

Supporting Information

Acyloxymethyl and alkoxy-carbonyloxymethyl prodrugs of a fosmidomycin surrogate as antimalarial and antibacterial agents.

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Biological Details

Antiplasmodial susceptibility testing²⁷.

Chloroquine resistant *P. falciparum*-K1 parasites were cultured in human erythrocytes (O+) at 37 °C under a low oxygen atmosphere (3% O₂, 4% CO₂, and 93% N₂) in RPMI-1640, supplemented with 10% human serum. Infected human red blood cells (200 µL, 1% parasitaemia, 2% haematocrit) were added to each well and incubated for 72 h. After incubation, test plates were frozen at -20°C. Parasite multiplication was measured by the Malstat method. One hundred microliters of Malstat™ reagent was transferred in a new plate and mixed with 20 µL of the hemolysed parasite suspension for 15 min at room temperature. Then, 20 µL of nitro blue tetrazolium chloride (NBT) at 2 mg/mL/PES at 0.1 mg/mL solution was added and the plate was incubated again for 2 h at room temperature in the dark. Absorbance was read at 655 nm in a Biorad 3550-UV microplate reader. As a positive control, chloroquine was included (IC₅₀ = 0.13 µM).

Antitubercular susceptibility testing²⁸.

In vitro antimycobacterial activity was evaluated by a colorimetric resazurin microtiter assay based on a *M. tuberculosis* H37Rv laboratory strain. Volumes of 100 µL of 7H9-Supplemented broth (Middlebrook 7H9 broth + 10% OADC + 0.5% glycerol + 0.1% casitone) were dispensed in each well of a sterile, flat-bottomed 96-well plate. A 2-fold serial dilution of each compound was made directly in the plate with final concentrations ranging from 50 µM to 3.12 µM. Bacterial isolates were freshly subcultured on LJ medium. The inoculum was prepared in 7H9-S broth, adjusted spectrophotometrically to a no. 1 McFarland tube standard, and further diluted 1:10 in 7H9-S broth for the test. A volume of 100 µL of bacteria was added to each well. A growth control (medium and bacteria), a drug control (medium and compound) and a sterile control (only medium) were also included. All of the outer-perimeter wells were filled with 200 µL of sterile deionized water to minimize evaporation of the medium in the test wells during incubation. The plate was subsequently covered, sealed in a plastic bag, and incubated at 37°C under a normal atmosphere. After 7 days of incubation, 30 µL of resazurin solution was added to each well, and the plate was reincubated overnight. A change in color from blue to pink indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in color. Bacterial replication was analyzed by visual reading.

In vitro cytotoxicity assay¹.

The in vitro cytotoxicity on the MRC-5 *Homo sapiens* long fibroblast cell line (ATCC® CCL-171™) was assessed for each analogue by a resazurin-based cytotoxicity assay. Briefly, the MRC-5 cells were cultured in 75 cm² sterile Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum in a 5% CO₂ atmosphere at 37°C. When a semi-confluent layer of cells was formed, the cells were trypsinized, washed with sterile PBS, seeded into a transparent, flat-bottomed 96-well plate at a density of 4 x 10⁴ cells per well and left for recovery at 37°C, 5% CO₂ for at least 24 hours. For each compound, a two-fold serial dilution was made in complete DMEM with final concentrations ranging from 128 µM to 0.5 µM. Subsequently, the MRC-5 cells were exposed to the compounds by adding 100 µL of the serial dilutions to the wells. Test plates were incubated for 24 hours in an atmosphere of 5% CO₂ at 37°C. For the resazurin assay, the cells were washed 2 times with 200 µL PBS and 100 µL resazurin working solution was added per well. Subsequently, the plates were left for incubation at 37°C, 5% CO₂ for 3 hours. The irreversible reduction of resazurin to resorufin is proportional to aerobic respiration and the quantity of resorufin produced is proportional to the number of viable cells. To monitor the viable cell number after compound exposure, each well was analyzed using a microplate fluorometer equipped with a 560 nm excitation / 590 nm emission filter set. Tamoxifen was included as positive control (IC₅₀ = 11.09 µM).

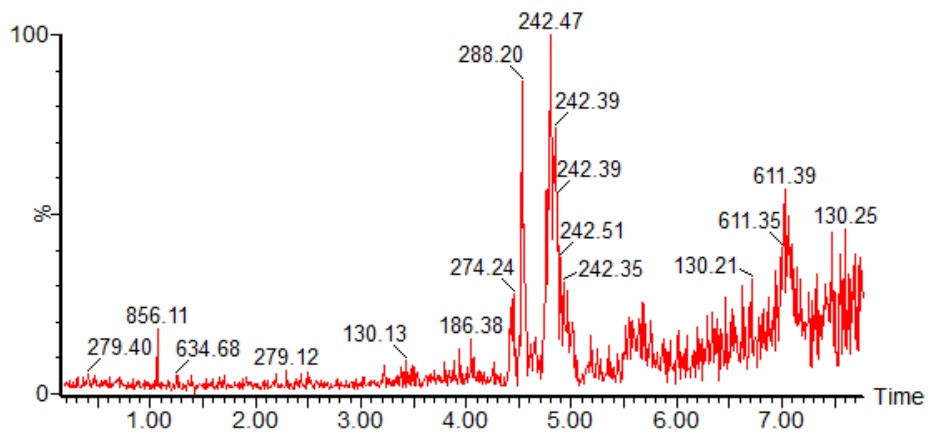
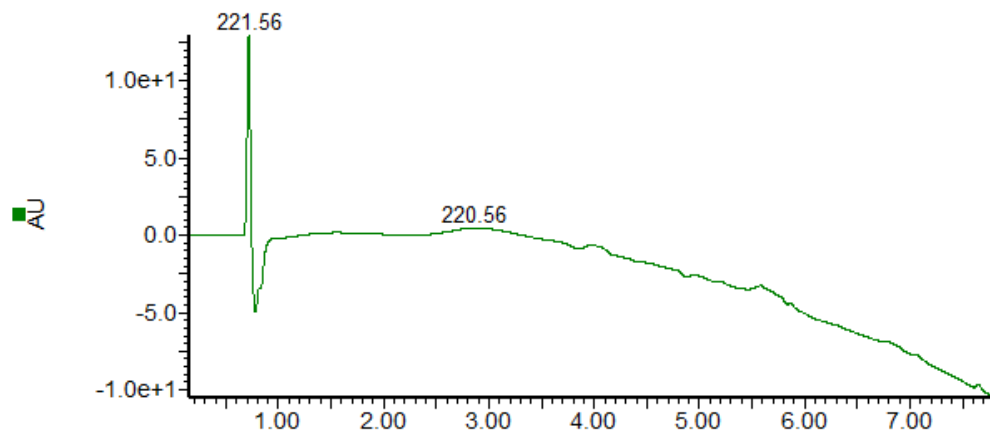
References

- (1) Cos, P.; Vlietinck, A. J.; Berghe, D. V.; Maes, L. Anti-Infective Potential of Natural Products: How to Develop a Stronger in Vitro 'Proof-of-Concept.' *J. Ethnopharmacol.* **2006**, *106* (3), 290–302. <https://doi.org/10.1016/j.jep.2006.04.003>.
- (2) Martin, A.; Camacho, M.; Portaels, F.; Palomino, J. C. Resazurin Microtiter Assay Plate Testing of Mycobacterium Tuberculosis Susceptibilities to Second-Line Drugs: Rapid, Simple, and Inexpensive Method. *Antimicrob. Agents Chemother.* **2003**, *47* (11), 3616–3619. <https://doi.org/10.1128/AAC.47.11.3616-3619.2003>.

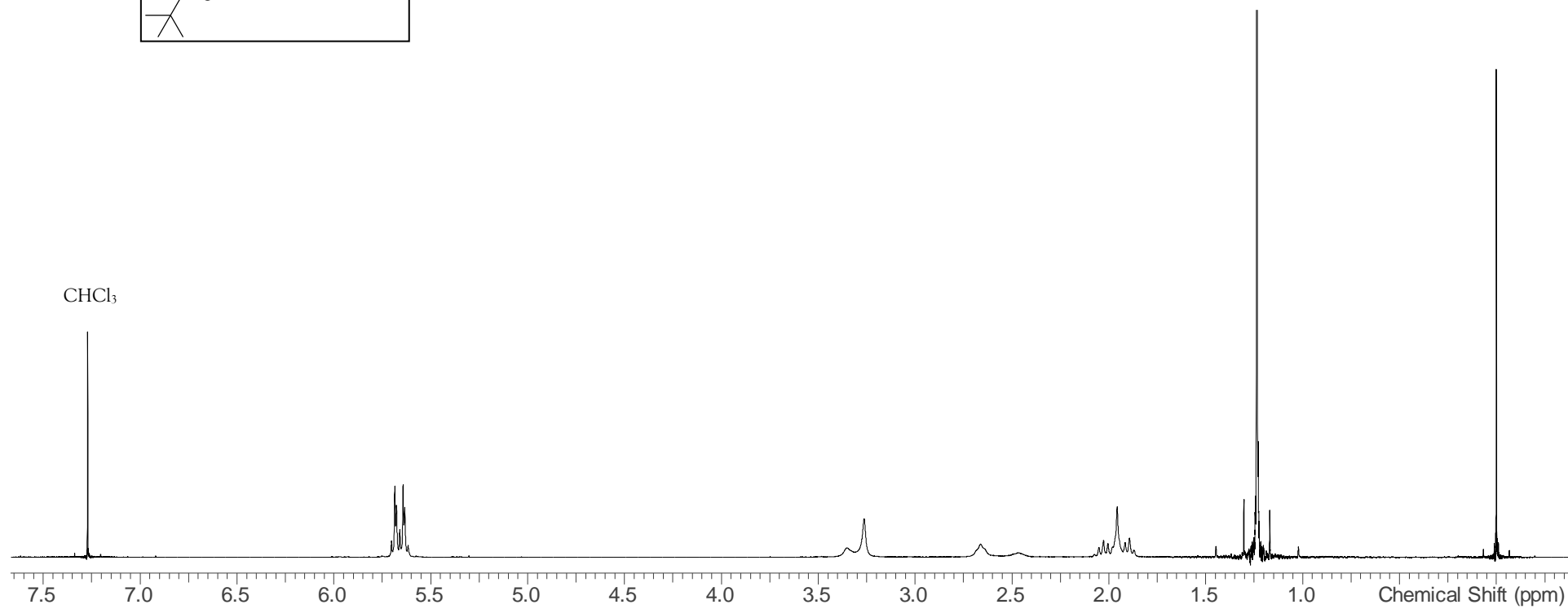
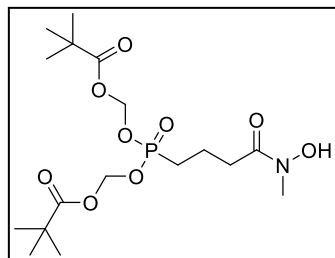
Purity confirmation of final compounds

For the final compounds described in this manuscript, LC-MS analyses are provided to confirm purity. LC-MS analyses were carried out on a Waters AutoPurification System equipped with PDA and ESI-MS detection and using a Waters CORTECS C18 Column (4.6×100 mm, 2.7 μm) and a water/acetonitrile/formic acid linear gradient system at a flow rate of 1.44 mL/min.

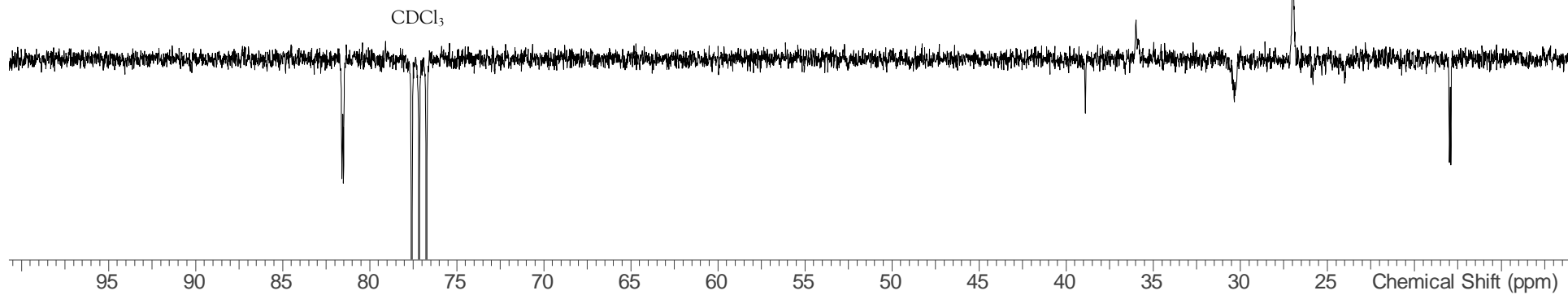
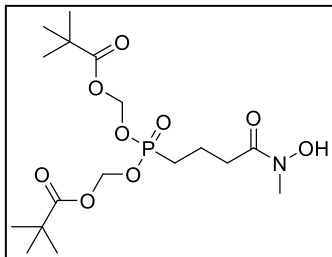
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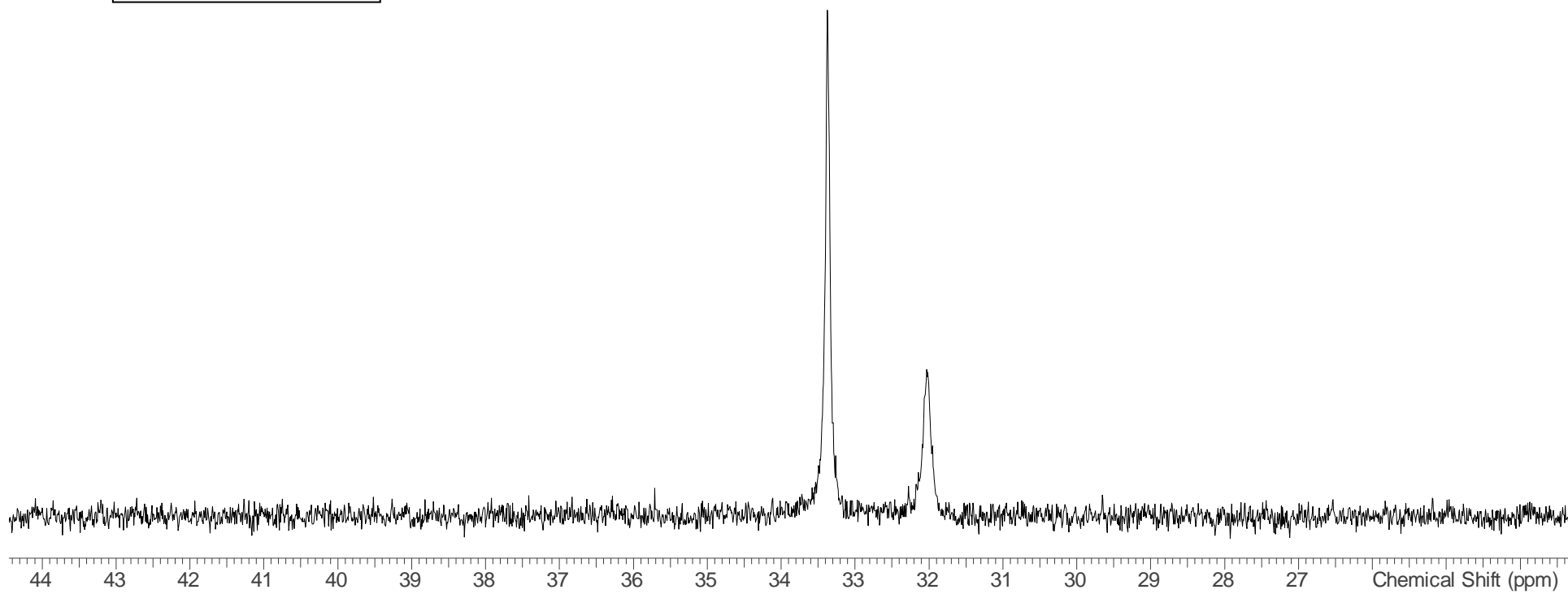
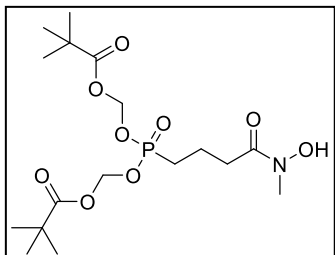
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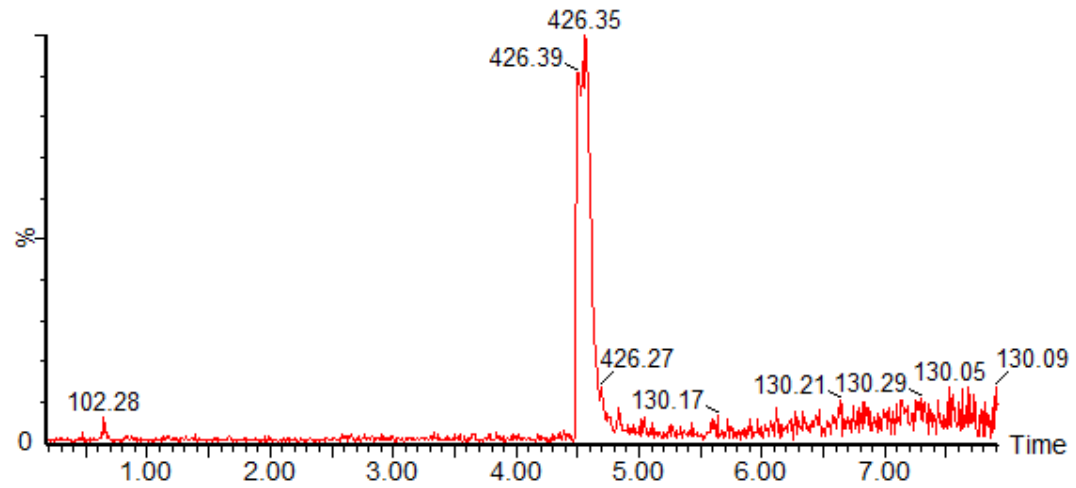
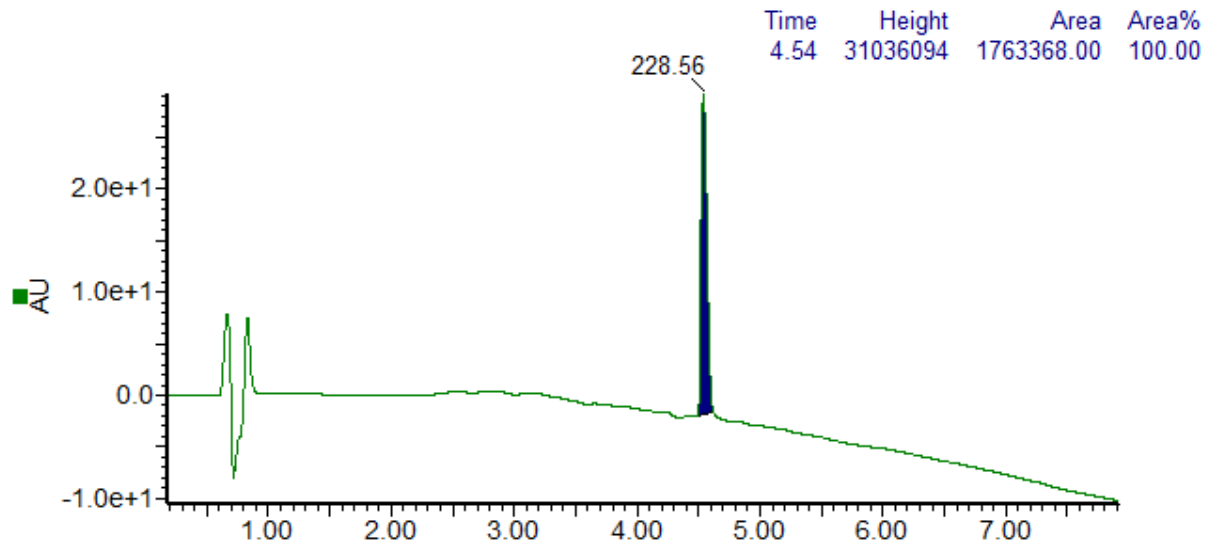
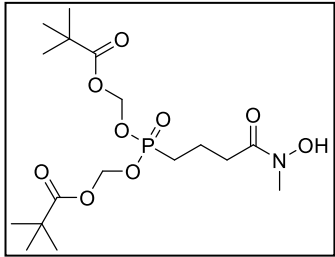


APT NMR spectrum of 13a (75MHz, CHLOROFORM-d)

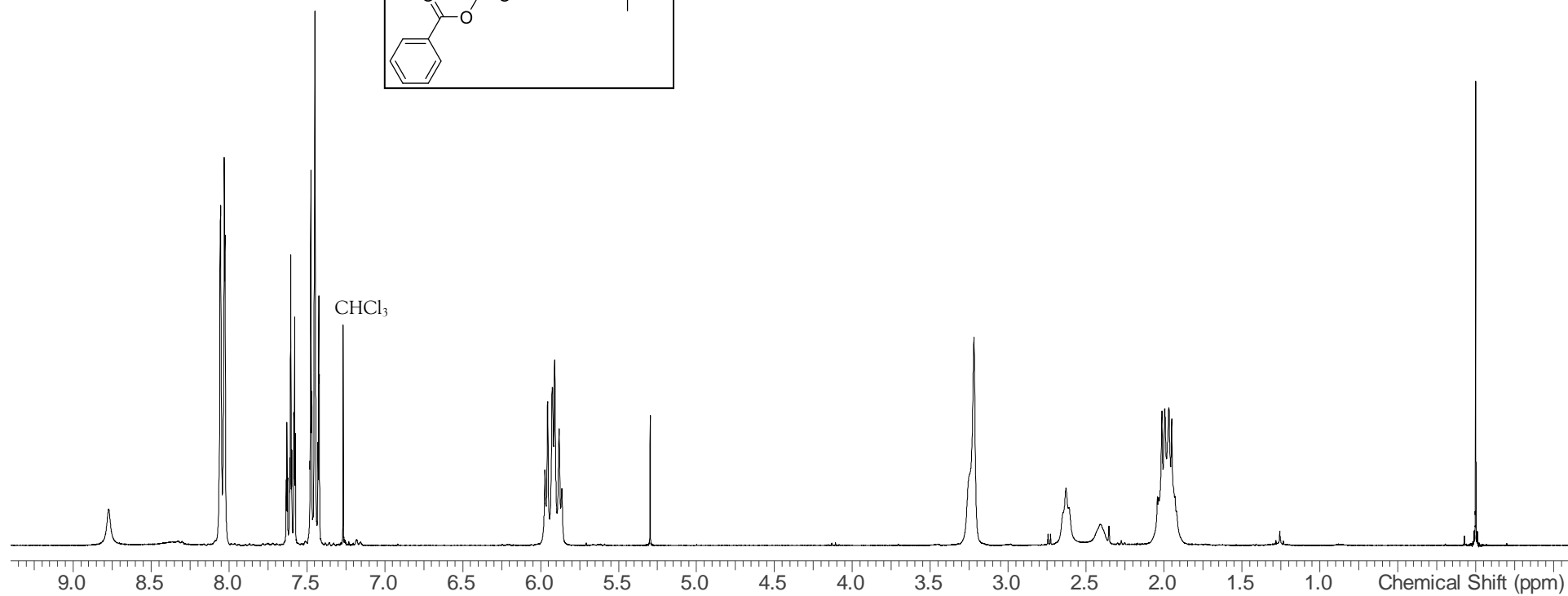
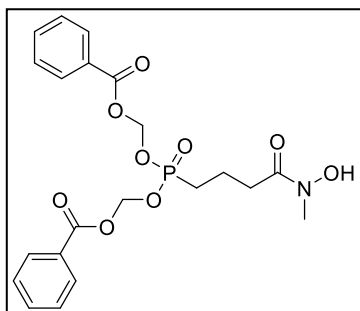


^{31}P NMR spectrum of 13a (121MHz, CHLOROFORM-*d*)

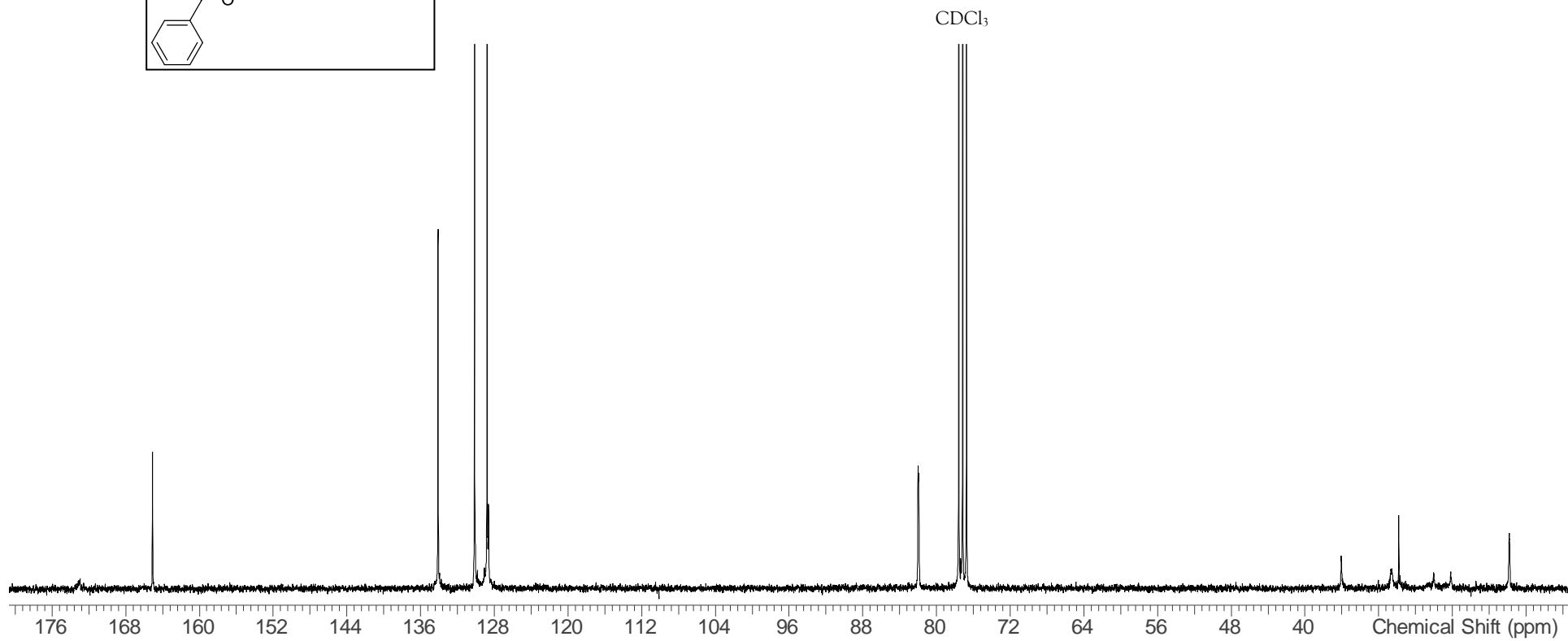
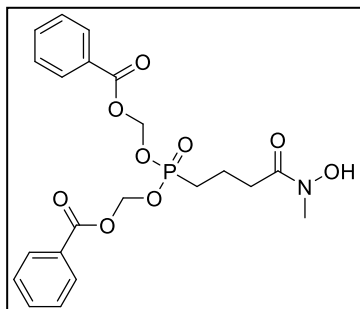




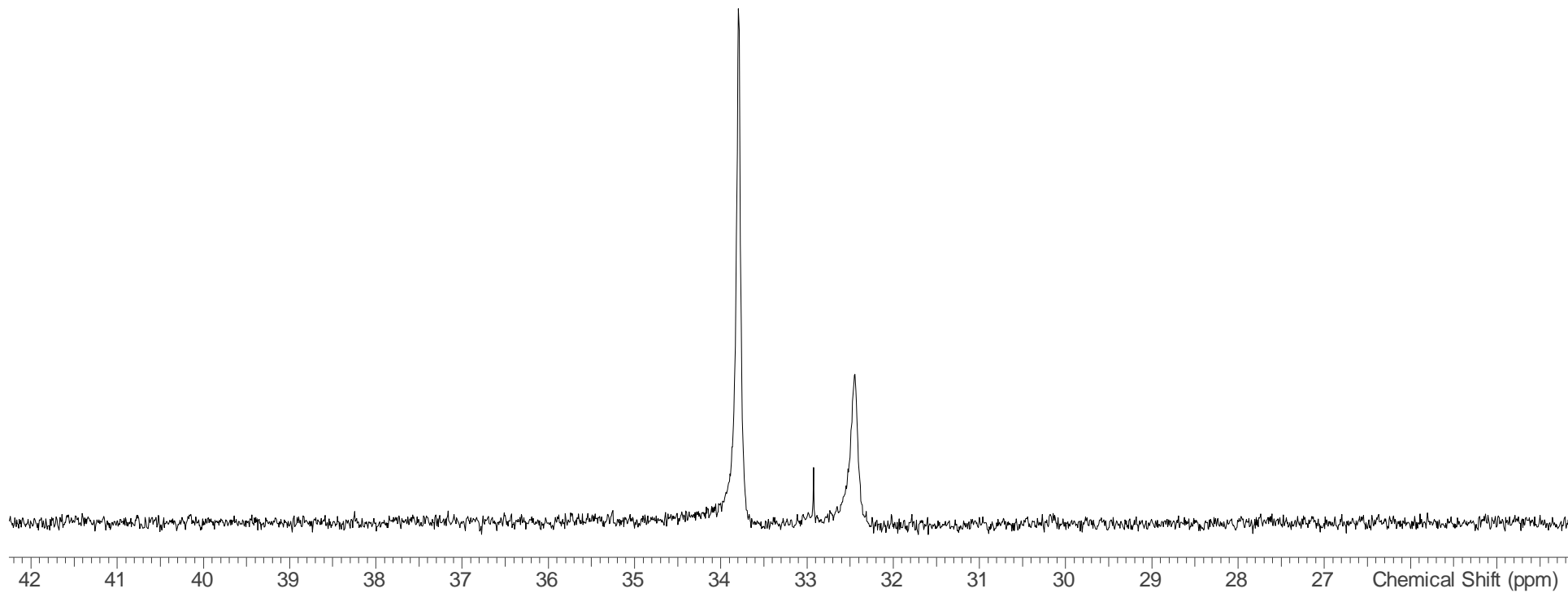
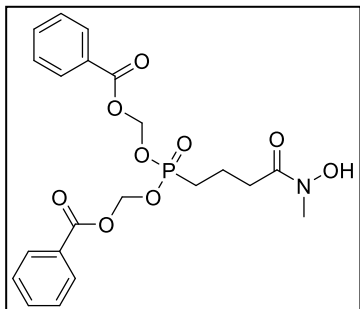
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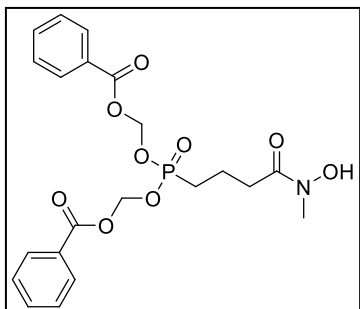


^{13}C NMR spectrum of **13b** (75MHz, CHLOROFORM-*d*)

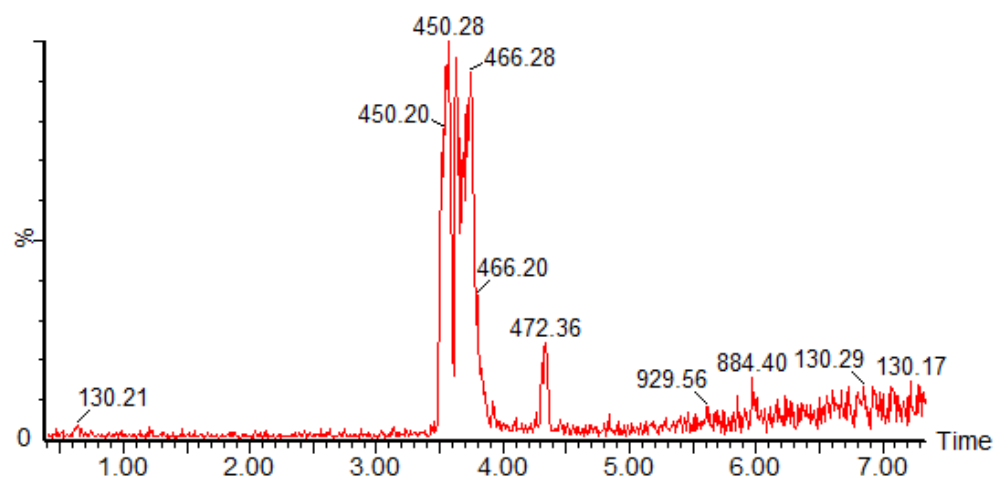
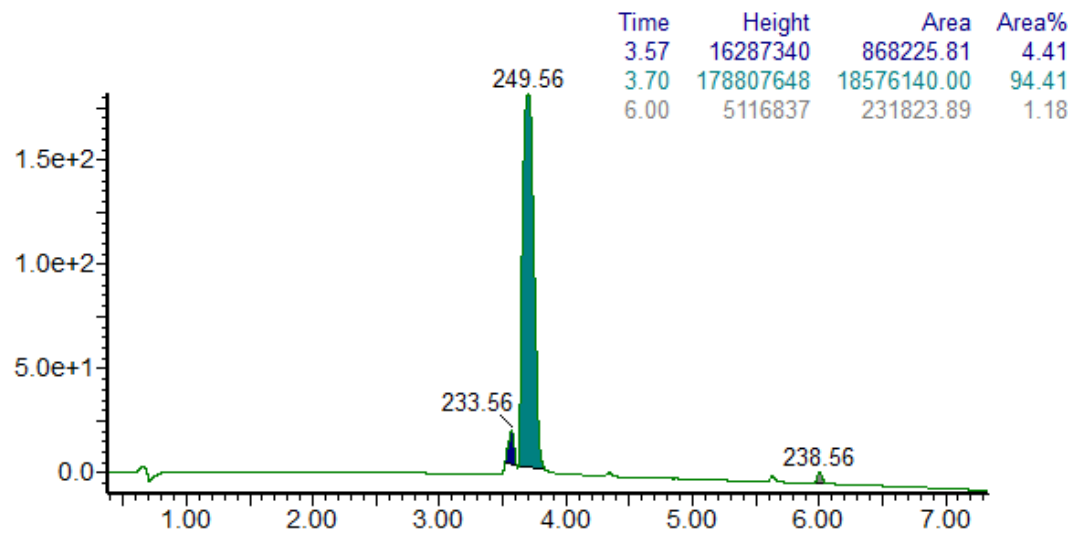


^{31}P NMR spectrum of **13b** (121MHz, CHLOROFORM-*d*)

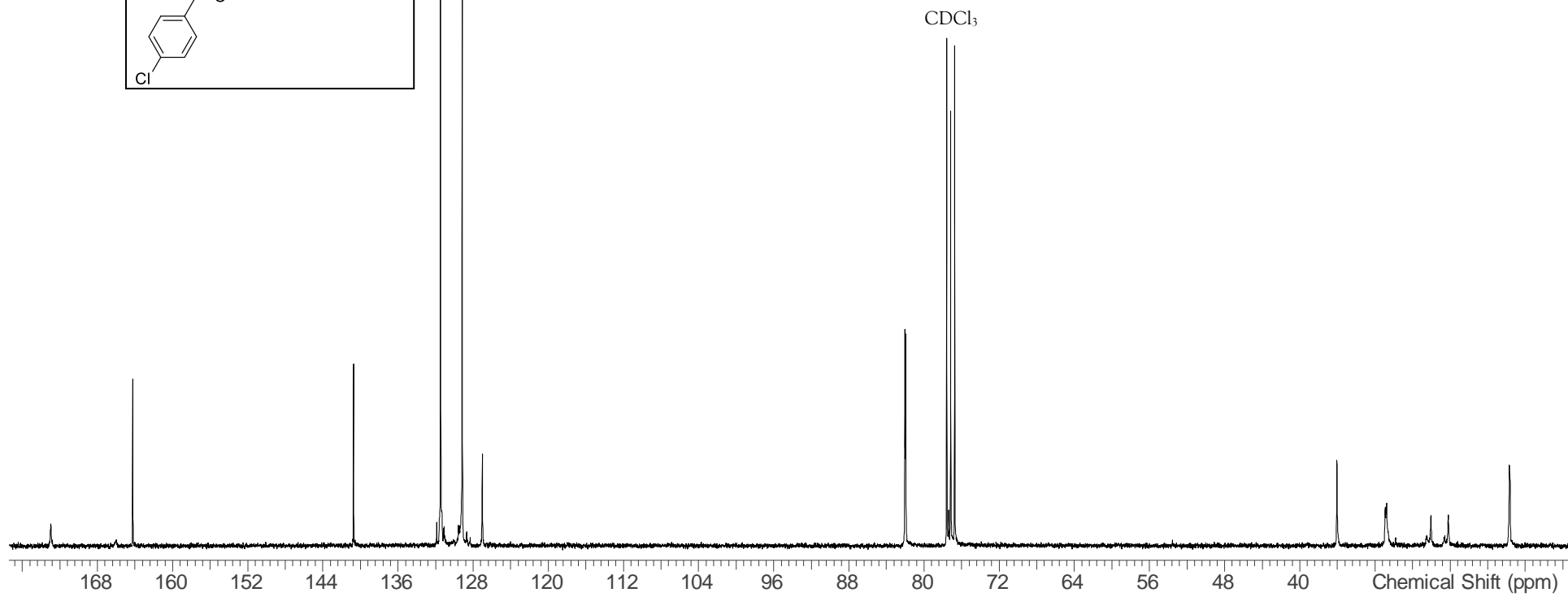
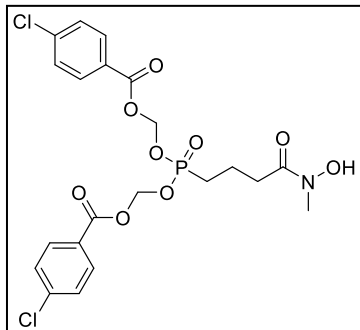




