Supplementary Information

Potent Inhibition of Ice Recrystallization by Low Molecular Weight Carbohydrate-Based Surfactants and Hydrogelators

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Supplemental IRI Activity of *N*-Octyl-D-Gluconamide (NOGlc, 5)

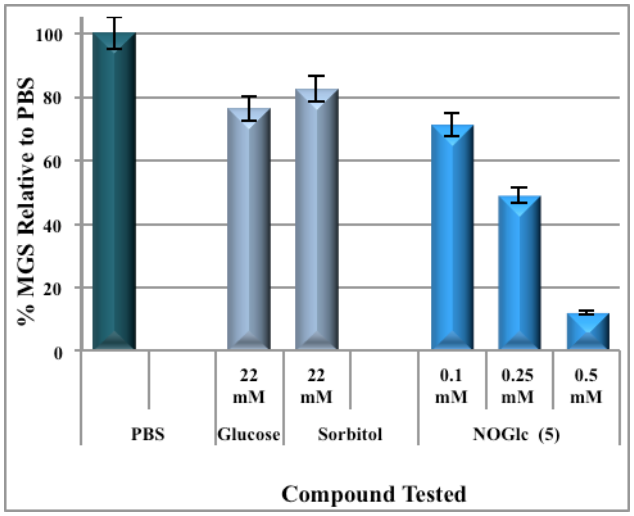


Fig. S1 IRI activity of D-glucose, D-sorbitol and low molecular weight hydrogelator **5** (NOGlc). All compounds are represented as a % MGS (mean grain size) of ice crystals relative to the PBS positive control.

Supplemental Solid-State NMR Data

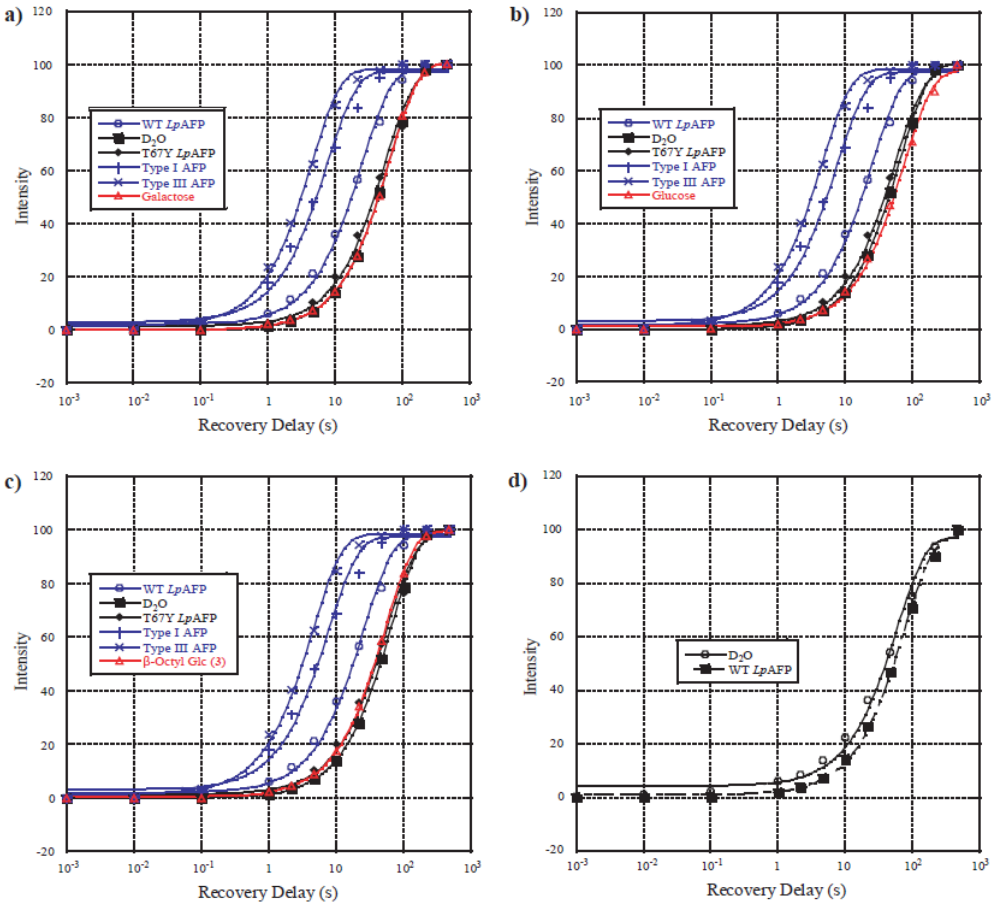


Fig. S2 Saturation recovery curves of frozen D_2O with AFPs or carbohydrate derivatives. The concentration of all carbohydrate derivatives was corrected to a total overall proton concentration of 1234 mM, unless stated otherwise. The ^2H relaxation rate for frozen D_2O was $R_1 = 0.0154 \pm 0.0002$ s^{-1}. (a) AFP controls and D-galactose ($R_1 = 0.0154 \pm 0.0003$ s^{-1}). The concentration at which D-galactose was measured was 103 mM. (b) AFP controls and D-glucose ($R_1 = 0.0132 \pm 0.0001$ s^{-1}). The concentration at which D-glucose was measured was 103 mM. (c) AFP controls and derivative **3** ($R_1 = 0.0190 \pm 0.0002$ s^{-1}). The concentration at which **3** was measured was 44 mM. (d) WT *Lp*AFP ($R_1 = 0.0133 \pm 0.0005$ s^{-1}) and frozen D_2O. The concentration at which WT *Lp*AFP was measured was 0.5 mM, which corresponds to a total overall proton concentration of 433 mM.

Assessment of Antifreeze Activity

Thermal Hysteresis (TH) Assay

Nanoliter osmometry was performed using a Clifton nanoliter osmometer (Clifton Technical Physics, Hartford, NY), as described by Chakrabartty and Hew.[1] All of the measurements were performed in doubly distilled water. Ice crystal morphology was observed through a Leitz compound microscope equipped with an Olympus 20× (infinity-corrected) objective, a Leitz Periplan 32X photo eyepiece, and a Hitachi KPM2U CCD camera connected to a Toshiba MV13K1 TV/VCR system. Still images were captured directly using a Nikon CoolPix digital camera.

Ice Recrystallization Inhibition (IRI) Assay

Sample analysis for IRI activity was performed using the "splat cooling" method as previously described.[2] In this method, the analyte was dissolved in phosphate buffered saline (PBS) solution and a 10 μL droplet of this solution was dropped from a micropipette through a two meter high plastic tube (10 cm in diameter) onto a block of polished aluminum precooled to approximately -80 °C. The droplet froze instantly on the polished aluminum block and was approximately 1 cm in diameter and 20 μm thick. This wafer was then carefully removed from the surface of the block and transferred to a cryostage held at -6.4 °C for annealing. After a period of 30 min, the wafer was photographed between crossed polarizing filters using a digital camera (Nikon CoolPix 5000) fitted to the microscope. A total of three images were taken from each wafer. During flash freezing, ice crystals spontaneously nucleated from the supercooled solution. These initial crystals were relatively homogeneous in size and quite small. During the annealing cycle, recrystallization occurred, resulting in a dramatic increase in ice crystal size. A quantitative measure of the difference in recrystallization inhibition of two compounds X and Y is the difference in the dynamics of the ice crystal size distribution. Image analysis of the ice wafers was performed using a novel domain recognition software (DRS) program.[3] This processing employed the Microsoft Windows Graphical User Interface to allow a user to visually demarcate and store the vertices of ice domains in a digital micrograph. The data was then used to calculate the domain areas. All data was plotted and analyzed using Microsoft Excel. The mean grain (or ice crystal) size (MGS) of the sample was compared to the MGS of the control PBS solution for that same day of testing. IRI activity is reported as the percentage of the MGS (% MGS) relative to the PBS control, and the % MGS for each sample was plotted along with its standard error of the mean. Large percentages represent a large MGS, which is indicative of poor IRI activity.

Recombinant Expression and Purification of *Lp*AFP

WT and T67Y *Lp*AFP were recombinantly expressed in *E. coli* BL21 DE3 as described previously.[4] The gene sequences for His-tagged WT and T67Y *Lp*AFP in a pET 24a+ vector were verified by DNA sequencing (OHRI, Ottawa, ON, Canada). After transformation, WT or T67Y *Lp*AFP cultures were grown in 750 mL of LB media containing 50 μg/mL of kanamycin at 37 °C until an $OD_{600\ nm}$ of ~0.6 was reached. Growth was continued at 24 °C until the $OD_{600\ nm}$ reached ~1.0 before protein expression was

[1] A. Chakrabartty and C. L. Hew, *Eur. J. Biochem*., 1991, **202**, 1057.
[2] C. A. Knight, J. Hallett and A. L. DeVries, *Cryobiology*, 1988, **25**, 55.
[3] J. Jackman, M. Noestheden, D. Moffat, J. P. Pezacki, S. Findlay and R. N. Ben, *Biochem. Biophys. Res. Commun*., 2007, **354**, 340.
[4] A. J. Middleton, A. M. Brown, P. L. Davies and V. K. Walker, *FEBS Lett*., 2009, **583**, 815.

induced by the addition of IPTG to a final concentration of 0.2 mM. After overnight growth at 24 °C, the cells were harvested by centrifugation at 4 °C and resuspended in 50 mL of lysis buffer (50 mM Tris-HCl and 100 mM NaCl). The resuspended cells were lysed by boiling for 8 min and allowed to cool to 4 °C on ice. The lysate was clarified by centrifugation at 8000 RPM for 1 h at 4 °C and the resulting supernatant was purified by nickel affinity chromatography. The supernatant was incubated with Ni-NTA superflow (QIAGEN) for 1 h at 4 °C, then loaded to the column and the flow-through was collected. After washing the column with elution buffer (100 mM Tris and 300 mM NaCl), the protein was eluted with elution buffer and an imidazole gradient (20 mM, then 2 X 100 mM, then 3 X 250 mM). Fractions containing WT or T67Y *Lp*AFP were collected and purified by centrifugation with an Amicon Ultra-15 Filter Device (Millipore) by washing five times with H_2O (15 mL). The protein solution was then concentrated to a final volume of 1 mL. Purity was analyzed using SDS-PAGE. Pure WT or T67Y *Lp*AFP were then lyophilized and stored as a white powder at -78 °C. WT or T67Y *Lp*AFP were dissolved in D_2O to the desired concentration for the solid-state NMR measurements. Prior to measuring the saturation recovery curves of frozen D_2O with these proteins, TH activity was measured in D_2O and TH values for both WT *Lp*AFP and T67Y *Lp*AFP were consistent with what has previously been reported.[4]

Solid-State NMR Spectroscopy

All experiments were conducted at a magnetic field of 9.39 T ($\nu_0(^2H)$ = 61.422 MHz) and a temperature of -25 °C using a Bruker 7 mm double resonance wideline probe. The D_2O solutions were pipetted into a Bruker 7 mm o.d. rotors and frozen prior to use. ^2H spin-lattice relaxation times (R_1) were measured using a saturation recovery sequence. The signal was detected using a quadrupolar echo sequence (90° - τ - 90° - τ - acquire) and was saturated, prior to a recovery delay, with the use of fifty 45° saturation pulses. The 90° pulse length was of 3.88 μs and the echo delay (τ) was set to 30 μs.[5]

Rheology

Dynamical shear stress measurements were performed on Physica MCR 301 (Anton Paar, Graz, Austria) rheometer using a 25mm diameter plate-plate geometry with a 1mm gap. Plate surfaces were roughened to minimize hydrogel slip and pre-heated to 80 °C. The analyte was dissolved in either de-ionied water or phosphate buffered saline (PBS) solution heated to 95 °C to achieve a homogeneous fluid solution. After loading between rheometer plates, the solutions were cooled to 37 ^oC at a rate of 10 ^oC/min and the resulting gel phase was allowed to equilibrate for an additional 5 min at 37 ^oC before measurements commenced. Dynamic oscillation mode was used to measure the storage and loss components of the dynamic shear modulus of the hydrogels at a shear strain amplitude of 0.25% from 0.1 to 10 rads/s. All tests were made in a controlled humidity and temperature cell at 37 ^oC and saturated vapor conditions.

[5] A. B. Siemer, K.-Y. Huang and A. E. McDermott, *Proc. Natl. Acad. Sci. U.S.A.*, 2010, **107**, 17580

Scanning Transmission Electron Microscopy

Negatively stained gel solutions for the electron microscopy study were prepared by adding 1% phosphotungstic acid to the hydrogel solutions at their desired concentrations (1% w/v for NOGlc (**5**) and 0.5% w/v for NOGal (**6**)). Carbon film coated grids were dipped into the stained gel solutions, which were heated in a water bath until solutions became clear. The dipped grids were subsequently dried at room temperature. Bright field scanning transmission electron micrographs were obtained using a JEOL JSM-7500F field emission scanning electron microscope, operating under transmission electron imaging mode.

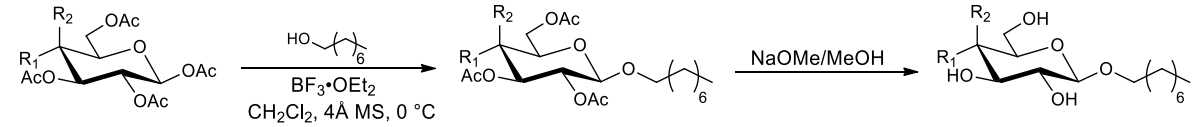
Experimental Section

General Experimental

All anhydrous reactions were performed in flame-dried glassware under a positive pressure of dry argon. Air or moisture-sensitive reagents and anhydrous solvents were transferred with oven-dried syringes or cannulae. All flash chromatography was performed with E. Merck silica gel 60 (230-400 mesh). All solution phase reactions were monitored using analytical thin layer chromatography (TLC) with 0.2 mm pre-coated silica gel aluminum plates 60 F254 (E. Merck). Components were visualized by illumination with a short-wavelength (254 nm) ultra-violet light and/or staining (ceric ammonium molybdate, potassium permanganate, or phosphomolybdate stain solution). All solvents used for anhydrous reactions were distilled. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium/benzophenone under nitrogen. Dichloromethane (DCM) was distilled from calcium hydride. *N,N*-dimethylformamide (DMF) was stored over activated 4Å molecular sieves under argon. ^1H (400 or 500 MHz) and ^{13}C NMR (100 or 125 MHz) spectra were recorded at ambient temperature on a Bruker Avance 400, Bruker Avance 500, or Varian Inova 500 spectrometer. Deuterated chloroform ($CDCl_3$), methanol (CD_3OD), or water (D_2O) were used as NMR solvents, unless otherwise stated. Chemical shifts are reported in ppm downfield from trimethylsilane (TMS) or the solvent residual peak as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet and br, broad. Low resolution mass spectrometry (LRMS) was performed on a Micromass Quatro-LC Electrospray spectrometer with a pump rate of 20 μL/min using electrospray ionization (ESI).

