ELECTRONIC SUPPLEMENTARY INFORMATION

Synthesis of a non-natural glucose-2-phosphate ester able to dupe the *acc* system of *Agrobacterium fabrum*

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 # Equal contribution

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Table 1. Crystallographic data and refinement parameters

	AccA-G2LP
Space group Cell parameters (Å,°)	I222 *a* = 78.3 *b* = 108.3 *c* = 108.2 *β* = 113.6
Resolution (Å)	42-1.8 (1.91-1.8)
No. of observed reflections	274443 (42603)
No. of unique reflections	45083 (7249)
R_{sym} (%)	9 (121.5)
Completeness (%)	99.5 (98.2)
I/σ	12.9 (1.6)
$CC_{1/2}$	99.8 (58.6)
R_{cryst} (%)	17.1
R_{free} (%)	19.5
rms bond deviation (Å)	0.01
rms angle deviation (°)	1.0
Average B (Å^2) protein ligand solvent	 38.8 28.3 40

Values for the highest resolution shell are in parentheses

2D NMR Characterization of Glucose-2-lactate phosphate ammonium

Glucose-2-lactate phosphate ammonium salt was characterized by 2D-NMR. A mixture of α/β with 5/3 ratio was calculated from the integration of H-1 signals. H-1α at 5.36 ppm with $J_{1,2}$ = 3.6 Hz, and H-1β at 4.68 ppm with $J_{1,2}$ = 7.9 Hz, partially hidden by deuterium oxide. With the help of ^{1}H-^{1}H COSY, we found H-2α as a multiplet at 3.95 ppm and H-2β at 3.77 ppm, impaired by H-3α, H-5α. In addition, the α and β CH- group of lactic acid as multiplets were found at 4.5 ppm, and the corresponding α and β CH_3- are doublet at 1.40 ppm. Decomposition of G2P lactate can be easily traced signals at 4.5 ppm and 1.4 ppm for free lactic acid (**Fig 1**).

Like G2P (ref 12), HSQC shows coupling with phosphorus for ^{13}C-NMR signals. The carbon of α and β CH- group appears as doublet (δ=73.1 ppm, $J_{C\text{-}P}$ = 5.7 Hz), and the same splitting is also overserved for C-1, C-2 and C-3 (**Fig 2**). The differences are shown in the **Table 2**.

In comparison with glucose-2-phosphate (ref 12), the carbon shift of C-1, C-2 and C-3 of G2P lactate move to high fields slightly, and the α-anomer locates in a higher field than β-anomer in both cases. The coupling constant is almost the same except $J_{C2\text{-}P}$ of glucose-2-lactate phosphate which is stronger than that of glucose-2-phosphate due to the introduction of lactic acid. This could be explained by the conformation adjustment. Among those three carbons, the *J*-coupling of C-2 is the strongest one since the coupling of C-2 and phosphorus is the second-order coupling whereas others are third-order coupling. More interestingly, for α and β glucose phosphate, in both case, the *J*-coupling of C-1 and C-3 exhibit distinct differences.

2D NMR of the ^{31}P-^{1}H correlation shows the ^{31}P-NMR of H-2α and CHα appears at -1.16 ppm, and H-2β and CHβ at -0.86 ppm. Theoretically, four phosphorus signals (C2α, C2β, CHα, CHβ) should be observed. The proximity of ^{31}P-NMR chemical signals of CH and H-2 resulted in only two peaks, and the high resolution NMR spectroscopy could give more details (**Fig 3**).

Table 2. Partial ^{1}H-NMR and ^{13}C-NMR of G2P lactate

α-anomer				β-anomer			
H-1	5.36 (*J* = 3.6 Hz)	C-1	90.9 (*J* = 2.0 Hz)	H-1	4.68 (*J* = 7.9 Hz)	C-1	95.0 (*J* = 5.1 Hz)
H-2	3.95	C-2	75.7 (*J* = 6.8 Hz)	H-2	3.79	C-2	78.9 (*J* = 6.6 Hz)
H-3	3.75 - 3.81	C-3	71.5 (*J* = 5.6 Hz)	H-3	3.63 – 3.56	C-3	75.0 (*J* = 2.7 Hz)
H_{CH}	4.56	C_{CH}	73.1 (*J* = 5.7 Hz)	H_{CH}	4.49	C_{CH}	73.1 (*J* = 5.7 Hz)

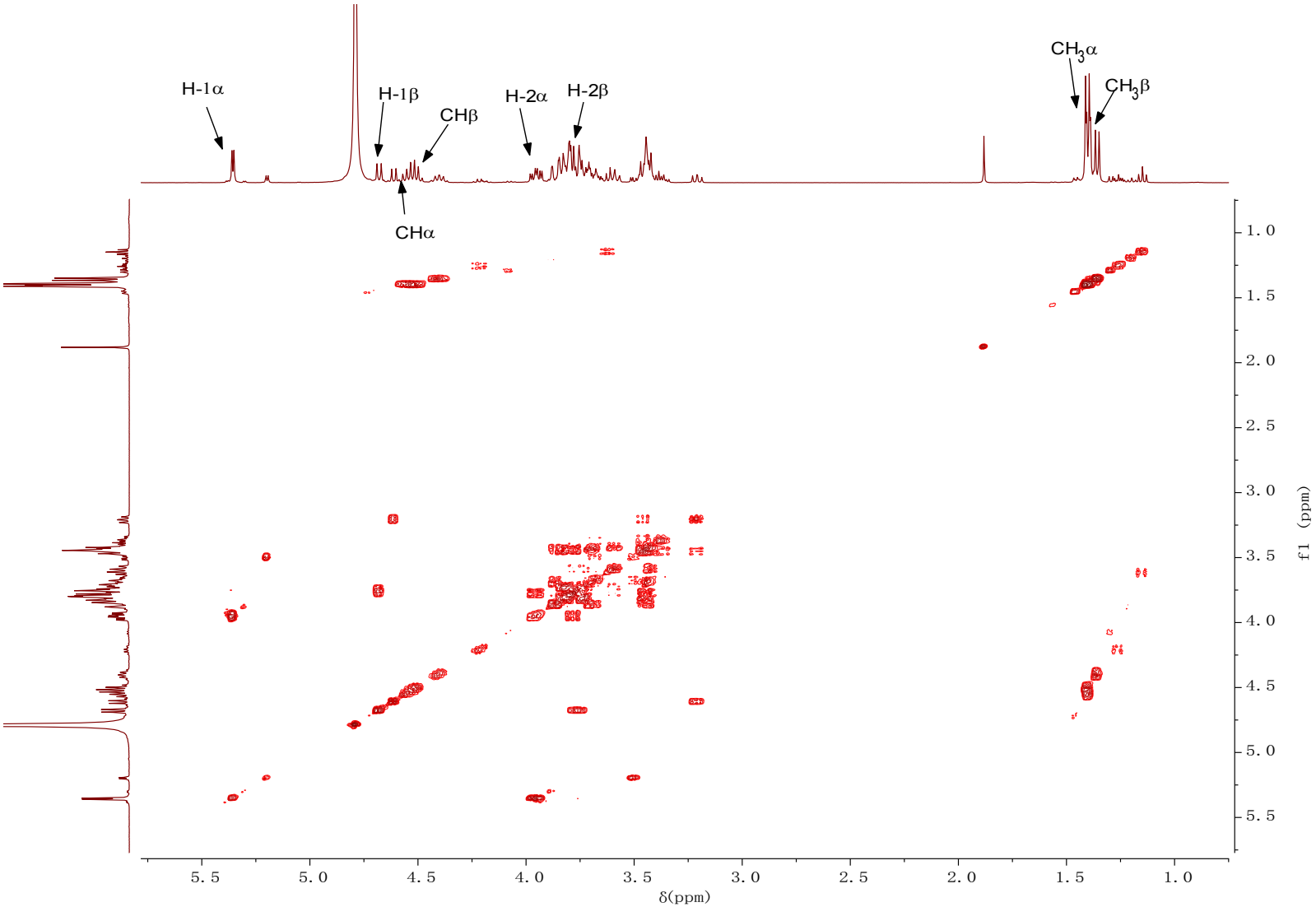


Fig 1. ^{1}H-^{1}H COSY of G2P lactate (400 MHz, D_2O)