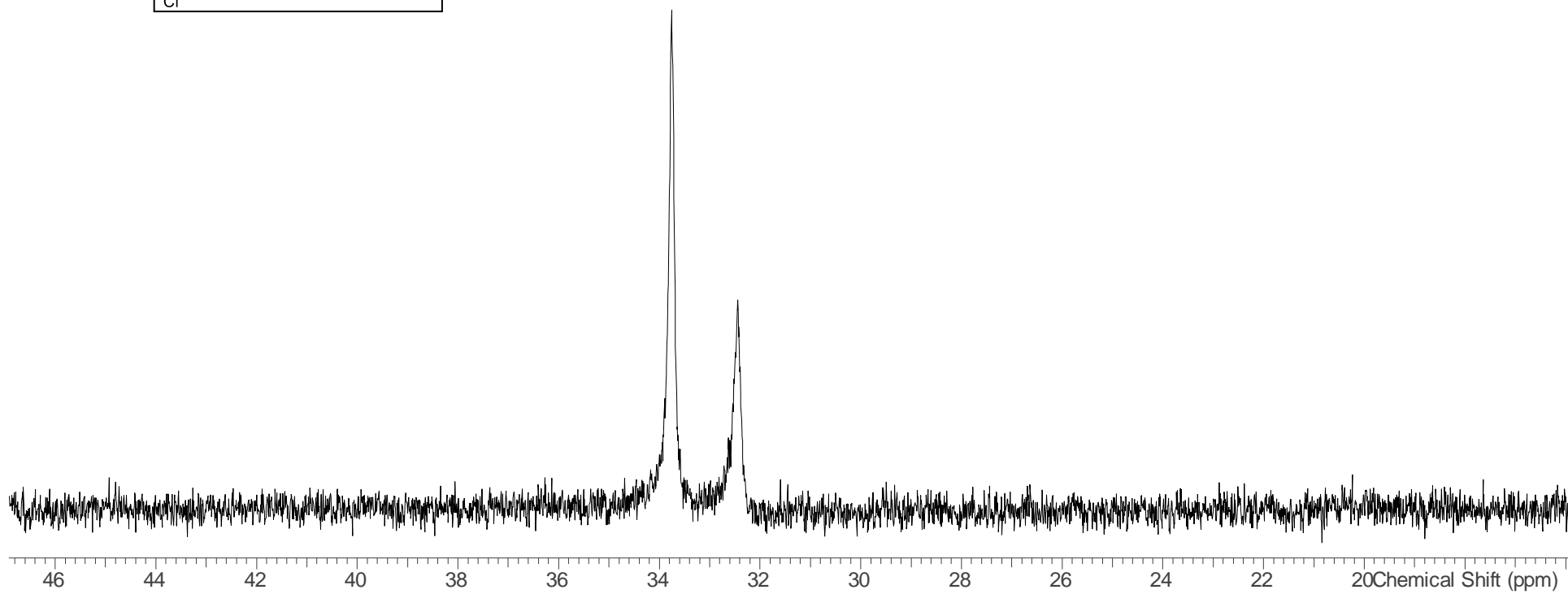
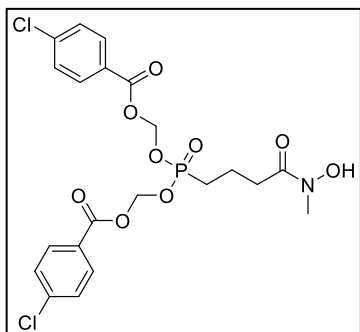
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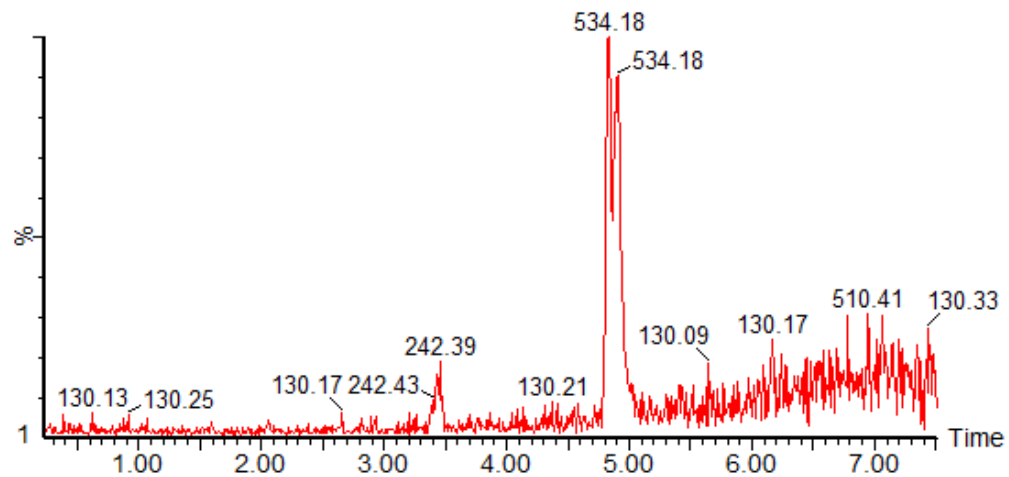
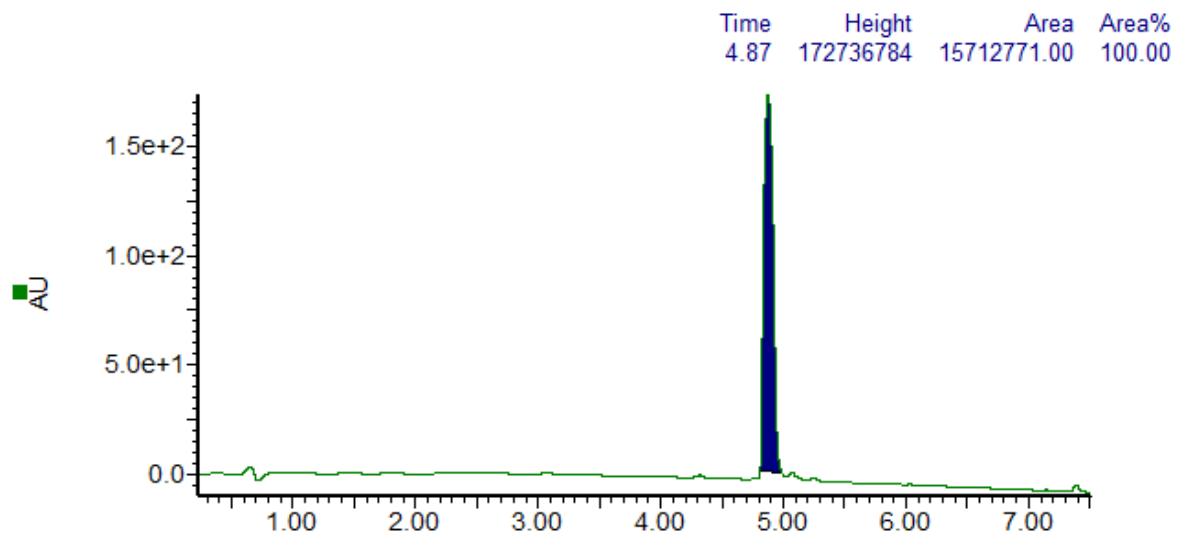
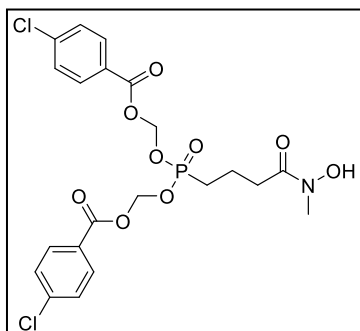


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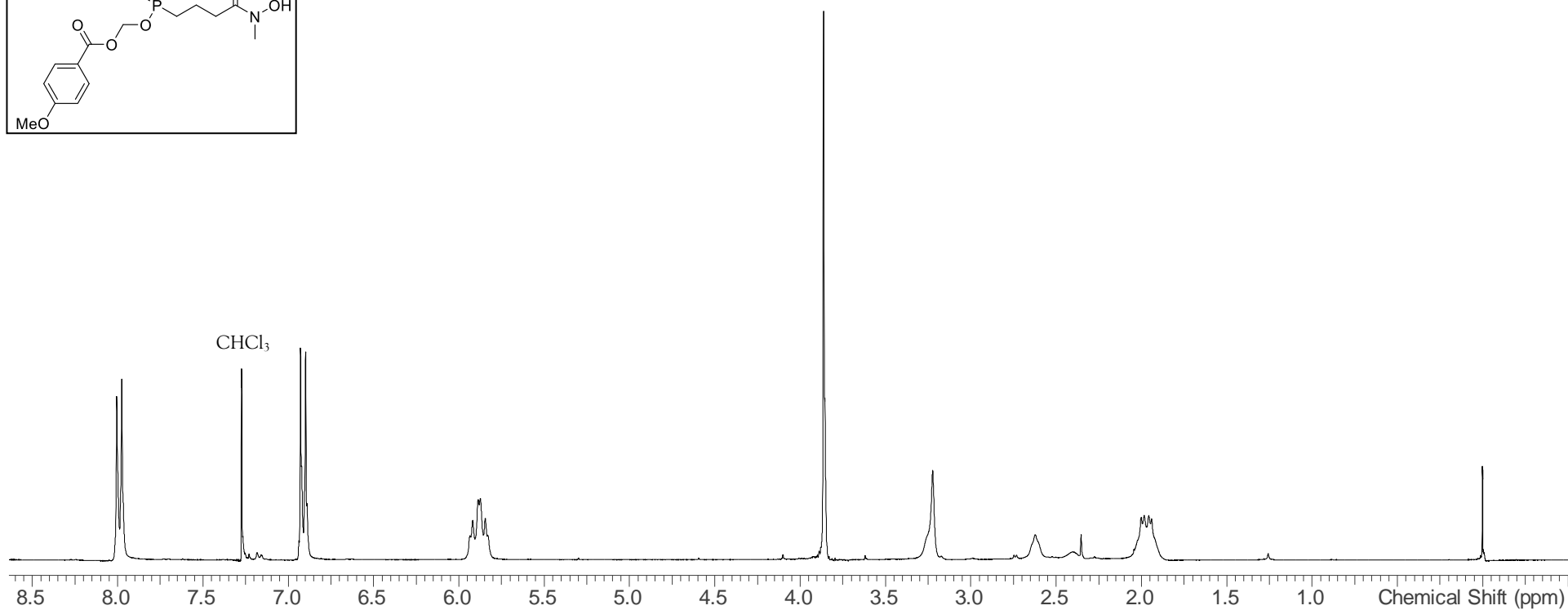
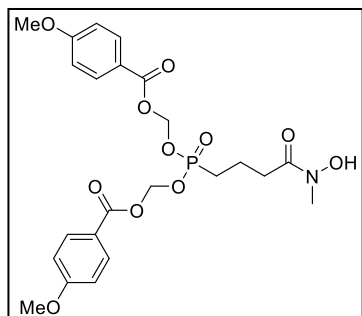


^{31}P NMR spectrum of 13c (121MHz, CHLOROFORM-*d*)

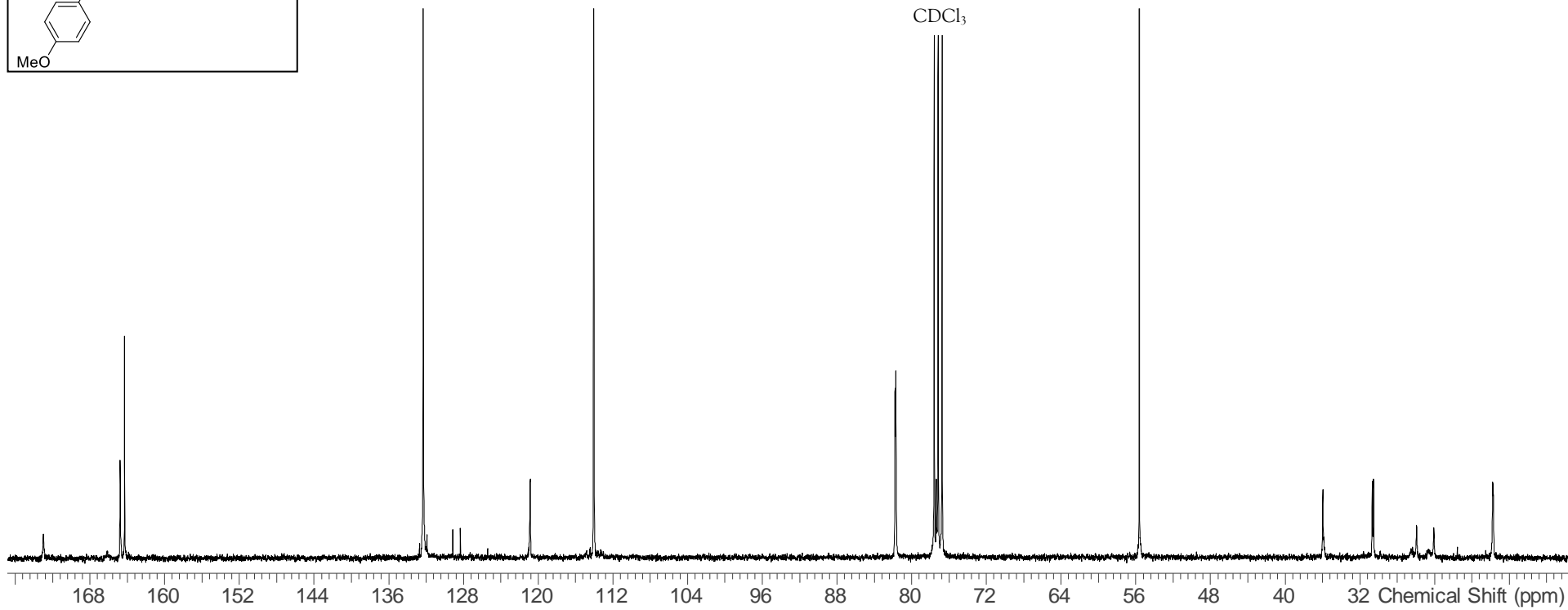
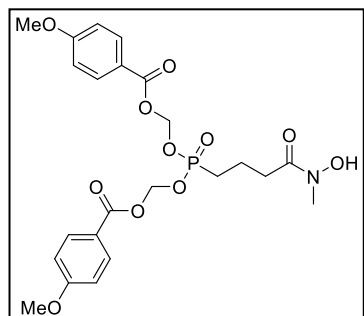




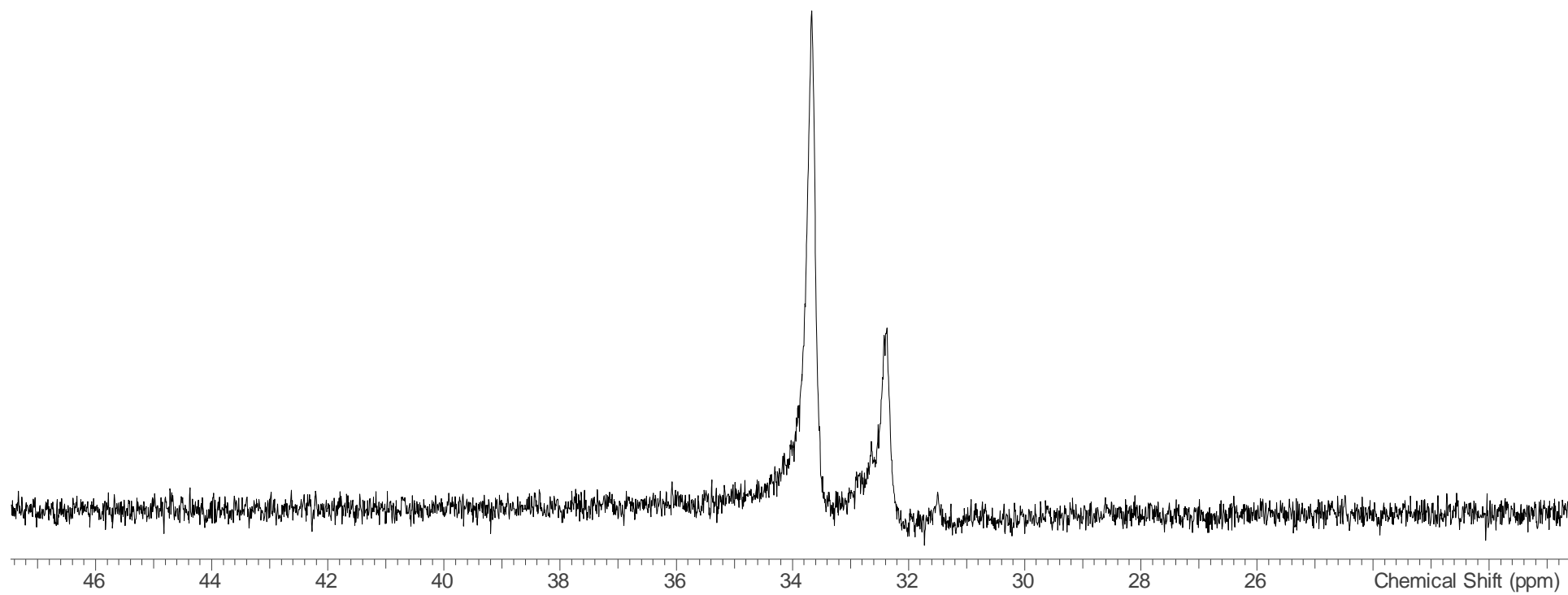
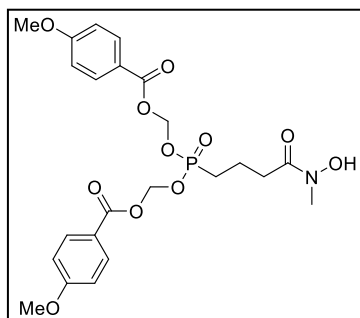
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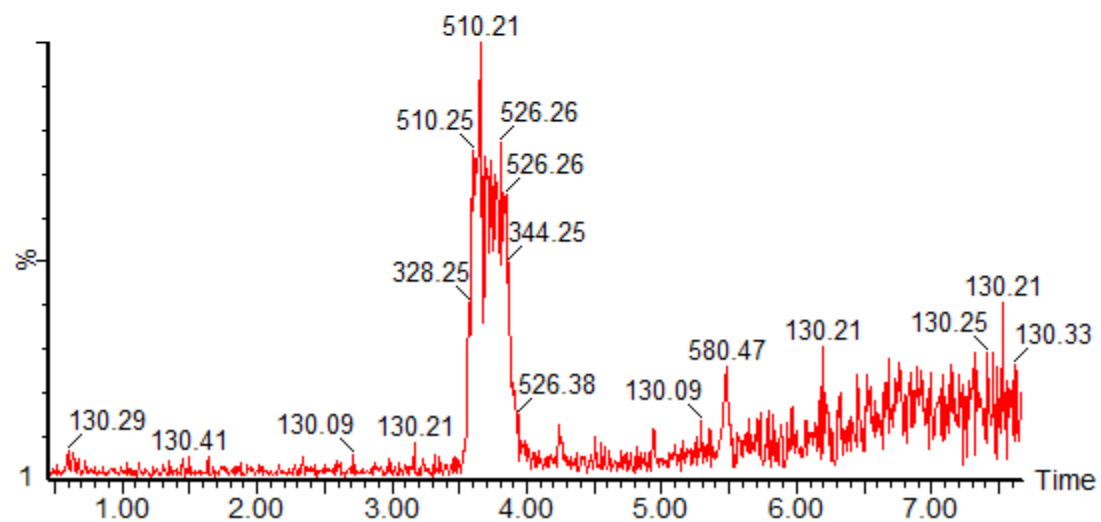
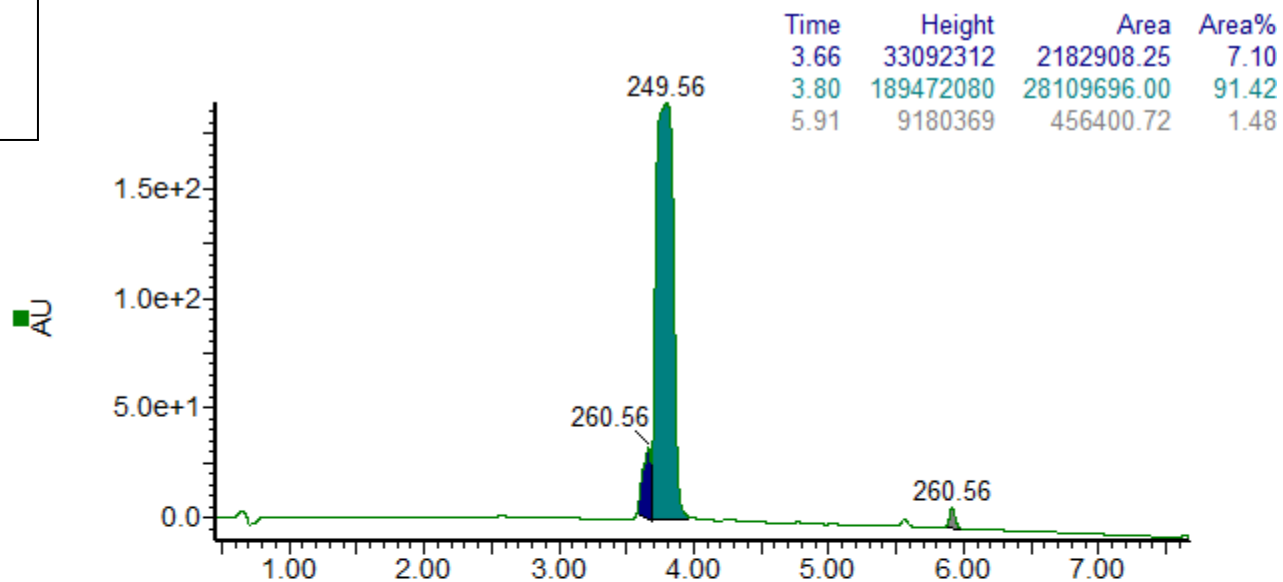
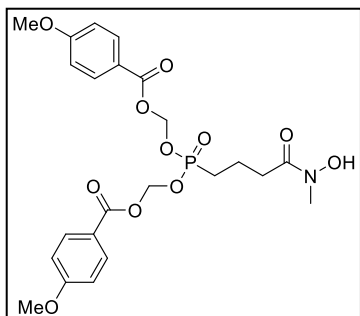


^{13}C NMR spectrum of **13d** (75MHz, CHLOROFORM-*d*)

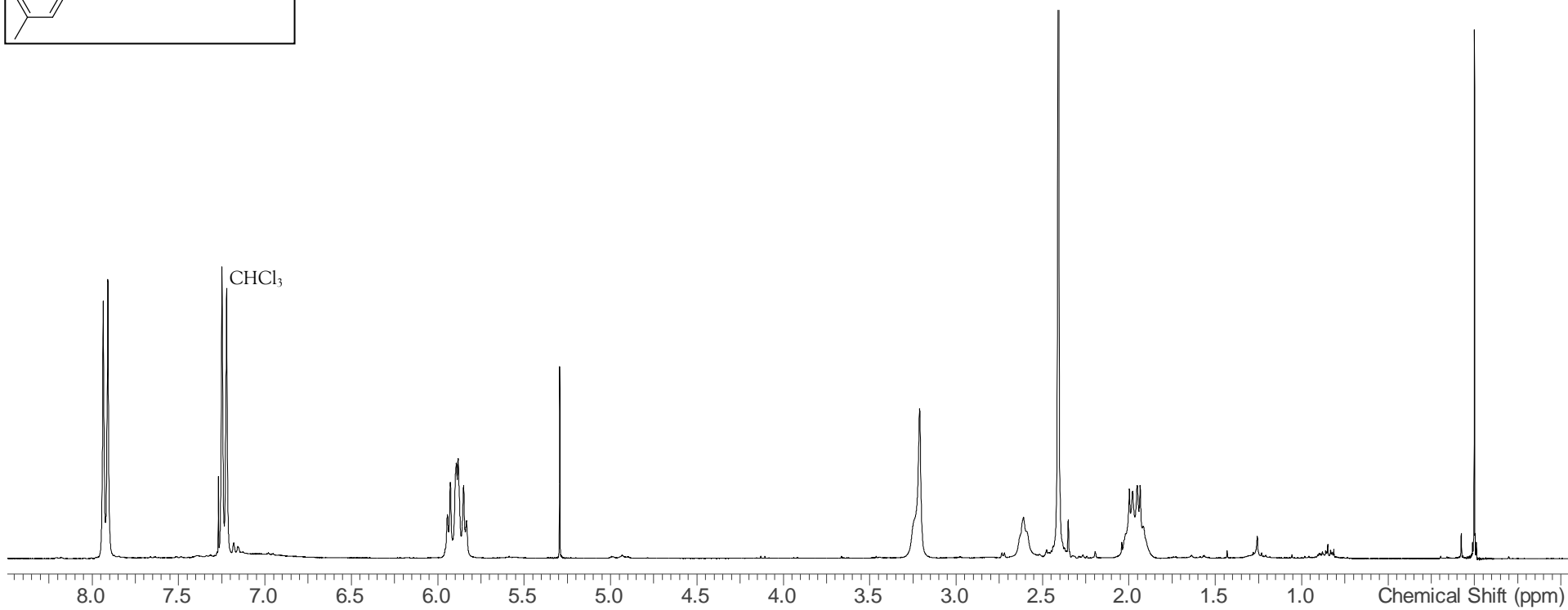
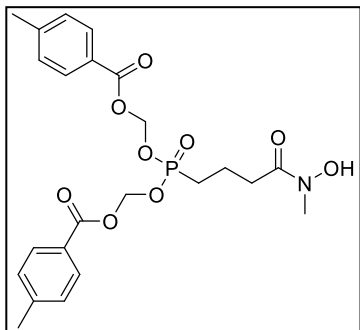


^{31}P NMR spectrum of **13d** (121MHz, CHLOROFORM-*d*)

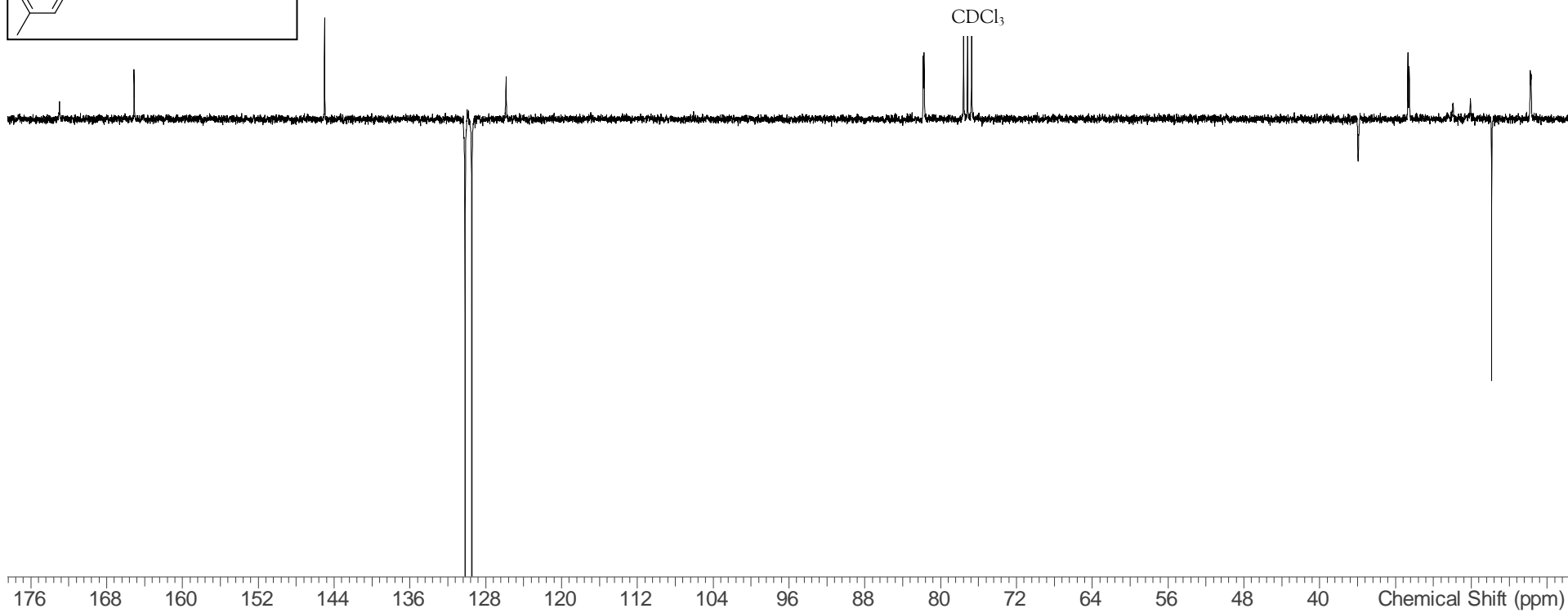
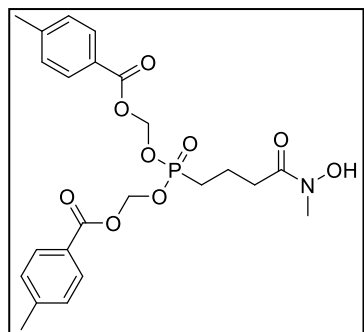




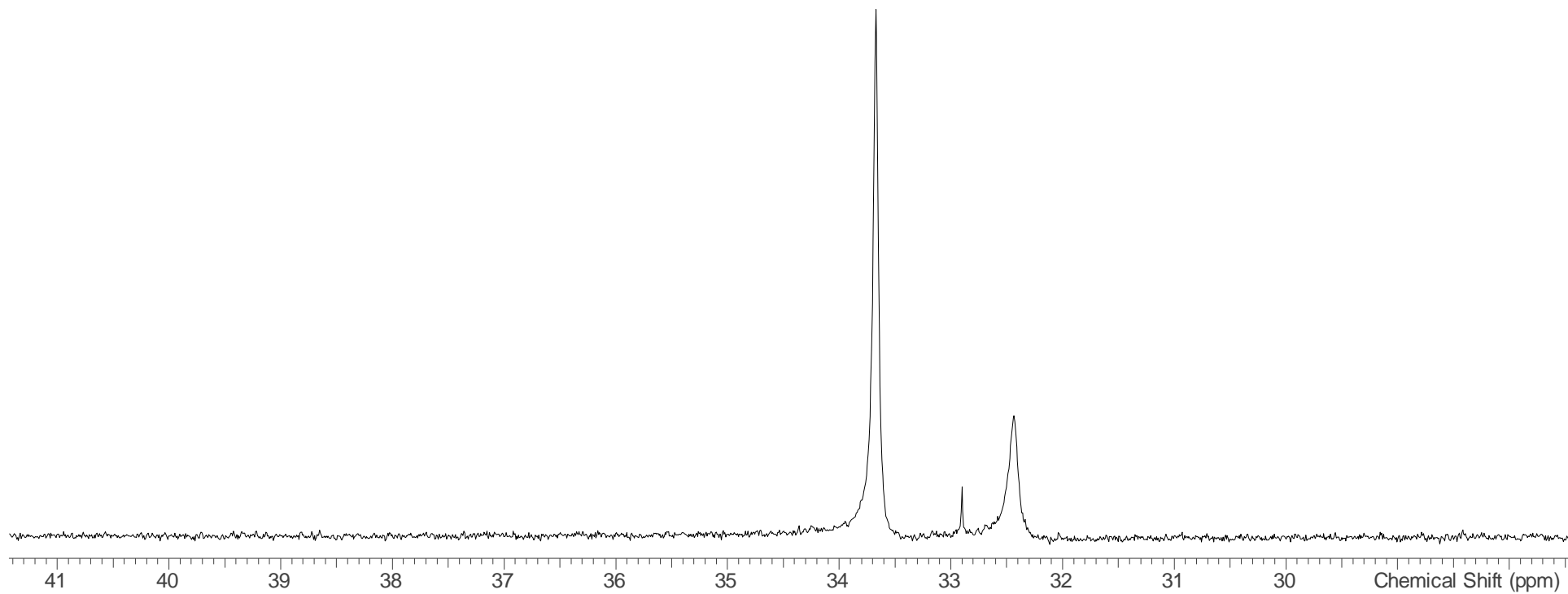
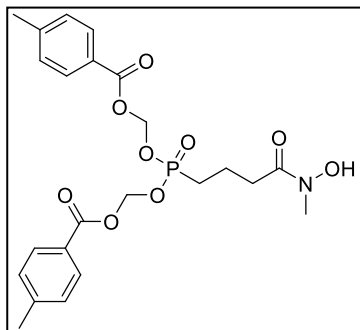
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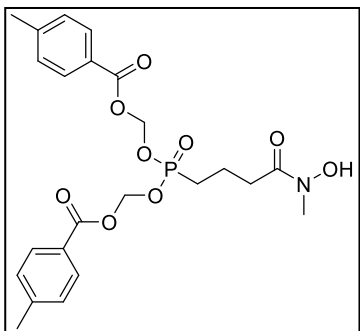


APT NMR spectrum of 13e (75MHz, CHLOROFORM-*d*)

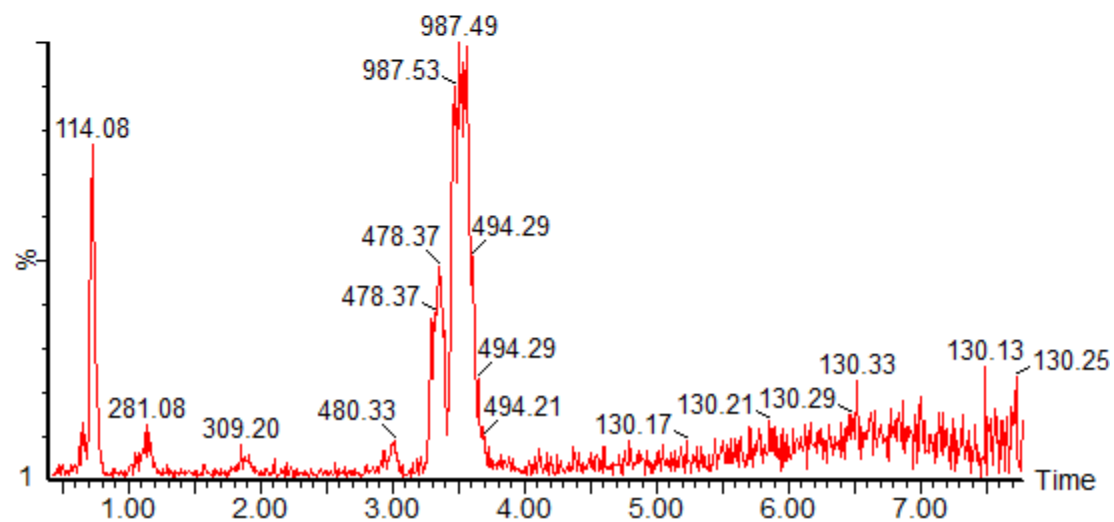
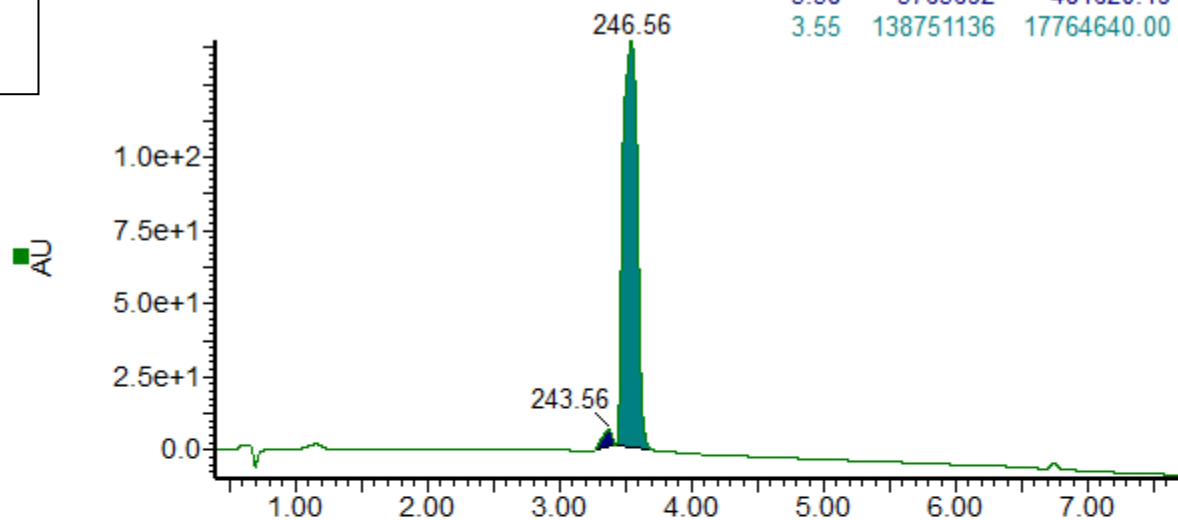


^{31}P NMR spectrum of **13e** (121MHz, CHLOROFORM-*d*)

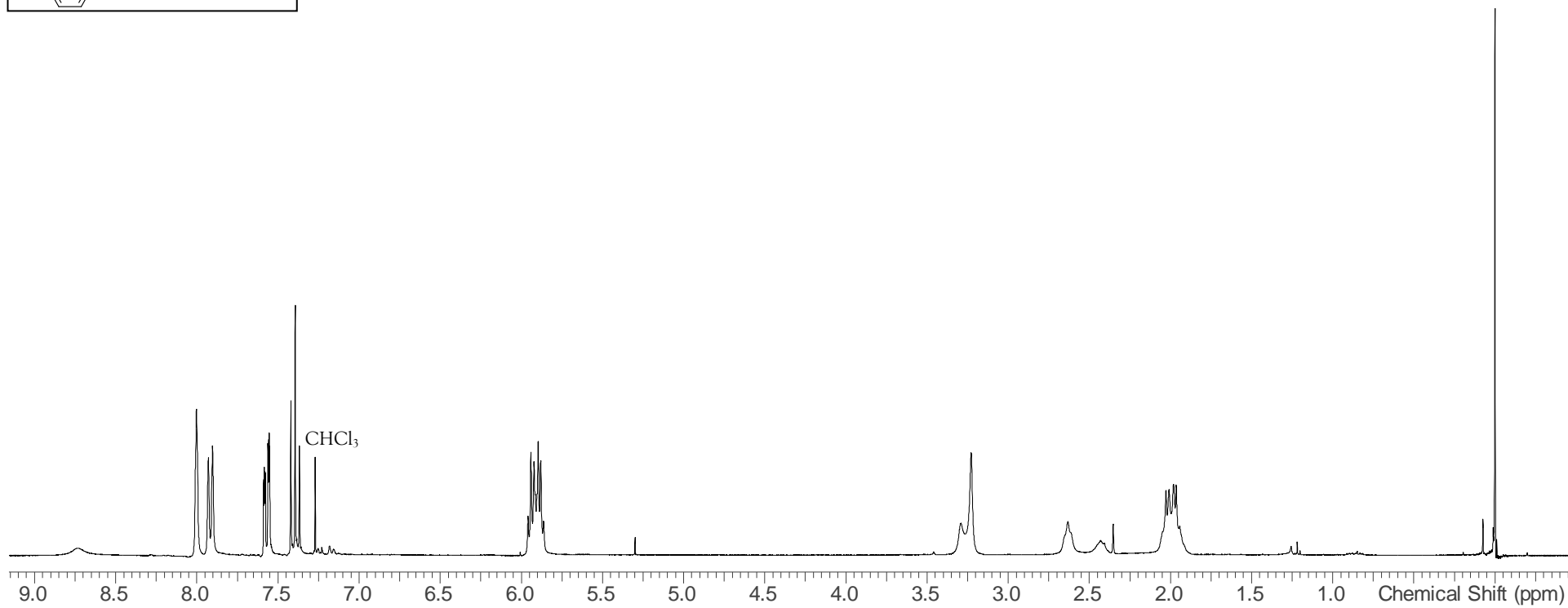
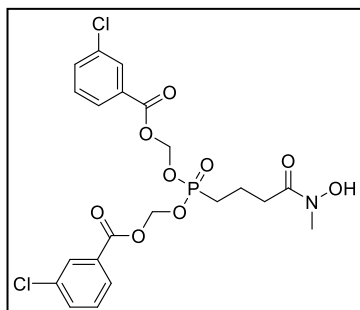




