Influence of Gb_3 glycosphingolipids differing in their fatty acid chain on the phase behavior of solid supported membranes: Chemical syntheses and impact of Shiga toxin binding

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Materials

Sphingomyelin from bovine brain, cholesterol and perylene, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), Oregon Green DHPE, β-BODIPY 500/510 C12-HPC (Bodipy-PC), Texas Red DHPE were purchased commercially. STxB and STxB labeled with Cy3 (STxB-Cy3) were purified as described previously.[1] STxB concentrations refer to the pentamer. Ultrasharp CSC38/no Al cantilevers were purchased from MikroMasch (Tallinn, Estonia).

Solid supported bilayers

Lipids and fluorescence dye stock solutions in chloroform or chloroform/methanol were mixed in glass test tubes to obtain the desired ratios. Solvent was removed under a constant stream of nitrogen at 55 °C to form lipid films, which were further dried for 3 h under reduced pressure at 55 °C and stored at 4 °C. The lipid film was rehydrated in buffer (20 mM TRIS/HCl, 100 mM NaCl, 1 mM $CaCl_2$, pH 7.4) for 30 min at 55 °C followed by three cycles of vortexing (30 s) in 5 min intervals. To obtain unilamellar vesicles, the multilamellar vesicle suspension (nominal lipid concentration 0.3 mg mL^{-1}) was extruded at 55 °C using a mini extruder equipped with a 50 nm polycarbonate membrane. Freshly cleaved mica sheets were placed into custom made PTFE chambers and preheated to 55 °C. After the vesicle suspension was added, the calcium ion concentration was increased to 10 mM for 10 min to induce spreading and subsequently lowered to 1 mM. After 2 h incubation, samples were slowly cooled to 20 °C and rinsed with 100 mL PBS buffer (1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 136.9 mM NaCl, pH 7.4). STxB or STxB-Cy3 (60 nM) was added to the bilayer, and incubated for 1 h at 20 °C. Unbound protein was removed by rinsing with PBS buffer. For each Gb_3 derivative membranes with two different l_d marker dyes were investigated to rule out a dye specific alteration of the phase behavior. Overall phase behavior and percentages of the phases were independent of the dye used (Table S2).

Fluorescence Microscopy

Fluorescence images were recorded using epifluorescence microscopy. I) An Axiotech Vario (Carl Zeiss, Jena, Germany) equipped with water immersion objectives (Achroplan 40×/0.8 W, Achroplan 63×/0.95 W, Zeiss), an Illuminator HXP 120 C lamp and filter set 44 (BP 475/40, FT 500, BP 530/50) for Bodipy-PC and Oregon Green DHPE, filter set 45 (BP 560/40, FT 585, BP 630/75) for STxB-Cy3 and Texas Red DHPE and filter set 47 (BP 436/20, FT 455, BP 480/40) for perylene was used. II) An Olympus BX51 (Olympus, Tokio, Japan) with water immersion objectives (LumPlan FLN 40×/0.80 W, LumPlan FLN 100×/1.00 W), an XCite Series 120 Q lamp and filter set U-MNB2 (excitation 470-490 nm, emission >520 nm, dichroic mirror 500 nm) for Bodipy-PC and Oregon Green DHPE, U-MNG2 (excitation 530-550 nm, emission >590 nm, dichroic mirror 570 nm) for STxB-Cy3 and Texas Red DHPE, and filter set U-MNUA2 (excitation 360-370 nm, emission 420-460 nm, dichroic mirror 400 nm) for perylene was used. III) An Examiner Z1 with laser scanning head LSM 710 (Carl Zeiss, Jena, Germany) equipped with an objective W Plan-Apochromat 63×, NA 1,0 (Zeiss). Bodipy-PC and Oregon Green fluorescence were excited at 488 nm and recorded at 496-575 nm; Texas Red DHPE and Cy3 were excited at 561 nm and detected at 574-620 nm), and perylene was excited at 405 nm, and recorded at 408-479 nm). All images were taken at 20 °C. Percentages of ordered and disordered phases were determined by manually thresholding image sections of fluorescence micrographs using ImageJ.[2] Values are given as mean ± standard deviation (number of images) of the percentages extracted. The minimum number of independent vesicle preparations was four for each Gb_3 derivative. These vesicles were used to independently prepare solid supported membranes and at least seven solid supported membranes were analyzed.

Atomic force microscopy

Images were taken using a JPK NanoWizard I (JPK Instruments, Berlin, Germany) at 20 °C in PBS with cantilevers with a nominal spring constant of k = 0.03 N m^{-1} (Ultrasharp CSC38/no al). Images were obtained in contact mode with a scan speed of 30 µm/s (512 × 512 pixels). Images were analyzed using JPK Data Processing Software 4.0 (JPK Instruments, Berlin, Germany) or Gwyddion 2.26 (http://gwyddion.net/).[3] Height differences between the phases were extracted by fitting Gaussian func-

tions to custom-binned height histograms of manually chosen regions in the images. Values are given as mean ± standard deviation (number of histograms) of the heights extracted. The minimum number of independent vesicle preparations was three for each Gb_3 derivative. These vesicles were used to independently prepare solid supported membranes and at least four solid supported membranes were analyzed. Values for single histograms shown in the Figures are given as (difference of mean values of Gaussians) ± (sum of standard deviations of the Gaussians).

Fatty acid distribution of Gb_3 in cells

Table S1. Molecular species of Gb_3 in different cells. Data for erythrocytes and HeLa cells are taken from Figure S4 of Römer et al.[4] Gb_3 derivatives investigated in this study are printed in bold.

	% of total Gb_3 in	
Fatty Acid	Erythrocytes	HeLa
C16:0	3	3
C18:0	2	0
C18:1	2	0
C20:0	2	0
C22:0	17	3
C23:0	1	7
C24:0	**29**	**6**
C24:1	**5**	**1**
C22:0 2-OH	3	10
C23:0 2-OH	1	0
C24:0 2-OH	**19**	**29**
C24:1 2-OH	**10**	**41**
Others	6	0

Fatty acid distribution of bovine brain sphingomyelin

Table S2. Molecular species of bovine brain sphingomyelin. Data taken from Shaw et al.[5]

Fatty Acid	% of total SM
C16:0	2
C18:0	49
C20:0	5
C22:0	8
C24:0	6
C24:1	20
Others	10

Additional Results

Table S3. Comparison of the area percentages observed itemized by the l_d fluorescence dye.

		-STxB			+STxB		
		l_o			l_o		
Compound		l_o	l_i	n	l_o	l_i	n
27	Texas Red DHPE	70 ± 9		22	78 ± 11		23
	Oregon Green DHPE	75 ± 6		62	75 ± 11		29
28	Texas Red DHPE	40 ± 6	37 ± 8	58	42 ± 8	32 ± 8	55
	Bodipy-PC	42 ± 7	33 ± 8	15	44 ± 5	27 ± 6	9
29	Texas Red DHPE	56 ± 5		46	78±5		52
	Bodipy-PC	56 ± 3		10	74±3		10
30	Texas Red DHPE	73 ± 6		29	89 ± 6		24
	Oregon Green DHPE	66 ± 7		22	83 ± 8		18

DOPC/sphingomyelin/cholesterol (40:40:20) membranes. Membranes composed of DOPC/SM/Chol (40:40:20) form bright dye (Bodipy-PC) enriched liquid-disordered (l_d) domains and darker liquid-ordered (l_o) domains (Figure S1A). The l_o phase accounts for 66 ± 6 % (n = 35). The integrity of the membrane is analyzed using the dye perylene, which partitions more equally between the l_o and l_d phase (Figure S1B).

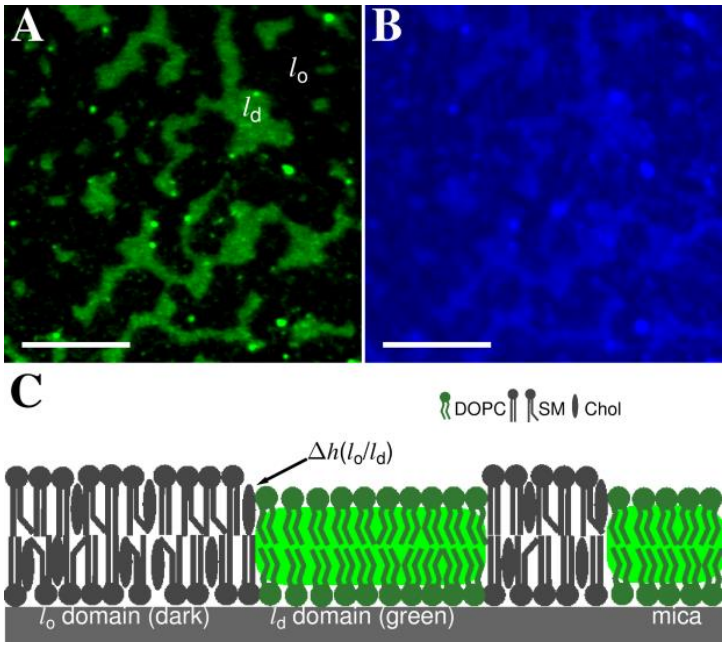


Fig. S1. Domain organization of membranes composed of DOPC/SM/Chol (40:40:20) doped with perylene (0.5 mol%) and Bodipy-PC (0.1 mol%). (A) Fluorescence micrograph. Membranes segregate into liquid-ordered (l_o, dark) and liquid-disordered domains (l_d, green). Scale bar: 10 µm. (B) Perylene fluorescence shows that the dark areas in (A) are membrane covered. Scale bar: 10 µm. (C) Schematic side view of the proposed organization of membranes composed of DOPC/SM/Chol (40:40:20) on mica. Membrane segregate into liquid-disordered (l_d) domains enriched in a fluorescent dye shown in green and dark liquid-ordered domains (dark) enriched in cholesterol and sphingomyelin. Phases differ in height due to different chain packing. (C) Schematic drawing showing the membranes segregating into liquid-disordered (l_d) domains enriched in a fluorescent dye shown in green and dark liquid-ordered domains (dark) enriched in cholesterol and sphingomyelin. The height difference due to different chain packing can be detected by AFM.

After STxB incubation (60 nM STxB-Cy3 for 1 h) the phase behavior of membranes composed of DOPC/SM/Chol (40:40:20) remain constant with an area percentage of l_o domains of 67 ± 5 % (n = 25). In the Cy3-fluorescence channel no specific Cy3-fluorescence was detected demonstrating that STxB does not bind to DOPC/SM/Chol membranes.

DOPC/sphingomyelin/cholesterol membranes doped with Gb_3-C24:0-2OH

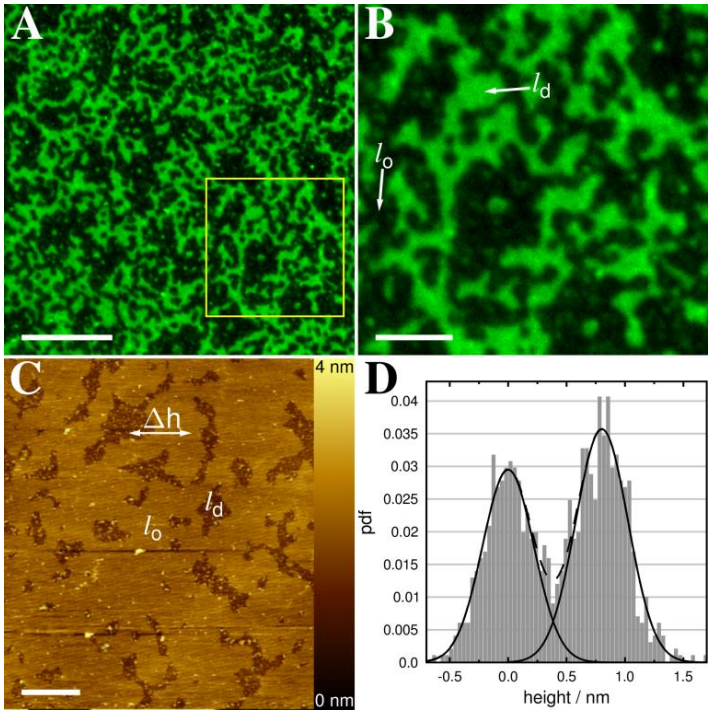


Fig. S2. Solid supported membranes composed of DOPC/SM/Chol/Gb_3-C24:0-2OH (40:35:20:5) doped with perylene (0.5 mol%) and Texas Red DHPE (0.1 mol%) on mica. (A) Fluorescence micrograph of Texas Red DHPE (false colored in green). Membranes segregate into liquid-ordered (l_o, dark) and liquid-disordered domains (l_d, green). Scale bar: 15 µm. (B) Magnification of the region marked in (A). Scale bar: 5 µm. (C) AFM image showing a higher l_o phase and a lower l_d phase (darker color coding). Scale bar: 5 µm. (D) Histogram analysis from which the height difference $\Delta h(l_o/l_d) = 0.8 \pm 0.4$ nm was extracted.

DOPC/sphingomyelin/cholesterol membranes doped with Gb_3-C24:0-2OH after STxB binding

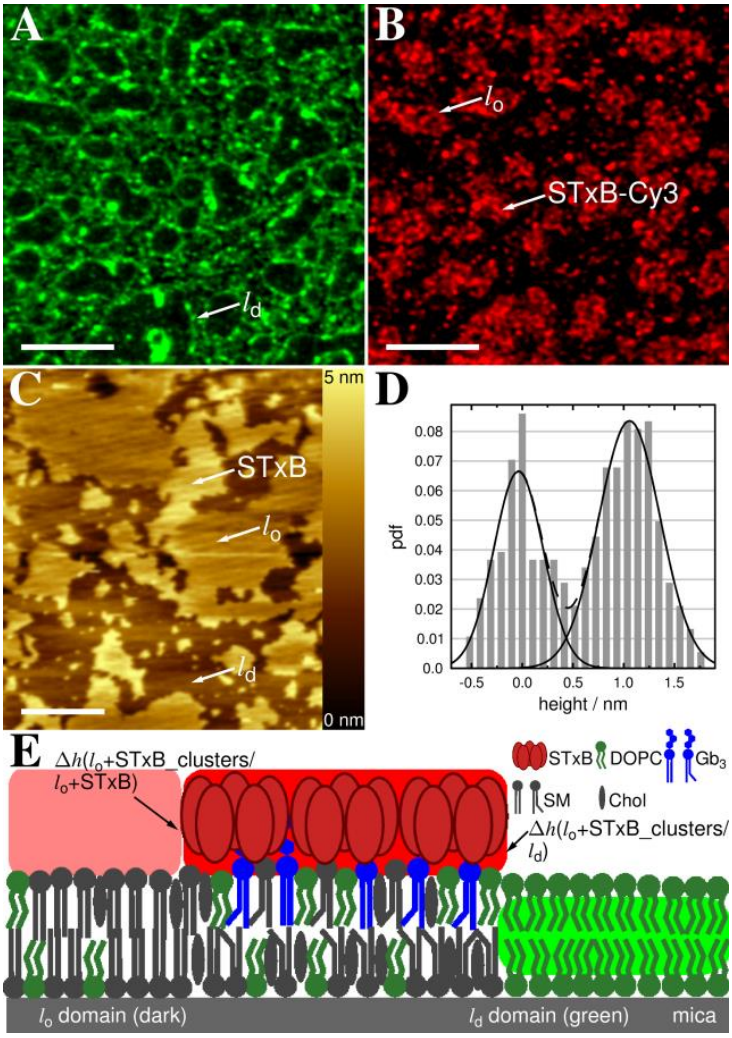


Fig. S3. Solid supported membranes composed of DOPC/SM/Chol/Gb_3-C24:0-2OH (40:35:20:5) doped with perylene (0.5 mol%) and Bodipy-PC (0.1 mol%) after incubation with Cy3-labeled STxB (60 nM for 1 h). (A) Fluorescence micrograph of Bodipy-PC. Membranes segregate into liquid-ordered (l_o, dark) and liquid-disordered domains (l_d, green). Scale bar: 7 µm. (B) Fluorescence micrograph of STxB-Cy3. The inhomogeneous red fluorescence of the labeled protein is inverse to the Bodipy-PC labeled l_d phase. Scale bar: 7 µm. (C) Atomic force microscopy image showing a lower l_d phase (dark color coding), higher l_o phase and STxB clusters on top of the ordered phase. Scale bar: 3 µm. (D) Height difference between STxB clusters and l_o is determined to $\Delta h(l_{o+STxB\_clusters}/l_{o+STxB}) = 1.1 \pm 0.5$ nm. (E) Schematic side view of the proposed organization of membranes composed of DOPC/SM/Chol/Gb_3 (40:35:20:5) on mica after STxB binding. Membranes segregate into l_d domains enriched in the bulky fluorescent dye (green) and dark l_o domains. STxB selectively binds to Gb_3 resulting in membrane reorganization. Using fluorescently labeled protein allows to localize Gb_3 (red).

DOPC/sphingomyelin/cholesterol membranes doped with Gb_3-C24:1-2OH

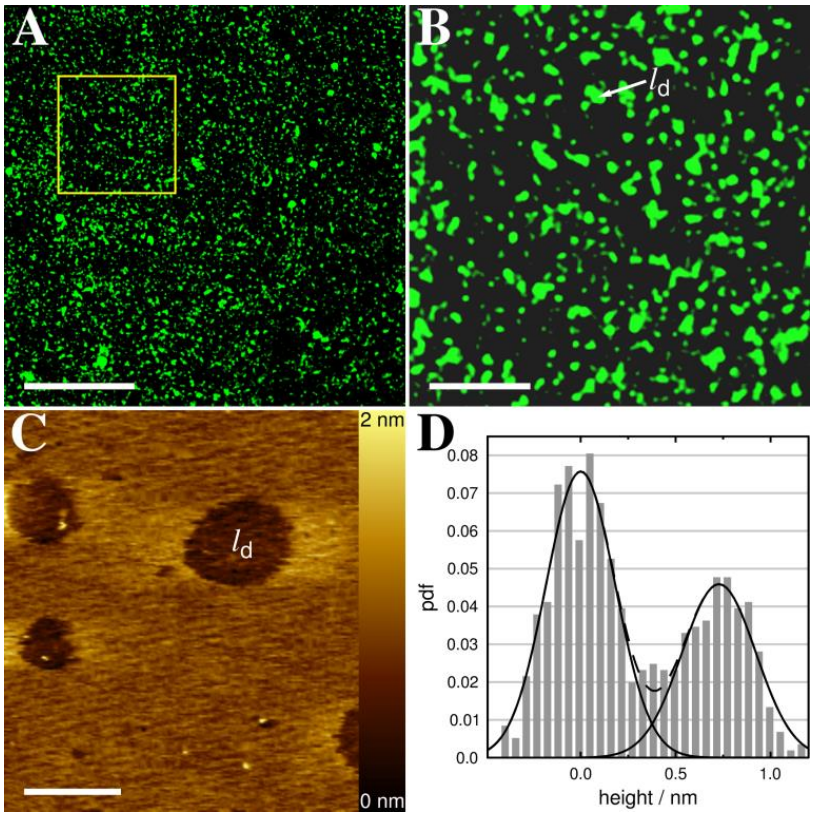


Fig. S4. Solid supported membranes composed of DOPC/SM/Chol/Gb_3-C24:1-2OH (40:35:20:5) doped with perylene (0.5 mol%) and Oregon Green DHPE (0.1 mol%). (A) Fluorescence micrograph. Membranes segregate into liquid-ordered (l_o, dark) and liquid-disordered domains (l_d, green). Scale bar: 30 µm. (B) Magnification of the region marked in (A). Scale bar: 8 µm. (C) Atomic force microscopy image showing a higher l_o phase and a lower l_d phase (darker color coding). Scale bar: 3 µm. (D) Height difference is determined to $\Delta h(l_o/l_d) = 0.7 \pm 0.4$ nm.

Composition of l_o and l_d phase

To obtain an estimate of the domain composition and area fractions, one can use tie lines in the ternary phase diagram. As stated by Dimova and coworkers[6] a detailed phase diagram for the ternary mixture of DOPC/brain SM/Chol is as yet not available. However, the size and position of the two-phase region is very similar to the one provided by Dimova and coworkers[6] using egg SM. They used fluorescence microscopy to extract tie lines for different ratios of DOPC/egg SM/Chol (Fig. S5).

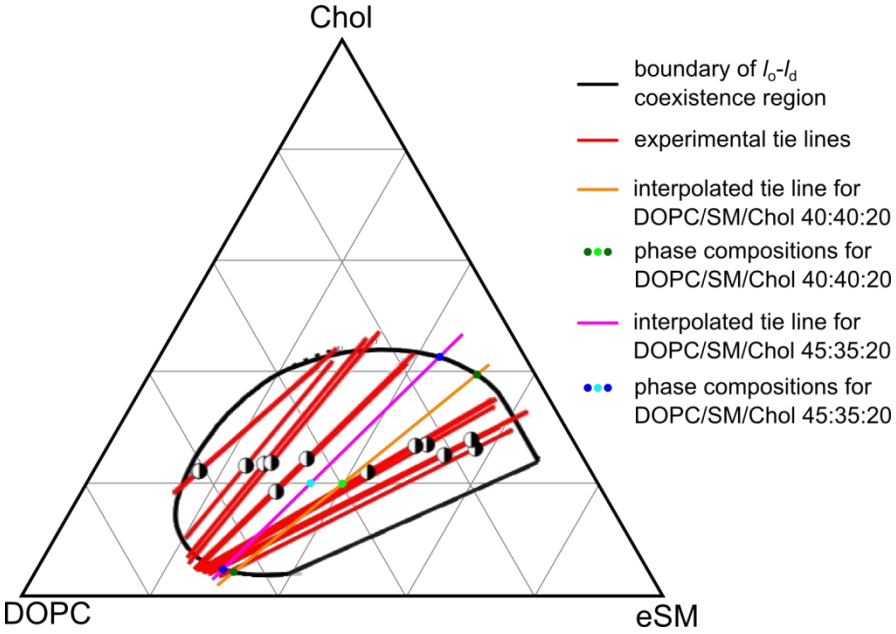


Fig. S5. Phase diagram of DOPC/egg SM/Chol. The figure has been modified after Bezlyepkia et al.[6] All lines and points used in this calculations are shown a given in the figure legend.

We used this phase diagram to estimate the phase compositions. The inclination angle of the tie line for our lipid composition (DOPC/SM/Chol, 40:40:20, green dot) is not given. We estimated the angle to 39° (orange line) given the fact that the angle increases linearly in the range of 20-50 mol% DOPC. Using this tie line, the composition of the l_o is: DOPC/SM/Chol 9:51:40 and for the l_d phase: DOPC/SM/Chol 65:31:4 (dark green dots). The fractional areas of the phases can be calculated using equations (7d), (9a), (10a) (Supporting Information, Dimova et al.[6]) taking the sizes of the three lipids into account, which are given in Table S1 (Supporting Information, Dimova et al.[6]). In this case, 63 % of the total area is in the liquid disordered phase. Compared to the value we found in our solid supported membrane system of 34 %, this area fraction is larger indicating the influence of the solid substrate as discussed in the manuscript. Now assuming that Gb_3-C24:1 would add to the DOPC component and not to the SM component would result in the composition shown as cyan dot (DOPC/SM/Chol, 45:35:20) being still in the centre of the two-phase region of the phase diagram. The resulting composition of the l_o phase is then: DOPC/SM/Chol 13:44:44, while the composition of the l_d phase is: DOPC/SM/Chol 66:29:5 with an area fraction of the l_d phase of 67 %. Translating this relative change in l_d phase fraction for our solid supported system would mean that upon exchanging 5 mol% of SM with C24:1-Gb_3 would result in an *increase* in l_d area fraction from 34 % to 36 %. We observe a *decrease* in l_d phase from 34 % to 24 % indicating that the large Gb_3 extends the area of the more ordered phase.

STxB surface coverage

Membranes are composed of DOPC/SM/Chol/Gb_3 (40:35:20:5). By taking the capability of STxB to bind up to 15 Gb_3 molecules into account it is possible to roughly estimate the number of Gb_3 molecules bound and the area occupied by STxB. For the calculations, the following assumptions have been made taking the given values into account:

i) The total area occupied by one approximately pentagonally shaped STxB can be estimated to 2500 Å^2 using the crystallographic dimensions.[7]

ii) Pentagons can only be packed up to a surface density of Γ = 0.921 with the actual density most probably being lower.[8, 9] Random sequential adsorption of circular objects on a two dimensional surface predicts $\Gamma \approx 0.547$.[10]

iii) The areas of the lipid molecules are taken from Bezlyepkina et al.[6] The area per molecule including condensations effects by cholesterol are DOPC: 66.0 Å^2, sphingomyelin: 49.0 Å^2, cholesterol: 27 Å^2. The molecular area of Gb_3 is 80 Å^2.[11]

iv) The affinity of STxB to Gb_3 is approx. K_D = 1 nM.[12] Adsorption behavior is taken as Langmuir like.

Receptor coverage. Upon incubation of the Gb_3 containing membranes with 60 nM STxB, the protein surface coverage (Θ) with respect to the available Gb_3 can be calculated using the Langmuir isotherm (Eq. S1).

$$\Theta = \frac{\left(c_{\mathrm{STxB}}\, K_{\mathrm{D}}^{-1}\right)}{\left(1 + c_{\mathrm{STxB}}\, K_{\mathrm{D}}^{-1}\right)} \qquad \text{(S1)}$$

A surface coverage of Θ = 0.984 for c_{STxB} = 60 nM is calculated using the K_D value given in (iv) if the number of binding sites were not a limiting factor.

Minimum area occupied by STxB. Using the lipid composition of the membrane and the lipid areas given in (iii), Gb_3 accounts for 6.6 % of the total membrane area. The minimum area of the membrane that is covered by STxB thus corresponds to the product of Gb_3 area fraction and the ratio of STxB area (i) and the area of 15 Gb_3 molecules (iii). Neglecting the highest possible packing density on the surface (ii) STxB therefore requires a minimum fraction of 16 % of the total membrane area. This lower limit of STxB coverage is in the same range as that found for Gb_3-C24:0 containing membranes (Figure 3, clusters of proteins with a surface coverage of 15 %).

Minimum number of Gb_3 molecules bound to STxB in case of high surface coverage. The number of Gb_3 molecules bound to STxB is calculated using the number of Gb_3 molecules and the number of STxB molecules per unit area. After STxB binding, the number of Gb_3 molecules per unit area is calculated assuming that all Gb_3s are located in the phase, to which STxB has been bound (l_o), which effectively increases the Gb_3 concentration in this area. The number of STxB per unit area is calculated using the molecular area of STxB (i) and the maximum coverage possible for the two cases: Γ = 0.921 and 0.547 (ii). These are combined with three different cases of protein coverage. Full coverage with STxB (100 %), the maximum percentage of l_o phase in case of full coverage found for Gb_3-C24:1-OH (87 %) and the minimum area homogenously covered by protein in case of Gb_3-C24:1 (43 %). The limiting case of 100 % l_o phase with bound protein still results in a reasonable number of Gb_3 molecules bound to STxB.

Table S4. Theoretical number of Gb_3 molecules bound to STxB.

Gb_3 species	l_o area fraction / %	Γ	Gb_3/STxB
-	100	0.921	2.6
		0.547	4.3
Gb_3-C24:1-2OH (**30**)	87	0.921	2.9
		0.547	5.0
Gb_3-C24:1 (**28**)	43	0.921	6.0
		0.547	10.0

General experimentals: Chemical syntheses

All reactions were performed in flame-dried glassware under an argon atmosphere. The solvents were dried by standard procedures and distilled prior to use. ^{1}H- and ^{13}C-NMR spectra were obtained with 300 MHz, 500 MHz and 600 MHz spectrometers using the solvent as internal standard. In the case of solvent mixtures CD_3OD was used as internal standard. Assignments of the respective signals were made by the combination of H,H-COSY, HSQC and HMBC experiments. Unsecured assignments are characterized with the index*. ESI-HRMS mass spectrometry was carried out on a FTICR instrument. IR spectra were measured on a FT/IR-4100 spectrometer with a Pike GladiATR unit. Optical rotations were obtained using a common polarimeter. Dialysis was performed in deionized water using Spectra/Por Float-A-Lyzer G2 with a molecular weight cut off of 500 g/mol. For gel permeation HPLC a system type (recycling system) was used.

S1. Trichloroacetimidate and sphingosine were dissolved in dry CH_2Cl_2/hexane (1:1), molecular sieves 4 Å were added and the mixture stirred at r.t. for 30 min. Afterwards $BF_3{\cdot}OEt_2$ was added at 0 °C and the solution stirred at r.t. between 16 h and 2 d. The reaction was quenched by addition of pyridine, molecular sieves were filtered off using silica gel and the solvents were removed under reduced pressure.

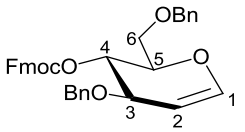
S2. To a suspension of sphingosine in benzene and water PPh_3 was added and the reaction mixture was stirred at 60 °C for 16 h. The solvents were removed under reduced pressure and the residue was azeotroped with toluene (3 × 1.0 mL). The amine was dried in high vacuum for 1 h and dissolved in dry THF. To a solution of fatty acid in dry THF were added HOBt, EDCI and diisopropylamine at 0 °C. The mixture was stirred at 0 °C for 10 min, then the amine was added dropwise and the solution stirred at r.t. for 16 h. The reaction was quenched by addition of water. The organic layer was washed with sat. NH_4Cl and sat. $NaHCO_3$ solution, dried over Na_2SO_4 and the solvents were removed under reduced pressure.

S3. To a suspension of sphingosine in benzene and water PPh_3 was added and the reaction mixture was stirred at 60 °C for 16 h. The solvents were removed under reduced pressure and the residue was azeotroped with toluene (3 × 1.0 mL). The amine was dried in high vacuum for 1 h and dissolved in dry THF. Fatty acid and DIPEA were added at r.t., followed by HATU dissolved in DMF. The reaction mixture was stirred at r.t. for 2 h and EtOAc was added. The organic layer was washed with brine (4 × 5.0 mL) and the combined water layers were once re-extracted with EtOAc. The organic layers were dried over Na_2SO_4 and the solvents were removed under reduced pressure.

S4. To a solution of the protected glycosphingolipids in CH_3OH/CH_2Cl_2 (3:1) NaOMe (1.6 M or 5.2 M in CH_3OH) was added till a pH greater than 12 was reached. The reaction mixture was stirred at r.t. or 50 °C between 4 h and 2 d. The solution was neutralized with amberlite®, filtered and the solvents were removed under reduced pressure.

Globotriaose

3,6-Di-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-D-glucal (**1a**)

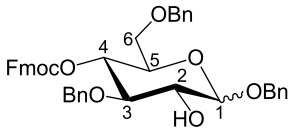


To a solution of 3,6-di-*O*-benzyl-D-glucal (7.3 g, 22 mmol, 1.0 eq.) in pyridine (100 mL) was added FmocCl (11.5 g, 44.5 mmol, 2.0 eq) and the mixture stirred at r.t. for 2 h. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel (pentane/EtOAc, 10:1 → 4:1). The protected glucal **1a** was isolated as a colorless oil in 11.5 g (95%).

***R*$_f$**: 0.37 (hexane/EtOAc, 4:1); $[\alpha]^{D}_{20}$ = -9.0° (*c* 0.53, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.71 (dd, *J* = 10.5 Hz, 4.5 Hz, 1 H, 6-H_a), 3.78 (dd, *J* = 10.5 Hz, 6.4 Hz, 1 H, 6-H_b), 4.08-4.13 (m, 1 H, 3-H),

4.19-4.26 (m, 1 H, CH-Fmoc), 4.33-4.40 (m, 1 H, 5-H), 4.39-4.45 (m, 2 H, CH_2-Fmoc), 4.51 (d, *J* = 12.2 Hz, 1 H, CH_2-Ph), 4.56 (d, *J* = 12.2 Hz, 1 H, CH_2-Ph), 4.59 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.63 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.91 (ddt, *J* = 6.2 Hz, 3.6 Hz, 0.6 Hz, 1 H, 2-H), 5.15-5.21 (m, 1 H, 4-H), 6.48 (dd, *J* = 6.2 Hz, 1.2 Hz, 1 H, 1-H), 7.20-7.36 (m, 12 H, Ph-H, Ar-H), 7.37-7.44 (m, 2 H, Ar-H), 7.57-7.62 (m, 2 H, Ar-H), 7.74-7.79 (m, 2 H, Ar-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 46.7 (CH-Fmoc), 67.9 (C-6), 70.1 (CH_2-Fmoc), 70.1 (CH_2-Ph), 70.8 (C-3), 72.2 (C-4), 73.4 (CH_2-Ph), 74.9 (C-5), 99.2 (C-2), 120.0, 125.0, 127.1, 127.1, 127.6, 127.6, 127.6, 127.8, 128.3, 128.3 (C_{tert}-Ar, C_{tert}-Ph), 137.6, 137.9 (C_{quart}-Ph), 141.2, 141.2, 143.1, 143.1 (C_{quart}-Ar), 144.6 (C-1), 154.2 (CO-Fmoc); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2865, 1744, 1646, 1497, 1477, 1450, 1385, 1248, 1100, 1066; **MS** (ESI): *m*/*z* (%) = 571.2 $[M+Na]^+$, 1119.4 $[2M+Na]^+$; **HRMS (ESI)** for $C_{35}H_{32}O_6$ (548.63): calcd. 571.2091 $[M+Na]^+$, found 571.2076.

Benzyl 3,6-di-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-D-glucopyranoside (**1b**)



To a solution of glucal **1a** (2.0 g, 3.6 mmol, 1.0 eq) in dry CH_2Cl_2 (40 mL) was added dropwise DMDO (0.07 M in acetone, 77 mL, 5.39 mmol, 1.5 eq) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, the solvents were removed under reduced pressure at 10 °C and the residue dried in high vacuum for 10 min. The epoxide was dissolved in dry CH_2Cl_2 (60 mL) and benzyl alcohol (20 mL, 192 mmol, 53 eq) and the reaction mixture was stirred at r.t. for 16 h. The solvents were removed under reduced pressure and the residue was purified by column chromatography on silica gel (pentane/EtOAc, 5:1 → 3:1 → 1:1). Both isomers were isolated as colorless foams in 1.87 g (77%, β-isomer, **1b-a**) and 270 mg (11%, α-isomer, **1b-b**) yield.

Analytical data for **1b-a** (β-isomer):

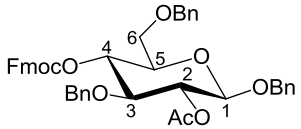
***R*$_f$**: 0.24 (hexane/EtOAc, 3:1); $[\alpha]_D^{20}$ = -13.1° (*c* 0.78, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.40 (s_{br}, 1 H, 2-OH), 3.60-3.72 (m, 4 H, 2-H, 4-H, 5-H, 6-H_2), 4.07-4.17 (m, 1 H, CH-Fmoc), 4.29-4.35 (m, 2 H, CH_2-Fmoc), 4.39 (d, *J* = 7.6 Hz, 1 H, 1-H), 4.53 (d, *J* = 11.8 Hz, 1 H, CH_2-Ph), 4.57 (d, *J* = 11.8 Hz, 1 H, CH_2-Ph), 4.64 (d, *J* = 11.5 Hz, 1 H, CH_2-Ph), 4.70 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.83 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.88 (dd, *J* = 9.3 Hz, 9.1 Hz, 1 H, 3-H), 4.94 (d, *J* = 11.5 Hz, 1 H, CH_2-Ph), 7.15-7.43 (m, 19 H, Ph-H, Ar-H), 7.50-7.61 (m, 2 H, Ar-H), 7.72-7.80 (m, 2 H, Ar-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 46.7 (CH-Fmoc), 69.5 (C-6), 70.0 (CH_2-Fmoc), 71.2 (CH_2-Ph), 73.3 (CH_2-Ph), 73.6, 74.3 (C-2, C-5)*, 74.6 (CH_2-Ph), 75.2 (C-4), 81.5 (C-3), 101.4 (C-1), 120.0, 120.0, 125.0, 125.0 (C_{tert}-Ar), 127.1, 127.1, 127.5, 127.5, 127.5, 127.6, 127.6, 127.6, 127.8, 128.0, 128.1, 128.2, 128.2, 128.2, 128.2, 128.4 (C_{tert}-Ph), 136.7, 137.8, 138.1 (C_{quart}-Ph), 141.1, 141.2, 143.1, 143.2 (C_{quart}-Ar), 154.3 (CO-Fmoc); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3463, 2920, 2852, 1749, 1660, 1497, 1450, 1385, 1251, 1065; **MS** (ESI): *m*/*z* (%) = 695.2 $[M+Na]^+$, 1367.5 $[2M+Na]^+$; **HRMS (ESI)** for $C_{42}H_{40}O_8$ (672.76): calcd. 695.2615 $[M+Na]^+$, found 695.2609.

Analytical data for **1b-b** (α-isomer):

***R*$_f$**: 0.21 (hexane/EtOAc, 3:1); $[\alpha]_D^{20}$ = +25.5° (*c* 0.30, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.53-3.59 (m, 2 H, 6-H_2), 3.78 (dd, *J* = 9.0 Hz, 3.5 Hz, 1 H, 2-H), 3.84 (dd, *J* = 9.9 Hz, 8.7 Hz, 1 H, 4-H), 3.96-4.04 (m, 1 H, 5-H), 4.11 (t, *J* = 6.9 Hz, 1 H, CH-Fmoc), 4.28-4.34 (m, 2 H, CH_2-Fmoc), 4.50 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.54 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.58 (d, *J* = 11.6 Hz, 1 H, CH_2-Ph), 4.69 (d, *J* = 11.5 Hz, 1 H, CH_2-Ph), 4.78 (d, *J* = 11.5 Hz, 1 H, CH_2-Ph), 4.85 (d, *J* = 11.6 Hz, 1 H, CH_2-Ph), 4.94 (dd, *J* = 9.9 Hz, 9.0 Hz, 1 H, 3-H), 5.03 (d, *J* = 3.5 Hz, 1 H, 1-H), 7.15-7.42 (m, 19 H, Ph-H, Ar-H), 7.50-7.58 (m, 2 H, Ar-H), 7.73-7.78 (m, 2 H, Ar-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 46.7 (CH-Fmoc), 68.9, 69.0, 70.0, 72.5 (C-2, C-4, C-5, C-6)*, 69.9 (CH_2-Fmoc), 73.6 (CH_2-Ph), 74.8 (2 x CH_2-Ph), 80.6 (C-3), 97.5 (C-1), 120.0, 120.0, 125.0, 125.0 (C_{tert}-Ar), 127.1, 127.1, 127.5, 127.5,

127.5, 127.5, 127.6, 127.8, 128.1, 128.2, 128.2, 128.2, 128.5 (C_{tert}-Ph), 136.7, 137.7, 138.2 (C_{quart}-Ph), 141.1, 141.2, 143.1, 143.2 (C_{quart}-Ar), 154.2 (CO-Fmoc); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3445, 2899, 2870, 1749, 1605, 1497, 1451, 1384, 1250, 1054; **MS** (ESI): *m/z* (%) = 695.3 $[M+Na]^+$, 1367.6 $[2M+Na]^+$; **HRMS (ESI)** for $C_{42}H_{40}O_8$ (672.76): calcd. 695.2615 $[M+Na]^+$, found 695.2605.

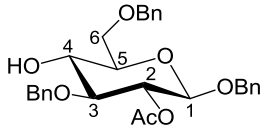
Benzyl 2-*O*-acetyl-3,6-di-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-β-D-glucopyranoside (**1c**)



To a solution of **1b-a** (540 mg, 0.80 mmol, 1.0 eq) in pyridine (3.8 mL) was added acetic anhydride (3.0 mL, 32 mmol, 40 eq) and the mixture stirred at r.t. for 16 h. The solvents were removed under reduced pressure and the residue was purified by column chromatography on silica gel (pentane/EtOAc, 5:1 → 3:1). The acetyl protected glucopyranoside **1c** was obtained in 545 mg (95%) as a colorless foam.

***R*f**: 0.42 (hexane/EtOAc, 2:1); $[\alpha]^D_{20}$ = -27.9° (*c* 0.38, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.96 (s, 3 H, O(CO)CH_3), 3.63-3.69 (m, 3 H, 5-H, 6-H_2), 3.72 (dd, *J* = 9.4 Hz, 9.3 Hz, 1 H, 3-H), 4.09-4.18 (dd, *J* = 7.0 Hz, 7.0 Hz, 1 H, CH-Fmoc), 4.30-4.37 (m, 2 H, CH_2-Fmoc), 4.46 (d, *J* = 8.0 Hz, 1 H, 1-H), 4.51-4.66 (m, 5 H, CH_2-Ph), 4.90 (d, *J* = 12.4 Hz, 1 H, CH_2-Ph), 4.90-5.00 (m, 1 H, 4-H), 5.13 (dd, *J* = 9.3 Hz, 8.0 Hz, 1 H, 2-H), 7.15-7.44 (m, 19 H, Ph-H, Ar-H), 7.51-7.60 (m, 2 H, Ar-H), 7.73-7.79 (m, 2 H, Ar-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 20.9 (O(CO)*C*H_3), 46.7 (CH-Fmoc), 69.5 (C-6), 70.0 (CH_2-Fmoc), 70.4 (CH_2-Ph), 72.4 (C-2), 73.2 (C-5), 73.6 (CH_2-Ph), 74.0 (CH_2-Ph), 75.5 (C-4), 80.0 (C-3), 99.5 (C-1), 120.0, 124.9, 125.0 (C_{tert}-Ar), 127.1, 127.5, 127.5, 127.6, 127.6, 127.7, 127.7, 127.8, 128.2, 128.2, 128.3 (C_{tert}-Ph), 136.9, 137.6, 137.8 (C_{quart}-Ph), 141.2, 141.2, 143.0, 143.1 (C_{quart}-Ar), 154.1 (CO-Fmoc), 169.0 (O(*C*O)CH_3); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2868, 1751, 1606, 1498, 1453, 1363, 1263, 1220, 1094; **MS** (ESI): *m/z* (%) = 737.3 $[M+Na]^+$, 1452.5 $[2M+Na]^+$; **HRMS (ESI)** for $C_{44}H_{42}O_9$ (714.80): calcd. 737.2721 $[M+Na]^+$, found 737.2718.

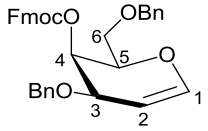
Benzyl 2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (**1**)



To a solution of glucopyranoside **1c** (2.88 g, 4.00 mmol, 1.0 eq) in DMF (80 mL) was added piperidine (32 mL, 323 mmol, 81 eq) at r.t. The solution was stirred for 30 min, then the solvents were evaporated and the residue purified by column chromatography on silica gel (pentane/EtOAc, 5:1 → 3:1). The desired product **1** was obtained in 1.96 g (99%) as a colorless oil.

***R*f**: 0.47 (hexane/EtOAc, 2:1); $[\alpha]^D_{20}$ = -35.7° (*c* 0.87, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.99 (s, 3 H, O(CO)CH_3), 2.82 (s_{br}, 1 H, 4-OH), 3.47 (dd, *J* = 9.6 Hz, 9.3 Hz, 1 H, 4-H), 3.50 (dd, *J* = 9.3 Hz, 9.3 Hz, 1 H, 3-H), 3.71-3.81 (m, 3 H, 5-H, 6-H_2), 4.45 (d, *J* = 7.9 Hz, 1 H, 1-H), 4.59 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.60 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 4.64 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 4.71 (d, *J* = 12.2 Hz, 1 H, CH_2-Ph), 4.75 (d, *J* = 12.2 Hz, 1 H, CH_2-Ph), 4.88 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 5.07 (dd, *J* = 9.3 Hz, 7.9 Hz, 1 H, 2-H), 7.24-7.39 (m, 15 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 20.9 (O(CO)*C*H_3), 70.3 (C-6), 70.3 (CH_2-Ph), 72.0 (C-5), 72.7 (C-2), 73.7 (CH_2-Ph), 74.1 (C-4), 74.3 (CH_2-Ph), 82.3 (C-3), 99.6 (C-1), 127.5, 127.6, 127.7, 127.7, 127.7, 128.2, 128.3, 128.4 (C_{tert}-Ph), 137.1, 137.6, 138.1 (C_{quart}-Ph), 169.3 (O(*C*O)CH_3); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3438, 2870, 1744, 1722, 1657, 1453, 1366, 1228, 1052; **MS** (ESI): *m/z* (%) = 515.2 $[M+Na]^+$, 1007.4 $[2M+Na]^+$; **HRMS (ESI)** for $C_{29}H_{32}O_7$ (492.56): calcd. 515.2040 $[M+Na]^+$, found 515.2056.

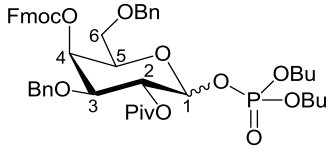
3,6-Di-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-D-galactal (**2a**)



To a solution of 3,6-di-*O*-benzyl-D-galactal[13] (2.5 g, 7.7 mmol, 1.0 eq) in pyridine (50 mL) was added FmocCl (4.0 g, 15 mmol, 2.0 eq) and the mixture stirred at r.t. for 3 h. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel (pentane/EtOAc, 6:1 → 4:1). The protected galactal **2a** was obtained in 3.48 g (83%) yield as a colorless oil.

***R*f**: 0.44 (hexane/EtOAc, 3:1); $[\alpha]_D^{20}$ = +13.0° (*c* 0.87, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.65 (dd, *J* = 9.8 Hz, 6.4 Hz, 1 H, 6-H_a), 3.73 (dd, *J* = 9.8 Hz, 6.6 Hz, 1 H, 6-H_b), 4.18-4.33 (m, 4 H, 3-H, 5-H, CH_2-Fmoc), 4.41 (dd, *J* = 9.7 Hz, 6.5 Hz, 1 H, CH-Fmoc), 4.50 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.53 (d, *J* = 12.2 Hz, 1 H, CH_2-Ph), 4.58 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.70 (d, *J* = 12.2 Hz, 1 H, CH_2-Ph), 4.81 (dt, *J* = 6.4 Hz, 2.0 Hz, 1 H, 2-H), 5.41-5.45 (m, 1 H, 4-H), 6.44 (dd, *J* = 6.4 Hz, 1.9 Hz, 1 H, 1-H), 7.18-7.44 (m, 14H, Ph-H, Ar-H), 7.54-7.66 (m, 2 H, Ar-H), 7.72-7.78 (m, 2 H, Ar-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 46.7 (CH-Fmoc), 67.6 (C-6), 68.3 (CH_2-Fmoc), 69.5 (CH_2-Ph), 70.3 (C-3), 71.1 (C-4), 73.7 (CH_2-Ph), 74.2 (C-5), 100.8 (C-2), 119.9, 119.9, 125.1, 125.4, 127.1, 127.1, 127.4, 127.5, 127.7, 127.7, 127.8, 127.8, 128.2, 128.4 (C_{tert}-Ar, C_{tert}-Ph), 137.5, 137.9 (C_{quart}-Ph), 141.2, 141.2, 143.1, 143.5 (C_{quart}-Ar), 144.4 (C-1), 155.0 (CO-Fmoc); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2865, 1744, 1646, 1497, 1477, 1450, 1385, 1248, 1100, 1066; **HRMS (ESI)** for $C_{35}H_{32}O_6$ (548.63): calcd. 571.2091$[M+Na]^+$, found 571.2084.

3,6-Di-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-2-*O*-pivaloyl-α/β-D-galactopyranosyl Dibutyl Phosphate (**2**)

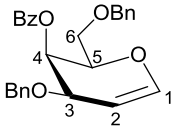


To a solution of galactal **2a** (796 mg, 1.45 mmol, 1.0 eq) in dry CH_2Cl_2 (40 mL) was added dropwise DMDO (0.07 M in acetone, 31 mL, 2.2 mmol, 1.5 eq) at 0 °C. The solution was stirred at 0 °C for 30 min, the solvents were removed under reduced pressure at 10 °C and the residue dried in high vacuum for 10 min. The epoxide was redissolved in dry CH_2Cl_2 (40 mL) and dibutyl phosphate (350 µL, 1.7 mmol, 1.2 eq) dissolved in dry CH_2Cl_2 (1 mL) was added dropwise at -78 °C. After 30 min the mixture was warmed up to 20 °C. DMAP (706 mg, 5.78 mmol, 4.0 eq) and pivaloyl chloride (360 µL, 2.9 mmol, 2.0 eq) were added and the mixture was warmed to -10 °C over 1 h. A mixture of hexane/EtOAc (2:1, 90 mL) was added and the slurry filtered over silica gel. The solvents were removed under reduced pressure and the residue purified by column chromatography on silica gel (pentane/EtOAc, 4:1 → 2:1). Dibutyl phosphate **2** was obtained in 1.10 g (72%) yield as α/β-mixture.

