

Supplementary information

Internucleotidic bond formation using *H*-phosphoramidate derivatives and acidic activators

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Table of contents

1. Experimental procedure and data	S3
2. ^{31}P NMR analysis of the effect of acidic activators on the condensation reaction in MeCN. (Table1)	S8
3. ^{31}P NMR analysis of the effect of solvents on the condensation reaction. (Table2)	S13
4. ^{31}P NMR analysis of the effect of leaving groups on the condensation reaction. (Table3)	S19
5. ^{31}P NMR analysis of the effect of acidic activators on the condensation reaction in pyridine. (Table5)	S23
6. ^{31}P NMR analysis of the crude mixture of <i>S</i> -cyanoethyl phosphorothioate diester 10 (Table 8)	S32
7. ^1H , ^{13}C , ^{31}P NMR spectra of isolated compounds	S36

1. Experimental section

General information

All reactions were conducted under an Ar atmosphere. Dry organic solvents were prepared by appropriate procedures. Additionally, ^1H NMR spectra were recorded at 400 or 500 MHz with tetramethylsilane (δ 0.0) as the internal standard in CDCl_3 , CD_3CN , or pyridine- d_5 . Further, ^{13}C NMR spectra were recorded at 126 MHz with CD_3CN , which was used as the internal standard at δ 118.3. Furthermore, ^{31}P NMR spectra were recorded at 162 or 202 MHz with H_3PO_4 (δ 0.0) as the external standard in CDCl_3 , CD_3CN , or pyridine- d_5 . IR spectra were obtained using an ATR-IR spectrometer. Analytical thin-layer chromatography was performed on commercial glass plates with a 0.25 mm-thick silica gel layer. Column chromatography was carried out on Yamazen UNIVERSAL Premium column (M size) using automated flash chromatography system W-prep 2XY (Yamazen Corporation).

DFT Calculations

All DFT molecular orbital calculations were carried out using the GAMESS programs on NEC Co. Geometry optimizations were carried out at the B3LYP/6-31G* level.

Synthesis of compounds

N-(Methyl)triazolium Trifluoromethanesulfonate (NMTRT).

Trifluoromethanesulfonic acid (1.1 mL, 12 mmol) was added dropwise to a stirred solution of *N*-(methyl)triazole (0.68 mL, 12 mmol) in dry MeOH–Et₂O (13.6 mL, 1:1, v/v) at 0 °C. The mixture was stirred for 10 min at 0 °C. Thereafter, the mixture was added to Et₂O (55 mL). The resultant precipitate was collected by filtration, washed with dry Et₂O (80 mL), and dried under vacuum to afford NMTRT (1.41 g, 6.0 mmol, 50%) as a white crystalline solid.

IR (neat, cm^{-1}): 3164, 3116, 2941, 2839, 1577, 1542, 1434, 1419, 1371, 1358, 1282, 1249, 1224 ^1H NMR (500 MHz, CD_3CN) δ 9.30 (s, 1H (H-5)), 8.61 (s, 1H (H-3)), 4.06 (d, J = 0.7 Hz, 3H (1-Me)); ^{13}C { ^1H } NMR (126 MHz, CDCl_3) δ 143.4 (C-3), 142.0 (C-5), 121.8 (q, $^1J_{\text{CF}}$ = 320 Hz, - CF_3) 39.5 (1- CH_3).

ESI-MS m/z calcd for $\text{C}_3\text{H}_6\text{N}_3$ [M-OTf]⁺, 84.0556; found 84.0554.

Investigation of the effect of acidic activators on the condensation reaction in MeCN. (Table 1)

5'-*O*-Dimethoxytritylthymidine 3'-thiomorpholino *H*-phosphonamidate **1** (0.0208 g,

0.030 mmol) and a thymidine derivative **2** (0.0120 g, 0.025 mmol) were dissolved in CD₃CN (0.50 mL) with MS3A (0.4 g). An acidic activator (0.15 mmol) was added to the solution at rt and the mixture was stirred for 2 h at rt. The solution was transferred into an NMR sample tube (5 mm × 180 mm) and a spectrum was recorded. The formation of **3** was confirmed by ³¹P NMR spectra (δ 9.3, 8.5 ppm, ¹J_{PH} = 720, 726 Hz) (Fig. S1–S5).

Investigation of the effect of solvents on the condensation reaction. (Table 2)

5'-*O*-Dimethoxytritylthymidine 3'-thiomorpholino *H*-phosphoramidate **1** (0.0208 g, 0.030 mmol) and a thymidine derivative **2** (0.0120 g, 0.025 mmol) were dissolved in a solvent (for entry 1, CH₂Cl₂, for entry 2, THF, for entry 3, 4, MeCN, for entry 5, pyridine, for entry 6, 2,6-lutidine, 0.50 mL) with MS4A (0.4 g). For entry 4, 6 equivalents of pyridine were added to the mixture. 1*H*-Tetrazole (0.0105 g, 0.15 mmol) was added to the solution at rt and the mixture was stirred for 1 h at rt. To the mixture, a deuterated solvent (for entry 1, CD₃CN, for entry 2, pyridine-*d*₅, for entry 3, CDCl₃, 0.20 mL) was added. Thereafter, the solution was transferred into an NMR sample tube (5 mm × 180 mm) and a spectrum was recorded. The formation of **3** was confirmed by ³¹P NMR spectra (MeCN: δ 9.3, 8.5 ppm, ¹J_{PH} = 720, 726 Hz, pyridine: δ 10.2, 8.6 ppm, ¹J_{PH} = 723, 724 Hz, 2,6-lutidine: δ 8.7, 6.9 ppm) (Fig. S6–S8).

Investigation of the effect of leaving groups on the condensation reaction. (Table 3)

H-Phosphoramidate monomer **1**, **4**, **5**, or **6** (0.030 mmol) and a thymidine derivative **2** (0.0120 g, 0.025 mmol) were dissolved in pyridine-*d*₅ (0.50 mL) with MS4A (0.4 g). 1*H*-Tetrazole (0.15 mmol) was added to the solution at rt and the mixture was stirred for 1 h at rt. The solution was transferred into an NMR sample tube (5 mm × 180 mm) and a spectrum was recorded. The formation of **3** was confirmed by ³¹P NMR spectra (δ 10.2, 8.6 ppm, ¹J_{PH} = 723, 724 Hz) (Fig. S9–S12).

Investigation of the effect of acidic activators on the condensation reaction in pyridine. (Table 4)

5'-*O*-Dimethoxytritylthymidine 3'-thiomorpholino *H*-phosphoramidate **1** (0.0208 g, 0.030 mmol) and a thymidine derivative **2** (0.0120 g, 0.025 mmol) were dissolved in dry pyridine (0.50 mL) with MS4A (0.4 g). An acidic activator (0.15 mmol) was added to the solution at rt and the mixture was stirred for 10 min at rt. Thereafter, *S*-(cyanoethyl) methanesulfonothioate (**7**, 0.015 mL, 0.125 mmol) and *N*, *O*-bis (trimethylsilyl) acetamide (0.031 mL, 0.125 mmol) in pyridine-*d*₅ (0.2 mL) was added to the mixture. The solution was transferred into an NMR sample tube (5 mm × 180 mm) and a spectrum

was recorded. The formation of **8** was confirmed by ^{31}P NMR spectra (δ 27.7, 27.4 ppm) (Fig. S13–S20).

Synthesis of dimers

T_{PSCE}T dimer (**10tt**)

5'-O-Dimethoxytritylthymidine 3'-morpholino *H*-phosphonamidate **1** (0.0416 g, 0.060 mmol) and a thymidine derivative **2** (0.0192 g, 0.040 mmol) were dissolved in dry pyridine (0.80 mL) with MS4A (0.8 g). *N*-Cyanomethyl pyrrolidinium triflate (CMPT, 0.0625 g, 0.24 mmol) was added to the mixture. After the mixture was stirred for 30 min at rt, *S*-(cyanoethyl) methanesulfonylthioate (**7**) in dry pyridine (0.8 mL, 0.048 mmol) was added to the mixture. The mixture was stirred for 1 h at rt. Thereafter, pyridine was removed by repeated coevaporations with toluene. The residue was dissolved in CHCl_3 (2 mL) and 2% TFA solution in CHCl_3 (2 mL) was added. After the mixture was stirred for 5 min at rt, MeOH (2 mL) and CHCl_3 (30 mL) were successively added to the mixture, and the mixture was washed with saturated aqueous solutions of NaHCO_3 (3×20 mL). The aqueous layers were combined and back-extracted with CHCl_3 (1×30 mL). The organic layers were combined, dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column chromatography was performed with a linear gradient of 0%–10% MeOH in CHCl_3 to afford **10tt** as a colorless foam (0.0265 g, 0.031 mmol, 78%).

The ^1H and ^{31}P NMR spectra corresponded to the previously reported one¹.

A_{PSCEA} dimer (**10aa**)

5'-O-Dimethoxytrityl-*N*⁶-benzoyldeoxyadenosine 3'-thiomorpholino *H*-phosphonamidate **1a** (0.0484 g, 0.060 mmol) and a deoxyadenosine derivative **2a** (0.0238 g, 0.040 mmol) were dissolved in dry pyridine (0.80 mL) with MS4A (0.8 g). CMPT (0.0625 g, 0.24 mmol) was added to the mixture. After the mixture was stirred for 30 min at rt, *S*-(cyanoethyl) methanesulfonylthioate (**7**) in dry pyridine (0.8 mL, 0.048 mmol) was added to the mixture. The mixture was stirred for 1 h at rt. Thereafter, pyridine was removed by repeated coevaporations with toluene. The residue was dissolved in CHCl_3 (2 mL), and 2% trifluoroacetic acid (TFA) solution in CHCl_3 (2 mL) was added. After the mixture was stirred for 5 min at rt, MeOH (2 mL) and CHCl_3 (30 mL) were successively added to the mixture, and the mixture was washed with saturated aqueous solutions of NaHCO_3 (3×20 mL). The aqueous layers were combined and back-extracted with CHCl_3 (1×30 mL). The organic layers were combined, dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The residue was purified by silica gel column

chromatography. Column chromatography was performed with a linear gradient of 0%–10% MeOH in CHCl₃ to afford **10aa** as a colorless foam (0.0328 g, 0.030 mmol, 76%). The ¹H and ³¹P NMR spectra corresponded to the previously reported one¹.

C_{PSCE}C dimer (10cc)

5'-*O*-Dimethoxytrityl-*N*⁴-benzoyldeoxycytidine 3'-thiomorpholino *H*-phosphoramidate diester **1c** (0.0449 g, 0.060 mmol) and a deoxycytidine derivative **2c** (0.0214 g, 0.040 mmol) were dissolved in dry pyridine (0.80 mL) with MS4A (0.8 g). CMPT (0.0625 g, 0.24 mmol) was added to the mixture. After the mixture was stirred for 30 min at rt, *S*-(cyanoethyl) methanesulfonylthioate (**7**) in dry pyridine (0.8 mL, 0.048 mmol) was added to the mixture. The mixture was stirred for 1 h at rt. Thereafter, pyridine was removed by repeated coevaporations with toluene. The residue was dissolved in CHCl₃ (2 mL) and 2% TFA solution in CHCl₃ (2 mL) was added. After the mixture was stirred for 10 min at rt, MeOH (2 mL) and CHCl₃ (30 mL) were successively added to the mixture, and the mixture was washed with saturated aqueous solutions of NaHCO₃ (3 × 20 mL). The aqueous layers were combined and back-extracted with CHCl₃ (1 × 30 mL). The organic layers were combined, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column chromatography was performed with a linear gradient of 0%–10% MeOH in CHCl₃ for two times to afford **10cc** as a colorless foam (0.0287 g, 0.030 mmol, 75%).

The ¹H and ³¹P NMR spectra corresponded to the previously reported one¹.

G_{PSCE}G dimer (10gg)

Firstly, 5'-*O*-dimethoxytrityl-*N*²-isobutyryldeoxyguanosine 3'-thiomorpholino *H*-phosphoramidate diester **1g** (0.0473 g, 0.060 mmol) and a deoxyguanosine derivative **2g** (0.0230 g, 0.040 mmol) were dissolved in dry pyridine (0.80 mL) with MS4A (0.8 g). CMPT (0.0625 g, 0.24 mmol) was added to the mixture. After the mixture was stirred for 30 min at rt, *S*-(cyanoethyl) methanesulfonylthioate (**7**) in dry pyridine (0.8 mL, 0.048 mmol) was added to the mixture. The mixture was stirred for 1 h at rt. Thereafter, pyridine was removed by repeated coevaporations with toluene. The residue was dissolved in CHCl₃ (2 mL) and 2% TFA solution in CHCl₃ (2 mL) was added. After the mixture was stirred for 10 min at rt, MeOH (2 mL) and CHCl₃ (30 mL) were successively added to the mixture, and the mixture was washed with saturated aqueous solutions of NaHCO₃ (3 × 20 mL). The aqueous layers were combined and back-extracted with CHCl₃ (1 × 30 mL). The organic layers were combined, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography. Column

chromatography was performed with a linear gradient of 0%–10% MeOH in CHCl₃ to afford **10gg** as a colorless foam (0.0310 g, 0.030 mmol, 74%)

The ¹H and ³¹P NMR spectra corresponded to the previously reported one¹.

Reference for SI

1. T. Tsurusaki, K. Sato and T. Wada, *Organic & Biomolecular Chemistry*, 2023, **21**, 2486-2492.

2. ^{31}P NMR analysis of the effect of acidic activators on the condensation reaction in MeCN. (Table1)

NMR conversion was calculated by (sum of all product peaks) / (sum of all peaks / 1.2).

Table 1, entry 1

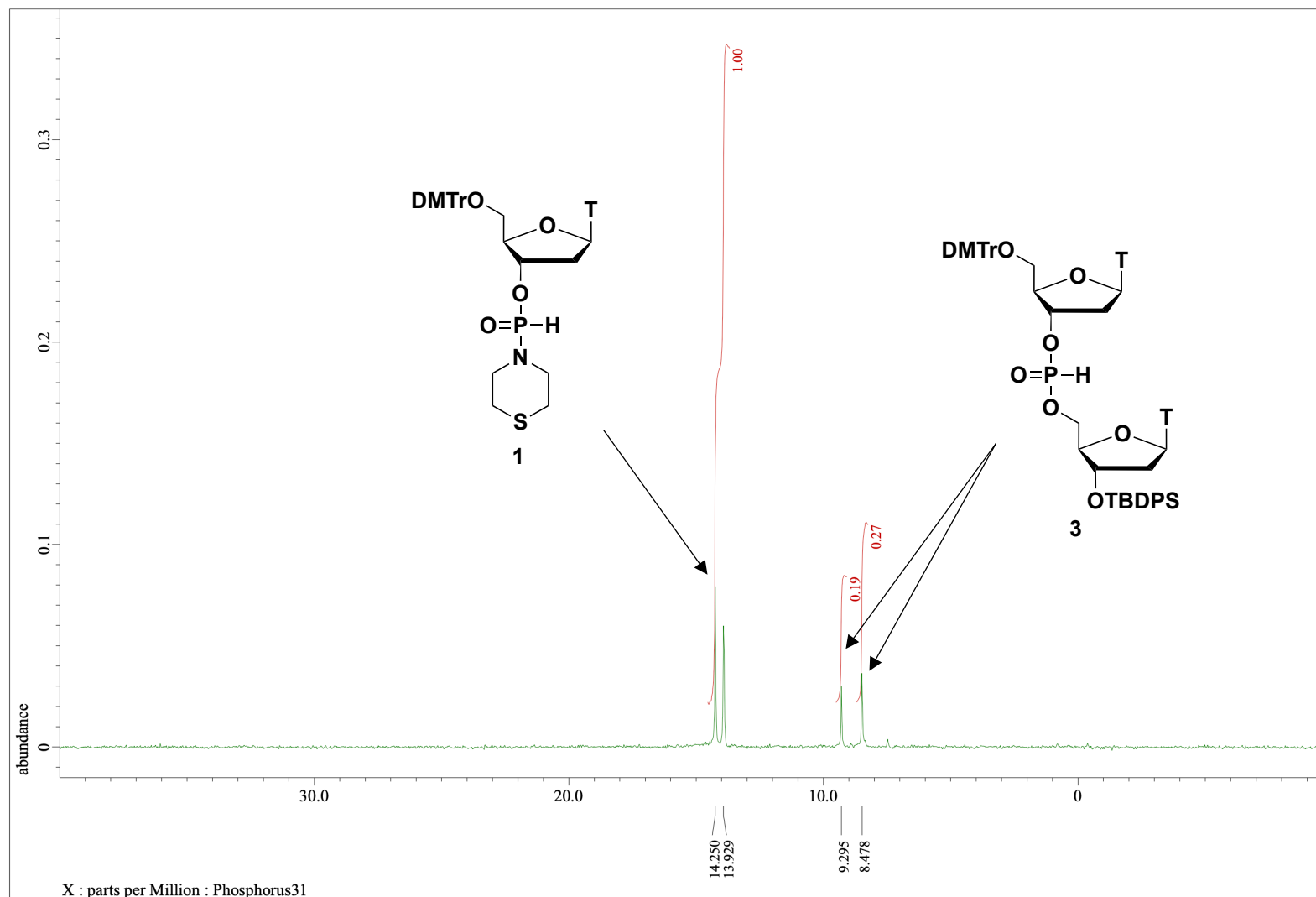


Figure S1 ^{31}P NMR spectrum (CD_3CN , 162 MHz) of the reaction mixture (acidic activator: ETT).

Table 1, entry 2

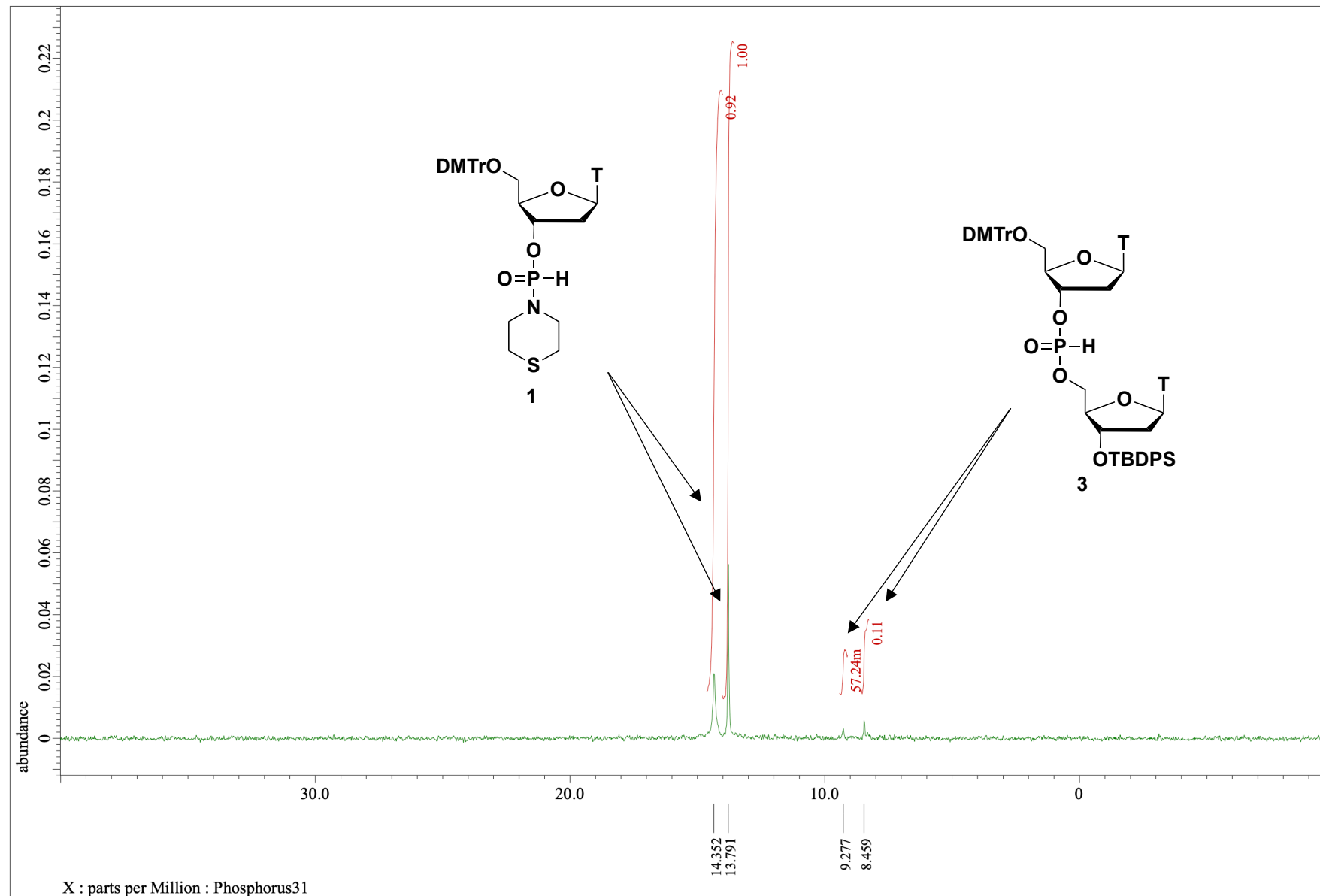


Figure S2 ^{31}P NMR spectrum (CD_3CN , 162 MHz) of the reaction mixture (acidic activator: CMPT).

