Supporting Information

Multiple Deuteration of Triphenylphosphine and Live-Cell Raman Imaging of Deuterium-incorporated Mito-Q

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Contents;

1. Generals

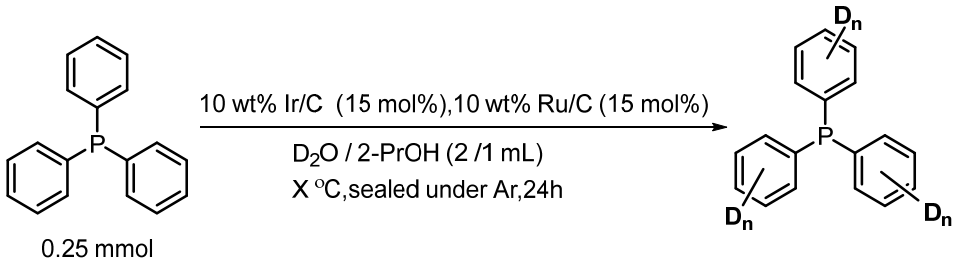
10 wt% Pt/C, 10 wt% Ru/C, 10 wt% Pd/C, 10 wt% Rh/C and 10 wt% Ir/C catalysts were obtained from the N.E. Chemcat Corporation. D_2O (>99.9% D atom) was purchased from Spectra Gases, Inc. All other reagents were purchased from commercial sources and used without purification. Flash column chromatography was performed with Silica Gel 60 N (Kanto Chemical Co., Inc., 40-50 μm spherical, neutral). ^1H, ^2H, ^{13}C and ^{31}P NMR spectra were recorded on a JEOL AL 400, ECS 400 (^1H: 400 MHz, ^{13}C: 100 MHz, ^{31}P: 162 MHz) or ECA 500 spectrometer (^1H: 500 MHz, ^2H: 77 MHz, ^{13}C: 125 MHz, ^{31}P: 203 MHz) at room temperature in $CDCl_3$, as a solvent and an internal standard (^1H NMR: δ = 7.26 for $CHCl_3$, ^2H NMR: δ = 7.26 for $CDCl_3$,^{13}C NMR: δ = 77.0 for $CDCl_3$, ^{31}PNMR: δ = 0.00 for H_3PO_4, or -5.36 for PPh_3). The deuterium contents were determined by ^1H NMR (1,4-dioxane as an internal standard).

2. General procedures for the deuteration

Phosphine (0.25 mmol), 10 wt% Ir/C (72.0 mg, 0.0375 mmol), 10 wt% Ru/C (37.9 mg, 0.0375 mmol), 2-PrOH (1.0 mL) and D_2O (2.0 mL) were added to a 18 mL test tube, which was sealed by septum, and the inside gas was immediately replaced by Ar using vacuum pump (×10). The reaction mixture was stirred at 100 °C for 24 h, then filtered through a membrane filter (Milipore, Millex®-LH, 0.45 μm) together with Et_2O (20 mL) and H_2O (10 mL) to remove the catalyst. The combined filtrates were extracted with Et_2O (20 mL ×3), and the organic layers were dried over Na_2SO_4. Na_2SO_4 is filtered off and the residue was concentrated in vacuo. The crude product was purified by column chromatography. The product was analyzed by ^1H NMR in $CDCl_3$ and ^2H NMR in $CHCl_3$ including slight amount of $CDCl_3$. Deuterium contents were determined by integral value in ^1H NMR spectrum by using 1,4-dioxane as an internal standard and the yield of obtained product.[1]

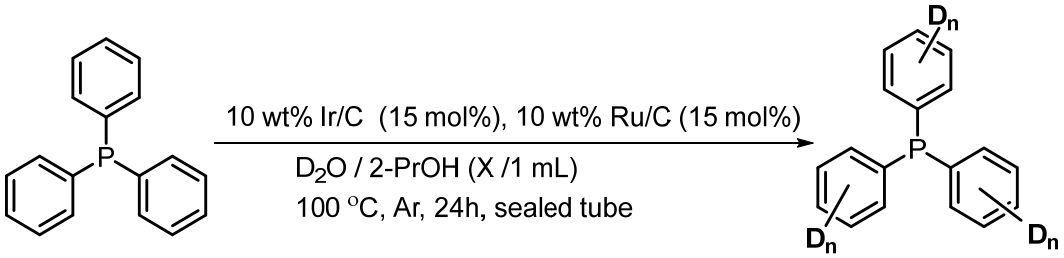
3. Optimization

3-1. Effect of reaction temperature



Entry	X (°C)	D contents (%)	Yield (%)
1	80	84	93
2	100	87	85
3	120	81	64
4	140	74	61
5	160	82	7

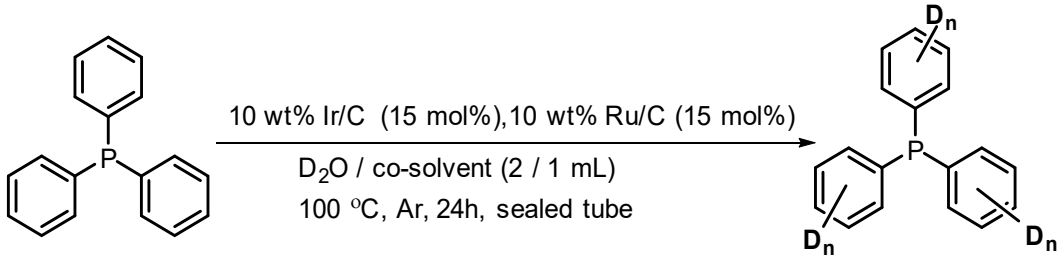
3-2. Usage of D_2O



Entry	X (mL)	D contents (%)	Yield (%)
1	2	87	85
2	3	87	87
3	4	82	80

3-3. Co-solvent effect

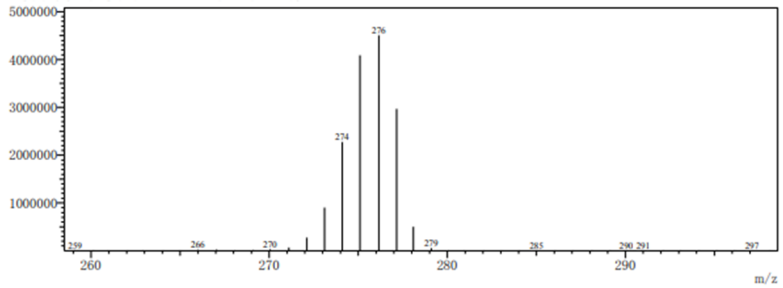
2-PrOH was an important co-solvent. The deuterium contents were compared in the reactions without co-solvent (b) and with co-solvent (2-PrOH (a), MeOH (c), or *t*-BuOH (d)) by GC/MS analysis. The dehydrogenation of 2-PrOH generated hydrogen, which can be activator of platinum metal (see the reaction mechanism on page S7). Additionally, 2-PrOH improved the solubility of PPh_3.



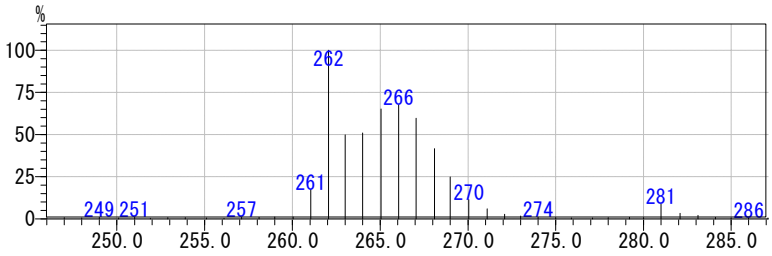
MW: 262

GC/MS analysis of obtained PPh_3.

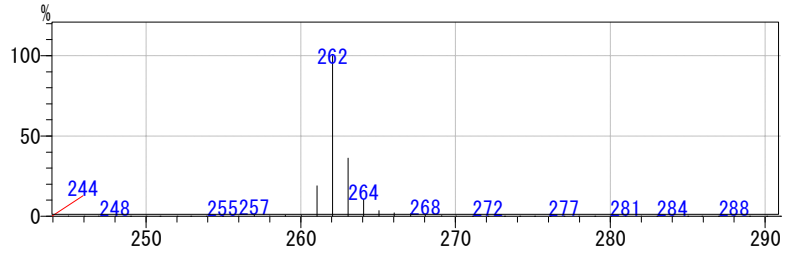
a) With 2-PrOH as co-solvent (reaction conditions shown in Table 1, entry 7)



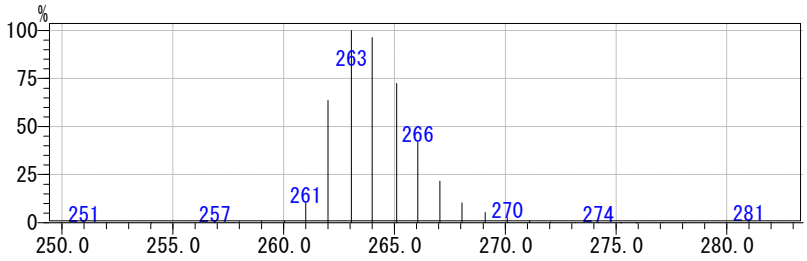
b) Without co-solvent



c) With MeOH as co-solvent instead of 2-PrOH



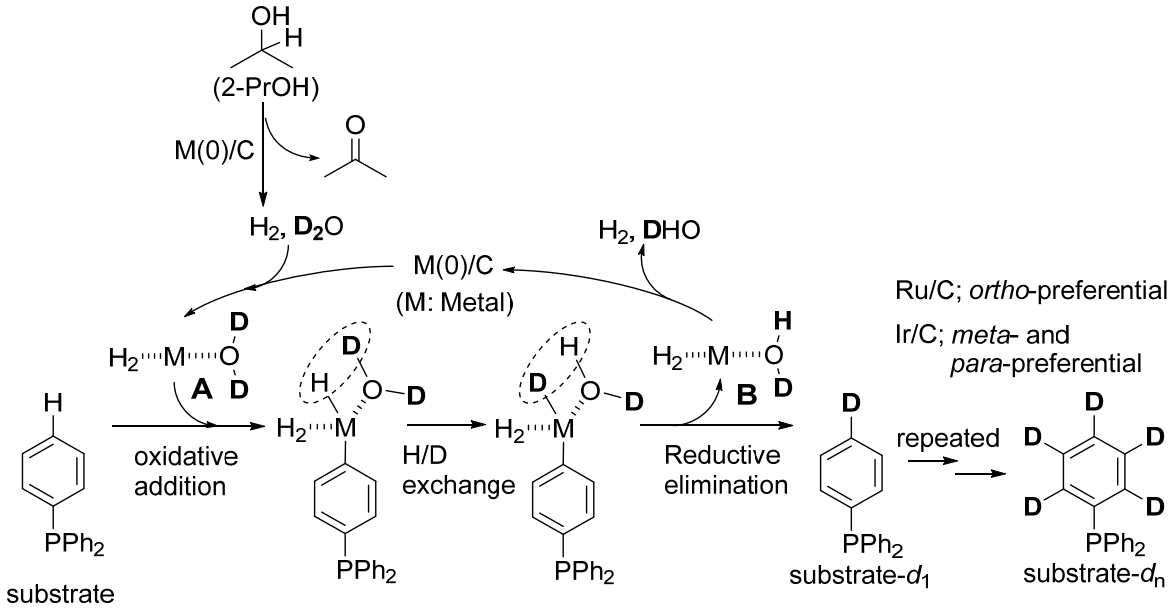
d) With *t*-BuOH as co-solvent instead of 2-PrOH



4. Reaction mechanism of deuteration

The proposed reaction mechanism, based on reference 2, is shown in Scheme S1.

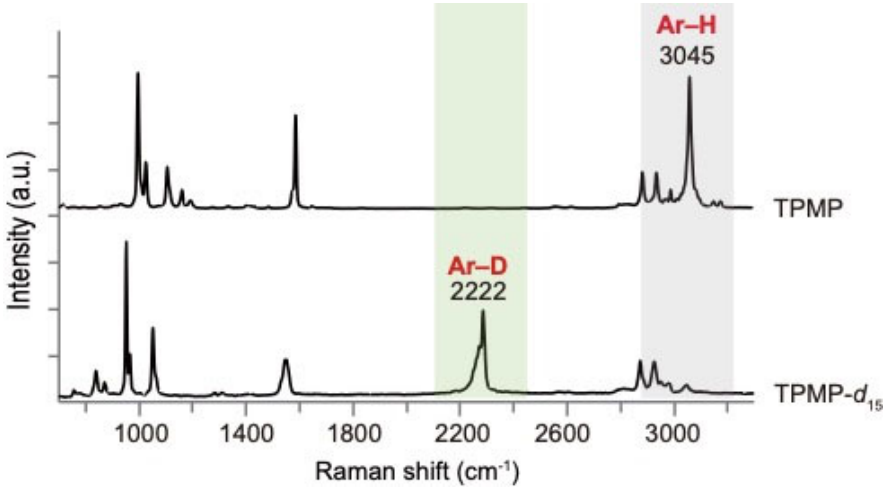
Zero-valent platinum-group metals (M) are activated with hydrogen, generated by dehydrogenation of 2-PrOH, to form the reactive intermediate **A**. The oxidative addition of **A** to PPh_3, the H/D exchange reaction, and subsequent reductive elimination produces mono-deuterated PPh_3. Similar deuterations repeat on all positions of phenyl groups on PPh_3. Ru metal strongly coordinated to the phosphine atom of PPh_3 and deuterated the *ortho*-position preferentially in a similar way to *ortho*-selective deuteration using Ru nanoparticles.[3] On the other hand, Ir/C is suitable for deuteration of *meta* and *para* positions with less steric hindrance as in the reported work.[1]



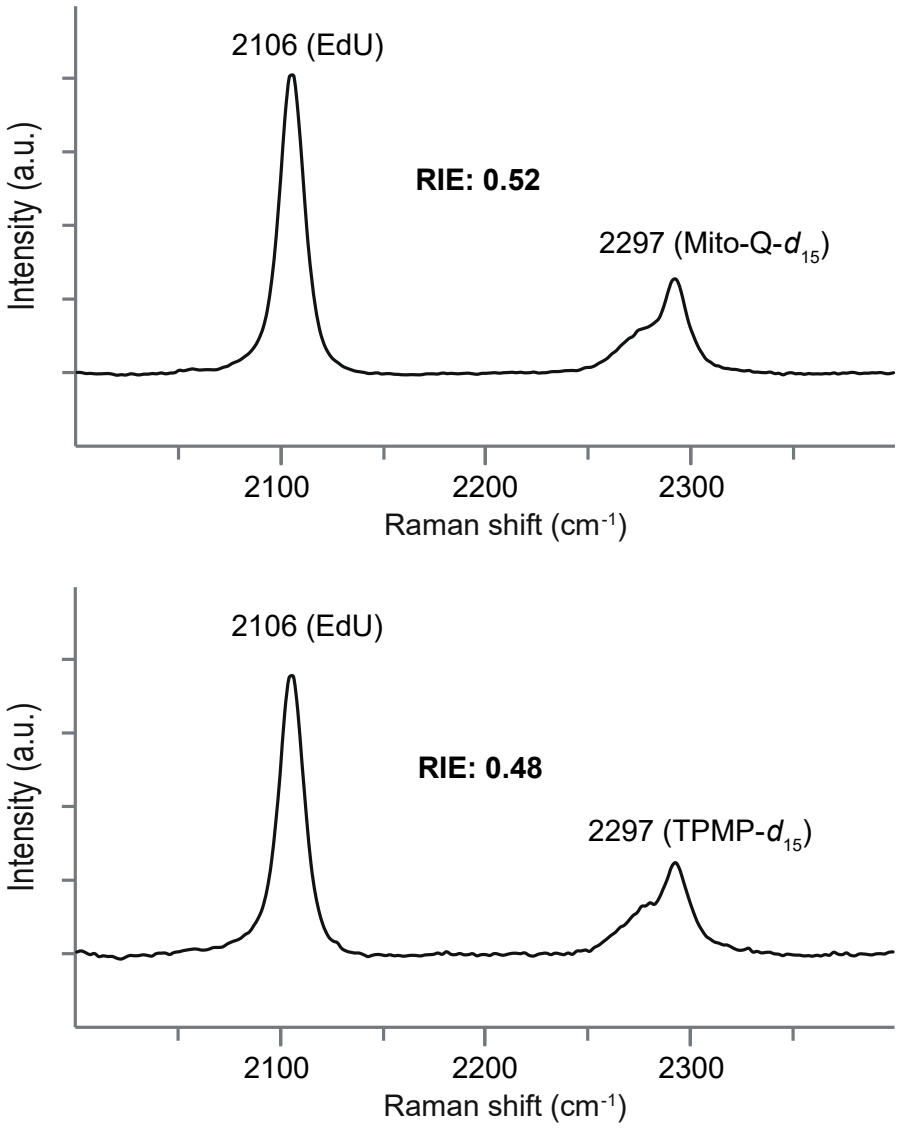
Scheme S1. Reaction mechanism of deuteration.

5. Live-cell Raman imaging

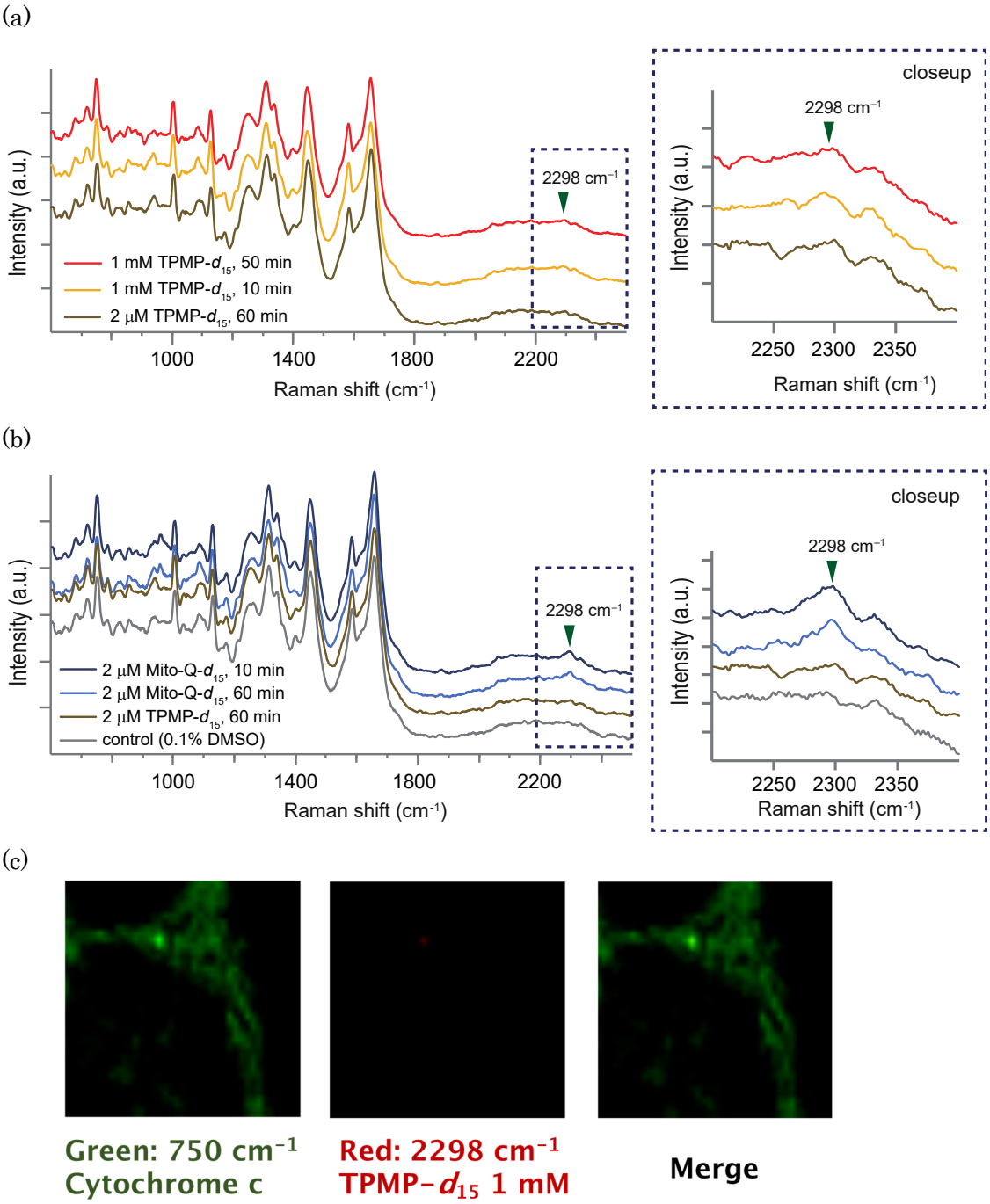
5-1. Supplementary figures



Supplementary Fig. 1. Raman spectra of TPMP and deuterium-incorporated TPMP (TPMP-d_{15}) as a representative result of Ph_3P^+-d_{15}. Powder samples were used for the measurement of Raman spectra.



Supplementary Fig. 2. Relative Raman intensity vs. 5-ethynyl-2'-deoxyuridine (EdU) (RIE) values of nitriles. (a) Mito-Q-d_{15} (b)TPMP-d_{15}. The laser wavelength was 532 nm. According to reference 4, the RIE values were calculated from the ratios of the peak areas using mixtures of phosphonium salt-d_{15} and EdU diluted in DMSO.



Supplementary Fig. 3. Average Raman spectra obtained from the cytoplasm of HeLa cells. (a) Raman spectra of the cytoplasm with 1 mM or 2 μM TPMP-d_{15}. The final concentration of DMSO was 0.5%. (b) Raman spectra using 2 μM phosphonium salt-d_{15} or control (without compound). The final concentration of DMSO was 0.1%. (c) Live-cell Raman imaging of HeLa cells with 0.1% DMSO as a control experiment. a.u., arbitrary units.

5-2. Experimental procedures

Cell culture

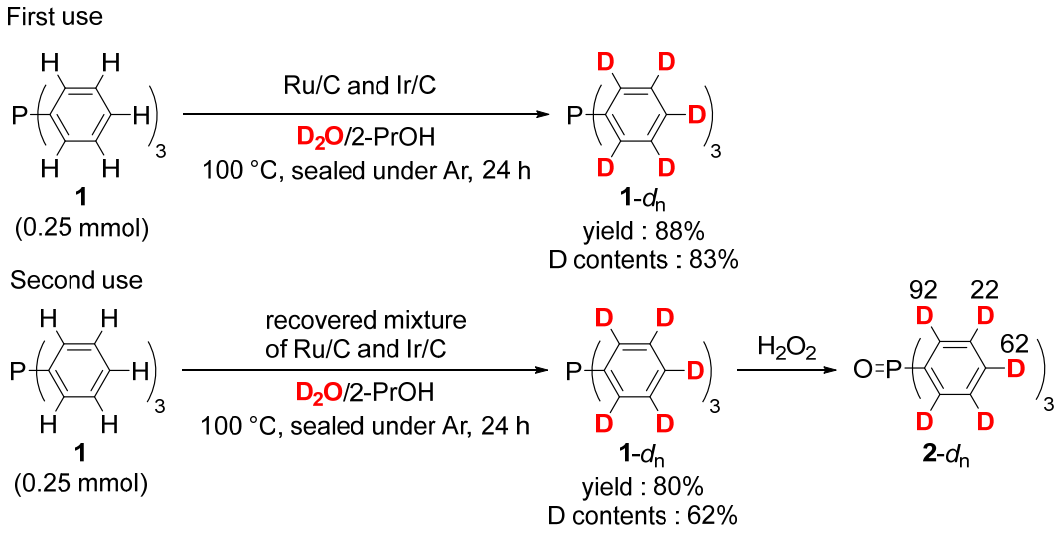
HeLa human cervical cancer cells were cultured in Dulbecco's modified Eagle's medium (043-30085, Wako) supplemented with 10% fetal bovine serum and 5×10^4 U/L penicillin G, and 50 mg/L streptomycin sulfate (15070-063, Gibco) at 37 °C and 5% CO_2.

Raman analysis

Raman spectra and images were obtained using a home-made inverted confocal Raman microscope. HeLa cells were cultured on a glass-bottom dish (Matsunami D11130H), and the culture medium was washed twice with Hank's balanced salt solution (HBSS) (H8264, Sigma) and then replaced with HBSS containing a phosphonium salt-d_{15} immediately before measurements were taken. Loading concentration of phosphonium salt-d_{15} and incubation times are indicated in the figure captions. This dish was equipped on an inverted microscope (Nikon, eclipse Ti2). A 532 nm CW laser beam was focused on a sample using a 60x objective (NA=1.27, water immersion, Nikon). The Raman signal passing through the same objective lens and a pinhole providing a confocal condition was dispersed by a polychromator (MS3804) and detected by a thermoelectric cooled CCD (Andor, DU-970-BVF). All measurements were carried out at room temperature. A single Raman image was constructed using 40 × 40 points at intervals of 0.5 μm. The laser-irradiation intensity and exposure time per point were 50 mW and 0.1 s, respectively. The SVD technique was applied for the spectra in the 600–2500 cm^{-1} for noise reduction.[5] SVD analyses were performed by Igor Pro 8 (Wavemetrics). Owing to the differing autofluorescence-background signals present at each point in the Raman spectrum, we used a modified polynomial-fitting technique[6] to determine the autofluorescence-baseline signal, which was subtracted from the original Raman spectrum. Finally, a Raman image was constructed by displaying the intensity of each vibrational band of interest at each pixel.

