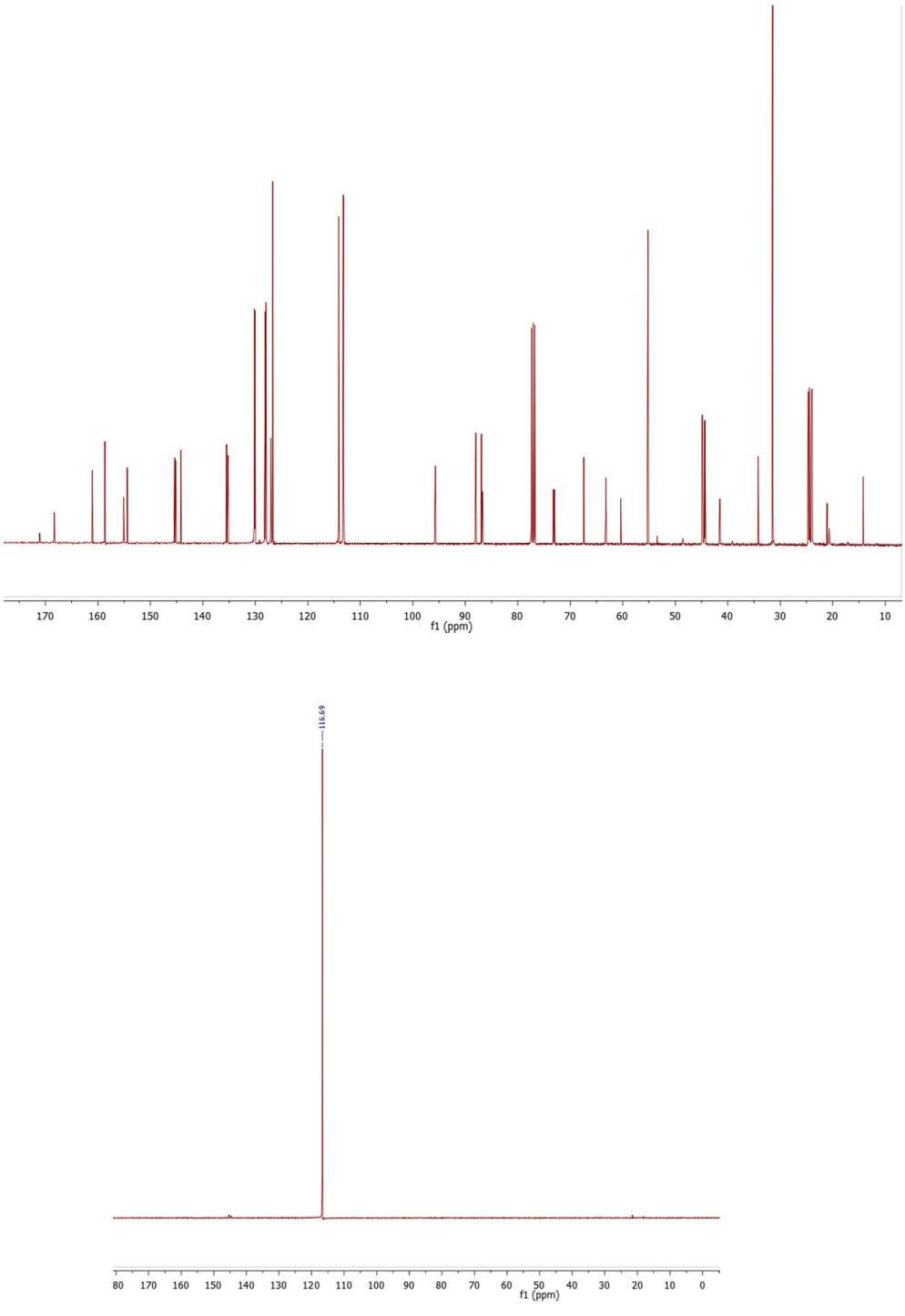
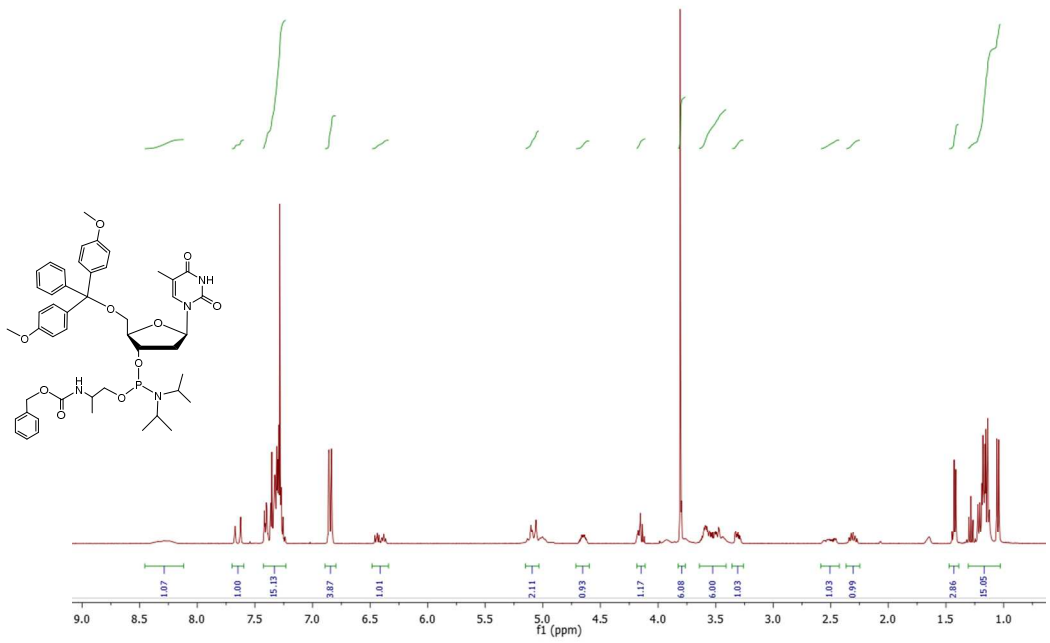


Figure S2. ^{1}H, ^{13}C and ^{31}P NMR of compound 6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3.87
1.01
2.11
0.93
1.17
6.08
6.00
1.03
1.03
0.99
2.86
15.05
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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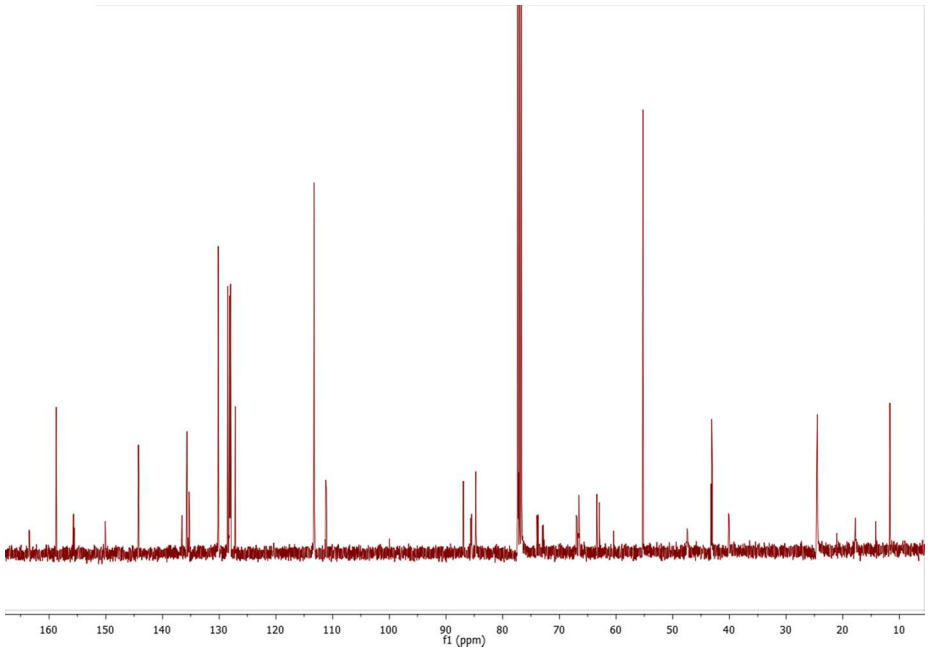
2.5
2.0
1.5
1.0
f1 (ppm)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100
90
80
70
60
50
40
30
20
10
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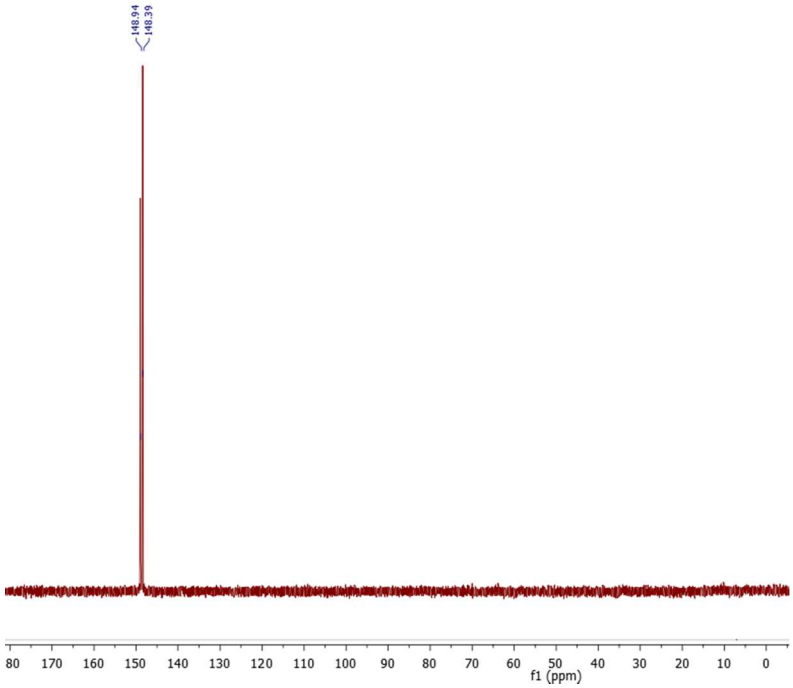
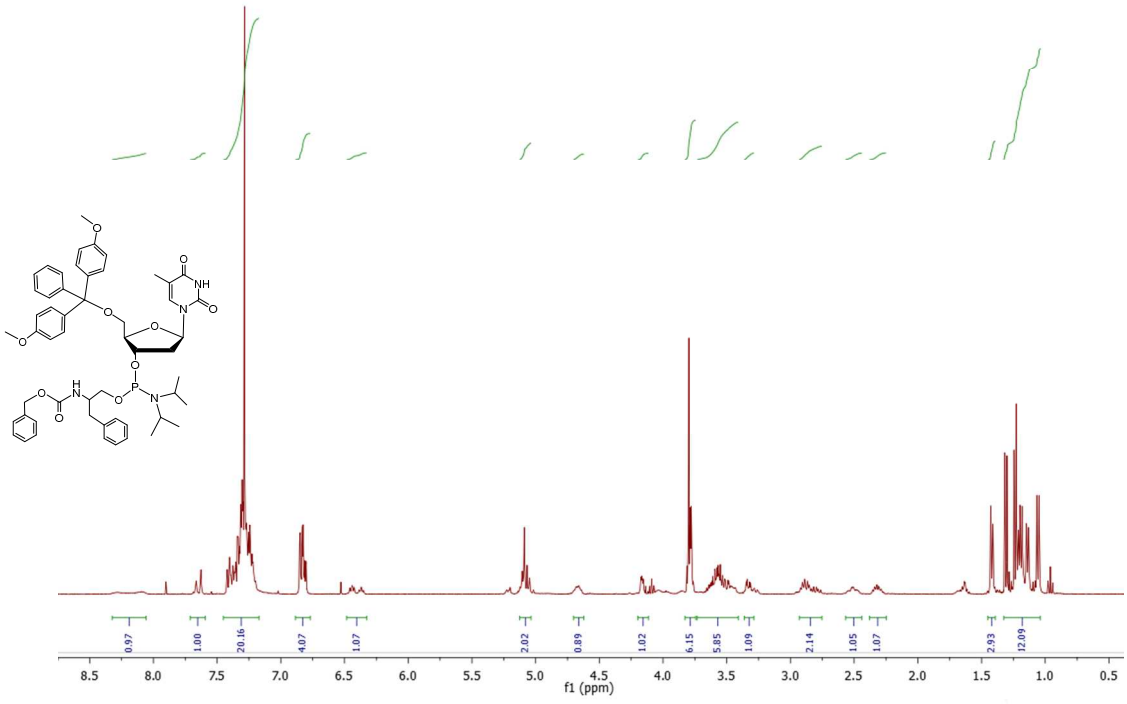


Figure S3. ^{1}H, ^{13}C and ^{31}P NMR of compound 7a



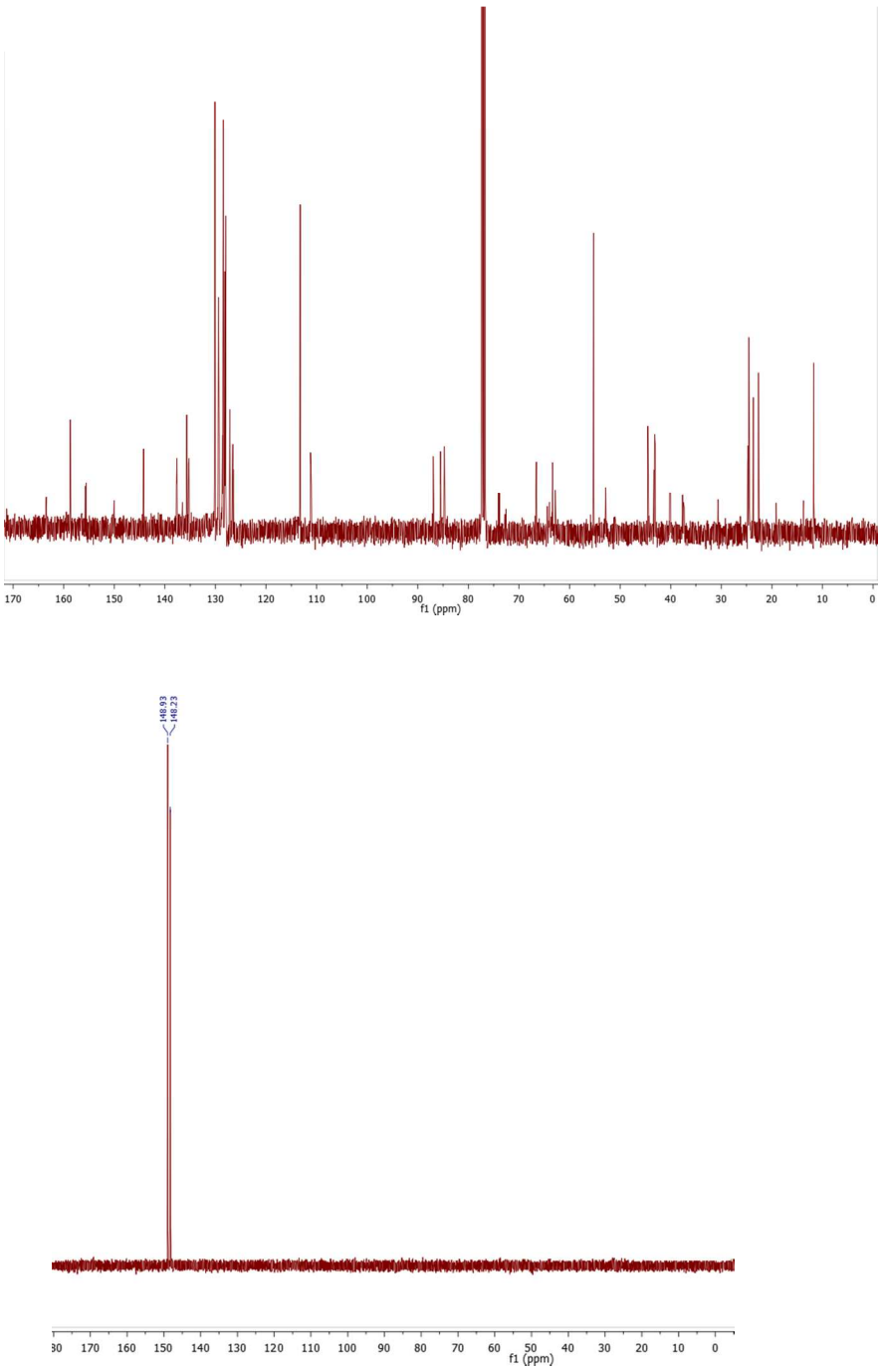
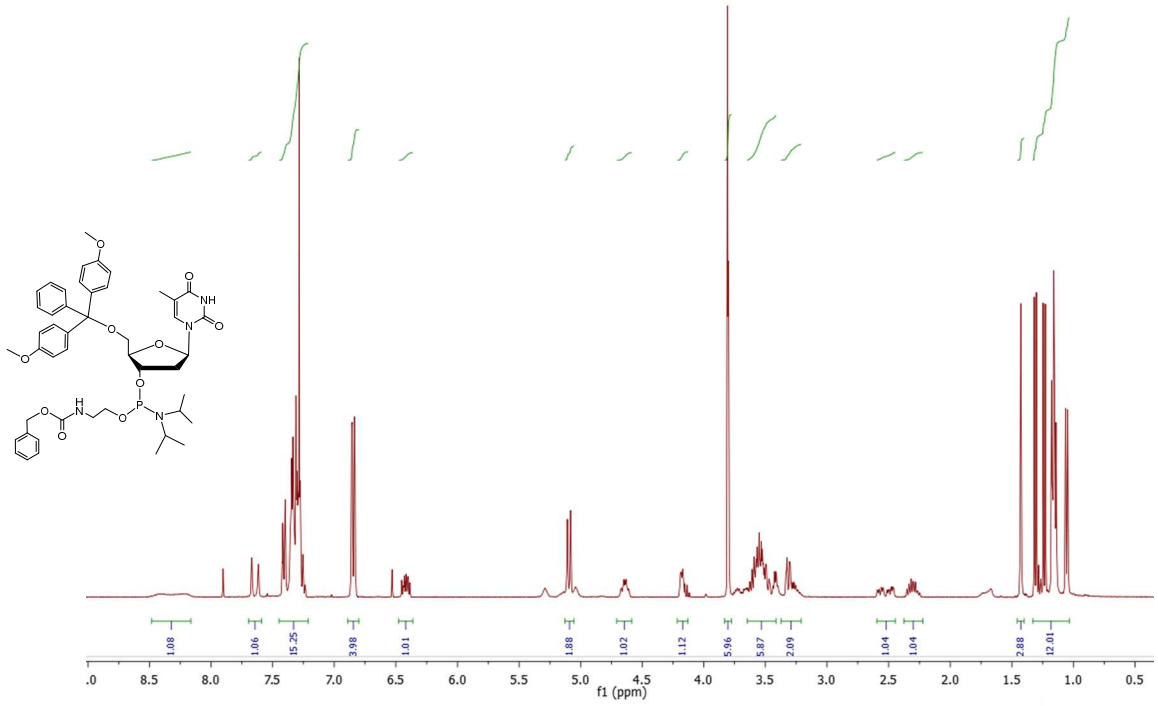


Figure S4. ^{1}H, ^{13}C and ^{31}P NMR of compound 7b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1.01
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5.87
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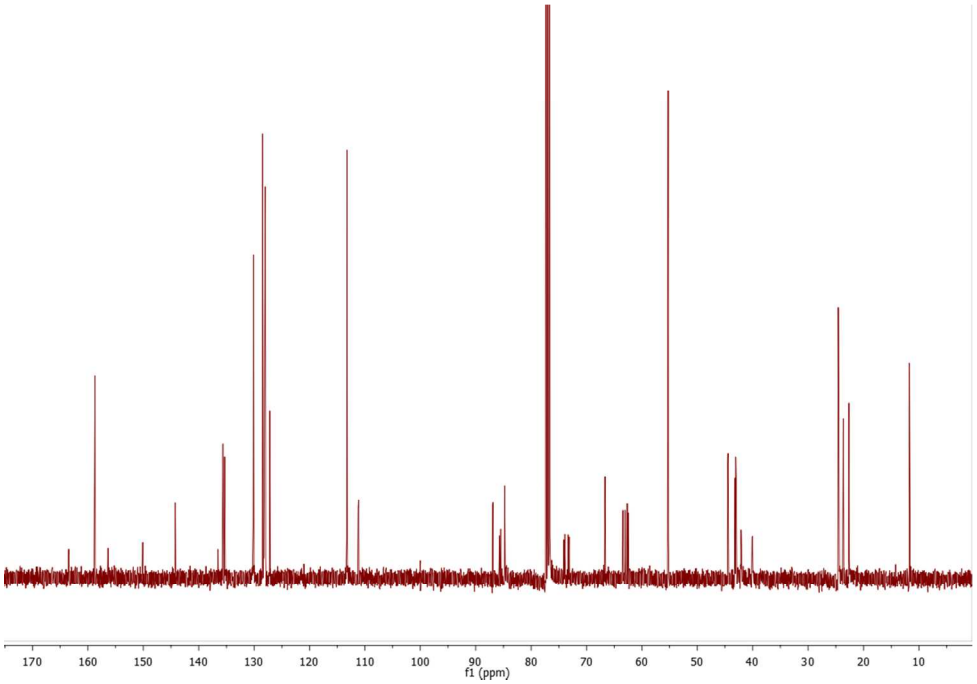
1.04
1.04
2.88
12.01
f1 (ppm)


f1 (ppm)

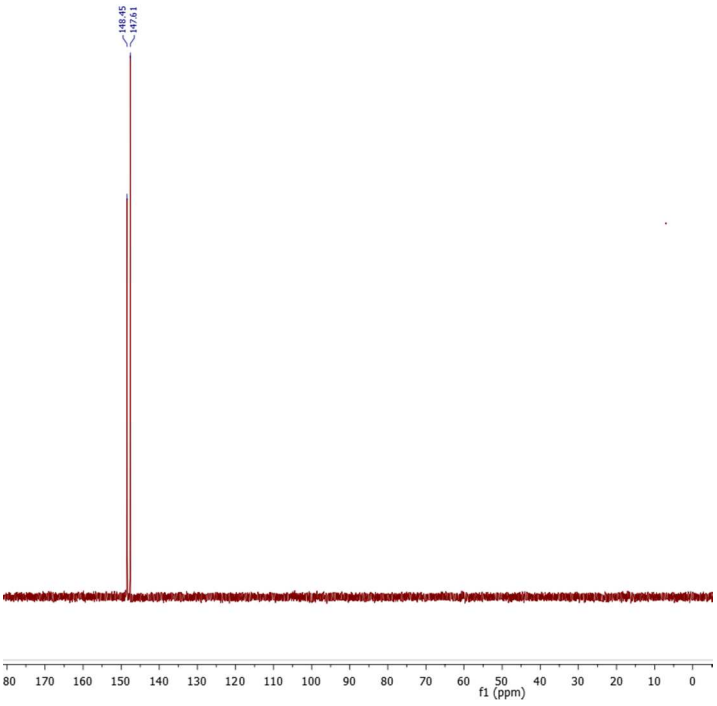
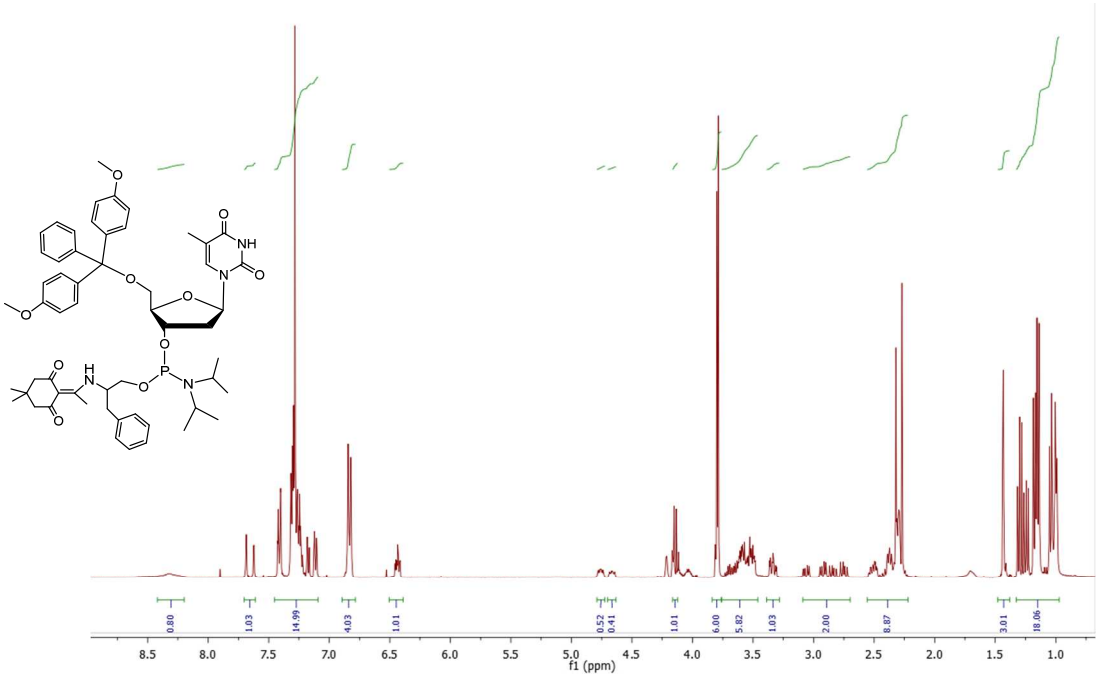
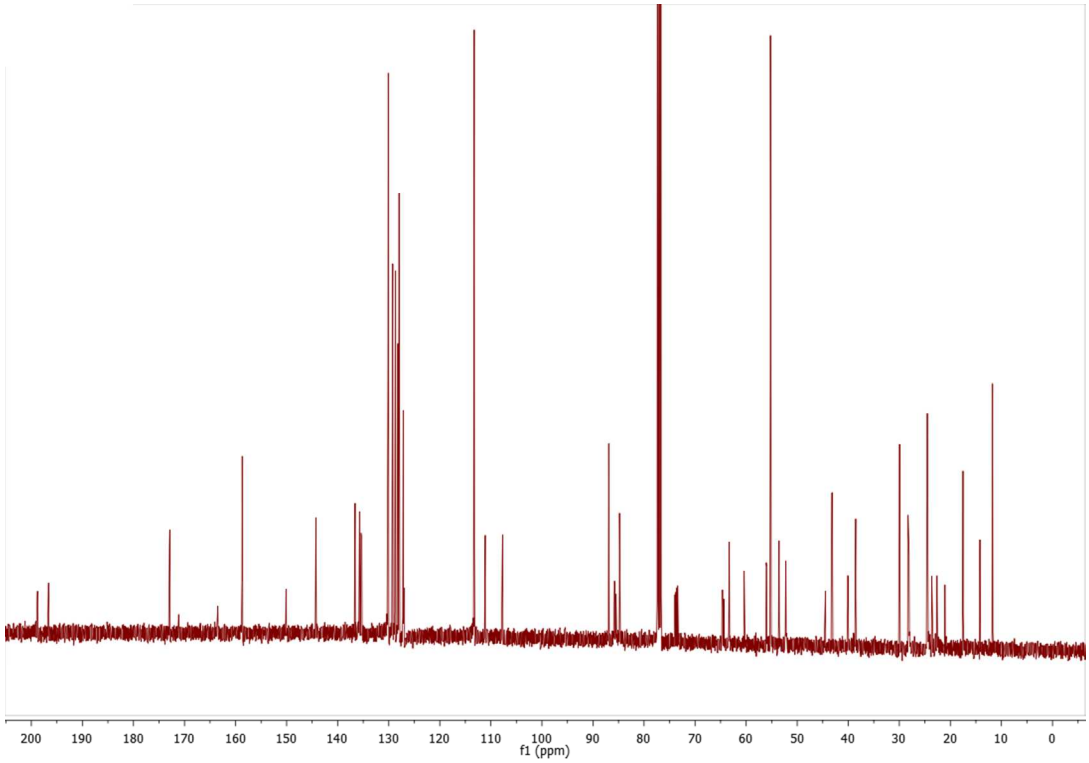