***R*f**: 0.23 (α), 0.09 (β) (hexane/EtOAc, 3:1). Analytical data for β-isomer: $[\alpha]_D^{20}$ = +20.7° (*c* 0.81, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.85-0.94 (m, 6 H, $O(CH_2)_3CH_3$), 1.18 (s, 9 H, $OC(O)C(CH_3)_3$), 1.30-1.42 (m, 4 H, $O(CH_2)_3CH_3$), 1.53-1.67 (m, 4 H, $O(CH_2)_3CH_3$), 3.56-3.70 (m, 4 H, $O(CH_2)_3CH_3$), 3.89-4.21 (m, 7 H, 3-H, 5-H, 6-H_2, CH-Fmoc, CH_2-Fmoc), 4.38-4.50 (m, 3 H, CH_2-Ph), 4.75 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 5.24 (dd, *J* = 8.0 Hz, 7.0 Hz, 1 H, 1-H), 5.39 (dd, *J* = 9.9 HZ, 8.0 Hz, 1 H, 2-H), 5.49 (dd, *J* = 3.2 Hz, 0.8 Hz, 1 H, 4-H), 7.09 (m, 14 H, Ph-H, Ar-H), 7.52-7.66 (m, 2 H, Ph-H), 7.71-7.79 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 13.6 (d, $J_{C,P}$ = 4.2 Hz, $O(CH_2)_3CH_3$), 18.7 (d, $J_{C,P}$ = 3.7 Hz, $O(CH_2)_3CH_3$), 27.2 ($OC(O)C(CH_3)_3$), 32.1 (d, $J_{C,P}$ = 5.9 Hz, $O(CH_2)_3CH_3$), 32.2 (d, $J_{C,P}$ = 6.0 Hz, $O(CH_2)_3CH_3$), 38.9 ($OC(O)C(CH_3)_3$), 46.5 (CH-Fmoc), 67.9 (d, $J_{C,P}$ = 6.4 Hz, $O(CH_2)_3CH_3$), 68.1 (d, $J_{C,P}$ = 6.4 Hz, $O(CH_2)_3CH_3$), 67.4, 67.4, 70.0, 70.0, 70.3, 71.9, 72.7, 73.7, 77.2 (C-2, C-3, C-4, C-5, C-6, CH_2-Ph, CH-Fmoc, CH_2-Fmoc), 96.8 (d, $J_{C,P}$ = 5.1 H, C-1),

119.8, 119.8, 125.1, 125.6, 127.1, 127.2, 127.6, 127.7, 127.7, 127.7, 127.8, 127.8, 127.8, 128.1, 128.3, 128.4 (C_{tert}-Ar, C_{tert}-Ph), 136.9, 137.3 (C_{quart}-Ph), 141.0, 141.1, 143.0, 143.5 (C_{quart}-Ar), 154.7 (CO-Fmoc), 176.6 (O*C*(O)C(CH_3)$_3$); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3064, 2932, 2873, 1743, 1606, 1497, 1478, 1452, 1396, 1257, 1054; **HRMS (ESI)** for $C_{48}H_{59}O_{12}P$ (858.95): calcd. 881.3625 $[M+Na]^+$, found 881.3636.

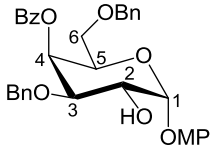
4-*O*-Benzoyl-3,6-di-*O*-benzyl-D-galactal (**11**)



To a solution of 3,6-di-*O*-benzyl-D-galactal[13] (**10**) (4.69 g, 14.4 mmol, 1.0 Äq.) in pyridine (45 mL) was added dropwise benzoyl chloride (2.5 mL, 22 mmol, 1.5 Äq.) and a catalytic amount of DMAP. The mixture was stirred at r.t. for 16 h, and then the solvent was removed under reduced pressure. After column chromatography on silica gel (pentane/EtOAc, 12:1 → 10:1 → 5:1) 5.00 g (81%) of **11** were obtained.

***R*$_f$**: 0.50 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$ = +29.3° (*c* 0.45, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.65 (dd, *J* = 10.2 Hz, 6.2 Hz, 1 H, 6-H_a), 3.73 (dd, *J* = 10.2 Hz, 6.8 Hz, 1 H, 6-H_b), 4.32 (dd, *J* = 6.8 Hz, 6.2 Hz, 1 H, 5-H), 4.33-4.37 (m, 1 H, 4-H), 4.49 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.52 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.58 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.73 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.82 (ddd, *J* = 6.4 Hz, 1.9 Hz, 1.8 Hz, 1 H, 2-H), 5.81-5.86 (m, 1 H, 3-H), 6.49 (dd, *J* = 6.4 Hz, 1.7 Hz, 1 H, 1-H), 7.20-7.36 (m, 10 H, Ph-H), 7.40-7.47 (m, 2 H, Ph-H), 7.52-7.60 (m, 1 H, Ph-H), 8.05-8.12 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 63.9 (C-3), 68.6 (C-6), 69.7 (C-4), 71.0 (CH_2-Ph), 73.6 (CH_2-Ph), 74.5 (C-5), 101.2 (C-2), 127.4, 127.5, 127.7, 127.8, 128.2, 128.3, 129.7, 129.9, 130.0 (C_{tert}-Ph), 133.0, 137.4, 137.9 (C_{quart}-Ph), 144.3 (C-1), 165.9 (O(*C*O)Ph); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2920, 2850, 1720, 1601, 1452, 1361, 1262, 1175, 1091; **MS** (ESI): *m*/*z* (%) = 453.2 $[M+Na]^+$, 883.3 $[2M+Na]^+$; **HRMS (ESI)** for $C_{27}H_{26}O_5$ (430.49): calcd. 453.1672 $[M+Na]^+$, found 453.1665.

p-Methoxyphenyl 4-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-galactopyranoside (**12**)

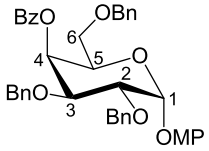


To a solution of galactal **11** (3.70 g, 8.60 mmol, 1.0 eq) in dry CH_2Cl_2 (150 mL) was added dropwise DMDO (0.07 M in acetone, 184 mL, 12.9 mmol, 1.5 eq) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, the solvents were removed under reduced pressure and the residue dried in high vacuum for 10 min. The epoxide was dissolved in dry CH_2Cl_2 (150 mL), *p*-methoxyphenol (10.7 g, 86.2 mmol, 10 eq) was added and the mixture stirred at r.t. for 16 h. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel (pentane/EtOAc, 10:1 → 8:1 → 5:1). The α-isomer **12** was obtained in 4.42 g (90%) yield as a colorless oil.

***R*$_f$**: 0.31 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$ = +100.0° (*c* 0.55, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.38 (d, *J* = 6.3 Hz, 1 H, 2-OH), 3.57-3.62 (m, 2 H, 6-H_2), 3.76 (s, 3 H, OCH_3), 4.05 (dd, *J* = 10.0 Hz, 3.2 Hz, 1 H, 3-H), 4.22 (ddd, *J* = 10.0 Hz, 6.3 Hz, 3.7 Hz, 1 H, 2-H), 4.38 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 4.43 (ddd, *J* = 7.6 Hz, 6.4 Hz, 0.7 Hz, 1 H, 5-H), 4.47 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 4.55 (d, *J* = 11.2 Hz, 1 H, CH_2-Ph), 4.93 (d, *J* = 11.2 Hz, 1 H, CH_2-Ph), 5.58 (d, *J* = 3.7 Hz, 1 H, 1-H), 5.95 (dd, *J* = 3.2 Hz, 0.7 Hz, 1 H, 4-H), 6.76-6.84 (m, 2 H, Ph-H), 7.04-7.11 (m, 2 H, Ph-H), 7.17-7.37 (m, 10 H, Ph-H), 7.40-7.49 (m, 2 H, Ph-H), 7.54-7.61 (m, 1 H, Ph-H), 8.05-8.11 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 55.6 (OCH_3), 67.6 (C-4), 68.5 (C-6), 68.5 (C-2), 69.1 (C-5), 71.5 (CH_2-Ph), 73.5 (CH_2-Ph), 76.6 (C-3), 98.7 (C-1), 114.6, 118.8, 127.6, 127.7, 127.8, 128.1, 128.3, 128.4, 128.4, 129.8, 129.8 (C_{tert}-Ph), 133.2, 137.6, 137.6, 150.6, 155.4 (C_{quart}-Ph), 165.7 (O(*C*O)Ph); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3478,

2922, 2853, 1719, 1602, 1505, 1452, 1267, 1177, 1092; **MS** (ESI): *m*/*z* (%) = 593.2 $[M+Na]^+$, 1163.5 $[2M+Na]^+$; **HRMS (ESI)** for $C_{34}H_{34}O_8$ (570.63): calcd. 593.2146 $[M+Na]^+$, found 593.2136.

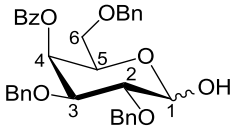
p-Methoxyphenyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-α-D-galactopyranoside (**12a**)



To a solution of galactopyranoside **12** (3.09 g, 5.42 mmol, 1.0 eq) in DMF (60 mL) was added sodium hydride (60% in mineral oil, 260 mg, 6.50 mmol, 1.2 eq) at 0 °C and stirred for 30 min. Subsequently benzyl bromide (7.7 mL, 6.5 mmol, 1.2 eq) was added dropwise at 0 °C and the reaction mixture stirred at r.t. for 16 h. The reaction was quenched with water and extracted with EtOAc. The organic layer was washed with sat. $NaHCO_3$ solution and brine twice and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel (pentane/EtOAc, 8:1 → 5:1). **12a** was obtained in 3.14 g (90%) yield as a colorless oil.

***R*$_f$**: 0.58 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$ = +81.0° (*c* 0.39, $CHCl_3$); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 3.55-3.57 (m, 2 H, 6-H_2), 3.77 (s, 3 H, OCH_3), 4.04 (dd, *J* = 9.9 Hz, 3.5 Hz, 1 H, 2-H), 4.29 (dd, *J* = 9.9 Hz, 3.3 Hz, 1 H, 3-H), 4.37 (d, *J* = 11.8 Hz, 1 H, CH_2-Ph), 4.41 (ddd, *J* = 6.4 Hz, 6.4 Hz, 0.7 Hz, 1 H, 5-H), 4.43 (d, *J* = 11.8 Hz, 1 H, CH_2-Ph), 4.65 (d, *J* = 11.2 Hz, 1 H, CH_2-Ph), 4.70 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.86 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.91 (d, *J* = 11.2 Hz, 1 H, CH_2-Ph), 5.45 (d, *J* = 3.5 Hz, 1 H, 1-H), 5.95 (dd, *J* = 3.3 Hz, 0.7 Hz, 1 H, 4-H), 6.79-6.84 (m, 2 H, Ph-H), 7.06-7.10 (m, 2 H, Ph-H), 7.17-7.38 (m, 15 H, Ph-H), 7.43-7.48 (m, 2 H, Ph-H), 7.57-7.62 (m, 1 H, Ph-H), 8.02-8.06 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 55.6 (OCH_3), 68.6, 68.6, 68.7 (C-4, C-5, C-6)*, 72.0 (CH_2-Ph), 73.4 (CH_2-Ph), 73.5 (CH_2-Ph), 75.1 (C-2), 76.4 (C-3), 97.8 (C-1), 114.5, 118.7, 127.4, 127.5, 127.6, 127.6, 127.6, 127.8, 127.9, 128.1, 128.2, 128.2, 128.3, 129.8 (C_{tert}-Ph), 133.0, 137.6, 138.1, 138.2, 150.9, 155.1 (C_{quart}-Ph), 165.6 (O(*C*O)Ph); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2922, 2853, 1720, 1601, 1505, 1452, 1353, 1267, 1211, 1093; **MS** (ESI): *m*/*z* (%) = 683.3 $[M+Na]^+$, 1343.5 $[2M+Na]^+$; **HRMS (ESI)** for $C_{41}H_{40}O_8$ (660.75): calcd. 683.2615 $[M+Na]^+$, found 683.2597.

4-*O*-Benzoyl-2,3,6-tri-*O*-benzyl-D-galactopyranose (**13**)[14]

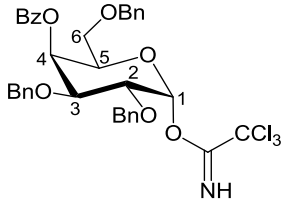


To a solution of the galactopyranoside (56 mg, 87 µmol, 1.0 eq) in CH_3CN (2.5 mL) and water (2.5 mL) was added ceric ammonium nitrate (151 mg, 275 µmol, 3.2 eq) and the reaction mixture stirred at r.t. for 2 h. Ice water and CH_2Cl_2 were added and the organic layer washed with brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. After column chromatography on silica gel (pentane/EtOAc, 3:1 → 2:1) 41 mg (86%) of **13** were obtained as yellowish oil.

***R*$_f$**: 0.30 (hexane/EtOAc, 2:1); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 3.06 (d, *J* = 2.1 Hz, 1 H, 1_α-OH), 3.40 (d, *J* = 5.2 Hz, 1 H, 1_β-OH), 3.51 (dd, *J* = 9.7 Hz, 6.4 Hz, 1 H, 6_α-H_a), 3.55 (dd, *J* = 9.7 Hz, 6.4 Hz, 1 H, 6_α-H_b), 3.56 (dd, *J* = 9.7 Hz, 6.4 Hz, 1 H, 6_β-H_a), 3.63 (dd, *J* = 9.7 Hz, 6.6 Hz, 1 H, 6_β-H_b), 3.63 (dd, *J* = 9.5 Hz, 7.6 Hz, 1 H, 2_β-H), 3.67 (dd, *J* = 9.5 Hz, 3.3 Hz, 1 H, 3_β-H), 3.86 (ddd, *J* = 6.6 Hz, 6.4 Hz, 0.8 Hz, 1 H, 5_β-H), 3.88 (dd, *J* = 9.8 Hz, 3.4 Hz, 1 H, 2_α-H), 4.04 (dd, *J* = 9.8 Hz, 3.4 Hz, 1 H, 3_α-H), 4.41 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 4.41 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.41-4.44 (m, 1 H, 5_α-H), 4.50 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.52 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 4.55 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 4.57 (d, *J* = 11.6 Hz, 1 H, CH_2-Ph), 4.67 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 4.76 (dd, *J* = 7.6 Hz, 5.2 Hz, 1 H, 1_β-H), 4.79 (d, *J* = 11.0 Hz, 1 H, CH_2-Ph), 4.83 (d, *J* = 11.6 Hz, 1 H, CH_2-Ph), 4.83 (d, *J* = 11.3 Hz, 1 H, CH_2-Ph), 4.84 (d, *J* = 11.0 Hz, 1 H, CH_2-Ph), 4.85 (d, *J* = 11.3 Hz, 1 H, CH_2-Ph), 5.34 (dd, *J* = 3.4 Hz, 2.1 Hz, 1 H, 1_α-H), 5.79 (dd, *J* = 3.3 Hz, 0.8 Hz, 1 H, 4_β-H), 5.85 (dd, *J* = 3.4 Hz,

1.0 Hz, 1 H, 4_α-H), 7.16-7.35 (m, 30 H, Ph-H), 7.41-7.48 (m, 4 H, Ph-H), 7.55-7.63 (m, 2 H, Ph-H), 7.99-8.10 (m, 4 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 67.5 (C-4_β), 68.2 (C-5_α), 68.4 (C-4_α), 68.6 (C-6_β), 68.6 (C-6_α), 71.7 (CH_2-Ph), 71.9 (CH_2-Ph), 72.6 (C-5_β), 73.6 (CH_2-Ph), 73.7 (CH_2-Ph), 73.8 (CH_2-Ph), 75.3 (CH_2-Ph), 75.4 (C-2_α), 76.1 (C-3_α), 79.4 (C-2_β), 79.8 (C-3_β), 92.2 (C-1_α), 97.4 (C-1_β), 127.5. 127.5, 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 127.9, 128.0, 128.0, 128.1, 128.2, 128.2, 128.3, 128.3, 128.3, 128.4, 128.5, 129.7, 129.8, 129.9, 129.9 (C_{tert}-Ph), 133.0, 133.1, 137.3, 137.5, 137.6, 137.9, 138.0, 138.3 (C_{quart}-Ph), 165.6, 165.6 (O(*C*O)Ph).

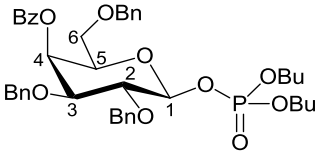
4-*O*-Benzoyl-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl trichloroacetimidate (**3a**)[15]



To a solution of galactopyranose **13** (507 mg, 0.91 mmol, 1.0 eq) in dry CH_2Cl_2 (12 mL) were added dropwise at 0 °C Cl_3CCN (1.8 mL, 18 mmol, 20 eq) and DBU (20 µL, 0.13 mmol, 0.15 eq). The reaction mixture was stirred at 0 °C for 1 h and concentrated under reduced pressure. After column chromatography on silica gel (pentane/EtOAc, 3:1) 611 mg (96%) of **3a** were obtained as a yellow foam.

***R*$_f$**: 0.50 (hexane/EtOAc, 2:1); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.53 (dd, *J* = 9.6 Hz, 7.3 Hz, 1 H, 6-H_a), 3.57 (dd, *J* = 9.6 Hz, 6.1 Hz, 1 H, 6-H_b), 4.06 (dd, *J* = 9.9 Hz, 3.3 Hz, 1 H, 2-H), 4.13 (dd, *J* = 9.9 Hz, 2.7 Hz, 1 H, 3-H), 4.35-4.43 (m, 1 H, 5-H), 4.37 (d, *J* = 11.6 Hz, 1 H, CH_2-Ph), 4.45 (d, *J* = 11.6 Hz, 1-H, CH_2-Ph), 4.60 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 4.70 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 4.75 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 4.86 (d, *J* = 11.7 Hz, 1 H, CH_2-Ph), 5.96 (dd, *J* = 2.7 Hz, 1.1 Hz, 1 H, 4-H), 6.56 (d, *J* = 3.3 Hz, 1 H, 1-H), 7.14-7.34 (m, 15 H, Ph-H), 7.39-7.50 (m, 2 H, Ph-H), 7.54-7.61 (m, 1 H, Ph-H), 7.97-8.04 (m, 2 H, Ph-H), 8.58 (s, 1 H, NH); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 67.9 (C-4, C-6)*, 70.7 (C-5), 71.7 (CH_2-Ph), 73.2 (CH_2-Ph), 73.6 (CH_2-Ph), 74.7 (C-2), 75.4 (C-3), 91.2 (O(C(NH))*C*Cl_3) 95.0 (C-1), 127.4, 127.6, 127.6, 127.8, 127.9, 127.9, 127.9, 128.0, 128.1, 128.1, 128.2, 128.2, 128.3, 128.3, 129.8. 129.8 (C_{tert}-Ph), 133.0, 137.4, 137.8, 138.1 (C_{quart}-Ph), 161.1 (O(*C*(NH))CCl_3), 165.5 (O(*C*O)Ph).

4-*O*-Benzoyl-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl Dibutyl Phosphate (**3**)

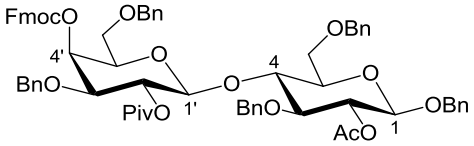


A solution of **3a** (611 mg, 0.87 mmol, 1.0 eq) in dry CH_2Cl_2 (10 mL) was cooled to 0 °C and dibutyl phosphate (210 µL, 1.05 mmol, 1.2 eq) dissolved in dry CH_2Cl_2 (1.0 mL) was added. The reaction mixture was stirred at 0 °C for 1 h and the solvents were removed under reduced pressure. After column chromatography on silica gel (pentane/EtOAc, 3:1 → 1:1) 416 mg (69%) of **3** were obtained as a colorless oil.

***R*$_f$**: 0.31 (hexane/EtOAc, 2:1); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.86 (t, *J* = 7.5 Hz, 3 H, O(CH_2)$_3$CH_3), 0.89 (t, *J* = 7.4 Hz, 3 H, O(CH_2)$_3$CH_3), 1.22-1.44 (m, 4 H, O(CH_2)$_3$CH$_3$), 1.49-1.68 (m, 4 H, O(CH_2)$_3$CH$_3$), 3.57 (dd, *J* = 9.3 Hz, 7.2 Hz, 1 H, 6-H_a), 3.64 (dd, *J* = 9.3 Hz, 5.7 Hz, 1 H, 6-H_b), 3.72 (dd, *J* = 9.6 Hz, 3.0 Hz, 1 H, 3-H) 3.77 (dd, *J* = 9.6 H, 7.0 Hz, 1 H, 2-H), 4.42 (d, *J* = 11.8 Hz, 1 H, CH_2-Ph), 3.92-4.00 (m, 1 H, 5-H), 3.99-4.15 (m, 4 H, O(CH_2)$_3$CH$_3$), 4.49 (d, *J* = 11.8 Hz, 1 H, CH_2-Ph), 4.55 (d, *J* = 11.4 Hz, 1 H, CH_2-Ph), 4.87 (d, *J* = 11.1 Hz, 1 H, CH_2-Ph), 4.83 (d, *J* = 11.1 Hz, 1 H, CH_2-Ph), 4.86 (d, *J* = 11.4 Hz, 1 H, CH_2-Ph), 5.22 (dd, *J* = 7.4 Hz, 7.0 Hz, 1 H, 1-H), 5.88 (dd, *J* = 3.0 Hz, 0.9 Hz, 1 H, 4-H), 7.17-7.38 (m, 15 H, Ph-H), 7.42-7.52 (m, 2 H, Ph-H), 7.55-7.64 (m, 1 H, Ph-H), 8.06-8.12 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 13.6 ($J_{C,P}$ = 2.1 Hz, O(CH_2)$_3$CH_3),

18.6 ($J_{C,P}$ = 5.5 Hz, O(CH_2)$_3$$CH_3$), 32.1 ($J_{C,P}$ = 4.0 Hz, O(CH_2)$_3$$CH_3$), 32.2 ($J_{C,P}$ = 4.2 Hz, O(CH_2)$_3$$CH_3$), 66.9 (C-4), 67.7 ($J_{C,P}$ = 5.6 Hz, O(CH_2)$_3$$CH_3$), 67.8 (C-6), 67.9 ($J_{C,P}$ = 5.6 Hz, O(CH_2)$_3$$CH_3$), 72.0 ($CH_2$-Ph), 73.6 ($CH_2$-Ph), 75.3 ($CH_2$-Ph), 78.7 ($J_{C,P}$ = 9.2 Hz, C-2), 79.4 ($J_{C,P}$ = 1.9 Hz, C-3), 98.8 ($J_{C,P}$ = 6.3 Hz, C-1), 127.4, 127.6, 127.6, 127.7, 127.7, 127.7, 127.8, 127.9, 128.0, 128.1, 128.1, 128.2, 128.2, 128.3, 128.3, 129.7, 129.8, 129.9 (C_{tert}-Ph), 133.1, 137.3, 137.4, 138.1 (C_{quart}-Ph), 165.5 (O(*C*O)Ph); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2959, 2931, 2872, 1723, 1602, 1453, 1267, 1174, 1095, 1023; **MS** (ESI): *m*/*z* (%) = 769.3 [M+Na]$^+$, 1515.6 [2M+Na]$^+$; **HRMS (ESI)** für $C_{42}H_{51}O_{10}P$ (746.82): ber. 769.3112 [M+Na]$^+$, gef. 769.3112.

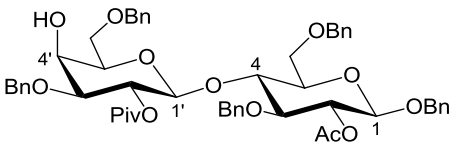
Benzyl 3,6-Di-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-2-*O*-pivaloyl-β-D-galactopyranosyl-(1→4)-2-*O*-acetyl-3,4-di-*O*-benzyl-β-D-glucopyranoside (**13a**)



Galactosyl phosphate **2** (178 mg, 0.21 mmol, 1.3 eq) and glucopyranoside **1** (79 mg, 0.16 mmol, 1.0 eq) were azeotroped with toluene (3 × 1.0 mL) and dried in high vacuum for 1 h. The monosaccharides were dissolved in dry CH_2Cl_2 (3.0 mL), cooled to -40 °C and TMSOTf (38 µL, 0.21 mmol, 1.3 eq) was added. The solution was stirred at -40 °C for 2 h and then quenched by the addition of pyridine (1.0 mL). The solvents were removed under reduced pressure and the residue purified by column chromatography on silica gel (pentane/EtOAc, 5:1 → 3:1). Disaccharide **13a** was obtained in 190 mg (99%) yield as a colorless foam.

***R*$_f$**: 0.27 (hexane/EtOAc, 3:1); $[\alpha]_D^{20}$ = -2.3° (*c* 0.27, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.17 (s, 9 H, O(CO)C(CH_3)$_3$), 1.98 (s, 3 H, O(CO)CH_3), 3.30-3.37 (m, 1 H, 5-H), 3.43 (dd, *J* = 7.5 Hz, 3.2 Hz, 1 H, 3′-H), 3.43-3.48 (m, 1 H, 6′-H$_a$), 3.57 (dd, *J* = 9.2 Hz, 8.6 Hz, 1 H, 3-H), 3.57 (dd, *J* = 11.3 Hz, 2.5 Hz, 1 H, 6′-H$_b$), 3.71-3.77 (m, 1 H, 6-H$_a$), 3.80 (dd, *J* = 11.0 Hz, 2.9 Hz, 1 H, 6-H$_b$), 4.09 (dd, *J* = 9.4 Hz, 9.2 Hz, 1 H, 4-H), 4.11-4.21 (m, 2 H, 5′-H, CH-Fmoc), 4.30 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.37 (d, *J* = 7.9 Hz, 1 H, 1-H), 4.39-4.51 (m, 7 H, 1′-H, CH_2-Ph, CH_2-Fmoc), 4.60 (d, *J* = 12.4 Hz, 1 H, CH_2-Ph), 4.63 (d, *J* = 11.4 Hz, 1 H, CH_2-Ph), 4.78 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.89 (d, *J* = 12.4 Hz, 1 H, CH_2-Ph), 4.97 (d, *J* = 11.1 Hz, 1 H, CH_2-Ph), 5.04 (dd, *J* = 9.2 Hz, 7.9 Hz, 1 H, 2-H), 5.30 (dd, *J* = 9.5 Hz, 7.5 Hz, 1 H, 2′-H), 5.43 (d, *J* = 3.2 Hz, 1 H, 4′-H), 7.11-7.41 (m, 29 H, Ph-H), 7.52-7.61 (m, 2 H, Ar-H), 7.70-7.78 (m, 2 H, Ar-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 21.0 (O(CO)*C*H_3), 27.3 (O(CO)C(*C*H_3)$_3$), 38.8 (O(CO)*C*(CH_3)$_3$), 46.5 (CH-Fmoc), 67.3 (C-6′), 67.9 (C-6), 70.2 (C-4′), 70.2 (CH_2-Fmoc), 70.3 (CH_2-Ph), 70.9 (C-2′), 71.5 (CH_2-Ph), 72.0 (C-4), 72.6 (C-2), 73.6 (CH_2-Ph), 73.6 (CH_2-Ph), 74.7 (CH_2-Ph), 75.1 (C-5), 75.4 (C-5′), 77.8 (C-3′), 80.3 (C-3), 99.7 (C-1′), 99.8 (C-1), 119.7, 119.8, 125.1, 125.6 (C_{tert}-Ar), 127.1, 127.2, 127.4, 127.4, 127.4, 127.6, 127.6, 127.7, 127.7, 127.8, 127.9, 128.0, 128.0, 128.0, 128.2, 128.3, 128.4 (C_{tert}-Ph), 137.3, 137.3, 137.7, 137.9, 138.7 (C_{quart}-Ph), 141.0, 141.1, 143.0, 143.5 (C_{quart}-Ar), 154.8 (CO-Fmoc), 169.3 (O(*C*O)CH_3), 176.4 (O(*C*O)C(CH_3)$_3$); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2922, 2852, 1741, 1497, 1452, 1366, 1255, 1230, 1054; **MS** (ESI): *m*/*z* (%) = 1163.5 [M+Na]$^+$; **HRMS (ESI)** for $C_{69}H_{72}O_{15}$ (1141.30): calcd. 1163.4763 [M+Na]$^+$, found 1163.4758.

Benzyl 3,6-Di-*O*-benzyl-2-*O*-pivaloyl-β-D-galactopyranosyl-(1→4)-2-*O*-acetyl-3,4-di-*O*-benzyl-β-D-glucopyranoside (**13b**)

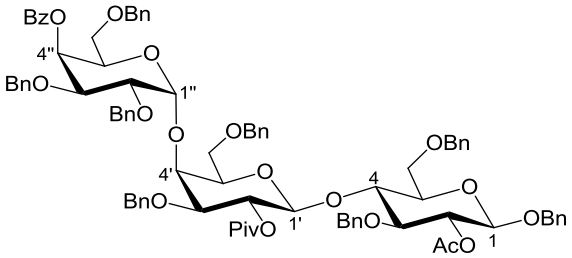


To a solution of disaccharide **13a** (1.09 g, 0.96 mmol, 1.0 eq) in DMF (30 mL) was added piperidine (13 mL, 0.13 mol, 137 eq) at r.t. and the mixture stirred for 30 min. The solvents were removed under

reduced pressure and the residue purified by column chromatography on silica gel (pentane/EtOAc, 5:1 → 3:1). The partially deprotected disaccharide **13b** was obtained in 752 mg (85%) yield as a colorless oil.

***R*f**: 0.30 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$ = 0.0° (*c* 0.38, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.15 (s, 9 H, O(CO)C(CH_3)$_3$), 1.91 (s, 3 H, O(CO)CH_3), 2.36 (s_{br}, 1 H, 4′-OH), 3.26-3.40 (m, 3 H, 3-H, 5-H, 5′-H), 3.50 (dd, *J* = 9.6 Hz, 2.5 Hz, 1 H, 3′-H), 3.52 (dd, *J* = 9.5 Hz, 5.0 Hz, 1 H, 6′-H_a), 3.64 (dd, *J* = 9.5 Hz, 6.6 Hz, 1 H, 6′-H_b), 3.69-3.74 (m, 1 H, 6-H_a), 3.75 (dd, *J* = 11.0 Hz, 3.7 Hz, 1 H, 6-H_b), 3.99-4.02 (m, 1 H, 4′-H), 4.04 (dd, *J* = 9.6 Hz, 8.9 Hz, 1 H, 4-H), 4.37 (d, *J* = 7.9 Hz, 1 H, 1′-H), 4.37 (d, *J* = 12.1 Hz, 1 H, CH_2-Ph), 4.44 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.44 (d, *J* = 7.7 Hz, 1 H, 1-H), 4.48 (d, *J* = 11.6 Hz, 1 H, CH_2-Ph), 4.55 (d, *J* = 11.4 Hz, 1 H, CH_2-Ph), 4.56 (d, *J* = 12.6 Hz, 1 H, CH_2-Ph), 4.62 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.73 (d, *J* = 12.1 Hz, 1 H, CH_2-Ph), 4.85 (d, *J* = 12.6 Hz, 1 H, CH_2-Ph), 4.90 (d, *J* = 11.6 Hz, 1 H, CH_2-Ph), 4.95 (d, *J* = 11.4 Hz, 1 H, CH_2-Ph), 4.99 (dd, *J* = 9.6 Hz, 7.9 Hz, 1 H, 2′-H), 5.17 (dd, *J* = 9.8 Hz, 7.7 Hz, 1 H, 2-H), 7.16-7.38 (m, 25 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 20.9 (O(CO)*C*H_3), 27.4 (O(CO)C(*C*H_3)$_3$), 38.8 (O(CO)*C*(CH$_3$)$_3$), 65.7 (C-4′), 68.0 (C-6), 68.5 (C-6′), 70.2 (CH_2-Ph), 71.0 (C-2), 71.5 (CH_2-Ph), 72.5 (C-2′), 73.2, 75.2, 75.2 (C-4, C-5, C-5′)*, 73.6 (CH_2-Ph), 73.6 (CH_2-Ph), 74.6 (CH_2-Ph), 79.3 (C-3), 80.4 (C-3′), 99.6 (C-1′), 99.8 (C-1), 127.1, 127.4, 127.6, 127.6, 127.6, 127.7, 127.9, 127.9, 127.9, 128.0, 128.2, 128.3, 128.4, 128.4 (C_{tert}-Ph), 137.2, 137.3, 138.0, 138.0, 138.8 (C_{quart}-Ph), 169.3 (O(*C*O)CH_3), 176.7 (O(*C*O)C(CH_3)$_3$); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3469, 2920, 2870, 1745, 1730, 1454, 1366, 1237, 1059; **MS** (ESI): *m*/*z* (%) = 941.4 $[M+Na]^+$, 1860.8 $[2M+Na]^+$; **HRMS (ESI)** for $C_{54}H_{62}O_{13}$ (919.06): calcd. 941.4083 $[M+Na]^+$, found 941.4082.

Benzyl 4-*O*-Benzoyl-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-pivaloyl-β-D-galactopyranosyl-(1→4)-2-*O*-acetyl-3,4-di-*O*-benzyl-β-D-glucopyranoside (**14**)

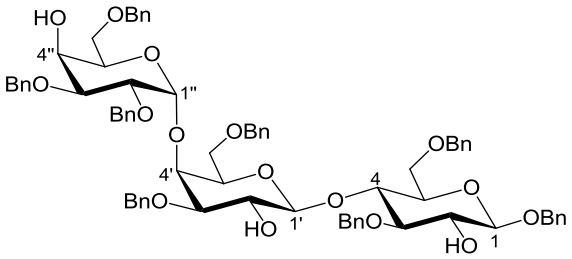


Galactosyl phosphate **3** (379 mg, 0.51 mmol, 1.5 eq) and disaccharide **13b** (311 mg, 0.34 mmol, 1.0 eq) were azeotroped with toluene (3 × 1.0 mL) and dried in high vacuum for 1 h. Mono- and disaccharide were dissolved in dry CH_2Cl_2 (10 mL), cooled to -60 °C and TMSOTf (92 µL, 0.5 mmol, 1.5 eq) was added. The solution was allowed to warm to -20 °C over 2.5 h and quenched with pyridine (1.0 mL). The solvents were removed under reduced pressure and the residue purified by column chromatography on silica gel (pentane/EtOAc, 5:1 → 3:1). Trisaccharide **14** was obtained in 465 mg (94%) yield as a colorless foam.

***R*f**: 0.46 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$ = +26.6° (*c* 0.56, $CHCl_3$); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 1.15 (s, 9 H, O(CO)C(CH_3)$_3$), 1.80 (s, 3 H, O(CO)CH_3), 3.02 (dd, *J* = 8.9 Hz, 4.9 Hz, 1 H, 6″-H_a), 3.17 (dd, *J* = 8.9 Hz, 8.7 Hz, 1 H, 6″-H_b), 3.28 (ddd, *J* = 9.6 Hz, 3.8 Hz, 1.8 Hz, 1 H, 5-H), 3.30 (dd, *J* = 10.5 Hz, 2.6 Hz, 1 H, 3′-H), 3.41 (dd, *J* = 8.8 Hz, 5.4 Hz, 1 H, 5′-H), 3.47 (dd, *J* = 9.3 Hz, 9.2 Hz, 1 H, 3-H), 3.59 (dd, *J* = 9.2 Hz, 5.4 Hz, 1 H, 6′-H_a), 3.73 (dd, *J* = 10.8 Hz, 1.8 Hz, 1 H, 6-H_a), 3.77 (dd, *J* = 10.8 Hz, 3.8 Hz, 1 H, 6-H_b), 3.87 (d, *J* = 12.2 Hz, 1 H, CH_2-Ph), 3.87 (dd, *J* = 10.2 Hz, 3.3 Hz, 1 H, 2″-H), 3.93 (d, *J* = 10.2 Hz, 1 H, CH_2-Ph), 4.07 (dd, *J* = 10.2 Hz, 3.0 Hz, 1 H, 3″-H), 4.10 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.10 (dd, *J* = 9.6 Hz, 9.2 Hz, 1 H, 4-H), 4.15 (d, *J* = 2.6 Hz, 1 H, 4′-H), 4.18 (dd, *J* = 9.2 Hz, 8.8 Hz, 1 H, 6′-H_b), 4.28 (d, *J* = 11.9 Hz, 1 H, CH_2-Ph), 4.33 (d, *J* = 7.9 Hz, 1 H, 1-H), 4.35 (d, *J* = 11.8 Hz, 1 H, CH_2-Ph), 4.38 (d, *J* = 12.5 Hz, 1 H, CH_2-Ph), 4.44 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.48 (d, *J* = 7.9 Hz, 1 H, 1′-H), 4.51 (d, *J* = 11.8 Hz, 1 H, CH_2-Ph), 4.54 (d, *J* = 12.5 Hz, 1 H, CH_2-Ph), 4.61 (d, *J* = 12.0 Hz, 1 H, CH_2-Ph), 4.65 (d, *J* = 10.2 Hz, 1 H, CH_2-Ph), 4.65-4.68 (m, 1 H, 5″-H), 4.72 (d,

J = 12.6 Hz, 1 H, CH_2-Ph), 4.74 (d, J = 12.2 Hz, 1 H, CH_2-Ph), 4.81 (d, J = 12.5 Hz, 1 H, CH_2-Ph), 4.83 (d, J = 12.5 Hz, 1 H, CH_2-Ph), 4.94 (dd, J = 9.3 Hz, 7.9 Hz, 1 H, 2-H), 4.94 (d, J = 12.6 Hz, 1 H, CH_2-Ph), 5.02 (d, J = 3.3 Hz, 1 H, 1″-H), 5.35 (dd, J = 10.5 Hz, 7.9 Hz, 1 H, 2′-H), 5.90 (dd, J = 3.0 Hz, 1.0 Hz, 1 H, 4″-H), 6.89-6.92 (m, 2 H, Ph-H), 7.02-7.38 (m, 40 H, Ph-H), 7.48-7.52 (m, 1 H, Ph-H), 7.88-7.92 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 20.9 (O(CO)CH_3), 27.4 (O(CO)C(CH_3)$_3$), 38.8 (O(CO)C(CH_3)$_3$), 67.2 (C-6′), 67.6 (C-6″), 67.8 (C-5″), 68.1 (C-6), 68.6 (C-4″), 70.2 (CH_2-Ph), 71.3 (C-2′), 71.6 (CH_2-Ph), 71.7 (CH_2-Ph), 72.3 (C-2), 73.0 (CH_2-Ph), 73.1 (CH_2-Ph), 73.3 (C-5′), 73.5 (CH_2-Ph), 73.9 (C-4′), 74.0 (CH_2-Ph), 74.2 (CH_2-Ph), 74.9 (C-2″), 75.1 (C-5), 75.3 (C-4), 77.5 (C-3″), 79.4 (C-3), 79.7 (C-3′), 99.6 (C-1′), 99.8 (C-1), 100.9 (C-1″), 126.7, 126.9, 127.1, 127.4, 127.4, 127.5, 127.6, 127.6, 127.6, 127.7, 127.7, 127.7, 127.8, 127.9, 128.0, 128.0, 128.1, 128.1, 128.3, 128.3, 128.4, 128.7, 129.7, 130.4 (C_{tert}-Ph), 132.5, 137.3, 137.8, 137.8, 138.0, 138.0, 138.2, 138.3, 139.0 (C_{quart}-Ph), 165.3 (O(CO)Ph), 169.3 (O(CO)CH_3), 176.5 (O(CO)C(CH_3)$_3$); **IR** (ATR): $\tilde{\nu}$ (cm^{-1}) = 2923, 2856, 1738, 1723, 1453, 1364, 1270, 1233, 1091, 1053; **MS** (ESI): m/z (%) = 750.8 $[M+2Na]^{2+}$, 1477.6 $[M+Na]^+$; **HRMS (ESI)** for $C_{88}H_{94}O_{19}$ (1455.68): calcd. 1477.6282 $[M+Na]^+$, found 1477.6307.

Benzyl 2,3,6-Tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,4-di-*O*-benzyl-β-D-glucopyranoside (**15**)

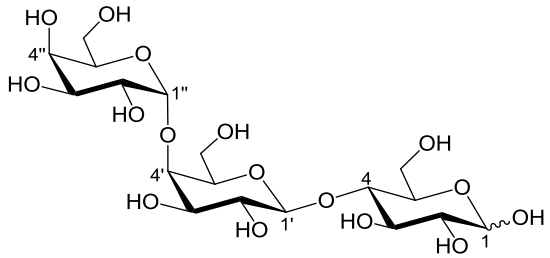


To a solution of trisaccharide **14** (460 mg, 0.32 mmol, 1.0 eq) in CH_3OH (12 mL) and CH_2Cl_2 (4.0 mL) was added NaOMe (5.2 M in methanol, 3.0 mL, 16 mmol, 49 eq) at r.t. The solution was stirred at r.t. for 2 d and then at 50 °C for 2 d. The solvents were removed under reduced pressure and the residue purified by column chromatography on silica gel (pentane/EtOAc, 4:1 → 3:1). Trisaccharide **15** was obtained in 339 mg (88%) yield as a colorless oil.

***R*$_f$**: 0.26 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$ = +39.8° (*c* 0.31, $CHCl_3$); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 2.30 (s_{br}, 1 H, 2-OH), 2.83 (s_{br}, 1 H, 4″-OH), 2.99 (s_{br}, 1 H, 2′-OH), 3.19 (dd, J = 9.8 Hz, 2.3 Hz, 1 H, 3′-H), 3.31-3.36 (m, 2 H, 5′-H, 6′-H_a) 3.37 (dd, J = 9.5 Hz, 4.3 Hz, 1 H, 6″-H_a) 3.50 (ddd, J = 9.5 Hz, 3.7 Hz, 1.6 Hz, 1 H, 5-H), 3.52-3.57 (m, 2 H, 2-H, 3-H), 3.58 (dd, J = 9.6 Hz, 7.6 Hz, 1 H, 6″-H_b), 3.76 (dd, J = 9.8 Hz, 7.8 Hz, 1 H, 2′-H), 3.83 (dd, J = 11.5 Hz, 1.6 Hz, 1 H, 6-H_a), 3.92 (dd, J = 9.4 Hz, 3.0 Hz, 1 H, 3″-H), 3.95 (dd, J = 9.4 Hz, 2.9 Hz, 1 H, 2″-H), 3.97 (dd, J = 11.5 Hz, 3.7 Hz, 1 H, 6-H_b), 3.98 (dd, J = 9.5 Hz, 9.1 Hz, 1 H, 4-H), 4.01 (d, J = 2.3 Hz, 1 H, 4′-H), 4.07 (dd, J = 7.8 Hz, 7.8 Hz, 1 H, 6′-H_b), 4.13 (d, J = 11.6 Hz, 1 H, CH_2-Ph), 4.16 (dd, J = 3.0 Hz, 1.4 Hz, 1 H, 4″-H), 4.17 (d, J = 11.6 Hz, 1 H, CH_2-Ph), 4.27 (d, J = 12.0 Hz, 1 H, CH_2-Ph), 4.33-4.37 (m, 1 H, 5″-H), 4.34 (d, J = 7.6 Hz, 1 H, 1-H), 4.37 (d, J = 12.0 Hz, 1 H, CH_2-Ph), 4.50 (d, J = 12.3 Hz, 1 H, CH_2-Ph), 4.51 (d, J = 7.8 Hz, 1 H, 1′-H), 4.52 (d, J = 11.4 Hz, 1 H, CH_2-Ph), 4.55 (d, J = 12.0 Hz, 1 H, CH_2-Ph), 4.57 (d, J = 11.4 Hz, 1 H, CH_2-Ph), 4.62 (d, J = 11.8 Hz, 1 H, CH_2-Ph), 4.65 (d, J = 11.8 Hz, 1 H, CH_2-Ph), 4.67 (d, J = 12.0 Hz, 1 H, CH_2-Ph), 4.77 (d, J = 11.8 Hz, 1 H, CH_2-Ph), 4.78 (d, J = 12.3 Hz, 1 H, CH_2-Ph), 4.84 (d, J = 11.9 Hz, 1 H, CH_2-Ph), 4.91 (d, J = 11.8 Hz, 1 H, CH_2-Ph), 4.94 (d, J = 11.9 Hz, 1 H, CH_2-Ph), 5.05 (d, J = 2.9 Hz, 1 H, 1″-H), 7.14-7.39 (m, 40 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 67.5 (C-6″), 67.8 (C-4″), 68.4 (C-5″), 68.7 (C-6), 69.2 (C-6′), 71.0 (CH_2-Ph), 71.8 (C-2′), 72.0 (CH_2-Ph), 72.0 (CH_2-Ph), 73.0 (CH_2-Ph), 73.5 (CH_2-Ph), 73.5 (CH_2-Ph), 73.7 (C-5′), 73.9 (C-4′), 74.0 (CH_2-Ph), 74.2 (C-2), 74.5 (CH_2-Ph), 74.9 (C-5), 76.1 (C-2″), 76.9 (C-4), 78.3 (C-3″), 80.6 (C-3′), 83.0 (C-3), 100.4 (C-1″), 101.7 (C-1), 103.5 (C-1′), 127.4, 127.5, 127.6, 127.6, 127.6, 127.6, 127.7, 127.8, 128.0, 128.2, 128.2, 128.3, 128.3, 128.3, 128.4, 128.4 (C_{tert}-Ph), 137.1, 137.9, 137.9, 138.1, 138.2, 138.2, 138.6, 139.0 (C_{quart}-Ph); **IR** (ATR): $\tilde{\nu}$ (cm^{-1}) = 3450, 2918, 2868, 1715, 1496, 1453, 1363, 1207, 1074, 1048; **MS**

(ESI): *m*/*z* (%) = 635.3 $[M+2Na]^{2+}$, 1247.5 $[M+Na]^{+}$; **HRMS (ESI)** for $C_{74}H_{80}O_{16}$ (1225.42): calcd. 1247.5339 $[M+Na]^{+}$, found 1247.5342.

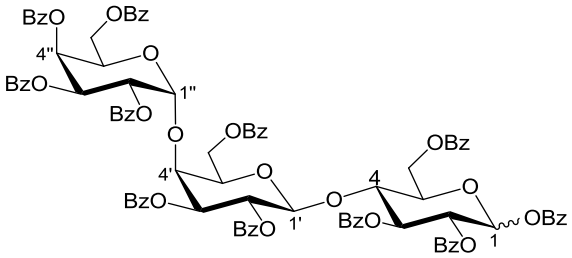
α-D-Galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-D-glucopyranose (**15a**)



To a solution of trisaccharide **15** (212 mg, 0.17 mmol, 1.0 eq) in CH_3OH (21 mL) and CH_2Cl_2 (9.0 mL) was added $Pd(OH)_2$/C (10% Pd, 2.4 mg, 17 µmol, 0.1 eq). The argon atmosphere was exchanged by an hydrogen atmosphere and the mixture stirred at r.t. for 16 h. The catalyst was filtered off using Celite® and the solvents were removed under reduced pressure. The completely deprotected trisaccharide **15a** was obtained in 86 mg (quant) yield as a colorless oil.

^{1}H-NMR (300 MHz, CD_3OD): δ = 3.19 (dd, *J* = 8.5 Hz, 8.3 Hz, 1 H), 3.27-3.32 (m, 2 H), 3.42 (dd, *J* = 9.6 Hz, 3.4 Hz, 1 H), 3.47-3.61 (m, 4 H), 3.63-3.95 (m, 11 H), 3.97-4.00 (m, 1 H), 4.23-4.31 (m, 1 H), 4.39-4.44 (m, 1 H), 4.50 (d, *J* = 7.9 Hz, 1 H), 4.95 (d, *J* = 3.1 Hz, 1 H), 5.10 (d, *J* = 3.5 Hz, 1 H); **^{13}C-NMR** (126 MHz, CD_3OD): δ = 61.5, 62.0, 62.0, 62.6, 70.5, 70.5, 71.0, 71.2, 71.4, 72.6, 72.6, 72.7, 72.8, 73.2, 73.5, 74.6, 76.0, 76.4, 76.5, 79.6, 79.7, 81.1, 81.4 (C-2, C-3, C-4, C-5, C-6, C-2′, C-3′, C-4′, C-5′, C-6′, C-2″, C-3″, C-4″, C-5″, C-6″), 93.6, 98.0 (C-1), 102.5, 102.5, 105.2, 105.3 (C-1′, C-1″); **MS** (ESI): *m*/*z* (%) = 503.2 $[M-H]^-$, 539.1 $[M+Cl]^-$; **HRMS (ESI)** for $C_{18}H_{32}O_{16}$ (504.44): calcd. 503.1618 $[M-H]^-$, found 503.1608.

Benzoyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-D-glucopyranoside (**15b**)

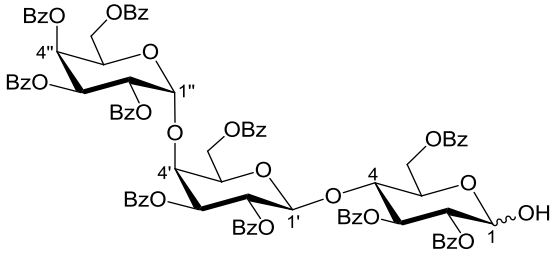


To a solution of trisaccharide **15a** (195 mg, 0.39 mmol, 1.0 eq) in pyridine (8.0 mL) was added benzoyl chloride (680 µL, 5.90 mmol, 15 eq) and a catalytic amount of DMAP. The reaction mixture was stirred at r.t. for 4 d and the solvents were removed under reduced pressure. The perbenzoylated trisaccharide **15b** was obtained after gel permeation HPLC in 582 mg (91%) yield.