Table 1, entry 3

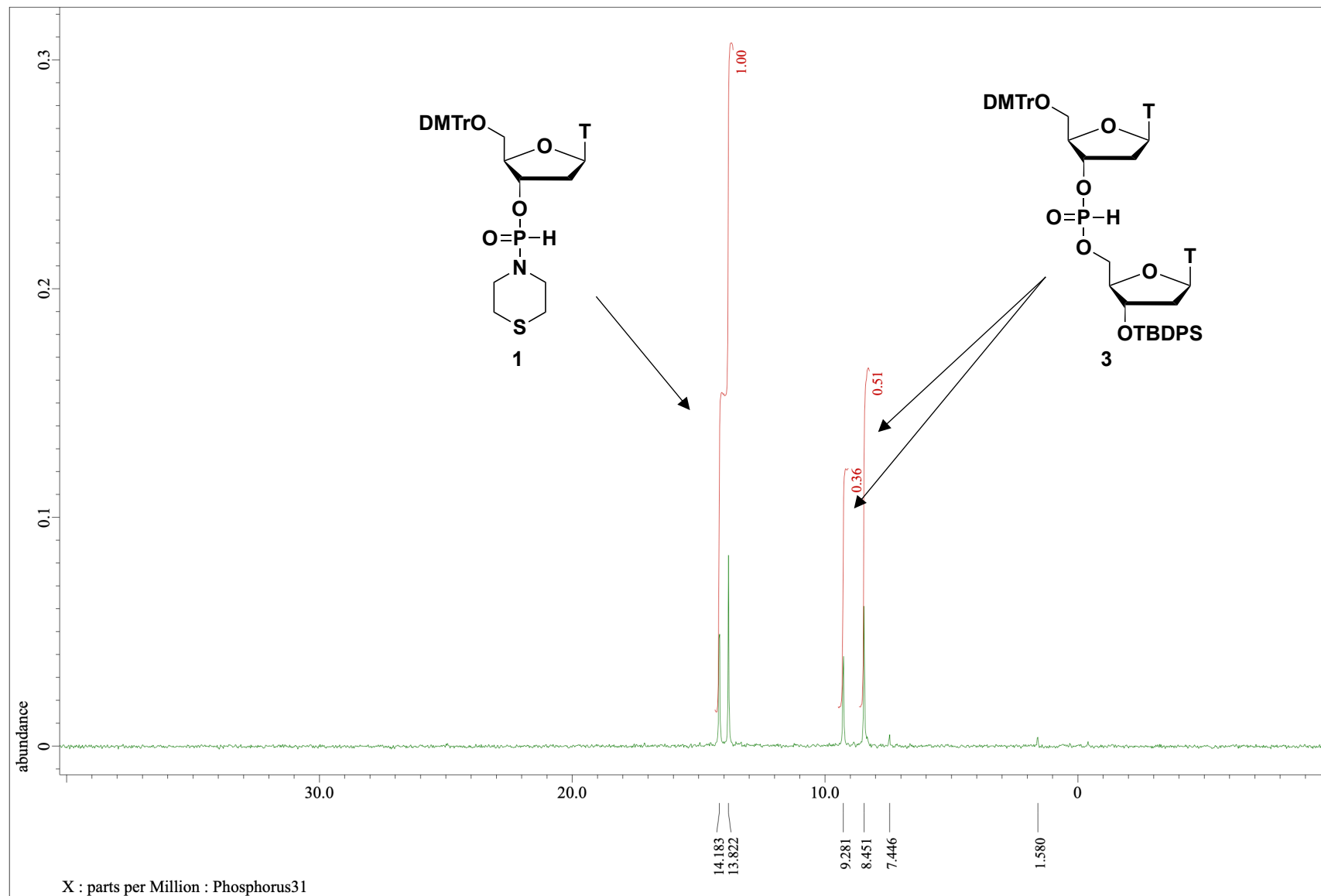


Figure S3 ^{31}P NMR spectrum (CD_3CN , 162 MHz) of the reaction mixture (acidic activator: TET).

Table 1, entry 4

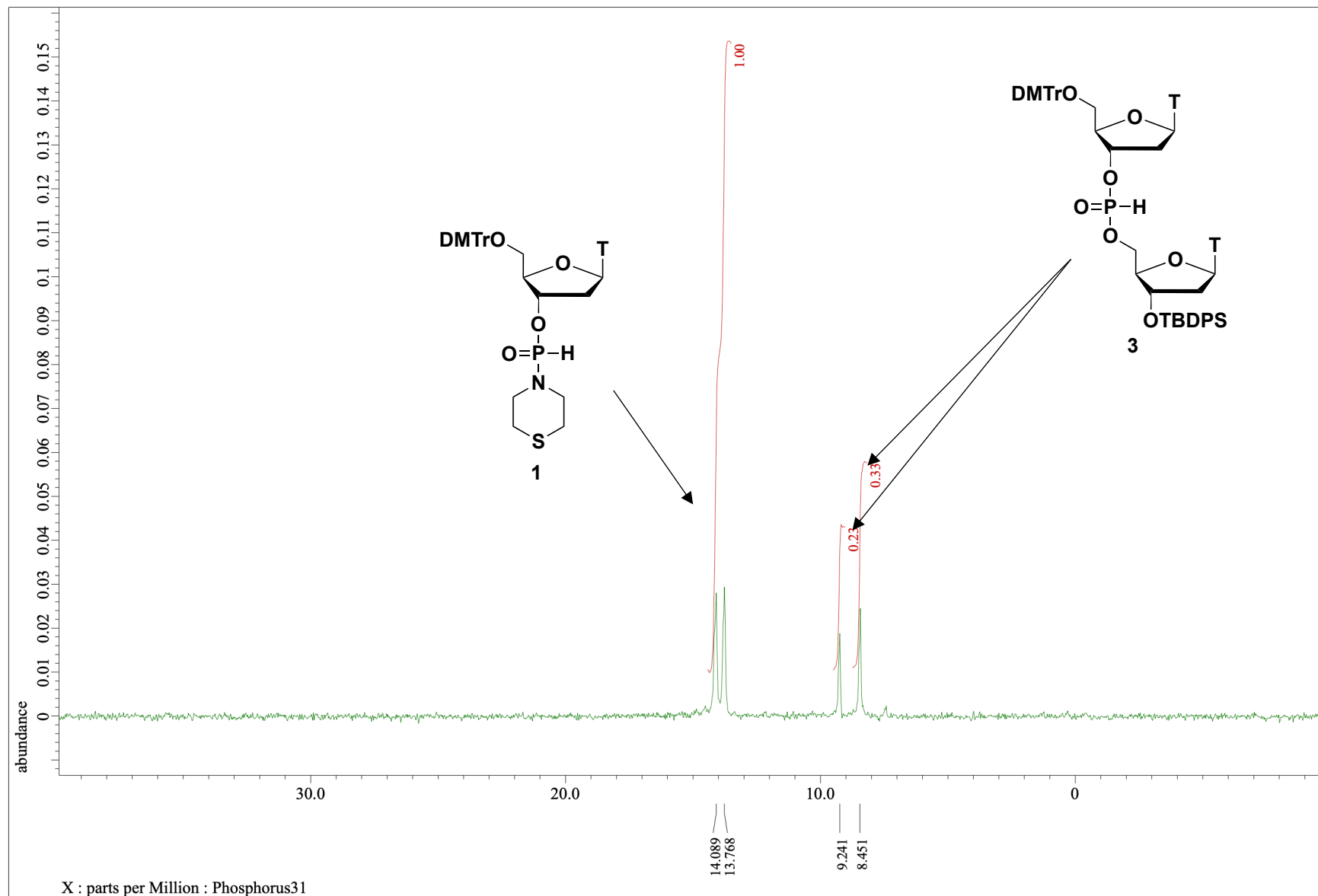
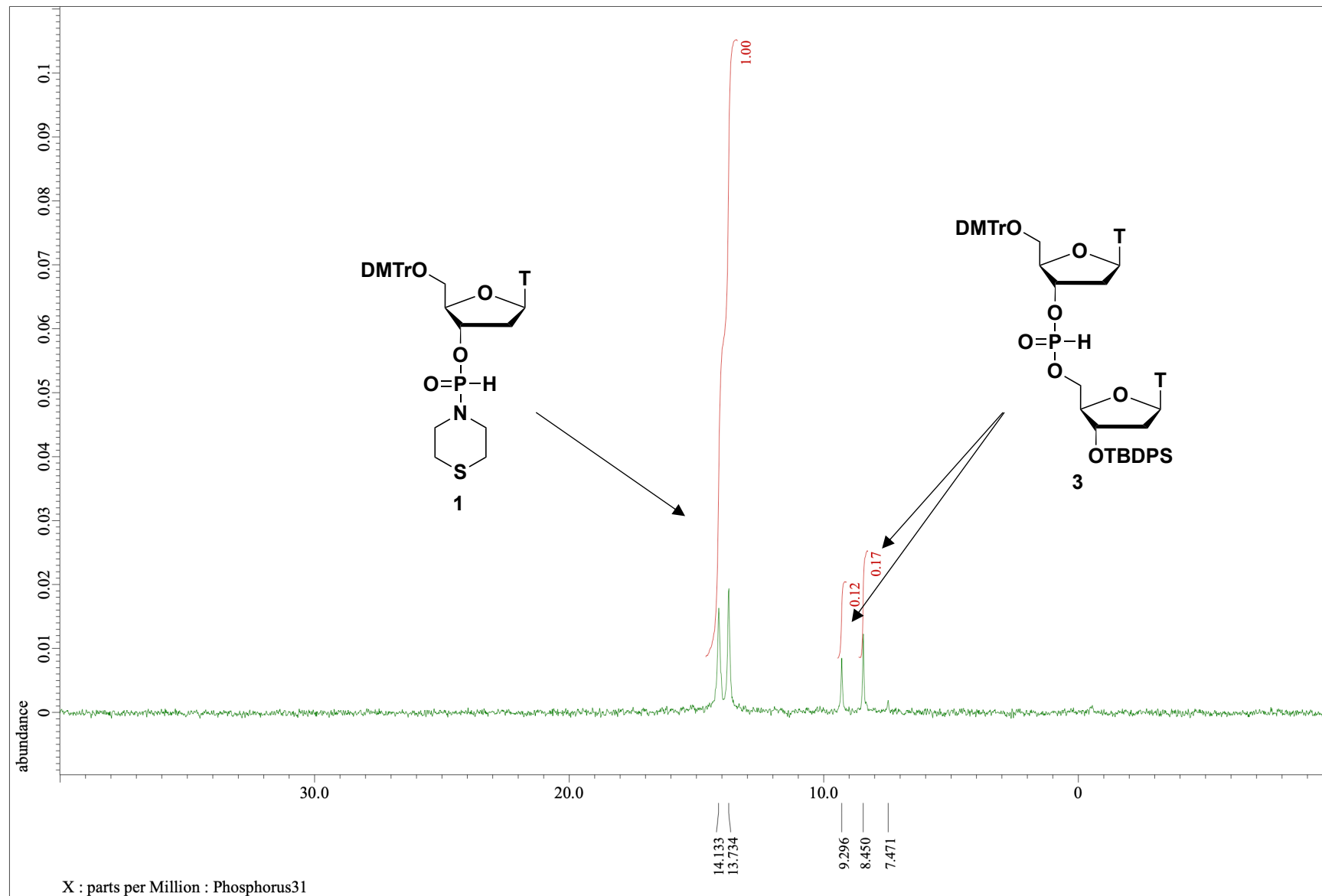


Figure S4 ^{31}P NMR spectrum (CD_3CN , 162 MHz) of the reaction mixture (acidic activator: DCI).

Figure S5 ^{31}P NMR spectrum (CD_3CN , 162 MHz) of the reaction mixture (acidic activator: PhIMT).

3. ^{31}P NMR analysis of the effect of solvents on the condensation reaction. (Table2)

Table 2, entry 1

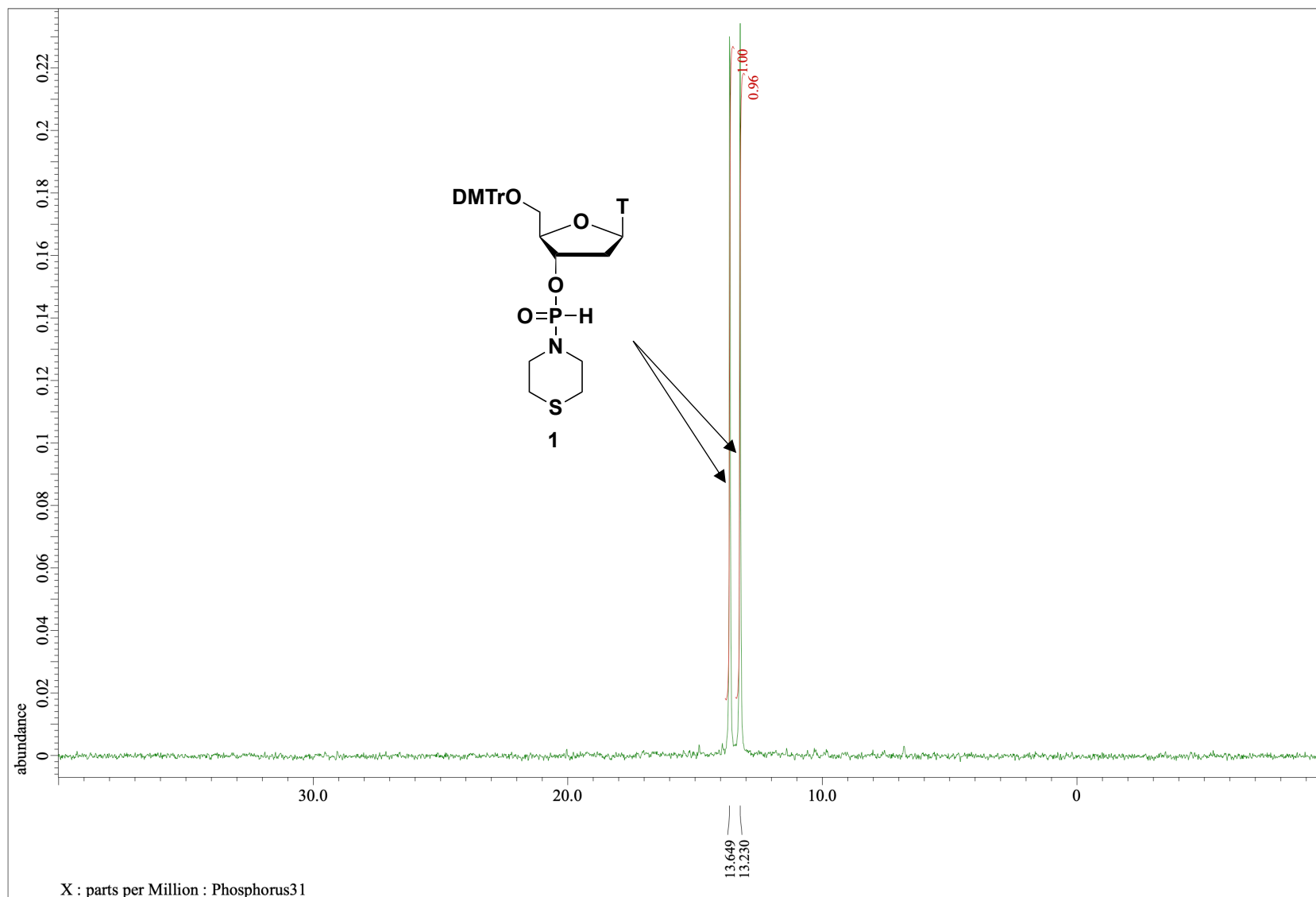


Figure S 6 ^{31}P NMR spectrum (CDCl_3 , 162 MHz) of the reaction mixture (solvent: CH_2Cl_2).

Table 2, entry 2

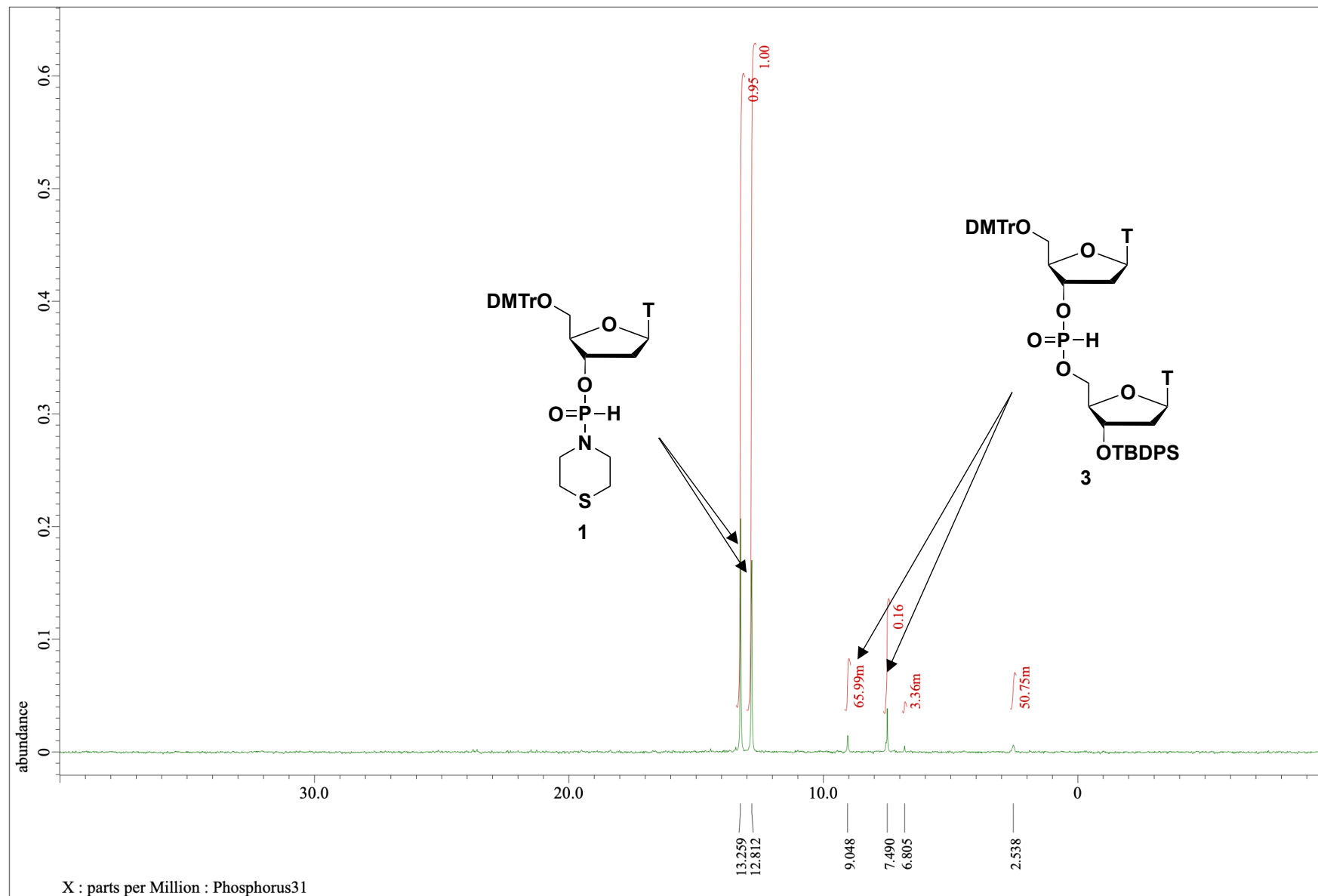


Figure S 7 ^{31}P NMR spectrum (CDCl_3 , 162 MHz) of the reaction mixture (solvent: THF).

