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Supplementary information

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Supplementary Information

Structure and mechanism of the ER-based glucosyltransferase ALG6

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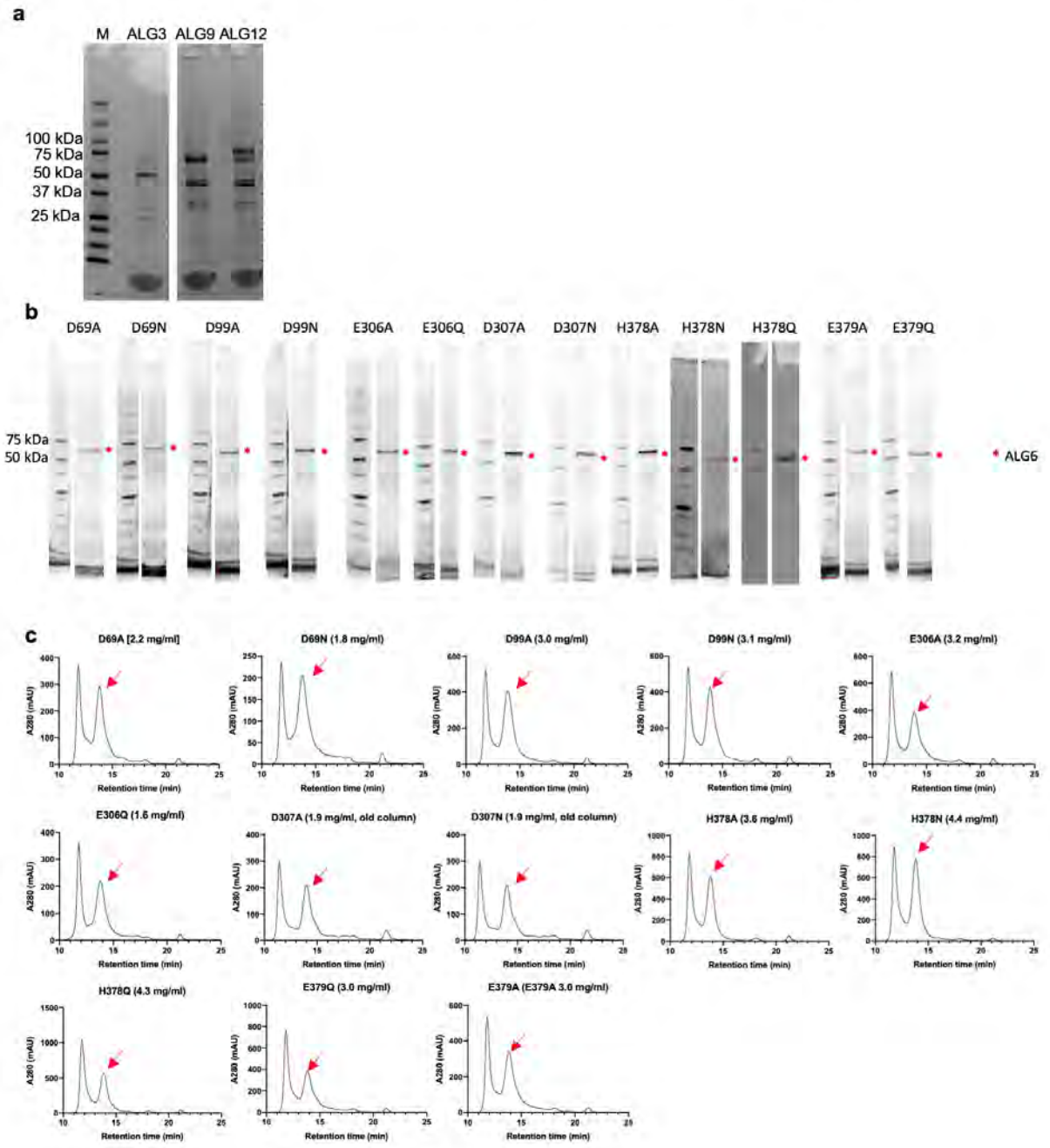
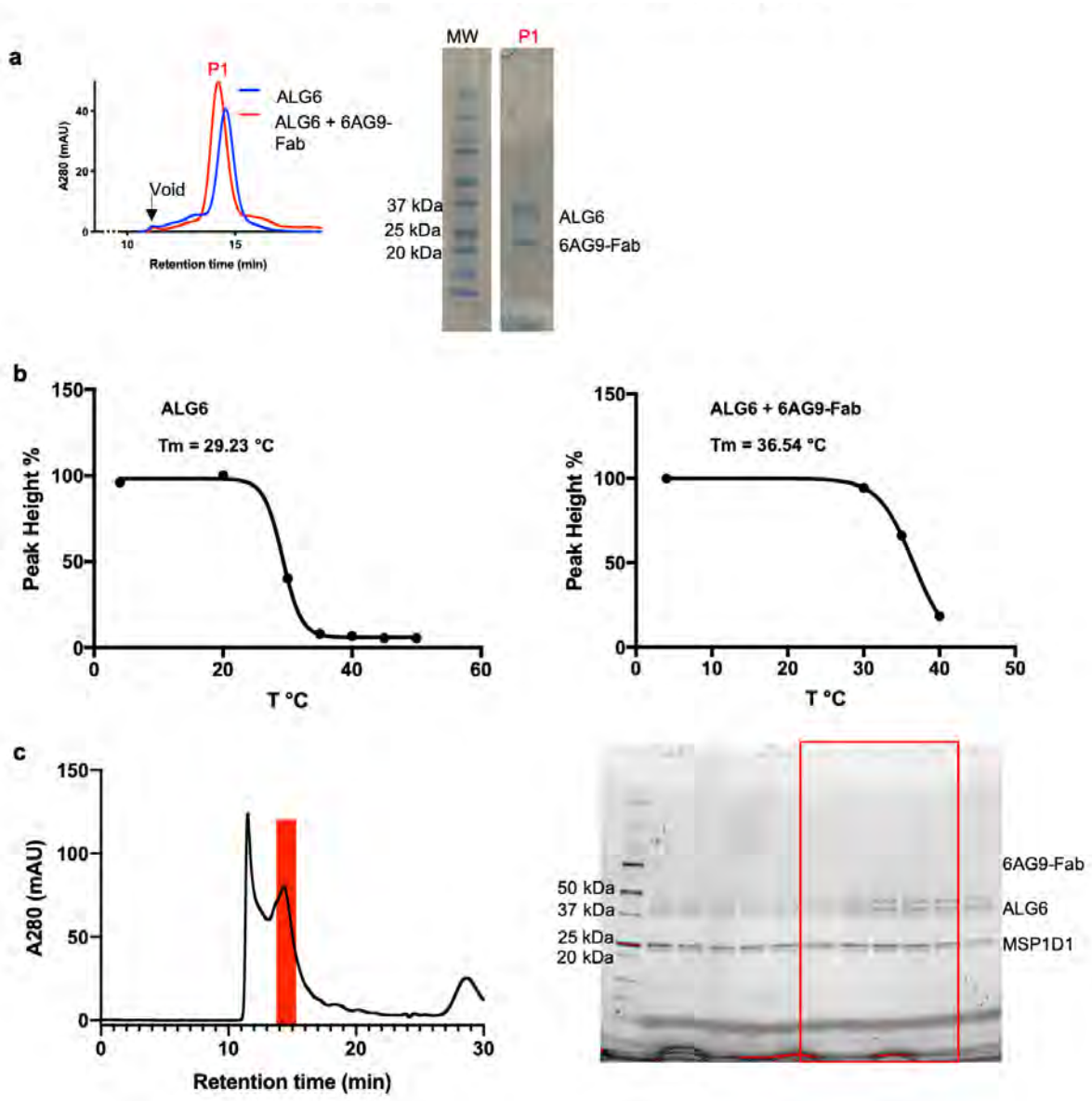
Supplementary information figures:

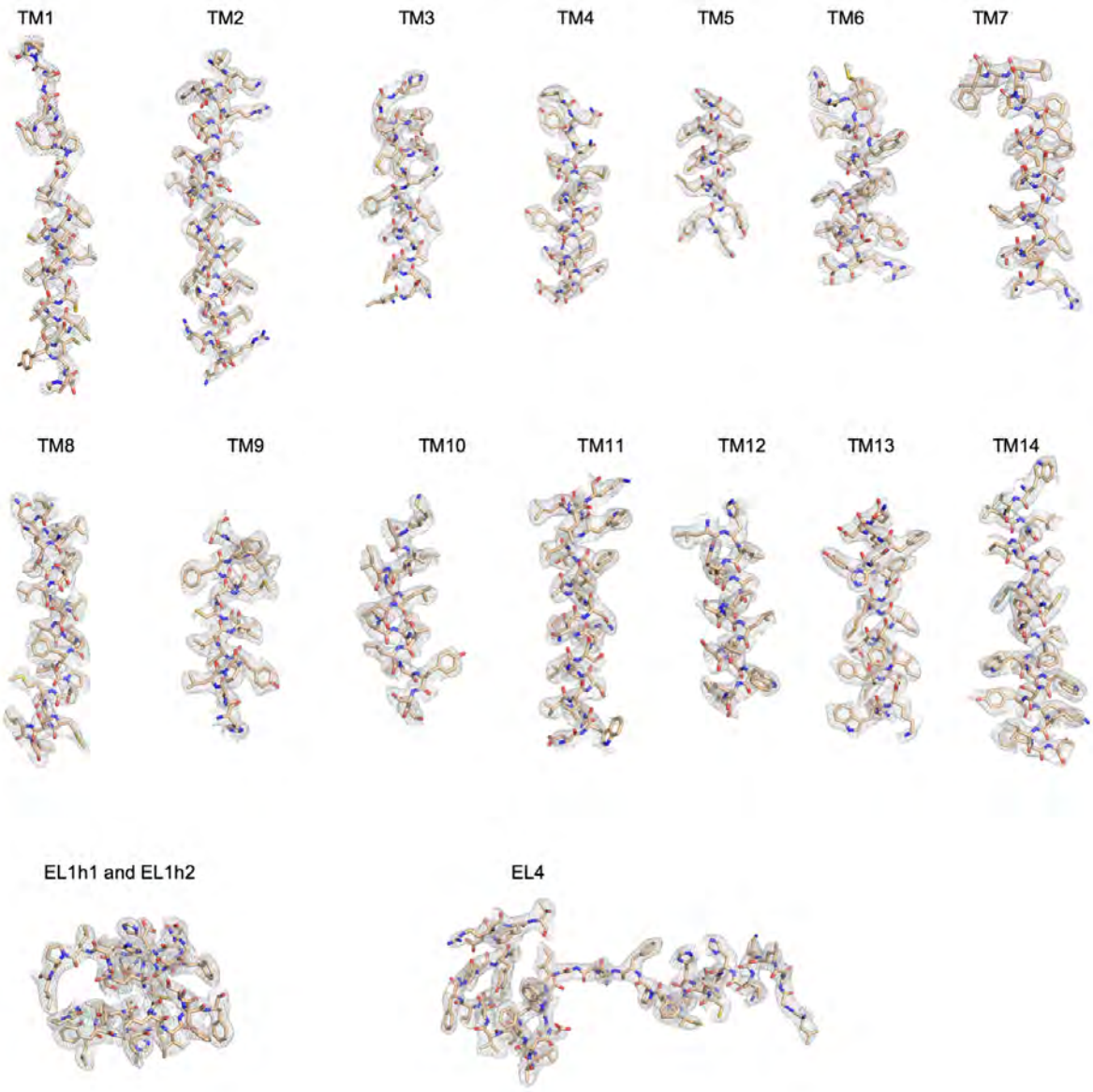
Supplementary information methods:



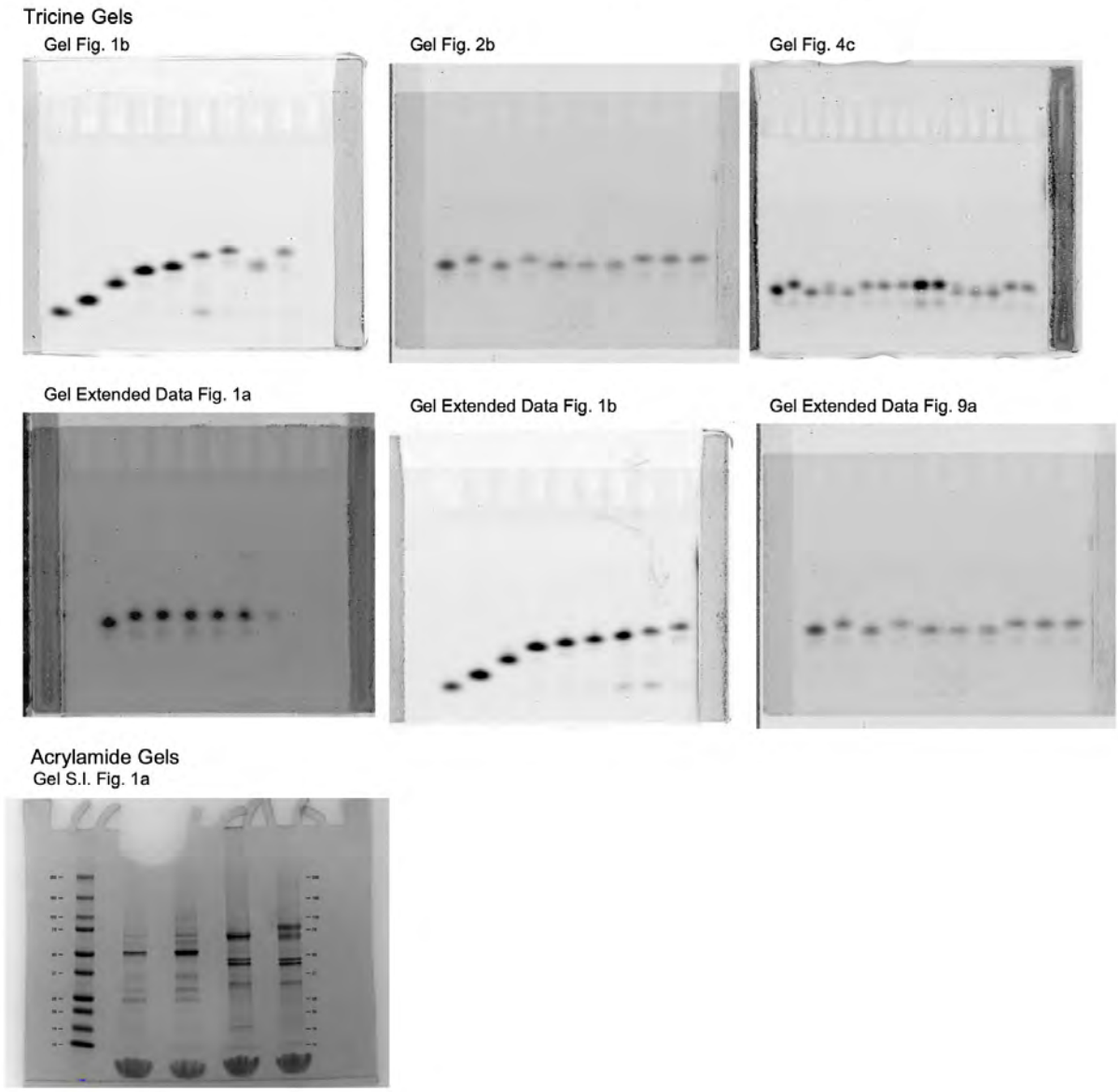
SI Figure 1 | Purification of ALG3, ALG9, ALG12 and ALG6 variants. a, SDS-PAGE analysis of purified enzymes ALG3, ALG9 and ALG12. **b,** SDS-PAGE analysis of purified ALG6 variants. The red asterisks indicate ALG6 **c,** Size exclusion chromatography analysis (TSKgel G3000WXL, 0.5 ml/min) of purified ALG6 variants in detergent. The red arrows indicate the peak fractions that were used for functional assays. Note that ALG6 variants D307A and D307N were purified with an different column, leading to a slightly shifted elution peak.



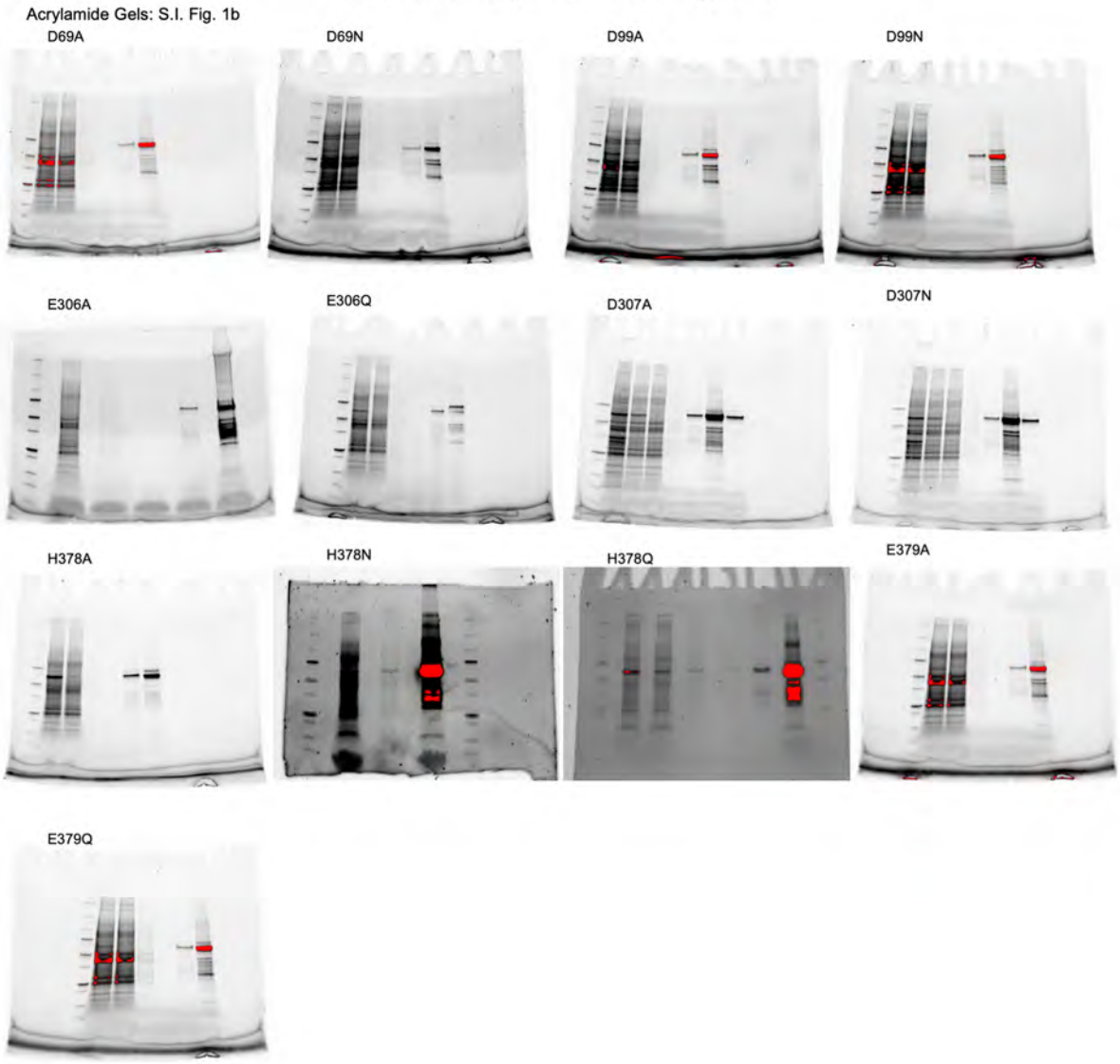
SI Figure 2 | Characterization on of 6AG9-Fab binding interactions with ALG6 and sample preparation for cryo-EM analysis. a, SEC profile of ALG6 in the presence and absence of 6AG9-Fab, as shown in Fig. 2a in the main manuscript (left) and reducing SDS-PAGE analysis of SEC elution peak of the ALG6-6AG9-Fab complex (right). **b,** Thermostability analysis[1] of wild type ALG6 in detergent solution and in the presence or absence of 6AG9-Fab, performed by comparing the heights of SEC elution peaks after heating samples for 10 min at the respective temperatures prior to SEC analysis. **c,** SEC profile (TSKgel G3000WXL, 0.5 ml/min) of ALG6 reconstituted in lipid nanodiscs and in complex with 6AG9-Fab (left) and non-reducing SDS-PAGE analysis of corresponding peak fractions (right). The red box indicates the fractions that were pooled and used for cryo-EM analysis.



SI Figure 3 | EM density for different segments of the structure of substrate-free ALG6-6AG9-Fab complex in lipid nanodiscs at 3.0 Å resolution. ALG6 is depicted in stick representation. Maps are displayed at a contour level of 7.0 rmsd and were carved at 1.6.



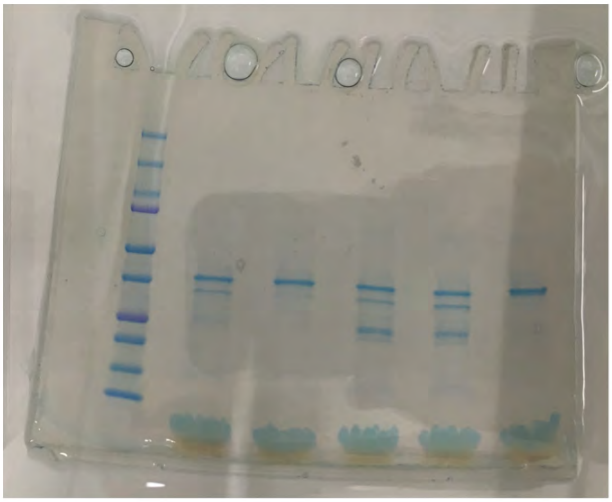
SI Figure 4 | Raw and uncropped gels 1.



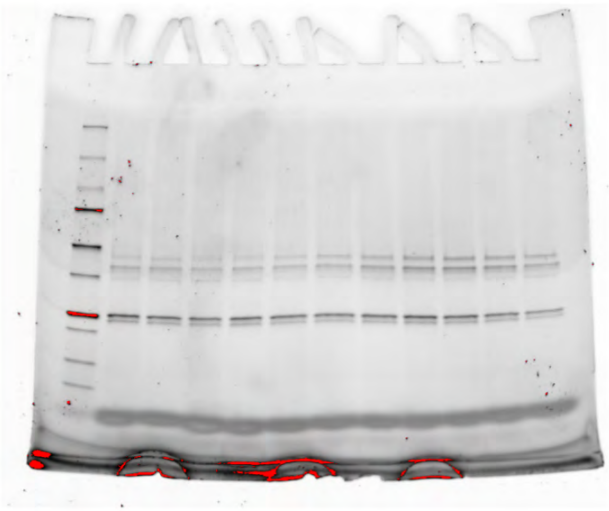
SI Figure 5 | Raw and uncropped gels 2.

Acrylamide Gels

SI Fig. 2a



SI Fig. 2c

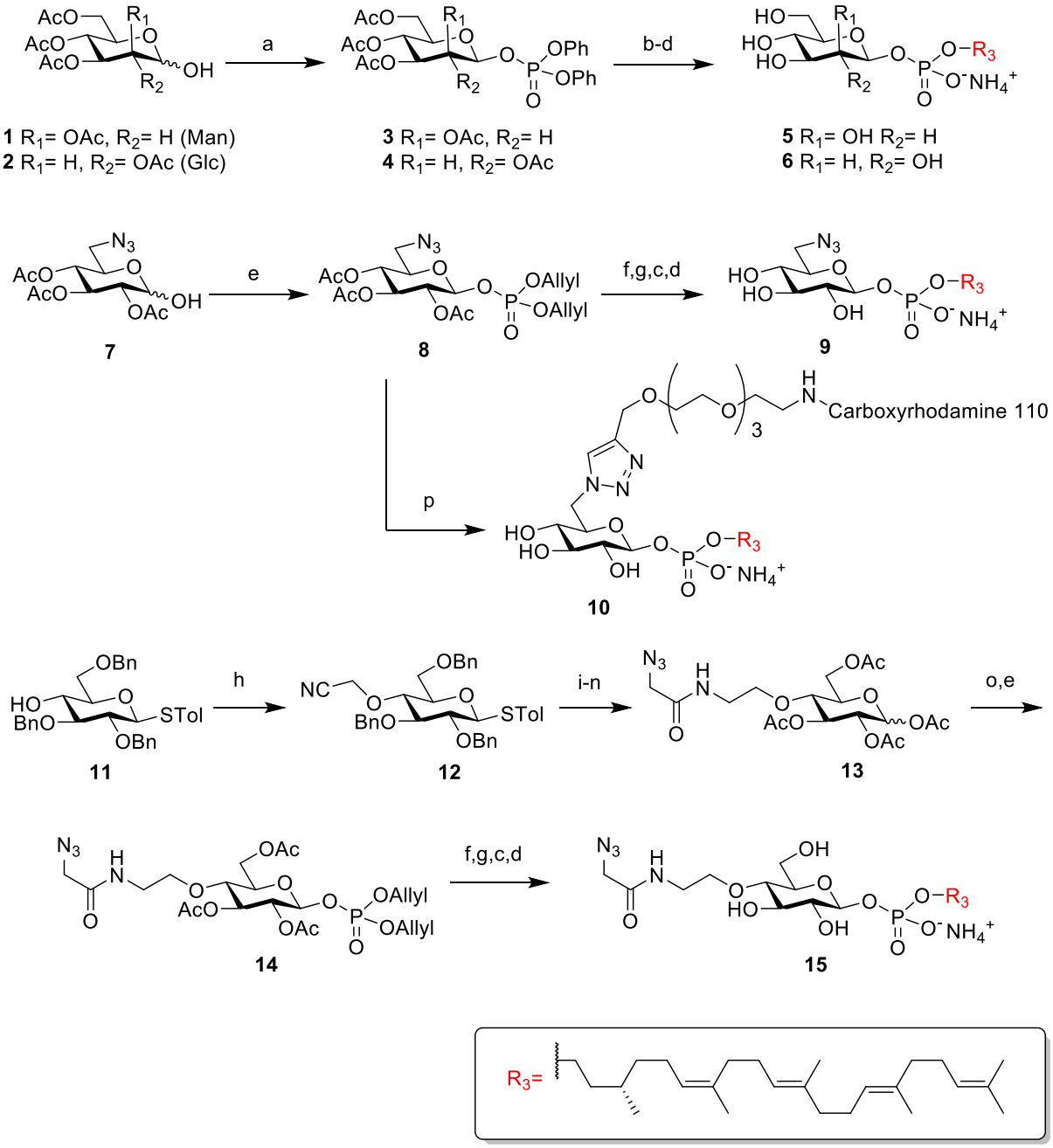


SI Figure 6 | Raw and uncropped gels 3.