***R*$_f$**: 0.49 (hexane/EtOAc, 1:1); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 3.55 (dd, *J* = 8.3 Hz, 5.6 Hz, 1 H), 3.58 (dd, *J* = 7.6 Hz, 5.7 Hz, 1 H), 3.95 (dd, *J* = 8.2 Hz, 6.4 Hz, 1 H), 3.97 (dd, *J* = 8.2 Hz, 6.4 Hz, 1 H), 4.04 (dd, *J* = 9.9 Hz, 5.7 Hz, 1 H), 4.06 (dd, *J* = 9.5 Hz, 5.3 Hz, 1 H), 4.10 (dd, *J* = 7.3 Hz, 7.1 Hz, 1 H), 4.11 (ddd, *J* = 9.9 Hz, 3.9 Hz, 2.3 Hz, 1 H), 4.17 (dd, *J* = 8.4 Hz, 8.3 Hz, 1 H), 4.19 (dd, *J* = 8.7 Hz, 8.5 Hz, 1 H), 4.32-4.40 (m, 6 H), 4.47 (dd, *J* = 12.3 HZ, 3.9 Hz, 1 H), 4.51 (dd, *J* = 12.3 Hz, 2.3 Hz, 1 H), 4.54-4.58 (m, 2 H), 4.61 (dd, *J* = 12.5 Hz, 2.1 Hz, 1 H), 4.90 (d, *J* = 6.5 Hz, 6.5 Hz, 1 H), 4.91 (d, *J* = 7.9 Hz, 1 H), 4.93 (dd, *J* = 2.8 Hz, 1.0 Hz, 1 H), 4.94 (d, *J* = 7.7 Hz, 1 H), 5.14 (dd, *J* = 10.8 Hz, 2.5 Hz, 1 H), 5.16 (dd, *J* = 10.8 Hz, 2.5 Hz, 1 H), 5.43 (d, *J* = 3.4 Hz, 1 H), 5.46 (d, *J* = 3.4 Hz, 1 H), 5.53 (dd, *J* = 10.2 Hz, 3.8 Hz, 1 H), 5.67 (dd, *J* = 10.9 Hz, 3.4 Hz, 1 H), 5.69 (dd, *J* = 8.6 Hz, 7.9 Hz, 1 H), 5.80 (d, *J* = 7.8 Hz, 1 H), 5.82 (dd, *J* = 9.3 Hz, 1.6 Hz, 1 H), 5.84 (dd, *J* = 9.5 Hz, 9.4 Hz, 1 H), 5.87 (dd, *J* = 8.7 Hz, 8.6 Hz, 1 H), 5.99 (dd, *J* = 10.9 Hz, 3.3 Hz, 1 H), 6.01 (dd, *J* = 10.9 Hz, 3.3 Hz, 1 H), 6.13

(d, *J* = 7.8 Hz, 1 H), 6.14-6.16 (m, 2 H), 6.19-6.24 (m, 1 H), 6.71 (d, *J* = 3.6 Hz, 1 H), 7.14-7.54 (m, 43 H), 7.55-7.60 (m, 1 H), 7.63-7.79 (m, 8 H), 7.84-7.89 (m, 16 H), 8.06-8.16 (m, 4 H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 60.2, 60.4, 61.5, 61.8, 62.1, 67.7, 67.7, 68.0, 69.0, 69.6, 69.6, 69.9, 70.4, 70.8, 70.9, 71.0, 72.5, 73.3, 73.5, 73.6, 73.8, 75.6, 75.8, 76.5, 76.6, 89.7 (C-2, C-3, C-4, C-5, C-6, C-2′, C-3′, C-4′, C-5′, C-6′, C-2″, C-3″, C-4″, C-5″, C-6″), 92.2 (C-1), 98.7 (C-1), 98.9, 101.4, 101.8 (C-1′, C-1″), 128.0, 128.0, 128.1, 128.1, 128.1, 128.1, 128.2, 128.2, 128.2, 128.2, 128.3, 128.3, 128.4, 128.4, 128.4, 128.4, 128.6, 128.7, 128.7, 128.9, 128.9, 129.1, 129.1, 129.1, 129.3, 129.3, 129.4, 129.4, 129.4, 129.5, 129.5, 129.6, 129.6, 129.7, 129.7, 129.8, 129.9, 129.9 (C_{tert}-Ph), 132.7, 132.8, 132.9, 132.9, 133.0, 133.1, 133.1, 133.2, 133.5, 133.6 (C_{quart}-Ph), 164.2, 164.3, 164.6, 164.7, 164.7, 164.8, 164.9, 165.0, 165.0, 165.0, 165.2, 165.3, 165.4, 165.5, 165.5, 165.9, 165.9, 166.1, 166.1 (O(*C*O)Ph); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2958, 2923, 1724, 1601, 1492, 1451, 1315, 1262, 1176, 1091; **MS** (ESI): *m*/*z* (%) = 847.2 $[M+2Na]^{2+}$, 1672.4 $[M+Na]^+$; **HRMS (ESI)** for $C_{95}H_{76}O_{27}$ (1649.60): calcd. 1671.4466 $[M+Na]^+$, found 1671.4425.

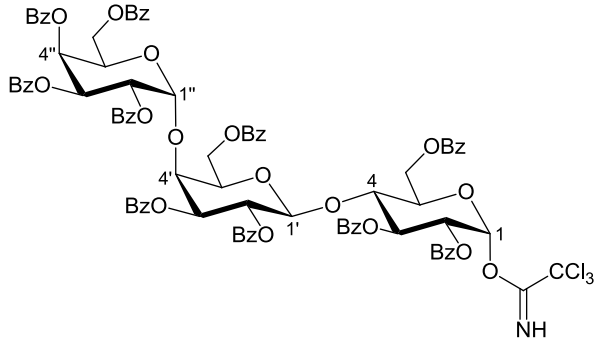
2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-D-glucopyranose (**16**)



The perbenzoylated trisaccharide **15b** (391 mg, 0.24 mmol, 1.0 eq) was dissolved in dry THF (12 mL) in a pressure flask and methylamine (8 M in ethanol, 0.50 mL, 4.0 mmol, 17 eq) was added. The solution was stirred at r.t. for 16 h. After TLC monitoring again methylamine (8 M in Ethanol, 0.50 mL, 4.0 mmol, 17 eq) was added and the reaction mixture stirred at r.t. for 16 h. The solvent was removed under reduced pressure and the residue purified by gel permeation HPLC. Trisaccharide **16** was obtained in 306 mg (84%) yield as a colorless oil.

***R*$_f$**: 0.48 (hexane/EtOAc, 1:1); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 2.38 (d, *J* = 8.7 Hz, 1 H, OH), 2.94 (dd, *J* = 4.6 Hz, 1.7 Hz, 1 H, OH); 3.57-3.63 (m), 3.95-4.01 (m), 4.04 (dd, *J* = 10.7 Hz, 8.2 Hz, 1 H), 4.07-4.12 (m), 4.14 (dd, *J* = 11.1 Hz, 5.7 Hz, 1 H), 4.17 (dd, *J* = 10.9 Hz, 8.6 Hz, 1 H), 4.20-4.26 (m), 4.32 (dd, *J* = 11.2 Hz, 5.3 Hz, 1 H), 4.35 (d, *J* = 1.7 Hz, 1 H), 4.38 (d, *J* = 1.3 Hz, 1 H), 4.40-4.50 (m), 4.57-4.64 (m), 4.87-4.98 (m), 4.99 (d, *J* = 7.9 Hz, 1 H), 5.14-5.26 (m), 5.47-5.50 (m), 5.57 (d, *J* = 3.4 Hz, 1 H), 5.66-5.72 (m), 5.79-5.88 (m), 6.00-6.06 (m, H), 6.14-6.21 (m), 7.09-7.56 (m), 7.66-8.17 (m); **^{13}C-NMR** (126 MHz, CDCl3): δ = 60.4, 60.5, 61.5, 62.2, 62.3, 64.3, 67.6, 67.7, 68.0, 68.0, 68.3, 69.0, 69.0, 69.7, 69.7, 69.7, 69.9, 70.3, 72.4, 72.5, 72.7, 73.2, 73.6, 73.7, 74.4, 75.4, 75.7, 76.8, 76.9 (C-2, C-3, C-4, C-5, C-6, C-2′, C-3′, C-4′, C-5′, C-6′, C-2″, C-3″, C-4″, C-5″, C-6″), 90.1, 95.5 (C-1), 98.6, 98.7, 101.2, 101.3 (C-1′, C-1″), 128.1, 128.2, 128.2, 128.2, 128.2, 128.3, 128.3, 128.4, 128.5, 128.5, 128.6, 128.8, 129.0, 129.0, 129.1, 129.2, 129.2, 129.2, 129.4, 129.5, 129.5, 129.5, 129.6, 129.6, 129.7, 129.7, 129.7, 129.8, 129.9, 129.9 (C_{tert}-Ph), 132.8, 133.0, 133.0, 133.1, 133.1, 133.2, 133.3, 133.3, 133.4, 133.5, 133.6 (C_{quart}-Ph), 164.9, 164.9, 165.0, 165.0, 165.1, 165.4, 165.7, 165.7, 165.8, 165.9, 165.9, 166.1, 166.2, 166.8 (O(*C*O)Ph); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3440, 2965, 1720, 1601, 1491, 1450, 1315, 1261, 1176, 1091; **MS** (ESI): *m*/*z* (%) = 795.2 $[M+2Na]^{2+}$, 1568.5 $[M+Na]^+$; **HRMS (ESI)** for $C_{88}H_{72}O_{26}$ (1545.50): calcd. 1567.4204 $[M+Na]^+$, found 1567.4249.

2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**17**)



To a solution of trisaccharide **16** (150 mg, 97.1 µmol, 1.0 eq) in dry CH_2Cl_2 (5.0 mL) was added dropwise Cl_3CCN (78.0 µL, 776 µmol, 8.0 eq) and DBU (5.0 µL, 33 µmol, 0.3 eq) at 0 °C. The solution was stirred at 0 °C for 1 h and then the solvents were removed under reduced pressure. After filtration over silica gel 162 mg (99%) of the desired compound **17** were obtained as brown foam.

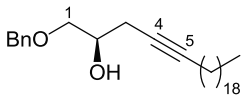
***R*f**: 0.57 (hexane/EtOAc, 1:1); $[\alpha]_D^{20}$ = +85.2° (*c* 0.25, $CHCl_3$); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.57 (dd, *J* = 8.1 Hz, 5.9 Hz, 1 H), 4.03 (dd, *J* = 10.9 Hz, 2.6 Hz, 1 H), 4.08 (dd, *J* = 11.1 Hz, 5.8 Hz, 1 H), 4.15 (dd, *J* = 10.9 Hz, 8.3 Hz, 1 H), 4.30 (dd, *J* = 11.1 Hz, 5.6 Hz, 1 H), 4.33-4.47 (m, 3 H), 4.52 (dd, *J* = 11.9 Hz, 3.2 Hz, 1 H), 4.66 (dd, *J* = 12.1 Hz, 1.5 Hz, 1 H),4.91-4.99 (m, 1 H), 5.01 (d, *J* = 8.1 Hz, 1 H, 1′-H), 5.21 (dd, *J* = 11.1 Hz, 2.8 Hz, 1 H), 5.47 (d, *J* = 3.5 Hz, 1 H, 1″-H), 5.50 (dd, *J* = 10.1 Hz, 3.5 Hz, 1 H), 5.73 (dd, *J* = 11.0 Hz, 3.5 Hz, 1 H), 5.87 (dd, *J* = 10.9 Hz, 7.7 Hz, 1 H), 6.03 (dd, *J* = 10.7 Hz, 3.3 Hz, 1 H), 6.17 (dd, *J* = 3.4 Hz, 1.2 Hz,1 H), 6.23 (dd, *J* = 10.0 Hz, 8.8 Hz, 1 H), 6.71 (d, *J* = 3.8 Hz, 1 H, 1-H), 7.11-7.60 (m, 30 H), 7.67-8.04 (m, 18 H), 8.10-8.18 (m, 2 H), 8.56 (s, 1 H, NH); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 60.1, 61.5, 61.8, 67.6, 68.0, 69.0, 69.6, 69.7, 70.7, 70.8, 71.2, 72.5, 73.7, 75.4, 76.5 (C-2, C-3, C-4, C-5, C-6, C-2′, C-3′, C-4′, C-5′, C-6′, C-2″, C-3″, C-4″, C-5″, C-6″), 90.6 (O(C(NH))*C*Cl_3), 93.0 (C-1), 98.8, 101.7 (C-1′, C-1″), 128.1, 128.1, 128.1, 128.2, 128.3, 128.3, 128.4, 128.4, 128.4, 128.5, 128.5, 128.6, 129.0, 129.1, 129.2, 129.4, 129.4, 129.5, 129.5, 129.5, 129.5, 129.6, 129.6, 129.7, 129.7, 129.7, 129.8, 129.9 (C_{tert}-Ph), 132.7, 132.9, 133.0, 133.1, 133.1, 133.1, 133.3, 133.3, 133.4, 133.5 (C_{quart}-Ph), 160.5 (O(*C*(NH))CCl_3), 164.6, 164.6, 164.9, 165.0, 165.2, 165.4, 165.4, 165.5, 165.9, 166.1 (O(*C*O)Ph); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2923, 1720, 1601, 1584, 1451, 1315, 1262, 1176, 1091, 1067; **MS** (ESI): *m*/*z* (%) = 1712.5 $[M+Na]^+$; **HRMS (ESI)** for $C_{90}H_{72}Cl_3NO_{26}$ (1689.88): calcd. 1710.3300 $[M+Na]^+$, found 1710.3302.

Modified Fatty Acids

(*R*)-2-(Benzoyloxy)-1-(benzyloxy)-4-tetracosin (**20**)[16]

To a solution of 1-Heneicosin (**18**) (965 mg, 3.30 mmol, 1.0 eq) in dry THF (3.0 mL) was added dropwise *n*BuLi (1.6 M in hexane, 2.1 mL, 3.3 mmol, 1.0 eq) at r.t. for 20 min. Afterwards HMPA (7.1 mL, 40 mmol, 12 eq) was added within 20 min, followed by (*R*)-benzyl glycidyl ether (**19**) (542 mg, 3.3 mmol, 1.0 eq) within 10 min and the solution was stirred at r.t. for 16 h. The reaction mixture was quenched with water and extracted with Et_2O (2x). The combined organic layers were dried over Na_2SO_4 and the solvents were removed under reduced pressure. It was not possible to separate the secondary alcohol and the remaining (*R*)-benzyl glycidyl ether by column chromatography on silica gel (pentane/Et_2O, 20:1 → 15:1 → 4:1). Therefore the mixture was used without further purification in the next step.

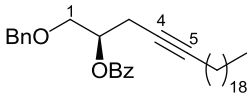
Analytical data for (*R*)-1-(benzyloxy)-4-tetracosin-2-ol: (**20a**)



^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.79 (t, *J* = 6.8 Hz, 3 H, 24-H_3), 1.11-1.42 (m, 34 H, 7-H_2 – 17-H_2), 2.00-2.08 (m, 2 H, 6-H_2), 2.29-2.35 (m, 2 H, 3-H_2), 2.42 (s_{br}, 1 H, OH), 3.39 (dd, *J* = 9.7 Hz, 6.6 Hz, 1 H, 1-H_a), 3.51 (dd, *J* = 9.7 Hz, 3.7 Hz, 1 H, 1-H_b), 3.77-3.88 (m, 1 H, 2-H), 4.45-4.49 (m, 2 H, CH_2-Ph), 7.15-7.29 (m, 5 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.1 (C-24), 18.7 (C-6), 22.6 (C-23), 23.9 (C-3), 28.9, 28.9, 29.1, 29.3, 29.5, 29.6, 29.6, 29.7, 31.9 (C-7 – C-22), 69.1 (C-2), 73.0 (C-1), 73.3 (CH_2-Ph), 75.4 (C-4), 82.9 (C-5), 127.6, 127.7, 128.4 (C_{tert}-Ph), 137.9 (C_{quart}-Ph).

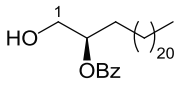
The secondary alcohol **20a** and the benzyl ether were dissolved in pyridine (16 mL) and a catalytic amount of DMAP and benzoyl chloride (230 µL, 3.39 mmol, 1.6 eq) were added. The reaction mixture was stirred at r.t. for 16 h. The solvents were removed under reduced pressure and the residue was purified **20** was obtained in 827 mg (45%) yield as a colorless oil over two steps.

Analytical data for (*R*)-2-(benzoyloxy)-1-(benzyloxy)-4-tetracosin (**20**):



***R*$_f$**: 0.67 (hexane/EtOAc, 4:1); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.4 Hz, 3 H, 24-H_3), 1.18-1.47 (m, 34 H, 7-H_2 – 17-H_2), 2.06-2.15 (m, 2 H, 6-H_2), 2.63-2.71 (m, 2 H, 3-H_2), 3.77-3.81 (m, 2 H, 1-H_2), 4.57 (d, *J* = 12.2 Hz, 1 H, CH_2-Ph), 4.63 (d, *J* = 12.2 Hz, 1 H, CH_2-Ph), 5.28-5.38 (m, 1 H, 2-H), 7.23-7.36 (m, 5 H, Ph-H), 7.40-7.48 (m, 2 H, Ph-H), 7.53-7.60 (m, 1 H, Ph-H), 8.04-8.10 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.1 (C-24), 18.7 (C-6), 21.3 (C-3), 22.7 (C-23), 28.8, 28.9, 29.2, 29.3, 29.5, 29.6, 29.7, 29.7, 31.9 (C-7 – C-22), 69.9 (C-1), 71.8 (C-2), 73.3 (CH_2-Ph), 74.8 (C-4), 82.7 (C-5), 127.6, 127.6, 128.3, 128.3, 129.8, 130.3 (C_{tert}-Ph), 132.9, 138.1 (C_{quart}-Ph), 165.9 (O(*C*O)Ph).

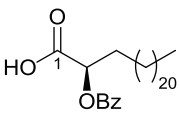
(*R*)-2-(Benzyloxy)-tetracosan-1-ol (**21**)



To a solution of alkyne **20** (33.0 mg, 58.8 µmol, 1.0 eq) in CH_3OH (3.0 mL) and CH_2Cl_2 (1.0 mL) was added under argon atmosphere a spatula point of Pd/C (10%). The argon atmosphere was exchanged by a hydrogen atmosphere and the mixture stirred at r.t. for 16 h. The catalyst was filtered off using Celite® and the solvents were removed under reduced pressure. The primary alcohol **21** was obtained in 27.9 mg (quant.) yield as a colorless oil.

***R*$_f$**: 0.10 (hexane/EtOAc, 15:1); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.86 (t, *J* = 6.7 Hz, 3 H, 24-H_3), 1.16-1.46 (m, 40 H, 4-H_2 – 23-H_2), 1.62-1.76 (m, 2 H, 3-H_2), 2.19 (s_{br}, 1 H, OH), 3.69-3.85 (m, 2 H, 1-H_2), 5.09-5.19 (m, 1 H, 2-H), 7.37-7.47 (m, 2 H, Ph-H), 7.50-7.59 (m, 1 H, Ph-H), 7.99-8.09 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.1 (C-24), 22.7 (C-23), 25.3 (C-4), 29.3, 29.4, 29.5, 29.5, 29.6, 29.6, 29.7 (C-5 – C-21), 30.7 (C-3), 31.9 (C-22), 65.0 (C-1), 76.5 (C-2), 128.4, 129.7, 130.2 (C_{tert}-Ph), 133.0 (C_{quart}-Ph), 166.9 (O(*C*O)Ph).

(*R*)-2-(Benzyloxy)-tetracosanoic acid (**22**)

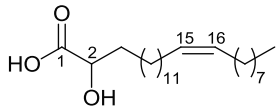


A suspension of alcohol **21** (250 mg, 0.53 mmol, 1.0 eq) in acetone (7.0 mL) and sat. aqueous $NaHCO_3$ solution (1.9 mL) was cooled to 0 °C and sodium bromide (5.0 mg, 48 µmol, 0.09 eq) and TEMPO

(1.7 mg, 11 µmol, 0.02 eq) were added. Afterwards TCCA (247 mg, 1.06 mmol, 2.0 eq.) was added in small portions. The reaction mixture was stirred at r.t. for 16 h and the solvents were removed under reduced pressure. Sat. aqueous NH_4Cl solution and EtOAc were added and the organic layer was washed with 1 M aqueous HCl solution and dried over Na_2SO_4. The solvents were removed under reduced pressure and the residue was purified by column chromatography on silica gel (pentane/EtOAc, 2:1, 1% HCOOH). The fatty acid **22** was obtained in 156 mg (73%) yield as a white solid.

***R*f**: 0.23 (hexane/EtOAc, 2:1); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.86 (t, *J* = 6.4 Hz, 3 H, 24-H_3), 1.17-1.41 (m, 38 H, 5-H_2 – 23-H_2), 1.43-1.58 (m, 2 H, 4-H_2), 1.94-2.05 (m, 2 H, 3-H_2), 5.24 (t, *J* = 5.5 Hz, 1 H, 2-H), 7.39-7.48 (m, 2 H, Ph-H), 7.51-7.61 (m, 1 H, Ph-H), 8.02-8.11 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.1 (C-24), 22.7 (C-23), 25.2 (C-4), 29.1, 29.4, 29.5, 29.6, 29.7, 29.7 (C-5 – C-21), 31.1 (C-3), 31.9 (C-22), 76.8 (C-2), 128.4, 129.4, 129.9 (C_{tert}-Ph), 133.4 (C_{quart}-Ph), 166.1 (O(*C*O)Ph); **MS** (ESI): *m*/*z* (%) = 487.4 $[M-H]^-$, 511.4 $[M+Na]^+$; **HRMS (ESI)** für $C_{31}H_{52}O_4$ (488.74): ber. 511.3758 $[M+Na]^+$, gef. 511.3758.

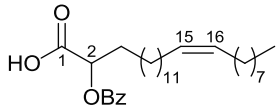
(15*Z*)-2-(Hydroxy)-15-tetracosenoic acid (**9**)



To a solution of diisopropylamine (350 µL, 2.49 mmol, 4.5 eq) in dry THF (3.0 mL) was added dropwise *n*BuLi (2.5 M in hexane, 870 µL, 2.18 mmol, 4.0 eq) at -55 °C. The solution was stirred at -55 °C for 30 min, and then warmed up to 0 °C. In dry THF (2.0 mL) dissolved nervonic acid (200 mg, 0.55 mmol, 1.0 eq) was added dropwise, followed by HMPA (95 µL, 0.55 mmol, 1.0 eq) after 15 min. The mixture was stirred at r.t. for 6 h, cooled to 0 °C and oxygen was bubbled through the solution for 1 h. The mixture was poured onto water, 1 M aq. HCl solution (26 mL) was added and extracted with Et_2O. The organic layer was washed with 1% H_2SO_4 solution and brine and dried over $MgSO_4$. The solvents were removed under reduced pressure and the residue purified by column chromatography on silica gel (pentane/EtOAc, 4:1 → 2:1, 1% HCOOH). Fatty acid **9** was obtained as a racemic mixture in 118 mg (56%) yield.

***R*f**: 0.25 (hexane/EtOAc, 2:1, 1% HCOOH); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.86 (t, *J* = 6.9 Hz, 3 H, 24-H_3), 1.18-1.52 (m, 32 H, 4-H_2 – 13-H_2, 18-H_2 – 23-H_2), 1.59 (m, 1 H, 3-H_a), 1.76-1.89 (m, 1 H, 3-H_b), 1.93-2.04 (m, 4 H, 14-H_2, 17-H_2), 4.25 (dd, *J* = 7.3 Hz, 4.0 Hz, 1 H, 2-H), 5.26-5.36 (m, 2 H, 15-H, 16-H), 5.64-6.72 (s_{br}, 1 H, OH), 6.18 (s_{br}, 1 H, COOH); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.2 (C-24), 22.8 (C-23), 24.9, 27.3, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 29.8, 32.0, 34.3 (C-3 – C-14, C-17 – C-22), 70.3 (C-2), 129.8, 129.8 (C-15, C-16), 179.7 (C-1); **IR** (ATR): $\tilde{\nu}$ (cm^{-1}) = 3542, 2916, 2846, 1687, 1462, 1405, 1377, 1262, 1136, 1092; **MS** (ESI): *m*/*z* (%) = 381.3 $[M-H]^-$, 405.3 $[M+Na]^+$; **HRMS (ESI)** for $C_{24}H_{46}O_3$ (382.62): calcd. 381.3374 $[M-H]^-$, found 381.3375.

(15*Z*)-2-(Benzoyloxy)-15-tetracosenoic acid (**24**)



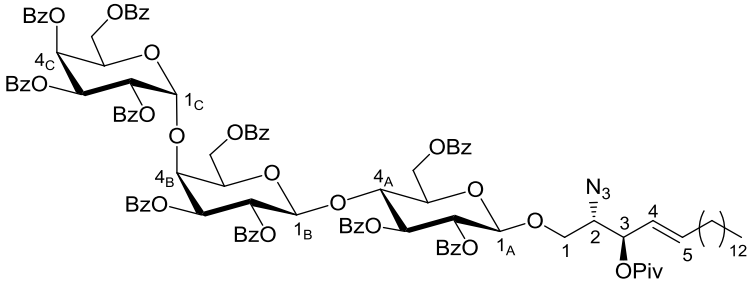
To a solution of fatty acid **9** (113 mg, 0.30 mmol, 1.0 eq) in pyridine (10 mL) was added benzoyl chloride (38 µL, 0.33 mmol, 1.1 eq) and the mixture stirred at r.t. for 5 d. The solvent was removed under reduced pressure and the residue dissolved in EtOAc. The organic layer was washed with sat. $NaHCO_3$ solution twice, once with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel (pentane/EtOAc, 2:1, 1% HCOOH). Fatty acid **24** was obtained in 106 mg (74%) yield as white solid.

***R*f**: 0.21 (hexane/EtOAc, 2:1, 1% HCOOH); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.87 (t, *J* = 6.8 Hz, 3 H, 24-H_3), 1.20-1-40 (m, 32 H, 4-H_2 – 13-H_2, 18-H_2 – 23-H_2), 1.46-1.58 (m, 2 H, 3-H_2), 1.95-2.06 (m, 4 H,

14-H_2, 17-H_2), 5.25 (t, *J* = 6.1 Hz, 1 H, 2-H), 5.32-5.39 (m, 2 H, 15-H, 16-H), 7.41-7.50 (m, 2 H, Ph-H), 7.54-7.63 (m, 1 H, Ph-H), 8.05-8.12 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, DMSO-d_6): δ = 14.0 (C-24), 22.2 (C-23), 24.9, 26.6, 26.6, 28.5, 28.6, 28.7, 28.8, 28.8, 28.9, 28.9, 29.0, 29.0, 29.1, 29.1, 30.8, 31.3 (C-3 – C-14, C-17 – C-22), 73.2 (C-2), 128.7, 129.1, 129.6 (C_{tert}-Ph), 133.3 (C_{quart}-Ph), 165.3 (O(*C*O)Ph), 171.3 (C-1); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3460, 2921, 1720, 1604, 1463, 1452, 1433, 1346, 1270, 1175; **MS** (ESI): *m*/*z* (%) = 485.4 $[M-H]^-$, 509.4 $[M+Na]^+$; **HRMS (ESI)** for $C_{31}H_{50}O_4$ (486.73): calcd. 509.3601 $[M+Na]^+$, found 509.3598.

Globosides

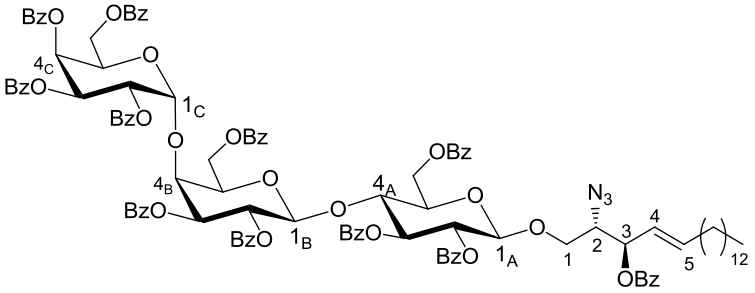
O-(2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-pivaloyl-4-octadecen-1,3-diol (**25**)



Glycosidation of trichloroacetimidate **17** (100 mg, 59.3 µmol, 1.0 eq) with sphingosine **4** (46.0 mg, 113 µmol, 1.9 Äq.) was performed according to **S1** with $BF_3{\cdot}OEt_2$ (1.5 µL, 12 µmol, 0.2 eq) in dry CH_2Cl_2/hexane (6.0 mL). The reaction mixture was stirred at r.t. for 16 h. After column chromatography on silica gel (pentane/EtOAc, 5:1 → 3:1) 55.0 mg (48%) of **25** were obtained as a colorless oil.

***R*$_f$**: 0.47 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$ = +49.7° (*c* 0.57, $CHCl_3$); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 0.80 (t, *J* = 6.7 Hz, 3 H, 18-H_3), 1.03 (s, 9 H, O(CO)C(CH_3)$_3$), 1.06-1.80 (m, 22 H, 7-H_2 – 17-H_2), 1.75-1.80 (m, 2 H, 6-H_2), 3.37 (dd, *J* = 10.2 Hz, 5.6 Hz, 1 H, 1-Ha), 3.51 (dd, *J* = 7.6 Hz, 6.6 Hz, 1 H, 5_B-H), 3.61 (ddd, *J* = 9.7 Hz, 7.0 Hz, 5.6 Hz, 1 H, 2-H), 3.67 (dd, *J* = 10.2 Hz, 7.0 Hz, 1 H, 1-H_b), 3.84 (ddd, *J* = 9.6 Hz, 4.4 Hz, 2.6 Hz, 1 H, 5_A-H), 3.92 (dd, *J* = 11.1 Hz, 7.8 Hz, 1 H, 6_B-H_a), 3.98 (dd, *J* = 11.1 Hz, 5.8 Hz, 1 H, 6_B-H_b), 4.14 (dd, *J* = 11.0 Hz, 8.4 Hz, 1 H, 6_C-H_a), 4.20 (dd, *J* = 9.6 Hz, 9.1 Hz, 1 H, 4_A-H), 4.27 (d, *J* = 2.6 Hz, 1 H, 4_B-H), 4.31 (dd, *J* = 11.0 Hz, 5.1 Hz, 1 H 6_C-H_b), 4.38 (dd, *J* = 12.0 Hz, 4.4 Hz, 1 H, 6_A-H_a), 4.56 (dd, *J* = 12.0 Hz, 2.0 Hz, 1 H, 6_A-H_b), 4.63 (d, *J* = 7.5 Hz, 1 H, 1_A-H), 4.84 (d, *J* = 7.9 Hz, 1 H, 1_B-H), 4.88 (ddd, *J* = 8.4 Hz, 5.1 Hz, 1.0 Hz, 1 H, 5_C-H), 5.10 (dd, *J* = 8.0 Hz, 2.6 Hz, 1 H, 3_B-H), 5.11-5.13 (m, 1 H, 3-H), 5.16-5.21 (m, 1 H, 4-H), 5.33 (dd, *J* = 9.0 Hz, 7.5 Hz, 1 H, 2_A-H), 5.39 (d, *J* = 3.6 Hz, 1 H, 1_C-H), 5.49 (dt, *J* = 15.3 Hz, 7.2 Hz, 1 H, 5-H), 5.62 (dd, *J* = 11.0 Hz, 3.6 Hz, 1 H, 2_C-H), 5.71 (dd, *J* = 9.1 Hz, 9.0 Hz, 1 H, 3_A-H), 5.73 (dd, *J* = 10.9 Hz, 7.9 Hz, 1 H, 2_B-H), 5.95 (dd, *J* = 11.0 Hz, 3.3 Hz, 1 H, 3_C-H), 6.10 (dd, *J* = 3.3 Hz, 1.0 Hz, 1 H, 4_C-H), 7.07-7.18, 7.20-7.36, 7.39-7.46, 7.61-7.64, 7.66-7.73, 7.78-7.90, 8.01-8.03 (m, 50 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.1 (C-18), 22.7, 28.6, 29.0, 29.3, 29.6, 29.6, 29.6, 29.7, 31.9 (C-7 – C-17), 27.0 (O(CO)C(*C*H_3)$_3$), 32.2 (C-6), 38.7 (O(CO)*C*(CH_3)$_3$), 60.5 (C-6_B), 61.5 (C-6_C), 62.3 (C-6_A), 63.3 (C-2), 67.7 (C-5_C), 68.0 (C-3_C), 68.0 (C-1), 69.1 (C-4_C), 69.7 (C-2_B), 69.9 (C-2_C), 71.9 (C-2_A), 72.6 (C-5_B), 73.0 (C-5_A), 73.3 (C-3_A), 73.6 (C-3), 73.8 (C-3_B), 75.8 (C-4_B), 76.8 (C-4_A), 98.8 (C-1_C), 100.4 (C-1_A), 101.4 (C-1_B), 122.7 (C-4), 128.2, 128.2, 128.2, 128.3, 128.3, 128.4, 128.4, 128.5, 128.5, 128.5, 128.6, 128.7, 129.1, 129.1, 129.2, 129.3, 129.3, 129.5, 129.5, 129.6, 129.6, 129.6, 129.7, 129.8, 129.8, 129.9, 129.9 (C_{tert}-Ph), 132.8, 133.0, 133.0. 133.1, 133.1, 133.2, 133.2, 133.3, 133.6 (C_{quart}-Ph), 138.2 (C-5), 164.8, 165.0, 165.0, 165.0, 165.2, 165.4, 165.7, 165.7, 166.1, 166.3 (O(*C*O)Ph), 176.6 (O(*C*O)C(CH_3)$_3$); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2926, 2360, 2341, 1723, 1451, 1261, 1090; **MS** (ESI): *m*/*z* (%) = 990.86 $[M+2Na]^{2+}$, 1979.71 [M-2H+2Na]; **HRMS (ESI)** for $C_{111}H_{113}N_3O_{28}$ (1937.09): calcd. 990.8648 $[M+2Na]^{2+}$, found 990.8649.

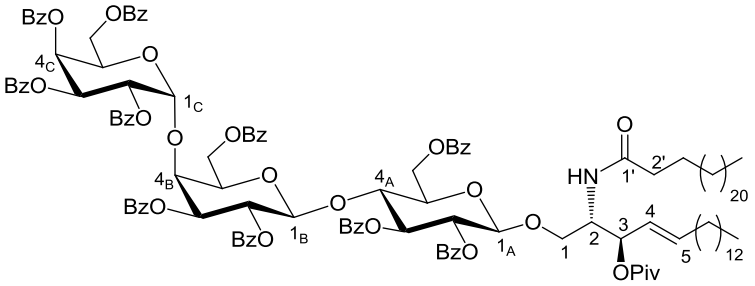
O-(2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecen-1,3-diol (**26**)



Glycosidation of **17** (26.6 mg, 15.7 µmol, 1.0 eq) with sphingosine **5** (20.0 mg, 46.6 µmol, 3.0 eq) was performed according to **S1** with $BF_3{\cdot}OEt_2$ (0.4 µL, 3.2 µmol, 0.2 eq) in dry CH_2Cl_2/hexane (3.0 mL). The reaction mixture was stirred at r.t. for 2 d. After gel permeations HPLC 12.5 mg (41%) of **26** were obtained as a colorless oil.

***R*f**: 0.27 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$ = +38.6° (*c* 0.28, $CHCl_3$); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 0.86 (t, *J* = 7.2 Hz, 3 H, 18-H_3), 1.11-1.31 (m, 22 H, 7-H_2 – 17-H_2), 1.82-1.88 (m, 2 H, 6-H_2), 3.48 (dd, *J* = 10.2 Hz, 5.0 Hz, 1 H, 1-Ha), 3.57 (dd, *J* = 8.0 Hz, 6.0 Hz, 1 H, 5_B-H), 3.79 (dd, *J* = 10.2 Hz, 6.7 Hz, 1 H, 1-H_b), 3.85 (ddd, *J* = 9.0 Hz, 6.7 Hz, 5.0 Hz, 1 H, 2-H), 3.91 (ddd, *J* = 9.7 Hz, 4.3 Hz, 2.0 Hz, 1 H, 5_A-H), 3.98 (dd, *J* = 10.9 Hz, 8.0 Hz, 1 H, 6_B-H_a), 4.03 (dd, *J* = 10.9 Hz, 6.0 Hz, 1 H, 6_B-H_b), 4.18 (dd, *J* = 11.1 Hz, 8.4 Hz, 1 H, 6_C-H_a), 4.27 (dd, *J* = 9.7 Hz, 8.9 Hz, 1 H, 4_A-H), 4.33 (d, *J* = 2.7 Hz, 1 H, 4_B-H), 4.35 (dd, *J* = 11.1 Hz, 5.2 Hz, 1 H 6_C-H_b), 4.42 (dd, *J* = 12.0 Hz, 4.3 Hz, 1 H, 6_A-H_a), 4.61 (dd, *J* = 12.0 Hz, 2.0 Hz, 1 H, 6_A-H_b), 4.71 (d, *J* = 7.3 Hz, 1 H, 1_A-H), 4.91 (d, *J* = 7.7 Hz, 1 H, 1_B-H), 4.93 (ddd, *J* = 8.4 Hz, 5.2 Hz, 1.1 Hz, 1 H, 5_C-H), 5.17 (dd, *J* = 10.8 Hz, 2.7 Hz, 1 H, 3_B-H), 5.34-5.39 (m, 1 H, 4-H), 5.40 (dd, *J* = 8.8 Hz, 7.3 Hz, 1 H, 2_A-H), 5.45 (d, *J* = 3.6 Hz, 1 H, 1_C-H), 5.48 (dd, *J* = 9.0 Hz, 4.2 Hz, 1 H, 3-H) 5.64 (dt, *J* = 15.2 Hz, 6.8 Hz, 1 H, 5-H), 5.67 (dd, *J* = 11.0 Hz, 3.5 Hz, 1 H, 2_C-H), 5.77 (dd, *J* = 8.9 Hz, 8.8 Hz, 1 H, 3_A-H), 5.79 (dd, *J* = 10.8 Hz, 7.7 Hz, 1 H, 2_B-H), 6.01 (dd, *J* = 11.0 Hz, 3.4 Hz, 1 H, 3_C-H), 6.15 (dd, *J* = 3.4 Hz, 1.1 Hz, 1 H, 4_C-H), 7.11-7.5, 7.66-7.78, 7.83-7.97, 8.07-8.10 (m, 55 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.2 (C-18), 22.8, 28.6, 29.2, 29.4, 29.5, 29.6, 29.7, 29.7, 29.7, 32.0, (C-7 – C-17), 32.3 (C-6), 60.6 (C-6_B), 61.6 (C-6_C), 62.3 (C-6_A), 63.4 (C-2), 67.7 (C-5_C), 68.1 (C-1, C-3_C), 69.1 (C-4_C), 69.7 (C-2_B), 69.9 (C-2_C), 72.0 (C-2_A), 72.6 (C-5_B), 73.0 (C-5_A), 73.4 (C-3_A), 73.6 (C-3_B), 74.8 (C-3), 75.8 (C-4_B), 77.2 (C-4_A), 98.8 (C-1_C), 100.3 (C-1_A), 101.4 (C-1_B), 122.3 (C-4), 128.1, 128.1, 128.2, 128.2, 128.3, 128.4, 128.4, 128.4, 128.5, 128.6, 129.1, 129.2, 129.3, 129.4, 129.4, 129.5, 129.6, 129.6, 129.7, 129.7, 129.8, 129.8, 129.8, 129.9(C_{tert}-Ph), 132.7, 132.9 133.0, 133.1. 133.1, 133.3, 133.5 (C_{quart}-Ph), 138.8 (C-5), 164.7, 164.8, 164.9, 164.9, 164.9, 165.0, 165.3, 165.5, 165.6, 166.0, 166.1 (O(*C*O)Ph); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2922, 2852, 1723, 1602, 1451, 1376, 1261, 1092; **MS** (ESI): *m*/*z* (%) = 1001.4 $[M+2Na]^{2+}$, 1979.8 $[M+Na]^+$; **HRMS (ESI)** for $C_{113}H_{109}N_3O_{28}$ (1957.68): calcd. 1978.7090 $[M+Na]^+$, found 1978.7113.

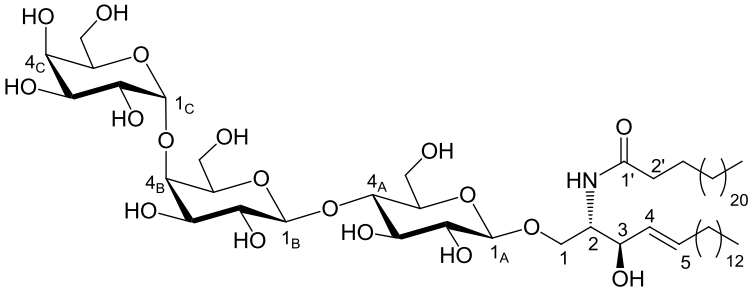
O-(2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galacto-pyranosyl)-(1→4)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-pivaloyl-2-(tetracosanamido)-4-octadecen-1,3-diol (**27a**)



Reduction of azidosphingosine **25** (21.0 mg, 10.8 µL, 1.0 eq) in benzene (2.0 mL) and water (27 µL) using PPh_3 (6.5 mg, 25 µmol, 2.3 eq) was performed according to **S3**. The corresponding amine was dissolved in dry THF (1.0 mL) and HATU (5.3 mg, 14 µmol, 1.3 eq) dissolved in DMF (0.5 mL), DIPEA (2.4 µL, 14 µmol, 1.3 eq) and tetracosanoic acid (5.0 mg, 14 µmol, 1.3 eq) were added. The reaction mixture was stirred for 3 h. After column chromatography on silica gel (pentane/EtOAc, 6:1 → 4:1 → 2:1) 14.6 mg (60%) of **27a** were obtained as a colorless oil.

***R*$_f$**: 0.43 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$ = +35.0° (*c* 0.32, $CHCl_3$); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 0.86 (t, *J* = 7.2 Hz, 3 H, 18-H_3), 0.86 (t, *J* = 7.2 Hz, 3 H, 24′-H_3), 1.00 (s, 9 H, O(CO)C(CH_3)$_3$), 1.10-1.34 (m, 64 H, 7-H_2 — 17-H_2, 3′-H_2 — 23′-H_2), 1.59-1.67 (m, 2 H, 2′-H_2), 1.86-1.93 (m, 2 H, 6-H_2), 3.40 (dd, *J* = 9.8 Hz, 3.5 Hz, 1 H, 1-H_a), 3.57 (dd, *J* = 7.7 Hz, 6.0 Hz, 1 H, 5$_B$-H), 3.85 (ddd, *J* = 9.6 Hz, 5.0 Hz, 1.9 Hz, 1 H 5$_A$-H), 3.94 (dd, *J* = 11.1 Hz, 7.7 Hz, 1 H 6$_B$-H_a), 3.98 (dd, *J* = 9.8 Hz, 2.9 Hz, 1 H, 1-H_b), 4.06 (dd, *J* = 11.1 HZ, 6.0 Hz, 1 H, 6$_B$-H_b), 4.19 (dd, *J* = 9.6 Hz, 9.2 Hz, 1 H, 4$_A$-H), 4.19-4.23 (m, 1 H, 2-H), 4.21 (dd, *J* = 11.5 Hz, 8.2 Hz, 1 H, 6$_C$-H_a), 4.32 (d, *J* = 2.6 Hz, 1 H, 4$_B$-H), 4.41 (dd, *J* = 11.6 Hz, 5.0 Hz, 1 H, 6$_A$-H_a), 4.43 (dd, *J* = 11.5 Hz, 4.8 Hz, 1 H, 6$_C$-H_b), 4.57 (dd, *J* = 11.6 Hz, 1.9 Hz, 1 H, 6$_A$-H_b), 4.59 (d, *J* = 7.7 Hz, 1 H, 1$_A$-H), 4.89 (d, *J* = 8.0 Hz, 1 H, 1$_B$-H), 4.93 (ddd, *J* = 8.2 Hz, 4.8 Hz, 1.4 Hz, 1 H, 5$_C$-H), 5.11 (dd, *J* = 7.5 Hz, 7.4 Hz, 1 H, 3-H), 5.15 (dd, *J* = 10.8 Hz, 2.6 Hz, 1 H, 3$_B$-H); 5.25 (ddt, *J* = 15.4 Hz, 7.5 Hz, 1.5 Hz, 1 H, 4-H), 5.33 (dd, *J* = 9.4 Hz, 7.7 Hz, 1 H, 2$_A$-H), 5.45 (d, *J* = 4.5 Hz, 1 H, 1$_C$-H), 5.45 (d, *J* = 8.0 Hz, 1 H, NH), 5.65 (dt, *J* = 15.4 Hz, 6.8 Hz, 1 H, 5-H), 5.66 (dd, *J* = 10.9 Hz, 3.5 Hz, 1 H, 2$_C$-H), 5.78 (dd, *J* = 10.8 Hz, 8.0 Hz, 1 H, 2$_B$-H), 5.79 (dd, *J* = 9.4 Hz, 9.2 Hz, 1 H, 3$_A$-H), 5.99 (dd, *J* = 11.0 Hz, 3.3 Hz, 1 H, 3$_C$-H), 6.16 (dd, *J* = 3.3 Hz, 1.4 Hz, 1 H, 4$_C$-H), 7.11-7.57 (m, 31 H, Ph-H), 7.64-7.80 (m, 6 H, Ph-H), 7.82-8.00 (m, 11 H, Ph-H), 8.05-8.12 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.2 (C-18, C-24′), 22.7, 25.5, 29.0, 29.2, 29.4, 29.5, 29.6, 29.6, 29.7, 29.7, 29.8, 32.0, 32.3 (C-6 — C-17, C-2′ — C-23′), 27.0 (O(CO)C(*C*H_3)$_3$), 38.7 (O(CO)*C*(CH_3)$_3$), 50.2 (C-2), 60.5 (C-6$_B$), 61.6 (C-6$_C$), 62.4 (C-6$_A$), 67.7 (C-1), 67.8 (C-5$_C$), 68.0 (C-3$_C$), 69.1 (C-4$_C$), 69.7 (C-2$_B$), 70.0 (C-2$_C$), 72.5 (C-2$_A$), 72.6 (C-5$_B$), 72.9 (C-5$_A$), 73.0 (C-3), 73.1 (C-3$_A$), 73.6 (C-3$_B$), 75.9 (C-4$_B$), 77.0 (C-4$_A$), 98.8 (C-1$_C$), 100.9 (C-1$_A$), 101.3 (C-1$_B$), 125.0 (C-4), 128.1, 128.1, 128.2, 128.2, 128.3, 128.3, 128.4, 128.4, 128.5, 128.5, 129.0, 129.0, 129.2, 129.2, 129.4, 129.5, 129.5, 129.6, 129.6, 129.7, 129.7, 129.8, 129.8 (C_{tert}-Ph), 132.7, 132.9, 133.0, 133.0, 133.1, 133.1, 133.2, 133.3, 133.5 (C_{quart}-Ph), 136.6 (C-5), 164.7, 164.8, 164.8, 165.0, 165.1, 165.3, 165.5, 165.6, 166.0, 166.1, (O(*C*O)Ph), 172.2 (C-1′), 176.6 (O(*C*O)C(CH_3)$_3$); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2922, 2852, 2103, 1964, 1724, 1683, 1584, 1450, 1263, 1092; **MS** (ESI): *m*/*z* (%) = 1153.6 $[M+2Na]^{2+}$, 2260.0 $[M-H]^-$, 2284.2 $[M+Na]^+$; **HRMS (ESI)** for $C_{135}H_{161}NO_{29}$ (2261.71): calcd. 2283.1046 $[M+Na]^+$, found 2283.1044.

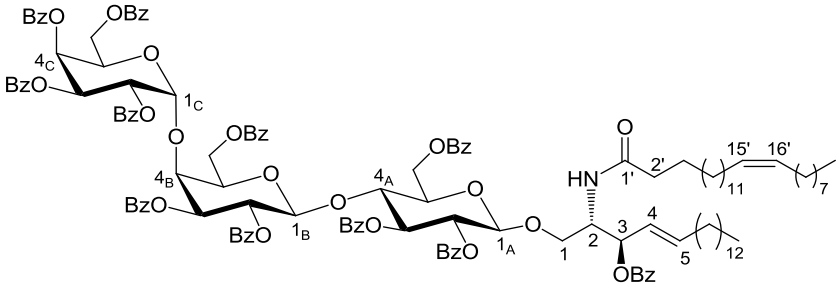
O-(α-D-Galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-(tetracosanamido)-4-octadecen-1,3-diol (**27**)



Global deprotection of **27a** (8.4 mg, 37 µmol, 1.0 eq) in CH_3OH/CH_2Cl_2 (4.0 mL) using NaOMe was performed according to **S4**. The reaction mixture was stirred at 50 °C for 16 h. After dialysis for 2 d and lyophilisation 3.5 mg (83%) of **27** were obtained as a white solid.

***R*$_f$**: 0.51 ($CHCl_3/CH_3OH/H_2O$, 2:1:0.15); $[\alpha]_D^{20}$ = +12.0° (*c* 0.1, $CHCl_3/CH_3OH/H_2O$, 1.6:1.0:0.2); **^{1}H-NMR** (600 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2): δ = 0.86 (t, *J* = 7.1 Hz, 3 H, 18-H_3), 0.87 (t, *J* = 7.1 Hz, 3 H, 24′-H_3), 1.21-1.37 (m, 62 H, 7-H_2 – 17-H_2, 4′-H_2 – 23′-H_2), 1.50-1.60 (m, 2 H, 3′-H_2), 1.96-2.02 (m, 2 H, 6-H_2), 2.14 (t, *J* = 7.1 Hz, 2 H, 2′-H_2), 3.27-3.31 (m, 1 H), 3.39-3.43 (m, 1 H), 3.48-3.61 (m, 5 H), 3.62-3.71 (m, 4 H), 3.74-3.89 (m, 5 H), 3.91-3.97 (m, 3 H), 4.03 (dd, *J* = 7.8 Hz, 7.7 Hz, 1 H), 4.16 (dd, *J* = 9.8 Hz, 4.6 Hz, 1 H), 4.22 (dd, *J* = 6.3 Hz, 5.9 Hz, 1 H), 4.31 (d, *J* = 7.9 Hz, 1 H), 4.40 (d, *J* = 7.9 Hz, 1 H), 4.89-4.91 (m, 1 H), 5.40 (dd, *J* = 15.3 Hz, 7.8 Hz, 1 H, 4-H), 5.67 (dt, *J* = 15.3 Hz, 6.7 Hz, 1 H, 5-H); **^{13}C-NMR** (126 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2): δ = 14.6, 14.6 (C-18, C-24′), 23.3, 26.7, 30.0, 30.0, 30.1, 30.2, 30.3, 30.4, 30.4, 30.5, 32.6, 32.6, 33.1, 37.1 (C-6 — C-17, C-2′ — C-23′), 53.8 (C-2), 60.9, 61.0, 61.8, 69.5, 69.5, 70.1, 70.2, 71.8, 72.0, 72.3, 73.5, 73.8, 73.8, 75.3, 75.6, 75.8, 77.3, 77.5, 79.0, 80.0 (C-1, C-3, C-2$_A$, C-3$_A$, C-4$_A$, C-5$_A$, C-6$_A$, C-2$_B$, C-3$_B$, C-4$_B$, C-5$_B$, C-6$_B$, C-2$_C$, C-3$_C$, C-4$_C$, C-5$_C$, C-6$_C$), 101.6, 103.4, 104.3 (C-1$_A$, C-1$_B$, C-1$_C$), 129.8 (C-4), 135.3 (C-5), 175.3 (C-1′); **IR** (ATR): $\tilde{\nu}$ (cm^{-1}) = 3308, 2917, 2849, 2361, 2341, 1736, 1646, 1541, 1466, 1375; **MS** (ESI): *m*/*z* (%) = 1134.8 $[M-H]^-$, 1158.8 $[M+Na]^+$; **HRMS (ESI)** für $C_{60}H_{113}NO_{18}$ (1136.54): ber. 1158.7850 $[M+Na]^+$, gef. 1158.7850.

