Supporting Information

Study of Uridine 5′-Monophosphate (UDP)-Galactopyranose Mutase Using UDP-5-Fluoro-Galactopyranose As a Probe: Incubation Results and Mechanistic Implications

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S1. Experimental Methods

General. NMR spectra were recorded using a Varian Direct Drive 600 MHz, a Varian Inova 500 MHz or a Varian Direct Drive 400 MHz NMR spectrometer at the Nuclear Magnetic Resonance Facility at the University of Texas at Austin. ^{13}C and ^{31}P NMR spectra were recorded with proton broad-band decoupling, and deuterated solvents were used as the internal reference. NMR spectra were processed and plotted using MestReNova 10 (Mestrelab Research S.L.). Electrospray ionization mass spectra (ESI-MS) were acquired at the Mass Spectrometry Facility (MSF) in the Department of Chemistry of the University of Texas at Austin. High-performance liquid chromatography (HPLC) was performed using a Beckman System Gold 125 Solvent Module with a 166 detector equipped with a Dionex anion exchange column (CarboPac PA1, Thermo Scientific, 4 × 250 mm or 9 × 250 mm). Silica gel column chromatography was carried out using SiliaFlash P60 (230–400 mesh, Silicycle).

Reagents. All chemical reagents and anhydrous solvents were purchased from commercial sources and used without further purification unless otherwise specified. UDP-5F-Gal*p* (**7**) was prepared according to Scheme 3. Detailed procedures are described in section S10. Tetrahydrofuran was distilled in the presence of sodium benzophenone ketyl under argon. Dichloromethane was dried over CaH_2 and then distilled. UDP-Gal*f* (**2**) was synthesized as previously reported.[1] The concentration of each UDP-sugar was determined by measuring its absorption at 262 nm and calibrated based on the extinction coefficient of uracil at 262 nm (ε_{262} = 10,000 $cm^{-1}M^{-1}$). Solutions of sodium dithionite ($Na_2S_2O_4$) were freshly prepared prior to its addition to the assays.

Enzymes. The *glf* gene from *Escherichia coli* K-12 which encodes UDP-galactopyranose mutase (UGM) was cloned into the pET-24b(+) vector.[2] The resulting pQZ-1 plasmid was used to transform *E. coli.* BL21 Star (DE3) cells in which UGM was overexpressed. Purification of UGM was carried out according to reported procedures.[3] Preparation of *apo*-UGM and reconstitution to *holo*-UGM with FAD or 5-deaza-FAD was done according to established protocols.[3] Protein concentrations were determined by the Bradford assay using bovine serum albumin (BSA) as standard,[4] and protein purity was assessed by SDS-PAGE. Enzyme stock solutions contained 100 mM potassium phosphate buffer (pH = 7.5) with 15% glycerol and stored at –80 °C.

Enzyme assays. Unless otherwise specified, enzymatic assays were typically performed in potassium phosphate (KP_i) buffer (pH 7.5) at 37 °C containing freshly-prepared $Na_2S_2O_4$ (200 μM for anaerobic assay, and 20 mM for aerobic assay) and initiated by the addition of either substrate or substrate analogue. For assays longer than 2 h, the same amount of freshly-prepared $Na_2S_2O_4$ was added every 2 h. Reactions were terminated by the addition of MeOH (3-fold in volume). The protein precipitates were removed by centrifugation, and the supernatant was evaporated using a speed vacuum concentrator. The residue was redissolved in water and frozen at –80 °C before HPLC analysis. Anaerobic assays were carried out the same way in a Coy anaerobic glovebox under an atmosphere of > 98% N_2 and ~ 1.5% H_2 with < 1 ppm O_2. Buffers used for anaerobic assays were stirred open in the glovebox overnight to equilibrate with the anaerobic atmosphere.

HPLC Methods. HPLC analysis/purification was performed using Dionex columns (CarboPac PA1, Thermo Scientific, 4 × 250 mm for analytical and 9 × 250 mm for preparative applications) and the samples were eluted with a two-solvent system of A, H_2O, and B, 1 M NH_4OAc. Three methods were developed and the elution was monitored by a UV-detector set at 262 nm. The flow rate was either 1 mL/min (for the 4 × 250 mm column) or 4 mL/min (for the 9 × 250 mm column). The elution conditions for each method and the respective retention time of each species of interest are listed in Table S1 and Table S2.

Table S1. HPLC methods. The numbers are the percentage of B (1 M NH_4OAc) in the eluent

Method A	Method B	Method C
0 – 5 min: 20% isocratic	0 – 15 min: 20 – 90% linear	0 – 20 min: 3 – 22% linear
5 – 25 min: 20 – 50% linear	15 – 20 min: 90% isocratic	20 – 40 min: 22 – 38% linear
25 – 35 min: 50 – 90% linear	20 – 25 min: 90 – 20% linear	40 – 60 min: 38% isocratic
35 – 40 min: 90% isocratic	25 – 30 min: 20% isocratic	60 – 65 min: 38 – 3% linear
40 – 45 min: 90 – 20% linear		
45 – 50 min: 20% isocratic		

Table S2. Retention time of each species under different HPLC methods

	Method A	Method B	Method C
UMP	7.3 min	5.1 min	20.0 min
UDP-Gal*p* (**1**)	13.3 min	7.4 min	26.3 min
UDP-Gal*f* (**2**)	15.8 min	8.4 min	n.d.[a]
UDP-5F-Gal*p* (**7**)	17.0 min	8.8 min	31.3 min
UDP-5,6-F_2-Gal*p* (**26**)	n.d.[a]	n.d.[a]	34.0 min
UDP	24.1 min	10.6 min	48.5 min
FAD	30.5 min	14.7 min	n.d.[a]

[a]not determined.

S2. Structural Assignment of 21 and 5-*epi*-21

As described in the text, compound **20** was subjected to epoxidation followed by fluoridolysis to introduce fluoride at C5 position. Only two of four possible products (fluorination at C5 or C6 and the corresponding epimers at C5) were isolated after these two steps. The C5 signals of both isomers are doublet, each with a coupling constant around 220~230 Hz, which is typical for $^1J_{C,F}$.[5] These results unambiguously demonstrated that fluorination occurred at C5 (Figure S1), not at C6, and the two products were epimers of 5-fluoro products. However, the assignment of the stereochemistry at C5 (i.e., D-*galacto*, **21**, or L-*altro*, 5-*epi*-**21**) was not straightforward. Attempt to detect NOE was unsuccessful since key signals in both isomers are either overlapping or very close to each other. Changing NMR solvent or derivatization of C6–OH also failed to resolve this problem. Nevertheless, the large coupling constant between 4-H and 5-F (J = 25.2 Hz) in the minor isomer suggests a diaxial relationship between the two.[5] The fact that only 5-*epi*-**21** could have its 4-H and 5-F in a diaxial configuration through ring-flipping (5-*epi*-**21**-$^1C_4$) prompted us to assign the minor isomer as 5-*epi*-**21** and the major isomer as **21**. The 5-*epi*-**21** should exist predominantly in $^1C_4$ conformation (5-*epi*-**21**-$^1C_4$) based on the $J_{H4,F}$ coupling constant. The observed small coupling constant between 2-H and 3-H (J = 4.5 Hz) when C6–OH of the minor product was acetylated (data not shown) is consistent with its assignment as 5-*epi*-**21**.

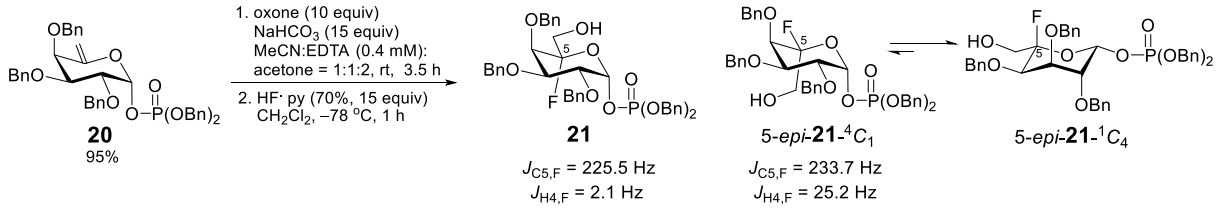

Figure S1. Structural analysis of **21** and 5-*epi*-**21**.

S3. Product Identification of Reaction between 7 and UGM

A solution of 200 μM UDP-5F-Gal*p* (**7**) was incubated with UGM and 20 mM $Na_2S_2O_4$ at 37 °C in 50 μL 100 mM KP_i buffer (pH 7.5). Depletion of **7** was observed when the enzyme concentration and reaction time were increased to 20 μM and 1 h, respectively. The two new peaks in the HPLC traces (trace *b*, Figure S2) of the reaction workups were determined to be UDP (24.1 min) and FAD (30.5 min) based on co-elution with standards (traces *e* and *f*). In the absence of enzyme, formation of UDP and consumption of **7** was also observable, but only over extended incubation time (24 h) (traces *c*, *d* and *g*).

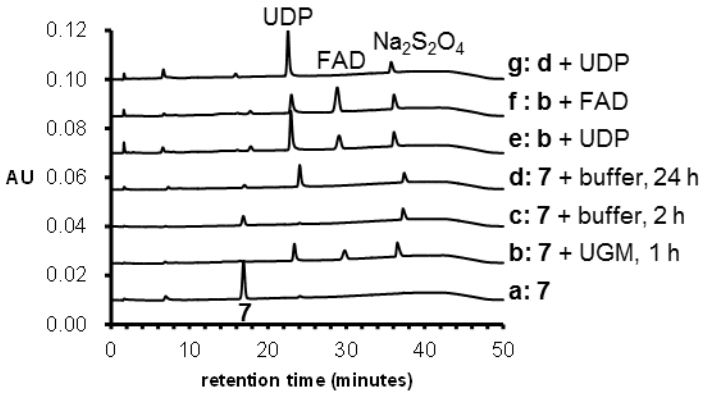

Figure S2. HPLC traces of the incubation of UDP-5F-Gal*p* (**7**) with UGM. Trace *a*: synthetic **7**, and reaction of 200 μM **7** with *b*: 20 μM UGM for 1 h, *c*: buffer for 2 h, and *d*: buffer for 24 h. To verify the identities of the new peaks, samples of trace *b* and trace *d* were coinjected with commercially available standards as shown, *e*: trace *b* with UDP, *f*: trace *b* with FAD, and *g*: trace *d* with UDP. The HPLC traces were generated using HPLC Method A.

To further confirm the identities of peaks at 24.1 and 30.5 min on trace *b* (Figure S2), the reaction was repeated at 2.5 mL scale, and the peaks at 24.1 and 30.5 min were collected, lyophilized and characterized by ESI-MS. As shown in Figure S3, the observed *m/z* of peak at 24.1 min is 403.0, which matches that of UDP (calcd. for $C_9H_{14}N_2O_{12}P_2$, 403.0 $[M - H]^-$). For the peak at 30.5 min, the observed *m/z* was 784.2, which matches that of FAD (calcd for $C_{27}H_{33}N_9O_{15}P_2$, 784.2 $[M - H]^-$). In addition, the collected peak at 30.5 min showed light-yellow color providing further support of the identity of this species.

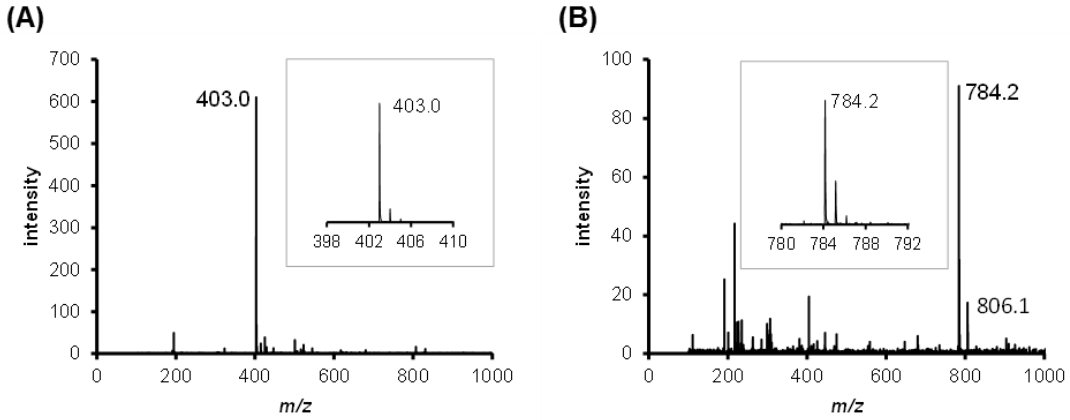

Figure S3. Mass spectra of peaks at **(A)** 24.1 min and **(B)** 30.5 min on trace *b* of Figure S2.

S4. Participation of UGM in the Hydrolysis of UDP-5F-Gal*p* (7)

We noticed in the preliminary studies that excess sodium dithionite ($Na_2S_2O_4$) could complicate the HPLC analysis. To minimize its interference on the time-course experiments for quantitative analysis, the incubations were conducted anaerobically in the presence of an internal standard. Under this condition, the dosage of $Na_2S_2O_4$ could be greatly reduced but still enough to keep enzyme active. Specifically, UGM (0.0, 0.8, 2, and 5 μM) was incubated with 200 μM UDP-5F-Gal*p* (**7**), 200 μM $Na_2S_2O_4$, and 25 μM internal standard (adenosine diphosphate for enzymatic reaction and adenosine for non-enzymatic reaction) in 360 μL KP_i buffer (100 mM, pH 7.5) anaerobically at 37 ºC for up to 24 h. Reaction aliquots were taken at various time intervals and analyzed by HPLC to follow the consumption of **7** and formation of UDP.

Exemplary HPLC traces from the reaction between 200 μM **7** and 5 μM UGM are shown in Figure S4A, and the controls without UGM are shown in Figure S4B. No significant change in the relative amount of uridine containing species (i.e., **7** and UDP) versus internal standard was observed in both cases (insets in Figure S4). Similar observations were also made when 2 or 0.8 μM UGM was used. These results revealed that no uridine-containing species other than UDP was produced, indicating that UDP is the only enzymatic product bearing uridine in the reaction of **7** with UGM. The residual concentration of UDP-5F-Gal*p* (**7**) can therefore be estimated based on the relative areas under peaks corresponding to **7** and UDP on HPLC traces. Residual concentration of **7** was plotted against time under different enzyme concentration as shown in Figure S5

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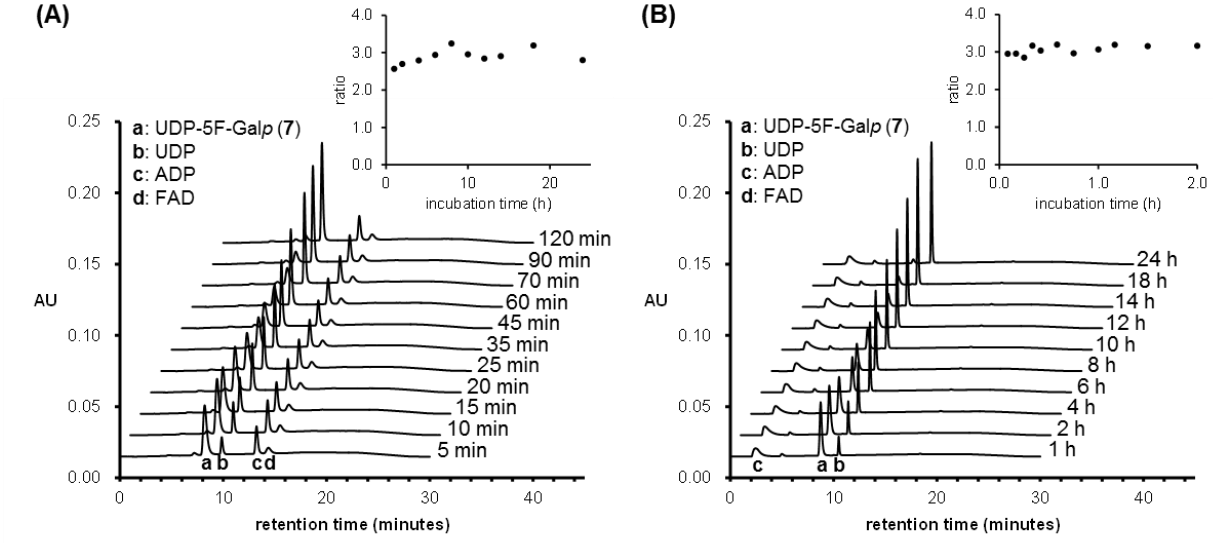


Figure S4. HPLC traces of reaction of 200 μM **7** in the presence of **(A)** 5 μM and **(B)** 0 μM of UGM. The incubation time is indicated next to each trace. The inset of each figure shows the area ratio between the sum of **7** and UDP versus the internal standard. These traces were generated using HPLC method B.

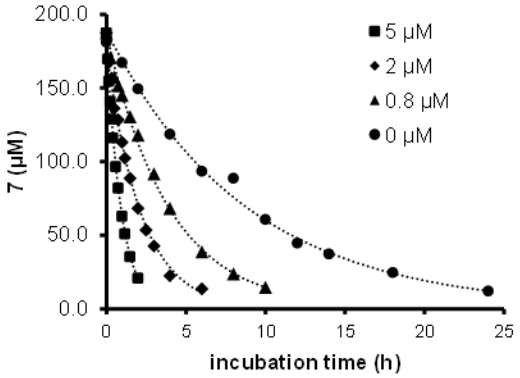


Figure S5. Residual concentration of 7 versus reaction time when 200 µM of 7 was incubated with UGM of different concentrations.

S5. Participation of Reduced FAD

Assay mixtures containing 200 μM **7** and 20 mM $Na_2S_2O_4$ in 50 μL KP_i buffer (100 mM, pH 7.5) at 37 ºC with either no UGM, 20 μM *apo*-UGM, 20 μM *apo*-UGM reconstituted with FAD or 20 μM *apo*-UGM reconstituted with 5-deaza-FAD. The reaction after 3 h incubation was analyzed by HPLC. As shown in Figure S6A, only the trial with FAD-reconstituted UGM (traces *b*) significantly differed from the trial with no enzyme (traces *d*), producing more UDP.

To examine the requirement of reductant for the observed enzyme activity, reaction containing 200 μM UDP-5F-Gal*p* (**7**) and 5 μM of as-isolated UGM in 50 μL KPi buffer (100 mM, pH 7.5) was incubated with (trace *a*, Figure S6B) or without (trace *c*) 20 mM $Na_2S_2O_4$ at 37 ºC for 1 h. Reaction containing reductant but without enzyme was also included as a control. When reductant ($Na_2S_2O_4$) was absent in the reaction mixture, the extent of hydrolysis of **7** (trace *c*) is not significantly different from its self-decomposition (trace *b*).

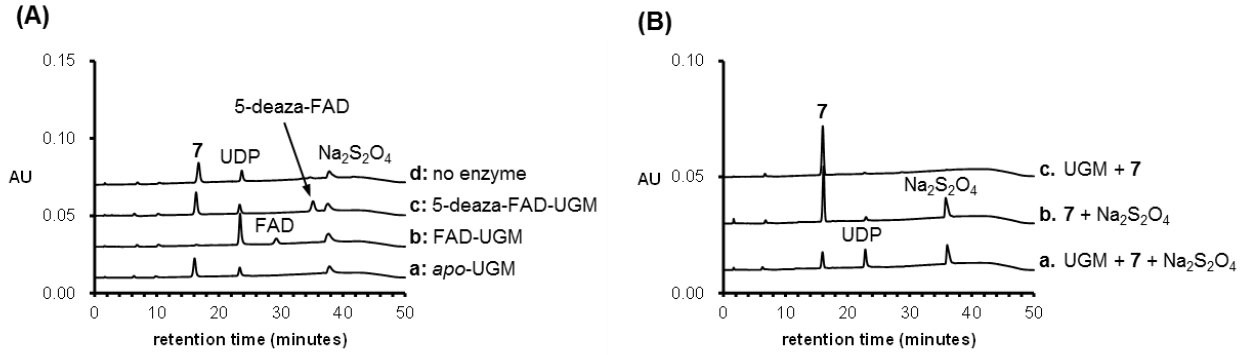


Figure S6. (A) HPLC traces (Method A) of the reactions of 200 μM **7** with differently reconstituted UGM (20 μM): *a*: *apo*-UGM, *b*: *apo*-UGM reconstituted with FAD, *c*: *apo*-UGM reconstituted with 5-deaza-FAD, *d*: no enzyme control. **(B)** HPLC traces (Method A) of incubation of 200 μM UDP-5F-Gal*p* (**7**) with 5 μM UGM for 1 h (trace *a*); without UGM (trace *b*), and without reductant ($Na_2S_2O_4$) (trace *c*).

S6. Identification of FAD-Analogue Adduct

A mixture containing 2.4 mM of UDP-5F-Gal*p* (**7**), 240 μM UGM, 20 mM $Na_2S_2O_4$ and 240 mM $NaBH_3CN$ in 200 μL KP_i buffer (100 mM, pH 7.5) was incubated anaerobically at 37 °C for 1 h.[6] The enzyme was precipitated by adding 30 μL of 6 N HCl and the supernatant was neutralized with 500 mM KP_i buffer at pH 7.5 until the pH of the mixture was returned to 7. The denatured protein was pelleted twice by centrifugation and washed once with 200 μL of 1 M NaCl. The combined supernatants were treated with freshly-prepared $Na_2S_2O_4$ (25 mM) and analyzed by LC-MS with negative ion ESI-MS detection.

