Electronic Supplementary Material (ESI) for RSC Advances.

The NKT cell TCR repertoire can accommodate structural modifications to the lipid and orientation of the terminal carbohydrate of iGb3

SUPPORTING INFORMATION

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Contents

Biological analysis

Mice

C57BL/6 mice were bred in-house at the Department of Microbiology and Immunology Animal House. C57BL/6 *Tcra-J*^{tm1Tg} (referred to herein as Jα18$^{-/-}$) mice were bred in-house (originally provided by M. Taniguchi; backcrossed 10 times). All procedures were approved by the University of Melbourne Animal Ethics Committee and all methods were carried out in accordance with the relevant guidelines and regulations.

Glycolipids

α-GalCer (KRN7000) and iGb3 (C26) were purchased from Enzo Life Sciences. α-GalCer (C24:1)/PBS-44 was kindly provided by Prof. Paul Savage, Brigham Young University. iGb3 analogues were produced in-house at Victoria University of Wellington according to the methods in the current and previous publications.[1] For *in vitro* cultures, glycolipid stocks were prepared using Tween 20-based [0.5% (v/v) Tween 20, sucrose (56 mg/ml), L-histidine (7.5 mg/ml) in PBS] vehicle reagent. For CD1d-tetramer production α-GalCer (PBS-44) was prepared in Tyloxapol-based vehicle (0.5% Tyloxapol in TRIS-buffered saline) reagent.

Flow cytometry and antibodies

Antibody cocktails included α-GalCer (PBS-44)-loaded CD1d-tetramer (Brilliant Violet 421), 7-aminoactinomycin D (Sigma) and anti-CD16-CD32 (clone 2.4G2; grown in-house). mAbs against βTCR (clone H57-597; Alexa Fluor 647) and CD19 (clone 1D3; Brilliant Violet 786) were purchased from BD Biosciences. Flow cytometry experiments were performed using a BD LSR Fortessa and analysed using FlowJo software (TreeStar).

In vitro proliferation assays

Splenocytes from Jα18$^{-/-}$ (C57BL/6) mice were pulsed overnight with iGb3 analogues (1 μg/ml), α-GalCer (100 ng/ml) or a vehicle control in 48-well flat-bottom plates (3 x 10^6 cells per well), harvested and then washed with fresh media. Wild-type (C57BL/6) thymocytes were enriched for NKT cells by complement-mediated depletion of anti-CD24 and anti-CD8-labelled cells, with dead cells being removed via Histopaque-1083 (Sigma) gradient centrifugation. Thymic NKT cells were then labelled with 1 μM CFSE (Molecular Probes) and incubated for 10 min at 37 °C. Jα18$^{-/-}$ (C57BL/6) splenocytes (3 x 10^5 per well) and NKT cell-enriched thymocytes (5 x 10^4 per well) were then co-cultured in 96-well round-bottom plates for 72 h.

Cytokine analysis

Cell culture supernatants were collected after 72 h co-culture of glycolipid-pulsed splenocytes (Jα18$^{-/-}$ C57BL/6) and NKT cell-enriched thymocytes (C57BL/6) and then analysed using Cytometric Bead Array (CBA) flex sets for mice (BD Biosciences).

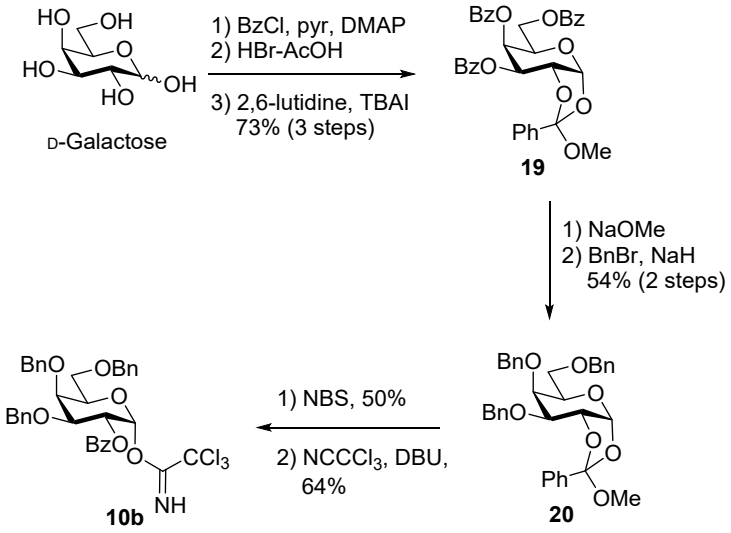
Synthetic chemistry materials and methods

General procedures

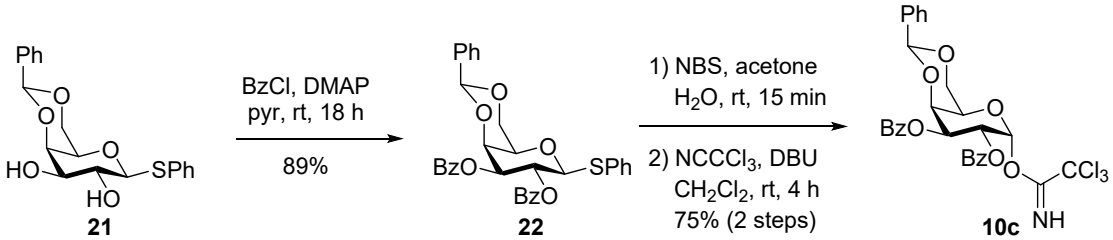
Unless otherwise stated all reactions were performed under argon. Prior to use, THF (Pancreac) was distilled from sodium and benzophenone, pyridine was distilled and dried over 4Å molecular sieves (4Å MS), CH_2Cl_2, (Pancreac) was distilled from P_2O_5, and H_2O and benzene (Fisher Scientific) were distilled. $BF_3.OEt_2$ (Janssen Chimica) was distilled prior to use. Benzaldehyde dimethyl acetal (Aldrich), $Me_2C(OMe)_2$ (Aldrich), NBS (Aldrich), DBU (Merck), CSA (Acros), PPh_3 (Aldrich), TFA (Aldrich), BnBr (Fluka), TMSOTf (Aldrich), NaOMe (Janssen Chimica), trichloroacetonitrile (Aldrich), $C_{25}H_{51}COOH$ (Acros), BzCl (Aldrich, distilled and stored under argon), EDCI (Aldrich), DMAP (Merck), sodium (Aldrich), trimethyl orthoacetate (Aldrich), HBr-AcOH (Aldrich), 2,6-lutidine (Aldrich), EtOAc (Pancreac), $Na_2S_2O_3$ (Panreac), NaH (Avocardo Research Chemicals, 60% dispersion in mineral oil), HCl (Panreac), NH_4Cl (Labserv), hexanes (Fisher Scientific), petroleum ether (Pure Science), MeOH (Pure Science), EtOH (absolute, Pure Science), $NaHCO_3$ (Pure Science), NaCl (Pancreac), NH_3 (BOC gasses), were used as received. (2*S*,3*R*,4*E*)-2-Azido-1-(4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-octadec-4-ene (**8**),[1] 1-*O*-(2,3,4,6-tetra-*O*-benzoyl-D-glucopyranosyl) trichloroacetimidate (**10a**),[2] and phenyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**21,** Scheme S1)[3] were prepared according to previously published procedures. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC-analysis on Macherey-Nagel silica gel coated plastic sheets (0.20 mm, with fluorescent indicator UV254) with detection by UV-absorption (short wave UV – 254 nm; long wave UV – 366 nm), by dipping in 10% H_2SO_4 in EtOH followed by charring at ~150 °C, by dipping in I_2 in silica, or by dipping into a solution of ninhydrin in EtOH followed by charring at ~150 °C. Column chromatography was performed on Pure Science silica gel (40-63 micron). AccuBOND II ODS-C18 (Agilent) was used for reverse phase chromatography while Sephadex® LH-20 (Aldrich) was used for size exclusion chromatography. Infrared spectra were recorded as thin films using a Bruker Tensor 27 FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) sampling accessory and are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance spectra were recorded at

20 °C in CD_3OD, $CDCl_3$, or pyridine-d_5 (which is a particularly good NMR solvent for the amphiphilic glycolipid final products) using either a Varian INOVA operating at 500 MHz or Varian VNMRS operating at 600 MHz. Chemical shifts are given in ppm (δ) relative to TMS reference. NMR peak assignments were made using COSY, HSQC and HMBC 2D experiments. Mass spectrometry was performed by submitting samples in a methanol/acetonitrile (1/1) solvent system to electrospray ionization using an Agilent LCMS QTOF (model 6530). Both iGb3 (**2**) and 1,3-β-Gal-LacCer (**3**) were tested to be endotoxin-free at a sensitivity of 0.125 EU/mL with an endotoxin kit (Pyrotell, Limulus Amebocyte Lysate).

Schemes for the synthesis of imidate donors

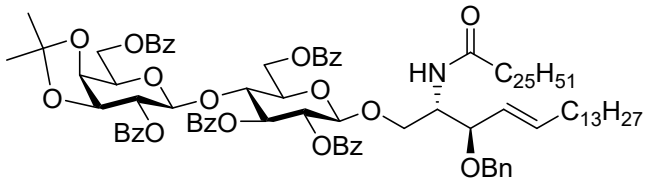


Scheme S1. Synthesis of armed imidate donor **10b**.



Scheme S2. Synthesis of benzylidene-protected imidate donor **10c**.

Experimental data for individual compounds



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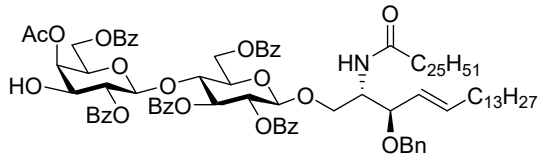
(2*S*,3*R*,4*E*)-1-(4-*O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-hexacosanoylamido-octadec-4-ene (14): To a solution of (2*S*,3*R*,4*E*)-2-azido-1-(4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-octadec-4-ene (**8**)[1] (100 mg, 0.077 mmol) in toluene (1 mL), was added triphenylphosphine (40 mg, 0.15 mmol) and distilled water (10 drops). The solution was then warmed to 80 °C and stirred overnight before being cooled to room temperature, diluted with EtOAc, washed with sat. aq. NH_4Cl, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a colourless oil. The oil was co-evaporated twice with dry toluene then suspended in CH_2Cl_2 (2 mL), and EDCI (73 mg, 0.383 mmol), DMAP (30 mg, 0.246 mmol) and hexacosanoic acid (151 mg, 0.383 mmol) were added, and the resulting solution stirred 3 days at room temperature. The reaction mixture was then purified directly by gradient flash chromatography (petroleum ether/EtOAc, 10/1 to 2/1, v/v) to give the title compound (**14**) as a colourless oil (92 mg, 0.056 mmol, 72% over two steps). R_f: 0.55 (PE/EA, 2/1, v/v); $[\alpha]_D^{22}$ = +16.0° (c = 1.0, CH_2Cl_2); IR (film) 3323, 3063, 3034, 2921, 2852, 2361, 2341, 1971, 1724, 1646, 1602, 1531, 1452, 1267, 1112, 1068, 1027, 707 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.3 Hz, 2H, CH-*o*, OBz), 8.02 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.3 Hz, 2H, CH-*o*, OBz), 8.00 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.6 Hz, 2H, CH-*o*, OBz), 7.96 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.5 Hz, 2H, CH-*o*, OBz), 7.92 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.3 Hz, 2H, CH-*o*, OBz), 7.62–7.11 (m, 20H, H_{arom}), 5.73 (t, $J_{2',3'}$ = 9.4 Hz, 1H, H-3′), 5.49 (dt, $J_{4,5}$ = 15.3 Hz, $J_{5,6}$ = 6.9 Hz, 1H, H-5), 5.393 (dd, $J_{2',3'}$ = 9.6 Hz, $J_{1',2'}$ = 8.0 Hz, 1H, H-2′), 5.392 (d, $J_{NH,2}$ = 9.1 Hz, 1H, NH C_{26}), 5.24 (dd, $J_{4,5}$ = 15.3 Hz, $J_{3,4}$ = 8.5 Hz, 1H, H-4), 5.14 (t, $J_{1'',2''}$ = 7.2 Hz, 1H, H-2′′), 4.60–4.55 (m, 3H, H-1′, H-1′′, H-6′a), 4.48 (dd, $J_{6'a,6'b}$ = 12.3 Hz, $J_{5',6'}$ = 4.0 Hz, 1H, H-6′b), 4.44 (d, $J_{a,b}$ = 11.5 Hz, 1H, CH-a, 3-*O*-Bn), 4.26–4.19 (m, 5H, H-1a, H-4′, H-3′′, H-6′′a, CH-b, 3-*O*-Bn), 4.09–4.05 (m, 2H, H-2, H-4′′), 3.81–3.79 (m, 1H, H-5′′), 3.75–3.69 (m, 3H, H-5′, H-6′′b, H-3), 3.47 (dd, $J_{1a,1b}$ = 9.5 Hz, $J_{1b,2}$ = 3.4 Hz, 1H, H-1b), 1.95–1.92 (m, 2H, H-6), 1.71–1.61 (m, 2H, CH_2-α), 1.52 (s, 3H, CH_3 *i*Pr), 1.55–1.03 (m, 71H, H-7–H-17, H-β–H-(ω-1), CH_3 *i*Pr), 0.88 t ($J_{17,18}$ = $J_{\omega-1,\omega}$ = 6.9 Hz, 6H, H-18, H-ω); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4 (HNC=O), 166.0 (C=O, 6′′-*O*-Bn), 165.8 (C=O, 6′-*O*-Bn), 165.6 (C=O, 3′-*O*-Bn), 165.3 (C=O, 2′-*O*-Bn), 165.0 (C=O, 2′′-*O*-Bn), 138.3 (C-*i*, 3-*O*-Bn), 137.0 (C-5), 133.5, 133.4, 133.3, 133.2, 133.1 (C-*p*, 5 x OBz),

129.9, 129.81, 129.78, 129.7, 129.6, 129.5, 129.3, 129.1, 128.7, 128.6, 128.5, 128.4, 128.2, 127.6, 127.4 (30 x CH_{arom}), 127.3 (C-4), 110.9 (C_q *i*Pr), 101.3 (C-1′), 100.1 (C-1′′), 79.1 (C-3), 77.0 (C-3′′), 75.2 (C-4′), 73.6 (C-2′′), 73.1 (C-4′′), 73.0 (C-5′), 72.4 (C-3′), 72.3 (C-2′), 71.3 (C-5′′), 70.3 (CH_2, 3-*O*-Bn), 68.3 (C-1), 62.8 (C-6′′), 62.6 (C-6′), 51.2 (C-2), 36.4 (C-α), 32.2 (C-6), 31.9, 29.75, 29.73, 29.71, 29.70, 29.66, 29.56, 29.54, 29.40, 29.38, 29.37, 29.27, 29.23, 22.70 (C-7–C-17, C-γ–C-(ω−1)), 27.4, 26.1 (2 x CH_3 *i*Pr), 25.5 (C-β), 14.1 (C-18, C-ω); HRMS(ESI) m/z calcd. for $[C_{101}H_{137}NO_{18}+Na]^+$: 1674.9728, obsd.: 1674.9717. Spectral data matched that previously reported.[1]



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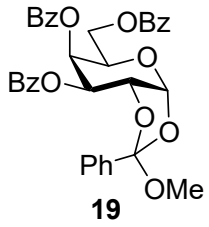
(2*S*,3*R*,4*E*)-1-(4-*O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-*O*-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-hexacosanoylamido-octadec-4-ene (15): to a solution of fully protected lactosyl ceramide (**14**) (226 mg, 0.14 mmol) in CH_2Cl_2 (5 mL) was added TFA/H_2O solution (1/1, v/v, 0.5 mL) and the resulting solution was stirred at room temperature for 12 h. The solution was diluted with ethyl acetate and the organic layer was then washed with sat. aq. $NaHCO_3$ and brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The colourless oil was then purified by gradient flash chromatography (petroleum ether/EtOAc, 5/1 to 1/1, v/v) to give the corresponding diol as a colourless oil (213 mg, 0.13 mmol, 96%).[1] The diol (181 mg, 0.11 mmol) was then co-evaporated with toluene (x3) and dissolved in dry CH_2Cl_2 (1.1 mL). Trimethyl orthoacetate (84 μL, 0.67 mmol) and CSA (52 mg, 0.22 mmol) were added and the reaction mixture was stirred at rt for 16 h. The reaction mixture was diluted with EtOAc (30 mL), washed with 1M HCl solution (3 x 30 mL), sat. aq. $NaHCO_3$ (30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by gradient flash chromatography (petroleum ether/EtOAc, 10/1 to 1/1, v/v) to afford the title compound (**15**) as a clear oil (170 mg, 0.26 mmol, 92%). R_f: 0.24 (PE/EA, 2/1, v/v); $[\alpha]_D^{24}$ = +4.0 (c = 1.0, CH_2Cl_2); IR (film) 3325, 3064, 2923, 2853, 2359, 1966, 1726, 1452, 1372, 1265, 1177, 1108, 1094, 1069, 1027, 975, 736, 708 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.8 Hz, 2H, CH-*o*, OBz), 8.03 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.6 Hz, 4H, 2 x CH-*o*, OBz), 7.96 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.3 Hz, 2H, CH-*o*, OBz), 7.96 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.8 Hz, 2H, CH-*o*, OBz), 7.60–7.13 (m, 20H, H_{arom}), 5.72 (t, $J_{2',3'} = J_{3',4'}$ = 9.7 Hz, 1H, H-3′), 5.50 (dt, $J_{4,5}$ = 15.6 Hz, $J_{5,6}$ = 6.6 Hz, 1H, H-5), 5.41 (dd, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 9.7 Hz, 1H, H-2′), 5.37 (d, $J_{NH,2'}$ = 9.3 Hz, 1H, NH), 5.26–5.22 (m, 1H, H-4), 5.22 (d, $J_{3'',4''}$ = 3.5 Hz, 1H, H-4′′), 5.16 (dd, $J_{2'',3''}$ = 9.8 Hz, $J_{1'',2''}$ = 8.0 Hz, 1H, H-2′′), 4.65 (d, $J_{1'',2''}$ = 8.0 Hz, 1H, H-1′′), 4.61 (d, $J_{1',2'}$ = 8.0 Hz, 1H, H-1′), 4.60–4.51 (m, 2H, H-6′a, H-6′b), 4.44 (d, $J_{a,b}$ = 11.5 Hz, 1H, CH-a, 3-*O*-Bn), 4.25 (d, $J_{a,b}$ = 11.5 Hz, 1H,