6. Reuse test

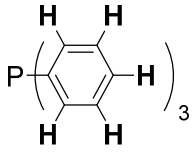
PPh_3 (65.6 mg, 0.25 mmol), 10 wt% Ir/C (72.0 mg, 0.0375 mmol), 10 wt% Ru/C (37.9 mg, 0.0375 mmol), 2-PrOH (1.0 mL) and D_2O (2.0 mL) were added to a 18 mL test tube, which was sealed by septum, and the inside gas was immediately replaced by Ar using vacuum pump (×10). The reaction mixture was stirred at 100 °C for 24 h, then filtered through a membrane filter (Milipore, Millex®-LH, 0.45 μm) together with Et_2O (20 mL) and H_2O (10 mL) to recover catalyst. The combined filtrates were extracted with Et_2O (20 mL ×3), and the organic layers were dried over Na_2SO_4. Na_2SO_4 is filtered off and the residue was concentrated in vacuo to give the crude products. The crude products were purified by column chromatography. The recovered catalyst was dried for 36 hours with vacuum pump at room temperature and used in the next run (PPh_3 (65.6 mg, 0.25 mmol), 2-PrOH (1.0 mL) and D_2O (2.0 mL)). Compared with the deuterium contents of **1**-d_n in the first and second runs, the deuterium contents (average 62%D) in second run reduced (Scheme S2). The deuterium contents of each position on phenyl group of **1**-d_n in the second run was determined after oxidation with H_2O_2 according to the procedure of synthesis of triphenylphosphine-oxide-d_{15} as shown in the section 7. Consequently, the deuterium content of *meta*-position was low and the *para*-position was moderately deuterated.



Scheme S2. Deuteration using recovered catalysts.

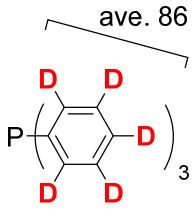
7. Synthetic procedures and spectroscopic data

Triphenylphosphine (1; authentic sample)



^{1}H NMR (500 MHz, $CDCl_3$); δ 7.36–7.30 (m, 15H); ^{31}P NMR (203 MHz, $CDCl_3$) δ −5.35 (brs).

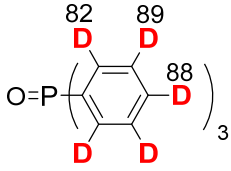
Triphenylphosphine-d_{15} (**1**-d_{15}; Table 1, entry 6)



1-d_{15} was synthesized according to General Procedure. **1** (65.6 mg, 0.25 mmol) was used as a substrate. Triphenylphosphine-d_{15} (**1**-d_{15}, 76% yield, 52.6 mg) was obtained after silica-gel column chromatography (*n*-hexane / EtOAc = 20/1). Colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.34–7.30 (m, 2.10H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.38.

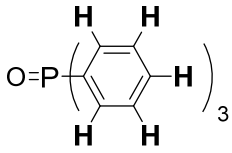
Triphenylphosphine-oxide-d_{15} (**2**-d_{15}; Table 1, entry 6)

The deuterium contents at each aromatic C-D position were determined after the transformation of **1**-d_{15} using hydrogen peroxide to triphenylphosphine oxide (**2**-d_{15}). Procedure; According to literature,[7] **1**-d_{15} (53 mg), 2.0 mL tetrahydrofuran (THF), and 30% hydrogen peroxide (0.6 mL) were charged into a 50 mL flask with a magnetic stirrer and stirred 12 h at room temperature. Saturated $Na_2S_2O_3$ aq. was dropwised to the reaction mixture at 0 °C. The mixture was extracted with EtOAc, and the organic layers were dried over Na_2SO_4. Na_2SO_4 is filtered off and the residue was concentrated in vacuo.



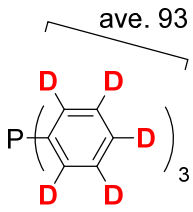
2-d_{15} was obtained (57.4 mg, quant.). Colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$); δ 7.65 (m, 1.08H), 7.53 (s, 0.36H), 7.44 (s, 0.66H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.69, 7.57, 7.49.

Triphenylphosphine oxide (2; authentic sample)



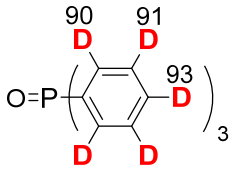
^{1}H NMR (500 MHz, $CDCl_3$); δ 7.68–7.64 (m, 6H), 7.54–7.51 (m, 3H), 7.46–7.43 (m, 6H); ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.23 (brs).

Triphenylphosphine-*d*$_{15}$ (1-*d*$_{15}$; Table 1, entry 7)



The reaction was repeated twice, according to Procedure of Table 1, entry 6. Colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$); δ 7.34–7.32 (m, 1.09H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.40; ^{31}P NMR (203 MHz, $CDCl_3$) δ −5.79, −5.89, −5.91, −6.01 (Some peaks were observed, because various products bearing different numbers of deuterium atom were mixed. GC/MS data of **1**-*d*$_{15}$ was measured in Table 1, entry 8. See page S15. Similarly, many peaks were observed in ^{13}C NMR, which was described in page S25, ^{13}CNMR (125 MHz, $CDCl_3$) δ 127.77, 127.96, 128.20, 128.26, 128.36, 128.45, 132.98, 133.18, 133.33, 133.505, 133.66, 136.87, 136.96).

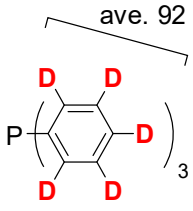
Triphenylphosphine-oxide-*d*$_{15}$ (2-*d*$_{15}$; Table 1, entry 7)



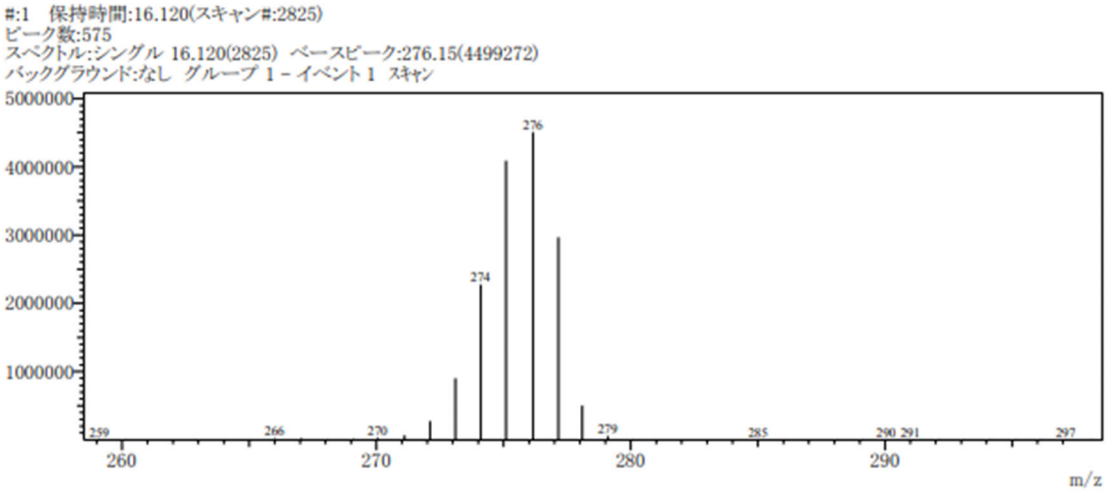
2-***d*$_{15}$ was synthesized, according to Procedure of Table 1, entry 6. Colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$); δ 7.65 (m, 0.60H), 7.53 (s, 0.21H), 7.44 (s, 0.54H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.70, 7.58, 7.50.

Triphenylphosphine-d_{15} (1-d_{15}; Table 1, entry 8)

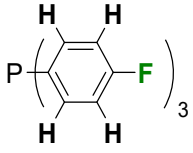
Scale-up reaction of PPh_3



Triphenylphosphine (656 mg, 2.5 mmol), 10 wt% Ir/C (720 mg, 0.375 mmol), 10 wt% Ru/C (379 mg, 0.375 mmol), D_2O (20 mL) and 2-PrOH (10 mL) were used. The deuteration was operated according to General Procedure and the procedure was repeated twice. ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.35−7.34 (m, 1.20H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.39.

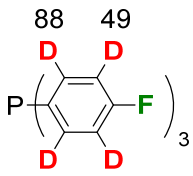


Tris(4-fluorophenyl)phosphine (3; authentic sample)



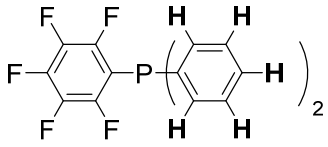
^{1}H NMR (400 MHz, $CDCl_3$); δ 7.27–7.23 (m, 6H), 7.07–7.03 (m, 6H); ^{31}P NMR (162 MHz, $CDCl_3$) δ −8.5 (brs).

Tris(4-fluorophenyl)phosphine-d_n (3-d_n)



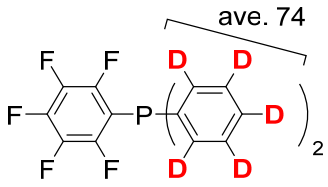
3-d_n was synthesized according to General Procedure. **3** (79.1 mg, 0.25 mmol) was used as a substrate. Tris(4-fluorophenyl)-phosphine-d_n (**3**-d_n, 78% yield, 62.9 mg) was obtained after silica-gel column chromatography (*n*-hexane / EtOAc = 20/1). Colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.28–7.22 (m, 0.72H), 7.05 (d, J = 8.7 Hz, 3.06H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.29, 7.09; ^{31}P NMR (203 MHz, $CDCl_3$) δ −9.39, −9.41, −9.51, −9.62 (Some peaks were observed, because various products bearing different numbers of deuterium atom were mixed. Similarly, many peaks were observed in ^{13}C NMR, which was described in page S30).

(Pentafluorophenyl)diphenylphosphine (4; authentic sample)



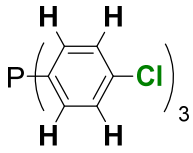
^{1}H NMR (400 MHz, $CDCl_3$); δ 7.46–7.36 (m, 10H); ^{31}P NMR (162 MHz, $CDCl_3$) δ −24.70 (t, J = 39.2 Hz).

(Pentafluorophenyl)diphenylphosphine-d_n (4-d_n)



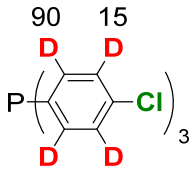
4-d_n was synthesized according to General Procedure. **4** (88.1 mg, 0.25 mmol) was used as a substrate. (Pentafluorophenyl)diphenylphosphine-d_n (**4**-d_n, 59% yield, 52.6 mg) was obtained after silica-gel column chromatography (*n*-hexane / EtOAc = 50/1). Colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.43–7.37 (m, 2.60H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.46, 7.42; ^{31}P NMR (162 MHz, $CDCl_3$) δ −24.19, −24.30, −24.41, −24.54, −24.65, −24.78, −24.89, −25.00, −25.13 (Some peaks were observed, because various products bearing different numbers of deuterium atom were mixed. Similarly, many peaks were observed in ^{13}C NMR, which was described in page S33).

Tris(4-chlorophenyl)phosphine (5; authentic sample)



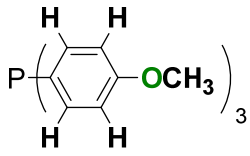
^{1}H NMR (400 MHz, $CDCl_3$); δ 7.34–7.32 (m, 6H), 7.21–7.17 (m, 6H); ^{31}P NMR (162 MHz, $CDCl_3$) δ −8.06 (brs).

Tris(4-chlorophenyl)phosphine-*d*$_n$ (5-*d*$_n$)



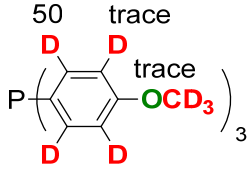
5-*d*$_n$ was synthesized according to General Procedure. **5** (91.4 mg, 0.25 mmol) was used as a substrate. Tris(4-chlorophenyl)-phosphine-*d*$_n$ (**5**-*d*$_n$, 64% yield, 59.6 mg) was obtained after silica-gel column chromatography (*n*-hexane / EtOAc = 20/1). Colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.32 (s, 5.10H), 7.21–7.17 (m, 0.60H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.31, 7.18; ^{31}P NMR (162 MHz, $CDCl_3$) δ −8.92, −9.00, −9.04, −9.14 (Some peaks were observed, because various products bearing different numbers of deuterium atom were mixed. Similarly, many peaks were observed in ^{13}C NMR, which was described in page S36).

Tris(4-methoxyphenyl)phosphine (6; authentic sample)



^{1}H NMR (400 MHz, $CDCl_3$); δ 7.24–7.20 (m, 6H), 6.88–6.86 (m, 6H), 3.79 (s, 9H); ^{31}P NMR (162 MHz, $CDCl_3$) δ −10.35 (brs).

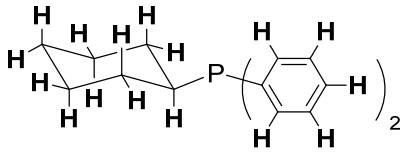
Tris(4-methoxyphenyl)phosphine-*d*$_n$ (6-*d*$_n$)



6-*d*$_n$ was synthesized according to General Procedure. **6** (88.1 mg, 0.25 mmol) was used as a substrate. Tris(4-methoxyphenyl)-phosphine-*d*$_n$ (**6**-*d*$_n$; 77%

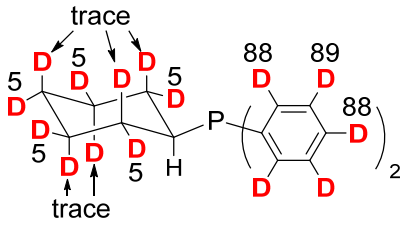
yield, 67.9 mg) was obtained after silica-gel column chromatography (*n*-hexane / EtOAc = 5/1). Colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.24–7.20 (m, 3.00H), 6.88–6.86 (m, 6.00H), 3.78 (s, 9.00H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.22, 6.90, 3.77; ^{31}P NMR (203 MHz, $CDCl_3$) δ −10.33, −10.37, −10.40, −10.44, −10.47, −10.84, −10.94, −10.98, −11.07 (Some peaks were observed, because various products bearing different numbers of deuterium atom were mixed. Similarly, many peaks were observed in ^{13}C NMR, which was described in page S39).

Cyclohexyldiphenylphosphine (7; authentic sample)



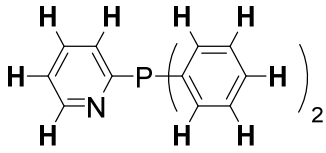
^{1}H NMR (400 MHz, $CDCl_3$); δ 7.53–7.47 (m, 4H), 7.37–7.30 (m, 6H), 2.27–2.19 (m, 1H), 1.78–1.70 (m, 5H), 1.36–1.18 (m, 5H); ^{31}P NMR (162 MHz, $CDCl_3$) δ −3.88 (brs).

Cyclohexyldiphenylphosphine-d_n (7-d_n)



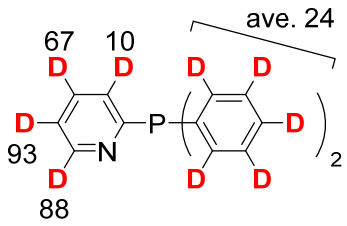
7-d_n was synthesized according to General Procedure. **7** (67.1 mg, 0.25 mmol) was used as a substrate. Cyclohexyldiphenylphosphine-d_n (**7**-d_n; 72% yield, 49.6 mg) was obtained after silica-gel column chromatography (*n*-hexane / EtOAc = 20/1). Colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.51−7.48 (m, 0.44H), 7.34−7.32 (m, 0.72H), 2.25−2.18 (m, 1H), 1.77−1.69 (m, 4.75H), 1.35−1.18 (m, 5.00H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.54, 7.39, 1.76, 1.23; ^{31}P NMR (162 MHz, $CDCl_3$) δ −4.10, −4.20, −4.32 (Some peaks were observed, because various products bearing different numbers of deuterium atom were mixed. Similarly, many peaks were observed in ^{13}C NMR, which was described in page S42).

Diphenyl-2-pyridylphosphine (8; authentic sample)



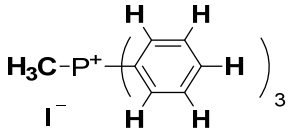
^{1}H NMR (400 MHz, $CDCl_3$); δ 8.73–8.72 (m, 1H), 7.55 (tt, J = 7.8, 1.8 Hz, 1H), 7.44–7.33 (m, 10H), 7.18–7.15 (m, 1H), 7.08 (dd, J = 7.8, 0.9 Hz, 1H); ^{31}P NMR (162 MHz, $CDCl_3$) δ −3.99 (brs).

Diphenyl-2-pyridylphosphine-d_n (8-d_n)



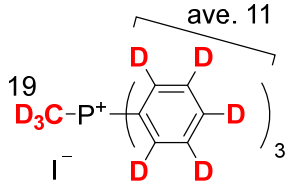
8-d_n was synthesized according to General Procedure. **8** (67.1 mg, 0.25 mmol) was used as a substrate. Diphenyl-2-pyridylphosphine-d_n (**8**-d_n was synthesized according to General Procedure, 79% yield, 52.9 mg) was obtained after silica-gel column chromatography (*n*-hexane / EtOAc = 5/1). Colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ 8.71 (brs, 0.12H), 7.59–7.53 (m, 0.33H), 7.40–7.33 (m, 7.60H), 7.16 (brs, 0.07H), 7.07 (t, J = 3.7 Hz, 0.90H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 8.76, 7.60, 7.40, 7.22; ^{31}P NMR (162 MHz, $CDCl_3$) δ −4.18 (brs) (Various products bearing different numbers of deuterium atom were mixed. Therefore, many peaks were observed in ^{13}C NMR, which was described in page S45).

Methyl-triphenylphosphonium iodide (9)



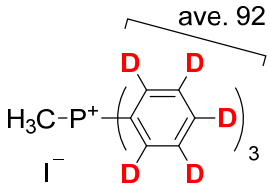
9 was synthesized according to previous work.[8] Methyl iodide (283.8 mg, 2 mmol) was added dropwise for 1 min to the stirring mixture of triphenylphosphine (1 mmol) in anhydrous tetrahydrofuran (2 mL) at room temperature. After the mixture was stirred at room temperature for 18 h, the mixture was evaporated in vacuo. The residue was washed using Et_2O on filter and dried. The product was obtained as a colorless solid. (389 mg, 0.96 mmol, 96 %). ^{1}H NMR (500 MHz, $CDCl_3$); δ 7.77–7.64 (m, 15H), 3.09 (d, J = 13.2 Hz, 3H); ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.90 (brs).

Methyl-triphenylphosphonium iodide-d_n (9-d_n; Scheme 2)



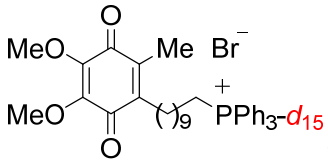
9 (0.25 mmol), 10 wt% Ir/C (72.0 mg, 0.0375 mmol), 10 wt% Ru/C (37.9 mg, 0.0375 mmol), 2-PrOH (1.0 mL) and D_2O (2.0 mL) were added to a 18 mL test tube, which was sealed by septum, and the inside gas was immediately replaced by Ar using vacuum pump (×10). The reaction mixture was stirred at 100 °C for 24 h, then filtered through a membrane filter (Milipore, Millex®-LH, 0.45 μm) together with $CHCl_3$ (20 mL) and H_2O to remove the catalyst. The combined filtrates were extracted with $CHCl_3$, and the organic layers were dried over Na_2SO_4, and concentrated in vacuo. The crude was washed using Et_2O on filter and dried. Methyl-triphenylphosphonium iodide-d_n (**9**-d_n, 89% yield, 90.1 mg) was obtained. Light-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.81–7.65 (m, 13.35H), 3.17 (d, J = 13.3 Hz, 2.43H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.71, 3.09; ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.33, 22.06, 21.98, 21.87, 21.85, 21.79, 21.71, 20.15 (Some peaks were observed, because various products bearing different numbers of deuterium atom were mixed. Similarly, many peaks were observed in ^{13}C NMR, which was described in page S48).

Methyl-triphenylphosphonium iodide-d_{15} (TPMP-d_{15}; Scheme 3)



TPMP-d_{15} was synthesized according to previous work.[8] Methyl iodide (14.2 mg, 0.1 mmol) was added dropwise for 1 min to the stirring mixture of triphenylphosphine (0.05 mmol) in anhydrous tetrahydrofuran (1.0 mL) at room temperature. After the mixture was stirred at room temperature for 18 h, the mixture was evaporated in vacuo. The residue was washed using Et_2O on filter and dried. The product was obtained. (11.1 mg, 0.027 mmol, 53 %). Light-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ 7.81–7.70 (m, 1.13H), 3.24 (d, J = 13.3 Hz, 3H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.75; ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.71 (Various products bearing different numbers of deuterium atom were mixed. Therefore, many peaks were observed in ^{13}C NMR, which was described in page S50).

Mito-Q-d_n

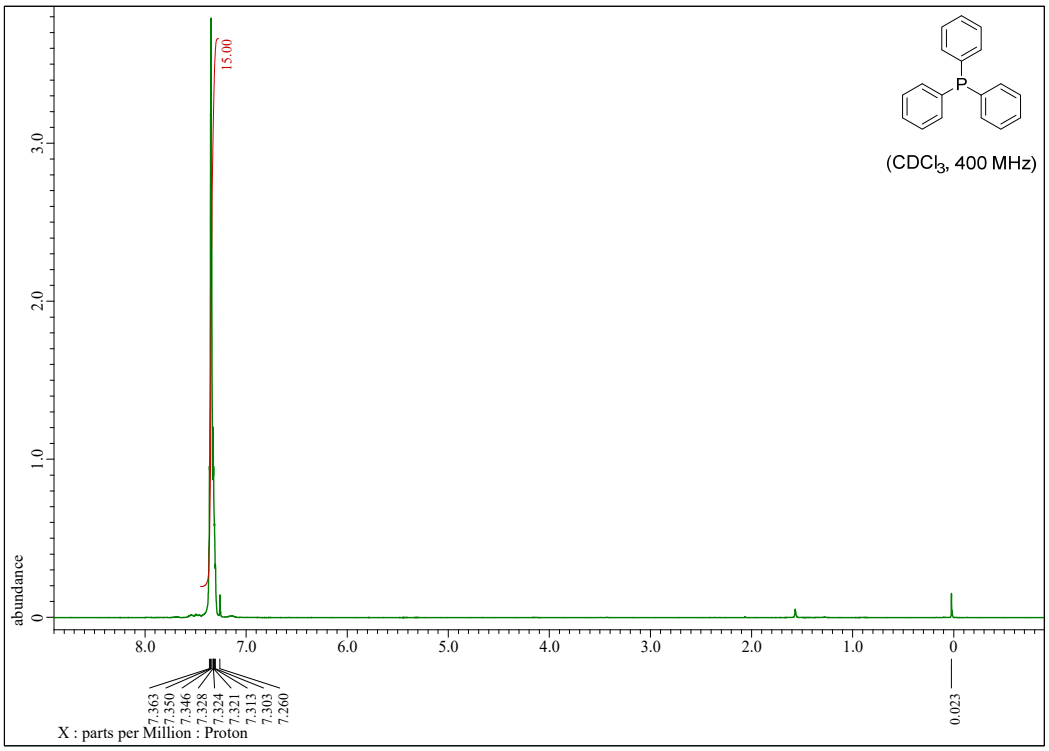


Mito-Q-d_n was synthesized according to previous work.[9] To a solution of 2,3-Dimethoxy-6-methyl-5-(10-bromodecyl)-1,4-benzoquinone (218 mg, 0.54 mmol) in MeOH (2.2 mL) was added $NaBH_4$ (62 mg, 1.6 mmol). After stirring for 5 min, the reaction was quenched by 1N aqueous HCl, and Et_2O and H_2O were added. The organic layer was separated, and aqueous layer was extracted with Et_2O two times. The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. A mixture of the crude and PPh_3-d_{15} (165 mg, 0.60 mmol) in EtOH (1.1 mL) was sealed and heated at 90 °C for 3.5 days. After concentration and incubation for 1 day, the residue was purified by flash silica gel column chromatography ($CHCl_3$ / MeOH = 9 / 1) to give Mito-Q-d_n (273 mg, 74%). Orange solid. ^{1}H NMR (500 MHz, $CDCl_3$); δ 7.87–7.84 (m, 0.49H), 7.79 (brs, 0.11H), 7.71–7.70 (m, 0.96H), 3.98 (s, 6H), 3.84–3.80 (m, 2H), 2.42 (t, J = 8.6, 6.9 Hz, 2H), 2.00 (s, 3H), 1.39–1.20 (m, 16H); ^{2}H NMR (77 MHz, $CHCl_3$); δ 7.85, 7.75.