Addition of $NaBH_3CN$ in the enzymatic reaction allowed us to detect reduced covalent intermediates **22** and **23** (peaks **I** and **II** in Figure S7). Carbonyl group is known to be relatively inert toward $NaBH_3CN$ reduction under neutral pH as compared to iminium ion.[7] However, since the reaction was quenched by 6 N HCl, the temporary low pH could possibly facilitate the reduction of carbonyl group, which could explain the observation of both singly (**22**) and doubly reduced (**23**) adducts. In addition, peaks **III** and **IV** were found to have identical *m/z*, corresponding to that of unmodified FAD_{ox}. But, careful inspection showed that the isotopic patterns of **III** and **IV** are different. Peak **IV** has a slightly stronger signal at *m/z* 786.1, which matches the predicted *m/z* of FAD_{red}. We therefore reasoned that FAD_{ox} and FAD_{red} were eluted from the LC column at different retention time (peaks **III** and **IV**, respectively). However, FAD_{red} is oxidized rapidly under the ionization condition resulting in almost identical mass spectra.

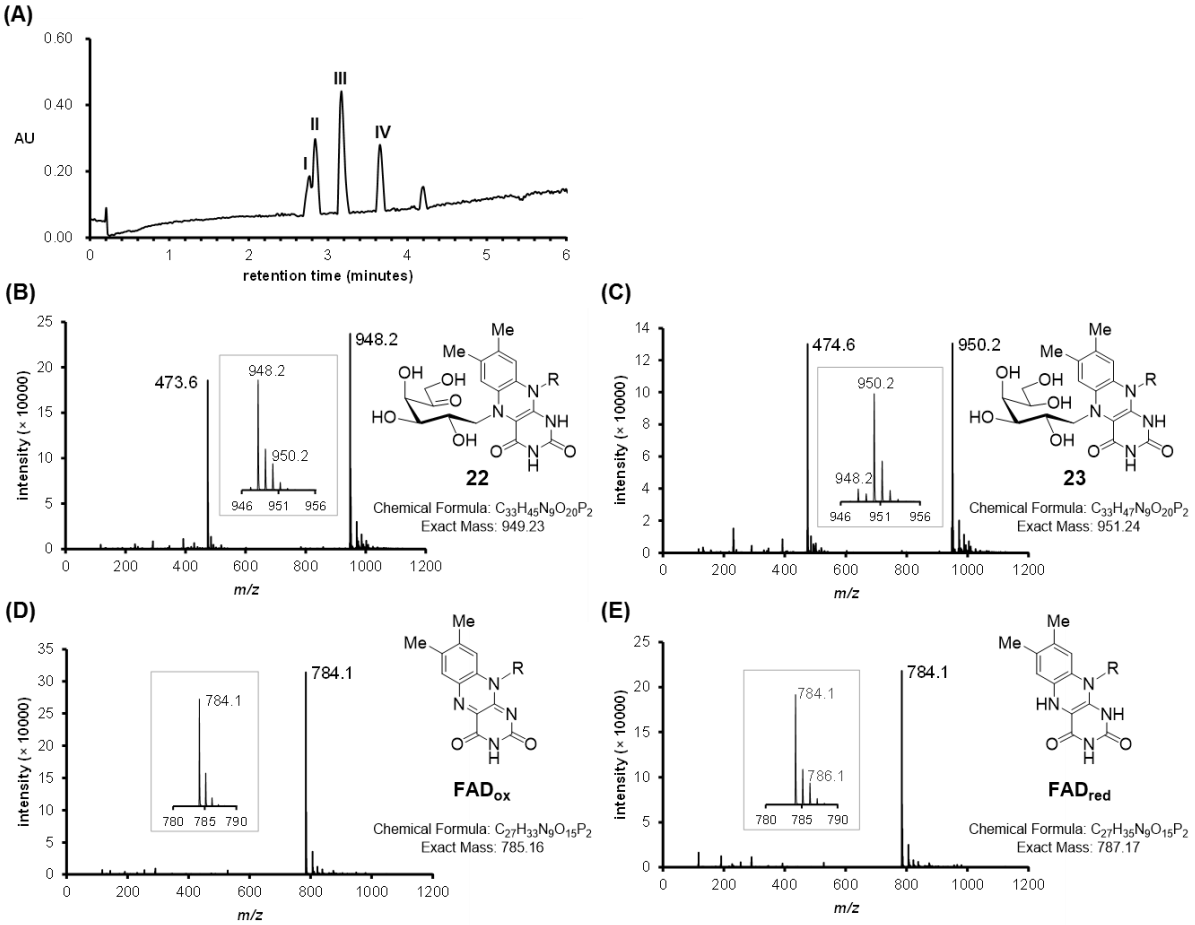


Figure S7. LC-ESI-MS of the reaction between **7** and UGM in the presence of $NaBH_3CN$. **(A)** LC trace, and negative-mode mass spectra of **(B)** peak **I** **(C)** peak **II** **(D)** peak **III** and **(E)** peak **IV**, along with the assigned structures. The insets show the expanded regions around the predicted *m/z* of target molecules.

S7. Time-Dependent Release of Free Fluoride Anion

UGM stock was buffer-exchanged with 20 mM KP_i buffer in D_2O (pD 7.9) and used to prepare an anaerobic assay mixture containing 5 μM UGM, 700 μM UDP-5F-Gal*p* (**7**), and 2 mM $Na_2S_2O_4$ in 700 μL of 20 mM KP_i buffer in D_2O (pD 7.9). The mixture was immediately transferred to an NMR tube, sealed, and frozen prior to NMR analysis at which point the reaction mixture was thawed, and the temperature was raised to 37 °C. ^{19}F NMR spectra were then recorded every 15 min over 12 h at 37 °C. The spectra of the first 135 min were compiled in Figure 3B.

We also monitored the non-enzymatic hydrolysis of 700 μM of **7** in 700 μL of 20 mM KP_i buffer in D_2O (pD 7.9) using ^{19}F NMR (acquired every 2 h) over 12 h. Fluoride release was observed non-enzymatically (Figure S8) as evidenced by the reduction of the 5-F triplet signal from **7** at –119 ppm and the appearance of a free fluoride singlet signal at –122 ppm.[8,9] However, **7** was not completely consumed even after 12 h. These results are consistent with the time-course experiments shown in Figure S5.

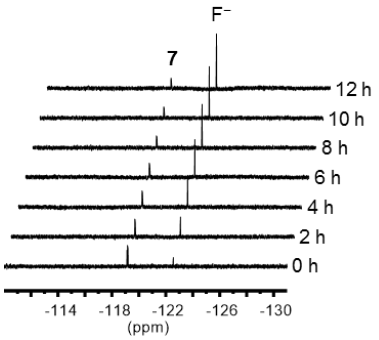
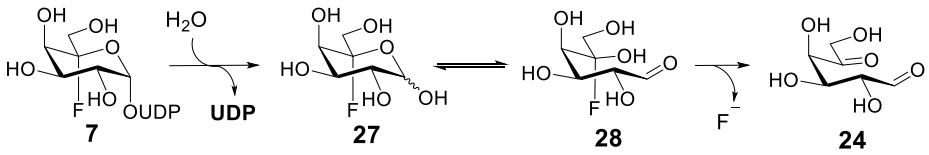


Figure S8. Time-course ^{19}F NMR of non-enzymatic hydrolysis of **7**. The spectra were recorded every 2 h.

The release of fluoride in the non-enzymatic hydrolysis could be explained by the following scenario (Scheme S1). Compound **7** first undergoes hydrolysis to release UDP. The resulting hexose (**27**) existing in hemiacetal form is in equilibrium with its linear form (**28**), which also possesses a *gem*-fluorohydrin moiety at C5. Rapid elimination of fluoride from this moiety generates a signal at –122 ppm in ^{19}F NMR and **24**. In the presence of UGM, FAD_{red} in the active site is more efficient in displacing UDP of **7** resulting in more rapid release of fluoride.

Scheme S1. Proposed mechanism for the non-enzymatic hydrolysis of 7.



S8. Characterization of the Turnover Product from Incubation of UDP-5F-Gal*p* with UGM

A mixture containing 5 μM UGM, 200 μM **7** and 2 mM $Na_2S_2O_4$ in 100 μL water was incubated at 37 °C anaerobically for 3 h, after which the protein was filtered off using YM-10 membrane. The lyophilized filtrate was then incubated with 100 μL of *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) solution in pyridine (5 mg/mL) at 80 °C for 20 min, followed by the treatment of 100 μL acetic anhydride (Figure S9A).[10] After another 20-min incubation at 80 °C, solvents were evaporated under reduced pressure. The residue was dissolved in 500 μL EtOAc, and was subsequently washed with 1 N HCl (100 μL × 2) and water (100 μL). As shown in Figure S9B and C, LC-ESI-MS analysis of the EtOAc layer in positive mode revealed the existence of two new species with *m/z* ratios of 564.1 and 759.1, consistent with mono- and di-*O*-pentafluorobenzyl oxime acetates (**24a**, 564.1 for $[M + Na]^+$ and **24b**, 759.1 for $[M + Na]^+$) of 5-oxo-D-galactose (**24**), respectively.

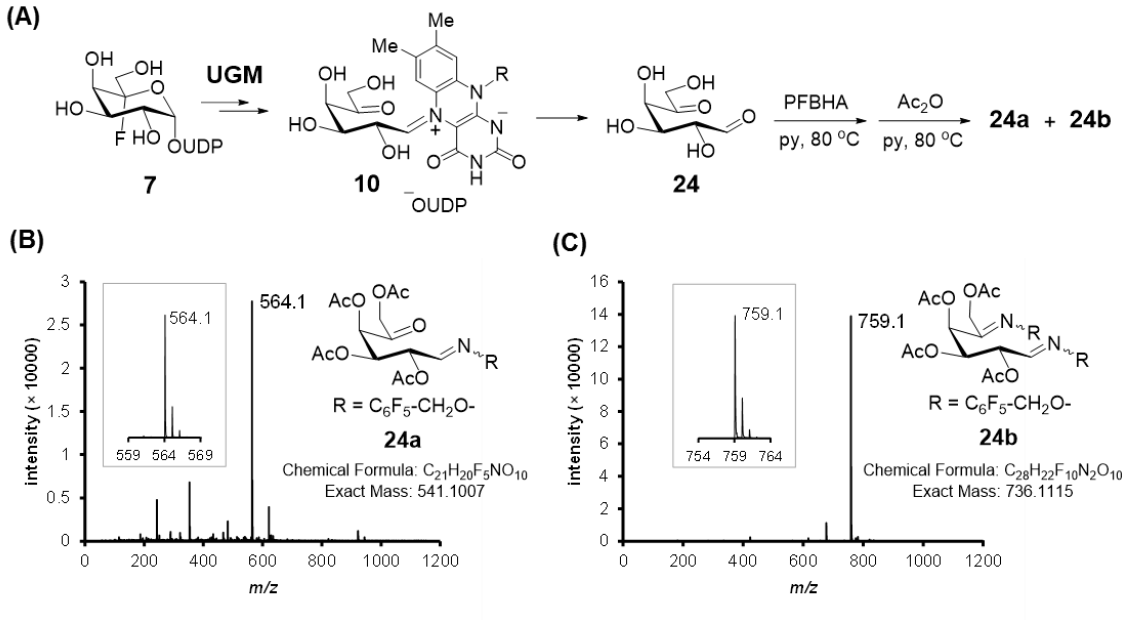


Figure S9. (A) Derivatization of the turnover product **24** generated in the reaction of **7** with UGM using PFBHA and Ac_2O. **(B & C)** Proposed structures of derivatized **24** along with their predicted molecular masses. The observed signals from LC-ESI-MS are consistent with $[M + Na]^+$ ions of **24a** and **24b**.

S9. Hydrolysis of UDP-Gal*p* (1) by UGM

Inspired by the studies of UDP-5F-Gal*p* (**7**), we also want to know whether UGM is inherently capable of catalyzing hydrolysis of Schiff base intermediate (**5** or **10**). Reactions containing 1 to 20 μM of UGM, 200 μM UDP-Gal*p* (**1**), and 2 mM $Na_2S_2O_4$ in 30 μL KP_i buffer (100 mM, pH 7.5) were incubated for 2 h at 37 ºC at which point they were analyzed by HPLC for the production of UDP. Although not as prominent as the hydrolysis of **7** by UGM, UDP was indeed produced from UDP-Gal*p* (**1**) and dependent on the amount of enzyme used (Figure S10A).

To ensure that the observed hydrolysis activity of UGM was not from the instability of either UDP-Gal*p* (**1**) or UDP-Gal*f* (**2**), each substrate was subjected to the same reaction condition (200 μM substrate, 2 mM $Na_2S_2O_4$, 100 mM KP*i* buffer, pH 7.5) without enzyme for 2 to 6 h at 37 ºC. As shown in Figure S10B and C, there was no significant hydrolysis of either substrate. The stock of **2** contained UDP contaminant, but it did not increase significantly over time. These results again support the hypothesis that UGM possesses hydrolysis activity.

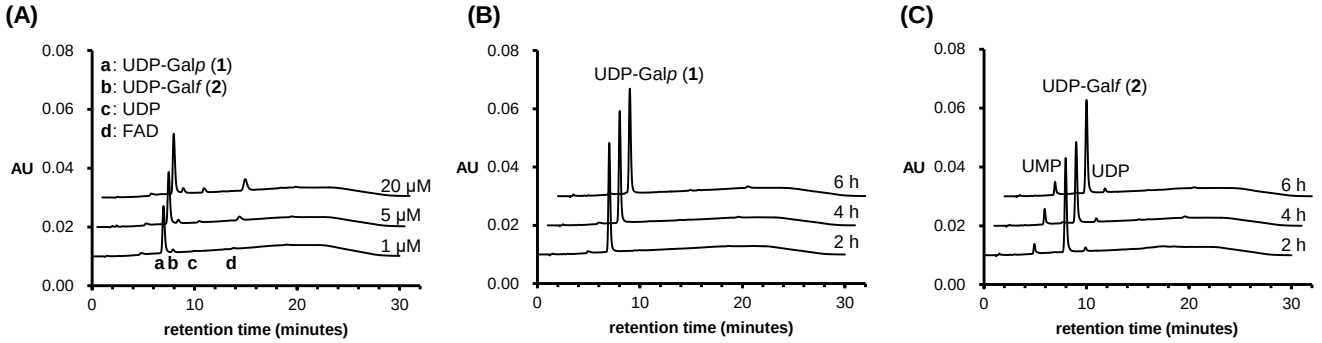


Figure S10. (A) HPLC traces of reactions of UGM with UDP-Gal*p* (**1**) for 2 h. The amount of enzyme used was indicated next to each trace. HPLC traces of hydrolysis of **(B)** UDP-Gal*p* (**1**) and **(C)** UDP-Gal*f* (**2**) in the buffer over 6 h. The incubation time was indicated next to each trace.

Initial rates of hydrolysis of UDP-Gal*p* (**1**) were also measured using 5 μM of UGM, 20 to 2500 μM UDP-Gal*p* (**1**), and 200 μM $Na_2S_2O_4$ in 60 μL of KP_i buffer (100 mM, pH 7.5) anaerobically at 37 ºC. Aliquots of 30 μL were withdrawn at 1 and 2 h, worked up and analyzed by HPLC to determine the fractional conversion based on HPLC peak integrations. Reactions were run in triplicate and pseudo-random order, and the non-linear regression function of R was used to fit the data to the Michaelis-Menten equation (Figure S11). k_{cat} and k_{cat}/K_M of this hydrolysis reaction were determined to be 0.636 ± 0.031 h^{-1} and 0.0114 ± 0.0026 h^{-1} μM^{-1}, respectively.

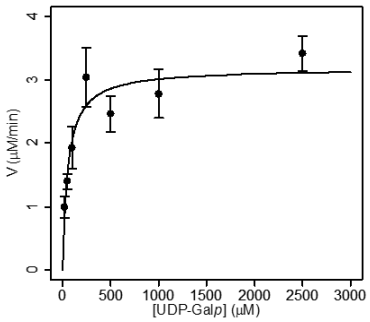


Figure S11. Initial rates of hydrolysis of UDP-Gal*p* (**1**) by UGM, plotted against the concentration of UDP-Gal*p* (**1**). Each initial rate is based on three replicate trials and error bars denote ± one standard error about the mean initial rates.

S10. Chemical Syntheses

Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-phenylselenyl-α-D-galactopyranoside (17). To a solution of $(SePh)_2$ (1.82 g, 5.83 mmol) in absolute ethanol (15 mL) cooled at 0 ºC was added $NaBH_4$ (0.92 g, 24.3 mmol) portion-wise over 10 min. The resulting yellowish mixture was stirred at 0 ºC for 30 min, and the solution gradually turned into a clear solution. To this clear solution was added **16** (2.55 g, 4.85 mmol) in DMF (10 mL) dropwise over 20 min. The resulting pale-yellow suspension solution was stirred at 0 ºC for 5 min, then at 100 ºC for 3.5 h. The reaction mixture was cooled down to room temperature, diluted with CH_2Cl_2, and quenched with $NH_4Cl_{(aq)}$. Solvent was removed under reduced pressure. The obtained residue was partitioned between CH_2Cl_2 (100 mL × 3) and water (100 mL), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated to give a yellow oil, which was chromatographed on silica gel (EtOAc/Hex = 1/9 to 15/85) to afford **17** as a pale yellow oil (2.88 g, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.79 (dd, *J* = 12.4, 6.8 Hz, 1H, 6–H), 3.09 (dd, *J* = 12.4, 7.2 Hz, 1H, 6–H), 3.30 (s, 3H, OMe), 3.78 (brt, *J* = 7.0 Hz, 1H, 5–H), 3.88 (dd, *J* = 10.0, 2.8 Hz, 1H, 3–H), 3.94 (brd, *J* = 2.8 Hz, 1H, 4–H), 4.01 (dd, *J* = 10.0, 3.6 Hz, 2–H), 4.53 (d, *J* = 11.6 Hz, 1H, OCH_2Ph), 4.63 (d, *J* = 3.6 Hz, 1H, 1–H), 4.66 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.73 (d, *J* = 11.6 Hz, 1H, OCH_2Ph), 4.81 (d, *J* = 12.4 Hz, 1H, OCH_2Ph), 4.87 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.98 (d, *J* = 11.6 Hz, 1H, OCH_2Ph), 7.18–7.42 (m, 20H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.0, 55.2, 73.5, 74.7, 75.7, 76.1, 79.3, 98.8, 126.8, 127.5, 127.5, 127.7, 127.7, 128.0, 128.3, 128.4, 128.4, 129.1, 130.2, 132.1, 138.3, 138.4, 138.7. HRMS (ESI) *m/z* calcd for $C_{34}H_{36}O_5Se$ ($[M + Na]^+$) 627.1623; found 627.1628.

2,3,4-Tri-*O*-benzyl-6-deoxy-6-phenylselenyl-α/β-D-galactopyranose (18). A solution of **17** (2.88 g, 4.77 mmol) in acetic acid (90 mL) and H_2SO_4 (3 M, 11.1 mL) was stirred at 85 ºC for 2 h. The solution was cooled down to 0 ºC, and mixed with Na_2CO_3 (3 g). The resulting mixture was partitioned between CH_2Cl_2 (100 mL × 3) and 1 N NaOH (200 mL). Combined organic layers were washed with brine (200 mL), dried over $MgSO_4$, filtered and concentrated to give yellow oil, which was subsequently chromatographed on silica gel (EtOAc/Hex = 25/75) to afford **18** as a yellow oil (1.98 g, 71%, α : β = 1.6 : 1). Spectroscopy characterization of the α-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.92 (dd, *J* = 12.6, 8.4 Hz, 1H, 6–H), 3.08 (dd, *J* = 12.6, 6.2 Hz, 1H, 6–H), 3.88 (dd, *J* = 9.8, 2.6 Hz, 1H, 3–H), 3.98–4.10 (m, 3H, 2–H, 4–H, 5–H; overlapped with 4–H of the β-anomer), 4.52 (d, *J* = 11.6 Hz, 1H, OCH_2Ph), 4.69 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.71–4.85 (m, 3H, OCH_2Ph, overlapped with 3H of OCH_2Ph of the β-anomer), 4.98 (d, *J* = 11.6 Hz, 1H, OCH_2Ph), 5.24 (d, *J* = 3.6 Hz, 1H, 1–H), 7.10–7.53 (m, 20H). Spectroscopy characterization of the β-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.95 (dd, *J* = 12.8, 8.2 Hz, 1H, 6–H), 3.12 (dd, *J* = 12.8, 5.8 Hz, 1H, 6–H), 3.27 (brs, 1H, OH), 3.43 (br dd, *J* = 8.2, 5.8 Hz, 5–H), 3.50 (dd, *J* = 9.6, 2.8 Hz, 3–H), 3.74 (dd, *J* = 9.6, 7.2 Hz, 2–H), 3.98–4.10 (m, 1H, 4–H; overlapped with 2–H, 4–H, and 5–H of the α-anomer), 4.54 (d, *J* = 11.6 Hz, 1H, OCH_2Ph), 4.57 (brd, *J* = 7.2 Hz, 1–H), 4.71–4.85 (m, 3H, OCH_2Ph, overlapped with 3H of OCH_2Ph of the α-anomer), 4.90 (d, *J* = 11.2 Hz, 1H, OCH_2Ph), 5.00 (d, *J* = 11.6 Hz, 1H, OCH_2Ph), 7.15–7.51 (m, 20H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.5, 27.7, 70.5, 73.1, 73.2, 73.5, 74.4, 74.6, 74.7, 74.9, 75.1, 76.4, 79.1, 80.5, 82.5, 91.9, 97.7, 127.0, 127.1, 127.5, 127.6, 127.6, 127.7, 127.7, 127.7, 127.8, 128.0, 128.1, 128.3, 128.3, 128.3, 128.4, 129.1, 129.2, 129.9, 132.4, 132.6, 138.1, 138.3, 138.3, 138.5. HRMS (ESI) *m/z* calcd for $C_{33}H_{34}O_5Se$ ($[M + Na]^+$) 613.1466; found 613.1466.

Dibenzyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-phenylselenyl-α-D-galactopyranosyl phosphate (19). To a solution of dibenzyl phosphite (2.0 mL, 9.0 mmol) in anhydrous toluene (30 mL) was added *N*-chlorosuccinimide (1.62 g, 12.1 mmol). After stirring at room temperature for 2 h, the mixture was filtered to remove white precipitate. The filtrate was concentrated to give dibenzyl chlorophosphate as a pale-yellow oil. To a solution of **18** (3.98 g, 6.7 mmol) in anhydrous THF (30 mL) cooled at –78 ºC was added BuLi (4 mL, 2.5 M in hexane, 10 mmol) dropwise over 10 min. The resulting yellowish solution was stirred at the same temperature for another 15 min, and was subsequently treated with the above described freshly prepared dibenzyl chlorophosphate in anhydrous THF (15 mL) dropwise over 15 min. The reaction mixture was stirred at –78 ºC for 2 h and quenched with $NH_4Cl_{(aq)}$. The mixture was then partitioned between ether (150 mL × 2) and water (150 mL). The combined organic layers were washed with brine (150 mL), dried over MgSO4, and concentrated to give crude yellow oil, which was chromato-

graphed on silica gel (EtOAc/Hex = 25/75) to afford **19** as a bright-yellow oil (2.98 g, 48%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.93 (dd, *J* = 12.4, 8.4 Hz, 1H, 6–H), 3.01 (dd, *J* = 12.4, 5.8 Hz, 1H, 6–H), 3.83 (dd, *J* = 10.0, 3.2 Hz, 1H, 3–H), 4.03 (br dd, *J* = 8.4, 5.8 Hz, 1H, 5–H), 4.07–10 (m, 1H, 4–H), 4.11 (dt, *J* = 10.0 , 3.2 Hz, 1H, 2–H), 4.47 (d, *J* = 11.2 Hz, 1H, OCH_2Ph), 4.71 (d, *J* = 11.2 Hz, 1H, OCH_2Ph), 4.71 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.77 (d, *J* = 11.2 Hz, 1H, OCH_2Ph), 4.81 (d, *J* = 11.6 Hz, 1H, OCH_2Ph), 4.89–5.05 (m, 5H, OCH_2Ph), 5.94 (dd, *J* = 6.8, 3.2 Hz, 1H, 1–H), 7.06–7.44 (m, 30H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.1, 69.0 (CH_2, d, *J* = 5.3 Hz), 69.1 (CH_2, d, *J* = 5.7 Hz), 72.7, 73.2, 73.2, 74.9, 75.5 (d, *J* = 6.4 Hz), 78.4, 96.5 (d, *J* = 6.0 Hz), 127.0, 127.4, 127.6, 127.7, 127.8, 128.1, 128.2, 128.3, 128.4, 128.4, 128.5, 129.2, 129.8, 132.1, 135.8 (d, *J* = 7.6 Hz), 135.9 (d, *J* = 8.1 Hz), 137.9, 138.2, 138.4. ^{31}P NMR ($CDCl_3$, 162MHz) δ –2.18. HRMS (ESI) *m/z* calcd for $C_{47}H_{47}O_8PSe$ ($[M + Na]^+$) 873.2058; found 873.2071.

Dibenzyl 2,3,4-tri-*O*-benzyl-6-deoxy-α-D-*xylo*-hex-5-enopyranosyl phosphate (20). To a solution of **19** (477.1 mg, 0.561 mmol) in MeOH/H_2O (16.8 mL, v/v = 6/1) was added $NaHCO_3$ (75.5 mg, 0.90 mmol) and $NaIO_4$ (240.3 mg, 1.12 mmol). The suspension solution was stirred at room temperature for 2 h, and MeOH was removed under reduced pressure. The residue was then partitioned between CH_2Cl_2 (30 mL × 3) and water (30 mL). The combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated to afford the corresponding selenoxide as a yellow oil (487.3 mg). The obtained selenoxide was re-dissolved in 2,3-dihydropyran (10 mL) and refluxed for 2.5 h. The resulting bright yellow solution was concentrated, and chromatographed on silica gel (EtOAc/Hex = 25/75 to 3/7) to afford **20** as a yellow oil (369.3 mg, 95%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.89 (dd, *J* = 10.0, 3.3 Hz, 1H, 3–H), 4.08 (d, *J* = 3.3 Hz, 1H, 4–H), 4.28 (dt, *J* = 10.0, 3.0 Hz, 1H, 2–H), 4.41 (d, *J* = 12.5 Hz, 1H, OCH_2Ph), 4.45 (s, 1H, 6–H), 4.58 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.63 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.72 (d, *J* = 12.5 Hz, 1H, OCH_2Ph), 4.76 (s, 1H, 6–H), 4.78 (d, *J* = 11.0 Hz, 1H, OCH_2Ph), 4.82 (d, *J* = 11.0 Hz, 1H, OCH_2Ph), 4.95 (d, *J* = 7.0 Hz, 2H, OCH_2Ph), 5.03 (dd, *J* = 12.0, 7.5 Hz, 1H, OCH_2Ph), 5.06 (dd, *J* = 12.0, 7.0 Hz, 1H, OCH_2Ph), 6.03 (dd, *J* = 7.0, 3.0 Hz, 1H, 1–H), 7.12–7.40 (m, 25H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 69.1 (d, *J* = 5.4 Hz), 69.2 (d, *J* = 5.4 Hz), 69.4, 72.2, 73.8, 74.2, 74.9 (d, *J* = 6.9 Hz), 75.7, 96.1 (d, *J* = 6.1 Hz), 103.1, 127.6, 127.6, 127.6, 127.6, 127.7, 128.0, 128.1, 128.2, 128.3, 128.3, 128.3, 128.4, 128.4, 128.4, 128.5, 129.8, 135.7 (d, *J* = 7.8 Hz), 135.8 (d, *J* = 8.1 Hz), 137.6, 138.0, 138.2, 151.0. ^{31}P NMR ($CDCl_3$, 202 MHz) δ –1.9. HRMS (ESI) *m/z* calcd for $C_{41}H_{41}O_8P$ ($[M + Na]^+$) 715.2431; found 715.2431.

Dibenzyl 2,3,4-tri-*O*-benzyl-5-fluoro-α-D-galactopyranosyl phosphate (21) and Dibenzyl 2,3,4-tri-*O*-benzyl-5-fluoro-β-L-idopyranosyl phosphate (5-*epi*-21). To a solution of **20** (1.47 g, 2.1 mmol) in MeCN/EDTA (4×10^{-4} M)/acetone (84 mL, 1:1:2) was added a mixture of oxone (6.52 g, 21 mmol) and $NaHCO_3$ (2.67 g, 32 mmol) in one portion. The suspension solution was stirred at room temperature for 3.5 h. It was then partitioned between CH_2Cl_2 (200 mL × 2) and water (100 mL). The pooled organic layers were dried over Na_2SO_4, filtered, and concentrated to give the desired epoxide as a white slurry. The epoxide was re-dissolved in andydrous CH_2Cl_2 (21 mL), cooled to –78 ºC, and treated with hydrogen fluoride pyridine (70%, 827 mL, 32 mmol), which was added dropwise over 5 min. The resulting yellowish solution was stirred at –78 ºC for another 1 h. After that, it was quenched with $NaHCO_{3(aq)}$ at 0 ºC. The mixture was partitioned between CH_2Cl_2 (100 mL × 2) and water (50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give a pale-yellow slurry, which was chromatographed on silica gel (EtOAc/Hex = 35/65) repeatedly to afford **21** (475.7 mg, 31%) and 5-*epi*-**21** (75 mg, 5%), both as colorless oil. Spectroscopic characterization of **21**: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.56 (t, *J* = 12.0 Hz, 1H, C6–H), 3.72 (dd, *J* = 23.7, 12.0 Hz, 1H, C6–H), 4.18 (t, *J* = 2.1 Hz, 1H, C4–H), 4.22 (dt, *J* = 10.2, 2.1 Hz, 1H, 3–H), 4.24 (ddd, *J* = 10.2, 3.6, 2.4 Hz, 1H, 2–H), 4.68 (d, *J* = 11.4 Hz, 1H, OCH_2Ph), 4.71 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.74 (d, *J* = 11.1 Hz, 1H, OCH_2Ph), 4.78 (d, *J* = 11.1 Hz, 1H, OCH_2Ph), 4.84 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.93 (d, *J* = 11.4 Hz, 1H, OCH_2Ph), 4.96 (dd, *J* = 12.0, 6.6 Hz, 1H, OCH_2Ph), 4.99 (dd, *J* = 12.0, 7.2 Hz, 1H, OCH_2Ph), 5.03 (dd, *J* = 11.7, 6.9 Hz, 1H, OCH_2Ph), 5.08 (dd, *J* = 11.7, 6.9 Hz, 1H, OCH_2Ph), 6.01 (dd, *J* = 8.1, 3.6 Hz, 1H, 1–H), 7.15–7.37 (m, 25H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 63.3 (d, *J* = 25.1 Hz, C-6), 69.1 (dd, *J* = 5.7, 1.4 Hz), 69.2 (d, *J* = 5.6 Hz), 73.5, 73.5, 74.2–74.6 (m, C-2, C-3 and C-4), 75.2, 95.5 (d, *J* = 6.0 Hz, C-1), 113.4 (d, *J* = 225.5 Hz, C-5), 127.5, 127.6, 127.7, 127.7, 127.8, 128.1, 128.2, 128.3, 128.3, 128.3, 128.4, 128.4, 128.4, 128.4, 128.5, 135.9 (d, *J* = 8.1 Hz), 135.9 (d, *J* = 8.3 Hz), 137.7, 137.7,

138.2. ^{31}P NMR ($CDCl_3$, 243 MHz) δ –2.9. ^{19}F NMR (CDCl3, 565 MHz) δ –116.9 (dd, *J* = 23.7, 12.0 Hz). HRMS (ESI) *m/z* calcd for $C_{41}H_{42}FO_9P$ ($[M + Na]^+$) 751.2443; found 751.2443. Spectroscopic characterization of the 5-*epi*-**21**: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.56 (dd, *J* = 12.0, 4.8 Hz, 1H, 6–H), 3.74–3.81 (m, 3H, 2–H, 3–H, and 6–H), 4.11 (dd, *J* = 25.2, 4.2 Hz, 1H, 4–H), 4.41 (d, *J* = 11.4 Hz, 1H, OCH_2Ph), 4.51 (d, *J* = 12.6 Hz, 1H, OCH_2Ph), 4.53 (s, 2H, OCH_2Ph), 4.58 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.62 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.98–5.09 (m, 4H, OCH_2Ph), 5.91 (dd, *J* = 6.6, 1.8 Hz, 1H, 1–H), 7.12–7.37 (m, 25H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 62.9 (d, *J* = 37.4 Hz, C-6), 69.7 (d, *J* = 5.7 Hz), 69.8 (d, *J* = 5.4 Hz), 70.3 (d, *J* = 22.1 Hz, C-4), 72.9, 73.2, 73.4 (C-3), 73.4, 76.0 (d, *J* = 7.4 Hz, C-2), 93.0 (d, *J* = 5.4 Hz, C-1), 112.4 (d, *J* = 233.7 Hz, C-5), 127.7, 127.8, 127.9, 127.9, 128.0, 128.1, 128.3, 128.4, 128.4, 128.6, 128.6, 128.6, 135.4 (d, *J* = 6.9 Hz), 135.5 (d, *J* = 6.9 Hz), 137.2, 137.4, 137.8. ^{31}P NMR ($CDCl_3$, 243 MHz) δ –2.8. ^{19}F NMR ($CDCl_3$, 565 MHz) δ –128.9 (brs). HRMS (ESI) *m/z* calcd for $C_{41}H_{42}FO_9P$ ($[M + Na]^+$) 751.2443; found 751.2440.

UDP-5-fluoro-α-D-galactopyranose (7). A Solution of **21** (210 mg, 0.29 mmol) in MeOH was stirred under hydrogen atmosphere (1 atm) in the presence of Pd/C (10%) at room temperature for 3 h. The catalyst was filtered off through a Celite pad. The filtrate was concentrated and re-dissolved in water (10 mL). This solution was treated with DOWEX (50WX8, Bu_3NH^+ form), and the resulting suspension solution was stirred at 4 ºC overnight. After filtering off the resin, the filtrate was lyophilized to give tributylammonium 5-fluoro-α-D-galactopyranosyl-1-phosphate. This phosphate compound was mixed with UMP-morpholidate (296.7 mg, 0.43 mmol), and co-evaporated with anhydrous pyridine three times. 1*H*-tetrazole (60.6 mg, 0.87 mmol) was also co-evaporated with anhydrous pyridine separately. Both of these mixtures/compounds were dried under high vacuum for 2 h, dissolved in anhydrous pyridine (3 mL), and then combined together. The resulting yellowish solution was stirred at room temperature for 3 d. The reaction mixture was partitioned between water (30 mL) and Et_2O (20 mL × 3). Since the target compound is unstable at low and high pH, the aqueous layer was neutralized with 1 M NH_4HCO_3 to bring the pH back to ~ 6. The crude product was purified by HPLC using Dionex column (CarboPac PA1, 9 × 250 mm, HPLC method C, see Section S1). The collected fractions were pooled and lyophilized several times to give **7** as a white to pale-yellow powder (15.9 mg, 9.4%). NH_4OAc could not be fully removed, so the amount of product was determined based on the UV absorbance of the uracil moiety in the structure. ^{1}H NMR (D_2O, 600 MHz) δ 3.58 (dd, *J* = 16.8, 12.6 Hz, 1H, 6″ –H), 3.66 (dd, *J* = 20.4, 12.6 Hz, 1H, 6″–H), 3.84 (ddd, *J* = 10.8, 4.2, 3.0 Hz, 1H, 2″–H), 3.98–4.06 (m, 3H, 3″–H, 4″–H and 5′–H), 4.07–4.15 (m, 2H, 4′–H and 5′–H), 4.19 (t, *J* = 4.8 Hz, 1H, 3′–H), 4.21 (t, *J* = 4.8 Hz, 1H, 2′–H), 5.58 (dd, *J* = 8.4, 4.2 Hz, 1H, 1″–H), 5.81 (d, *J* = 8.4 Hz, 1H, 5–H), 5.82 (d, *J* = 5.4 Hz, 1H, 1′–H), 7.77 (d, *J* = 8.4 Hz, 1H, 6–H). ^{31}P NMR (D_2O, 243 MHz) δ –11.5 (d, *J* = 19.1 Hz), –13.1 (d, *J* = 19.1 Hz). ^{19}F NMR (D_2O, 565 MHz) δ –120.0 (t, *J* = 18.5 Hz). HRMS (ESI) *m/z* calcd for $C_{15}H_{23}FN_2O_{17}P_2$ ($[M – H]^–$) 583.0383; found 582.0381.

Dibenzyl 2,3,4-tri-*O*-benzyl-6-deoxy-5,6-difluoro-α-D-galactopyranosyl phosphate (25). To a solution of **21** (112.6 mg, 0.16 mmol) in anhydrous pyridine/CH_2Cl_2 (v/v = 1:10, 1.65 mL) cooled at –40 ºC was added diethylaminosulfur trifluoride (204 μL, 1.6 mmol) dropwise over 5 min. The resulting solution was gradually warmed to room temperature, and was stirred for another 12 h. The reaction was then brought to reflux for 2 h, which was then cooled down to 0 ºC, and subsequently quenched by $NaHCO_{3(aq)}$. The quenched solution was then partitioned between CH_2Cl_2 (10 mL × 2) and water (10 mL). The pooled organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated to give pale yellow oil. The crude oil was then chromatographied with silica gel to give **25** as pale yellow oil (36.9 mg, 32.7%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.21 (ddd, *J* = 46.8, 11.6, 10.0 Hz, 1H, 6–H), 4.18–4.27 (m, 3H, 2–H, 3–H and 4–H), 4.54 (ddd, *J* = 45.6, 26.4, 10.0 Hz, 1H, 6–H), 4.63 (d, *J* = 10.8 Hz, 1H, OCH_2Ph), 4.74 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.75 (d, *J* = 11.2 Hz, 1H, OCH_2Ph), 4.79 (d, *J* = 11.2 Hz, 1H, OCH_2Ph), 4.85 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.96 (d, *J* = 11.2 Hz, 1H, OCH_2Ph), 4.97 (dd, *J* =12.0, 5.6 Hz, 1H, OCH_2Ph), 5.01 (dd, *J* = 12.0, 6.8 Hz, 1H, OCH_2Ph), 5.04 (dd, *J* = 11.6, 6.8 Hz, 1H, OCH_2Ph), 5.09 (dd, *J* = 11.6, 6.8 Hz, 1H, OCH_2Ph), 6.01 (dd, *J* = 8.2, 2.4 Hz, 1H, 1–H), 7.15–7.40 (m, 25H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 69.1, 69.2, 69.2, 73.4, 73.5, 73.9, 74.1, 74.1, 74.2, 74.5, 76.0, 81.2 (dd, *J* = 173.9, 24.6 Hz, C-6), 95.5 (d, *J* = 5.9 Hz, C-1), 111.7 (d, *J* = 229.6, 24.8 Hz, C-5), 127.4, 127.5, 127.7, 127.8, 128.0, 128.1, 128.2, 128.3, 128.3, 128.4, 128.4, 135.7 (d, *J* = 8.3 Hz), 135.8 (d, *J* = 8.2 Hz), 137.5, 137.5, 138.1. ^{31}P NMR ($CDCl_3$, 162 MHz) δ –3.0. ^{19}F NMR (CDCl3, 376 MHz) δ –116.2 (dt, *J* = 26.4, 12.8 Hz, 5–F), –236.2 (td, *J* = 46.2, 14.3

Hz, 6–F). HRMS (ESI) *m/z* calcd for $C_{41}H_{41}F_2O_8P$ ([M + Na]$^+$) 753.23990; found 753.23840.

UDP-6-deoxy-5,6-difluoro-α-D-galactopyranose (26). A Solution of **25** (67.7 mg, 0.093 mmol) in MeOH was stirred under hydrogen atmosphere (1 atm) in the presence of Pd/C (10%) at room temperature until the TLC indicated the complete consumption of starting material. The catalyst was filtered off through a Celite pad. The filtrate was concentrated and re-dissolved in water (10 mL). This solution was treated with DOWEX (50WX8, Bu_3NH^+ form), and the resulting suspension solution was stirred at 4 ºC overnight. After filtering off the resin, the filtrate was lyophilized to give tributylammonium 6-deoxy-5,6-difluoro-α-D-galactopyranosyl-1-phosphate (1:2 Bu_3NH^+ salt). This phosphate compound was mixed with UMP-morpholidate (190.1 mg, 0.28 mmol), and co-evaporated with anhydrous pyridine three times. 1*H*-tetrazole (39.2 mg, 0.87 mmol) was also co-evaporated with anhydrous pyridine separately. Both of these mixtures/compounds were dried under high vacuum for 1 h, dissolved in anhydrous pyridine (3 mL), and then combined together. The resulting yellowish solution was stirred at room temperature for 22 h. The reaction mixture was partitioned between water (10 mL) and Et_2O (10 mL × 3). The crude product was purified by HPLC using Dionex column (CarboPac PA1, 9 × 250 mm, HPLC method C, see Section S1). The desired product was found to co-elute with UPPU. The collected fractions containing both **26** and UPPU were pooled, lyophilized, and repurified with C18 column (Agilent ZORBAX ODS, 5 μM, 9.4 × 250 mm). The desired product was eluted at 3 min and was well-resolved from UPPU (elued at 6.5 min) at 4 mL/min flow rate with isocratic elution of 0.5% MeCN in 1% $NH_4OAc_{(aq)}$. Fractions containing product were pooled and lyophilized several times to give **26** as white powder (2.3 mg, 4.4%). ^{1}H NMR (D_2O, 600 MHz) δ 3.46 (ddd, *J* = 10.8, 4.2, 2.4 Hz, 1H, 2″–H), 4.04 (ddd, *J* = 10.8, 3.6, 1.8 Hz, 1H, 3″–H), 4.02–4.08 (m, 2H, 4″–H and 5′–H), 4.10 (ddd, *J* = 12.0, 4.2, 2.4 Hz, 1H, 5′–H), 4.13 (ddd, *J* = 5.4, 4.2, 2.4 Hz, 1H, 4′–H), 4.22 (t, *J* = 5.4 Hz, 1H, 3′–H), 3.83 (dd, *J* = 5.4, 4.2 Hz, 1H, 2′–H), 4.38 (ddd, *J* = 46.2, 12.3, 10.5 Hz, 1H, 6″–H), 4.48 (ddd, *J* = 45.6, 24.6, 10.5 Hz, 1H, 6″–H), 5.60 (dd, *J* = 8.1, 4.2 Hz, 1H, 1″–H), 5.83 (d, *J* = 4.2 Hz, 1H, 1′–H), 5.83 (d, *J* = 7.8 Hz, 1H, 5–H), 7.82 (d, *J* = 7.8 Hz, 1H, 6–H). ^{31}P NMR (D_2O, 243 MHz) δ –11.3 (d, *J* = 20.9 Hz), –13.2 (d, *J* = 20.9 Hz). ^{19}F NMR (D_2O, 565 MHz) δ –119.0 (dt, *J* = 24.6, 12.3 Hz, 5–F), –237.9 (td, *J* = 45.9, 12.3 Hz, 6–F). HRMS (ESI) *m/z* calcd for $C_{15}H_{22}F_2N_2O_{16}P_2$ ([M – H]$^-$) 585.03400; found 585.03500.