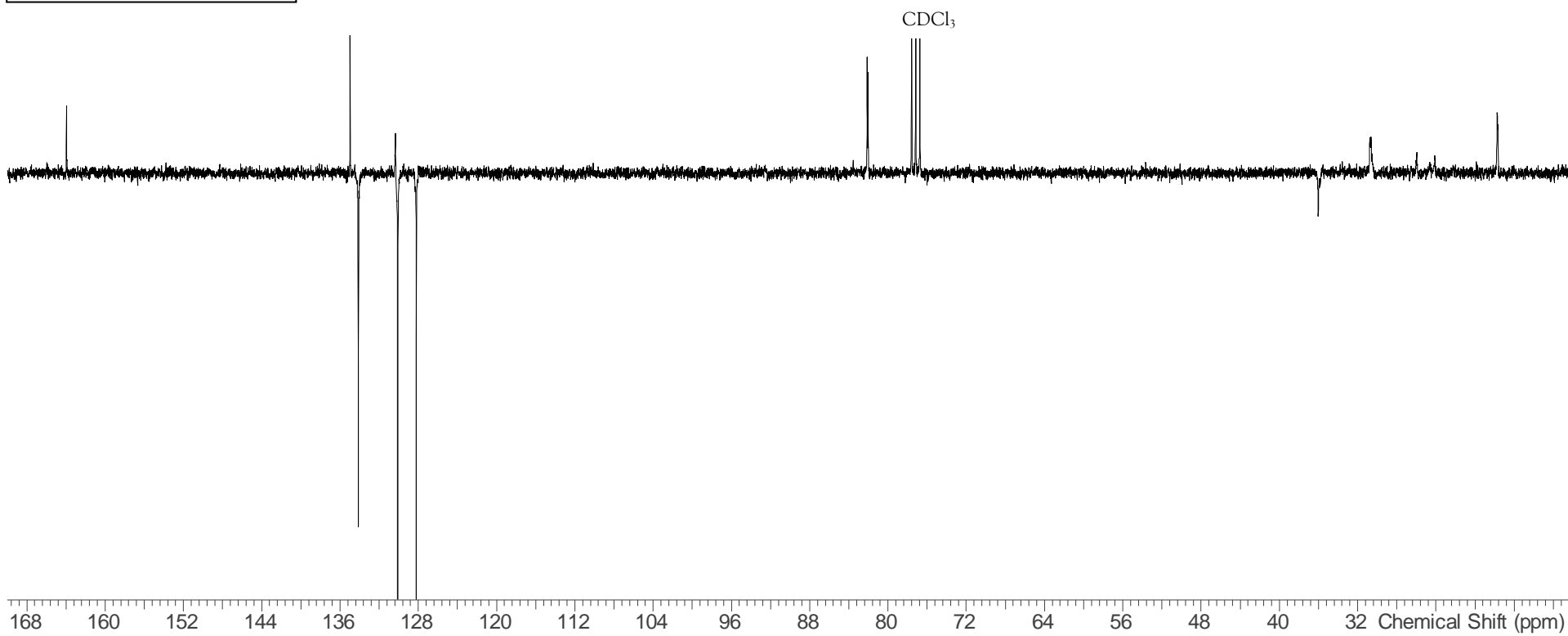
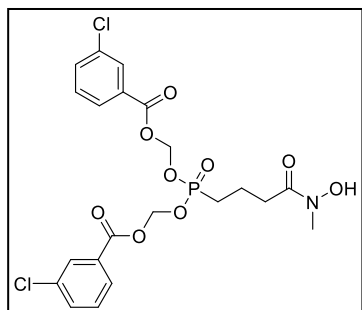
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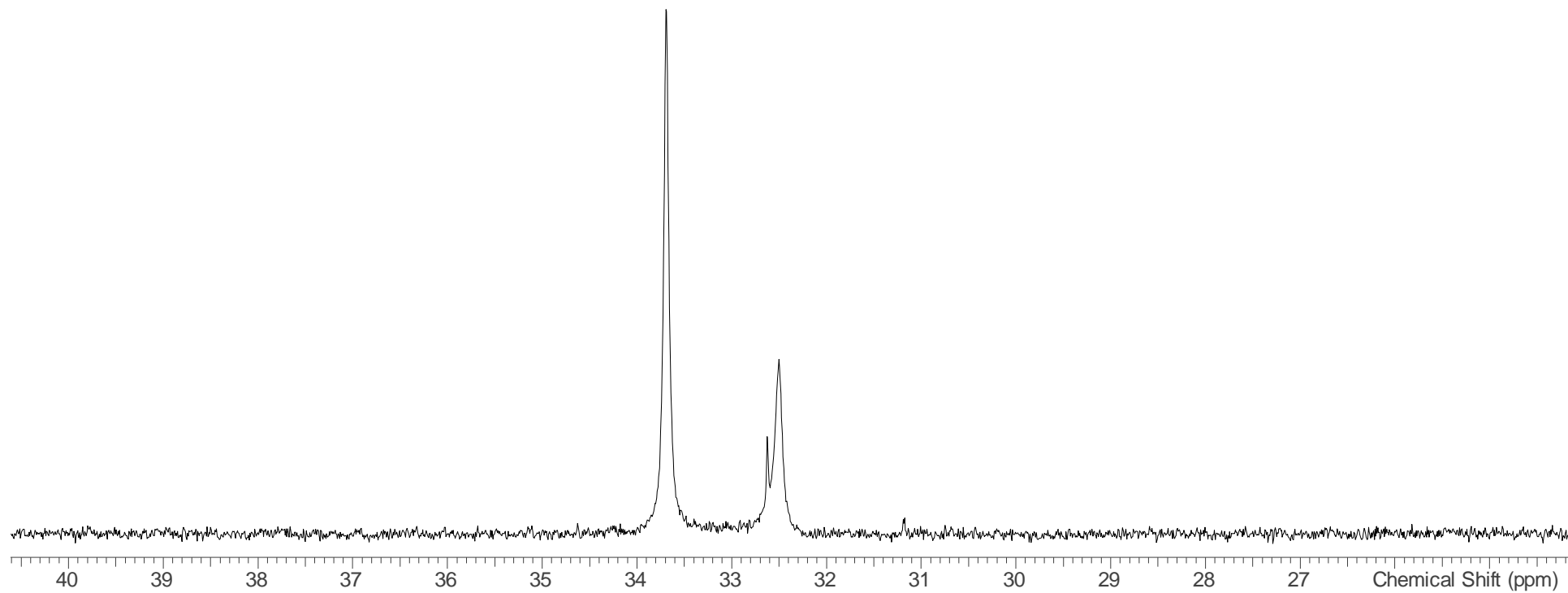
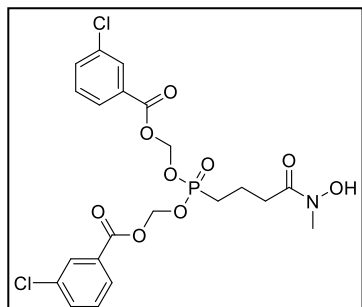
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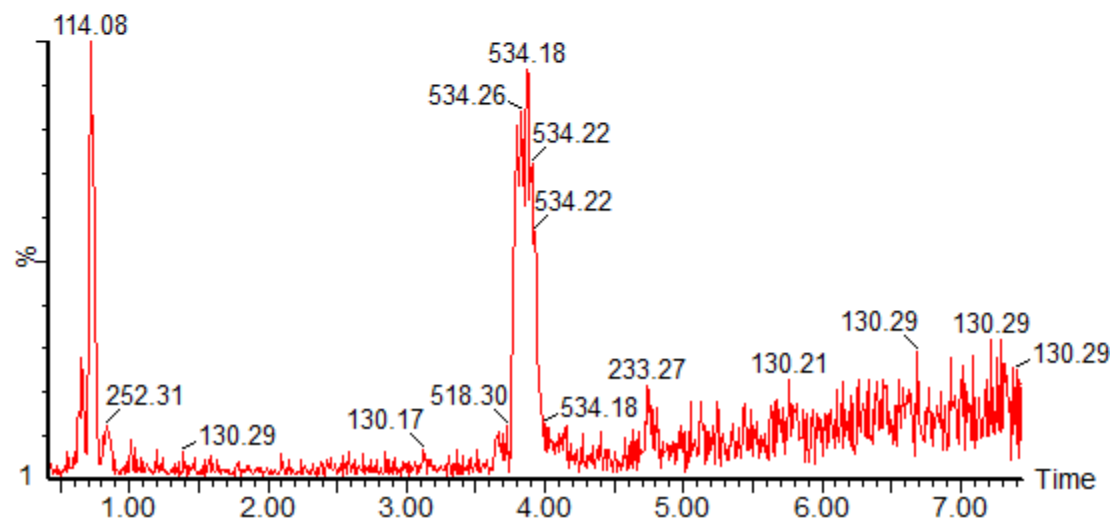
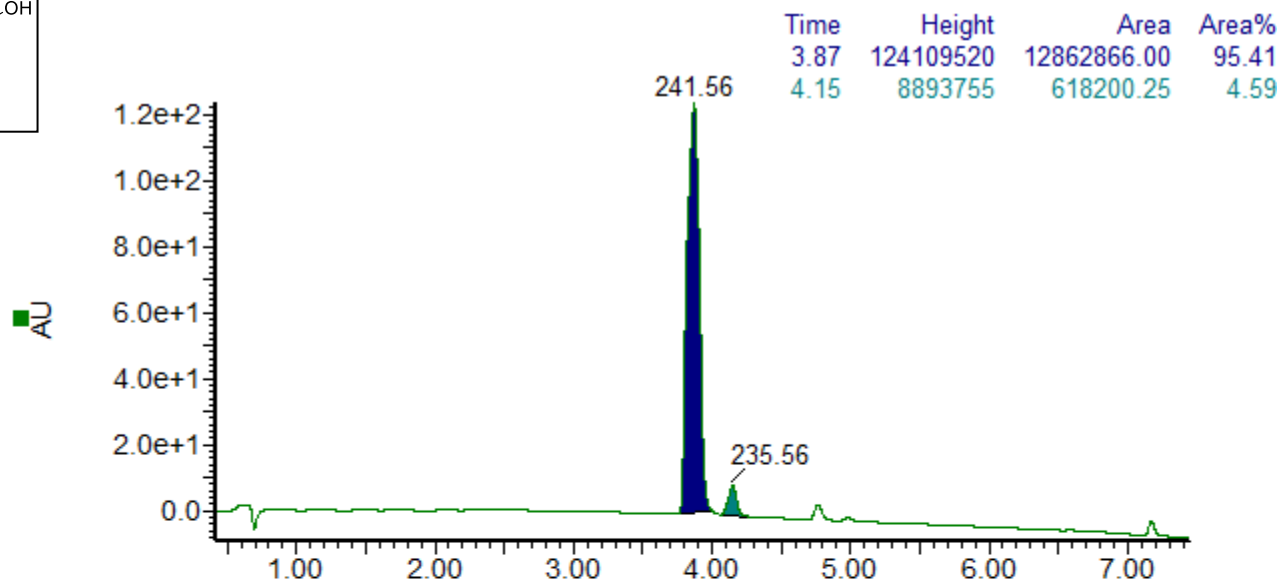
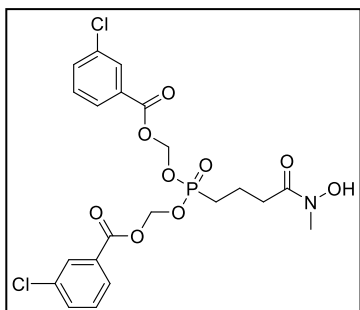


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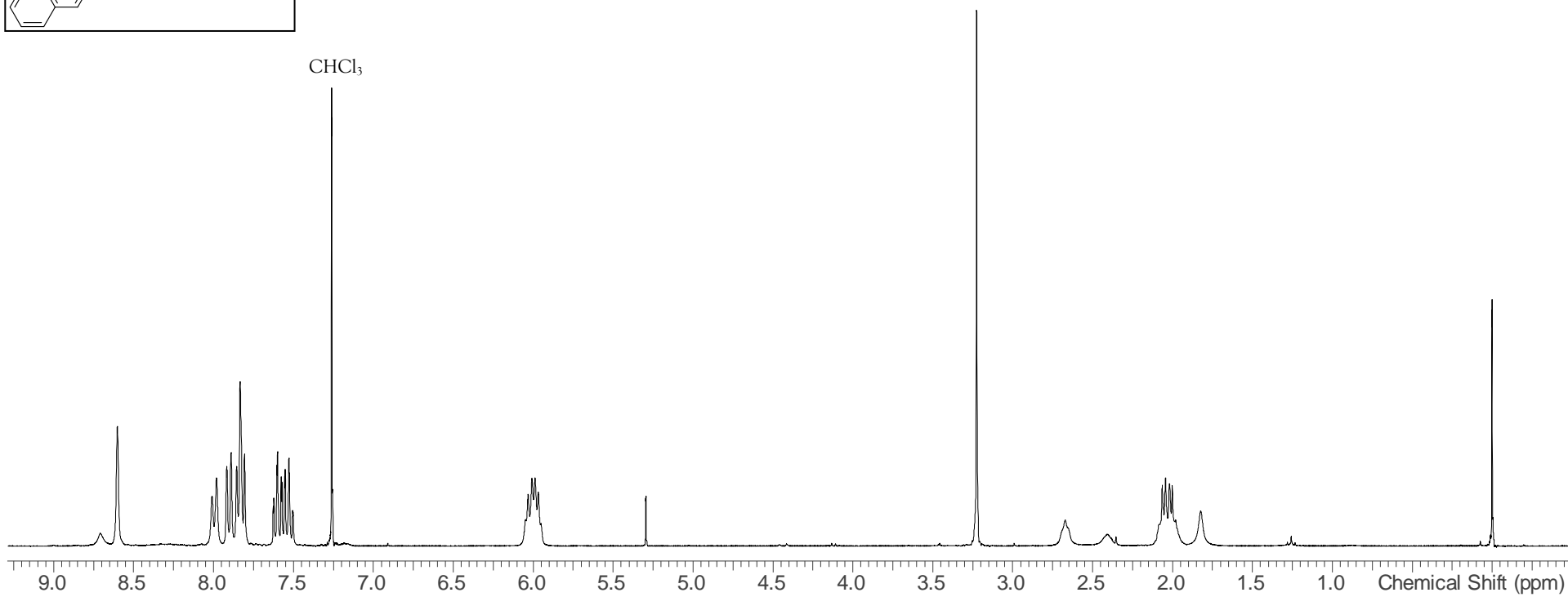
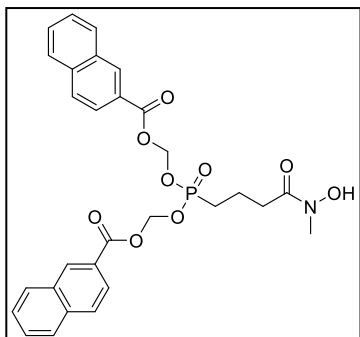


^{31}P NMR spectrum of **13f** (121MHz, CHLOROFORM-*d*)

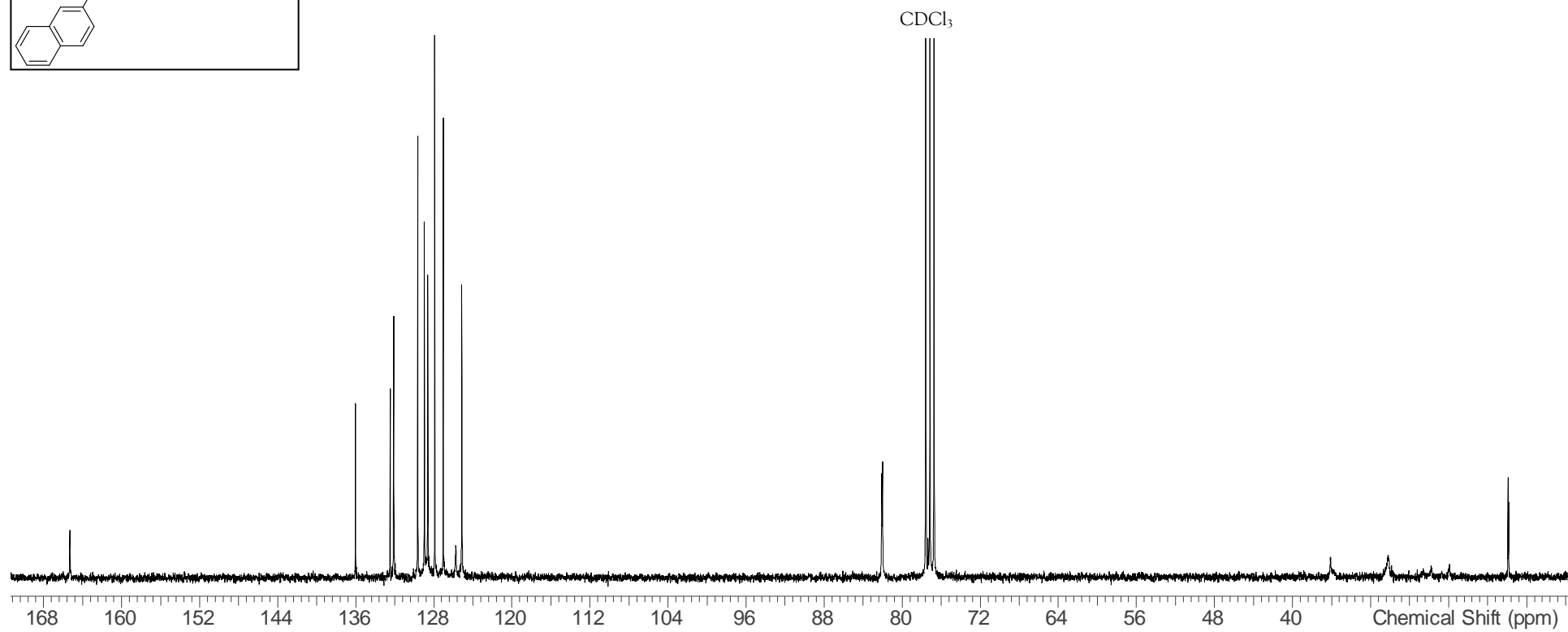
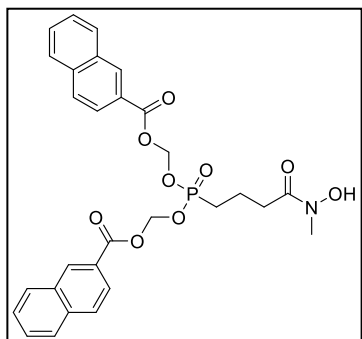




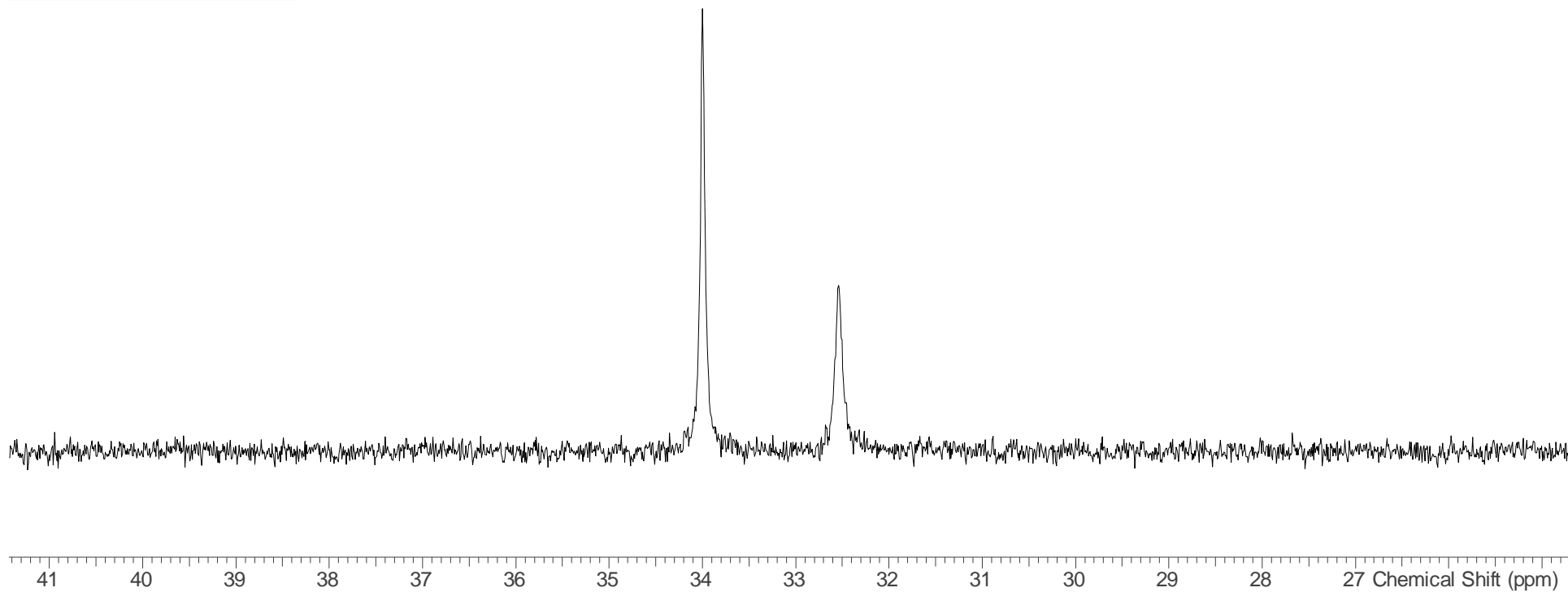
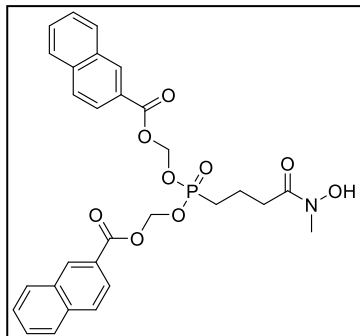
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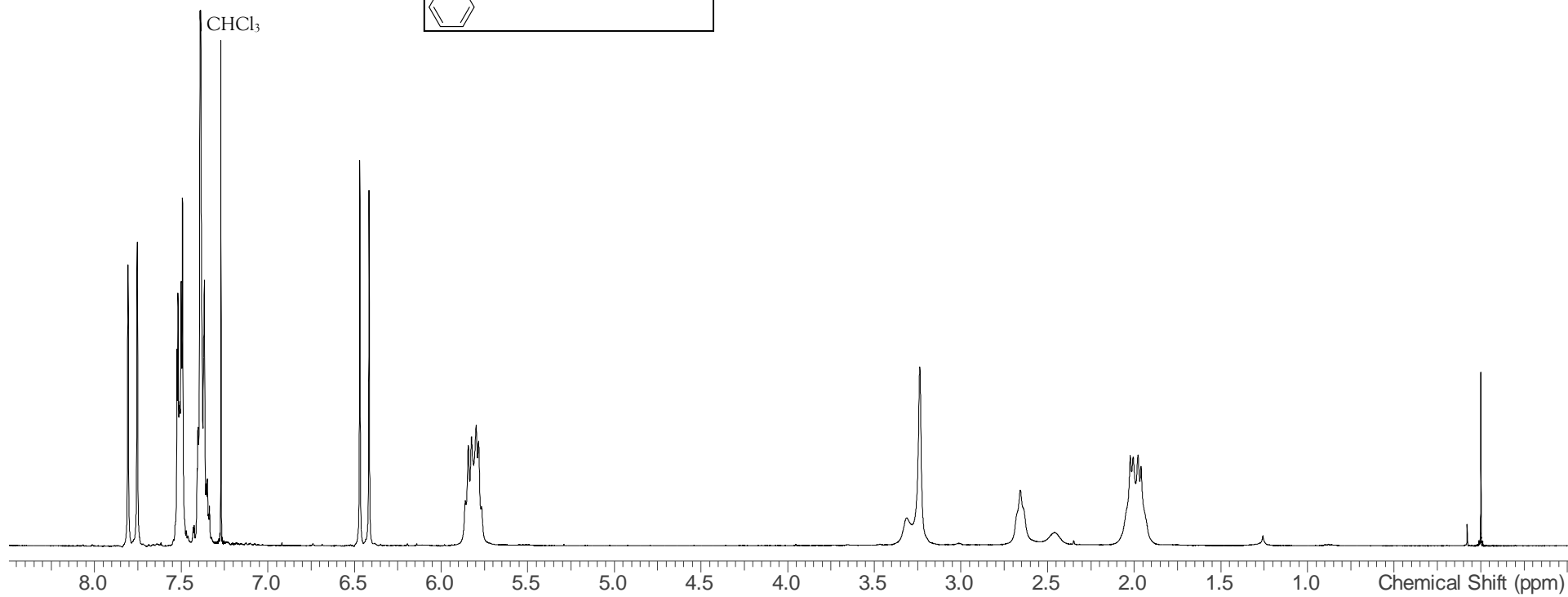
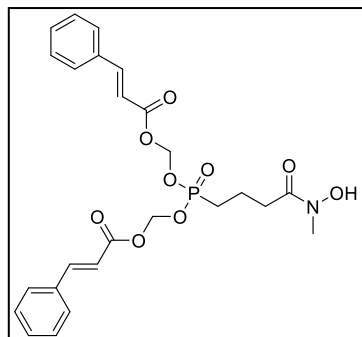
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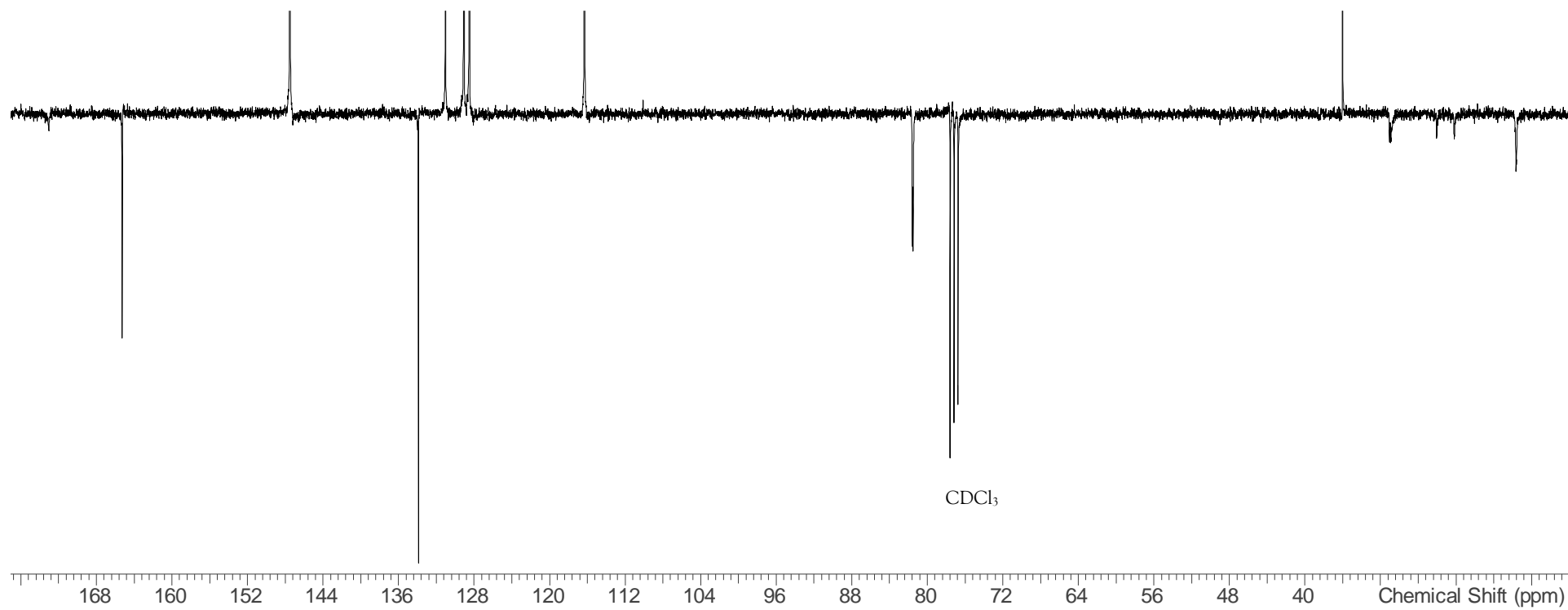
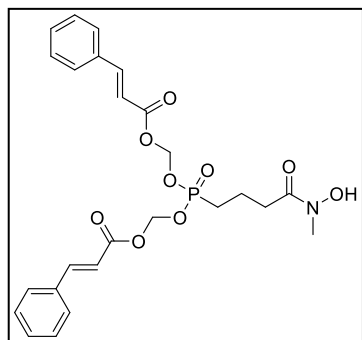
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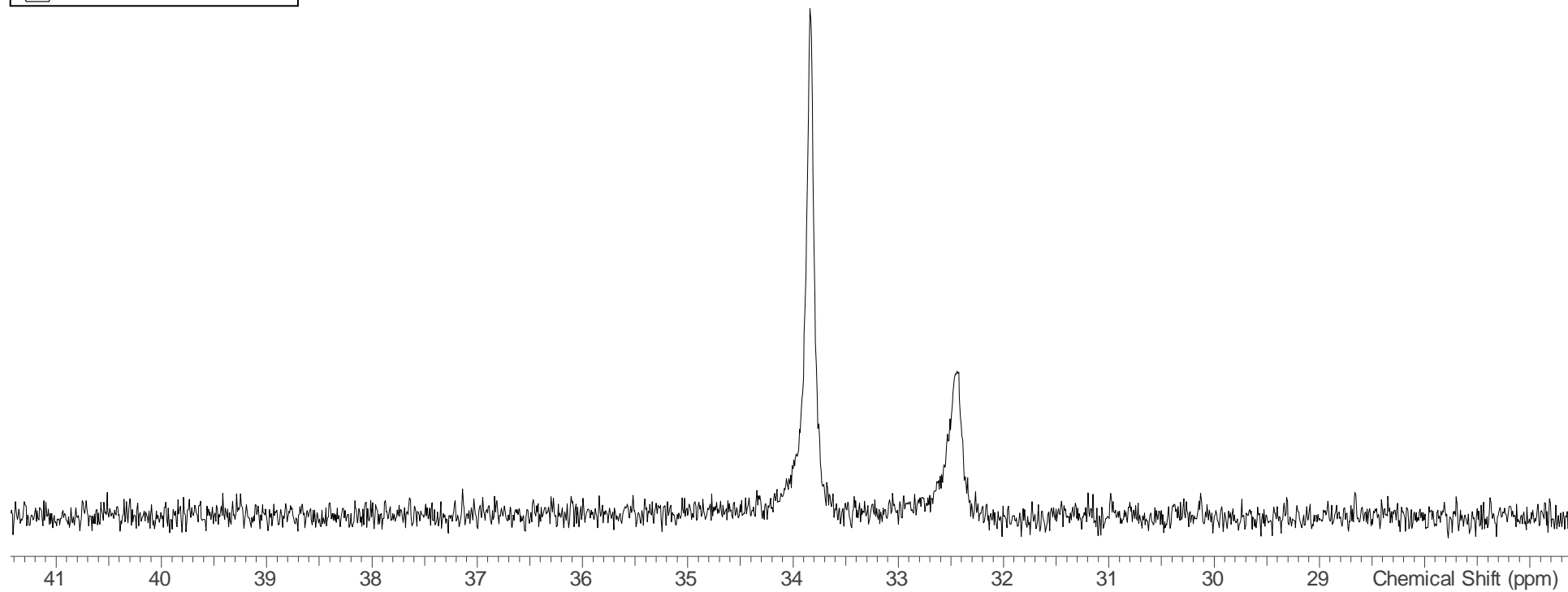
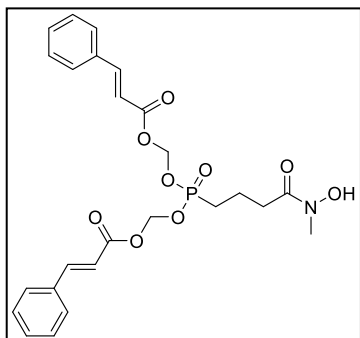
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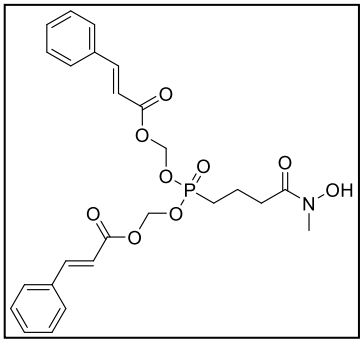


APT NMR spectrum of **13h** (75MHz, CHLOROFORM-*d*)

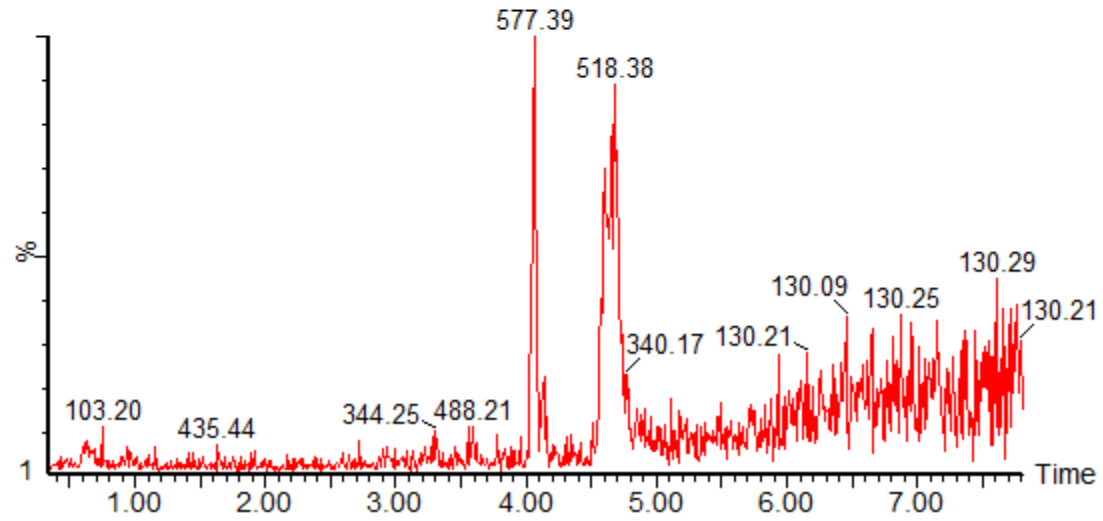
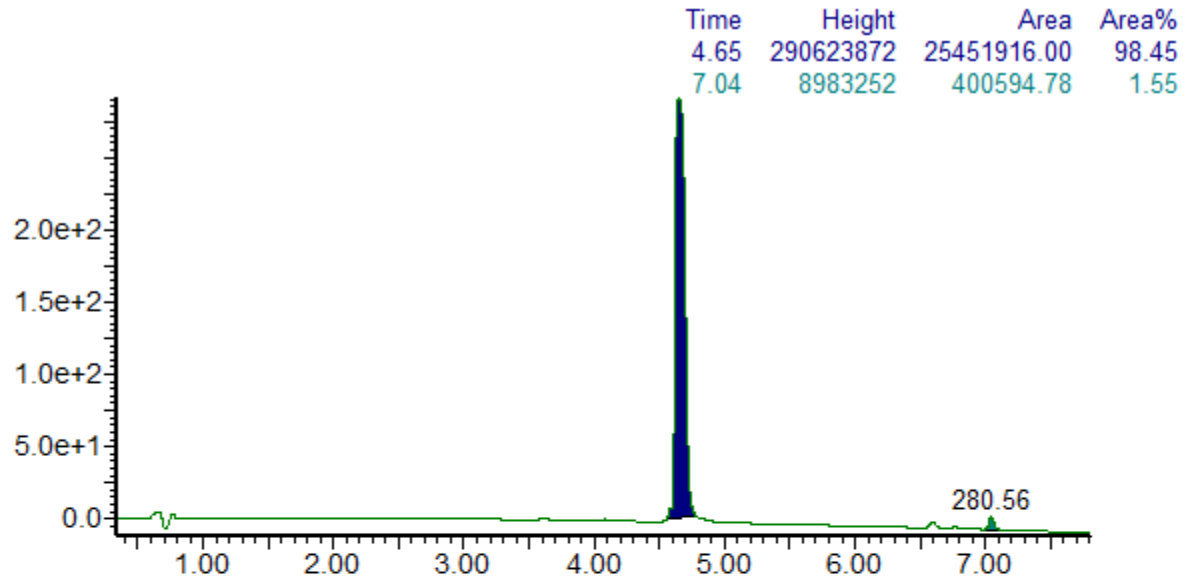


^{31}P NMR spectrum of **13h** (121MHz, CHLOROFORM-*d*)