Figure S5. ^{1}H, ^{13}C and ^{31}P NMR of compound 7c





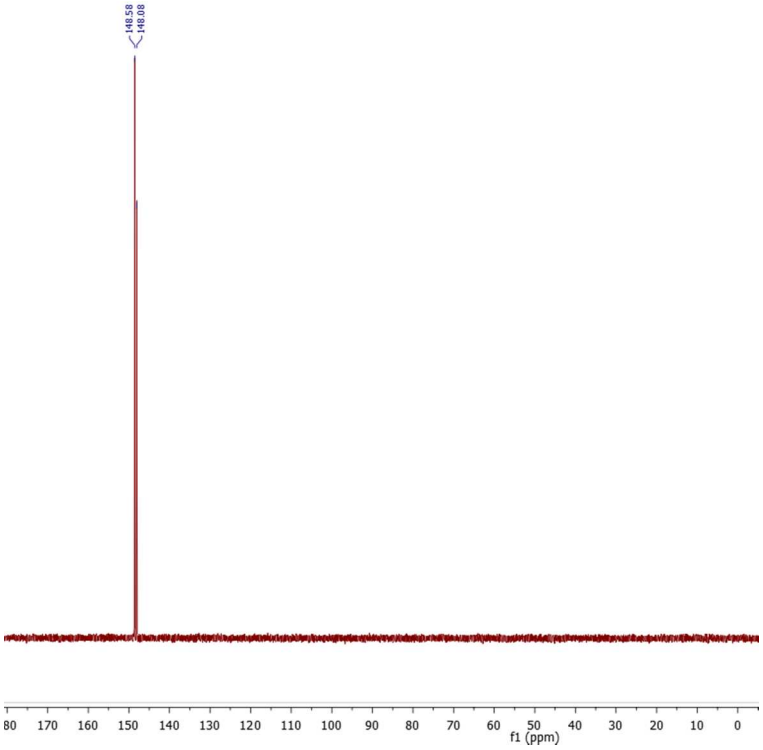
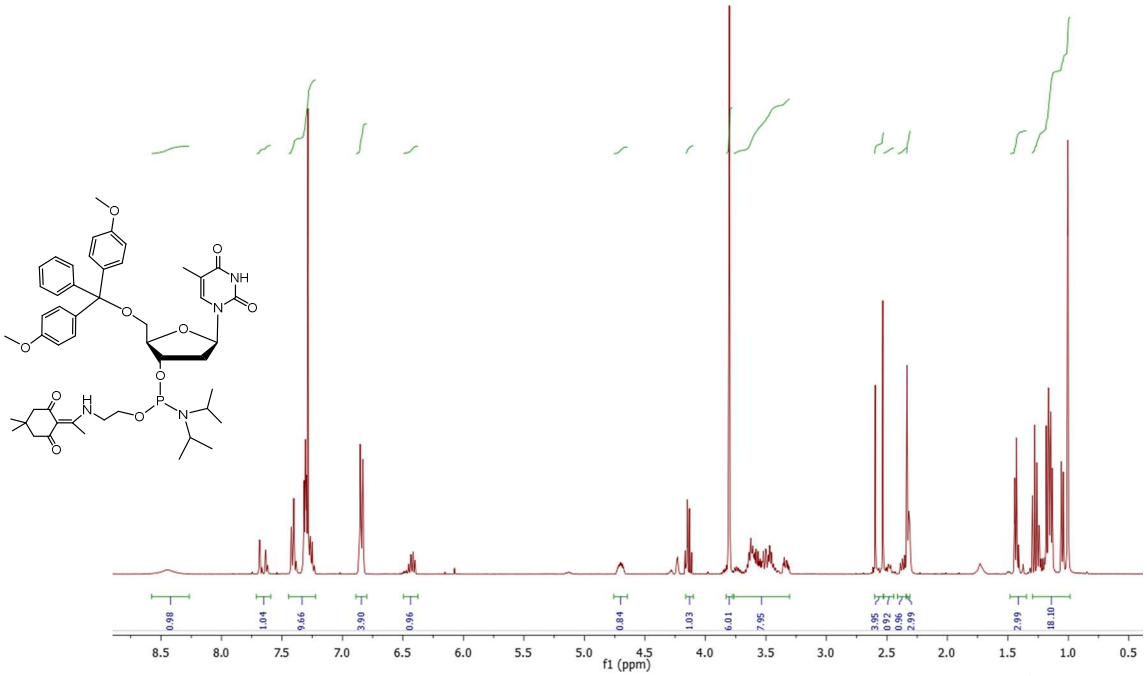


Figure S6. ^{1}H, ^{13}C and ^{31}P NMR of compound 7d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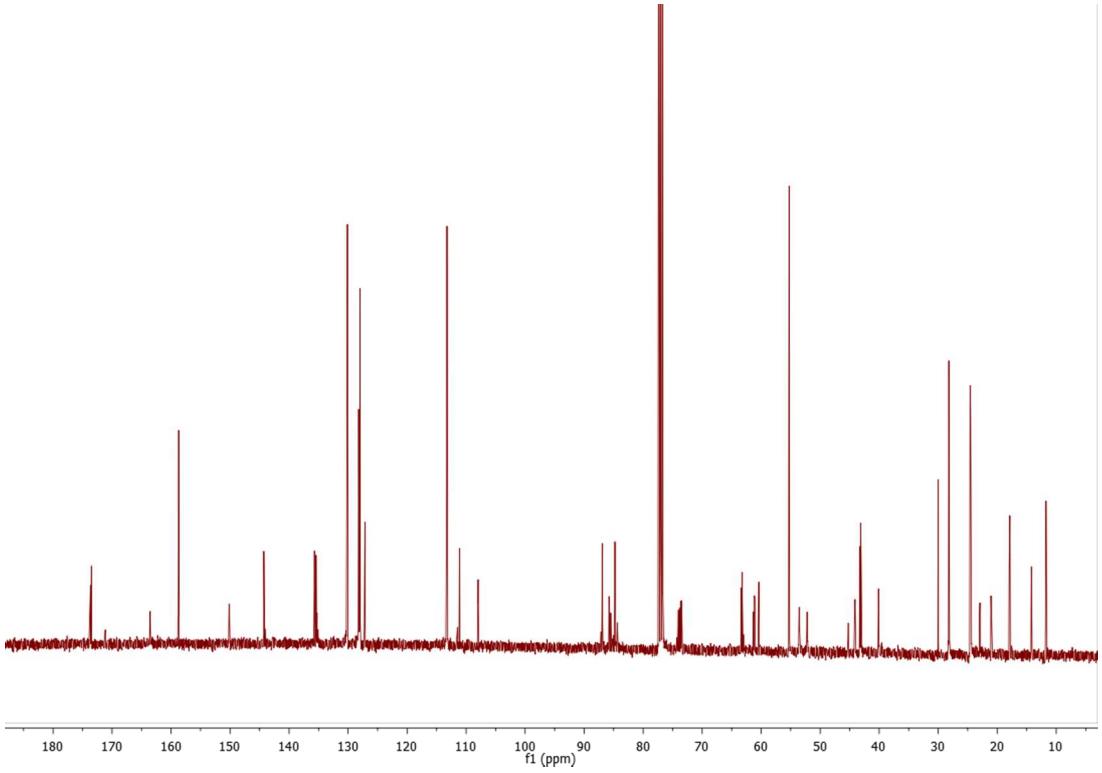
0.96
2.99
2.99
18.10
f1 (ppm)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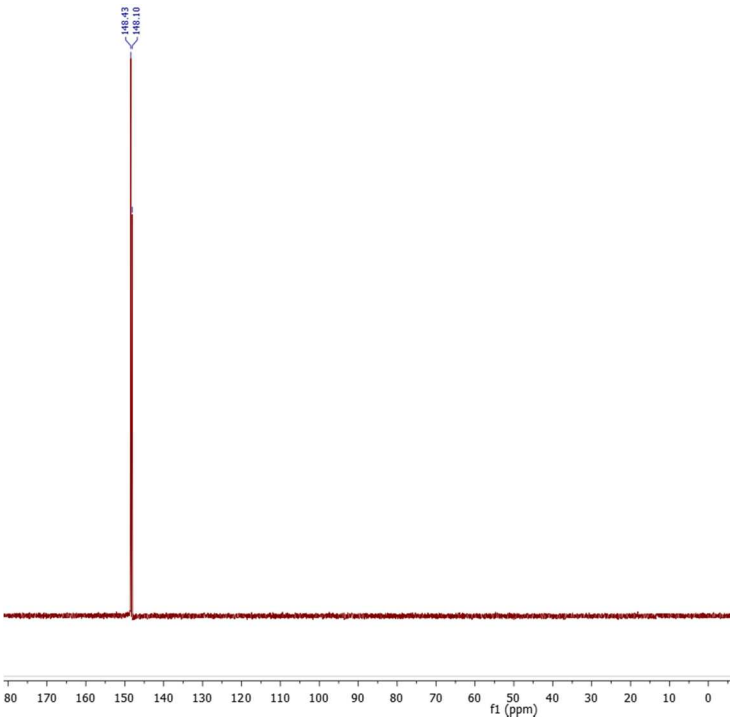
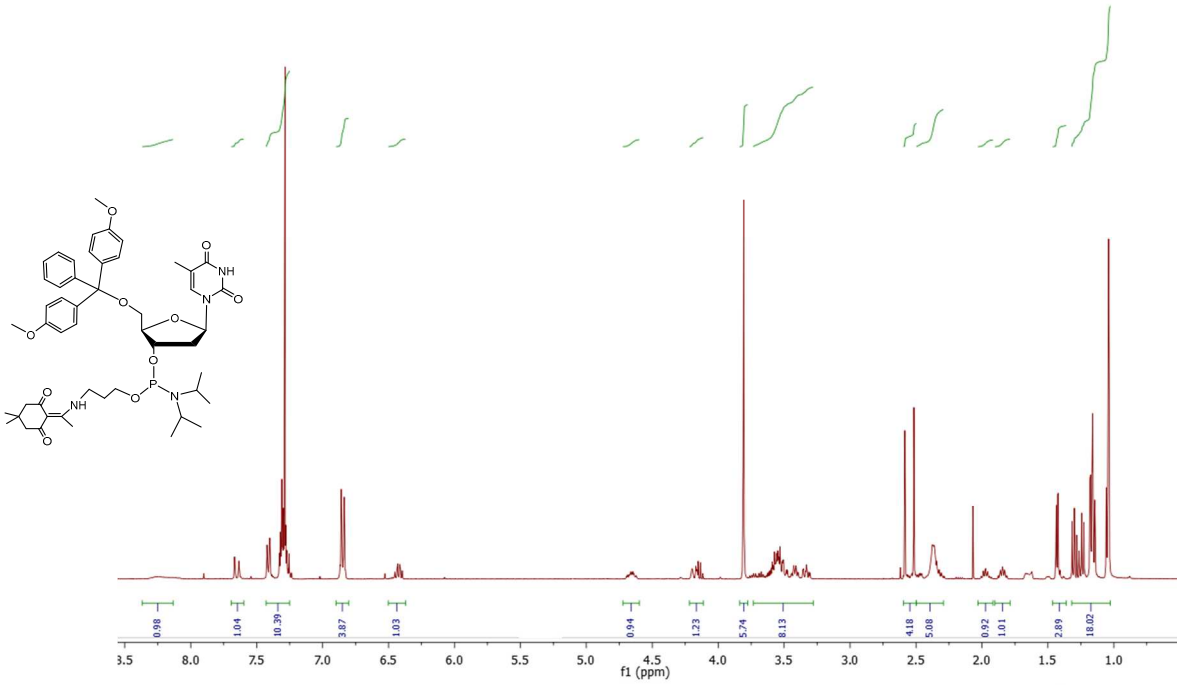
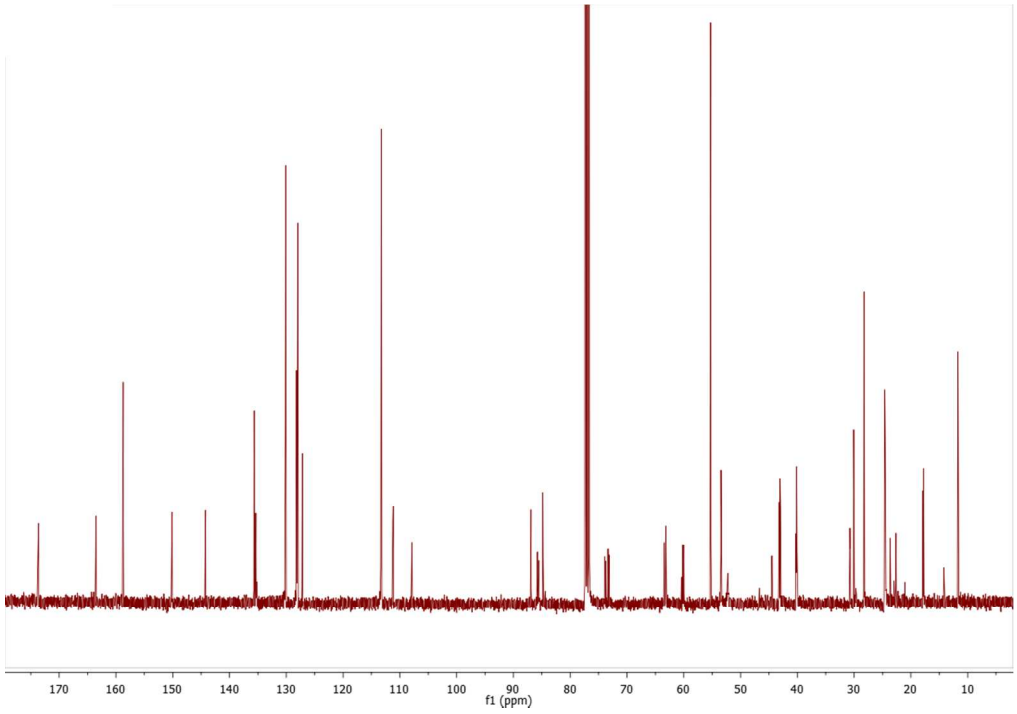


Figure S7. ^{1}H, ^{13}C and ^{31}P NMR of compound 7e





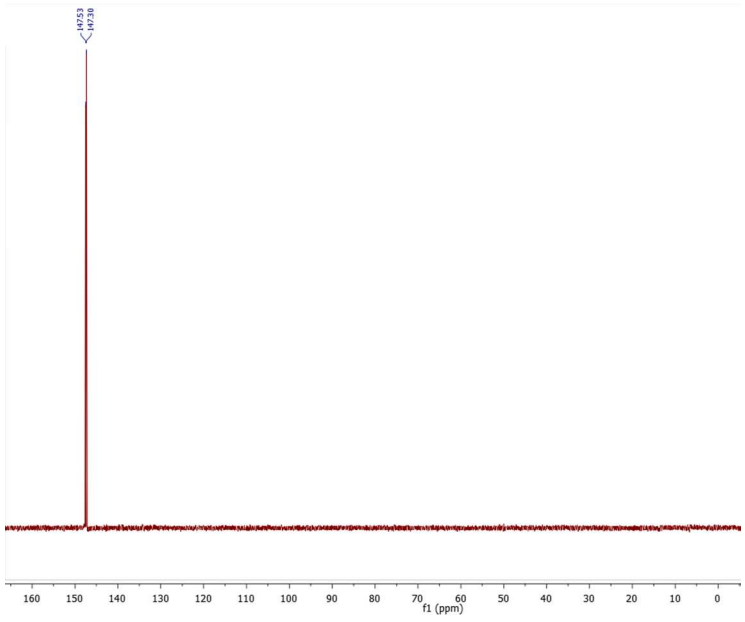
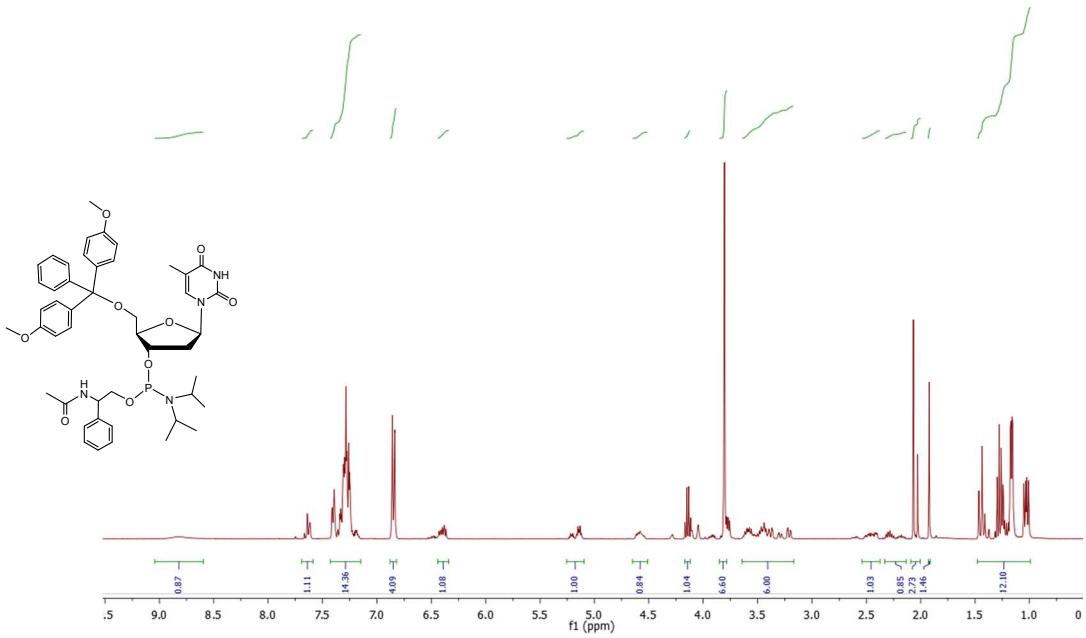


Figure S8. . ^{1}H, ^{13}C and ^{31}P NMR of compound 7f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4.09
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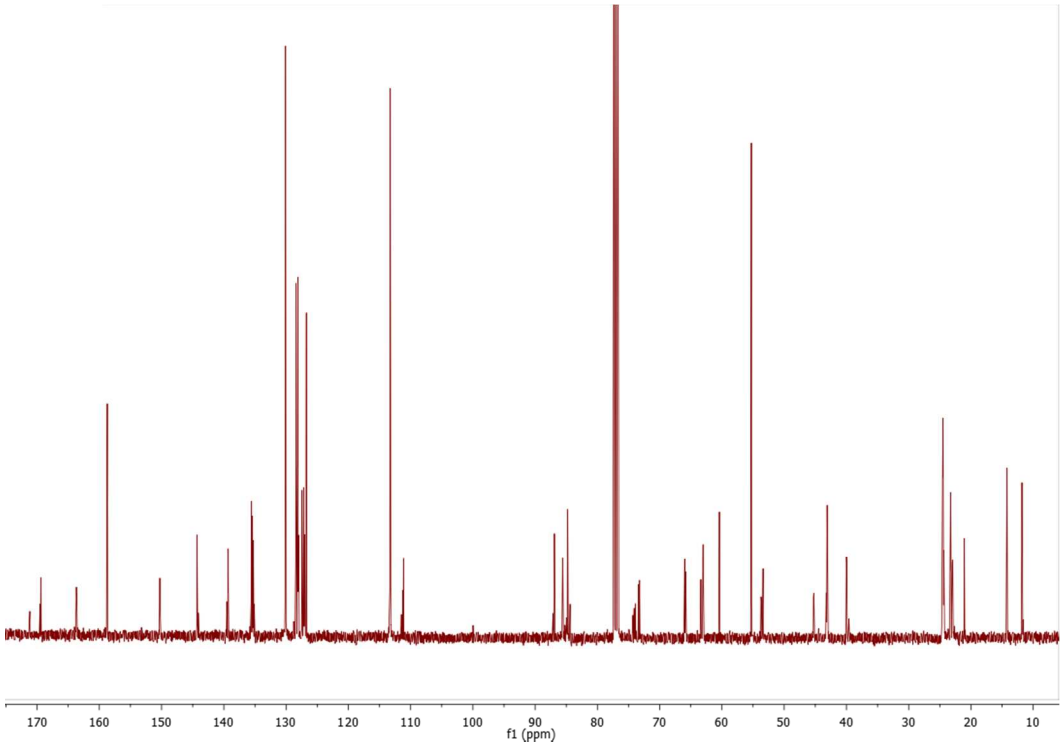
1.46
12.10
f1 (ppm)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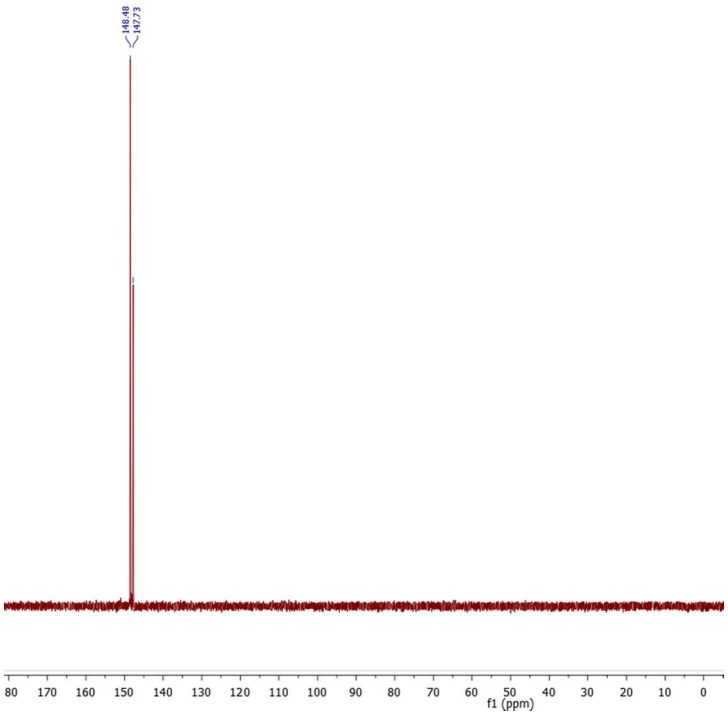
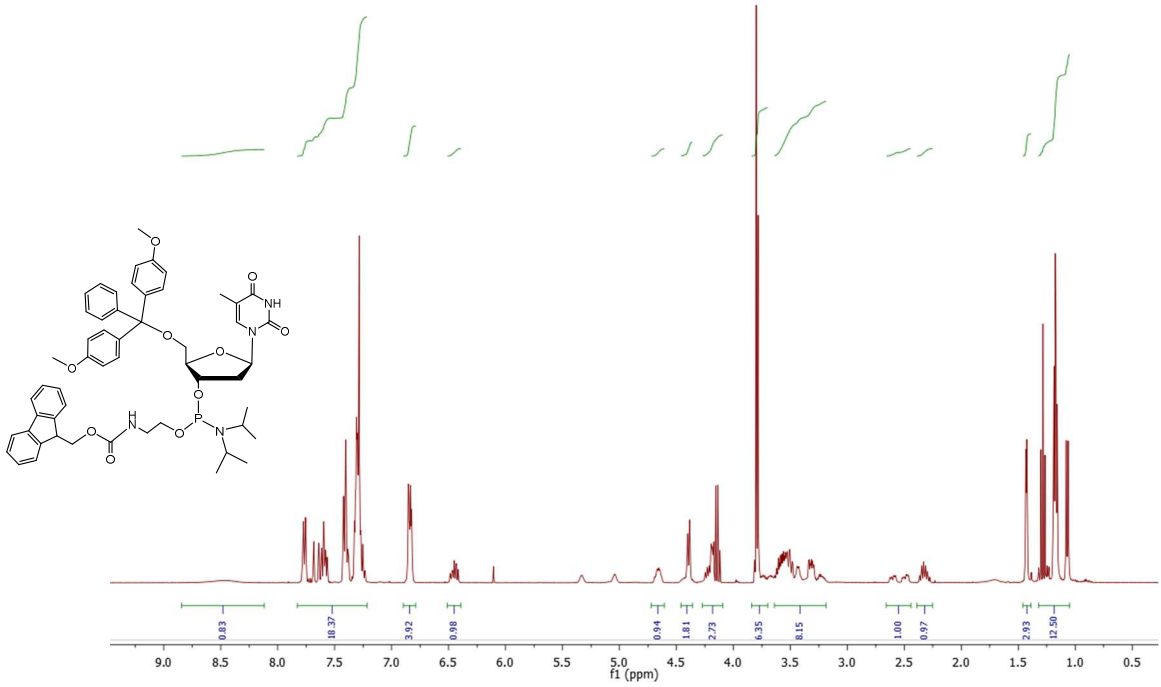