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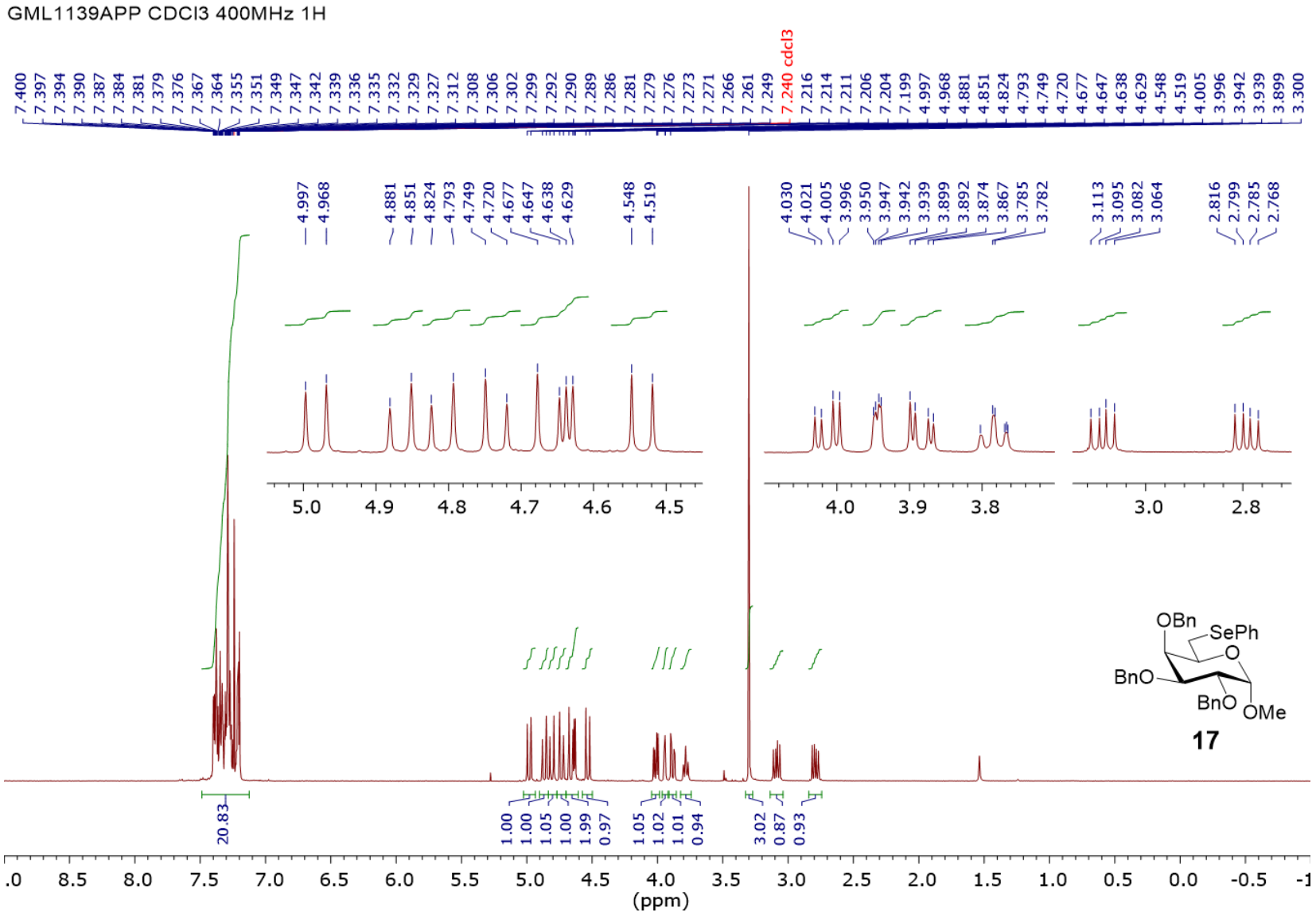
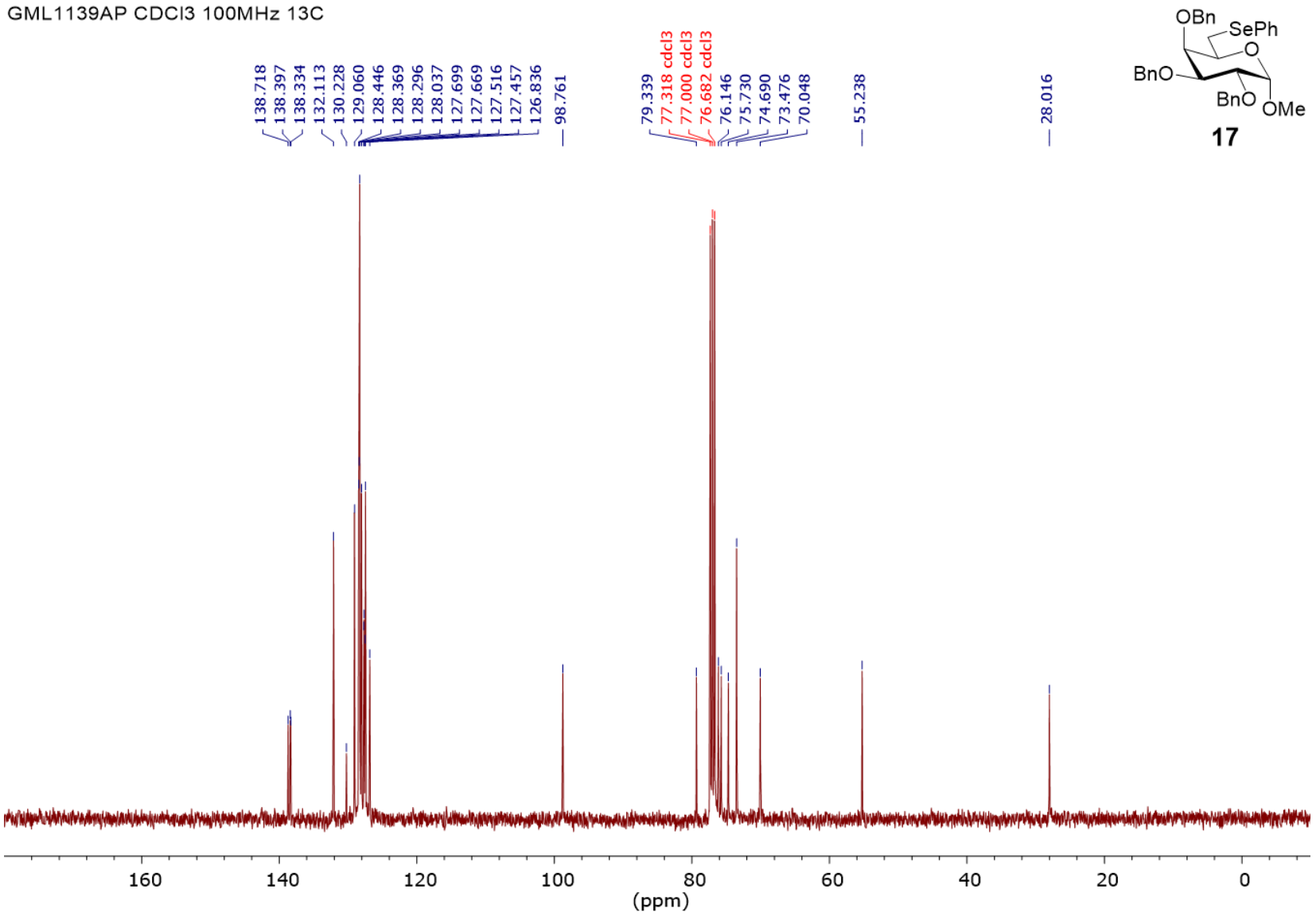
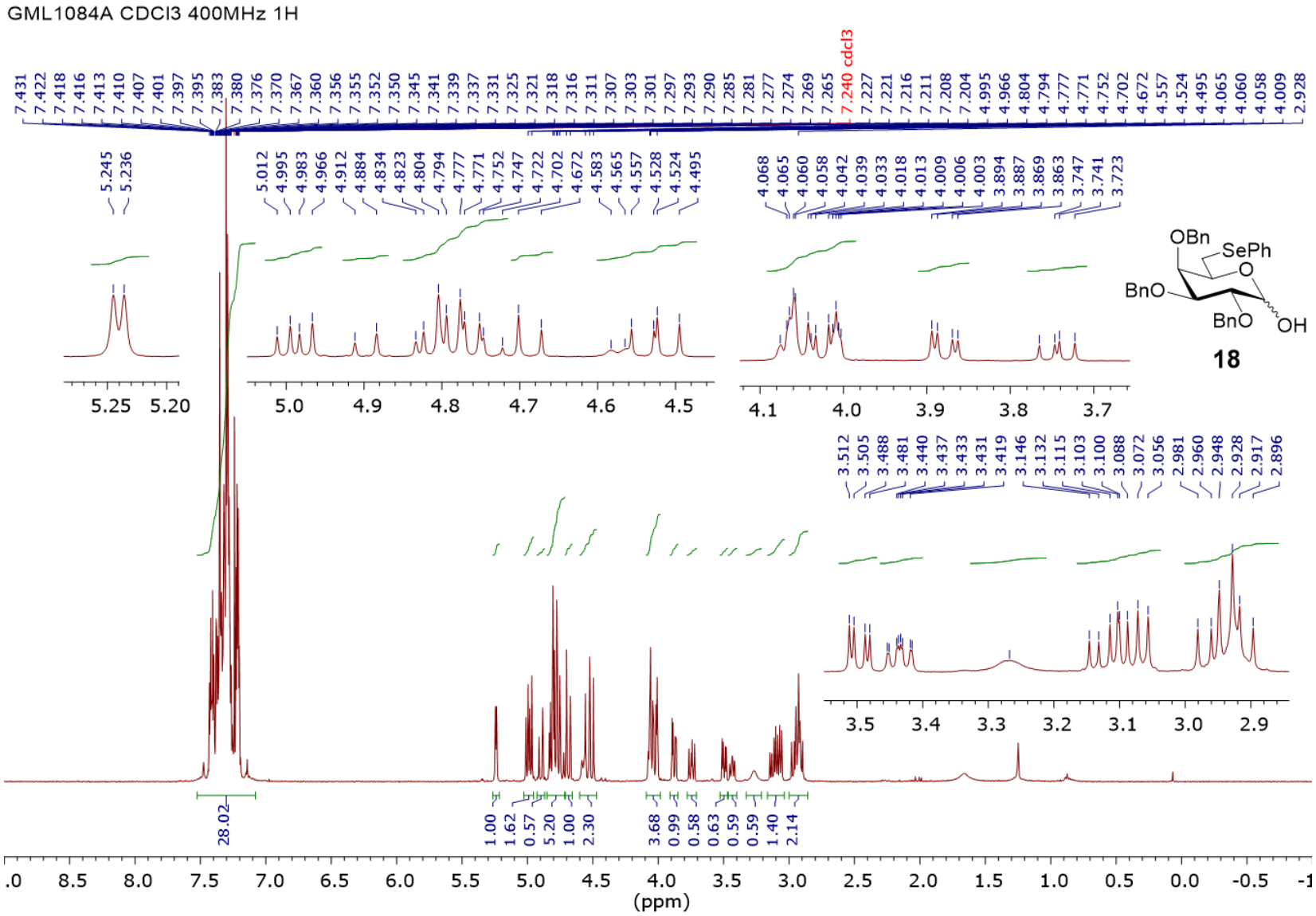
Appendix: NMR Spectra



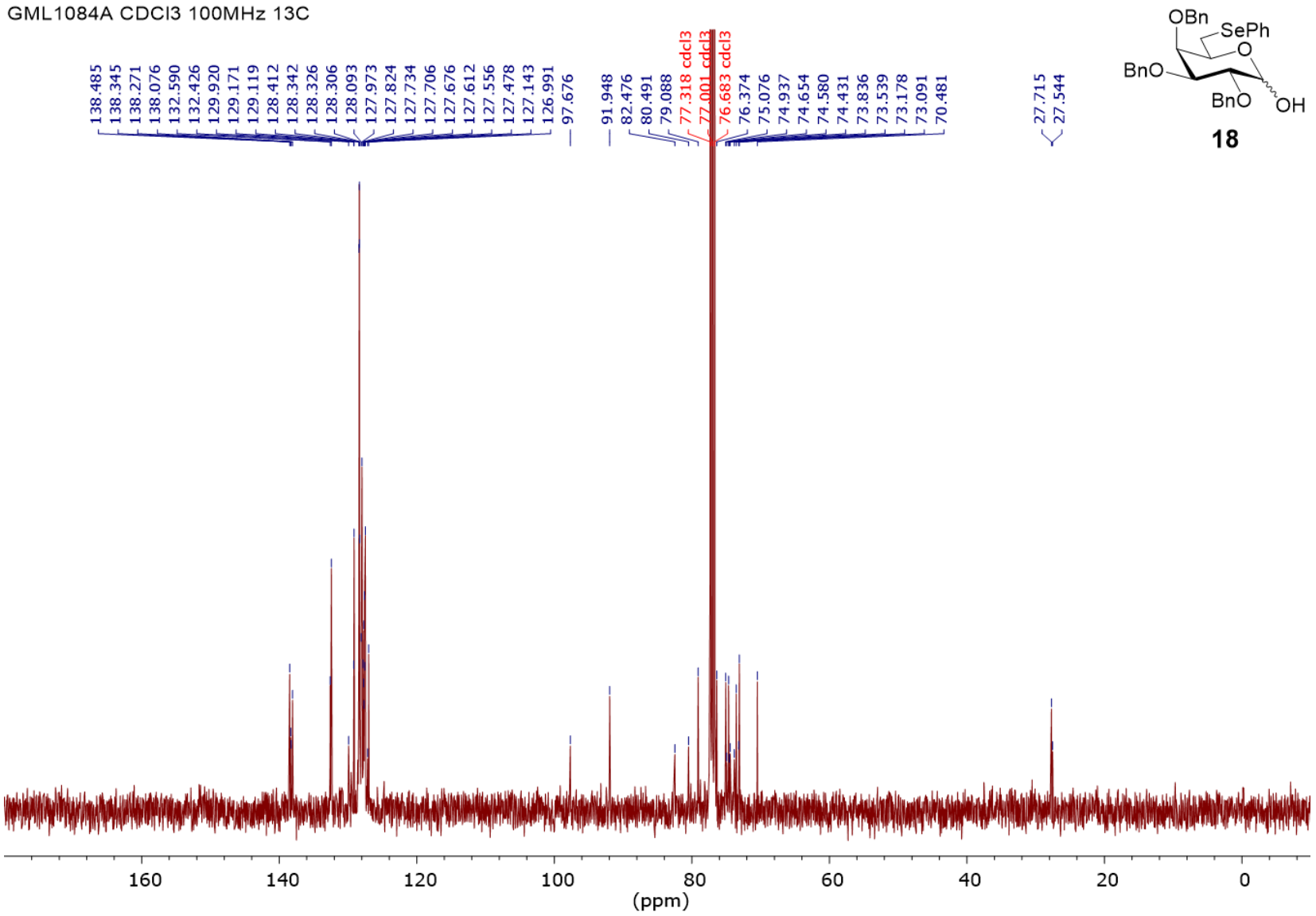
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **17**



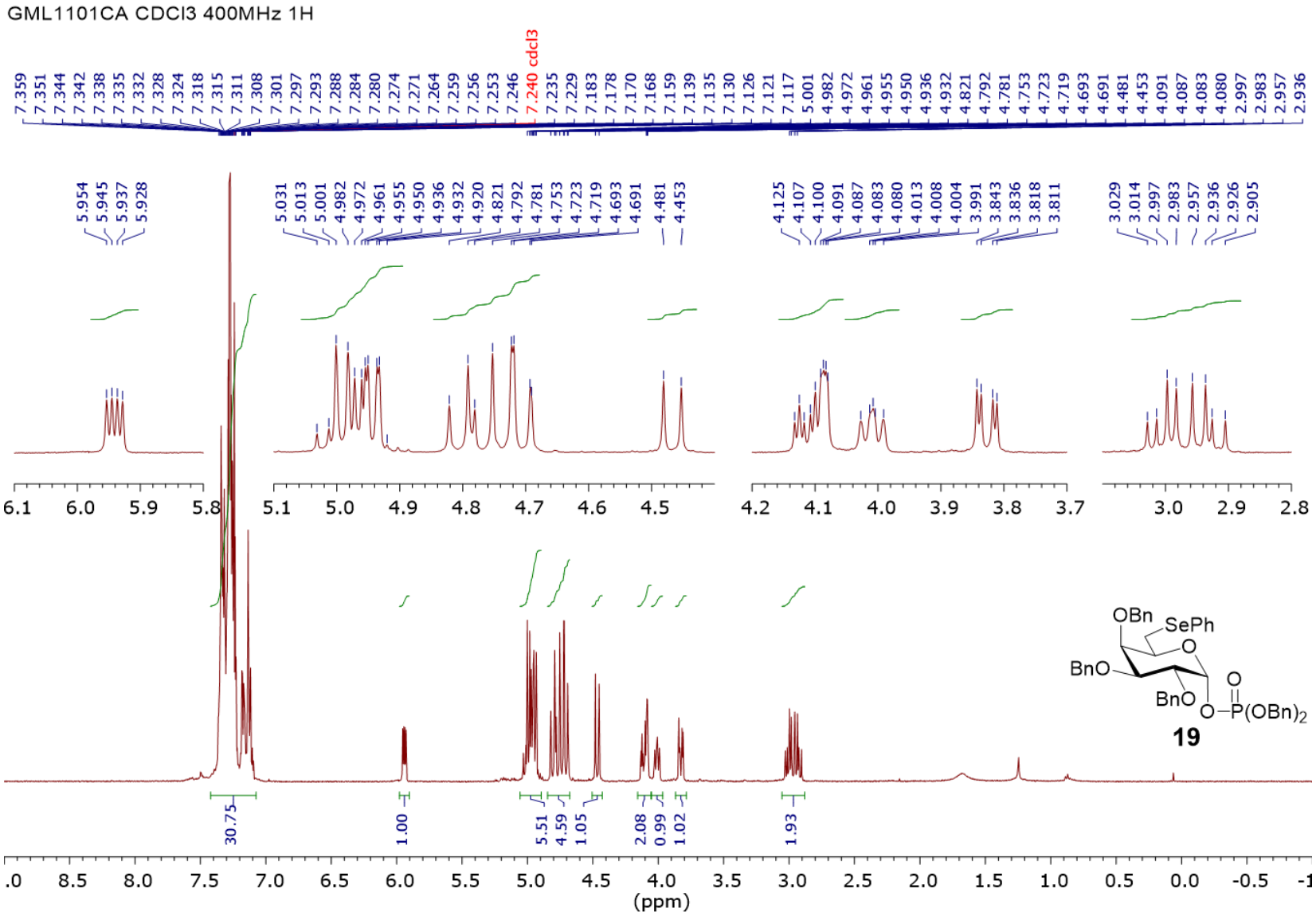
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **17**



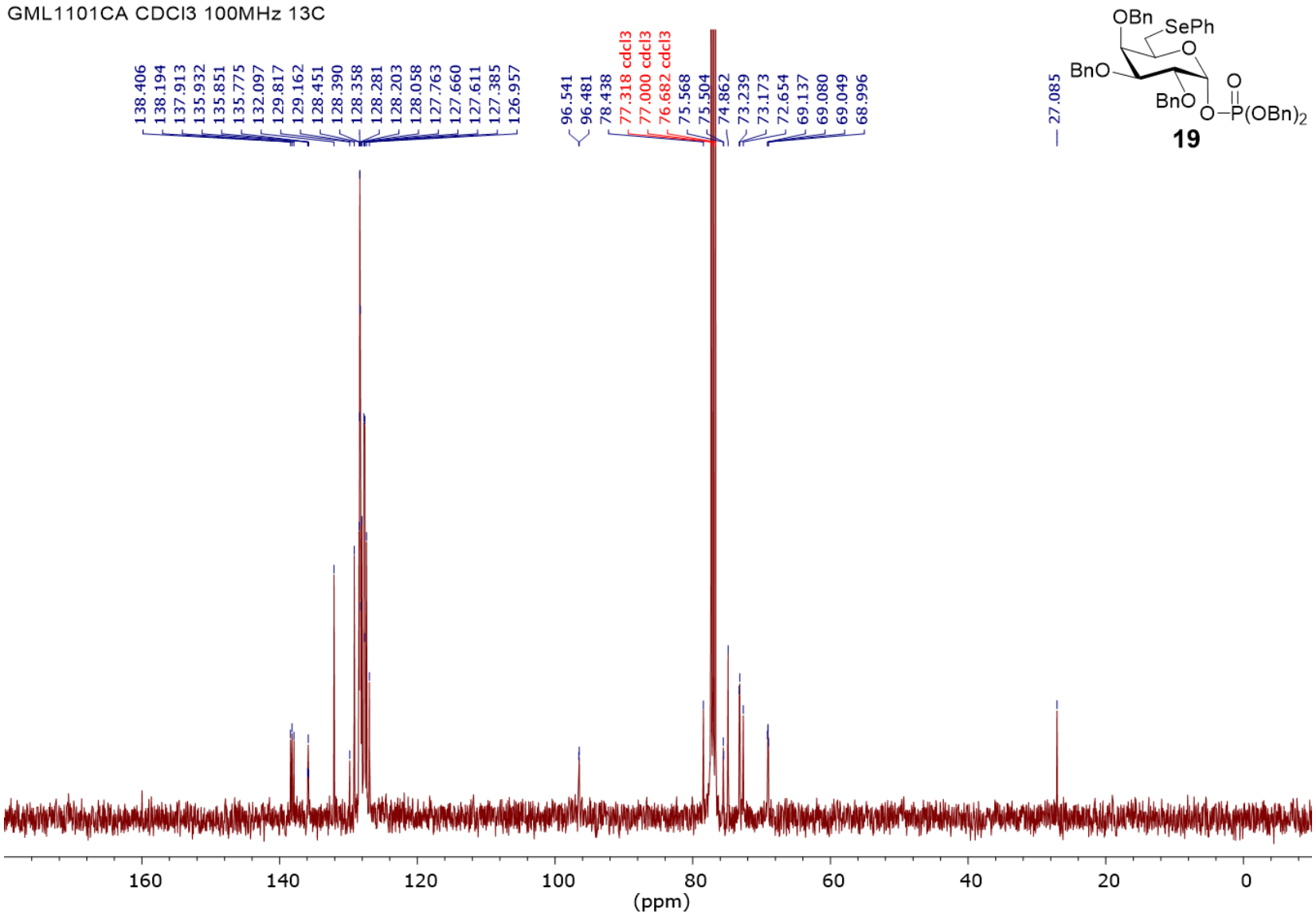
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **18**