O-(2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*,15′*Z*)-3-*O*-pivaloyl-2-(15′-tetracosenamido)-4-octadecen-1,3-diol (**28a**)

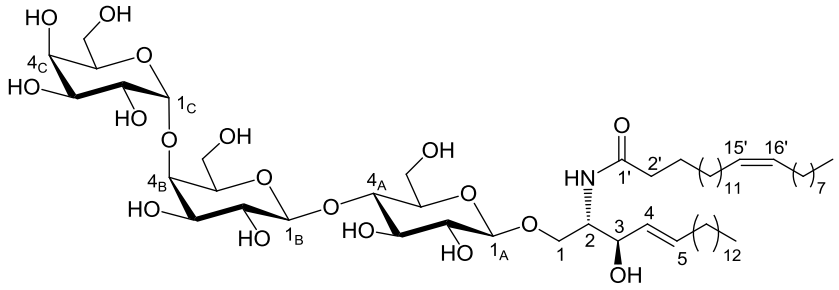


Reduction of azidosphingosine **26** (7.2 mg, 3.7 µL, 1.0 eq) in benzene (3.0 mL) and water (10 µL) using PPh_3 (2.6 mg, 9.9 µmol, 2.8 eq) was performed according to **S3**. The amine was dissolved in dry THF (2.0 mL) and HATU (2.0 mg, 5.3 µmol, 1.4 eq) dissolved in DMF (0.5 mL), DIPEA (1.0 µL, 5.9 µmol, 1.6 eq) and nervonic acid (2.0 mg, 5.5 µmol, 1.5 eq) were added. The reaction mixture was stirred at r.t. for 16 h. After column chromatography on silica gel (pentane/EtOAc, 5:1→3:1) and gel permeations HPLC 3.1 mg (31%) of **28a** were obtained as a colorless oil.

***R*$_f$**: 0.58 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$ = +39.7° (*c* 0.31, $CHCl_3$); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 0.84-0.88 (m, 6 H, 18-H_3, 24′-H_3), 1.02-1.40 (m, 26 H, 7-H_2 — 17-H_2, 2′-H_2 — 13′-H_2, 18′-H_2 — 23′-H_2), 1.72-1.79 (m, 2 H, 1′-H_2), 1.86-1.92 (m, 2 H, 6-H_2), 1.96-2.02 (m, 2 H, 14′-H_2, 17′-H_2)*, 3.51 (dd,

J = 9.8 Hz, 3.9 Hz, 1 H, 1-H_a), 3.55 (dd, *J* = 7.7 Hz, 6.3 Hz, 1 H, 5_B-H), 3.81 (m_c, 1 H, 5_A-H), 3.93, (dd, *J* = 10.9 Hz, 7.7 Hz, 1 H, 6_B-H_a), 4.01-4.07 (m, 2 H, 6_B-H_b, 1-H_b), 4.15, (dd, *J* = 9.4 Hz, 9.4 Hz, 1 H, 4_A-H), 4.20 (dd, *J* = 10.8 Hz, 8.0 Hz, 1 H, 6_C-H_a), 4.31 (d, *J* = 2.0 Hz, 1 H, 4_B-H), 4.32-4.42 (m, 4 H, 6_A-H_2, 6_C-H_b, 2-H), 4.60 (d, *J* = 7.6 Hz, 1 H, 1_A-H), 4.85 (d, *J* = 7.8 Hz, 1 H, 1_B-H), 4.92 (dd, *J* = 8.0 Hz, 5.6 Hz, 1 H, 5_C-H), 5.13 (dd, *J* = 10.8 Hz, 2.0 Hz, 1 H, 3_B-H), 5.31-5.39 (m, 4 H, 2_A-H, 4-H, 15′-H, 16′-H), 5.44 (d, *J* = 3.7 Hz, 1 H, 1_C-H), 5.44-5.46 (m, 1 H, 3-H), 5.62 (d, *J* = 9.4 Hz, 1 H, NH), 5.65 (dd, *J* = 11.2 Hz, 3.7 Hz, 1 H, 2_C-H), 5.74 (dt, *J* = 15.1 Hz, 7.3 Hz, 1 H, 5-H), 5.75 (dd, *J* = 10.8 Hz, 7.8 Hz, 1 H, 2_B-H), 5.76 (dd, *J* = 9.4 Hz, 9.2 Hz, 1 H, 3_A-H), 5.98 (dd, *J* = 11.2 Hz, 3.0 Hz, 1 H, 3_C-H), 6.15 (d, *J* = 3.0 Hz, 1 H, 4_C-H), 7.13-7.52 (m, 31 H, Ph-H), 7.66 (m, 2 H, Ph-H), 7.71 (m, 2 H, Ph-H), 7.76 (m, 2 H, Ph-H), 7.81-7.96 (m, 16 H, Ph-H), 8.08 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.2, 14.2 (C-18, C-24′), 22.8, 25.6, 27.3 27.3, 29.0, 29.3, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6,29.6, 29.7, 29.7, 29.8, 29.8, 29.9, 32.0, 32.0, 32.3, 36.5 (C-6 — C-17, C-2′ — C-14′, C-17′ — C-23′), 50.5 (C-2), 60.5 ($C\text{-}6_B$), 61.6 ($C\text{-}6_C$), 62.3 ($C\text{-}6_A$),67.5 (C-1), 67.7 ($C\text{-}5_C$), 68.0 ($C\text{-}3_C$), 69.1 ($C\text{-}4_C$), 69.6 ($C\text{-}2_B$), 70.0 ($C\text{-}2_C$), 72.5 ($C\text{-}2_A$), 72.6 ($C\text{-}5_B$), 73.0 ($C\text{-}3_A$), 73.1 ($C\text{-}5_A$), 73.6 ($C\text{-}3_B$), 74.2 (C-3), 75.9 ($C\text{-}4_B$), 77.2 ($C\text{-}4_A$), 98.8 ($C\text{-}1_C$), 100.7 ($C\text{-}1_A$), 101.2 ($C\text{-}1_B$), 124.7 (C-4), 128.1, 128.1, 128.2, 128.2, 128.3, 128.4, 128.4, 128.5, 128.6, 129.0, 129.2, 129.3, 129.4, 129.5, 129.5, 129.5, 129.6, 129.6, 129.6, 129.7 (C_{tert}-Ph), 129.8, 129.8 (C-15′, C-16′), 130.1, 132.7, 132.9, 133.0, 133.1, 133.2, 133.3, 133.3, 133.5 (C_{quart}-Ph), 137.1 (C-5), 164.7, 164.8, 165.0, 165.2, 165.3, 165.5, 165.6, 166.0, 166.1 (O(*C*O)Ph), 172.4 (C-1′); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2922, 2852, 1725, 1672, 1602, 1584, 1492, 1451, 1315, 1176; **MS** (ESI): *m*/*z* (%) = 1162.8 $[M+2Na]^{2+}$, 2302.5 $[M+Na]^+$; **HRMS (ESI)** for $C_{137}H_{155}NO_{29}$ (2279.69): calcd. 2301.0577 $[M+Na]^+$, found 2301.0624.

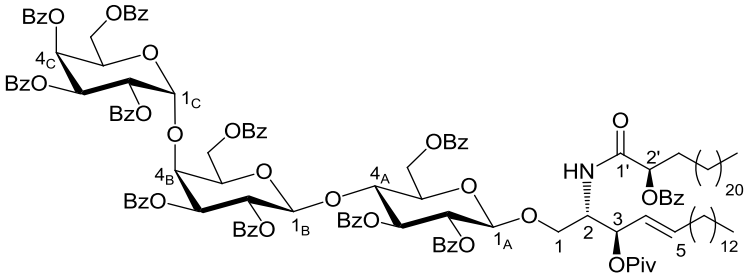
O-(α-D-Galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*,15′*Z*)-2-(15′-tetracosenamido)-4-octadecen-1,3-diol (**28**)



Global deprotection of **28a** (16.0 mg, 7.08 µmol, 1.0 eq) in CH_3OH/CH_2Cl_2 (2.0 mL) using NaOMe was performed according to **S4**. The reaction mixture was stirred at r.t. for 16 h. After dialysis for 2 d and lyophilisation 8.0 mg (99%) of **28** were obtained as a white solid.

***R*$_f$**: 0.79 ($CHCl_3/CH_3OH/H_2O$, 1.6:1:0.2); $[\alpha]_D^{20}$ = +19.0° (*c* 0.10, $CHCl_3/CH_3OH/H_2O$, 1.6:1.0:0.2); **^{1}H-NMR** (600 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2): δ = 0.81-0.92 (m, 6 H, 18-H_3, 24′-H_3), 1.16-1.38 (m, 58 H, 7-H_2 — 17-H_2, 3′-H_2 — 14′-H_2, 18′-H_2 — 23′-H_2)*, 1.92-2.06 (m, 6 H, 6-H_2, 1′-H_2, 17′-H_2)*, 2.09-2.19 (m, 2 H, 14′-H_2 od. 17′-H_2)*, 3.36-3.47 (m, 1 H), 3.49-4.09 (m, 12 H), 4.17 (dd, *J* = 10.1 Hz, 4.1 Hz, 1 H), 4.24 (dd, *J* = 6.4 Hz, 5.6 Hz, 1 H), 4.32 (d, *J* = 7.7 Hz, 1 H), 4.34-4.84 (m, 9 H), 4.89-4.92 (m, 1 H), 5.30-5.36 (m, 2 H, 15′-H, 16′-H), 5.40 (dd, *J* = 15.2 Hz, 7.8 Hz, 1 H, 4-H), 5.68 (dt, *J* = 15.2 Hz, 6.4 Hz, 1 H, 5-H); **^{13}C-NMR** (126 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2): δ = 13.4, 13.4 (C-18, C-24′), 22.1, 22.1, 25.5, 26.6, 26.6, 28.7, 28.8, 28.8, 28.8, 28.9, 29.0, 29.0, 29.1, 29.1, 29.2, 29.2, 29.3, 31.4, 31.4, 31.9, 35.9 (C-6 — C-17, C-2′ — C-14′, C-17′ - C-23′), 52.6 (C-2), 59.7, 59.9, 60.6, 68.3, 68.4, 68.9, 69.1, 70.7, 70.8, 71.2, 72.4, 72.7, 74.1, 74.4, 74.7, 77.9, 78.9 (C-1, C-3, $C\text{-}2_A$, $C\text{-}3_A$, $C\text{-}4_A$, $C\text{-}5_A$, $C\text{-}6_A$, $C\text{-}2_B$, $C\text{-}3_B$, $C\text{-}4_B$, $C\text{-}5_B$, $C\text{-}6_B$, $C\text{-}2_C$, $C\text{-}3_C$, $C\text{-}4_C$, $C\text{-}5_C$, $C\text{-}6_C$), 100.5, 102.3, 103.2 ($C\text{-}1_A$, $C\text{-}1_B$, $C\text{-}1_C$), 128.8 (C-4), 129.4, 129.4 (C-15′, C-16′), 134.3 (C-5), 174.3 (C-1); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3310, 2918, 2849, 2361, 1737, 1647, 1558, 1466, 1364, 1217; **MS** (ESI): *m*/*z* (%) = 1132.7 $[M-H]^-$, 1156.8 $[M+Na]^+$; **HRMS (ESI)** for $C_{60}H_{111}NO_{18}$ (1134.52): calcd. 1156.7693 $[M+Na]^+$, found 1156.7696.

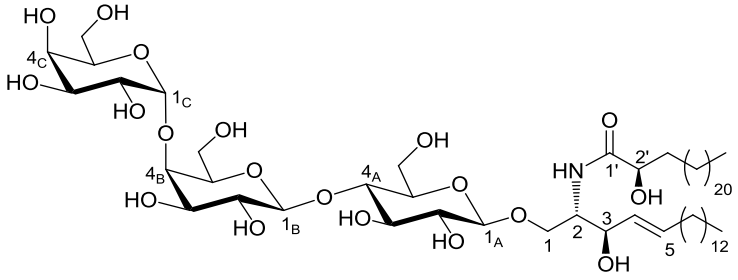
O-(2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*,2′*R*)-2-(2′-benzoyloxy-tetracosanamido)-3-*O*-pivaloyl-4-octadecen-1,3-diol (**29a**)



Reduction of azidosphingosine **25** (16.5 mg, 8.52 µmol, 1.0 eq) in benzene (1.0 mL) and water (3 µL) using PPh_3 (5.0 mg, 19 µmol, 2.2 eq) was performed according to **S2**. The amine was dissolved in dry THF (0.5 mL) and HOBt (1.8 mg, 14 µmol, 1.6 eq), EDCI (2.6 mg, 14 µmol, 1.6 eq), DIPEA (2.3 µL, 14 µmol, 1.6 eq) and fatty acid **22** (6.7 mg, 14 µmol, 1.6 eq) dissolved in dry THF (1.0 mL) were added. After column chromatography on silica gel (pentane/EtOAc, 3:1 → 2:1) 12.2 mg (60%) of **29a** were obtained as a colorless oil.

***R*f**: 0.47 (hexane/EtOAc, 2:1); $[\alpha]_D^{20}$ = +28.0° (*c* 0.61, $CHCl_3$); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 0.86 (t, *J* = 7.0 Hz, 3 H, 18-H_3), 0.86 (t, *J* = 6.9 Hz, 3 H, 24′-H_3), 1.00 (s, 9 H, O(CO)C(CH_3)$_3$), 1.08-1.30 (m, 64 H, 7-H_2 — 17-H_2, 3′-H_2 — 23′-H_2), 1.72-1.80 (m, 2 H, 6-H_2), 3.45 (dd, *J* = 10.3 Hz, 5.3 Hz, 1 H, 1-H_a), 3.55 (dd, *J* = 7.8 Hz, 6.0 Hz, 1 H, 5_B-H), 3.69 (ddd, *J* = 9.4 Hz, 4.2 Hz, 2.1 Hz, 1 H 5_A-H), 3.87 (dd, *J* = 10.3 Hz, 4.3 Hz, 1 H, 1-H_b), 3.93, (dd, *J* = 11.2 Hz, 7.8 Hz, 1 H, 6_B-H_a), 4.02 (dd, *J* = 11.2 HZ, 6.0 Hz, 1 H, 6_B-H_b), 4.11 (dd, *J* = 9.4 Hz, 9.1 Hz, 1 H, 4_A-H), 4.21 (dd, *J* = 11.1 Hz, 8.5 Hz, 1 H, 6_C-H_a), 4.24 (m_c, 1 H, 2-H), 4.32, (d, *J* = 2.4 Hz, 1 H, 4_B-H), 4.38 (dd, *J* = 12.2 Hz, 4.2 Hz, 1 H, 6_A-H_a), 4.40 (dd, *J* = 11.1 Hz, 5.0 Hz, 1 H, 6_C-H_b), 4.48 (dd, *J* = 12.2 Hz, 2.1 Hz, 1 H, 6_A-H_b), 4.57 (d, *J* = 7.7 Hz, 1 H, 1_A-H), 4.84 (d, *J* = 7.8 Hz, 1 H, 1_B-H), 4.92 (m_c, 1 H, 5_C-H), 5.11 (t, *J* = 6.1 Hz, 1 H, 2′-H), 5. 14 (dd, *J* = 10.8 Hz, 2.4 Hz, 1 H, 3_B-H); 5.21 (dd, *J* = 7.2 Hz, 6.5 Hz, 1 H, 3-H), 5.24 (m_c, 1 H, 4-H), 5.27 (dd, *J* = 9.4 Hz, 7.7 Hz, 1 H, 2_A-H), 5.44 (d, *J* = 3.6 Hz, 1 H, 1_C-H), 5.53 (dt, *J* = 14.0 Hz, 6.6 Hz, 1 H, 5-H), 5.66 (dd, *J* = 9.4 Hz, 9.1 Hz, 1 H, 3_A-H), 5.66 (dd, *J* = 10.0 Hz, 3.6 Hz, 1 H, 2_C-H), 5.77 (dd, *J* = 10.8 Hz, 7.8 Hz, 1 H, 2_B-H), 5.99 (dd, *J* = 10.0 Hz, 3.3 Hz, 1 H, 3_C-H), 6.15 (dd, *J* = 3.3 Hz, 1.2 Hz, 1 H, 4_C-H), 6.31 (d, *J* = 9.0 Hz, 1 H, NH), 7.14-7.25 (m, 9 H, Ph-H), 7.28-7.43 (m, 18 H, Ph-H), 7.44-7.52 (m, 5 H, Ph-H), 7.54-7.61 (m, 2 H, Ph-H), 7.65-7.78 (m, 6 H, Ph-H), 7.84-7.90 (m, 7 H, Ph-H), 7.91-7.98 (m, 6 H, Ph-H), 8.04-8.08 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.1, 14.1 (C-18, C-24′), 22.7, 24.8, 28.8, 29.2, 29.4, 29.4, 29.5, 29.6, 29.7, 29.7, 31.6, 31.9, 32.1 (C-6 — C-17, C-3′ — C-23′), 26.9 (O(CO)C(*C*H_3)$_3$), 38.6 (O(CO)*C*(CH_3)$_3$), 50.7 (C-2), 60.5 (C-6_B), 61.5 (C-6_C), 62.3 (C-6_A), 66.9 (C-1), 67.7 (C-5_C), 68.0 (C-3_C), 69.1 (C-4_C), 69.6 (C-2_B), 70.0 (C-2_C), 72.0 (C-2_A), 72.6 (C-5_B), 73.0 (C-5_A), 73.0 (C-3), 73.2 (C-3_A), 73.6 (C-3_B), 74.6 (C-2′), 75.9 (C-4_B), 76.8 (C-4_A), 98.8 (C-1_C), 100.4 (C-1_A), 101.4 (C-1_B), 124.3 (C-4), 128.2, 128.2, 128.3, 128.3, 128.4, 128.4, 128.5, 128.5, 128.5, 128.6, 128.7, 129.1, 129.2, 129.3, 129.3, 129.5, 129.6, 129.6, 129.7, 129.8, 129.9, 129.9 (C_{tert}-Ph), 132.8, 133.0, 133.0, 133.1, 133.2, 133.2, 133.3, 133.6, 133.6 (C_{quart}-Ph), 136.7 (C-5), 164.9, 165.0, 165.0, 165.1, 165.2, 165.4, 165.7, 165.8, 166.2, 166.3, 166.3 (O(*C*O)Ph), 169.3 (C-1′), 176.8 (O(*C*O)C(CH_3)$_3$); **IR** (ATR): $\tilde{\nu}$ (cm^{-1}) = 2958, 2852, 2361, 2341, 1724, 1450, 1260, 1090; **MS** (ESI): *m*/*z* (%) = 1213.7 $[M+2Na]^{2+}$, 2404.3 $[M+Na]^+$; **HRMS (ESI)** for $C_{142}H_{165}NO_{31}$ (2381.82): calcd. 2403.1258 $[M+Na]^+$, found 2403.1266.

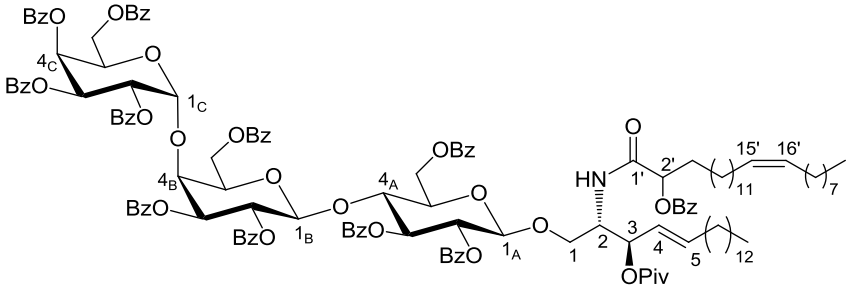
O-(α-D-Galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*,2′*R*)-2-(2′-hydroxyltetracosanamido)-4-octadecen-1,3-diol (**29**)



Global deprotection of **29a** (12.0 mg, 5.04 µmol, 1.0 eq) in CH_3OH/CH_2Cl_2 (2.0 mL) using NaOMe was performed according to **S4**. The reaction mixture was stirred at r.t. for 16 h. After dialysis for 2 d and lyophilisation 5.0 mg (86%) of **29** were obtained as a white solid.

***R*f**: 0.50 ($CHCl_3/CH_3OH/H_2O$, 1.6:1:0.2); $[\alpha]_D^{20}$ = +11.6° (*c* 0.19, $CHCl_3/CH_3OH$, 1.6:1.0); **^{1}H-NMR** (600 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2): δ = 0.87 (t, *J* = 6.8 Hz, 3 H, 18-H_3), 0.87 (t, *J* = 6.8 Hz, 3 H, 24-H_3), 1.19-1.80 (m, 64 H, 7-H_2 – 17-H_2, 3-H_2 – 23-H_2), 1.93-2.06 (m, 2 H, 6-H_2), 3.24-3.31 (m, 1 H), 3.39-4.15 (m, 22 H), 4.23 (dd, *J* = 6.0 Hz, 6.0 Hz, 1 H), 4.34 (m, *J* = 7.3 Hz, 1 H), 4.89-4.94 (m, 1 H), 5.40 (ddt, *J* = 15.3 Hz, 7.4 Hz, 1.3 Hz, 1 H, 4-H), 5.71 (dt, *J* = 15.3 Hz, 6.5 Hz, 1 H, 5-H); **^{13}C-NMR** (126 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2): δ = 14.6, 14.6 (C-18, C-24′), 23.3, 25.8, 29.8, 29.9, 30.0, 30.0, 30.1, 30.3, 30.3, 30.4, 30.4, 30.5, 32.5, 32.6, 33.1, 35.3 (C-6 — C-17, C-3′ — C-23′), 53.6 (C-2), 60.9, 61.0, 61.8, 69.3, 69.5, 70.0, 70.2, 71.8, 72.0, 72.2, 72.3, 72.7, 73.5, 73.7, 75.2, 75.6, 75.8, 77.9, 78.6, 79.0, 79.8 (C-1, C-3, C-2′, C-2_A, C-3_A, C-4_A, C-5_A, C-6_A, C-2_B, C-3_B, C-4_B, C-5_B, C-6_B, C-2_C, C-3_C, C-4_C, C-5_C, C-6_C), 101.6, 103.3, 104.2 (C-1_A, C-1_B, C-1_C), 129.4 (C-4), 135.4 (C-5), 176.4 (C-1′); **IR** (ATR): $\tilde{\nu}$ (cm^{-1}) = 2920, 2849, 2364, 2337, 2150, 2013, 1455; **MS** (ESI): *m*/*z* (%) = 1150.7 $[M-H]^-$; **HRMS (ESI)** for $C_{60}H_{113}NO_{19}$ (1152.53): calcd. 1150.7834 $[M-H]^-$, found 1150.7830.

O-(2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*,15′*E*)-2-(2′-benzoyloxy-15′-tetracosenamido)-3-*O*-pivaloyl-4-octadecen-1,3-diol (**30a**)

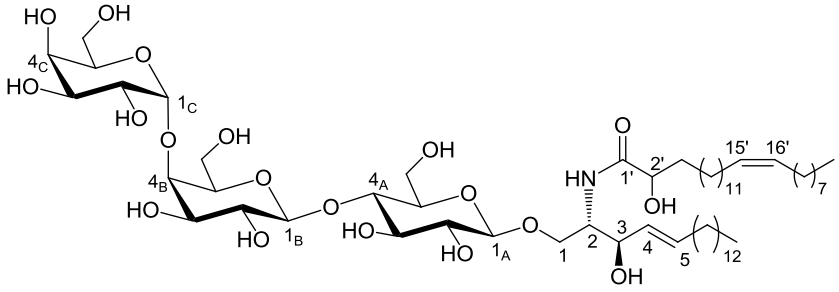


Reduction of azidosphingosine **25** (40 mg, 20.7 µmol, 1.0 eq) in benzene (4.0 mL) and water (52 µL) using PPh_3 (12.6 mg, 47.4 µmol, 2.3 eq) was performed according to **S2**. The amine was dissolved in dry THF (4.0 mL) and HATU (10 mg, 27 µmol, 1.3 eq) dissolved in DMF (1.0 mL), DIPEA (4.6 µL, 27 µmol, 1.3 eq) and α-hydroxylated nervonic acid (**24**) (13 mg, 27 µmol, 1.3 eq) were added. The reaction mixture was stirred at r.t. for 16 h. After column chromatography on silica gel (pentane/EtOAc, 5:1→3:1) and gel permeations HPLC 32 mg (65%) of **30a** were obtained as a colorless oil.

***R*f**: 0.42 (hexane/EtOAc, 2:1); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ = 0.85 (t, *J* = 7.1 Hz, 3 H, 18-H_3), 0.85 (t, *J* = 7.2 Hz, 3 H, 24′-H_3), 0.96 (s, 9 H, O(CO)C(CH_3)$_3$), 1.09-1.33 (m, 60 H, 7-H_2 — 17-H_2, 3′-H_2 — 14′-H_2, 17′-H_2 — 23′-H_2), 1.96-2.02 (m, 2 H, 6-H_2), 3.46 (dd, *J* = 9.9 Hz, 4.2 Hz, 1 H, 1-H_a), 3.54-3.60 (m, 1 H, 5_B-H), 3.86 (ddd, *J* = 9.8 Hz, 4.7 Hz, 1.9 Hz, 1 H, 5_A-H), 3.91 (dd, *J* = 11.2 Hz, 7.9 Hz, 1 H, 6_B-H_a), 3.96 (dd, *J* = 9.9 Hz, 3.1 Hz, 1 H, 1-H_b), 4.01-4.05 (m, 1 H, 6_B-H_b), 4.18 (dd, *J* = 9.8 Hz, 9.2 Hz, 1 H, 4_A-H), 4.20 (dd, *J* = 10.5 Hz, 8.2 Hz, 1 H, 6_C-H_a), 4.22-4.25 (m_c, 1 H, 2-H),

4.31 (d, J = 2.2 Hz, 1 H, 4_B-H), 4.40 (dd, J = 10.5 Hz, 5.0 Hz, 1 H, 6_C-H_b), 4.44 (dd, J = 12.2 Hz, 4.7 Hz, 1 H, 6_A-H_a), 4.53 (dd, J = 12.2 Hz, 1.9 Hz, 1 H, 6_A-H_b), 4.70 (d, J = 7.8 Hz, 1 H, 1_A-H), 4.87 (d, J = 7.9 Hz, 1 H, 1_B-H), 4.93 (ddd, J = 8.2 Hz, 5.0 Hz, 1.1 Hz, 1 H, 5_C-H), 4.99 (dd, J = 8.6 Hz, 4.6 Hz, 1 H, 2′-H), 5. 10-5.17 (m, 3 H, 3_B-H, 3-H, 4-H), 5.31-5.34 (m, 2 H, 15′-H, 16′-H), 5.37 (dd, J = 9.3 Hz, 7.8 Hz, 1 H, 2_A-H), 5.38 (dt, J = 15.3 Hz, 7.8 Hz, 1 H, 5-H), 5.44 (d, J = 3.6 Hz, 1 H, 1_C-H), 5.66 (dd, J = 11.0 Hz, 3.6 Hz, 1 H, 2_C-H), 5.77 (dd, J = 10.9 Hz, 7.9 Hz, 1 H, 2_B-H), 5.80 (dd, J = 9.3 Hz, 9.2 Hz, 1 H, 3_A-H), 5.98 (dd, J = 11.0 Hz, 3.3 Hz, 1 H, 3_C-H), 6.09 (d, J = 9.5 Hz, 1 H, NH), 6.15 (dd, J = 3.3 Hz, 1.1 Hz, 1 H, 4_C-H), 7.13-7.57 (m, 35 H, Ph-H), 7.64-7.78 (m, 6 H, Ph-H), 7.83-7.99 (m, 12 H, Ph-H), 8.06-8.09 (m, 2 H, Ph-H); **^{13}C-NMR** (126 MHz, $CDCl_3$): δ = 14.2, 14.2 (C-18, C-24′), 22.7, 22.8, 27.3, 28.8, 29.2, 29.4, 29.4, 29.4, 29.5, 29.5, 29.6, 29.7, 29.7, 29.7, 29.7, 29.7, 29.8, 29.8, 29.9, 32.0, 32.0, 32.0, 32.1 (C-7 – C-17, C-3′ – C-14′, C-17′ – C-23′), 26.9 (O(CO)C(CH_3)$_3$), 27.3 (C-6), 38.6 (O(CO)C(CH_3)$_3$), 50.4 (C-2), 60.5 (C-6_B), 61.6 (C-6_C), 62.5 (C-6_A), 67.4 (C-1), 67.4 (C-5_C), 68.0 (C-3_C), 69.1 (C-4_C), 69.6 (C-2_B), 70.0 (C-2_C), 72.3 (C-2_A), 72.6 (C-5_B), 72.6 (C-3), 73.1 (C-5_A), 73.2 (C-3_A), 73.6 (C-3_B), 74.5 (C-2′), 75.9 (C-4_B), 76.9 (C-4_A), 98.8 (C-1_C), 100.7 (C-1_A), 101.3 (C-1_B), 124.2 (C-4), 128.1, 128.1, 128.2, 128.2, 128.3, 128.4, 128.4, 128.4, 128.5, 128.5, 129.0, 129.1, 129.2, 129.2, 129.4, 129.5, 129.6, 129.6, 129.6, 129.7, 129.7, 129.8 (C_{tert}-Ph), 129.8, 129.8 (C-15′, C-16′), 132.7, 132.9, 133.0, 133.0, 133.1, 133.2, 133.3, 133.3, 133.5 (C_{quart}-Ph), 136.6 (C-5), 164.7, 164.8, 164.8, 165.0, 165.0, 165.0, 165.3, 165.6, 165.6, 166.0, 166.1 (O(CO)Ph), 169.7 (C-1′), 176.6 (O(CO)C(CH_3)$_3$); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 2958, 2852, 2348, 1726, 1601, 1548, 1451, 1315, 1260; **MS** (ESI): m/z (%) = 1212.6 $[M+2Na]^{2+}$, 2402.2 $[M+Na]^+$; **HRMS (ESI)** for $C_{142}H_{163}NO_{31}$ (2379.8): calcd. 1212.0497 $[M+2Na]^{2+}$, found 1212.0500.

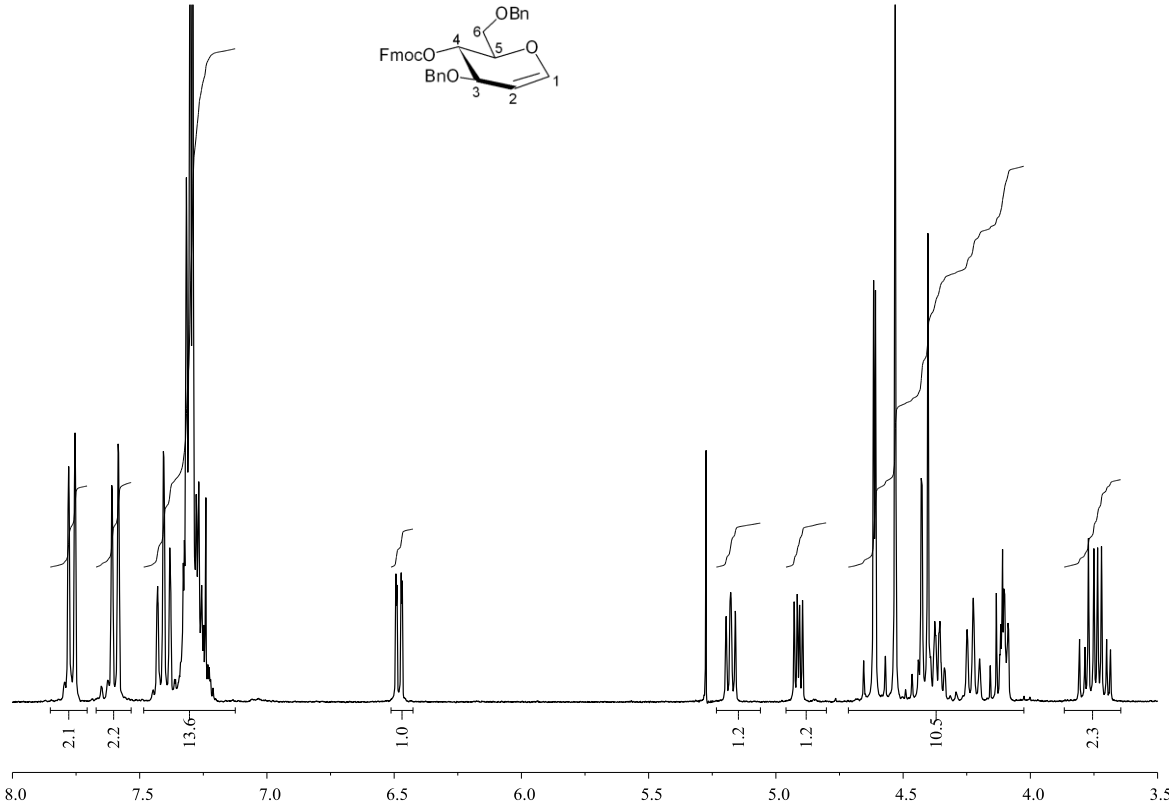
O-(α-D-Galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*,15′*E*)-2-(2′-hydroxyl-15′-tetracosenamido)-4-octadecen-1,3-diol (**30**)



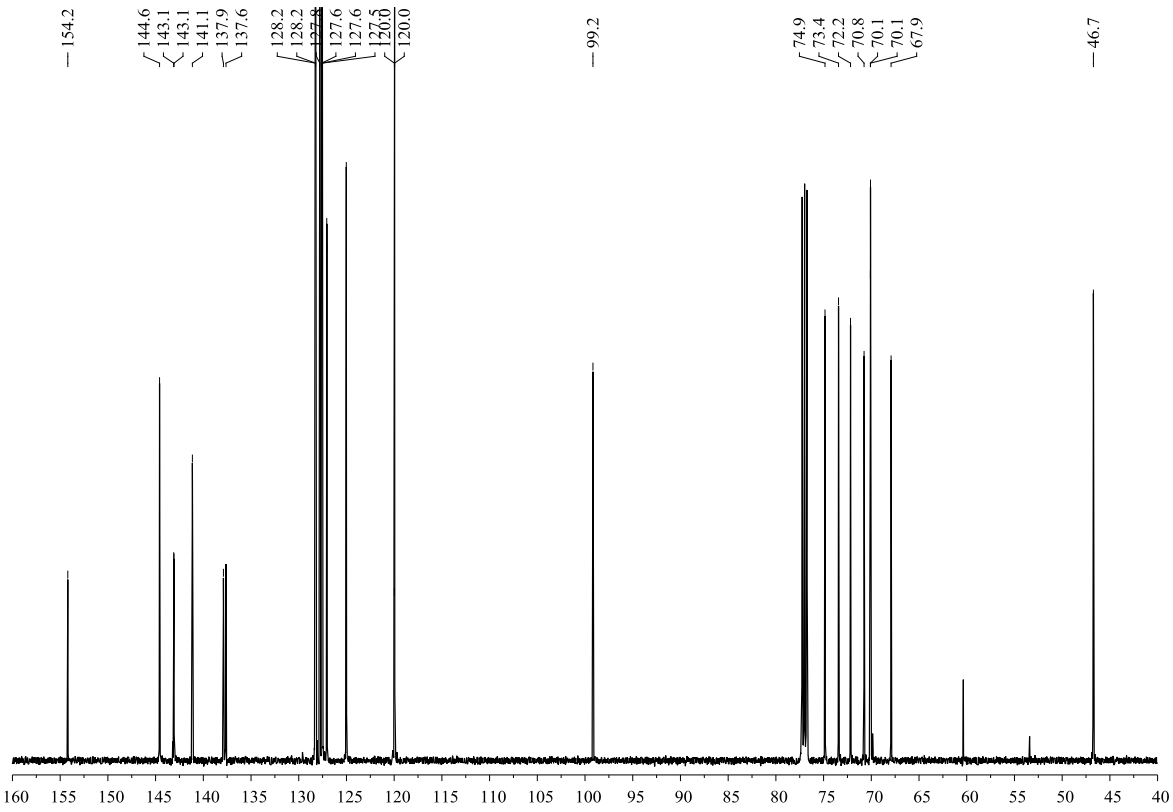
Global deprotection of **30a** (30 mg, 12.6 μmol, 1.0 eq) in CH_3OH/CH_2Cl_2 (4.0 mL) using NaOMe was performed according to **S4**. The reaction mixture was stirred at 50 °C for 16 h. After dialysis for 4 d and lyophilisation 11 mg (62%) of **30** were obtained as a white solid.

***R*$_f$**: 0.51 ($CHCl_3/CH_3OH/H_2O$, 1.6:1.0:0.2); **^{1}H-NMR** (600 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2): δ = 0.78-0.90 (m, 6 H, 18-H_3, 24′-H_3), 1.17-1.79 (m, 58 H, 6-H_2 – 17-H_2, 3′-H_2 – 13′-H_2, 18′-H_2 – 23′-H_2), 1.96-2.02 (m, 2 H, 17′-H_2)*, 3.25-3.33 (m, 1 H), 3.39-3.43 (m, 1 H), 3.47-3.60 (m, 3 H), 3.62-3.72 (m, 2 H), 3.74-3.89 (m, 3 H), 3.90-3.99 (m, 4 H), 4.02-4.19 (m, 3 H), 4.19-4.24 (m, 1 H), 4.28-4.34 (m, 1 H), 4.37-4.67 (m, 8 H), 4.88-4.91 (m, 1 H), 5.30-5.35 (m, 2 H, 15′-H, 16′-H), 5.41 (dd, J = 15.3 Hz, 7.7 Hz, 1 H, 4-H), 5.70 (dt, J = 15.3 Hz, 6.5 Hz, 1 H, 5-H); **^{13}C-NMR** (126 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2): δ = 14.6, 14.6 (C-18, C-24′), 23.3, 29.8, 29.9, 29.9, 30.0, 30.1, 30.2, 30.3, 30.6, 32.6, 33.1 (C-6 – C-17, C-2′ – C-14′, C-17′ – C-23′), 54.1 (C-2), 61.0, 61.8, 69.5, 70.0, 70.2, 71.8, 72.0, 73.7, 75.2, 75.6, 75.8, 79.1, 79.9 (C-1, C-3, C-2_A, C-3_A, C-4_A, C-5_A, C-6_A, C-2_B, C-3_B, C-4_B, C-5_B, C-6_B, C-2_C, C-3_C, C-4_C, C-5_C, C-6_C), 101.6, 103.4, 104.3 (C-1_A, C-1_B, C-1_C), 129.2 (C-4), 130.4, 130.4 (C-15′, C-16′), 135.2 (C-5); **IR** (ATR): $\tilde{\upsilon}$ (cm^{-1}) = 3374, 2953, 2919, 2850, 2360, 1632, 1465, 1260, 1083; **MS** (ESI): m/z (%) = 1172.8 $[M+Na]^+$; **HRMS (ESI)** for $C_{60}H_{111}NO_{19}$ (1150.52): calcd. 1172.7643 $[M+Na]^+$, found 1172.7623.

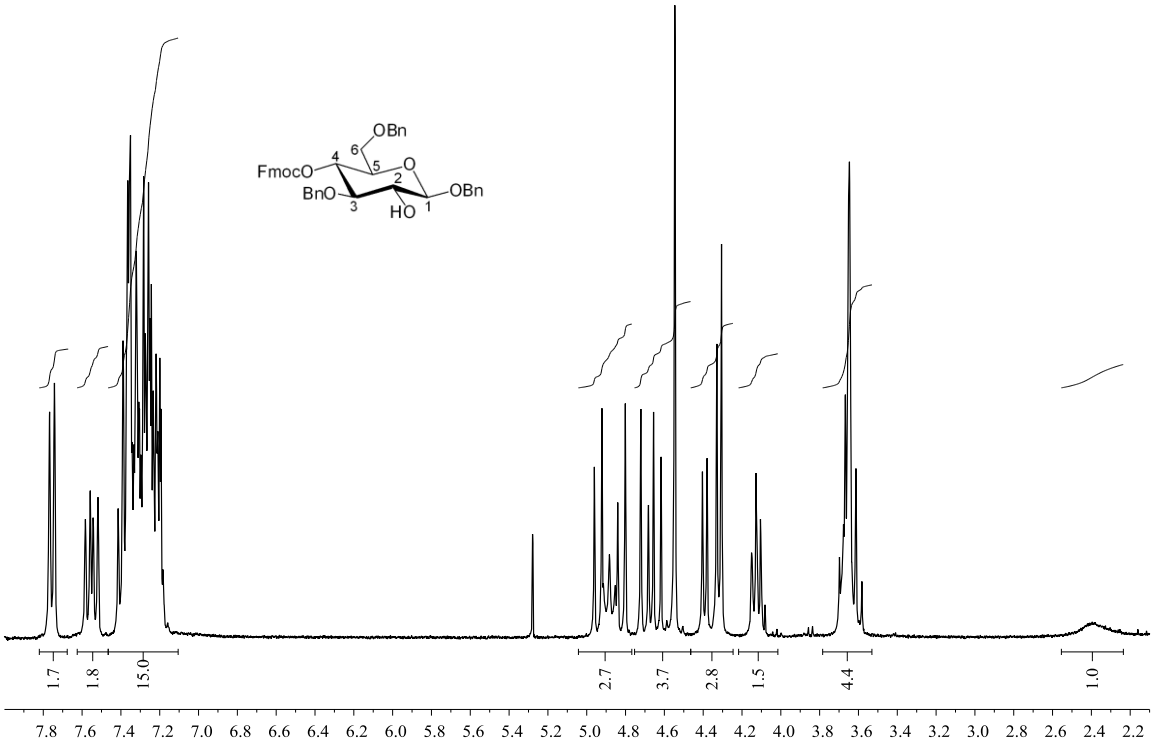
^{1}H-NMR of **1a** (300 MHz, $CDCl_3$)



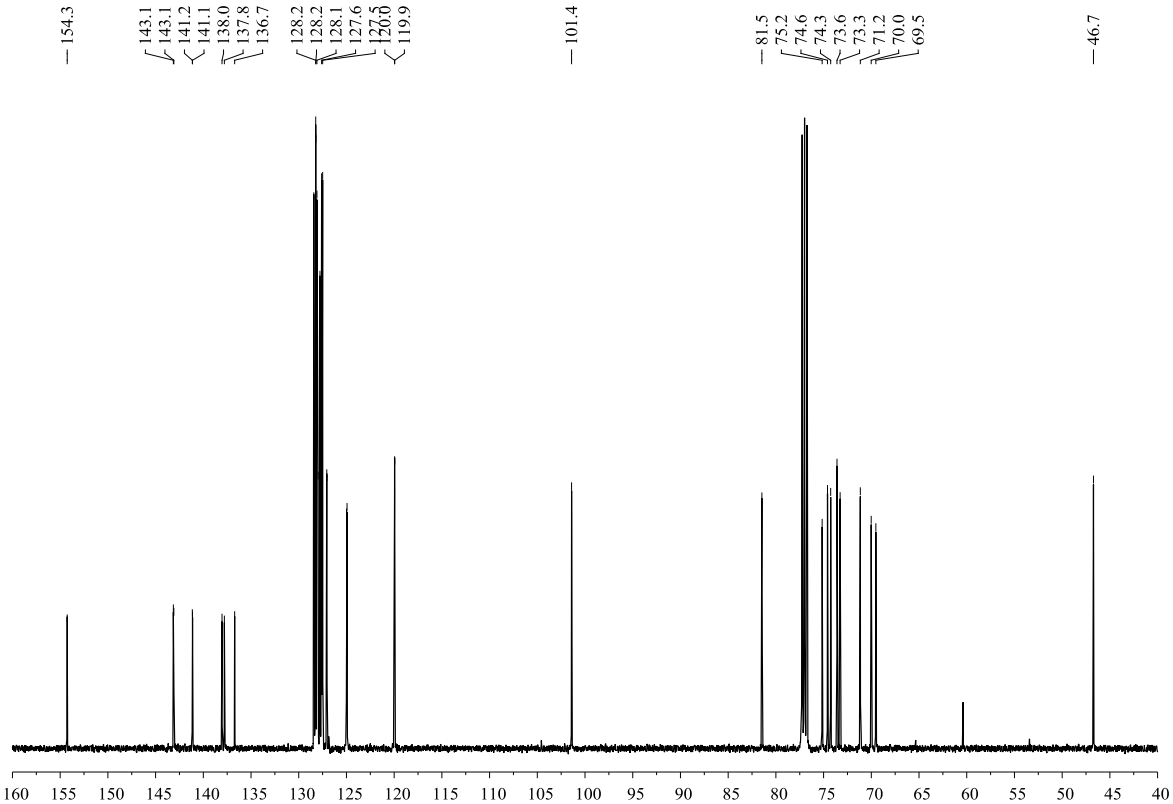
^{13}C-NMR of **1a** (126 MHz, $CDCl_3$)



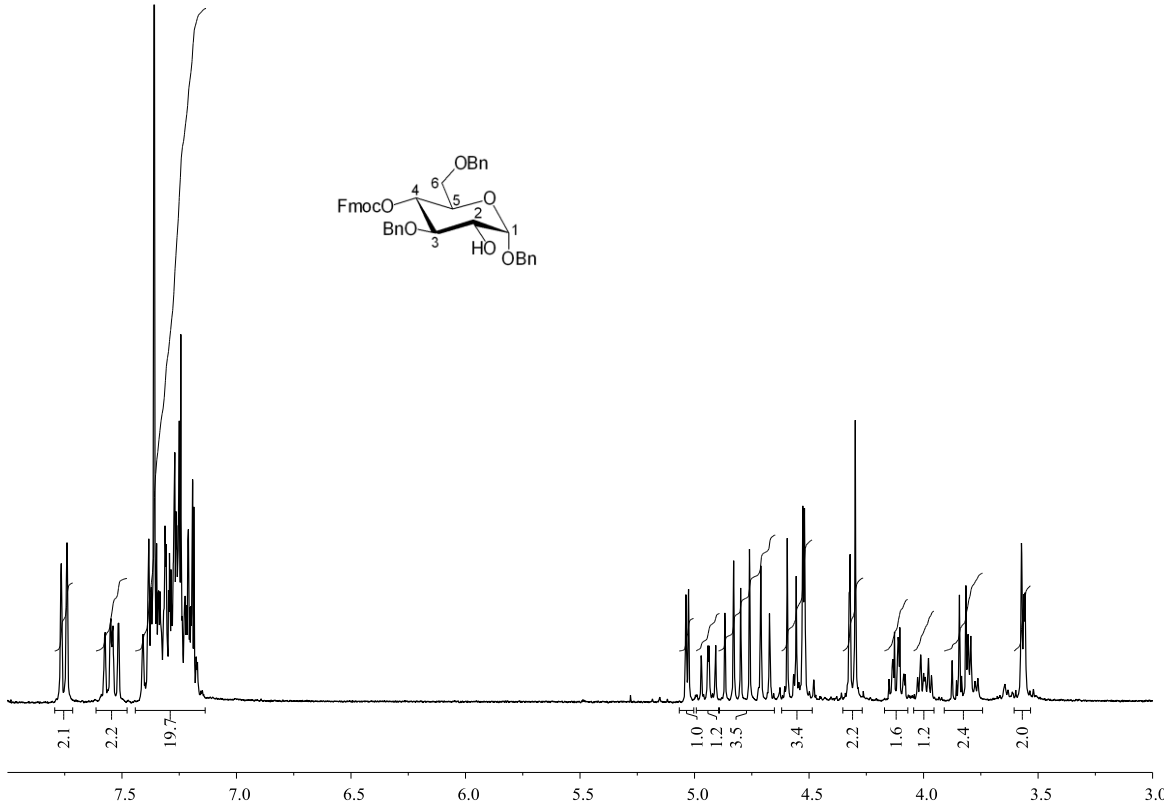
^{1}H-NMR of **1b-a** (300 MHz, $CDCl_3$)



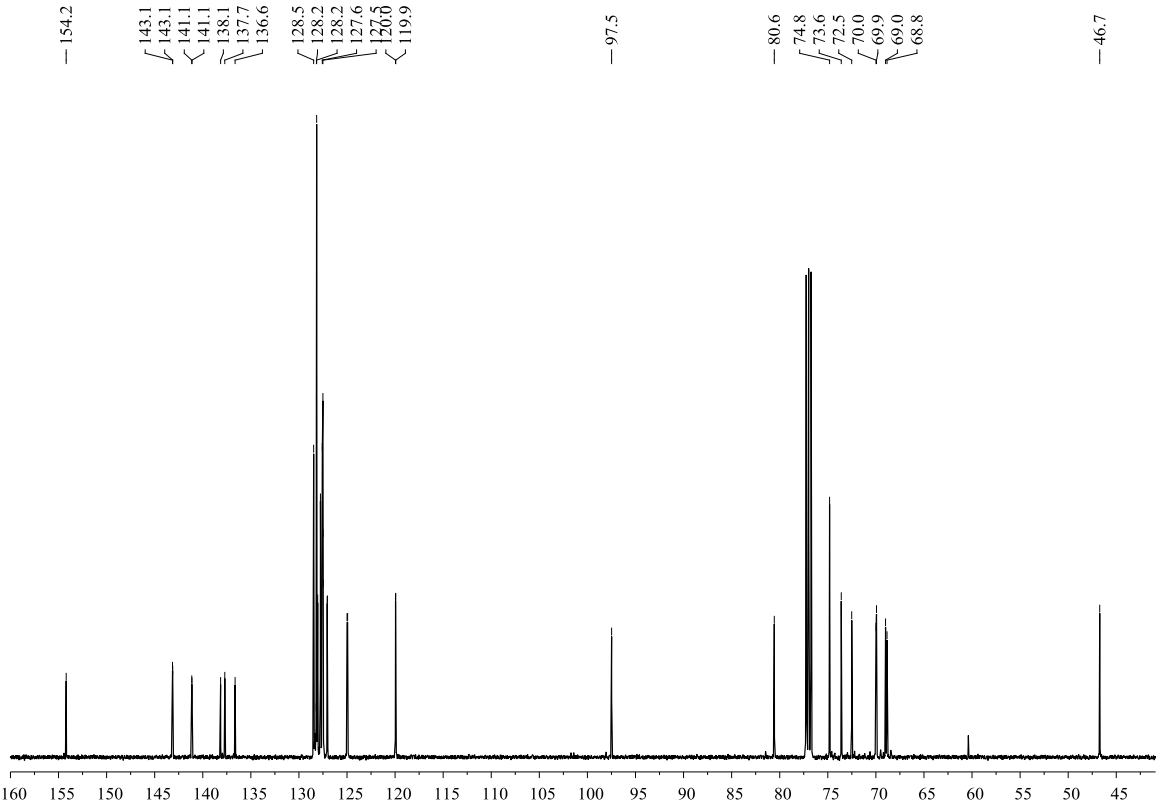
^{13}C-NMR of **1b-a** (126 MHz, $CDCl_3$)



