CHEMOSELECTIVE STAUDINGER-PHOSPHITE REACTION OF SYMMETRICAL GLYCOSYL-PHOSPHITES WITH AZIDO-PEPTIDES AND POLYGYCEROLS

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SUPPORTING INFORMATION

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GENERAL REMARKS

Reagents and chemicals were purchased from Sigma (Deisenhofen, Germany), Roth (Karlsruhe, Germany), AppliChem (Darmstadt, Germany), Merck (Darmstadt, Germany), ACROS (Geel, Belgium), ABCR (Karlsruhe, Germany) and Boehringer Mannheim (Mannheim, Germany).

Water or air sensitive reactions were performed in dry flask under argon atmosphere using standard *Schlenck*-techniques. Dry tetrahydrofurane, dichloromethane and toluene were obtained from a Glass Contour 6 – solvent purification system. Dry dimethylformamide and methanol were purchased from ACROS. Pyridine and triethylamine were dried and stored on potassium hydroxide. Phosphorus trichloride was distilled freshly before use. Column chromatography was performed on silica gel 60 µM (Merck). TLC were run on Merck TLC plates (DC Kieselgel 60 F_{254}) evaluated in UV light (254 nm) and stained with appropriate stains. Technical grade solvents for column chromatography were distilled prior to use.

Dialysis was performed with benzoylated cellulose tubing, MWCO 3500 Da purchased from sigmaaldrich.

^{1}H-NMR, ^{13}C-NMR were recorded on a Jeol ECX/400, in $CDCl_3$, MeOD or D_2O (Deutero GmbH). The chemical shifts were determined relatively to TMS using the residual solvent peak as internal calibration signal. IR spectra were measured a Nexus FT-IR spectrometer equipped with a Nicolet Smart DuraSampleIR ATR.

HPLC-HRMS and conversion studies of azido-peptide A were performed on an Agilent 6210 TOF LC/MS system, Agilent Technologies, Santa Clara, CA, USA. Spray voltage was set to 4 kV. Drying gas flow rate was set to 25 psi. Separation of the sample was performed on a Luna 5u C18(2) 100 A column (5 µm, 4.6×150 mm) at a flow rate of 0.6 mL/min. The following solvent (A=1% AcOH in H2O, B=1% AcOH in MeCN) gradient was applied: 0% B 0-5 min; 0-10% B 5-6 min; 10-60% B 6-31 min; 60-100% B 31-34 min; 100% B 34-40 min. An analogous peptide containing deuterated alanine was added before the measurements as an internal standard and conversion was calculated by integration of both. HPLC- Fluorescence spectra were recorded on a Waters 600S controller with a Jasco FP 2020 Plus fluorescence detector connected to the waters system. The excitation wavelength was 470 nm, the emission wavelength 530 nm. Separation was performed on an Agilent eclipse XDB C18 5µm column (5µm, 4.6 x 180 mm) at a flow rate of 1 mL/min. The following solvent (A=1% AcOH in H2O, B=1% AcOH in MeCN) and gradient was applied: 0% B 0-5 min; 0-10% B 5-6 min; 10-60% B 6-31 min; 60-100% B 31-34 min; 100% B 34-40 min.

Peptide synthesis: Peptides **10** and **12** were synthesized on an ABI 433A peptide synthesizer using standard Fmoc based SPPS (0,1 mmol scale), amide coupling conditions HBTU/HOBt utilizing preloaded wang resin (Novabiochem) analogously to our previously published protocol (R. Serwa et al. J. Pept. Sci. 2010, see ref. 13 in manuscript). Fmoc-*p*azido-Phe-OH was coupled manually (3 eq). Cleavage from the resin was performed with trifluoroacetic acid (95%), and triisopropylsilane/water 1:1 as scavenger for 2 h. Coupling and cleavage procedures are described by Novabiochem. Fmoc-*p*azido-Phe-OH was obtained from BACHEM.

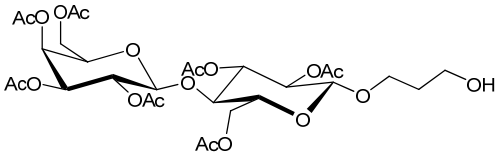
SPR measurement: SPR measurements were carried out at 25 °C on a Biacore X instrument (GE Healthcare, Freiburg, Germany) as described in Artner *et al.*, *Chem. Commun.*, 2012, **48**, 522-524. Changes of note were the use of a different running buffer which was containing 20 mM HEPES, pH 7.4; 150 mM NaCl and 1 mM $CaCl_2$. Binding analyses were performed with running buffer at a flow rate of 20 µl/min. To measure peanut agglutinin (PNA) interaction to immobilized TF antigen, a 500 nM solution of PNA (Axxora GmbH, Loerrach, Germany) was used and the resulting RU value was set to 100% binding (positive control). For all competitive measurements PNA was preincubated for 18 min at room temperature with the functionalized polyglycerols at a final concentration of 100 µM polyglycerol before injection. The resulting RU values were calculated as X% binding of the control and converted to % inhibition.

SYNTHESIS OF CARBOHYDRATE PHOSPHITES

*Synthesis of **5**, **6** and **7**:*

Compounds **5**, **6** and **7** were synthesized according to previously published protocols.[1-4]

*Synthesis of **14**[1]:*



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A solution of the peracetylated lactose-1-bromide (1.0 g, 1.43 mmol) was suspended in 1,3-propanediol (20 mL), treated with Ag_2CO_3 (710 mg, 2.57 mmol), and stirred at room temperature. Silver mirror started to form within 30 minutes. After stirring for 15 h, the mixture was washed with toluene (5 x 20 mL). The combined washings were evaporated and purified by column chromatography. Flash chromatography (silicagel, EtOAc/n-hexanes 7:3). Yield 596 mg (60% yield). For further details, refer to [1].

^{1}H–NMR (400 MHz; $CDCl_3$)): δ 5.32 (dd, *J* = 3.4 Hz, 1 H); 5.17 (t, *J* = 9.3 Hz, 1 H); 5.08 (dd, *J* = 10.4 Hz, 7.9, 1 H); 4.93 (dd, *J* = 10.4 Hz, 3.4, 1 H); 4.86 (dd, *J* = 9.5, 7.9 Hz, 1 H); 4.51 (dd, *J* = 11.9, 2.0 Hz, 1 H); 4.47 (dd, *J* = 7.9, 0.7 Hz, 2 H); 4.13-4.03 (m, 3 H); 3.94 (dt, *J* = 9.9, 6.0 Hz, 1 H); 3.85 (t, *J* = 6.3 Hz, 1 H); 3.77 (t, *J* = 9.4, 1 H); 3.73-3.63 (m, 3 H); 3.59 (ddd, *J* = 9.9, 5.1, 2.1 Hz, 1 H); 2.13, 2.11, 2.04, 2.02, 2.02, 1.94, 1.90 (7 s, 21 H); 1.91 (s (br.), OH); 1.78 (quintet, *J* = 5.7 Hz, 2 H).

^{13}C–NMR (100 MHz; $CDCl_3$)): δ 170.53, 170.43, 170.22, 170.13, 169.84, 169.82, 169.16 (7 s); 100.14, 100.56, 76.34, 72.73, 71.66, 71.02, 70.74, 69.15, 66.66 (9 d); 67.76, 61.93, 60.86, 60.06, 32.13 (5 t); 20.91, 20.86, 20.75, 20.70, 20.57 (5 q, peaks overlapping).

IR (ATR): 3541*w*, 2945*w*, 2880*w*, 2363*w*, 2330*w*, 1740*s*, 1431*w*, 1368*m*, 1212*s*, 1169*m*, 1131*m*, 1035*s*, 978*m*, 955,, 902*m*, 834*w*, 738*w*.

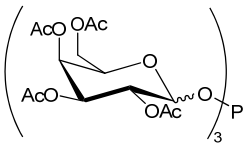
SYNTHESIS OF THE SYMMETRICAL GLYCOSYL PHOSPHITES

TLC was conducted on silica gel plates pretreated with the solvent containing 2% Et_3N.

General procedure for the synthesis of the sugar phosphites:

A solution of the alcohol **5**,[2] **6**,[3] **7**,[1, 4] or **14**[1, 4] (0.615 mmol) in THF (2 mL) was cooled to 0°C, treated with Et_3N (172 µL, 1.23 mmol, 2.0 eq.) followed by PCl_3 (17.3 µL, 0.199 mmol, 0.3 eq.) that was added slowly (transferred *via* an oven dried 100 µL glass syringe). The mixture was stirred vigorously while allowing to warm up to room temperature overnight. TLC showed formation of a new spot slightly above the starting alcohol. After completion of the reaction (monitored by ^{31}P–NMR), the mixture was cooled to 0°, treated with cold Et_2O (3 mL) to facilitate complete precipitation of $Et_3N^{\cdot}HCl$, which was removed by centrifugation and filtration. The filtrate was evaporated under reduced pressure strictly below 30°, and purified by fast column chromatography on a short pad of silica gel. FC (EtOAc/n-hexane/Et_3N 30:20:1 → 70:30:2).

ANALYTICAL DATA OF THE PHOSPHITES:



1

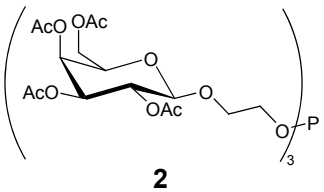
The phosphite was used as a crude product due to decomposition during purification. Assignment of ^{1}H and ^{13}C–NMR was cumbersome due to the overlapping signals from both α/β anomers and due to the 4 possible diastereomers resulting from the mixture.

^{1}H NMR (*crude*, 400 MHz, Methanol-d4) δ 5.85 (dd, *J* = 8.4, 3.5 Hz, 1 H, C(1)H, minor diastereomer), 5.81 (dd, *J* = 7.9, 3.4 Hz, 1H, C(1)H, major diastereomer), 5.47 (dd, *J* = 3.5, 1.3 Hz, 1 H, minor diastereomer), 5.41-5.31 (m, 7H), 5.29-5.17 (m, 4H), 5.16 – 5.01 (m, 6H), 4.76 (d, *J* = 7.3 Hz, 1H), 4.46 (ddd, *J* = 7.7, 6.1, 1.3 Hz, 2H), 4.20 (d, *J* = 5.4 Hz, 5H, C(6)H_2), 4.16 – 3.98 (m, 5H, C(6)H_2), 2.16 – 1.91 (m, 61H, Ac).

^{31}P–NMR: (400 MHz, CD_3OD): 141.00.

IR (ATR): 2943*w*, 2832*w*, 1745*s*, 1434*w*, 1370*m*, 1214*s*, 1129*w*, 1063*m*, 1023*s* (br.), 935*m*, 971*m*, 808*w*, 732*w*.

HR-ESI-MS: 1095.2572, $[M + Na]^+$, calc. for $C_{42}H_{57}O_{30}PNa$ = 1095.2570.



2

Following the general procedure for the synthesis of symmetrical phosphites 132 mg of **2** (109 mmol) were obtained after purification, which corresponds to a yield of 55%.

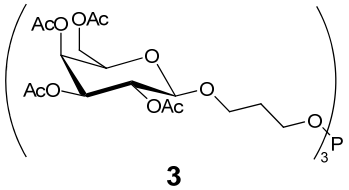
^{1}H–NMR (400 MHz, CD_3OD): δ 5.38 (d, *J* = 3.0 Hz, H–C(4)); 5.14-5.06 (m, 2 H); 4.71 (d, *J* = 7.4 Hz, H–C(1)); 4.16-4.09 (m, 3 H); 3.97-3.93 (m, 3 H); 3.79–3.72 (m, H–C(5)); 2.14 (s, 3H); 2.06 (s, 3H); 2.01 (s, 3H); 1.93 (s, 3H).

^{13}C–NMR (100 MHz, CD_3OD): δ 170.75, 170.69, 170.19, 170.13 (4s), 100.97 (d), 71.04 (d), 70.47 (d), 69.56, 69.51 (2 t), 69.08 (d), 67.53 (d), 61.21 (t), 19.68, 19.32, 19.26, 19.20 (4q).

^{31}P–NMR: (400 MHz, CD_3OD): 139.85.

IR (ATR): 2940*w*, 2880*w*, 1741*s*, 1432*w*, 1367*m*, 1212*s*, 1174*m*, 1134*m*, 1033*s* (br.), 952*m*, 915*m*, 738*m*.

HR-ESI-MS: 1205.3532, $[M + H]^+$, calc. for $C_{48}H_{70}O_{33}P$ = 1205.3537.



3

Following the general procedure for the synthesis of symmetrical phosphites 149 mg of **3** (119 mmol) were obtained after purification, which corresponds to a yield of 60%.

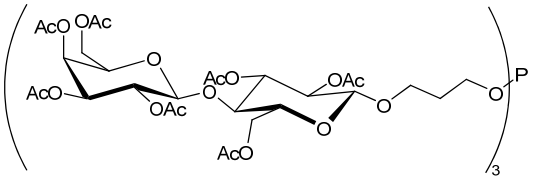
^{1}H–NMR (400 MHz, CD_3OD): δ 5.37 (dd, *J* = 3.2, 0.4 Hz, H–C(4)); 5.12 (dd, *J* = 10.4, 3.3 Hz, H–C(3)); 5.07 (dd, *J* = 10.4, 7.5 Hz, H–C(2)); 4.64 (d, *J* = 7.5 Hz, H–C(1)); 4.15–4.07 (m, 3 H); 3.95 (dt, *J* = 10.0, 5.7 Hz, 1 H); 3.90–3.81 (m, 2 H); 3.64 (dt, *J* = 9.9, 6.3 Hz, 1 H); 2.13, 2.05, 2.01, 1.93 (4 s, 12 H); 1.85 (quintet, *J* = 6.4 Hz, 2 H).

^{13}C–NMR (100 MHz, CD_3OD): δ 170.73, 170.70, 170.19, 170.03 (4 s); 100.97 (d); 71.03, 70.42 (2 d); 69.17 (d); 67.53 (d); 66.01 (t); 61.21 (t); 58.51 (t); 30.98 (t); 19.61, 19.33, 19.26, 19.21 (4 q).

^{31}P–NMR: (400 MHz, CD_3OD): 139.94.

IR (ATR): 2944*w*, 2885*w*, 2832*w*, 1743*s*, 1650*w*, 1492*w*, 1368*m*, 1215*s*, 1172*m*, 1134*m*, 1024*s* (br.), 975*m*, 955*m*, 906*m*, 812*w*, 735*m*.

HR-ESI-MS: 1247.3992 $[M + H]^+$, calc. for $C_{51}H_{76}O_{33}P$ = 1247.4006.



4

Following the general procedure for the synthesis of symmetrical phosphites 235 mg of **4** (111 mmol) were obtained after initial chromatographic purification, which corresponds to a yield of 56%, that contained *ca.* 10% of the unreacted alcohol **11**. Repeated flash chromatography resulted in decomposition as well as in the reduction of recovery of the desired phosphite.

^{1}H–NMR (700 MHz, $CDCl_3$)): δ 5.33 (d, *J* = 2.8 Hz, H–C(4')); 5.18 (t, *J* = 9.0 Hz, H–C(3)); 5.10 (dd, J = 10.4, 7.8 Hz, H–C(2')); 5.00 (dd, *J* = 10.4, 3.4 Hz, H–C(3')); 4.87 (dd, *J* = 9.5, 8.0 Hz, H–C(2)); 4.60 (d, *J* = 8.0, H–C(1')); 4.53-4.45 (m, H–C(1), H_a–C(6)); 4.31–3.84 (several m, 10 H); 2.16, 2.11, 2.10, 2.05, 2.04, 2.03, 1.97 (7s, 21 H), 1.80 (quintet, *J* = 6.0 Hz, 2 H).