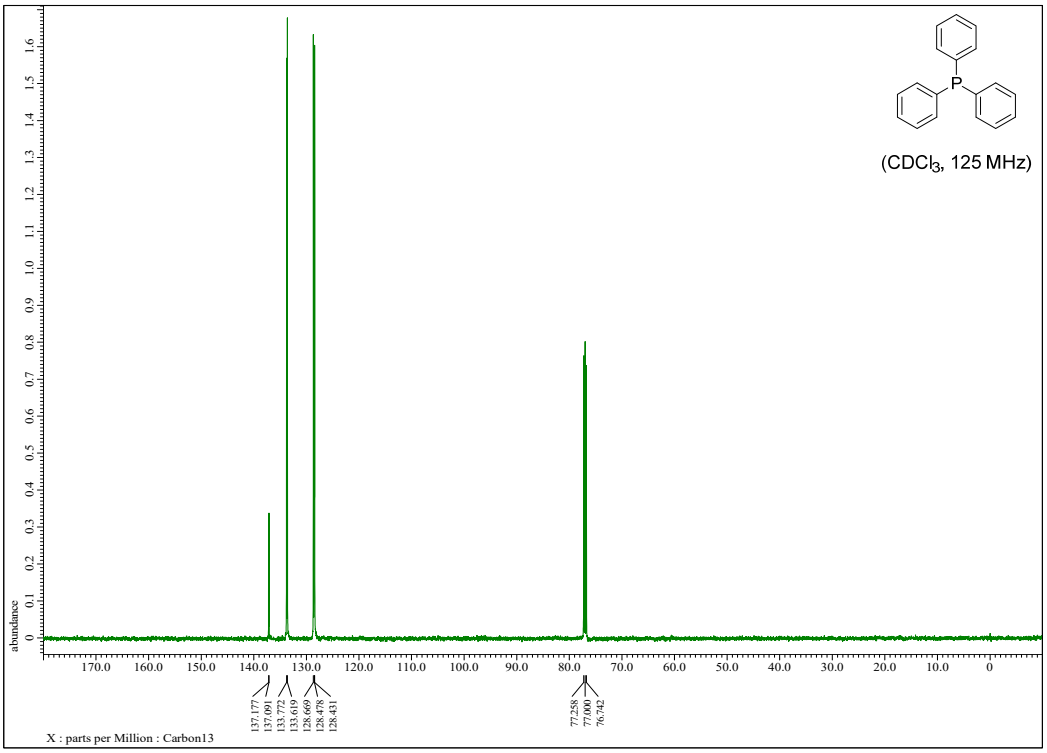
The data are compared to reported work (Literature data; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.88-7.68 (m, 15H), 3.98 (s, 6H), 3.82 (m, 2H), 2.56 (dd, J = 8.2, 8.0 Hz, 2H), 2.13 (s, 3H), 1.62–1.21 (m, 16H).[10]

8. ^{1}H, ^{31}P NMR spectra of representative substrates and ^{1}H, ^{2}H and ^{31}P NMR spectra of deuterated products.

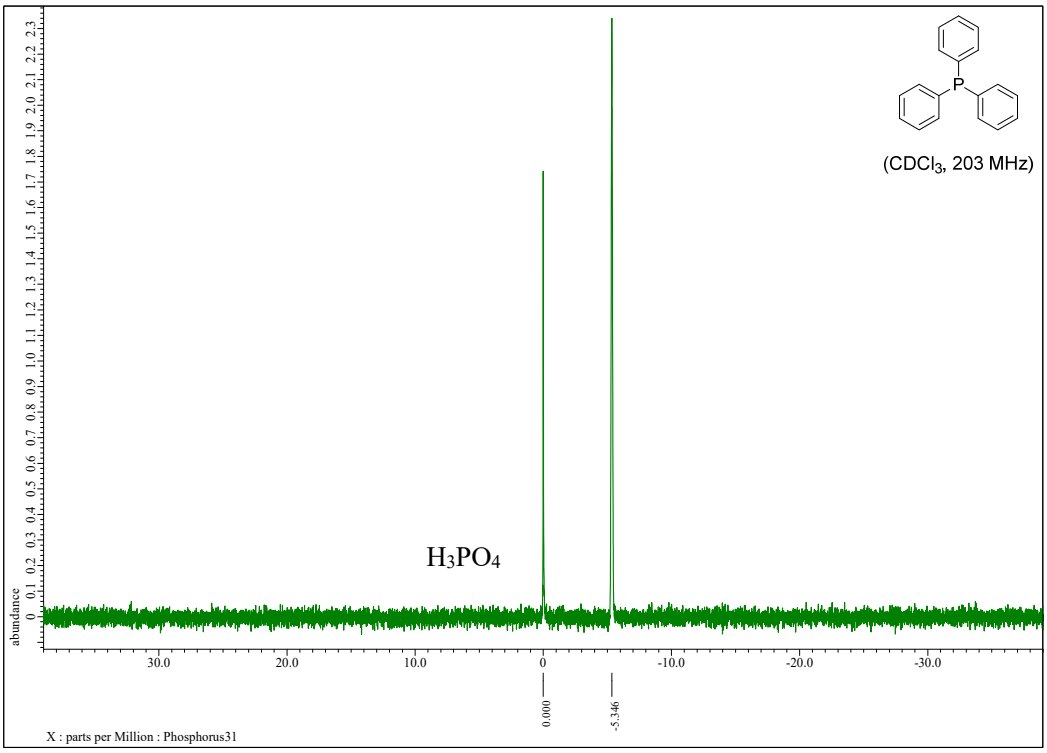
^{1}H NMR of triphenylphophine (**1**; authentic sample)



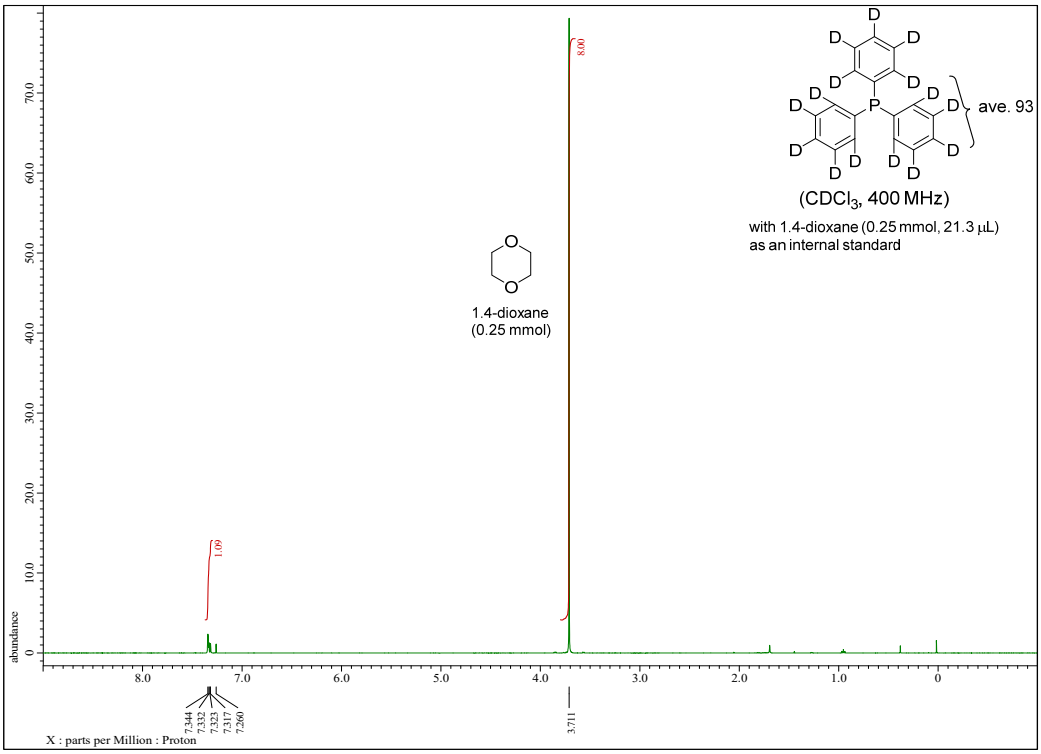
^{13}C NMR of triphenylphophine (**1**; authentic sample)



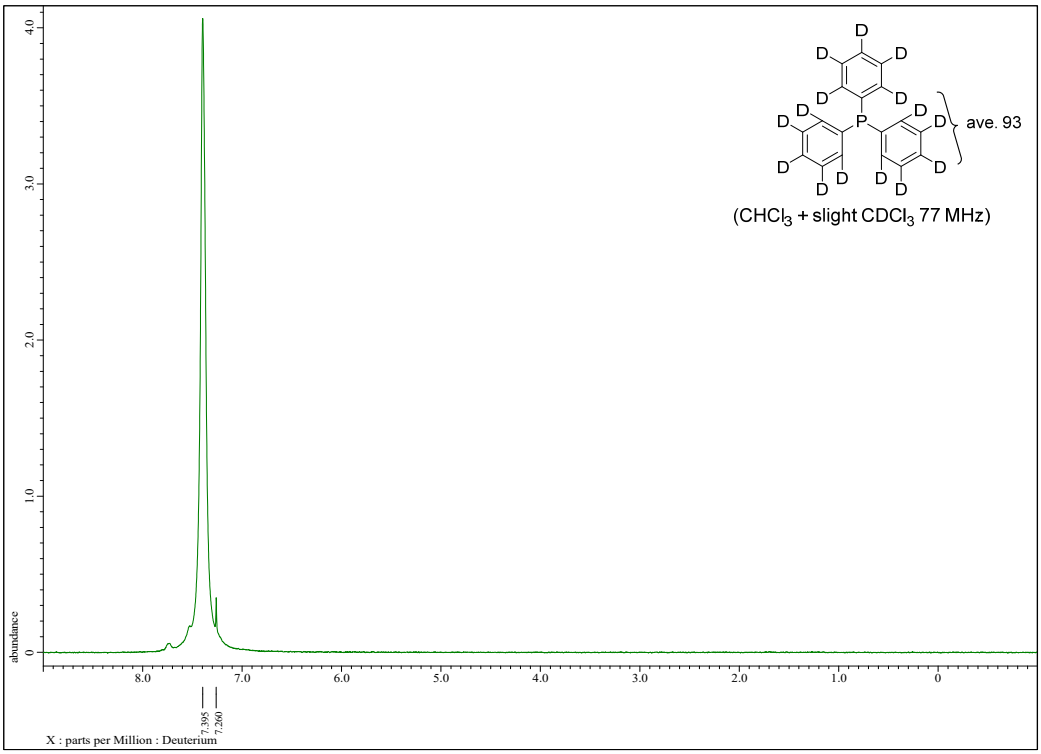
^{31}P NMR of triphenylphophine (**1**; authentic sample)



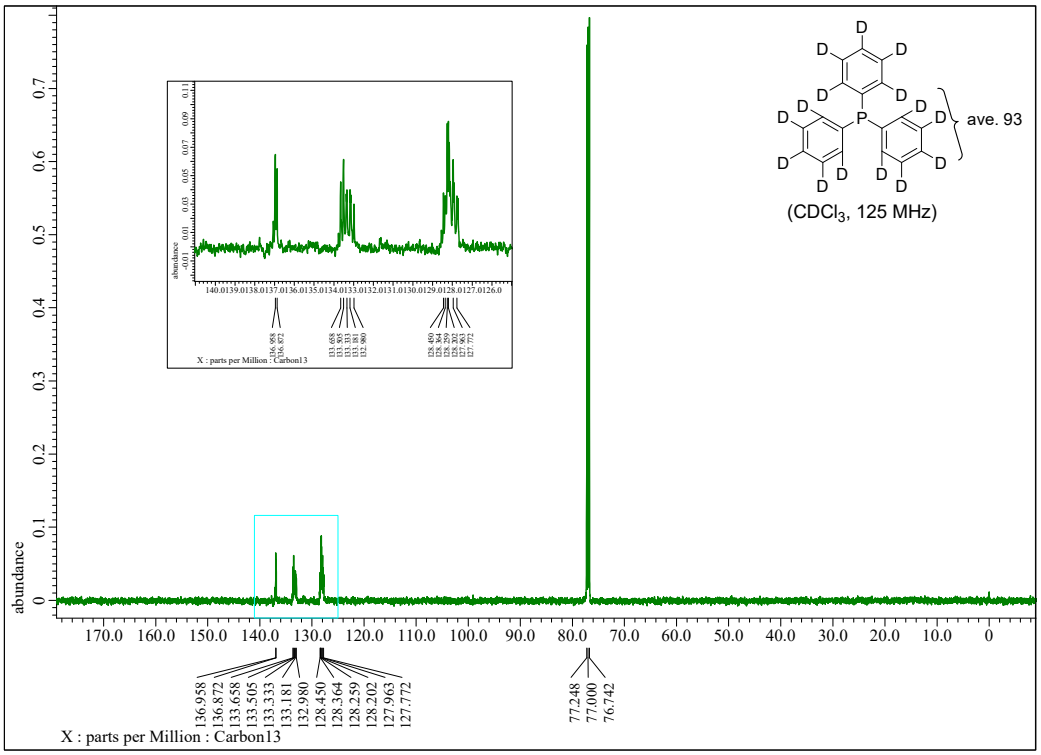
^{1}H NMR of triphenylphophine-d_{15} (**1**-d_{15}; Table 1, entry 7)



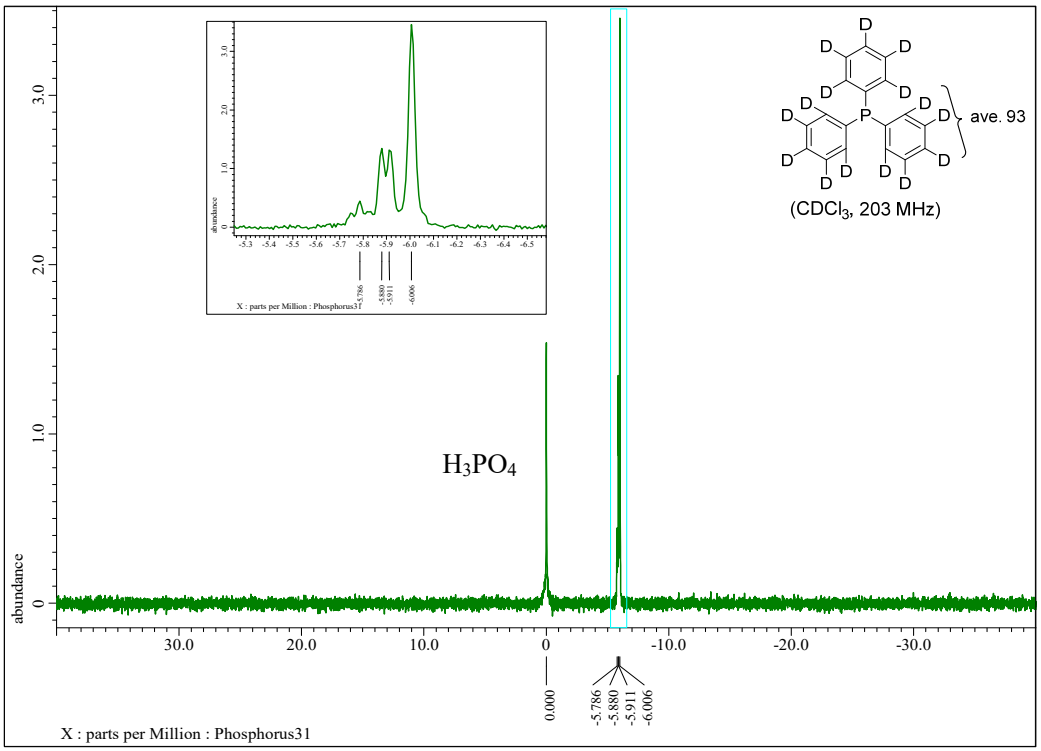
^{2}H NMR of triphenylphophine-d_{15} (**1**-d_{15}; Table 1, entry 7)



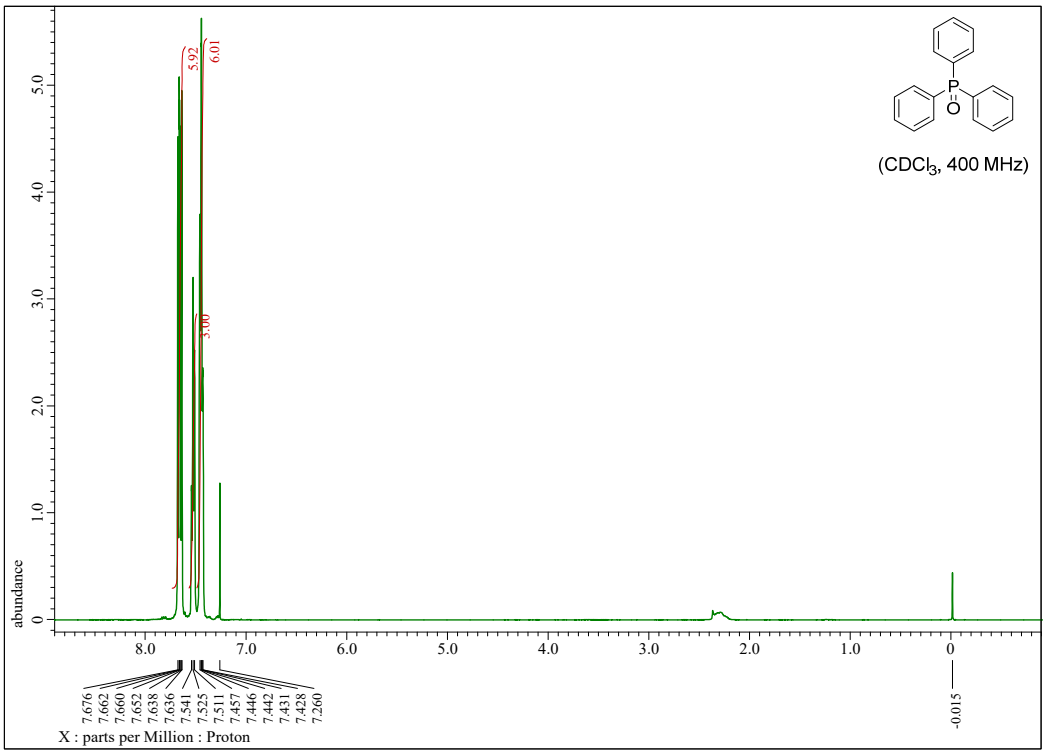
^{13}C NMR of triphenylphophine-d_{15} (**1**-d_{15}; Table 1, entry 7)



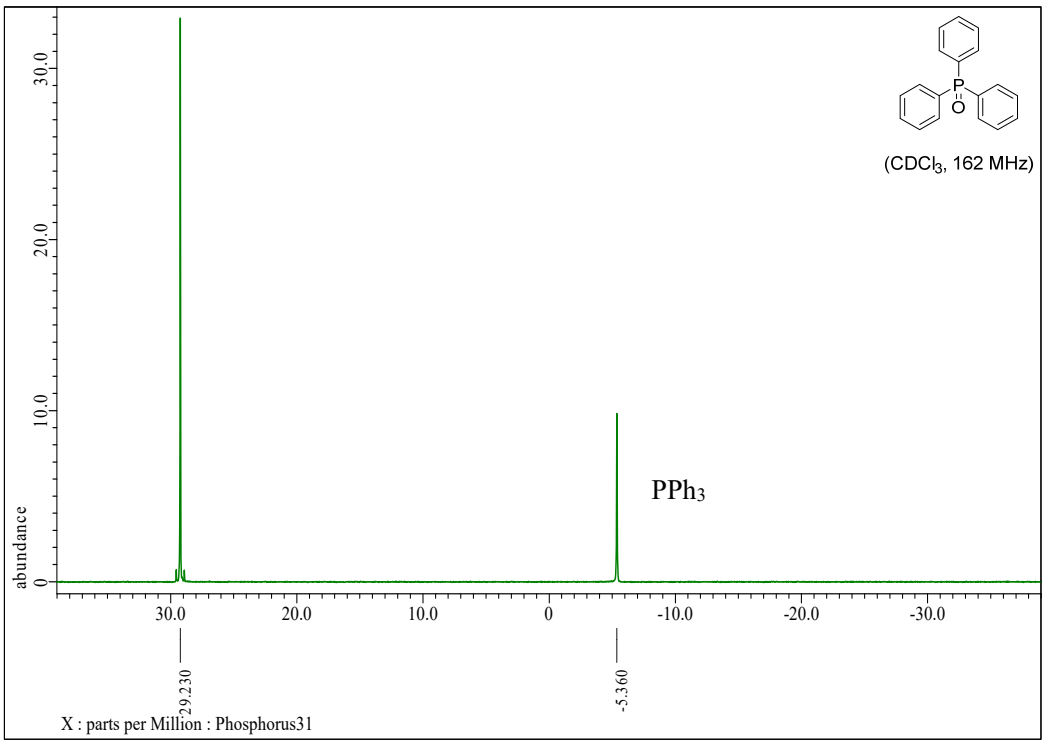
^{31}P NMR of triphenylphophine-d_{15} (**1**-d_{15}; Table 1, entry 7)



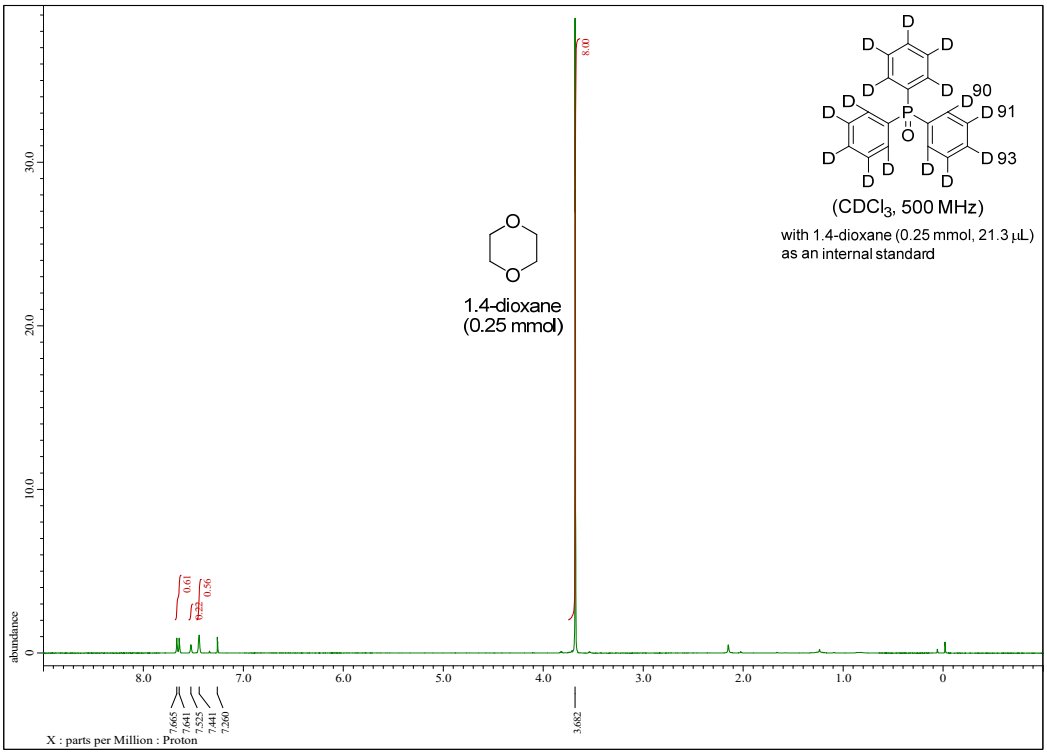
^{1}H NMR of triphenylphophine oxide (**2**; authentic sample)



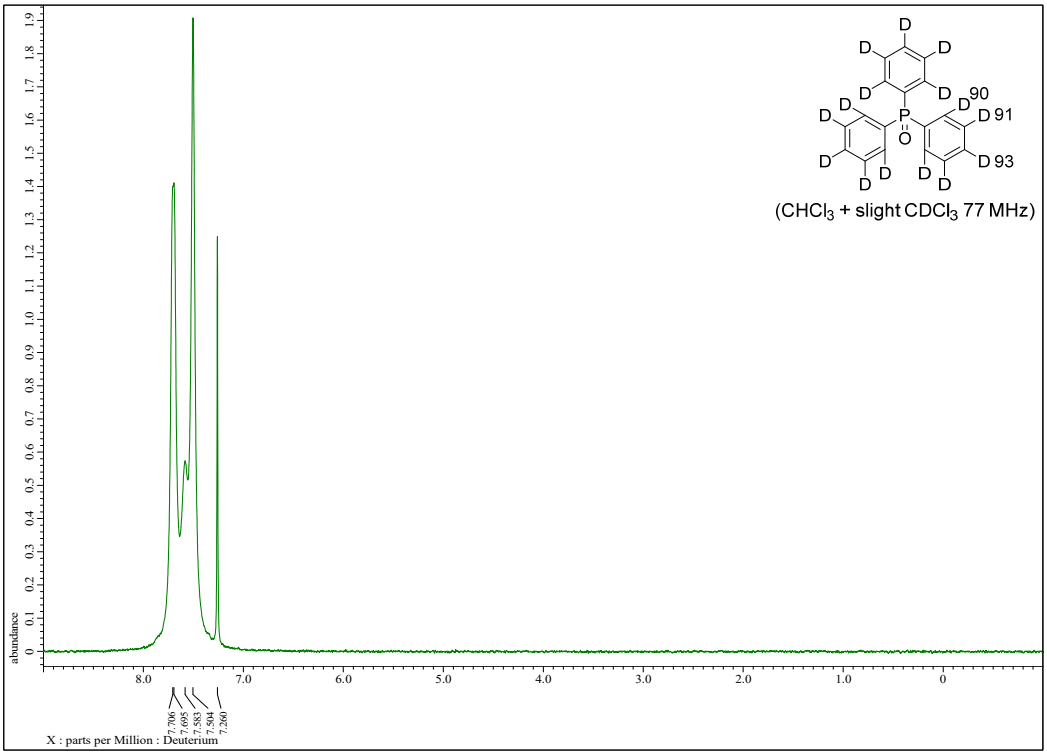
^{31}P NMR of triphenylphophine oxide (**2**; authentic sample)



