Solid-Phase Synthesis of Boranophosphate/Phosphorothioate/Phosphate Chimeric Oligonucleotides and Their Potential as Antisense Oligonucleotides

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Support information

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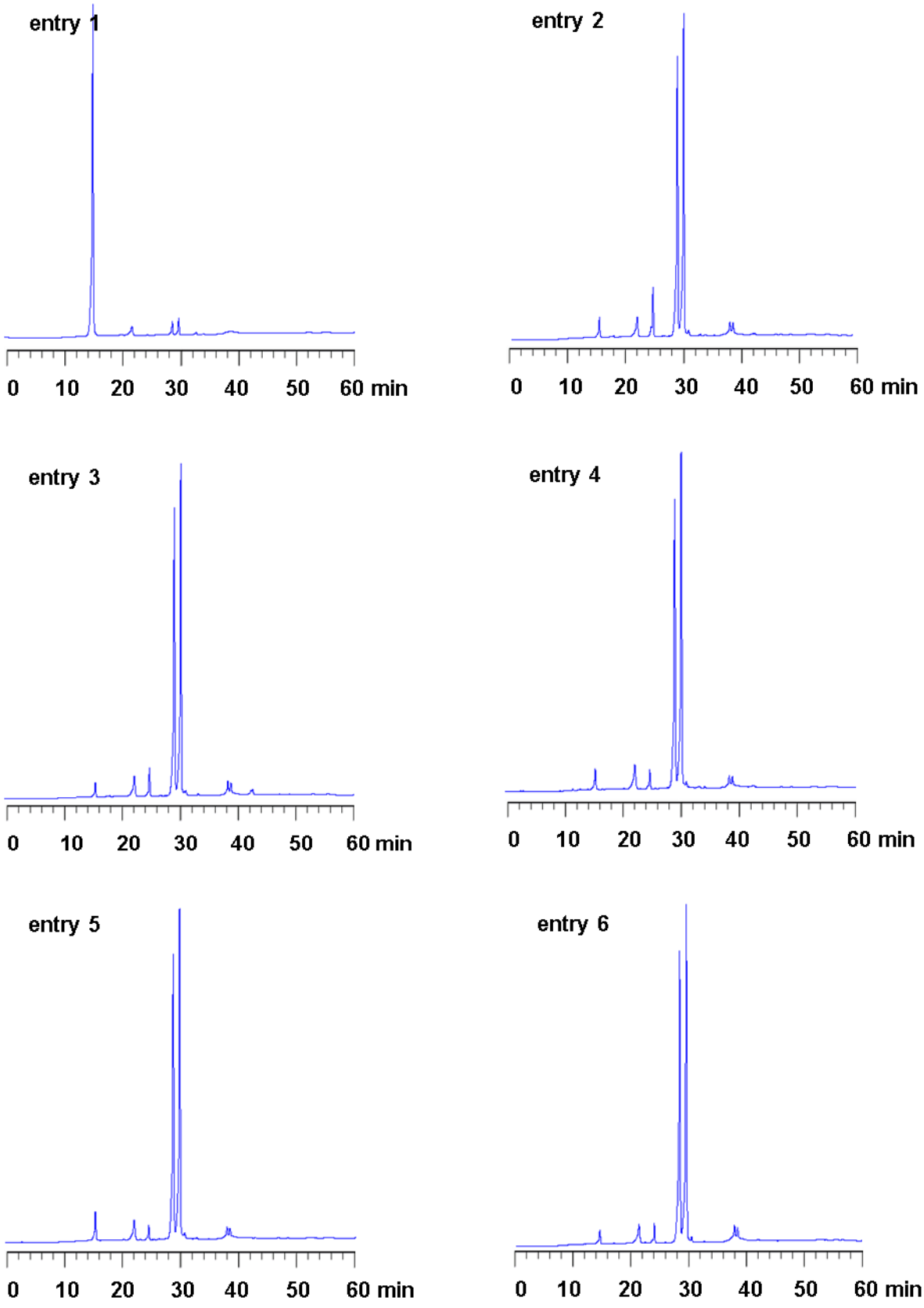
RP-HPLC profiles of $T_{PS}T$ dimer (Table 1)



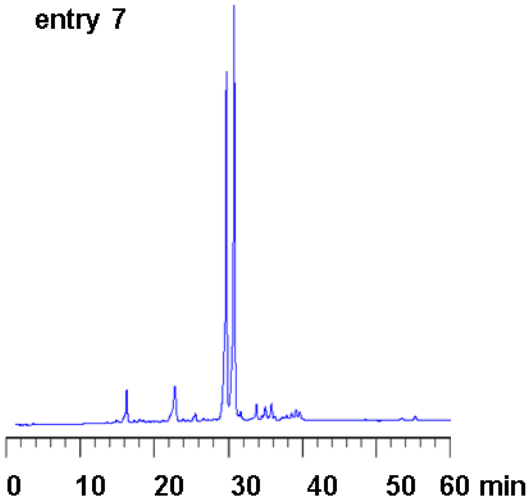
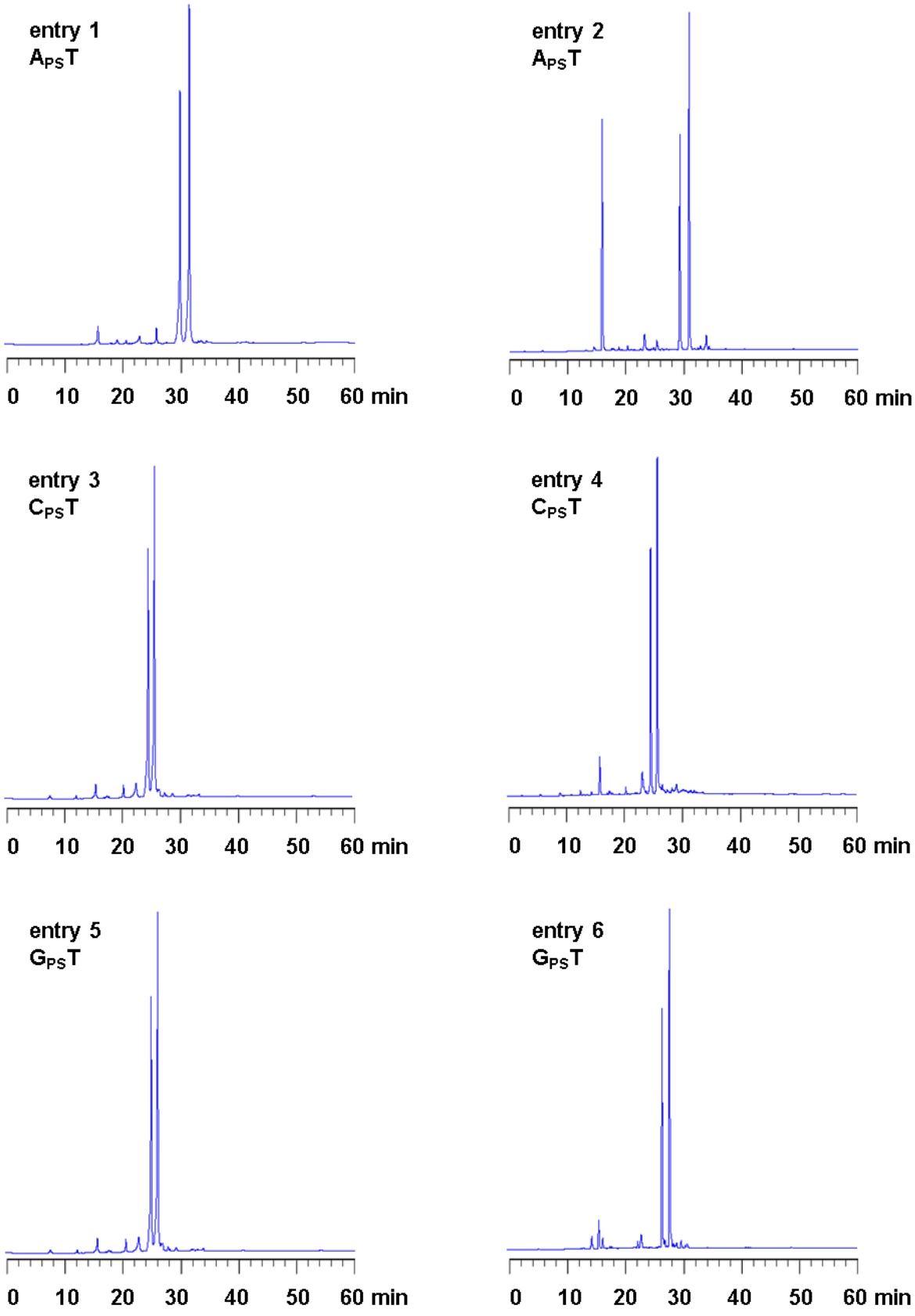


Figure S1. RP-HPLC profiles of crude $T_{PS}T$. RP-HPLC was performed with a linear gradient of 0%–30% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 ℃ at a flow rate of 0.5 mL/min using a C18 column.

RP-HPLC profiles of $B_{PS}T$ dimers (Table 2)



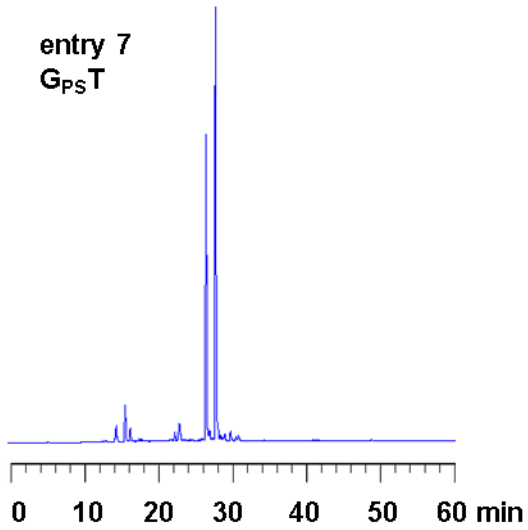


Figure S2. RP-HPLC profiles of crude $B_{PS}T$. RP-HPLC was performed with a linear gradient of 0%–30% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a C18 column.

RP-HPLC profiles of $\underline{B}_{PB}T$ dimers (Table 3)

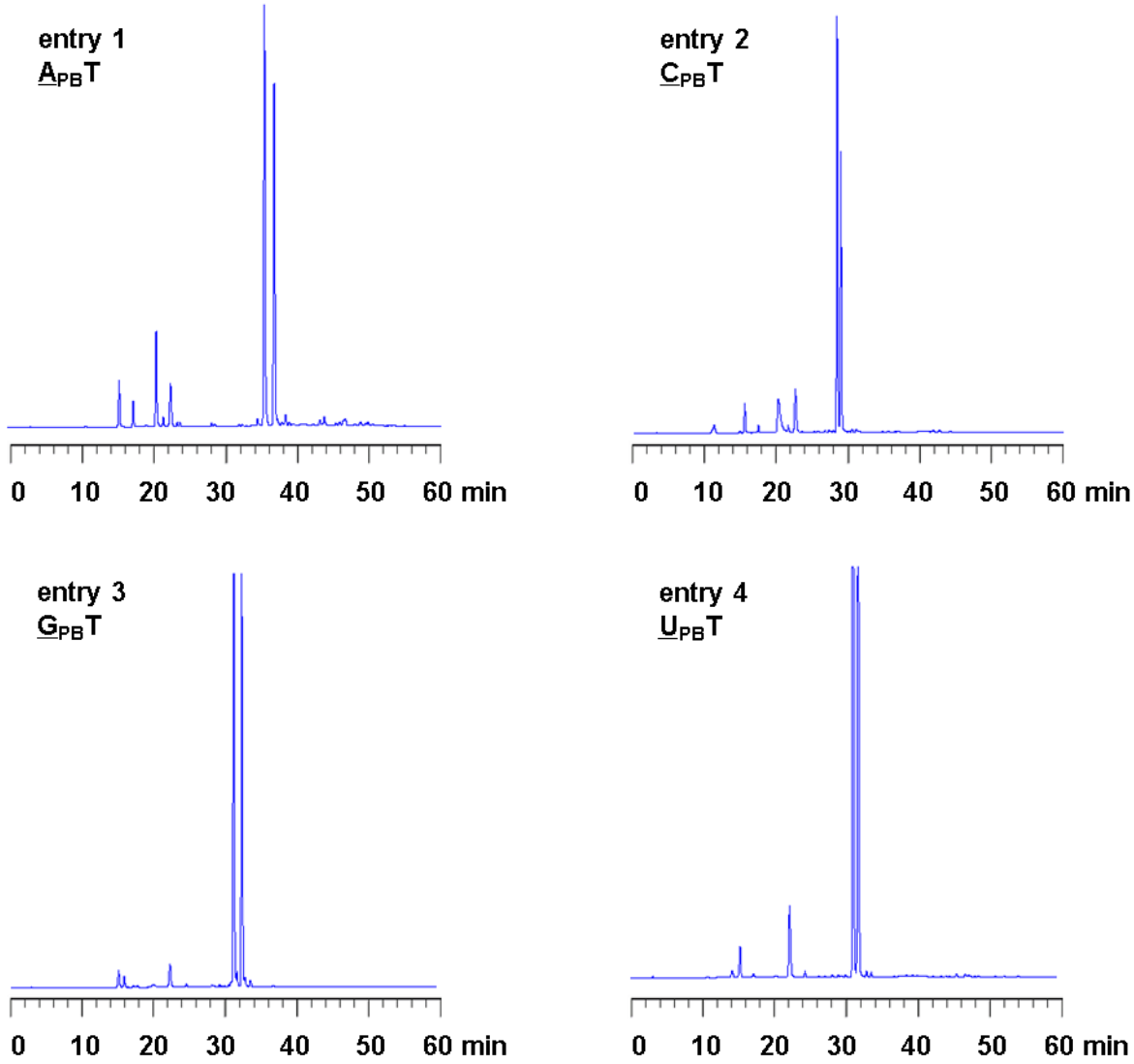


Figure S3. RP-HPLC profiles of crude $\underline{B}_{PB}T$. RP-HPLC was performed with a linear gradient of 0%–30% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a C18 column.

RP-HPLC profiles of tetramers (Table 4, entries 1 and 2)

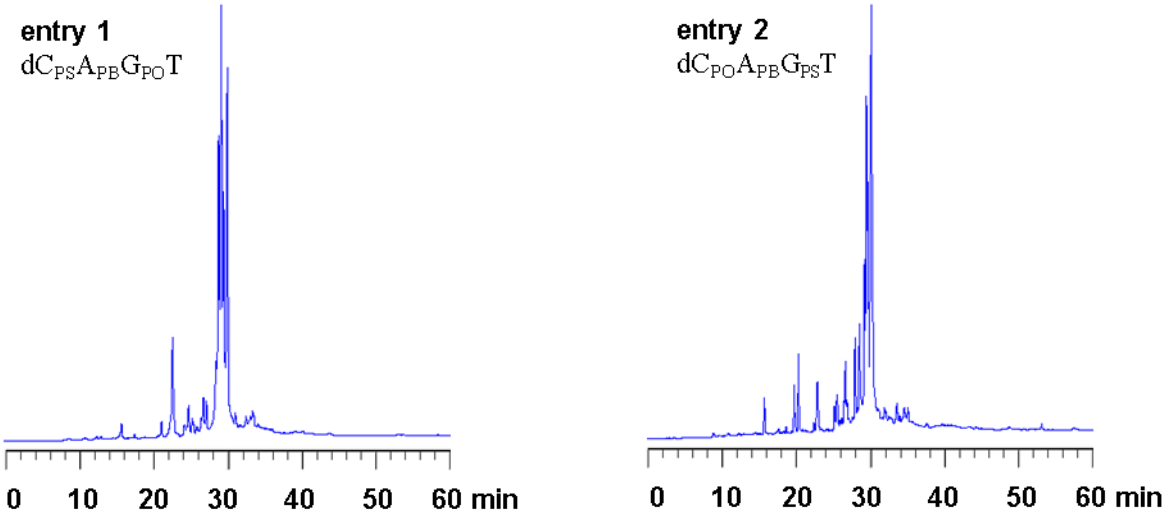


Figure S4. RP-HPLC profiles of crude tetramers ($dC_{PS}A_{PB}G_{PO}T$ and $dC_{PO}A_{PB}G_{PS}T$). RP-HPLC was performed with a linear gradient of 0%–30% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a C18 column.

HPLC profiles of dodecamers (Table 4 entry 3)

crude
$dC_{PS}A_{PS}G_{PS}T_{PS}C_{PB}A_{PB}G_{PB}T_{PB}C_{PO}A_{PO}G_{PO}T$

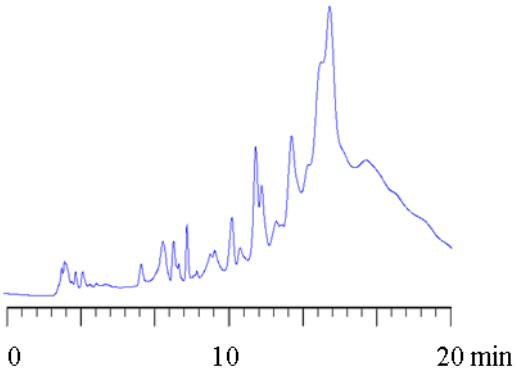


Figure S5. RP-HPLC profile of crude $dC_{PS}A_{PS}G_{PS}T_{PS}C_{PB}A_{PB}G_{PB}T_{PB}C_{PO}A_{PO}G_{PO}T$. RP-HPLC was performed with a linear gradient of 5%–40% MeOH in 100 mM HFIP, 8 mM TEA for 20 min at 60 °C at a flow rate of 0.5 mL/min using a C18 column.

pure
$dC_{PS}A_{PS}G_{PS}T_{PS}C_{PB}A_{PB}G_{PB}T_{PB}C_{PO}A_{PO}G_{PO}T$
RP-HPLC

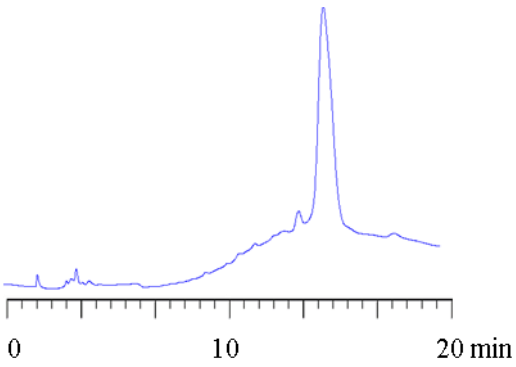


Figure S6. RP-HPLC profile of pure $dC_{PS}A_{PS}G_{PS}T_{PS}C_{PB}A_{PB}G_{PB}T_{PB}C_{PO}A_{PO}G_{PO}T$. RP-HPLC was performed with a linear gradient of 5%–40% MeOH in 100 mM HFIP, 8 mM TEA for 20 min at 60 °C at a flow rate of 0.5 mL/min using a C18 column.

