Design, synthesis and biological evaluation of carbohydrate-functionalized cyclodextrins and liposomes for hepatocyte-specific targeting

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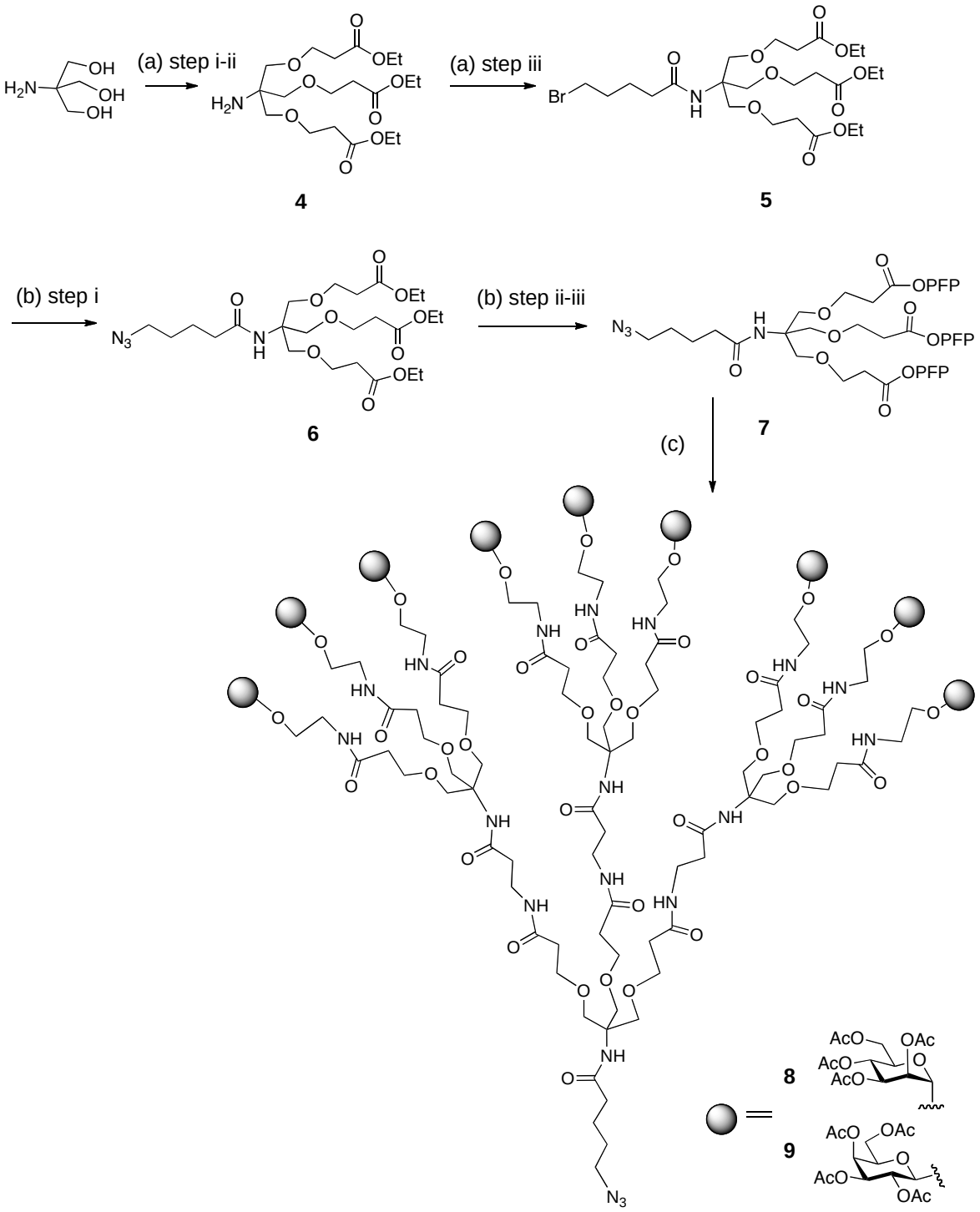
1. General Information

All chemicals were reagent grade and used as supplied except where noted. Dichloromethane (CH_2Cl_2) was purified by a Cycle-Tainer Solvent Delivery System. Triethylamine was distilled over CaH_2 prior to use. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in CAN solution followed by heating. Flash column chromatography was carried out using force flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh).

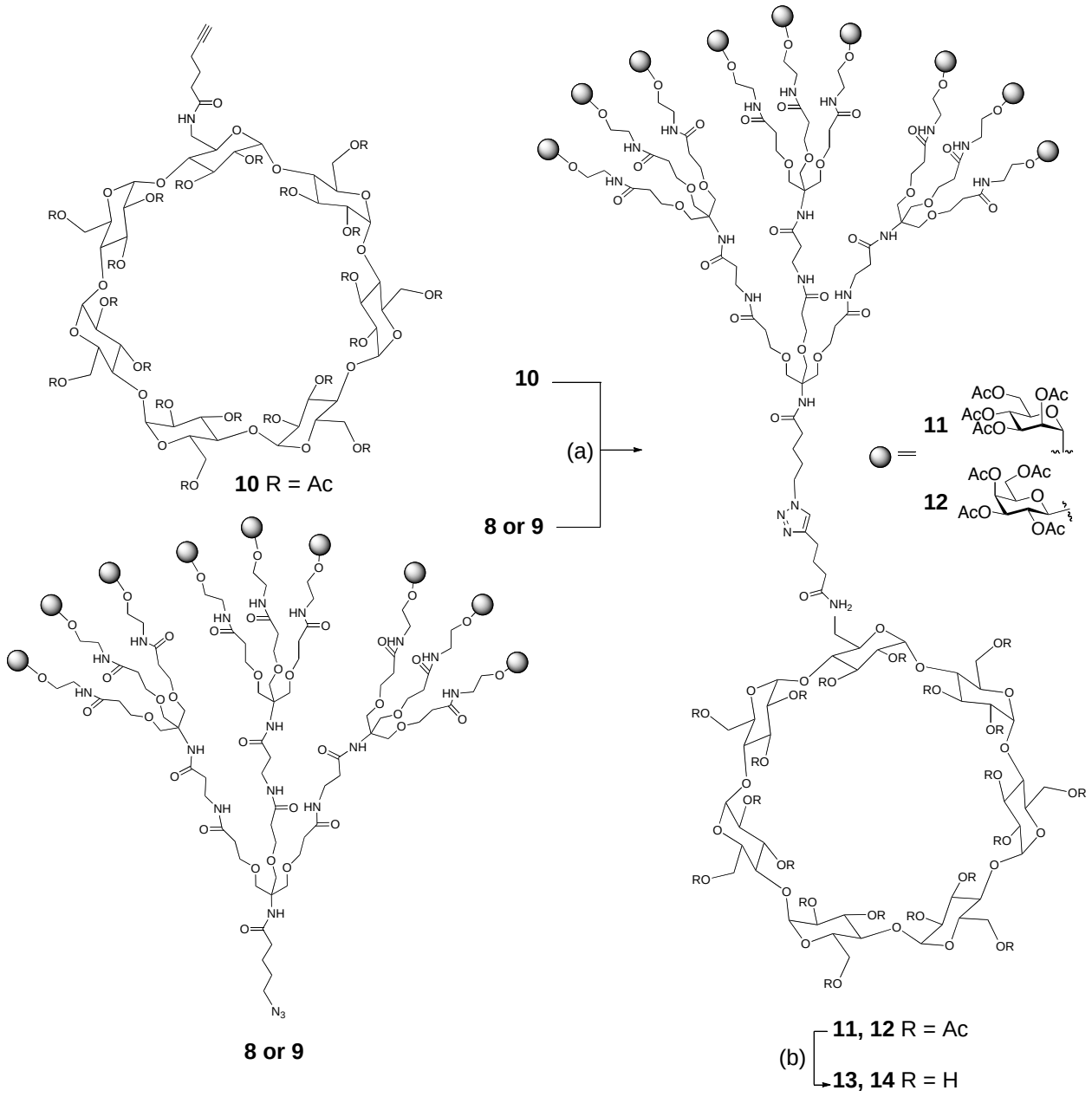
^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300 (300 MHz) or Varian VXR700 (700 MHz) spectrometer. High-resolution mass spectra (HR-MALDI MS) and ESI-MS were performed by the Mass Spectrometry-service at the MPI Berlin. ESI-MS were run on an Agilent 1100 Series LC/MSD instrument. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotation measurements were conducted using a Perkin-Elmer 241 polarimeter.

Absorption spectra were recorded using a Varian CARY 50 spectrophotometer fitted with Hellma optical fibers (Hellma, 041.002-UV) and an immersion probe made of quartz suprazil (Hellma, 661.500-QX). Fluorescence emission spectra were recorded on a Perkin-Elmer LS-50B spectrofluorometer.

2. Synthesis of *β*-cyclodextrin glycodendrimers 13, 14

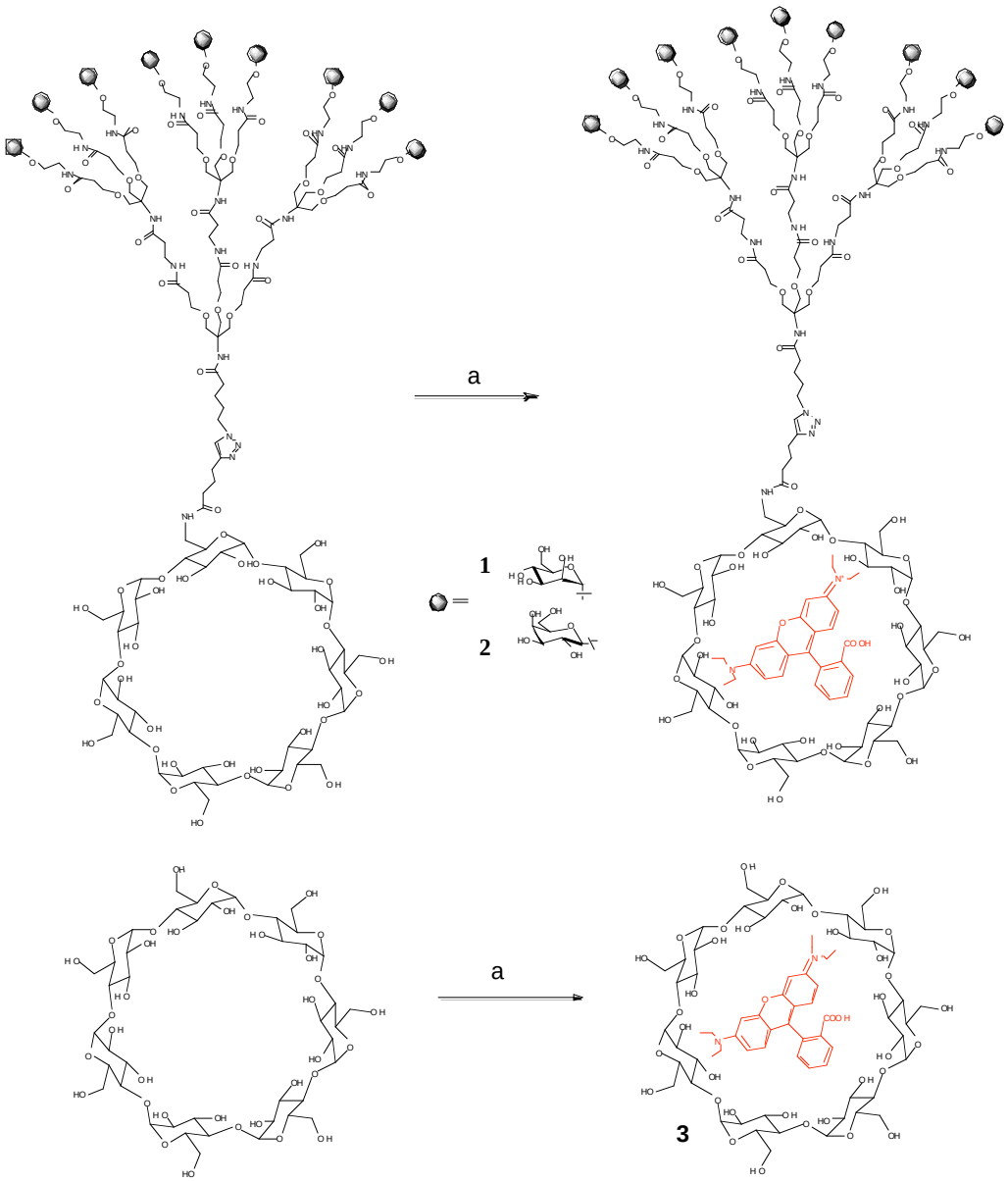


Scheme 1. Reagents and Conditions**:** (a) (i) Acrylonitrile/NaOH (40%); (ii) conc. HCl/EtOH, 51%; (iii) 5-bromovaleric acid, DIC, HOBT, DCM, 69%; (b) (i) NaN_3, DMF, 72%; (ii) NaOH/MeOH; (iii) pentafluorophenol, DIC, HOBT, DCM, 86% (2 steps); (c) tripod-mannose or tripod-galactose, DCM, TEA, 52-59%.