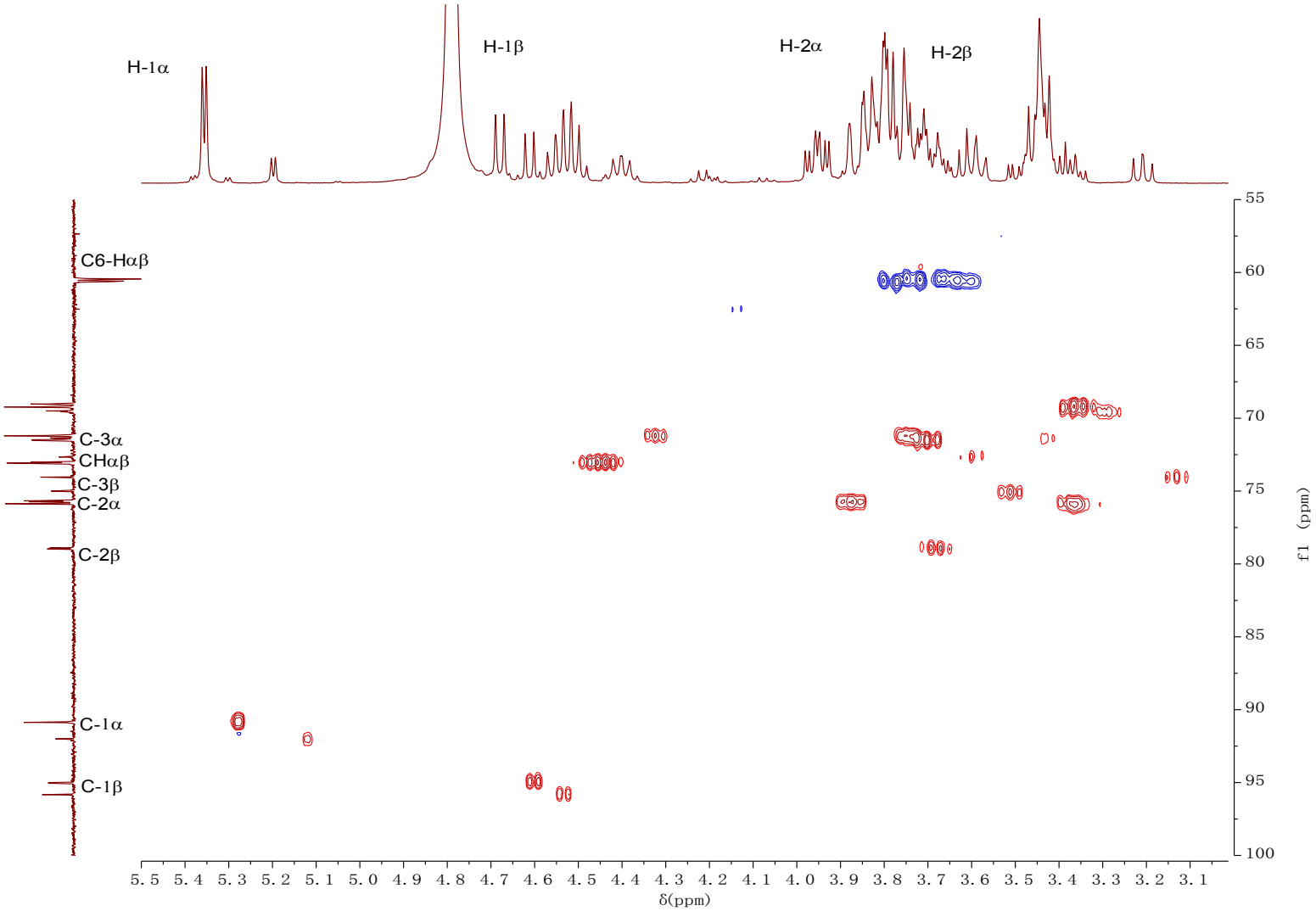


Fig 2. HSQC of G2P lactate (400 MHz, D_2O)