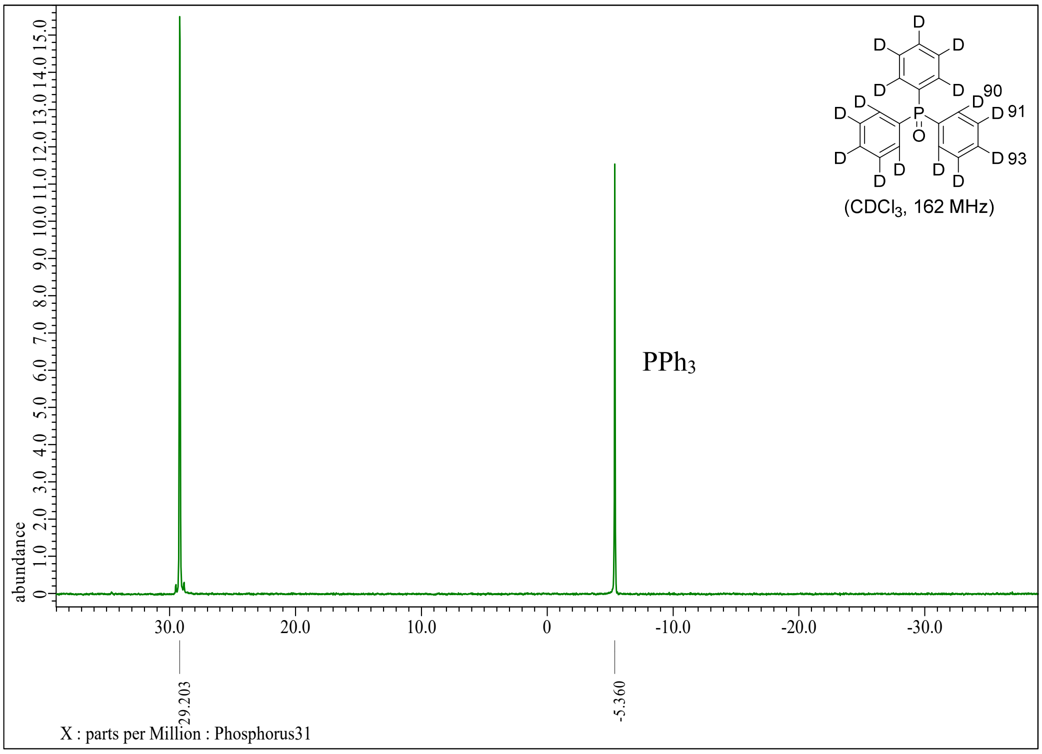
^{1}H NMR of triphenylphophine oxide-d_{15} (**2**-d_{15}; Table 1, entry 7)



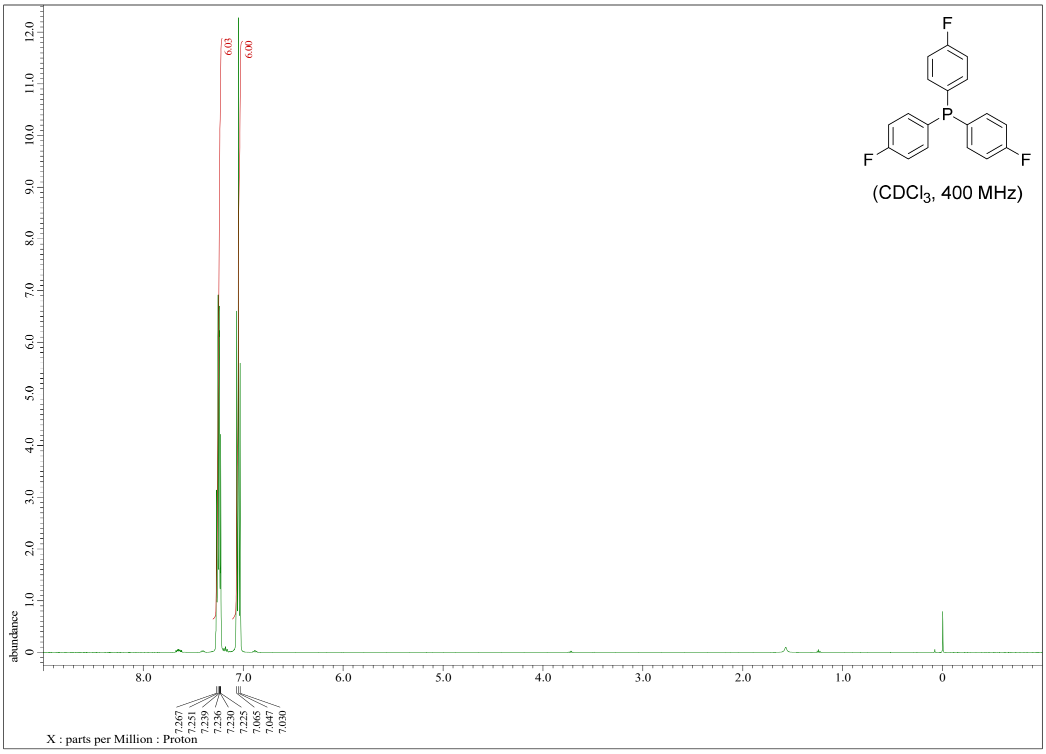
^{2}H NMR of triphenylphophine oxide-d_{15} (**2**-d_{15}; Table 1, entry 7)



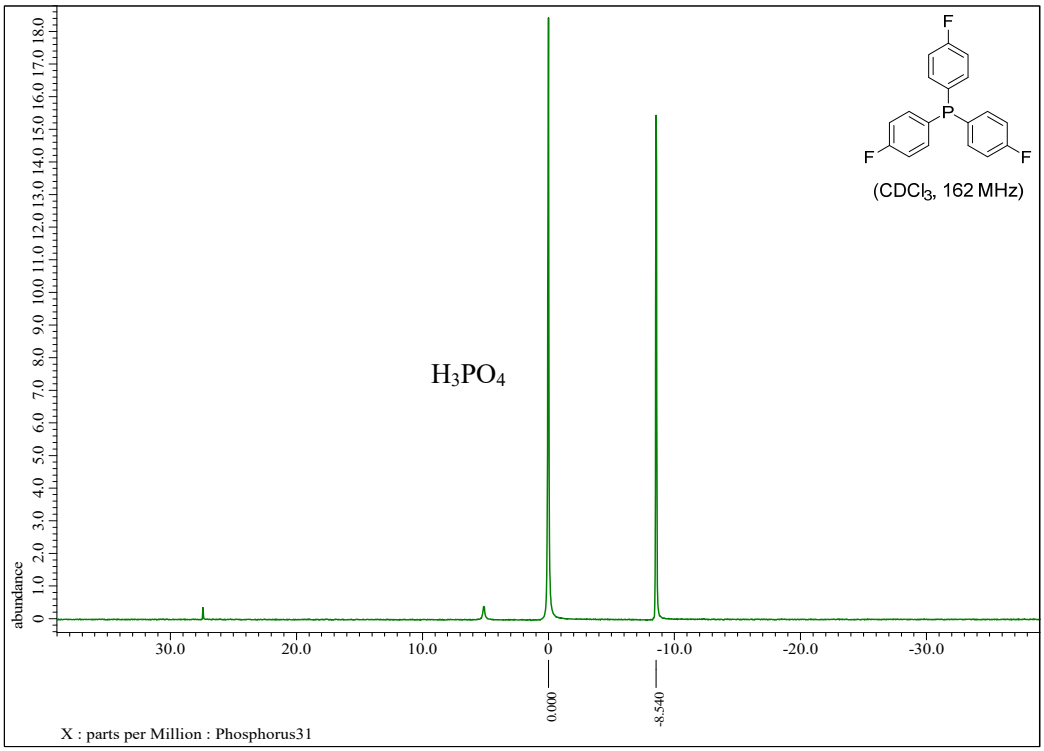
^{31}P NMR of triphenylphophine oxide-d_{15} (**2**-d_{15}; Table 1, entry 7)



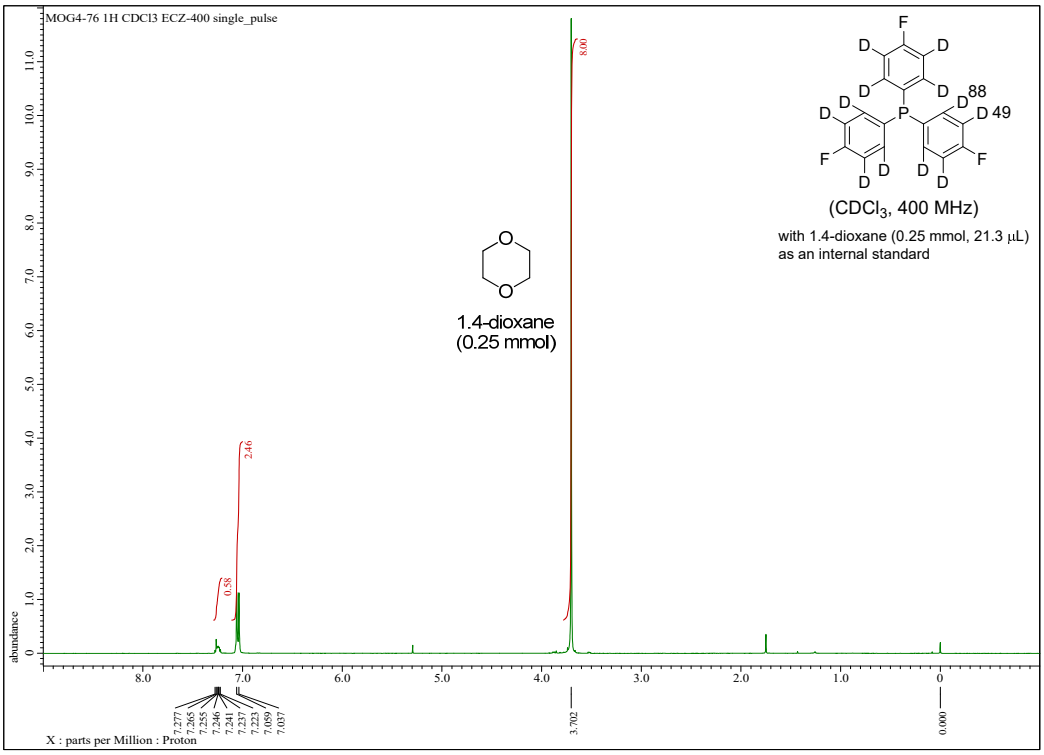
^{1}H NMR of tris(4-fluorophenyl)-phosphine (**3**; authentic sample)



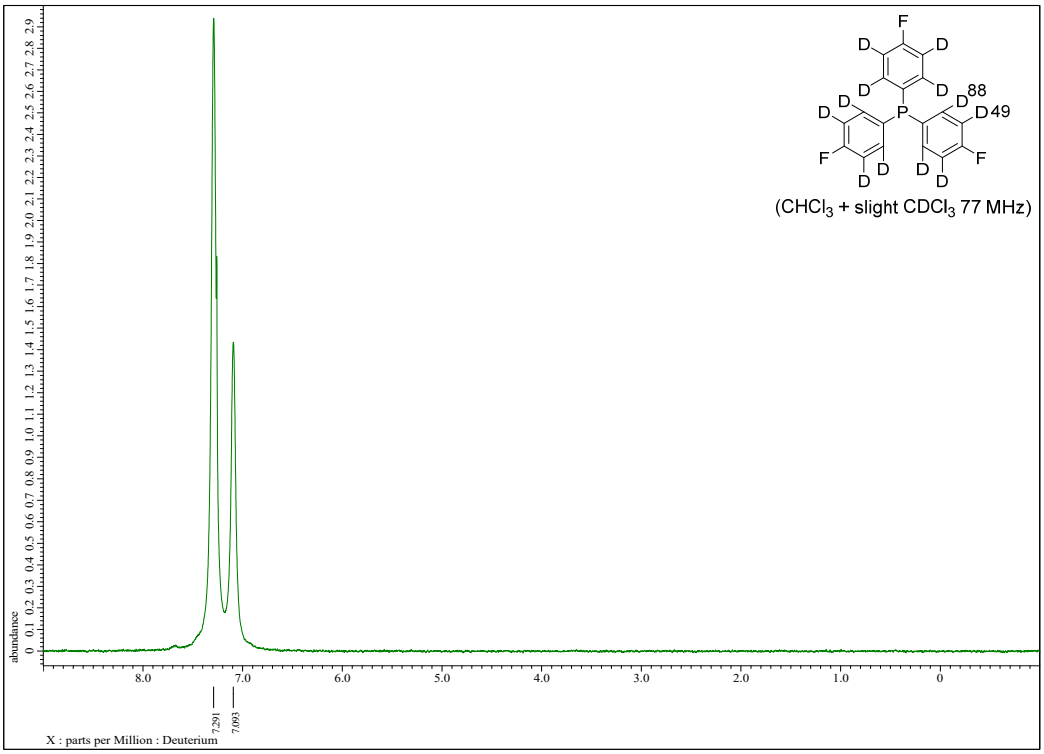
^{31}P NMR of tris(4-fluorophenyl)-phosphine (**3**; authentic sample)



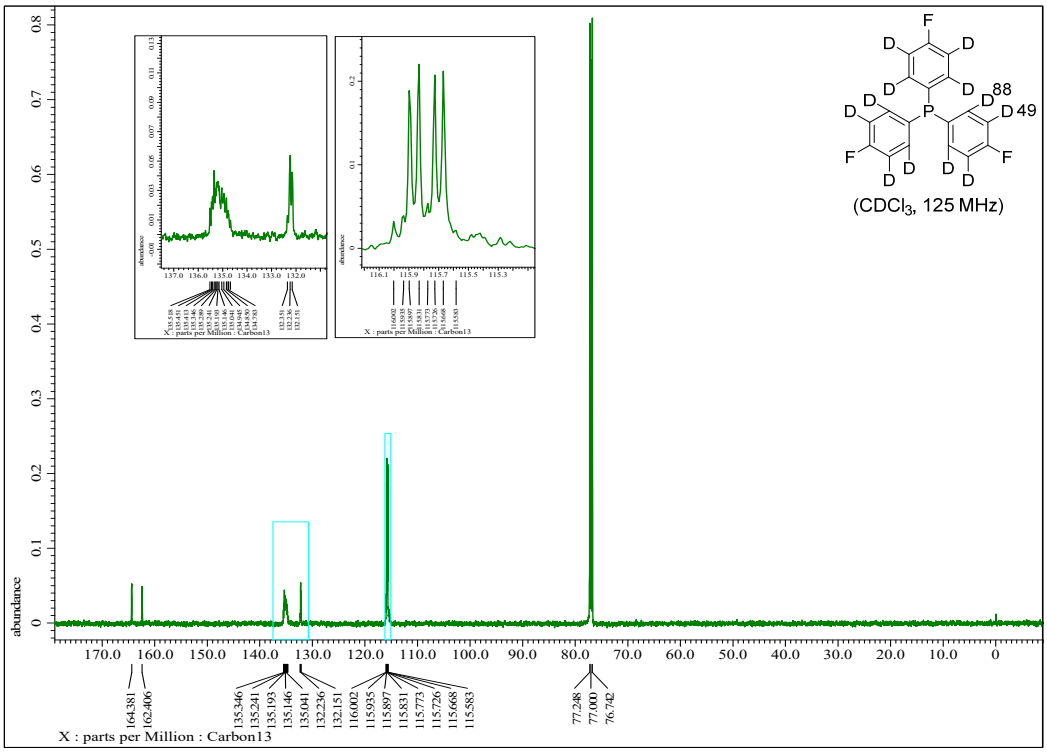
^{1}H NMR of tris(4-fluorophenyl)-phosphine-d_n (**3**-d_n)



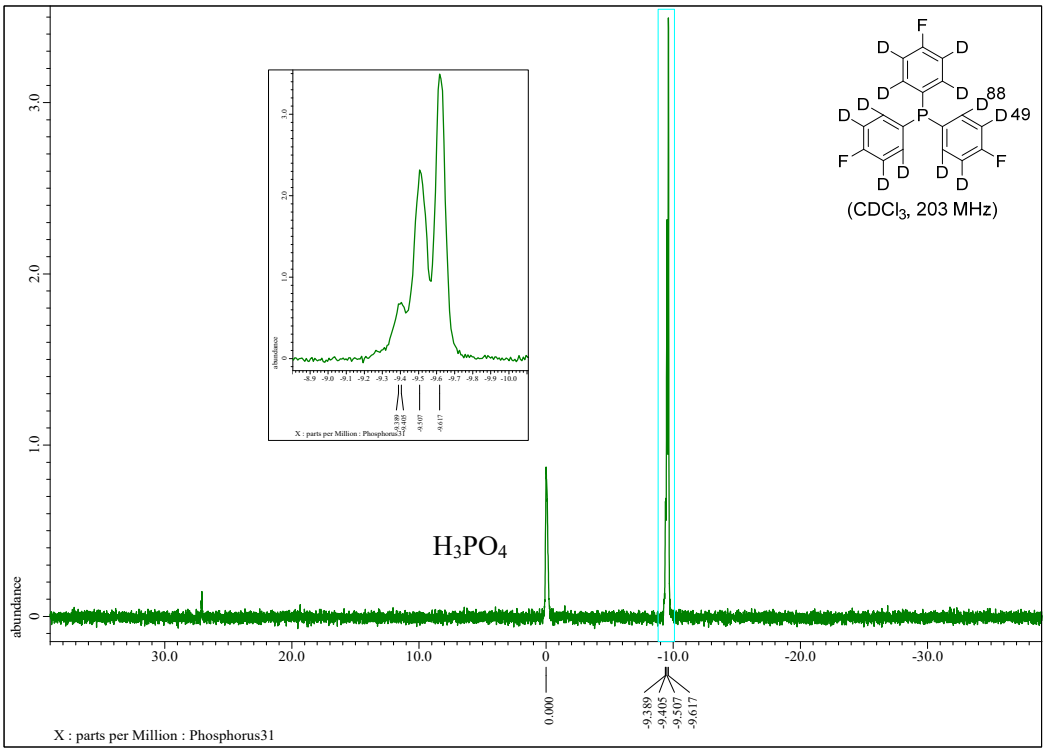
^{2}H NMR of tris(4-fluorophenyl)-phosphine-d_n (**3**-d_n)



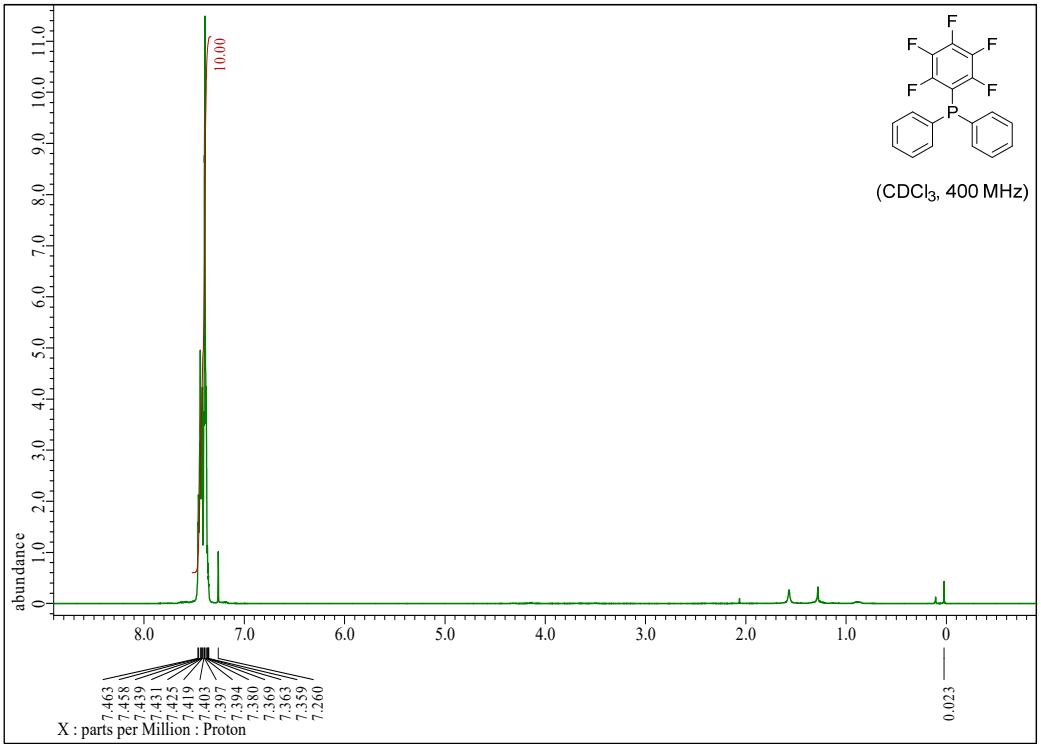
^{13}C NMR of tris(4-fluorophenyl)-phosphine-d_n (**3**-d_n)



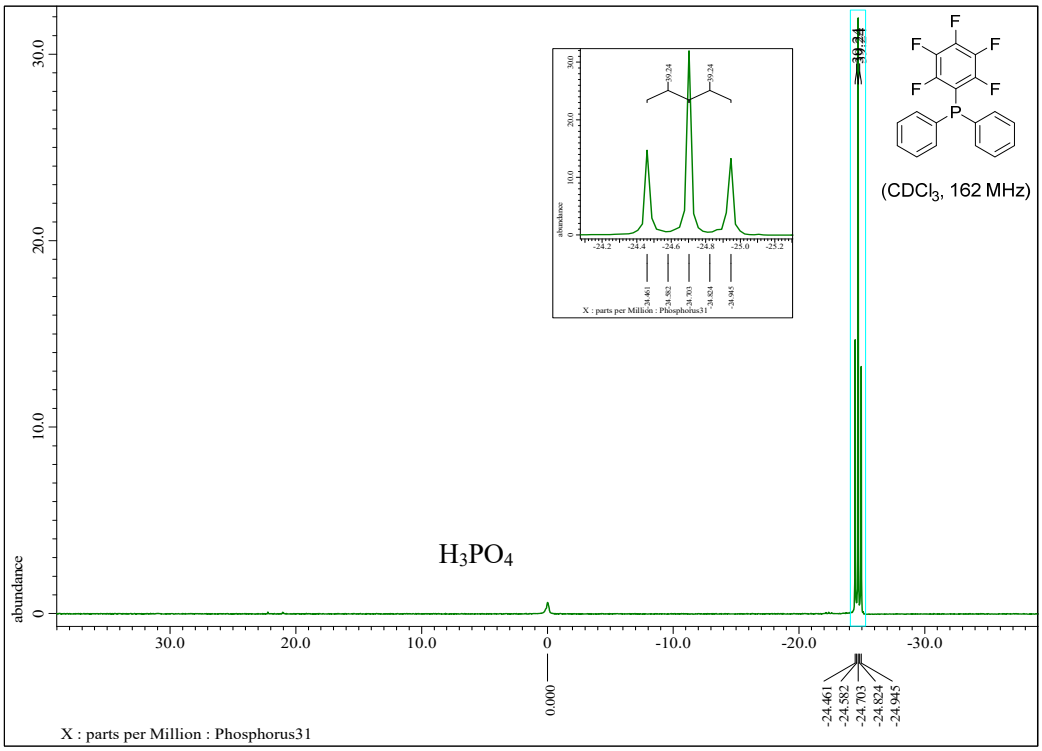
^{31}P NMR of tris(4-fluorophenyl)-phosphine-d_n (**3**-d_n)



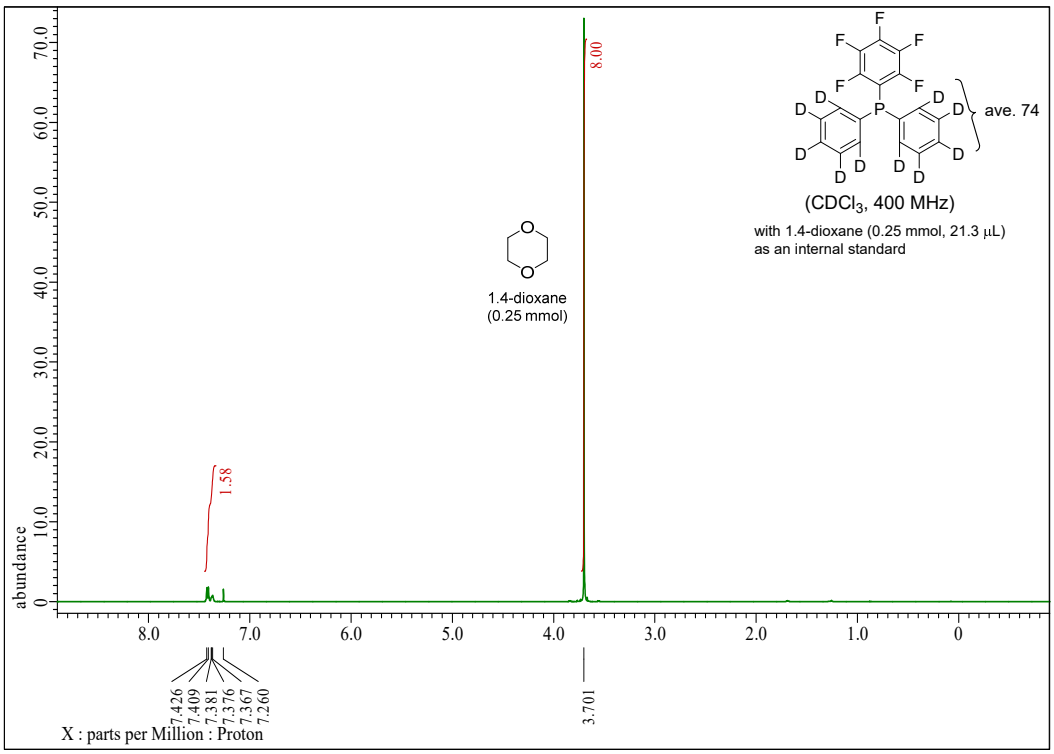
^{1}H NMR of (pentafluorophenyl)diphenylphosphine (**4**; authentic sample)