CH-b, 3-*O*-Bn), 4.19 (t, $J_{3',4'}$ = 9.7 Hz, 1H, H-4′), 4.07 (m, 1H, H-2), 3.82 (dd, $J_{2'',3''}$ = 9.8 Hz, $J_{3'',4''}$ = 3.5 Hz, 1H, H-3′′), 3.76 (d, $J_{4',5'}$ = 7.8 Hz, 1H, H-5′), 3.73–3.68 (m, 2H, H-3, H-6′′a), 3.64–3.59 (m, 2H, H-5′′, H-6′′b), 3.50 (dd, $J_{1a,1b}$ = 9.8 Hz, $J_{1b,2}$ = 3.5 Hz, 1H, H-1b), 1.99 (s, 3H, OAc), 1.98–1.92 (m, 2H, H-6), 1.69–1.60 (m, 2H, CH_2-α), 1.37–1.03 (m, 68H, H-7–H-17, H-β–H-(ω−1)), 0.88 t ($J_{17,18}$ = $J_{\omega-1,\omega}$ = 6.9 Hz, 6H, H-18, H-ω); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4 (HN<u>C</u>=O), 170.6 (C=O, OAc), 166.5 (C=O, 2′′-*O*-Bn), 165.9 (C=O, 6′-*O*-Bn), 165.6 (C=O, 6′′-*O*-Bn), 165.3 (C=O, 2′-*O*-Bn), 165.3 (C=O, 3′-*O*-Bn), 138.3 (C-*i*, 3-*O*-Bn), 137.0 (C-5), 133.6, 133.5, 133.4, 133.2 (C-*p*, 5 x OBz), 129.89, 120.82, 129.76, 129.62, 129.51, 129.41, 129.06, 128.9, 128.6, 128.58, 128.26, 128.19, 127.63, 127.40, 127.30 (30 x CH_{arom}), 101.3 (C-1′), 100.3 (C-1′′), 79.1 (C-3), 75.3 (C-4′), 73.5 (C-2′′), 73.0 (C-5′), 72.4 (C-3′), 72.1 (C-2′), 71.5 (C-3′′), 71.1 (C-5′′), 70.3 (CH_2, 3-*O*-Bn), 69.3 (C-4′′), 68.3 (C-1), 62.5 (C-6′), 61.2 (C-6′′), 21.1 (C-2), 36.4 (C-α), 32.2 (C-6), 31.92, 29.72, 29.70, 29.68, 29.66, 29.55, 29.54, 29.39, 29.37, 29.36, 29.26, 29.22, 25.45, 22.69 (C-7–C-17, C-β–C-(ω−1)), 20.6 (OAc), 14.1 (C-18, C-ω); HRMS(ESI) m/z calcd. for $[C_{100}H_{135}NO_{19}+Na]^+$: 1676.9521, obsd.: 1676.9526. Spectral data matched that previously reported.[1]



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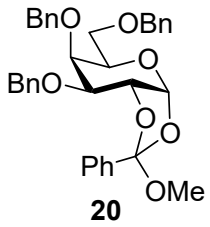
3,4,6-Tri-*O*-benzoyl-1,2-*O*-(α-methoxybenzylidene)-α-D-galactopyranose (19): To a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactosyl bromide[4] (2.31 g, 3.50 mmol) in CH_2Cl_2 (12 mL), was added MeOH (580 µL) and 2,6-lutidene (580 µL, 1.4 equiv.) and the reaction was stirred at room temperature for 2 days then at reflux overnight. The solution was then diluted with Et_2O (200 mL), washed with water (2 x 150 mL), and the combined organic layers were washed with a solution of sat. aq. $Na_2S_2O_3$ (150 mL), brine (150 mL), dried over $NaSO_4$, filtered and the solvent removed under reduced pressure. The residue was then purified by silica gel flash column chromatography (petroleum ether/EtOAc, 10:1 to 5:1, v/v) to give the title compound **19** as a clear oil (73%, 1.56 g, 2.56 mmol). R_f = 0.52 (petroleum ether/EtOAc, 2/1, v/v); $[\alpha]_D^{23}$ = +65.6 (c = 1.0, $CHCl_3$); IR (film) 3064, 3033, 2943, 2854, 1724, 1601, 1451, 1267, 1177, 1160, 1091, 1068, 1025, 975, 763, 707 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98-7.92 (m, 6H, H_{arom}), 7.60-7.57 (m, 2H, H_{arom}), 7.57-7.53 (m, 3H, H_{arom}), 7.42-7.37 (m, 9H, $H_{arom)}$, 6.21 (d, $J_{1,2}$ = 5.0 Hz, 1H, H-1), 5.82 (dd, $J_{3,4}$ = 4.1 Hz, $J_{4,5}$ = 2.6 Hz, 1H, H-4), 5.53 (dd, $J_{2,3}$ = 6.2 Hz, $J_{3,4}$ = 4.1 Hz, 1H, H-3), 4.79 (dd, $J_{2,3}$ = 6.1 Hz, $J_{1,2}$ = 5.1 Hz, 1H, H-2), 4.60 (dd, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6a}$ = 6.8 Hz, 1H, H-6a), 4.52 (td, $J_{5,6a}$ = $J_{5,6b}$ = 6.3 Hz, $J_{4,5}$ = 2.5 Hz, 1H, H-5), 4.38 (dd, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6b}$ = 5.6 Hz, 1H, H-6b), 3.25 (s, 3H, OMe); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.0, 165.23, 165.21 (C=O Bz), 136.3 (C-*i* Ph), 133.6, 133.4, 133.2 (C-*i* Bz), 129.83, 129.81, 129.77, 129.75, 129.6, 129.4, 129.1, 129.0, 128.63, 128.55, 128.5, 128.39, 128.36, 126.08,

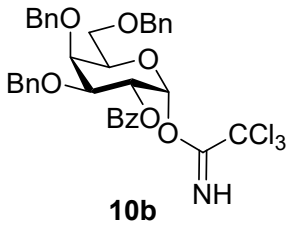
126.05 (CH_{arom}), 120.7 (C_q), 98.3 (C-1), 73.6 (C-2), 70.2 (C-3), 68.9 (C-5), 66.6 (C-4), 62.4 (C-6), 51.1 (OMe); HRMS(ESI) m/z calcd. for $[C_{35}H_{30}O_{10}+NH_4]^+$: 628.2177, obsd.: 628.2165.



20

3,4,6-Tri-*O*-benzyl-1,2-*O*-(α-methoxybenzylidene)-α-D-galactopyranose (20). 3,4,6-Tri-*O*-benzoyl-1,2-*O*-(α-methoxybenzylidene)-α-D-galactopyranose (**19**) (1.43 g, 2.34 mmol) was dissolved in CH_2Cl_2 (1.2 mL) to which MeOH (4.8 mL) and NaOMe (63.3 mg in 2.34 mL of MeOH) was added dropwise. The resulting solution was stirred at room temperature for 17 h. The solution was neutralized by the addition of Dowex-H^+, filtered, and the solvent removed under reduced pressure to give 1,2-*O*-(α-methoxybenzylidene)-α-D-galactopyranose as a clear oil, which was used without further purification. To a solution of 1,2-*O*-(α-methoxybenzylidene)-α-D-galactopyranose (698 mg, 2.34 mmol) in DMF (10 mL), cooled to 0 °C, was added benzyl bromide (1.11 mL, 9.36 mmol), followed by the portion-wise addition of NaH (468 mg of a 60% dispersion in mineral oil). The resulting suspension was allowed to warm to room temperature overnight. Excess NaH was then carefully quenched by the addition of MeOH (2 mL) and the solution was diluted with Et_2O (150 mL). The combined organic layers were then washed twice with sat. aq. $NaHCO_3$ solution (100 mL), twice with water (100 mL), once with brine (100 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified by gradient silica gel flash column chromatography (petroleum ether/EtOAc, 10/1 to 5/1, v/v) to give 3,4,6-tri-*O*-benzyl-1,2-*O*-(α-methoxybenzylidene)-α-D-galactopyranose (**20**) as a colourless oil (52%, 691 mg, 1.22 mmol) over the two steps. R_f = 0.73 (petroleum ether/EtOAc, 1/1, v/v); $[\alpha]_D^{23}$ = +21.2 (c = 1.0, $CHCl_3$); IR (film) 3031, 2923, 2857, 1496, 1366, 1278, 1190, 1099, 1068, 1027, 991, 920, 764, 737, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.60-7.57 (m, 2H, H_{arom}), 7.42-7.37 (m, 18H, H_{arom}), 5.97 (d, $J_{1,2}$ = 4.8 Hz, 1H, H-1), 4.88 (d, $J_{a,b}$ = 11.7 Hz, 1H, CHa 4-*O*-Bn), 4.65 (d, $J_{a,b}$ = 11.7 Hz, 1H, CHa 3-*O*-Bn), 4.64 (dd, $J_{2,3}$ = 7.0 Hz, $J_{1,2}$ = 4.8 Hz, 1H, H-2), 4.57 (d, $J_{a,b}$ = 11.7 Hz, 1H, CHb 4-*O*-Bn), 4.48 (d, $J_{a,b}$ = 11.8 Hz, 1H, CHa 6-*O*-Bn), 4.47 (d, $J_{a,b}$ = 11.7 Hz, 1H, CHb 3-*O*-Bn), 4.41 (d, $J_{a,b}$ = 11.8 Hz, 1H, CHb 6-*O*-Bn), 3.97 (ddd, $J_{5,6b}$ = 7.3 Hz, $J_{5,6a}$ = 5.9 Hz, $J_{4,5}$ = 1.4 Hz, 1H, H-5), 3.84 (dd, $J_{3,4}$ = 2.3 Hz, $J_{4,5}$ = 1.9 Hz, 1H, H-4), 3.61 (dd, $J_{6a,6b}$ = 9.2 Hz, $J_{5,6a}$ = 5.8 Hz, 1H, H-6a), 3.58 (dd, $J_{6a,6b}$ = 9.2 Hz, $J_{5,6b}$ = 7.6 Hz, 1H, H-6b), 3.45 (dd, $J_{2,3}$ = 7.1 Hz, $J_{3,4}$ = 2.6 Hz, 1H, H-3), 3.28 (s, 3H, OMe); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 138.3, 138.0, 137.7 (C-*i*), 129.0, 128.4, 128.34, 128.28, 128.25, 128.1, 127.9, 127.8, 127.7, 127.61, 127.57 (CH_{arom}), 125.9 (CH-*o* Ph), 119.8 ($PhCO_3$), 98.7 (C-1), 79.3 (C-3), 78.0 (C-2), 74.4 (CH_2 4-*O*-Bn), 73.5 (CH_2 6-*O*-Bn), 72.6 (C-

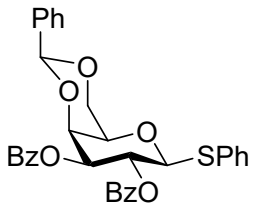
5), 72.4 (C-4), 71.8 (CH_2 3-*O*-Bn), 68.2 (C-6), 50.6 (OMe); HRMS(ESI) m/z calcd. for $[C_{35}H_{36}O_7+Na]^+$: 591.2353, obsd.: 591.2355.



10b

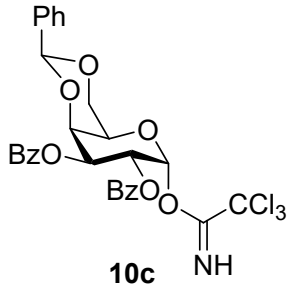
***O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl) trichloroacetimidate (10b).** To a solution of 3,4,6-tri-*O*-benzyl-1,2-*O*-(α-methoxybenzylidene)-α-D-galactopyranose (**20**) (98.9 mg, 0.174 mmol) in MeCN (18 mL) at 0 °C, were added water (1.8 mL) and NBS (92.0 mg, 0.523 mmol), and the solution was stirred at room temperature for 12 h. The solution was then diluted with EtOAc (50 mL), and the organic layers were then washed with a 10% solution of $Na_2S_2O_3$ (2 x 50 mL), sat. aq. $NaHCO_3$ solution (50 mL) and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified by silica gel flash column chromatography (petroleum ether/EtOAc, 10:1 to 5:1, v/v) to give 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranose as a colourless oil [50%, 48.2 mg, 0.087 mmol, R_f: 0.55 (petroleum ether/EtOAc, 1/1, v/v)] and the 1-*O*-benzoyl isomer [44%, 42.4 mg, 0.077 mmol, R*f* : 0.45 (Pet Ether/EtOAc, 1/1, v/v)]. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.3, 2H, CH-*o* Bz), 7.59-7.55 (m, 1H, CH-*p* Bz), 7.47-7.42 (m, 2H, CH-*m* Bz), 7.36-7.24 (m, 15H, CH_{arom} Bn), 5.60 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 5.58 (dd, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.6 Hz, 1H, H-2), 4.96 (d, $J_{a,b}$ = 11.6 Hz, 1H, CHa 4-*O*-Bn), 4.72 (d, $J_{a,b}$ = 12.0 Hz, 1H, CHa 3-*O*-Bn), 4.69 (d, $J_{a,b}$ = 12.0 Hz, 1H, CHb 3-*O*-Bn), 4.59 (d, $J_{a,b}$ = 11.6 Hz, 1H, CHb 4-*O*-Bn), 4.50 (d, $J_{a,b}$ = 11.8 Hz, 1H, CHa 6-*O*-Bn), 4.43 (d, $J_{a,b}$ = 11.8 Hz, 1H, CHb 6-*O*-Bn), 4.23 (t, $J_{5,6a} = J_{5,6b}$ = 6.2 Hz, 1H, H-5), 4.14 (dd, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 2.9 Hz, 1H, H-3), 4.01 (dd, $J_{3,4}$ = 2.0 Hz, 1H, H-4), 3.60 (dd, $J_{6a,6b}$ = 9.4 Hz, $J_{5,6a}$ = 6.6 Hz, 1H, H-6a), 3.49 (dd, $J_{6a,6b}$ = 9.4 Hz, $J_{5,6b}$ = 6.2 Hz, 1H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.0 (C=O), 138.3, 138.1, 137.7 (C-*i*), 133.0 (C-*p* Bz), 129.8 (C-*o* Bz), 128.42, 128.38, 128.36, 128.35, 128.29, 128.27, 128.0, 127.8, 127.73, 127.68, 127.65 (CH_{arom}), 91.0 (C-1), 76.2 (C-3), 74.7 (CH_2 4-*O*-Bn), 74.5 (C-4), 73.6 (CH_2 6-*O*-Bn), 72.7 (CH_2 3-*O*-Bn), 71.7 (C-2), 69.5 (C-5), 69.3 (C-6). HRMS(ESI) m/z calcd. for $[C_{34}H_{34}NO_7+Na]^+$: 572.2643, obsd.: 572.2657. The lactol, 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranose (58.0 mg, 0.105 mmol), was then dissolved in CH_2Cl_2 (1 mL) and cooled to -0 °C. To the solution was then added trichloroacetonitrile (100 µL, 1.05 mmol) and DBU (15.6 µL, 0.105 mmol) and the solution was stirred for 1 hour. The solvent was removed under reduced pressure and the resulting pale yellow oil purified by silica gel flash column chromatography (Pet ether/EtOAc, 5/1 to 2/1, v/v) to give 1-*O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl) trichloroacetimidate (**10b**) (64%, 46.7 mg, 0.067 mmol). R*f* (α-anomer): 0.64 (petroleum ether/EtOAc, 1/1, v/v); R*f* (β-anomer): 0.50 (petroleum ether/EtOAc, 1/1, v/v). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (s, 1H, NH),