^{13}C–NMR (100 MHz, CD_3OD): δ 170.68, 170.37, 170.34, 170.07, 170.04, 169.43, 169.18 (7 s); 102.40, 101.07 (2 d); 76.26 (d); 72.67, 71.67, 70.95, 70.68, 69.95, 68.80 (6 d); 67.67 (t); 66.61 (d); 63.40, 60.97, 60. 56 (3 t); 32. 09 (t); 20.89, 20.82, 20.69, 20.61, 20.53, 20.49 (6 q, peaks overlapping).

^{31}P–NMR: (400 MHz, CD_3OD): 140.07.

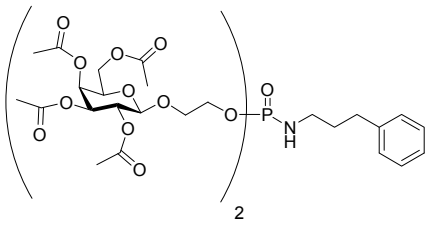
IR (ATR): 2965*w*, 2926*w*, 2853*w*, 1743*s*, 1650*w*, 1432*w*, 1369*m*, 1217*s*, 1170*m*, 1133*m*, 1044*s* (br.), 978*m*, 954*m*, 902*m*, 839*w*, 739*m*.

HR-ESI-MS: 2111.6491, $[M + H]^+$, calc. for $C_{87}H_{124}O_{57}P$ = 2111.6542.

STAUDINGER REACTION WITH SMALL MOLECULES

GENERAL PROCEDURE

A solution of the corresponding phosphite (1 equiv.) in acetonitrile was treated with (3-azidopropyl)benzene (1.5 equiv.) and heated to 45°C. After 50 hours the reaction was cooled to room temperature, some drops of water were added and the reaction mixture was stirred for additional 6 hours. The solvent was removed under reduced pressure und the crude product was purified by column chromatography (EtOAc/*c*-hexane 3: 1 → 9:1) to yield the corresponding phosphoramidate.



8

Phosphite **2**: 49.8 mg, 41.3 µmol.

Phosphoramidate **8**: 37.5 mg, 38.9 µmol, 94%.

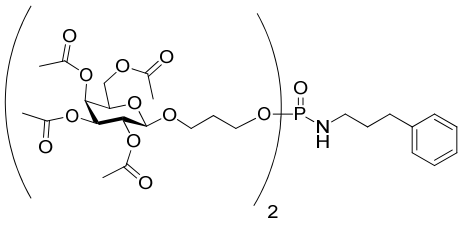
^{1}H NMR (500 MHz, CD_3CN) δ 7.35 – 7.16 (m, 5H), 5.36 – 5.32 (m, 2H), 5.09 – 5.00 (m, 4H), 4.66 – 4.60 (m, 2H), 4.14 (dd, *J* = 11.2, 6.9 Hz, 2H), 4.10 – 3.99 (m, 8H), 3.97 – 3.91 (m, 2H), 3.77 – 3.71 (m, 2H), 3.42 (dt, *J* = 11.1, 6.8 Hz, 1H), 2.89 (dq, *J* = 10.4, 7.0 Hz, 2H), 2.70 – 2.63 (m, 2H), 2.09 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.00 – 1.99 (m, 6H), 1.92 (s, 6H), 1.82 – 1.75 (m, 2H).

^{13}C NMR (126 MHz, CD_3CN) δ 171.2 (*C*=O), 171.2 (*C*=O), 170.8 (*C*=O), 170.7 (*C*=O), 143.2 (C_q), 129.4 (*C*H), 129.4 (*C*H), 126.8 (*C*H), 101.9 (*C*H), 71.7 (*C*H), 71.6 (*C*H), 69.8 (CH_2, d, *J* = 7.4 Hz), 69.8 (*C*H), 68.4 (*C*H), 65.8 (CH_2), 62.3 (CH_2), 41.7 (CH_2), 34.4 (CH_2, d, *J* = 5.9 Hz), 33.5 (CH_2), 21.1 (CH_3), 21.1 (CH_3), 20.9 (CH_3), 20.8 (CH_3).

^{31}P–NMR: (202 MHz, CD_3CN): 5.69.

IR (ATR): 2929*w*, 2036*w*, 1746*s*, 1432*w*, 1369*m*, 1220*s*, 1175*m*, 1045*s* (br.), 979*m*, 957*m*, 919*w*.

HR-ESI-MS: 986.3029, $[M + Na]^+$, calc. for $C_{41}H_{58}NNaO_{23}P$ = 986.3125.



9

Phosphite **3**: 48.0 mg, 38.5 µmol.

Phosphoramidate **9**: 36.6 mg, 36.9 µmol, 96%.

^{1}H NMR (500 MHz, Acetonitrile-d3) δ 7.32 – 7.13 (m, 5H), 5.35 – 5.31 (m, 2H), 5.07 – 4.97 (m, 4H), 4.59 – 4.54 (m, 2H), 4.13 (ddd, *J* = 11.2, 6.8, 1.0 Hz, 2H), 4.06 (ddd, *J* = 11.2, 6.0, 1.4 Hz, 2H), 3.99 (ddd, *J* = 7.0, 5.7, 1.2 Hz, 2H), 3.93 (ddd, *J* = 7.4, 6.9, 5.6 Hz, 4H), 3.88 (dtd, *J* = 10.2, 5.9, 3.3 Hz, 2H), 3.61 (dtd, *J* = 10.2, 6.4, 2.5 Hz, 2H), 3.35 (dt, *J* = 10.8, 6.8 Hz, 1H), 2.86 (dq, *J* = 10.6, 7.0 Hz, 2H), 2.68 – 2.60 (m, 2H), 2.10 – 2.07 (m, 6H), 2.03 – 2.01 (m, 6H), 1.99 (s, 6H), 1.92 (s, 6H), 1.89 – 1.83 (m, 4H), 1.77 (dt, *J* = 14.7, 7.4 Hz, 2H).

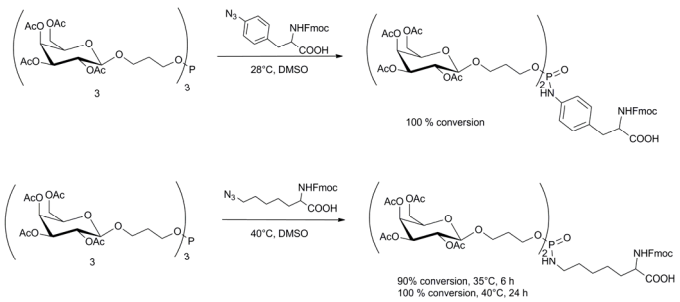
^{13}C NMR (126 MHz, Acetonitrile-d3) δ 171.2 (*C*=O), 171.2 (*C*=O), 170.8 (*C*=O), 170.6 (*C*=O), 143.2 (C_q), 129.9 (*C*H), 129.4 (*C*H), 129.4 (*C*H), 129.2 (*C*H), 126.8 (*C*H), 126.3 (*C*H), 101.9 (*C*H), 71.7 (*C*H), 71.6 (*C*H), 69.9 (*C*H), 68.4 (*C*H), 66.9 (CH_2, d, *J* = 7.8 Hz), 63.4 (CH_2), 62.4 (CH_2), 41.7 (CH_2), 34.4 (CH_2, d, *J* = 5.6 Hz), 33.5 (CH_2), 31.4 (CH_2), 21.0 (CH_3), 20.9 (CH_3), 20.8 (CH_3), 20.8 (CH_3).

^{31}P–NMR: (202 MHz, CD_3CN): 5.55.

IR (ATR): 2937*w*, 2358*w*, 2036*w*, 1980*w*, 1748*s*, 1431*w*, 1370*m*, 1221*s*, 1174*m*, 1054*s* (br.), 907*w*.

HR-ESI-MS: 1014.3342, $[M + Na]^+$, calc. for $C_{41}H_{58}NNaO_{23}P$ = 1014.3439.

STAUDINGER REACTION WITH AMINOACIDS



Scheme S1: Synthesis of small glycosyl phosphoreamidate amino acids via Staudinger-phosphite reaction

The phosphite **3** was reacted against the Fmoc protected alkyl and aryl azidoaminoacids to test for the general reactivity of the phosphites. The reaction was conducted in analytical grade DMSO. No additional H_2O was added. Solutions of the aminoacid (10 µl, 50 mM) and the phosphite (10 µl, 150 mM) were added into DMSO (80 µl) in an Ependorf vial, vortexed, centrifuged and shaken at 28° for 6 h. As expected the aryl azide Fmoc-*p*azido-Phe-OH reacted completely and exclusively into the desired phosphoramidite in 6 h at 28 °C, whereas, the alkyl azide Fmoc-*p*azido-Lys-OH needed elevated temperature (40 °C) and longer reaction time (> 24 h) for the 100% conversion. The extent of the conversion was determined by the UV-trace in LC-MS. The results lead to the application of the Staudinger-phosphite reaction for the chemoselective functionalization of aryl and alkyl azidopeptides, dendrimers, and oligonucleotides.

STAUDINGER REACTION WITH PEPTIDES

A stock solution of the peptide (40 mM in DMSO) and glycosyl phosphite **3** (40 mM in DMSO) was prepared. The reaction was performed by mixing peptide stock solution (10 µL) and glycosyl phosphite stock solution (30 µL) and incubated at room temperature for 6 h.

Peptide 10:

Retention time: 18.223 min.

HRMS: ($C_{46}H_{66}N_{13}O_{12}$:$[M+H^+]$ calcd: 992.4948, found: 992.4929.

HRMS: ($C_{46}H_{60}D_6N_{13}O_{12}$:$[M+H^+]$ calcd: 998.5325, found: 998.5338.

Peptide 11:

Retention time: 21.786 min.

HRMS: ($C_{80}H_{117}N_{11}O_{35}P^+$:[M+H$^+$] calcd: 1822.7446, found: 1822.7479.

To the crude reaction mixture was added one equivalent of a deuterated analog of peptide **10** (both alanines were replaced with D3-alanine), conversion was then analyzed by HPLC-MS.

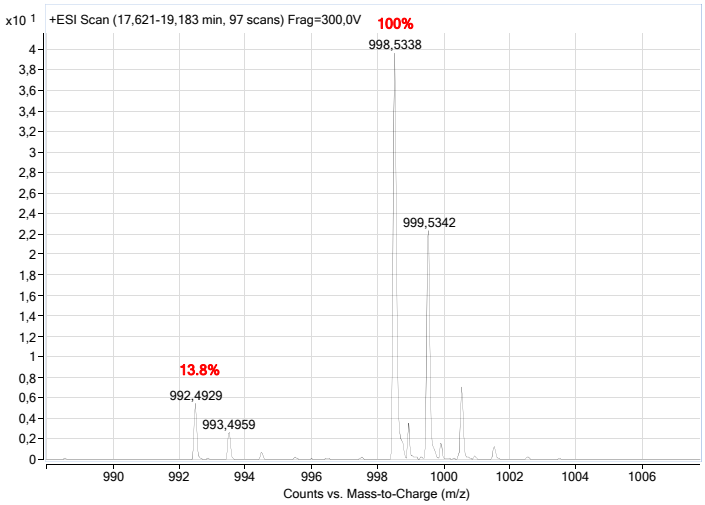


Figure S1: HPLC-MS spectra of azido-peptide **10** after Staudinger-phosphit reaction

Peptide 12:

Retention time: 16.017 min.

HRMS: ($C_{49}H_{72}N_{17}O_{17}$:[M+H$^+$] calcd: 1170.5287, found: 1170.5258.

Peptide 13:

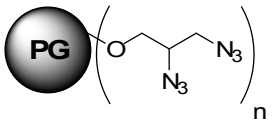
The crude reaction mixture was analyzed by fluorescence HPLC (530/470 nm).

Retention time: 20.695 min.

HRMS: ($C_{83}H_{123}N_{15}O_{40}P$+:[M+H$^+$] calcd: 2000.7784, found: 2000.7763.

DENDRITIC OLIGOSACCHARIDES

SYNTHESIS OF POLYGLYCEROL AZIDES[5]



core: 7.7 kDa

A: DF 100%
13.5 mmol N_3/g

C: DF.: 30%
4.05 mmol N_3/g

core 12.6 kDa

B: DF 98 %
13.2 mmol N_3/g

core 10 kDa

D: DF.: 32%
4.32 mmol N_3/g

Synthesis of O-Mesylpolyglycerol. Polyglycerol was dissolved in dry pyridine in a three necked flask under inert conditions. The solution was cooled to 0°C and then methanesulfonyl chloride was added dropwise that the temperature did not exceed 5°C. After stirring for one hour, the temperature was thereafter allowed to rise to 25 °C and the red mixture was stirred for 16 h. Solvent was removed under vacuum and the crude product was dissolved and dialysed in acetone to give a yellow honey-like product. Conversion: 30%.^{1}H-NMR (400 MHz, MeOD): δ = 5.16 − 4.74 (functionalised secondary PG-groups), 4.63−4.20 (functionalised primary PG-groups), 3.17 (Me), 0.89 (PG-starter); ^{13}C-NMR (400 MHz, MeOD): δ = 83.1 − 69.0 (PG), 38.2 (Me); IR (KBr): ν = 3030, 2941, 2361, 1709, 1457, 1362, 1184, 971, 813, 753 cm^{-1}.

Synthesis of Polyglycerolazide. O-Mesylpolyglycerol was dissolved in dry DMF in a one-necked flask with reflux condenser and magnetic stirrer. After addition of NaN_3 the resulting suspension was heated at 60°C for 72 h. After cooling, filtration delivered a yellowish filtrate and a white residue of excess NaN_3. The filtrate was concentrated *in vacuo* at 60°C and was dissolved and dialysed in methanol. Conversion: quant.; yield: 560 mg, 96%; ^{1}H-NMR (400 MHz, MeOD): δ = 4.23–2.87 (PG), 1.81 (PG-starter), 0.85 (PG-starter); ^{13}C-NMR (62.5 MHz, MeOD): δ = 81.9–67.5 (PG), 60.5 (functionalised secondary PG-groups), 51.5 (functionalised primary PG-groups); IR (KBr): ν = 2873, 2361, 2102 (N_3), 1457, 1273, 1122, 668 cm^{-1}.

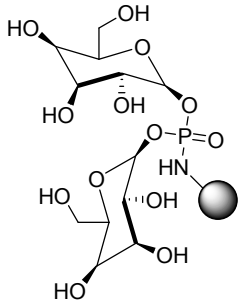
GENERAL PROCEDURE FOR THE PREPARATION OF GLYCOSYLATED DENDRITIC POLYMERS VIA THE STAUGINGER-PHOSPHITE REACTION

0.4 µmol with respect to the azides of the dendritic polymer in DMSO (10 µL/0.39 mM (7.7 kDa) or 8 µL/0.54 mM (12.6 kDa)) was treated with 50 µL of a 10 mM solution of the corresponding phosphite in DMSO in an Eppendorf tube, shortly vortexed, spinned down and shaked at 40°C /1000 RPM for 20 h, while adding a second portion of 50 µL after 5 h (1.3 eq in total). The reaction mixture was diluted with chloroform and dialysed versus chloroform.