Scheme 2. Reagents and Conditions: (a) **8** or **9**/$CuSO_4$/ascorbic acid/THF:H_2O (1:1) 82-84%; (b) NaOMe/MeOH 77-78%;

3. Synthesis of complexes 1-3



Scheme 3. Reagent and solvent: (a) **13** or **14** or *β*-cyclodextrin/rhodamine B/H_2O.

4. Photophysical Properties.

The photo-physical properties of dendrimers **1-3** were compared with the reference compound rhodamine B. The emission properties of all compounds showed the characteristic luminescence of the RhB core. Minor differences related to the different chemical composition were noted. Complexes **1-2** showed a bathochromic shift of 10-12 nm compared with the reference complex due the presence of dendrimer. Quantum yields of all compounds were calculated by using a standard formula with RhB as a reference. Quantum yields of complexes **1** and **2** are slightly higher than the quantum yield of **3**. This alteration in photophysical properties may be due to the high sugar density around the fluorescent probe.

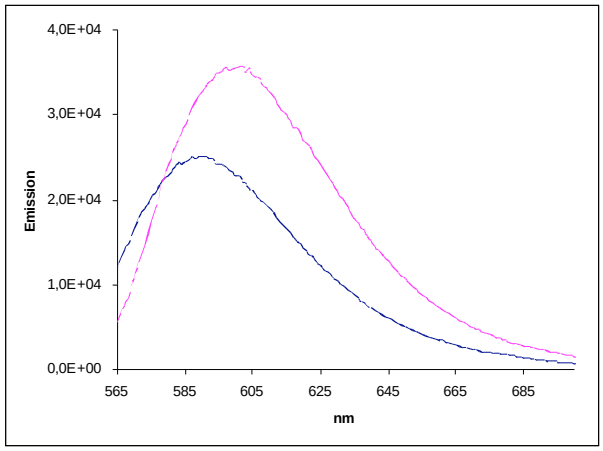


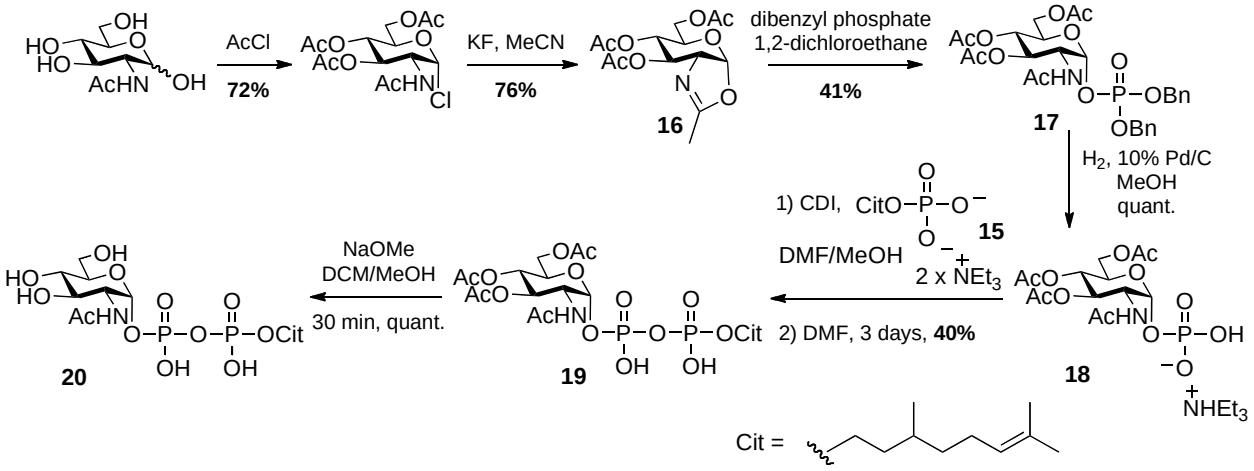
Fig 1. Fluorescent spectra of 1 (pink) and RhB (blue line) excited at λ_{max} 514 nm

Compound	λ_{max}(nm)	Φ
1	597	0.33
2	597	0.37
3	595	0.37
RhB	585	0.31

Table S1. Photophysical properties of complexes **1-3.**

5. Synthesis of Glycolipids

Synthesis of GlcNAc-diphosphate citronellyl 20 (negative control)



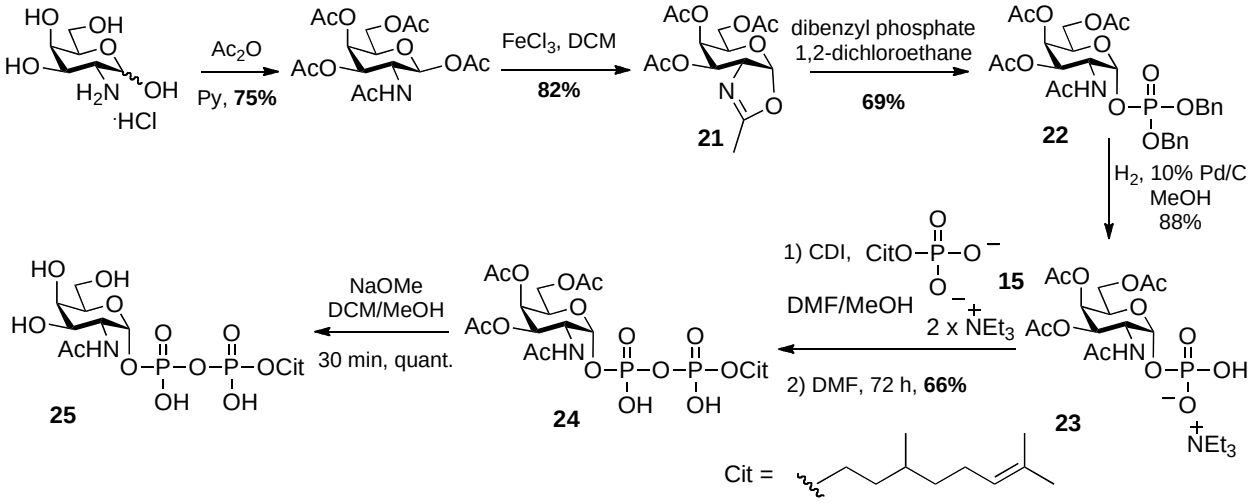
Scheme 4.

3,4,6-Tri-*O*-acetyl-2-*N*-acetylamido-2-deoxy-α-D-glucopyranosyl chloride. Acetyl chloride (40 mL, 563 mmol) was added through an air condenser into a round bottom flask containing 2-acetylamido-2-deoxy-D-glucose (20.0 g, 90.41 mmol). The reaction mixture was heated for 1 h until a color change to pink was observed and the reaction mixture was stirred vigorously overnight. After 16 hours, TLC (petrol:ethyl acetate, 1:2) indicated formation of a product (R_f 0.3) with complete consumption of the starting material (R_f 0). The reaction mixture was diluted with DCM (150 mL) and then poured on to ice water (100 mL). The organic layer was washed with ice cold sodium bicarbonate (3 x 100 mL) until no more gas was evolved. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Recrystallization from diethyl ether/DCM yielded 3,4,6-tri-*O*-acetyl-2-*N*-acetylamido-2-deoxy-α-D-glucopyranosyl chloride as a crystalline solid (23.95 g, 72%); m.p. 120-122 ºC (diethyl ether/DCM) [Lit. 122-123 ºC] ***[ref. 1]***; $[\alpha]_D^{18}$ +127.0 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{26}$ +120.6 (c, 1.03 in $CHCl_3$)] ***[ref. 2]***; ^{1}H-NMR(400 MHz, $CDCl_3$) δ 6.19 (d, *J* = 3.7 Hz,1H). 5.83 (d, *J* = 8.6 Hz, 1H), 5.32 (d, *J* = 10.0 Hz, 1H), 5.22 (d, *J* = 9.8 Hz, 1H), 4.53 (ddd, *J* = 3.7, 10.7, 8.9 Hz, 1H), 4.31-4.23 (m, 2H), 4.14-4.05 (m, 1H), 2.11 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H),

2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)[1,2-*d*]-oxazoline 16. A suspension of potassium fluoride (1.27 g, 21.87 mmol) and 3,4,6-tri-*O*-acetyl-2-*N*-acetylamido-2-deoxy-α-D-glucopyranosyl chloride (2.00 g, 5.47 mmol) in anhydrous acetonitrile (10 mL) was refluxed at 82 ºC for 15 h under an atmosphere of argon. After cooling the mixture to room temperature, the solid materials were removed by filtration and the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate) yielding 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)[1,2-*d*]-oxazoline **16** (1.36 g, 76%) as a yellow foam; $[\alpha]_D^{18}$ +12.2 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{22}$ +17.2 (c, 1.6 in $CHCl_3$)] ***[ref. 3]***; ^{1}H-NMR (400 MHz, $CDCl_3$) 5.96 (d, *J* = 7.4 Hz, 1H). 5.25 (d, *J* = 2.4 Hz, 1H), 4.92 (d, *J* = 1.2, 9.2 Hz, 1H), 4.17-4.15 (m, 2H), 4.12 (m, 1H), 3.59 (m, 1H), 2.10 (s, 3H), 2.09 (s, 3H), 2.08 (d, *J* = 1.8 Hz, 3H), 2.07 (s, 3H),

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose 1-dibenzylphosphate 17. 2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)[1,2-*d*]-oxazoline **16** (0.50 g, 1.52 mmol) was dissolved in anhydrous 1,2-dichloroethane (10 mL) and stirred under an atmosphere of argon. A solution of dibenzyl phosphate (0.47 g, 1.67 mmol) in 1,2-dichloroethane (4 mL) was added dropwise to the sugar solution. After 24 h, TLC (diethyl ether:methanol, 95:2) indicated the formation of a major product (R_f 0.4). The reaction was concentrated *in vacuo* and the resulting residue purified by flash column chromatography (diethyl ether:methanol, 95:5) to afford 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose 1-dibenzylphosphate **17** (0.38 g, 41%) as a colourless gum; $[\alpha]_D^{25}$ +4.8 (c, 1 in $CHCl_3$); FTIR (thin film) 3443, 1642, 1236 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40-7.30 (m, 10H), 5.96 (br d, *J* = 9.0 Hz, 1H), 5.66 (dd, *J* = 3.3, 6.1 Hz, 1H), 5.30-5.21 (m, 2H), 5.18-4.99 (m, 4H), 4.39-4.33 (m, 1H), 4.09 (dd, *J* = 4.7, 12.0 Hz, 1H), 4.01 (ddd, *J* = 10.1, 2.0, 4.1 Hz, 1H), 3.91 (dd, *J* = 2.1, 12.5 Hz, 1H), 2.02 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.70 (s, 3H); ^{31}P-NMR (162 MHz, $CDCl_3$) δ -2.5; *m/z* (ESI) 630 $[M+Na]^+$; HRMS (ESI) Calcd. For $C_{28}H_{34}NNaO_{12}P$ $[M+Na]^+$ 630.1711. Found: 630.1710; spectroscopic data was identical to that previously reported ***[ref. 4]***.

Synthesis of GalNAc-diphosphate citronellyl 25



Scheme 5.

1,3,4,6-Tetra-*O*-acetyl-2-*N*-acetylamido-2-deoxy-β-D-galactopyranoside. Galactosamine hydrochloride (0.32 g, 1.49 mmol) was dissolved in pyridine (9 mL) and acetic anhydride (6 mL) added to the solution at 0 ºC. The reaction mixture was allowed to warm to room temperature and stirred overnight. TLC (ethyl acetate) indicated formation of a product (R_f 0.3) with complete consumption of the starting material (R_f 0). The reaction mixture was co-evaporated three times with toluene and the solid recrystallized from methanol to give the title compound (0.43 g, 75%) as a white crystalline solid; m.p. 230-232 ºC (MeOH) [Lit. 234-236 ºC (ethyl acetate] ***[ref. 5]***; $[\alpha]_D^{25}$ +2.6 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{27}$ +9.2 (c, 0.5 in $CHCl_3$)] ***[ref. 5]***; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.70 (d, *J* = 8.8 Hz, 1H); 5.83 (d, *J* = 8.6 Hz, 1H), 5.39 (m, 2H), 5.07 (dd, *J* = 11.3, 3.3 Hz, 1H), 4.45 (dt, *J* = 9.2, 11.2 Hz, 1H), 4.20-4.10 (m, 2H), 4.04-3.99 (m, 1H), 2.17 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H), spectroscopic data was identical to that previously reported ***[ref. 6]***.

