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Supporting Information

Lipopolysaccharide from Gut-Associated Lymphoid-Tissue-Resident *Alcaligenes faecalis*: Complete Structure Determination and Chemical Synthesis of Its Lipid A

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Experimental section

Characterization of *A. faecalis* LOS and LPS

Isolation and purification of *A. faecalis* LOS and LPS

The LOS material of *A. faecalis* was isolated from dried bacterial cells by using the phenol/chloroform/light petroleum extraction protocol.[1] The LPS was instead isolated through the hot phenol/water extraction method.[2] The extracts were extensively dialyzed (cutoff 12–14 kDa) against distilled water and cell material was removed by means of enzymatic digestion with nucleases (5 h, 37 °C) and proteases (16 h, 56 °C) followed by extensive dialysis (cutoff 12–14 kDa) against distilled water and ultracentrifugation (100,000 × *g*, 4 °C, 16 h). The nature of the purified extracts was evaluated by SDS-PAGE after gel staining with silver nitrate.[3] The LOS and the LPS were then further purified by means of size-exclusion chromatography on Sephacryl HR-S300 and HR-S400 columns (GE Healthcare Life Sciences) respectively, both eluted with 50 mM ammonium bicarbonate.

Compositional analysis of *A. faecalis* LOS and LPS

Monosaccharides were detected as acetylated *O*-methylglycoside derivatives obtained by methanolysis (1.25 M hydrogen chloride in methanol, 85 °C, 16 h) followed by acetylation with acetic anhydride in pyridine (85 °C, 30 min).[4] The absolute configuration was defined through anaysis of the *O*-octylglycoside derivatives.[4] The linkage pattern of the saccharide moiety of *A. faecalis* LOS and LPS was defined by analysis of the partially methylated and acetylated alditol derivatives of the monosaccharide units.[4,5] Briefly, the sample was methylated with iodomethane, treated with sodium tetradeuteroborate ($NaBD_4$) to transforms the methyl ester functions of Kdo in a hydroxymethyl group with two deuterium atoms, then hydrolyzed with trifluoroacetic acid (TFA; 2 M, 120 °C, 1 h), carbonyl-reduced using again sodium tetradeuteroborate ($NaBD_4$), acetylated with acetic anhydride and pyridine, and analyzed by gas-liquid chromatography/mass spectrometry (GLC-MS).[4-6]

The total fatty acid content was established by treating an aliquot of pure LOS and LPS with HCl (4 M,100 °C, 4 h), followed by a treatment with NaOH (5 M, 100 °C, 30 min). After adjustment of the pH, fatty acids were extracted in chloroform and methylated with diazomethane and analyzed by means of GLC-MS. The ester-bound fatty acids were released after treatment with 0.5 M aqueous NaOH/methanol (1:1, v/v, 85 °C, 2 h), and the product was then acidified, extracted in chloroform, methylated with diazomethane, and analyzed by means of GLC-MS.

In parallel, an aliquot of LPS and LOS was also treated with hydrogen chloride in methanol (1 M, 80 °C, 16 h). The mixture was extracted with hexane containing the fatty acids as methyl esters derivatives that were analyzed by means of GLC-MS.

The absolute configuration of fatty acids was established as previously reported.[7] The 3-hydroxyfatty acids were released by treatment with NaOH (4 M, 100 °C, 5 h), converted into the 3- methoxy acid L-phenylethylamides, and then analyzed by GLC-MS. Comparing retention times of authentic L-phenylethylamides of various standard fatty acids with those

derived from *A. faecalis* LOS/LPS it was possible to establish that the configuration of all the fatty acids was *R*. Analyses were all executed on an Agilent Technologies gas chromatograph (6850A) equipped with a mass-selective detector (5973N) and a Zebron ZB-5 capillary column (Phenomenex, 30 m × 0.25 mm internal diameter, flow rate 1 $mLmin^{-1}$, He as carrier gas).

Isolation of the core OS and the O-antigen fraction

In order to define the complete structure of the core OS, an aliquot of the LOS fraction underwent a full deacylation procedure. Briefly, LOS was treated with anhydrous hydrazine (1.5 mL), stirred at 37 °C for 90 min, cooled, poured into ice-cold acetone (15 mL) and allowed to precipitate. The precipitate was then centrifuged (4,500 x *g*, 30 min), washed with ice-cold acetone, dried, dissolved in water and lyophilized. The *O*-deacylated product was then *N*-deacylated with 4 M KOH (120 °C). Salts were removed by gel-filtration chromatography on a Sephadex G-10 column (Pharmacia, 50 x 1.5 cm) eluted with distelled water. The fully deacylated product was then further purified on a Toyopearl TSK HW-40S column (Tosoh Bioscience). In order to complete the analysis on the core OS, an aliquot of LOS fraction underwent also a mild acid hydrolysis with acetate buffer (pH 4.4, 2 h, 100 °C) followed by a centrifugation step (8,000 x *g*, 30 min, 4 °C) that allowed the separation of the saccharide component in the supernatant which was then further purified by means of a gel-filtration chromatography Toyopearl TSK HW-40S column (Tosoh Bioscience).

The above acetate buffer treatment was also performed on 10 mg of the LPS fraction derived from the hot phenol/water extraction, to isolate di O-antigen moiety. The O-antigen fraction was then purified through gel-filtration chromatography on a Sephacryl HR-S100 column (GE Healthcare Life Sciences) eluted with 50 mM ammonium bicarbonate. Both the saccharidic fractions, i.e. the core OS and the O-antigen, were analyzed by means of 1D and 2D NMR spectroscopy.

NMR spectroscopy

1D and 2D ^{1}H NMR spectra were recorded in D_2O at 298 K at pD = 7 with a Bruker 600 DRX spectrometer equipped with a cryoprobe. The spectra were calibrated with internal acetone (δ_H = 2.225 ppm; δ_C = 31.45 ppm). ^{31}P NMR experiments were carried out with a Bruker DRX-400 spectrometer; aqueous 85 % phosphoric acid was used as external reference (δ = 0.00 ppm). Total correlation spectroscopy (TOCSY) experiments were recorded with spinlock times of 100 ms by using data sets (t1 × t2) of 4096 × 256 points. Rotating frame Overhauser enhancement spectroscopy (ROESY) and Nuclear Overhauser enhancement spectroscopy (NOESY) experiments were performed by using data sets (t1 × t2) of 4096 × 256 points and by using mixing times between 100 and 300 ms, acquiring 16 scans. Double-quantum-filtered phase sensitive correlation spectroscopy (DQF-COSY) experiments were carried out by using data sets of 4096 × 512 points. The data matrix in all the homonuclear experiments was zero-filled in both dimensions to give a matrix of 4K × 2K points and was resolution-enhanced in both dimensions by a cosine-bell function before

Fourier transformation. Coupling constants were determined by 2D phase-sensitive DQF-COSY.[8,9] Heteronuclear single quantum coherence ($^{1}H,^{13}C$ HSQC) and heteronuclear multiple bond correlation ($^{1}H,^{13}C$ HMBC) experiments were executed in ^{1}H-detection mode by single-quantum coherence with proton decoupling in the ^{13}C domain using data sets of 2048 × 256 points. $^{1}H,^{13}C$ HSQC was performed using sensitivity improvement and in the phase-sensitive mode using Echo/Antiecho gradient selection, with multiplicity editing during selection step.[10] $^{1}H,^{13}C$ HMBC was optimized on long range coupling constants, with low-pass *J*-filter to suppress one-bond correlations, using gradient pulses for selection. Moreover, a 60 ms delay was used for the evolution of long-range correlations. $^{1}H,^{13}C$ HMBC was optimized for a 6 Hz coupling constant, and $^{1}H,^{31}P$ HSQC was optimized for an 8 Hz coupling constant. The data matrix in all the heteronuclear experiments was extended to 2048 × 1024 points by using forward linear prediction extrapolation.[11]

Isolation of the lipid A

Another little aliquot (3 mg) of both LOS and LPS was treated with acetate buffer (pH 4.4, 2 h, 100 °C) to isolate lipid A from the saccharide moiety. A mixture of chloroform and methanol was added to the hydrolysis product to obtain a chloroform/methanol/hydrolysate (2:2:1.8, v/v/v). The mixture was then extensively shaken and centrifuged. The chloroform phase, containing lipid A, was collected and washed with the water phase of a freshly prepared Bligh–Dyer mixture (2:2:1.8 chloroform/methanol/water).[12] The organic phases were pooled, dried, and analyzed by means of MALDI-TOF-MS (SCIEX, Concord, ON, Canada).

MALDI-TOF mass spetrometry (MS) and MS^2 analysis

The MS investigation was performed on an Applied Biosystems MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer, equipped with delayed extraction technology. Ions formed by a pulsed UV laser (Nd:YAG laser, λ= 355 nm) were accelerated by 24 kV. For lipid A analysis the matrix solution was prepared by dissolving trihydroxyacetophenone (THAP) dissolved in methanol/0.1% TFA/acetonitrile (7:2:1, v/v/v) at a concentration of 75 $mgmL^{-1}$. The lipid A fraction was dissolved in chloroform/methanol (1:1, v/v), as previously described.[13,14] Lipid A preparation and matrix solution were deposited on the MALDI plate and left to dry at room temperature. Each spectrum was the result of the accumulation of 1500 laser shots, whereas 6000–7000 shots were summed for the MS^2 data acquisitions. All reported experiments were acquired in both negative and positive polarity, reflectron mode.

Results

Isolation and compositional analyses of LOS and LPS from *A. faecalis*

Crude LOS and LPS were isolated from dried bacterial cells of *A. faecalis* through the phenol/chloroform/light petroleum and the hot phenol/water extraction protocols respectively.[1,2] The nature of the extracted material was defined by the SDS-PAGE analysis after silver nitrate gel staining[3] that revealed a run to the bottom of the gel in the case of the LOS indicative of its low molecular mass; on the contrary, a ladder-like pattern was clearly apparent for the LPS fraction diagnostic for the presence of a polysaccharide moiety, i.e. the O-antigen. Both the LPS and LOS fractions were purified from cell contaminants by enzymatic digestion followed by dialysis, ultracentrifugation and size exclusion chromatography. After purification, a set of chemical analyses were performed to define the *A. faecalis* LOS and LPS monosaccharide and fatty acid composition.

Monosaccharide analysis of the LOS fraction revealed the presence of terminal D-galactose (Gal), 6-substituted D-glucose (Glc), terminal D-glucosamine (GlcN), 6-substituted D-GlcN, terminal D-galactosamine (GalN), 4-substituted D-GalN, 3,4-disubstituted L,D-heptose (Hep), 2,7-disubstituted L,D-Hep and 5-substituted Kdo. On the other hand, the monosaccharide analysis of the LPS fraction showed the occurrence mainly of terminal-D-xylose (Xyl), 2-substituted D-rhamnose (Rha), 3-substituted D-Rha and 2,3,4-trisubstituted D-Rha, as previously reported.[25] All sugar residues were in the pyranose form.

Total fatty acid content analysis highlighted the occurrence of the same acyl chain moieties for the LOS and LPS. The main fatty acids detected were (*R*)-3-hydroxytetradecanoic acid (14:0(3-OH)) with ester and amide linkages, and (*R*)-3-hydroxydodecanoic acid (12:0(3-OH)), decanoic (10:0), dodecanoic (12:0) and tetradecanoic (14:0) acids in ester linkage.

Isolation and characterization of the core OS from *A. faecalis* LOS

In order to characterize the structure of the core OS, a complete deacylation of the LOS was executed using a mild hydrazinolysis followed by strong alkaline treatment.[26] The obtained product after purification underwent an extensive 2D NMR analysis (Figure S1-S4). The complete establishment of all spin systems was achieved by tracing the spin connectivity derived from the double quantum filtered correlated spectroscopy (DQF-COSY) and the total correlation spectroscopy (TOCSY) spectra; the precise identification of each carbon atom was attained through the heteronuclear single quantum coherence (^{1}H,^{13}C HSQC) spectrum. The designation of the anomeric configuration of each monosaccharide was gained by the *intra*-residual NOE correlations visible in the nuclear Overhauser effect spectroscopy (NOESY) spectrum and the $^{3}J_{H1,H2}$ coupling constants attained from the DQF-COSY spectrum, whereas the vicinal $^{3}J_{H,H}$ coupling constants guided the determination of the relative configuration of each monosaccharide. Finally, the merging of data from NOESY and heteronuclear multiple bond correlation (^{1}H,^{13}C HMBC), allowed the establishment of the complete oligosaccharide structure. Finally, ^{31}P and ^{31}P,^{1}H HSQC experiments were also recorded to define the phosphorylated positions revealing the occurrence of three different monophosphate ester groups in the range of chemical shift between -0.76 ppm and 3.60

ppm, all of which were correlated with sugar proton signals (Table S1). All sugar units were present as pyranose rings, according to the ^{13}C chemical shift values and in agreement with chemical analyses.

The ^{1}H and the $^{1}H,^{13}C$ HSQC spectra of the fully deacylated oligosaccharide (Figure S1), revelead the presence of anomeric proton signals (**A**-**L**) between 5.54 and 4.41 ppm (Figure S1a,d and Table S1) relative to twelve spin systems. Moreover, the upfield-shifted signals at 1.90/2.16 ppm were attributed to the H-3 methylene protons of the 3-deoxy-D-*manno*-oct--ulosonic (Kdo, **K**) unit (Table S1, Figure S1c).

The spin systems **A** (H-1/C-1 signals at 5.54/90.7 ppm, Table S1) and **H** (H-1/C-1 signals at 4.82/99.2 ppm, Table S1) were identified as Glc*p*N1*P* and Glc*p*N4*P* of the lipid A due to their H-2 protons that showed a correlation with nitrogen-bearing carbon atoms at 54.1 and 55.4 ppm, respectively; the *gluco* configuration of both units was outlined by the high $^{3}J_{H,H}$ ring proton values (8–10 Hz). The occurrence of an *inter*-residue contact between H-1 **H** and H-6 **A** in the NOESY spectrum (Figure S3), further corroborated the assignment of residues **A** and **H** as the lipid A sugar backbone. Phosphorylation at positions O-1 **A** and O-4 **H** was determined by the observation of downfield shifts for C-1/H-1 of residue **A** (δ_C/δ_H = 90.7/5.54 ppm) and C-4/H-4 **H** (δ_C/δ_H = 73.9/3.64 ppm), in addition to the correlations in the $^{31}P,^{1}H$ HSQC spectrum (not shown) with a signal at δ = 2.01 ppm for residue **H** and δ = 3.60 ppm for **A**.

The residues **D** and **G** were identified as α-*manno* configured units as proven by the low coupling constant $^{3}J_{H1,H2}$ and $^{3}J_{H2,H3}$ values (below 3 Hz), which are diagnostic of H-2 equatorial orientation; furthermore, the possibility to assign, in the TOCSY spectrum, from H-2 all the other resonances of the ring protons, led the identification of both units as α-heptose residues. Finally, in accordance also to compositional analyses, both residues were attributed to L-*glycero*-D-*manno*-heptoses as also proven by the chemical shift values of their C-6 (below 71 ppm) (Table S1).

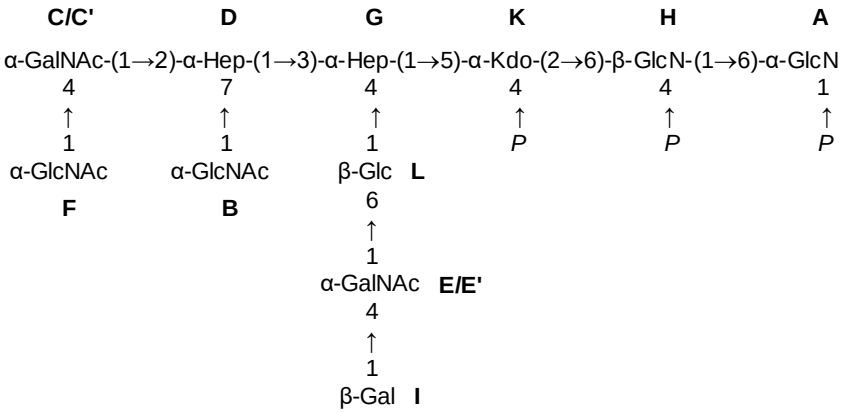
Spin systems **C**, **C'**, **E**, **E'** and **I** were all recognized as *galacto*-configured residues based on their low $^{3}J_{H-3,H-4}$ and $^{3}J_{H-4,H-5}$ values (3 and 1 Hz, respectively). The $^{1}J_{C-1,H-1}$ and $^{3}J_{H-1,H-2}$ values (170 and 3.6 Hz, respectively) and the *intra*-residual NOE correlation observed between H-1 and H-2 determined the assignment of **C**, **C'**, **E** and **E'** as α-configured sugar units, whereas **I** was identified as a β-configured residue. Finally, the occurrence of signals for C-2 of **C**, **C'**, **E** and **E'** at 54.2 ppm and 50.8 ppm also indicated that C-2 of these sugar residues were nitrogen-bearing carbon atoms, thus allowing their final identification as α-GalN units.

Residues **B**, **F** and **L** were all attributed to *gluco*-configured residues due to their high $^{3}J_{H,H}$ ring proton values; moreover, due to the occurrence of a correlation of H-2 with a nitrogen-bearing carbon signal at 54.1 ppm, **B** and **F** were identified as α-GlcN units, whereas **I** was assigned to a β-configured glucose due to the chemical shifts of H-1 and C-1 (4.41 and 102.8 ppm respectively), the *intra*-residue NOE contact of H-1 with H-3 and H-5, and the $^{3}J_{H-1,H-2}$ value (8.1 Hz).

Finally, the α-anomeric configuration of Kdo residue was attributed on the basis of the chemical shift values of H-3 and of the $^{3}J_{H7,H8a}$ and $^{3}J_{H7,H8b}$ coupling constants.[27] Furthermore, Kdo was found phosphorylated at position O-4, based on the downfield displacement of C-4/H-4 (δ_C/δ_H = 70.2/4.46 ppm) and on the correlation of H-4 with a signal at δ = - 0.76 ppm observed in the $^{31}P,^{1}H$ HSQC spectrum (not shown).

Downfield shift of carbon resonances (Figure S1b) was crucial to identify glycosylated positions at O-3 and O-4 of residue **G**, O-2 and O-7 of residue **D**, O-6 of **A**, **H** and **L**, O-4 of **C** and **E**, O-5 of **K**; whereas **B**, **C'**, **E'**, **F** and **I** were all identified as terminal sugar units, in full accordance with linkage analysis. The primary sequence of the core OS was inferred by using NOE contacts of the NOESY spectrum (Figure S3) and by long-range correlations visible in the HMBC (Figure 1a and S4). Briefly, starting from the lipid A sugar backbone, built up of residues **A** and **H**, the latter was found to be substituted at position O-6 by Kdo unit. This was further confirmed by the weak low-field shift of signal of C-6 of **H**, matching with the expected α-(2→6) ketosidic linkage of Kdo with the β-GlcN residue. Kdo **K** unit was, in turn, substituted at O-5 by α-Hep **G** validated by the *inter*-residual NOE correlation of H-1 **G** with H-5 **K** (Figure S3) and by the corresponding long-range correlation observed in the HMBC spectrum (Figure 1a). Residue **G** was substituted at positions O-3 and O-4 by α-Hep **D** and β-Glc **L** respectively, as proven by the NOE contact between the signal H-1 **D** (5.27 ppm) and H-3 **G** (4.10 ppm), and between H-1 **L** (4.41 ppm) and H-4 **G** (4.03 ppm) (Figure S3); these were also corroborated by the observation of the related long-range correlations visible in the HMBC spectrum (Figure 1a). Residue **L** was, in turn, substituted at its O-6 by terminal α-GalN unit **E/E'** proved by the long-range correlation between H-1 **E/E'** (5.15/5.12 ppm, Table S1) and C-6 of **L** (66.2 ppm). Residue **E** was found to be not-stoichiometrically substituted at position O-4 by β-Gal **I** as proven by the long-range connectivity between H-1 **I** (4.51 ppm) and C-4 of **E** (73.6 ppm). The α-Hep **D** was substituted at O-2 by α-GalN **C**/**C'** and at O-7 by α-GlcN residue **B**, as proven by the NOE contacts visible between anomeric proton signal of **C**/**C'** (5.33/5.38 ppm) and H-2 of **D** (4.02 ppm), and H-1 **B** (5.34 ppm) with H-7 of **D** (3.75 ppm) (Figure S3 and 1a). Finally, residue **C** was found to be not-stoichiometrically substituted at its position O-4 by α-GlcN residue **F** due to the NOE correlation between H-1 **F** (5.16 ppm) and H-4 of **C** (4.00 ppm) (Figure S3).

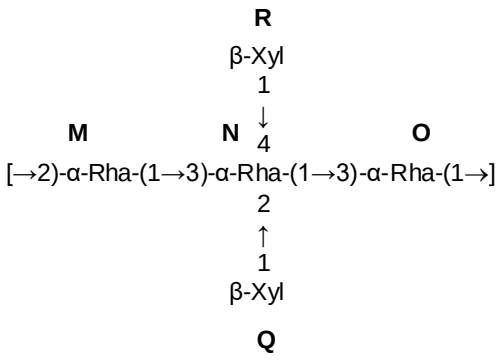
All the above data were further confirmed by the NMR investigation of the core OS obtained after mild acid hydrolysis of the LOS fraction (^{1}H NMR spectrum is reported in Figure S5). This procedure selectively cleaved the linkage between the Kdo unit and the non-reducing glucosamine of the lipid A moiety thus enabling the isolation, after proper purification, of both the lipid A and the core OS moieties. The NMR analysis showed the occurrence of four *N* acetyl groups, as suggested by the signals in the range 1.99-1.87 ppm (Figure S3), and the relative NOE correlations between the H-2 of each amino sugar and the methyl protons resonating in the above range of ppm. Therefore all the NMR data, in conjugation with chemical analyses, highlighted that the core OS was a *mono*-phosphorylated, heptose containing nonasaccharide built up of four *N*-acetyl-hexosamines as sketched in Figure 2a and Scheme S1.



Scheme S1. Core OS structure including the glucosamine disaccharide backbone of the lipid A (**H** and **A**) achieved by combination of 1D and 2D NMR analysis of the fully deacylated and the acid hydrolysis products of the *A. faecalis* LOS fraction. Monosaccharide lettering is indicated as in Table S1. "*P*" indicates the phosphate group.

Isolation and structural characterization of the O-antigen from *A. faecalis* LPS

The O-antigen moiety was isolated by mild acid hydrolysis of the LPS followed by centrifugation and size-exclusion chromatography purification. A complete set of 1D and 2D NMR experiements (DQF-COSY, TOCSY, NOESY, HSQC and HMBC) was recorded in order to define the structure of such a polysaccharide moiety (Figures S6-S9). The latter turned out to be made up of a branched pentasaccharide repeating unit where the linear chain was a rhamnan, while xylose residues were attached to one of the rhamnoses as the two branches, as previously reported.[25] Briefly, the proton and HSQC spectra revealed the occurrence of seven main anomeric signals indicative of seven different spin systems (**M**–**R**, Table S2, Figure S6a), five of which (**M**, **N**, **O**, **O'** and **P**) showed correlations in the TOCSY spectrum with methyl signals at 1.33, 1.39, 1.28, 1.28 and 1.33 ppm, respectively (Figure S8b). Residues **M** (H-1/C-1 signals at 5.25/100.3 ppm), **N** (H-1/C-1 signals at 5.11/102.3 ppm), **O** (H-1/C-1 signals at 4.94/102.0), and **O'** (H-1/C-1 signals at 4.96/102.0 ppm) (Table S2) were all identified as α-rhamnose units. Residue **P** (H-1/C-1 signals at 4.83/100.2) was assigned to a β-configured rhamnose as proven by the NOE contact of H-1 with H-3 and H-5 in the NOESY spectrum, further confirmed by the H-5 chemical shift at 3.47 ppm and the C-5 chemical shift at 71.9 ppm, namely at a higher (δ_H) and lower (δ_C) fields than those observed for α-configured rhamnose units.[28,29] Finally, residues **Q** and **R** were identified as β-xylose units in full agreement with chemical analyses. Downfield displacement of the chemical shifts visible in the HSQC spectrum (Figure S6b) was indicative of glycosylation at position O2 for residue **M** and **P**, O-3 for **O** and **O'**, O-2,O-3 and O-4 for **N**, whereas **Q** and **R** were assigned to terminal sugar units. The combination of data attained from NOESY (Figure S8) and HMBC (Figure S9) spectra yielded the sugar sequence in the repeating unit of the *A. faecalis* O-antigen, as sketeched below in Scheme S2.



Scheme S2. The main structure of the reapeating unit composing the O-antigen of *A. faecalis* LPS as deduced by 1D and 2D NMR investigation of the mild acid hydrolysis executed on the LPS fraction. Monosaccharide lettering is indicated as in Table S2; all residues are in D configuration.

Briefly, a linear rhamnan chain was defined as composed of the sequence **M**1→3**N**1→3**O**1→2**M** (Figure 1d) as proven by the relative long-range and *inter*-residue NOE correlations shown in Figure 1c,d, S8 and S9. The β-Xyl units **Q** and **R** were found sitting at positions O-2 and O-4 of unit **N** respectively, as proven by the long-range correlations between H-1 of **Q** and C-2 of **N**, and H-1 of **R** with C-4 of **N**, in addition to the corresponding *inter*-residue NOE connectivities (Figures 1c,d and S8). As stated above, this branched repeating unit was identical to the one reported by Knirel *et al*. (1986),[25] nevertheless, a minor substructure was also indetified. Information about the latter could be drawn by the occurrence of the long-range correlations between H-1 of **P** and C-2 of **M**, in addition to the one between H-1 of **O'** and C-2 of **P**, suggesting the existance of the substructure **O'**1→2**P**1→2**M**, i.e. α-Rha-(1→2)-β-Rha-(1→2)-α-Rha.

Isolation and structural characterization of *A. faecalis* lipid A

Purification through Bligh–Dyer extraction[12] executed after the mild acid hydrolysis of LOS and LPS, allowed the isolation of the lipid A fractions that underwent a detailed MALDI-TOF MS and MS^2 investigation. The results showed that the LPS and the LOS express an identical lipid A structure, as expected.

The reflectron MALDI-TOF mass spectrum, recorded in negative polarity, of the lipid A is reported in Figure S10. The spectrum showed, in the range *m/z* 1347.8–1784.9, a complex pattern of peaks relative to deprotonated $[M-H]^-$ lipid A species differing both in the nature and number of acyl chains and phosphate content. Three distinct families of peaks around *m/z* 1375.8, 1601.9 and 1756.1 were clearly identified and matched with tetra-, penta-, and hexa-acylated lipid A species respectively, whose heterogeneous nature was also clearly apparent due to the occurrence of mass differences of 28 amu (-$(CH_2)_2$-unit) , which are indicative of lipid A species that differ in the length of their fatty acid chains (Figure S10). In addition differences of 16 amu were also detected and suggested the substitution or absence of hydroxylated acyl chains in both tetra-, penta- and hexa-acylated lipid A species. In detail, the peaks at *m/z* 1521.9 and 1601.9 were attributed to *mono*- and *bis*-phosphorylated penta-acylated lipid A species carrying four 14:0 (3-OH) and one 12:0 (3-OH). In the mass range *m/z* 1347.8-1387.9, tetra-acylated lipid A species were detected and

the peak at *m/z* 1375.8 was assigned to a *bis*-phosphorylated tetra-acylated lipid A species carrying three 14:0 (3-OH) and one 12:0 (3-OH). *Mono*- and *bis*-phosphorylated hexa-acylated lipid A forms carrying four 14:0 (3-OH), one 12:0 (3-OH) and one 10:0 were assigned to peaks at *m/z* 1676.3 and 1756.1 respectively.

To establish the detailed structure of *A. faecalis* lipid A, revealing the exact location of the acyl chains as well as of the phosphate units with respect to the glucosamine disaccharide backbone, a negative-ion MS^2 analysis on several ion peaks was conducted (Figures S11-S12). In detail, the MS^2 spectrum of the precursor ion at *m/z* 1375.8 (Figure S11a), corresponding to a *bis*-phosphorylated tetra-acylated lipid A species, showed an intense peak at *m/z* 1131.6 attributed to an ion that was derived from the loss of a 14:0 (3-OH) moiety. Less intense peaks were detected at *m/z* 1033.6 and 1159.6 matching with a lipid A fragment avoid of both one phosphate and one 14:0 (3-OH) (*m/z* 1033.6), and one 12:0 (3-OH) (*m/z* 1159.6). Moreover, peak at *m/z* 915.4 was attributed to an ion derived from the loss of both one 12:0 (3-OH) and one 14:0 (3-OH), whereas peak at *m/z* 817.4 indicated a product ion derived from the loss of one 12:0 (3-OH), one 14:0 (3-OH) and one phosphate group. An important peak originating from the cleavage of the glycosidic linkage (Y_1)[30] at *m/z* 710.4 (Figure S11a) was also detected. This product ion led the definition of the acyl chains decorating the reducing glucosamine unit, namely two 14:0 (3-OH), and specularly those decorating the non-reducing glucosamine, that is one 14:0 (3-OH) and 12:0 (3-OH). More information crucial for the structural characterization derived from the negative-ion MS^2 experiments executed on peaks at *m/z* 1601.9 (Figure S11b) and *m/z* 1676.3 (Figure S11c). Briefly, MS^2 spectrum of precursor ion at *m/z* 1601.9 (Figure S11b), relative to a *bis*-phosphorylated penta-acylated lipid A species, revealed two main product ions at *m/z* 1357.7 and 1259.7 matching with lipid A fragments avoid of one 14:0 (3-OH) (*m/z* 1357.7), and of one 14:0 (3-OH) and one phosphate (*m/z* 1259.7). The occurrence of Y_1 ion at *m/z* 710.4 was pivotal to further corroborate the structural hypothesis of the 12:0 (3-OH) unit as an acyl moiety decorating the non-reducing glucosamine unit. On the other hand, MS^2 analysis of precursor ion at *m/z* 1676.3 (Figure S11c), corresponding to a *mono*-phosphorylated hexa-acylated lipid A species, showed besides product ions derived from the loss of one 14:0 (3-OH) (*m/z* 1432.0), or one 12:0 (3-OH) (*m/z* 1460.0), or two 14:0 (3-OH) units (*m/z* 1187.7), also important ions originating from sugar ring fragmentations $^{0,2}A_2$ (*m/z* 1236.7) and $^{0,4}A_2$ (*m/z* 950.6) (Figure S11c). The latter fragmentations were key to define the location of the 10:0 as a secondary acyl substituent only of the reducing glucosamine unit. Moreover, the above fragmentations, in addition to the absence of an Y_1 ion, were also helpful to define the position of the phosphate group in such a lipid A species that is on the non-reducing glucosamine. Finally, the absence of fragments matching with the loss of a whole unit of a hydroxylated fatty acid with the secondary 10:0 acyl substituent, as well as the occurrence of a fragment derived from the sequential loss of two 14:0 (3-OH) moieties (*m/z* 1187.7) from the precursor ion, indicated that the secondary 10:0 was bound to the *N*-linked primary acyl moiety of the reducing glucosamine. This structural deduction was also confirmed by analysis of the MS^2 spectra recorded on precursor ions at *m/z* 1097.8 (related to a *mono*-phosphorylated tri-acylated lipid A species, Figure S12a), at *m/z* 1521.9 (*mono*-phosphorylated penta-acylated lipid A species, Figure S12b), and at *m/z* 1756.1 (*bis*-phosphorylated hexa-acylated lipid A species, Figure S12c).

The analysis of all the above negative-ion MS^2 spectra suggested that *A. faecalis* lipid A was decorated by 14:0 (3-OH) moieties as the primary fatty acids, whereas 12:0 (3-OH) and

10:0 were the secondary acyl chains. In order to unequivocally define the nature and position of secondary fatty acids, a reflectron MALDI-TOF MS experiment in positive polarity was recorded and the spectrum (not shown) confirmed the presence of the molecular mass of the lipid A species with Na^+ counterions. The peak at *m/z* 1320.2, relative to a sodiated *mono*-phosphorylated tetra-acylated lipid A species carrying three 14:0 (3-OH) and one 12:0 (3-OH), underwent a positive-ion MS^2 investigation. The MS^2 spectrum of precursor ion at *m/z* 1320.2 (Figure S13), among other product ions, showed a very diagnostic peak for structural elucidation at *m/z* 688.4 attributed to the oxonium ion. This fundamental fragment ion arose from the cleavage of the glycosydic linkage and gave a first indication of the 12:0 (3-OH) moiety linked as a secondary acyl chain to the primary amide bound 14:0 (3-OH) of the non-reducing glucosamine. In support of this hypothesis, the ion at *m/z* 852.7 (Figure S13), derived from a loss of 467 mass units, matched with the sequential loss of the phosphate group plus 368 mass units; the observation of such a peak led to the conclusion that a rearrangement, promoted by the free 3-OH groups on both primary 14:0 (3-OH), drove the loss of 184 mass units ($C_{12}H_{24}O$) from each primary acyl chain.[14,31] Because such a fragmentation can only occur if the fatty acids have a free 3-OH group, this further suggested that the 12:0 (3-OH) was a secondary acyl moiety of the primary *N*-linked 14:0 (3-OH) of the non-reducing glucosamine unit. Finally, the MS^2 spectra recorded in positive polarity on precursor ions at *m/z* 1546.1 (Figure S14a) and *m/z* 1700.2 (Figure S14b) related to *mono*-phosphorylated penta- and hexa-acylated lipid A species, also supported and confirmed the above structural evaluation. Therefore, combining fatty acid analysis with both positive- and negative-ion MALDI-TOF MS and MS^2 data on isolated lipid A, it was possible to define the *A. faecalis* LOS/LPS as expressing a blend of lipid A species, whose main component with the highest degree of acylation is reported in Figure 1a.

Table S1.

(600 MHz, 298 K, D_2O) ^{1}H,^{13}C (*italic*) and ^{31}P (**bold**) chemical shifts (ppm) of fully deacylated LOS from *A. faecalis*.

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
A	^{1}H	5.54	3.26	3.80	3.37	4.05	4.17/3.91		
6-α-D-GlcN1*P*	^{13}C	*90.7*	*54.1*	*69.7*	*69.6*	*72.1*	*69.0*		
	^{31}P	**3.60**							
B	^{1}H	5.34	3.27	3.82	3.42	3.69	3.88/3.79		
t-α-D-GlcN	^{13}C	*95.9*	*54.1*	*69.7*	*69.5*	*72.2*	*60.2*		
C	^{1}H	5.33	3.30	3.83	4.00	3.89	3.67		
4-α-D-GalN	^{13}C	*97.2*	*54.2*	*69.8*	*75.6*	*71.4*	*60.9*		
C'	^{1}H	5.38	3.27	3.83	3.70	3.89	3.67		
t-α-D-GalN	^{13}C	*95.9*	*54.2*	*69.8*	*69.7*	*71.4*	*60.7*		
D	^{1}H	5.27	4.02	3.92	3.75	3.65	4.06	3.75	
2,7-α-L,D-Hep	^{13}C	*100.0*	*77.7*	*70.1*	*69.2*	*72.2*	*69.3*	*70.7*	
E	^{1}H	5.15	3.42	3.93	3.98	4.02	3.66		
4-α-D-GalN	^{13}C	*94.8*	*50.8*	*71.9*	*73.6*	71.7	60.8		
E'	^{1}H	5.18	3.42	3.91	3.97	4.07	3.66		
t-α-D-GalN	^{13}C	*94.8*	*50.8*	*68.0*	*66.6*	*71.7*	*60.8*		
F	^{1}H	5.16	3.22	3.82	3.37	3.69	3.77/3.68		
t-α-D-GlcN	^{13}C	*95.9*	*54.1*	*69.7*	*69.5*	*72.0*	*60.2*		
G	^{1}H	5.05	3.98	4.10	4.03	3.67	3.80	3.65/3.48	
3,4-α-L,D-Hep	^{13}C	*100.0*	*70.2*	*76.3*	*72.1*	*69.9*	*69.8*	*62.5*	
H	^{1}H	4.82	2.98	3.76	3.64	3.43	3.62/3.44		
6-β-D-GlcN4*P*	^{13}C	*99.2*	*55.4*	*71.8*	*73.9*	*72.0*	*62.3*		
	^{31}P				**2.01**				
I	^{1}H	4.51	3.54	3.75	3.99	3.66	3.69		
t-β-D-Gal	^{13}C	*103.7*	*72.2*	*73.5*	*70.4*	*75.7*	*60.3*		
L	^{1}H	4.41	3.21	3.36	3.35	3.50	3.88		
6-β-D-Glc	^{13}C	*102.8*	*73.3*	*75.8*	*69.5*	*74.4*	*66.2*		
K	^{1}H	-	-	1.90/2.16	4.46	4.18	3.69	3.95	3.86/3.56
5-α-D-Kdo4*P*	^{13}C	*171.7*	*95.6*	*34.2*	*70.2*	*71.2*	*72.1*	*70.1*	*63.6*
	^{31}P				**-0.76**				

Table S2.

(600 MHz, 2983 K, D_2O) ^{1}H and ^{13}C (*italic*) chemical shifts (ppm) of the O-antigen isolated through mild acid hydrolysis of the *A. faecalis* LPS fraction.

		1	2	3	4	5	6
M	^{1}H	5.25	4.01	4.06	3.52	3.88	1.33
2-α-D-Rha	^{13}C	*100.3*	*78.5*	*69.3*	*72.1*	*69.1*	*16.6*
N	^{1}H	5.11	4.16	4.03	3.65	3.93	1.39
2,3,4-α-D-Rha	^{13}C	*102.3*	*79.3*	*75.5*	*79.7*	*69.5*	*16.6*
O	^{1}H	4.94	4.12	3.99	3.51	3.81	1.28
3-α-D-Rha	^{13}C	*102.0*	*69.4*	*80.2*	*72.9*	*69.0*	*16.5*
O'	^{1}H	4.96	4.19	3.99	3.51	3.79	1.28
3-α-D-Rha	^{13}C	*102.0*	*70.0*	*80.2*	*72.9*	*69.0*	*16.5*
P	^{1}H	4.83	4.17	3.72	3.71	3.47	1.33
2-β-D-Rha	^{13}C	*100.2*	*79.4*	*76.6*	*72.3*	*71.9*	*16.7*
Q	^{1}H	4.47	3.37	3.43	3.49	3.98/3.22	
t-β-D-Xyl	^{13}C	*105.2*	*73.4*	*75.5*	*72.0*	*64.9*	
R	^{1}H	4.48	3.32	3.38	3.48	3.98/3.27	
t-β-D-Xyl	^{13}C	*103.0*	*73.1*	*75.7*	*72.0*	*64.9*	

Figure S1.

(600 MHz, 298 K, D_2O, Table S1). Superimposition of ^1H and HSQC spectra of the fully deacylated LOS from *A. faecalis*. Zoom of (**a**) the anomeric, (**b**) the carbinolic, and (**c**) the Kdo methylene densities regions. Key heteronuclear one-bond correlations are indicated. Numbering of sugar residues is as reported in Table S1. The full proton and HSQC spectra are reported in (**d**).

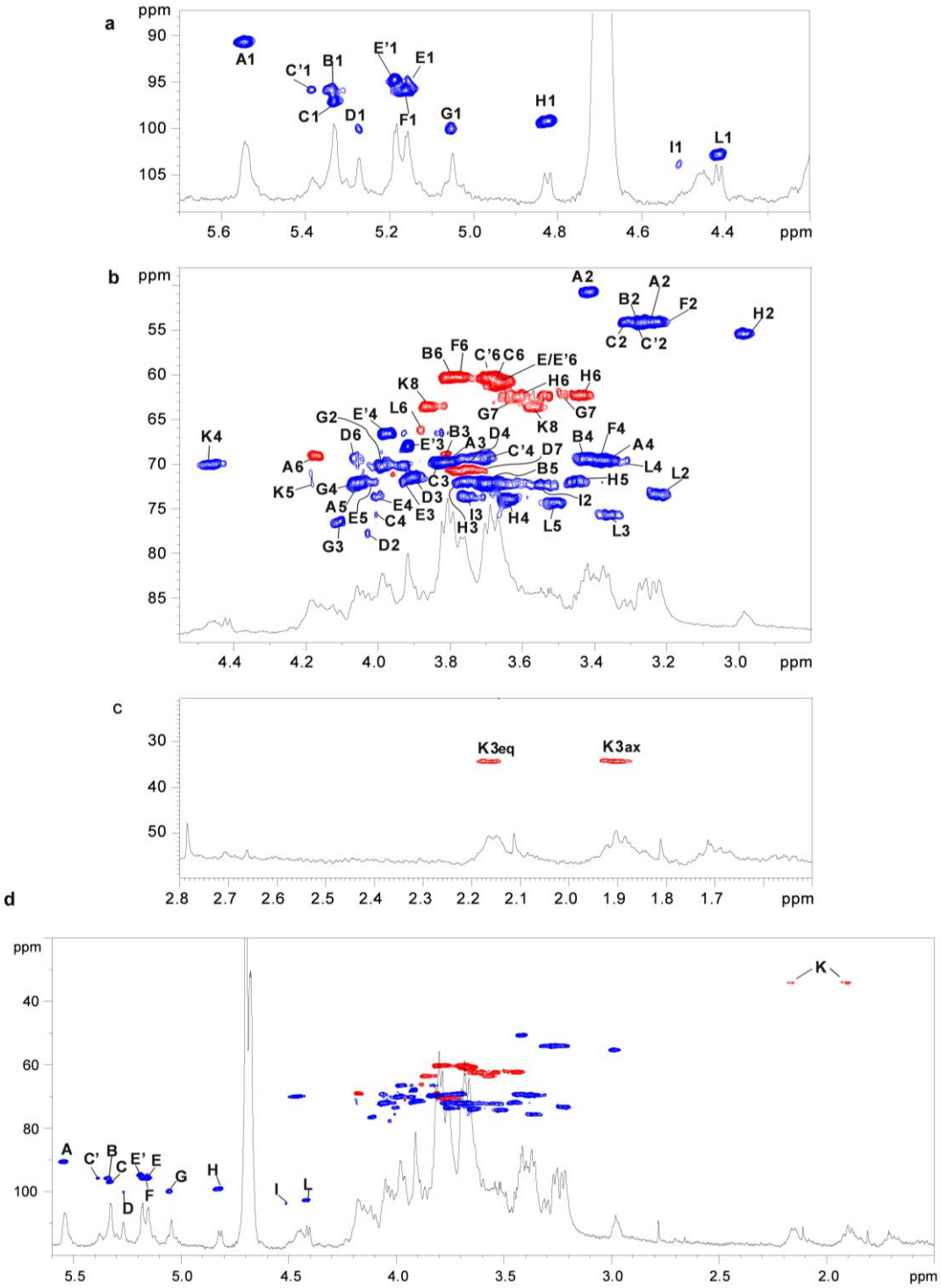


Figure S2.

(600 MHz, 298 K, D_2O, Table S1). COSY spectrum of the fully deacylated LOS from *A. faecalis*. The The H-1-H-2 correlations are indicated following numbering of sugar residues as reported in Table S1.

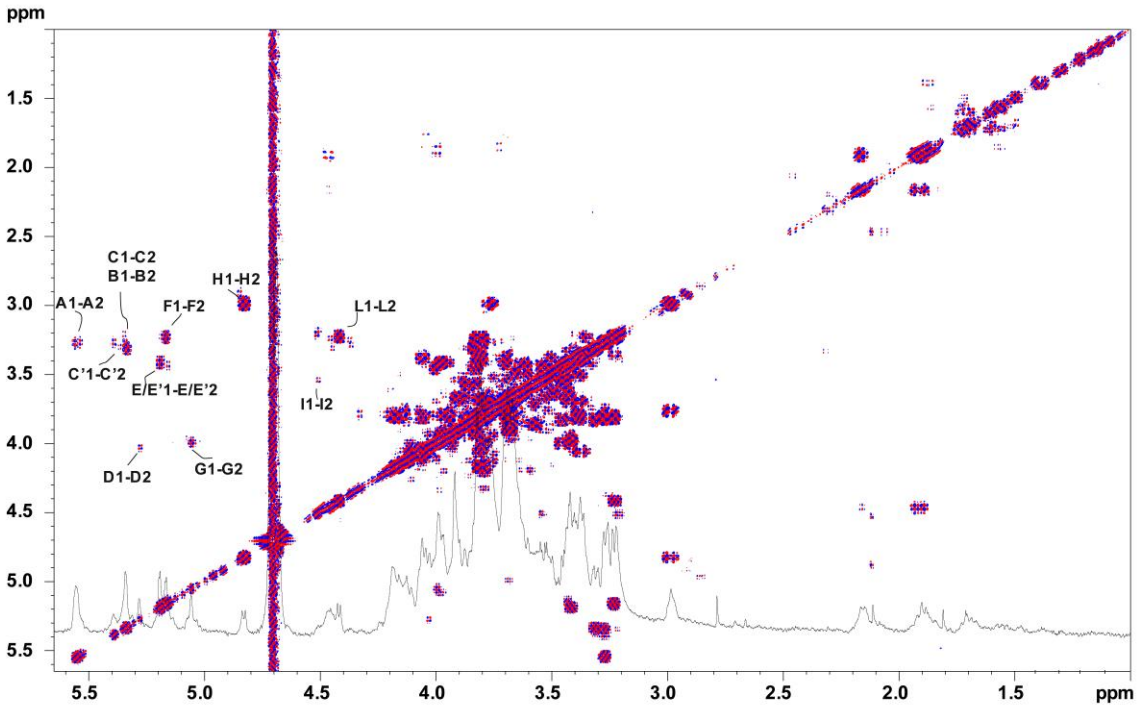


Figure S3.

(600 MHz, 298 K, D_2O, Table S1). **a)** Zoom of the overlapped ^1H, NOESY (red) and TOCSY (black) spectra of the fully deacylated LOS from *A. faecalis*. The key *inter*-residue NOE contacts that furnished structural information on the primary sequence of the core OS are indicated. The superimposition of the full proton, NOESY (red) and TOCSY (black) spectra is reported in **b**.

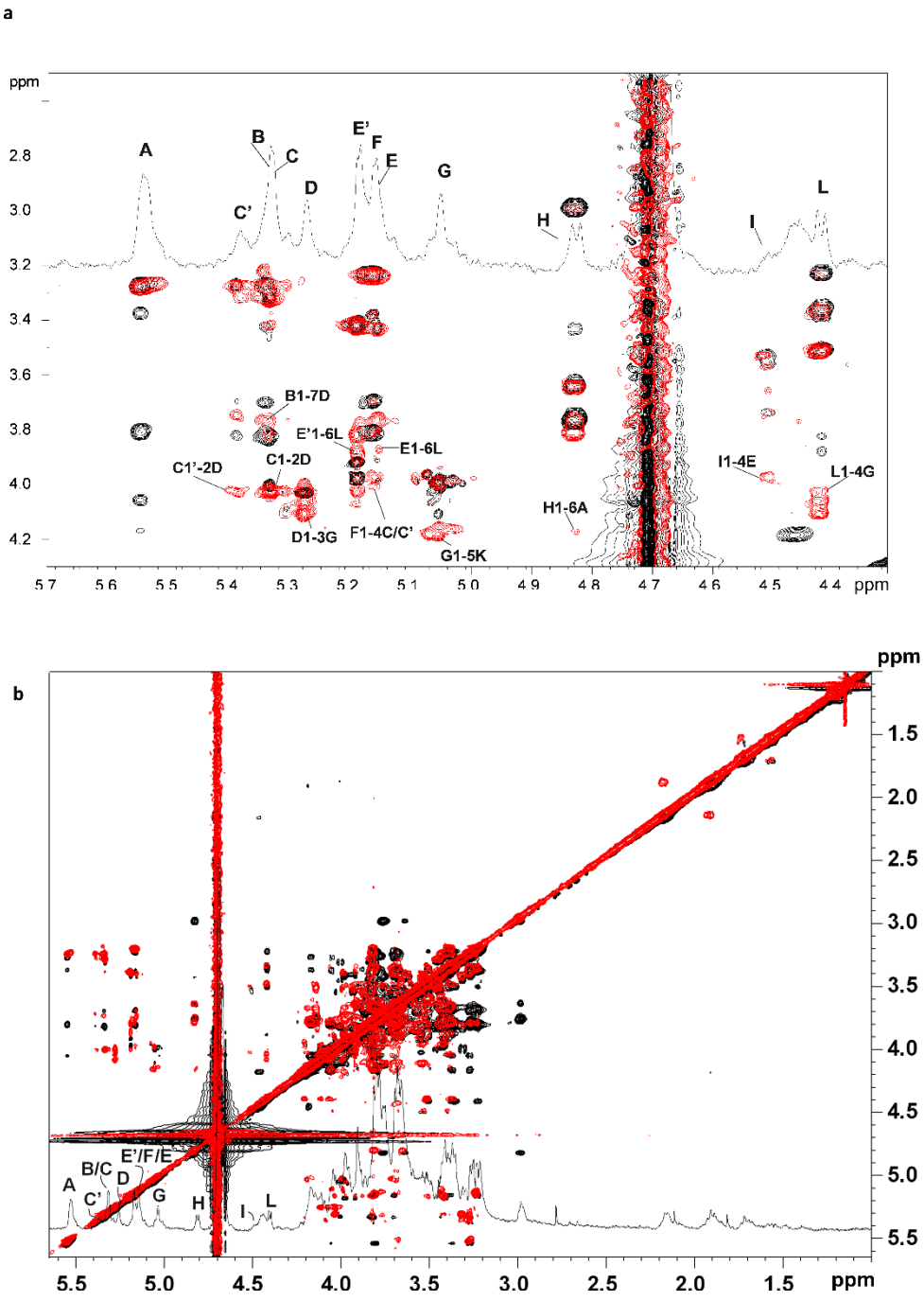


Figure S4.

(600 MHz, 298 K, D_2O, Table S1). Superimposition of proton and HMBC full spectra recorded for the deacylated LOS from *A. faecalis*. Key *inter*-residue long-range correlations are indicated. Numbering of sugar residues is as reported in Table S1 and Figure 2. Zoom of the anomeric region is reported in Figure 1a.

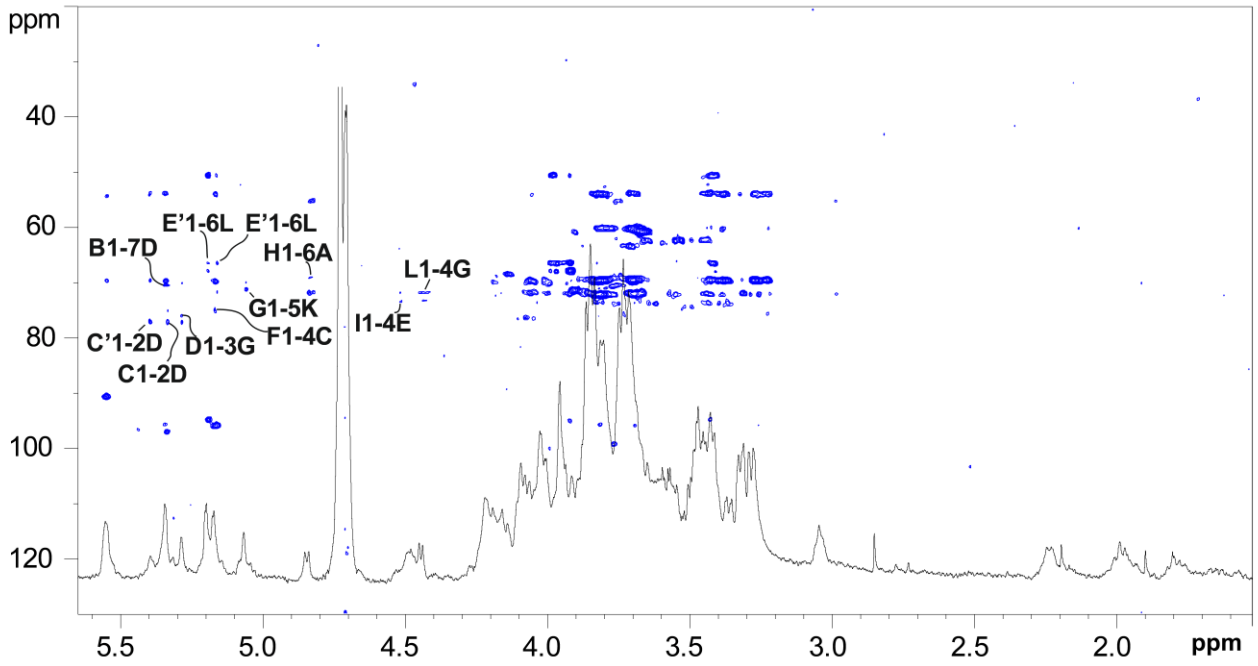


Figure S5.

(600 MHz, 298 K, D_2O). ^1H NMR spectrum of the core OS obtained after mild acid hydrolysis of the *A. faecalis* LOS fraction. The signals relative to the proton of the *N*-acetyl groups (**NHAc**) are indicated.

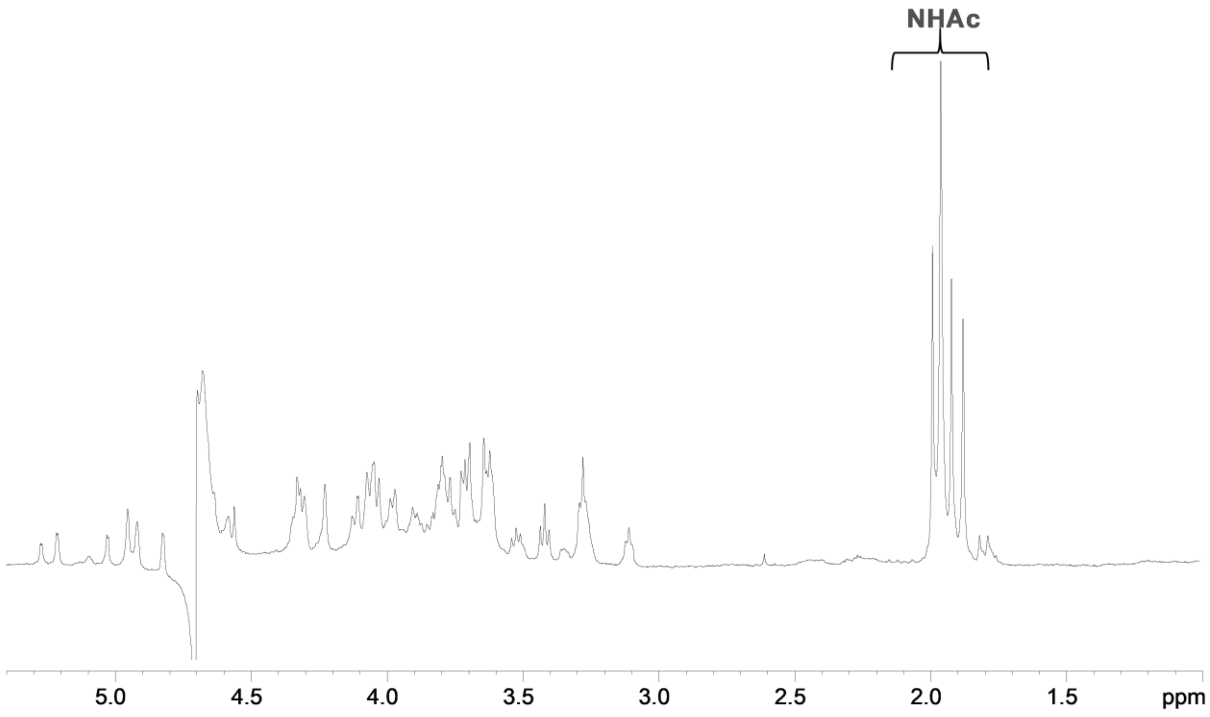


Figure S6.

(600 MHz, 298 K, D_2O, Table S2). HSQC spectrum recorded for the O-antigen from *A. faecalis* LPS fraction. (**a**-**c**) Zoom of the overlapped ^1H and HSQC spectra, one-bond heteronuclear correlation are indicated; the overlapped proton and HSQC full spectra are reported in **d**.

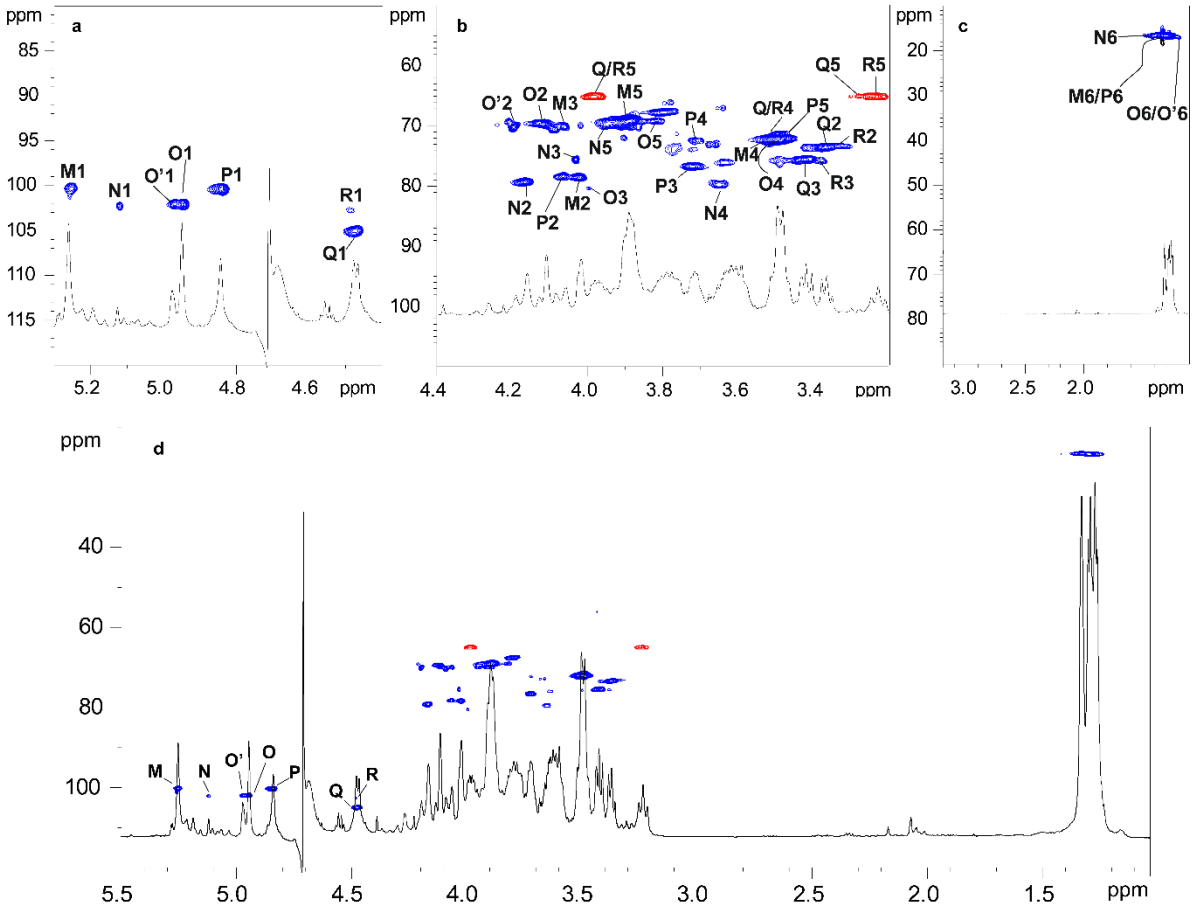


Figure S7

(600 MHz, 298 K, D_2O, Table S2). COSY spectrum recorded for the O-antigen from *A. faecalis* LPS fraction. The H-1-H-2 correlations are indicated following numbering of sugar residues as reported in Table S2.

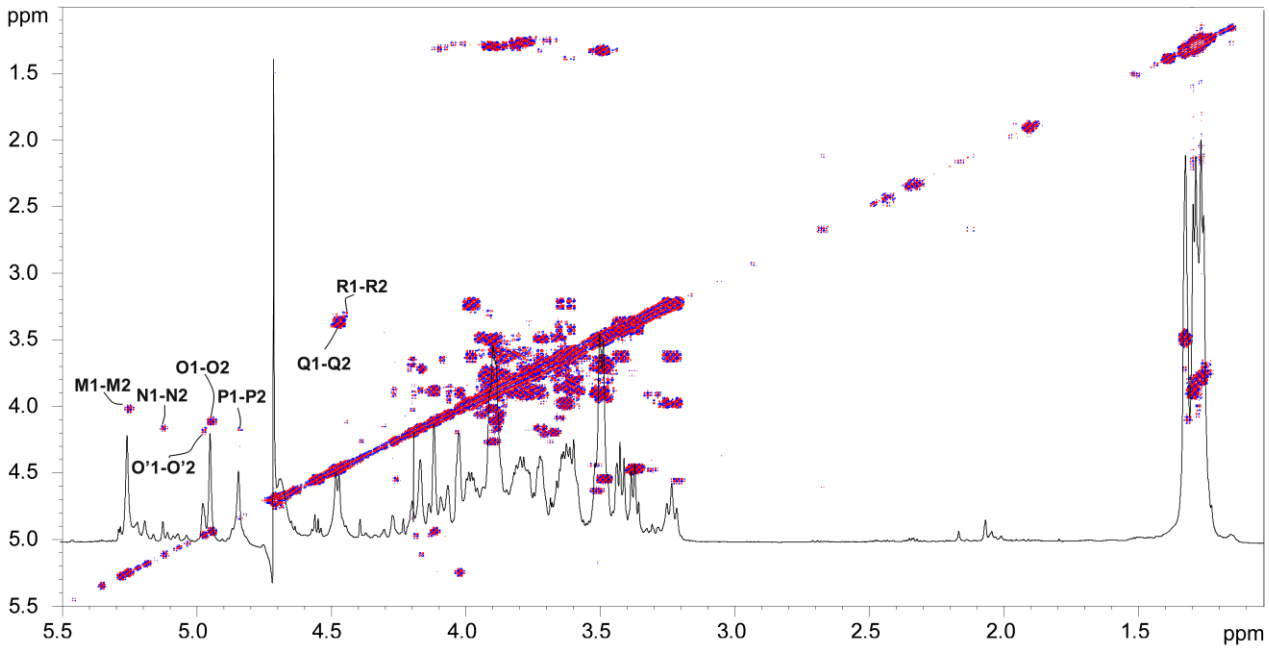


Figure S8.

(600 MHz, 298 K, D_2O, Table S2). **a)** Zoom of the overlapped ^1H, TOCSY (red) and NOESY (blue) spectra recorded for the O-antigen from *A. faecalis* LPS fraction, the most important *inter*-residue contacts are marked; the related full spectra are reported in **b**.