The solvent was evaporated. The degree of functionalisation could be determined via ^{1}H-NMR by integrating isolated carbohydrate signals (e.g. acetyl groups) in comparison to the PG backbone. Total conversion was then calculated with respect to the degree of functionalization with azido groups. The relative intensity of the residual N_3-signal in IR was concordant with the latter.

The solution for analysis in dry Chloroform was directly transferred to a round bottom flask for the following deprotection. The solution was diluted up to a content of 50% methanol. 3 µL NaOMe (30wt% in MeOH) were added and the reaction was stirred at room temperature for 20-30 min (2h for lactose). The solvent was evaporated, the residue dissolved in MeOH/H_2O (1/1) and dialysed versus Millipore water. After removal of the solvent, 1-3 mg glycosylated dendritic polymer were obtained.

ANALYTICAL DATA OF THE FUNCTIONALISED POLYMERS:



functionalisation
(determined via ^{1}H-NMR)

1A : 29 %
1B : 29 %

Figure S2: calculated degree of functionalisation of **PG1 A+B.**

PG1A-peracetylated

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 5.39 (d, *J* = 2.26 Hz, 2 H), 5.23-5.16 (m, 1 H), 5.16-5.05 (m, 1,6 H), 5.05-5.00 (m, 1,5 H), 4.65-4.58 (m, 1,75 H), 4.55 (d, *J* = 7.95 Hz, 1,0 H), 4.52 (dd, *J* = 15.49, 7.95 Hz, 0,3 H), 4.21-3.56 (m, 5,6 H), 4.09 (ddd, *J* = 76.69, 39.47, 5.34 Hz, 5,8 H), 3.87-3.20 (m, 5,6 H), 2.15 (broad, *J* = 1.68 Hz, 4,7 H), 2.06 (broad, *J* = 9.77, 9,5 Hz, 1H), 1.98 (broad, *J* = 6.30 Hz, 1H).

^{31}P NMR (*crude*, 400 MHz, $CDCl_3$) δ (ppm) = 8.9, 10.1, 10.8 (broad signals).

IR (ATR): 2924 (m), 2906 (m), 2111 (vw), 1748 (vs), 1437 (m), 1368 (s), 1224 (vs), 1038 (s), 957 (m), 916 (m), 734 (m).

PG1A-deprotected

^{1}H NMR (400 MHz, D_2O) δ (ppm) = 5.34 (broad, 2 H), 5.15 (d, *J* = 4.7 Hz, 2 H), 4.40 (dd, *J* = 50.1, 7.8 Hz, 8 H), 4.23 – 4.09 (m, 5 H), 4.09 – 3.93 (m, 5 H), 3.90 – 3.16 (m, 52 H).

IR (ATR): 3353 (broad), 2923 (m), 2853 (w), 2359 (m), 2342 (m), 2108 (vw), 1635 (m), 1597 (s), 1550 (s), 1454 (w), 1420 (m), 1327 (w), 1070 (s), 1033 (s), 921 (vw), 844 (w), 709 (m), 680 (w).

PG1B-peracetylated

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 5.80 (dd, *J* = 8.5, 3.3 Hz, 2 H), 5.57 – 4.92 (m, 30 H), 4.44 – 4.36 (m, 2 H), 4.25 – 3.93 (m, 21 H), 3.62 (broad, 39 H), 2.24 – 1.90 (m, 84 H).

PG1B - deprotected

IR (ATP): 3708 (m), 2922 (m), 2864 (w), 2358 (vs), 2342 (s), 1599 (vw), 1558 (w), 1456 (vw), 1215 (vw), 1055 (s), 1033 (vs), 1012 (m), 668 (m).

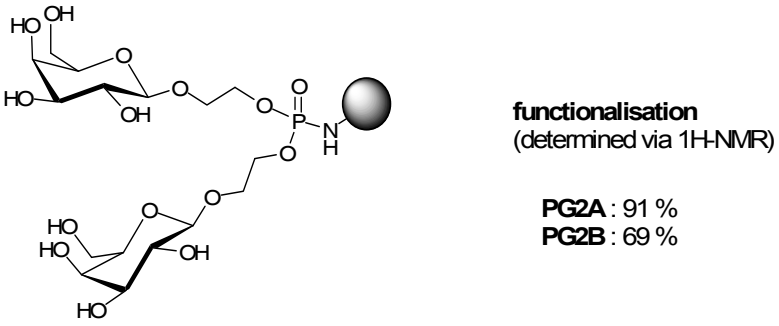


Figure S3: calculated degree of functionalisation of **PG2 A+B**.

PG2-peracetylated

1H NMR (400 MHz, $CDCl_3$) δ (ppm) = 5.80 (dd, *J* = 8.34, 3.36 Hz, 0.58 H), 5.50 (d, *J* = 5.50-5.48 Hz, 0.34 H), 5.47 (dd, *J* = 3.08, 1.22 Hz, 0.48 H), 5.43-5.39 (m, 1.23 H), 5.35-5.30 (m, 0.85 H), 5.27-5.15 (m, 2 H), 5.10-4.96 (m, 2 H), 4.51-4.34 (m, 0.76 H), 4.25-4.05 (m, 4 H), 4.06-3.97 (m, 6 H), 3.65-3.56 (broad, 6 H), 2.15-2.96 (m, 12 H).

IR (ATR): 2928 (w), 2101 (m), 1750 (vs), 1432 (m), 1371 (s), 1222 (vs), 1070 (s), 946 (m), 810 (w), 747 (m)

PG2A-deprotected

IR (ATP): 3372 (broad), 2923 (m), 2853 (w), 2358 (m), 2342 (m), 2068 (vw), 1646 (w), 1597 (s), 1550 (s), 1454 (w), 1428 (m), 1326 (w), 1234 (w), 1096 (w), 1069 (w), 1033 (w), 921 (vw), 823 (vw), 709 (m), 686 (w).

PG2B-peracetylated

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 5.80 (dd, J = 8.5, 3.3 Hz, 2 H), 5.57 – 4.92 (m, 30 H), 4.44 – 4.36 (m, 2 H), 4.25 – 3.93 (m, 21 H), 3.62 (broad, 39 H), 2.24 – 1.90 (m, 84 H).

PG2B-deprotected

^{1}H NMR (500 MHz, METHANOL-D3) δ (ppm) = 4.36 (broad, 2 H), 4.24 (broad, 3 H), 4.16-4.05 (m, 1 H), 3.94 – 3.38 (m, 20 H), 3.07 – 2.93 (m, 1 H).

IR (ATR): 3342 (broad), 2952 (m), 2923 (m), 2885 (m), 2360 (m), 2341 (w), 2105 (w), 1683 (w), 1644 (w), 1598 (s), 1550 (vs), 1455 (s), 1429 (m), 1327 (w), 1207 (w), 1186 (w), 1131 (m), 1093 (m), 1057 (s), 1033 (s), 1016 (m), 840 (w), 803 (w), 711 (m).

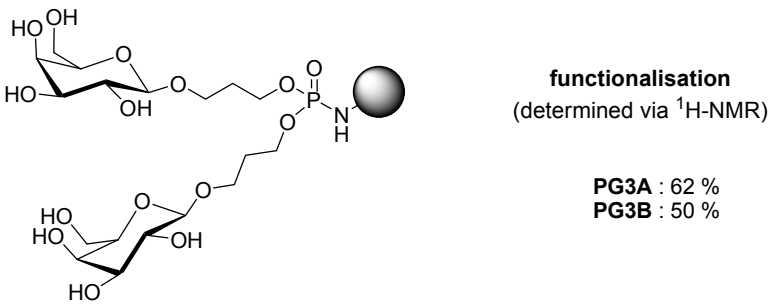


Figure S4: calculated degree of functionalisation of **PG3 A+B.**

PG3A-peracetylated

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 5.38 (d, *J* = 3.2 Hz, 2 H), 5.29 (s, 1 H), 5.18 (dd, *J* = 10.5, 8.0 Hz, + broad, 2 H), 5.02 (dd, *J* = 10.5, 3.4 Hz, + broad, 2 H), 4.55 – 4.49 (broad, 1 H), 4.47 (d, *J* = 7.9 Hz, 1.31 H), 4.24 – 3.87 (m, 10 H), 3.87 – 3.26 (m, 10 H), 2.05 – 1.92 (m, 25 H), 1.91 – 1.80 (m, 2.4 H).

IR (ATR): 2939 (w), 2100 (m), 1747 (vs), 1433 (m), 1369 (s), 1220 (vs), 1174 (m), 1053 (s), 956 (m), 909 (m), 734 (m),

PG3B-1-peracetylated

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 5.46 – 5.36 (m, 2 H), 5.17 – 5.12 (m, 1 H), 5.07 – 5.02 (m, 1 H), 4.51 (broad, 1 H), 3.96 (s, 13 H), 3.84 – 3.26 (broad, 21 H), 2.21 – 1.85 (m, 30 H).

PG3B-2-peracetylated

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 5.44 – 5.35 (m, 2 H), 5.29 (s, 1 H), 5.17 (dd, *J* = 10.5, 7.9 Hz, + broad, 2 H), 5.08 – 4.98 (m, 1 H), 5.02 (dd, *J* = 10.5, 3.4 Hz, 2 H), 4.57 – 4.50 (m, 1 H), 4.47 (d, *J* = 7.9 Hz, 1 H), 4.22 – 3.76 (m, 12 H), 3.69 – 3.24 (m, 5 H), 2.18 – 1.95 (m, 20 H), 1.95 – 1.77 (m, 4 H).

IR (ATR): 2960 (w), 2130 (w), 1745 (vs), 1369 (m), 1217 (vs), 1173 (m), 1134 (m), 1048 (s), 956 (w), 909 (w), 733 (m).

PG3A-deprotected

^{1}H NMR (400 MHz, D_2O) δ 4.28 (broad d, J = 7.5 Hz, 2 H), 4.07 (broad, 3 H), 3.98 - 3.86 (m, 2 H), 4.53 – 3.51 (m, 27 H), 3.82 (broad, 2 H), 3.72 – 3.26 (m, 19 H), 3.24 (s, 1 H), 1.94 (broad, 4 H).

IR (ATR): 3338 (broad), 2932 (m), 2359 (m), 2342 (m), 2049 (w), 1639 (w), 1598 (s), 1550 (vs), 1446 (s), 1419 (s), 1323 (m), 1058 (s), 1033 (s), 922 (w), 825 (w), 711 (m)

PG3B-deprotected

^{1}H NMR (500 MHz, METHANOL-D3/D_2O) δ (ppm) = 4.36 – 4.26 (m, 2H), 4.23 – 4.11 (m, 4H), 4.07 – 3.95 (m, 2H), 3.89 (s, 2H), 3.86 (d, J = 2.1 Hz, 1H), 3.82 – 3.46 (m, 20H), 2.02 (d, J = 5.4 Hz, 4H).

IR (ATR): 3339 (broad), 2922 (m), 2361 (w), 2337 (w), 2103 (m), 1700 (vw), 1652 (vw), 1419 (w), 1373 (w), 1215 (m), 1504 (vs), 1033 (vs), 1019 (vs), 892 (m), 757 (m), 668 (m).

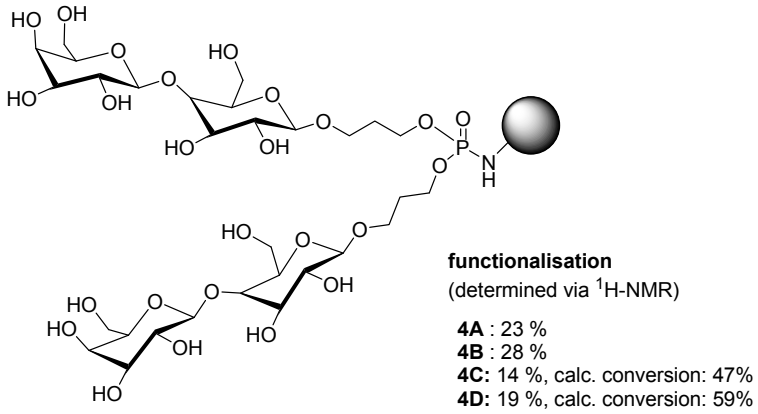


Figure S5: calculated degree of functionalisation of **PG4 A, B, C+D**.

PG4A-peracetylated

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 5.63 (t, *J* = 4.7 Hz, 2 H), 5.50 (broad, 2 H), 5.34 (dd, *J* = 13.3, 3.3 Hz, 5 H), 5.22 – 4.77 (m, 14 H), 4.59 (d, *J* = 7.9 Hz, 2 H), 4.54 – 4.40 (m, 7 H), 4.32 – 4.19 (m, 4 H), 4.18-4.02 (m, 15 H), 3.96 – 3.74 (m, 16 H), 3.65-3.50 (m, 13 H), 2.21 – 1.92 (m, 81 H), 1.92 – 1.76 (m, 9 H), 1.72-1.60 (m, 8 H).

IR (ATR): 2941 (w), 2101 (w), 1743 (s), 1431 (m), 1369 (s), 1217 (vs), 1170 (m), 1134 (m), 1045 (s), 914 (s), 732 (s),

PG4B-peracetylated

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 5.63 (t, *J* = 4.7 Hz, 2 H), 5.50 (broad, 2 H), 5.34 (dd, *J* = 13.4, 3.1 Hz, 5 H), 5.28 (s, 1 H), 5.17 (s, 7 H), 5.01 – 4.80 (m, 7 H), 4.59 (d, *J* = 8.0 Hz, 2 H), 4.53 – 4.40 (m, 7 H), 4.31 – 4.19 (m, 4 H), 4.16 – 4.00 (m, 17 H), 3.98 – 3.22 (m, 36 H), 2.23 – 1.92 (m, 88 H), 1.91 – 1.74 (m, 10 H).

IR (ATR): 2943 (w), 2103 (w), 1743 (vs), 1432 (w), 1369 (s), 1217 (vs), 1170 (m), 1134 (m), 1045 (vs), 913 (m), 732 (m)

PG4A-deprotected

^{1}H NMR (400 MHz, xMeOD/D_2O) δ 5.73 – 5.67 (m, 2 H), 5.35 (s, 1 H), 4.48 – 4.31 (m, 11 H), 4.28 – 4.21 (m, 2 H), 4.19 – 3.96 (m, 8 H), 3.96 – 3.33 (m, 79 H), 3.27 – 3.15 (m, 3 H), 2.01 – 1.82 (m, 9 H), 1.65 (broad, 7 H).

IR (ATR): 3345 (broad), 2923 (m), 2359 (s), 2342 (m), 1653 (m), 1558 (w), 1456 (w), 1319 (m), 1054 (vs), 1033 (vs), 1017 (s), 910 (w), 830 (s), 780 (s) 668 (m).

PG4B-deprotected

^{1}H NMR (700 MHz, MeOD/D_2O) δ (ppm) 4.45 – 4.36 (m, 2 H), 4.28 – 4.09 (m, 2 H), 4.07 – 3.45 (m, 19 H), 2.07-1.99 (m, 1 H), 1.99-1.88 (m, 1 H), 1.89 – 1.83 (m, 1 H).

IR (ATR): 3342 (broad), 2922 (m), 2874 (w), 2360 (m), 2342 (m), 2103 (m), 1653 (vw), 1436 (w), 1394 (w), 1223 (w), 1052 (vs), 1033 (vs), 1019 (vs), 893 (vw), 783 (w).