Figure S9. ^{1}H, ^{13}C and ^{31}P NMR of compound 7g



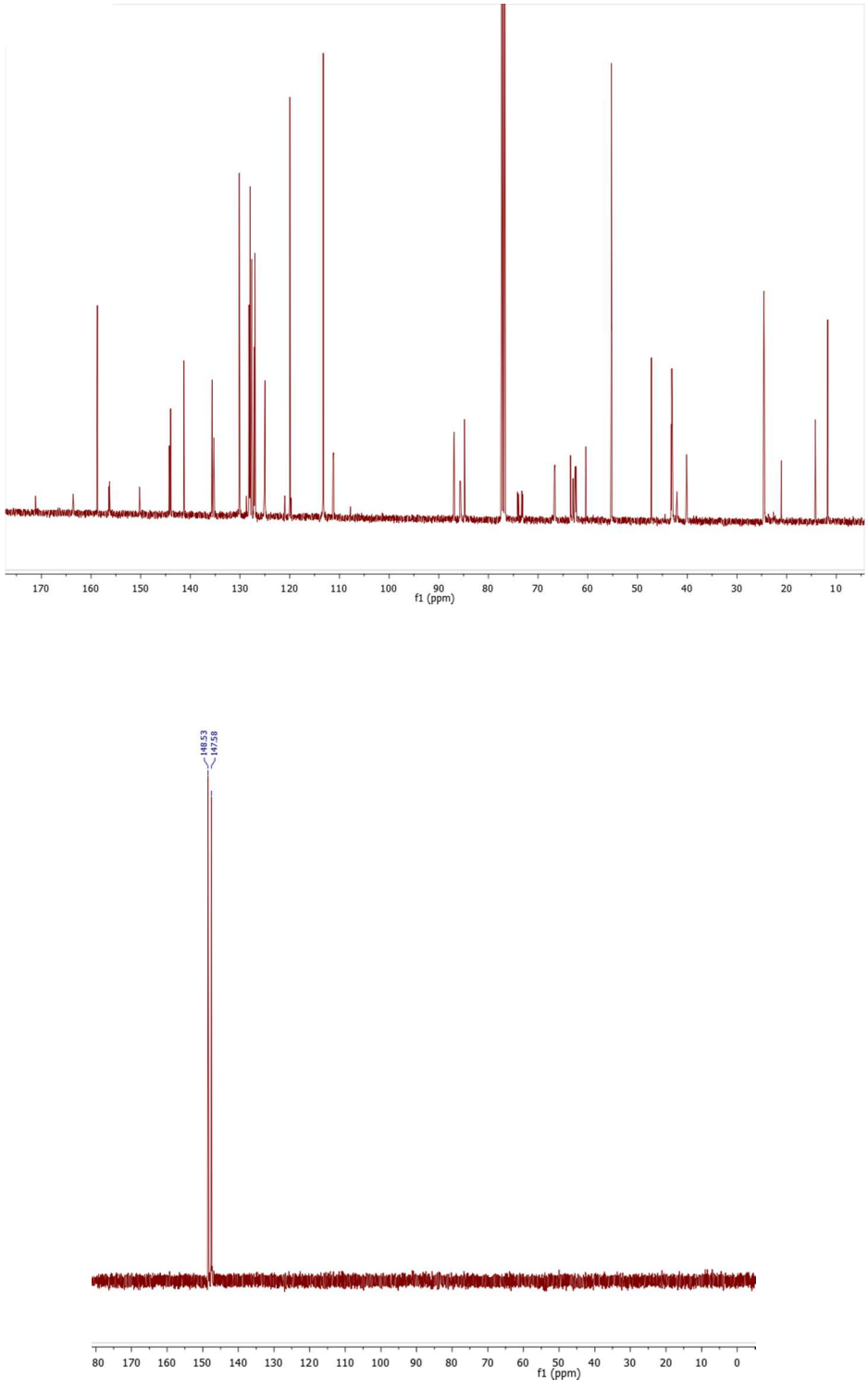
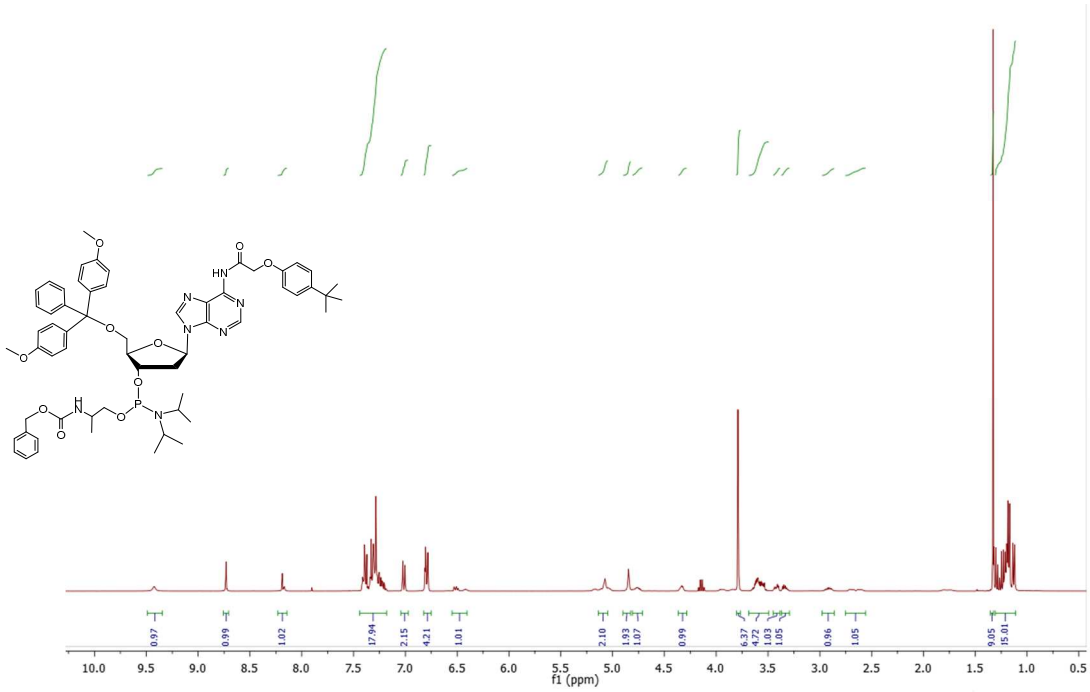


Figure S10. ^{1}H, ^{13}C and ^{31}P NMR of compound 7h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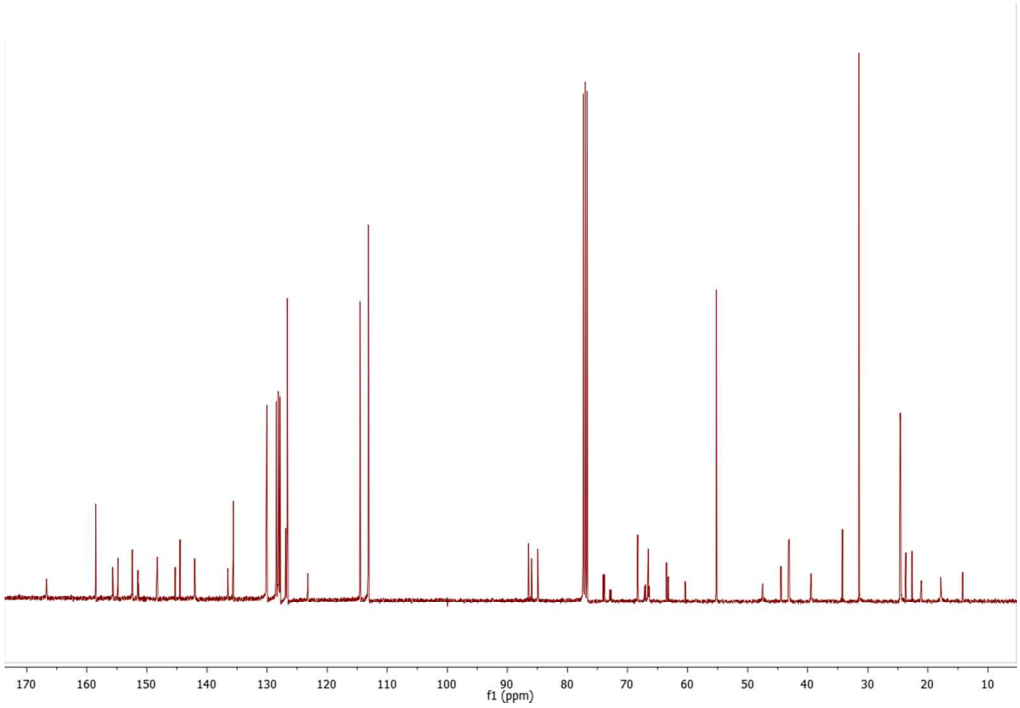
2.10
1.93
1.07
0.99
6.37
4.72
1.03
1.05
0.96
1.05
9.05
15.01
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)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f1 (ppm)

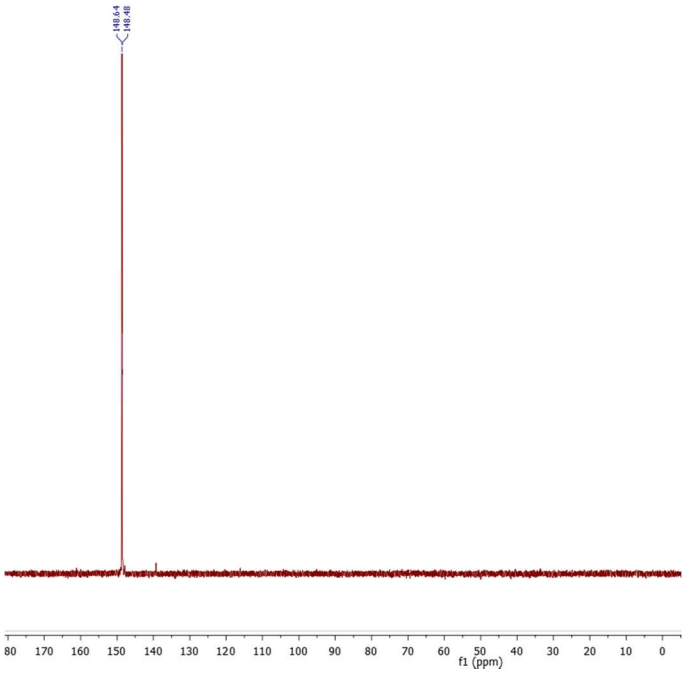
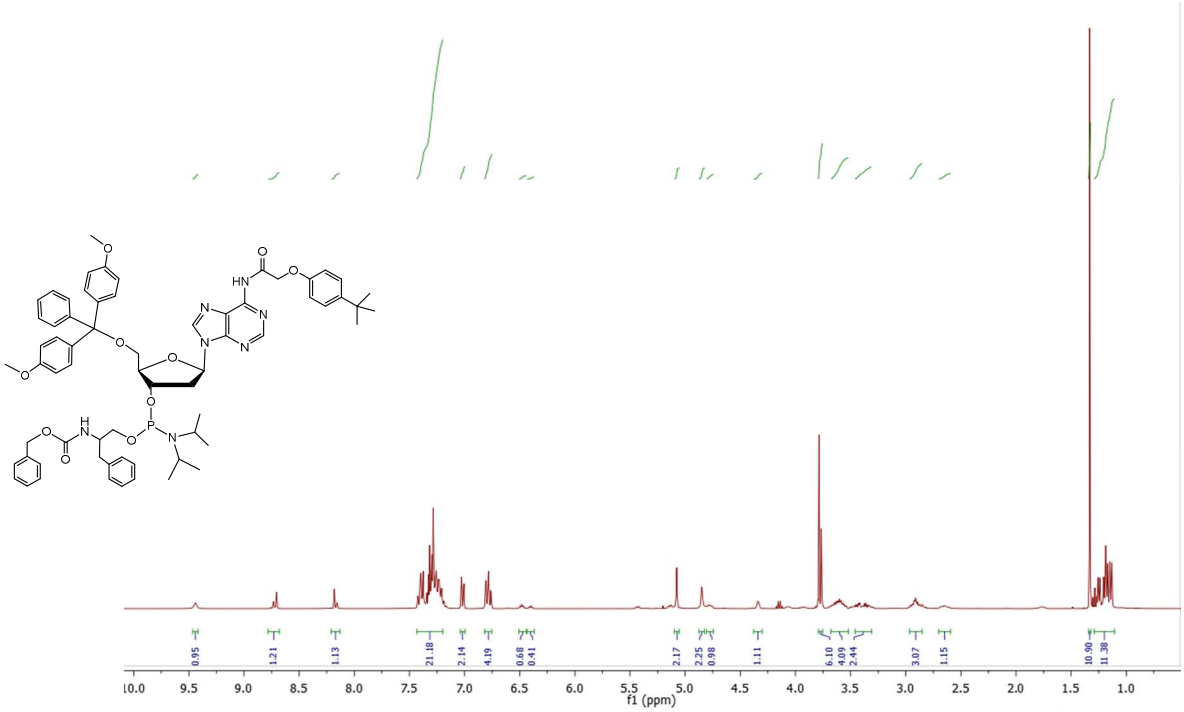
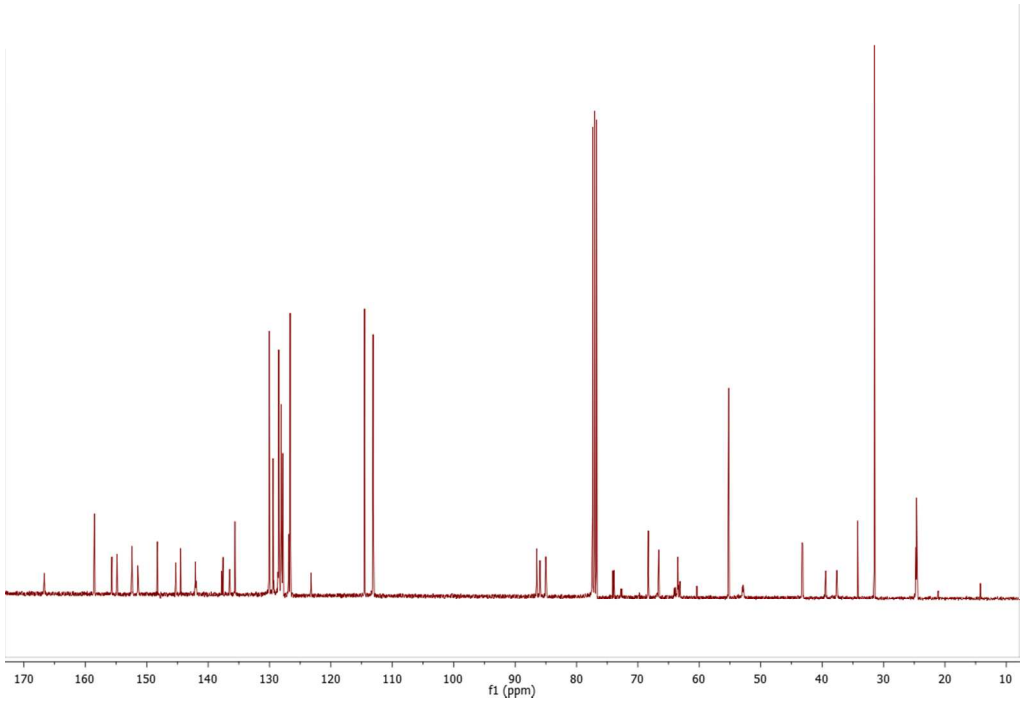


Figure S11. ^{1}H, ^{13}C and ^{31}P NMR of compound 8a





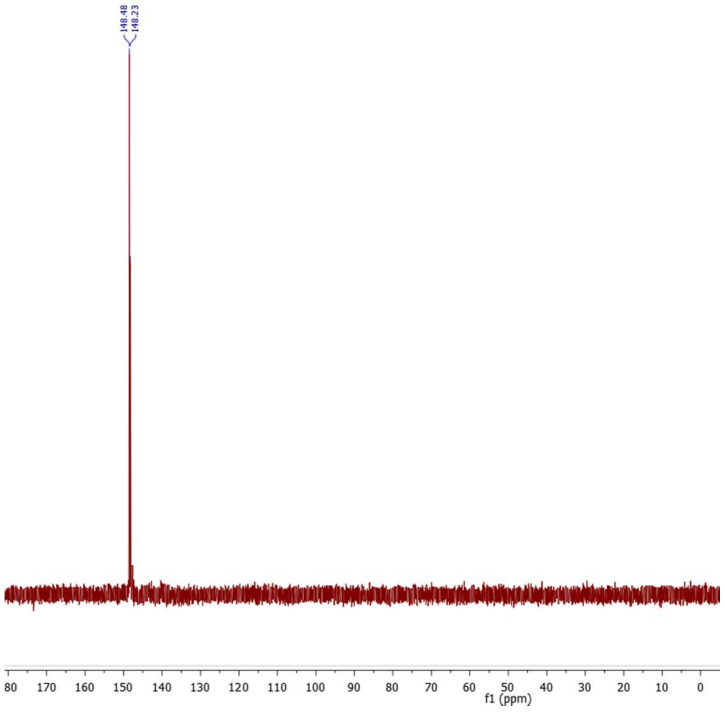
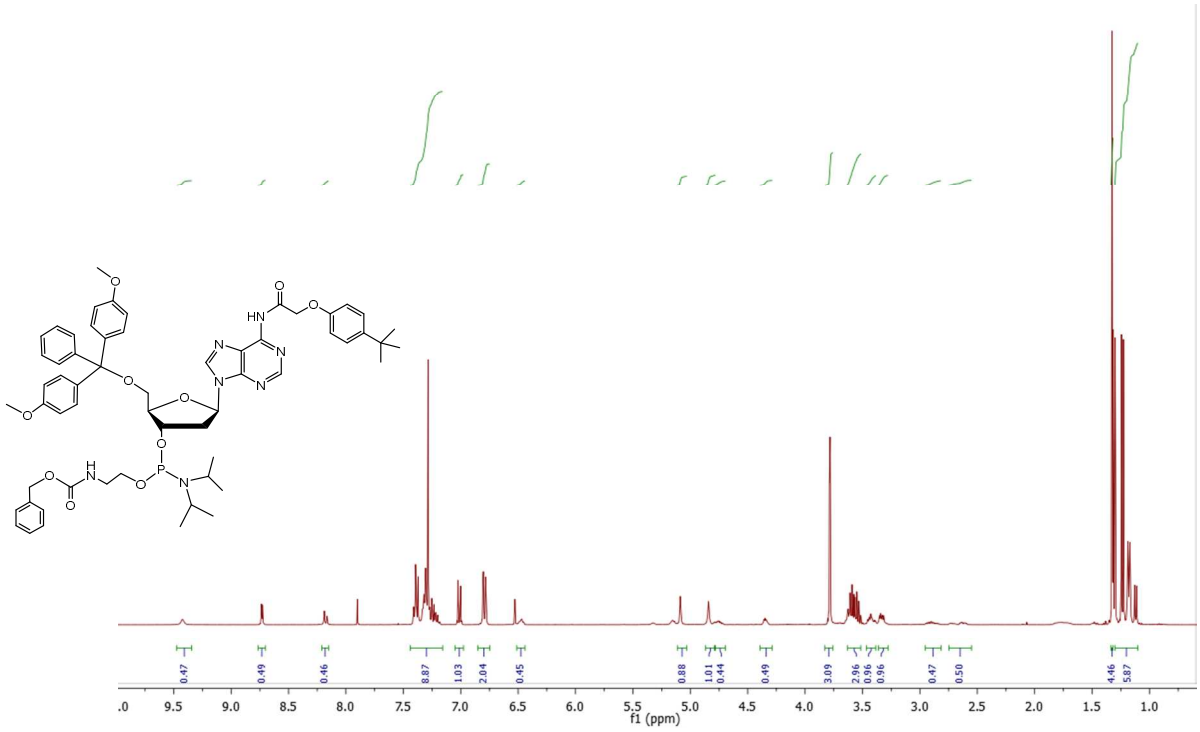


Figure S12. ^{31}H, ^{13}C and ^{1}P NMR of compound 8b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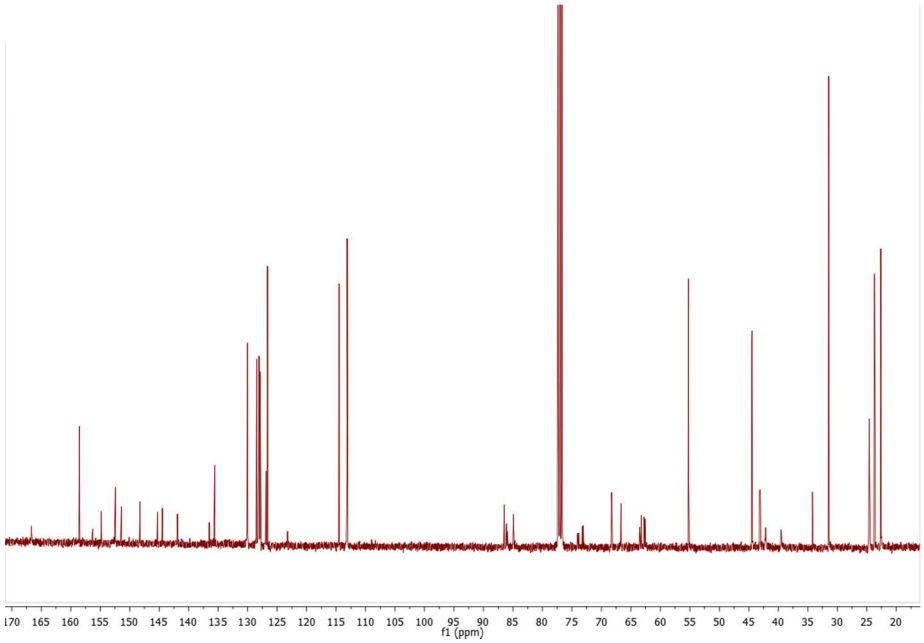
0.96
0.96
0.47
0.50
4.46
5.87
f1 (ppm)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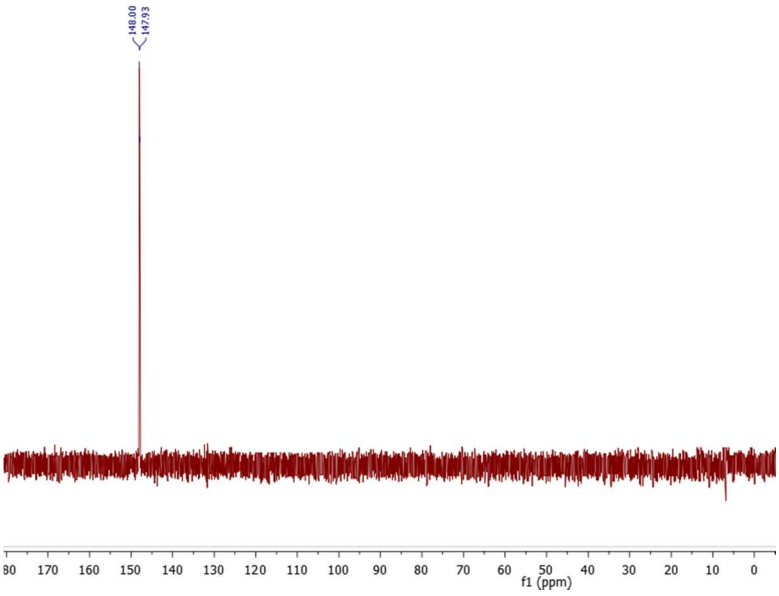
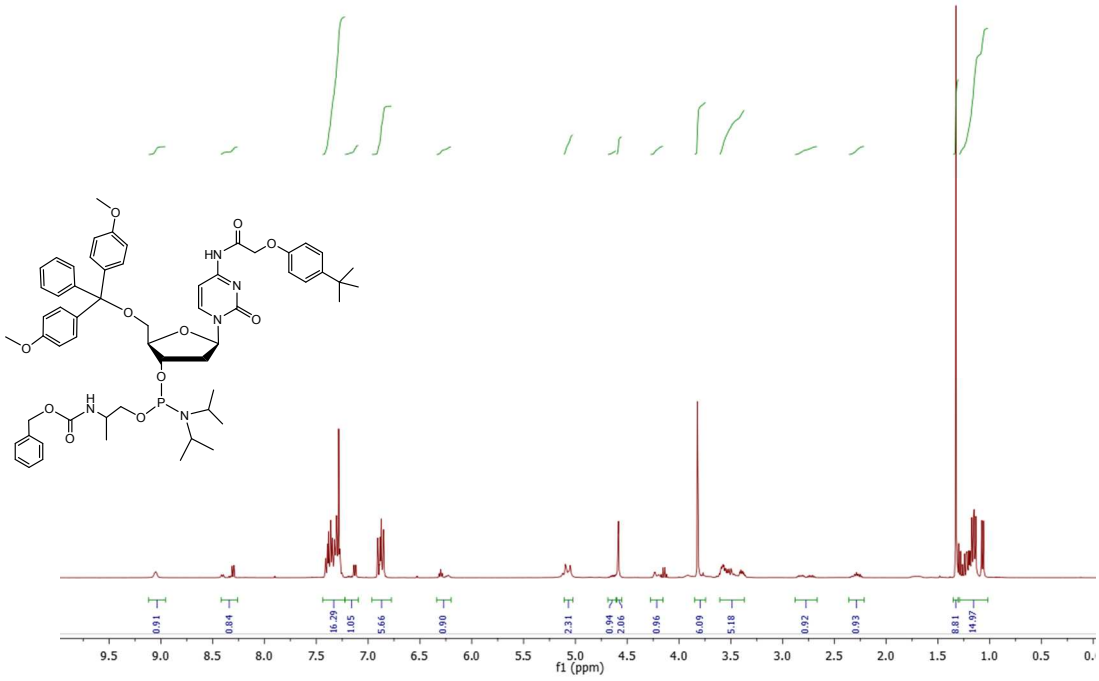


Figure S13. ^{31}H, ^{13}C and ^{31}P NMR of compound 8c



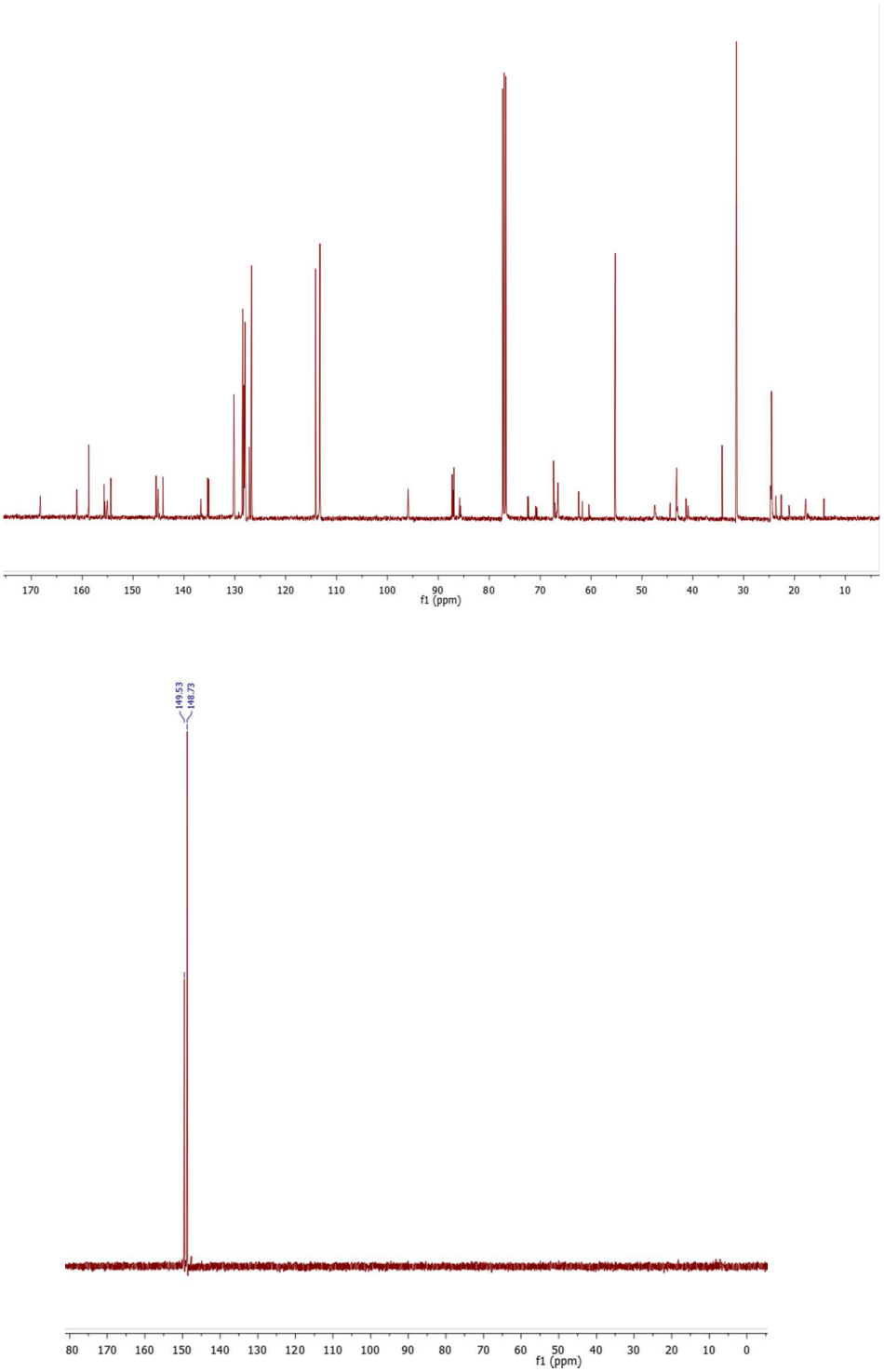
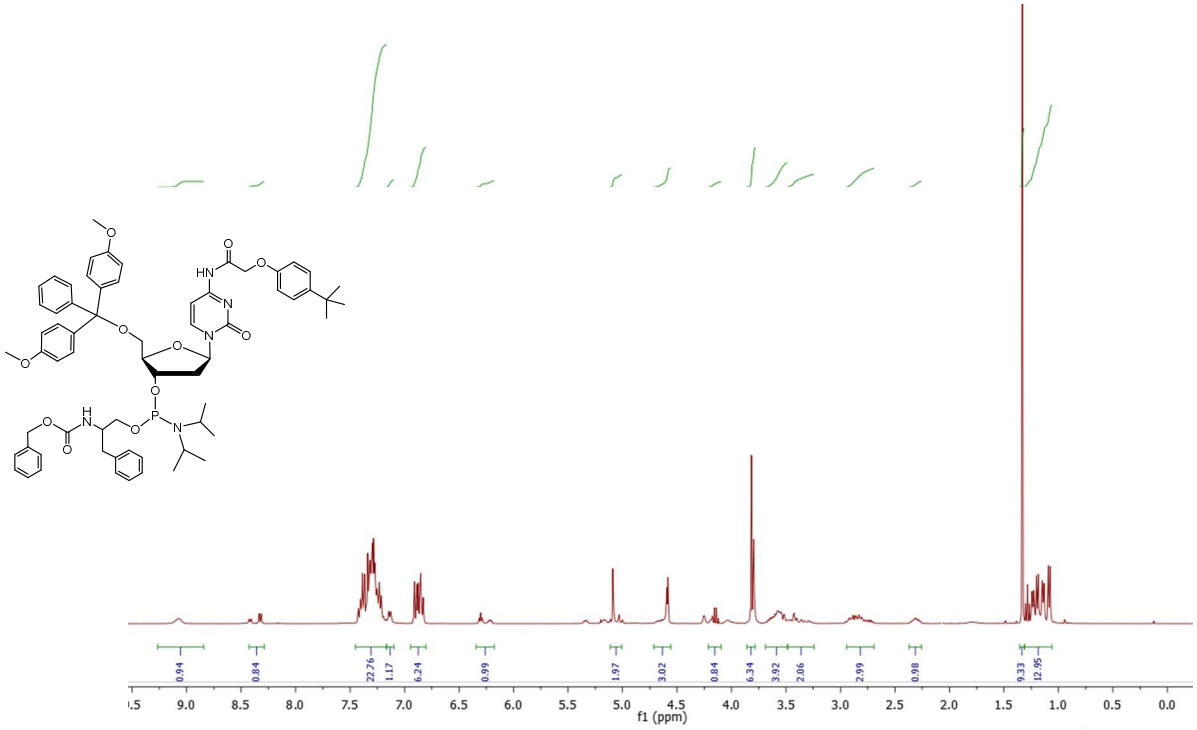