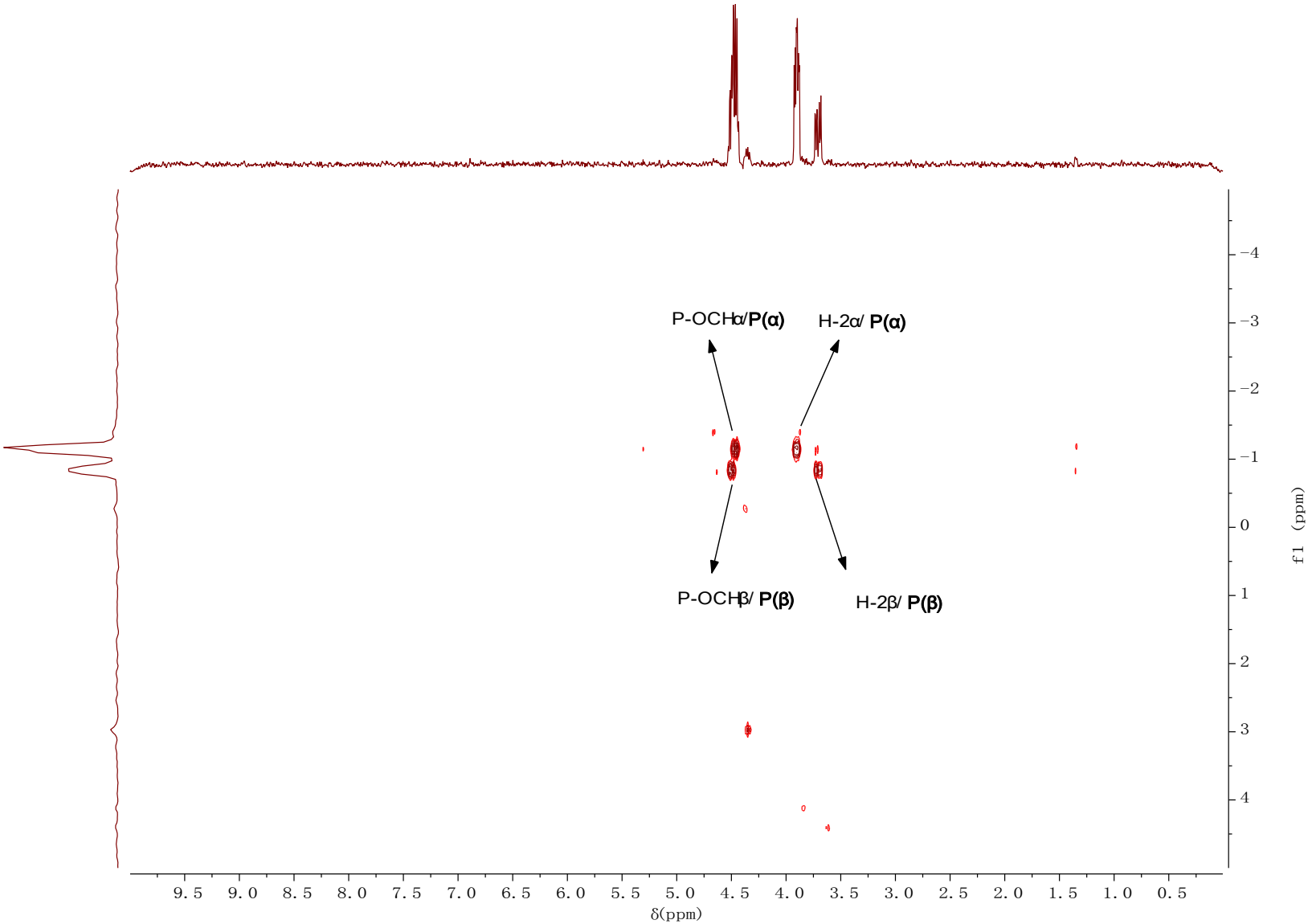
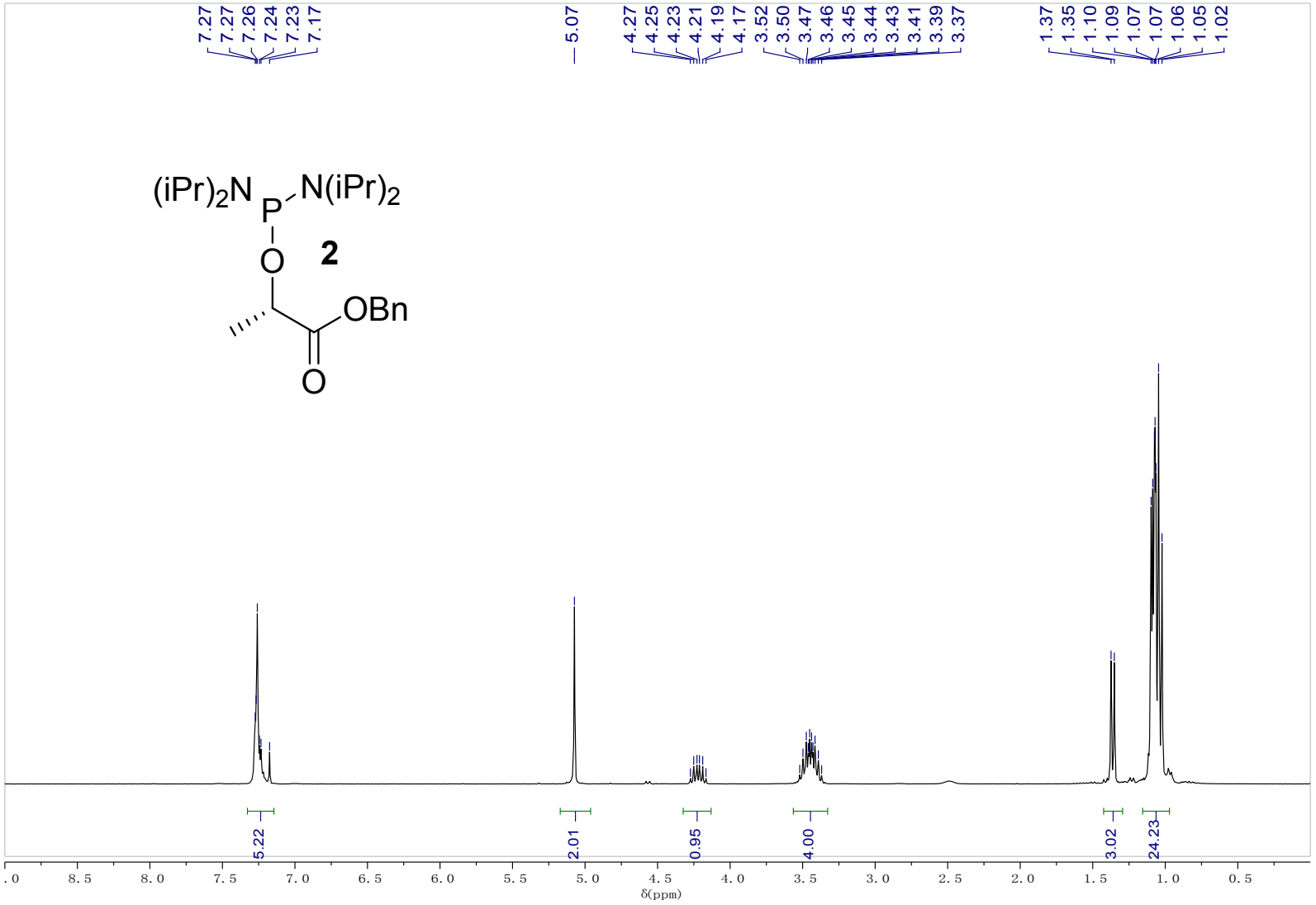
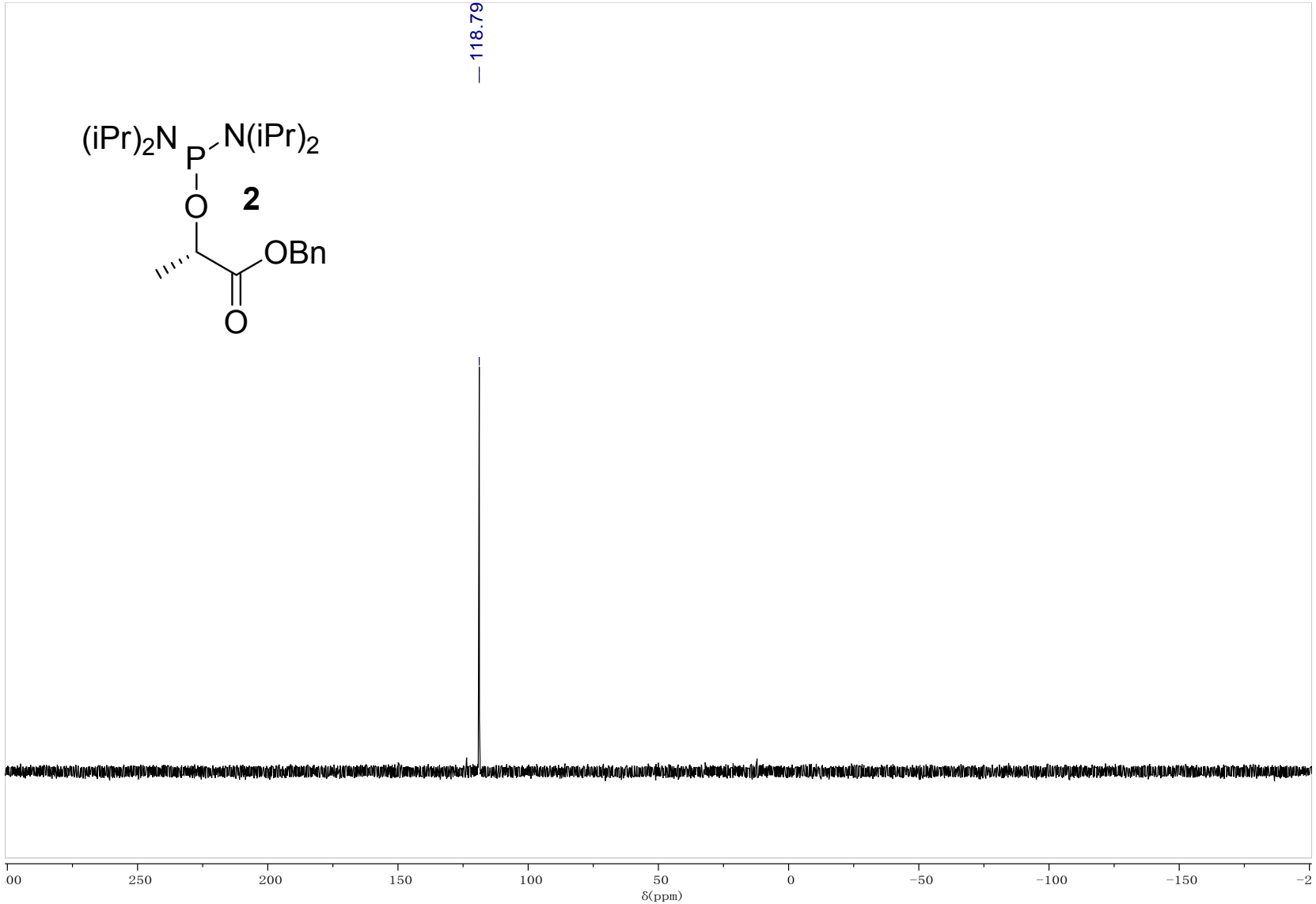


Fig 3. ^{31}P-^{1}H correlation of G2P lactate (400 MHz, D_2O)

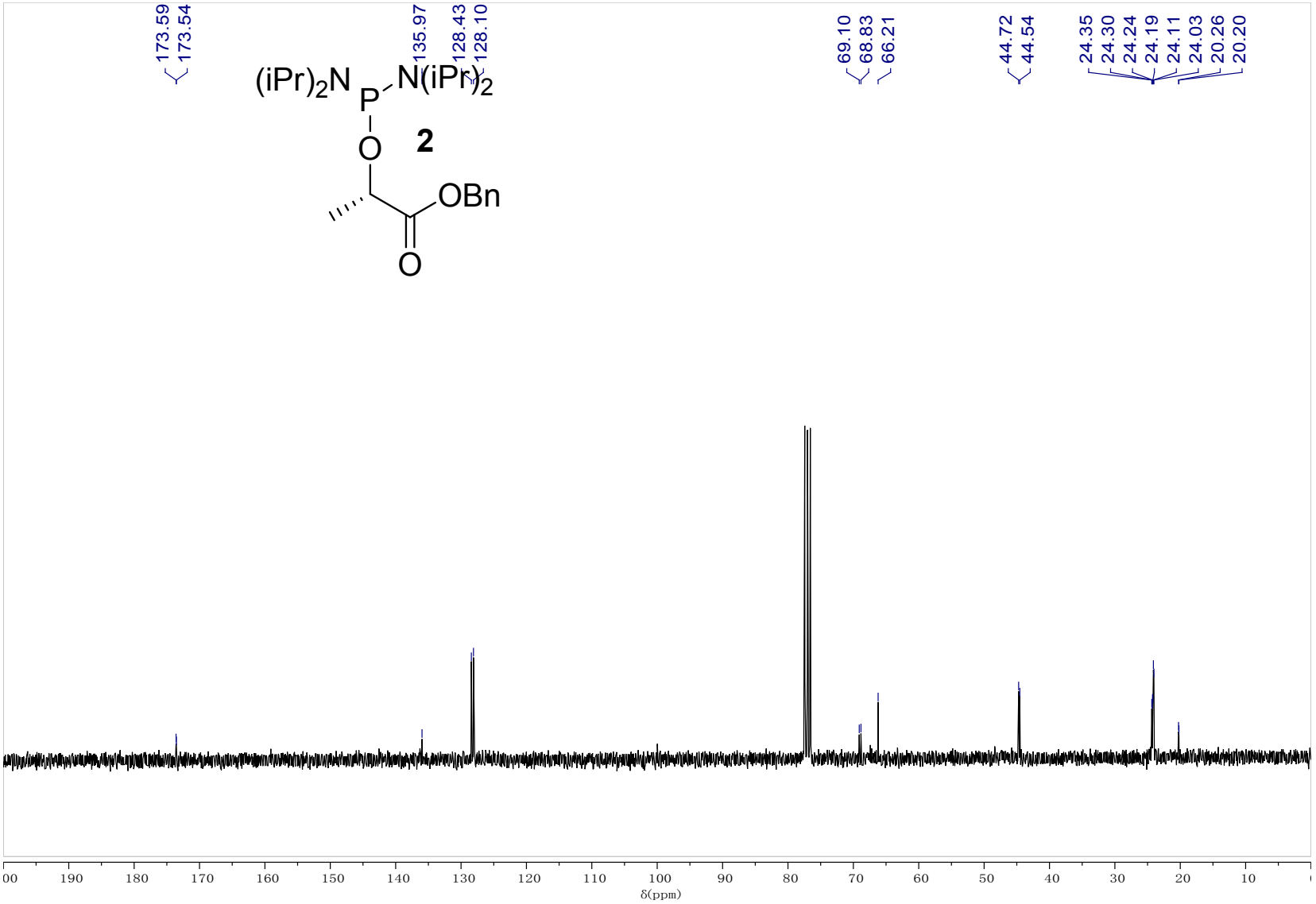
Benzyl (S)-2-((bis(diisopropylamino)phosphanyl)oxy)propanoate (2)