Compounds below are in order of appearance in the manuscript. Intermediates that were not numbered in the manuscript received numbers beginning with **11.**

*Synthesis of n-octyl-β-D-glucopyranoside (**3**) and n-octyl-β-D-galactopyranoside (**4**)*



11a: Glc: R_1=OAc; R_2=H
11b: Gal: R_1=H; R_2=OAc

12a: Glc: R_1=OAc; R_2=H
12b: Gal: R_1=H; R_2=OAc

3: Glc: R_1=OH; R_2=H
4: Gal: R_1=H; R_2=OH

n-Octyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (12a)

To a mixture of 1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranose (**11a**, 275 mg, 0.70 mmol), 1-octanol (215 μL, 1.36 mmol) and 4 Å MS in anhydrous CH_2Cl_2 (6 mL) stirring at 0 °C under Ar, was slowly added boron trifluoride diethyl etherate (160 μL, 1.27 mmol). The reaction mixture was stirred overnight, then diluted with CH_2Cl_2 and quenched with sodium bicarbonate. The solution was filtered through Celite®, then extracted with CH_2Cl_2. The organic layer was washed with sodium bicarbonate, water, saturated brine, then dried over $MgSO_4$ and concentrated. Flash column chromatography (7:3 hexanes/EtOAc) afforded **12a** as a white powder (103 mg, 32%). Characterization data is consistent with that previously reported in the literature.[6,7] ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.20 (t, *J* = 9.5 Hz, 1H), 5.09 (t, *J* = 9.7 Hz, 1H), 4.98 (dd, *J* = 9.6, 8.0 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.26 (dd, *J* = 12.3, 4.7 Hz, 1H), 4.13 (dd, *J* = 12.3, 2.5 Hz, 1H), 3.87 (dt, *J* = 9.6, 6.4 Hz, 1H), 3.67 (ddd, *J* = 10.0, 4.7, 2.5 Hz, 1H), 3.47 (dt, *J* = 9.6, 6.8 Hz, 1H), 2.09 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.64-1.48 (m, 2H), 1.34-1.21 (m, 10H), 0.88 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.3, 169.4, 169.3, 100.8, 72.9, 71.7, 71.4, 70.3, 68.5, 62.0, 31.8, 29.4, 29.3, 29.2, 25.8, 22.6, 20.7, 20.6, 20.6, 20.6, 14.1. LRMS (ESI): *m/z* calcd. for $C_{22}H_{40}NO_{10}$ $[M+NH_4]^+$ 478.5; found, 478.4.

n-Octyl-β-D-glucopyranoside (3)

Compound **12a** (103 mg, 0.22 mmol) was dissolved in a solution of sodium methoxide in methanol (5 mL) and stirred for one hour at room temperature. The solution was then neutralized with Amberlite® IR-120 (H^+) ion-exchange resin, filtered and concentrated. The product was purified by column chromatography (9:1 CH_2Cl_2/MeOH) to afford **3** as a white powder (64 mg, 98%). ^{1}H NMR (400 MHz, D_2O): δ 4.44 (d, *J* = 8.0 Hz, 1H), 3.95-3.87 (m, 2H), 3.75-3.62 (m, 2H), 3.50-3.33 (m, 3H), 3.24 (dd, *J* = 9.24, 8.03 Hz, 1H), 1.62 (quint, *J* =7.1 Hz, 2H), 1.38-1.24 (m, 10H), 0.86 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, D_2O): δ 102.1, 75.8, 75.7, 73.0, 70.6, 69.5, 60.7, 31.1, 28.7, 28.5, 28.4, 25.1, 22.0, 13.4. LRMS (ESI): *m/z* calcd. for $C_{14}H_{28}NaO_6$ $[M+Na]^+$ 315.4; found, 315.3.

n-Octyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (12b)

To a mixture of 1,2,3,4,6-Penta-*O*-acetyl-β-D-galactopyranose (**11b**, 500 mg, 1.28 mmol), 1-octanol (280 μL, 1.79 mmol) and 4 Å MS in anhydrous CH_2Cl_2 (10 mL) stirring at 0 °C under Ar, was slowly added boron trifluoride diethyl etherate (210 μL, 1.66 mmol). The reaction mixture was stirred overnight, then diluted with CH_2Cl_2 and quenched with sodium bicarbonate. The solution was filtered through Celite®, then extracted with CH_2Cl_2. The organic layer was washed with sodium bicarbonate, water, saturated

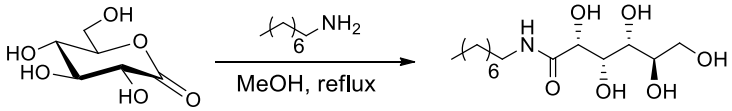
[6] D. S. K. Tsui and P. A. J. Gorin, *Carbohydr. Res*., 1985, **144**, 137.
[7] H. Akita, E. Kawahara and K. Kato, *Tetrahedron: Asymmetry*, 2004, **15**, 1623.

brine, then dried over $MgSO_4$ and concentrated. Flash column chromatography (7:3 hexanes/EtOAc) afforded **12b** as a white powder (228 mg, 39%). Characterization data is consistent with that previously reported in the literature.[6,8] ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.38 (dd, *J* = 3.4, 0.9 Hz, 1H), 5.20 (dd, *J* = 10.5, 7.9 Hz, 1H), 5.01 (dd, *J* = 10.5, 3.4 Hz, 1H), 4.45 (d, *J* = 8.0 Hz, 1H), 4.23-4.08 (m, 2H), 3.93-3.84 (m, 2H), 3.47 (dt, *J* = 9.6, 6.9 Hz, 1H), 2.15 (s, 3H), 2.05 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.62-1.53 (m, 2H), 1.34-1.22 (m, 10H), 0.88 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 169.4, 101.4, 71.0, 70.6, 70.3, 68.9, 67.1, 61.3, 31.8, 29.4, 29.3, 29.2, 25.8, 22.6, 20.7, 20.7, 20.7, 20.6, 14.1. LRMS (ESI): *m/z* calcd. for $C_{22}H_{36}KO_{10}$ $[M+K]^+$ 499.6; found, 499.4.

n-Octyl-β-D-galactopyranoside (4)

Compound **12b** (183 mg, 0.40 mmol) was dissolved in a solution of sodium methoxide in methanol (5 mL) and stirred for one hour at room temperature. The solution was then neutralized with Amberlite® IR-120 (H^+) ion-exchange resin, filtered and concentrated. The product was purified by column chromatography (9:1 CH_2Cl_2/MeOH) to afford **4** as a white powder (109 mg, 94%). ^{1}H NMR (400 MHz, D_2O): δ 4.38 (d, *J* = 7.9 Hz, 1H), 3.95-3.89 (m, 2H), 3.81-3.72 (m, 2H), 3.70-3.61 (m, 3H), 3.49 (dd, *J* = 9.9, 7.9 Hz, 1H), 1.62 (quint, *J* =7.0 Hz, 2H), 1.39-1.24 (m, 10H), 0.86 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, D_2O): δ 102.7, 75.0, 72.7, 70.7, 70.6, 68.5, 60.8, 31.0, 28.7, 28.4, 28.3, 25.0, 21.9, 13.3. LRMS (ESI): *m/z* calcd. for $C_{14}H_{28}NaO_6$ $[M+Na]^+$ 315.4; found, 315.3.

*Synthesis of N-octyl-D-gluconamide (**5**)*



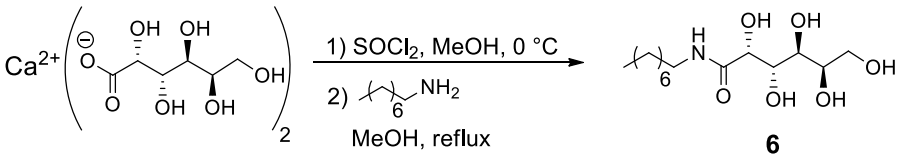
5

N-Octyl-D-gluconamide (5)

To a solution of D-gluconic acid-δ-lactone (1.4 g, 7.86 mmol) in MeOH (30 mL) was added *n*-octylamine (1.3 mL, 7.86 mmol). The mixture was refluxed for 1 hour then cooled in an ice bath. The precipitate was filtered off and washed with cold MeOH to afford **5** as a white powder (1.45 g, 60%). Characterization data is consistent with that previously reported in the literature.[9,10] ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.59, (t, *J* = 6.0 Hz, 1H), 5.34 (d, *J* = 5.1 Hz, 1H), 4.53 (t, *J* = 4.8 Hz, 1H), 4.47 (d, *J* = 5.1 Hz, 1H), 4.39 (d, *J* = 7.2 Hz, 1H), 4.33 (d, *J* = 5.8 Hz, 1H), 3.97 (dd, *J* = 4.9, 3.8 Hz, 1H), 3.89 (ddd, *J* = 7.2, 3.7, 2.2 Hz, 1H), 3.57 (m, 1H), 3.46 (m, 2H), 3.37 (m, 1H), 3.06 (m, 2H), 1.40 (quint, *J* = 6.6 Hz, 2H), 1.31-1.18 (m, 10H), 0.86 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.2, 73.6, 72.4, 71.5, 70.1, 63.4, 38.3, 31.3, 29.2, 28.8, 28.7, 26.4, 22.1, 14.0. LRMS (ESI): *m/z* calcd. for $C_{14}H_{30}NO_6$ $[M+H]^+$ 308.2; found 308.3.

[8] X.-B. Li, M. Ogawa, T. Monden, T. Maeda, E. Yamashita, M. Naka, M. Matsuda, H. Hinou and S.-I. Nishimura, *Angew. Chem. Int. Ed.*, 2006, **45**, 5652.
[9] S. Svenson, A. Schafer and J.-H. Fuhrhop, *J. Chem. Soc. Perkin Trans 2*, 1994, 1023.
[10] S. Svenson, B. Kirste and J.-H. Fuhrhop, *J. Am Chem. Soc.*, 1994, **116**, 11969.

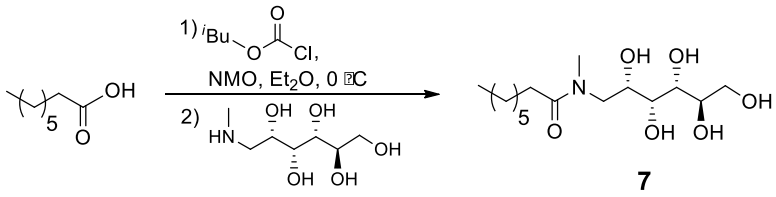
*Synthesis of N-octyl-D-galactonamide (**6**)*



N-Octyl-D-galactonamide (6)

A solution of calcium galactonate (200 mg, 0.46 mmol) in methanol (2 mL) was cooled to 0 °C and $SOCl_2$ (70 μL, 0.93 mmol) was added dropwise. The solution was slowly warmed to room temperature and was stirred overnight. The mixture was then evaporated and dried *in vacuo* to give 200 mg of a white powder. This powder was then dissolved in methanol (2 mL) and *n*-octylamine (230 μL, 1.44 mmol) was added. The mixture was refluxed for 2 hours then cooled in an ice bath. The precipitate was filtered off and washed with cold methanol to afford **6** as a white powder (112 mg, 38%). Characterization data is consistent with that previously reported in the literature.[9,10] ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.53, (t, J = 6.0 Hz, 1H), 5.07 (d, J = 7.2 Hz, 1H), 4.44 (t, J = 5.6 Hz, 1H), 4.28 (d, J = 8.1 Hz, 1H), 4.17 (d, J = 6.6 Hz, 1H), 4.12 (d, J = 7.5 Hz, 1H), 4.08 (d, J = 8.1 Hz, 1H), 3.78 (t, J = 8.7 Hz, 1H), 3.69 (q, J = 5.6 Hz, 1H), 3.45-3.37 (m, 3H), 3.07 (m, 2H), 1.40 (m, 2H), 1.31-1.18 (m, 10H), 0.86 (t, J = 6.6 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.3, 70.9, 70.7, 69.8, 69.1, 63.2, 38.3, 31.3, 29.3, 28.8, 28.7, 26.4, 22.1, 14.0. LRMS (ESI): *m/z* calcd. for $C_{14}H_{30}NaNO_6$ $[M+Na]^+$ 330.2; found 330.2.

Synthesis of N-octanoyl-*N*-methyl-D-glucamine (**7**)

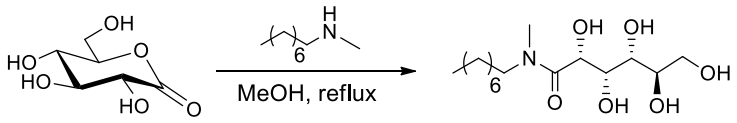


N-Octanoyl-*N*-methyl-D-glucamine (7)

A solution of octanoic acid (0.89 mL, 5.63 mmol) in Et_2O (10 mL) was cooled to 0 ºC and isobutyl chloroformate (0.74 mL, 5.63 mmol) was added. After stirring for 5 min at 0 ºC, *N*-methylmorpholine (0.68 mL, 5.63 mmol) was added dropwise and the mixture was stirred for 10 min. The precipitate was removed by filtration trough Celite® and the filter cake washed with 10 mL of Et_2O. The solution of the crude mixed anhydride in Et_2O was then added by cannula to a cooled solution (0 ºC) of *N*-methyl-D-glucamine (500 mg, 2.56 mmol) in MeOH (10 mL). The mixture was warmed to room temperature, stirred for 1 hour and concentrated *in vacuo*. Recrystallization in MeOH afforded **7** (250 mg, 30%) as a white powder. ^{1}H NMR (500 MHz, DMSO-d_6, present as a 1.3:1 mixture of rotamers): δ 4.87 (d, J = 5.2 Hz, 1H, major), 4.72 (d, J = 5.1 Hz, 1H, minor), 4.51 (d, J = 5.4 Hz, 1H, major), 4.47 (d, J = 5.5 Hz, 1H,

minor), 4.41-4.26 (m, *3*H), 3.74 (m, 1H), 3.56 (m, 2H), 3.52-3.35 (m, 4H), 3.29 (dd, *J* = 14.4, 3.7 Hz, 1H, major), 3.21 (dd, *J* = 13.2, 8.1 Hz, 1H, minor), 2.99 (s, 3H, minor), 2.80 (s, 3H, major), 2.34 (m, 2H, major), 2.26 (t, *J* = 7.4 Hz, 2H, minor), 1.46 (m, 2H), 1.24 (m, 8H), 0.86 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6, present as a mixture of rotamers): δ (major) 172.4, 71.5, 71.5, 70.8, 69.9, 63.3, 51.9, 33.5, 32.2, 31.2, 28.9, 28.7, 24.9, 22.1, 14.0; δ (minor) 172.6, 72.5, 71.4, 71.3, 69.3, 63.3, 50.8, 36.7, 32.7, 31.2, 28.8, 28.7, 24.9, 22.1, 14.0. HRMS (ESI): *m/z* calcd. for $C_{15}H_{32}NO_6$ $[M+H]^+$ 322.223; found 322.285.