Figure S14. ^{31}H, ^{13}C and ^{31}P NMR of compound 9a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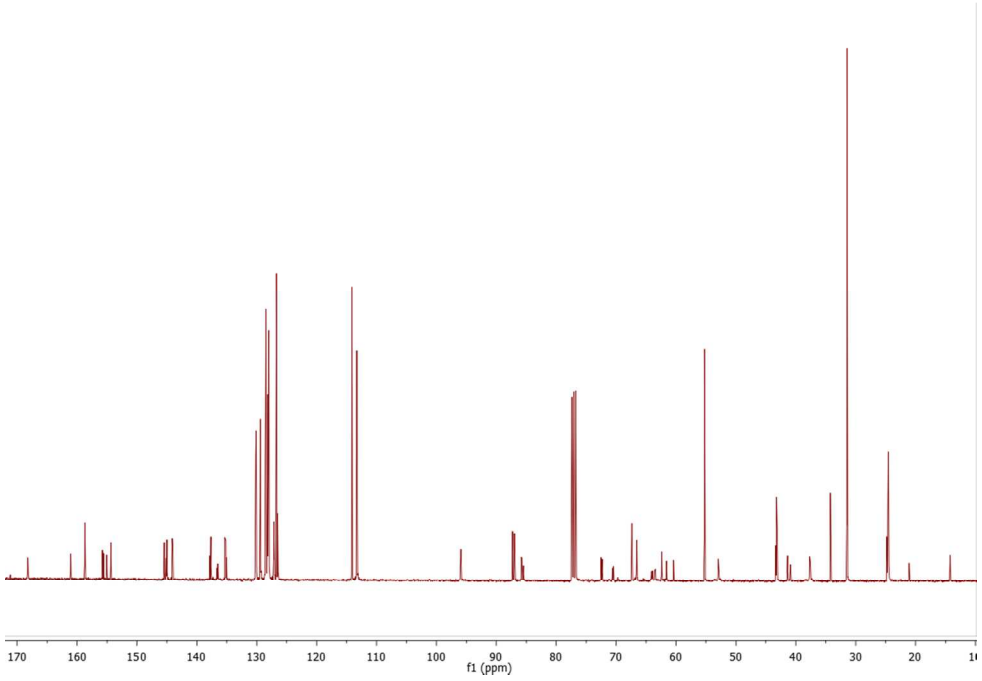
0.84
22.76
1.17
6.24
0.99
1.97
3.02
0.84
6.34
3.92
2.06
2.99
0.98
9.33
12.95
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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90
80
70
60
50
40
30
20
f1 (ppm)

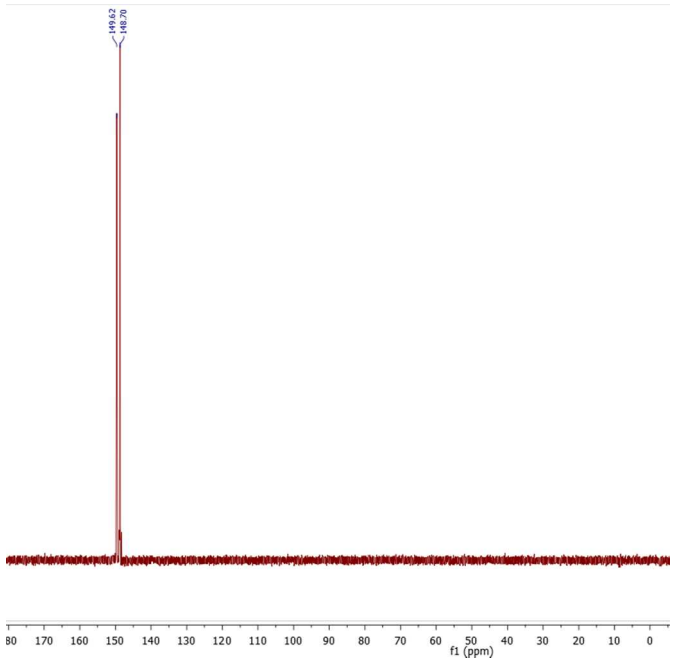
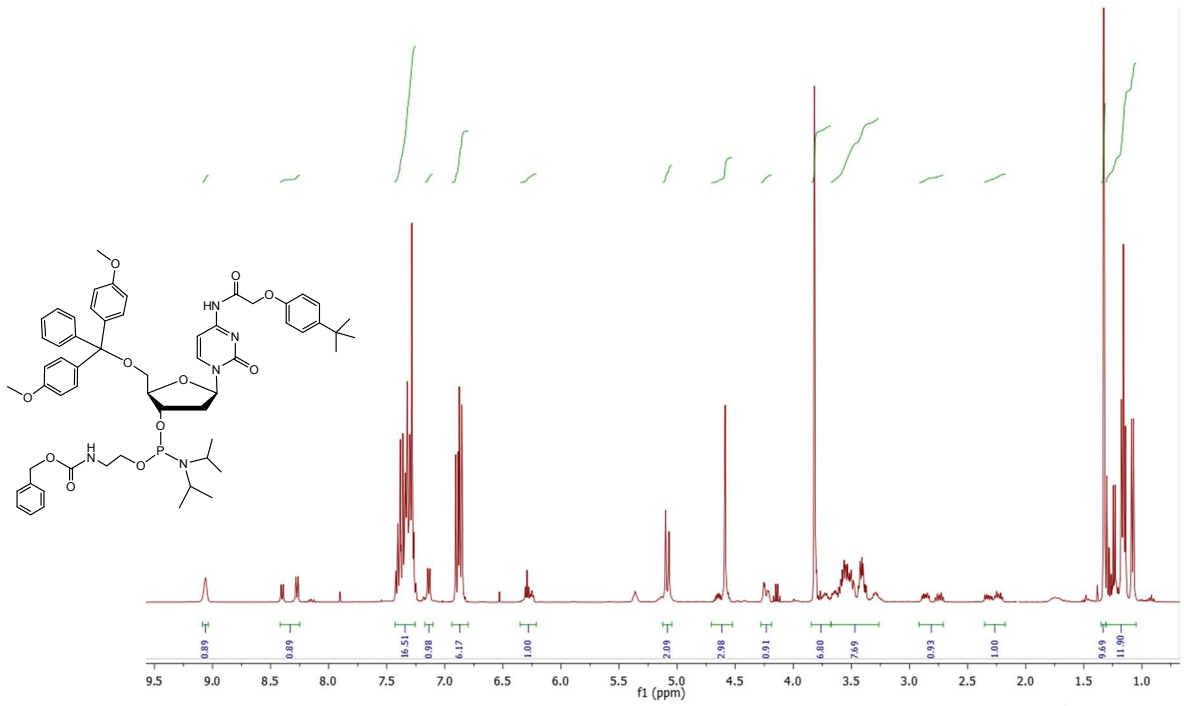
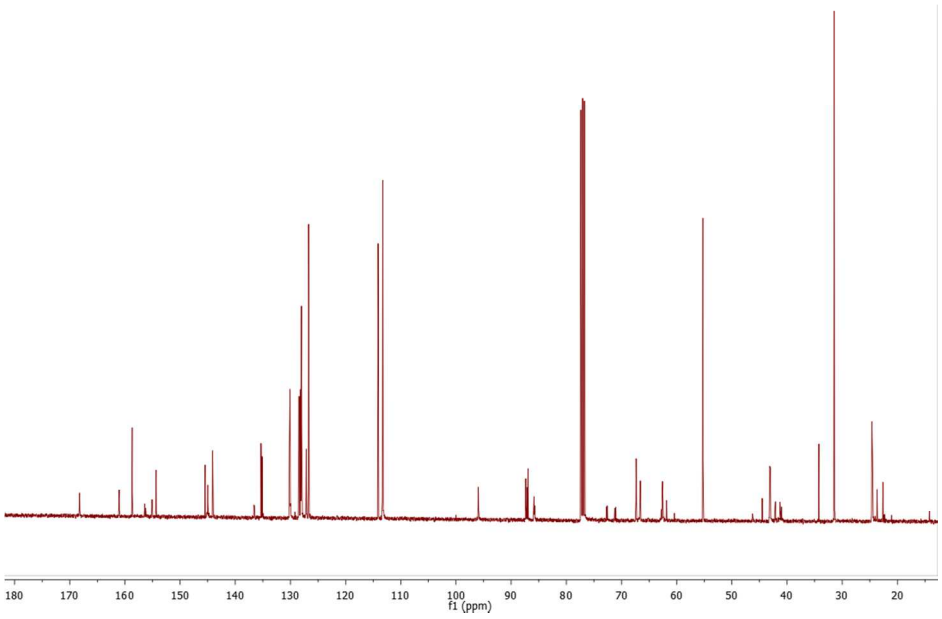


Figure S15. ^{31}H, ^{13}C and ^{31}P NMR of compound 9b





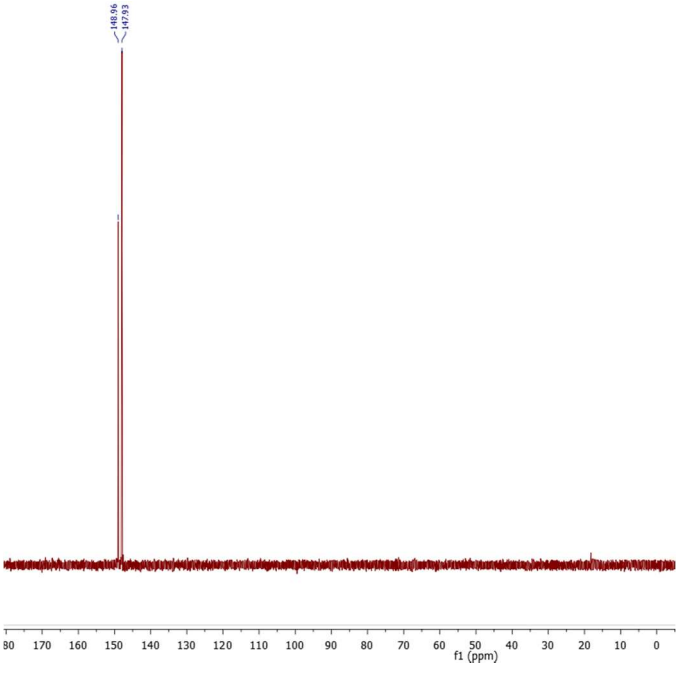


Figure S16. ^{331}H, ^{13}C and ^{1}P NMR of compound 9c

2) LC-MS conditions and spectra

LC-MS analyses were performed on a Q-TOF LC/MS in negative mode. A reverse phase C18, 1.7 µm, 2.1 X 100nm column was used with a gradient of 0-80% Buffer B over 45 min with a flow rate of 0.2 mL/min (Buffer A was 1:80:9.5:9.5 of 500 mM dibutylammonium acetate:water:isopropanol:acetonitrile; Buffer B was 1:10:44.5:44.5 of 500 mM dibutylammonium acetate: water: isopropanol:acetonitrile).

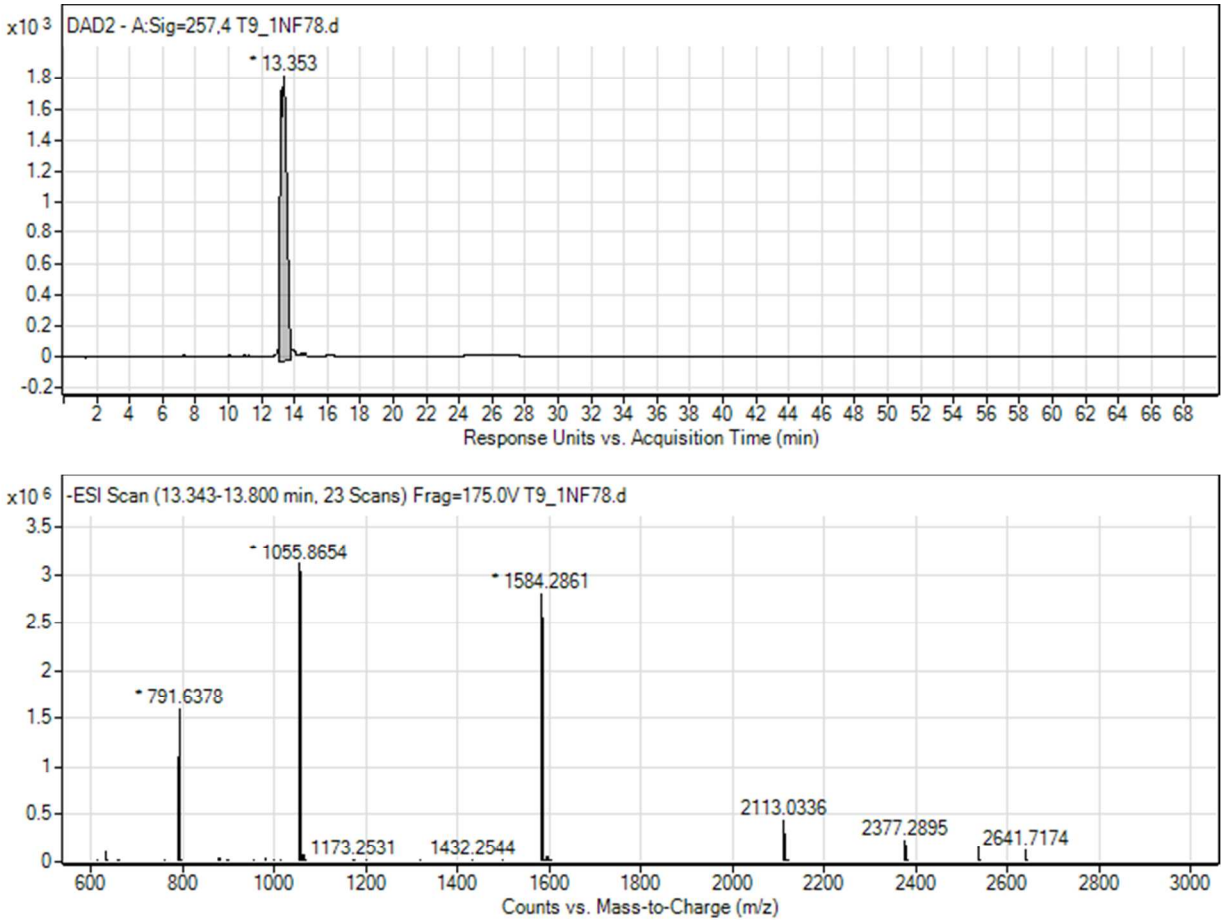


Figure S17. LC-MS analysis of the crude reaction mixture of ODN 1. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.

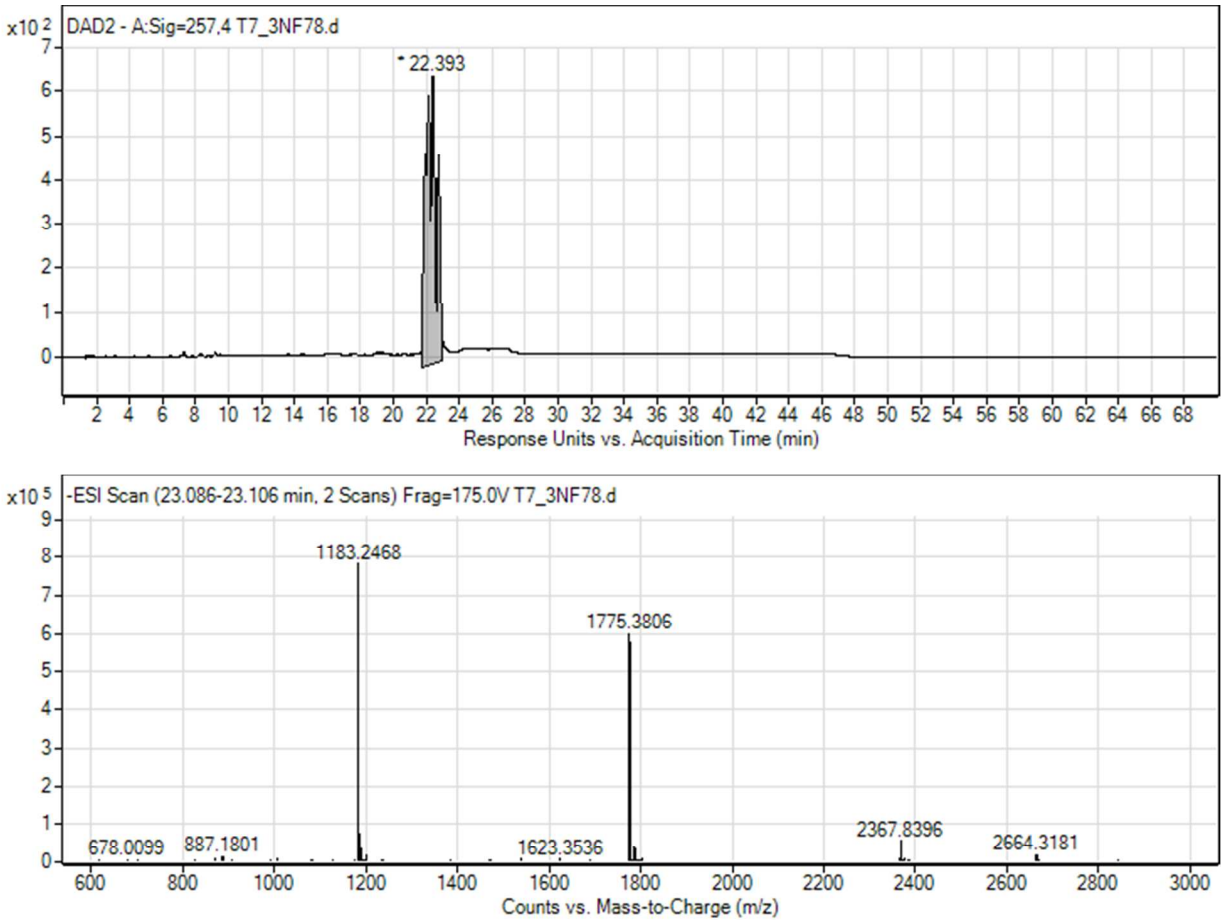


Figure S18. LC-MS analysis of the crude reaction mixture of ODN 2. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.

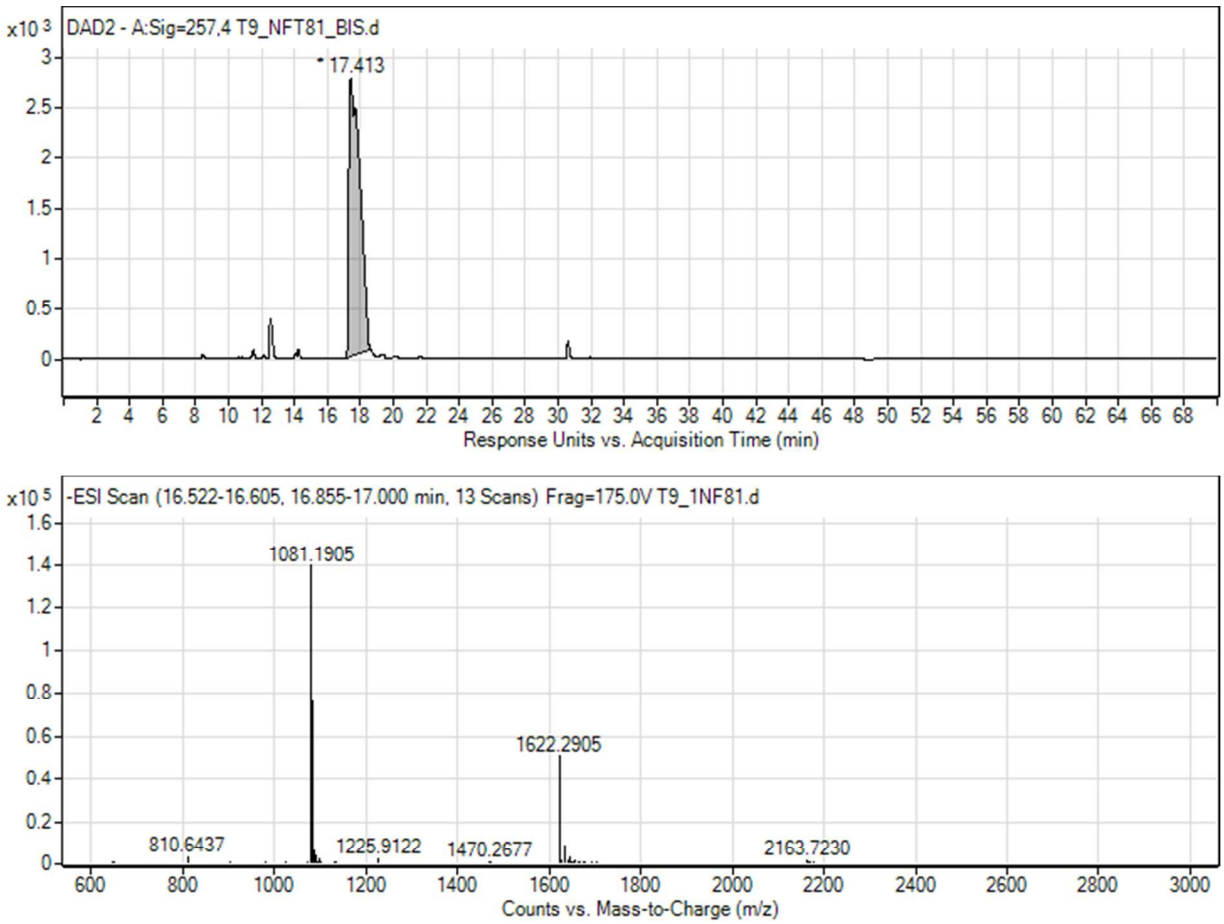
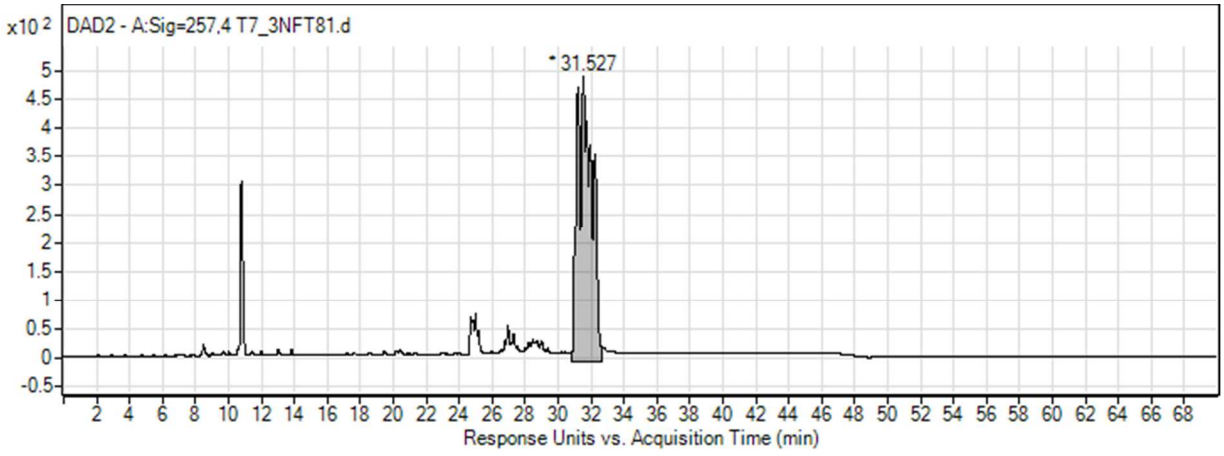


Figure S19. LC-MS analysis of the crude reaction mixture of ODN 3. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.



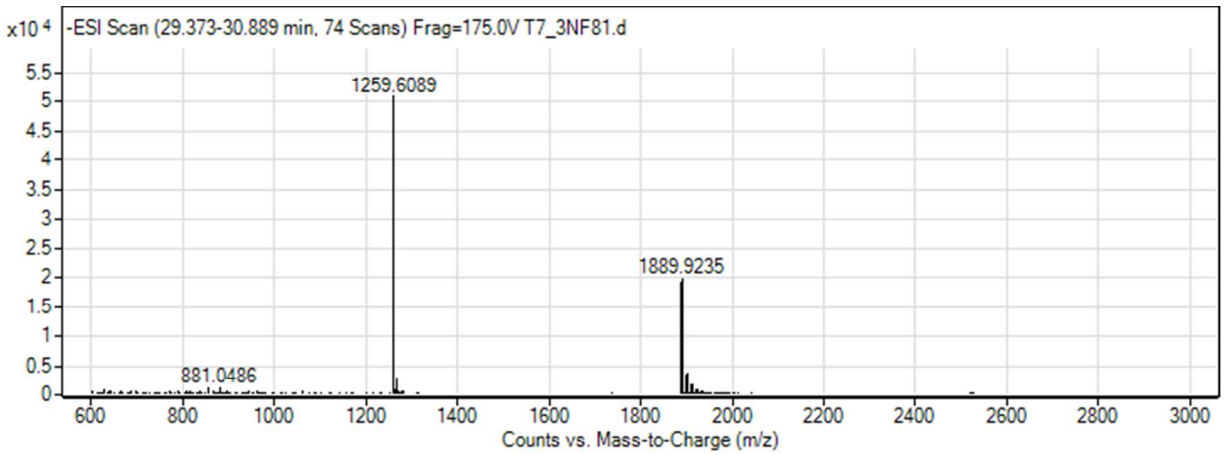


Figure S20. LC-MS analysis of the crude reaction mixture of ODN 4. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.