Supplementary information methods: Substrate analog synthesis

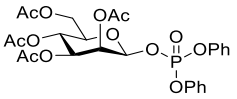
General procedures. All reagents were purchased from commercial sources and used without further purifications unless otherwise stated. All reactions were carried out in flame-dried round-bottomed-flask under an argon atmosphere, except if specified. Room temperature (rt) refers to ambient temperature. Temperatures of 0 °C were maintained using an ice-water, -78°C with acetone/dry ice bath and the other temperatures using a cryostat. Dry solvents were obtained by passing commercially available pre-dried, oxygen-free solvents through activated alumina columns. Hydrogenation was performed at room pressure using H_2 filled balloon. Chromatographic purifications were performed with silica gel high purity grade, pore size 60 Å, 230-400 mesh particle size (sigma-aldrich). Thin layer chromatography (TLC) was performed using ALUGRAM Xtra Sil G/UV on pre-coated aluminium sheets, using UV light as a visualizing, and basic aqueous potassium permanganate solution and ceric ammonium molybdate (CAM) as developing agents. When applicable, reactions were followed by analytical reverse-phase ultra-high performance liquid chromatography (RP-UPLC) on a Dionex Ultimate 3000 RSLC System (DAD-3000 RS Photodiode Array Detector) and a Dionex Acclaim RSLC 120 column (C18, 3.0 x 50 mm, particle size 2.2 µm, 120 Å pore size) at a flow rate of 1.2 mL min^{-1}. Compounds were detected by UV absorption at 214 nm. Data recording and processing was done with Dionex Chromeleon Management System Version 6.8, and X-calibur (version 2.2, Thermo Scientific). Eluents for analytical RP-UPLC were as follow: A: milliQ-deionized water with 0.05 % TFA and D: HPLC-grade MeCN/milliQ-deionized water (9:1) with 0.05 % TFA. Conditions for analytical RP-UPLC were as follow: in 4.5 min from 100 % A to 100 % D, then staying at 100 % D or in 7.5 min from 100 % A to 100 % D, then staying at 100 % D. NMR spectra for ^{1}H, ^{13}C, DEPT, ^{31}P, COSY, HSQC, HMBC and NOE were recorded at room temperature with a Bruker AV (400 MHz ^{1}H). Spectra were and processed using TopSpin 3.6.1 software. Chemical shifts are reported in δ (ppm) relative units to residual solvent peaks $CDCl_3$ (7.26 ppm for ^{1}H and 77.2 ppm for ^{13}C) and MeOD (3.31 ppm for ^{1}H and 49.00 ppm for ^{13}C). Splitting patterns are assigned as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), multiplet (m), dd (doublet of doublets), and td (triplet of doublets). High resolution mass spectra (HRMS) and matric-assisted laser desorption/ionization time-of-flight mass spectra (MALDI-TOF-MS) were provided by the "Service of Mass Spectrometry" at the Department of Chemistry and Biochemistry in Bern and were obtained by electron spray ionization (ESI) in positive or negative mode recorded on a Thermo Scientific LTQ OrbitrapXL.

Abbreviations. EtOAc = ethyl acetate, THF = tetrahydrofuran, $Pd(PPh_3)_4$ = Tetrakis(triphenylphosphine)palladium, DMF = N,N-dimethylformamide. DMAP = dimethylaminopyridine, TFA = trifluoroacetic acid



Scheme S1 Synthesis of Man-ß-P-farnesylcitronellyl **5**, Glc-ß-P-farnesylcitronellyl **6**, 6-Azido-ß-Glc-P-farnesylcitronellyl **9,** 6-triazole-peg-Rhodamine110-6-deoxyl-Glc-ß-P-farnesylcitronellyl **10,** 4-O-(2-azidoacetylaminoethyl)-Glc-ß-P-farnesylcitronellyl **15.** 6-triazole-peg-Rhodamine110-6-deoxyl-Glc-ß-P-farnesylcitronellyl Reaction conditions: (a) $ClPO(OPh)_2$, DMAP; (b) H_2, PtO_2; (c) R_3-OH, CCl_3CN; (d) MeOH NH_4OH; (e) $ClPO(OAllyl)_2$, DMAP; (f) $Pd(PPh_3)_4$, Et_2NH; (g) Dowex®50WX8; (h) bromoacetonitrile, NaH; (i) BH_3•SMe_2, THF; (j) chloroacetic anhydride, DIPEA; (k) NBS, acetone/water; (l) H_2, Pd/C; (m) Ac_2O, pyr; (n) NaN_3, DMF; (o) N_2H_4•AcOH, (p) Fluor 488-Alkyne, $CuSO_4$, sodium ascorbate, TBTA.

Diphenyl(2,3,4-6-tetra-O-acetyl-β-D-manno-pyranosyl)phosphate **3**[2].



2,3,4-6-tetra-O-acetyl- α/β -D-mannopyranose (700 mg, 2.01 mmol, 1 eq) and DMAP (1.23 g, 10.05 mmol, 5 eq) were dissolved in 70 ml DCM. Diphenyl phosphorylchloride (1.25 ml, 6.03 mmol, 3 eq) was dissolved in 70 ml DCM and added dropwise overnight *via* syringe pump (injection rate of 1.6 ml per hour in a 20-ml syringe) at rt. When the addition was finished, the reaction mixture was quenched by addition of saturated $NaHCO_3$ and the aqueous phase was extracted 3 times with DCM, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel basified with Et_3N (EtOAc/hexane 3:8 to 4:6, R_f = 0.26 in EtOAc/hexane 1:1) to yield **3** (641 mg, 1.10 mmol, 55 %) as a colorless waxy solid.

^{1}H NMR (400 MHz, CDCl3) δ = 7.35-7.15 (m, 10 H, Ar-H), 5.60 (dd, *J* = 7.2 Hz, 1.6 Hz, 1H, H-1), 5.48 (d, *J* = 1.2 Hz, 1H, H-2), 5.25 (t, *J* = 10.0 Hz, 1H, H-4), 5.07 (dd, *J* = 9.6 Hz, 3.2 Hz, 1H, H-3), 4.27 (dd, *J* = 12.4 Hz, 5.6 Hz, 1H, H-6a), 4.10-4.13 (m, 1H, H-6b), 3.78 (ddd, *J* = 9.2 Hz, 5.6 Hz, 3.2 Hz, 1H, H-5), 2.09, 2.04, 2.04, 1.98 (4x s, 4x 3H, 4x OAc).

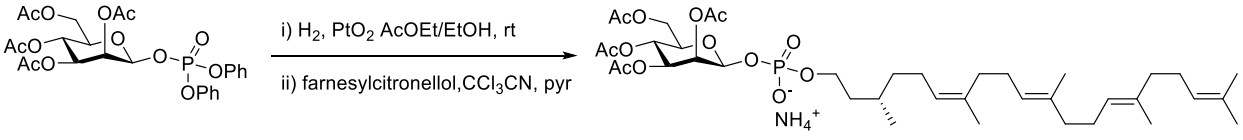
^{13}C NMR (101 MHz, CDCl3) δ = 170.6, 169.9, 169.8, 169.6 (4xs, 4×OC=O), 150.5, 150.1 (2x d, 2x *J* = 7.6 Hz, 2x Ar-C), 130.0, 129.8, (2x d, 2x *J* = 9.1 Hz, 2x ArC), 126.0, 125.8, (2x d, 2x *J* = 1.2 Hz, 2x Ar-C), 120.5, 120.3 (2x d, 2x *J* = 4.0 Hz, 2x Ar-C), 94.9 (d, *J* = 4.5 Hz, C-1), 73.3 (s, C-5), 70.3 (s, C-3), 68.2 (d, *J* = 9.1 Hz, C-2), 65.5 (s, C-4), 62.2 (s, C-6), 20.8, 20.8, 20.7, 20.6 (4xs, 4xOC=OCH3).

^{31}P NMR (122 MHz, CDCl3) δ = -13.9.

ESI-MS (+) *m/z* calc 581.14 for $C_{26}H_{30}O_{13}P^+$ (M+[H^+]), found 581.14.

Data consistent with that previously reported.

Farnesylcitronellylphosphoryl-2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside **S1**



To a solution of **3** (113 mg, 0.20 mmol, 1 eq) in 3 mL of a mixture of EtOH/EtOAc 1:1, PtO_2 (44 mg, 0.20 mmol, 1 eq) was added and the suspension stirred under a H_2 atmosphere for 4 h. The reaction mixture was filtered over a pad of celite and concentrated to dryness to yield a waxy solid which was used without further purification. The resulting intermediate phosphoric acid (83 mg, 0.19 mmol, 1 eq) and farnesylcitronellol[3] (280 mg, 0.78 mmol, 4.00 eq) were dissolved in 4 ml pyridine and trichloroacetonitrile (0.19 ml, 1.94 mmol, 10.00 eq) was added at rt under an Ar atmosphere. The reaction mixture was warmed up to 65 °C and stirred overnight. The reaction mixture was concentrated to dryness and purified by flash column chromatography (EtOAc/MeOH 9:1 to 8:2 + 0.5% NH_4OH, R_f = 0.20 in EtOAc/MeOH 8:2) to yield **S1** (111 mg, 0.14 mmol, 73 %) as a colorless waxy solid.

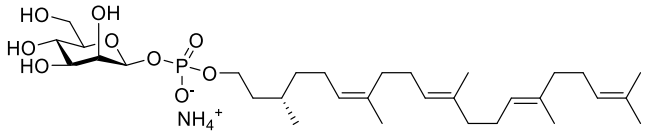
^{1}H NMR (400 MHz, MeOD) δ = 5.48 (d, *J* = 1.2 Hz, 1H, H-2), 5.38 (d, *J* = 8.8 Hz, 1H, H-1), 5.22-5.20 (m, 2H, H-3, H-4), 5.15-5.09 (m, 4H, H-6', H-10', H-14', H-18'), 4.27 (dd, *J* = 12.0 Hz, 4.4 Hz, 1H, H-6a), 4.15 (dd, *J* = 12.4 Hz, 2.4 Hz, 1H, H-6b), 3.93-3.84 (m, 3H, H-5, H-1'), 2.16 (s, 3H, OAc), 1.97-2.10 (m, 20H, 2x OAc, H-5', H8', H-9', H-12', H-13', H-16', H-17'), 1.95 (s, 3H, OAc), 1.68-1.67 (m, 7H, H-2'a, H-21', H22'), 1.62 (s, 3H, H-24'), 1.60 (m, 7H, H-3', H-23', H-25'), 1.46 -1.29 (m, 2H, H-2'b, H-4'a), 1.19-1.15 (m, 1H, H-4'b), 0.92 (d, *J* = 6.8 Hz, 3H, H-20').

^{13}C NMR (101 MHz, MeOD) δ = 172.3, 171.9, 171.5, 171.2 (4x s, 4xOC=O), 136.0 (s, C-7'), 135.9 (s, C-11'), 135.9 (s, C-15'), 132.0 (s, C-19'), 126.7 (s, C-18'), 125.5 (s, C-14'), 125.5 (s, C-10'), 125.5 (s, C-6'), 94.9 (d, *J* = 4.1 Hz, C-1), 73.7 (s, C-5), 72.6 (s, C-3), 71.1 (d, *J* = 7.1 Hz, C-2), 67.0 (s, C-4), 65.4 (d, *J* = 5.9 Hz, C-1'), 63.4 (s, C-6), 40.9 (s, C-8'), 40.8 (s, C-12'), 38.9 (s, C-2'), 38.8 (s, C-9'), 38.7 (s, C-4'), 32.9 (s, C-13'), 30.5 (s, C-3'), 27.8 (s, C-16'), 27.6 (s, C-5'), 27.6 (s, C-17'), 26.4 (s, C-21'), 23.7 (s, C-22'), 20.7, 20.7, 20.6, 20.5 (4x s, 4x OC=OCH3), 19.9 (s, C-20'), 17.8 (s, C23'), 16.2 (s, C-24'), 16.1 (s, C-25').

^{31}P NMR (122 MHz, CDCl3) δ = -2.2.

ESI-HRMS (-) *m/z* calc 769.3934 for $C_{39}H_{62}O_{13}P^-$, found 769.3964.

Farnesylcitronellylphosphoryl-β-mannopyranoside **5**



To a solution of **S1** (90 mg, 0.11 mmol) in 2 mL of methanol, an excess of NH_4OH (200 eq per acetyl) was added. The mixture was stirred for 16 h at rt and then dried *in vacuo* to afford **5** (70.50 mg, 0.11 mmol, quant.) as colorless waxy solid. (R_f = 0.50 in EtOAc/MeOH 4:6).

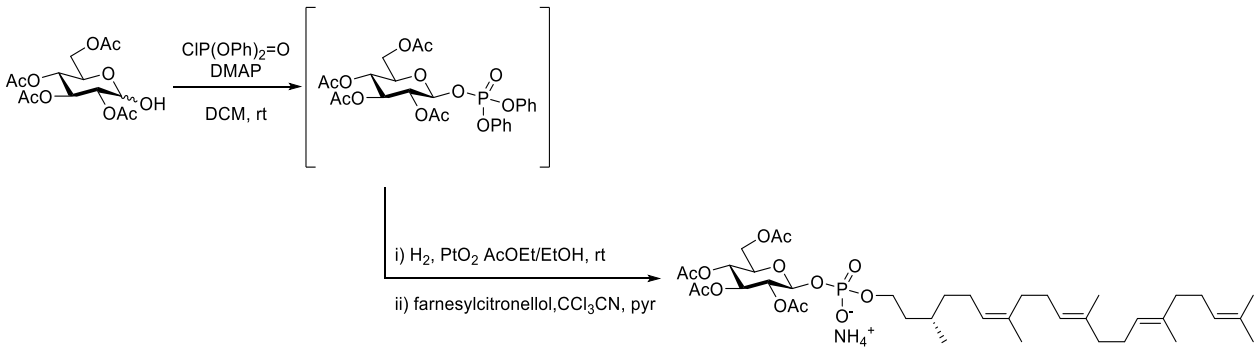
1H NMR (400 MHz, MeOD) δ = 5.15-5.07 (m, 5H, H-1, H-6', H-10', H-14', H-18'), 3.98-3.93 (m, 2H, H-1'), 3.91-3.86 (m, 2H, H-2, H-6a), 3.72 (dd, *J* = 11.6 Hz, 6.0 Hz, 1H, H-6b), 3.56-3.53 (m, 1H, H-4), 3.51-3.48 (m, 1H, H-3), 3.29-3.27 (m, 1H, H-5), 2.12-1.94 (m, 14H, H-5', H-8', H-9', H-12', H-13', H16', H-17'), 1.71-1.67 (m, 7H, H-2'a, H-21', H-22'), 1.62-1.60 (m, 10H, H-3', H-23', H-24', H-25'), 1.48-1.33 (m, 2H, H-2'b, H-4'a), 1.22-1.14 (m, 1H, H-4'b), 0.93 (d, *J* = 6.8 Hz, 3H, H20').

13C NMR (101 MHz, MeOD) δ = 136.0 (s, C-7'), 135.9 (s, C-11'), 135.9 (s, C-15'), 132.0 (s, C-19'), 126.6 (s, C-18'), 125.5 (s, C-14'), 125.4 (s, C-10'), 125.44 (s, C-6'), 97.1 (d, *J* = 4.9 Hz, C-1), 78.8 (s, C-5), 74.9 (s, C-3), 72.9 (d, *J* = 7.1 Hz, C-2), 68.1 (s, C-4), 65.4 (d, *J* = 5.8 Hz, C-1'), 62.7 (s, C-6), 40.9 (s, C-8'), 40.8 (s, C-12'), 39.0 (s, C-2'), 38.9 (s, C-9'), 38.7 (s, C4'), 32.9 (s, C-13'), 30.5 (s, C-3'), 27.8 (s, C-16'), 27.6 (s, C-5'), 27.6 (s, C-17'), 25.9 (s, C21'), 23.7 (s, C-22'), 19.8 (s, C-20'), 17.8 (s, C-23'), 16.1 (s, C-24'), 16.1 (s, C-25').

31P NMR (122 MHz, CDCl3) δ = -1.4.

ESI-HRMS (-) *m/z* calc 601.3511 for $C_{31}H_{54}O_9P^-$. found 601.3547.

Farnesylcitronellylphosphoryl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside **S2**



To a solution of *2,3,4,6-tetra-O-acetyl-α/β-glucopyranose* (250 mg, 0.72 mmol) in 25 mL of dry DCM, DMAP (875mg, 7.2 mmol, 10 eq) was added and the solution stirred for 15 min. Diphenylchlorophosphate (1.11 ml, 504mmol, 7.5eq) was dissolved in 22 mL of DCM and added *via* syringe pump over 16h (1.4 mL/h). After the addition was complete the reaction mixture was quenched with $NaHCO_3$, diluted with 50mL of DCM, washed 3 times with

$NaHCO_3$, dried over Na_2SO_4, filtered. and evaporated *in vacuo* to give a mixture of β/α anomers in 10:1 ratio. Purification by flash chromatography (hexanes/ethyl acetate 3:2 to 1:1) afforded Diphenyl(2,3,4,6-tetra-O-acetyl -β-D-gluco-pyranosyl)phosphate **4** as a colorless oil (200mg, 51%). *Note that this purification should be carried out in short time in order to minimize anomerization and decomposition on silica; this unstable intermediate was not completely characterized*. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37-7.18 (m, 10H, Ar-H), 5.47-5.42 (m, 1H, H-1), 5.19-5.12 (m, 3H), 4.22 (dd, J^1 = 12.46 Hz, J^2 = 4.46 Hz, 1H, H-6'), 4.02 (dd, J^1 = 12.46 Hz, J^2 = 2.26 Hz, 1H, H-6), 3.83-3.78 (m, 1H- H-5), 2.05, 2.02, 1.98 (3x s, 3H, O=CC$\underline{H_3}$). ^{31}P NMR (122 MHz, $CDCl_3$) δ = -13.8. The diphenyl phosphate intermediate was immediately dissolved in 10mL of EtOAc/EtOH 1:1 and stirred under hydrogen atmosphere in presence of PtO_2 (77mg, 0.33 mmol, 1 eq) for 3h. The mixture was filtered over celite and concentrated *in vacuo*. A portion of the resulting phosphoric acid (80 mg, 0.19 mmol) was dissolved in 6 mL of dry pyridine together with farnesylcitronellol (136 mg, 0.38 mmol, 2 eq) and CCl_3CN (190 µL 1.9mmol, 10 eq) and the mixture heated to 65°C. After 16h, the mixture was concentrated to dryness *in vacuo* and purified by flash column chromatography (EtOAc/MeOH 4:1 + 0.5% NH_4OH of a 25% solution) to afford **S2** (69 mg, 88 µmol 33%) as a colorless waxy solid.

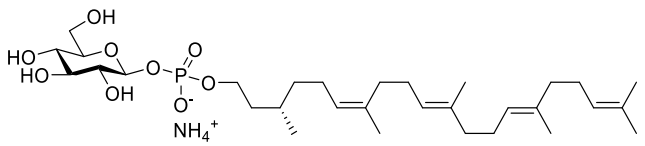
^{1}H NMR (400 MHz, MeOD) δ = 5.29-5.24 (m, 1H, H-3), 5.22-5.18 (m, 1H, H-1), 5.15-5.03 (m, 5H, H-4), 4.97-4.92 (m, 1H)4.29 (dd, J^1 = 12.43 Hz J^2 = 4.14 Hz, 1H, H-6'), 4.15 (dd, J^1 = 12.43 Hz J^2 = 2.07 Hz, 1H, H-6), 3.92-3.89 (m, 3H), 2.10-1.96 (m, 26H), 1.68-1.60 (m, 16H), 1.45-1.30 (m, 4H), 1.19-1.14 (m, 1H), 0.91 (d, J^1 = 6.81 Hz, 3H).

^{13}C NMR (101 MHz, MeOD) δ = 172.2, 171.5, 171.2, 136.0, 135.9, 135.9, 132.0, 126.6, 125.5, 125.5, 125.5, 96.9 (d, J = 4.98 Hz), 74.4, 73.4 (d, J = 8.69 Hz), 73.2, 69.6, 65.2 (d, J = 5.75 Hz), 63.0, 40.8, 40.8, 38.9, 38.7, 38.6, 32.9, 30.5, 27.8, 27.6, 27.6, 26.4, 25.9, 23.7, 20.8, 20.7, 20.6, 20.5, 19.9, 17.8, 16.2, 16.1.

^{31}P NMR (122 MHz, $CDCl_3$) δ = -2.76.

ESI-HRMS (-): *m/z* calc for $C_{39}H_{62}O_{13}P^-$ 769.3934 found 769.3942.

Farnesylcitronellylphosphoryl-β-glucopyranoside **6**



To a solution of **S2** (25 mg, 30.6 µmol) in 1 mL of methanol, an excess of NH_4OH (200 eq per acetyl) was added. The mixture is stirred for 16 h at rt and then dried *in vacuo* to afford **6** (19 mg, 30.6 µmol quant.) as colorless waxy solid. (Rf = 0.50, EtOAc/MeOH 4:6).

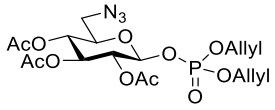
^{1}H NMR (400 MHz, MeOD) δ = 5.15-5.07 (m, 4H), 4.84-4.83 (m, 1H), 4.05-3.94 (m, 2H), 3.89-3.86 (m, 1H), 3.67-3.62 (m, 1H), 3.41-3.33 (m, 2H), 3.27-3.22 (m, 2H), 2.10-1.94 (m, 14H), 1.68-1.62 (m, 16H), 1.47-1.29 (m, 4H), 1.21-1.12 (m, 1H), 0.92 (d, *J* = 6.59 Hz, 3H).

^{13}C NMR (101 MHz, MeOD) δ = 134.6, 134.5, 130.6, 125.2, 124.1, 124.1, 98.0 (d, *J* = 5.55 Hz), 77.2, 76.3, 74.4 (d, *J* = 8.05 Hz), 70.2, 64.0 (d, *J* = 5.78 Hz), 61.6, 39.5, 39.5, 37.5, 37.4, 37.3, 31.5, 29.1, 26.4, 26.2, 26.2, 25.00, 24.5, 22.3, 20.7, 18.4, 26.4, 14.7, 14.7.

^{31}P NMR (122 MHz, $CDCl_3$) δ = -1.45.

ESI-HRMS (-): *m/z* calc for $C_{31}H_{54}O_9P^-$ 601.3511, found 601.3523.

Diallyl(2,3,4-tri-O-acetyl-6-azido-6-deoxy-β-D-gluco-pyranosyl)phosphate **8**[4]



Hydrazine acetate (270 mg, 2.929 mmol, 1.1eq) was added to a solution of 1,*2,3,4-tetra-O-acetyl-6-azido-6-deoxy-α/β-D-Glucopyranose*[5] (994 mg, 2.663 mmol, 1eq) in 7.6 mL of dry DMF. The mixture was stirred at 55°C and monitored by TLC (hex/EtOAc : 7/3), after 1.5h the mixture was diluted in EtOAc and washed with $NaHCO_3$, dried over Na_2SO_4 filtered and evaporated. The resulting hemiacetal **7** was used without further purification. DMAP (774 mg, 6.34 mmol, 10 eq) was added to a solution of the hemiacetal (210 mg 0.63 mmol, 1eq) in 21 mL of dry DCM and stirred for 15 min. A solution of diallyl phosphoryl chloride (928 mg, 4.72 mmol, 7.5 eq) in 16 mL of DCM was added to the reaction mixture *via* syringe pump at 1.6 mL/min. After the addition was completed the mixture was stirred 15min, then diluted in DCM and washed with $NaHCO_3$, the organic phase was evaporated and purified with a short flash column chromatography (hex/EtOAc : 2/8) yielding **8** as single anomer (201 mg 40.9 mmol, 77%).

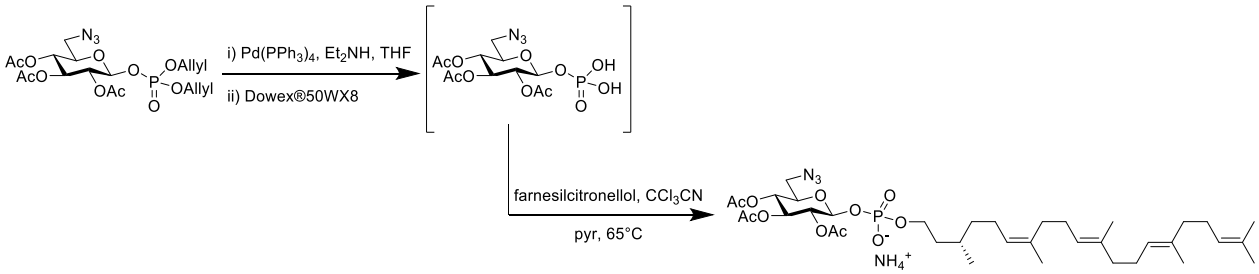
^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.96-5.84 (m, 2H, CH_2=C<u>H</u>-CH_2-), 5.38-5.32 (m, 3H, H-1, CH=C<u>H^a</u>$_2$), 5.26-5.18 (m, 3H, H-3, CH=C<u>H^b</u>$_2$), 5.11-5.05 (m, 2H, H-4, H-2), 4.59-4.48 (m, 4H, -OC<u>H$_2$</u>CH=CH_2), 3.80- 3.75 (m, 1H, H-5), 3.41 (dd, J^1 = 13.57 Hz, J^2 = 2.80 Hz, H-6), 3.32 (dd, J^1 = 13.57 Hz, J^2 = 5.61 Hz, H-6'), 2.03 (s, 3H, O=CC<u>H$_3$</u>), 2.02 (s, 3H, O=CC<u>H$_3$</u>), 1.99 (s, 3H, O=CC<u>H$_3$</u>).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.1, 169.5, 169.3 (3xs, O=<u>C</u>CH_3), 132.0 (m, CH_2=<u>C</u>H-CH_2), 118.7 (d, *J* =7.22 Hz), 96.3 (d, *J* =4.42 Hz, C-1), 73.8, 72.4, 71.3, 71.2, 68.8 (d, *J* = 3.5 Hz <u>*C*</u>H_2*C*H=CH_2), 68.7 (d, *J* = 3.5 Hz <u>*C*</u>H_2*C*H=CH_2), 50.9, 20.7, 20.7, 20.6 (3xs, O=C<u>C</u>H_3).

