SUPPLEMENTARY INFORMATION

Uptake of unnatural trehalose analogs as a reporter for *Mycobacterium tuberculosis*

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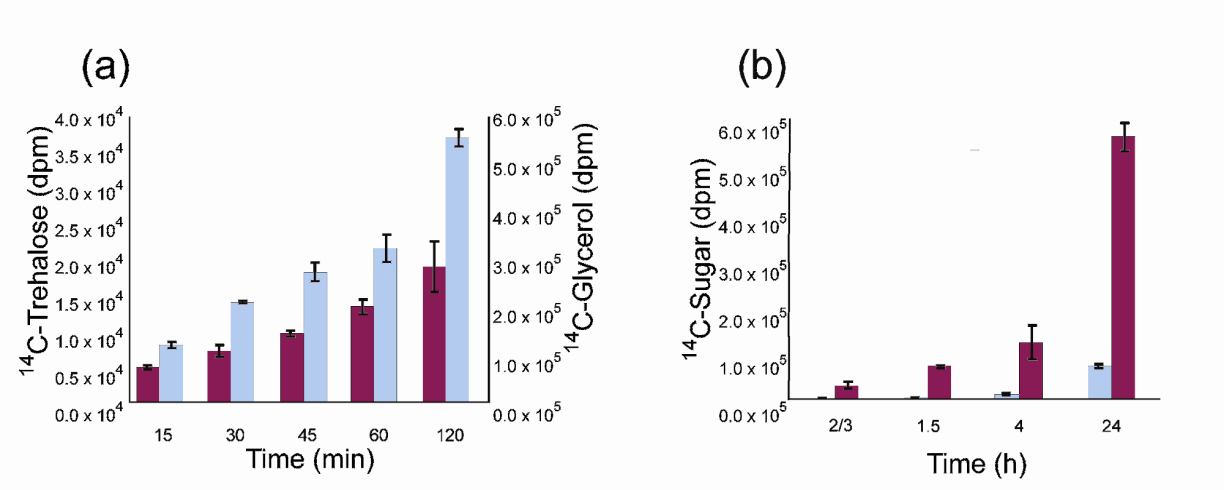
Table of Contents

Nature Chemical Biology: doi:10.1038/nchembio.539

Nature Chemical Biology: doi:10.1038/nchembio.539

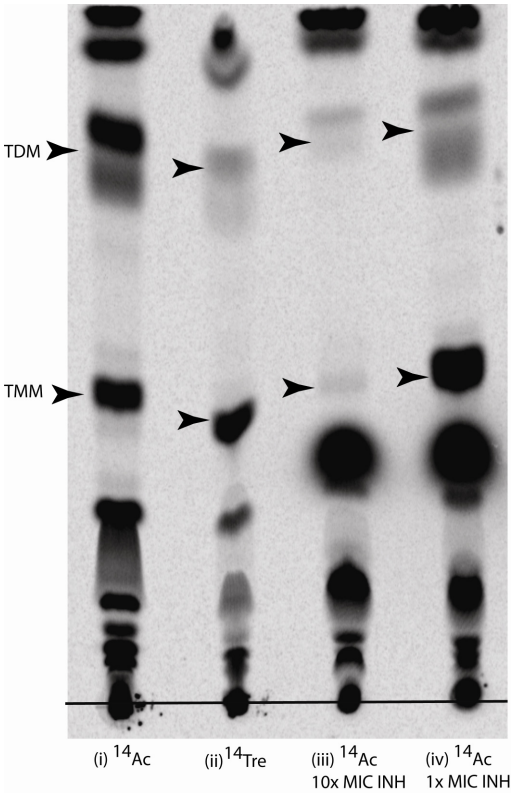
Supplementary Results

Supplementary Figure 1. (a) Uptake of ^{14}C-trehalose in *Mtb* culture over 2 hours, purple-^{14}C-trehalose, blue-^{14}C–glycerol. (b) Uptake over 24 hour period purple-^{14}C-trehalose, blue-^{14}C–Glucose.



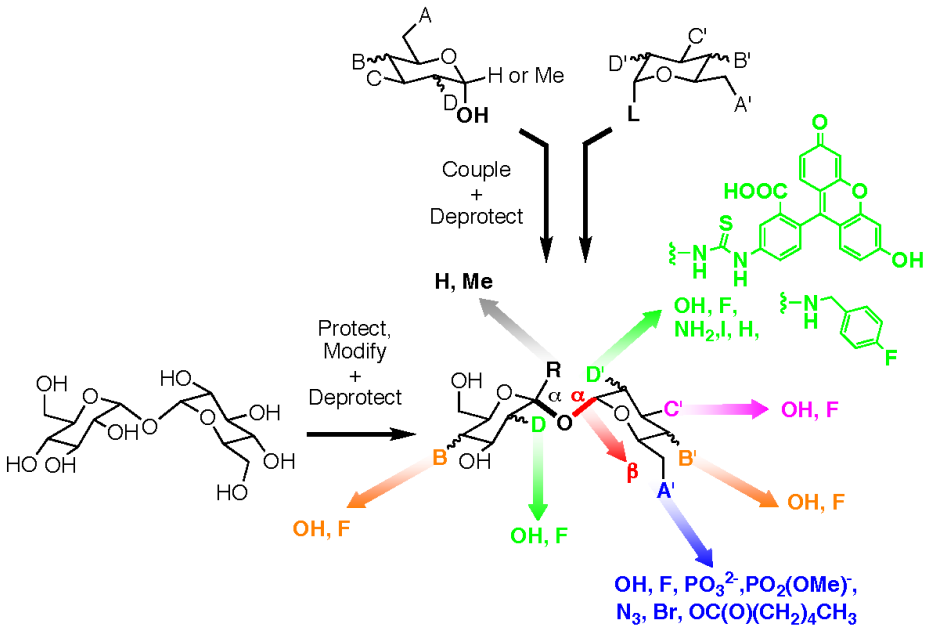
Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Figure 2. ^{14}C-trehalose is incorporated into TMM and TDM in *Mtb*. Arrowheads show the migration position of authentic samples of TMM and TDM. In the first lane the culture was labeled for 24 hr with ^{14}C-acetate to label all fatty acids and then extracted as described in Methods. In the second lane the culture was labeled with ^{14}C-trehalose for 24 hr and then extracted. In the third and fourth lanes the culture was treated with 10X and 1X MIC concentrations of isoniazid (INH) to block the synthesis of mycolic acids and thereby inhibit formation of TMM and TDM. ^{14}C-Trehalose labels spots that co-migrate with TMM and TDM, the synthesis of which is blocked by INH.

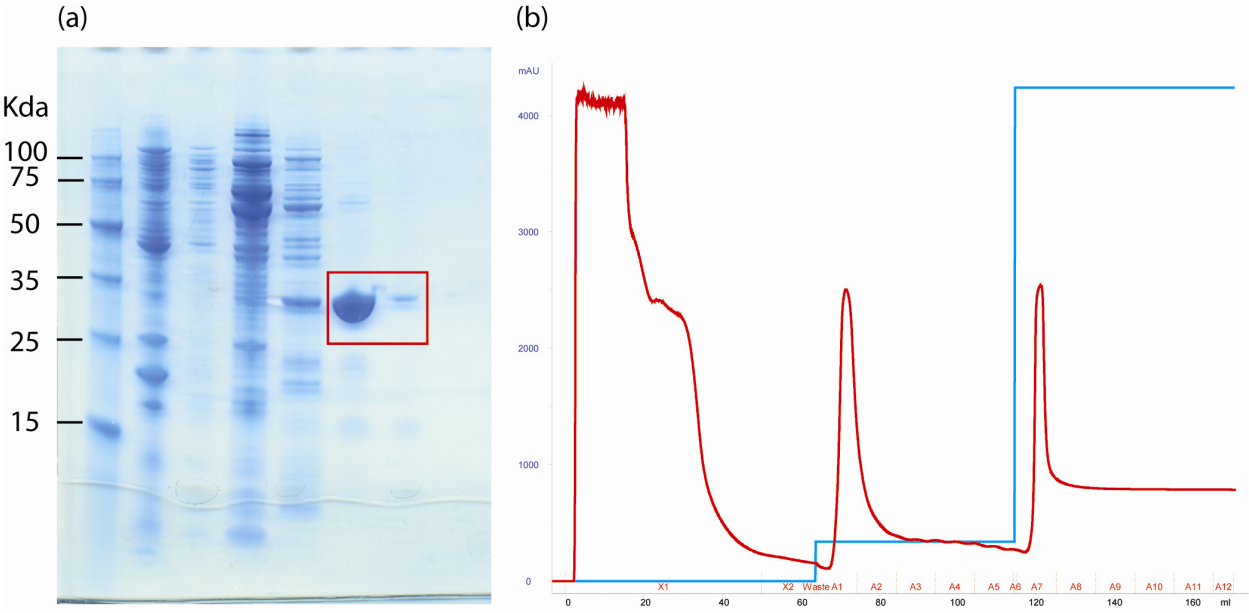


Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Scheme 1. Representative strategies for the synthesis of the trehalose library. Arrows indicate sites and nature of systematic modifications introduced either through (i) directly creating the 1,1-linkage (couple and deprotect) or (ii) alteration of the trehalose scaffold (protect, modify and deprotect).

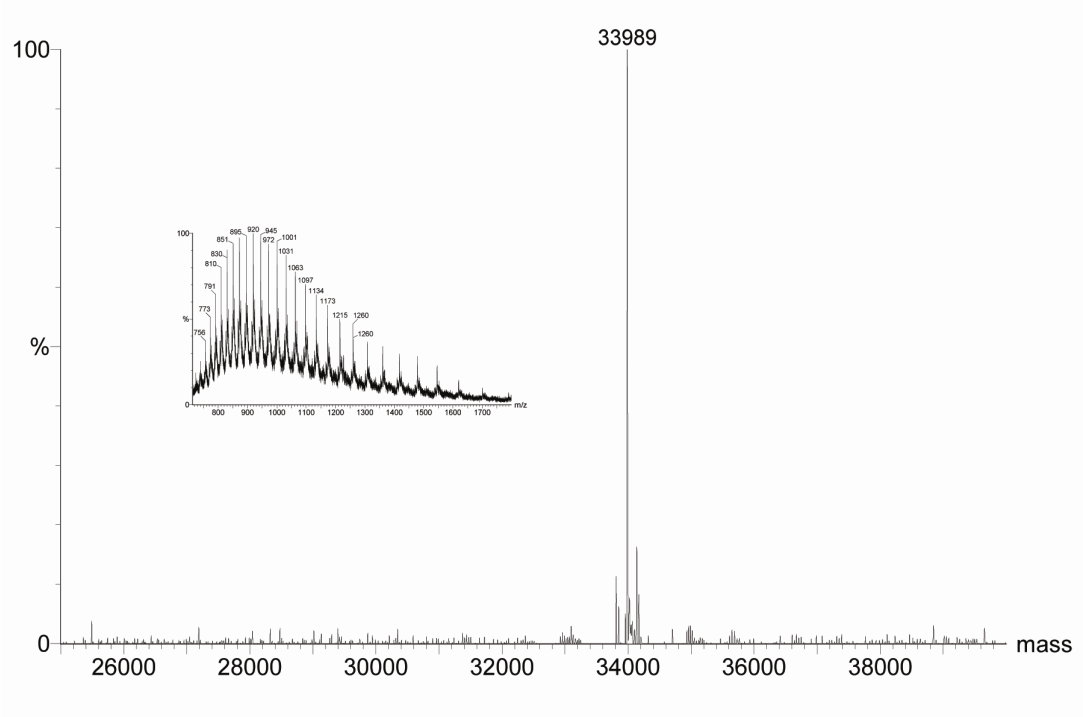


Supplementary Figure 3. (a) SDS page gel of Ag85A, lane 1- novagen perfect protein makers 15-150 Kda, box indicates fractions collected (b) FPLC trace of protein expression red- protein UV absorbance, blue- gradient of imidazole. (5 mM to 1M)

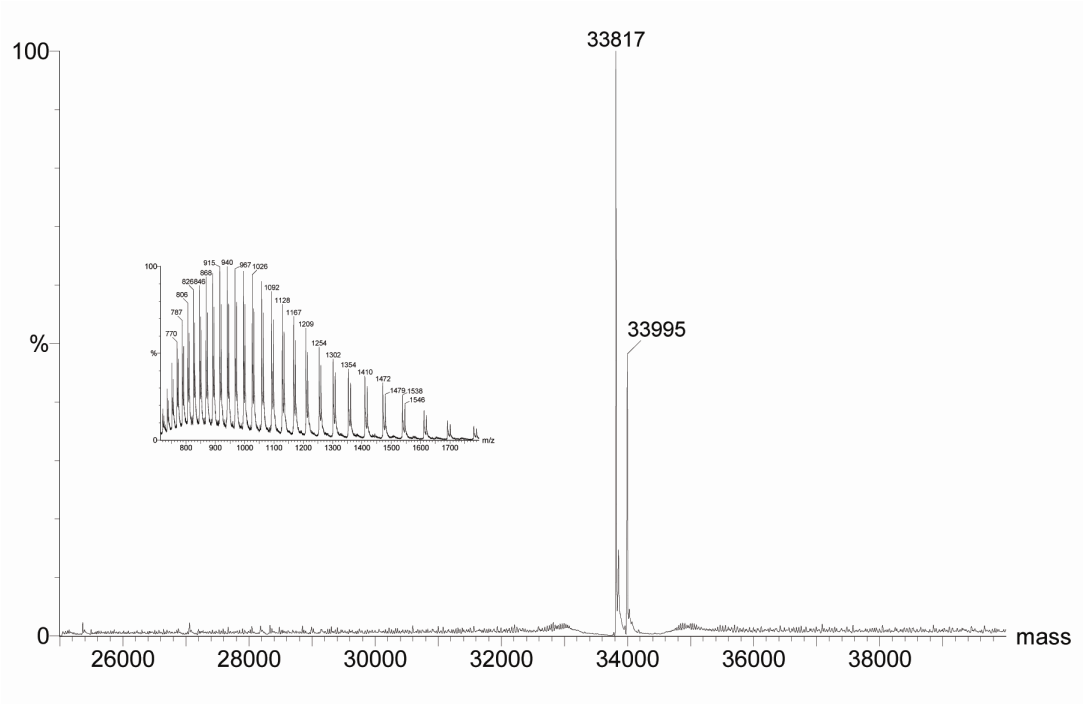


Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Figure 4. Mass spectrum of Ag85A with gluconoylation. Inset shows protein spectrum before deconvolution.

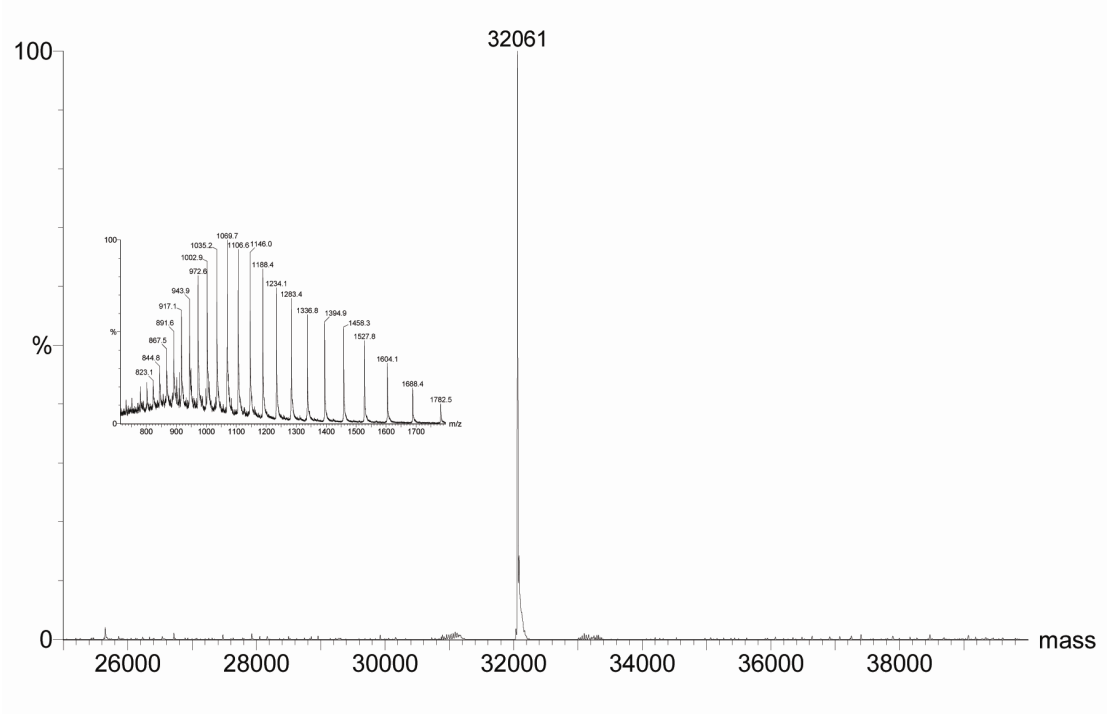


Supplementary Figure 5. Mass spectrum of Ag85A as a mixture with and without gluconoylation. Inset shows protein spectrum before deconvolution.



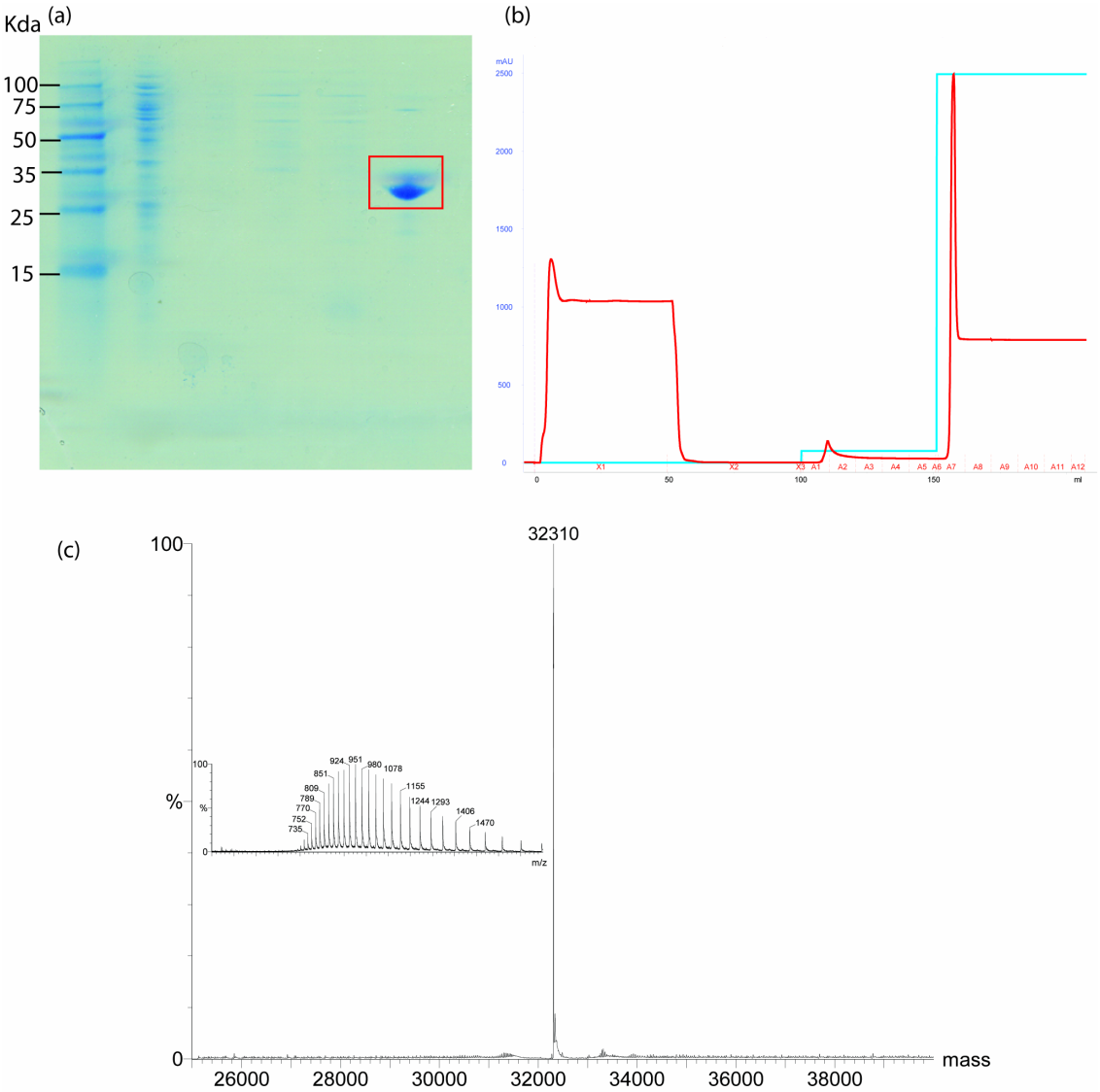
Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Figure 6. Mass spectrum of Ag85A treated with thrombin. Inset shows protein spectrum before deconvolution.



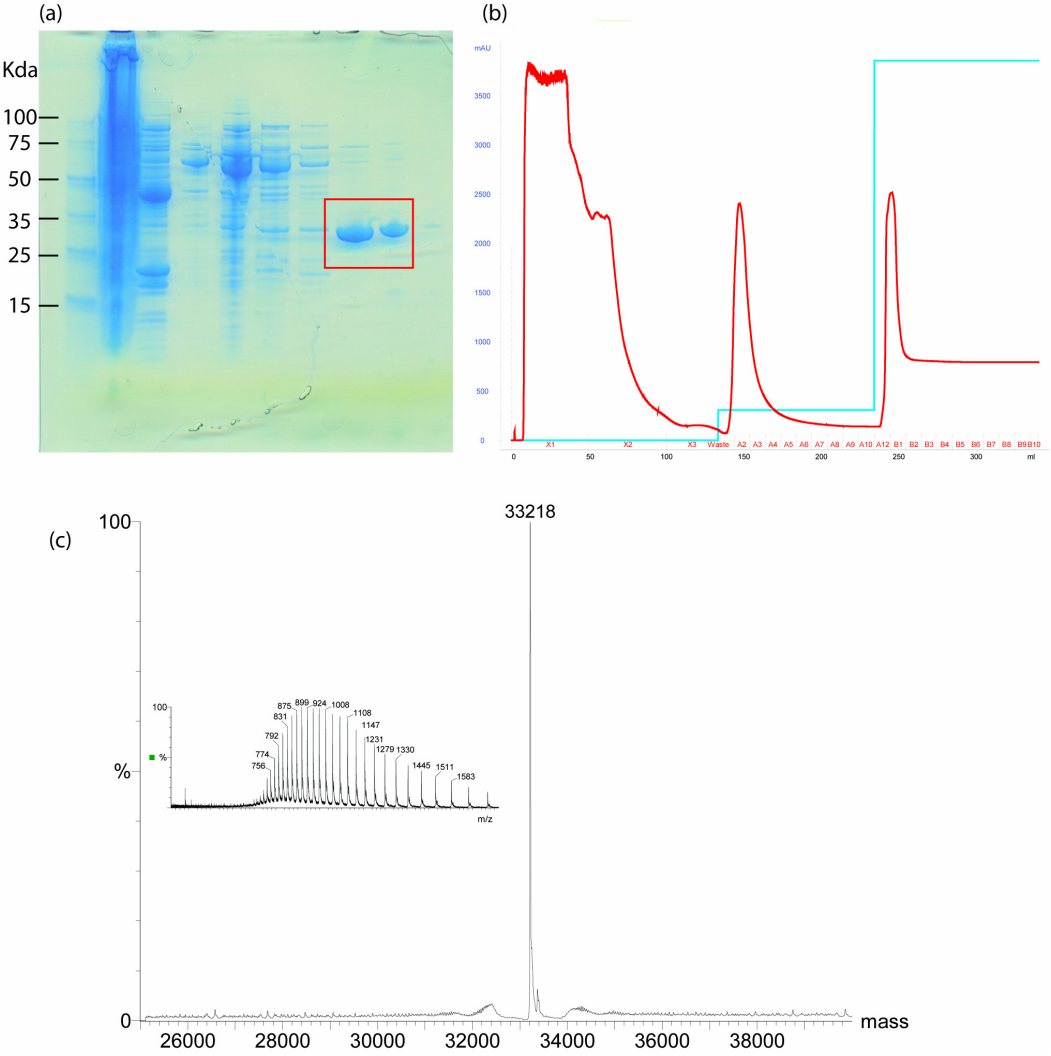
Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Figure 7. (a) SDS page gel of Ag85B, lane 1- novagen perfect protein makers 15-150 Kda, box indicates fractions collected (b) FPLC trace of protein expression red- protein absorbance, blue- gradient of imidazole. (5mM to 1M) (c) Mass spectrum of Ag85 B. Inset shows protein spectrum before deconvolution.



Nature Chemical Biology: doi:10.1038/nchembio.539

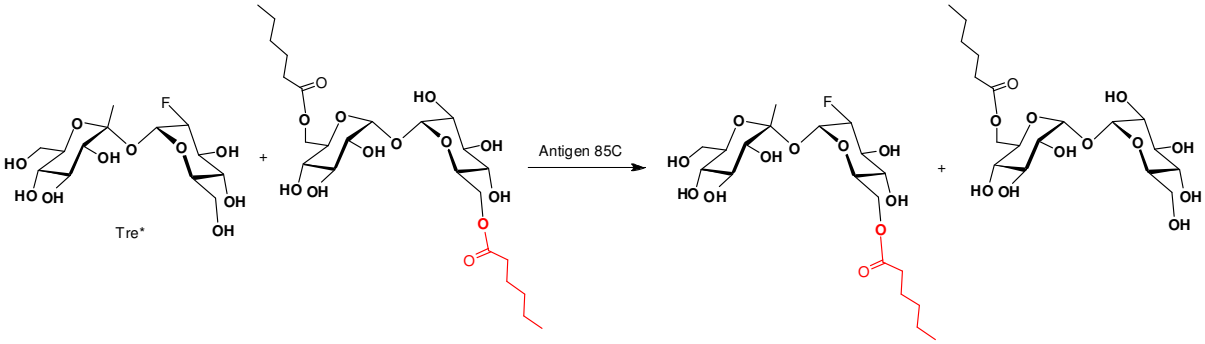
Supplementary Figure 8. (a) SDS page gel of Ag85 C = lane 1- novagen perfect protein makers 15-150 Kda, box indicates fractions collected (b) FPLC trace of protein expression red- protein absorbance, blue- gradient of imidazole. (5mM to 1M) (c) Mass spectrum of Ag85 C. inset shows protein spectrum before deconvolution.



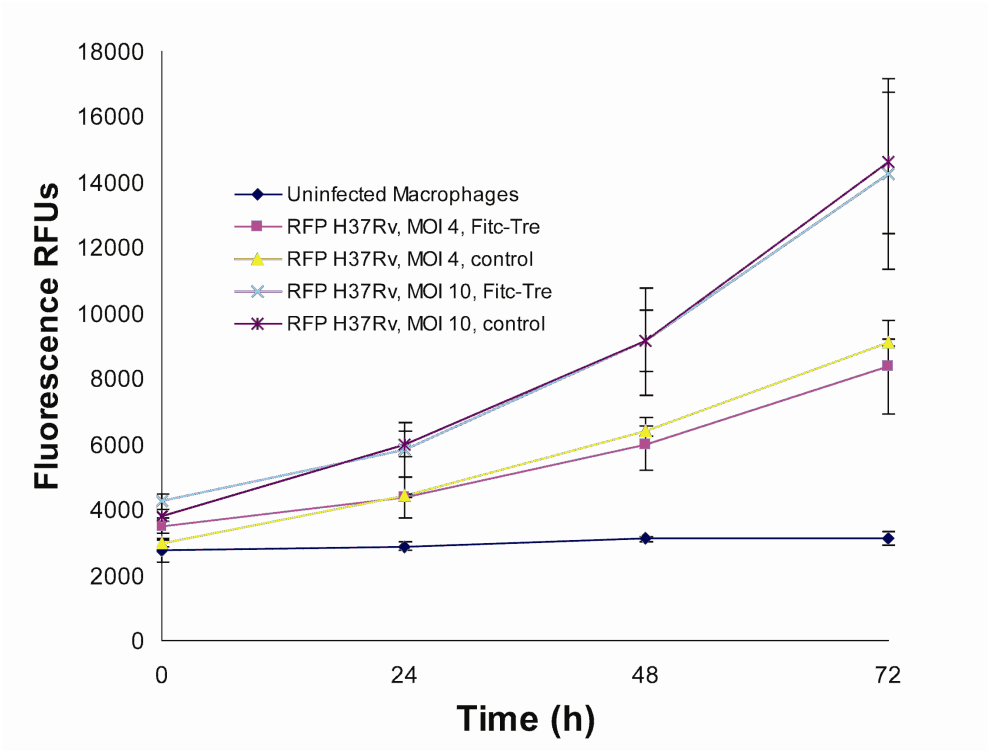
Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Scheme 2.

Simplified Antigen 85 turnover assay. Tre* represents any trehalose analogue. Mass spectrometry is utilized to quantify the amount of acyl-transfer to the unnatural trehalose by Antigen 85.



Supplementary Figure 9. Growth inhibition curves of RFP (red fluorescent protein) H37Rv *Mtb* residing within J774 macrophages. RFP fluorescence was monitored (excitation 594 nm, emission 612 nm). Error bars reflect the standard deviation for 4 experiments. MOI- multiplicity of infection. RFUs- relative units of fluorescence



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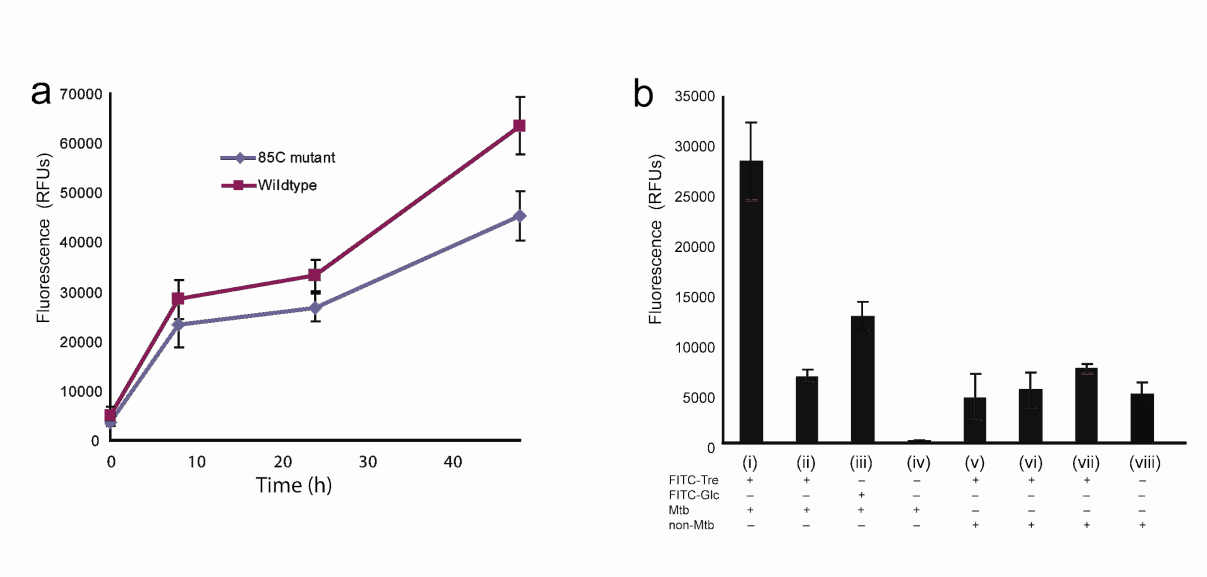
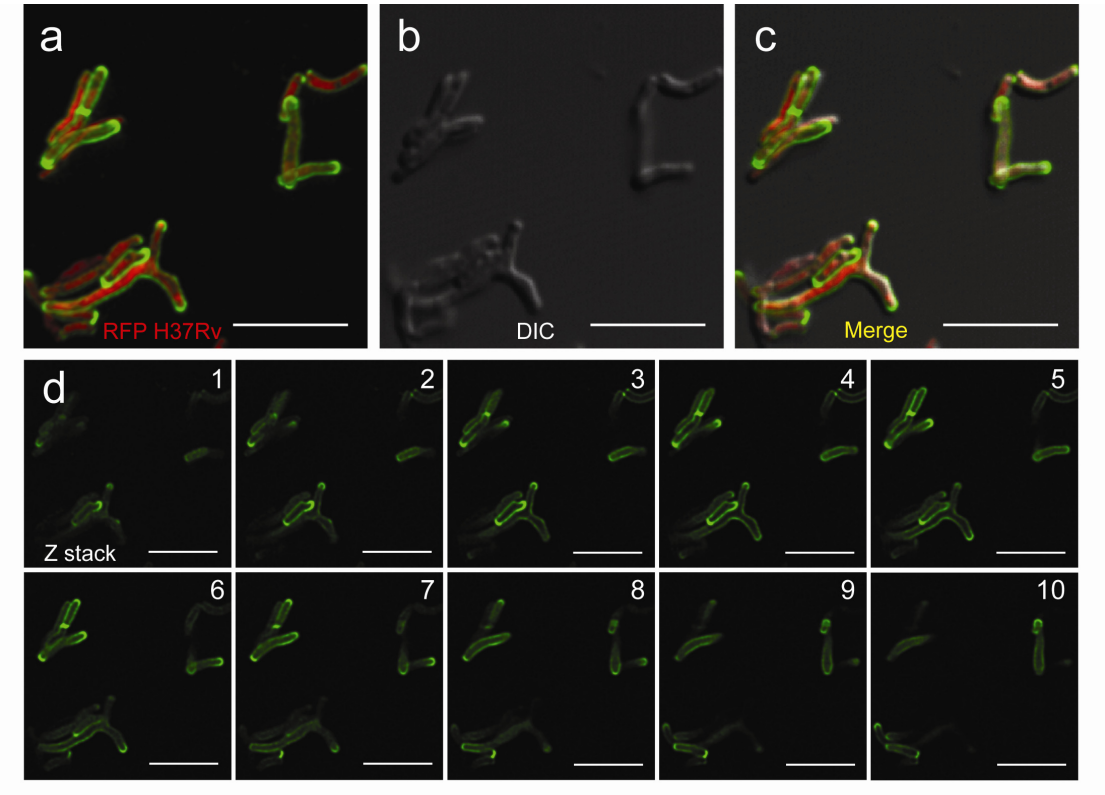
Supplementary Figure 10. FITC-Tre is incorporated by *Mtb* into FITC-TDM through the action of the Ag85 enzymes. (a) Uptake of FITC-Tre into wildtype CDC 1551 (purple squares) or 85C mutant, known to have a 40% reduction in mycolic acid incorporation into the cell wall (blue diamonds). (b) Uptake of FITC-Tre or FITC-Glc into (i , iii, and iv) live *Mtb,* (ii) heat killed *Mtb,* (v) *Staphylococcus aureus,* (vi) *Pseudomonas aeruginosa,* (vii) *Haemophilus influenza,* (viii) Mean autoflorescence of *H. influenza as* representative of autofluorescence of non-mycobacterial species.



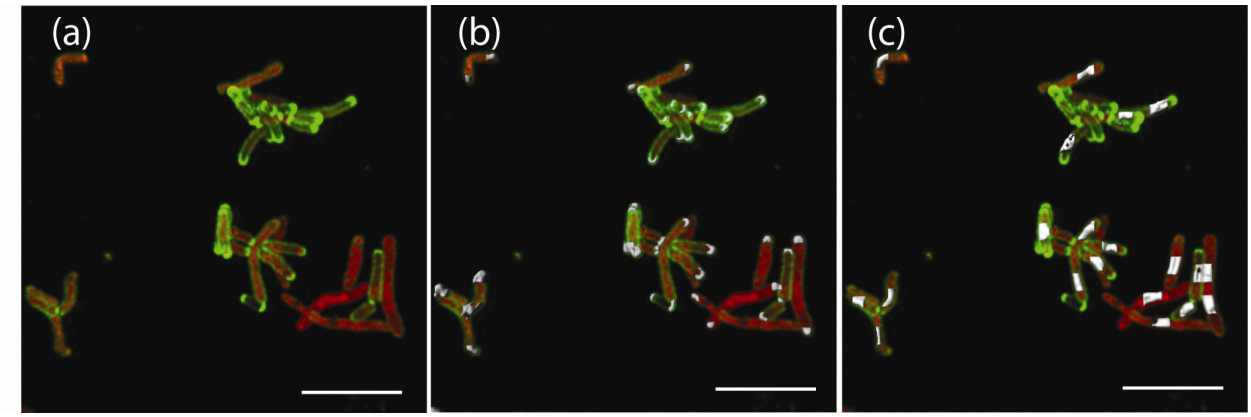
Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Figure 11. Polar labeling by FITC-Tre *in vitro* (a) shows FITC-Tre labeling of the RFP expressing strain of H37Rv and (b) is the DIC image of (a), while (c) is an overlay of (a-b) and (d) depicts the Z stack (1-10) through the same image (step size of 0.13 μm) and demonstrates the FITC-Tre localization to the poles and membrane.

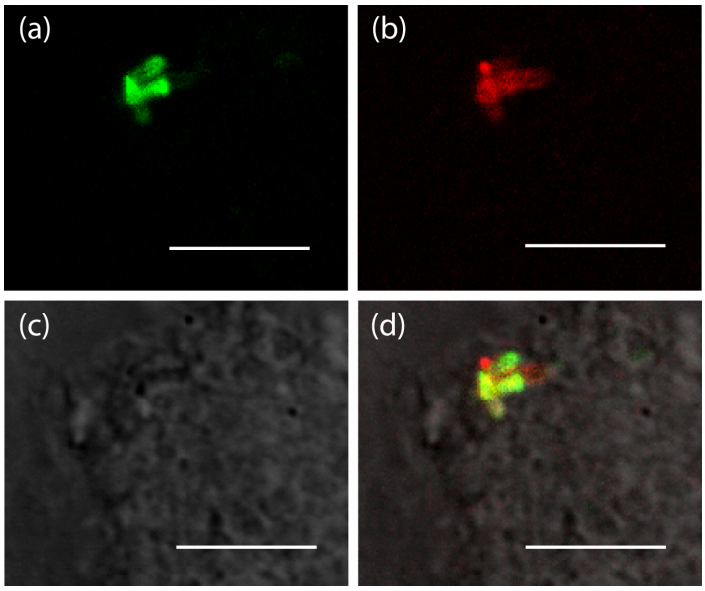


Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Figure 12. The quantification of the intensity of the poles and mid-sections of H37Rv *Mtb* (a) shows a representative image of H37Rv *Mtb,* labeled with FITC-Tre (green) and expressing RFP (red). (b) indicates the regions of interest (ROIs) at the mycobacterial poles in white, while (c) shows the ROIs of the bacterial mid-sections in white.