^{1}H-NMR (300 MHz, Chloroform-*d*)

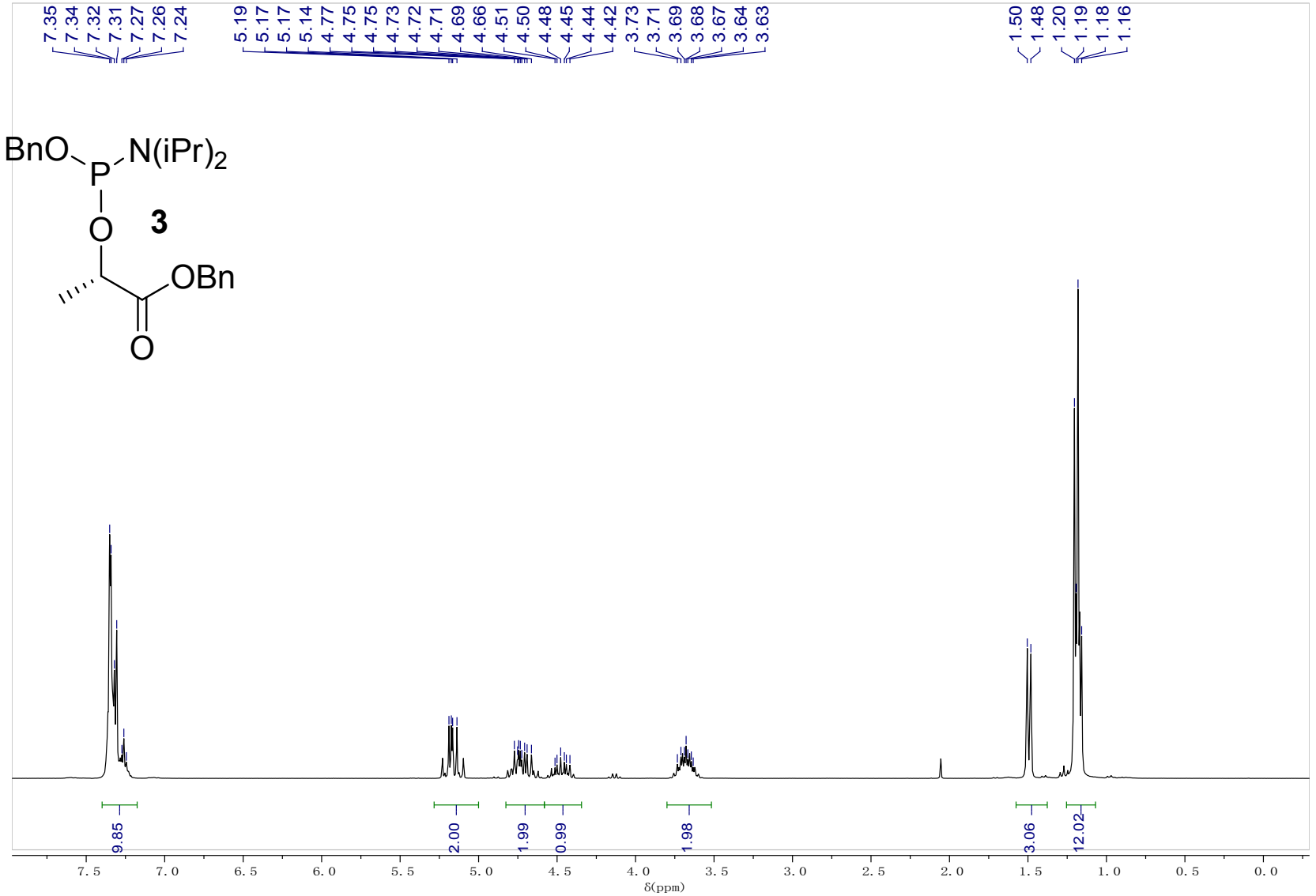


^{31}P-NMR (122 MHz, Chloroform-*d*)

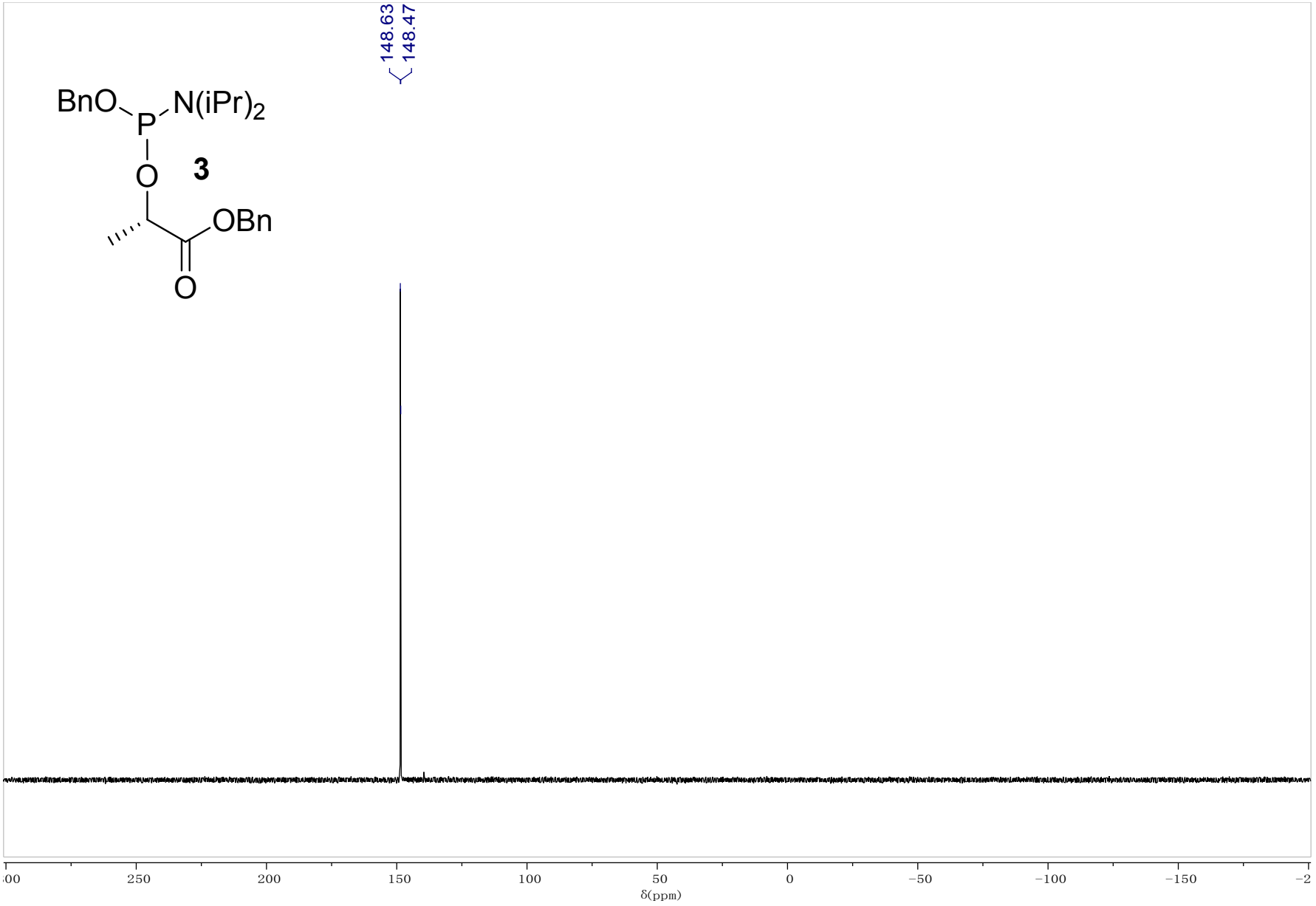


^{13}C-NMR (75 MHz, Chloroform-*d*)