^{31}P NMR (122 MHz, $CDCl_3$) δ = 3.17.

ESI-HRMS (+): *m/z* calc 491.1305 for $C_{18}H_{26}N_3O_{11}P$ found 514.1187 $[M+Na]^+$.

<u>Farnesylcitronellylphosphoryl-6-azido-6-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranoside **S3**</u>



To a solution of **8** (90.4 mg, 0.184 mmol, 1 eq) and $Pd(PPh_3)_4$ (10 mg, 92 µmol, 0.05 eq) in 5 mL of dry THF, diethylamine (94 µL, 0.92 mmol, 5 eq) was added and the mixture was stirred for 2.5 h. The mixture was dried under reduced pressure, loaded on an ion exchange resin (Dowex 50WX8, ammonium form) and eluted with MeOH, the organic phase was evaporated to dryness. To a solution of the resulting phosphoric acid (20.15 mg 490 µmol) and farnesylcitronellol (56 mg, 1.47 mmol, 3 eq) in 1 mL of pyridine, CCl_3CN (49 µL, 0.49mmol, 10 eq) was added and heated at 65°C overnight. The reaction mixture was concentrated to dryness and purified by flash column chromatography (EtOAc/MeOH : 4/1 + 0.5% of NH_4OH solution 25% in water) to yield **S3** (11.16 mg, 1.42 mmol, 32% over two steps) as a colorless oil.

^{1}H NMR (400 MHz, MeOD) δ = 5.27-5.19 (m, 2H, H-2, H-1), 5.15-5.08 (m, 4H), 5.08-5.03 (m, 1H, H-4), 4.97-4.92 (m, 1H, H-2), 3.96-3.90 (m, 2H), 3.87-3.83 (m, 1H, H-5), 3.59 (dd, 1H, J^1 = 13.55 Hz, J^2 = 2.90 Hz, H-6'), 3.38-3.33 (m, 1H, H-6), 2.10-1.96 (m, 21H), 1.68-1.60 (m, 16H), 1.46-1.29 (m, 5H), 1.21-1.14 (m, 1H), 0.92 (d, *J* = 6.23 Hz, 3H).

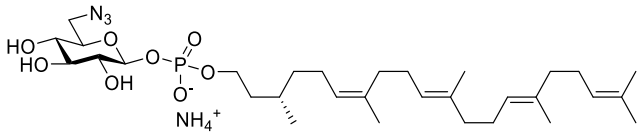
^{13}C NMR (101 MHz, MeOD) δ = 171.6, 171.3, 171.2 (3x s, O=<u>C</u>CH_3), 136.0, 135.9, 135.9, 132.0, 126.6, 125.5, 125.5, 96.9 (d, *J* = 4.86 Hz, 1C, C-1), 74.4 (C-3), 74.0, 73.6 (d, *J* = 8.3 Hz, C-2), 70.5 (C-4). 65.4 (d, *J* = 6.0 Hz), 40.9, 40.8, 39.0, 38.9, 38.7, 32.9, 30.5, 27.8, 27.6, 26.4, 25.9, 23.7, 20.8, 20.6, 20.5, 19.9, 17.8, 16.2, 16.1.

^{31}P NMR (122 MHz, MeOD) δ = -2.83.

ESI-HRMS (-): *m/z* calc 752.3893 for $C_{37}H_{59}O_{11}N_3P^-$ found 752.3889.

note that the β configuration was confirmed by ROESY, see spectrum.

Farnesylcitronellylphosphoryl-6-azido-6-deoxyl-β-glucopyranoside **9**



To a solution of **S3** (2.1 mg, 2.79 µmol) in 0.5 mL of methanol, an excess of NH_4OH (25% solution 200 eq per acetyl) was added. The mixture is stirred for 16 h at rt and then dried *in vacuo* to afford **9** (1.68 mg, 2.6 µmol 96%) as colorless waxy solid. (Rf = 0.58, EtOAc/MeOH 4:6).

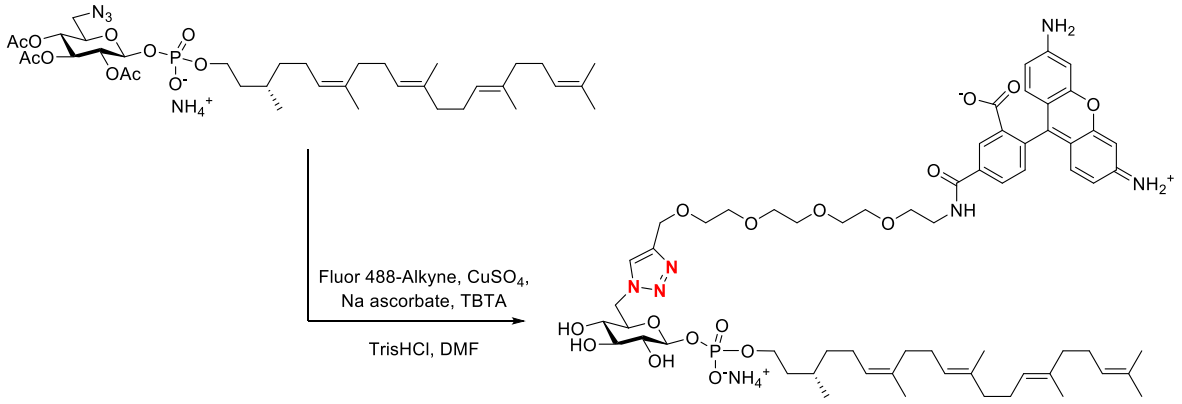
^{1}H NMR (400 MHz, MeOD) δ = 5.03-5.01 (m, 4H), 3.92-3.82 (m, 2H), 3.55-3.51 (m, 1H), 3.33-3.13 (m, 5H), 2.01-1.84 (m, 14H), 1.58-1.57 (m, 7H), 1.52-1.50 (m, 9H), 1.38-1.19 (m, 4H), 1.11-1.04 (m, 1H), 0.83-0.81 (m, 3H).

^{13}C NMR (101 MHz, MeOD) δ = 136.0, 135.9, 135.8, 132.0, 126.7, 125.7, 125.5, 125.5, 99.6 (d, *J* = 5Hz, C1), 77.5, 76.7, 75.7 (d, *J* = 7.37 Hz), 71.8, 65.3 (d, *J* = 5.82 Hz), 52.6 40.9, 40.8 38.9, 38.9, 30.7, 32.9, 30.5, 27.8, 27.6, 27.5, 26.4, 25.9, 23.7, 19.9, 17.8, 16.1, 16.1.

^{31}P NMR (122 MHz, MeOD) δ = -1.17.

ESI-HRMS (-): *m/z* calc 626.3576 for $C_{31}H_{53}N_3O_8P^-$, found 626.3578.

Farnesylcitronellylphosphoryl-6-triazole-peg-rhodamine110-6-deoxyl-β-glucopyranoside **10**



A suspension of Fluor 488-Alkyne (4.3mg, 7.4 µmol, 1.2 eq, sigma-aldrich, cas. 761621) in 500 µL of TrisHCl (100 mM pH 7.5), was added to a solution of **S3** (4.6 mg, 6.2 mmol) in 0.5 mL of DMF. Aqueous solutions of $CuSO_4$ (40 µL, 100mM, 0.6 eq), sodium

ascorbate (45 µL, 100mM 0.75 eq) and TBTA (0.7 mg, 1.4 µmol, 0.22 eq) were sequentially added. The mixture was stirred 6h at rt and then lyophilized. The mixture was purified by preparative TLC (sigma-aldrich, 20cm x 20cm, 2mm thickness) on a 20x20 plate eluted with EtOAc/MeOH 1:1 + 0.5% NH_4OH. The band containing the desired product was scratched and washed with MeOH to obtain **10** as a pink powder (3.8 mg, 3.1 µmol 51%).

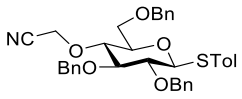
^{1}H NMR (400 MHz, MeOD) δ = 8.55 (s, 1H), 8.17-8.08 (m, 3H), 7.71 (m, 1H), 7.16-7.14 (m, 2H), 6.80-6.79 (m, 3H),5.11-5.08 (m, 3H), 4.84-4.79 (m, 1H, H-6), 4.52-4.46 (m, 1H, H-6'), 4.00-3.84 (m, 3H), 3.68-3.49 (m, 15H, H-5), 3.42-3.37 (m, 1H, H-3), 3.22-3.17 (m, 1H, H-2), 3.09-3.05 (m, 1H, H-4), 2.09-1.89 (m, 16H) 1.66-1.59 (m, 14H), 1.43-1.27 (m, 6H), 1.20-1.09 (m, 1H), 0.88 (d, *J* = 6.5 Hz, 3H).

^{13}C NMR (101 MHz, MeOD) δ = 180.3, 172.5, 170.3, 168.9, 160.9, 159.6, 145.6, 144.4, 136.4, 136.0, 135.9, 134.0, 133.5, 132.1, 131.0, 129.6, 129.5, 126.6, 126.5, 125.5, 125.5, 125.5, 117.5, 115.0, 99.5 (d, *J* = 5.9Hz), 98.4, 77.4, 75.6, 75.6 (d, *J* = 8.4 Hz), 72.1, 71.3, 71.2, 71.2, 71.2, 71.2, 70.9, 70.5, 65.1 (d, *J* = 5.6 Hz), 64.9, 52.1, 41.0, 40.9, 40.8, 39.0, 38.9, 38.7, 32.9, 30.8, 30.5, 27.8, 27.6, 26.4, 25.9, 24.2, 23.7, 19.8, 17.8, 16.1, 16.1.

^{31}P NMR (122 MHz, MeOD) δ = -1.16.

ESI-HRMS (-): *m/z* calc 1213.5843 for $C_{63}H_{86}N_6O_{16}P^-$, found 1213.5849.

p-Methylphenyl-2,3,6-tri-O-benzyl-4-O-(cyanometyl) -1-deoxy-1-thio-β-D-glucopyranoside **12**[6]



To a solution of methylphenyl-2,3,6-tri-O-benzyl-1-deoxy-1-thio-β-D-glucopyranoside **11**[7] (2.79 g, 5.01 mmol, 1 eq) in 25 mL of acetonitrile, NaH (1.00 g of 60% dispersion in mineral oil, 25.05 mmol, 5eq) was added an stirred at rt for 30 min. Bromoacetonitrile (1.84 mL, 27.55 mmol, 5.5 eq) was added dropwise at -20°C and after 5 h the mixture was allowed to warm up to rt overnight. The reaction mixture was concentrated to dryness under reduced pressure. The residue was suspended in DCM, filtered and the organic phase was evaporated *in vacuo.* The residue was purified by flash column chromatography (Hexanes/EtOAc, 9:1) to afford **12** as a colorless oil (2.51 g, 5.3 mmol, 84%).

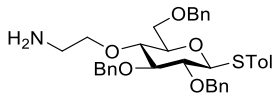
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40-7.38 (m, 2H), 7.32-7.20 (m, 15H), 6.97-6.95 (m, 2H), 4.86-4.81 (m, 2H, -C$\underline{H}_2$Ph), 4.66-4.59 (m, 2H, -C$\underline{H}_2$Ph), 4.55-4.46 (m, 3H, -C$\underline{H}_2$Ph, H-1), 4.22

(s, 2H, -C<u>H</u>$_2$CN), 3.73-3.64 (m, 2H, H-6), 3.57-3.53 (m, 1H, H-3), 3.48-3.44 (m, 1H, H-4), 3.39-3.34 (m, 1H, H-2), 3.33-3.29 (m, 1H, H-5), 2.23 (s, 3H, PhC<u>H</u>$_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.2, 138.1, 138.0, 138.0, 133.0, 129.9, 129.5, 128.7, 128.6, 128.5, 128.3, 128.1, 128.1, 127.9, 127.8, 116.2 (-CH_2<u>C</u>N), 87.8 (C-1), 86.2 (C-3), 81.0 (C-2), 78.6 (C-4), 78.2 (C-5), 75.9 (-<u>C</u>H_2Ph), 75.4 (-<u>C</u>H_2Ph), 73.6 (-<u>C</u>H_2Ph), 68.8 (C-6), 57.5 (-<u>C</u>H_2CN), 21.2 (Ph<u>C</u>H_3).

ESI-HRMS (+): *m/z* calc 618.2285 for $C_{36}H_{37}NO_5SNa^+$ [M + Na]$^+$, found 618.2261.

<u>p-Methylphenyl-2,3,6-tri-O-benzyl-4-O-(aminoethyl)-1-deoxy-1-thio-β-D-glucopyranoside **S4**</u>



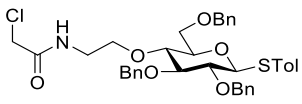
To a refluxing solution of **12** (2.51g, 4.21 mmol) in 50 mL of THF, BH_3•SMe_2 (6.3 mL, of a solution 2 M in THF, 3 eq) was added and the mixture was refluxed for 16 h. The mixture was then cooled to rt, quenched with 3 mL of MeOH and stirred for 24h. The volatiles are evaporated *in vacuo* and the residue was filtered on a short silica plug (2% of MeOH in $CHCl_3$ + 0.5% NH_4OH) that gave the amine **S4** as a colorless oil (1.94g, 4.07 mmol, 77%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.49-7.47 (m, 2H), 7.40-7.28 (m, 15H), 7.04-7.02 (m, 2H), 4.90-4.87 (m, 2H), 4.82-4.79 (m, 1H), 4.72-4.69 (m, 1H), 4.65-4.54 (m, 3H), 3.81-3.71 (m, 3H), 3.63-3.58 (m, 1H), 3.55-3.50 (m, 1H), 3.46-3.42 (m, 3H), 2.78-2.67 (m, 2H), 2.30 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 136.6, 138.5, 138.3, 137.8, 132.8, 129.8, 128.6, 125.5, 125.5, 125.4, 128.0, 127.9, 127.9, 127.8, 127.7, 87.8, 86.8, 81.0, 79.4, 78.2, 75.9, 75.5, 75.3, 73.6, 69.2, 42.6, 21.2.

ESI-HRMS (+): *m/z* calc 600.2778 for $C_{36}H_{42}NO_5S^+$ [M + H]$^+$ found 600.2749.

<u>p-Methylphenyl-2,3,6-tri-O-benzyl-4-O-(2-chloroacetylaminoethyl)-1-thio-β-D-glucopyranoside **S5**</u>



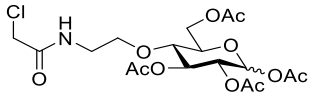
To a solution of **S4** (1.937 g, 3.23 mmol) and DIPEA (1.34 mL, 8.08 mmol, 2.5eq) in 16 mL of DCM, chloroacetyl chloride (0.31 mL, 3.87 mmol, 1.2 eq) was added dropwise at 0°C and stirred for 5h. The reaction mixture was diluted with 100 mL of DCM and washed with saturated solution of K_2CO_3, 1 M HCl, and brine. The organic phase was dried over Na_2SO_4, filtered, concentrated in vacuo and the resulting brown oil was purified by flash column chromatography (hexanes/EtOAc 7:3) to yield **S5** as white amorphous solid (1.856g, 3.35 mmol, 85% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.41-7.18 (m, 16H), 6.98-6.96 (m, 2H), 4.86-4.83 (m, 2H), 4.96-4.46 (m, 5H), 3.82-6.68 (m, 4H), 3.57-3.51 (m, 2H), 3.44-3.23 (m, 5H), 2.23 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ =165.9, 138.3, 138.2, 138.1, 138.0, 132.9, 129.8, 128.7, 128.6, 125.5, 128.4, 128.0, 128.0, 127.9, 127.8, 127.7, 87.9, 86.7, 81.0, 79.1, 77.8, 75.9, 75.5, 73.8, 71.1, 68.9, 42.7 40.2, 29.8, 21.2.

ESI-HRMS (+): *m/z* calc 676.2494 for $C_{38}H_{43}O_6NClS^+$ $[M + H]^+$ found 676.2490.

1,2,3,6-tetra-O-acetyl-4-O-(2-chloroacetylaminoethyl)-α-β-D-glucopyranoside **S6**

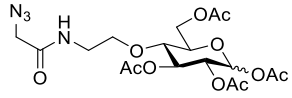


To a solution of **S5** (400 mg, 0.59 mmol) in 6 mL of acetone/water 4:1, NBS (200 mg, 1.12 mmol, 1.9 eq) was added at 0°C and the mixture was stirred for 2h at rt. Next, the reaction mixture was transferred in a separatory funnel, diluted with EtOAc (40 mL) and washed with sat. $Na_2S_2O_3$, $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 filtered and evaporated. The mixture was filtered over a silica pad eluted with hexanes/EtOAc 1:1 , the fraction containing the lactol (R_f= 0.5 in hexanes/EtOAc 1:1) where collected and evaporated *in vacuo*. The resulting oil was dissolved in 8 mL of *i*PrOH/EtOAc mixture (1:1) and stirred under a H_2 atmosphere in presence of Pd/C (50 mg, 20% loading) for 2h. The mixture was then filtered over celite and evaporated under reduced pressure. The residue was then dissolved in 5 mL of pyridine and Ac_2O (0.5 mL, 5.4 mmol) was added. The mixture was stirred for 16 h and then diluted in EtOAc (25 mL), washed with $NaHCO_3$, HCl 0.1 M, and brine. The organic layer dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc/hexanes 8:2) to yield **S6** (α/β 1:1) compound as a white foam (161 mg, 0.345 mmol, 58 % over three steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.83 (bs, 1H, N-H), 6.26 (d, *J* = 6.20 Hz, α H-1), 5.68 (d, *J* = 8.30 Hz, β H-1) 5.49-5.45 (m, 1H, α H-3), 5.26-5.22 (m, 1H, β H-3), 5.04-4.98 (m, 2H, α and β H-2), 4.36-4.24 (m, 2H, -HN-CH_2-CH_2-O), 4.05-.4.04 (m, 2H, Cl-CH_2C=O), 3.72-3.69 (m, 2H, H-6), 3.62-3.58 (m, 1H, H-5), 3.54-3.48 (m, 1H, H-4), 3.53-3.42 (bm, 2H, HN-CH_2-CH_2-O), 2.17, 2.11, 2.11, 2.09, 2.08, 2.07, 2.02, 2.00 (8x s, 3H, α and β).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.8, 170.2, 170.2, 170.0, 169.7, 169.0, 169.0 (7x s OC=O), 166.4 (NC=O), 89.7 (C-1, α), 89.3 (C-1, β), 75.9 (C-4), 75.8, 74.9, 73.8, 71.9, 71.1, 71.0, 70.9, 70.7, 69.6, 62.2, 62.1, 42.7, 42.7, 40.1, 40.0, 21.1, 21.0, 20.9, 20.7, 20.6.

1,2,3,6-tetra-O-acetyl-4-O-(2-azidoacetylaminoethyl)-α-β-D-glucopyranoside **13**



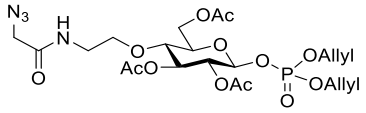
To a solution of **S6**, (230 mg, 0.49 mmol) in 1 mL of degassed DMF, NaN_3 (160 mg, 2.46 mmol, 5 eq) was added and the mixture stirred at 55°C for 1.5 h. The mixture was then diluted in EtOAc, washed with $NaHCO_3$ and the water layer was extracted 3 times with EtOAc. The combined organic phase was dried over Na_2SO_4, filtered and evaporated in vacuo. The residue was purified by flash column chromatography (Hex/EtOAc, 3:7) to yield title compound **13** as colorless oil (206 mg, 0.440 mmol, 89% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.6 (bs, 1H, N-H), 6.27 (d, *J* = 3.79 Hz, 1H, α H-1), 5.68 (d, *J* = 8.45 β H-1), 5.5-5.45 (m, 1H, α H-3), 5.27-5.22 (m, 1H, β H-3), 5.04-4.98 (m, 2H, α and β H-2), 4.35-4.26 (m, 2H, -HN-CH_2-CH_2-O), 3.95-3.95 (m, 2H, N_3-CH_2C=O), 3.70-3.37 (m, 6H, H-5, H-4, HN-CH_2-CH_2-O), 2.17, 2.12, 2.11, 2.10, 20.8, 2.07, 2.02, 2.01 (8x s, 3H, α and β OC=OCH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.8, 170.3, 170.2, 170.0, 169.6, 169.0, 169.0, (7xs OC=O), 167.2, (NC=O) 91.8 (C1, α), 89.3, (C-1, β), 76.0, 75.9, 75.0, 73.9, 72.0, 71.1, 71.1, 71.1, 70.7, 69.6, 62.2, 62.2, 52.8, 52.8, 39.7, 39.7, 21.1, 21.0, 21.0, 20.9, 20.7, 20.6.

ESI-HRMS (+): *m/z* calc 497.1490 for $C_{18}H_{26}N_4O_{11}Na^+$ $[M + Na]^+$ found 497.1481.

Diallyl(2,3,6-tri-O-acetyl-4-O-(2-azidoacetylaminoethyl)-β-D-gluco-pyranosyl)phosphate **14**



To a solution of **13** (172 mg, 0.36 mmol) in 1 mL of DMF, hydrazine acetate (37 mg, 0.40 mmol, 1.1 eq) was added and the mixture stirred at 55°C for 1.5 h. Then, the reaction mixture was diluted with EtOAc (10 mL) and washed with $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 filtered and evaporated *in vacuo.* Part of the resulting hemiacetal (115 mg, 0.27 mmol) was dissolved in 9 mL of DCM and DMAP (330 mg, 2.7 mmol, 10 eq) was added and the mixture stirred for 15 min. Then, a solution of diallylphosphoril chloride in 10 mL of DCM was added *via* syringe pump (1.6 mL/h). After the addition was completed the mixture was diluted in DCM (10 mL) and washed with $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The resulting oil was purified by flash column chromatography (hexanes/ EtOAc, 2:8), to yield compound **14** as colorless oil (61 mg, 0.103 mmol, 41% yield over two steps).

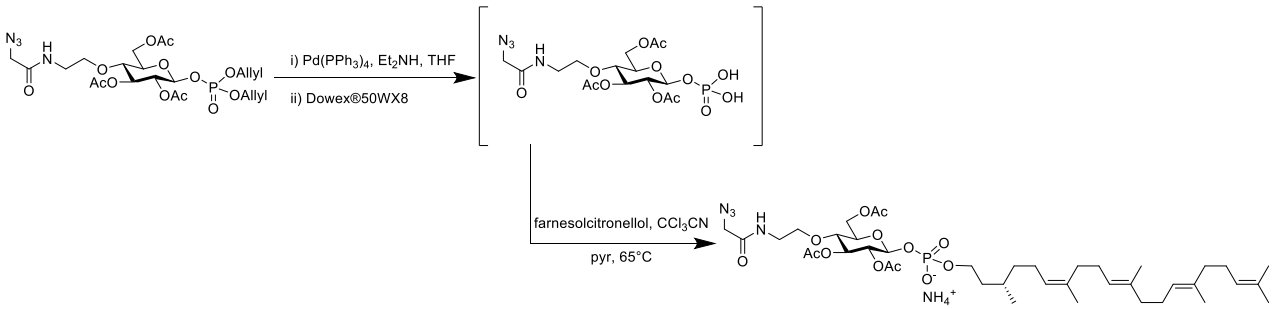
^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.59 (bs, 1H, N-H), 5.95-5.85 (m, 2H, CH_2=CH-CH_2-), 5.38-5.18 (m, 6H, CH=CH_2, H-1, H-3), 5.00-4.96 (m, 1H, H-2), 4.57-4.48 (m, 4H, -OCH_2CH=CH_2), 4.41 (dd, *J* = 12.17 Hz, *J* = 1.97 Hz, 1H, H-6), 4.23 (dd, *J* = 12.17 Hz, *J* = 4.80 Hz, 1H, H-6'), 3.96 (m, 2H, N_3-CH_2C=O), 3.70-3.65 (m, 2H, H-5, HN-CH_2-CH'_2-O), 3.60-3.56 (m, 1H, HN-CH_2-CH_2-O), 3.52-3.36 (m, 3H, H-3, HN-CH_2-CH_2-O), 2.09, 2.06, 2.03, (3x s OC=OCH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.6, 170.1, 169.6, (3x s, OC=O), 167.2 (NC=O), 132.3 (d, *J* = 7.7 Hz, CH_2-CH=CH_2), 132.0 (d, *J* = 7.7 Hz, CH_2-CH=CH_2), 118.7 (d, *J* = 11.7 Hz, CH_2-CH=CH_2), 96.2 (d, *J* = 4.8 Hz, C-1), 75.9 (C-4), 74.5 (C-3), 73.8 (C-5), 71.6 (d, *J* = 9.3 Hz, C-2), 71.1 (HN-CH_2-CH_2-O), 68.7 (d, *J* = 5.8 Hz - CH_2-CH=CH_2), 68.6 (d, *J* = 5.8 Hz - CH_2-CH=CH_2), 62.2 (C-6), 52.7 (N_3-CH_2C=O), 39.6 (HN-CH_2-CH_2-O), 20.9, 20.9, 20.7 (3x s, OC=OCH_3).

^{31}P NMR (122 MHz, MeOD) δ = -3.0.

ESI-HRMS (+): *m/z* calc 615.1674 for $C_{22}H_{33}N_4O_{13}PNa^+$ $[M + Na]^+$ found 615.1668.