2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-galactopyrano)[1,2-*d*]-oxazoline 21. A suspension of ferric fluoride (3.02 g, 18.6 mmol) and 1,3,4,6-tetra-*O*-acetyl-2-*N*-acetylamido-2-deoxy-β-D-galactopyranoside (2.9 g, 7.45 mmol) in anhydrous DCM (50 mL) was stirred at room temperature under argon. After one hour, TLC (ethyl acetate) indicated formation of a product (R_f 0.3 – visualized with sulphuric acid TLC stain) with complete consumption of the starting material (R_f 0.2.). The reaction mixture was poured into ice cold water and extracted

with DCM (3 x 50 mL). The combined organics phases were washed with sodium hydrogen carbonate (80 mL of a saturated aqueous solution), and water (80 mL), and dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (ethyl acetate) yielding **21** (2.04 g, 82%) as a foam; $[\alpha]_D^{23}$ +25.8 (c, 1 in $CHCl_3$) [Lit. $[\alpha]_D^{20}$ +26 (c, 1.0 in $CHCl_3$)] ***[ref. 6]***; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.97 (d, *J* = 7.4 Hz, 1H), 5.24 (d, *J* = 3.1 Hz, 1H), 4.89 (dd, *J* = 3.3, 7.4 Hz, 1H), 4.24-4.07 (m, 3H), 3.99-3.96 (m, 1H), 2.10 (s, 6H), 2.05 (s, 3H), 2.03 (d, *J* = 1.3 Hz, 3H) spectroscopic data was identical to that previously reported ***[ref. 6]***.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranose 1-dibenzylphosphate 22. 2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-galactopyrano)[1,2-*d*]-oxazoline **21** (1.41 g, 4.29 mmol) was dissolved in anhydrous 1,2-dichloroethane (30 mL) and stirred under an atmosphere of argon. Dibenzyl phosphate (1.67 g, 6.00 mmol) was added as a solid to the sugar solution. After 24 h, TLC (diethyl ether:methanol, 97:3) indicated the formation of a product (R_f 0.4). The reaction was concentrated *in vacuo* and the resulting residue purified by flash column chromatography (diethyl ether:methanol, 97:3) to afford **22** (1.79 g, 69 %) as a colorless gum; FTIR (thin film) 3441, 1645, 1238, cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40-7.30 (m, 10H), 5.72 (dd, *J* = 3.3, 5.8 Hz, 1H), 5.55 (br d, *J* = 9.4 Hz, 1H), 5.37 (dd, *J* = 1.4, 3.2 Hz, 1H), 5.14-5.03 (m, 5H), 4.66-4.54 (m, 1H), 4.26 (dt, *J* = 0.9, 6.6, 6.6 Hz, 1H), 4.09 (dd, *J* = 6.6, 12.3 Hz, 1H), 3.93 (dd, *J* = 6.6. 12.3 Hz, 1H), 2.14 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H), 1.73 (s, 3H), δ_P^{31} (162 MHz, $CDCl_3$) -2.3; *m/z* (ESI) 630 $[M+Na]^+$; HRMS (ESI) Calcd. For $C_{28}H_{34}NNaO_{12}P$ $[M+Na]^+$ 630.1711. Found: 630.1724; spectroscopic data was identical to that previously reported ***[ref. 7]***.

6. References

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7. ^{1}H, ^{13}C-NMR, ^{31}P-NMR

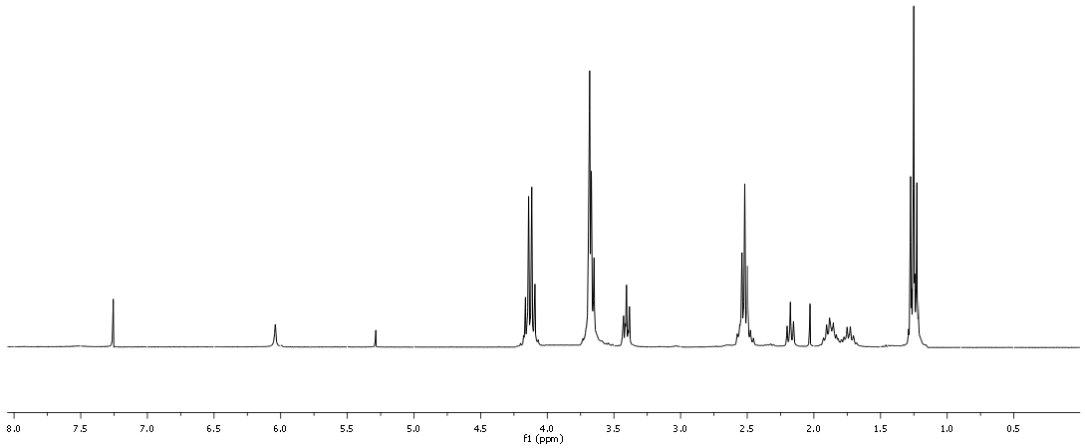


Figure 2. ^{1}H NMR of **5**.

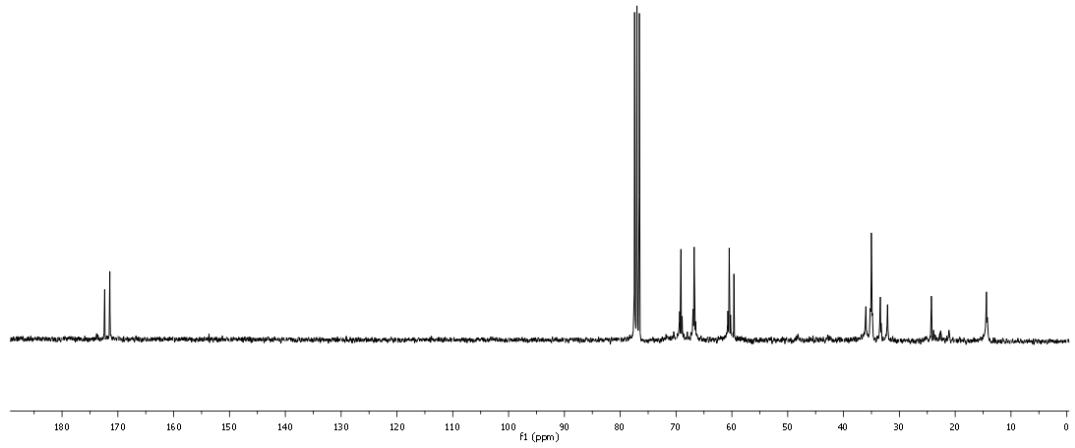
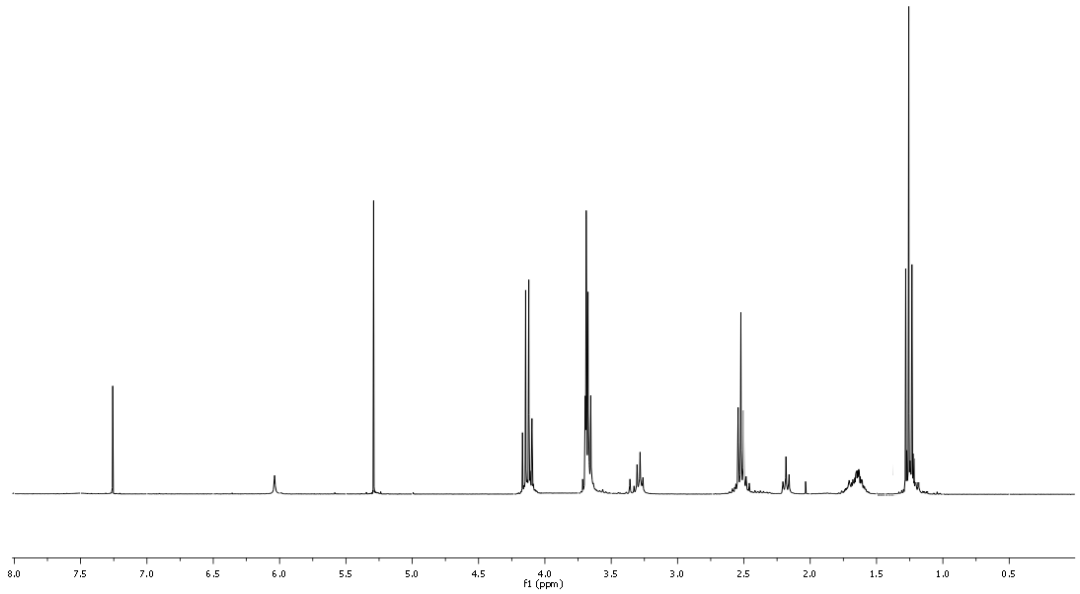


Figure 3. ^{13}C NMR of **5**.



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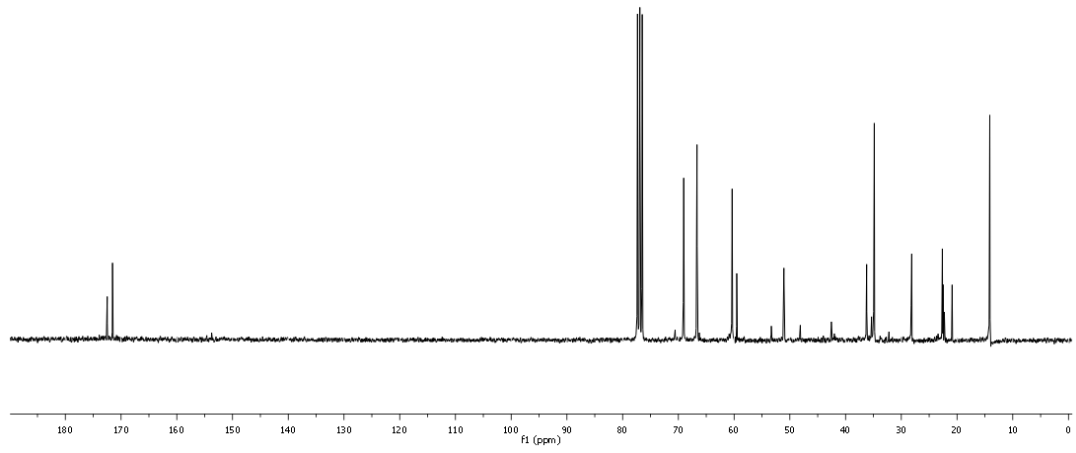

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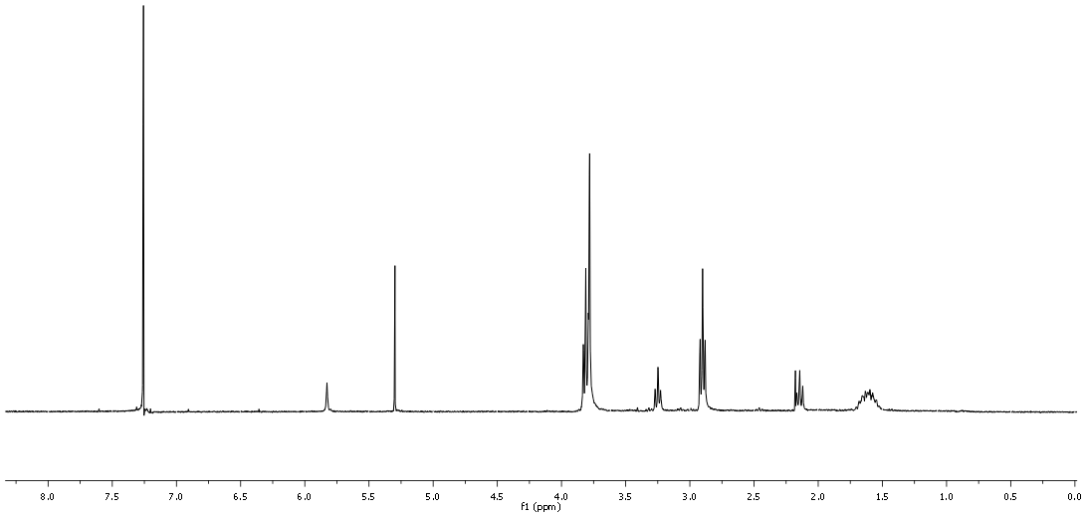