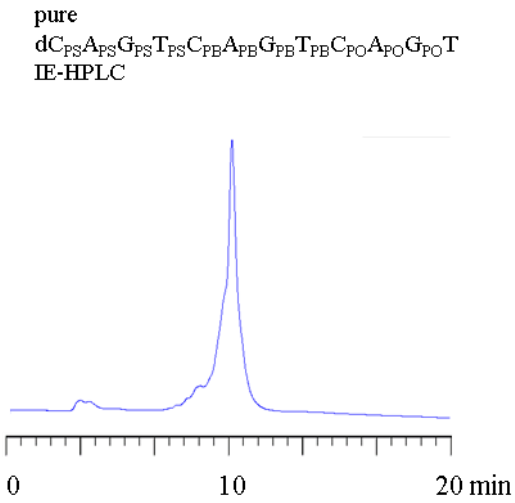


Figure S7. IE-HPLC profile of pure $dC_{PS}A_{PS}G_{PS}T_{PS}C_{PB}A_{PB}G_{PB}T_{PB}C_{PO}A_{PO}G_{PO}T$. IE-HPLC was performed with a linear gradient of 0 M–1M $NaClO_4$ in 50%–25% CH_3CN, 10 mM Tris-HCl (pH 7.5) for 20 min at 30 °C with a flow rate of 0.4 mL/min using a quaternary ammonium anion exchange resin column.

HPLC profiles of dodecamers (Table 4 entry 4)

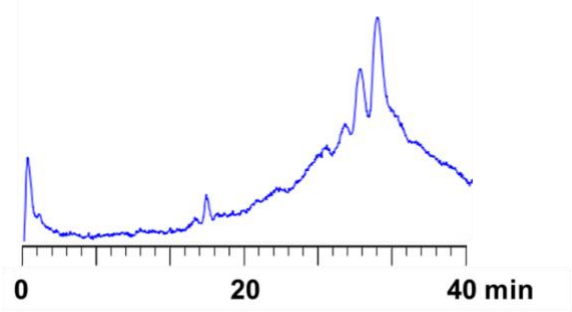


Figure S8. IE-HPLC profile of crude $dG_{PB}C_{PS}A_{PB}T_{PO}T_{PO}G_{PO}G_{PO}T_{PS}A_{PB}T_{PS}T_{PB}C$. IE-HPLC was performed with a linear gradient of 0 M–1 M NaCl in 30% *i*PrOH, 10 mM Tris-HCl (pH 7.5) for 40 min at a flow rate of 0.4 mL/min using a quaternary ammonium anion exchange resin column.

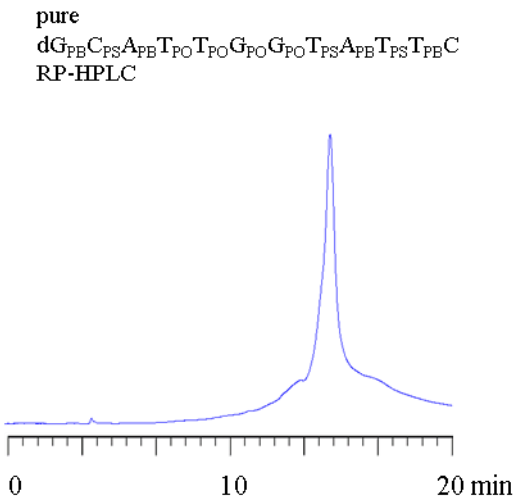


Figure S9. RP-HPLC profile of pure $dG_{PB}C_{PS}A_{PB}T_{PO}T_{PO}G_{PO}G_{PO}T_{PS}A_{PB}T_{PS}T_{PB}C$. RP-HPLC was performed with a linear gradient of 5%–40% MeOH in 100 mM HFIP, 8 mM TEA for 20 min at 60 °C at a flow rate of 0.5 mL/min using a C18 column.

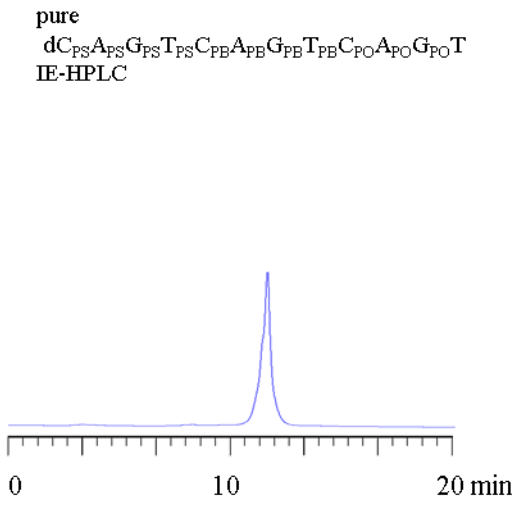


Figure S9. IE-HPLC profile of pure $dG_{PB}C_{PS}A_{PB}T_{PO}T_{PO}G_{PO}G_{PO}T_{PS}A_{PB}T_{PS}T_{PB}C$. IE-HPLC was performed with a linear gradient of 0 M–1 M $NaClO_4$ in 50%–25% CH_3CN, 10 mM Tris-HCl (pH 7.5) for 20 min at 30 °C with a flow rate of 0.4 mL/min using a quaternary ammonium anion exchange resin column.

HPLC profiles of dodecamers (Table 4 entry 5)

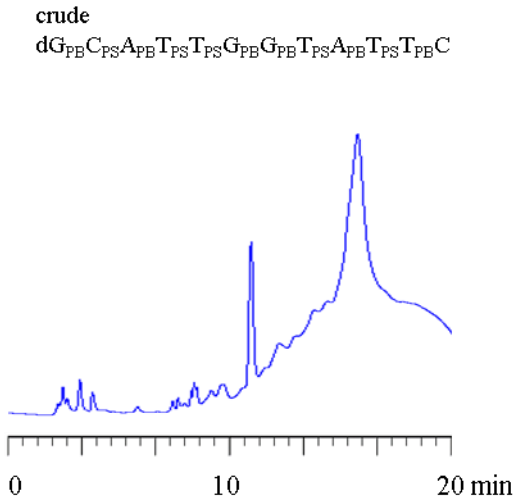


Figure S10. RP-HPLC profile of crude dG$_{PB}$C$_{PS}$A$_{PB}$T$_{PS}$T$_{PS}$G$_{PB}$G$_{PB}$T$_{PS}$A$_{PB}$T$_{PS}$T$_{PB}$C. RP-HPLC was performed with a linear gradient of 5%–40% MeOH in 100 mM HFIP, 8 mM TEA for 20 min at 60 °C at a flow rate of 0.5 mL/min using a C18 column.

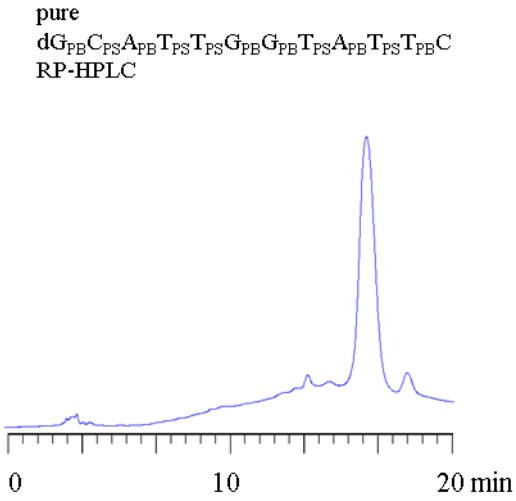


Figure S11. RP-HPLC profile of pure dG$_{PB}$C$_{PS}$A$_{PB}$T$_{PS}$T$_{PS}$G$_{PB}$G$_{PB}$T$_{PS}$A$_{PB}$T$_{PS}$T$_{PB}$C. RP-HPLC was performed with a linear gradient of 5%–40% MeOH in 100 mM HFIP, 8 mM TEA for 20 min at 60 °C at a flow rate of 0.5 mL/min using a C18 column.

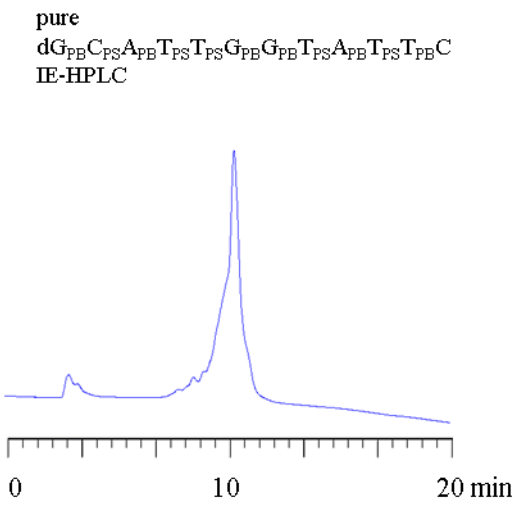


Figure S12. IE-HPLC profile of pure dG$_{PB}$C$_{PS}$A$_{PB}$T$_{PS}$T$_{PS}$G$_{PB}$G$_{PB}$T$_{PS}$A$_{PB}$T$_{PS}$T$_{PB}$C. IE-HPLC was performed with a linear gradient of 0 M–1 M $NaClO_4$ in 50%–25% CH_3CN, 10 mM Tris-HCl (pH 7.5) for 20 min at 30 °C with a flow rate of 0.4 mL/min using a quaternary ammonium anion exchange resin column.

HPLC profiles of dodecamers (Table 4 entry 6)

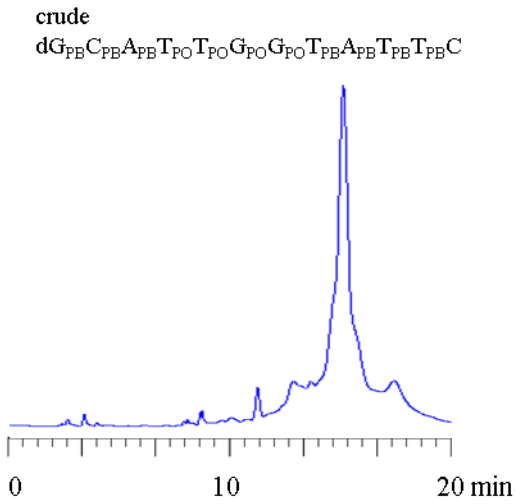


Figure S13. RP-HPLC profile of crude dG$_{PB}$C$_{PB}$A$_{PB}$T$_{PO}$T$_{PO}$G$_{PO}$G$_{PO}$T$_{PB}$A$_{PB}$T$_{PB}$T$_{PB}$C. RP-HPLC was performed with a linear gradient of 5%–40% MeOH in 100 mM HFIP, 8 mM TEA for 20 min at 60 °C at a flow rate of 0.5 mL/min using a C18 column.

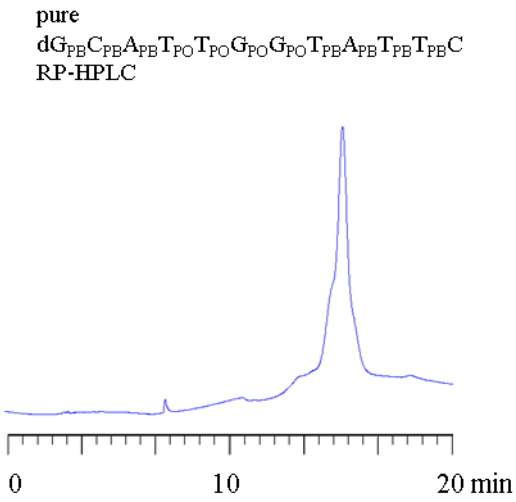


Figure S14. RP-HPLC profile of pure dG$_{PB}$C$_{PB}$A$_{PB}$T$_{PO}$T$_{PO}$G$_{PO}$G$_{PO}$T$_{PB}$A$_{PB}$T$_{PB}$T$_{PB}$C. RP-HPLC was performed with a linear gradient of 5%–40% MeOH in 100 mM HFIP, 8 mM TEA for 20 min at 60 °C at a flow rate of 0.5 mL/min using a C18 column.

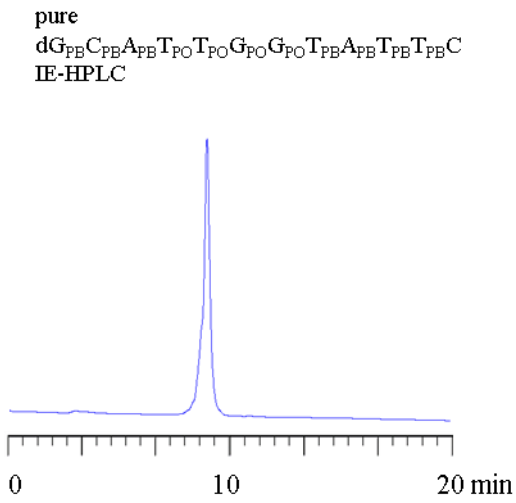


Figure S15. IE-HPLC profile of pure dG$_{PB}$C$_{PB}$A$_{PB}$T$_{PO}$T$_{PO}$G$_{PO}$G$_{PO}$T$_{PB}$A$_{PB}$T$_{PB}$T$_{PB}$C. IE-HPLC was performed with a linear gradient of 0 M–1 M $NaClO_4$ in 50%–25% CH_3CN, 10 mM Tris-HCl (pH 7.5) for 20 min at 30 °C with a flow rate of 0.4 mL/min using a quaternary ammonium anion exchange resin column.

HPLC profiles of dodecamers (Table 4 entry 7)

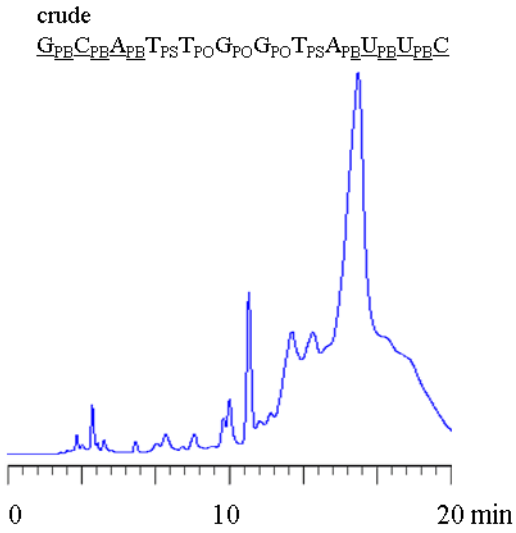


Figure S16. RP-HPLC profile of crude GPBCPBAPBTPSTPOGPOGPOTPSAPBUPBUPBC. RP-HPLC was performed with a linear gradient of 5%–40% MeOH in 100 mM HFIP, 8 mM TEA for 20 min at 60 °C at a flow rate of 0.5 mL/min using a C18 column.

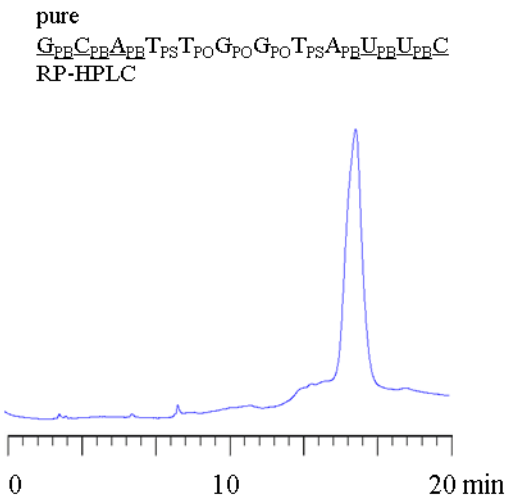


Figure S17. RP-HPLC profile of pure GPBCPBAPBTPSTPOGPOGPOTPSAPBUPBUPBC. RP-HPLC was performed with a linear gradient of 5%–40% MeOH in 100 mM HFIP, 8 mM TEA for 20 min at 60 °C at a flow rate of 0.5 mL/min using a C18 column.

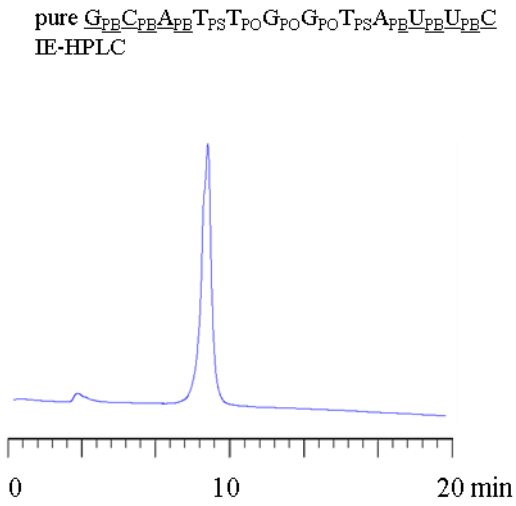


Figure S18. IE-HPLC profile of pure $G_{PB}C_{PB}A_{PB}T_{PS}T_{PO}G_{PO}G_{PO}T_{PS}A_{PB}U_{PB}U_{PB}C$. IE-HPLC was performed with a linear gradient of 0 M–1 M $NaClO_4$ in 50%–25% CH_3CN, 10 mM Tris-HCl (pH 7.5) for 20 min at 30 °C with a flow rate of 0.4 mL/min using a quaternary ammonium anion exchange resin column.