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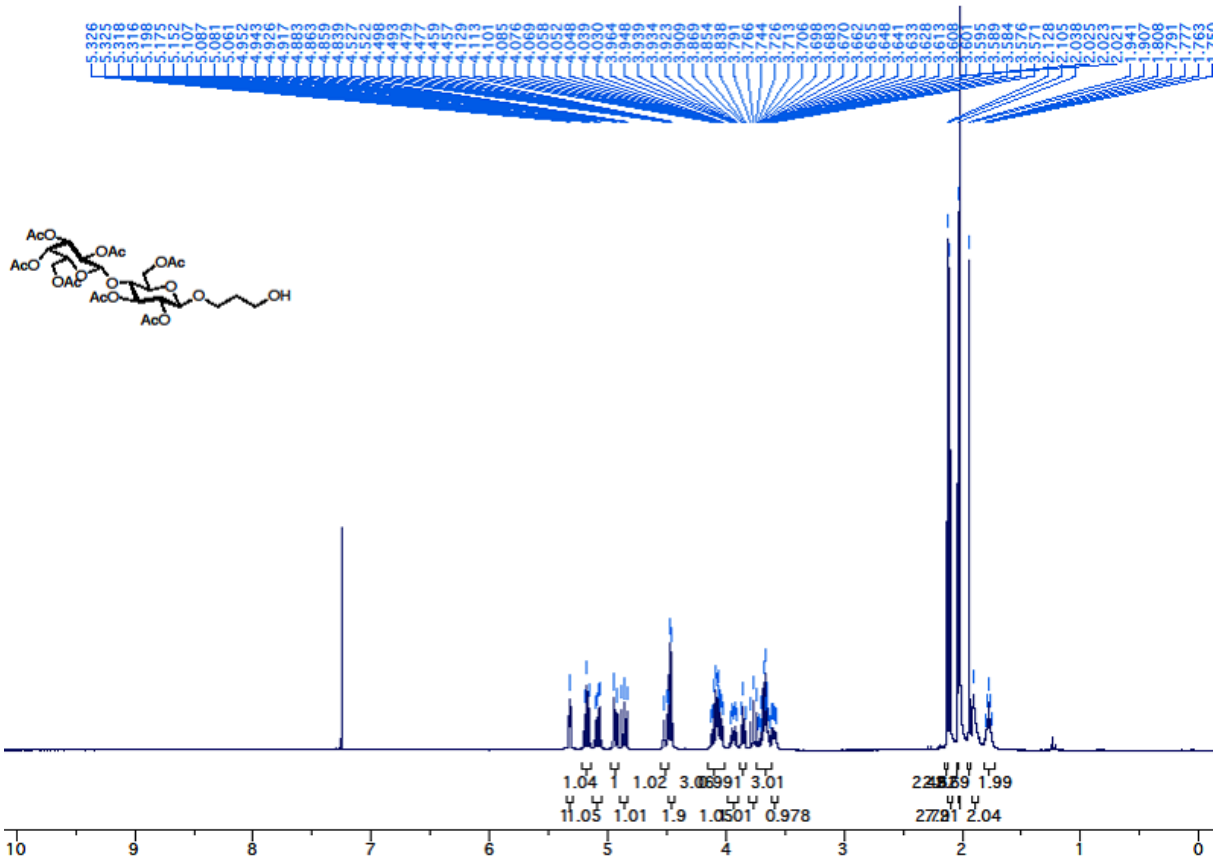
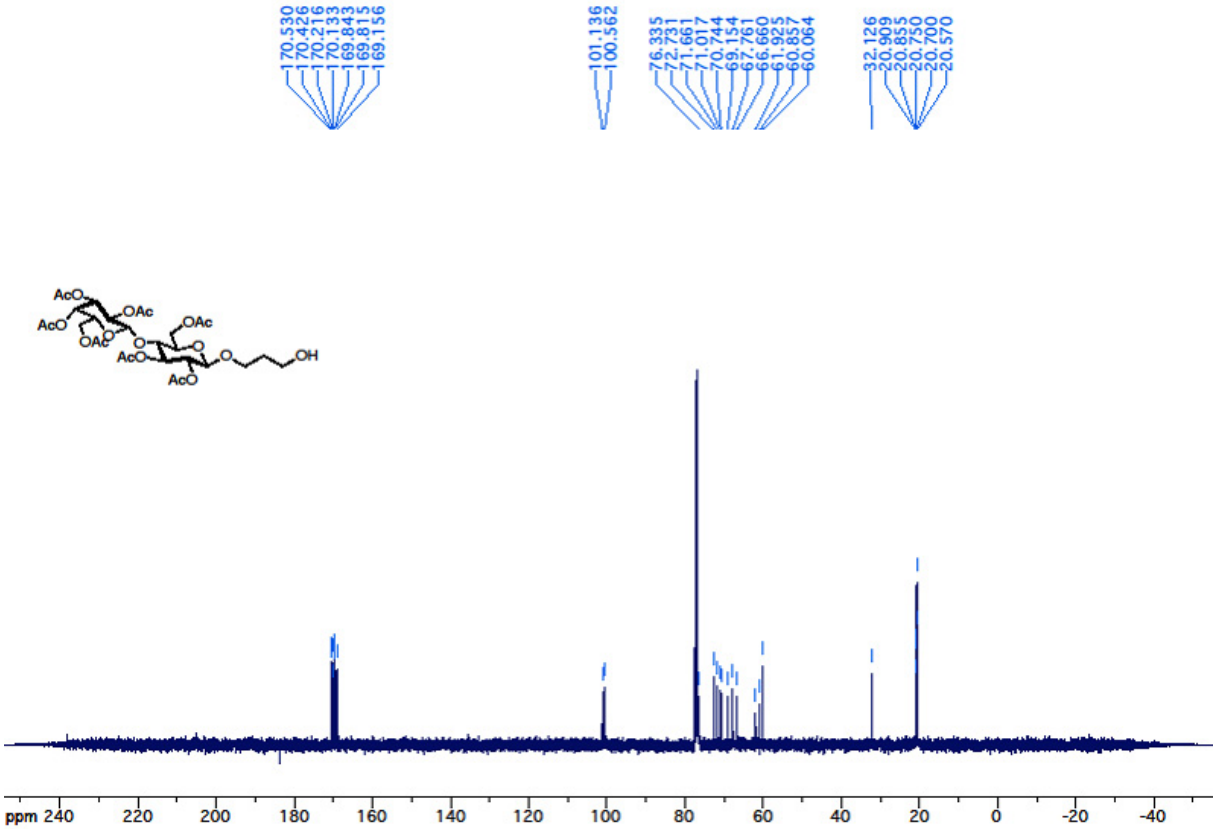
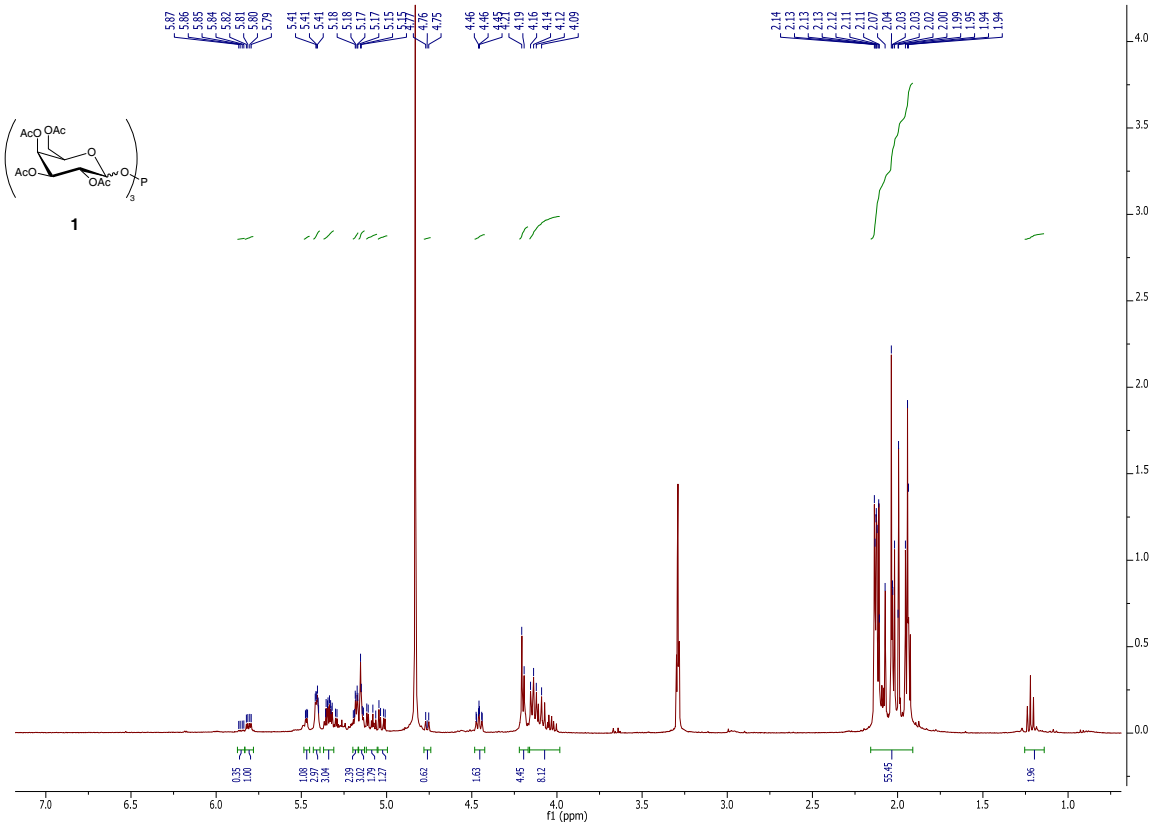
5. S. Roller, H. Zhou and R. Haag, Molecular Diversity, 2005, 9, 305-316.