Farnesylcitronellylphosphoryl-2,3,6-tri-O-acetyl-4-O-(2-azidoacetylaminoethyl)-β-D-glucopyranosidee **S7**



To a solution of **14**. (26.5 mg, 44.7 µmol, 1 eq) and $Pd(PPh_3)_4$ (2.6 mg, 2.2 µmol, 0.05 eq) in 0.4 mL of dry THF, diethylamine (25 µL, 225 µmol, 5 eq) was added and the mixture was stirred for 16 h. The mixture was dried under reduced pressure, loaded on an ion exchange resin (Dowex 50WX8 ammonium form) and eluted with MeOH, the organic phase was evaporated to dryness. The residue was coevaporated with pyridine 3 times, then dissolved in 0.9 mL of pyridine together with farnesylcitronellol (48 mg, 130 µmol, 3eq), To this solution CCl_3CN (44 µL, 440 µmol, 10 eq) was added and heated at 65°C overnight. The reaction mixture was concentrated to dryness and the resulting oil purified by flash column chromatography (EtOAc/MeOH : 3/1 + 0.5% of NH_4OH solution 25% in water) to yield **S7** (15.6 mg, 17.9 µmol, 41% over two steps) as a colorless oil.

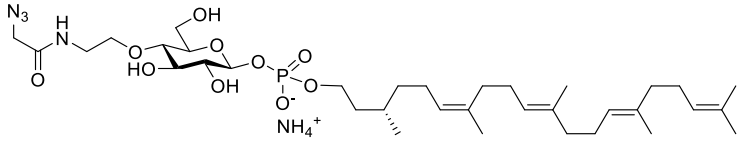
^{1}H NMR (400 MHz, MeOD) δ = 5.24-5.19 (m, 1H, H-3), 5.17-5.09 (m, 5H, H-1), 4.84-4.81 (m, 1H, H-2), 4.42-4.39 (m, 1H, H-6), 4.28-4.24 (m, 1H, H-6'), 3.92-3.89 (m, 4H), 3.73-3.69 (m, 2H), 3.73-3.69, 3.62-3.56 (m, 2H), 2.09-1.95 (m, 23H), 1.68-1.60 (m, 14H), 1.46-1.35 (m, 4H), 1.22-1.11 (m, 1H), 0.91 (d, *J* = 6.51 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.4, 171.7, 171.4, (3xs, O<u>C</u>=O), 170.3 (N<u>C</u>=O), 136.1, 136.0, 135.9, 132.1, 126.5, 125.5, 125.4, 96.9 (d, *J* = 5.3 Hz, C-1), 77.2, 76.3, 74.5, 73.9, (d, *J* = 8.3 Hz, C-2), 71.9, 65.4 (d, *J* = 6.17 Hz), 63.7, 53.0, 40.9, 40.7, 38.9 (d, *J* = 8.0 Hz) 38.6, 32.9, 30.5, 27.8, 27.8, 27.6, 26.4, 25.9, 23.7, 20.9, 20.9, 20.8, 20.8, 19.8, 17.8, 16.1, 16.1.

^{31}P NMR (122 MHz, MeOD) δ = -4.2.

ESI-HRMS (+): *m/z* calc 853.4369 for $C_{41}H_{66}N_4O_{13}P^-$, found 853.4390.

<u>Farnesylcitronellylphosphoryl-4-O-(2-azidoacetylaminoethyl)-β-D-glucopyranoside **15**</u>



To a solution of **S7** (5.5 mg, 6.6 µmol) in 0.5 mL of methanol, an excess of NH_4OH (200 eq per acetyl of a 25% solution) was added. The mixture is stirred for 16 h at rt and then dried *in vacuo*. The residue was dissolved in 0.5 mL of a mixture MeOH/NH_4HCO_3 (100 mM solution) 1:1 and loaded on a RediSepRf gold C18 (5.5 g) column preequilibrated with the same solvent mixture. The column was eluted with 20 mL of 1:1 MeOH/ NH_4HCO_3 solution, 20 mL of 4:1, 20 mL of 9:1 and 20 mL of MeOH. Pure fractions (identified by HPLC-MS) were collected and evaporated to yield compound **15** as a colorless oil (3.2 mg, 4.3 µmol 67% yield).

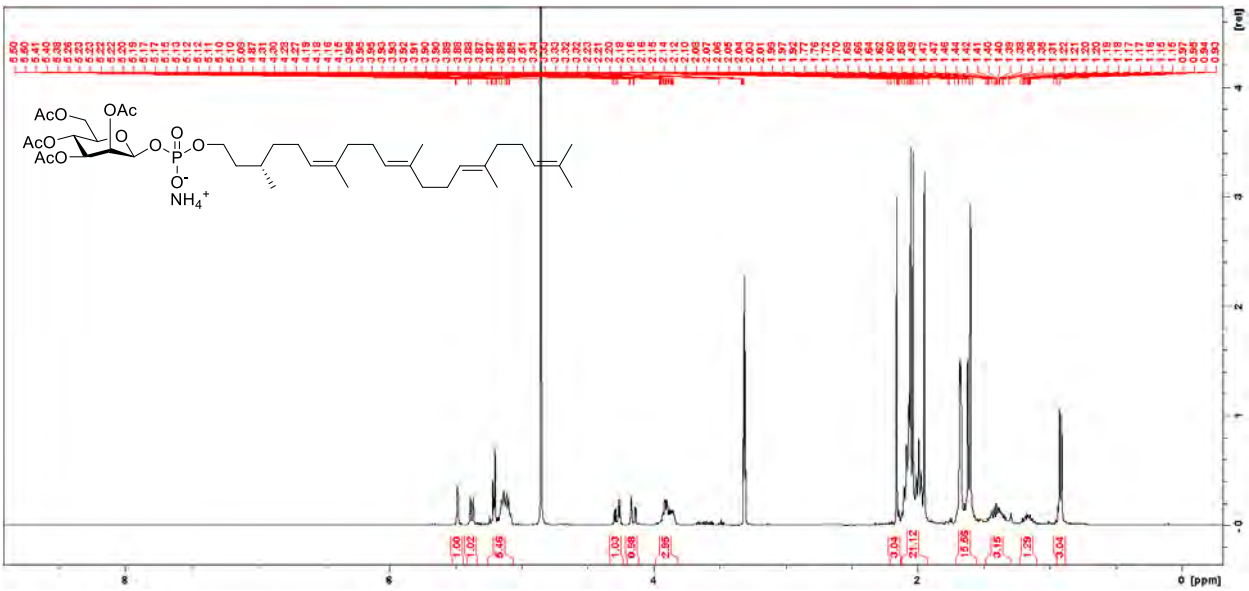
^{1}H NMR (400 MHz, MeOD) δ = 5.15-5.09 (m, 4 H), 4.83-4.79 (m, 1H, H-1), 3.1-3.84 (m, 5H), 3.82-3.79 (m, 1H), 3.77-3.71 (m, 1H), 3.68-3.64 (m, 1H), 3.53-3.38 (m, 3H), 3.28-3.20 (m, 2H), 2.10 -1.95 (m, 13H), 1.68- 1.60 (m, 16H), 1.45-1.33 (m, 4H), 1.21-1.12 (m, 1H), 0.92 (d, *J* = 6.50 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.2 (N<u>C</u>=O), 136.0, 135.9, 135.9, 132.0, 126.7, 125.5, 125.5, 99.5 (d, *J* = 6.2 Hz, C-1), 79.7 (C-4), 77.7 (C-3), 77.6 (C-5), 76.1 (d, *J* = 7.7 Hz, C-2), 72.0 (-CH_2<u>C</u>H_2-O), 65.3 (d, *J* = 5.16 Hz), 62.4 (C-6), 53.0 (N_3<u>C</u>H_2C=O), 41.0, 40.9, 40.8, 38.9, 38.9, 38.7, 32.9, 27.8, 27.6, 27.6, 26.4, 25.9, 23.7, 19.8, 17.8, 16.1, 16.1.

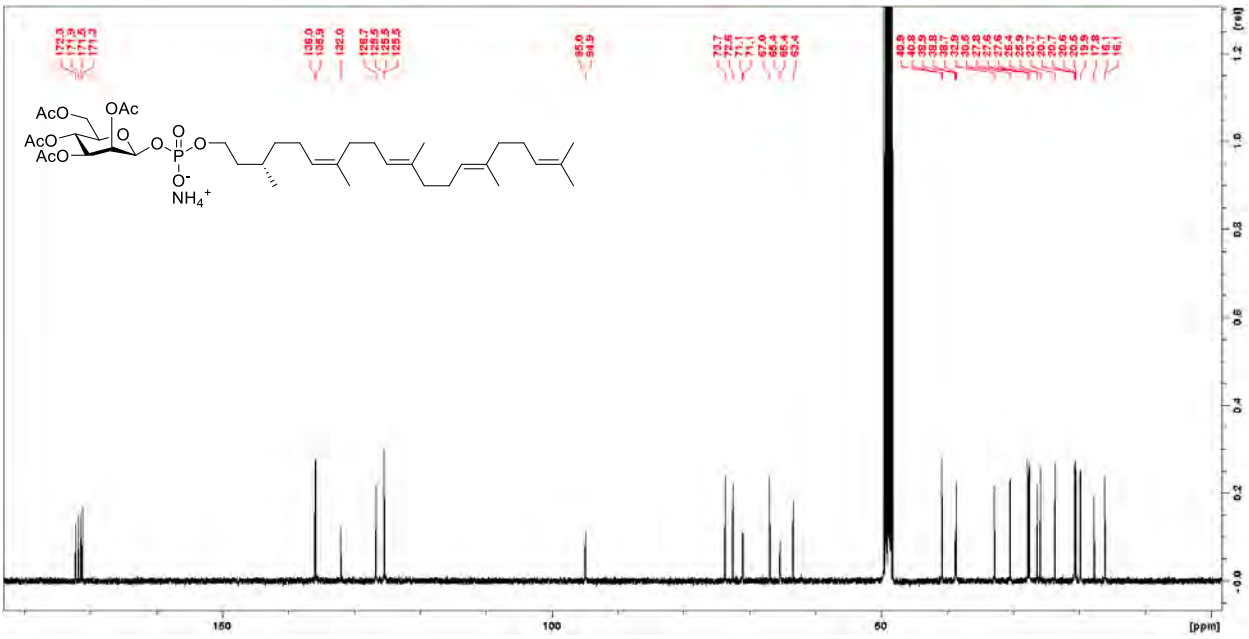
^{31}P NMR (122 MHz, MeOD) δ = -1.0.

ESI-HRMS (+): *m/z* calc 727.4053 for $C_{35}H_{60}N_4O_{10}P^-$ found 727.4040.

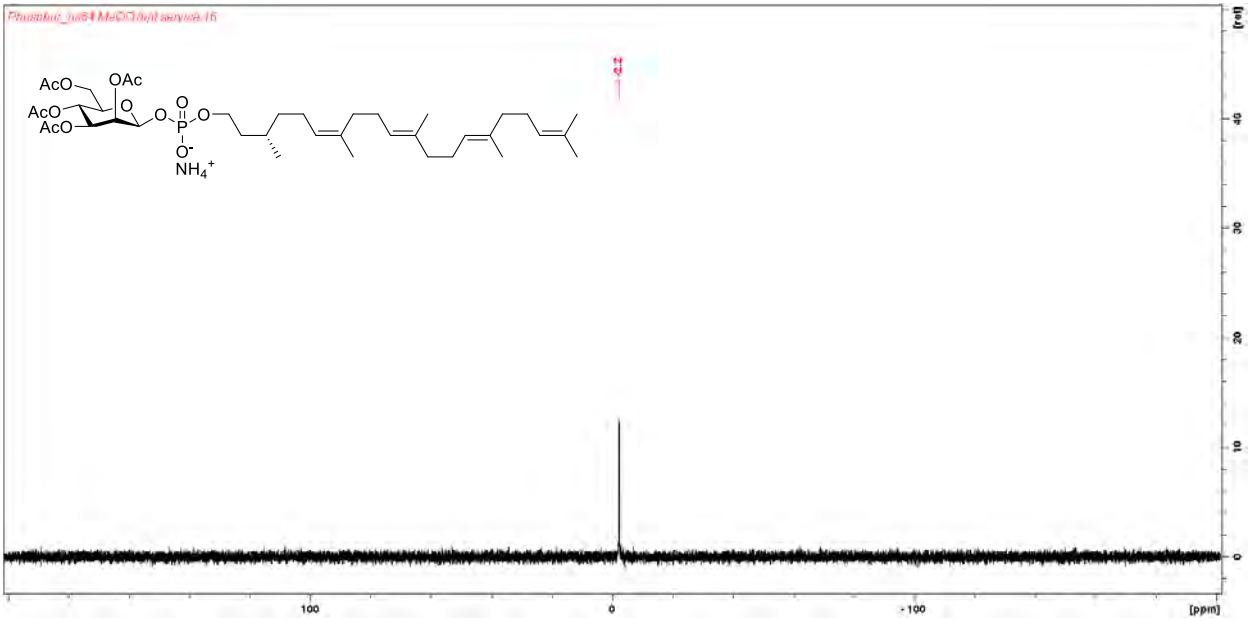
Farnesylcitronellylphosphoryl-2,3,4,6-tetra-O-acetyl-β-D-mannopyranoside **S1** ^{1}H NMR



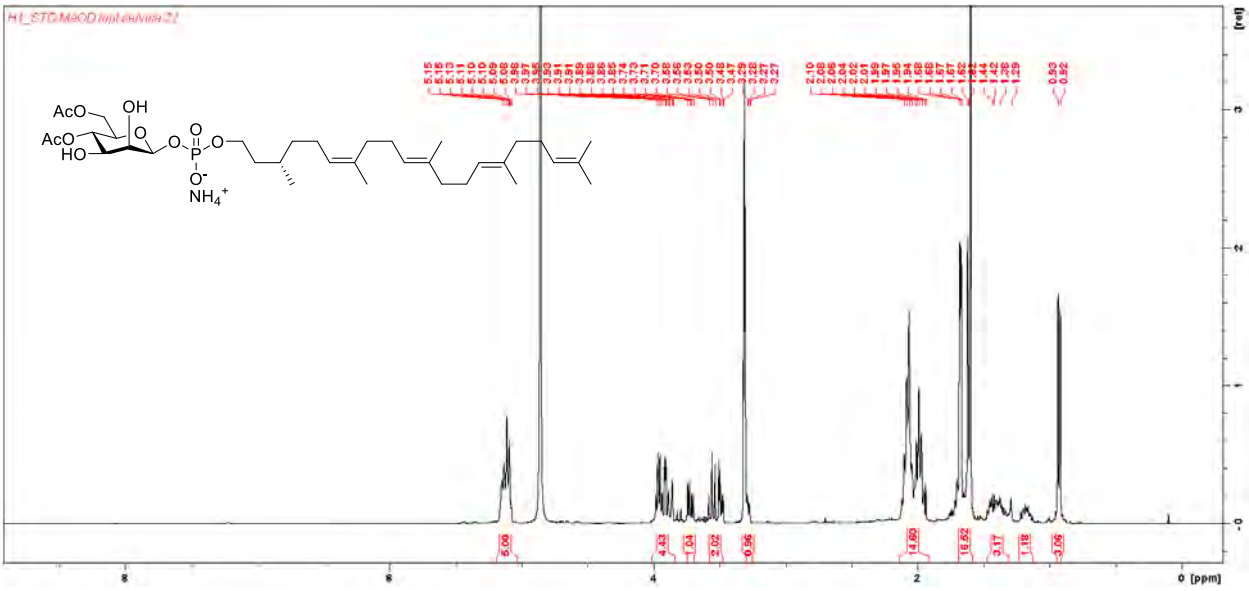
^{13}C NMR



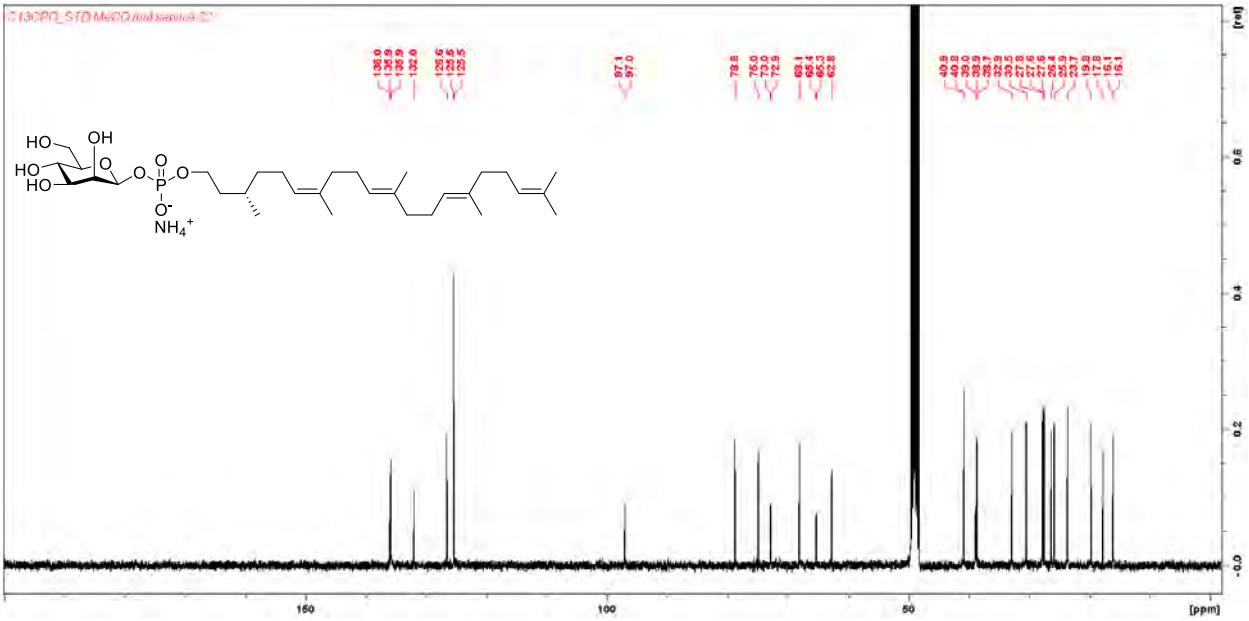
^{31}P NMR



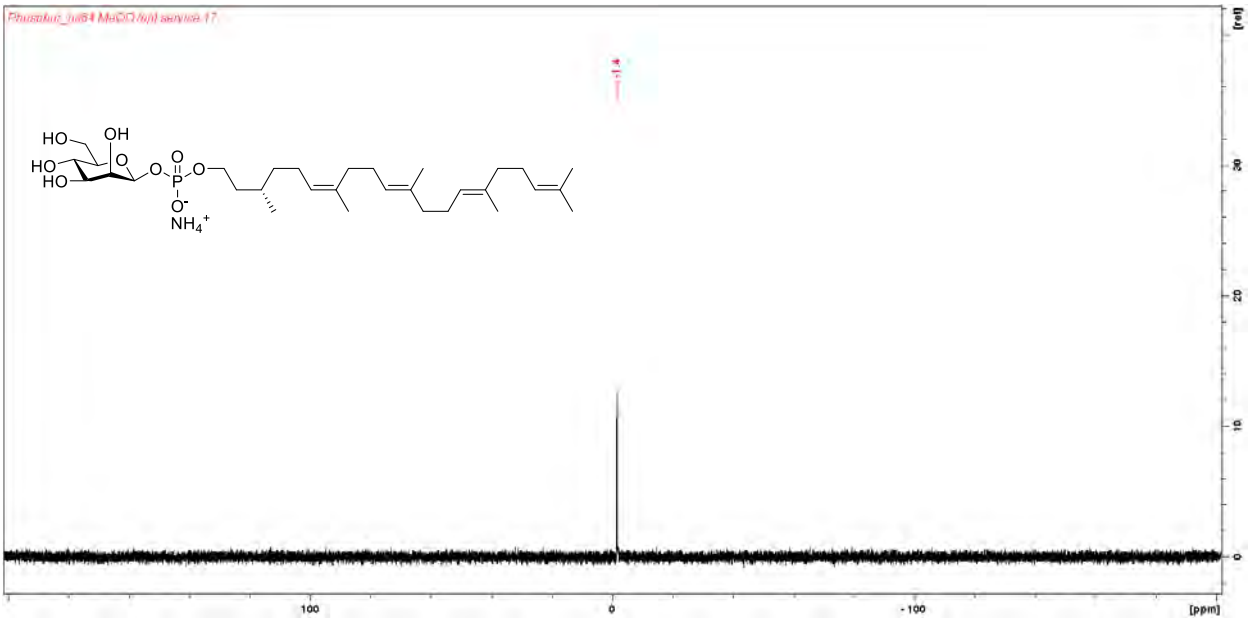
Farnesylcitronellylphosphoryl-β-mannopyranoside **5** ^{1}H NMR



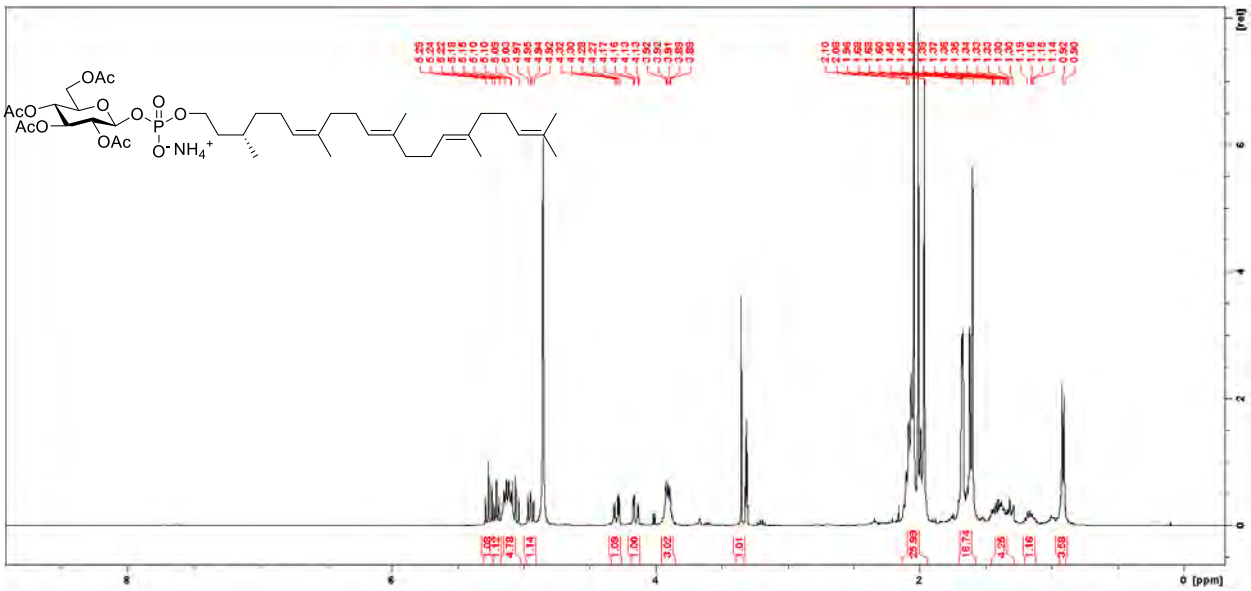
^{13}C NMR



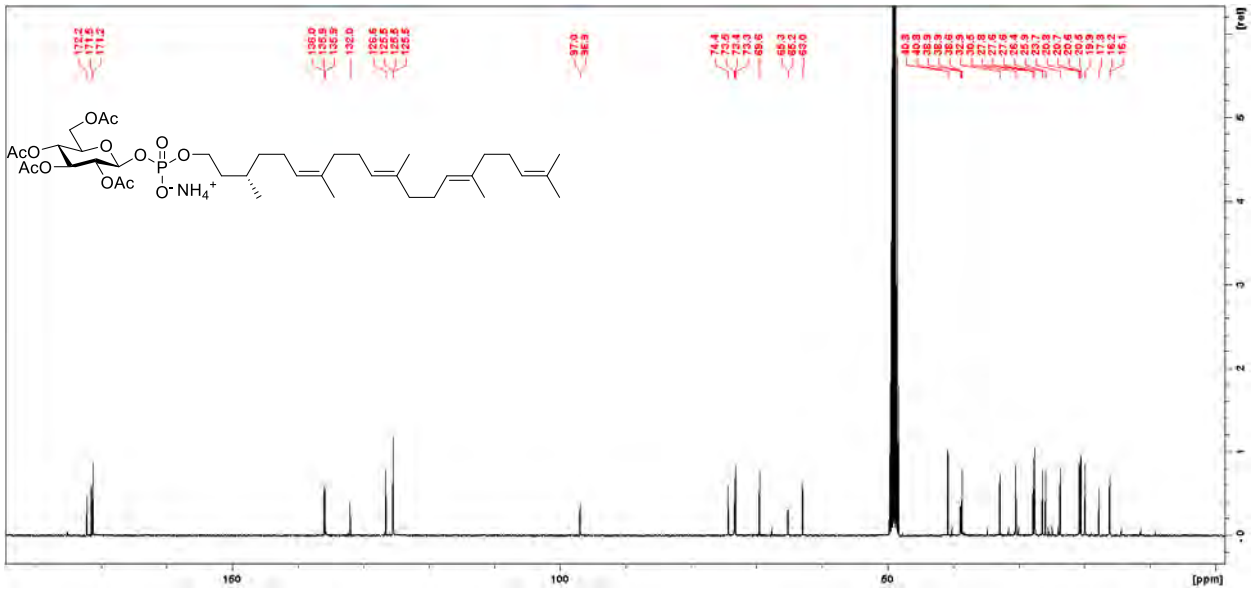
^{31}P NMR



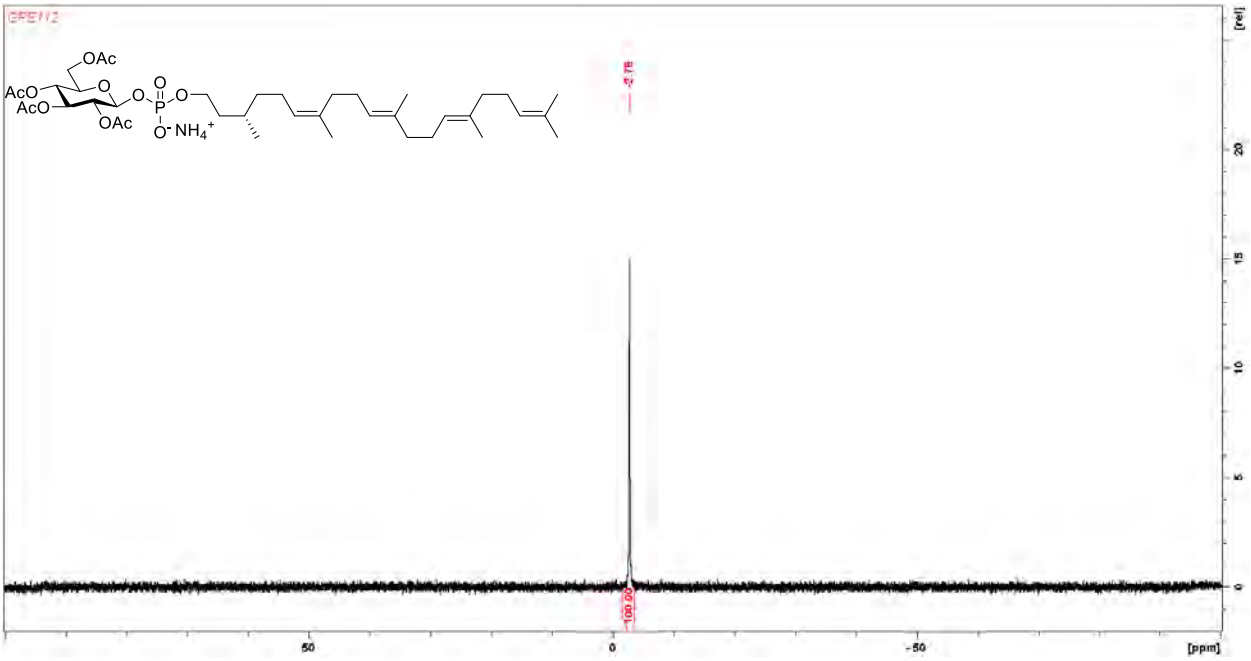
Farnesylcitronellylphosphoryl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside **S2** ^{1}H NMR