Thermal denaturation test

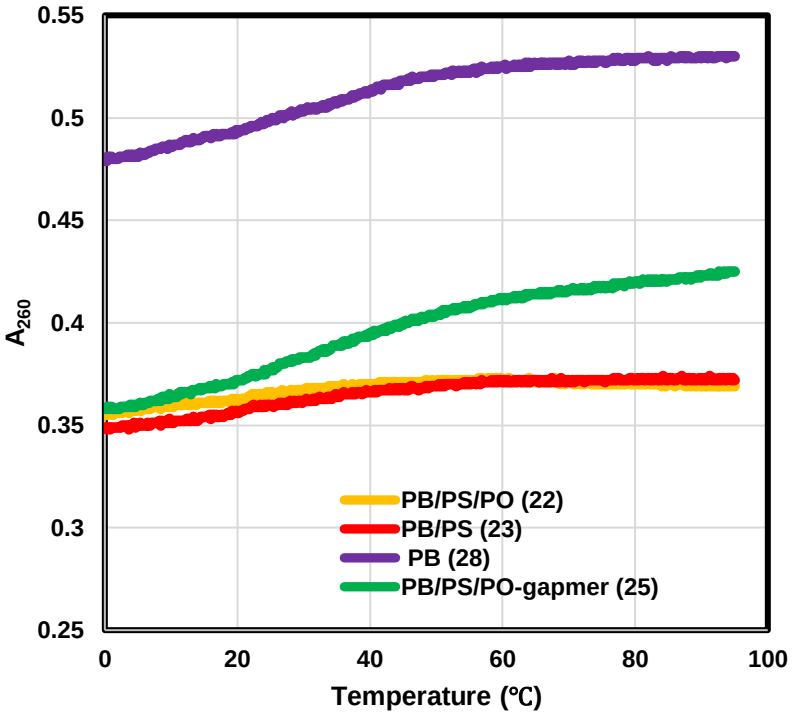


Figure S19. UV Melting curves of single stranded ODNs **22**, **23**, **25**, and **28**.

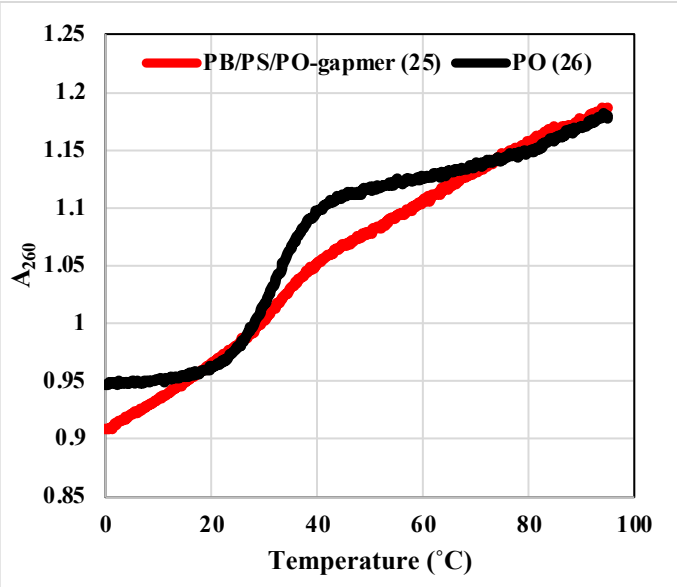
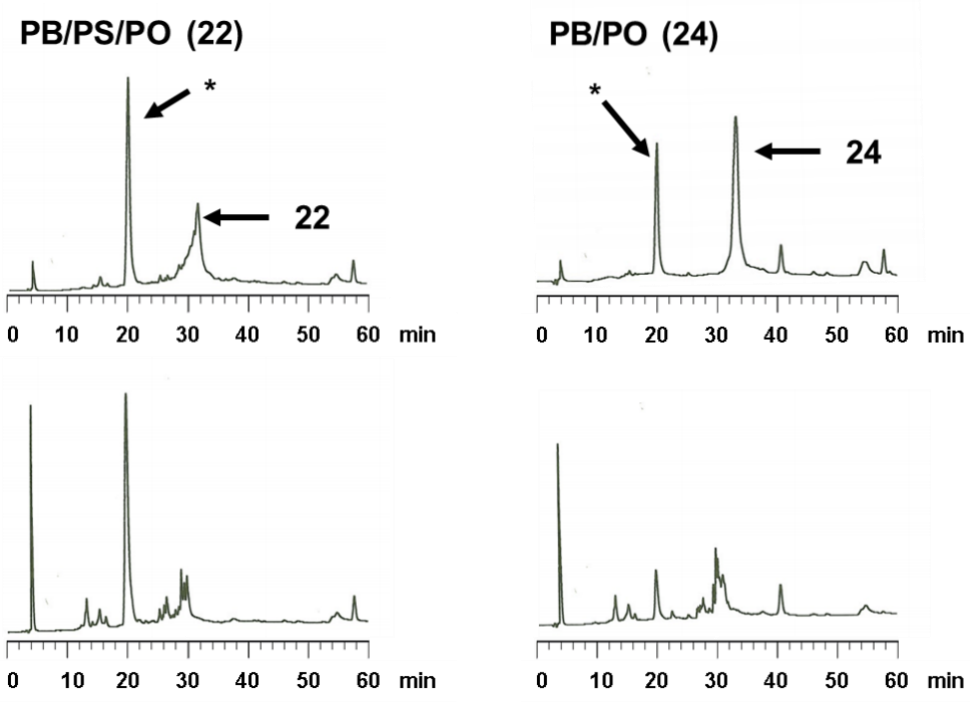
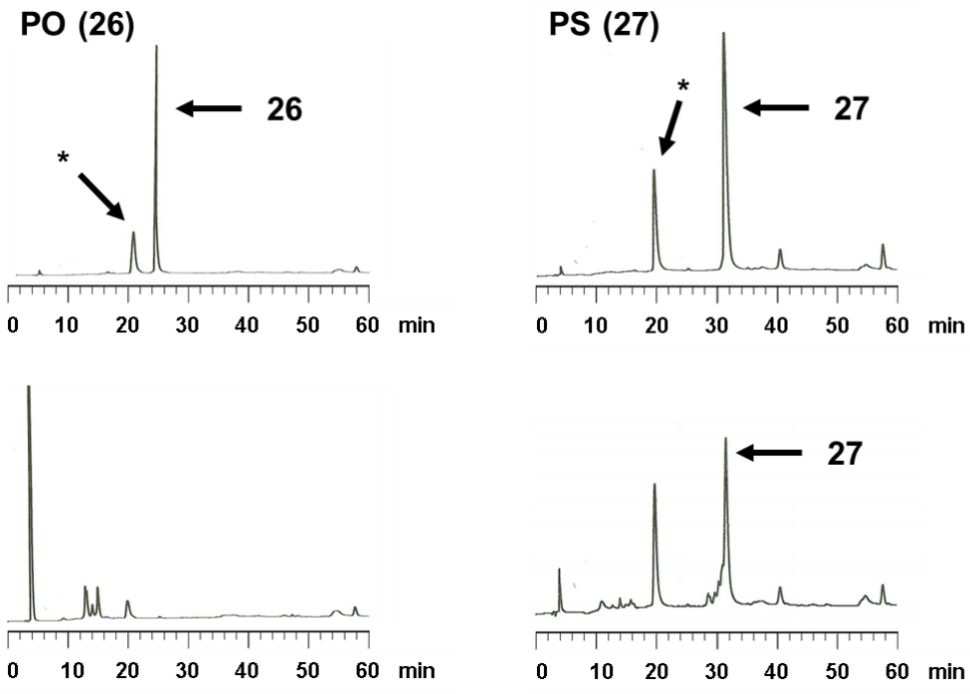
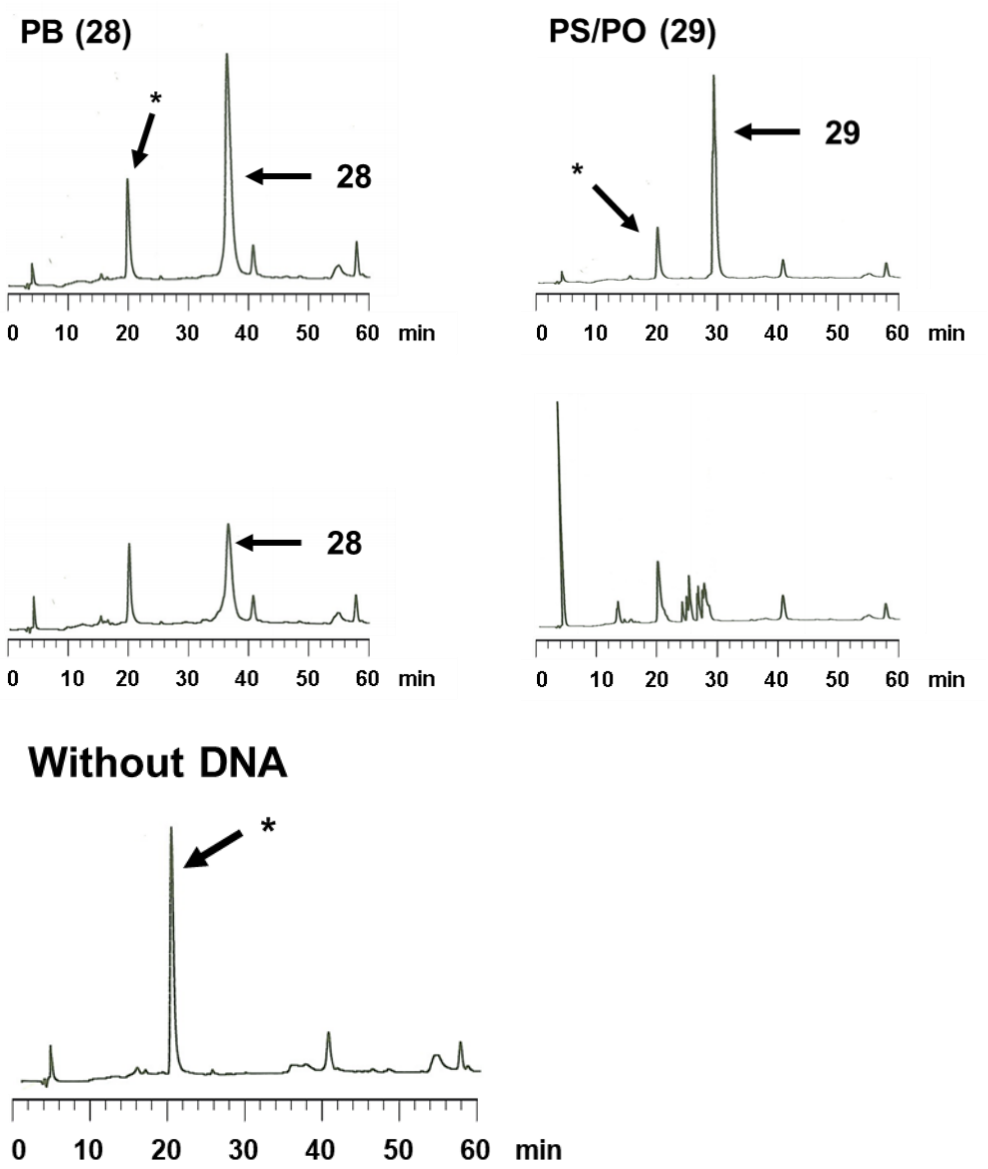


Figure S20. UV Melting curves of double stranded ODNs **25** and **26** with cRNA with a mismatched base.

Nuclease resistance



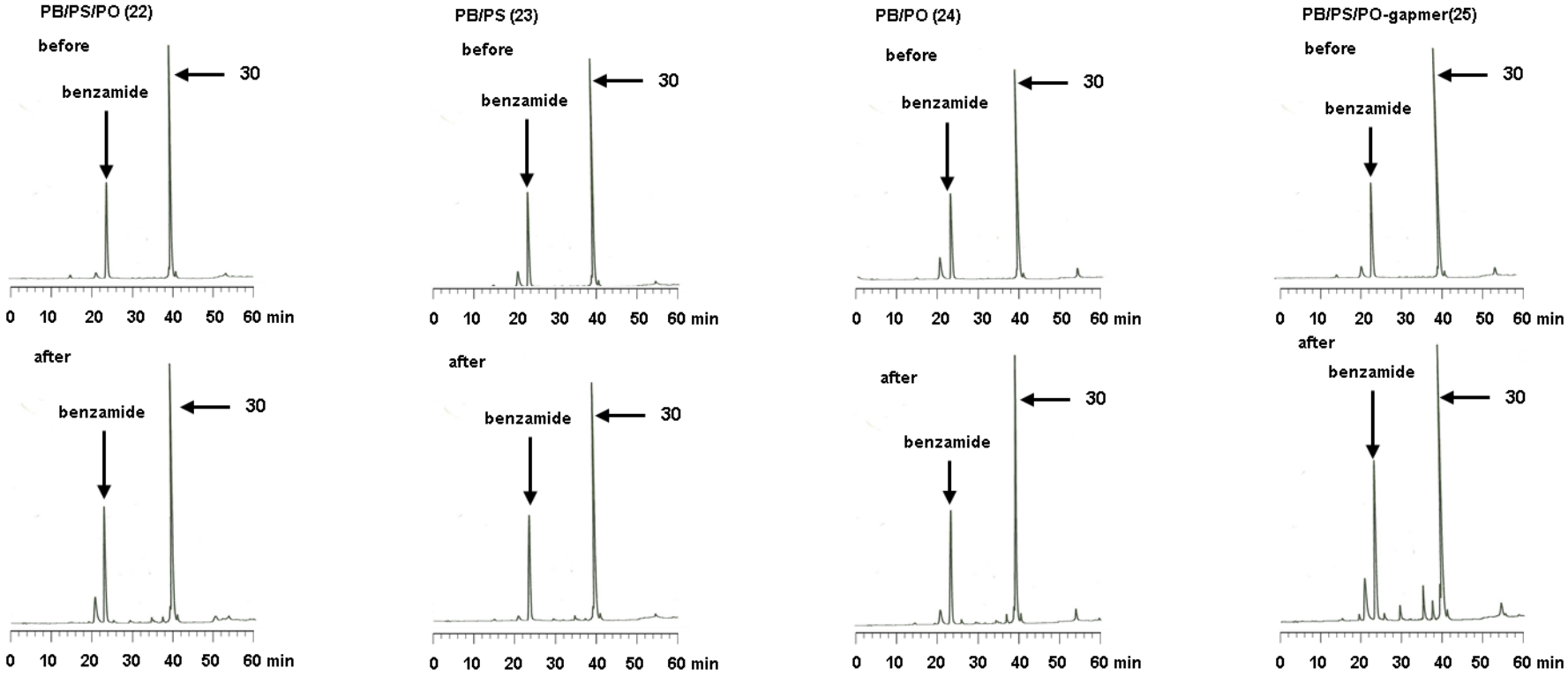




* indicates an artifact peak

Figure S21. RP-HPLC profiles of ODN before (upper) and after (lower) the treatment with snake venom phosphodiesterase (SVPDE) for 12 h at 37°C. RP-HPLC was performed with a linear gradient of 0%–40% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 ℃ at a flow rate of 0.5 mL/min using a C18 column

RNase H activity (25 U/mL)



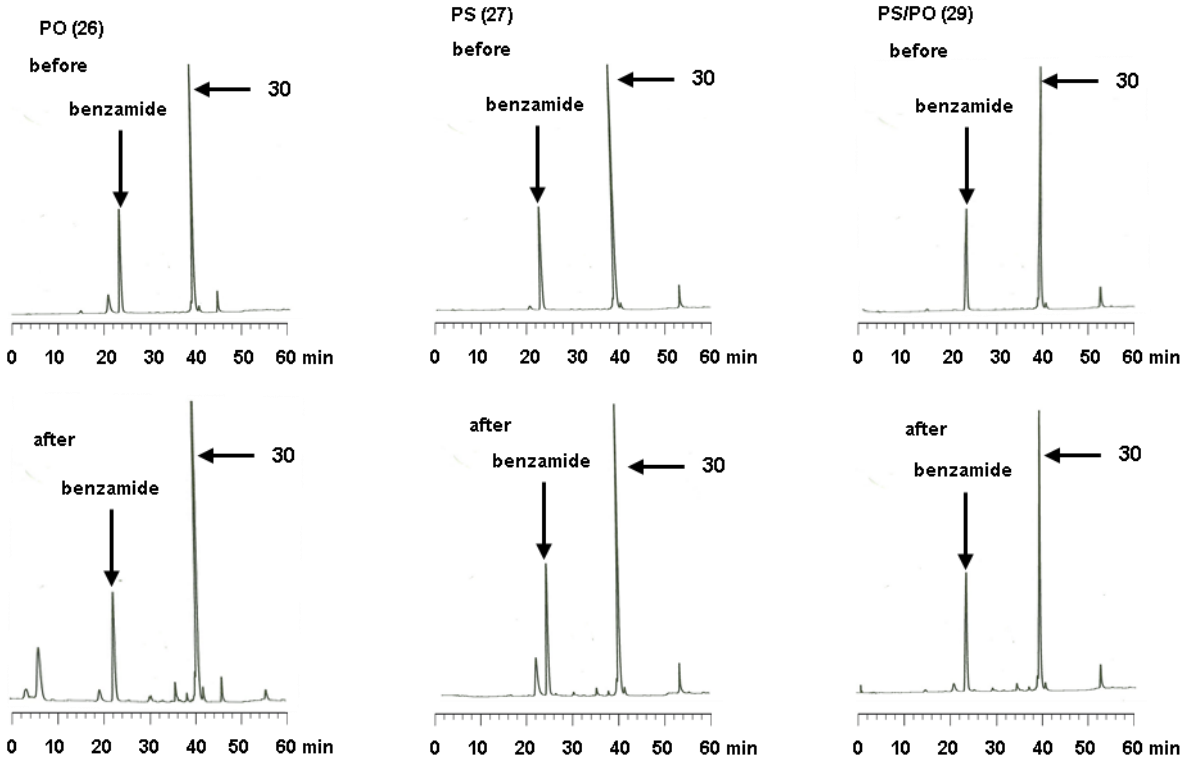
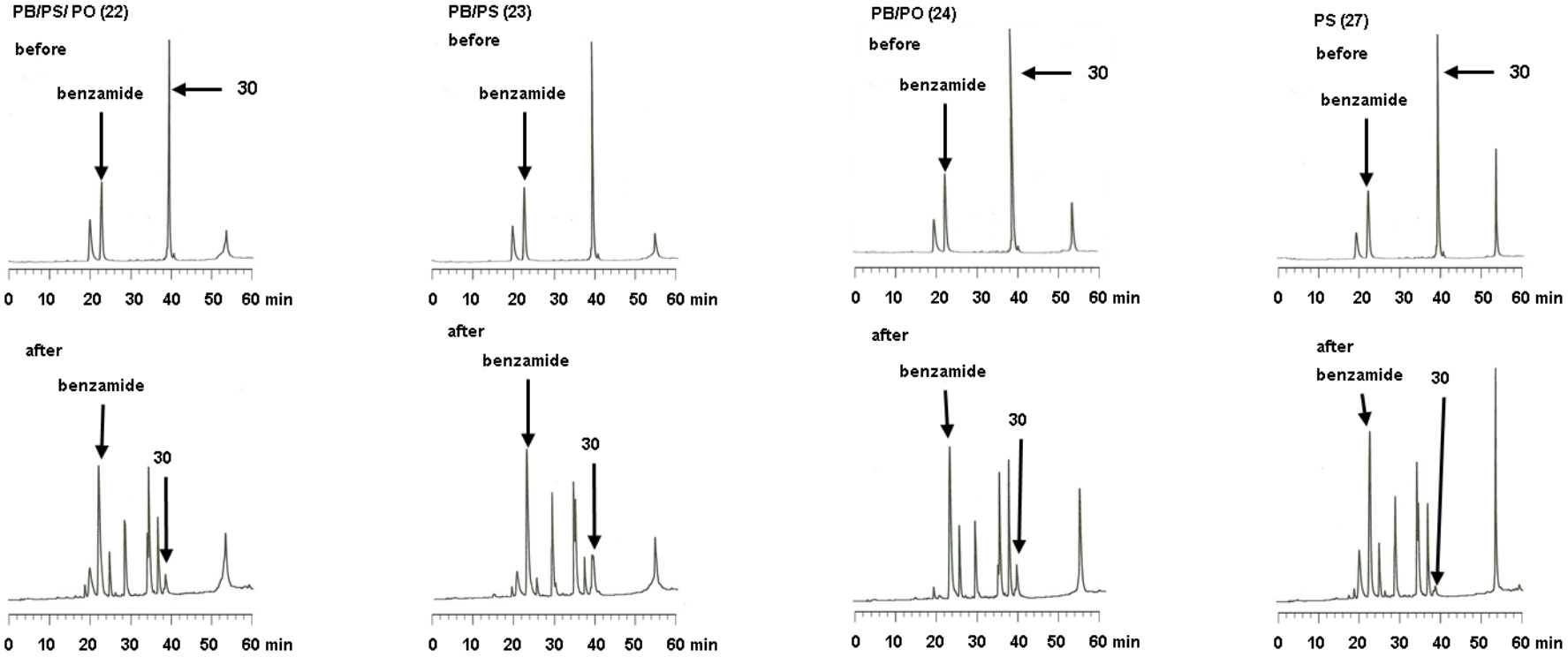


Figure S22. RP-HPLC profiles of ODN/cRNA duplexes before (upper) and after (lower) the treatment with 25 U/mL RNase H for 30 min at 37 °C RP-HPLC was performed with a linear gradient of 0%−11% MeCN over 44 min followed by 11−40% over 16 min in 0.1 M TEAA buffer (pH 7.0) at 50 °C with a flow rate of 0.5 mL/min.