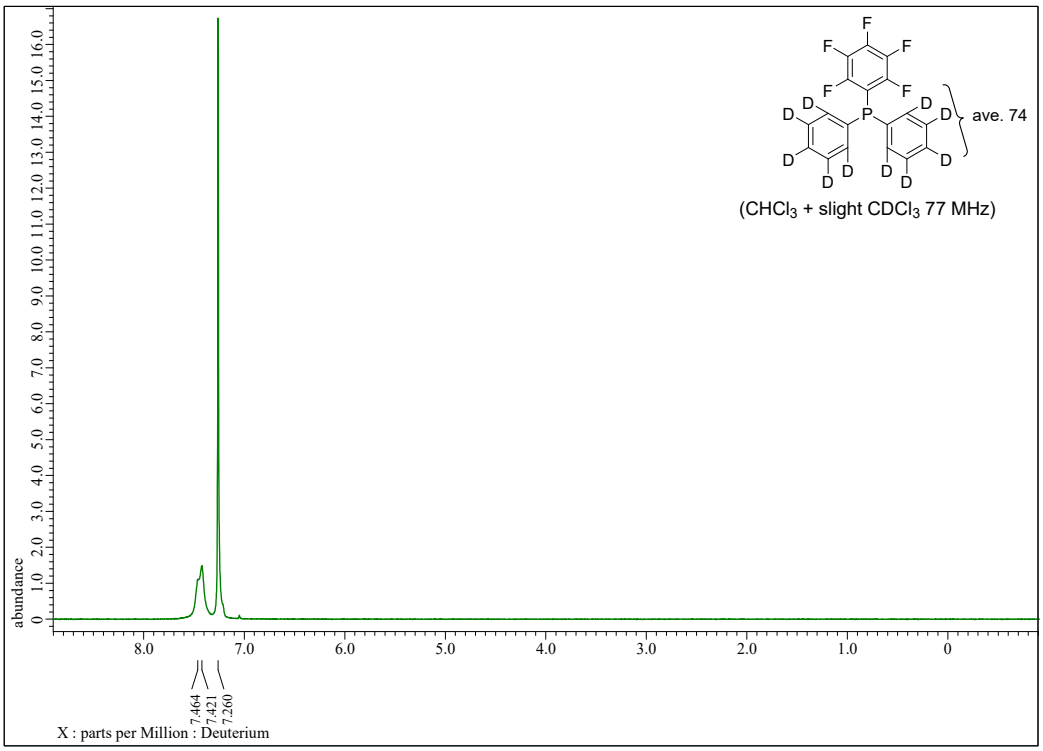
^{31}P NMR of (pentafluorophenyl)diphenylphosphine (**4**; authentic sample)



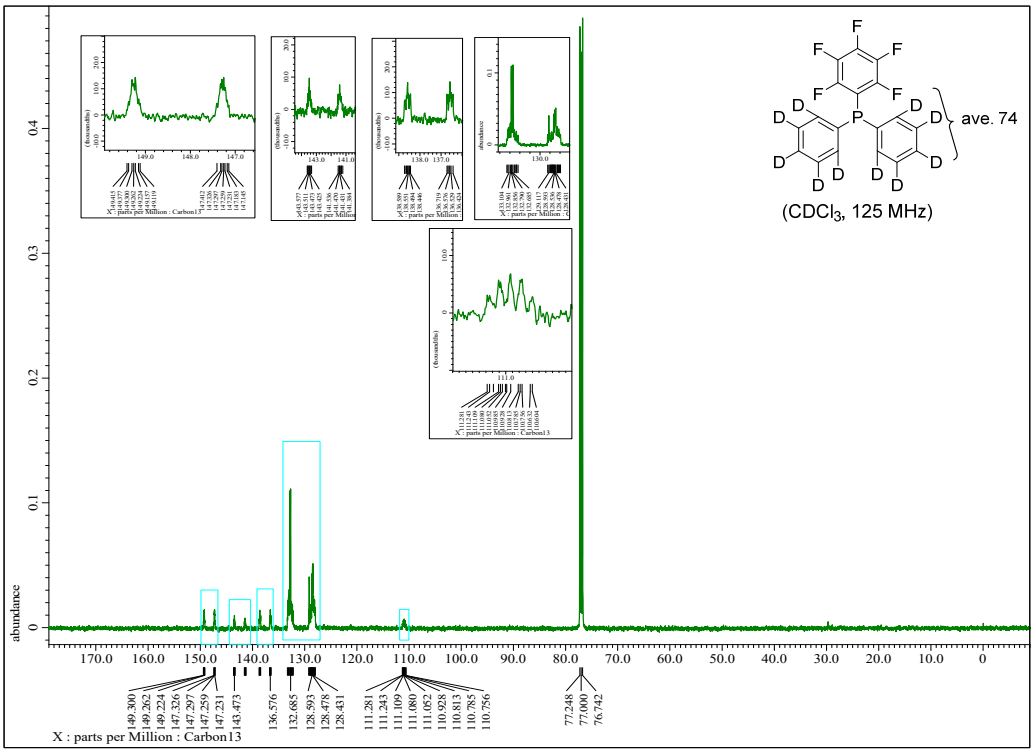
^{1}H NMR of (pentafluorophenyl)diphenylphosphine-d_n (**4**-d_n)



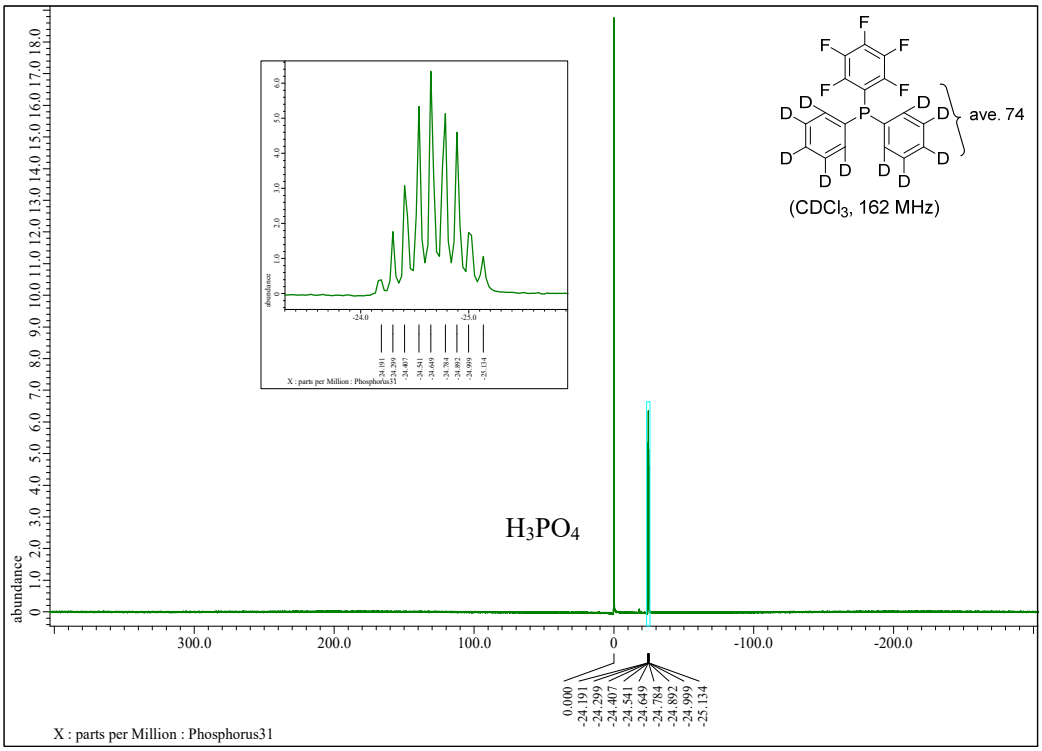
^{2}H NMR of (pentafluorophenyl)diphenylphosphine-d_n (**4**-d_n)



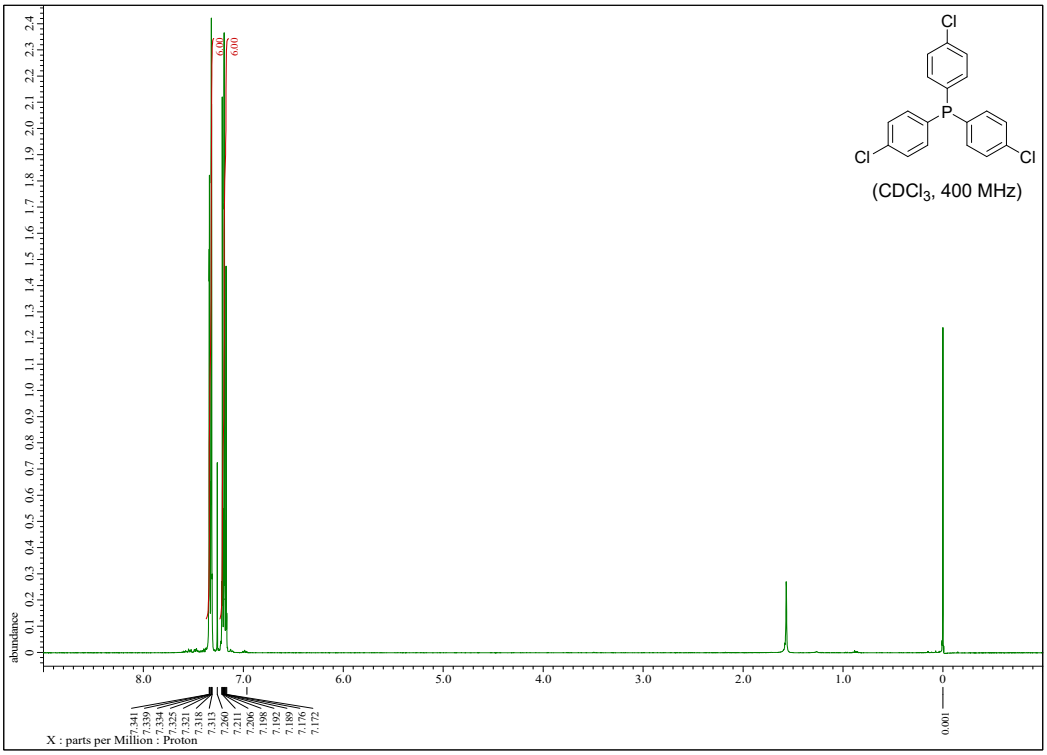
^{13}C NMR of (pentafluorophenyl)diphenylphosphine-d_n (**4**-d_n)



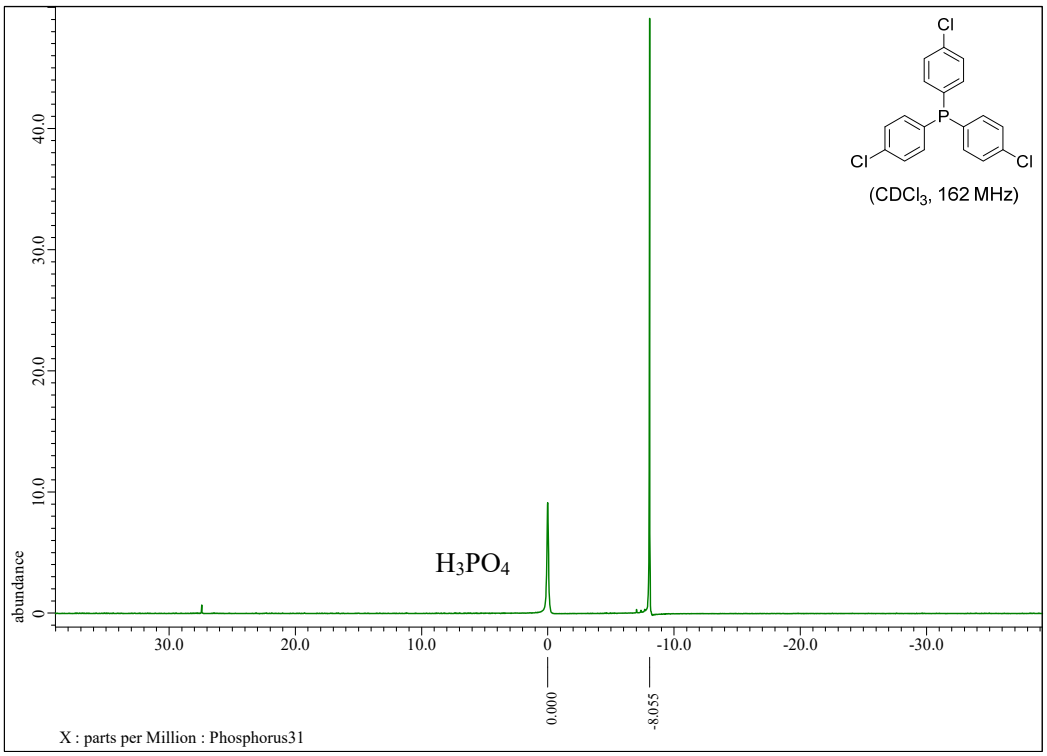
^{31}P NMR of (pentafluorophenyl)diphenylphosphine-d_n (**4**-d_n)



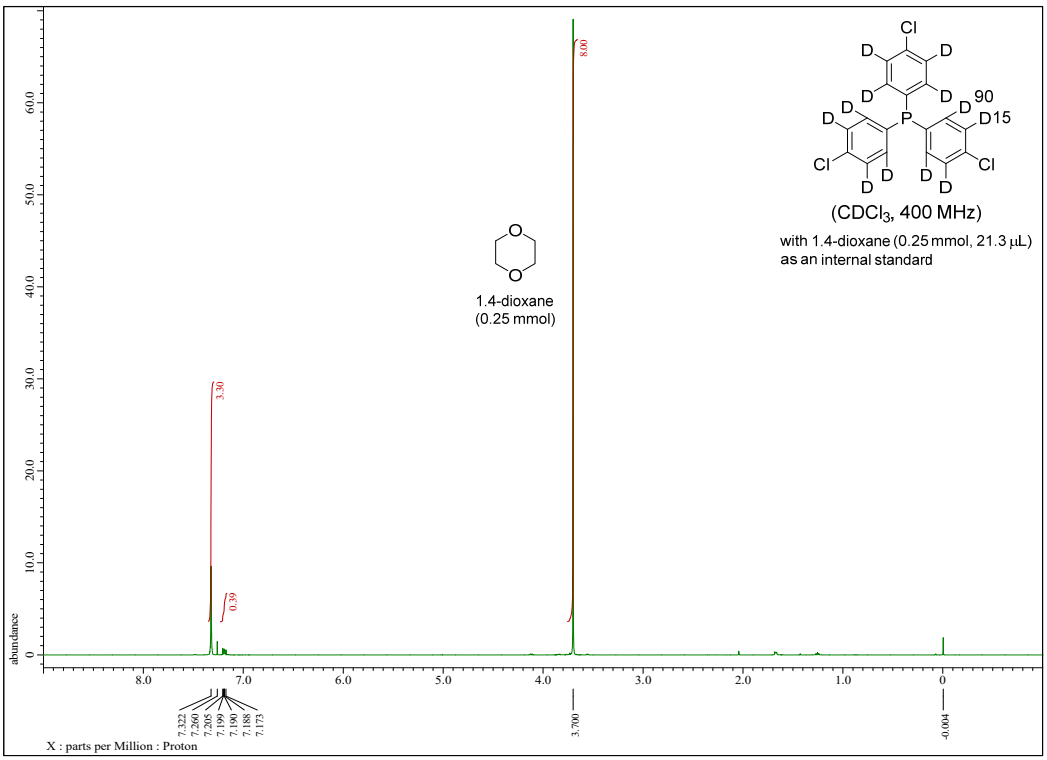
^{1}H NMR of tris(4-chlorophenyl)-phosphine (**5**; authentic sample)



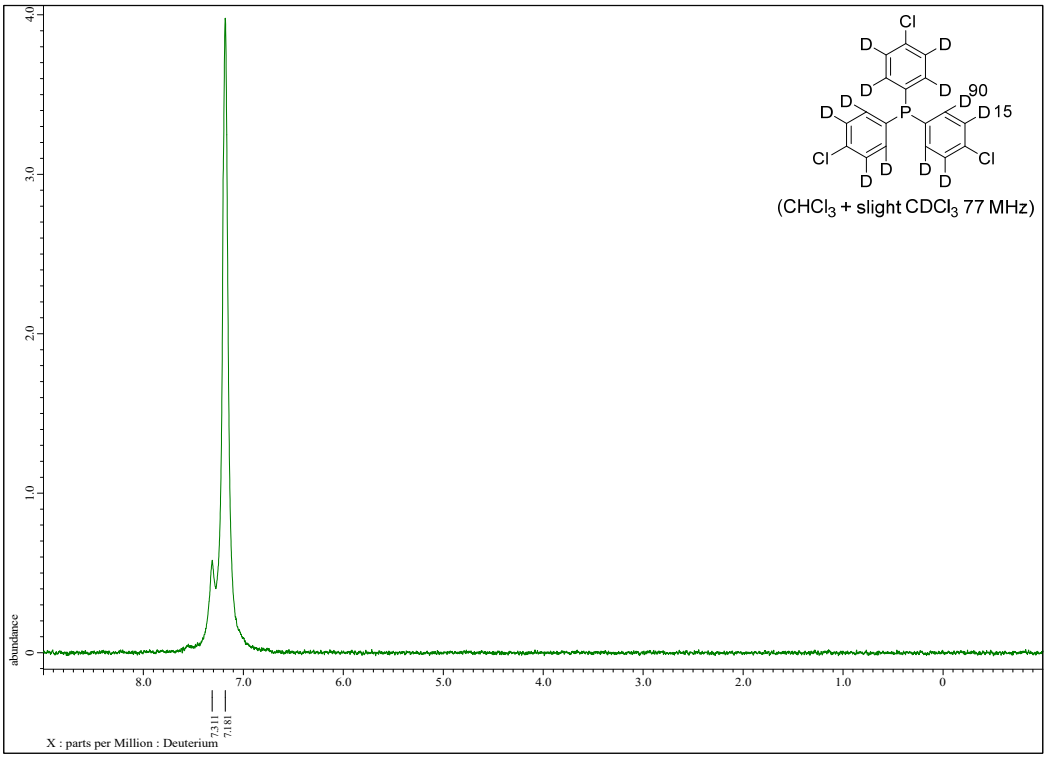
^{31}P NMR of tris(4-chlorophenyl)-phosphine (**5**; authentic sample)



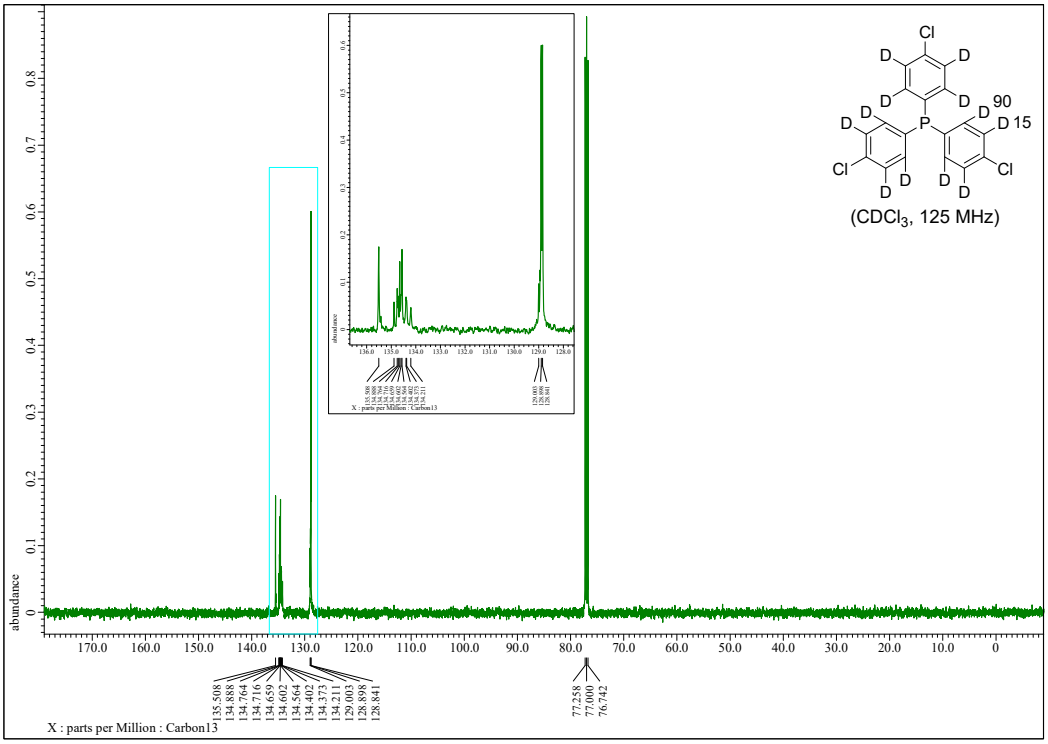
^{1}H NMR of tris(4-chlorophenyl)-phosphine-d_n (**5**-d_n)



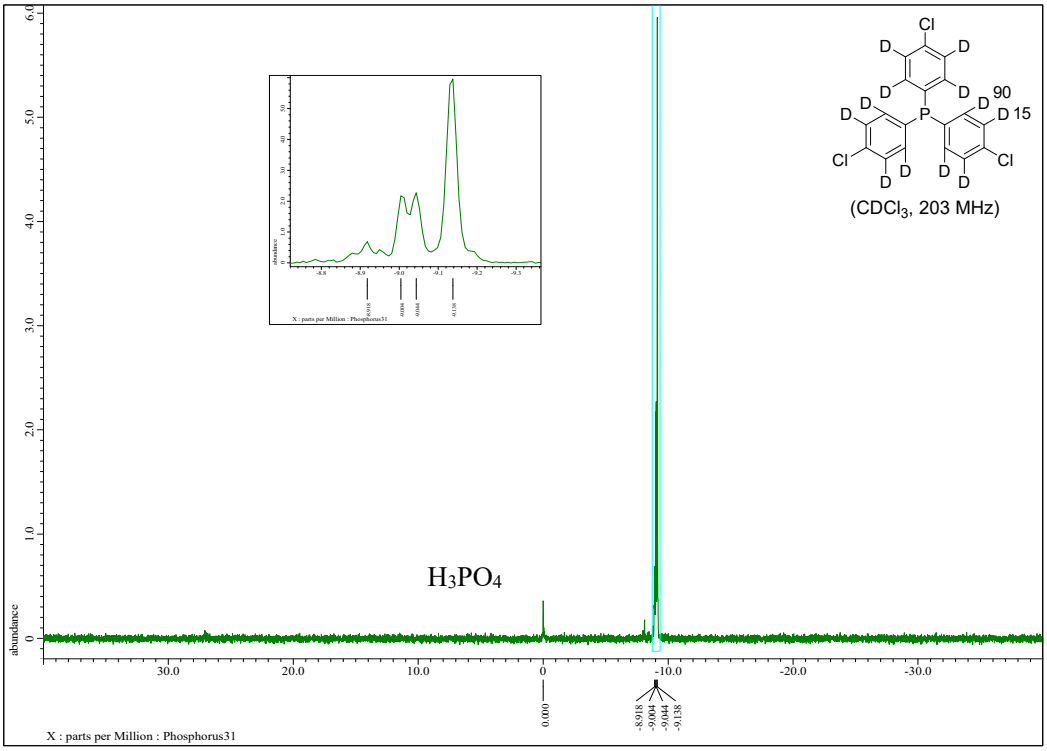
^{2}H NMR of tris(4-chlorophenyl)-phosphine-d_n (**5**-d_n)



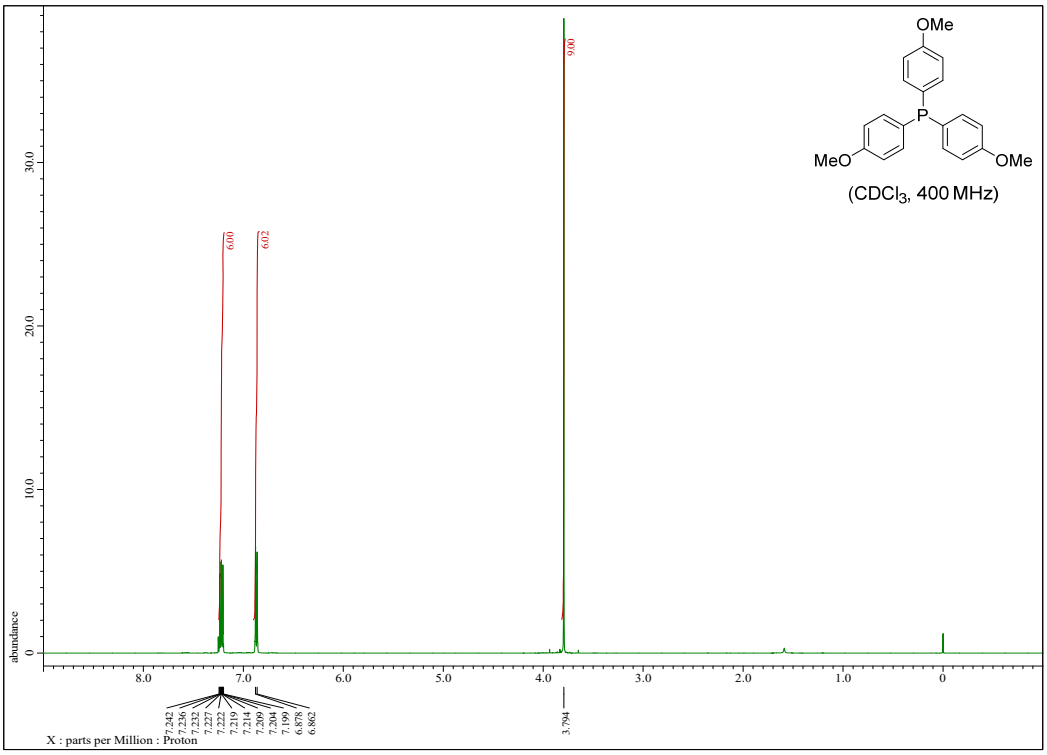
^{13}C NMR of tris(4-chlorophenyl)-phosphine-d_n (**5**-d_n)