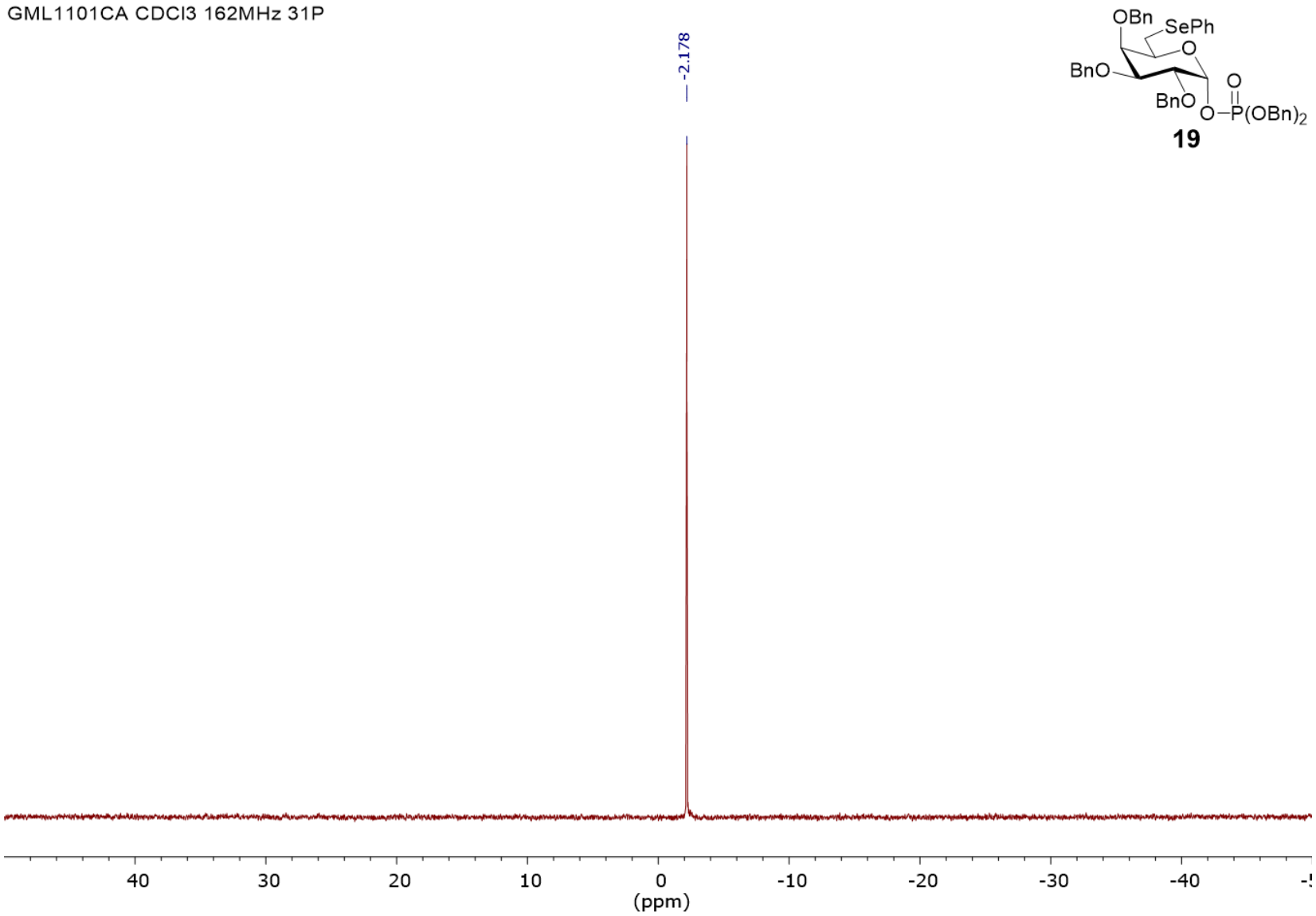
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **18**



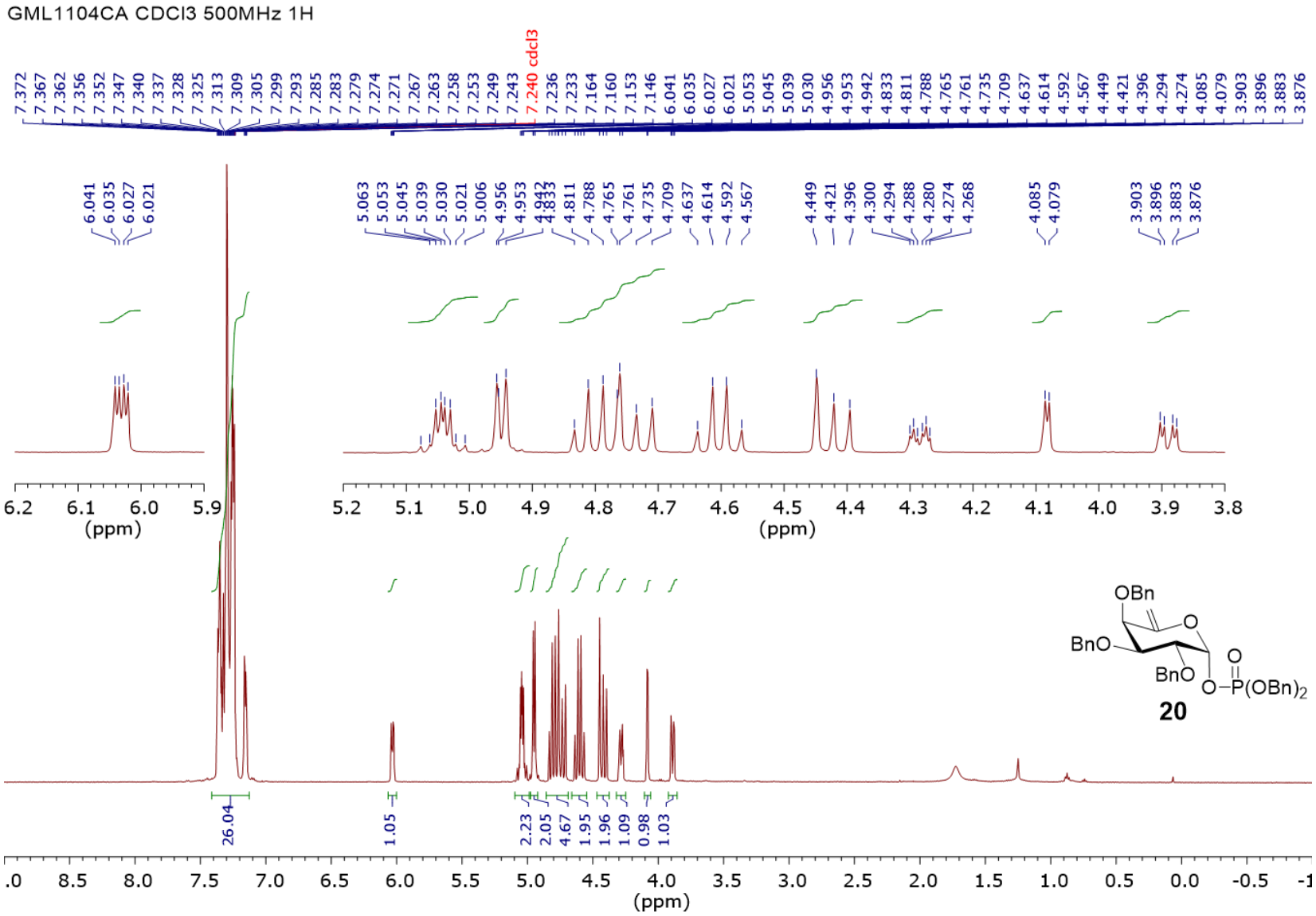
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **19**



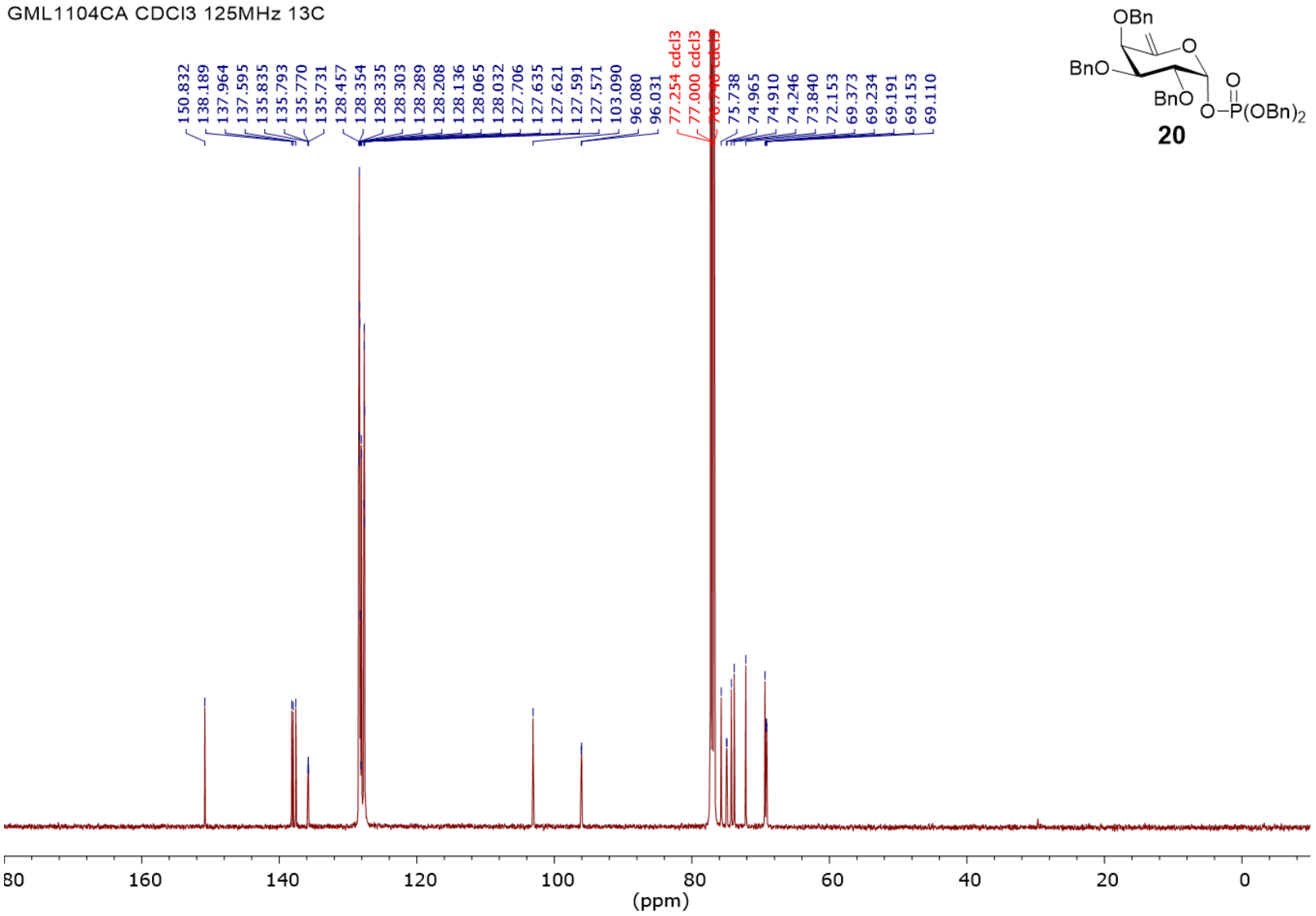
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **19**



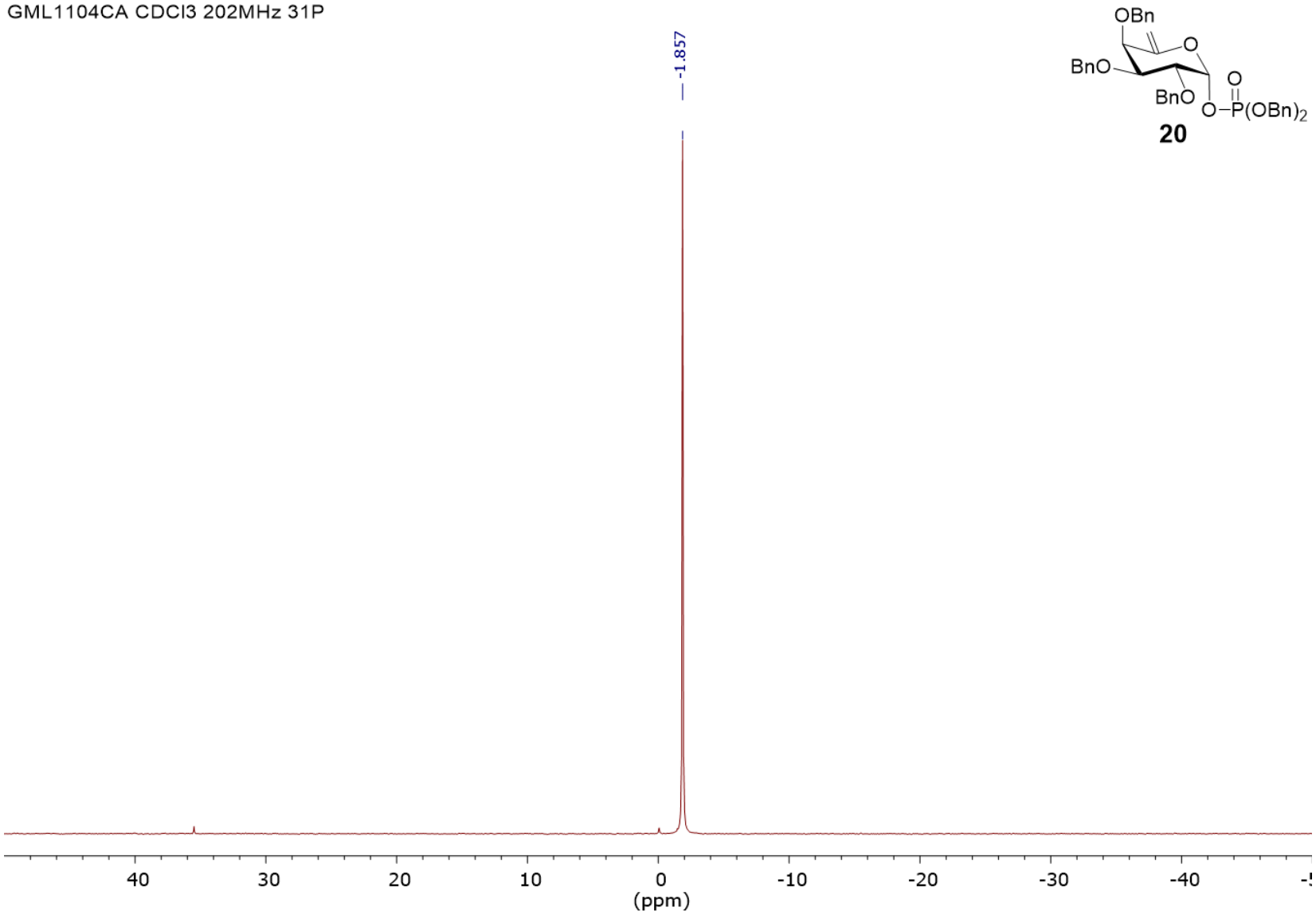
^{31}P NMR (162 MHz, $CDCl_3$) spectrum of **19**



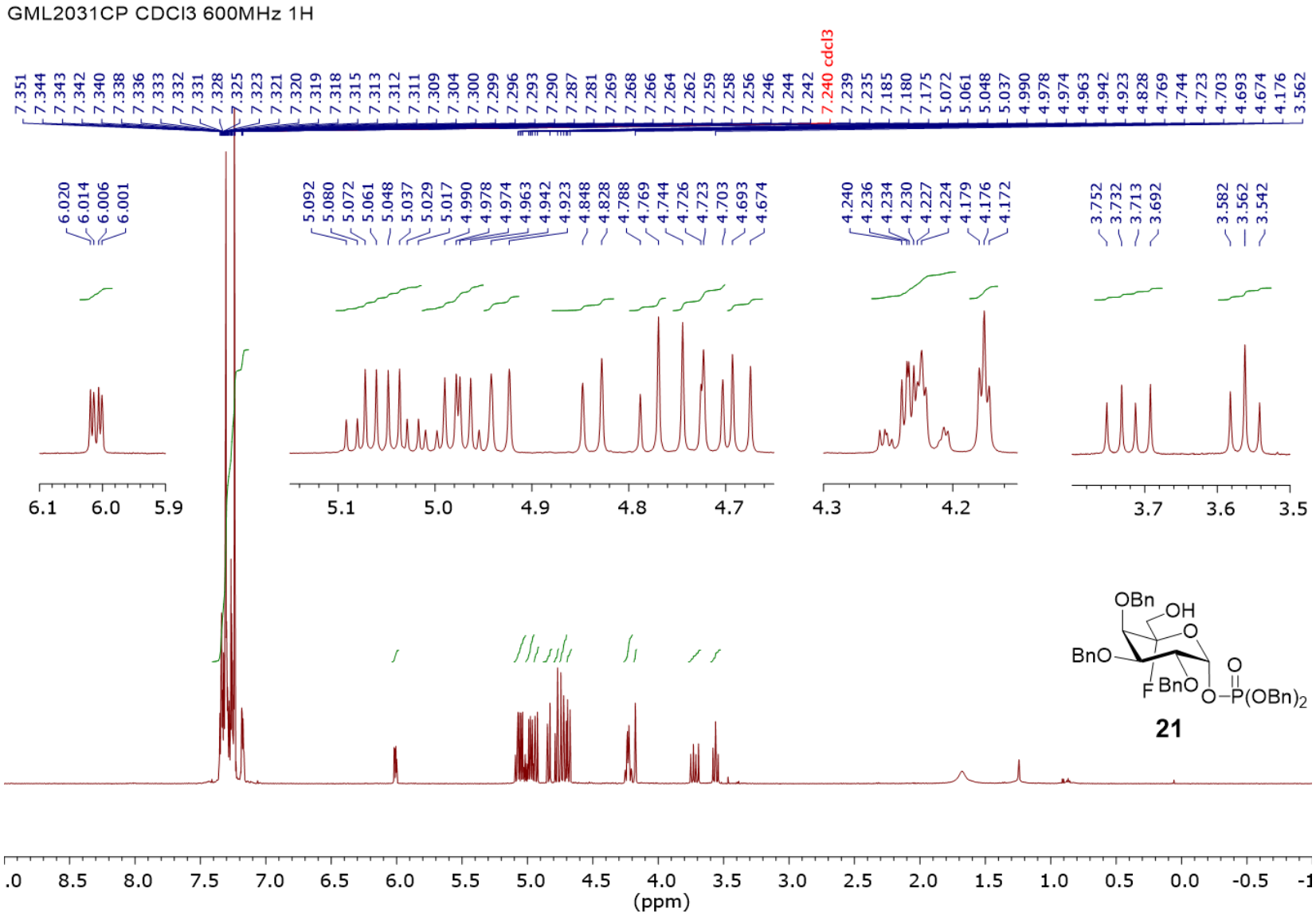
^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **20**