RNase H activity (50 U/mL)



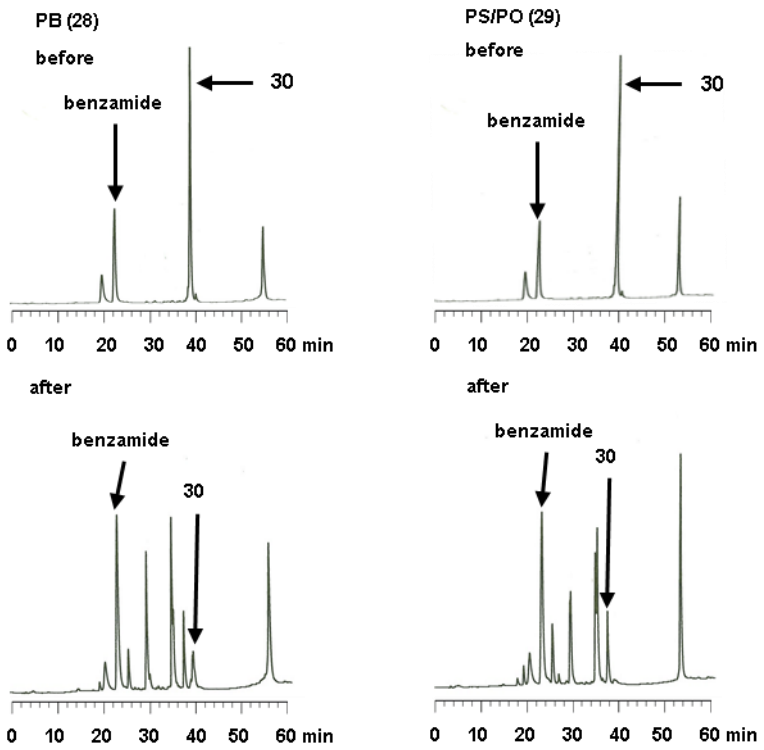
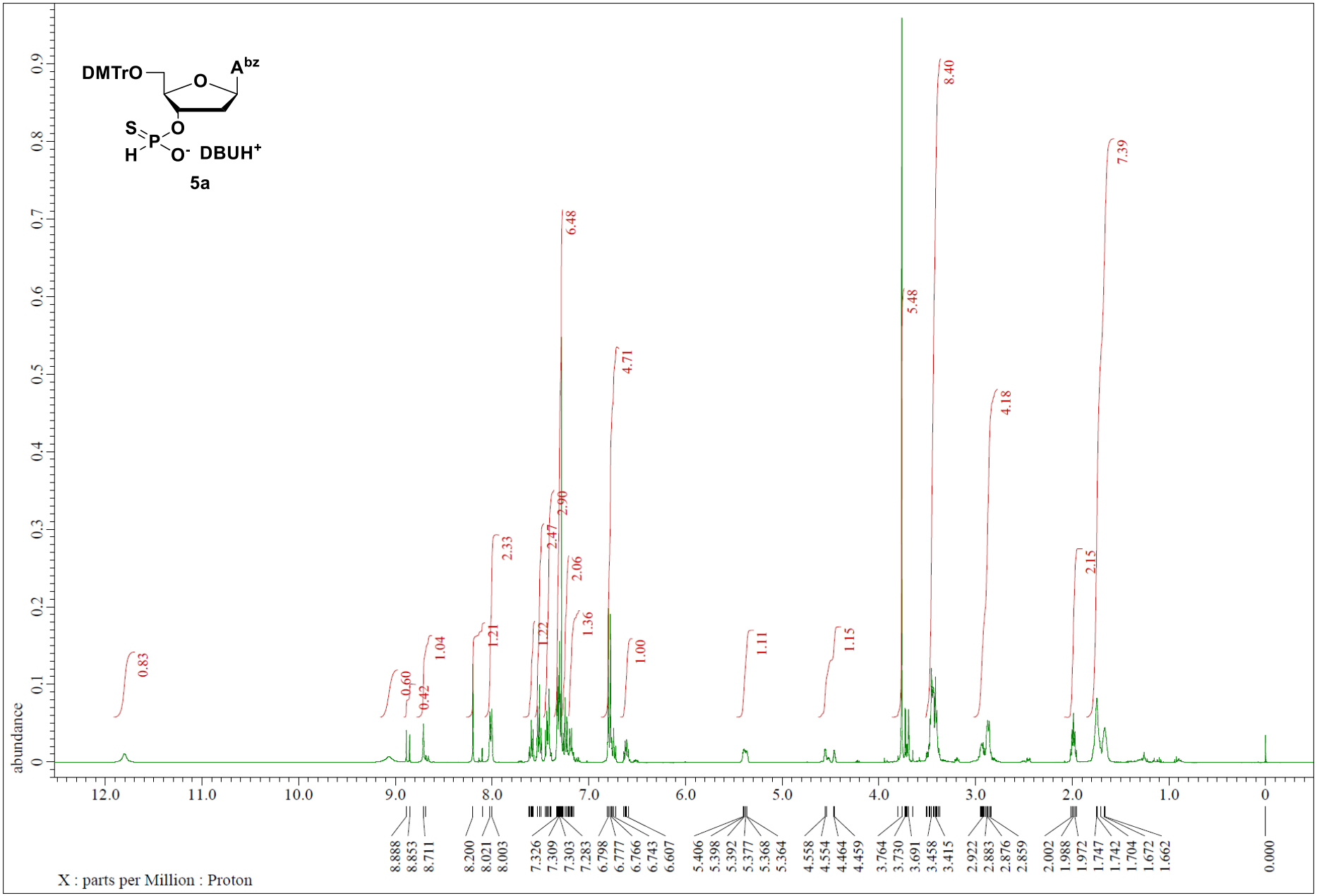


Figure S23. RP-HPLC profiles of ODN/cRNA duplexes before (upper) and after (lower) the treatment with 50 U/mL RNase H for 30 min at 37 °C RP-HPLC was performed with a linear gradient of 0%−11% MeCN over 44 min followed by 11%−40% over 16 min in 0.1 M TEAA buffer (pH 7.0) at 50 °C with a flow rate of 0.5 mL/min.

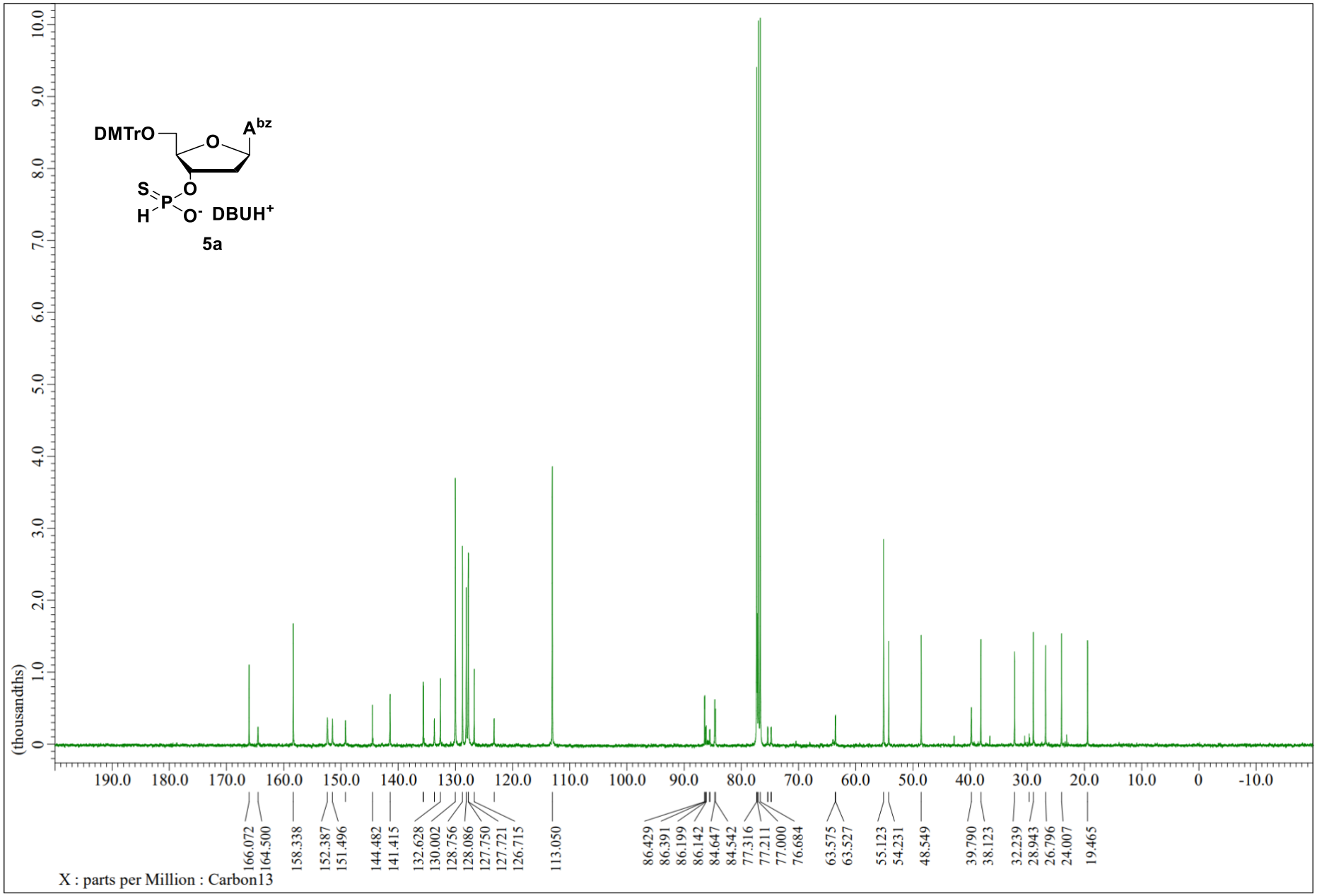
^{1}H, ^{13}C, ^{31}P NMR spectra

NMR spectra

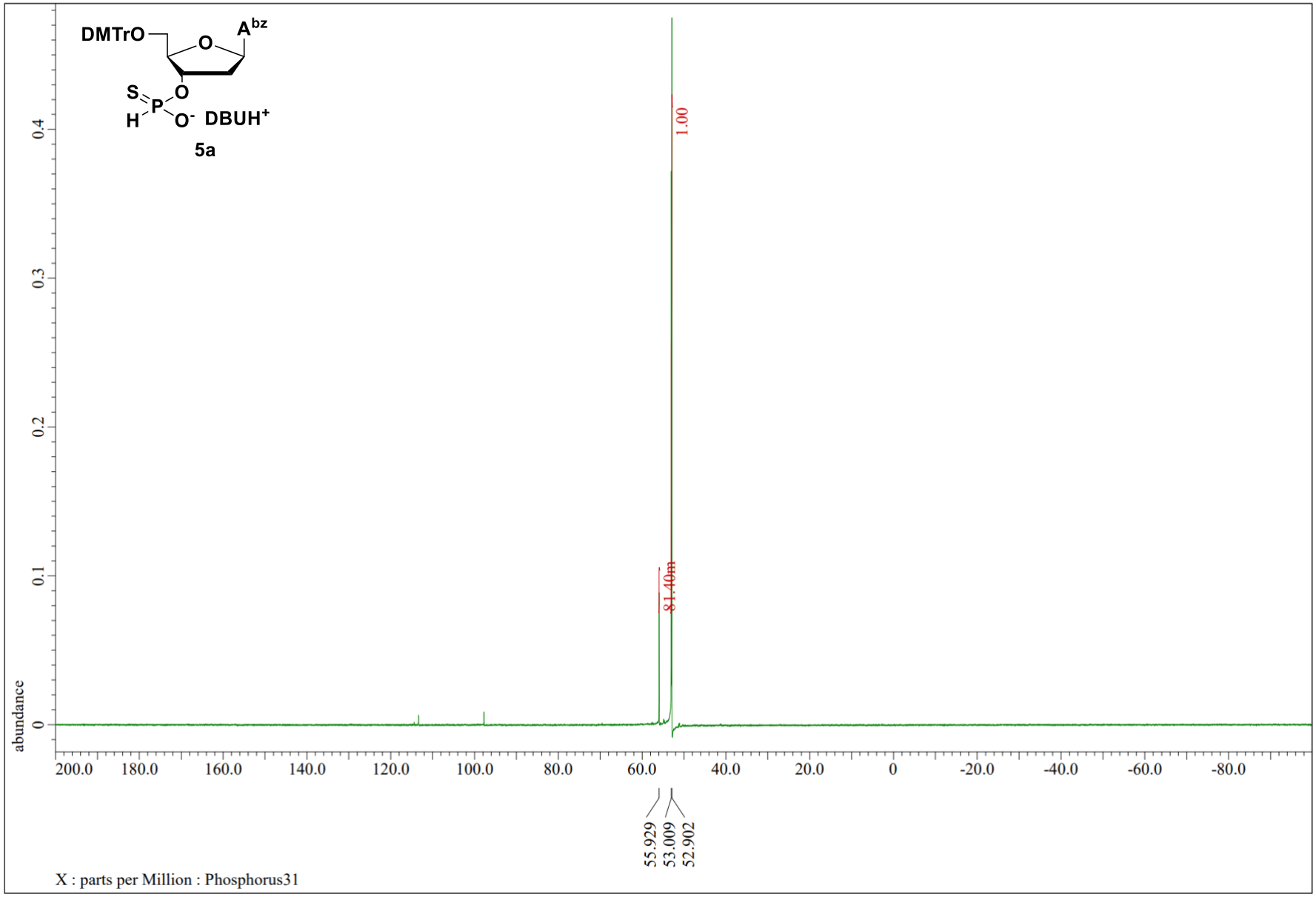
^{1}H-NMR (400 MHz, $CDCl_3$)



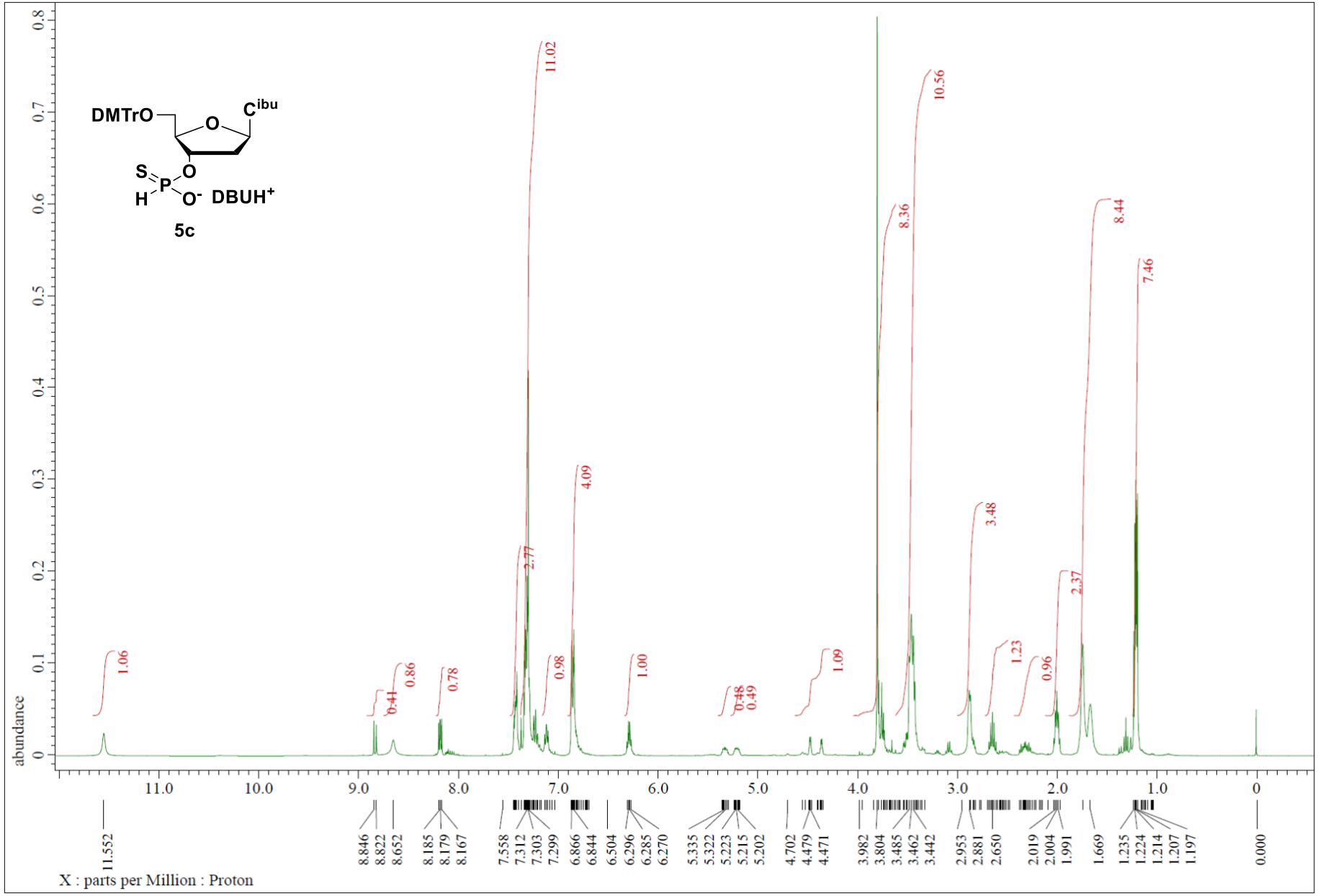
$^{13}C\{H\}$ NMR (100 MHz, $CDCl_3$)