Table 2, entry 3

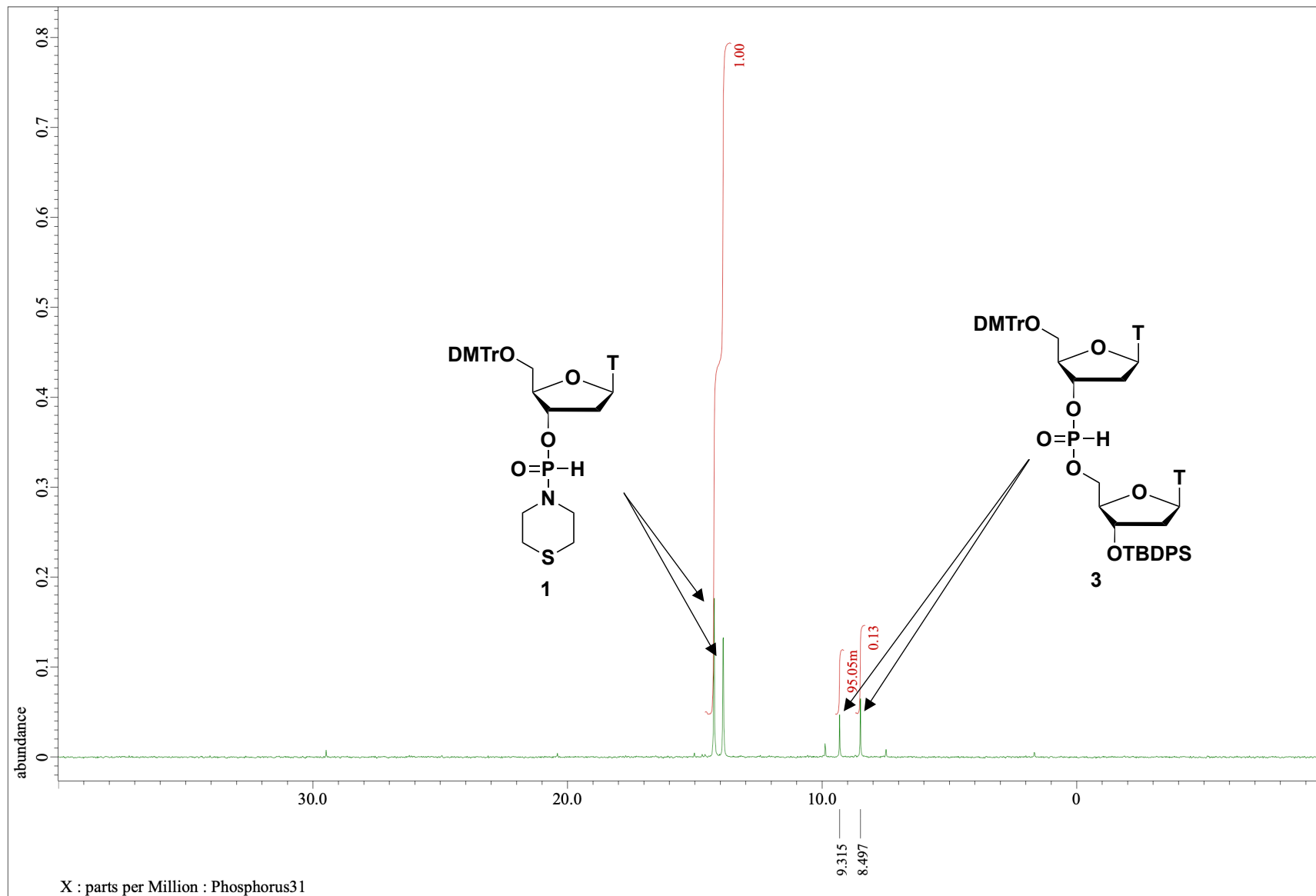
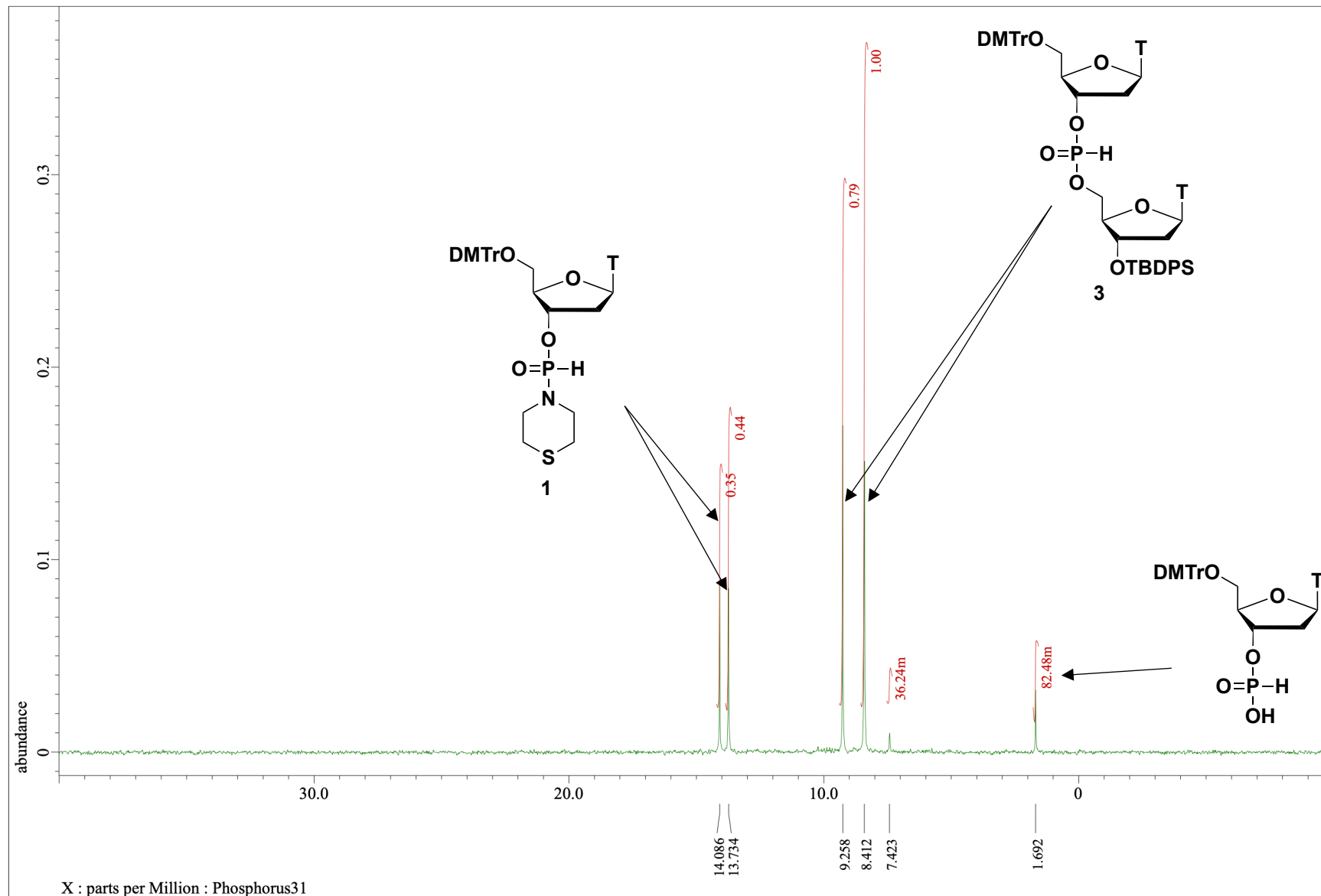


Figure S8 ^{31}P NMR spectrum (CD_3CN , 162 MHz) of the reaction mixture (solvent: MeCN).

Figure S 9 ^{31}P NMR spectrum (CD_3CN , 162 MHz) of the reaction mixture (solvent: MeCN with 6 equivalents of pyridine).

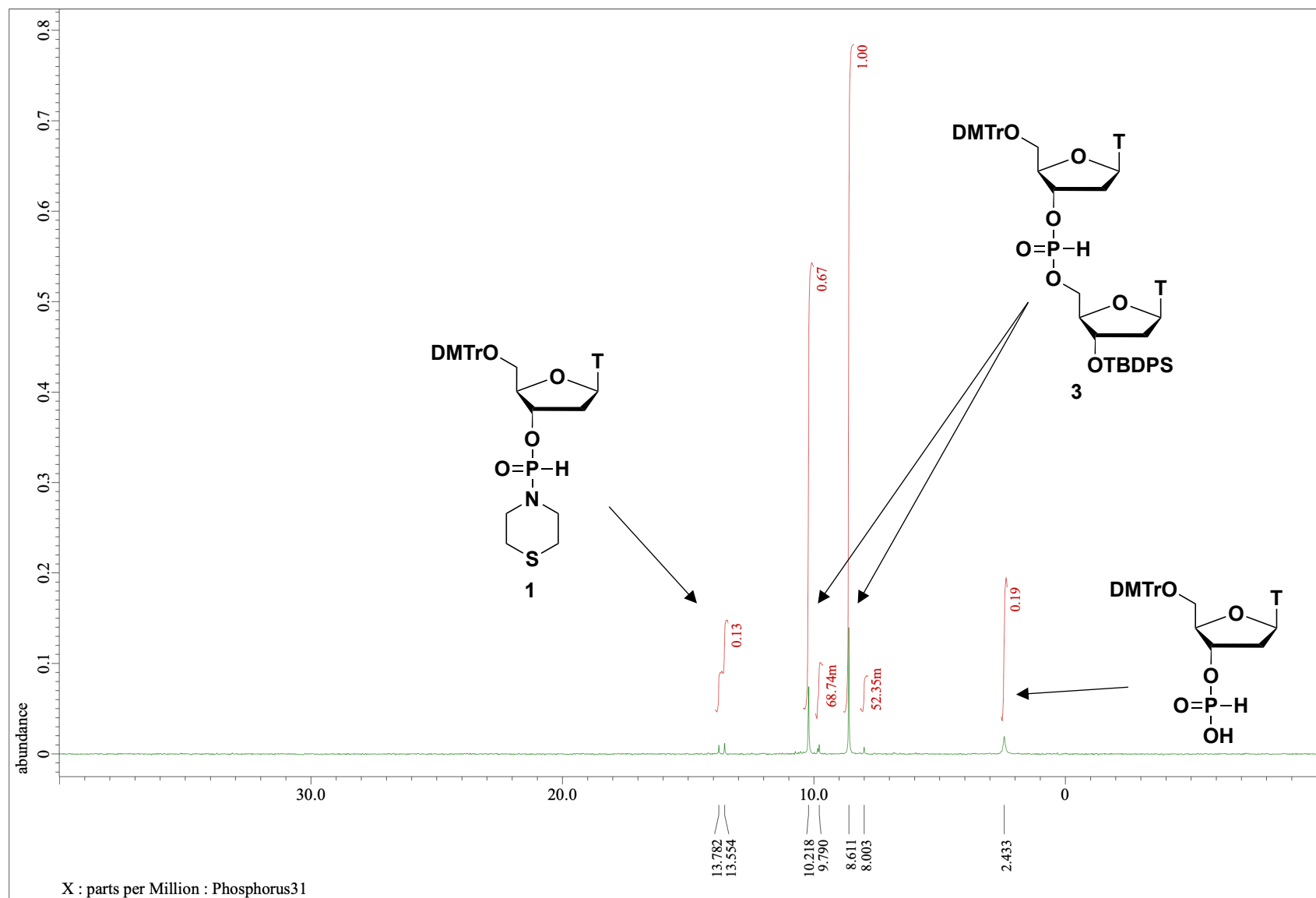
Figure S10 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (solvent: pyridine).

Table 2, entry 6

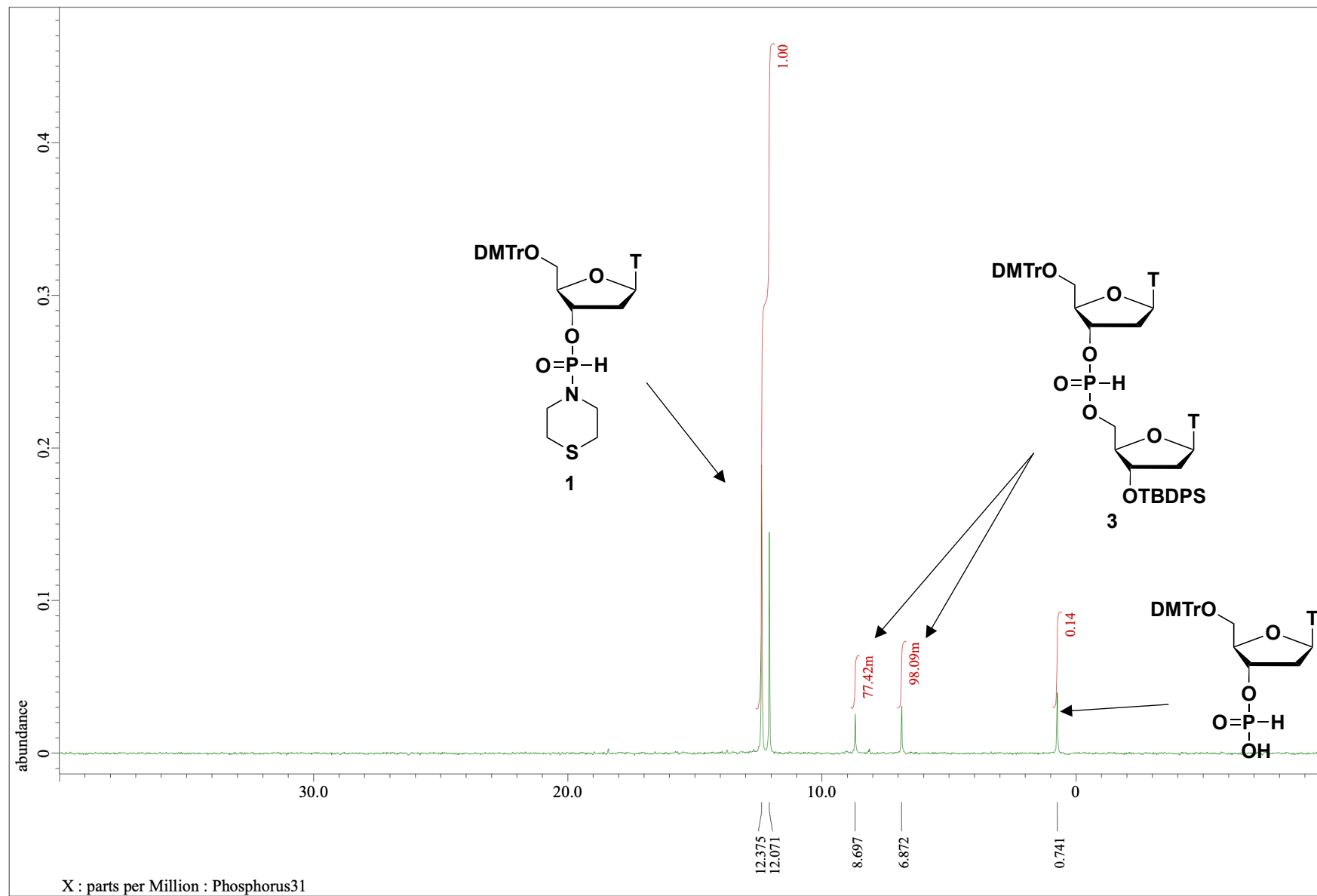


Figure S11 ^{31}P NMR spectrum (CDCl_3 , 162 MHz) of the reaction mixture (solvent: 2,6-lutidine).

4. ^{31}P NMR analysis of the effect of leaving groups on the condensation reaction. (Table3)

Table 3, entry 1

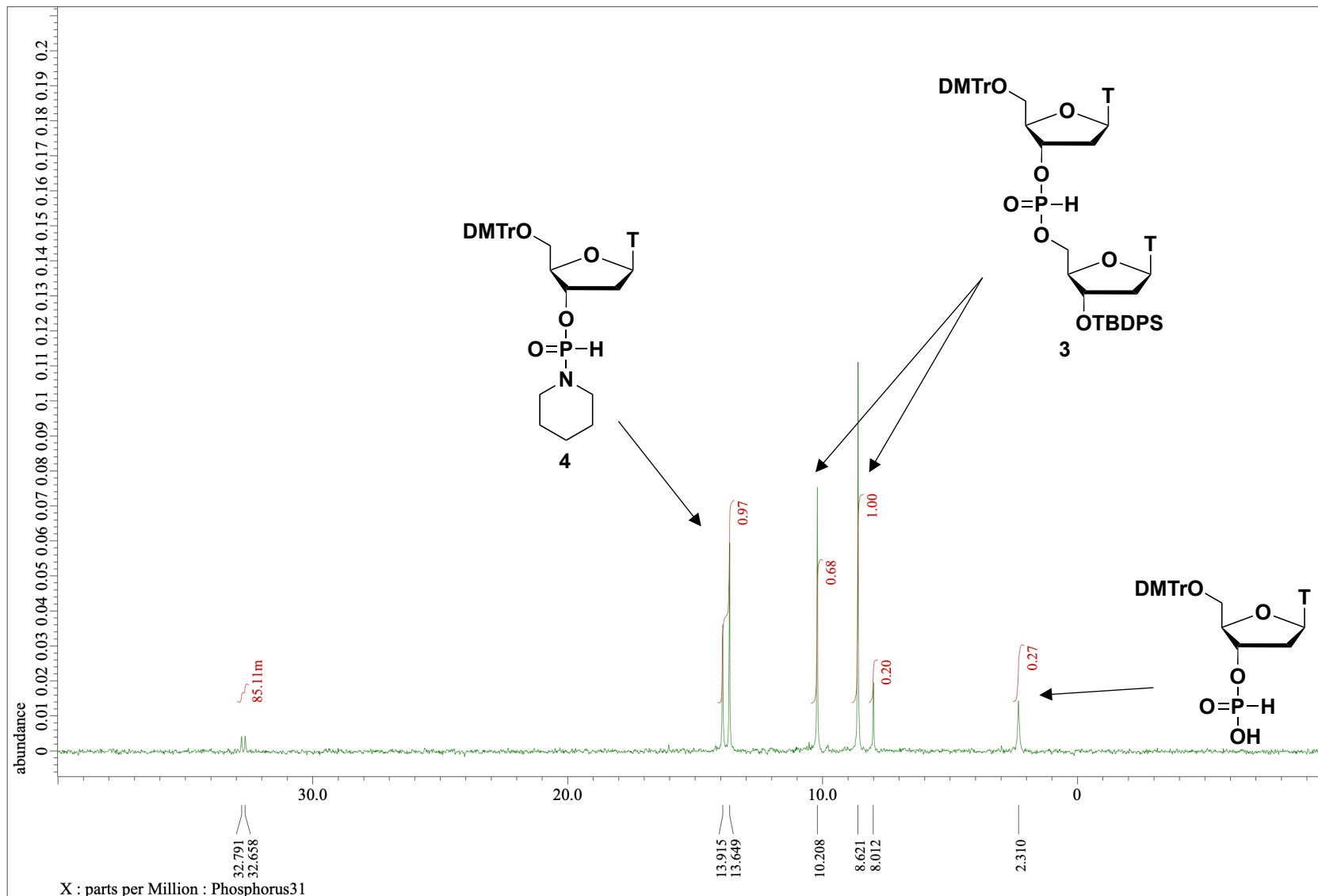


Figure S12 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (leaving group: piperidine).