SPECTRA



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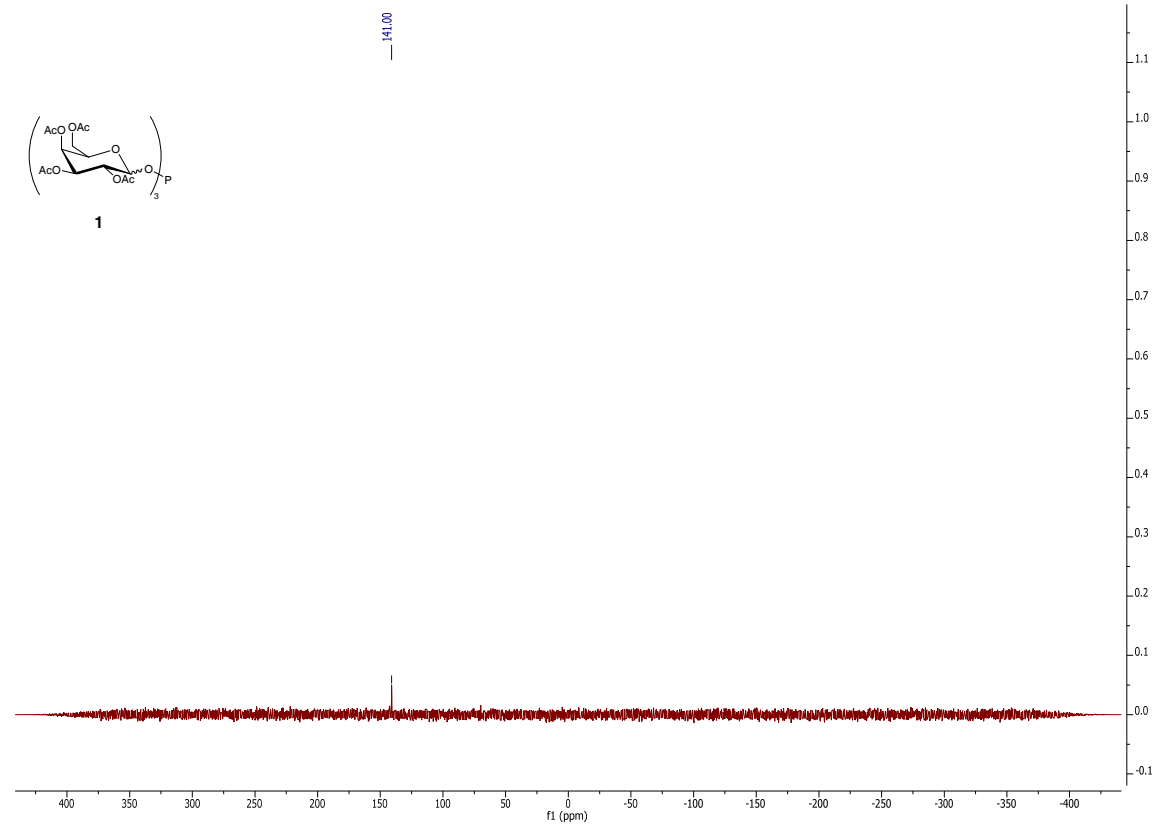
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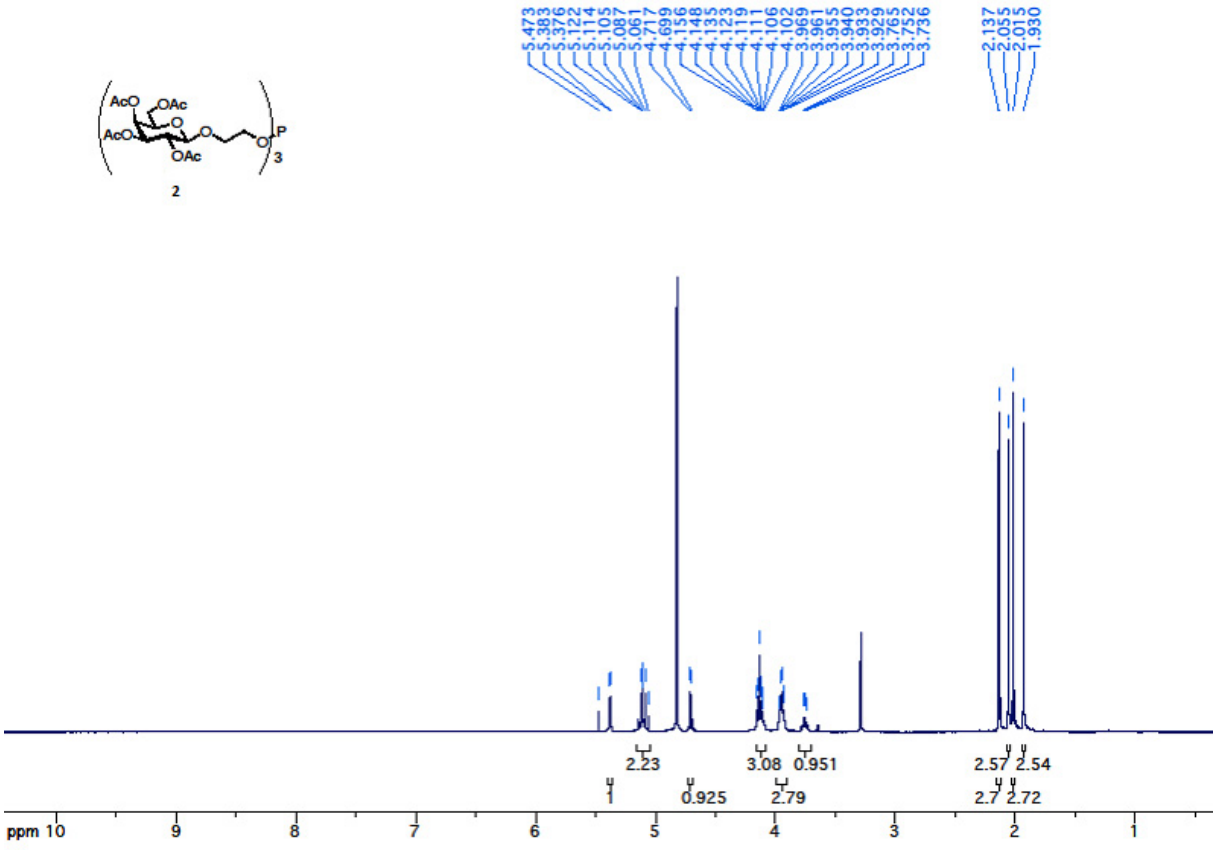
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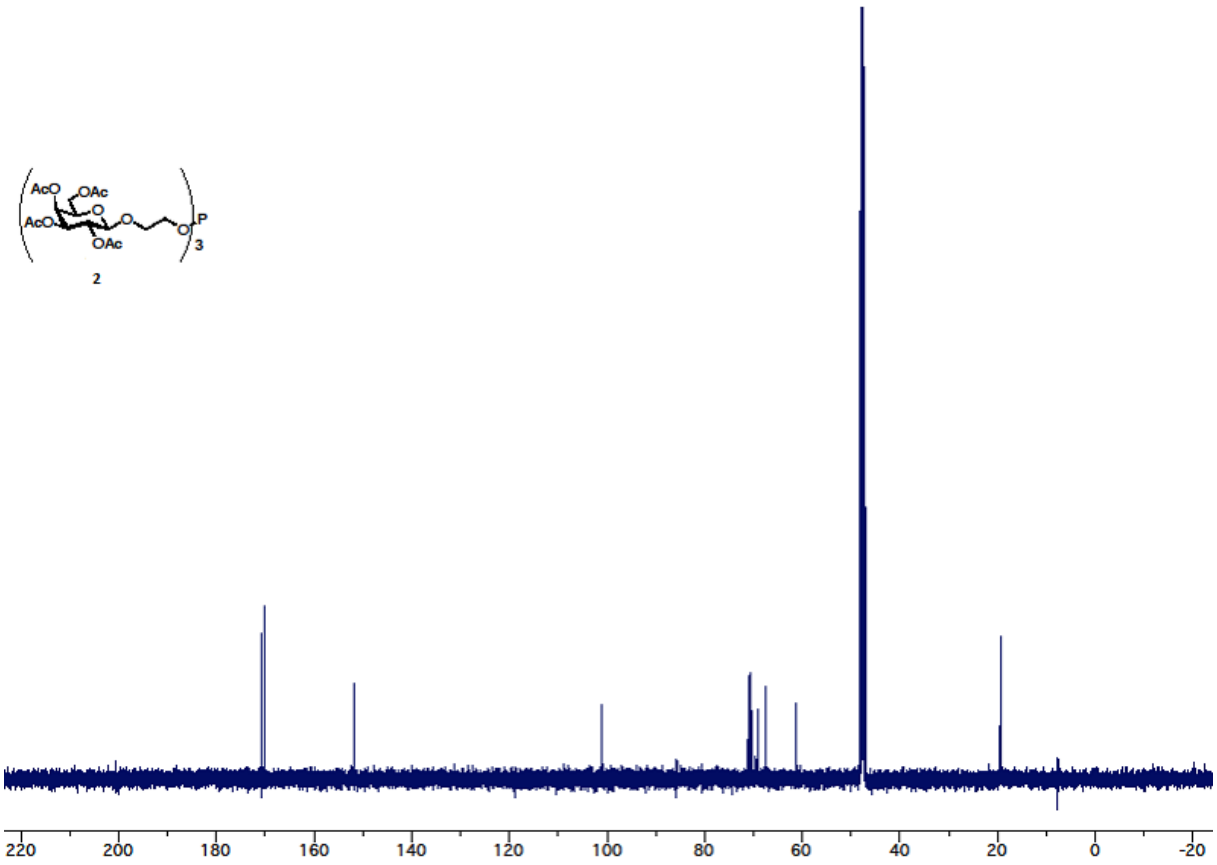
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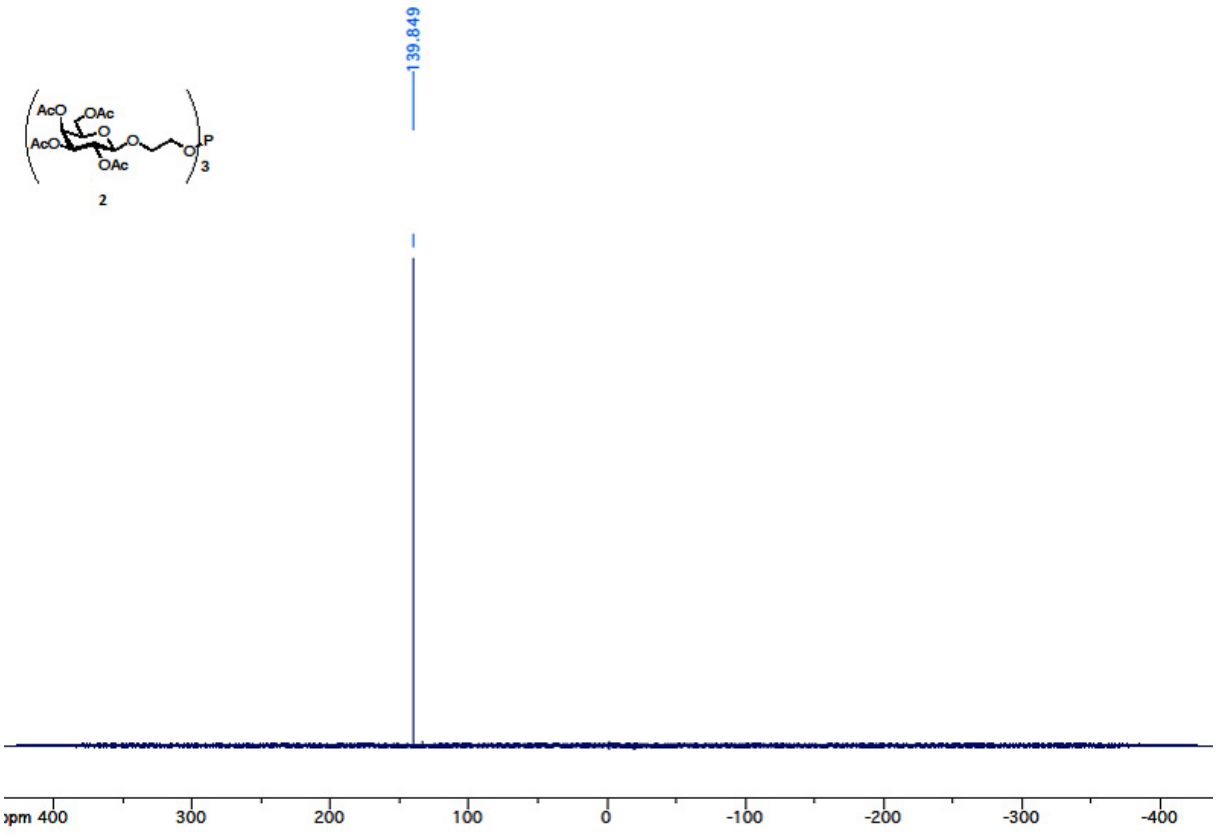