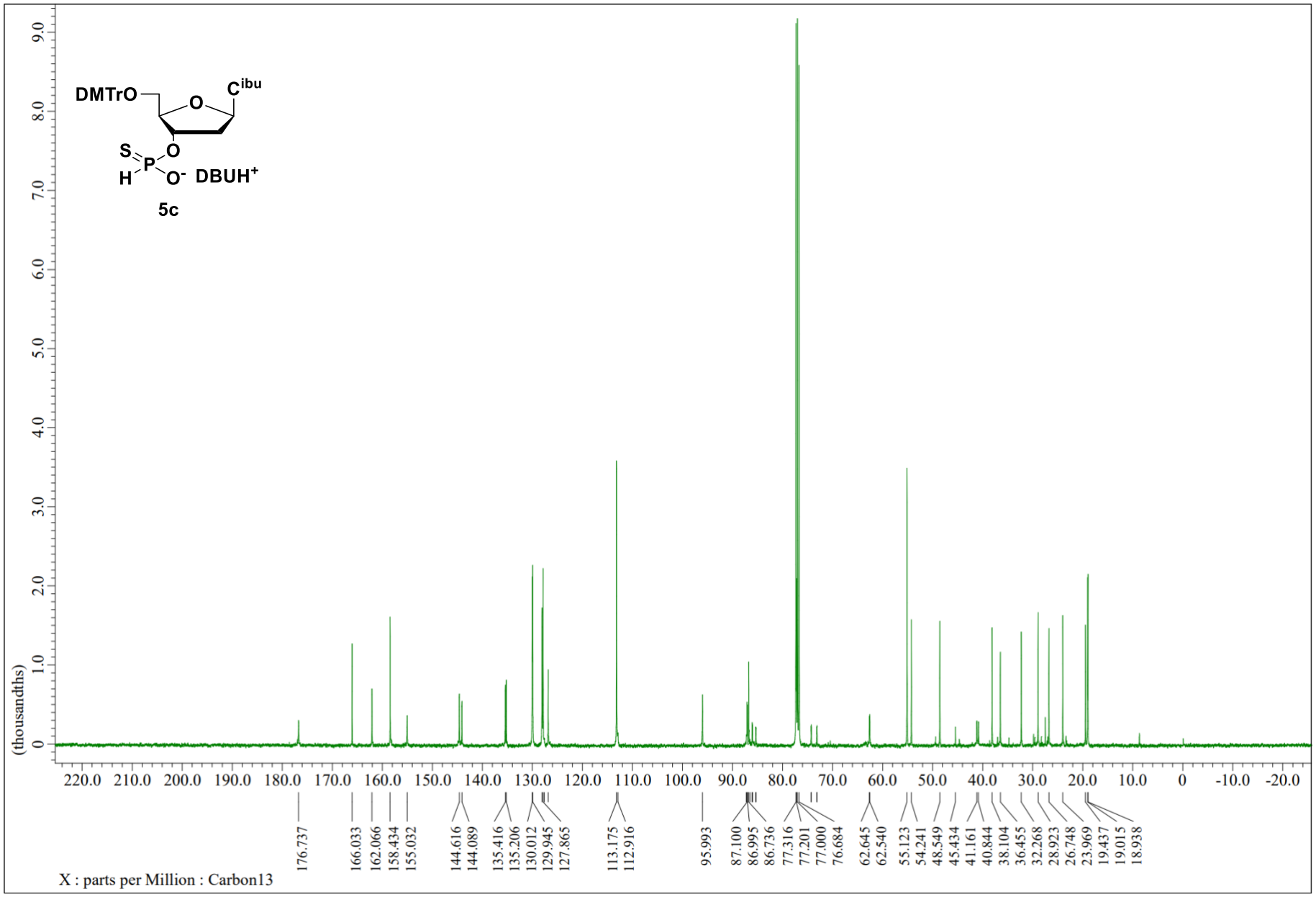
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



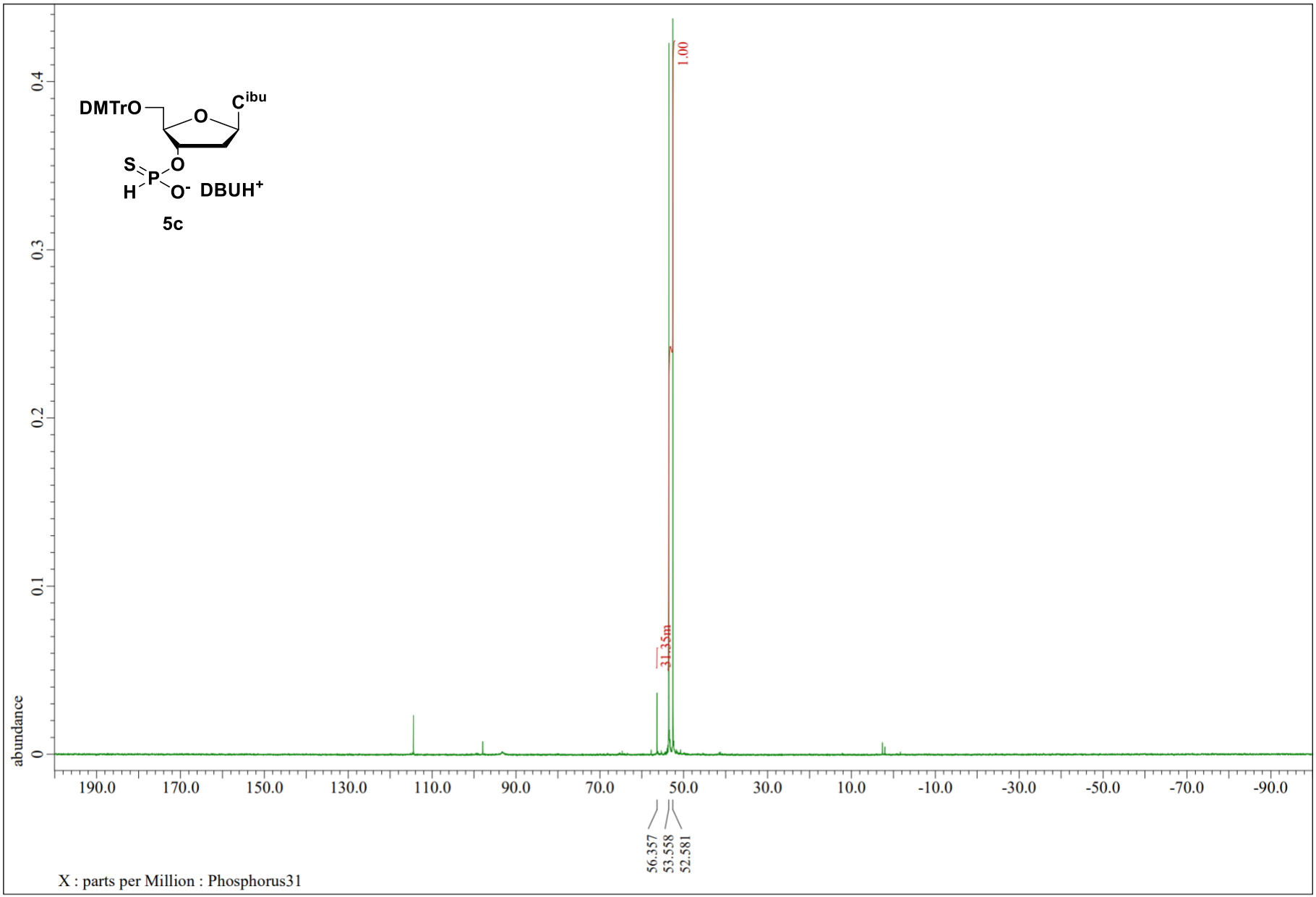
^{1}H-NMR (400 MHz, $CDCl_3$)



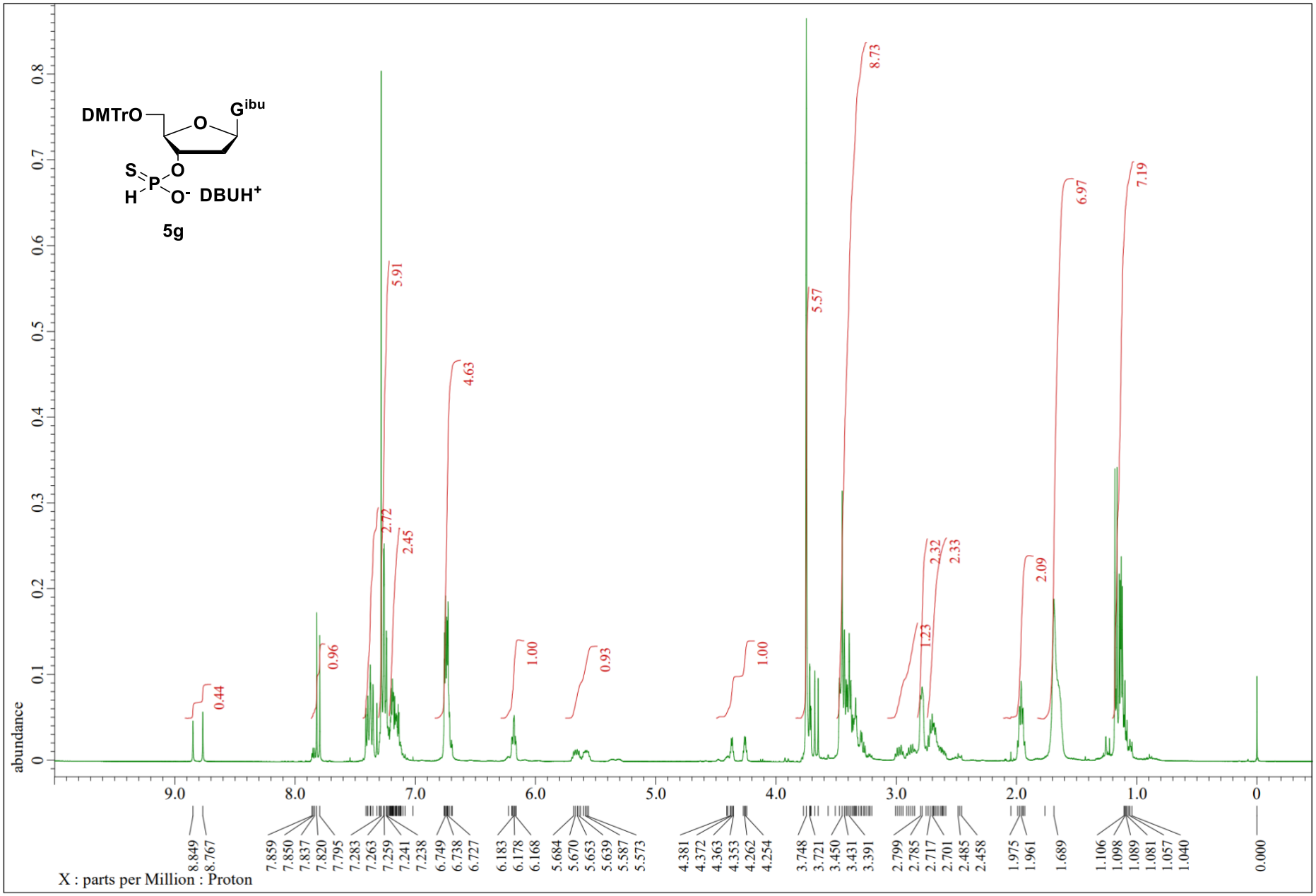
$^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$)



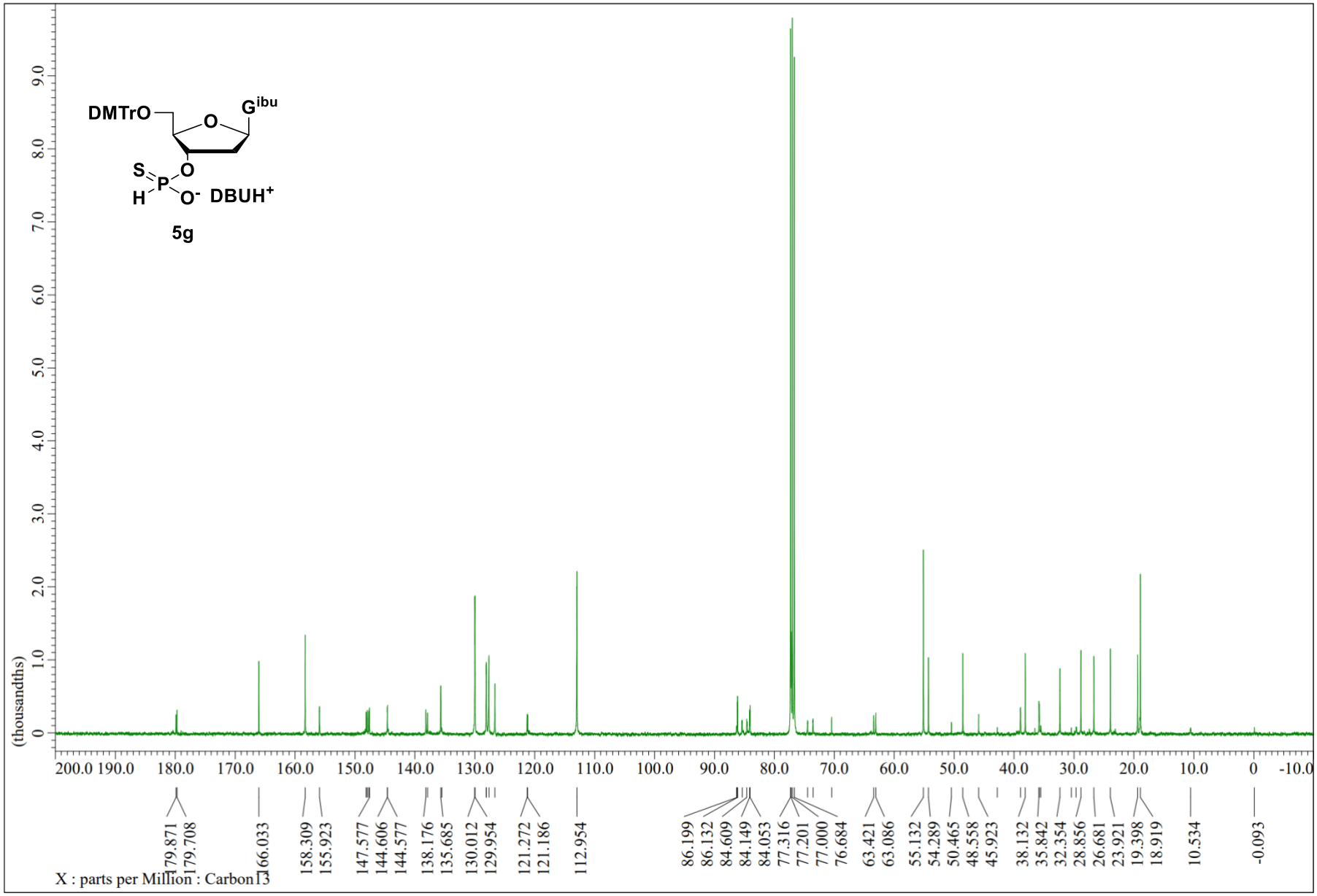
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