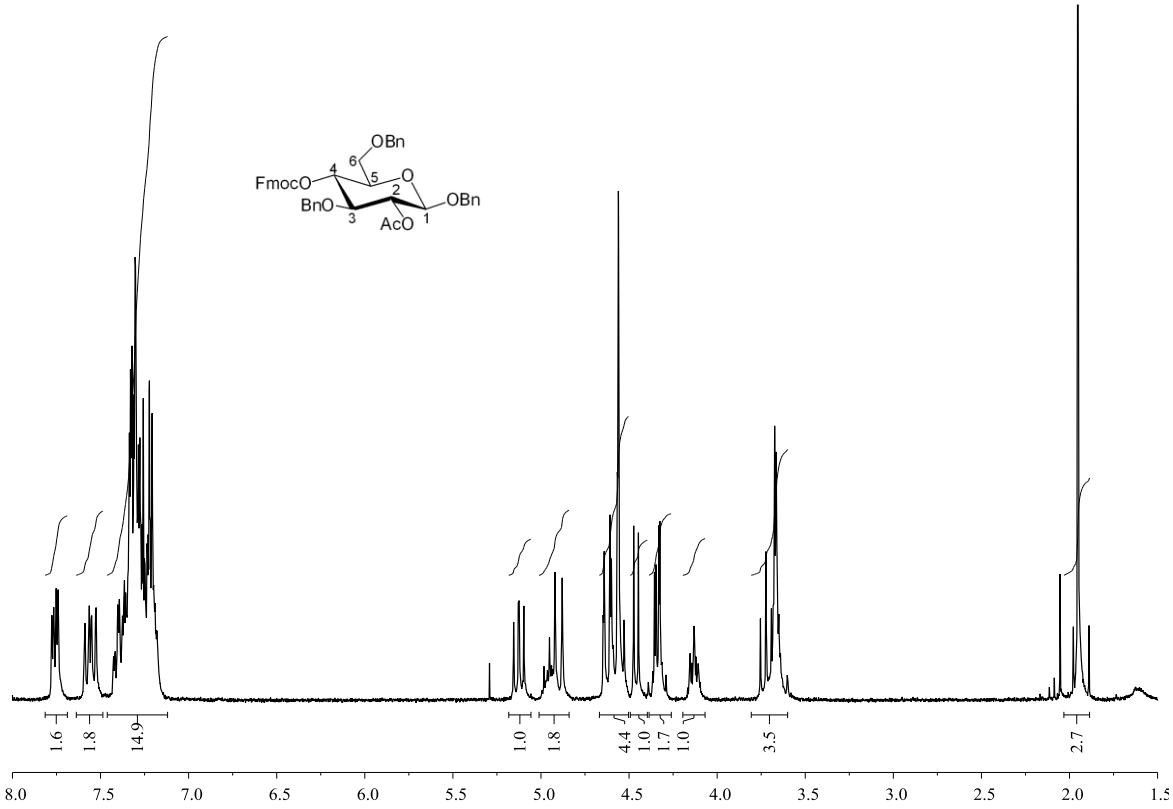
^{1}H-NMR of **1b-b** (300 MHz, $CDCl_3$)



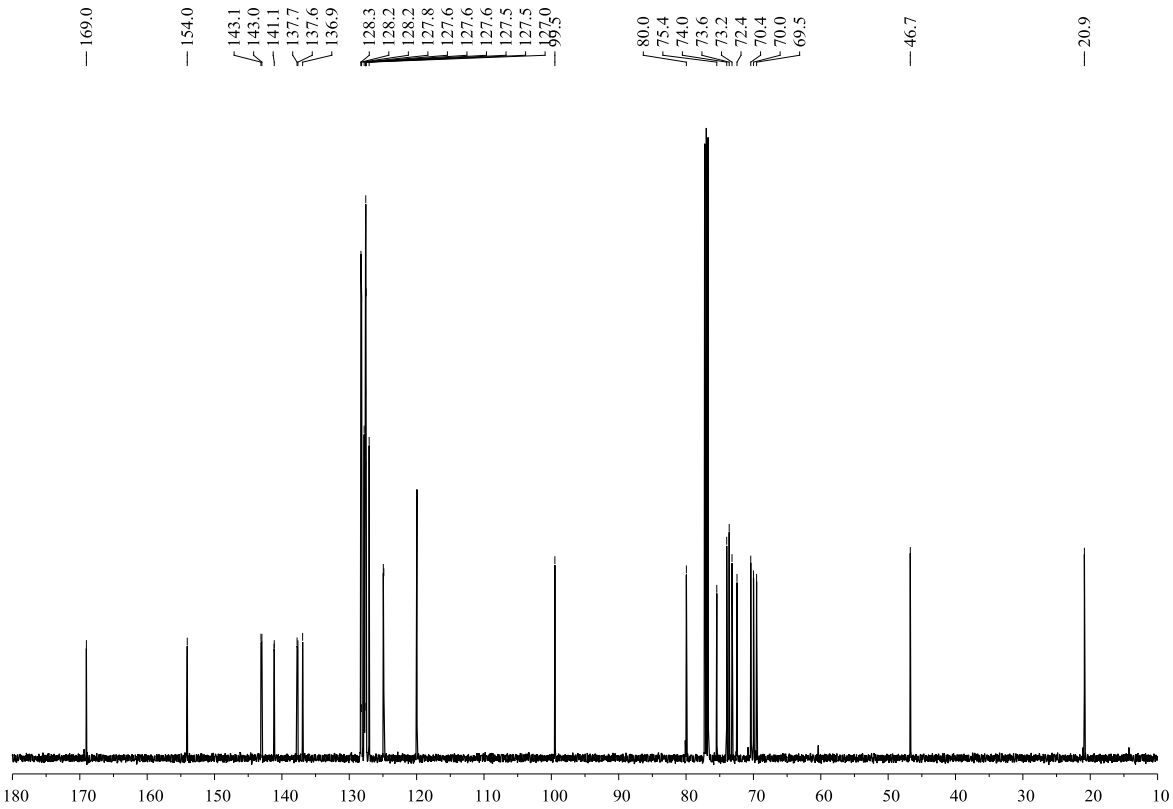
^{13}C-NMR of **1b-b** (126 MHz, $CDCl_3$)



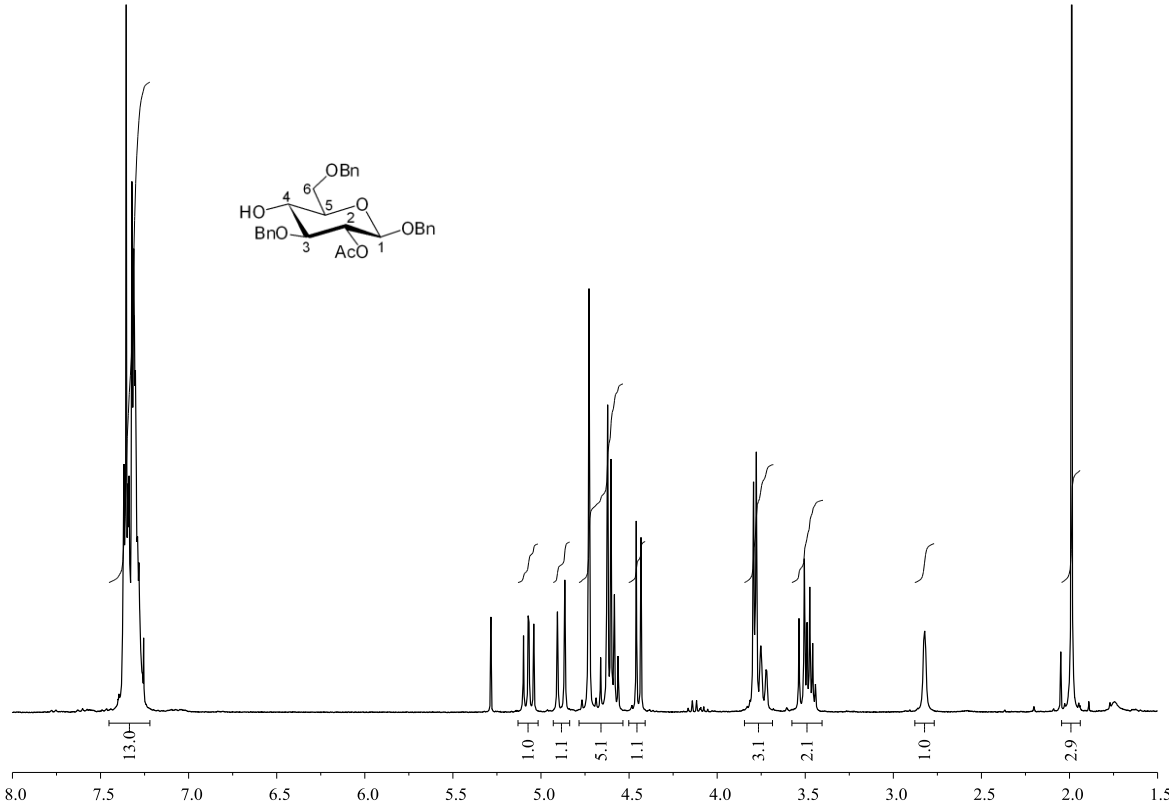
^{1}H-NMR of **1c** (300 MHz, $CDCl_3$)



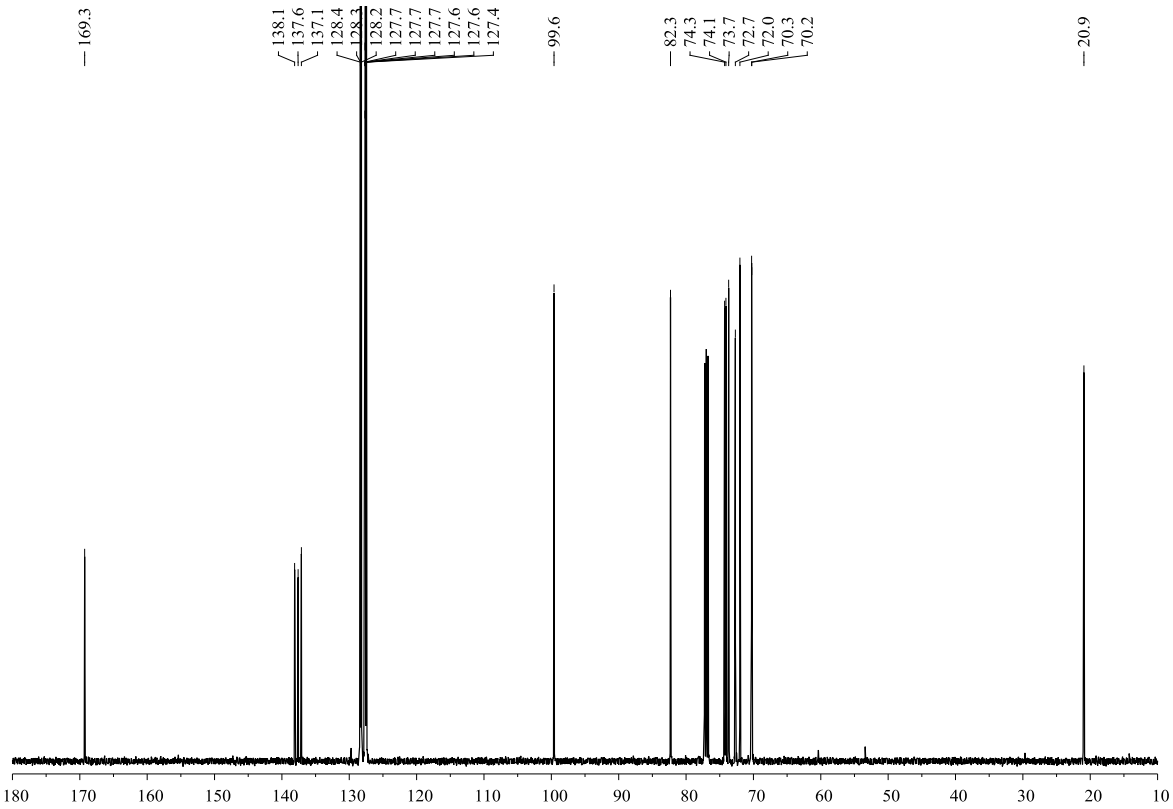
^{13}C-NMR of **1c** (126 MHz, $CDCl_3$)



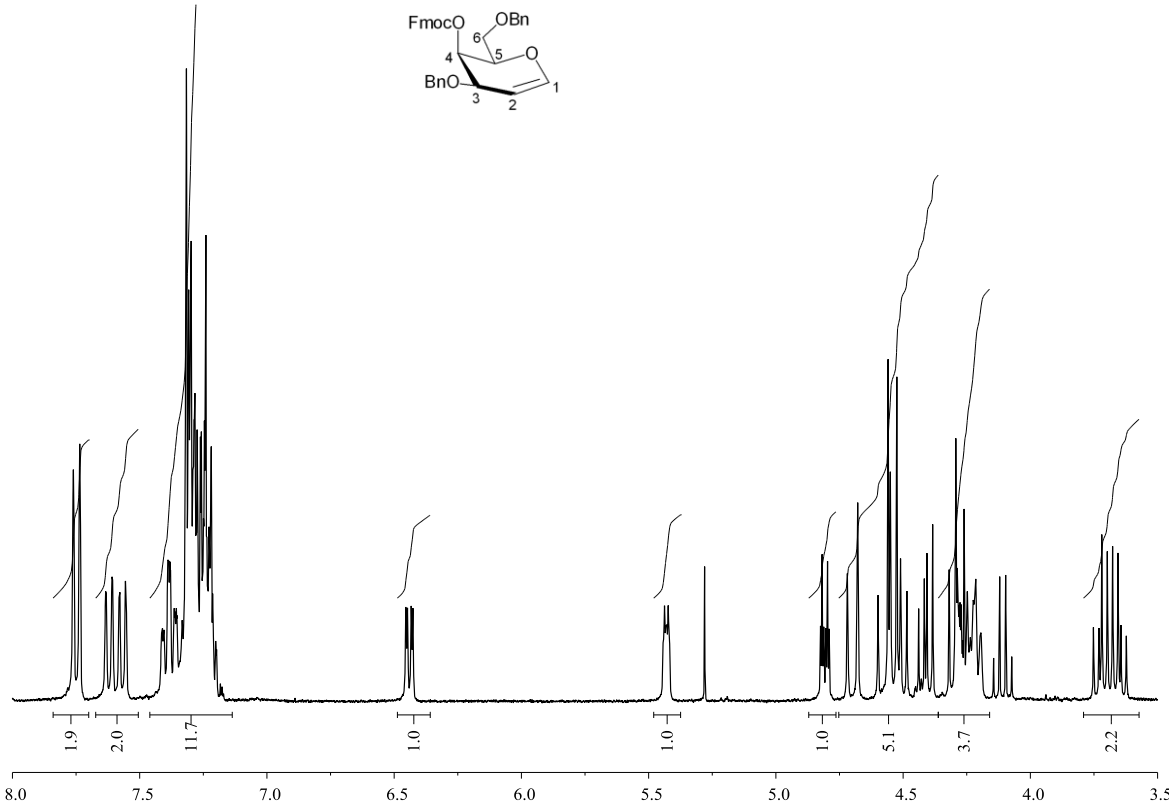
^{1}H-NMR of **1** (300 MHz, $CDCl_3$)



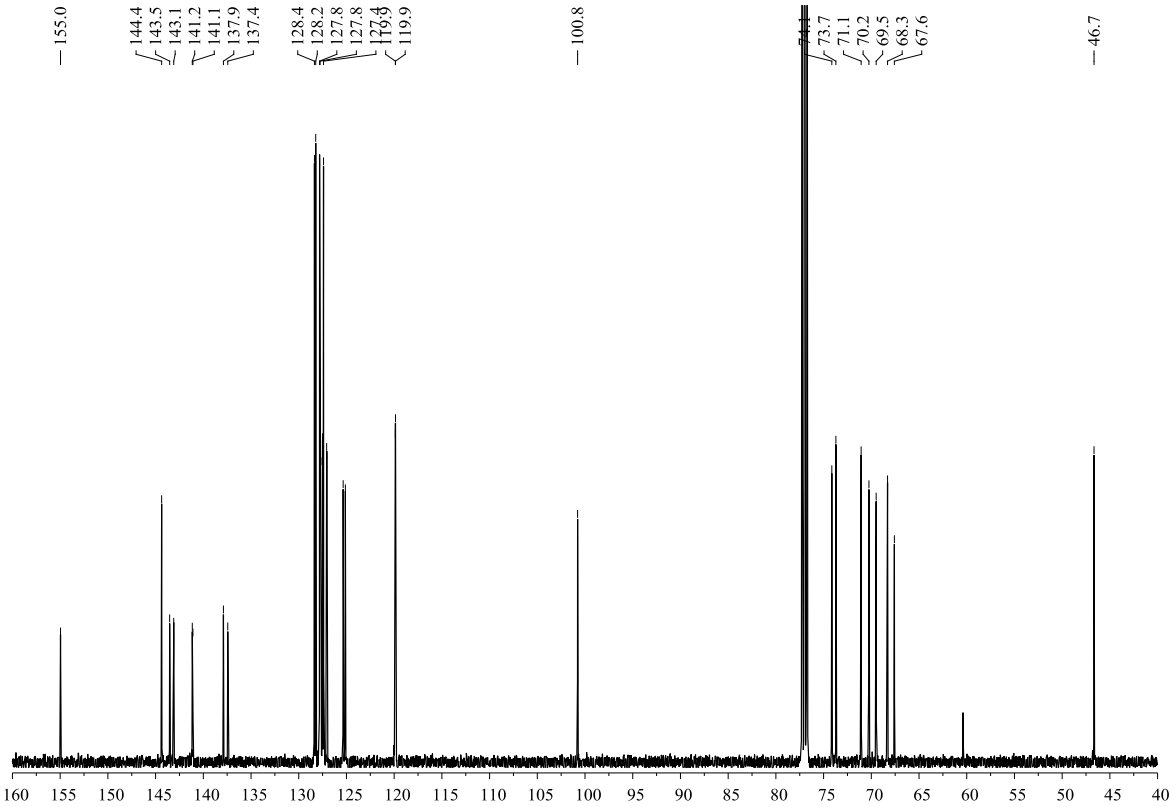
^{13}C-NMR of **1** (126 MHz, $CDCl_3$)



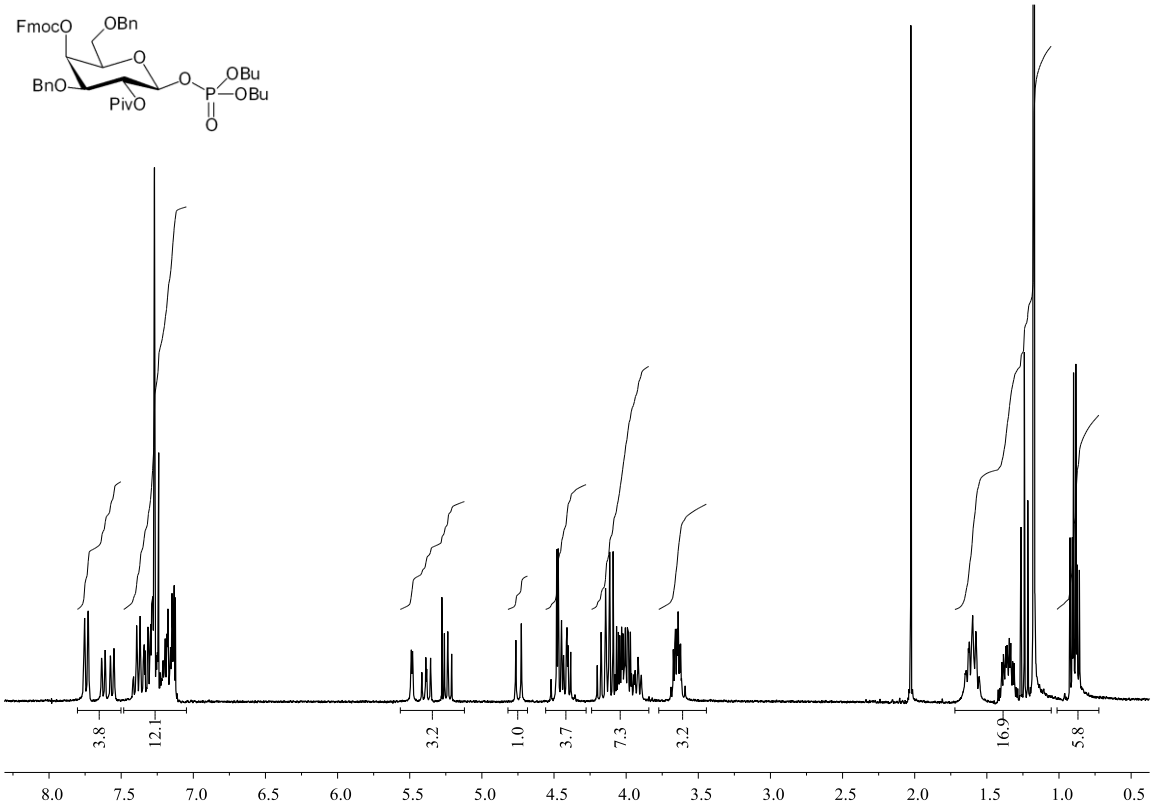
^{1}H-NMR of **2a** (300 MHz, $CDCl_3$)



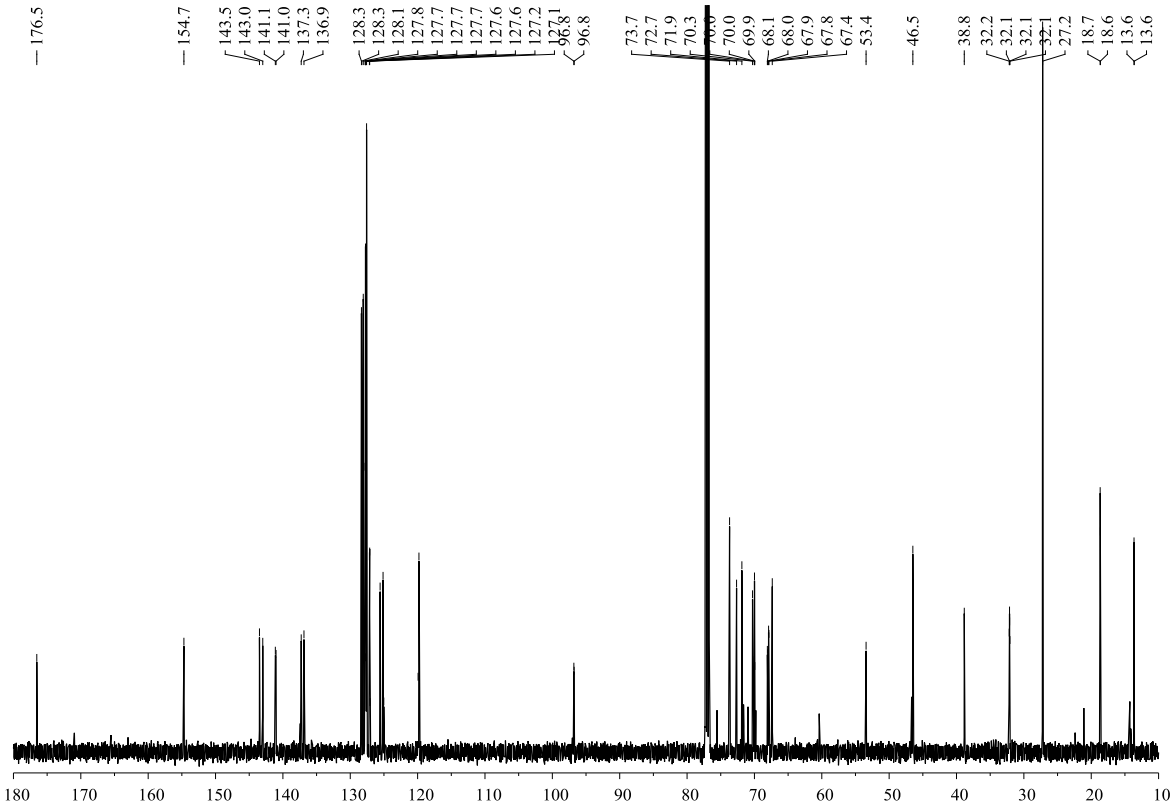
^{13}C-NMR of **2a** (126 MHz, $CDCl_3$)



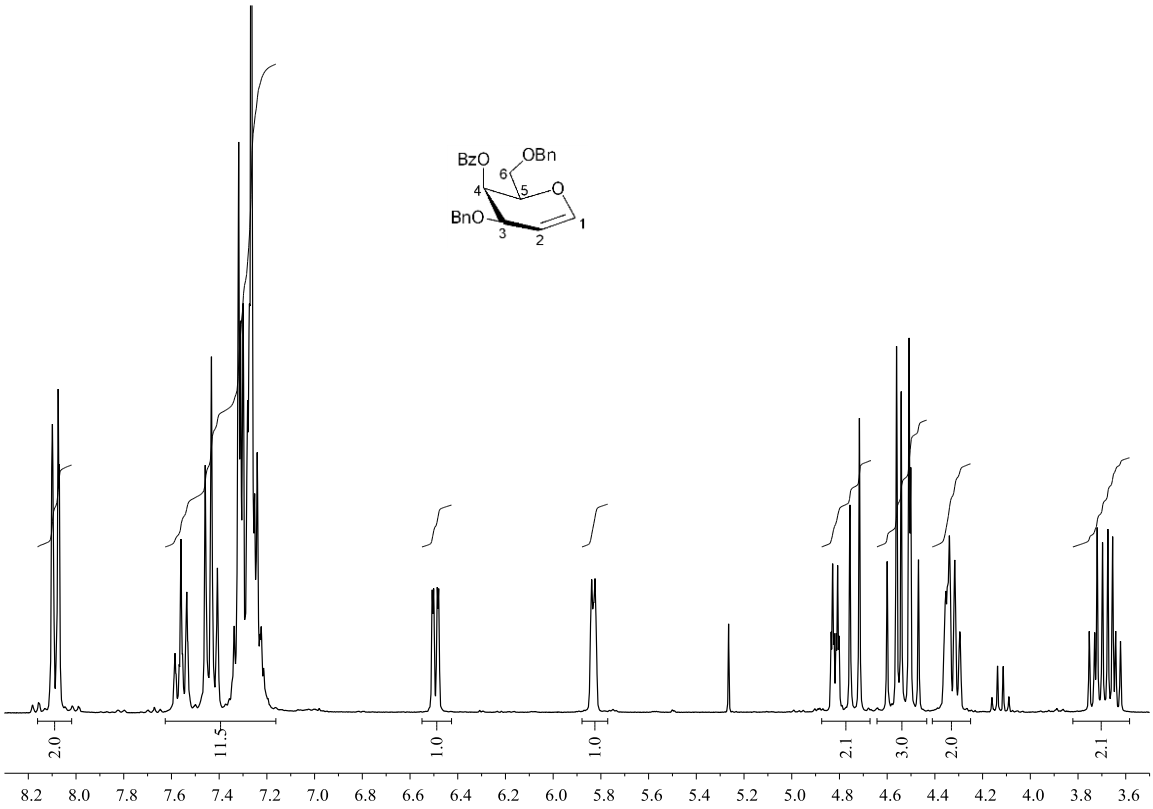
^{1}H-NMR of **2** (300 MHz, $CDCl_3$)



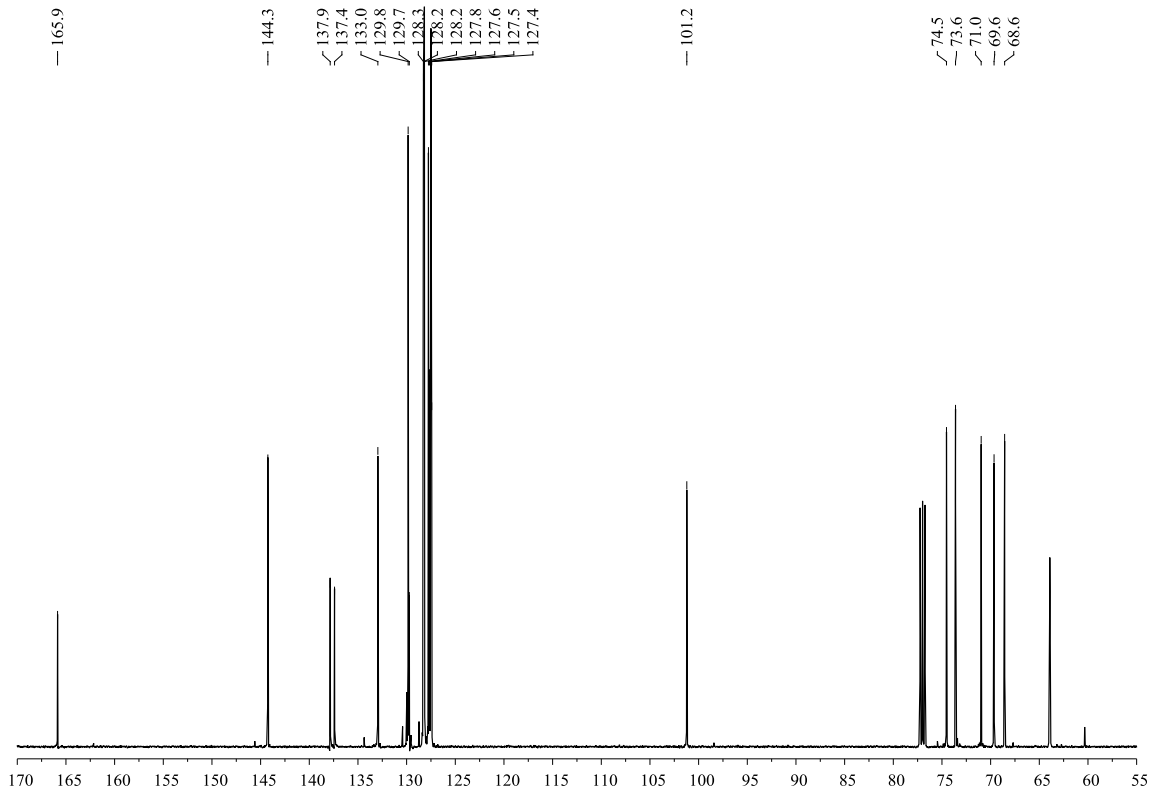
^{13}C-NMR of **2** (126 MHz, $CDCl_3$)



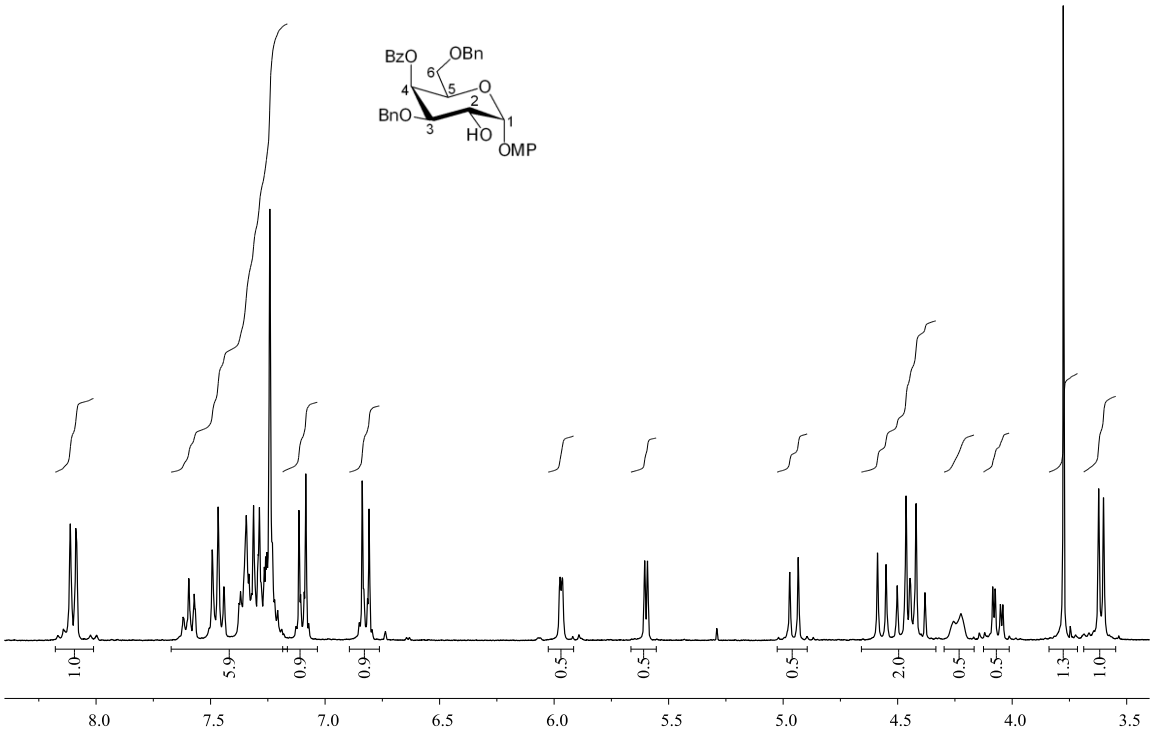
^{1}H-NMR of **11** (300 MHz, $CDCl_3$)



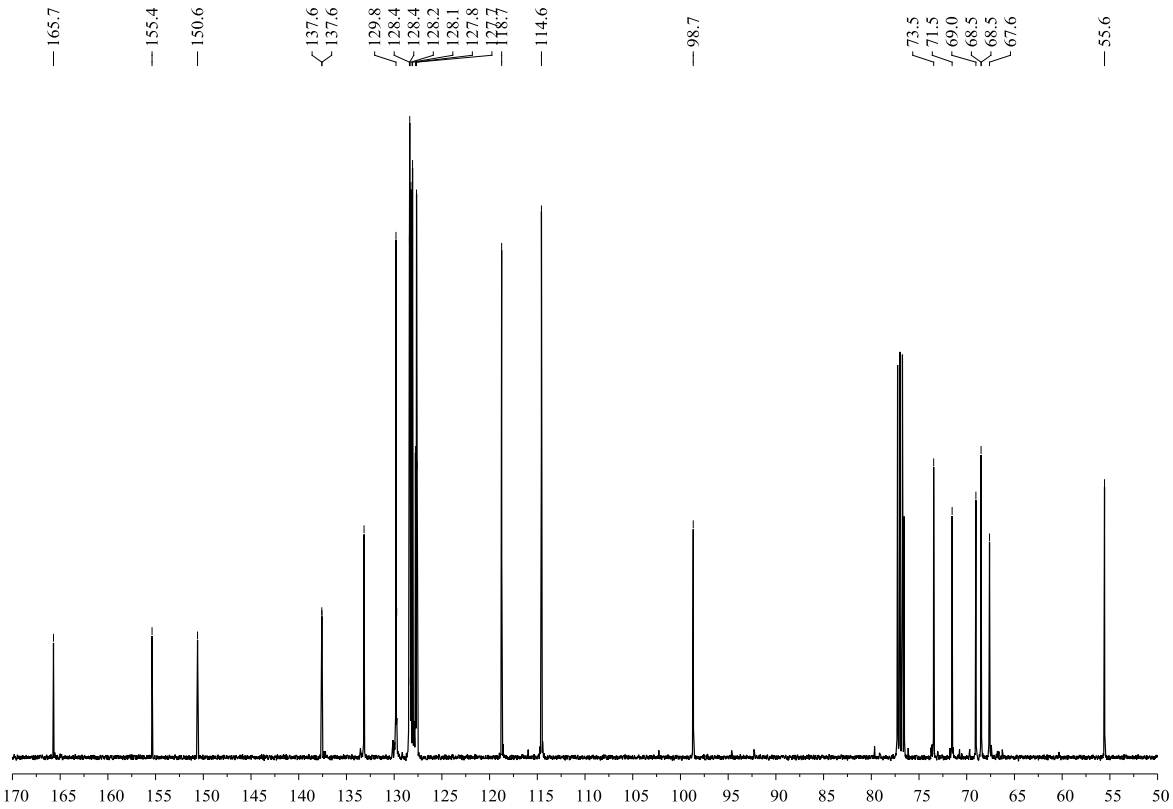
^{13}C-NMR of **11** (126 MHz, $CDCl_3$)



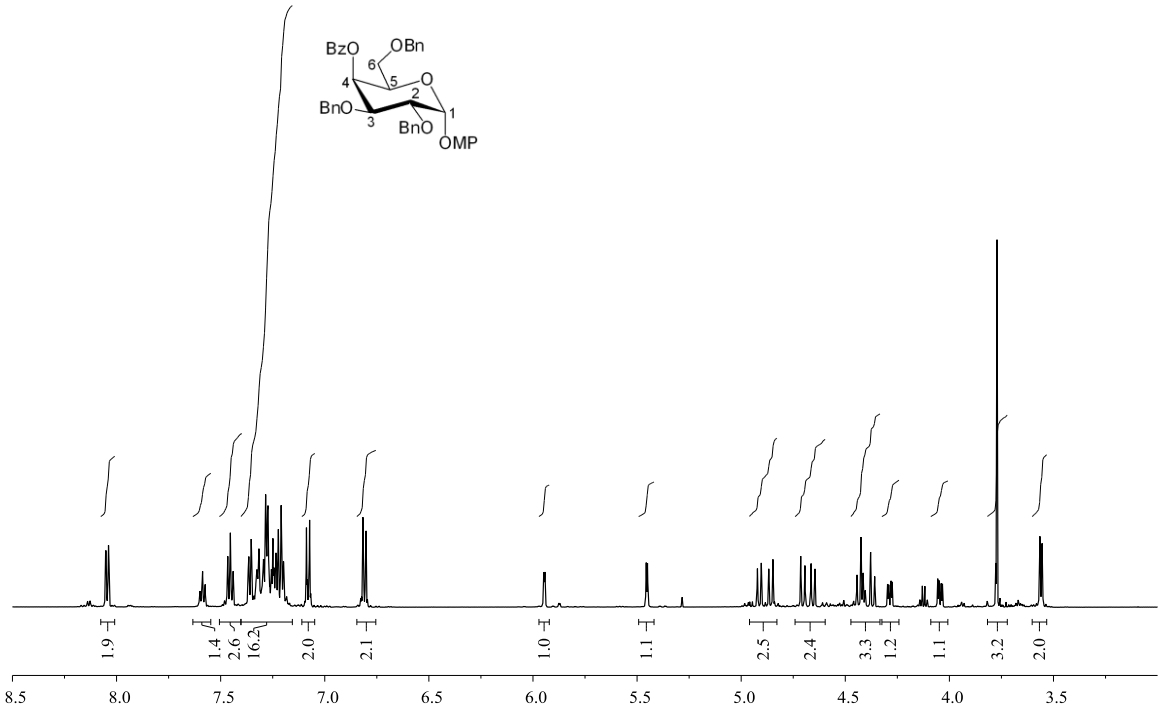
^{1}H-NMR of **12** (300 MHz, $CDCl_3$)



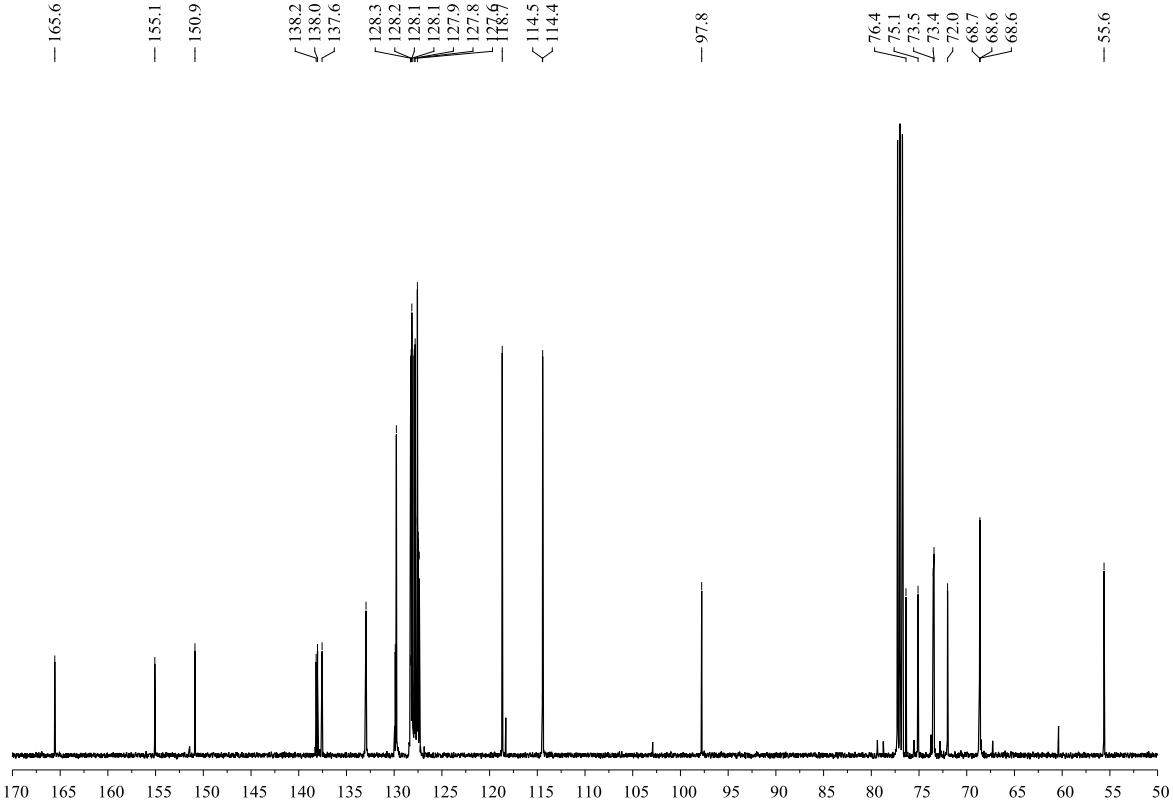
^{13}C-NMR of **12** (126 MHz, $CDCl_3$)



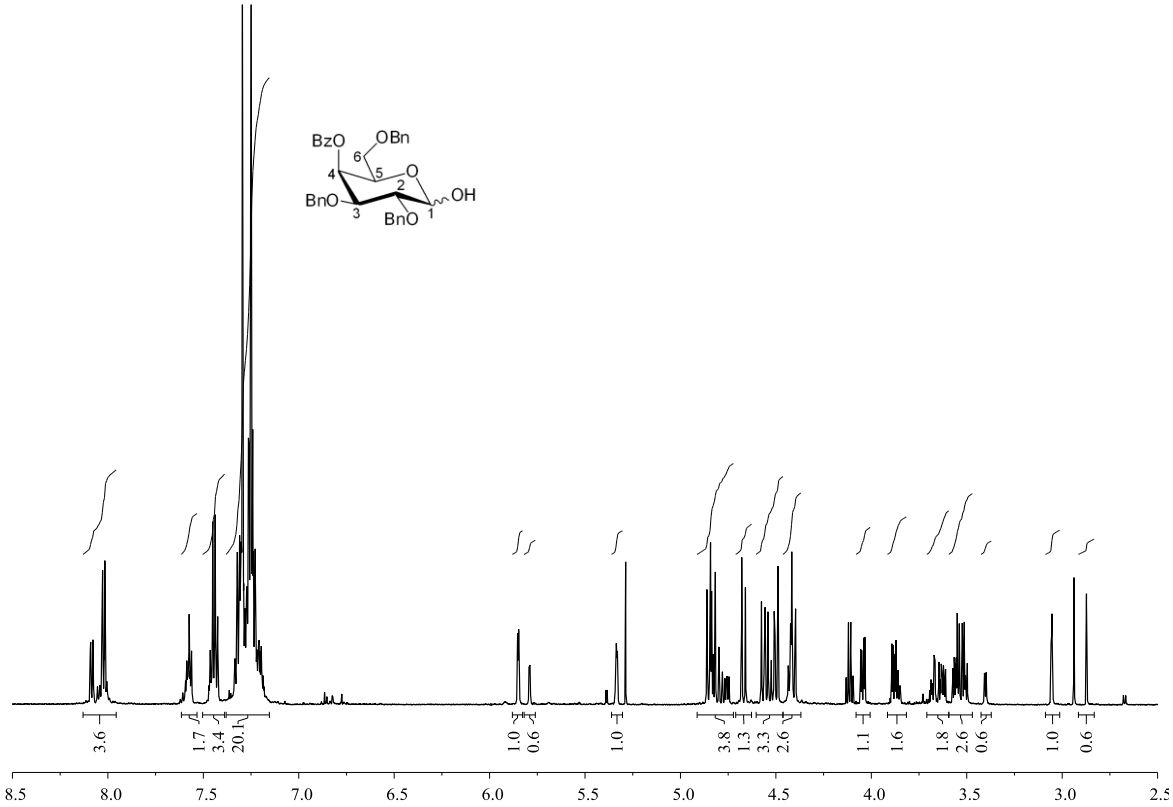
^{1}H-NMR of **12a** (600 MHz, $CDCl_3$)



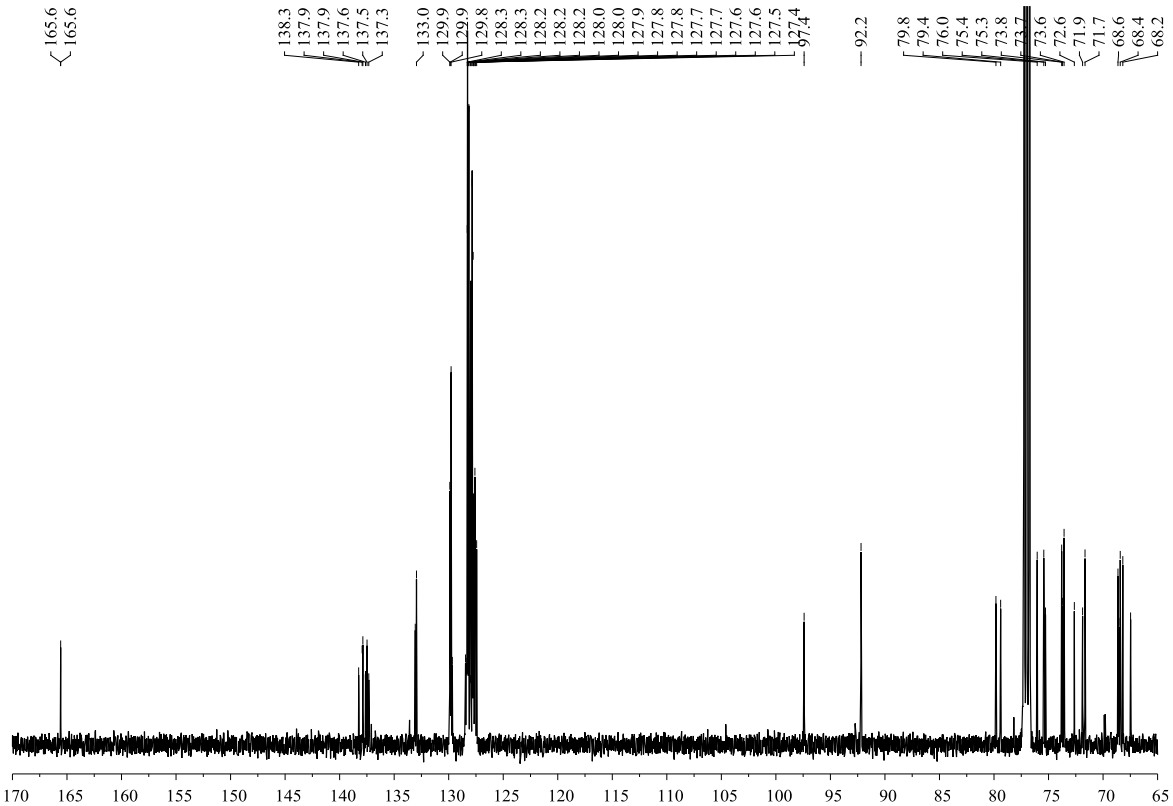
^{13}C-NMR of **12a** (126 MHz, $CDCl_3$)