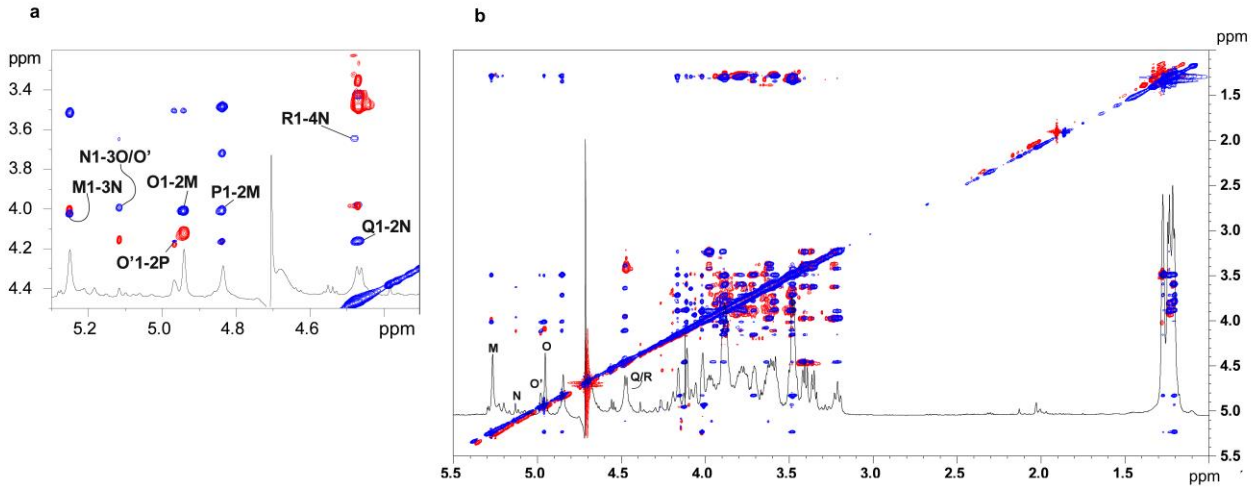


Figure S9.

(600 MHz, 298 K, D_2O, Table S2). **a**) Superimposition of the overlapped ^1H and HMBC full spectra recorded for the O-antigen from *A. faecalis* LPS fraction. **b**) Zoom of the overlapped ^1H, HMBC (blue) and HSQC (red) spectra, the key *inter*-residue long-range correlations are indicated.

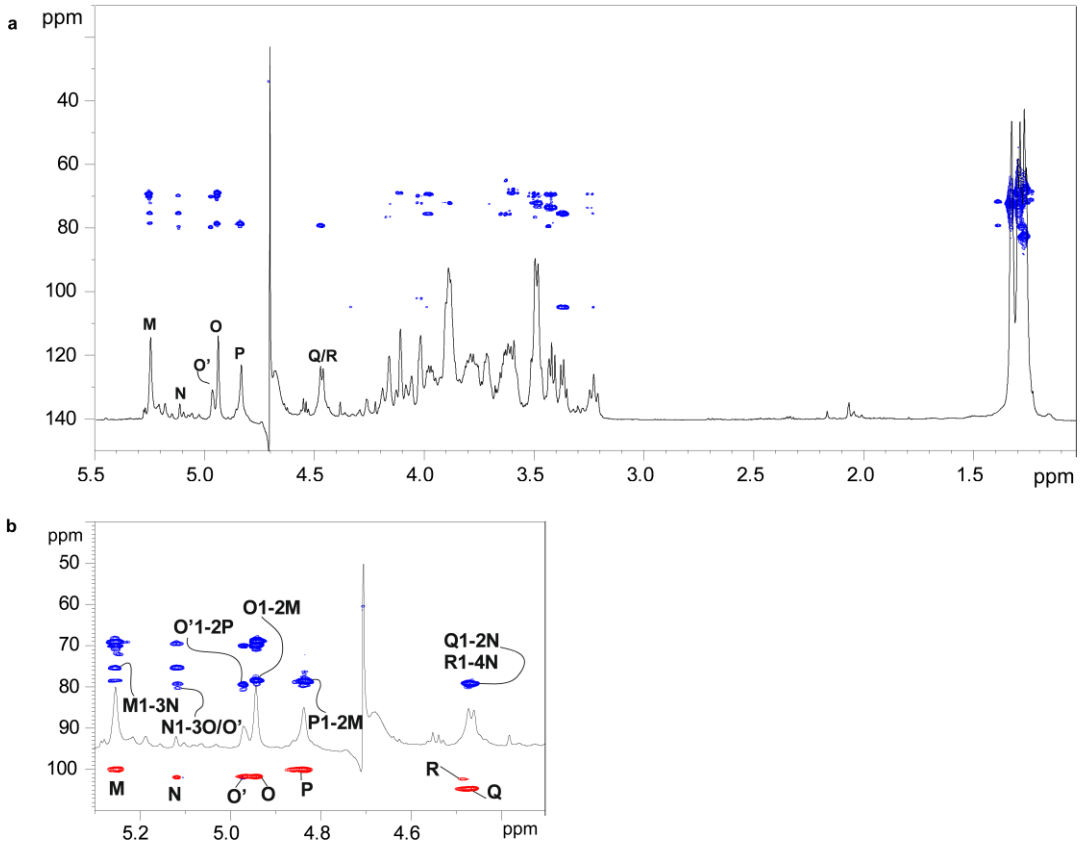


Figure S10.

Reflectron MALDI-TOF mass spectrum, measured in negative polarity, of lipid A from *A. faecalis* LPS/LOS obtained after acetate buffer treatment. Only deprotonated ions $[M-H]^-$ are formed. The lipid A species (i.e. Tetra-, Penta- and Hexa-acylated species) are indicated as Tetra LipA, Penta LipA and Hexa LipA. Differences of 16 and 28 amu are also reported. **P** indicates phosphate group.

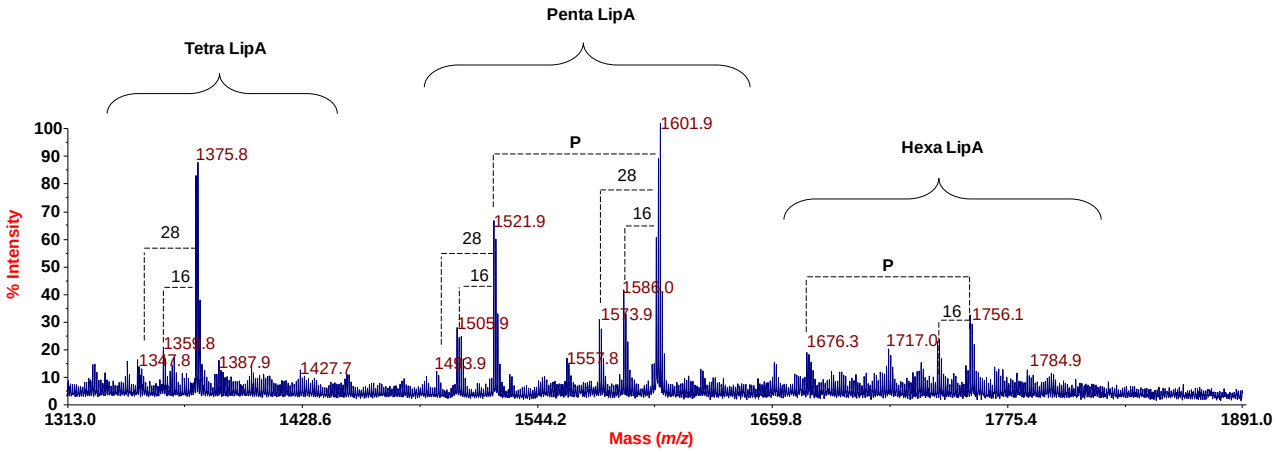


Figure S11.

Negative-ion MS^2 analysis of lipid A from *A. faecalis* LPS/LOS. (**a**) MALDI-TOF MS^2 spectrum of the *bis*-phosphorylated tetra-acylated lipid A species at *m/z* 1375.8. (**b**) MALDI-TOF MS^2 spectrum of the *bis*-phosphorylated penta-acylated lipid A species at *m/z* 1601.9. (**c**) MALDI-TOF MS^2 spectrum of the *mono*-phosphorylated hexa-acylated lipid A species at *m/z* 1676.3. The fragment assignments are indicated. The proposed structures for the lipid A species are sketched in each inset.

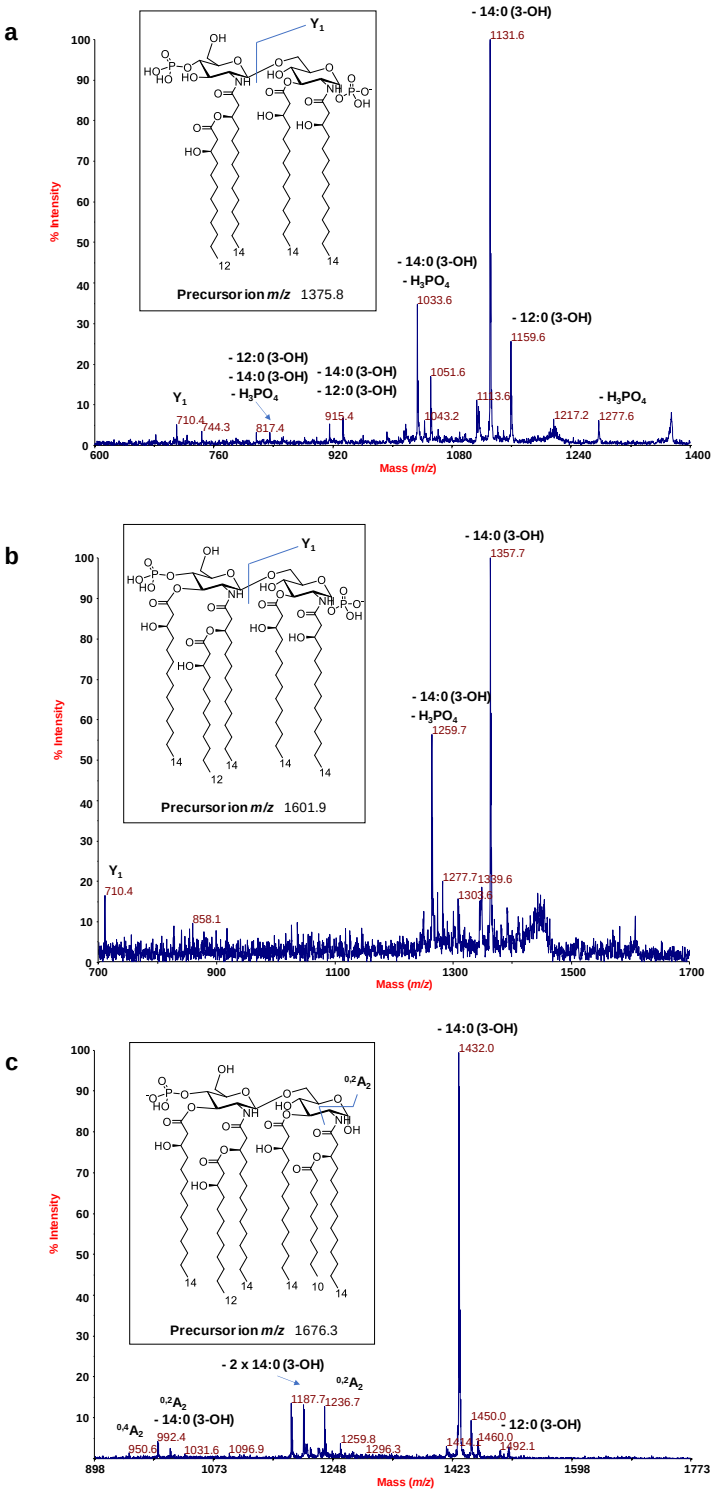


Figure S12.

Negative-ion MS^2 analysis of lipid A from *A. faecalis* LPS/LOS. (**a**) MALDI-TOF MS^2 spectrum of the *mono*-phosphorylated tri-acylated lipid A species at *m/z* 1097.8. (**b**) MALDI-TOF MS^2 spectrum of the *mono*-phosphorylated penta-acylated lipid A species at *m/z* 1521.9. (**c**) MALDI-TOF MS^2 spectrum of the *bis*-phosphorylated hexa-acylated lipid A species at *m/z* 1756.1. The fragment assignments are indicated. The proposed structures for the lipid A species are sketched in each inset.

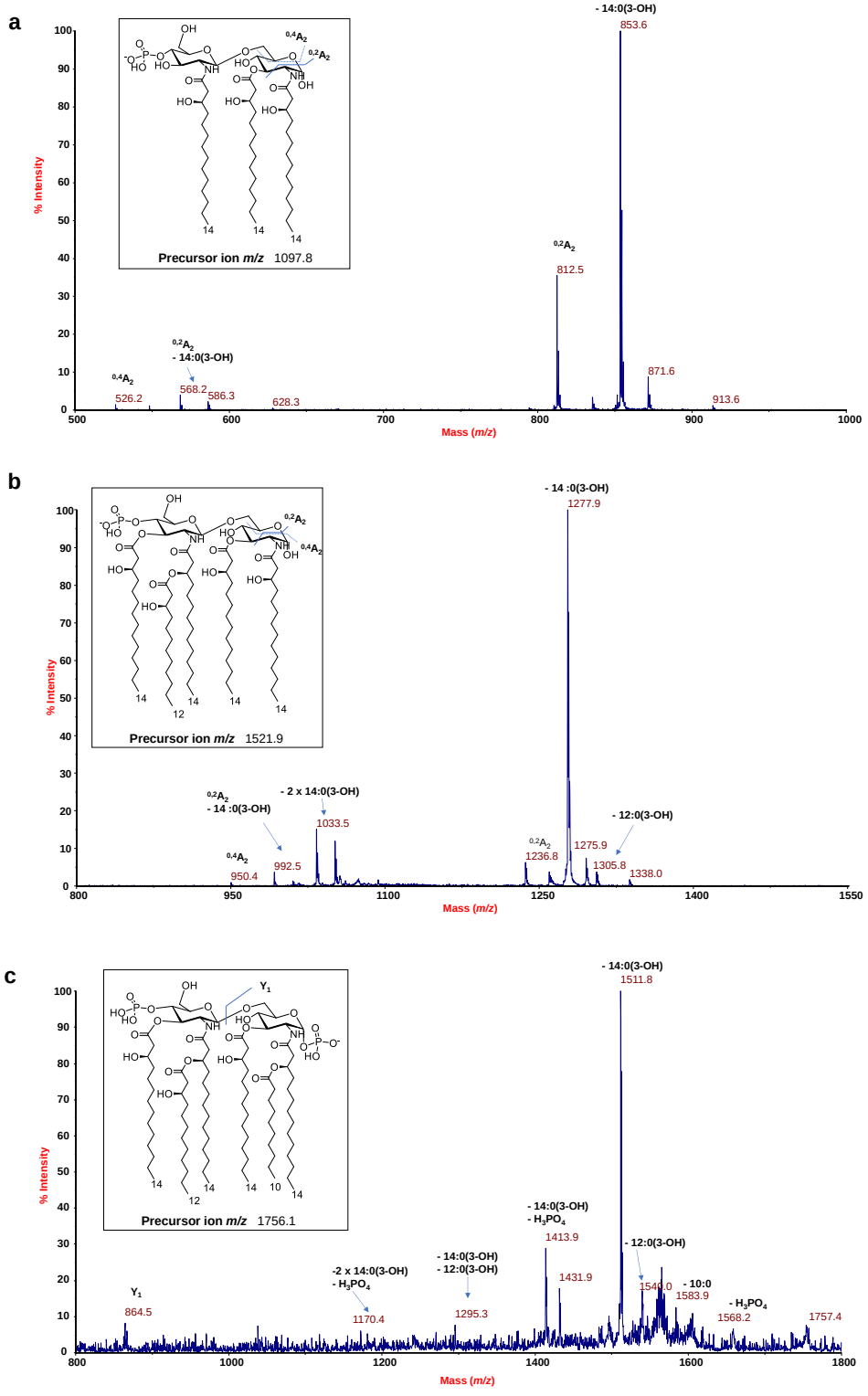


Figure S13.

Positive-ion MALDI-TOF MS^2 analysis of precursor ion at *m/z* 1320.2 relative to a *mono*-phosphorylated lipid A species from *A. faecalis* LOS/LPS. The proposed structure for the tetra-acylated lipid A species is sketched in the inset. The structure for the oxonium ion identified at *m/z* 688.4 is also reported.

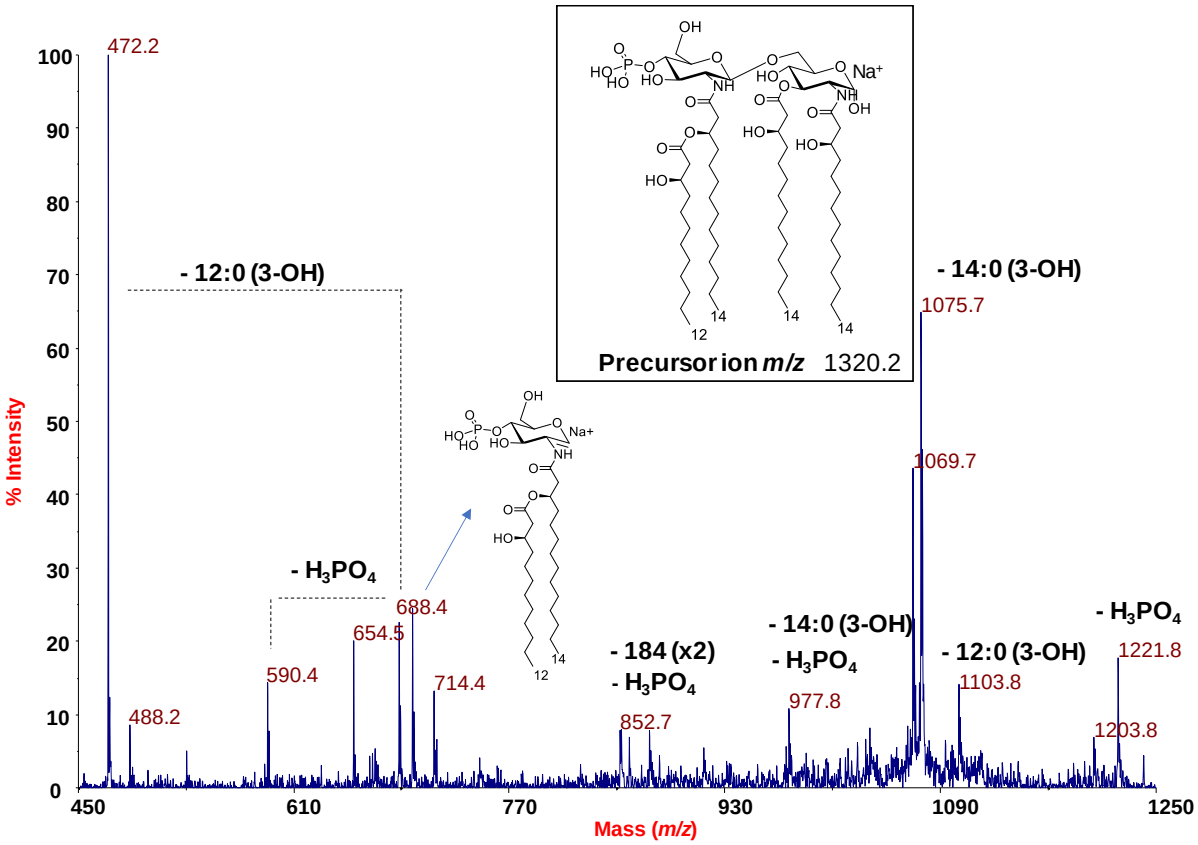
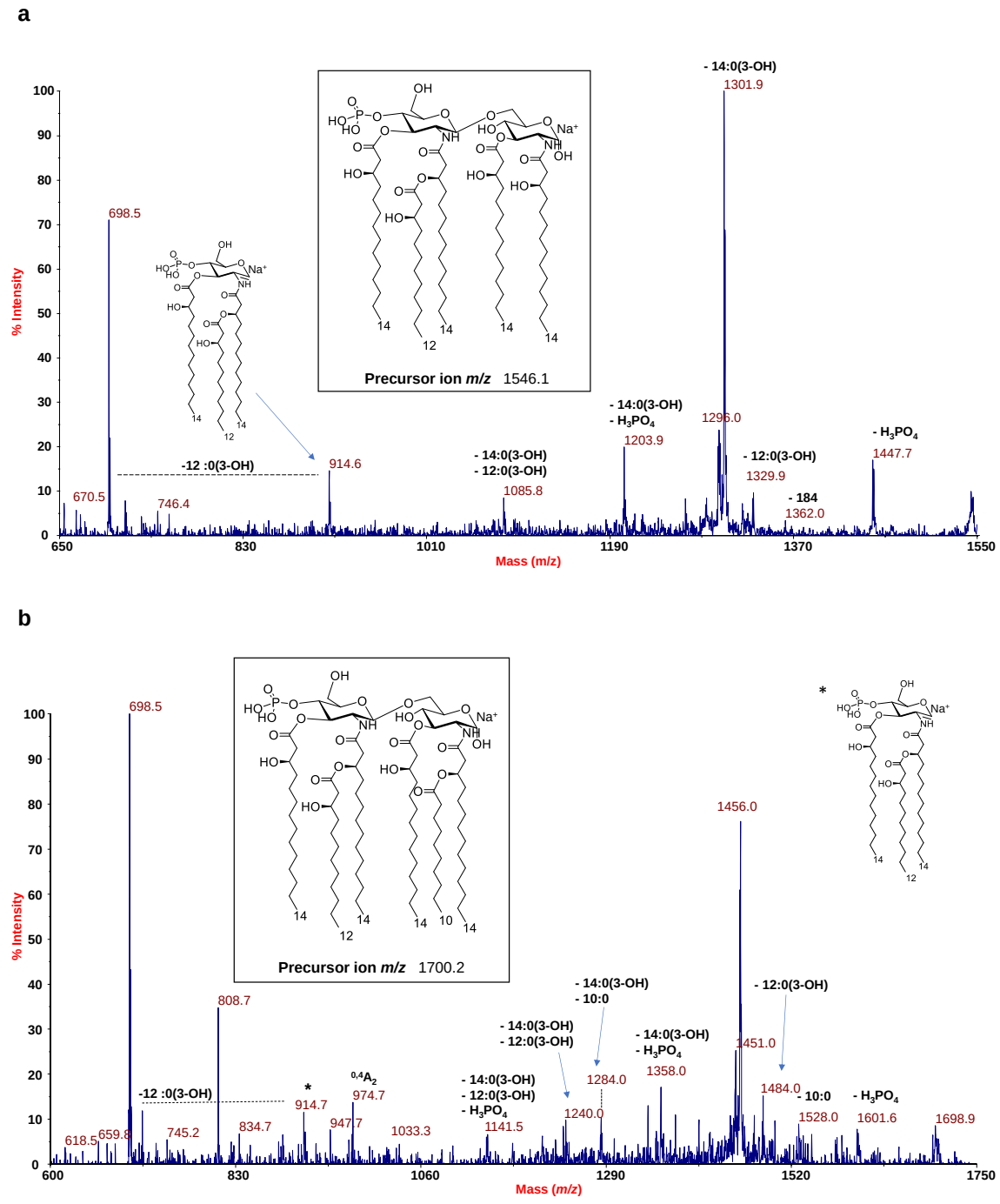


Figure S14.

a) Positive-ion MALDI-TOF MS^2 analysis of precursor ion at *m/z* 1546.1 relative to a *mono*-phosphorylated penta-acylated lipid A species from *A. faecalis* LOS/LPS. The proposed structure is sketched in the inset. The structure for the oxonium ion identified at *m/z* 914.6 is also reported. **b**) Positive-ion MALDI-TOF MS^2 analysis of precursor ion at *m/z* 1700.2 relative to a *mono*-phosphorylated hexa-acylated lipid A species from *A. faecalis* LOS/LPS. The proposed structure is reported in the inset. *indicates the oxonium ion, whose structure is also sketched in the spectrum.



Chemical Synthesis of AfLA

General procedure

^{1}H spectra were measured at 30 °C with a JEOL ECA500 or AVANCE700 NMR spectrometers. Chemical shifts were represented as δ-values relative to the internal standard TMS. Mass spectra were obtained on Thermo LTQ-Orbitrap XL^{TM} (ESI-Orbitrap). Analytical thin layer chromatography (TLC) was performed on Kieselgel 60F_{254} Plates (Merck, 0.25 mm thickness) and compound visualized by UV (254 nm). Silica-gel column chromatography was performed using Kieselgel 60 (Merck, 0.040-0.063 mm) or Silica Gel 60 N (Kanto Chemical co., spherical, natural, 0.040-0.050 mm) at medium pressure (0.2-0.4 MPa) using the indicated solvent system. Unless otherwise noted, non-aqueous reactions were carried out under Ar. Anhydrous dichloromethane, tetrahydrofuran (THF) and dimethylformamide (DMF) were purchased from Kanto Chemical Co. Distilled dichloromethane was distilled from calcium hydride. Optical rotations were measured with a Perkin-Elmer 241Polarimeter.

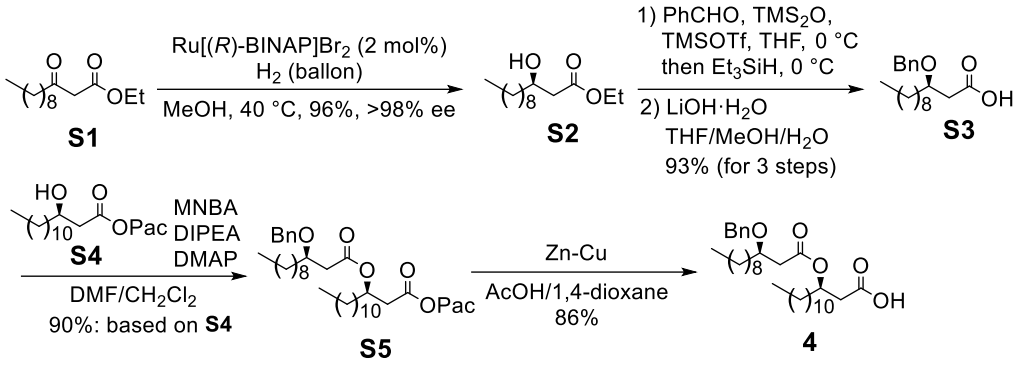
Synthesis procedure and compounds data

Synthesis of fatty acids

The hexa-acylated lipid A species from *A. faecalis* (**hexa-AfLA**) comprises a (*R*)-3-hydroxy fatty acid [i.e. 12:0 (3-OH)] as a secondary fatty acid in an acyloxyacyl amide moiety whose presence has been rarely reported,[15-17] and there is no synthetic report of natural lipid A including this structure. Therefore, this fatty acid was synthesized as shown in Scheme S3. First, enantiomerically enriched β-hydroxy esters **S2** was synthesized via asymmetric hydrogenation[18,19] of β-keto esters **1** (Scheme S3). The enantiomeric excesses of β-hydroxy ester **S2** was determined to be > 98% ee as judged by ^{1}H NMR analysis using a chiral shift reagent, europium tris[3-(heptafluoropropylhydroxymethlene)-(+)-camphorate], Eu(hfc)$_3$.[20,21]

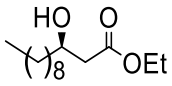
Compound **S2** was converted to 3-*O*-Bn derivative by using reductive benzylation reaction,[22] which was improved from Nishizawa's method.[23] The subsequent hydrolysis of ethyl ester afforded β-hydroxycarboxylic acid **S3** in good yield.

Compound **S3** was conjugated with phenacyl esters **S4** (Scheme S3) in the presence of 2-methyl-6-nitrobenzoic anhydride (MNBA),[24] *N*,*N*-diisopropylethylamine (DIPEA) and 4-dimethyl-aminopyridine (DMAP), to give **S5**. Cleavage of the phenacyl group by Zn-Cu in AcOH and 1,4-dioxane gave **4**.



Scheme S3. The synthesis of the [14:0 (3-O12:0 (3-OH)] acyl moiety

Ethyl (*R*)-3-hydroxy-dodecanoate (S2)



S2

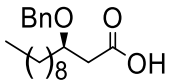
(*R*)-BINAP (502.1 mg, 0.806 mmol) and (cod)Ru(2-methylallyl)$_2$ (213.1 mg, 0.667 mmol) were placed in a 200 mL flask and the vessel was purged with argon. Anhydrous acetone (20 mL) was added. To this suspension 2.64 mL of a methanolic HBr solution (0.5-1.0 M, 1.32-2.64 mmol) were added and the suspension was stirred 30 min at room temperature. A brown solid precipitated. Subsequently, the solvent was thoroughly removed *in vacuo*. To this suspension were added β-keto ester **S1** (8.0 g, 33.0 mmol) and anhydrous methanol (40 mL). The argon atmosphere was replaced with 1 atm of hydrogen and the mixture was stirred at 40 °C for 1.5 h and at 30 °C for 18 h. The precipitate was filtered off and washed by *n*-hexane, and the filtrate was evaporated *in vacuo*. The residue was purified by silica-gel flash column chromatography (Hexane:EtOAc = 19/1→4/1) to give **S2** as a brown oil (7.71 g, 96%, > 99% ee).

$[\alpha]_D^{20}$ -16 (*c* 1.0 g/mL, $CHCl_3$),

^{1}H NMR (500 MHz, $CDCl_3$); δ: 4.17 (q, *J* = 7.1 Hz, 2H, C$\underline{H_2}$ of ethyl group), 4.03-3.98 (m, 1H, β-C$\underline{H}$ of acyl chain), 2.95 (br, 1H, O$\underline{H}$), 2.50 (dd, *J* = 16.3, 3.2 Hz, 1H, α-C$\underline{H_2}$ of acyl chain), 2.40 (dd, *J* = 16.3, 9.0 Hz, 1H, α-C$\underline{H_2}$ of acyl chain), 1.55-1.50 (m, 1H, γ-C$\underline{H_2}$ of acyl chain), 1.47-1.40 (m, 2H, γ-C$\underline{H_2}$ of acyl chain, $\underline{CH_2}$ of acyl chain), 1.37-1.20 (m, 16H, $\underline{CH_2}$ of acyl chain, $\underline{CH_3}$ of ethyl group), 0.87 (t, *J* = 7.0 Hz, 3H, $\underline{CH_3}$ of acyl chain). ^{13}C NMR (125 MHz, $CDCl_3$); δ: 173.1, 68.0, 60.6, 41.3, 36.5, 31.9, 29.53, 29.50, 29.3, 25.4, 22.6, 14.1, 14.0

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{14}H_{29}O_3$ $[M+H]^+$: 245.2111, Found 245.2110.

(*R*)-3-(benzyloxy)dodecanoic acid (S3)



S3

To a solution of **S2** (1.0 g, 4.09 mmol) in anhydrous THF (20 ml) were added hexamethyldisiloxane (5.22 mL, 24.6 mmol), TMSOTf (1.48 mL, 8.18 mmol) and benzaldehyde (1.25 mL, 12.3 mmol) dropwise at 0 °C. After the mixture was stirred for 3 h, triethylsilane (1.96 mL, 12.3 mmol) was added and the stirring was continued for 4 h at 0 °C. The solution was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica-gel flash column chromatography (Toluene/EtOAc = 99/1→4/1). The compound was used for next reaction without further purification.

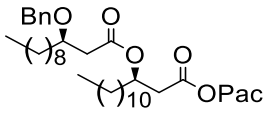
To a solution of the crude in THF/MeOH/H_2O = 3/2/1 (6 mL) was added $LiOH \cdot H_2O$ (723 mg, 17.2 mmol) and stirred for 1 d. The reaction was quenched with 1 N HCl aq. and extracted with EtOAc. The organic layer was washed with 1 N HCl aq. and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (Toluene/EtOAc = 19/1→1/1) to give **S3** as a yellow oil (0.980 g, 93% for 2 steps).

$[\alpha]_D^{20}$ -11 (*c* 0.001 g/mL, $CHCl_3$),

^{1}H NMR (500 MHz, $CDCl_3$); δ: 7.33-7.23 (m, 5H, $C_6\underline{H_5}$), 4.57 (d, *J* = 11.5 Hz, 1H, C_6H_5-$C\underline{H_2}$), 4.54 (d, *J* = 11.5 Hz, 1H, C_6H_5-$C\underline{H_2}$), 3.87 (quin, *J* = 6.1 Hz, 1H, β-C<u>H</u> of acyl chain), 2.63 (dd, 15.3, 7.2 Hz, 1H, α-CH_2 of acyl chain), 2.53 (dd, *J* =15.4, 5.2 Hz, 1H, α-CH_2 of acyl chain) ,1.68-1.60 (m, 1H, γ-CH_2 of acyl chain), 1.60-1.52 (m, 1H, γ-CH_2 of acyl chain), 1.43-1.22 (m, 14H, CH_2 of acyl chain), 0.88 (t, *J* = 6.9 Hz, 3H, CH_3 of acyl chain). ^{13}C NMR (125 MHz, $CDCl_3$); δ: 177.6, 138.3, 128.4, 127.8, 127.7, 75.8, 71.6, 39.7, 34.2, 31.9, 29.60, 29.57, 29.55, 29.3, 25.1, 22.7, 14.1

HRMS (ESI-LIT-orbitrap MS, negative) Calcd. for $C_{19}H_{29}O_3$ $[M-H]^-$: 305.2122, Found 305.2122.

Phenacyl (*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)-tetradecanoate (S5)



S5

To a stirred solution of **S4** (100.3 mg, 277 μmol) and **S3** (142.2 mg, 464 μmol) in CH_2Cl_2 (10 mL) and DMF (2.5 mL) was added DIPEA (72.1 μL, 414 μmol), MNBA (293 mg, 851

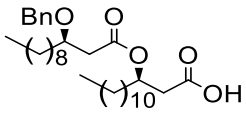
μmol) and DMAP (2.90 mg, 2.37 μmol) and stirred at room temperature for 21 h. The reaction mixture was quenched with 10% aqueous citric acid and extracted with $CHCl_3$. The organic layer was washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) to give **S5** as a white solid (161 mg, 90%).

$[\alpha]_D^{20}$ +0.6 (*c* 0.01 g/mL, $CHCl_3$),

^{1}H NMR (500 MHz, $CDCl_3$); δ: 7.85 (dd, *J* = 8.1, 0.9 Hz, 2H, $C_6\underline{H_5}$-CO), 7.60 (t, *J* = 7.4 Hz, 1H, $C_6\underline{H_5}$-CO), 7.47 (t, *J* = 7.8 Hz, 2H, $C_6\underline{H_5}$-CO), 7.32-7.22 (m, 5H, $C_6\underline{H_5}$-CH_2), 5.33 (quin, *J* = 6.4 Hz, 1H, β-CH of main chain), 5.27 (s, 2H, C_6H_5-CO-$C\underline{H_2}$), 4.58 (d, *J* = 11.4 Hz, 1H, C_6H_5-$C\underline{H_2}$), 4.51 (d, *J* = 11.4 Hz, 1H, C_6H_5-$C\underline{H_2}$), 3.85 (quin, *J* = 6.1 Hz, 1H, β-CH of side chain), 2.78 (dd, *J* = 15.5, 7.5 Hz, 1H, α-CH_2 of mian chain), 2.72 (dd, *J* = 15.5, 7.5 Hz, 1H, α-CH_2 of mian chain), 2.66 (dd, *J* = 15.2, 6.9 Hz, 1H, α-CH_2 of side chain), 2.50 (dd, *J* = 15.2, 5.7 Hz, 1H, α-CH_2 of side chain), 1.69-1.51 (m, 4H, γ-CH_2 of acyl chain), 1.43-1.25 (m, 32H, CH_2 of acyl chain), 0.88 (t, *J* = 6.8 Hz, 6H, CH_3 of acyl chain). ^{13}C NMR (125 MHz, $CDCl_3$); *δ*: 191.7, 171.2, 169.8, 138.7, 134.2, 133.8, 128.8, 128.3, 127.73, 127.66, 127.4, 76.1, 71.4, 70.6, 66.1, 40.0, 38.9, 34.5, 34.0, 31.90, 31.89, 29.7, 29.64, 29.62, 29.60, 29.56, 29.5, 29.4, 29.34, 29.31, 25.2, 25.1, 22.7, 14.1

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{41}H_{62}O_6$ $[M+Na]^+$: 673.4439, Found 673.4435.

(*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)-tetradecanoic acid (4)



4

A suspension of zinc powder (2 g) in ion-exchanged water was sonicated for 1 h. To the suspension of activated zinc was added aqueous copper sulfate (II) and formed zinc-copper couple. After washing with water, zinc-copper couple was filtered as a black solid. To a solution of compound **S5** (944 mg, 1.45 mmol) in acetic acid (10 mL) and 1,4-dioxane (10 mL) was added zinc-copper couple at room temperature. After stirred vigorously for 14 h, the precipitate was filtered off and the filtrate was co-evaporated with toluene. The residue was purified by silica-gel column chromatography (Toluene/EtOAc = 19/1→9/1) to give **4** as colorless oil (662 mg, 86%).

$[\alpha]_D^{20}$ +0.7 (*c* 0.01 g/mL, $CHCl_3$),

^{1}H NMR (500 MHz, $CDCl_3$); δ: 7.32 (d, J = 4.4 Hz, 4H, $C_6\underline{H_5}$), 7.28-7.23 (m, 1H, $C_6\underline{H_5}$) 5.20 (quin, *J* = 6.2 Hz, 1H, β-CH of main chain), 4.53 (d, *J* = 11.4 Hz, 1H, C_6H_5-$C\underline{H_2}$), 4.49 (d, *J* = 11.4 Hz, 1H, C_6H_5-$C\underline{H_2}$), 3.85 (quin, *J* =6.1 Hz, 1H, β-CH of side chain), 2.64-2.50 (m, 3H, α-CH_2 of side chain and mian chain), 2.47 (dd, *J* = 15.0, 5.6 Hz, 1H, α-CH_2 of side chain),

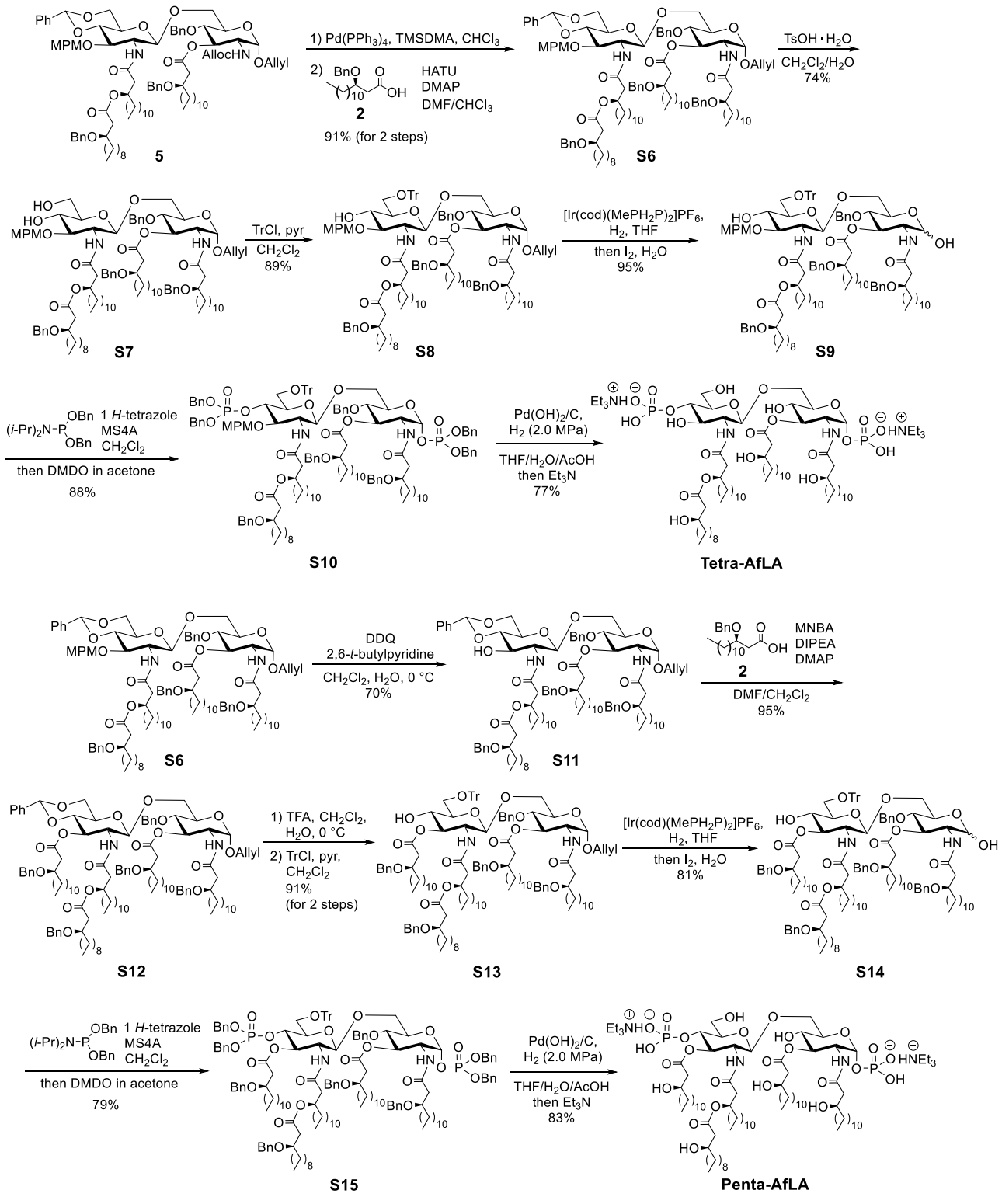
1.63-1.50 (m, 4H, γ-CH_2 of acyl chain), 1.47-1.40 (m, 32H, CH_2 of acyl chain), 0.88 (t, *J* = 6.9 Hz, 6H, CH_3 of acyl chain). ^{13}C NMR (125 MHz, $CDCl_3$); *δ*: 171.2, 138.5, 128.3, 127.7, 127.5, 76.1, 71.4, 70.4, 39.9, 34.4, 33.9, 31.89, 31.88, 29.62, 29.60, 29.59, 29.57, 29.54, 29.46, 29.33, 29.31, 29.29, 25.1, 22.7, 14.1

HRMS (ESI-LIT-orbitrap MS, negative) Calcd. for $C_{33}H_{55}O_5$ $[M-H]^-$: 531.4055, Found 531.4058.

Synthesis of Tetra-AfLA and Penta-AfLA

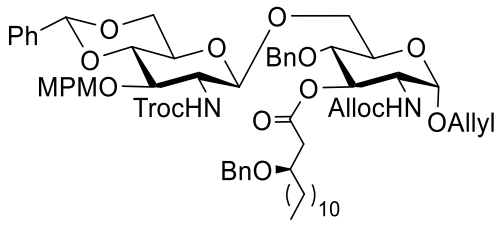
Chemical synthesis of **Tetra-AfLA** and **Penta-AfLA** were accomplished as illustrated in Scheme S4. The 2-*N*-Alloc group of **5** was removed by $Pd(PPh_3)_4$ and TMSDMA, and then the introduction of benzyl-protected β-hydroxy fatty acid **2** to the free 2-amino group using HATU and DMAP gave **S6** with 91% yield. Next, the cleavage of the 4',6'-*O*-benzylidene group by *p*-toluenesulfonic acid monohydrate ($TsOH\cdot H_2O$) gave **S7** with 74% yield, and subsequently the Tr group was selectively introduced to the primary hydroxyl group with 89% yield. The anomeric allyl group was then isomerized to the 1-propenyl group with the Ir complex, and the resulting 1-propenyl group was then removed by iodine and water to yield the 1,4'-dihydroxyl disaccharide **S9** with 95% yield. The simultaneous phosphitylation of 1 and 4'-position using phosphoramidite in the presence of 1*H*-tetrazole and MS4A gave the desired 1, 4'-*O*-diphosphite, which was then oxidized to 1,4'-*O*-diphosphorylated **S10** using DMDO with 88% yield. After hydrogenolysis of **S10** with $Pd(OH)_2$/C under H_2 (2.0 MPa) in THF/H_2O/AcOH, the first chemical synthesis of **Tetra-AfLA** was accomplished with 77% yield.

After the cleavage of the MPM group at 3-position of **S6** by DDQ oxidation, β-hydroxy fatty acid **2** was introduced using MNBA to obtain **S12** with 95% yield. Next, the 4',6'-*O*-benzylidene group was cleaved by TFA, and then the trityl group was selectively introduced to the primary hydroxyl group. The anomeric allyl group was then isomerized to the 1-propenyl group with the Ir complex, and the resulting 1-propenyl group was then removed by iodine and water to yield the 1,4'-dihydroxyl disaccharide **S14** with 81%. The simultaneous phosphitylation of 1 and 4'-position using phosphoramidite in the presence of 1*H*-tetrazole and MS4A gave the desired 1, 4'-*O*-diphosphite, which was then oxidized to 1,4'-*O*-diphosphorylated **S15** using DMDO with 79% yield. After hydrogenolysis of **S15** with $Pd(OH)_2$/C under H_2 (2.0 MPa) in THF/H_2O/AcOH, the first chemical synthesis of **Penta-AfLA** was accomplished with 83% yield.



Scheme S4. Synthesis of Tetra-AfLA and Penta-AfLA

Allyl 2-allyloxycarbonylamino-4-*O*-benzyl-6-*O*-[4,6-*O*-benzylidene-2-deoxy-3-*O*-(4-methoxyphenylmethyl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-α-D-glucopyranoside (3)



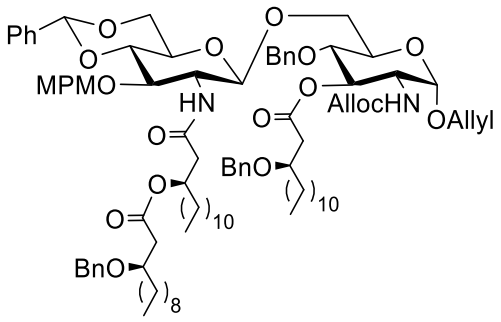
3

To a stirred solution of **1** (500 mg, 532 μmol) and **2** (267 mg, 799 μmol) in CH_2Cl_2 (10 mL) and DMF (10 mL) was added DIPEA (139 μL, 799 μmol), MNBA (367 mg, 1.07 mmol) and DMAP (6.59 mg, 53.9 μmol) and stirred at room temperature for 15 h. The reaction mixture was quenched with 10% aqueous citric acid and extracted with $CHCl_3$. The organic layer was washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (Toluene/EtOAc = 97/3) to give **3** as a white solid (662 mg, 99%).

^{1}H NMR (500 MHz, $CDCl_3$); *δ*: 7.50 (dd, *J* = 7.7, 1.7 Hz, 2H, $C_6\underline{H_5}$-CH), 7.42-7.19 (m, 15H, $C_6\underline{H_5}$-CH, $C_6\underline{H_5}$-CH_2, CH_3O-$C_6\underline{H_4}$), 6.86 (d, *J* = 8.4 Hz, 2H, CH_3O-$C_6\underline{H_4}$), 5.89-5.79 (m, 2H, OCH_2-C<u>H</u>=CH_2 of Alloc and Allyl group), 5.57 (s, 1H, C_6H_5-C<u>H</u>), 5.37 (dd, *J* = 10.7, 9.3 Hz, 1H, H-3), 5.29-5.24 (m, 2H, OCH_2-CH=C$\underline{H_2}$ of Alloc and Allyl group), 5.20-5.16 (m, 2H, OCH_2-CH=C$\underline{H_2}$ of Alloc and Allyl group), 5.01 (d, *J* = 9.9 Hz, 1H, 2-NH), 4.94 (br s, 1H, 2-NH'), 4.87 (d, *J* = 3.6 Hz, 1H, H-1), 4.81 (d, *J* = 11.5 Hz, 1H, CH_3O-C_6H_4-C$\underline{H_2}$), 4.64-4.43 (m, 10H, CH_3O-C_6H_4-C$\underline{H_2}$-, OC$\underline{H_2}$-CH=CH_2 of Alloc group, -COO-C$\underline{H_2}$-CCl_3, C_6H_5-C$\underline{H_2}$, H-1'), 4.33 (dd, *J* = 11.5, 4.9 Hz, 1H, H-6a'), 4.15 (dd, *J* = 12.9, 5.3 Hz, 1H, OC$\underline{H_2}$-CH=CH_2 of allyl group), 4.05 (d, *J* = 9.3 Hz, 1H, H-6a), 3.96-3.90 (m, 3H, OC$\underline{H_2}$-CH=CH_2 of allyl group, H-3', H-2), 3.86-3.77 (m, 3H, H-5, H-2, β-CH of acyl chain), 3.79 (s, 3H, C$\underline{H_3}$O-C_6H_4-CH_2) 3.73-3.65 (m, 3H, H-6b, H-4, H-4'), 3.42-3.33 (m, 2H, H-5', H-2'), 2.58 (dd, *J* = 15.9, 7.0 Hz, 1H, α-CH_2 of acyl chain), 2.42 (dd, *J* = 16.0, 5.4 Hz, 1H, α-CH_2 of acyl chain) 1.57-1.45 (m, 2H, γ-CH_2 of acyl chain), 1.40-1.20 (m, 18H, CH_2 of acyl chain), 0.88 (t, *J* = 6.9 Hz, 3H, CH_3 of acyl chain). ^{13}C NMR (125 MHz, $CDCl_3$); *δ*: 171.9, 159.4, 159.4, 155.7, 153.8, 138.6, 137.9, 137.3, 133.3, 132.6, 130.0, 129.0, 128.4, 128.3, 128.2, 127.83, 127.71, 127.4, 126.0, 117.9, 117.6, 113.8, 101.2, 96.7, 82.4, 75.7, 75.5, 74.5, 74.4, 74.0, 73.8, 71.5, 69.9, 68.6, 68.4, 66.2, 65.6, 55.2, 54.1, 39.7, 34.3, 31.9, 29.64, 29.61, 29.58, 29.3, 25.1, 22.7, 14.1.

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{65}H_{83}Cl_3N_2O_{16}$ $[M+Na]^+$: 1275.4700, Found 1275.4695.

Allyl 2-allyloxycarbonylamino-4-*O*-benzyl-6-*O*-[4,6-*O*-benzylidene-2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-2-deoxy-3-*O*-(4-methoxyphenylmethyl)-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-α-D-glucopyranoside (5)



5

A suspension of zinc powder (1.5 g) in water was sonicated for 1 h. To the suspension of activated zinc was added aqueous copper sulfate (II) and formed zinc-copper couple. After washing with water, zinc-copper couple was filtered as a black solid. To a solution of **3** (200 mg, 159 μmol) in acetic acid (10 mL) and 1,4-dioxane (10 mL) was added zinc-copper couple at room temperature. After stirred vigorously for 7 h, the precipitate was filtered off and the filtrate was co-evaporated with toluene. The residue was extracted with EtOAc and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give crude as a white solid.

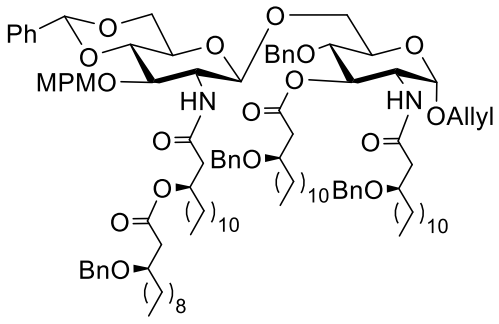
To a stirred solution of the crude and **4** (93.3 mg, 175 μmol) in $CHCl_3$ (10 mL) and DMF (10 mL) was added HATU (90.9 mg, 239 μmol) and DMAP (1.95 mg, 15.9 μmol) and stirred at room temperature for 19 h. The reaction mixture was quenched with 10% aqueous citric acid and extracted with $CHCl_3$. The organic layer was washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) to give **5** as a white solid (223 mg, 88% for 2 steps).

^{1}H NMR (500 MHz, $CDCl_3$); *δ*: 7.49-7.45 (m, 2H, $C_6\underline{H_5}$-CH), 7.40-7.19 (m, 20H, $C_6\underline{H_5}$-CH, $C_6\underline{H_5}$-CH_2, CH_3O-$C_6\underline{H_4}$-), 6.79 (d, *J* = 8.6 Hz, 2H, CH_3O-$C_6\underline{H_4}$-), 5.90-5.80 (m, 3H, OCH_2-$C\underline{H}$=CH_2 of allyl and Alloc groups, 2-NH'), 5.52 (s, 1H, C_6H_5-$C\underline{H}$), 5.35 (dd, *J* = 10.5, 9.5 Hz, 1H, H-3), 5.28 (dd, *J* = 17.2, 1.4 Hz, 1H, OCH_2-CH=$C\underline{H_2}$ of allyl group), 5.25 (d, *J* = 16.5 Hz, 1H, OCH_2-CH=$C\underline{H_2}$ of Alloc group) 5.20-5.15 (m, 2H, OCH_2-CH=$C\underline{H_2}$ of allyl and Alloc groups) 5.10-5.06 (m, 1H, β-CH of acyl chain) 5.02 (d, *J* = 9.9 Hz, 1H, NH-2), 4.97 (d, *J* = 8.0, 1H, H-1'), 4.85 (d, *J* = 3.6 Hz, 1H, H-1), 4.76 (d, *J* = 11.0 Hz, CH_3O-C_6H_4-$C\underline{H_2}$), 4.61-4.42 (m, 9H, C_6H_5-$C\underline{H_2}$, CH_3O-C_6H_4-$C\underline{H_2}$, $OC\underline{H_2}$-CH=CH_2 of Alloc group), 4.31 (dd, J = 10.5, 4.9 Hz, 1H, H-6a'), 4.22 (t, *J* = 9.4 Hz, 1H, H-3'), 4.16 (dd, *J* = 12.8, 5.2 Hz, 1H, OCH_2-CH=$C\underline{H_2}$ of allyl group), 4.01-3.88 (m, 3H, H-6a, OCH_2-CH=$C\underline{H_2}$ of allyl group, H-2), 3.86-3.77 (m, 3H, H-5, β-CH of acyl chain), 3.77-3.65 (m, 2H, H-6b, H-6b'), 3.74 (s, 3H, $C\underline{H_3}O$-C_6H_4-CH_2), 3.66 (t, *J* = 9.1 Hz, 1H, H-4'), 3.63 (t, *J* = 9.5 Hz, 1H, H-4), 3.47 (td, *J* = 9.7, 5.0 Hz, 1H, H-5'), 3.38 (q, *J* = 8.4 Hz, 1H, H-2'), 2.57 (dd, *J* = 16.0, 6.8 Hz, 1H, α-CH_2 of acyl

chain), 2.50 (dd, *J* = 14.9, 7.4 Hz, 1H, α-CH_2 of acyl chain), 2.43-2.31 (m, 3H, α-CH_2 of acyl chain), 2.23 (dd, *J* = 15.0, 5.7 Hz, 1H, α-CH_2 of acyl chain), 1.64-1.49 (m, 6H, γ-CH_2 of acyl chain), 1.40-1.20 (m, 50H, CH_2 of acyl chain), 0.90-0.85 (m, 9H, CH_3 of acyl chain). ^{13}C NMR (175 MHz, $CDCl_3$); *δ*: 171.9, 171.4, 169.8 (2×CO, 1×CONH of acyl chain), 159.2 (CH_3O-$\underline{C}_6H_4$-), 155.7 (CO of Alloc group), 138.6, 138.5, 137.7, 137.4 ($\underline{C}_6H_5$- or CH_3O-$\underline{C}_6H_4$-), 133.4 (OCH_2-$\underline{C}H$=CH_2 of allyl group), 132.6 (OCH_2-$\underline{C}H$=CH_2 of Alloc group), 132.2, 131.0, 130.5, 130.1, 129.7, 129.5, 129.0, 128.5, 128.4, 128.3, 128.2, 127.9, 127.75, 127.72, 127.6, 127.5, 126.1 ($\underline{C}_6H_5$- or CH_3O-$\underline{C}_6H_4$-), 117.9 (OCH_2-CH=$\underline{C}H_2$ of allyl group), 117.6 (OCH_2-CH=$\underline{C}H_2$ of Alloc group), 113.7 (CH_3O-$\underline{C}_6H_4$-), 100.2 (C_6H_5-$\underline{C}H$), 100.4 (C-1'), 96.2 (C-1), 76.6 (C-3'), 76.3 (β-CH of acyl chain), 76.0 (C-4), 75.4 (β-CH of acyl chain), 74.5 (C_6H_5-$\underline{C}H_2$), 73.9 (CH_3O-C_6H_4-$\underline{C}H_2$) 73.6 (C-3), 71.5 (C_6H_5-$\underline{C}H_2$), 71.3 (C_6H_5-$\underline{C}H_2$ and β-CH of acyl chain), 70.1 (C-5), 68.8 (C-6'), 68.3 ($O\underline{C}H_2$-CH=CH_2 of allyl group), 68.1 (C-6), 66.1 (C-5'), 65.7 ($O\underline{C}H_2$-CH=CH_2 of Alloc group), 57.6 (C-2'), 55.2 ($\underline{C}H_3O$-C_6H_4-CH_2), 54.2 (C-2), 41.4, 39.9, 39.7 (3×α-CH_2 of acyl chain), 34.4, 34.1, 33.8 (3×γ-CH_2 of acyl chain), 31.9, 29.7, 29.6, 29.4, 29.3, 28.6, 25.4, 25.2, 25.1, 22.7 (CH_2 of acyl chain), 14.1 (CH_3 of acyl chain).

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{95}H_{136}N_2O_{18}$ $[M+Na]^+$: 1615.9680, Found 1615.9675.

Allyl 4-*O*-benzyl-6-*O*-[4,6-*O*-benzylidene-2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-2-deoxy-3-*O*-(4-methoxyphenylmethyl)-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (S6)



S6

To a solution of **5** (189 mg, 118 umol) in $CHCl_3$ (12 mL) was added TMSDMA (186 uL. 1.18 mmol) and tetrakis(triphenylphosphine)palladium(0) (13.6 mg, 11.8 umol). After stirred for 3 h, the reaction was quenched by addition of water and the mixture was extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$, and dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give deprotected product as a yellow solid.

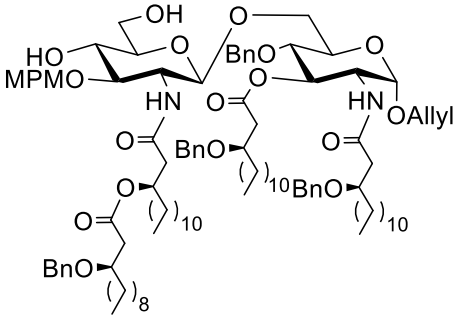
To a stirred solution of the deprotected product (118 umol) and **2** (59.3 mg, 177 umol) in $CHCl_3$ (6.0 mL) and DMF (6.0 mL) was added HATU (67.6 mg, 178 umol), DIPEA (62 uL,

360 umol), and DMAP (2.7 mg, 22 umol) and stirred at room temperature for 12 h. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous NH_4Cl, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) and gel permeation chromatography to give **S6** as a white solid (195.6 mg, 91% for 2 steps).

^{1}H-NMR (500MHz, $CDCl_3$) δ: 7.46 (dd, *J* = 7.5, 2.1 Hz, 2H, $C_6\underline{H_5}$-CH), 7.40-7.18 (m, 25H, $C_6\underline{H_5}$-CH, $C_6\underline{H_5}$-CH_2, CH_3O-$C_6\underline{H_4}$-), 6.80 (d, *J* = 8.7 Hz, 2H, CH_3O-$C_6\underline{H_4}$-), 6.19 (d, *J* = 9.3 Hz, 1H, 2-NH), 5.86 (d, 1H, *J* = 7.6 Hz, 2-NH'), 5.75-5.66 (m, 1H, OCH_2-C$\underline{H}$=CH_2 of allyl group), 5.52 (s, 1H, C_6H_5-C$\underline{H}$), 5.36 (dd, *J* = 10.7, 9.2 Hz, 1H, H-3), 5.18 (dd, *J* = 17.2, 1.6 Hz, 1H, OCH_2-CH=C$\underline{H_2}$ of allyl group), 5.10-5.05 (m, 2H, OCH_2-CH=C$\underline{H_2}$ of allyl group, β-CH of acyl chain), 4.97 (d, *J* = 8.0 Hz, 1H, H-1'), 4.83-4.78 (m, 2H, H-1, C_6H_5-C$\underline{H_2}$), 4.61-4.42 (m, 9H, C_6H_5-C$\underline{H_2}$, CH_3O-C_6H_4-C$\underline{H_2}$), 4.32-4.20 (m, 3H, H-6'a, H-2, H-3'), 4.02 (dd, *J* = 10.7, 5.4 Hz, 1H, OC$\underline{H_2}$-CH=CH_2 of allyl group), 3.98 (dd, *J* = 10.9, 1.6 Hz, 1H, H-6a), 3.85-3.80 (m, 4H, β-CH of acyl chain, H-5), 3.78-3.70 (m, 3H, H-6b, H-6b', OC$\underline{H_2}$-CH=CH_2 of allyl group), 3.74 (s, 3H, C$\underline{H_3}$O-C_6H_4-), 3.66 (t, *J* = 8.9 Hz, 1H, H-4'), 3.64 (t, *J* = 9.1 Hz, 1H, H-4), 3.47 (td, *J* = 9.7, 5.0 Hz, 1H, H-5'), 3.39-3.30 (m, 1H, H-2'), 2.56 (dd, *J* = 16.0, 7.2 Hz, 1H, α-CH_2 of acyl chain), 2.50 (dd, *J* = 14.9, 7.4 Hz, 1H, α-CH_2 of acyl chain), 2.42-2.33 (m, 3H, α-CH_2 of acyl chain), 2.28 (d, *J* = 5.9 Hz, 2H, α-CH_2 of acyl chain), 2.23 (dd, *J* = 15.0, 5.7 Hz, 1H, α-CH_2 of acyl chain), 1.56-1.37 (m, 8H, γ-CH_2 of acyl chain), 1.37-1.17 (m, 68H, CH_2 of acyl chain), 0.90-0.85 (m, 12H, CH_3 of acyl chain). ^{13}C NMR (500MHz, $CDCl_3$); δ: 171.9, 171.4, 171.1, 169.8, 159.2, 138.6, 138.5, 137.7, 137.4, 133.4, 130.6, 129.5, 128.9, 128.5, 128.3, 128.23, 128.19, 127.9, 127.73, 127.69, 127.59, 127.56, 127.5, 127.4, 126.1, 117.7, 113.7, 101.2, 100.4, 96.5, 82.2, 76.6, 76.3, 76.0, 75.4, 74.5, 73.8, 73.6, 71.4, 71.3, 71.2, 70.1, 68.8, 68.3, 68.1, 66.0, 57.7, 55.1, 52.1, 41.9, 41.3, 39.9, 39.7, 34.3, 34.15, 34.11, 33.8, 31.91, 31.88, 29.72, 29.67, 29.65, 29.64, 29.62, 29.60, 29.55, 29.38, 29.35, 29.29, 25.3, 25.24, 25.19, 25.1, 22.7, 14.1

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{112}H_{164}N_2O_{18}$ $[M+Na]^+$: 1848.1871, found 1848.1848

Allyl 4-*O*-benzyl-6-*O*-[2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-2-deoxy-3-*O*-(4-methoxyphenylmethyl)-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (S7)



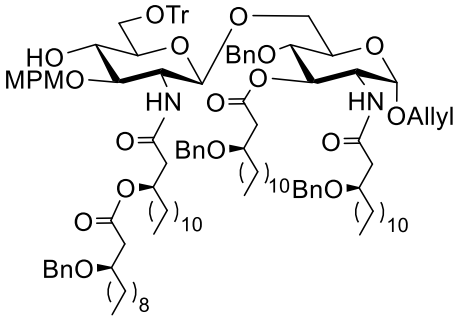
S7

To a solution of **S6** (40.0 mg, 21.9 umol) in $CHCl_3$ (2.2 mL) and MeOH (1.1 mL) was added $TsOH \cdot H_2O$ (12.6 mg, 66.2 umol) and stirred at room temperature for 6 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$/Acetone = 19/1→4/1) to give **S7** as a white solid (28.1 mg, 74%).