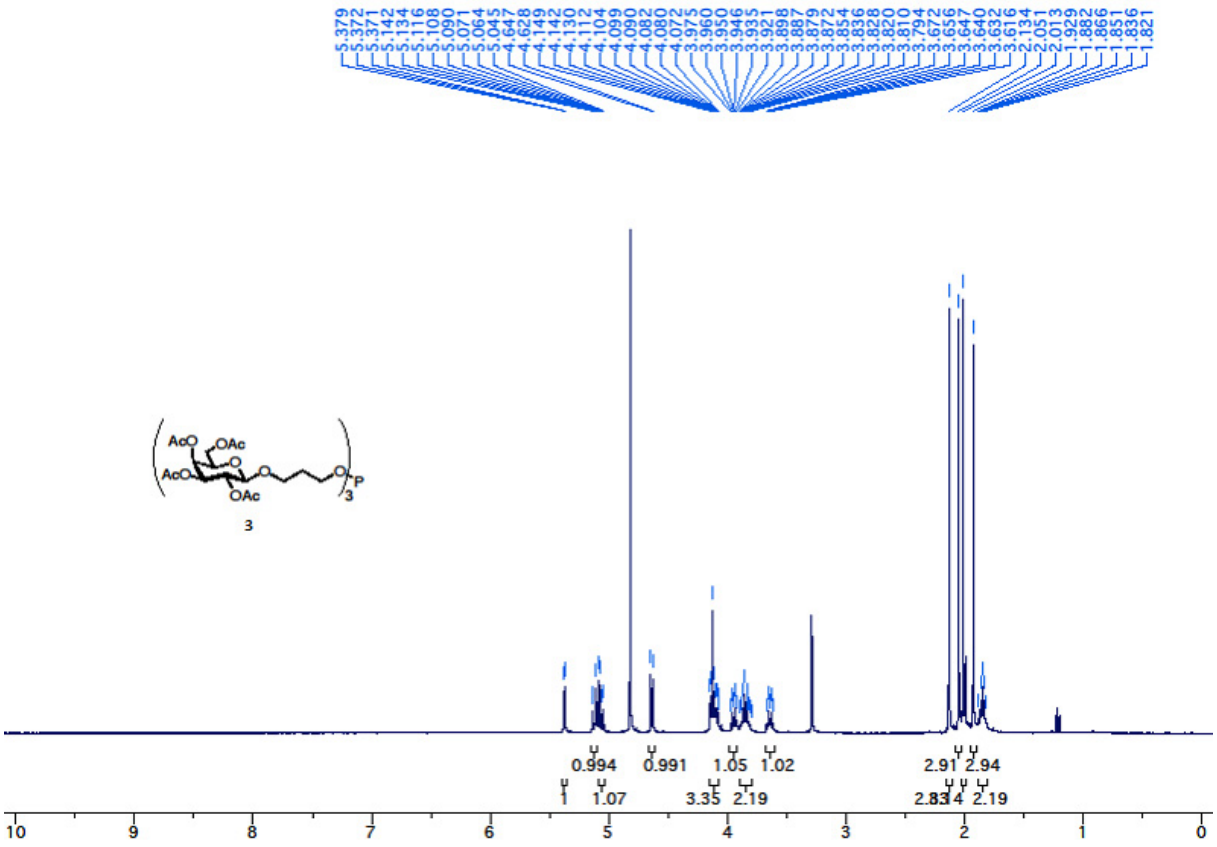

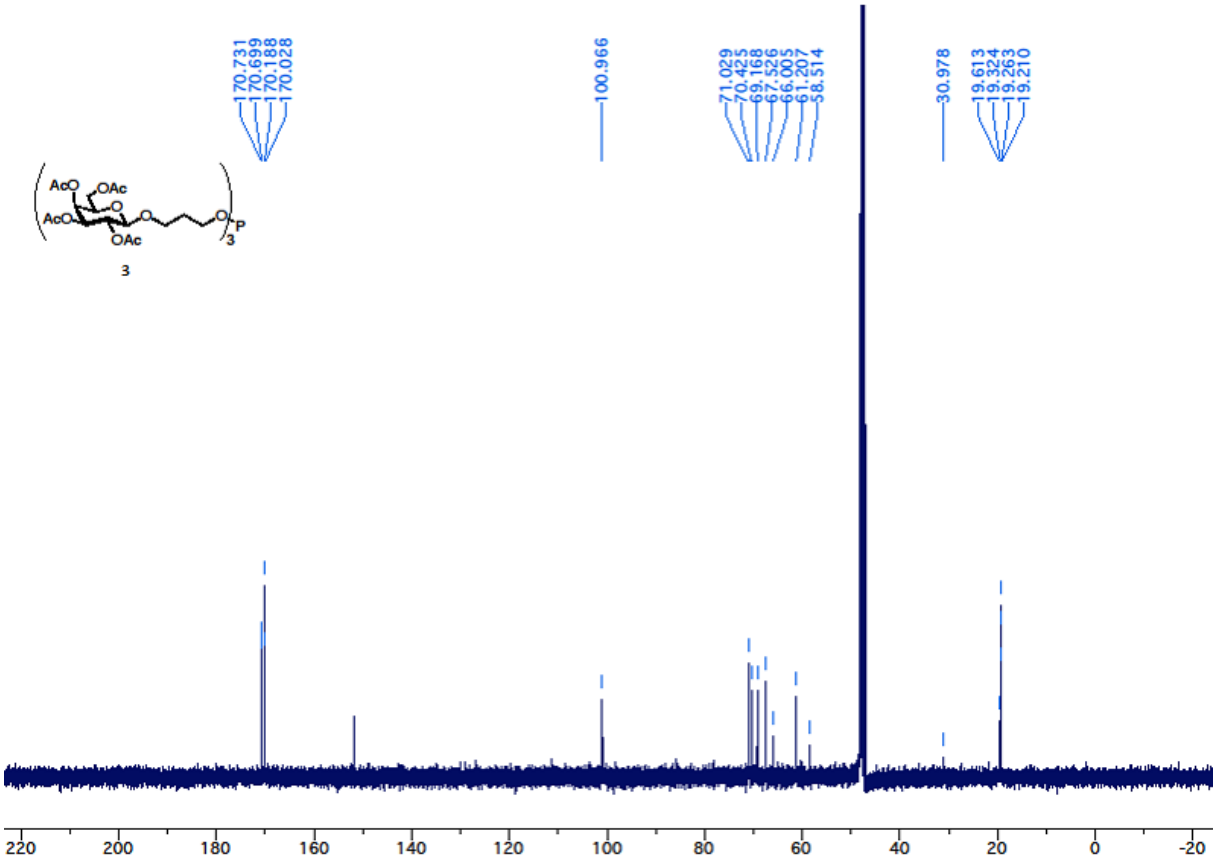

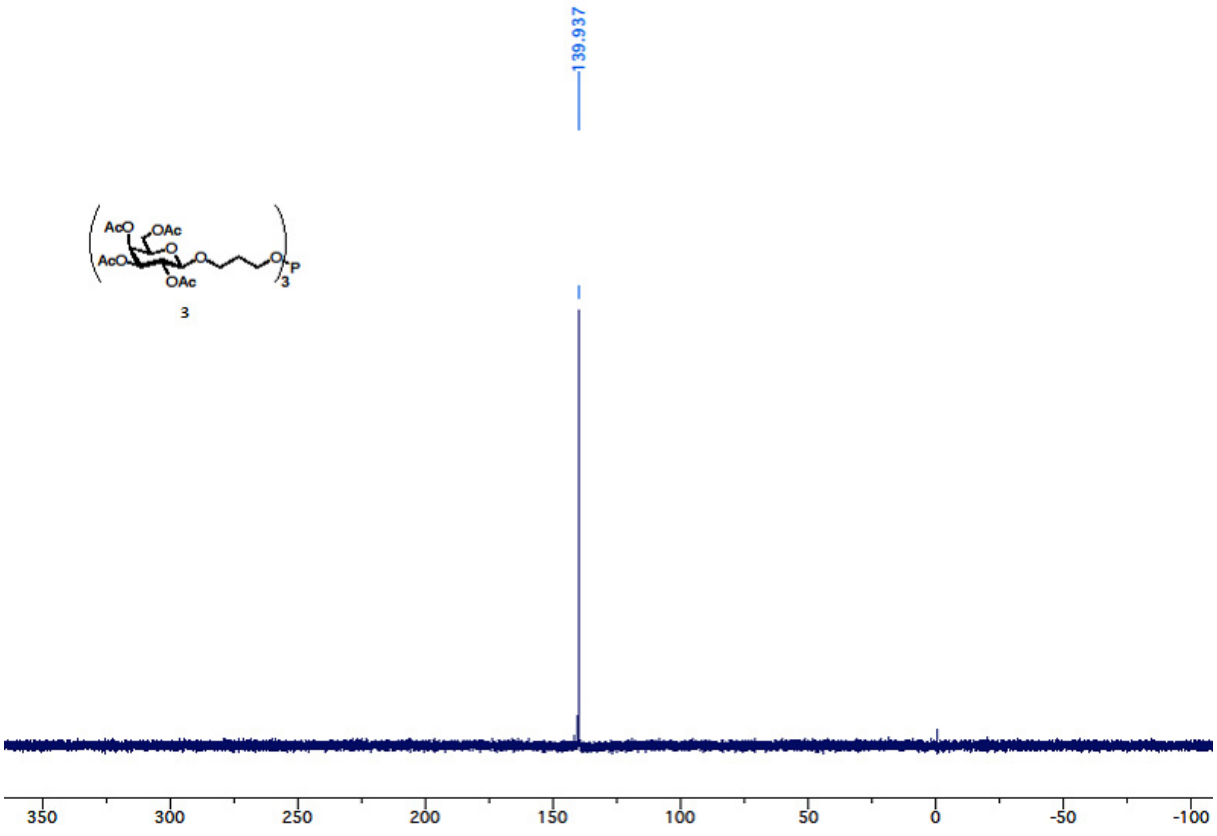

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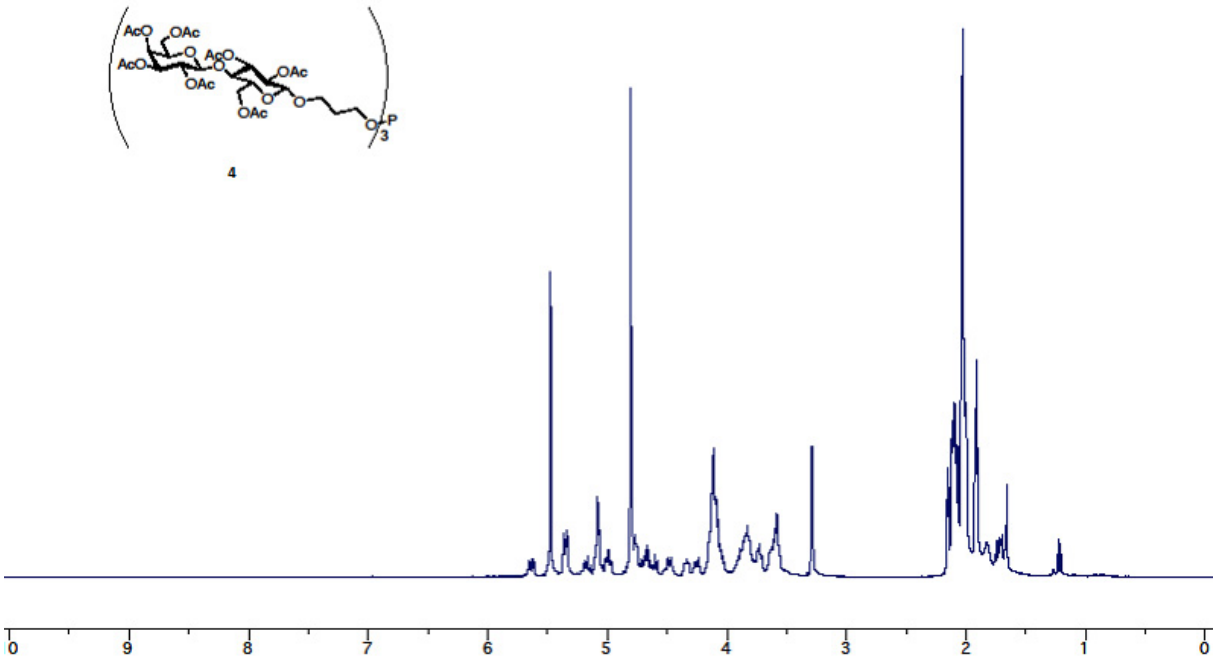

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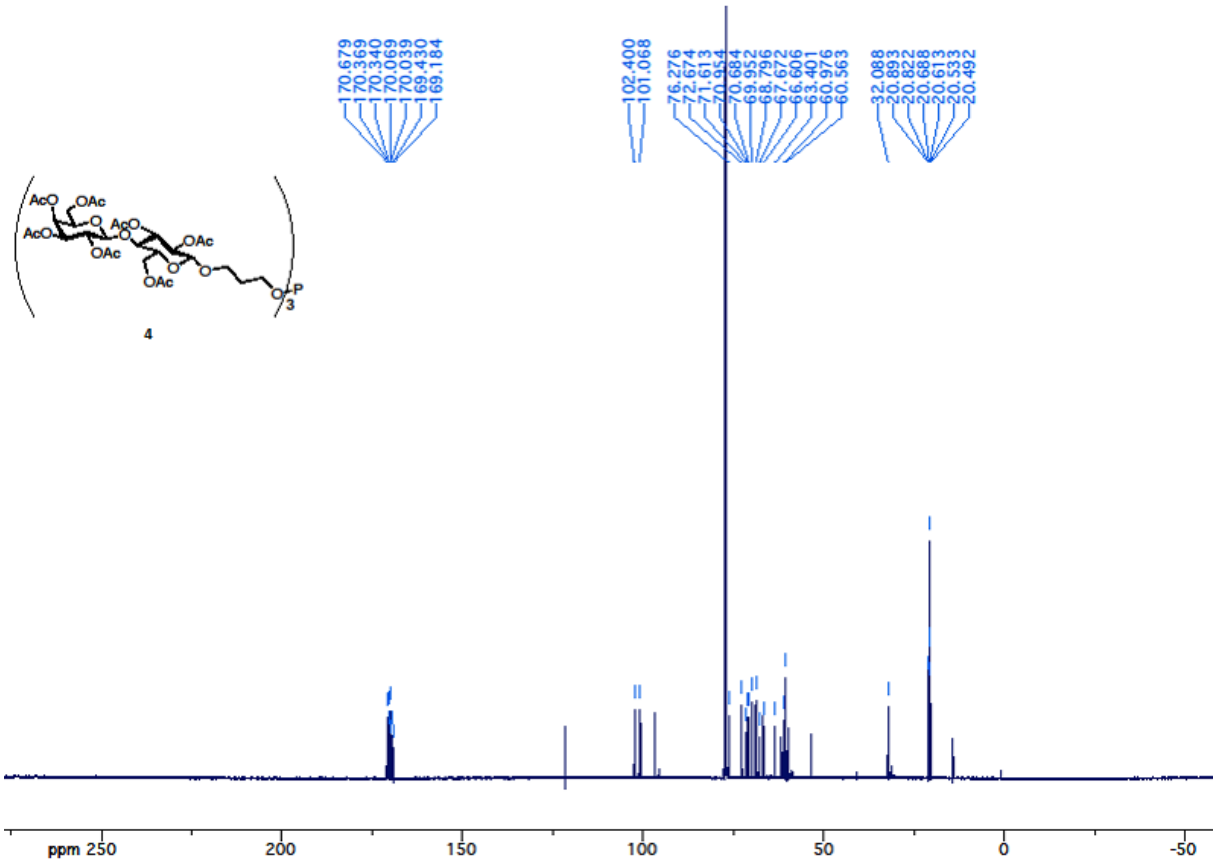