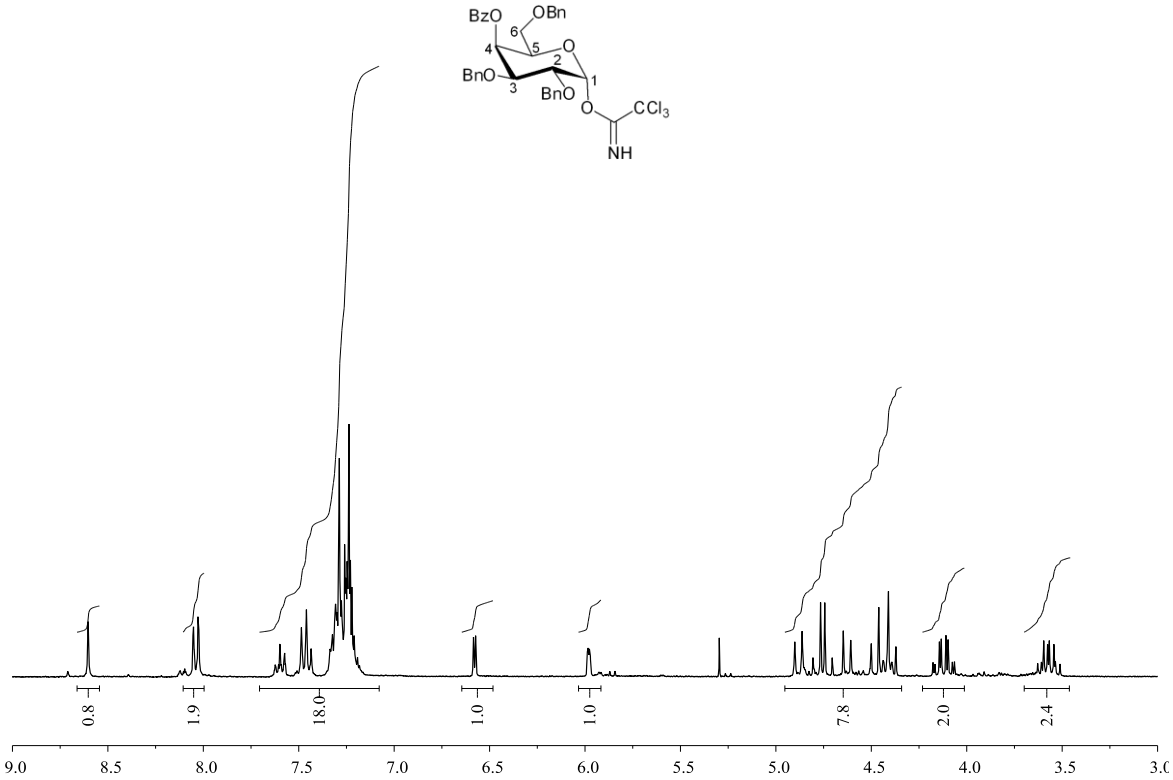
^{1}H-NMR of **13** (600 MHz, $CDCl_3$)



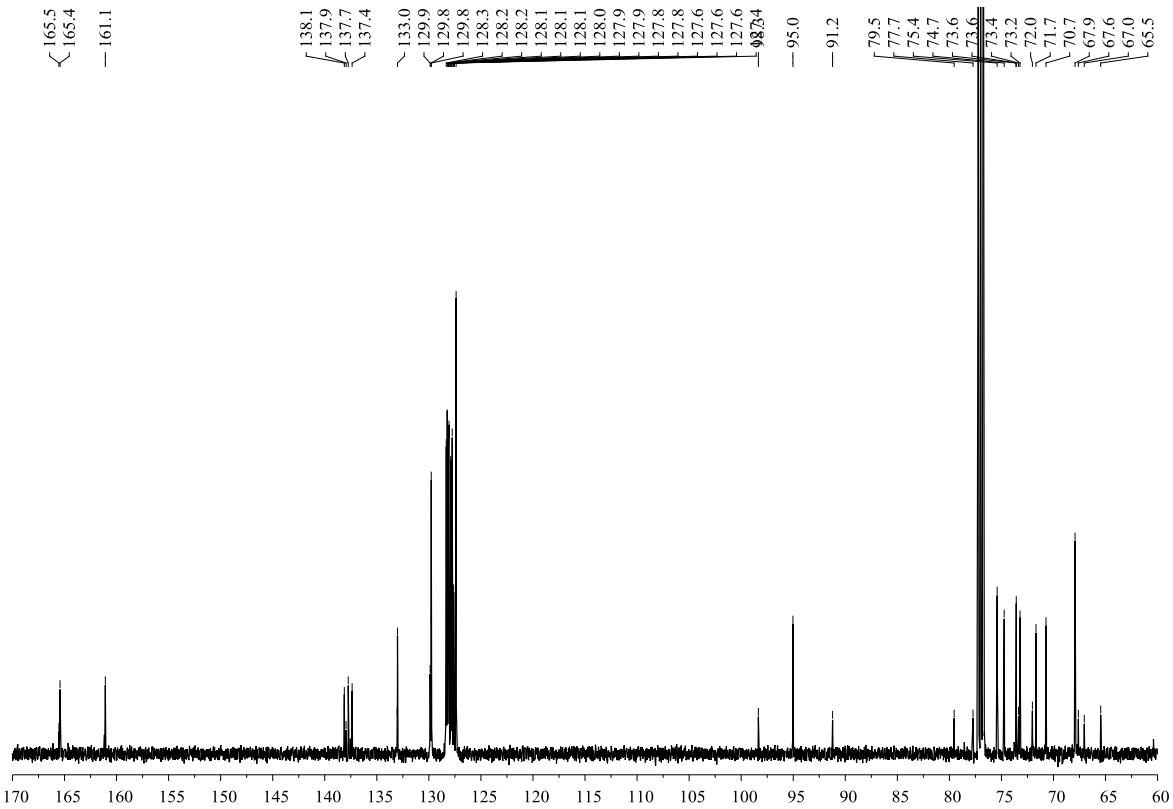
^{13}C-NMR of **13** (126 MHz, $CDCl_3$)



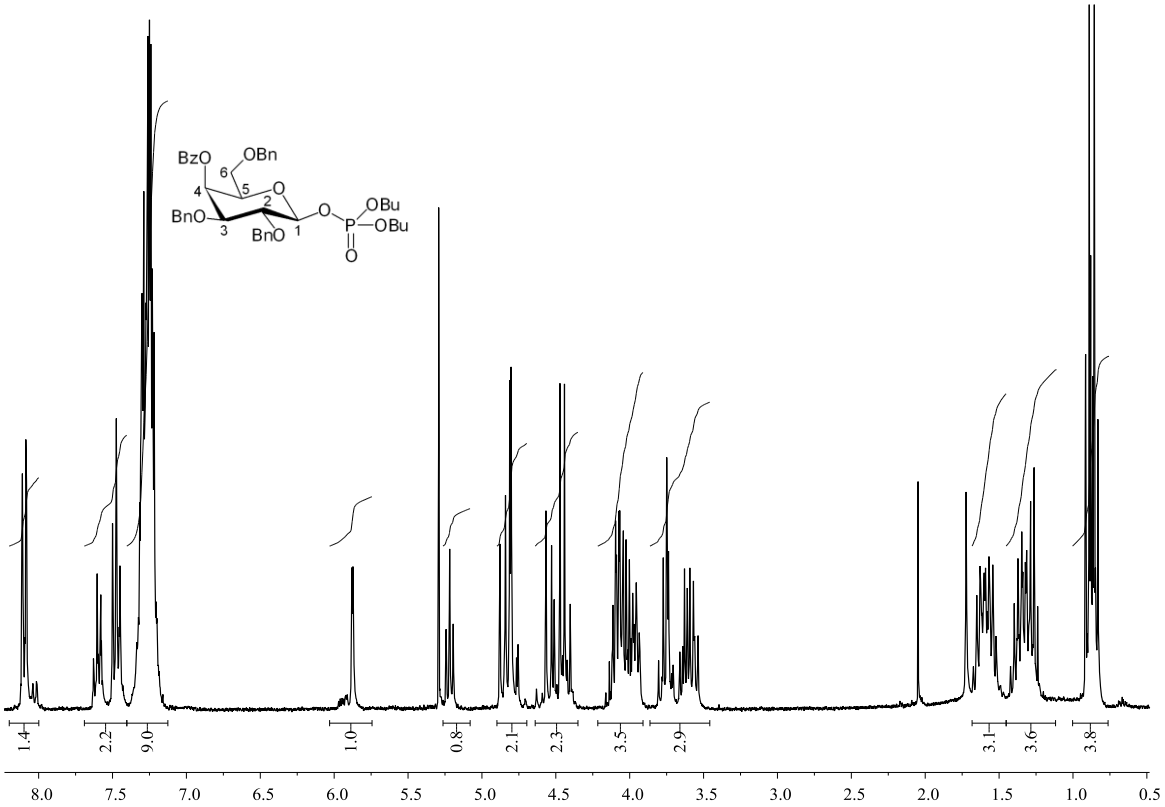
^{1}H-NMR of **3a** (300 MHz, $CDCl_3$)



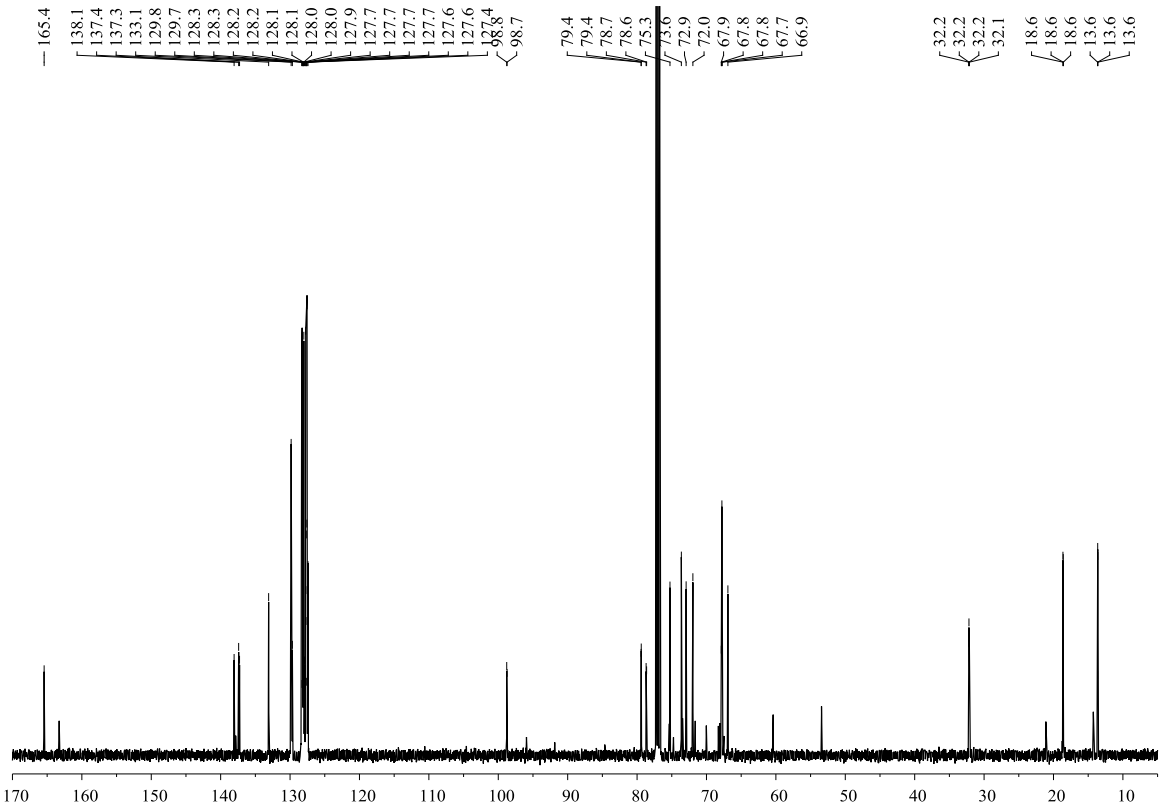
^{13}C-NMR of **3a** (126 MHz, $CDCl_3$)



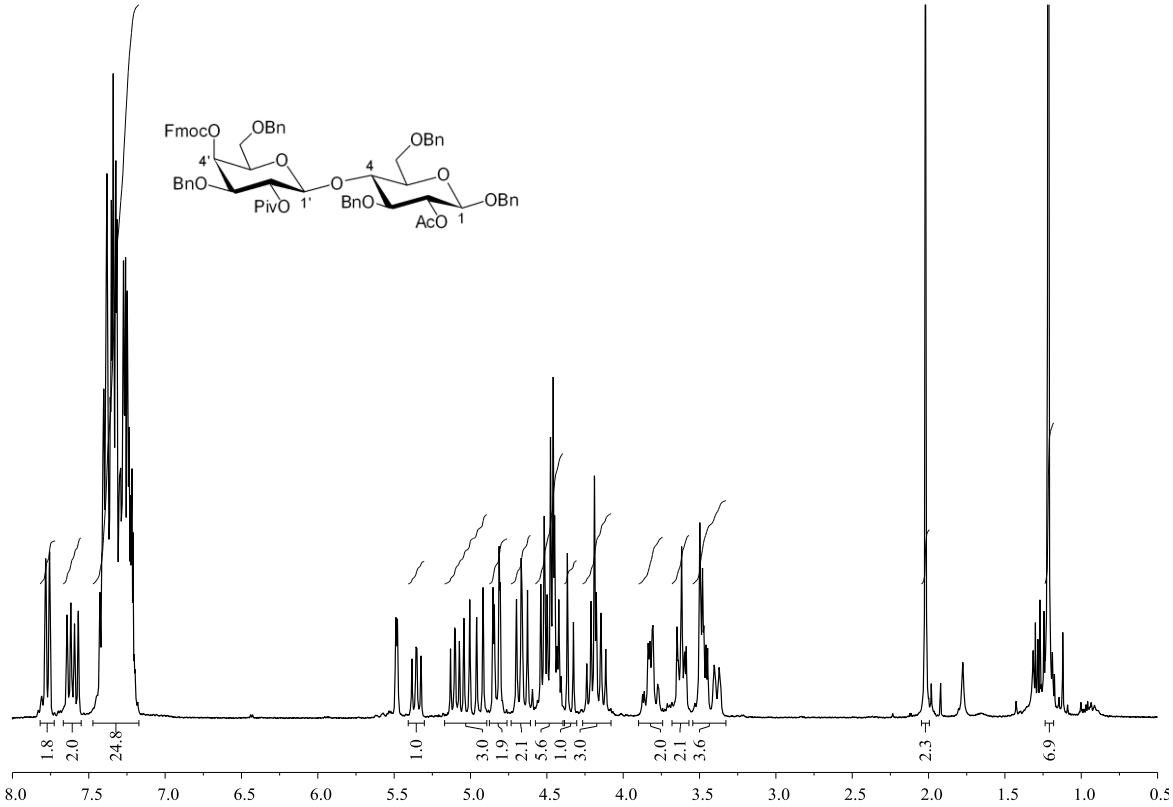
^{1}H-NMR of **3** (300 MHz, $CDCl_3$)



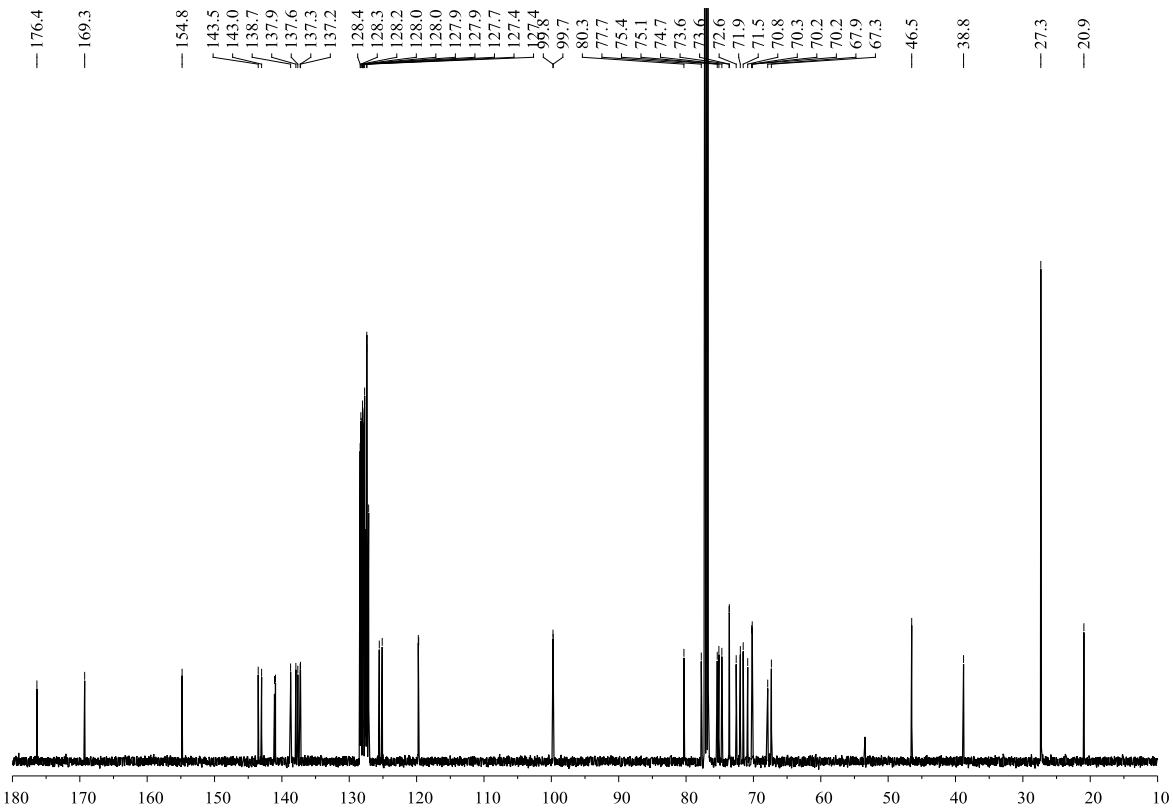
^{13}C-NMR of **3** (126 MHz, $CDCl_3$)



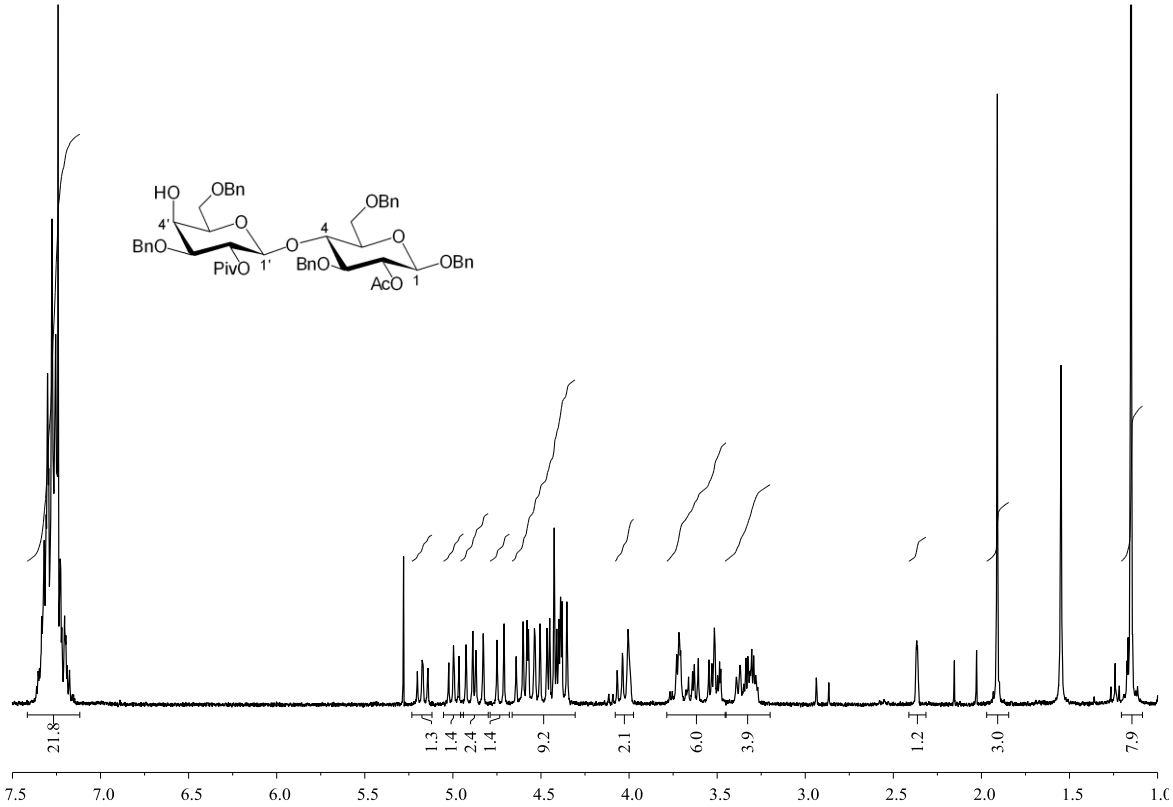
^{1}H-NMR of **13a** (300 MHz, $CDCl_3$)



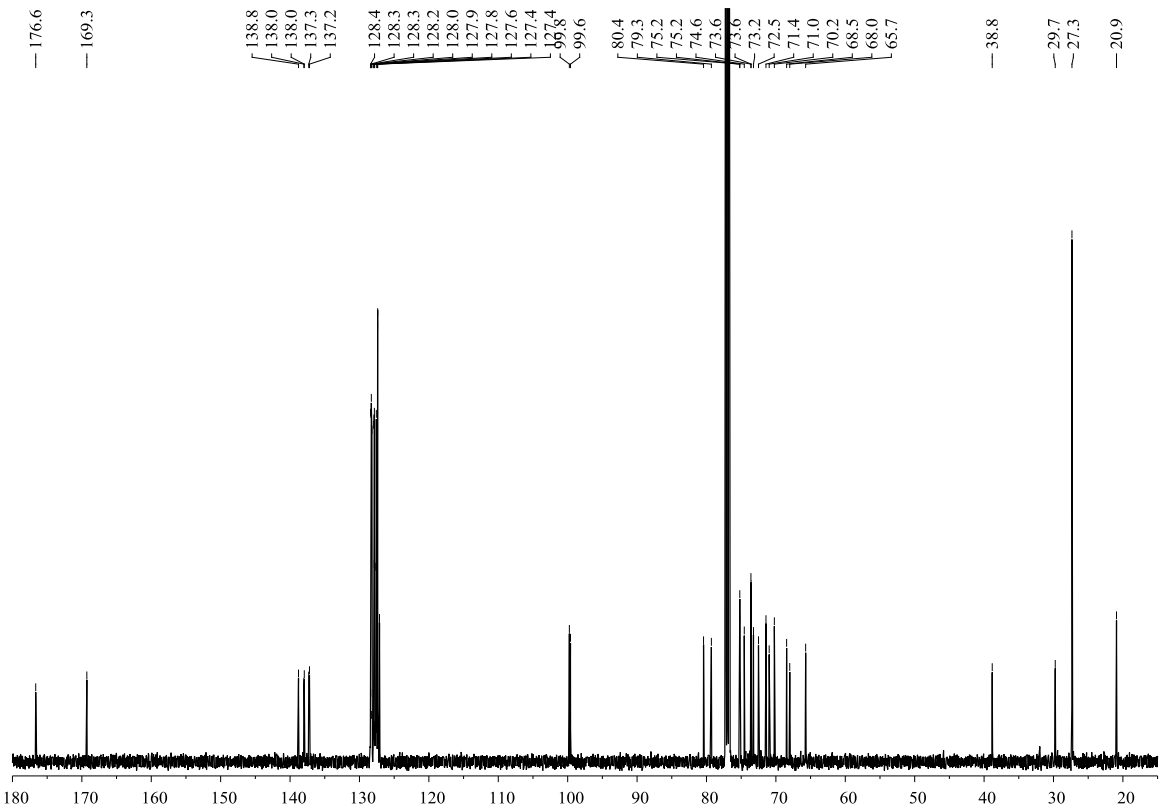
^{13}C-NMR of **13a** (126 MHz, $CDCl_3$)



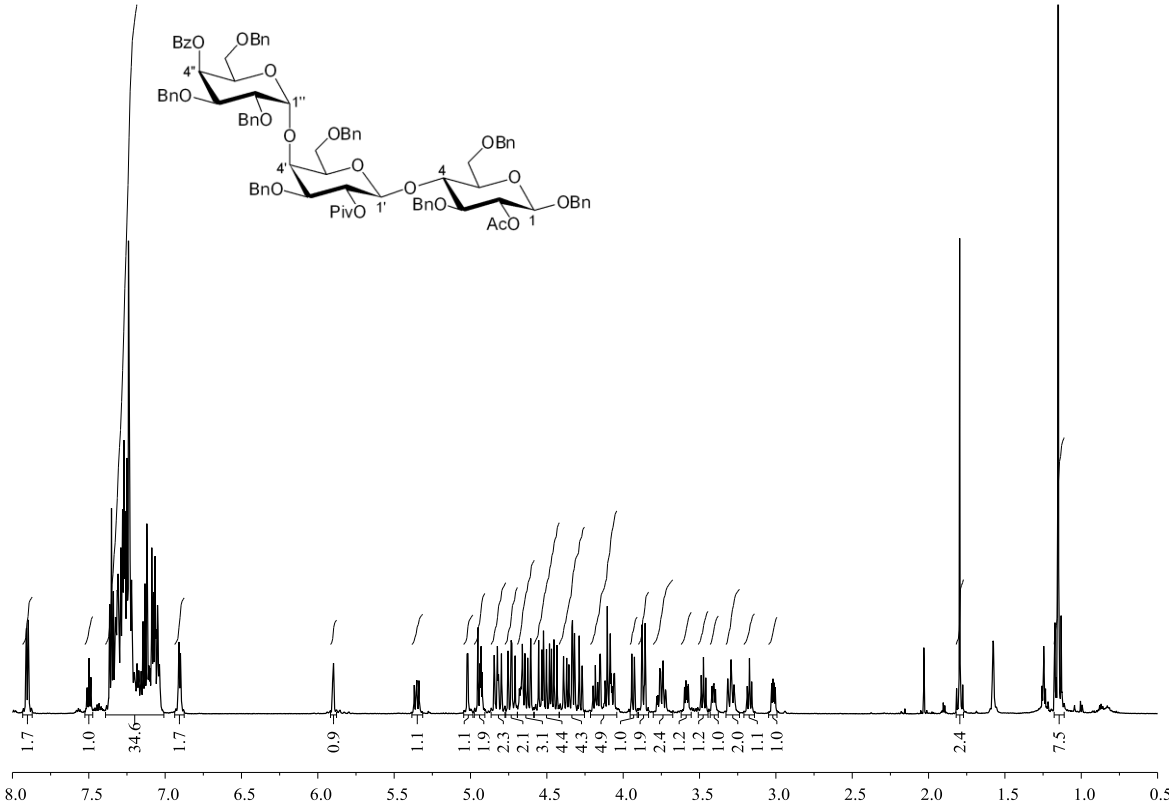
^{1}H-NMR of **13b** (300 MHz, $CDCl_3$)



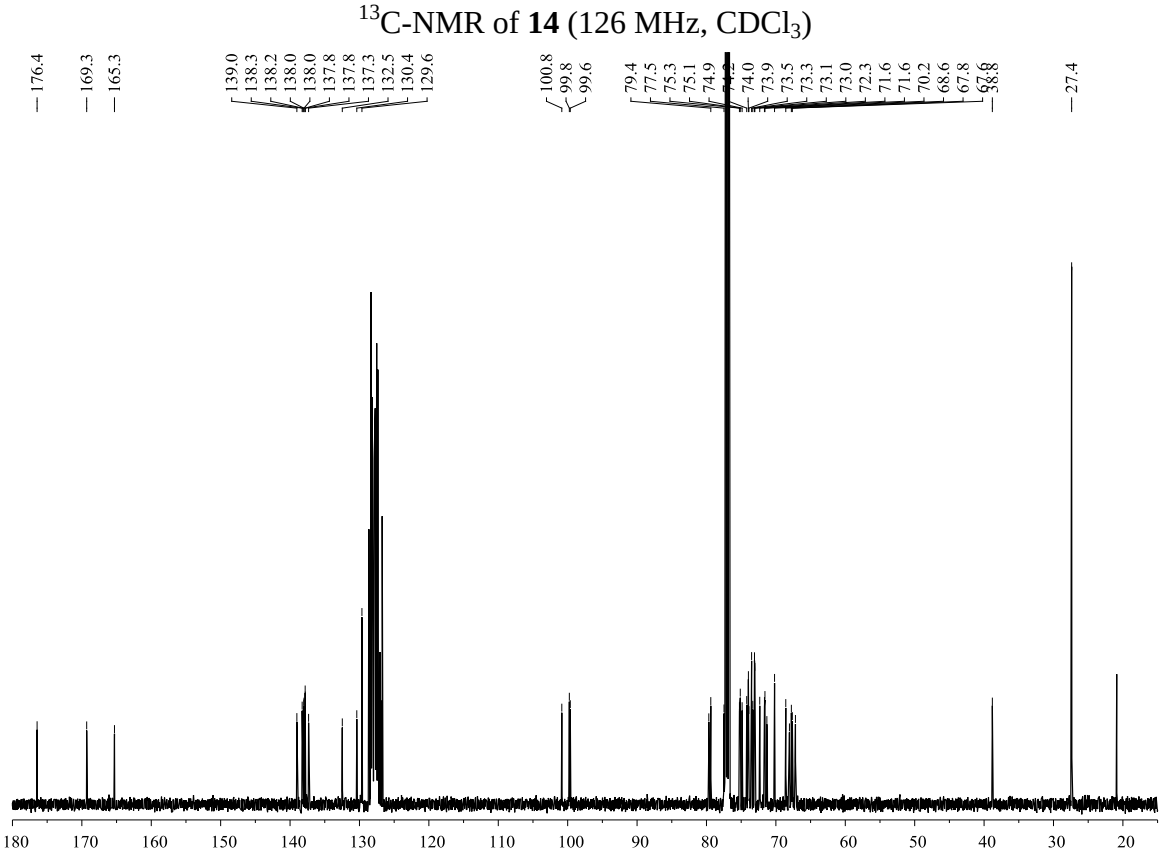
^{13}C-NMR of **13b** (126 MHz, $CDCl_3$)



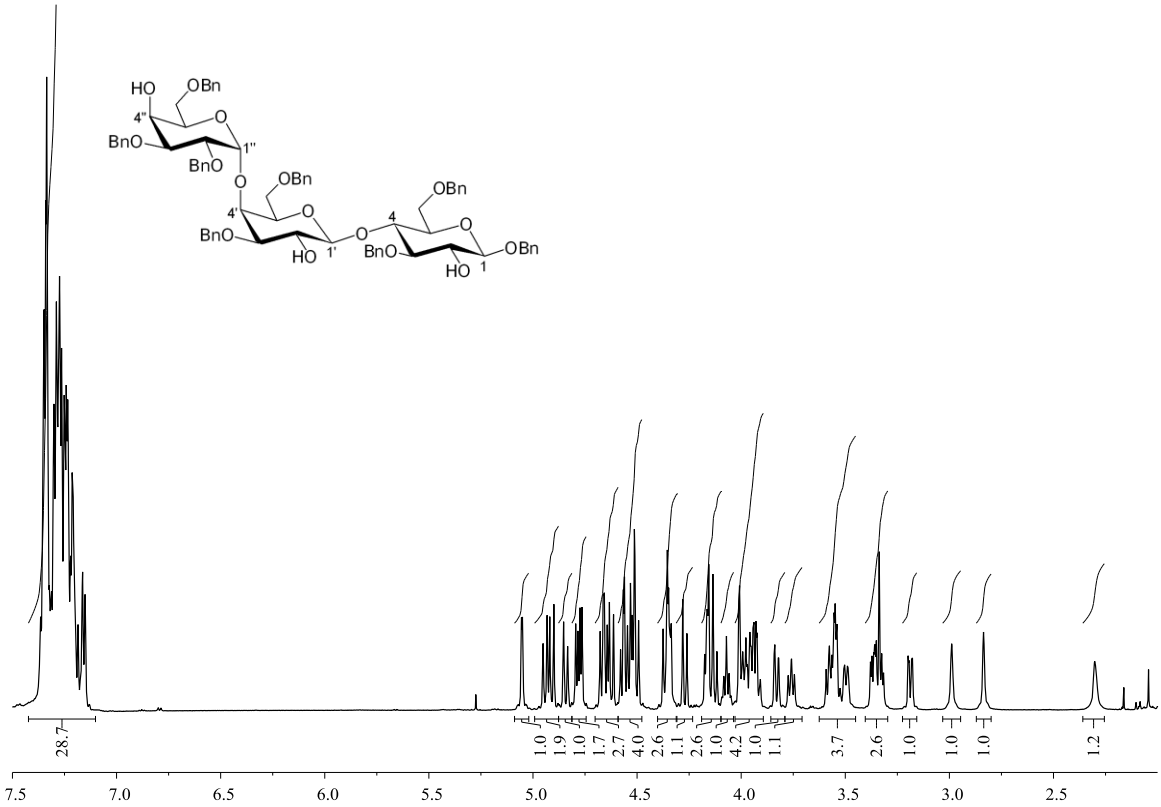
^{1}H-NMR of **14** (600 MHz, $CDCl_3$)



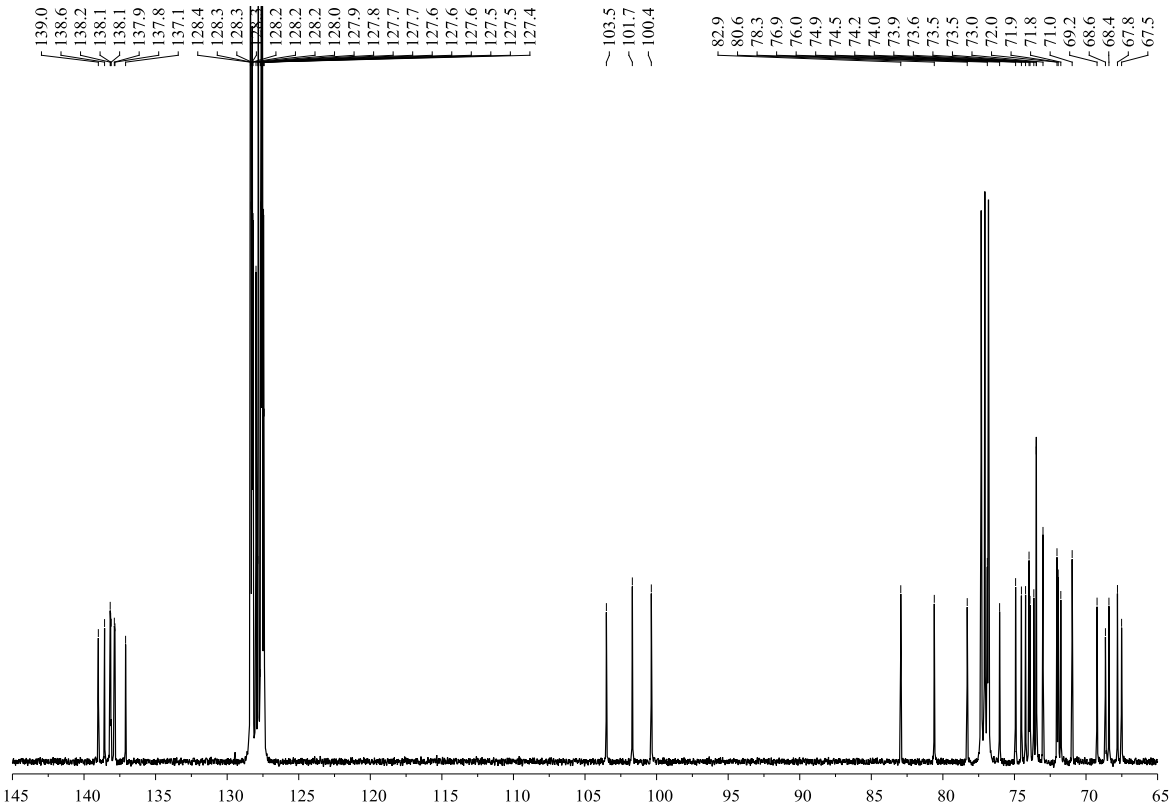
^{13}C-NMR of **14** (126 MHz, $CDCl_3$)



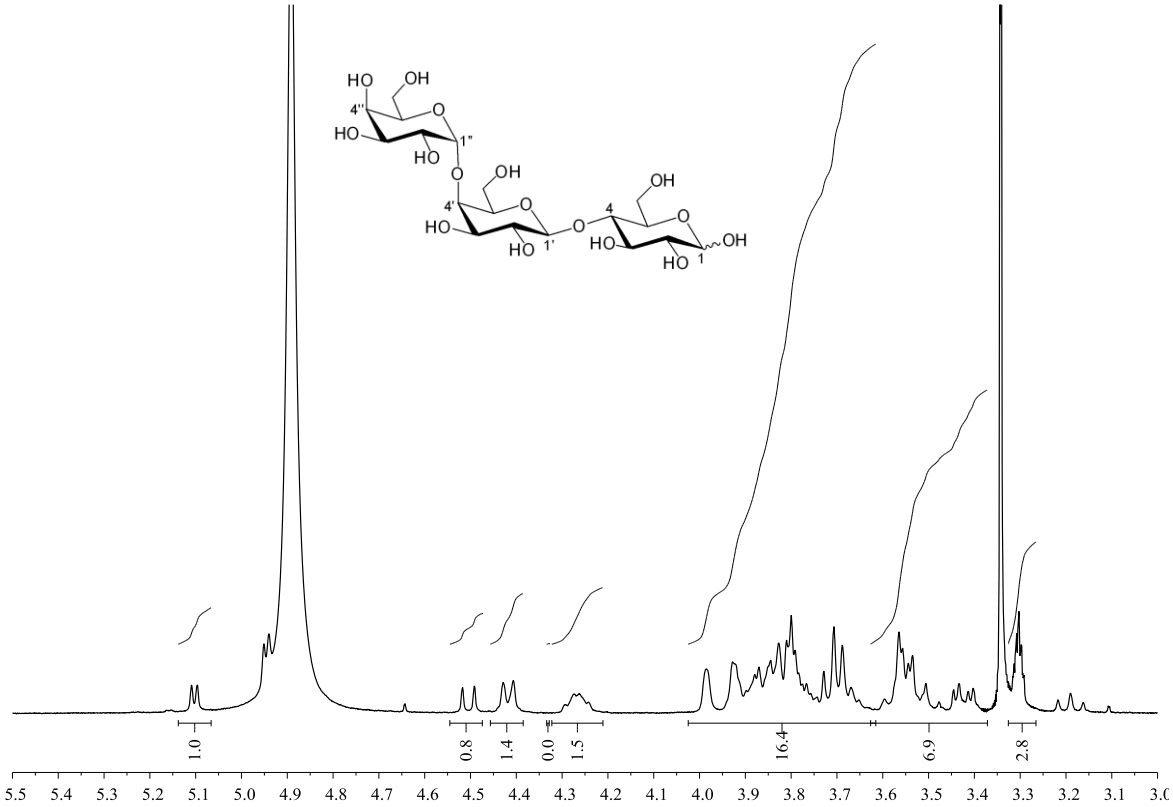
^{1}H-NMR of **15** (600 MHz, $CDCl_3$)



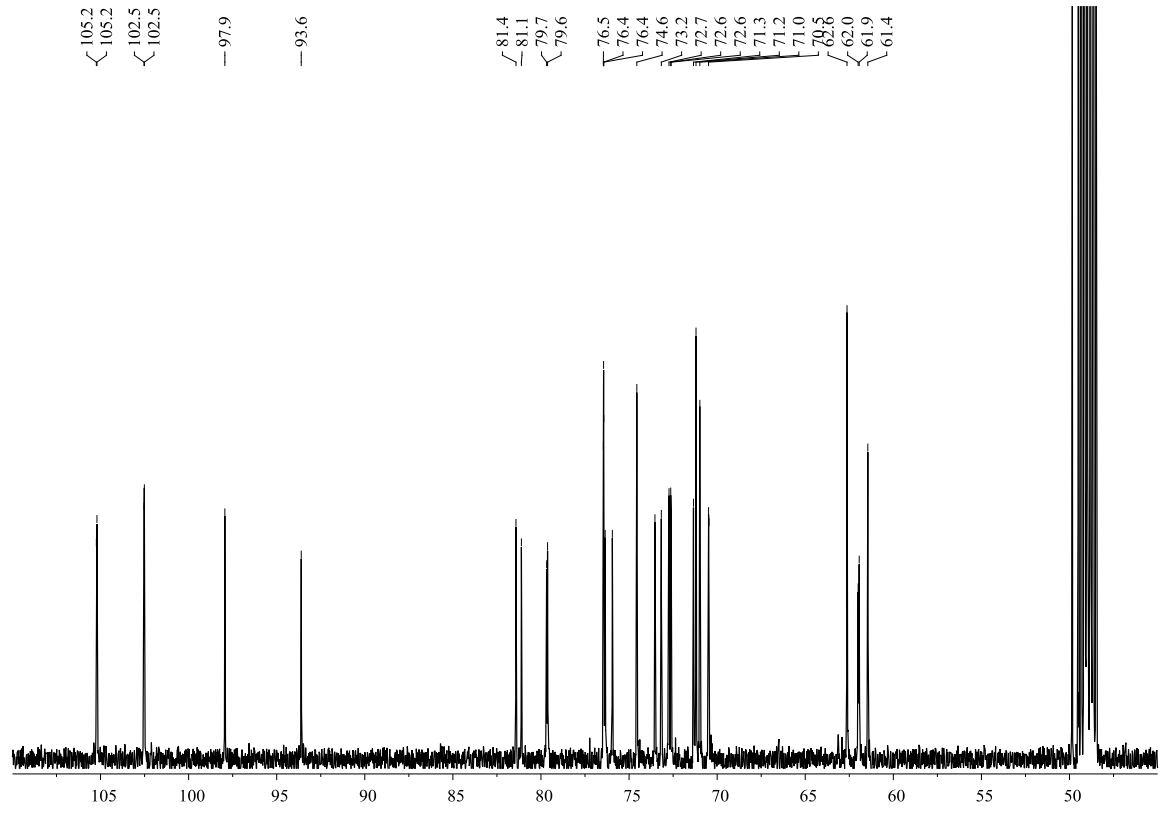
^{13}C-NMR of **15** (126 MHz, $CDCl_3$)



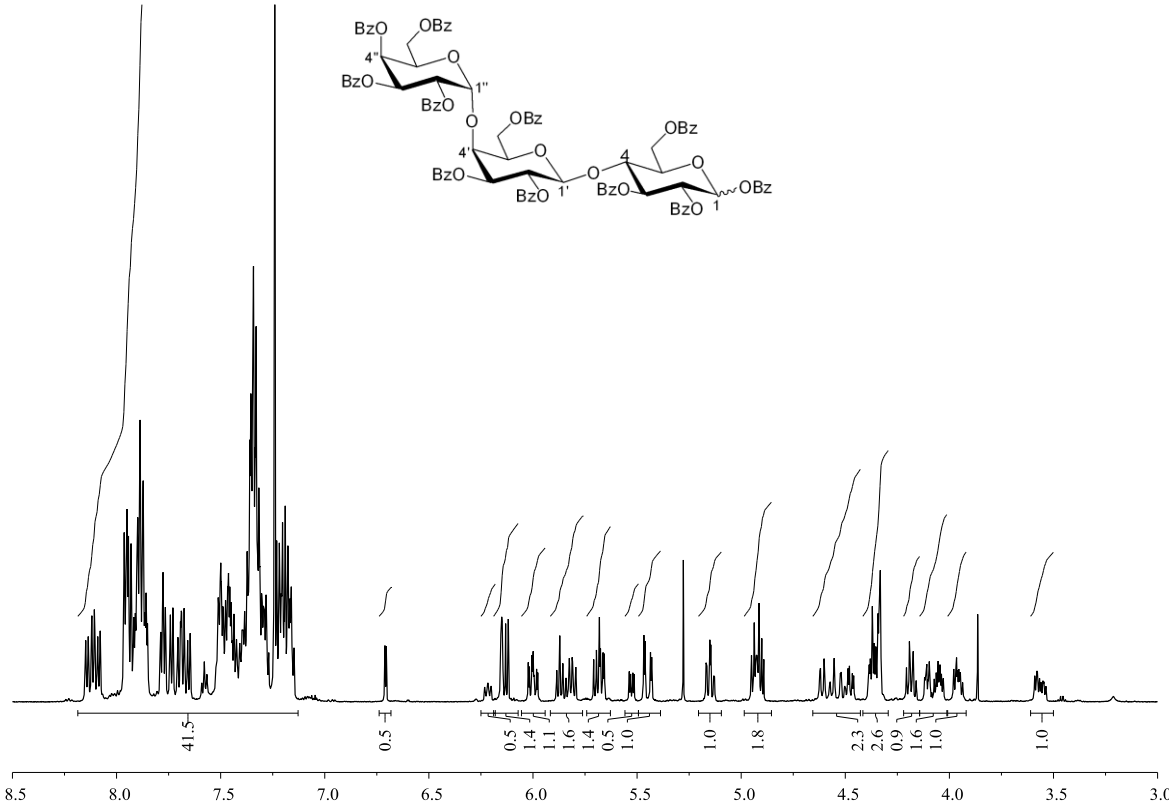
^{1}H-NMR of **15a** (300 MHz, CD_3OD)



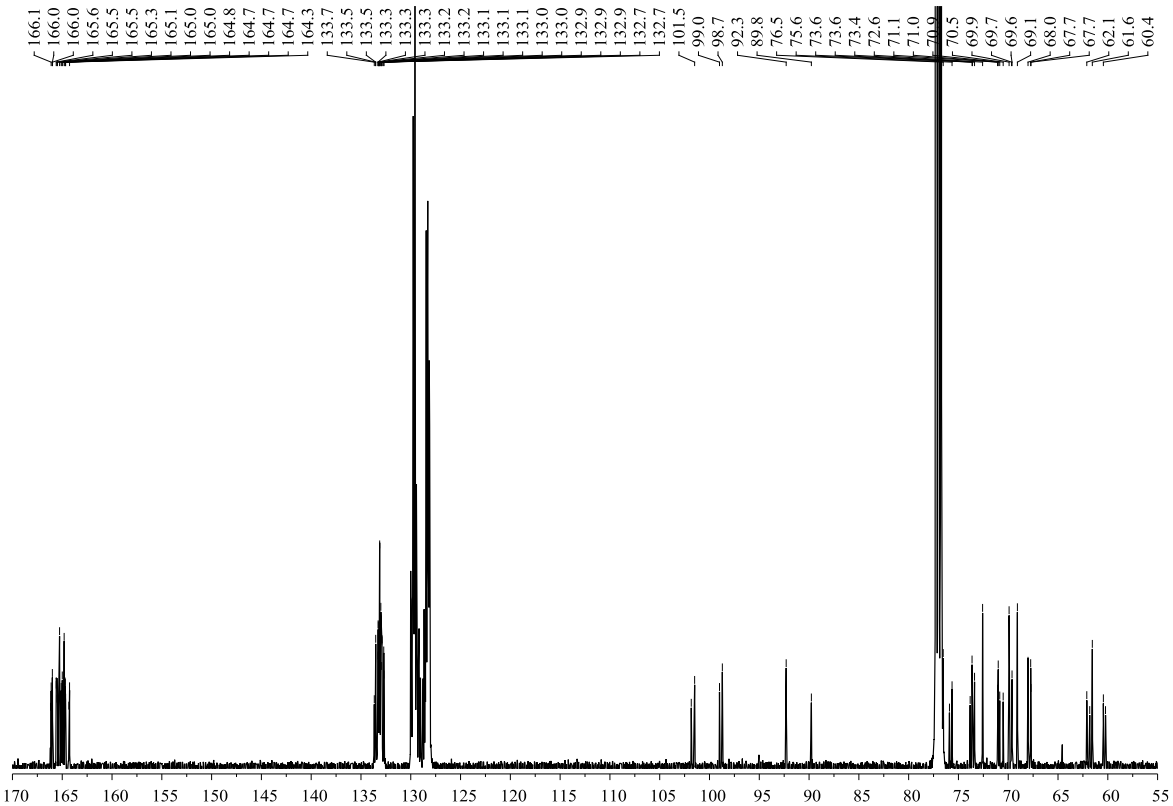
^{13}C-NMR of **15a** (126 MHz, CD_3OD)



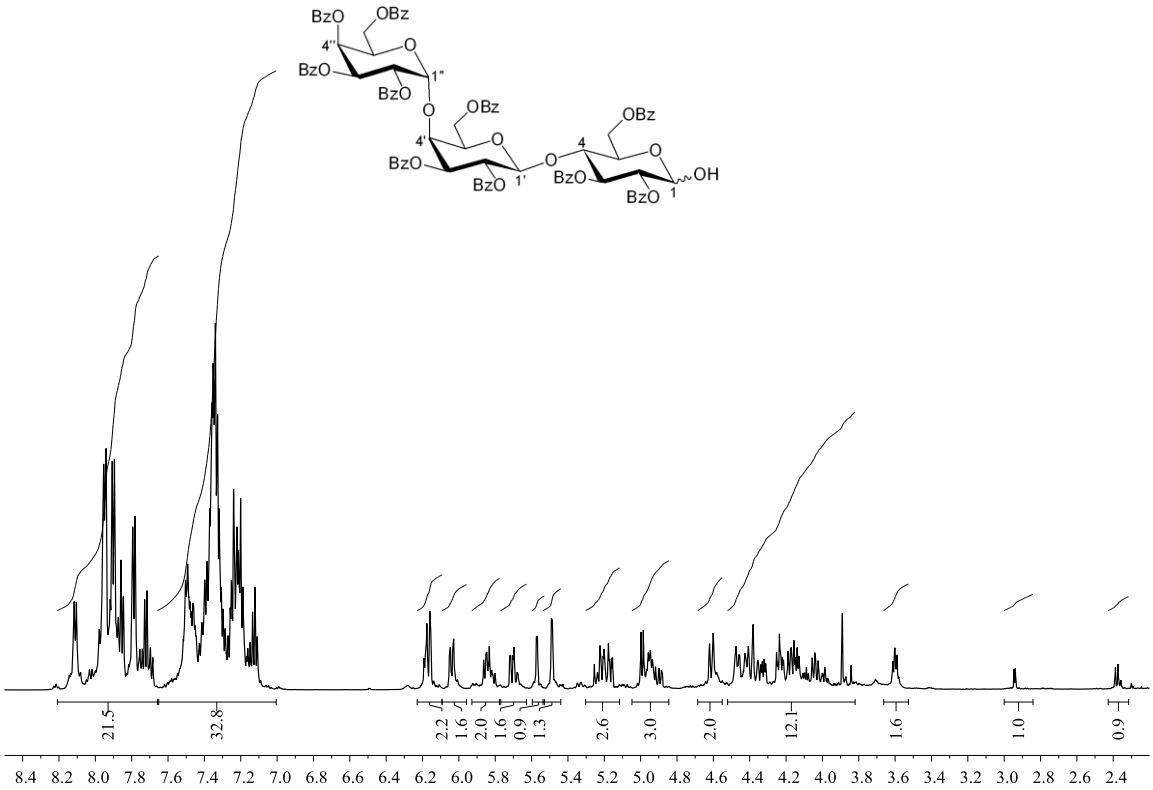
^{1}H-NMR of **15b** (600 MHz, $CDCl_3$)