8.06 (d, *J* = 8.0, 2H, CH-*o* Bz), 7.56 (t, 1H, *J* = 7.4, CH-*p* Bz), 7.41 (t, 2H, *J* = 7.6, CH-*m* Bz), 7.38-7.21 (m, 15H, CH_{arom} Bn), 6.66 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 5.82 (dd, $J_{2,3}$ = 9.7 Hz, $J_{1,2}$ = 3.3 Hz, 1H, H-2), 5.01 (d, $J_{a,b}$ = 11.4 Hz, 1H, CHa 4-*O*-Bn), 4.72 (d, $J_{a,b}$ = 12.2 Hz, 1H, CHa 3-*O*-Bn), 4.65 (d, $J_{a,b}$ = 11.4 Hz, 1H, CHb 4-*O*-Bn), 4.64 (d, $J_{a,b}$ = 12.2 Hz, 1H, CHb 3-*O*-Bn), 4.50 (d, $J_{a,b}$ = 11.6 Hz, 1H, CHa 6-*O*-Bn), 4.45 (d, $J_{a,b}$ = 11.6 Hz, 1H, CHb 6-*O*-Bn), 4.23 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.8 Hz, 1H, H-5), 4.21-4.18 (m, 2H, H-3 and H-4), 3.71 (t, $J_{5,6a}$ = $J_{6a,6b}$ = 8.4 Hz, 1H, H-6a), 3.62 (dd, $J_{6a,6b}$ = 9.4 Hz, $J_{5,6b}$ = 5.4 Hz, 1H, H-6b); HRMS(ESI) m/z calcd. for $[C_{36}H_{34}Cl_3NO_7+Na]^+$: 720.1293, obsd.: 720.1300. Data matched that previously reported.[2]



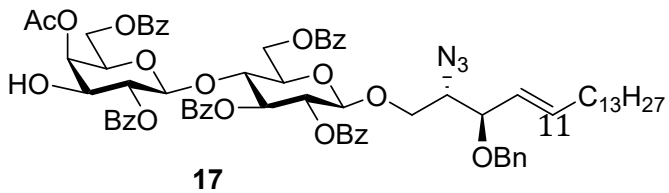
22

Phenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (22). To phenyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**21**) (300 mg, 0.832 mmol), which was prepared according to published procedures,[3] was added pyridine (8.3 mL), followed by benzoyl chloride (0.39 mL, 3.33 mmol) and DMAP (20.3 mg, 0.166 mmol) and the resulting solution stirred at room temperature for 18 h. EtOAc (70 mL) was then added, and the organic layers were then washed with $NaHCO_3$ (2 x 50 mL), water (50 mL), brine (50 mL) and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified by silica gel flash column chromatography (petroleum ether/EtOAc, 1/1, v/v) to give a colourless oil. The product was then crystalised from Pet ether/EtOAc (2/1, v/v) to give the title compound (**22**) as a white solid (89%, 421 mg, 0.74 mmol). R*f*: 0.38 (Petroleum ether/EtOAc, 1/1, v/v); $[\alpha]_D^{27}$ = +64.6 (c = 1.0, $CHCl_3$); IR (film) 3063, 2866, 1722, 1601, 1584, 1451, 1272, 1176, 1130, 1091, 1070, 1026, 993, 905, 737, 708 cm^{-1}; ^1H NMR (500 MHz, cdcl$_3$) δ 7.98 (d, *J* = 7.3 Hz, 2H, CH-*o* 2-*O*-Bz), 7.93 (d, *J* = 7.3 Hz, 2H, CH-*o* 3-*O*-Bz), 7.62 (d, *J* = 7.2 Hz, 2H, CH-*o* SPh), 7.53 (t, *J* = 7.4 Hz, 1H, CH-*p* 2-*O*-Bz), 7.47 (t, *J* = 7.4 Hz, 1H, CH-*p* 3-*O*-Bz), 7.43-7.30 (m, 10H, H_{arom}), 7.27-7.24 (m, 2H, CH-*m* SPh), 5.81 (t, $J_{1,2}$ = $J_{2,3}$ = 9.9 Hz, 1H, H-2), 5.52 (s, 1H, $PhCHO_2$), 5.37 (dd, $J_{2,3}$ = 10.0, $J_{3,4}$ = 3.3 Hz, 1H, H-3), 4.97 (d, $J_{1,2}$ = 9.8 Hz, 1H, H-1), 4.60 (d, $J_{3,4}$ = 3.2 Hz, 1H, H-4), 4.46 (dd, $J_{6a,6b}$ = 12.3, $J_{5,6a}$ = 1.2 Hz, 1H, H-6a), 4.11 (dd, $J_{6a,6b}$ = 12.3, $J_{5,6b}$ = 1.3 Hz, 1H, H-6b), 3.77 (s, 1H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2 (C=O 3-*O*-Bz), 164.9 (C=O 2-*O*-Bz), 137.5 (C-*i* $PhCHO_2$), 133.9 (C-*o* SPh), 133.3 (C-*p* 3-*O*-Bz), 133.1 (C-*p* 2-*O*-Bz), 131.0 (C-*i* SPh), 130.0 (C-*o* 3-*O*-Bz), 129.8 (C-*o* 2-*O*-Bz), 129.6 (C-*i* 2-*O*-Bz), 129.04 (C-*i* 2-*O*-Bz), 129.03 (C-*p* $PhCHO_2$), 128.8 (C-*m* SPh), 128.36, 128.35 (C-*m* 2- and 3-*O*-Bz), 128.1 (C-*p* SPh), 126.4 (C-*m* $PhCHO_2$), 100.9 ($PhCHO_2$), 85.3 (C-1), 74.1 (C-3), 73.6 (C-4), 69.9 (C-5), 69.1 (C-6), 67.0 (C-7); HRMS(ESI) m/z calcd. for $[C_{33}H_{28}O_7S+NH_4]^+$: 586.1894, obsd.: 586.1891.



10c

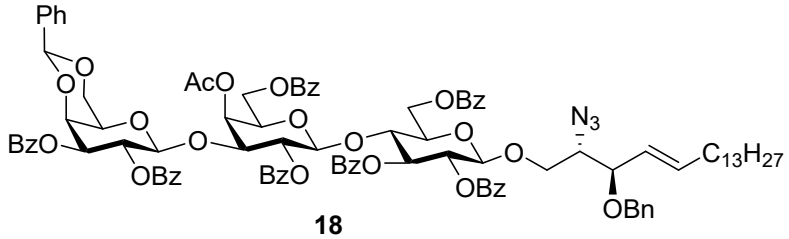
2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-a-D-galactosyl trichloroacetimidate (10c): To a solution of thioglycoside **22** (444 mg, 0.781 mmol) in acetone (4.5 mL) and water (0.5 mL), was added NBS (556 mg, 3.12 mmol) and the reaction was stirred at room temperature for 30 min. The reaction mixture was diluted with EtOAc (45 mL), and washed sat. aq. $Na_2S_2O_3$ (50 mL), water (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting oil was purified by gradient flash chromatography (petroleum ether/EtOAc, 5/1 to 1/1, v/v) to give 2,3-di-*O*-benozyl-4,6-*O*-benzylidene-β-D-galactopyranoside (297 mg, 0.623 mmol) as a colourless oil. To a stirred solution of 2,3-di-*O*-benozyl-4,6-*O*-benzylidene-β-D-galactopyranoside (297 mg, 0.623 mmol, co-evaporated 3 times with dry toluene) in dry CH_2Cl_2 (6 mL) at 0 °C was added trichloroacetonitrile (0.63 mL, 900 mg, 6.23 mmol) followed by DBU (93 μL, 94.9 mg, 0.623 mmol) and the resulting solution was allowed to warm to room temperature overnight. The solution was then was concentrated under reduced pressure and purified by gradient flash column chromatography (petroleum ether/EtOAc/, 95/5 to 1/1, v/v with 1% NEt_3) to give trichloroacetimidate **10c** as a colourless oil (94%, 365 mg, 0.586 mmol). R*f* = 0.63 (petroleum ether/EtOAc, 1/1, v/v with 1% NEt_3); $[\alpha]_D^{27}$ = +139.0 (c = 1.0, $CHCl_3$); IR (film) 3472, 3432, 3342, 3068, 3035, 2918, 2857, 1721, 1674, 1270, 1249, 1109, 1071,1027, 979, 754, 708 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.61 (s, 1H, NH), 8.02 (d, $J_{CH\text{-}o,\ CH\text{-}m}$ = 8.2 Hz, 2H, CH-*o*, OBz), 7.98 (d, $J_{CH\text{-}o,\ CH\text{-}m}$ = 8.2 Hz, 2H, CH-*o*, OBz), 7.56-7.50 (m, 4H, H_{arom}), 7.41-7.34 (m, 7H, H_{arom}), 6.89 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 6.07 (dd, $J_{2,3}$ = 10.7 Hz, $J_{1,2}$ = 3.5 Hz, 1H, H-2), 5.76 (dd, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.3 Hz, 1H, H-3), 5.61 (s, 1H, PhC*H*O_2), 4.79 (d, $J_{3,4}$ = 3.3 Hz, 1H, H-4), 4.42 (d, $J_{3,4}$ = 12.7 Hz, 1H, H-6a), 4.17-4.14 (m, 2H, H-5, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2 (C=O, 3-*O*-Bz), 165.6 (C=O, 2-*O*-Bz), 160.6 (C=NH), 137.3, 133.5, 133.4, 129.9, 129.8, 129.2, 129.1, 129.0, 128.5, 128.4, 128.3, 128.2, 126.2 (18 x CH_{arom}), 100.8 (CH-benzylidene), 94.7 (C-1), 91.0 (CCl_3), 73.7 (C-4), 69.2 (C-3), 68.8 (C-6), 67.5 (C-2), 65.0 (C-5); HRMS(ESI) m/z calcd. for $[C_{29}H_{24}Cl_3NO_8+K]^+$: 658.0199, obsd.: 658.0177.



17

(2*S*,3*R*,4*E*)-1-(4-*O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-*O*-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-azido-octadec-4-ene (17): To a solution of fully protected azido lactosyl ceramide **8** (328 mg, 0.252 mmol) in CH_2Cl_2 (8 mL) was added TFA/H_2O solution (1/1, v/v, 0.5 mL) and the resulting solution was stirred at room temperature for 12 h. The solution was diluted with EtOAc (60 mL) and the organic layer was then washed with sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The colourless oil was then purified by silica gel gradient flash chromatography (petroleum ether/EtOAc, 10/1 to 1/1, v/v) to give (2*S*,3*R*,4*E*)-1-(4-*O*-(2,6-di-*O*-benzoyl-*O*-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-azido-octadec-4-ene as a colourless oil (305 mg, 0.242 mmol, 96%). R_f = 0.08 (PE/EA, 2/1, v/v). Spectral data matched that previously reported.[20] (2*S*,3*R*,4*E*)-1-(4-*O*-(2,6-Di-*O*-benzoyl-*O*-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-azido-octadec-4-ene (305 mg, 0.242 mmol) was then co-evaporated with toluene (x 3) and dissolved in dry CH_2Cl_2 (3.6 mL). Trimethyl orthoacetate (91 μL, 0.726 mmol) and CSA (28.1 mg, 0.121 mmol) were added and the reaction mixture was stirred at rt for 16 h. The reaction mixture was diluted with EtOAc (60 mL), washed with 1M HCl solution (3 x 50 mL), sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by silica gel gradient flash chromatography (petroleum ether/EtOAc, 10/1 to 1/1, v/v) to afford acetate **17** as a clear oil (91%, 286 mg, 0.220 mmol). R_f: 0.63 (PE/EA, 1/1, v/v); $[\alpha]_D^{25}$ = +2.0° (c = 1.0, $CHCl_3$); IR (film) 3475, 2102, 1727, 1602, 1452, 1371, 1315, 1269, 1177, 1110, 1095, 1070, 1028, 976, 709 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 8.3 Hz, 2H, CH-*o*, 2´´-*O*-Bz), 8.04–8.02 (m, 4H, 2 x CH-*o*, 3´-*O*-Bz, 6´´-*O*-Bz), 7.98 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.8 Hz, 2H, CH-*o*, 6´-*O*-Bz), 7.95 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.8 Hz, 2H, CH-*o*, 2´-*O*-Bz), 7.62–7.19 (m, 20H, H_{arom}), 5.72 (t, $J_{2',3'} = J_{3',4'}$ = 9.6 Hz, 1H, H-3´), 5.46 (dd, $J_{2',3'}$ = 9.6 Hz, $J_{1',2'}$ = 7.9 Hz, 1H, H-2´), 5.41 (dt, $J_{4,5}$ = 15.7 Hz, $J_{5,6}$ = 6.6 Hz, 1H, H-5), 5.26 (dd, $J_{4,5}$ = 15.7 Hz, $J_{3,4}$ = 8.6 Hz, 1H, H-4), 5.21 (d, $J_{3'',4''}$ = 3.4 Hz, 1H, H-4´´), 5.17 (dd, $J_{2'',3''}$ = 9.8 Hz, $J_{1'',2''}$ = 8.0 Hz, 1H, H-2´´), 4.69 (d, $J_{1',2'}$ = 7.9 Hz, 1H, H-1´), 4.65 (d, $J_{1'',2''}$ = 8.0 Hz, 1H, H-1´´), 4.62 (d, $J_{6'a,6'b}$ = 12.0 Hz, 1H, H-6´a), 4.53 (dd, $J_{6'a,6'b}$ = 12.0 Hz, $J_{5,6'b}$ = 4.3 Hz, 1H, H-6´b), 4.41 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-a, 3-*O*-Bn), 4.21 (t, $J_{3',4'}$ = 9.6 Hz, 1H, H-4´), 4.15 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-b, 3-*O*-Bn), 3.92 (dd, $J_{1a,1b}$ = 10.0 Hz, $J_{1a,2}$ = 5.9 Hz, 1H, H-1a), 3.83–3.81 (m, 2H, H-5´, H-3´´), 3.76–3.72 (m, 2H, H-3, H-6´´a), 3.61–3.56 (m, 2H, H-2, H-5´´), 3.55–3.50 (m, 2H, H-6´´b, H-1b), 2.01 (s, 3H, OAc), 1.98–1.91 (m, 2H, H-6), 1.32–1.21 (m, 22H, H-7–H-17), 0.88 (t, $J_{17,18}$ = 6.9 Hz, 3H, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (C=O, OAc), 166.6

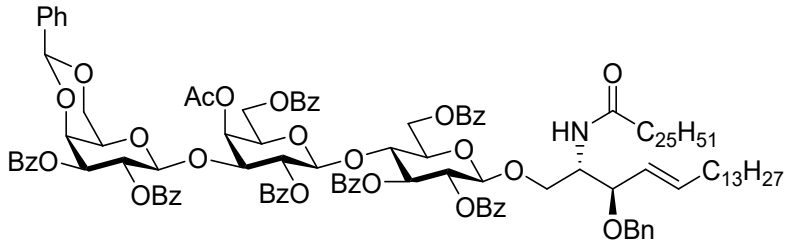
(C=O, 2´´-*O*-Bn), 166.1 (C=O, 6´-*O*-Bn), 165.8 (C=O, 6´´-*O*-Bn), 165.5 (C=O, 3´-*O*-Bn), 165.1 (C=O, 2´-*O*-Bn), 138.4 (C-5), 138.2 (C-*i*, 3-*O*-Bn), 133.7, 133.6, 133.5, 133.4, 133.3 (C-*p*, 5 x OBz), 130.02, 130.30, 129.88, 129.74, 129.67, 129.65, 129.60, 129.43, 129.02, 128.80, 128.71, 128.70, 128.50, 128.40, 128.37, 127.58 (30 x CH_{arom}), 125.6 (C-4), 101.2 (C-1´), 100.5 (C-1´´), 79.7 (C-3), 75.6 (C-4´), 73.6 (C-2´´), 73.1 (C-5´), 72.8 (C-3´), 71.8 (C-2´), 71.7 (C-3´´), 71.3 (C-5´´), 70.1 (CH_2, 3-*O*-Bn), 69.4 (C-4´´), 68.7 (C-1), 63.9 (C-2), 62.7 (C-6´), 61.3 (C-6´´), 32.4 (C-6), 32.0, 29.83, 29.81, 29.80, 29.79, 29.76, 29.55, 29.49, 29.29, 29.03, 22.8 (C-7–C-17), 20.7 (OAc), 14.3 (C-18); HRMS(ESI) m/z calcd. for $[C_{72}H_{83}N_3O_{18}+NH_4]^+$: 1319.6010, obsd.: 1319.5966.



