Supplementary information

Solution-phase synthesis of oligodeoxyribonucleotides using the *H*-phosphonate method with *N*-unprotected 5′-phosphite monomers

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1. ^{31}P NMR analysis of the synthesis of 5′-phosphite monomers (Table 1)

Table 1, entry 2

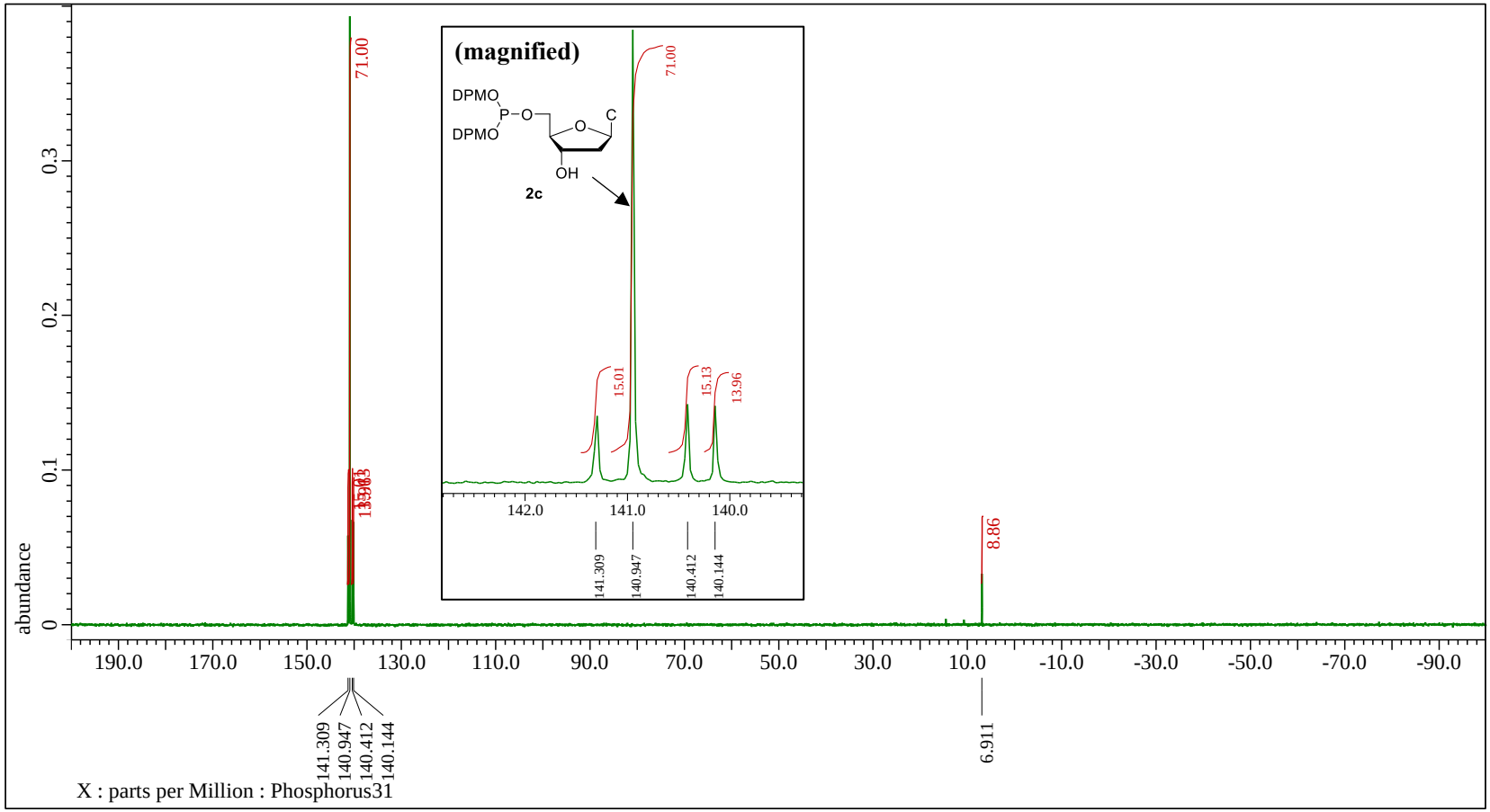


Fig. S1 ^{31}P NMR spectrum (pyridine-d_5, 162 MHz) of the reaction mixture in the phosphitylation of 2′-deoxycytidine.

Table 1, entry 4

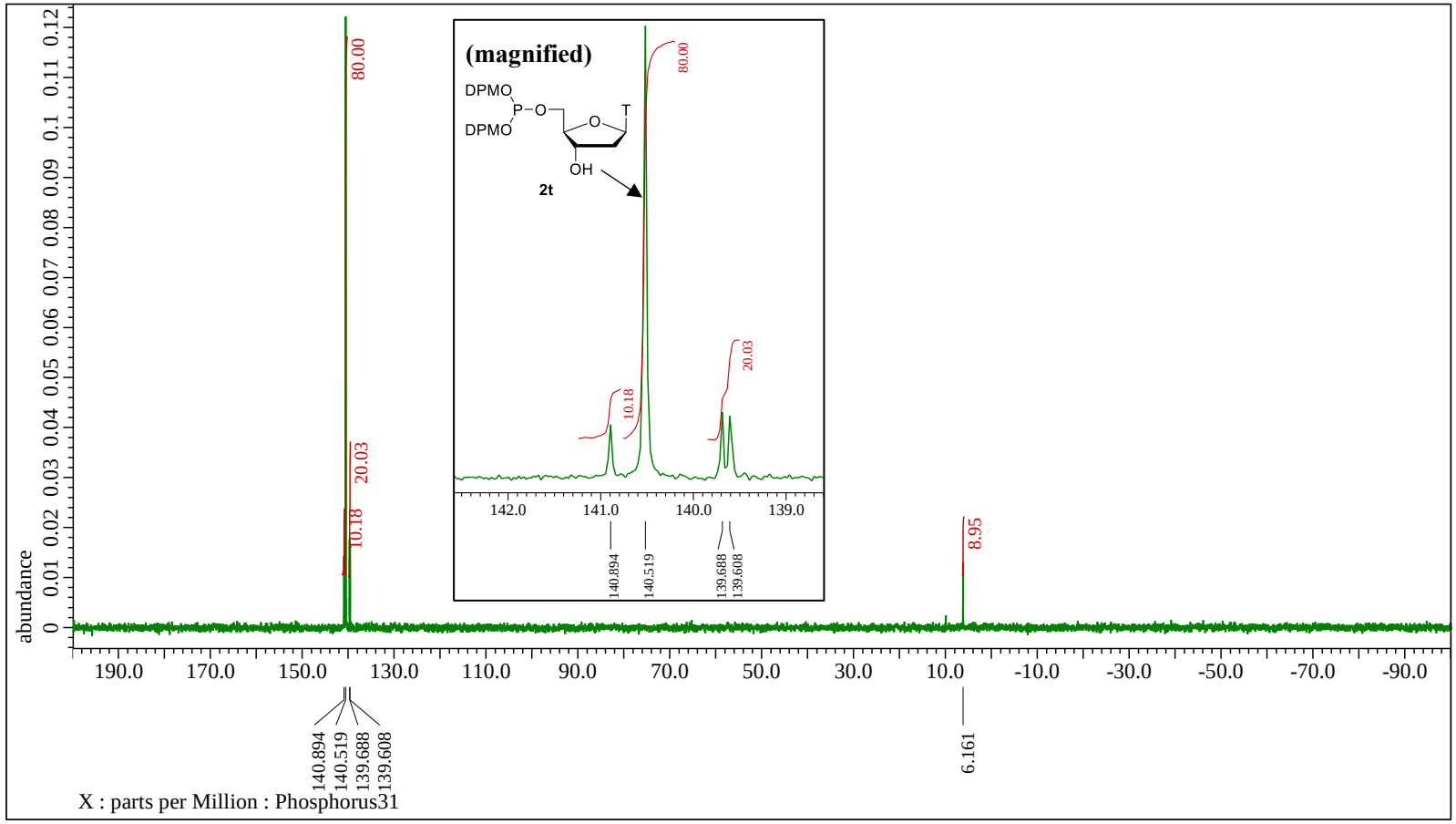
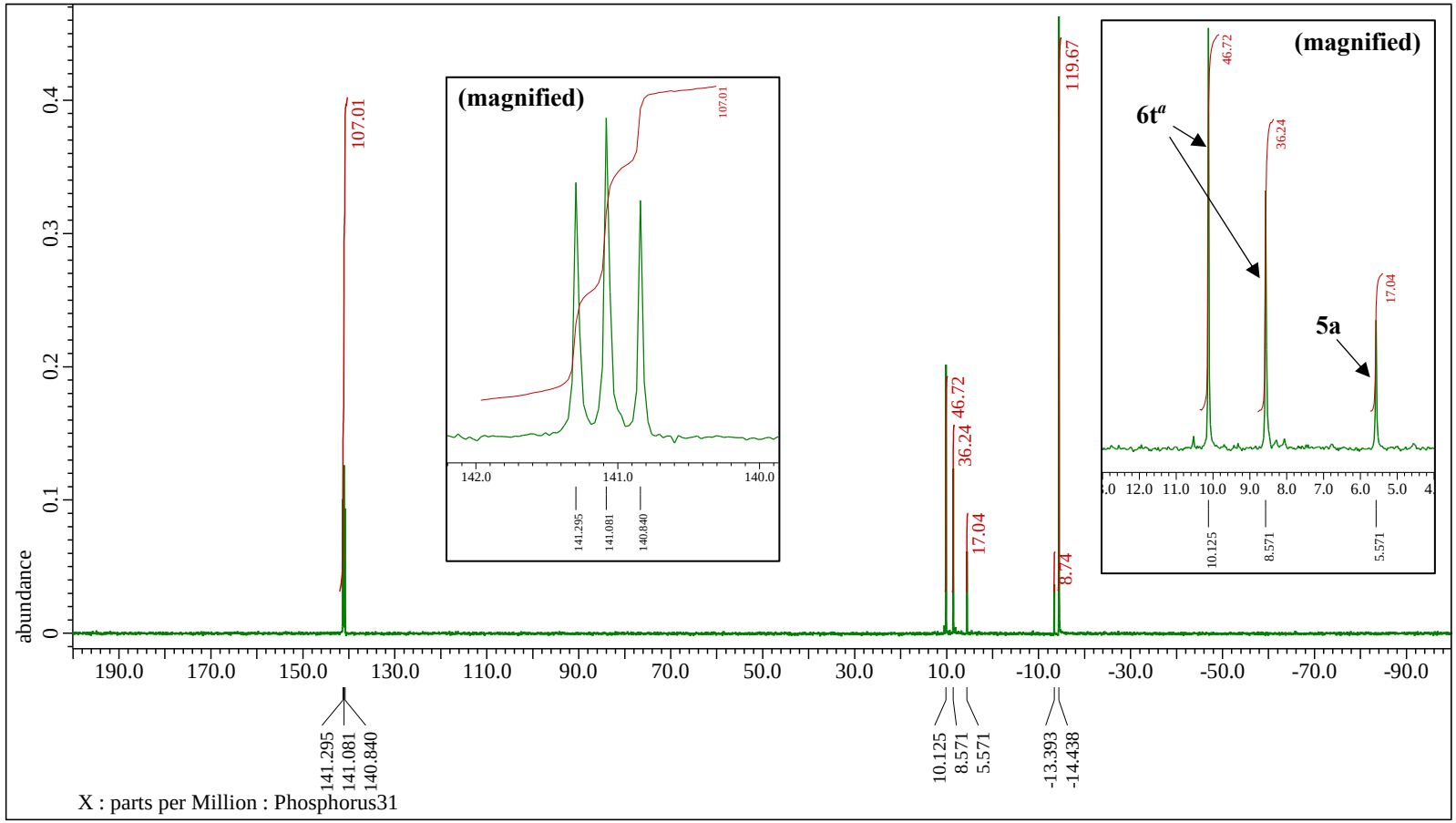


Fig. S2 ^{31}P NMR spectrum (pyridine-d_5, 162 MHz) of the reaction mixture in the phosphitylation of thymidine.

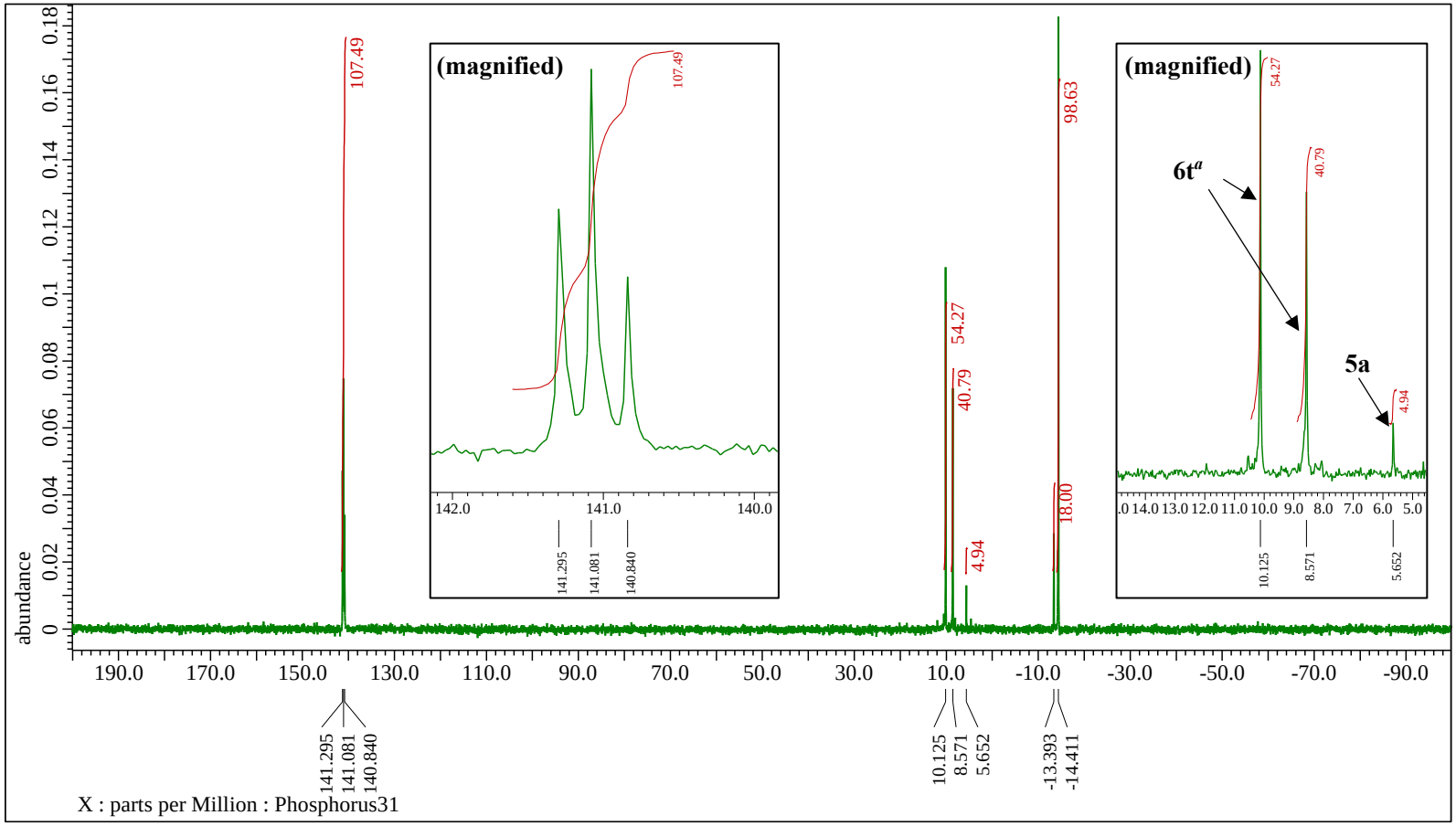
2. ^{31}P NMR analysis of the condensation of 5′-phosphite monomers with *H*-phosphonate monoesters (Table 2)

Table 2, entry 1

(A) ^{31}P NMR spectrum of the reaction mixture 30 min after BOP-Cl (2.0 equiv) was added.



(B) ^{31}P NMR spectrum of the reaction mixture 1.5 h after extra BOP-Cl (2.0 equiv) was added (the total addition of BOP-Cl: 4.0 equivalents).



(C) ^{31}P NMR spectrum of the reaction mixture 1.5 h after extra BOP-Cl (2.0 equiv) was added again (the total addition of BOP-Cl: 6.0 equivalents).

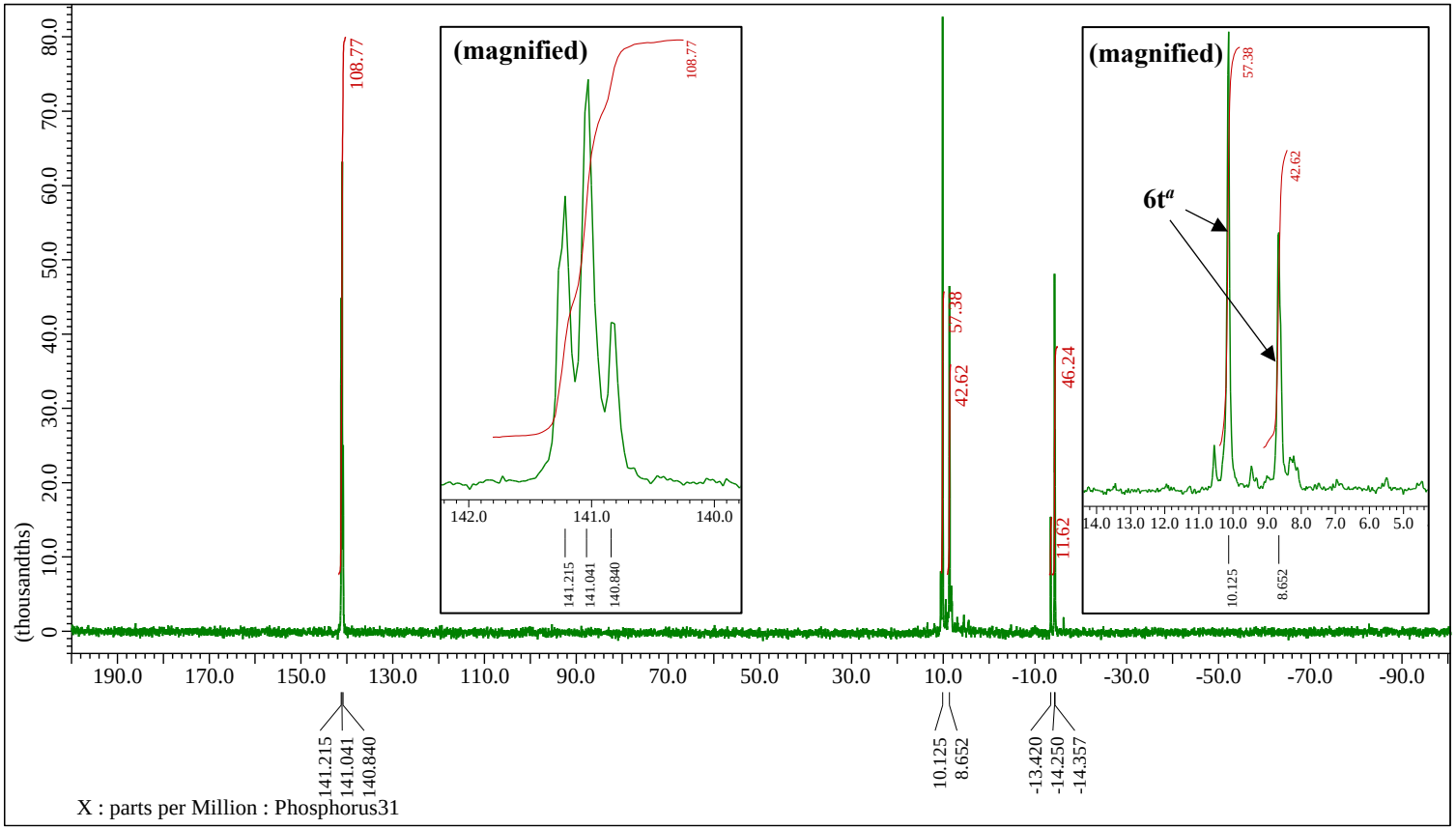


Fig. S3 ^{31}P NMR spectra (pyridine-d_5, 162 MHz) of the reaction mixtures in the condensation between **2t** and **5a** in pyridine. The mixtures were analyzed (A) 30 min after BOP-Cl (2.0 equiv) was added, (B) 1.5 h after extra BOP-Cl (2.0 equiv) was added, and (C) 1.5 h after extra BOP-Cl (2.0 equiv) was added again.

[a]The peaks were derived from the diastereomeric *H*-phosphonate diester of **6t**.

Table 2, entry 2

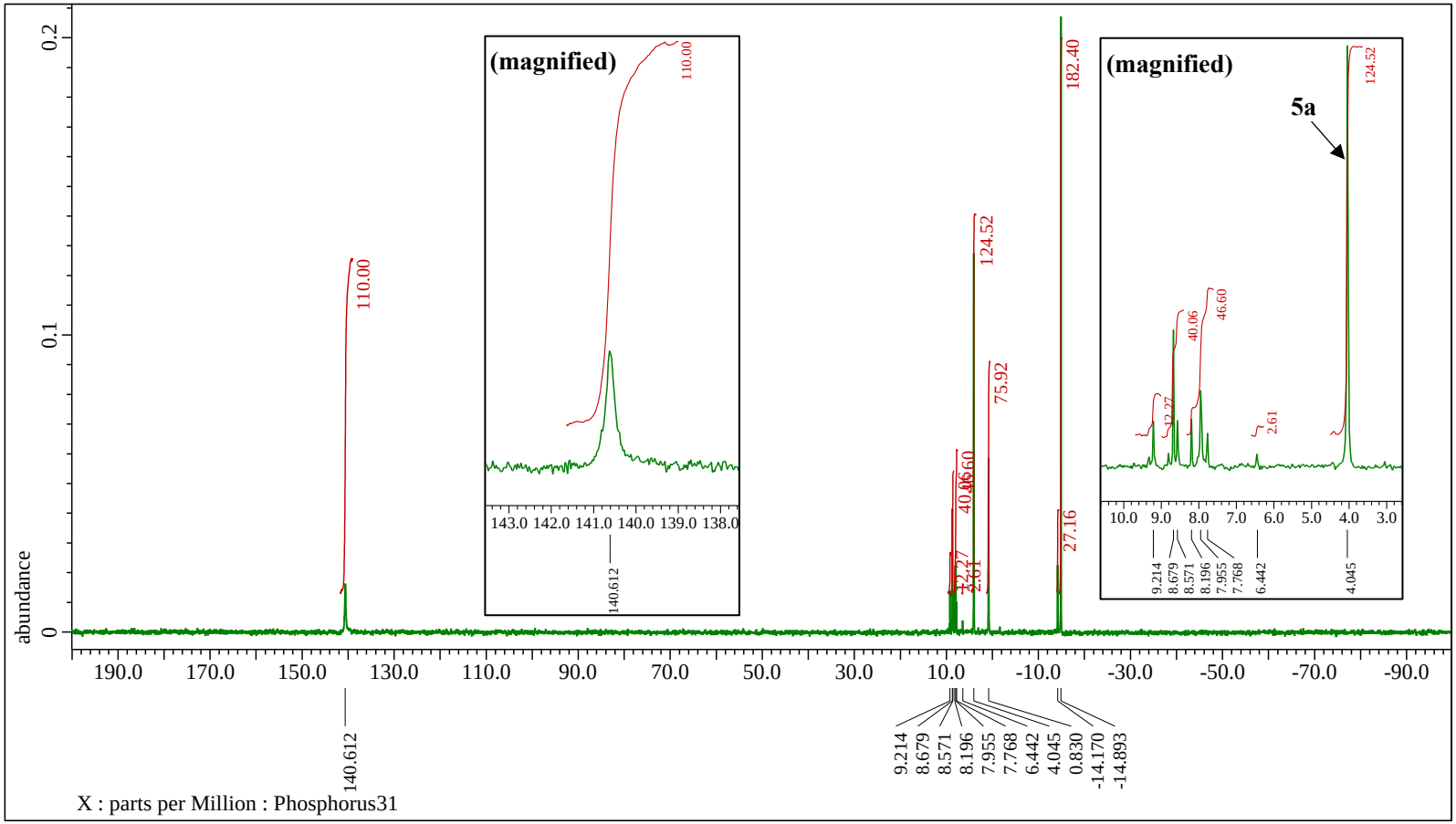
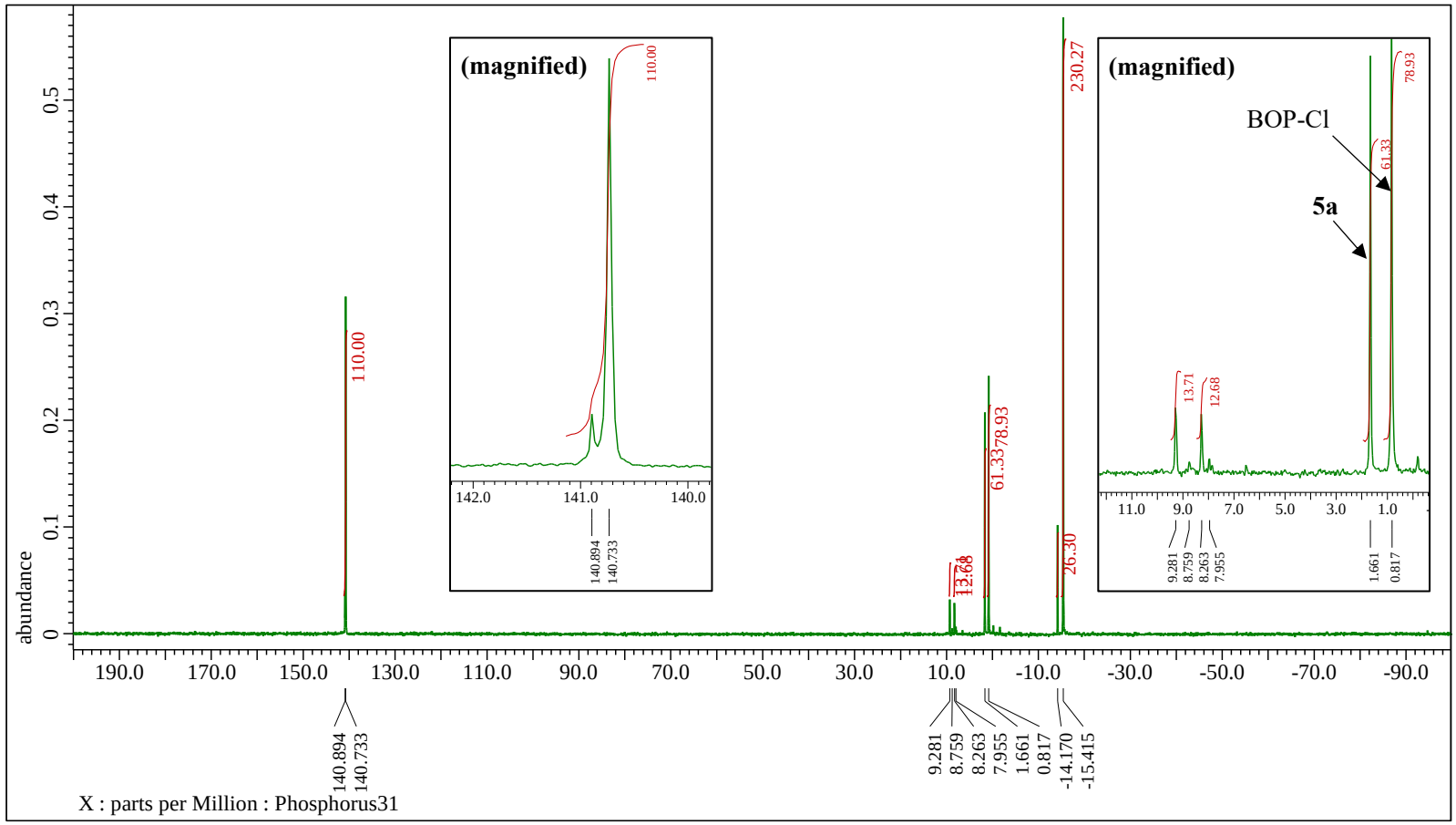


Fig. S4 ^{31}P NMR spectrum (CD_3CN, 162 MHz) of the reaction mixture in the condensation between **2t** and **5a** in CH_3CN solvent with pyridine (10 equiv) 30 min after BOP-Cl (2.0 equiv) was added.