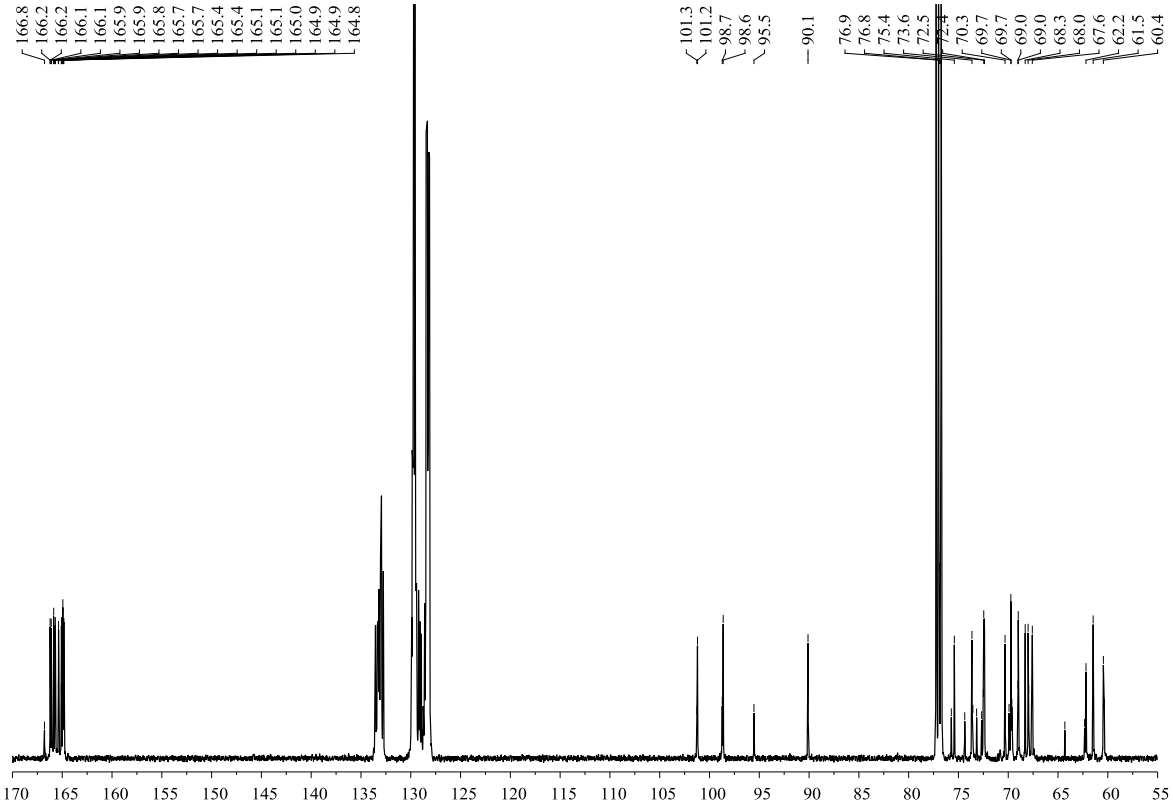
^{13}C-NMR of **15b** (126 MHz, $CDCl_3$)



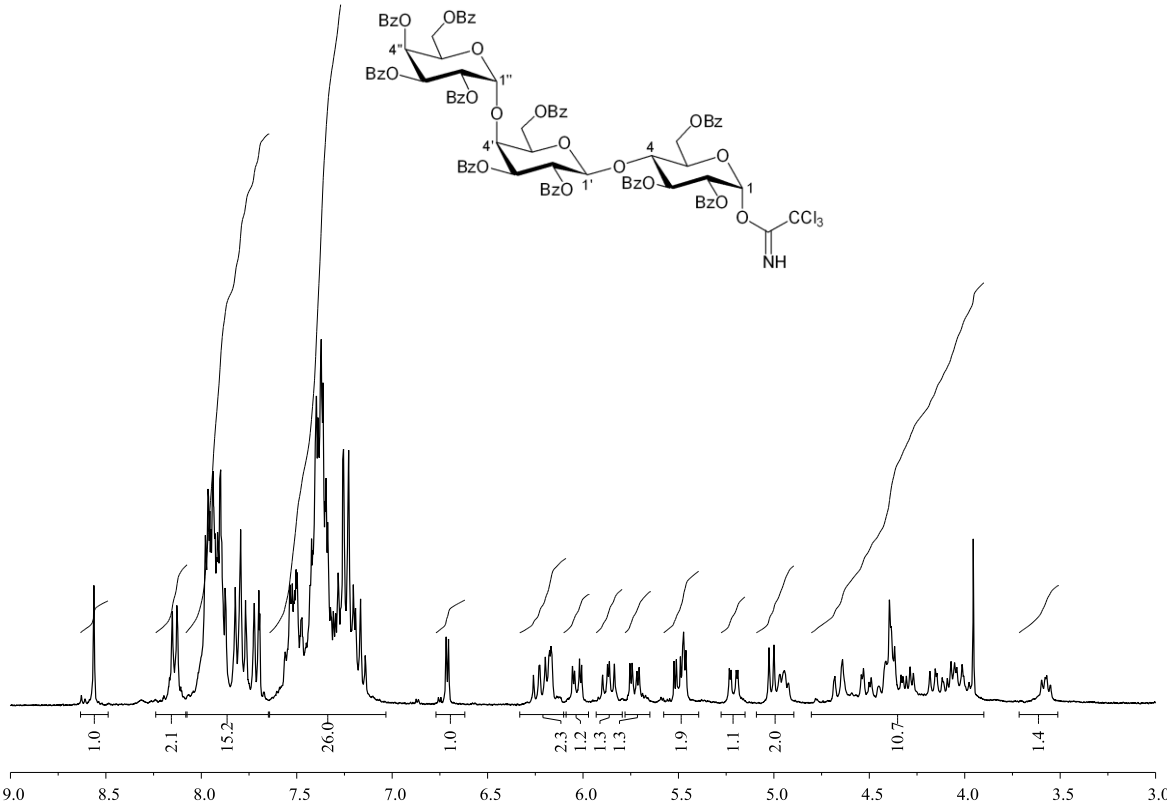
^{1}H-NMR of **16** (600 MHz, $CDCl_3$)



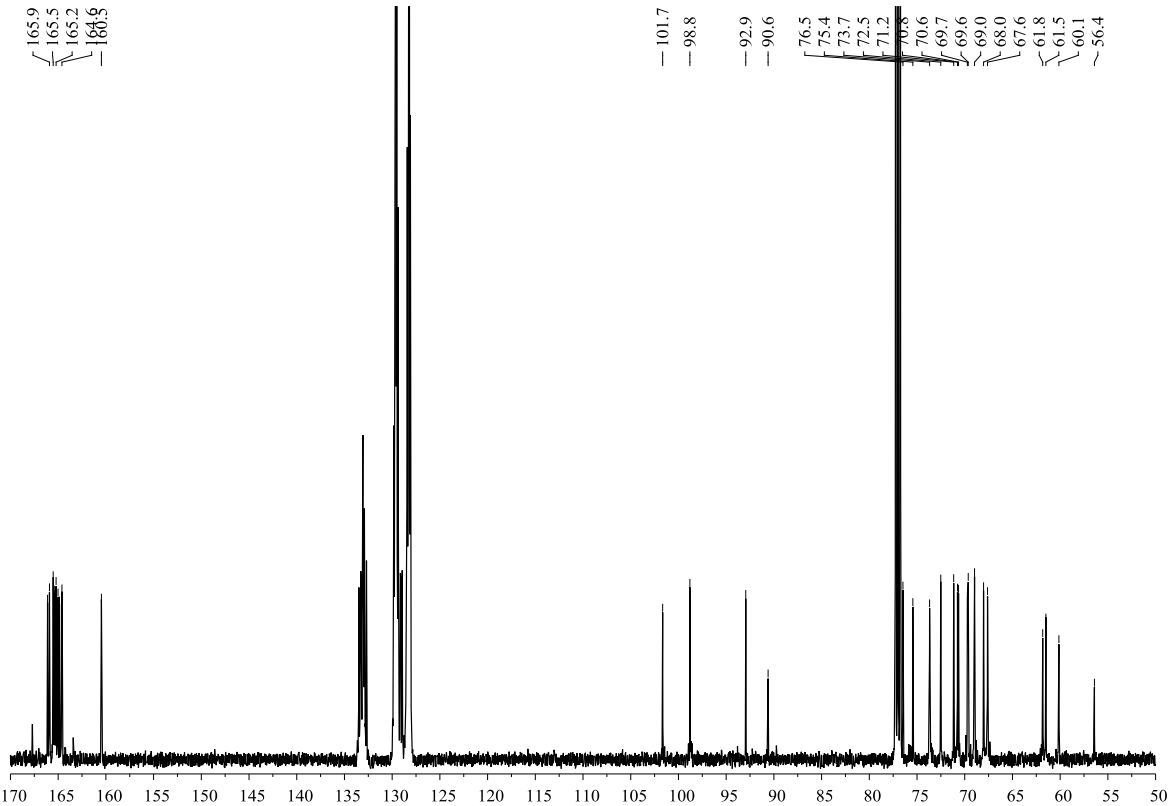
^{13}C-NMR of **16** (126 MHz, $CDCl_3$)



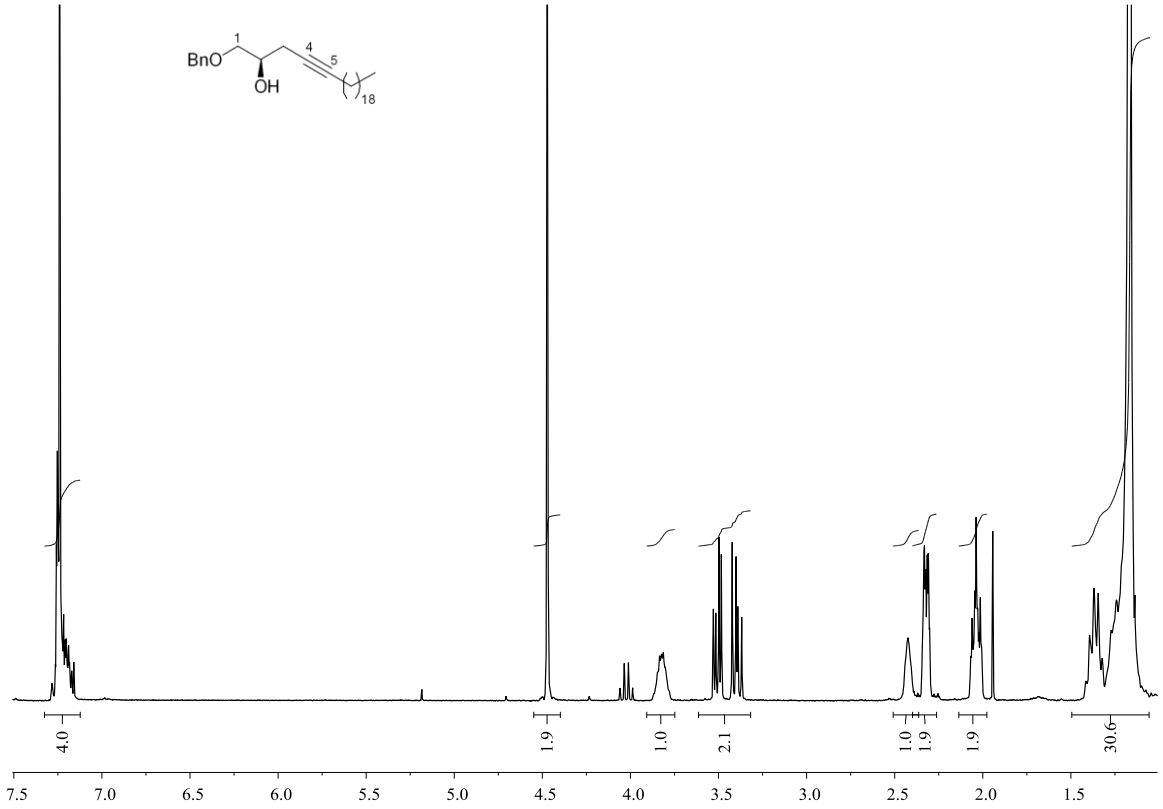
^{1}H-NMR of **17** (300 MHz, $CDCl_3$)



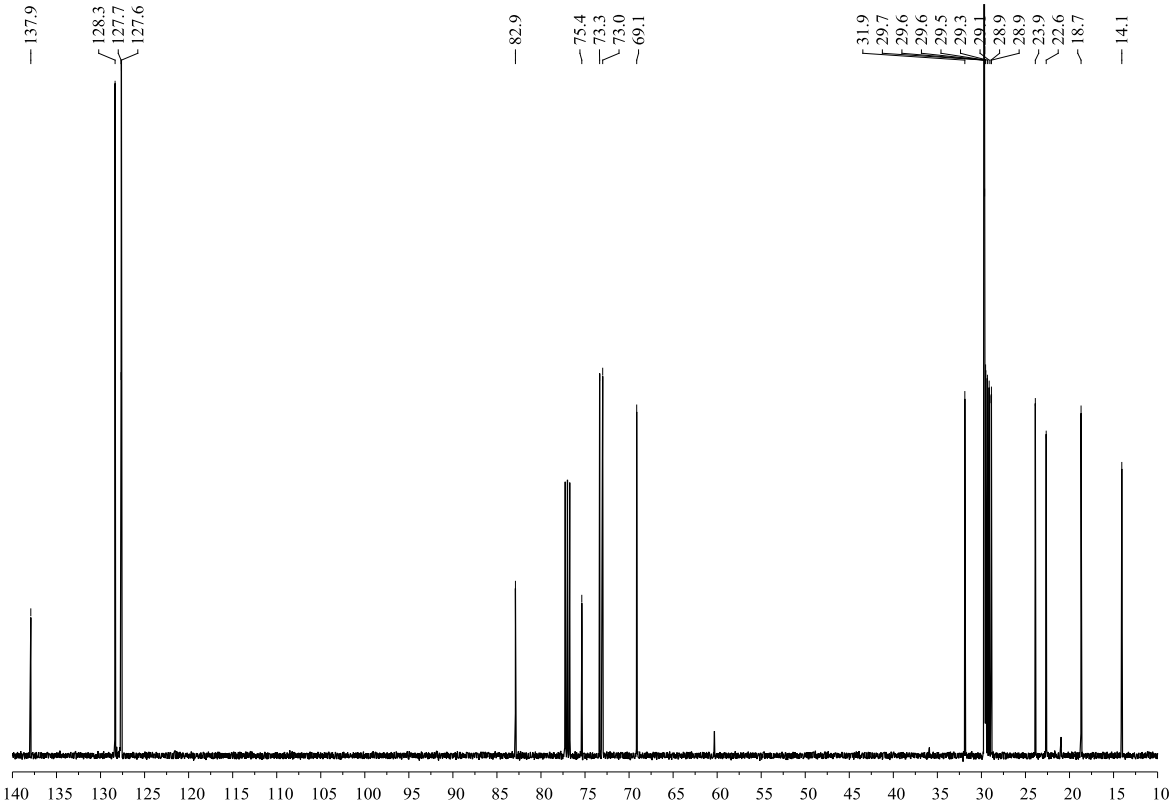
^{13}C-NMR of **17** (126 MHz, $CDCl_3$)



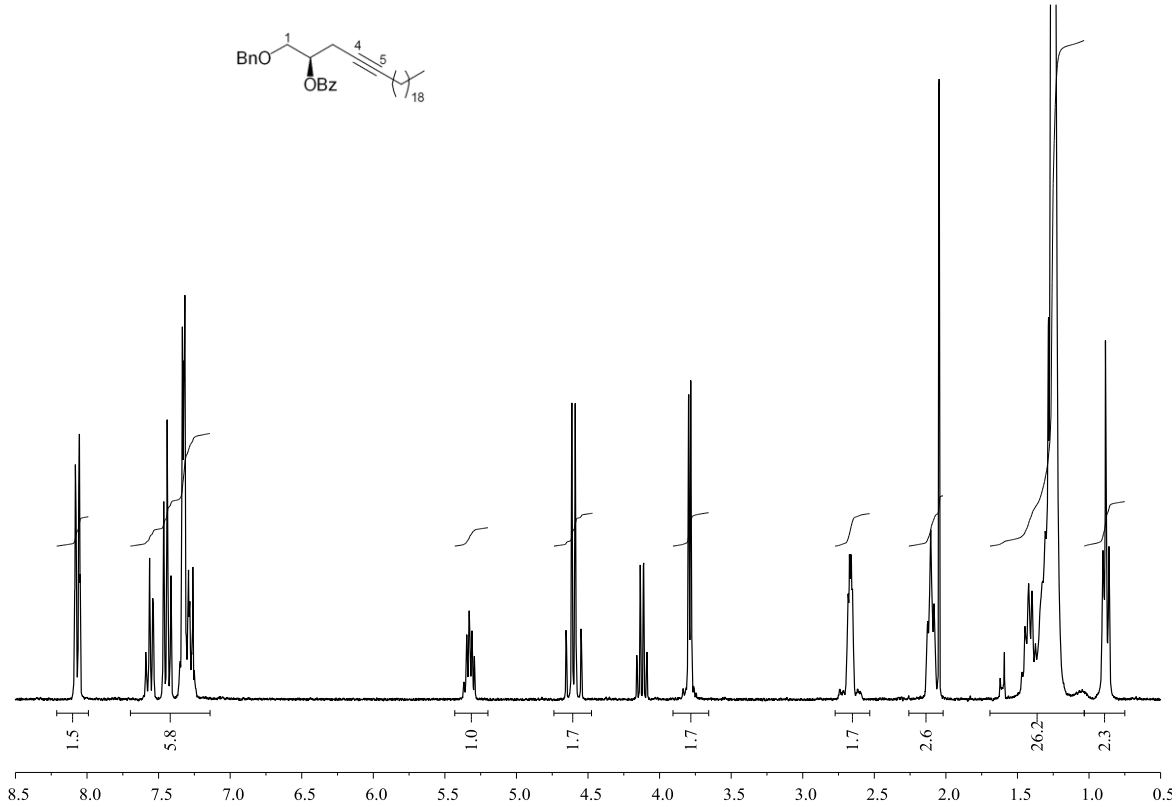
^{1}H-NMR of **20a** (300 MHz, $CDCl_3$)



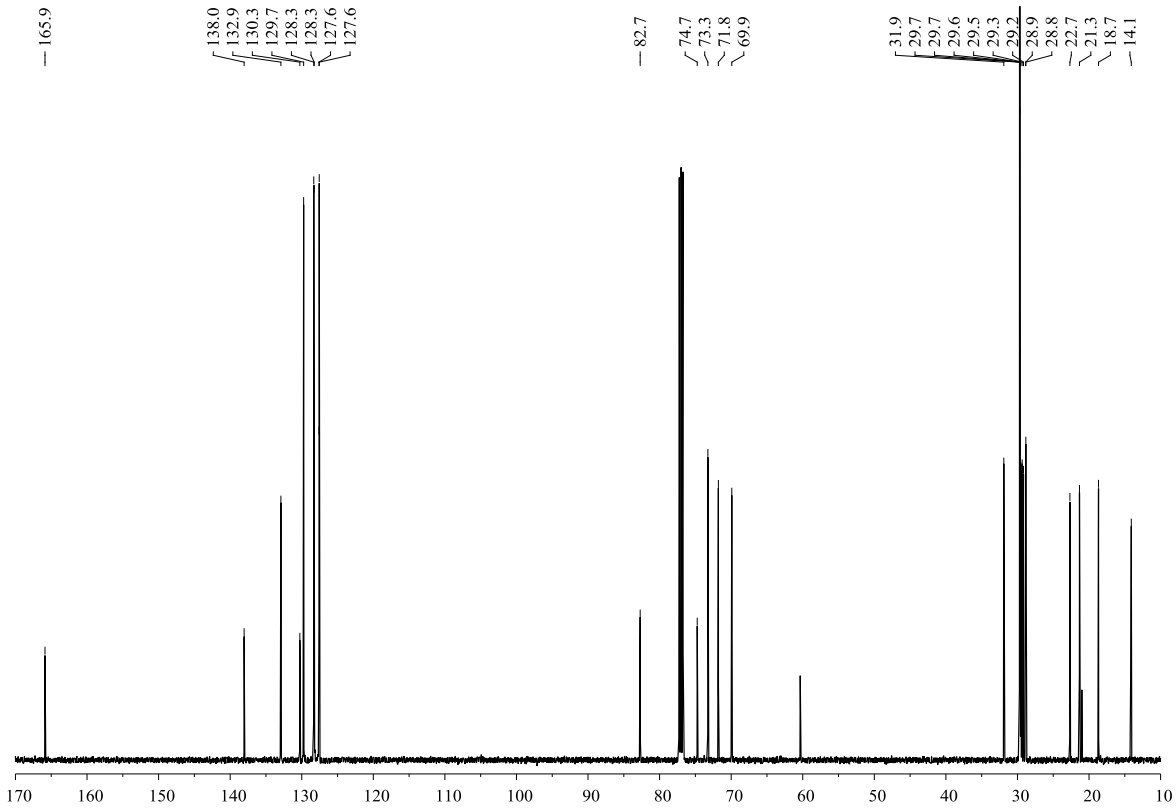
^{13}C-NMR of **20a** (126 MHz, $CDCl_3$)



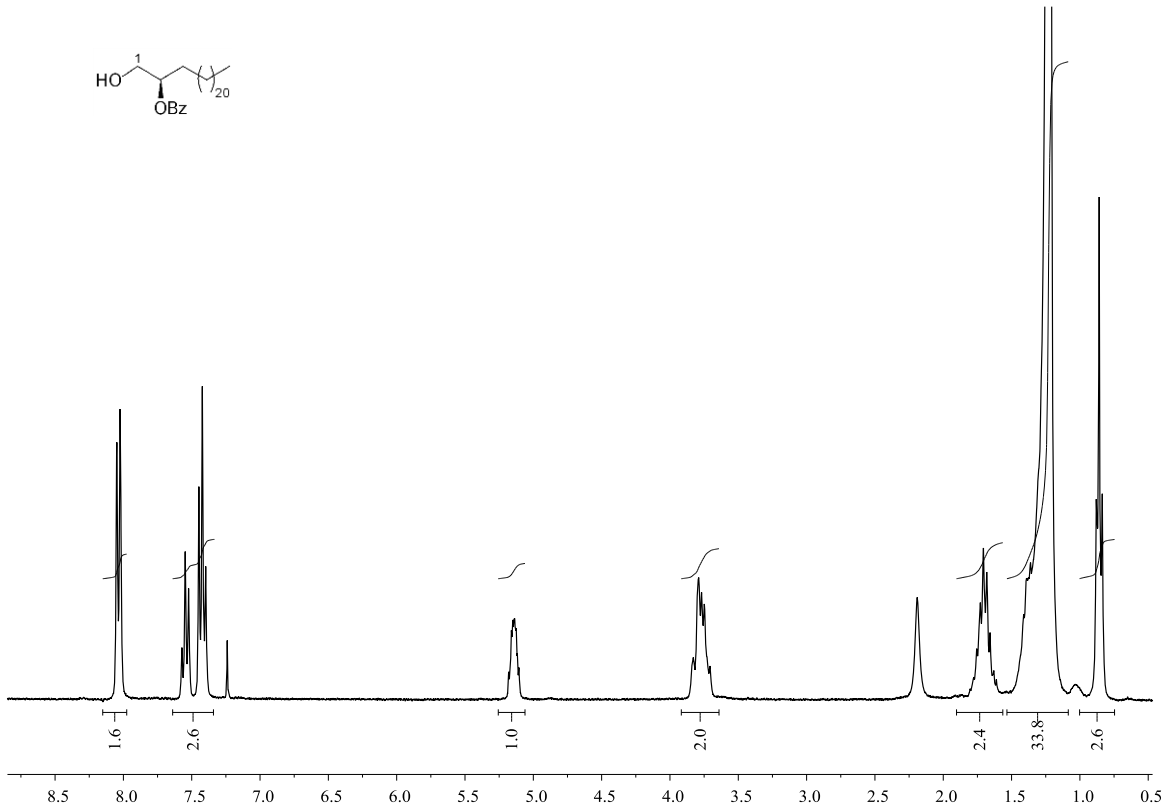
^{1}H-NMR of **20** (300 MHz, $CDCl_3$)



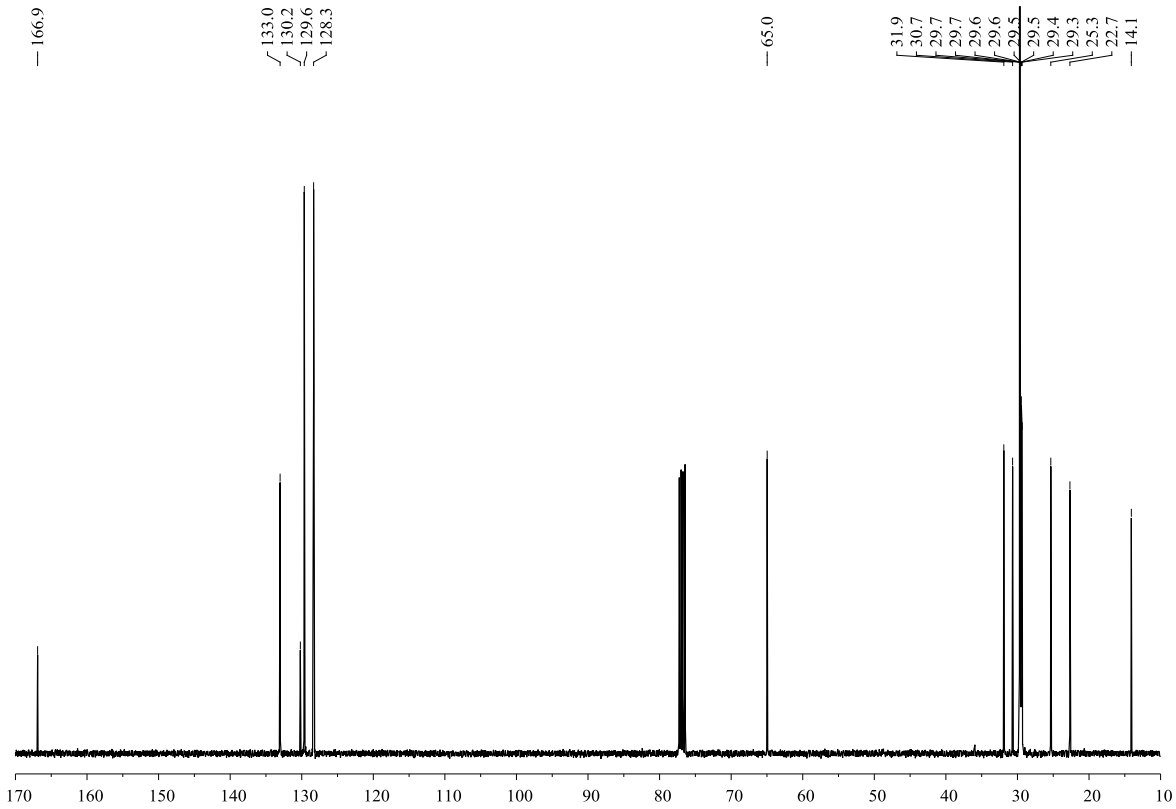
^{13}C-NMR of **20** (126 MHz, $CDCl_3$)



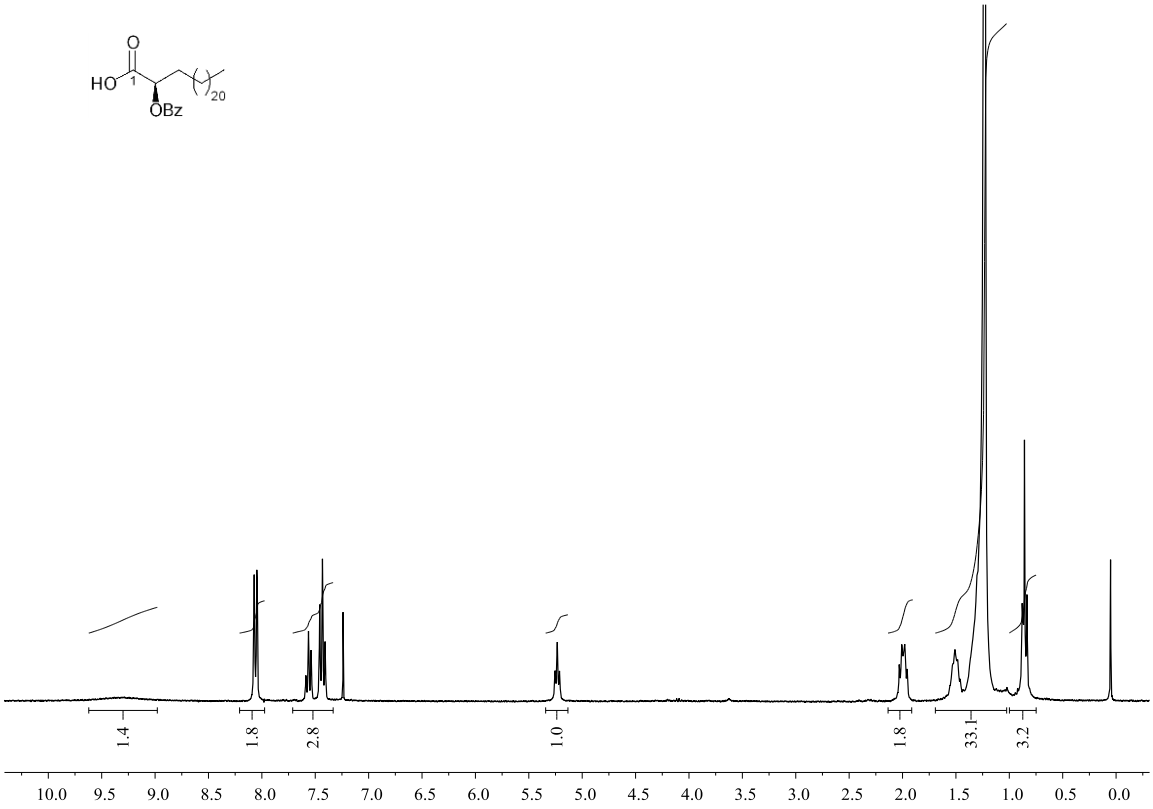
^{1}H-NMR of **21** (300 MHz, $CDCl_3$)



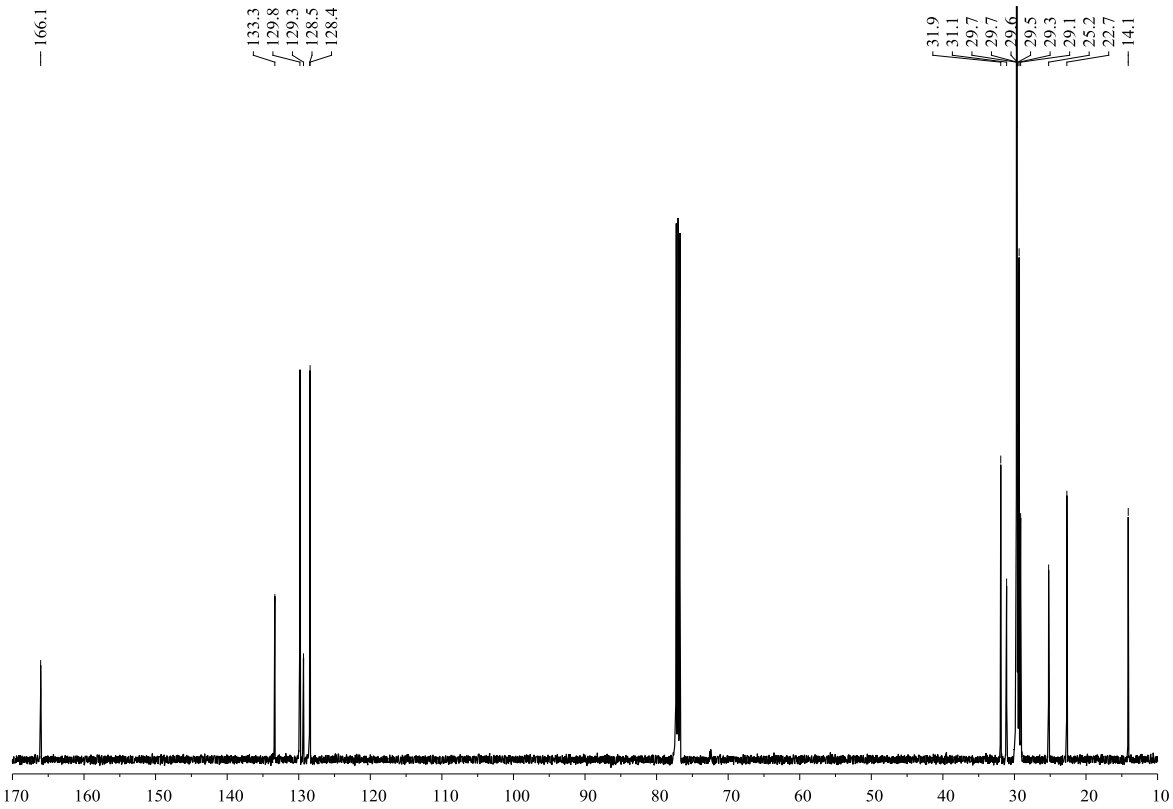
^{13}C-NMR of **21** (126 MHz, $CDCl_3$)



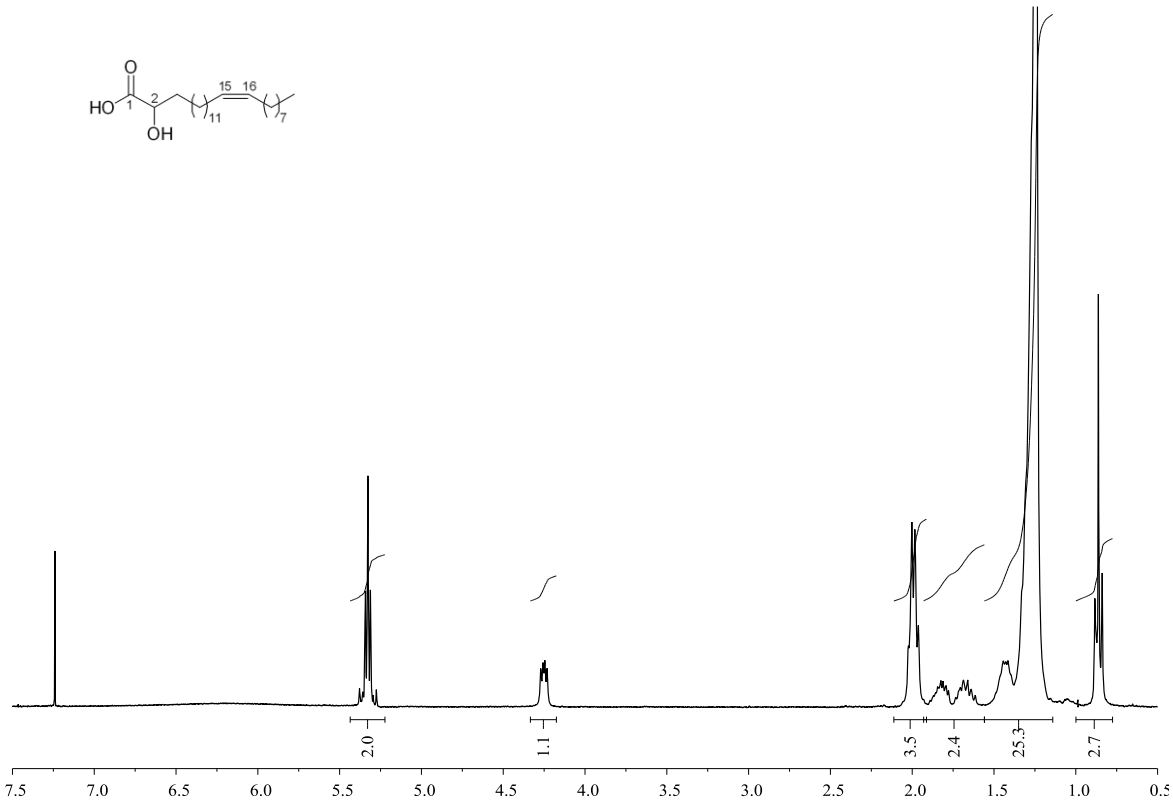
^{1}H-NMR of **22** (300 MHz, $CDCl_3$)



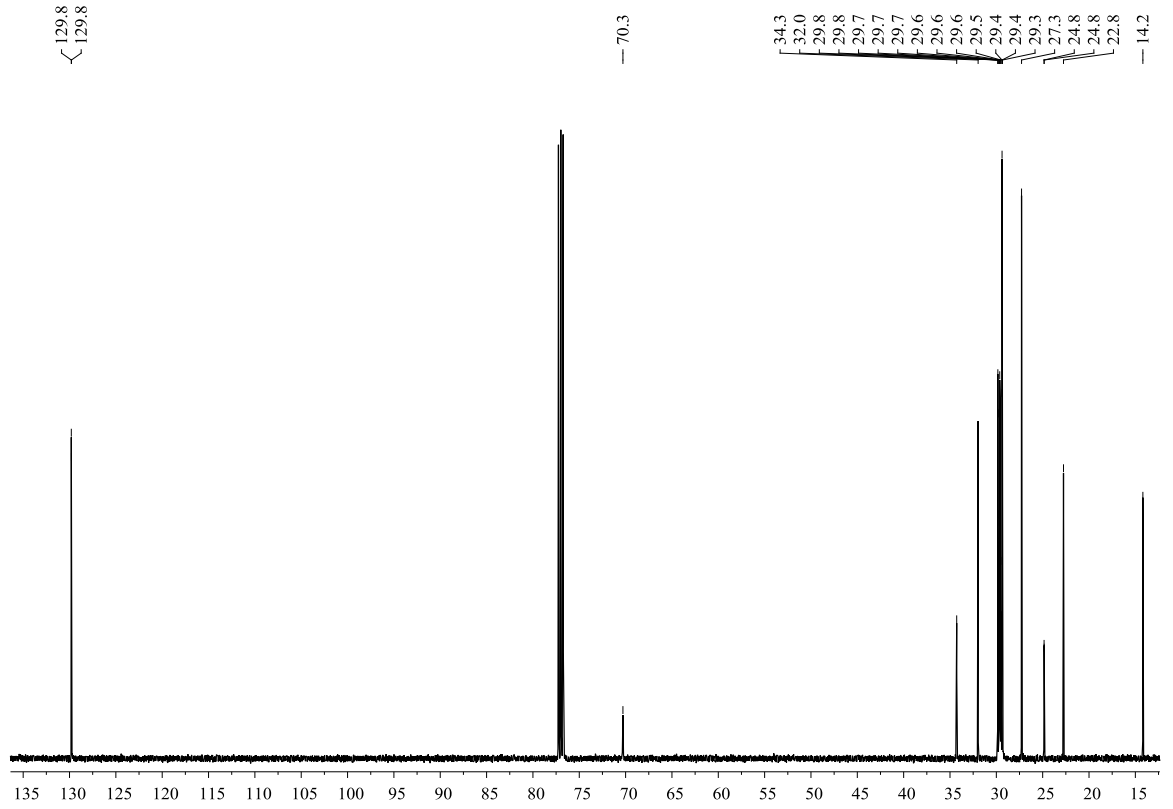
^{13}C-NMR of **22** (126 MHz, $CDCl_3$)



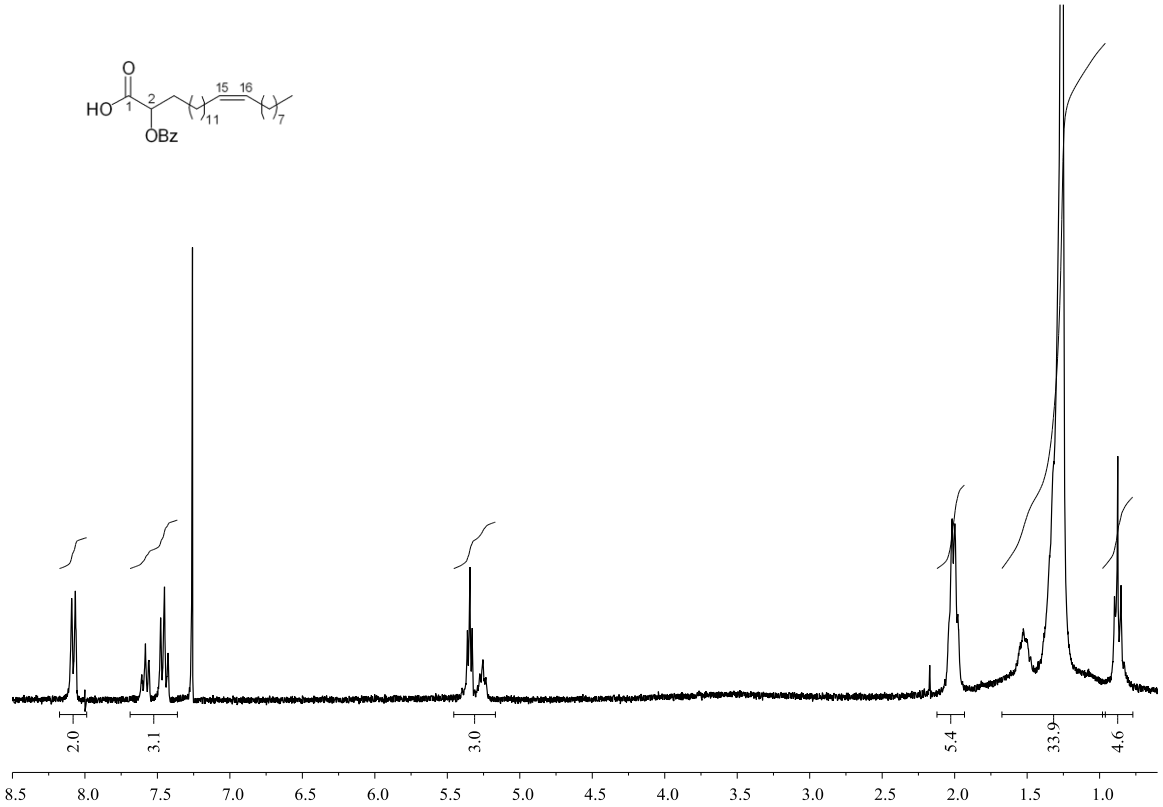
^{1}H-NMR of **23** (300 MHz, $CDCl_3$)



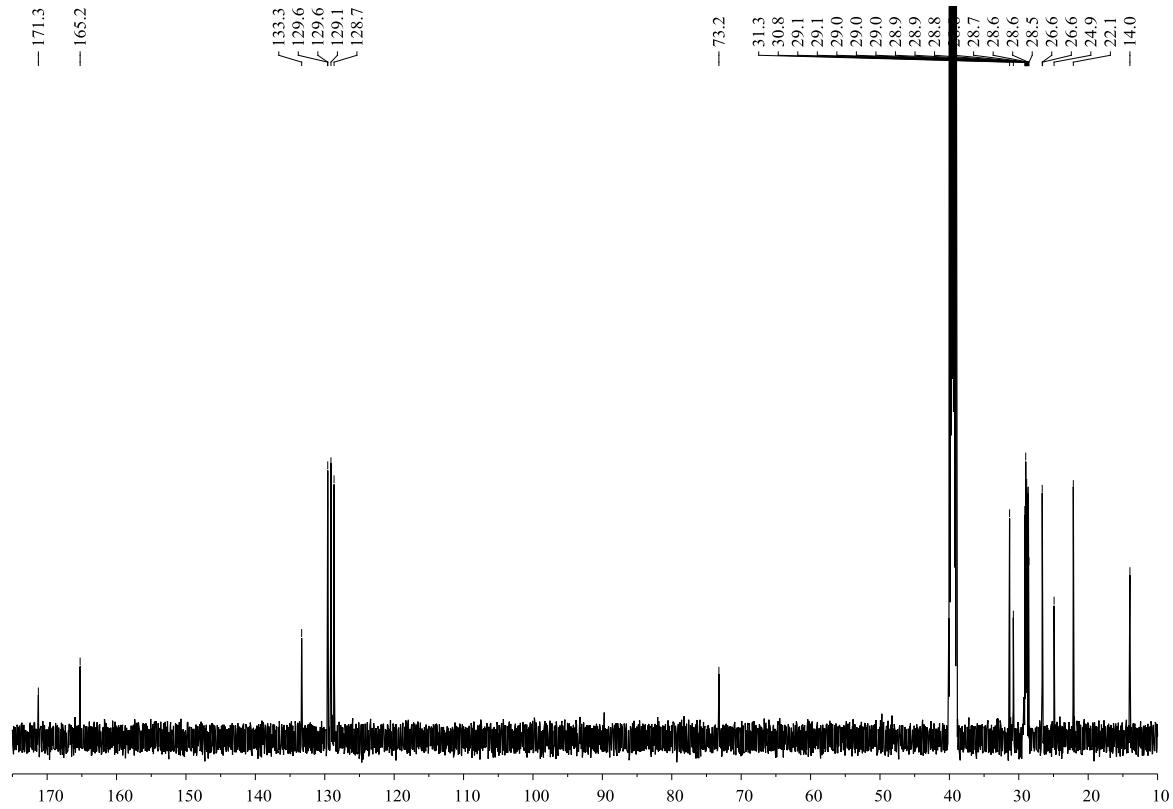
^{13}C-NMR of **23** (126 MHz, $CDCl_3$)



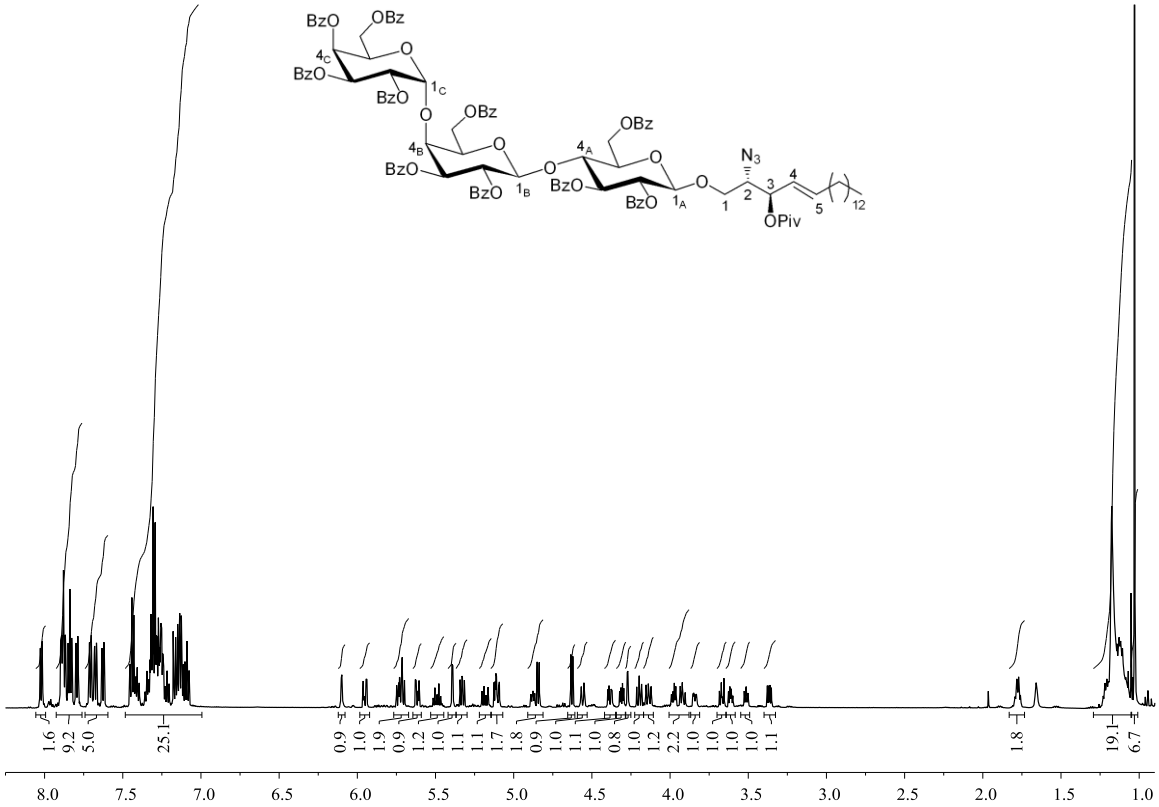
^{1}H-NMR of **24** (300 MHz, $CDCl_3$)



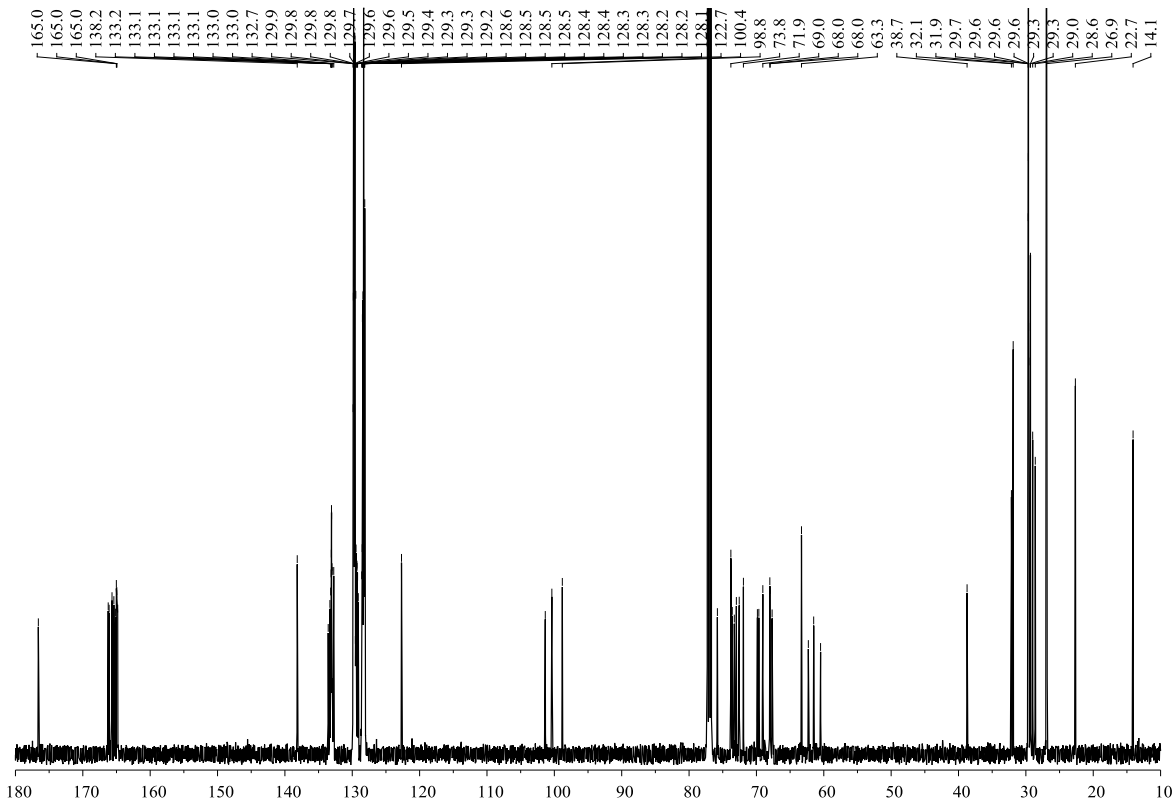
^{13}C-NMR of **24** (126 MHz, DMSO-d_6)