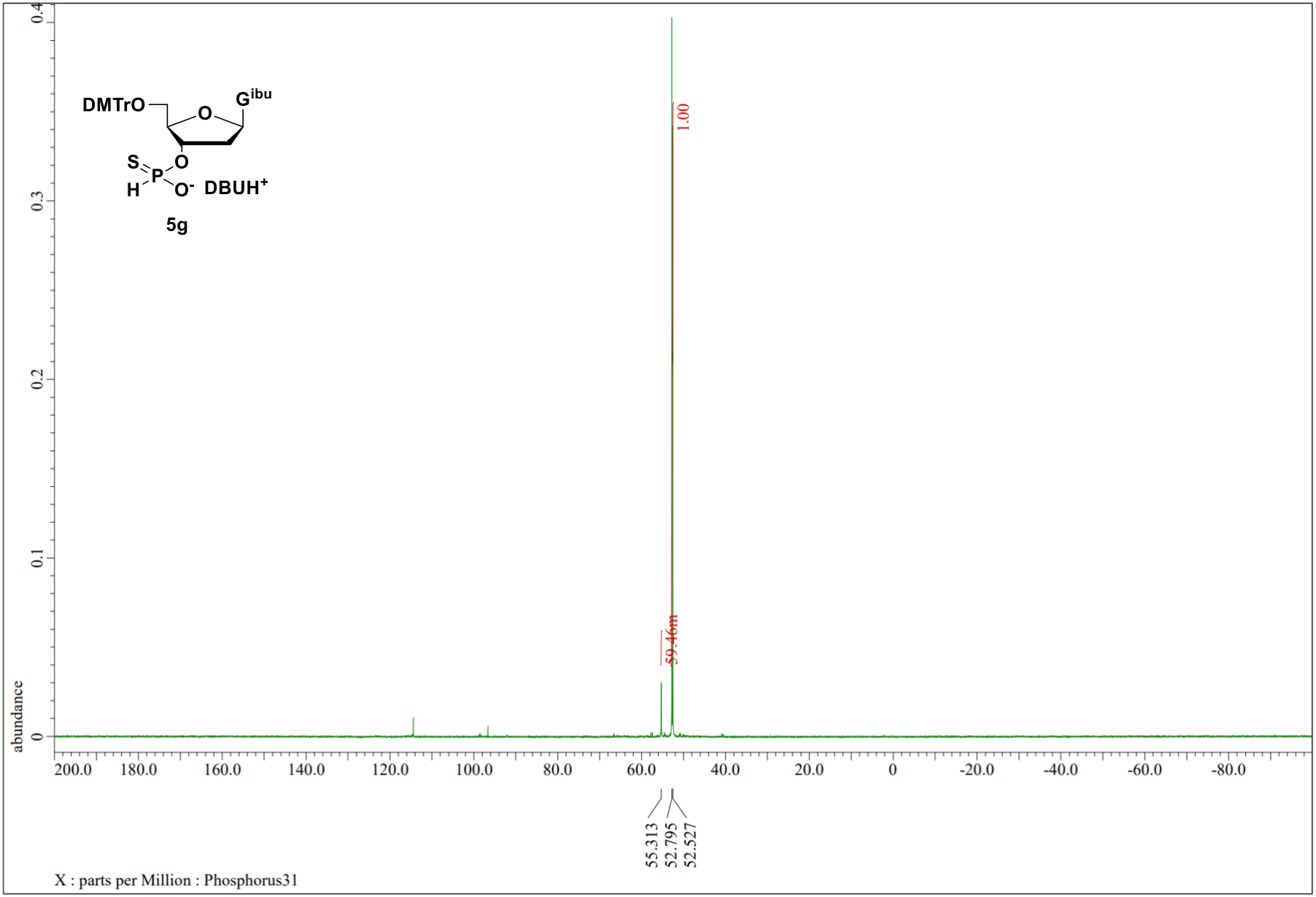
^{1}H-NMR (400 MHz, $CDCl_3$)



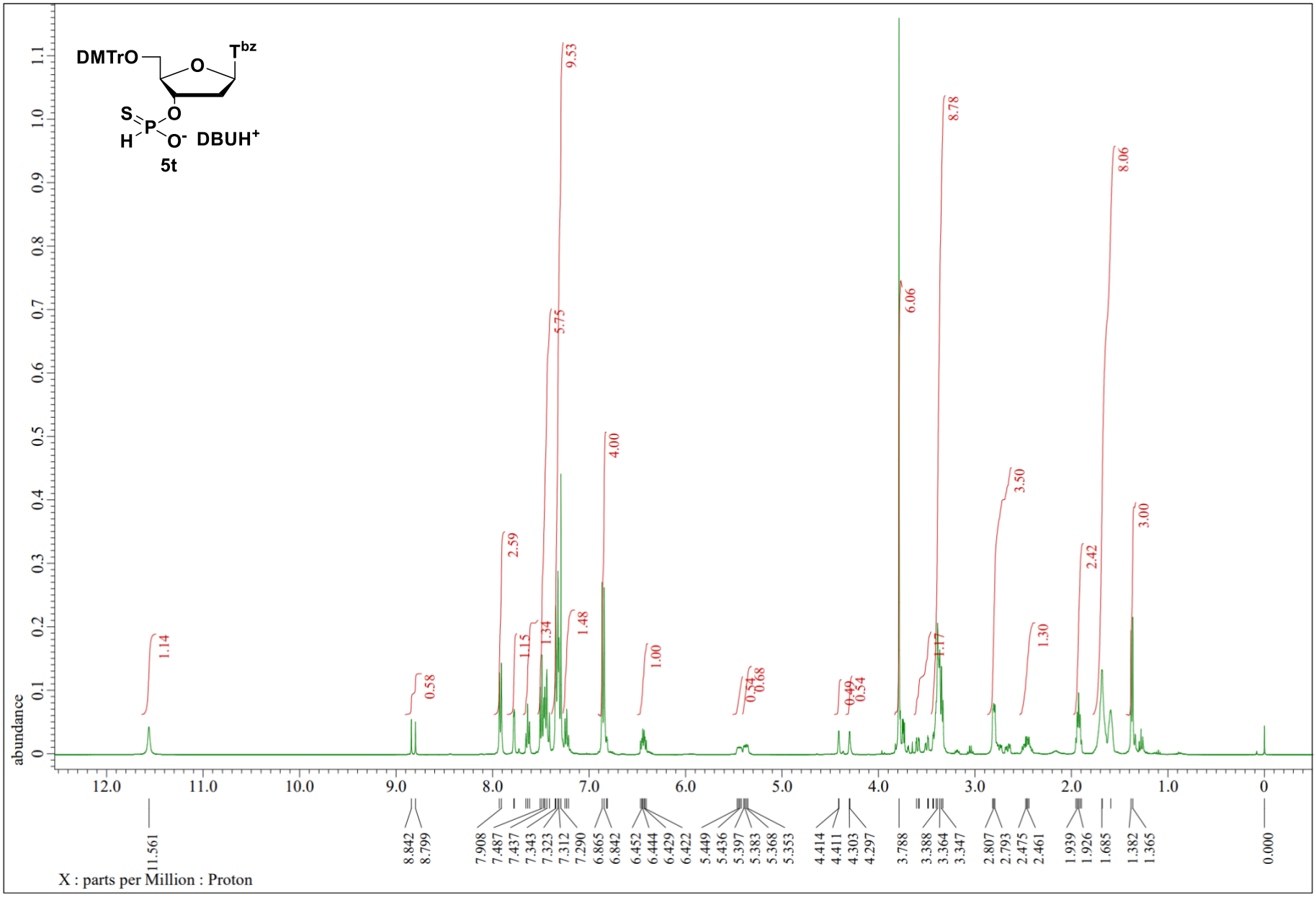
$^{13}C\{H\}$ NMR (100 MHz, $CDCl_3$)



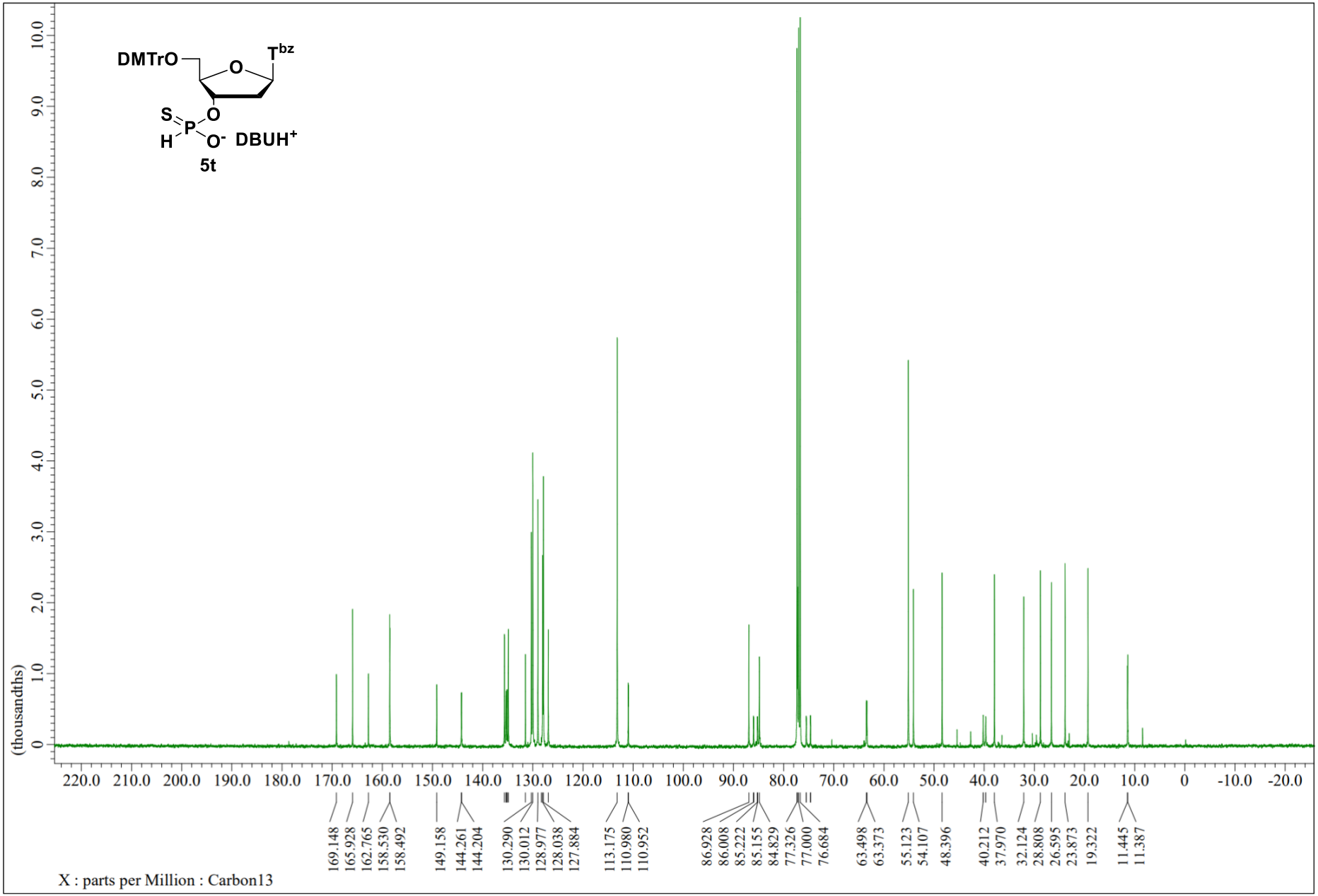
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



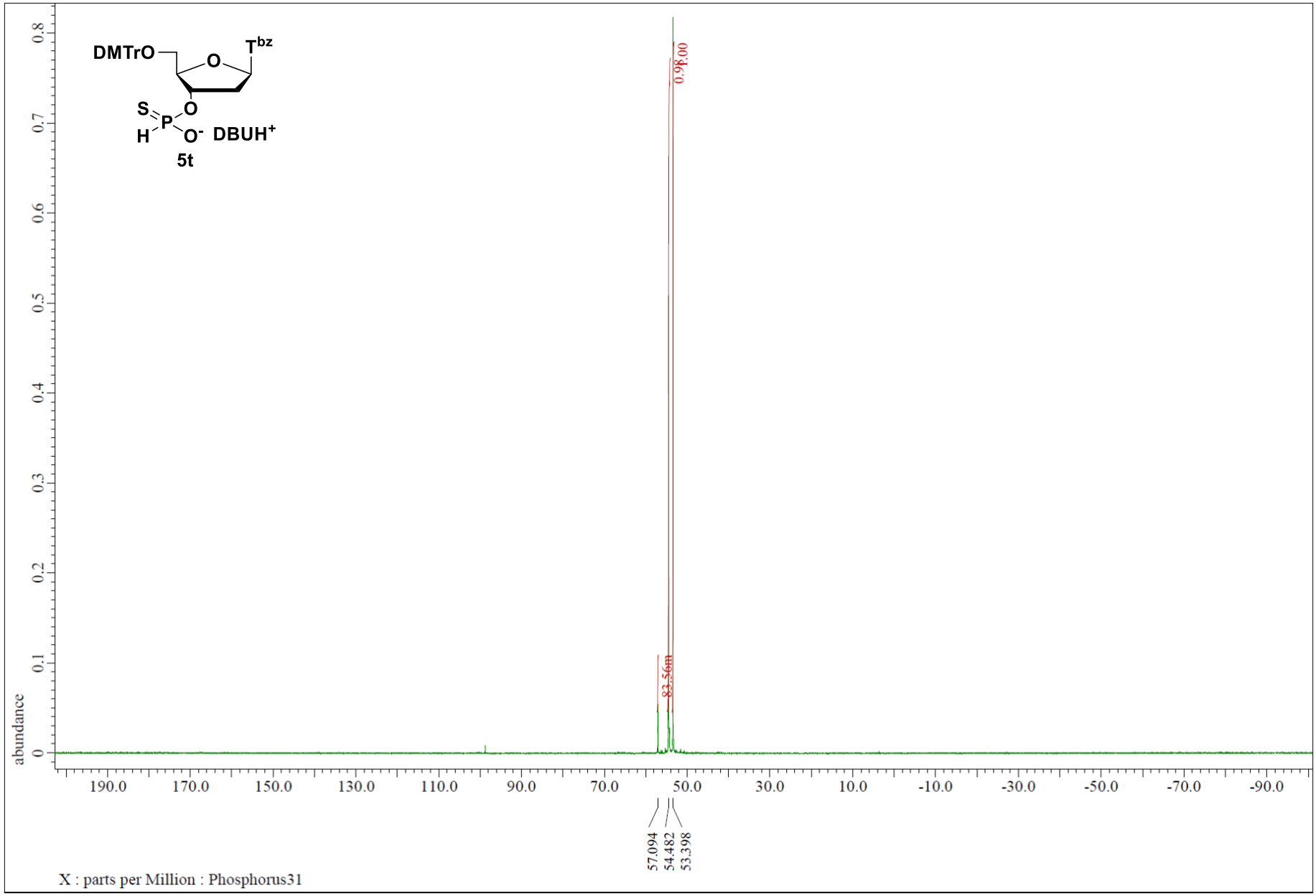
^{1}H-NMR (400 MHz, $CDCl_3$)



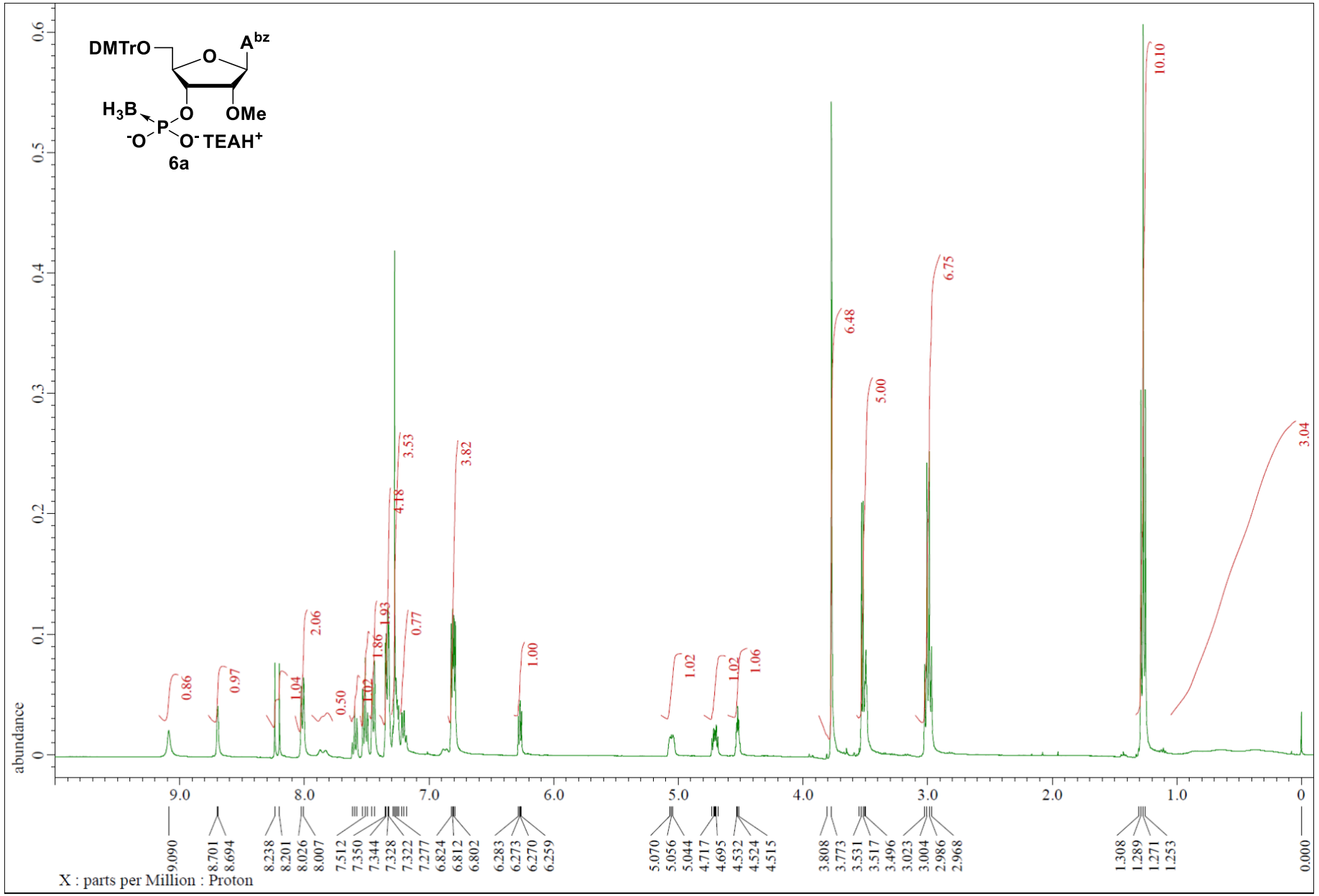
$^{13}C\{H\}$ NMR (100 MHz, $CDCl_3$)