*Synthesis of N-methyl-N-octyl-D-gluconamide (**8**)*

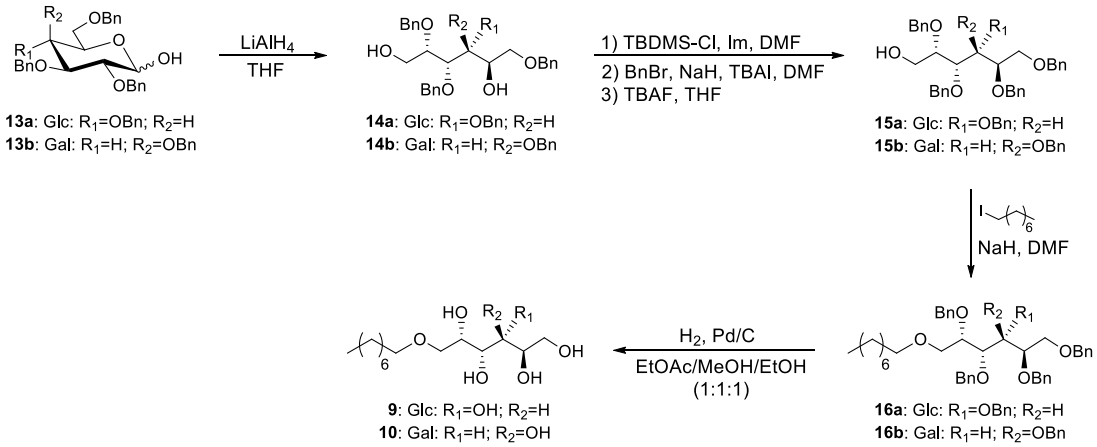


8

N-Methyl-*N*-octyl-D-gluconamide (8)

To a solution of D-gluconic acid-*δ*-lactone (950 mg, 5.3 mmol) in MeOH (20 mL) was added *N*-methyl-*N*-octylamine (760 mg, 5.3 mmol). The mixture was stirred under reflux for 1 hour. The clear solution was cooled to room temperature and filtered successively through Dowex-1X8 resin (OH^-) followed by Amberlite IR-120 (H^+). The solvent was evaporated and the residue was dried *in vacuo* to afford **8** as a waxy solid (800 mg, 47%). ^{1}H NMR (500 MHz, DMSO-d_6, present as a 1.1:1 mixture of rotamers): δ 4.77 (d, *J* = 7.2 Hz, 1H, minor), 4.71 (d, *J* = 7.1 Hz, 1H, major), 4.50-4.25 (m, 5H), 3.82 (m, 1H), 3.57 (m, 1H), 3.47 (m, 1H), 3.42-3.20 (m, 4H), 2.99 (s, 3H, major), 2.80 (s, 3H, minor), 1.52 (quint, *J* = 6.7 Hz, 2H, minor), 1.43 (quint, *J* = 6.7 Hz, 2H, major), 1.25 (m, 10H), 0.86 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6, present as a mixture of rotamers): δ (major) 171.5, 71.6, 71.5, 70.4, 69.5, 63.4, 47.2, 34.4, 31.2, 28.8, 28.7, 26.4, 26.2, 22.1, 14.0; δ (minor) 171.6, 71.7, 71.1, 70.9, 69.2, 63.4, 48.5, 33.1, 31.2, 28.8, 28.7, 26.4, 26.2, 22.1, 14.0. HRMS (ESI): *m/z* calcd. for $C_{15}H_{31}NaNO_6$ $[M+Na]^+$ 322.223; found 322.260.

*Synthesis of 1-O-octyl-D-glucitol (**9**) and 1-O-octyl-D-galactitol (**10**)*



13a: Glc: R_1=OBn; R_2=H
13b: Gal: R_1=H; R_2=OBn

14a: Glc: R_1=OBn; R_2=H
14b: Gal: R_1=H; R_2=OBn

15a: Glc: R_1=OBn; R_2=H
15b: Gal: R_1=H; R_2=OBn

16a: Glc: R_1=OBn; R_2=H
16b: Gal: R_1=H; R_2=OBn

9: Glc: R_1=OH; R_2=H
10: Gal: R_1=H; R_2=OH

2,3,4,6-tetra-*O*-benzyl-D-glucitol (14a)

To a stirred solution of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside[11] (**13a**, 200 mg, 0.37 mmol) in THF was added $LiAlH_4$ (56 mg, 1.5 mmol). After stirring overnight at room temperature, the mixture was cooled to 0 °C and the excess of $LiAlH_4$ was carefully quenched with a few drops of water. The mixture was diluted with Et_2O and the organic phase was washed successively with 1% HCl, water and brine. The organic layer was dried over $MgSO_4$ and evaporated. Flash column chromatography (7:3 Pet. Ether/EtOAc) afforded **14a** (200 mg, quant.) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.18 (m, 20H), 4.70 (d, *J* = 11.7 Hz, 1H), 4.66 (d, *J* = 11.7 Hz, 1H), 4.65 (d, *J* = 11.7 Hz, 1H), 4.62 (d, *J* = 11.7 Hz, 1H), 4.58 (d, *J* = 11.2 Hz, 1H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.53 (d, *J* = 11.2 Hz, 1H), 4.50 (d, *J* = 11.7 Hz, 1H), 4.02 (m, 1H), 3.88 (m, 1H), 3.77 (m, 2H), 3.72 (dd, J = 11.8, 4.5 Hz, 1H), 3.63 (m, 2H), 3.55 (dd, *J* = 11.7, 4.5 Hz, 1H), 2.27 (br, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.1, 137.9, 137.8, 137.8, 128.5, 128.4, 128.4, 128.4, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 79.4, 79.1, 77.3, 74.5, 73.4, 73.2, 73.1, 71.1, 70.7, 61.8. LRMS (ESI) *m/z* calcd. for $C_{34}H_{42}NO_6$ $[M+NH_4]^+$ 560.3; found 560.3.

2,3,4,5,6-penta-*O*-benzyl-D-glucitol (15a)

To a solution of **14a** (200 mg, 0.37 mmol) in DMF (2 mL) was added imidazole (60 mg, 0.89 mmol) and TBDMS-Cl (66 mg, 0.44 mmol), successively. After stirring overnight, the reaction mixture was diluted with Et_2O and washed with 1M $KHSO_4$, water and brine. The solvent was evaporated and crude material dried under high vacuum for several hours. The resulting alcohol was re-dissolved in DMF (5 mL) and NaH (18 mg, 0.44 mmol) was added. The mixture was stirred for 30 min and then benzyl bromide (66 μL, 0.55 mmol) and TBAI (14 mg, 0.04 mmol) were added. After stirring overnight, the mixture was diluted with water and organic phase was extracted with Et_2O. The organic layer was washed with water and brine, dried over $MgSO_4$ and the solvent evaporated. This crude material was re-dissolved in THF (2 mL) and TBAF (400 μL, 0.4 mmol) were added and the reaction stirred for 2 hours at room temperature. The mixture was diluted with Et_2O and the organic layer was washed with water and brine, and the solvent was evaporated. Flash column chromatography (4:1 Pet. Ether/EtOAc) afforded **15a** (120 mg, 51%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33-7.19 (m, 25H), 4.74 (d, *J* = 11.4 Hz, 1H), 4.66 (d, *J* = 11.6 Hz, 1H), 4.63 (d, *J* = 12.0 Hz, 2H), 4.61 (d, *J* = 11.4 Hz, 1H), 4.57 (s, 2H), 4.50 (d, *J* = 11.9 Hz, 1H), 4.47 (d, *J* = 11.9 Hz, 1H), 4.43 (d, *J* = 11.6 Hz, 1H), 3.95 (t, *J* = 4.7 Hz, 1H), 3.86 (ddd, *J* = 8.3, 4.8, 3.5 Hz, 2H), 3.83 (dd, *J* = 5.4, 4.8 Hz, 1H), 3.70 (dd, *J* = 11.3, 6.0 Hz, 1H), 3.67 (m, 2H), 3.49 (q, *J* = 6.9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 138.6, 138.4, 138.3, 138.2, 138.2, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 127.9, 127.7, 127.7, 127.6, 127.5, 127.4, 79.3, 79.3, 78.9, 78.6, 74.7, 73.8, 73.3, 72.7, 71.9, 69.6, 61.8. LRMS (ESI) *m/z* calcd. for $C_{41}H_{48}NO_6$ $[M+NH_4]^+$ 650.4; found 650.5.

2,3,4,5,6-penta-*O*-benzyl-1-*O*-octyl-D-glucitol (16a)

To a solution of **15a** (95 mg, 0.15 mmol) in DMF (2 mL) was added NaH (12 mg, 0.30 mmol). The solution was stirred for 30 min then 1-iodooctane (54 μL, 0.30 mmol) was added and the mixture was stirred overnight. The following day, NaH (6 mg, 0.15 mmol) and 1-iodooctane (27 μL, 0.15 mmol) were added and the mixture was stirred for an additional 24 hours. The reaction mixture was then carefully quenched with water and extracted with Et_2O. The organic layer was washed with water and brine, dried

[11] Compound **13a** was prepared as previously described: S. Dasgupta and M. Nitz, *J. Org. Chem.*, 2011, **76**, 1918.

over $MgSO_4$ and the solvent evaporated. Flash column chromatography (20:1 Pet. Ether/EtOAc) afforded **16a** (64 mg, 57%) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7-20 (m, 25H), 4.72 (d, *J* = 11.9 Hz, 1H), 4.70 (d, *J* = 11.9 Hz, 1H), 4.62 (d, *J* = 11.9 Hz, 1H), 4.60 (s, 2H), 4.59 (d, *J* = 11.9 Hz, 1H), 4.59 (d, *J* = 11.9 Hz, 1H), 4.57 (d, *J* = 11.9 Hz, 1H), 4.42 (s, 2H), 3.96 (m, 3H), 3.89 (dd, *J* = 9.3, 4.7 Hz, 1H), 3.67 (dd, *J* = 10.0, 5.5 Hz, 1H) 3.64 (dd, *J* = 10.0, 4.7 Hz, 1H) 3.61 (dd, *J* = 10.1, 5.6 Hz, 1H) 3.57 (dd, *J* = 10.1, 4.7 Hz, 1H), 3.30 (t, *J* = 6.7 Hz, 2H), 1.50 (quint, *J* = 6.7 Hz, 2H), 1.32-1.20 (m, 10H), 0.87 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.9, 138.9, 138.8, 138.7, 138.2, 128.3, 128.2, 128.2, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 127.3, 78.9, 78. 9, 78.4, 78.3, 73.7, 73.7, 73.2, 72.8, 72.6, 71.5, 71.0, 70.5, 31.8, 29.7, 29.5, 29.3, 26.2, 22.6, 14.1. LRMS (ESI) *m/z* calcd. for $C_{49}H_{64}NO_6$ $[M+NH_4]^+$ 762.5; found 762.6.

1-*O*-octyl-D-glucitol (9)

A solution **16a** (60 mg, 0.13 mmol) in 3 mL of 1:1:1 mixture of EtOAc/EtOH/MeOH and 5% Pd/C (33 mg, 0.013 mmol) was stirred for 6 hours under an atmosphere of H_2. The flask was purged with N_2 and the catalyst removed by filtration through Celite®. The solvents were removed *in vacuo* and the solid was carefully washed with EtOAc to afford **9** (17 mg, 75%) as a white solid. ^{1}H NMR (500 MHz, D_2O): δ 3.90 (dt, *J* = 10.2, 6.1 Hz, 1H), 3.80-3.77 (m, 2H), 3.74-3.71 (m, 1H), 3.62-3.58 (m, 3H), 3.55-3.47 (m, 3H), 1.54 (quint, *J* = 6.7 Hz, 2H), 1.30-1.23 (m, 10H), 0.82 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, D_2O): δ 72.0, 71.8, 71.7, 71.6, 71.4, 70.0, 63.2, 31.7, 29.3, 29.2, 29.1, 25.9, 22.5, 13.8. LRMS (ESI) *m/z* calcd. for $C_{14}H_{30}NaO_6$ $[M+Na]^+$ 317.2; found 317.2.

2,3,4,6-tetra-*O*-benzyl-D-galactitol (14b)

This compound was prepared from 2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside[12] (**13b**, 500 mg, 0.93 mmol) following the same procedure as described for the preparation of **13a**. Flash column chromatography (7:3 Pet. Ether/EtOAc) afforded **14b** (500 mg, quant.) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35-7.20 (m, 20H), 4.74 (d, *J* = 11.2 Hz, 1H), 4.67 (d, *J* = 11.2 Hz, 1H), 4.66 (d, *J* = 11.6 Hz, 1H), 4.63 (d, *J* = 11.2 Hz, 1H), 4.58 (d, *J* = 11.6 Hz, 1H), 4.48 (d, *J* = 11.2 Hz, 1H), 4.46 (d, *J* = 11.8 Hz, 1H), 4.41 (d, *J* = 11.8 Hz, 1H), 4.04 (m, 1H), 3.88 (m, 2H), 3.79 (dd, J = 12.8, 5.8 Hz, 1H), 3.71 (m, 2H), 3.54 (dd, J = 9.3, 5.8 Hz, 1H), 3.49 (dd, *J* = 9.3, 6.7 Hz, 1H), 2.60 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.9, 137.9, 137.9, 137.8, 128.5, 128.4, 128.4, 128.4, 128.1, 127.9, 127.9, 127.8, 127.7, 80.1, 79.04, 77.4, 74.3, 73.8, 73.3, 72.3, 70.8, 69.8, 60.9. LRMS (ESI) *m/z* calcd. for $C_{34}H_{42}NO_6$ $[M+NH_4]^+$ 560.3; found 560.4.