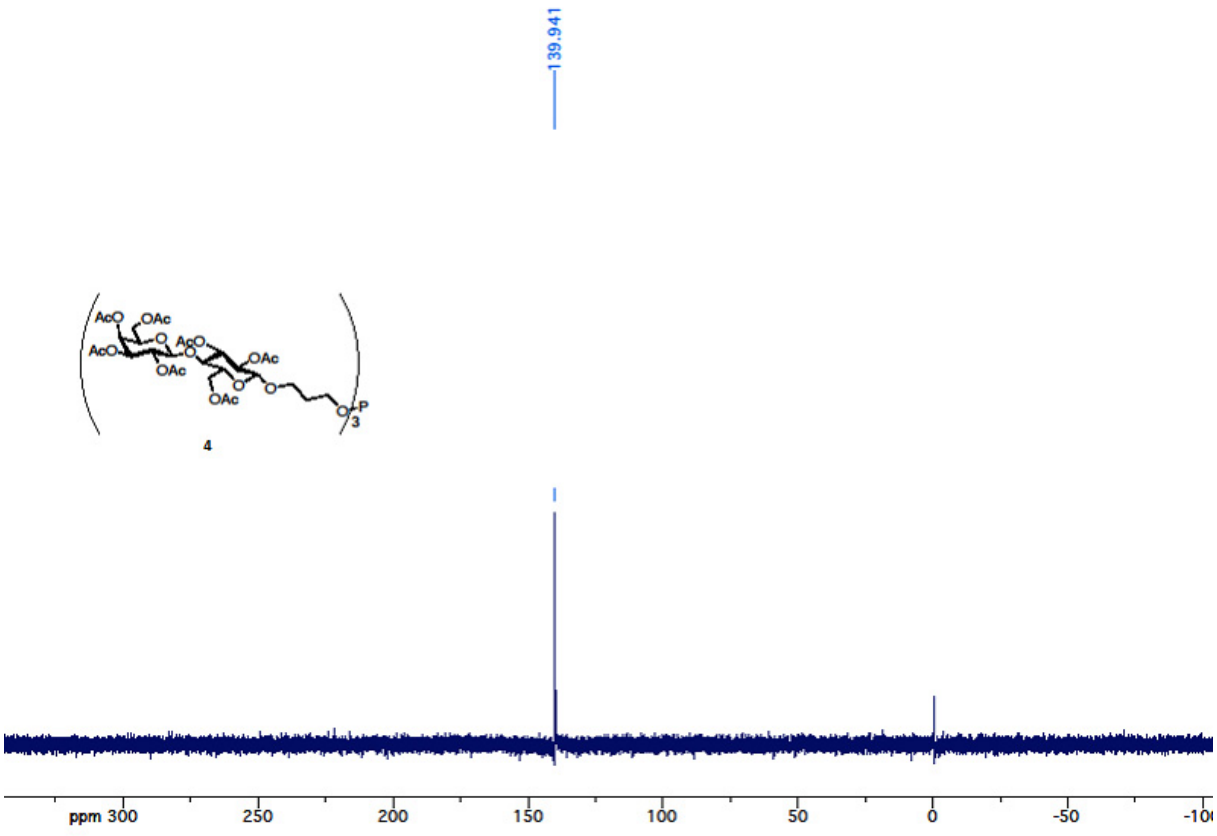

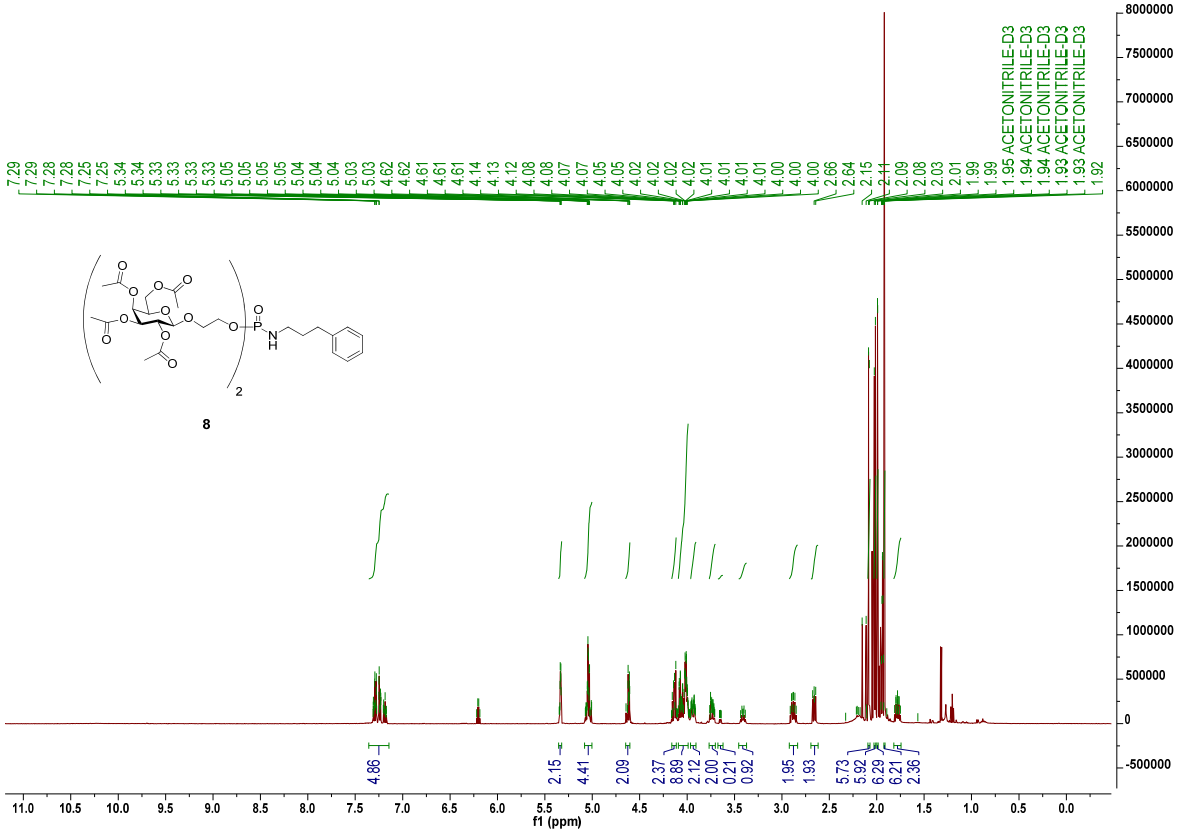
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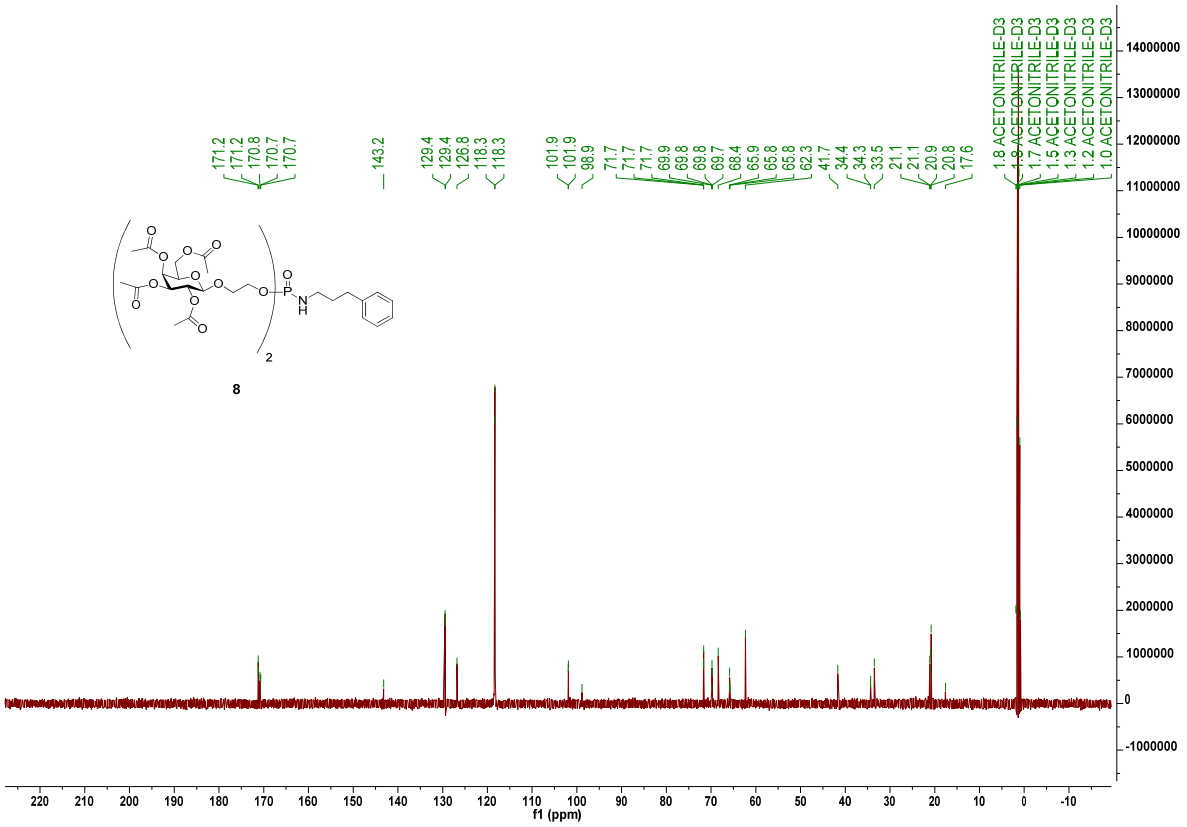
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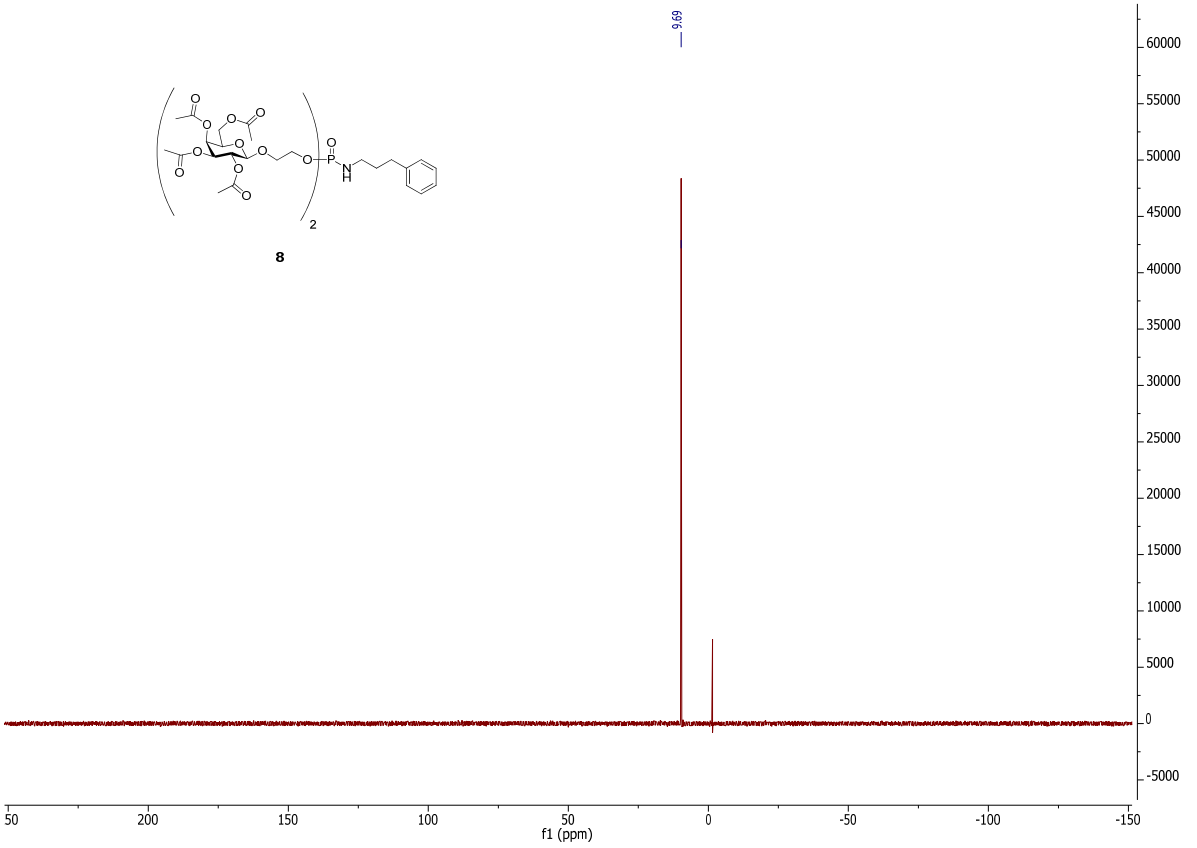
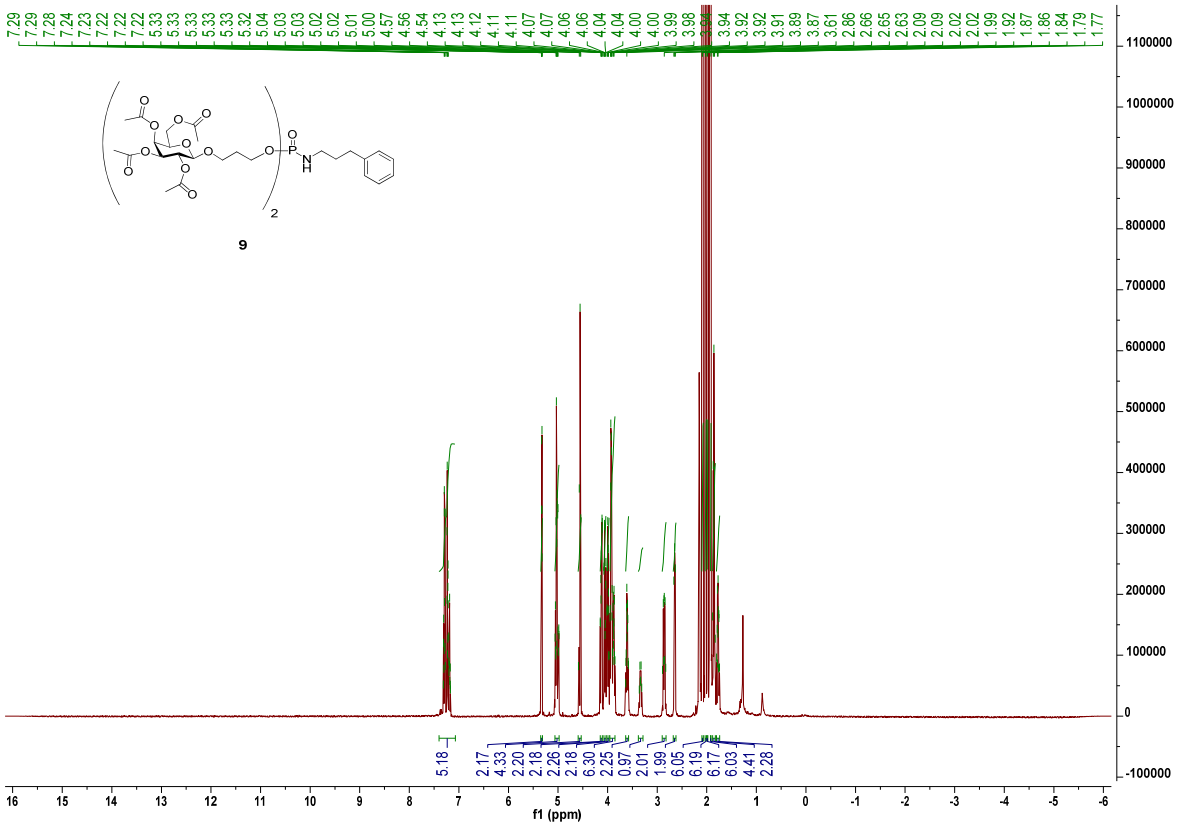
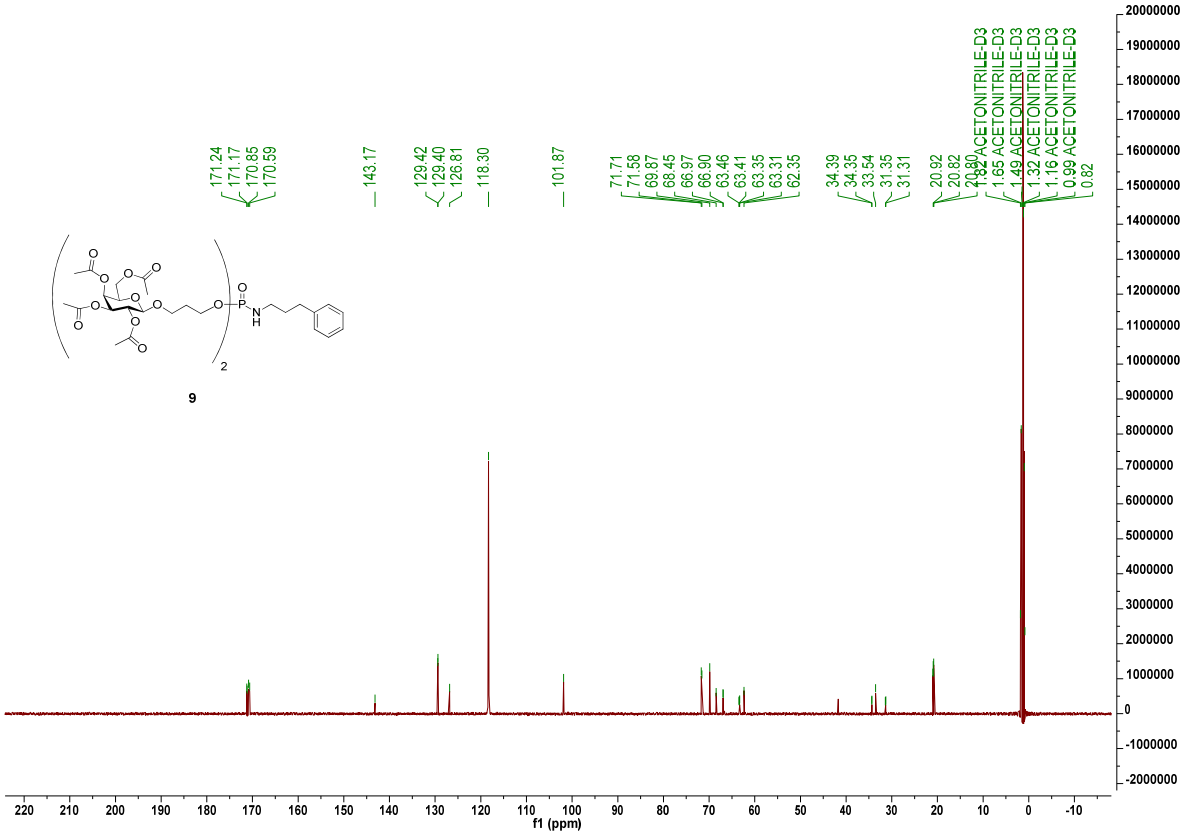
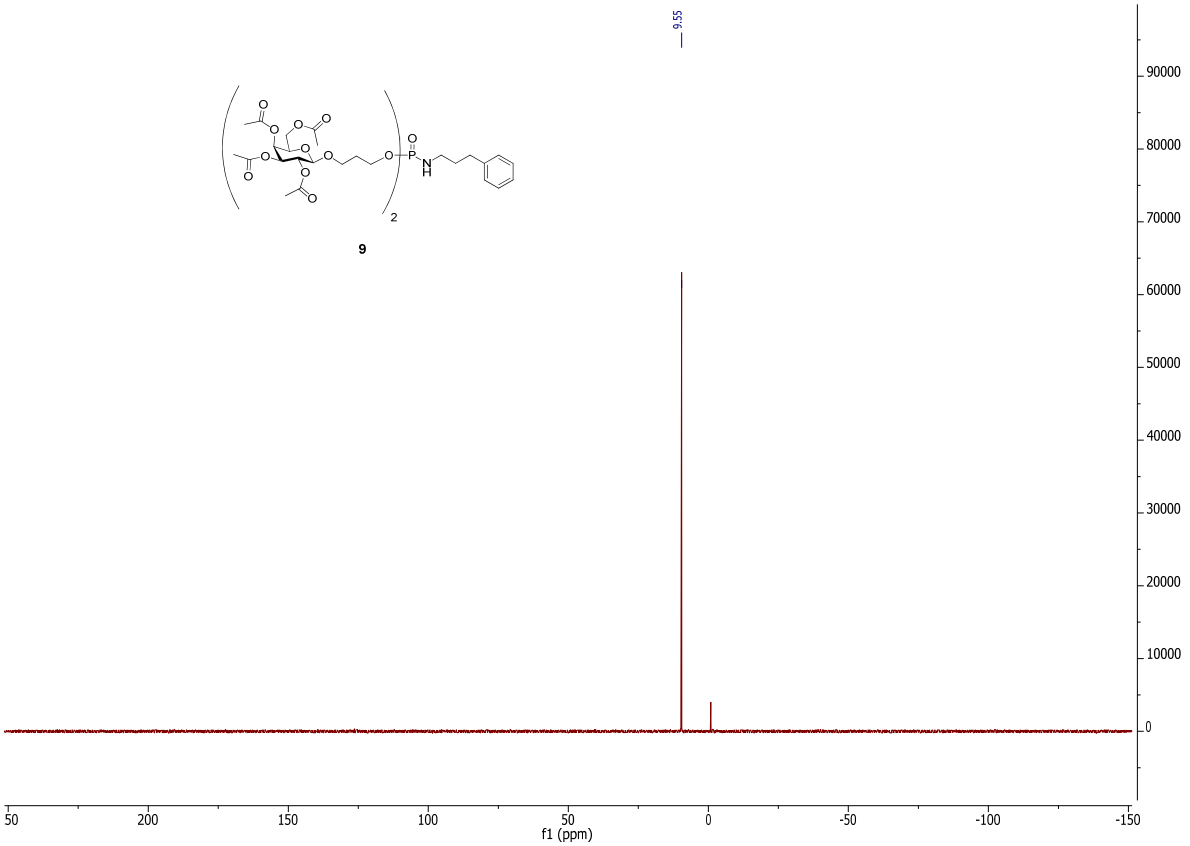
17.6
1.8 ACETONITRILE-D3
1.8 ACETONITRILE-D3
1.7 ACETONITRILE-D3
1.5 ACETONITRILE-D3
1.3 ACETONITRILE-D3
1.2 ACETONITRILE-D3
1.0 ACETONITRILE-D3
8
f1 (ppm)