18

(2*S*,3*R*,4*E*)-1-(4-*O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-azido-octadec-4-ene (18). A solution of galactose imidate **10c** (103 mg, 0.166 mmol) and glycolipid **17** (108 mg, 0.0829 mmol), co-evaporated 3 times with dry toluene, was dissolved in dry CH_2Cl_2 (0.5 mL) and 4 Å molecular sieves were added. This mixture was cooled to -20 °C and 45 µL (0.0249 mmol) of a stock solution of TMSOTf (20 µL of TMSOTf in 180 µL of CH_2Cl_2) was added slowly drop wise. The resulting solution was allowed to warm to 0 °C over 45 minutes, at which point TLC analysis revealed a ca. 1:1 ratio of product **18**:acceptor **17**. The solution was then cooled to -20 °C again, and donor **10c** (50 mg, 0.083 mmol in 0.25 mL of CH_2Cl_2) was added slowly over ten minutes. The solution was then allowed to warm to 0 °C and stirred for 1 h, after which time the solution was diluted with EtOAc (40 mL), washed with sat. aq. $NaHCO_3$ (2 x 30 mL), brine (30 mL), dried $MgSO_4$, filtered and concentrated under reduced pressure. The resulting oil was purified by gradient flash column chromatography (0-0.3% MeOH in CH_2Cl_2). The product was then further purified by LH20 size exclusion chromatography (CH_2Cl_2/MeOH, 1/1, v/v) to give the trisaccharide **18** as a colourless oil (51%, 74 mg, 0.0042 mmol). R_f = 0.46 (Tolune/EtOAc, 4/1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 6.8 Hz, 2H, CH-*o*, OBz), 8.03 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.6 Hz, 2H, CH-*o*, OBz), 7.97 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.3 Hz, 2H, CH-*o*, OBz), 7.92 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.3 Hz, 2H, CH-*o*, OBz), 7.86 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.4 Hz, 2H, CH-*o*, OBz), 7.75 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.4 Hz, 2H, CH-*o*, OBz), 7.64–7.10 (m, 33H, H_{arom}), 5.72 (t, $J_{2',3'}$ = $J_{3',4'}$ = $J_{1''',2'''}$ = $J_{2''',3'''}$ = 9.4 Hz, 2H, H-3′, H2′′′), 5.47–5.38 (m, 5H, PhC*H*O_2, H-4′′, H-2′, H-2′′, H-5),

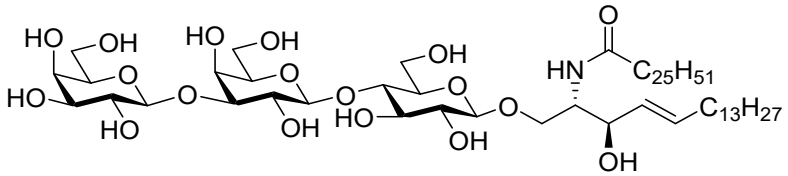
5.27 (dd, $J_{4,5}$ = 15.5 Hz, $J_{3,4}$ = 8.5 Hz, 1H, H-4), 5.13 (dd, $J_{2''',3'''}$ = 10.4 Hz, $J_{3''',4'''}$ = 3.4 Hz, 1H, H-3'''), 4.78 (d, $J_{1''',2'''}$ = 8.0 Hz, 1H, H-1'''), 4.65 (d, $J_{1',2'}$ = 7.9 Hz, 1H, H-1'), 4.52 (d, $J_{1'',2''}$ = 8.0 Hz, 1H, H-1''), 4.47–4.31 (m, 5H, H-4''', CH-a, 3-*O*-Bn, H-6'a, H-6'-b, H-6'''a), 4.19–4.07 (m, 3H, CH-b, 3-*O*-Bn, H-4', H-6''a), 4.03–3.97 (m, 2H, H-5', H-6'''b), 3.90 (dd, $J_{1a,1b}$ = 10.2 Hz, $J_{1a,2}$ = 5.7 Hz, 1H, H-1a), 3.76–3.58 (m, 4H, H-2, H-3, H-3'', H-5''), 3.52 (s, 1H , H-5'''), 3.50–3.46 (m, 1H, H-1b), 3.28 (dd, $J_{6'''a,6''b}$ = 11.5 Hz, $J_{5,6''b}$ = 8.2 Hz, 1H, H-6''b), 1.95 (s, 3H, OAc), 1.94–1.92 (m, 2H, H-6a, H-6b), 1.30 (m, 22H, H-7–H-17), 0.88 (t, $J_{17,18}$ = 6.7 Hz, 3H, H-18); HRMS(ESI) m/z calcd. for $[C_{101}H_{105}N_3O_{25}+NH_4]^+$:1777.7375, obsd.: 1777.7346.



16c

(2*S*,3*R*,4*E*)-1-(4-*O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-(hexacosanoylamido)-octadec-4-ene (16c). To a solution of glycolipid azide **18** (69mg, 0.0392 mmol) in toluene (1.2 mL) were added triphenylphosphine (20.6 mg, 0.0784 mmol) and the solution stirred at 45 °C for 30 minutes. Distilled water (30 μL, 0.784 mmol) was then added and the solution was stirred for 15 h at 45 °C. The reaction mixture was then cooled to rt, diluted with EtOAc (30 mL), washed quickly with sat. aq. NH_4Cl (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a colourless oil which was used without further purification. The oil was co-evaporated twice with dry toluene then suspended in CH_2Cl_2 (2 mL), EDCI (22.5 mg, 0.1175 mmol), DMAP (19.1 mg, 0.157 mmol), and hexacosanoic acid (46.6 mg, 0.1175 mmol) were added and the resulting solution stirred at rt for 2 days. The reaction mixture was then diluted with EtOAc (30 mL), washed with sat. aq. $NaHCO_3$ (2 x 30 mL), water (30 mL), brine (30 mL) and then dried over $MgSO_4$. The product was then purified by silica gel gradient flash chromatography (petroleum ether/EtOAc, 10/1 to 1/1, v/v) to give fully protected β-Gal-LacCer **16c** as a colourless oil (70%, 58.3 mg, 0.0192 mmol, over two steps). R_f = 0.59 (Petroleum ether/EtOAc, 1/1, v/v); $[\alpha]_D^{27}$ = +43.0 (c = 1.0, $CHCl_3$); IR (film) 3067, 3033, 2923, 2853, 1731, 1670, 1270, 1109, 1094, 1069, 755, 709 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.15 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.1 Hz, 2H, CH-*o*, 6'-*O*-Bz/6''-*O*-Bz), 8.01 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.1 Hz, 2H, CH-*o*, 2'''-*O*-Bz/3'-*O*-Bz), 7.96 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.1 Hz, 2H, CH-*o*, 2''-*O*-Bz), 7.88 (d, $J_{CH\text{-}o,CH\text{-}m}$ =

7.1 Hz, 2H, CH-*o*, 6′-*O*-Bz/6′′-*O*-Bz), 7.85 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.0 Hz, 2H, CH-*o*, 6′′′-*O*-Bz), 7.74 (d, $J_{CH\text{-}o,CH\text{-}m}$ = 7.1 Hz, 2H, CH-*o*, 2′-*O*-Bz), 7.60– 7.09 (m, 33H, H_{arom}), 5.71–5.67 (m, 2H, H-2′′′, H-3′), 5.48 (dt, $J_{4,5}$ = 15.4 Hz, $J_{5,6}$ = 7.3 Hz, 1H, H-5), 5.44 (s, 1H, PhC*H*O_2), 5.42–5.35 (m, 4H, H-4′′, H-2′, H-2′′, NH), 5.23 (dd, $J_{4,5}$ = 15.4 Hz, $J_{3,4}$ = 8.6 Hz, 1H, H-4), 5.11 (dd, $J_{2''',3'''}$ = 10.4 Hz, $J_{3''',4'''}$ = 3.5 Hz, 1H, H-3′′′), 4.76 (d, $J_{1''',2'''}$ = 7.8 Hz, 1H, H-1′′′), 4.57 (d, $J_{1',2'}$ = 7.8 Hz, 1H, H-1′), 4.53 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, H-1′′), 4.45 (s, 1H, H-4′′′), 4.44–4.39 (m, 2H, H-6′a, CH-a, 3-*O*-Bn), 4.33–4.29 (m, 2H, H-6′b, H-6′′′a), 4.23 (d, $J_{a,b}$ = 11.7 Hz, 1H, CH-b, 3-*O*-Bn), 4.22 (dd, $J_{1a,1b}$ = 9.7 Hz, $J_{1a,2}$ = 2.8 Hz, 1H, H-1a), 4.13 (t, $J_{3',4'}$ = $J_{4',5'}$ = 2.8 Hz, 1H, H-4′), 4.09–3.95 (m, 4H, H-2, H-6′′a, 3′′, 6′′′b), 3.69 (t, $J_{2,3}$ = $J_{3,4}$ = 8.5 Hz, 1H, H-3), 3.64 (dd, $J_{5'',6''}$ = 7.8 Hz, $J_{4'',5''}$ = 5.3 Hz, 1H, H-5′′), 3.61–3.59 (m, 1H, H-5′), 3.49 (s, 1H, H-5′′′), 3.46 (dd, $J_{1a,1b}$ = 9.7 Hz, $J_{1b,2}$ = 3.7 Hz, 1H, H-1b), 3.36 (dd, , $J_{6''a,6''b}$ = 11.6 Hz, $J_{5,6''b}$ = 7.8 Hz, 1H, H-6′′b), 1.95 (s, 3H, OAc), 1.94–1.92 (m, 2H, H-6), 1.69–1.61, 1.32–1.04 (2 x m, 70H, H-7–H-17, H-α–H-(ω-1)), 0.90 (t, $J_{17,18}$ = $J_{Hω\text{-}H\text{-}(ω\text{-}1)}$ = 6.7 Hz, , 6H, H-18, H-ω); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3 (HN<u>C</u>=O), 170.4 (C=O, OAc), 166.1 (C-3′′′), 165.93 (C-6′′), 165.90 (C-6′), 165.3 (C-2′′), 165.2, 164.6 (C2′′′, C3′), 164.1 (C-2′), 137.0 (C-5), 127.40 (C-4), 138.3, 137.9, 137.6, 133.5, 133.4, 133.35, 133.29, 133.1, 133.0, 132.6, 132.1, 131.9, 130.0, 129.89, 129.87, 129.76, 129.66, 129.47, 129.41, 129.38, 129.09, 129.05, 128.98, 128.93, 128.71, 128.58, 128.54, 128.47, 128.37, 128.30, 128.24, 128.19, 128.09, 128.00, 127.64, 127.35, 126.49, 125.32 (C_{arom}), 101.4 (C-1′), 101.00, 100.96 (C-1′′′, CH-benzylidene), 100.55 (C-1′′), 79.1 (C-3), 76.9 (C-3′′), 74.8 (C-4′), 73.1 (C-4′′′), 72.9 (C-5′), 72.5 (C-3′′′), 72.2 (C-3′), 72.1 (C-2′), 71.9 (C-5′′), 71.4(C-2′′), 70.2 (CH_2, 3-*O*-Bn), 69.0 (C-2′′′), 68.8 (C-4′′), 68.4 (C-1 & C-6′′′), 66.4 (C-5′′′), 62.4 (C-6′), 62.2 (C-6′′), 51.2 (C-2), 36.4, 32.2, 32.0, 29.8, 29.75, 29.73, 29.70, 29.68, 29.57, 29.56, 29.41, 29.40, 29.39, 29.27, 29.24, 25.5, 22.7, 21.5 (C-6–C-17, C-α–C-(ω-1)), 20.6 (OAc), 14.2 (C-18, C-ω); HRMS(ESI) m/z calcd. for $[C_{127}H_{157}NO_{26}+H]^+$: 2113.1067, obsd.: 2113.1055.



1,3-β-Gal-LacCer (βG-iGb3, **3**)

(2*S*,3*R*,4*E*)-2-(Hexacosanoylamido)-1-(4-*O*-(3-*O*-(β-D-galactopyranosyl)-β-D-galactopyranosyl)-β-D-glucopyranosyloxy)-3-hydroxy-octadec-4-ene (β-Gal-LacCer, 3): To a solution of fully protected β-Gal-LacCer **16c** (56.6 mg, 0.0265 mmol) in THF (2 mL), liquid NH_3 (15 mL) was added at -35 °C, followed by the careful addition of small pieces of Na (s) until the blue colour of the solution remained. The solution was stirred for 30 min under refluxing NH_3, quenched

via the addition of 4 drops of MeOH and then small pieces of Na (s) were added until the solution turned blue. After stirring the solution for a further 30 min under refluxing NH_3, the reaction mixture was quenched via the addition of MeOH (10 mL) and allowed to warm to room temperature so that the excess NH_3 could evaporate. The solution was then diluted with MeOH (10 mL) and CH_2Cl_2 (5 mL) and neutralised by the careful addition of Dowex H^+ until pH 7. The mixture was then filtered, with washing of the filtrate with pyridine (2 x 20 mL), and the solvent removed under reduced pressure to give the product as a clear oil that was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH, 20/1 to 3/1, v/v) then further purified by LH-20 size exclusion chromatography (CH_2Cl_2/MeOH, 1/1, v/v) to give β-Gal-LacCer (**3**) as an amorphous white solid (41%, 12.8 mg, 0.0109 mmol). R_f = 0.18 (CH_2Cl_2/MeOH, 1/5, v/v); $[\alpha]_D^{22}$ = +8.61 (c = 1.0, MeOH); IR (film) 3450, 2922, 2852, 1727, 1646, 1451, 1401, 1315, 1271, 1096, 1068, 1026, 711 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ 8.48 (d, $J_{NH,2}$ = 7.8 Hz 1H, NH), 6.04 (dd, $J_{4,5}$ = 15.4 Hz, $J_{3,4}$ = 6.6 Hz, 1H, H-4), 5.92 (dt, $J_{4,5}$ = 15.4 Hz, $J_{5,6}$ = 6.6 Hz, 1H, H-5), 5.30 (d, $J_{1''',2'''}$ = 7.8 Hz, 1H, H-1´´´), 5.09 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, H-1´´), 4.90 (d, $J_{1',2'}$ = 7.8 Hz, 1H, H-1´), 4.83–4.78 (m, 3H, H-2, H-3, H-1a), 4.68 (s, 1H, H-4´´´), 4.66 (t, $J_{2'',3''}$ = 8.3 Hz, 1H, H-2´´), 4.60 (d, $J_{3'',4''}$ = 2.9 Hz, 1H, H-4´´), 4.56–4.40 (m, 6H, H-2´´´, H-6´a, H-6´´a, H-6´´b, H-6´´´a, H-6´´´b), 4.31–4.12 (m, 6H, H-4´, H-6´b, H-3´, H-3´´, H-3´´´, H-1b), 4.10–4.06 (m, 3H, H-5´´, H-5´´´, H-2), 3.88–3.84 (H-5´), 2.46 (t, $J_{\alpha,\beta}$ = 7.5 Hz, 2H, H-α), 2.07 (q, $J_{6,7}$ = 7.1 Hz, 2H, H-6), 1.88 – 1.81 (m, 2H, H-β), 1.41 – 1.26 (m, 66H, H-7–H-17, H-γ–H-(ω-1)), 0.90 – 0.86 (m, 6H, H-18, H-ω); ^{13}C NMR (125 MHz, pyridine-d_5) δ 172.0 (HNC=O), 131.3 (C-5), 130.9 (C-4), 105.8 (C-1´´´), 104.0 (C-1´), 103.9 (C-1´´), 83.3 (C-3´´), 80.5 (C-4´), 75.7 (C-5´´ & C-5´´´), 75.2 (C-3´ & (C-5´), 73.8 (C-3´´´), 73.4 (C-2´), 71.8 (C-2´´´), 71.3 (C-3), 70.1 (C-2´´) 69.1 (C-1), 68.8 (C-4´´)68.1 (C-4´´´), 60.9, 60.6, 60.3 (C-6´, C-6´´, C-6´´´), 53.5 (C-2), 35.5 (C-α), 31.4 (C-6), 25.0 (C-β), 30.74, 30.72, 28.64, 28.62, 28.60, 28.51, 28.41, 28.33, 28.24, 28.23, 28.21, 21.60, 21.54 (C-7–C-17, C-γ–C-(ω-1)), 12.9 (C-18, C-ω); HRMS(ESI) m/z calcd. for $[C_{62}H_{117}NO_{18}+H]^+$: 1164.8343, obsd.: 1164.8315.

NMR Spectra

^{1}H NMR, $CDCl_3$, 500 MHz

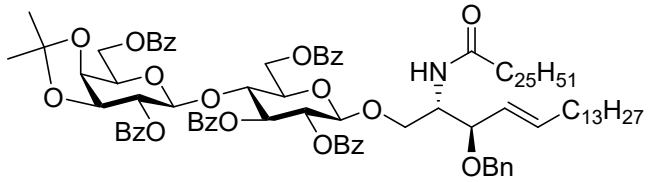


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Figure S1: ^{1}H NMR of (2*S*,3*R*,4*E*)-1-(4-*O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)- 2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-hexacosanoylamido-octadec-4-ene (**14**).