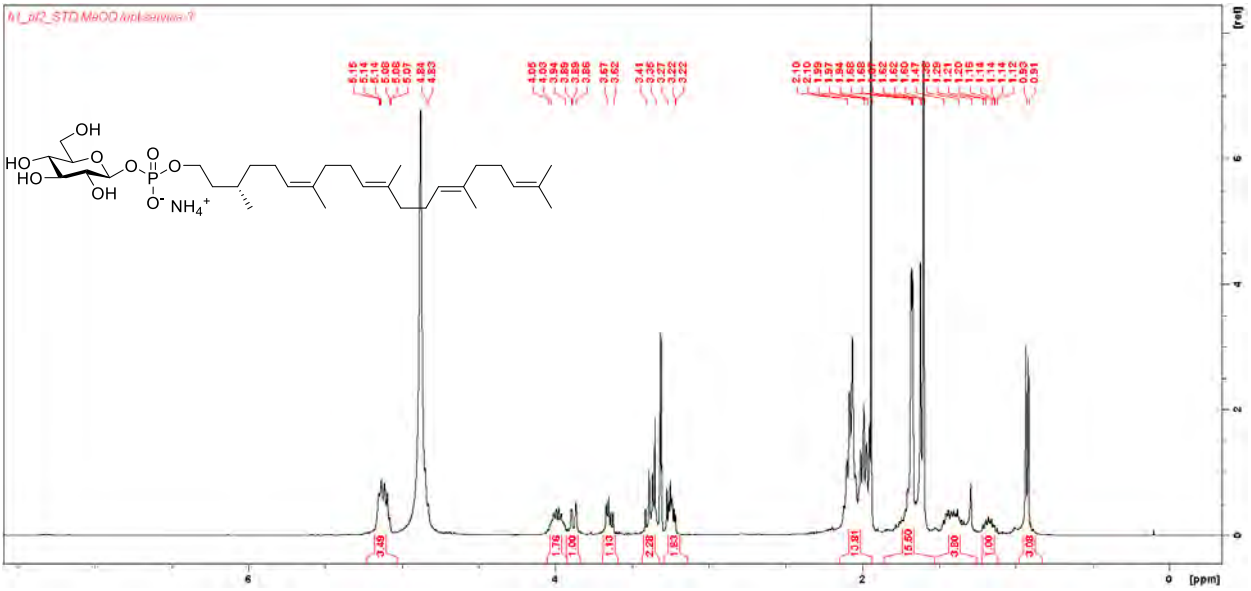
^{13}C NMR



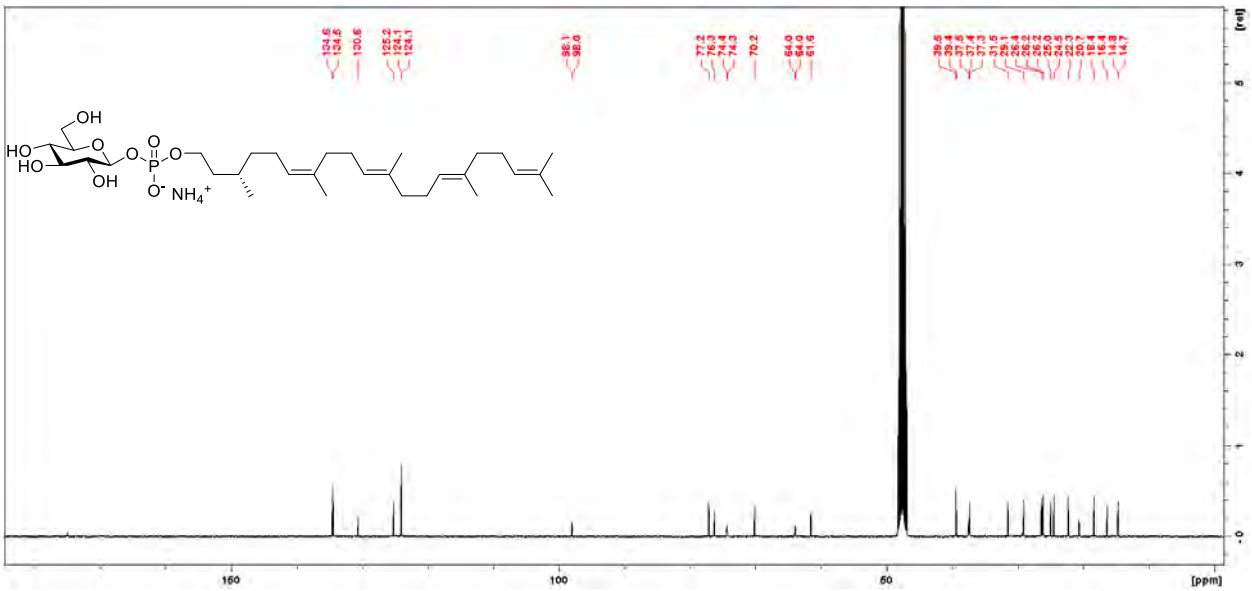
^{31}P NMR



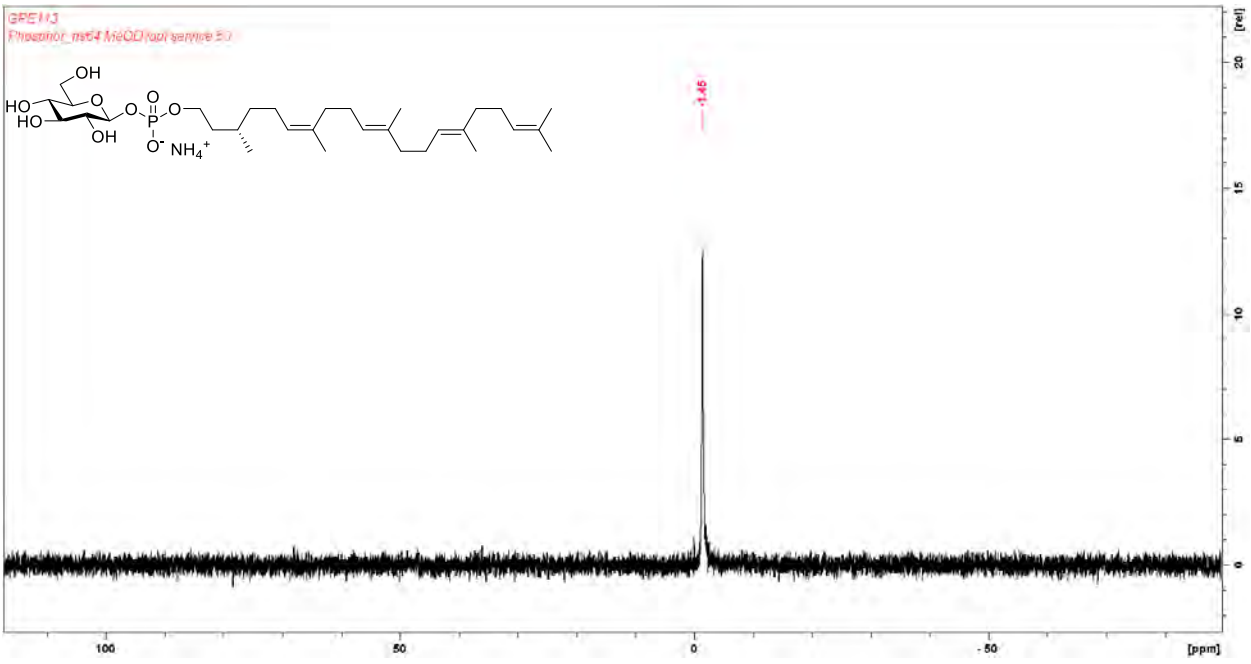
Farnesylcitronellylphosphoryl-β-glucopyranoside **6** ^{1}H NMR



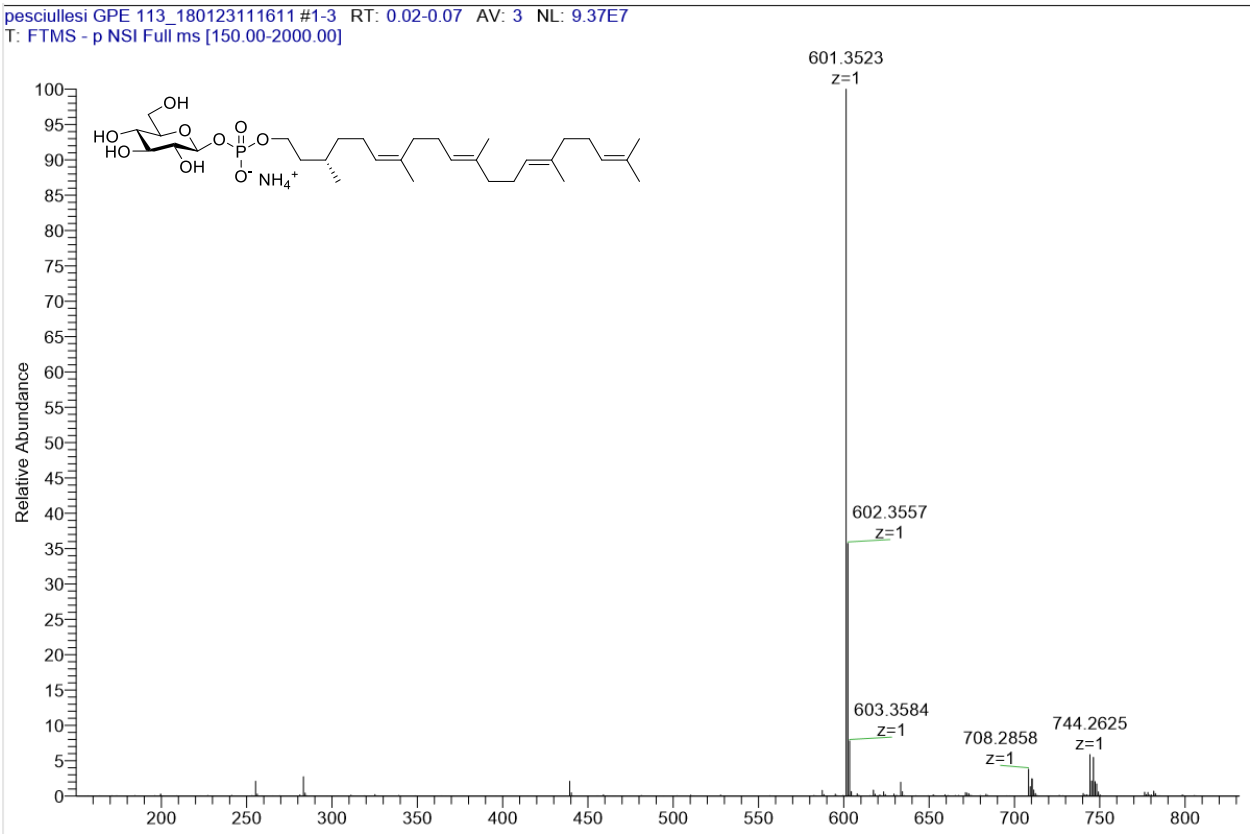
^{13}C NMR



^{31}P NMR



HR-MS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601.3523
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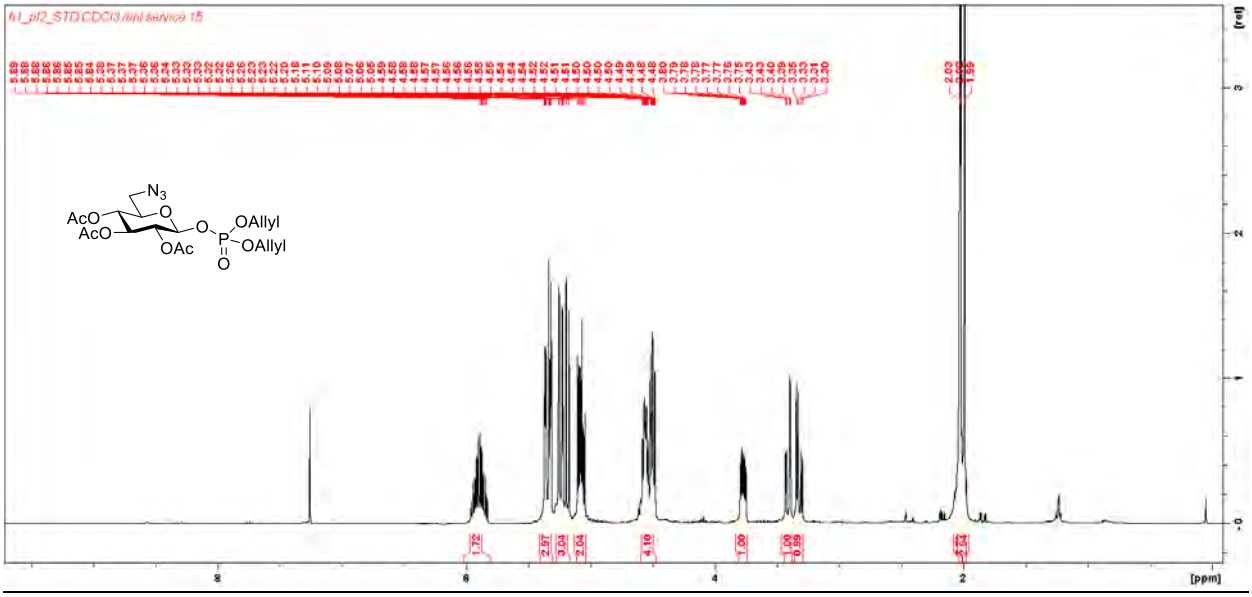
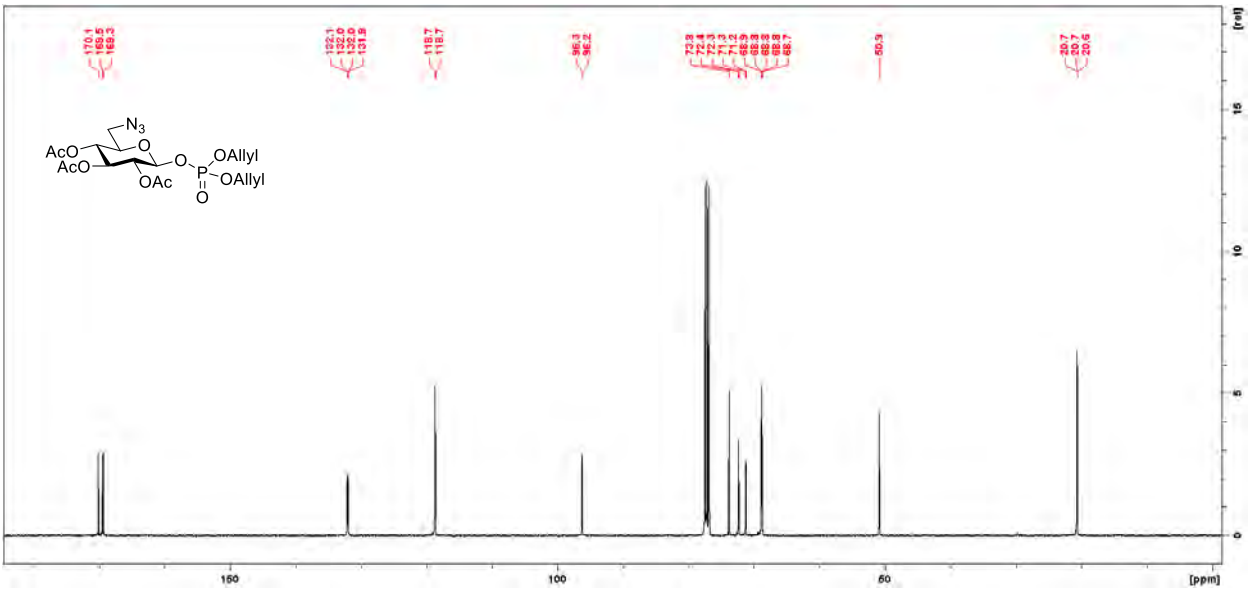
Relative Abundance
OH
HO
HO
OH
O
O
O
P
O
O
O
NH4+

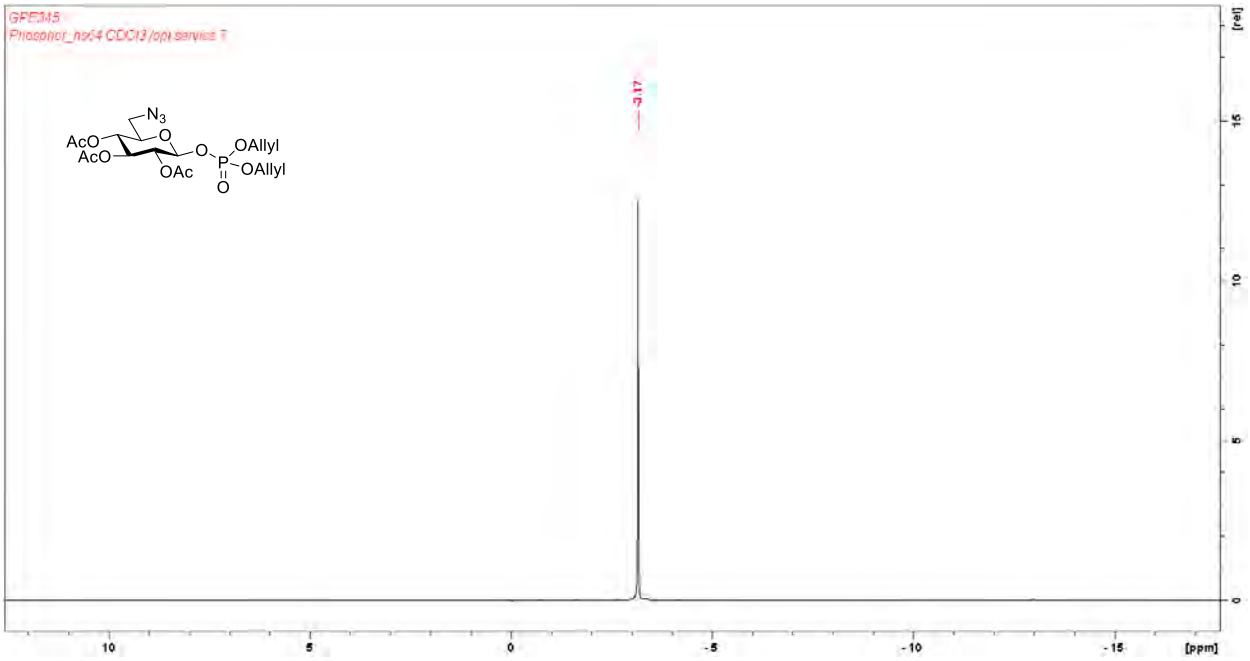
Diallyl(2,3,4-tri-O-acetyl-6-azido-6-deoxy-β-D-gluco-pyranosyl)phosphate **8** ^{1}H NMR



^{13}C NMR

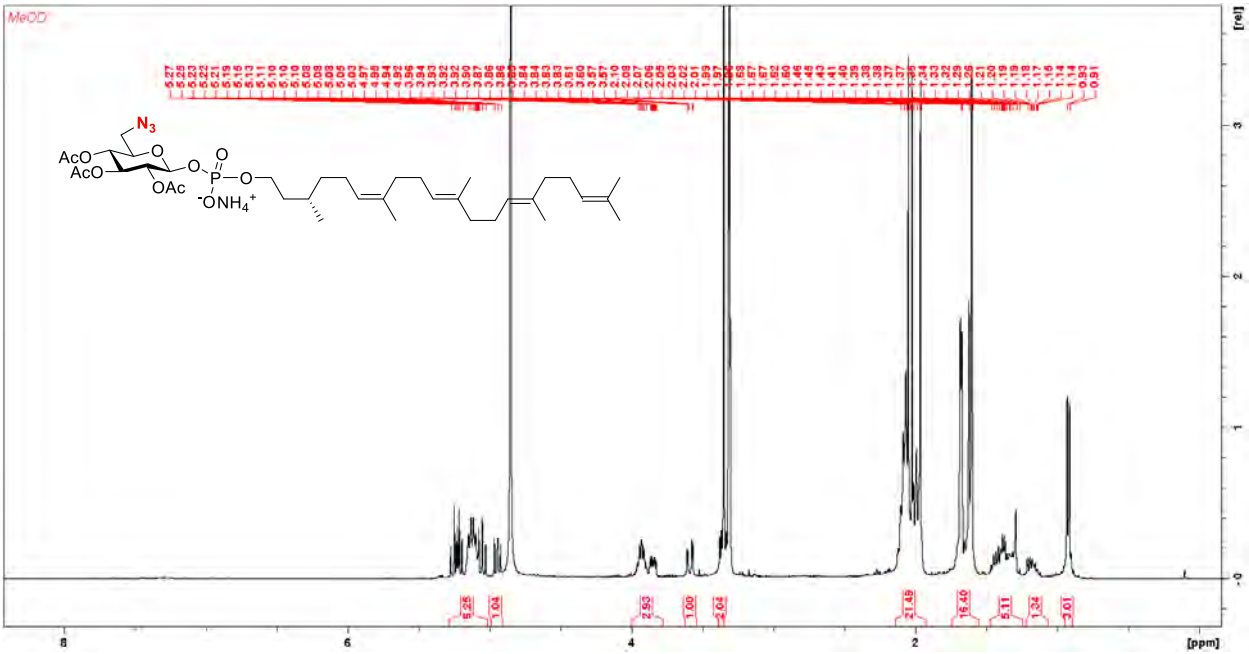


^{31}P NMR

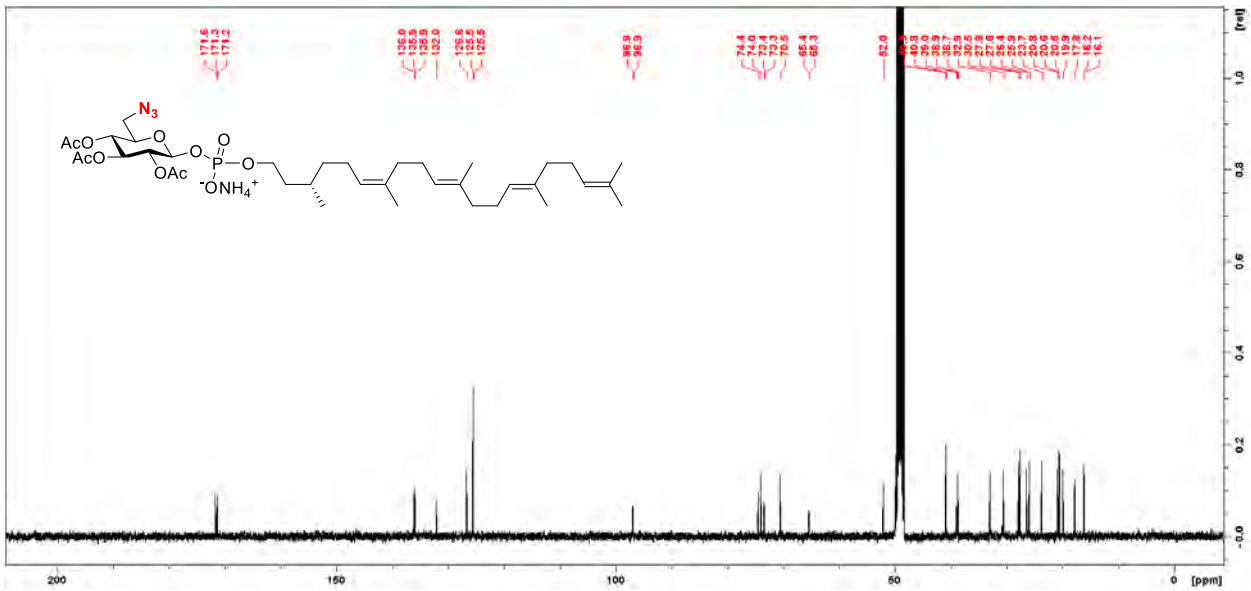


Farnesylcitronellylphosphoryl-6-azido-6-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranoside **S3**