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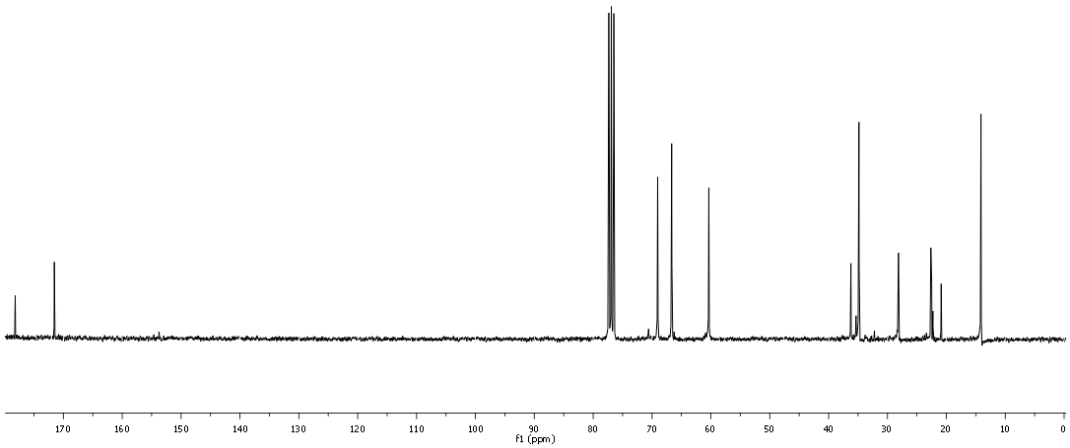

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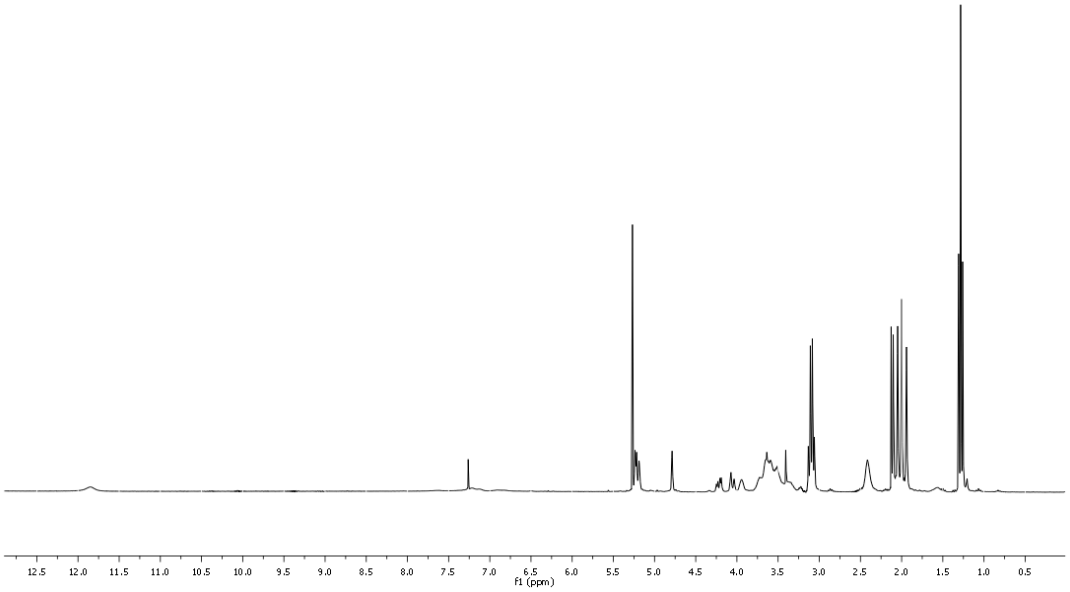

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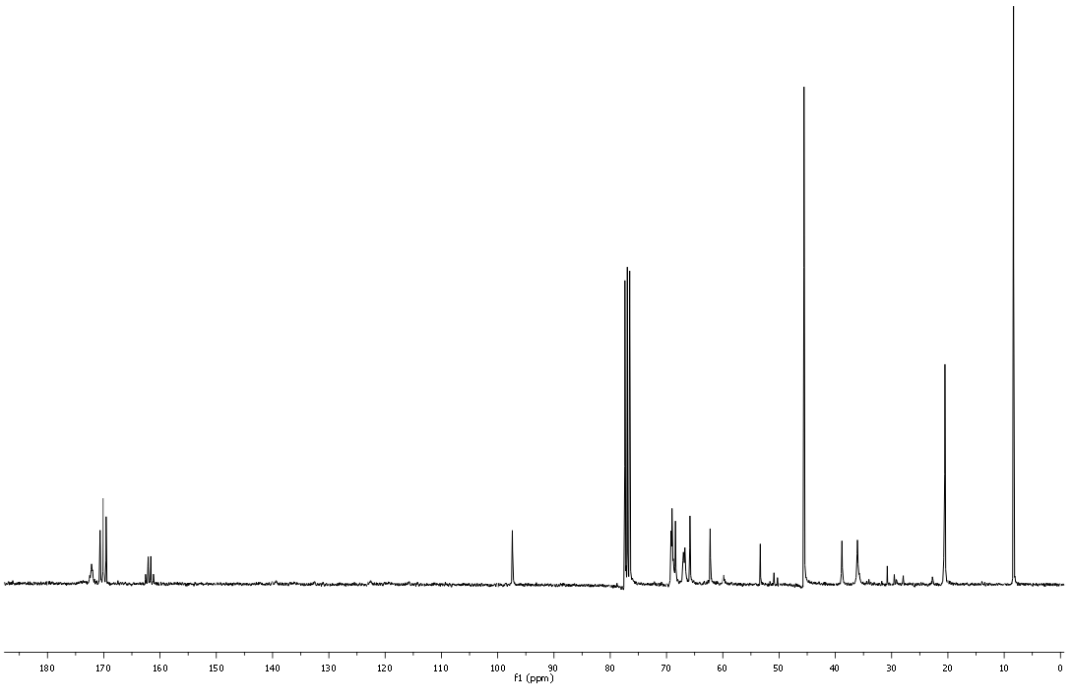

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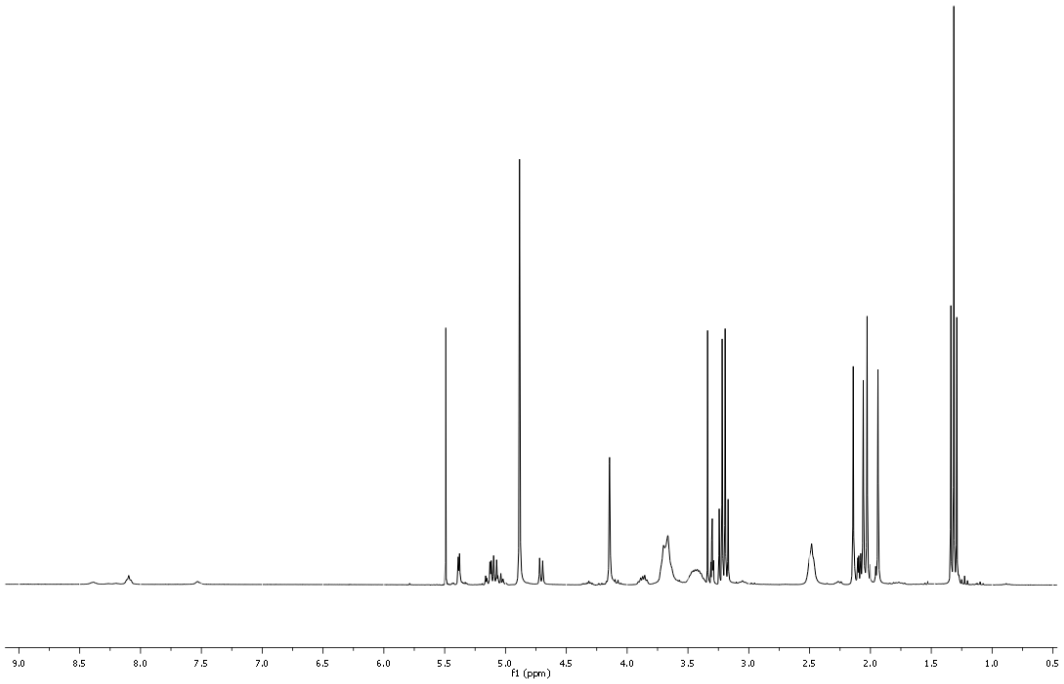

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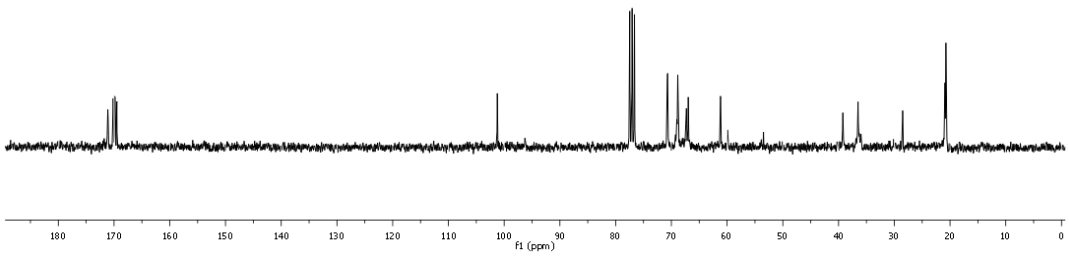

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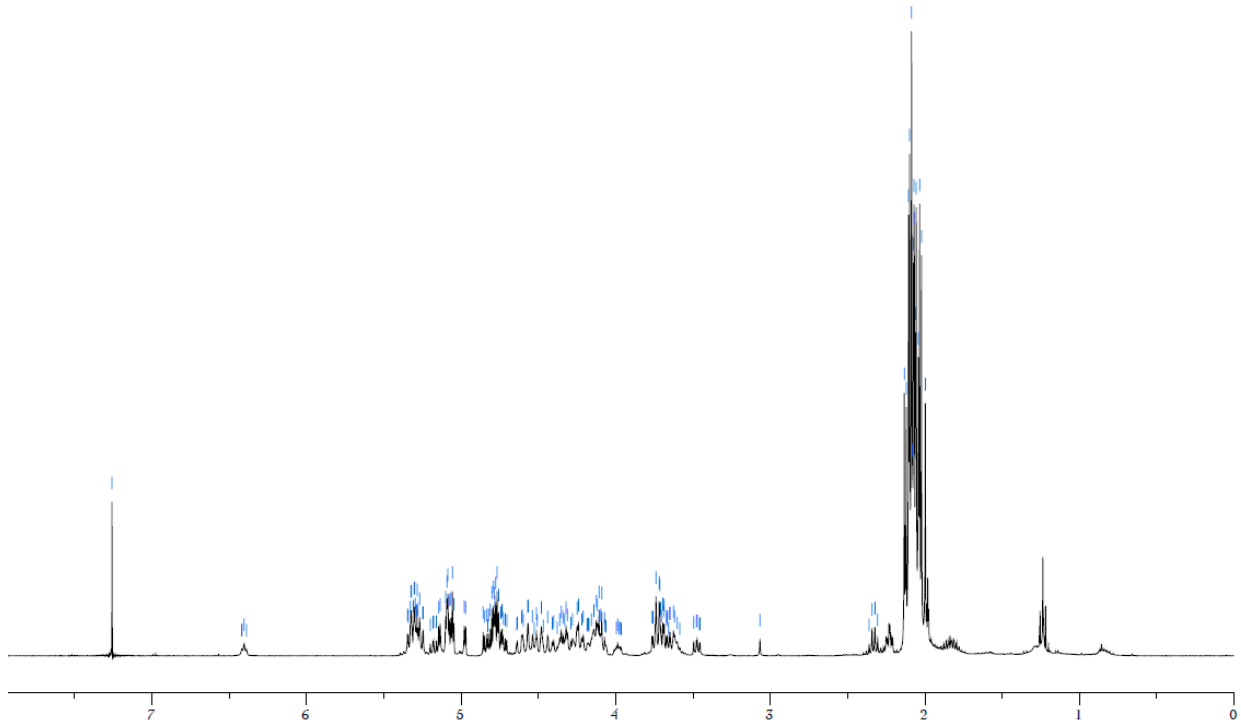