Table 2, entry 3

(A) ^{31}P NMR spectrum of the reaction mixture 30 min after BOP-Cl (4.0 equiv) was added.



(B) ^{31}P NMR spectrum of the reaction mixture 5.5 h after BOP-Cl (4.0 equiv) was added.

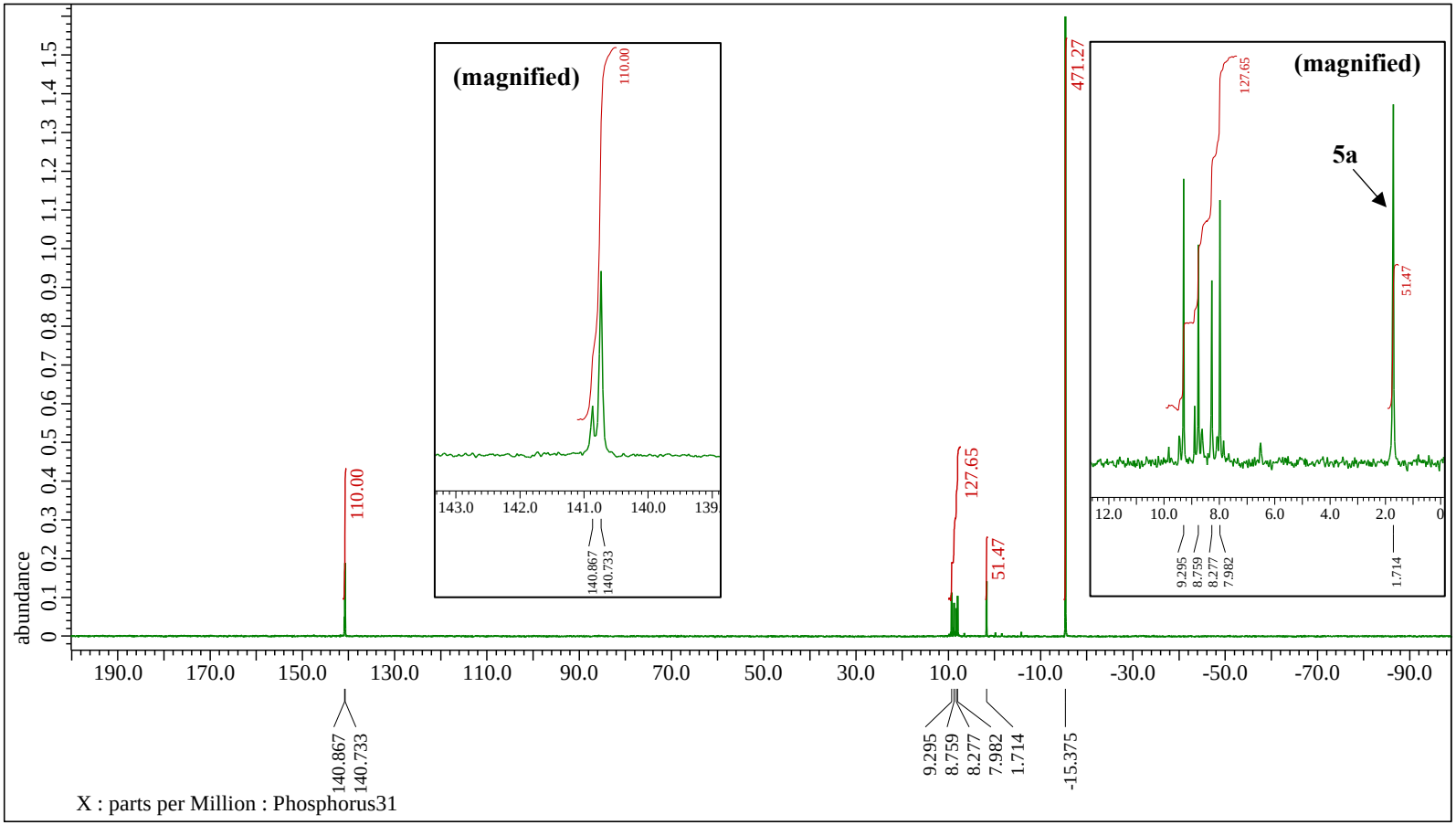


Fig. S5 ^{31}P NMR spectra (CD_3CN, 162 MHz) of the reaction mixtures in the condensation between **2t** and **5a** in CH_3CN solvent with 2,6-lutidine (10 equiv). The mixtures were analyzed (A) 30 min and (B) 5.5 h after BOP-Cl (4.0 equiv) was added.

Table 2, entry 4

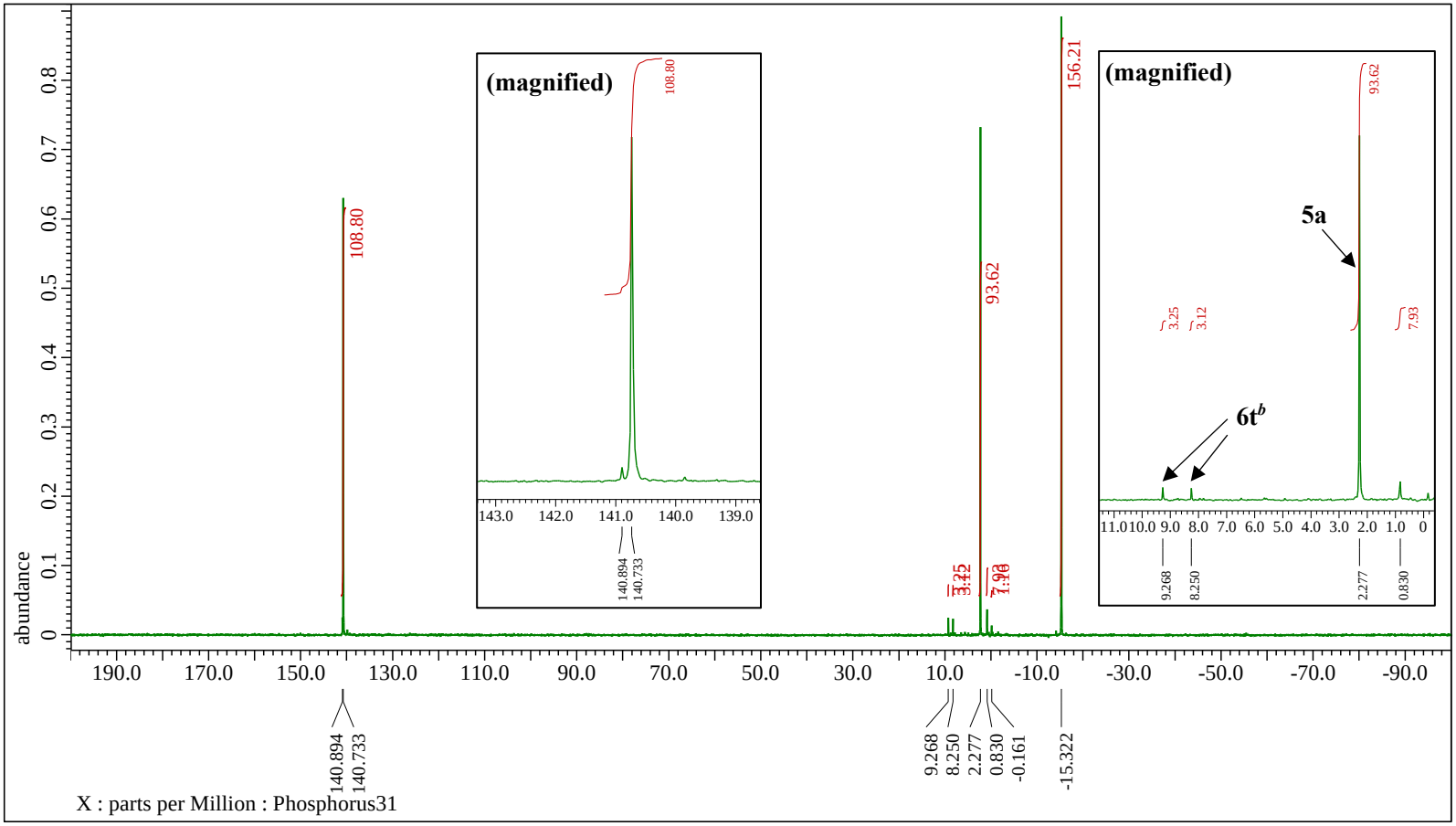


Fig. S6 ^{31}P NMR spectrum (CD_3CN, 162 MHz) of the reaction mixture in the condensation between **2t** and **5a** in CH_3CN solvent with Et_3N (10 equiv) 30 min after BOP-Cl (2.0 equiv) was added..

[b]The peaks were derived from the diastereomeric *H*-phosphonate diester of **6t**.

Table 2, entry 5

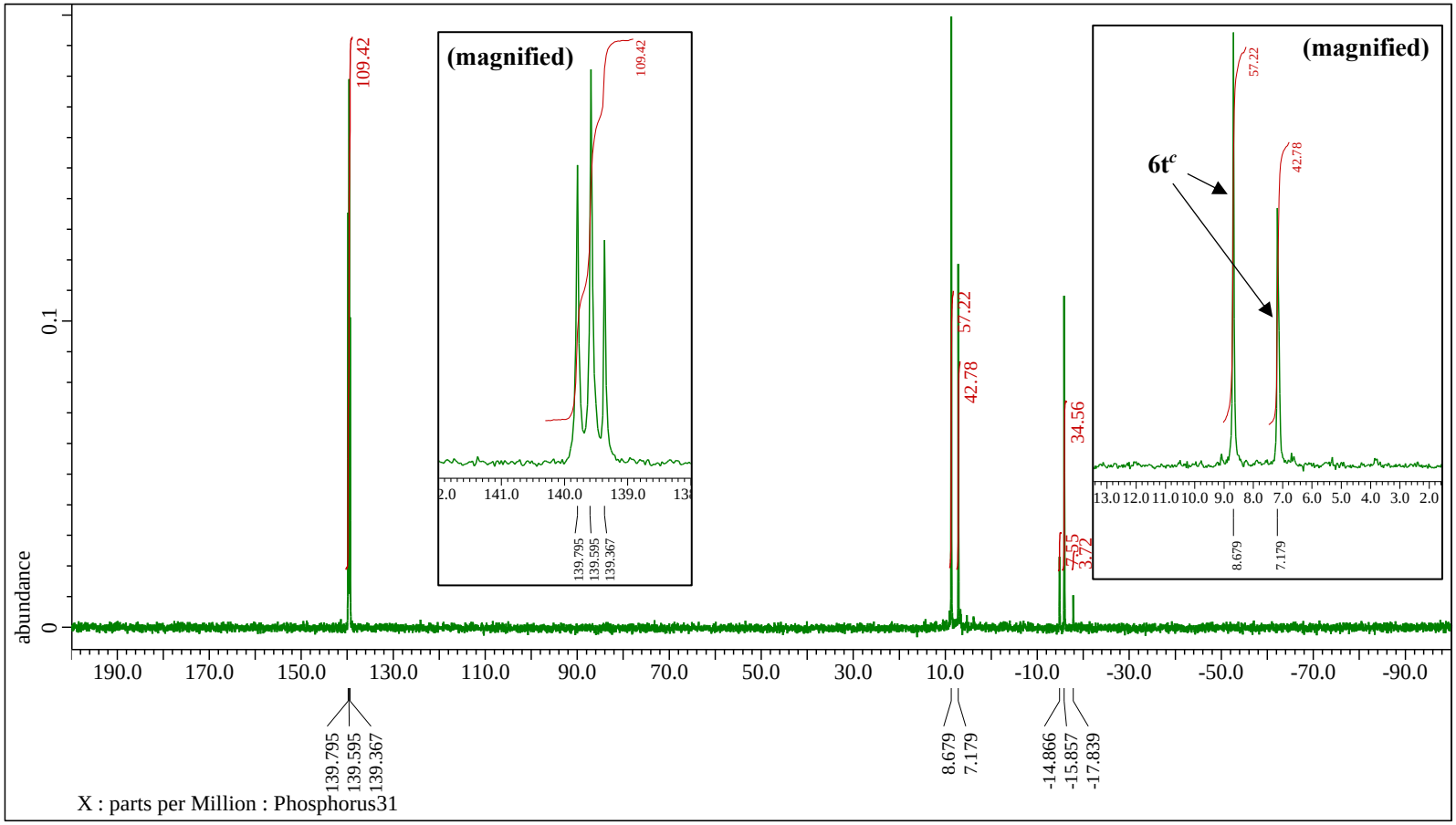


Fig. S7 ^{31}P NMR spectrum (pyridine-d_5, 162 MHz) of the reaction mixture in the condensation between **2t** and **5b** in pyridine 30 min after BOP-Cl (2.0 equiv) was added.

[c]The peaks were derived from the diastereomeric *H*-phosphonate diester of **6t**.

Table 2, entry 6

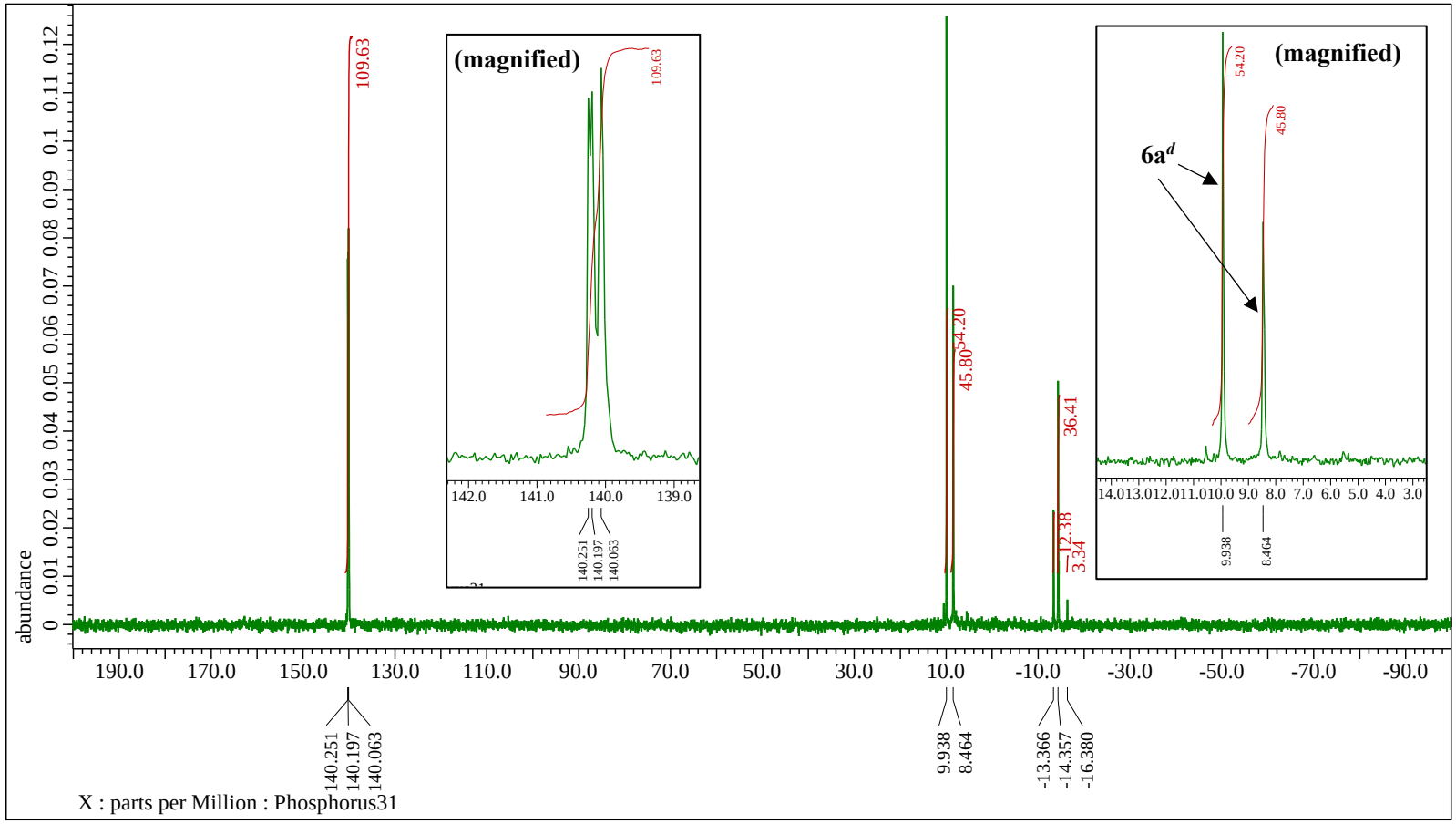


Fig. S8 ^{31}P NMR spectrum (pyridine-d_5, 162 MHz) of the reaction mixture in the condensation between **2a** and **5b** in pyridine 30 min after BOP-Cl (2.0 equiv) was added.

[d]The peaks were derived from the diastereomeric *H*-phosphonate diester of **6a**.

Table 2, entry 7

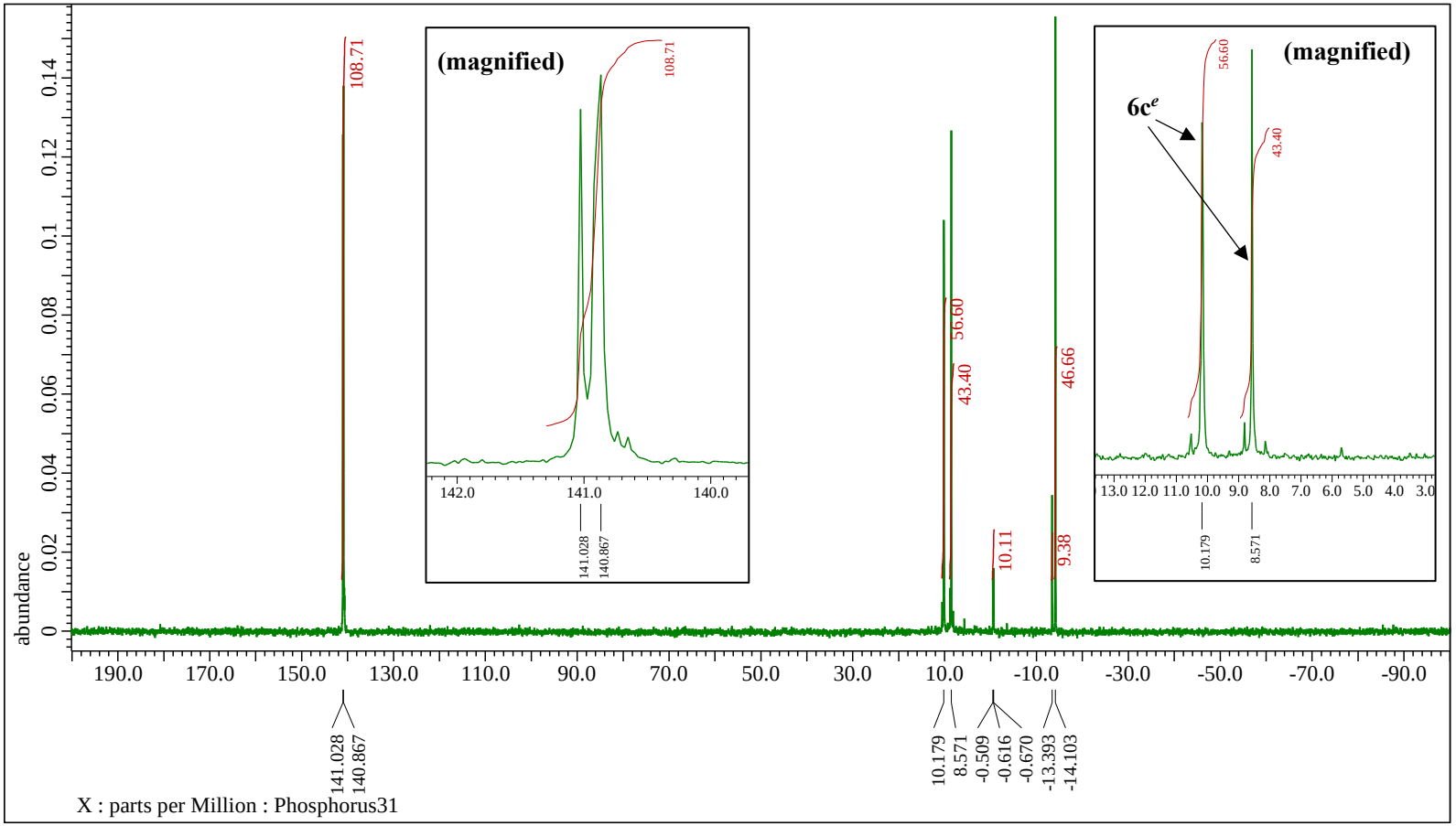


Fig. S9 ^{31}P NMR spectrum (pyridine-d_5, 162 MHz) of the reaction mixture in the condensation between **2c** and **5b** in pyridine 30 min after BOP-Cl (2.0 equiv) was added.

[e]The peaks were derived from the diastereomeric *H*-phosphonate diester of **6c**.

Table 2, entry 8

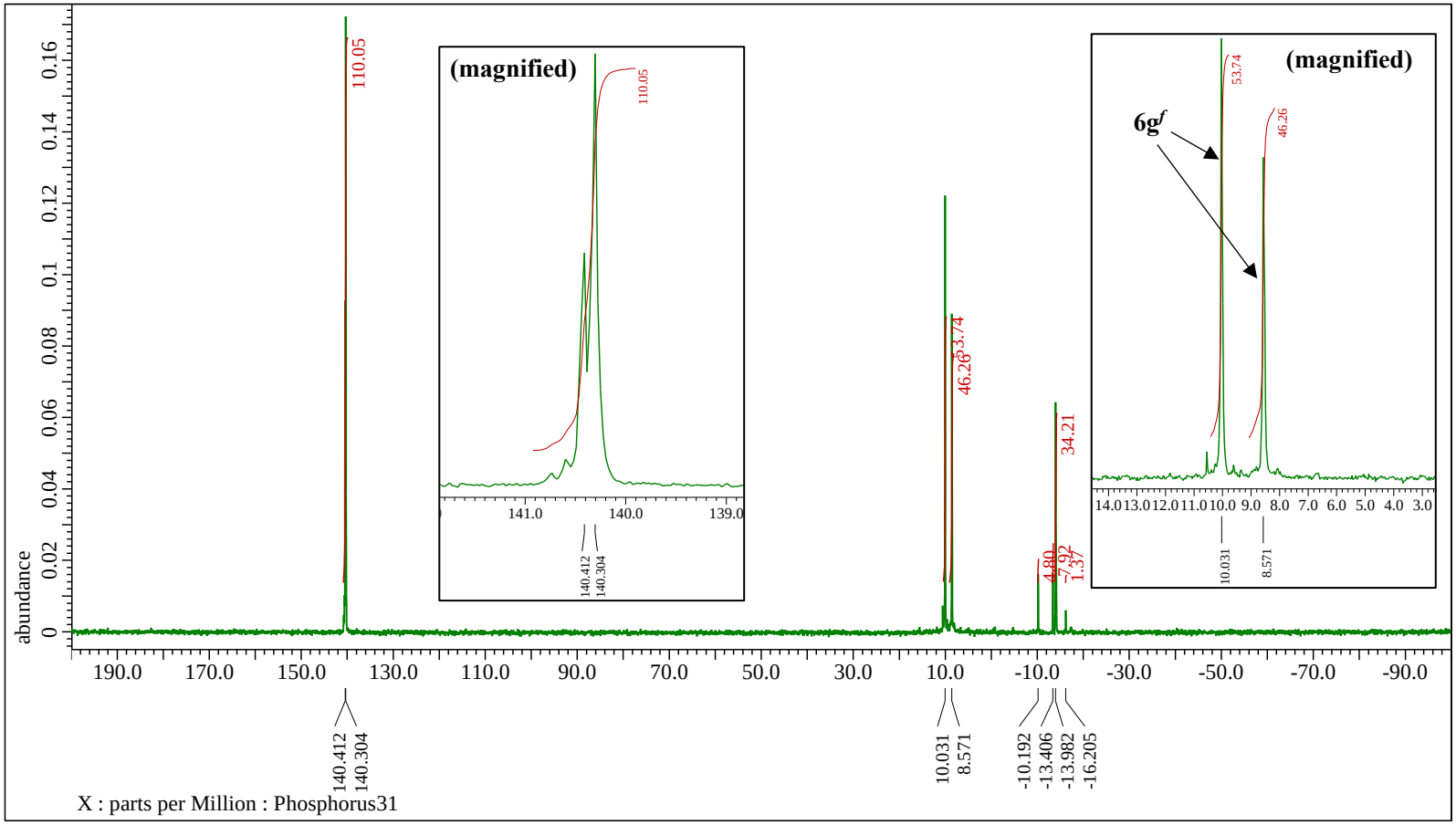


Fig. S10 ^{31}P NMR spectrum (pyridine-d_5, 162 MHz) of the reaction mixture in the condensation between **2g** and **5b** in pyridine 30 min after BOP-Cl (2.0 equiv) was added.

[f]The peaks were derived from the diastereomeric *H*-phosphonate diester of **6g**.