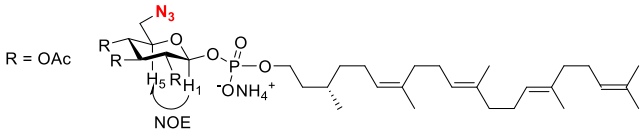
^{1}H NMR

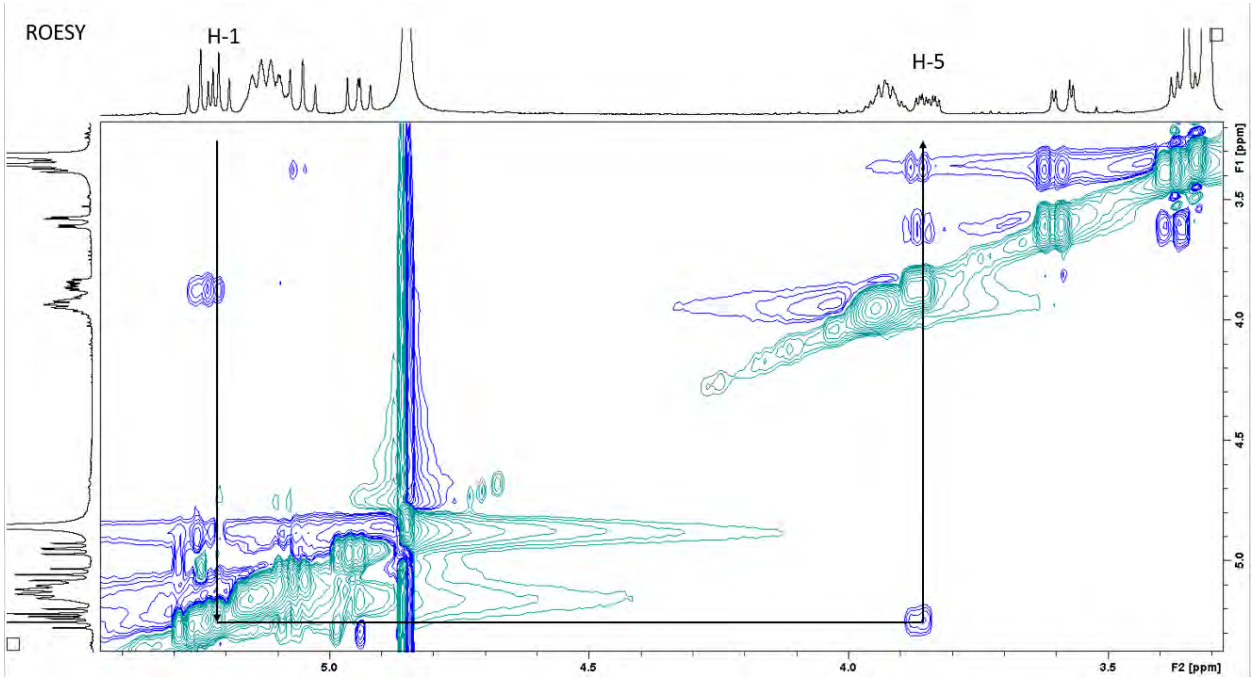


^{13}C NMR

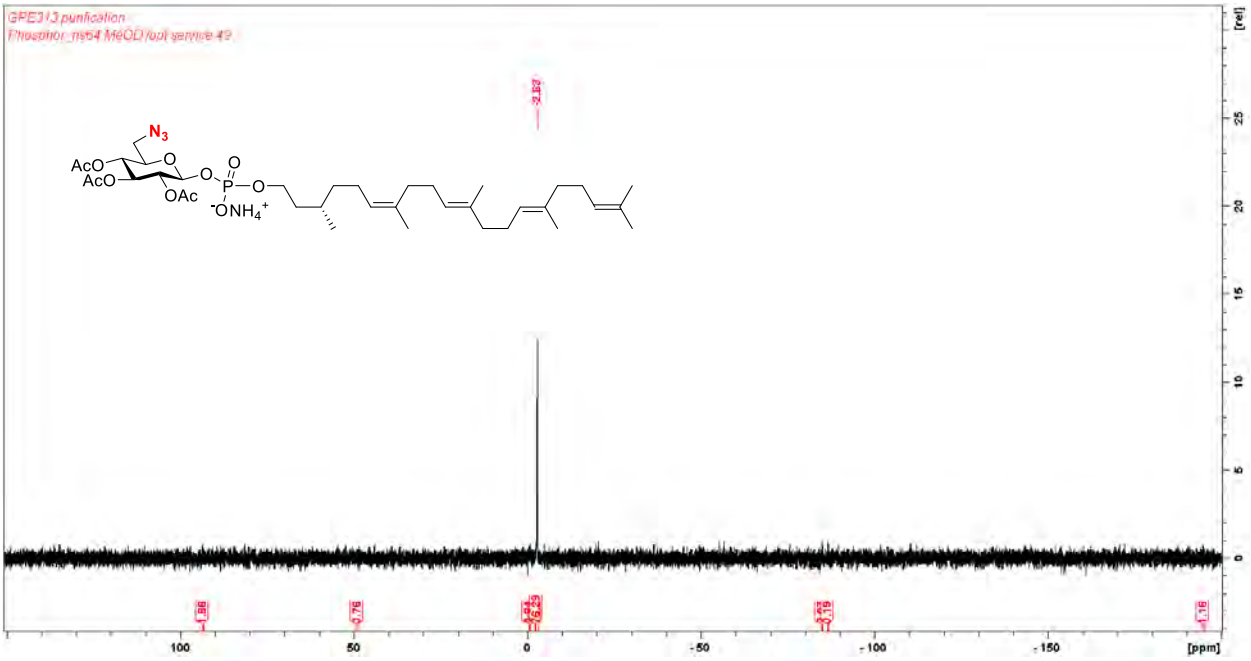


ROESY EXPERIMENT

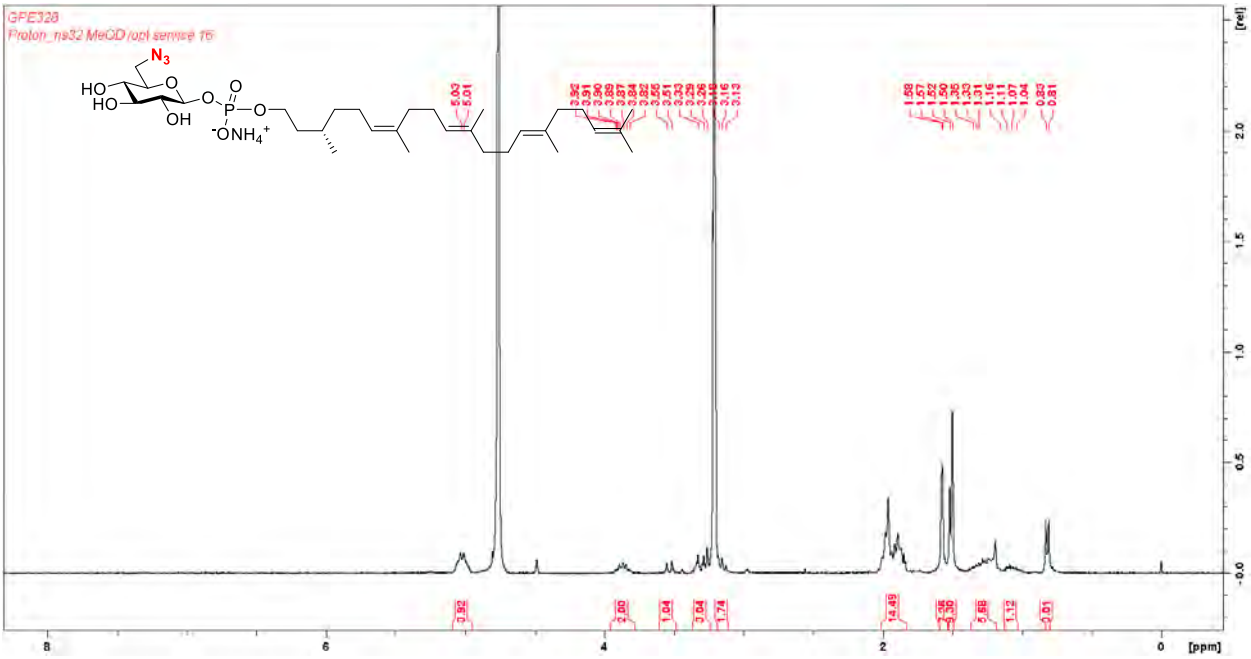




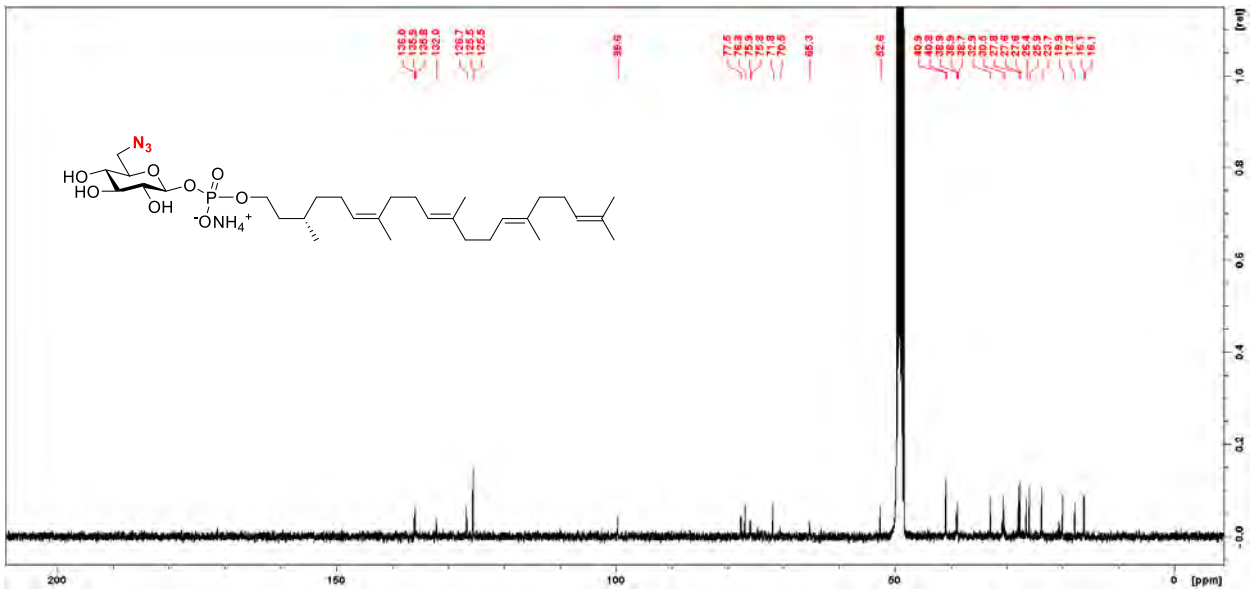
^{31}P NMR



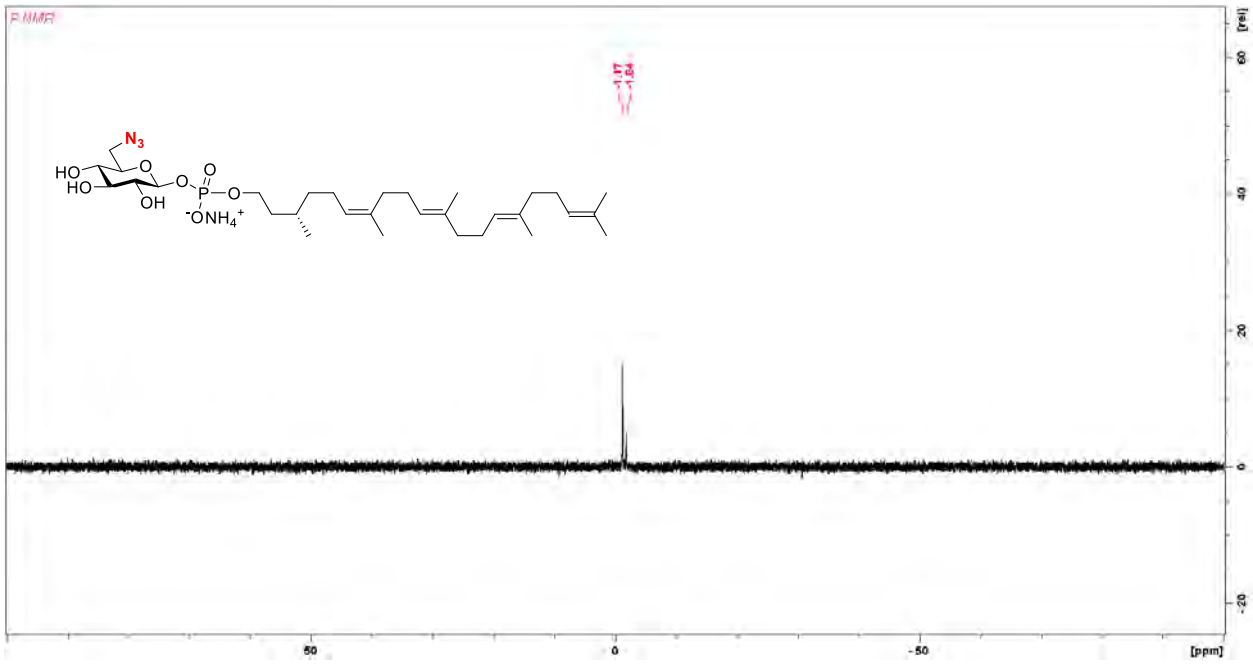
Farnesylcitronellylphosphoryl-6-azido-6-deoxyl-β-glucopyranoside **9** ^{1}H NMR



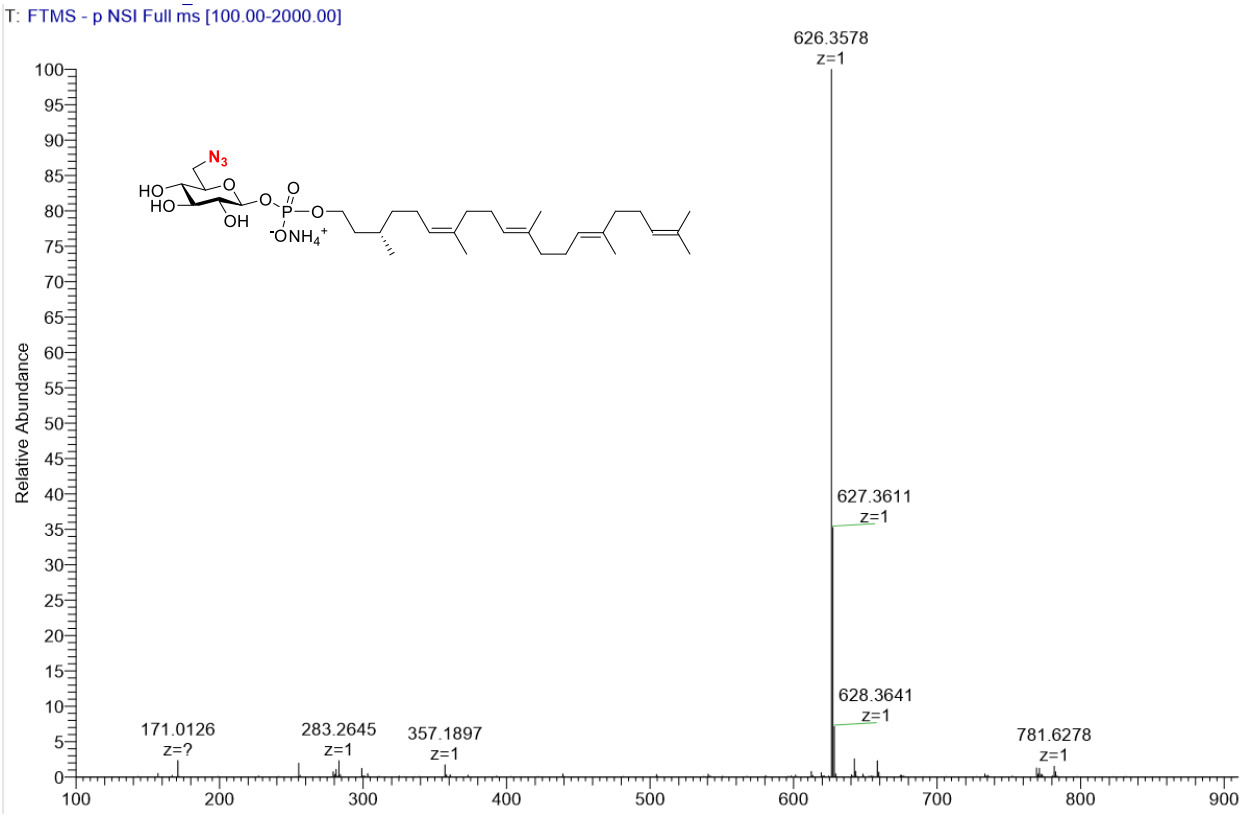
^{13}C NMR



^{31}P NMR

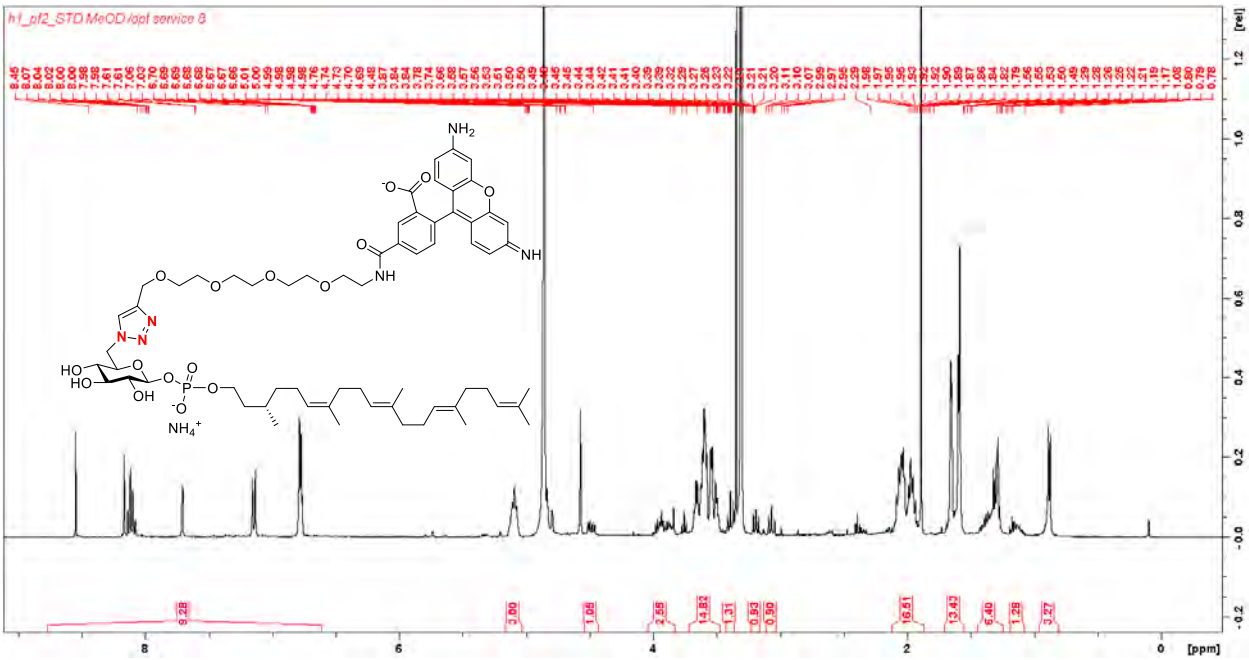


HRMS

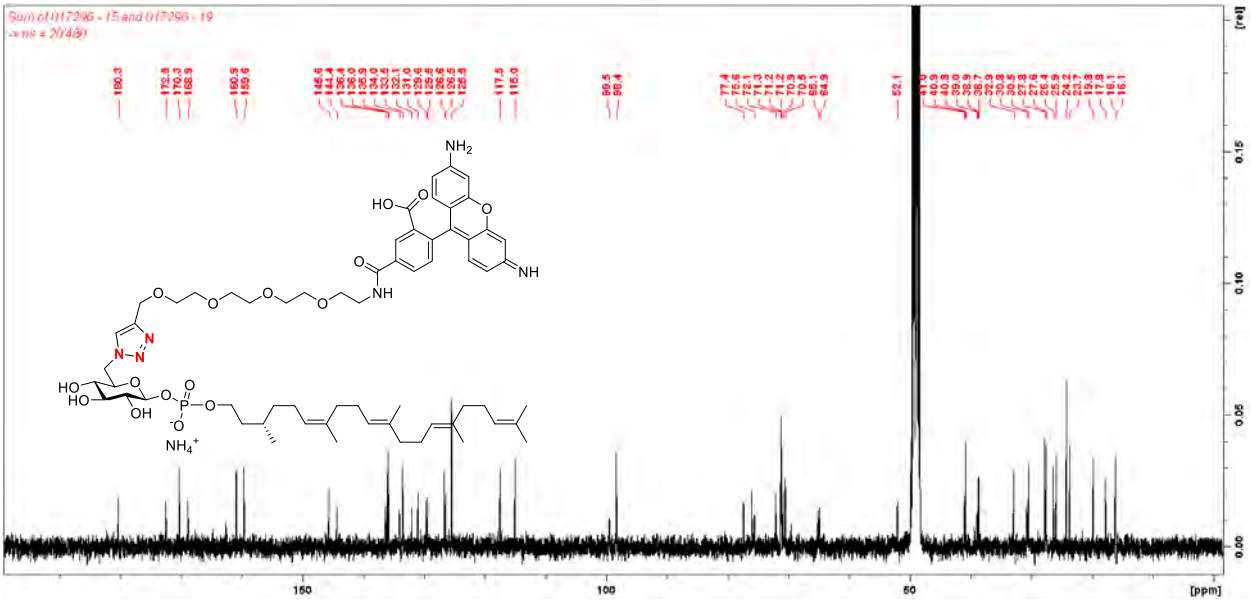


Farnesylcitronellylphosphoryl-6-triazole-peg-Rhodamine110-6-deoxyl-β-glucopyranoside **10**