^{1}H-NMR (500MHz, $CDCl_3$) δ: 7.40-7.18 (m, 22H, $C_6\underline{H_5}$-CH_2, CH_3O-$C_6\underline{H_4}$-), 6.86 (d, *J* = 8.6 Hz, 2H, CH_3O-$C_6\underline{H_4}$-), 6.20 (d, *J* = 9.5 Hz, 1H, 2-NH), 5.91 (d, 1H, *J* = 7.7 Hz, 2-NH'), 5.75-5.66 (m, 1H, OCH_2-$C\underline{H}$=CH_2 of allyl group), 5.35 (dd, *J* = 10.7, 9.3 Hz, 1H, H-3), 5.17 (dd, *J* = 17.2, 1.4 Hz, 1H, OCH_2-CH=$C\underline{H_2}$ of allyl group), 5.15-5.10 (m, 1H, β-CH of acyl chain), 5.08 (dd, *J* = 10.5, 1.0 Hz, 1H, OCH_2-CH=$C\underline{H_2}$ of allyl group), 4.76 (d, *J* = 8.0 Hz, 1H, H-1'), 4.75 (d, *J* = 3.6 Hz, 1H, H-1), 4.66 (d, *J* = 11.3 Hz, 1H, C_6H_5-$C\underline{H_2}$), 4.60 (d, *J* = 11.0 Hz, 1H, C_6H_5-$C\underline{H_2}$), 4.53-4.42 (m, 8H, C_6H_5-$C\underline{H_2}$, CH_3O-C_6H_4-$C\underline{H_2}$), 4.29 (td, *J* = 10.1, 3.1 Hz, 1H, H-2), 4.00 (dd, *J* = 12.7, 5.4 Hz, 1H, $OC\underline{H_2}$-CH=CH_2 of allyl group), 3.90 (d, *J* = 10.0 Hz, 1H, H-6a), 3.89-3.72 (m, 10H, $OC\underline{H_2}$-CH=CH_2 of allyl group, β-CH of acyl chain, H-3', H-5, H-6b, H-6), 3.77 (s, 3H, $C\underline{H_3}O$-C_6H_4-), 3.68 (t, *J* = 9.5 Hz, 1H, H-4), 3.54 (t, *J* = 8.9 Hz, 1H, H-4'), 3.43 (q, *J* = 8.4 Hz, 1H, H-2'), 3.34-3.30 (m, 1H, H-5'), 3.39-3.30 (m, 1H, H-2'), 2.56 (dd, *J* = 16.0, 7.0 Hz, 1H, α-CH_2 of acyl chain), 2.42-2.27 (m, 6H, α-CH_2 of acyl chain), 1.58-1.36 (m, 8H, γ-CH_2 of acyl chain), 1.36-1.18 (m, 68H, CH_2 of acyl chain), 0.88 (t, *J* = 6.9 Hz, 12H, CH_3 of acyl chain). ^{13}C NMR (125MHz, $CDCl_3$); δ: 171.9, 171.5, 171,2, 169.9, 159.5, 138.6, 138.53, 138.52, 137.8, 133.4, 130.4, 129.6, 128.5, 128.41, 128.36, 128.3, 127.9, 127.8, 127.73, 127.69, 127.64, 127.56, 127.4, 117.8, 114.1, 100.3, 96.7, 80.2, 76.4, 76.3, 75.9, 75.45, 75.41, 74.6, 73.6, 72.8, 71.43, 71.38, 71.3, 70.6, 70.4, 68.4, 68.1, 62.7, 56.5, 55.2, 52.1, 42.0, 41.4, 39.9, 39.7, 34.4, 34.14, 34.10, 33.8, 31.95, 31.94, 31.9, 29.8, 29.73, 29.72, 29.71, 29.68, 29.67, 29.66, 29.63, 29.61, 29.58, 29.40, 29.38, 29.37, 29.3, 25.4, 25.3, 25.2, 25.1, 22.70, 22.69, 14.1

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{105}H_{160}N_2O_{18}$ $[M+Na]^+$: 1760.1558, found 1760.1538

Allyl 4-*O*-benzyl-6-*O*-[2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-2-deoxy-3-*O*-(4-methoxyphenylmethyl)-6-*O*-trithyl-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (S8)



S8

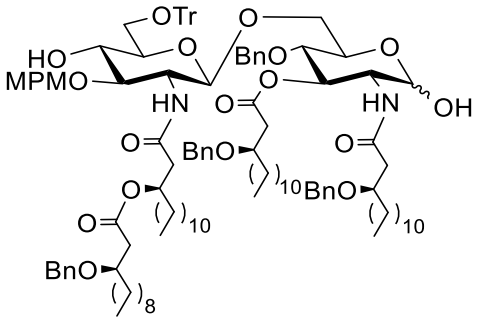
To a solution of **S7** (28.1 mg, 16.3 umol) in CH_2Cl_2 (1.6 mL) was added pyridine (13.1 uL, 162 umol) and TrCl (7.1 mg, 25 umol) and stirrerd at room temperature for 4.5 h. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous NH_4Cl and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) to give **S8** as a white solid (28.5 mg, 89%).

^{1}H-NMR (500MHz, $CDCl_3$) δ: 7.45-7.43 (m, 6H, $C_6\underline{H_5}$-C), 7.31-7.15 (m, 31H, $C_6\underline{H_5}$-CH_2, CH_3O-$C_6\underline{H_4}$-, $C_6\underline{H_5}$-C), 6.83 (d, *J* = 8.7 Hz, 2H, CH_3O-$C_6\underline{H_4}$-), 6.17 (d, *J* = 9.5 Hz, 1H, 2-NH), 5.81 (d, 1H, *J* = 7.7 Hz, 2-NH'), 5.72-5.63 (m, 1H, OCH_2-C$\underline{H}$=CH_2 of allyl group), 5.36 (dd, *J* = 10.7, 9.3 Hz, 1H, H-3), 5.13-5.09 (m, 1H, β-CH of acyl chain), 5.12 (dd, *J* = 17.2, 1.3 Hz, 1H, OCH_2-CH=C$\underline{H_2}$ of allyl group), 5.02 (dd, *J* = 10.3, 1.1 Hz, 1H, OCH_2-CH=C$\underline{H_2}$ of allyl group), 4.83 (d, *J* = 8.0 Hz, 1H, H-1'), 4.76 (d, *J* = 3.6 Hz, 1H, H-1), 4.63-4.42 (m, 10H, C_6H_5-C$\underline{H_2}$, CH_3O-C_6H_4-C$\underline{H_2}$), 4.31-4.26 (m, 1H, H-2), 4.05 (dd, *J* = 10.7, 1.6 Hz, 1H, H-6a), 3.99-3.90 (m, 2H, OC$\underline{H_2}$-CH=CH_2 of allyl group, H-3'), 3.85-3.77 (m, 4H, β-CH of acyl chain, H-5), 3.75 (s, 3H, C$\underline{H_3}$O-C_6H_4-), 3.73-3.60 (m, 4H, OC$\underline{H_2}$-CH=CH_2 of allyl group, H-6b, H-4, H-4'), 3.44-3.30 (m, 4H, H-5', H-2', H-6'), 2.55 (dd, *J* = 16.1, 6.9 Hz, 1H, α-CH_2 of acyl chain), 2.49 (dd, *J* = 15.0, 7.5 Hz, 1H, α-CH_2 of acyl chain), 2.41-2.20 (m, 4H, α-CH_2 of acyl chain), 2.27 (d, *J* = 5.9 Hz, 2H, α-CH_2 of acyl chain), 1.55-1.39 (m, 8H, γ-CH_2 of acyl chain), 1.35-1.15 (m, 68H, CH_2 of acyl chain), 0.90-0.85 (m, 12H, CH_3 of acyl chain). ^{13}C NMR (125MHz, $CDCl_3$); δ: 171.9, 171.4, 171.0, 169.8, 159.3, 143.7, 138.64, 138.58, 138.5, 137.7, 133.4, 130.6, 129.5, 128.7, 128.43, 128.35, 128.3, 128.2, 127.9, 127.78, 127.75, 127.72, 127.70, 127.61, 127.58, 127.5, 127.4, 127.1, 117.8, 113.9, 99.7, 96.5, 87.0, 80.2, 76.35, 76.29, 76.0, 75.4, 74.5, 74.1, 73.7, 73.3, 72.4, 71.4, 71.32, 71.29, 71.2, 70.0, 68.3, 67.3, 64.3, 56.8, 55.2,

52.1, 42.0, 39.8, 39.7, 34.4, 34.2, 34.1, 33.8, 31.92, 31.89, 29.74, 29.70, 29.67, 29.65, 29.64, 29.62, 29.59, 29.57, 29.38, 29.37, 29.3, 25.33, 25.26, 25.2, 25.1, 22.68, 22.67, 14.1

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{124}H_{174}N_2O_{18}$ $[M+Na]^+$: 2002.2654, found 2002.2643

4-*O*-Benzyl-6-*O*-[2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-2-deoxy-3-*O*-(4-methoxyphenylmethyl)-6-*O*-trithyl-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-D-glucopyranoside (S9)



S9

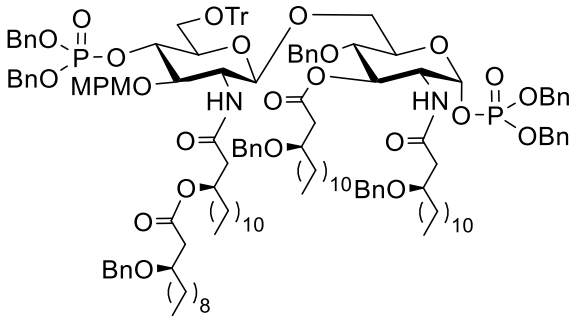
$[Ir(cod)(PMePh_2)_2]PF_6$ (0.9 mg, 1 umol) was suspended in THF (720 uL) under Ar atmosphere and the Ar was replaced with H_2 to activate the Ir complex. After the Ir complex was activated when the color of the mixture was changed from red to yellow, filled again with Ar. To a solution of **S8** (7.2 mg, 3.6 umol) in THF (360 uL) was added a solution of activated Ir complex in THF. After the mixture was stirred for 20 h, water (72 uL) and iodine (3.2 mg, 13 umol) were added and the reaction mixture was stirred for 30 min. Excess of iodine was quenched with 10% aqueous $Na_2S_2O_3$ and then the mixture was extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous $Na_2S_2O_3$, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) to give **S9** as a white solid (6.7 mg, 95%, α/β = 73/27).

^{1}H-NMR (500MHz, $CDCl_3$) δ: 7.43 (d, *J* = 7.2 Hz, 6H, $C_6\underline{H_5}$-C), 7.36-7.10 (m, 31H, $C_6\underline{H_5}$-CH_2, CH_3O-$C_6\underline{H_4}$-, $C_6\underline{H_5}$-C), 6.83 (d, *J* = 8.6 Hz, 2H, CH_3O-$C_6\underline{H_4}$-), 6.23 (d, *J* = 9.5 Hz, 1H, 2-NH), 5.94 (d, 1H, *J* = 7.6 Hz, 2-NH'), 5.36 (dd, *J* = 10.5, 9.2 Hz, 1H, H-3), 5.21 (d, *J* = 8.0 Hz, 1H, H-1'), 5.13-5.05 (m, 2H, β-CH of acyl chain, H-1), 4.70-4.40 (m, 10H, C_6H_5-$C\underline{H_2}$, CH_3O-C_6H_4-$C\underline{H_2}$), 4.21-4.15 (m, 1H, H-2), 4.07 (t, *J* = 8.7 Hz, 1H, H-5), 3.86 (dd, *J* = 11.0 Hz, 1H, H-6a), 3.86-3.75 (m, 4H, β-CH of acyl chain, H-3'), 3.76 (s, 3H, $C\underline{H_3}O$-C_6H_4-), 3.66 (dd, *J* = 11.9, 7.2 Hz, 1H, H-6b), 3.64-3.59 (m, 1H, H-4'), 3,45-3.31 (m, 4H, H-5', H-6', H-4), 3.22 (q, *J* = 8.7 Hz, 1H, H-2'), 2.60-2.28 (m, 8H, α-CH_2 of acyl chain), 1.60-1.39 (m, 8H, γ-CH_2 of acyl chain), 1.35-1.15 (m, 68H, CH_2 of acyl chain), 0.90-0.85 (m, 12H, CH_3 of acyl chain). ^{13}C NMR (125MHz, $CDCl_3$); δ: 171.8, 171.7, 171.3, 159.4, 143.6, 143.4, 138.65, 138.61, 138.4, 137.5, 130.6, 129.9, 129.6, 128.7, 128.4, 128.3, 128.2, 128.0, 127.9, 127.84, 127.75,127.7, 127.61, 127.58, 127.4, 127.3, 127.2, 114.0, 91.4, 87.1, 80.3, 76.5, 76.3, 76.1,

75.5, 74.4, 74.0, 73.9, 73.3, 71.7, 71.6, 71.4, 71.2, 64.5, 55.2, 52.5, 42.1, 41.7, 39.8, 34.4, 34.3, 34.2, 34.0, 31.94, 31.92, 29.8, 29.7, 29.6, 29.44, 29.38, 29.3, 25.3, 25.22, 25.19, 25.1, 22.7, 14.1

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{121}H_{170}N_2O_{18}$ $[M+Na]^+$: 1962.2341, found 1962.2307

4-*O*-Benzyl-6-*O*-[2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-4-*O*-bis(benzyloxy)phosphoryl-2-deoxy-3-*O*-(4-methoxyphenylmethyl)-6-*O*-trithyl-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-1-*O*-bis(benzyloxy)phosphoryl-2-deoxy-α-D-glucopyranoside (S10)



S10

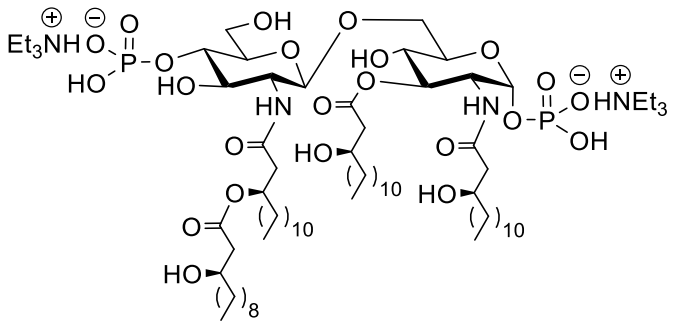
To a solution of **S9** (6.7 mg, 3.5 μmol) in CH_2Cl_2 (800 μL) was added MS4A, 1*H*-tetrazole (3.3 mg, 47 μmol) and dibenzyl-*N,N*-diisopropylphosphoramidite (11.5 μL, 34.5 μmol) and stirred at room temperature for 1.5 h. To the reaction mixture was added DMDO (1.0 mL, 0.02 M in acetone, 20 μmol) and stirred at room temperature for 30 min. The reaction was quenched with saturated aqueous $NaHCO_3$ and then the mixture was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (CH_2Cl_2/acetone/Et_3N = 95/5/0.1) to give **S10** as a white solid (7.5 mg, 88%).

^{1}H-NMR (500MHz,$CDCl_3$) δ: 7.44 (d, *J* = 7.4 Hz, 6H, $C_6\underline{H_5}$-C), 7.31-7.09 (m, 37H, $C_6\underline{H_5}$-C, $C_6\underline{H_5}$-CH_2, CH_3O-$C_6\underline{H_4}$-), 7.05 (d, *J* = 6.4 Hz, 2H, $C_6\underline{H_5}$-CH_2), 6.97 (d, *J* = 7.0 Hz, 2H, $C_6\underline{H_5}$-CH_2), 6.73 (d, *J* = 7.0 Hz, 1H, 2-NH'), 6.22 (d, *J* = 7.0 Hz, 1H, 2-NH), 5.77 (dd, *J* = 5.0, 3.4 Hz, 1H, H-1), 5.31 (dd, *J* = 11.0, 9.4 Hz, 1H, H-3), 5.20 (quin, *J* = 6.2 Hz, 1H, β-CH of acyl chain), 4.96-4.84 (m, 5H, H-1, C_6H_5-$C\underline{H_2}$), 4.71-4.34 (m, 15H, CH_3O-C_6H_4-$C\underline{H_2}$, C_6H_5-$C\underline{H_2}$, H-4'), 4.34-4.27 (m, 1H, H-2'), 4.08-4.03 (m, 2H, H-5, H-6a), 3.98 (t, *J* = 9.3 Hz, 1H, H-3'), 3.83-3.73 (m, 3H, β-CH of acyl chain, H-6b), 3.71-3.65 (m, 1H, H-2'), 3.69 (s, 1H, $C\underline{H_3}O$-C_6H_4-), 3.64-3.59 (m, 1H, H-5'), 3.55 (t, 1H, *J* = 9.6 Hz, H-4), 3.51 (d, 1H, *J* = 10.2 Hz, H-6a'), 3.41 (dd, 1H, *J* = 10.2, 7.0 Hz, H-6'b), 2.53 (dd, 1H, *J* = 16.1, 7.4 Hz, 1H, α-CH_2 of acyl chain), 2.46 (dd, 1H, *J* = 15.0, 7.0 Hz, 1H, α-CH_2 of acyl chain), 2.42-2.12 (m, 6H, α-CH_2 of

acyl chain), 1.57-1.33 (m, 8H, γ-CH_2 of acyl chain), 1.32-1.08 (m, 68H, CH_2 of acyl chain), 0.90-0.84 (m, 12H, CH_3 of acyl chain).

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{149}H_{196}N_2O_{24}P_2$ $[M+Na]^+$: 2482.3545, found 2482.3494

2-Deoxy-6-*O*-[2-deoxy-2-((*R*)-3-((*R*)-3-hydroxydodecanoyloxy)tetradecanoylamino)-β-D-glucopyranosyl]-3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-α-D-glucopyranose 1,4'-Bisphosphate (triethylammonium salt) (Tetra-AfLA)



Tetra-AfLA

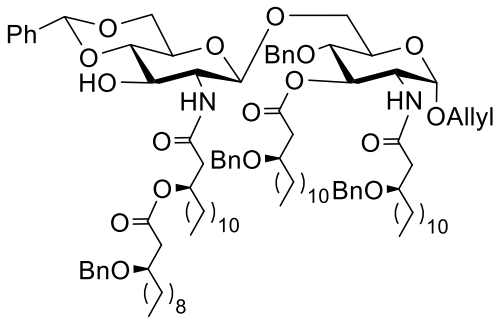
To a solution of **S10** (7.5 mg, 3.0 μmol) in dist. THF (2.0 mL), water (200 μL) and acetic acid (100 μL) was added $Pd(OH)_2/C$ (16.9 mg). The mixture was stirred under 2.0 MPa of H_2 at room temperature for 2 d. The mixture was neutralized with Et_3N (200 μL) and Pd catalyst was removed by filtration. After removal of the solvent *in vacuo*, the residue was purified by gel filtration chromatography and liquid-liquid partition chromatography[32] ($CHCl_3$:MeOH:H_2O:*i*PrOH:Et_3N = 29:29:38:4:1) to give **Tetra-AfLA** as a white solid (3.7 mg, 77%).

^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$ = 1/1); *δ*: 5.39 (dd, *J* = 7.2, 3.4 Hz, 1H, H-1), 5.17 (quin, *J* = 6.3 Hz, 1H, β-CH of acyl chain), 5.10 (t, *J* = 10.0 Hz, 1H, H-3), 4.58 (d, *J* = 7.7 Hz, 1H, H-1'), 4.10-4.00 (m, 2H, H-2, H-5), 3.98-3.86 (m, 4H, H-6a, H-4', β-CH of acyl chain), 3.84-3.68 (m, 4H, H-6b', β-CH of acyl chain, H-6a, H-6b'), 3.67-3.56 (m, 2H, H-2', H-3'), 3.40 (t, *J* = 9.6 Hz, 1H, H-4), 3.30-3.26 (m, 1H, H-5'), 3.08 (q, *J* = 7.4 Hz, 12H, CH_2 of Et_3N), 2.50 (d, *J* = 6.3 Hz, 1H, α-CH_2 of acyl chain), 2.41 (dd, *J* = 15.3, 3.8 Hz, 1H, α-CH_2 of acyl chain), 2.38-2.30 (m, 3H, α-CH_2 of acyl chain), 2.23 (dd, *J* = 14.5, 3.0 Hz, 1H, α-CH_2 of acyl chain), 2.15 (dd, *J* = 14.5, 9.3 Hz, 1H, α-CH_2 of acyl chain), 1.61-1.53 (m, 2H, γ-CH_2 of acyl chain), 1.42-1.10 (m, H, γ-CH_2 of acyl chain, CH_2 of acyl chain), 0.79 (t, *J* = 6.9 Hz, 12H, CH_3 of acyl chain). ^{13}C NMR (175 MHz, $CDCl_3/CD_3OD$ = 1/1); δ: 173.4, 172.7, 172.5, 172.4, 171.6 (2×CO, 2×CONH of acyl chain), 101.7 (C-1'), 93.2 (C-1), 75.3 (C-5'), 73.5 (C-3 and C-3'), 73.1 (C-4'), 72.5 (C-5), 71.8 (β-CH of acyl chain), 68.6 (β-CH of acyl chain), 68.4 (C-4), 68.3 (C-6), 68.1 (β-CH of acyl chain), 67.5 (β-CH of acyl chain), 60.2 (C-6'), 55.3 (C-2'), 51.98(C-

2), 46.5 (CH_2 of Et_3N), 43.4, 42.24, 42.20, 41.0 (4×α-CH_2 of acyl chain), 39.7, 37.4, 37.0, 36.9, 34.2, 31.8, 29.54, 29.51, 29.4, 29.33, 29.29, 29.22, 29.17, 29.0, 25.5, 25.4, 25.2 25.1, 24.9, 22.5 (CH_2 of acyl chain), 13.7, 13.6 (CH_3 of acyl chain), 8.4 (CH_3 of Et_3N), ^{31}P NMR (243 MHz, $CDCl_3/CD_3OD$ = 1/1); δ: 2.88, 0.12

HRMS (ESI-LIT-orbitrap MS, negative) Calcd. $C_{66}H_{126}N_2O_{23}P_2$ $[M-2H]^{2-}$: 687.4040, found 687.4039

Allyl 4-*O*-benzyl-6-*O*-[4,6-*O*-benzylidene-2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-2-deoxy-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (S11)



S11

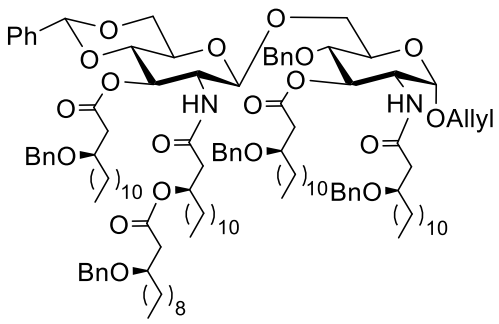
To a solution of **S6** (40.6 mg, 22.2 umol) in CH_2Cl_2 (2.2 mL) was added 2,6-di-*tert*-butylpyridine (15 uL, 67 umol) and stirred at -20 °C. To the mixture was added DDQ (7.6 mg, 33 umol) and water (110 uL) and stirred at -20 °C for 17 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) to give **S11** as a white solid (26.4 mg, 70%).

^{1}H-NMR (500MHz, $CDCl_3$) δ: 7.42 (dd, *J* = 7.5, 1.8 Hz, 2H, $C_6\underline{H_5}$-CH), 7.35-7.13 (m, 23H, $C_6\underline{H_5}$-C, $C_6\underline{H_5}$-CH_2), 6.20 (d, *J* = 9.5 Hz, 1H, 2-NH), 5.77 (d, 1H, *J* = 6.9 Hz, 2-NH'), 5.78-5.67 (m, 1H, OCH_2-C<u>H</u>=CH_2 of allyl group), 5.50 (s, 1H, C_6H_5-C<u>H</u>), 5.37 (dd, *J* = 10.6, 9.3 Hz, 1H, H-3), 5.17 (dd, *J* = 17.2, 1.4 Hz, 1H, OCH_2-CH=C<u>H_2</u> of allyl group), 5.15-5.10 (m, 1H, β-CH of acyl chain), 5.09 (dd, *J* = 10.4, 1.2 Hz, OCH_2-CH=C<u>H_2</u> of allyl group), 4.99 (d, *J* = 8.3 Hz, 1H, H-1'), 4.78 (d, *J* = 3.6 Hz, 1H, H-1), 4.62-4.43 (m, 8H, C_6H_5-C<u>H_2</u>), 4.35-4.24 (m, 4H, 3-OH', H-6'a, H-2, H-3'), 4.03-3.99 (m, 2H, H-6a, OC<u>H_2</u>-CH=CH_2 of allyl group), 3.91-3.78 (m, 4H, β-CH of acyl chain, H-5), 3.76-3.64 (m, 4H, H-4, H-6b, H-6b', OC<u>H_2</u>-CH=CH_2 of allyl group), 3.49 (t, *J* = 9.2 Hz, 1H, H-4'), 3.43 (td, *J* = 9.6, 4.8 Hz, 1H, H-5'), 3.19 (q, *J* = 8.3 Hz, 1H, H-2'), 2.60-2.53 (m, 2H, α-CH_2 of acyl chain), 2.44 (d, *J* = 4.7 Hz, 1H, α-CH_2 of acyl chain) 2.41 (d, *J* = 4.6 Hz, 1H, α-CH_2 of acyl chain), 2.28 (d, *J* = 5.9 Hz, 2H, α-CH_2 of acyl chain), 2.18-2.14 (m, 2H, α-CH_2 of acyl chain), 1.68-1.45 (m, 8H, γ-CH_2

of acyl chain), 1.35-1.20 (m, 68H, CH_2 of acyl chain), 0.90-0.84 (m, 12H, CH_3 of acyl chain). ^{13}C NMR (125MHz, $CDCl_3$); δ: 171.9, 171.8, 171.1, 170.8, 138.6, 138.5, 138.1, 137.7, 137.2, 133.3, 129.0, 128.5, 128.4, 128.3, 128.2, 128.1, 127.93, 127.89, 127.8, 127.70, 127.68, 127.6, 127.5, 127.4, 126.4, 117.8, 101.9, 100.0, 96.6, 81.6, 76.3, 75.8, 75.6, 75.4, 74.6, 73.7, 72.0, 71.4, 71.3, 71.1, 70.0, 69.3, 68.4, 66.3, 59.5, 52.0, 42.4, 42.0, 39.74, 39.70, 34.40, 34.35, 34.1, 33.5, 31.92, 31.90, 31.86, 29.73, 29.69, 29.684, 29.675, 29.65, 29.64, 29.62, 29.61, 29.58, 29.56, 29.49, 29.46, 29.4, 29.34, 29.33, 29.29, 29.25, 25.24, 25.20, 25.18, 24.7, 22.67, 22.65, 14.084, 14.075

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{104}H_{156}N_2O_{17}$ $[M+Na]^+$: 1728.1296, found 1728.1262

Allyl 4-*O*-benzyl-6-*O*-[4,6-*O*-benzylidene-2-*O*-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (S12)



S12

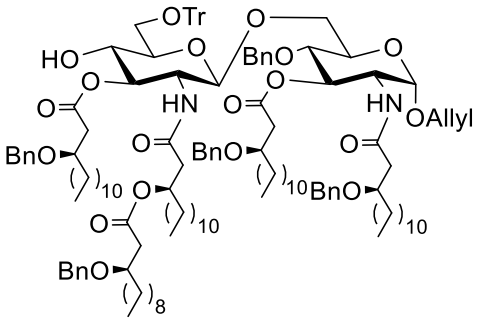
To a stirred solution of **S11** (59.0 mg, 34.6 μmol) and **2** (17.3 mg, 51.9 μmol) in CH_2Cl_2 (1.7 mL) and DMF (1.7 mL) was added DIPEA (18.1 μL, 104 μmol), MNBA (18.6 mg, 54.0 umol) and DMAP (1.3 mg, 11 μmol) and stirred at room temperature for 18 h. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous NH_4Cl and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified with gel permeation chromatography to give **S12** as a white solid (66.3 mg, 95%).

^{1}H-NMR (500MHz, $CDCl_3$) δ: 7.36 (d, *J* = 7.7 Hz, 2H, C_6H_5-CH), 7.34-7.17 (m, 28H, $C_6\underline{H_5}$-CH, $C_6\underline{H_5}$-CH_2), 6.17 (d, *J* = 9.5 Hz, 1H, 2-NH), 5.81 (d, 1H, *J* = 8.4 Hz, 2-NH'), 5.73-5.65 (m, 1H, OCH_2-$C\underline{H}$=CH_2 of allyl group), 5.40 (t, *J* = 9.7 Hz, 1H, H-3'), 5.39 (s, 1H, C_6H_5-$C\underline{H}$), 5.35 (dd, *J* = 10.6, 9.3 Hz, 1H, H-3), 5.19 (dd, *J* = 17.2, 1.4 Hz, 1H, OCH_2-CH=$C\underline{H_2}$ of allyl group), 5.09 (dd, *J* = 10.4, 1.2 Hz, OCH_2-CH=$C\underline{H_2}$ of allyl group), 5.00 (q, *J* = 6.0, 1H, β-CH of acyl chain), 4.75 (d, *J* = 3.2 Hz, 1H, H-1), 4.75 (d, *J* = 8.9 Hz, 1H, H-1'), 4.58-4.37 (m, 10H, C_6H_5-$C\underline{H_2}$), 4.32-4.25 (m, 2H, H-6'a, H-2), 4.02 (dd, *J* = 12.7, 5.3 Hz, 1H, $OC\underline{H_2}$-CH=CH_2 of allyl group), 3.96 (d, *J* = 9.3 Hz, 1H, H-6a,), 3.88 (q, *J* = 6.2 Hz, 1H, β-CH of acyl chain), 3.85-3.70 (m, 7H, β-CH of acyl chain, H-2', H-5, H-6b', $OC\underline{H_2}$-CH=CH_2 of allyl group),

3.69 (dd, *J* = 10.7, 4.7 Hz, 1H, H-6b), 3.64 (t, *J* = 9.4 Hz, 1H, H-4'), 3.61 (t, *J* = 9.5 H, 1H, H-4), 3.47 (td, *J* = 9.7, 5.0 Hz, 1H, H-5'), 2.69 (dd, *J* = 15.0, 7.3 Hz, 1H, α-CH_2 of acyl chain), 2.63 (dd, *J* = 14.9, 6.3 Hz, 1H, α-CH_2 of acyl chain), 2.57 (dd, *J* = 16.0, 7.2 Hz, 1H, α-CH_2 of acyl chain), 2.49 (dd, *J* = 14.8, 5.5 Hz, 1H, α-CH_2 of acyl chain), 2.45 (dd, *J* = 14.8, 5.4 Hz, 1H, α-CH_2 of acyl chain), 2.41 (dd, *J* = 15.9, 5.2 Hz, 1H, α-CH_2 of acyl chain), 2.32-2.24 (m, 3H, α-CH_2 of acyl chain), 2.11 (dd, *J* = 14.8, 5.2 Hz, 1H, α-CH_2 of acyl chain), 1.68-1.45 (m, 10H, γ-CH_2 of acyl chain), 1.35-1.20 (m, 86H, CH_2 of acyl chain), 0.90-0.84 (m, 15H, CH_3 of acyl chain). ^{13}C NMR (125MHz, $CDCl_3$); δ: 171.9, 171.7, 171.1, 170.9, 169.5, 138.6, 138.5, 138.4, 137.7, 136.9, 136.4, 129.0, 128.5, 128.4, 128.3, 128.2, 128.1, 127.8, 127.73, 127.70, 127.65, 127.63, 127.61, 127.57, 127.55, 127.51, 127.4, 126.1, 117.8, 101.4, 101.2, 96.5, 78.9, 76.4, 76.3, 75.9, 75.7, 75.4, 74.5, 73.7, 71.4, 71.3, 71.2, 71.1, 70.9, 70.0, 68.6, 68.2, 67.9, 66.3, 55.2, 52.1, 42.0, 41.3, 39.7, 39.6, 34.4, 34.3, 34.1, 33.7, 31.9, 29.69, 29.65, 29.5, 29.35, 29.30, 25.33, 25.25, 25.19, 25.17, 22.7, 14.1

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{125}H_{188}N_2O_{19}$ $[M+Na]^+$: 2044.3699, found 2044.3663

Allyl 4-*O*-benzyl-6-*O*-[2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-6-*O*-trithyl-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (S13)



S13

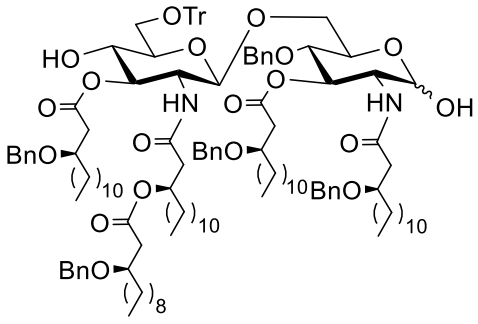
To a solution of **S12** (26.9 mg, 13.3 umol) in CH_2Cl_2 (6.6 mL) and water (660 uL) was added TFA (660 uL) and stirred at room temperature for 1 h. The reaction mixture was co-evaporated with toluene. To the residue was added CH_2Cl_2 (1.3 mL), pyridine (10.7 uL, 133 umol) and TrCl (9.3 mg, 33 umol) and stirrerd at room temperature for 23 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$/TEA = 100/0.1) to give **S13** as a white solid (26.6 mg, 92% for 2 steps).

^{1}H-NMR (500MHz,$CDCl_3$) δ: 7.43 (d, *J* = 7.7 Hz, 6H, $C_6\underline{H_5}$-C), 7.35-7.10 (m, 34H, $C_6\underline{H_5}$-C, $C_6\underline{H_5}$-CH_2), 6.16 (d, *J* = 9.3 Hz, 1H, 2-NH), 5.72 (d, 1H, *J* = 8.6 Hz, 2-NH'), 5.71-5.63 (m, 1H,

OCH_2-C<u>H</u>=CH_2 of allyl group), 5.34 (t, *J* = 10.0 Hz, 1H, H-3), 5.13 (d, *J* = 17.5 Hz, 1H, OCH_2-CH=C<u>H_2</u> of allyl group), 5.09 (t, *J* = 9.9 Hz, 1H, H-3'), 5.03 (d, *J* = 11.2 Hz, OCH_2-CH=C<u>H_2</u> of allyl group), 5.03-4.98 (m, 1H, β-CH of acyl chain), 4.72 (d, *J* = 3.4 Hz, 1H, H-1), 4.60 (d, *J* = 8.2 Hz, 1H, H-1'), 4.58-4.40 (m, 10H, C_6H_5-C<u>H_2</u>), 4.27 (td, *J* = 10.1, 3.4 Hz, 1H, H-2), 4.03 (d, *J* = 9.7 Hz, 1H, H-6a), 3.97 (dd, *J* = 12.7, 5.2 Hz, 1H, OC<u>H_2</u>-CH=CH_2 of allyl group), 3.88-3.77 (m, 6H, β-CH of acyl chain, H-5, H-2'), 3.70 (dd, *J* = 12.7, 6.0 Hz, 1H, OC<u>H_2</u>-CH=CH_2 of allyl group), 3.63-3.55 (m, 3H, H-6b, H-4, H-4'), 3.47-3.44 (m, 1H, H-5'), 3.37-3.33 (m, 2H, H-6'), 2.74 (s, 1H, 4-OH'), 2.66 (dd, *J* = 15.0, 7.2 Hz, 1H, α-CH_2 of acyl chain), 2.62 (dd, *J* = 15.0, 7.5 Hz, 1H, α-CH_2 of acyl chain), 2.54 (dd, *J* = 15.8, 6.5 Hz, 1H, α-CH_2 of acyl chain), 2.51 (dd, *J* = 14.1, 5.5 Hz, 1H, α-CH_2 of acyl chain), 2.45 (dd, *J* = 15.0, 5.3 Hz, 1H, α-CH_2 of acyl chain), 2.38 (dd, *J* = 16.0, 5.4 Hz, 1H, α-CH_2 of acyl chain), 2.31-2.25 (m, 3H, α-CH_2 of acyl chain), 2.12 (dd, *J* = 14.7, 5.7 Hz, 1H, α-CH_2 of acyl chain), 1.64-1.44 (m, 10H, γ-CH_2 of acyl chain), 1.35-1.18 (m, 86H, CH_2 of acyl chain), 0.88 (t, *J* = 6.8 Hz, 15H, CH_3 of acyl chain). ^{13}C NMR (125MHz, $CDCl_3$); δ: 172.1, 171.9, 171.6, 171.0, 169.3, 146.9, 143.6, 138.6, 138.6, 138.5, 138.2, 137.7, 133.3, 128.6, 128.38, 128.36, 128.35, 128.3, 128.2, 127.92, 127.90, 127.8, 127.74, 127.70, 127.62, 127.56, 127.5, 127.4, 127.2, 127.1, 117.8, 100.5, 96.4, 87.0, 82.0, 76.5, 76.3, 76.1, 75.9, 75.4, 74.4, 74.2, 73.7, 71.4, 71.3, 71.2, 71.1, 70.9, 69.9, 68.2, 67.2, 64.2, 64.1, 52.1, 42.0, 41.3, 39.8, 39.7, 34.4, 34.25, 34.15, 34.1, 33.6, 31.92, 31.89, 29.73, 29.68, 29.66, 29.6, 29.5, 29.37, 29.35, 29.3, 25.4, 25.3, 25.19, 25.17, 22.7, 14.1

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{137}H_{198}N_2O_{19}$ $[M+Na]^+$: 2198.4481, found 2198.4446

4-*O*-Benzyl-6-*O*-[2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-6-*O*-trithyl-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-D-glucopyranoside (S14)



S14

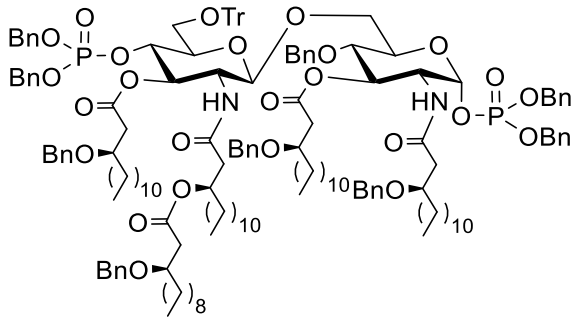
[Ir(cod)($PMePh_2$)$_2$]PF_6 (7.4 mg, 8.8 umol) was suspended in THF (2.1 mL) under Ar atmosphere and the Ar was replaced with H_2 to activate the Ir complex. After the Ir complex was activated when the color of the mixture was changed from red to yellow, filled again with Ar. To a solution of **S13** (45.0 mg, 20.7 umol) in THF (4.2 mL) was added a solution of

activated Ir complex in THF. After the mixture was stirred for 4 h, water (420 uL) and iodine (15.2 mg, 59.9 umol) were added and the reaction mixture was stirred for 40 min. Excess of iodine was quenched with 10% aqueous $Na_2S_2O_3$ and then the mixture was extracted with $CHCl_3$. The organic layer was washed with 10% aqueous $Na_2S_2O_3$, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) to give **S14** as a white solid (35.7 mg, 81%).

^{1}H-NMR (500MHz,$CDCl_3$) δ: 7.42 (d, *J* = 7.4 Hz, 6H, $C_6\underline{H_5}$-C), 7.35-7.12 (m, 34H, $C_6\underline{H_5}$-C, $C_6\underline{H_5}$-CH_2), 6.22 (d, *J* = 9.3 Hz, 1H, 2-NH), 5.75 (d, 1H, *J* = 8.4 Hz, 2-NH'), 5.34 (t, *J* = 9.9 Hz, 1H, H-3), 5.08-5.00 (m, 3H, H-1, H-3', β-CH of acyl chain), 4.85 (d, *J* = 8.3 Hz, 1H, H-1'), 4.58-4.40 (m, 10H, C_6H_5-$C\underline{H_2}$), 4.19 (td, *J* = 10.2, 3.0 Hz, 1H, H-2), 4.03 (d, *J* = 10.7 Hz, 1H, H-6a), 3.86-3.79 (m, 4H, β-CH of acyl chain), 3.70 (dd, *J* = 12.7, 6.0 Hz, 1H, H-2'), 3.63-3.55 (m, 2H, H-4', H-6b), 3.47-3.44 (m, 1H, H-5'), 3.37-3.33 (m, 3H, H-6', H-4), 2.79 (d, *J* = 2.0 Hz, 1H, 4-OH'), 2.66-2.17 (m, 10H, α-CH_2 of acyl chain), 1.64-1.44 (m, 10H, γ-CH_2 of acyl chain), 1.35-1.20 (m, 86H, CH_2 of acyl chain), 0.90-0.85 (m, 15H, CH_3 of acyl chain). ^{13}C NMR (125MHz, $CDCl_3$); δ: 172.1, 171.8, 171.2, 170.0, 143.5, 138.62, 138.55, 138.3, 138.2, 137.6, 128.6, 128.5, 128.4, 128.3, 128.2, 127.9, 127.83, 127.80, 127.3, 127.71, 127.66, 127.64, 127.60, 127.56, 127.4, 127.2, 100.2, 91.4, 87.2, 76.39, 76.35, 76.2, 75.9, 75.5, 75.3, 74.22, 74.16, 73.6, 71.5, 71.3, 71.2, 71.1, 70.9, 66.8, 64.3, 54.3, 52.5, 42.0, 41.4, 39.9, 39.8, 39.7, 34.4, 34.3, 34.1, 33.9, 31.92, 31.90, 29.72, 29.69, 29.65, 29.62, 29.58, 29.5, 29.4, 29.3, 25.23, 25.19, 25.16, 25.1, 22.7, 14.1

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{134}H_{194}N_2O_{19}$ $[M+H]^+$: 2136.4349, found 2136.4336

4-*O*-Benzyl-6-*O*-[2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-4-*O*-bis(benzyloxy)phosphoryl-2-deoxy-6-*O*-trithyl-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-1-*O*-bis(benzyloxy)phosphoryl-2-deoxy-α-D-glucopyranoside (S15)



S15

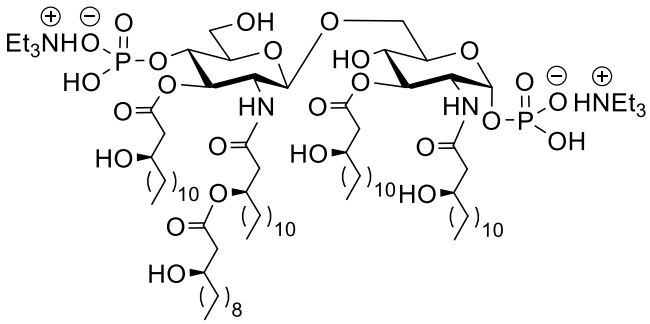
To a solution of **S14** (7.5 mg, 3.5 μmol) in CH_2Cl_2 (1.0 mL) was added MS4A, 1*H*-tetrazole (3.2 mg, 46 μmol) and dibenzyl-*N,N*-diisopropylphosphoramidite (17.5 μL, 52.7 μmol) and stirred at room temperature for 100 min. To the reaction mixture was added DMDO (1.0 mL,

0.02 M in acetone, 20 μmol) and stirred at room temperature for 40 min. The reaction was quenched with saturated aqueous $NaHCO_3$ and then the mixture was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (CH_2Cl_2/acetone/Et_3N = 95/5/0.1→ 90/10/0.1) to give **S15** as a white solid (7.4 mg, 79%).

^{1}H-NMR (500MHz,$CDCl_3$) δ: 7.44 (d, *J* = 7.4 Hz, 6H, $C_6\underline{H_5}$-C), 7.30-7.04 (m, 34H, $C_6\underline{H_5}$-C, $C_6\underline{H_5}$-CH_2), 6.34 (d, *J* = 7.4 Hz, 1H, 2-NH'), 6.22 (d, *J* = 8.6 Hz, 1H, 2-NH), 5.77 (dd, 1) 5.40 (t, *J* = 9.7 Hz, 1H), 5.32 (dd, *J* = 10.9, 9.3 Hz, 1H), 5.20 (d, J = 8.3 Hz, 1H, H-1'), 5.11 (quin, J = 6.2 Hz, 1H, β-CH of acyl chain), 4.99-4.81 (m, 4H, C_6H_5-$C\underline{H_2}$), 4.65-4.31 (m, 16H, C_6H_5-$C\underline{H_2}$, H-4', H-2), 4.06-4.01 (m, 2H, H-6a, H-5), 3.87-3.79 (m, 3H, β-CH of acyl chain, H-6b), 3.76-3.68 (m, 2H, β-CH of acyl chain), 3.66-3.60 (m, 1H, H-2'), 3.57 (t, 1H, J = 9.7 Hz, H-4), 3.53-3.43 (m, 2H, H-5', H-6a'), 3.37 (dd, 1H, J = 10.2, 6.8 Hz, H-6'b), 2.69 (dd, 1H, *J* = 15.3, 6.1 Hz, 1H, α-CH_2 of acyl chain), 2.56 (dd, 1H, *J* = 16.0, 7.3 Hz, 1H, α-CH_2 of acyl chain) 2.50-2.37 (m, 4H, α-CH_2 of acyl chain), 2.30-2.14 (m, 3H, α-CH_2 of acyl chain), 2.01 (dd, 1H, J = 15.4, 4.9 Hz, α-CH_2 of acyl chain) 1.64-1.44 (m, 10H, γ-CH_2 of acyl chain), 1.35-1.20 (m, 86H, CH_2 of acyl chain), 0.90-0.85 (m, 15H, CH_3 of acyl chain).

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{162}H_{220}N_2O_{25}P_2$ $[M+H]^+$: 2656.5553, found 2656.5552

2-Deoxy-6-*O*-[2-deoxy-2-((*R*)-3-((*R*)-3-hydroxydodecanoyloxy)tetradecanoylamino)-3-*O*-((*R*)-3-hydroxytetradecanoyl)-β-D-glucopyranosyl]-3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-α-D-glucopyranose 1,4'-Bisphosphate (triethylammonium salt) (Penta-AfLA)



Penta-AfLA

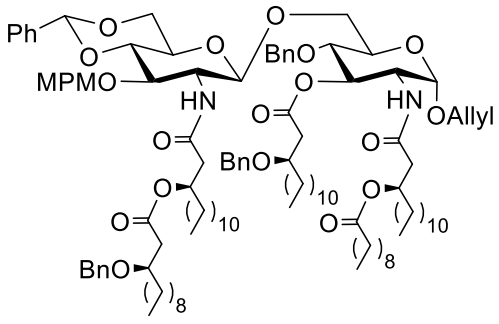
To a solution of **S15** (19.5 mg, 7.34 μmol) in dist. THF (5.5 mL), water (550 μL) and acetic acid (275 μL) was added $Pd(OH)_2$/C (39.5 mg). The mixture was stirred under 2.0 MPa of H_2 at room temperature for 16 h. The mixture was neutralized with Et_3N (550 μL) and Pd catalyst was removed by filtration. After removal of the solvent *in vacuo*, the residue was purified by gel filtration chromatography and liquid-liquid partition chromatography[32]

($CHCl_3$:MeOH:H_2O:*i*PrOH:Et_3N = 30:30:36:4:1) to give **Penta-AfLA** as a white solid (11.0 mg, 83%).

^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD = 1/1); *δ*: 5.36 (dd, *J* = 7.2, 3.4 Hz, 1H, H-1), 5.10 (t, J = 10.0 Hz, 2H, H-3, H-3'), 5.08-5.02 (m, 1H, β-CH of acyl chain), 4.60 (d, *J* = 8.6 Hz, 1H, H-1'), 4.11 (q, *J* = 9.9 Hz, 1H, H-4'), 4.04 (dt, *J* = 10.7, 2.7 Hz, 1H, H-2), 4.02-3.84 (m, 6H, β-CH of acyl chain, H-5, H-2', H-6a', H-6a), 3.83-3.77 (m, 1H, β-CH of acyl chain), 3.74 (dd, *J* = 12.0, 5.1 Hz, 1H, H-6b), 3.66 (d, *J* = 12.2 Hz, 1H, H-6b'), 3.43 (t, *J* = 9.7 Hz, 1H, H-4), 3.29 (m, 1H, H-5'), 3.07 (q, *J* = 7.3 Hz, 12H, CH_2 of Et_3N), 2.46-2.30 (m, 8H, α-CH_2 of acyl chain), 2.22 (dd, *J* = 14.4, 3.1 Hz, 1H, α-CH_2 of acyl chain), 2.14 (dd, J = 14.4, 9.4 Hz, 1H, α-CH_2 of acyl chain), 1.52-1.10 (m, 116H, CH_2 of acyl chain, CH_3 of Et_3N), 0.80 (t, J = 6.7 Hz, 15H, CH_3 of acyl chain), ^{13}C NMR (175 MHz, $CDCl_3$/CD_3OD = 1/1); δ: 173.4, 172.5, 172.4, 171.9, 171.6 (3×CO, 2×CONH of acyl chain), 101.4 (C-1'), 93.7 (C-1), 75.5 (C-5'), 73.7 (C-3), 73.5 (C-3'), 72.6 (C-5), 71.2 (β-CH of acyl chain), 71.0 (C-4'), 68.8 (β-CH of acyl chain), 68.5 (β-CH of acyl chain), 68.4 (β-CH of acyl chain), 68.1 (C-4), 67.8 (C-6), 60.2 (C-6'), 53.6 (C-2'), 51.9 (C-2), 46.4 (CH_2 of Et_3N), 43.4, 42.3, 42.2, 41.8, 40.7 (5×α-CH_2 of acyl chain), 37.4, 37.2, 37.1, 33.5 (γ-CH_2 of acyl chain), 31.8, 29.60, 29.57, 29.55, 29.5, 29.4, 29.3, 29.2, 25.49, 25.48, 25.43 25.38, 22.5 (CH_2 of acyl chain), 13.6 (CH_3 of acyl chain), 8.3 (CH_3 of Et_3N), ^{31}P NMR (243 MHz, $CDCl_3$/CD_3OD = 1/1); δ: 1.41, -0.52

HRMS (ESI-LIT-orbitrap MS, negative) Calcd. for $C_{80}H_{152}N_2O_{25}P_2$ $[M-2H]^{2-}$: 800.5006, found 800.5006

Allyl 4-*O*-benzyl-6-*O*-[4,6-*O*-benzylidene-2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-2-deoxy-3-*O*-(4-methoxyphenylmethyl)-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-(decanoyloxy)tetradecanoylamino)-2-deoxy-α-D-glucopyranoside (7)



7

To a solution of **5** (210 mg, 132 μmol) in $CHCl_3$ (3 mL) was added TMSDMA (68.6 μL, 434 μmol) and tetrakis(triphenylphosphine)palladium(0) (16.8 mg, 14.5 μmol). After stirred for 80 min, the reaction was quenched by addition of water and the mixture was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give crude as a yellow solid.

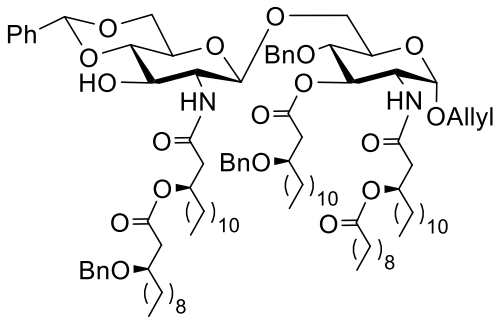
To a stirred solution of the crude and **6** (57.8 mg, 145 μmol) in $CHCl_3$ (3 mL) and DMF (3 mL) was added HATU (75.1 mg, 198 μmol) and DMAP (1.61 mg, 13.2 μmol) and stirred at room temperature for 18 h. The reaction mixture was quenched with 10 % aqueous citric acid and extracted with $CHCl_3$. The organic layer was washed with 10 % aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) to give **7** as a white solid (193 mg, 78 % for 2 steps).

^{1}H NMR (500 MHz, $CDCl_3$); *δ*: 7.47 (m, 2H, $C_6\underline{H_5}$-CH), 7.46-7.17 (m, 20H, $C_6\underline{H_5}$-CH, $C_6\underline{H_5}$-CH_2, CH_3O-$C_6\underline{H_4}$-), 6.79 (d, *J* = 8.0 Hz, 2H, CH_3O-$C_6\underline{H_4}$-), 5.88-5.79 (m, 3H, 2-NH, OCH_2-$C\underline{H}$=CH_2 of allyl group, 2-NH'), 5.52 (s, 1H, C_6H_5-$C\underline{H}$), 5.35-5.25 (m, 2H, H-3, OCH_2-CH=$C\underline{H_2}$ of allyl group), 5.19 (d, *J* = 11.8 Hz, 1H, OCH_2-CH=$C\underline{H_2}$ of allyl group), 5.07 (m, 2H, β-CH of acyl chain), 4.97 (d, *J* = 8.0 Hz, 1H, H-1'), 4.81 (d, *J* = 3.5, 1H, H-1), 4.76 (d, *J* = 11.0 Hz, 1H, CH_3O-C_6H_4-$C\underline{H_2}$), 4.61-4.42 (m, 7H, CH_3O-C_6H_4-$C\underline{H_2}$, C_6H_5-$C\underline{H_2}$), 4.31 (dd, *J* = 10.5, 5.0 Hz, 1H, H-6a'), 4.24-4.20 (m, 2H, H-2, H-3'), 4.16 (dd, *J* = 12.0, 5.0 Hz, 1H, $OC\underline{H_2}$-CH=CH_2 of allyl group), 3.98 (d, *J* = 10.0 Hz, 1H, H-6a), 3.93 (dd, *J* = 9.0, 5.0 Hz, 1H, $OC\underline{H_2}$-CH=CH_2 of allyl group), 3.88-3.77 (m, 3H, H-5, β-CH of acyl chain), 3.80 (s, 3H, $C\underline{H_3}O$-C_6H_4), 3.77-3.70 (m, 2H, H-6b, H-6b'), 3.70-3.60 (m, 2H, H-4', H-4), 3.47 (td, *J* = 9.3, 5.2 Hz, 1H, H-5'), 3.38 (q, *J* = 8.3 Hz, 1H, H-2'), 2.56 (dd, *J* = 16.0, 7.1 Hz, 1H, α-CH_2 of acyl chain), 2.50 (dd, *J* = 14.8, 7.5 Hz, 1H, α-CH_2 of acyl chain), 2.43 (dd, *J* = 16.1, 4.8 Hz, 1H, α-CH_2 of acyl chain), 2.39-2.32 (m, 3H, α-CH_2 of acyl chain), 2.30-2.21 (m, 4H, α-CH_2 of acyl chain), 1.61-1.49 (m, 10H,), 1.40-1.20 (m, 82H), 0.88 (t, *J* = 7.0 Hz, 15H). ^{13}C NMR (125 MHz, $CDCl_3$); *δ*: 173.1, 172.2, 171.4, 169.8, 169.5, 159.2, 138.6, 138.5, 137.7, 137.4, 133.5, 130.6, 129.5, 129.0, 128.5, 128.4, 128.3, 128.2, 127.9, 127.75, 127.72, 127.6, 127.5, 126.1, 117.9, 113.8, 101.3, 100.5, 96.3, 82.3, 76.3, 76.0, 75.4, 74.5, 73.9, 73.7, 71.3, 70.9, 70.2, 68.8, 68.3, 66.1, 57.7, 55.2, 52.4, 41.43,41.38, 39.9, 39.7, 34.5, 34.3, 34.2, 33.8, 31.94, 31.89, 29.76, 29.68, 29.61, 29.48, 29.45, 29.42, 29.38, 29.34, 29.30, 29.2, 25.4, 25.24, 25.21, 25.1, 25.0, 22.7, 14.1

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{115}H_{176}N_2O_{19}$ $[M+Na]^+$: 1912.2760, Found 1912.2749.

Allyl 4-*O*-benzyl-6-*O*-[4,6-*O*-benzylidene-2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-2-deoxy-β-D-glucopyranosyl]-3-*O*-

((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-(decanoyloxy)tetradecanoylamino)-2-deoxy-α-D-glucopyranoside (S16)



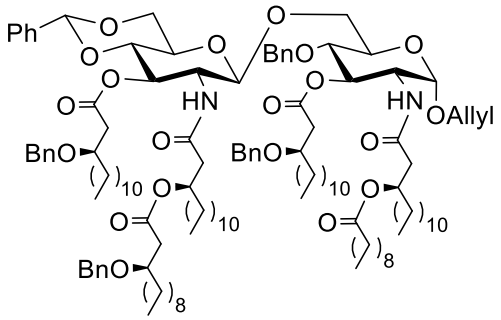
S16

To a solution of **7** (150 mg, 79.4 μmol) in CH_2Cl_2 (3 mL) was added 2,6-di-*tert*-butylpyridine (53.4 μL, 238μL) and stirred at 0 °C for 30 min. To the mixture was added DDQ (27.0 mg, 119 μmol) and water (100 μL) and stirred at 0 °C for 20 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) to give intermediate **S16** as a white solid. (113 mg, 80%)

^{1}H-NMR (500 MHz, $CDCl_3$); *δ*: 7.42 (dd, *J* = 7.5, 1.9 Hz, 2H, $C_6\underline{H_5}$-CH), 7.31-7.16 (m, 18H, $C_6\underline{H_5}$-CH, $C_6\underline{H_5}$-CH_2), 5.94 (d, *J* = 9.3 Hz, 1H, 2-NH), 5.91-5.81 (m, 1H, OCH_2-C$\underline{H}$=CH_2 of allyl group), 5.84 (d, *J* = 7.0 Hz, 1H, 2-NH'), 5.50 (s, 1H, C_6H_5-C$\underline{H}$), 5.34 (dd, *J* = 10.7, 9.2 Hz, 1H, H-3), 5.28 (dd, *J* = 17.2, 1.6 Hz, 1H, OCH_2-CH=C$\underline{H_2}$ of allyl group), 5.20 (dd, *J* = 10.3, 1.3 Hz, 1H, OCH_2-CH=C$\underline{H_2}$ of allyl group), 5.16-5.04 (m, 2H, β-CH of acyl chain), 4.98 (d, *J* = 8.3 Hz, 1H, H-1'), 4.83 (d, *J* = 3.6 Hz, 1H, H-1), 4.61-4.42 (m, 6H, C_6H_5-C$\underline{H_2}$), 4.30-4.22 (m, 3H, H-3', H-6a', H-2), 4.15 (dd, *J* = 12.8, 5.2 Hz, 1H, OCH_2-CH=C$\underline{H_2}$ of allyl group), 4.03 (dd, *J* = 10.7, 1.6 Hz, 1H, H-6a), 3.95-3.81 (m, 4H, OCH_2-CH=CH_2 of allyl group, β-CH of acyl chain, H-5), 3.76-3.72 (m, 2H, H-6b, H-6b'), 3.67 (t, *J* = 9.5 Hz, 1H, H-4), 3.49 (t, *J* = 9.2 Hz, 1H, H-4'), 3.43 (td, *J* = 9.6, 4.9 Hz, 1H, H-5'), 3.21 (q, *J* = 8.4 Hz, 1H, H-2'), 2.61-2.53 (m, 1H, α-CH_2 of acyl chain), 2.46 (dd, *J* = 12.5, 4.4 Hz,1H, α-CH_2 of acyl chain), 2.43 (dd, *J* = 11.5, 4.1 Hz, 1H, α-CH_2 of acyl chain), 2.36 (dd, *J* = 14.9, 7.3 Hz, 1H, α-CH_2 of acyl chain), 2.29 (dd, *J* = 10.1, 4.8 Hz, 1H, α-CH_2 of acyl chain), 2.26 (d, *J* = 7.6 Hz, 2H, α-CH_2 of acyl chain), 2.18-2.15 (m, 2H, α-CH_2 of acyl chain), 1.63-1.32 (m, 10H, γ-CH_2 of acyl chain), 1.31-1.12 (m, 82H, CH_2 of acyl chain), 0.90-0.85 (m, 15H, CH_3 of acyl chain). ^{13}C NMR (125 MHz, $CDCl_3$); *δ*: 173.1, 172.2, 171.8, 170.9, 169.6, 138.6, 138.1, 137.7, 137.3, 133.4, 129.0, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.73, 127.70, 127.5, 126.4, 118.0, 102.0, 100.1, 96.4, 81.6, 75.8, 75.7, 75.4, 74.7, 73.7, 72.0, 71.3, 71.2, 70.9, 70.0, 69.5, 68.7, 68.4, 67.7, 66.3, 59.4, 52.3, 42.4, 41.5, 39.7, 34.5, 34.4, 34.3, 34.2, 33.5, 31.94, 31.89, 29.8, 29.7, 29.6, 29.53, 29.50, 29.46, 29.4, 29.34, 29.30, 29.2, 25.2, 25.0, 24.8, 22.70, 22.68, 14.1

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{107}H_{168}N_2O_{18}$ $[M+Na]^+$: 1792.2184, Found 1792.2167.

Allyl 4-*O*-benzyl-6-*O*-[4,6-*O*-benzylidene-2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-(decanoyloxy)tetradecanoylamino)-2-deoxy-α-D-glucopyranoside (8)



8

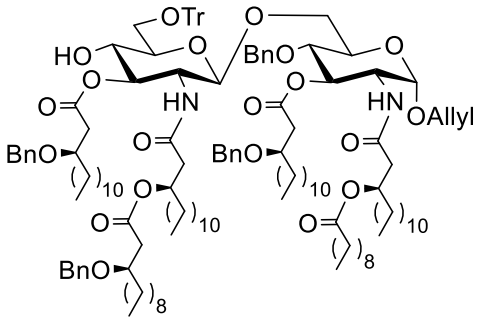
To a stirred solution of intermediate **S16** (110 mg, 62.1 μmol) and **2** (24.9 mg, 74.6 μmol) in CH_2Cl_2 (2 mL) and DMF (2 mL) was added DIPEA (21.7 μL, 124 μmol), MNBA (32.1 mg, 93.2 μmol) and DMAP (0.76 mg, 6.2 μmol) and stirred at 40 °C for 22 h. The reaction mixture was quenched with 10% aqueous citric acid and extracted with $CHCl_3$. The organic layer was washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) to give **8** as a white solid (108 mg, 83%).