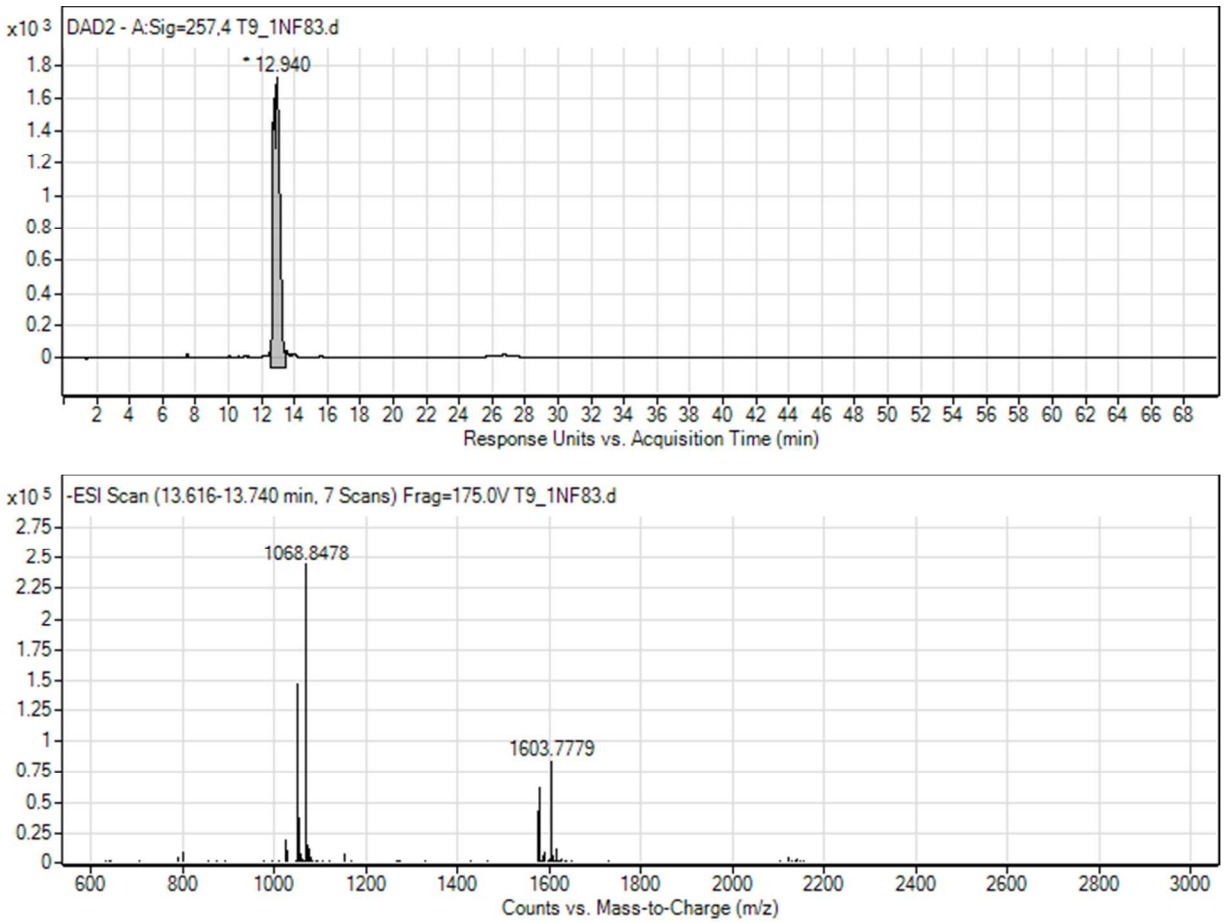


Figure S21. LC-MS analysis of the crude reaction mixture of ODN 5. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.

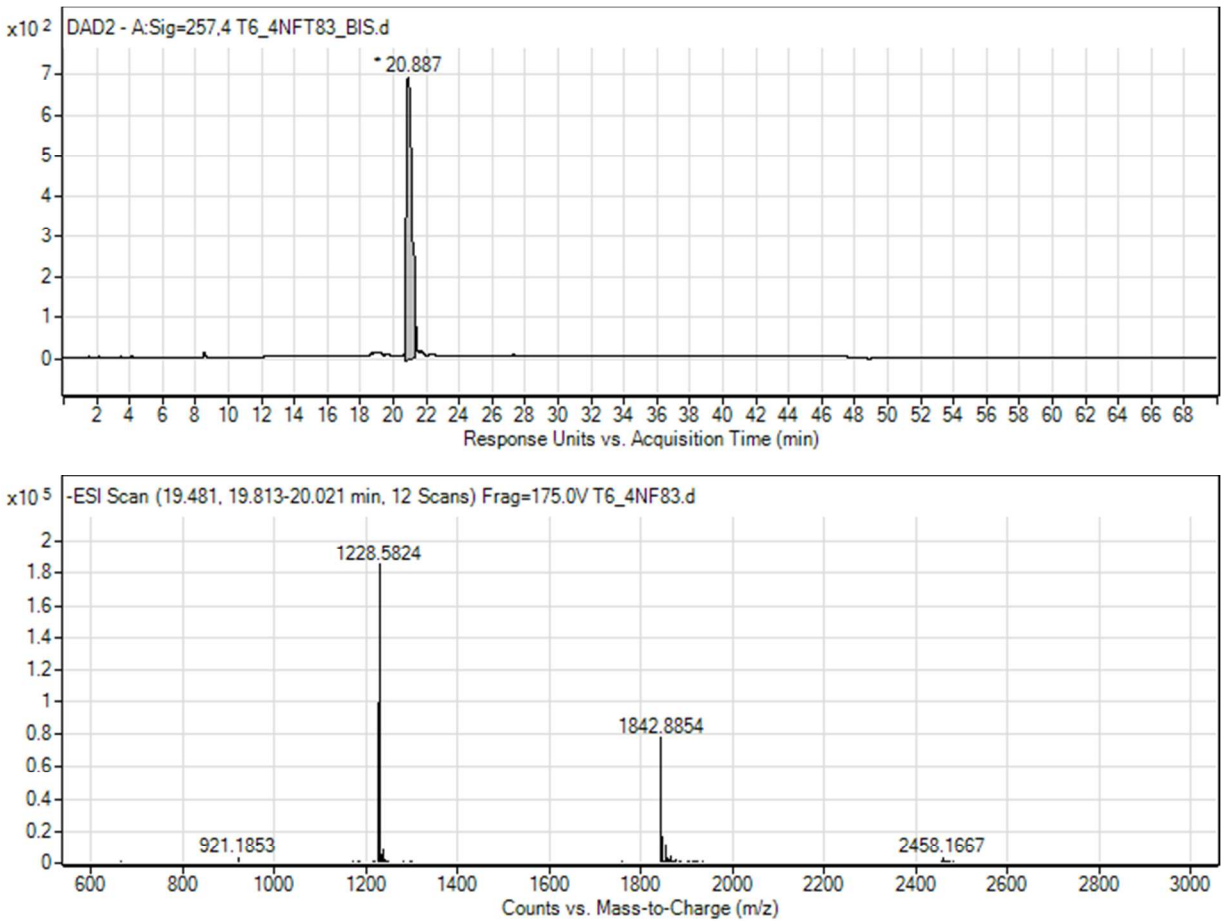
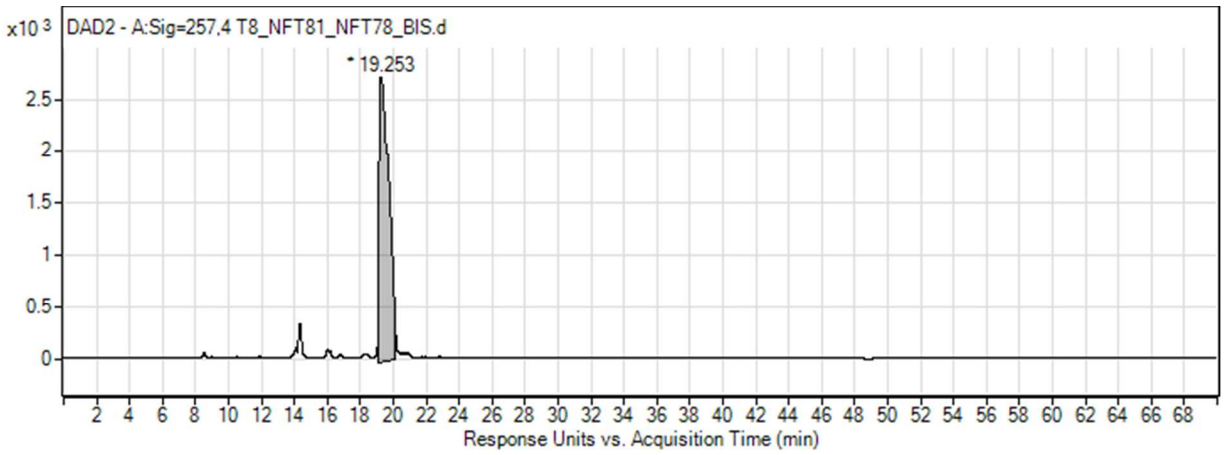


Figure S22. LC-MS analysis of the crude reaction mixture of ODN 6. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.



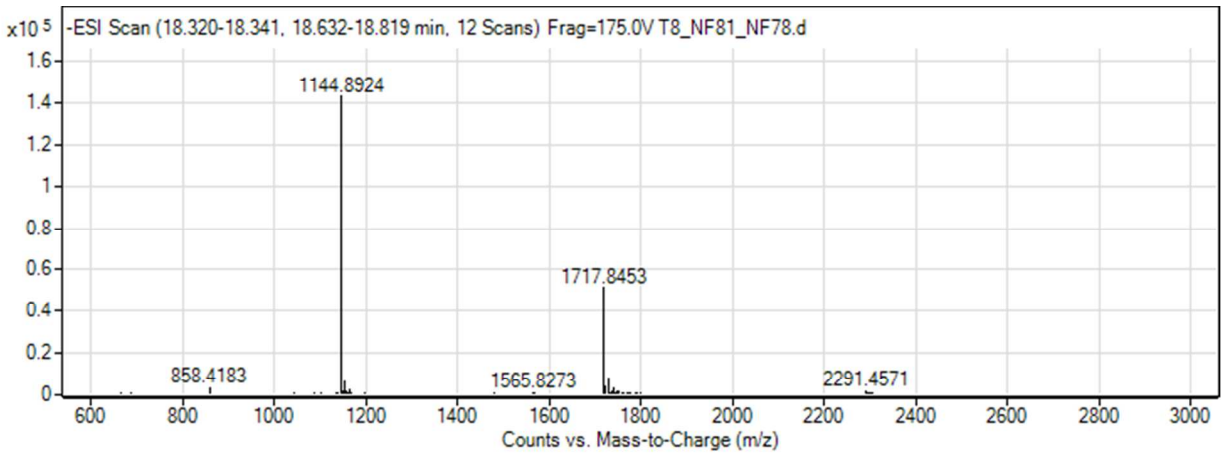


Figure S23. LC-MS analysis of the crude reaction mixture of ODN 7. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.

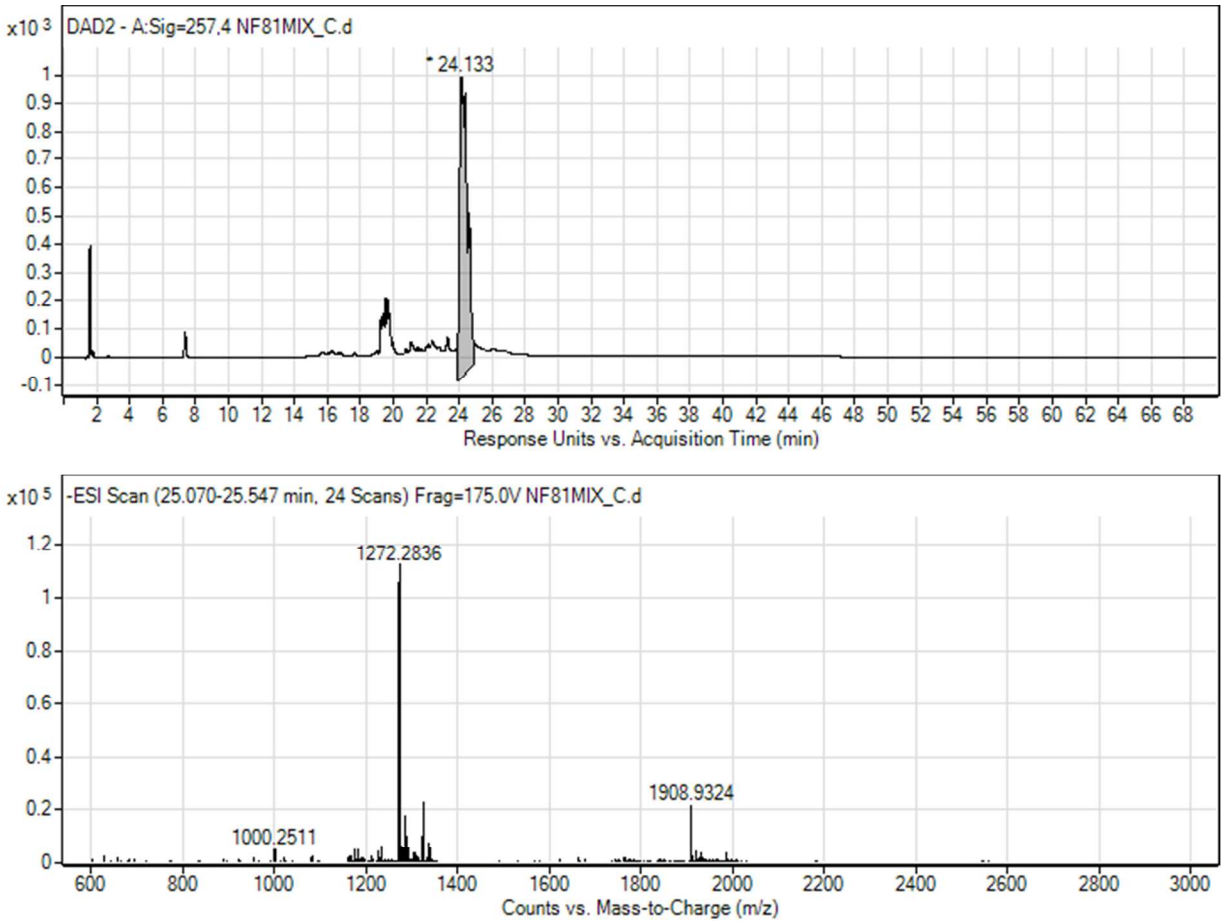


Figure S24. LC-MS analysis of the crude reaction mixture of ODN 8. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.

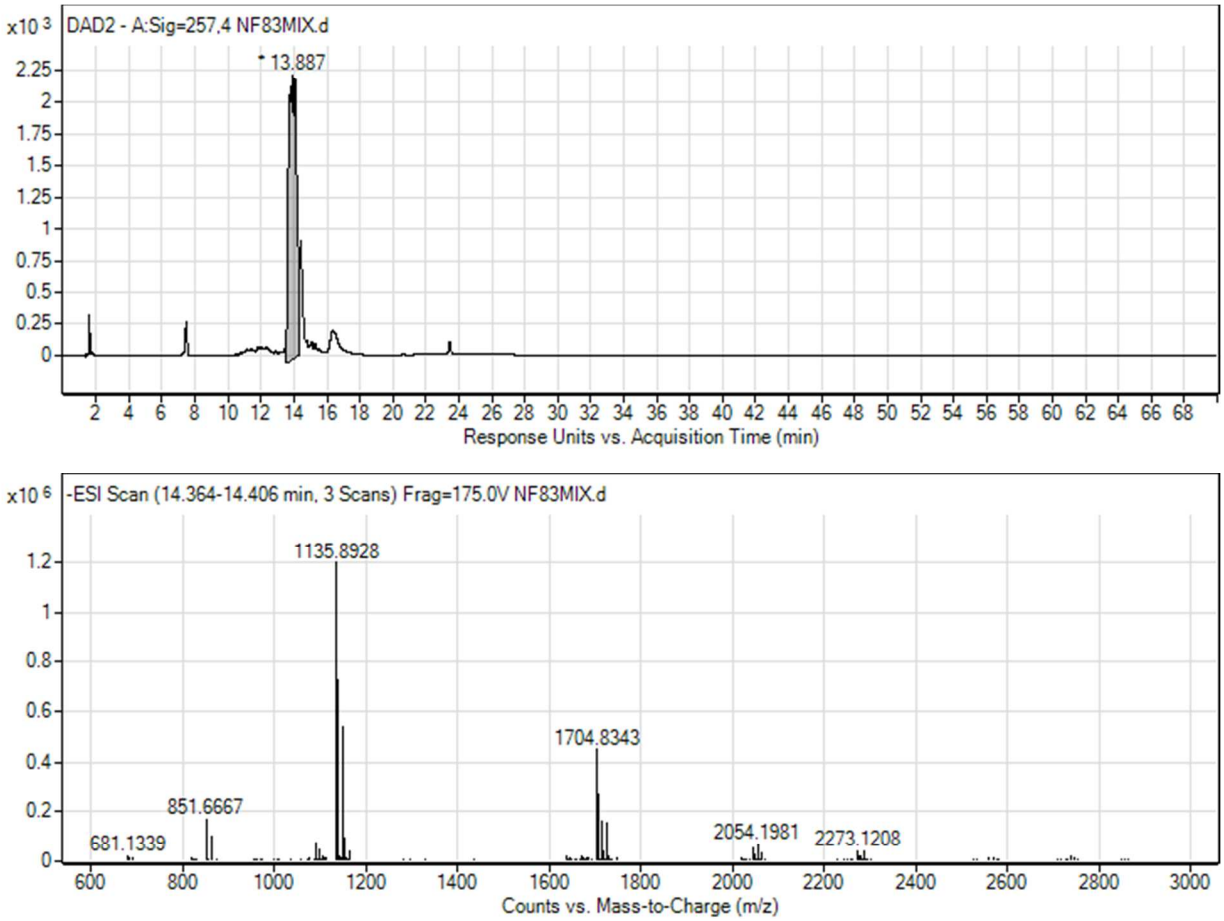
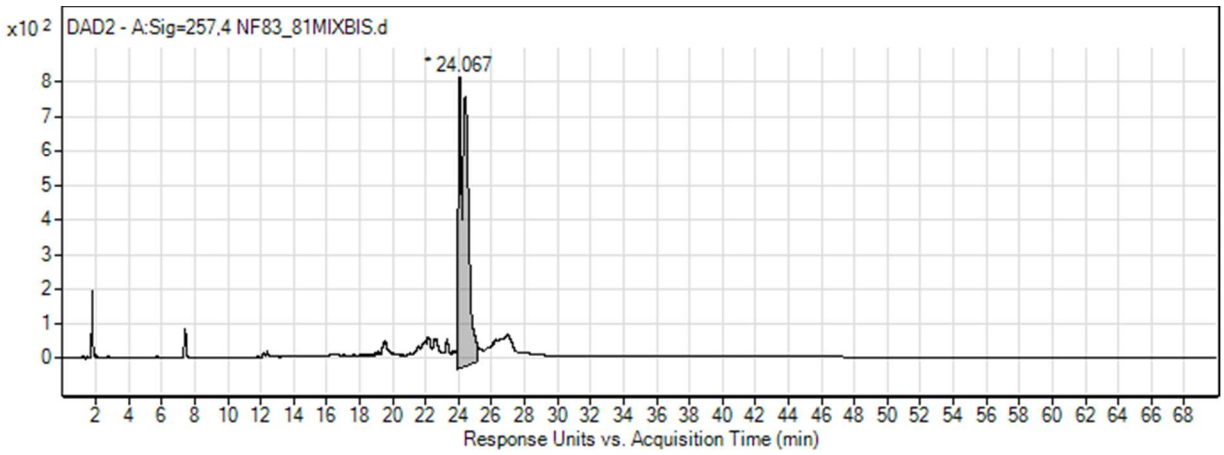


Figure S25. LC-MS analysis of the crude reaction mixture of ODN 9. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.



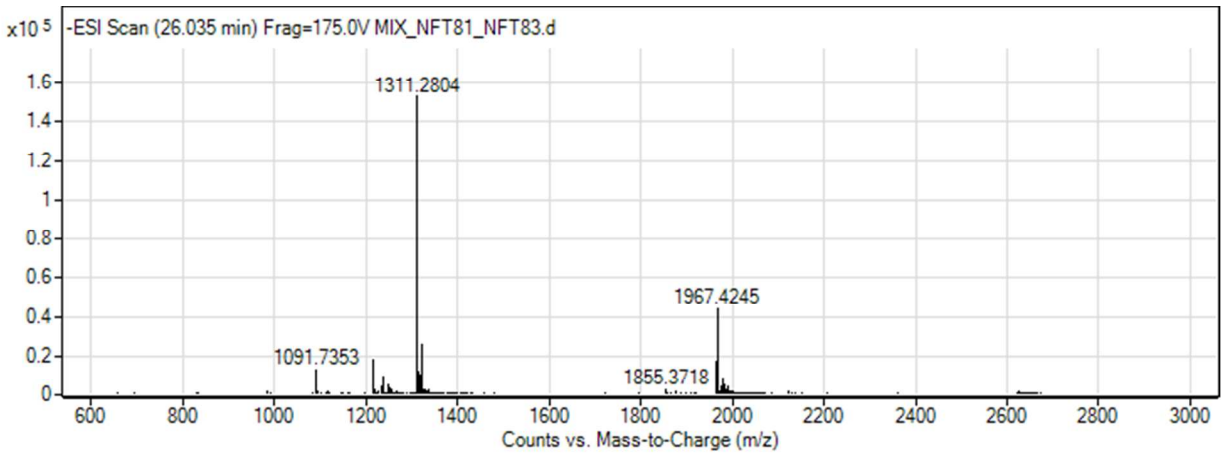
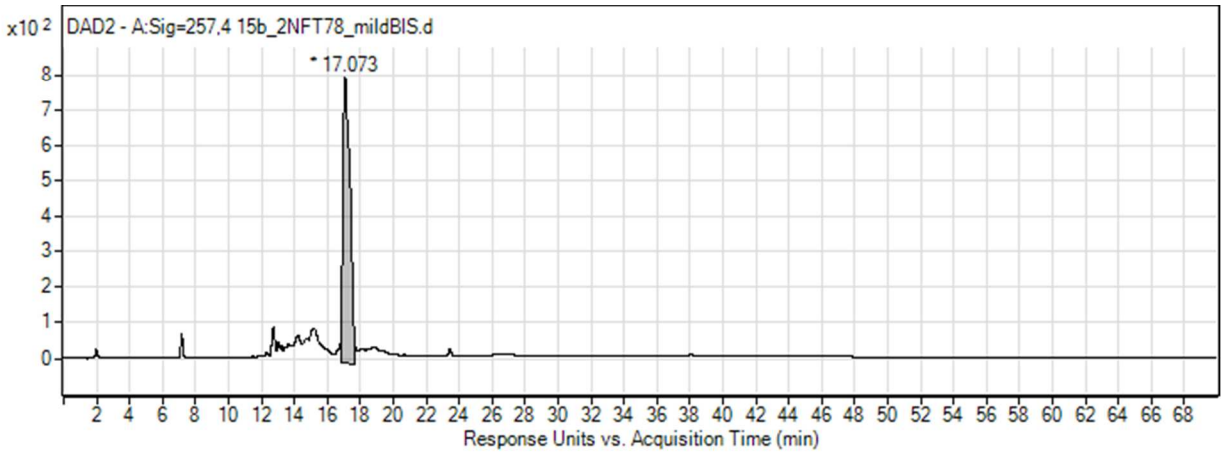


Figure S26. LC-MS analysis of the crude reaction mixture of ODN 10. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -2 charged peak.