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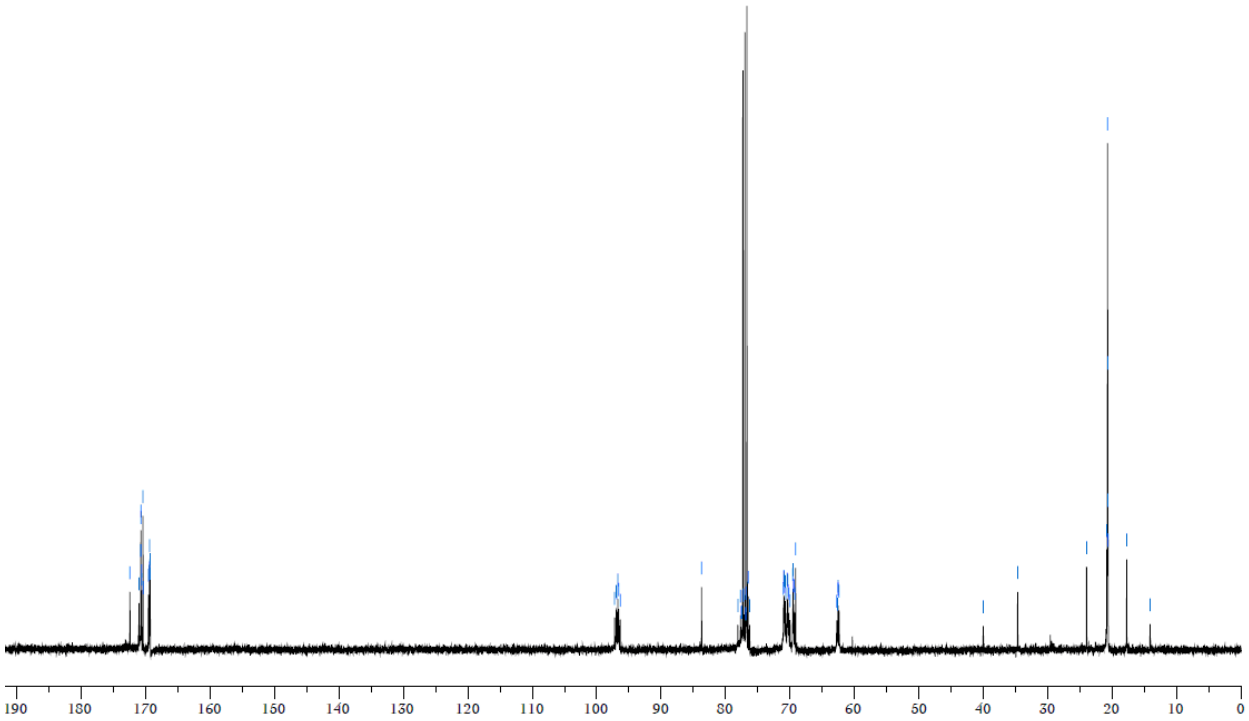

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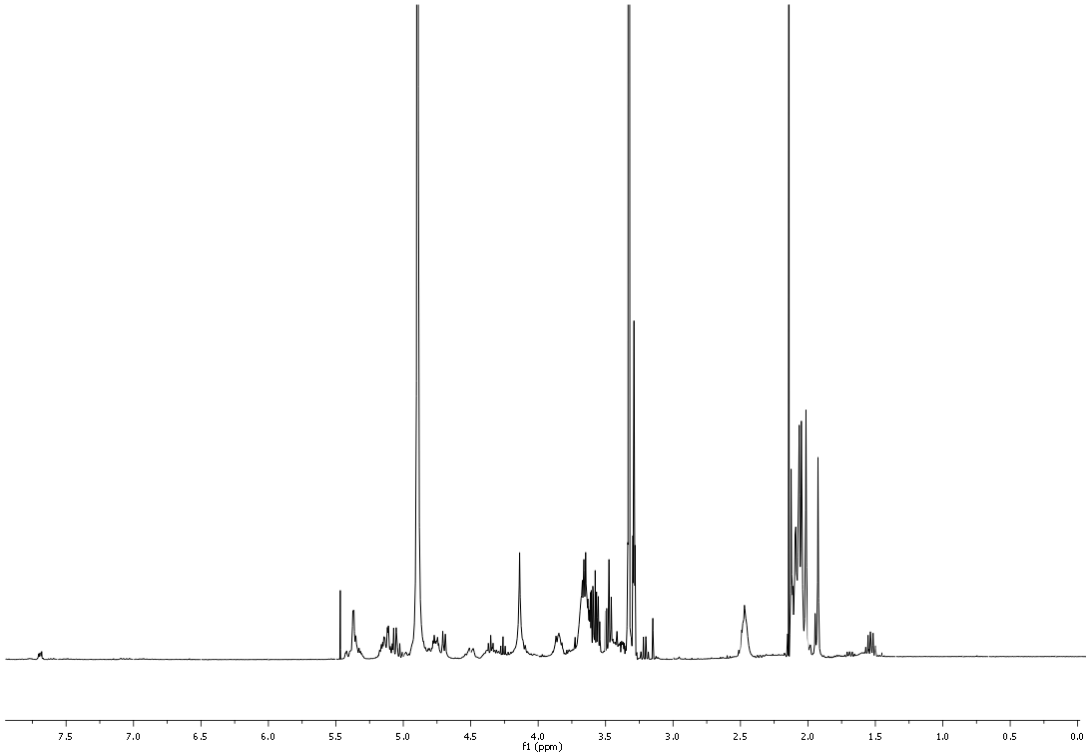
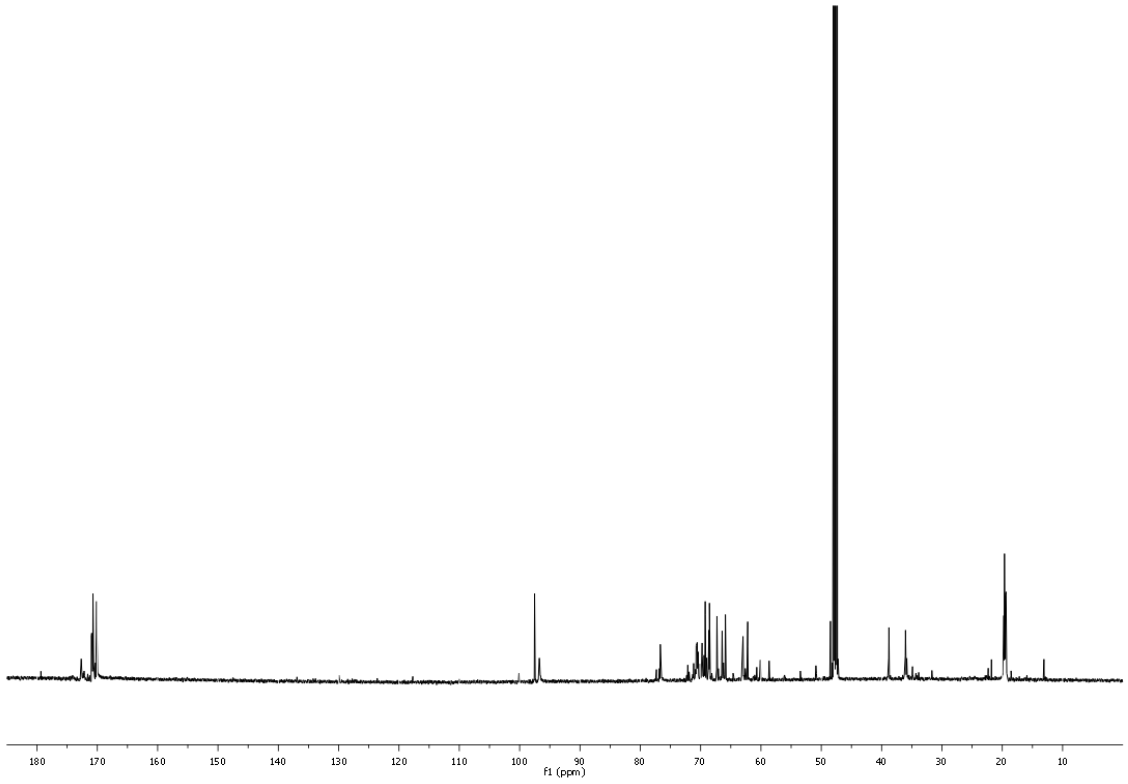


Figure 14. ^{1}H NMR of **11**.



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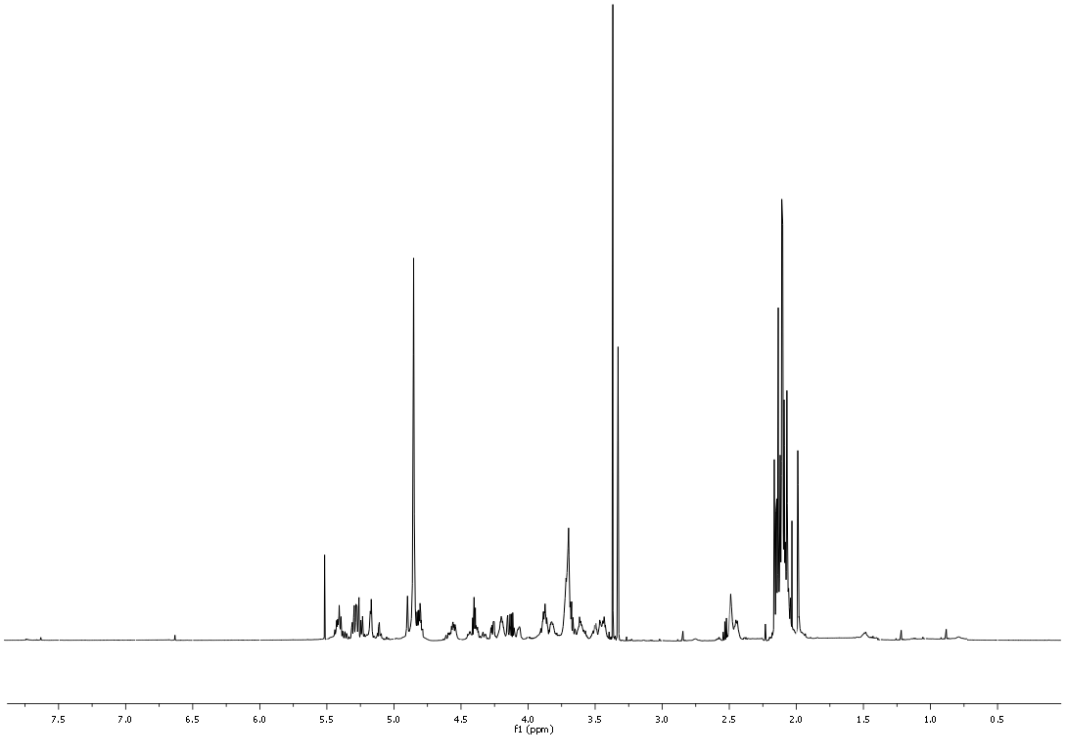

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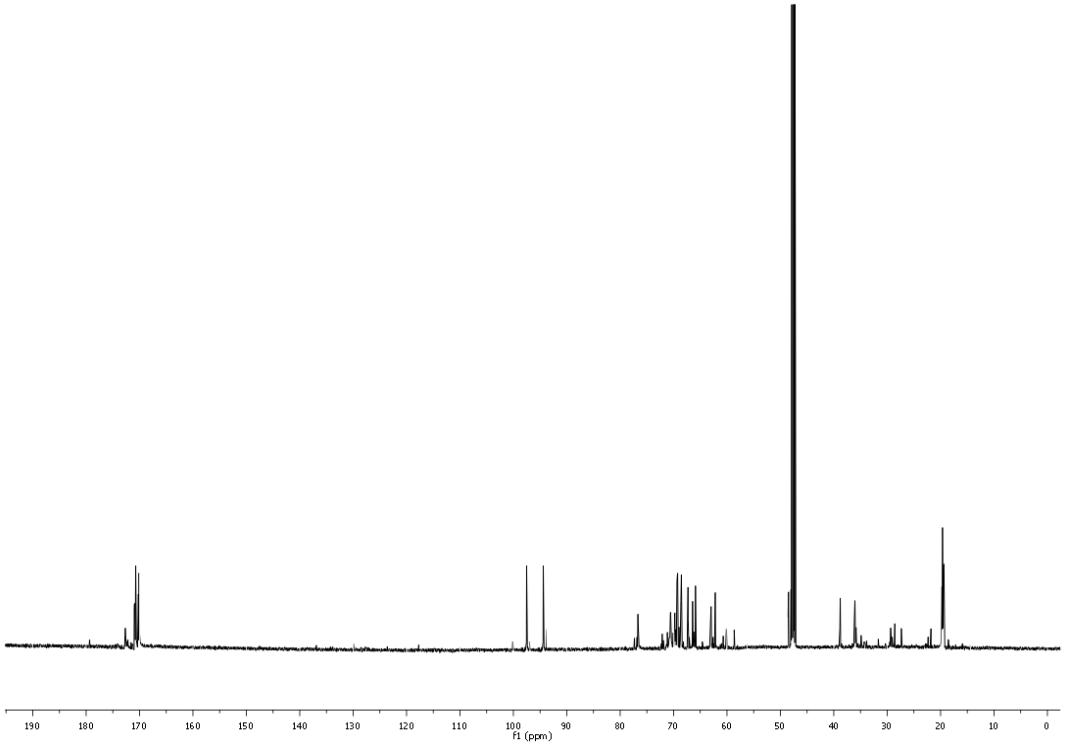