2,3,4,5,6-penta-*O*-benzyl-D-galactitol (15b)

This compound was prepared from **14b** (300 mg, 0.55 mmol) following the same procedure as described for the preparation of **15a**. Flash column chromatography (4:1 Pet. Ether/EtOAc) afforded **15b** (160 mg, 46%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33-7.20 (m, 25H), 4.70 (d, *J* = 11.7 Hz, 1H), 4.69 (s, 2H), 4.61 (d, *J* = 11.7 Hz, 2H), 4.57 (d, *J* = 11.7 Hz, 1H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.46 (d, *J* = 12.0 Hz, 1H), 4.40 (d, *J* = 12.0 Hz, 1H), 4.03 (dd, *J* = 6.0, 4.2 Hz, 1H), 3.96 (dt, *J* = 5.9, 4.2 Hz, 1H) 3.83 (dd, *J* = 5.3, 4.2 Hz, 1H), 3.77-3.71 (m, 3H), 3.66 (dd, *J* = 10.3, 4.5 Hz, 1H),

[12] Compound **13b** was prepared as previously described: M. Adinolfi, G. Barone, A. Iadonisi and L. Mangoni, *Tetrahedron Lett.,* 1998, ***39***, 2021.

3.63 (dd, *J* = 10.3, 4.1 Hz, 1H), 1.59 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.7, 138.4, 138.3, 138.3, 138.1, 128.3, 128.3, 128.3, 128.3, 128.2, 127.9, 127.9, 127.8, 127. 8, 127.7, 127.6, 127.6, 127.5, 127.4, 79.8, 79.4, 79.3, 78.8, 74.6, 73.3, 73.3, 73.0, 72.2, 70.1, 61.1. LRMS (ESI) *m/z* calcd. for $C_{41}H_{48}NO_6$ $[M+NH_4]^+$ 650.4; found 650.4.

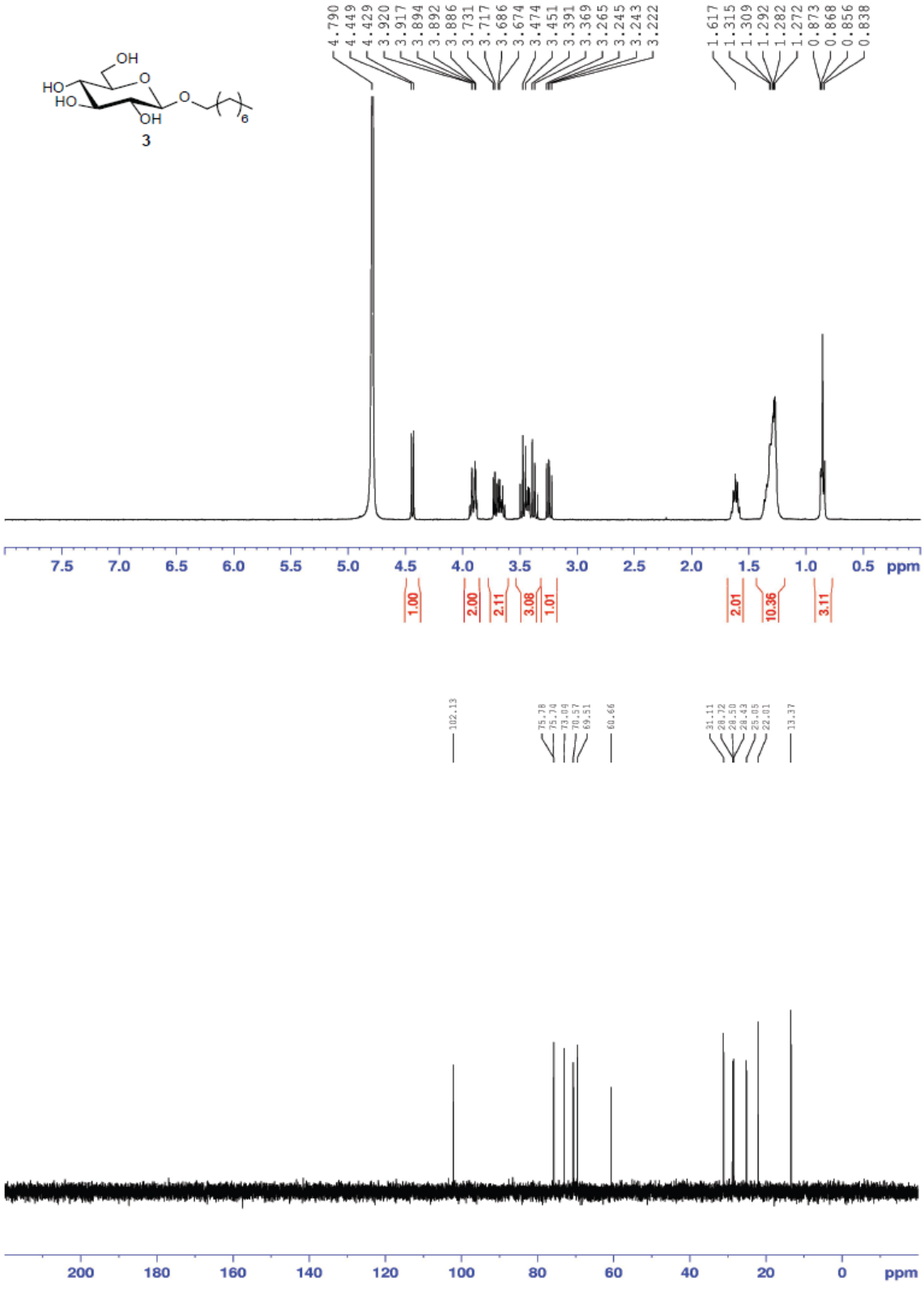
2,3,4,5,6-penta-*O*-benzyl-1-*O*-octyl-D-galactitol (16b)

This compound was prepared from **15b** (120 mg, 0.19 mmol) following the same procedure as described for the preparation of **16a**. Flash column chromatography (20:1 Pet. Ether/EtOAc) afforded **16b** (80 mg, 57%) as a viscous oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32-7.19 (m, 25H), 4.74 (d, *J* = 11.8 Hz, 1H), 4.70 (d, *J* = 11.8 Hz, 1H), 4.67 (d, *J* = 11.8 Hz, 1H), 4.63 (s, 2H), 4.59 (d, *J* = 11.8 Hz, 1H) 4.55 (d, *J* = 11.8 Hz, 1H) 4.51 (d, *J* = 11.8 Hz, 1H) 4.47 (d, *J* = 11.8 Hz, 1H) 4.44 (d, *J* = 11.8 Hz, 1H), 4.01 (t, *J* = 4.2 Hz, 1H), 3.87-3.77 (m, 4H), 3.71 (m, 1H), 3.52 (dd, *J* = 10.1, 3.1 Hz, 1H), 3.45 (dd, *J* = 10.1, 4.8 Hz, 1H), 3.26 (dt, *J* = 6.7, 2.0 Hz, 2H), 1.48 (quint, *J* = 6.7 Hz, 2H), 1.32-1.17 (m, 10H), 0.87 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.8, 138.8, 138.7, 138.7, 138.4, 128.3, 128.2, 128.2, 128.2, 128.0, 127.9, 127.6, 127.4, 127.4, 127.4, 127.4, 127.3, 79.3, 79.3, 79.2, 78.6, 74.7, 74.1, 73.2, 72.8, 71.9, 71.4, 71.1, 70.0, 31.8, 29.7, 29.4, 29.3, 26.2, 22.7, 14.1. LRMS (ESI) *m/z* calcd. for $C_{49}H_{61}O_6$ $[M+H]^+$ 745.5; found 745.6.

1-*O*-octyl-D-galactitol (10)

This compound was prepared from **16b** (60 mg, 0.081 mmol) following the same procedure as described for the preparation of **9**. Compound **10** (12 mg, 50%) was obtained as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 4.63 (s, 2H), 4.04 (dd, *J* = 6.6, 5.8 Hz, 1H), 3.92 (dd, *J* = 6.6, 5.8 Hz, 1H), 3.64 (m, 4H), 3.57 (dd, *J* = 9.6, 5.8 Hz, 1H), 3.53 (dd, *J* = 9.6, 6.7 Hz, 1H), 3.49 (t, *J* = 6.6 Hz, 2H), 1.58 (quint, *J* = 6.7 Hz, 2H), 1.41-1.25 (m, 10H), 0.90 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (125 MHz, Pyridine-d_5): δ 75.3, 73.2, 73.0, 73.0, 72.8, 70.9, 66.5, 33.2, 31.4, 30.9, 30.7, 27.7, 24.1, 15.5. LRMS (ESI) *m/z* calcd. for $C_{14}H_{30}NaO_6$ $[M+Na]^+$ 317.2; found 317.3.