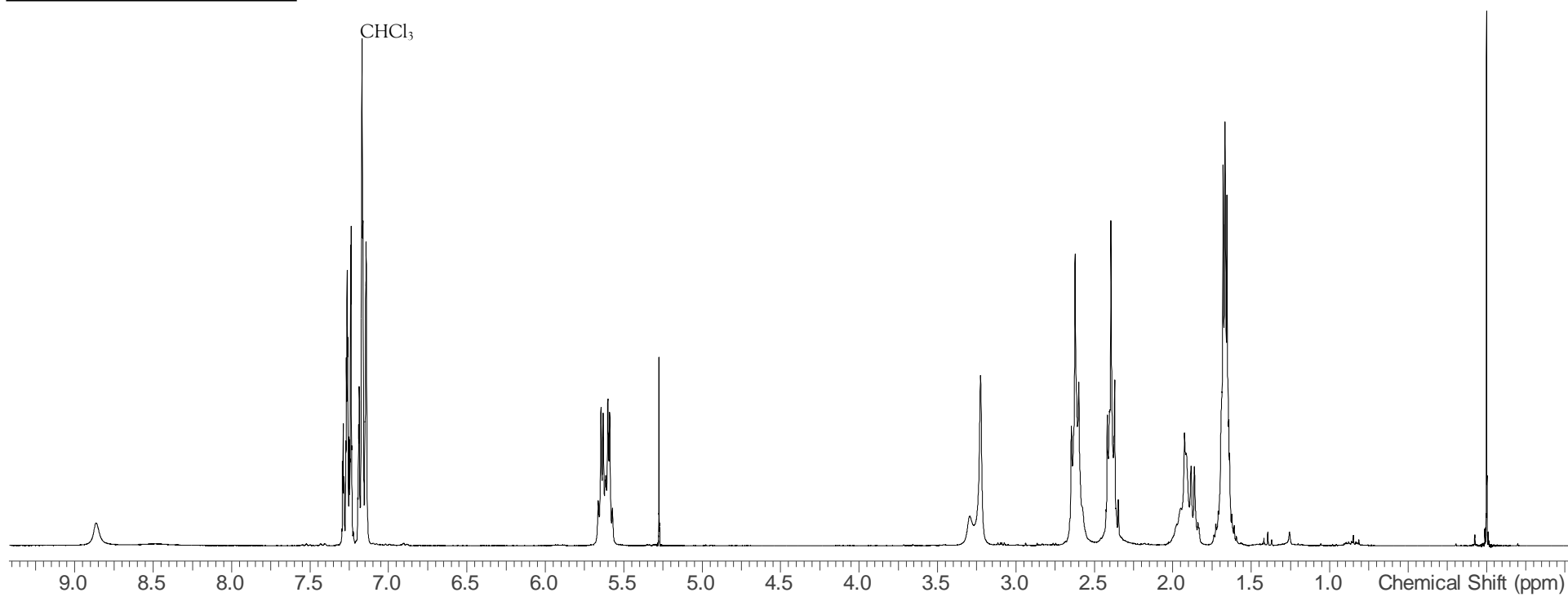
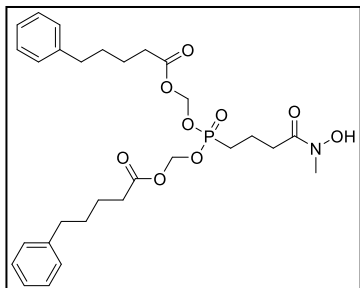




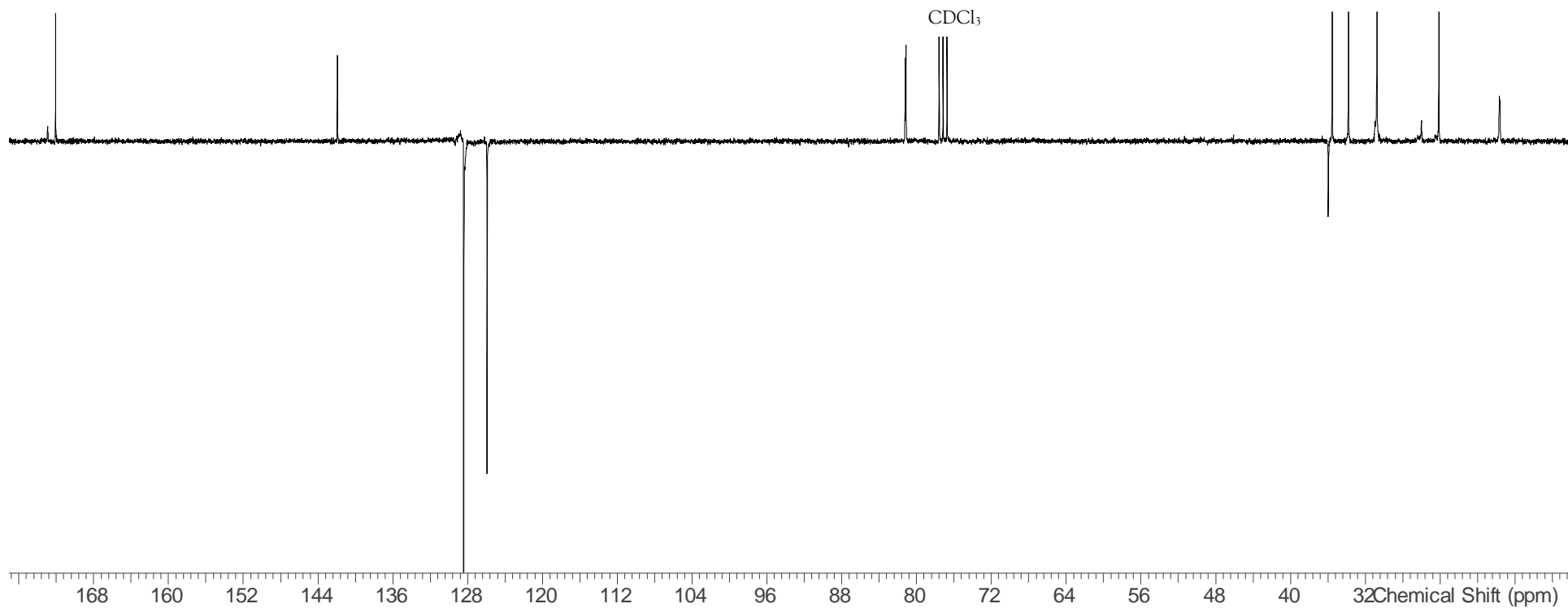
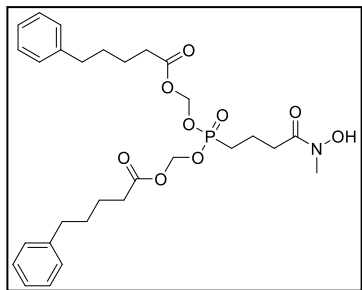
AU



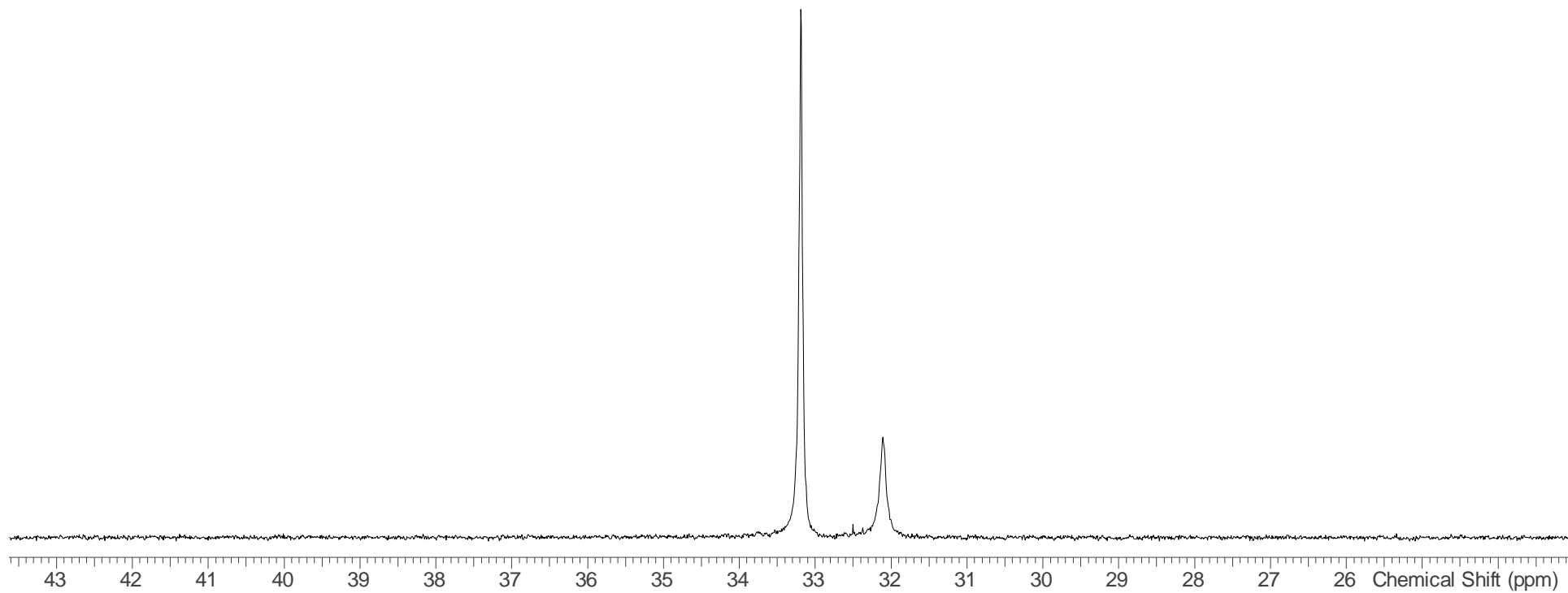
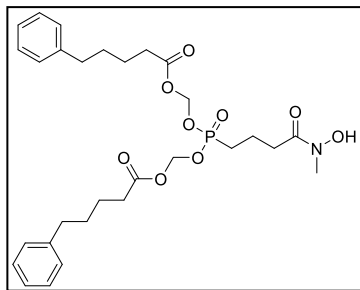
^1H NMR spectrum of **13i** (300MHz, CHLOROFORM-*d*)

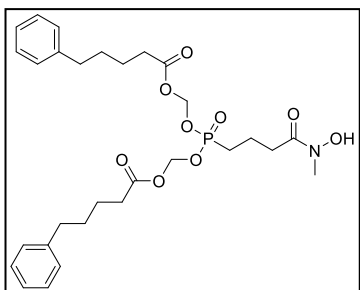


APT NMR spectrum of 13i (75MHz, CHLOROFORM-d)

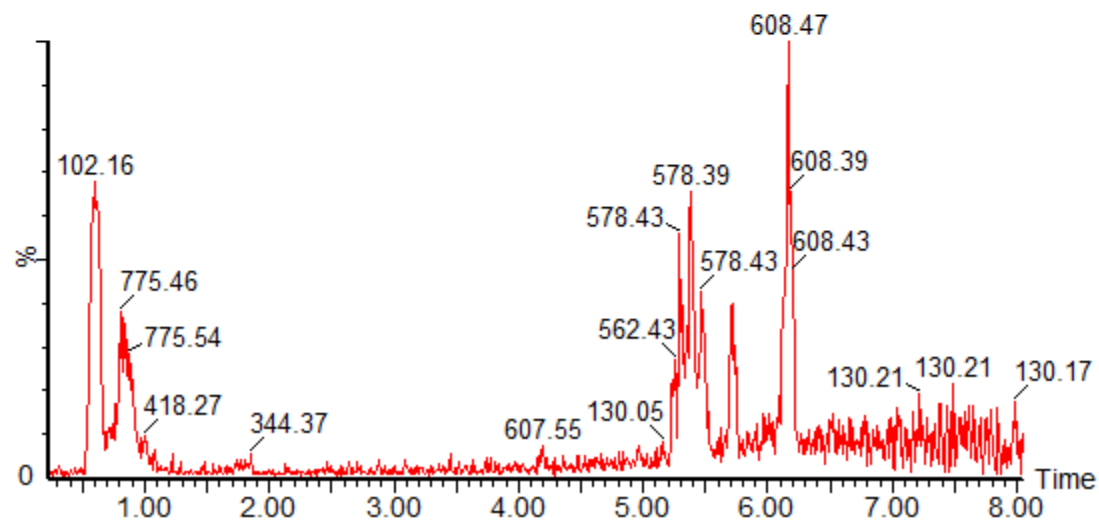
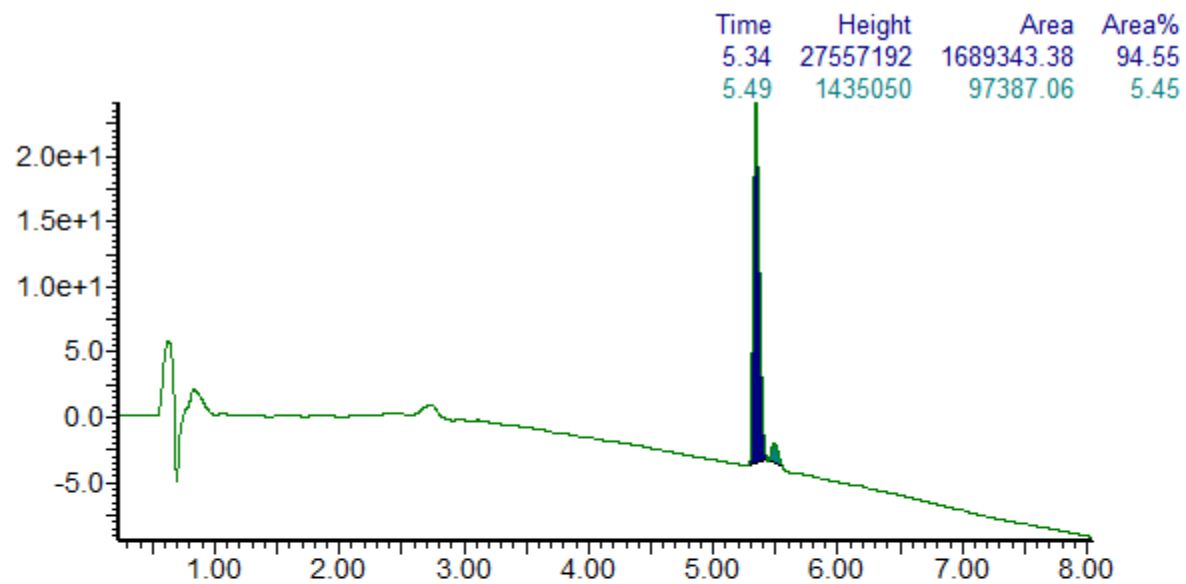


^{31}P NMR spectrum of **13i** (121MHz, CHLOROFORM-*d*)

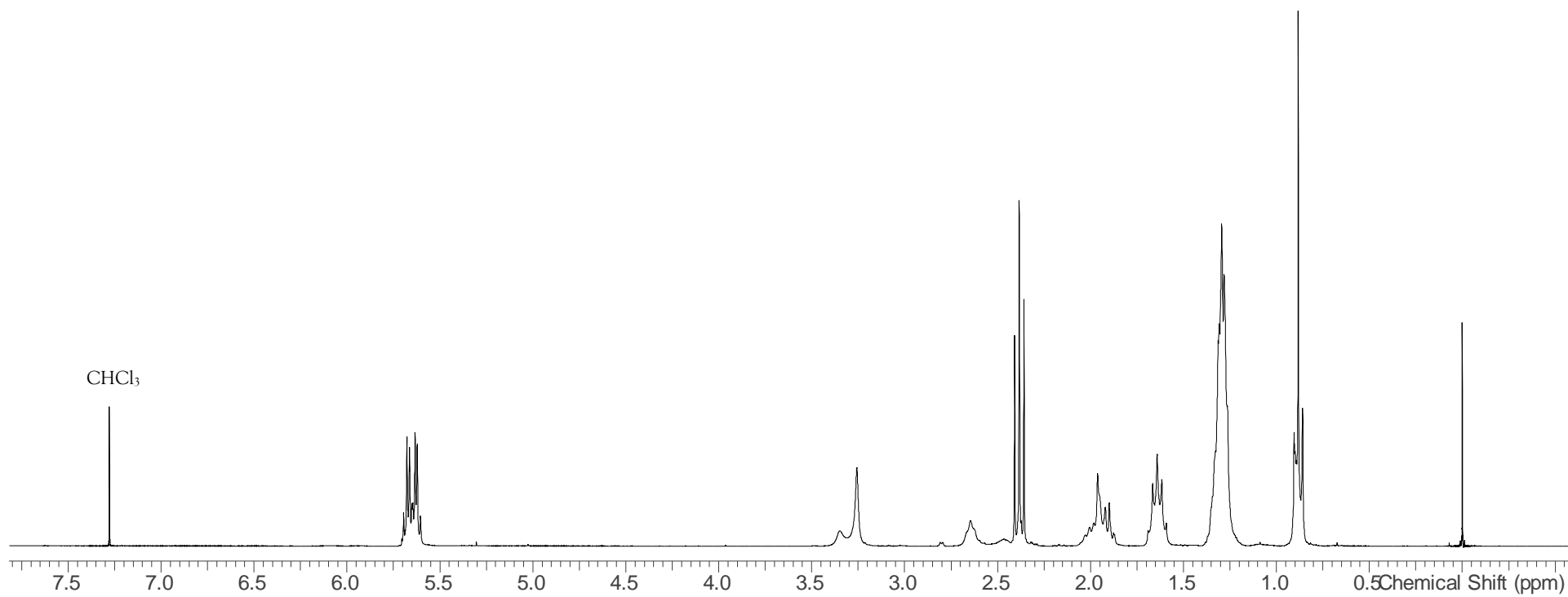
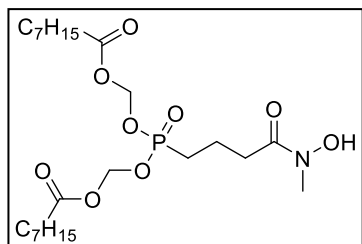




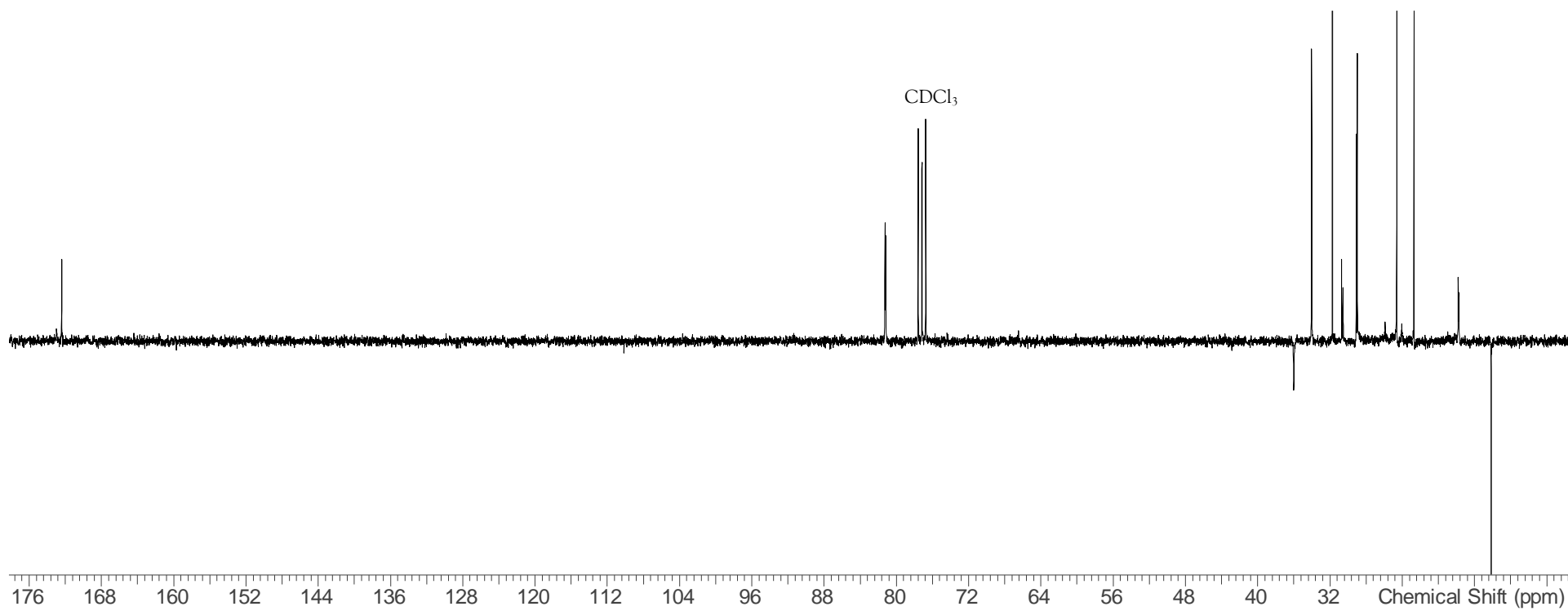
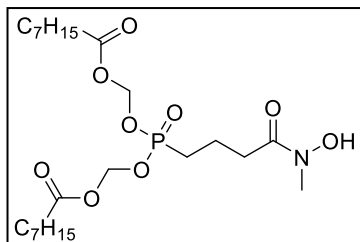
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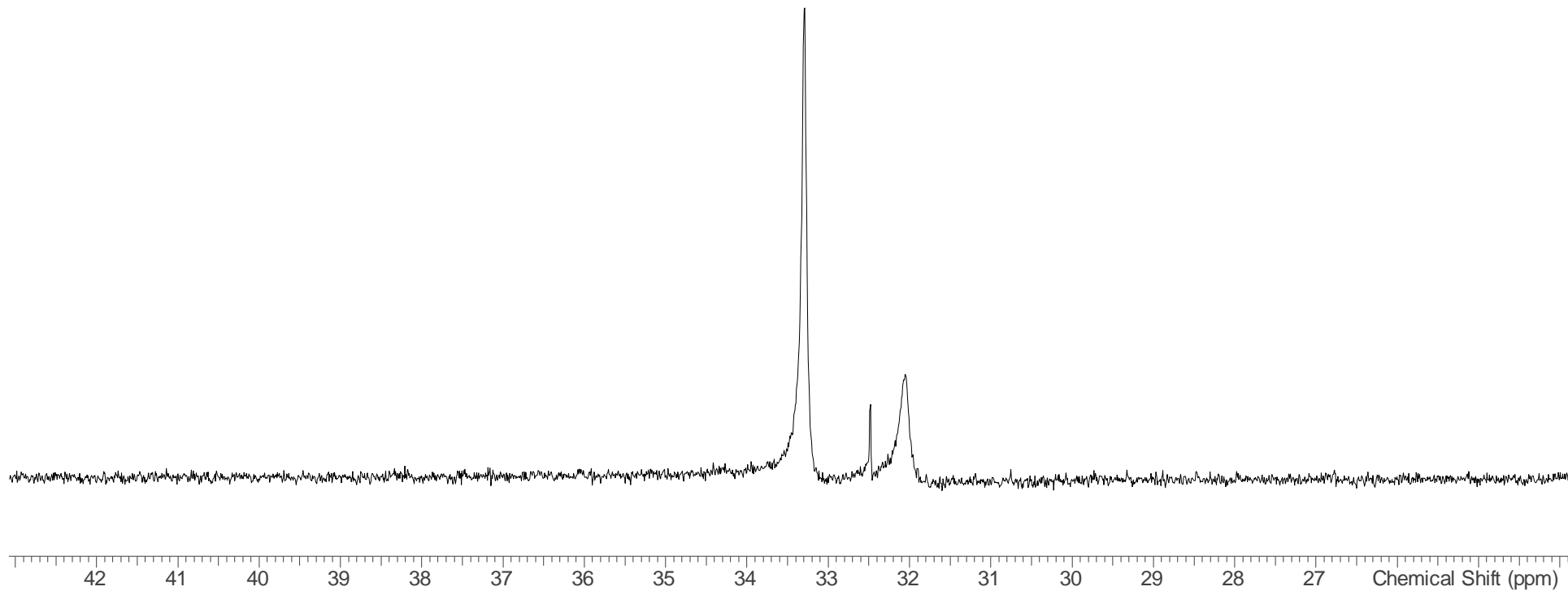
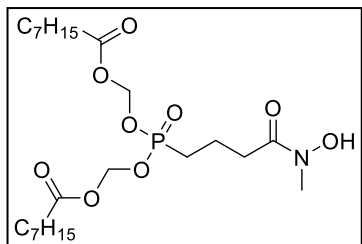
^1H NMR spectrum of **13j** (300MHz, CHLOROFORM-*d*)

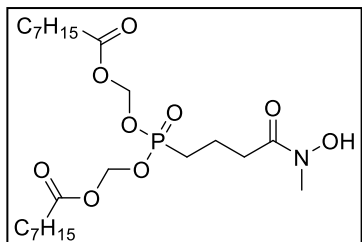


APT NMR spectrum of 13j (75MHz, CHLOROFORM-*d*)



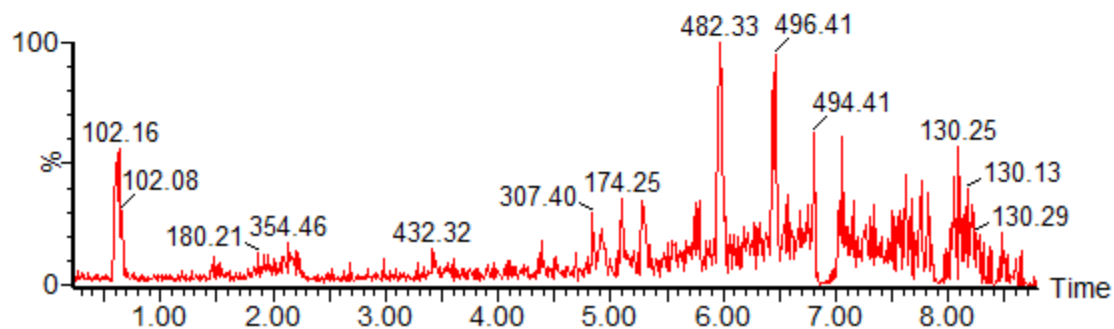
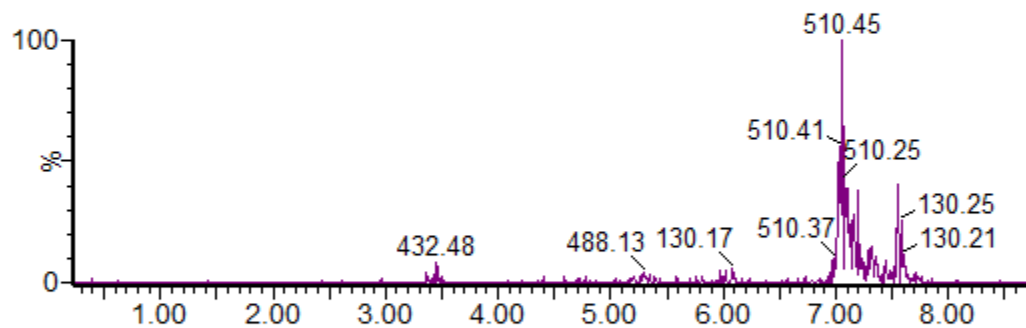
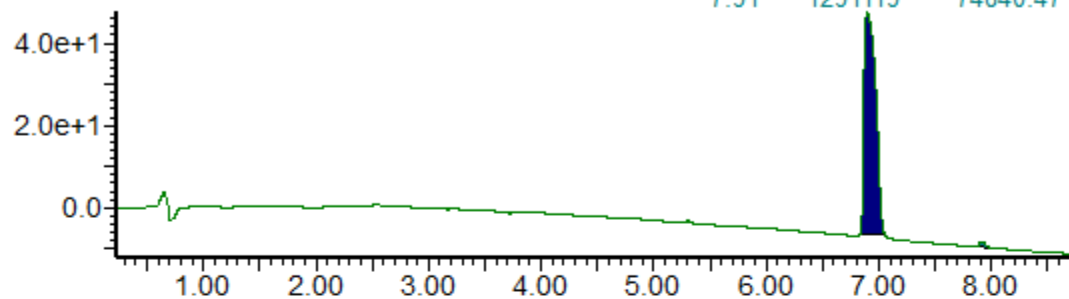
^{31}P NMR spectrum of **13j** (121MHz, CHLOROFORM-*d*)



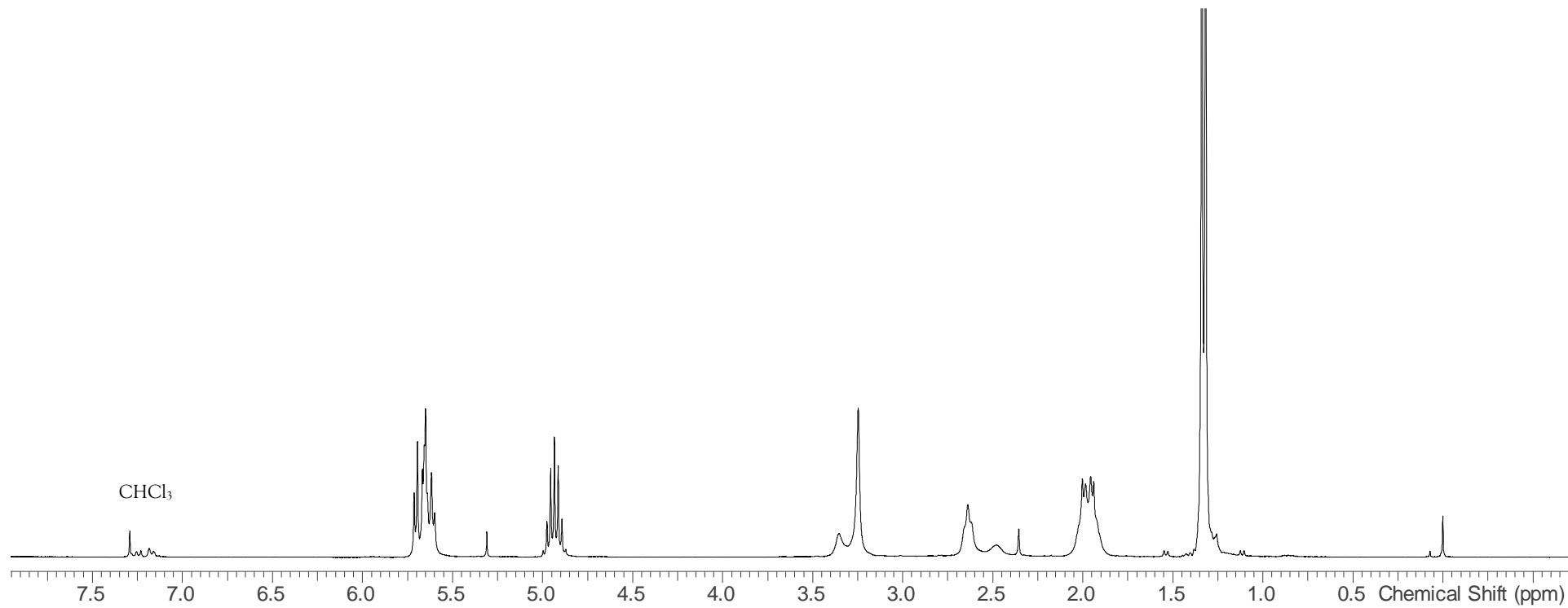
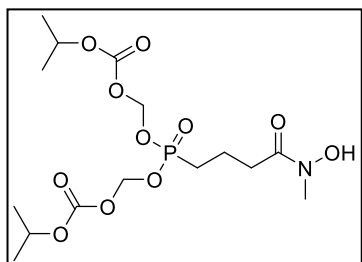


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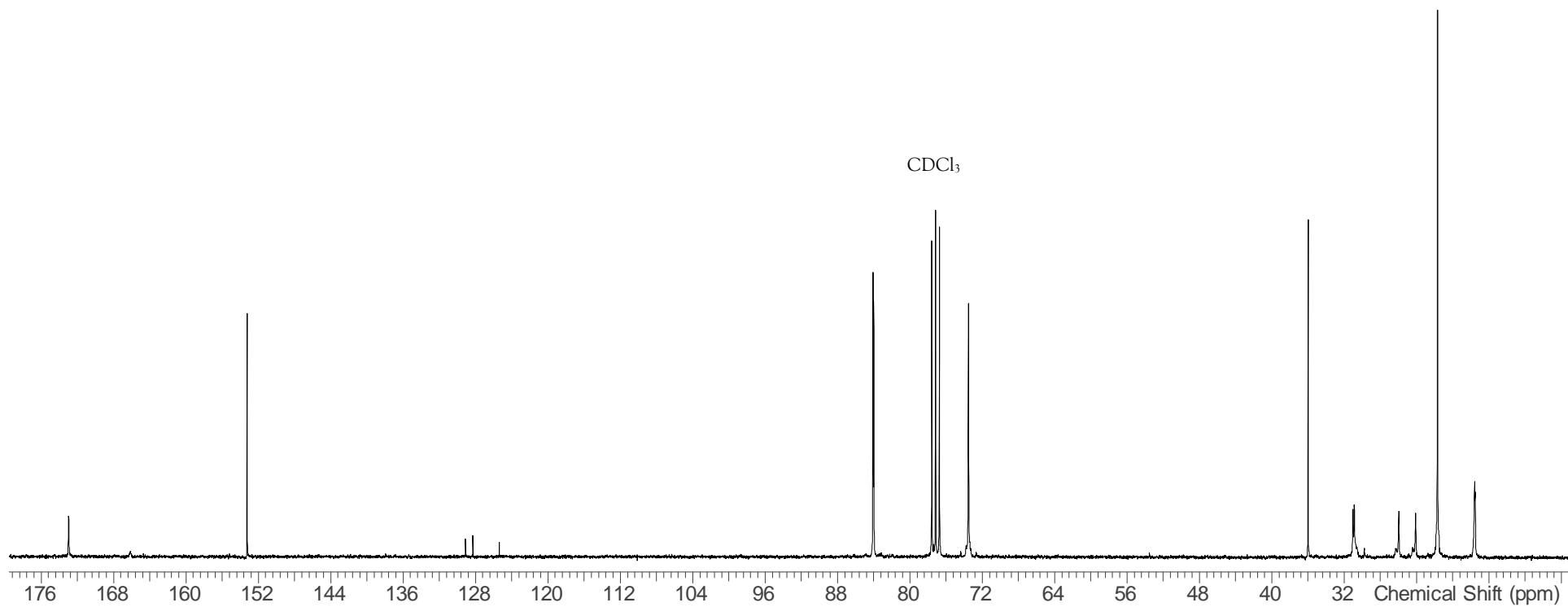
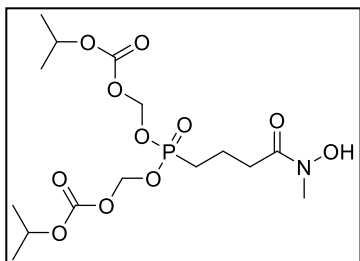
Time	Height	Area	Area%
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7.91	1291119	74840.47	1.13



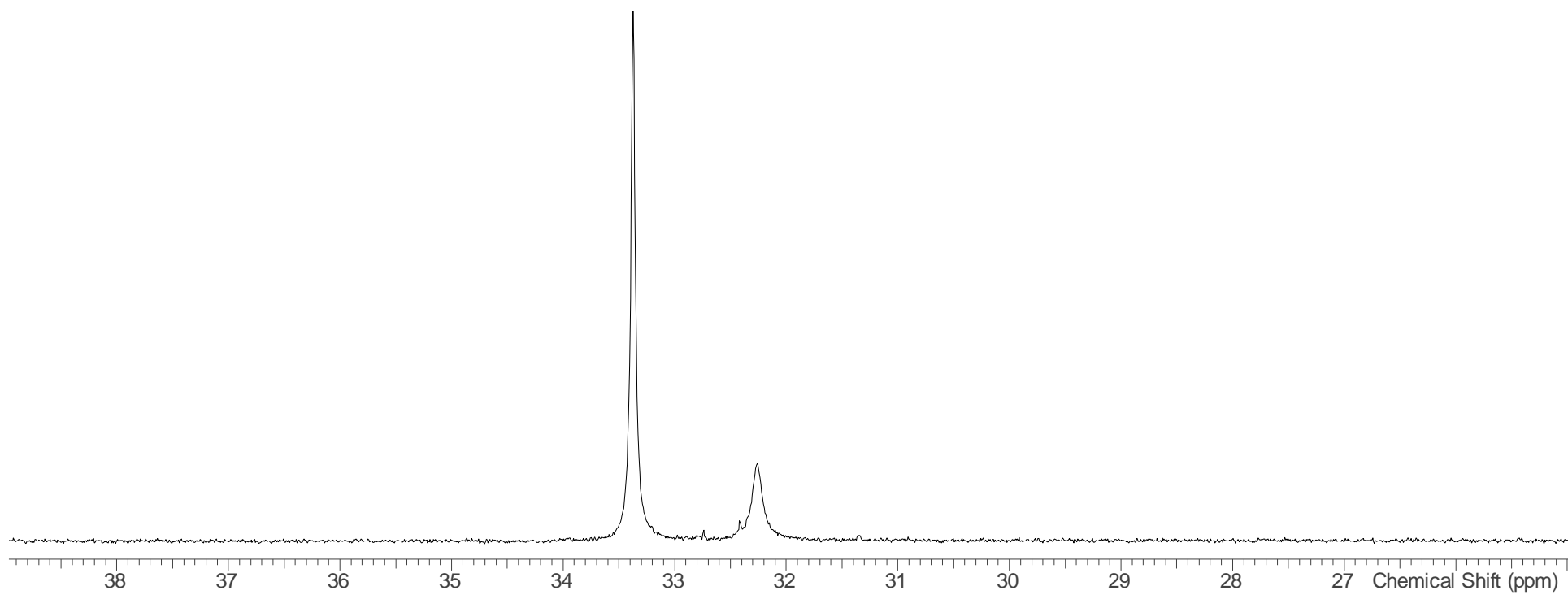
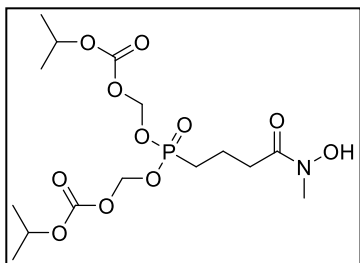
¹H NMR spectrum of **13k** (300MHz, CHLOROFORM-*d*)

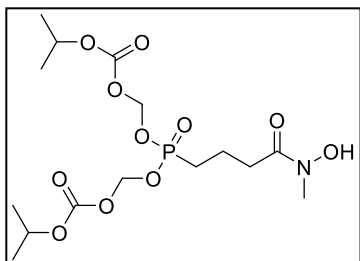


^{13}C NMR spectrum of **13k** (75MHz, CHLOROFORM-*d*)