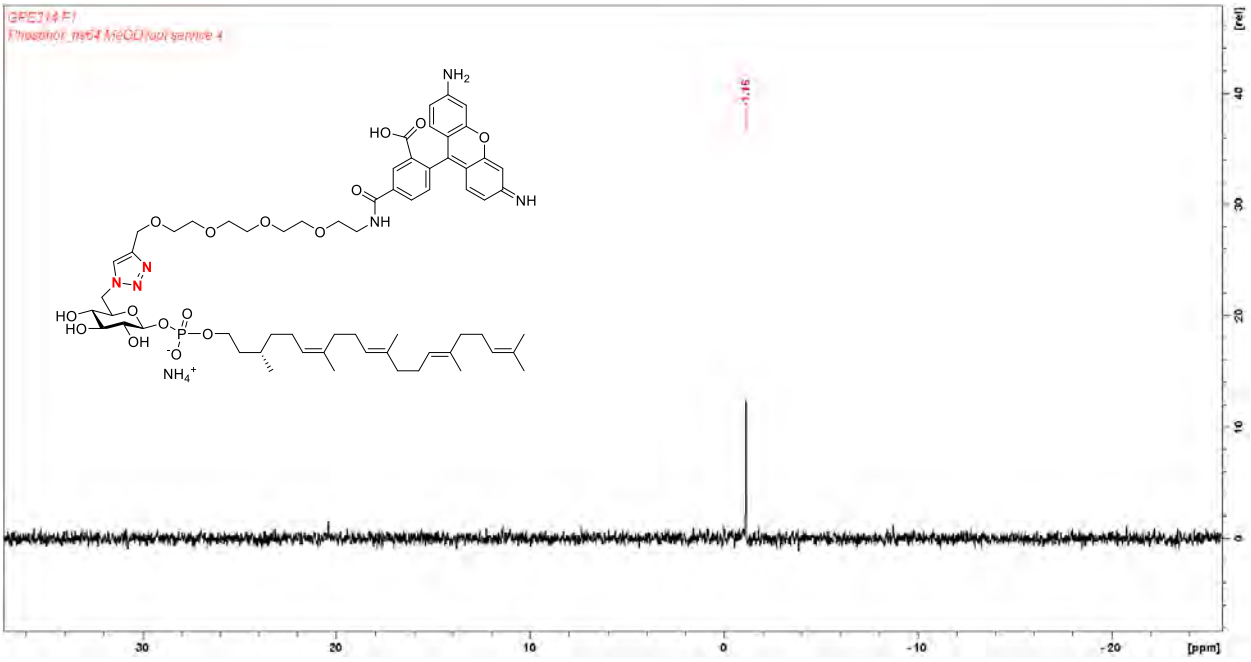
^{1}H NMR



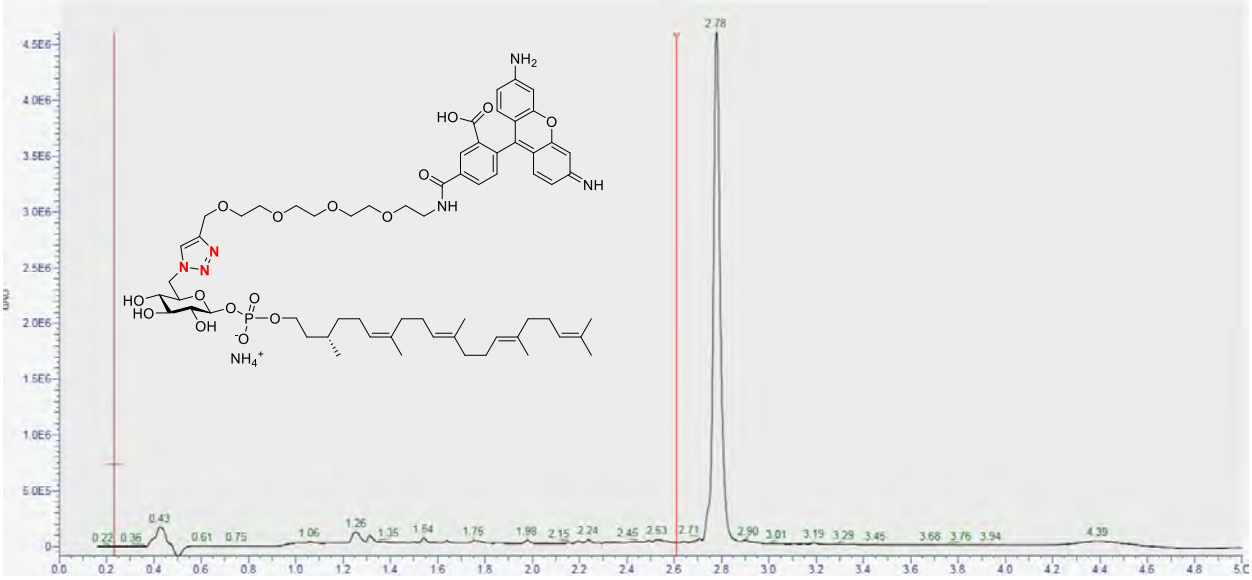
^{13}C NMR



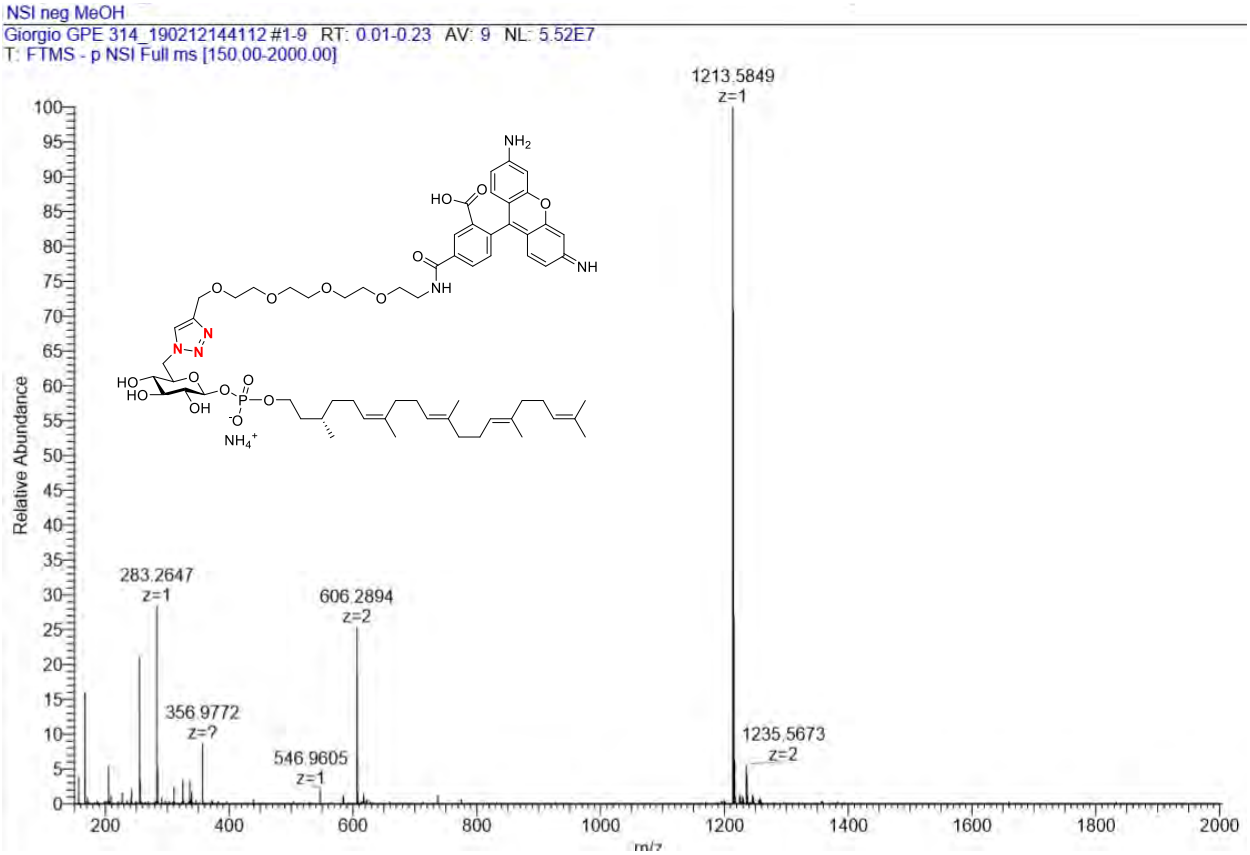
^{31}P NMR



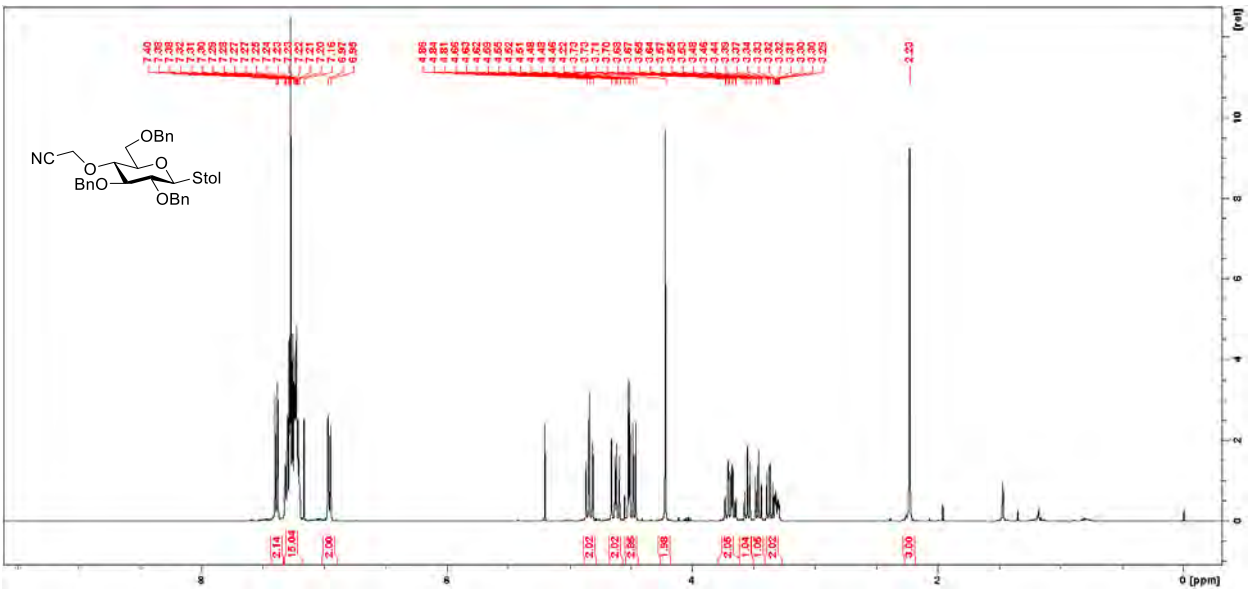
HPLC chromatogram on C_{18} Column



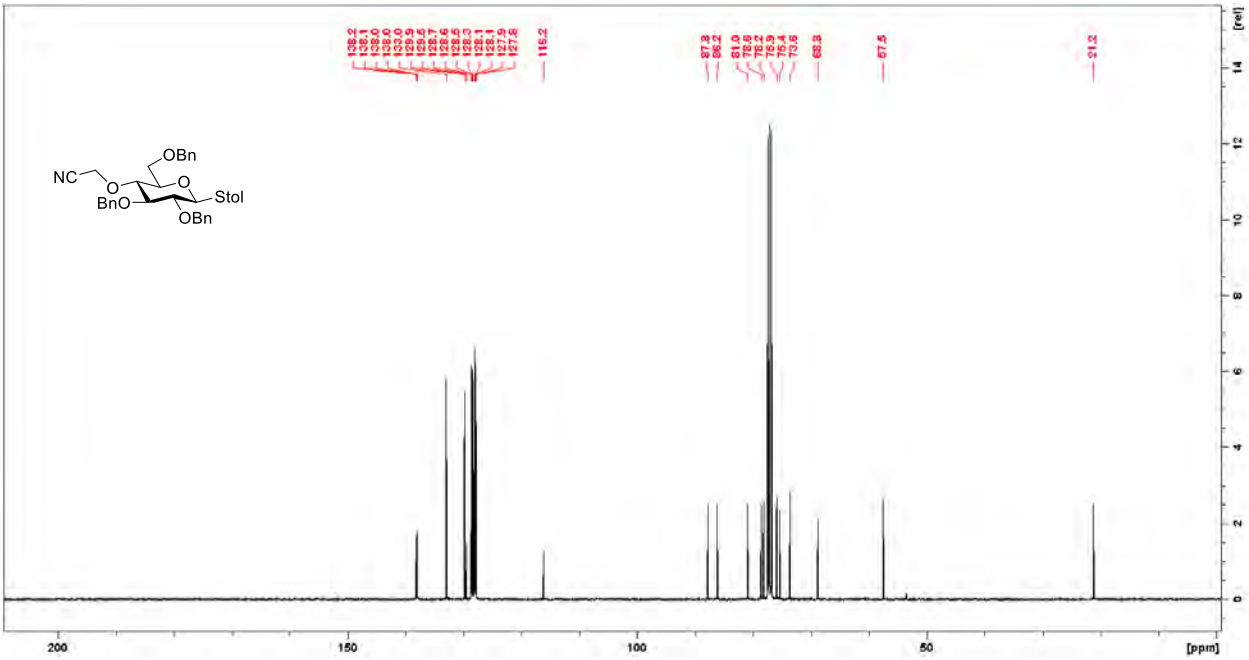
|HRMS



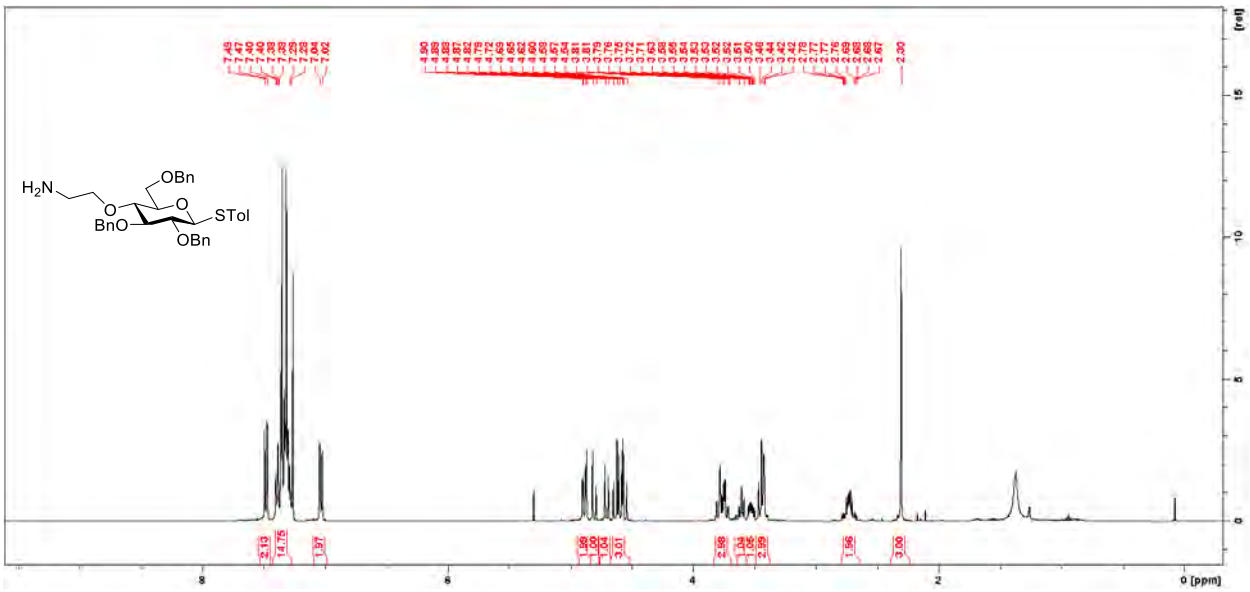
p-methylphenyl-2,3,6-tri-O-benzyl-4-O-(cyanometyl)-1-thio-β-D-glucopyranoside **12** ^{1}H NMR



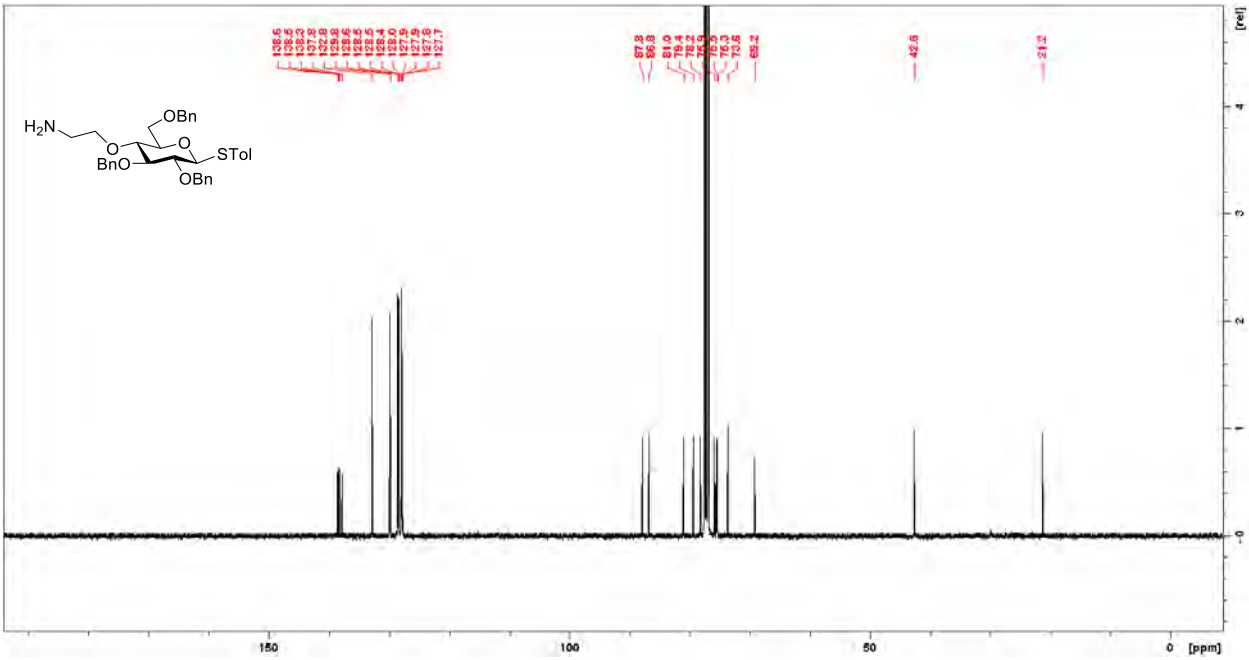
^{13}C NMR



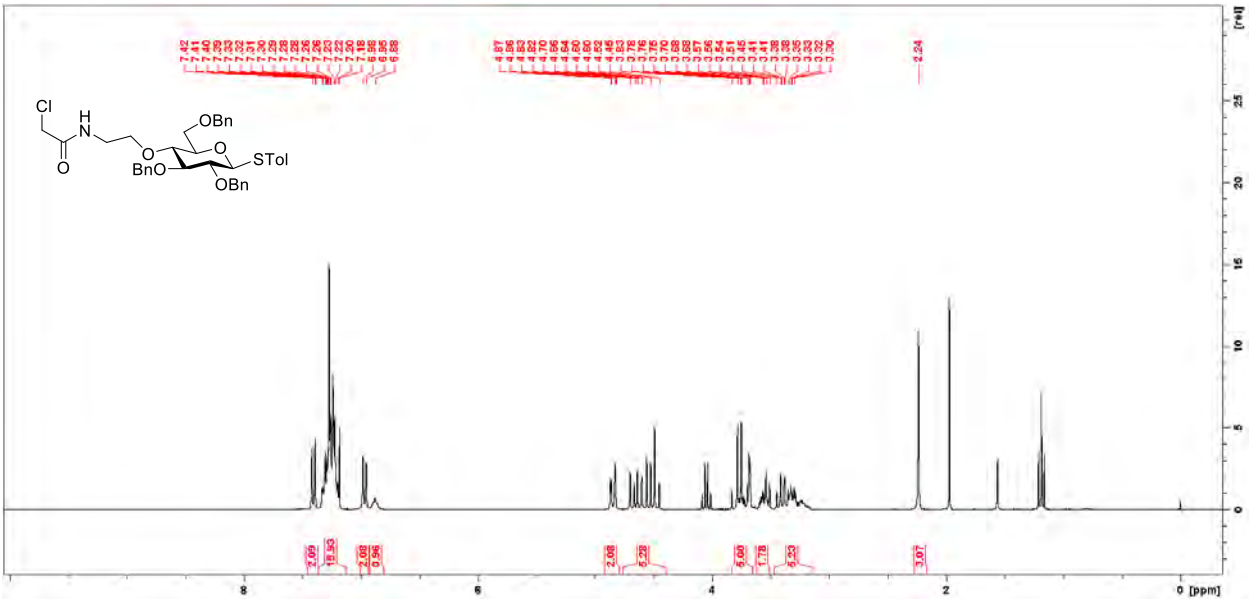
p-methylphenyl-2,3,6-tri-O-benzyl-4-O-(aminoethyl)-1-thio-β-D-glucopyranoside **S4** ^{1}H NMR.



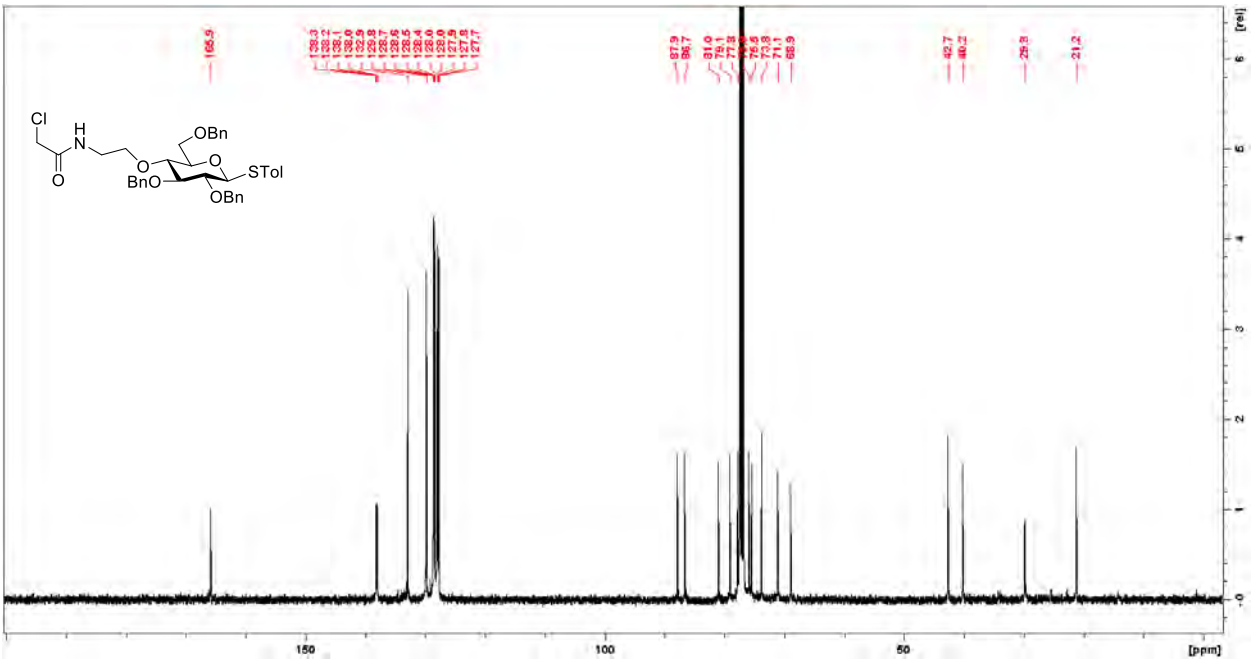
^{13}C NMR



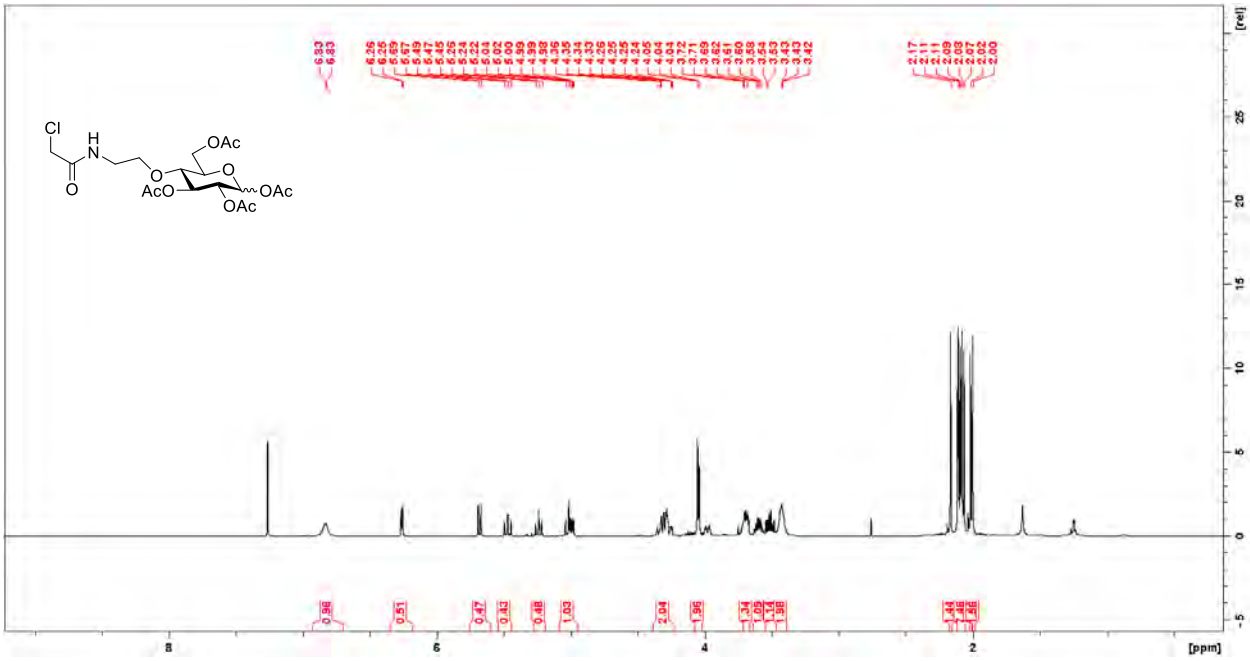
p-methylphenyl-2,3,6-tri-O-benzyl-4-O-(2-chloroacetylaminoethyl)-1-thio-β-D-glucopyranoside **S5** ^{1}H NMR.



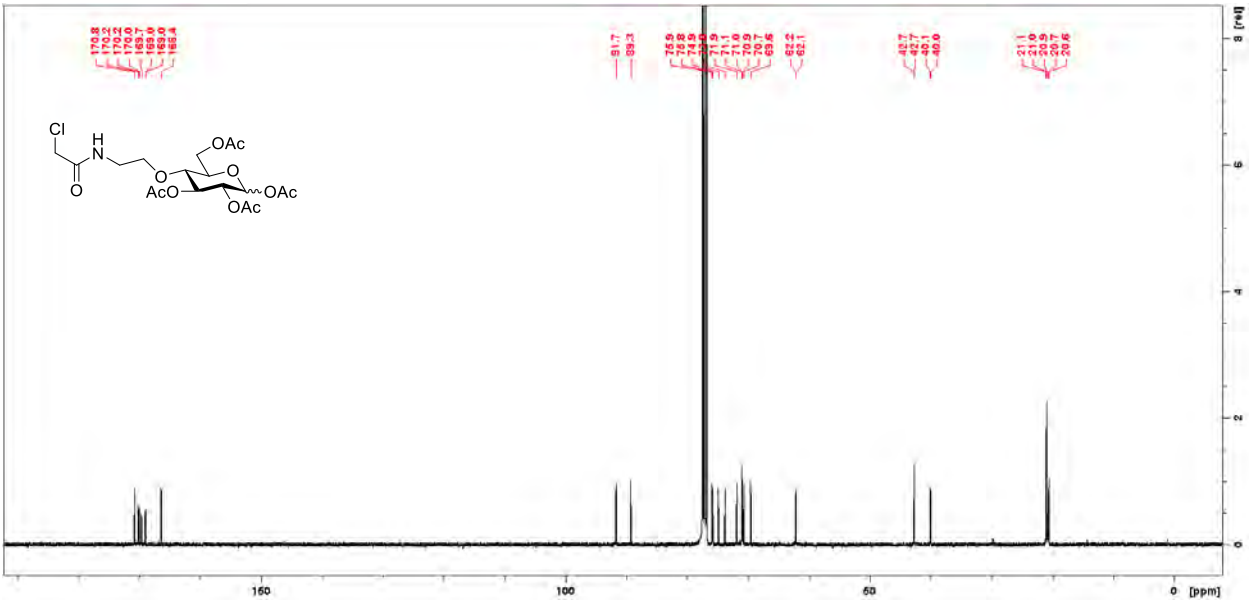
^{13}C NMR



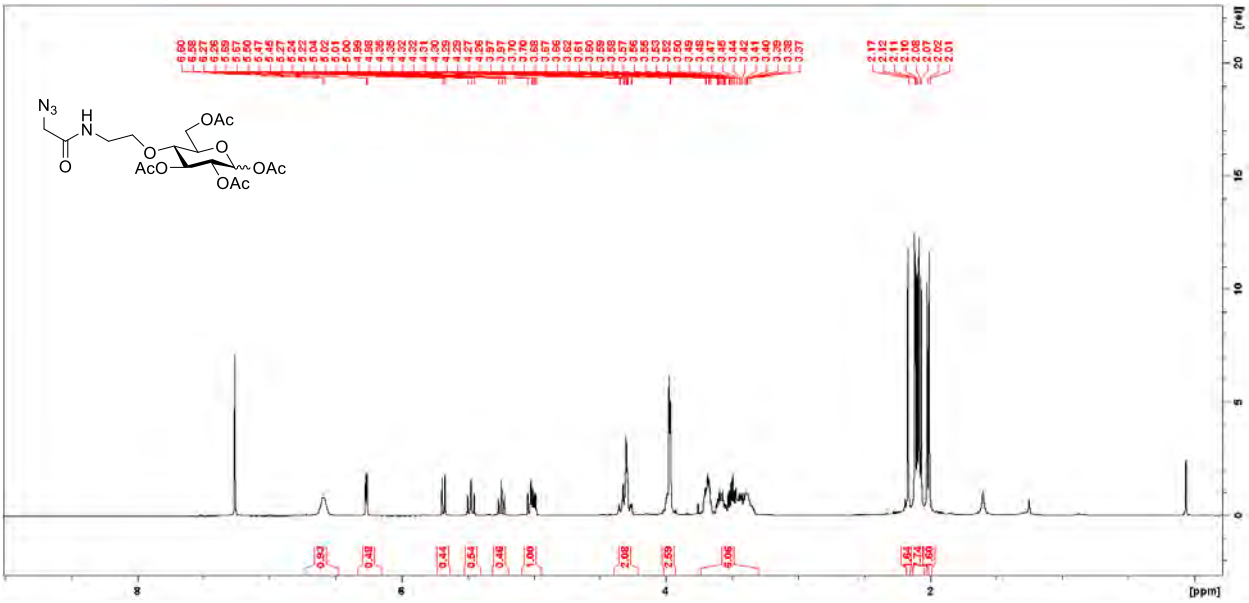
1,2,3,6-tetra-O-acetyl-4-O-(2-chloroacetylaminoethyl)-α-β-D-glucopyranoside **S6** ^{1}H NMR.