NMR Spectra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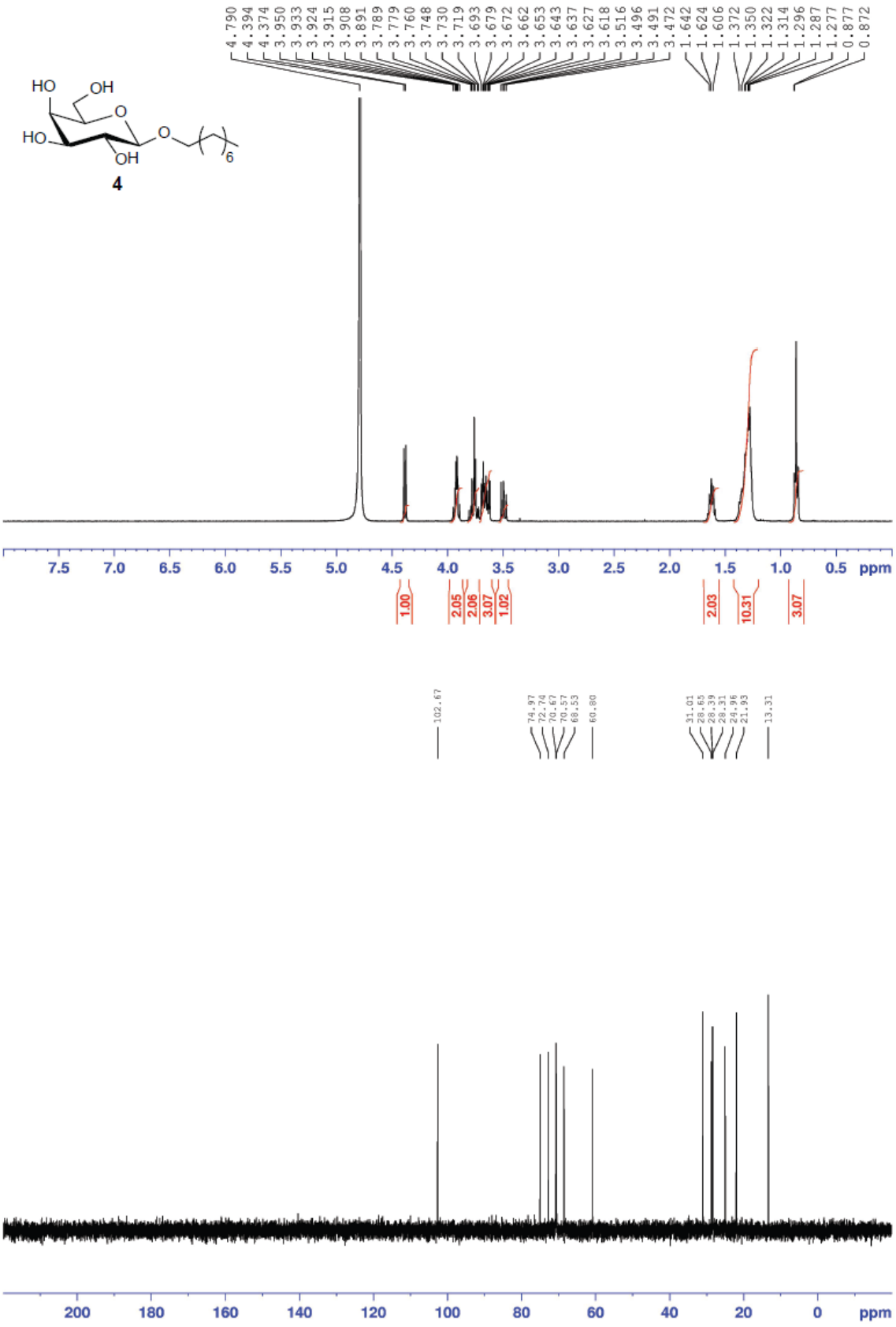
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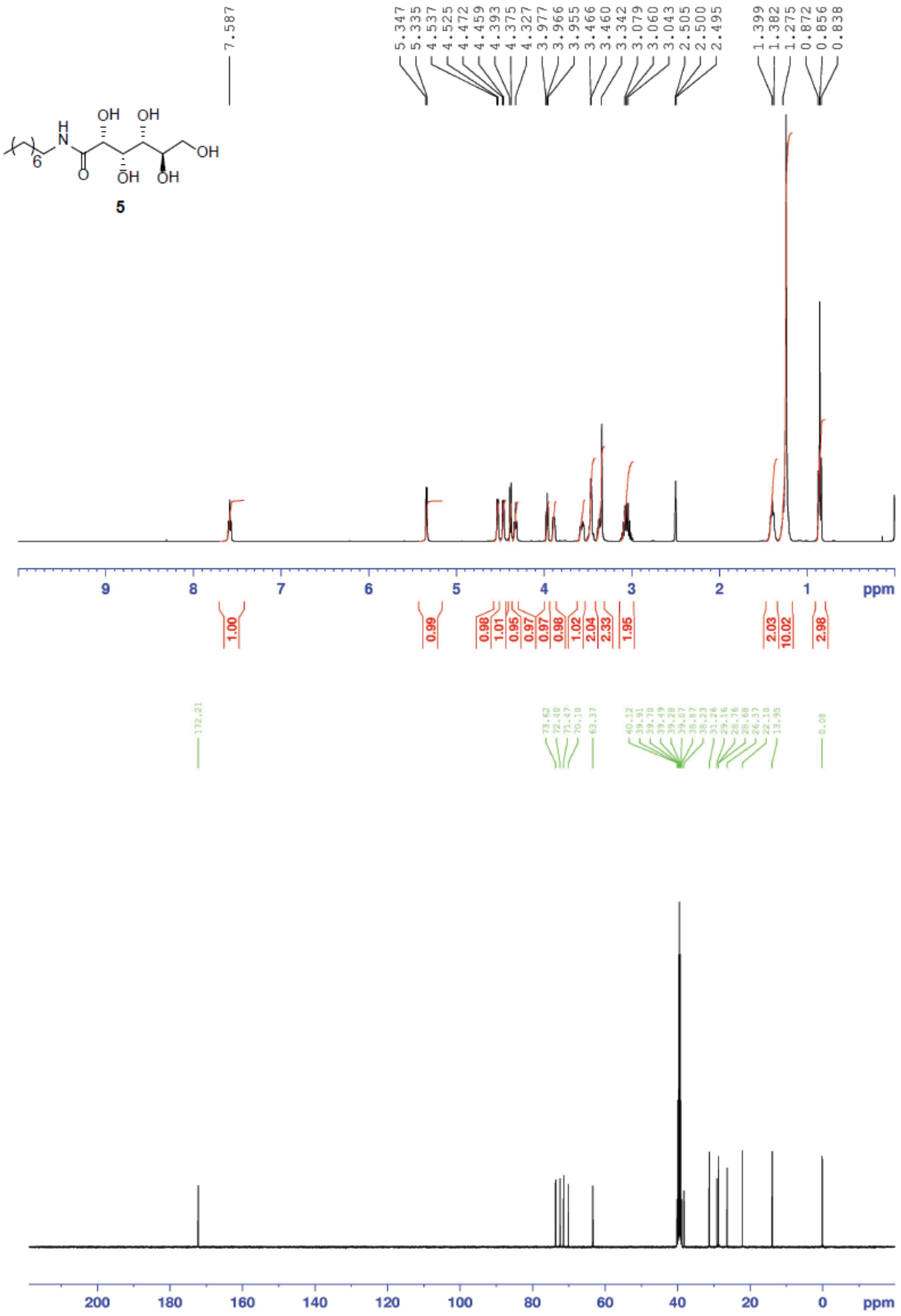
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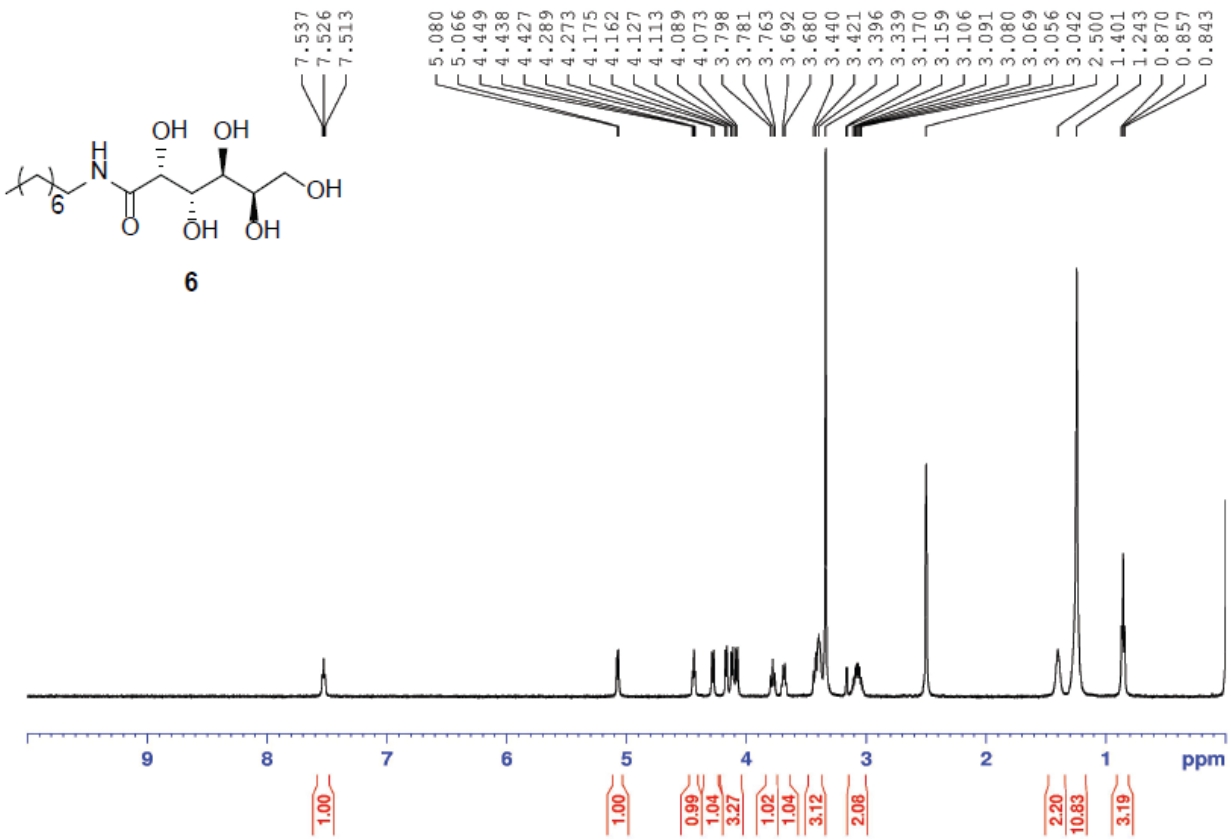
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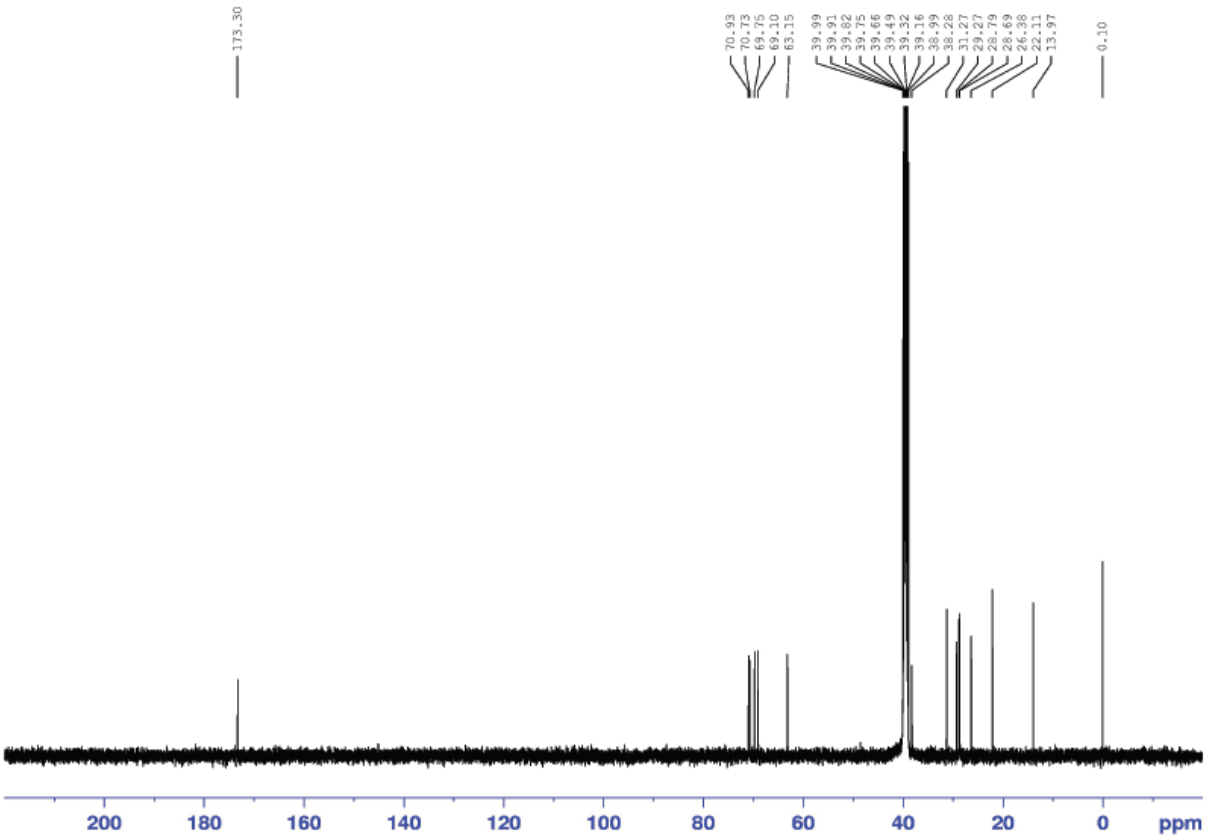
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120
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80
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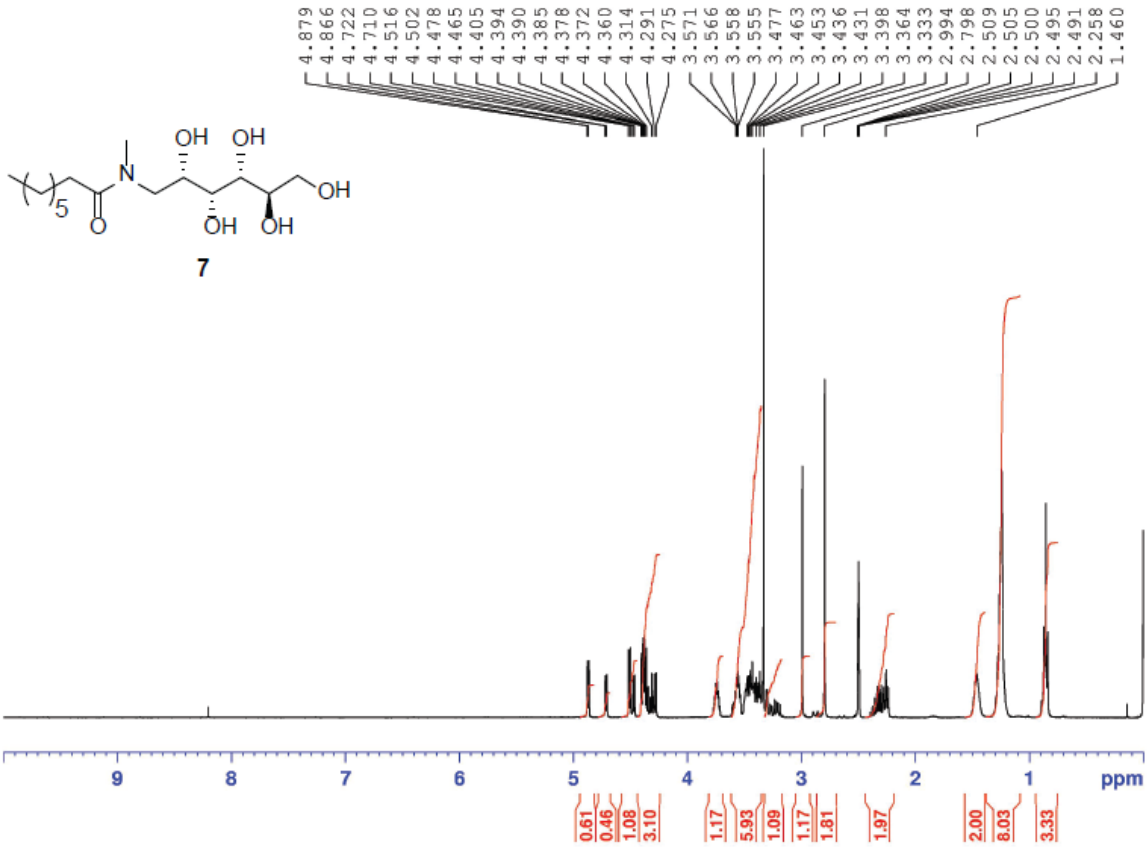
0
ppm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4.722
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4.478
4.465
4.405
4.394
4.390
4.385
4.378
4.372
4.360
4.314
4.291
4.275
3.571
3.566
3.558
3.555
3.477
3.463
3.453
3.436
3.431
3.398
3.364
3.333
2.994
2.798
2.509
2.505
2.500
2.495
2.491
2.258
1.460
OH
OH
N
5
O
OH
OH
OH
9
8
7
6
5
4
3
2
1
ppm
0.61
0.46
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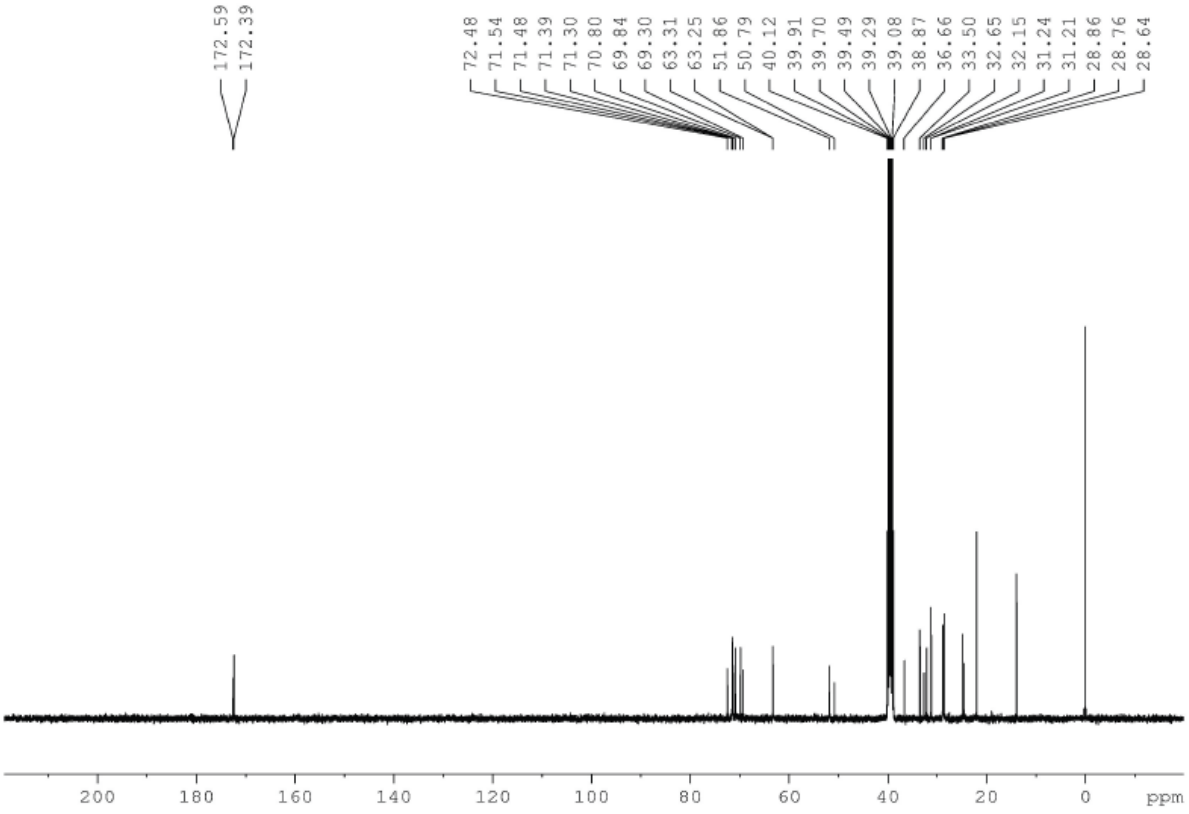
1.17
5.93
1.09
1.17
1.81
1.97
2.00
8.03
3.33