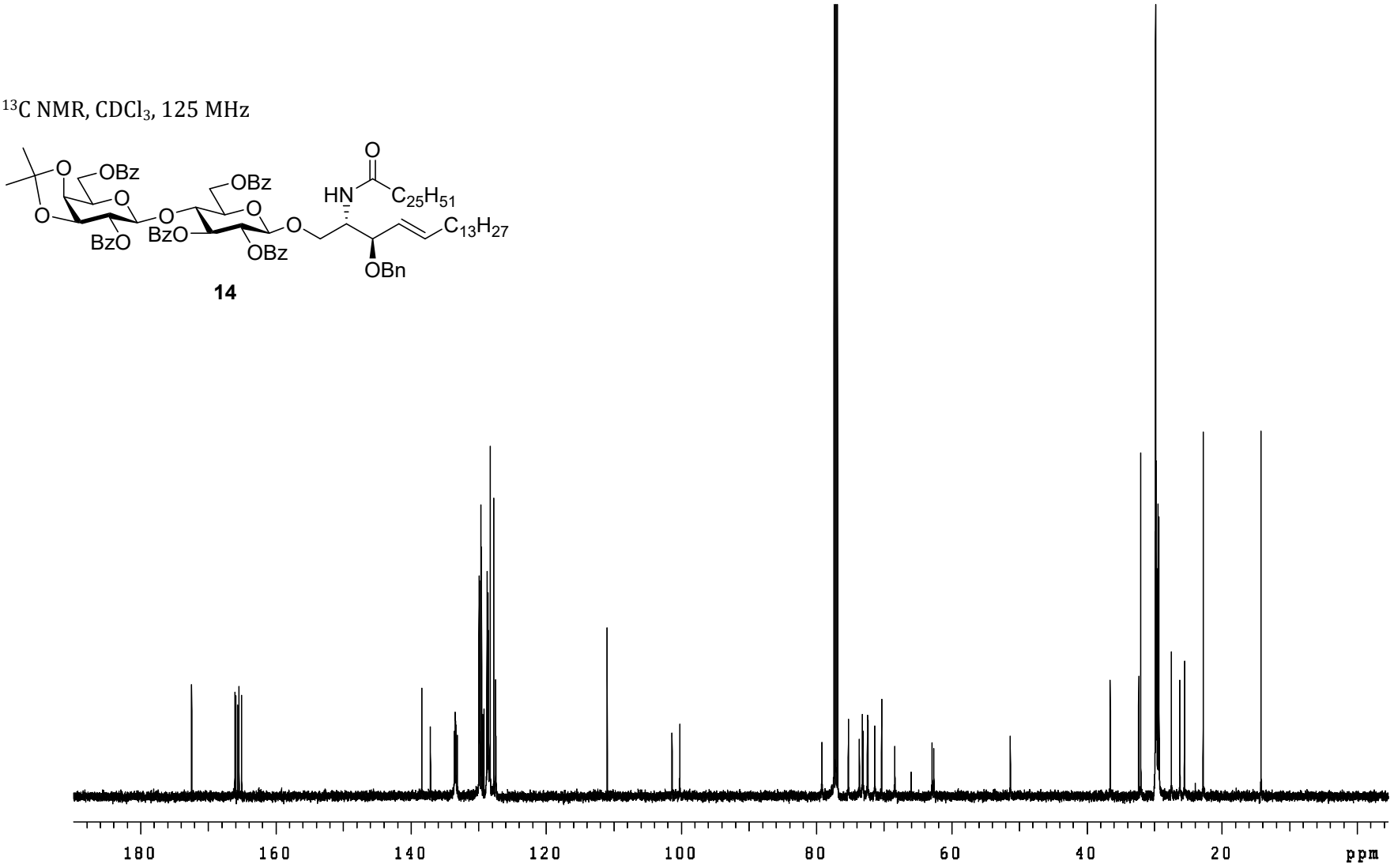

Figure S2: ^{13}C NMR of (2*S*,3*R*,4*E*)-1-(4-*O*-(2,6-Di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)- 2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-hexacosanoylamido-octadec-4-ene (**14**).

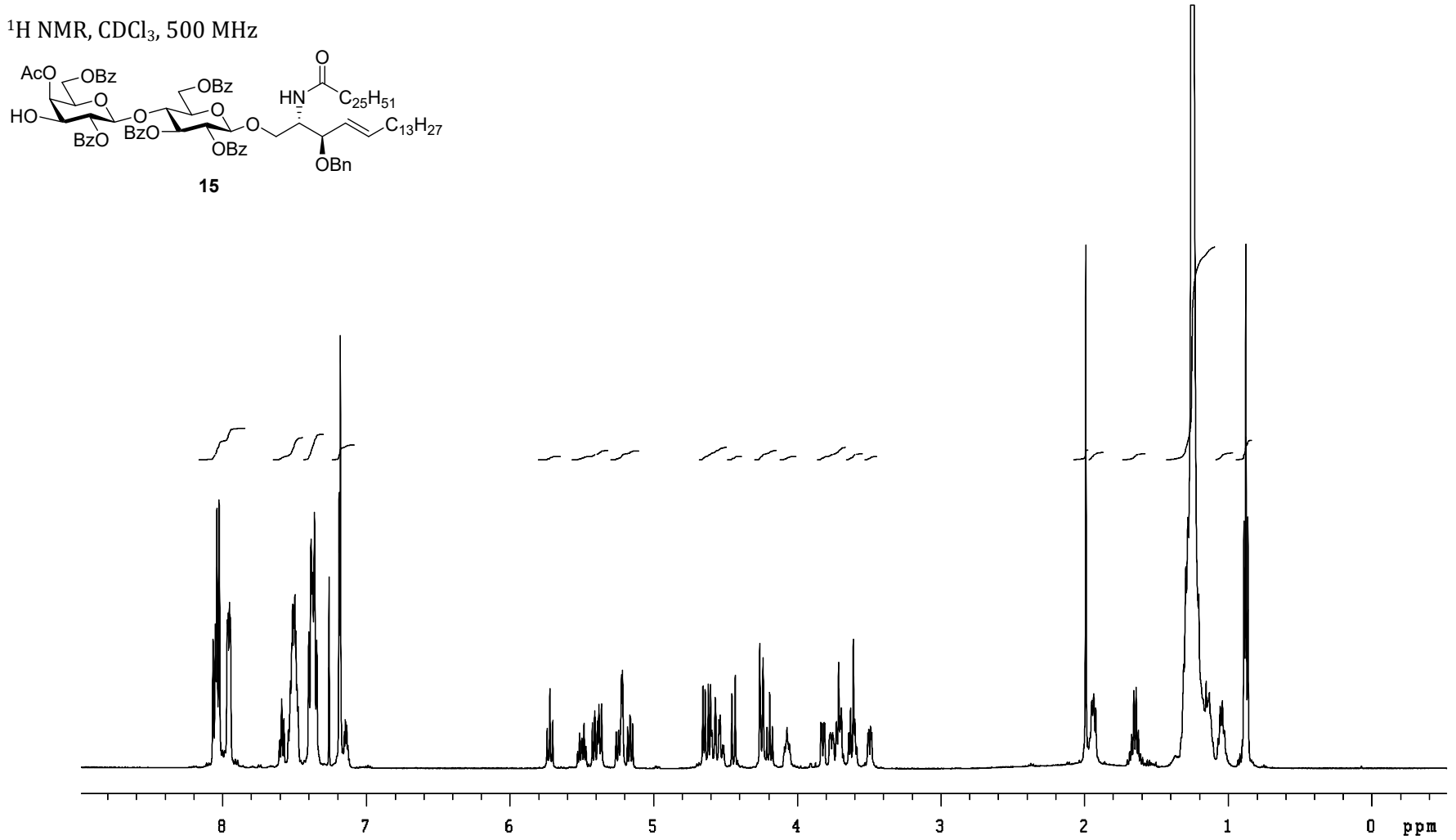

Figure S3: ^{1}H NMR of (2*S*,3*R*,4*E*)-1-(4-*O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranosyloxy)-3-benzyloxy-2-hexacosanoylamido-octadec-4-ene (**15**).

^{13}C NMR, $CDCl_3$, 125 MHz

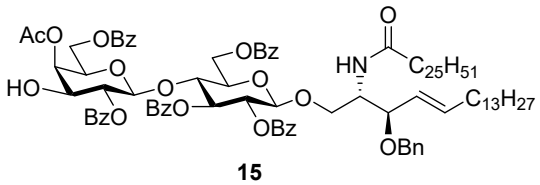

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Figure S4: ^{13}C NMR of (2*S*,3*R*,4*E*)-1-(4-*O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranosyloxy)-3-benzyloxy-2-hexacosanoylamido-octadec-4-ene (**15**).

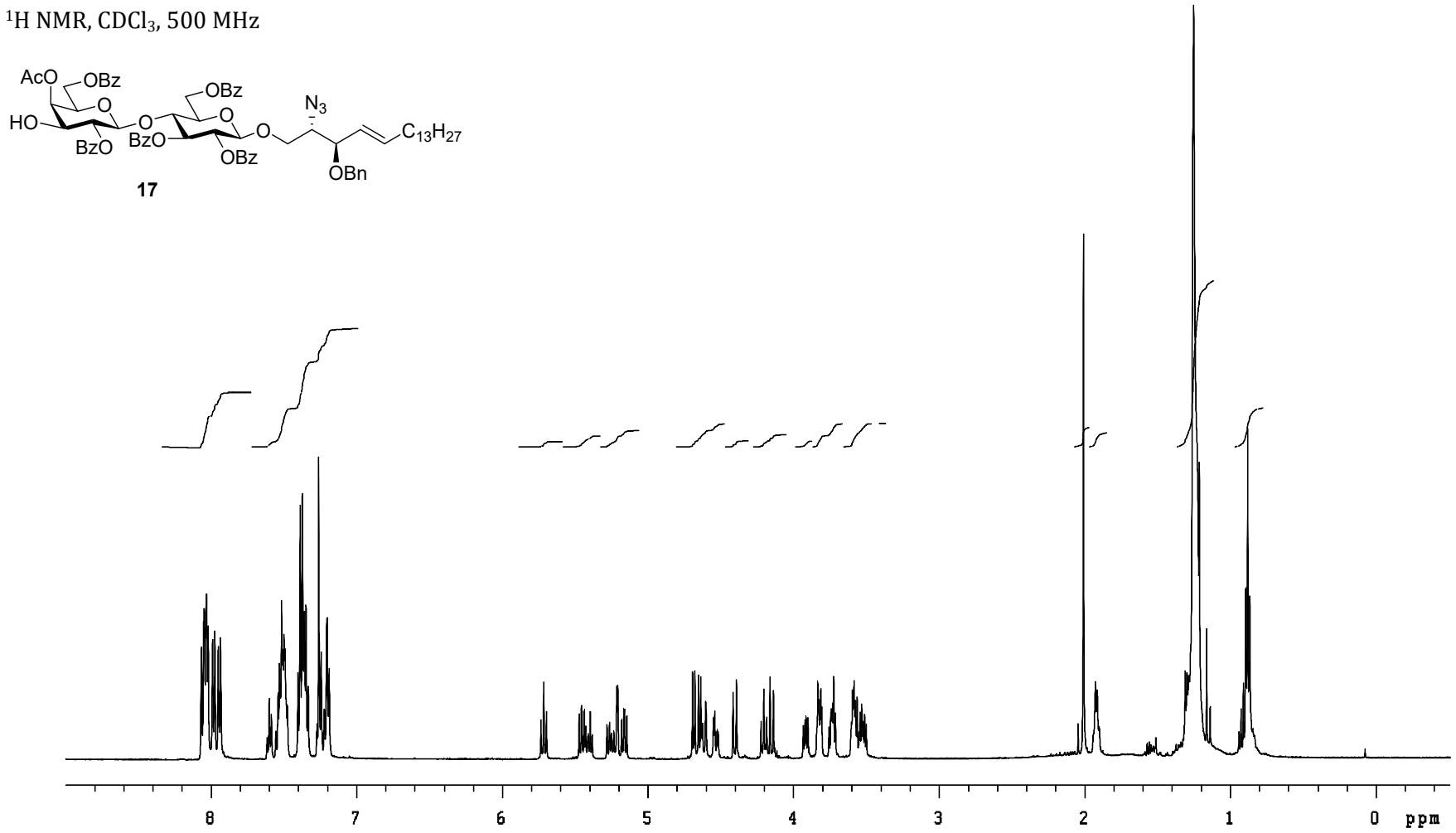

Figure S5: ^{1}H NMR of (2*S*,3*R*,4*E*)-1-(4-*O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-azido-octadec-4-ene (**17**).

^{13}C NMR, $CDCl_3$, 125 MHz

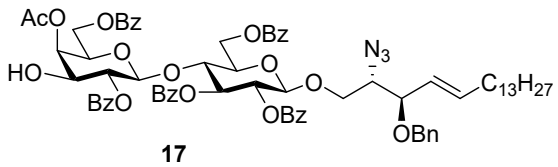

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Figure S6: ^{13}C NMR of (2*S*,3*R*,4*E*)-1-(4-*O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-azido-octadec-4-ene (**17**).

^{1}H NMR, $CDCl_3$, 300 MHz

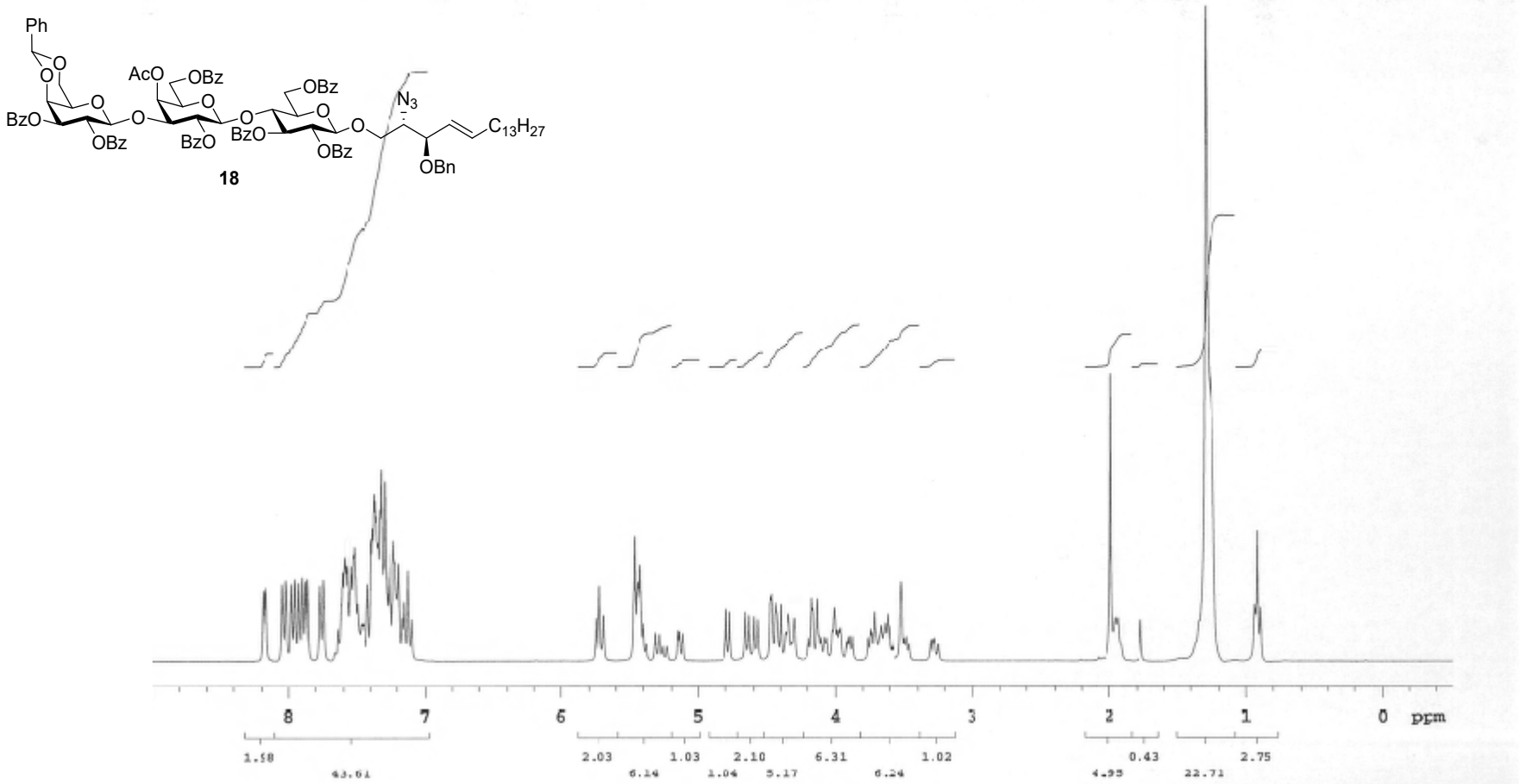


Figure S7: ^{1}H NMR of (2*S*,3*R*,4*E*)-1-(4-*O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-azido-octadec-4-ene (**18**).