3. ^{31}P NMR spectra of the intermediates in the synthesis of TTT

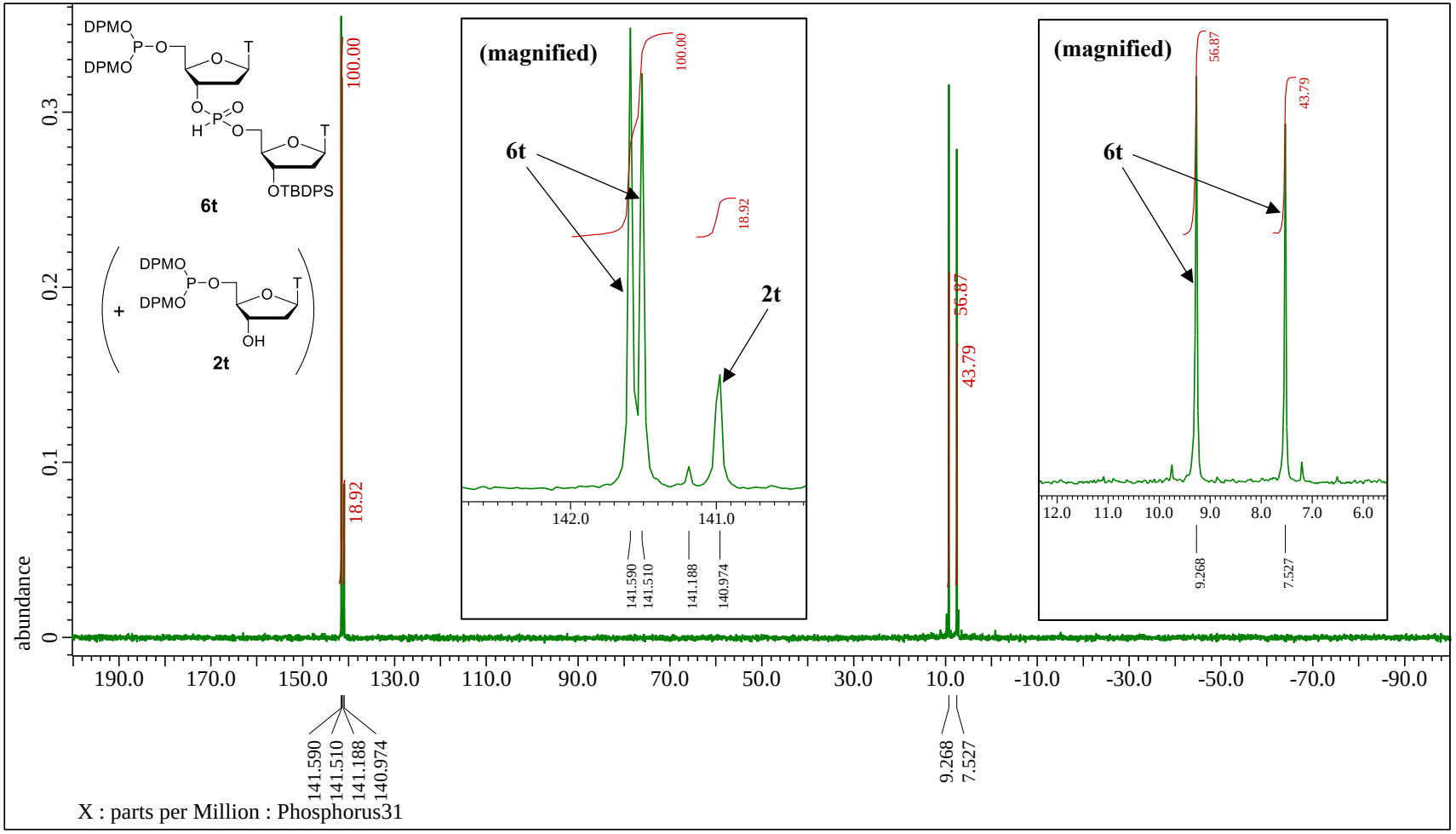


Fig. S11 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of crude **6t**.

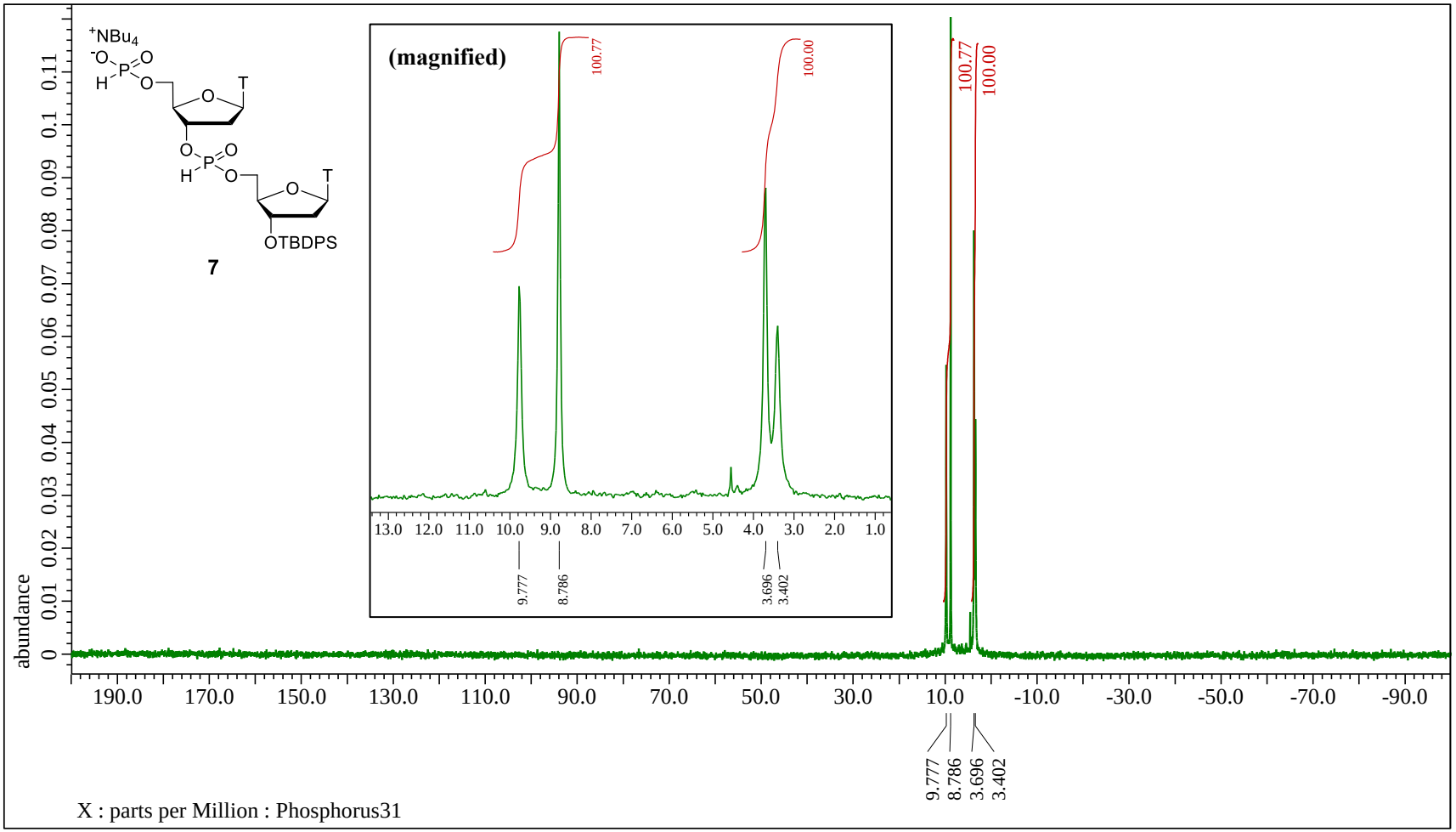


Fig. S12 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of crude **7**.

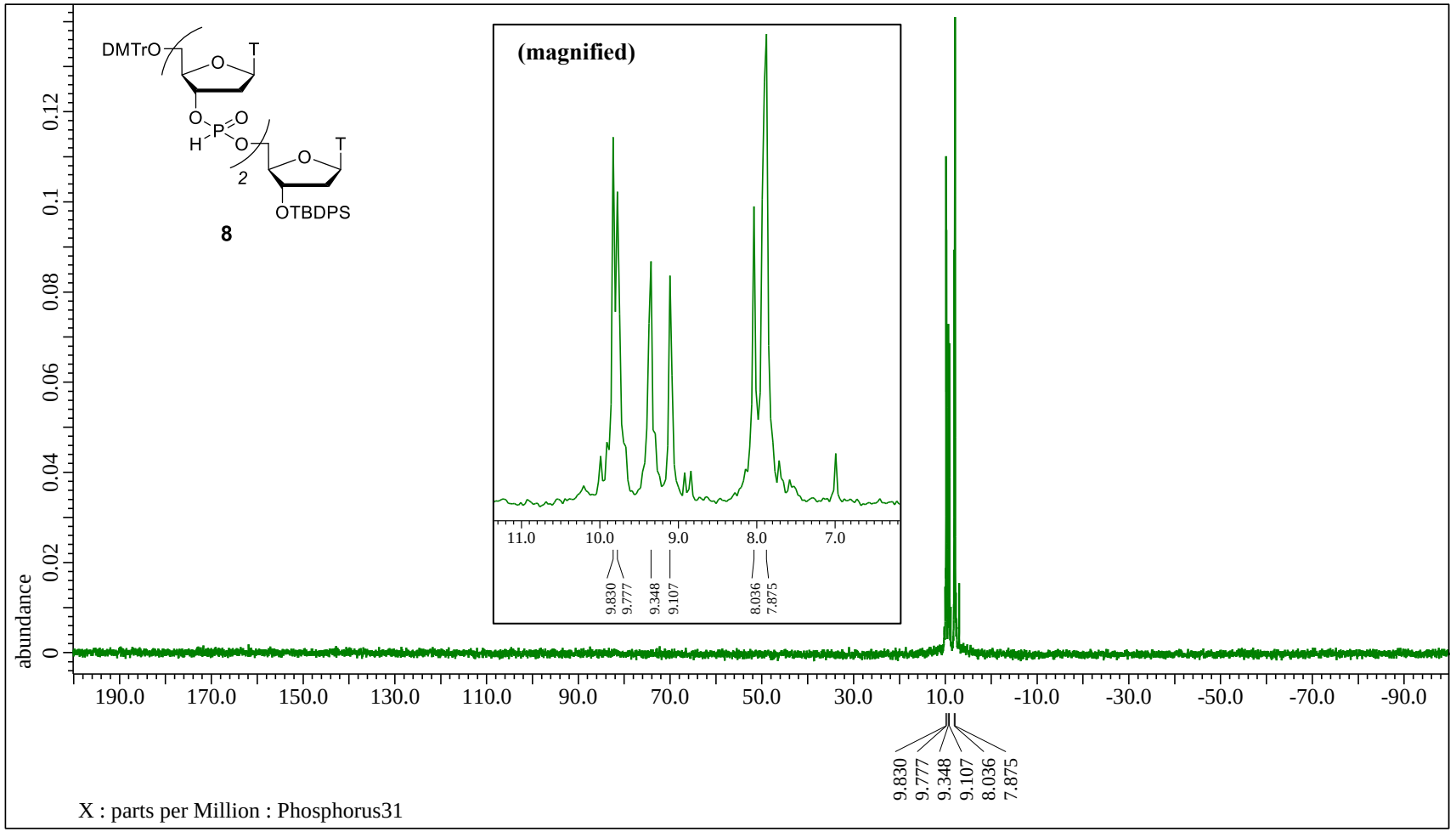
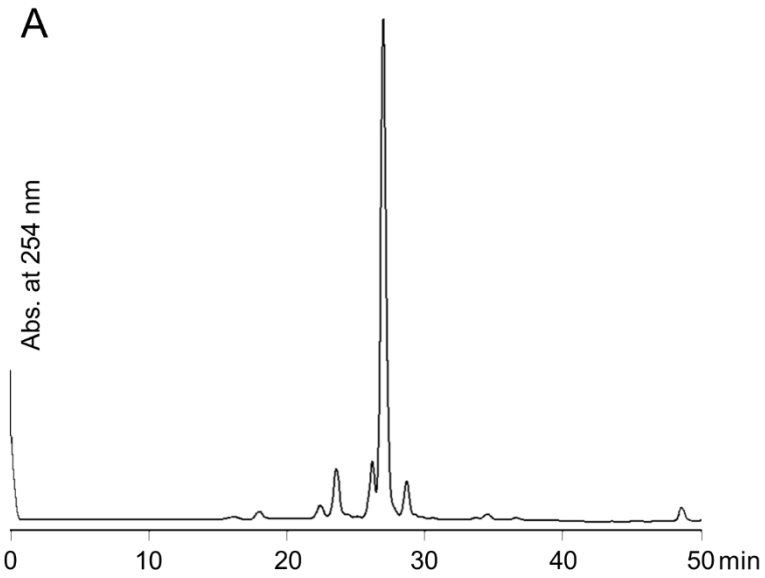


Fig. S13 ^{31}P NMR spectrum ($CDCl_3$, 162 MHz) of crude **8**.

4. RP-HPLC profiles of oligomers

Crude TTT



Purified TTT

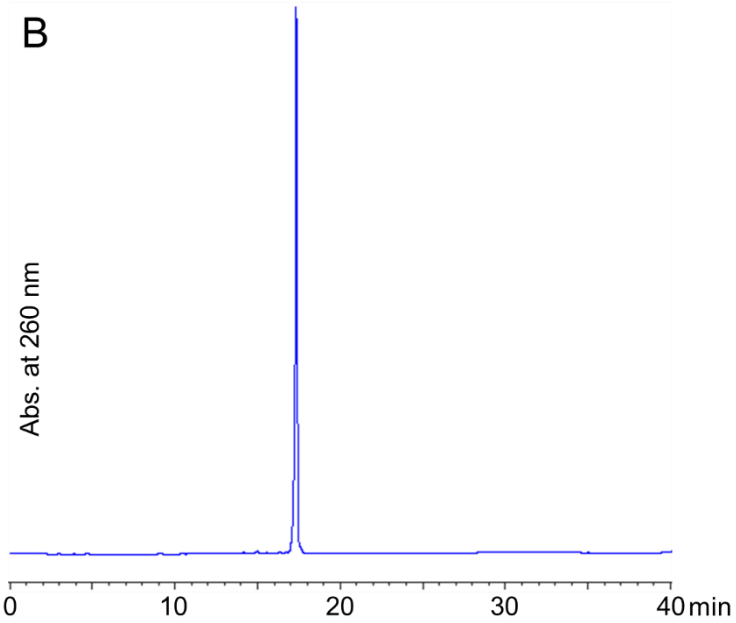
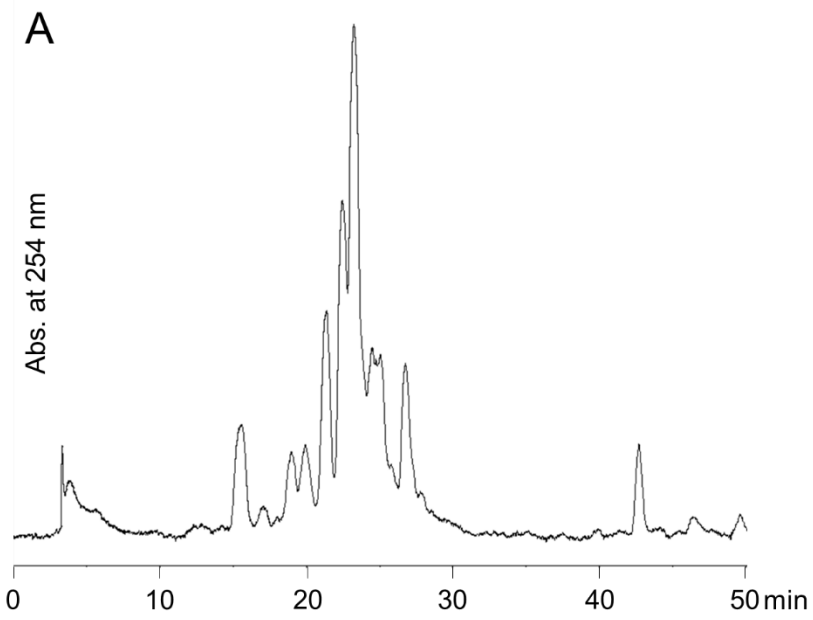


Fig. S14 RP-HPLC profiles of TTT: (A) crude TTT, (B) purified TTT. RP-HPLC was performed with a linear gradient of 0%–24% CH_3CN in 0.1 M TEAA buffer (pH 7.0) over 48 min at rt at a rate of 1.0 mL/min for analysis of (A) and with a linear gradient of 0%–40% CH_3CN in 0.1 M TEAA buffer (pH 7.0) over 40 min at 50 °C at a rate of 0.5 mL/min for analysis of (B).

Crude d(CGAT)



Crude d(GCAT)

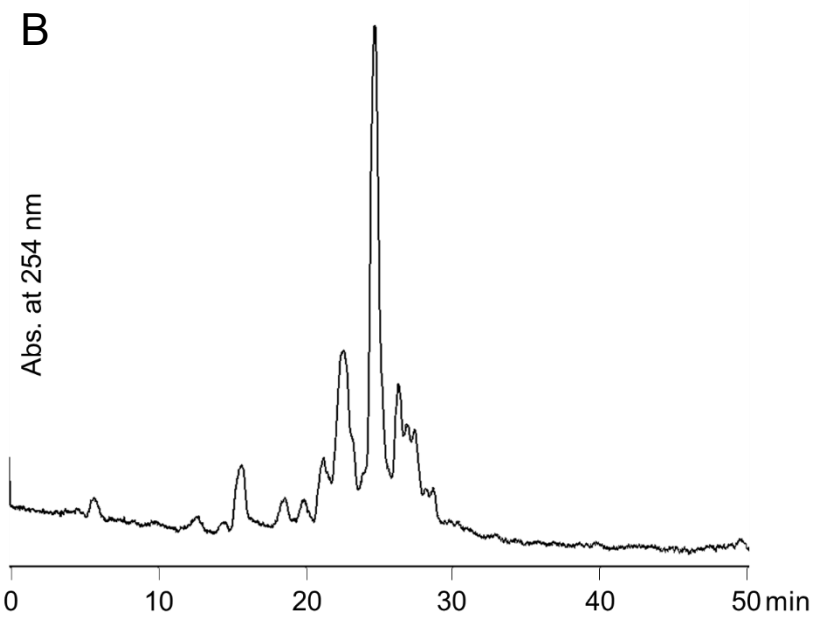
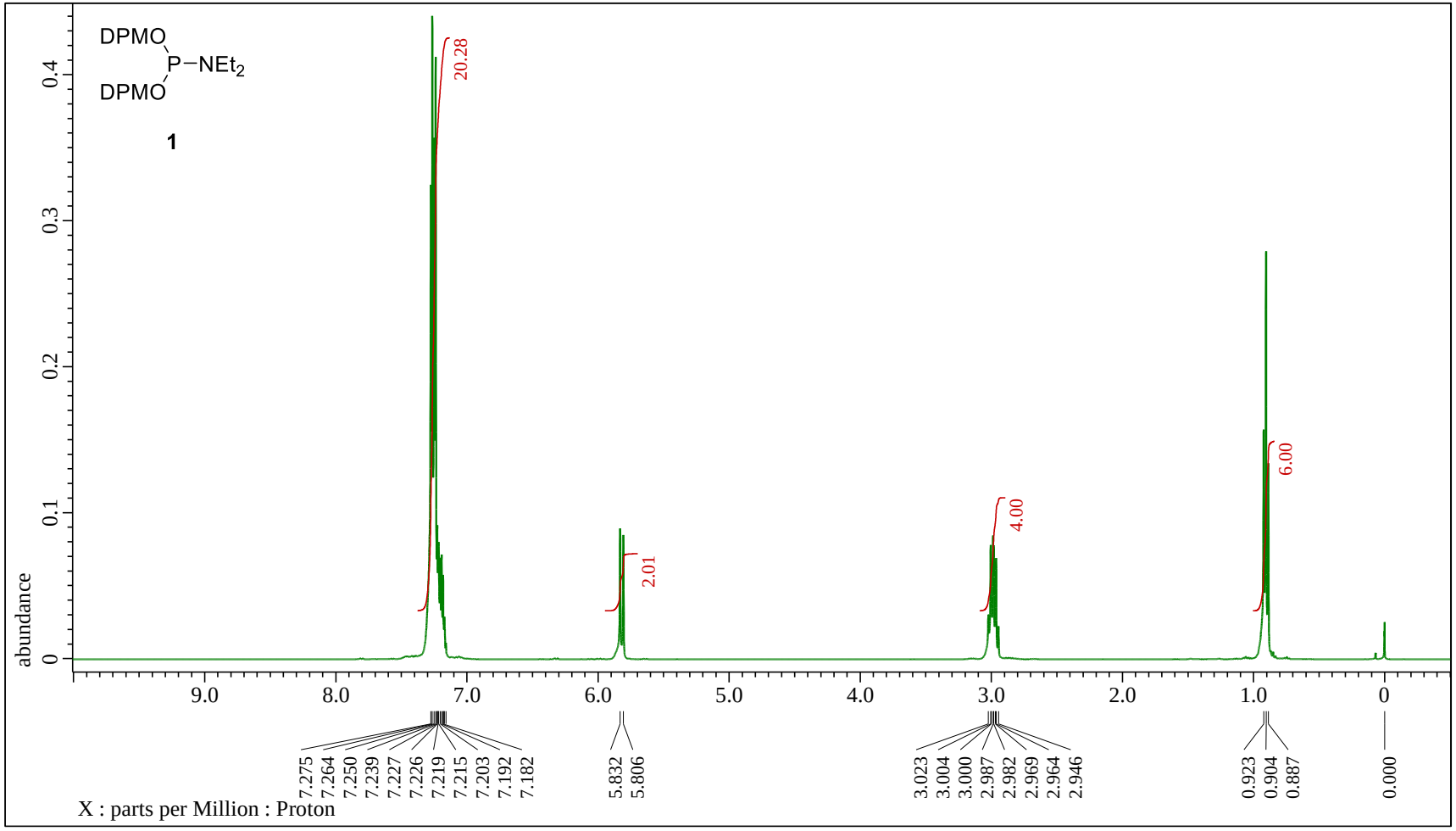


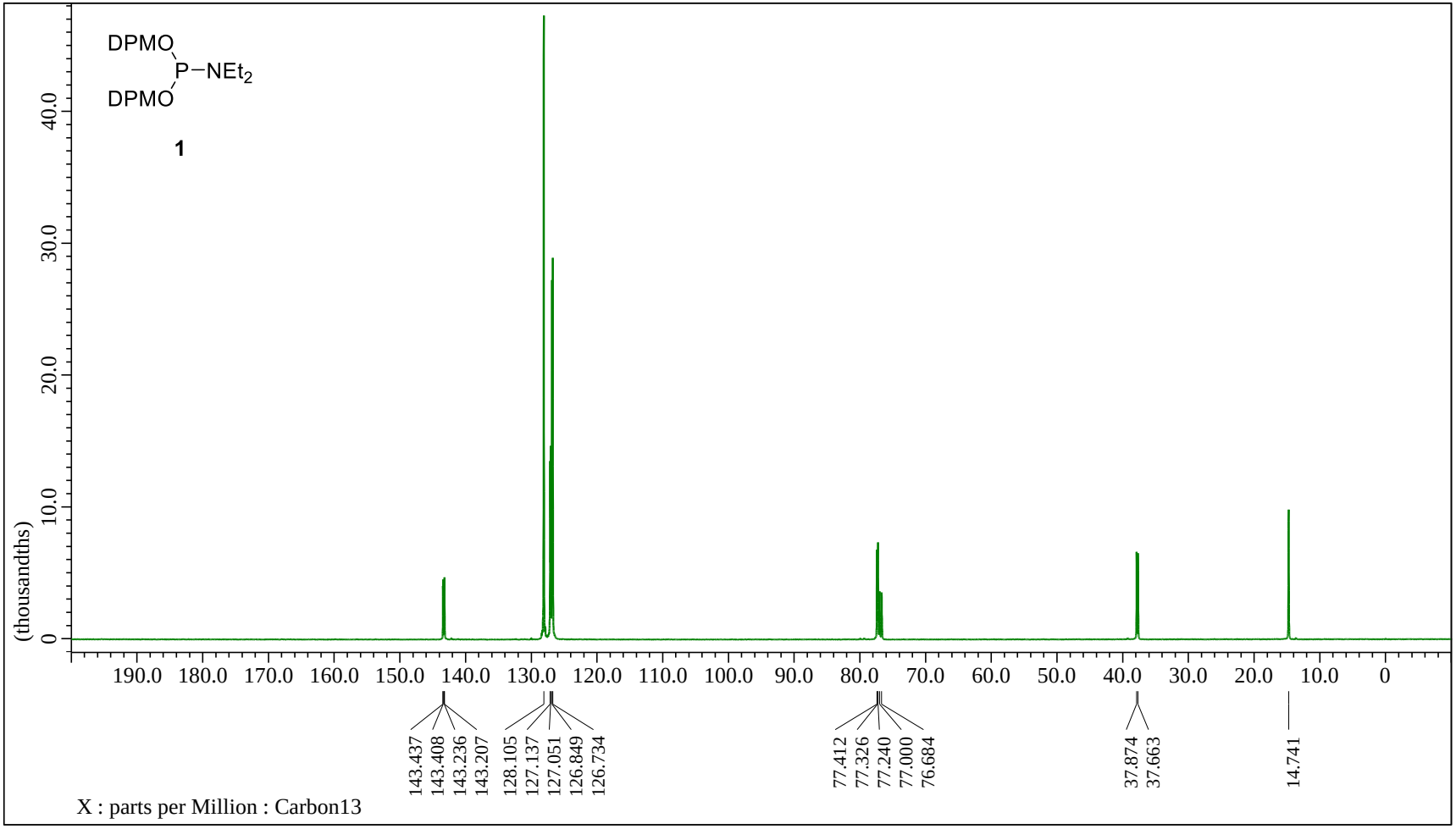
Fig. S15 RP-HPLC profiles of (A) crude d(CGAT) and (B) crude d(GCAT). RP-HPLC was performed with a linear gradient of 0%–24% CH_3CN in 0.1 M TEAA buffer (pH 7.0) over 48 min at rt at a rate of 1.0 mL/min.