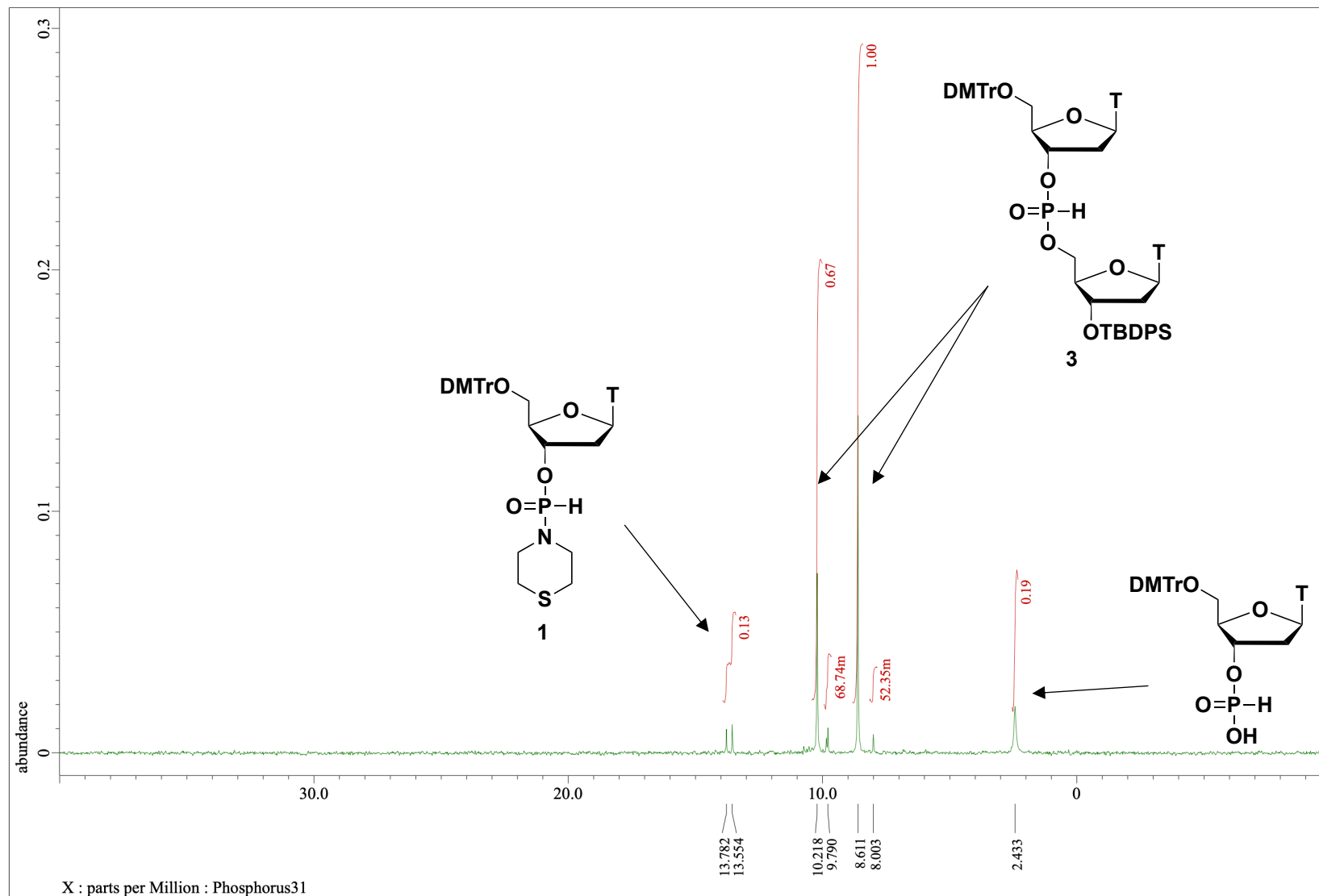
Figure S13 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (leaving group: thiomorpholine).

Table 3, entry 3

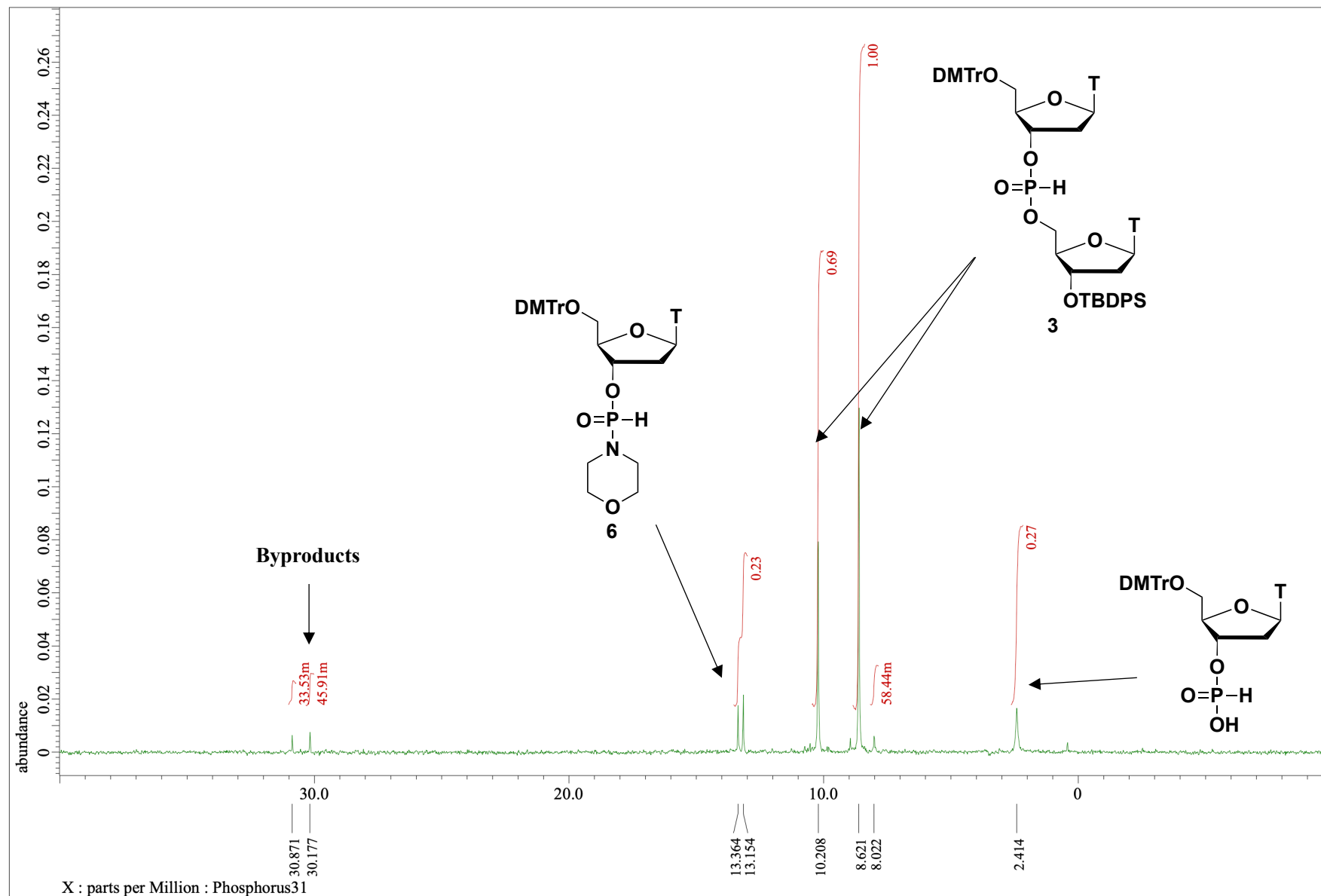


Figure S14 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (leaving group: morpholine).

5. ^{31}P NMR analysis of the effect of acidic activators on the condensation reaction in pyridine. (Table5)

Table 5, entry 1

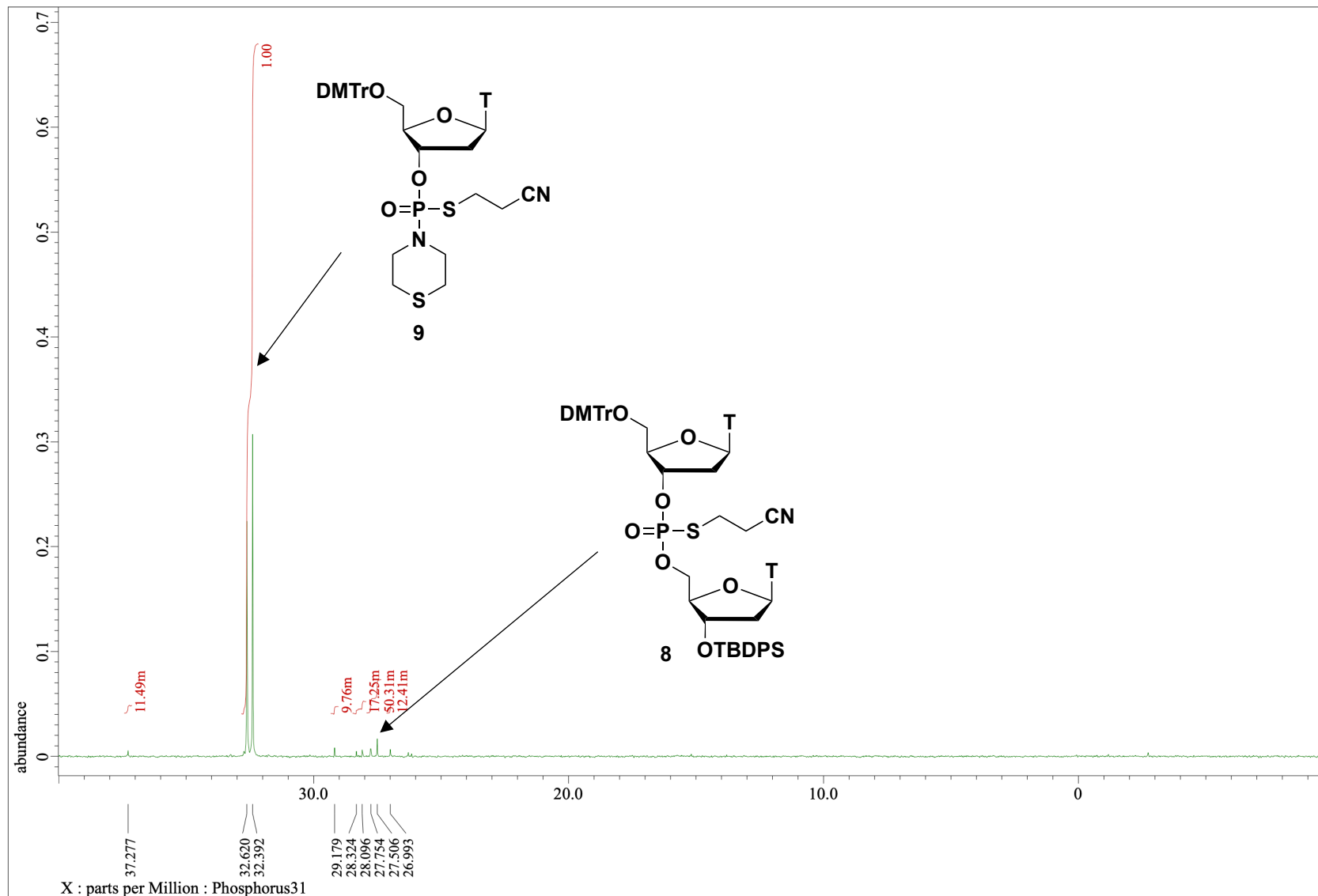


Figure S15 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (acidic activator: TRI).

Table 5, entry 2

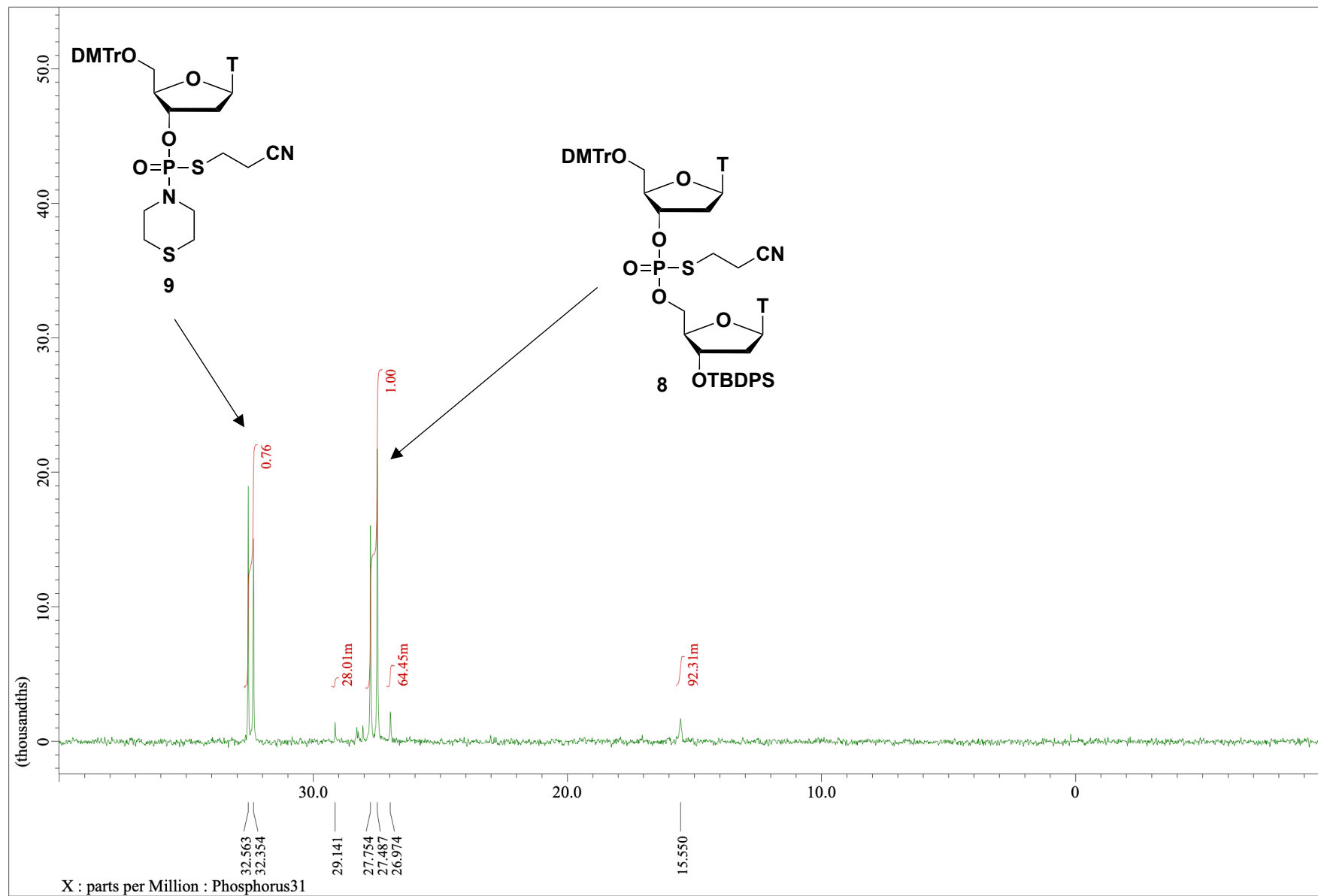


Figure S16 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (acidic activator: PhIMT).

Table 5, entry 3

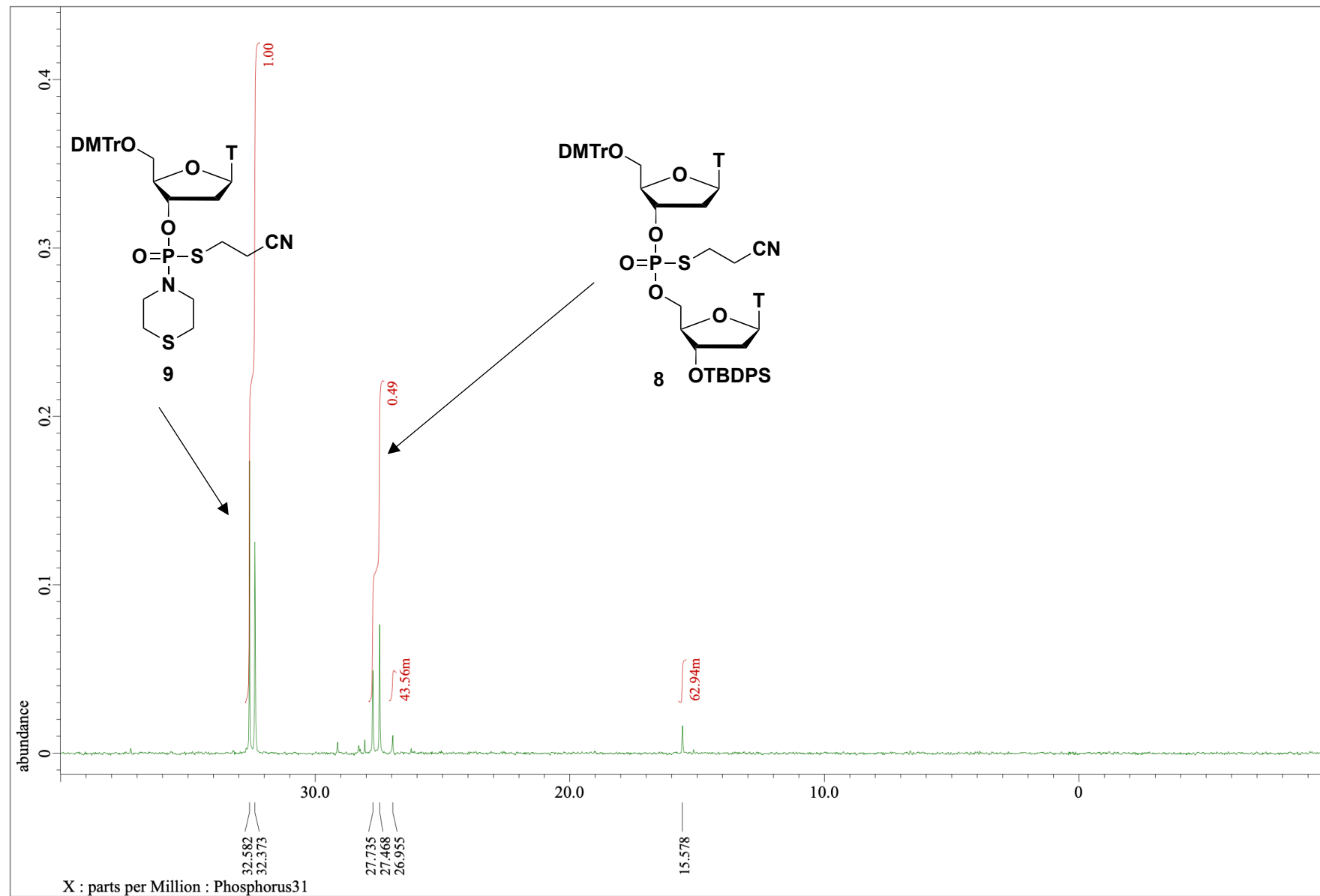


Figure S17 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (acidic activator: NT).