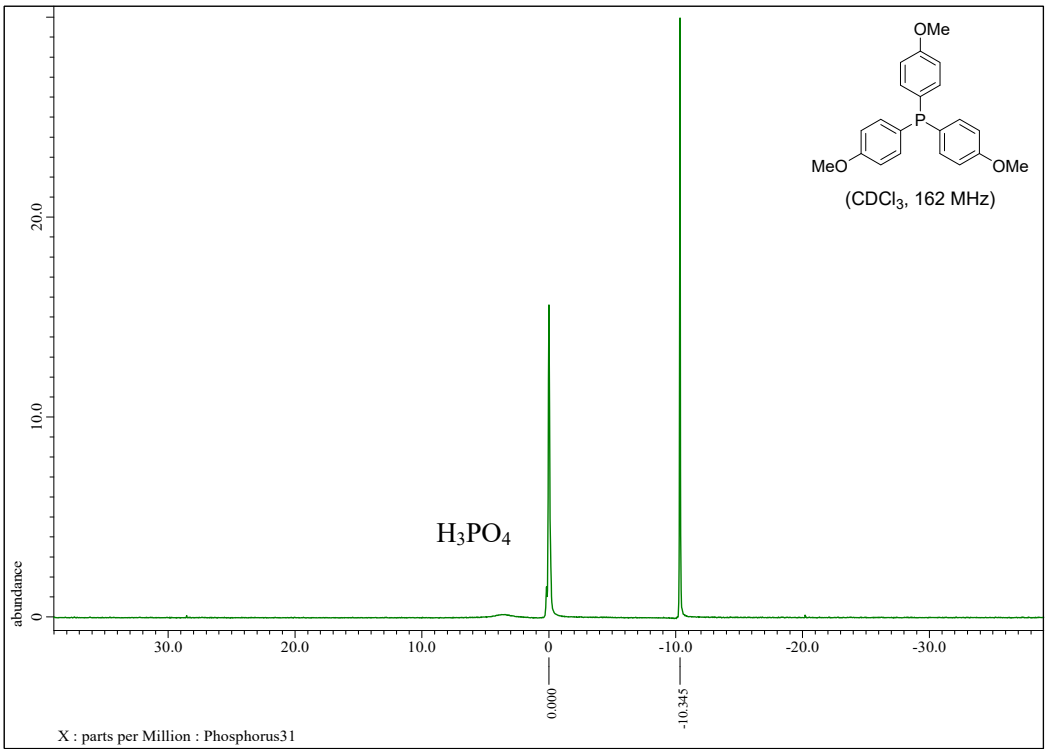
^{31}P NMR of tris(4-chlorophenyl)-phosphine-d_n (**5**-d_n)



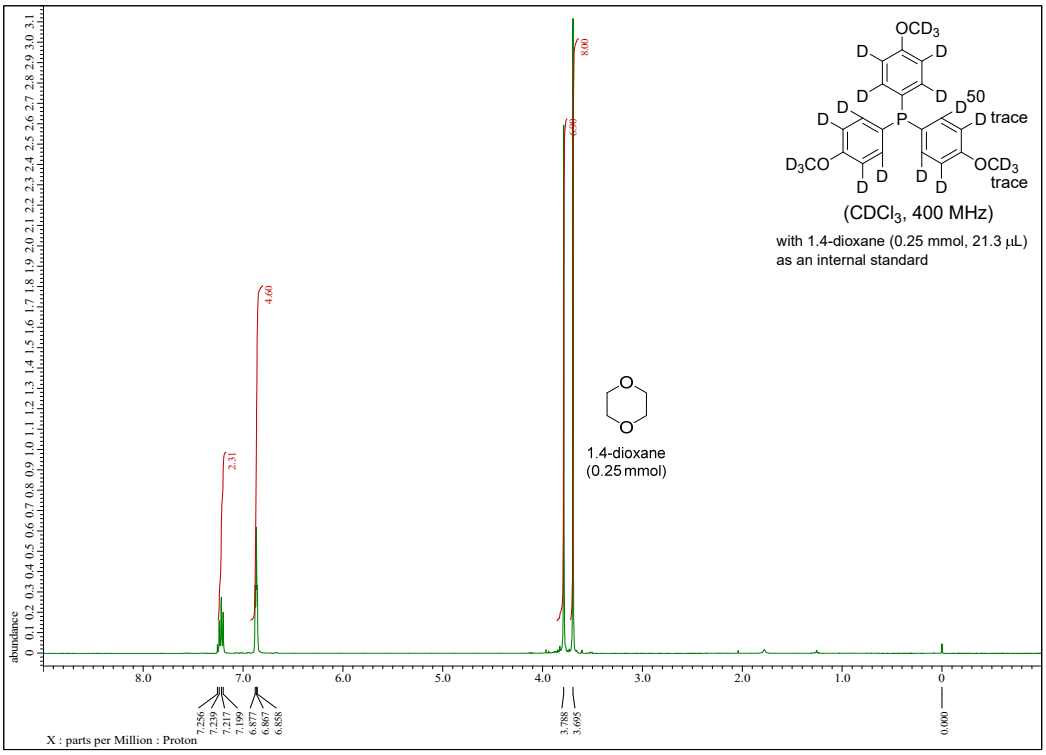
^{1}H NMR of tris(4-methoxyphenyl)-phosphine (**6**; authentic sample)



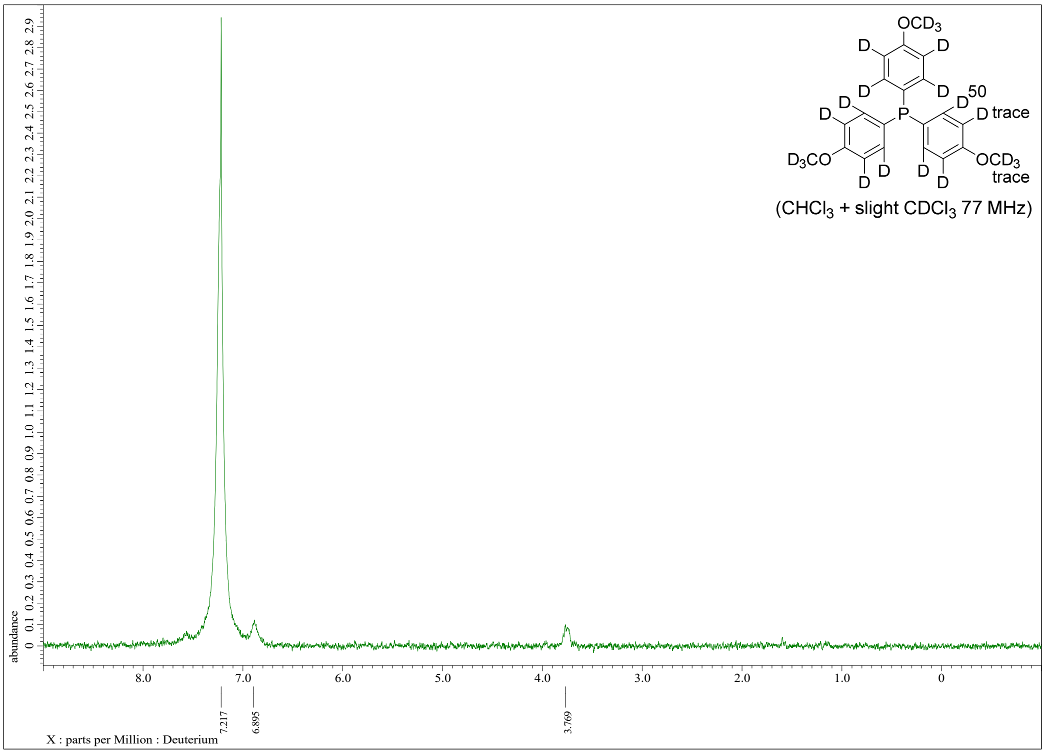
^{31}P NMR of tris(4-methoxyphenyl)-phosphine (**6**; authentic sample)



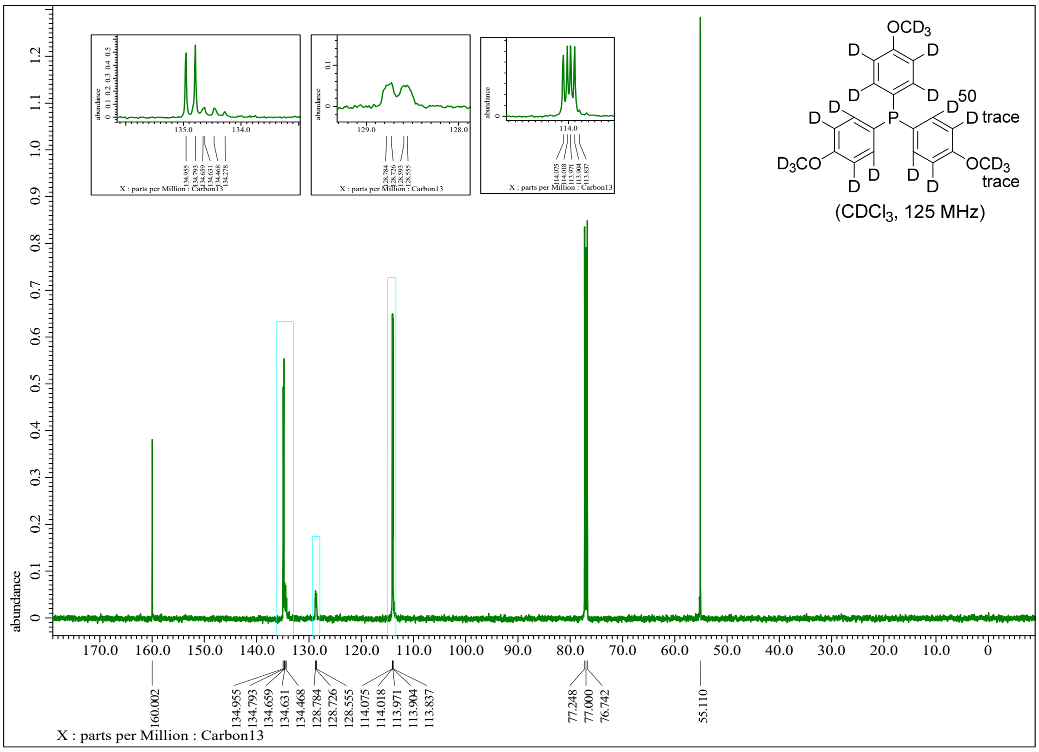
^{1}H NMR of tris(4-methoxyphenyl)-phosphine-d_n (**6**-d_n)



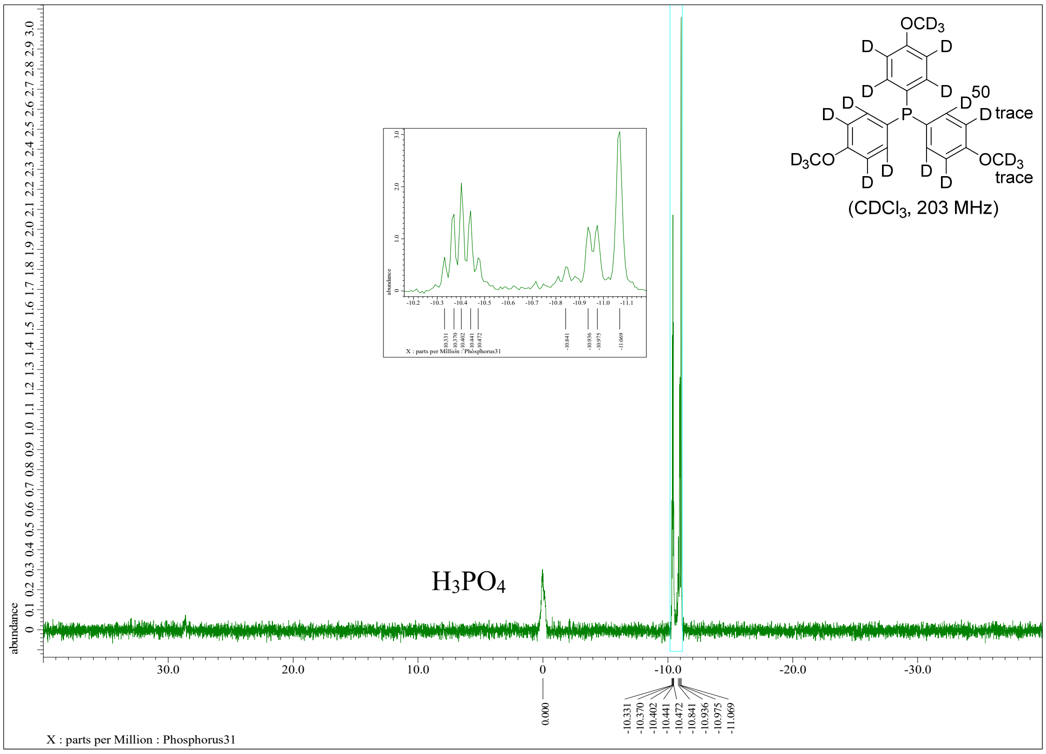
[2]H NMR of tris(4-methoxyphenyl)-phosphine-d_n (**6**-d_n)



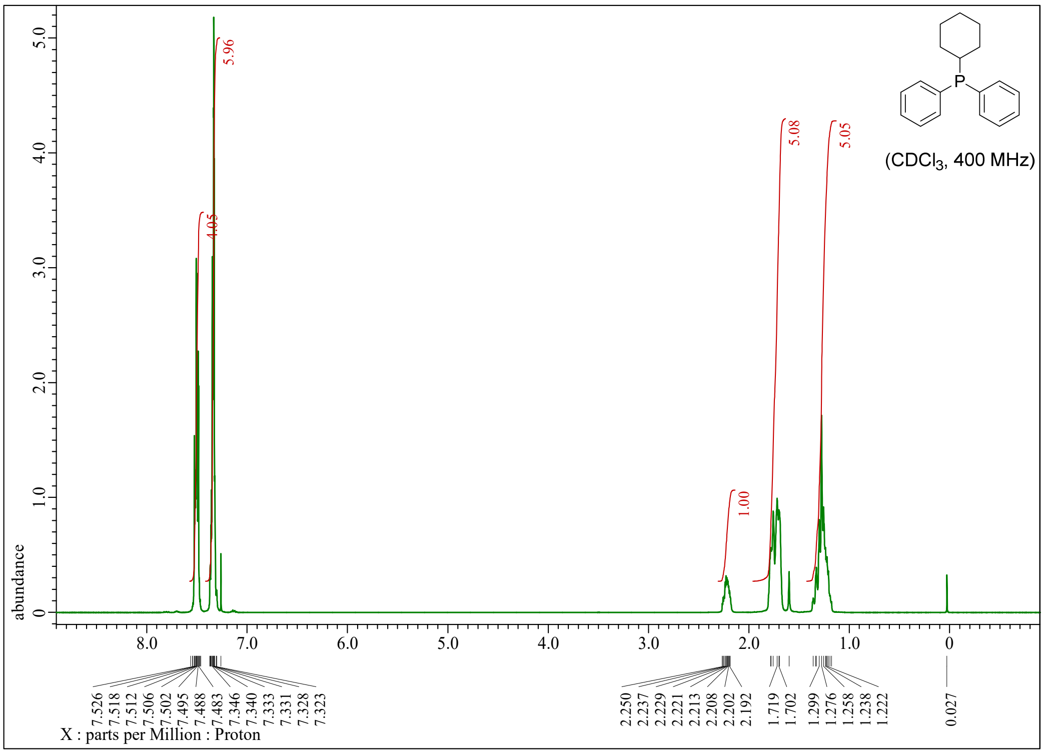
[13]C NMR of tris(4-methoxyphenyl)-phosphine-d_n (**6**-d_n)



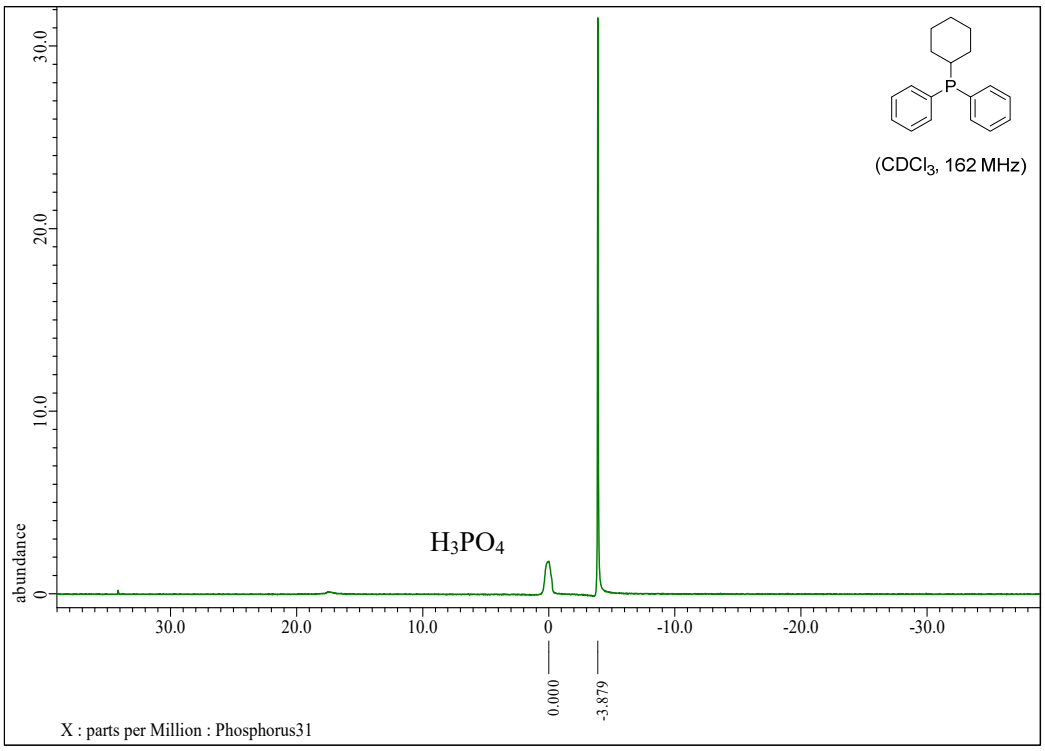
^{31}P NMR of tris(4-methoxyphenyl)-phosphine-d_n (**6**-d_n)



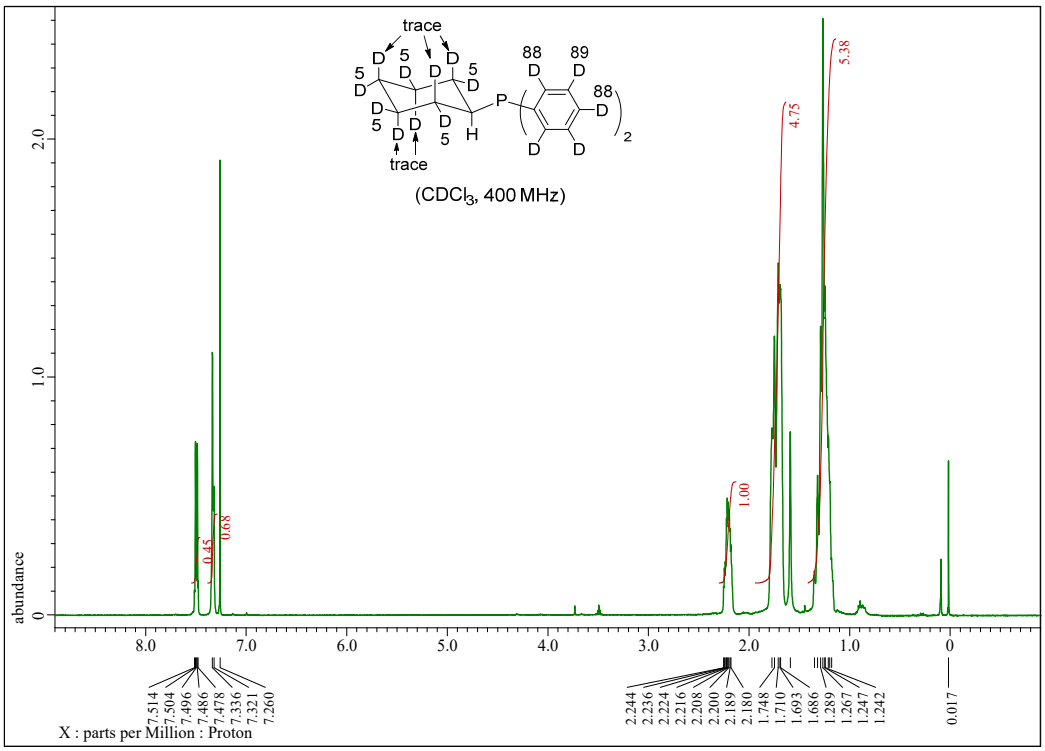
^{1}H NMR of cyclohexyldiphenylphosphine (**7**; authentic sample)



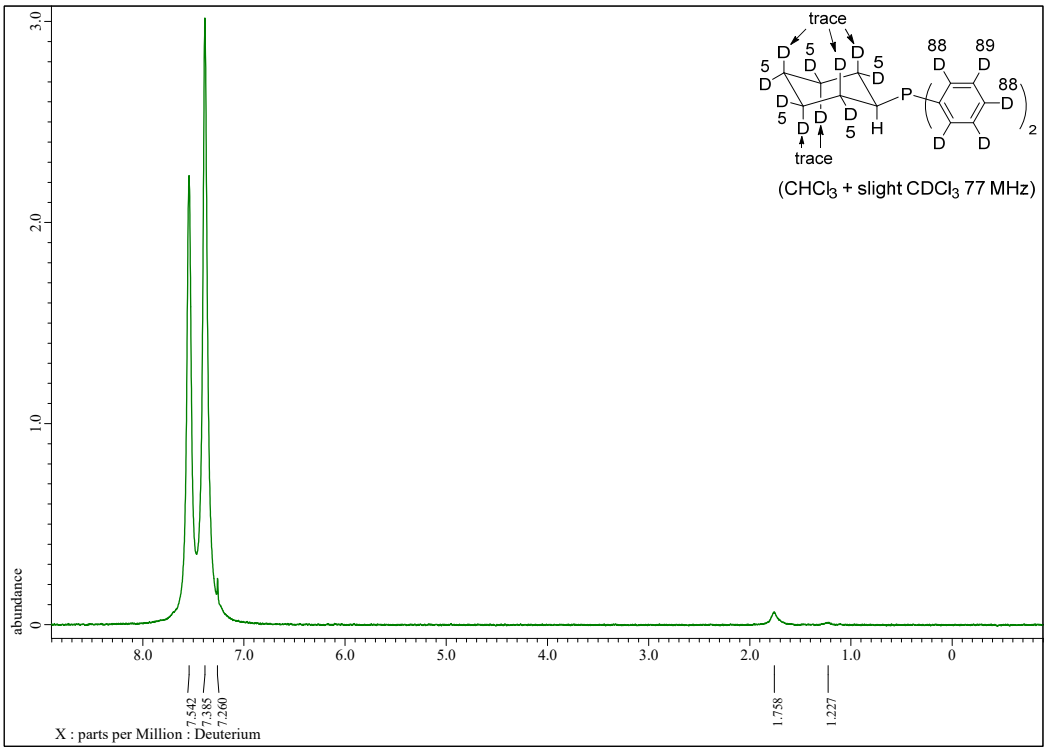
^{31}P NMR of cyclohexyldiphenylphosphine (**7**; authentic sample)