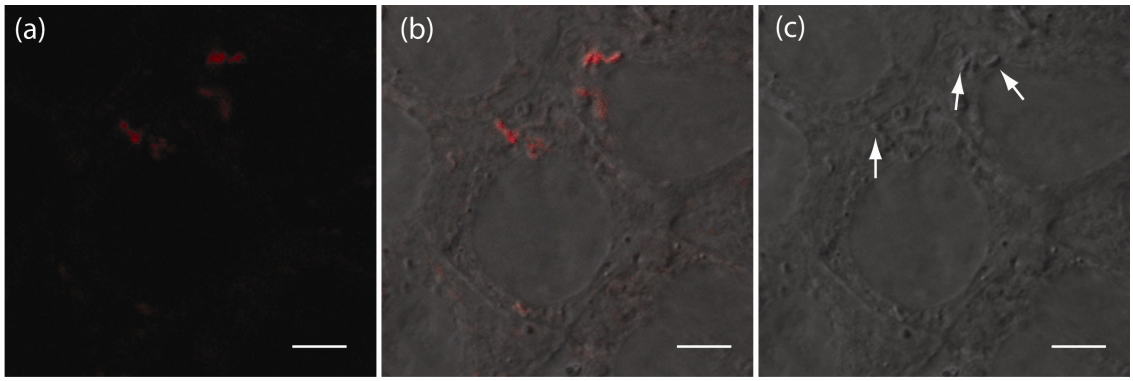


Supplementary Figure 13. (a) H37Rv *Mtb* are labeled with FITC-Tre and (b) treated with anti-*Mtb* antibody (AbCam ab905) while (c) shows the differential interference contrast (DIC) and (d) is an overlay of all three channels and shows colocalization of FITC-Tre and antibody**.** Scale bars 5 µm.

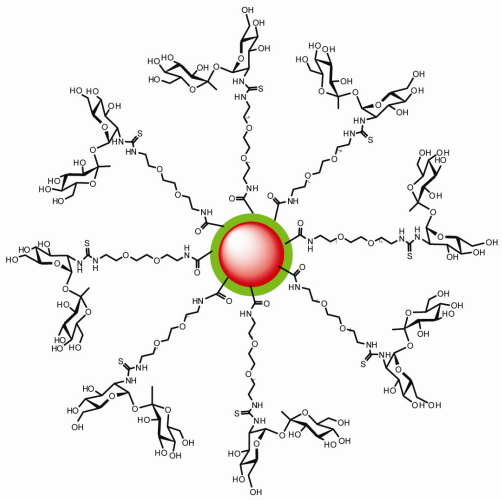


Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Figure 14. Trehalose-Quantum dot (Tre-QD) labeled *Mtb*. (a) QD labeled *Mtb* (excitation 562 nm. emission 585nm – 634 nm) (b) Overlay of QD image with DIC (c) DIC image of infected macrophages treated with Tre-QDs. Arrows indicate putative *Mtb* identified in DIC image (d) Tre-QDs with ~110 sugars/dot. Scale bars 5 µm.



(d)



25

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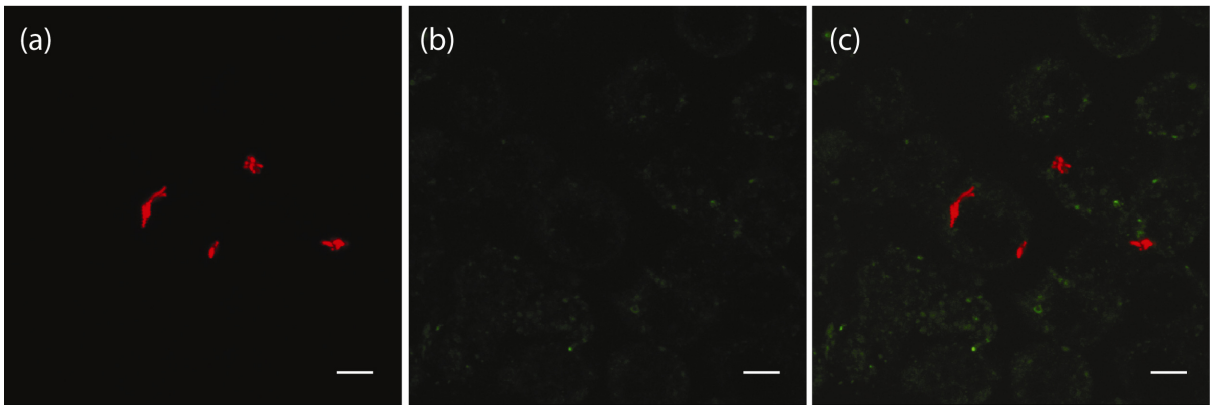
Supplementary Figure 15. FITC-Glc labeling in infected macrophages (a) RFP BCG (excitation 594 nm and emission spectra recorded at 602-651 nm) (b) FITC-Glc label (excited at 488 nm and emission spectra were recorded at 497-557 nm) (c) overlay of RFP and FITC with FITC gain enhanced to show that there is no colocalization. Scale bars 5 µm.



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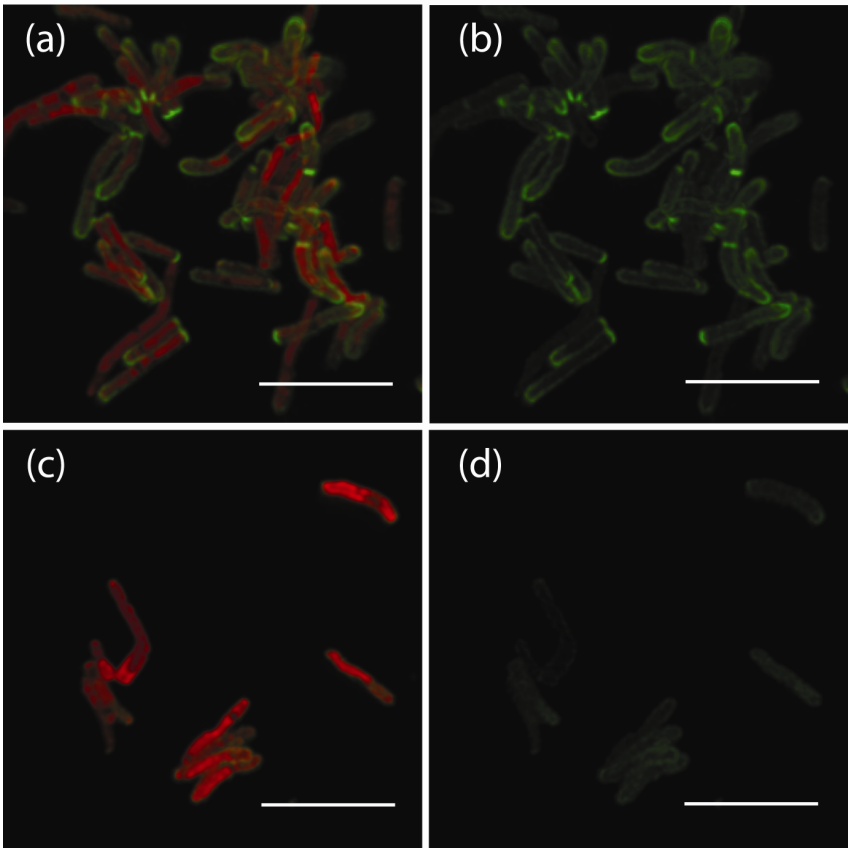
Supplementary Figure 16. Trehalose saturation of FITC-Tre treated H37Rv *Mtb* expressing RFP shows no FITC-tre labeling *in vitro.* (a-b) FITC-Tre labeled H37Rv *Mtb* expressing RFP (b) FITC-Tre channel of overlay shown in (a). (c-d) *Mtb* treated as in (a-b), except grown in the presence of saturating concentrations of trehalose. (c) shows RFP channel, while (d) shows the disappearance of FITC-Tre labeling in the presence of trehalose (100 mM).



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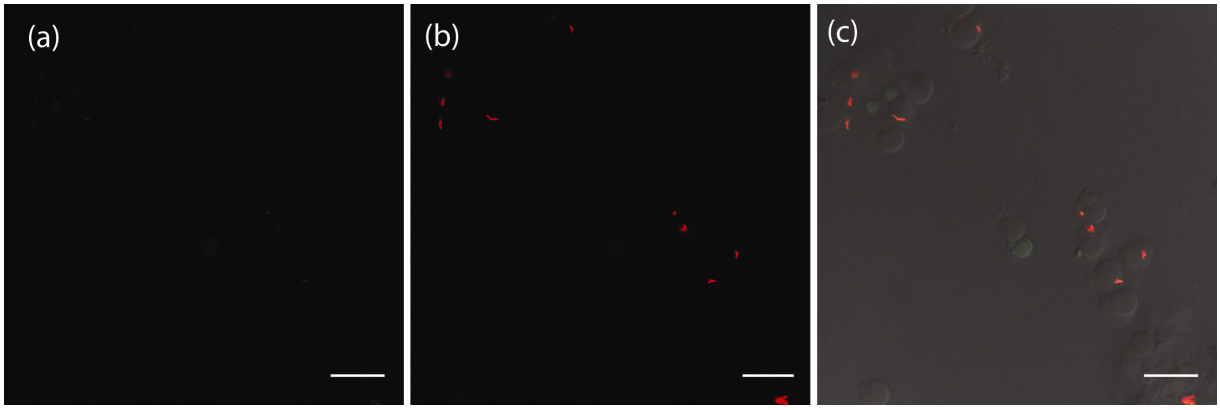
Supplementary Figure 17. Trehalose saturation of FITC-Tre treated BCG shows no FITC-Tre labeling *in vivo*. (a) FITC-Tre treated BCG expressing RFP shows no green fluorescence (b) RFP channel of image in (a) and (c) shows an overlay of FITC-Tre, RFP and DIC images. Scale bars 25 µm.



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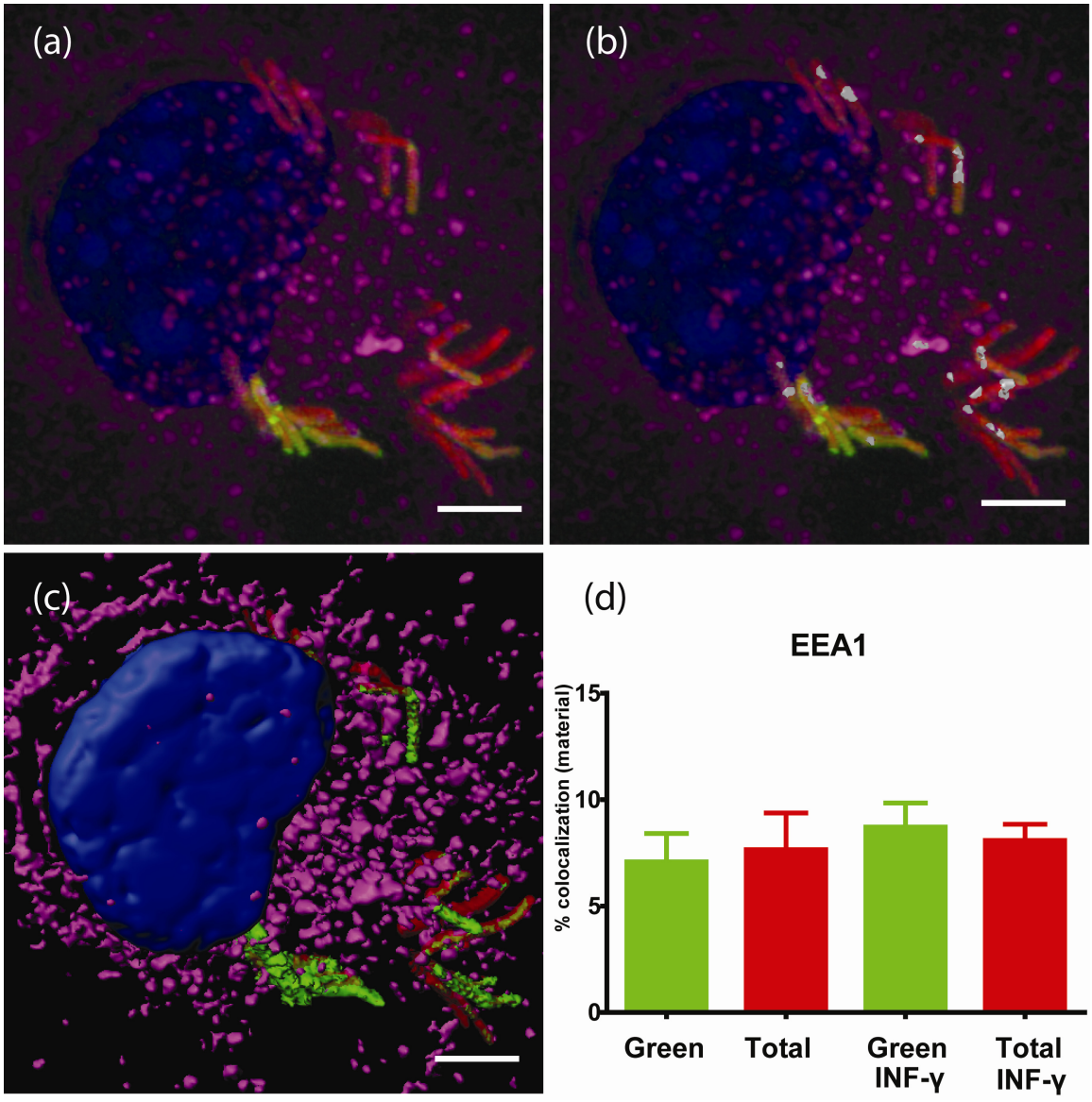
Supplementary Figure 18. (a) Representative image for EEA-1 (interferon treated), green – FITC-Tre, red- RFP H37Rv, Pink –Anti-EEA-1, blue- Nucleus (Dapi) (b) white indicates colocalization (c) 3D reconstruction (d) colocalization statistics (Values are SEM for a minimum of 3 separate experiments)



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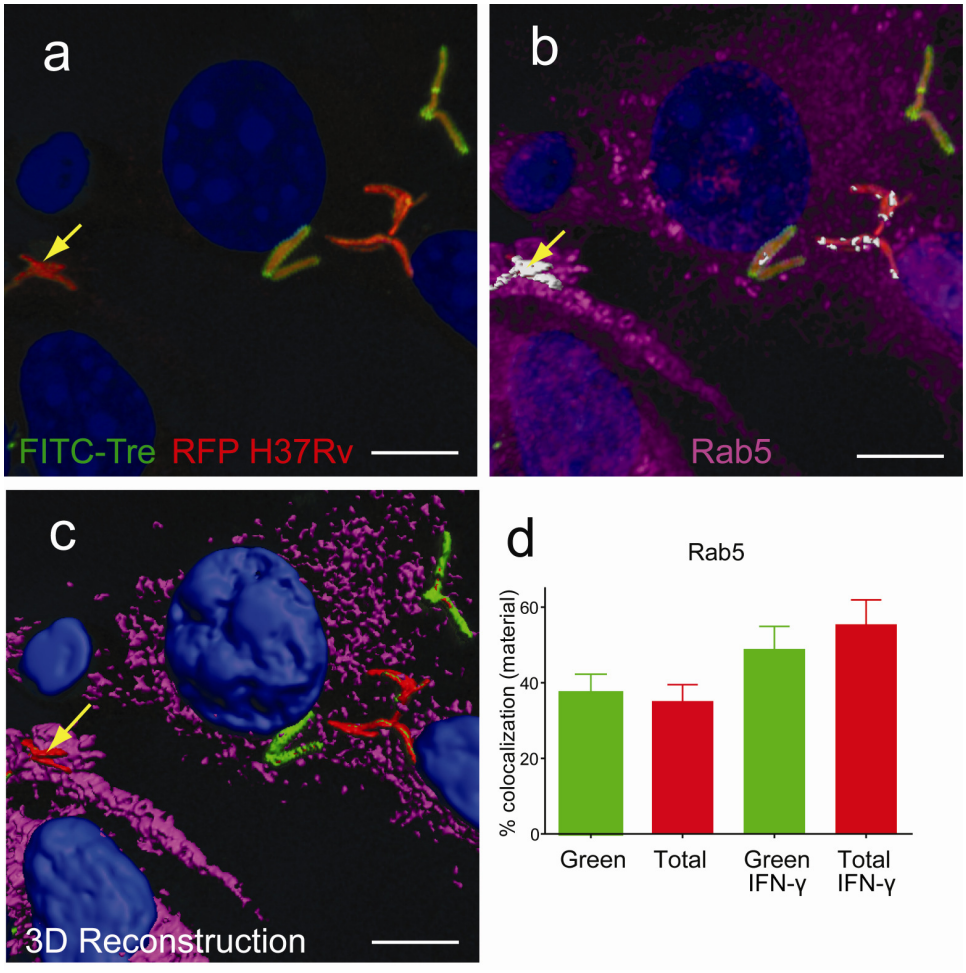
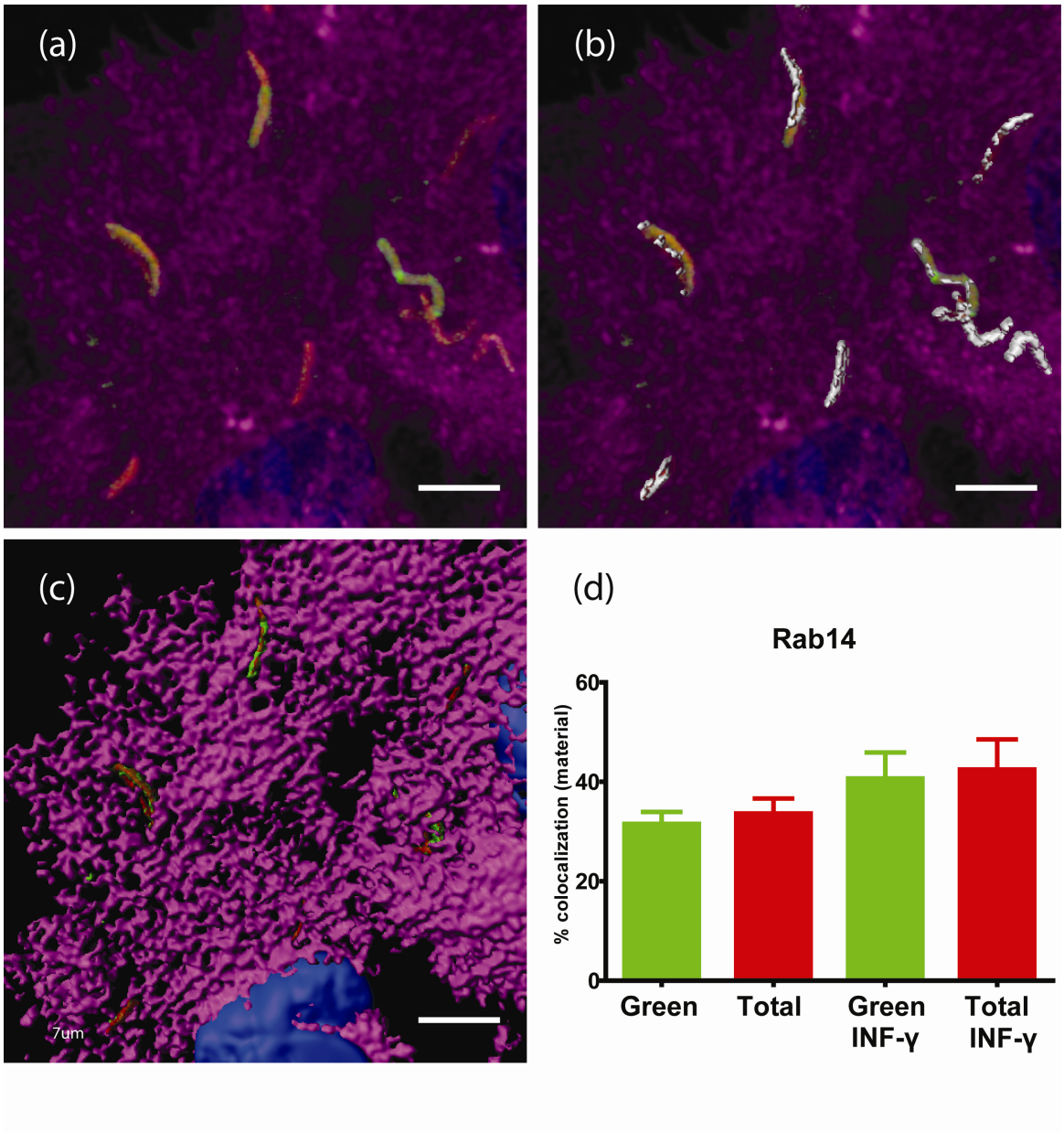
Supplementary Figure 19. Labeling studies with anti-Rab5 in infected bone marrow macrophages (a-d) illustrates triple labeling colocalization studies with RFP, FITC-Tre and anti-LAMP antibody in bone marrow macrophages (BMMs) activated with IFN-γ. Yellow arrows indicate red bacteria that are not green, ie do not take up FITC-Tre and that colocalize to a high degree with Rab5. (a) shows the merged RFP and FITC images, (b) the labeling with anti-Rab5 (magenta) with colocalization indicated in white (c) a three dimensional reconstruction of with all three labels superimposed. (d) shows quantitation of colocalization of Rab5 with bacteria that were labeled with FITC-Tre (green bars) or were red (total population). P-Values: Green vs. Total (ns), Green vs. Green+IFN-γ (0.07), Green vs. Total+IFN-γ (0.02), Total vs. Green + IFN-γ (0.04), Total vs Total+ IFN-γ (0.008) and Green+IFN-γ vs. Total+ IFN-γ (ns). Error bars are standard error of the mean (SEM) for a minimum of 3 experiments. P value > 0.1 are notated not significant (ns). Scale bars 5µm.



Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Figure 20. (a) Representative image for Rab14 , green – FITC-Tre, red- RFP H37Rv, Pink –Anti-Rab14, blue- Nucleus (Dapi) (b) white indicates colocalization (c) 3D reconstruction (d) colocalization statistics (Values are mean SEM for a minimum of 3 separate experiments). Scale bars 5 µm.



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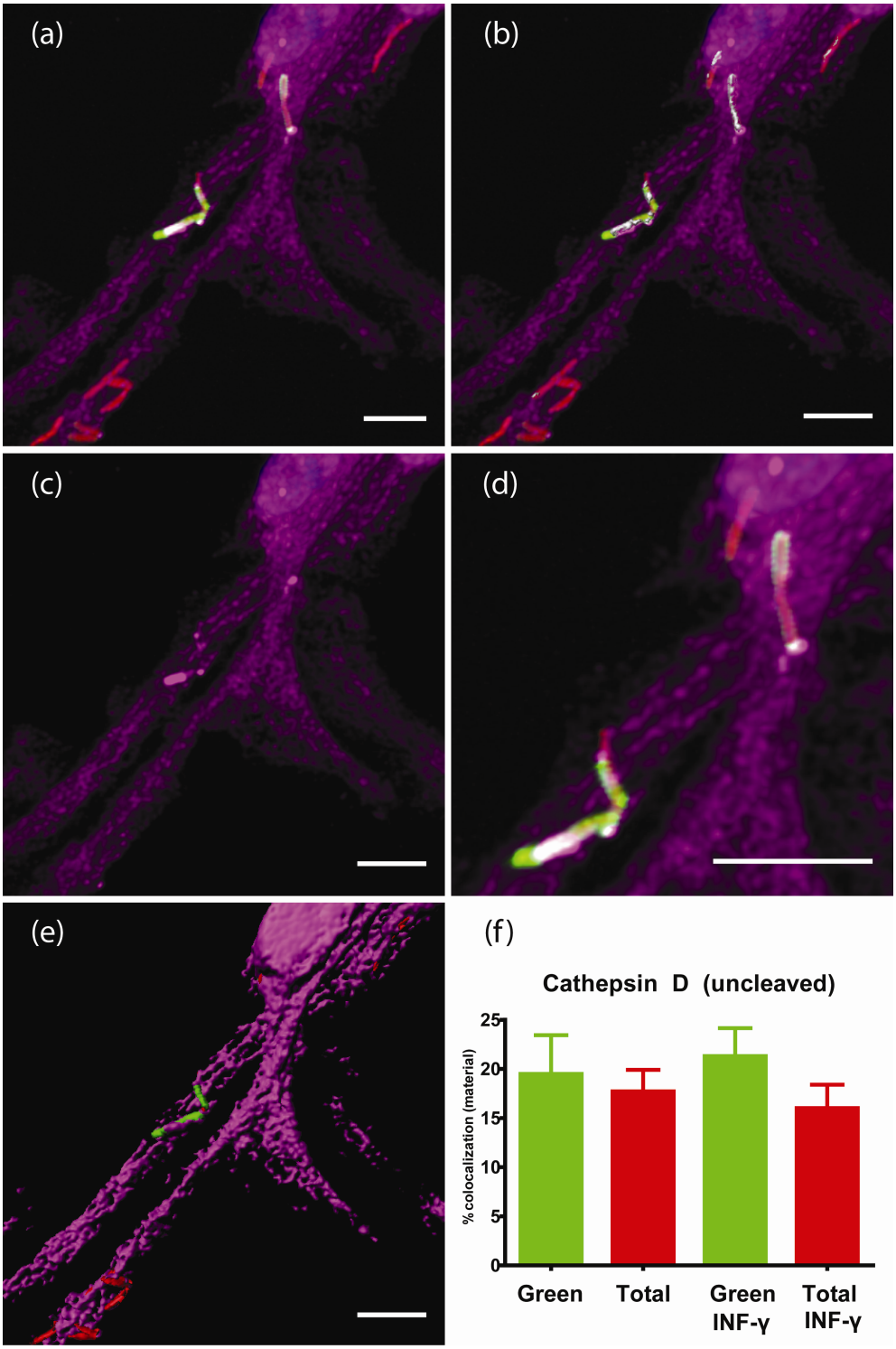
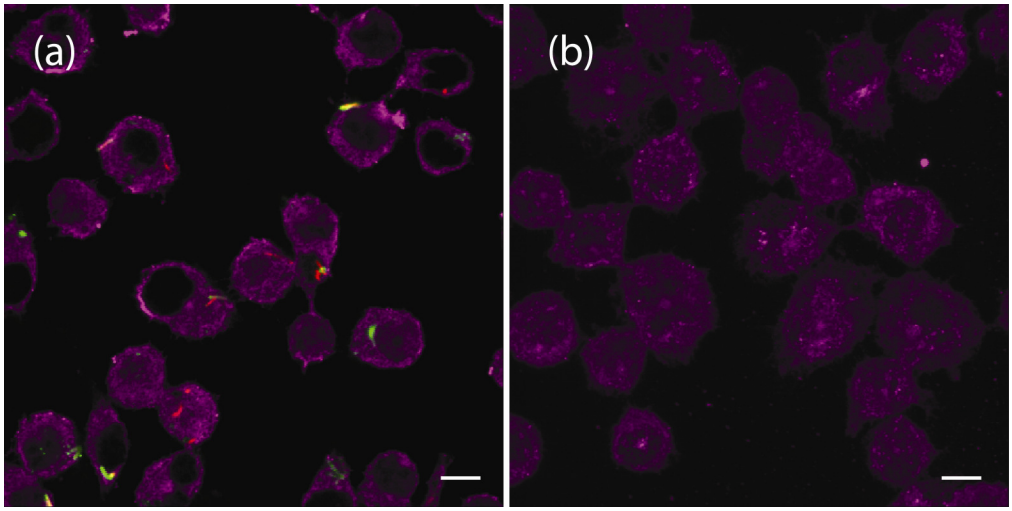
Supplementary Figure 21. (a) Representative image for Pro-Cathepsin D, green – FITC-Tre, red- RFP H37Rv, Pink –Anti-Cathepsin D-1, blue- Nucleus (Dapi) (b) white indicates colocalization (c) Only CathD (d) zoomed in (e) 3D reconstruction (f) colocalization statistics (Values are mean SEM for a minimum of 3 separate experiments). Scale bars 5 µm.

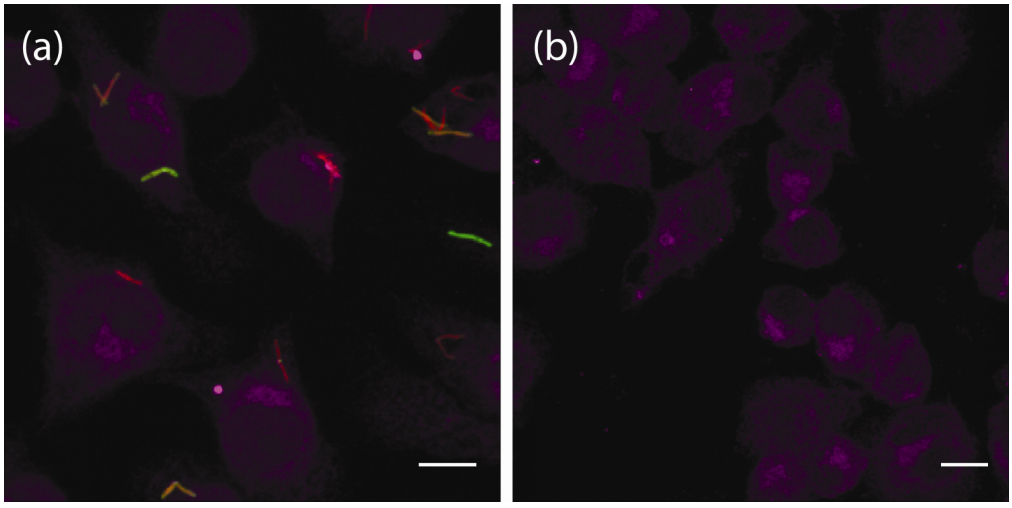


Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Figure 22. (a) J774 macrophages infected with RFP H37Rv and labeled with FITC-Tre and Ag85 antibody (Colorado state). (b) uninfected macrophages labeled with Ag85 antibody (Colorado state). Scale bars 5 µm.



Supplementary Figure 23. (a) J774 macrophages infected with RFP H37Rv and labeled with FITC-Tre and Ag85A antibody (Abcam). (b) uninfected macrophages labeled with Ag85A antibody. Scale bars 5 µm.



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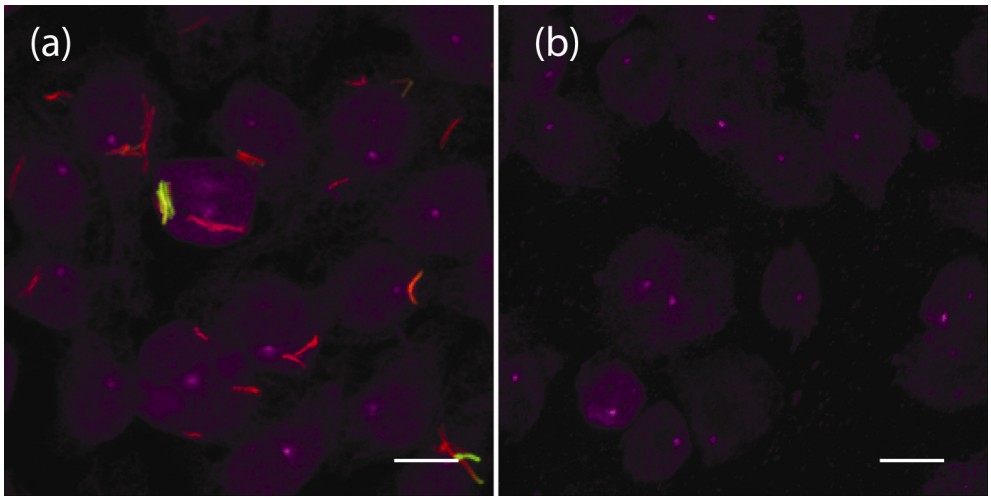
Supplementary Figure 24. (a) J774 macrophages infected with RFP H37Rv and labeled with FITC-Tre and Ag85B antibody (Abcam). (b) unifected macrophages labeled with Ag85A antibody. Scale bars 5 µm.



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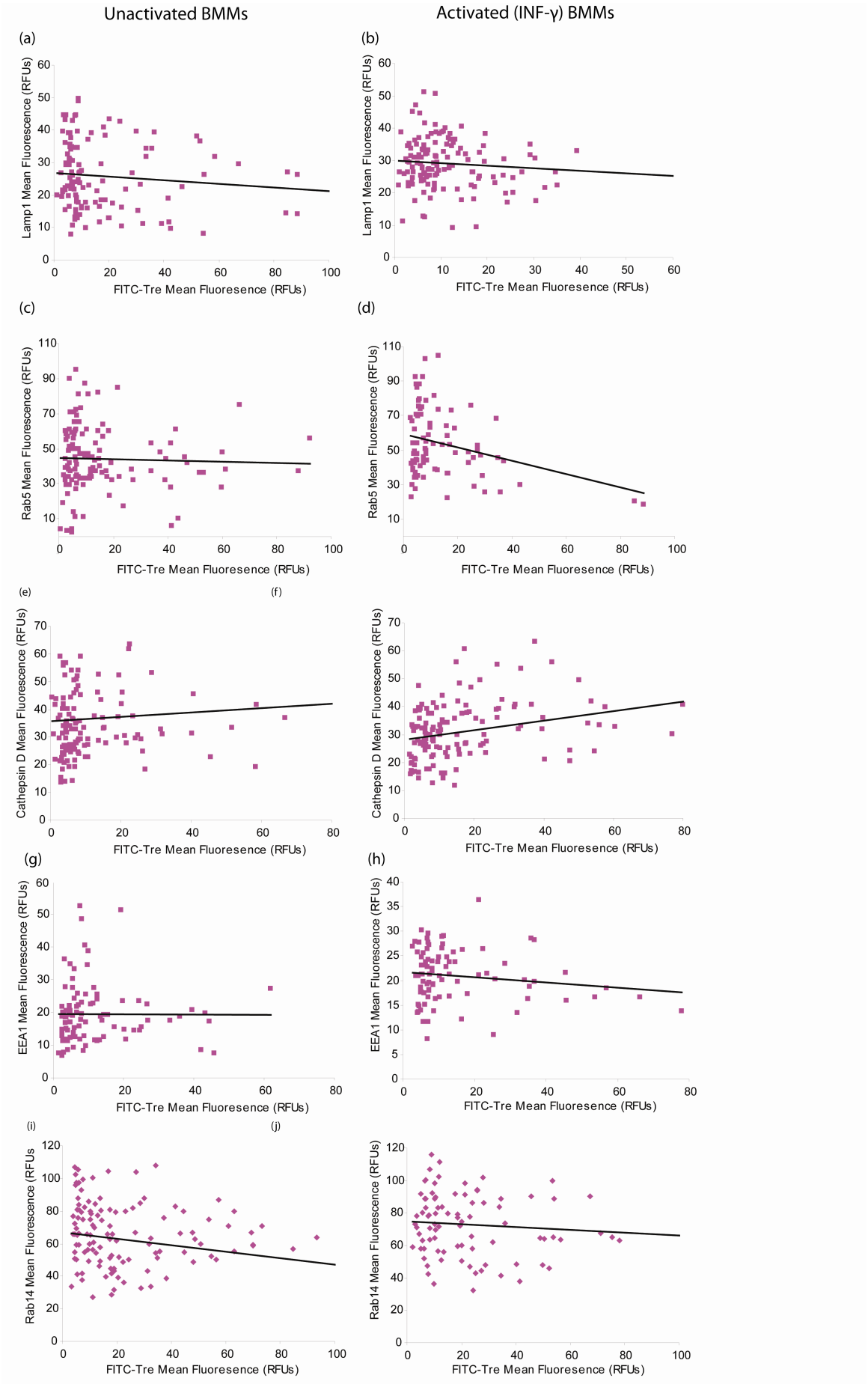
Supplementary Figure 25. Mean antibody fluorescence and FITC-Tre fluorescence for regions of interest (ROIs) identified by RFP expressing *Mtb.* Each point reflects an individual ROI for red fluorescent protein (RFP) expressing H37Rv residing within unactivated or activated bone marrow Macrophages (BMMs). (a,b) LAMP-1 (c,d) Rab5 (e,f) Cathepsin D (g,h) EEA-1 (i,j) Rab14

Nature Chemical Biology: doi:10.1038/nchembio.539



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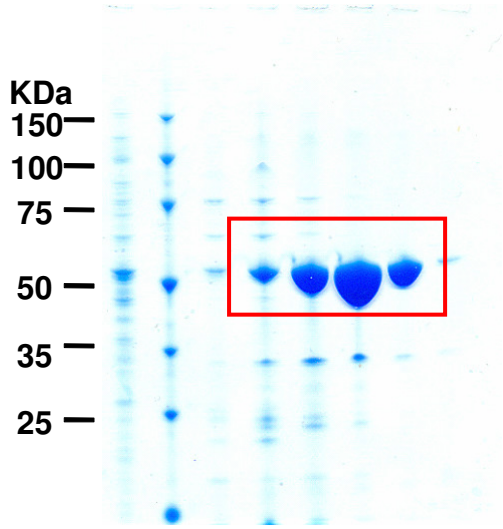
Supplementary Figure 26. (a) SDS page gel of OtsA , lane 1- novagen perfect protein makers 15-150 Kda, box indicates fractions collected



Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Methods

General Experimental

Green Amber Red Screen was conducted on a Waters Quattro Micro-MS with electrospray ionization operating in negative mode (ESI-) interfaced with a Waters 1525µ HPLC system and Waters 2777 sampler fitted with a 4-valve injector module. MS analysis was under the control of Micromass Masslynx 4.1 software and data processed using Masslynx4.1, QuantLynx, Microsoft Excel 2003, SigmaPlot 11.0 and Origin 7.5.

Protein concentrations were calculated using standard BCA assay or with a Labtek ND-1000 Nanodrop.

High Performance Liquid Chromatography was conducted on a Dionex UltiMate 3000 HPLC system at ambient temperature, with an in line variable UV absorbance detector, or a Varian PLS400 Evaporative Light Scattering detector (ELSD) parallel to the main flow path.

Hydrogenations were either performed manually or with a Thales Nano H Cube®.

Protein purification was performed on an AKTA Prime FPLC system (GE Healthcare).

Fluorescence and radioactivity of lipid extractions was read on a Phosphorimager: Typhoon 9410 Variable Mode Imager by GE Healthcare Bio-Sciences.

Nature Chemical Biology: doi:10.1038/nchembio.539

Scintillation counting was conducted on an LS 6500 Multi-purpose scintillation counter by Beckman Coulter.

Fluorescence readings of *Mtb* were conducted on a FLUOstar Optima by BMG Labtech.

Images of stained cells were obtained by confocal microscopy (Leica SP2, Leica Microsystems, Exton, PA) using a 63× oil immersion objective NA 1.4. All data were processed by Leica LAS AF Lite and Adobe Illustrator Software. DAPI was first excited with 405 diode laser and emission spectra were recorded at 413-470 nm prior to other fluorophores. FITC-Tre was excited at 488 nm and emission spectra were recorded at 497-557 nm. RFP was excited at 594 and emission spectra recorded at 602-651 nm. Alexa 633 was excited at 633 and emission spectra recorded at 651-759nm**.**

Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1.0 dm and are reported with implied units of 10^{-1} deg cm^2 g^{-1}. Concentrations (c) are given in g/100 mL.

Melting points (m.p.) were recorded on a Leica Galen III hot stage microscope eqpped with a Testo 720 thermocouple probe and are uncorrected.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker DPX400 (400 MHz), a Bruker AV400 (400 MHz) or a Bruker AVII500 (500 MHz) spectrometer, as indicated. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker AV400 (100 MHz) spectrometer or on a Bruker AVII500 (125 MHz) spectrometer, as indicated. NMR Spectra were fully assigned using COSY, HSQC, HMBC and DEPT 135. All chemical shifts are quoted on the δ scale in ppm using residual solvent as the internal

standard (^{1}H NMR: $CDCl_3$ = 7.26, CD_3OD = 4.87; DMSO-d_6 = 2.50 and ^{13}C NMR: $CDCl_3$ = 77.0; CD_3OD= 49.0; DMSO-d_6 = 39.5). Coupling constants (*J*) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, and a = apparent.

Infrared (IR) spectra were recorded on a Bruker Tensor 27 Fourier Transform spectrophotometer using thin films on NaCl plates for liquids and oils and KBr discs for solids and crystals. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}) and classified as strong (s) or broad (br).