5. ^{1}H, ^{13}C, ^{31}P NMR spectra of compounds

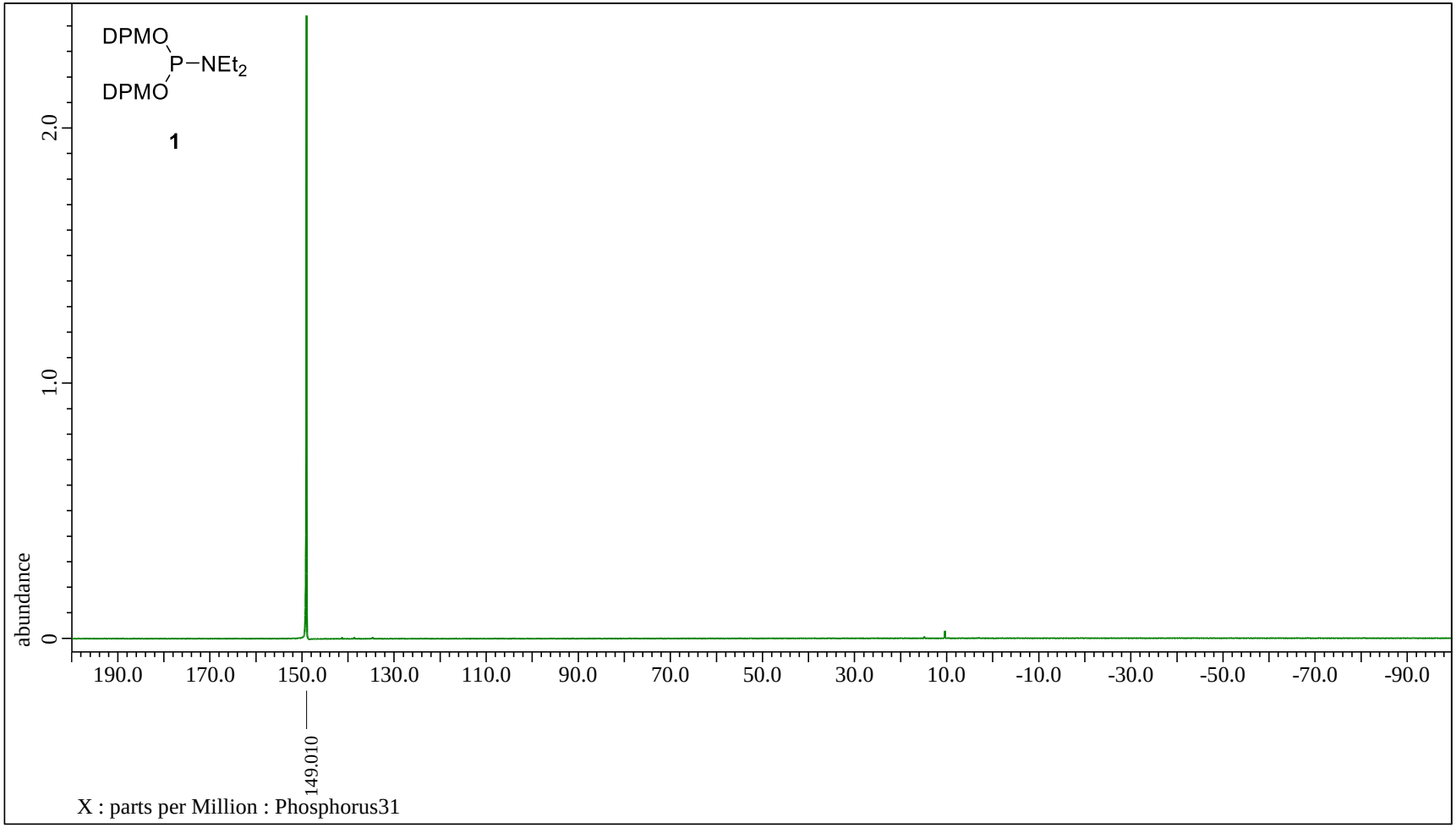
^{1}H NMR ($CDCl_3$, 400 MHz)



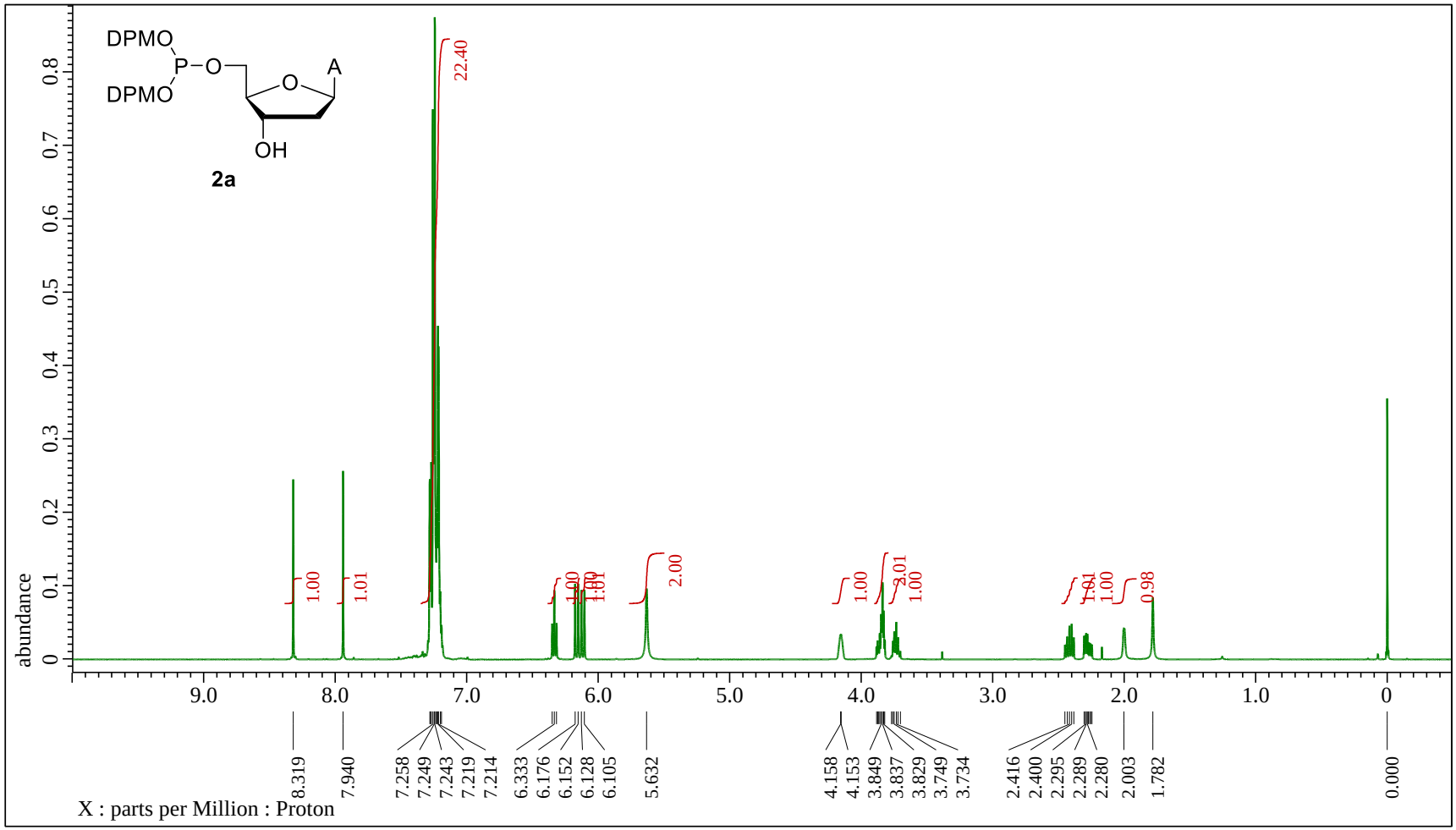
^{13}C NMR ($CDCl_3$, 100 MHz)



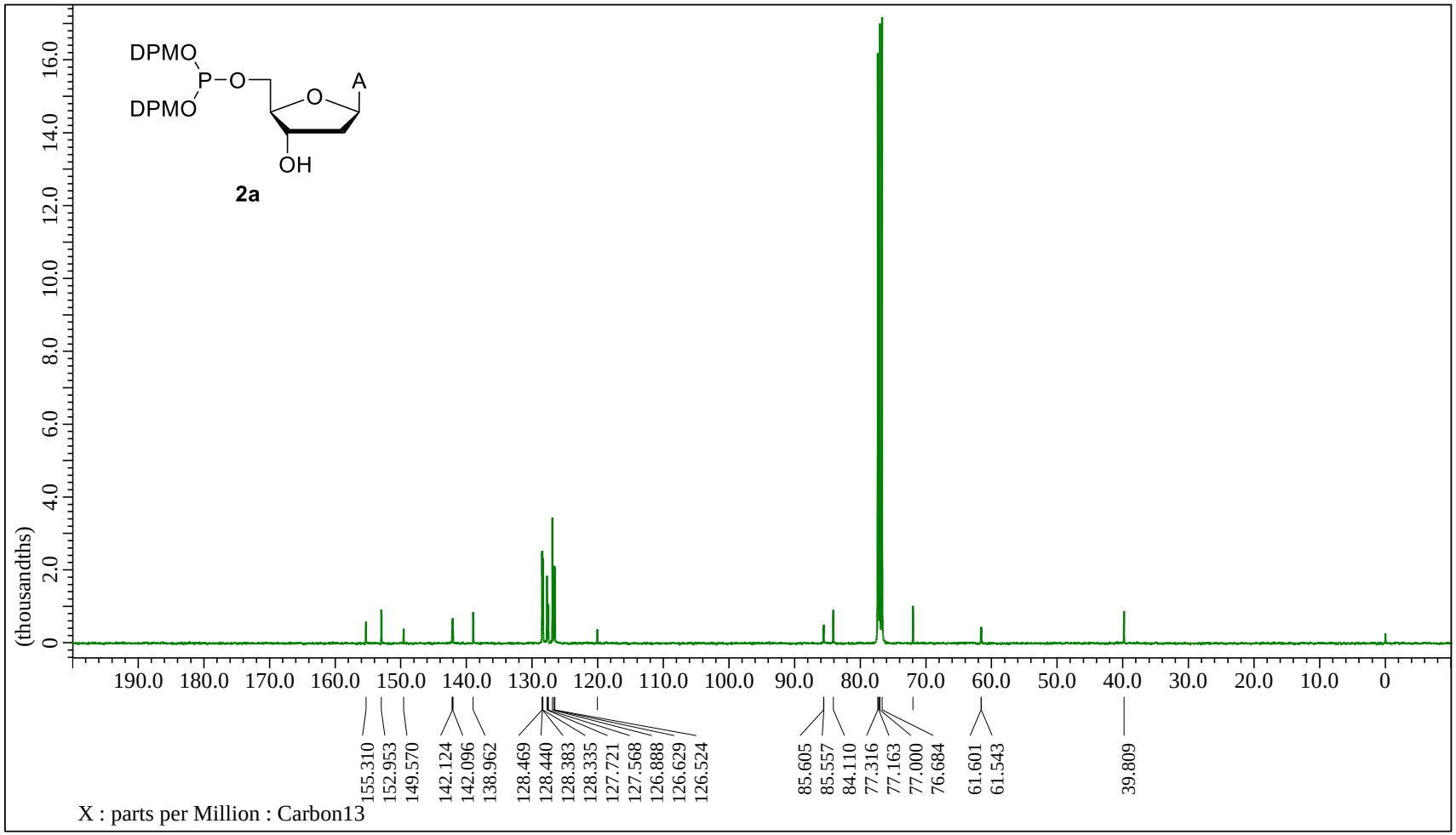
^{31}P NMR ($CDCl_3$, 162 MHz)



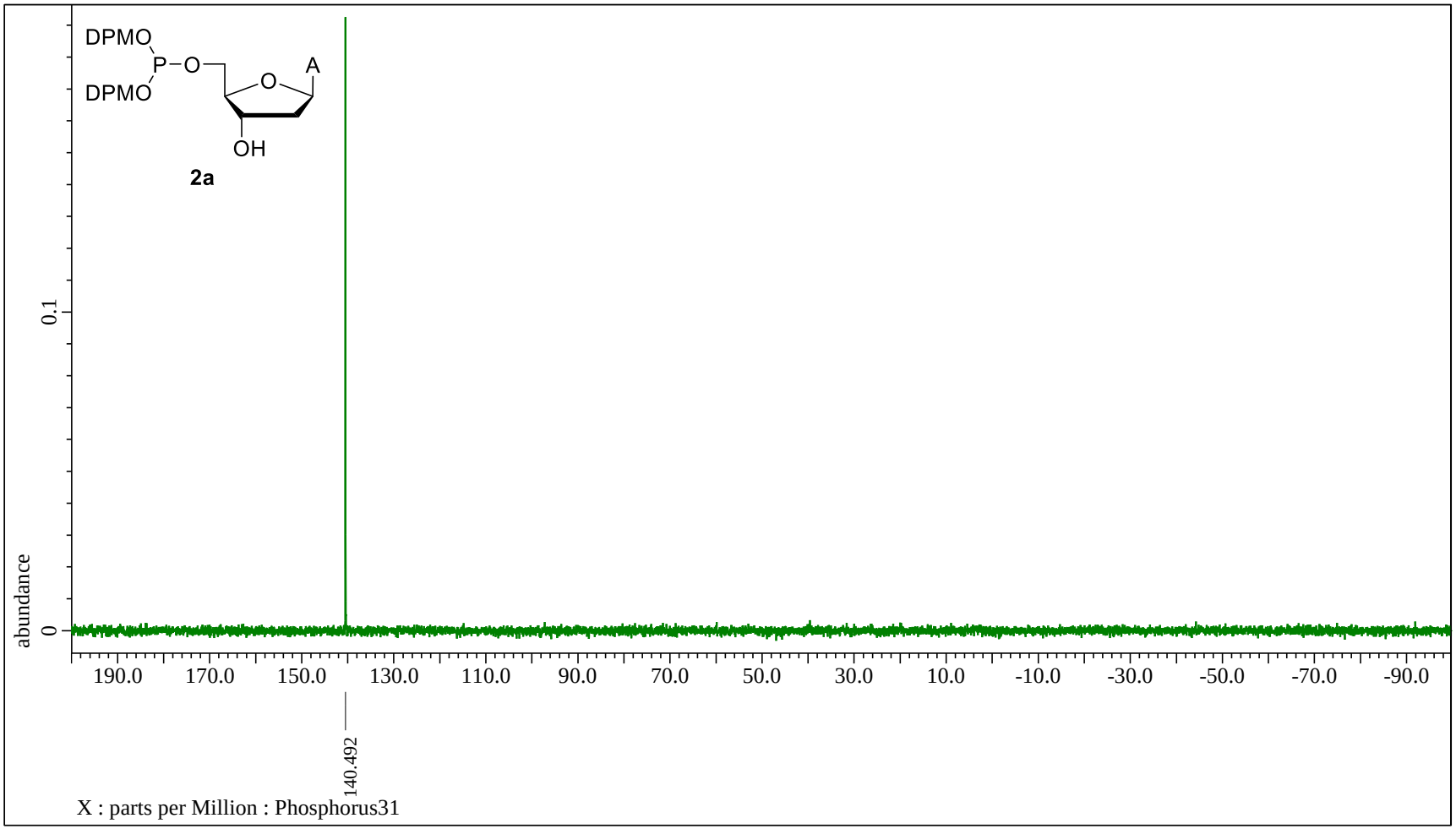
^{1}H NMR ($CDCl_3$, 400 MHz)



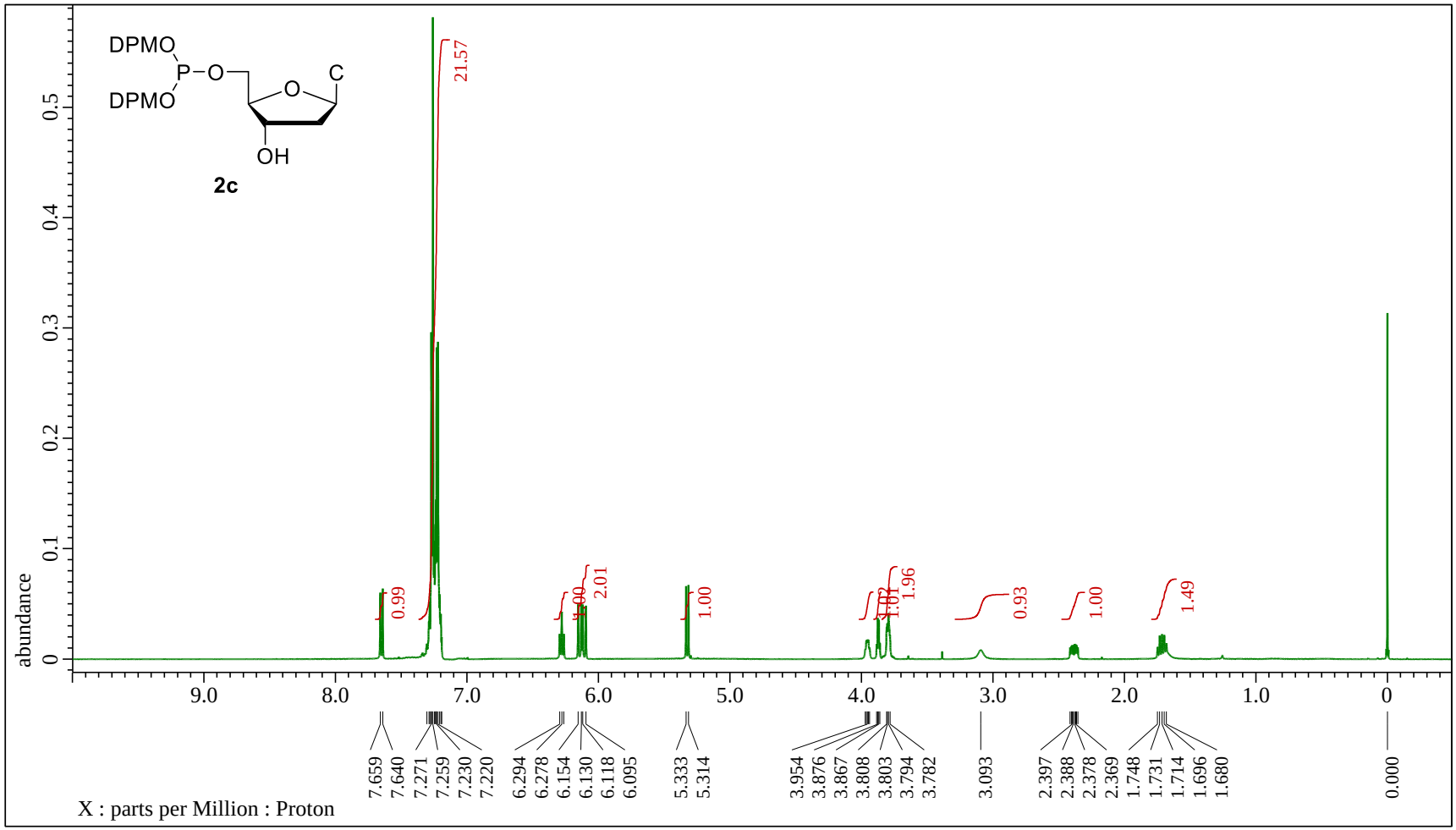
^{13}C NMR ($CDCl_3$, 100 MHz)



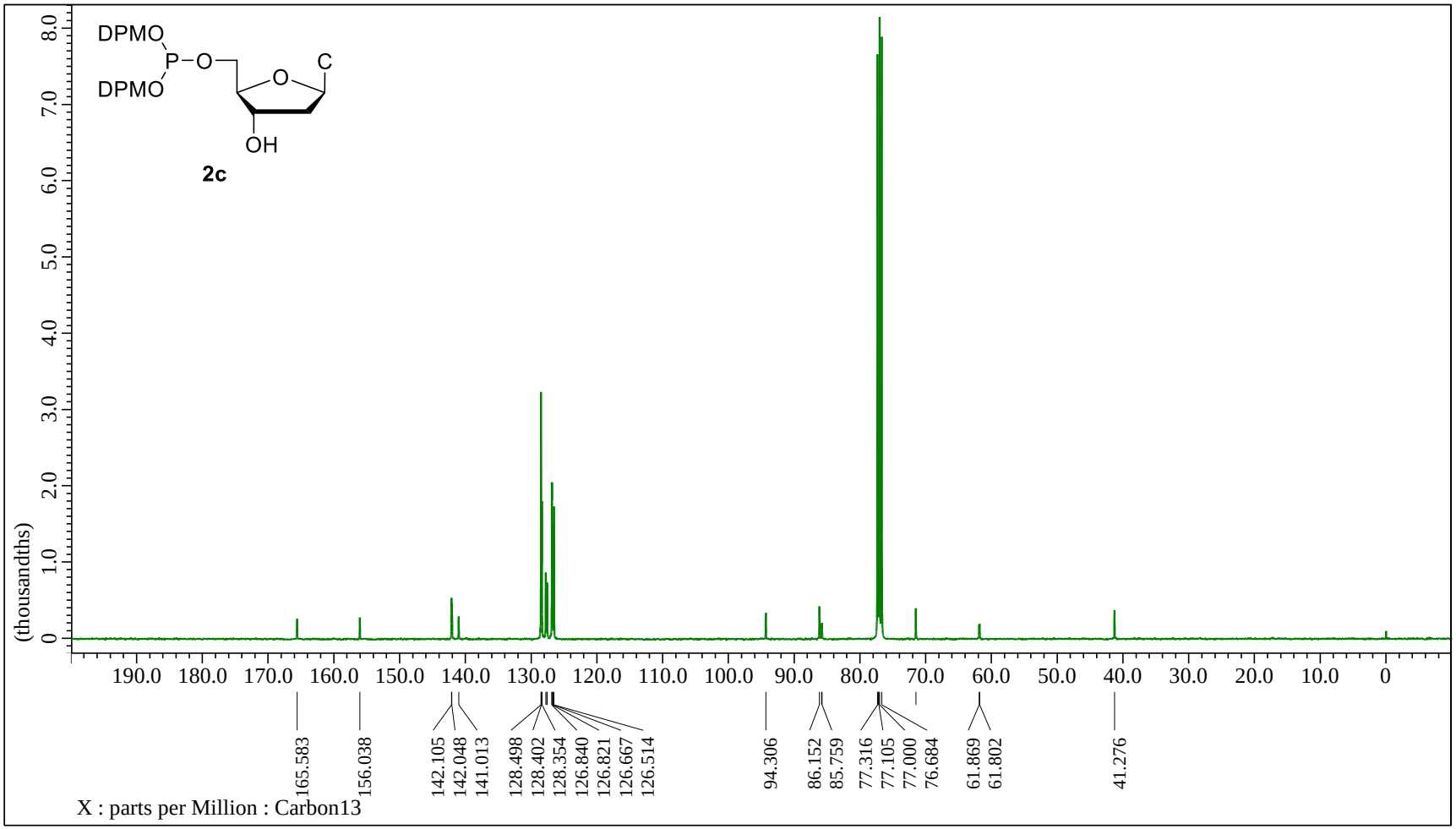
^{31}P NMR ($CDCl_3$, 162 MHz)



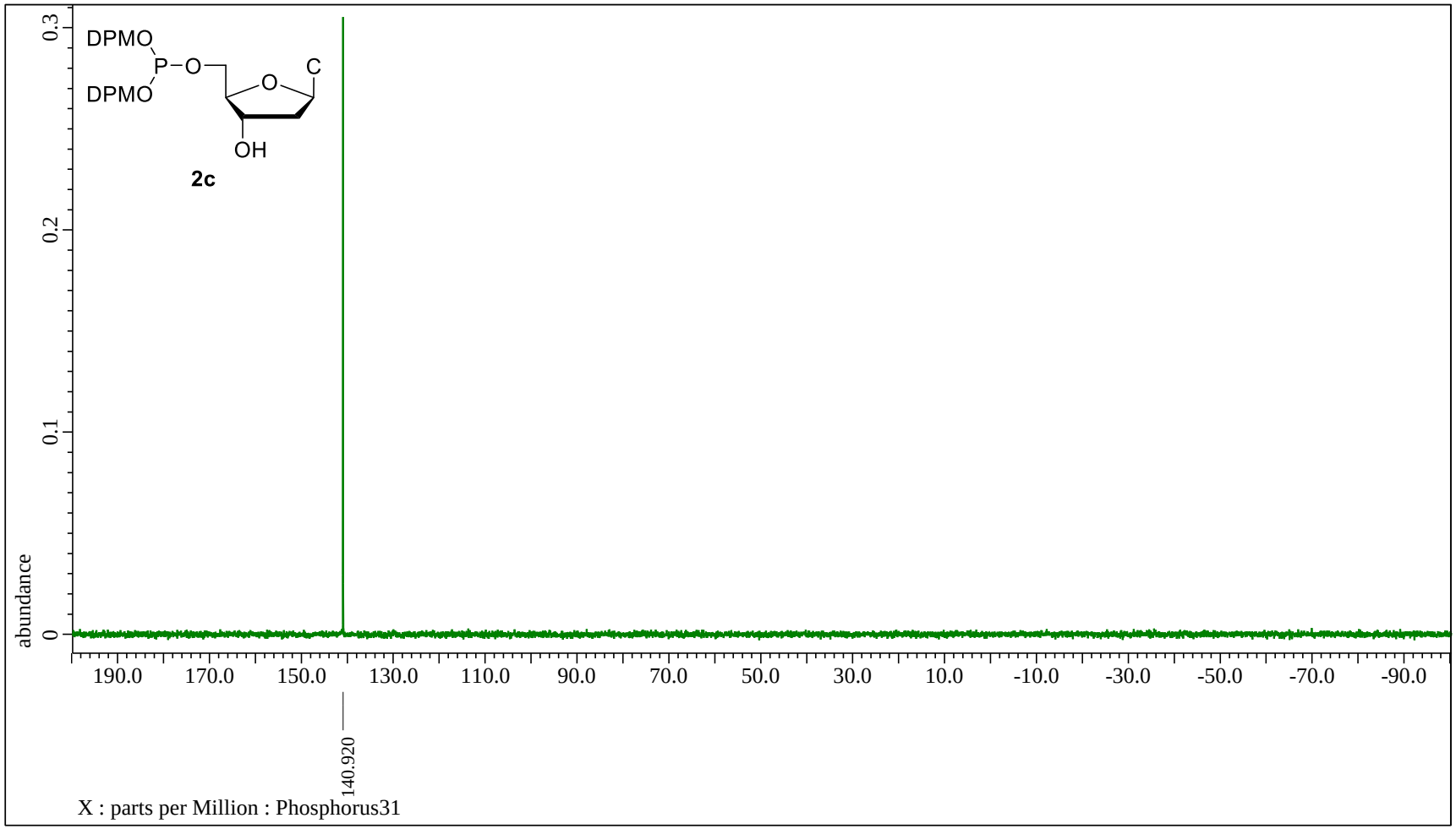
^{1}H NMR ($CDCl_3$, 400 MHz)