7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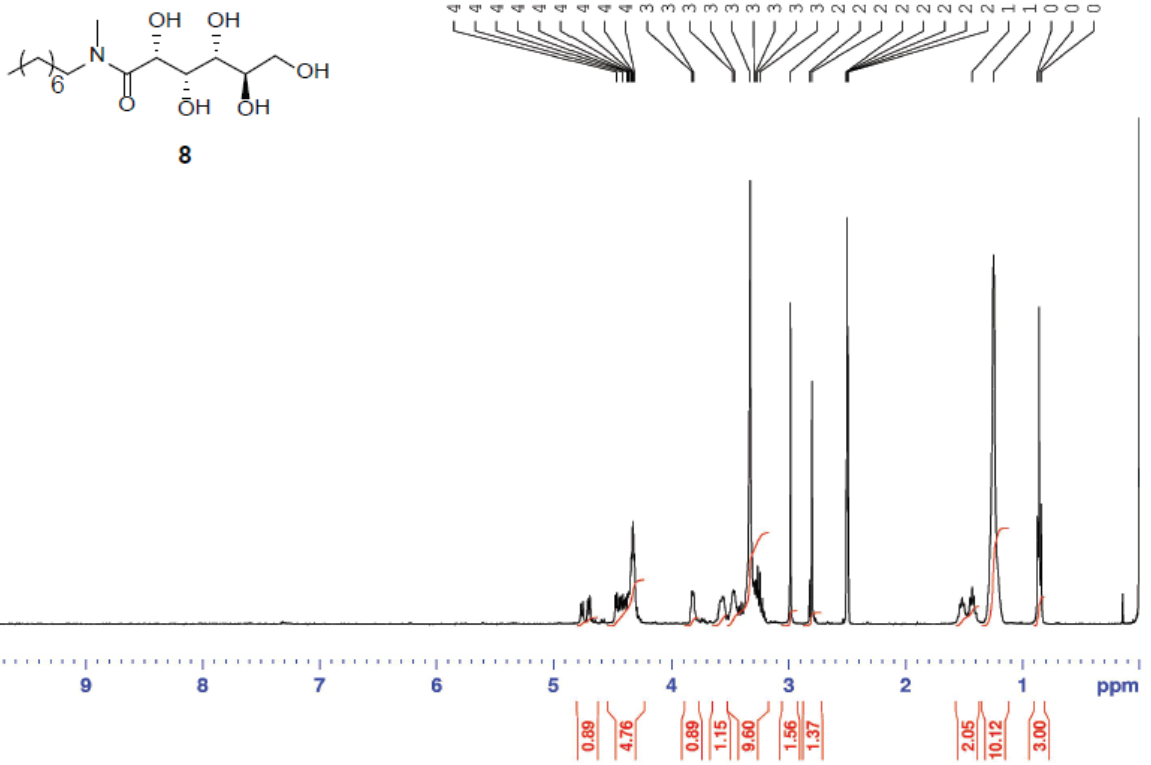
172.39
72.48
71.54
71.48
71.39
71.30
70.80
69.84
69.30
63.31
63.25
51.86
50.79
40.12
39.91
39.70
39.49
39.29
39.08
38.87
36.66
33.50
32.65
32.15
31.24
31.21
28.86
28.76
28.64
200
180
160
140
120
100
80
60
40
20
0
ppm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4.420
4.379
4.370
4.364
4.345
4.336
4.331
4.321
3.825
3.817
3.475
3.461
3.332
3.299
3.281
3.266
3.245
2.987
2.824
2.802
2.509
2.504
2.500
2.495
2.491
1.433
1.247
0.874
0.857
0.840
OH
OH
OH
OH
OH
N
6
O
9
8
7
6
5
4
3
2
1
ppm
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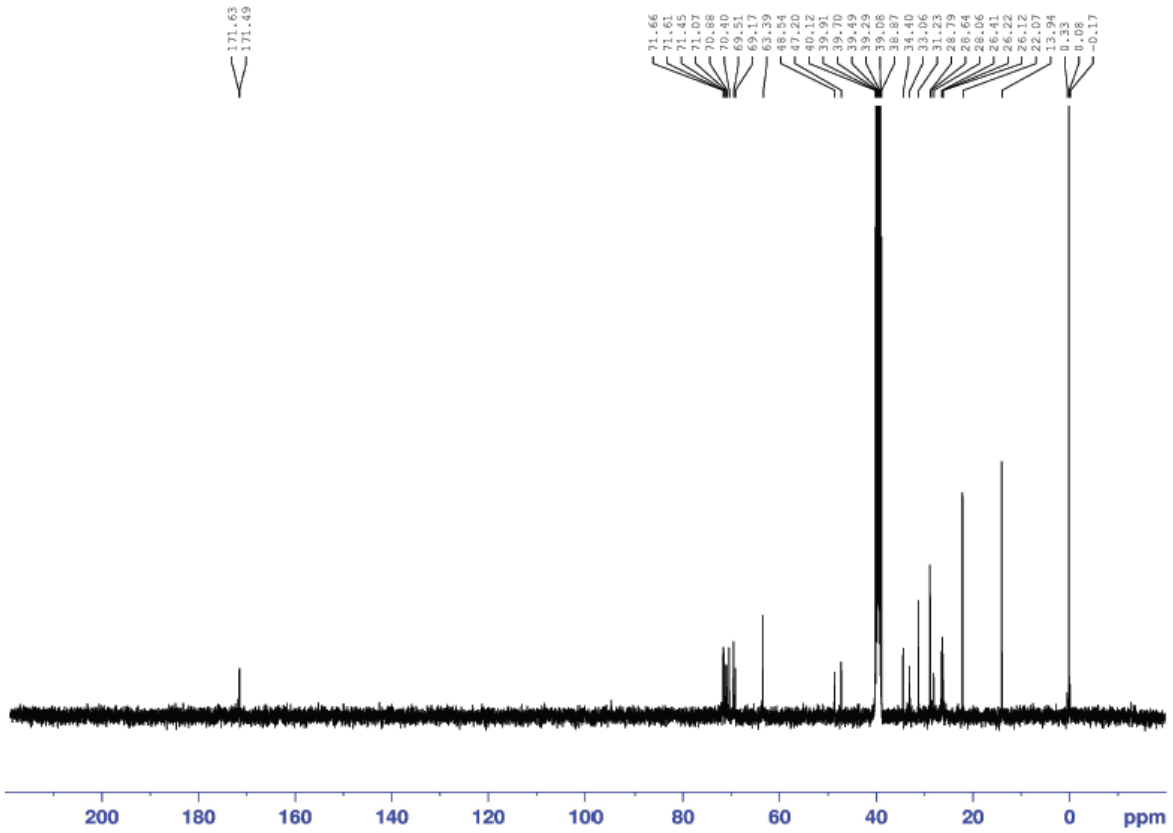
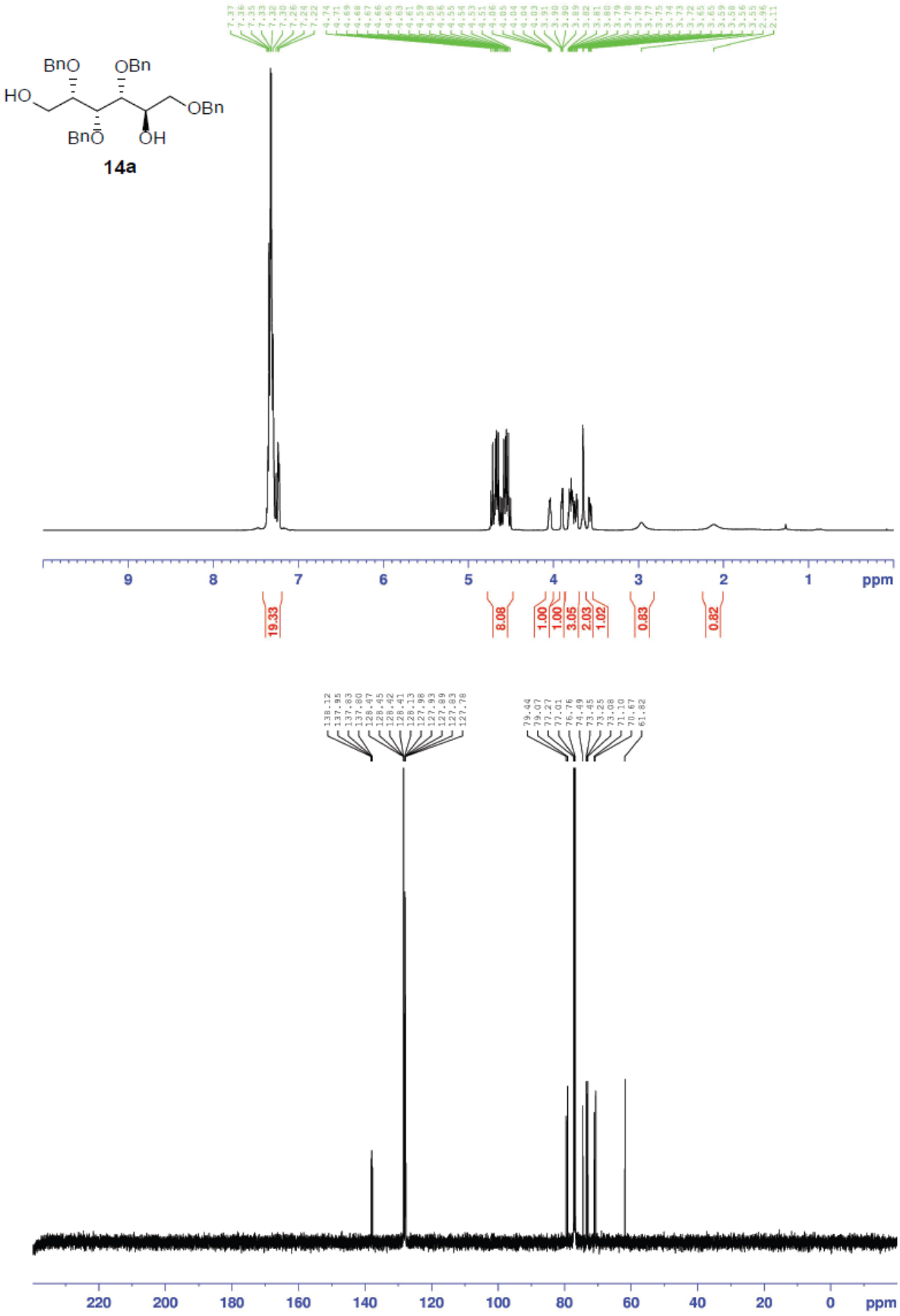
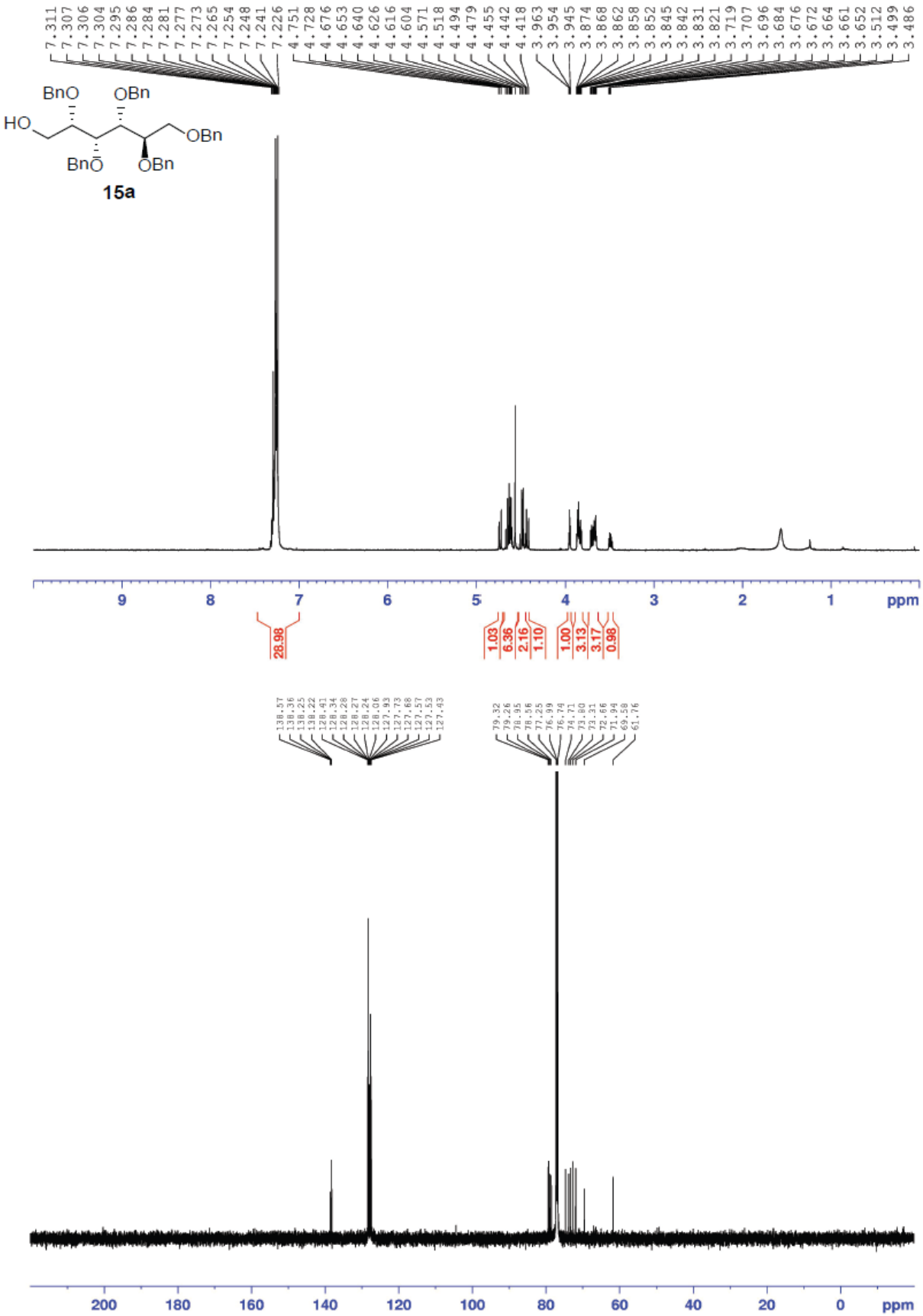
4.76
0.89
1.15
9.60
1.56
1.37
2.05
10.12
3.00