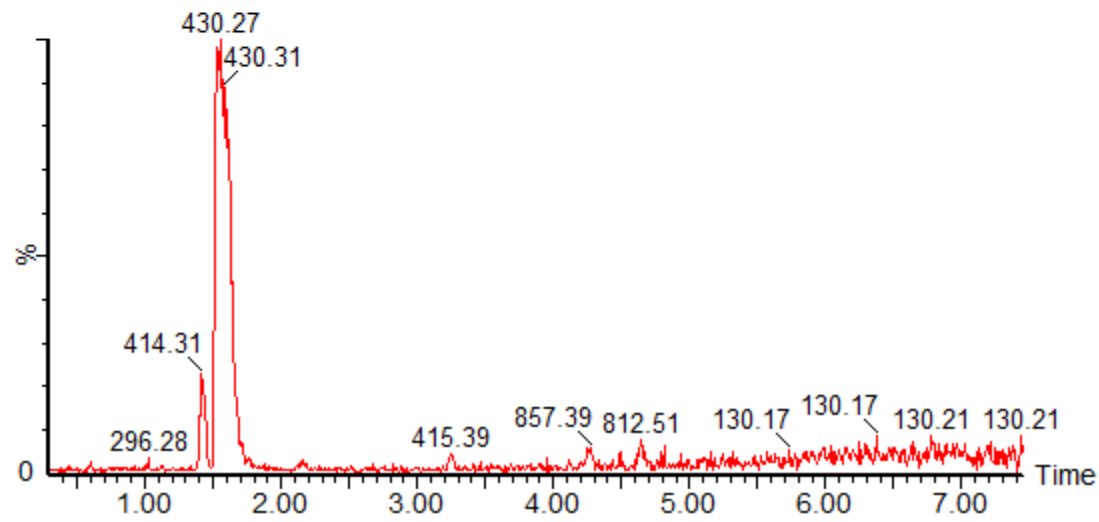
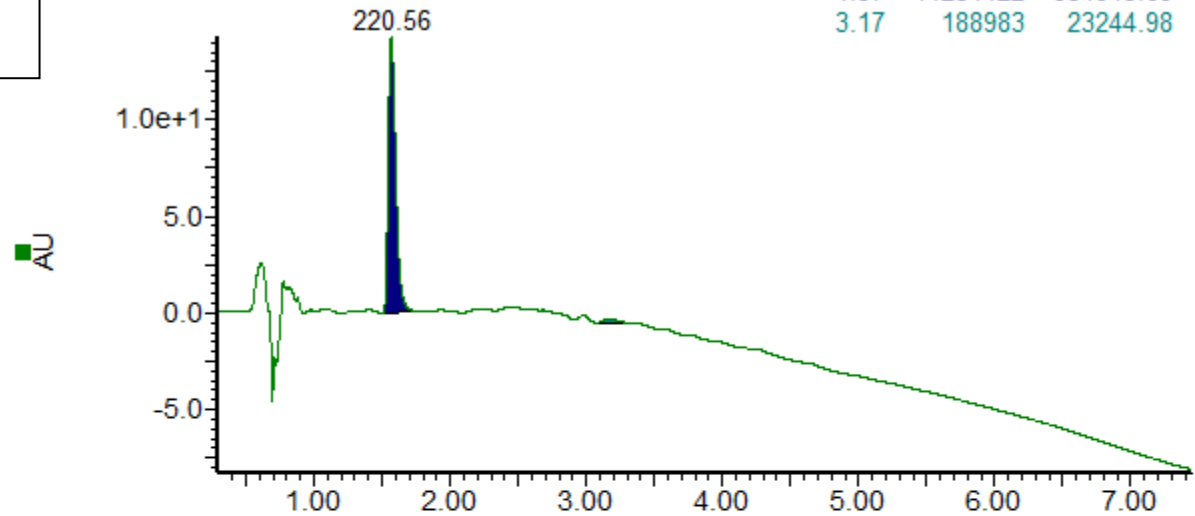


^{31}P NMR spectrum of **13k** (121MHz, CHLOROFORM-*d*)

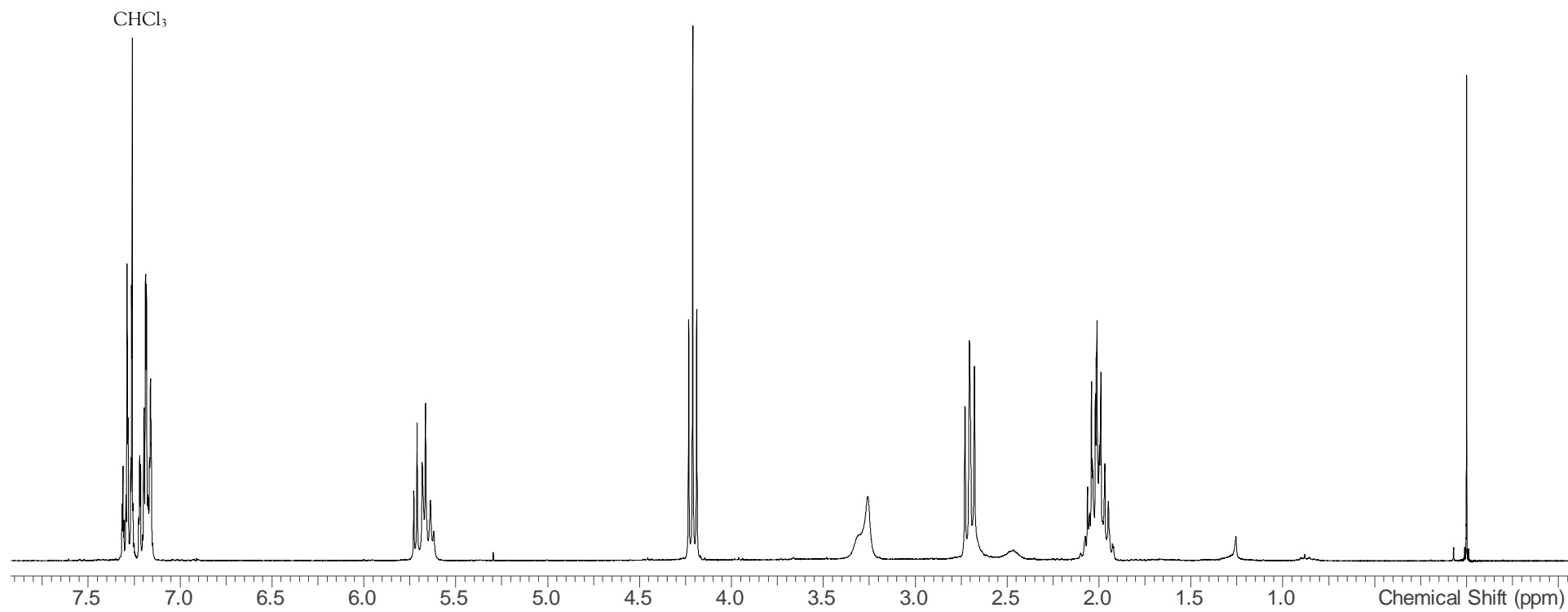
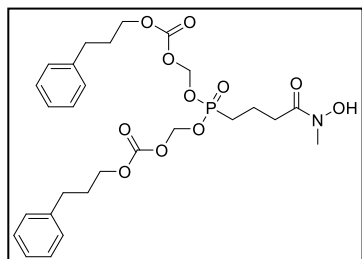




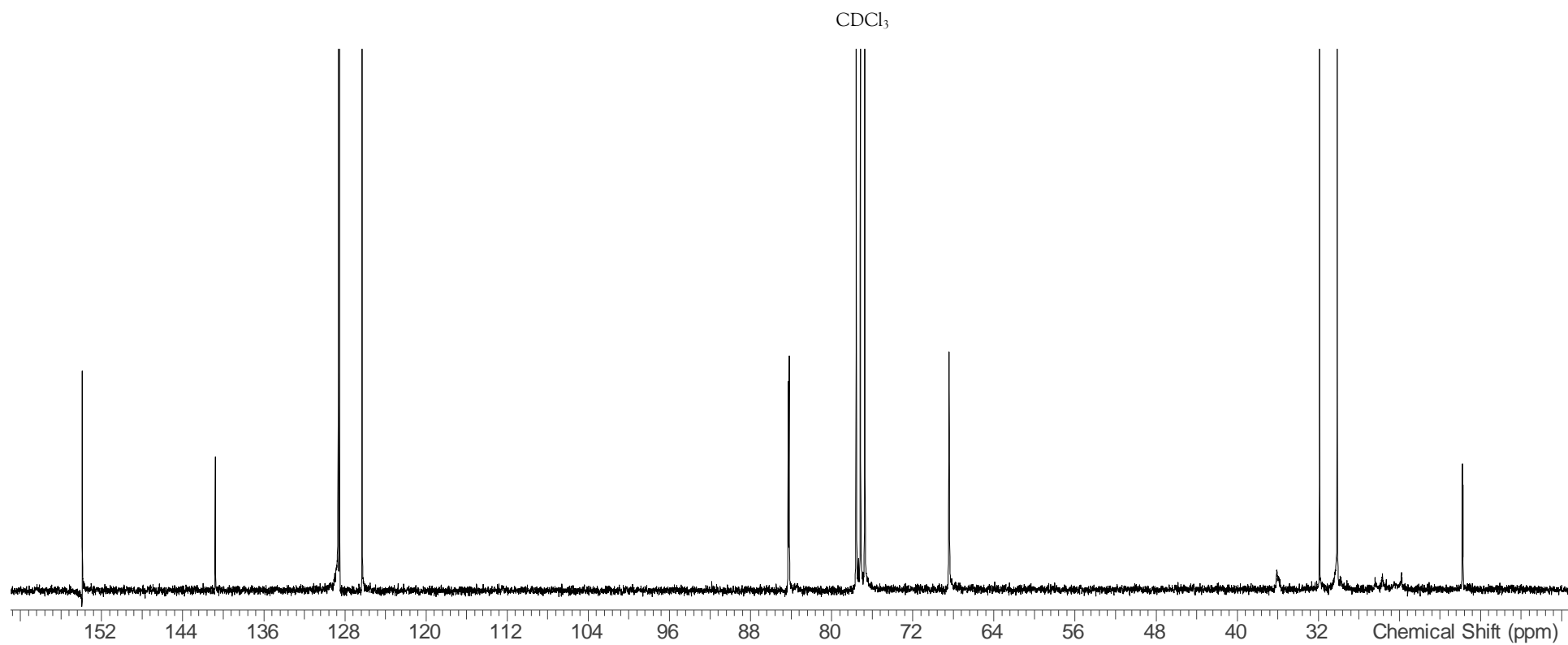
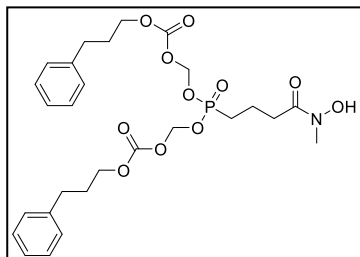
Time	Height	Area	Area%
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3.17	188983	23244.98	2.43



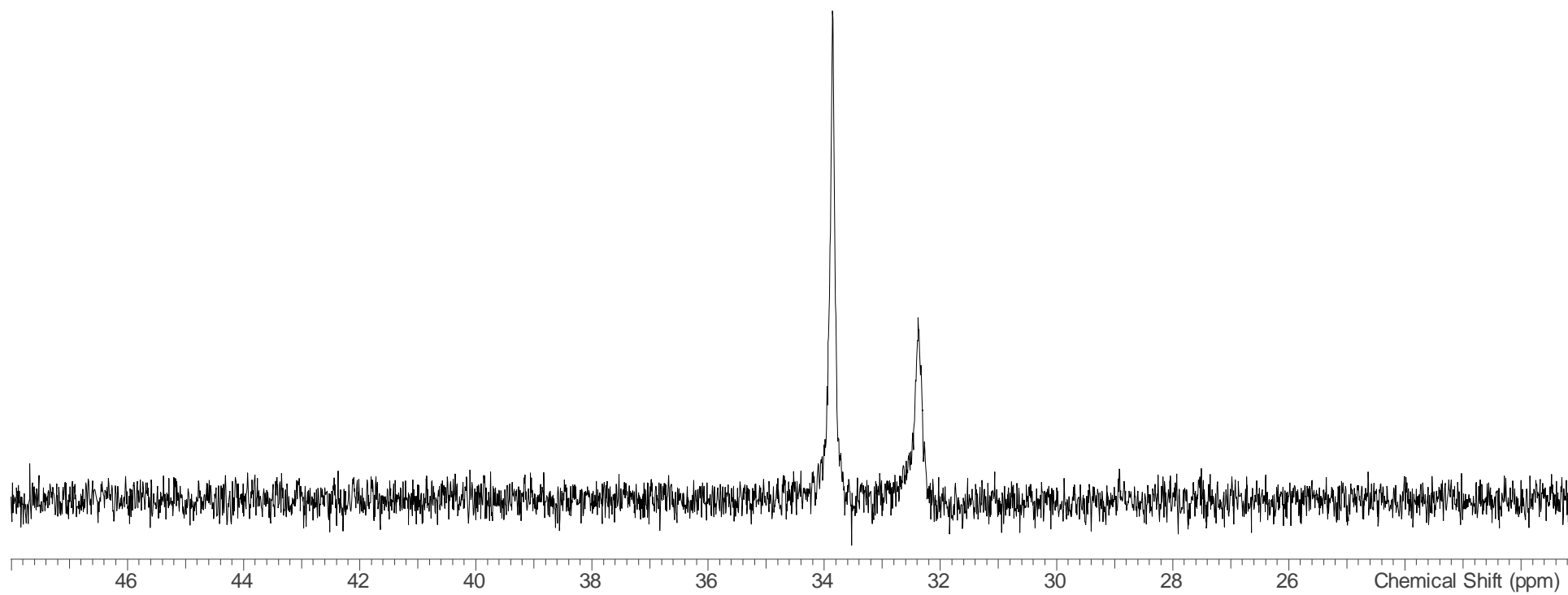
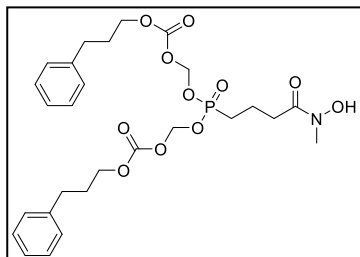
^1H NMR spectrum of **131** (300MHz, CHLOROFORM-*d*)

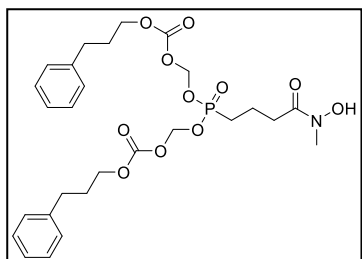


^{13}C NMR spectrum of **13I** (75MHz, CHLOROFORM-*d*)

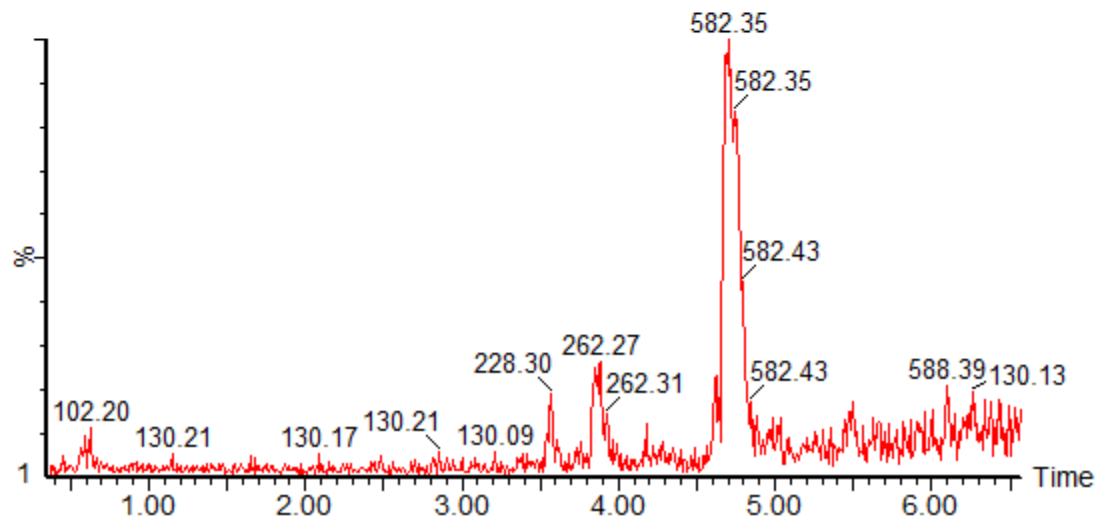
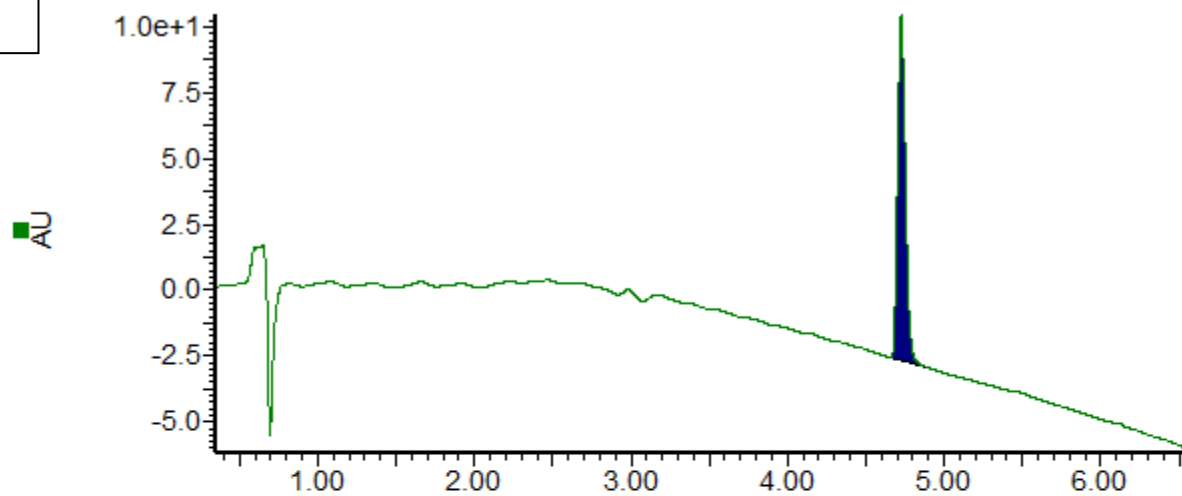


^{31}P NMR spectrum of **13I** (121MHz, CHLOROFORM-*d*)

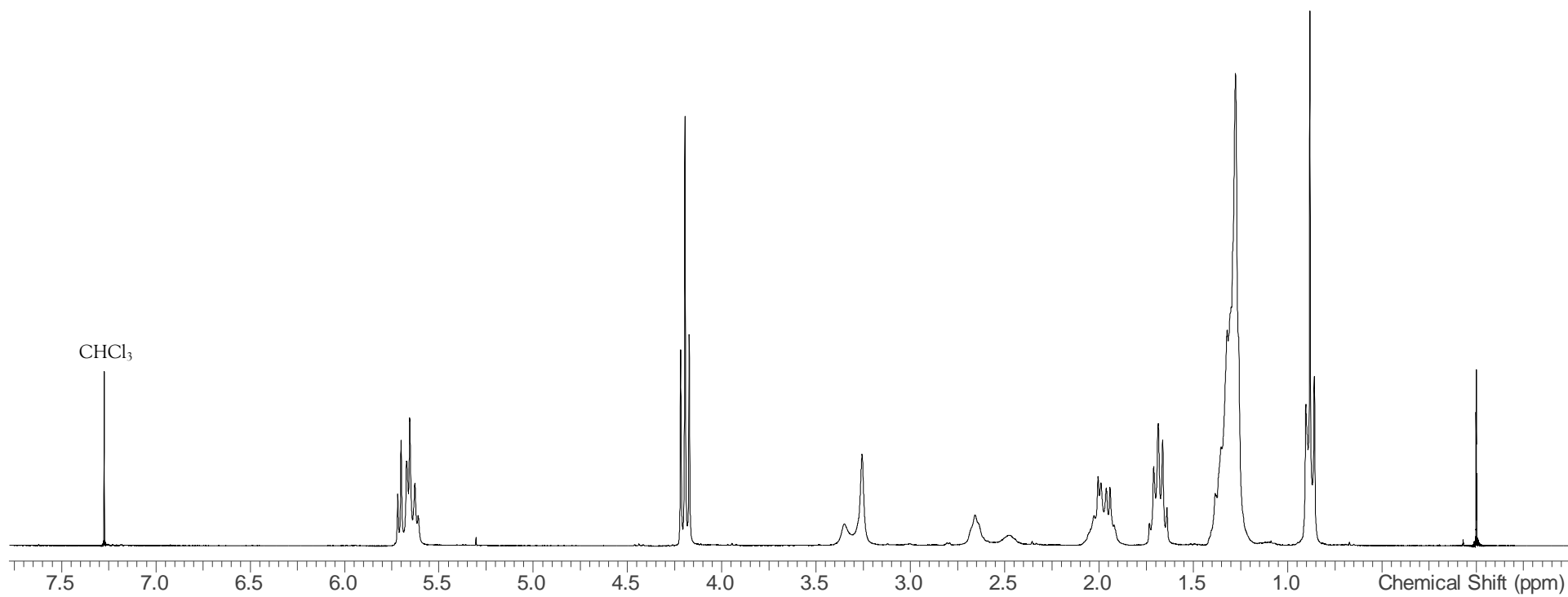
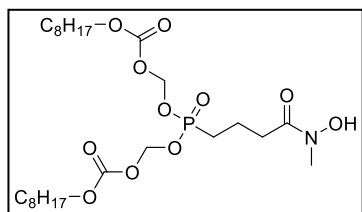




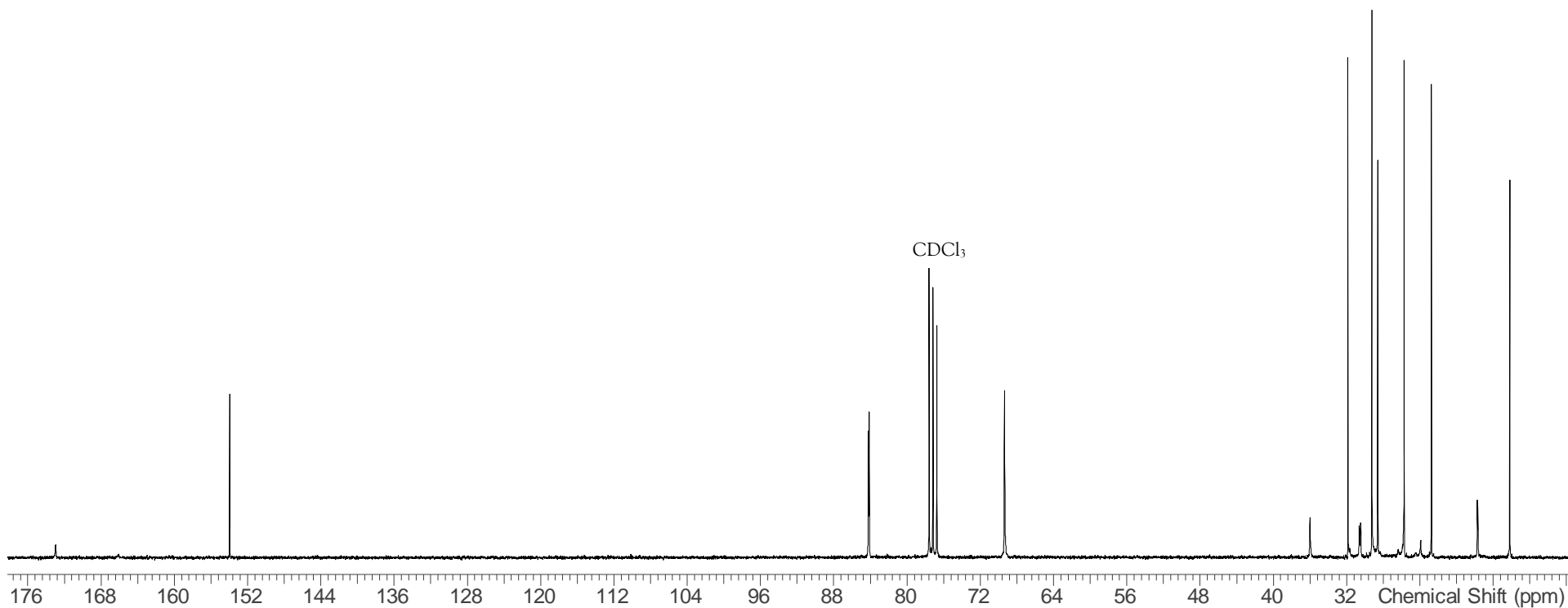
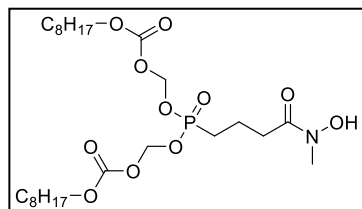
Time	Height	Area	Area%
4.73	13123807	767159.38	100.00



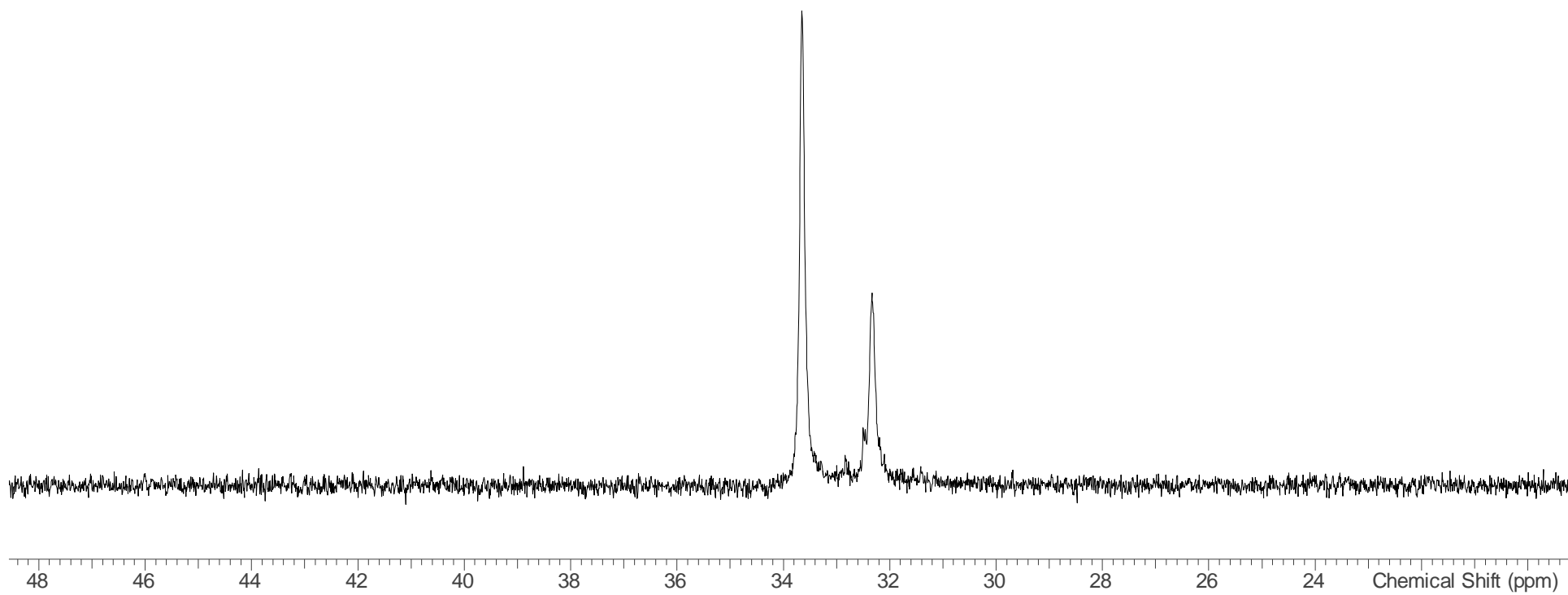
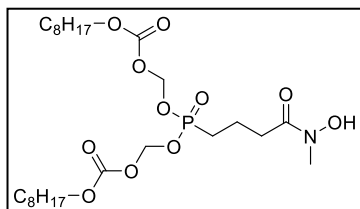
^1H NMR spectrum of **13m** (300MHz, CHLOROFORM-*d*)

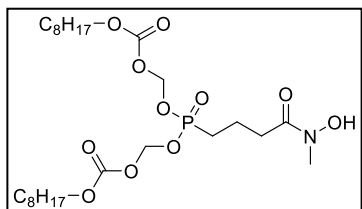


^{13}C NMR spectrum of **13m** (75MHz, CHLOROFORM-*d*)

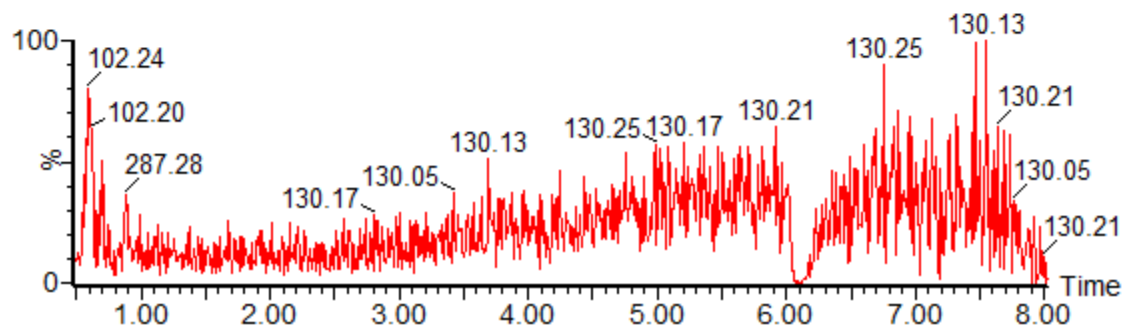
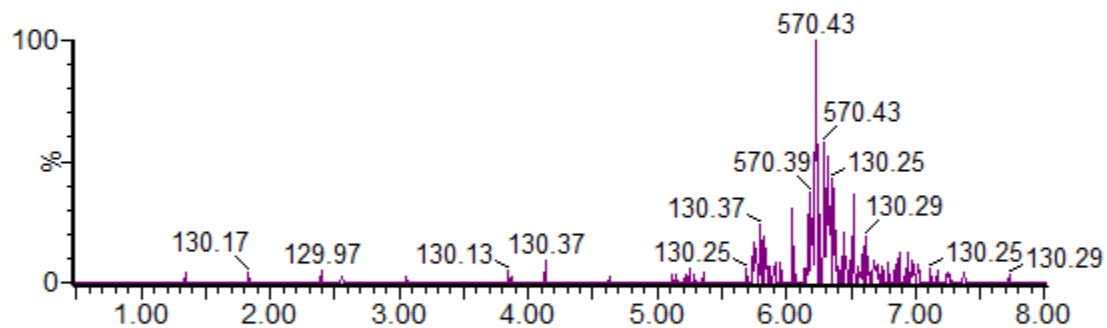
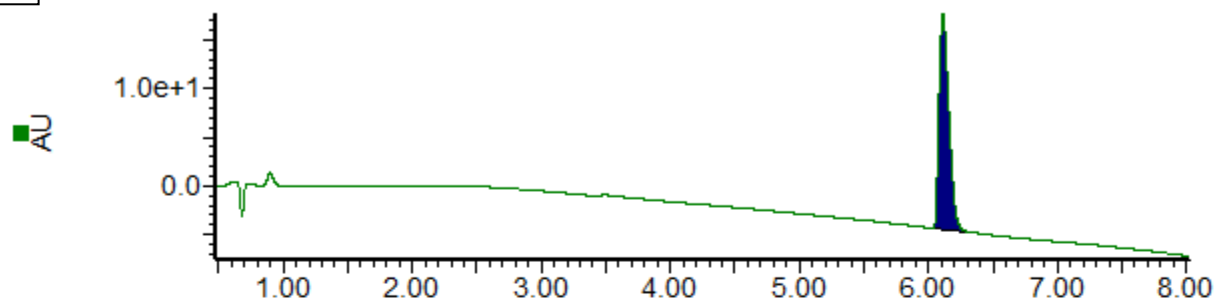


^{31}P NMR spectrum of **13m** (121MHz, CHLOROFORM-*d*)

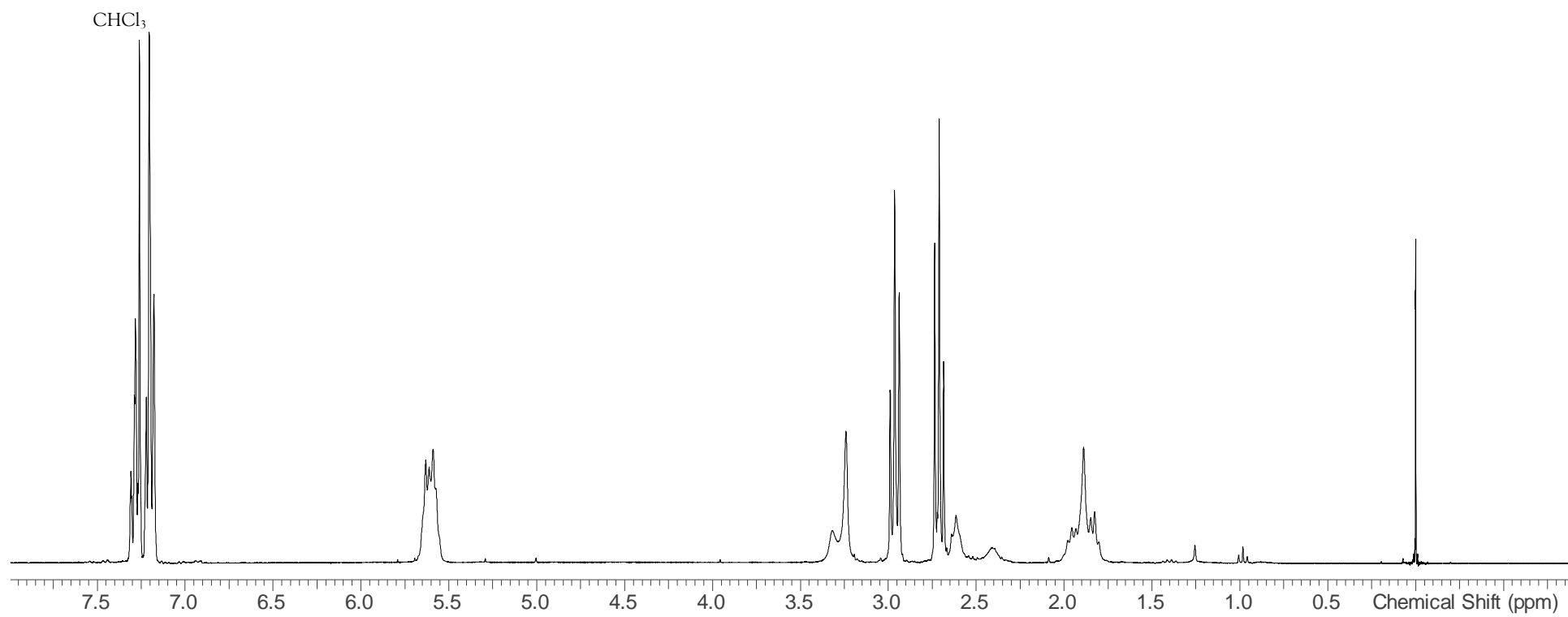
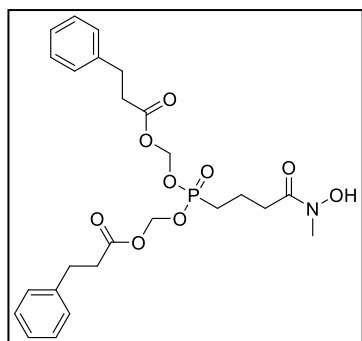




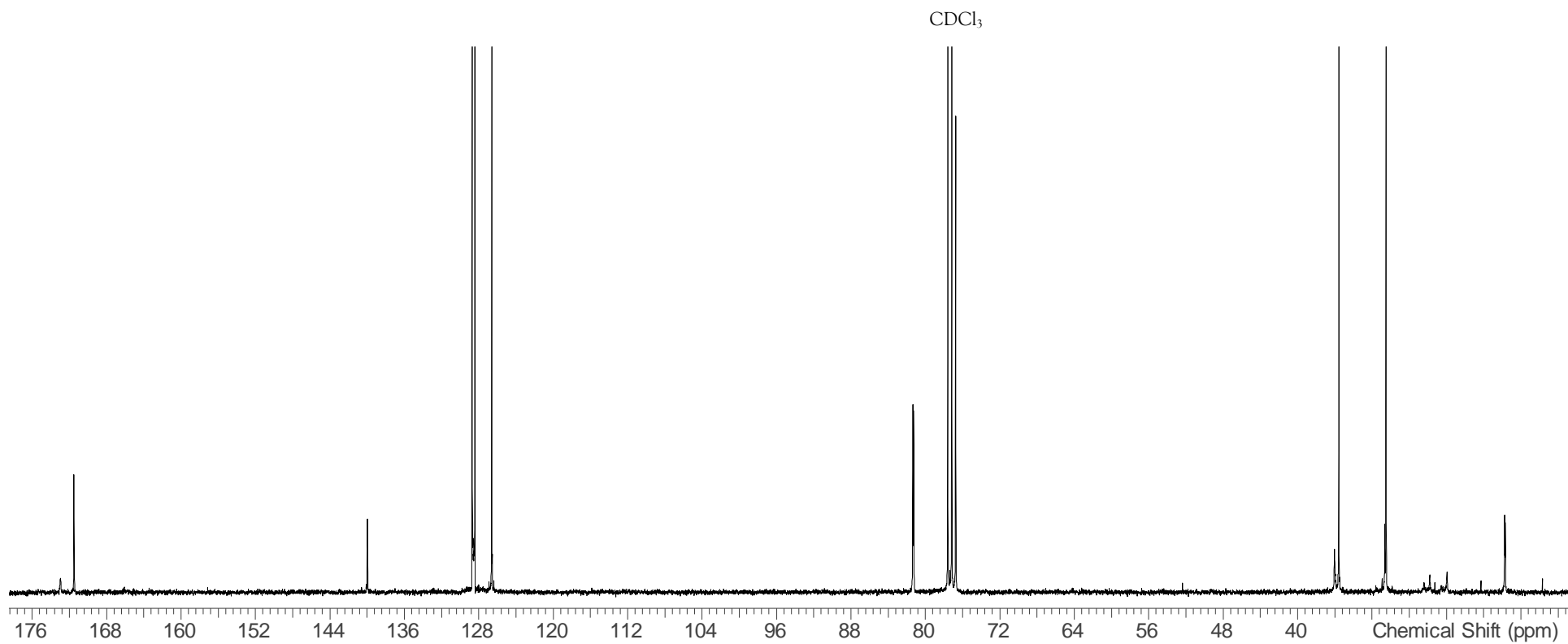
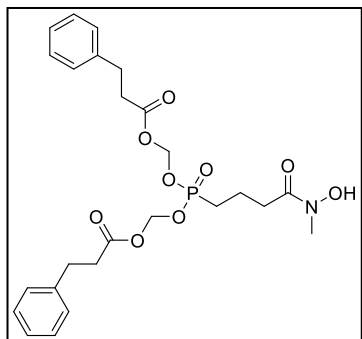
Time	Height	Area	Area%
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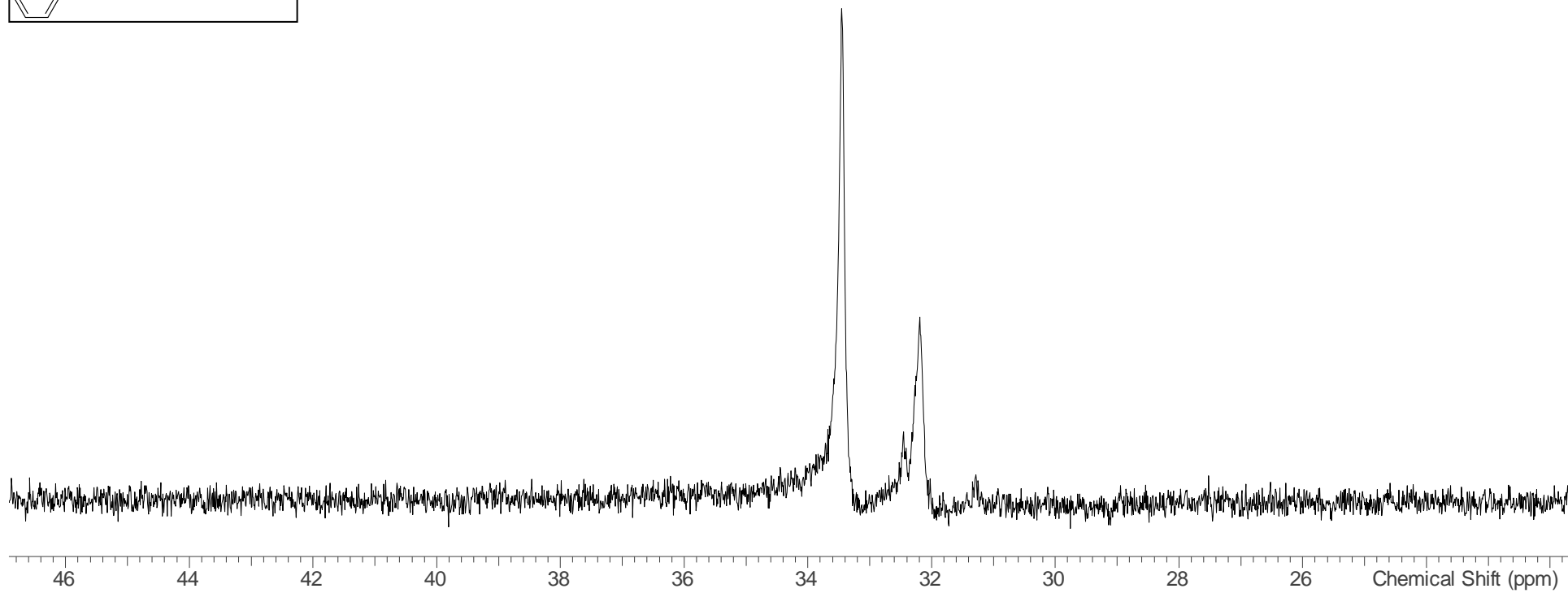
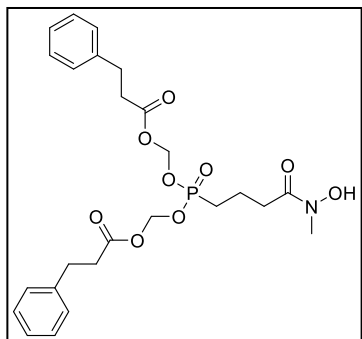
^1H NMR spectrum of **13n** (300MHz, CHLOROFORM-*d*)