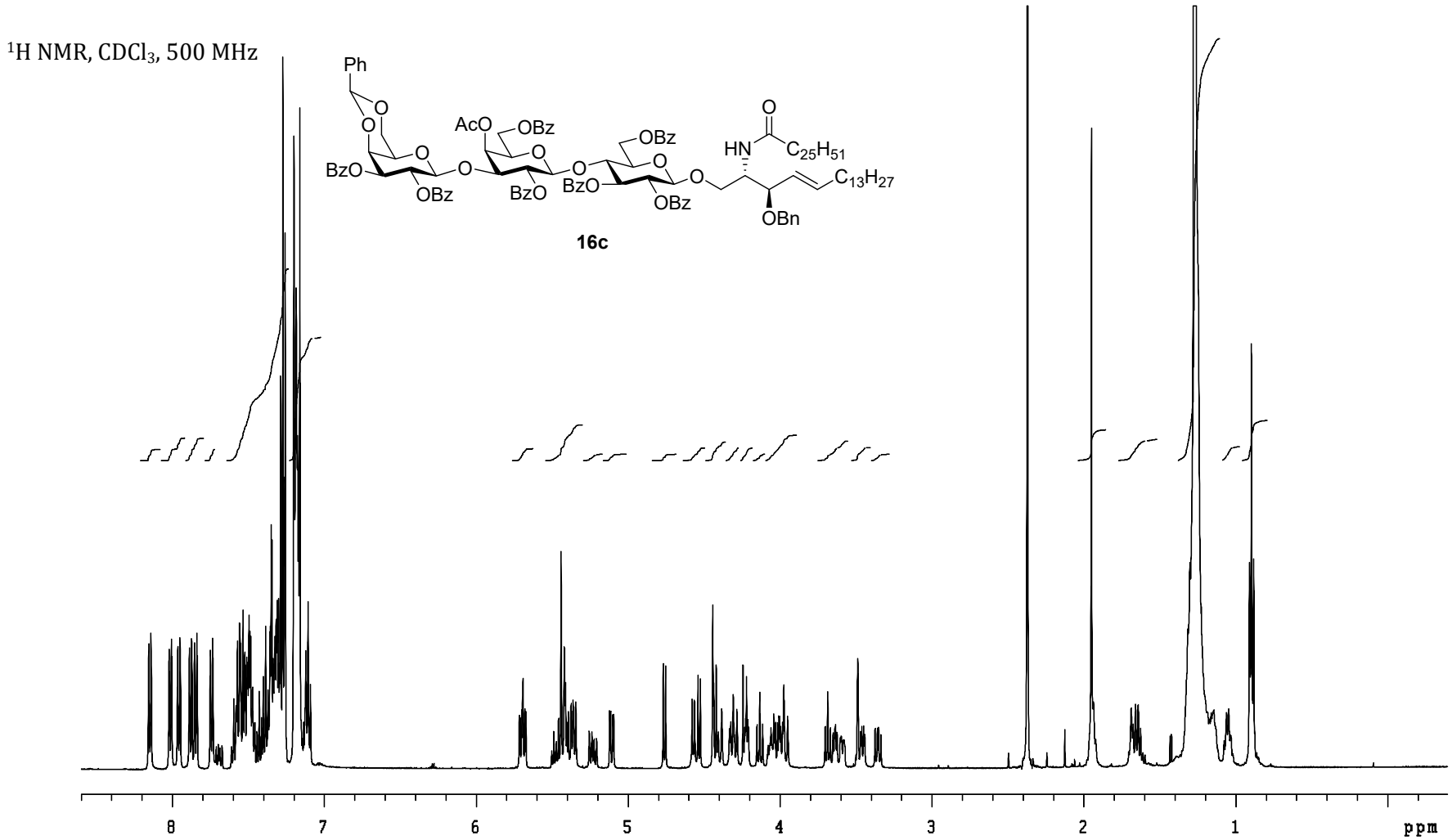


Figure S8: ^{1}H NMR of (2*S*,3*R*,4*E*)-1-(4-*O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-(hexacosanoylamido)-octadec-4-ene (**16c**).

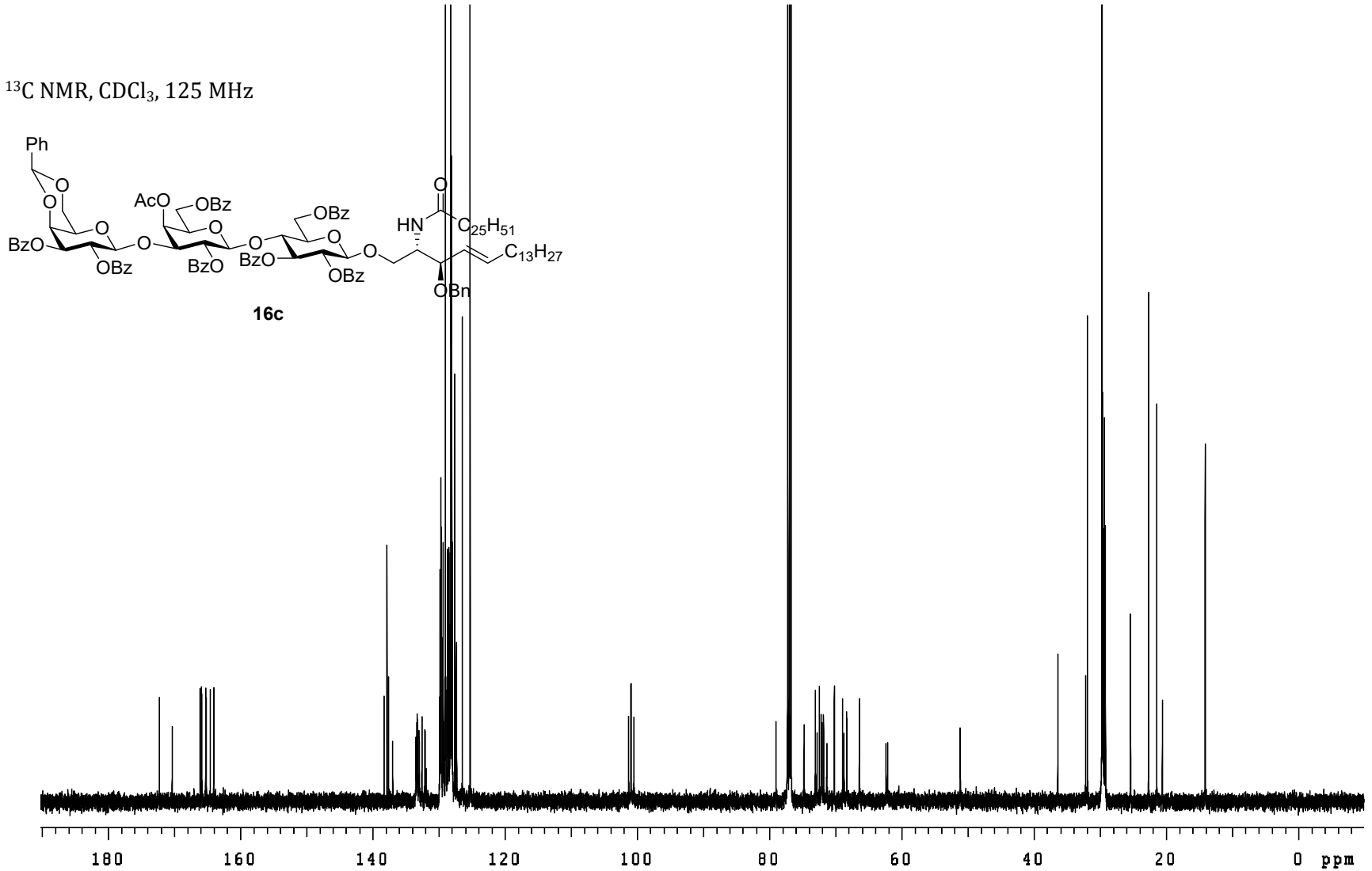


Figure S9: ^{13}C NMR of (2*S*,3*R*,4*E*)-1-(4-*O*-(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyloxy)-3-benzyloxy-2-(hexacosanoylamido)-octadec-4-ene (**16c**).

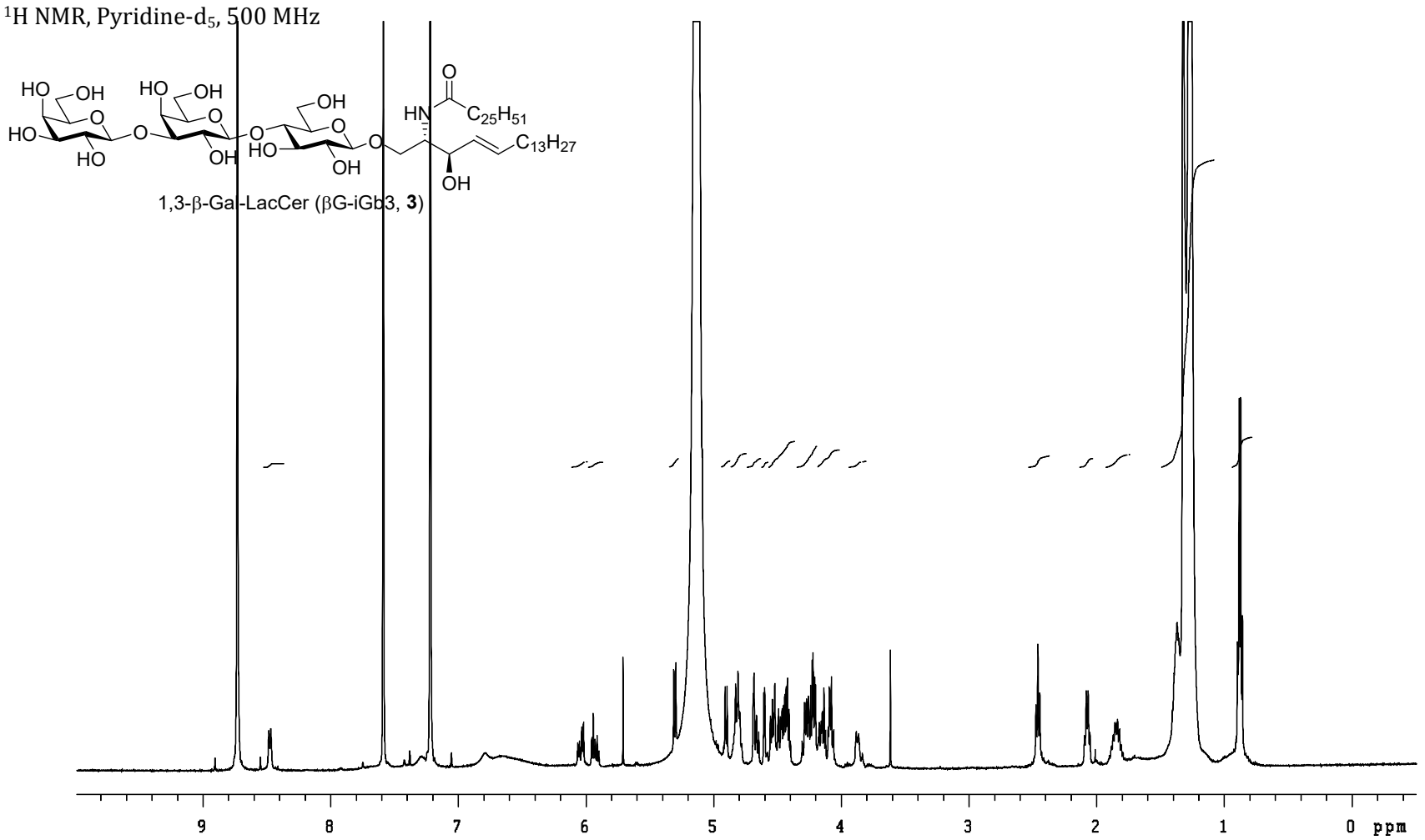


Figure S10: ^{1}H NMR of (2*S*,3*R*,4*E*)-2-(Hexacosanoylamido)-1-(4-*O*-(3-*O*-(β-D-galactopyranosyl)-β-D-galactopyranosyl)-β-D-glucopyranosyloxy)-3-hydroxy-octadec-4-ene (β-Gal-LacCer, **3**).

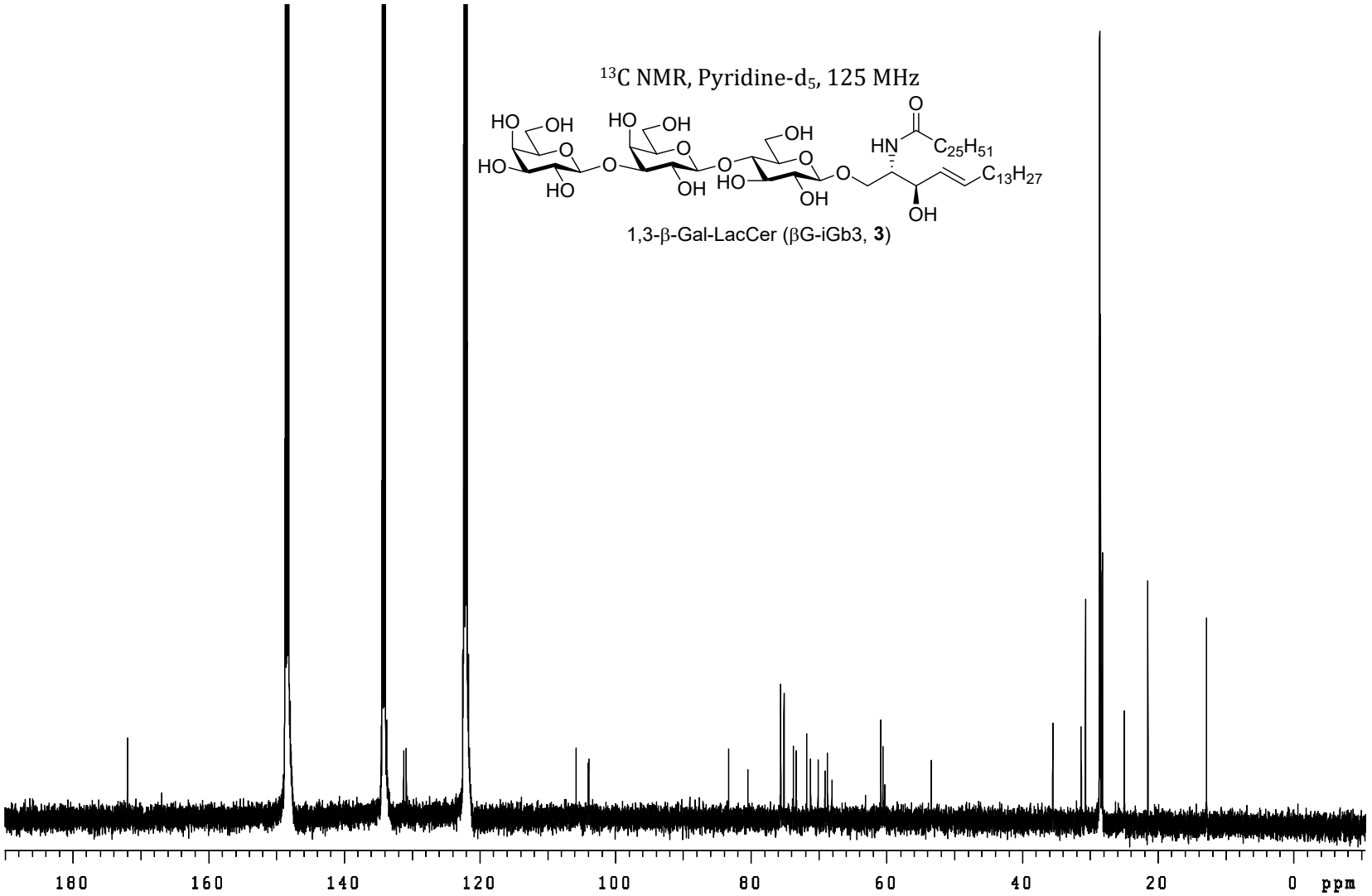


Figure S11: ^{13}C NMR of (2*S*,3*R*,4*E*)-2-(Hexacosanoylamido)-1-(4-*O*-(3-*O*-(β-D-galactopyranosyl)-β-D-galactopyranosyl)-β-D-glucopyranosyloxy)-3-hydroxy-octadec-4-ene (β-Gal-LacCer, **3**).

^{1}H NMR, $CDCl_3$, 500 MHz

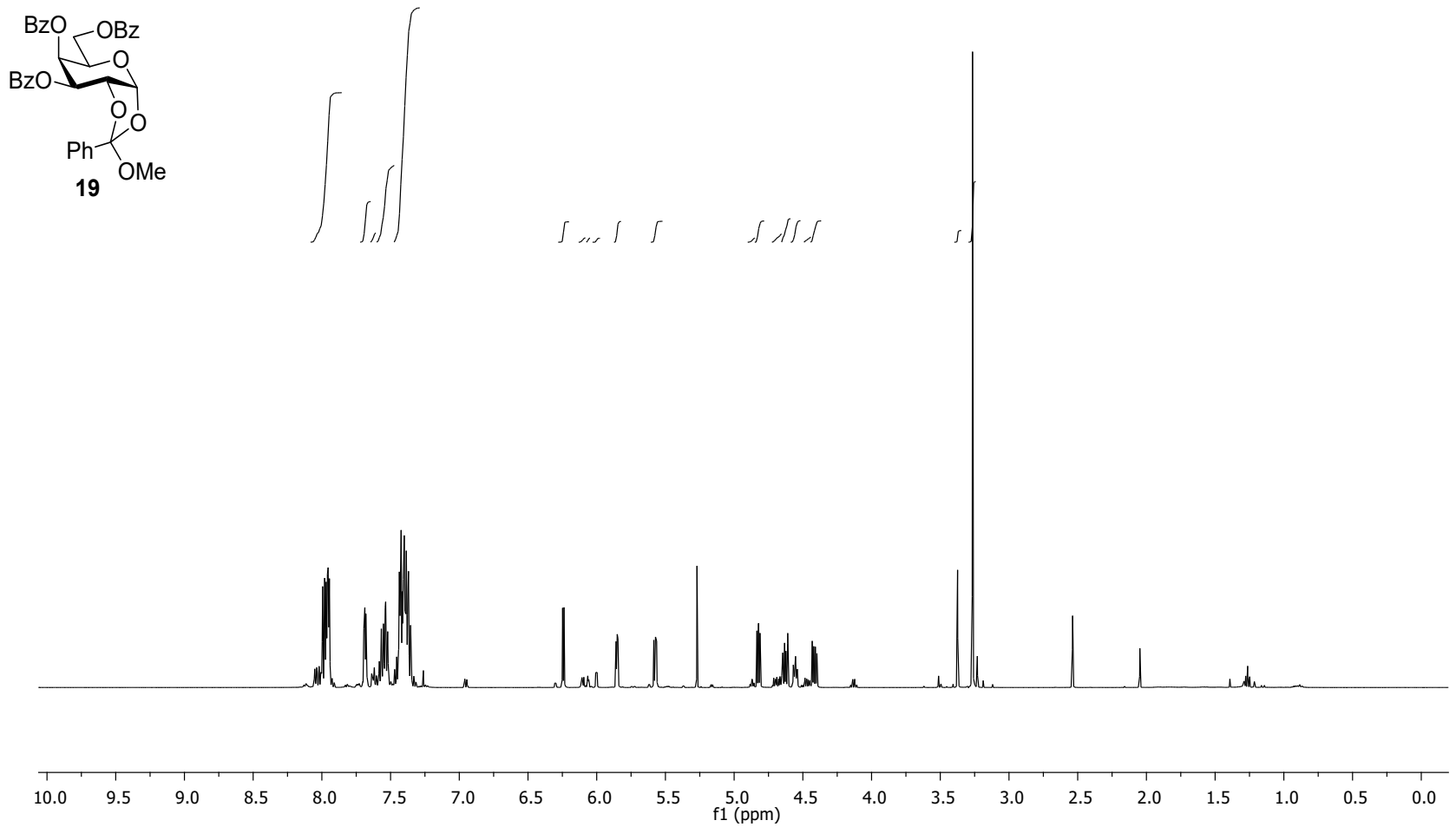
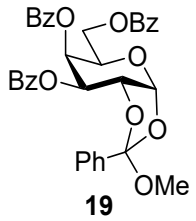


Figure S12: ^{1}H NMR of 3,4,6-Tri-*O*-benzoyl-1,2-*O*-(α-methoxy)benzylidene-α-D-galactopyranose (**19**).