8


171.63
171.49
71.66
71.61
71.45
71.07
70.88
70.40
69.51
69.17
63.39
48.54
47.20
40.12
39.91
39.70
39.49
39.29
39.08
38.87
34.40
33.06
31.23
28.79
28.64
28.06
26.41
26.22
26.12
22.07
13.94
0.33
0.08
-0.17
200
180
160
140
120
100
80
60
40
20
0
ppm


BnO
OBn
HO
OBn
BnO
OH
14a
ppm
ppm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7.307
7.306
7.304
7.295
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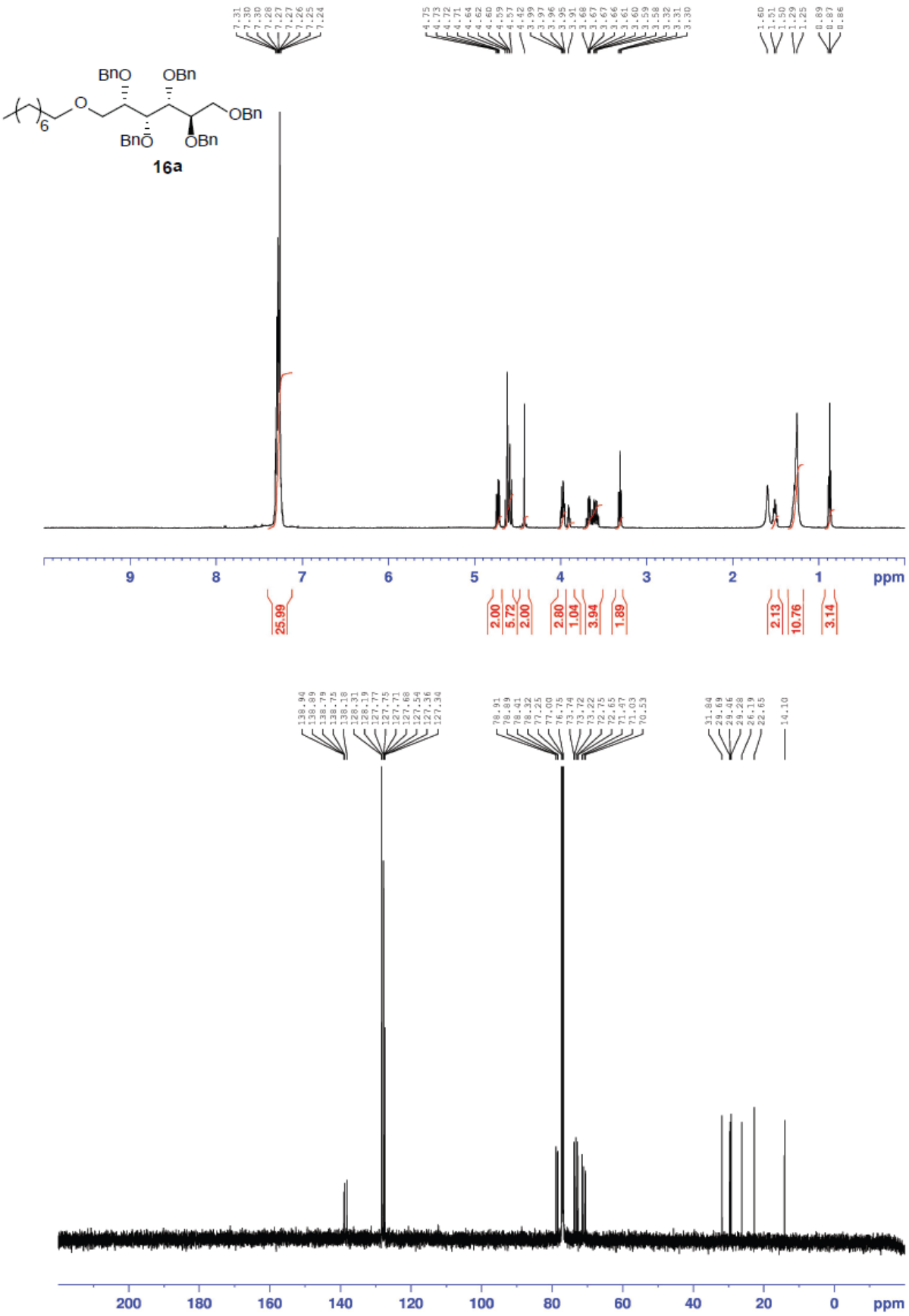
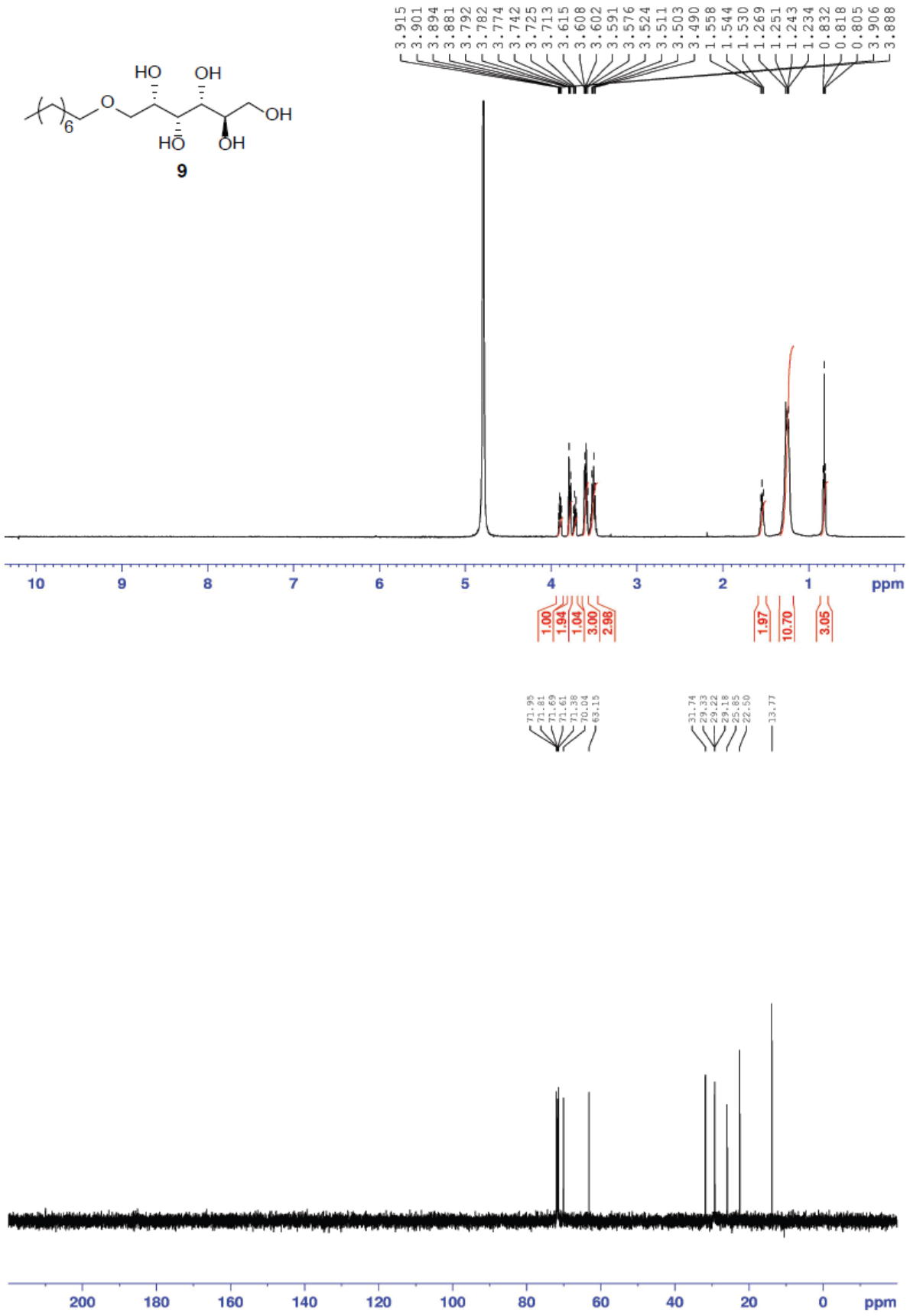
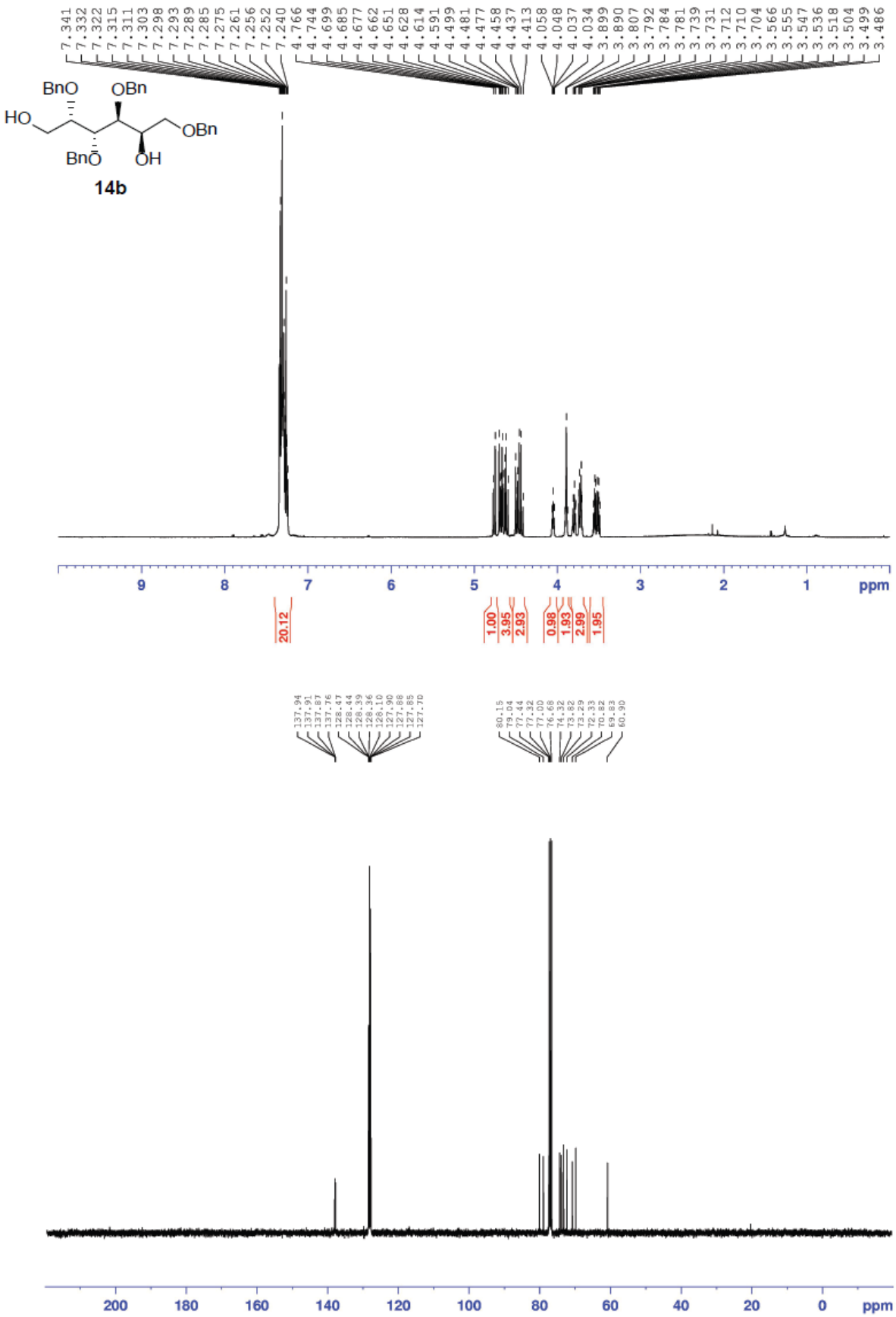
7.284
7.281
7.277
7.273
7.265
7.254
7.248
7.241
7.226
4.751
4.728
4.676
4.653
4.640
4.626
4.616
4.604
4.571
4.518
4.494
4.479
4.455
4.442
4.418
3.963
3.954
3.945
3.874
3.868
3.862
3.858
3.852
3.845
3.842
3.831
3.821
3.719
3.707
3.696
3.684
3.676
3.672
3.664
3.661
3.652
3.512
3.499
3.486
BnO
OBn
HO
OBn
BnO
OBn
15a
9
8
7
6
5
4
3
2
1
ppm
28.98
1.03
6.36
2.16
1.10
1.00
3.13
3.17
0.98
138.57
138.36
138.25
138.22
128.41
128.34
128.28
128.27
128.24
128.06
127.93
127.73
127.68
127.57
127.53
127.43
79.32
79.26
78.95
78.56
77.25
76.99
76.74
74.71
73.80
73.31
72.66
71.94
69.58
61.76
200
180
160
140
120
100
80
60
40
20
0
ppm