^{1}H NMR (500 MHz, $CDCl_3$); *δ*: 7.38 (m, 2H, $C_6\underline{H}_5$-CH), 7.31-7.18 (m, 23H, $C_6\underline{H}_5$-CH, $C_6\underline{H}_5$-CH_2), 5.90 (d, *J* = 9.5 Hz, 1H, 2-NH), 5.88-5.83 (m, 1H, OCH_2-C$\underline{H}$=CH_2 of allyl group), 5.77 (d, *J* = 8.5 Hz, 1H, 2-NH') 5.39 (s, 1H, C_6H_5-C$\underline{H}$), 5.39 (t, *J* = 10.0 Hz, 1H, H-3'), 5.34-5.18 (m, 2H, H-3, OCH_2-CH=C$\underline{H}_2$ of allyl group), 5.20 (dd, *J* = 10.4, 1.4 Hz, 1H, OCH_2-CH=C$\underline{H}_2$ of allyl group), 5.07 (quin, *J* = 6.0 Hz, 1H, β-CH of acyl chain), 5.00 (quin, *J* = 6.0 Hz, 1H, β-CH of acyl chain), 4.80 (d, *J* = 4.0 Hz, 1H, H-1), 4.74 (d, *J* = 8.0 Hz, 1H, H-1'), 4.59-4.38 (m, 8H, C_6H_5-C$\underline{H}_2$), 4.30 (dd, *J* = 10.5, 5.5 Hz, 1H, H-6a'), 4.20 (dt, *J* = 12.0, 5.0 Hz, 1H, H-2), 4.15 (dd, *J* = 12.0, 5.0 Hz, 1H, OCH_2-CH=C$\underline{H}_2$ of allyl group), 3.97 (d, *J* = 9.5 Hz, 1H, H-6a), 3.93 (dd, *J* = 9.0, 5.0 Hz, 1H, OCH_2-CH=C$\underline{H}_2$ of allyl group), 3.94-3.77 (m, 5H, β-CH of acyl chain, H-5, H-2'), 3.73 (t, *J* = 10.2 Hz, 1H, H-6b') 3.68 (dd, *J* = 10.8, 4.9 Hz, 1H, H-6b), 3.64 (t, *J* = 9.5 Hz, H-4'), 3.59 (t, *J* = 9.5 Hz, 1H, H-4), 3.47 (dt, *J* = 12.0, 5.0 Hz, 1H, H-5'), 2.68 (dd, *J* = 15.0, 7.4 Hz, 1H, α-CH_2 of acyl chain), 2.63 (dd, *J* = 14.8, 6.2 Hz, 1H, α-CH_2 of acyl chain), 2.57 (dd, *J* = 16.0, 7.2 Hz, 1H, α-CH_2 of acyl chain), 2.51-2.41 (m, 3H, α-CH_2 of acyl chain), 2.36 (dd, *J* = 14.8, 7.2 Hz, 1H, α-CH_2 of acyl chain), 2.30-2.25 (m, 4H, α-CH_2 of acyl chain), 2.11 (dd, *J* = 14.8, 5.4 Hz, 1H, α-CH_2 of acyl chain), 1.61-1.41 (m, 12H, γ-CH_2 of acyl chain), 1.40-1.20 (m, 100H, CH_2 of acyl chain), 0.88 (m, 18H, CH_3 of acyl chain). ^{13}C NMR (125 MHz, $CDCl_3$); *δ*: 173.1, 172.2, 171.7, 171.0, 169.5, 138.63, 138.59, 138.5, 137.7, 136.9, 133.5, 129.0, 128.5, 128.38, 128.34, 128.27, 128.2, 127.9, 127.73, 127.68, 127.65, 127.59, 127.5, 126.1, 117.9, 101.4, 101.2, 96.3, 79.0, 76.5, 75.9, 75.8, 75.4, 74.6, 73.7, 71.3, 71.3, 71.20, 71.18, 70.9, 70.1, 68.6, 68.3, 67.9, 66.4, 55.2, 52.4, 41.4, 41.3, 39.8, 39.7, 39.6, 34.5,

34.4, 34.35, 34.28, 34.2, 33.8, 31.94, 31.89, 29.71, 29.68, 29.64, 29.62, 29.48, 29.4, 29.34, 29.30, 29.2, 25.4, 25.3, 25.24, 25.21, 25.0, 22.7, 14.1

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{128}H_{200}N_2O_{20}$ $[M+Na]^+$: 2108.4587, Found 2108.4575

Allyl 4-*O*-benzyl-6-*O*-[2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-6-*O*-trityl-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-(decanoyloxy)tetradecanoylamino)-2-deoxy-α-D-glucopyranoside (9)



9

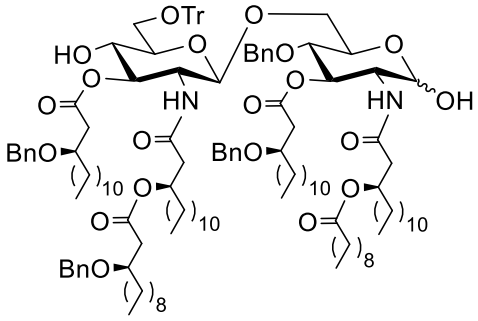
To a solution of **8** (20.0 mg, 9.58 μmol) in CH_2Cl_2 (1 mL) and water (0.1 mL) was added TFA (0.1 mL) and stirred at 0 °C for 20 min. The reaction mixture was co-evaporated with toluene. To the residue was added CH_2Cl_2 (1 mL), pyridine (7.7 μL, 95.8 μmol) and TrCl (2.94 mg, 10.5 μmol) and stirrerd at room temperature for 6 h. The reaction mixture was quenched with 10% aqueous citric acid and extracted with $CHCl_3$. The organic layer was washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) to give **9** as a white solid (14.6 mg, 83% for 2 steps).

^{1}H-NMR (500 MHz, $CDCl_3$); *δ*: 7.36 (d, *J* = 7.2 Hz, 6H, $C_6\underline{H_5}$-C), 7.31-7.03 (m, 33H, $C_6\underline{H_5}$-C, $C_6\underline{H_5}$-CH_2), 5.90 (d, *J* = 9.2 Hz, 1H, 2-NH), 5.86-5.77 (m, 1H, OCH_2-C<u>H</u>=CH_2 of allyl group), 5.76 (d, *J* = 8.6 Hz, 1H, 2-NH'), 5.31 (t, *J* = 10.0 Hz, 1H, H-3), 5,17 (dd, *J* = 17.2, 1.4 Hz, 1H, OCH_2-CH=C$\underline{H_2}$ of allyl group), 5.13 (d, *J* = 11.3 Hz, 1H, OCH_2-CH=C$\underline{H_2}$ of allyl group), 5.10-5.03 (m, 2H, H-3', β-CH of acyl chain), 5.00 (quin, *J* = 6.0 Hz, 1H, β-CH of acyl chain), 4.76 (d, *J* = 3.6 Hz, 1H, H-1), 4.59 (d, *J* = 8.3 Hz, 1H, H-1'), 4.57-4.41 (m, 8H, C_6H_5-C$\underline{H_2}$), 4.19 (dt, *J* = 10.6, 3.5 Hz, 1H, H-2), 4.09 (dd, *J* = 12.8, 5.2 Hz, 1H, OC$\underline{H_2}$-CH=CH_2 of allyl group), 4.04 (d, *J* = 9.2 Hz, 1H, H-6a), 3.89-3.76 (m, 6H, OC$\underline{H_2}$-CH=CH_2 of allyl group, H-2', H-5, β-CH of acyl chain), 3.65-3.40 (m, 3H, H-6b, H-4, H-4'), 3.48-3.43 (m, 1H, H-5'), 3.37-3.30 (m, 2H, H-6'), 2.77 (d, *J* = 2.8 Hz, 1H, 4-OH') 2.68-2.24 (m, 11H, α-CH_2 of acyl chain), 2.12 (dd, *J* = 14.9, 5.3 Hz, 1H, α-CH_2 of acyl chain), 1.63-1.42 (m, 12H, γ-CH_2 of acyl chain), 1.35-1.10 (m, 100H, CH_2 of acyl chain), 0.90-0.84 (m, 18H, CH_3 of acyl chain). ^{13}C NMR (125 MHz, $CDCl_3$); *δ*: 173.1, 172.2, 172.1, 171.6, 169.5, 169.4 (4×CO, 2×CONH of acyl chain), 143.6, 138.6, 138.2, 137.7 ($\underline{C_6}H_5$-C, $\underline{C_6}H_5$-CH_2), 133.4 (OCH_2-$\underline{C}$H=CH_2 of allyl group), 128.7,

128.41, 128.39, 128.3, 127.9, 127.80, 127.77, 127.73, 127.64, 127.58, 127.4, 127.2 ($\underline{C}_6H_5$-C, $\underline{C}_6H_5$-CH_2), 117.9 (OCH_2-CH=$\underline{C}H_2$ of allyl group), 100.6 (C-1'), 96.2 (C-1), 87.1 (C_6H_5-$\underline{C}$), 76.5 (β-CH of acyl chain), 76.1 (β-CH of acyl chain), 75.9 (C-4), 75.45 (β-CH of acyl chain), 75.39 (C-3'), 74.5 (C_6H_5-$\underline{C}H_2$), 74.3 (C-5'), 73.8 (C-3), 71.3 (C_6H_5-$\underline{C}H_2$), 71.19 (C_6H_5-$\underline{C}H_2$), 71.16 (C_6H_5-$\underline{C}H_2$), 71.0 (C-4'), 70.94 (β-CH of acyl chain), 70.89 (β-CH of acyl chain), 70.0 (C-5), 68.2 ($O\underline{C}H_2$-CH=CH_2 of allyl group), 67.3 (C-6), 64.2 (C-6'), 54.1 (C-2'), 52.4 (C-2), 41.4, 41.3, 39.8, 39.74, 39.70, 34.5 (6×α-CH_2 of acyl chain), 34.4, 34.3, 34.20, 34.17, 33.7, 31.94, 31.89, 29.76, 29.71, 29.69, 29.67, 29.64, 29.62, 29.49, 29.47, 29.39, 29.38, 29.35, 29.30, 29.2, 25.4, 25.3, 25.24, 25.20, 25.0, 22.70, 22.68 (CH_2 of acyl chain), 14.1 (CH_3 of acyl chain).

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{140}H_{210}N_2O_{20}$ $[M+H]^+$: 2240.5550, Found 2240.5535

4-*O*-Benzyl-6-*O*-[2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-6-*O*-trityl-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-(decanoyloxy)tetradecanoylamino)-2-deoxy-D-glucopyranose (10)



10

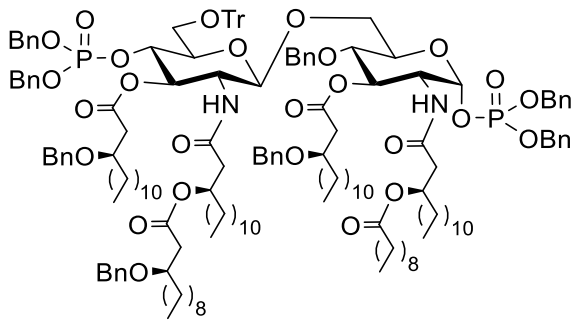
$[Ir(cod)(PMePh_2)_2]PF_6$ (0.74 mg, 0.90 μmol) was suspended in THF (0.5 mL) under argon atmosphere and the argon was replaced with elium to activate the Ir complex. After the Ir complex was activated when the color of the mixture was changed from red to yellow, filled again with argon. To a solution of **9** (10 mg, 4.49 μmol) in THF (0.5 mL) was added a solution of activated Ir complex in THF. After the mixture was stirred for 16 h, water (50 μL) and iodine (3.42 mg, 13.5 μmol) were added and the reaction mixture was stirred for 30 min. Excess of iodine was quenched with 10% aqueous $Na_2S_2O_3$ and then the mixture was extracted with $CHCl_3$. The organic layer was washed with 10% aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$) to give **10** as a white solid (11. 0mg, quant., α/β = 10/1).

α form

^{1}H NMR (500 MHz, ACETONE-D_6); δ: 7.40 (d, J = 7.6 Hz, 6H, $C_6\underline{H_5}$-C), 7.24-7.07 (m, 29H, $C_6\underline{H_5}$-C, $C_6\underline{H_5}$-CH_2), 6.97 (d, J = 8.7 Hz, 1H, 2'-NH), 6.53 (d, J = 9.3 Hz, 1H, 2-NH) , 5.29 (dd, J = 10.8, 9.4 Hz, 1H, H-3), 5.10-5.03 (m, 3H, H-3', β-CH of acyl chain), 5.03 (d, J = 3.6 Hz, 1H, H-1), 4.83 (d, J = 8.3 Hz, 1H, H-1'), 4.56 (d, J = 11.2 Hz, 1H, C_6H_5-C$\underline{H_2}$), 4.53 (d, J = 11.3 Hz, 1H, C_6H_5-C$\underline{H_2}$), 4.46 (d, J = 11.6 Hz, 2H, C_6H_5-C$\underline{H_2}$), 4.45 (d, J = 11.6 Hz, 1H, C_6H_5-C$\underline{H_2}$), 4.36 (d, J =11.6 Hz, 1H, C_6H_5-C$\underline{H_2}$), 4.35 (d, J = 11.6 Hz, 2H, C_6H_5-C$\underline{H_2}$), 4.07-3.97 (m, 3H, H-6a, H-2, H-5), 3.79-3.68 (m, 5H, β-CH of acyl chain, H-6a, H-2'), 3.60 (t, J = 9.6 Hz, 1H, H-4), 3.56 (t, J = 10.3 Hz, 1H, H-4'), 3.48-3.44 (m, 1H, H-5'), 3.31 (dd, J = 9.8, 1.8 Hz, 1H, H-6a'), 3.16 (dd, J = 9.9, 5.6 Hz, 1H, H-6b'), 2.56-2.48 (m, 3H, α-CH_2 of acyl chain), 2.42-2.26 (m, 6H, α-CH_2 of acyl chain), 2.18-2.13 (m, 3H, α-CH_2 of acyl chain), 1.50-1.24 (m, 12H, γ-CH_2 of acyl chain), 1.20-1.08 (m, 100H, CH_2 of acyl chain), 0.77-0.73 (m, 18H, CH_3 of acyl chain). ^{13}C NMR (125 MHz, ACETONE-D_6); δ: 173.2, 172.2, 172.1, 171.5, 170.1, 170.0, 145.2, 140.11, 140.08, 139.5, 129.6, 129.0, 128.94, 128.92, 128.7, 128.6, 128.49, 128.47, 128.4, 128.2, 128.1, 128.03, 128.01, 127.8, 101.5, 92.4, 87.1, 77.3, 76.8, 76.5, 76.4, 76.2, 75.1, 74.1, 71.63, 71.60, 71.56, 71.54, 71.50, 71.05, 70.1, 68.2, 64.3, 55.4, 53.5, 41. 6, 41.5, 40.4, 40.3, 40.2, 35.25, 35. 17, 34.9, 34.8, 32.68, 32.67, 32.66, 32.6, 30.52, 30.48, 30.46, 30.44, 30.41, 30.39, 30.37, 30.3, 30.21, 30.15, 30.11, 30.05, 30.03, 29.95, 29.9, 29.8, 29.6, 29.5, 29.3, 26.0, 25.9, 25.8, 23.34, 23.33, 14.4

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{137}H_{206}N_2O_{20}$ $[M+Na]^+$: 2222.5056, Found 2222.5033

4-*O*-Benzyl-6-*O*-[2-((*R*)-3-((*R*)-3-benzyloxydodecanoyloxy)tetradecanoylamino)-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-4-*O*-bis(benzyloxy)phosphoryl-2-deoxy-6-*O*-trityl-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-(decanoyloxy)tetradecanoylamino)-1-*O*-bis(benzyloxy)phosphoryl-2-deoxy-α-D-glucopyranose (11)



11

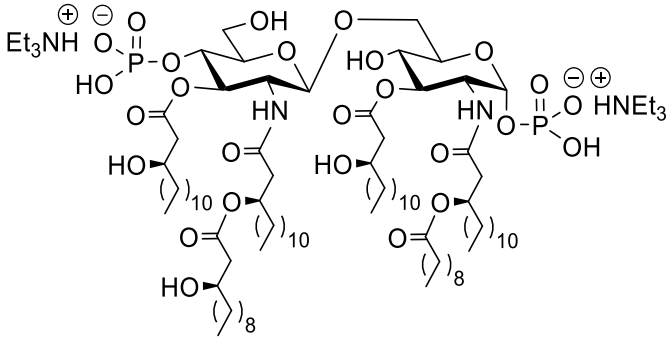
To a solution of **10** (6.0 mg, 2.73 μmol) in CH_2Cl_2 (1 mL) was added MS4A, 1*H*-tetrazole (1.3 mg, 19.1 μmol) and dibenzyl-*N,N*-diisopropylphosphoramidite (4.5 μL, 13.6 μmol) and stirred at room temperature for 16 h. To the reaction mixture was added DMDO (0.02 M in acetone) and stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and then the mixture was extracted with $CHCl_3$. The organic layer was

washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography ($CHCl_3$/acetone = 99/1) to give **11** as a white solid (5.9 mg, 80%).

^{1}H NMR (500 MHz, $CDCl_3$); *δ*: 7.43-7.30 (d, *J* = 7.3 Hz, 6H, $C_6\underline{H_5}$-C), 7.30-7.05 (m, 49H, $C_6\underline{H_5}$-C, $C_6\underline{H_5}$-CH_2), 6.30 (d, *J* = 7.6 Hz, 1H, 2-NH'), 6.03 (d, *J* = 8.6 Hz, 1H, 2-NH), 5.72 (dd, *J* = 5.1, 3.4 Hz, 1H, H-1), 5.42 (dd, *J* = 10.2, 9.0 Hz, 1H), 5.27 (dd, *J* = 10.6, 9.0 Hz, 1H), 5.20 (d, *J* = 8.3 Hz, 1H, H-1), 5.10 (quin, *J* = 6.2 Hz, 1H, β-CH of acyl chain), 5.07-4.90 (m, 5H, β-CH of acyl chain, C_6H_5-$C\underline{H_2}$), 4.69-4.35 (m, 13H, H-4', C_6H_5-$C\underline{H_2}$), 4.28-4.22 (m, 1H, H-2), 4.04 (d, *J* = 11.0 Hz, 1H, H-6a), 4.00 (dd, 10.5, 4.8 Hz, 1H, H-5), 3.87-3.78(m, 3H, β-CH of acyl chain, H-6b), 3.74(quin, *J* = 5.9 Hz, 1H, β-CH of acyl chain), 3.67-3.61(m, 1H, H-2'), 3.58 (t, *J* = 9.6 Hz, 1H, H-4), 3.48-3.42 (m, 2H, H-6'a, H-5'), 3.40-3.34 (m, 1H, H-6'b), 2.72-2.95 (m, 12H, α-CH_2 of acyl chain), 1.65-1.42 (m, 10H, γ-CH_2 of acyl chain), 1.40-1.20 (m, 100H, CH_2 of acyl chain), 0.88 (m, 18H, CH_3 of acyl chain).

HRMS (ESI-LIT-orbitrap MS, positive) Calcd. for $C_{165}H_{232}N_2O_{26}P_2$ $[M+Na]^+$: 2742.6261, Found 2742.6312

2-Deoxy-6-*O*-[2-deoxy-2-((*R*)-3-((*R*)-3-hydroxydodecanoyloxy)tetradecanoylamino)-3-*O*-((*R*)-3-hydroxytetradecanoyl)-β-D-glucopyranosyl]-2-((*R*)-3-(decanoyloxy)tetradecanoylamino)-3-*O*-((*R*)-3-hydroxytetradecanoyl)-α-D-glucopyranose 1,4'-Bisphosphate (Hexa-AfLA)



Hexa-AfLA

To a solution of **11** (7.0 mg, 2.6 μmol) in dist. THF (2.0 mL), water (200 μL) and acetic acid (100 μL) was added $Pd(OH)_2$/C (18.0 mg). The mixture was stirred under 2.0 MPa of H_2 at room temperature for 15 h. The mixture was neutralized with Et_3N (300 μL) and Pd catalyst was removed by filtration. After removal of the solvent *in vacuo*, the residue was purified by gel filtration chromatography to give **Hexa-AfLA** as a white solid (4.0 mg, 80%).

^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD = 1/1); *δ*: 5.31 (dd, *J* = 6.7, 3.4 Hz, 1H, H-1), 5.22-5.13 (m, 4H, β-CH of acyl chain, H-3, H-3'), 4.57 (d, *J* = 8.4 Hz, 1H, H-1'), 4.12 (q, *J* =10.0 Hz, 1H, H-4'), 4.04 (dt, *J* = 10.8, 2.8 Hz, 1H, H-2), 4.03-3.85 (m, 7H, β-CH of acyl chain, H-5, H-

2', H-6', H-6), 3.73 (dd, *J* = 11.9, 4.9 Hz, 1H, H-6b), 3.66 (d, *J* = 12.2 Hz, 1H, H-6b'), 3.40 (t, *J* = 9.7 Hz, 1H, H-4'), 3.30 (m, 1H, H-5'), 3.05 (q, *J* = 6.9 Hz, 12H, CH_2 of Et_3N), 2.50-2.30 (m, 10H, α-CH_2 of acyl chain), 2.20 (t, *J* = 7.3 Hz, 2H, α-C$\underline{H_2}$ of acyl chain), 1.65-1.15 (m, 128H, CH_2 of acyl chain, CH_3 of Et_3N), 0.89 (m, 18H, CH_3 of acyl chain), ^{13}C NMR (175 MHz, $CDCl_3/CD_3OD$ = 1/1); δ: 173.3, 172.1, 172.0, 171.7, 171.4, 170.9 (4×CO, 2×CONH of acyl chain), 101.6 (C-1'), 93.1 (C-1), 75.1 (C-5'), 73.2 (C-3), 72.8 (C-3'), 71.5 (C-5), 70.8 (β-CH of acyl chain), 70.4 (β-CH of acyl chain), 69.8 (C-4'), 68.3 (C-6), 68.0 (3×β-CH of acyl chain), 67.7 (C-4), 59.2 (C-6'), 52.9 (C-2'), 51.0 (C-2), 46.0 (CH_2 of Et_3N), 41.7, 41.6, 41.3, 40.4, 39.9 (5×α-CH_2 of acyl chain), 36.8, 36.7, 36.5 (CH_2 of acyl chain), 33.8 (α-CH_2 of acyl chain), 33.6, 33.52, 33.47, 33.0, 31.31, 31.28, 29.11, 29.09, 29.07, 29.04, 29.02, 28.94, 28.88, 28.82, 28.75, 28.73, 28.68, 28.5, 25.00, 24.99, 24.94, 24.88, 24.6, 24.4, 22.0 (CH_2 of acyl chain), 13.1 (CH_3 of acyl chain), 7.8 (CH_3 of Et_3N), ^{31}P NMR (243 MHz, $CDCl_3/CD_3OD$ = 1/1); δ: 1.69, -0.36

HRMS (ESI-LIT-orbitrap MS, negative) Calcd. for $C_{90}H_{170}N_2O_{26}P_2$ $[M-2H]^{2-}$: 877.5686, Found 877.5669

Immunological assays

Reagents

HEK-Blue hTLR4™ cells and HEK-Blue™ Null2 cells were purchased from Invivogen (San Diego, CA, USA). THP-1 cells were purchased from JCRB Cell Bank (Ibaraki, Osaka, Japan). Cell culture medium, antibiotic/antimycotic supplement and *E. coli* LPS (ultracentrifugation) were purchased from Wako Pure Chemical Co. (Osaka, Japan), and fetal bovine serum (FBS) from Gibco (Grand Island, NY, USA). Phorbol 12-myristate 13-acetate (PMA) was purchased from Focus Biomolecules (Plymouth Meeting, PA, USA).

Cell Culture

HEK-Blue hTLR4™ cells and HEK-Blue™ Null2 cells were cultured in Dulbecco's modified Eagle's medium (D-MEM) high-glucose medium supplemented with FBS (10%), L-glutamine (2 mM), penicillin (100 units/mL) and streptomycin (100 µg/mL). THP-1 cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with FBS (10%), L-glutamine (2 mM), penicillin (100 units/mL) and streptomycin (100 µg/mL). All cells were incubated in a humidified 5% CO_2 atmosphere at 37 °C.

Mice

Female Balb/c mice (age, 7 weeks) were purchased from Japan CLEA (Tokyo, Japan) and kept for one week before the experiments. According to the guideline, murine condition was checked at least once per day and mice were killed if their body weight reached to more than 20% reduction. All experiments were conducted in accordance with the guidelines of the Animal Care and Use Committee of National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN) and the Committee on the Ethics of Animal Experiments of NIBIOHN (approval no. DSR01-2R1).

SEAP Reporter Assay

HEK-Blue™ hTLR4 cells and HEK-Blue™ Null2 cells were seeded in a 96-well plate at a density of 2×10^4 cells/well and serum-starved for 20 h in a humidified 5% CO_2 atmosphere at 37 °C. After incubation, culture medium was removed from 96-well plate and cells were washed with saline. To each well was added D-MEM high-glucose medium (100 µL) supplemented with FBS (0.1%), L-glutamine (2 mM), penicillin (100 units/mL) and streptomycin (100 µg/mL) and treated with the appropriate concentration of test samples (dissolved in 25 µL of 5% DMSO in saline). After 20 h incubation (37 °C, 5% CO_2 atmosphere), supernatants were collected. 50 µL of each sample supernatant was added to p-Nitrophenylphosphate solution in PBS (0.8 mM, 100 µL) and incubated at room temperature in the dark. After 4 h, ODs at 405 nm were measured with microplate reader (TECAN infinite F50). Data represent averages of three repeated assays with standard deviations from individual experiments.

Quantification of cytokines *in vitro* using enzyme-linked immunosorbent assay (ELISA)

Prior to stimulation, THP-1 cells were seeded in a 96-well plate at a density of 6×10^4 cells/well and differentiated for 72 h in the presence of PMA (0.5 μM solution in DMSO). Nonadherent cells and culture medium were removed from 96-well plate and cells were washed with saline. To each well was added RPMI-1640 medium (100 μL) supplemented with FBS (10%), L-glutamine (2 mM), penicillin (100 units/mL) and streptomycin (100 μg/mL), and treated with the appropriate concentration of test samples (dissolved in 25 μL of 5% DMSO in saline). After 20 h incubation (37 °C, 5% CO_2 atmosphere), supernatants were collected. The induced cytokine levels in the supernatants were measured using ELISA which was performed in 96-well plate. ELISA for human IL-1β, IL-6, IL-18, TNF-α were performed using commercial kits (Invitrogen, Carlsbad, CA, USA) and according to manufacturer's protocol. For competition activities of test samples against *E. coli* LPS, the test samples and additional *E. coli* LPS (1 ng/mL) in RPMI-1640 medium supplemented with FBS (10%), L-glutamine (2 mM), penicillin (100 units/mL) and streptomycin (100 μg/mL) were incubated and measured by a method similar to that used for the inducing activities. Data represent averages of three repeated assays with standard deviations from individual experiments.

Quantification of cytokines *in vivo* using BD™ Cytometric Bead Array (CBA)

Synthesized *A. faecalis* lipid A (AfLA) was dissolved in PBS with 0.5% DMSO. AfLA solution (0.5 μg/mL or 5.0 μg/mL) or PBS with 0.5% DMSO (mock) was injected subcutaneously at 200 μL/mouse. After 6 hours blood was drawn and the cytokine induction activity (IL-6) was evaluated by BD™ Cytometric Bead Array. Assays were conducted twice with four mice in each group (total n=8).

Figure S15.

Penta-acylated *A. faecalis* lipid A (**penta-AfLA**) and tetra-acylated *A. faecalis* lipid A (**tetra-AfLA**) mediated NF-κB activation in HEK-Blue™ hTLR4 cells was evaluated by SEAP reporter assay. Results represent the mean ± standard deviation (SD) of three independent experiments.

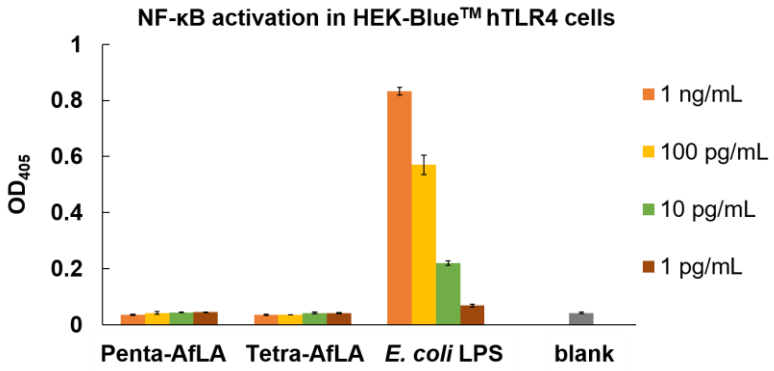


Figure S16.

Hexa-acylated *A. faecalis* lipid A (**hexa-AfLA**) mediated cytokine induction activity in PMA-differentiated THP-1 cells was evaluated by ELISA: (**a**) release of Tumor Necrosis Factor-α (TNF-α), (**b**) release of IL-1β, (**c**) release of IL-18. Results in (**a**)-(**c**) represent the mean ± standard deviation (SD) of three independent experiments. *p < 0.05, **p < 0.005 by Student's t test.

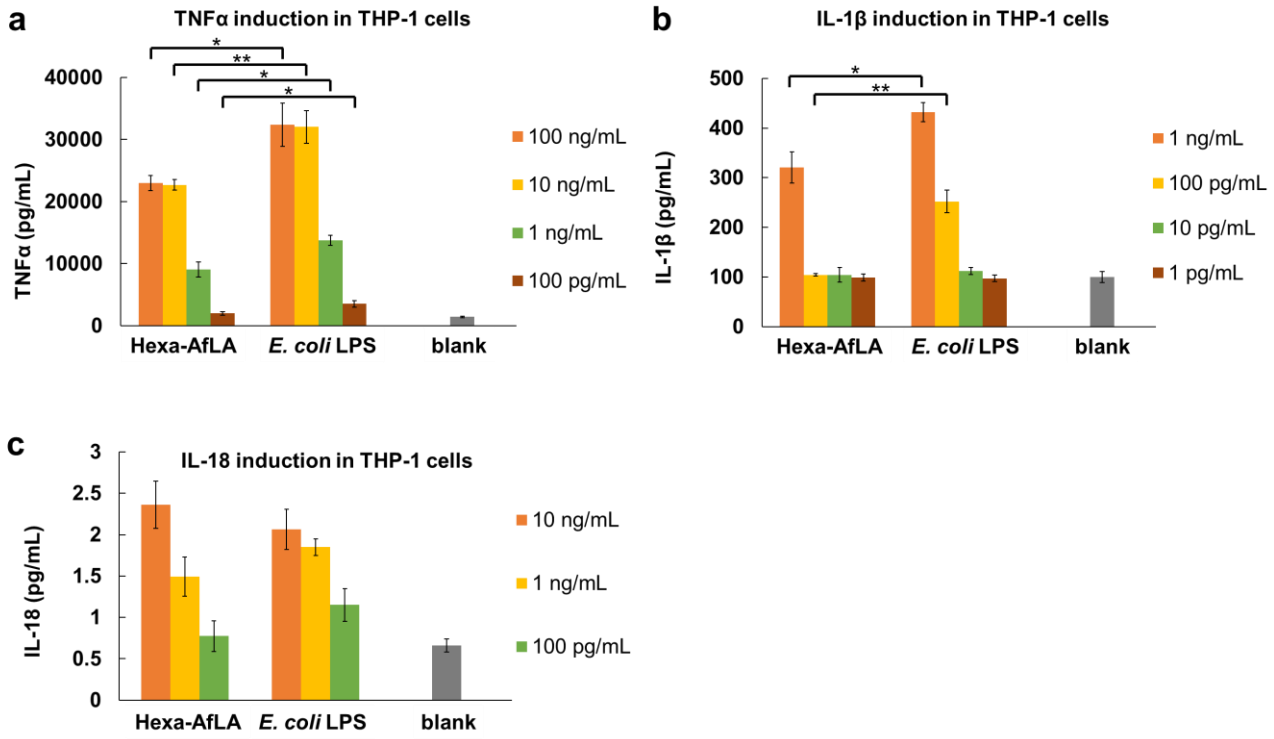
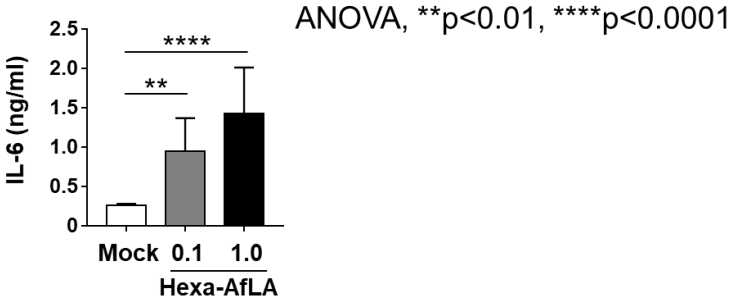


Figure S17.

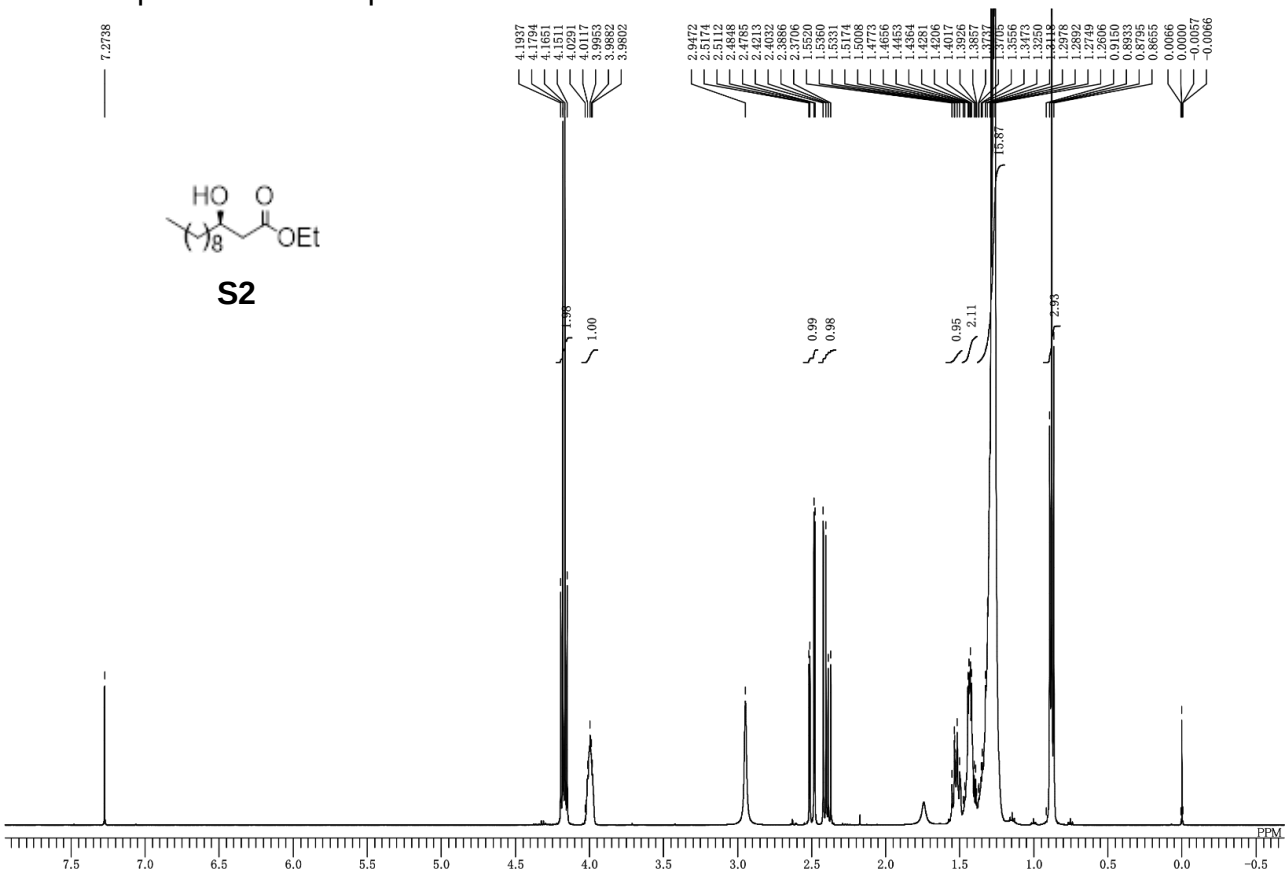
Hexa-acylated *A. faecalis* lipid A (**hexa-AfLA**) mediated cytokine induction activity in mice was evaluated by BD™ Cytometric Bead Array (CBA). Results represent the mean ± standard deviation (SD) of two independent experiments (n=8 per group).



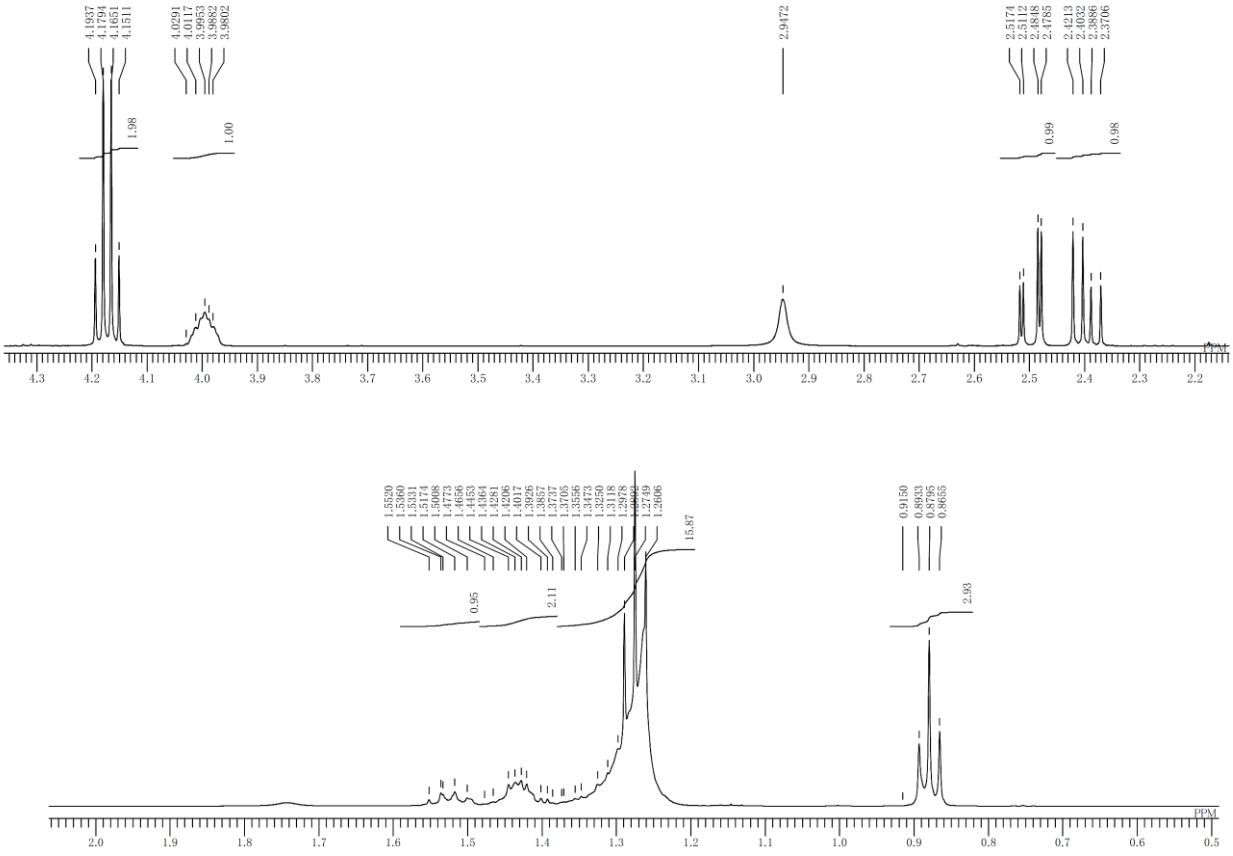
^{1}H and ^{13}C NMR spectra of synthesized compounds

Raw NMR data of synthesized compounds will be inserted.

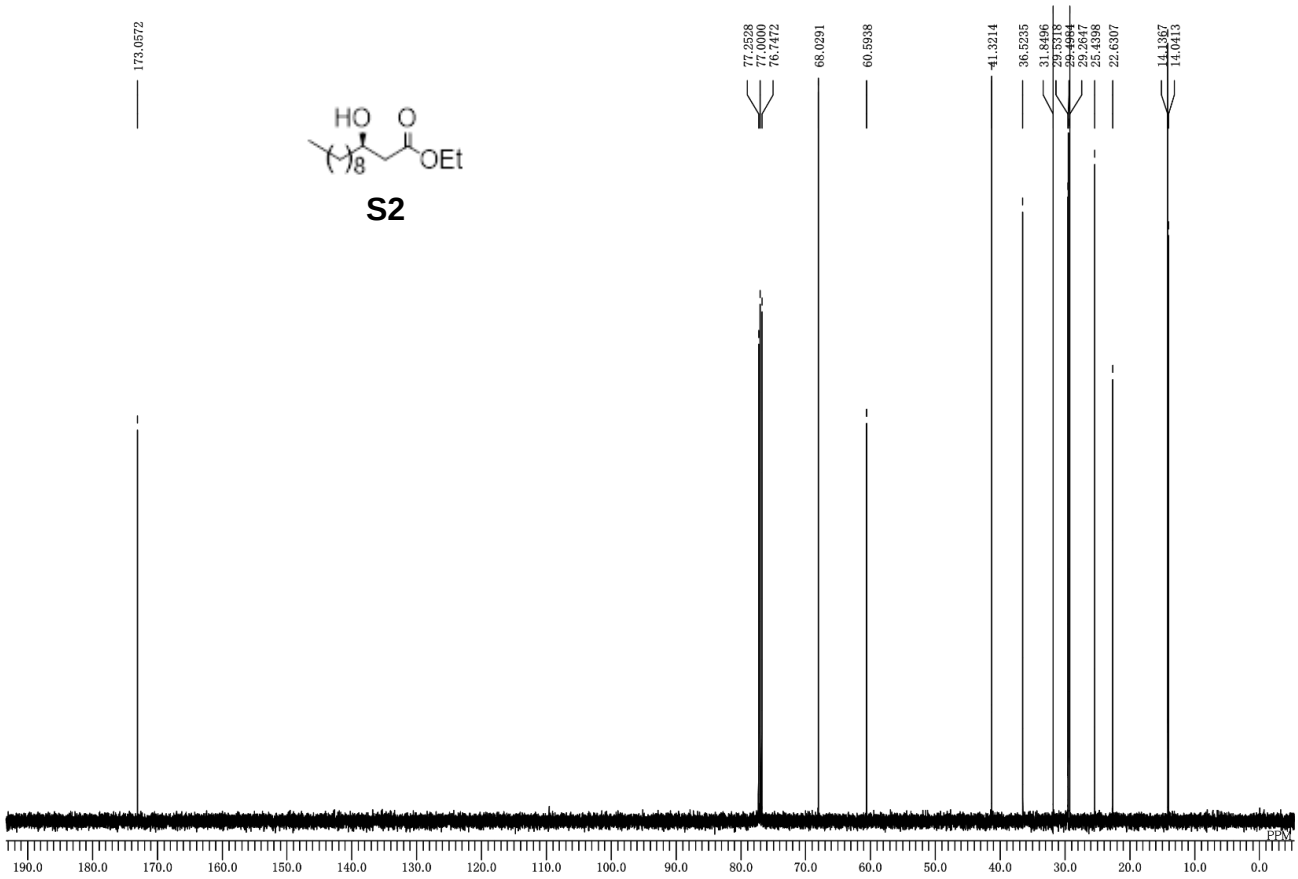
^{1}H NMR spectrum for compound **S2**



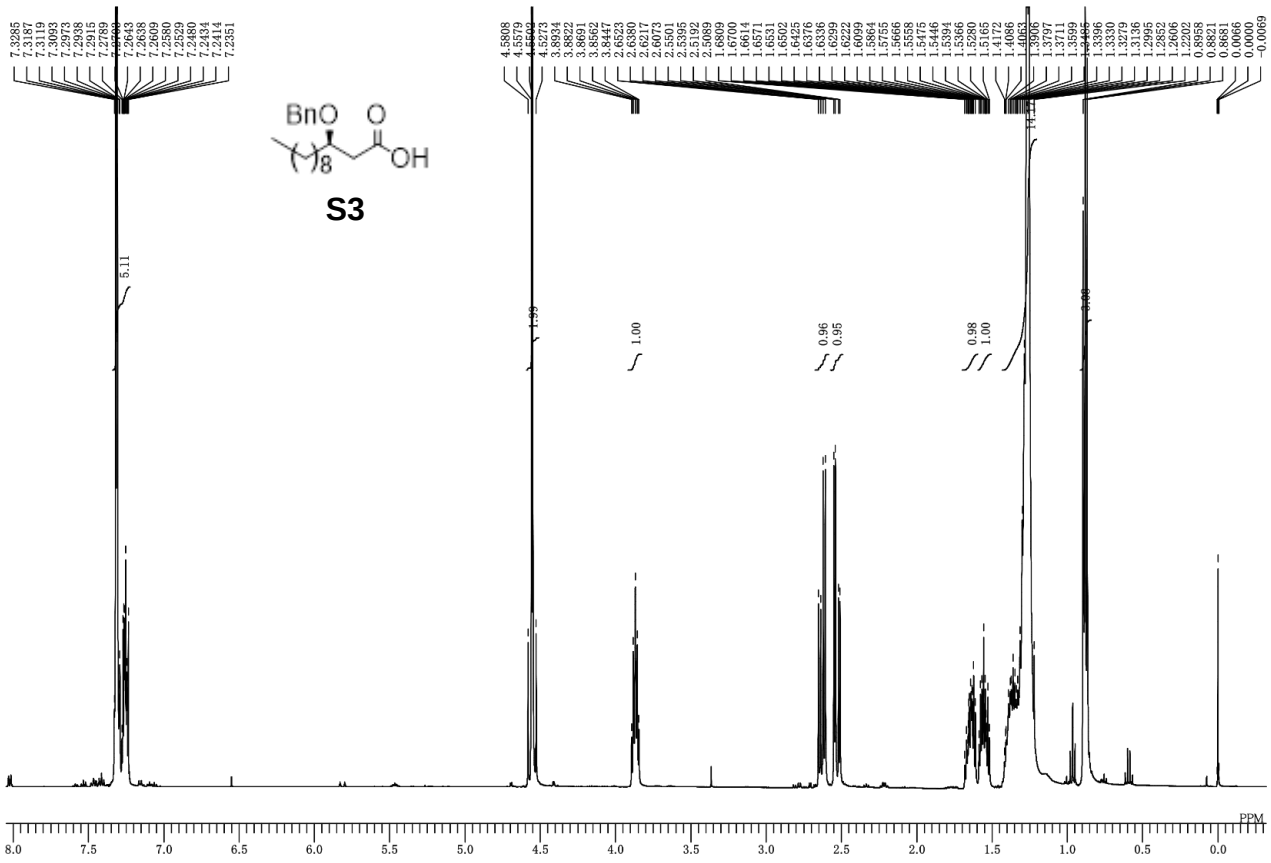
Enlarged view of ^{1}H NMR spectrum for compound **S2**