^{13}C NMR, $CDCl_3$, 125 MHz



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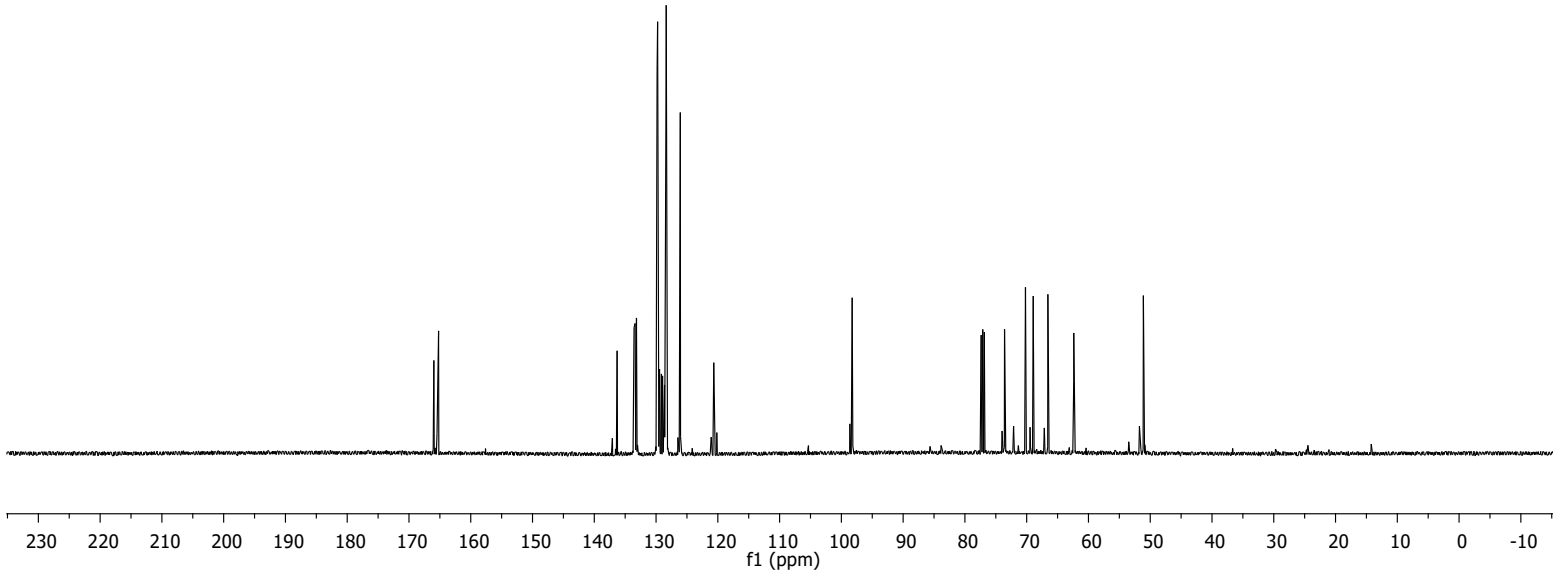

Figure S13: ^{13}C NMR of 3,4,6-Tri-*O*-benzoyl-1,2-*O*-(α-methoxy)benzylidene-α-D-galactopyranose (**19**).

^{1}H NMR, $CDCl_3$, 300 MHz

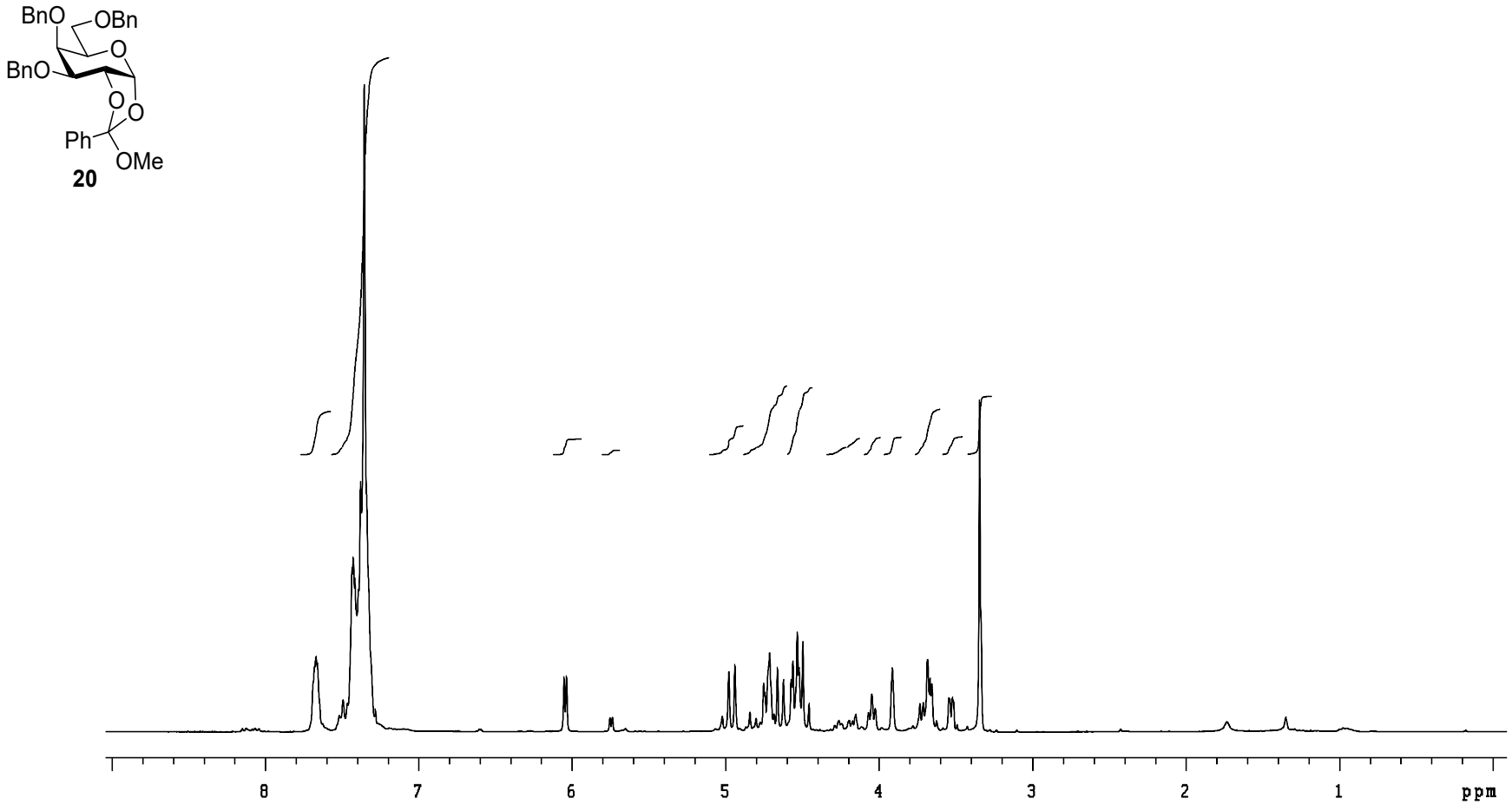

Figure S14: ^{1}H NMR of 3,4,6-Tri-*O*-benzyl-1,2-*O*-(α-methoxy)benzylidene-α-D-galactopyranose (**20**).

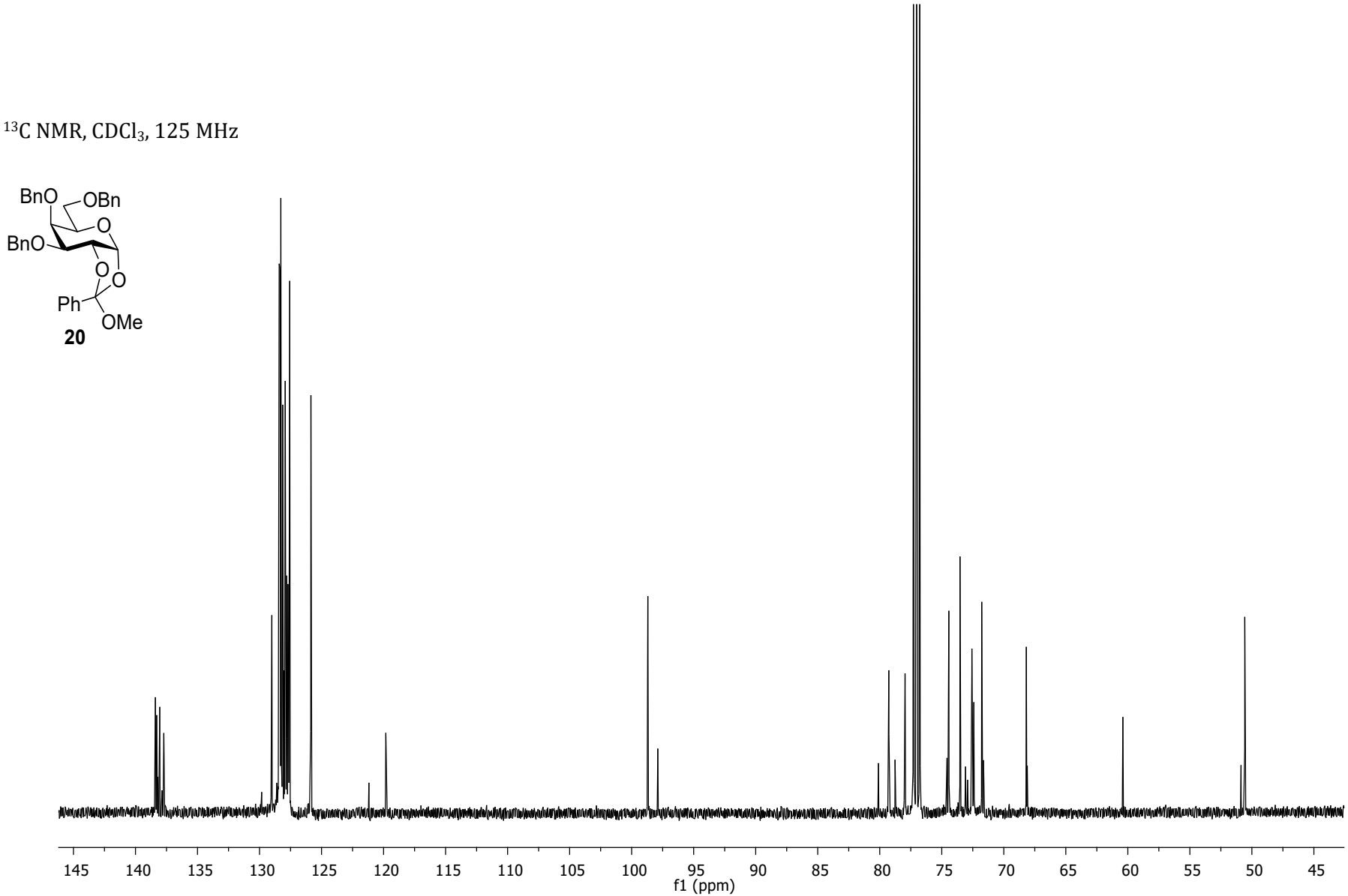

Figure S15: ^{13}C NMR of 3,4,6-Tri-*O*-benzyl-1,2-*O*-(α-methoxy)benzylidene-α-D-galactopyranose (**20**).

^{1}H NMR, $CDCl_3$, 500 MHz

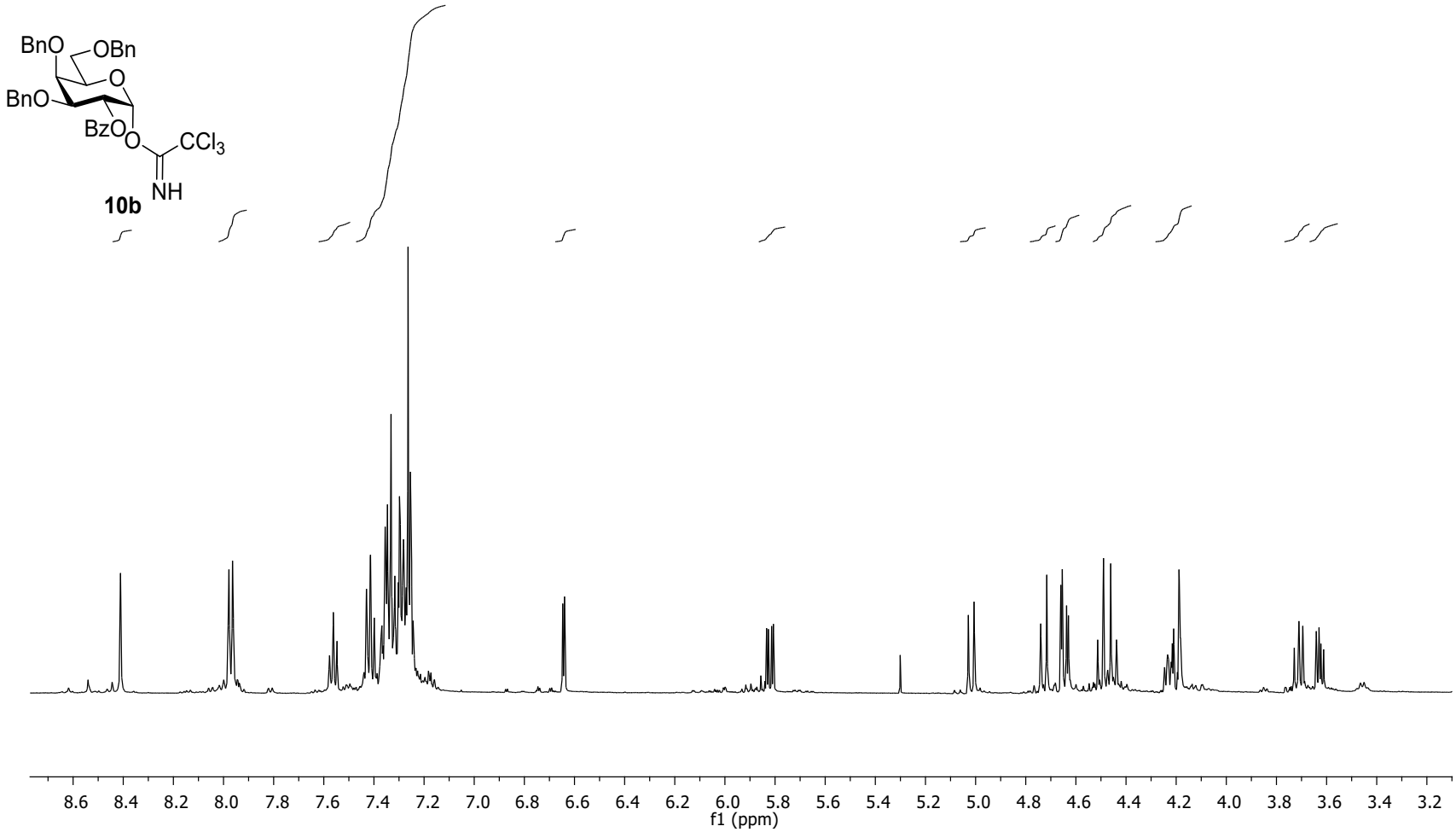

Figure S16: ^{1}H NMR of *O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-1,2-*O*-(α-methoxy)benzylidene-α-D-galactopyranosyl) trichloroacetimidate (**10b**).