Low resolution mass spectra (LRMS) were recorded on a Waters Micromass LCT Premier TOF spectrometer using electrospray ionization (ESI) and high resolution mass spectra (HRMS) were recorded on a Bruker MicroTOF ESI mass spectrometer. Nominal and exact m/z values are reported in Daltons.

Thin layer chromatography (TLC) was carried out using Merck aluminium backed sheets coated with $60F_{254}$ silica gel. Visualization of the silica plates was achieved using a UV lamp (λmax = 254 nm), and/or acid dip (1:1 MeOH/H_2O, 10% H_2SO_4) and/or ammonium molybdate 5% in 2M H_2SO_4, and/or potassium permanganate (5% $KMnO_4$ in 1M NaOH with 5% potassium carbonate). Column chromatography was carried out using BDH PROLAB® 40-63 mm silica gel (VWR). Mobile phases are reported in ratio of solvents (e.g. 4:1 petrol/ ethyl acetate)

Anhydrous solvents were purchased from Fluka or Acros with the exception of dichloromethane and THF, which were dried over Alumina cartiges. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Distilled water was

used for chemical reactions and Milli-Q™ purified water for protein manipulations. Reagents were purchased from Sigma Aldrich and used as supplied, unless otherwise indicated. D-Trehalose (>99%) and D-arabinose (>99%) were purchased from Fluka. D-Glucose (>99.5%), UDP-glucose, 2-fluoro-2-deoxy-D-glucose and 3-fluoro-3-deoxy-D-glucose were purchased from Sigma. D-Glucosamine hydrochloride was purchased from Carbosynth. Qdot® 655 ITK™ amino (PEG) quantum dots (8 μM solution) were purchased from Invitrogen. 'Petrol' refers to the fraction of light petroleum ether boiling in the range 40–60 ºC. All reactions using anhydrous conditions were performed using flame-dried apparatus under an atmosphere of argon or nitrogen. 3Å and 4Å molecular sieves were activated by heating in a 400 °C furnace and were also employed for anhydrous reactions. Basic alumina refers to basic aluminum oxide and was utilized during some hydrogenation reactions. Brine refers to a saturated solution of sodium chloride. Anhydrous magnesium sulfate ($MgSO_4$) or sodium sulfate (Na_2SO_4) were used as drying agents after reaction workup, as indicated. DOWEX 50WX8 (H^+ form) was conditioned as follows: 100 g of the commercial resin was placed in a 500 mL sintered filter funnel and allowed to swell with 200 mL of acetone for 5 minutes. The solvent was removed by suction and the resin was washed successively with 800 mL of acetone, 500 mL methanol, 500 mL 5M HCl, and then 1 L of water or until the pH of filtrate was ~ 7, as indicated by pH paper. The resin was partially dried on the filter and then stored and used as needed.

In addition to those specified above, the following abbreviations, designations, and formulas are used throughout the Supporting Information: Ar = Argon, MeOH = methanol, H_2O = water, Et_2O = diethyl ether, EtOH = ethanol, TFE = trifluoro ethanol, EtOAc = ethyl acetate, CH_2Cl_2 = DCM = dichloromethane, DMF = dimethylformamide, *i*PrOH = isopropanol, Et_3N = triethylamine, K_2CO_3 = potassium carbonate, $NaHCO_3$ = sodium bicarbonate, NaOH =

sodium hydroxide, NH_4Cl = ammonium chloride, NH_4OH = ammonium hydroxide, TFA = trifluoro acetic acid, aq. = aqueous, sat. = saturated, TMSOTf = Trimethylsilyl trifluoromethanesulfonate, FITC = fluorescein isothiocyanate, Bn = benzyl, Ac = acetyl, TBDPS = *tert*-butyldiphenylsilyl, TBAF = tetra-*n*-butylammonium fluoride, DAST = diethylaminosulfur trifluoride, DMAP = 4-Dimethylaminopyridine, PBS = phosphate buffered saline, TEA = triethanolamine, DAPI = 4',6-diamidino-2-phenylindole is a fluorescent stain that binds strongly to DNA, DIC = differential interference contrast image

Protein Mass Spectrometry (LC–MS) was performed on a Micromass LCT (ESITOF–MS) coupled to a Waters Alliance 2790 HPLC using a Phenomenex Jupiter C4 column (250x4.6 mm– 5μm) Water: acetonitrile, 95:5 (solvent A) and acetonitrile (solvent B), each containing 0.1% formic acid, were used as the mobile phase at a flow rate of 1.0 mL min^{-1}. The gradient was programmed as follows: 95% A (5 min isocratic) to 100% B after 15 min then isocratic for 5 min. The electrospray source was operated with a capillary voltage of 3.2 kV and a cone voltage of 25V. Nitrogen was used as the nebulizer and desolvation gas at a total flow of 600L h^{-1}. Spectra were calibrated using a calibration curve constructed from a minimum of 17 matched peaks from the multiply charged ion series of equine myoglobin obtained at a cone voltage of 25V. Total mass spectra were reconstructed from the ion series using the MaxEnt algorithm on MassLynx software (v. 4.0 from Waters) according to the manufacturer's instructions.

Supplementary Biological Materials and Methods

^{14}C-Trehalose, ^{14}C-glycerol and ^{14}C-glucose uptake into H37Rv

H37Rv *M tuberculosis* cells were grown in Middlebrook 7H9 media and were harvested at an OD_{650} of 0.5 by centrifugation (1250 *g* at 4°C for 10 min), washed once with buffer (Hepes 25 mM and 0.05M Tween at pH 7.2) and then resuspended in the same buffer. Radio-labelled ^{14}C-trehalose (0.1 μCi/mL), glycerol (1 μCi/mL), and corresponding nonlabelled sugars (50 mM) were mixed and added to the 16ml cell suspension. 1 mL aliquots were removed in at 15, 30, 45, 60 and 120 min and were filtered through GA-4 Metricel filter membranes (diameter, 2.54 cm; pore size, 0.8 μm; Gelman Instrument Co., Ann Arbor, Mich.) The membranes were washed (3 × 2 mL) with HEPES/Tween buffer and LiCl (0.1 M, 1 × 2mL). Filter papers were then placed in Ultimax gold scintillation fluid. Scintillation counting was conducted for 2 min per vial. All experiments were conducted in triplicate and samples without sugar were used as control. Uptake was measured over a 24 h time period with ^{14}C-glucose (0.9 μCi/mL), ^{14}C-Trehalose (0.6 μCi/mL) in 7H9 media, which contains 10 mM glucose. No unlabeled trehalose was added to the experiment. 1 mL aliquots were removed and counted as before with time points at 40, 90 mins, 4 and 24 h. **(Supplementary Figure 1)**

Isoniazid (INH) inhibition of mycolate production

H37Rv was grown in 7H9 media (15 mL) to an OD_{650} of 0.8 and InH was added to a concentration of 0.2 μg/mL (10 × MIC) or 0.02 μg/mL (1 × MIC). Culture was exposed to INH for 2 hours prior to addition of ^{14}C-acetate (150 μCi/tube). Cells were labeled with ^{14}C-acetate for 45 minutes and then harvested by centrifugation and extracted into 2 mL (2:1 chloroform:methanol). The organic layer was removed, concentrated and the residue was

resuspended in 200 µL (80:20 chloroform:methanol) and 50 µL was spotted onto silica TLC plates, which were developed for 1 hour (75:25:4 chloroform:methanol:water). Plates were scanned for fluorescence and exposed to a phosphor storage plate, which was scanned for radioactivity. **(Supplementary Figure 2)**

Mycolate analysis

Mycolate analysis were conducted based on previous reports[1]. A full size image of the TDM TLC plate was printed onto transparency film and was used to identify each spot on the plate. Each spot was then scraped and the silica was placed in a vial with 0.5 mL 20% TBAH and heated to 100 °C for 18 hours. To this was added dichloromethane (0.5 mL) and methyl iodide (50 µL) and reaction was shaken vigorously at room temperature of 2 hours. Organic layer was removed to a new vial and concentrated under a stream of argon. The resulting white crystals were extracted with diethyl ether (2 × 250 µL) and the extract was placed in a fresh vial and concentrated under a stream of argon. The resulting clear oil was re-dissolved in 50 µL (2:1 toluene:acetonitrile) and the entire reaction product was spotted on silica TLC plate and developed with three successive runs (3 × petroleum ether:diethyl ether 85:15). Plate was then exposed to storage phosphor plate, which was scanned for radioactivity.

Ag85 Expression.

General protein expression methods

Plasmids were obtained from Colorado State tuberculosis research contract HHSN266200400091c as Rv3804/Ag85A Rv1886c/Ag85B Rv0129c/Ag85C in pET23b vector containing a hexa-histidine tag and were expressed as has been described previously[2]. LB $Amp^{100}Cam^{38}$ (30 mL) was inoculated with desired plasmid in BL21 DE3 pLys strain and grown overnight at 37 ˚C with shaking to generate a starter culture, which was used to inoculate 4L of autoclaved $Amp^{100}Cam^{38}$ LB broth. Culture was grown at 37 ˚C with shaking

Nature Chemical Biology: doi:10.1038/nchembio.539

until it reaches an OD_{600} of 0.5 and removed from 37 ˚C and place in 25 ˚C incubator with shaking and allowed to cool to 25 ˚C. Expression was induced with isopropyl-β-D-thiogalactopyranoside (IPTG) (0.25mM) for 16 hours. Cells were harvested and resuspended in buffer (10mM Tris-HCl, pH 8) containing one tablets of Complete® protease inhibitor cocktail (Roche), lysozyme and DNAse I. Pellet was lysed by sonication and pelleted at 8,000 rpm for 60 min. Purification was carried out through Fast protein liquid chromatography (FPLC) via nickel affinity chromatography. Two 5 mL Ni NTA columns were connected and equilibrated with binding buffer (10mM Tris-HCl, 500mM NaCl, 5mM imidazole, pH 8). Lysate was applied to column slowly via 50 mL super loop and protein purification was conducted via FPLC using a stepwise gradient of elution buffer (10mM Tris-HCl, 1M Imidazole, pH 8). Eluant was then dialyzed into 20 mM TEA buffer pH 7.4 overnight. Perform SDS-page on all protein fractions. PD-10 desalting cartridges (Amersham) were utilized to remove endotoxins and to buffer exchange into 1mM TEA pH 7.4 for mass spectrometry assay. Protein concentrations were calculated by standard BCA page assay as well as by absorbance on a Nanodrop®. Mass spectrometry and sequencing analysis confirmed the correct identity of the proteins. **(Supplementary Figures 3-8)**

Expression of Ag85A

Ag85A was expressed as above and, upon dialysis, was obtained at a concentration of 260 μg/mL. During expression, it was found that both a protein of the expected mass as well as a protein of mass + 178 Da were obtained in the purified protein. **(Supplementary Figures 3-6)** Analysis indicates that this mass is caused by His tag phosphonogluconoylation[3] of a GSS–His sequence present in the protein His tag. Furthermore, when the his tag mixture of Ag85A was cleaved with thrombin, a single protein of the correct mass was obtained, confirming that the modification was in the His tag region. In separate expressions we obtained both a mixture of the gluconoylated and non-gluconoylated proteins as well as

purely gluconoylated protein. No significant difference in activity for the gluconoylated and non-gluconoylated protein was observed.

Sequence of Rv3804/Ag85A (PDB code 1SFR)

(Met)G S S H H H H H H S S G L V P R G S H M F S R P G L P V E Y L Q V P S P S M G
R D I K V Q F Q S G G A N S P A L Y L L D G L R A Q D D F S G W D I N T P A F E W
Y D Q S G L S V V M P V G G Q S S F Y S D W Y Q P A C G K A G C Q T Y K W E T F
L T S E L P G W L Q A N R H V K P T G S A V V G L S M A A S S A L T L A I Y H P Q
Q F V Y A G A M S G L L D P S Q A M G P T L I G L A M G D A G G Y K A S D M W G
P K E D P A W Q R N D P L L N V G K L I A N N T R V W V Y C G N G K P S D L G G
N N L P A K F L E G F V R T S N I K F Q D A Y N A G G G H N G V F D F P D S G T H
S W E Y W G A Q L N A M K P D L Q R A L G A T P N T G P A P Q G A

Calculated Masses:

Calculated average isotopic mass with glucose: 33995
Found average isotopic mass: 33995 or 33989 depending on calibration

Calculated average isotopic mass without N terminal methionine and gluconoylation: 33814
Found average isotopic mass: 33817

Calculated average isotopic mass after thrombin cleavage: 32063
Found average isotopic mass: 32061

Expression of Ag85B

Ag85B was expressed as above and, upon dialysis, was obtained at a concentration of 350 µg/mL **(Supplementary Figure 7)**

Sequence of Rv1886c/Ag85B (PDB codes 1F0N and 1F0P)

M F S R P G L P V E Y L Q V P S P S MG R D I K V Q F Q S G G N N S P A V Y L L D
G L R A Q D D Y N G W D I N T P A F E W Y Y Q S G L S I V M P V G G Q S S F Y S D
W Y S P A C G K A G C Q T Y K W E T F L T S E L P Q W L S A N R A V K P T G S A
A I G L S M A G S S A M I L A A Y H P Q Q F I Y A G S L S A L L D P S Q G M G P S L
I G L A M G D A G G Y K A A D M W G P S S D P A W E R N D P T Q Q I P K L V A N
N T R L W V Y C G N G T P N E L G G A N I P A E F L E N F V R S S N L K F Q D A Y
N A A G G H N A V F N F P P N G T H S W E Y W G A Q L N A M K G D L Q S S L G A
G K L A A A L E H H H H H H

Calculated Masses:

Calculated average isotopic mass: 32313
Found average isotopic mass: 32310

Nature Chemical Biology: doi:10.1038/nchembio.539

Expression of Ag85C

Ag85C was expressed as above and, upon dialysis, was obtained at a concentration of 1.6 mg/mL **(Supplementary Figure 8)**

Sequence of Rv0129c/Ag85C (PDB codes 1DQY, 1DQZ)

M F S R P G L P V E Y L Q V P S A S M G R D I K V Q F Q G G G P H A V Y L L D G L
R A Q D D Y N G W D I N T P A F E E Y Y Q S G L S V I M P V G G Q S S F Y T D W Y
Q P S Q S N G Q N Y T Y K W E T F L T R E M P A W L Q A N K G V S P T G N A A V
G L S M S G G S A L I L A A Y Y P Q Q F P Y A A S L S G F L N P S E S W W P T L I G
L A M N D S G G Y N A N S M W G P S S D P A W K R N D P M V Q I P R L V A N N T
R I W V Y C G N G T P S D L G G D N I P A K F L E G L T L R T N Q T F R D T Y A A
D G G R N G V F N F P P N G T H S W P Y W N E Q L V A M K A D I Q H V L N G A T
P P A A P A A P A A L E H H H H H H

Calculated Masses:

Calculated average isotopic mass: 33217
Found average isotopic mass: 33218

FITC-Tre and FITC-Glc uptake into Mtb

To CDC1551 *Mtb* or the Ag85C mutant of CDC1551(TBVTRM) in Middlebrook 7H9 medium (10 mL) at an OD_{650} of 0.44 was added FITC-Tre **9** in ethanol to a final concentration of 100µM. Heat-killed (80 ˚C, 60 min) bacteria (2 × 0.5 mL) at an OD_{650} of 0.6 were used as control. *Mtb* were incubated at 37 °C with shaking. After 8 hours, the culture (4 × 400 µL) was harvested by centrifugation (1 min, 12000 rpm) and washed (3 x 1 mL 7H9 medium) and resuspended (200 µL 7H9). Fluorescence measurements were conducted in 96-well format in appropriate plates (Nunc, Cat No 137103 (Roskilde, Denmark)). The background of the culture was obtained from cells treated in an identical fashion in the absence of FITC-Tre. Experiments were conducted in quadruplicate. Uptake was normalized for increase in OD_{650}. FITC-Glc **28** uptake (100µM) into CDC1551 and FITC-Tre **9** uptake into *H. influenza*e, *P. aeruginosa* and *S. aureus* was conducted in an analogous manner.

Nature Chemical Biology: doi:10.1038/nchembio.539

FITC-Tre uptake into other bacteria

H. influenzae (AMC 36-A-1, ATCC 10211) was rehydrated in standard Blood Heart Infusion (BHI) media with supplemented hemoglobin and IsoVitaliX (BD 211876) and plated on chocolate agar plates and grown at 37 °C under 5% CO_2. Single colony was picked and grown in supplemented BHI media for 8 hours in the presence of 100 μm FITC-Tre. After 8 hours cells were harvested in quadruplicate in a manner identical to FITC-Tre *Mtb* uptake.

*Pseudomonas auruginosa (P*AO1-LAC, ATCC 47085) was grown on standard blood agar. Growth in standard BHI in the presence of 100 μM FITC-tre was conducted for 7 hours to an OD of 0.55 and harvest and fluorescence analysis was conducted as with *Mtb*.

Staphylococcus aureus (ATCC 13801) grown on standard blood agar. Growth in standard BHI in the presence of 100 μM FITC-tre was conducted for 6 hours to an OD of 1.8 and cells were harvested and diluted to and OD of 0.55 and fluorescence analysis was conducted as with *Mtb*. Background fluorescence was measured in the absence of probe in an identical fashion and was averaged for all three bacteria.

Test of growth inhibition due to FITC-Tre in macrophages

J774 macrophages were grown to confluency in DMEM media and plated in black nunc 96 well plates (200 μL/well). Cells were infected with red fluorescent protein (RFP) expressing strain of H37Rv either 100 μL or 50 μL of bacteria in DMEM (OD 0.02), approximately 5-10 bacteria/macrophage. Infection was allowed to proceed for three hours upon which time, macrophages were washed with fresh DMEM and FITC-Tre (10mM stock in ethanol) was added to a concentration of 100 μM. Uninfected macrophages were treated in an identical fashion. As a second control infected macrophages were treated with the equivalent volume of ethanol. RFP fluorescence was monitored (excitation 594 nm, emission 612 nm) at 0, 24,

48 and 72 hours. Error bars reflect the standard deviation for 4 experiments. **(Supplementary Figure 9)**

Mtb antibody labeling

Primary *Mycobacterium tuberculosis* antibody (ab905, AbCam, Cambridge, MA, USA) was labeled utilizing Zenon Rabbit Polyclonal IgG labeling kit (Z-25302, Molecular Probes, Eugene, OR, USA). Antibody and Zenon Fab fragments were prepared according to kit specifications and diluted to 1 mL in PBS with goat serum. Coverslips were incubated with antibody solution for 30 min at 37 °C, were then washed (1 mL, 3 × 5 min, 0.1% Tween 20 in PBS) and were fixed in 5% formalin for 15 min. Coverslips were drained and mounted in anti-fade media (Invitrogen Prolong Gold). Cells treated with FITC-Tre and no antibody were exposed to the same mounting procedure, except second fixation step was omitted. *Alexa 594* labeled *Mtb* antibody and QDs were excited at 556 nm and emission spectra recorded at 594-665 nm. **(Supplementary Figure 13)**

Tre-Quantum dot labeling of Mtb infected macrophages

Tre Quantum dots (Tre-QDs) were treated in an analogous fashion to FITC-Tre and were added to give a final concentration of 7 nM (110 sugars/dot, 1 μM effective sugar concentration). Uncoated QDs were utilized as a control. Fixation, permeabalization, blocking, mounting and microscopy were conducted as with FITC-Tre. **(Supplementary Figure 14)**

FITC-Glc labeling of Mtb infected macrophages

Macrophages were grown to confluence on coverslips and infected with RFP expressing *Mtb* or BCG, using our standard protocol. FITC-Glc (1:1 ethanol/PBS stock solution) was added to a 100 μM concentration, and allowed to label overnight, upon which time cells were fixed,

Nature Chemical Biology: doi:10.1038/nchembio.539

blocked and mounted, using the standard protocol developed. FITC-Tre treated cells were analyzed in parallel to confirm that bacteria were labeling with the trehalose compound. For both RFP BCG and RFP H37Rv, no specific labeling was observed with FITC-Glc. **(Supplementary Figure 15)**

Trehalose inhibition of FITC-Tre labeling

In vitro, H37Rv *Mtb* expressing RFP were grown in 7H9 containing OADC to an OD 0.25 at which point FITC-Tre was added to a concentration of 200 μM and trehalose was added to a concentration of 100 mM and the culture was incubated with shaking for 24 hours. Culture was then harvested and washed (3 X 1 mL PBS) by centrifugation, fixed in 1:1 PBS: buffered formalin (10% in PBS) for 15 minutes. Culture was pelleted and resuspended in PBS (200 μL). Aliquots (10 μL) were placed on glass coverslips and the PBS was allowed to evaporate, following which 1 drop mounting media (invitrogen prolong gold) was added and the coverslip was placed on a glass slide. Z stacks (0.13 10 μm step size), zoom 8 were collected and images were deconvolved with Hugyens software and visualized with Imaris software. Bacteria grown in the absence of trehalose were treated in an identical fashion. **(Supplementary Figure 16)** *In vivo,* A sterile solution of D-trehalose in PBS was added to J774 macrophages infected with RFP BCG to a final concentration of 10 mM. Concurrent with the addition of trehalose, FITC-Tre was added to a final concentration of 200 μM. Fixation, permeabalization, blocking, mounting and microscopy were conducted as with FITC-Tre. **(Supplementary Figure 17)**

Polar labeling in vitro

H37Rv *Mtb,* expressing RFP were labeled and harvested in an identical fashion to the '*in vitro* trehalose inhibition labeling.' ROIs for the poles and mid-sections were quantitated using the 'marching cubes' tool in Imaris to manually generate surfaces from which statistics

Nature Chemical Biology: doi:10.1038/nchembio.539

were calculated for the regions of interest. Data were collected from 150 bacteria. **(Supplementary Figures 11-12)**

Minimum Inhibitory Concentration (MIC) Determination

Analysis was conducted as previously reported[4]. *Mtb* cells were grown in 7Hp media to an OD 0.2-0.3 (then dilute sample 1:1000). On a 96 well round-bottom plate, 50 µl/well of medium was added to all wells except first row. In the first row 100 µl drug of choice was added at twice the initial desired concentration. Using a multichannel pipettor, 50 µL was transferred to each next row starting with row 1 and ending with row 11. 50 uL was discarded after row 11. To each well was added 50 µl of the 1:1000 culture dilution, approximating 1 x 10^4 bacteria per well. The plate was incubated for 2 weeks at 37 °C inside a small zip-lock bag. The plate was read with an inverted plate reader and graded as either growth, no growth, or growth/no growth if <50%. The well contents were then resuspended and the OD_{600} of individual wells was read on a fluorostar plate reader. MIC_{50} was calculated by plotting concentration (µg/ml) versus percentage growth (absorbance MIC well/ absorbance wildtype well) and fitting curves in Origin Pro 8 to determine MIC_{50} values.

Primary bone marrow macrophages (BMM) isolation[5]

Six-week-old C57BL/6 female mice were used for these experiments. Mice were sacrificed and their femur bones and shoulders were removed by dissetction. Intact bones were then washed in 70% ethanol and placed in Dubelco's MEM media (DMEM) containing 20% fetal bovine serum (FBS), 1% hepes, 1% pyruvate and 1 mmol/L l-glutamine on ice until use. Ball joints were removed and marrow was flushed from bones use a 25 gauge needle and syringe filled with ice cold DMEM media. The sterile needle was inserted at both ends of the femur to ensure that openings were present and approximately 2 mL DMEM was used to flush marrow. An additional 2 mL was applied to the other end of the bone to ensure full removal

Nature Chemical Biology: doi:10.1038/nchembio.539

of marrow and process was repeated for all bones. Cell suspensions on ice were pipetted multiple times to break up aggregated cells. Then cell suspensions were passed through a 70 micron filter and resulting suspensions were counted on standard hematocytometer. To the cell suspension was added an equal volume of DMEM supplemented with 20% FBS, pyruvate, hepes and glutamine. DMEM also contained a 2× concentration recombinant mouse macrophage colony stimulating factor (M-CSF) Prospec (CYT-439) or biovision (4238-50) so that the final working concentration was 100 U/mL. DMEM al so contained 2× concentration penacillin-streptamycin amd 2× mercaptoethanol (10^{-4}M) so that final concentration of cell suspension was 1× for all additives. 15 mL cell suspension was plated in untreated greiner (100 × 20 mm) dishes. On day 3, 10 mL of DMEM complete with 20% FBS and M-CSF (100ng/mL) was added to each dish. After 5 or 6 days plates were washed with cold PBS (5 mL), followed by cold complete DMEM containing 10% Fetal bovine serum, 1% hepes, 1% pyruvate and 1 mmol/L l-glutamine (5 mL). Cells were then gently scraped in one direction and resulting cell suspension was centrifuged (8 min, 800 RPM). Cells were counted on standard hemocytometer. 160 x10^4 cells/well. Cell pellets were resuspended in 20 mL DMEM and plated on sterile coverslips. INF-γ was added to a concentration of 4 ng/mL. BMMs were then infected with RFP H37Rv, labeled with FITC-Tre (200 μM, 40 h), treated with various antibodies, mounted and imaged in the same manner as with J774 macrophage cell line.

Immunocytochemistry (ICC) procedure

Media was removed and cells were washed in PBS (1 mL, 3 × 2 min). Cells were fixed in 5% formalin (1:1 in PBS) for 15 minutes and then washed again with PBS (1 mL , 2 × 2 min). Cells were permeabalized (1 mL, 0.1% triton X-100 in PBS) for 6 minutes at room temperature and then washed with PBS (1 mL, 3 × 2 min). Non-specific protein interactions were blocked with protein blocker (1 mL) with 1 drop goat serum for 1 hour at RT. The

following primary antibodies were employed: Rabbit polyclonal to *LAMP1* (ab25170), Rabbit polyclonal to EEA1 (ab2900-100), rabbit polyclonal to Rab5 (ab18211) rabbit polyclonal to Rab14 (ab28639), chicken polyclonal to Ag85A (ab14073), rabbit polyclonal to Ag85B (ab43019) were obtained from Abcam. Rabbit polyclonal to Pro-cathepsin D (sc-10725) was obtained from Santa Cruz biotechnologies. Rabbit polyclonal to Ag85 was obtained from BEI resources (NR-13800). Antibodies were diluted 1:100 or 1:50 in PBS with 2 drops goat serum. Blocking agent was removed and coverslips were incubated with diluted antibodies (200 µL) for 16 hours at 4 °C. Coverslips were then washed (1 mL, 2 × 5 min, 0.1% Tween 20 in PBS) and then (1 mL, 5 min) in PBS. Secondary antibodies Goat anti rabbit IGG 633 (A21070) and goat anti chicken IGG 633 (A21103) were obtained from Invitrogen. Fresh 1:75 solutions of were made in PBS with 2 drops goat serum. Coverslips were labeled for 50 minutes with secondary antibody and then were washed (1 mL, 2 × 5 min 0.1% Tween 20 PBS) followed by DAPI (200 µL, 1 × 5 min, in PBS)and then PBS (1 mL, 5 min). Coverslips were then washed once further in PBS (1 mL, 5 min), drained and mounted in anti-fate media (Prolong GOLD, Invitrogen).

Image Collection and Processing

Multiple fields were sampled, and representative images were recorded. DAPI was first excited with 405 diode laser and emission spectra were recorded at 413-470 nm prior to other fluorophores. FITC-Tre was excited at 488 nm and emission spectra were recorded at 497-557 nm. RFP was excited at 594 and emission spectra recorded at 602-651 nm. Alexa 633 was excited at 633 and emission spectra recorded at 651-759nm. Essential sequential Z sections of stained cells were also recorded for generation of stacked images through cell 63X objective with a zoom of 4 and 512x512 image size. A 3-D volume was constructed from sequential z-sections of a cells assembled into a 3D volume in Imaris software (version 7.0.0,

Bitplane AG, Zurich, Switzerland). All collected images for analyses were deconvolved by Huygens Essential software (Version 3.4, Scientific Volume Imaging BV, Hilversum, The Netherlands). Identical deconvolution parameters were employed for each antibody dataset. **(Supplementary Figures 18-24)** Percentage colocalization was calculated using the colocalization function in Imaris, with thresholding by RFP channel. RFP channel was used to generate regions of interests (ROIs) as surfaces for all bacteria and statistic function was utilized to export for mean fluorescence and volume of all channels within the ROI. These values were plotted as mean antibody florescence (ex 633 em 651-759nm) versus mean FITC-Tre fluorescence (ex 488 em 497-557). **(Supplementary Figure 25)**

Ag85 ESI MS assay

Mass spectrometric assays were performed on a Waters QuattroMicro-MS with electrospray ionization operating in negative mode (ESI-) interfaced with a Waters 1525µ HPLC system fitted with a 1000psi backpressure regulator. A Waters 2777 auto-sampler fitted with a 4-port injector module and thermostatic sample trays was used to deliver samples. MS analysis was under the control of Micromass Masslynx 4.1 software and data processed using Masslynx4.1, QuantLynx, Microsoft Excel 2003 and Origin 7.5.

GAR screen: A 96-well plate was set up with each well containing TDH and one of the screen compounds in 1mM TEA buffer (pH = 7.2) at 37^{o}C. **(Supplementary Scheme 2)** To each well was added by automated injection (with mixing), 20uL of either Ag85a/b/c or buffer to give final concentrations of 500uM of each substrate and 2uM of Ag85 (or 0uM Ag85 in the case of negative control wells). The samples were incubated at 37 °C for 2h 40 min before injection of a 10uL aliquot directly into the ESI source. LC conditions: mobile phase: MeCN : H_2O (50 : 50); flow rate: 0.2 mL/min; isocratic method for 3 min. The resulting mass spectrum was measured in ESI- continuum mode (150-1000 Da). The mass

spectra were corrected for baseline subtraction and smoothed. The peak intensities for mono-hexanoylated product and the substrate were measured and the product/substrate ratio (ie Peak height$_{\text{acylated screen compound}}$/Peak height$_{\text{unacylated screen compound}}$) calculated for each well. The product/substrate ratio for each screen compound was normailsed against the product/substrate ratio for trehalose to give the relative reactivity ratio reported for each compound.

Expression of Trehalose-6-phosphate synthase

The plasmids coding for trehalose-6-phosphate synthase (OtsA), the pET22b:otsA (provided as a kind gift by Prof. Gideon Davies) was used to transform *E.coli* BL21 DE3 cells. Transformed cells were grown overnight in 50 ml of LB broth containing 100 μg ampicillin. One-liter cultures were then inoculated to an A_{600} of ~0.05. The cells were grown at 37°C to an A_{600} of 0.45. Protein expression was subsequently induced by the addition of 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and left to grow for a further 16 h at 30°C. Expression of the protein was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). All protein purification steps were carried out at 4°C. The cell pellets were resuspended in 50 ml 20 mM tris(hydroxymethyl)aminomethane (Tris), 200 mM NaCl, 15 mM imidazole, pH 7.8, containing one tablets of Complete® protease inhibitor cocktail (Roche), lysozyme and DNAse I. The cells were disrupted on ice by sonication. The suspension was then centrifuged (30,000 g for 40 min) to remove cell debris. The supernatant was then filtered using a 0.4 μm syringe filter. The filtered supernatant was applied to a preequilibrated (Buffer A; 20 mM Tris, 200 mM NaCl, 10 mM imidazole, pH 7.8) Pharmacia 5ml HisTrap Ni-NTA column. The column was washed at 1 ml/min with 20 column volumes of the same buffer and then eluted with a linear gradient of imidazole (10 mM to 500 mM over 25 column volumes) in Buffer A. Protein was detected with an on-line detector

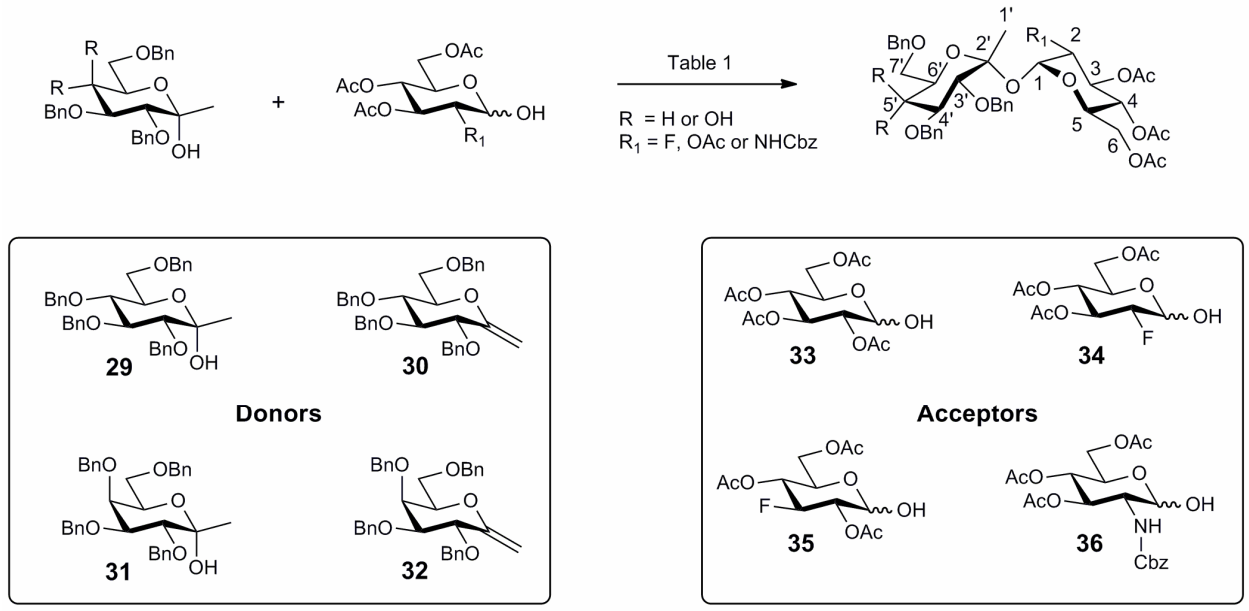
monitoring A_{280} and column fractions were collected and analyzed by SDS-PAGE. Fractions containing the ca. 54 KDa protein was pooled and dialyzed twice against 4 liters of 100 mM Hepes, 200 mM KCl, 20 mM $MgCl_2$, pH 7.5 and stored at 4°C. **(Supplementary Figure 26)**

Nature Chemical Biology: doi:10.1038/nchembio.539

Supplementary Chemistry Schemes, Synthesis and Characterization

Ketoside trehalose synthesis

Ketoside trehalose analogs are numbered in the supplementary information in the following manner. This numbering follows the precedent of analog number set by IUPAC nomenclature for ketosides. In the main text, for clarity, compounds are are referred to as methyl-trehalose and are numbered according to the convention for unmodified trehalose. Compounds **29**[6], **30**[7,8], **31**[9], **32**[8,10], **33**[11], **34**[12,13], **35**[14] and **36**[15] were synthesized as has been recorded previously and their characterization matched previously reported spectroscopic data.

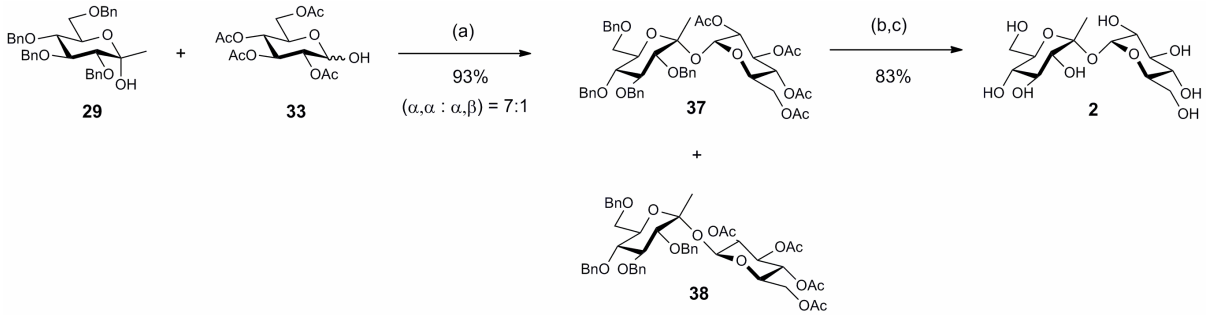


Entry	Donor	mmol	Acceptor	mmol	Catalyst	T ˚C	Time	Product	Yield	α,α: α,β
1	**29**	0.24	**33**	0.25	TMSOTf	–40	3 h	**37/38**	93%	7:1
2	**29**	0.14	**34**	0.22	TMSOTf	–40	2 h	**39**	82%	6:1
3	**30**	0.04	**34**	0.09	TMSOTf	–78	30 min	**40**	95%	1:1
4	**31**	0.32	**33**	0.42	TMSOTf	–40	30 min	**41**	88%	5:1
5	**31**	0.14	**34**	0.16	TMSOTf	–40	1 h	**42**	90%	6:1
6	**32**	0.31	**34**	0.58	TMSOTf	–78	30 min	**43**	30%	3:7
7	**29**	0.21	**35**	0.18	TMSOTf	–40	3 h	**44/45**	52%	4:1
8	**29**	0.31	**36**	0.38	TMSOTf	–78	15 min	**46/47**	81%	6:1

***Supplementary Table 1**: Glycosylation conditions*

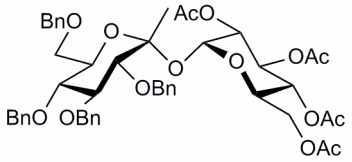
Nature Chemical Biology: doi:10.1038/nchembio.539

Synthesis of compound 2.