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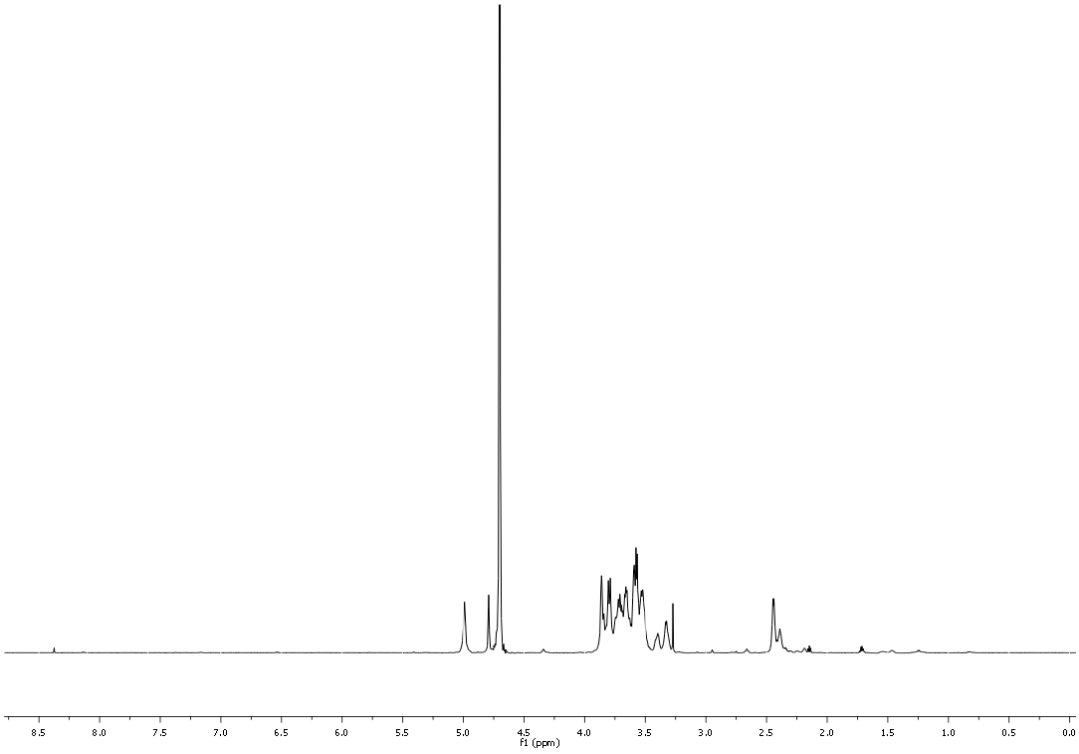

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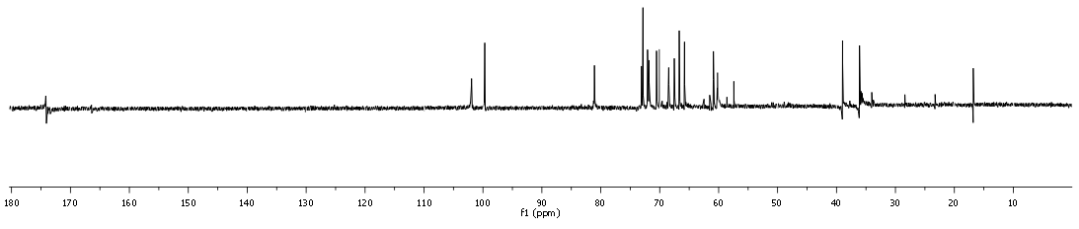

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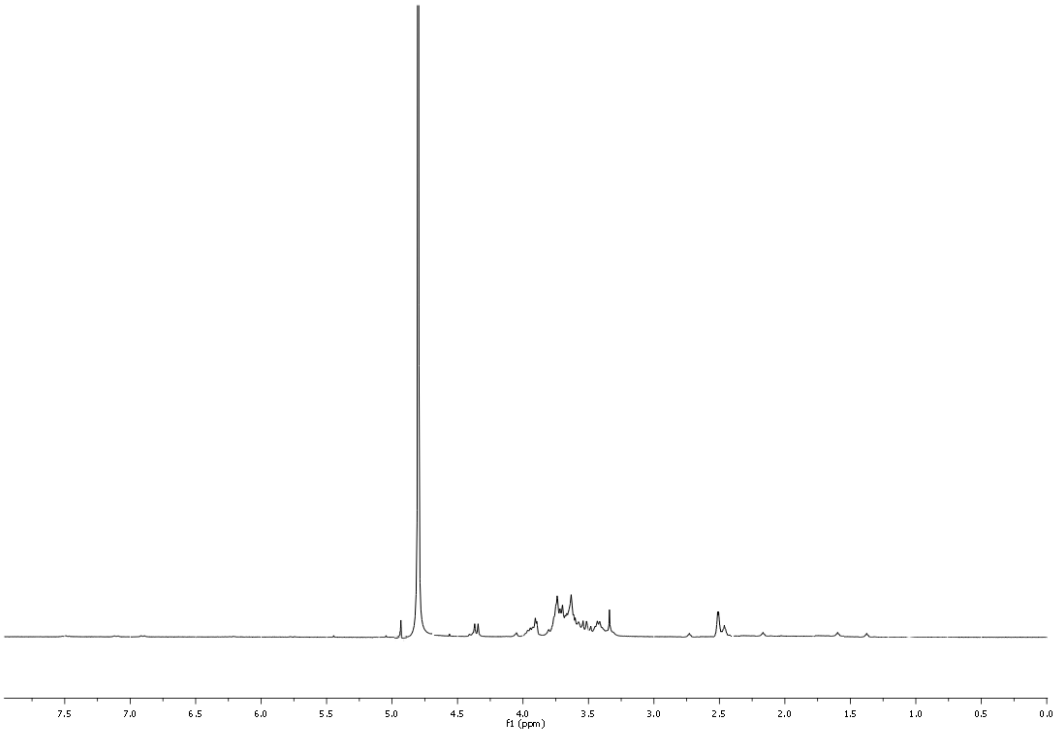

Figure 20. ^{1}H NMR of **14**.

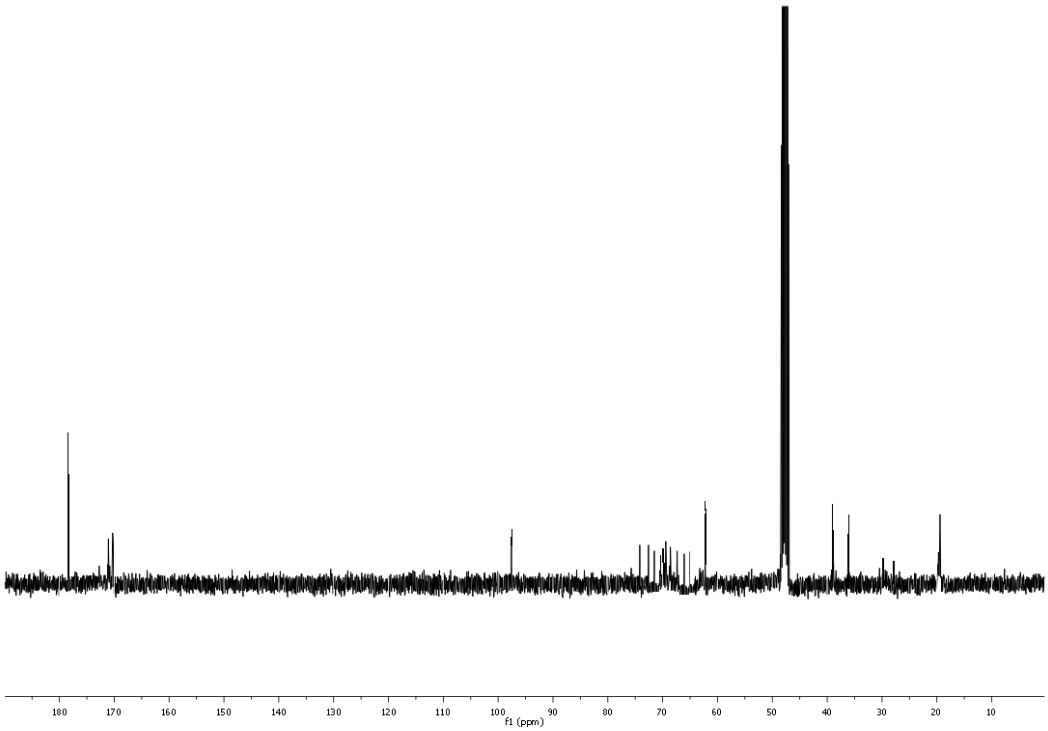

Figure 21. ^{13}C NMR of **14**.

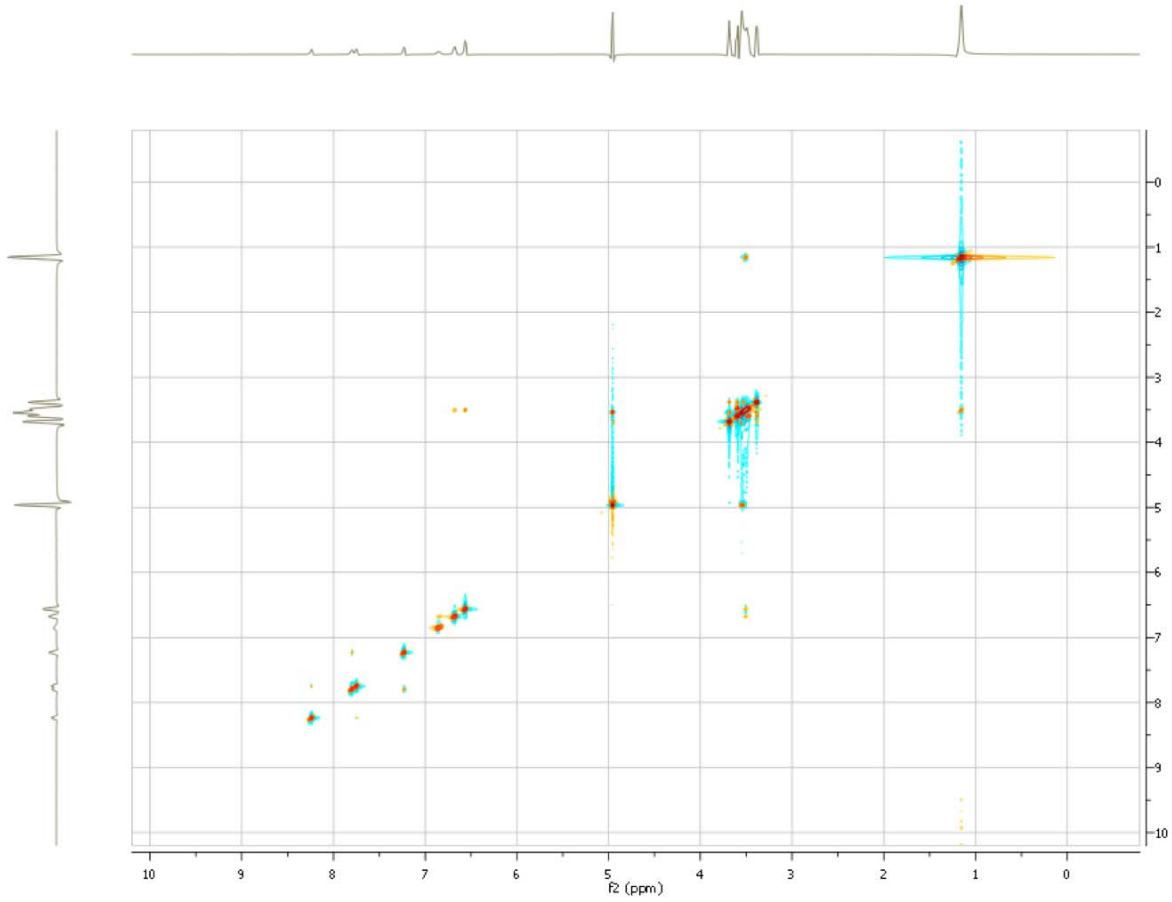

Figure 22. ^{1}H-NOESY NMR of **1**.