Table 5, entry 4

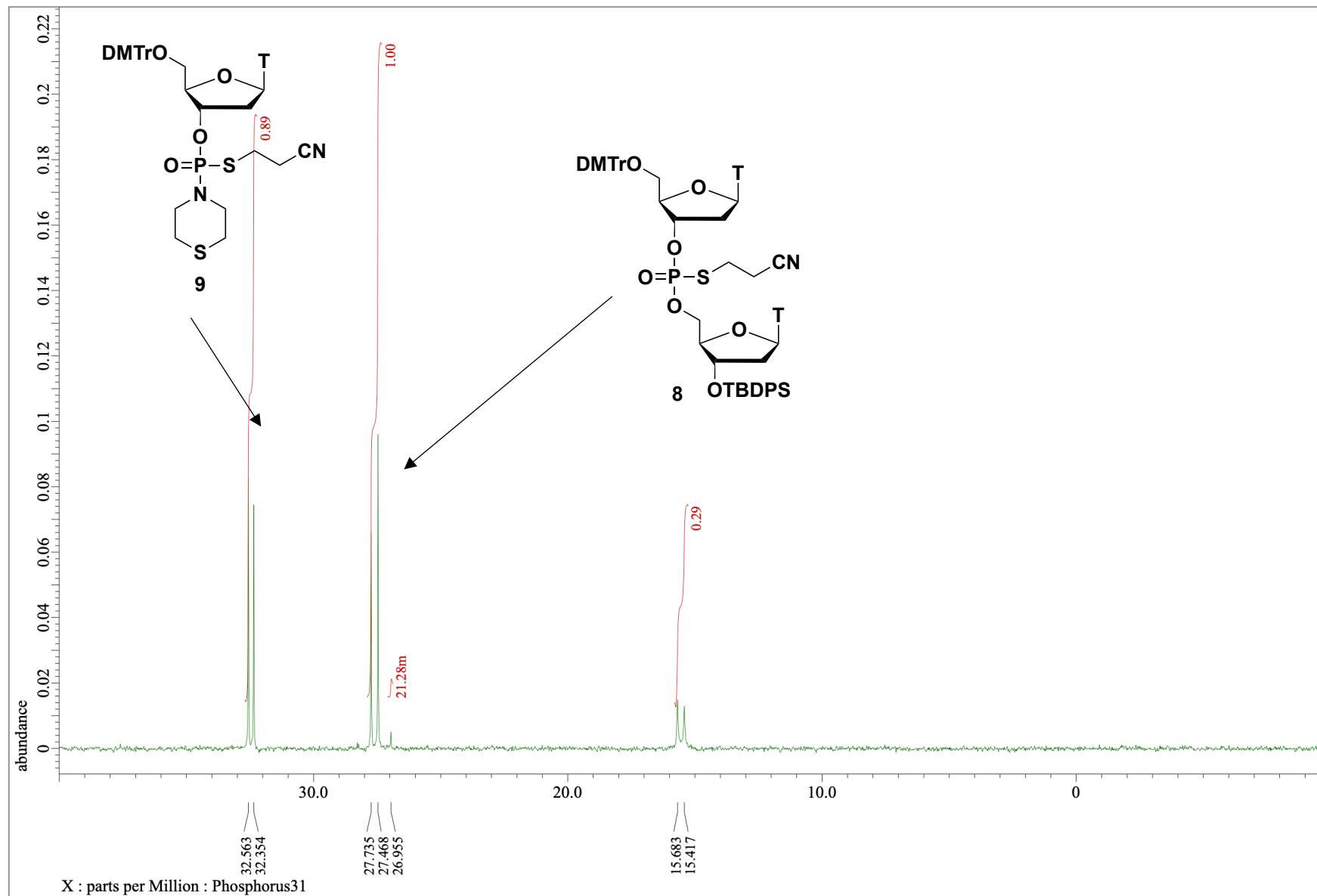


Figure S 18 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (acidic activator: PyT).

Table 5, entry 5

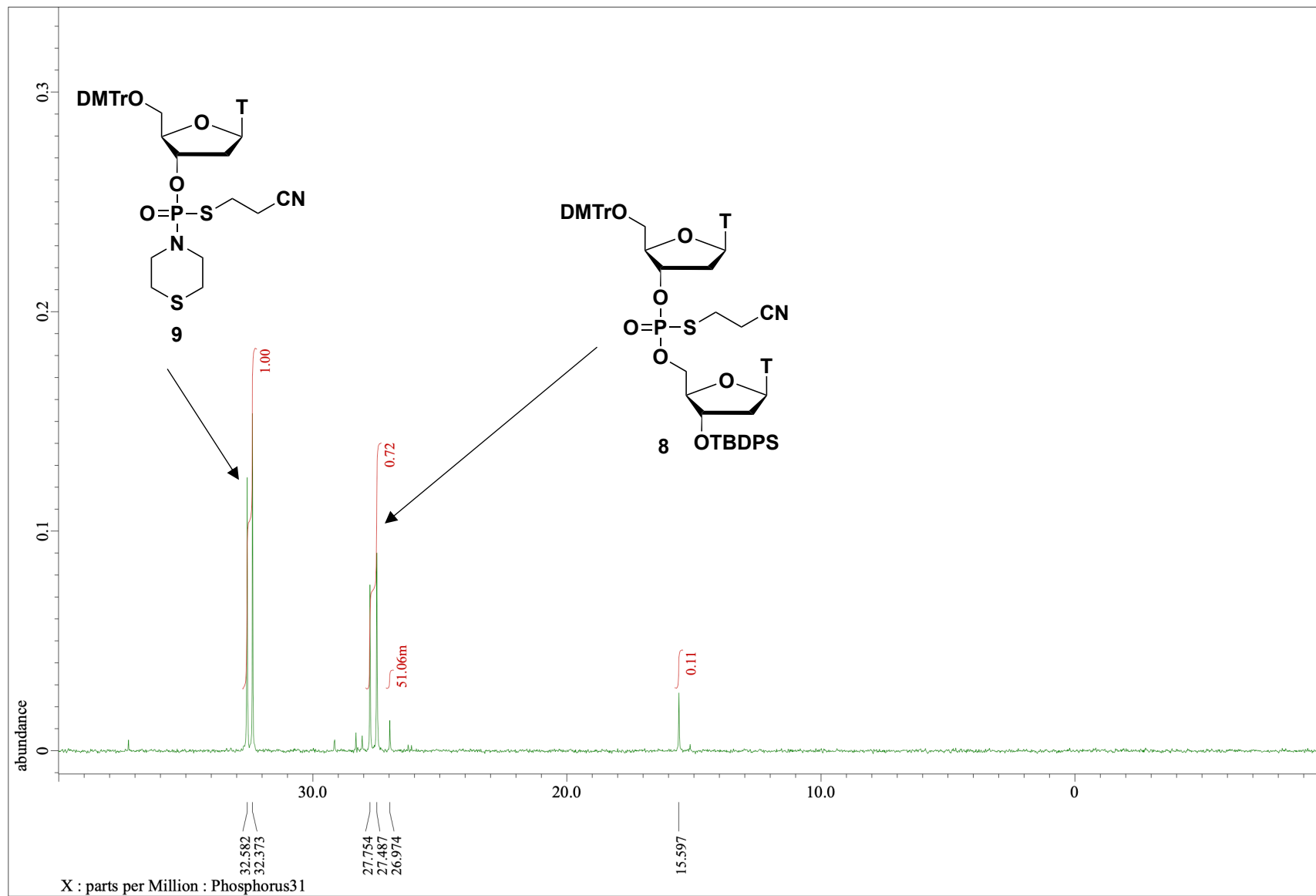


Figure S19 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (acidic activator: TET).

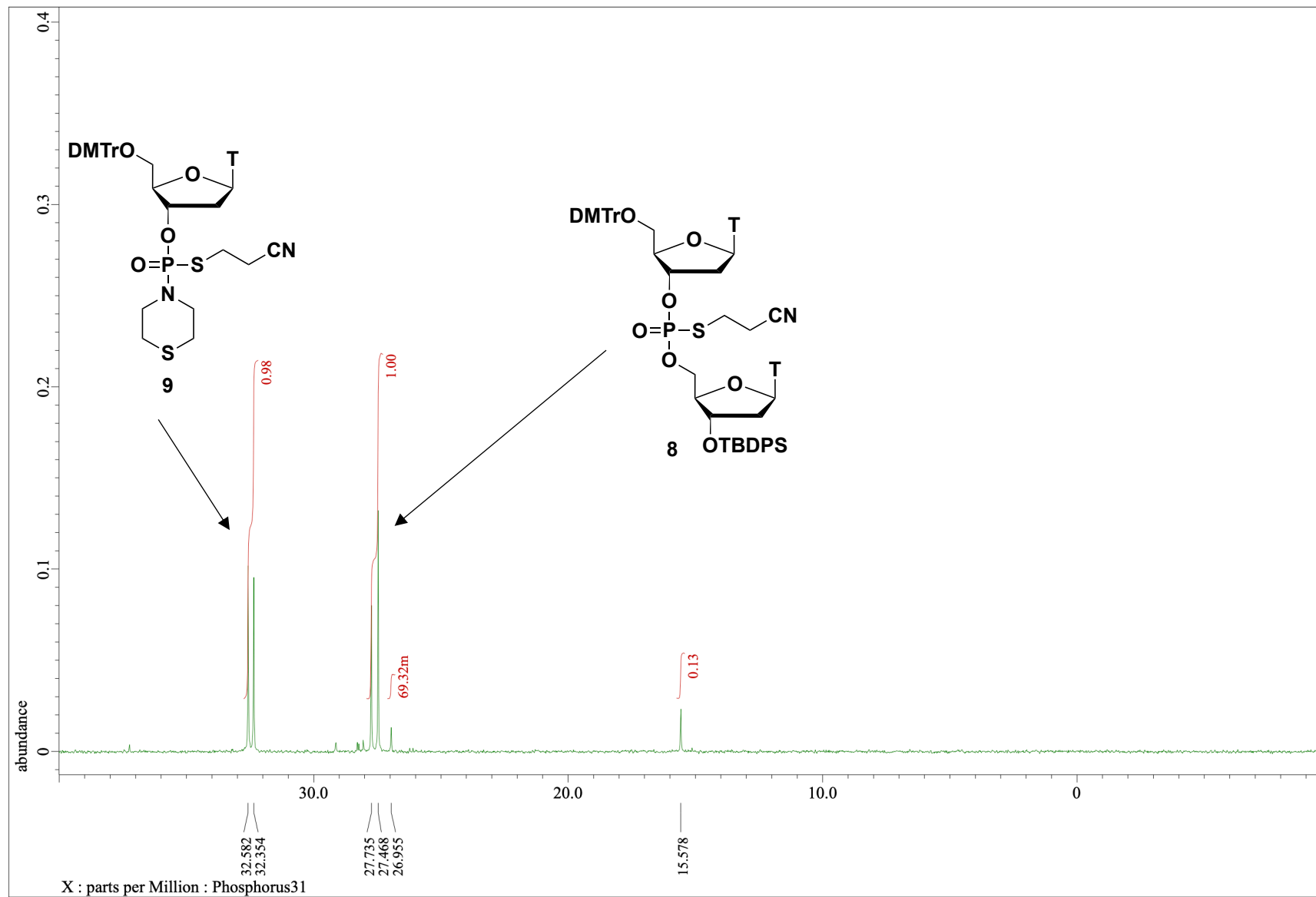
Figure S21 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (acidic activator: ETT).

Table 5, entry 8

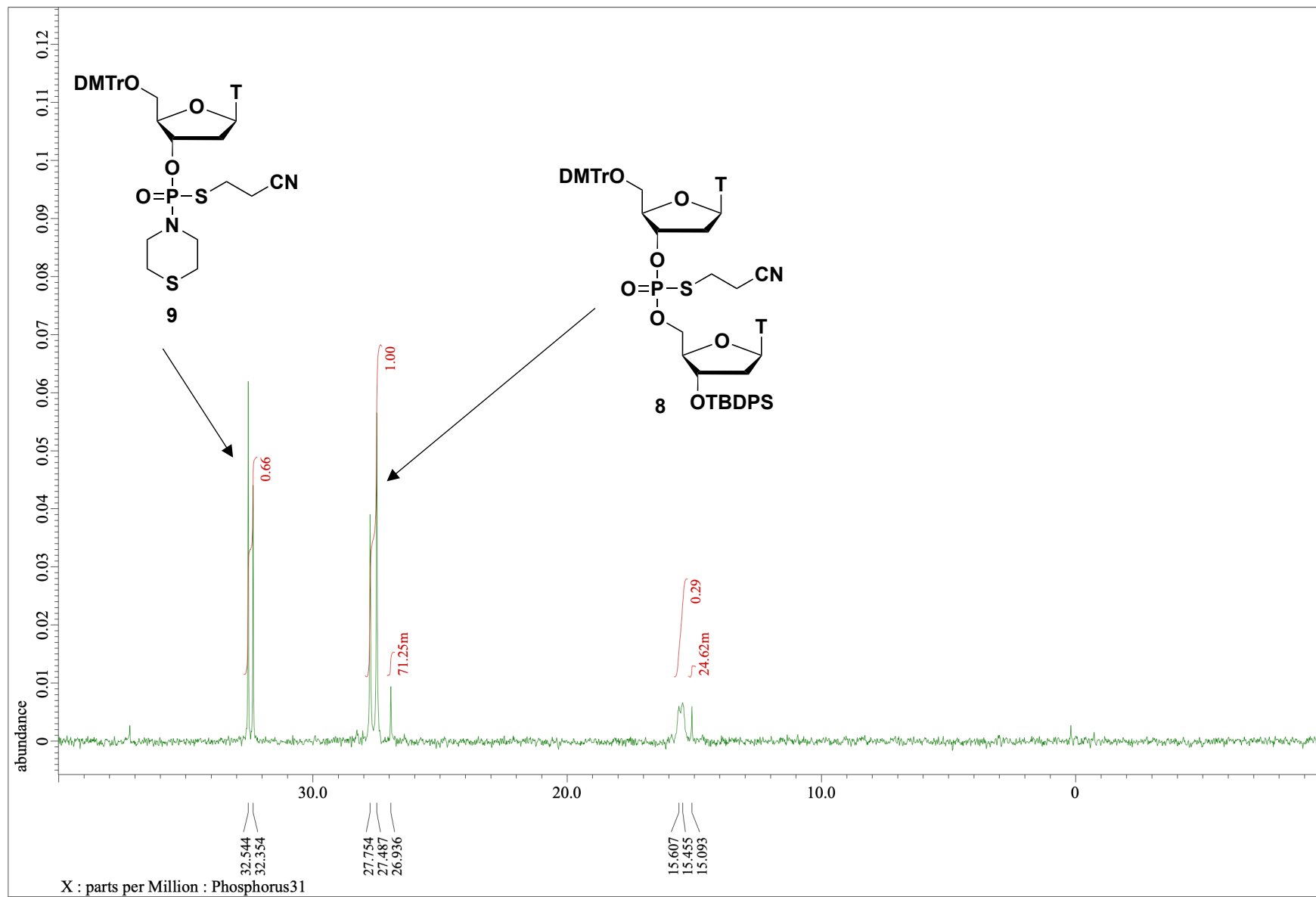


Figure S22 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (acidic activator: NMTRT).

Table 5, entry 9

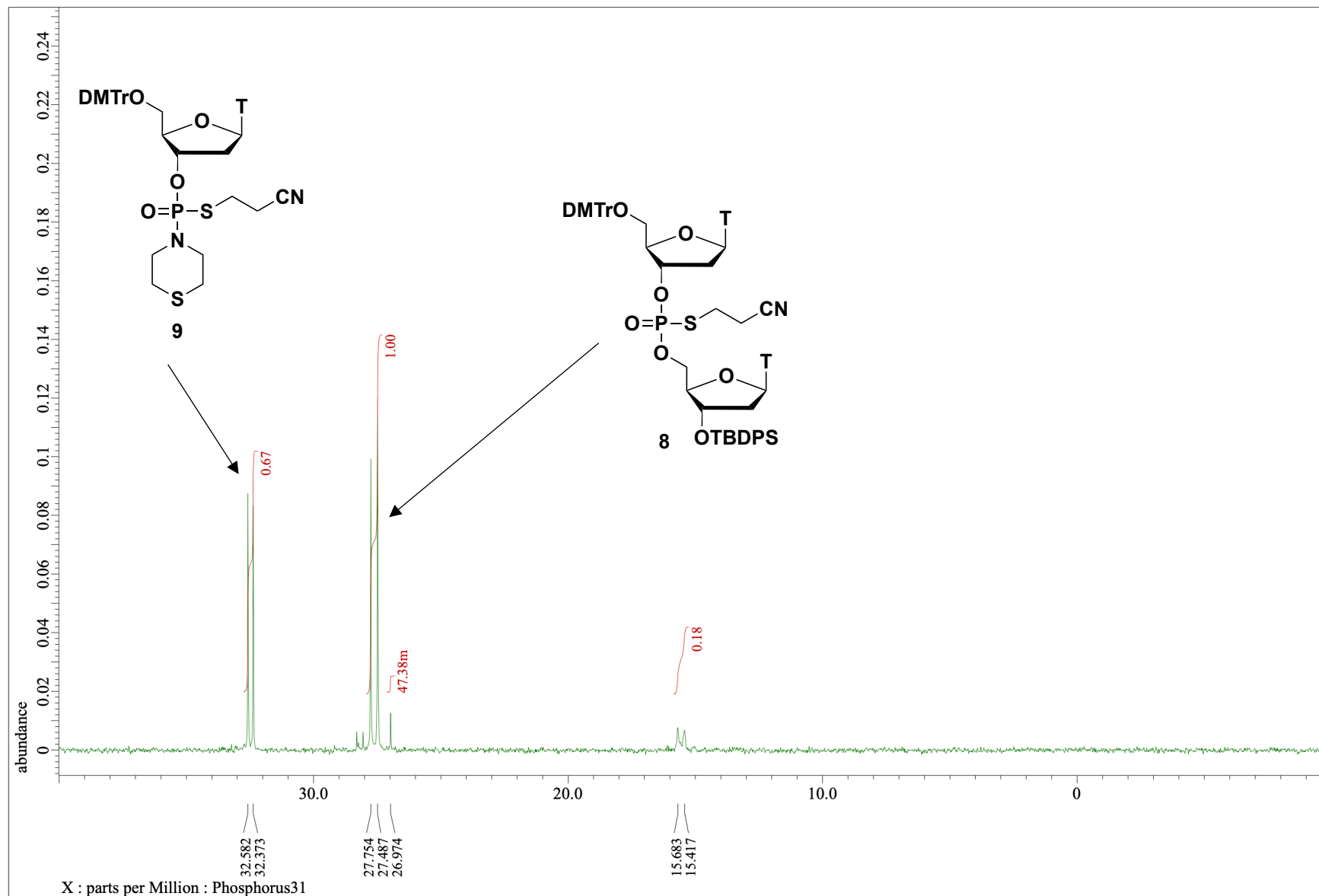


Figure S23 ^{31}P NMR spectrum (pyridine- d_5 , 162 MHz) of the reaction mixture (acidic activator: NBT).