Supplementary Scheme 3. *Reagents and conditions. (a) TMSOTf, 4Å molecular sieves, anhydrous DCM, –40 °C, 3 h (b) NaOMe, anhydrous MeOH, RT, 1 h (c) H_2 , $Pd(OH)_2/C$, basic alumina, RT, 72 h.*

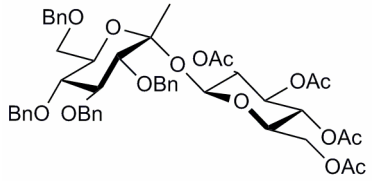
3,4,5,7-tetra-O-benzyl-1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-2,3,4,6-tetra-O-acetyl-α- D-glucopyranoside **(37)**



29[6] (134 mg, 0.24 mmol, 1 eq) and **33**[11] (87 mg, 0.25 mmol, 1.05 eq) were dried *in vacuo* for 1 hour and then dissolved in anhydrous CH_2Cl_2 (7 mL) and added to a dry flask in the presence of molecular sieves (ca. 100 mg). To this was added TMSOTf (10µl, 0.054 mmol, 0.2 eq) at –40 °C in the under an Ar atmosphere. The resulting mixture was stirred for 3 h. (2.5:1 petrol/ethyl acetate) revealed the production of two new product spots (R_f 0.3) and (R_f 0.25) and complete consumption of starting material. The reaction was then quenched by the addition 0.01mL triethylamine, filtered through Celite® and concentrated. The crude product was purified by column chromatography (5:2 petrol/ethyl acetate) to give colorless oil (189.5 mg, 93%) as a mixture of α,α and α,β products (7:1) that were separated during a second round of column chromatography (3:1 petrol/ethyl acetate) to afford pure the desired compound (162 mg, 85.6 %) as a clear oil. TLC (petrol:EtOAc, 5:2, v/v): R_f=0.30; $[\alpha]_D^{24}$ + 80.9 (c = 1 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (3 H, s, C-1'), 1.96 (3 H, s, 1 x

OCOC$\underline{H}_3$), 2.01 (6 H, s, 2 x OCOC$\underline{H}_3$), 2.05 (3 H, s, 1 x OCOC$\underline{H}_3$), 3.32 (1 H, d, $J_{3',4'}$ = 9.6 Hz, H-3'), 3.56 - 3.64 (3 H, m, H-$7_{a'}$, H-7_b', H-5'), 3.82 (1 H, dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6a}$ = 1.9 Hz, H-6_a), 3.88 (1 H, ddd, $J_{5,6}$ = 10.1 Hz, $J_{6,7a}$ = 3.8 Hz, $J_{6,7b}$ = 2.5 Hz H-6'), 4.05 - 4.11(2 H, m, H-4', H-6_b), 4.33 (1 H, ddd, $J_{4,5}$ = 10.4 Hz $J_{5,6a}$ = 4.3 Hz, $J_{5,6b}$ = 2.0 Hz, H-5), 4.48 (1 H, d, J = 12.3 Hz, 1x OCH_2Ph), 4.56 (1 H, d, J = 12.4 Hz, 1x OCH_2Ph), 4.56 (1 H, d, J = 11.0 Hz, 1x OCH_2Ph), 4.62 (1 H, d, J = 11.3 Hz 1 x OCH_2Ph), 4.85 (1 H, d, J = 11.1 Hz 1 x OCH_2Ph), 4.92 (1 H, d, J = 10.9 Hz, 1 x OCH_2Ph), 4.95-5.07 (4 H, m, H-4, 2 x OCH_2Ph, H-2), 5.37 (1 H, d, $J_{1,2}$ = 3.6 Hz, H-1), 5.55 (1 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, H-3), 7.08 - 7.40 (20 H, m, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 20.6, 20.6, 20.7, 20.7, (4 x OCO$\underline{C}H_3$) 22.6 (C-1'), 61.6 (C-6), 67.1 (C-5), 68.1 (C-7'), 68.6 (C-4), 70.2 (C-2), 70.7 (C-3), 72.0 (C-6'), 73.5 (OCH_2Ph), 74.8 (OCH_2Ph), 75.5 (2 x OCH_2Ph), 78.3 (C-5'), 82.6 (C-4'), 84.5 (C-3'), 89.2 (C-1), 101.4 (C-2'), 127.5, 127.6, 127.7, 127.9, 128.3, 128.3, 128.4, 128.8, 130.8 (4 x OCH_2Ph), 138.0, 138.2, 138.3, 138.5 (4 x 1 C, 4 x OCH_2Ph), 169.6, 169.8, 170.2, 170.5 (4 x C=O); IR (thin film): 3063, 3030, 2925, 1753 (C=O), 1496, 1454, 1367, 1222, 1133, 1090, 1038, 956, 916, 873, 737 cm^{-1}; HRMS (m/z) $[M+Na]^+$ calcd. for $C_{47}H_{53}O_{13}Na^+$, 907.3517;found, 907.3512; analysis (calcd., found for $C_{47}H_{53}O_{13}$): C (66.50,66.51), H (6.38,6.30).

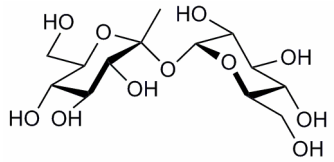
***3,4,5,7-tetra-O-benzyl-1- deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside* (38)**



The titled compound was purified as the lower spot by TLC (2:1 petrol/ethyl acetate) (R_f 0.25) from the reaction between **29** (134.4 mg, 0.242 mmol, 1 eq) and **33**[11] (87.2 mg, 0.25

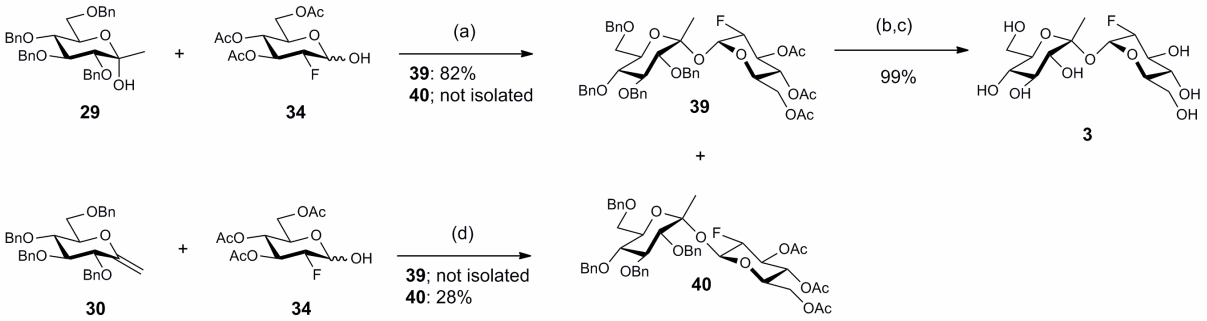
mmol, 1.05 eq) to produce **38** as a clear oil**.** (27 mg, 14.3 %). TLC (petrol:EtOAc, 5:2, v/v): R_f=0.30; $[\alpha]_D^{24}$ + 17.7 (c = 1 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (3 H, s, C-1'), 1.96 (1 H, s, 1 x OCOC$\underline{H}_3$), 2.00 (5 H, s, 5 x OCOC$\underline{H}_3$), 2.03 (5 H, m, 5 x OCOC$\underline{H}_3$), 2.04 (1 H, s, 1 x OCOC$\underline{H}_3$), 3.34 (1 H, d, $J_{3',4'}$ = 9.6 Hz, H-3'), 3.61 (1 H, ddd, $J_{4,5}$ = 12.4 Hz, $J_{5,6a}$ = 7.3 Hz, $J_{5,6b}$ = 2.5 Hz, H-5), 3.59-3.62 (1H, m, H-7_b'), 3.69-3.73 (2 H, m, H-5' H-7_a'), 4.03-4.07 (2 H, m, H-4', H-6_a), 4.12 (1 H, dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 4.8 Hz, H-6_b), 4.18 (1 H, ddd, $J_{5',6'}$ = 6.6 Hz, $J_{6',7a'}$ = 3.0 Hz, $J_{6',7b'}$ = 2.0 Hz, H-6'), 4.48 (1 H, d, J = 11.9 Hz, 1 x OCH_2Ph), 4.53 (1 H, d, J = 10.9 Hz, 1 x OCH_2Ph), 4.59 (1 H, dd, J = 11.4 Hz, 1 x OCH_2Ph), 4.61 (1 H, dd, J = 12.3 Hz, 1 x OCH_2Ph), 4.84 (1 H, d, J = 10.86 Hz, 1 x OCH_2Ph), 4.89 (1 H, d, $J_{1,2}$ = 8.2 Hz , H-1), 4.89 (2 H, d, J = 10.4 Hz, 2 x OCH_2Ph), 4.94 (1 H, d, J = 11.1 Hz, 1 x OCH_2Ph), 5.00-5.06 (2H, m, H-4, H-2), 5.19 (1 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 7.16-7.18 (2 H, m, Ar-H), 7.27-7.35 (18 H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 20.3, 20.6, 20.6, 20.7 (4 x OCO$\underline{C}H_3$), 22.1 (C-1'), 62.0 (C-6), 68.4 (C-4), 68.5 (C-7'), 70.9 (C-2), 72.0 (C-6' or C-5), 72.4 (C-6' or C-5), 72.9 (C-3), 73.4 (OCH_2Ph), 74.9 (OCH_2Ph), 75.5 (OCH_2Ph), 75.5 (OCH_2Ph), 78.2 (C-5'), 82.5 (C-4'), 84.5 (C-3'), 94.6 (C-1), 102.4 (C-2'), 127.5, 127.5, 127.6, 127.6, 127.8, 127.8, 128.3, 128.3, 128.4(4 x OCH_2Ph), 138.1, 138.2, 138.4, 138.6 (4 x 1 C, 4 x OCH_2Ph), 169.0, 169.4, 170.3, 170.6 (4x C=O); IR (thin film): 3029, 2931, 1755 (C=O), 1496, 1454, 1366, 1230, 1211, 1038, 908, 737 cm^{-1}; HRMS (m/z) $[M+Na]^+$ calcd. for $C_{47}H_{53}O_{13}Na^+$, 907.3517;found, 907.3512.

***1-Deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-α-D-glucopyranoside* (2)** [16]



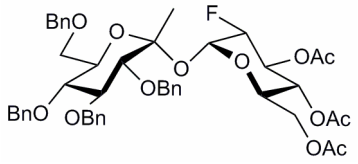
37 (120 mg, 0.13 mmol) was dissolved in 15 mL methanol with NaOMe (30 mg, 0.55 mmol, 4 eq) and stirred at room temperature for 1 hour until complete disappearance of starting material (R_f 0.55) and appearance of a new spot (R_f 0.05) was observed by TLC (1:1 petrol/ethyl acetate v/v) and deacetylated sugar was detected by ESI^+ *m/z* ($M+Na^+$): 741.3. Reaction was neutralized with DOWEX 50WX8 (H^+ form) ion exchange resin and concentrated *in vacuo*. The clear oil was redissolved in ethanol (15 mL) and to this was added basic alumina (70.1 mg) and 20% $Pd(OH)_2/C$ (80 mg) and palladium was activated by repeat purge flush cycles with hydrogen. Reaction was stirred under hydrogen atmosphere (balloon) at room temperature. After 72 h reaction was filtered through filter paper and filtrate was evaporated and purified by column chromatograph (9:5 ethyl acetate/methanol v/v) to give the desired, fully deprotected sugar, as a clear oil (40 mg, 83%). TLC (petrol/EtOAc 1:1 v/v) R_f=0.05; $[\alpha]_D^{25}$ +121.2 (0.32 in MeOH); [Lit. $[\alpha]_D^{27}$ +140.0 (c = 0.83 in MeOH)][16]; ^{1}H NMR (400 MHz, D_2O) δ 1.47 (3 H, s, C-1'), 3.24 (1 H, d, $J_{3,4}$ 9.9 Hz, H-3'), 3.35 (2 H, at, $J_{4,5}$ = $J_{5,6}$ = 9.6 Hz, H-5', H-4), 3.53 (1 H, dd, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.7 Hz, H-2), 3.59 (1 H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 5.5 Hz), 3.61 (1 H, dd, $J_{7a,7b}$ = 11.8 Hz, $J_{6,7a}$ = 5.1 Hz, H-7a), 3.67 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 10.8 Hz, H-4), 3.73 - 3.83 (5 H, m, H-6b, H-5, H-3, H-4' H-7b'), 3.99 (1 H, ddd, $J_{5,6}$ = 10.1 Hz, $J_{6,7a}$ = 5.3 Hz, $J_{6,7b}$ = 2.3 Hz, H-6'), 5.22 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1); ^{13}C NMR (126 MHz, D_2O) δ 22.5 (C-1'), 60.5 (C-6), 60.5 (C-7), 69.8 (C-4), 69.9 (C-5'), 71.4 (C-5), 71.8 (C-2), 72.3 (C-6'), 72.5 (C-3), 72.8 (1 C-4'), 76.4 (C-3'), 91.5 (C-1), 101.0 (C-2'); MS (m/z): $[M+Na]^+$ calcd. for $C_{14}H_{24}O_{11}Na^+$,379.1211; found 379.2; Spectroscopic data matches previously reported data[16]

Synthesis of compound 3.



Supplementary Scheme 4. *Reagents and conditions. (a) TMSOTf, 4 Å molecular sieves, anhydrous DCM, –40 °C, 2 h (b) NaOMe, anhydrous MeOH, RT, 3 h (c) H_2 , $Pd(OH)_2/C$, basic alumina, RT, 48h (d) TMSOTf, 4Å molecular sieves, anhydrous DCM, –78 °C, 30 min.*

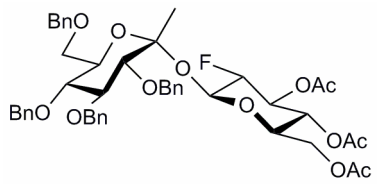
3,4,5,7-tetra-O-benzyl-1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-α-D-glucopyranoside **(39)**



29 (81 mg, 0.15 mmol, 1 eq) and **34** (69 mg, 0.22 mmol, 1.5 eq) were dried *in vacuo* for 1 hour and then dissolved in anhydrous CH_2Cl_2 (7 mL) and added to a dry flask in the presence of molecular sieves (ca. 100 mg). To this was added TMSOTf (10 µl, 0.054 mmol, 0.3 eq) at –40 °C under an Ar atmosphere. The resulting mixture was stirred for 2 h upon which time TLC (2.5:1 petrol/ethyl acetate) revealed the production of two very close new product spots (R_f 0.3) and (R_f 0.27) and complete consumption of starting material. The reaction was then quenched by the addition 0.01mL triethylamine, filtered through Celite® and concentrated to produce. The crude product was purified by column chromatography (2:1 etrol/ethyl acetate) to give the desired compound as a colorless oil (104 mg, 82%) and a 6:1 mixture of α,α (89.1 mg) to α,β. None of the α,β product was obtained to purity. TLC (petrol:EtOAc, 5:2, v/v): R_f=0.30; $[\alpha]_D^{24}$ + 65.5 (c = 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.48 (3 H, s, C-1'), 2.01 (3 H, s, 1 x OCOC<u>H</u>$_3$), 2.06 (3 H, s, 1 x OCOC<u>H</u>$_3$), 2.08 (3 H, s, 1 x OCOC<u>H</u>$_3$), 3.35

(1H, d, $J_{3',4'}$ = 9.4 Hz, H-3'), 3.62 (1 H, m, $J_{7a,7b}$ = 11.0 Hz, $J_{6,7a}$ =1.6 Hz, H-7b), 3.65 (1 H, at, $J_{5,6} = J_{4,5}$ = 9.8 Hz, H-5'), 3.71 (1 H, d, $J_{7a,7b}$ = 11.3 Hz, $J_{6,7a}$ = 4.1 Hz, H-7a), 3.83 (1 H, dd, $J_{6b,6a}$ = 12.5 Hz, $J_{6b,5}$ = 2.0 Hz, H-6b), 3.97 (1 H, ddd, $J_{5',6'}$ = 9.9 Hz, $J_{6',7a'}$ = 2.0 Hz, $J_{6',7b'}$ = 1.7 Hz, H-6'), 4.06 (1 H, at, $J_{3,4} = J_{4,5}$ = 9.4 Hz, H-4'), 4.08 (1 H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{6a,6b}$ = 4.5 Hz, H-6a), 4.33 (1 H, ddd, $J_{4,5}$ = 10.4 Hz, $J_{5,6a}$ = 4.7 Hz, $J_{5,6b}$ = 2.2 Hz, H-5), 4.49 (1 H, d, J = 12.3 Hz, 1 x OCH_2Ph),), 4.54 (6 H, ddd, $J_{2,F}$ = 48.0 Hz, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 4.57 (1 H, d, J = 11.1 Hz, 1 x OCH_2Ph), 4.60 (1 H, d, J = 12.3 Hz, 1 x OCH_2Ph), 4.62 (1 H, d, J = 10.4 Hz, 1 x OCH_2Ph), 4.83 (1 H, d, J = 10.9 Hz, 1 x OCH_2Ph), 4.93 (1 H, d, J = 11.4 Hz, 1 x OCH_2Ph), 4.96 (1 H, d, J = 12.3 Hz, 1 x OCH_2Ph), 4.99 (1 H, d, J = 11.4 Hz, 1 x OCH_2Ph) 4.99 (1 H, dt, $J_{4,5} = J_{3,4}$ = 9.6 Hz, $J_{4,F}$ =3.0 Hz), 5.46 (1 H, d, $J_{1,2}$ = 3.9 Hz, H-1), 5.61 (1 H, dt, $J_{3,F}$ = 12.2 Hz, $J_{3,4}$ = 9.4 Hz, $J_{2,3}$ = 9.4 Hz H-3), 7.16 - 7.37 (20 H, m, Ar-H); ^{13}C NMR (126 MHz, $CHCl_3$): δ 20.6, 20.6, 20.8 (3 x $OCO\underline{C}H_3$), 22.9 (C-1'), 61.6 (C-6), 67.0 (C-5), 68.2 (C-4), 68.5 (C-7'), 71.3 (1 C, d, $J_{C-3,F}$ = 26.5 Hz, C-3) 71.3 (C-6'), 73.1 (OCH_2Ph), 74.6 (OCH_2Ph), 75.5 (OCH_2Ph), 75.5 (OCH_2Ph), 78.2 (C-5'), 82.6 (C-4'), 84.5 (C-3'), 87.2 (1 C, d, $J_{C-2,F}$ = 195.5 Hz, C-2), 88.8 (1 C, d, $J_{C-1,F}$ = 21 Hz, C-1), 101.5 (C-2'), 127.5, 127.5, 127.6, 127.7, 127.8, 127.9, 127.9, 128.2, 128.3, 128.4(4 x OCH_2Ph), 138.2, 138.4, 138.6 (4 x 1 C, 4 x OCH_2Ph), 169.7, 170.1, 170.5 (3 x C=O); ^{19}F NMR (1H) (377 MHz, $CHCl_3$): δ -197.5; IR(thin film) 3064, 3030, 2940, 1754 (C=O), 1605, 1540, 1497, 1454, 1366, 1223, 1132, 1089, 1064, 974, 949, 912, 872, 751, 737 cm^{-}1; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{47}H_{53}FO_{13}Na^+$,867.3365;found: 867.3362.

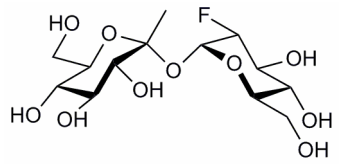
***3,4,5,7-tetra-O-benzyl-1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranoside* (40)**



Following the procedure in Li, 2001[9] a solution of **30** (24 mg, 0.045 mmol, 1 eq) and **34** (29 mg, 0.094 mmol, 2 eq) and molecular sieves 4A (MS 4A) (100 mg) in dry CH_2Cl_2 (5 mL) was stirred under nitrogen atmosphere at room temperature for 30 min. The solution was cooled to −78 °C and TMSOTf (5 µL, 0.027mmol, 0.5 eq) was added. The reaction mixture was stirred at −78 °C for 30 minutes. The reaction was monitored by TLC (2.5:1 petrol/ethyl acetate) and upon completion one broad product spot was visible (R_f 0.3) with disappearance of starting glycal **30** (R_f 0.8) and reaction was quenched with triethylamine (20 µl) and passed through Celite®. After the removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography (2.5:1 petrol/ ethyl acetate) as the eluent to afford the products (35 mg, 95%) 1:1 mixture of the α,α and α,β and gluco and manno sugars. From these only the titled compound could be isolated to purity as a clear oil (10 mg, 28%). TLC (petrol:EtOAc, 5:2, v/v): R_f=0.30; $[\alpha]_D^{24}$ + 39.7 (c = 0.37 in $CHCl_3$); ^{1}H NMR (500 MHz,$CDCl_3$): δ 1.42 (3 H, C-1'), 2.02 (3 H, s, 1 x OCOC<u>H</u>$_3$), 2.05 (3 H, s, 1 x OCOC<u>H</u>$_3$), 2.09 (3 H, 1 x OCOC<u>H</u>$_3$), 3.40 (1 H, d, $J_{3',4'}$ = 9.8 Hz, H-3'), 3.59 (1 H, ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 5.1 Hz, $J_{5,6b}$ = 2.5 Hz, H-5), 3.63-3.70 (3H, m, H-7a', H-7b', H-5'), 4.99-4.11 (2H, m, H-6b, H-4'), 4.09 (1 H, dd, $J_{6a,6b}$ = 11.8 Hz, $J_{6a,6b}$ = 5.5 Hz, H-6a), 4.21 (1 H, ddd, $J_{5',6'}$ = 10.1 Hz, $J_{6',7a'}$ = 3.9 Hz, $J_{6',7b'}$ = 1.42 Hz, H-6'), 4.37 (1 H, ddd, $J_{F,2}$ = 50.0 Hz, $J_{2,3}$ = 9.1 Hz, $J_{1,2}$ = 8.2 Hz, H-2) 4.50 (1 H, d, J = 12.0 Hz, 1 x OCH_2Ph), 4.54 (1 H, d, J = 10.7 Hz, 1 x OCH_2Ph), 4.59 (1 H, d, J = 12.0 Hz, 1 x OCH_2Ph), 4.71 (1 H, d, J = 11.7 Hz, 1 x OCH_2Ph), 4.85 (1 H, d, J = 11.0 Hz, 1 x OCH_2Ph), 4.87 (1 H, d, J = 11.0 Hz 1 x OCH_2Ph), 4.91 (1 H, d, J = 13.3 Hz, 1 x OCH_2Ph) 4.94-4.98 (2 H, m, H-4, 1 x OCH_2Ph), 5.01 (1 H, dd, $J_{1,2}$ = 7.7 Hz

$J_{1,F}$ = 2.4 Hz, H-1), 5.30 (1 H, dt, $J_{3,F}$ = 14.2 Hz, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 9.2 Hz, H-3), 7.16 - 7.34 (20 H, m, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 22.3, 22.6, 23.1 (3 x OCO<u>C</u>H$_3$), 28.8 (CH_3), 61.9 (C-6), 68.2 (1 C, d, $J_{C-4,F}$ = 5.0 Hz, C-4), 68.6 (C-7'), 72.1 (C-5), 72.6 (C-6'), 73.0 (1 C, d, $J_{C-3,F}$ = 18.9 Hz, C-3), 73.1 (OCH_2Ph), 73.5 (OCH_2Ph), 75.0 (OCH_2Ph), 75.5 (OCH_2Ph), 78.2 (C-5'), 82.7 (C-4'), 84.2 (C-3'), 88.9 (1 C, d, $J_{C-2,F}$ = 189 Hz, C-2), 94.0 (1 C, d, $J_{C-1,F}$ = 23.9 Hz, C-1), 102.6 (C-2'), 127.5, 127.69, 127.7, 127.7, 127.8, 127.8, 128.0, 128.3, (4 x OCH_2Ph), 138.1, 138.6 (4x 1 C. 4 x OCH_2Ph), 169.4, 170.13, 170.5 (3 x C=O); ^{19}F NMR (377 MHz, $CDCl_3$) δ -198.52; IR (thin film): 2921, 1754 (C=O), 1586, 1549, 1513, 1495, 1453, 1366, 1242, 1196, 1100, 1055, 1029, 735, 698 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{47}H_{53}FO_{13}Na^+$,867.3365;found: 867.3362.

1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-2-deoxy-2-fluoro-α-D-glucopyranoside **(3)**

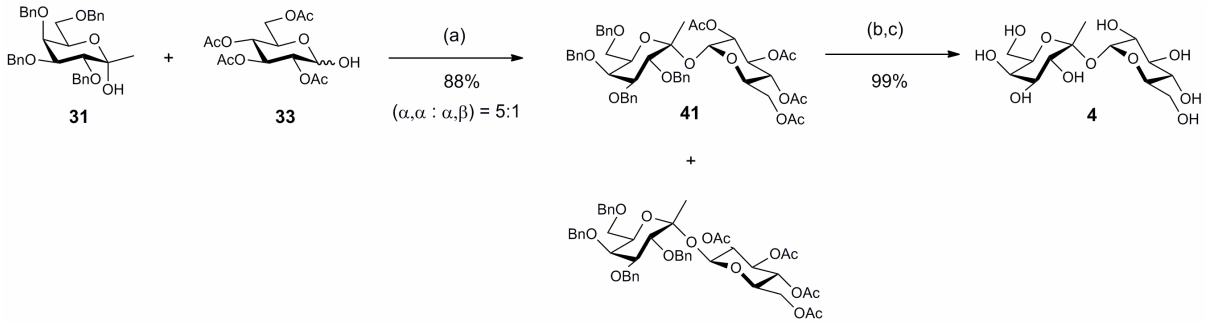


39 (66.6 mg, 0.078 mmol, 1 eq) was dissolved in 15 mL methanol with NaOMe (20 mg, 0.37 mmol, 4.8 eq) and stirred at room temperature for 3 hours until complete disappearance of starting material (R_f 0.55) and appearance of a new spot (R_f 0.05) was observed by TLC (1:1 petrol/ethyl acetate) and deacetylated sugar was detected by ESI$^+$ *m/z* ($M+Na^+$): 741.3. Reaction was neutralized with DOWEX 50WX8 (H^+ form) ion exchange resin and concentrated *in vacuo*. The clear oil was re-dissolved in methanol (10 mL) and to this was added basic alumina (5.7 mg) and 20% $Pd(OH)_2$/C (112 mg) and reaction was stirred under hydrogen atmosphere (balloon) at room temperature. After 48 h. reaction was filtered through filter paper and filtrate was evaporated and purified by column chromatograph (7:3 ethyl acetate/methanol) and Isolute SPE C18 cartridge to give the desired, fully deprotected sugar,

as a clear oil (27 mg, 98%). $[\alpha]_D^{25}$ + 95.5 (c = 0.42 in MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.45 (3 H, s, C-1'), 3.24 (1 H, d, $J_{3,4}$ = 9.8 Hz, H-3'), 3.33 (1 H, at, $J_{4,5}$ = $J_{5,6}$= 10.1 Hz, H-5'), 3.40 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.63 (1 H, dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 5.8 Hz, H-6a), 3.66 (1 H, dd, $J_{7a,7b}$ = 11.9 Hz, $J_{6,7a}$ = 5.7 Hz, H-7a'), 3.67 (1 H, t, J = 9.5 Hz, H-4'), 3.72 - 3.79 (3 H, m, H-6b, H-7b', H-6'), 3.80 (1 H, ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.4 Hz, H-5), 4.02 (1 H, dt, $J_{3,F}$ = 12.9, $J_{3,4}$ = 9.4 Hz, $J_{2,3}$ = 9.4 Hz, H-3), 4.36 (1 H, ddd, $J_{2,F}$ = 49.5 Hz, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 5.45 (1 H, d, $J_{1,2}$ = 4.1 Hz, H-1); ^{13}C NMR (126 MHz, D_2O): δ 22.27 (C-1'), 60.31 (C-6), 60.59 (C-7), 69.2 (1 C, d, $J_{C-4,F}$ = 7.5 Hz , C-4), 69.8 (C-5'), 71.2 (1 C, d, $J_{C-3,F}$ = 17.6 Hz, C-3), 71.9 (C-5), 72.5 (C-6'), 72.9 (C-4'), 76.1 (C-3'), 88.8 (C-1), 89.7 (1 C, d, J_{C2-F} = 189 Hz, C-2), 101.1 (C-2'); ^{19}F NMR (377 MHz, D_2O) δ -197.2 (dt, $J_{2,F}$ = 49.3 Hz, $J_{1,F}$ = $J_{3,F}$ = 12.6 Hz); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{13}H_{23}FO_{10}Na^+$,381.1173; found: 381.1167.

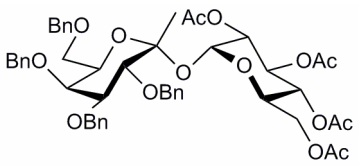
Nature Chemical Biology: doi:10.1038/nchembio.539

Synthesis of compound 4.



Supplementary Scheme 5. *Reagents and conditions. (a) TMSOTf, molecular sieves, anhydrous DCM, −40 °C, 30 min (b) NaOMe, anhydrous MeOH, RT, 1 h (c)* H_2 *,* $Pd(OH)_2/C$*, basic alumina, RT, 72 h.*

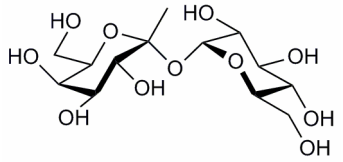
3,4,5,7-tetra-O-benzyl-1-deoxy-α-D-galacto-hept-2-ulopyranosyl-(2→1)-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside **(41)**



31 (176.1 mg, 0.317 mmol, 1 eq) and **33** (146.2 mg, 0.419 mmol, 1.3 eq) were dried *in vacuo* for 1 hour and then dissolved in anhydrous CH_2Cl_2 (8 mL) and added to a dry flask in the presence of molecular sieves (ca. 100 mg). To this was added TMSOTf (10µl, 0.54 mmol, 0.17 eq) at –40 °C under an Ar atmosphere. The resulting mixture was stirred for 30 min until TLC (2.5:1 petrol/ethyl acetate) revealed the production of two new product spots (R_f 0.3) and (R_f 0.27) and complete consumption of starting material. The reaction was then quenched by the addition 0.1 mL triethylamine, filtered through Celite® and concentrated to produce. The crude product was purified by column chromatography (5:2 petrol/EtOAc) to give as a colorless oil (246 mg, 88%) as a mixture of α,α and α,β products (5:1) that were separated during further chromatography (3:1 petrol/EtOAc) to yield the desired compound as exclusively α,α (180.7 mg, 65%)**.** TLC (petrol:EtOAc, 5:2, v/v): R_f=0.30; $[\alpha]_D^{24}$ + 72.0 (c =

1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46 (3 H, s, C-1'), 1.94 (3H, s, 1x OCOC$\underline{H}_3$) 2.01 (6 H, s, 2 x OCOC$\underline{H}_3$), 2.04 (3 H, s, 1 x OCOC$\underline{H}_3$), 3.40 (1 H, dd, $J_{7a,7b}$ 8.9 Hz, $J_{6,7a}$ 5.3 Hz, H-7b'), 3.56 (1 H, dd, $J_{7a,7b}$ = 8.4, $J_{6,7a}$ = 8.4 Hz, H-7a'), 3.75 (1 H, dd, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.83 (1 H, d, $J_{3,4}$ 9.9 Hz, H-3'), 4.01 (1 H, Br s, H-5'), 4.02 (1 H, dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 4.2 Hz, H-6a), 4.07 (1 H, dd, $J_{4,5}$ = 9.9, $J_{5,6}$ = 2.6 Hz, H-5'), 4.10 (1 H, ddd, J = 7.2, 4.3, 1.2 Hz, H-6') 4.34 (1 H, d, J = 11.8 Hz, 1 x OCH_2Ph), 4.38 (3 H, ddd, $J_{4,5}$ = 10.4 Hz, $J_{5,6a}$ = 4.3 Hz, $J_{5,6b}$ = 2.2 Hz, H-5), 4.40 (1 H, d, J = 11.6 Hz, 1 x OCH_2Ph), 4.57 (1 H, d, J = 11.6 Hz, 1 x OCH_2Ph), 4.61 (1 H, d, J = 11.1 Hz, 1 x OCH_2Ph), 4.82 (1 H, Br. s, 1 x OCH_2Ph), 4.95 (1 H, d, J = 11.4 Hz, 1 x OCH_2Ph), 4.99 (1 H, dd, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.6 Hz, H-2), 5.02 (1 H, at, $J_{1,2}$ = $J_{2,3}$ = 10.1, H-2), 5.04 (2 H, d, J = 10.41 Hz, 1 x OCH_2Ph), 5.06 (1 H, d, J = 10.2 Hz, 1 x OCH_2Ph), 5.33 (1 H, d, $J_{1,2}$ = 3.6 Hz, H-1), 5.53 (1 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 6.85 - 7.64 (20 H, m, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 20.3, 20.6, 20.6, 20.7 (4 x OCO$\underline{C}H_3$), 22.4 (C-1'), 61.6 (C-6), 67.0 (C-5), 68.4 (C-4), 68.4 (C-7) 70.5 (C-2), 70.5 (2 C, s, C-4 and C-6'), 72.6 (OCH_2Ph), 73.5 (OCH_2Ph), 74.2 (C-5'), 74.5 (OCH_2Ph), 75.6 (OCH_2Ph), 80.1 (C-4'), 80.86 (C-3'), 89.3 (C-1), 102.3 (C-2'), 127.5, 127.5, 127.6, 127.7, 127.8, 127.9, 128.1, 128.2, 128.2, 128.3, 128.4(4 x OCH_2Ph), 137.6, 138.4, 138.6, 138.7 (4 x 1 C, 4 x OCH_2Ph), 169.6, 169.9, 170.2, 170.6 (4 x C=O) IR (thin film): 3030, 2932, 2868, 1752 (C=O), 1497, 1454, 1368, 1221, 1139, 1100, 1038, 963, 922, 877, 845, 772.3, 753 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{47}H_{53}O_{13}Na^+$, 907.3517; found, 907.3511; analysis (calcd., found for $C_{47}H_{53}O_{13}$): C (66.81,66.59), H (6.37, 6.42).

***1-deoxy-α-D-galacto-hept-2-ulopyranosyl-(2→1)-α-D-glucopyranoside* (4)** [16]

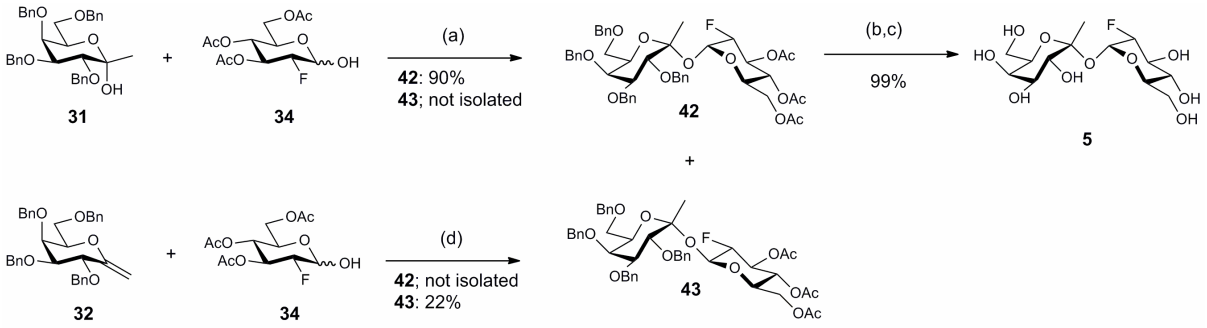


41 (163.2 mg, 0.184 mmol, 1 eq) was dissolved in 15 mL methanol with NaOMe (46.2 mg, 0.855 mmol) and stirred at room temperature for 1 hour until complete disappearance of starting material (R_f 0.55) and appearance of a new spot (R_f 0.05) was observed by TLC (1:1 petrol/ethyl acetate) and deacetylated sugar was detected by ESI^+ *m/z* (M+Na): 739.2. Reaction was neutralized with DOWEX 50WX8 (H^+ form)ion exchange resin and concentrated *in vacuo*. The resulting clear oil was redissolved in ethanol (15mL) and solution was degassed with nitrogen. To this was added basic alumina (50 mg) and 20% $Pd(OH)_2/C$ (70.6 mg) and reaction was stirred under hydrogen atmosphere (balloon) at room temperature. After 72 h. reaction was filtered through filter paper and filtrate was evaporated and purified by column chromatography (9:5 ethyl acetate/methanol) to give the desired, fully deprotected sugar, as a clear oil. (23 mg, 32%). TLC (petrol:EtOAc, 1:1, v/v): R_f=0.05; $[\alpha]_D^{25}$ + 156.5 (0.46 in MeOH); [Lit. $[\alpha]_D^{22}$ +164.9 (c = 0.64 in MeOH)][16]; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.51 (3 H, s, C-1'), 3.38 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.55 (1 H, d, $J_{3,4}$ = 10.1 Hz, H-3'), 3.56 (1 H, dd, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 3.66 (1 H, dd, $J_{7a,7b}$ = 12.0 Hz, $J_{6,7b}$ = 5.4 Hz, H-7b'), 3.69 (1 H, dd, $J_{7a,7b}$ = 12.0 Hz, $J_{6,7b}$ = 7.7 Hz, H-7a), 3.71 (1 H, dd, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6a}$ = 5.4 Hz, H-6a), 3.77 - 3.85 (3 H, m, H-3, H-5, H-6b), 3.94 (1 H, dd, $J_{4,5}$ = 3.5 Hz, $J_{5,6}$ = 1.1 Hz, H-5'), 3.95 (1 H, dd, $J_{3,4}$ = 10.7 Hz, $J_{4,5}$ = 3.5 Hz, H-4'), 4.22 (1 H, ddd, $J_{6,7a}$ = 7.1 Hz, $J_{6,7b}$ = 5.0 Hz, $J_{5,6}$ = 1.4 Hz, H-6'), 5.26 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1); ^{13}C NMR (126 MHz, $CDCl_3$): δ 25.2 (C-1'), 63.0 (C-6), 63.6 (C-7'), 71.8 (C-5'), 72.0 (C-4'), 72.4 (C-4), 73.8 (C-6'), 73.9 (C-2), 74.2 (C-5), 75.0 (C-3), 75.9 (C-3'), 94.0 (C-1),

103.7 (C-2'); MS (m/z): $[M+Na]^+$ calcd. for $C_{13}H_{24}O_{11}Na^+$, 379.1211; found: 379.2. Spectroscopic data matches with that previously reported [16]

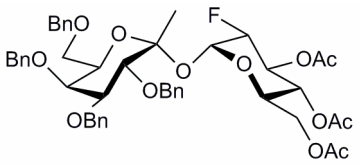
Nature Chemical Biology: doi:10.1038/nchembio.539

Synthesis of compound 5.



Supplementary Scheme 6. *Reagents and conditions. (a) TMSOTf, molecular sieves, anhydrous DCM, –40 °C, 1 h (b) NaOMe, anhydrous MeOH, RT, 3 h (c) H_2 , $Pd(OH)_2/C$, basic alumina, RT, 96 h (d) TMSOTf, 4Å molecular sieves, anhydrous DCM, –78 °C, 30 min.*

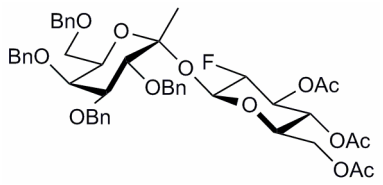
3,4,5,7-tetra-O-benzyl-1- deoxy-α-D-galacto-hept-2-ulopyranosyl-(2→1)-3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-α-D-glucopyranoside **(42)**



31 (88.7 mg, 0.16 mmol, 1.15 eq) and **34** (44.1 mg, 0.14 mmol, 1 eq) were dried *in vacuo* for 1 hour then dissolved in anhydrous CH_2Cl_2 (7 mL) and added to a dry flask in the presence of molecular sieves (ca. 100 mg). To this was added TMSOTf (5µl, 0.027 mmol, 0.18 eq) at –40 °C under an Ar atmosphere. The resulting mixture was stirred for 1 hour upon which time TLC (2.5:1 petrol/ethyl acetate) revealed the production of two new product spots (R_f 0.3) and (R_f 0.27) and complete consumption of starting material. The reaction was then quenched by the addition 0.01mL triethylamine, filtered through Celite® and concentrated. The crude product was purified by column chromatography (2:1 petrol/ethyl acetate) to give a 6:1 mixture of the α,α and α,β products (106 mg, 90%). Further purification in (3:1 petrol/ethyl acetate) yielded the desired compound (70.7 mg, 60%) as a colorless oil. α,β product was not obtained to purity through this method. TLC (petrol:EtOAc, 5:2, v/v): R_f=0.30; $[\alpha]_D^{24}$ + 46.6 (c = 0.78, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.47 (3 H, s, C-1'),

2.01 (3 H, s, 3 x OCOC$\underline{H}_3$), 2.06 (3H, s, 3 x OCOC$\underline{H}_3$), 2.09 (3H, s, 3 x OCOC$\underline{H}_3$), 3.52 (2 H, ad, *J* 6.6 Hz, H-7a', H-7b'), 3.83 (2 H, dd, $J_{6a,6b}$ 12.5 Hz, $J_{5,6b}$ 2.4 Hz, H-6b), 3.84 (1H, d, $J_{3,4}$ = 9.8 Hz, H-3'), 3.98 (1 H, dd, $J_{4,5}$ = 2.7 Hz, $J_{5,6}$ = 1.4 Hz, H-5'), 4.05 (1 H, dd, $J_{3,4}$ = 9.9 Hz, $J_{4,5}$ = 2.7 Hz, H-4'), 4.08 - 4.15 (2 H, m, H-6', H-6a), 4.33 (1 H, ddd, $J_{4,5}$ = 10.3 Hz, $J_{5,6a}$ = 4.8 Hz, $J_{5,6b}$ = 2.2 Hz, H-5), 4.42 (1 H, d, *J* = 12.0 Hz, 1 x OCH_2Ph), 4.47 (1 H, d, *J* = 11.9 Hz, 1 x OCH_2Ph), 4.53 (1 H, ddd, $J_{2,F}$ = 50.8 Hz $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.9 Hz, H-2), 4.58 (1 H, d, *J* = 11.3 Hz, 1 x OCH_2Ph), 4.63 (1 H, d, *J* = 11.3 Hz, 1 x OCH_2Ph), 4.76 (1 H, d, *J* = 11.9 Hz, 1 x OCH_2Ph), 4.79 (1 H, d, *J* = 12.0 Hz, 1 x OCH_2Ph), 4.93 (1 H, d, *J* = 11.3 Hz, 1 x OCH_2Ph), 5.00 (2 H, t, *J* = 9.7 Hz, H-4), 5.02 (1 H, d, *J* = 11.3 Hz, 1 x OCH_2Ph), 5.44 (1 H, d, $J_{1,2}$ = 4.1 Hz, H-1), 5.61 (1 H, adt, $J_{3,F}$ = 12.3 Hz, $J_{3,4}$ = $J_{2,3}$ = 9.5 Hz, H-3), 6.87 - 7.54 (20 H, m, Ar-H), 7.30; ^{13}C NMR (126 MHz, $CDCl_3$): δ 20.6, 20.6, 20.8 (3 x OCO$\underline{C}H_3$), 22.7 (C-1'), 61.63 (C-6), 67.0 (C-5) 68.0 (1 C, d, $J_{C-4,F}$ =7 Hz, C-4,), 68.7 (C-7'), 71.1 (1 C, d, $J_{C-3,F}$ = 18.9 Hz, C-3), 71.1 (C-6'), 72.6 (OCH_2Ph), 73.1 (OCH_2Ph), 74.4 (OCH_2Ph or C-5'), 74.5 (OCH_2Ph or C-5'), 75.5 (OCH_2Ph), 80.1 (C-4'), 80.6 (C-3') 87.5 (1 C, d, $J_{C-2,F}$ 194 Hz, C-2), 88.7 (1 C, d, $J_{C-1,F}$ = 21 Hz, C-1), 102.0 (C-2'), 127.5, 127.5, 127.6, 127.7, 127.7, 128.0, 128.2, 128.3, 128.4 (4 x OCH_2Ph), 138.1, 138.4, 138.7, 138.7 (4 x 1 C, 4 x OCH_2Ph), 169.7, 170.1, 170.5 (3 x C=O); ^{19}F NMR (1H) (377 MHz, $CDCl_3$): δ -197.6; IR (thin film): 2922, 2852, 2408, 1747 (C=O), 1496, 1453, 1367, 1220, 1055, 772 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{47}H_{53}FO_{13}Na^+$, 867.3365, found: 867.3362.