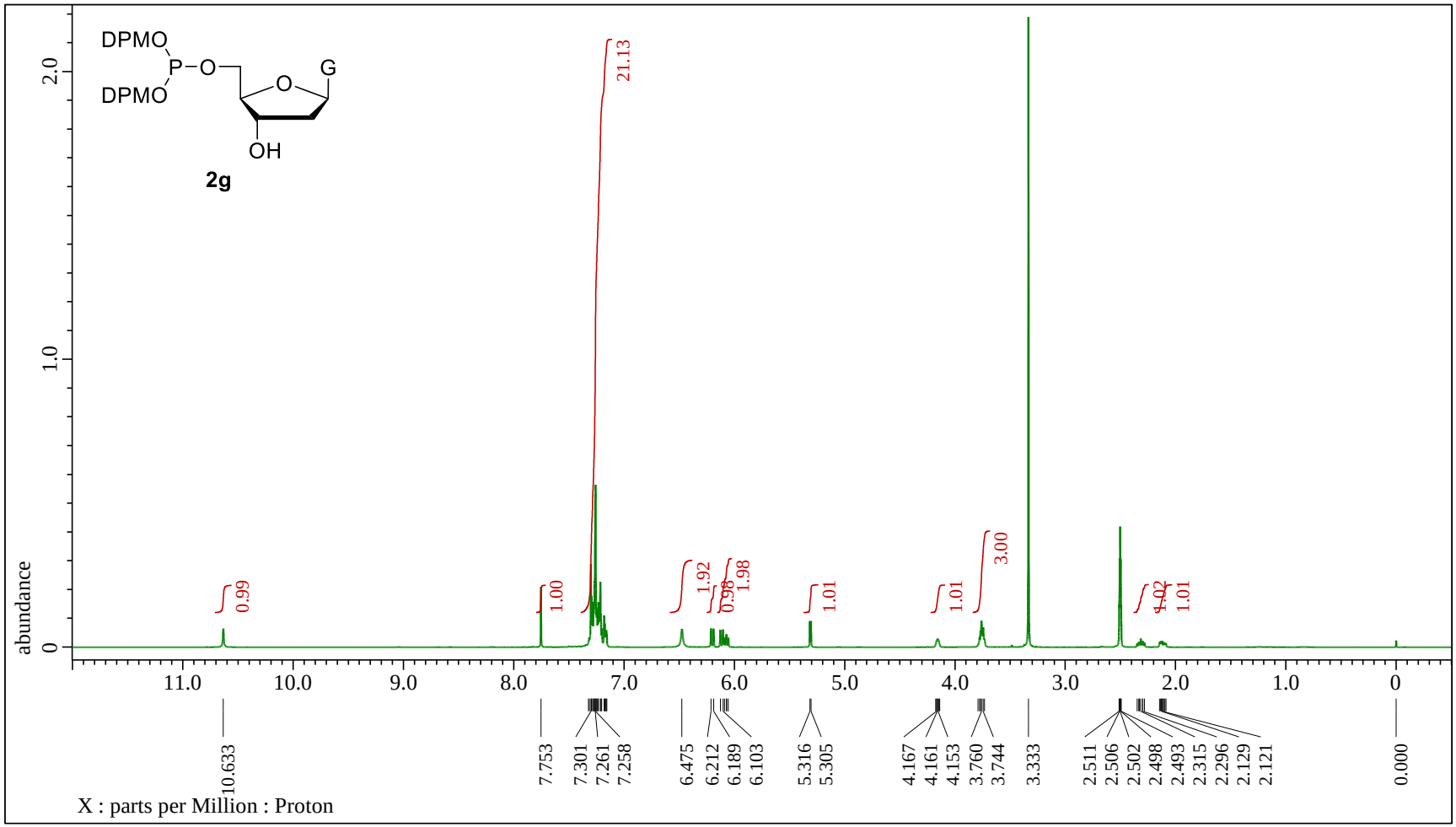
^{13}C NMR ($CDCl_3$, 100 MHz)



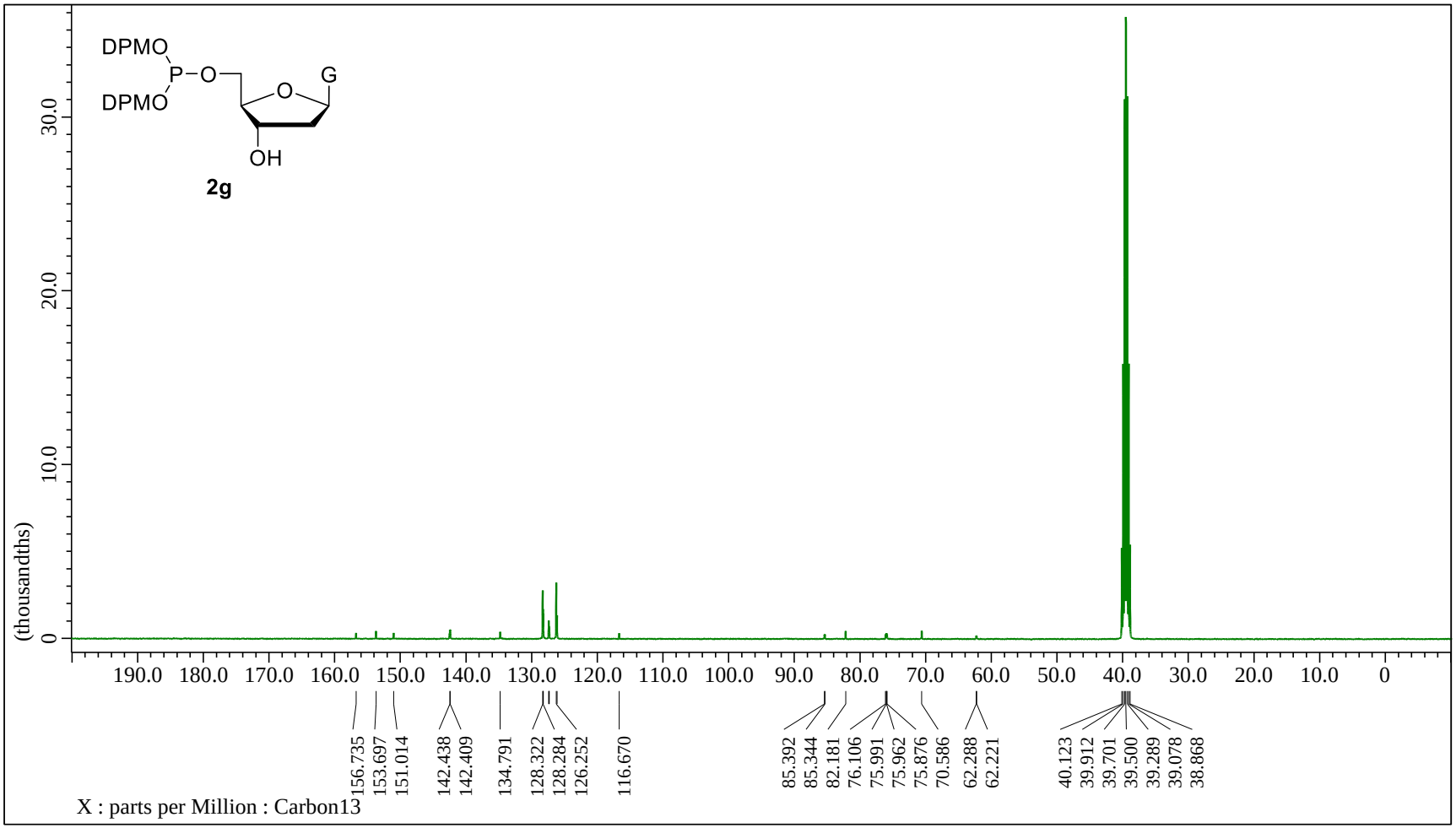
^{31}P NMR ($CDCl_3$, 162 MHz)



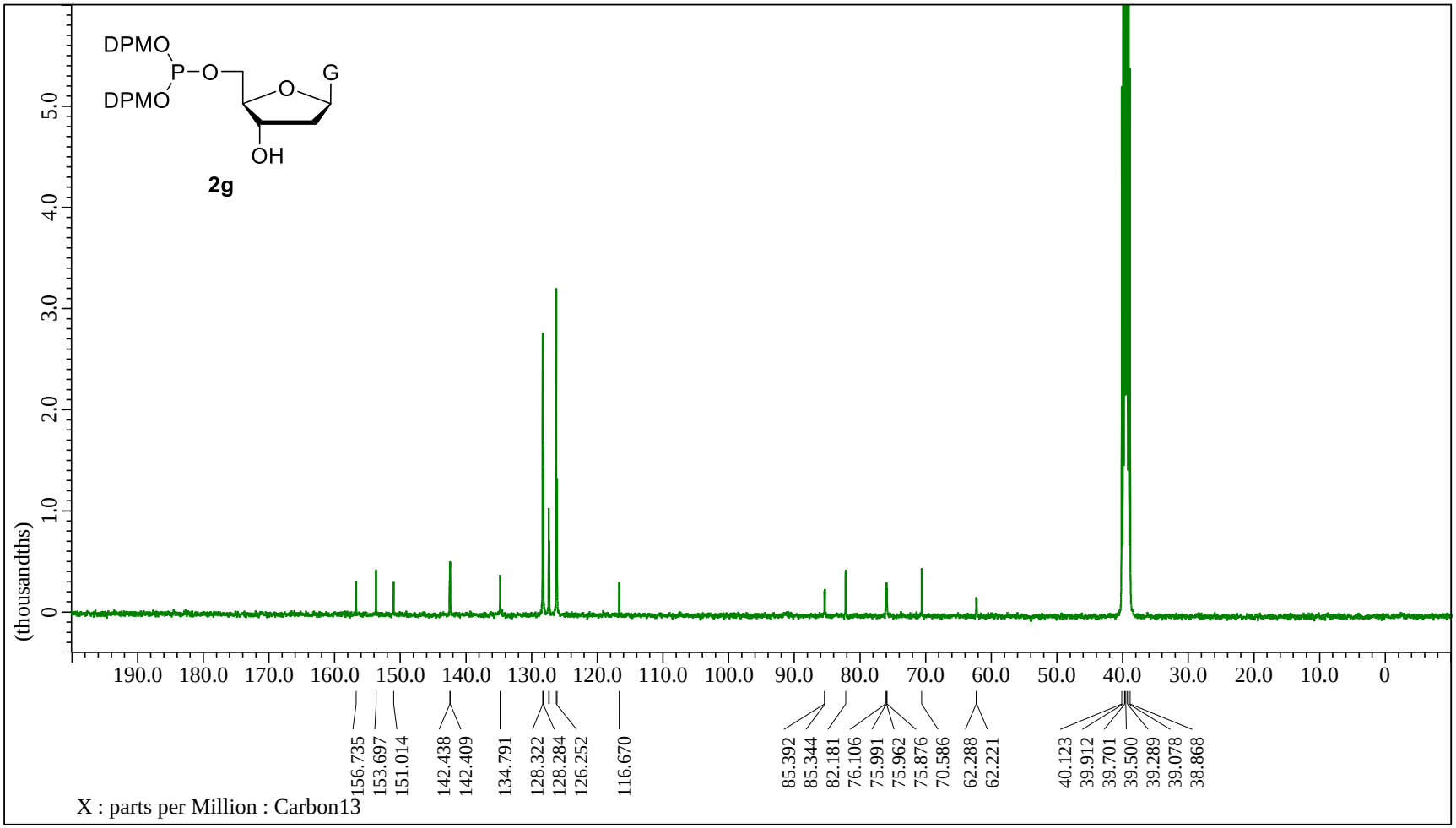
^{1}H NMR (DMSO-d_6, 400 MHz)



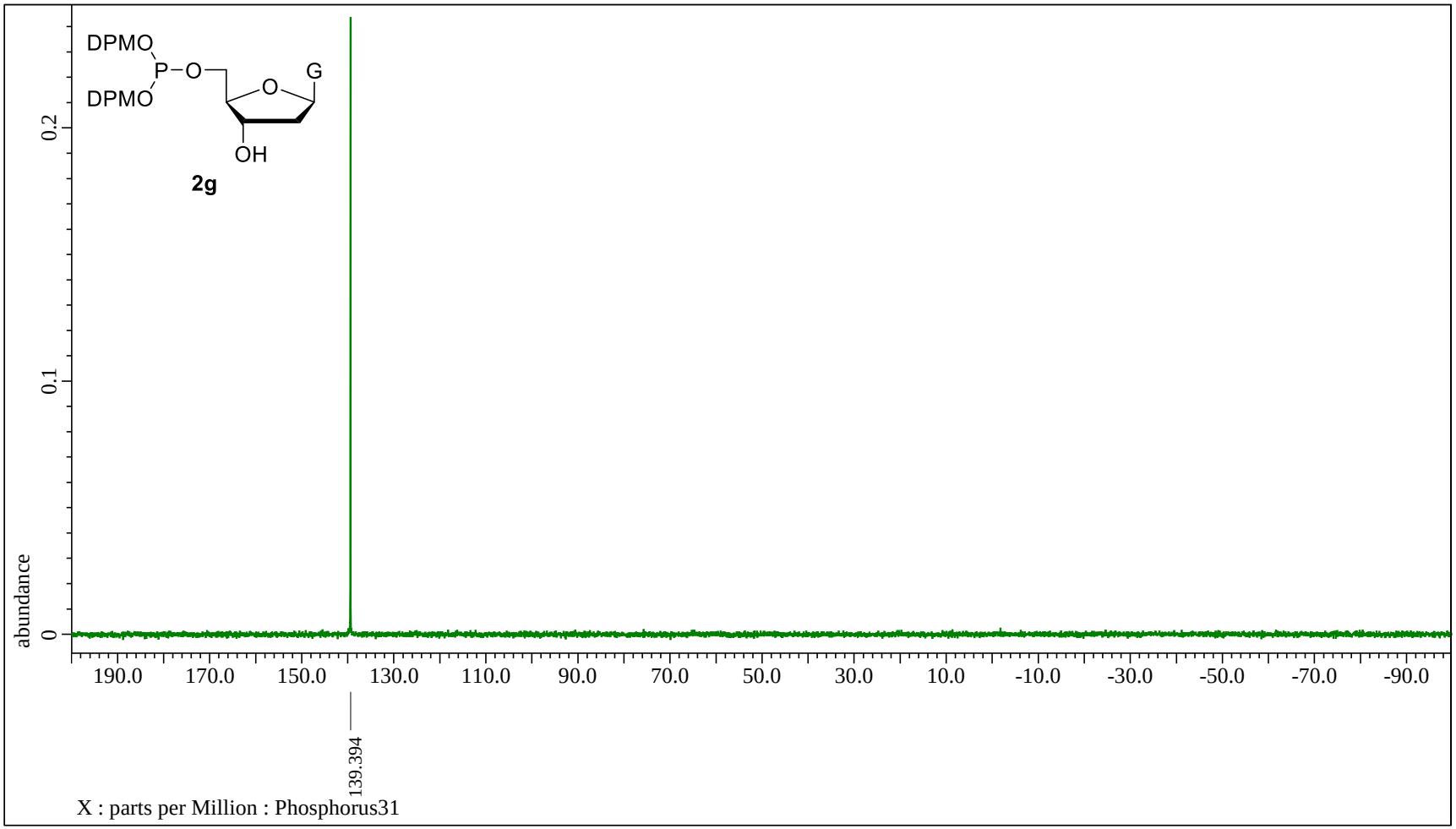
^{13}C NMR (DMSO-d_6, 100 MHz)



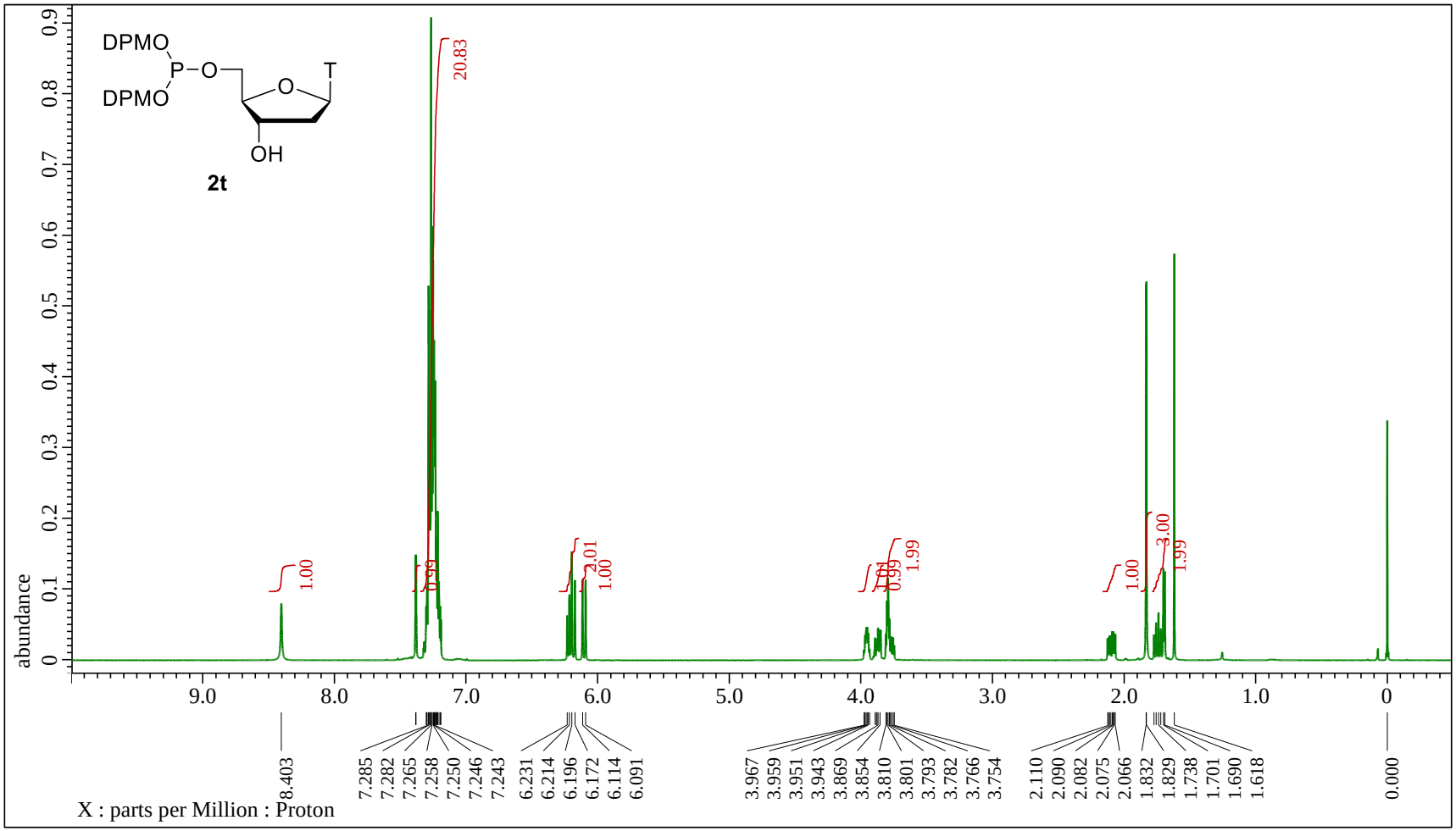
^{13}C NMR (DMSO-d_6, 100 MHz) (magnified)



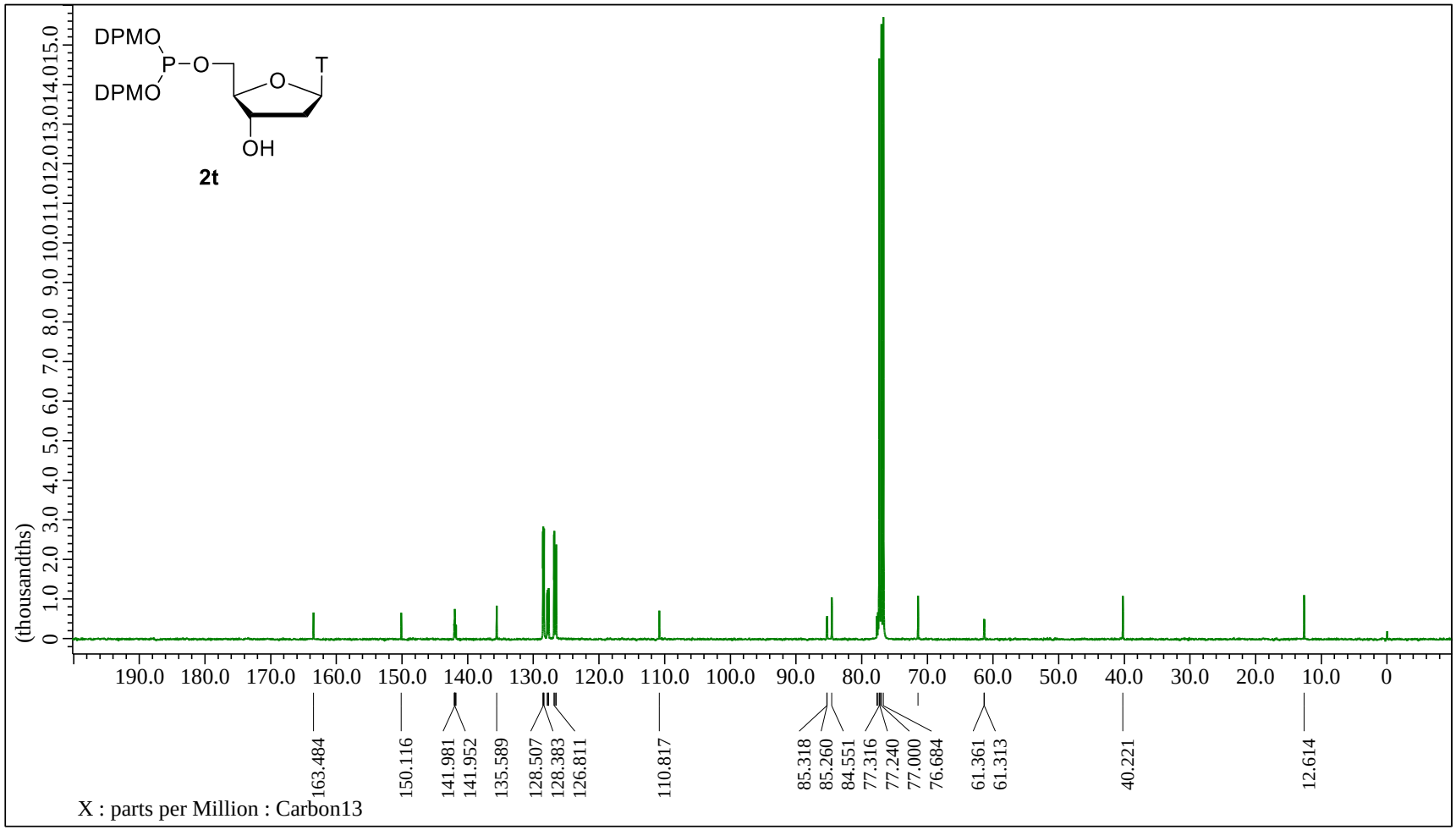
^{31}P NMR (DMSO-d_6, 162 MHz)



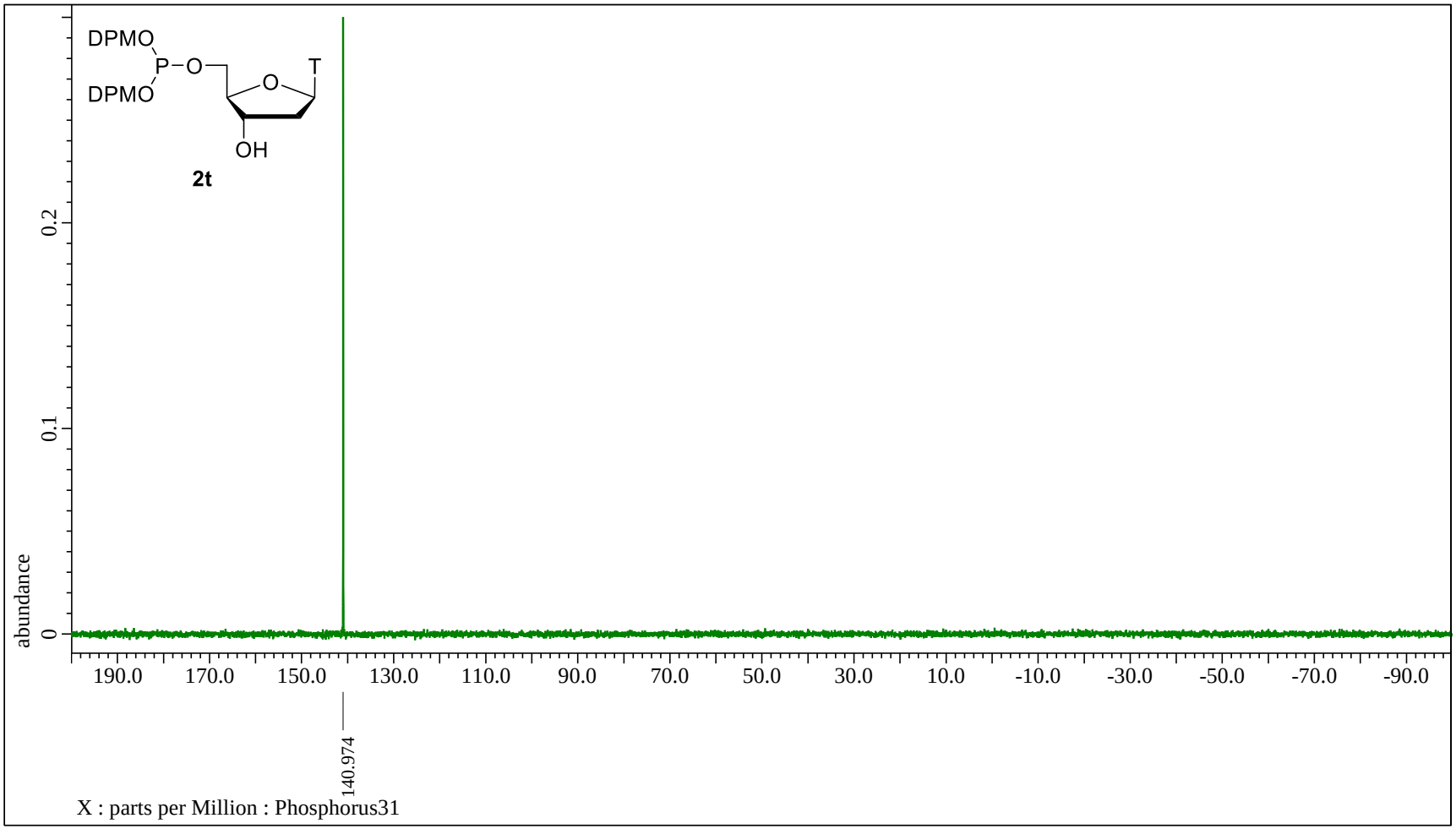
^{1}H NMR ($CDCl_3$, 400 MHz)