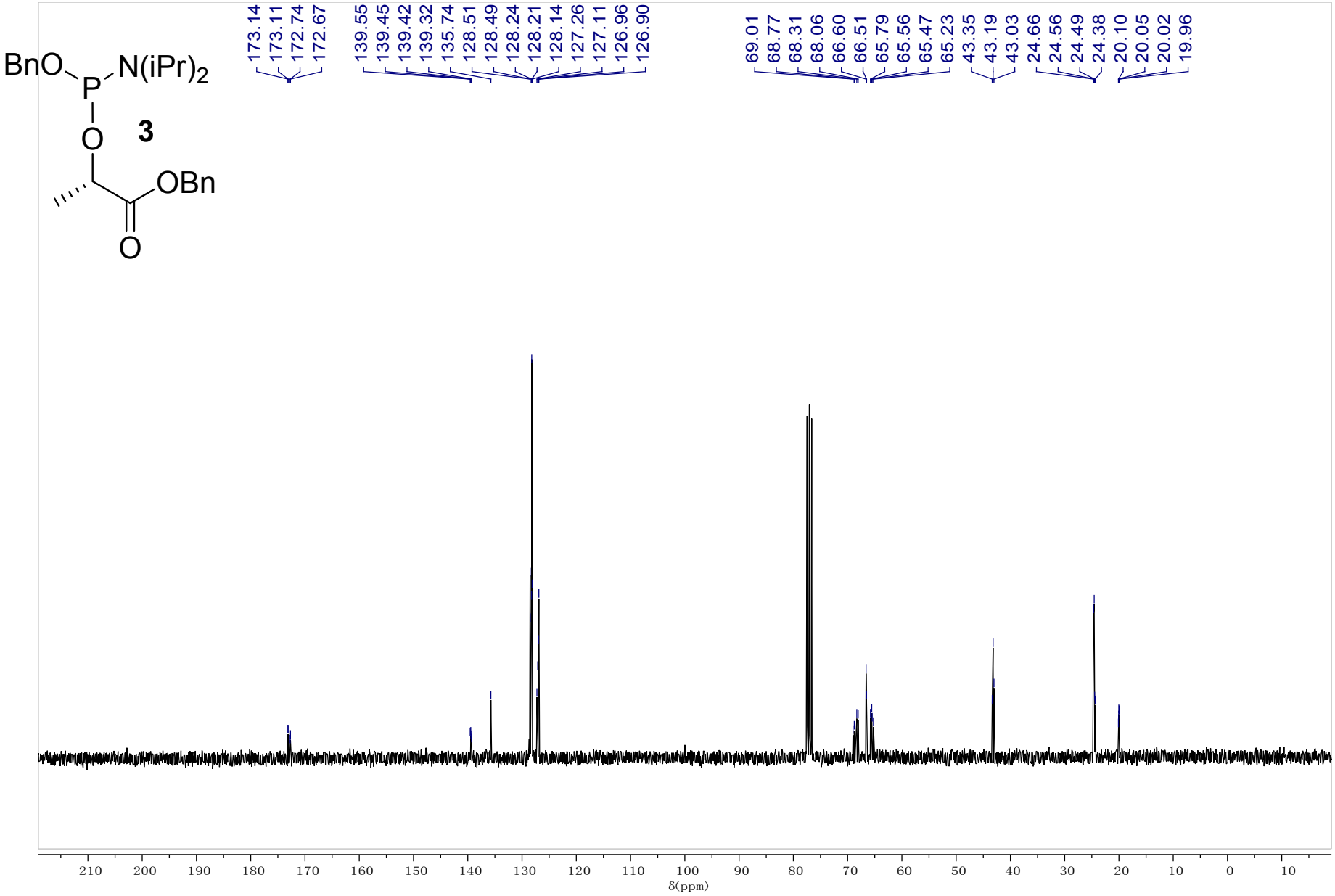
Benzyl (2S)-2-(((benzyloxy)(diisopropylamino)phosphanyl)oxy)propanoate (3)



^{1}H-NMR (300 MHz, Chloroform-*d*)



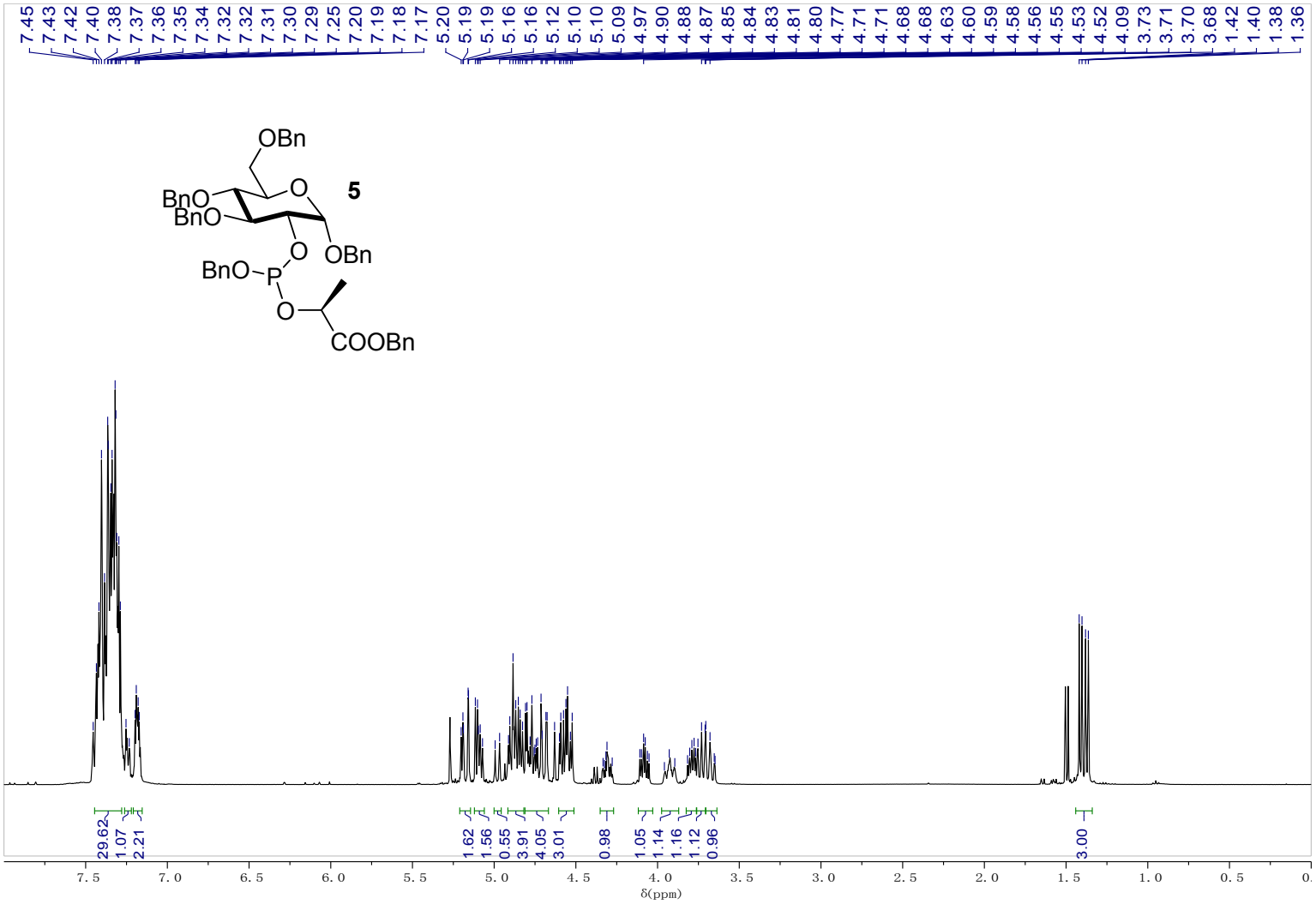
^{31}P-NMR (122 MHz, Chloroform-*d*)