6. ^{31}P NMR analysis of the crude mixture of *S*-cyanoethyl phosphorothioate diester 10 (Table 8)

Table 8, entry 1

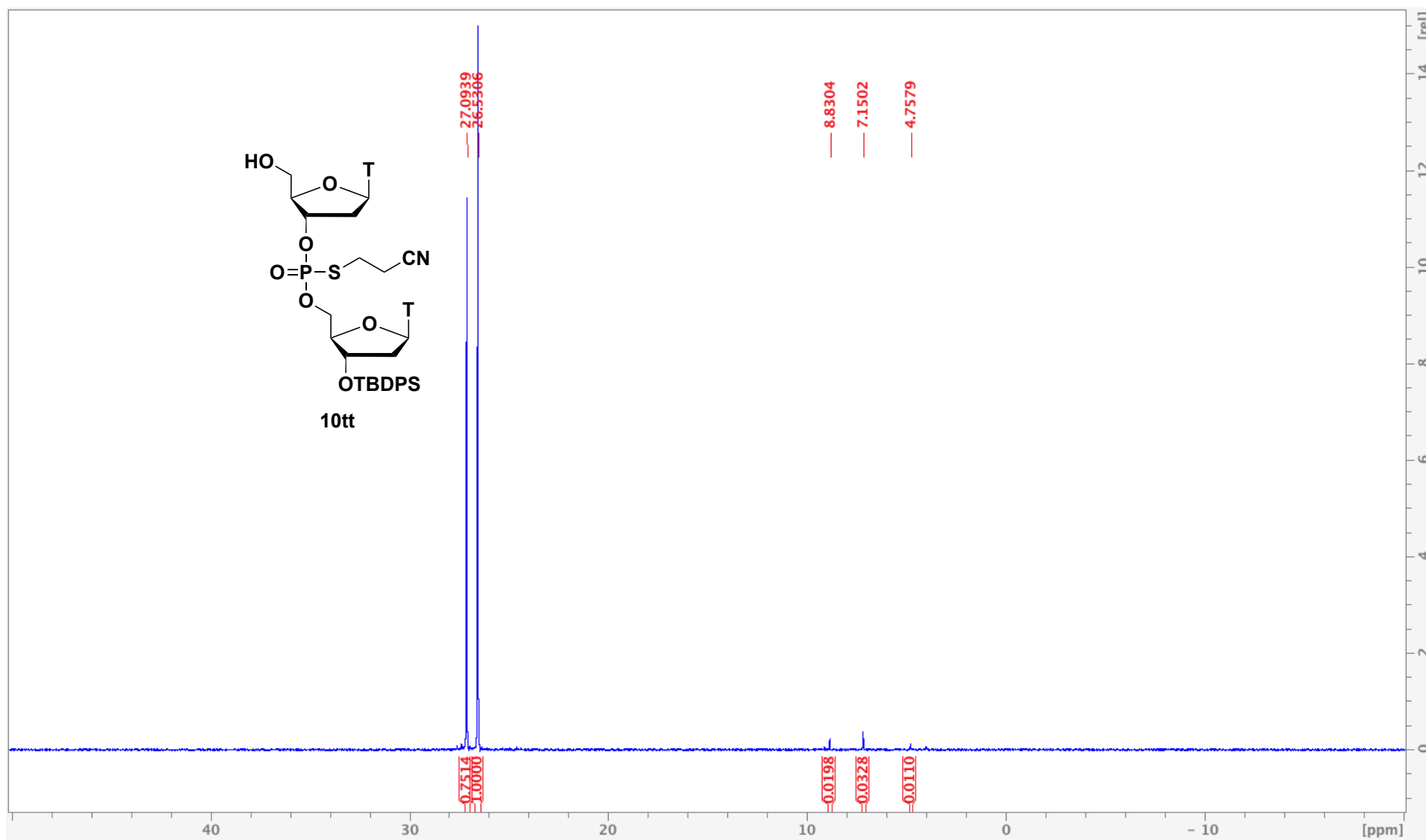


Figure S 24 ^{31}P NMR spectrum (CDCl_3 , 202 MHz) of the crude mixture (10tt).

Table 8, entry 2

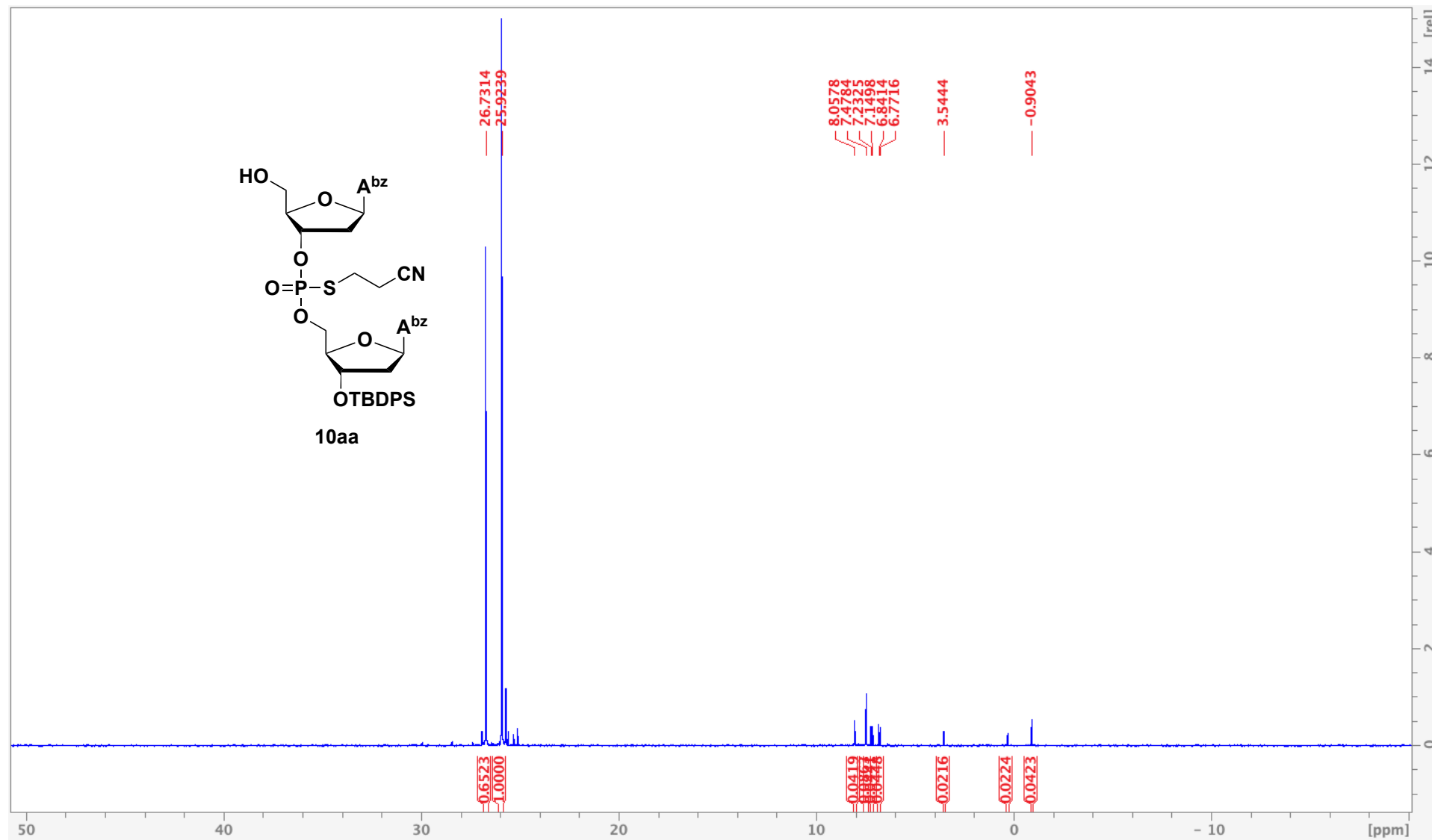


Figure S25 ^{31}P NMR spectrum (CDCl_3 , 202 MHz) of the crude mixture (10aa).

Table 8, entry 3

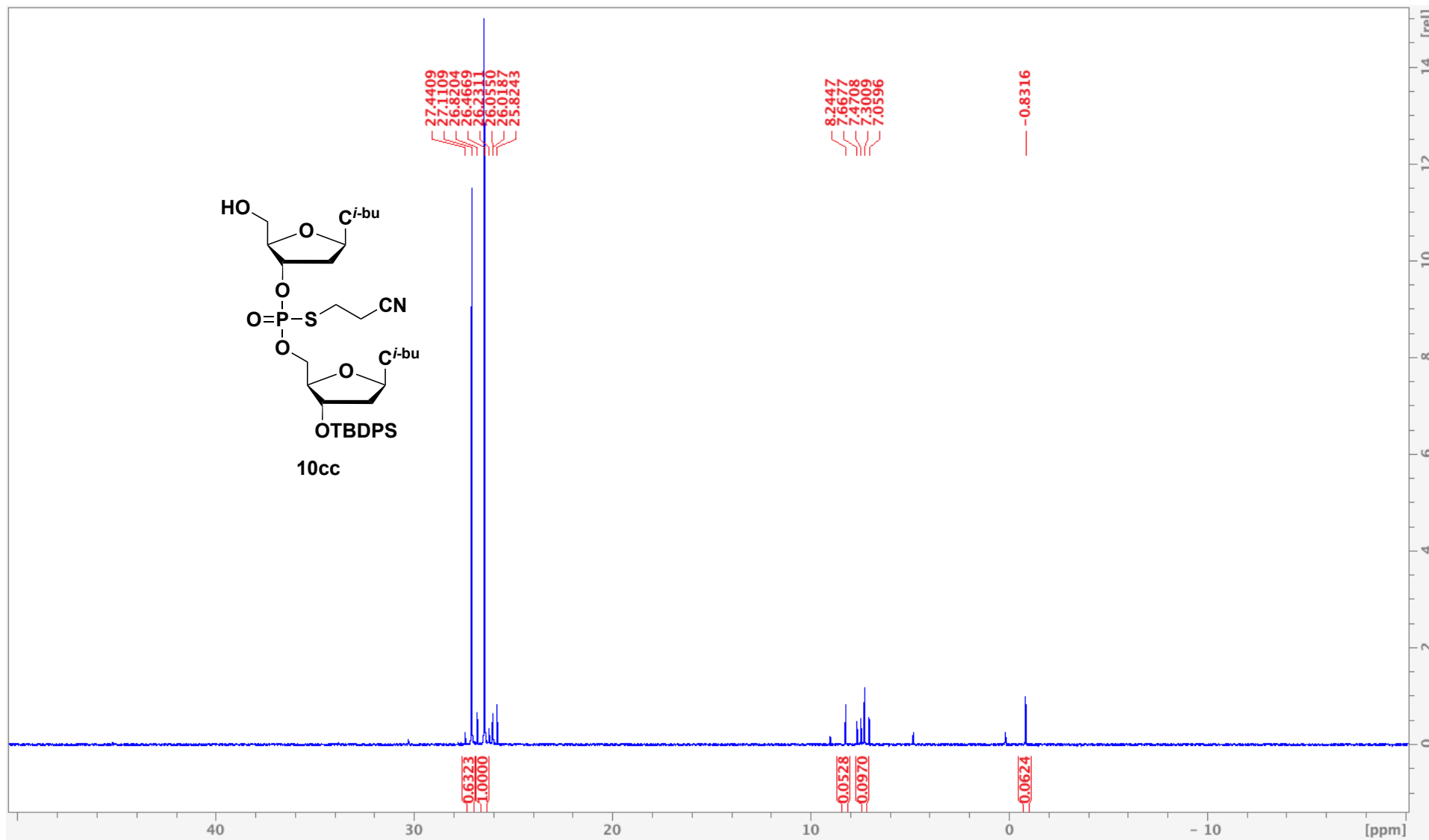


Figure S26 ^{31}P NMR spectrum (CDCl_3 , 202 MHz) of the crude mixture (10cc).

Table 8, entry 4

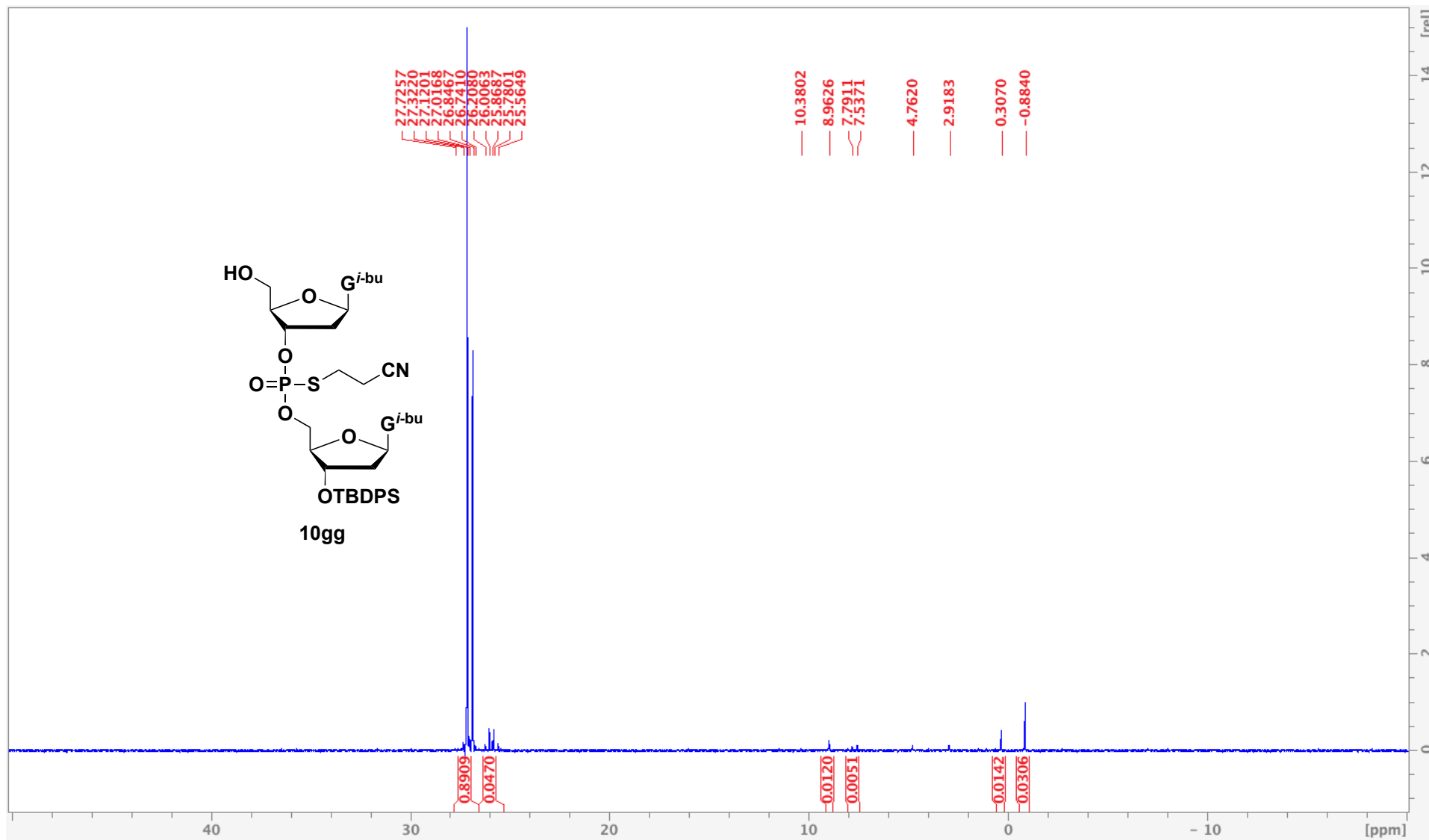
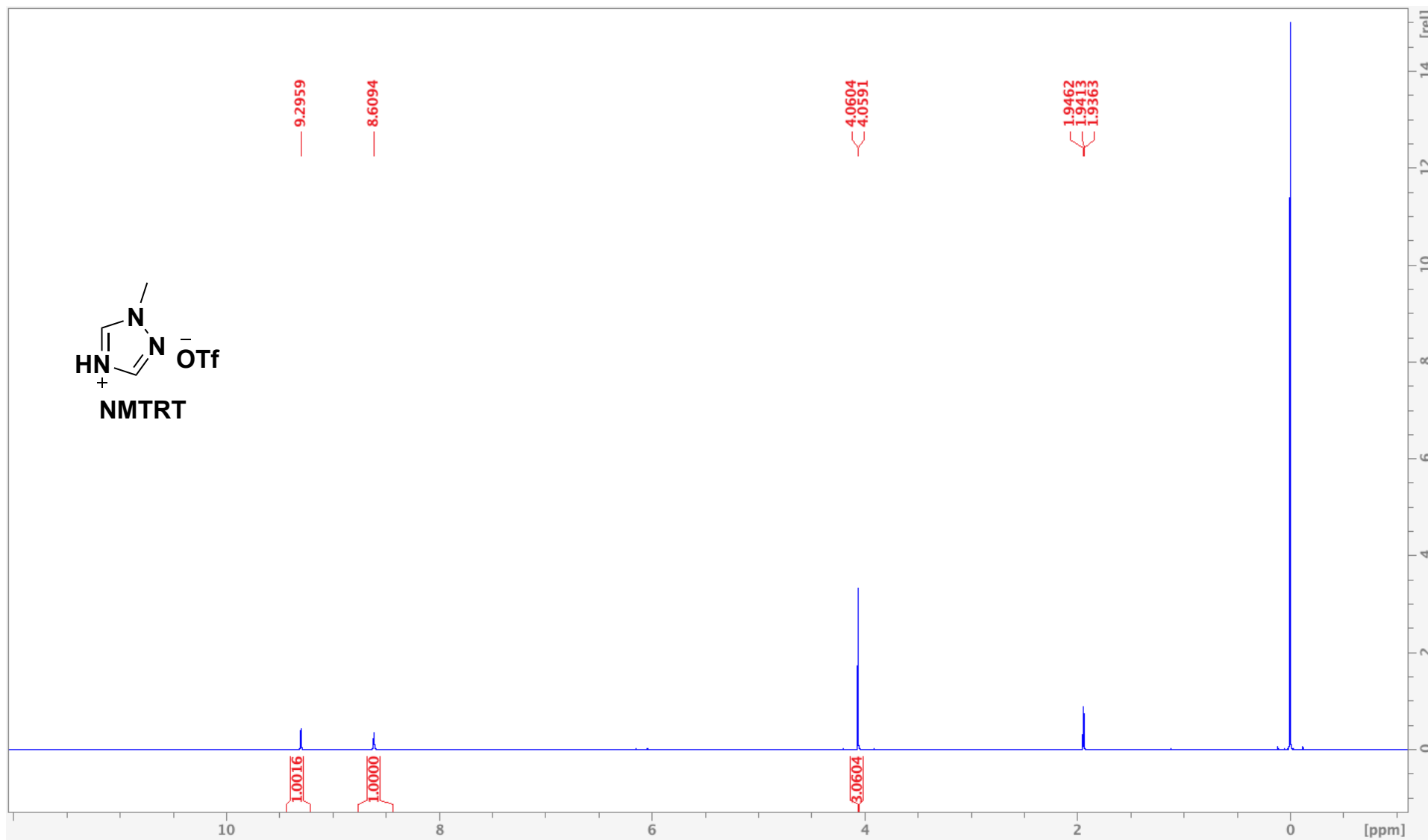