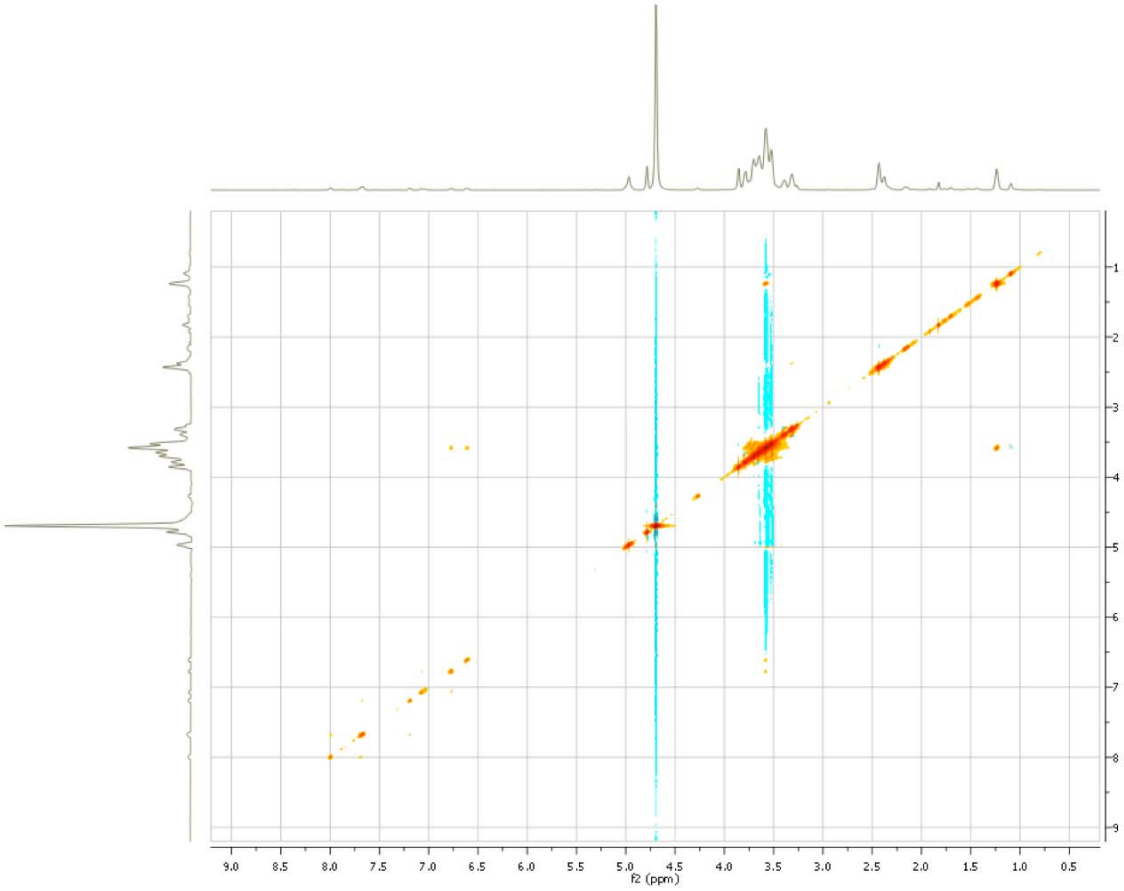

Figure 23. ^{1}H-NOESY NMR of **2**.

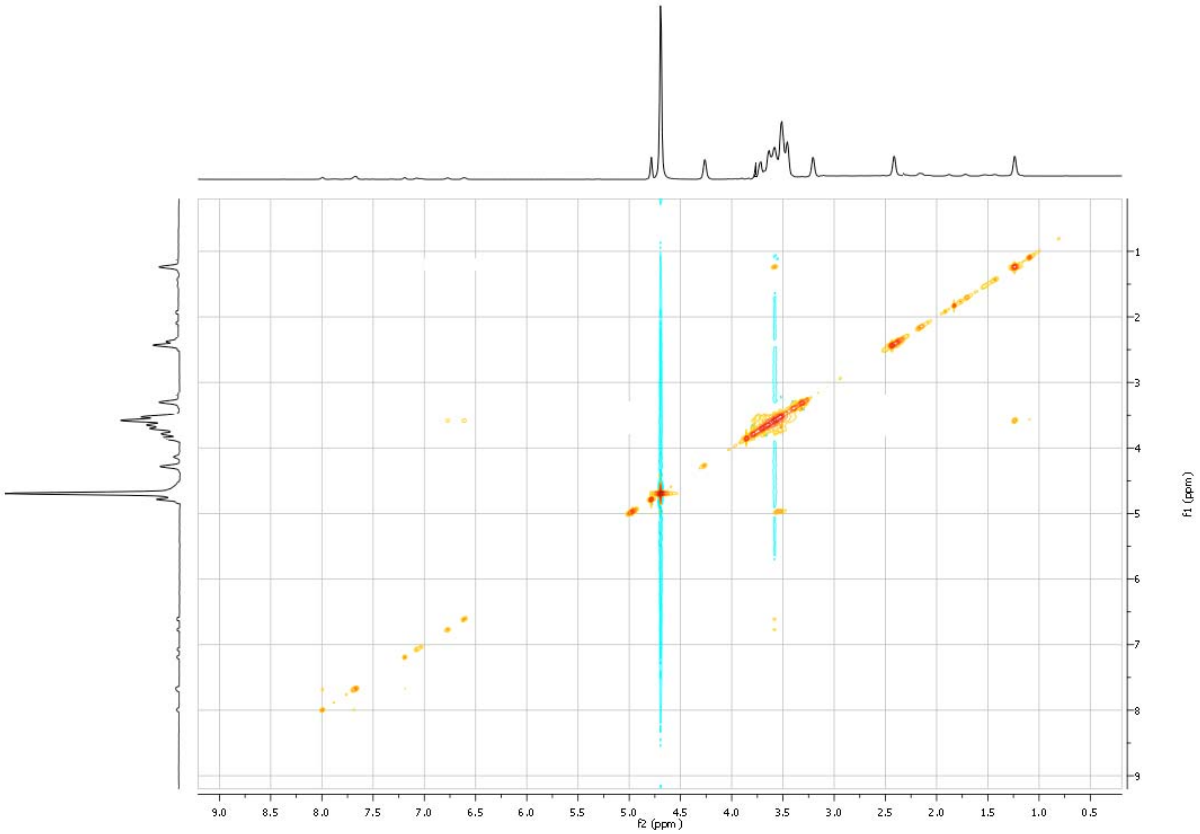

Figure 24. ^{1}H-NOESY NMR of **3**.

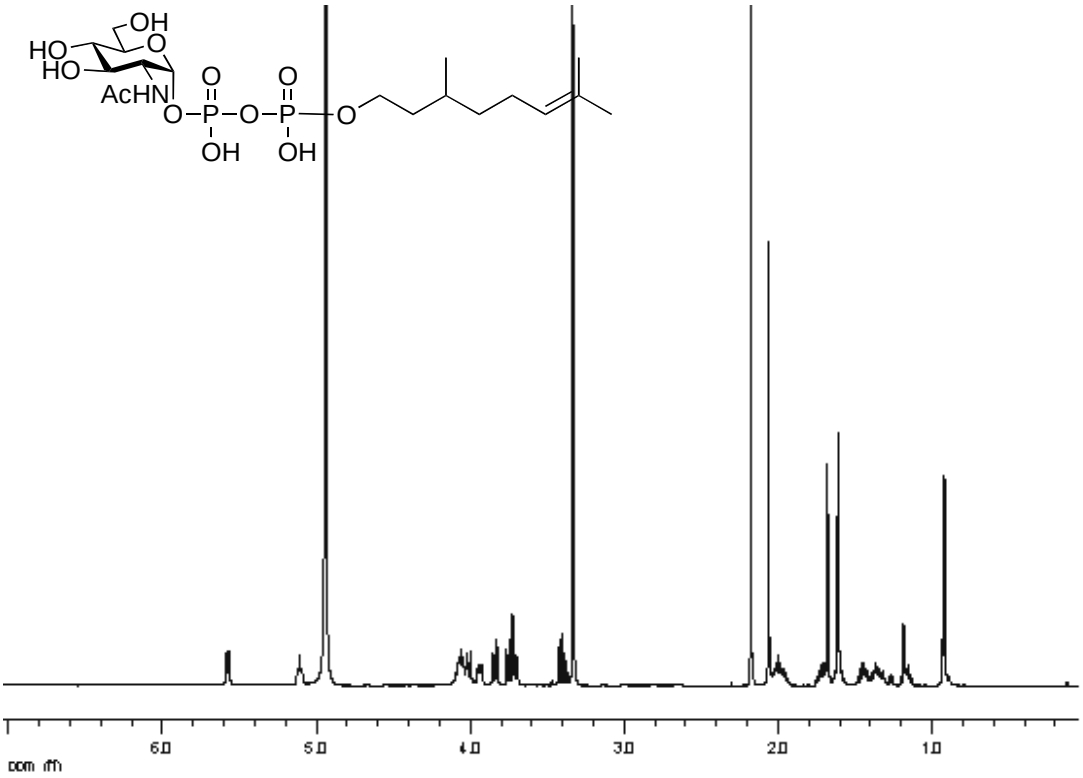

Figure 25. ^{1}H NMR of **20**.

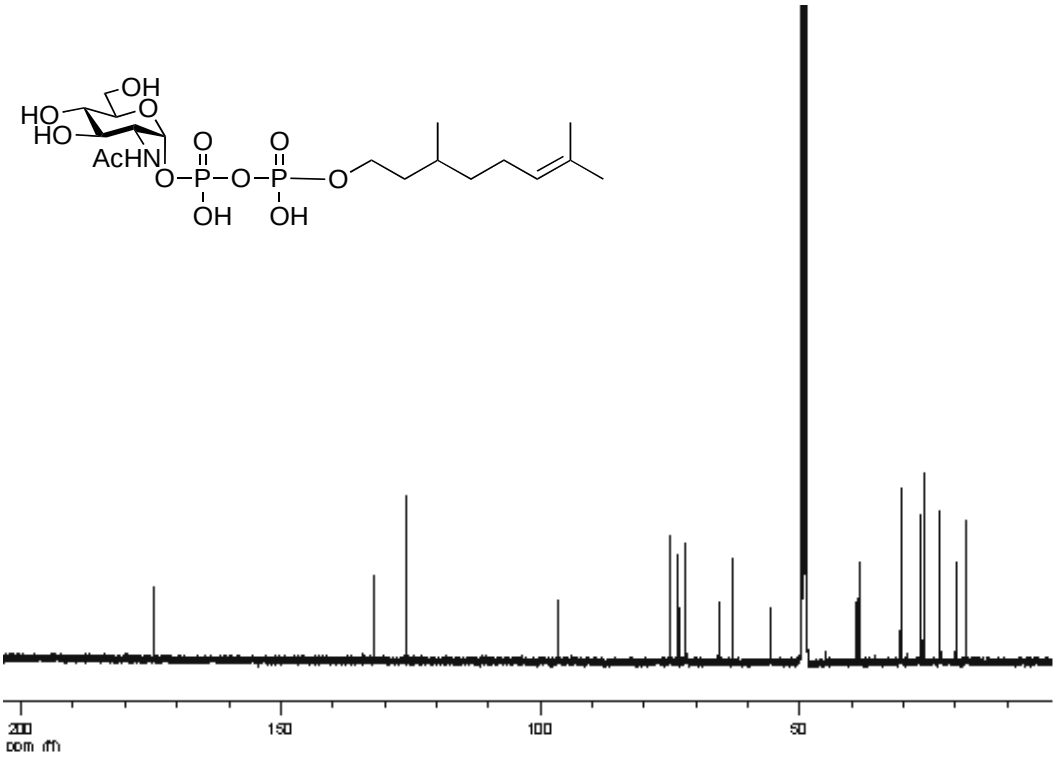

Figure 26. ^{13}C NMR of **20**.

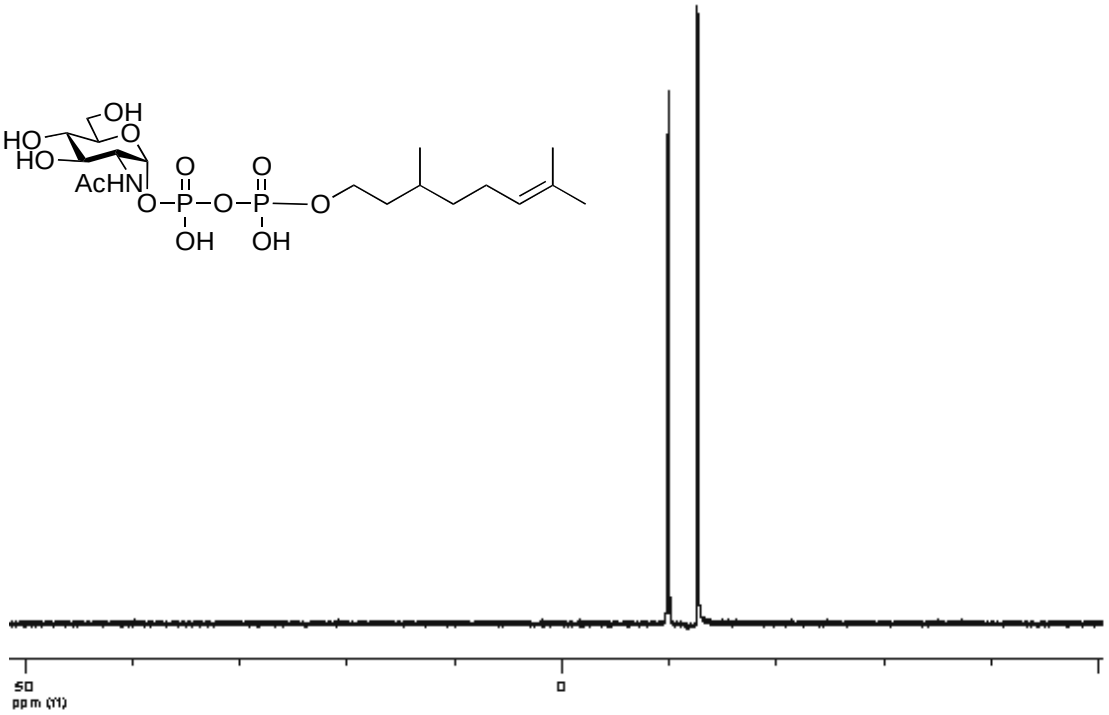

Figure 27. ^{31}P NMR of **20**.