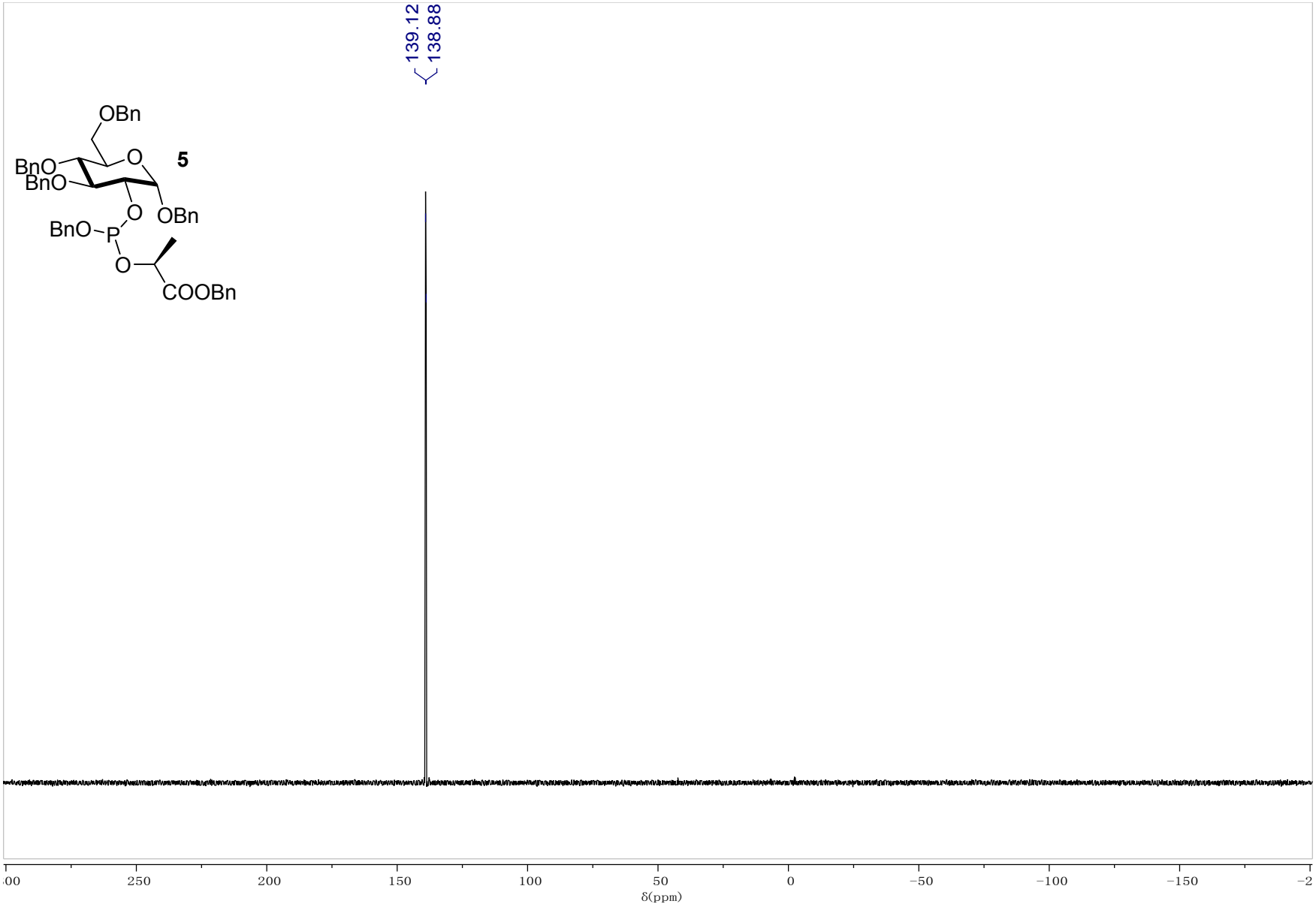
^{13}C-NMR (75 MHz, Chloroform-*d*)

Benzyl (2S)-2-(((benzyloxy)(((2S,3R,4S,5R,6R)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphanyl)oxy)propanoate

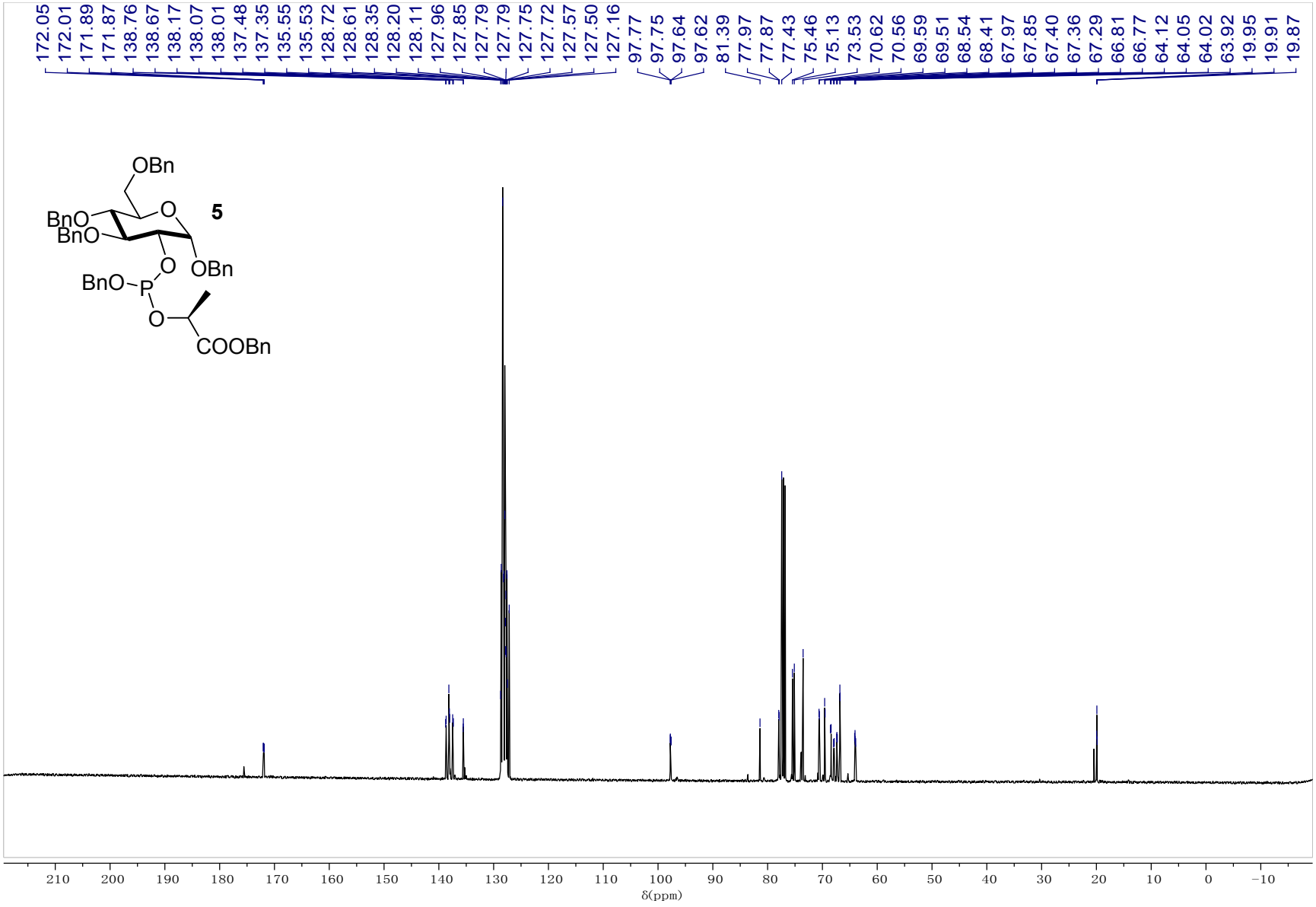
(5)



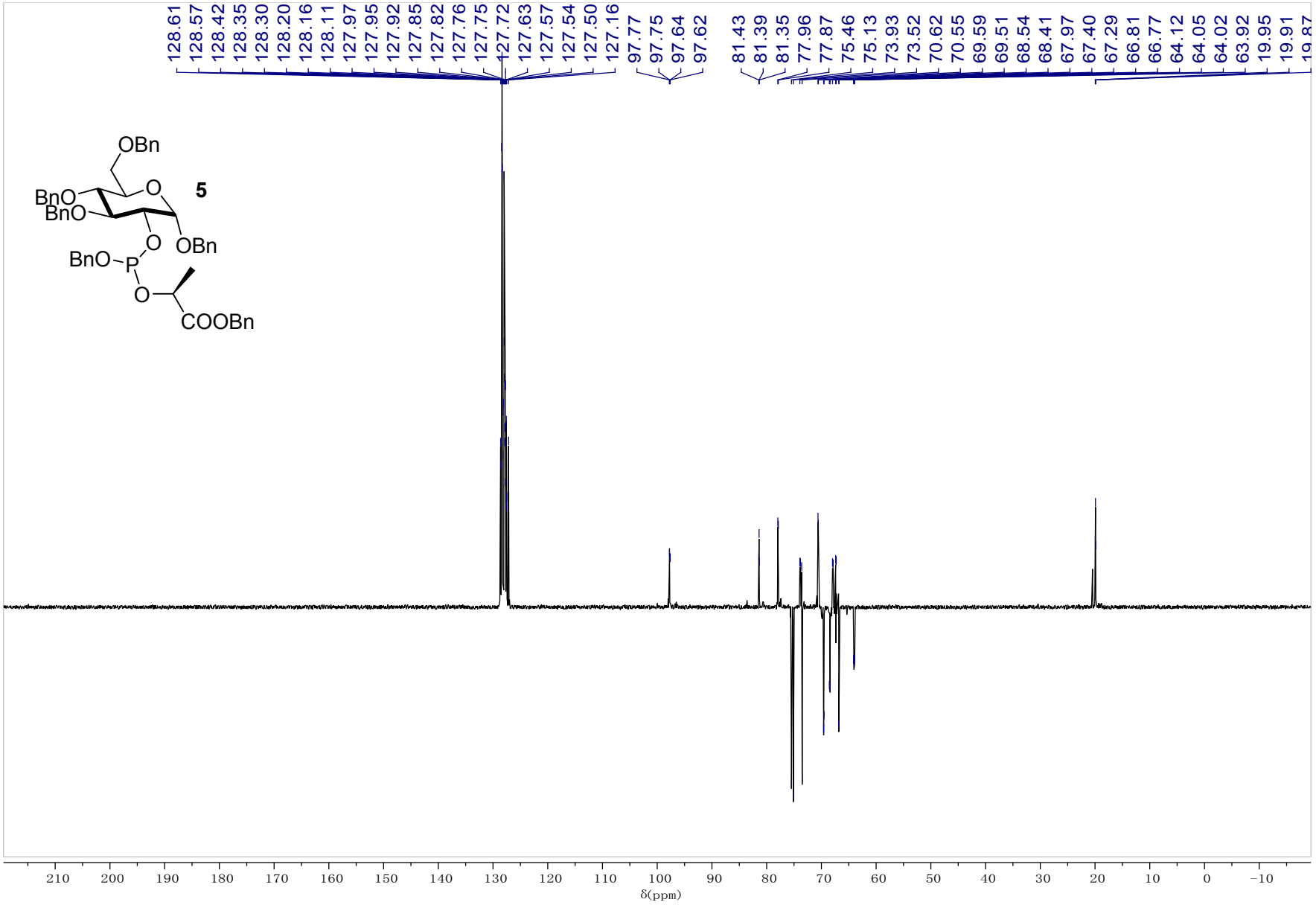
^{1}H-NMR (400 MHz, Chloroform-*d*)