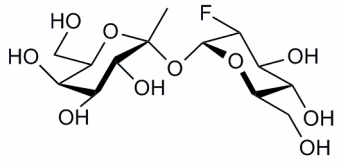
***3,4,5,7-tetra-O-benzyl-1-deoxy-α-D-galacto-hept-2-ulopyranosyl-(2→1)-3,4,6-tri-O-acetyl-2-deoxy-2-fluoro- β -D-glucopyranoside* (43)**



Following the procedure in Li, 2001[9]. A solution of **32** (168 mg, 0.31 mmol, 1 eq), and a **34** (178 mg, 0.58 mmol, 1.6 eq) and 4Å molecular sieves (ca.100 mg) in dry CH_2Cl_2 (5 mL) was stirred under nitrogen atmosphere at room temperature for 30 min. The solution was cooled to −78 °C and TMSOTf (5μL, 0.027 mmol, 0.05 eq.) was added. The reaction mixture was stirred at −78 °C for 30 minutes. The reaction was monitored by TLC (2.5:1 petrol/ethyl acetate) and upon completion, the appearance of one broad spot (R_f 0.3) and disappearance of starting glycal (R_f 0.8) was detected. Reaction was quenched with triethylamine (20 μ1) and passed through Celite® and concentrated. The residue was applied on a silica gel column chromatography (2:1 petrol/ethyl acetate) to afford the products as a 1:2 mixture of the α,α and α,β and gluco and manno sugars (75 mg, 30%). From these only the titled compound could be isolated to purity as a clear oil (57 mg, 22%). TLC (petrol:EtOAc, 5:2, v/v): R_f=0.30; $[\alpha]_D^{24}$ + 18.7 (c = 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.41 (3 H, s, C-1'), 2.00 (6 H, s, 2 x OCOC$\underline{H}_3$) 2.05 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.08 (3 H, s, 1 x OCOC$\underline{H}_3$), 3.41 (1 H, dt, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 3.2 Hz, $J_{5,6b}$ = 3.2 Hz H-5), 3.45 (1 H, dd, $J_{7a,7b}$ = 5.9 Hz, $J_{6,7b}$ = 3.1 Hz, H-7_b'), 3.50 - 3.55 (1 H, m, H-7_a'), 3.90 (1 H, d, $J_{3,4}$ = 9.8 Hz, H-3'), 3.96 (2 H, dd, J = 5.9 Hz, J = 3.0 Hz, H-6_a, H-6_b), 3.99 (1 H, at, $J_{4,5}$ = $J_{5,6}$ = 1.0 Hz, H-5'), 4.02 (1 H, dd, $J_{3,4}$ = 9.8 Hz, H-4'), 4.27 - 4.35 (1H, m, H-2, H-6'), 4.41 (1 H, d, J = 9.8 Hz, 1 x OCH_2Ph), 4.45 (1 H, d, J = 11.3 Hz, 1 x OCH_2Ph), 4.63 (1 H, d, J = 11.7 Hz, 1 x OCH_2Ph), 4.72 (1 H, d, J = 11.3 Hz, 1 x OCH_2Ph), 4.76 (2 H, s, 2 x OCH_2Ph), 4.93 - 4.97 (3 H, m, H-1, 2 x OCH_2Ph), 4.99 (1 H, dd, $J_{4,5}$ = 7.2 Hz $J_{3,4}$ = 4.9 Hz, H-4), 5.25 (1 H, dt, $J_{3,F}$ = 14.1 Hz, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz.H-3), 7.19 - 7.44 (20 H, m); ^{13}C NMR (126 MHz, $CDCl_3$) δ 20.4, 20.5, 20.6 (3 x

OCO<u>C</u>H$_3$), 22.3 (C-1'), 61.4 (C-6), 67.9 (1 C, d, $J_{C-4,F}$ = 7.6 Hz, C-4) 69.9 (C-7'), 71.8 (C-6'), 71.9 (C-5), 72.8 (OCH_2Ph), 73.3 (1 C, d, $J_{C-3,F}$ = 20.1 Hz, C-3), 73.7 (OCH_2Ph), 74.3 (C-5'), 74.6 (OCH_2Ph), 75.6 (OCH_2Ph), 80.1 (C-3'), 80.4 (C-4') 88.8 (C-2,d, $J_{C-2,F}$ = 190.8 Hz), 93.6 (C-1, d, $J_{C-1,F}$ = 22.9 Hz), 103.1 (C'-2), 127.4, 127.5, 127.5, 127.6, 127.7, 128.1, 128.1, 128.2, 128.2, 128.3, 128.4 (4 x OCH_2Ph) 138.0, 138.3, 138.5, 138.6 (4 x 1 C, 4 x OCH_2Ph), 170.1, 170.5, 171.1 (3 x C=O); ^{19}F NMR (377 MHz, $CDCl_3$) δ -198.73; IR (thin film): 3063, 3030, 2923, 2857, 1752 (C=O), 1604, 1548, 1496, 1454, 1367, 1230, 1212, 1139, 1100, 1054, 919, 808, 736 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{47}H_{53}O_{13}Na^+$,867.3365;found,867.3383.

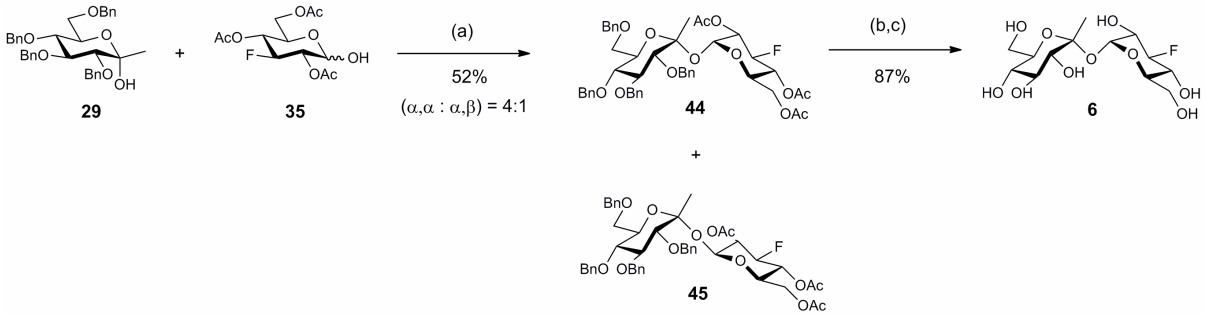
1-deoxy-α-D-galacto-hept-2-ulopyranosyl-(2→1)-2-deoxy-2-fluoro-α-D-glucopyranoside **(5)**



42 (42 mg, 0.05 mmol, 1 eq) was dissolved in 20 mL methanol with NaOMe (31 mg, 0.6 mmol, 12 eq) and stirred at room temperature for 3 hours until until complete disappearance of starting material (R_f 0.55) and appearance of a new spot (R_f 0.05) was observed by TLC (1:1 petrol/ethyl acetate) and deacetylated sugar was detected by ESI$^+$ *m/z* (M+Na): 741.3. Reaction was neutralized with DOWEX 50WX8 (H^+ form)ion exchange resin and concentrated *in vacuo*. The resulting clear oil was re-dissolved in methanol (10mL) and solution was degassed with nitrogen. To this was added basic alumina (31.0 mg) and 20% $Pd(OH)_2$/C (73 mg) and reaction was stirred under hydrogen atmosphere (balloon) at room temperature. After 96 h. reaction was filtered through filter paper and filtrate was evaporated. Further purification was obtained utilizing Isolute SPE C18 cartridge to give the desired, fully deprotected sugar, as a clear oil (20 mg, 100%). TLC (petrol:EtOAc, 1:1, v/v):

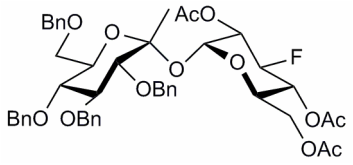
R_f=0.05; $[\alpha]_D^{25}$ +122.8 (0.42 in MeOH); ^{1}H NMR (500 MHz, D_2O) δ 1.47 (3 H, s, C-1'), 3.39 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.52 (1 H, d, $J_{3,4}$ = 10.4 Hz, H-3'), 3.61 (1 H, dd, $J_{7a,7b}$ = 12.0 Hz, $J_{6,7a}$ = 5.4 Hz, H-7b'), 3.64 (1 H, dd, $J_{7a,7b}$ = 12.0 Hz, $J_{6,7a}$ = 7.3 Hz, H-7°'), 3.66 (1 H, dd, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6a}$ = 5.4 Hz, H-6a), 3.76 (1 H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.79 (1 H, dd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 4.8 Hz, $J_{5,6b}$ = 2.6 Hz, H-5), 3.83 (1 H, dd, $J_{3,4}$ = 10.1, $J_{4,5}$ = 3.2 Hz, H-4'), 3.90 (1 H, dd, $J_{4,5}$ = 3.1 Hz, $J_{5,6}$ = 1.0 Hz, H-5'), 3.97 (1 H, ddd, $J_{6,7a}$ = 7.1, $J_{6,7b}$ = 5.5 Hz, $J_{5,6}$ = 1.0 Hz, H-6'), 4.02 (1 H, dt, $J_{3,F}$ = 13.0 Hz, $J_{3,4}$ =$J_{2,3}$ = 9.4 Hz, H-3), 4.37 (1 H, m, $J_{2,F}$ = 49.5 Hz, $J_{2,3}$ = 9.5 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 5.46 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1); ^{13}C NMR (126 MHz, D_2O): δ 22.44 (C-1'), 60.3 (C-6), 61.3 (C-7'), 69.3 (1 C, d, $J_{C\text{-}4,F}$ = 7.5 Hz, C-4), 69.3 (C-5'), 69.6 (C-4'), 71.2 (1 C, d, $J_{C\text{-}3,F}$ = 17.6 Hz, C-3), 71.6 (1 C, $J_{C\text{-}5,F}$ = 6.3 Hz, C-5), 71.8 (C-6'), 73.2 (C-3'), 88.8 (1 C, d, $J_{C\text{-}1,F}$ = 21.4 Hz, C-1), 89.9 (1 C, d, $J_{C2,F}$ = 189 Hz, C-2), 101.34 (C-2'); ^{19}F NMR (377 MHz, D_2O): δ -197.5 (dt, $J_{2,F}$ = 51.6, $J_{1,F}$ = $J_{3,F}$ =14.9 Hz); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{13}H_{23}FO_{10}Na^+$, 381.1173;found: 381.1165.

Synthesis of compound 6.



Supplementary Scheme 7. *Reagents and conditions. (a) TMSOTf, 4 Å molecular sieves, DCM, –40 °C, 5.5 h (b) i, NaOMe, anhydrous MeOH, RT, 1.5 h; ii, H Cube® 10% Pd/C cartridge at 70 bar, 25 °C, 1 hr.*

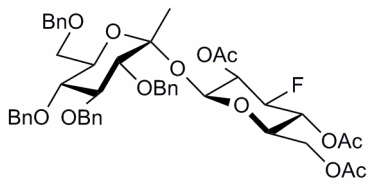
3,4,5,7-tetra-O-benzyl-1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-2,4,6-tri-O-acetyl-3-deoxy-3-fluoro-α-D-glucopyranoside **(44)**



29 (116 mg, 0.21 mmol, 1.16 eq) and **35** (56 mg, 0.18 mmol, 1 eq) were dried under reduced pressure for one hour and then dissolved in anhydrous DCM (8 mL) and added to activated molecular sieves (ca 100 mg). Sugars were stirred with sieves at RT for 1 hour and were then cooled to –40 °C. TMSOTf (10 µl, 0.054 mmol, 0.3 eq) was added and 1 hour later additional TMSOTf (10 µl, 0.054 mmol, 0.3 eq) was added to the reaction. Reaction was stirred for 5.5 h, upon which time conversion was detected by TLC (2:1 petrol/ethyl acetate) with conversion to product α,α (R_f 0.5) and α,β (R_f 0.48) and disappearance of starting sugars (Rf 0.6) and (Rf 0.05). Reaction was then quenched with 1 drop triethylamine, filtered through Celite® to remove molecular sieves, concentrated under reduced pressure and purified by column chromatography (2.5:1 petrol/ethyl acetate). The desired product was obtained as a clear oil (79 mg, 52%) α,α (63 mg) and α,β (16 mg) as well as recovered **35** (10 mg). TLC (petrol:EtOAc, 2:1, v/v): R_f=0.50; $[\alpha]_D^{25}$ = 66.3 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$):

δ 1.47 (3 H, s, C-1'), 2.02 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.04 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.13 (3 H, s, 1 x OCOC$\underline{H}_3$), 3.33 (1 H, d, $J_{3,4}$ = 9.6 Hz, H-3'), 3.58 - 3.70 (3 H, m, H-7a',H-7b', H-5'), 3.81 (1 H, dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 1.6 Hz, H-6b), 3.90 (1 H, ddd, $J_{5,6}$ = 10.1 Hz, $J_{6,7a}$ = 4.0 Hz, $J_{6,7b}$ = 2.3 Hz, H-6'), 4.02 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4'), 4.05 (1 H, dd, $J_{6a,6b}$ = 12.9 Hz, $J_{5,6a}$ = 4.5 Hz, H-6a), 4.25 (1 H, ddd, $J_{4,5}$ = 10.2 Hz, $J_{5,6a}$ = 4.2 Hz, $J_{5,6b}$ = 2.0 Hz, H-5), 4.50 (1 H, d, J = 12.1 Hz, 1 x OCH_2Ph), 4.58 (1 H, d, J = 9.3 Hz, 1 x OCH_2Ph), 4.60 (1 H, d, J = 12.1 Hz, 1 x OCH_2Ph), 4.64 (1 H, d, J = 11.4 Hz, 1 x OCH_2Ph), 4.87 (1 H, d, J = 10.9 Hz, 1 x OCH_2Ph), 4.90 (1 H, dt, $J_{3,F}$ = 54.0 Hz, $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 4.92 (2 H, m, 2 x OCH_2Ph), 4.99 (1 H, d, J = 11.1 Hz, 1 x OCH_2Ph), 5.08 (1 H, ddd, $J_{2,F}$ = 11.4 Hz, $J_{2,3}$ = 9.9 Hz, $J_{1,2}$ = 3.5 Hz, H-2), 5.21 (1 H, ddd, $J_{4,F}$ = 13.6 Hz, $J_{4,5}$ = 10.4 Hz, $J_{3,4}$ 9.1 Hz, H-4), 5.39 (1 H, at, $J_{1,2}$ = 3.4 Hz, H-1), 7.08 - 7.44 (20 H, m, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 20.6, 20.6, 20.7 (3 x OCO$\underline{C}H_3$), 22.5 (C-1'), 61.5 (C-6), 67.1 (1 C, d, $J_{C-5,F}$ = 6.3 Hz, C-5), 68.3 (1 C, d, $J_{C-4,F}$ = 17.4 Hz, C-4), 68.5 (C-7'), 70.7 (1 C, d, J_{C-2F} = 16.4 Hz, C-2) 72.1(C'-6), 73.5 (OCH_2Ph), 74.8 (OCH_2Ph), 75.5 (OCH_2Ph), 75.5 (OCH_2Ph), 78.2 (C-5'), 82.74 (C-4'), 84.5 (C-3'), 89.6 (1C = d, $J_{C-1,F}$ = 8.8 Hz C-1), 89.6 (1 C, d, $J_{C-3,F}$ = 189 Hz, C-3), 101.4 (C-2'), 127.5, 127.6, 127.7, 127.7, 127.8, 127.9, 127.9, 128.2, 128.3, 128.4 (4 x OCH_2Ph), 137.8, 138.2, 138.4, 138.6 (4 x 1 C, 4 x OCH_2Ph), 169.3, 169.7, 170.5 (3 x C=O) ;^{19}F{^{1}H} NMR(377 MHz, $CDCl_3$): δ -199.9; IR (thin film): 3063, 3030, 2923, 2864 (C=CH), 1751 (C=O), 1496, 1453, 1367, 1219, 1131, 1089, 1067, 1038, 736; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{47}H_{53}FO_{13}$ NH_4^+,867.3362;found: 867.3338; analysis (calcd., found for $C_{47}H_{53}O_{13}$): C (66.81,66.82), H (6.32,6.37).

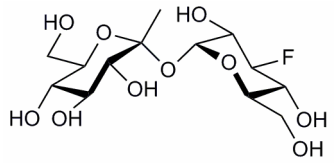
***3,4,5,7-Tetra-O-benzyl-1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-2,4,6-tri-O-acetyl-3-deoxy-3-fluoro-β-D-glucopyranoside* (45)**



45 was isolated as the lower spot TLC (2:1 petrol/ethyl acetate) (R_f 0.48) of the reaction between **29** and **35** as a clear oil (16 mg, 10%). TLC (petrol:EtOAc, 2:1, v/v): R_f=0.48; $[\alpha]_D^{25}$ = 12.4 (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (3 H, s, C-1'), 2.04 - 2.20 (9 H, m, 3 x OCOC<u>H</u>$_3$), 3.34 (1 H, d, $J_{3,4}$ = 9.8 Hz, H-3'), 3.52 (1 H, adt, $J_{4,5}$ = 9.9 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 3.3 Hz, H-5), 3.59 (1 H, dd, $J_{7a,7b}$ = 10.7, $J_{6,7b}$ = 1.9 Hz, H-7b'), 3.69 (1 H, at, $J_{4,5}$ = $J_{4,6}$ = 9.5 Hz, H-5'), 3.70 (1 H, dd, $J_{7a,7b}$ = 10.4 Hz, $J_{6,7a}$ = 3.8 Hz, H-7a'), 4.06 (2 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4'), 4.07 (2 H, dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b), 4.12 (2 H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 4.7 Hz, H-6a), 4.16 (1 H, ddd, $J_{5,6}$ = 10.2 Hz, $J_{6,7a}$ = 3.5 Hz, $J_{6,7b}$ = 2.0 Hz, H-6'), 4.51 (1 H, dt, $J_{3,F}$ = 53.0 Hz, $J_{2,3}$ = 9.1 Hz, $J_{3,4}$ = 9.1 Hz H-3), 4.47 (1 H, d, J = 12.0 Hz, 1 x OCH_2Ph), 4.53 (1 H, d, J = 11.0 Hz, 1 x OCH_2Ph), 4.59 (1 H, d, J = 12.3 Hz, 1 x OCH_2Ph), 4.60 (1 H, d, J = 11.3 Hz, 1 x OCH_2Ph), 4.81 (1 H, d, $J_{1,2}$ = 7.9 Hz, H-1), 4.84 (1 H, d, J = 10.7 Hz, 1 x OCH_2Ph), 4.86 - 4.90 (2 H, m, 2 x OCH_2Ph), 4.94 (1 H, d, J = 11.3 Hz, 1 x OCH_2Ph), 5.14 (2 H, m, H-4, H-2), 7.21 - 7.37 (20 H, m, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 20.4, 20.5, 20.6 (3 x OCO<u>C</u>H$_3$), 22.0 (C-1'), 61.9 (C-6), 68.4 (1 C, d, $J_{C-4,F}$ = 12.6 Hz, C-4), 68.5 (C-7') 70.9 (1 C, d, $J_{C-2,F}$ = 18.9 Hz, C-2), 71.2 (1 C, d, $J_{C-5,F}$ 7.5 Hz C-5), 72.4 (H-6'), 73.4 (O<u>C</u>H$_2$Ph), 74.9 (O<u>C</u>H$_2$Ph), 75.5 (O<u>C</u>H$_2$Ph), 75.6 (O<u>C</u>H$_2$Ph), 78.1 (H-5'), 82.5 (C-4'), 84.5 (C-3'), 91.7 (1 C, d, $J_{C-2,F}$ = 191.5 Hz, C-3), 94.2 (1 C, d, $J_{C-1,F}$ = 12.6 Hz, C-1), 102.4 (C-2'), 127.5, 127.6, 127.6, 127.6, 127.8, 128.3, 128.3, 128.4, 128.4 (4 x OCH_2Ph), 138.2, 138.2, 138.4, 138.6 (4x 1 C. 4 x OCH_2Ph), 168.8, 169.15, 170.6 (3 x C=O); IR (thin film): 3062, 3030, 2923, 2855 (C=CH), 1752 (C=O), 1496, 1453, 1368, 1218, 1151,

1126, 1063, 1043. 737; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{47}H_{53}FO_{13}Na^+$,867.3362;found: 867.3338.

1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-3-deoxy-3–fluoro-α-D-glucopyranoside **(6)**

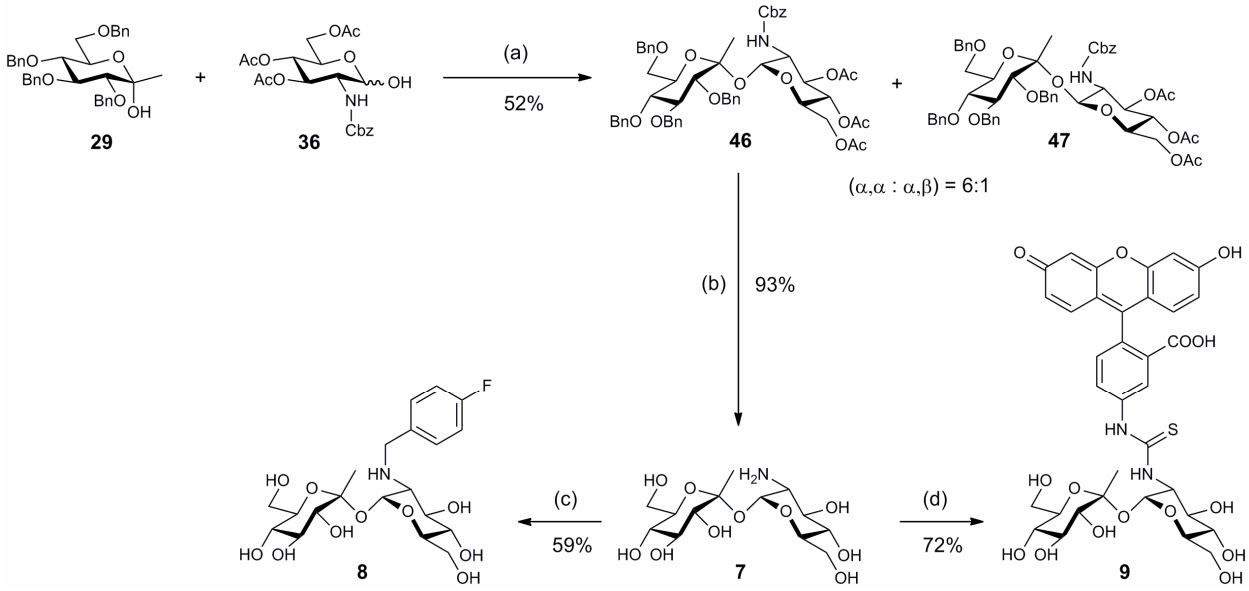


44 (42 mg, 0.049 mmol, 1 eq) was dissolved in anhydrous methanol (10 mL), to this was added sodium methoxide (20 mg, 0.37 mmol, 7.5 eq) and reaction was stirred under argon atmosphere for 1.5 hours until complete conversion to product was detected by TLC (2:1 petrol/ ethyl acetate) (R_f 0.0) with dissappearance of starting sugar (R_f 0.5). Reaction was neutralized with DOWEX 50WX8 (H^+ form) cation exchange resin. Resin was removed by filtration and filtrate was concentrated under reduced pressure and redissolved in ethanol (10 mL). Reaction mixture was circulated through a Thales Nano H Cube® 10% Pd/C cartridge at 70 bar, 25 °C for 1 hour. Near complete deprotection was detected by TLC (1:1 methanol/ethyl acetate) (R_f 0.45). Reaction mixture was partitioned between water and DCM and aqueous layer was lyophilized. Further purification was obtained utilizing Isolute SPE C18 cartridge to yield the desired product (15.6 mg, 87%) as a white, amorphous solid. TLC (MeOH:EtOAc, 1:1, v/v): R_f=0.45; $[\alpha]_D^{25}$ = 108.2 (c = 0.22, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.44 (3 H, s, C-1'), 3.23 (1 H, d, $J_{3,4}$ = 9.8 Hz, C-3'), 3.34 (1 H, at, $J_{4,5}$ = $J_{5,6}$ = 9.6 Hz, C-5'), 3.62 - 3.75 (6 H, m, H-6a, H-6b, H-7a, H-7b, H-4, H-4'), 3.79 (1 H, ddd, $J_{2,F}$ = 16.4 Hz, $J_{2,3}$ = 9.0 Hz, $J_{1,2}$ = 3.6 Hz, H-2), 3.80 (3 H, ddd, $J_{4,5}$ = 11.7 Hz, $J_{5,6a}$ = 6.3 Hz, $J_{5,6b}$ = 3.5 Hz, H-5'), 3.97 (2 H, ddd, $J_{5,6}$ = 10.1 Hz, $J_{6,7a}$ = 5.4 Hz, $J_{6,7b}$ = 2.2 Hz, H-6'), 4.68 (1 H, dt, $J_{3,F}$ = 55.0 Hz, $J_{3,4}$ = 9.1 Hz, $J_{2,3}$ = 9.1 Hz, H-3), 5.26 (2 H, at, J = 3.6 Hz, H-1);^{13}C NMR

(126 MHz, D_2O): δ 22.4 (C-1'), 60.1 (C-6), 60.5 (C-7'), 68.1 (1 C, d, $J_{C4,F}$ = 16.4 Hz, C-4), 69.8 (C-5'), 69.9 (1 C, d, $J_{C2,F}$ = 16.4 Hz, C-2), 71.3 (1 C, d, $J_{C5,F}$ = 7.5 Hz, C-5), 72.3 (C-6') 72.8 (C-4'), 76.3 (C-3'), 91.7 (1 C, d, $J_{C3,F}$ = 11.3 Hz, C-1), 94.5 (1 C, d, $J_{C3,F}$ = 180.1 Hz, C-3), 101.1 (C-2'); ^{19}F NMR (377 MHz, D_2O): δ -199.2 (dtt, $J_{3,F}$ = 55.1, $J_{2,F}$ = $J_{4,F}$ = 14.9 Hz, $J_{1,F}$ = $J_{5,F}$ = 3.4 Hz); HRMS (m/z): $[M-H]^-$ calcd. for $C_{13}H_{22}F\ O_{10}^-$, 357.1202;found: 357.1197.

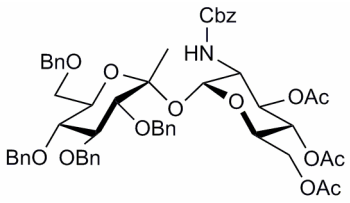
Nature Chemical Biology: doi:10.1038/nchembio.539

Synthesis of compounds 7-9.



Supplementary Scheme 8. *Reagents and conditions. (a) TMSOTf, molecular sieves, anhydrous DCM, –40 °C, 15 mins (b)i, NaOMe, anhydrous MeOH, RT, 1 h; ii, H Cube®, Pd/C cartridge, 70 bar, 10 h (c) i, 4-fluoro-benzaldehyde, MeOH, RT, 10 min; ii, sodium borohydride, MeOH, RT, 10 min (d) Fluorescein-isothiocyanate, 1:1 MeCN/$NaHCO_3$ buffer (pH 9), 50°C, 2 h.*

3,4,5,7-tetra-O-benzyl-1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-3,4,6-tetra-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside **(46)**

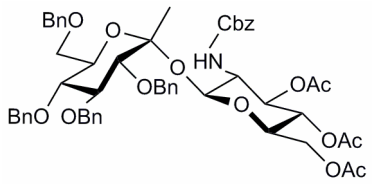


29 (173.8 g, 0.31 mmol, 1 eq) and **36** (168.0 mg, 0.38 mmol, 1.2 eq) were dried under reduced pressure for 1 hour and then dissolved in anhydrous DCM (10 mL) and added to a dry flask in the presence of molecular sieves (ca. 100 mg). Mixture was stirred with molecular sieves for 30 minutes at room temperature and then was cooled to –40 °C. To this was added TMSOTf (10 µl, 0.054 mmol. 0.15 eq) at –40 °C in the under an Ar atmosphere. The resulting mixture was stirred for 15 minutes until TLC (3:2 petrol/ethyl acetate) revealed the production of two new products (R_f 0.6 and R_f 0.15) and consumption of starting material

(R_f 0.35 and R_f 0.05). The reaction was then quenched by the addition triethylamine (0.1 mL), filtered through Celite® and concentrated. The crude product was purified by column chromatography (5:2 petrol/ethyl acetate) to yield the desired compound α,α (214 mg) as well as the α,β (32 mg) for a net yield (81%, 6:1 α,α: α,β) as well as recovered **29** (14.5 mg, 8%). TLC (petrol:EtOAc, 3:2, v/v): R_f=0.60; $[\alpha]_D^{25}$ = 67.3 (c = 0.94, $CHCl_3$).^{1}H NMR (500 MHz, $CDCl_3$): δ 1.48 (3 H, s, C-1'), 1.96 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.01 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.04 (3 H, s, 1 x OCOC$\underline{H}_3$), 3.33 (1 H, d, $J_{3,4}$ = 9.4 Hz, C-3'), 3.43 (1 H, dd, $J_{7a,7b}$ = 10.9 Hz, $J_{6,7b}$ = 1.42 Hz, H-7b'), 3.53 (1 H, dd, $J_{7a,7b}$ = 11.03 Hz, $J_{6,7a}$ = 2.8 Hz, H-7a'), 3.68 - 3.79 (3 H, m, H-5', H-6b, H-6'), 3.97 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, H-4'), 4.04 (2 H, dd, $J_{5,6a}$ = 12.6 Hz, $J_{6a,6b}$ = 4.41 Hz, H-6a), 4.05 (1 H, dd, $J_{2,3}$ = 10.7 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 4.26 (1 H, ddd, $J_{4,5}$ = 10.3 Hz, $J_{5,6a}$ 4.5 Hz, $J_{5,6b}$ = 2.21 Hz, H-5), 4.38 (1 H, d, J = 12.3 Hz, 1 x OCH_2Ph), 4.53 (1 H, d, J = 12.3 Hz, 1 x OCH_2Ph), 4.56 (1 H, d, J = 11.0 Hz, 1 x OCH_2Ph), 4.61 (1 H, d, J = 11.4 Hz, 1 x OCH_2Ph), 4.82 (1 H, d, J = 11.0 Hz, 1 x OCH_2Ph), 4.91 (1 H, d, J = 11.9 Hz, 1 x OCH_2Ph Cbz), 4.93 (1 H, d, J = 10.9 Hz, 1 x OCH_2Ph), 4.95 (1 H, d, J = 12.6 Hz, 1 x OCH_2Ph), 4.97 (1 H, d, J = 11.4 Hz, 1 x OCH_2Ph Cbz), 5.05 (1 H, d, J = 12.0 Hz, 1 x OCH_2Ph), 5.08 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, H-4), 5.30 (1 H, dd, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 5.36 (1 H, d, $J_{1,2}$ = 3.5 Hz, H-1), 6.98 - 7.41 (25 H, m, Ar-H); ^{13}C NMR (126MHz, $CDCl_3$): δ 20.6, 20.7 (3 x $OCOCH_3$), 23.1 (C-1'), 54.6 (C-2), 61.7 (C-6), 67.0 (O$\underline{C}H_2Ph$ Cbz), 67.2 (C-5), 68.1 (C-7'), 68.3 (C-4), 70.9 (C-3), 72.3 (C-5'), 73.4 (O$\underline{C}H_2Ph$), 74.9 (O$\underline{C}H_2Ph$), 75.5 (O$\underline{C}H_2Ph$), 75.7(O$\underline{C}H_2Ph$), 77.9 (C-6'), 82.8 (C-4'), 84.7 (C-3'), 90.7 (C-1), 101.6 (C-2'), 127.6, 127.6, 127.6, 127.8, 127.8, 128.1, 128.3, 128.4, 128.4 (5 x OCH_2Ph), 136.0, 138.1, 138.2, 138.3, 138.5 (5x 1C. 5 x OCH_2Ph), 155.6 (1 x C=O Cbz), 169.4, 170.6, 171.4 (3 x C=O) ; IR (thin film): 3346 (br. Amide), 3089, 2924, 2864 (C=CH), 1745 (C=O), 1720 (C=O), 1564, 1530, 1454, 1365, 1230, 1153, 1028, 737; HRMS (m/z): $[M+NH_4]^+$ calcd.

for $C_{55}H_{65}N_2O_{15}^+$, 993.4379; found: 993.4377; analysis (calcd., found for $C_{55}H_{61}NO_{15}$): C (67.68,67.61), H (6.30,6.21), N(1.44,1.44).

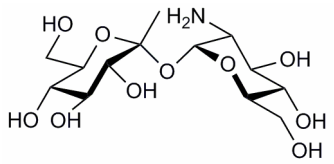
***3,4,5,7-tetra-O-benzyl-1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-3,4,6-tetra-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside* (47)**



47 was isolated as the lower spot (R_f 0.15) of the reaction between **29** and **36** (212 mg, 7%). TLC (petrol:EtOAc, 3:2, v/v): R_f=0.15 $[\alpha]_D^{25}$ = 37.7 (c = 1.0, $CHCl_3$).^{1}H NMR (500 MHz, $CDCl_3$): δ 1.36 (3 H, s, C-1'), 1.94 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.00 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.01 (3 H, s, 1 x OCOC$\underline{H}_3$), 3.35 (1 H, d, $J_{3,4}$ = 9.5 Hz, H-3'), 3.46 (1 H, br. s., H-2), 3.62 (2 H, adt, $J_{4,5}$ = 10.7 Hz , $J_{5,6a}$ = $J_{5,6b}$ = 1.1 Hz, H-5, H-7b'), 3.71 (2 H, at, $J_{4,5}$ = $J_{45,6}$ = 9.8 Hz, H-5'), 3.74 (1 H, dd, $J_{7a,7b}$ = 10.7 Hz, $J_{6,7a}$ = 3.2 Hz, H-7a'), 3.99 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4'), 4.05 (1 H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b), 4.12 (1 H, dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$= 4.7 Hz, H-6a), 4.17 (1 H, ddd, $J_{5,6}$ = 10.7 Hz, $J_{6,7a}$ = 3.3 Hz, $J_{6,7b}$ = 2.0 Hz, H-6'), 4.49 (1 H, d, J = 12.3 Hz, 1 x OCH_2Ph), 4.56 (1 H, d, J = 10.7 Hz, 1 x OCH_2Ph), 4.60 (1 H, d, J = 10.7 Hz, 1 x OCH_2Ph), 4.61 (1 H, d, J = 12.3 Hz, 1 x OCH_2Ph), 4.77 - 4.85 (3 H, m, 1 x OCH_2Ph Cbz, 2 x OCH_2Ph), 4.88 (2 H, d, J 11.0 Hz, 1 x OCH_2Ph), 4.95 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 5.06 - 5.18 (2 H, m, H-1, 1 x OCH_2Ph Cbz), 5.46 (1 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz H-3), 7.14 - 7.37 (35 H, m, Ar-H); ^{13}C NMR (126MHz, $CDCl_3$): δ 20.6, 20.7, 21.0 (3 x $OCOCH_3$), 22.1 (CH_3), 56.5 (C-2), 62.4 (C-6), 66.9 (O$\underline{C}H_2$Ph Cbz), 68.4 (C-4), 68.8 (C-7'), 71.85 (C-3), 72.0 (C-5), 72.3 (C-6'), 73.5 (O$\underline{C}H_2$Ph), 74.9 (O$\underline{C}H_2$Ph), 75.4 (O$\underline{C}H_2$Ph), 75.8(O$\underline{C}H_2$Ph), 78.2 (C-5'), 82.8 (C-4'), 84.7 (C-3'), 94.6 (C-1), 102.3 (C-2'), 127.5, 127.6, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5 (5 x OCH_2Ph), 136.2, 137.9,

138.2, 138.3, 138.7 (5x 1C. 5 x OCH_2Ph), 155.5 (1 x C=O Cbz), 169.6, 170.4, 170.6 (3 x C=O); IR (thin film): 3356 (br. amide), 3031, 2939 (C=CH), 1748 (C=O), 1587, 1454, 1367, 1231, 1043, 739; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{55}H_{61}NO_{15}Na^+$, 998.3933; found: 998.3905.