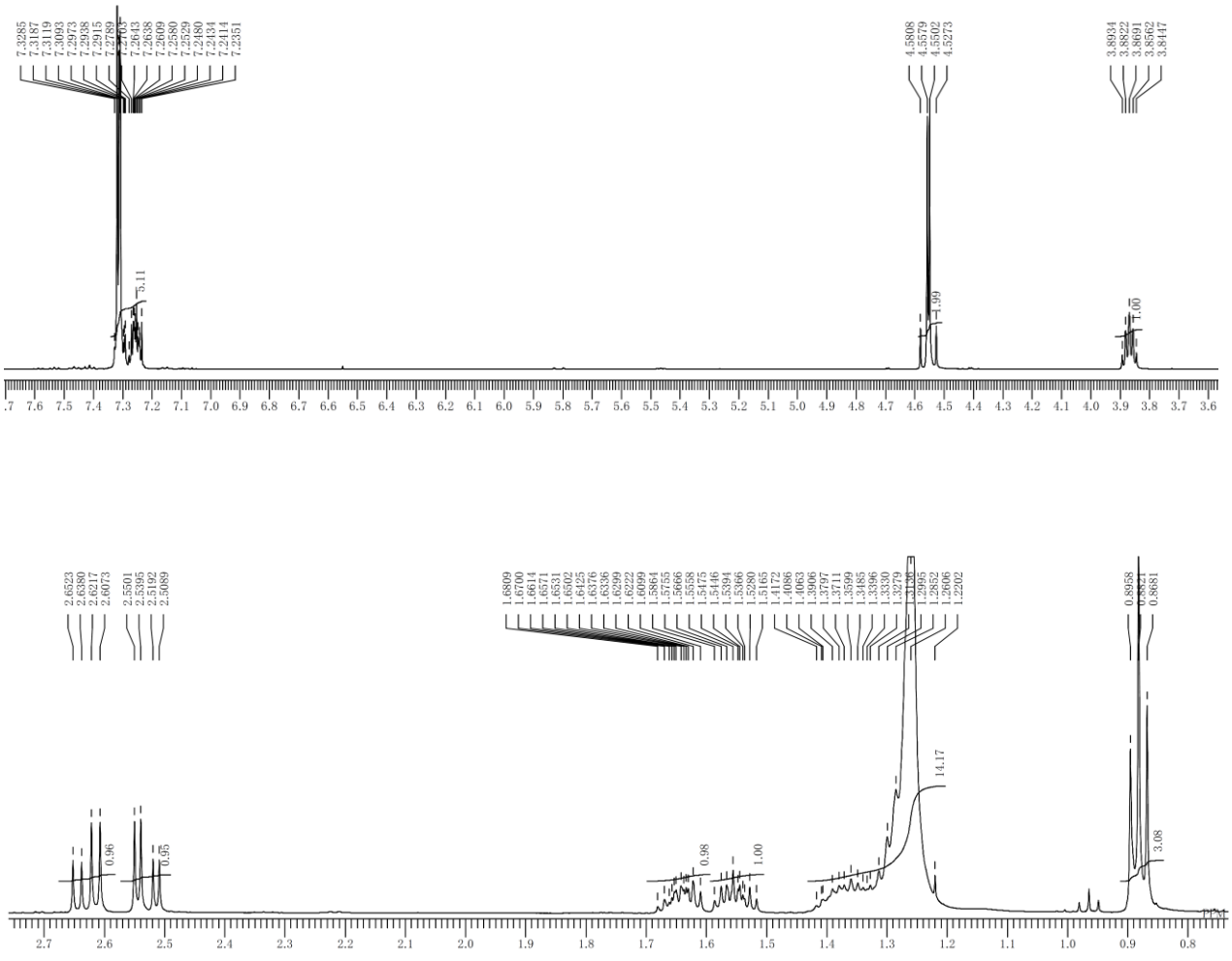
^{13}C NMR spectrum for compound **S2**



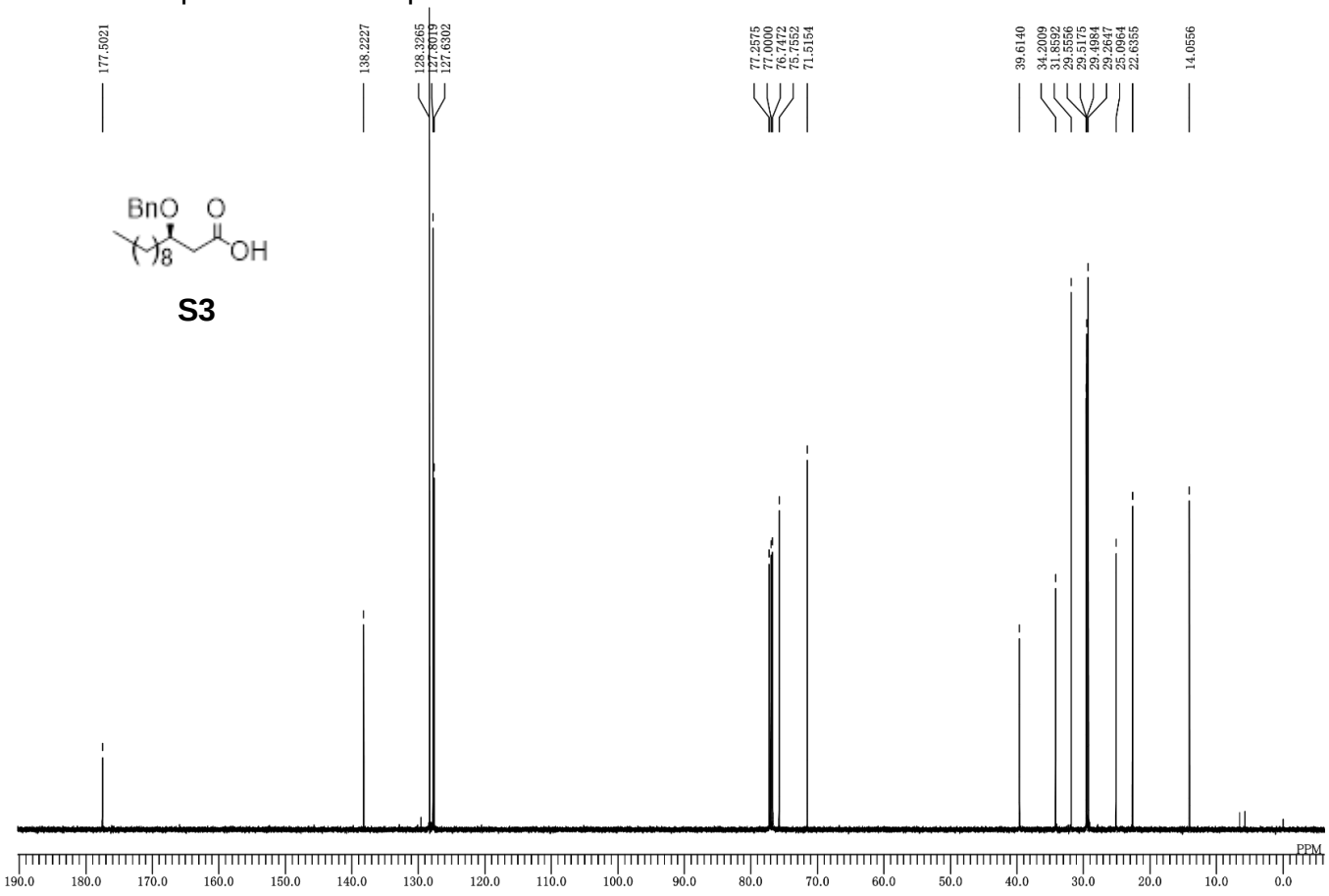
^{1}H NMR spectrum for compound **S3**



Enlarged view of ^{1}H NMR spectrum for compound **S3**

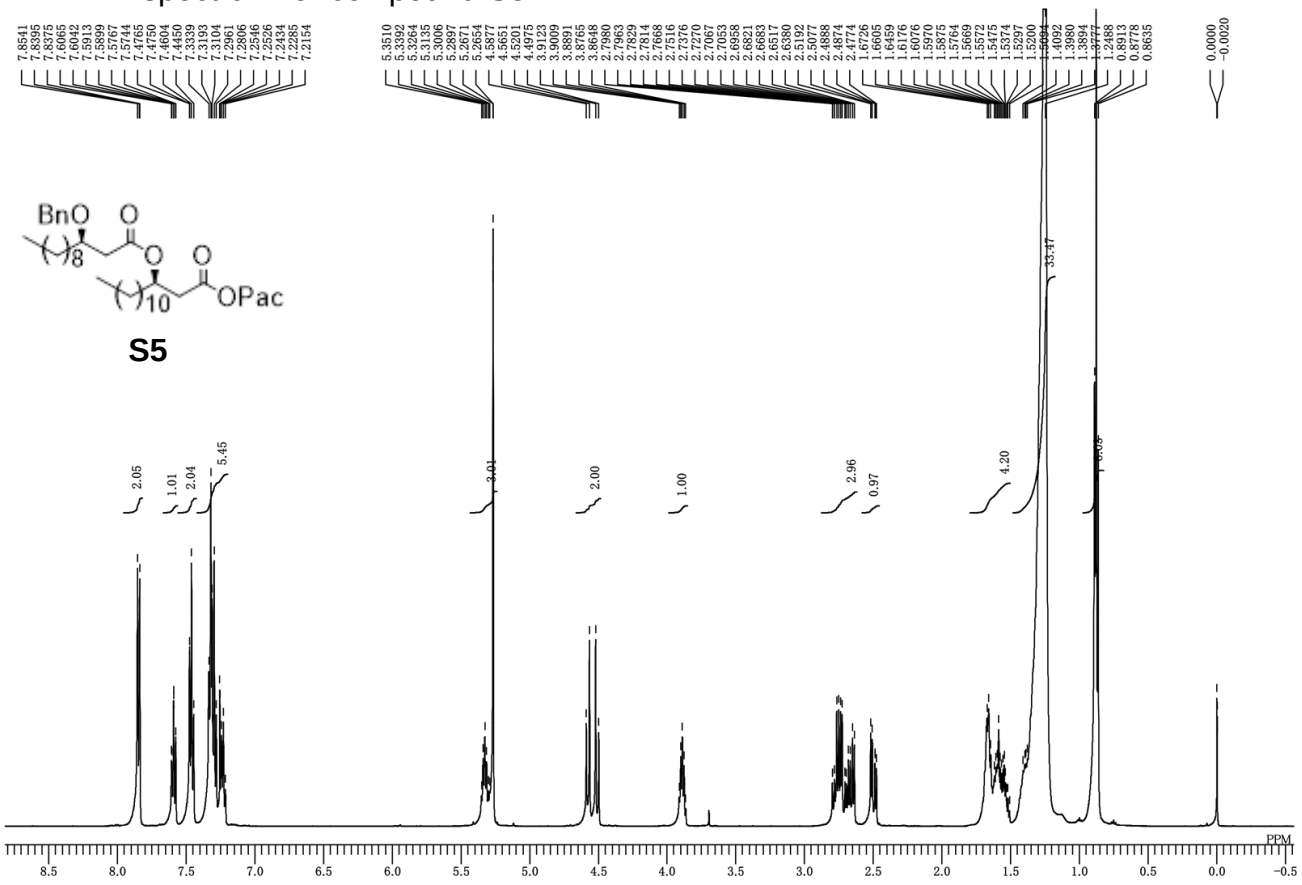


^{13}C NMR spectrum for compound **S3**

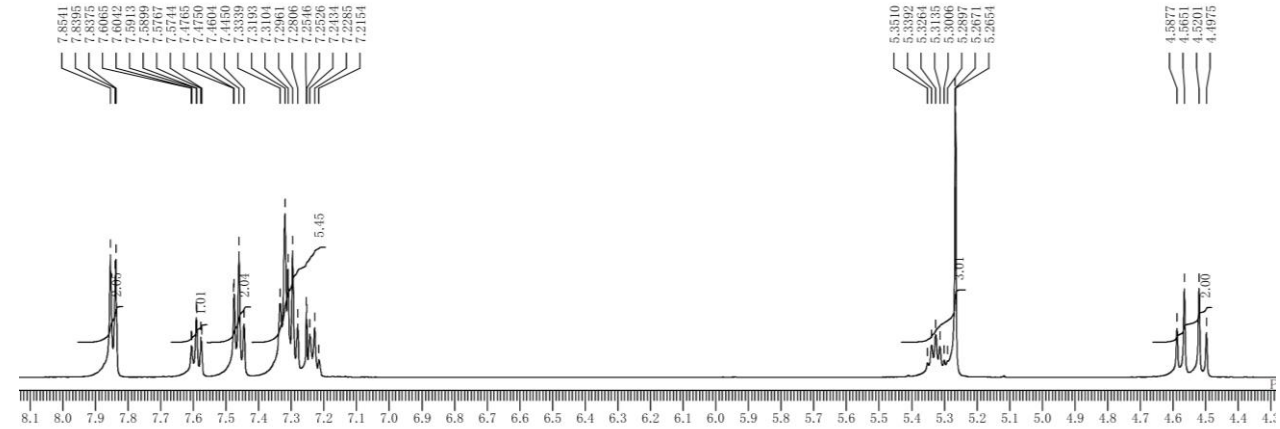


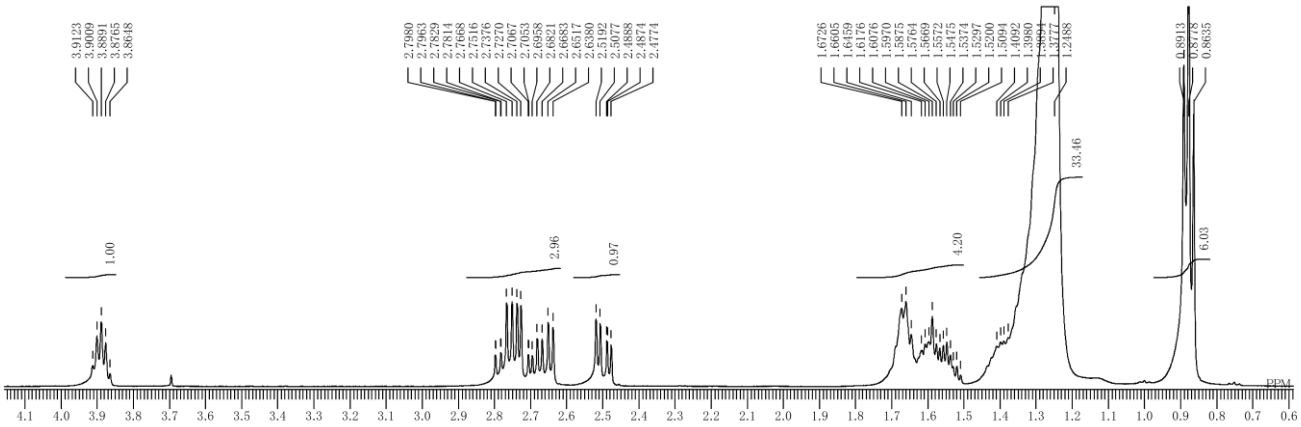
S3

^{1}H NMR spectrum for compound **S5**

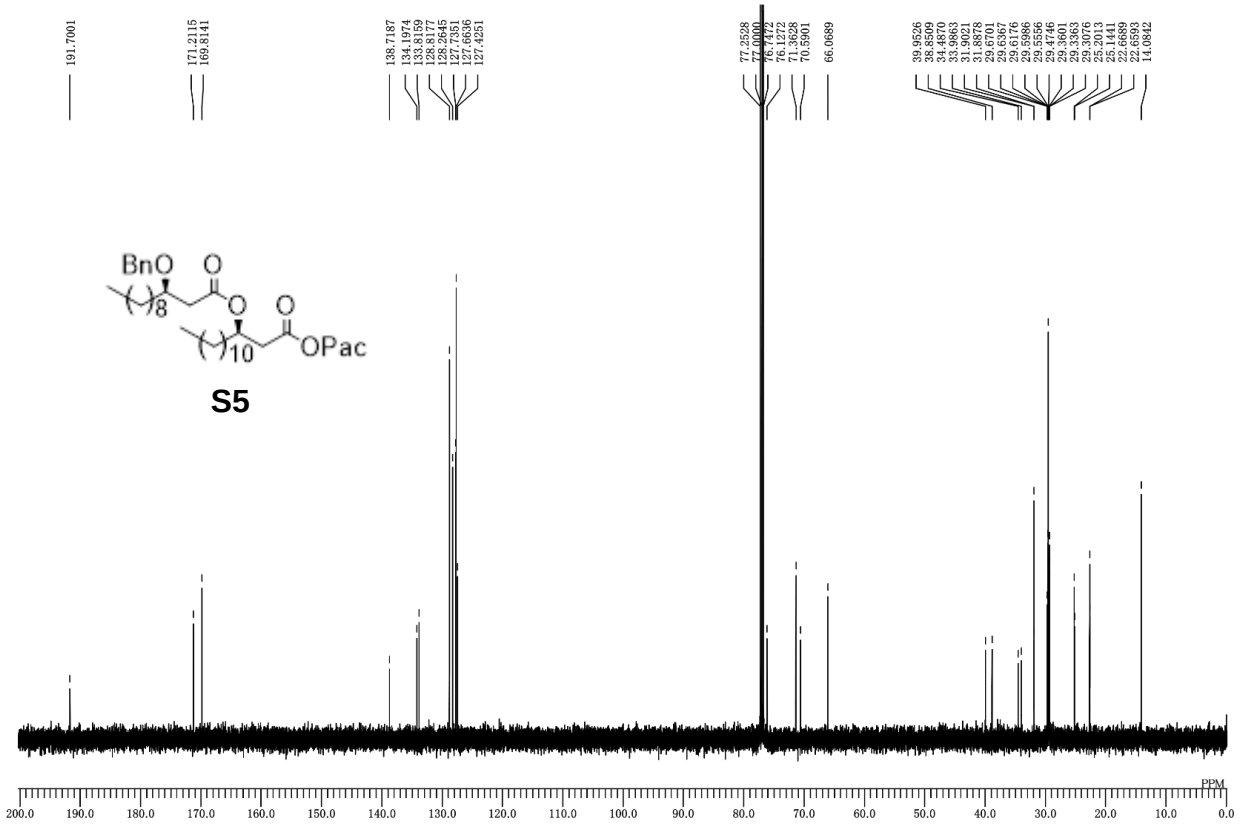


Enlarged view of ^{1}H NMR spectrum for compound **S5**

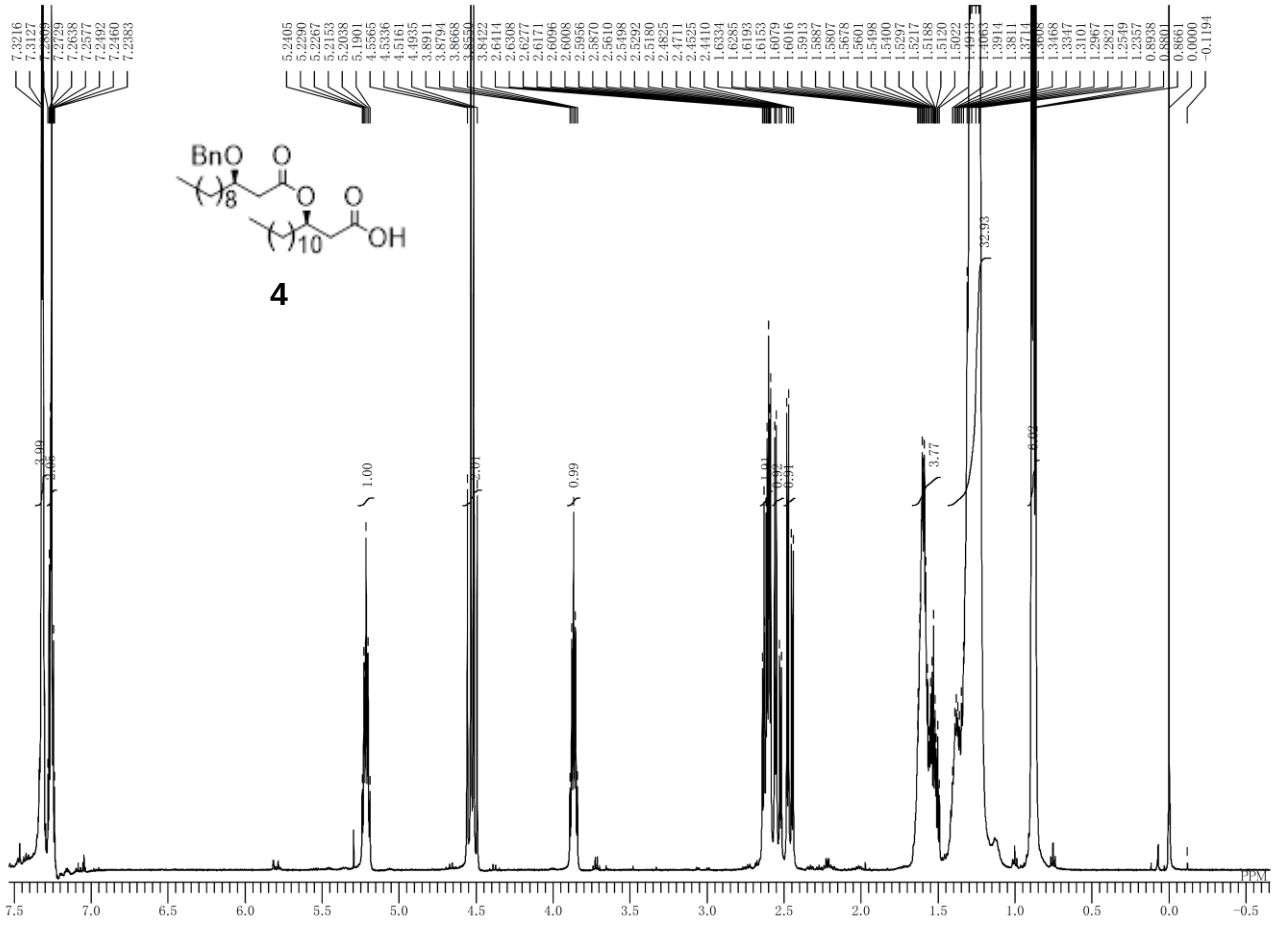




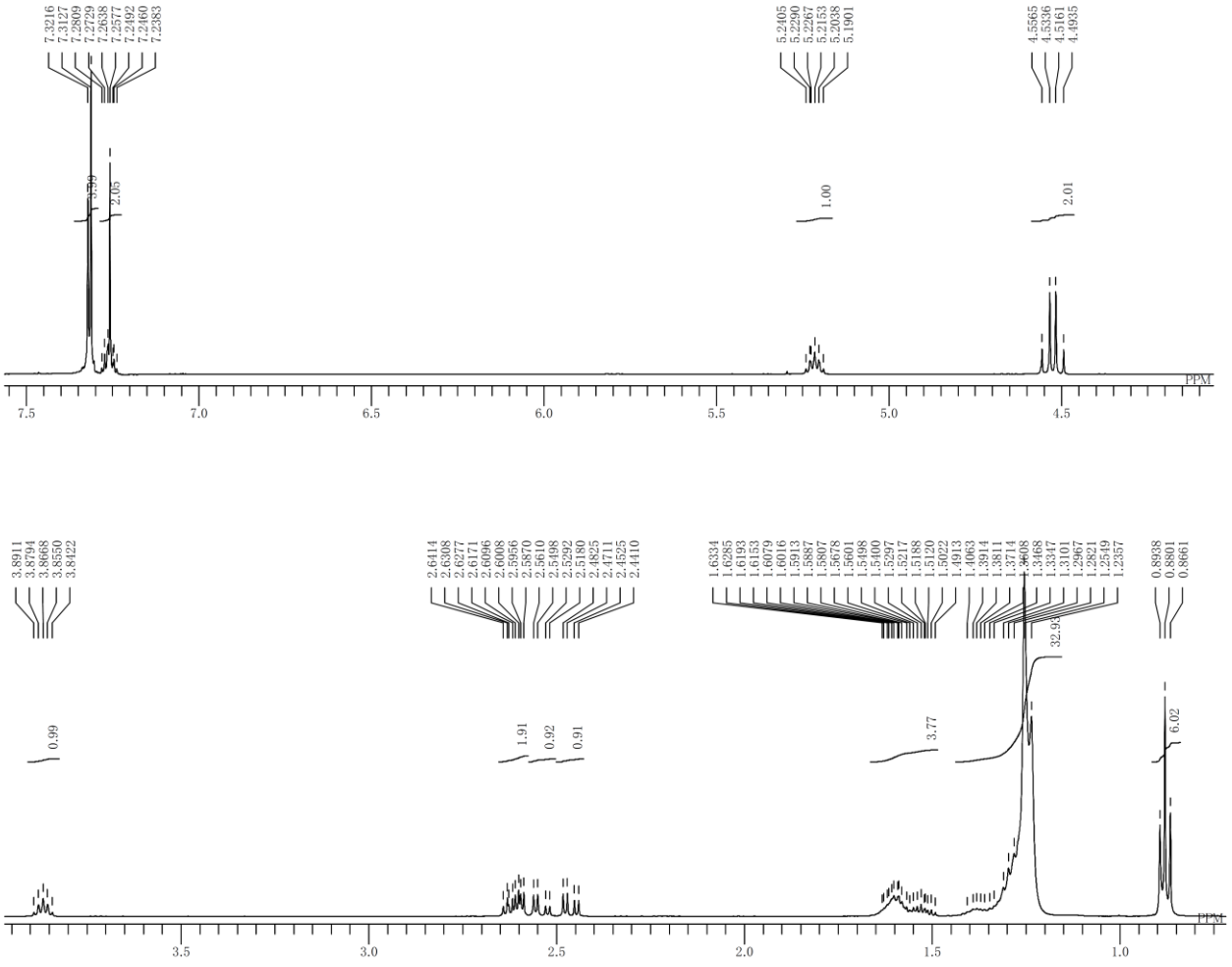
^{13}C NMR spectrum for compound **S5**



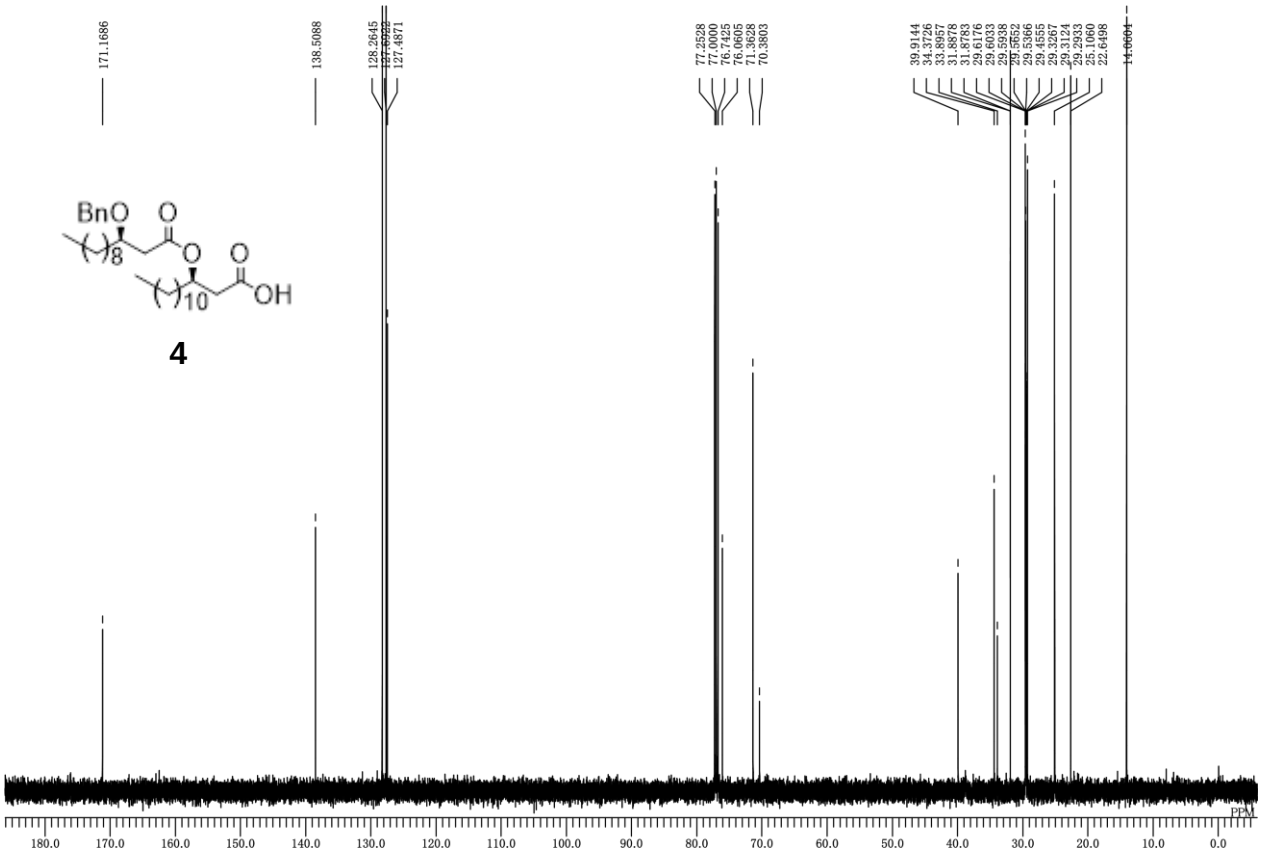
^{1}H NMR spectrum for compound **4**



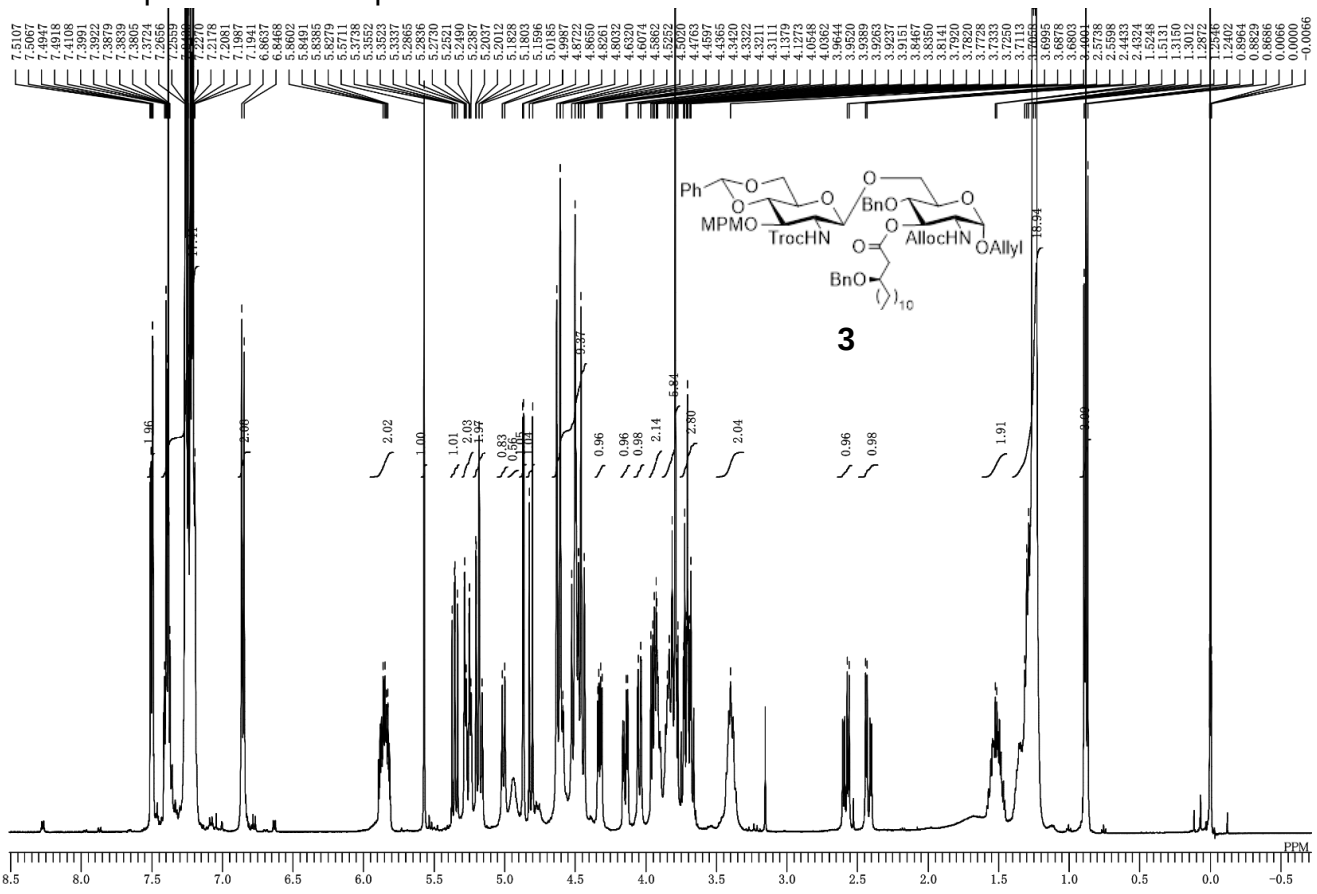
Enlarged view of ^{1}H NMR spectrum for compound **4**



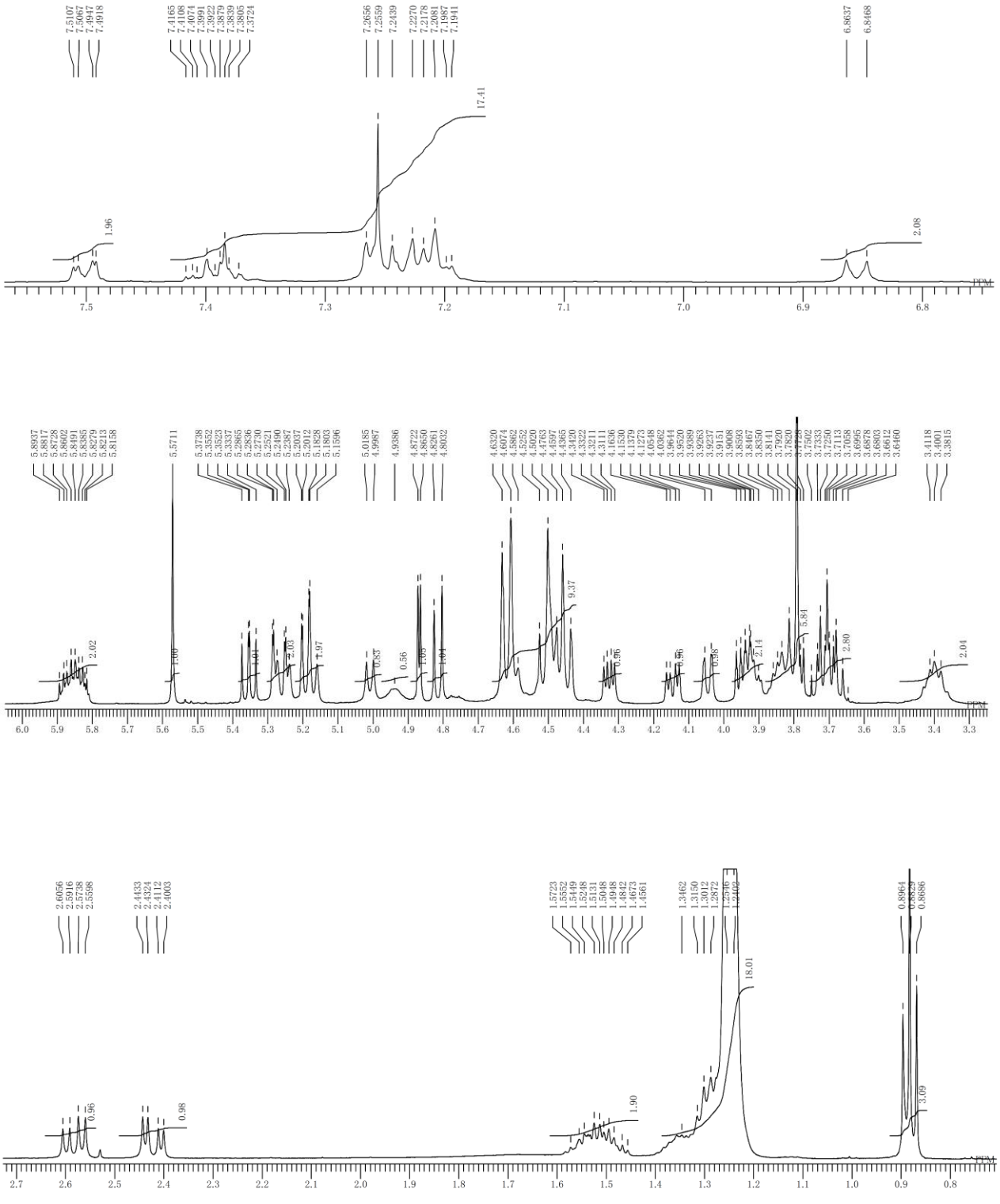
^{13}C NMR spectrum for compound **4**



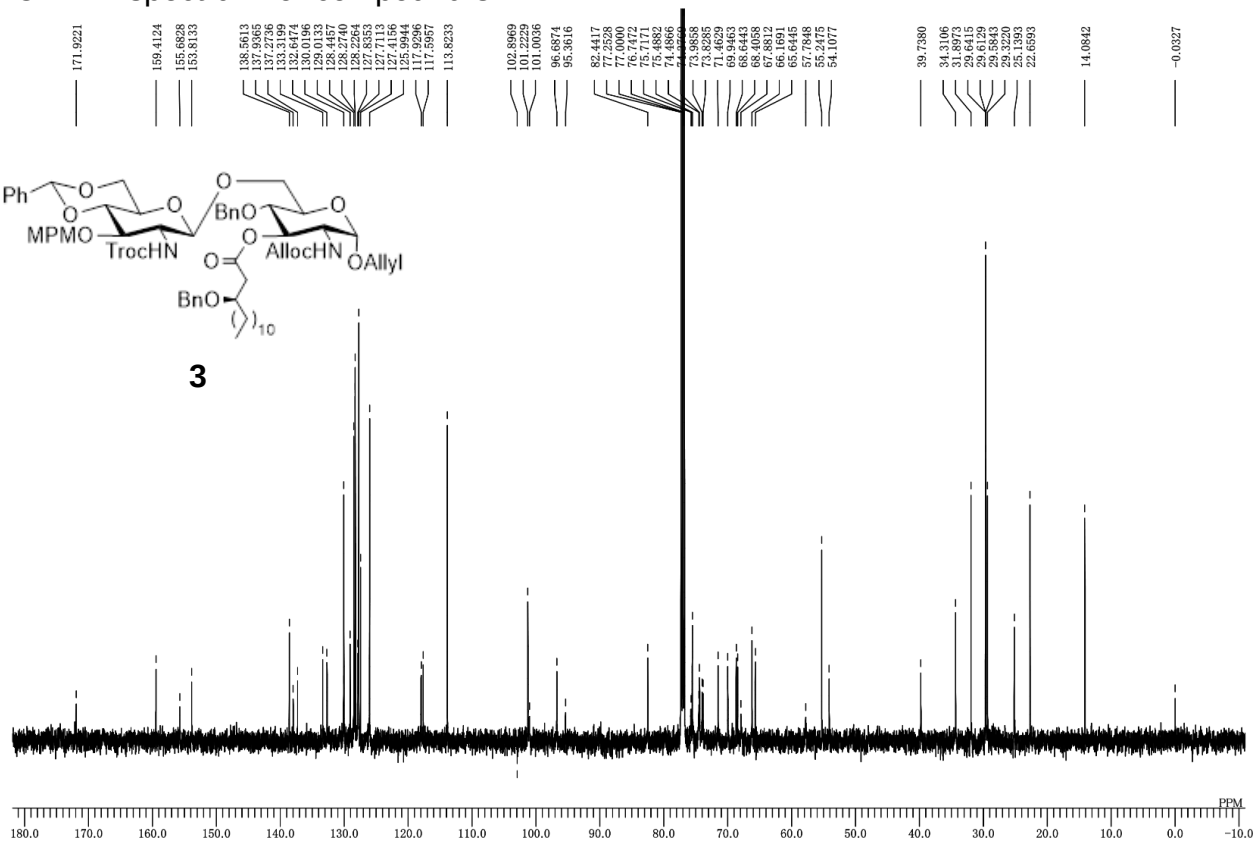
^{1}H NMR spectrum for compound **3**



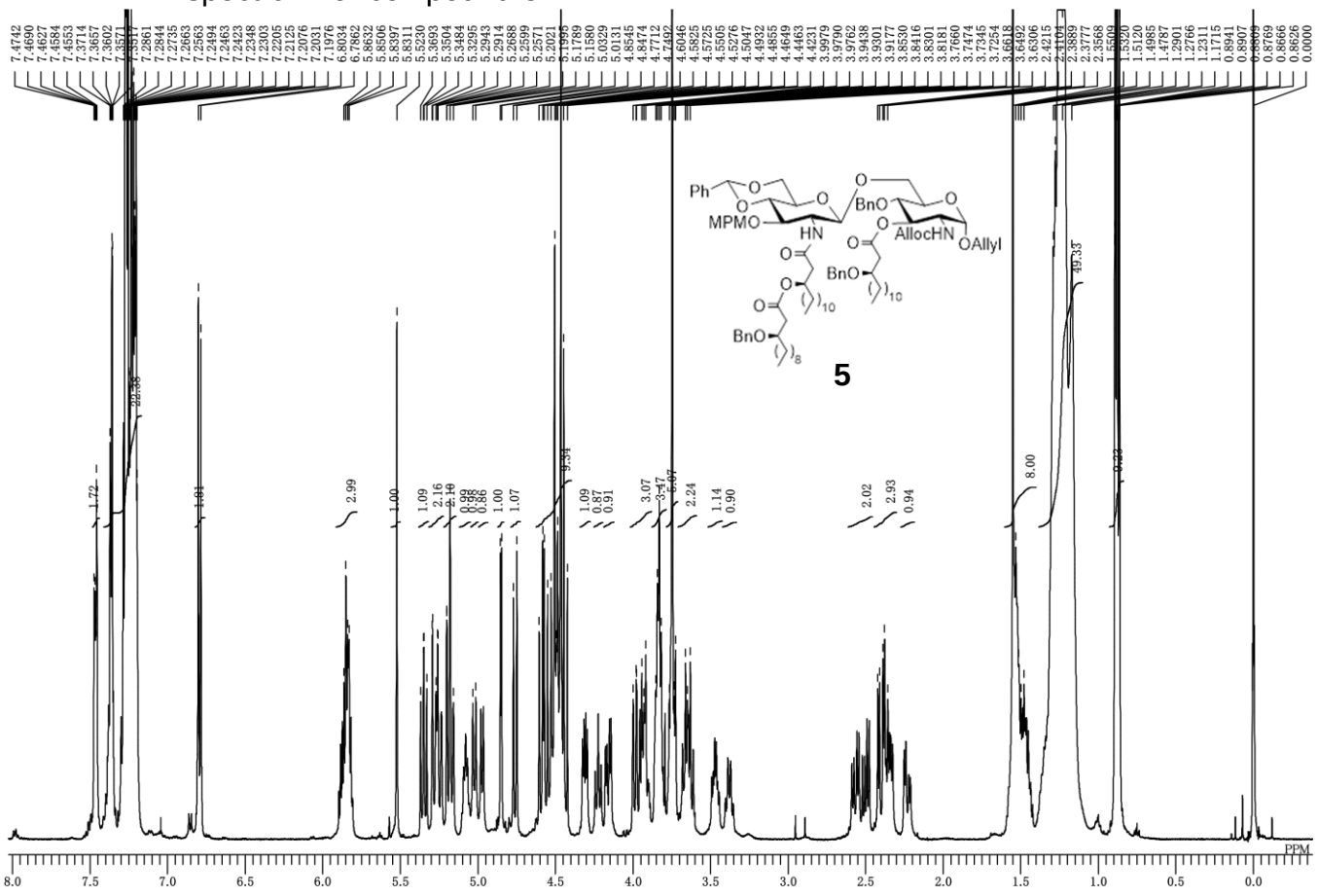
Enlarged view of ^{1}H NMR spectrum for compound **3**



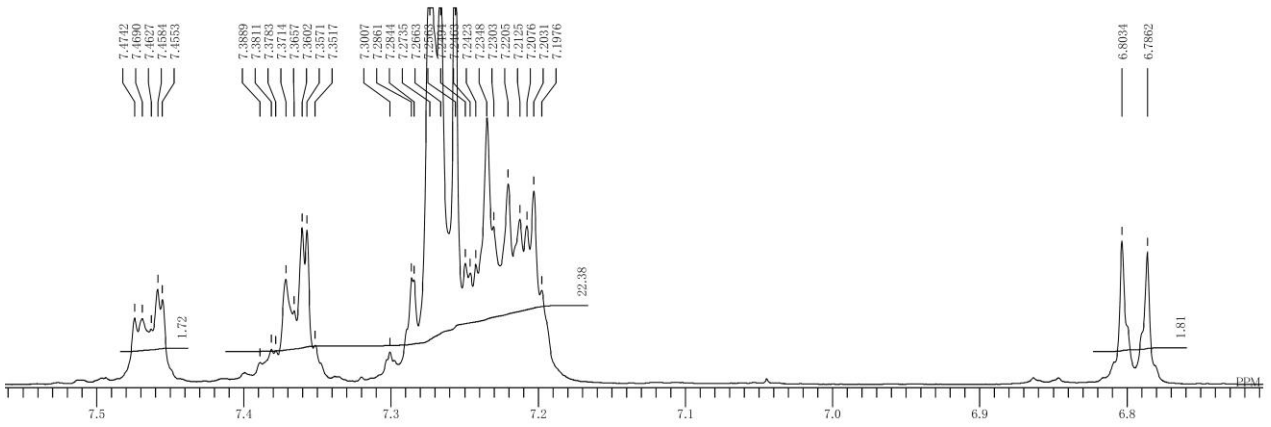
^{13}C NMR spectrum for compound **3**

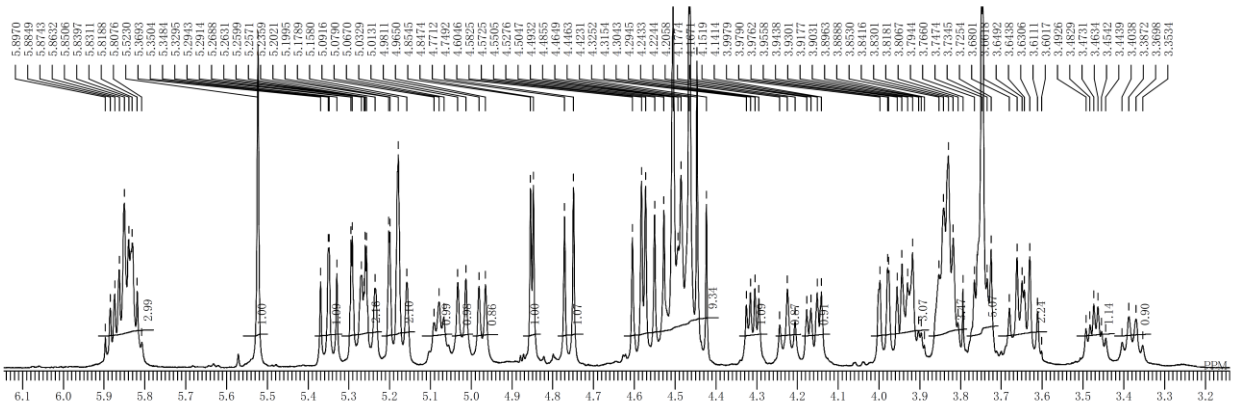


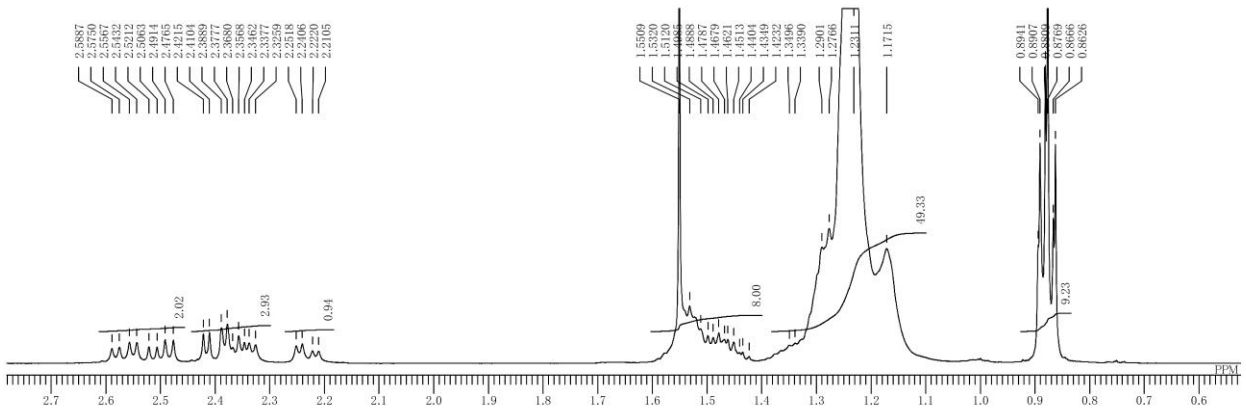
^{1}H NMR spectrum for compound **5**



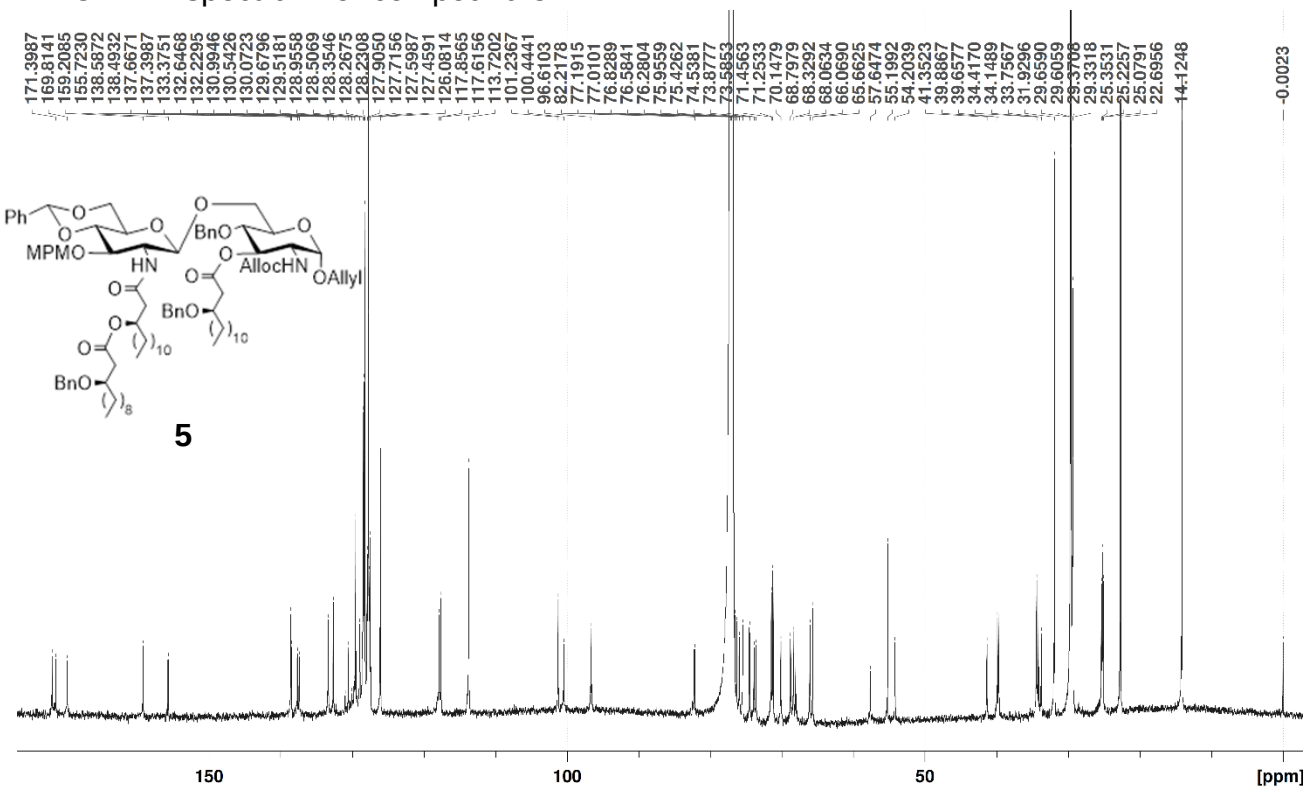
Enlarged view of ^{1}H NMR spectrum for compuond **5**





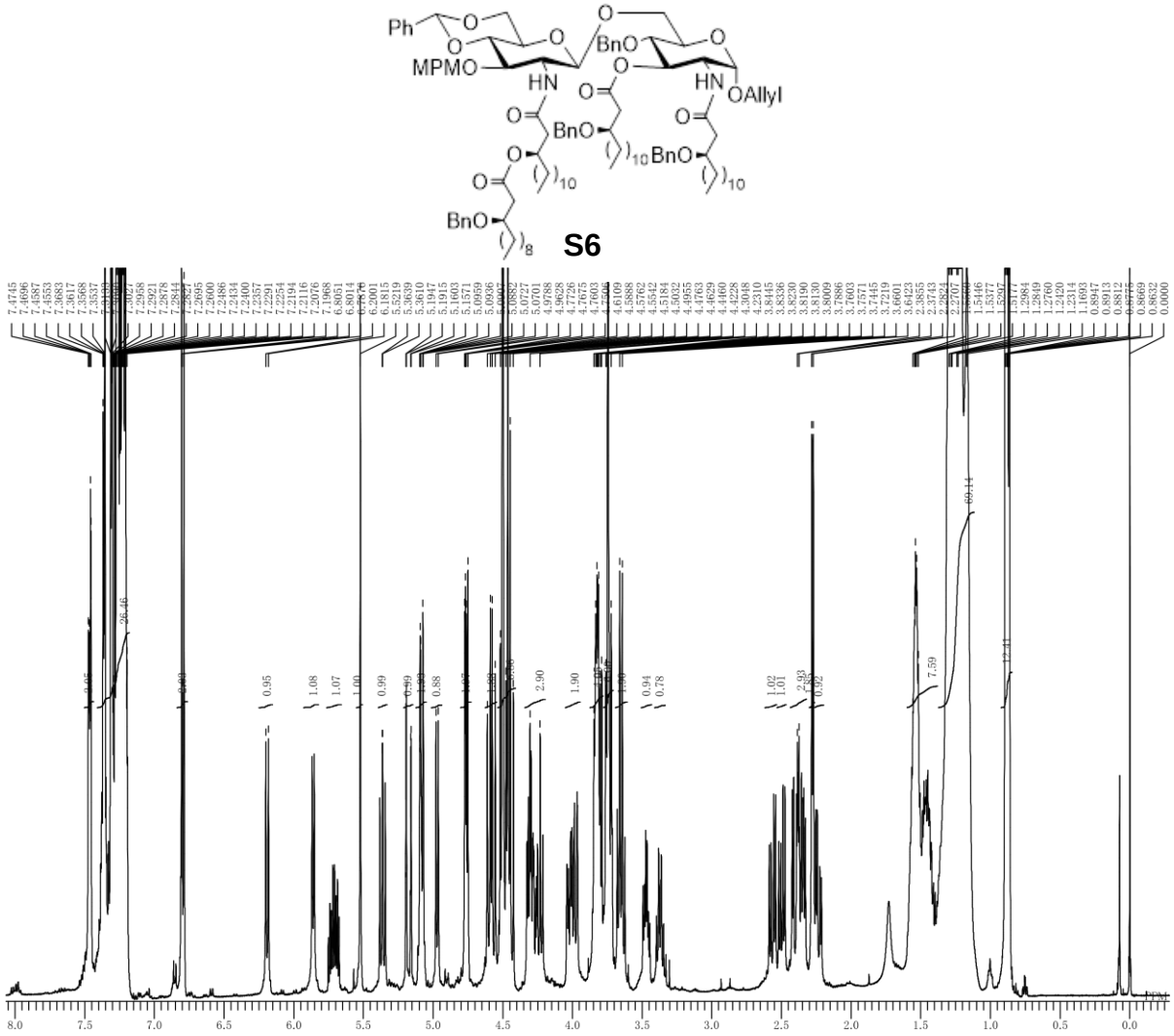


^{13}C NMR spectrum for compound **5**

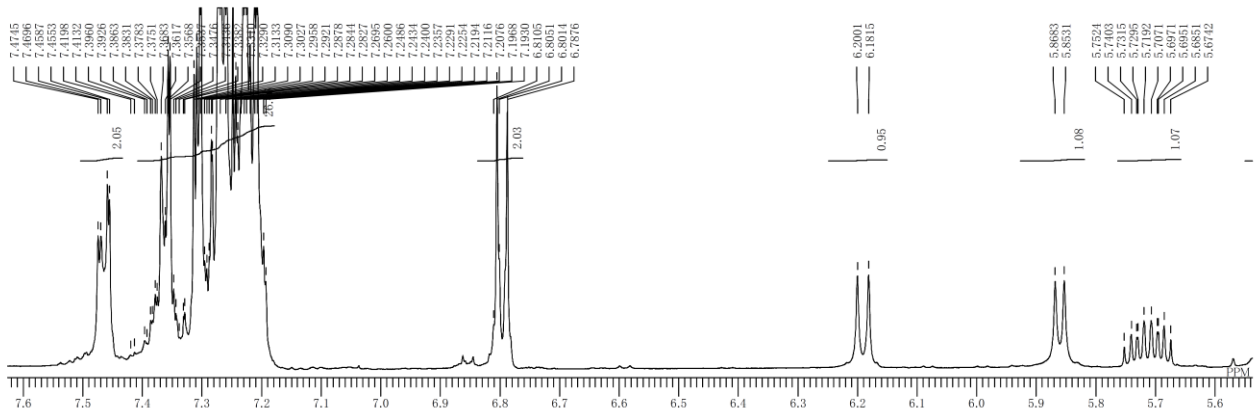


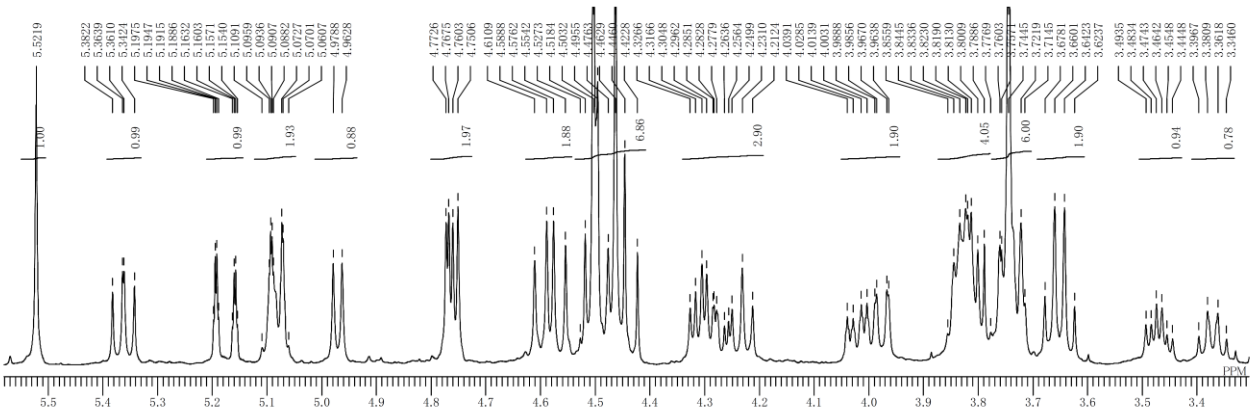
5

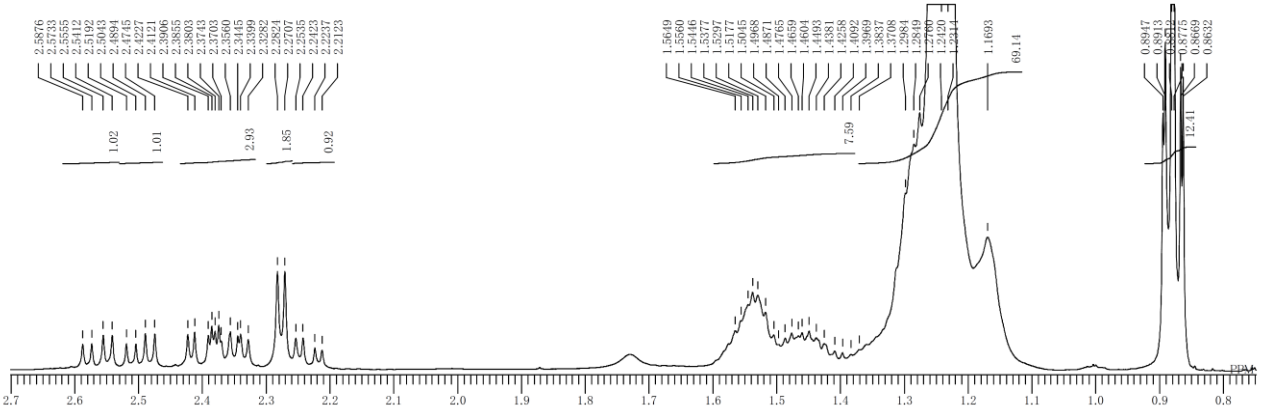
^{1}H NMR spectrum for compound **S6**



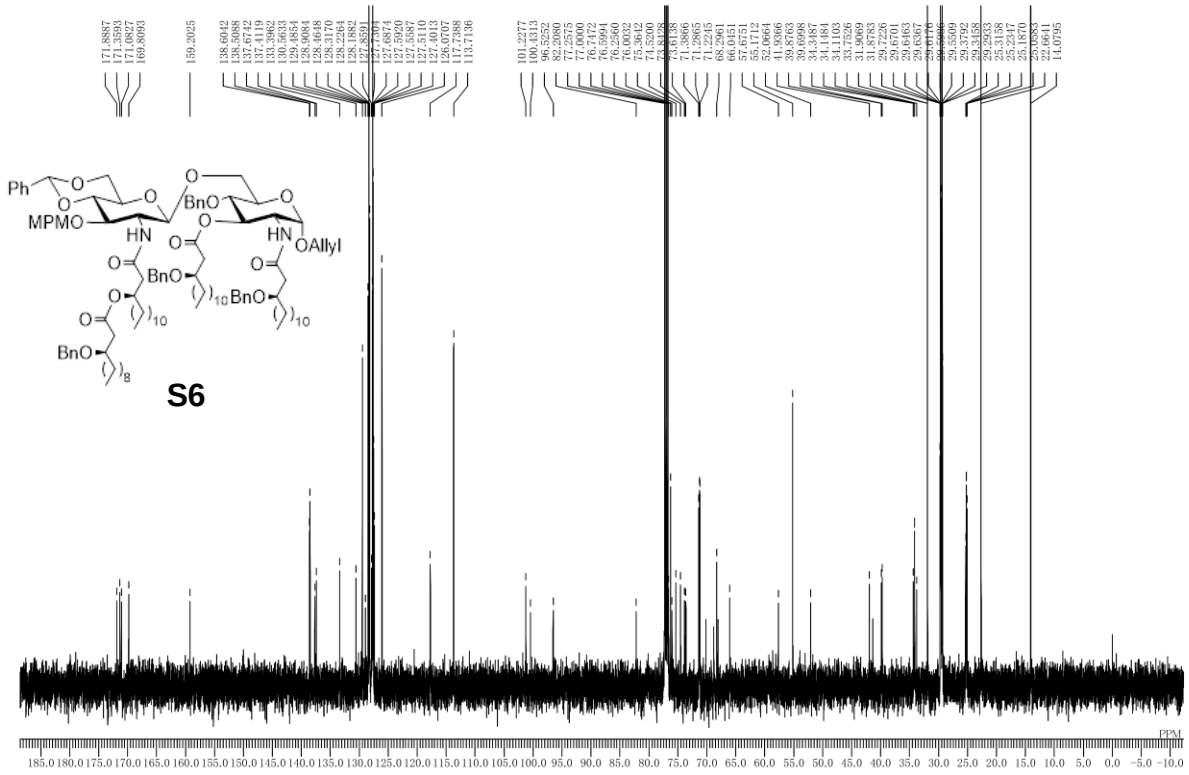
Enlarged view of ^{1}H NMR spectrum for compound **S6**



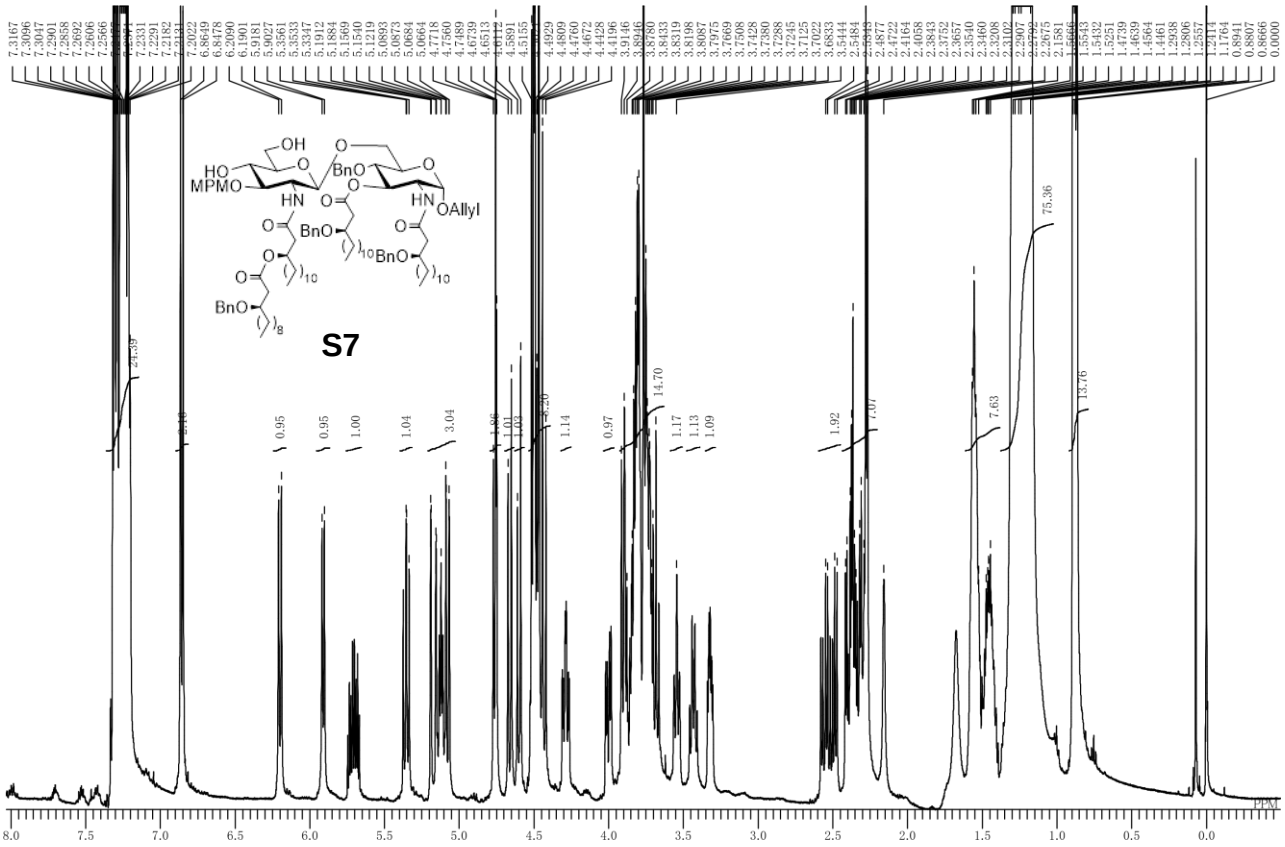




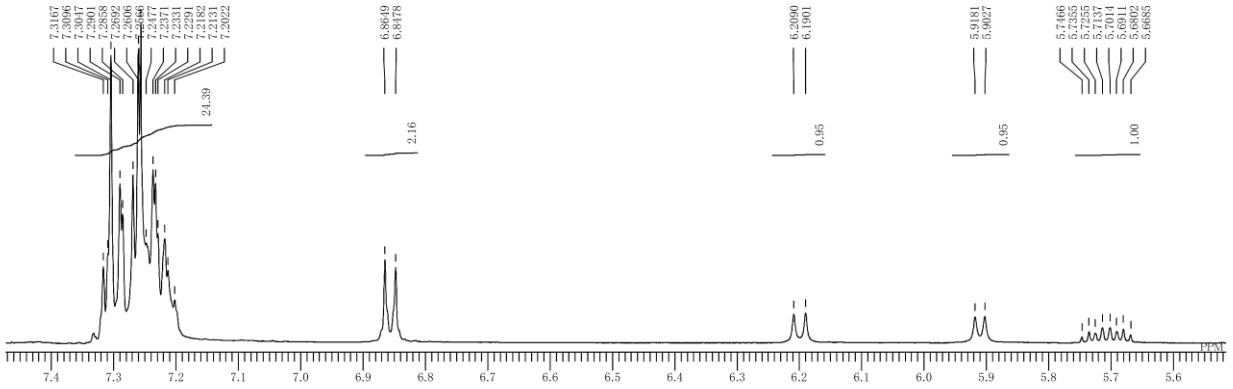
^{13}C NMR spectrum for compound **S6**

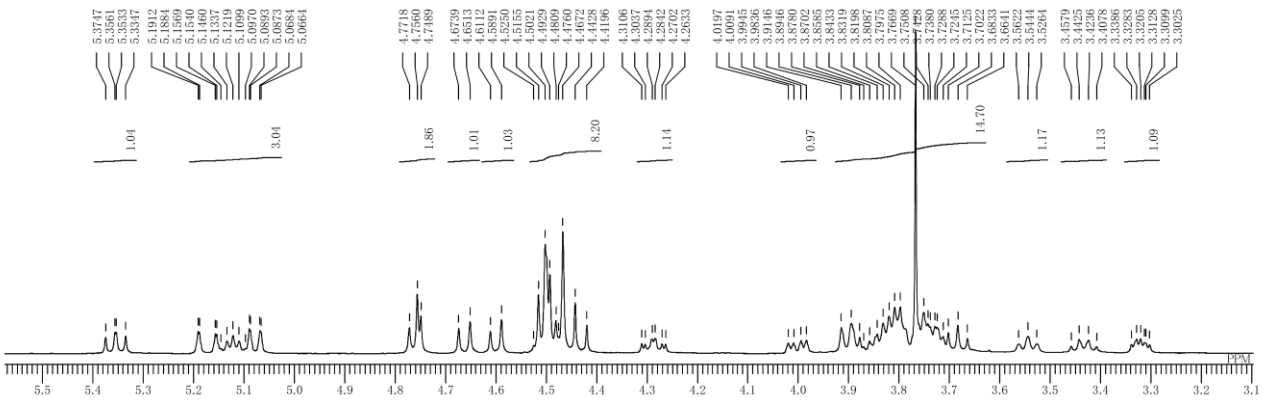


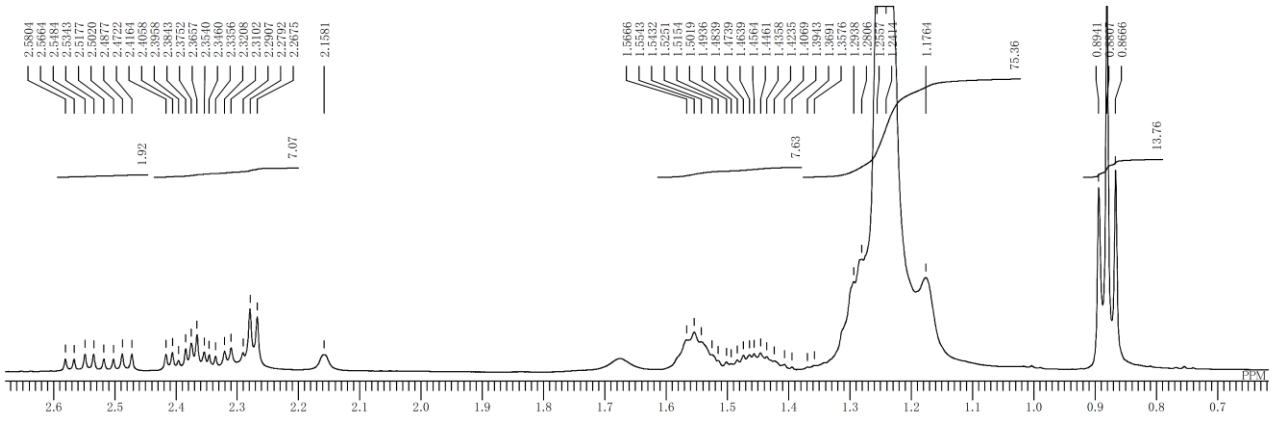
^{1}H NMR spectrum for compound **S7**



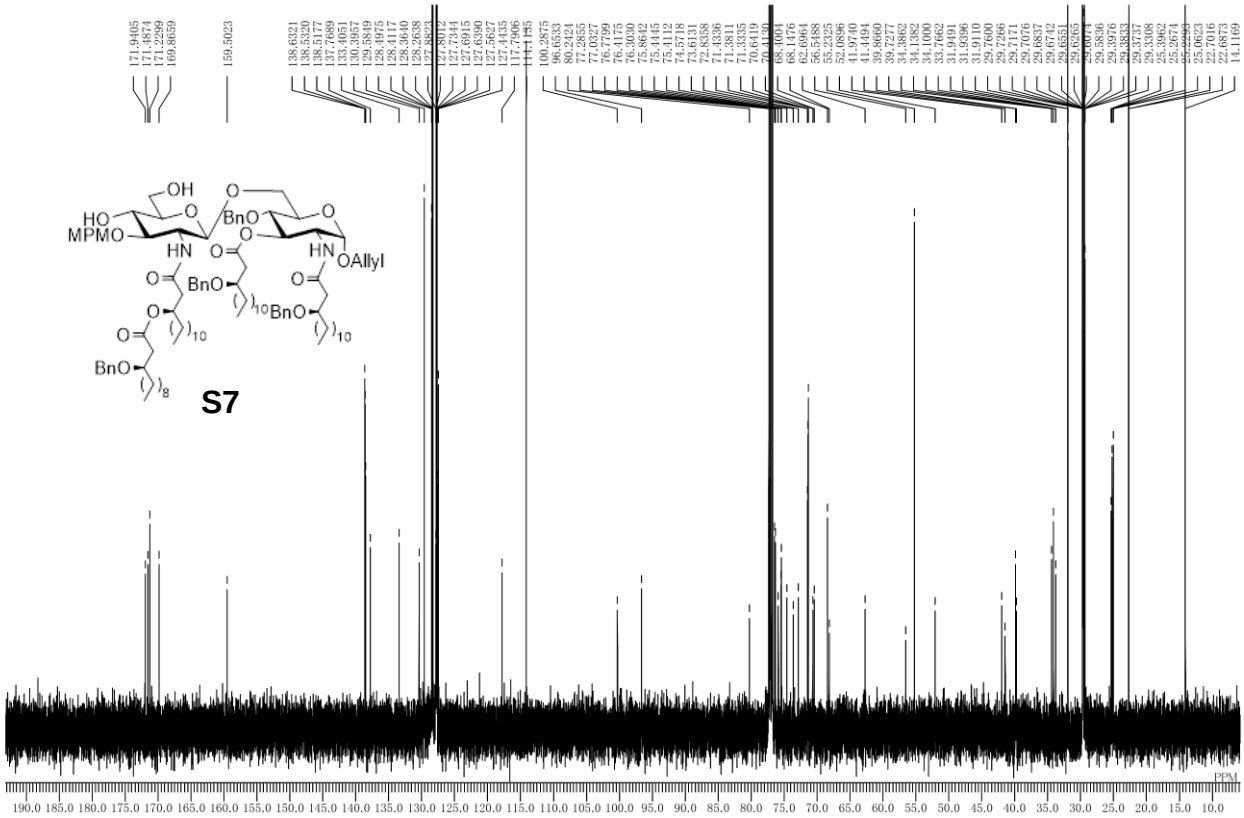
Enlarged view of ^{1}H NMR spectrum for compound **S7**