^{13}C NMR, $CDCl_3$, 125 MHz

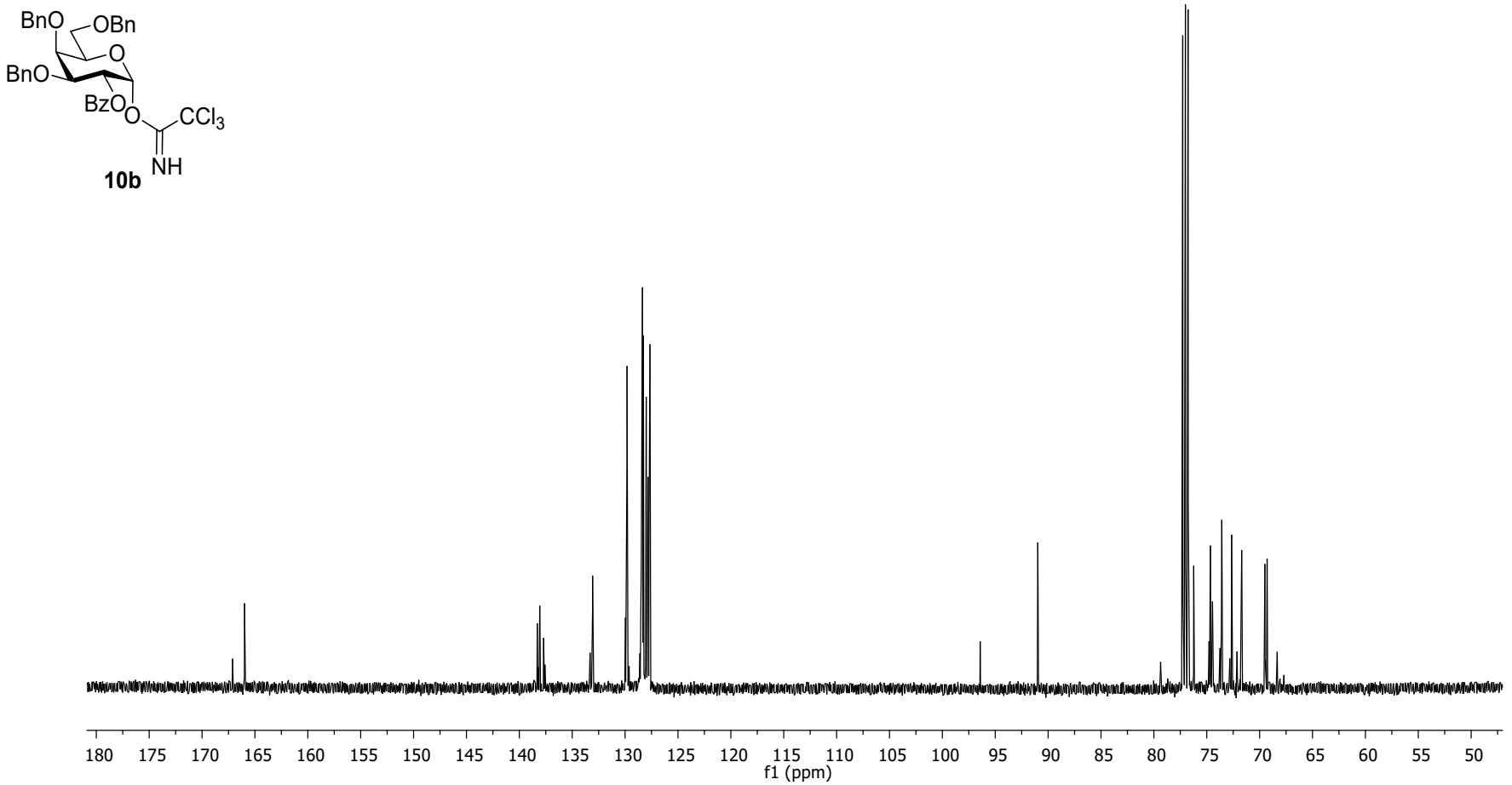

Figure S17: ^{13}C NMR of O-(2-O-Benzoyl-3,4,6-tri-O-benzyl-1,2-O-(α-methoxy)benzylidene-α-D-galactopyranosyl) trichloroacetimidate (**10b**).

^{1}H NMR, $CDCl_3$, 500 MHz

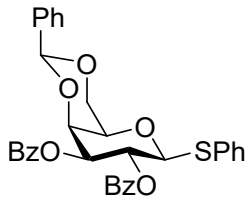

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Figure S18: ^{1}H NMR of Phenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-thiogalactopyranoside (**22**).

^{13}C NMR, $CDCl_3$, 125 MHz

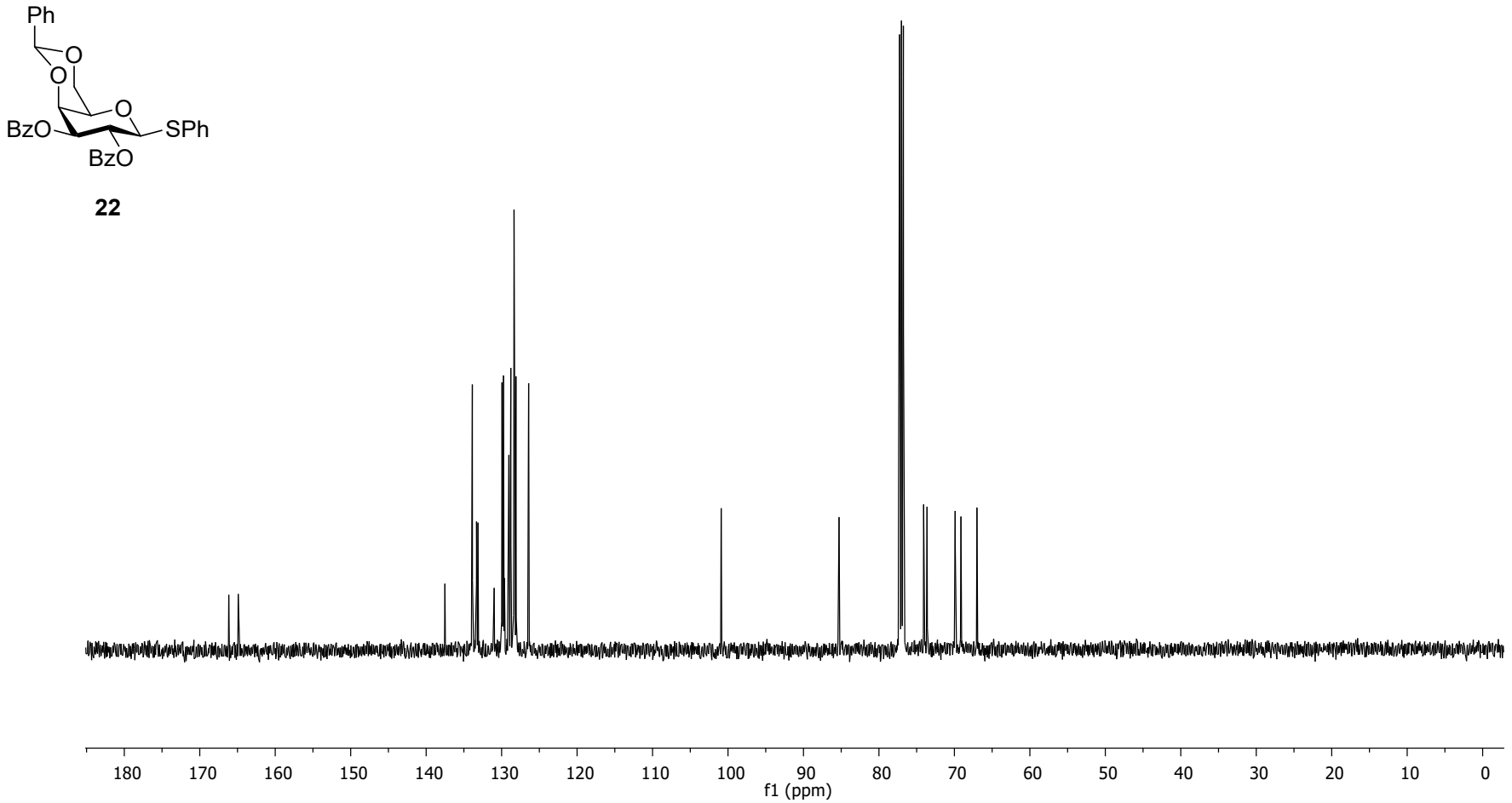

Figure S19: ^{13}C NMR of Phenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-thiogalactopyranoside (**22**).

^{1}H NMR, $CDCl_3$, 500 MHz

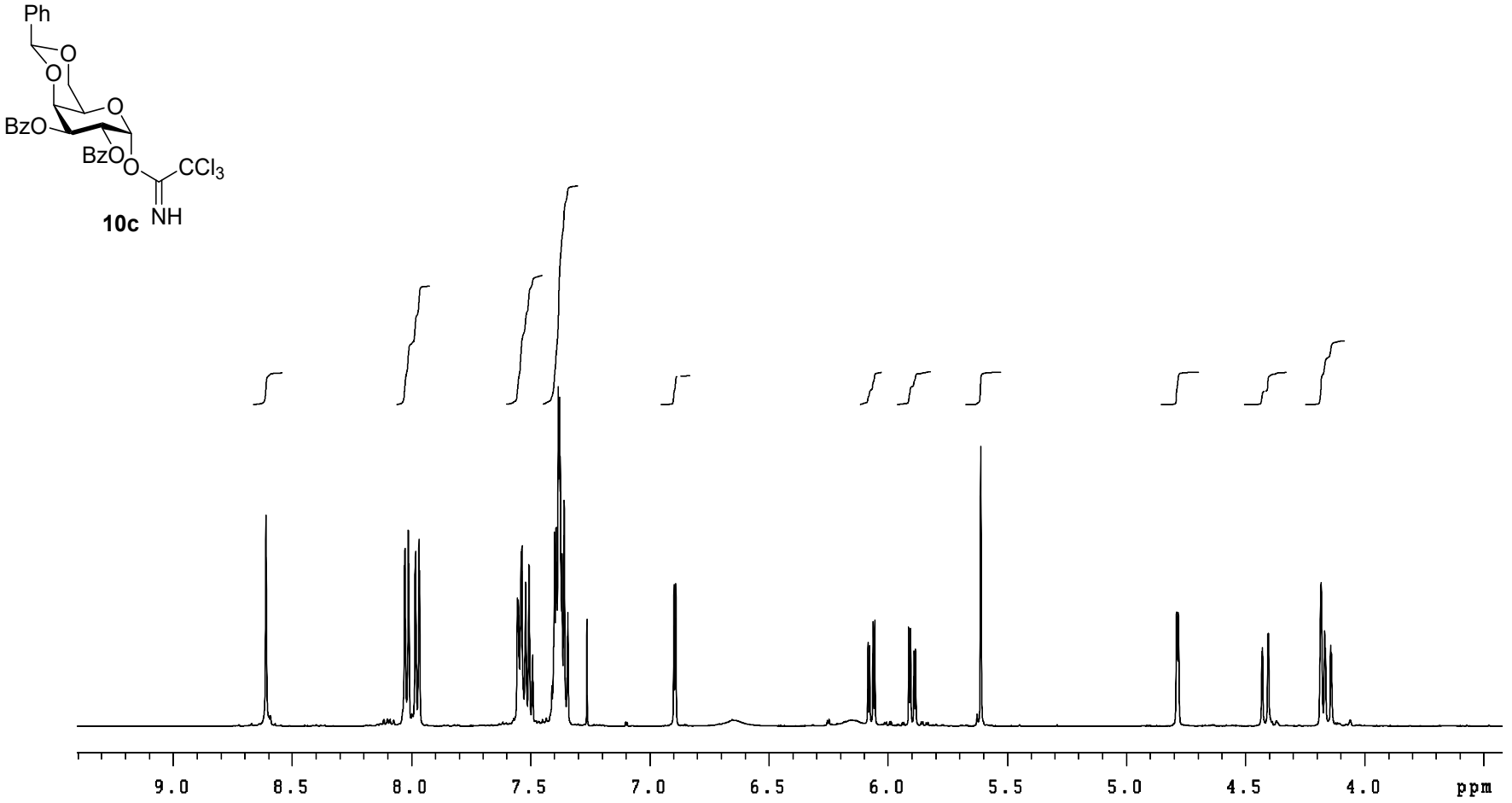

Figure S20: ^{1}H NMR of *O*-(2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-galactopyranosyl) trichloroacetimidate (**10c**).

^{13}C NMR, $CDCl_3$, 125 MHz

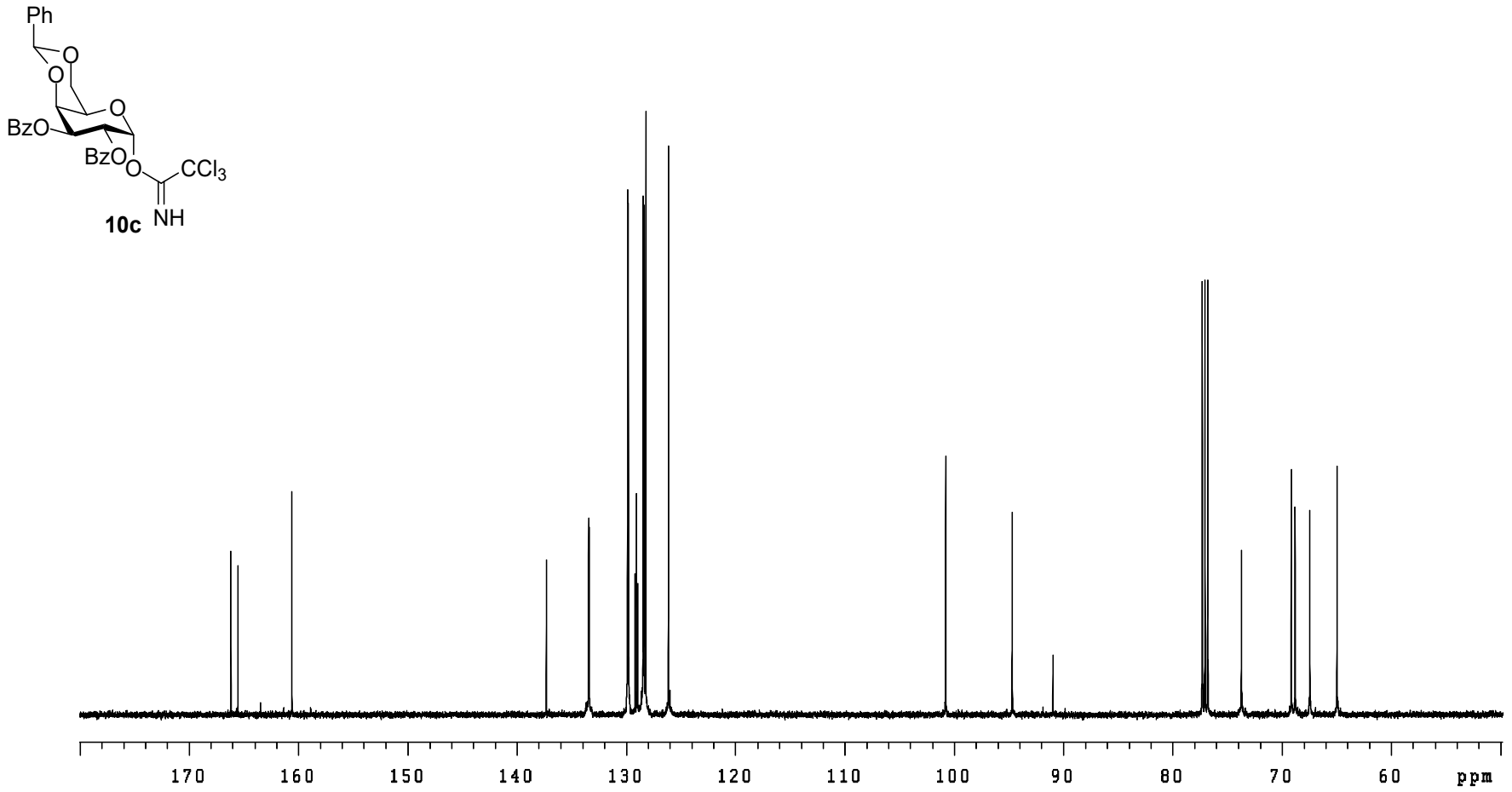

Figure S21: ^{13}C NMR *O*-(2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-galactopyranosyl) trichloroacetimidate (**10c**).

References

1. J. M. H. Cheng, E. M. Dangerfield, M. S. M. Timmer, B. L. Stocker. *Org. Bio. Chem.* **2014**, *12*, 2729-2736.
2. W. Pilgrim, P. V. Murphy, *J. Org. Chem.* **2010**, *75*, 6747–6755.
3. M. S. M. Timmer, B. L. Stocker, P. T. Northcote, B. A. Burkett BA. *Tetrahedron Lett.* **2009**, *50*, 7199-7204.
4. A. R. Parameswar, J. A. Hawkins, L. K. Mydock, M. S. Sands, A. V. Demchenko, *Eur. J. Org. Chem.* **2010**, *17*, 3269–3274.