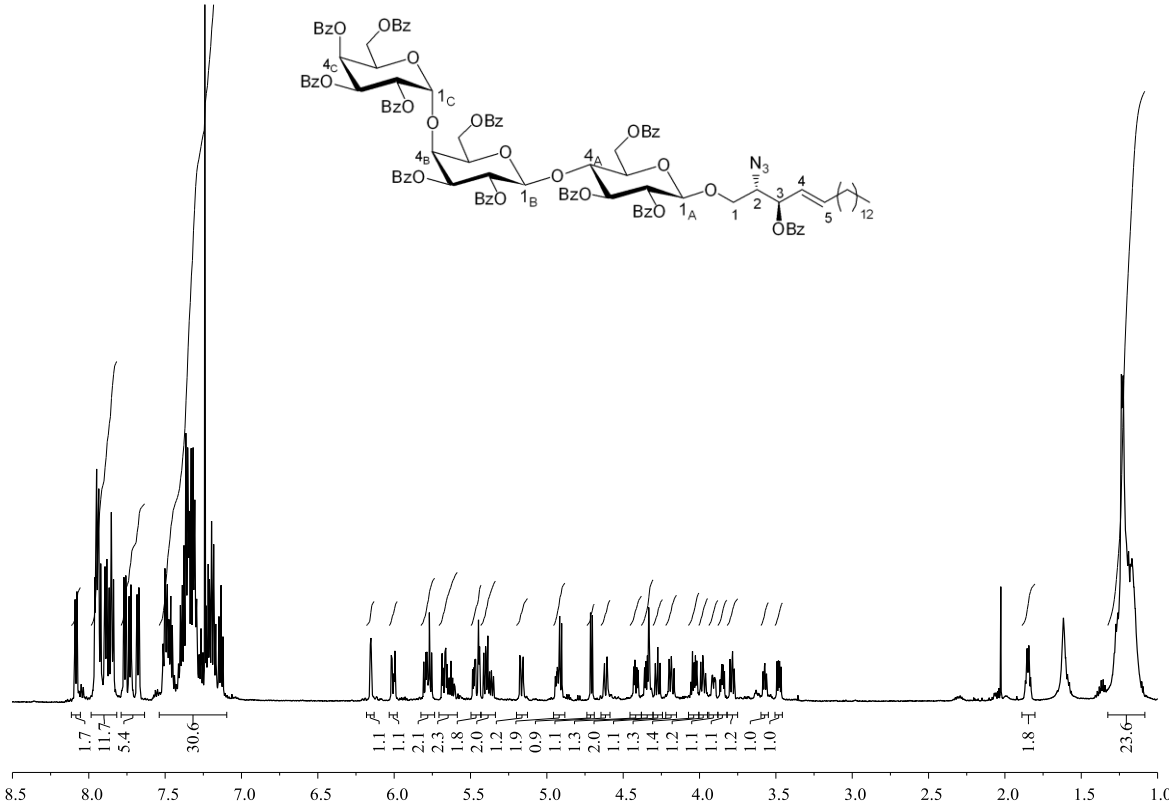
^{1}H-NMR of **25** (600 MHz, $CDCl_3$)



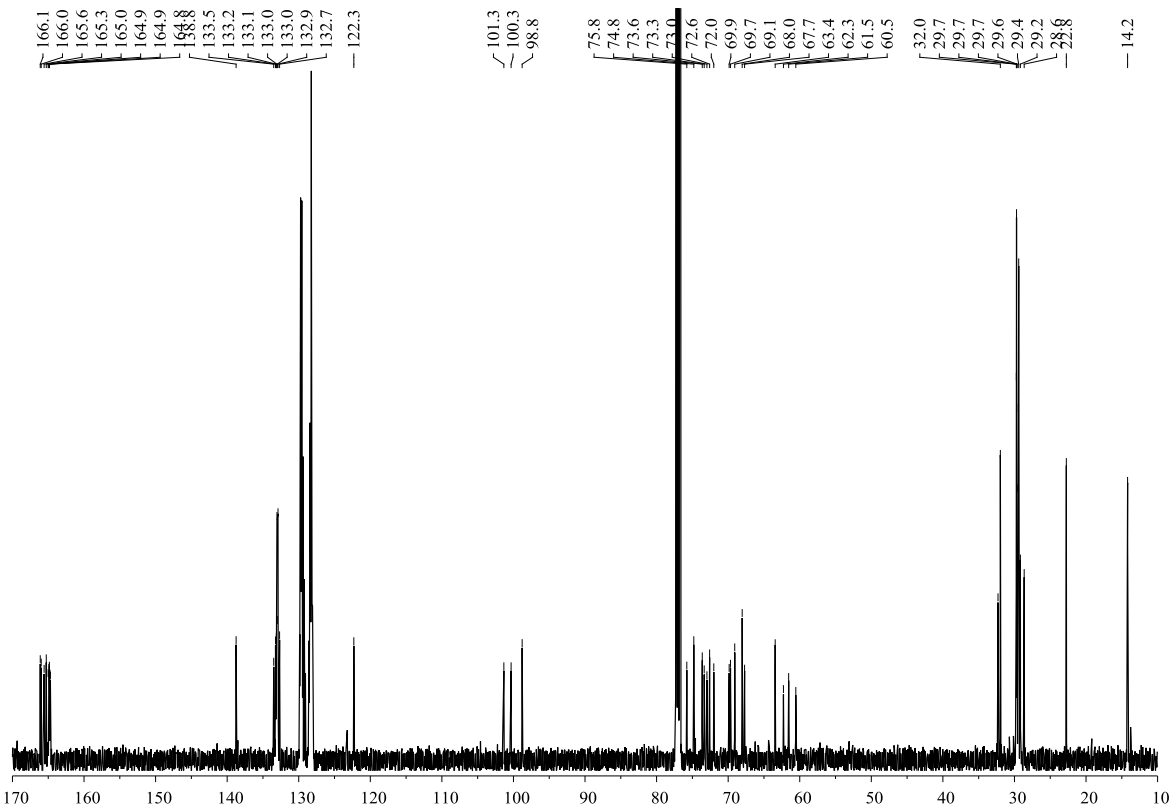
^{13}C-NMR of **25** (126 MHz, $CDCl_3$)



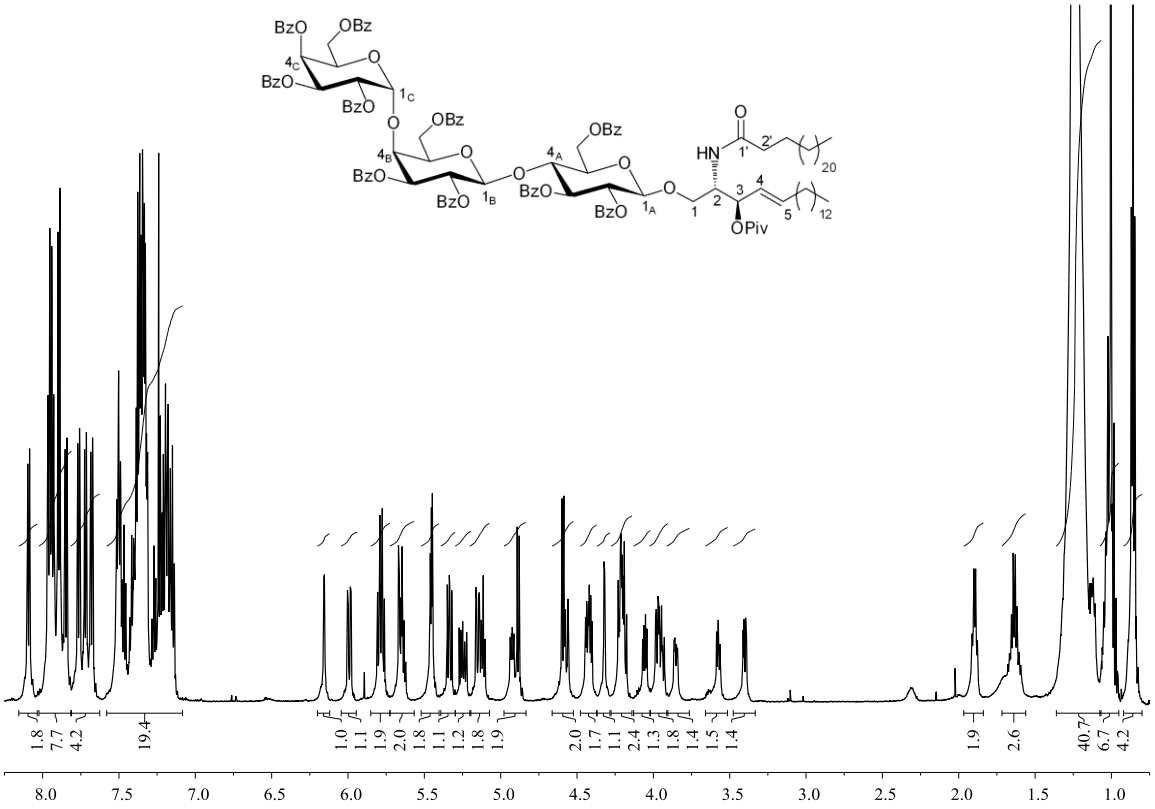
^{1}H-NMR of **26** (600 MHz, $CDCl_3$)



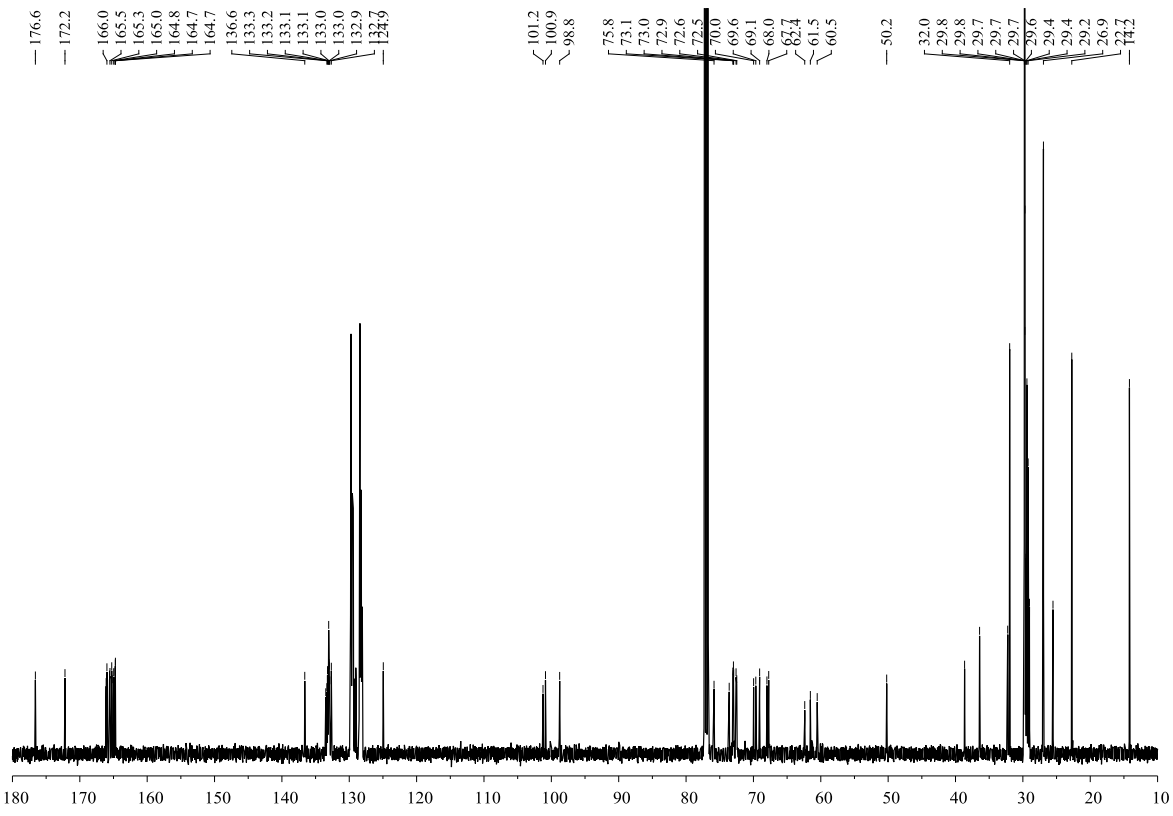
^{13}C-NMR of **26** (126 MHz, $CDCl_3$)



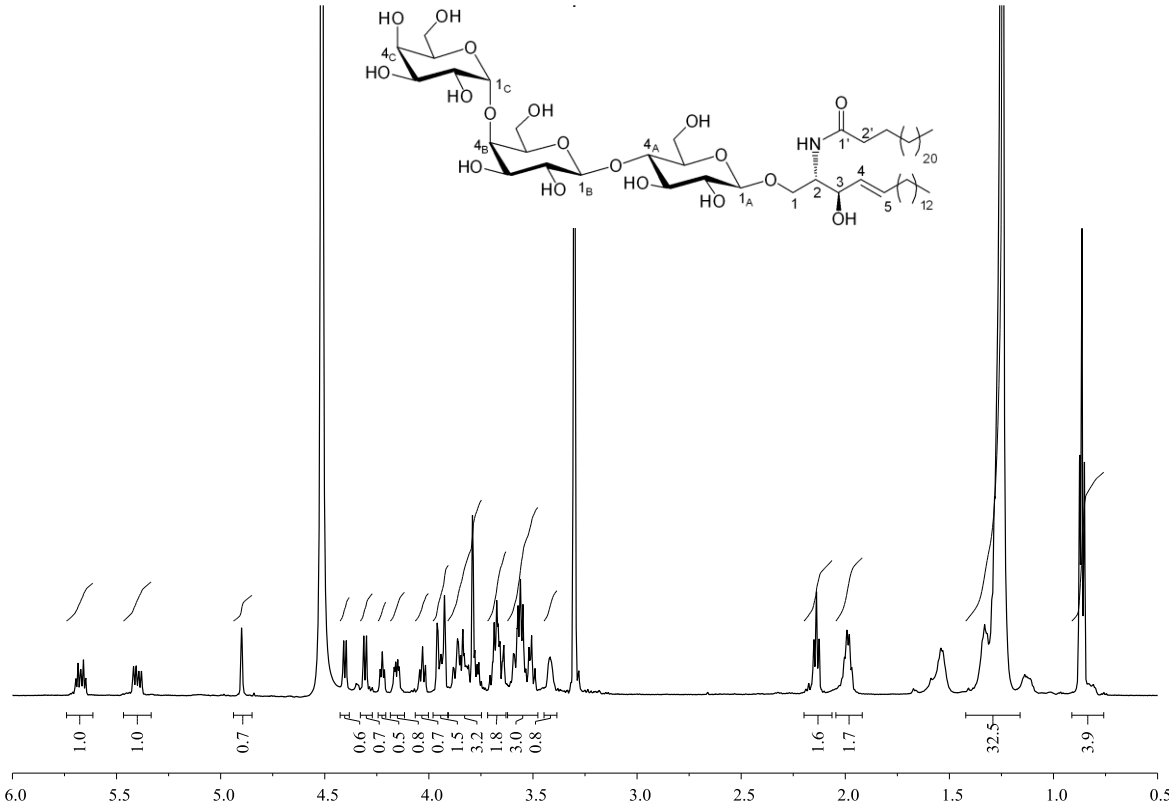
^{1}H-NMR of **27a** (600 MHz, $CDCl_3$)



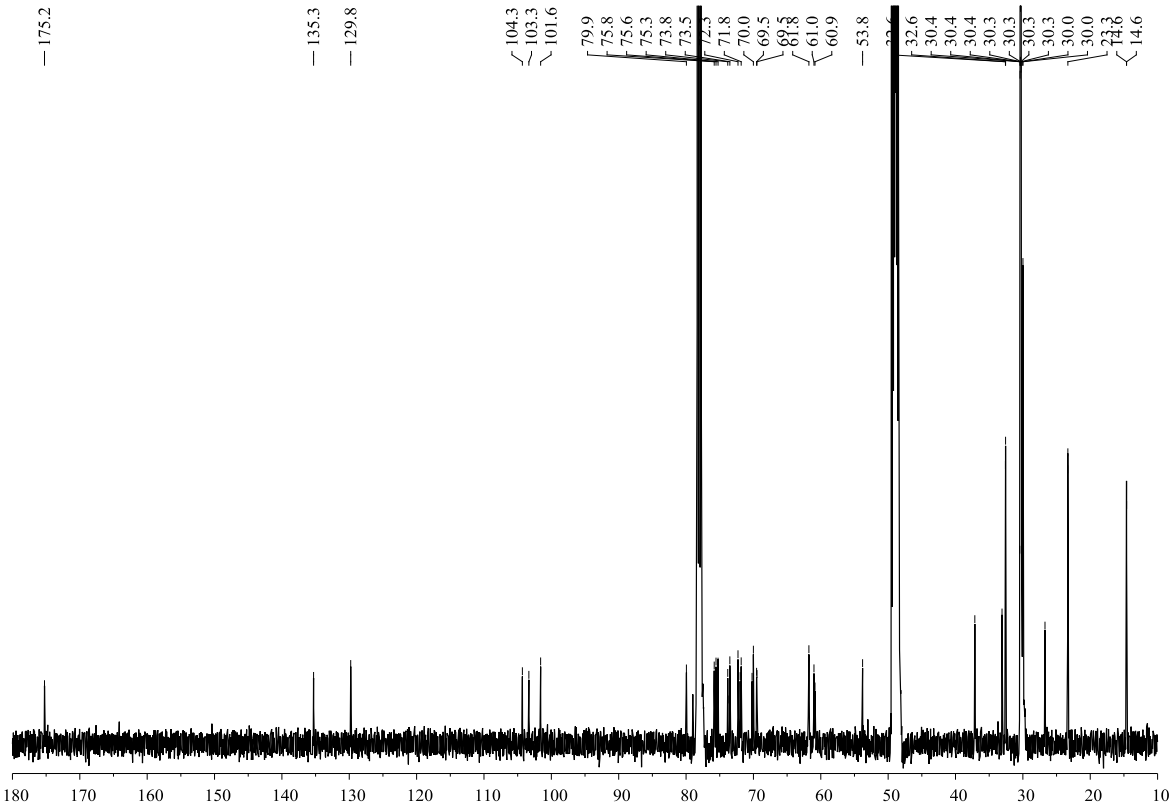
^{13}C-NMR of **27a** (126 MHz, $CDCl_3$)



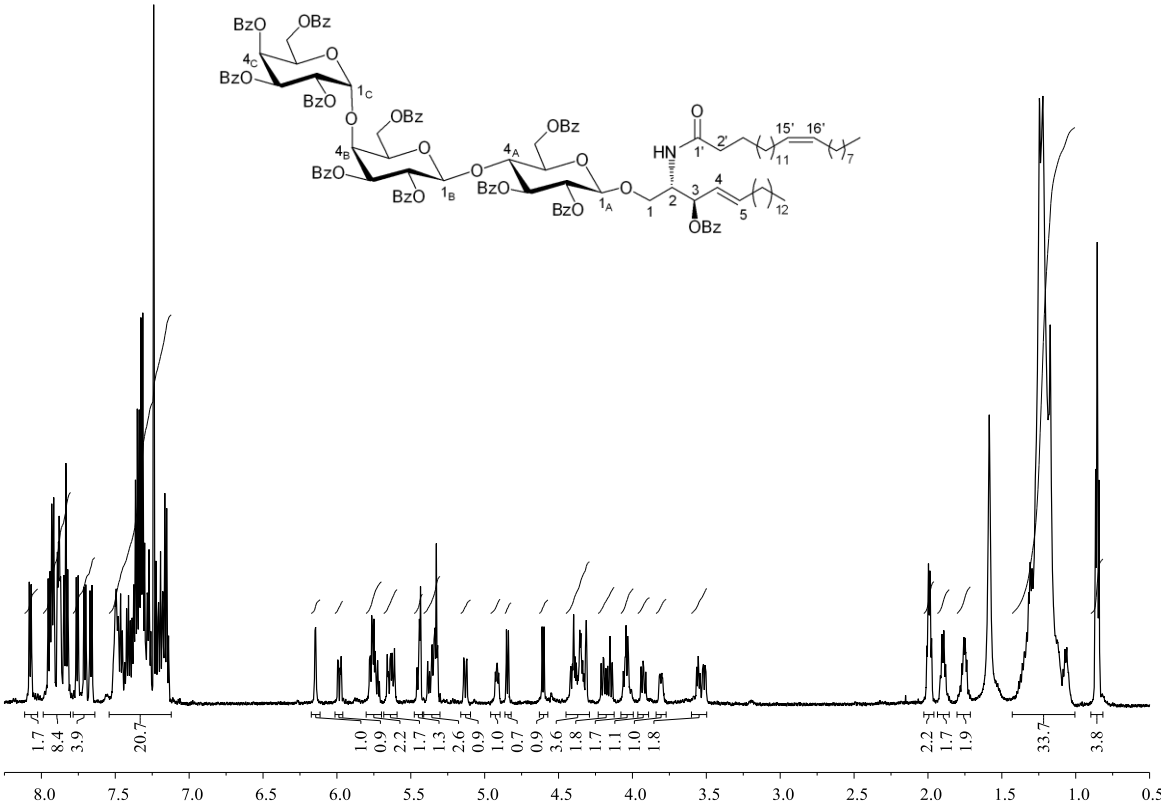
^{1}H-NMR of **27** (600 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2)



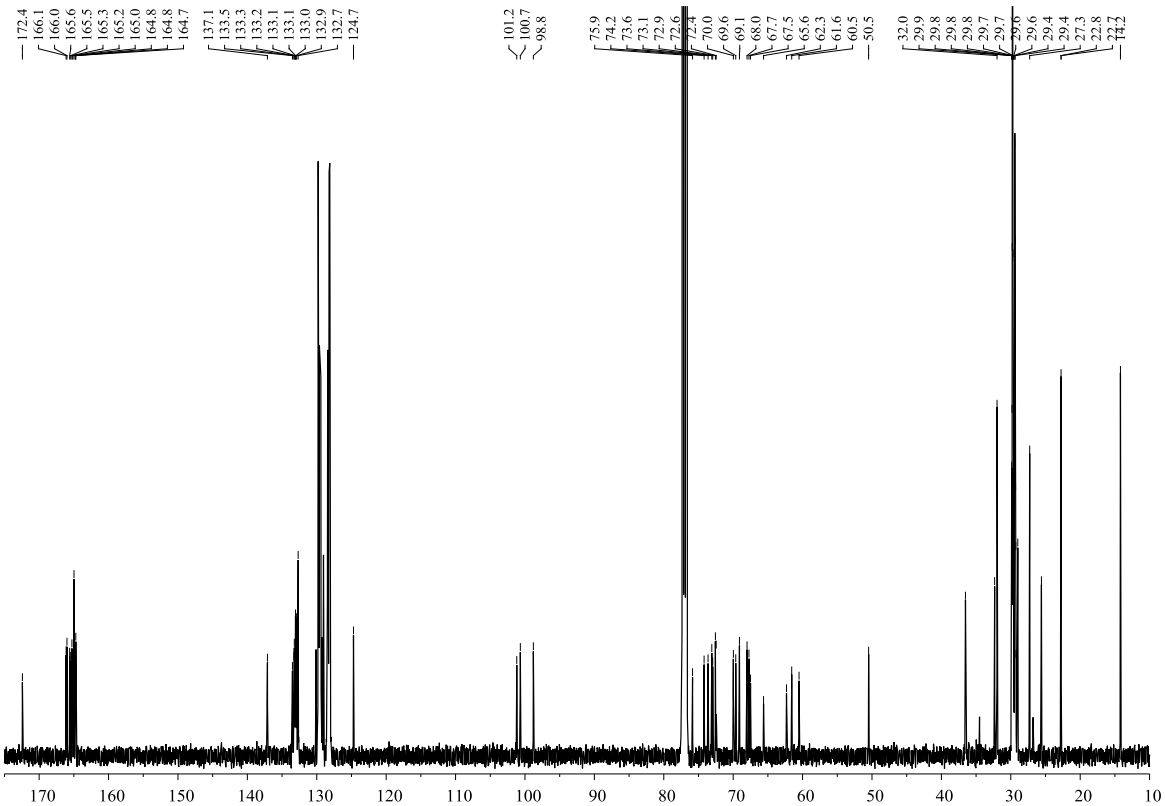
^{13}C-NMR of **27** (126 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2)



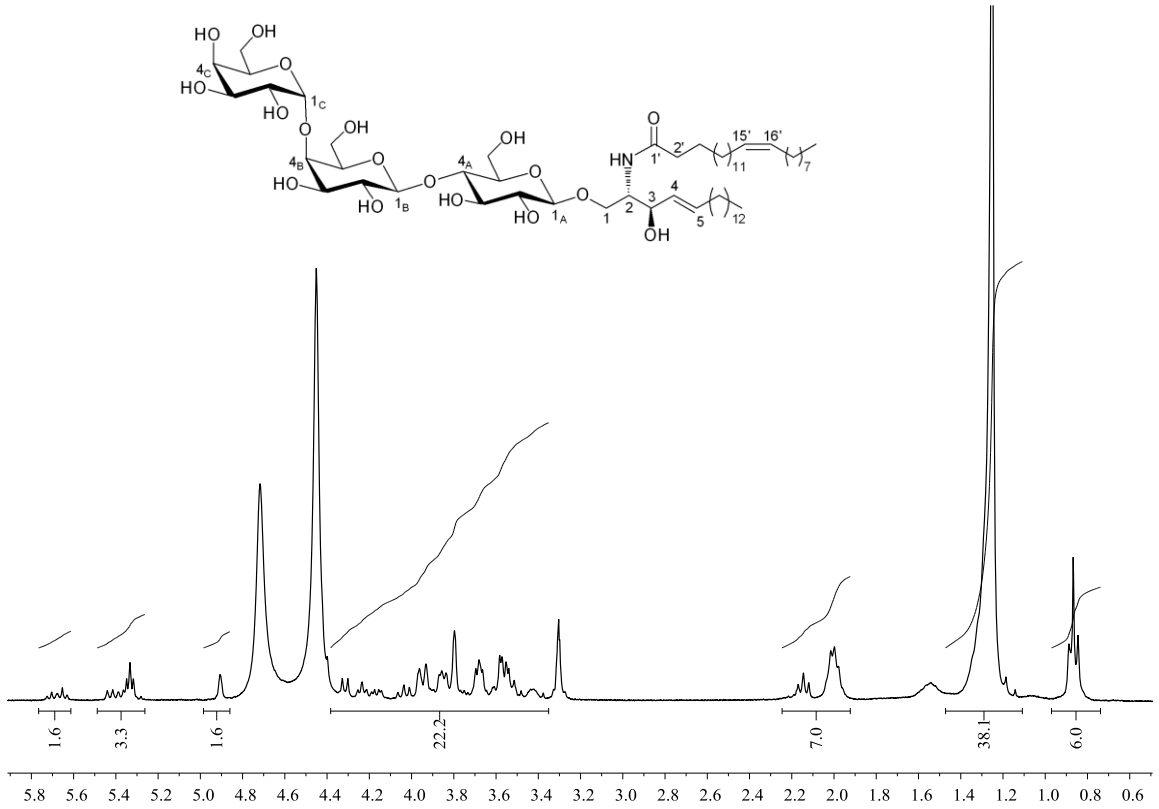
^{1}H-NMR of **28a** (600 MHz, $CDCl_3$)



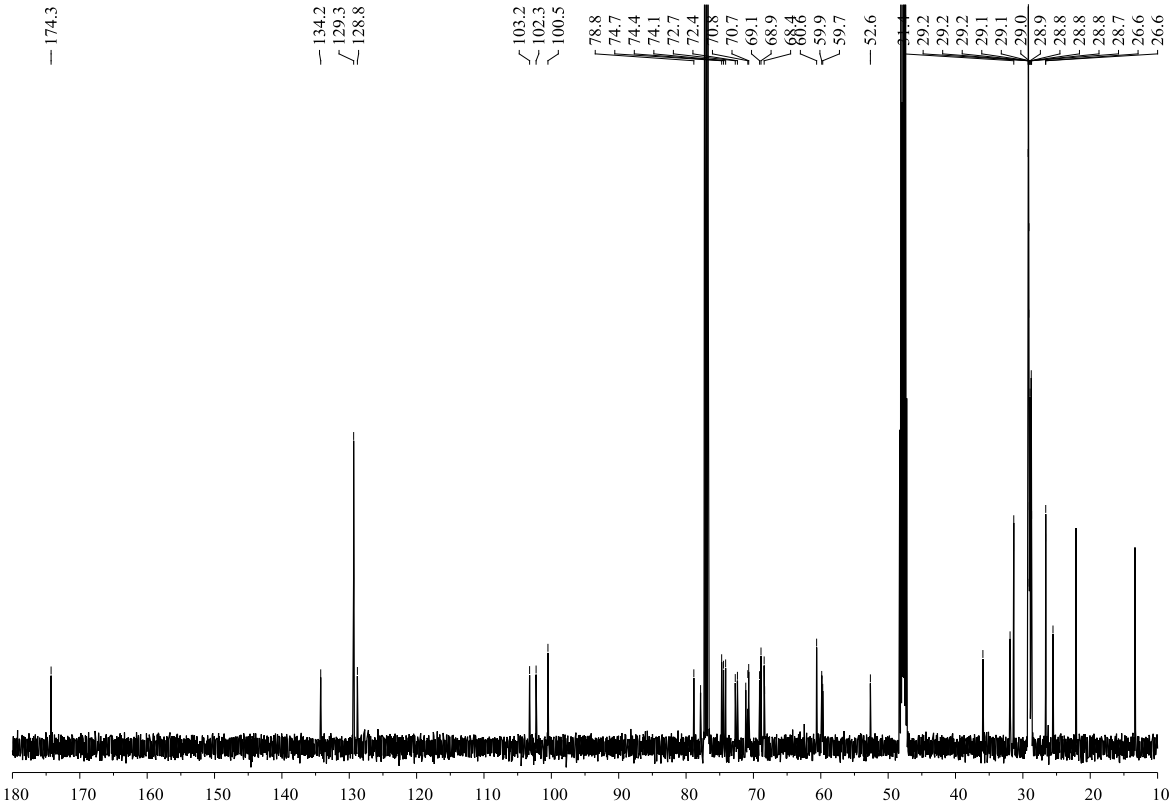
^{13}C-NMR of **28a** (126 MHz, $CDCl_3$)



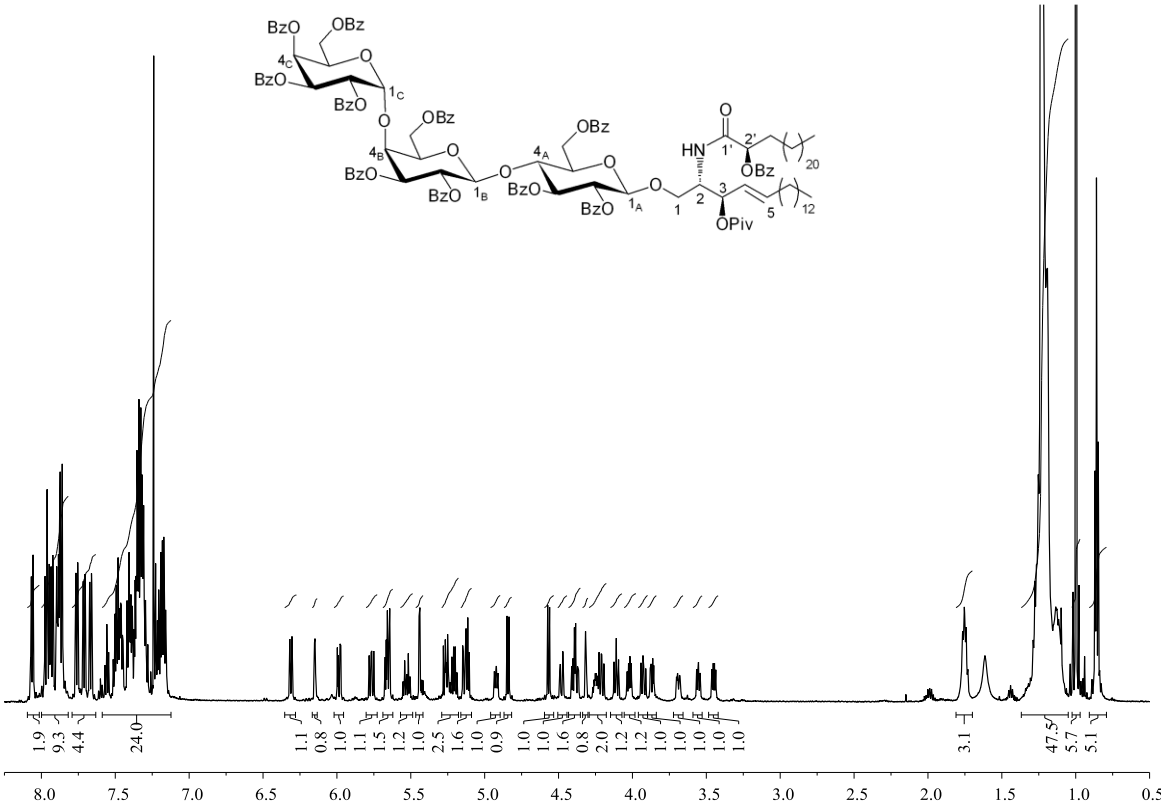
^{1}H-NMR of **28** (300 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2)



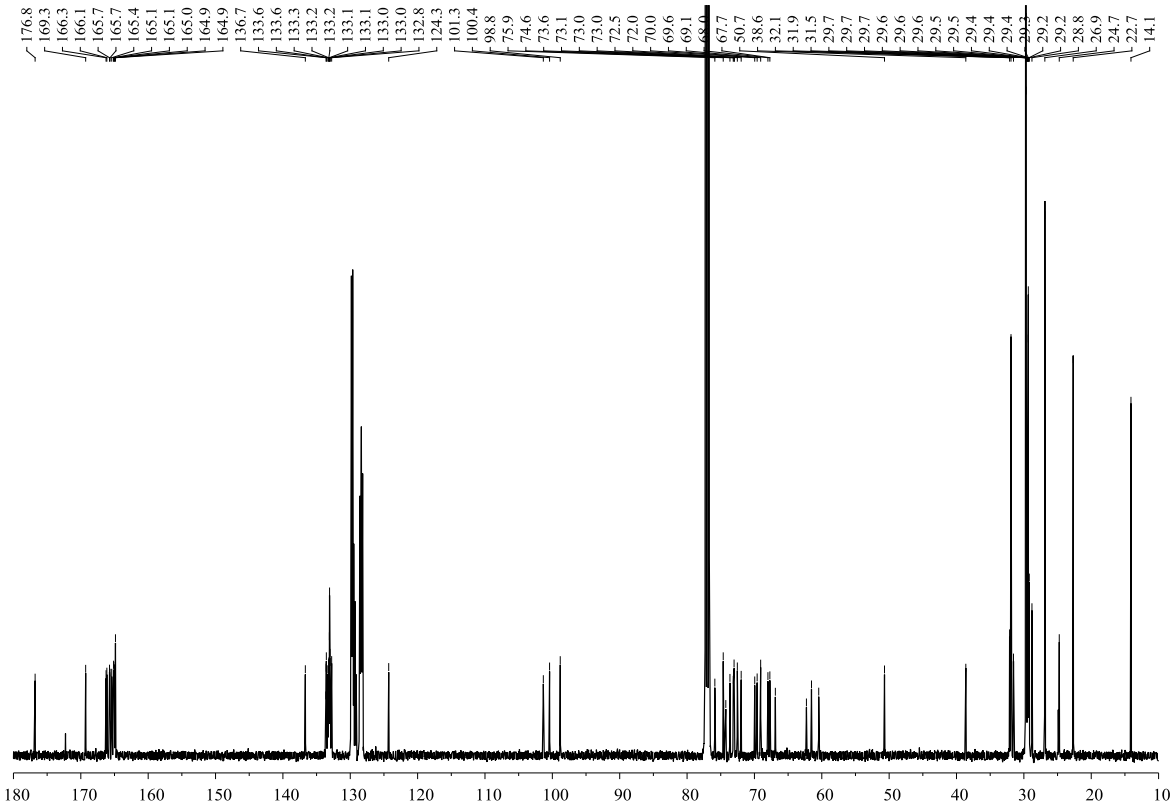
^{13}C-NMR of **28** (126 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2)



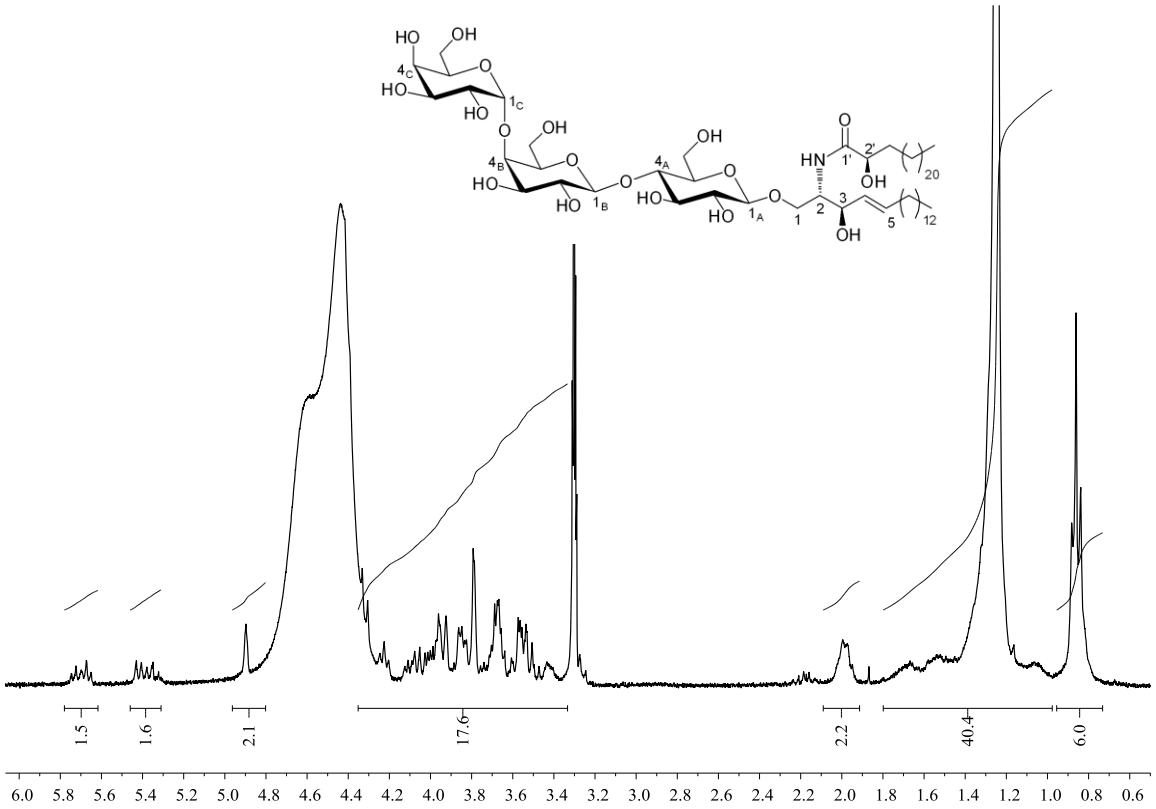
^{1}H-NMR of **29a** (600 MHz, $CDCl_3$)



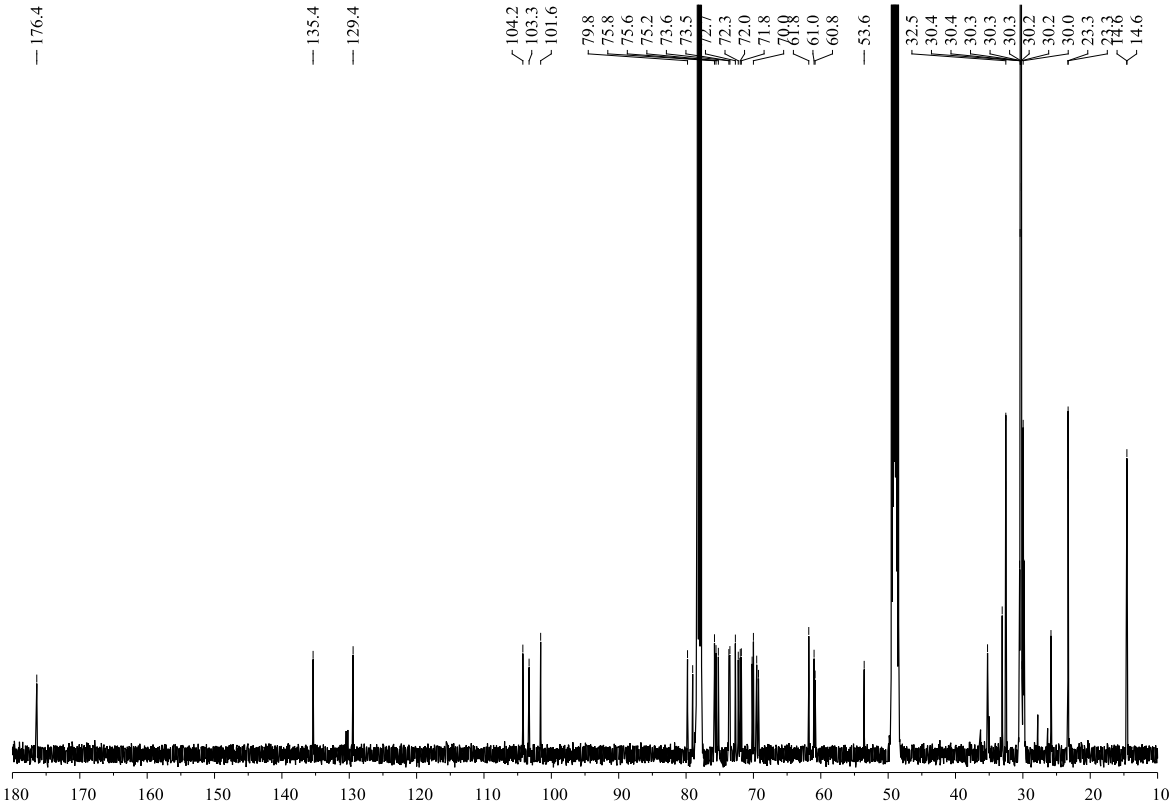
^{13}C-NMR of **29a** (126 MHz, $CDCl_3$)



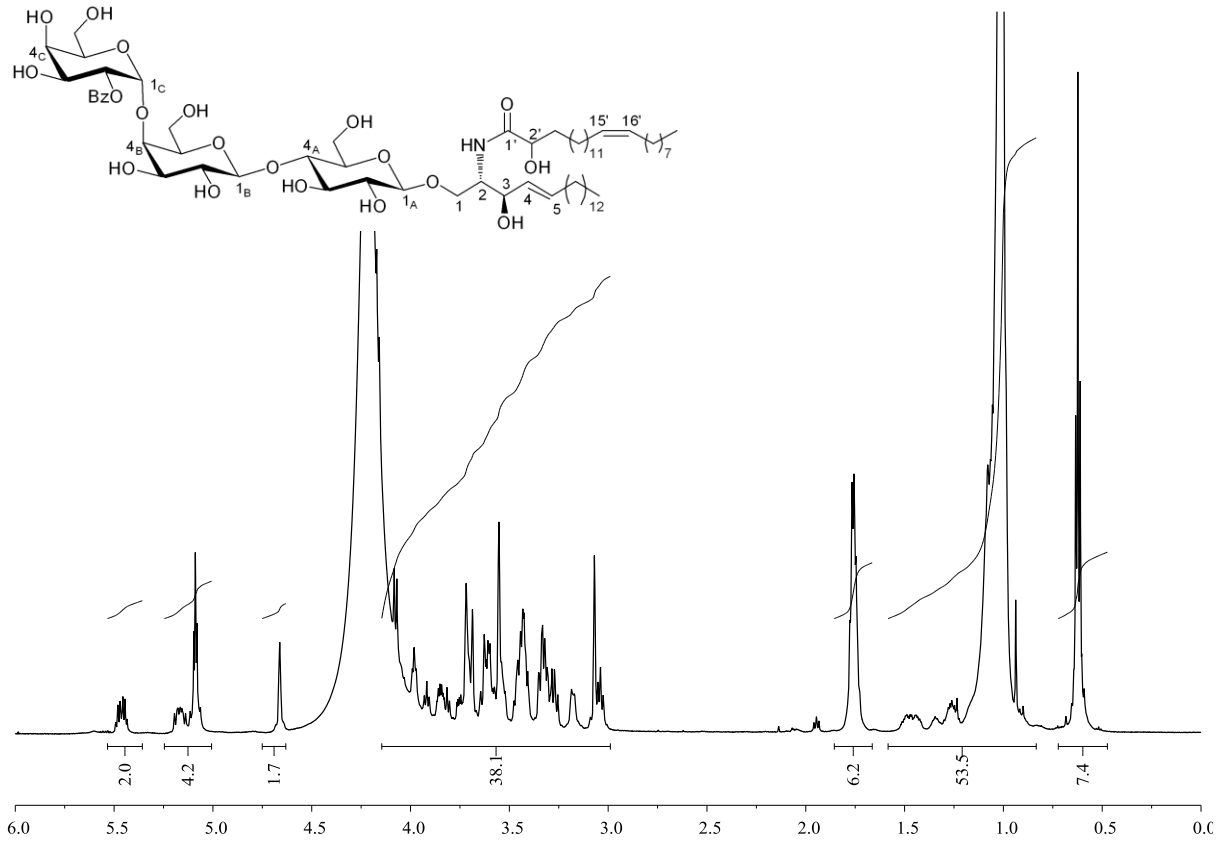
^{1}H-NMR of **29** (300 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2)



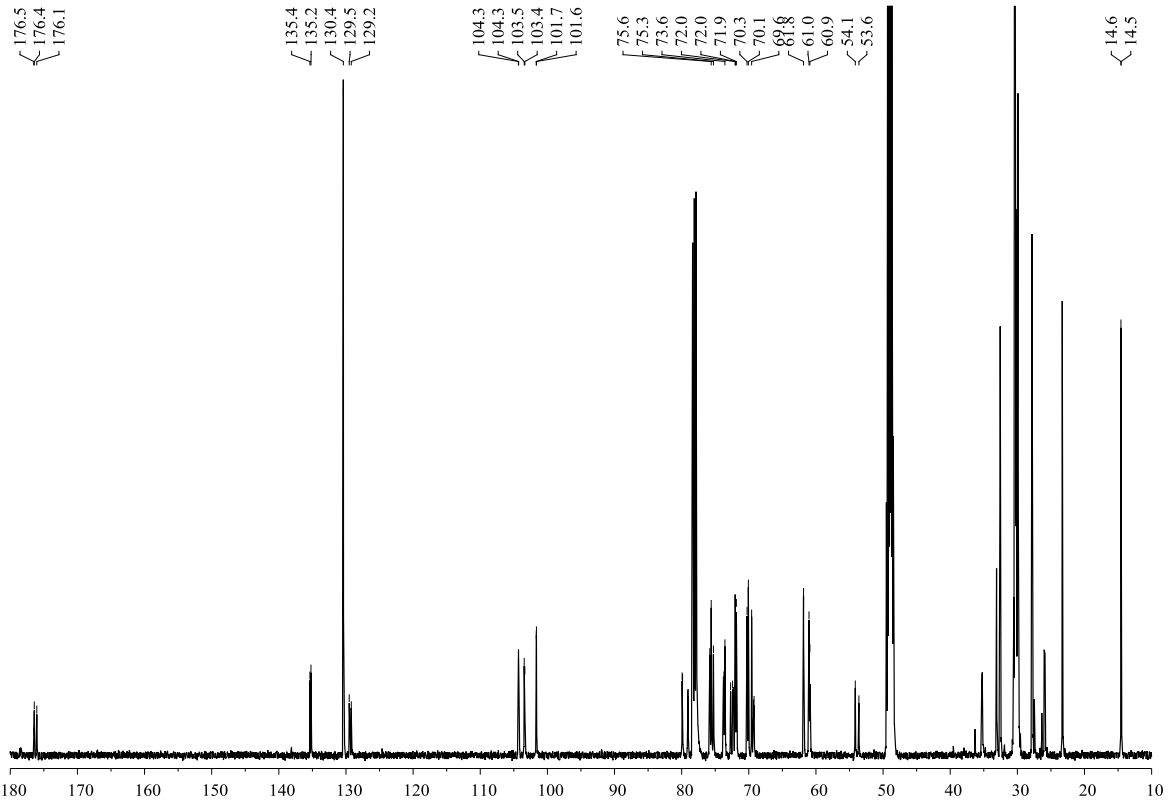
^{13}C-NMR of **29** (126 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2)



^{1}H-NMR of **30** (600 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2)



^{13}C-NMR of **30** (126 MHz, $CDCl_3/CD_3OD/D_2O$, 1.6:1.0:0.2)



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