^{31}P-NMR (122 MHz, Chloroform-*d*)



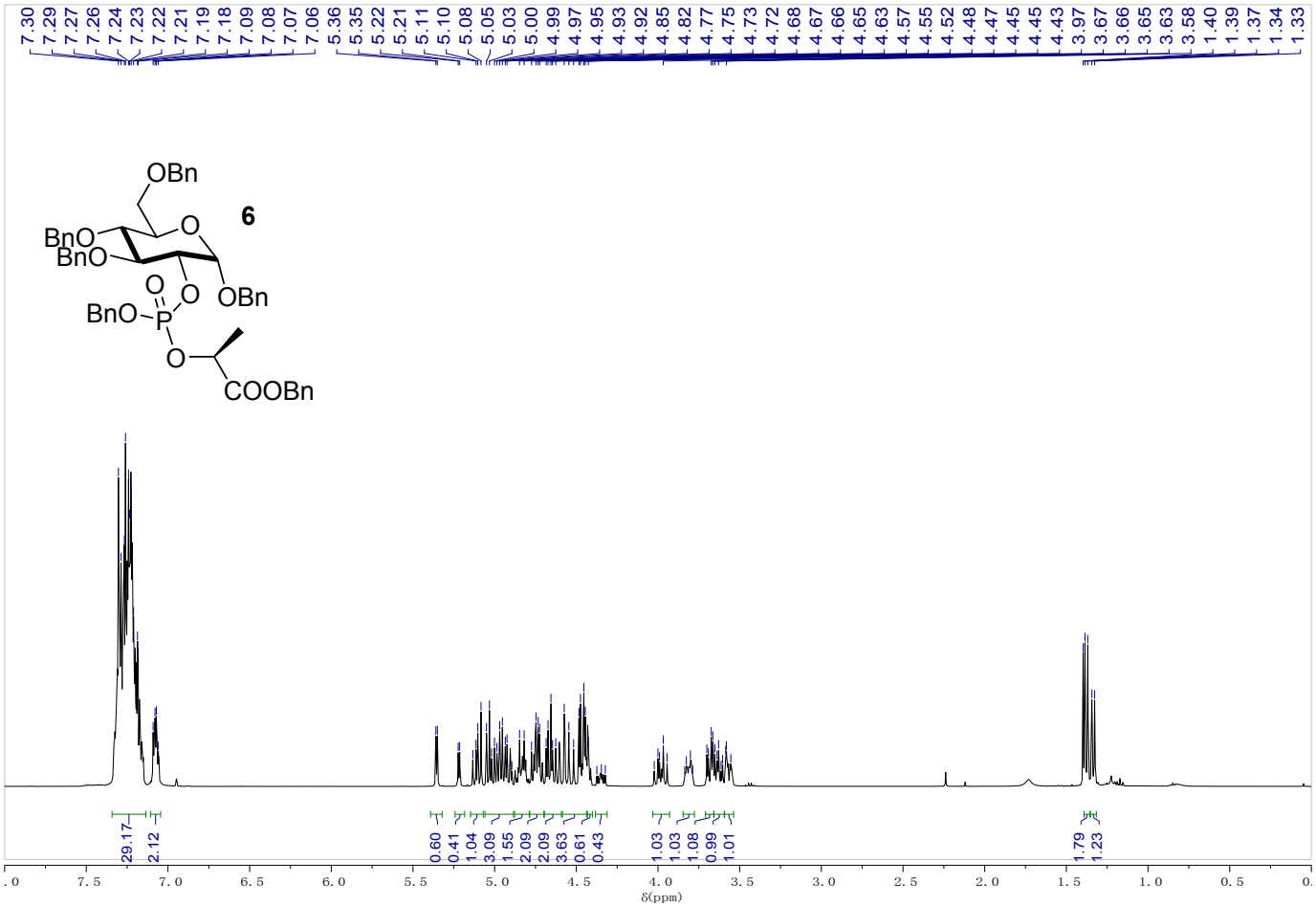
^{13}C-NMR (101 MHz, Chloroform-*d*)



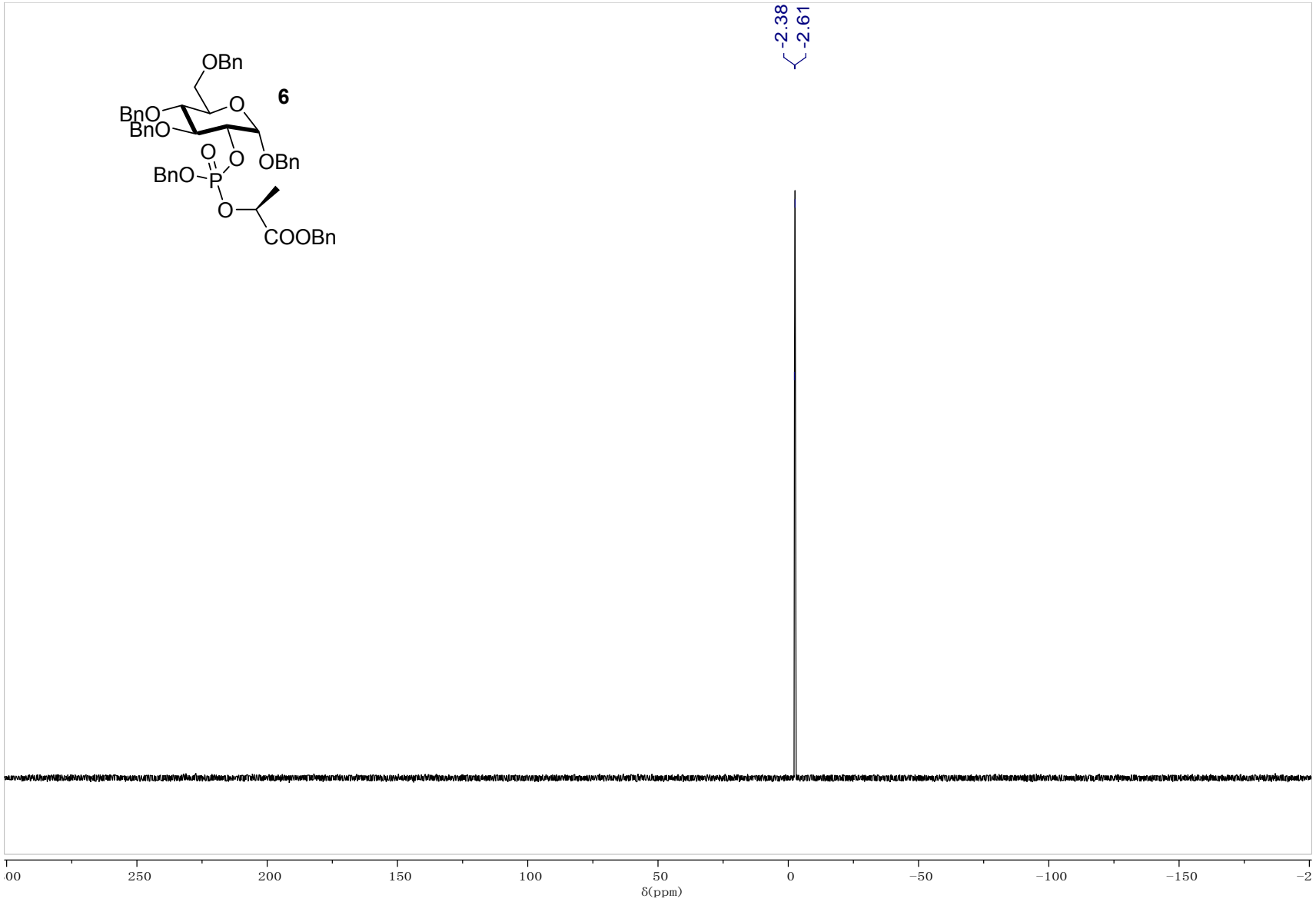
DEPT 135 ^{13}C-NMR (101 MHz, Chloroform-*d*)