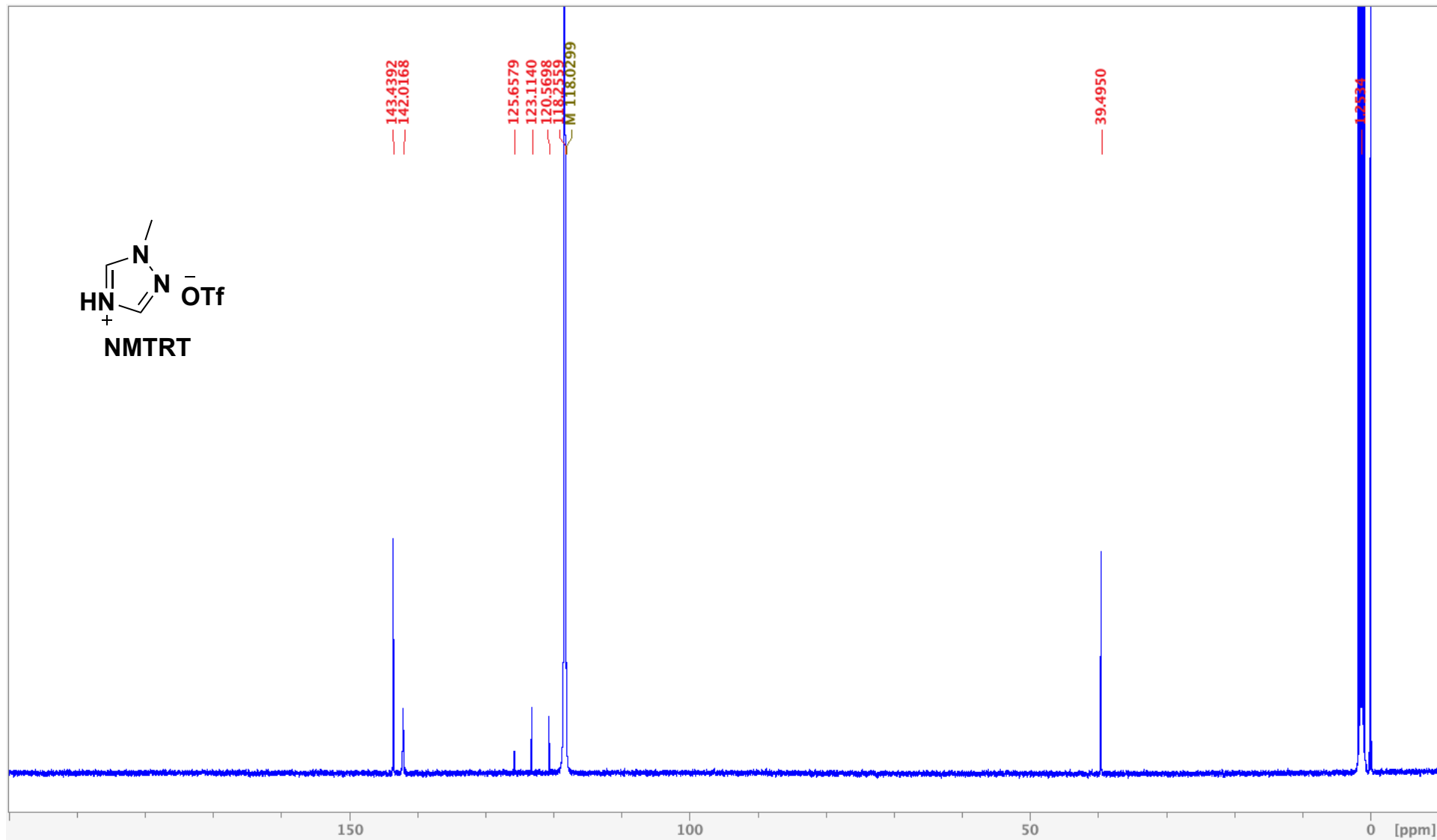
Figure S27 ³¹P NMR spectrum (CDCl₃, 202 MHz) of the crude mixture (10gg).

7. ^1H , ^{13}C , ^{31}P NMR spectra of isolated compounds.

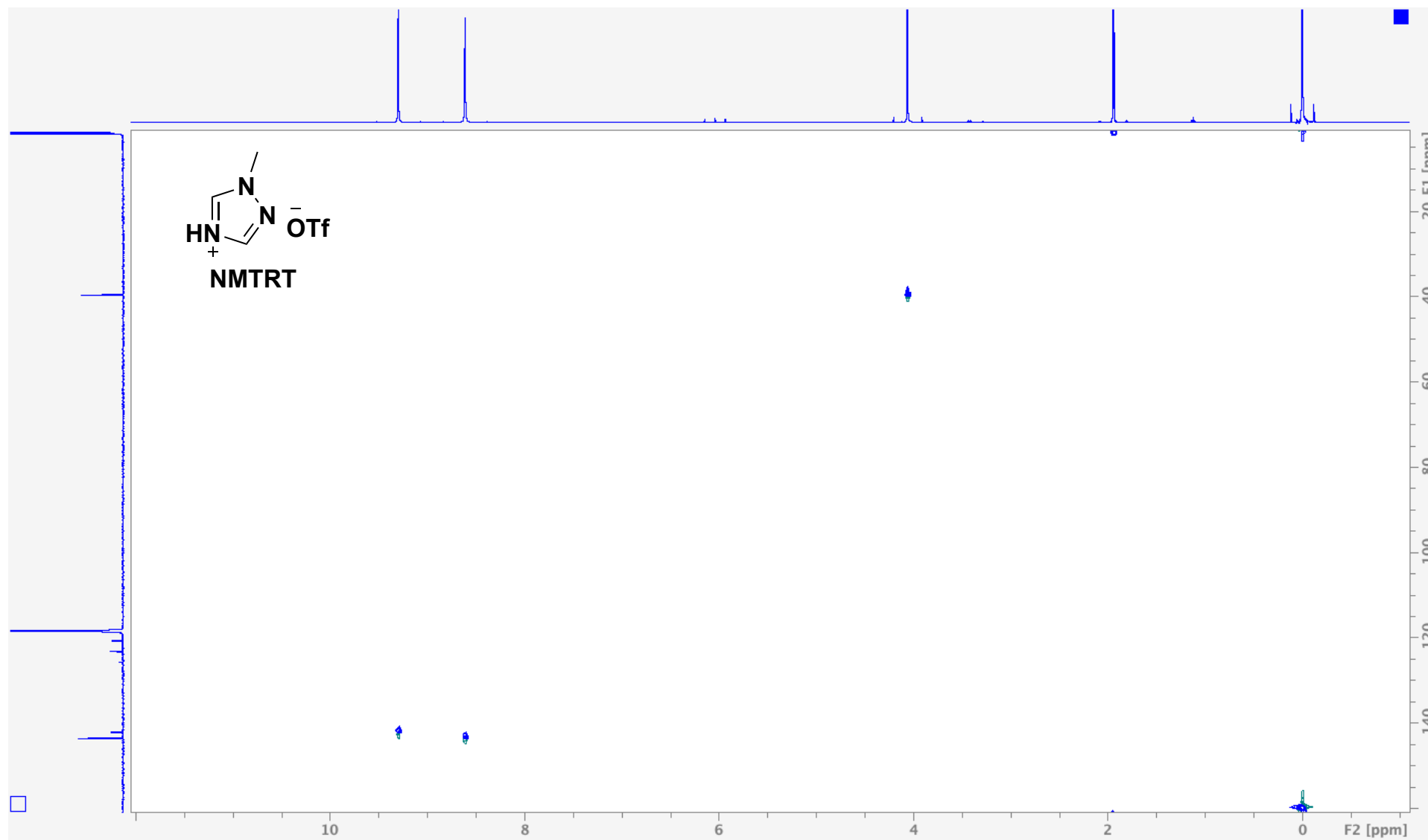
^1H NMR (500 MHz, CD_3CN)



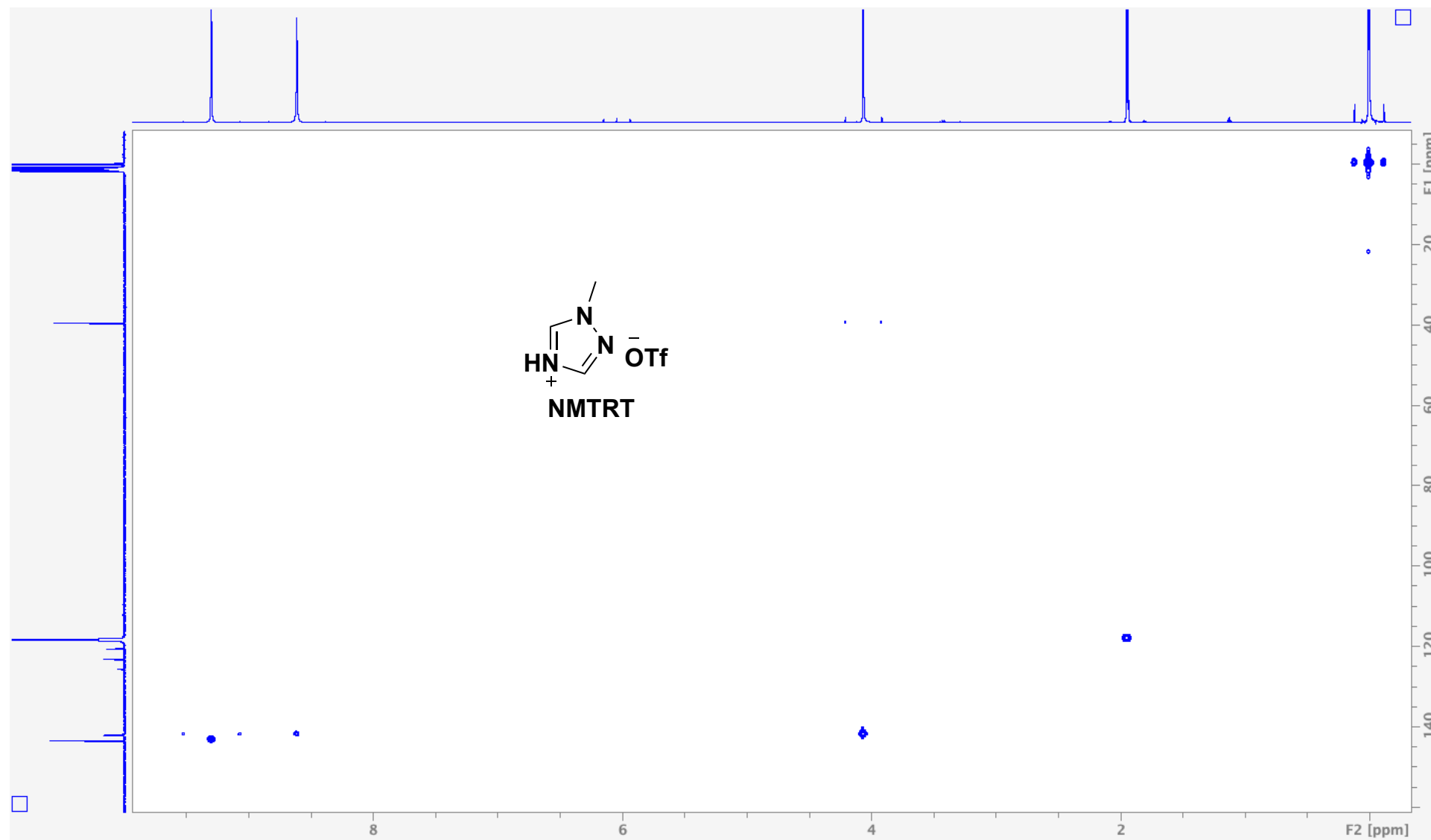
$^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, CD_3CN)



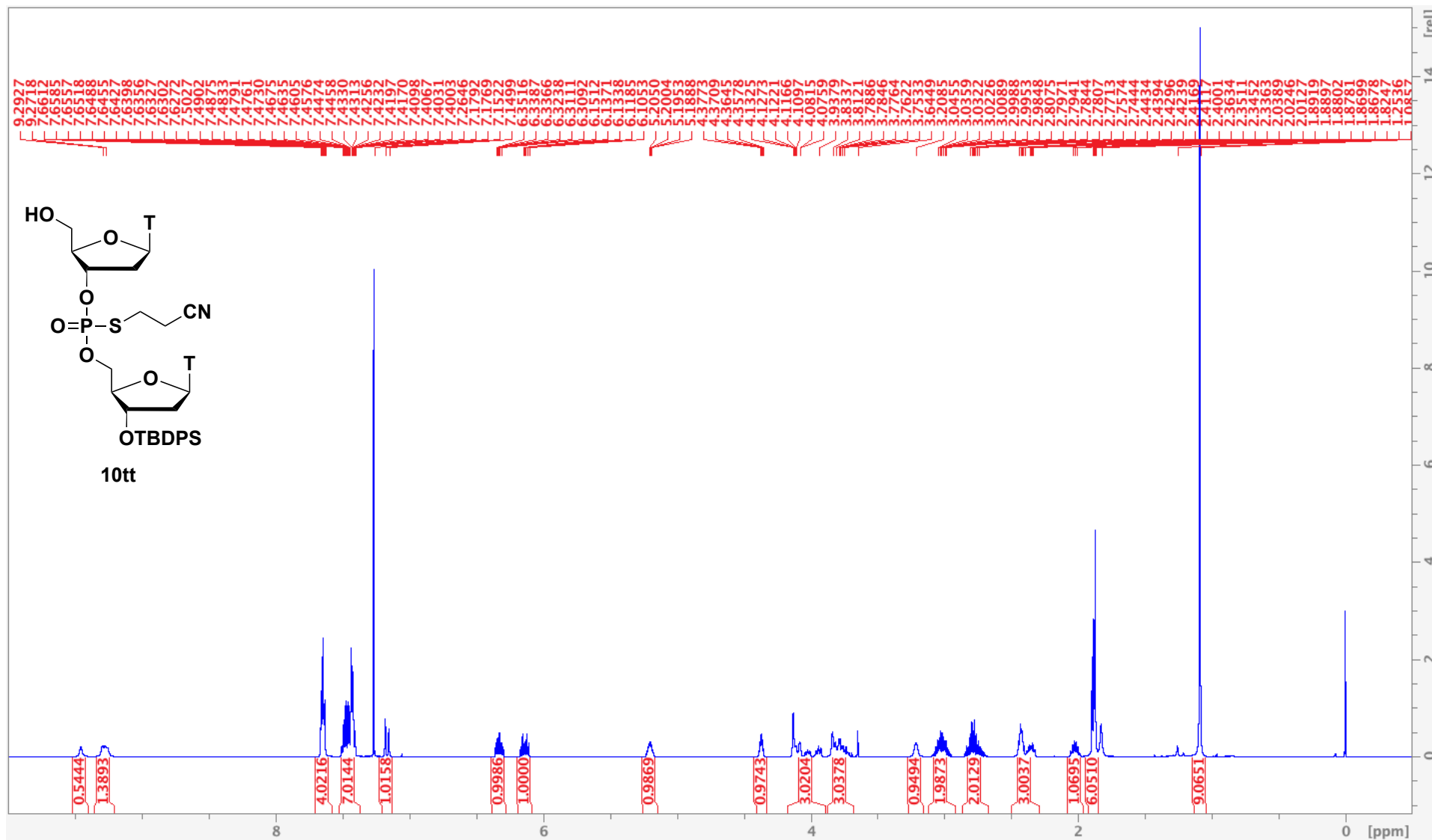
HSQC (CD₃CN)



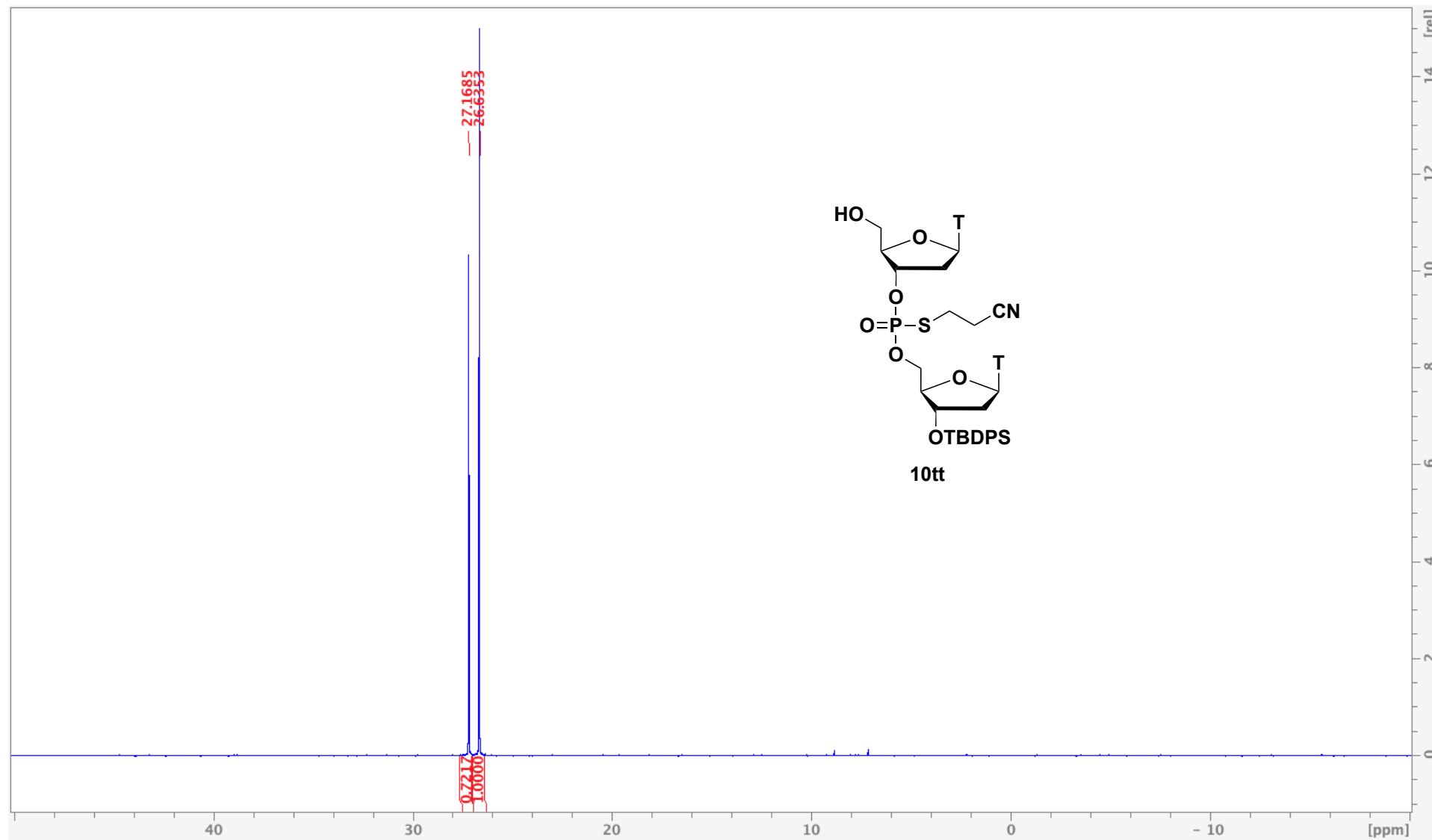
HMBC (CD₃CN)