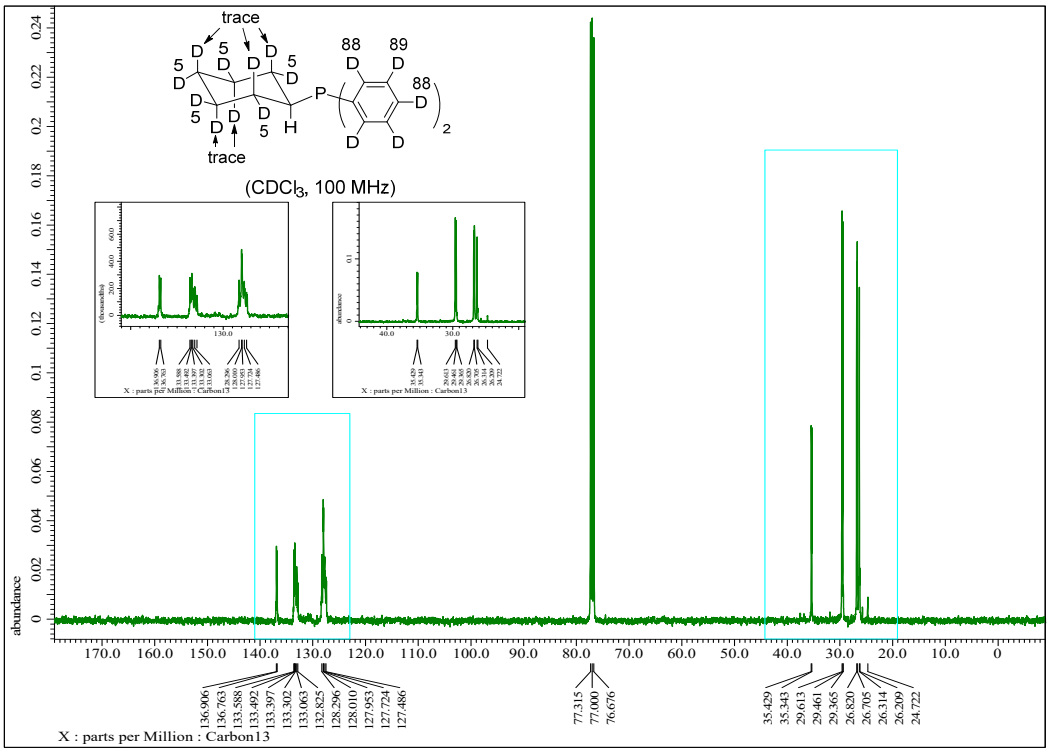
^{1}H NMR of cyclohexyldiphenylphosphine-d_n (**7**-d_n)



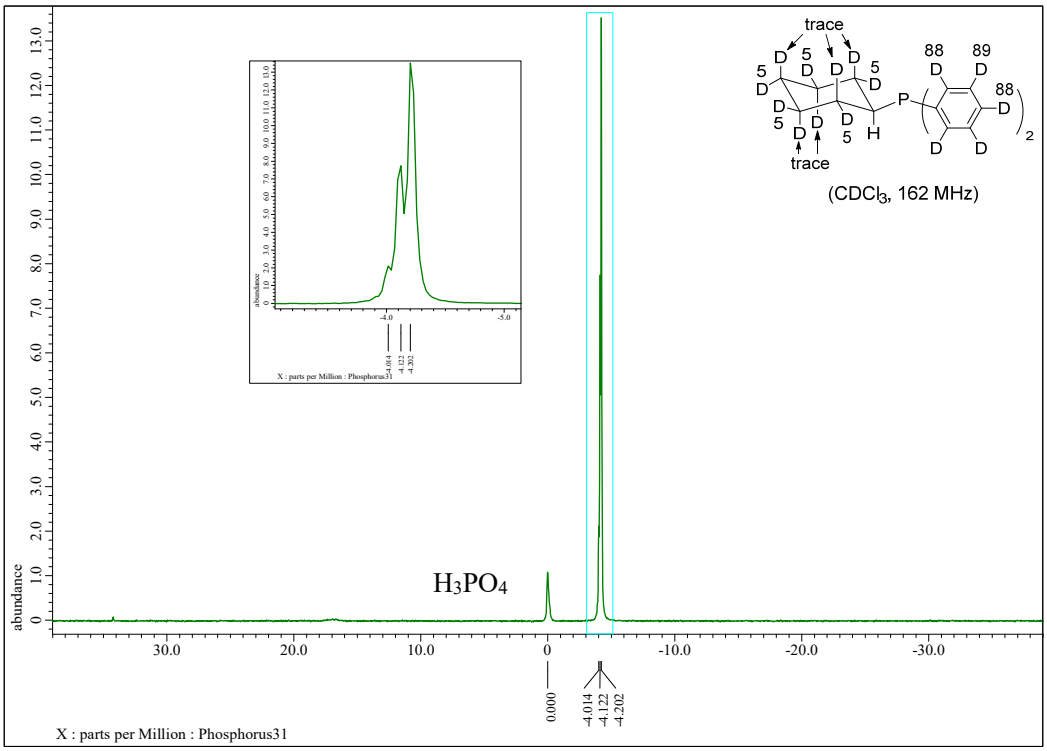
^{2}H NMR of cyclohexyldiphenylphosphine-d_n (**7**-d_n)



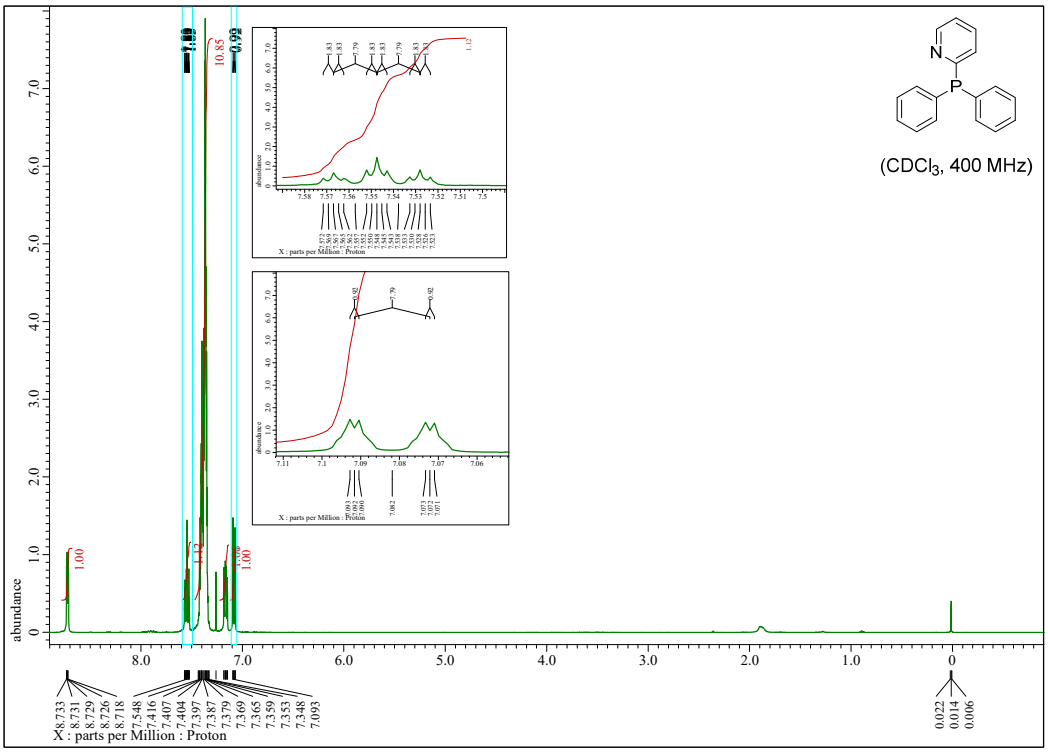
^{13}C NMR of cyclohexyldiphenylphosphine-d_n (**7**-d_n)



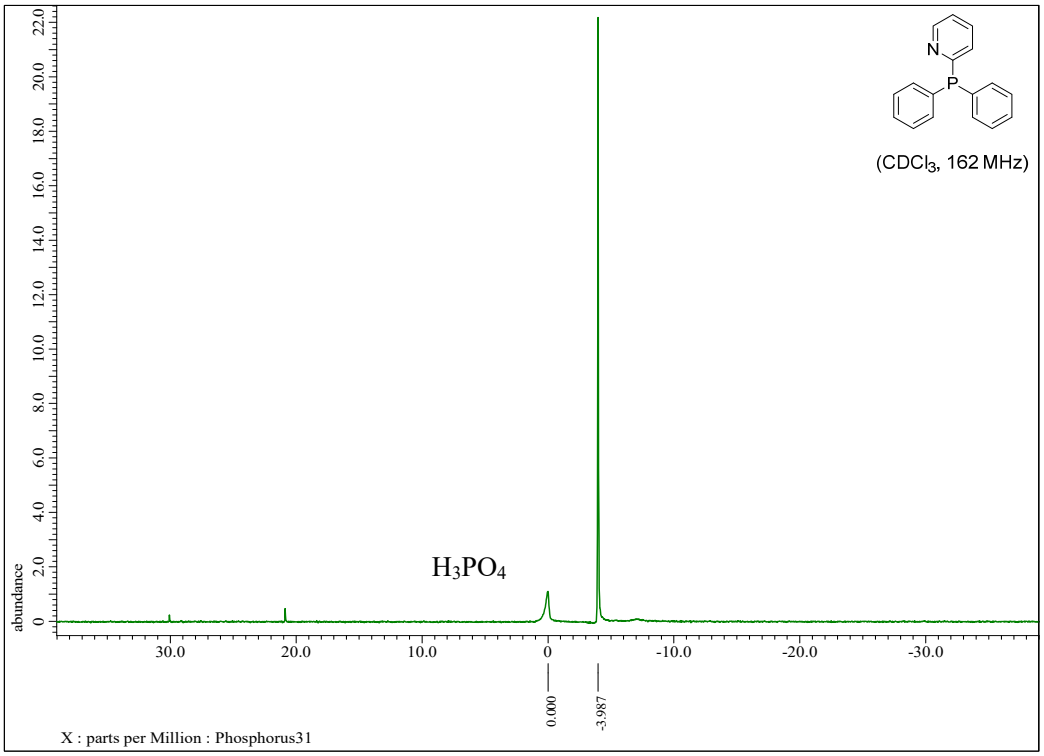
^{31}P NMR of cyclohexyldiphenylphosphine-d_n (**7**-d_n)



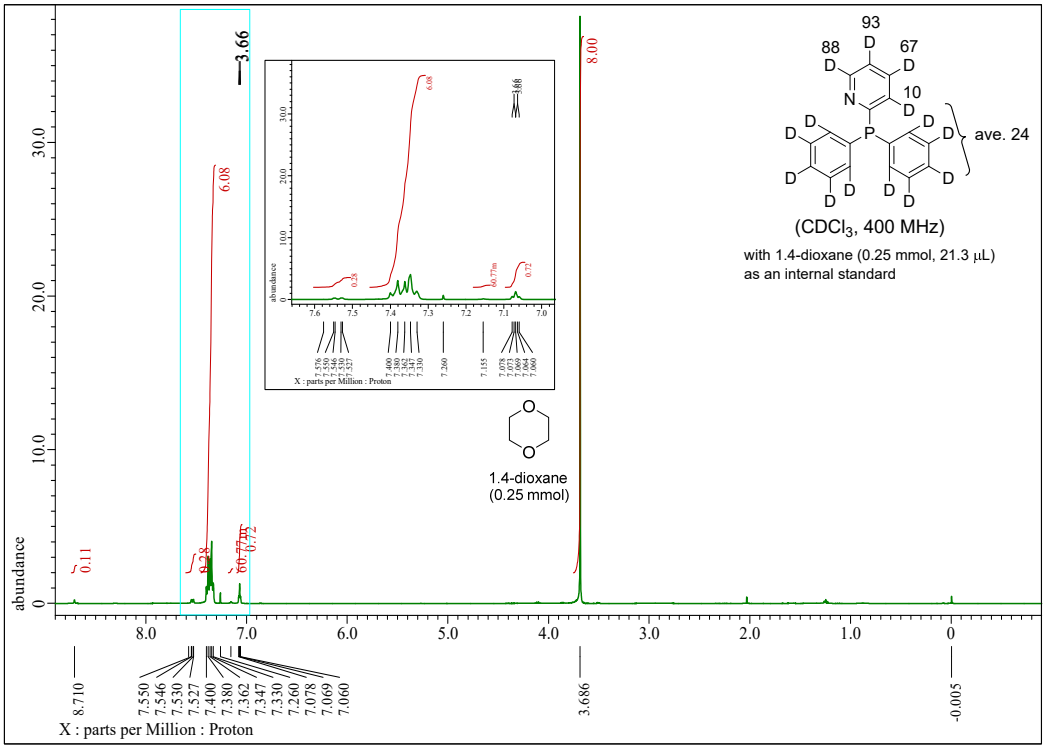
^{1}H NMR of diphenyl-2-pyridylphosphine (**8**; authentic sample)



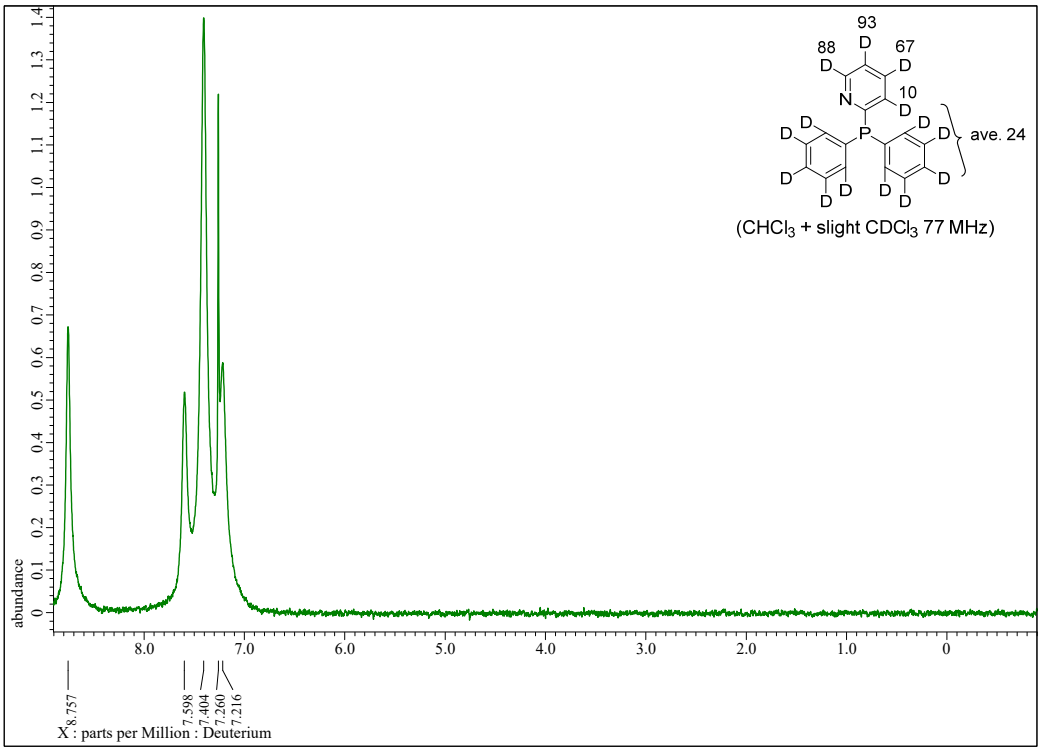
^{31}P NMR of diphenyl-2-pyridylphosphine (**8**; authentic sample)



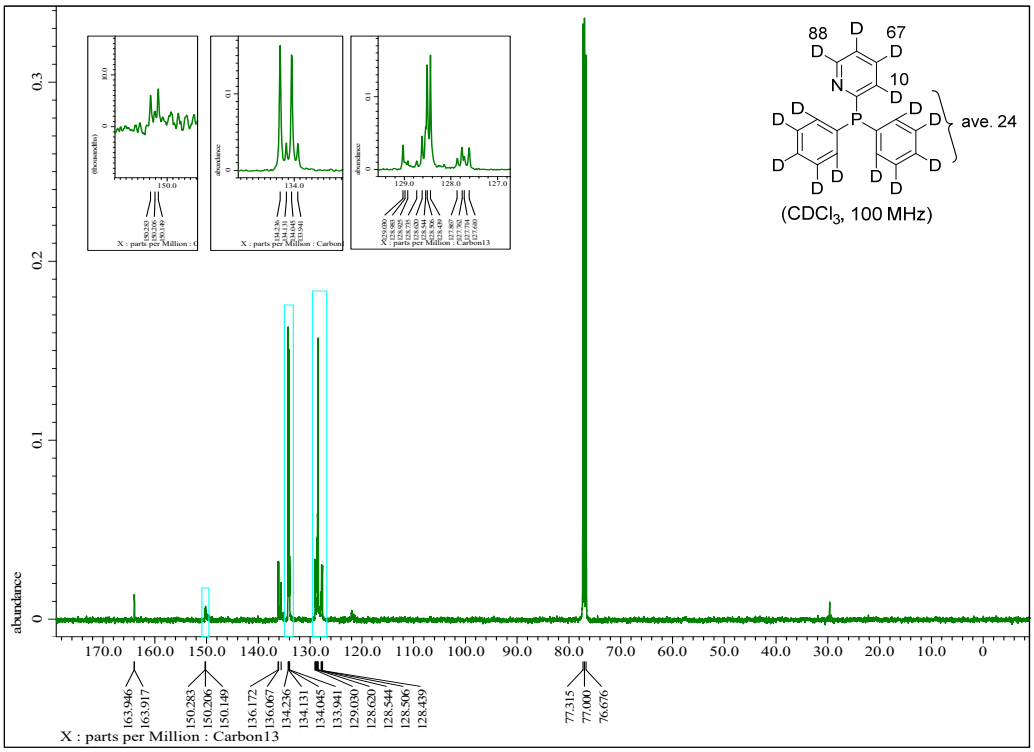
^{1}H NMR of diphenyl-2-pyridylphosphine-d_n (**8**-d_n)



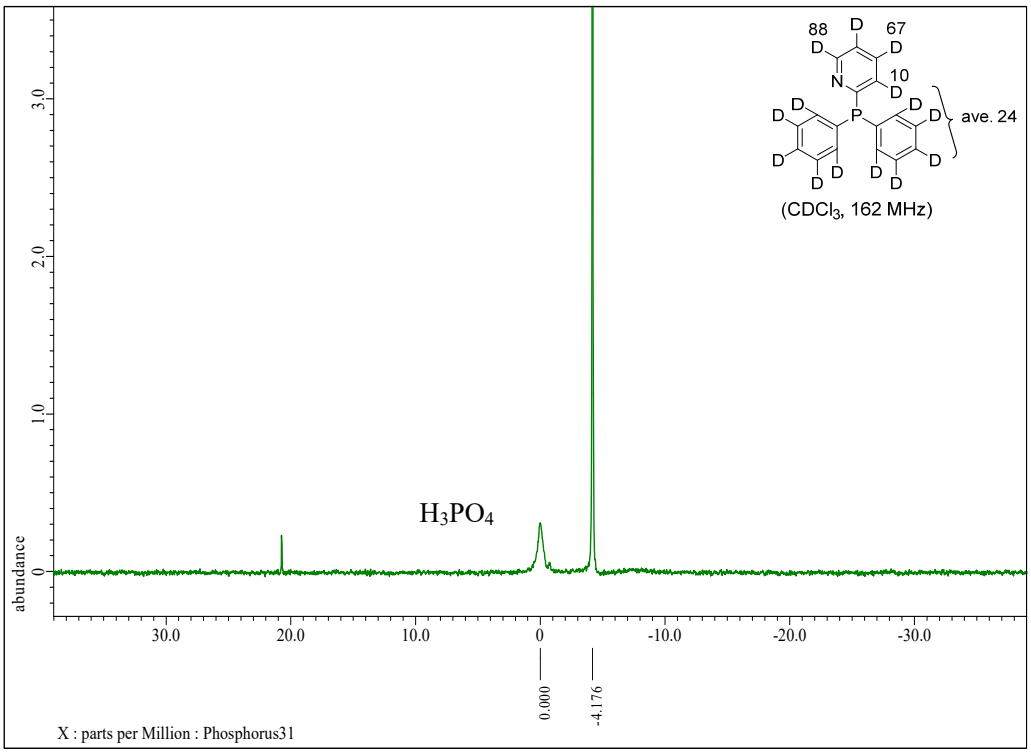
^{2}H NMR of diphenyl-2-pyridylphosphine-d_n (**8**-d_n)



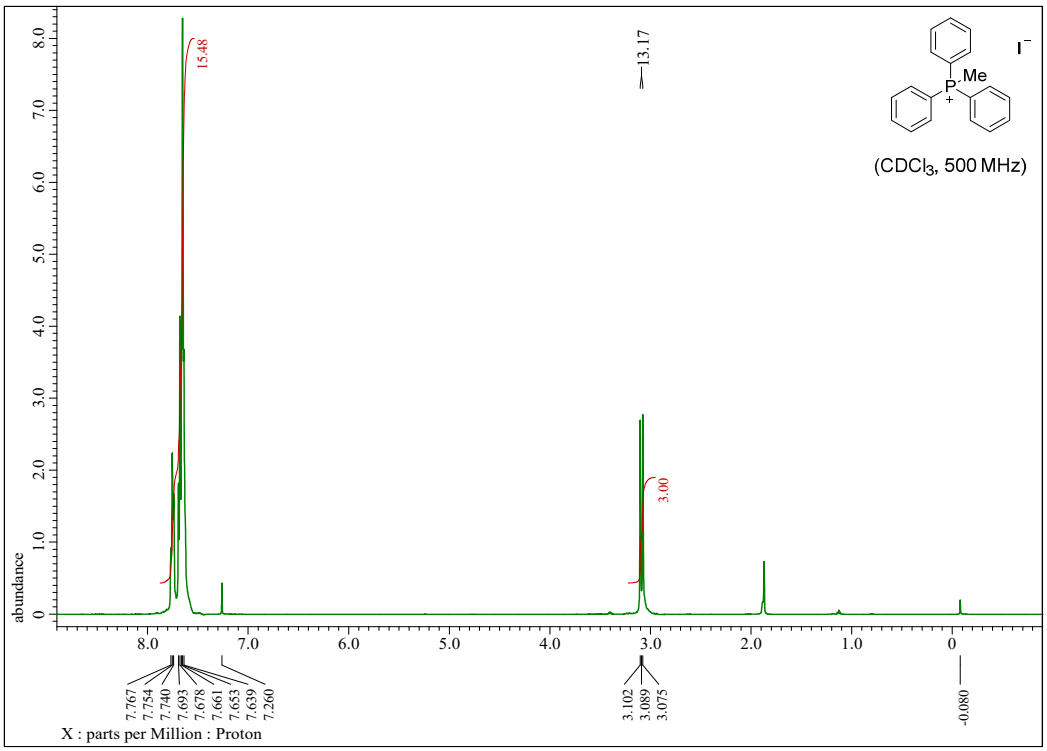
^{13}C NMR of diphenyl-2-pyridylphosphine-d_n (**8**-d_n)



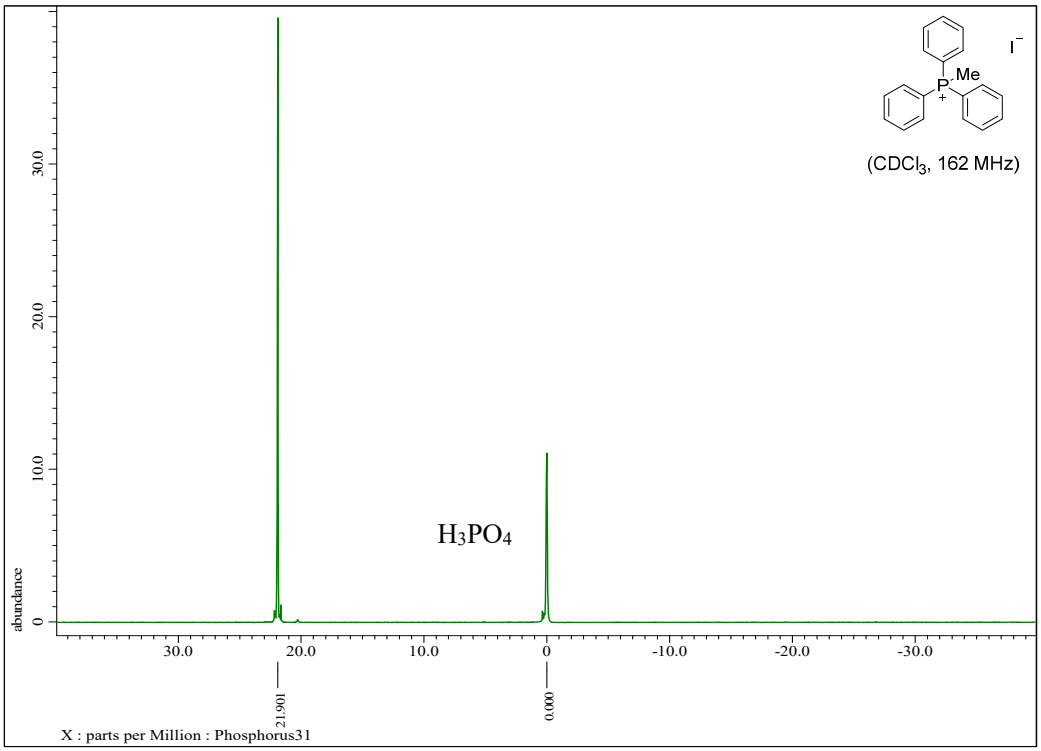
^{31}P NMR of diphenyl-2-pyridylphosphine-d_n (**8**-d_n)