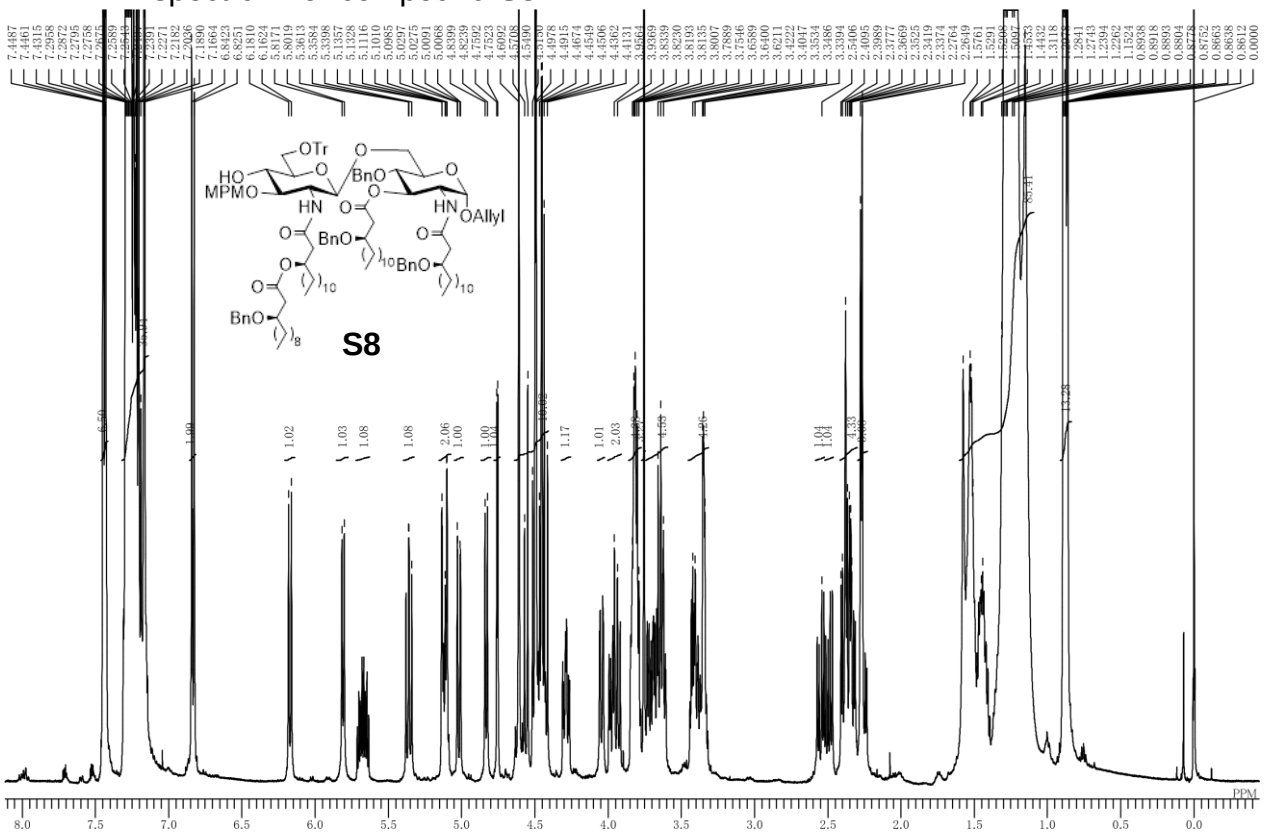




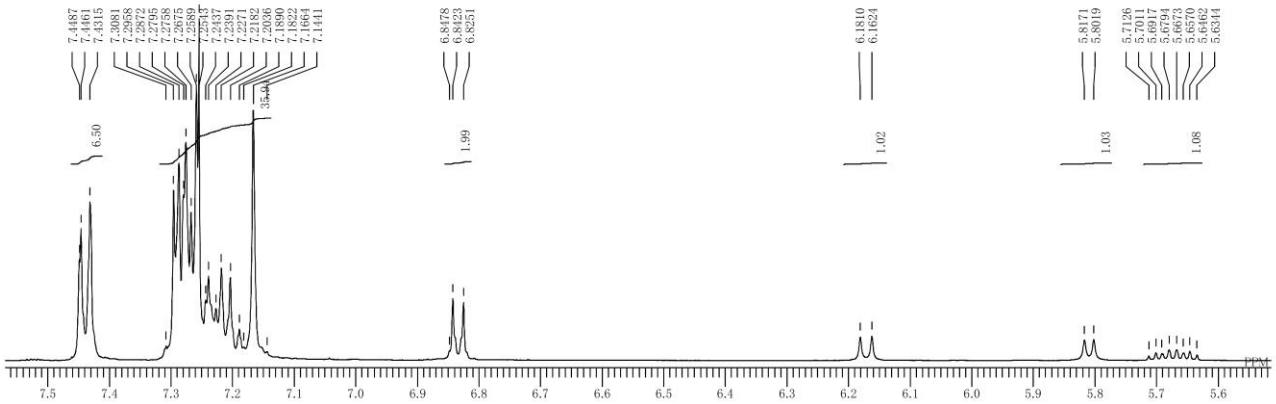
^{13}C NMR spectrum for compound **S7**

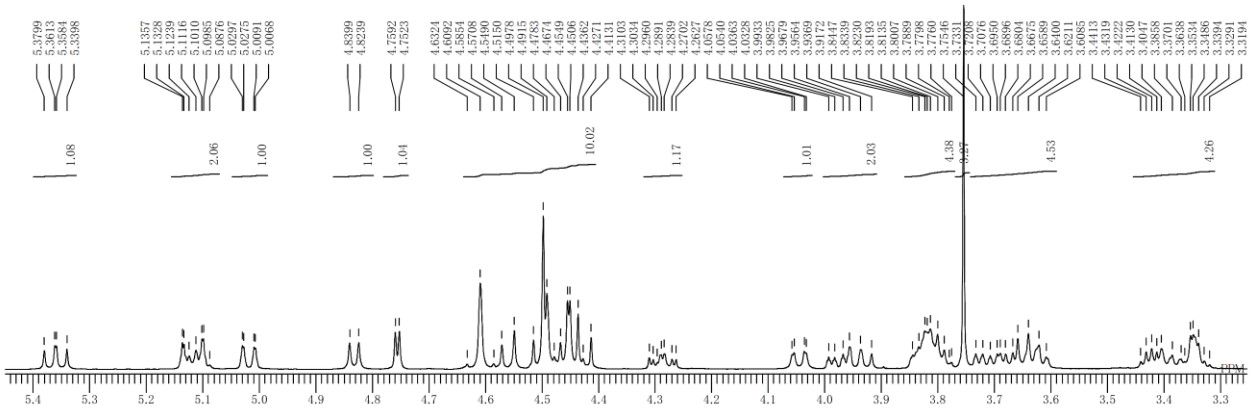


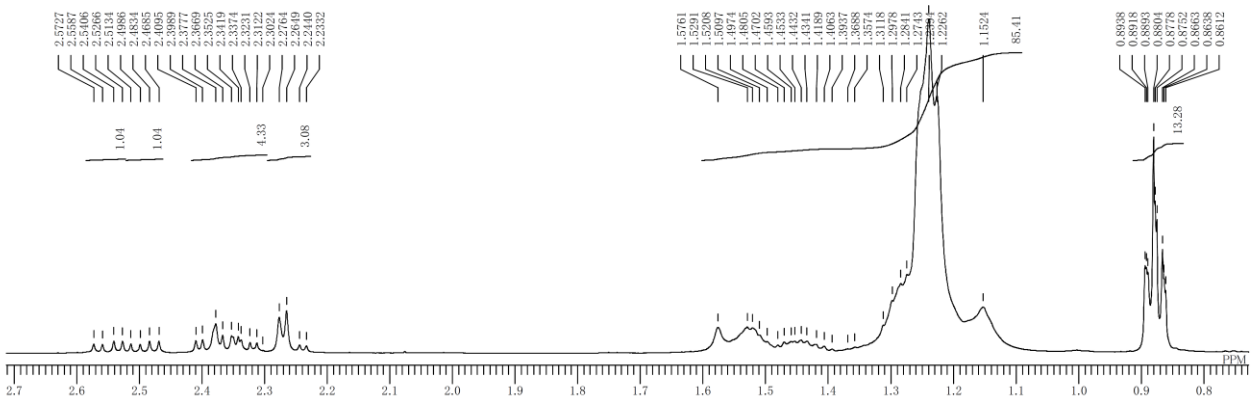
^{1}H NMR spectrum for compound **S8**



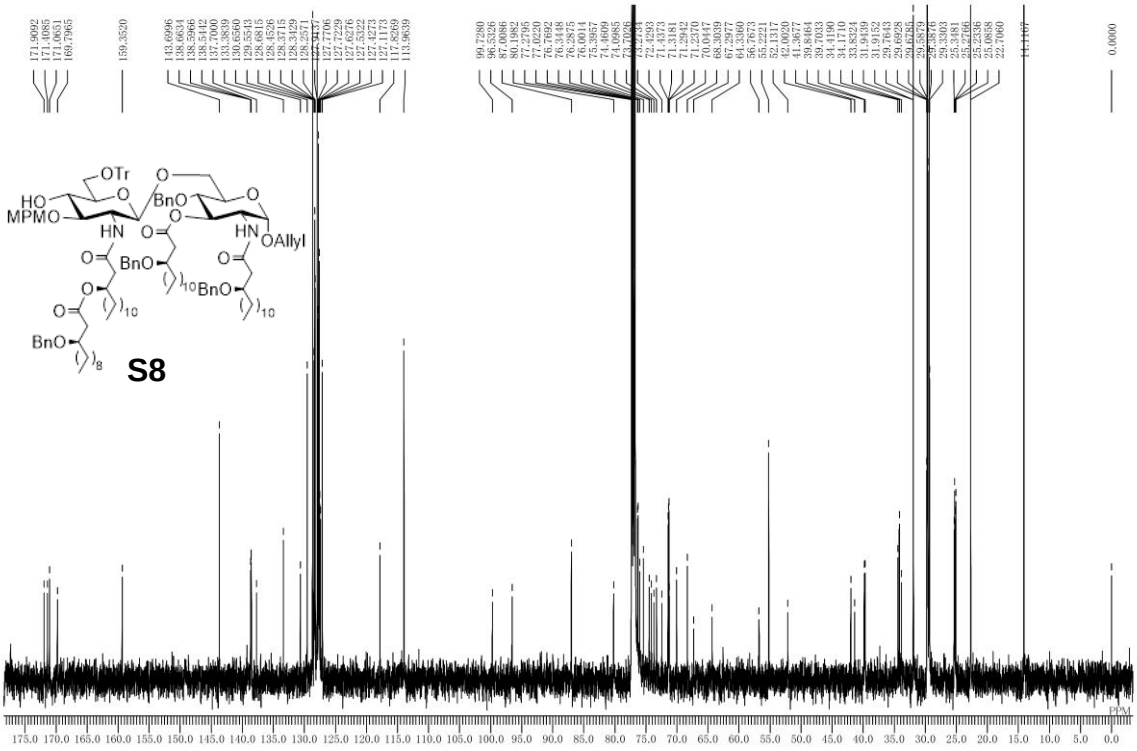
Enlarged view of ^{1}H NMR spectrum for compound **S8**



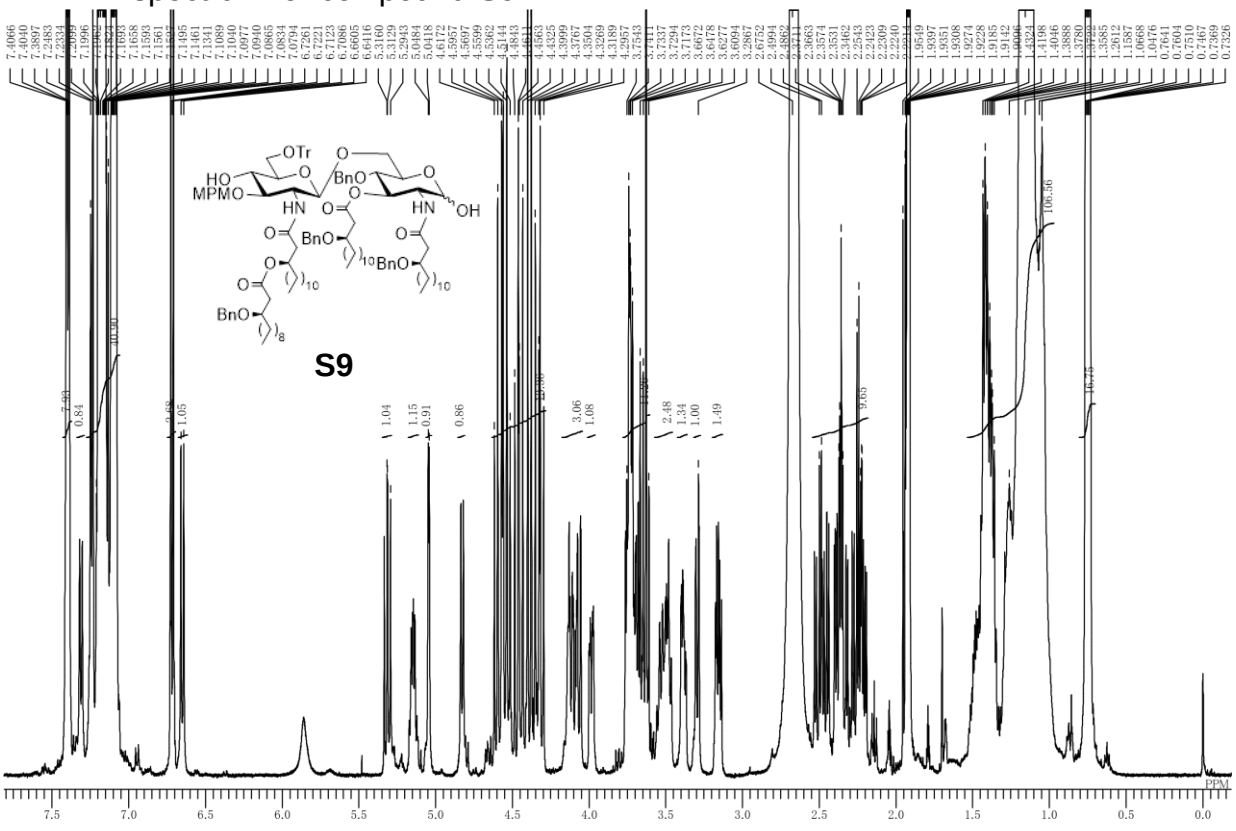




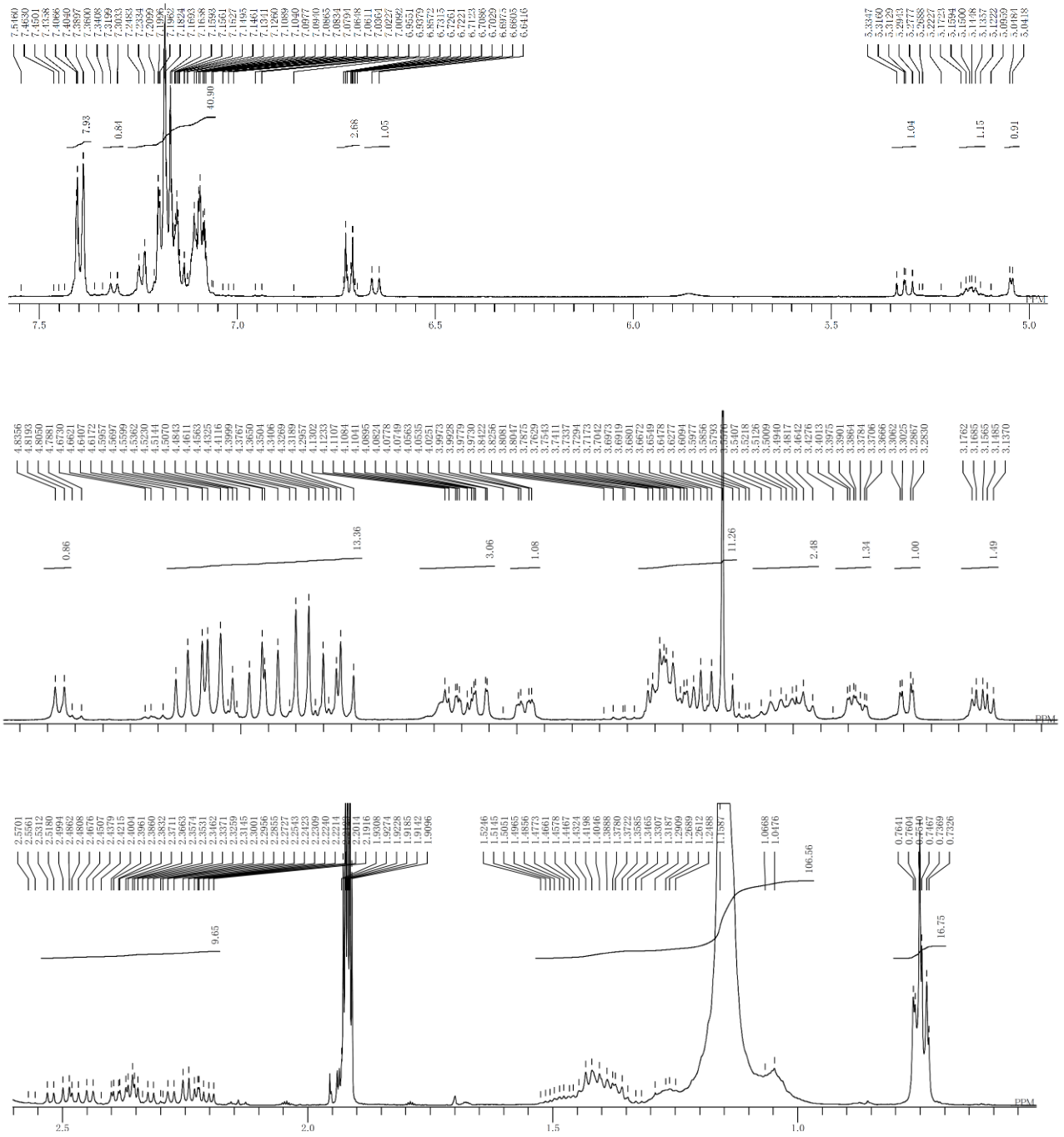
^{13}C NMR spectrum for compound **S8**



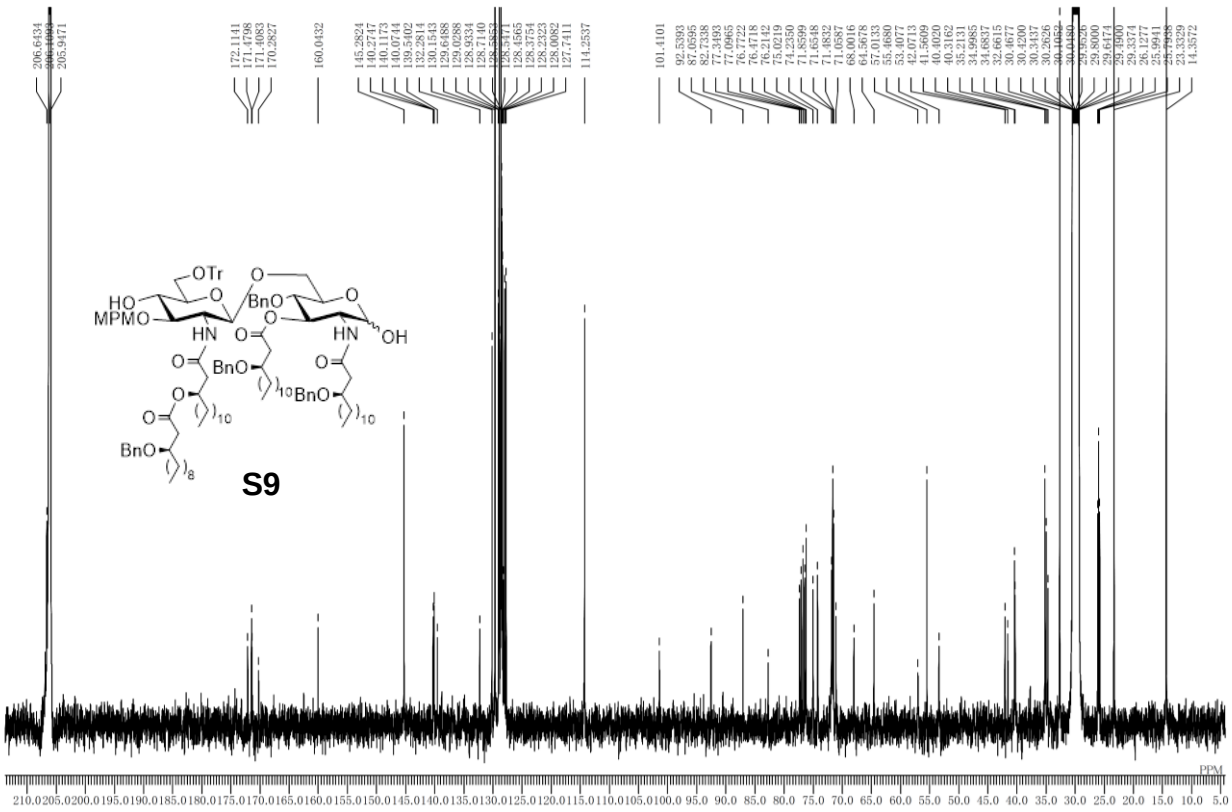
^{1}H NMR spectrum for compound **S9**



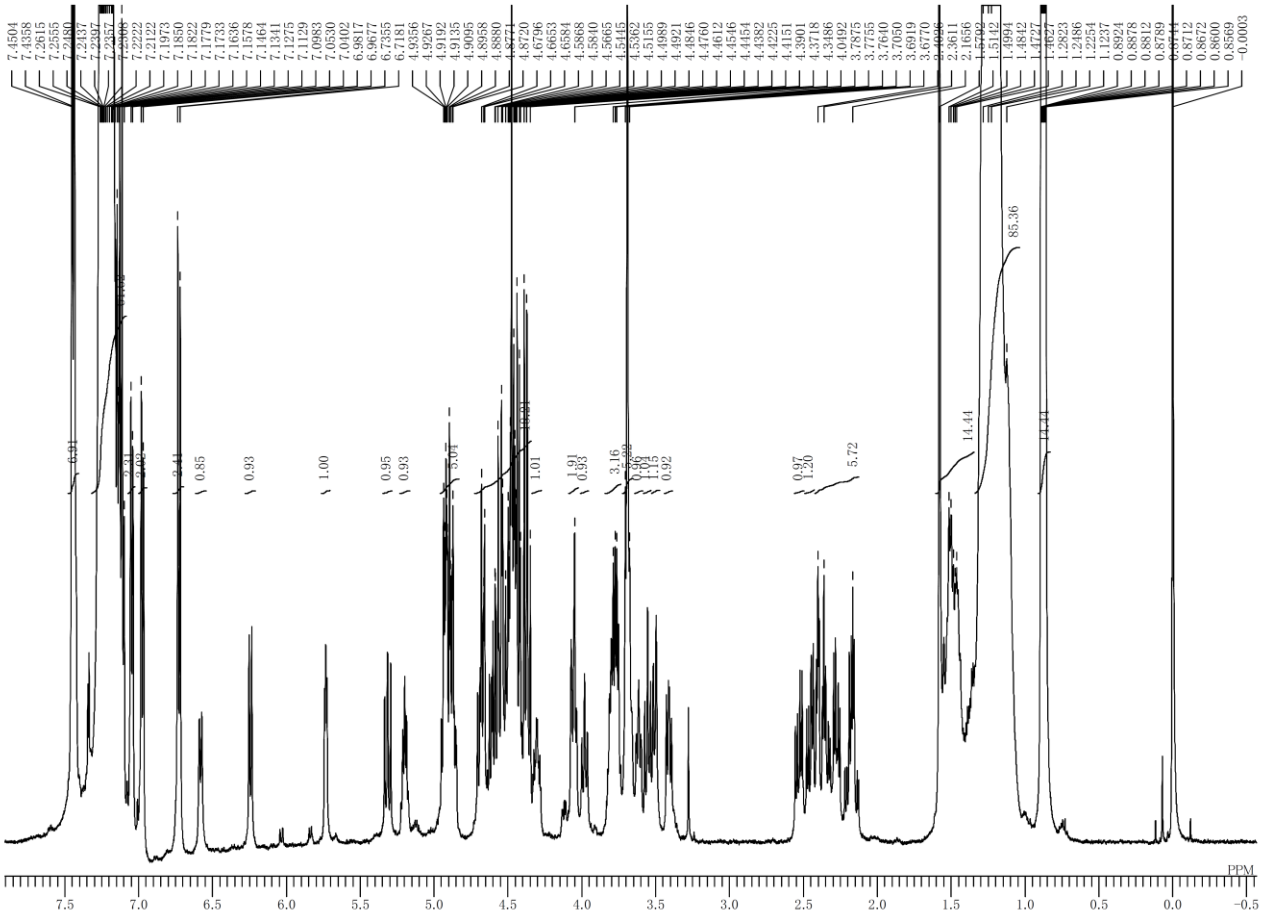
Enlarged view of ^{1}H NMR spectrum for compound **S9**



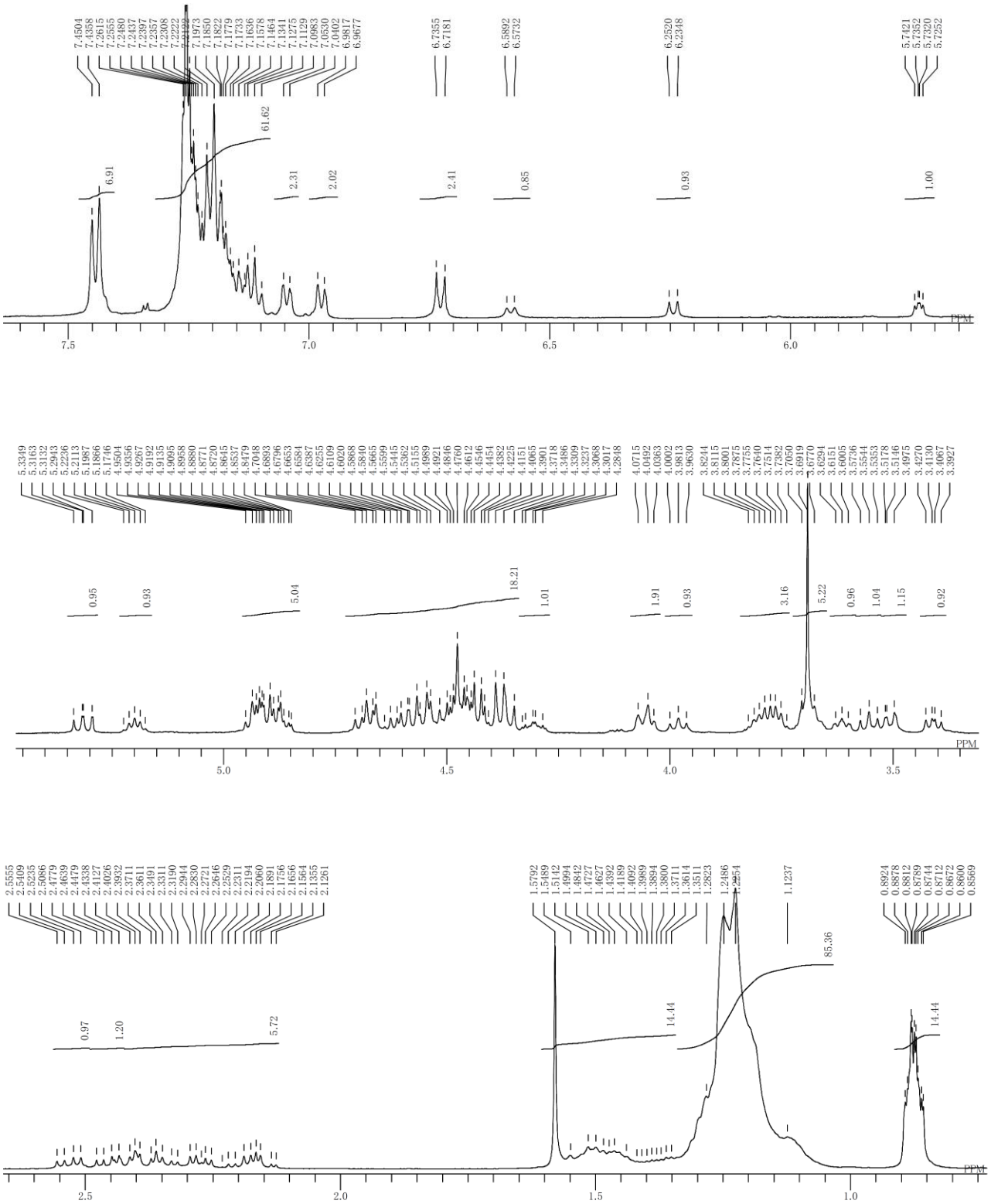
^{13}C NMR spectrum for compound **S9**



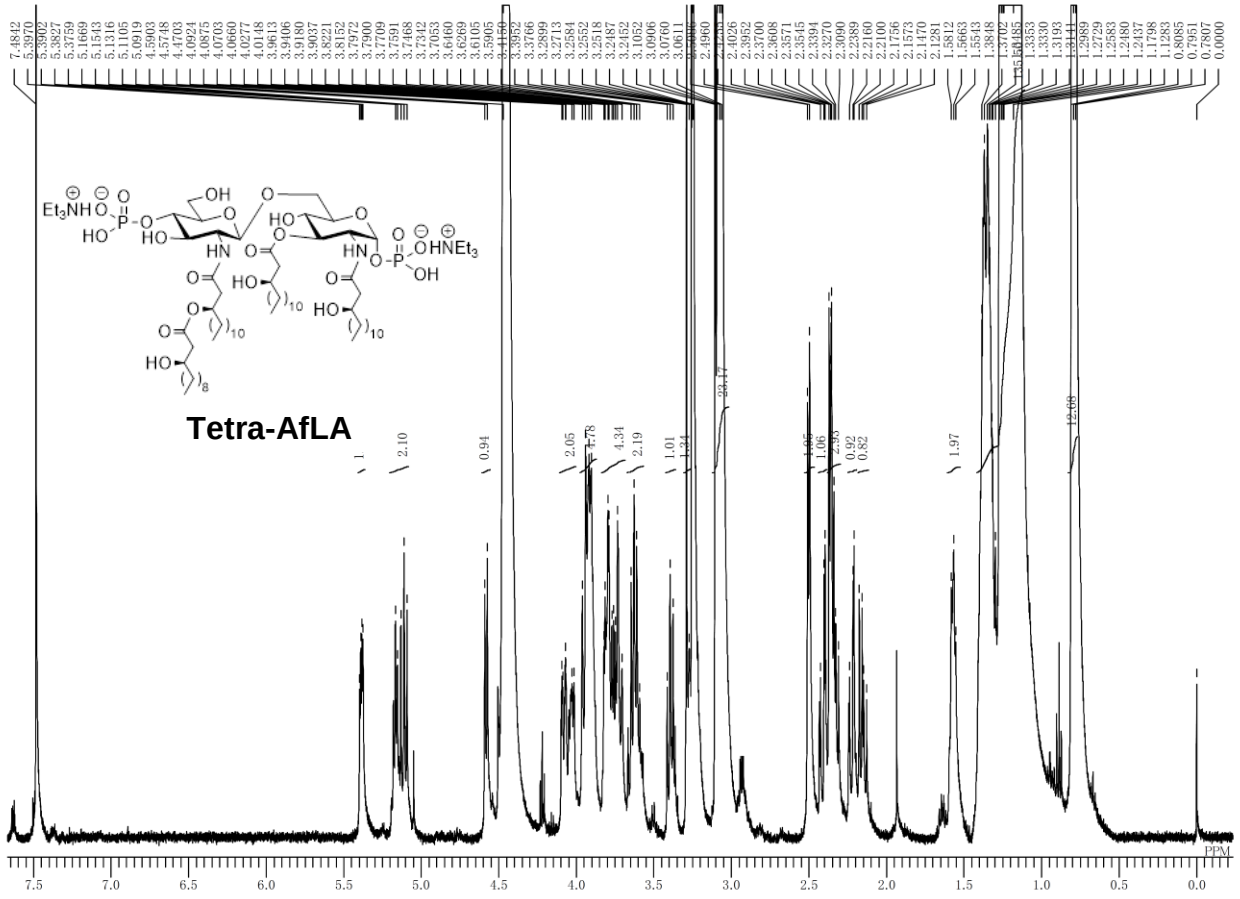
^{1}H NMR spectrum for compound **S10**



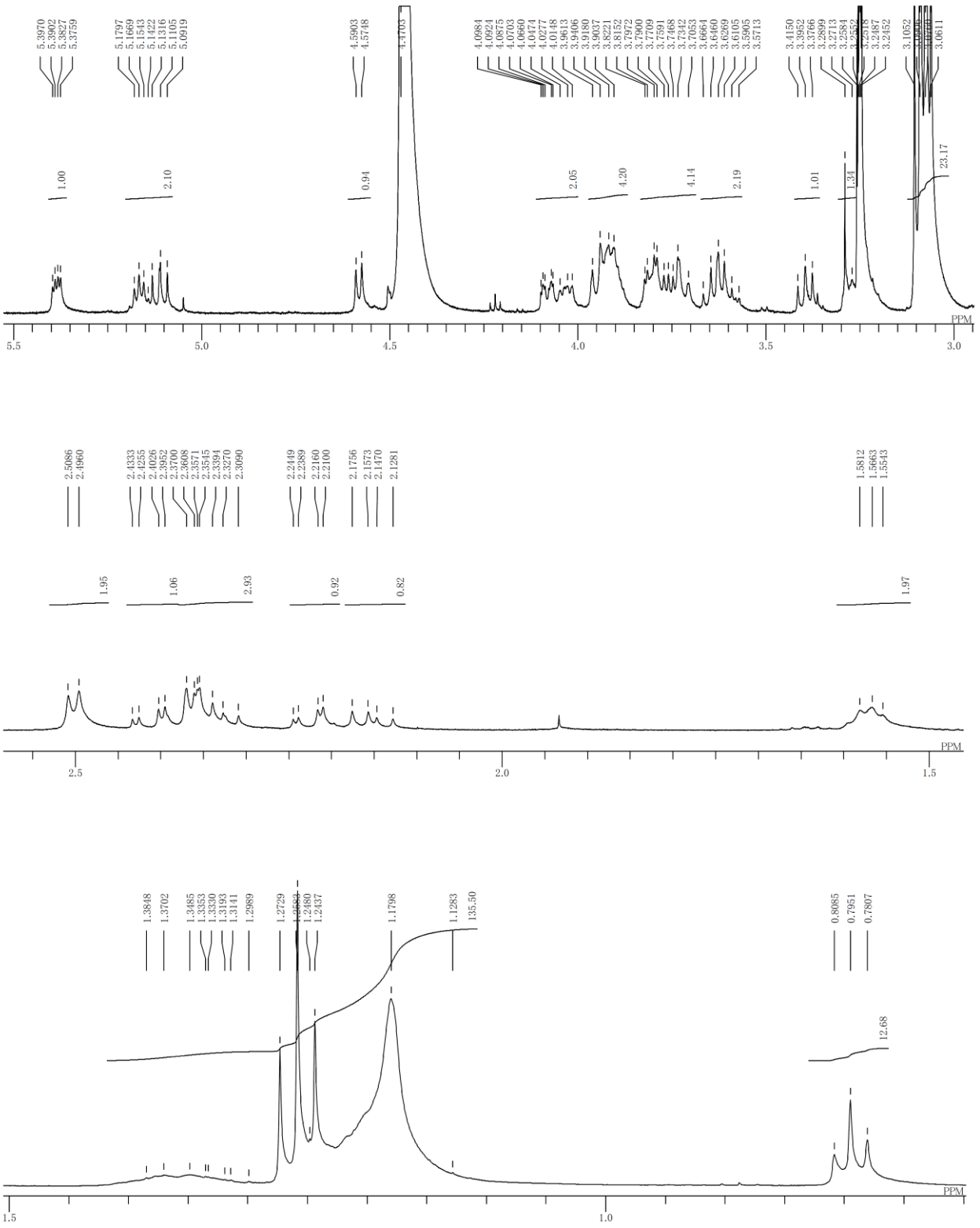
Enlarged view of ^{1}H NMR spectrum for compound **S10**



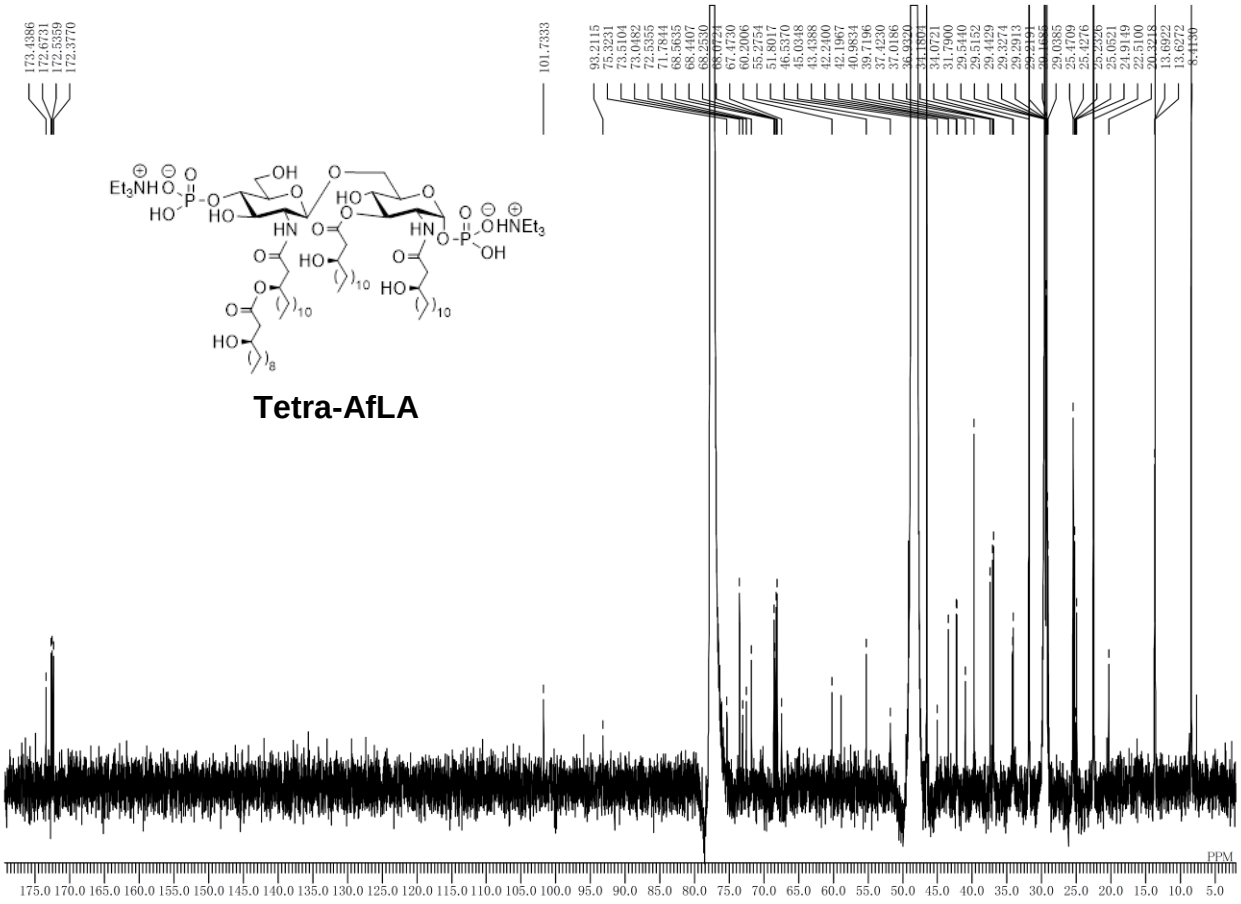
^{1}H NMR spectrum for **Tetra-AfLA**



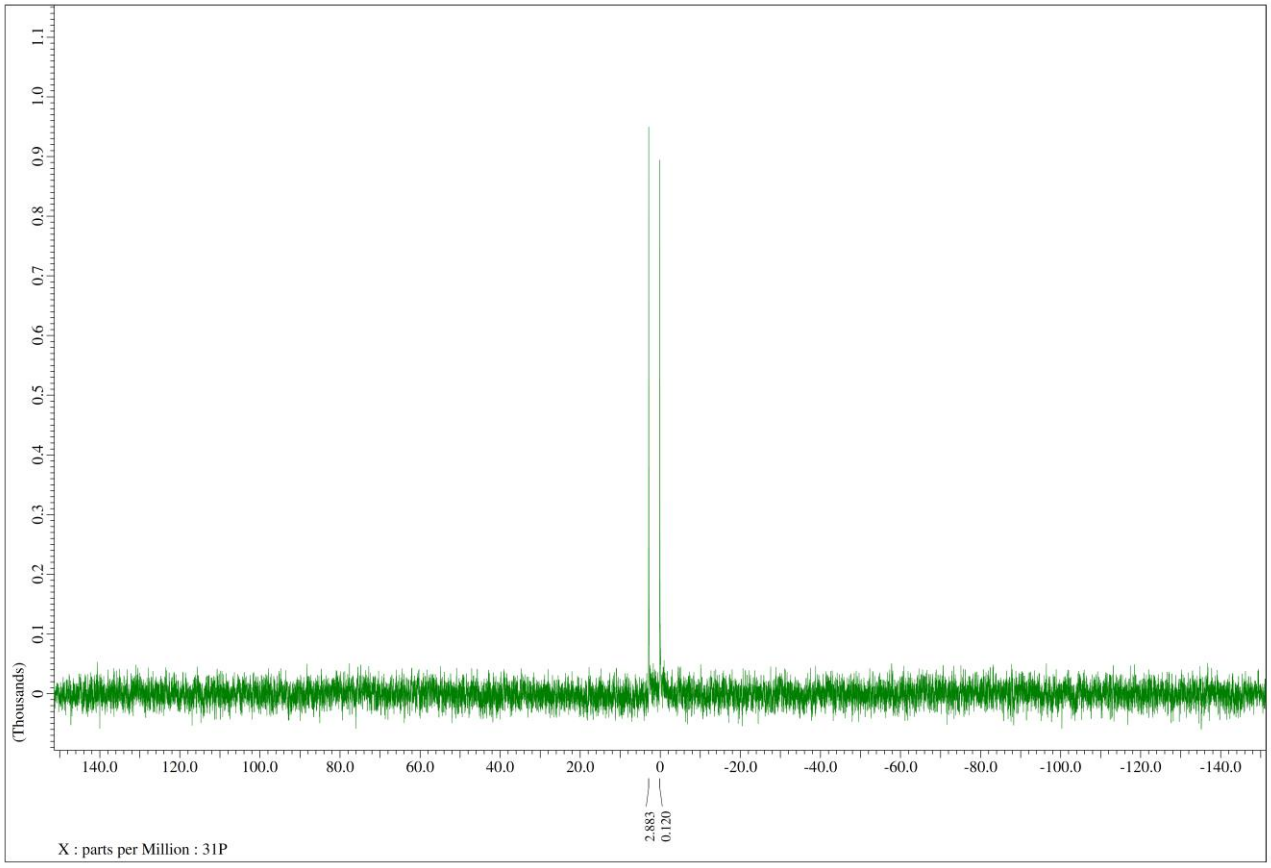
Enlarged view of ^{1}H NMR spectrum for **Tetra-AfLA**



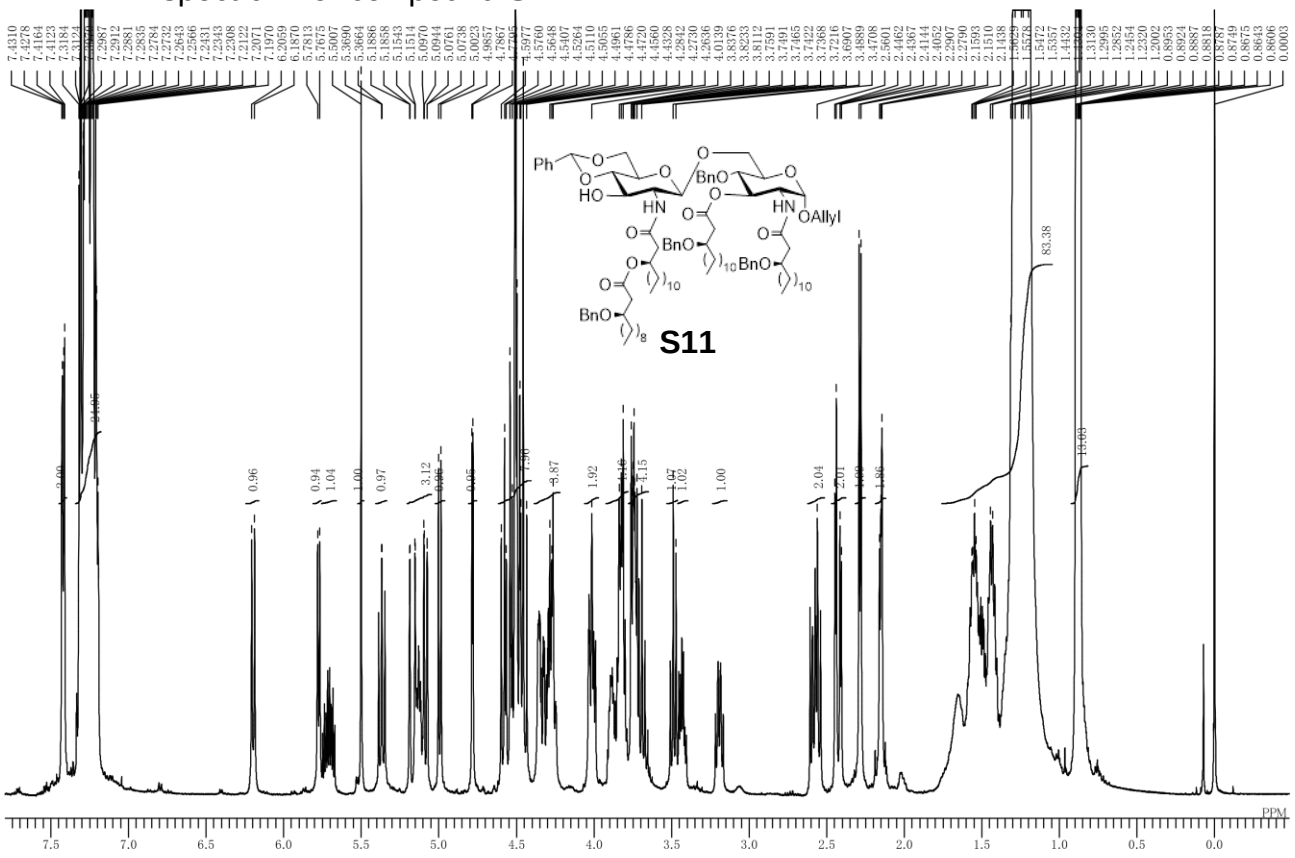
^{13}C NMR spectrum for **Tetra-AfLA**



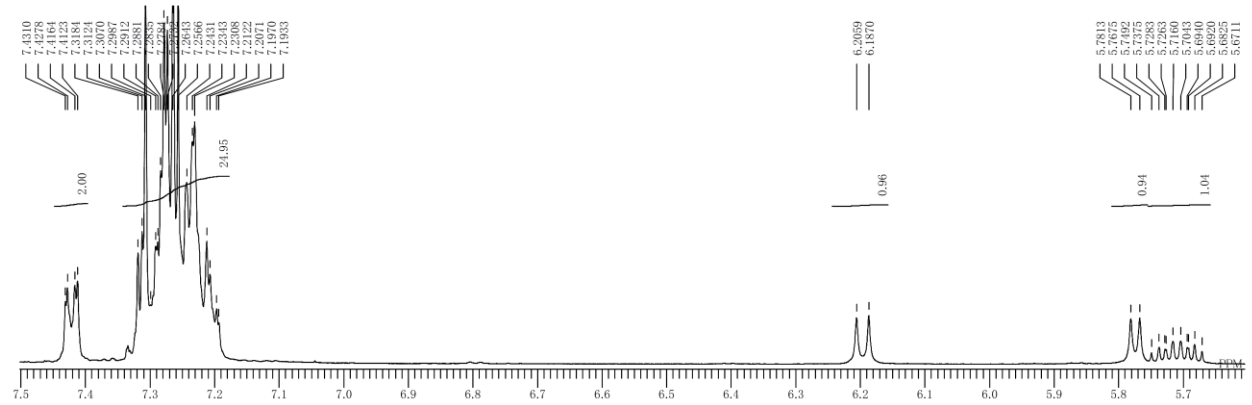
^{31}P NMR spectrum for **Tetra-AfLA**

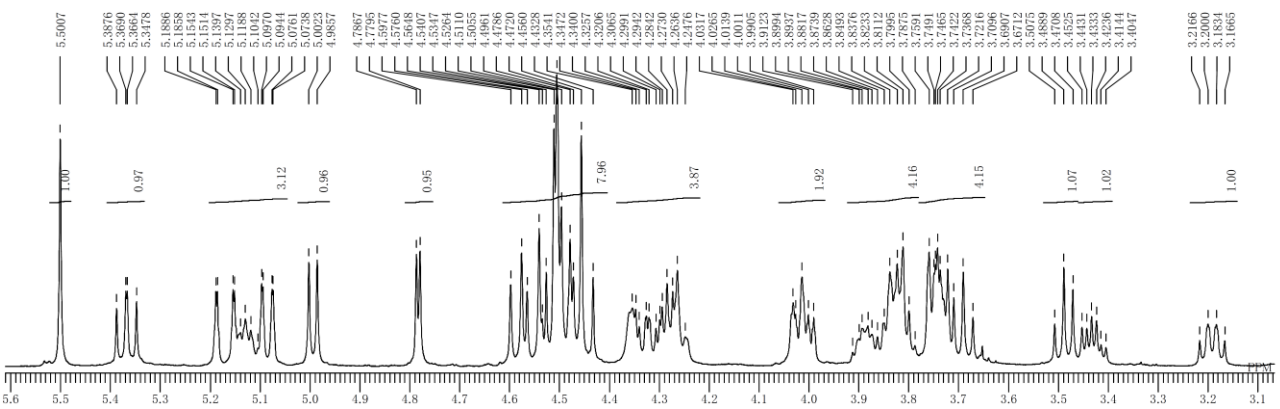


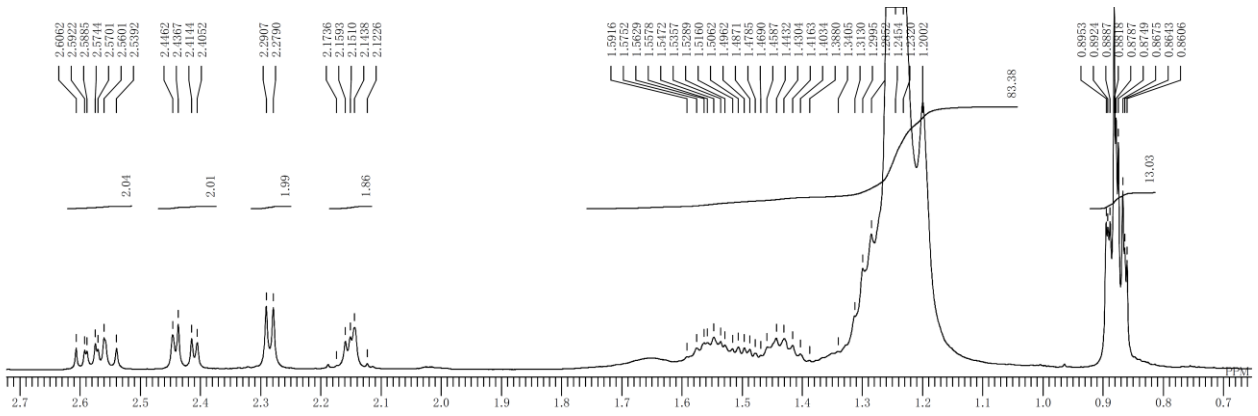
^{1}H NMR spectrum for compound **S11**



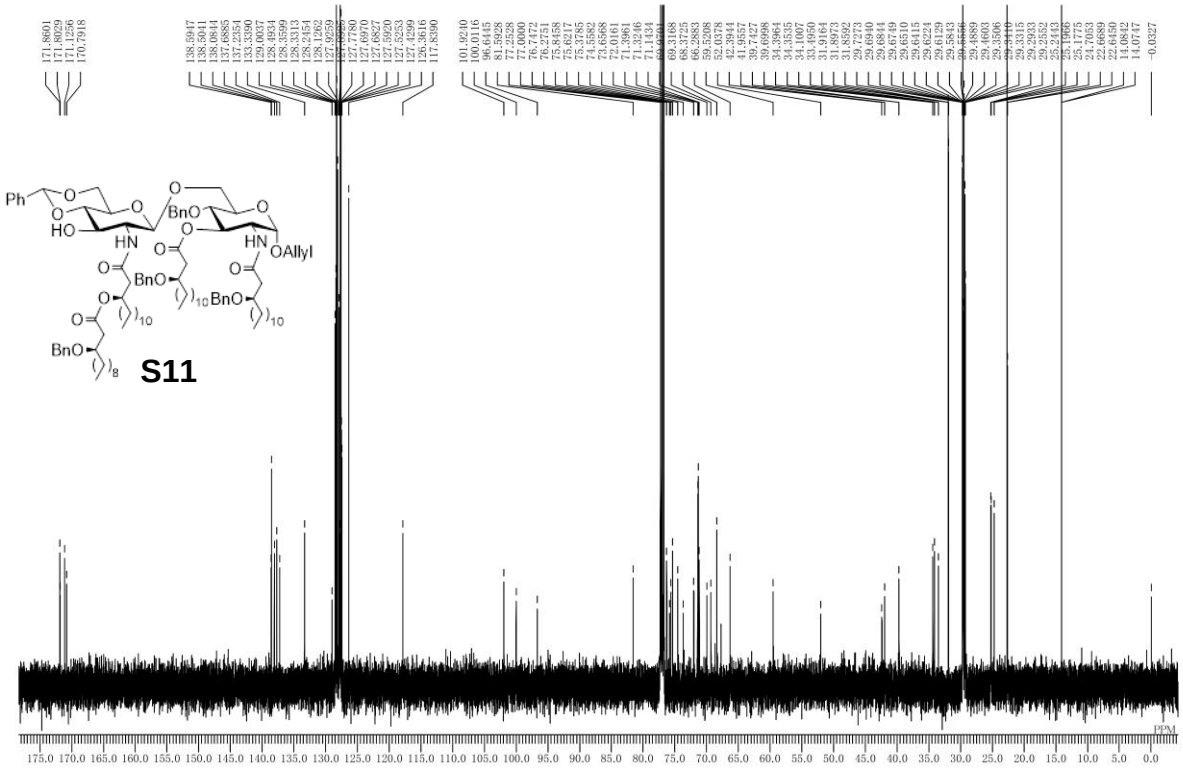
Enlarged view of ^{1}H NMR spectrum for compound **S11**



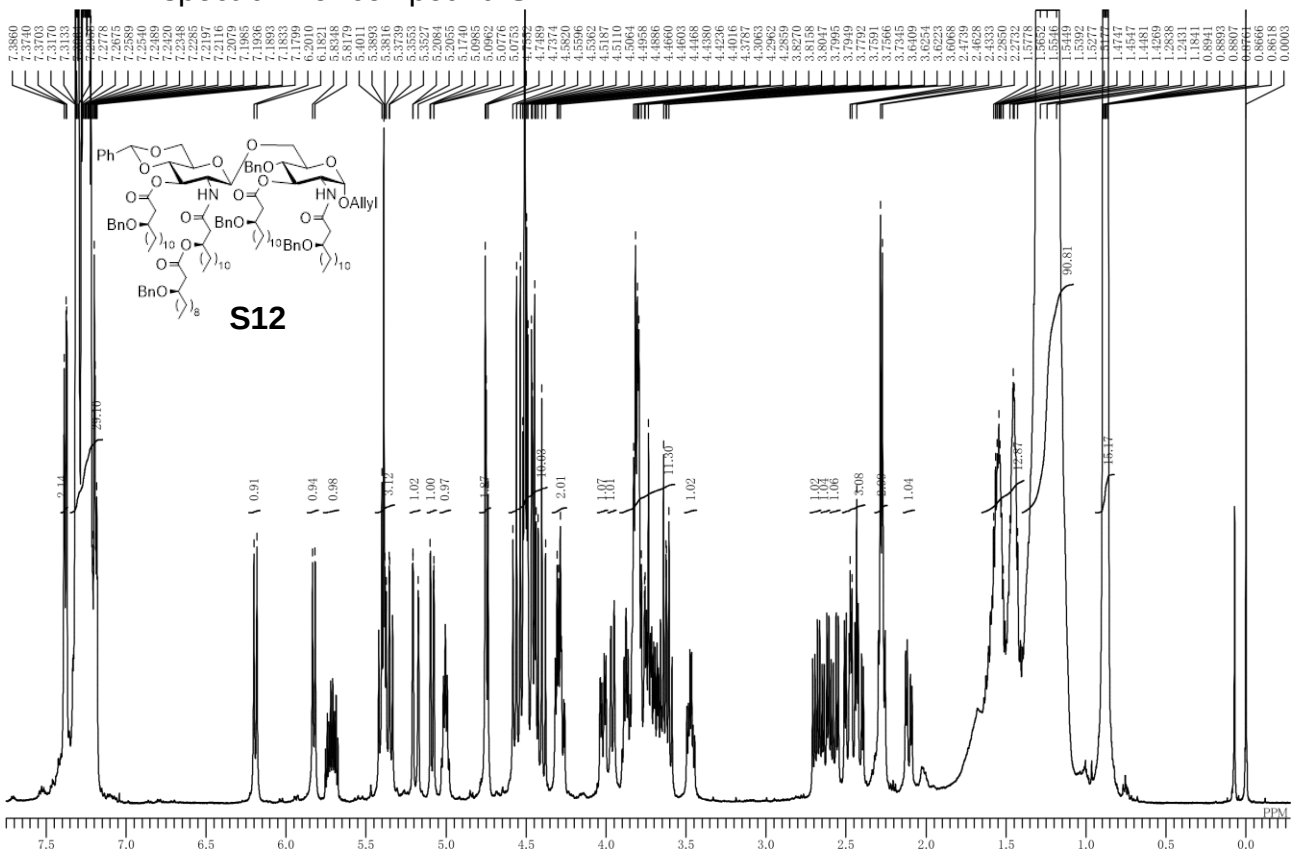




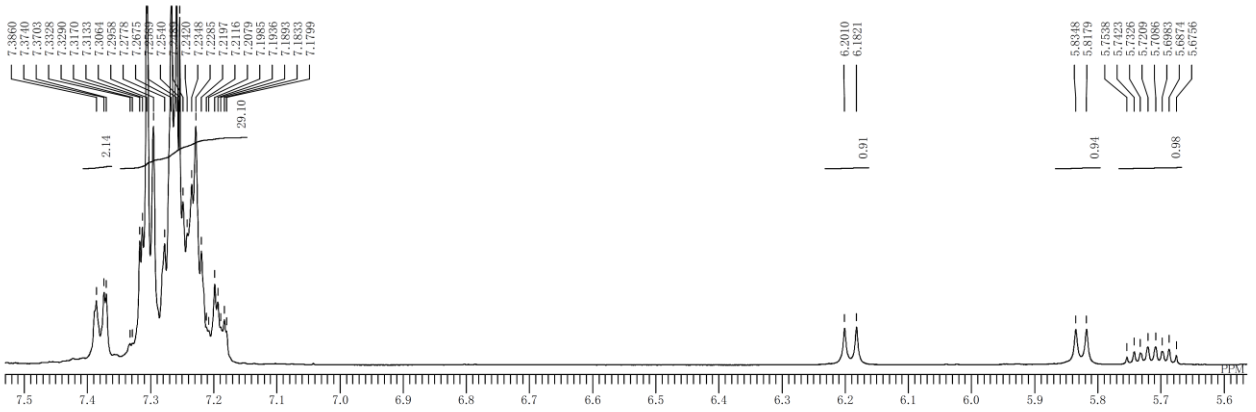
^{13}C NMR spectrum for compound **S11**

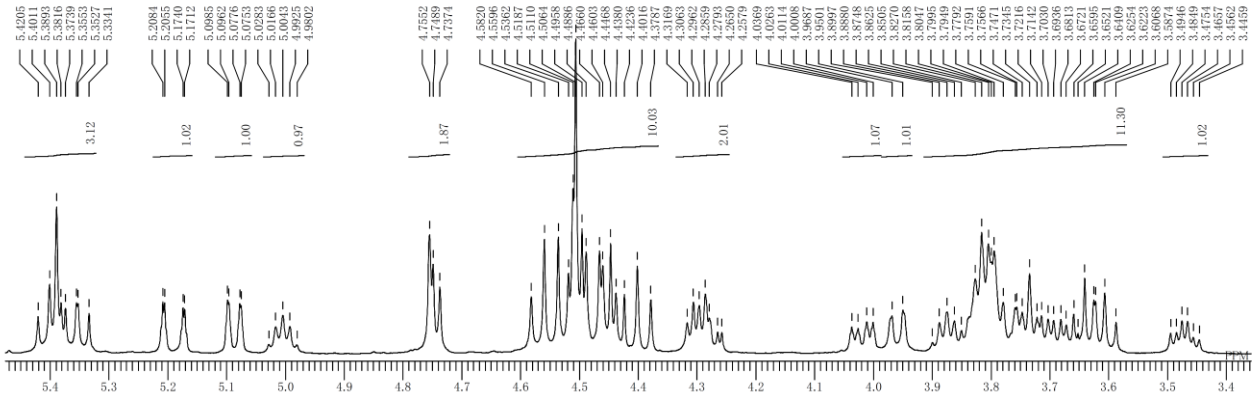


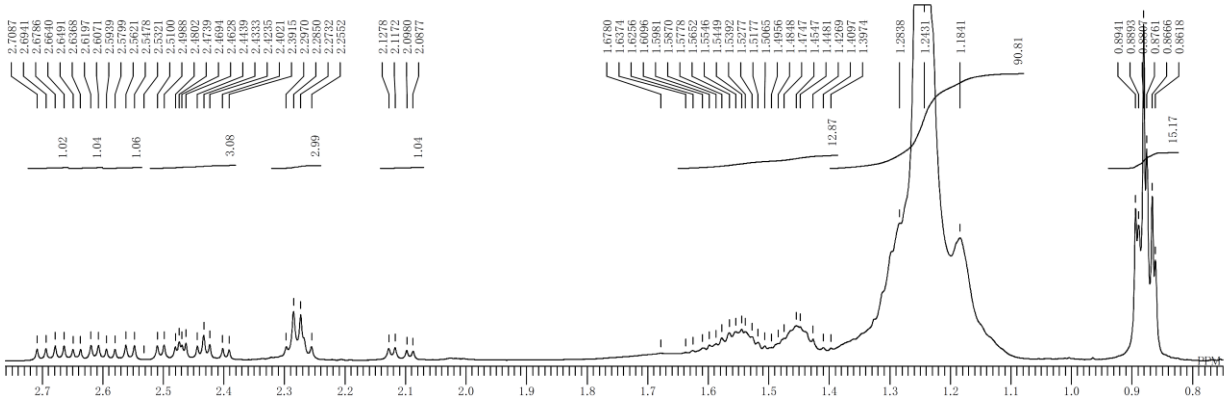
^{1}H NMR spectrum for compound **S12**



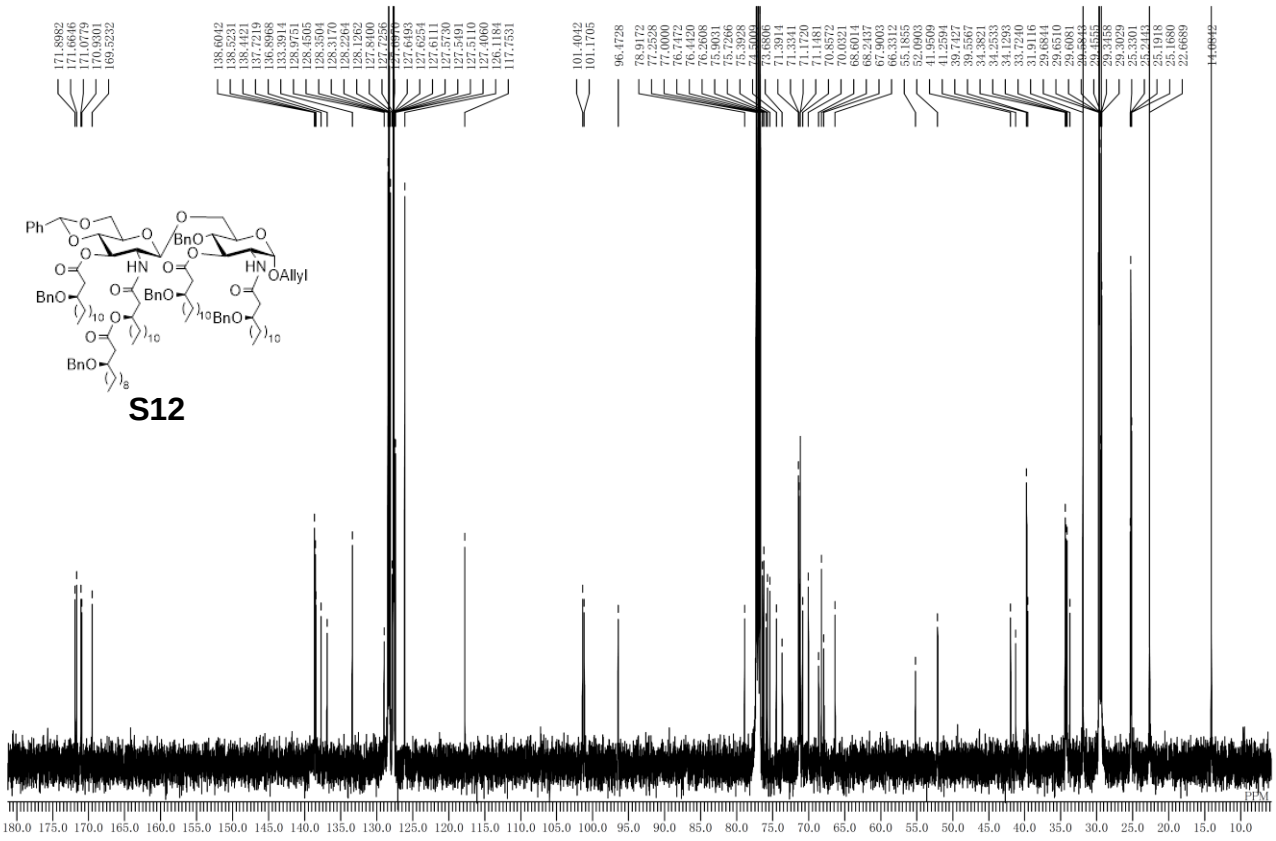
Enlarged view of ^{1}H NMR spectrum for compound **S12**