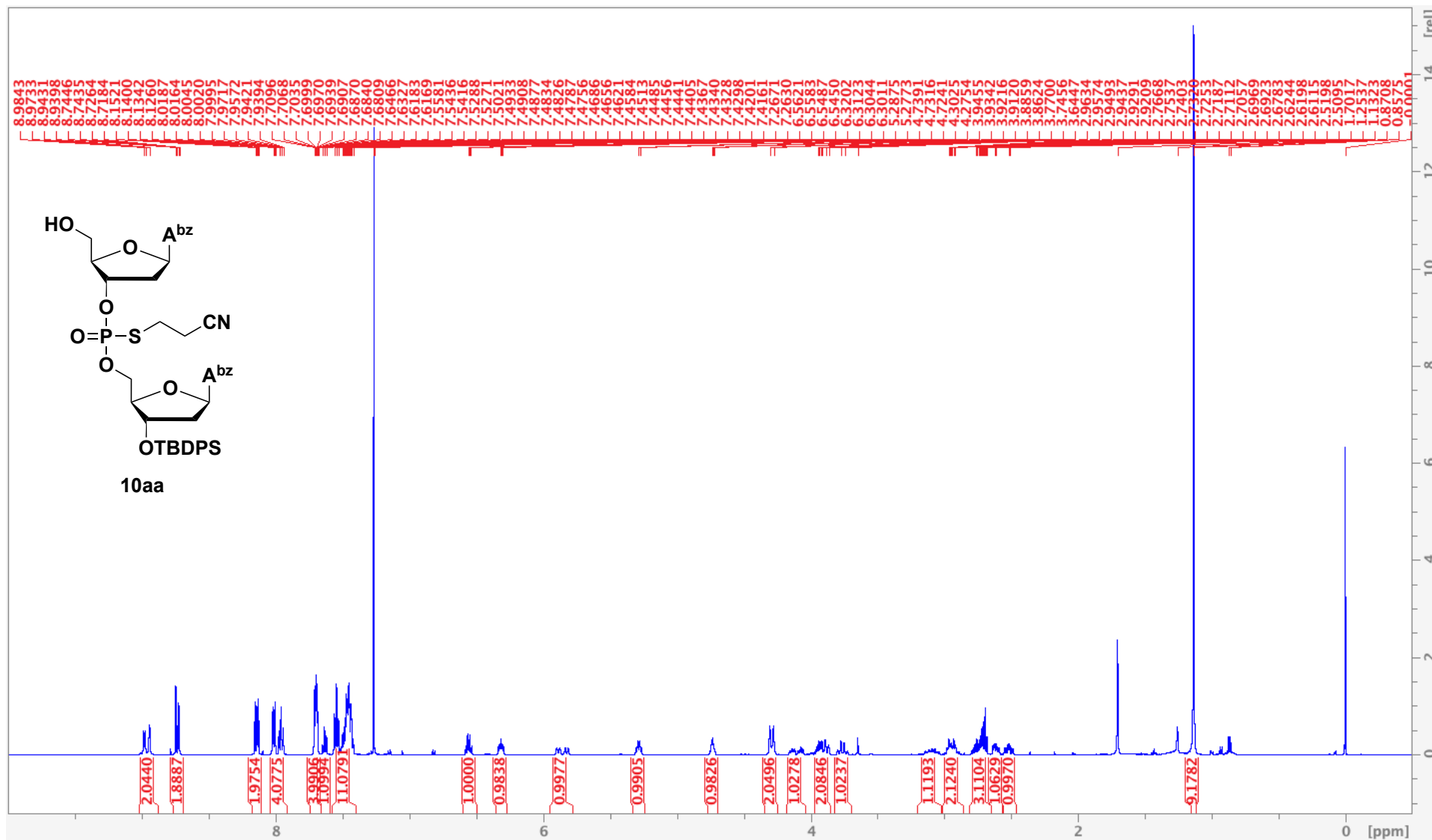
¹H NMR (500MHz, CDCl₃)



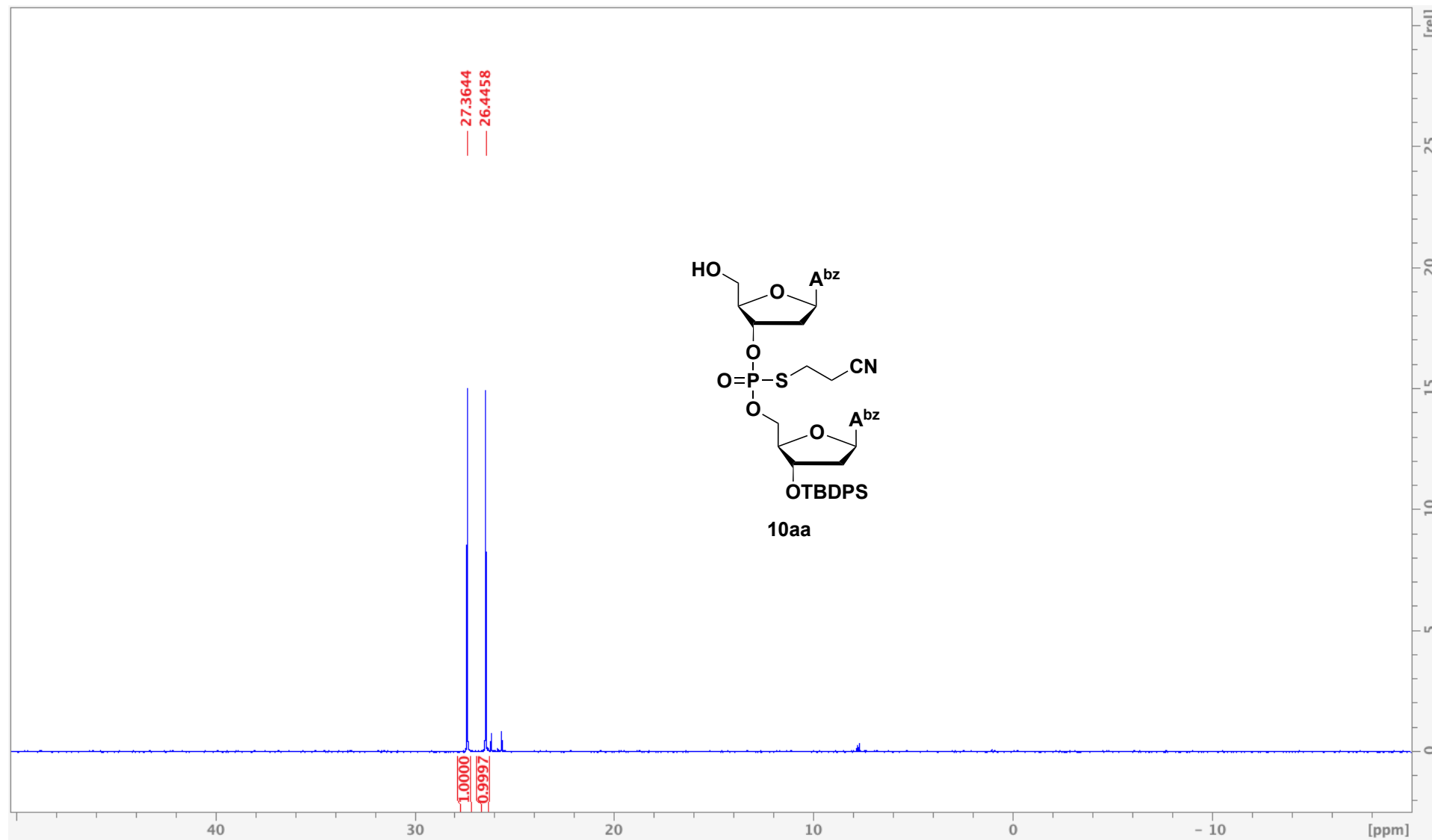
^{31}P NMR (202 MHz, CDCl_3)



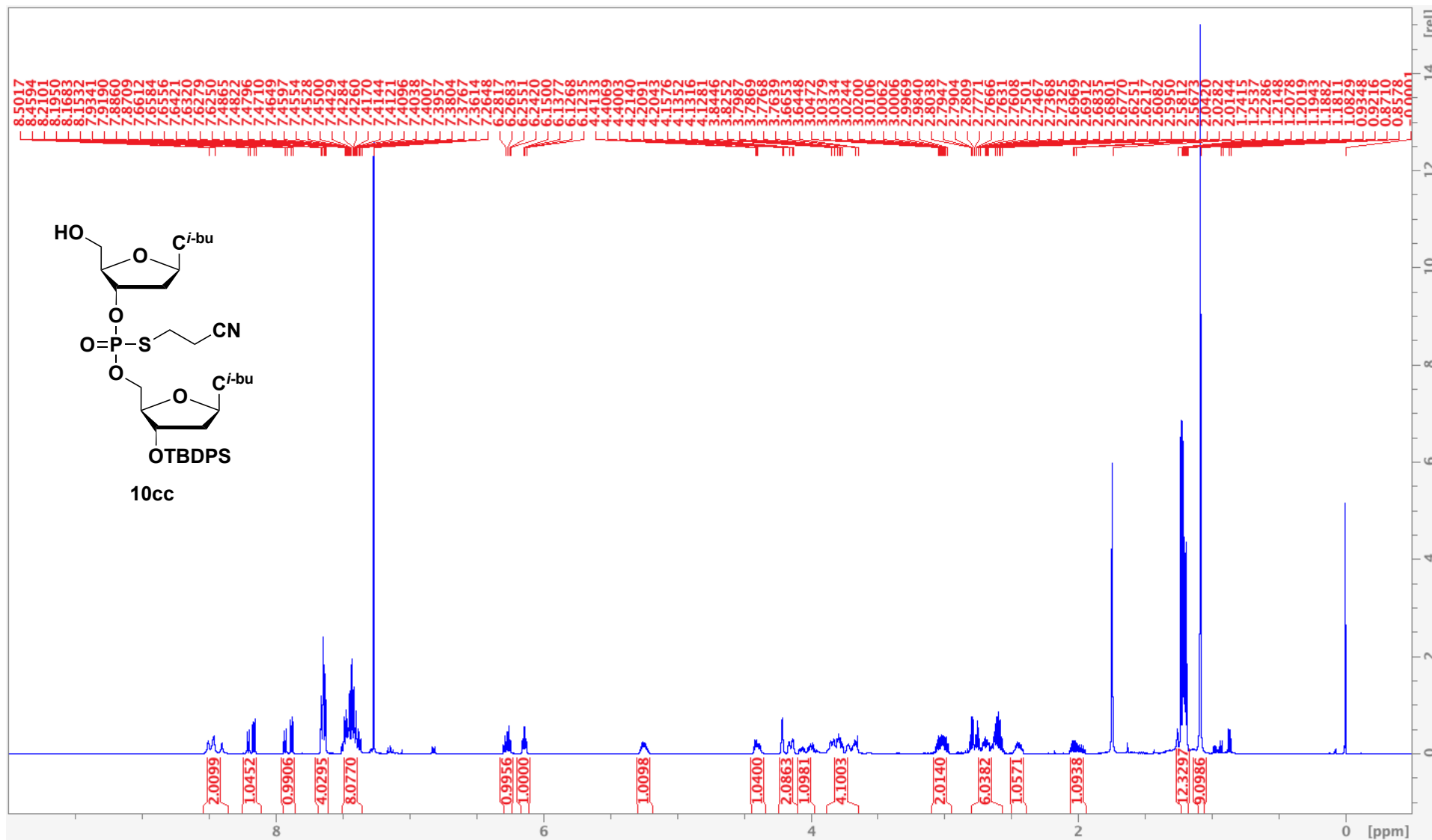
¹H NMR (500MHz, CDCl₃)



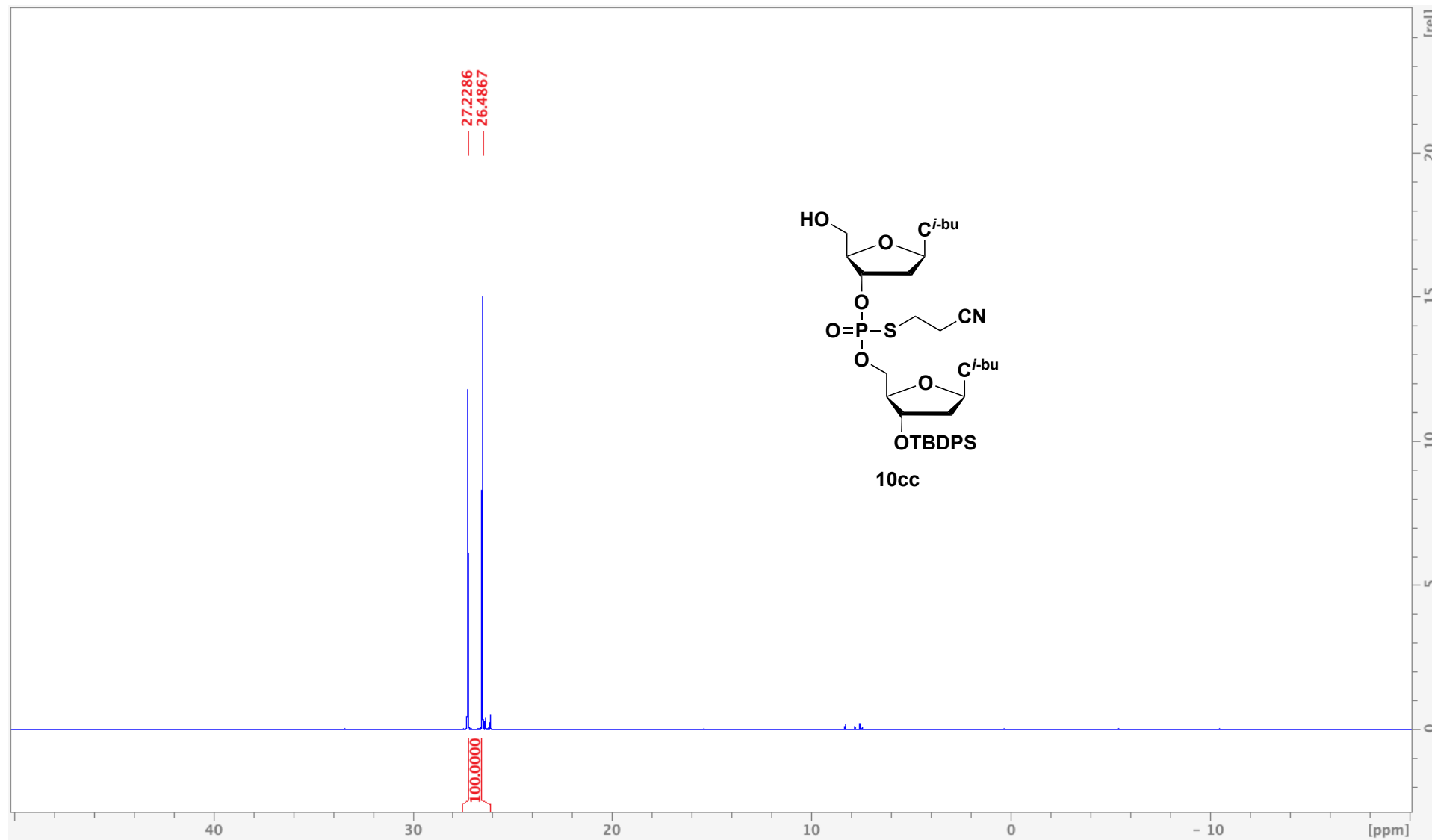
^{31}P NMR (202 MHz, CDCl_3)



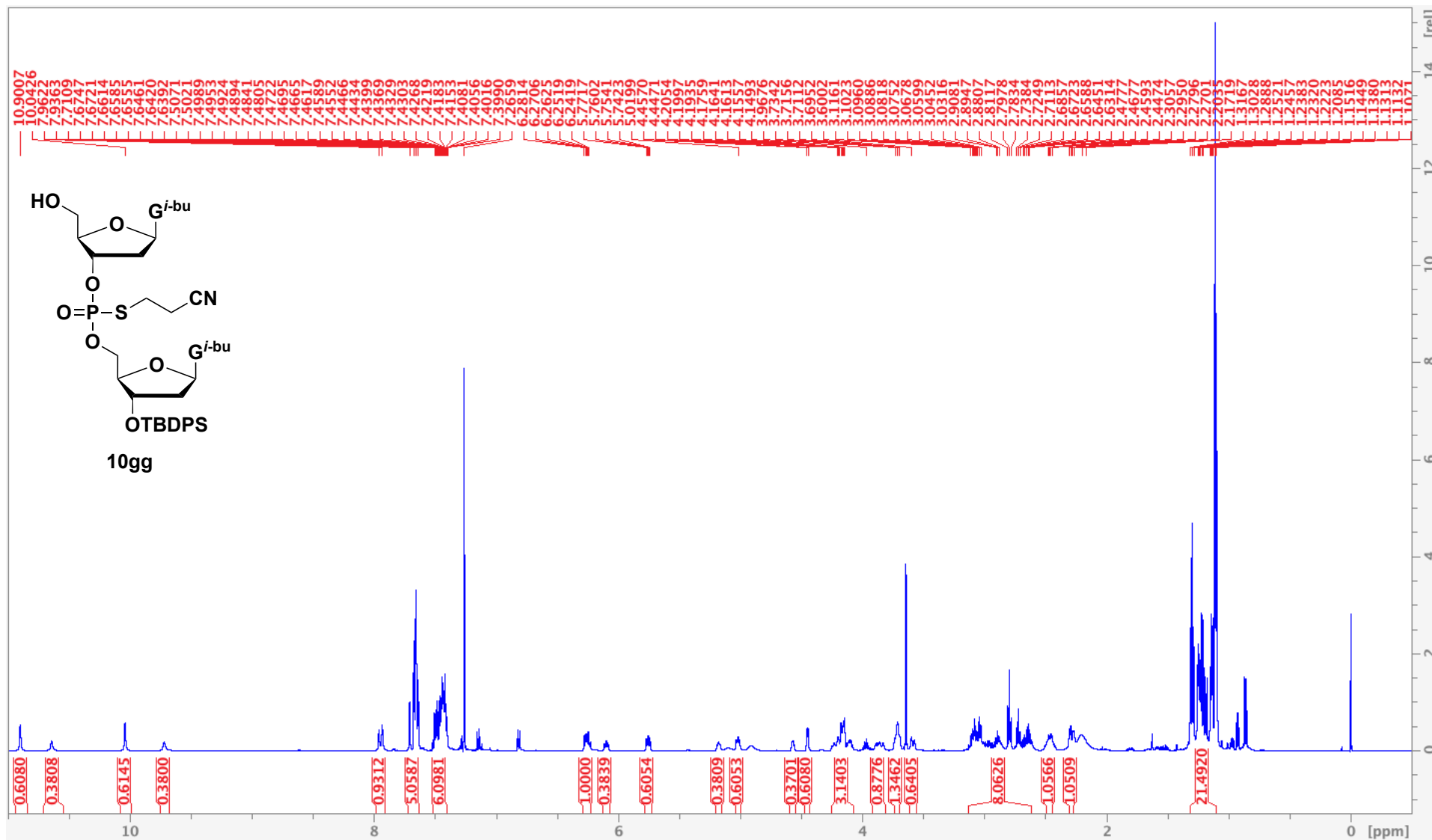
^1H NMR (500MHz, CDCl_3)



^{31}P NMR (202 MHz, CDCl_3)



¹H NMR (500MHz, CDCl₃)



^{31}P NMR (202 MHz, CDCl_3)