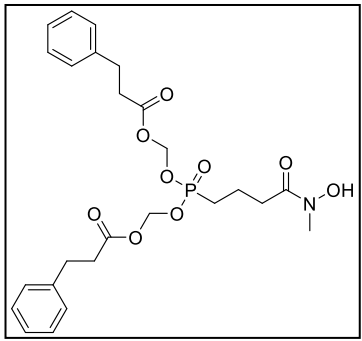


^{13}C NMR spectrum of **13n** (75MHz, CHLOROFORM-*d*)

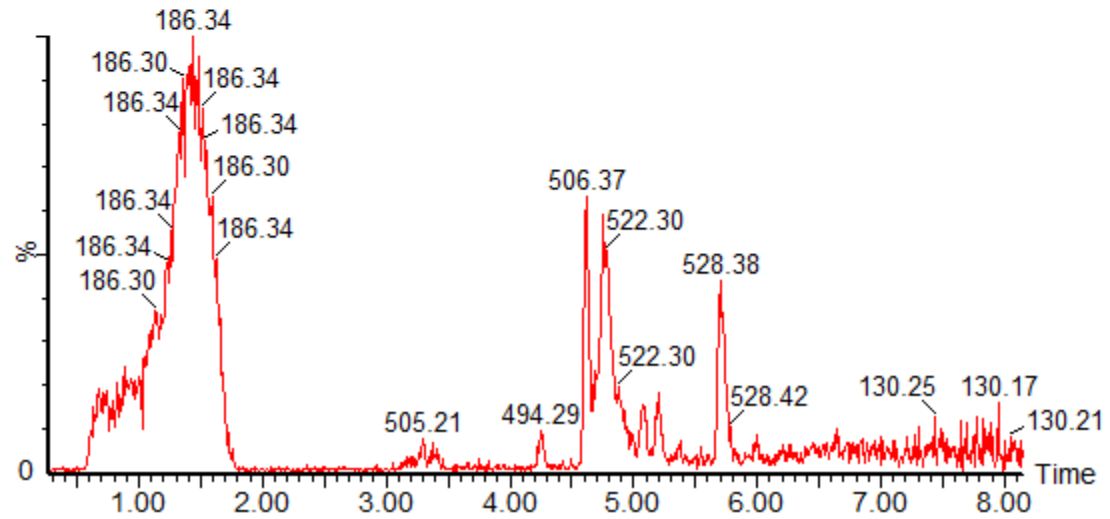
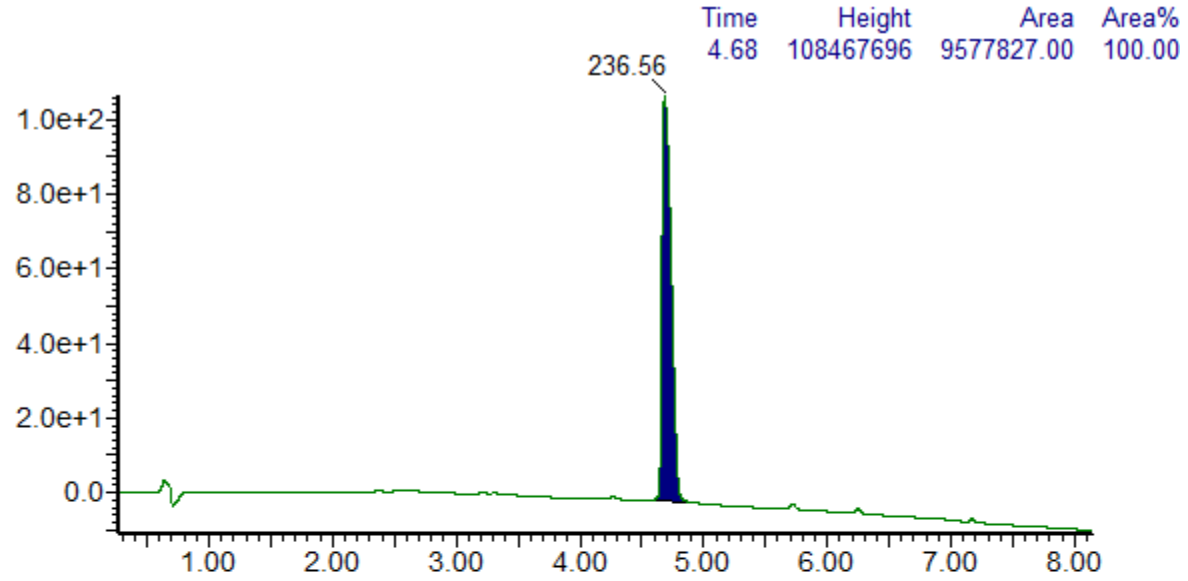


^{31}P NMR spectrum of **13n** (121MHz, CHLOROFORM-*d*)

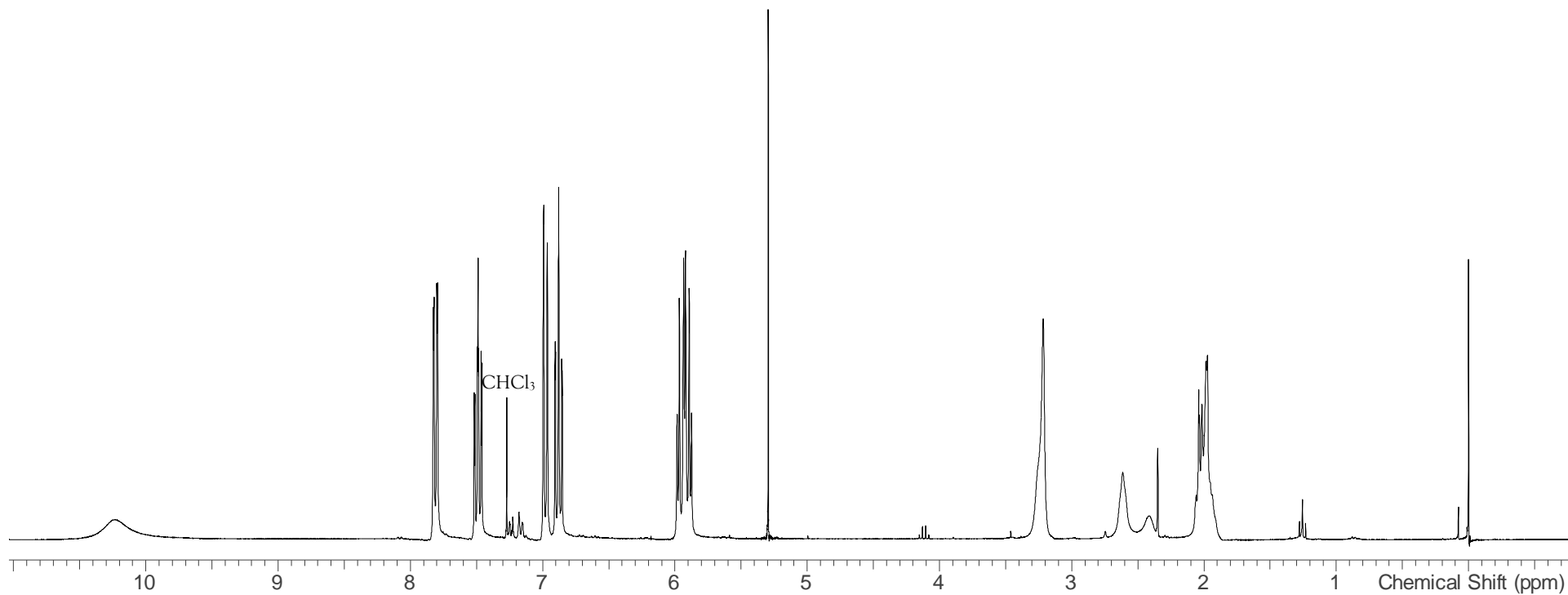
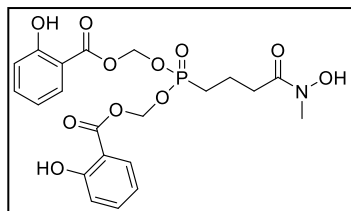




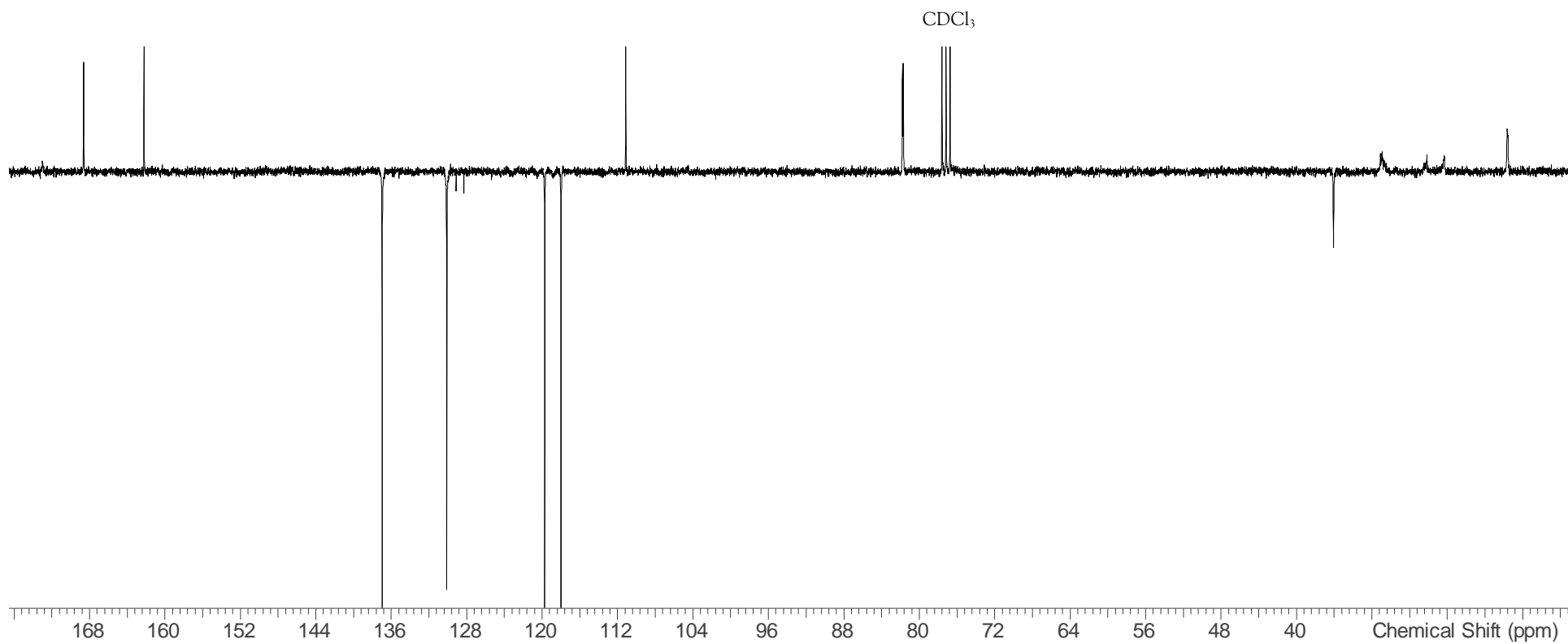
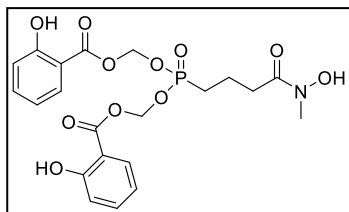
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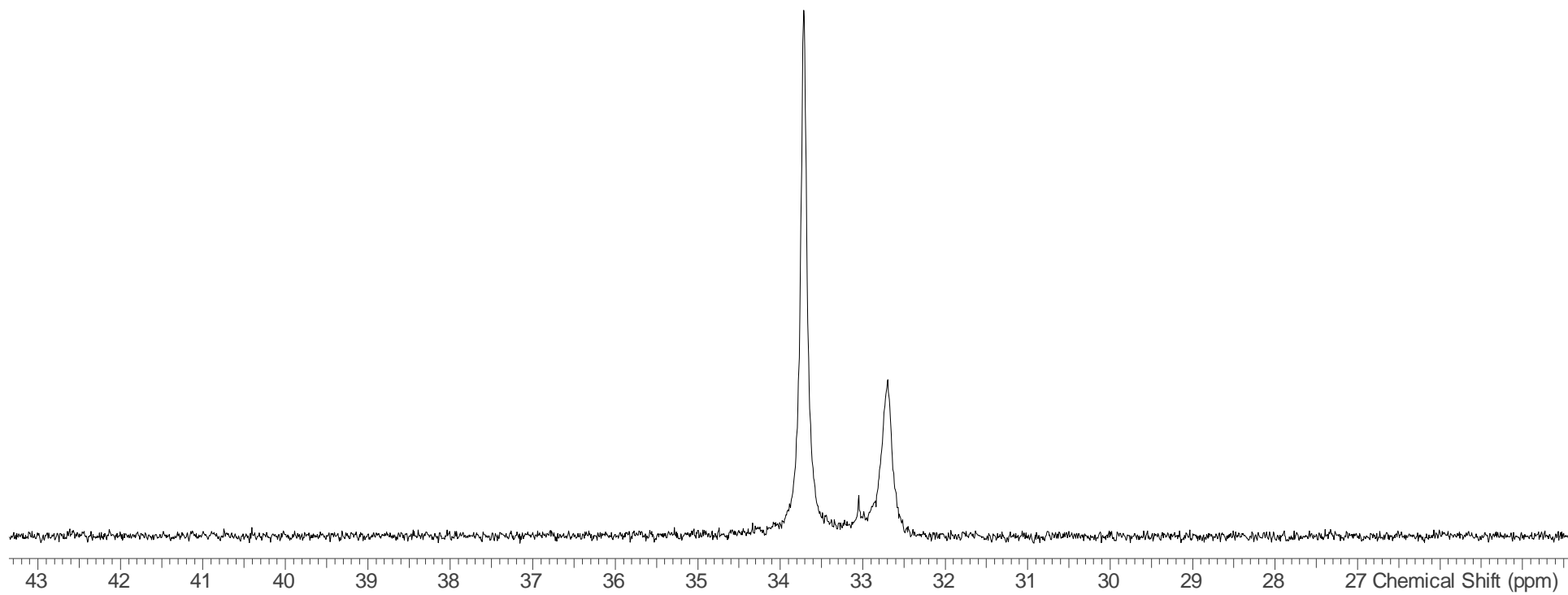
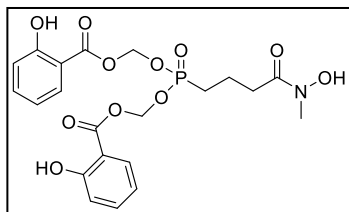
^1H NMR spectrum of 30A (300MHz, CHLOROFORM-*d*)

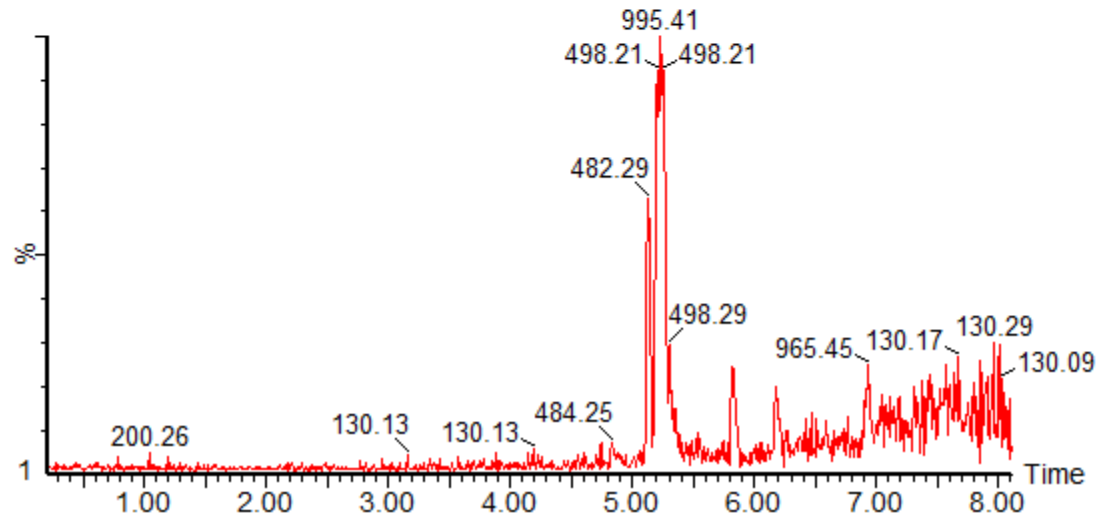
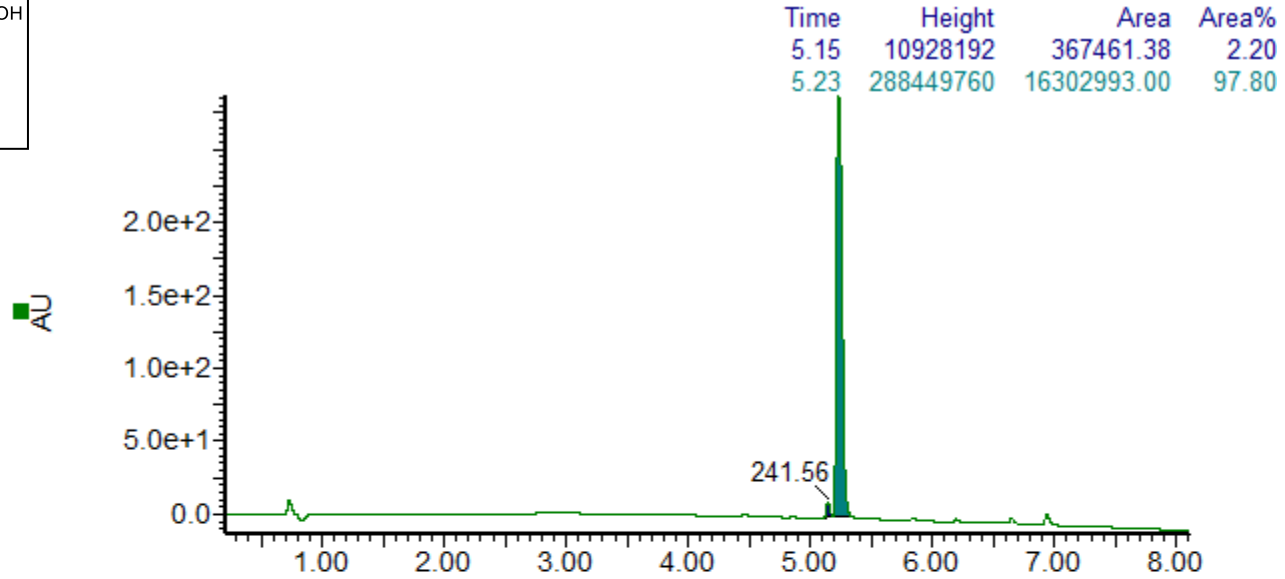
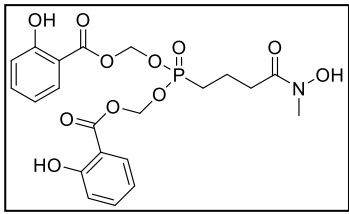


APT NMR spectrum of 30A (75MHz, CHLOROFORM-*d*)

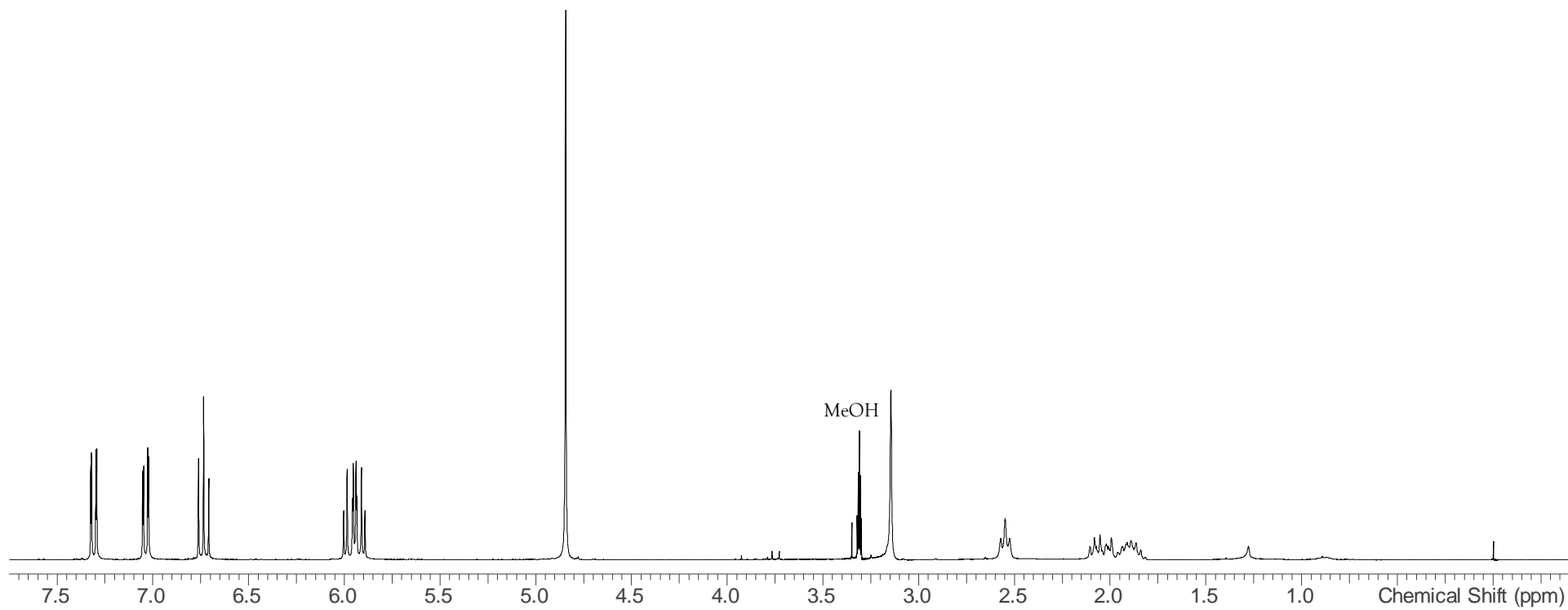
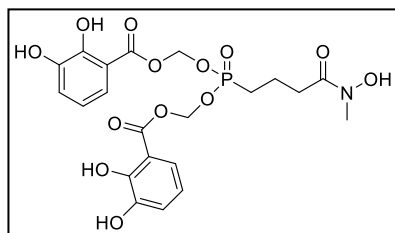


^{31}P NMR spectrum of 30A (121MHz, CHLOROFORM-*d*)

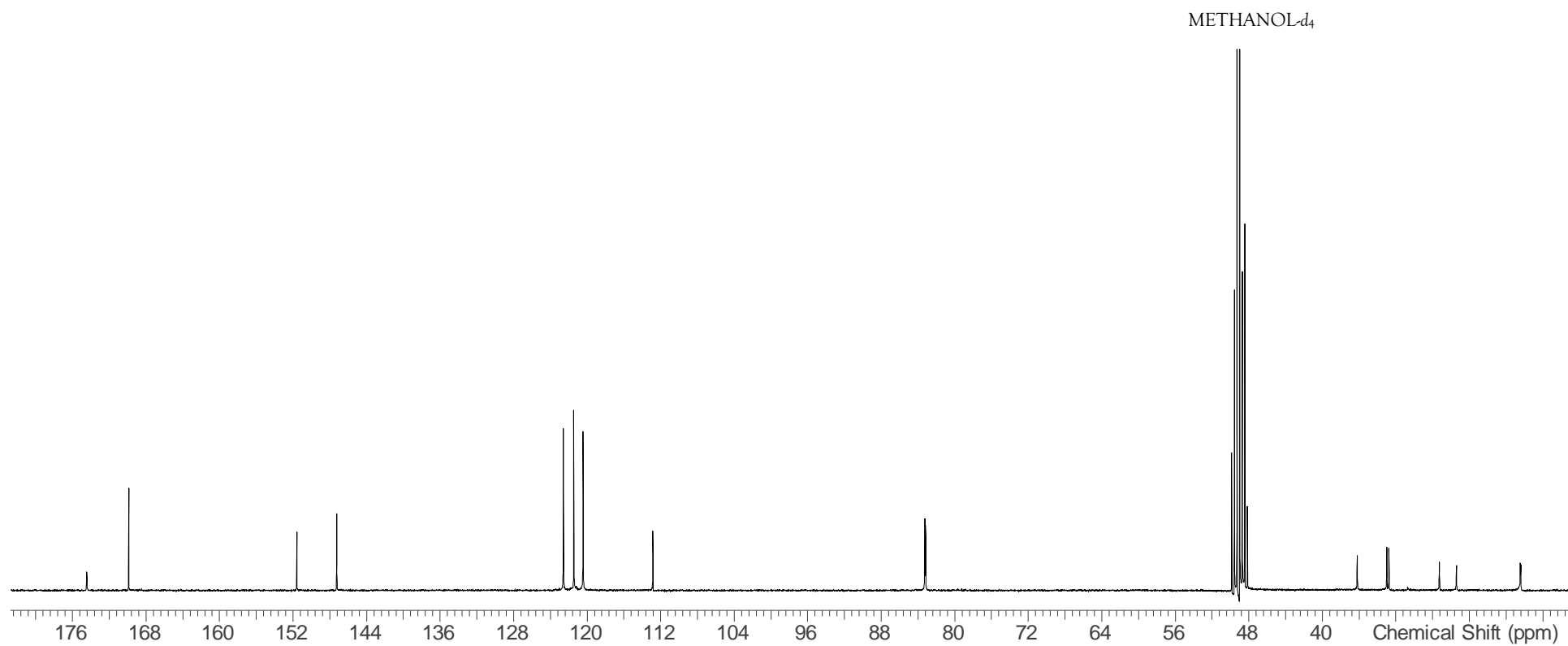
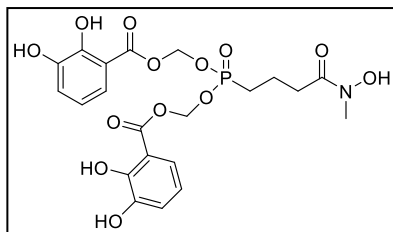


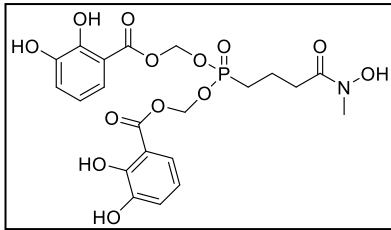


^1H NMR spectrum of **30B** (300MHz, METHANOL- d_4)

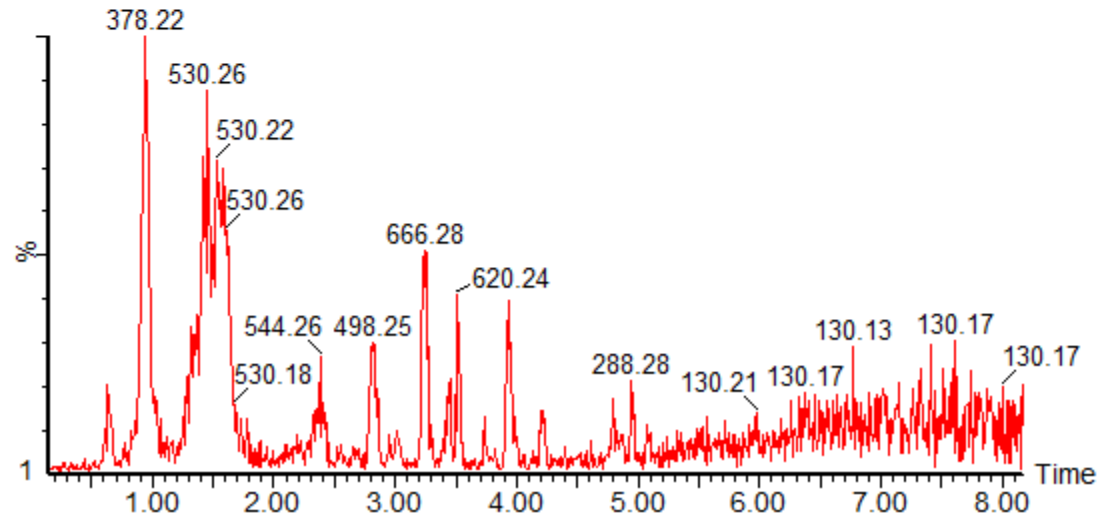
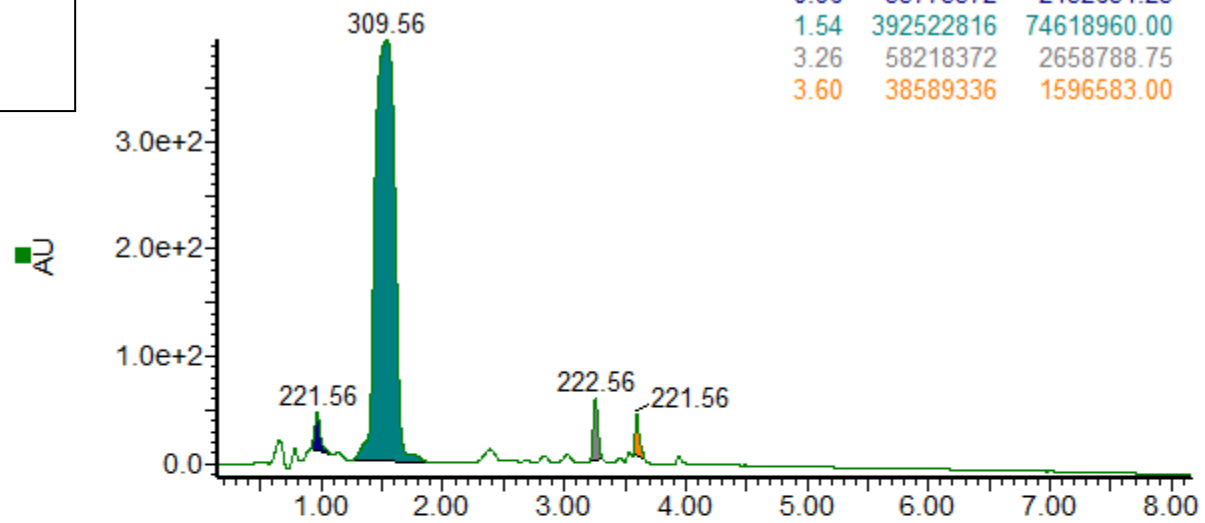


^{13}C NMR spectrum of **30B** (75MHz, METHANOL- d_4)

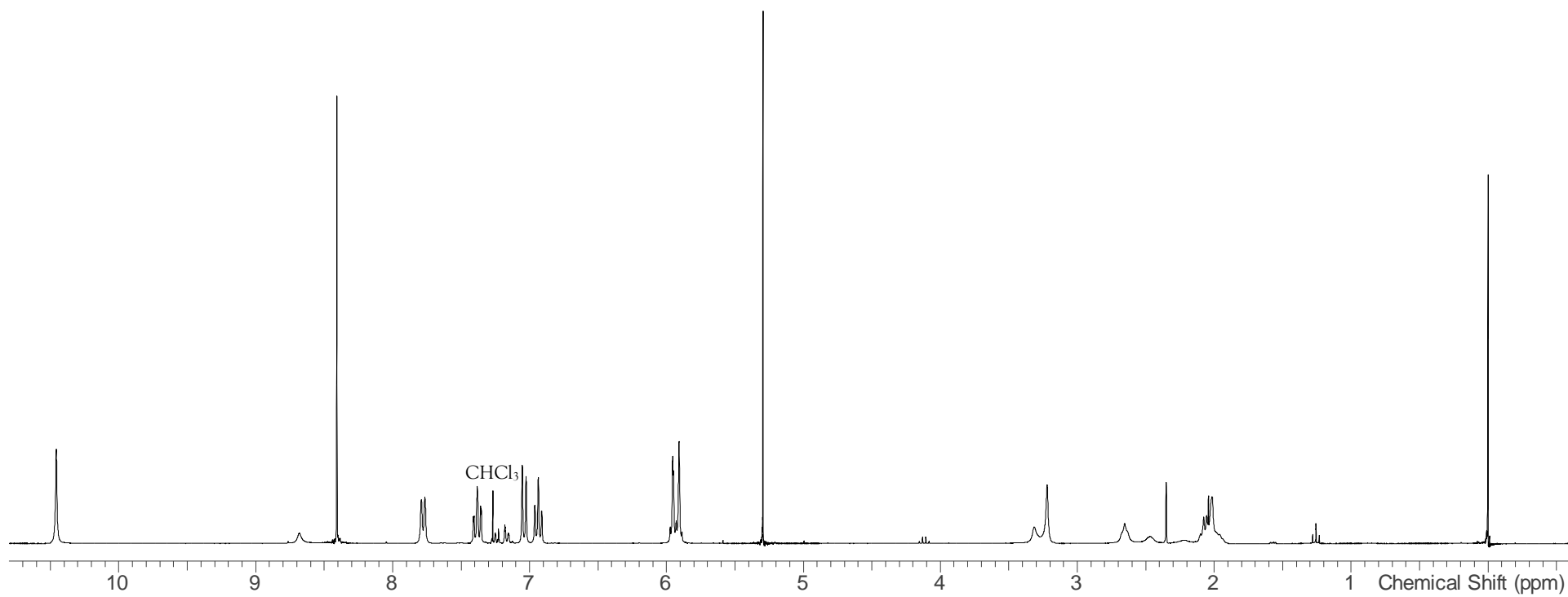
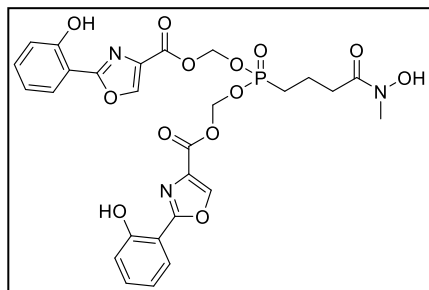




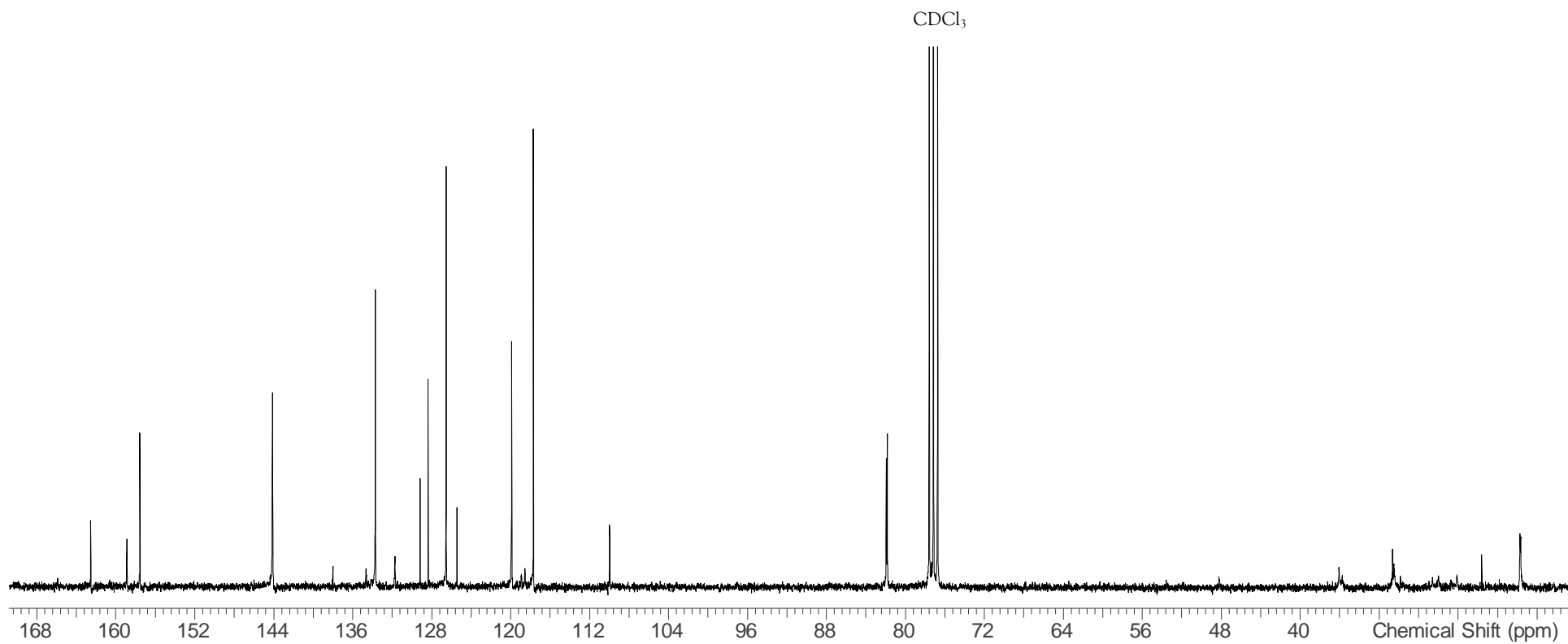
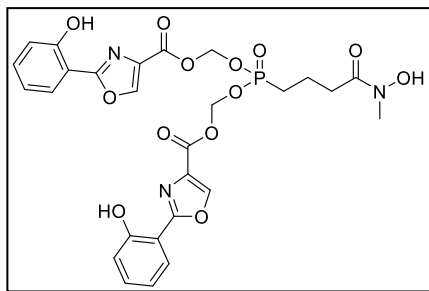
Time	Height	Area	Area%
0.96	35775572	2132631.25	2.63
1.54	392522816	74618960.00	92.11
3.26	58218372	2658788.75	3.28
3.60	38589336	1596583.00	1.97