^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **20**

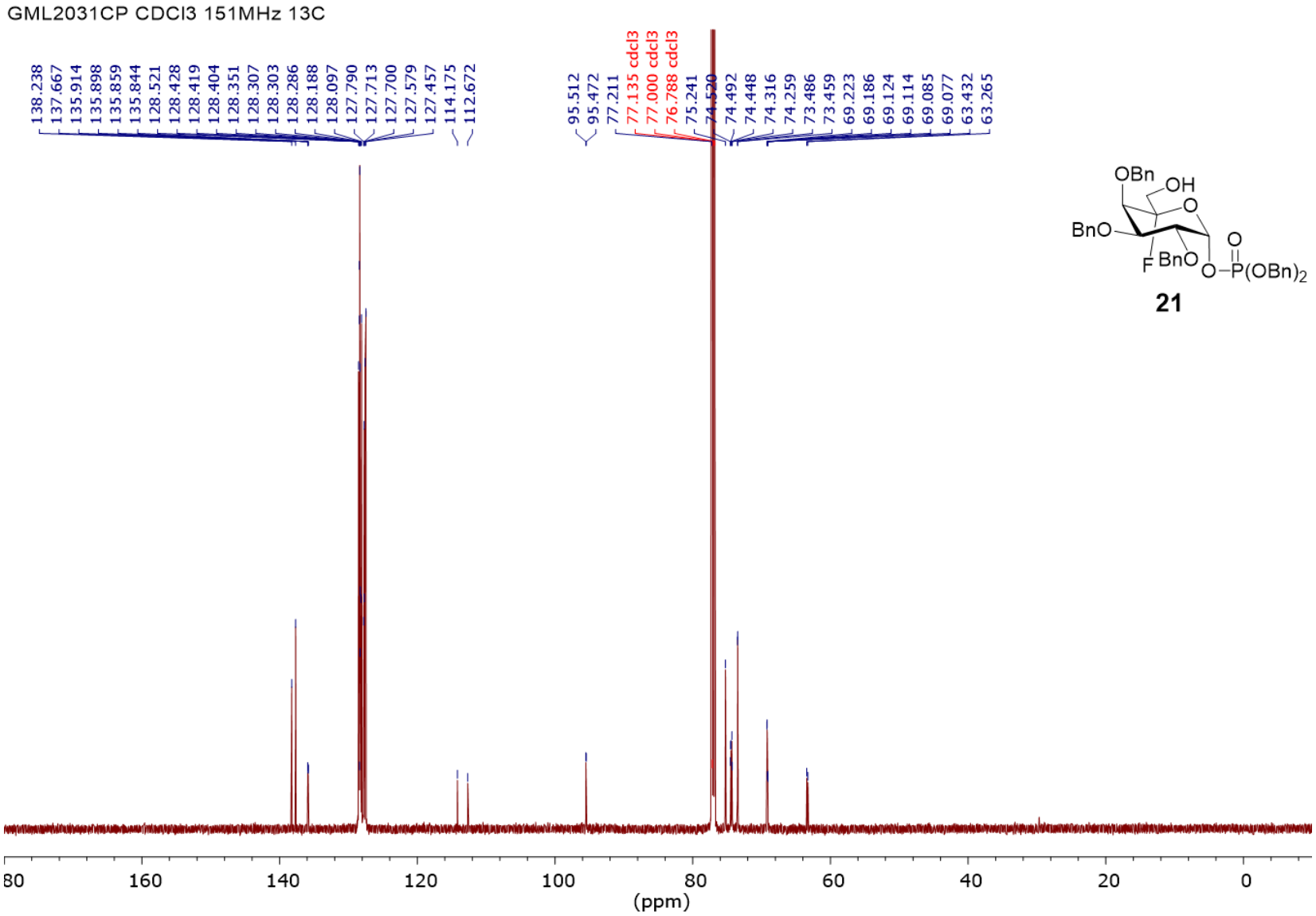


^{31}P NMR (202 MHz, $CDCl_3$) spectrum of **20**

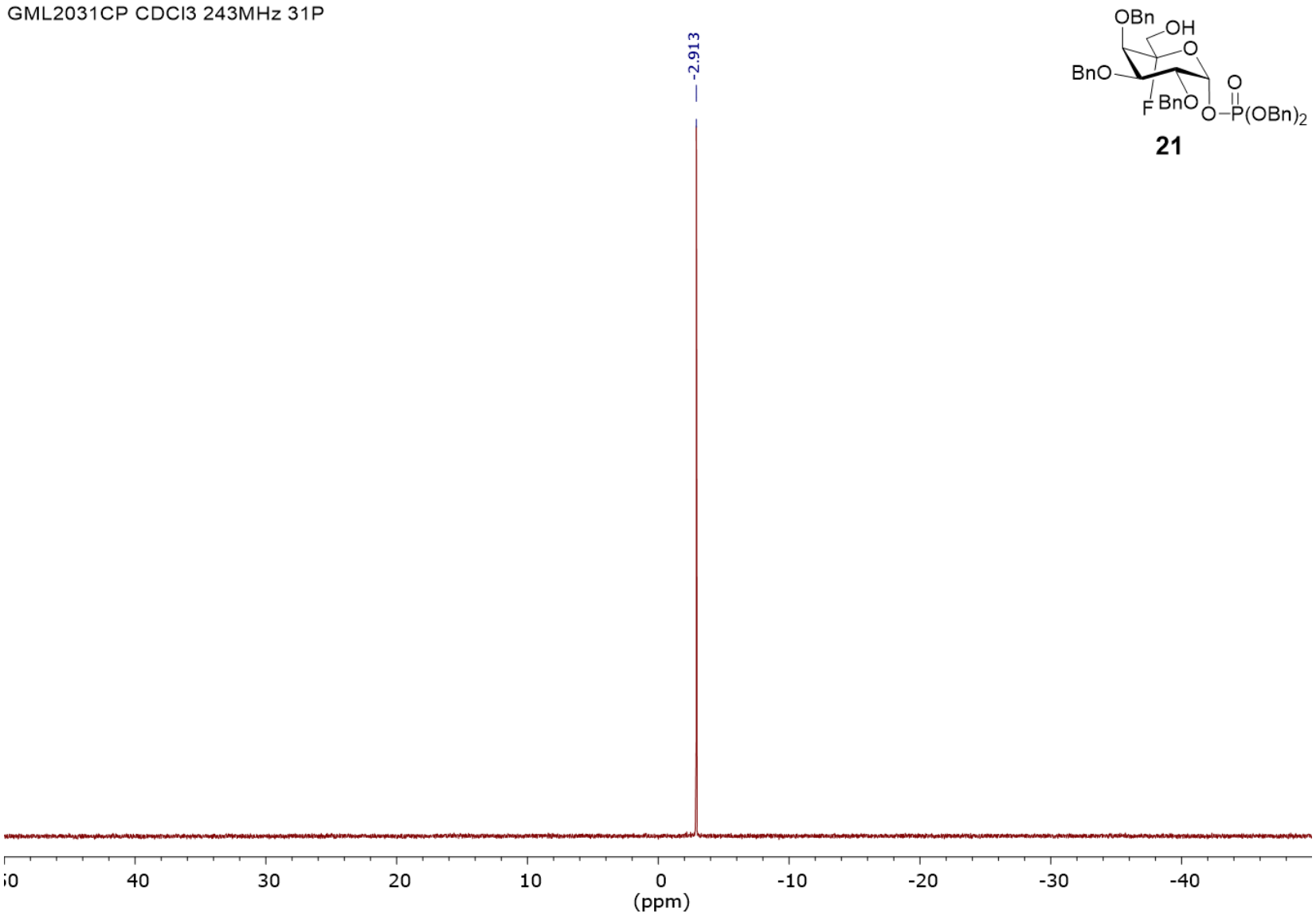


^{1}H NMR spectrum of **21**

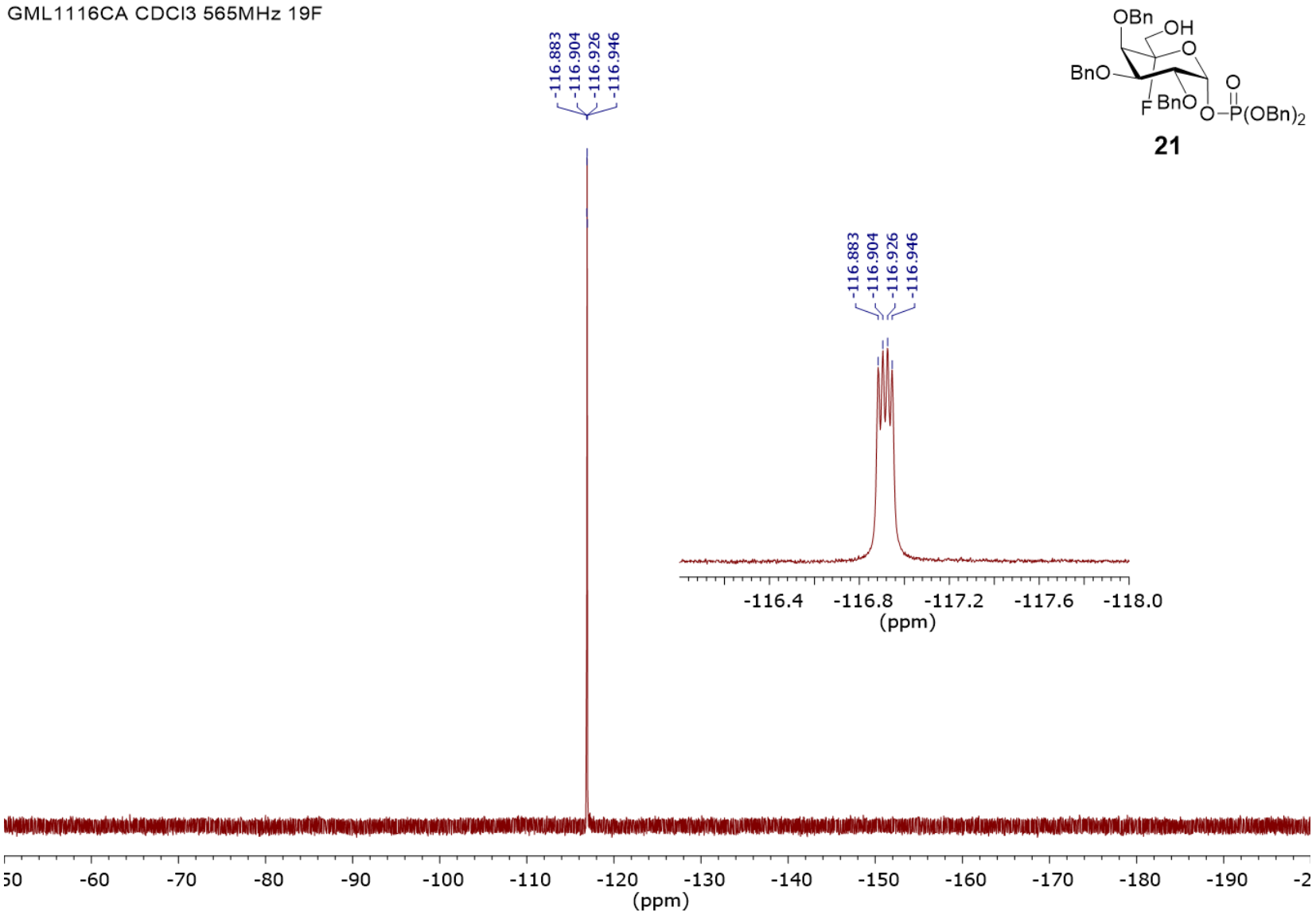
^{1}H NMR (600 MHz, $CDCl_3$) spectrum of **21**



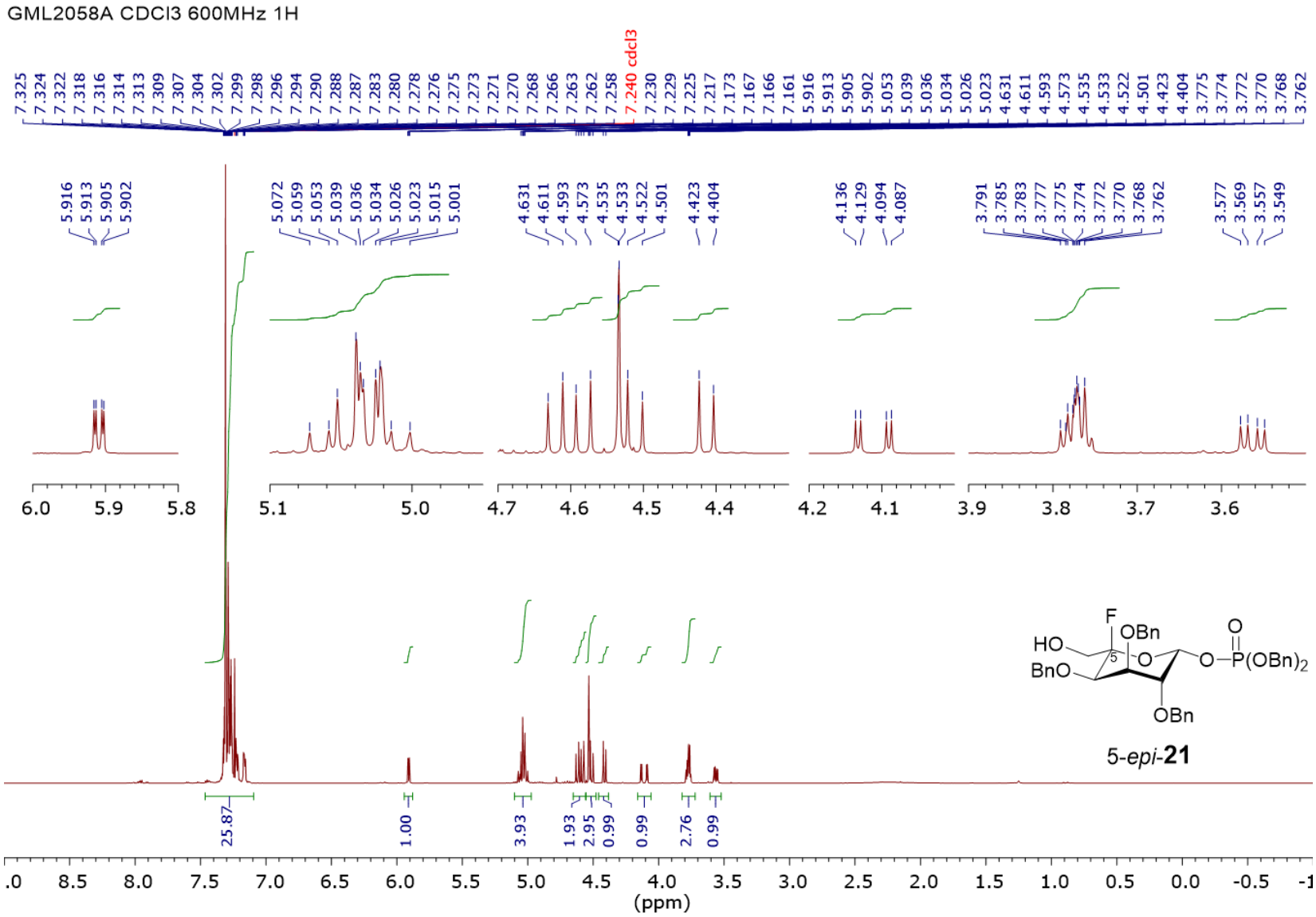
^{13}C NMR (151 MHz, $CDCl_3$) spectrum of **21**



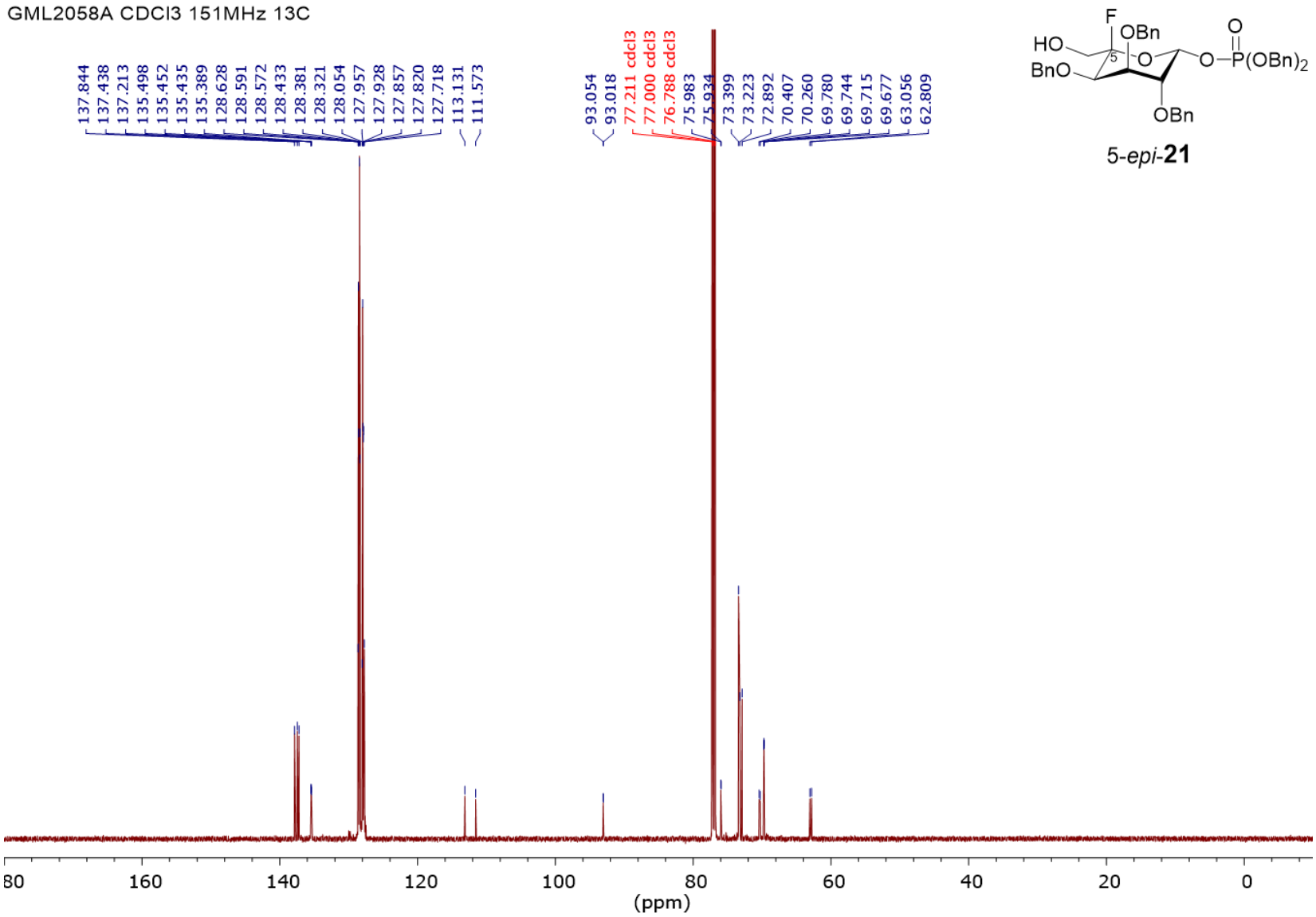
^{31}P NMR (243 MHz, $CDCl_3$) spectrum of **21**



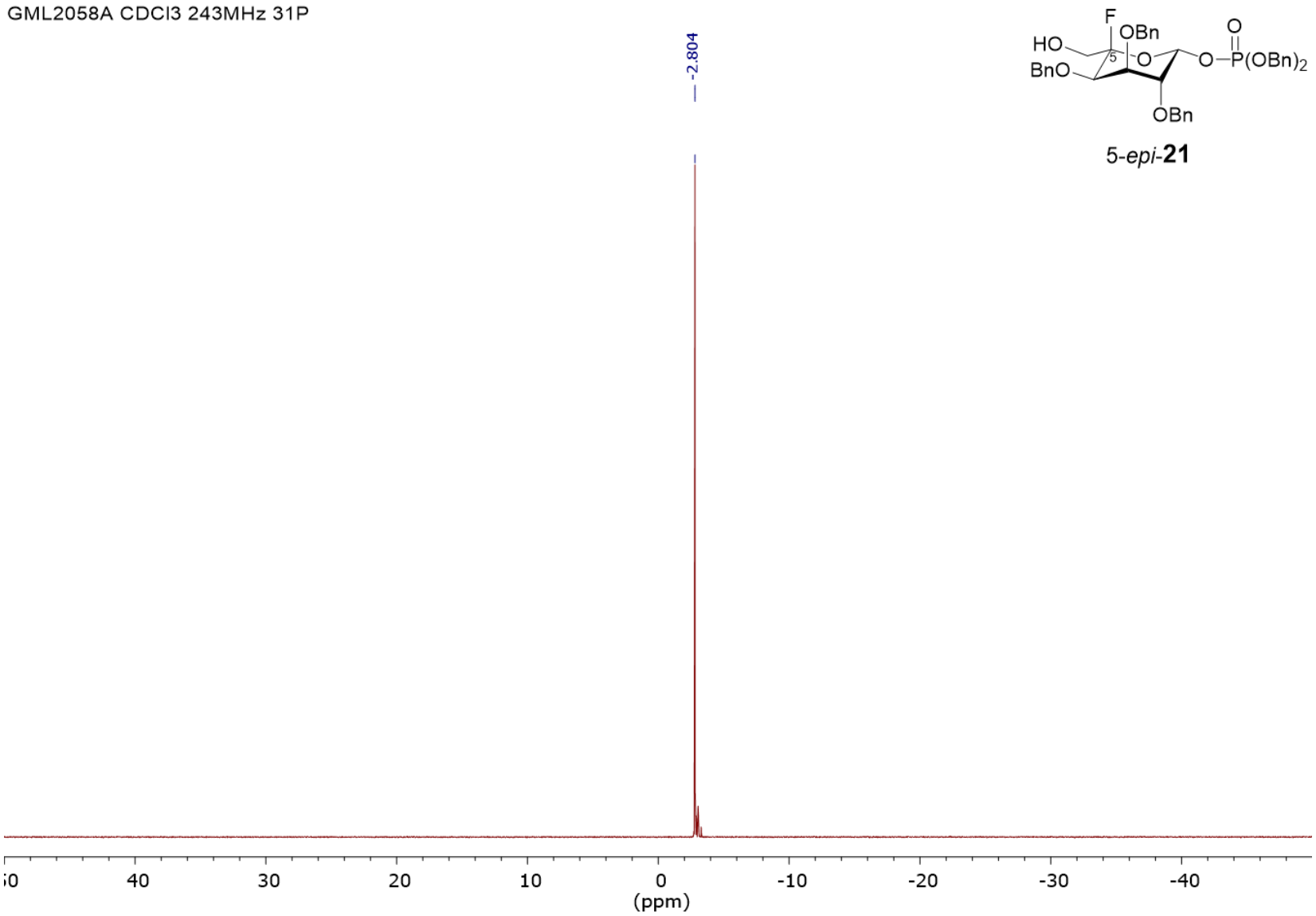
^{19}F NMR (565 MHz, $CDCl_3$) spectrum of **21**