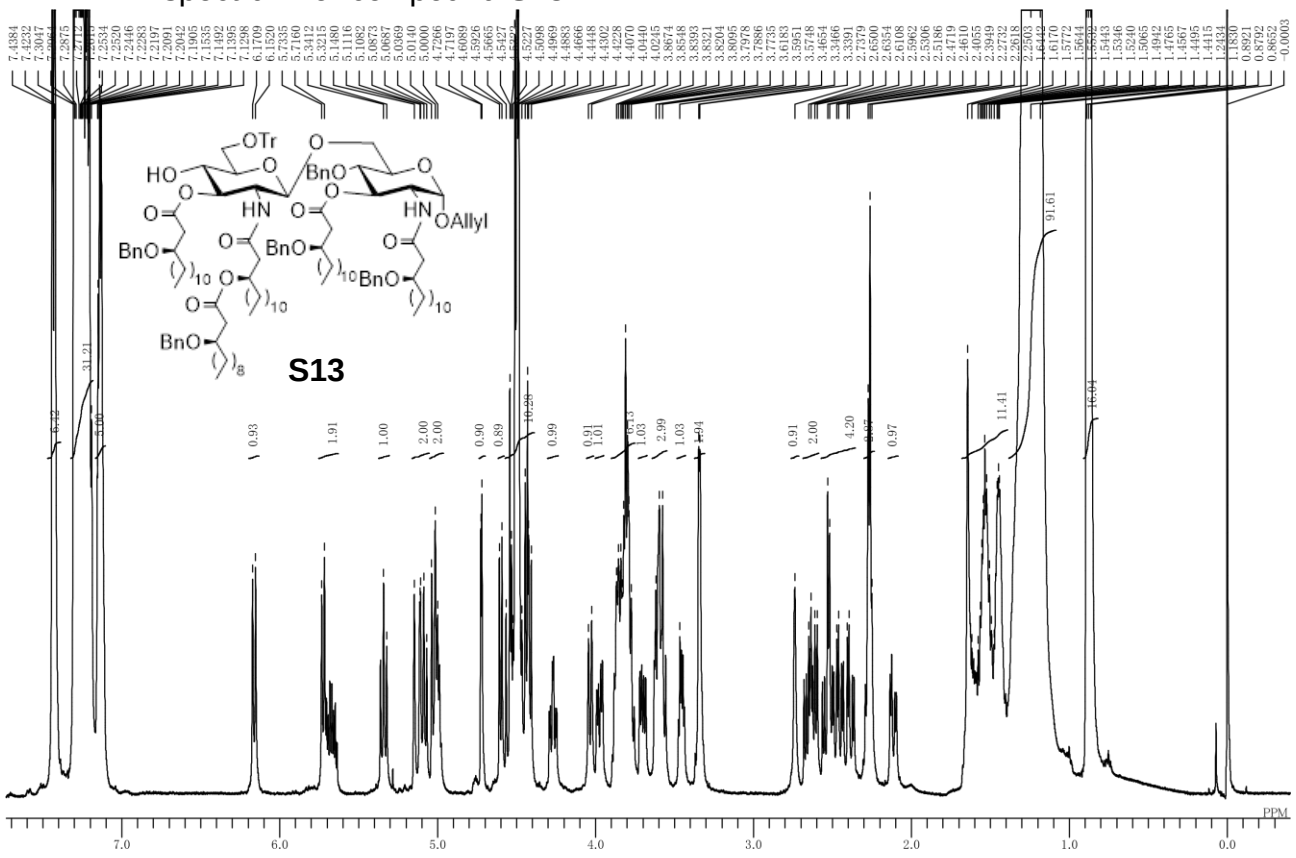




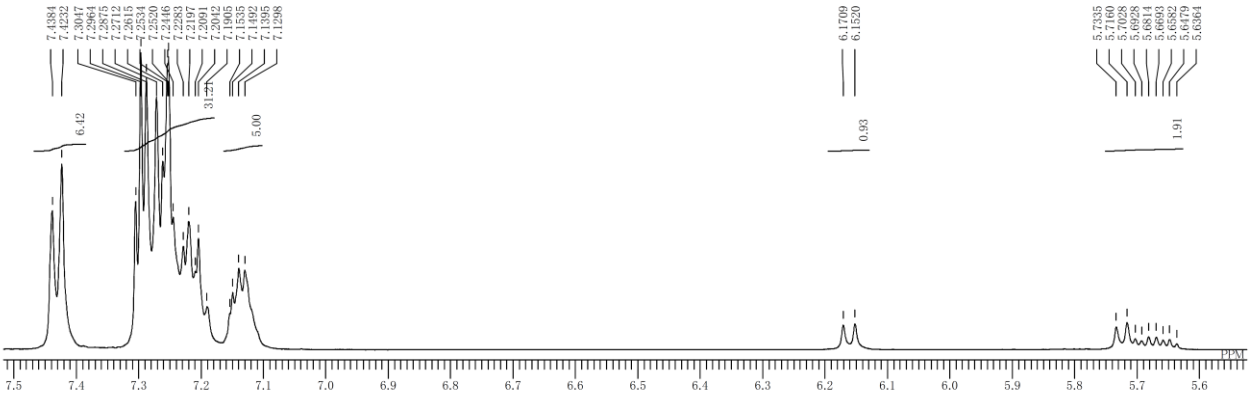
^{13}C NMR spectrum for compound **S12**

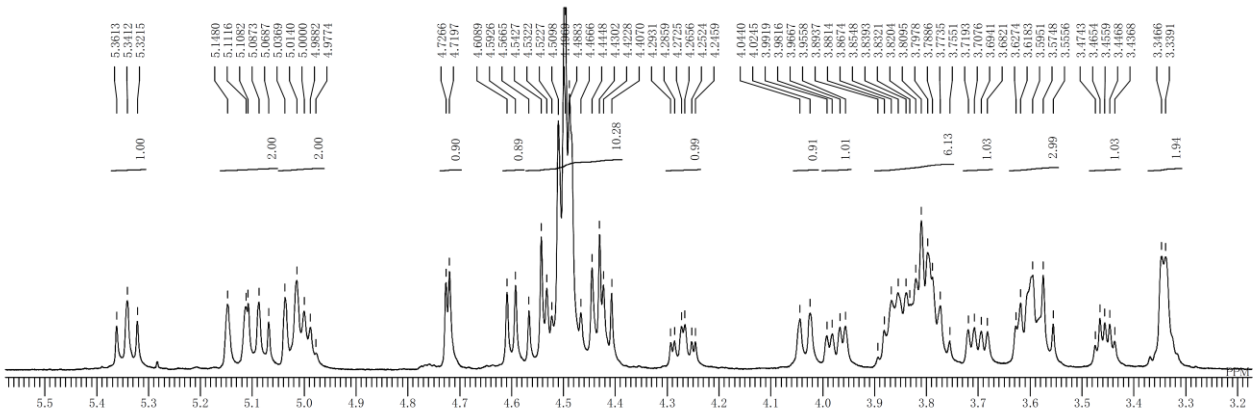


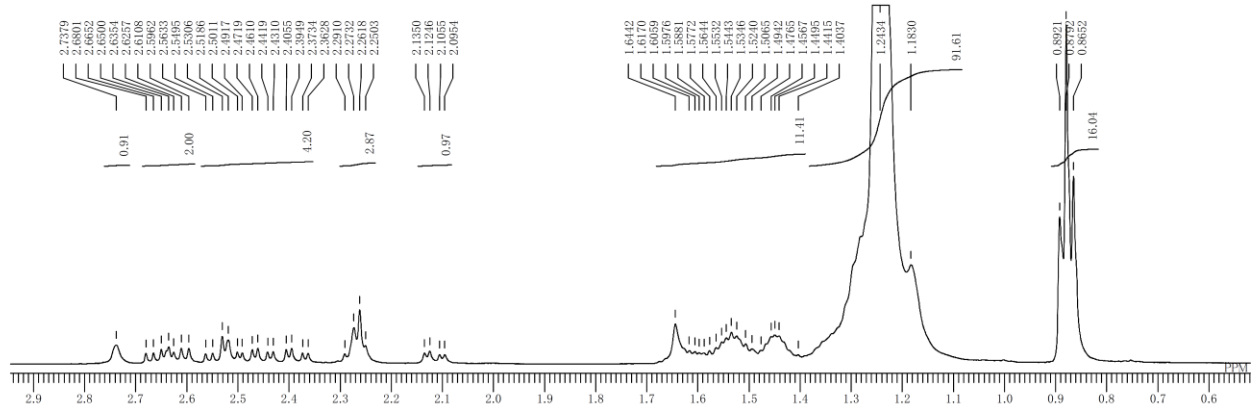
^{1}H NMR spectrum for compound **S13**



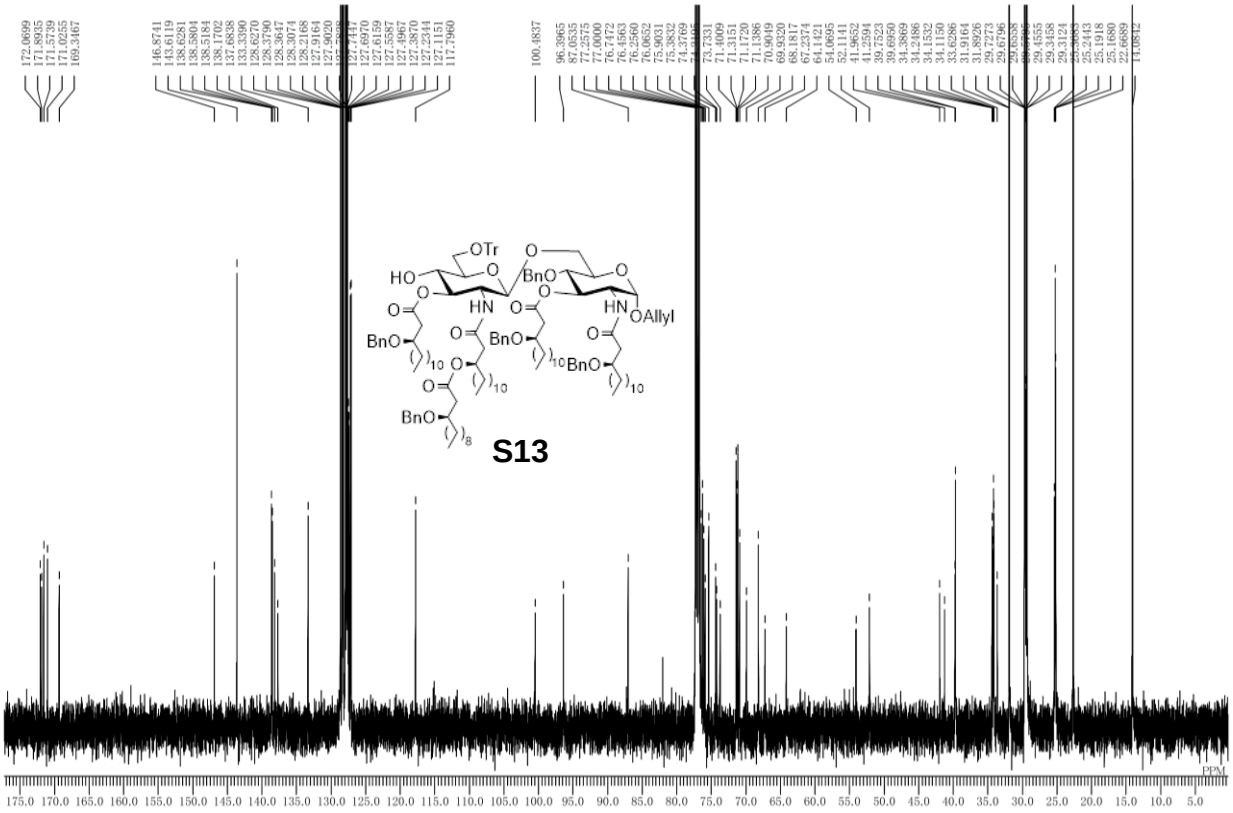
Enlarged view of ^{1}H NMR spectrum for compound **S13**



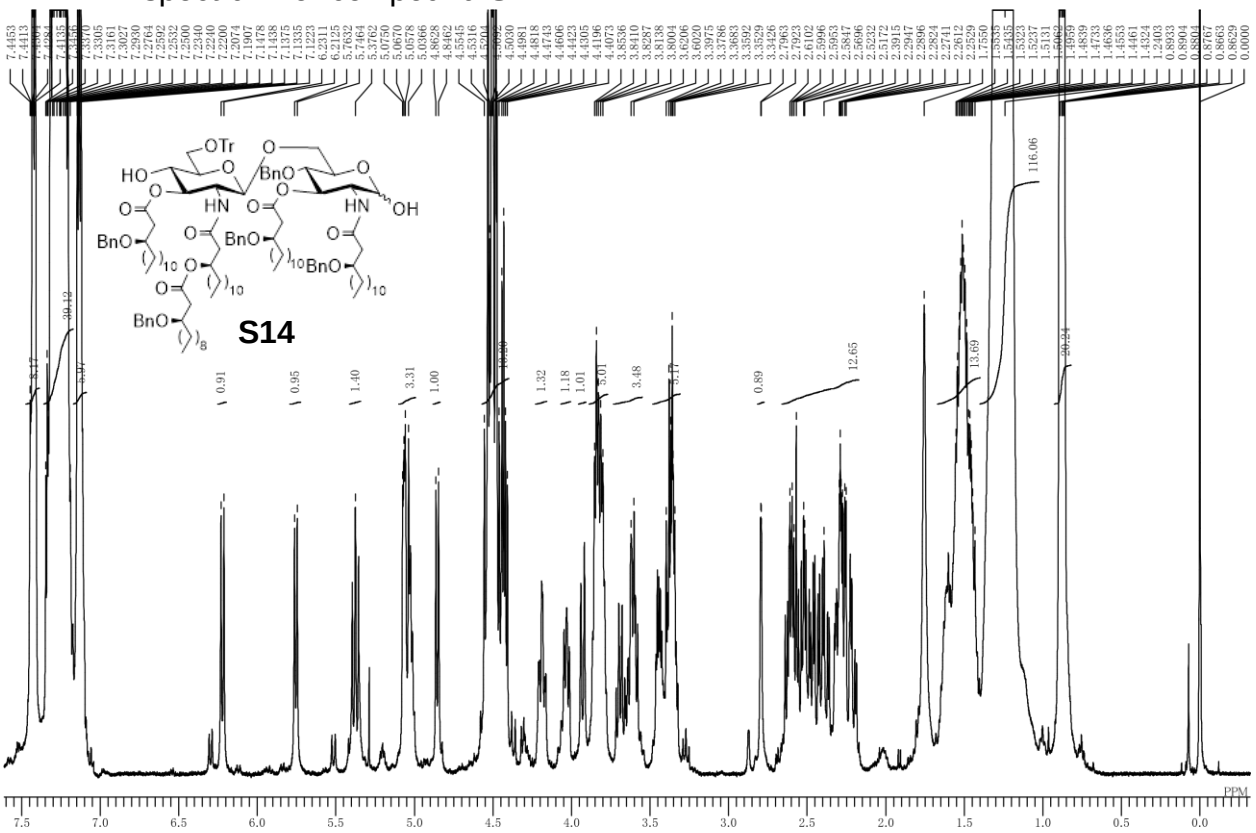




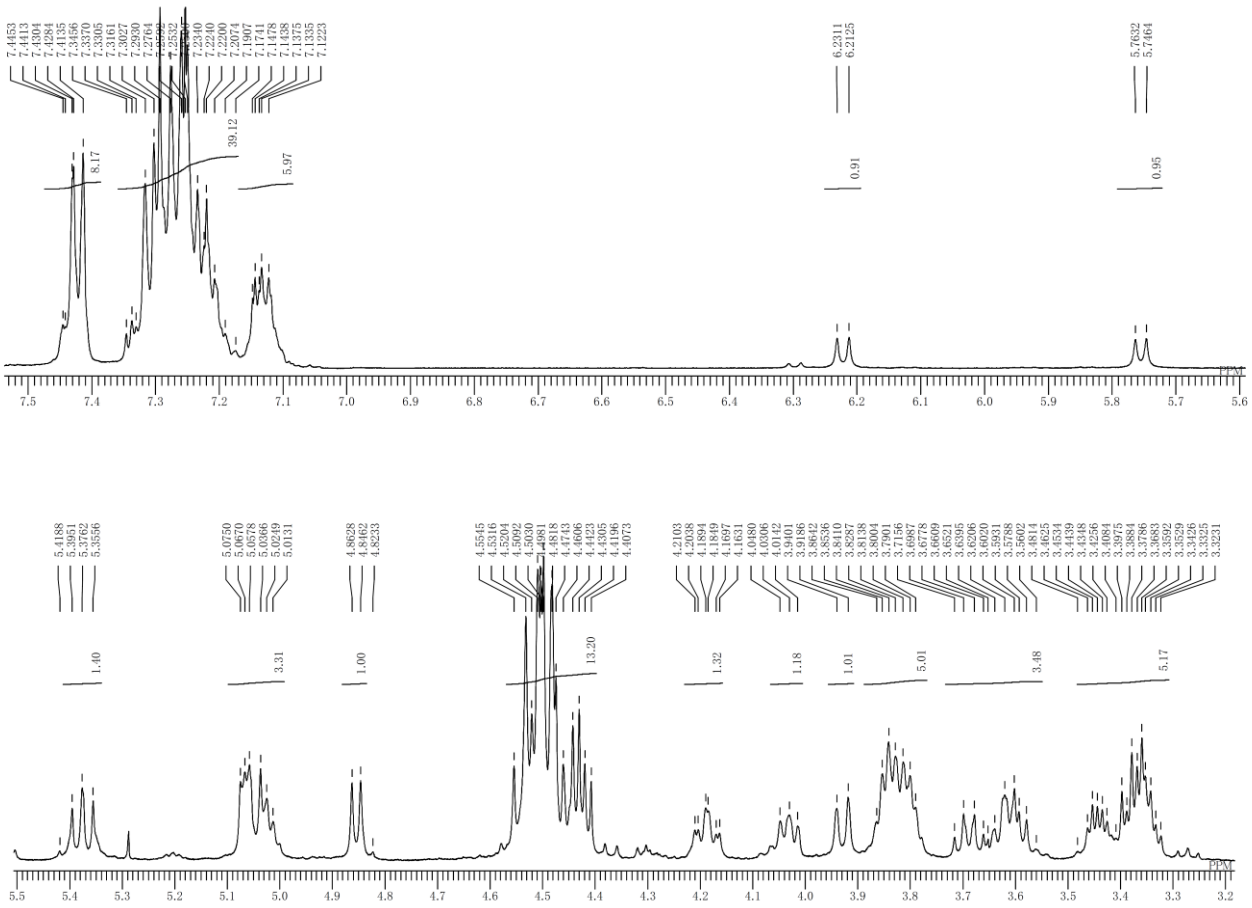
^{13}C NMR spectrum for compound **S13**



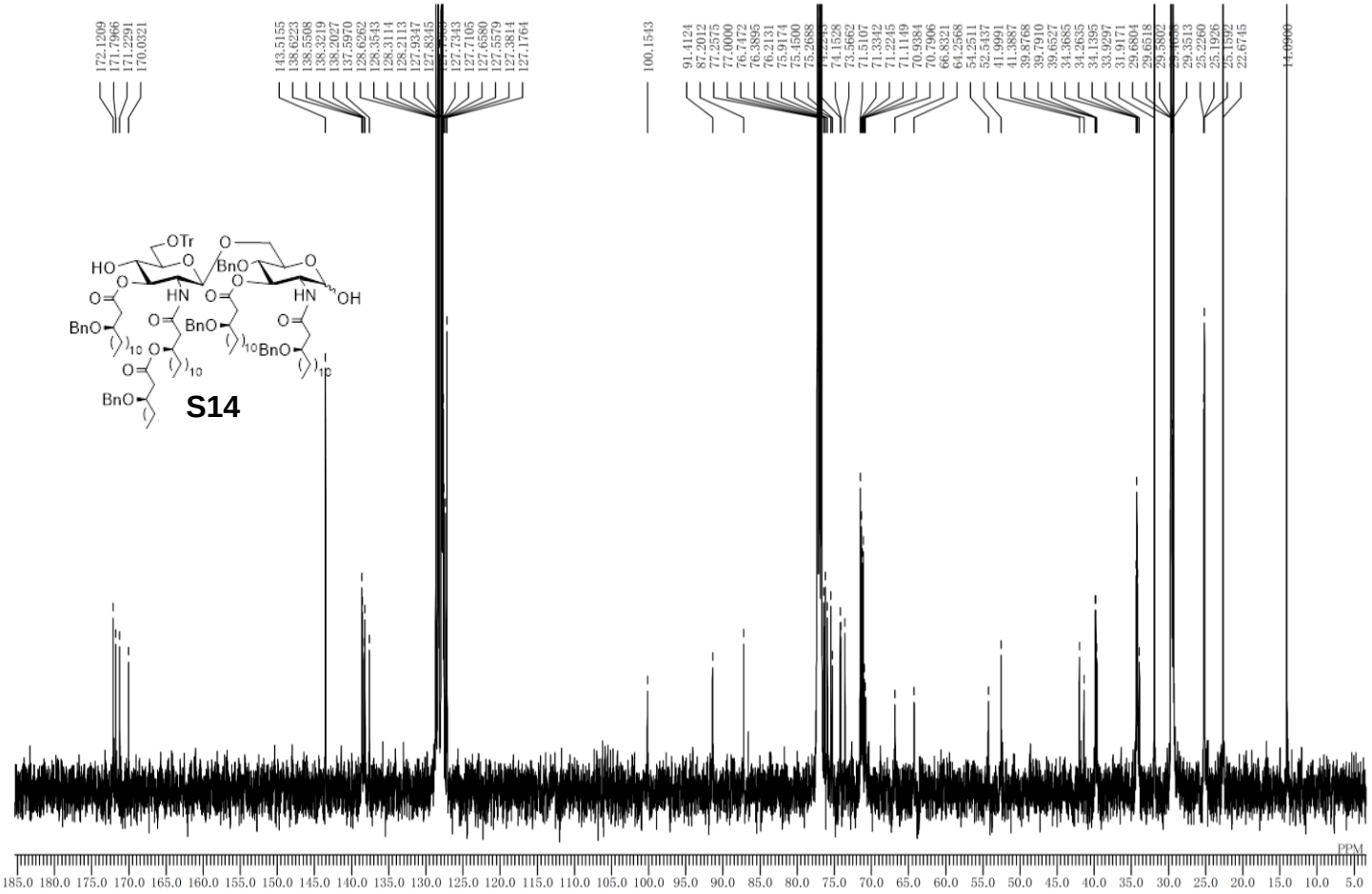
^{1}H NMR spectrum for compound **S14**



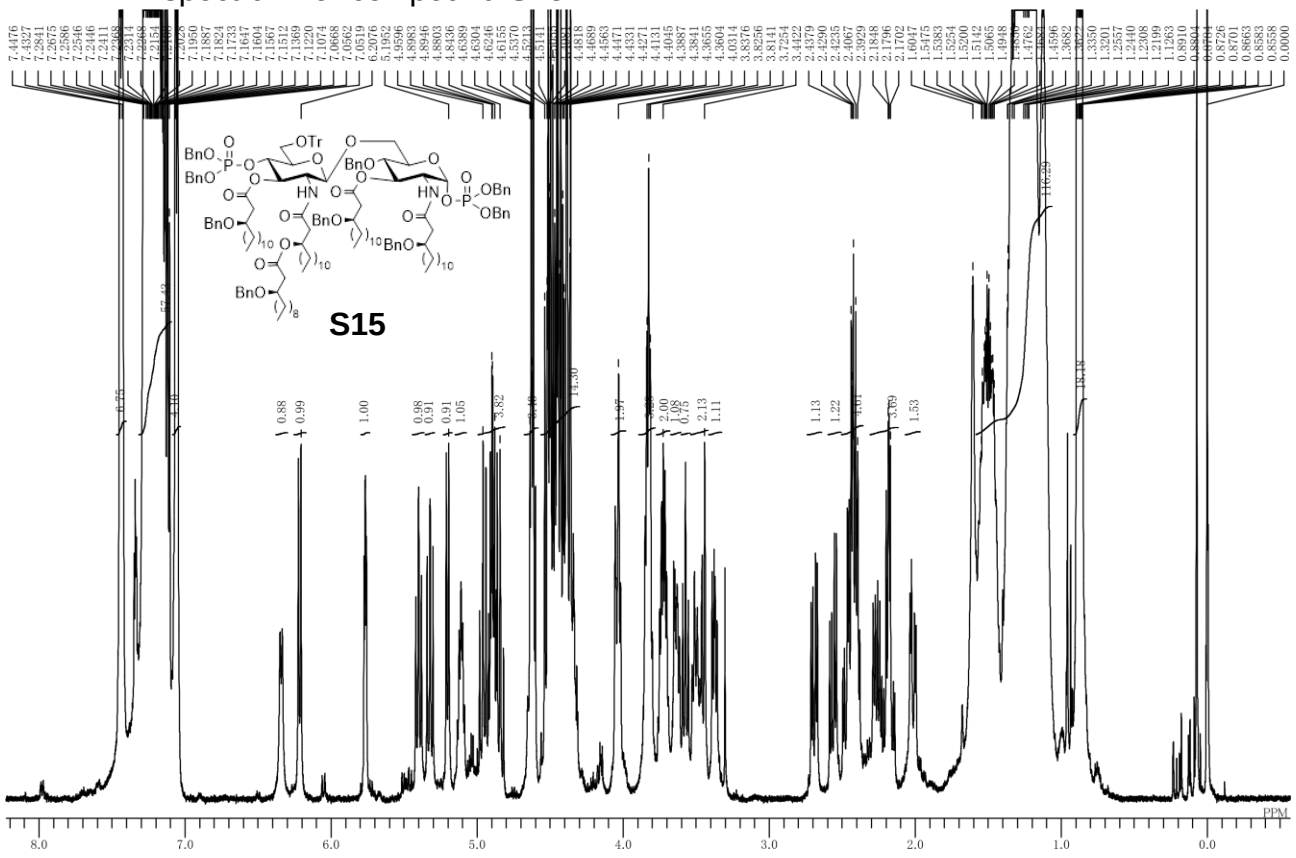
Enlarged view of ^{1}H NMR spectrum for compound **S14**



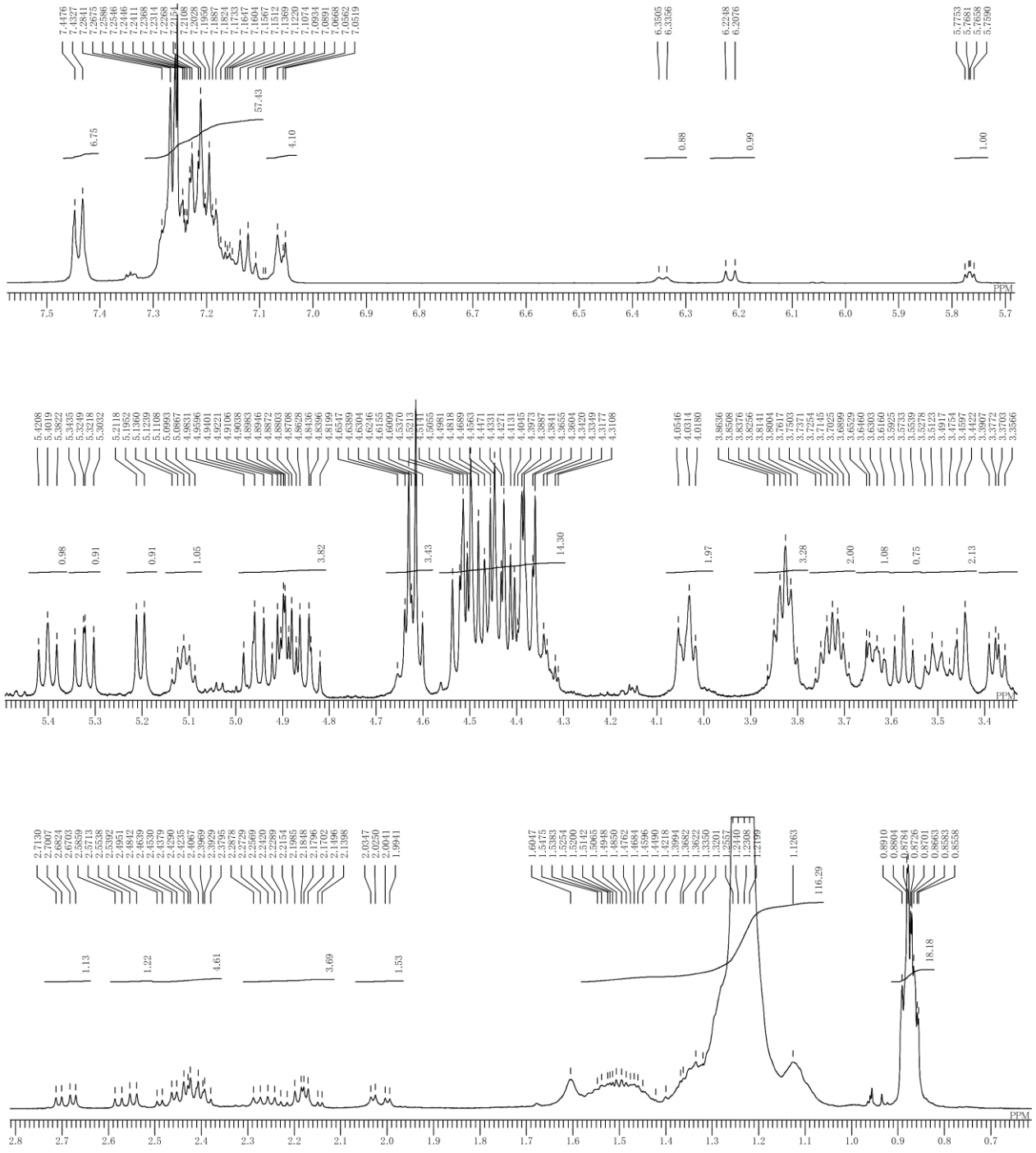
^{13}C NMR spectrum for compound **S14**



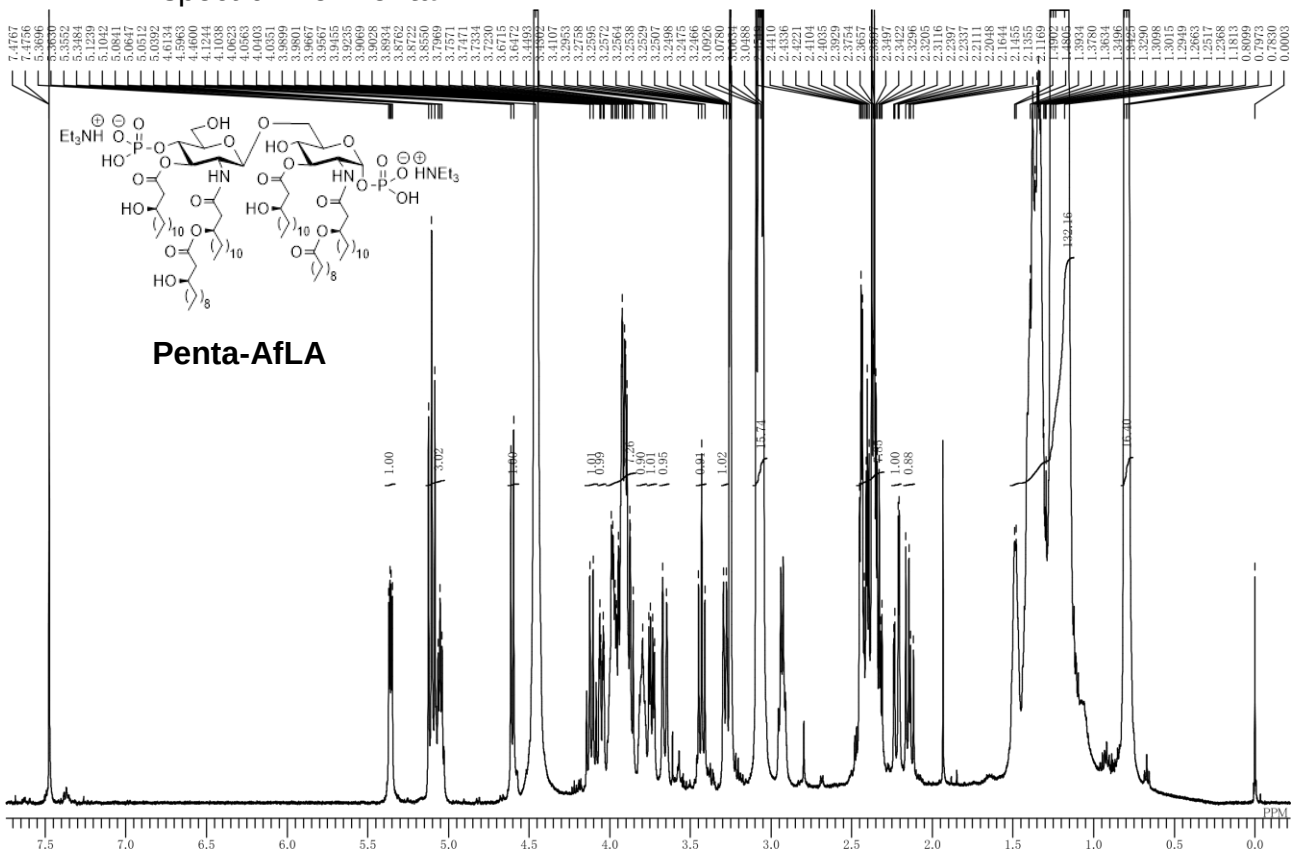
^{1}H NMR spectrum for compound **S15**



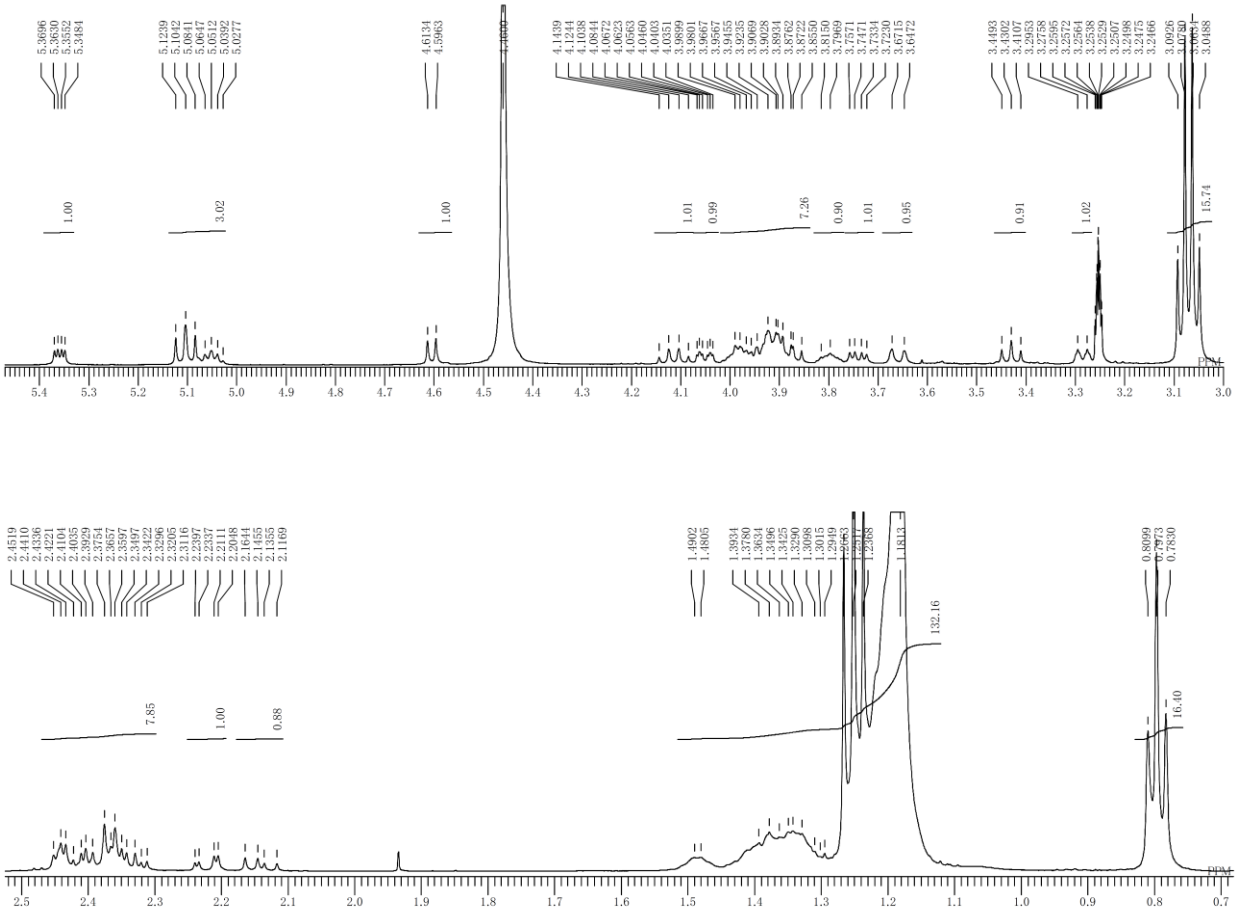
Enlarged view of ^{1}H NMR spectrum for compound **S14**



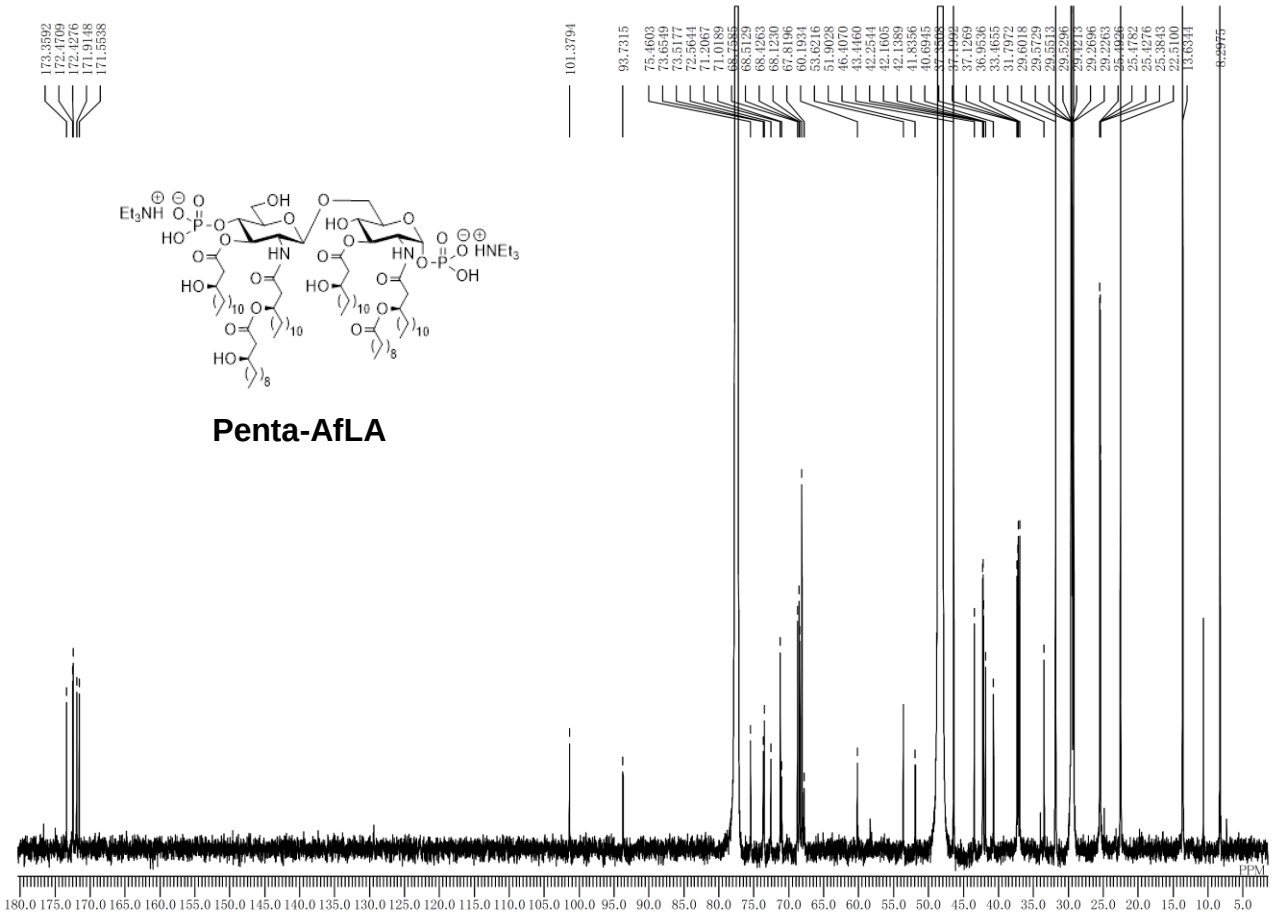
^{1}H NMR spectrum for **Penta-AfLA**



Enlarged view of ^{1}H NMR spectrum for **Penta-AfLA**

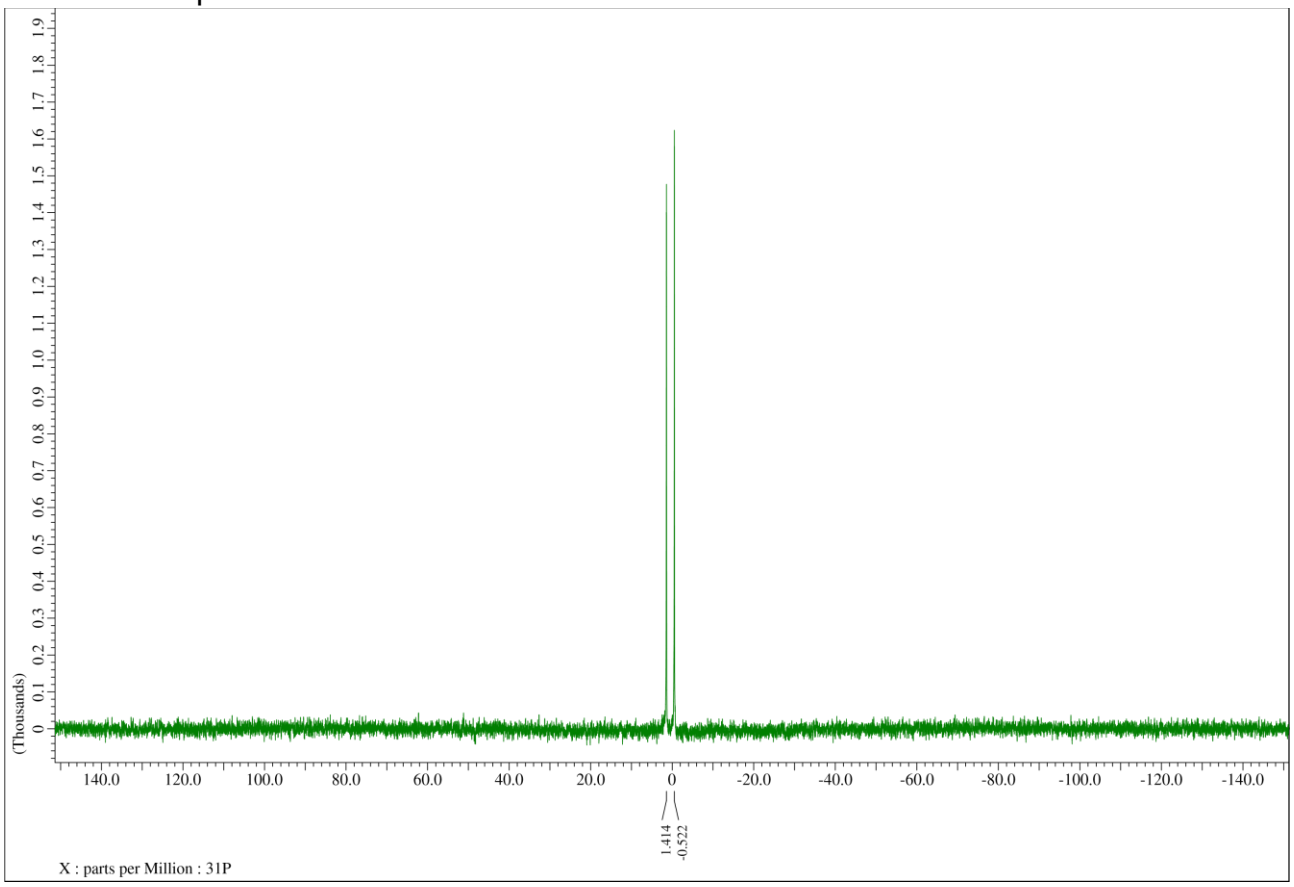


^{13}C NMR spectrum for **Penta-AfLA**

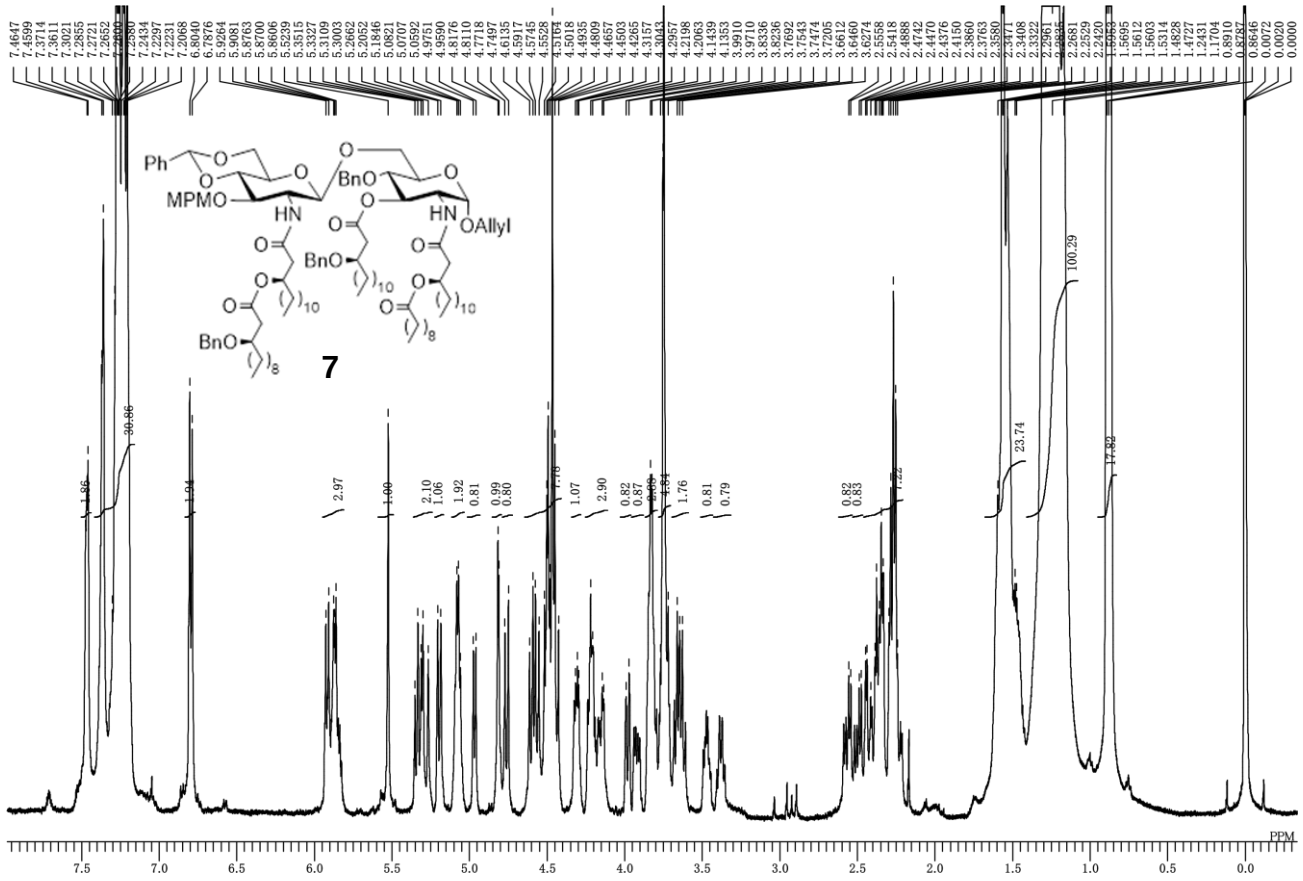


Penta-AfLA

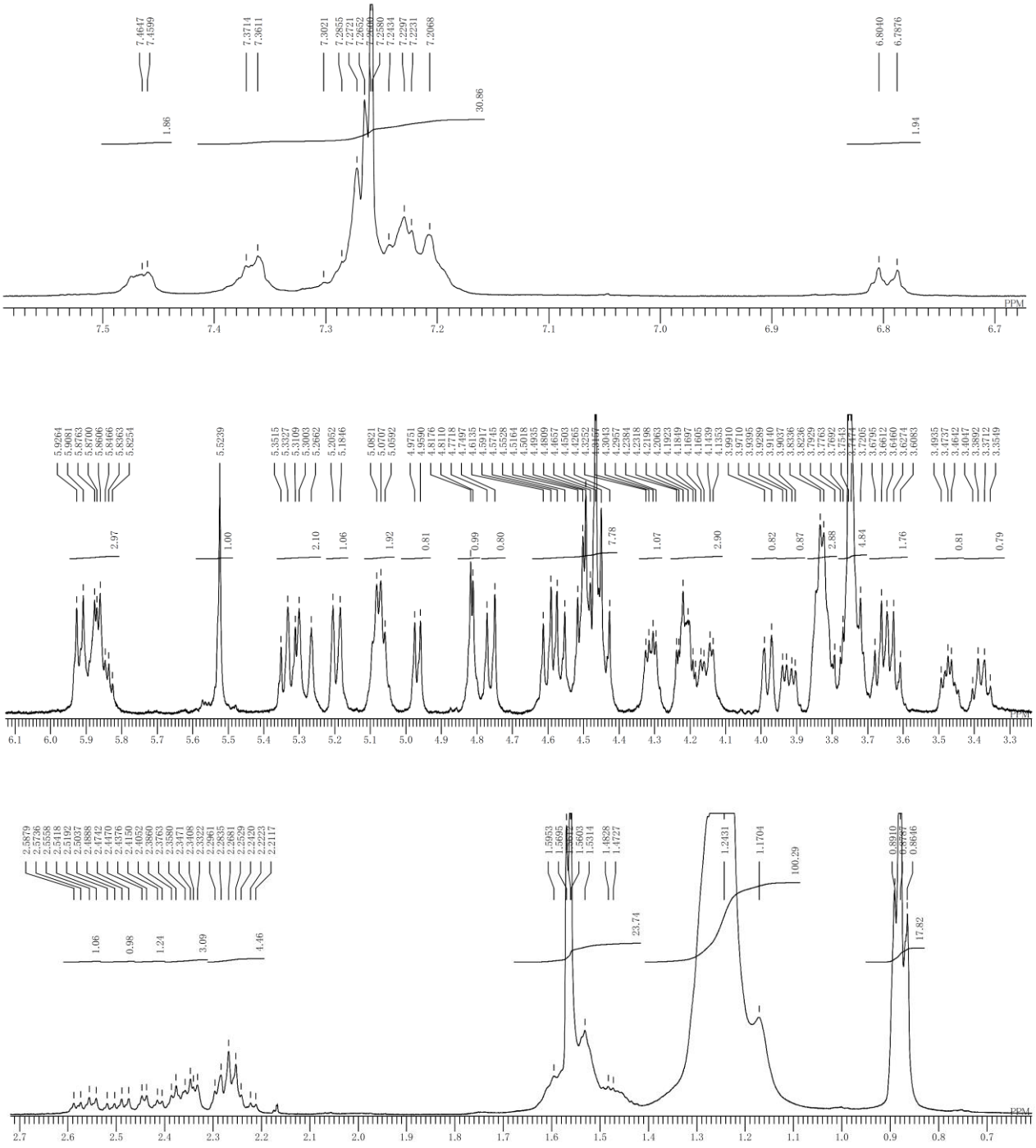
^{31} P NMR spectrum for **Penta-AfLA**



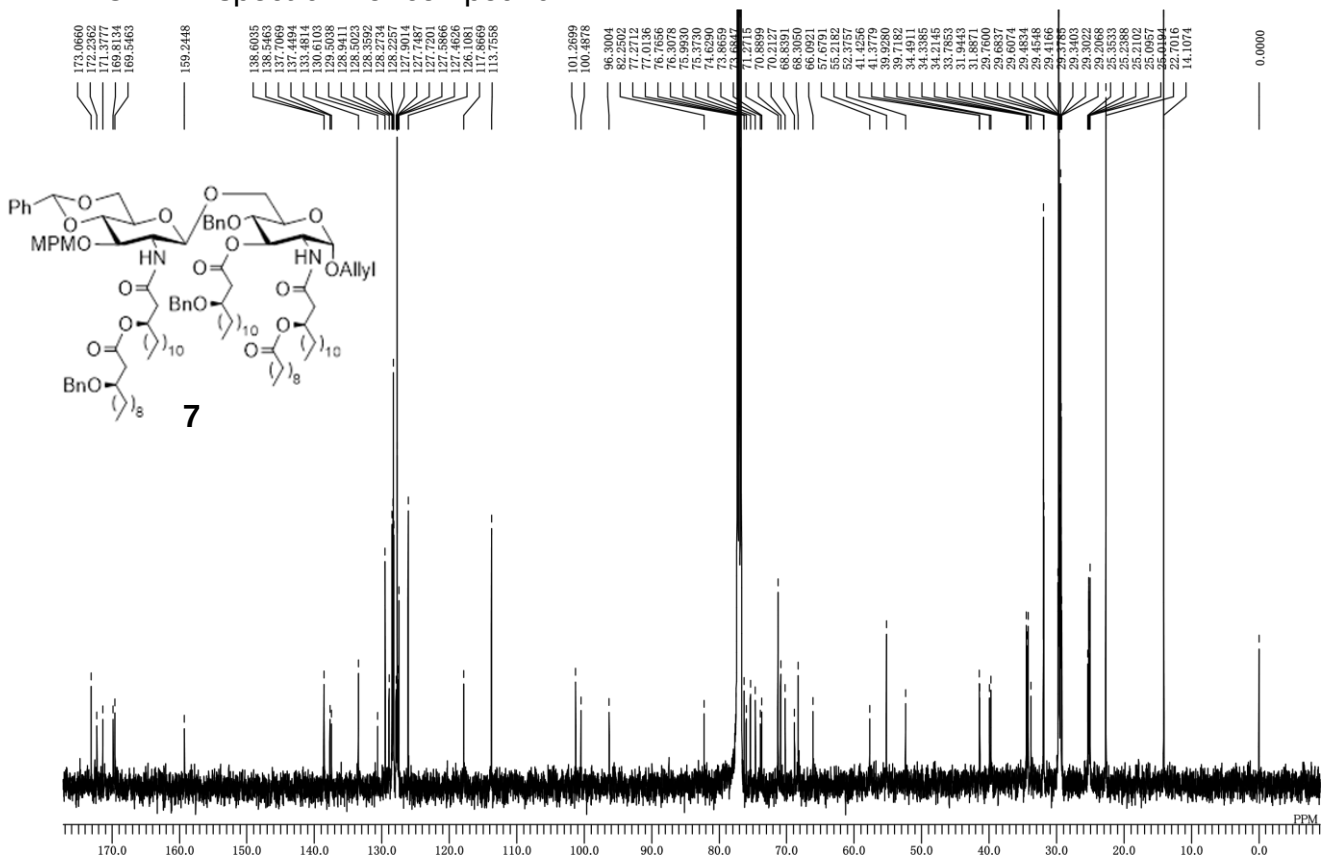
^{1}H NMR spectrum for compound **7**



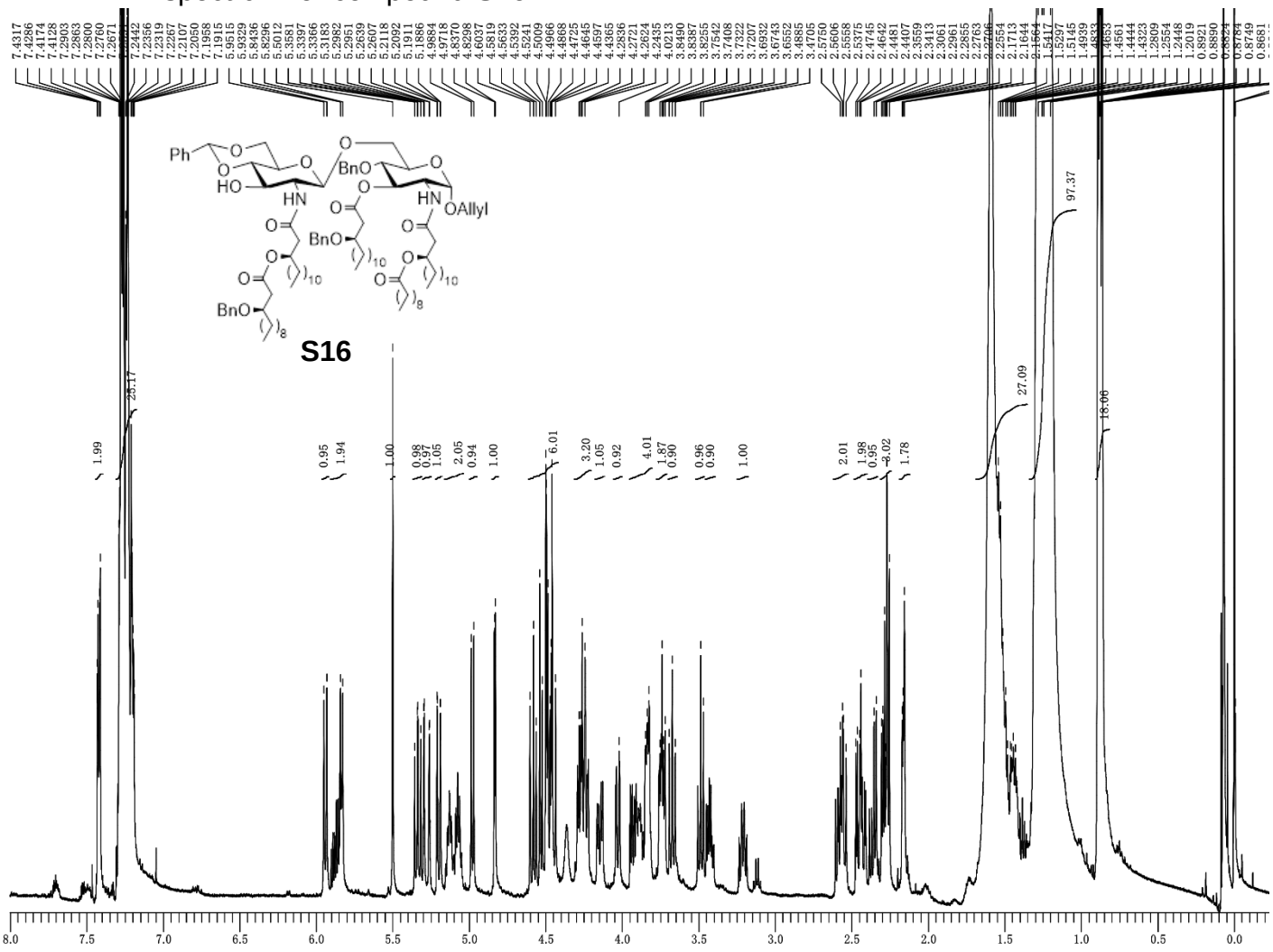
Enlarged view of ^{1}H NMR spectrum for compuond **7**



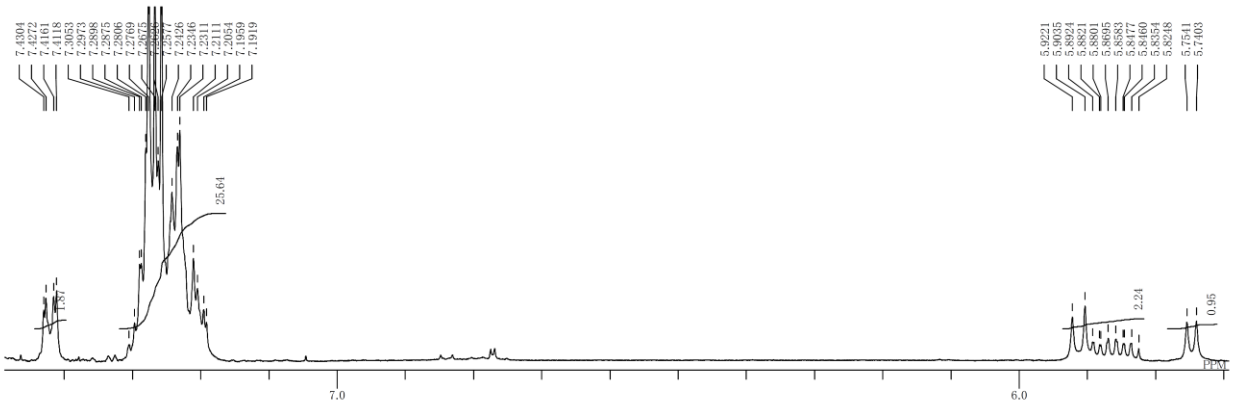
^{13}C NMR spectrum for compound **7**

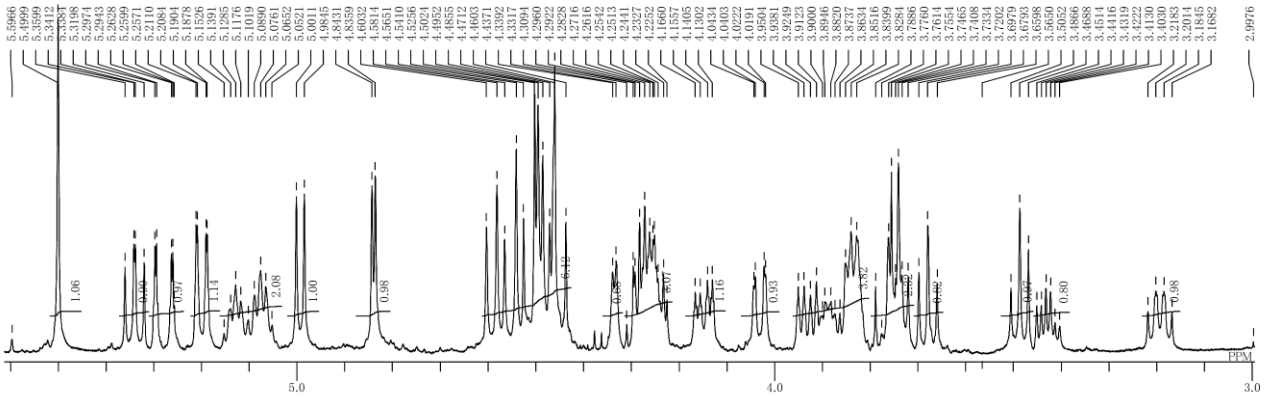


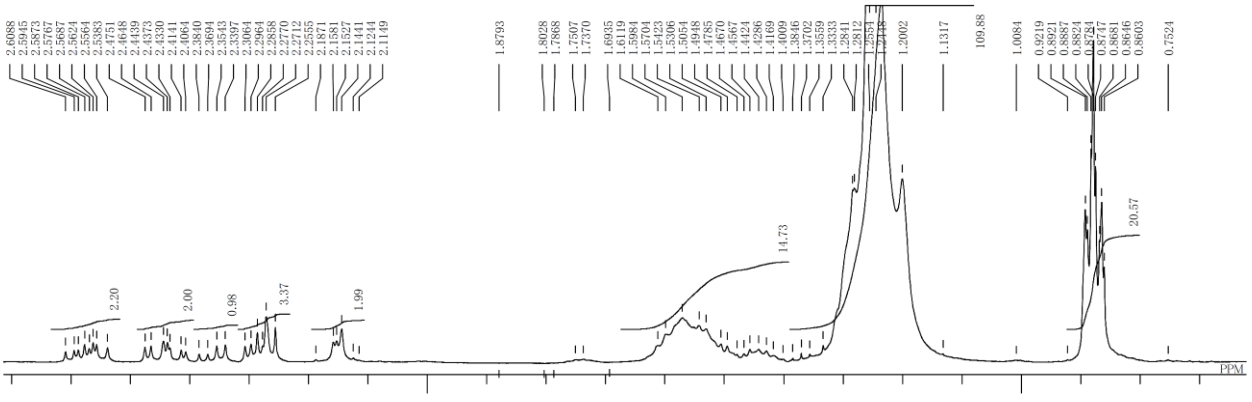
^{1}H NMR spectrum for compound **S16**



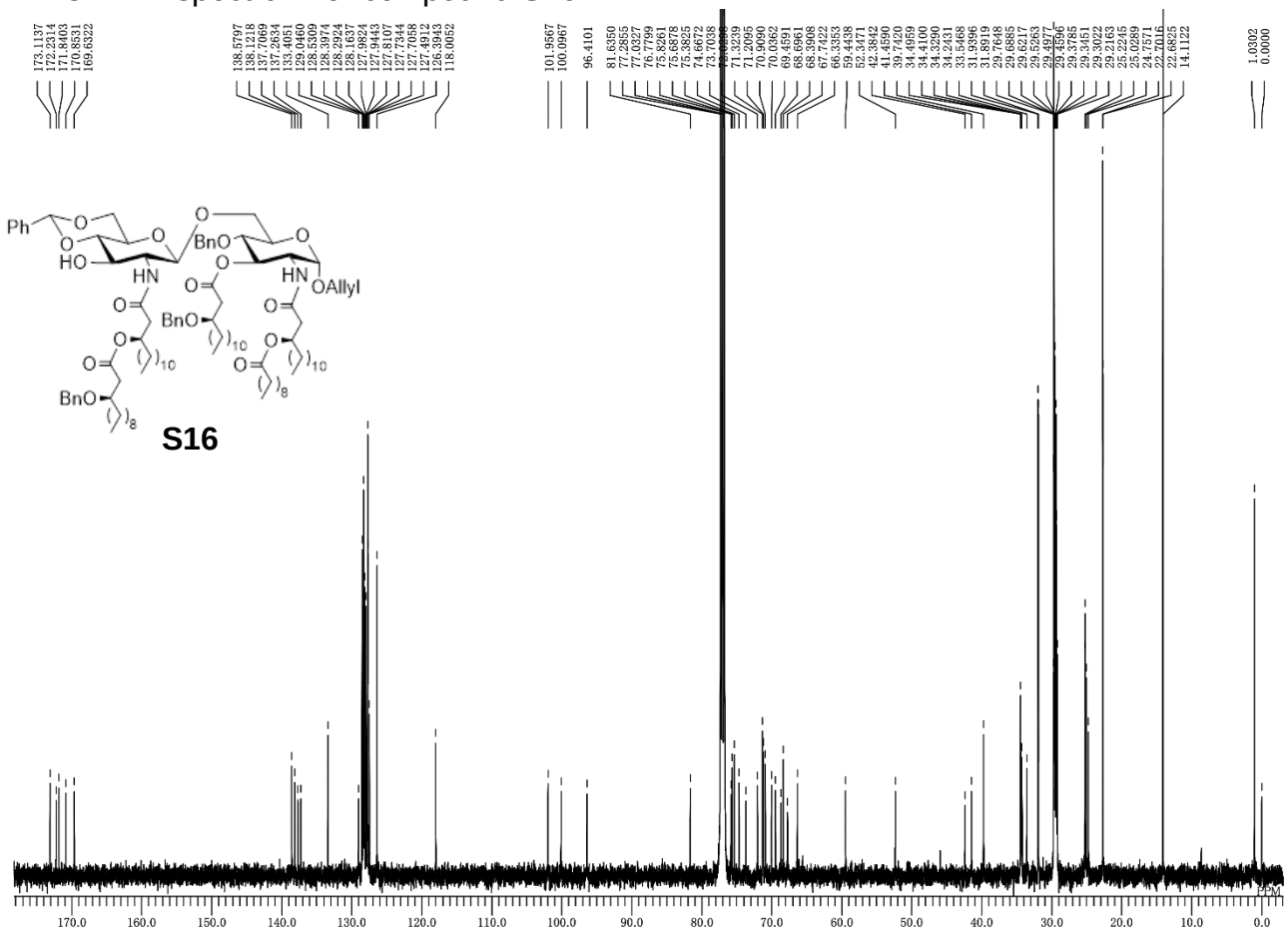
Enlarged view of ^{1}H NMR spectrum for compuond **S16**