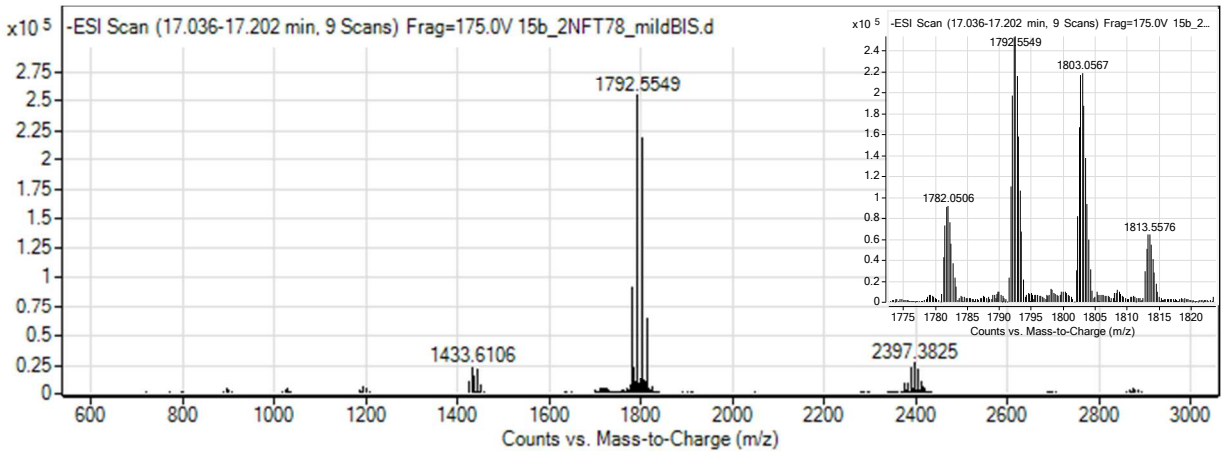


Figure S27. LC-MS analysis of the crude reaction mixture of ODN 11. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

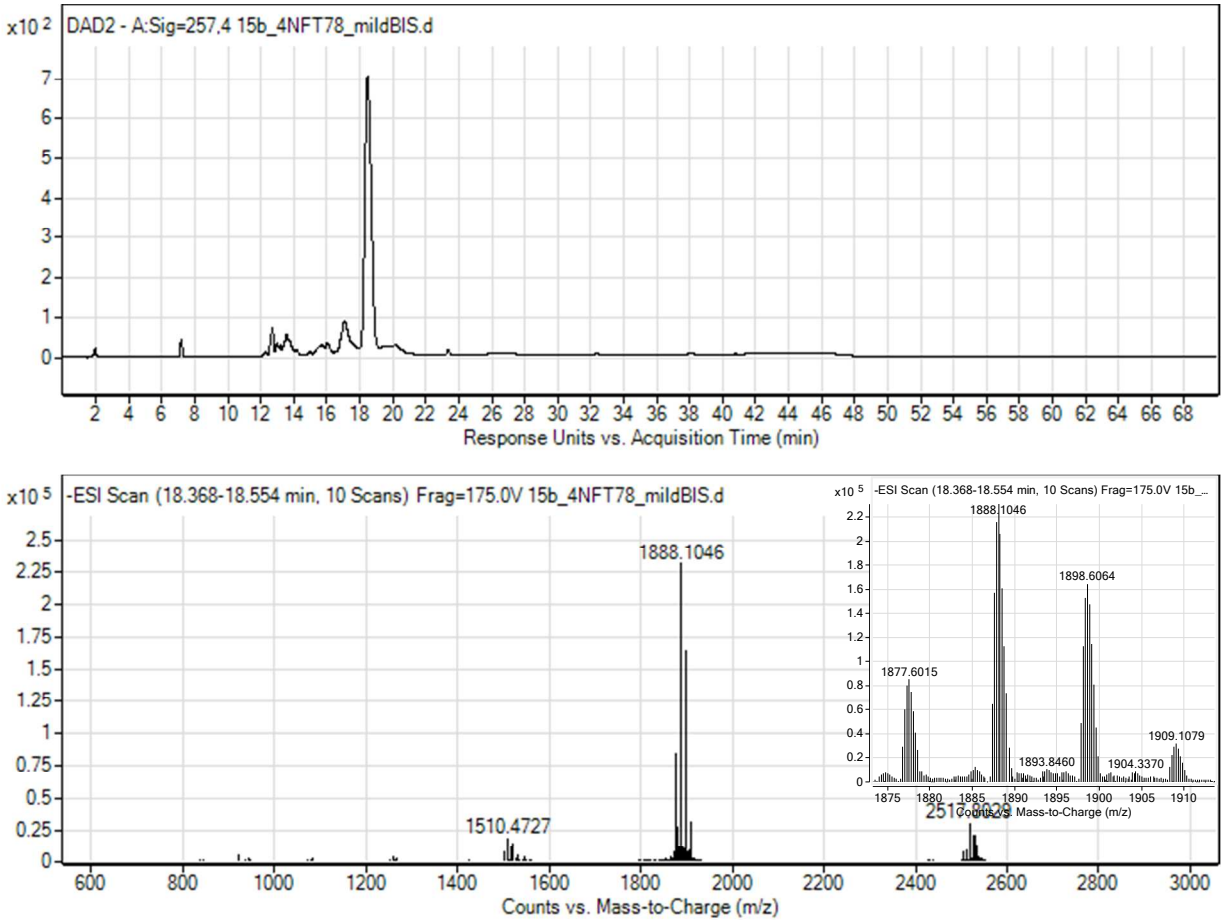


Figure S28. LC-MS analysis of the crude reaction mixture of ODN 12. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

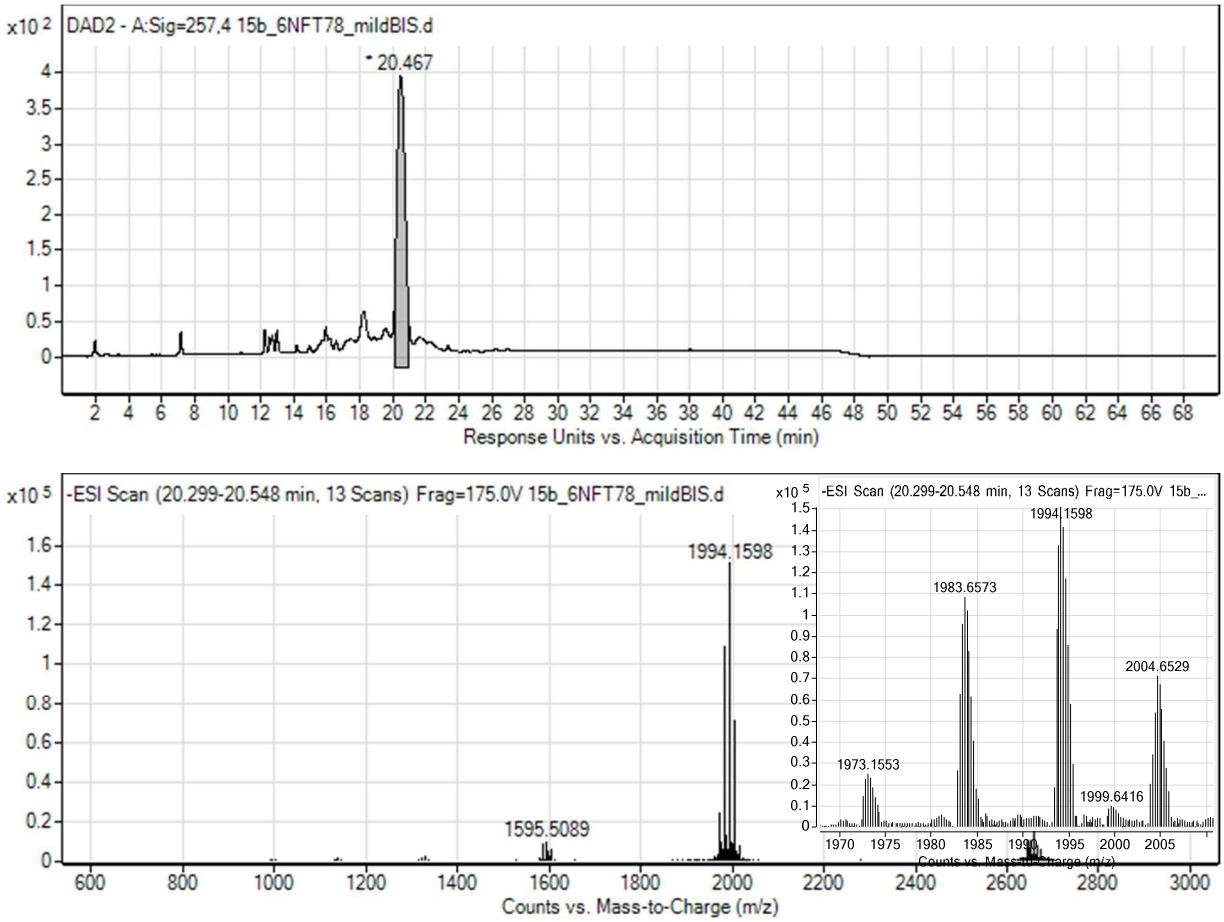
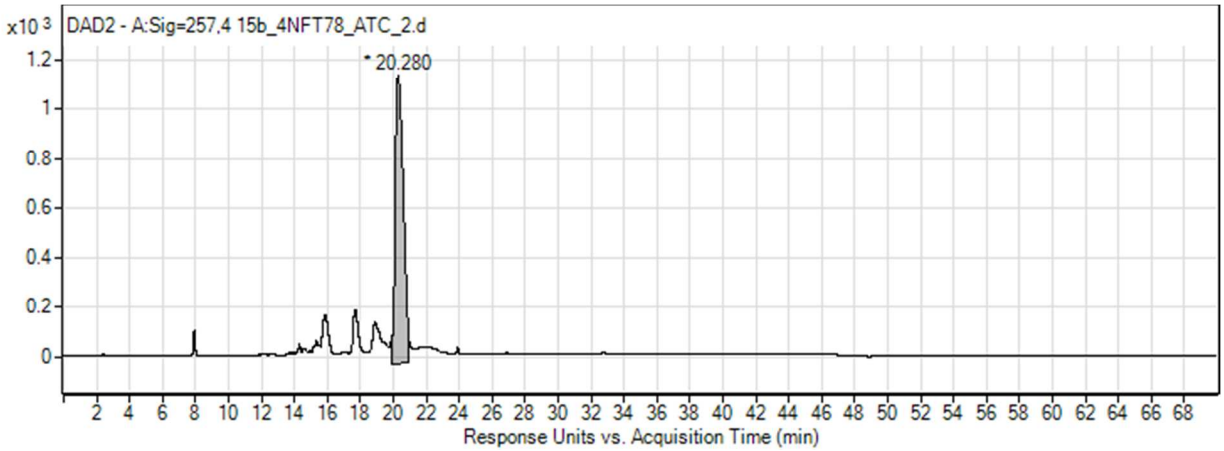


Figure S29. LC-MS analysis of the crude reaction mixture of ODN 13. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.



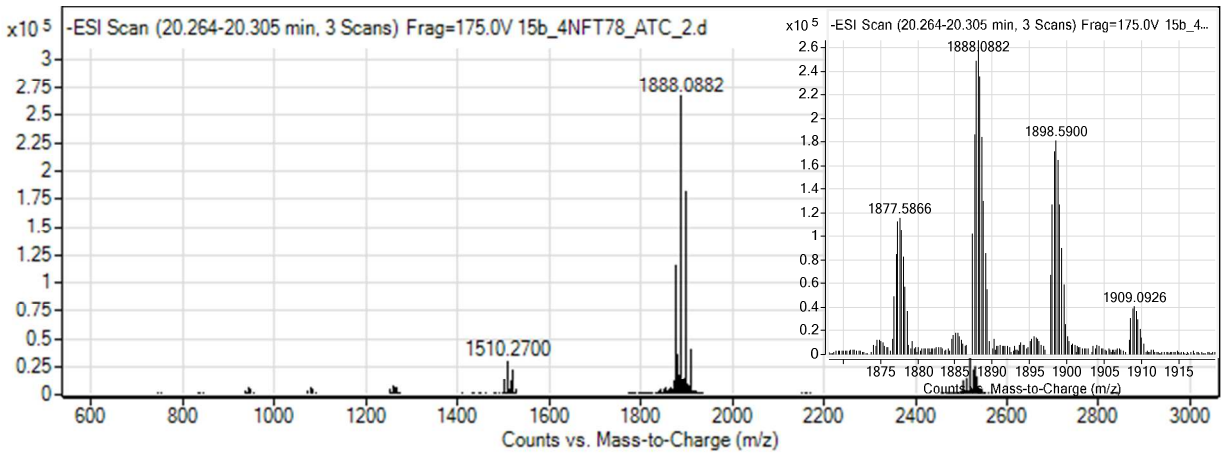


Figure S30. LC-MS analysis of the crude reaction mixtures of the crude reaction mixture of ODN 14. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

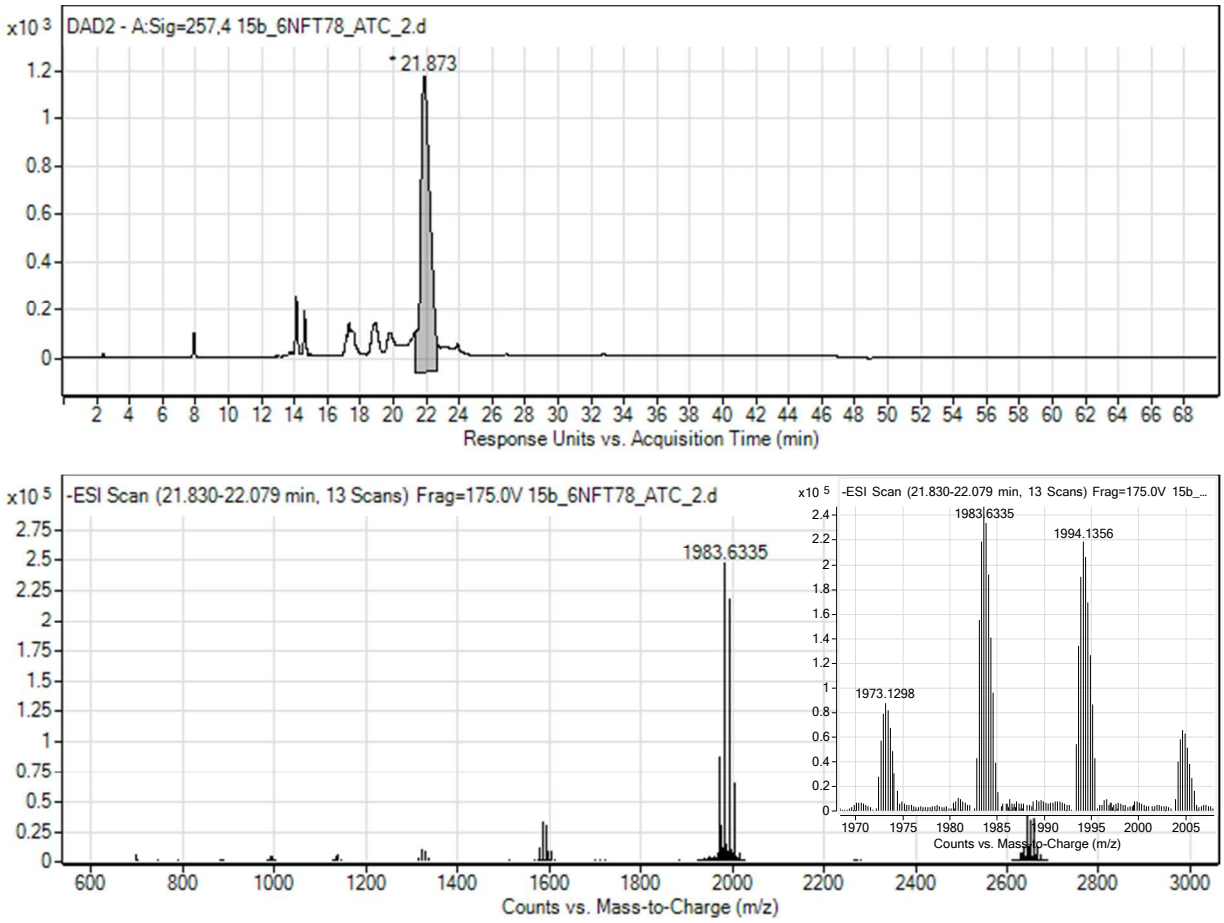


Figure S31. LC-MS analysis of the crude reaction mixture of ODN 15. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

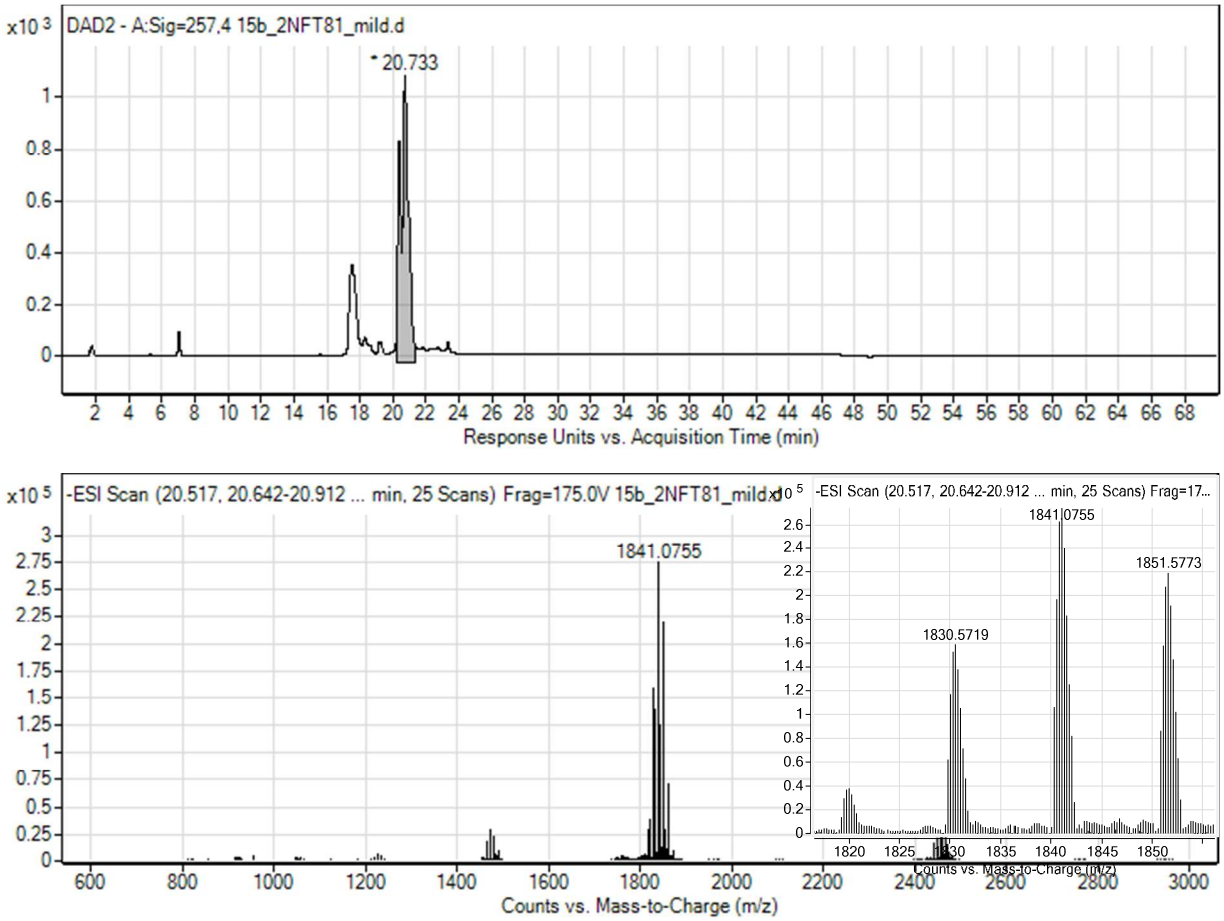
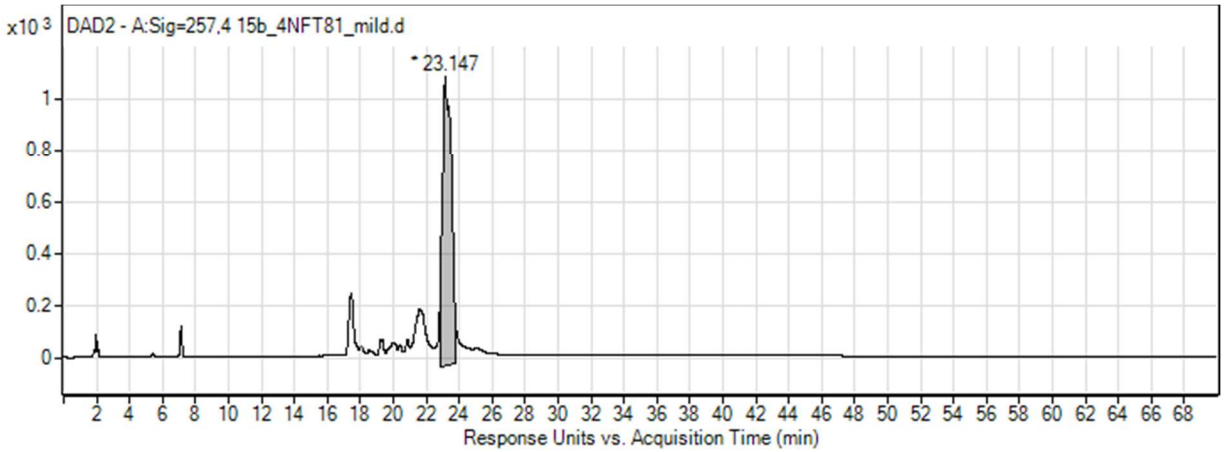


Figure S32. LC-MS analysis of the crude reaction mixture of ODN 16. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.



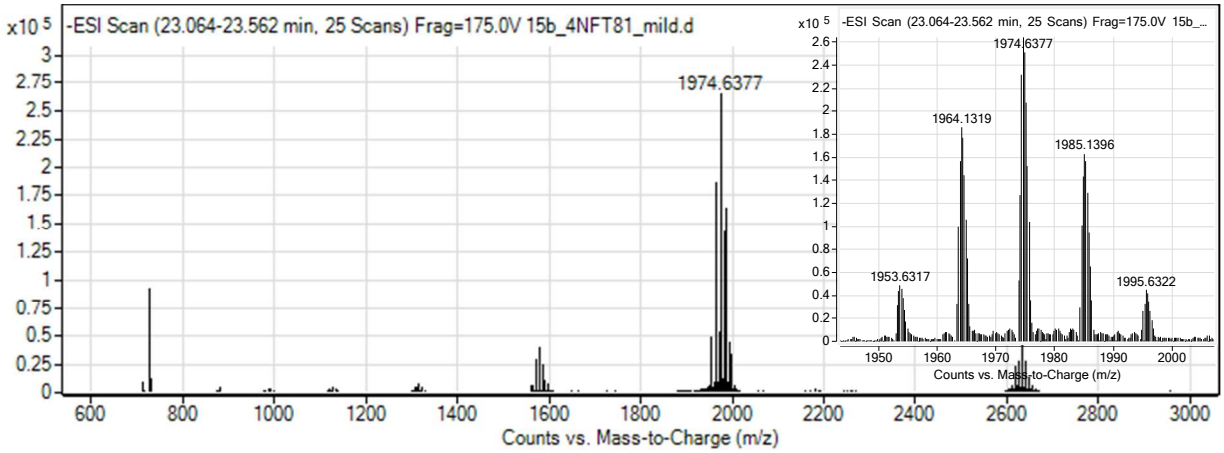


Figure S33. LC-MS analysis of the crude reaction mixture of ODN 17. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

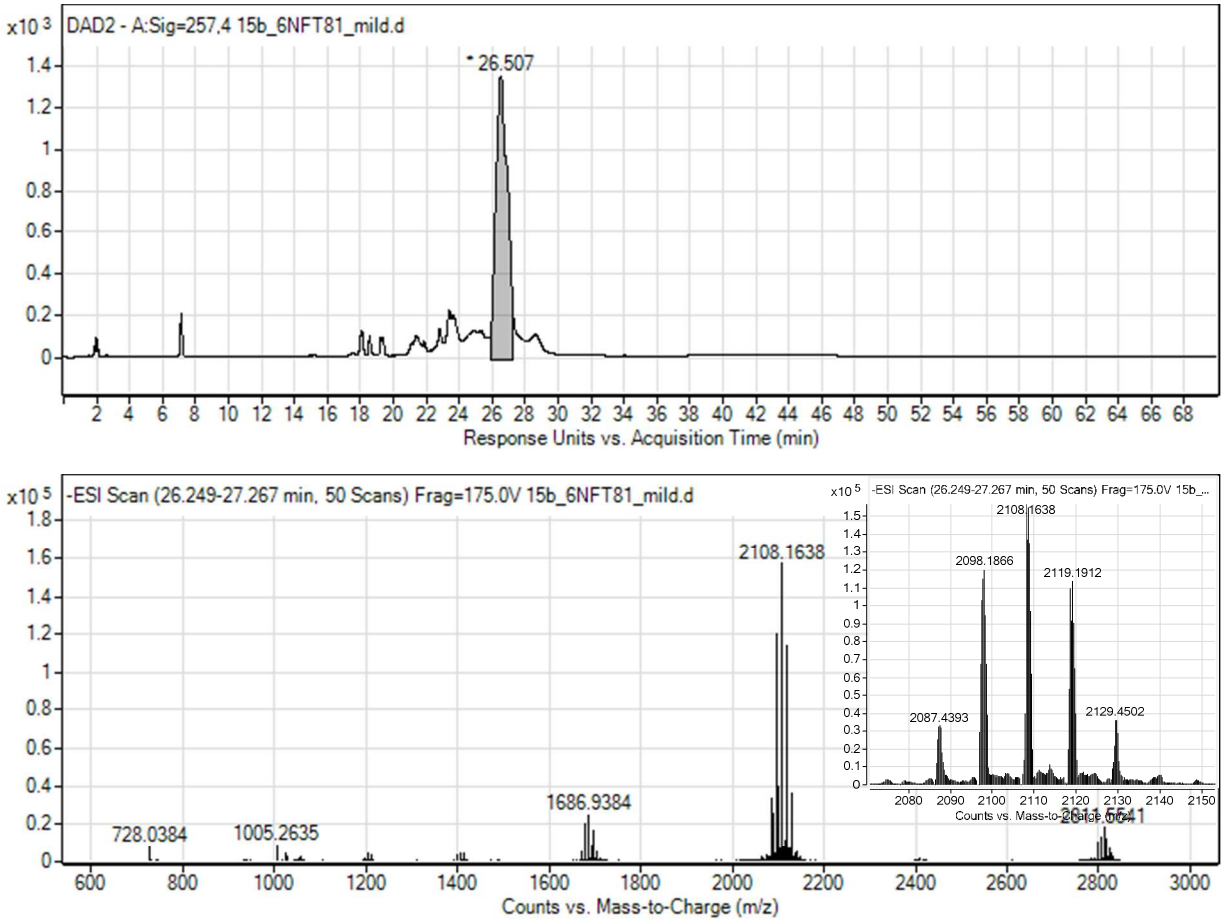


Figure S34. LC-MS analysis of the crude reaction mixtures of ODN 17. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

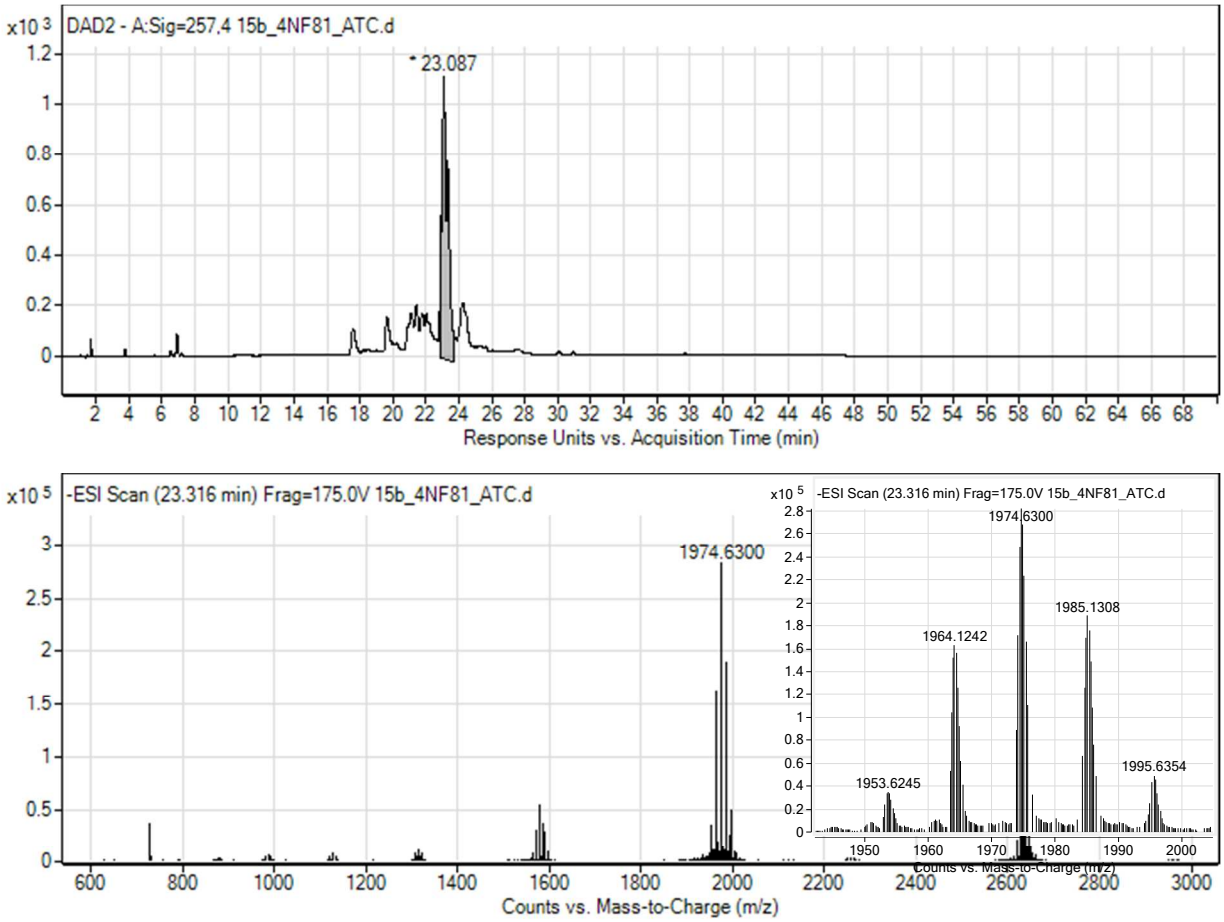
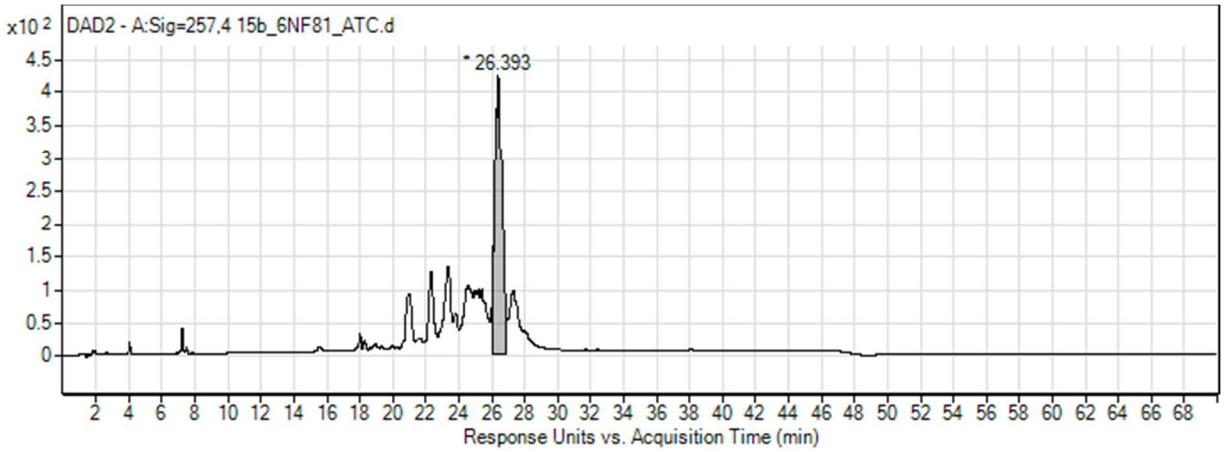


Figure S35. LC-MS analysis of the crude reaction mixture of ODN 19. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.