^{1}H NMR (600 MHz, $CDCl_3$) spectrum of 5-*epi*-**21**

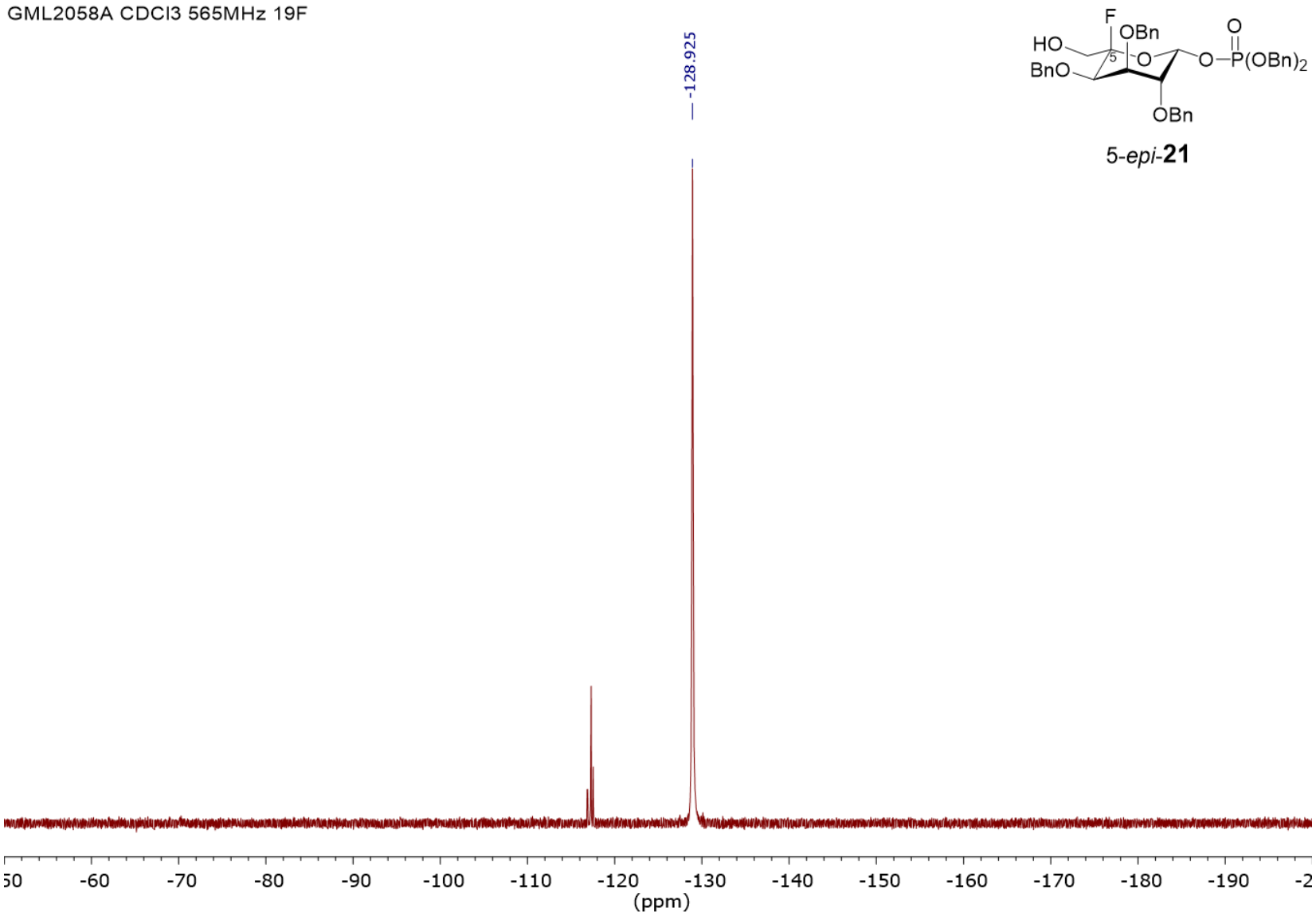


^{13}C NMR (151 MHz, $CDCl_3$) spectrum of 5-*epi*-**21**

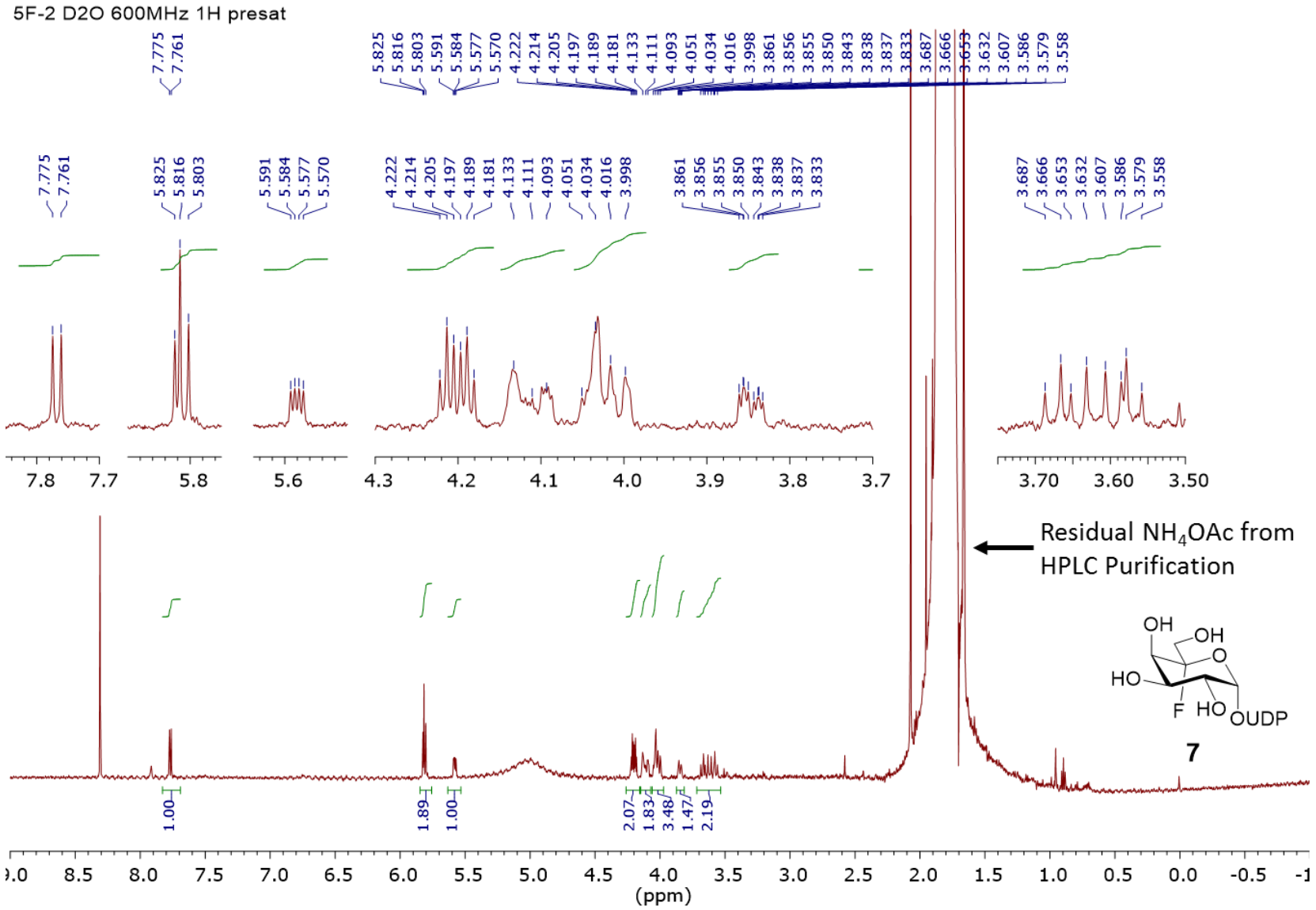


^{31}P NMR spectrum of 5-*epi*-**21**

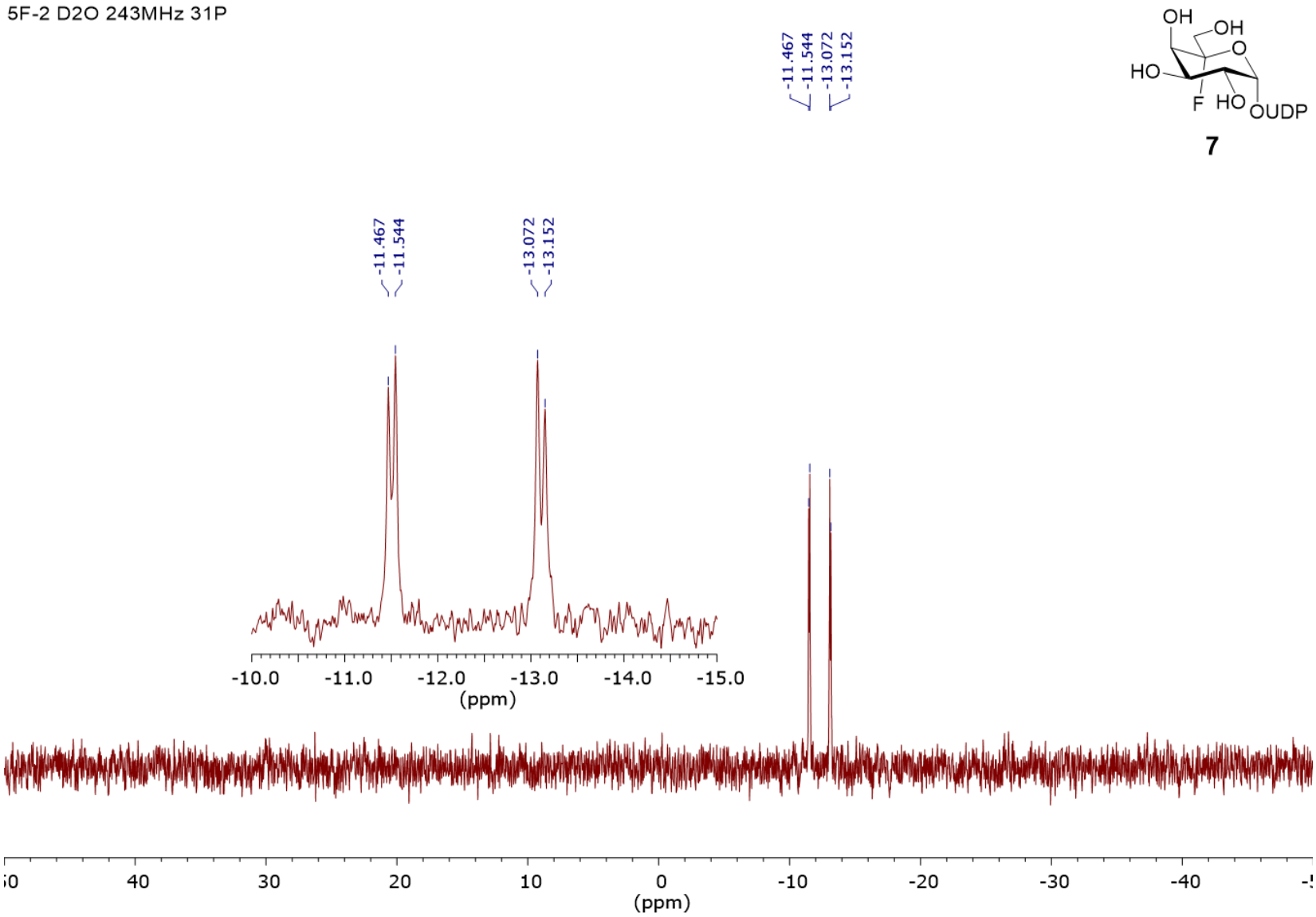
^{31}P NMR (243 MHz, $CDCl_3$) spectrum of 5-*epi*-**21**



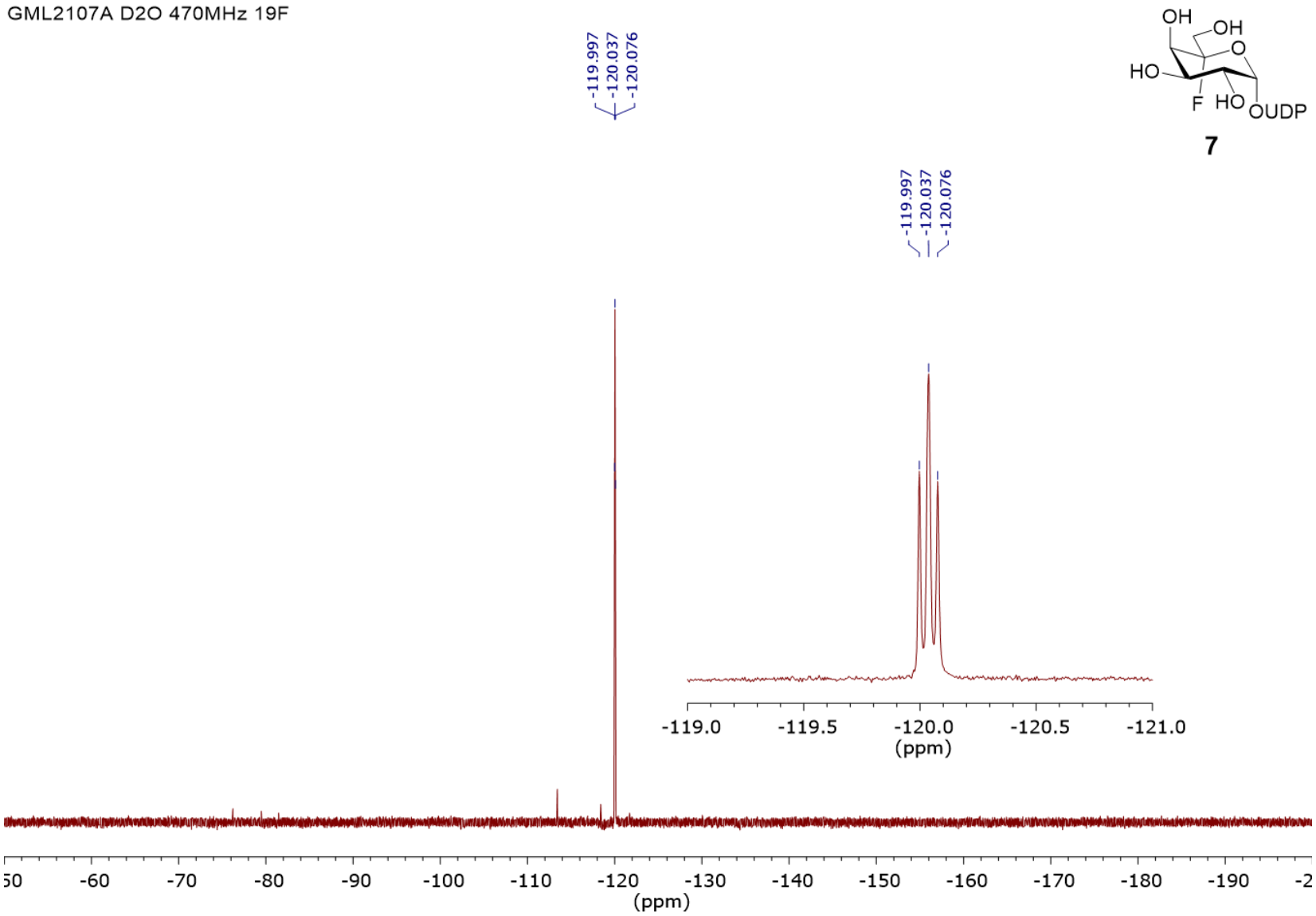
^{19}F NMR (565 MHz, $CDCl_3$) spectrum of 5-*epi*-**21**



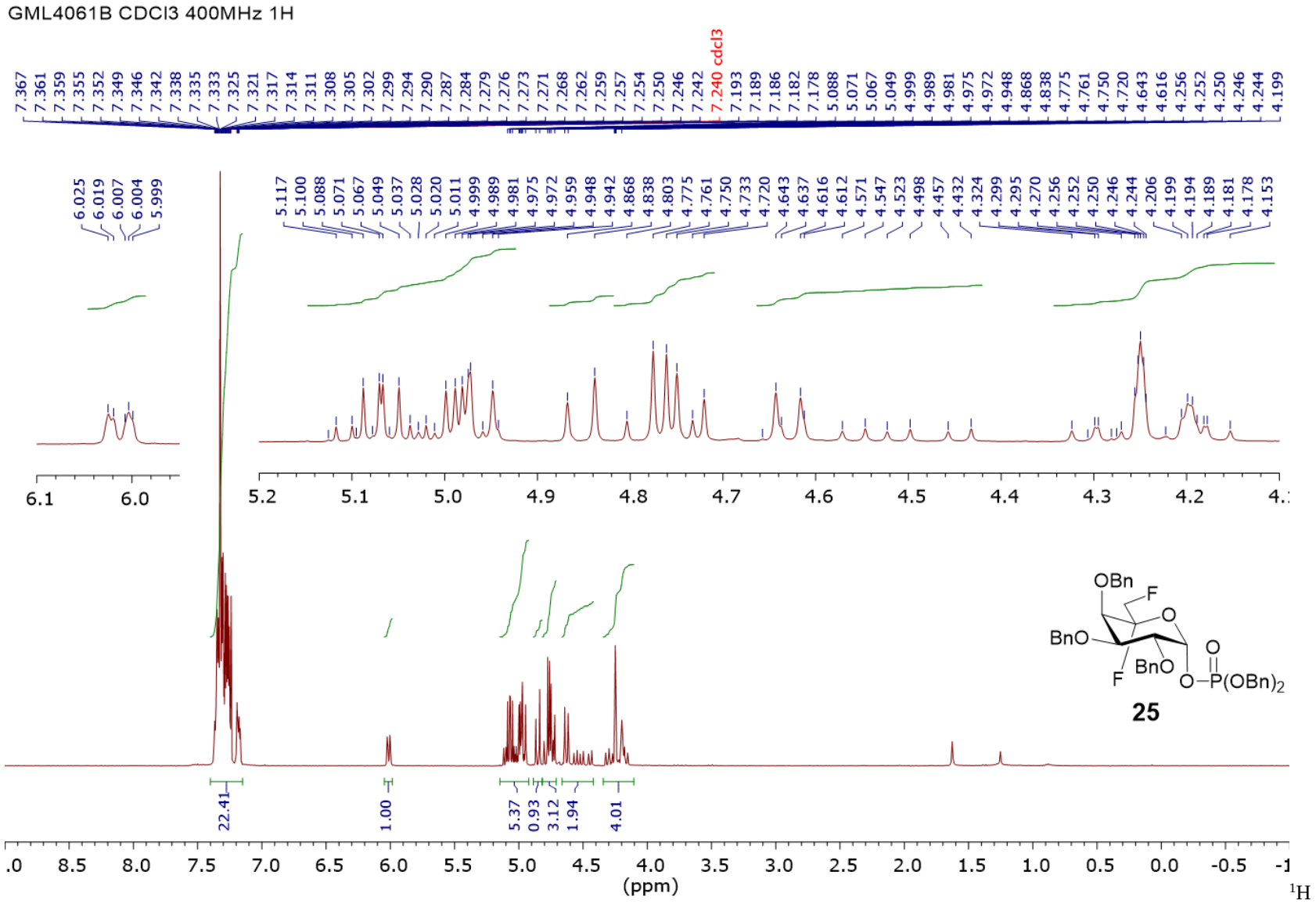
^{1}H NMR (600 MHz, D_2O) spectrum of **7**