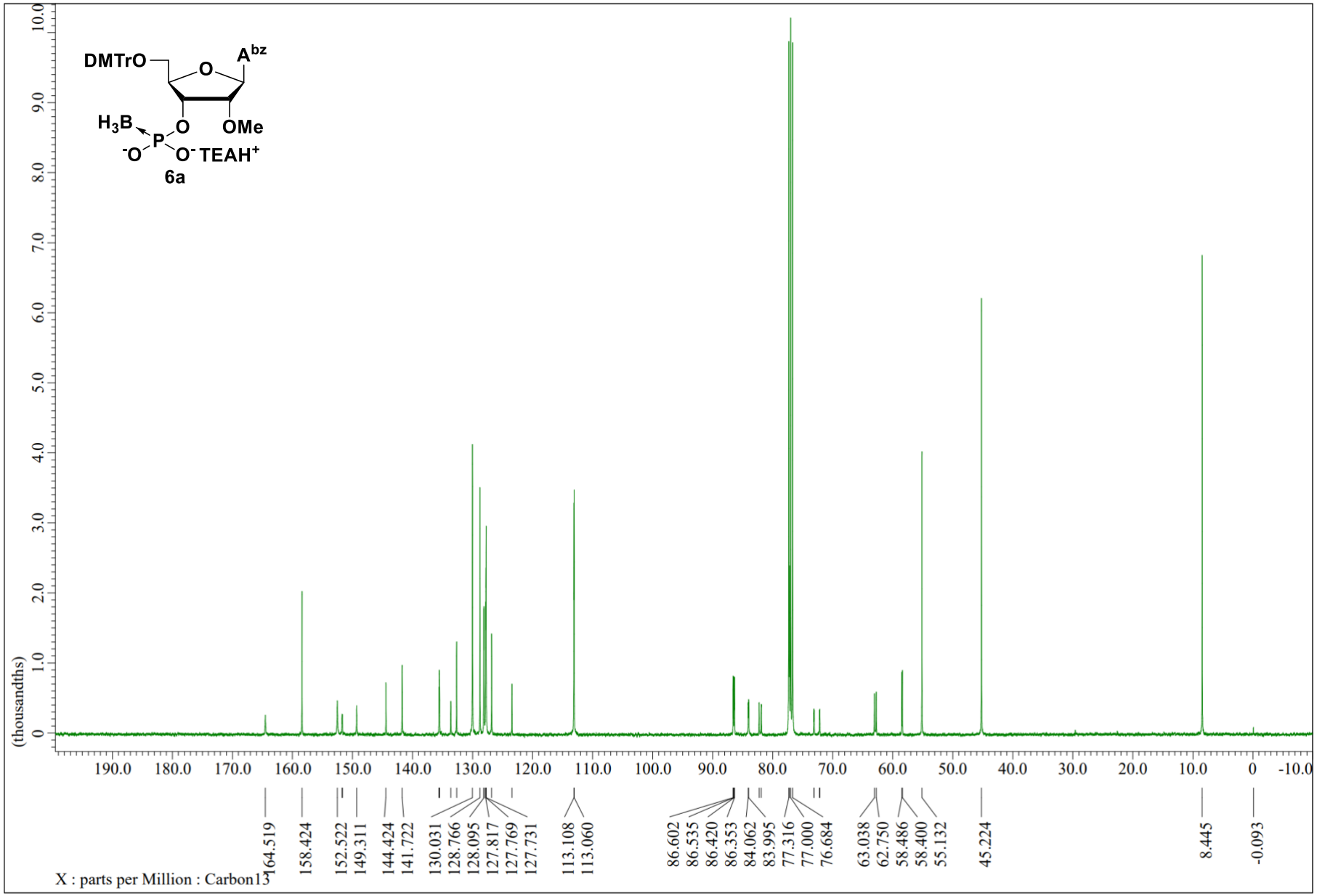
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



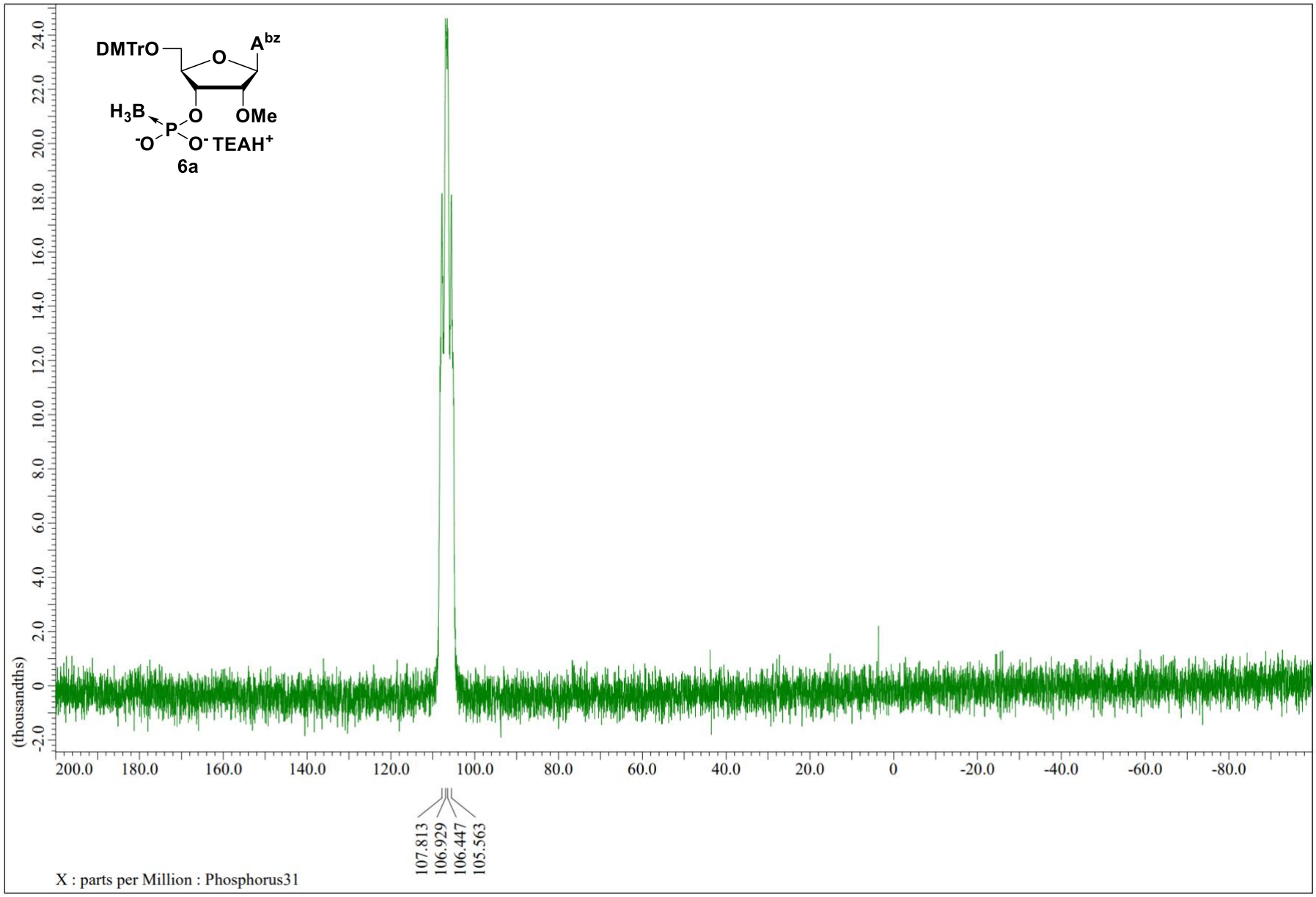
^{1}H-NMR (400 MHz, $CDCl_3$)



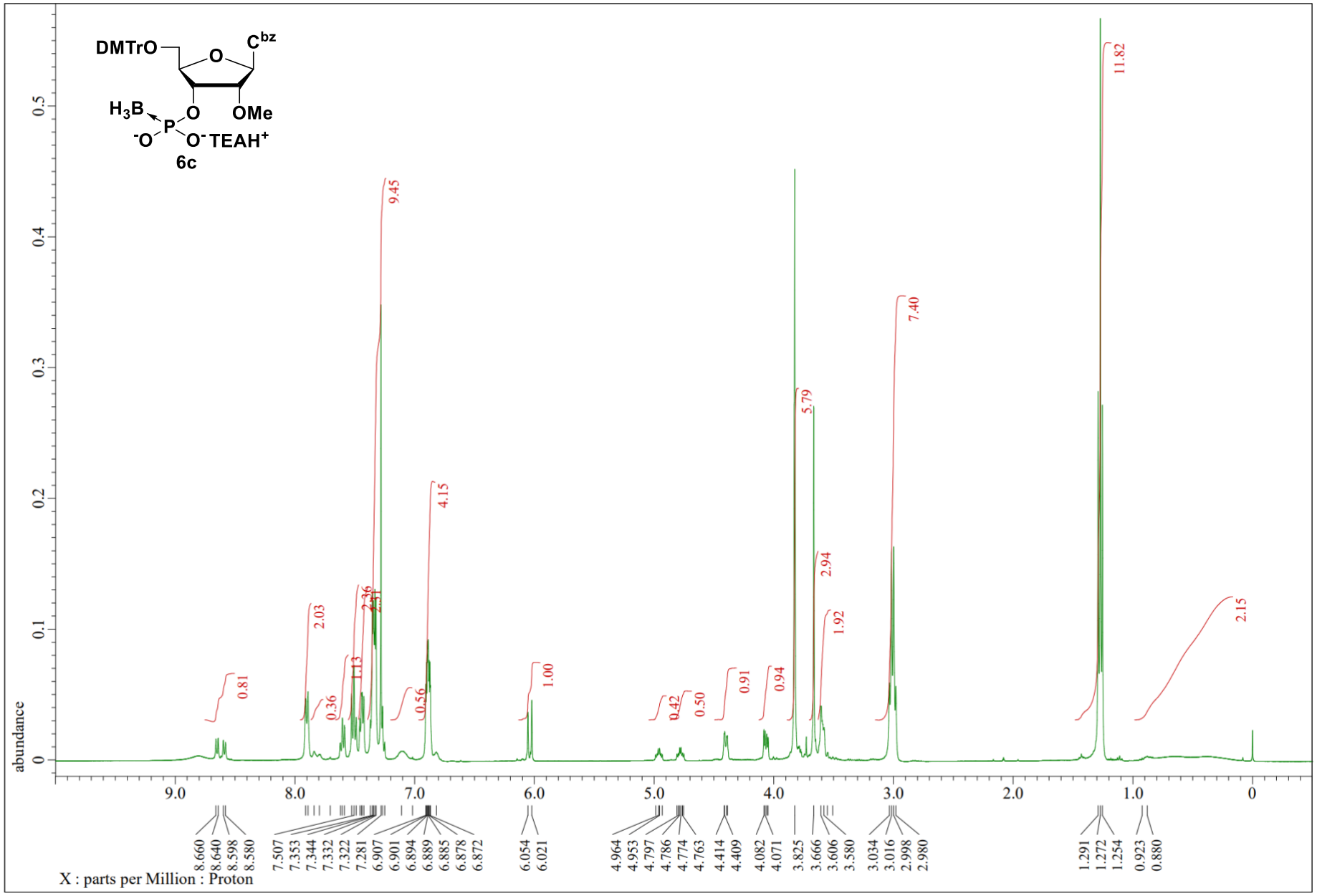
$^{13}C\{H\}$ NMR (100 MHz, $CDCl_3$)



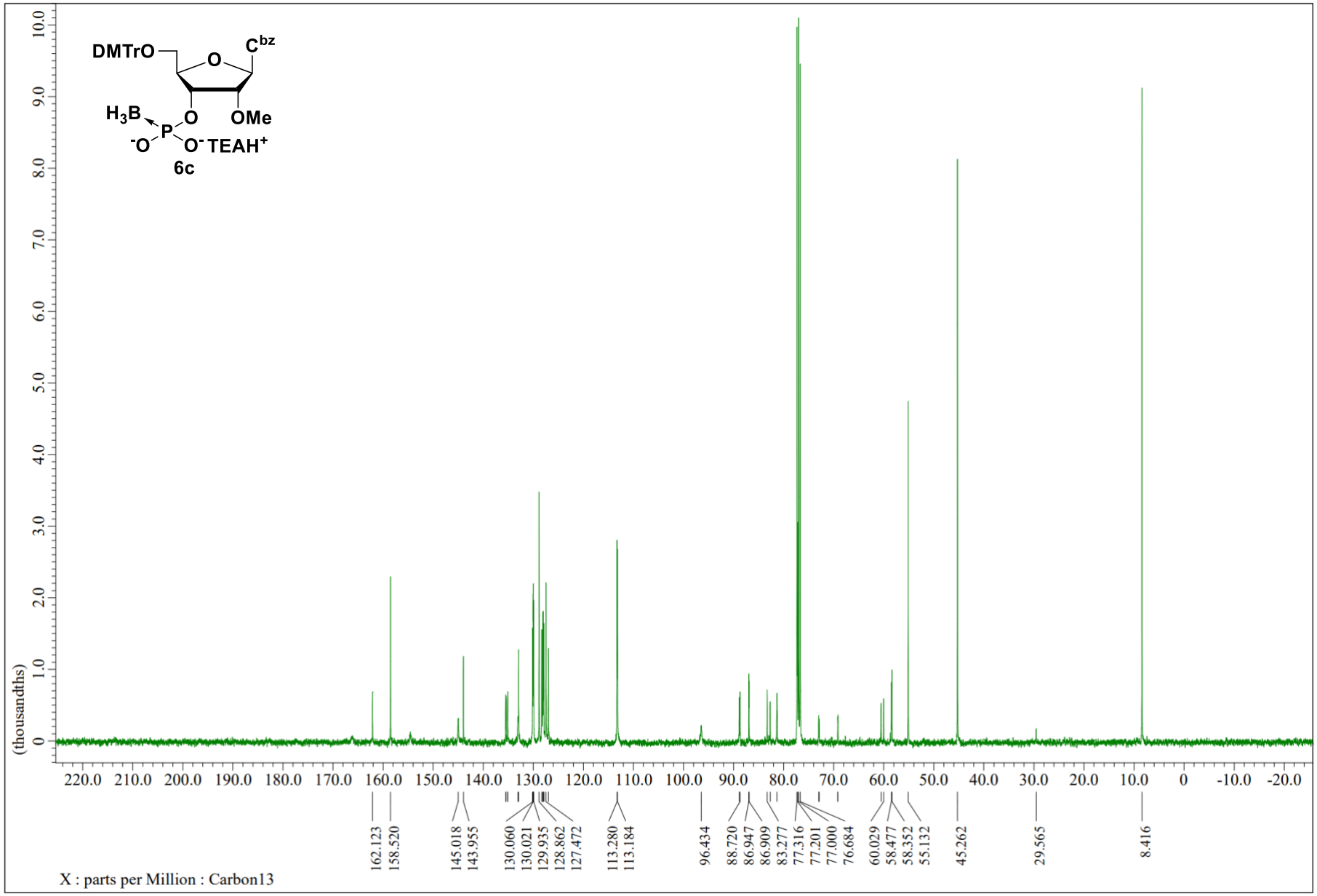
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



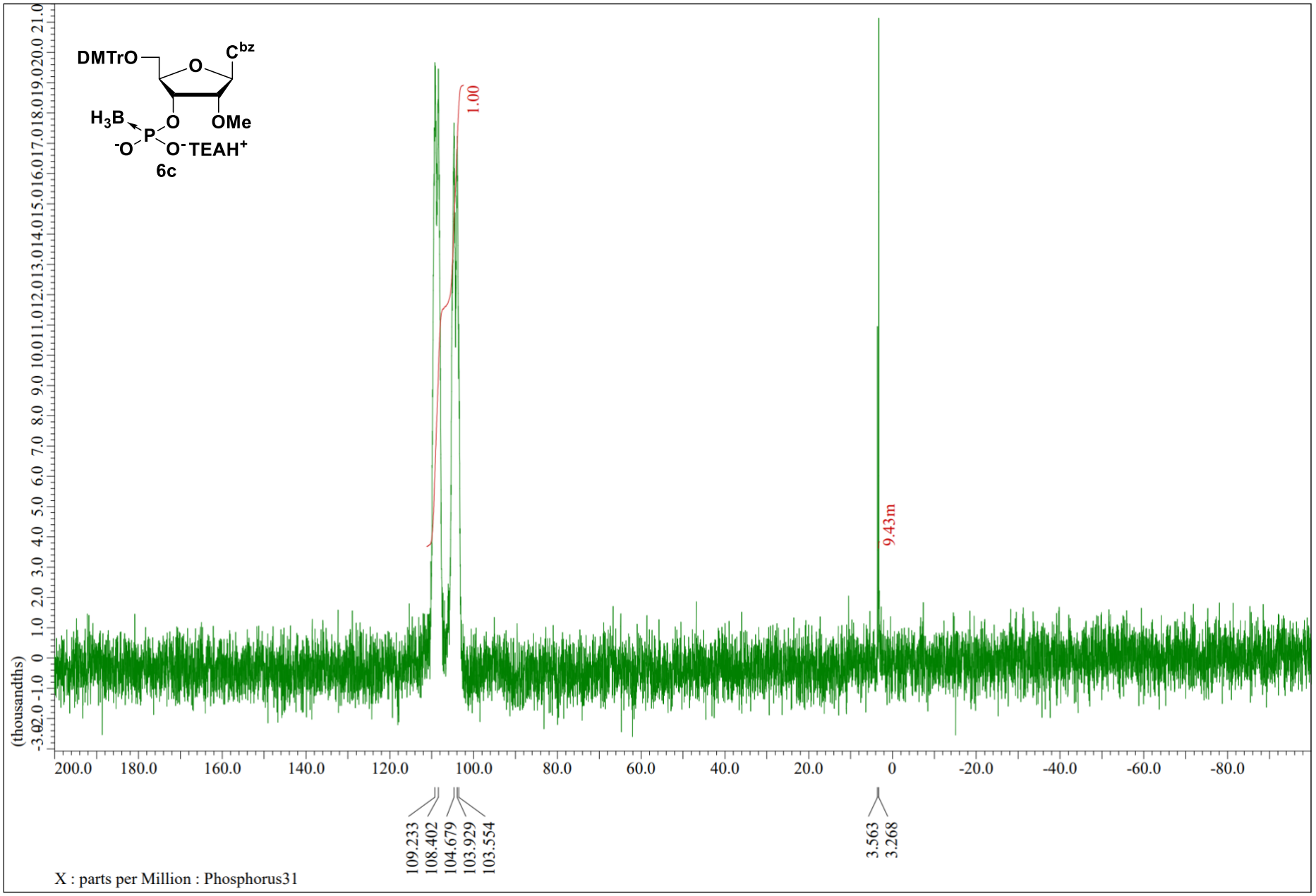
^{1}H-NMR (400 MHz, $CDCl_3$)