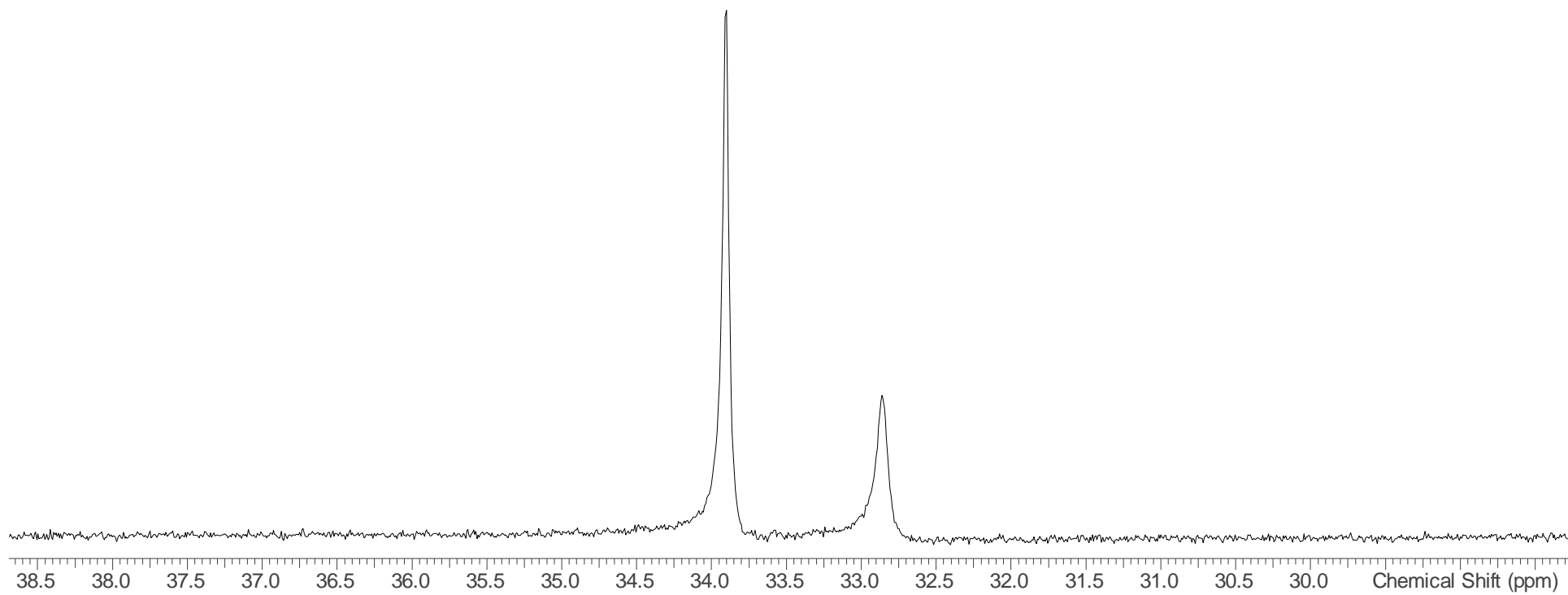
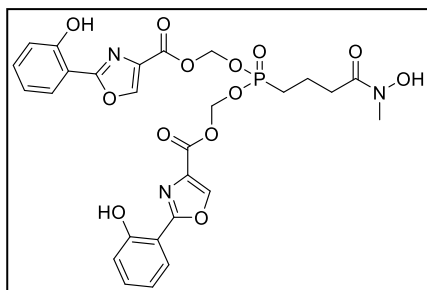
^1H NMR spectrum of 30C (300MHz, CHLOROFORM-*d*)

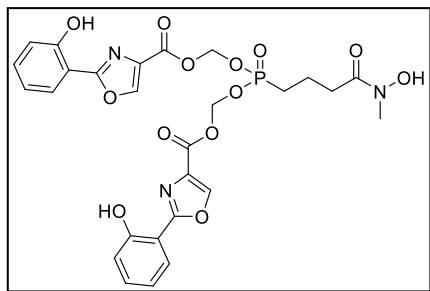


^{13}C NMR spectrum of 30C (75MHz, CHLOROFORM-*d*)

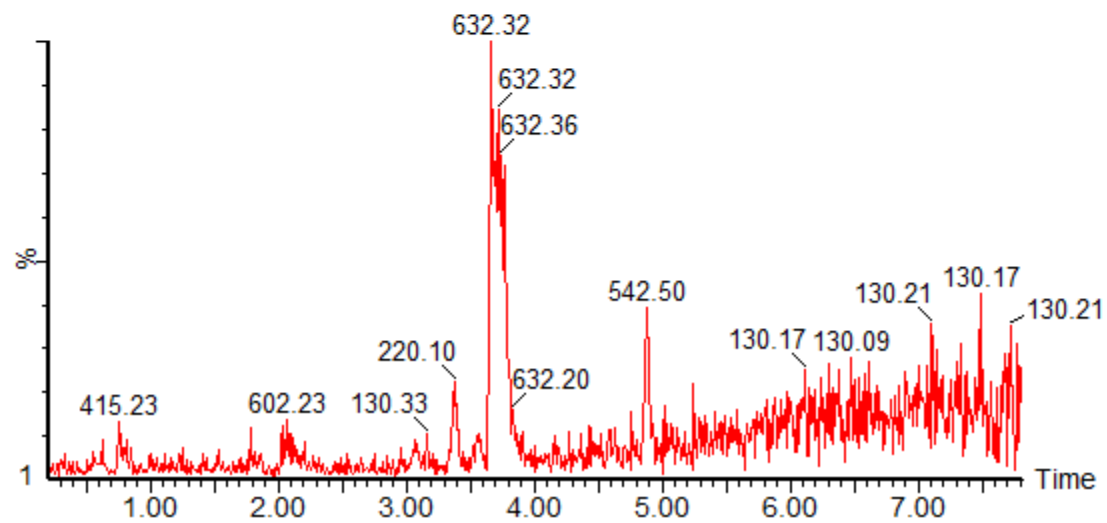
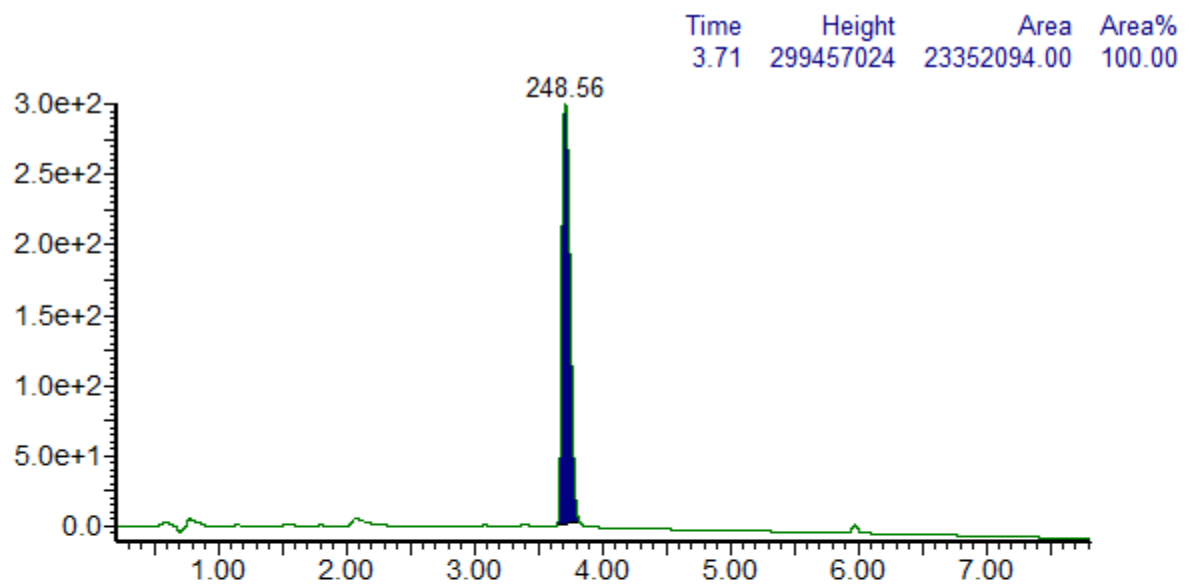


^{31}P NMR spectrum of 30C (121MHz, CHLOROFORM-*d*)

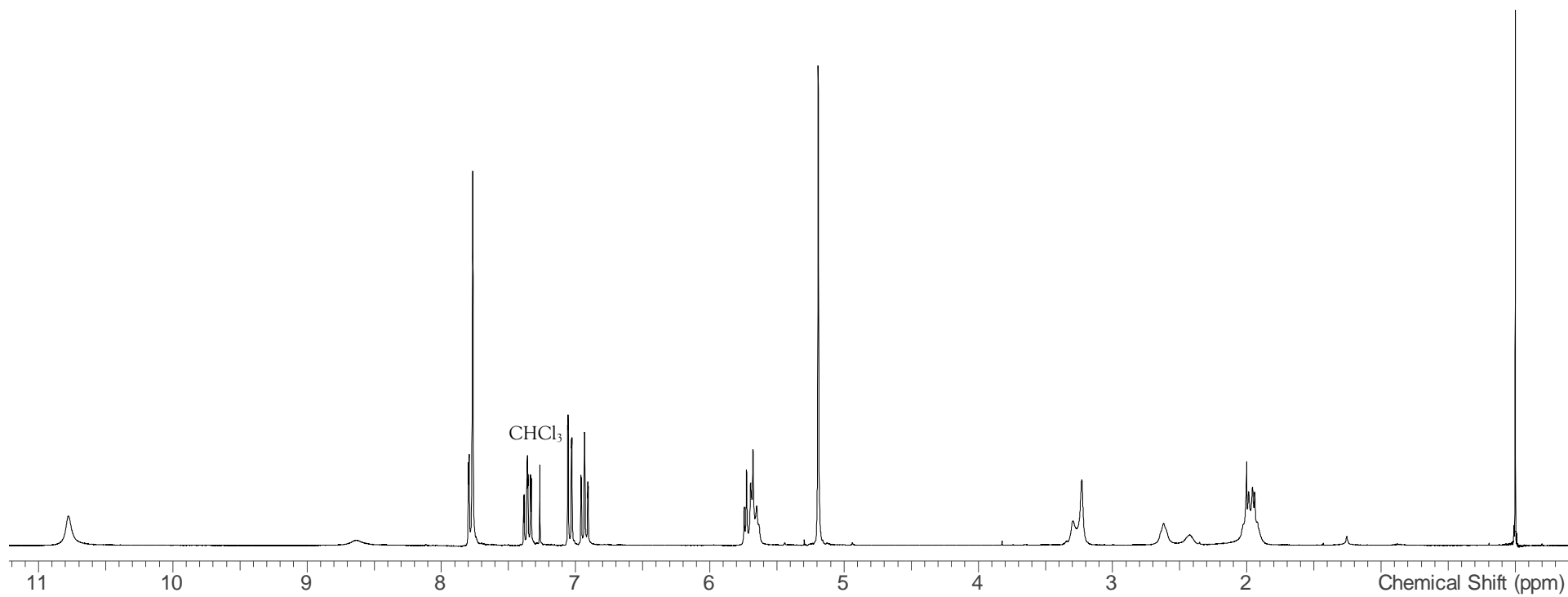
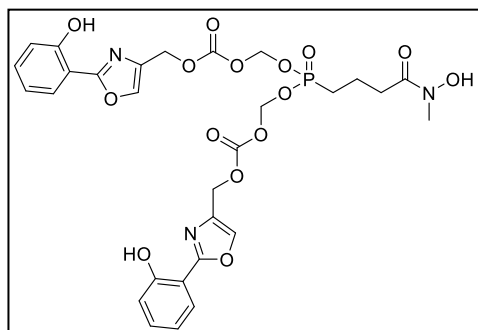




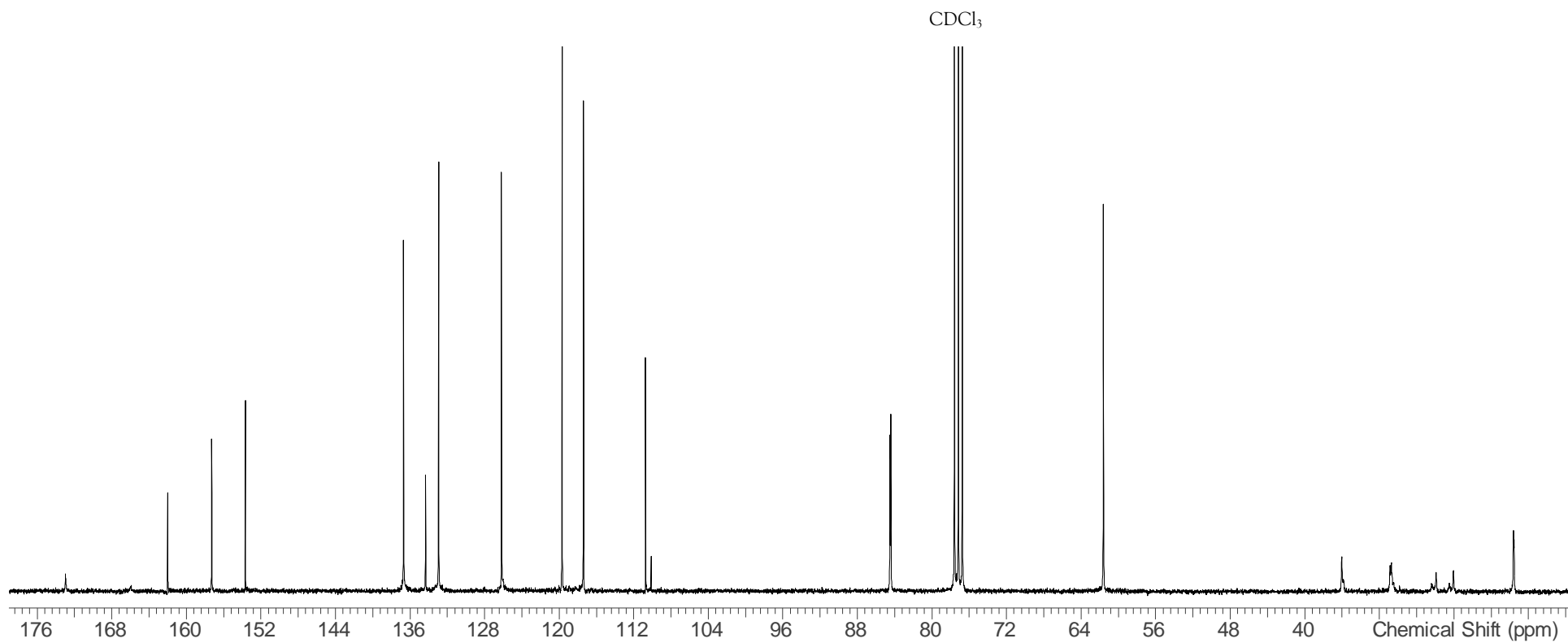
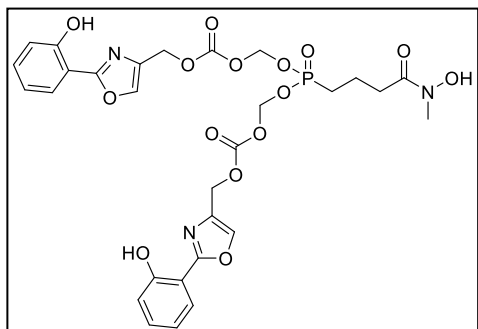
AU



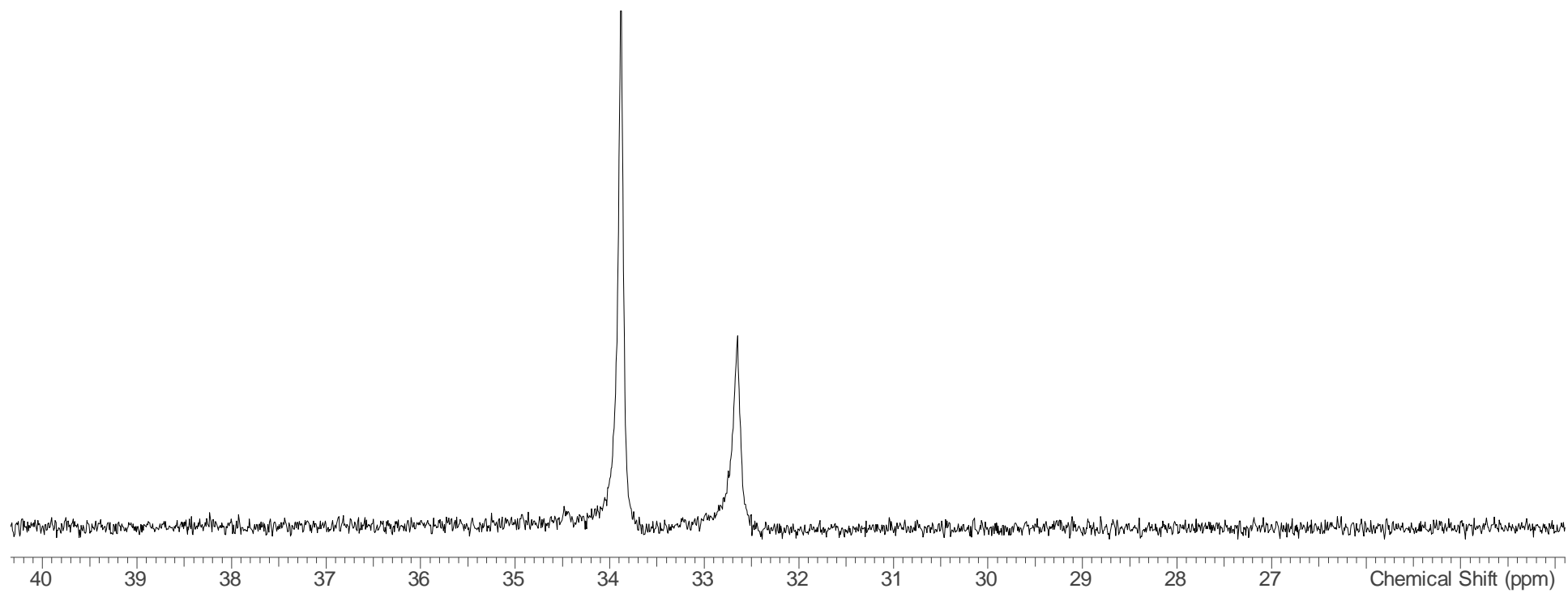
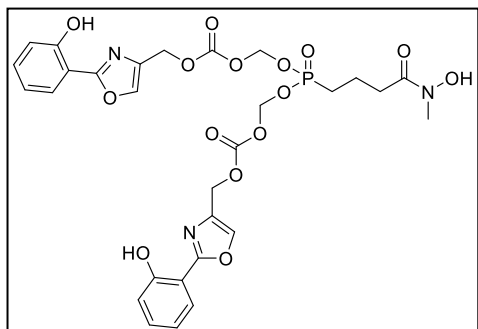
^1H NMR spectrum of 30D (300MHz, CHLOROFORM-*d*)

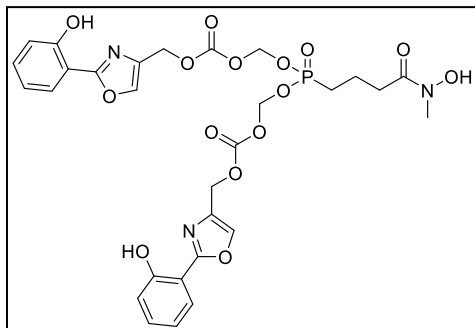


^{13}C NMR spectrum of **30D** (75MHz, CHLOROFORM-*d*)

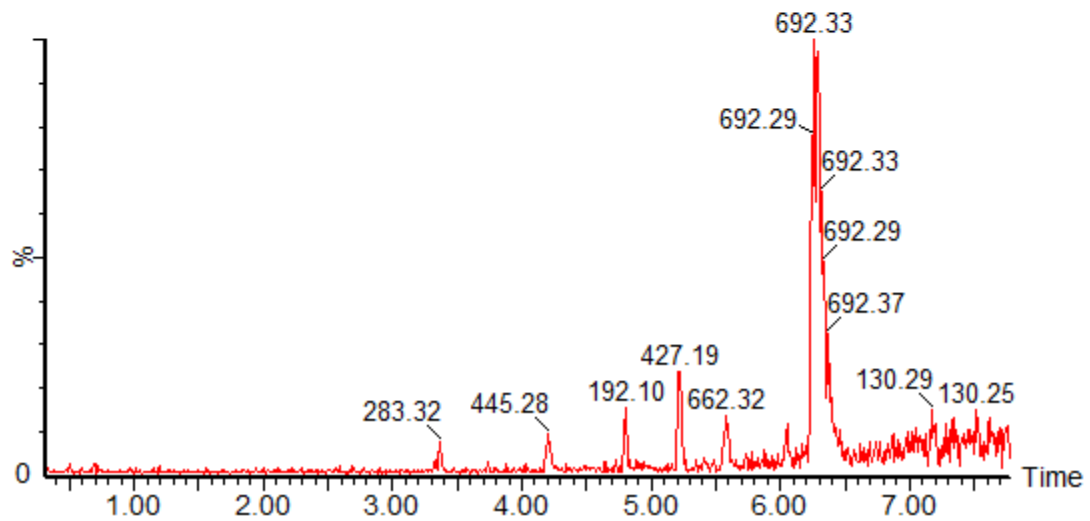
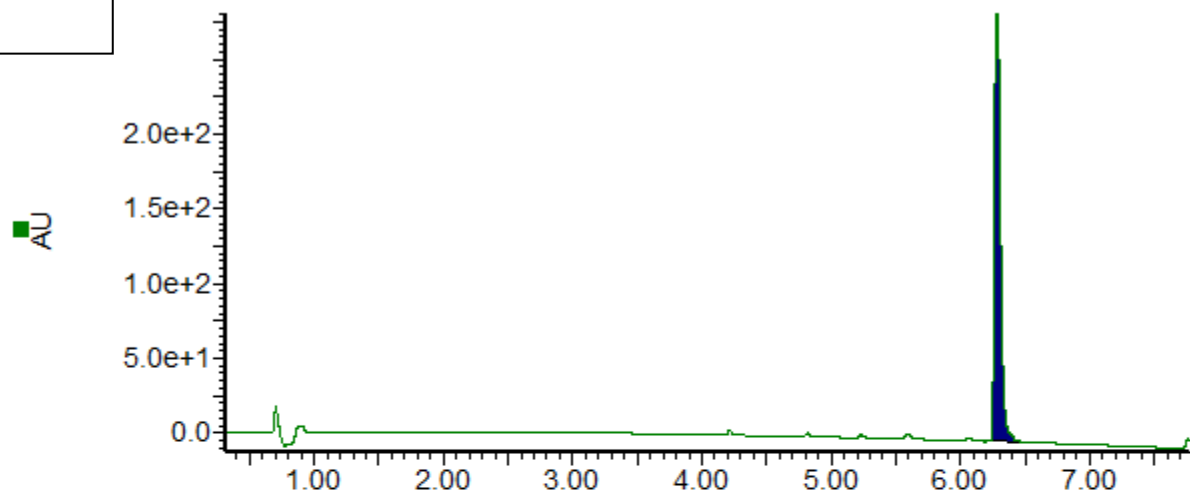


^{31}P NMR spectrum of **30D** (121MHz, CHLOROFORM-*d*)

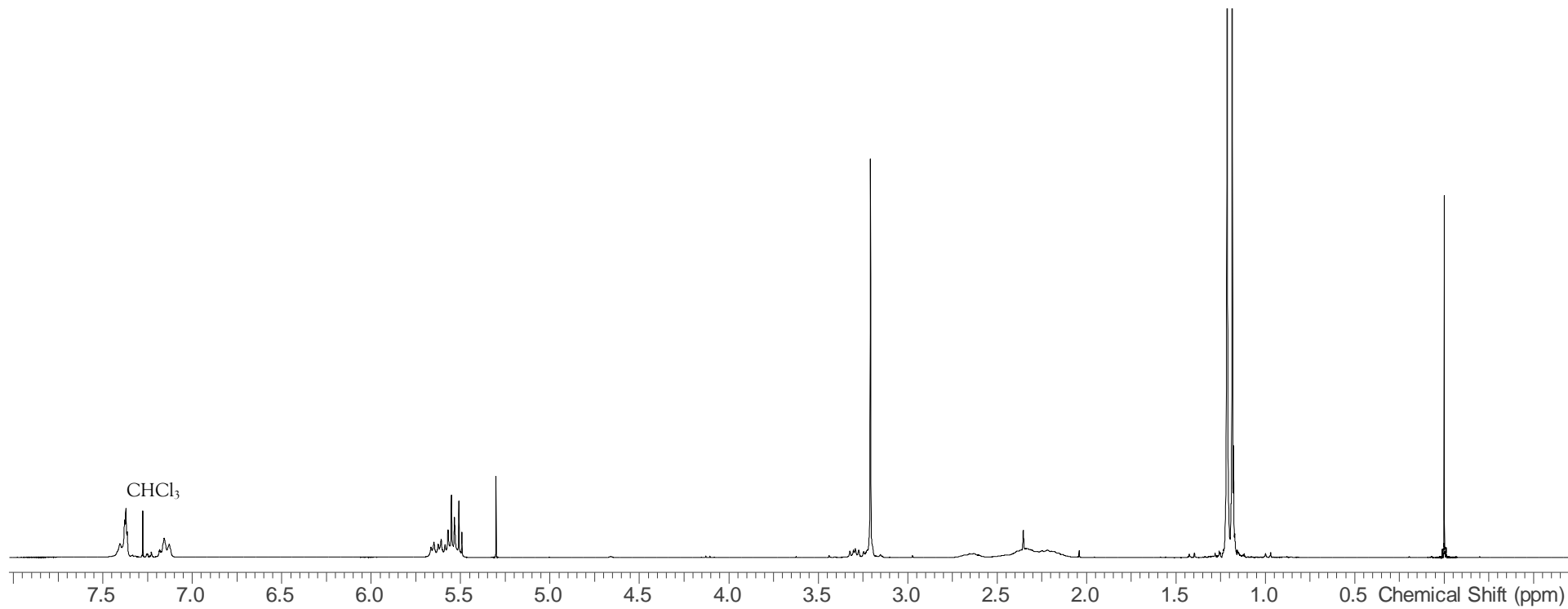
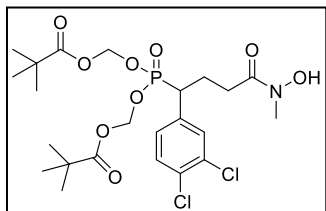




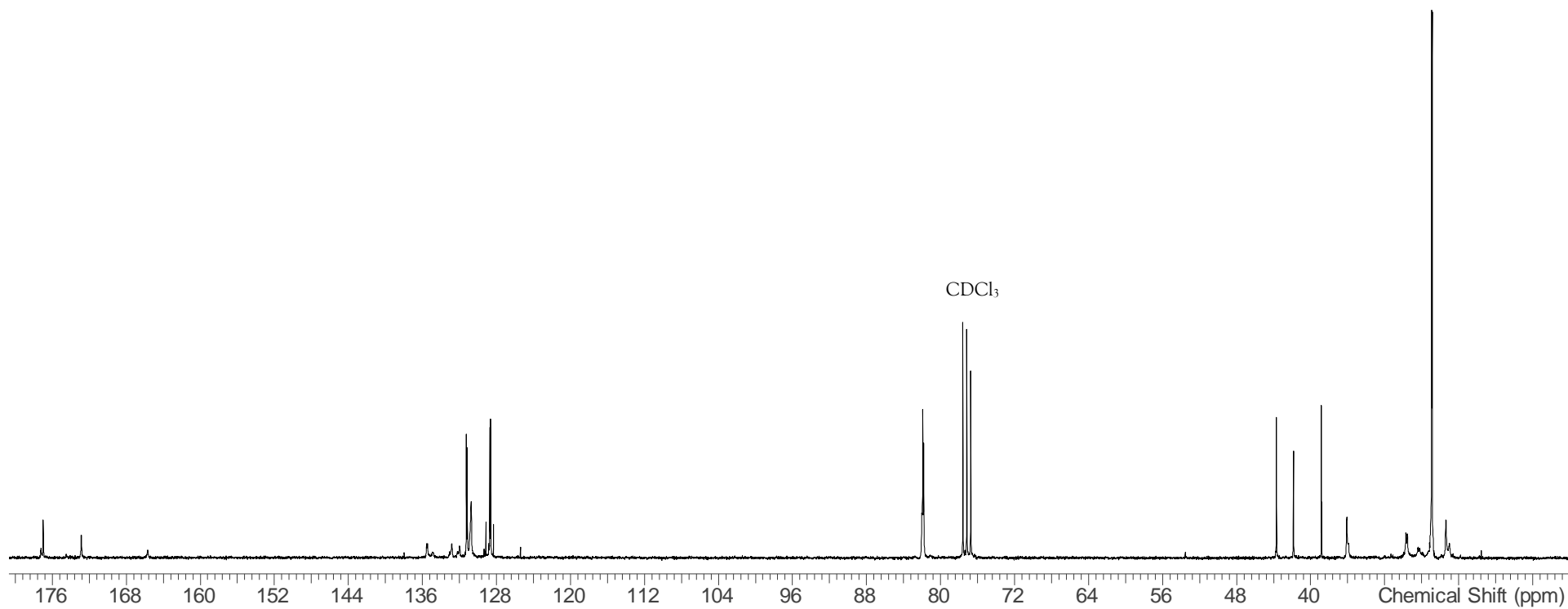
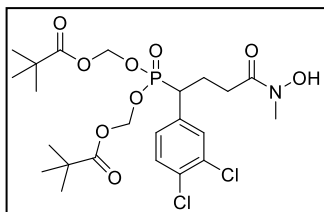
Time	Height	Area	Area%
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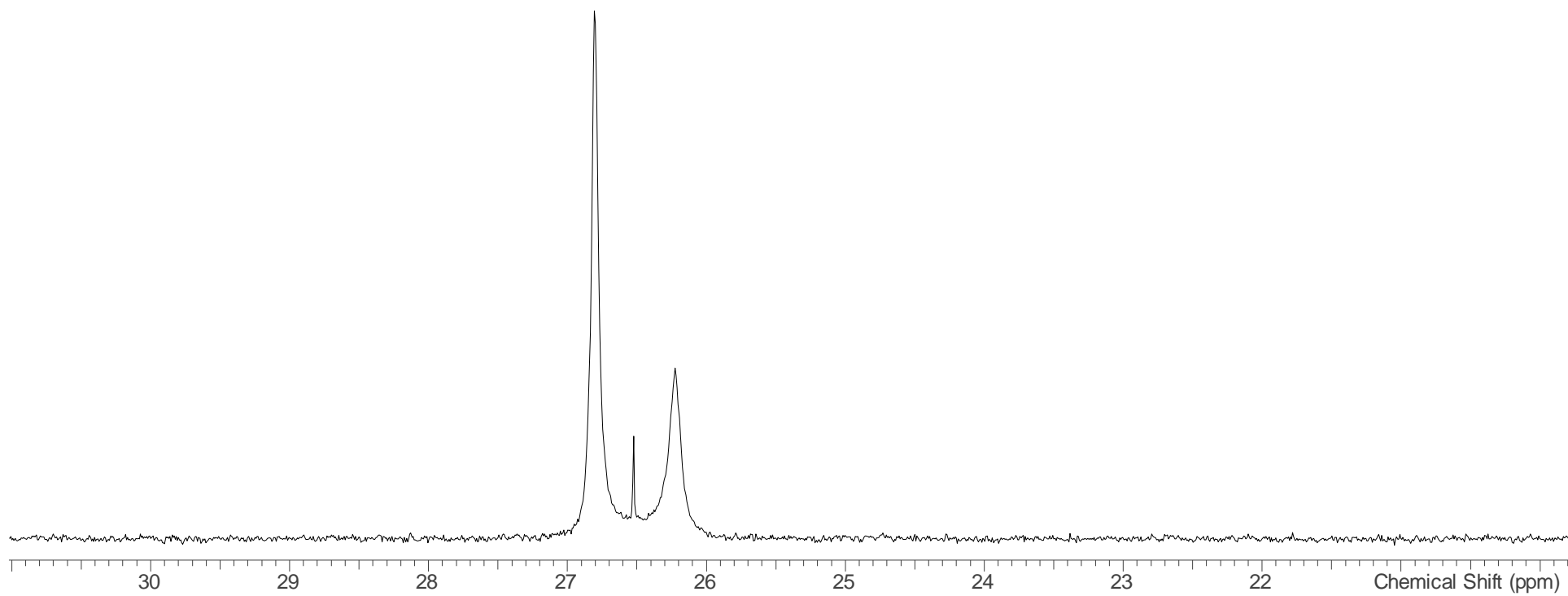
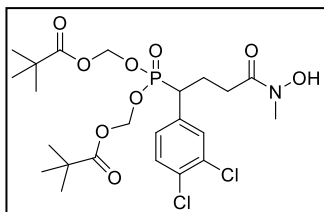
^1H NMR spectrum of **40a** (300MHz, CHLOROFORM-*d*)

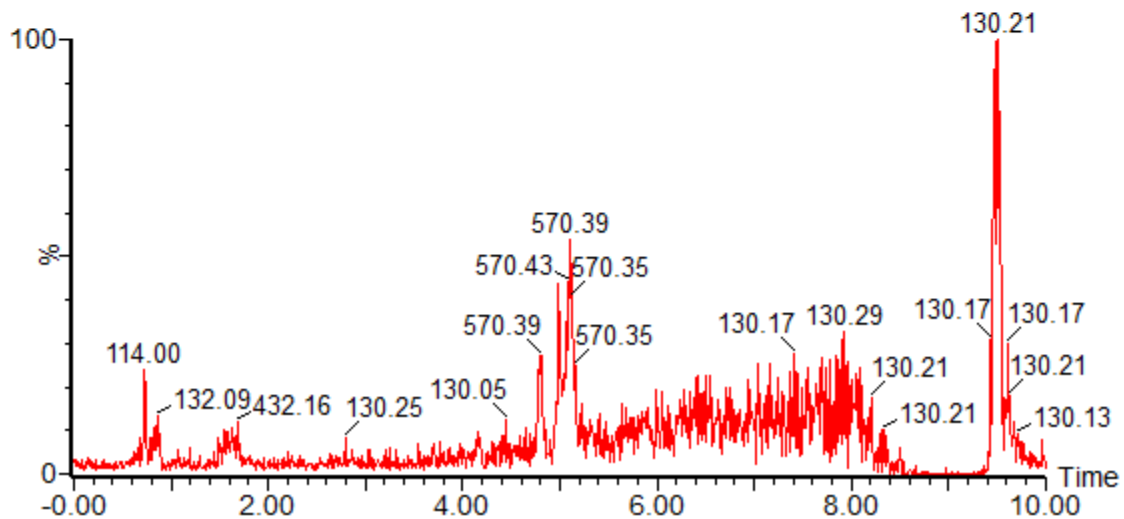
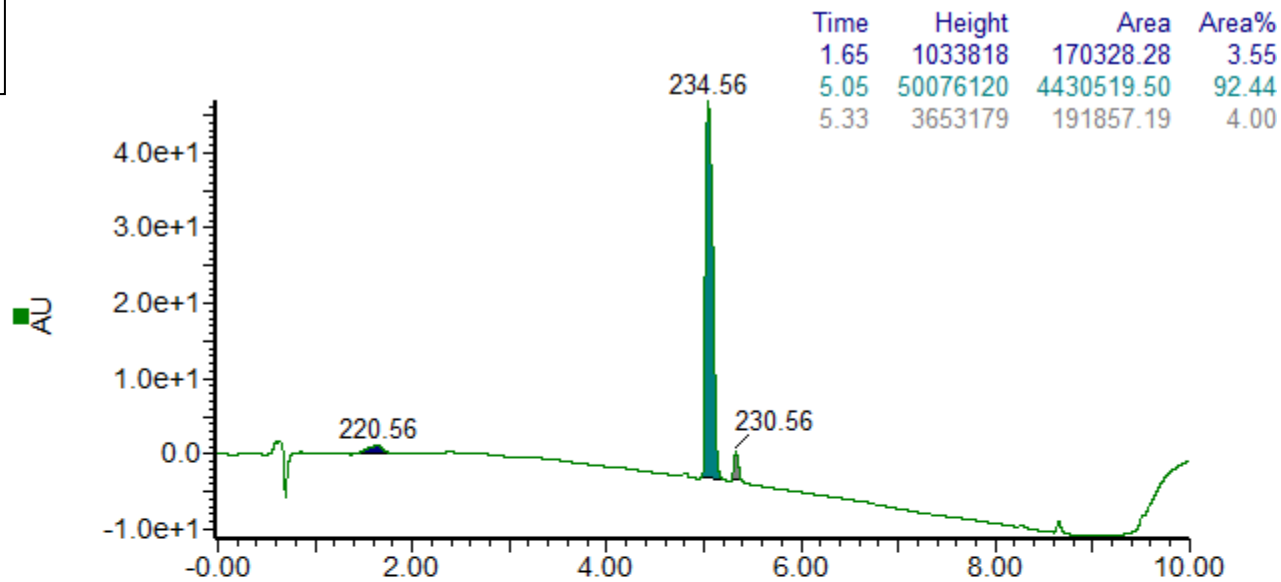
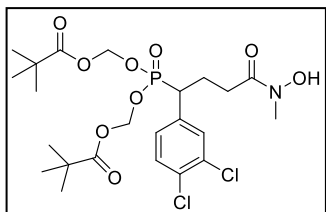