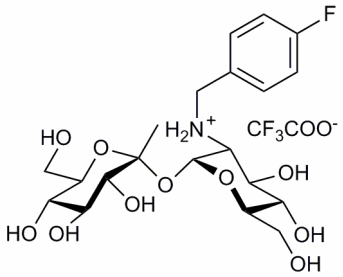
***1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-2–amino-2-deoxy-α-D-glucopyranoside glucopyranoside* (7)**



46 (578 mg, 0.59 mmol, 1 eq) was dissolved in anhydrous methanol (15 mL). To this was added sodium methoxide (25 mg, 0.46 mmol, 0.94 eq) and reaction was stirred for 1 hour, upon which time full conversion to product was detected by TLC (ethyl acetate) (R_f 0.75) and disappearance of starting sugar (R_f 1). Reaction was neutralized with DOWEX 50WX8 (H^+ form) cation exchange resin (ca 50 mg). DOWEX was removed by filtration and reaction was concentrated under reduced pressure to yield the deacetylated product (507 mg, 100%). This product was split into two portions and each portion was dissolved in 20 mL (1:1 trifluoroethanol/water) with formic acid (50 µl) and cycled through the Thales Nano H Cube® over a Pd/C cartridge at 70 bar for 10 hours. Upon completion product was detected by TLC (1:2:2 water/isopropanol/ethyl acetate) (R_f 0.05) with complete disappearance of starting sugar (Rf 1). Reactions were concentrated under reduced pressure, redissolved in water and lyophilized to yield the desired product as a brownish, amorphous solid as the formate salt. Amine was further purified by a 10 ml column of DOWEX 50WX8 (H^+ form) cation exchange resin. Amine was loaded onto resin, washed with water (20 ml), 0.1% NH_4OH (20 ml),eluted with 5 % NH_4OH (20 ml) and evaporated to dryness to yield the

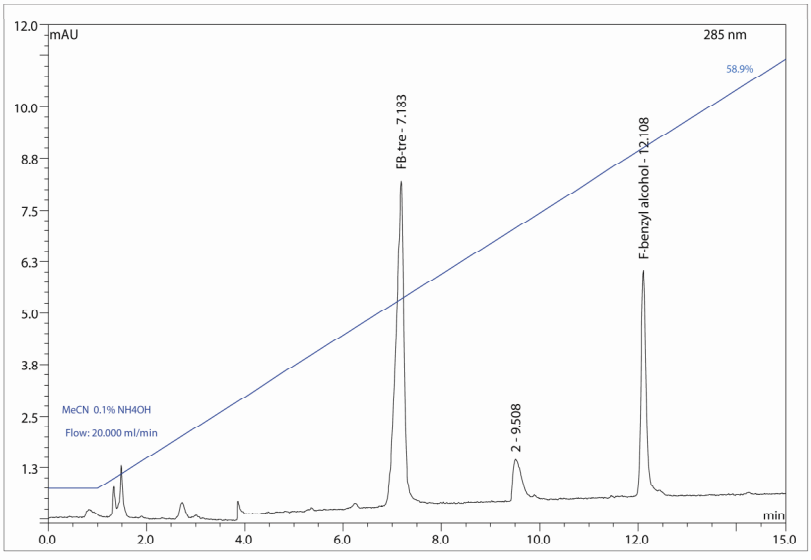
desired product as a white amorphous solid. (221 mg, 93%). TLC (water:isopropanol:ethyl acetate, 1:2:2, v/v): R_f=0.05; $[\alpha]_D^{25}$ = 83.8 (c = 0.21, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.48 (3 H, s, C-1'), 2.74 (1 H, d, *J* = 10.4 Hz, H-2), 3.23 (1 H, d, $J_{3,4}$ = 9.7 Hz, H-3'), 3.35 (2 H, atd, *J* = 9.6, 5.7 Hz, H-5, H-6'), 3.60 - 3.83 (8 H, m, H-7a', H-7b', H-6a, H-6b, H-4', H-5', H-3, H-4), 5.24 (1 H, d, $J_{1,2}$ = 3.2 Hz, H-1); ^{13}C NMR (126 MHz, D_2O): δ 22.9 (C-1'), 55.5 (C-2), 60.5 (C-6), 60.6 (C-7'), 69.7 (C-5 or C-6'), 69.8 (C-5 or C-6'), 72.1 (C-4), 72.6 (C-4' or C-5'), 72.7 (C-4' or C-5'), 73.5 (C-3) 76.4 (C-3'), 91.95 (C-1), 101.3 (C-2'); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{13}H_{25}NO_{10}Na^+$,378.1317;found: 378.1361

***1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-2-(4-fluorobenzylamino)-2-deoxy-α-D-glucopyranoside trifluoroacetic acid salt* (8)**



7 (11 mg, 0.03 mmol, 1 eq) was dissolved in methanol (1 mL). 4-fluoro-benzaldehyde (9.5 µl, 0.06 mmol, 2 eq) was added to the reaction. After 10 minutes, complete conversion to imine was detected by TLC (2:1 ethyl acetate/methanol) product (R_f 0.4). To this was added $NaBH_4$ (15.2 mg, 0.4 mmol, 13.1 eq) and reaction was allowed to stir for a further 10 minutes, upon which time reductive amination was detected by ESI mass spec as well as TLC (1:2:2 water/isopropanol/ethyl acetate) product (R_f 0.3). Upon conversion, reaction was purified by HPLC with a HPLC with a Phenomenex Synergi Hydro C18 column (150 mm x 21.2 mm, 4 µm) and an MeCN/H_2O gradient (5%/min) with 0.1% NH_4OH. Lyophilization yielded the desired compound as a off-white amorphous solid as the TFA salt. (8.4 mg, 59%).

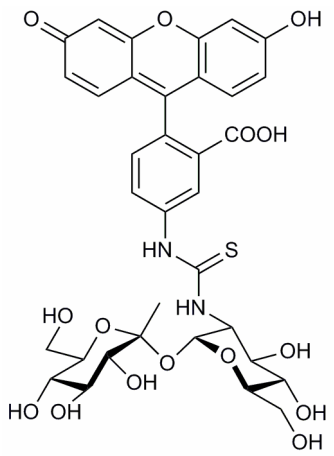
TLC (H_2O:*i*-PrOH:EtOAc, 1:2:2, v/v): R_f=0.30; $[\alpha]_D^{25}$ = 32.4 (c = 0.29, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.47 (3 H, s, C-1'), 2.63 (1 H, dt, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 2.8 Hz, $J_{2,NH}$ = 2.8 Hz, H-2), 3.22 (1 H, d, $J_{3,4}$ = 9.8 Hz, H-3'), 3.29 (1 H, at, $J_{4,5}$ = $J_{5,6}$ = 9.5 Hz, H-5'), 3.36 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.59 (1 H, dd, $J_{7a,7b}$ = 12.3 Hz, $J_{6,7a}$ = 5.0 Hz, H-7a'), 3.65 (1 H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 5.0 Hz, H-6a), 3.67 (1 H, dd, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6b}$ = 2.5 Hz, H-6b), 3.71 - 3.77 (3 H, m, H-7b', H-6', H-4'), 3.79 - 3.86 (3 H, m, H-3, 2 x NC$\underline{H_2}$Ph), 3.86 (1 H, ddd, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 4.4 Hz, $J_{5,6b}$ = 2.2 Hz, H-5), 5.36 (1 H, d, $J_{1,2}$ = 2.8 Hz, H-1), 7.05 (2 H, at, $J_{ortho,meta}$ = $J_{H,F}$ = 9.0 Hz, Ar-H_{ortho}), 7.30 (2 H, dd, $J_{ortho,meta}$= 8.4 Hz, $J_{H,F}$ = 5.8 Hz, Ar-H_{meta}); ^{13}C NMR (126 MHz, D_2O): δ 22.5 (C-1'), 50.0 (N$\underline{C}H_2$Ph-F), 60.3 (C-2), 60.6 (C-6), 60.7 (C-7'), 69.6 (C-4), 70.4 (C-5'), 71.8 (C-4'), 72.4 (C-3), 72.6 (C-6'), 72.7 (C-5), 76.5 (C-3'), 90.5 (C-1), 101.4 (C-2'), 115.1, 115.2, 115.3, 117.3, 117.5, 119.8, 129.7, 129.8 (Ar-C), 162.8, 162.9 (C=O, TFA);^{19}F NMR (1H) (377 MHz, D_2O): δ –75.6 (TFA), -116.3 (4-F-benzyl); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{20}H_{30}FNO_{10}Na^+$,486.1746;found, 486.1770



Supplementary Figure 27. *Preparative HPLC trace for the purification of* **8**. *Conditions: Phenomenex Synergi Hydro C18 column (150 mm x 21.2 mm, 4 µm), eluting with water with 0.1 % NH_4OH (A) and acetonitrile (B) at a flow rate of 20 mL/min; Gradient: 0 min B=%0, 1 min B=0%, 15 min B=60%; Compound* **8** *elutes at 7.2 min (labeled FB-tre) while side product 4-fluorobenzyl alcohol elutes at 12.1 min*

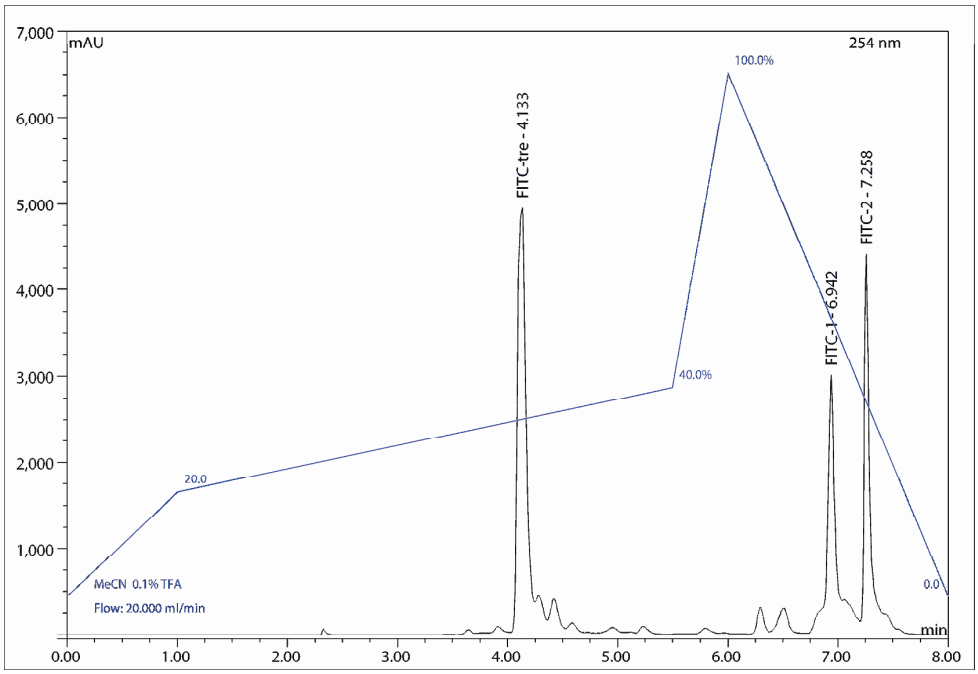
Nature Chemical Biology: doi:10.1038/nchembio.539

***1-deoxy-α-D-gluco-hept-2-ulopyranosyl-(2→1)-2-deoxy-2-(N'-(fluorescein-5-yl)-thioureido)-α-D-glucopyranoside* (9)**



7 (11 mg, 0.03 mmol, 1 eq) and fluorescein isothiocyanate (17 mg, 0.044 mmol, 1.4 eq) were dissolved in 75 mM $NaHCO_3$ buffer at pH 9 (1 mL) with acetonitrile (0.5 mL). Reaction was heated to 50 °C for 2 upon which time product (R_f 0.3) was detected by TLC (1:2:2 water/isopropanol/ethyl acetate) with near complete disappearance of fluorescein starting material (R_f 0.8) and starting amine (R_f 0.05). Reaction mixture was purified by HPLC with a Phenomenex Synergi Hydro C18 column (150 mm x 21.2 mm, 4 μm) and a acetonitrile gradient with 1% aqueous TFA. Lyophilization yielded the desired product as a yellow solid (16 mg, 72 %). TLC (H_2O:*i*-PrOH:EtOAc, 1:2:2, v/v): R_f=0.30; $[\alpha]_D^{25}$ = 72.2 (c = 0.18, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.48 (3 H, s, C-1'), 3.26 (1 H, d, $J_{3,4}$ = 9.8 Hz, H-3'), 3.49 (3 H, m, H4', H-5', H-4), 3.74 (6 H, m, H5, H-6', H-6a, H-6b, H-7a', H-7b'), 4.00 (1 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 4.41 (1 H, dd, $J_{2,3}$ = 10.9 Hz, $J_{1,2}$ = 2.9 Hz, H-2), 5.59 (1 H, d, $J_{1,2}$ = 3.6 Hz, H-1), 6.84 (4 H, t, *J* = 0.9 Hz), 7.12 - 7.18 (3 H, m), 7.68 (1 H, dt, *J* = 7.7, *J* = 0.9 Hz), 8.00 (1 H, d, *J*=1.0 Hz); ^{13}C NMR (126 MHz, D_2O) δ 22.79 (C-1'), 58.8 (C-2), 60.0 (C-7'), 60.56 (C-6), 68.9 (C-4), 70.4 (C-3, C-4'), 71.9 (C-6'), 72.8 (C-5), 72.8 (C-5'), 76.3 (C-3'), 89.6 (C-1), 101.5 (C-2'), 102.5, 113.9, 115.1, 117.1, 117.4, 128.3, 130.2, 131.4, 140.4, 156.2, 162.8, 163.1, 166.0, 170.2, 181.6; HRMS (m/z): $[M-H]^-$ calcd. for

$C_{34}H_{35}N_2O_{15}S^-$,743.1764;found: 743.1754; analysis (calcd., found for $C_{34}H_{36}N_2O_{15}S$): C (54.83,54.77), H (4.87,4.80), N (3.76,3.69), S(4.31, 4.26).

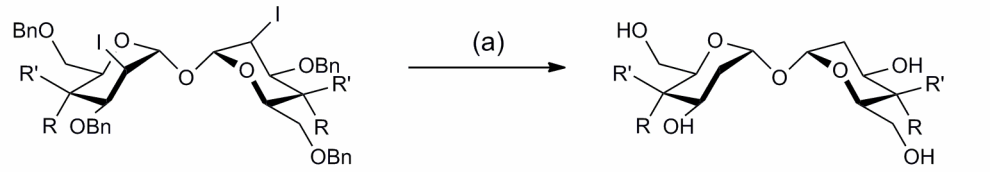


*Supplementary Figure 28***.** Preparative HPLC trace for the purification of **9**. Conditions: Phenomenex Synergi Hydro C18 column (150 mm x 21.2 mm, 4 µm), eluting with water with 0.1 % TFA (A) and acetonitrile (B) eluting at a flow rate of 20mL/min; Gradient: 0 min B=%0, 1 min B=20%, 5.5 min B=40%, 6 min B=100%, 8 min B=0%; Compound **9** elutes at 4.1 min (labeled FITC-Tre) while starting materials FITC elute at 6.9 and 7.3 min.

Nature Chemical Biology: doi:10.1038/nchembio.539

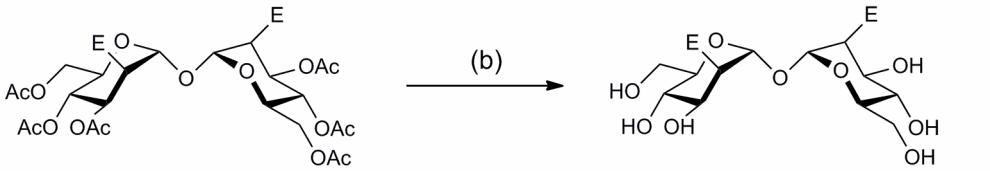
Synthesis of compounds 10-14.

Precursors **48-52** were prepared as described in the literature[17].



R = OBn, R' = H *Manno* **48**
R = H, R = OBn *Talo* **49**

R = OH, R' = H *Arabino* 95% **10**
R = H, R = OH *Lyxo* 90% **11**

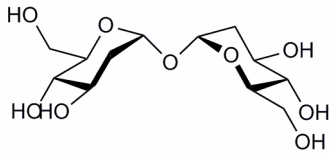


E = I, α,α **50**
E = F, α,α **51**
E = F, α,β **52**

E = I, α,α 98% **12**
E = F, α,α 91% **13**
E = F, α,β 93% **14**

Supplementary Scheme 9. *Reagents and conditions. (a) i, Bu_3SnH, Et_3B, toluene; ii, H_2, 10% Pd/C, 9:1 MeOH/AcOEt (b) 0.1M NaOMe, MeOH, 0 ºC.*

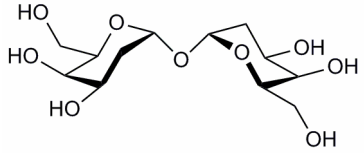
2-deoxy-α-D-arabino-hexopyranosyl-(1→1)-2-deoxy-α-D-arabino-hexopyranoside **(10)**[18]



A solution of **48**[17] (100 mg, 0.091 mmol, 1 eq), Bu_3SnH (151 μL, 0.544 mmol, 6 eq), and 1 M Et_3B in hexane (54 μL, 0.054 mmol, 0.6 eq) in dry toluene (910 μL) was stirred at room temperature for 5 h. The reaction was monitored by TLC (3:1 petrol/ ethyl acetate) and upon completion (R_f 0.22) was filtered through a short path of silica and concentrated under reduced pressure. The crude yellowish syrup was dissolved in 9:1 methanol/ethyl acetate (9.1 mL) at room temperature and subjected to hydrogenolysis (1 atm) using 10% Pd/C (725 mg). After stirring at the same temperature for 10 h, TLC (7:2:1 ethyl acetate/methanol/water) analysis indicated the presence of a highly polar compound (R_f 0.20). The reaction mixture

was filtered through Celite® and concentrated under reduced pressure. The residue was purified by column chromatography (7:2 ethyl acetate/methanol) to afford the titled compound (26.7 mg, 95% over two steps) as a white fluffy solid. TLC (H_2O:MeOH:EtOAc,1:2:7, v/v/v): R_f=0.20; ^{1}H NMR (400 MHz, D_2O): δ 1.64 (2 H, ddd, $J_{2az,2eq}$ = 13.2 Hz, $J_{2ax,3}$ = 12.5 Hz, $J_{1,2ax}$ = 3.3 Hz, H-2ax, H-2ax'), 2.07 (2 H, ddd, $J_{2az,2eq}$ = 13.2 Hz, $J_{2eq,3}$ = 5.1 Hz, $J_{1,2eq}$ = 1 Hz, H-2eq, H-2eq'), 3.28 (2H, dd, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4, H-4'), 3.55 (2 H, ddd, $J_{4,5}$ = 9.5 Hz, $J_{5,6b}$ = 2.2 Hz, $J_{5,6a}$ = 5.5 Hz, H-5, H-5'), 3.66 (2 H, dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6a}$ = 5.5 Hz, H-6a, H-6a'), 3.75 (2 H, dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b, H-6b'), 3.88 (2 H, ddd, $J_{2ax,3}$ = 12.5 Hz, $J_{3,4}$ = 9.5 Hz, $J_{2eq,3}$ = 5.1 Hz, H-3, H-3'), 5.15 (2 H, d, $J_{1,2ax}$ = 3.3 Hz, H-1, H-1'); ^{13}C NMR (101 MHz, D_2O): δ 37.0 (2 C, C-2, C-2'), 61.3 (2 C, C-6, C-6'), 68.8 (2 C. C-3, C-3'), 71.6 (2 C, C-4, C-4'), 73.4 (2C, C-5, C-5'), 93.1 (2 C, C-1, C-1'); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{12}H_{22}O_9Na^+$,333.1156;found, 333.1154. Spectroscopic data are in agreement with those reported[18].

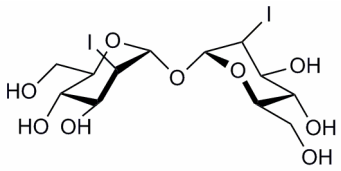
2-deoxy-α-D-lyxo-hexopyranosyl-(1→1)-2-deoxy-α-D-lyxo-hexopyranoside **(11)**



A solution of **49**[17] (200 mg, 0.18 mmol, 1 eq), Bu_3SnH (302 µL, 1.09 mmol, 6 eq), and 1M Et_3B in hexane (109 µL, 0.11 mmol, 0.6 eq) in dry toluene (1.8 mL) was stirred at room temperature for 24 h. The reaction was monitored by TLC (3:1 petrol/ethyl acetate) and upon completion (R_f 0.38) was filtered through a short path of silica and concentrated under reduced pressure. The crude yellowish syrup was dissolved in 9:1 methanol/ethyl acetate (18.1 mL) at room temperature and subjected to hydrogenolysis (1 atm) using 10% Pd/C (1.4 g). After stirring at the same temperature for 22 h, TLC (7:2:1 ethyl acetate/methanol/water)

analysis indicated the presence of a highly polar compound (R_f 0.15). The reaction mixture was filtered through Celite® and concentrated under reduced pressure. The residue was purified by column chromatography (7:2 ethyl acetate/methanol) to afford the titled compound (50.7 mg, 90% over two steps) as a white fluffy solid. TLC (H_2O:MeOH:EtOAc,1:2:7, v/v/v): R_f=0.15; $[\alpha]_D^{21}$ + 151.8 (c = 0.40 in MeOH); ^{1}H NMR (400 MHz, D_2O): δ 1.79 (2 H, ddd, $J_{2az,2eq}$ = 13.2 Hz, $J_{2ax,3}$ = 11.7 Hz, $J_{1,2ax}$ = 4.4 Hz, H-2ax, H-2ax'), 1.87 (2 H, ddd, $J_{2az,2eq}$ = 13.2 Hz, $J_{2eq,3}$ = 4.1 Hz, $J_{1,2eq}$ = 1 Hz, H-2eq, H-2eq'), 3.68–3.59 (2 H, m, H-6a, H-6a', H-6b, H-6b'), 3.76 (2 H, d, $J_{3,4}$ = 2.7 Hz, H-4, H-4'), 3.81 (2 H, dd, $J_{5,6b}$ = 4.8 Hz, $J_{5,6a}$ = 7.3 Hz, H-5, H-5'), 4.02 (2 H, ddd, $J_{2ax,3}$ = 11.7 Hz, $J_{2eq,3}$ = 4.1 Hz, $J_{3,4}$ = 2.7 Hz, H-3, H-3'), 5.19 (2 H, d, $J_{1,2ax}$ = 4.4 Hz, H-1, H-1'); ^{13}C NMR (101 MHz, D_2O): δ 31.3 (2 C, C-2, C-2'), 62.1 (2 C, C-6, C-6'), 65.1 (2 C, C-3, C-3'), 68.0 (2 C, C-4, C-4'), 71.8 (2 C, C-5, C-5'), 92.8 (2 C, C-1, C-1'); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{12}H_{22}O_9Na^+$,333.1156;found, 333.1158.

2-deoxy-2-iodo-α-D-mannopyranosyl-(1→1)-2-deoxy-2-iodo-α-D-mannopyranoside **(12)**

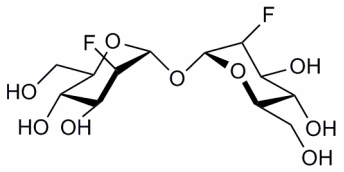


50 (20 mg, 0.025 mmol) was treated with 0.1M NaOMe in methanol (175 µL) at 0 ºC. The reaction mixture was stirred at the same temperature for 15 minutes and neutralized with DOWEX 50WX8 (H^+ form) cation exchange resin. The resin was filtered off and washed with methanol. TLC (7:1 ethyl acetate/methanol) analysis indicated the presence of a polar compound (R_f 0.30). The resulting solution was concentrated under reduced pressure to afford **12** (13.5 mg, 98%) as a white fluffy solid. TLC (MeOH:EtOAc,1:7, v/v): R_f=0.30;

$[\alpha]_D^{21}$ +97.1 (c = 0.7 in MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.10 (2 H, dd, $J_{3,4}$ = 8.5 Hz, $J_{2,3}$ = 4.1 Hz, H-3, H-3'), 3.61 (2 H, dd, $J_{3,4}$ = $J_{4,5}$ = 8.5 Hz, H-4, H-4'), 3.69–3.65 (4 H, m, H-5, H-6b, H-5', H-6b'), 3.84 (2 H, dd, $J_{6a,6b}$ = 14.1 Hz, $J_{5,6a}$ = 5.3 Hz, H-6a, H-6a'), 4.44 (2 H, d, $J_{2,3}$ = 4.1 Hz, H-2, H-2'), 5.48 (1 H, s, H-1); ^{13}C NMR (126 MHz, CD_3OD): δ 37.2 (2 C, C-2, C-2'), 63.0 (2 C, C-6, C-6'), 69.8 (2 C, C-3, C-3'), 70.9 (2 C, C-4, C-4'), 76.7 (2 C, C-5, C-5'), 99.3 (2 C, C-1, C-1'); IR (KBr): 3356, 2924, 1594, 1436 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{12}H_{20}I_2O_9Na^+$,584.9089;found, 584.9085.

2-deoxy-2-fluoro-α-D-mannopyranosyl-(1→1)-2-deoxy-2-fluoro-α-D-mannopyranoside

(13)

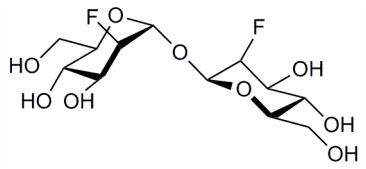


51 (20 mg, 0.033 mmol, 1 eq) was treated with 0.1M NaOMe in methanol (234 µL, 0.02 mmol, 0.6 equi) at 0 ºC. The reaction mixture was stirred at the same temperature for 15 minutes and neutralized with DOWEX 50WX8 (H^+ form) cation exchange resin. The resin was filtered off and washed with methanol. TLC (7:1 ethyl acetate/methanol) analysis indicated the presence of a polar compound (R_f 0.27). The resulting solution was concentrated under reduced pressure to afford **13** (10.6 mg, 91%) as a white fluffy solid. TLC (MeOH:EtOAc,1:7, v/v): R_f=0.27; $[\alpha]_D^{21}$ +109.9 (c = 0.16 in MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.57-3.77 (8 H, m, H-3, H-3', H-4, H-4', H-5, H-5', H-6b, H-6b'), 3.76 (2 H, d, $J_{6a,6b}$ = 11.7 Hz, H-6a, H-6a'), 4.64 (2 H, d, $J_{2,F}$ = 50.1 Hz, H-2, H-2'), 5.34 (2 H, d, $J_{1,F}$ = 7.6 Hz, H-1, H-1'); ^{13}C NMR (126 MHz, CD_3OD): δ 62.8 (2 C, C-6, C-6'), 68.7 (2 C, C-4, C-4'), 71.6 (2 C, d, $J_{3,F}$ = 13.4 Hz, C-3, C-3'), 76.0 (2 C, C-5, C-5'), 91.0 (2 C, d, $J_{2,F}$ = 175.5

Hz, C-2, C-2'), 94.4 (2 C, d, $J_{1,F}$ = 30.5 Hz, C-1, C-1'); ^{19}F NMR (470 MHz, CD_3OD): δ –206.5 (ddd, $J_{2,F}$ = 50.1 Hz, $J_{3,F}$ = 30.5 Hz, $J_{1,F}$ = 7.6 Hz); IR (KBr): 3355, 2926, 2855, 1417, 1066 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{12}H_{20}F_2O_9Na^+$,369.0968;found, 369.0967.

2-deoxy-2-fluoro-α-D-mannopyranosyl-(1→1)-2-deoxy-2-fluoro-β-D-mannopyranoside

(14)



52 (12.7 mg, 0.021 mmol, 1 eq) was treated with 0.1M NaOMe in methanol (149 µL, 0.014 mmol, 0.7 eq) at 0 ºC. The reaction mixture was stirred at the same temperature for 15 minutes and neutralized with DOWEX 50WX8 (H^+ form) cation exchange resin. The resin was filtered off and washed with methanol. TLC (7:1 ethyl acetate/methanol) analysis indicated the presence of a polar compound (R_f 0.11).The resulting solution was concentrated under reduced pressure to afford the titled compound (6.8 mg, 93%) as a white fluffy solid. TLC (MeOH:EtOAc,1:7, v/v): R_f=0.11; $[\alpha]_D^{21}$ +62.7 (c = 0.11 in MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.31 (1 H, overlapped, H-5'), 3.70–3.46 (5 H, m, H-3, H-4, H-6b, H-4',H-6b'), 3.80 (1 H, dd, $J_{3,F}$ = 31.0 Hz, $J_{3,4}$ = 12.3 Hz, $J_{2,3}$ = 2.5 Hz, H-3'), 3.89 (1 H, d, $J_{6a,6b}$ = 12.9 Hz, H-6a), 3.91 (1 H, d, $J_{6a,6b}$ = 11.7 Hz, H-6a'), 3.98 (1H, dd, $J_{4,5} = J_{5,6a}$ = 8.8 Hz, H-5), 4.65 (1 H, dd, $J_{2,F}$ = 49.3 Hz, $J_{2',3'}$ = 2.5 Hz, H-2'), 4.69 (1 H, dd, $J_{2,F}$ = 53.9 Hz, $J_{2,3}$ = 2.2 Hz, H-2), 4.86 (1 H, overlapped, H-1), 5.28 (1 H, d, $J_{1',F'}$ = 7.1 Hz, H-1'); ^{13}C NMR (126 MHz, CD_3OD) δ 63.2 (C-6'), 63.3 (C-6), 68.8 (C-4'), 69.1 (C-4), 71.6 (d, $J_{3,F}$ = 18.1 Hz, C-3'), 71.6 (d, $J_{3,F}$ = 18.1 Hz, C-3), 75.5 (C-5), 79.0 (C-5'), 90.9 (1 C, d, $J_{2,F}$ = 174.5 Hz, C-2'), 91.9 (1 C, d, $J_{2,F}$ = 184.1 Hz, C-2), 99.7 (1 C, d, $J_{1,F}$ = 30.5 Hz, C-1'), 99.9 (1 C, d, $J_{1,F}$ = 15.3 Hz,

C-1); ^{19}F NMR (377 MHz, CD_3OD) δ –206.9 (ddd, $J_{2',F'}$ = 49.3 Hz, $J_{3',F'}$ = 31.0 Hz, $J_{1',F'}$ = 7.1 Hz, F-2'α), –222.9 (ddd, $J_{2,F}$ = 53.9 Hz, $J_{3,F}$ = 29.8 Hz, $J_{1,F}$ = 19.5 Hz, F-2β); IR (KBr): 3355, 2926, 2855, 1417, 1066 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{12}H_{20}F_2O_9Na^+$,369.0968;found, 369.0969.

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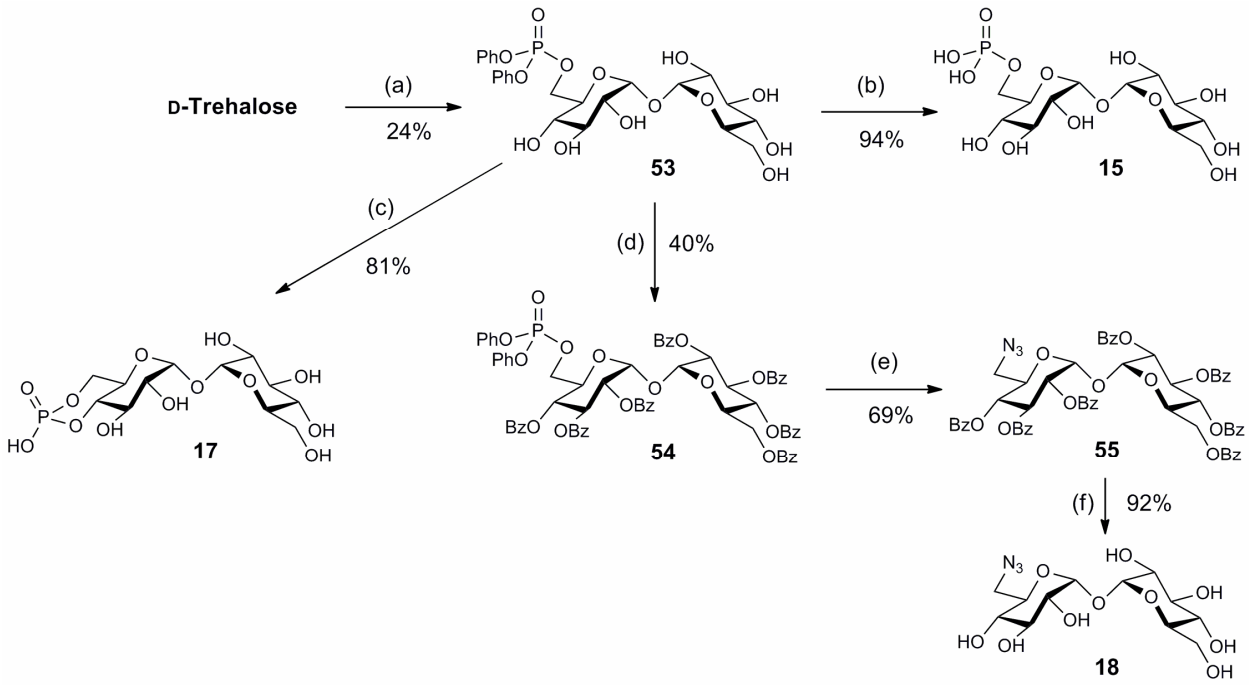
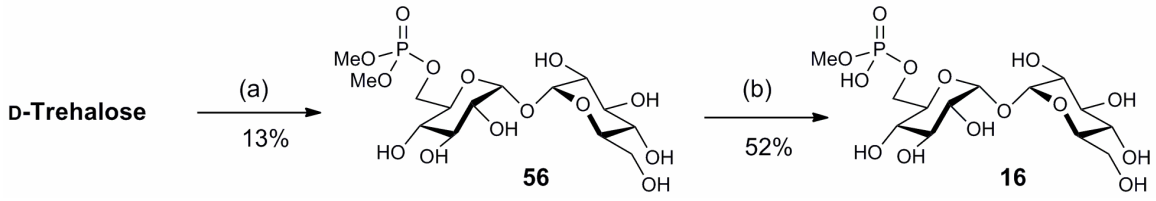
Synthesis of compounds 15-18.



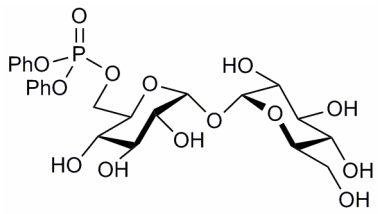
Supplementary Scheme 10. *Reagents and conditions. (a) diphenylchlorophosphate, pyridine, RT, 18 h. (b) H_2, PtO_2, ethanol, acetic acid, RT, 5 h. (c) ammonium hydroxide (28% in H_2O), RT, 18 h (d) Benzoyl chloride, pyridine, RT, 15 h. (e) Sodium azide, DMF, 90 °C, 18 h. (f) Sodium methoxide, methanol, RT, 13h.*



Supplementary Scheme 11. *Reagents and conditions. (a) dimethylchlorophosphate, pyridine, RT, 18 h (b) TMSBr, dioxane, RT, 18 h.*

Nature Chemical Biology: doi:10.1038/nchembio.539

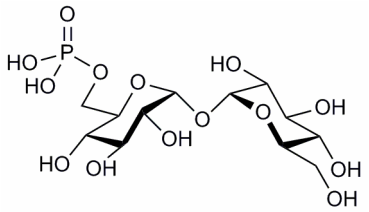
***6-O-diphenoxyphosphoryl-α,α-D-trehalose* (53)[19]**



To a suspension of D-trehalose (7.50 g, 21.9 mmol, 1 eq) in anhydrous pyridine (100 mL) was added dropwise diphenylchlorophosphate (4.54 mL, 21.9 mmol, 1 eq). TLC (1:4:4, water/isopropanol/ethyl acetate) after 18 hours showed the presence of two products (desired product R_f 0.7). The reaction was quenched with methanol (10 mL). The reaction mixture was concentrated *in vacuo*, and the residue co-evaporated with toluene to remove pyridine. Silica gel chromatography (1:3:13 water/isopropanol/ethyl acetate) allowed separation of the two products. Lyophilization yielded the desired compound (3.02 g, 24%) as a white amorphous solid. TLC (H_2O:*i*-PrOH:EtOAc,1:4:4, v/v/v): R_f=0.70; $[\alpha]_D^{22}$ +63.9 (c = 1.0 in MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.34 (1 H, at, $J_{3,4}$ = 9.1 Hz, $J_{4,5}$ = 9.1 Hz, H-4'), 3.38 (1 H, at, $J_{3,4} = J_{4,5}$ = 9.1 Hz, H-4), 3.43 (1 H, dd, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 3.5 Hz, H-2'), 3.48 (1 H, dd, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 3.70 (1 H, dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 5.4 Hz, H-6a'), 3.80 (1 H, at, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3'), 3.81 (1 H, t, $J_{2,3} = J_{3\text{-}4}$ = 9.1 Hz, H-3), 3.80-3.86 (2 H, m, H-6b', H-5'), 4.09 (1 H, dt, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 2.1 Hz, $J_{5,6b}$ = 2.1 Hz, H-5), 4.48 (1 H, ddd, $J_{6a,6b}$ = 11.5 Hz, $J_{P,6a}$ = 7.1 Hz, $J_{5,6a}$ = 3.5Hz, H-6a), 4.55 (1 H, ddd, $J_{6a,6b}$ = 11.5 Hz, $J_{P,6b}$ 6.8 Hz, $J_{5,6b}$ = 1.9 Hz, H-6b), 5.09 (1 H, d, $J_{1',2'}$ = 3.8 Hz, H-1'), 5.10 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1), 7.21-7.31 (3 H, m, Ar-H_{ortho}, Ar-H_{para}), 7.40-7.43 (2 H, m, Ar-H_{meta}); ^{13}C NMR (126 MHz, CD_3OD): δ 62.6 (C-6'), 69.8 (1 C, d, $J_{P,6}$ = 6.7 Hz, C-6), 71.2 (C-4'), 71.9 (C-4), 72.0 (1 C, d, $J_{P,5}$ = 6.7 Hz, C-5), 73.0 (C-5'), 73.2 (C-2'), 73.9 (C-2), 74.4 (C-3'), 74.6 (C-3), 95.2 (C-1'), 95.3 (C-1), 121.4 (2 C, d, $J_{P,C}$ = 4.8 Hz, Ar-C_{ortho}), 126.8 (Ar-C_{para}), 131.1 (Ar-C_{meta}), 151.9 (1 C, d, $J_{P,C}$ = 7.6 Hz, Ar-C_{ipso}), 151.9 (1 C, d, $J_{P,C}$ = 7.6 Hz, Ar-C_{ipso}); ^{31}P{^{1}H}

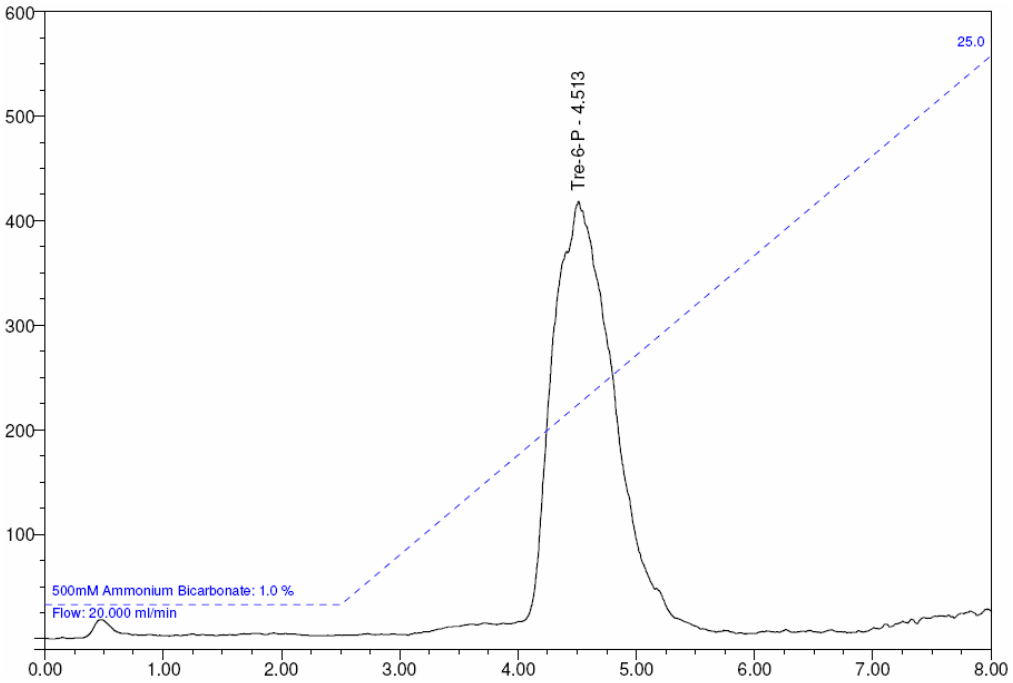
NMR (162 MHz, CD_3OD): δ -11.9; IR (KBr disc): 1287 (P=O), 3271 cm^{-1} (br OH); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{24}H_{31}O_{14}PNa^+$,596.1344;found, 596.1357.