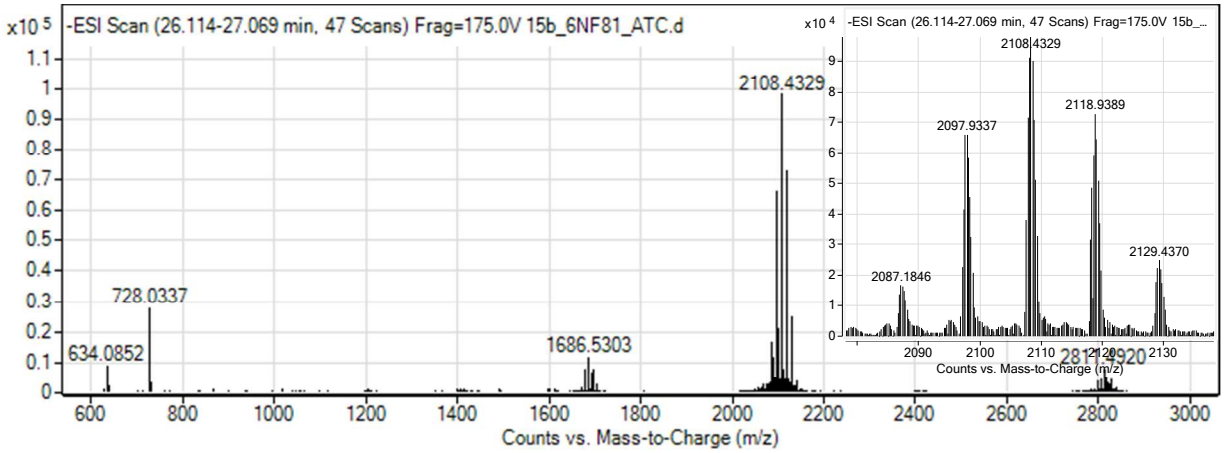


Figure S36. LC-MS analysis of the crude reaction mixture of ODN 20. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

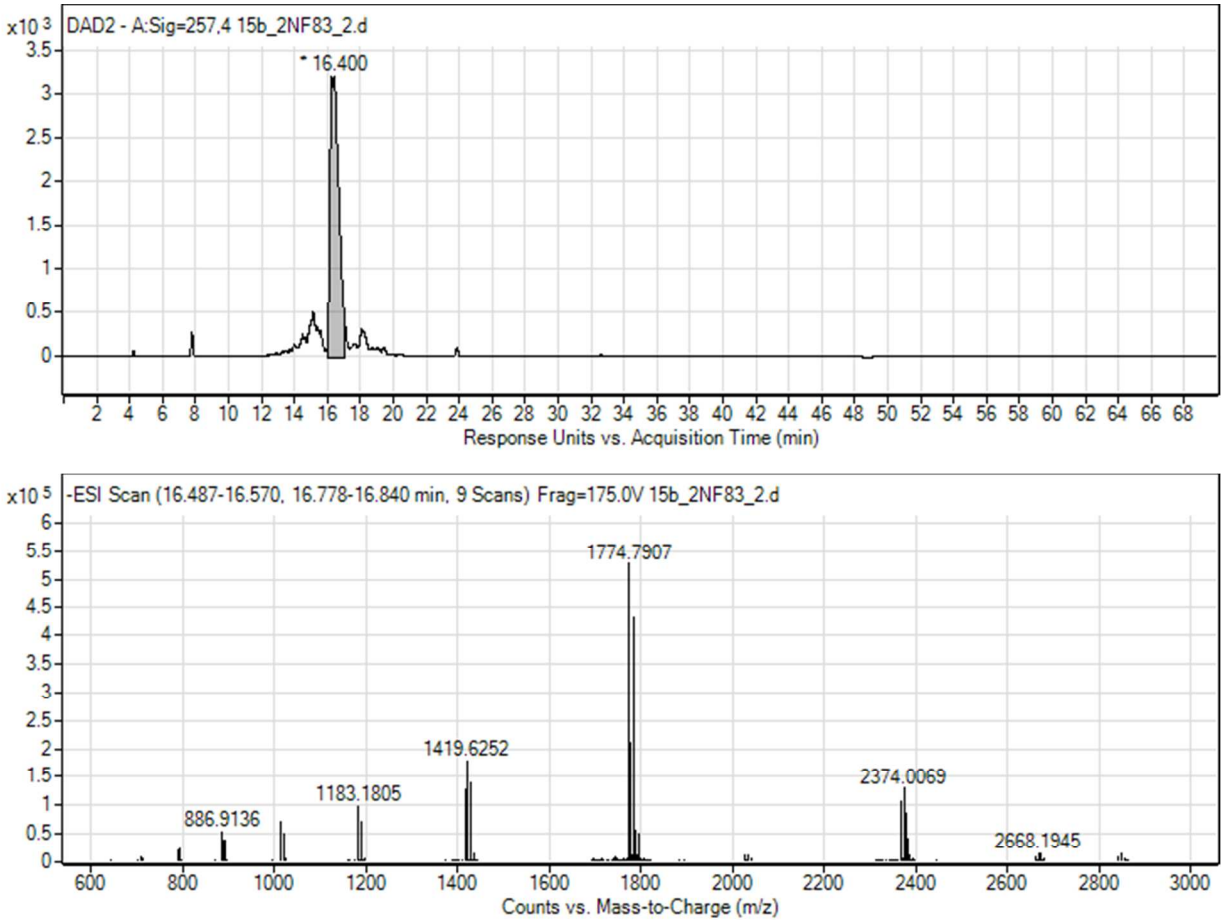


Figure S37. LC-MS analysis of the crude reaction mixture of ODN 21. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

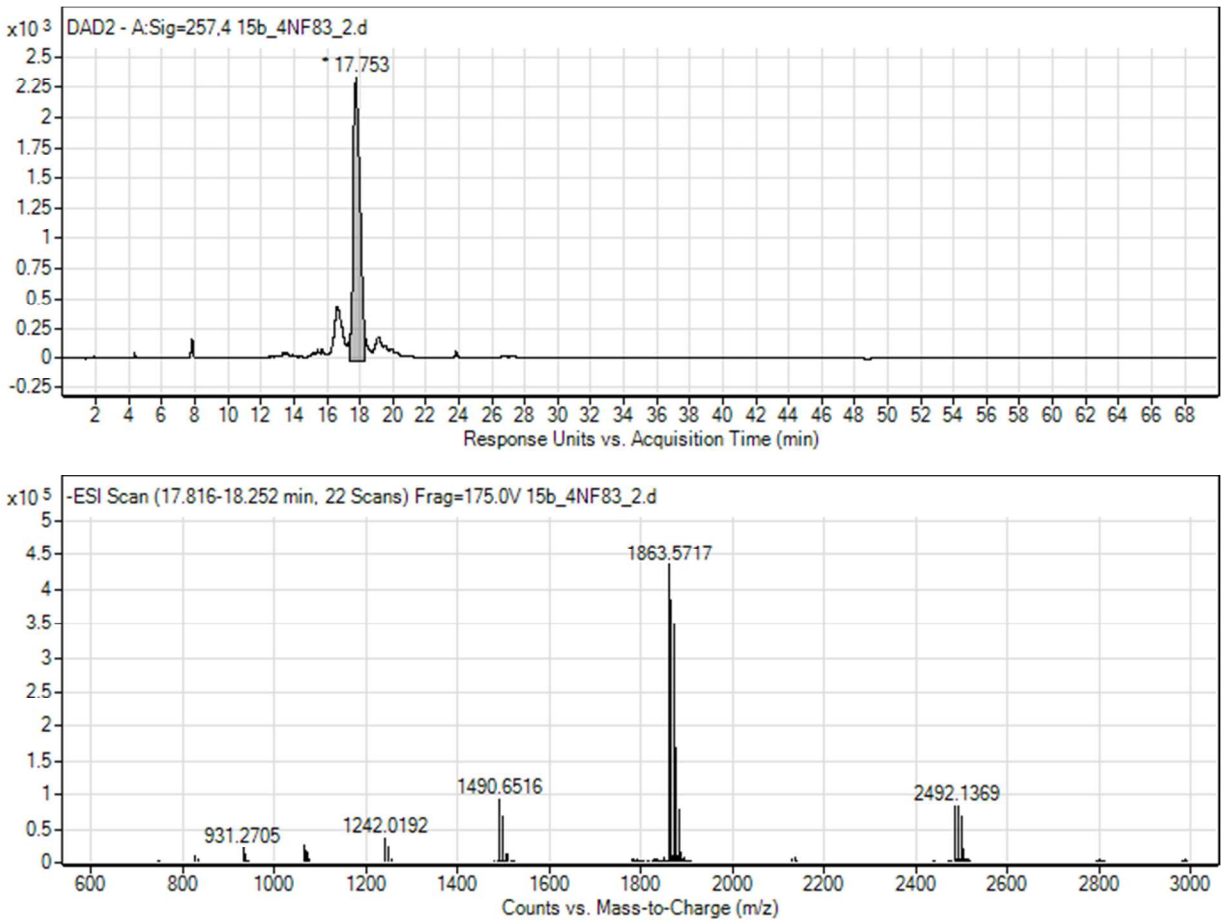
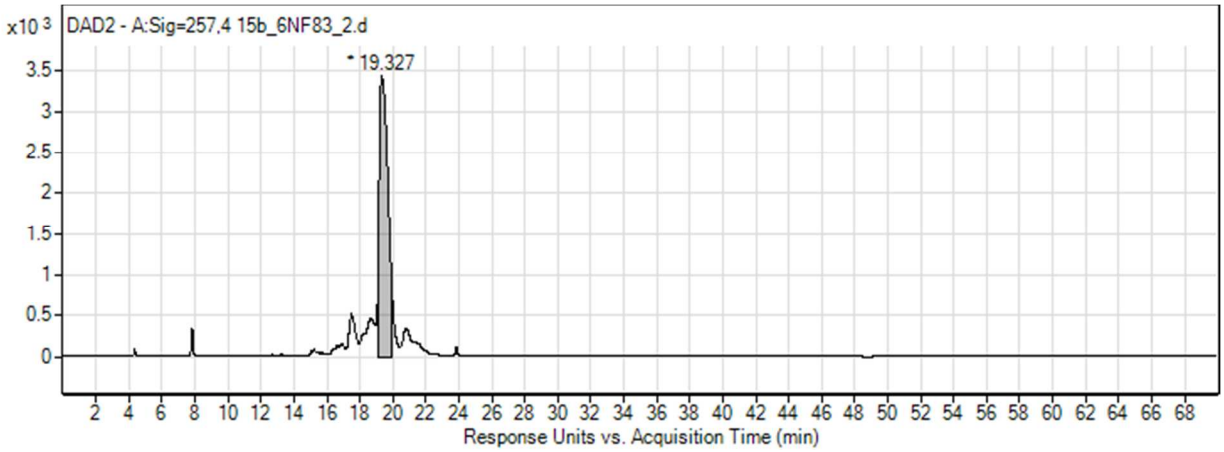


Figure S38. LC-MS analysis of the crude reaction mixture of ODN 22. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.



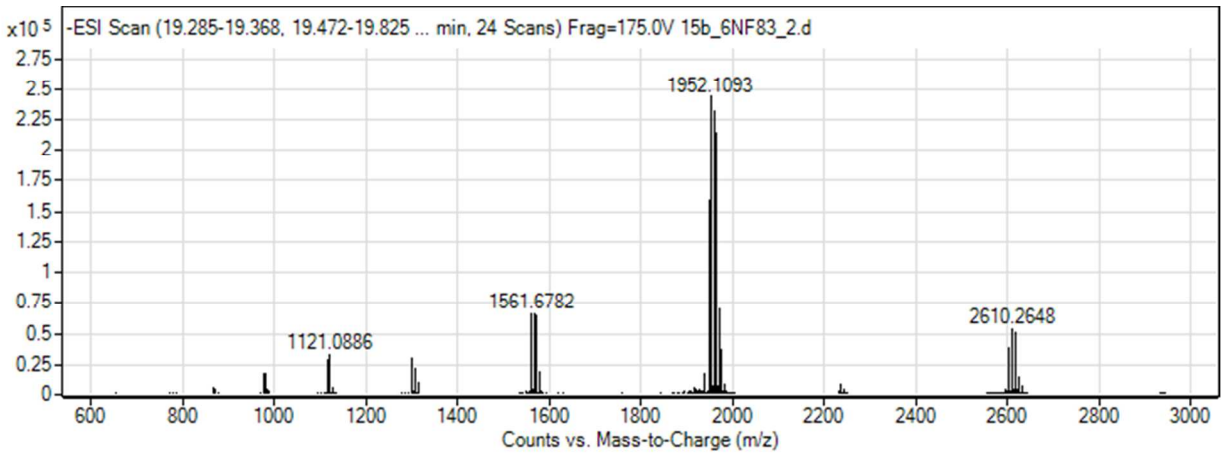


Figure S39. LC-MS analysis of the crude reaction mixture of ODN 23. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

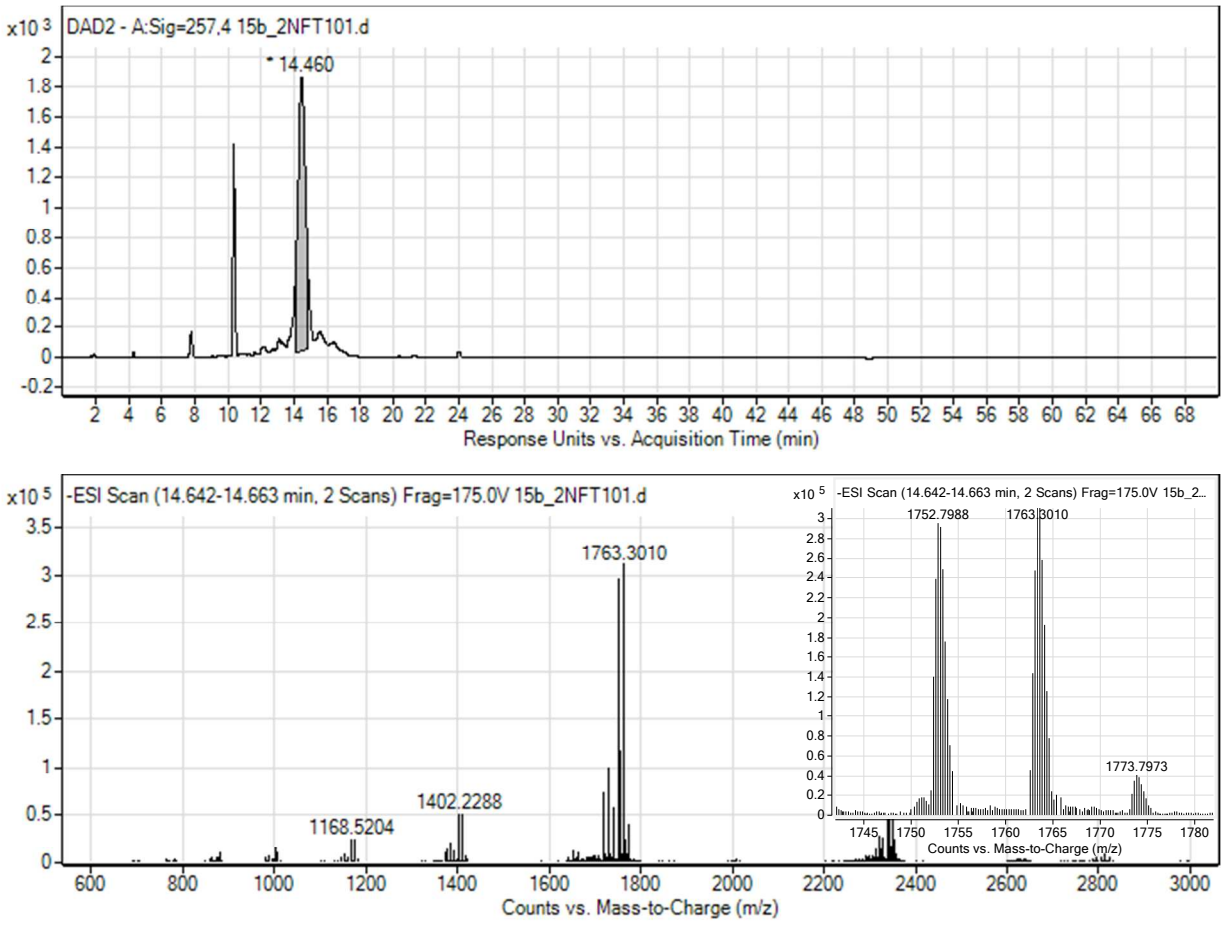


Figure S40. LC-MS analysis of the crude reaction mixture of ODN 24. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

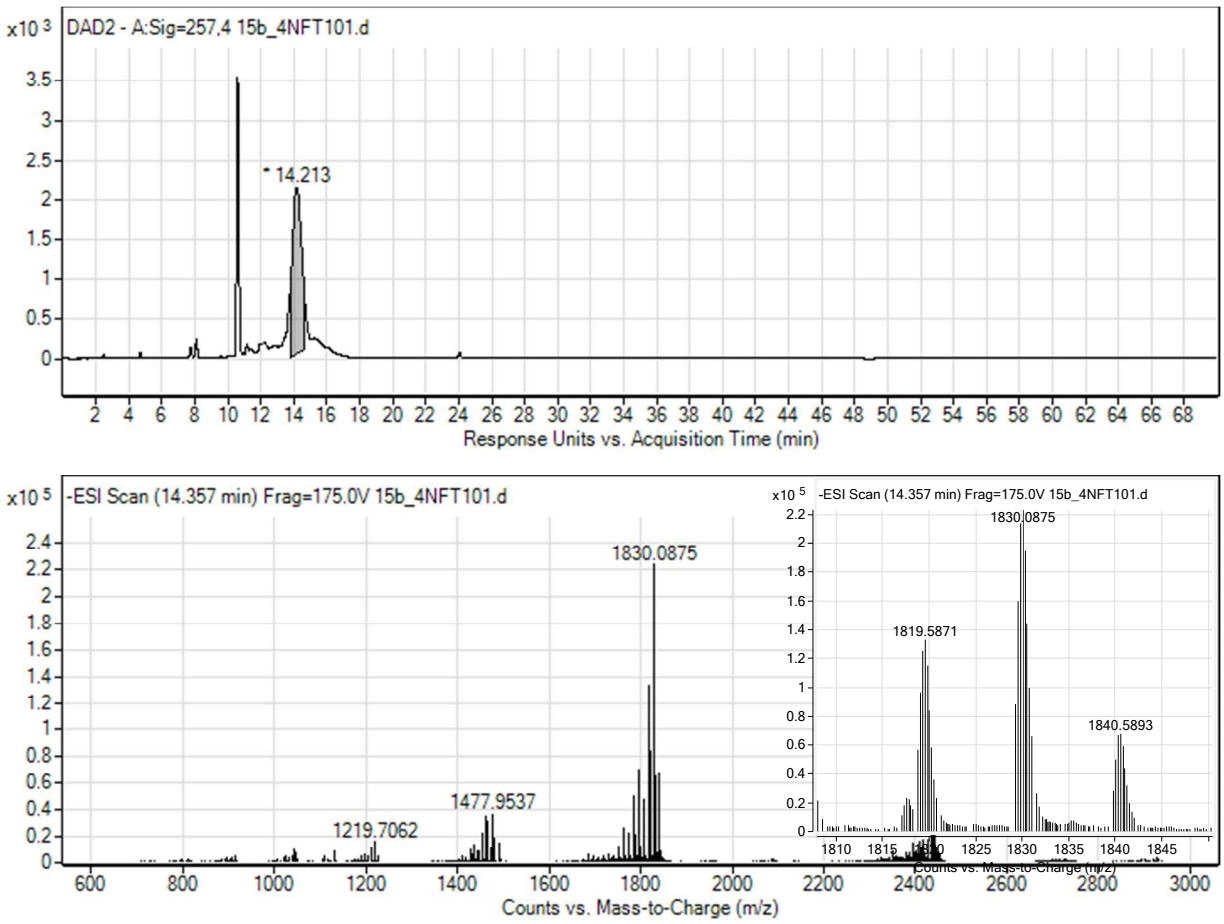
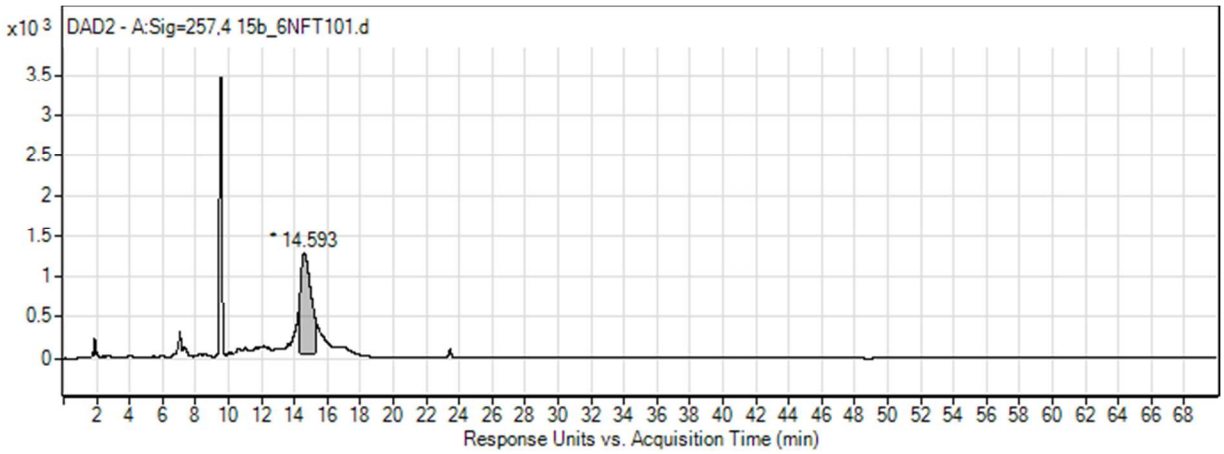


Figure S41. LC-MS analysis of the crude reaction mixture of ODN 25. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.



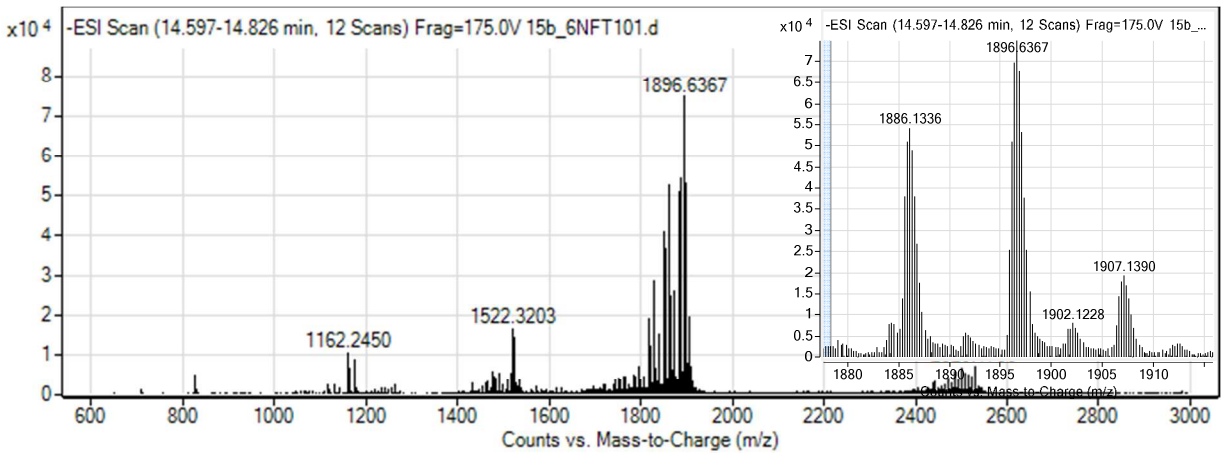


Figure S42. LC-MS analysis of the crude reaction mixture of ODN 26. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

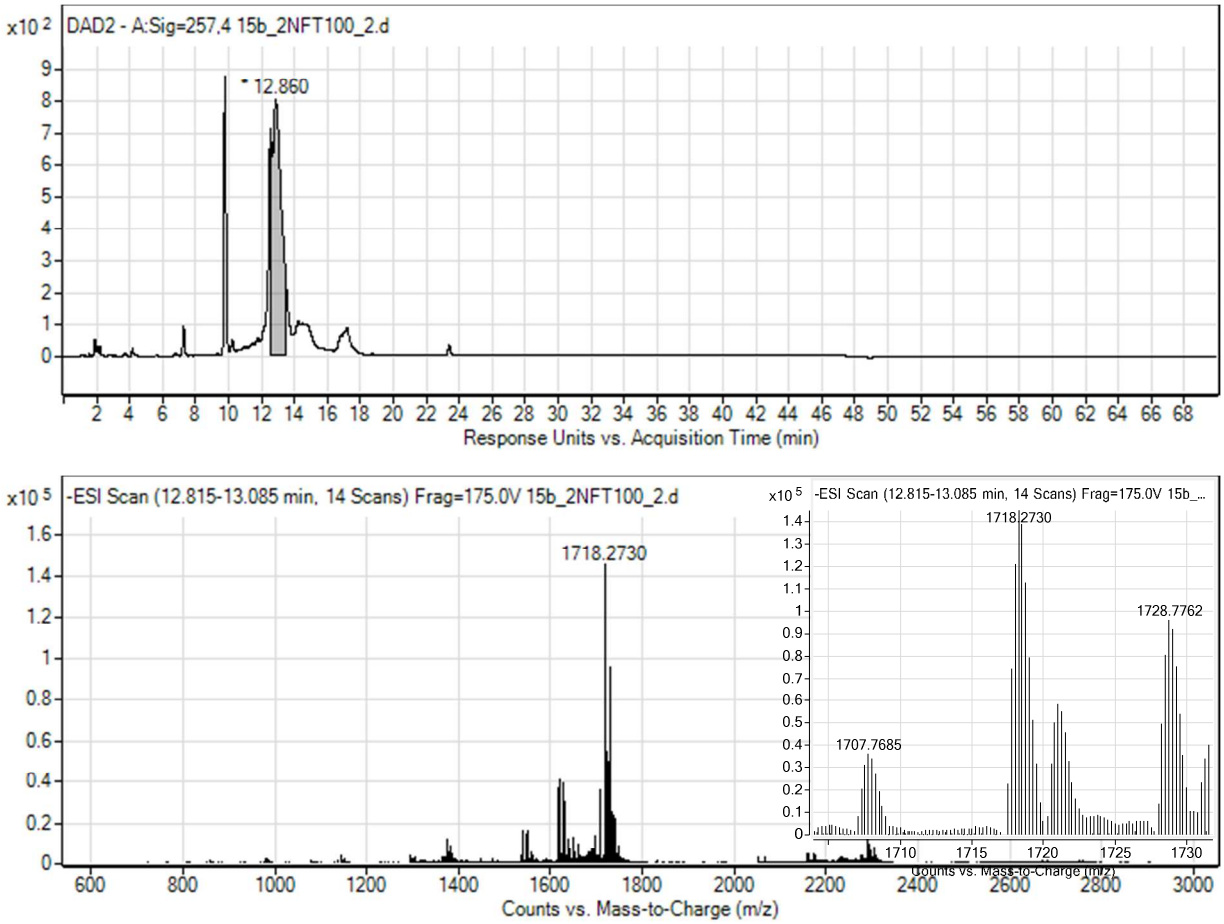


Figure S43. LC-MS analysis of the crude reaction mixture of ODN 27. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks