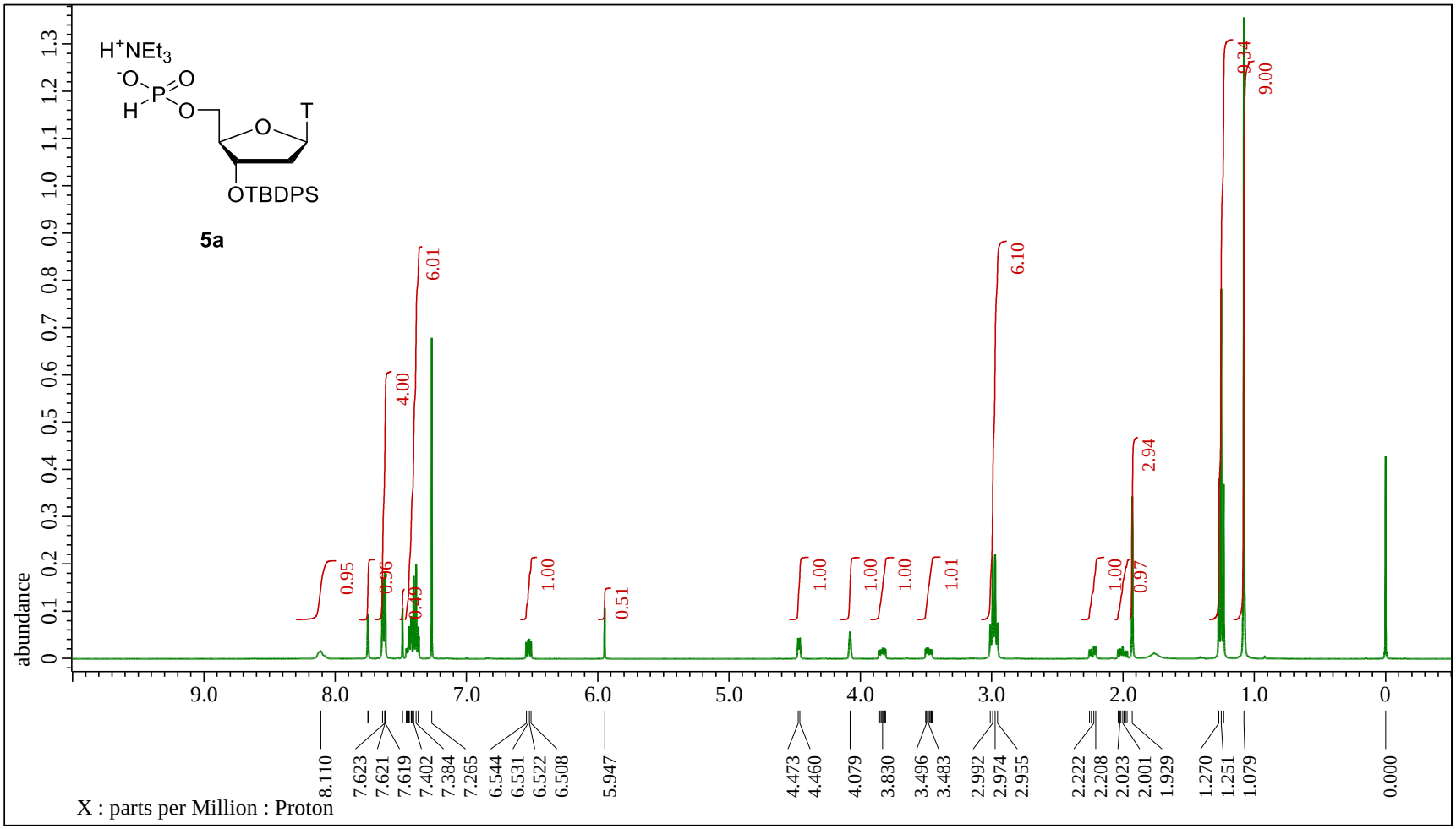
^{13}C NMR ($CDCl_3$, 100 MHz)



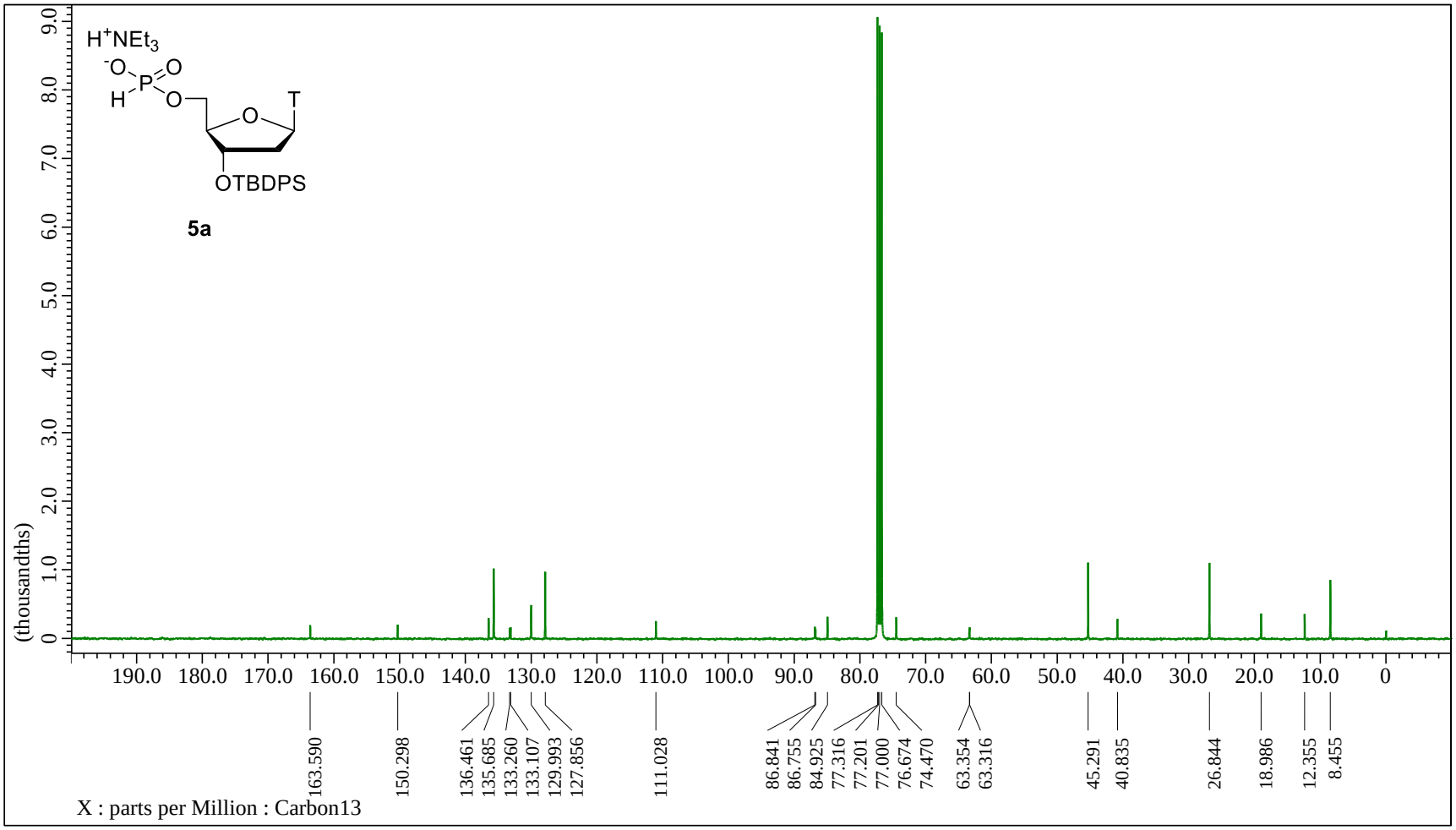
^{31}P NMR ($CDCl_3$, 162 MHz)



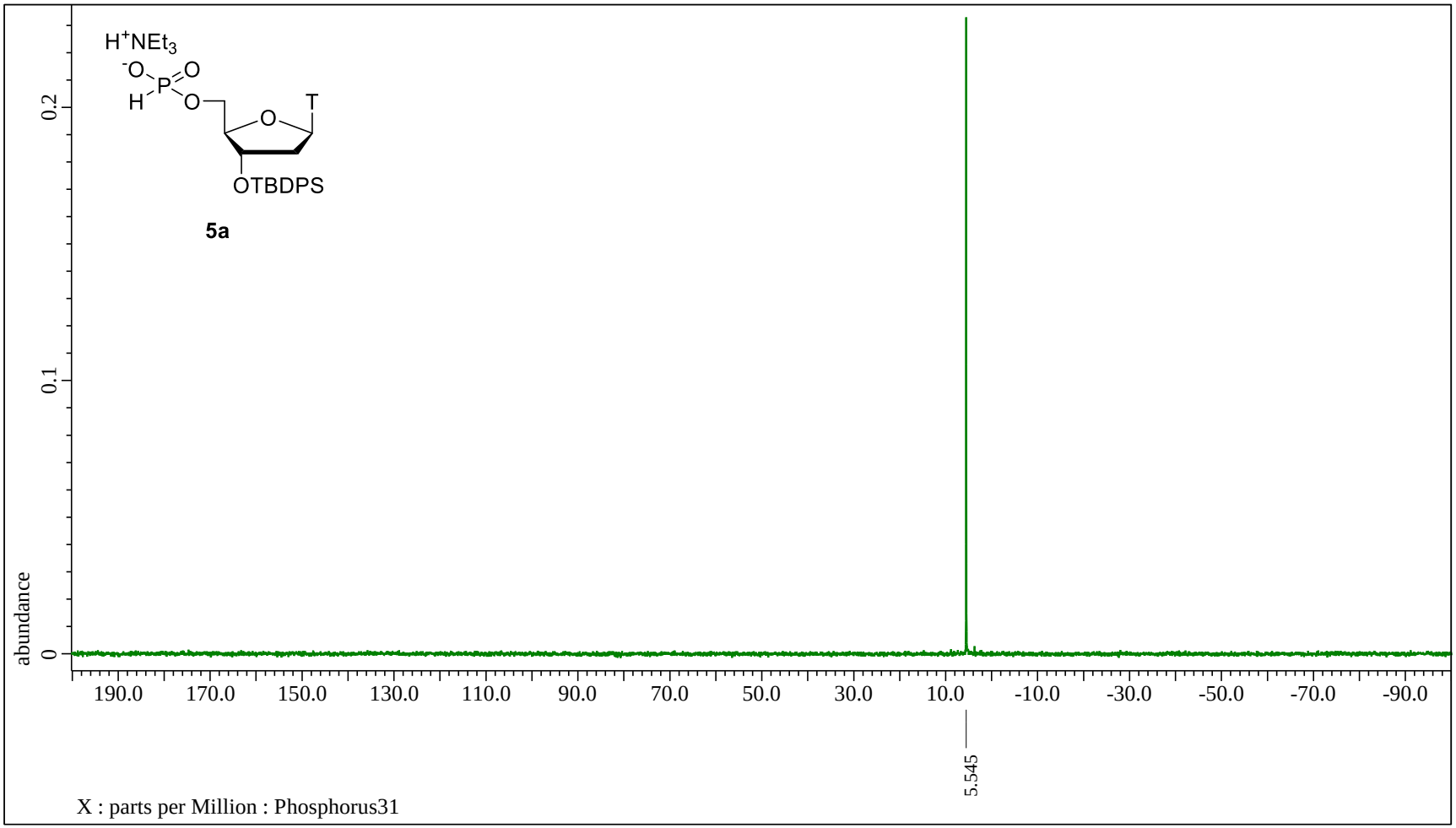
^{1}H NMR ($CDCl_3$, 400 MHz)



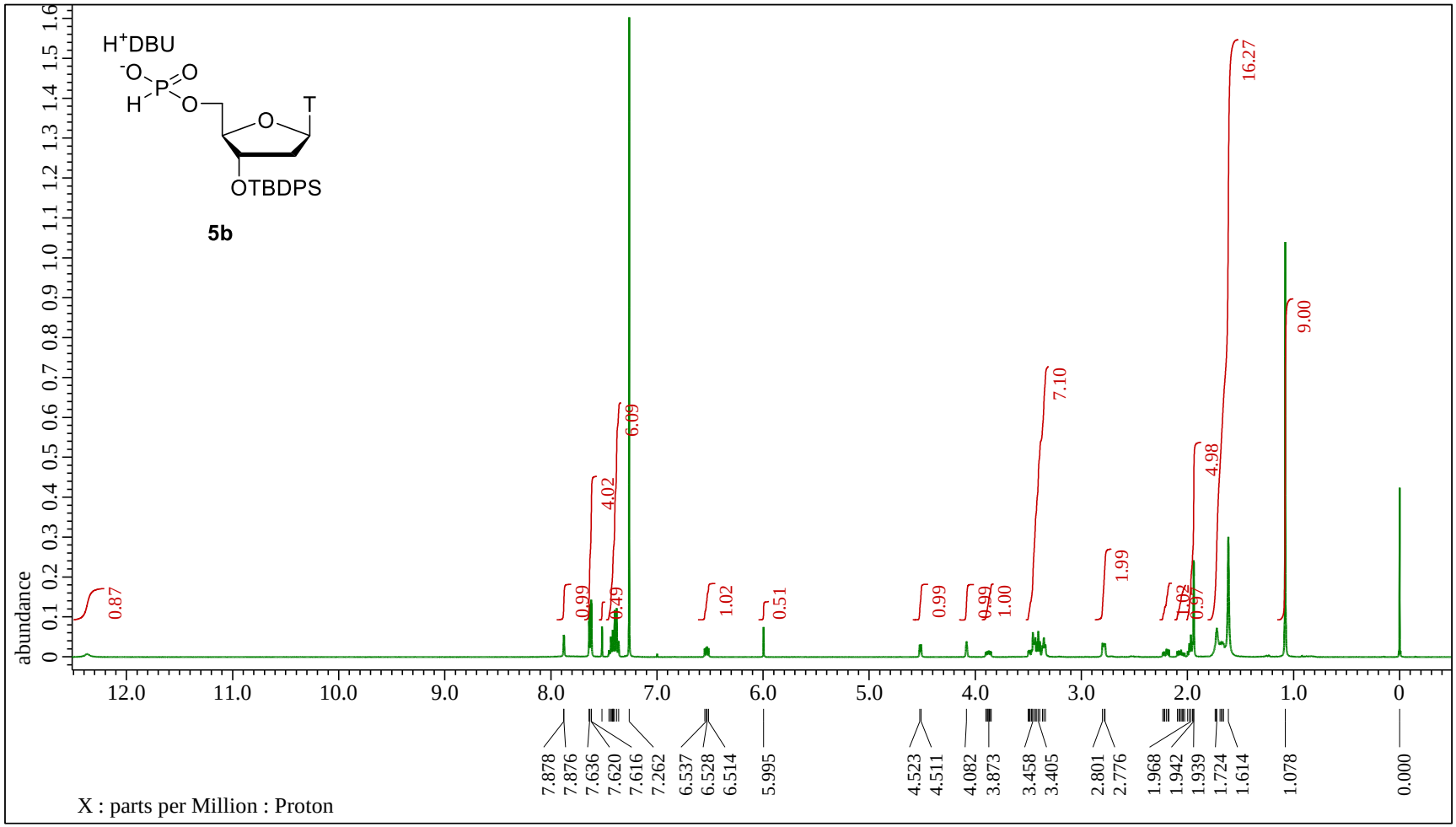
^{13}C NMR ($CDCl_3$, 100 MHz)



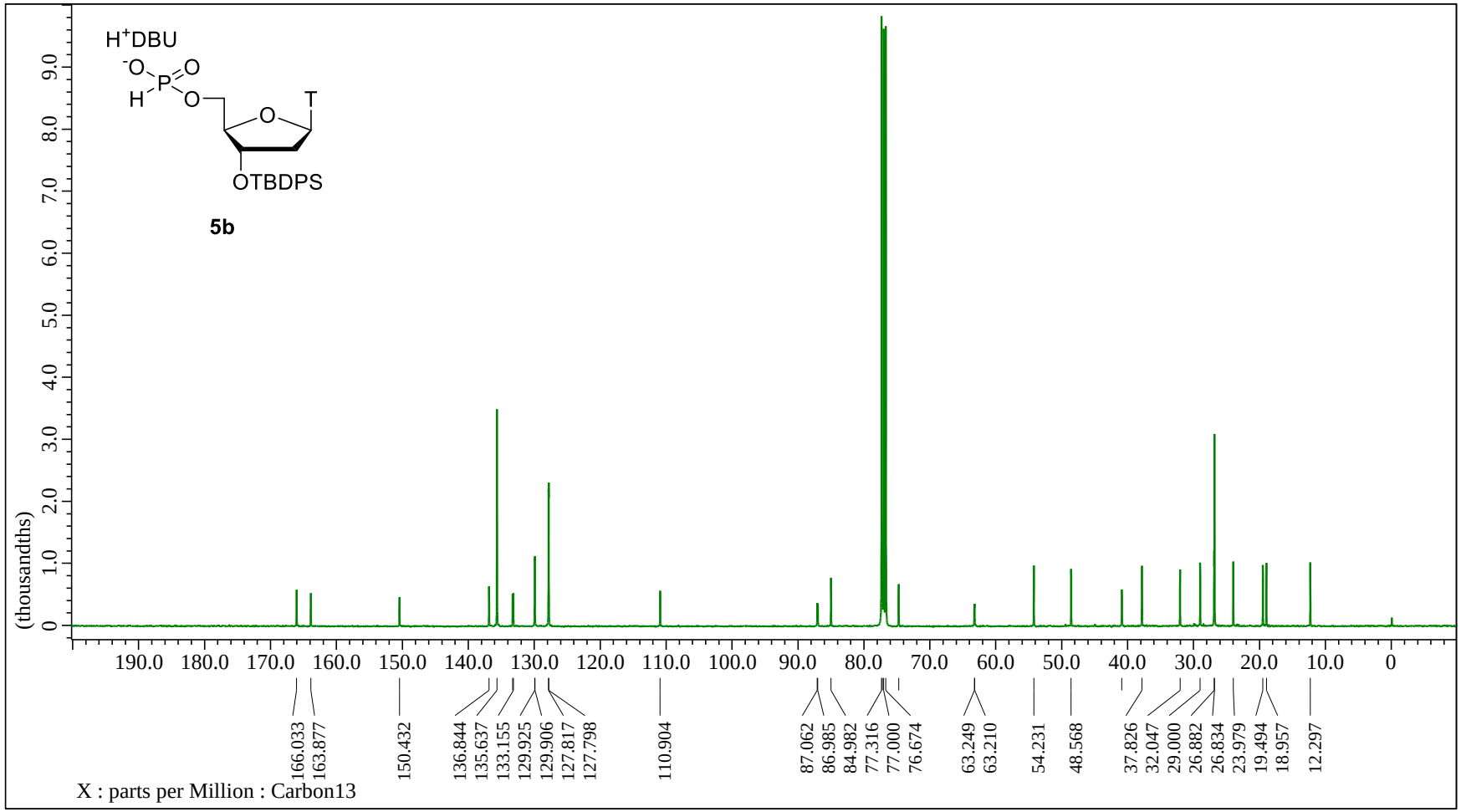
^{31}P NMR ($CDCl_3$, 162 MHz)



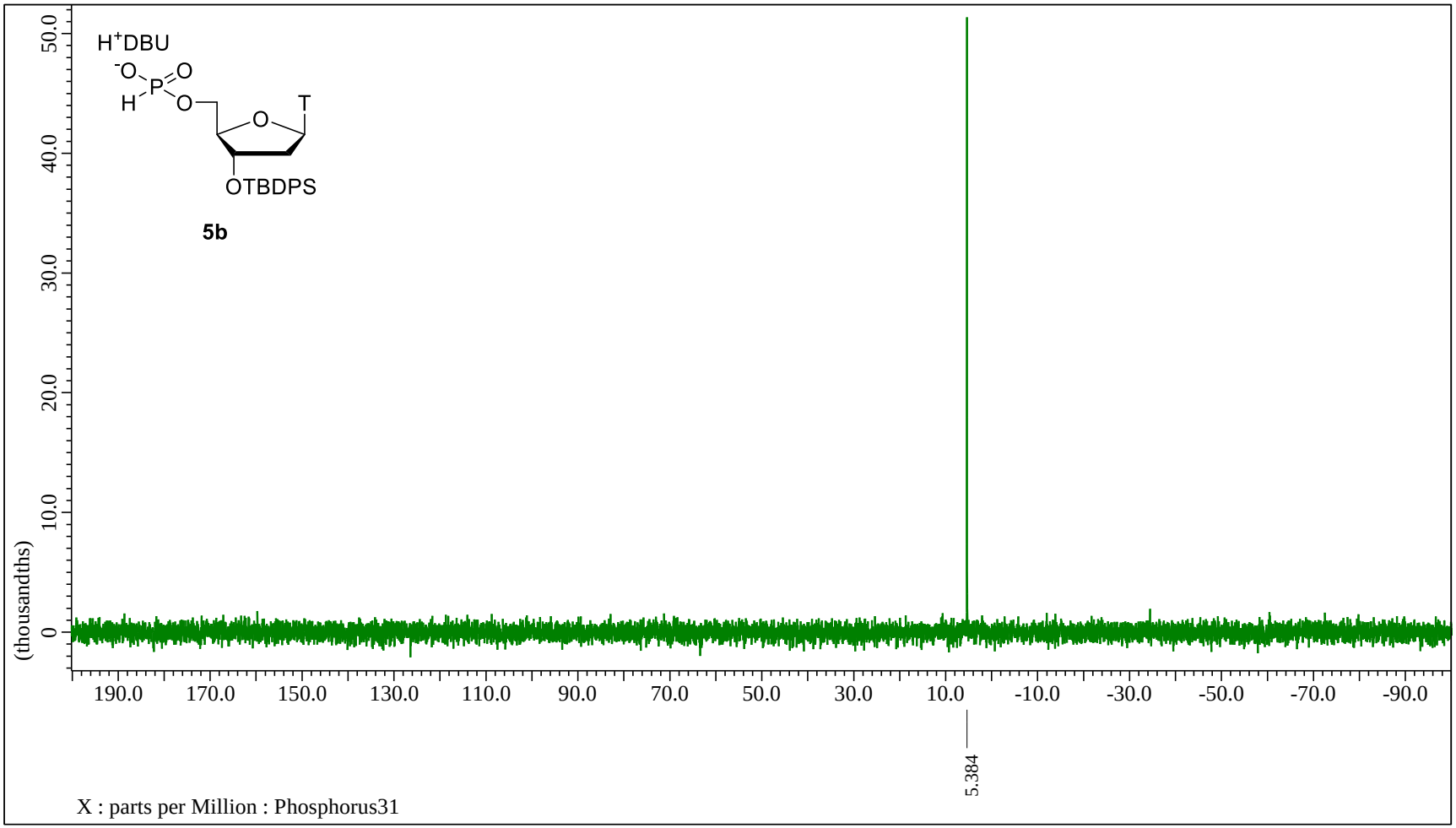
^{1}H NMR ($CDCl_3$, 400 MHz)



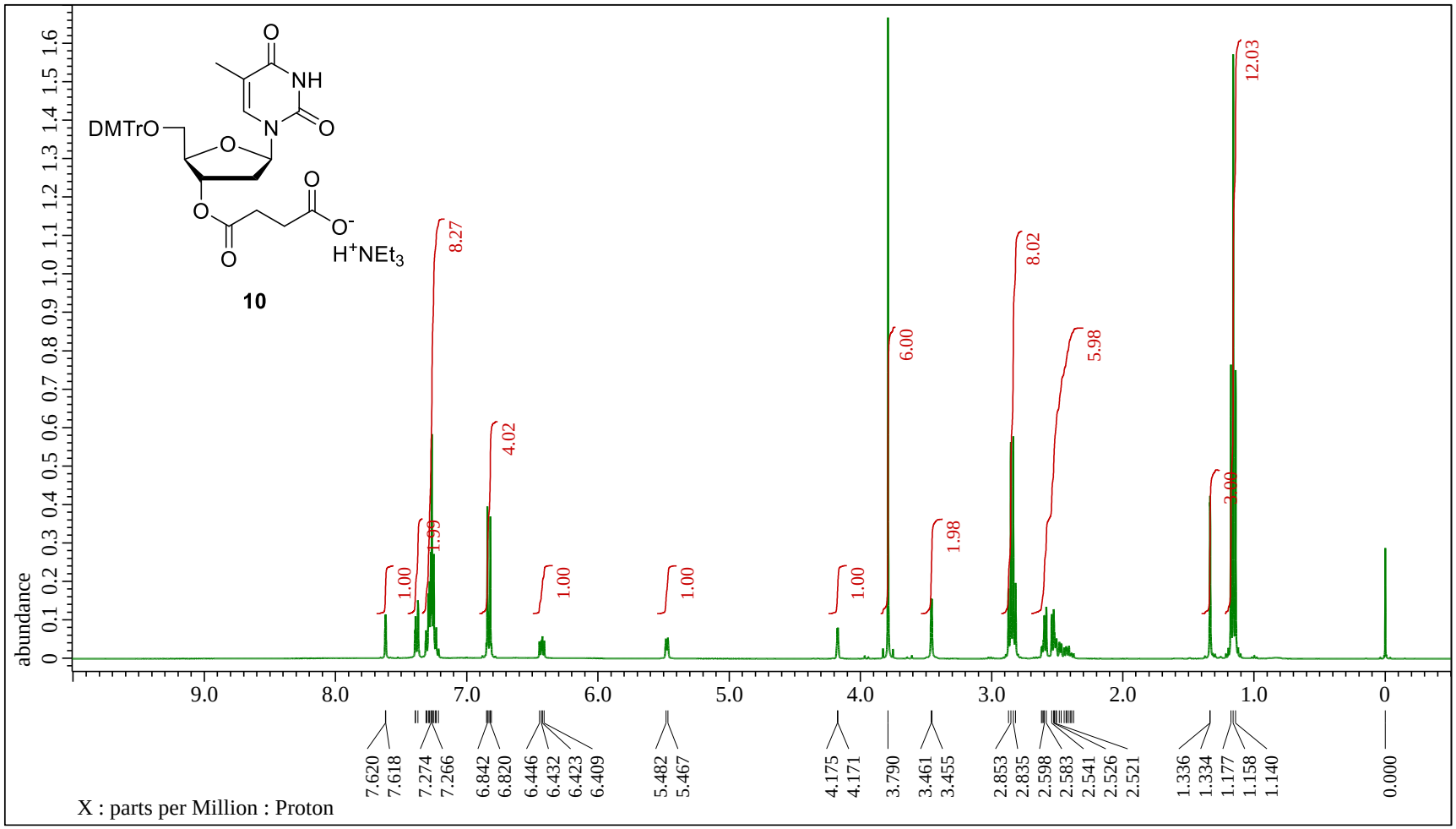
^{13}C NMR ($CDCl_3$, 100 MHz)