^{13}C NMR spectrum of **40a** (75MHz, CHLOROFORM-*d*)

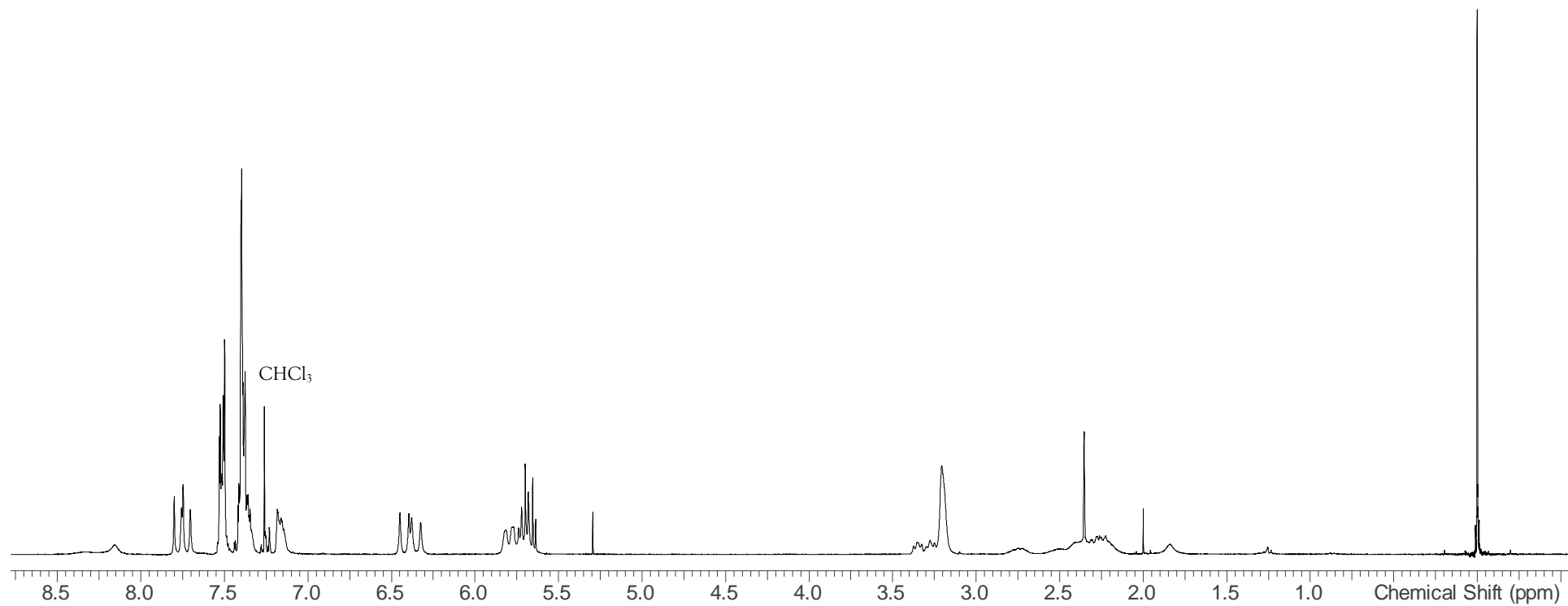
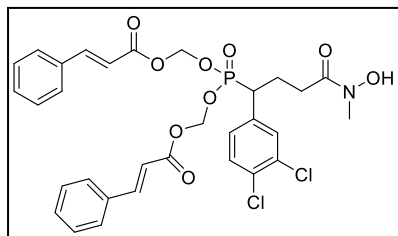


^{31}P NMR spectrum of **40a** (121MHz, CHLOROFORM-*d*)

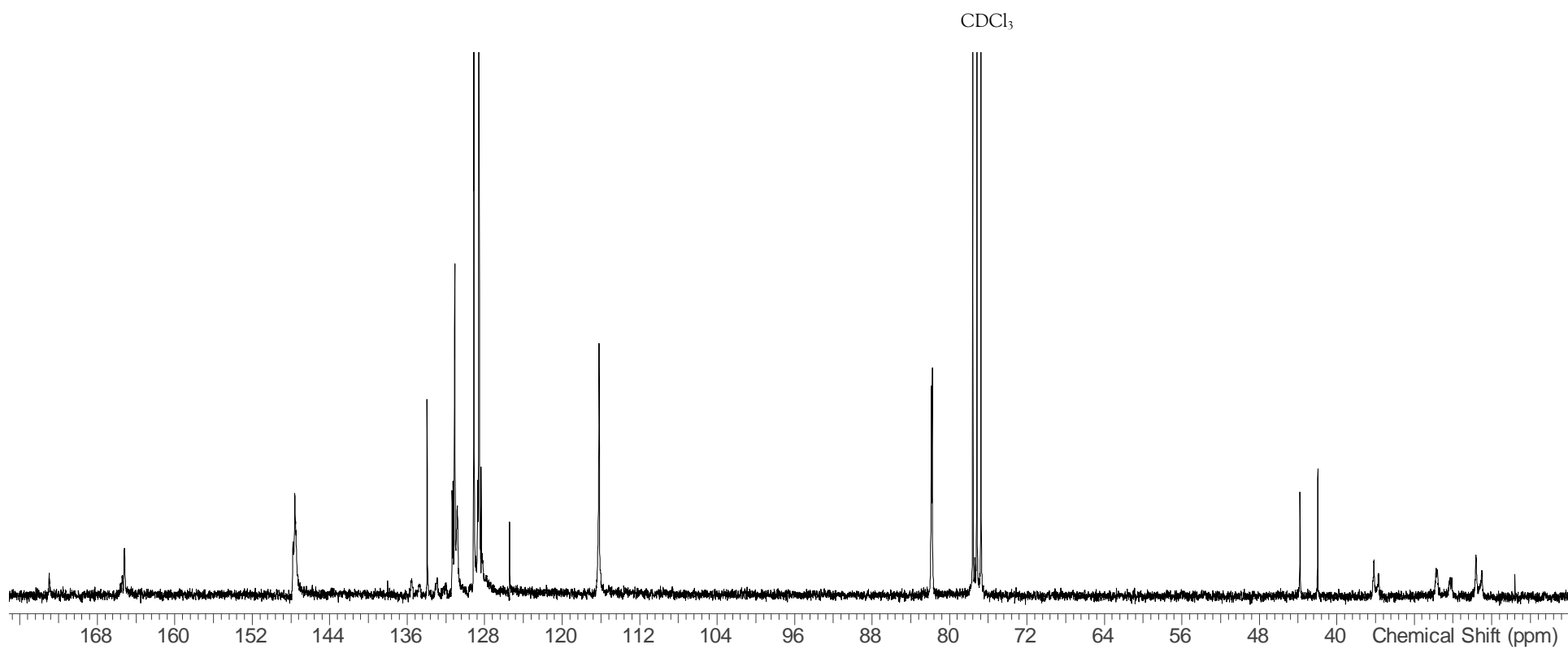
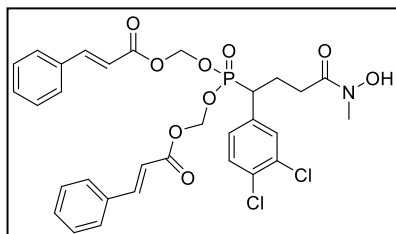




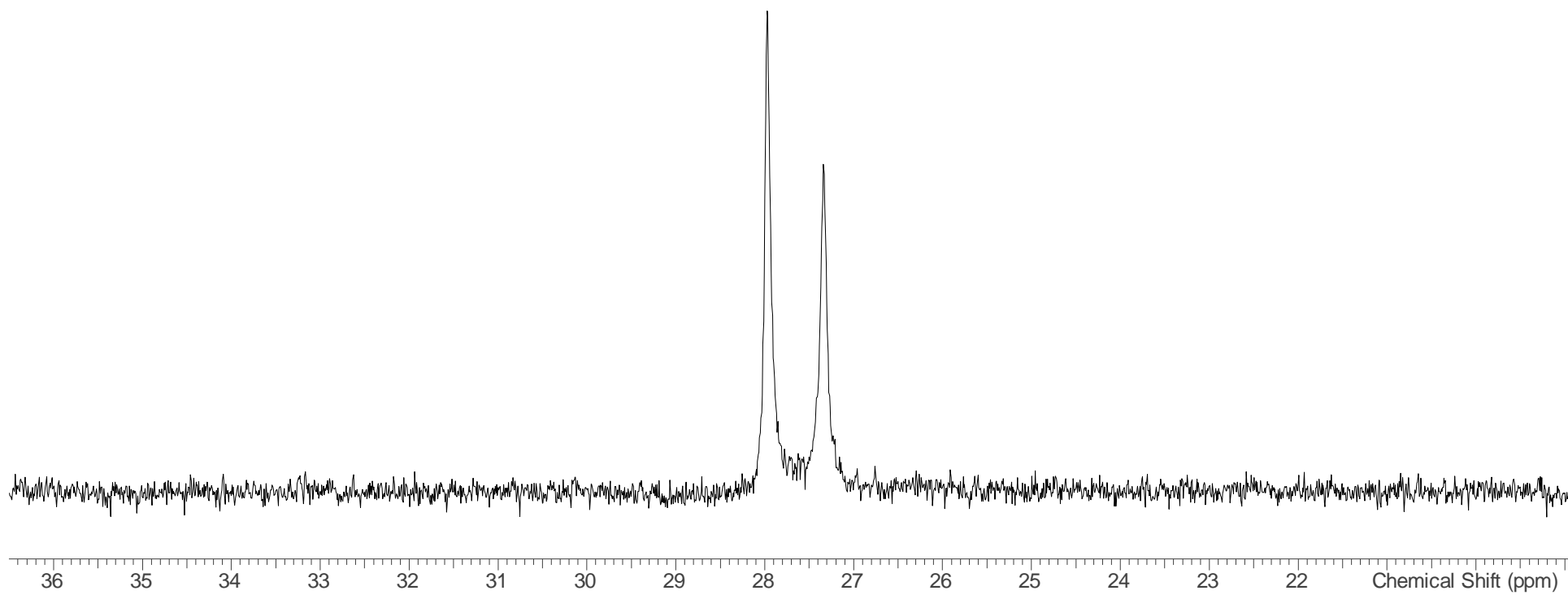
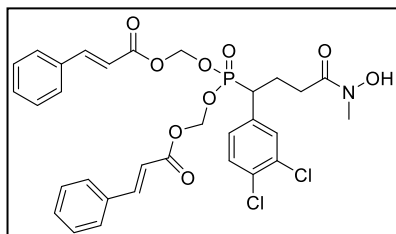
^1H NMR spectrum of **40h** (300MHz, CHLOROFORM-*d*)

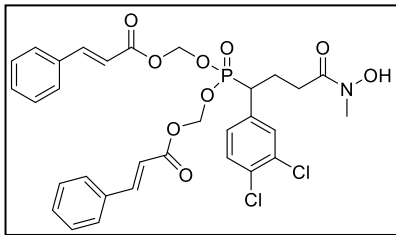


^{13}C NMR spectrum of **40h** (75MHz, CHLOROFORM-*d*)



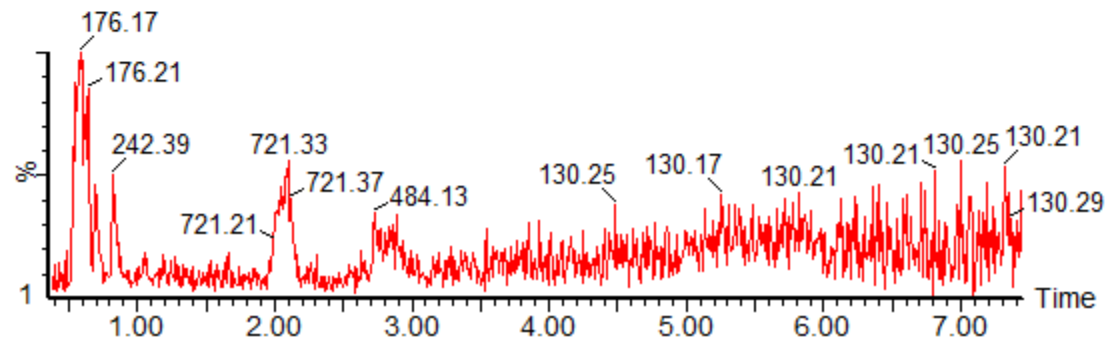
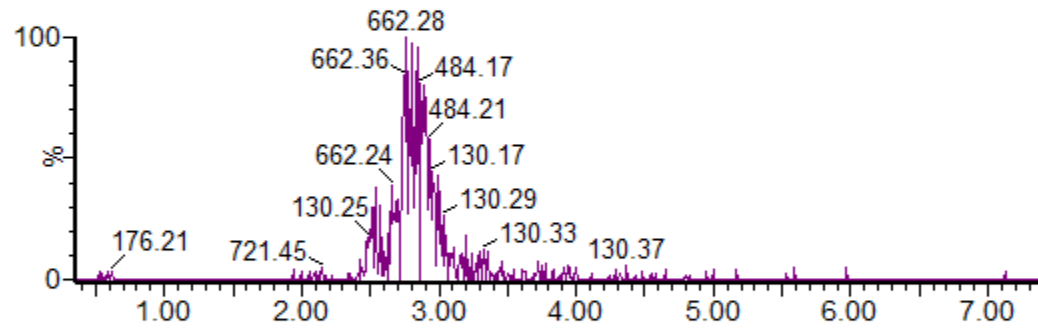
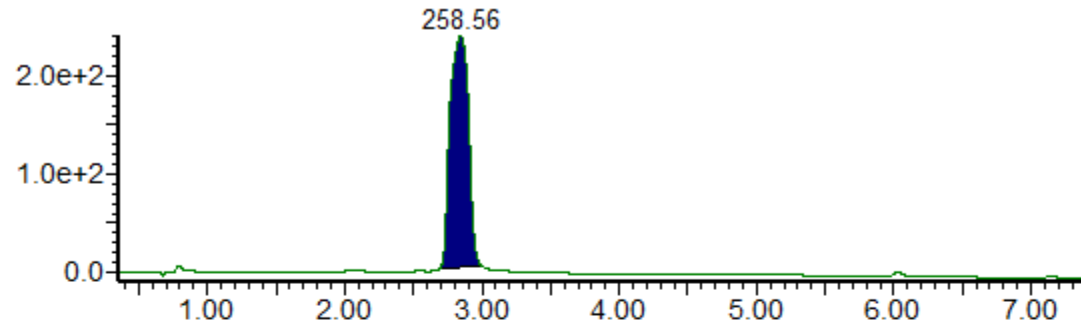
^{31}P NMR spectrum of **40h** (121MHz, CHLOROFORM-*d*)



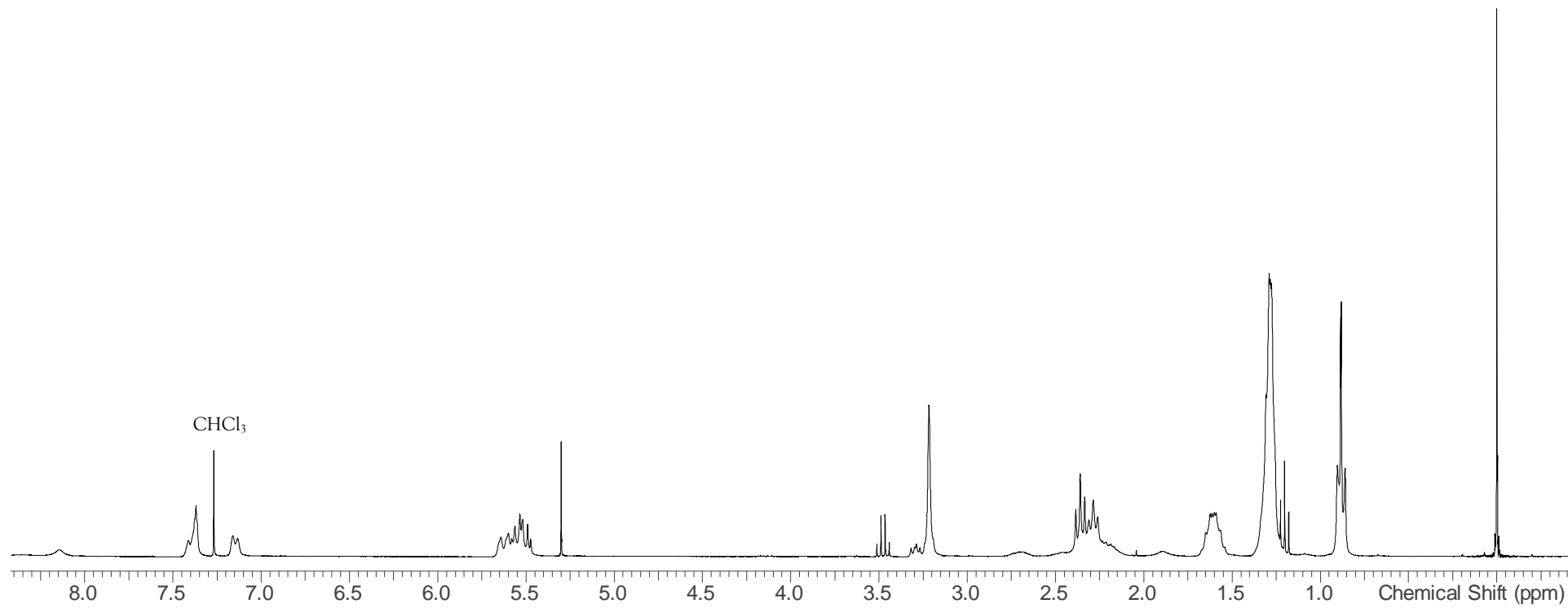
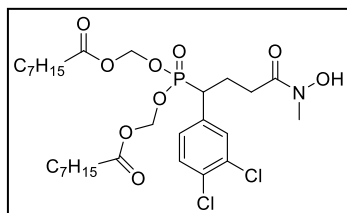


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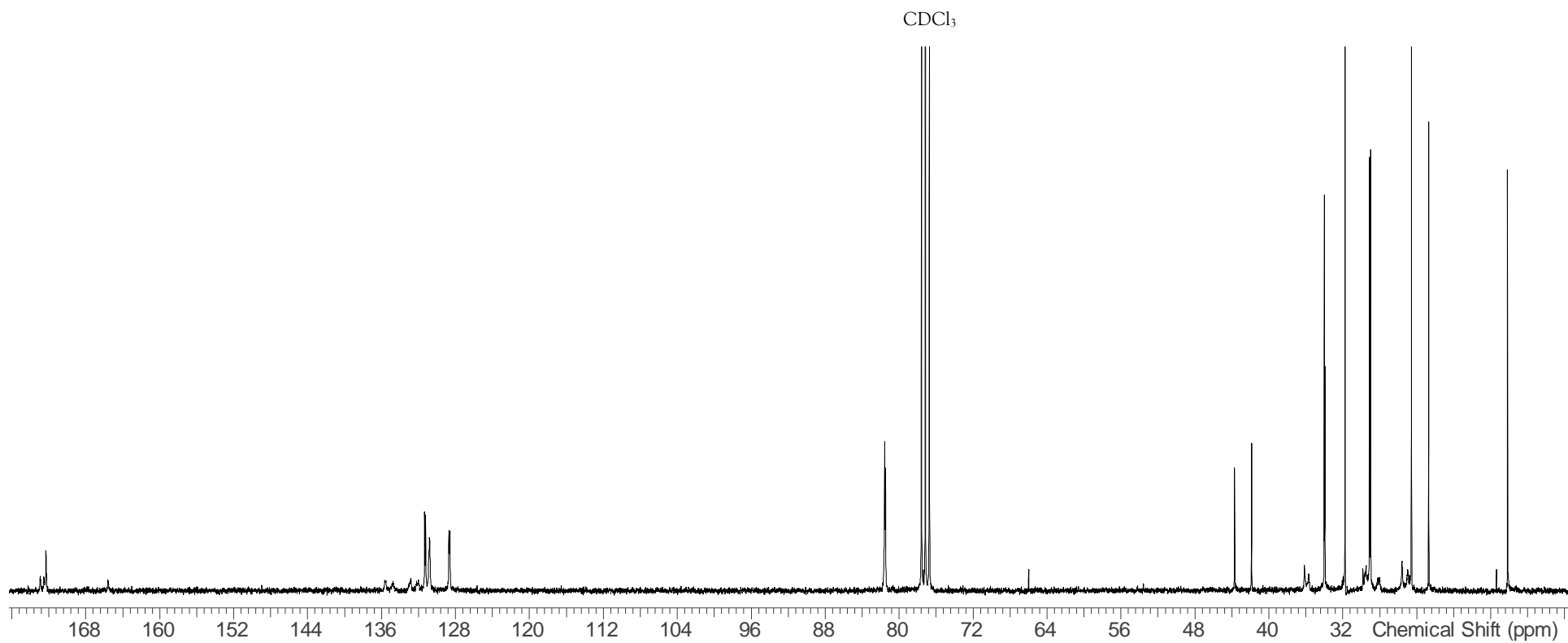
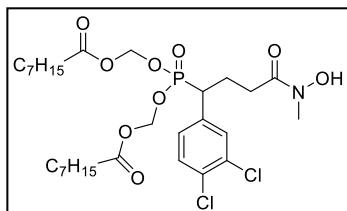
Time	Height	Area	Area%
2.85	235726880	37155180.00	100.00



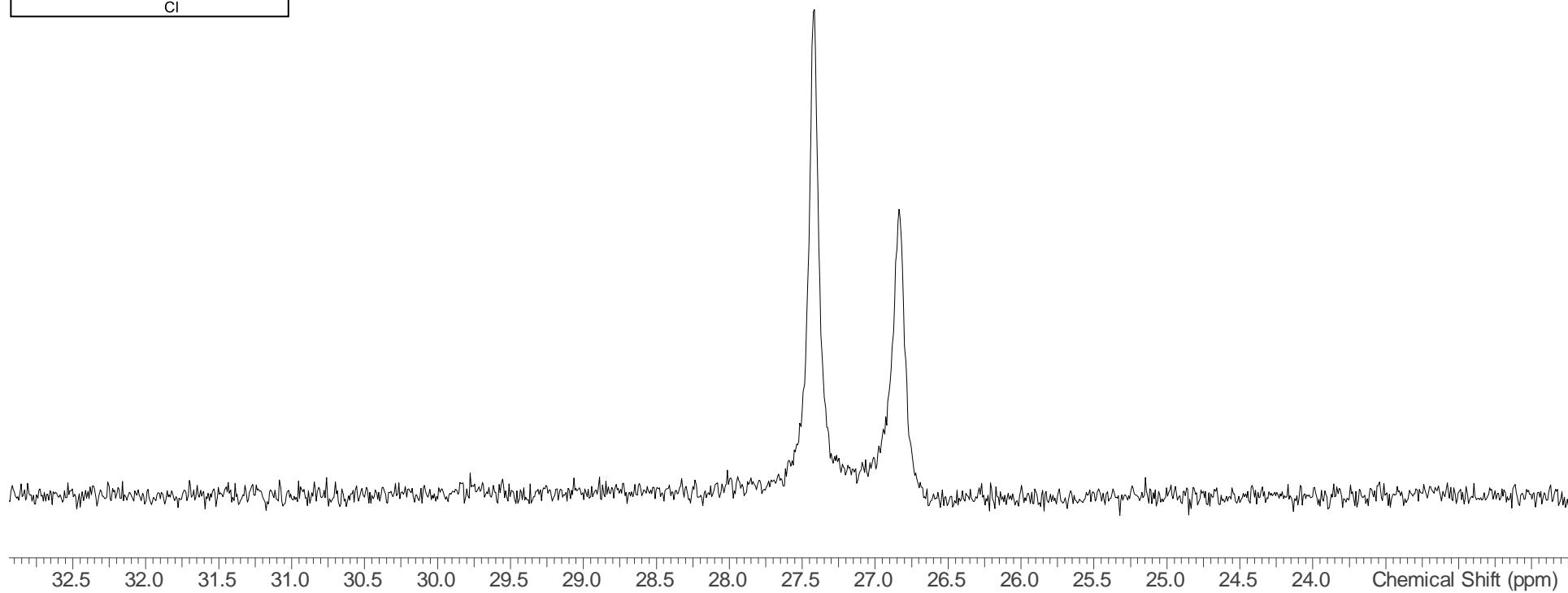
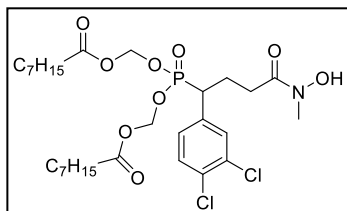
^1H NMR spectrum of **40j** (300MHz, CHLOROFORM-*d*)

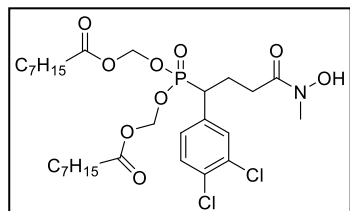


^{13}C NMR spectrum of **40j** (75MHz, CHLOROFORM-*d*)



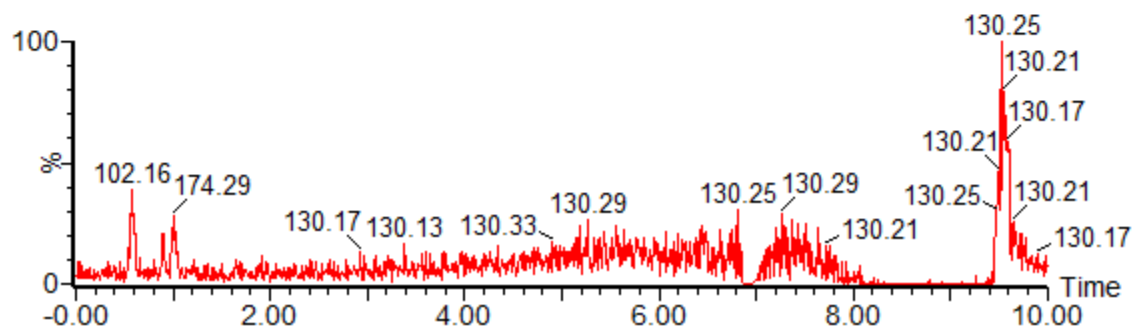
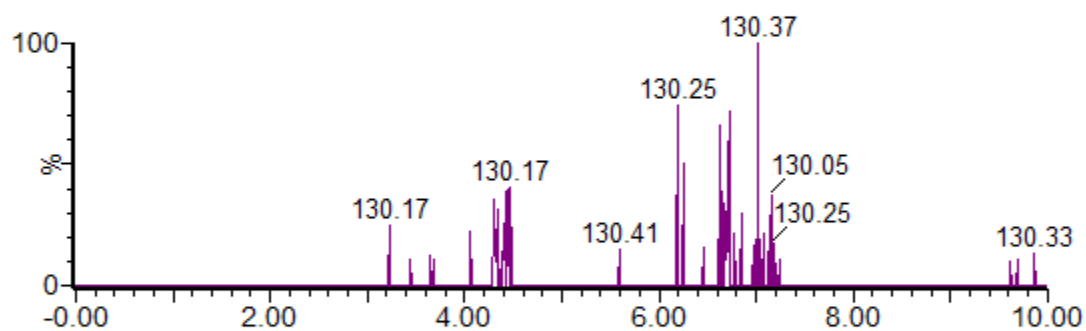
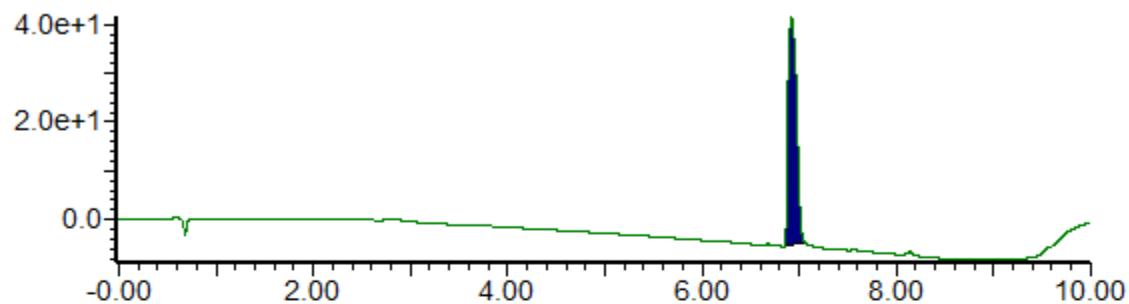
^{31}P NMR spectrum of **40j** (121MHz, CHLOROFORM-*d*)



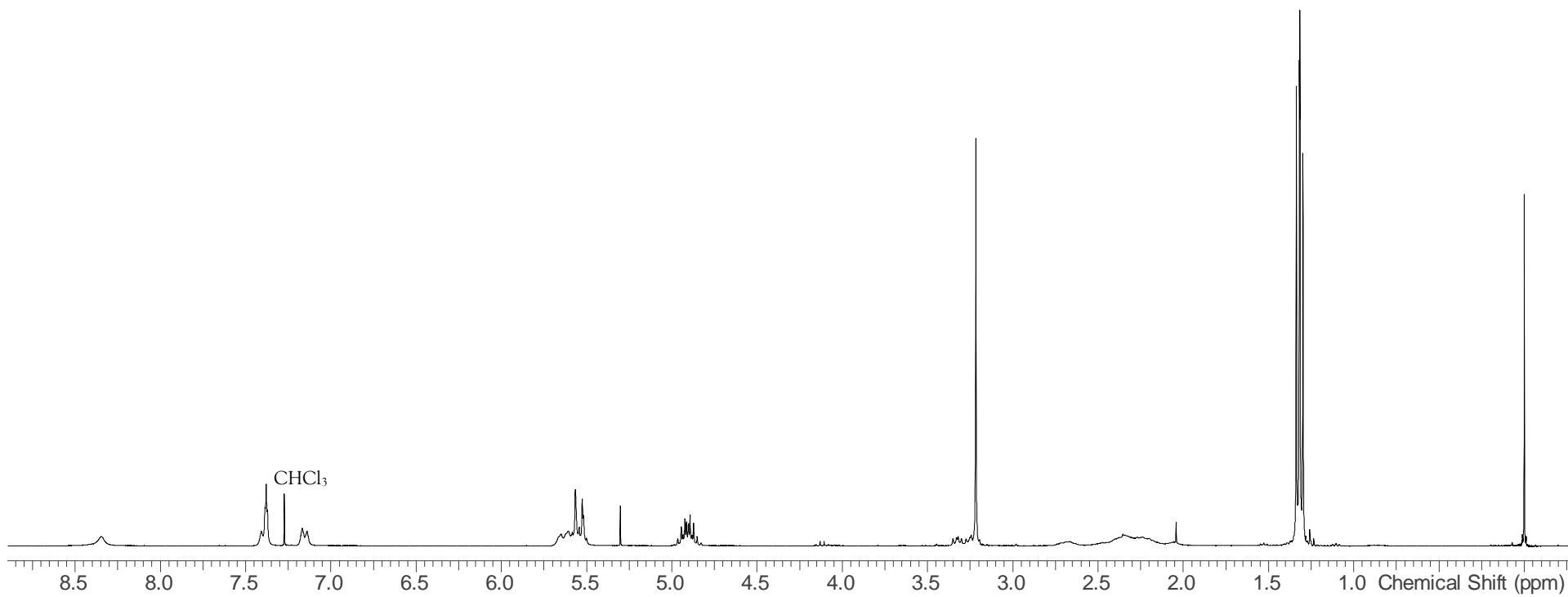
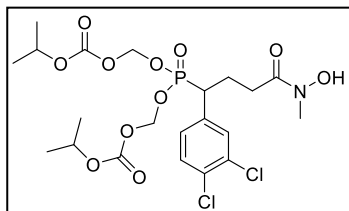


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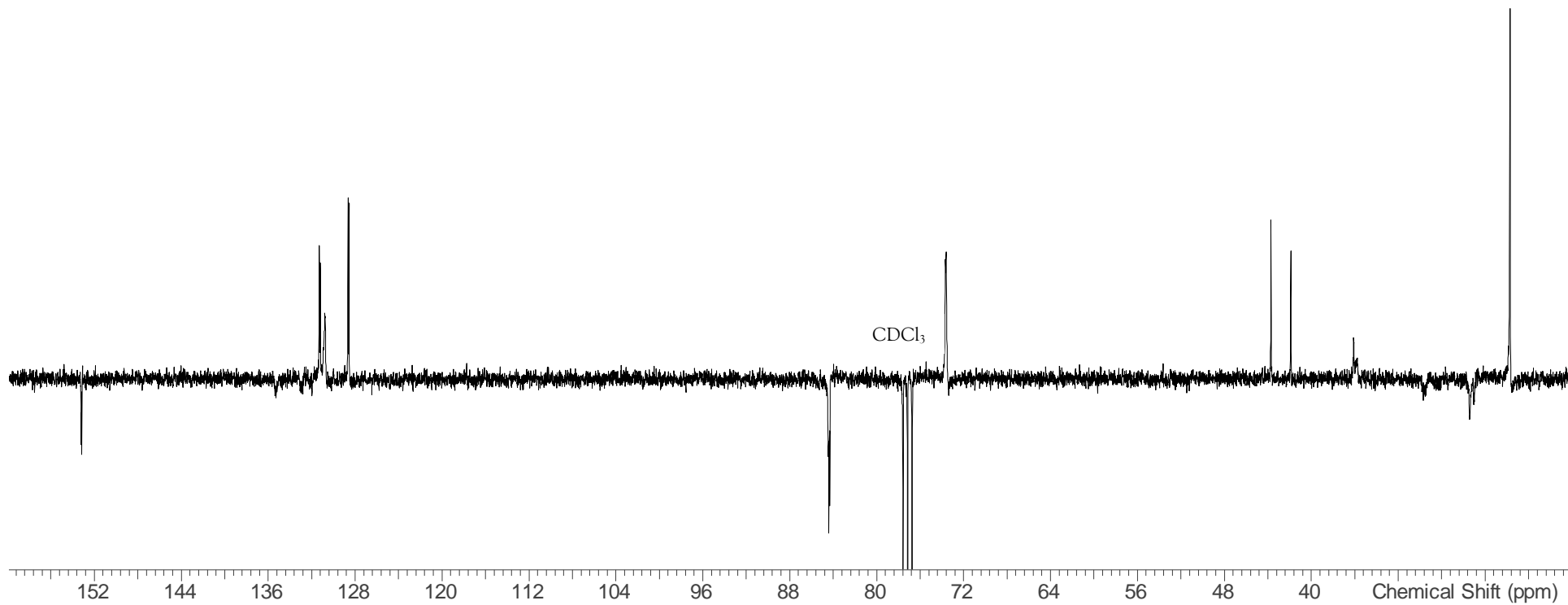
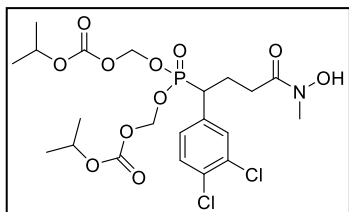
Time	Height	Area	Area%
6.92	46758752	4856267.00	100.00



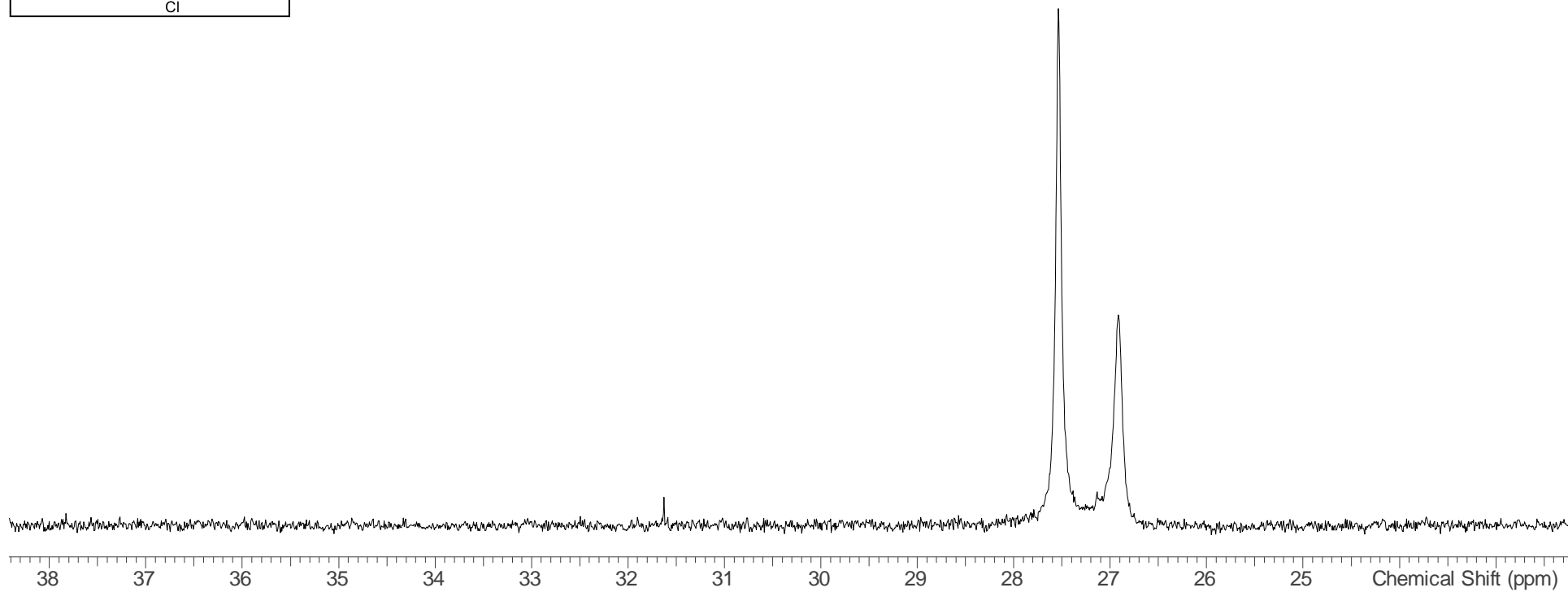
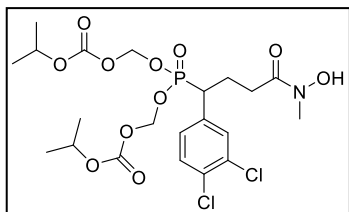
^1H NMR spectrum of 40k (300MHz, CHLOROFORM-*d*)

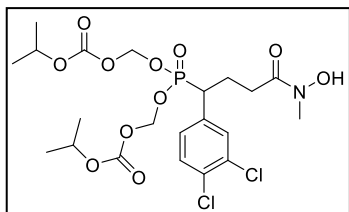


APT NMR spectrum of 40k (75MHz, CHLOROFORM-d)



^{31}P NMR spectrum of **40k** (121MHz, CHLOROFORM-*d*)





Time	Height	Area	Area%
4.17	45279984	4556901.00	100.00

AU