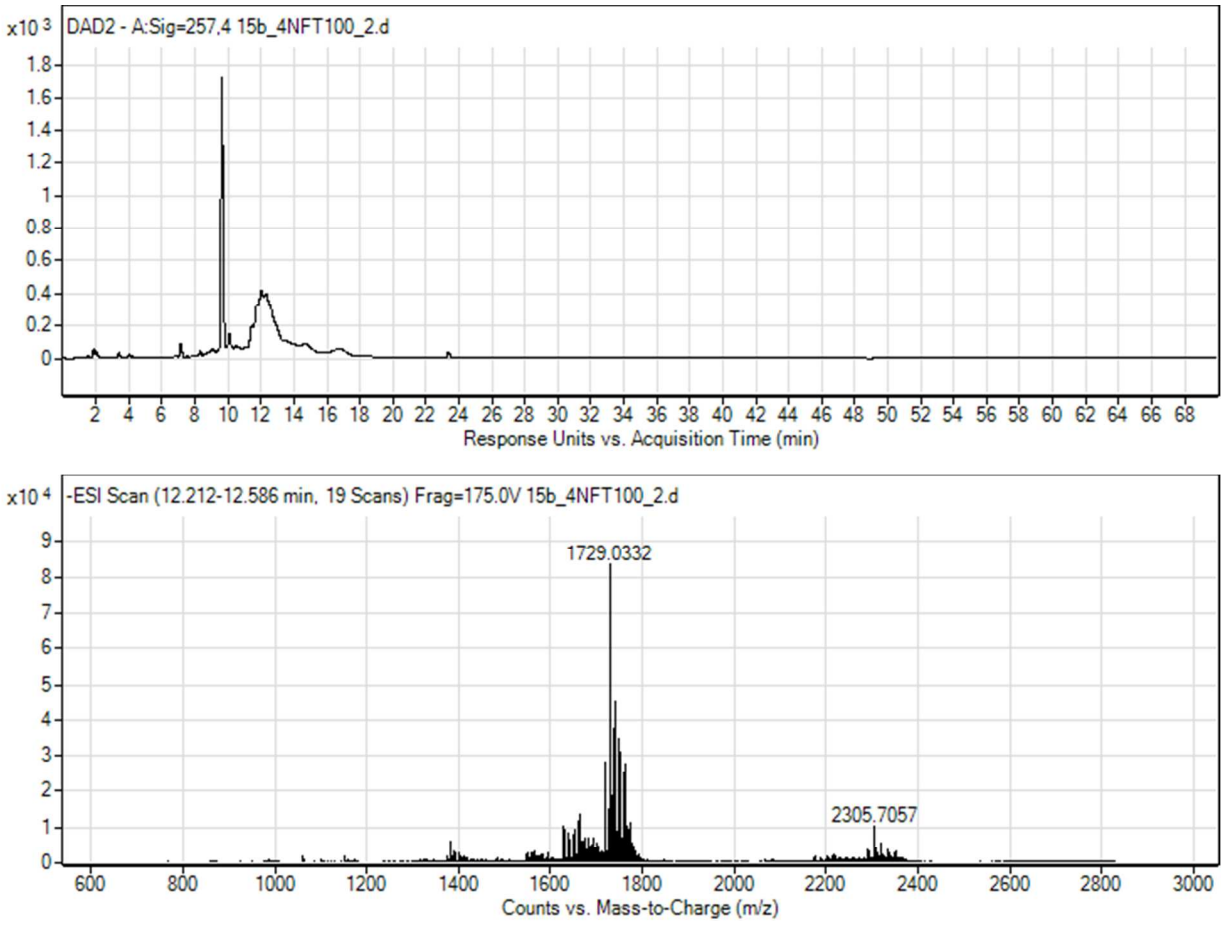


Figure S44. LC-MS analysis of the crude reaction mixture of ODN 28. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ picks.

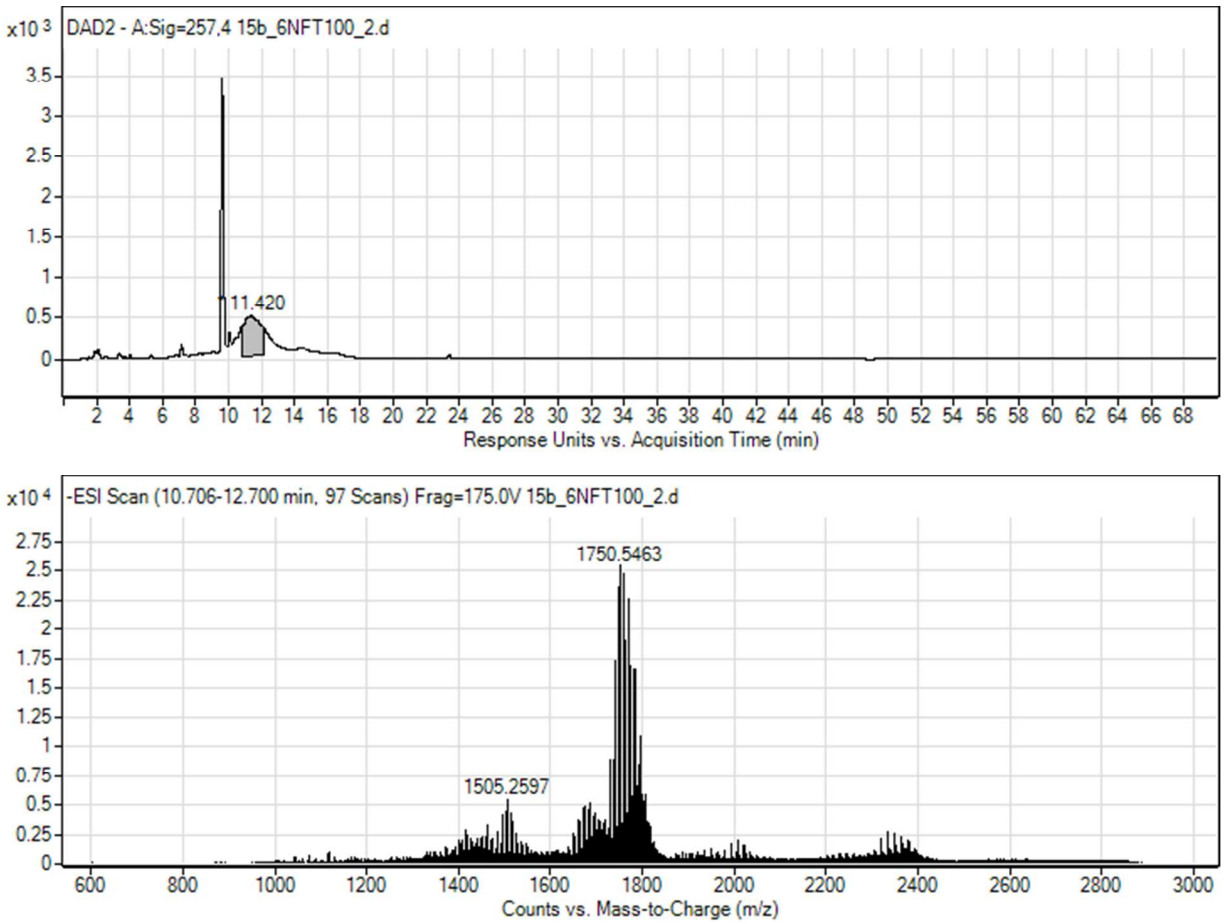
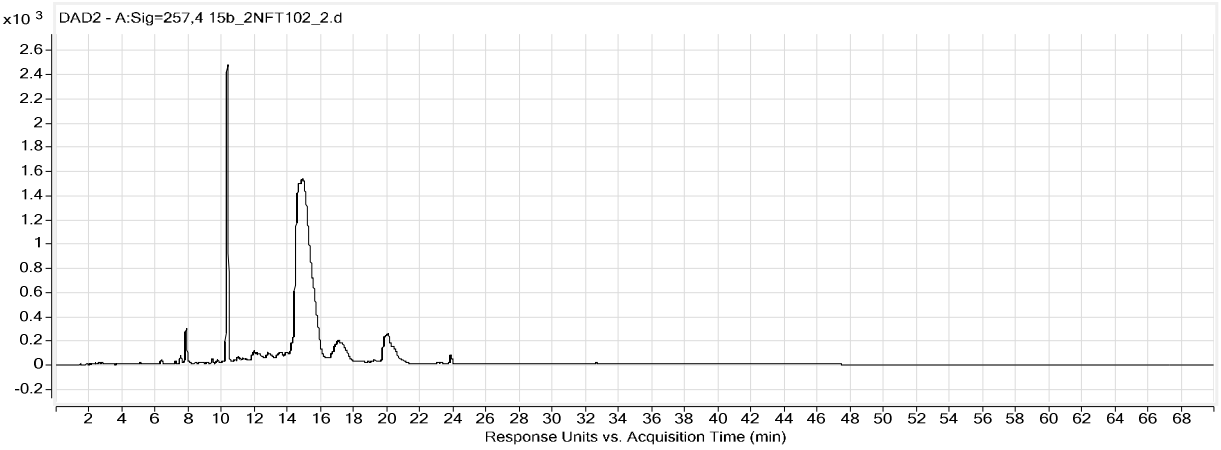


Figure S45. LC-MS analysis of the crude reaction mixture of ODN 29. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.



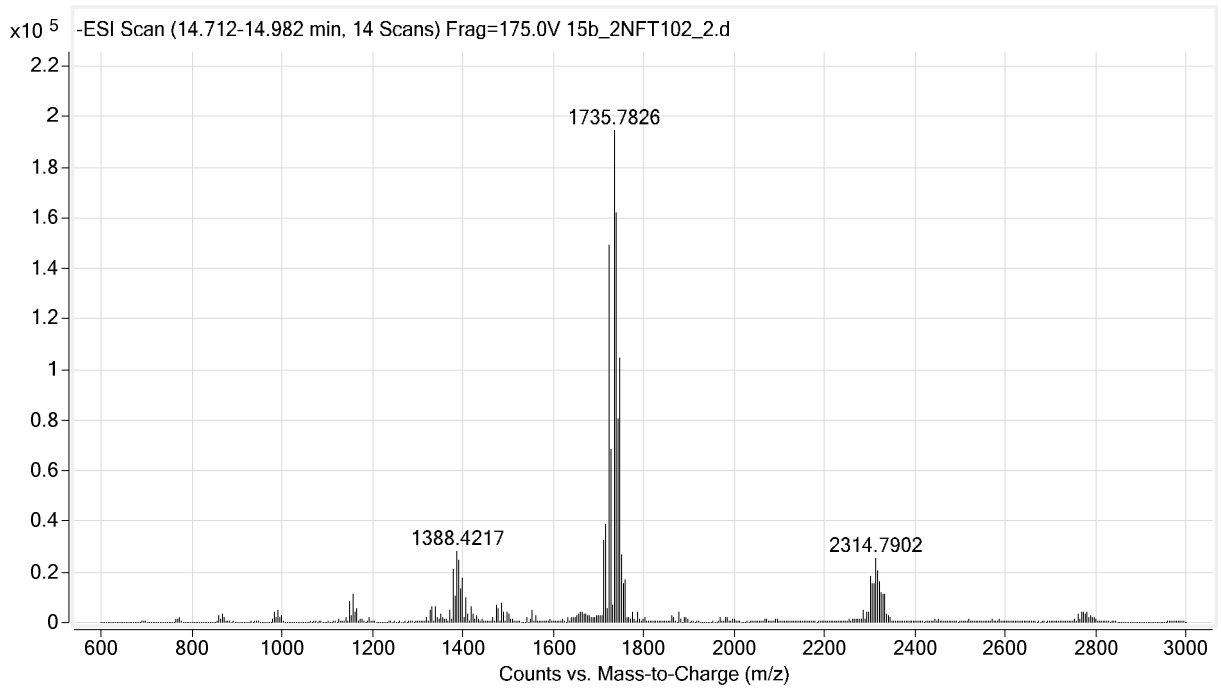
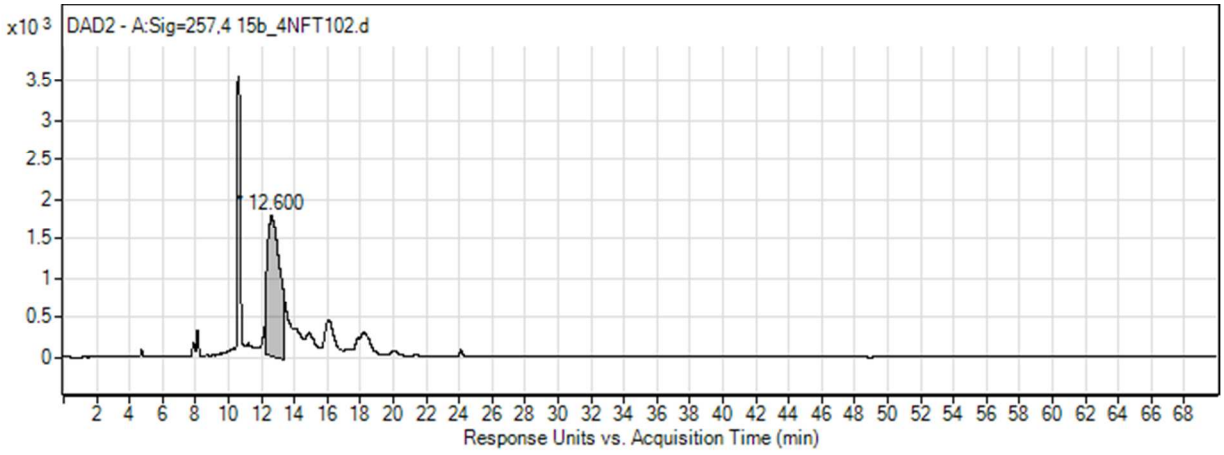


Figure S46. LC-MS analysis of the crude reaction mixture of ODN 30. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.



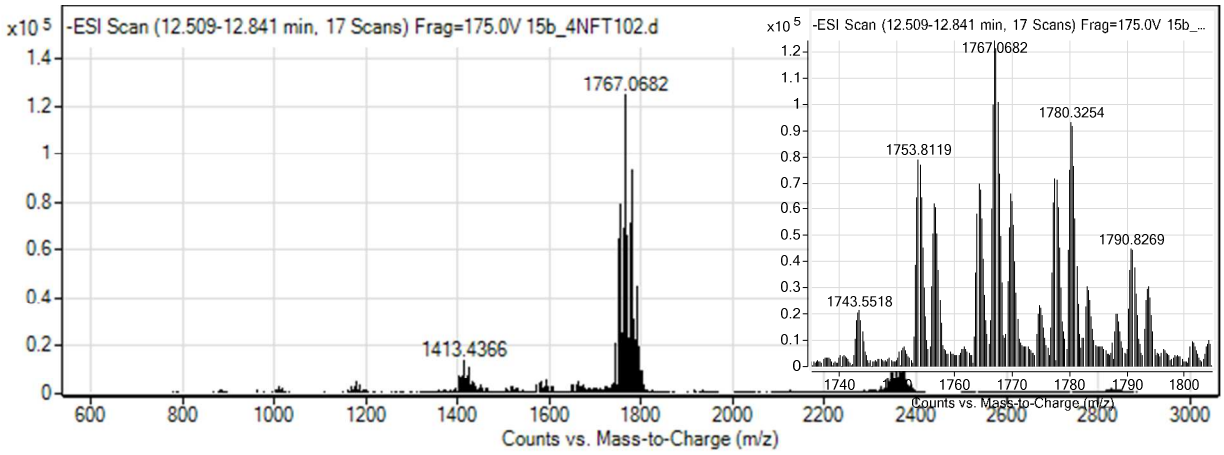


Figure S47. LC-MS analysis of the crude reaction mixture of ODN 31. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

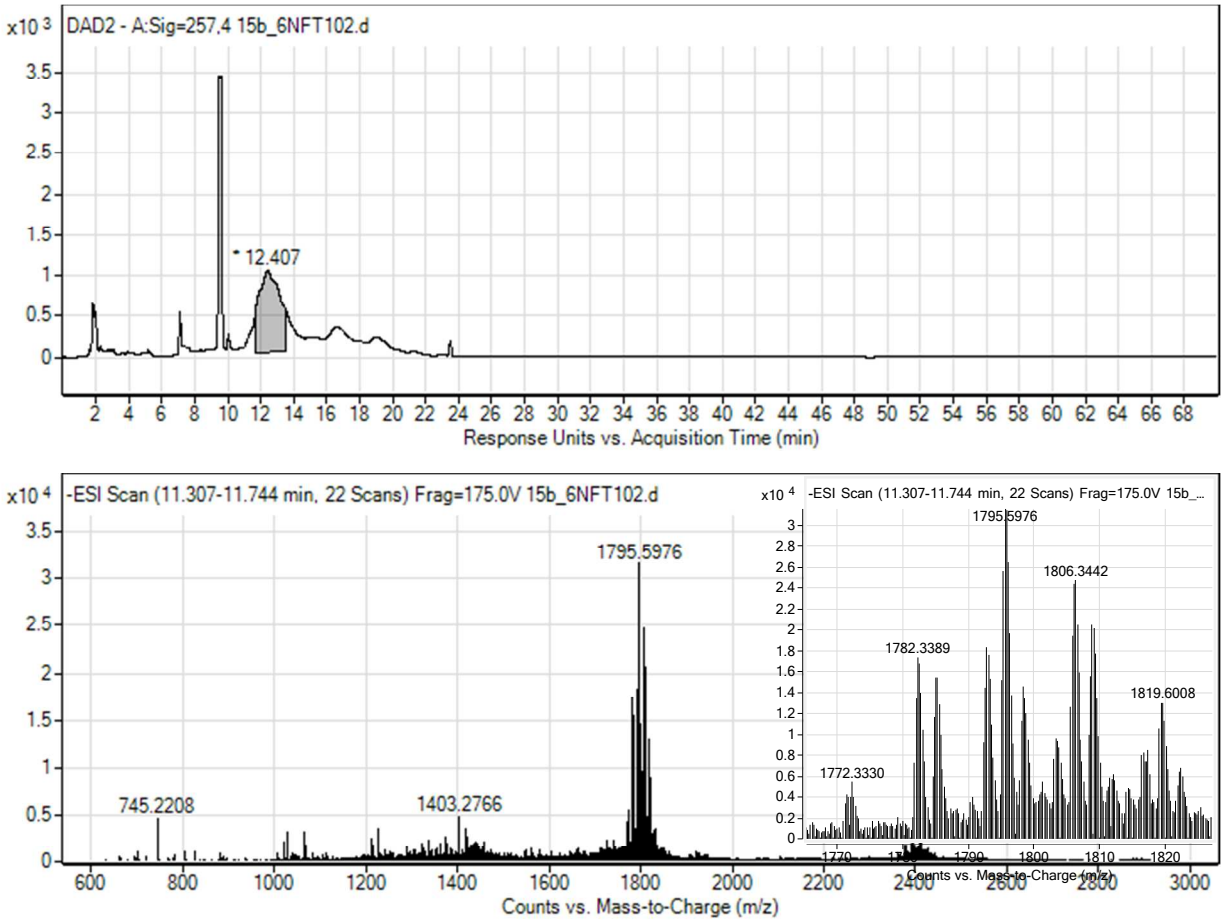


Figure S48. LC-MS analysis of the crude reaction mixture of ODN 32. UV absorbance chromatogram (A_{254}) and mass spectra corresponding to the peaks are shown. The base peak in the mass spectrum corresponds to the -4 charged peak and $(M-4+Na)^{4-}$, $(M-4+K)^{4-}$ peaks.

3) Melting temperature curves.

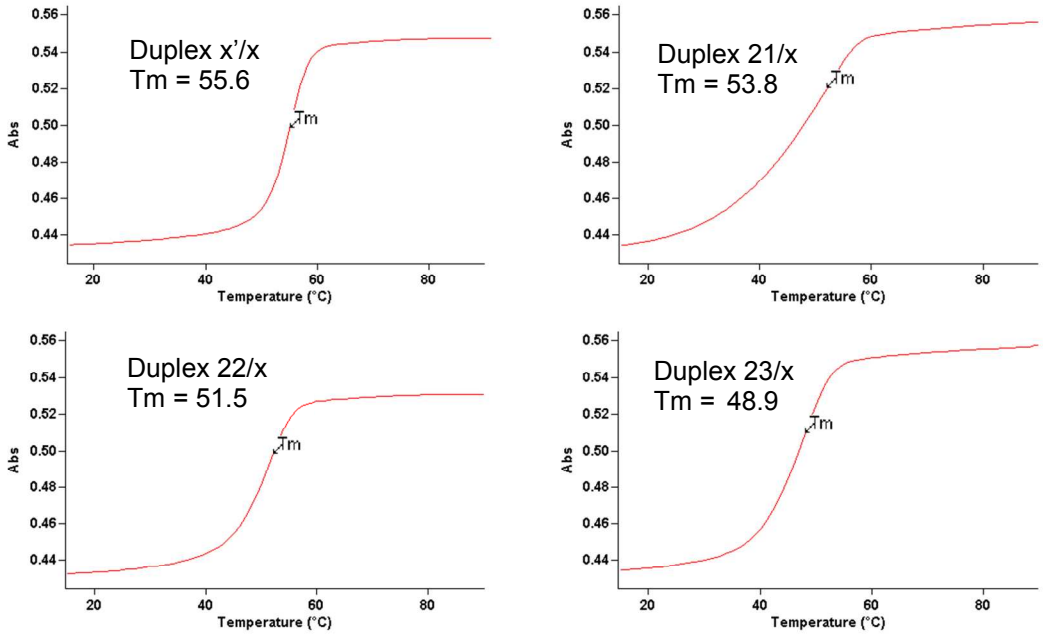


Figure S49. Melting curves of duplex x'/x, 21/x, 22/x and 23/x (See Manuscript, Table 4). x =5 ′-TAG CAG CAC ATC ATG GTT TAC A-3′; x′ = 5′-TGT AAA CCA TGA TGT GCT GCT A-3′;

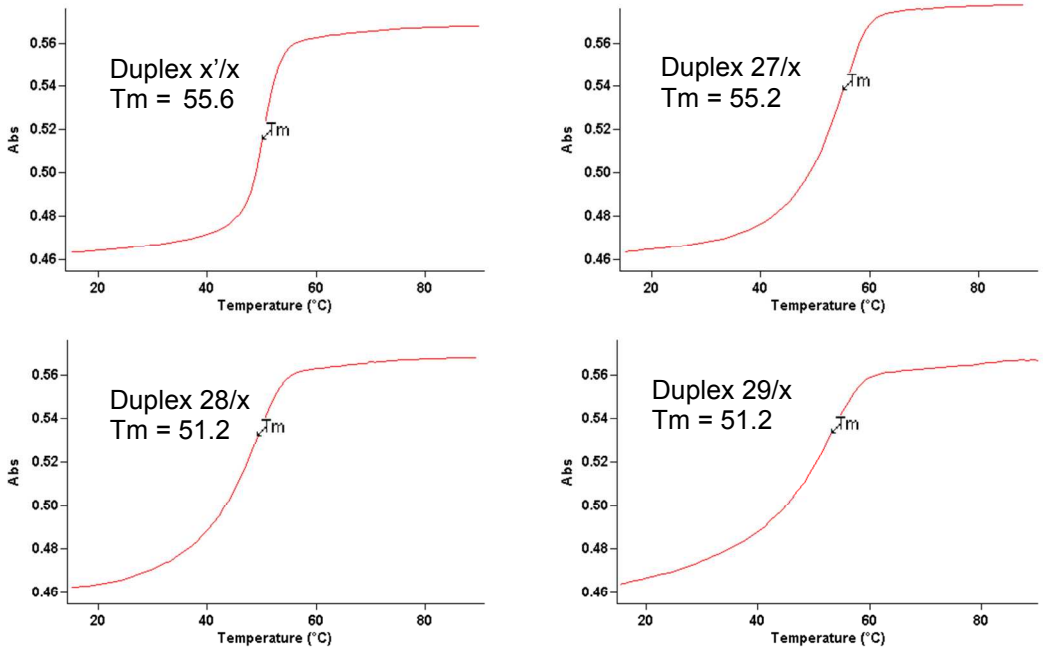


Figure S50. Melting curves of duplex x'/x, 27/x, 28/x and 29/x (See Manuscript, Table 4). x =5 ′-TAG CAG CAC ATC ATG GTT TAC A-3′; x′ = 5′-TGT AAA CCA TGA TGT GCT GCT A-3′;

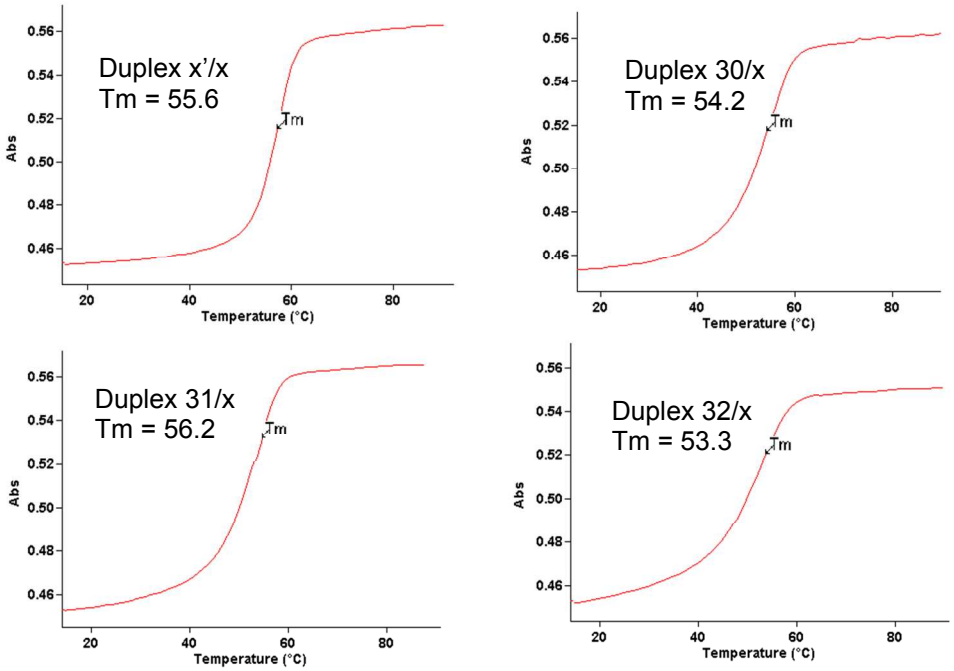


Figure S51. Melting curves of duplex x'/x, 30/x, 31/x and 32/x (See Manuscript, Table 4). x =5 ′-TAG CAG CAC ATC ATG GTT TAC A-3′; x′ = 5′-TGT AAA CCA TGA TGT GCT GCT A-3′;