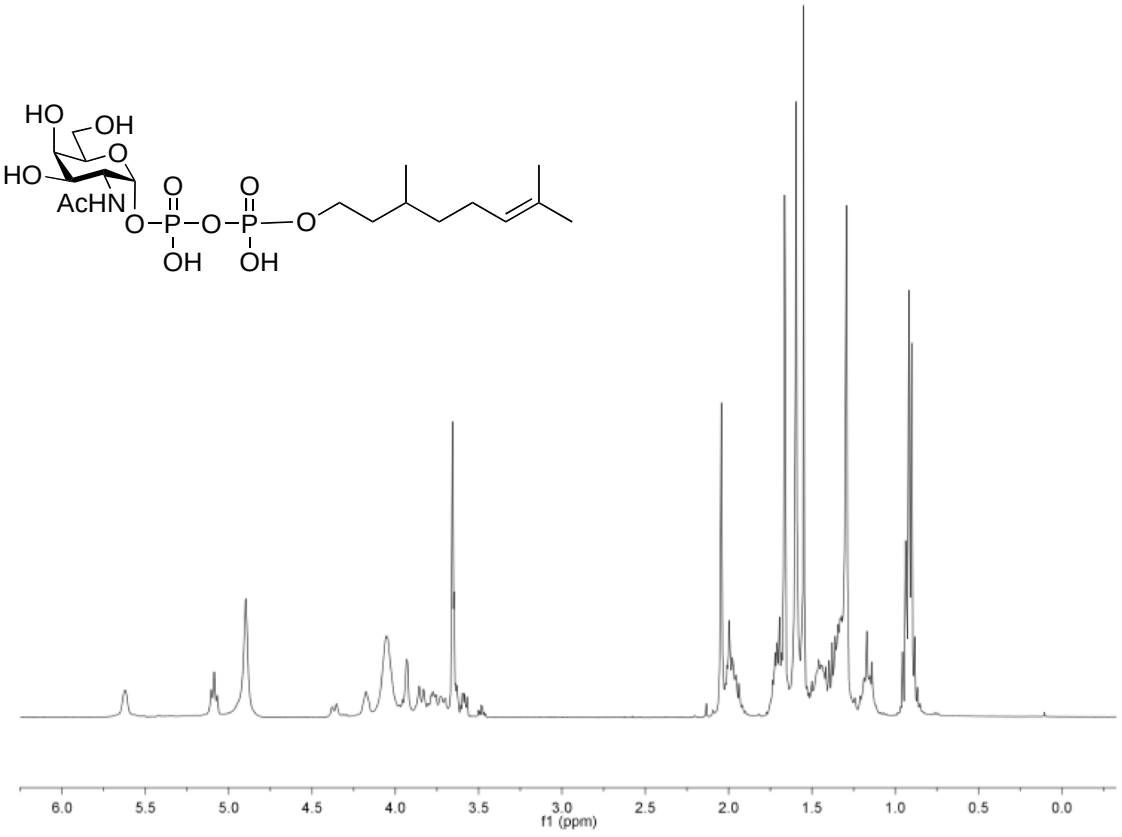

Figure 28. ^{1}H NMR of **25**.

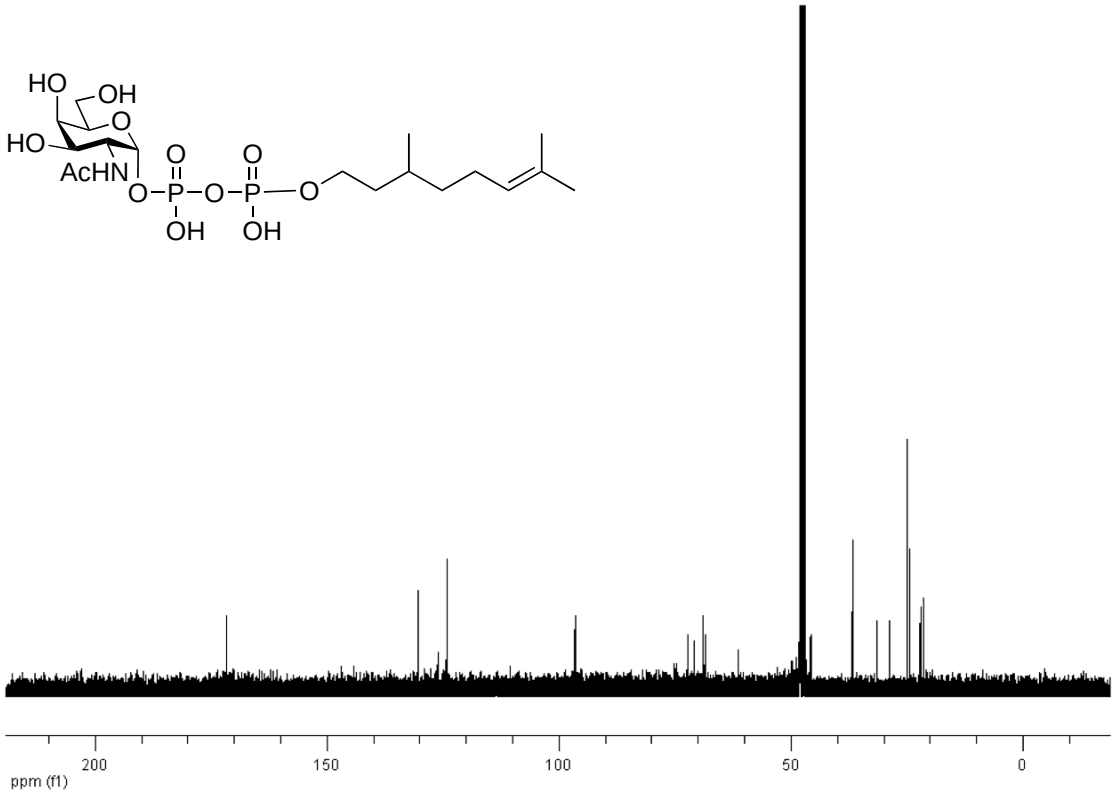

Figure 29. ^{13}C NMR of **25**.

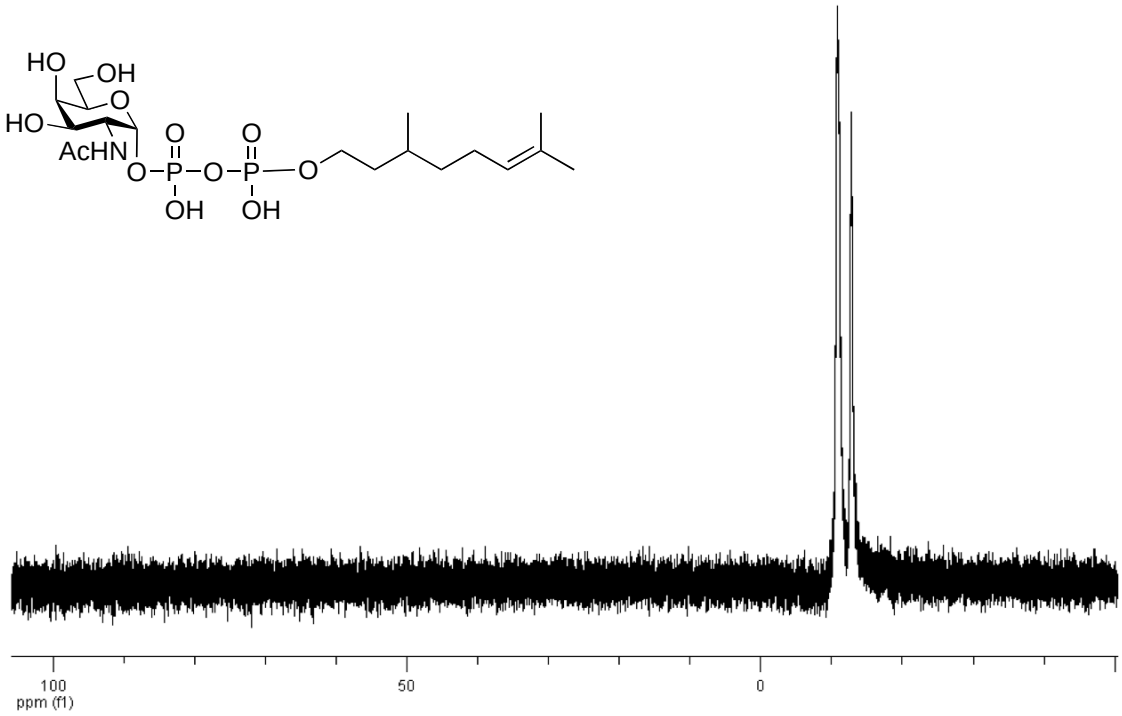

Figure 30. ^{31}P NMR of **25**.

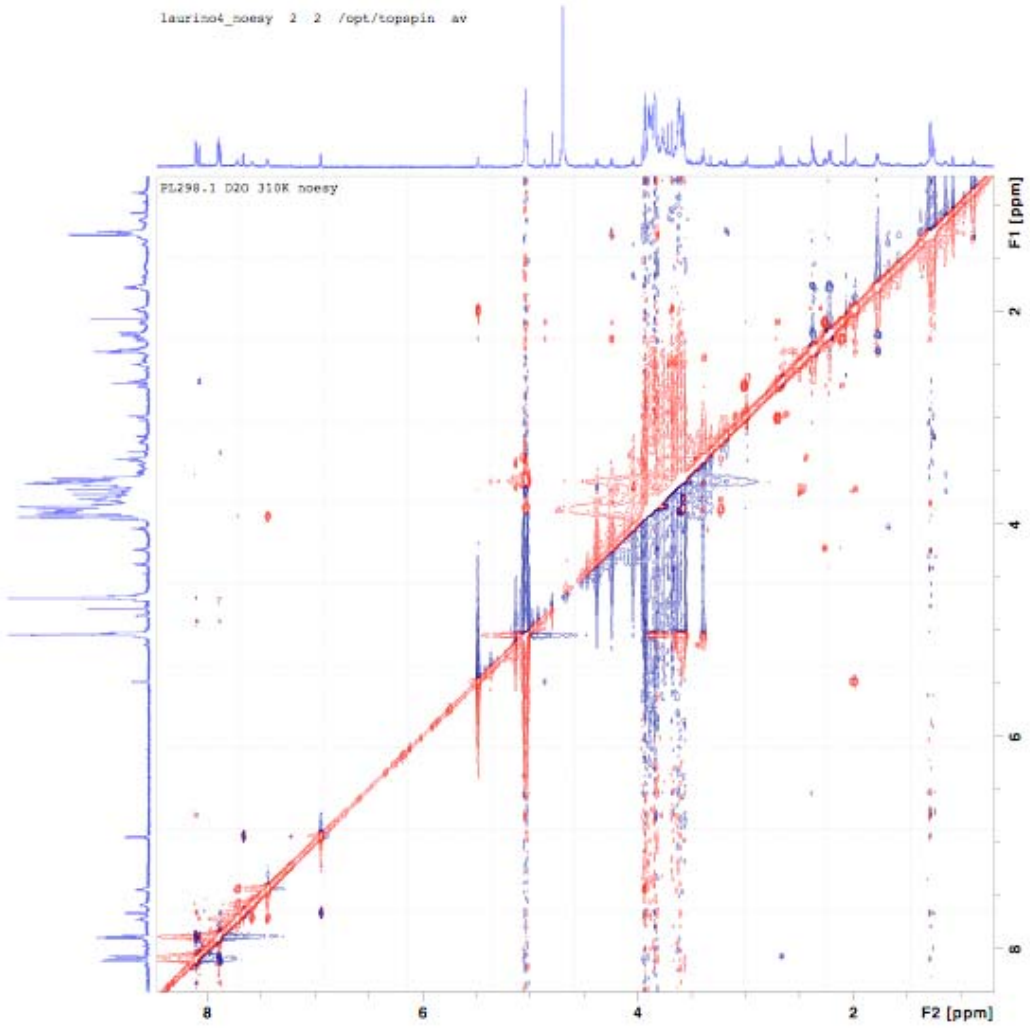

Figure 31. ^{1}H-NOESY NMR of **26**.