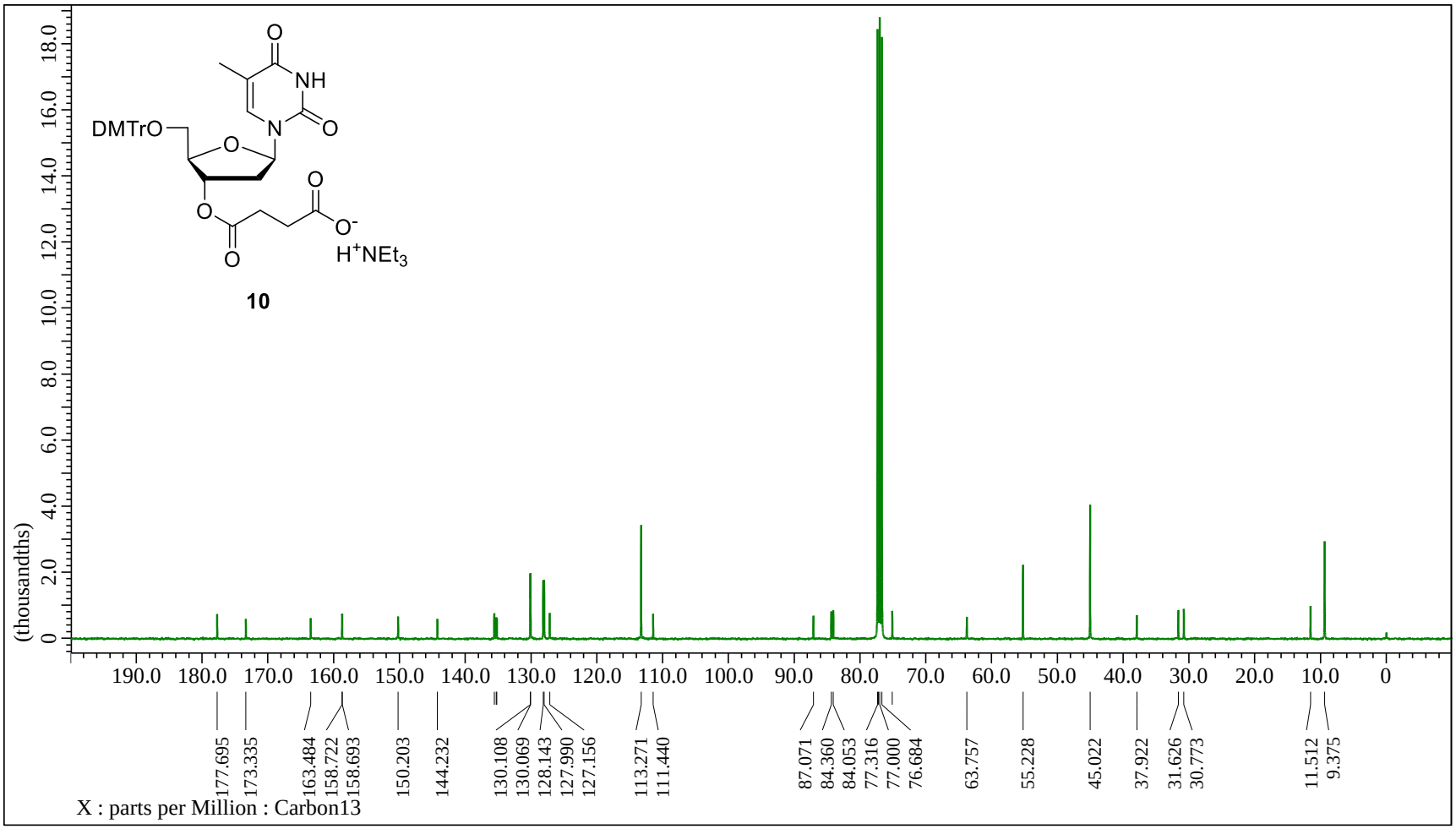
^{31}P NMR ($CDCl_3$, 162 MHz)



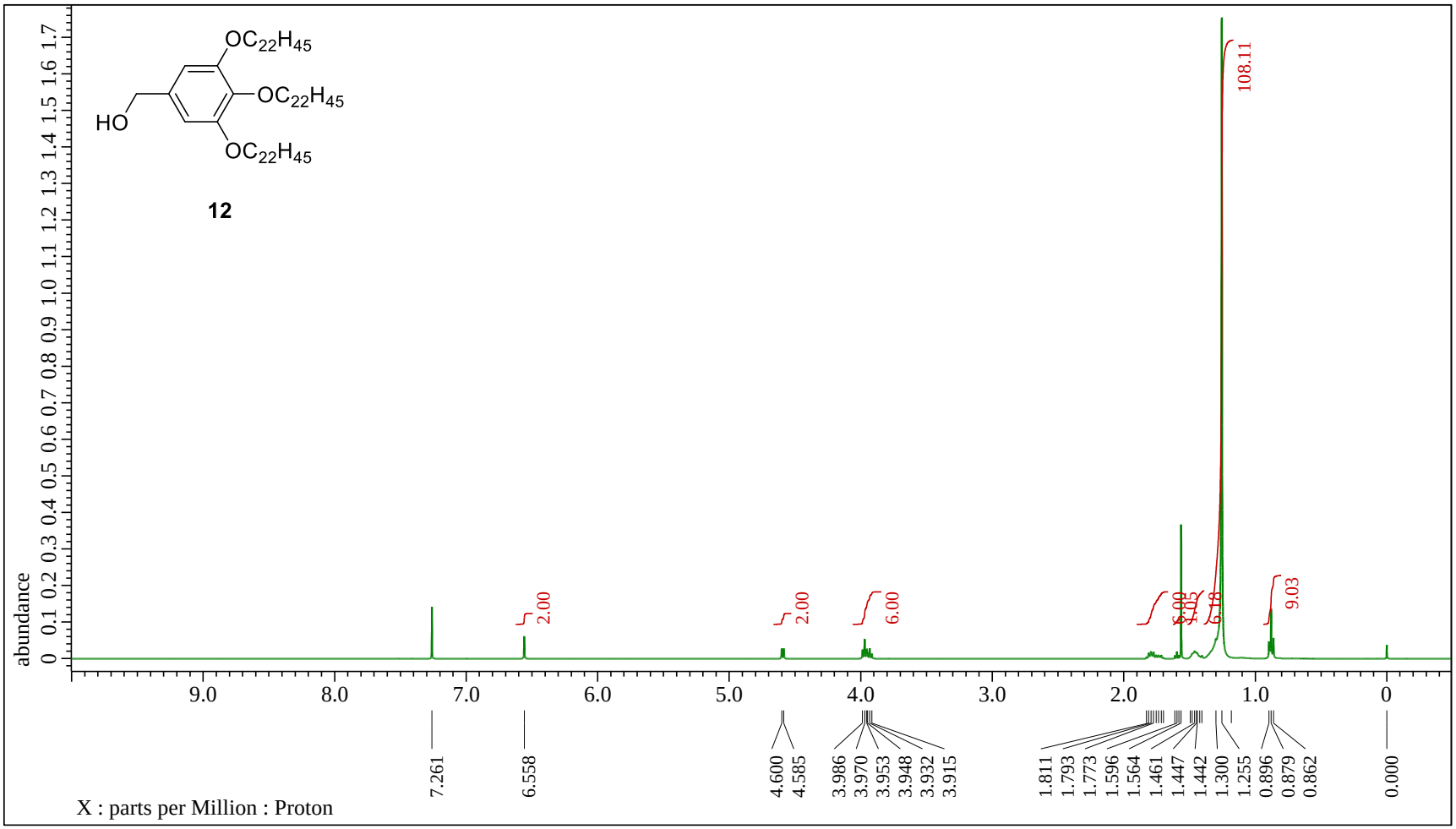
^{1}H NMR ($CDCl_3$, 400 MHz)



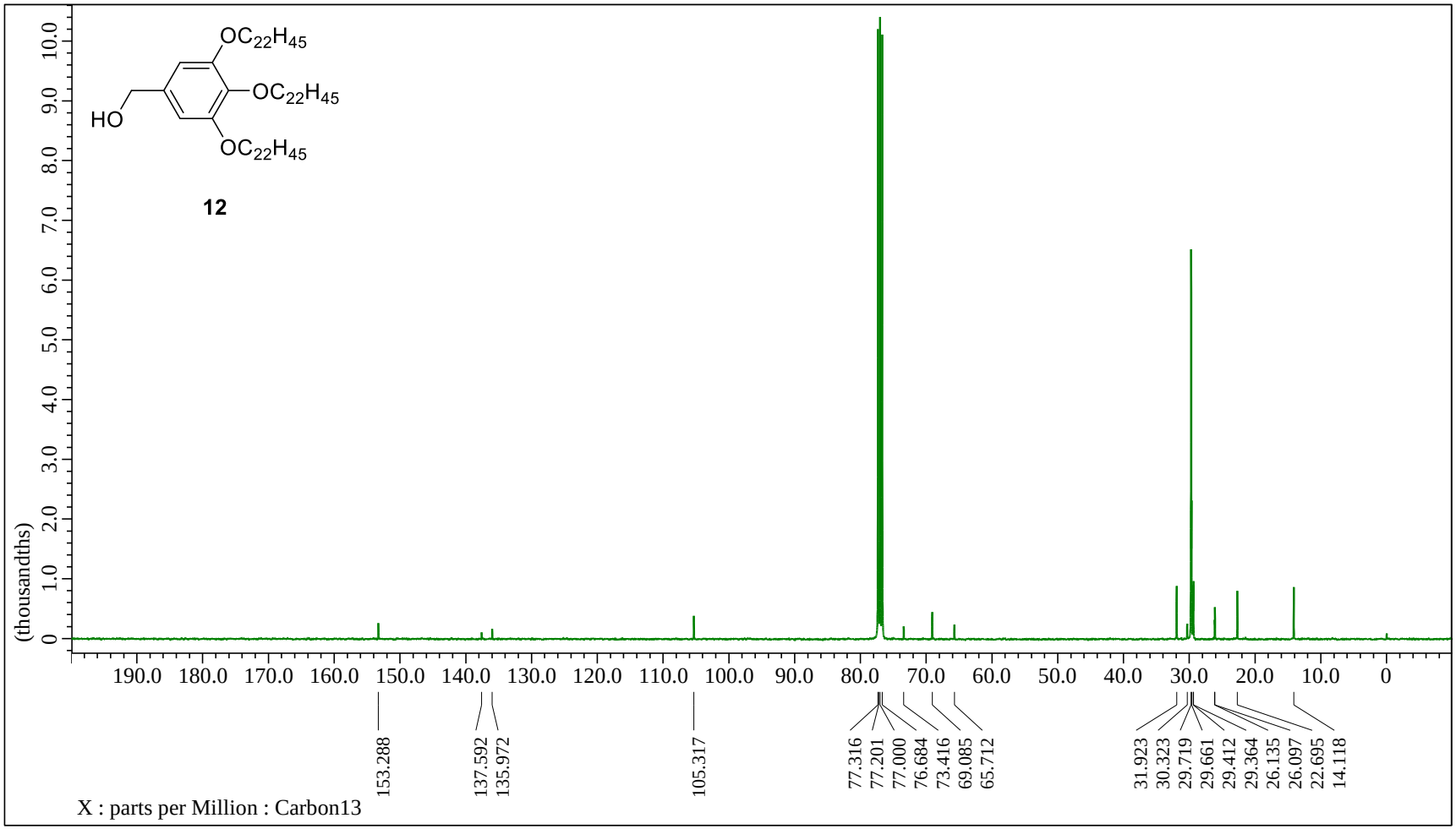
^{13}C NMR ($CDCl_3$, 100 MHz)



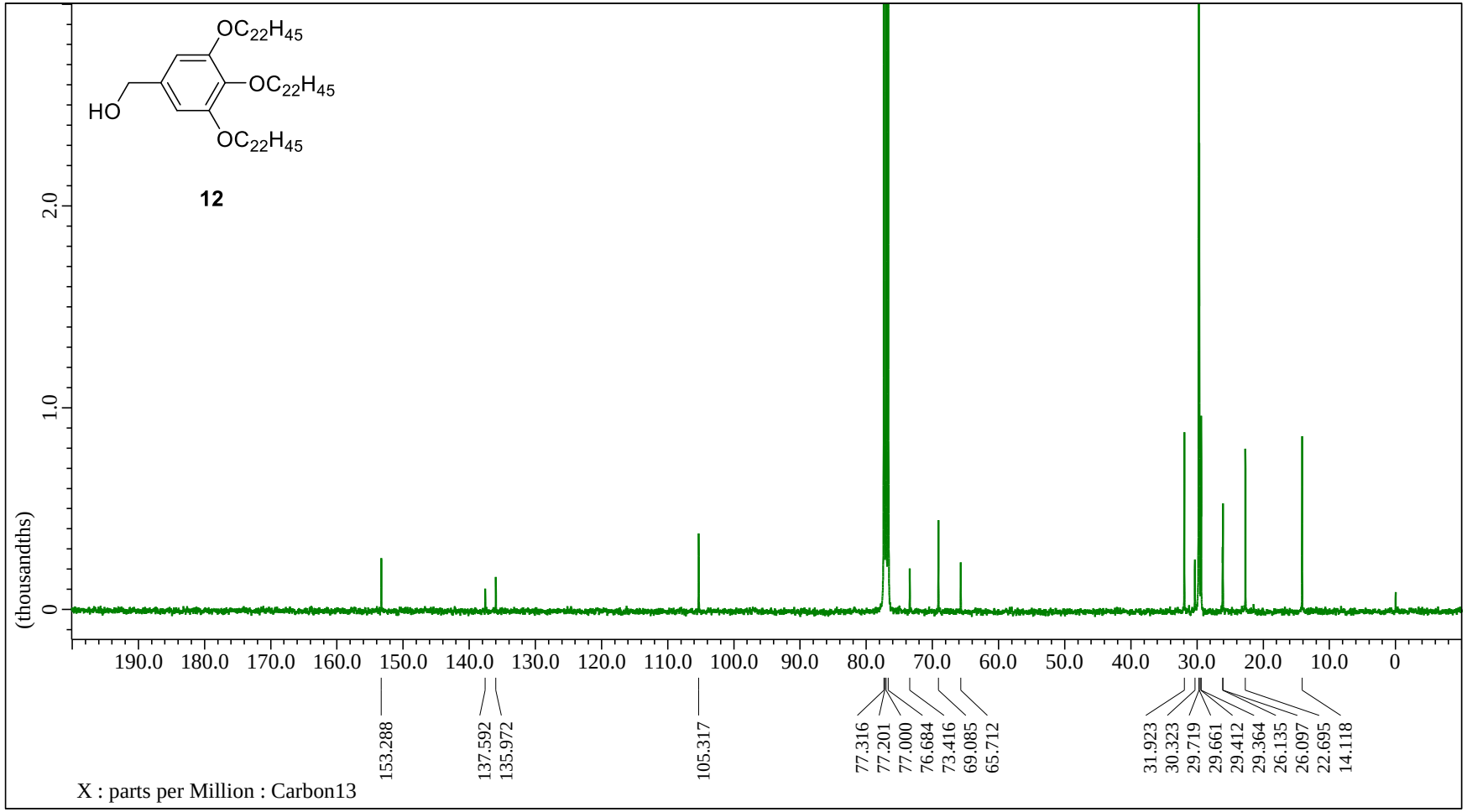
^{1}H NMR ($CDCl_3$, 400 MHz)



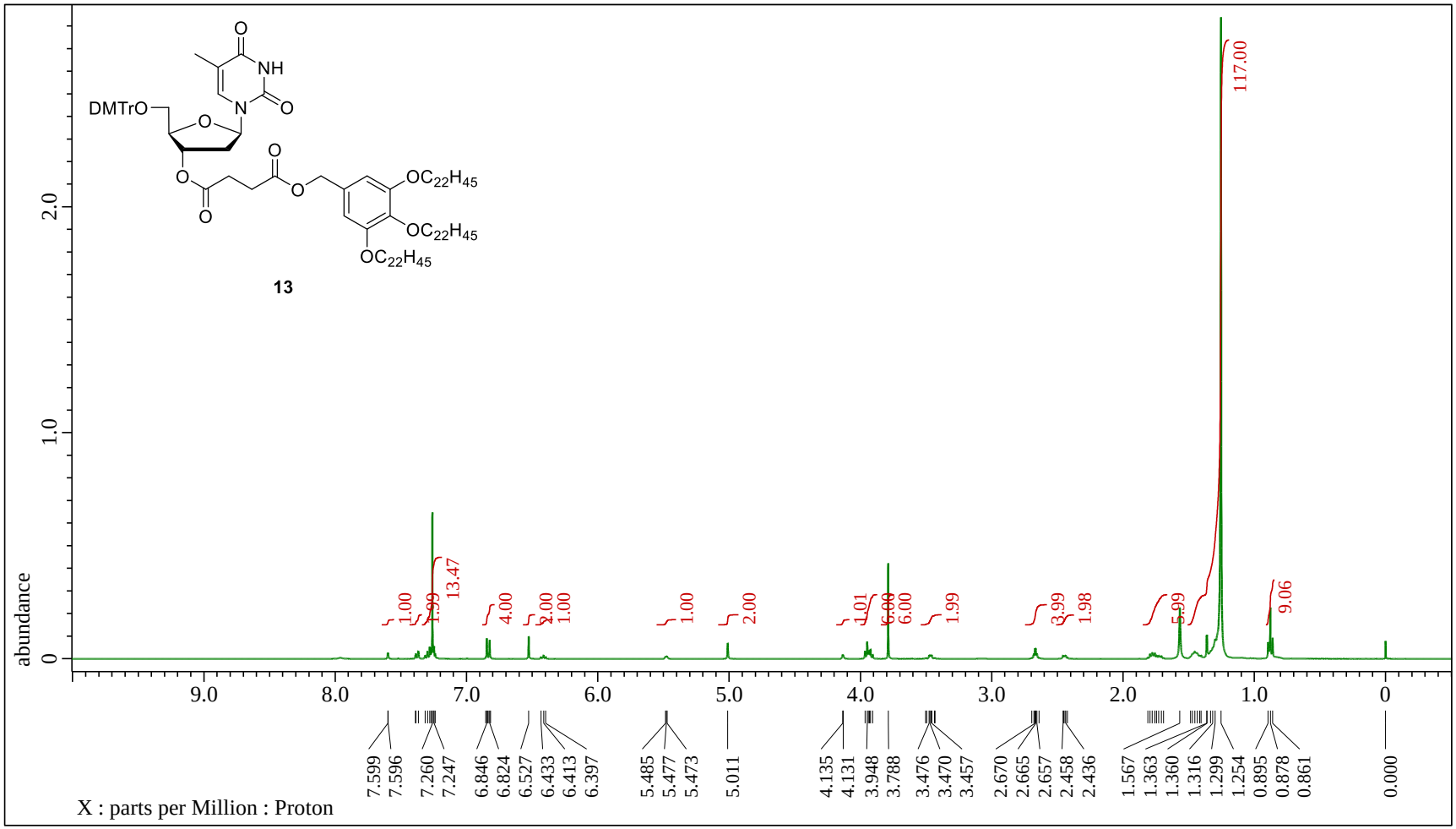
^{13}C NMR ($CDCl_3$, 100 MHz)



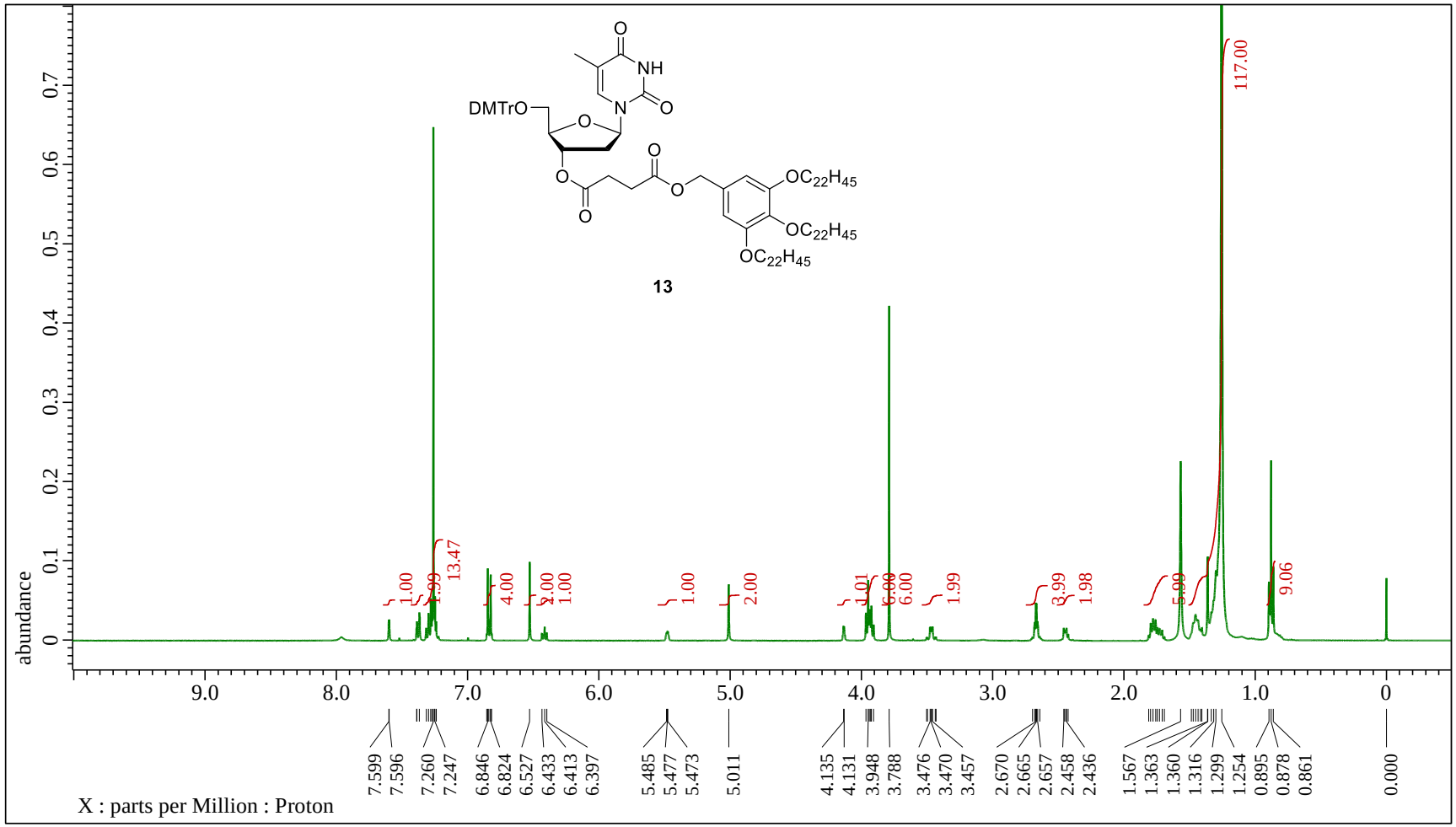
^{13}C NMR ($CDCl_3$, 100 MHz) (magnified)



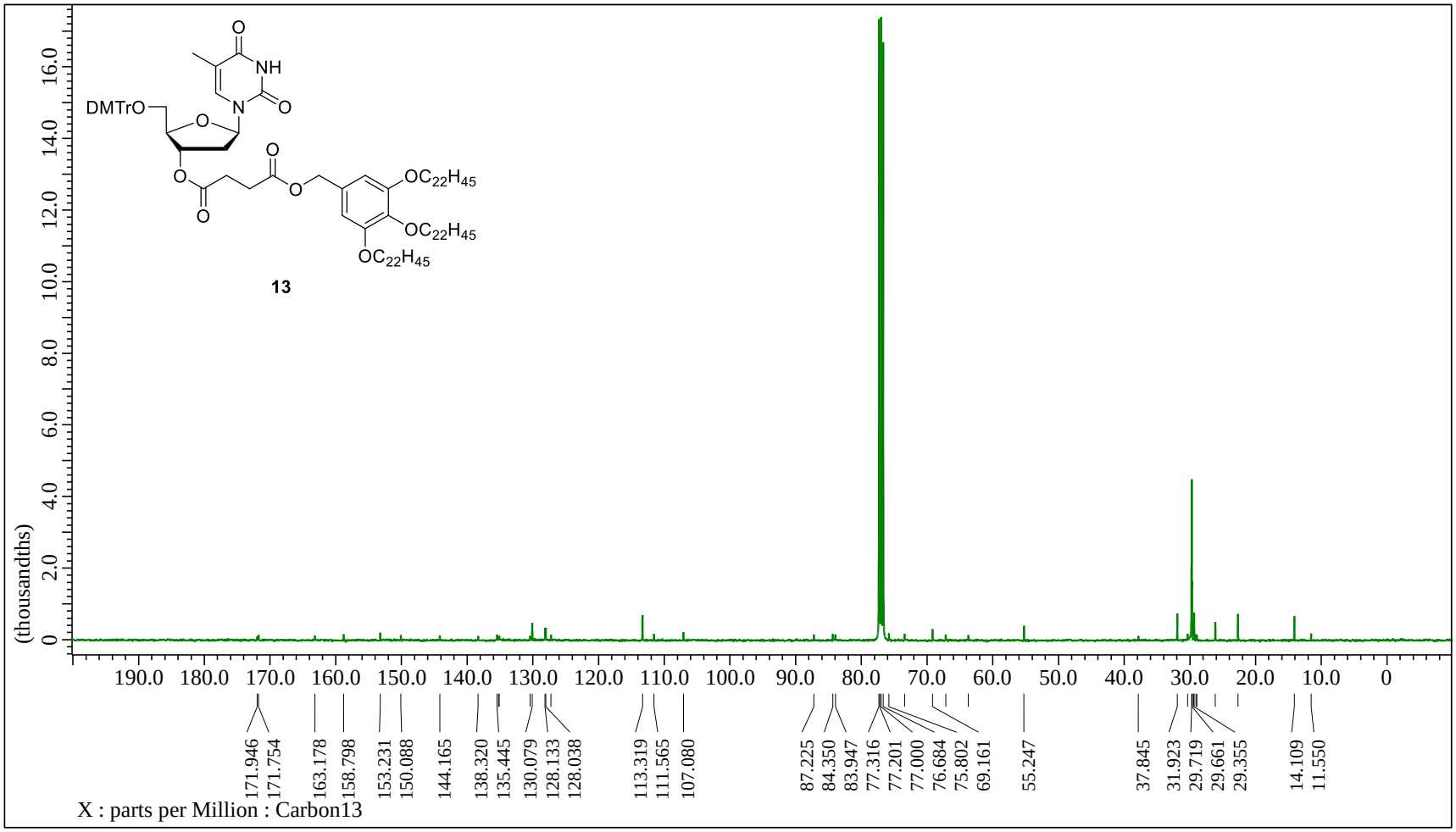
^{1}H NMR ($CDCl_3$, 400 MHz)