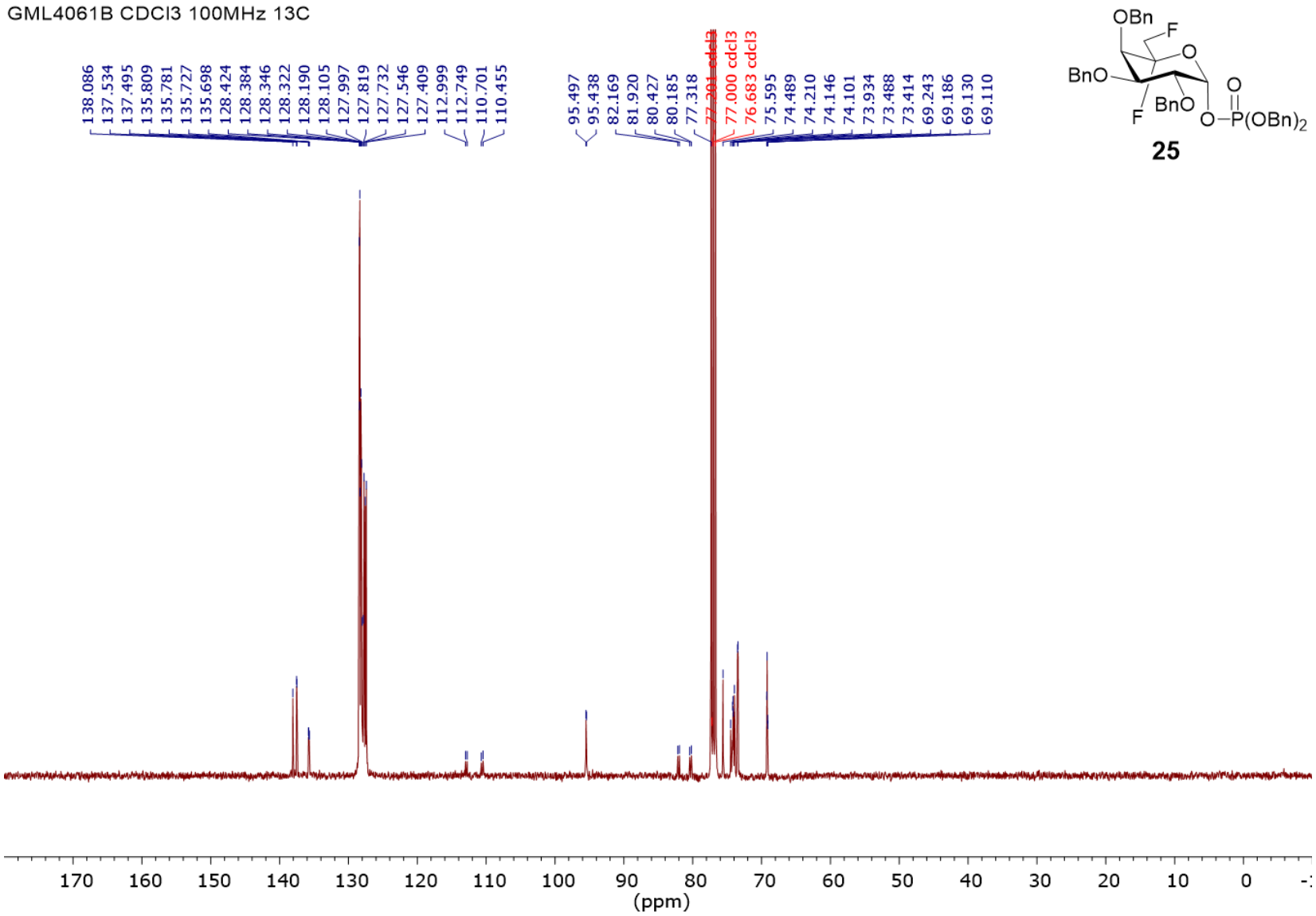
^{31}P NMR (243 MHz, D_2O) spectrum of **7**



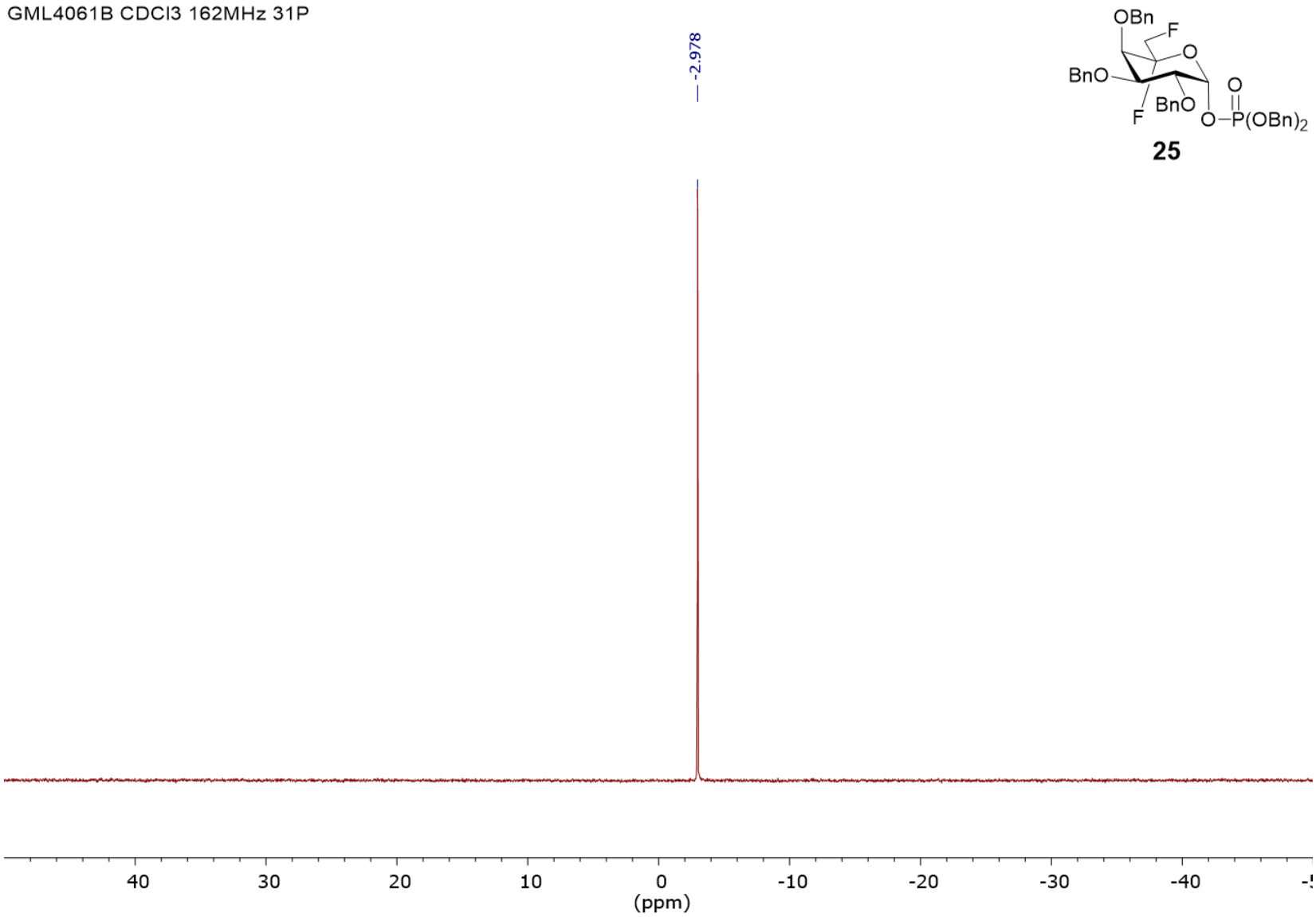
^{19}F NMR (470 MHz, D_2O) spectrum of **7**



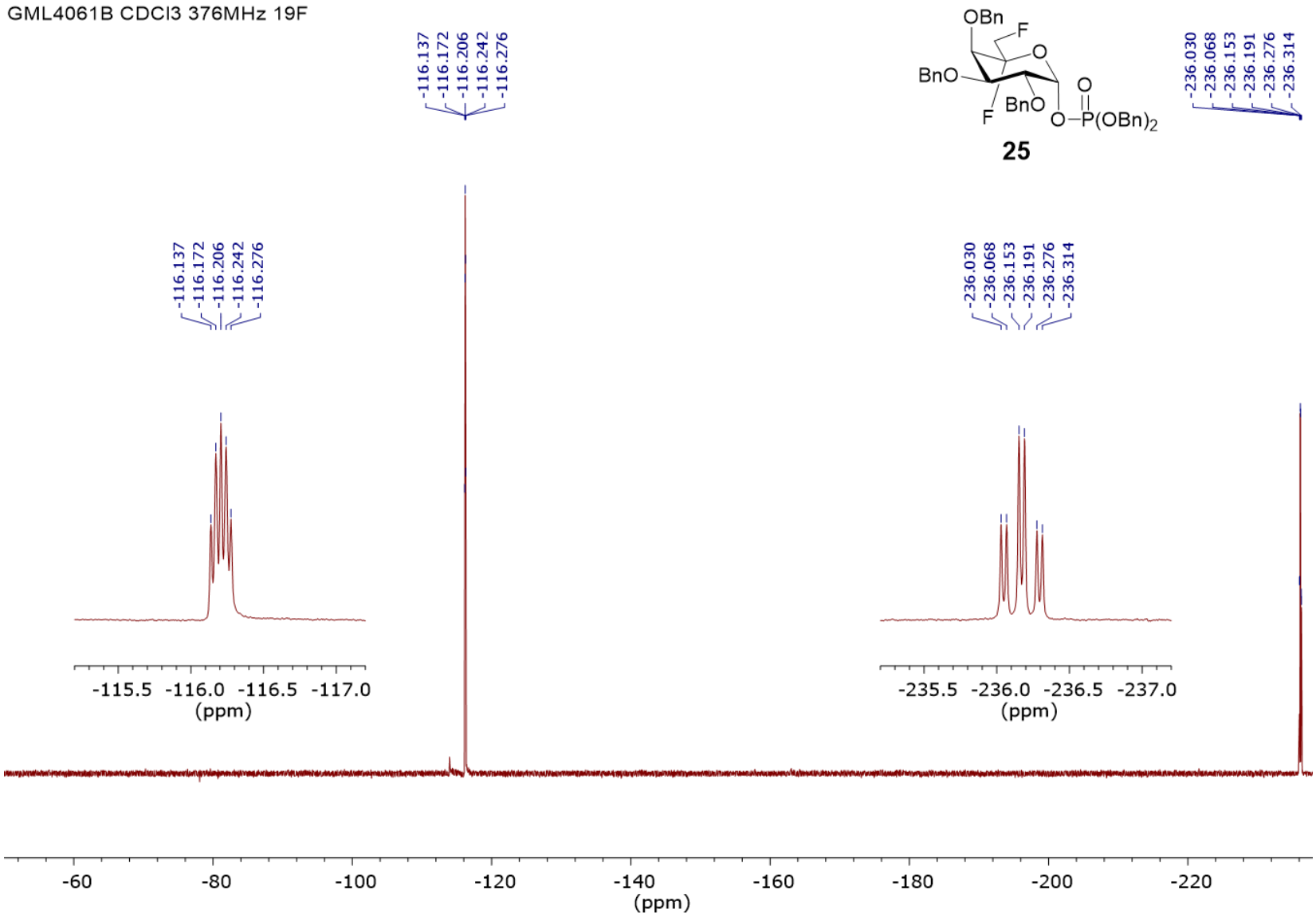
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **25**



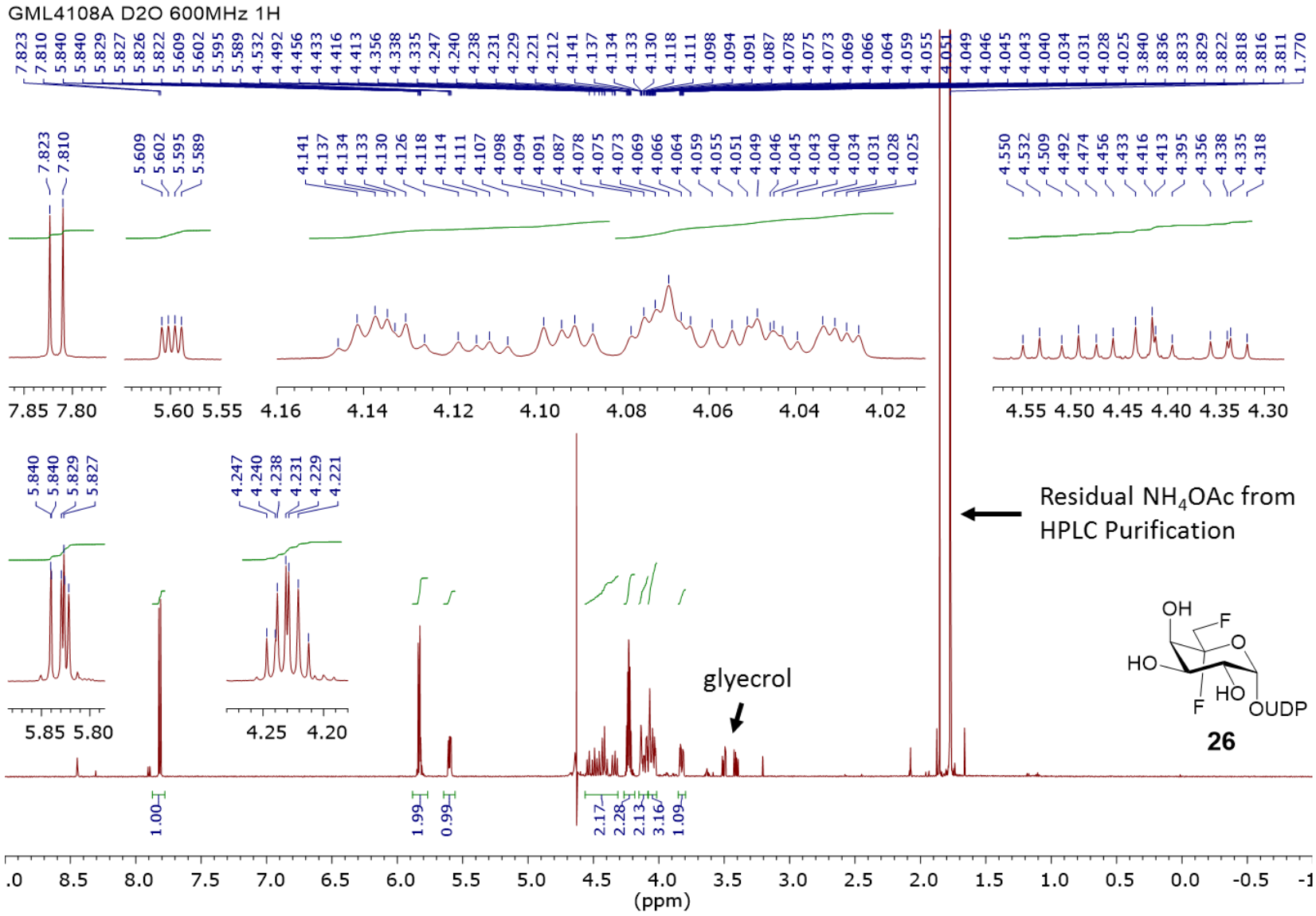
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **25**



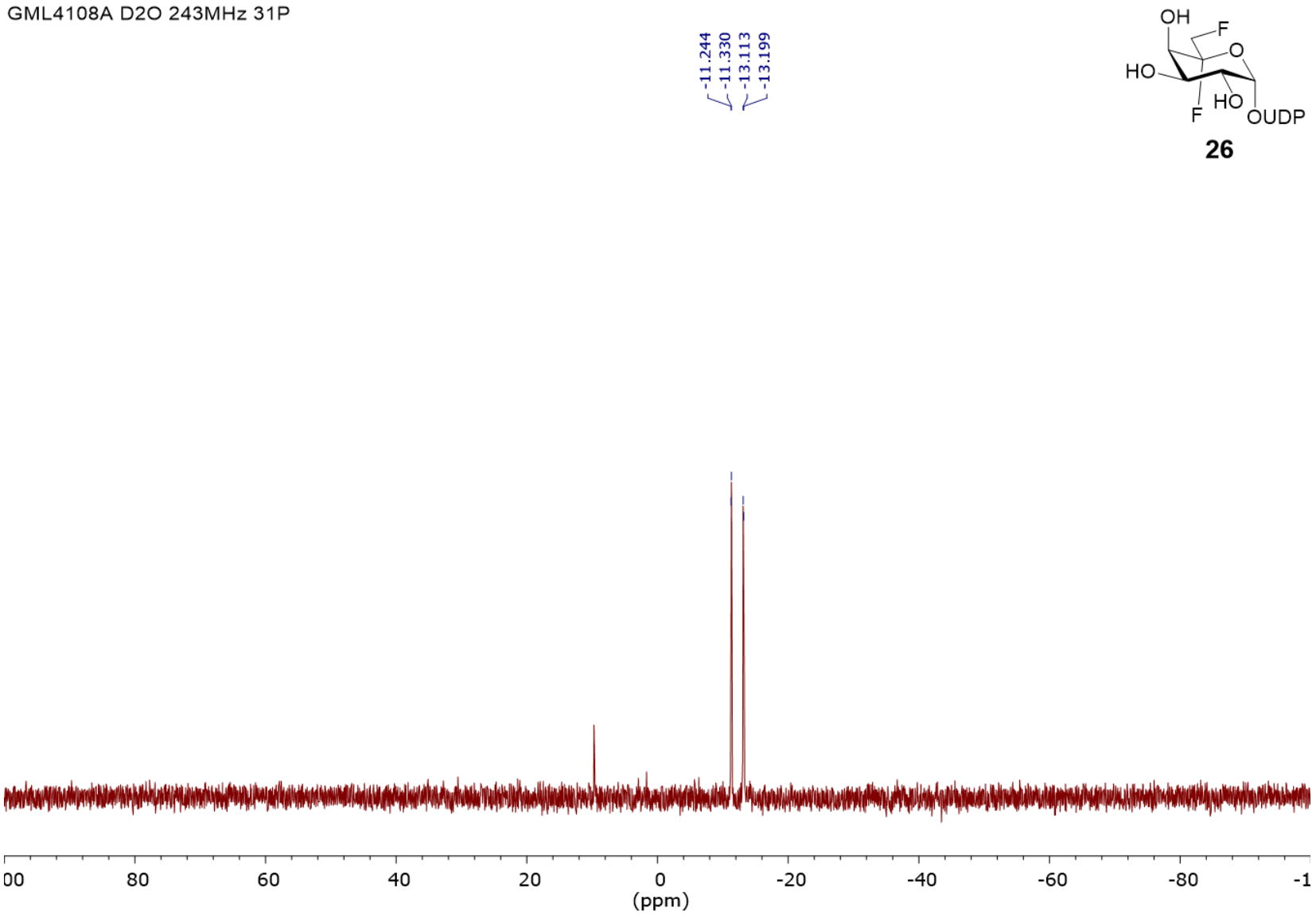
^{31}P NMR (162 MHz, $CDCl_3$) spectrum of **25**



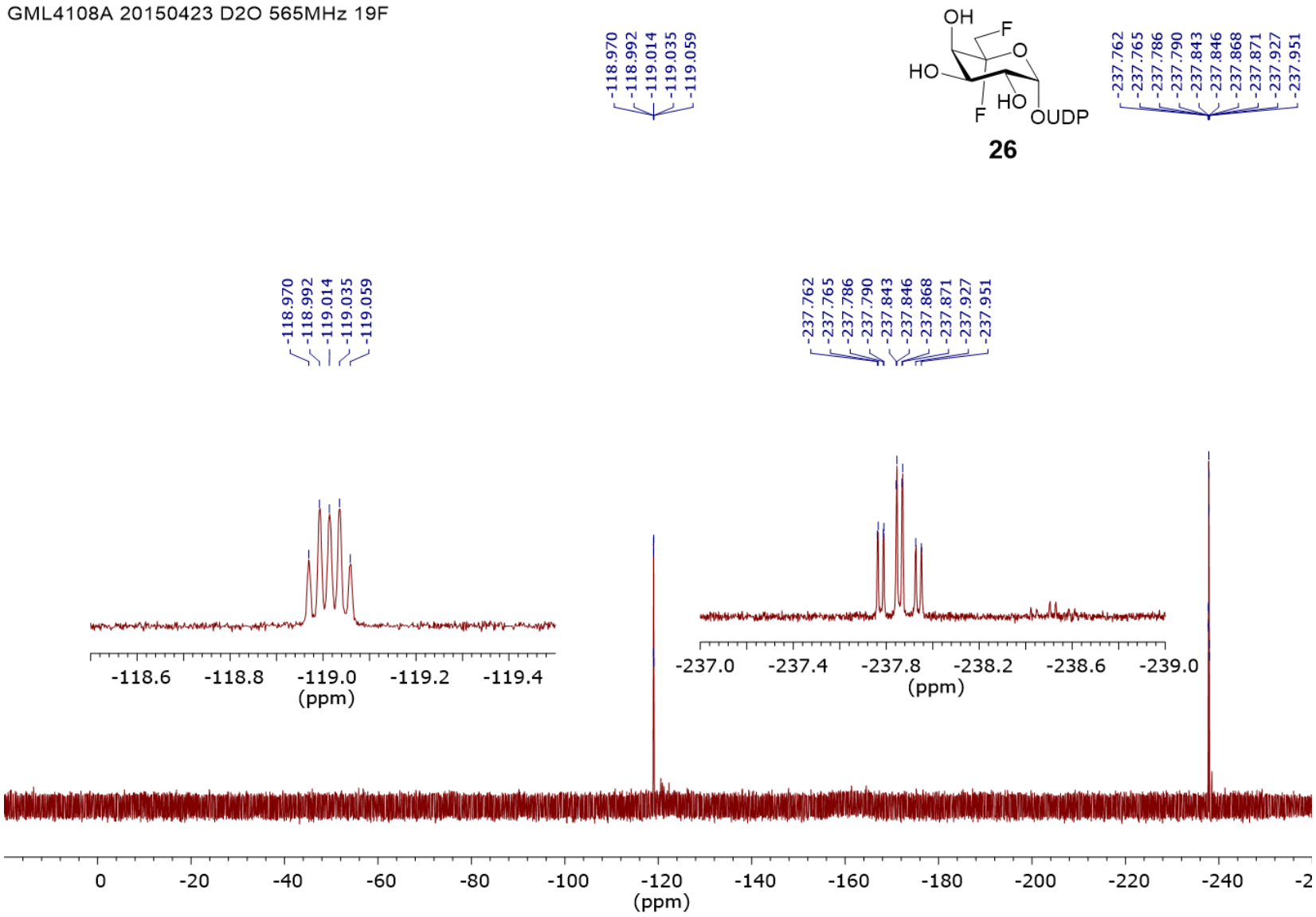
^{19}F NMR (376 MHz, $CDCl_3$) spectrum of **25**



^{1}H NMR (600 MHz, D_2O) spectrum of **26**



^{31}P NMR (243 MHz, D_2O) spectrum of **26**



^{19}F NMR (565 MHz, D_2O) spectrum of **26**