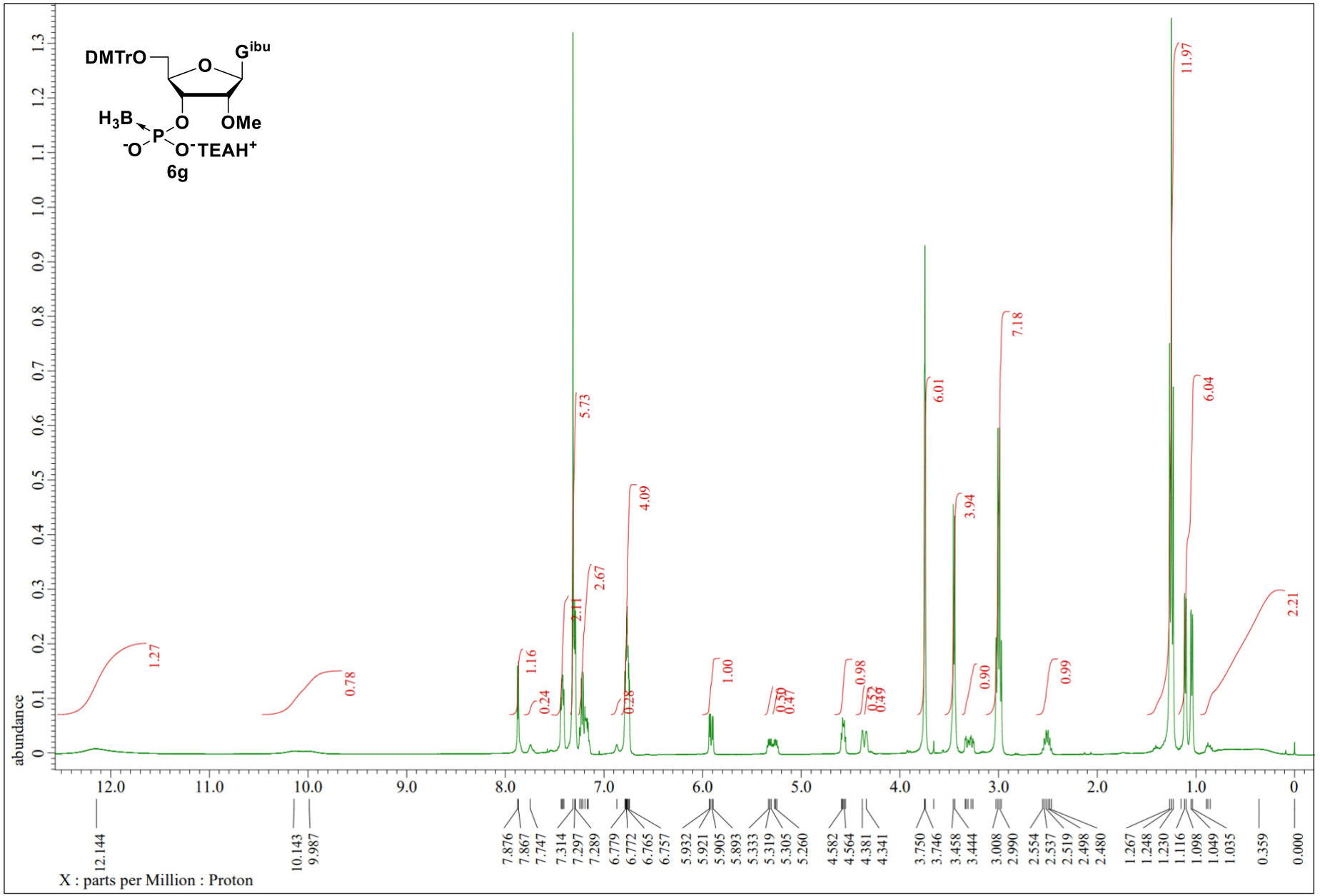
$^{13}C\{H\}$ NMR (100 MHz, $CDCl_3$)



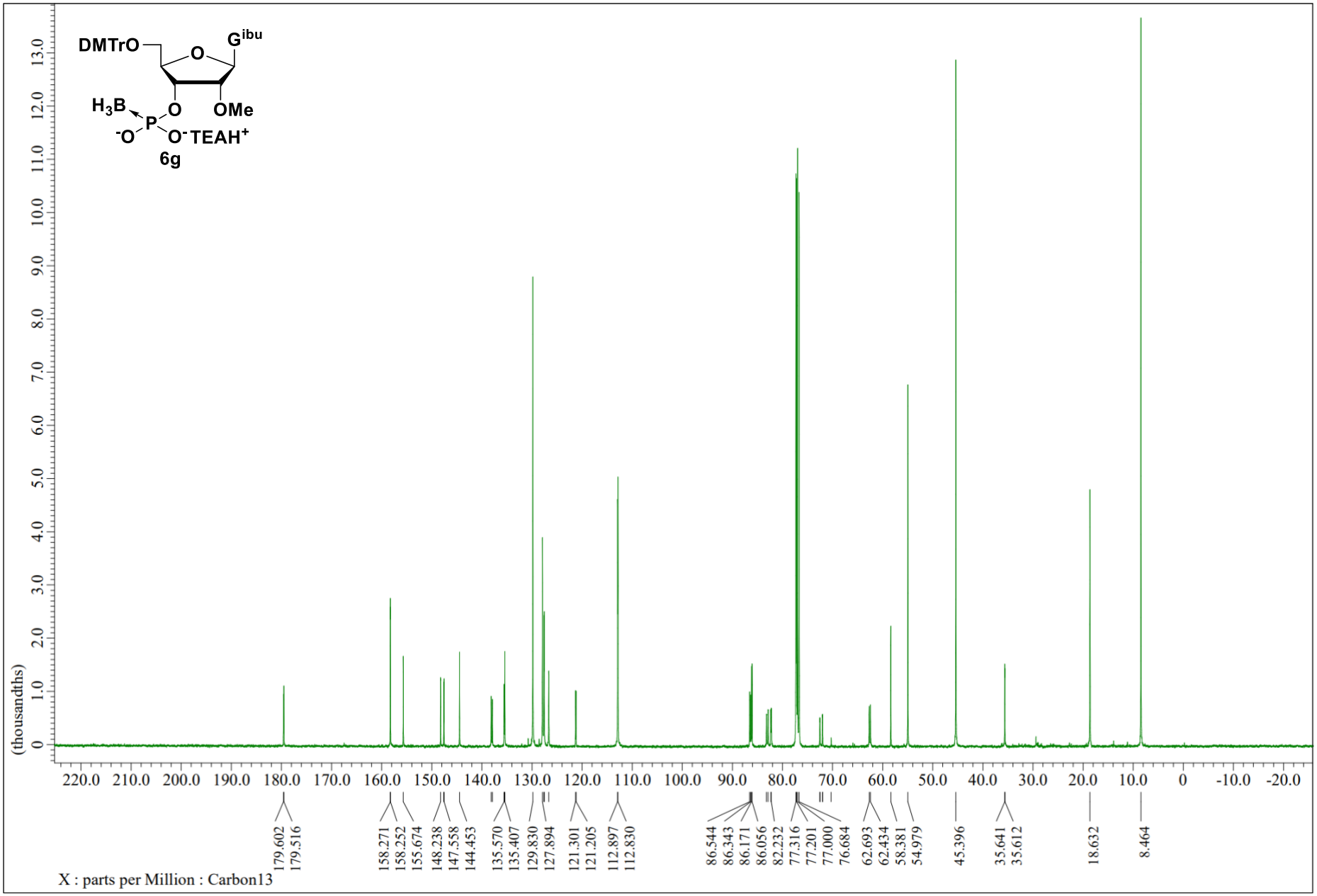
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



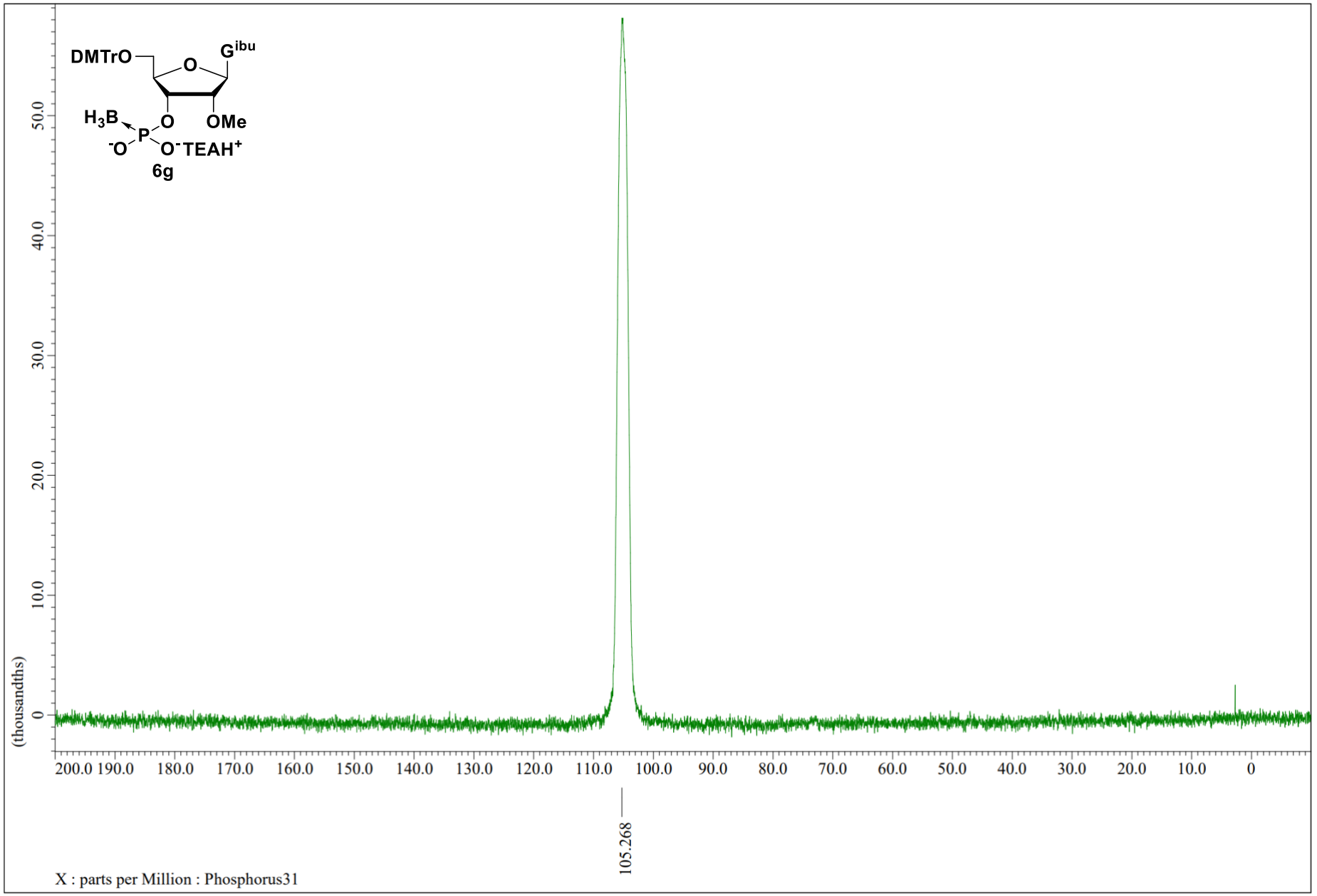
^{1}H-NMR (400 MHz, $CDCl_3$)



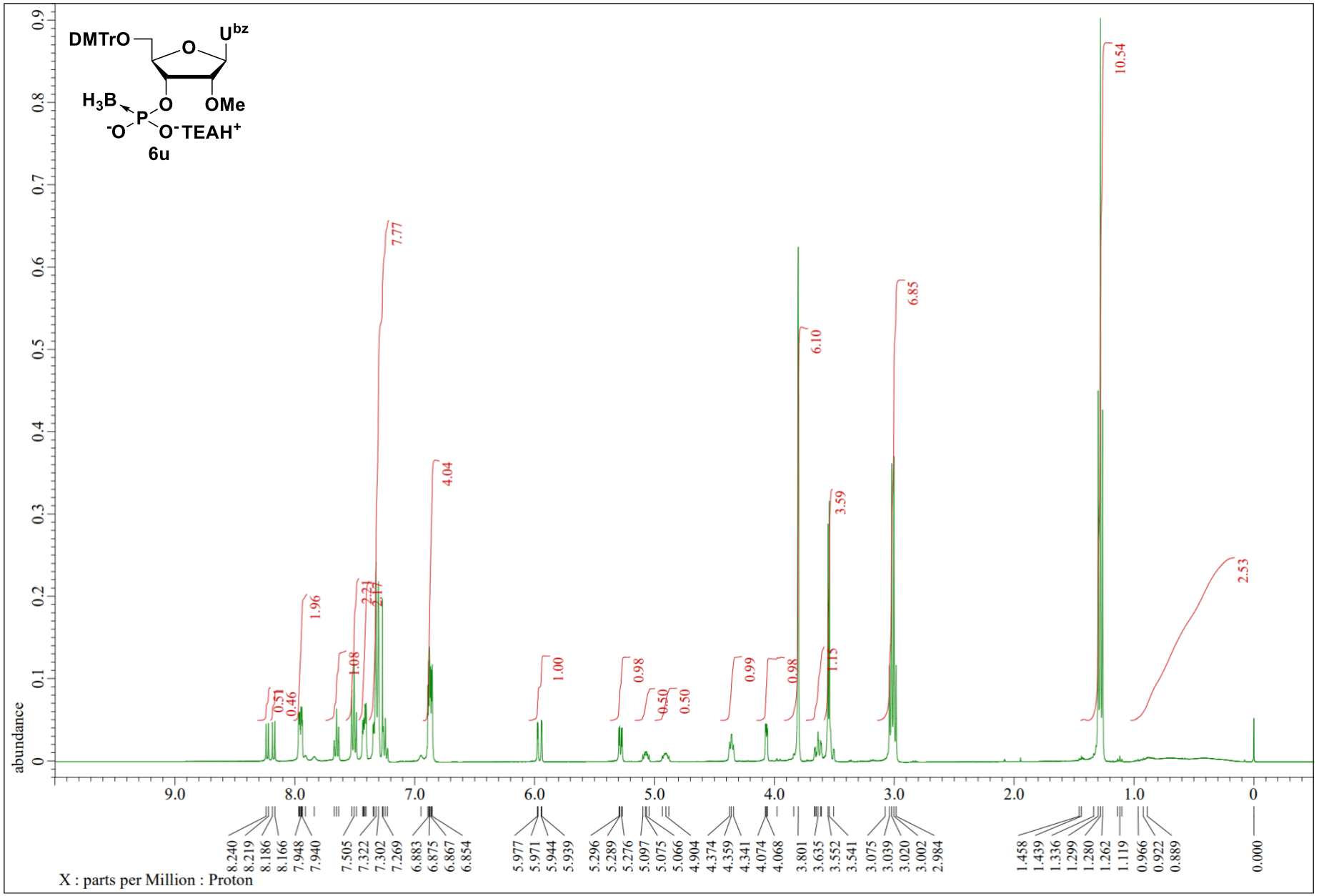
$^{13}C\{H\}$ NMR (100 MHz, $CDCl_3$)



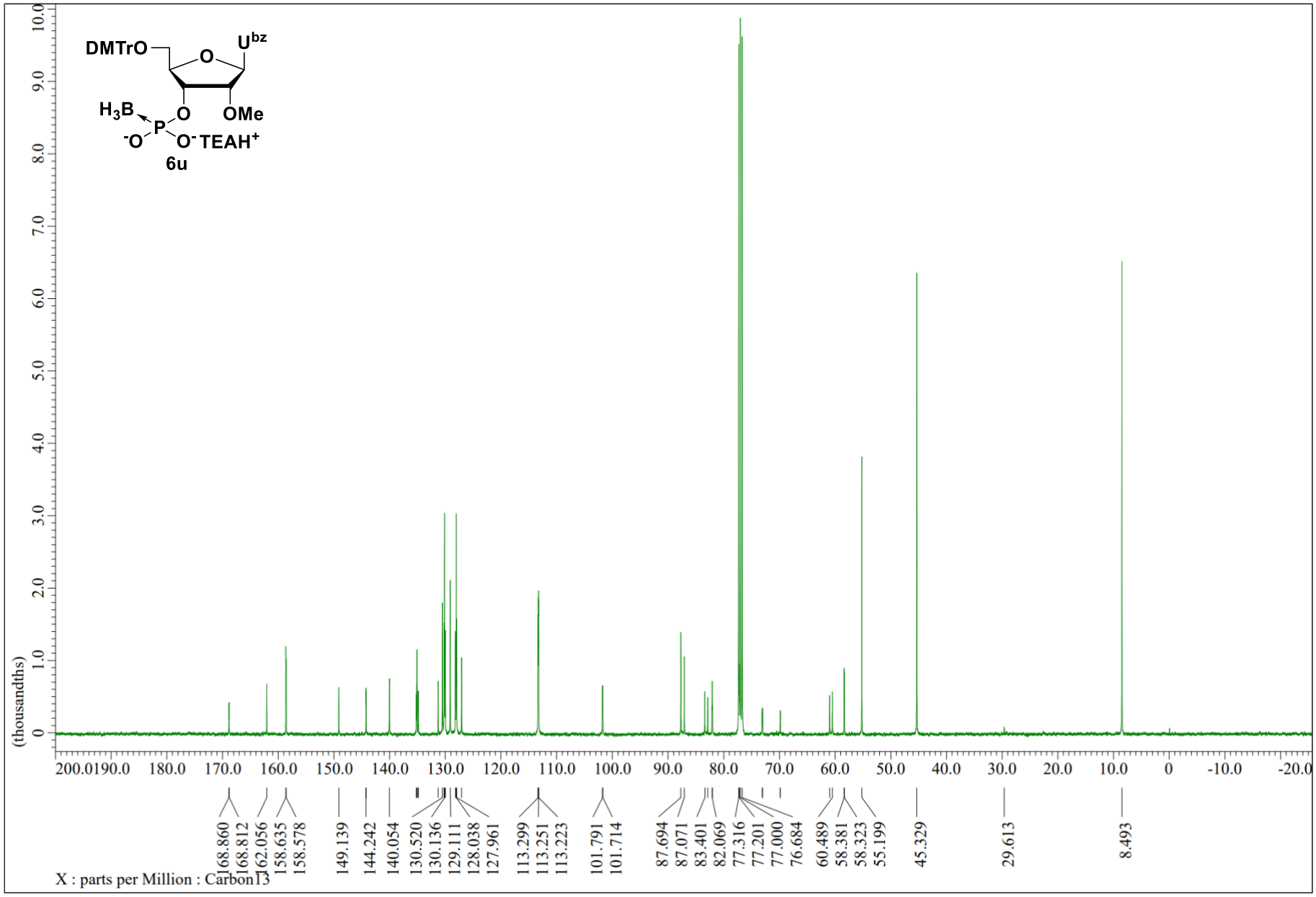
$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)



^{1}H-NMR (400 MHz, $CDCl_3$)



$^{13}C\{H\}$ NMR (100 MHz, $CDCl_3$)



$^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$)