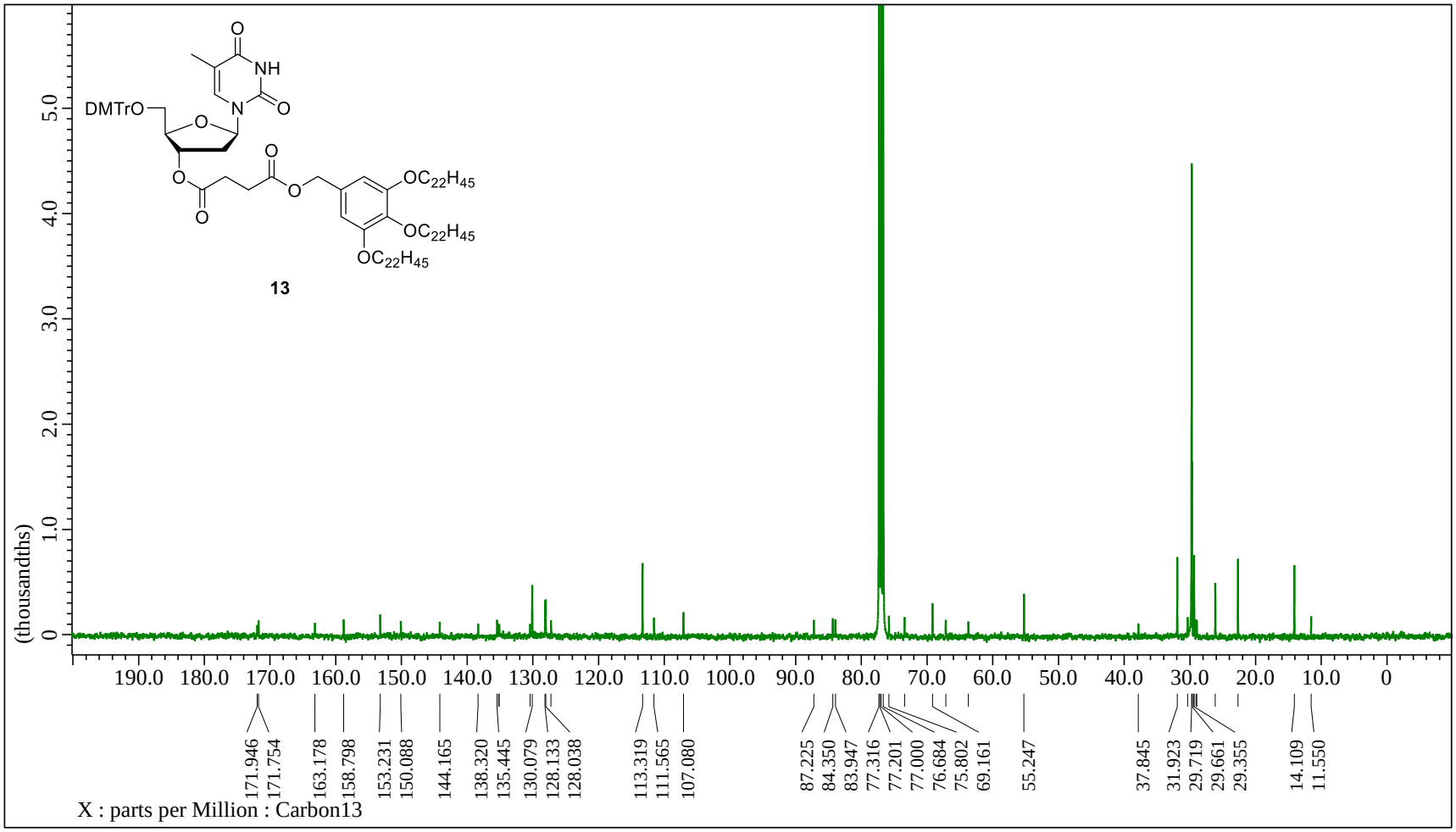
^{1}H NMR ($CDCl_3$, 400 MHz) (magnified)



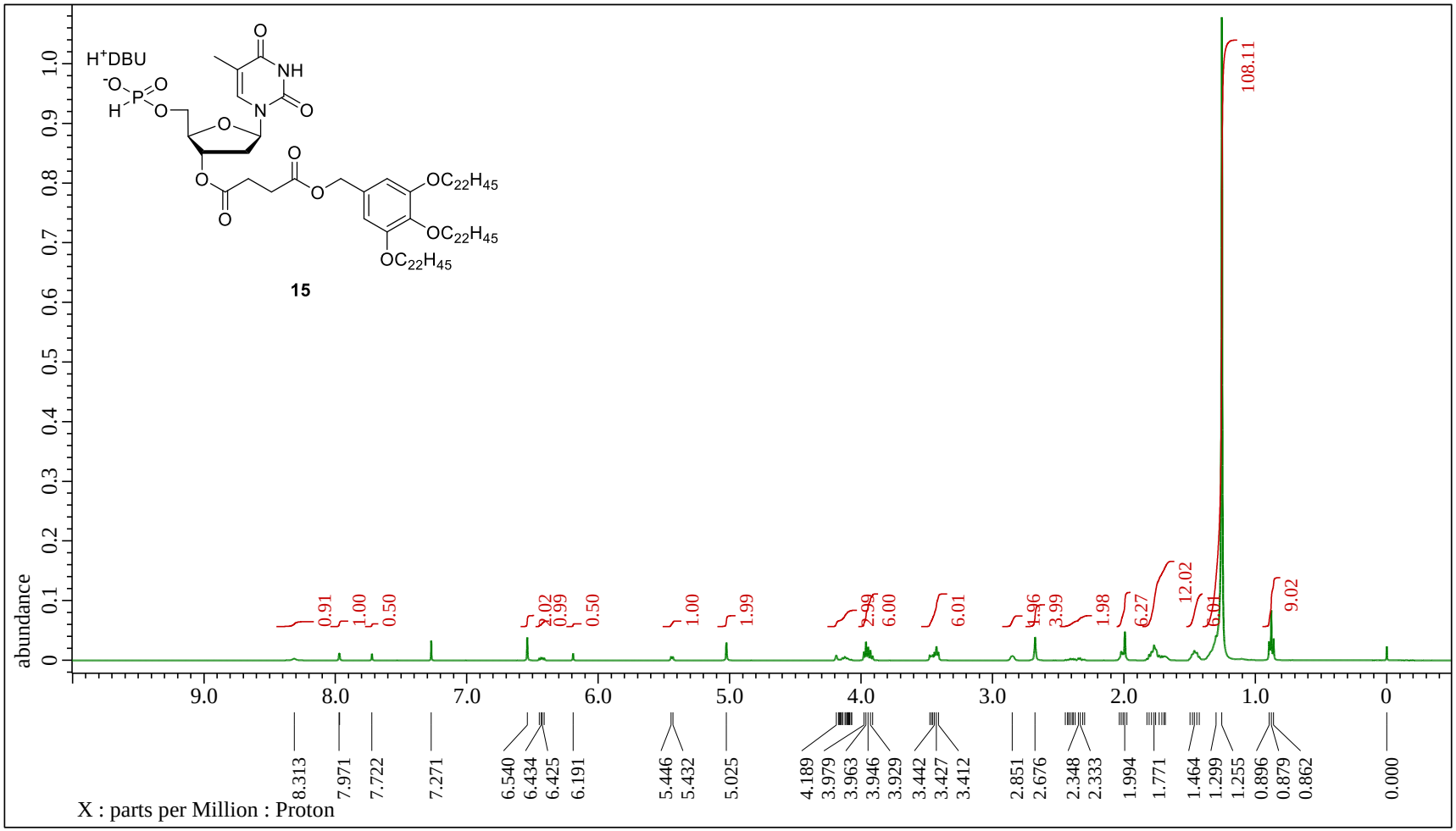
^{13}C NMR ($CDCl_3$, 100 MHz)



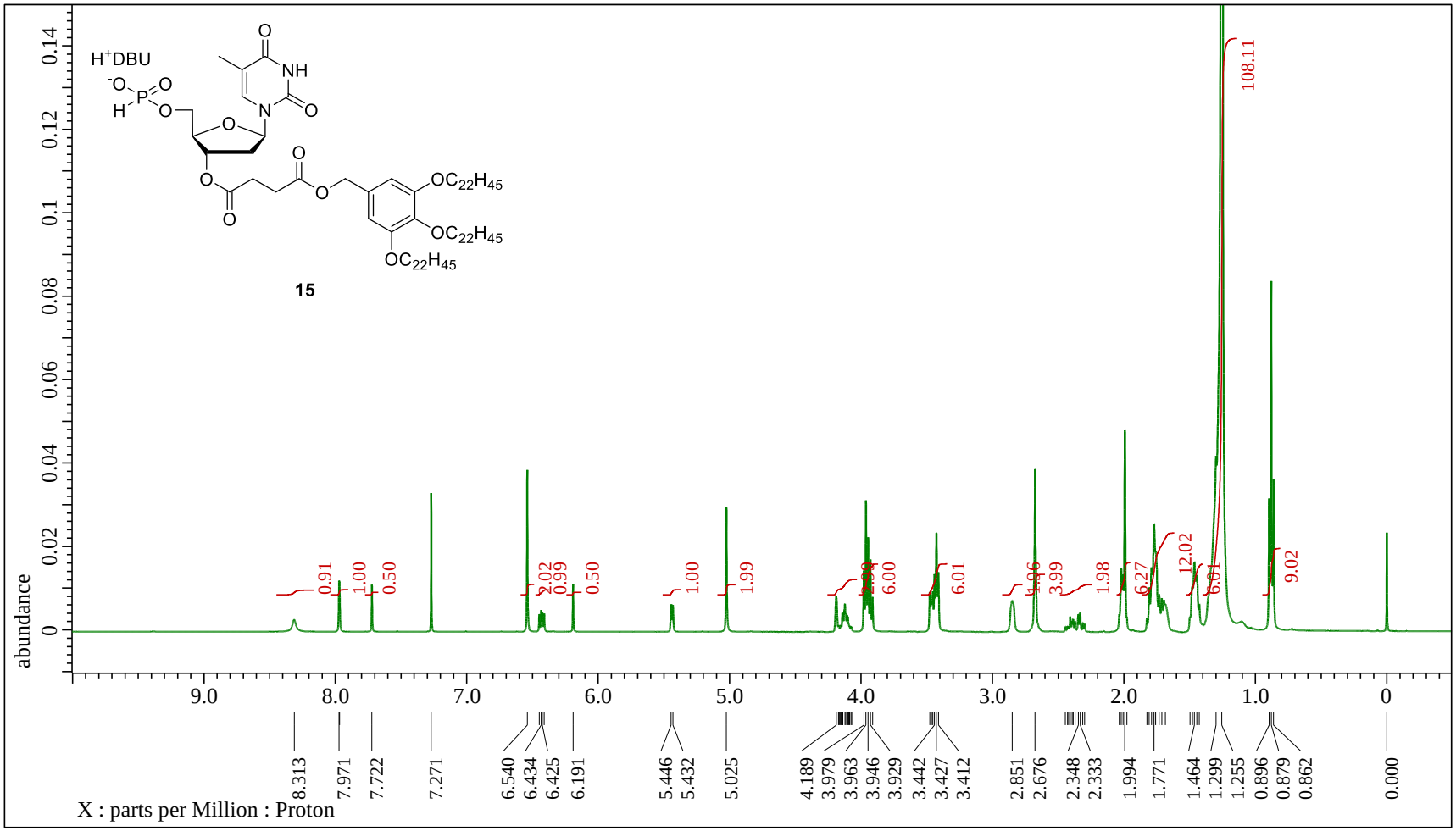
^{13}C NMR ($CDCl_3$, 100 MHz) (magnified)



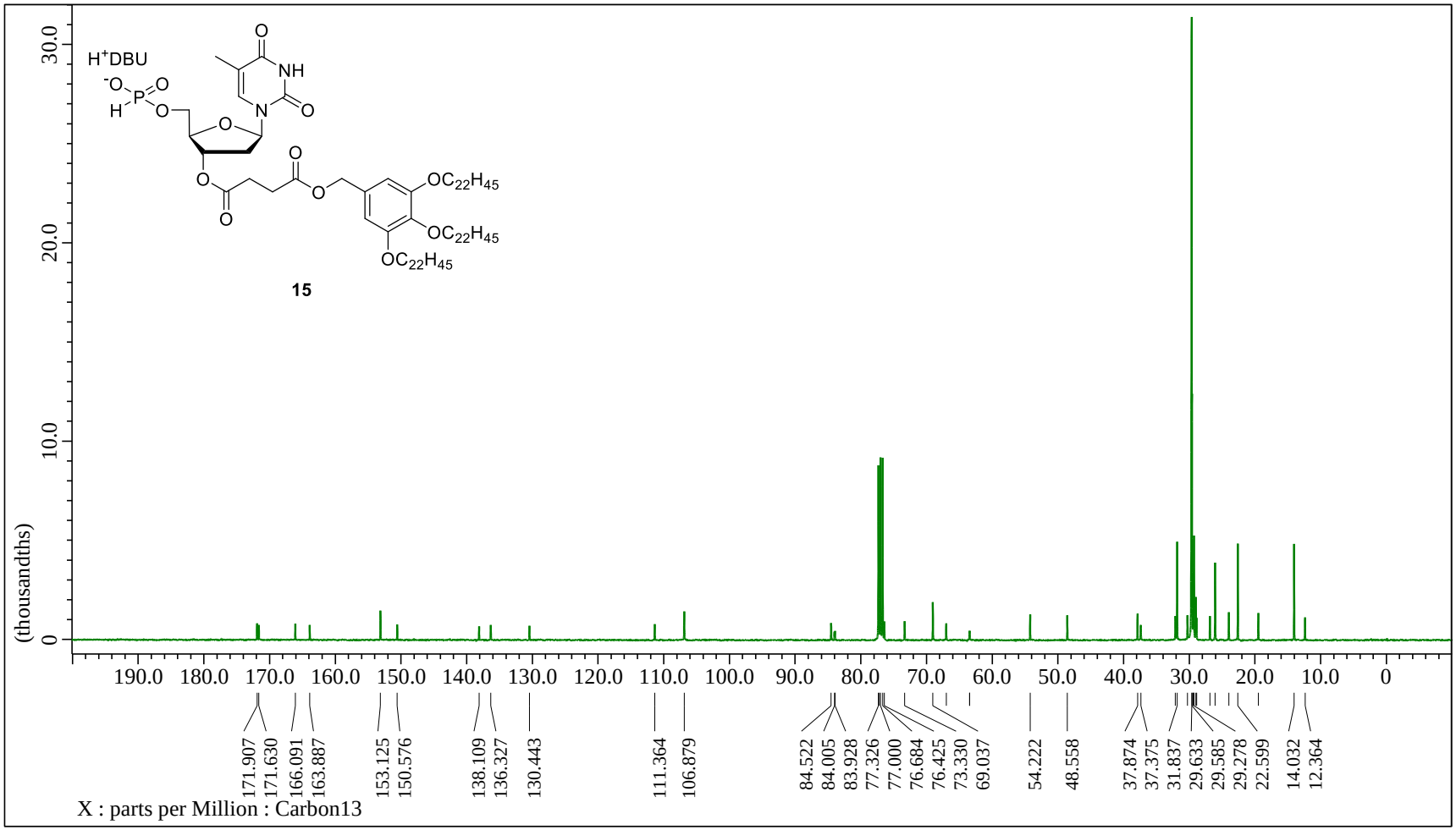
^{1}H NMR ($CDCl_3$, 400 MHz)



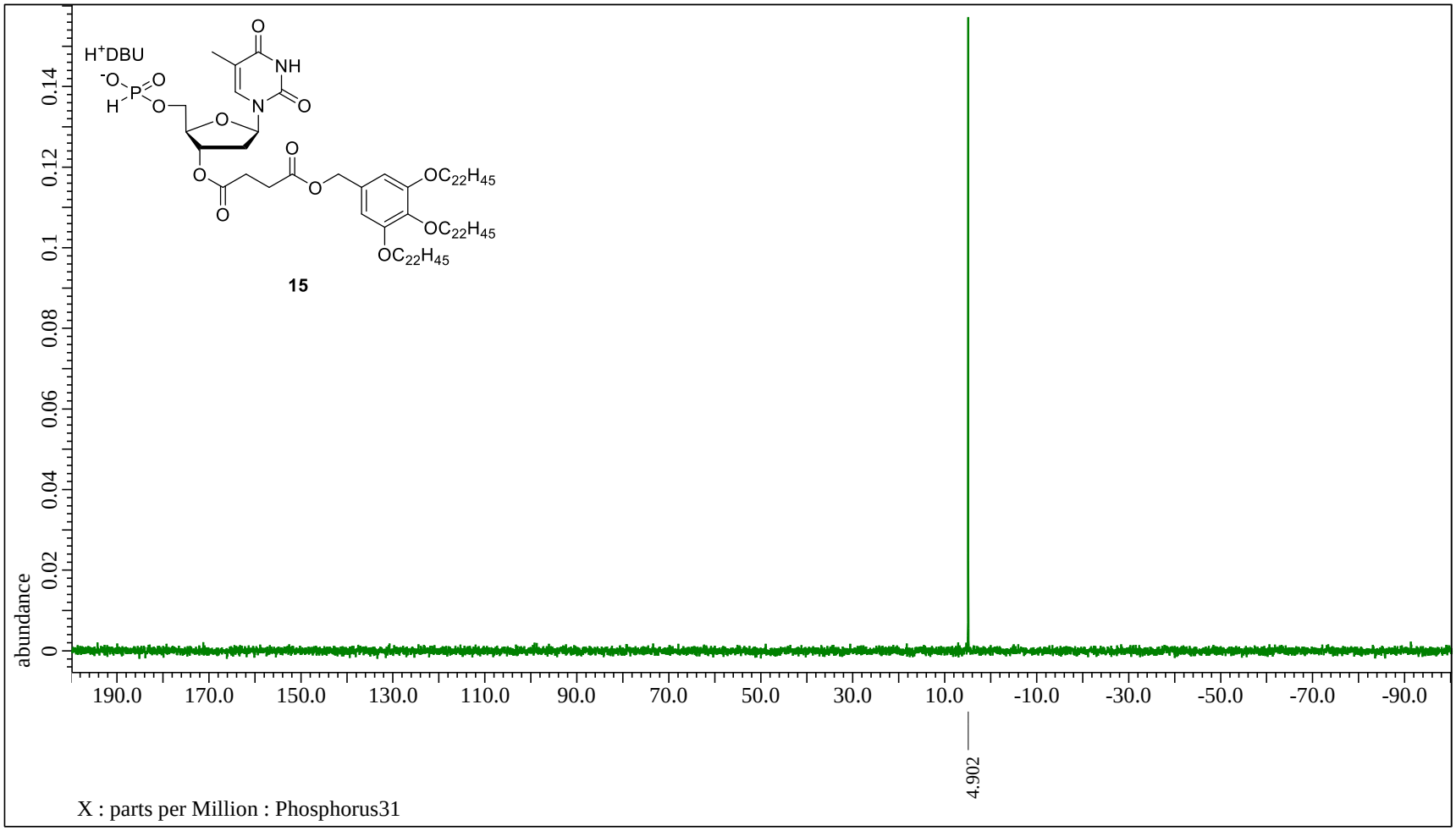
^{1}H NMR ($CDCl_3$, 400 MHz) (magnified)



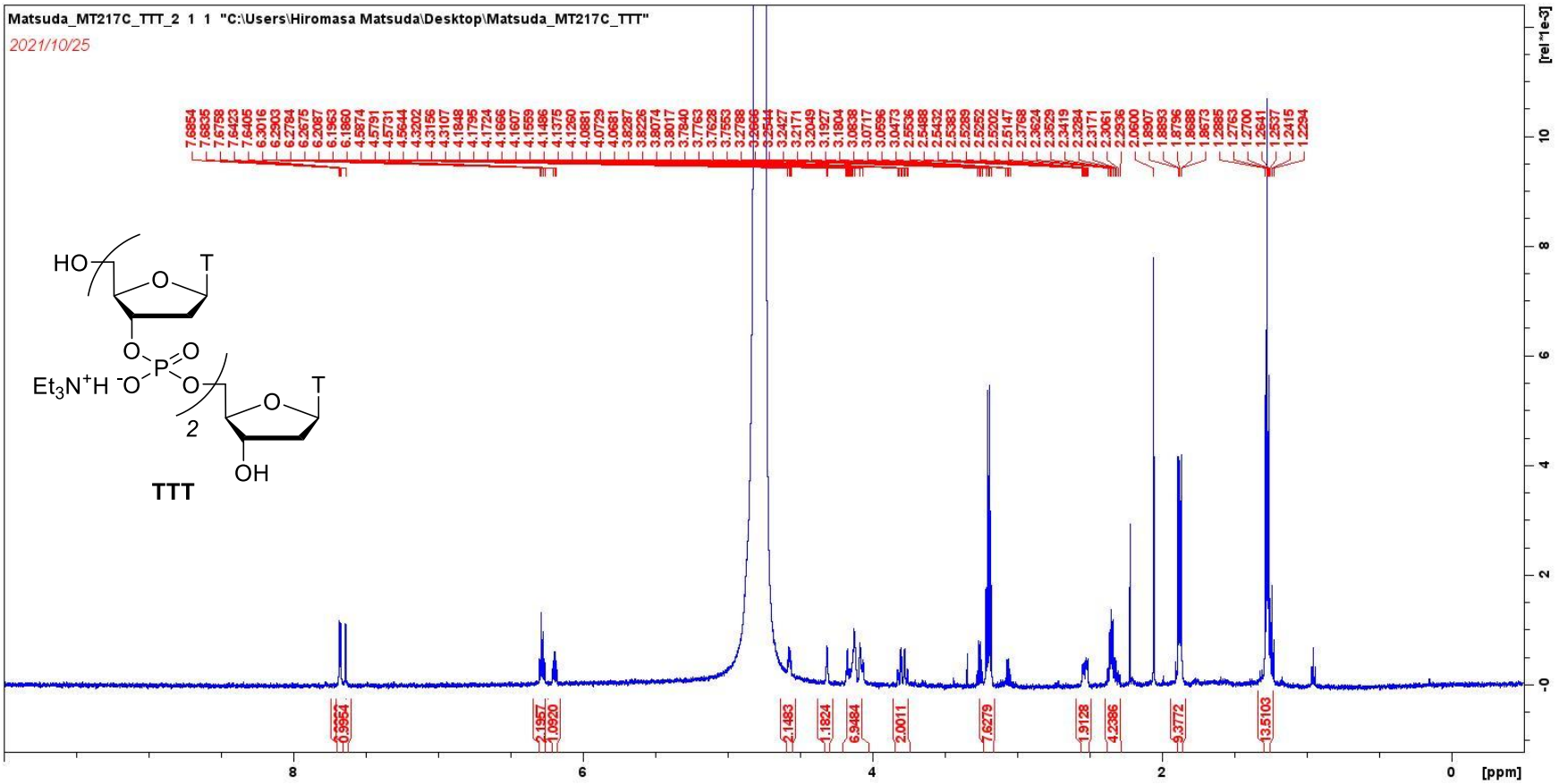
^{13}C NMR ($CDCl_3$, 100 MHz)



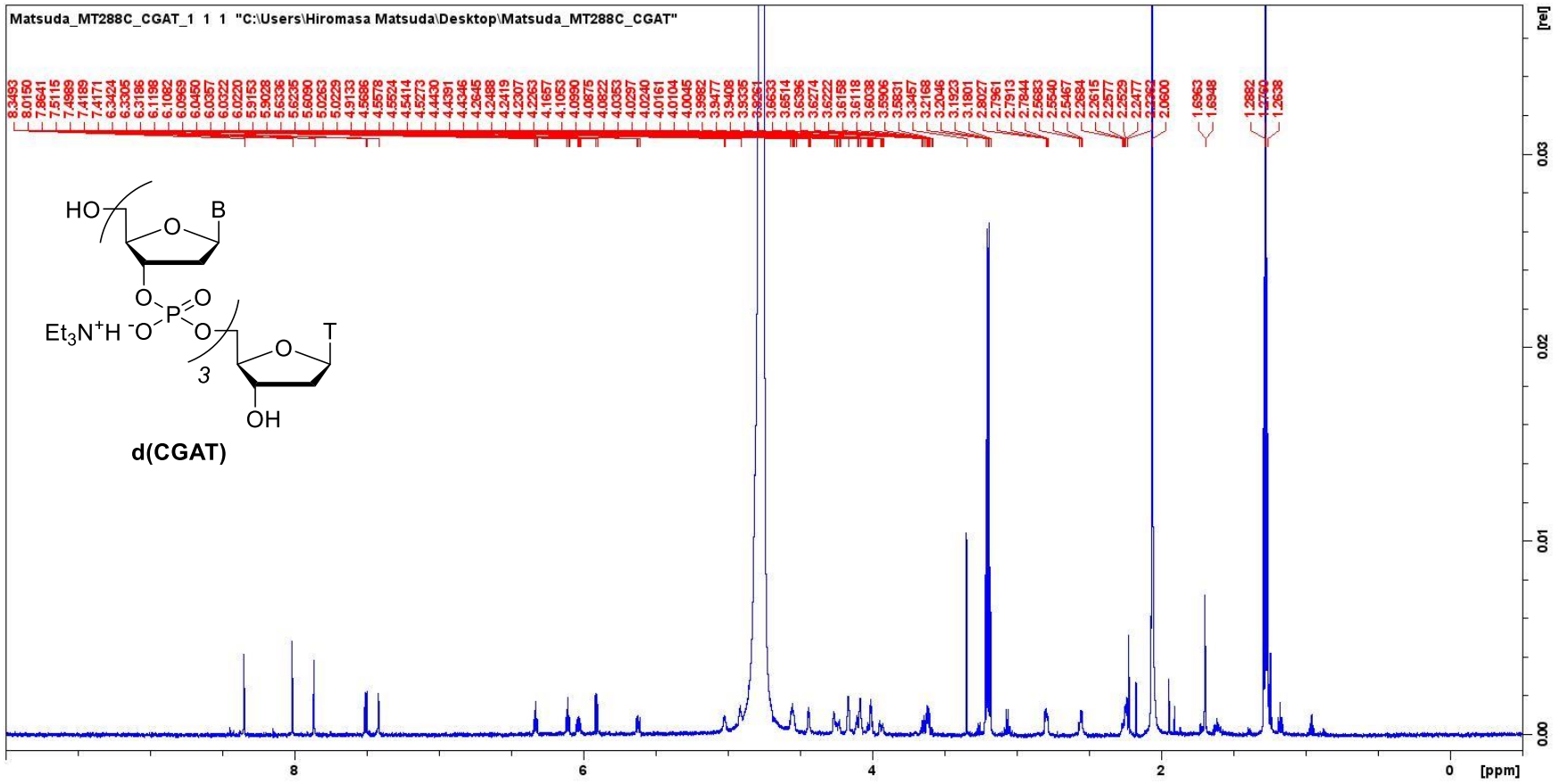
^{31}P NMR ($CDCl_3$, 162 MHz)



^{1}H NMR (D_2O, 600 MHz)



^{1}H NMR (D_2O, 600 MHz)



^{1}H NMR (D_2O, 600 MHz)