HO
OH
O
6
OH
HO
OH
9
3.915
3.901
3.894
3.881
3.792
3.782
3.774
3.742
3.725
3.713
3.615
3.608
3.602
3.591
3.576
3.524
3.511
3.503
3.490
1.558
1.544
1.530
1.269
1.251
1.243
1.234
0.832
0.818
0.805
3.906
3.888
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.94
1.04
3.00
2.98
1.97
10.70
3.05
71.99
71.81
71.69
71.61
71.38
70.04
63.15
31.74
29.33
29.22
29.18
25.85
22.50
13.77
200
180
160
140
120
100
80
60
40
20
0
ppm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7.322
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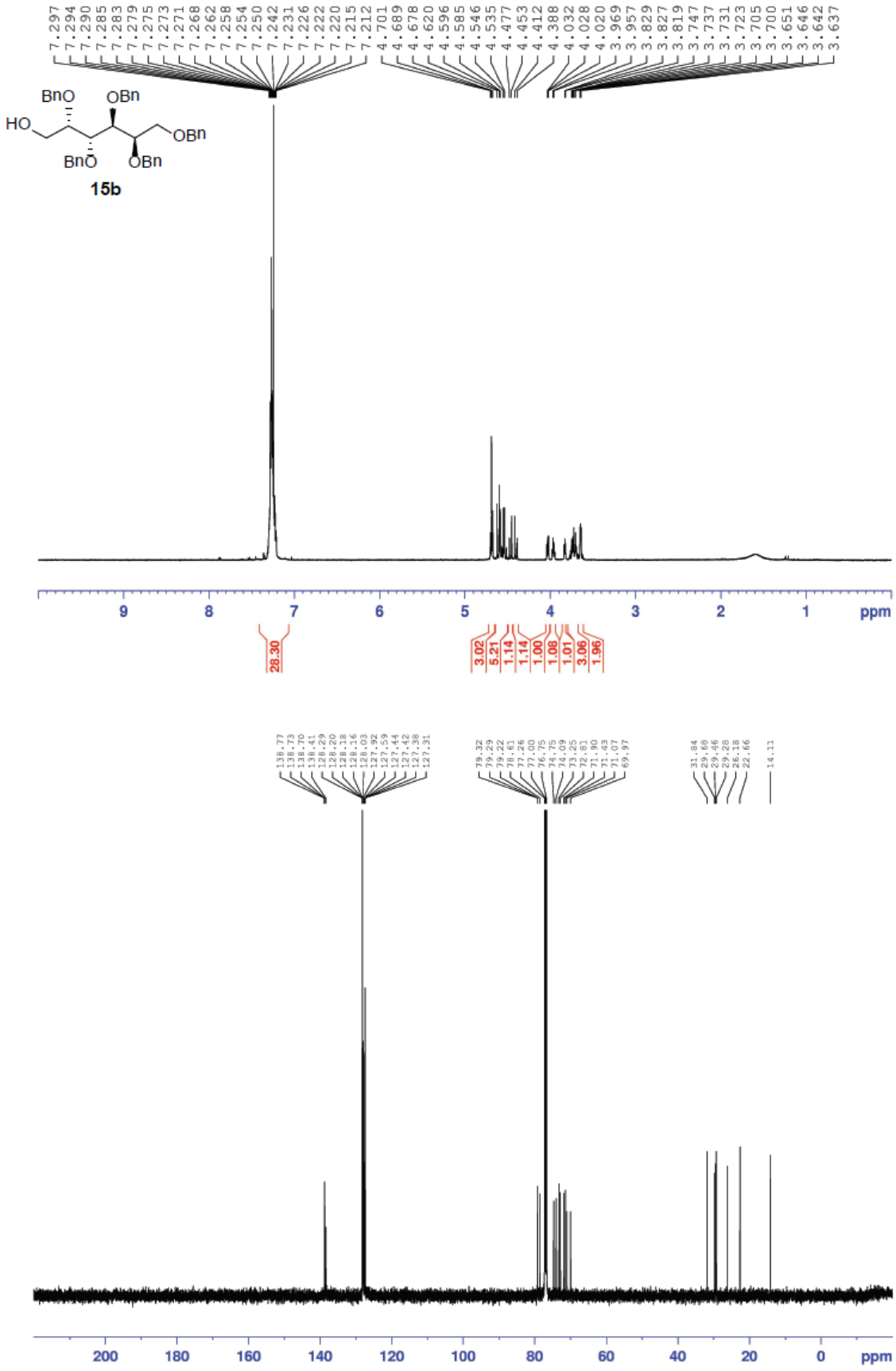
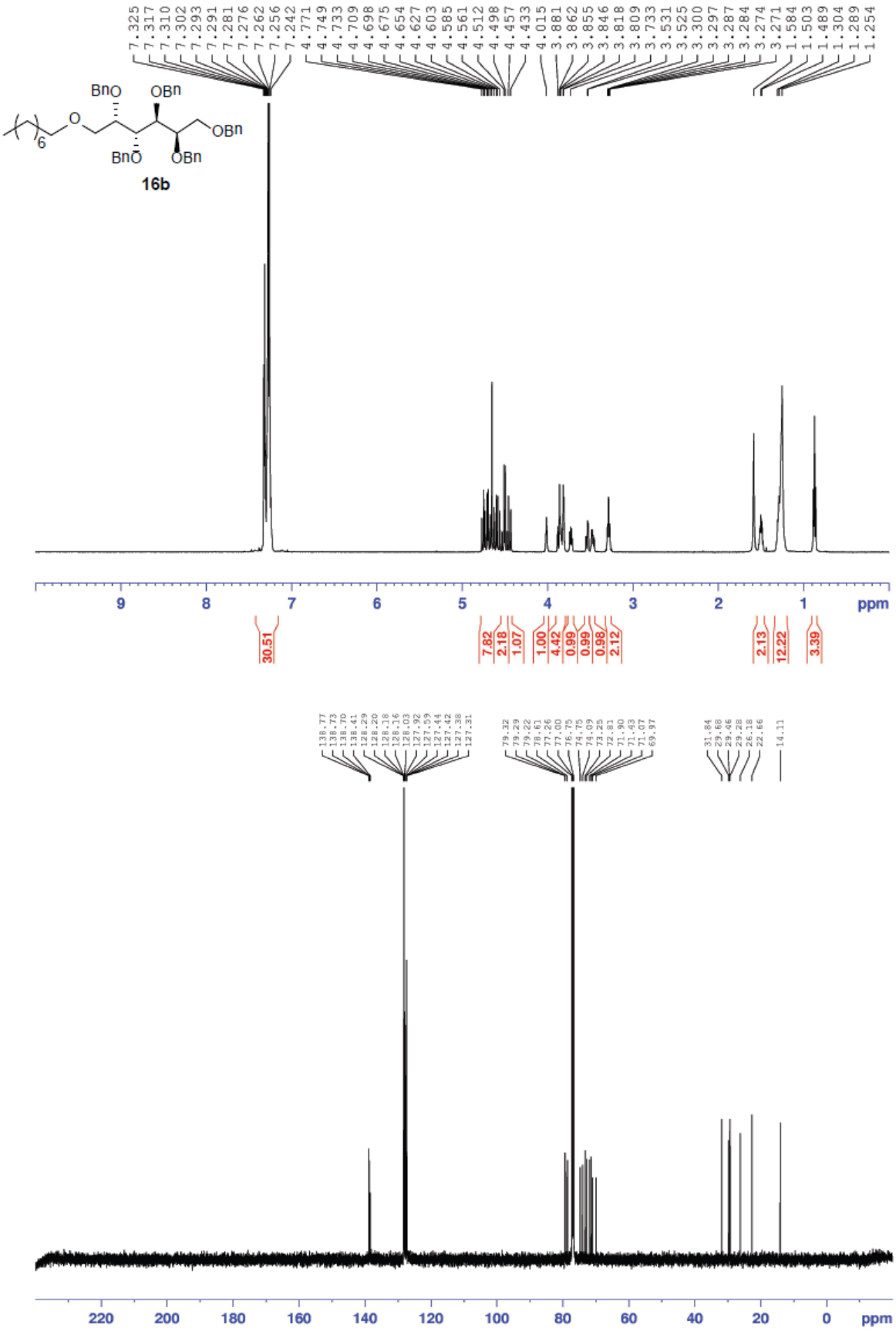
7.303
7.298
7.293
7.289
7.285
7.275
7.261
7.256
7.252
7.240
4.766
4.744
4.699
4.685
4.677
4.662
4.651
4.628
4.614
4.591
4.499
4.481
4.477
4.458
4.437
4.413
4.058
4.048
4.037
4.034
3.899
3.890
3.807
3.792
3.784
3.781
3.739
3.731
3.712
3.710
3.704
3.566
3.555
3.547
3.536
3.518
3.504
3.499
3.486
BnO
OBn
HO
OBn
BnO
OH
14b
9
8
7
6
5
4
3
2
1
ppm
20.12
1.00
3.95
2.93
0.98
1.93
2.99
1.95
137.94
137.91
137.87
137.76
128.47
128.44
128.39
128.36
128.10
127.90
127.88
127.85
127.70
80.15
79.04
77.44
77.32
77.00
76.68
74.32
73.82
73.29
72.33
70.82
69.83
60.90
200
180
160
140
120
100
80
60
40
20
0
ppm

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7.291
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7.276
7.262
7.256
7.242
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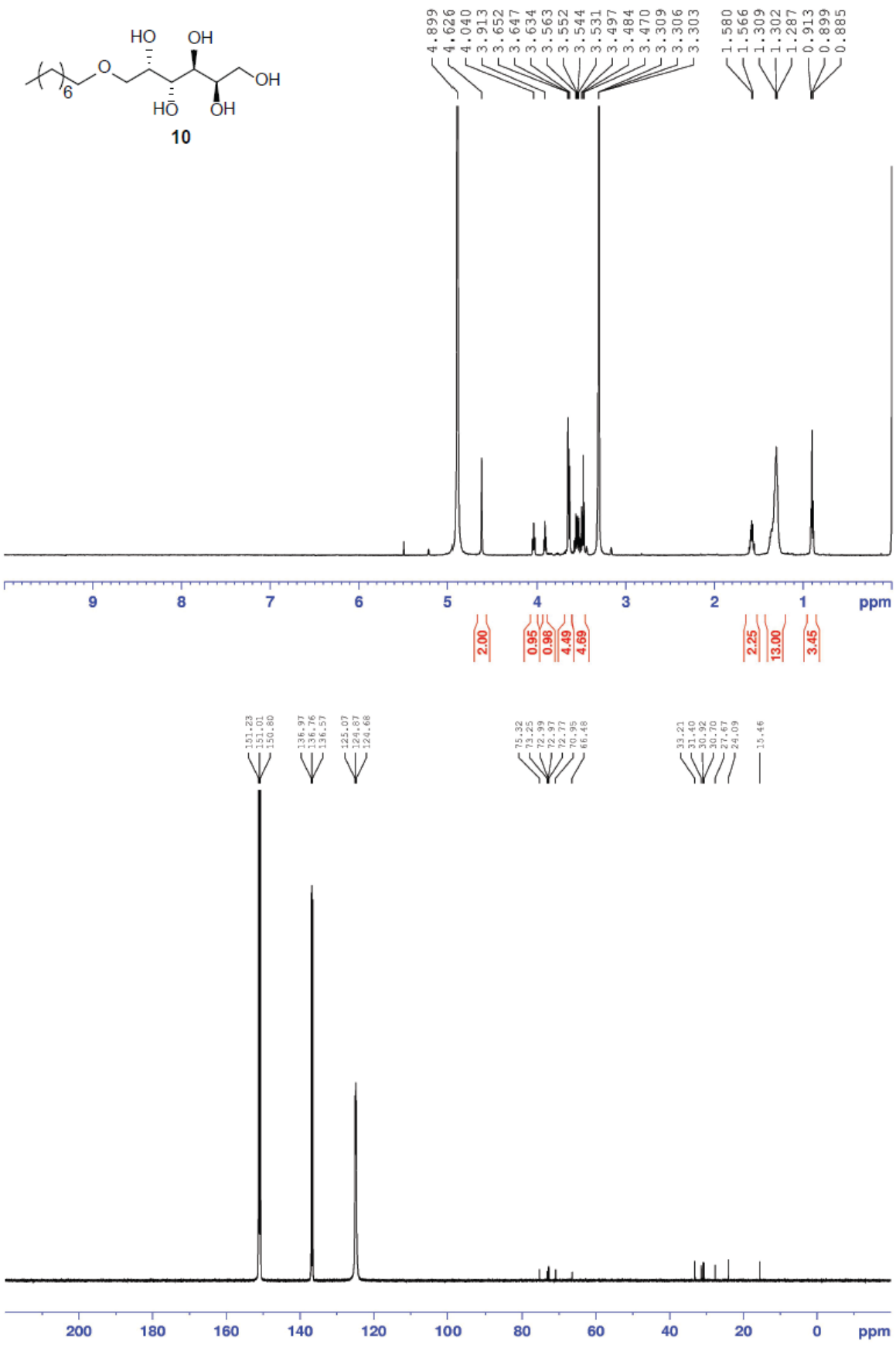
4.749
4.733
4.709
4.698
4.675
4.654
4.627
4.603
4.585
4.561
4.512
4.498
4.457
4.433
4.015
3.881
3.862
3.855
3.846
3.818
3.809
3.733
3.531
3.525
3.300
3.297
3.287
3.284
3.274
3.271
1.584
1.503
1.489
1.304
1.289
1.254
BnO
OBn
OBn
BnO
OBn
6
16b
9
8
7
6
5
4
3
2
1
ppm
30.51
7.82
2.18
1.07
1.00
4.42
0.99
0.99
0.98
2.12
2.13
12.22
3.39
138.77
138.73
138.70
138.41
128.29
128.20
128.18
128.16
128.03
127.92
127.59
127.44
127.42
127.38
127.31
79.32
79.23
79.22
78.61
77.26
77.00
76.75
74.75
74.09
73.25
72.81
71.90
71.43
71.07
69.97
31.84
29.68
29.46
29.28
26.18
22.66
14.11
220
200
180
160
140
120
100
80
60
40
20
0
ppm


HO
OH
O
6
OH
HO
OH
10
4.899
4.626
4.040
3.913
3.652
3.647
3.634
3.563
3.552
3.544
3.531
3.497
3.484
3.470
3.309
3.306
3.303
1.580
1.566
1.309
1.302
1.287
0.913
0.899
0.885
9
8
7
6
5
4
3
2
1
ppm
2.00
0.95
0.98
4.49
4.69
2.25
13.00
3.45
151.23
151.01
150.80
136.97
136.76
136.57
125.07
124.87
124.68
75.32
73.25
72.99
72.97
72.77
70.95
66.48
33.21
31.40
30.92
30.70
27.67
24.09
15.46
200
180
160
140
120
100
80
60
40
20
0
ppm