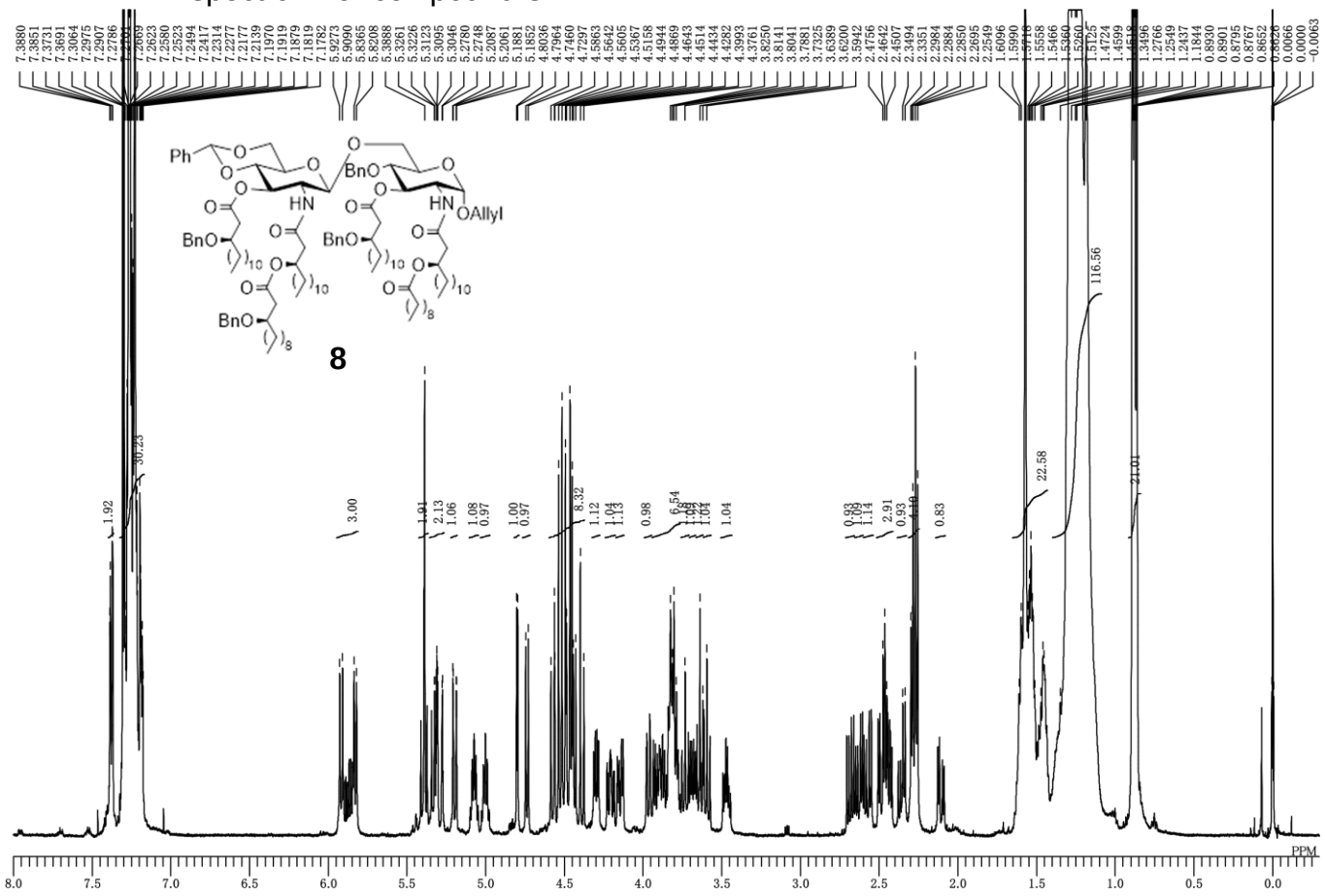




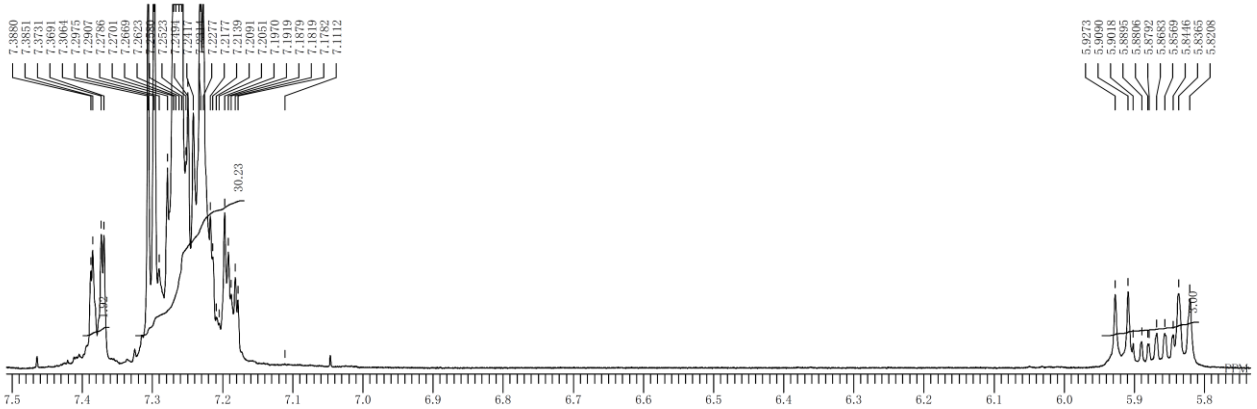
^{13}C NMR spectrum for compound **S16**

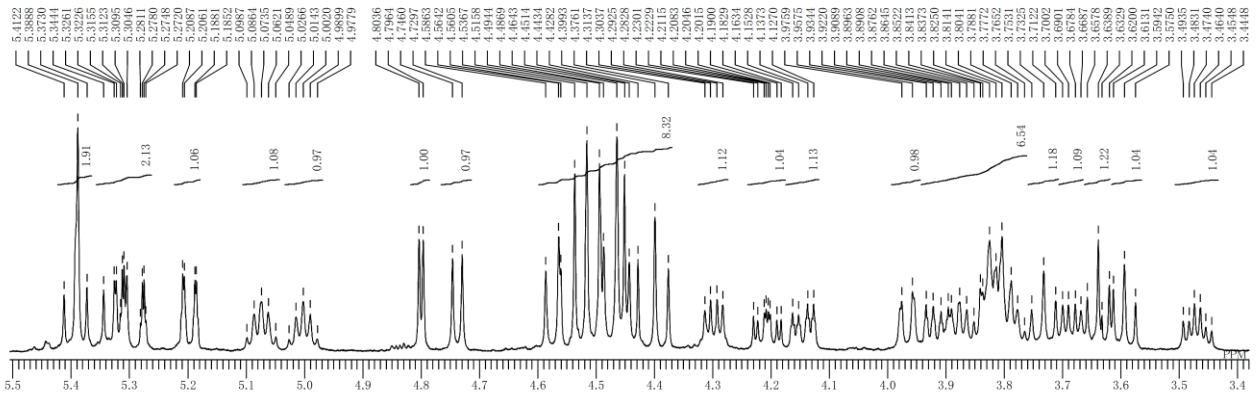


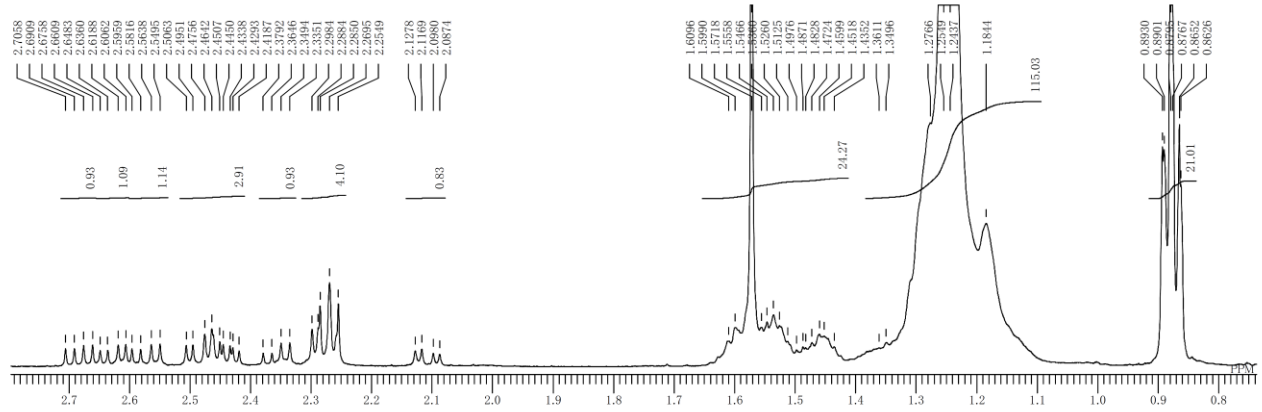
^{1}H NMR spectrum for compound **8**



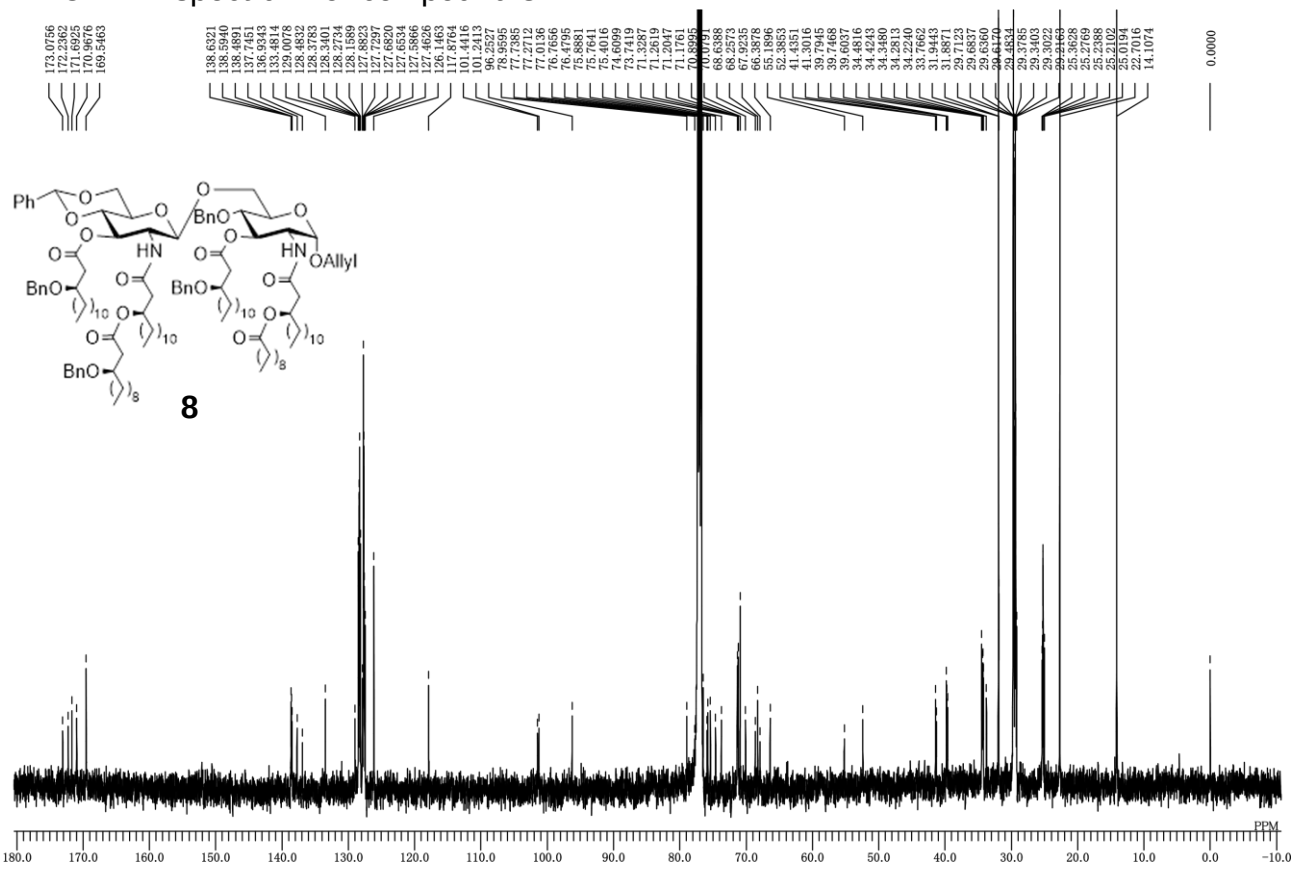
Enlarged view of ^{1}H NMR spectrum for compound **8**



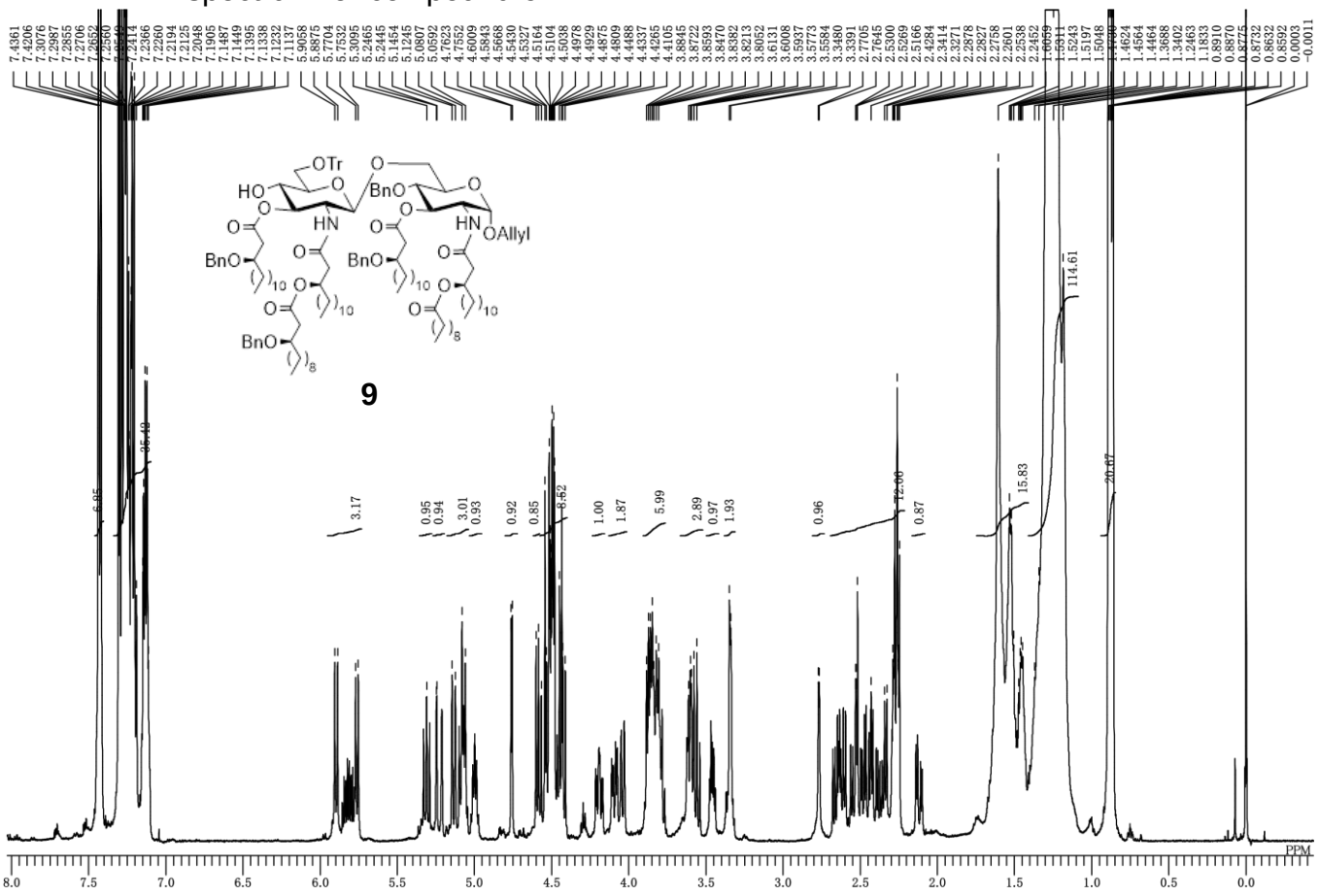




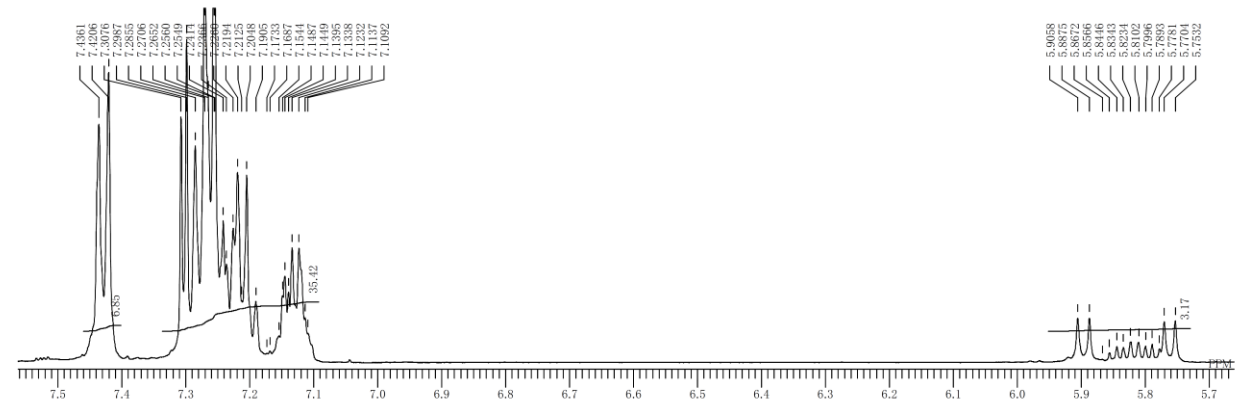
^{13}C NMR spectrum for compound **8**

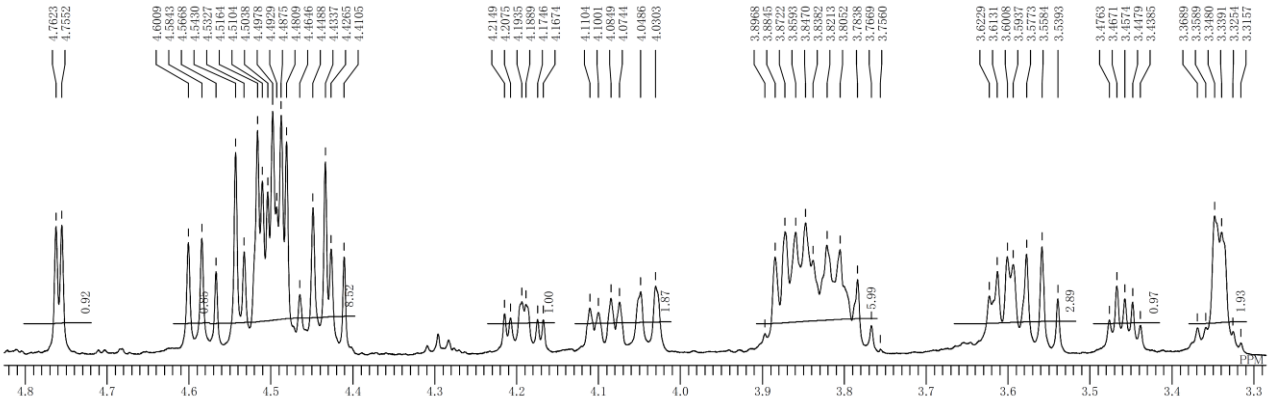


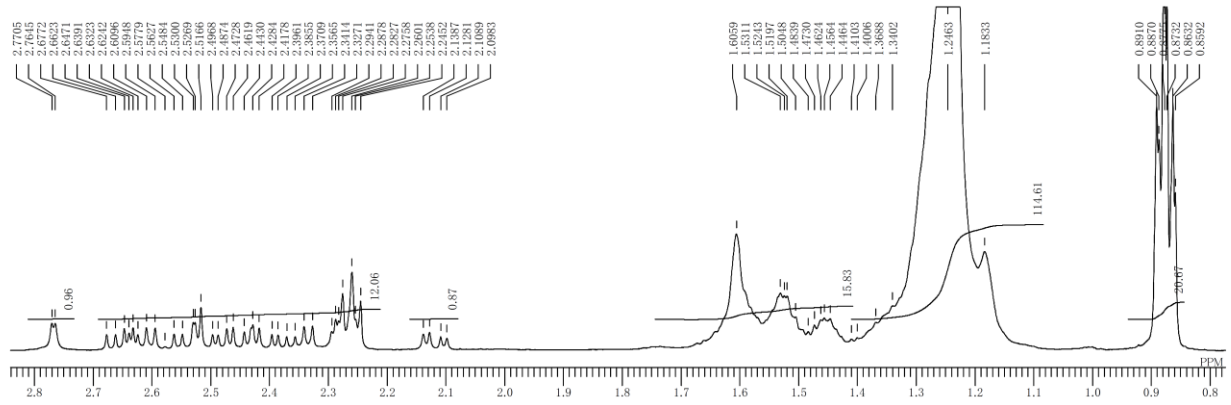
^{1}H NMR spectrum for compound **9**



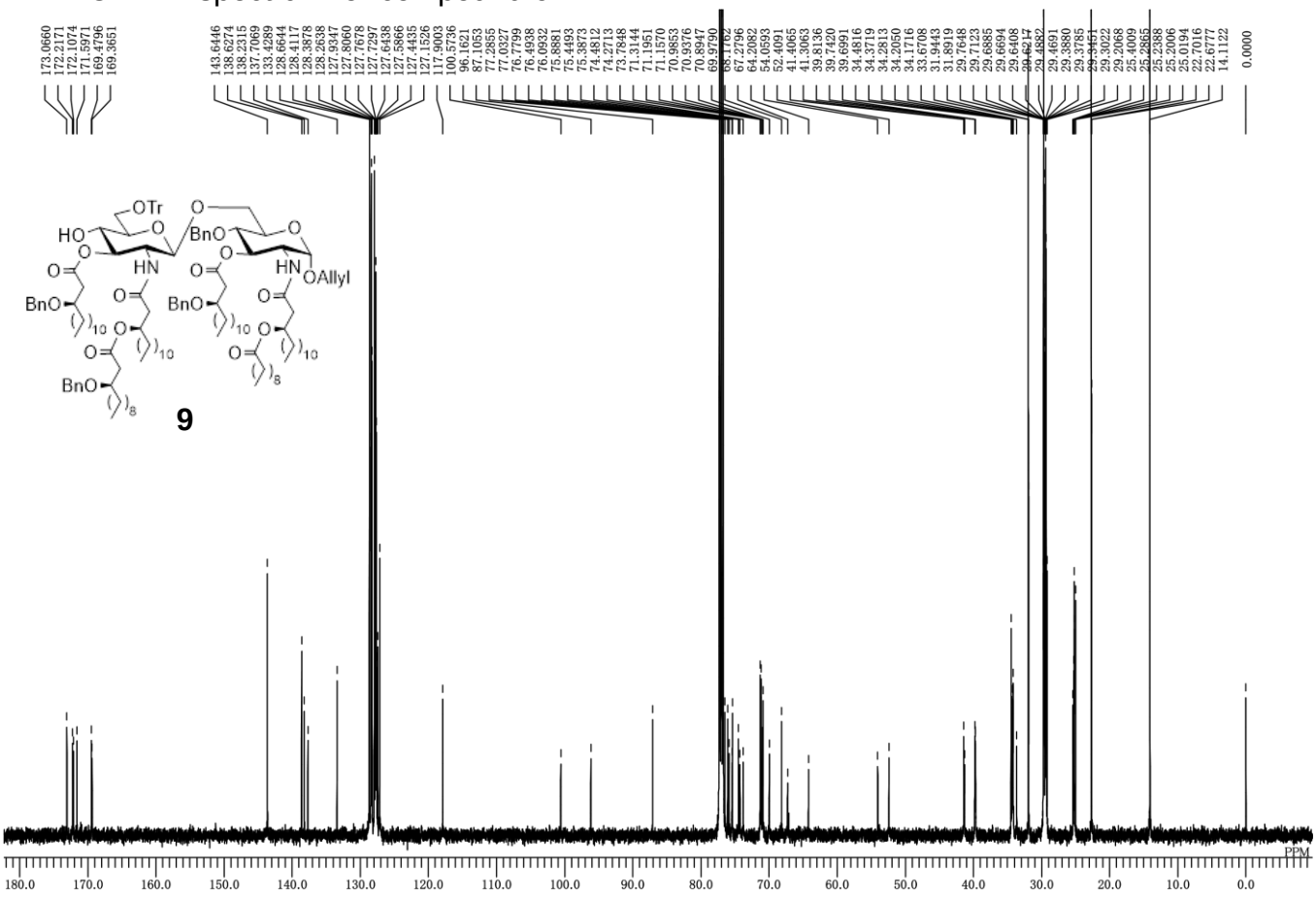
Enlarged view of ^{1}H NMR spectrum for compound **9**



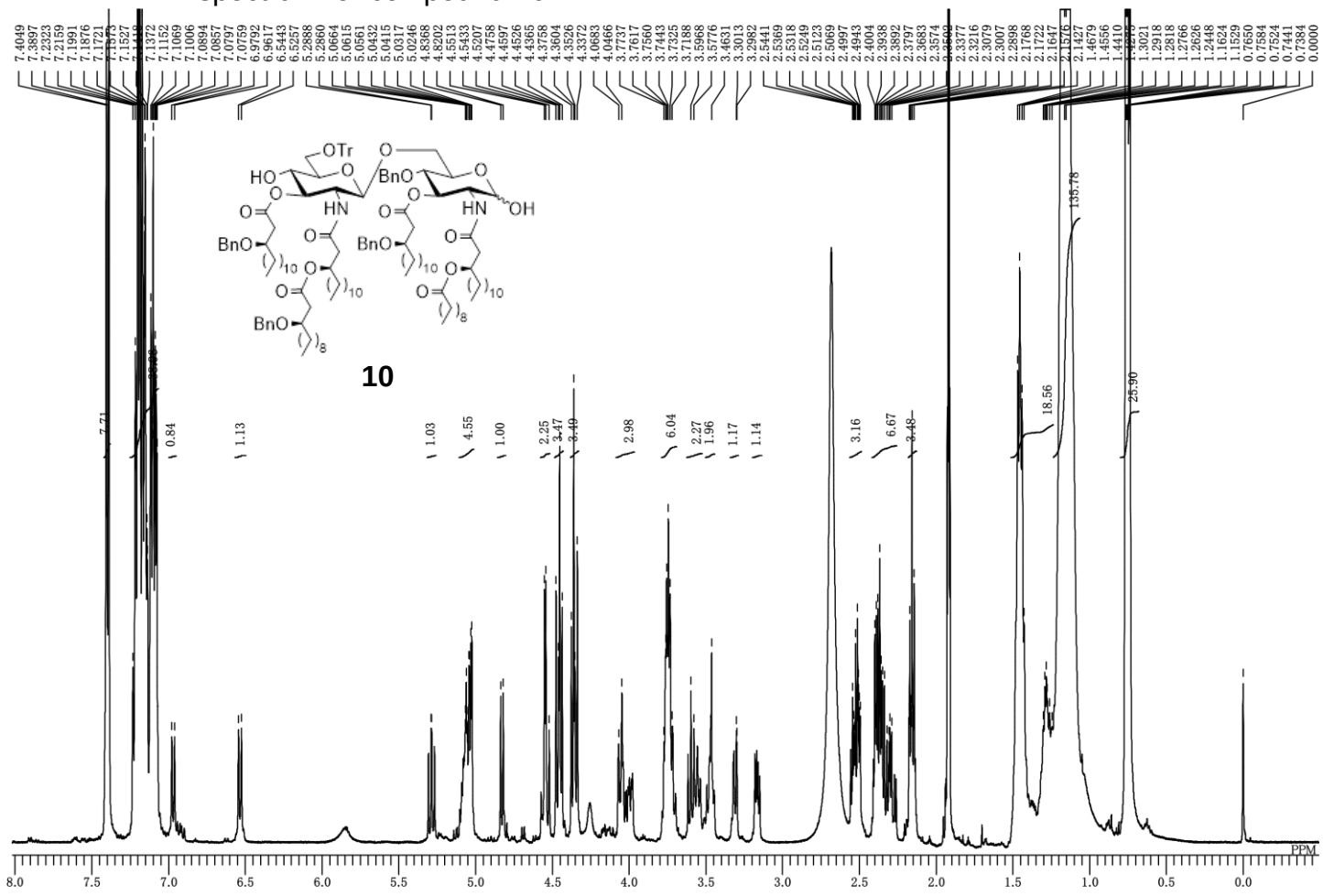




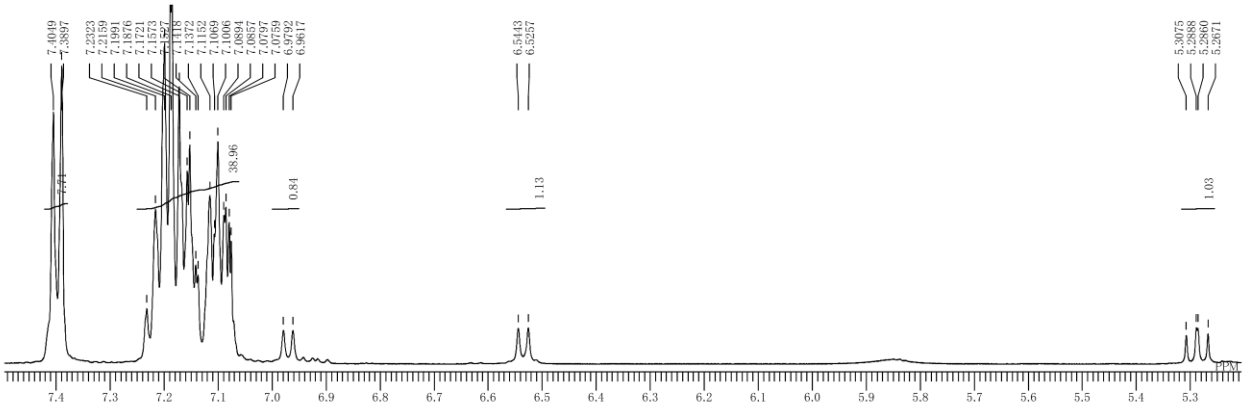
^{13}C NMR spectrum for compound **9**

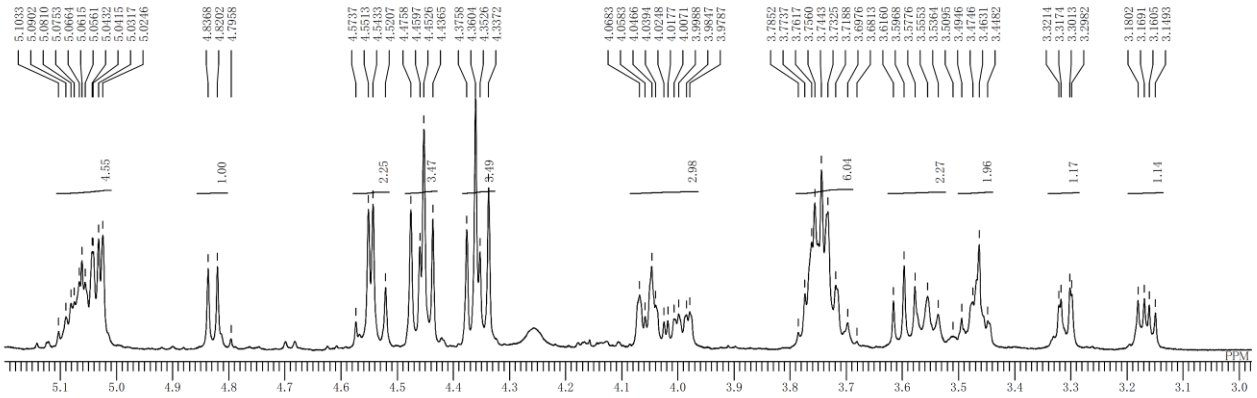


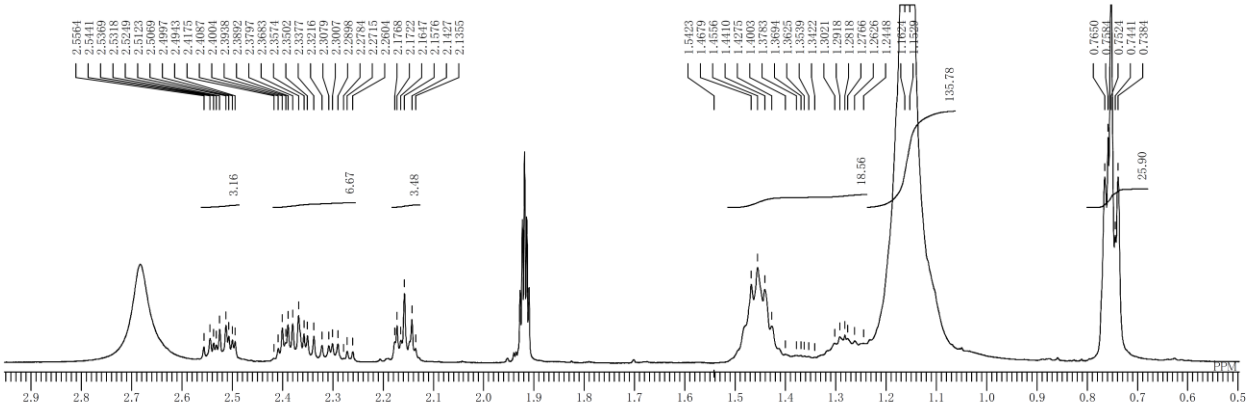
^{1}H NMR spectrum for compound **10**



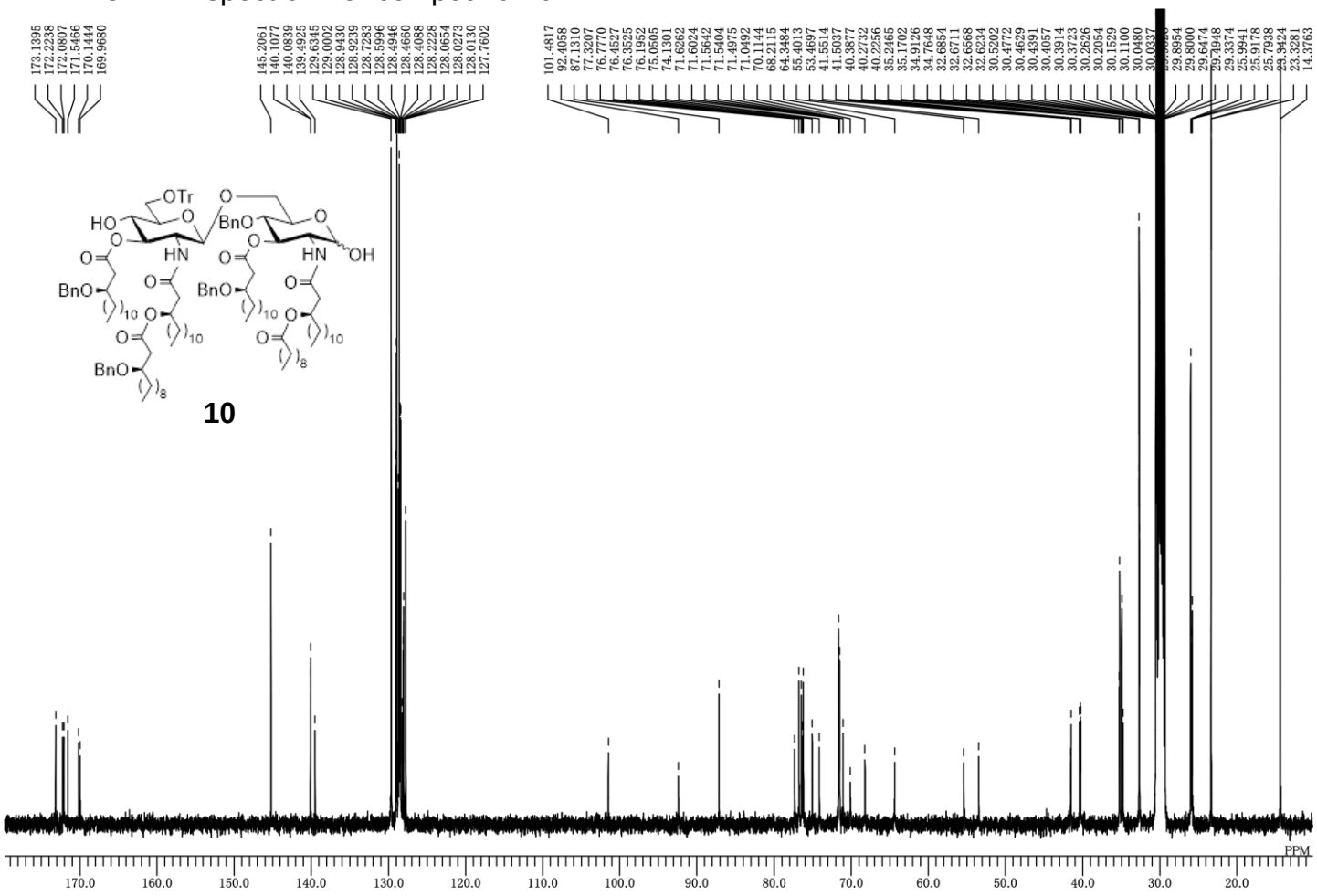
Enlarged view of ^{1}H NMR spectrum for compound **10**



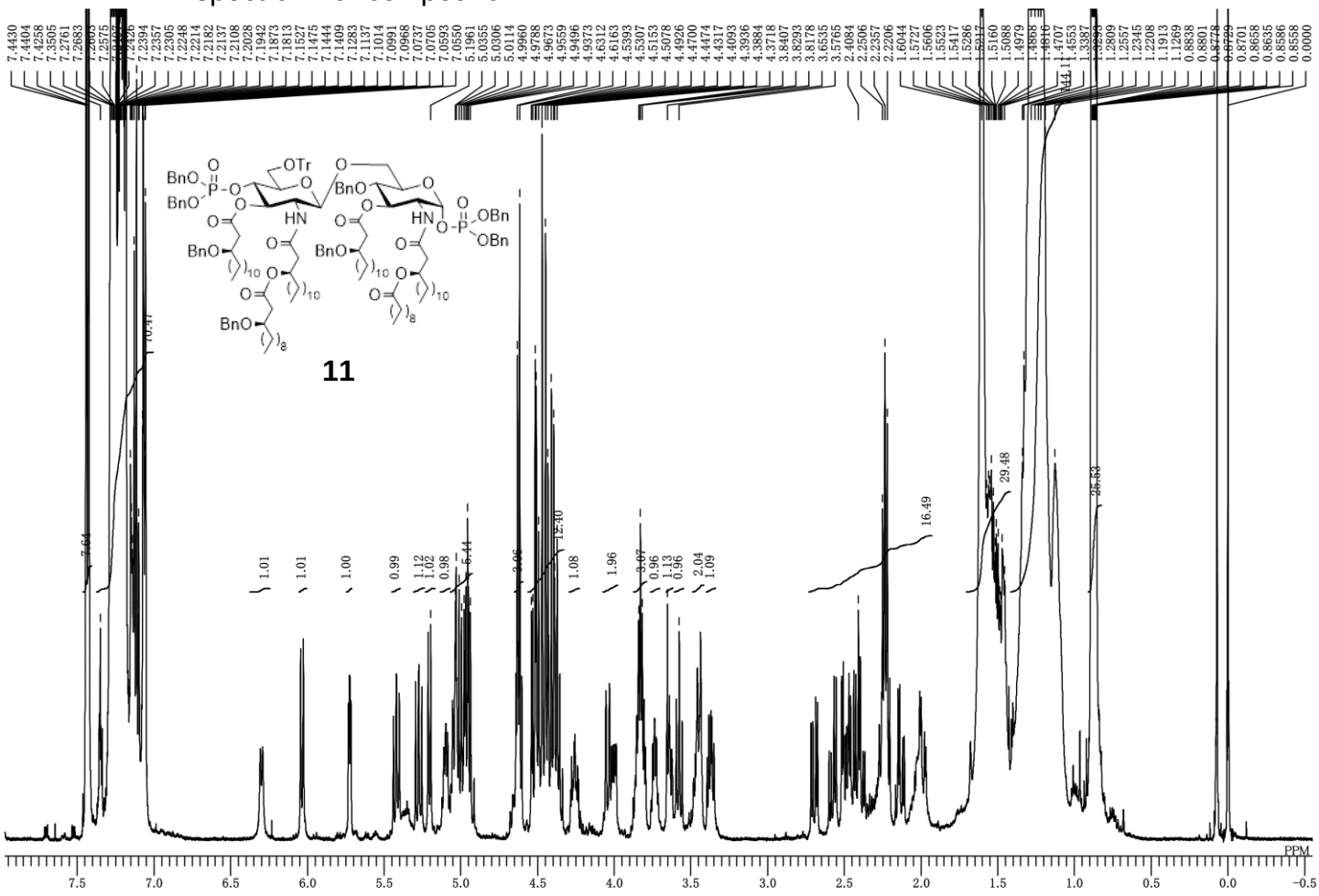




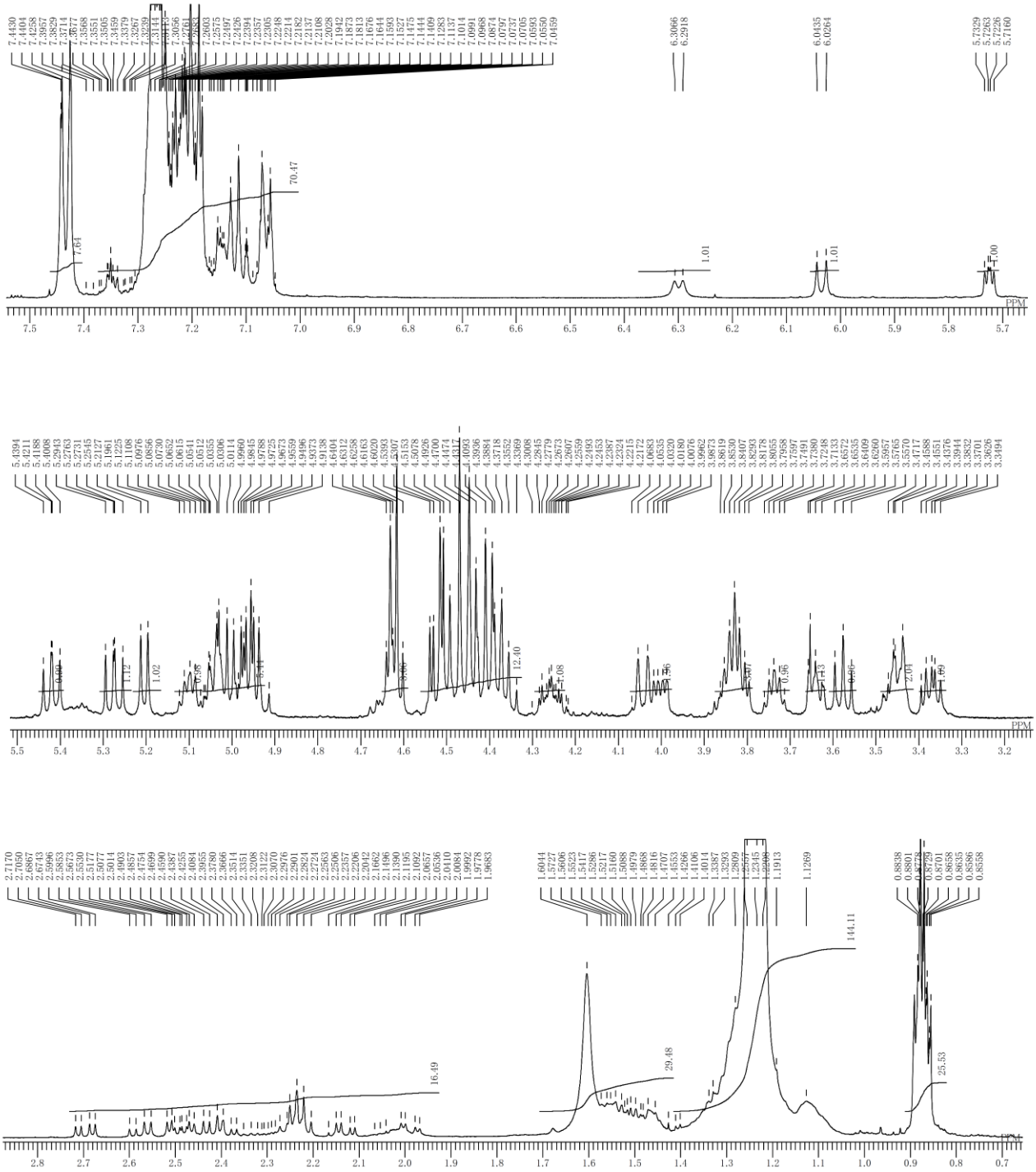
^{13}C NMR spectrum for compound **10**



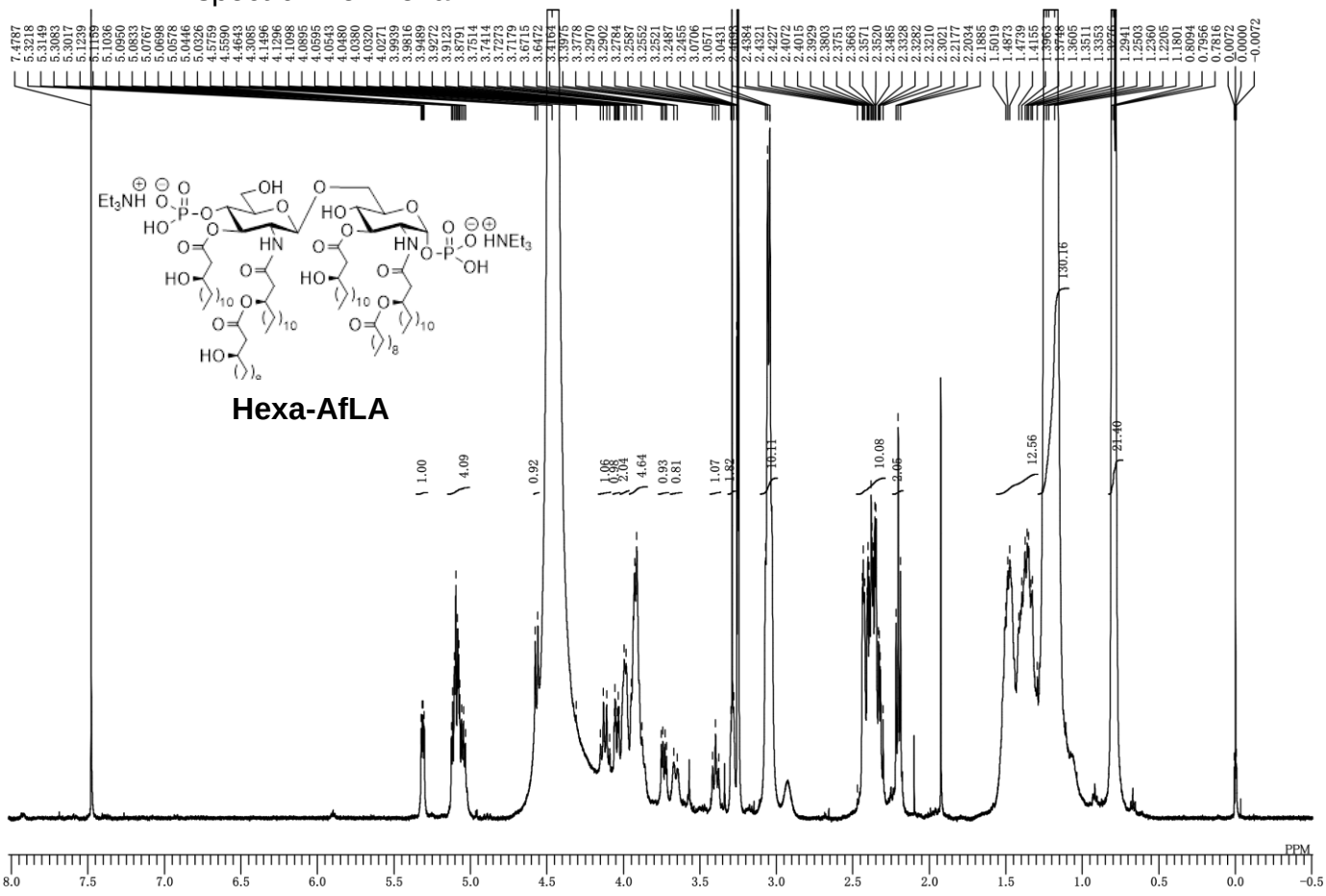
^{1}H NMR spectrum for compound **11**



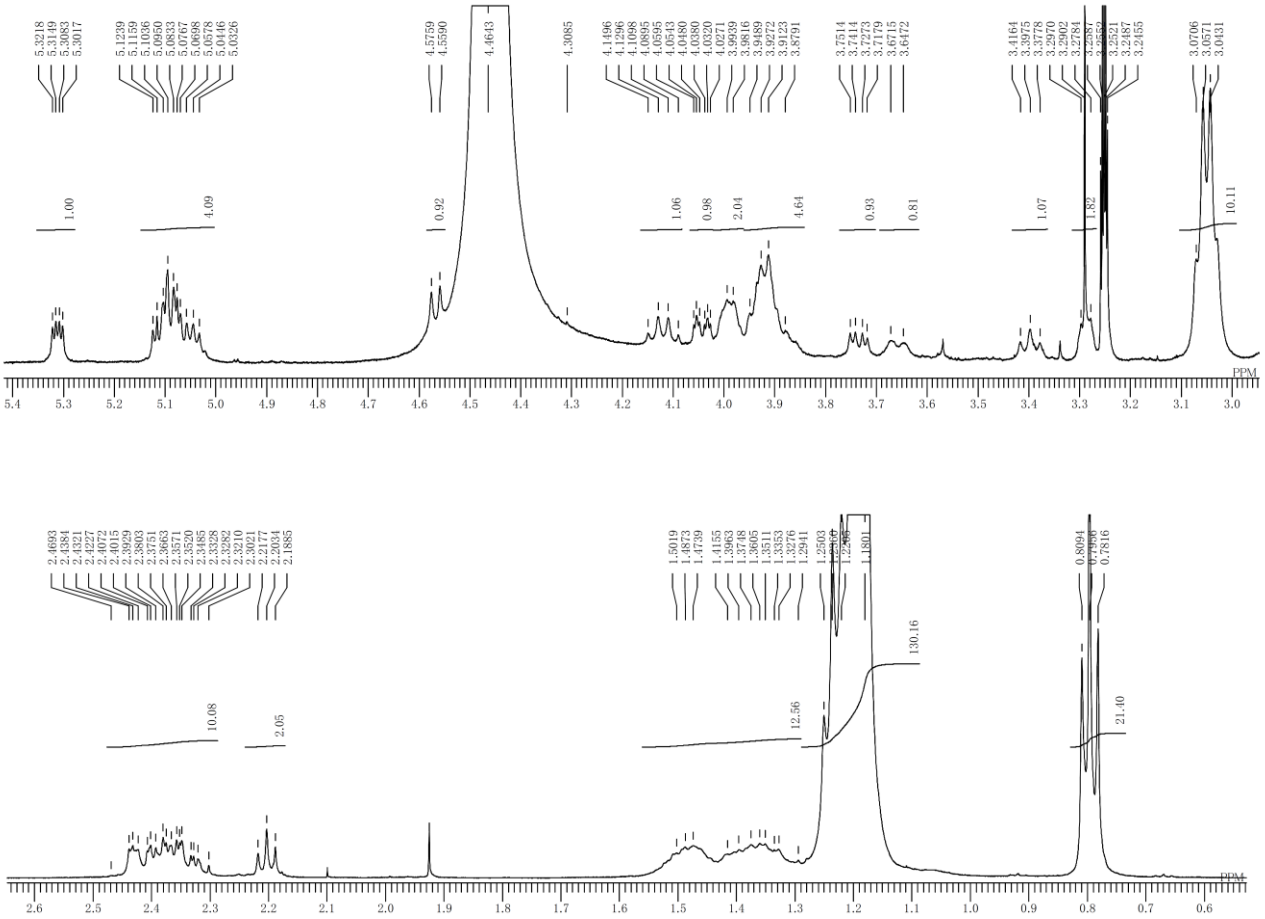
Enlarged view of ^{1}H NMR spectrum for compound **11**



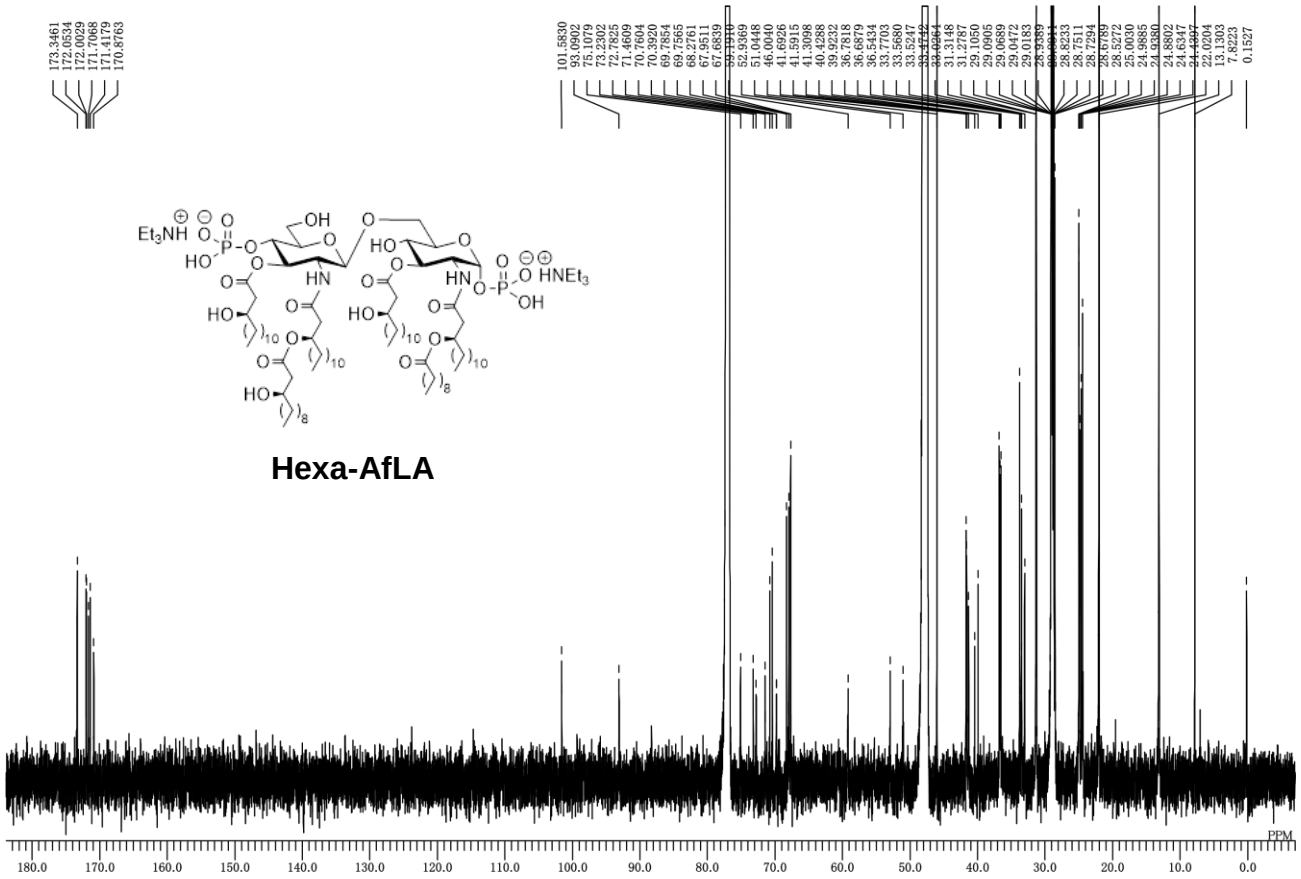
^{1}H NMR spectrum for **Hexa-AfLA**



Enlarged view of ^{1}H NMR spectrum for **Hexa-AfLA**

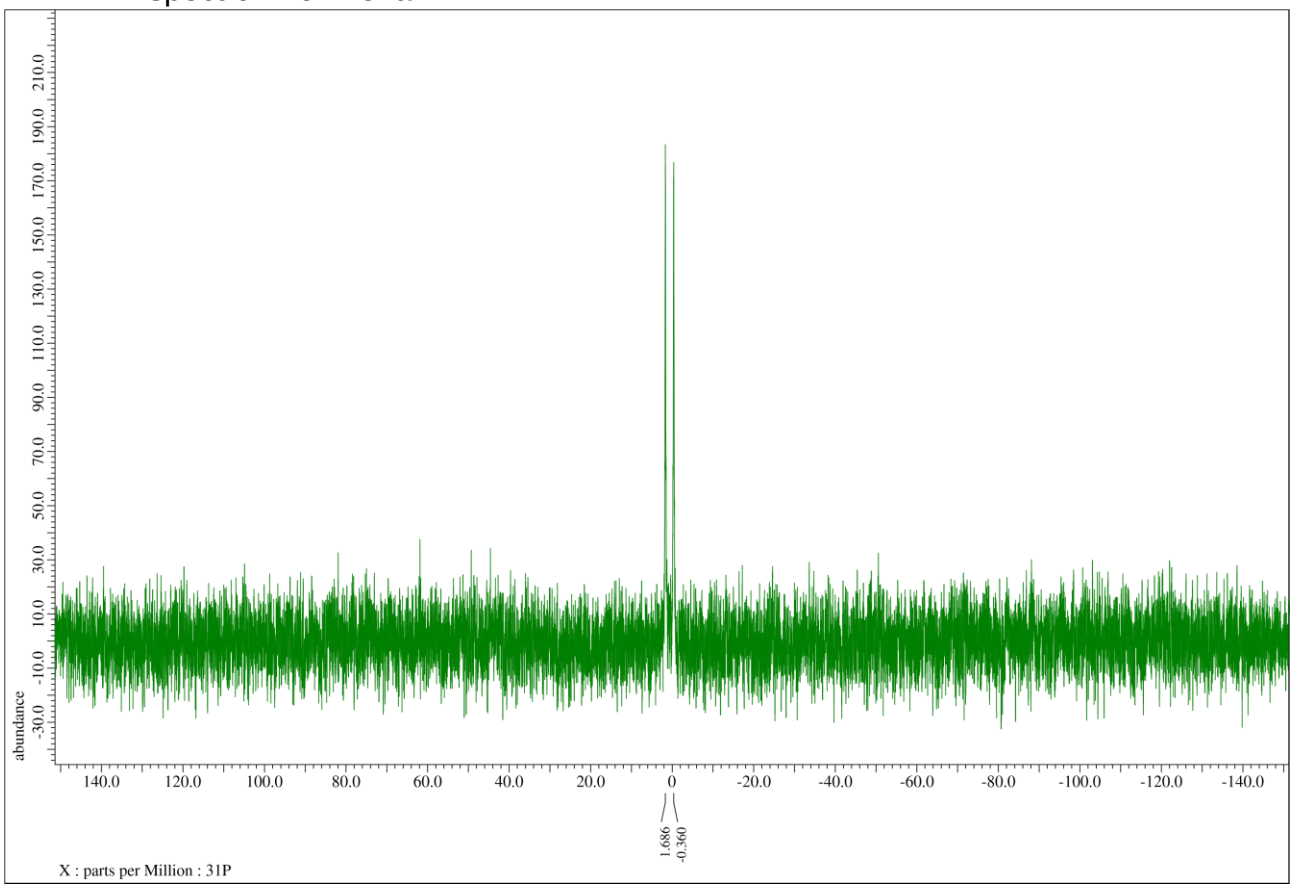


^{13}C NMR spectrum for **Hexa-AfLA**



Hexa-AfLA

^{31}P NMR spectrum for **Hexa-AfLA**



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