α,α-D-trehalose 6-(dihydrogenphosphate) **(15)**[20]



A suspension of **53** (50 mg, 0.072 mmol, 1 eq) and PtO_2 (5 mg, 0.02 mmol, 0.3 eq) in 75% aqueous ethanol (5 mL) with 0.5% glacial acetic acid (25 μL) was repeatedly degassed under high vacuum and the reaction vessel flushed with hydrogen. The reaction was maintained at RT with aggressive stirring under an atmospheric pressure of hydrogen for 5 hours after which TLC (1:4:4, water/isopropanol/ethyl acetate) showed the complete consumption of the starting material (R_f 0.7) and the formation of a single product (R_f 0). The reaction mixture was filtered through Celite® and the solvent was removed *in vacuo*. The crude solid was taken up in water (30 mL) and washed with ethyl acetate (2 x 20 mL). The aqueous layer was lyophilized and the compound purified using HPLC on an Applied Biosystems, Poros® HQ strongly basic anion exchange column (10 mm x 100 mm, 50 μm). A gradient from 0 mM to 500 mM aqueous NH_4HCO_3 was used as the mobile phase at a flow rate of 20 mL/min and eluants were detected with an Evaporative Light Scattering (ELSD) detector. Fractions containing the product were pooled and repeated lyophilization to removed residual NH_4HCO_3 afforded the title compound as a white amorphous solid (28.6 mg, 94%). $[\alpha]_D^{18}$ + 150.3 (c = 1.0 in H_2O), [lit. $[\alpha]_D^{21}$ + 151.2 (c = 0.8 in H_2O)][20]; ^1H NMR (500 MHz, D_2O): δ 3.36 (1 H, at, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4'), 3.50 (1 H, at, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 3.56 (1 H,

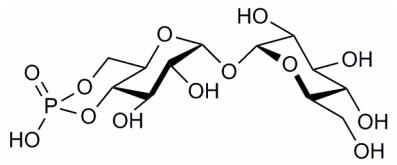
dd, $J_{2,3}$ =10.1 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 3.59 (1 H, dd, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 3.8 Hz, H-2'), 3.67 (1 H, dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 5.4 Hz, H-6a'), 3.71-3.83 (5 H, m, H-3, H-3', H-5', H-6b', H-5), 3.86 (1 H, ddd, $J_{6a,6b}$ = 12.1 Hz, $J_{P,6b}$ = 5.4 Hz, $J_{5,6b}$ = 1.7 Hz, H-6b), 3.94 (1 H, ddd, $J_{6a,6b}$ = 11.9 Hz, $J_{P,6a}$ = 7.7 Hz, $J_{5,6a}$ = 4.1 Hz, H-6a), 5.09 (1 H, d, $J_{1,2}$ = 4.1 Hz, H-1'), 5.12 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1); ^{13}C NMR (126 MHz, D_2O): δ 60.5 (C-6'), 63.0 (d, $J_{P,6}$ = 4.8 Hz, C-6), 69.1 (C-4'), 69.7 (C-4), 70.9 (C-2), 71.1 (C-2'), 71.6 (1 C, d, $J_{P,5}$ = 6.7 Hz, C-5), 72.1 (C-5'), 72.2 (C-3), 72.4 (C-3'), 93.3 (C-1), 93.4 (C-1'); ^{31}P{^{1}H} NMR (202 MHz, D_2O): δ 3.63; MS (m/z): $[M-H]^-$ calcd. for $C_{12}H_{22}O_{14}P^-$,421.1;found, 421.5.



Supplementary Figure 29. *Preparative HPLC trace for the purification of* **15**. *Conditions: Applied Biosystems, Poros® HQ strongly basic anion exchange column (10 mm x 100 mm, 50 µm). A gradient from 0 mM to 500 mM aqueous NH_4HCO_3 was used as the mobile phase at a flow rate of 20 mL/min. Compound* **15** *elutes at 4.5 min (Tre 6-P)*

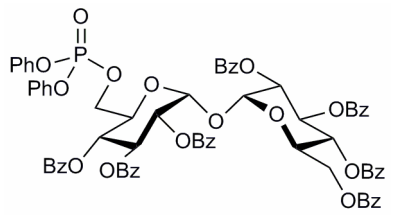
Nature Chemical Biology: doi:10.1038/nchembio.539

***4,6-di-O-(monohydrogen)phosphoryl-α,α-D-trehalose* (17)**



A solution of **53** (30 mg, 0.05 mmol, 1 eq) and ammonium hydroxide (28% in H_2O, 30 μL, 0.2 mmol, 4 eq) in water (4 mL) was stirred at RT for 14 hours after which TLC (1:1:1, water/isopropanol/ethyl acetate) showed the formation of a single product (R_f 0.3). The solvent and ammonium hydroxide were removed *in vacuo*. The crude solid was taken up in water (25 mL) and washed with DCM (3 x 10 mL). The aqueous layer was retained and the water removed under reduced pressure. The compounds were separated using silica gel chromatography (1 water : 2 isopropanol : 2 ethyl acetate). Lyophilization gave the title compound as an amorphous white solid (17 mg, 81 %). TLC (H_2O:*i*-PrOH:EtOAc,1:1:1, v/v/v): R_f=0.30;$[\alpha]_D^{19}$ + 52.6 (c = 1.0 in H_2O); ^{1}H NMR (500 MHz, D_2O): δ3.37 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4'), 3.56 (1 H, dd, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.8 Hz, H-2'), 3.67 (1 H, dd, $J_{2,3}$ = 9.2 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 3.68 (1 H, at, $J_{6a,6b}$ = $J_{5,6b}$ = 6.0 Hz, H-6'b), 3.73-3.80 (3 H, m, H-3', H-5', H-6'a), 3.89 (1 H, ddd, $J_{4,5}$ = 9.5 Hz, $J_{5,6ax}$ = 9.0 Hz, $J_{5,6eq}$ = 0.9 Hz, H-5), 3.92 (1 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.1 Hz, H-3), 4.01 (1 H, td, $J_{5,6ax}$ = 8.4 Hz, $J_{6ax,6eq}$ = 8.4 Hz, $J_{P,6ax}$ = 1.7 Hz, H-6ax), 4.02 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 4.13 (1 H, ddd, $J_{P,6eq}$ = 22.3 Hz, $J_{6ax,6eq}$ = 8.4 Hz, $J_{5,6eq}$ = 3.8 Hz, H-6eq), 5.10 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1'), 5.13 (1 H, d, $J_{1,2}$ = 4.1 Hz, H-1); ^{13}C NMR (126 MHz, D_2O): δ 60.4 (C-6'), 64.0 (d, $J_{P,4}$ = 4.8 Hz, C-4), 66.5 (d, $J_{P,6}$ = 5.7 Hz, C-6), 69.6 (C-4'), 70.4 (d, $J_{P,3}$ = 8.6 Hz, C-3), 70.8 (d, $J_{P,2}$ = 1.9Hz, C-2), 71.0 (C-2'), 72.2 (C-3'), 72.5 (C-5'), 78.2 (d, $J_{P,5}$ = 4.8 Hz, C-5), 93.7 (C-1'), 94.0 (C-1); ^{31}P{^{1}H} NMR (162 MHz, D_2O): δ -2.46; IR (KBr disc): 1139 (P=O), 3407 cm^{-1} (br OH); HRMS (m/z): $[M-H]^-$ calcd. for $C_{12}H_{20}O_{13}P^-$,403.0647;found, 403.0649.

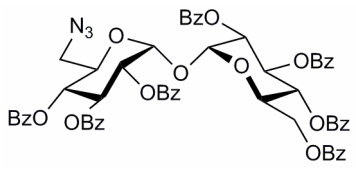
6-O-diphenoxyphosphoryl-2,2',3,3',4,4',6'-hepta-O-benzoyl-α,α-D-trehalose **(54)**



To a solution of **53** (100 mg, 0.18 mmol, 1 eq) in dry pyridine (5 mL) at RT was added dropwise benzoyl chloride (0.22 mL, 1.93 mmol, 11 eq). The reaction mixture was stirred for 15 hours after which TLC (1:1 petrol/ethyl) showed full consumption of starting material (R_f 0) and the formation of a single product (R_f 0.5). The reaction was quenched with methanol (1 mL) and the mixture was concentrated under reduced pressure. The resultant residue was partitioned between ethyl acetate (25 mL) and water (25 mL) and the aqueous layer was extracted with ethyl acetate (2 x 25 mL). The combined organics were washed with 1M HCl (3 x 25 mL), saturated $NaHCO_3$ (25 mL), brine (25 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Silica gel chromatography (1:1 petrol/ethyl) yielded the target compound as a colourless oil (0.091g, 40 %). TLC (petrol:EtOAc, 1:1, v/v): R_f=0.50; $[\alpha]_D^{19}$ +148.2 (c = 0.46 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.78 (1 H, ddd, $J_{6a,6b}$ = 11.4 Hz, $J_{P,6a}$ = 6.9 Hz, $J_{5,6a}$ = 4.0 Hz, H-6a), 3.87 (1 H, m, H-6b), 3.91 (1 H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 4.6 Hz, H-6'a), 4.03 (1 H, dd, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6b}$ = 2.7 Hz, H-6'b), 4.14 (1 H, m, H-5), 4.28 (1 H, ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 4.3 Hz, $J_{5,6b}$ = 2.9Hz, H-5'), 5.31 (1 H, dd, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 5.48 (1 H, dd, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.8 Hz, H-2'), 5.58 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.9 Hz, H-4), 5.62 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1), 5.65 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1'), 5.69 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.9 Hz, H-4'), 6.24 (1 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 6.29 (1 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.9 Hz, H-3'), 7.12-7.60 (31 H, m, Ar-H), 7.80, 7.81, 8.08 (3 x 2 H, 3 x d, $J_{ortho\text{-}meta}$ = 7.2 Hz, Bz Ar-H_{ortho}), 7.94 (4 H, d, $J_{ortho\text{-}meta}$ = 8.2 Hz, Bz Ar-H_{ortho}), 8.00, 8.12 (2 x 2 H, 2 x d, $J_{ortho\text{-}meta}$ = 7.3 Hz, Bz Ar-H_{ortho}); ^{13}C NMR (101 MHz, $CDCl_3$): δ 61.9 (C-6'), 65.6 (d, $J_{P,6}$ = 6.4 Hz, C-6), 67.7 (C-4'), 68.5 (C-4), 68.6 (C-5'), 68.7 (d, $J_{P,5}$ = 6.4 Hz, C-5), 70.3 (2C, C-3, C-3'),

71.0 (C-2'), 71.2 (C-2), 92.4 (C-1'), 92.4 (C-1), 120.1 (d, $J_{P,C}$ = 4.8 Hz, P-OPh Ar-C_{ortho}), 125.4 (d, $J_{P,C}$ = 7.2 Hz, P-OPh Ar-C_{para}), 128.4-129.9 (m, ArC), 133.2, 133.3, 133.3, 133.5, 133.6, 133.7, 133.8 (7 x Bz Ar-C_{para}), 150.3 (d, $J_{P,C}$ = 7.2 Hz, P-OPh Ar-C_{ipso}), 150.4 (d, $J_{P,C}$ = 6.4 Hz, P-OPh Ar-C_{ipso}), 164.6, 164.9, 165.2, 165.2, 165.6, 165.6, 165.9 (7 x C=O); IR (thin film) 1640 (C=O) cm^{-1}; MS (m/z): $[M+Na]^+$ calcd. for $C_{73}H_{59}O_{21}PNa^+$, 1325.32; found: 1325.3; Isotopic distribution: species observed $[M+Na]^+$, peaks observed 1325.27 (100%), 1326.28 (74%), 1327.28 (29%), 1328.29 (8%), 1329.29 (2%) peaks calculated 1325.32 (100%), 1326.32 (80%), 1327.32 (36%), 1328.32 (12%), 1329.33 (3%).

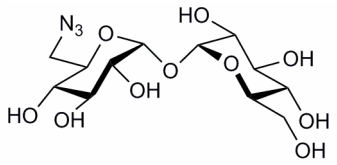
***6-azido-2,2',3,3',4,4',6'-hepta-O-benzoyl-6-deoxy-α,α-D-trehalose* (55)**



A solution of **54** (90 mg, 0.069 mmol, 1 equi) in DMF (3 mL) was heated to 90 °C with sodium azide (9 mg, 0.14 mmol, 2 equi). The reaction mixture was maintained at this temperature with aggressive stirring for 18 hours after which TLC (1:1 petrol/ethyl acetate) showed complete consumption of starting material (R_f 0.5) and the formation of a product (R_f 0.4). The reaction mixture was concentrated *in vacuo* and the residue partitioned between ethyl acetate (30 mL) and water (20 mL). The aqueous layer was extracted with ethyl acetate (2 x 20 mL) and the combined organics washed with brine (3 x 25 mL), dried over $MgSO_4$ and the solvent removed *in vacuo*. The compound was purified by silica gel chromatography (3:2 petrol/ethyl acetate) to afford the desired compound as a colourless oil (52 mg, 69 %). TLC (petrol:EtOAc, 1:1, v/v): R_f=0.40; $[\alpha]_D^{17}$ +210.4 (c = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.89-2.91 (2 H, m, H-6a, H-6b), 3.88 (1 H, dd, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 4.7 Hz, H-

6'a), 4.03 (1 H, dd, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6b}$ = 2.5 Hz, H-6'b), 4.14 (1 H, ddd, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 7.6 Hz, $J_{5,6b}$ = 3.0 Hz, H-5), 4.32 (1 H, ddd, $J_{4,5}$ = 10.4 Hz, $J_{5,6a}$ = 4.3 Hz, $J_{5,6b}$ = 2.8 Hz, H-5'), 5.50 (2 H, dd, $J_{2,3} = J_{2,3}$ = 10.1 Hz, $J_{1,2} = J_{1,2}$ = 3.8 Hz, H-2, H-2'), 5.57 (1 H, at, $J_{3,4} = J_{4,5}$ = 9.9 Hz, H-4), 5.70 (1 H, at, $J_{3,4} = J_{4,5}$ = 10.0 Hz, H-4'), 5.76 (1 H, d, $J_{1,2}$ = 4.0 Hz, H-1), 5.78 (1 H, d, $J_{1,2}$ = 4.0 Hz, H-1'), 6.26 (1 H, at, $J_{2,3} = J_{3,4}$ = 9.9 Hz, H-4), 6.30 (1 H, at, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3'), 7.32-7.60 (21 H, m, Ar-H), 7.83, 7.85 (2 x 2 H, 2 x dd, $J_{ortho\text{-}meta}$ = 8.2 Hz, $J_{ortho\text{-}ortho}$ = 1.3 Hz, Ar-H_{ortho}), 7.93, 7.94 (2 x 2 H, 2 x dd, $J_{ortho\text{-}meta}$ = 8.7 Hz, $J_{ortho\text{-}ortho}$ = 1.5 Hz, Ar-H_{ortho}), 7.96 (2 H, dd, $J_{ortho\text{-}meta}$ = 8.2 Hz, $J_{ortho\text{-}ortho}$ = 1.0 Hz, Ar-H_{ortho}), 8.08, 8.14 (2 x 2 H, 2 x dd, $J_{ortho\text{-}meta}$ = 7.4 Hz, $J_{ortho\text{-}ortho}$ = 1.3 Hz, Ar-H_{ortho}); ^{13}C NMR (101 MHz, $CDCl_3$): δ 50.0 (C-6), 61.9 (C-6'), 68.5 (C-5'), 68.6 (C-4'), 69.1 (C-4), 69.7 (C-5), 70.1 (C-3'), 70.2 (C-3), 71.1 (C-2'), 71.3 (C-2), 92.6 (C-1'), 92.7 (C-1), 128.4, 128.4, 128.7, 128.7 (4 x as, Ar-C_{meta}), 128.5, 128.7, 128.7, 128.8 129.0, 129.0, 129.4 (Ar-C_{ipso}), 129.8, 129.9, 129.9, 129.9 (4 x as, Ar-C_{ortho}), 133.1, 133.3, 133.3, 133.5, 133.6, 133.7, 133.8 (Ar-C_{para}), 164.9, 164.9, 165.2, 165.3, 165.5, 165.6, 165.8 (C=O); IR (thin film) 1732 (C=O), 2107 (N_3) cm^{-1}; MS (m/z): $[M+Na]^+$ calcd. for $C_{61}H_{49}N_3O_{17}Na^+$, 1118.30; found,1118.4; Isotopic distribution: peaks observed: ($M+Na^+$) peaks 1118.30 (100%), 11190.31 (66%), 1120.31 (24%), 1121.31 (7%), 1122.32 (2%) peaks calculated 1118.30 (100%), 1119.30 (68%), 1120.30 (26%), 1121.30 (7%), 1122.31 (2%).

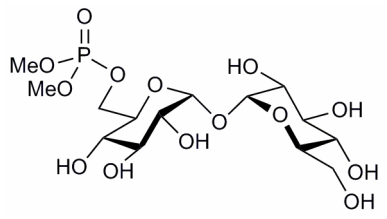
***6-azido-6-deoxy-α,α-D-trehalose* (18)[21]**



To a stirring solution of **55** (52 mg, 0.047 mmol, 1 equi) in methanol (3 mL) was added sodium methoxide (2.5 mg, 0.047 mmol, 1 equi). The reaction mixture was stirred for 13

hours at which point TLC (1:4:4 water/isopropanol/ethyl acetate) indicated complete consumption of the starting material (R_f 0) and the formation of a single product (R_f 0.3). The reaction was quenched with Dowex 50WX8 100-200 mesh (H^+ form), filtered and the solvent removed *in vacuo*. The compound was purified by silica gel chromatography (1:4:4 water/isopropanol/ethyl acetate). Lyophilization gave the title compound as a white amorphous solid (16 mg, 92 %). TLC (H_2O:*i*-PrOH:EtOAc,1:4:4, v/v/v): R_f=0.30; $[\alpha]_D^{25}$ + 167.3 (c = 0.26 in H_2O) [Lit. $[\alpha]_D$ + 149 (c = 0.81 in MeOH)][21] ;^{1}H NMR (500 MHz, D_2O): δ 3.35 (1 H, at, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4'), 3.37 (1 H, at, $J_{3,4} = J_{4,5}$ = 9.5, H-4), 3.47 (1 H, dd, $J_{6a,6b}$ = 13.6 Hz, $J_{5,6a}$ = 5.7 Hz, H-6a'), 3.56 (1 H, dd, $J_{2,3}$ = 10.4 Hz , $J_{1,2}$ = 3.8 Hz, H-2'), 3.58 (1 H, dd, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 3.59 (1 H, dd, $J_{6a,6b}$ = 13.7 Hz, $J_{5,6b}$ = 2.5 Hz, H-6b'), 3.67 (1 H, dd, $J_{6a,6b}$ = 11.7 Hz, $J_{5,6a}$ = 5.0 Hz, H-6a), 3.72 - 3.79 (4 H, m, H-5, H-3,H-3', H-6b), 3.88(1 H, ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 3.8 Hz, $J_{5,6b}$ = 2.2 Hz, H-5'), 5.10 (1 H, d, $J_{1,2}$ = 4.2 Hz, H-1), 5.11 (1 H, d, $J_{1,2}$ = 4.7 Hz, H-1'); ^{13}C NMR (126MHz, D_2O): δ 50.8 (C-6'-azide), 60.4 (C-6), 69.6 (C-4'), 70.4 (C-4), 70.9 (C-2'), 70.9 (C-2), 70.9 (C-5), 72.2 (C-5'), 72.3 (C-3'), 72.5 (C-3), 93.4 (C-1'), 93.6 (C-1); IR (KBr disc) ν = 2110 (N_3), 3456 cm^{-1}(br OH); MS (m/z): $[M+Na]^+$ calcd. for $C_{12}H_{21}N_3O_{10}Na^+$, 390.11; found,390.1.

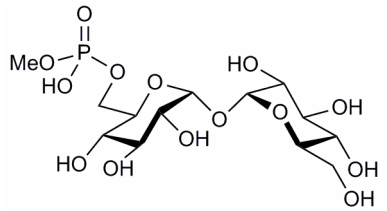
6-O-dimethoxyphosphoryl-α,α-D-trehalose **(56)**



To a suspension of D-trehalose (0.25 g, 0.73 mmol, 1 eq) in anhydrous pyridine (10 mL) was added dropwise dimethylchlorophosphate (79 μL, 0.73 mmol, 1 eq). TLC (1 water : 2 isopropanol : 4 ethyl acetate) after 18 hours showed the presence of several products (desired

product R_f 0.12). The reaction was quenched with methanol (5 mL). The reaction mixture was concentrated *in vacuo*, and the residue co-evaporated with toluene to remove pyridine. Silica gel chromatography (1:2:4, water/isopropanol/ethyl acetate) allowed isolation of the desired product as a white amorphous solid (43 mg, 13%). TLC (H_2O:*i*-PrOH:EtOAc,1:2:4, v/v/v): R_f=0.12; $[\alpha]_D^{18}$ + 83.3 (c = 1.0 in H_2O); ^{1}H NMR (500 MHz, D_2O): δ3.36 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4'), 3.43 (1 H, dd, $J_{3,4}$ = 10.1 Hz, $J_{4,5}$ = 9.1 Hz, H-4), 3.56 (1 H, dd, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 3.8 Hz, H-2'), 3.57 (1 H, t, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 3.67 (1 H, dd, $J_{6a,6b}$ = 11.5 Hz, $J_{5,6a}$ = 5.0 Hz, H-6a'), 3.72 (1 H, m, H-3'), 3.75 (3 H, d, $J_{P,H}$ = 11.0 Hz, OMe), 3.75-3.81 (3 H, m, H-3, H-5', H-6b'), 3.76 (3 H, d, $J_{P,H}$ = 11.0 Hz, OMe), 3.91 (1 H, dt, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 2.6 Hz, H-5), 4.25-4.29 (2 H, m, H-6a, H-6b), 5.10 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1'), 5.13 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1); ^{13}C NMR (126 MHz, D_2O): δ55.1 (d, $J_{P,C}$ = 8.8 Hz, 2 x OMe), 60.5 (C6'), 66.7 (d, $J_{P,6}$ = 5.7 Hz, C-6), 69.0 (C-4), 69.6 (C-4'), 70.5 (d, $J_{P,5}$ 6.7 Hz, C-5), 70.9 (C-2), 71.0 (C-2'), 72.2 (C-5'), 72.4 (C-3), 72.5 (C-3'), 93.4 (C-1), 93.5 (C-1'); IR (KBr disc): 1260 (P=O), 3486 cm^{-1} (br OH); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{14}H_{27}O_{14}PNa^+$,473.1031;found, 473.1027.

6-O-monomethoxyphosphoryl-α,α-D-trehalose **(16)**

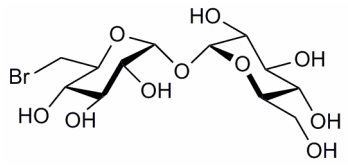


A suspension of **56** (11 mg, 25 μmol, 1 eq) in dioxane was briefly sonicated for 5 minutes. TMSBr (33 μL, 0.25 mmol, 10 eq) was added to this mixture at RT. The reaction was monitored by mass spectrometry (ESI^-) and TLC (1 water : 2 isopropanol : 2 ethyl acetate) and after 3 hours, two deprotected analogues were detected (desired product R_f 0.1) . The

reaction was quenched by the addition of water (1 mL) and the solvents removed *in vacuo*. The crude mixture was taken up in water (3 mL) and washed with ethyl acetate (3 x 1 mL). The aqueous layer was concentrated *in vacuo* and the products were separated by HPLC through an Applied Biosystems, Poros® HQ strongly basic anion exchange column (10 mm x 100 mm, 50 µm). A gradient from 0 mM to 500 mM aqueous NH_4HCO_3 was used as the mobile phase at a flow rate 20 mL/min and eluants were detected with an Evaporative Light Scattering (ELSD) detector. The title compound was not retained on the column and was immediately eluted whereas **15** was eluted at approximately 4.5 minutes (see previous chromatogram). Repeated lyophilization gave **16** (5.7 mg, 52%) and **15** (1.2 mg, 11%) as white amorphous solids. TLC (H_2O:*i*-PrOH:EtOAc,1:2:2, v/v/v): R_f=0.10; $[\alpha]_D^{25}$ + 38.8 (c = 0.24 in H_2O); ^{1}H NMR (500 MHz, D_2O): δ3.36 (1 H, t, $J_{3',4'}$ = 9.3 Hz, $J_{4',5'}$ = 9.3 Hz, H-4'), 3.45 (1 H, t, $J_{3,4}$ = 9.5 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.52 (3 H, d, $J_{P,H}$ = 11.0 Hz, OMe), 3.57 (1 H, dd, $J_{2',3'}$ = 8.3 Hz, $J_{1',2'}$ = 3.3 Hz, H-2'), 3.59 (1 H, dd, $J_{2,3}$ = 9.3 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 3.68 (1H, dd, $J_{6'a,6'b}$ = 11.2 Hz, $J_{5,6'b}$ = 3.9 Hz, H-6'b), 3.72 (1 H, m, H-5'), 3.76 (2 H, t, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.5 Hz, H-3, H-3'), 3.76 (1 H, m, H-6'a), 3.96 (1 H, m, H-5), 4.01 (2 H, m, H-6a, H-6b), 5.11 (1 H, d, $J_{1',2'}$ = 4.1 Hz, H-1'), 5.12 (1 H, d, $J_{1,2}$ = 4.4 Hz, H-1); ^{13}C NMR (126 MHz, D_2O): δ52.9 (d, $J_{P,C}$ = 5.7 Hz, OMe), 60.5 (C-6'), 64.1 (d, $J_{P,6}$ = 1.9 Hz, C-6), 69.2 (C-4), 69.7 (C-4'), 70.9 (C-2), 71.0 (C-2'), 71.7 (C-5), 72.1 (C-5'), 72.4 (C-3), 72.4 (C-3'), 93.3 (C-1), 93.4 (C-1'); IR (KBr disc) 1137 (P=O), 3440 cm^{-1} (br OH); HRMS (m/z): $[M-H]^-$ calcd. for $C_{13}H_{24}O_{14}P^-$,435.0909;found, 435.0923.

Synthesis of compound 19

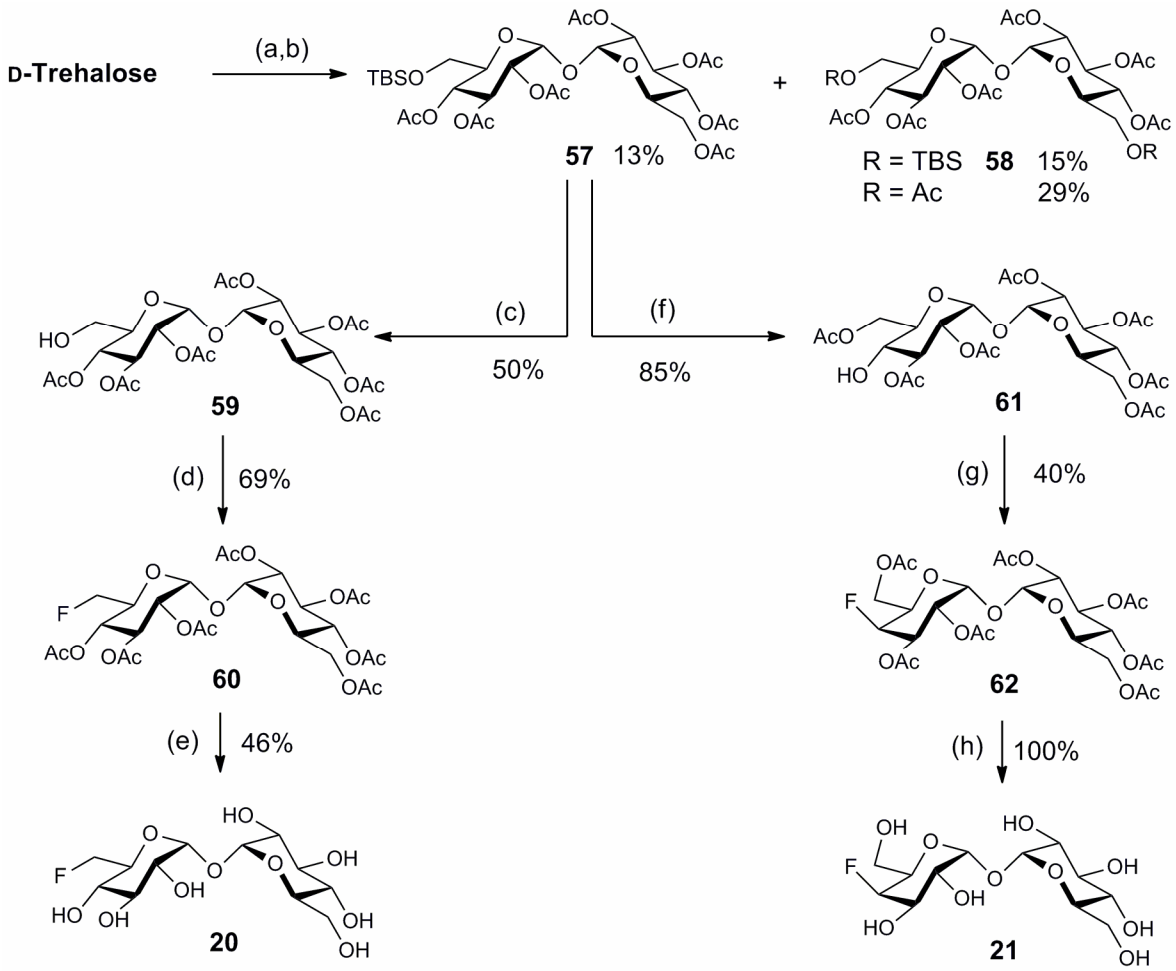
6-bromo-6-deoxy-α,α-D-trehalose **(19)** [22,23]



The compound was synthesized according to the literature[23]. In a 50 mL flask, anhydrous d-trehalose (1.52 g, 4.43 mmol, 1 eq) was dissolved in dry DMF (15mL). Triphenylphosphine (2.32 g, 8.87 mmol, 2 eq) was added followed by NBS (1.57 g, 8.87 mmol, 2 eq). The mixture was stirred overnight at room temperature and 24 h at +60 °C and evaporated to give 5.00g of a yellow oil containing a mixture of expected compound along with unreacted trehalose and corresponding dibromo. This oil was purified by column chromatography using (EtOAc: MeOH 100:0, 90:10 and 80:20) to give dibromo trehalose (408.3 mg, 20%) and compound **19** as a brownish, amorphous solid (594.0 mg, 33%). $[\alpha]_D^{21}$ + 134 (c = 1 in (H_2O) [Lit. $[\alpha]_D^{21}$ + 180.8 (C = 0.7 in CH_3OH)][22]; ^{1}H NMR (400 MHz D_2O): δ 3.33 (1 H, app t, $J_{4',5'} = J_{4',3'}$ = 9.8 Hz, H-4'), 3.38 (1 H, app t, $J_{4,5} = J_{4,3}$ = 9.8 Hz, H-4), 3.53 (1 H, dd, $J_{2,3}$ = 9.8 Hz, $J_{2,1}$ = 3.8 Hz, H-2), 3.56 (1 H, dd, $J_{2',3'}$ = 9.8 Hz, $J_{2',1'}$ = 3.8 Hz, H-2'), 3.56 (1 H, dd, $J_{6b,6a}$ = 11.4 Hz, $J_{6b,5}$ = 5.4 Hz, H-6b CH_2Br), 3.64 (1 H, dd, $J_{6b',6a'}$ = 11.6 Hz, $J_{6b',5'}$ = 5.0 Hz, H-6b' CH_2OH), 3.68 (1 H, dd, $J_{6a,6b}$ = 11.4 Hz, $J_{6a,5}$ = 2.8 Hz, H-6a CH_2Br), 3.70 (1H, app t , $J_{3',2'} = J_{3',4'}$ = 9.8 Hz, H-3'), 3.73 (1 H, dd, $J_{6a',6b'}$ = 11.4 Hz, $J_{6a',5'}$ = 2.6 Hz, H-6a' CH_2OH), 3.73 (1H, m, H-5'), 3.75 (1H, app t , $J_{3,2} = J_{3,4}$ = 9.8 Hz, H-3), 3.87 (1 H, ddd, $J_{5,4}$ = 9.8 Hz, $J_{5,6b}$ = 5.4 Hz, $J_{5,6a}$ = 2.8 Hz, H-5), 5.08 (1 H, d, $J_{1',2'}$ = 3.8 Hz, H-1'), 5.10 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1); ^{13}C NMR (126 MHz D_2O): δ 34.1 (C-6 CH_2Br), 60.8 (C-6' CH_2OH), 69.9 (C-4'), 71.0 (C-4), 71.2, 71.2 (C-2 and C-2'), 71.9 (C-5), 72.4 (C-3), 72.5 (C-5'), 72.8 (C-3'), 93.6 (C-1), 93.8 (C-1'); IR (KBr): 3421, 2925, 1653, 1636, 1419, 1261, 1149, 1103, 992, 939 cm^{-1};

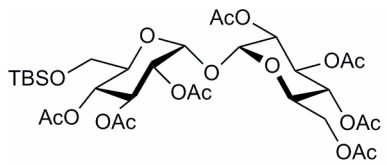
HRMS (m/z): $[M-H]^-$ calcd. for $C_{12}H_{20}O_{10}{}^{79}Br^-$, 403.0245; found, 403.0241; calcd. for $C_{12}H_{20}O_{10}{}^{81}Br^-$, 405.0227; found, 405.0219.

Synthesis of compounds 20 and 21



Supplementary Scheme 12. *Reagents and conditions (a) TBDMSCl, imidazole, RT, 20 min (b) Pyridine, acetic anhydride, 0 °C to RT, 16 h (c) HCl in MeOH RT, 140 min. (d) DAST, DMAP, CH_2Cl_2, RT, 18 h, 69%. (e) NaOMe, MeOH, RT, 18 h, 46% (f) TBAF, CH_3COOH, THF, RT, 48 h (g) DAST, DMAP CH_2Cl_2, RT, 64 h, 40% (h) NaOMe, MeOH, RT, 18 h, 99%.*

6-O-tert-butyldimethylsilyl-2,3,4-tri-O-acetyl-α-D-glucopyranosyl-(1→1)-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside **(57)**



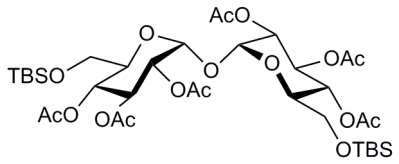
Anhydrous trehalose (3.42 g, 10.0 mmol, 1 eq) was added to 150mL of dry DMF at 50 °C.

The solution was cooled down to room temperature and imidazole (1.36g, 20.0 mmol, 2 eq)

and TBDMSCl (1.66 g, 11 mmol, 1.1 eq) were added. After 20 minutes, DMF was evaporated under high vacuum to give 8.53 g of yellow oil. This oil was dissolved in pyridine (100 mL) and DMAP (122 mg, 1.0 mmol, 0.1 eq) was added then the mixture was cooled to 0 °C and acetic anhydride (9.27 mL) was added slowly. The solution was stirred overnight at room temperature, upon which time TLC (4:1 chlorofom/ethyl acetate) revealed the appearance of three new spots (R_f 0.6), (R_f 0.5) and (R_f 0.4) then 100 mL of water was added and the solution extracted with ethyl acetate (3 x 100 mL). The organic layer was dried with $MgSO_4$, filtered and evaporated to give 7.46g of a yellow oil. This oil was purified by silica column chromatography (pure chloroform then 9:1 chloroform/ethyl acetate then 5:5 chloroform/ethyl acetate) to give 1.05g of pure **58** and 5.38g of a mixture of silyl trehalose acetates. This mixture was purified by a second by silica column chromatography (95:5 chloroform/ethyl acetate then 9:1 chloroform/ethyl acetate then 5:5 chlorofom/ethyl acetate) to give **58** (280 mg, 15% combined yield) followed by **57** (2.63g, 35% yield) and octa-acetyl-trehalose (2.0g , 29% yield). Data for compound **57**; TLC ($CHCl_3$:EtOAc, 4:1, v/v): R_f=0.50; $[\alpha]_D^{25}$ + 158 (c = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz $CDCl_3$): δ 0.01 (3 H, s, 1 x CH_3), 0.03 (3 H, s, 1 x CH_3), 0.86 (9 H, s, 1 x $C(CH_3)_3$), 2.03 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.03 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.04 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.05 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.08(3 H, s, 1 x OCOC$\underline{H}_3$), 2.09 (3 H, s, 1 x OCOC$\underline{H}_3$), 2.09 (3 H, s, 1 x OCOC$\underline{H}_3$), 3.63 (2 H, m, H-6a and H-6b CH_2OTBS), 3.93 (1H, ddd, $J_{5,4}$ = 10.0 Hz, $J_{5,6a}$ 4.4 Hz, $J_{5,6b}$ = 3.2 Hz, H-5), 4.00 (1 H, dd, $J_{6b',6a'}$ = 12.0 Hz, $J_{6b',5'}$ = 2.0 Hz, H-6b' OCOCH_3), 4.07 (1 H, ddd, $J_{5',4'}$ = 10.0 Hz, $J_{5',6a'}$ 5.6 Hz, $J_{5',6b'}$ = 2.0 Hz, H-5'), 4.24 (1 H, dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{6a',5'}$ = 5.6 Hz, H-6a' CH_2OAc), 4.99 (1 H, dd, $J_{2,3}$ = 10.0 Hz, $J_{2,1}$ = 3.6 Hz, H-2), 5.05 (1 H, dd, $J_{4',5'}$ = 10.0 Hz, $J_{4',3'}$ = 9.6 Hz, H-4'), 5.05 (1 H, dd, $J_{4,5}$ = 10.0 Hz, $J_{4,3}$ = 9.6 Hz, H-4), 5.09 (1 H, dd, $J_{2',3'}$ = 10.0 Hz, $J_{2',1'}$ = 4.0 Hz, H-2'), 5.25 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1), 5.28 (1H, d, $J_{1',2'}$ = 4.0 Hz, H-1'), 5.46 (2 H, dd, $J_{3,2}$ = 10.0 Hz, $J_{3,4}$ = 9.6 Hz, H-3 and H-3'); ^{13}C NMR (101 MHz $CDCl_3$):

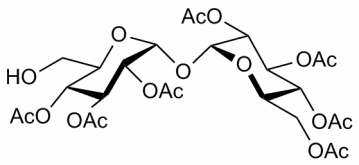
δ -5.5, -5.4 (CH_3), 18.3 (3 C, 1 x C($\underline{C}H_3)_3$), 20.6, 20.6, 20.6, 20.6, 20.6, 20.7, 20.8 (7 C, 7x OCO$\underline{C}H_3$), 25.8 (1 x $\underline{C}(CH_3)_3$), 61.8 (C-6', $OCOCH_3$), 61.9 (C-6 CH_2OTBS), 68.1 (C-4'), 68.5 (C-2'), 68.8 (C-4), 69.5 (C-2), 70.1 (C-5'), 70.2 (C-3'), 70.3 (C-5), 70.6 (C-3), 92.2, 92.3 (2x C-1), 169.5, 169.5, 169.5, 169.6, 169.7, 170.1, 170.6 (7x C=O); IR (thin film): 2956, 2855, 1755 (C=O), 1369, 1222, 1143, 1037, 838, 781 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{32}H_{50}O_{18}SiNa^+$,773.2659;found, 773.2651.