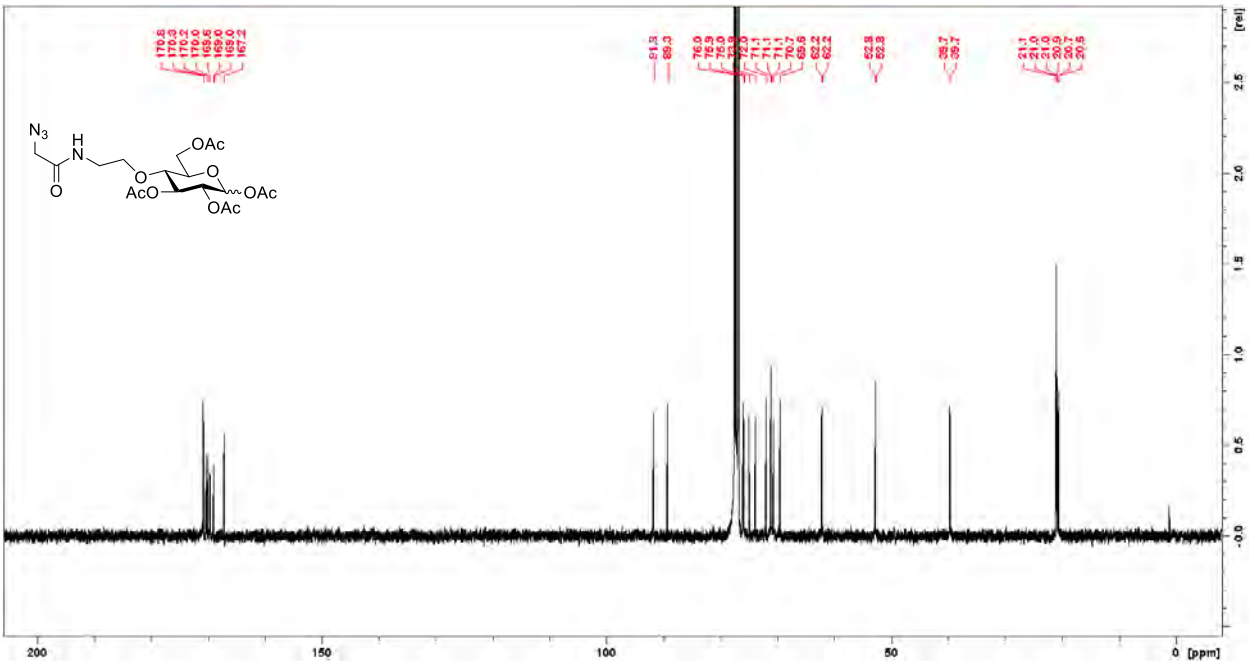
^{13}C NMR



1,2,3,6-tetra-O-acetyl-4-O-(2-azidoacetylaminoethyl)-α-β-D-glucopyranoside **13** ^{1}H NMR

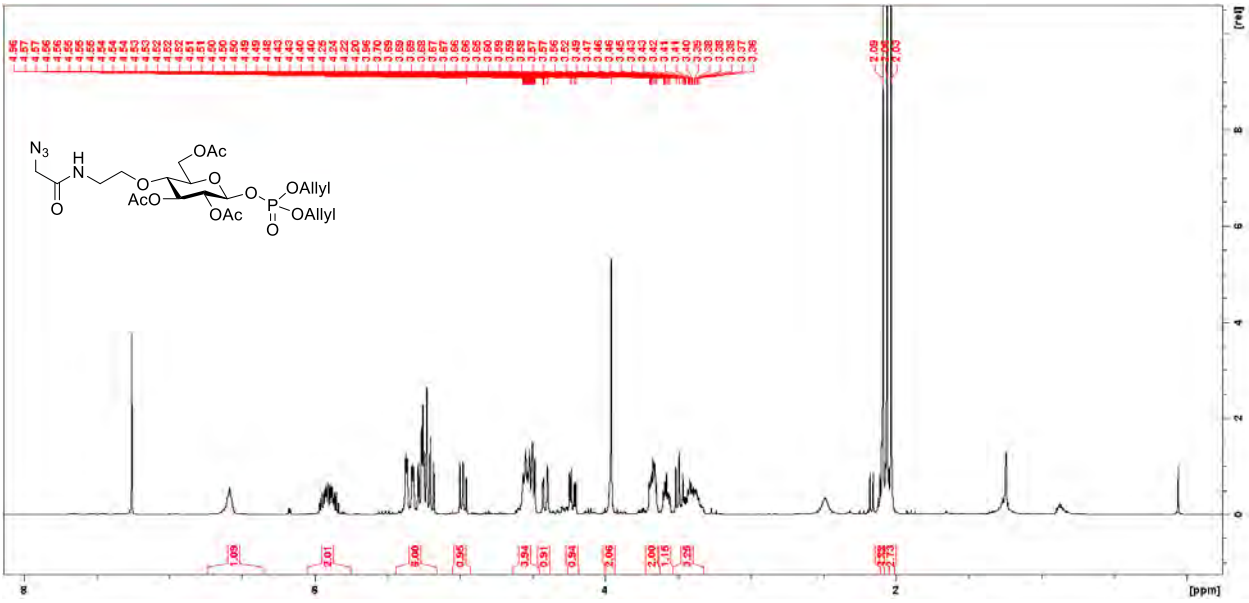


^{13}C NMR

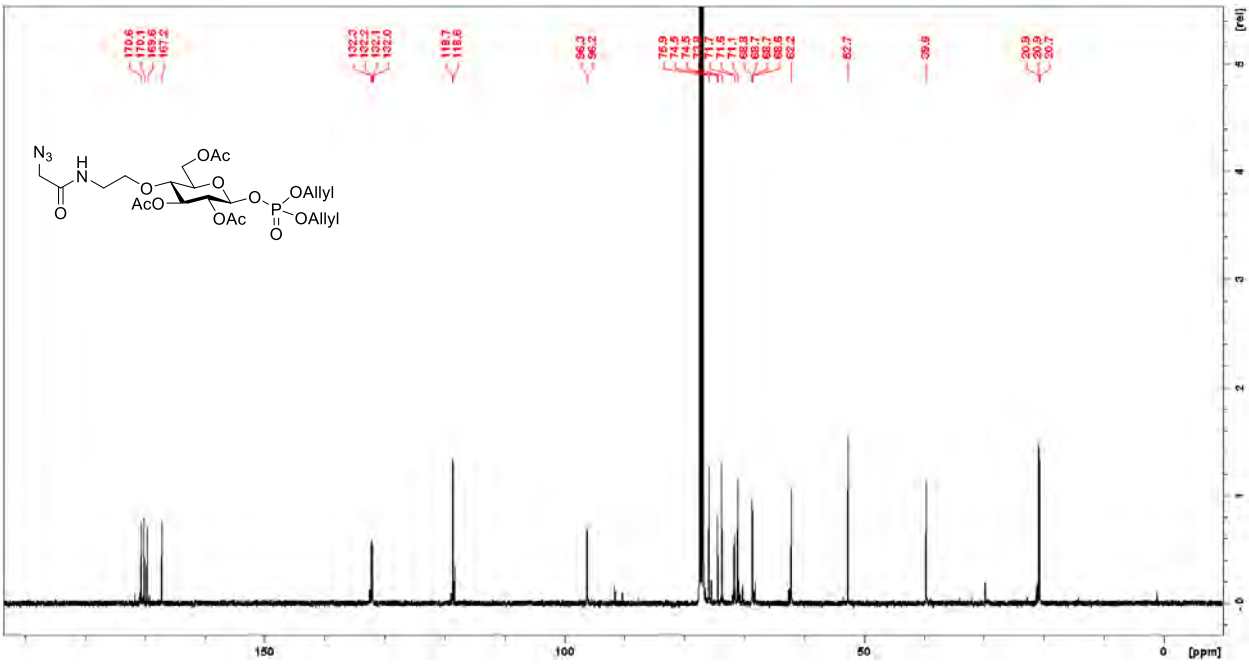


Diallyl(2,3,6-tri-O-acetyl-4-O-(2-azidoacetylaminoethyl)-β-D-gluco-pyranosyl)phosphate **14**

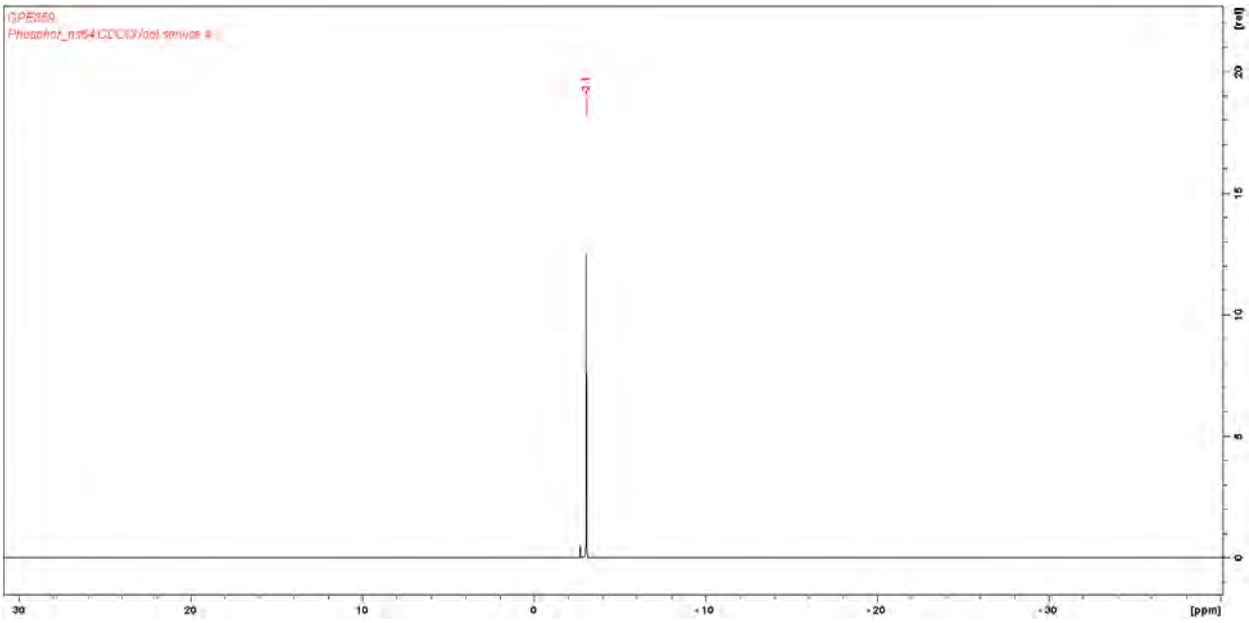
^{1}H NMR



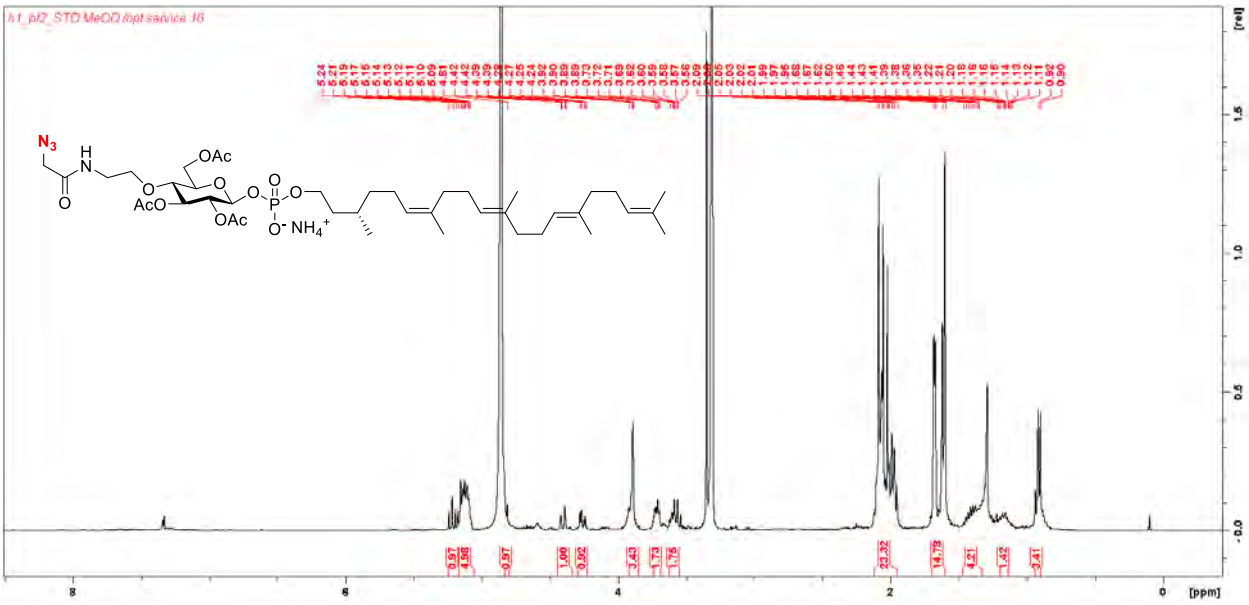
^{13}C NMR



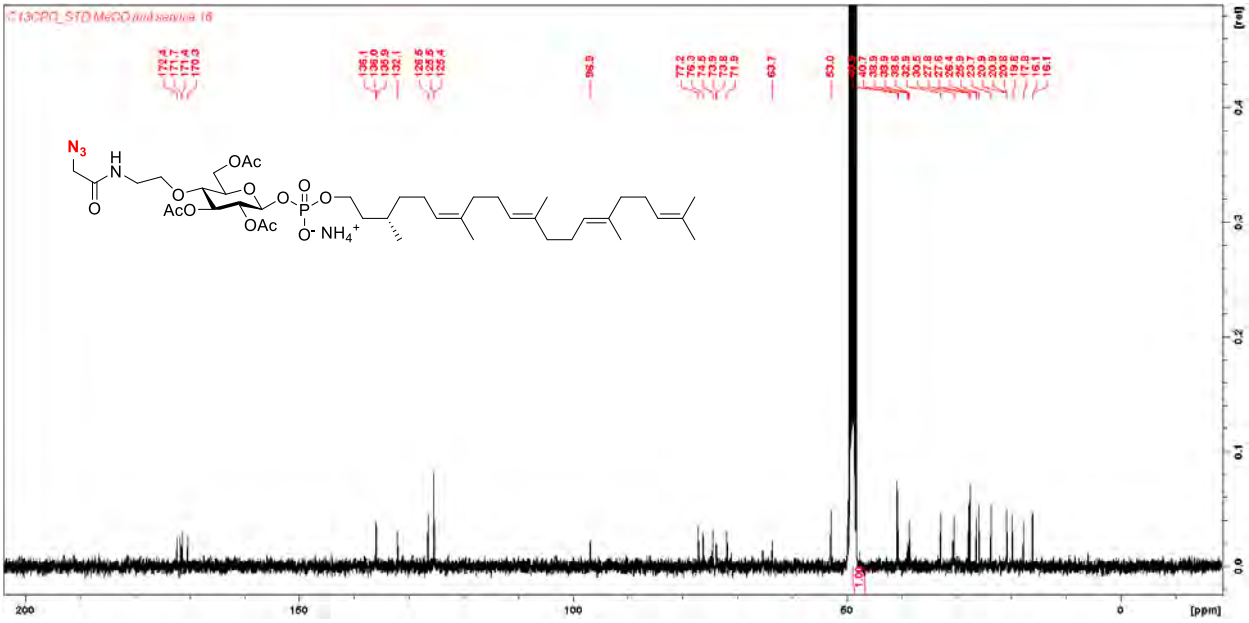
^{31}P NMR



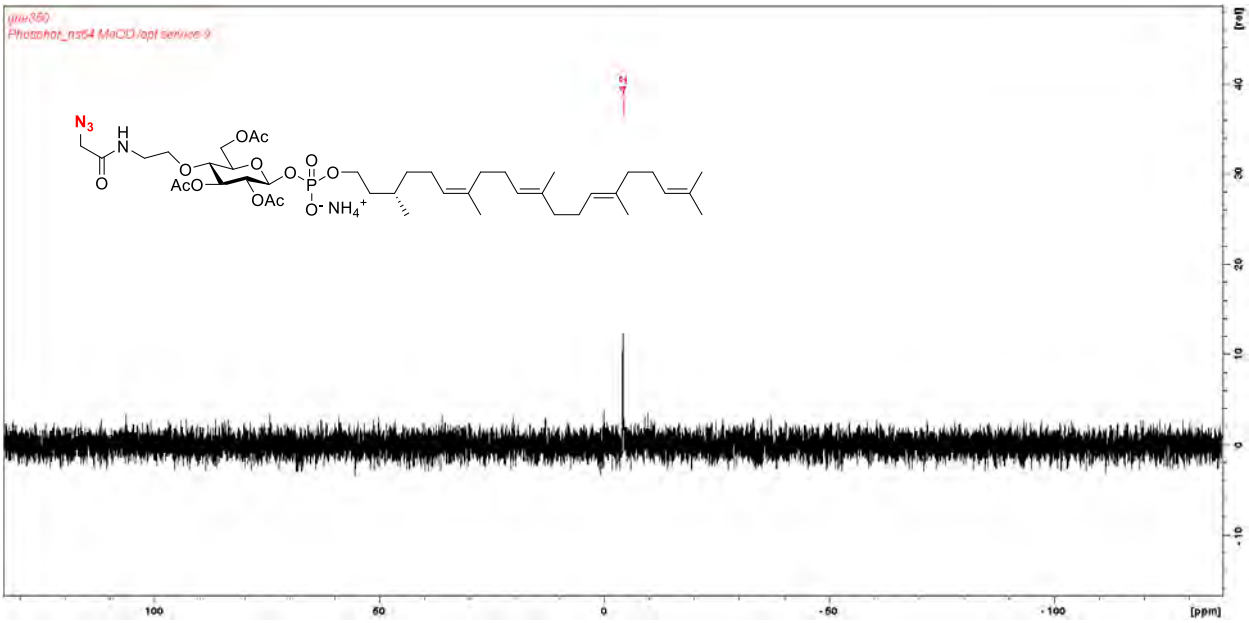
Farnesylcitronellylphosphoryl-2,3,6-tri-O-acetyl-4-O-(2-azidoacetylaminoethyl)-β-D-gluco-pyranoside **S7** ^{1}H NMR



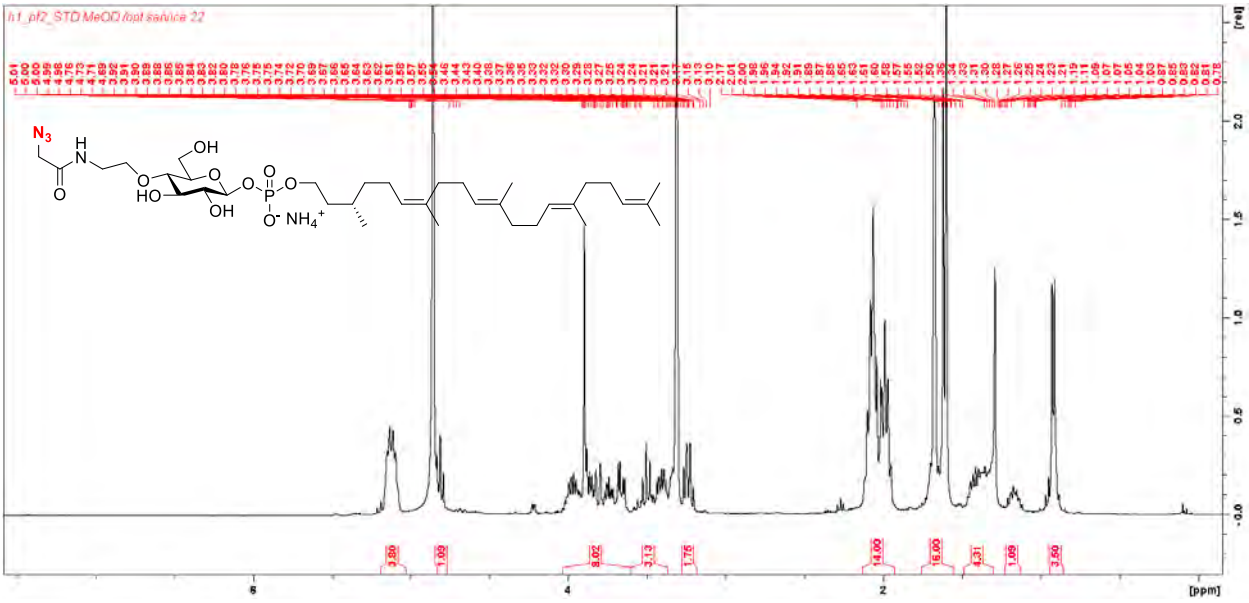
^{13}C NMR



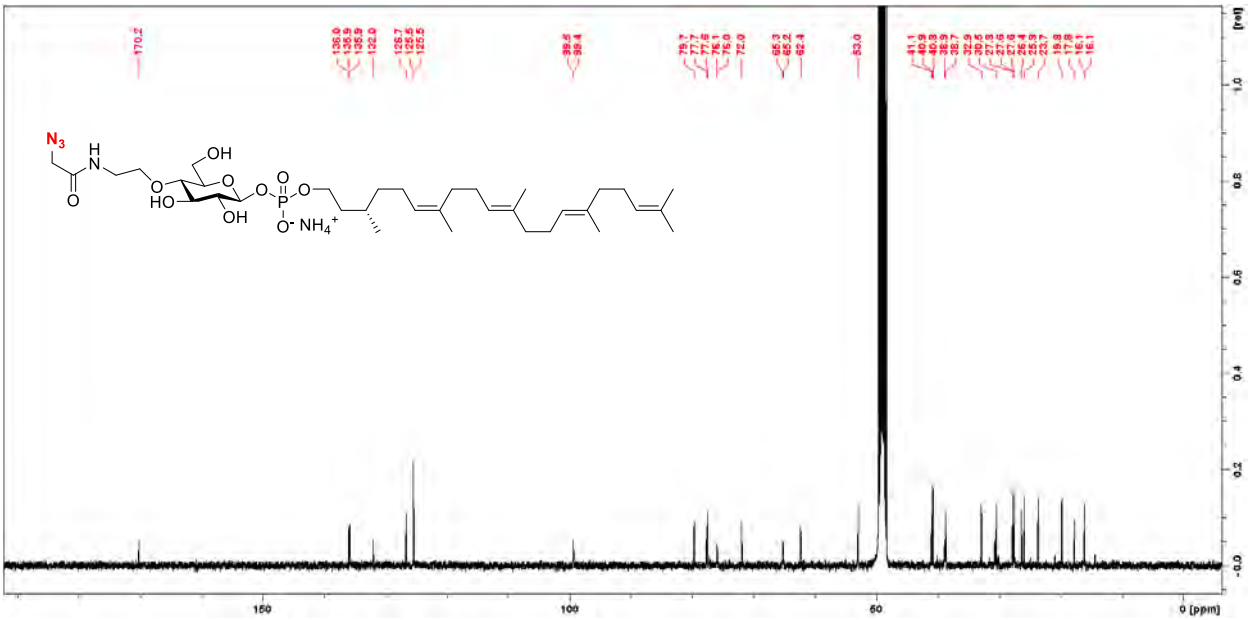
^{31}P NMR



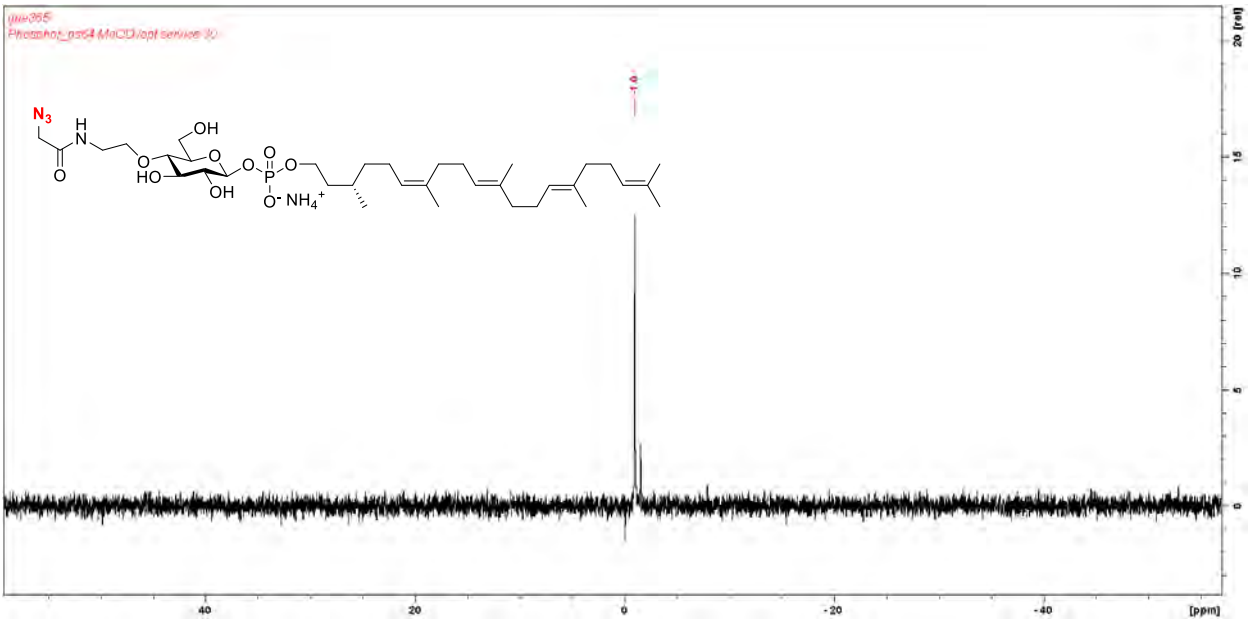
Farnesylcitronellylphosphoryl-4-O-(2-azidoacetylaminoethyl)-β-D-gluco-pyranoside **15** ^{1}H NMR



^{13}C NMR



^{31}P NMR



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