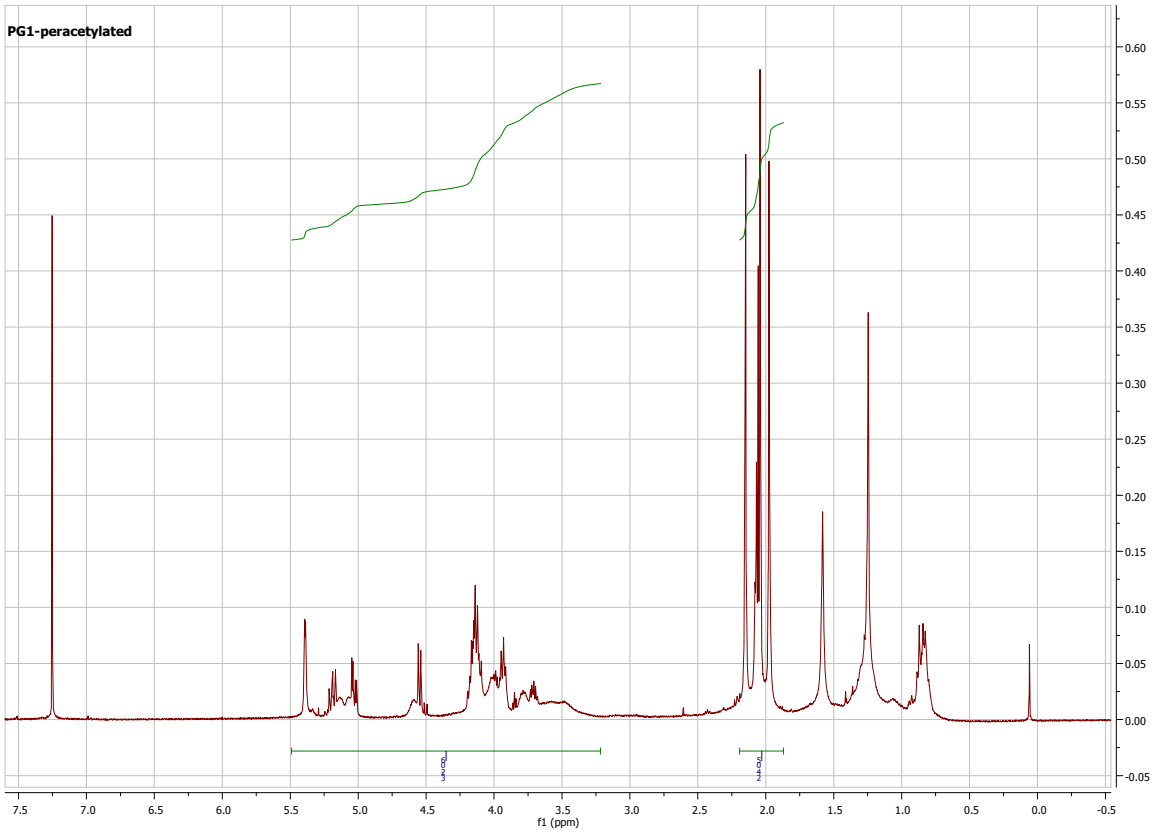

9.69
8
f1 (ppm)

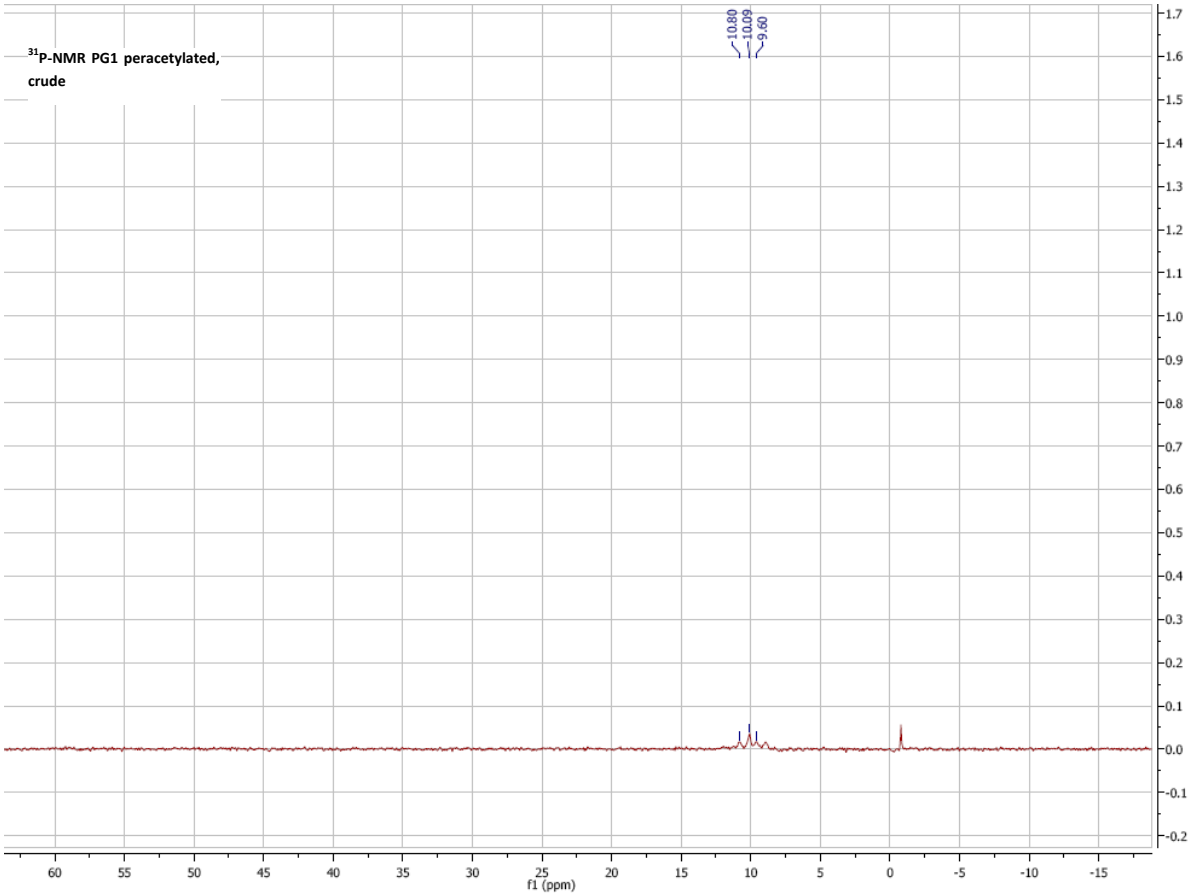

9
f1 (ppm)

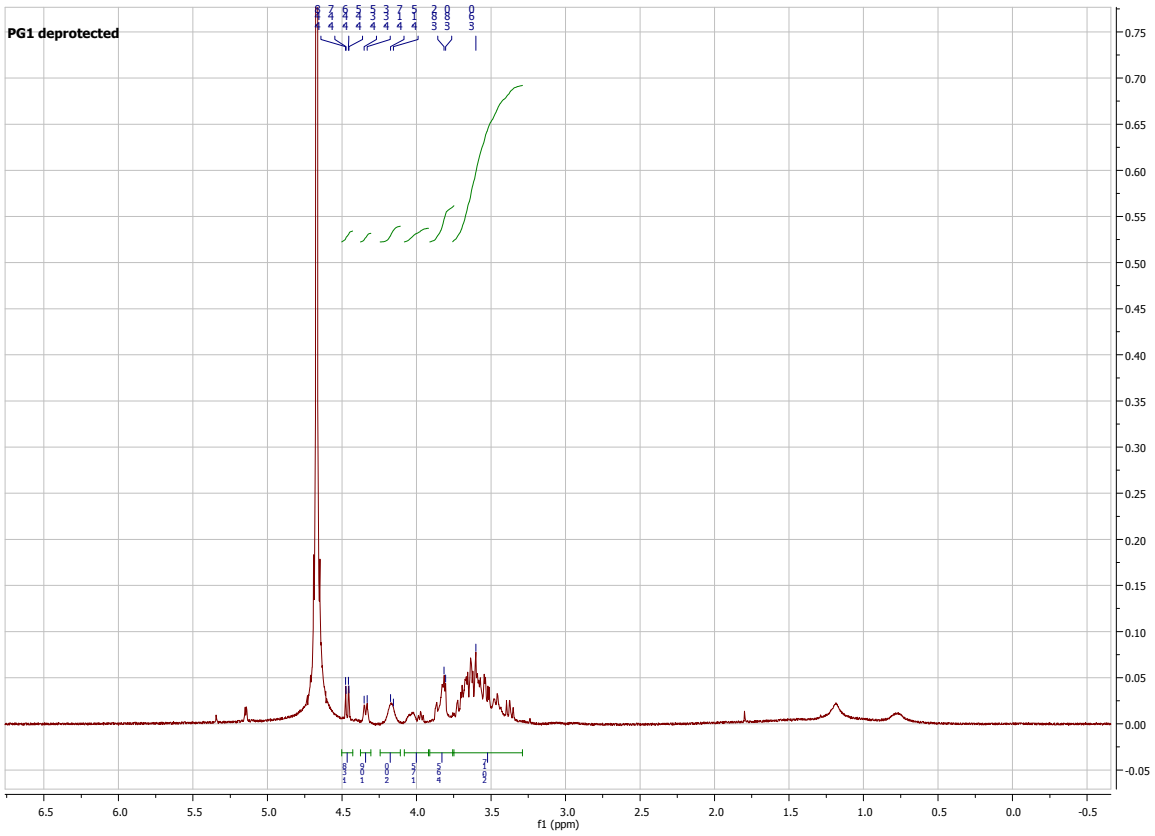

9
f1 (ppm)

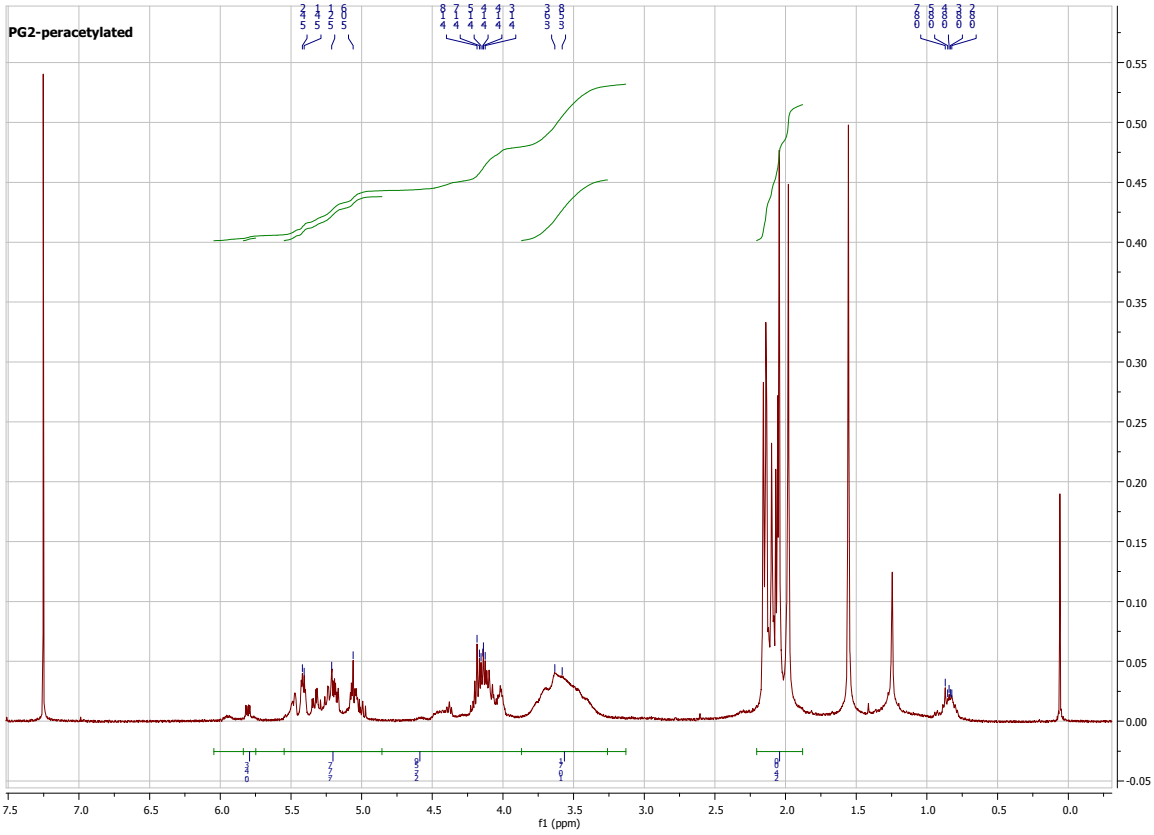


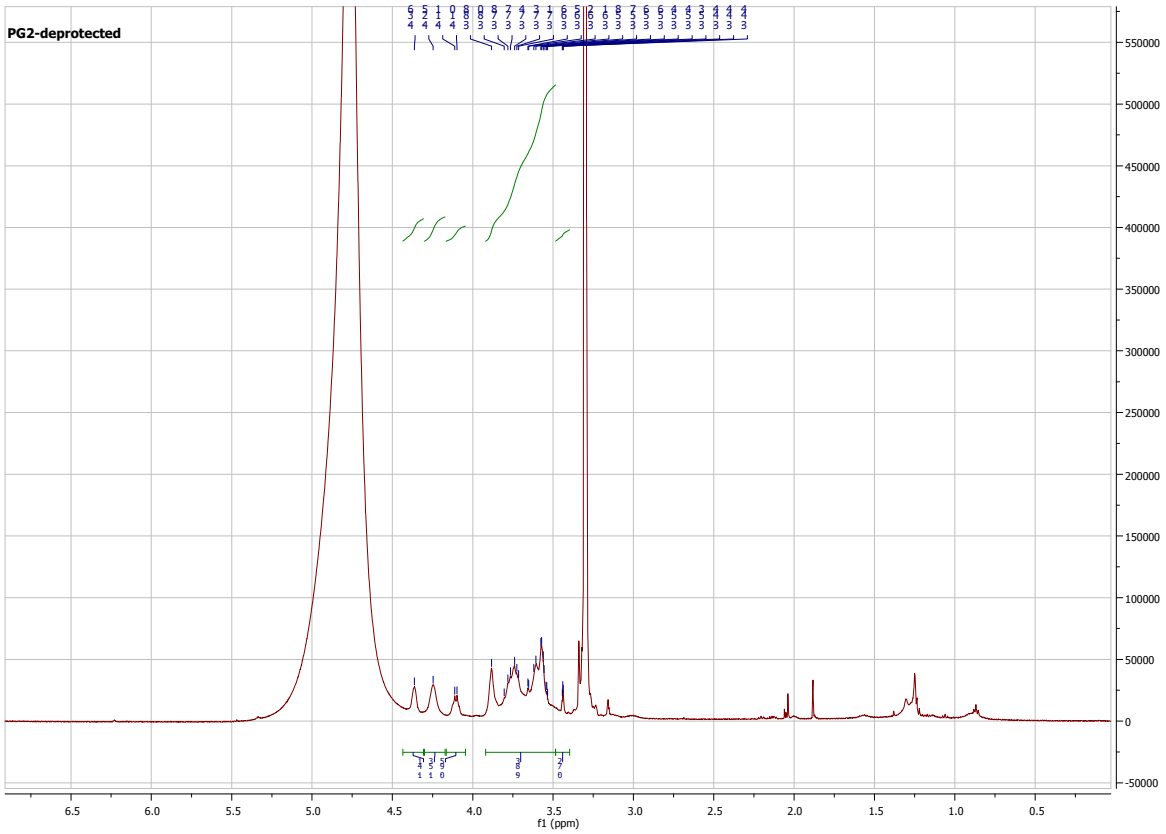
9




^{31}P-NMR PG1 peracetylated, crude
10.80
10.09
9.60
f1 (ppm)


PG1 deprotected
f1 (ppm)


PG2-peracetylated
f1 (ppm)


PG2-deprotected
f1 (ppm)