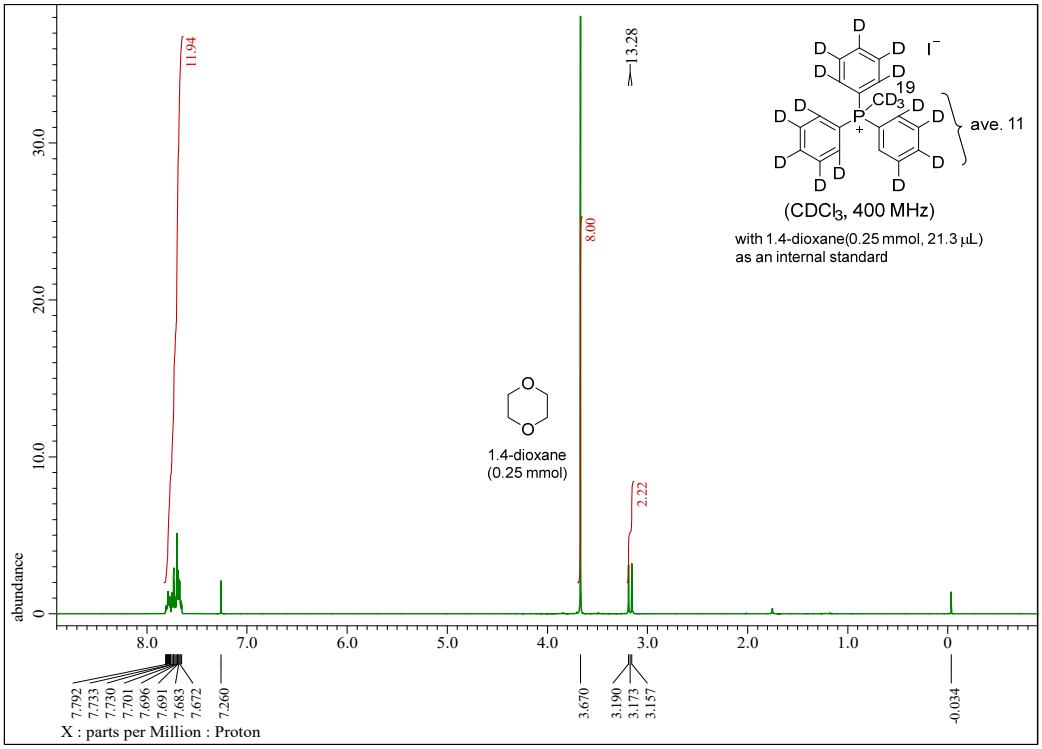
^{1}H NMR of methyl-triphenylphosphonium iodide (**9**)



^{31}P NMR of methyl-triphenylphosphonium iodide (**9**)



^{1}H NMR of methyl-triphenylphosphonium iodide-d_n (**9**-d_n)



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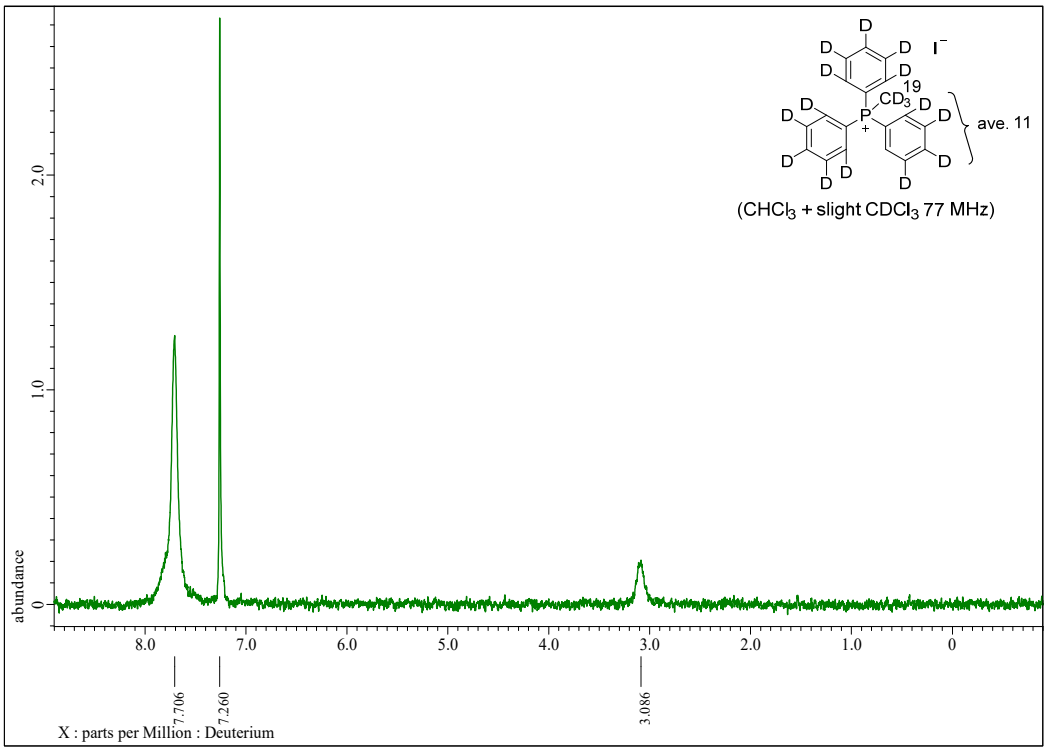
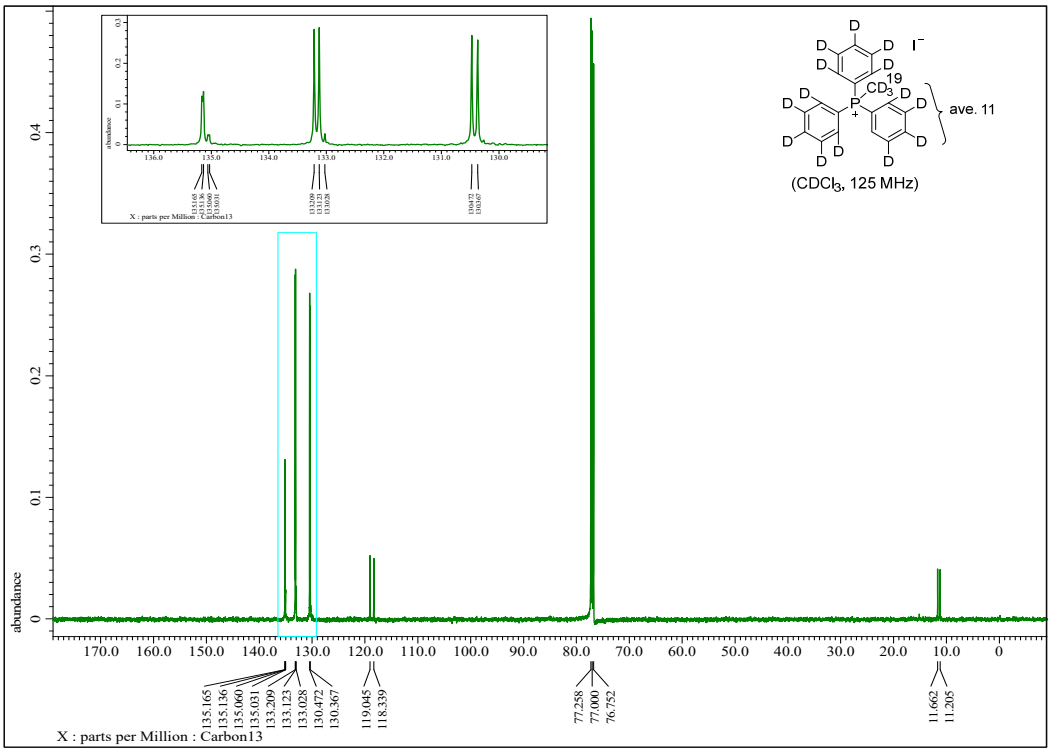
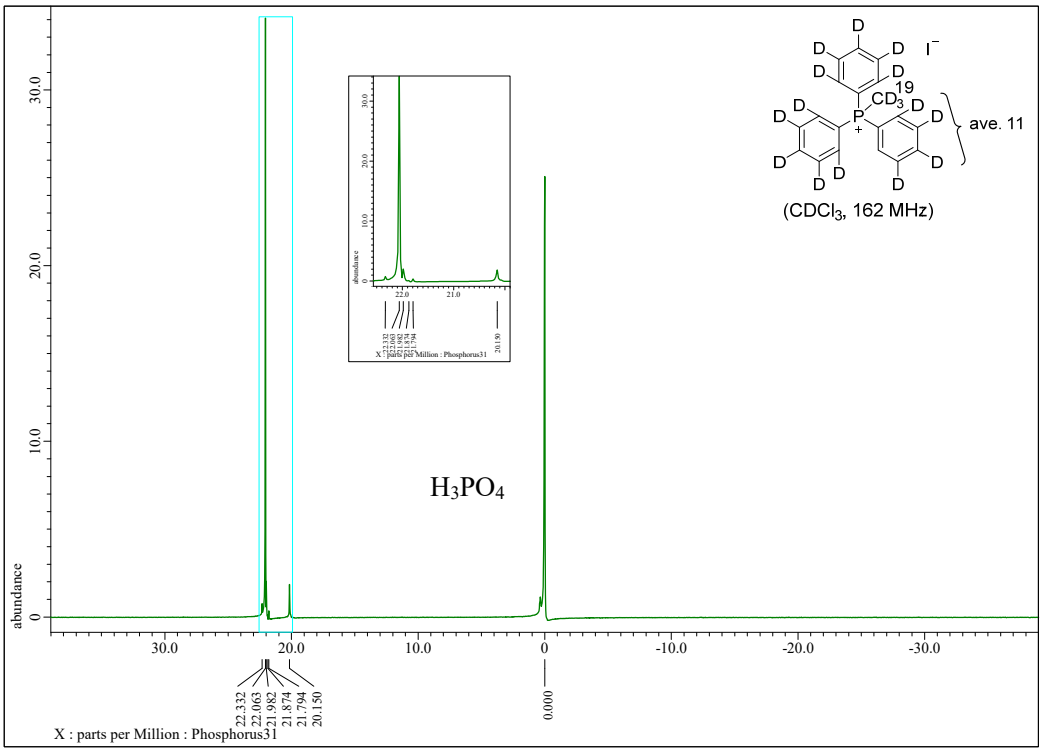


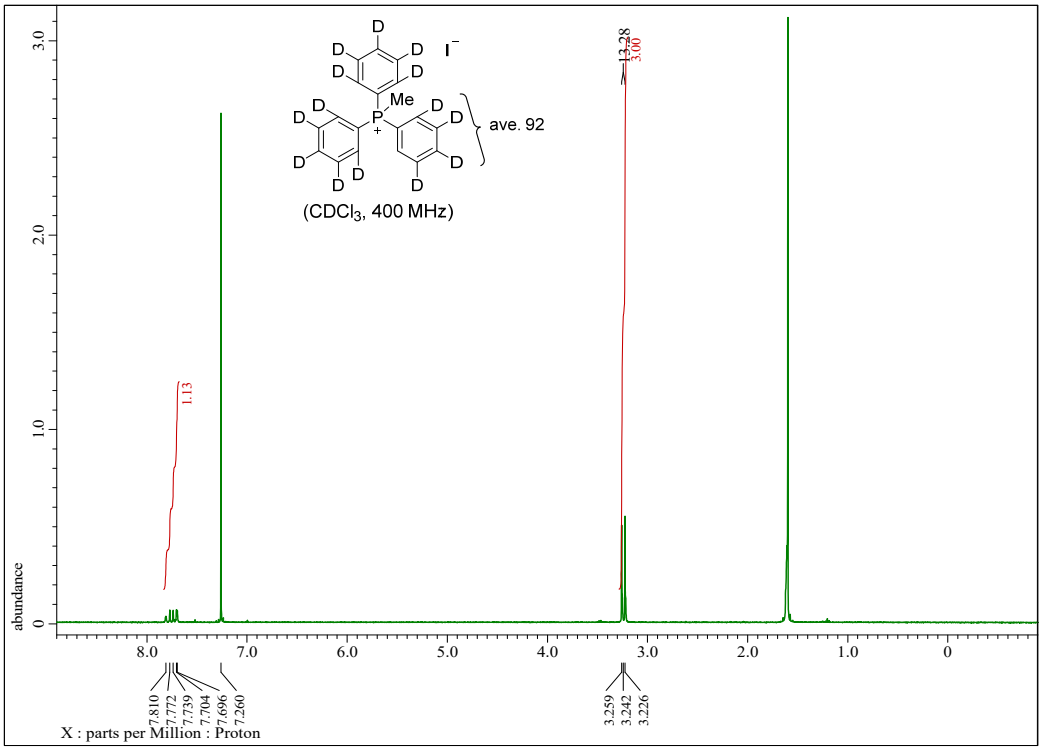
^{13}C NMR of methyl-triphenylphosphonium iodide-d_n (**9**-d_n)



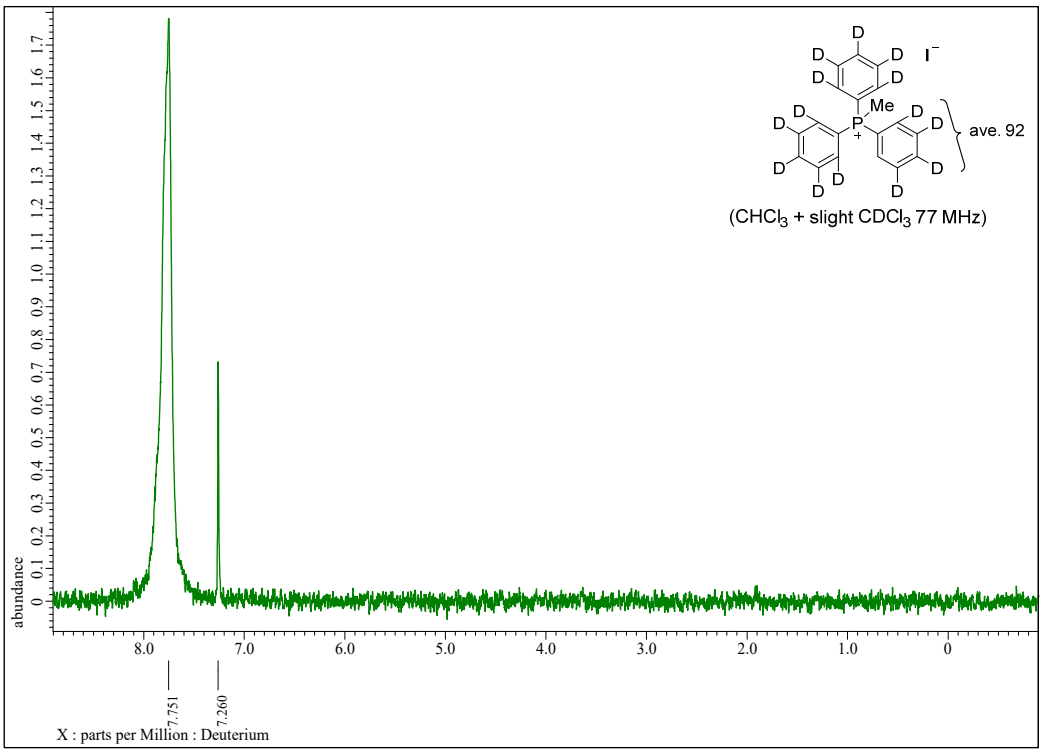
^{31}P NMR of methyl-triphenylphosphonium iodide-d_n (**9**-d_n)



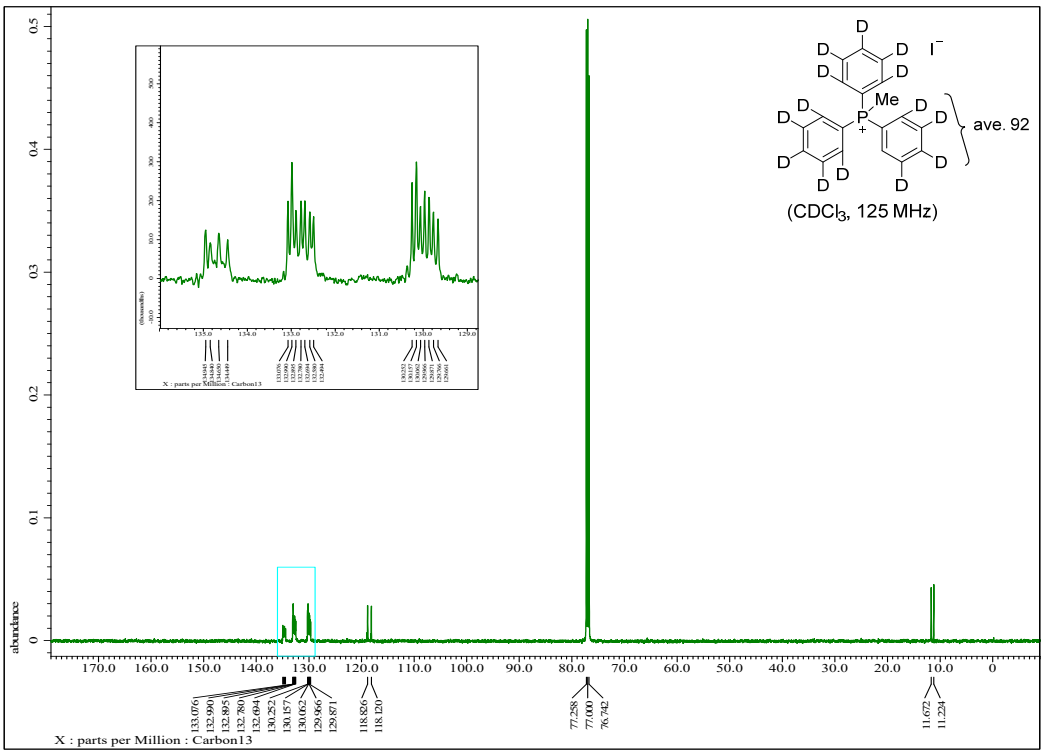
^{1}H NMR of methyl-triphenylphosphonium iodide-d_{15} (TPMP-d_{15}; Scheme 3)



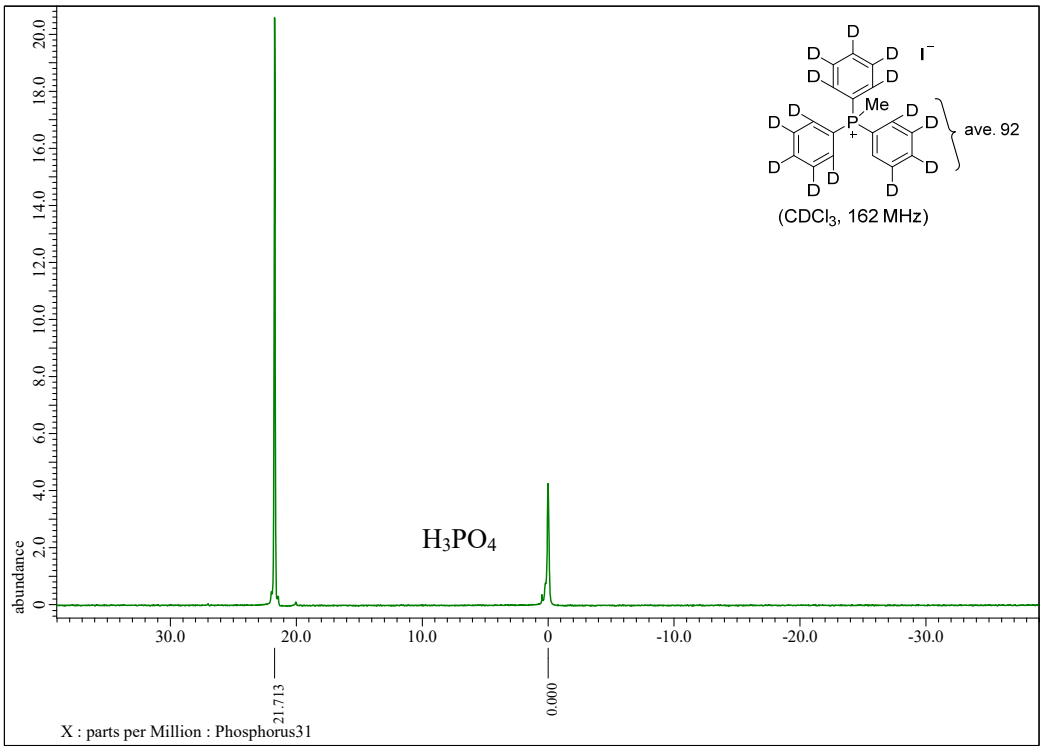
^{2}H NMR of methyl-triphenylphosphonium iodide-d_{15} (TPMP-d_{15}; Scheme 3)



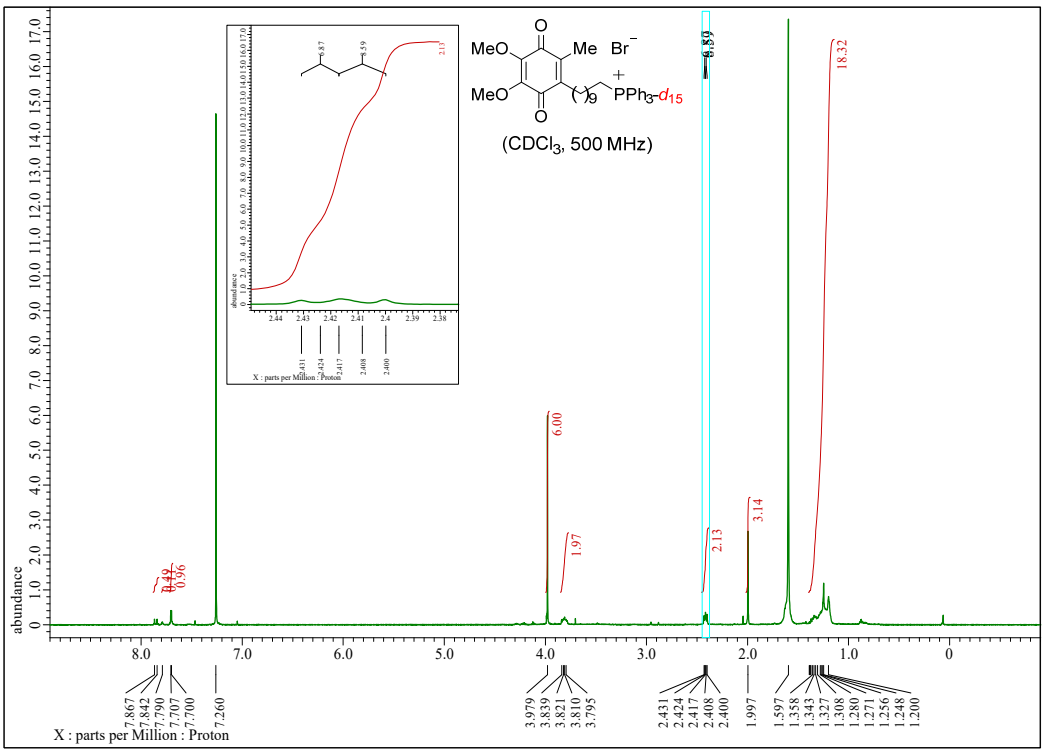
^{13}C NMR of methyl-triphenylphosphonium iodide-d_{15} (TPMP-d_{15}; Scheme 3)



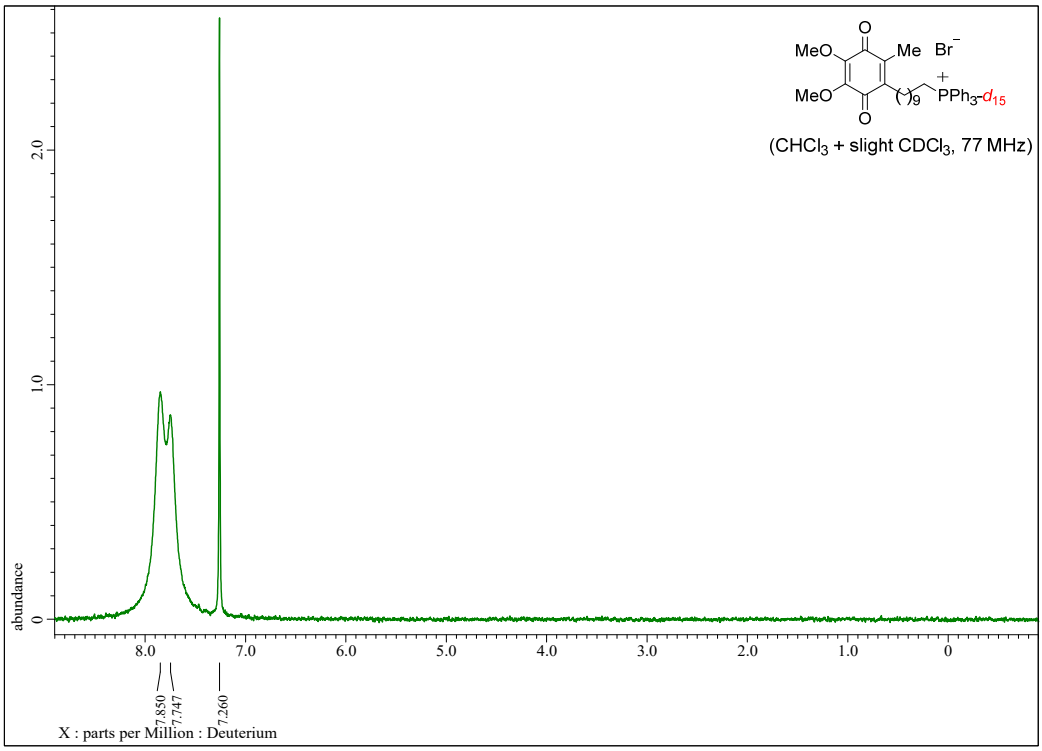
^{31}P NMR of methyl-triphenylphosphonium iodide-d_{15} (TPMP-d_{15}; Scheme 3)



^{1}H NMR of Mito-Q-d_n



^{2}H NMR of Mito-Q-d_n



9. References

1) The determination of D contents was performed according to the Supporting Information of literature, see; Y. Sawama, A. Nakano, T. Matsuda, T. Kawajiri, T. Yamada and H. Sajiki, *Org. Process Res. Dev.*, **2019**, *23*, 648−653.
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