Benzyl (2S)-2-(((benzyloxy)(((2S,3R,4S,5R,6R)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)phosphoryl)oxy)propanoate

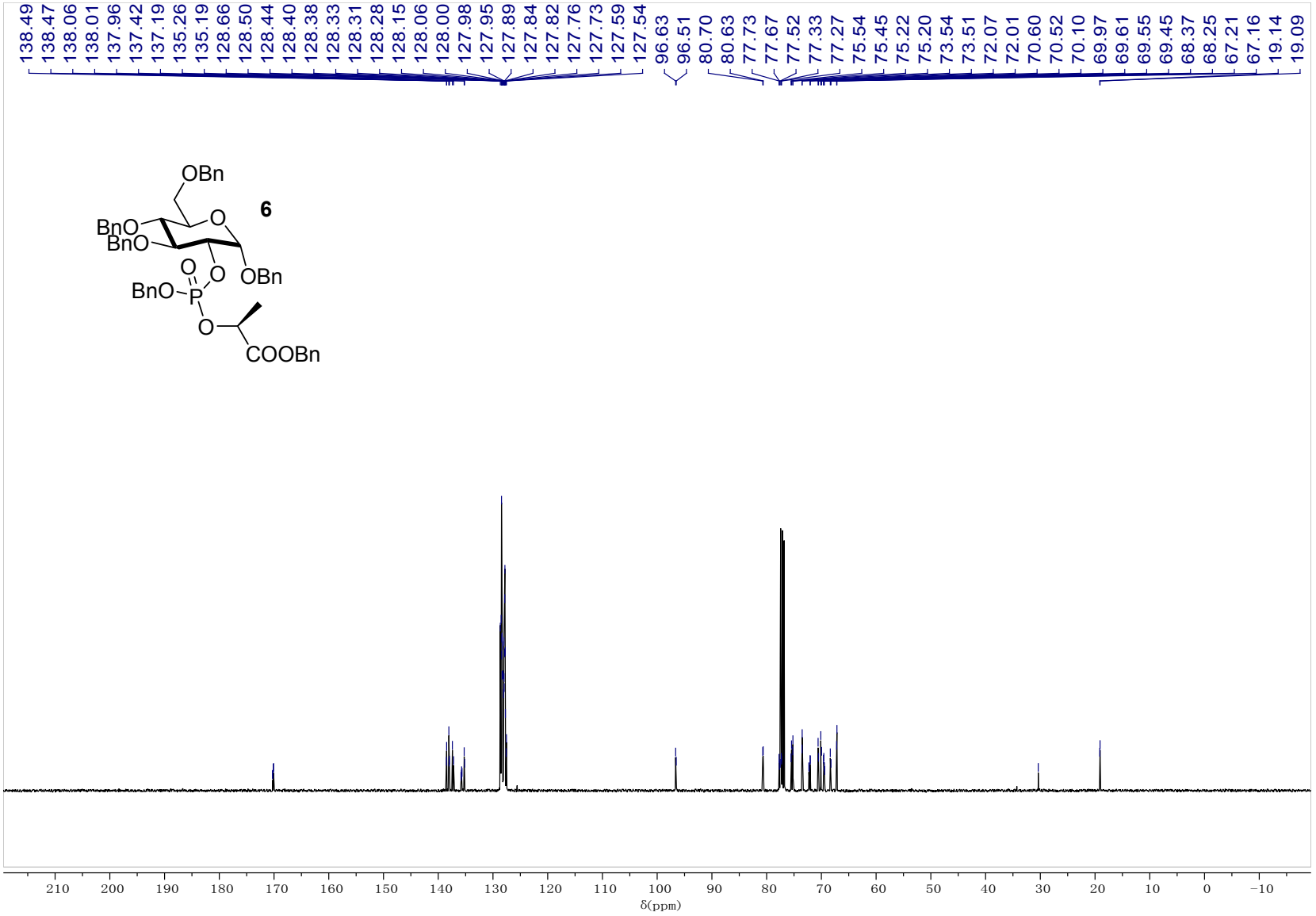
(6)



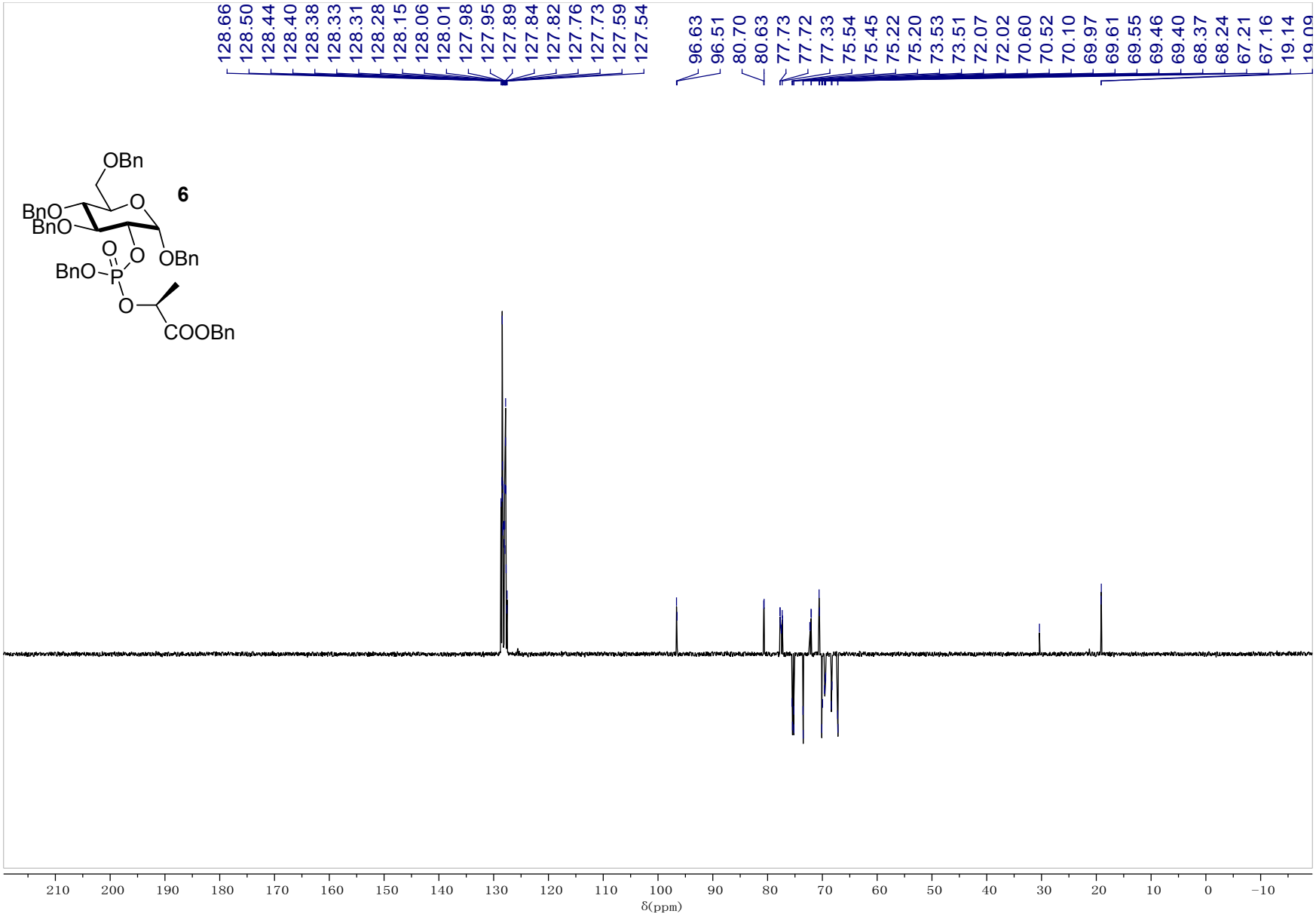
^{1}H-NMR (400 MHz, Chloroform-*d*)



^{31}P-NMR (122 MHz, Chloroform-*d*)



^{13}C-NMR (101 MHz, Chloroform-*d*)



DEPT 135 ^{13}C-NMR (101 MHz, Chloroform-*d*)