6-O-tert-butyldimethylsilyl-2,3,4-tri-O-acetyl-α-D-glucopyranosyl-(1→1)-6-O-tert-butyldimethylsilyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside **(58)**



58 was isolated as the upper spot (R_f 0.6) TLC (4:1 chloroform/ethyl acetate) of the reaction of *D*-Trehalose with TBDMS-Cl (*vide supra*) (280 mg, 15%). TLC ($CHCl_3$:EtOAc, 4:1, v/v): R_f=0.60; $[\alpha]_D^{25}$ + 157 (c = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz $CDCl_3$): δ 0.02 (6 H, s, 2 x CH_3), 0.02 (6 H, s, 2 x CH_3), 0.87 (18 H, s, 2 x C($C\underline{H}_3)_3$), 2.02 (6 H, 2 x OCOC$\underline{H}_3$), 2.04 (6 H, 2 x OCOC$\underline{H}_3$), 2.08 (6 H, 2 x OCOC$\underline{H}_3$), 3.63 (4 H, m, H-6a and H-6b CH_2OTBS), 3.93 (2 H, ddd, $J_{5,4}$ = 10.0 Hz, $J_{5,6a}$ 4.4 Hz, $J_{5,6b}$ = 3.2 Hz, H-5), 5.03 (2 H, dd, $J_{2,3}$ = 10.4 Hz, $J_{2,1}$ = 4.0 Hz, H-2), 5.07 (2 H, dd, $J_{4,5}$ = 10.0 Hz, $J_{4,3}$ = 9.6 Hz, H-4), 5.25 (2 H, d, $J_{1,2}$ = 4.0 Hz, H-1), 5.47 (2 H, dd, $J_{3,2}$ = 10.4 Hz, $J_{3,4}$ = 9.6 Hz, H-3); ^{13}C NMR (101 MHz $CDCl_3$): δ -5.5 (2 C, 2 x CH_3), -5.5 (2 C, 2 x CH_3), 18.3(2 C, 2 x C($\underline{C}H_3)_3$), 20.7, 20.7, 20.8 (6 C, 6 x $OCOCH_3$), 25.9 (2 C, 2 x C($\underline{C}H_3)_3$), 61.9 (2 C, C-6, C-6'), 68.8 (2 C, C-4 C-4'), 69.8 (2 C, C-2, C-2'), 70.5 (2 C, C-5,C-5'), 70.6 (2 C, C-3, C-3'), 92.2 (2 C, C-1, C-1'), 169.5, 169.6, 170.2 (6 x C=O); IR (thin film): 2955, 2858, 1756 (C=O), 1369, 1221, 1145, 1036, 837, 780 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{36}H_{62}O_{17}Si_2Na^+$,845.3418;found, 845.3401.

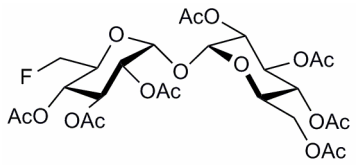
***2,3,4-tri-O-acetyl-α-D-glucopyranosyl-(1→1)-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside* (59)**



In a 200 mL round-bottomed flask was placed **57** (1.54 g, 2.06 mmol, 1 eq), dry methanol (62 mL) and dry dichloromethane (20 mL), and the solution was cooled to 0 °C. Acetyl chloride (293 μL, 4.12 mmol, 2 eq) was added and conversion was monitored by TLC every 10 minutes for 140 minutes. Upon completion, TLC (1:1 ethyl acetate/chloroform) showed disappearance of starting material (R_f 0.7) and appearance of a single product (R_f 0.5). Satd. $NaHCO_3$ solution (2 mL) was added, and the mixture was evaporated to dryness to give 1.33 g of oil. This oil was purified by silica column chromatography (99:1 chloroform/ethanol to 98:2 to 97:3 to 95:5) to give **59** as a white solid (654 mg, 50%). TLC ($CHCl_3$:EtOAc, 1:1, v/v): R_f=0.50; $[\alpha]_D^{25}$ + 164 (c, 1.0 in $CHCl_3$); [Lit. $[\alpha]_D^{21}$ + 167.5 (c = 0.7 in $CHCl_3$)][24]; ^{1}H NMR (400 MHz $CDCl_3$): δ 2.03 (3 H, 1 x OCOC$\underline{H}_3$), 2.03 (3 H, 1 x OCOC$\underline{H}_3$), 2.04 (3 H, 1 x OCOC$\underline{H}_3$), 2.05 (3 H, 1 x OCOC$\underline{H}_3$), 2.08 (3 H, 1 x OCOC$\underline{H}_3$), 2.09 (3 H, 1 x OCOC$\underline{H}_3$), 2.09 (3 H, 1 x OCOC$\underline{H}_3$), 3.60 (2 H, m, H-6a and H-6b CH_2OH), 3.91 (1 H, ddd, $J_{5,4}$ = 10.0 Hz, $J_{5,6a}$ = 4.6 Hz, $J_{5,6b}$ = 2.6 Hz, H-5), 3.99 (1 H, dd, $J_{6b',6a'}$ = 12.0 Hz, $J_{6b',5'}$ 2.0 Hz, H-6b' CH_2OAc), 4.10 (1 H, ddd, $J_{5',4'}$ = 10.4 Hz, $J_{5',6a'}$ = 5.6 Hz, $J_{5',6b'}$ = 2.0 Hz, H-5') , 4.27 (1 H, dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{6a',5'}$ = 5.6 Hz, H-6a' CH_2OAc), 5.01 (1 H, dd, $J_{2,3}$ = 10.0 Hz, $J_{2,1}$ = 3.6 Hz, H-2), 5.02 (1 H, dd, $J_{2',3'}$ = 10.0 Hz, $J_{1',2'}$ = 4.0 Hz, H-2'), 5.02 (1 H, dd, $J_{4,5}$ = 10.0 Hz, $J_{4,3}$ = 9.6 Hz, H-4), 5.06 (1 H, dd, $J_{4',5'}$ = 10.4 Hz, $J_{4',3'}$ = 9.6 Hz, H-4'), 5.29 (1 H, d, $J_{1,2}$ = 3.6 Hz, H-1), 5.30 (1 H, d, $J_{1',2'}$ = 4.0 Hz, H-1'), 5.50 (1 H, dd, $J_{3',2'}$ = 10.0 Hz, $J_{3',4'}$ 9.6 Hz, H-3'), 5.53 (1 H, dd, $J_{3,2}$ = 10.0 Hz, $J_{3,4}$ 9.6 Hz, H-3) ; ^{13}C NMR (101 MHz $CDCl_3$): δ 20.6, 20.6, 20.6, 20.6, 20.7, 20.7, 20.7 (7 x OCO$\underline{C}H_3$), 60.9 (C-6 OH), 61.7 (C-6' $OCOCH_3$), 68.1

(C-4'), 68.4 (C-2'), 68.8 (C-4), 69.7 (C-5'), 69.9 (2 C, C-2 and C-3'), 70.0 (C-3), 70.4 (C-5), 92.9 (C-1'), 93.0 (C-1), 169.6, 169.6, 169.9, 169.9, 170.0, 170.4, 170.6 (7x C=O acetates); IR (thin film): 3525, 3026, 2961, 1751, (C=O) 1370, 1222, 1164, 1037, 901, 757 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{26}H_{36}O_{18}Na^+$,659.1794;found, 659.1814.

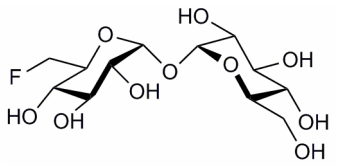
***2,3,4-tri-O-acetyl-6-deoxy-6-fluoro-α-D-glucopyranosyl-(1→1)-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside* (60)[25]**



In a 10 mL tube, **59** (111 mg, 0.174 mmol, 1 eq) was dissolved in dry dichloromethane (5 mL). DMAP (44.7 mg, 0.366 mmol, 2.1 eq) and DAST (46 µL, 0.348 mmol, 2.0 eq) were added and the solution was stirred overnight upon which time TLC (1:1 dichloromethane/ethyl acetate) revealed complete conversion to product (R_f 0.7) and dissappearance of starting material (R_f 0.5). The mixture was evaporated to dryness and the obtained oil was purified by silica column chromatography (pure chloroform then 95:5 chloroform/ethanol) to give 76.2 mg of the desired compound as a colorless oil (76 mg, 69%). TLC (CH_2Cl_2:EtOAc, 1:1, v/v): R_f=0.70; $[\alpha]_D^{25}$ + 164 (c = 1.0 in $CHCl_3$) [Lit. $[\alpha]_D^{21}$ + 166 (c = 0.65 in $CHCl_3$)][25]; ^{1}H NMR (400 MHz $CDCl_3$): δ 2.04 (3 H, 1 x OCOC$\underline{H}_3$), 2.05 (3 H, 1 x OCOC$\underline{H}_3$), 2.06 (3 H, 1 x OCOC$\underline{H}_3$), 2.08 (3 H, 1 x OCOC$\underline{H}_3$), 2.09 (3 H, 1 x OCOC$\underline{H}_3$), 2.09 (3 H, 1 x OCOC$\underline{H}_3$), 2.09 (3 H, 1 x OCOC$\underline{H}_3$), 3.99 (1 H, dd, $J_{6b',6a'}$ = 12.0 Hz, $J_{6b',5'}$ = 2.0 Hz, H-6b' $OCOCH_3$), 4.10 (1 H, ddd, $J_{5',4'}$ = 10.3 Hz, $J_{5',6a'}$ = 5.6 Hz, $J_{5',6b'}$ = 2.0 Hz, H-5'), 4.14 (1 H, dddd, $J_{5,F}$ = 21.5 Hz, $J_{5,4}$ = 10.3, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.8 Hz, H-5), 4.27 (1 H, dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{6a',5'}$ = 5.6 Hz, H-6a' $OCOCH_3$), 4.39 (1 H, ddd, $J_{6b,F}$ = 47.1 Hz, $J_{6b,6a}$ = 10.4 Hz, $J_{6b,5}$ = 2.8 Hz, H-6b CH_2F), 4.42 (1 H, ddd, $J_{6,F}$ = 47.1 Hz, $J_{6a,6b}$ = 10.4

Hz, $J_{5,6a}$ = 5.0 Hz, H-6a CH_2F), 5.02 (1 H, dd, $J_{4,5}$ = 10.3 Hz, $J_{4,3}$ = 9.5 Hz, H-4), 5.02 (1 H, dd, $J_{2',3'}$ = 10.3 Hz, $J_{2',1'}$ = 3.9 Hz, H-2'), 5.06 (1 H, dd, $J_{2,3}$ = 10.3 Hz, $J_{2,1}$ = 3.9 Hz, H-2), 5.07 (1 H, dd, $J_{4',5'}$ = 10.3 Hz, $J_{4',3'}$ = 9.5 Hz, H-4'), 5.28 (1 H, d, $J_{1,2}$ = 3.9 Hz, H-1), 5.29 (1 H, d, $J_{1',2'}$ = 3.9 Hz, H-1'), 5.48 (1 H, dd, $J_{3,2}$ = 10.3 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 5.50 (1 H, dd, $J_{3',2'}$ = 10.3 Hz, $J_{3',4'}$ = 9.5 Hz, H-3'); ^{13}C NMR (126 MHz, $CDCl_3$): δ 20.5, 20.6, 20.6, 20.6, 20.6, 20.7, 20.7 (7 x OCO<u>C</u>H_3), 61.7 (C-6' $OCOCH_3$), 68.4 (C-2'), 68.7 (1 C, d, $J_{C-4,F}$=7.0 Hz C-4), 69.0 (1 C, d, $J_{C-5,F}$ = 19 Hz C-5), 69.7 (C-5'), 69.8 (2 C, C-3 and C-3'), 70.0 (C-2), 81.3 (1 C, d, $J_{C-6,F}$ = 175 Hz, C-6), 92.9 (C-1'), 93.1 (C-1), 169.5, 169.5, 169.6, 169.7, 169.9, 170.0, 170.6 (7x C=O); ^{19}F NMR (377 MHz, $CDCl_3$): δ -230.6, dt, $J_{6,F}$ = 47.1 Hz, $J_{5,F}$ = 21.5 Hz; IR (thin film): 2959, 1752, 1643, 1558, 1538, 1435, 1370, 1219, 1039, 803 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{26}H_{35}O_{17}FNa^+$,661.1750;found, 661.1742.

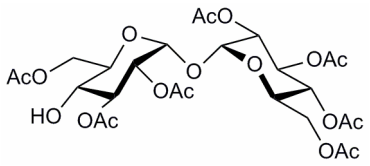
***6-deoxy-6-fluoro-α,α-D-trehalose* (20)[26]**



In a 25 mL flask, **60** (44.5 mg, 0.069 mmol, 1 eq) was dissolved in dry methanol (10mL). Dry sodium methoxide (21.6 mg, 0.4 mmol, 6 eq) was added and the solution stirred overnight upon which time TLC (1:4:4 water:isopropanol:ethyl acetate) showed complete conversion to a single product (R_f 0.4) and disappearance of starting material (R_f 1). Reaction was neutralized with DOWEX 50WX8 (H^+ form) cation exchange resin and then was filtered and evaporated to dryness to give 20.0 mg of a solid. This solid was purified by silica column chromatography (pure EtOAc then 1:4:4 water:isopropanol:ethyl acetate) to give a yellow compound that was discolored with activated charcoal, filtered and evaporated to afford the desired compound as a white, amorphous solid (11 mg, 46%) yield. TLC (H_2O:*i*-

PrOH:EtOAc,1:4:4, v/v/v): R_f=0.40; $[\alpha]_D^{25}$ + 124 (c = 0.2 in H_2O) [Lit. $[\alpha]_D^{21}$ + 174.2 (c = 1.0 in MeOH)][26]; ^{1}H NMR (400 MHz D_2O): δ 3.32 (1 H, dd, $J_{4',5'}$ = 10.4 Hz, $J_{4',3'}$ = 9.2 Hz, H-4'), 3.43 (1 H, dd, $J_{4,5}$ = 10.0 Hz, $J_{4,3}$ = 9.6 Hz, H-4), 3.55 (1 H, dd, $J_{2',3'}$ = 9.8 Hz, $J_{2',1'}$ = 4.0 Hz, H-2'), 3.58 (1 H, dd, $J_{2,3}$ = 9.8 Hz, $J_{2,1}$ = 4.0 Hz, H-2), 3.67 (1 H, dd, $J_{6b',6a'}$ = 12.0 Hz, $J_{6b',5'}$ = 5.0 Hz, H-6b'), 3.73 (1 H, m, H-5'), 3.75 (1 H, dd, $J_{3',2'}$ = 9.8 Hz, $J_{3',4'}$ = 9.2 Hz, H-3'), 3.76 (1 H, dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{6a',5'}$ = 2.6 Hz, H-6a'), 3.78 (1 H, dd, $J_{3,2}$ = 9.8 Hz, $J_{3,4}$ = 9.6 Hz, H-3'), 3.88 (1 H, dddd, $J_{5,F}$ = 28.7 Hz, $J_{5,4}$ = 10.0 Hz, $J_{5,6a}$ = 3.0 Hz, $J_{5,6b}$ = 1.8 Hz, H-5), 4.59 (1 H, ddd, $J_{6b,F}$ = 47.6 Hz, $J_{6b,6a}$ = 10.8 Hz, $J_{6b,5}$ = 1.8 Hz, H-6b), 4.65 (1 H, ddd, $J_{6a,F}$ = 47.6 Hz, $J_{6a,6b}$ = 10.8 Hz, $J_{6a,5}$ = 3.0 Hz, H-6a), 5.05 (1 H, d, $J_{1',2'}$ = 4.0 Hz, H-1'), 5.09 (1 H, d, $J_{1,2}$ = 4.0 Hz, H-1); ^{13}C NMR (101 MHz D_2O): δ 60.8 (C-6'), 68.1 (C-4'), 68.9 (1, C, d, $J_{C-4,F}$ = 6.2 Hz, C-4), 70.0 (C-4'), 71.2, 71.2 (C-2 and C-2'), 71.4 (1 C, d, $J_{C-5,F}$ = 12.0 Hz, C-5), 72.6 (C-5'), 72.7 (C-3), 72.8 (C-3'), 82.4 (1 C, d, $J_{C-6,F}$ = 168 Hz, C-6-F), 93.8 (C-1 or C-1'), 93.9 (C-1 or C-1'); ^{19}F NMR (377 MHz D_2O): δ -235.6 (dt, $J_{6,F}$ = 47.6 Hz, $J_{5,F}$ 28.7 Hz); IR (KBr): 3421 (OH), 2925, 1683, 1600, 1472, 1261, 1150, 1106, 1077, 1030, 990 cm^{-1}; HRMS (m/z): $[M-H]^-$ calcd. for $C_{12}H_{20}O_{10}F^-$,343.1046;found, 343.1045.

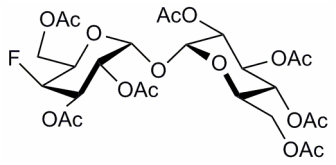
***2,3,6-tri-O-acetyl-α-D-glucopyranosyl-(1→1)-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside glucopyranoside* (61)**



In a 100 mL flask was dissolved **57** (1.5 g, 2.01 mmol, 1 eq) in dry THF (20 mL). Glacial acetic acid (230 µL, 4.0 mmol, 2 eq) was added followed a 1M TBAF solution in THF (4.0 mL, 4.03 mmol, 2 eq). After 24 h TLC (1:1 dichloromethane/ethyl acetate) showed some starting material (R_f 0.8) remaining and reaction is completed after 48 hours (R_f 0.5). The

mixture was evaporated and dried under high vacuum to give 4.0 g of oil. This oil was purified by silica column chromatography (98:2 chloroform/ethanol) to give compound **61** as a amorphous white solid (1.1 g, 85%). TLC (CH_2Cl_2:EtOAc, 1:1, v/v): R_f=0.50; $[\alpha]_D^{25}$ +150 (c = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz $CDCl_3$): δ 2.03 (3 H, 1 x OCOC$\underline{H}_3$), 2.05 (3 H, 1 x OCOC$\underline{H}_3$), 2.08 (3 H, 1 x OCOC$\underline{H}_3$), 2.09 (3 H, 1 x OCOC$\underline{H}_3$), 2.10 (3 H, 1 x OCOC$\underline{H}_3$), 2.11 (3 H, 1 x OCOC$\underline{H}_3$), 2.14 (3 H, 1 x OCOC$\underline{H}_3$), 3.56 (1 H, dd, $J_{4,5}$ = 10.0 Hz, $J_{4,3}$ = 9.2 Hz, H-4 OH), 3.91 (1 H, ddd, $J_{5,4}$ = 10.0 Hz, $J_{5,6a}$ 5.6 Hz, $J_{5,6b}$ = 2.0 Hz, H-5), 4.01 (1 H, dd, $J_{6b',6a'}$ = 12.0 Hz, $J_{6b',5'}$ = 2.0 Hz, H-6b'), 4.06 (1H, ddd, $J_{5',4'}$ = 10.0 Hz, $J_{5',6a'}$ = 5.6 Hz, $J_{5',6b'}$ = 2.0 Hz, H-5') , 4.23 (1 H, dd, $J_{6b,6a}$ = 12.0 Hz, $J_{6b,5}$ = 2.0 Hz, H-6b), 4.24 (1 H, dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{6a',5'}$ = 5.6 Hz, H-6a'), 4.41 (1 H, dd, $J_{6a,6b}$ = 12.0 Hz, $J_{6a,5}$ = 5.6 Hz, H-6a) 5.00 (1 H, dd, $J_{3,2}$ = 10.4 Hz, $J_{2,1}$ = 3.6 Hz, H-2), 5.02 (1 H, dd, $J_{3',2'}$ = 10.4 Hz, $J_{2',1'}$ = 4.0 Hz, H-2'), 5.04 (1 H, dd, $J_{4',5'}$ = 10.0 Hz, $J_{4',3'}$ = 9.2 Hz, H-4'), 5.24 (1 H, d, $J_{1,2}$ = 3.6 Hz, H-1), 5.30 (1 H, d, $J_{1',2'}$ = 4.0 Hz, H-1'), 5.31 (1 H, dd, $J_{3,2}$ = 10.4 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 5.49 (1 H, dd, $J_{3',2'}$ = 10.4 Hz, $J_{3',4'}$ = 9.2 Hz, H-3'); ^{13}C NMR (101 MHz $CDCl_3$): δ 20.6, 20.6, 20.6, 20.6, 20.7, 20.7, 20.9 (7x OCO$\underline{C}H_3$), 61.7 (C-6'), 62.6 (C-6), 68.1 (C-4'), 68.5 (C-2'), 69.5 (2 C, C-2 and C-5'), 69.8 (C-5), 69.9 (C-3'), 70.8 (C-3), 73.1 (C-4 OH), 91.9 (C-1), 92.2 (C-1'), 169.6, 169.6, 169.7, 170.0, 170.6, 171.3, 172.1 (7 x C=O acetates); IR (thin film): 3568, 3305, 3025, 2959, 1748, (C=O), 1370, 1223, 1161, 1039, 902, 757 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for for $C_{26}H_{36}O_{18}$ Na^+,659.1794;found, 659.1788.

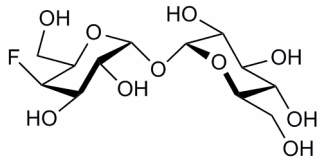
***2,3,6-tri-O-acetyl-4-deoxy-4-fluoro-α-D-galactopyranosyl-(1→1)-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside* (62)**[27]



In a 10mL tube, **61** (87.2 mg, 0.137 mmol, 1 eq) and DMAP (35.1 mg, 0.288 mmol, 2.1 eq) were dissolved in anhydrous dichloromethane (3 mL). The solution was cooled to -20 °C and DAST (36 µL , 0.274 mmol, 2 eq) was added. After 64 h, TLC (1:1 CH_2Cl_2:EtOAc) showed conversion to product (R_f = 0.72). The mixture was concentrated under reduced pressure and purified by silica column chromatography (98:2 $CHCl_3$:EtOH followed by 95:5 and 90:10) to give compound **62** (34.5 mg, 40%) as a clear oil. TLC (CH_2Cl_2:EtOAc, 1:1, v/v): R_f=0.72; $[\alpha]_D^{25}$ + 138 (c = 0.5 in $CHCl_3$) [Lit. $[\alpha]_D^{21}$ + 168 (c = 1.0 in $CHCl_3$)][27]; ^{1}H NMR (400 MHz $CDCl_3$): δ 2.04, 2.05, 2.06, 2.08, 2.09, 2.09, 2.09 (7x 3H, s, OCOC$\underline{H}_3$), 4.01 (1 H, dd, $J_{6b',6a'}$ = 12.0 Hz, $J_{6b',5'}$ = 2.0 Hz, H-6b'), 4.05 (1 H, ddd, $J_{5',4'}$ = 10.0 Hz, $J_{5',6a'}$ = 5.6 Hz, $J_{5',6b'}$ = 2.0 Hz, H-5'), 4.15 (2 H, m, H-5, H-6b), 4.24 (1 H, dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{6a',5'}$ = 5.6 Hz, H-6a'), 4.30 (1 H, m, H-6a), 5.02 (1 H, dd, $J_{4,F}$ = 50.2 Hz, $J_{4,3}$ = 2.4 Hz, H-4 F), 5.03 (1 H, dd, $J_{2',3'}$ = 10.0 Hz, $J_{1',2'}$ = 4.0 Hz, H-2'), 5.04 (1 H, app t, $J_{4',5'}$ = $J_{4',3'}$ = 10.0 Hz, H-4'), 5.25 (1 H, ddd, $J_{3,F}$ = 25.7 Hz $J_{3,2}$ = 10.0 Hz, $J_{3,4}$ = 2.4 Hz, H-3), 5.29 (1 H, d, $J_{1',2'}$ = 4.0 Hz, H-1'), 5.35 (3 H, d, $J_{1,2}$ = 3.8 Hz, H-1), 5.37 (1 H, dd, $J_{2,3}$ = 10.0 Hz , $J_{2,1}$ = 3.8 Hz, H-2), 5.47 (1H, dd, $J_{3',2'}$ = 10.0 Hz, $J_{3',4'}$ = 10.0 Hz, H-3') ; ^{13}C NMR (125 MHz $CDCl_3$): δ 20.5, 20.6, 20.6, 20.6, 20.6, 20.6, 20.8, (7x CH_3 acetates), 61.6 (1 C, d, $J_{C-6,F}$ = 6.3 Hz C-6), 61.7 (C-6'), 66.7 (C-2), 67.5 (1 C, d, $J_{C-5,F}$ = 18 Hz, C-5), 68.2 (C-4'), 68.3(1 C, d, $J_{C-3,F}$ = 18 Hz, C-3), 68.5 (C-2'), 69.7 (C-5'), 69.8 (C-3'), 86.4 (1 C, d, $J_{C-4,F}$ = 185 Hz, C-4, CH-F), 92.5 (C-1'), 92.9 (C-1), 169.5, 169.7, 170.0, 170.3, 170.3, 170.3, 170.6 (7x C=O acetates) ; ^{19}F {^{1}H} NMR (377 MHz, $CDCl_3$): δ -219.2, ddd, J_{F-H4} = 50.8 Hz, J_{F-H5} = 28.9 Hz, J_{F-H3} = 25.7 Hz; IR

(thin film): 2962, 1750, 1645, 1539, 1435, 1375, 1225, 1038, 734 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{26}H_{35}O_{17}F\ Na^+$,661.1750;found, 661.1765.

***4-deoxy-4-fluoro-α-D-galactopyranosyl-(1→1)-α-D-glucopyranoside* (21)**[27]



62 (16.2 mg, 0.047 mmol, 1 eq) was dissolved in dry methanol (5mL) and dry sodium methoxide (10.8 mg, 0.28 mmol, 6 eq) was added. After stirring overnight, TLC (4:4:1 ethyl acetate:isopropanol:water) showed the formation of a single compound (R_f 0.28). Reaction was neturalized with DOWEX 50WX8 (H^+ form) cation exchange resin and the solution was filtered and evaporated to give **21** as a clear oil (10.3 mg, 100%). TLC (H_2O:*i*-PrOH:EtOAc,1:4:4, v/v/v): R_f=0.28; $[\alpha]_D^{25}$ + 176 (c = 0.1 in H_2O) [Lit. $[\alpha]_D^{21}$ + 172.6 (C = 0.5 in CH_3OH)][27]; ^{1}H NMR (400 MHz D_2O): δ 3.33 (1 H, dd, $J_{4',5'}$ = 10.0 Hz, $J_{4',3'}$ = 9.2 Hz, H-4'), 3.53 (1 H, dd, $J_{2',3'}$ = 10.0 Hz, $J_{1',2'}$ = 4.0 Hz, H-2'), 3.67 (1H, dd, $J_{6b',6a'}$ = 12.0 Hz, $J_{6b',5'}$ = 5.6 Hz, H-6b'), 3.72 (2 H, m, H-6a and H-6b), 3.75 (1 H, dd, $J_{3',2'}$ = 10.0 Hz, $J_{3',4'}$ = 9.2 Hz, H-3'), 3.75 (1H, m, H-5'), 3.76 (1 H, dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{6a',5'}$ = 2.2 Hz, H-6a'), 3.85 (1 H, dd, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 4.00 (1 H, ddd, $J_{3,F}$ = 30.0 Hz, $J_{3,2}$ = 10.4 Hz $J_{3,4}$ = 2.8 Hz, H-3), 4.04 (1 H, dt, $J_{5,F}$ = 32.2 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz, H-5), 4.80 (1 H, dd, $J_{4,F}$ = 50.5 Hz, $J_{4,3}$ = 2.8 Hz, H-4), 5.08 (1 H, d, $J_{1',2'}$ = 4.0 Hz, H-1'), 5.15 (1 H, d, $J_{1,2}$ = 4.0 Hz, H-1); ^{13}C NMR (101 MHz D_2O): δ 60.4 (d, $J_{C-6,F}$ = 5.8 Hz C-6), 60.8 (C-6), 68.1 (d, J_{C-3F} = 17 Hz C-3), 68.2 (C-2), 70.0 (C-4'), 70.5 (d, J_{C-5F} = 18 Hz C-5), 71.3 (C-2'), 72.5 (C-5'), 72.8 (C-3'), 90.7 (d, J_{C-F} = 177 Hz, C-4-F), 93.8 (2x C-1); ^{19}F NMR (377 MHz D_2O): δ -219.7, ddd, J_{F-H4} = 50.5 Hz, J_{F-H5} = 32.2 Hz, J_{F-H3} = 30.0 Hz; IR (KBr): 3430 (OH), 2928,

1636, 1419, 1350, 1260, 1151, 1102, 1077, 1050, 1013 cm^{-1}; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{12}H_{21}O_{10}F\ Na^+$, 367.1011; found, 367.1008.

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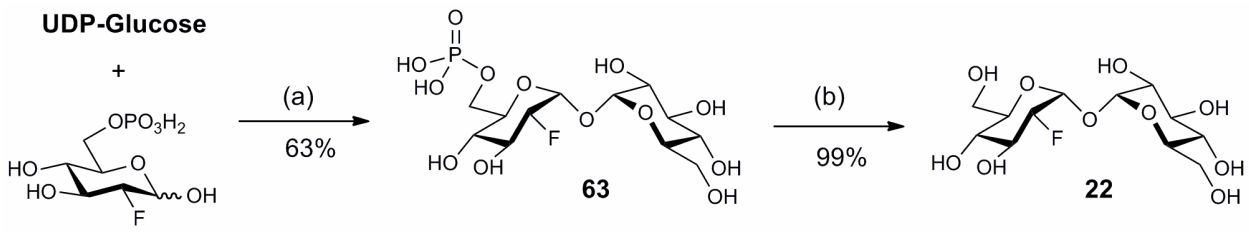
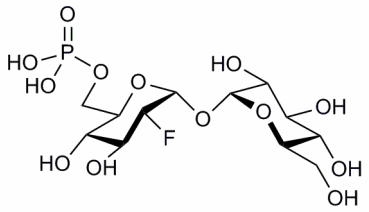
Synthesis of compound 22.



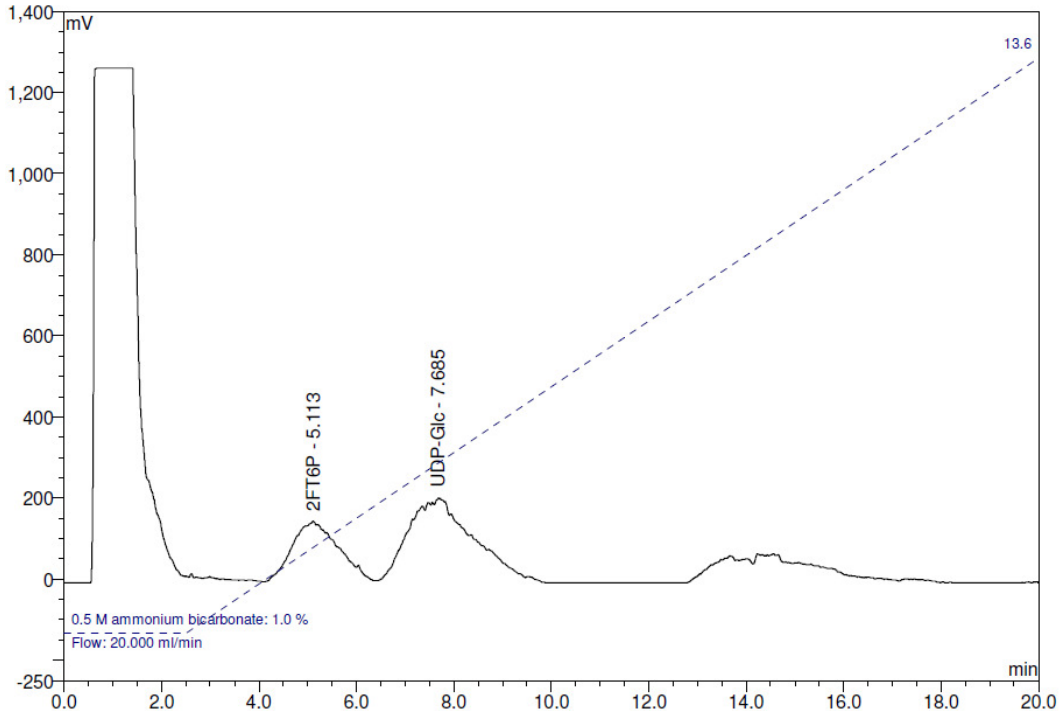
Supplementary Scheme 13. *Reagents and conditions. (a) Trehalose-6-phosphate synthase, Hepes buffer, pH 7.4, $MgCl_2$, 30 °C, 16 h. (b) Alkaline phosphatase, pH 8.0, 37 °C, 2 h.*

2-deoxy-2-fluoro-α,α-D-trehalose 6-(dihydrogenphosphate) **(63)**



2-Fluoro-2-deoxy-D-glucose-6-phosphate (prepared according to procedure[28]) was dissolved in 0.2 M, pH 7.4 Hepes buffer containing 10 mM $MgCl_2$ in the presence of 95 mg of UDP-glucose. To this mixture added trehalose-6-phosphate synthase (cloned and expressed from E. coli[29]) to a final concentration of 21.6 μM and pH was adjusted to 7.4. Total volume was 3 mL. The reaction mixture was incubated at 30 °C overnight. After confirming the reaction was completed by electrospray ionization mass spectrometer (ESI MS), the mixture was filtered using centrifugal concentrator Viva spin 500 (Sartorius) of nominal molecular weight cutoff 10,000 to remove proteins. Typically, overnight reaction was conducted to confirm the completion of the reaction. The filtrate was divided into three volumes and purified by the HPLC (Dionex Ultimate 3000) using a strong anion exchange column Applied Biosystems, Poros® HQ (10 mm x 100 mm, 50 μm). The HPLC was eluted with gradient of 0 – 500 mM ammonium bicarbonate at a flow rate of 20 mL/min and eluants were detected with an

evaporative light scattering detector (ELSD). The product was eluted at retention time of 5.11 minutes. Collected fractions were pooled and concentrated under reduced pressure, yielding a white solid (25 mg, 63%). ^{1}H NMR (400 MHz, D_2O): δ 3.29 (1 H, at, $J_{3,4} = J_{4,5} = 9.55$ Hz, H-4'), 3.52 (1 H, dd, $J_{2,3} = 10.0$ Hz, $J_{1,2} = 3.8$ Hz, H-2'), 3.55 (1 H, at, $J_{3,4} = J_{4,5} = 9.85$ Hz, H-4) 3.61 – 3.90 (7 H, m, H-5, H-6a, H-6b, H-3', H-5', H-6a', H-6b'), 3.98 (1 H, dt, $J_{3,F} = 13.1$ Hz, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3), 4.41 (1 H, ddd, $J_{2,F} = 49.0$ Hz, $J_{1,2} = 4.2$, $J_{2,3} = 9.5$ Hz, H-2), 5.09 (1 H, d, $J_{1,2} = 3.8$, H-1'), 5.28 (d, 1 H, $J_{1,2} = 4.2$ Hz, H-1), ^{13}C NMR (126 MHz, D_2O): δ 60.5 (C-6'), 62.2 (1 C, d, $J_{C-6,P} = 3.8$ Hz, C-6), 68.4 (C-4), 69.5 (C-4'), 70.7 (1 C, d, $J_{C-3,F} = 16.1$ Hz, C-3), 70.9 (C-2'), 71.9 (1 C, d, $J_{C-5,P} = 6.7$ Hz, C-5), 72.2 (C-5'), 72.5 (C-3'), 89.6 (d, $J_{C-2,F} = 187.9$ Hz, C-2), 91.4 (1 C, d, $J_{C-2,F} = 22.2$ Hz, C-1), 94.0 (C-1'); ^{19}F(^{1}H) NMR (376 MHz, D_2O) δ -201.2, ^{31}P{^{1}H} NMR (162 MHz, D_2O): δ 4.5; HRMS (m/z): [M-H]$^{-}$ calcd. for $C_{12}H_{21}FO_{13}P^{-}$,423.0709;found, 423.0709.



Supplementary Figure 30. *Preparative HPLC trace for the purification of* **63***. Conditions: HPLC (Dionex Ultimate 3000) using a strong anion exchange column Applied Biosystems, Poros® HQ (10 mm x 100 mm, 50 µm). The HPLC was eluted with gradient of 0 – 500 mM ammonium bicarbonate at a flow rate of 20 mL/min. Compound* **63** *elutes at 5.1 min (2FT6P)*

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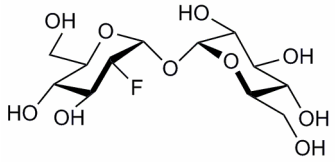
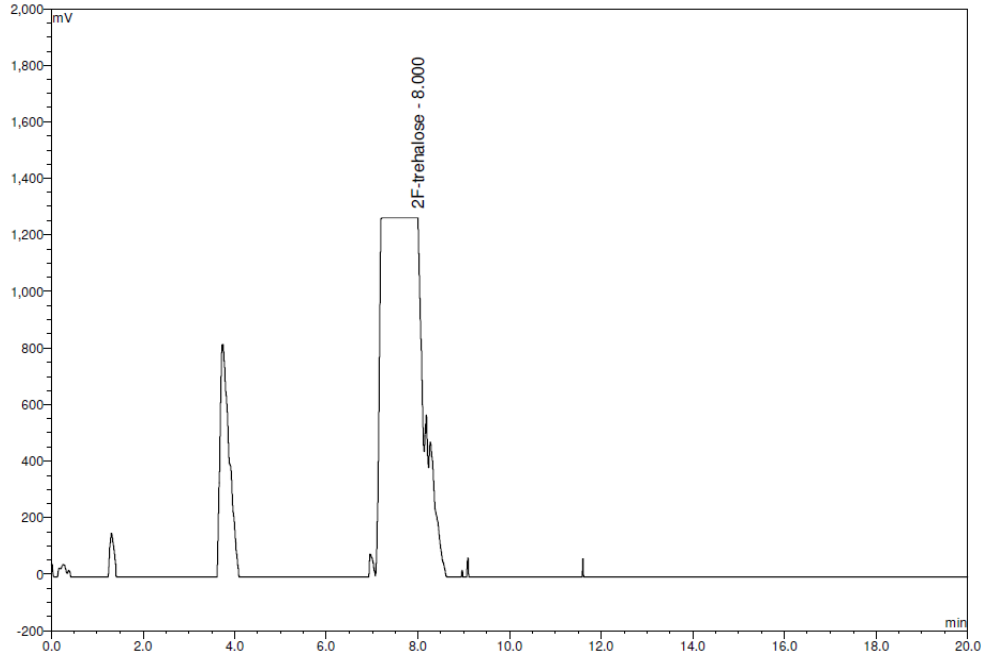
***2-deoxy-2-fluoro-α,α-D-trehalose* (22)**



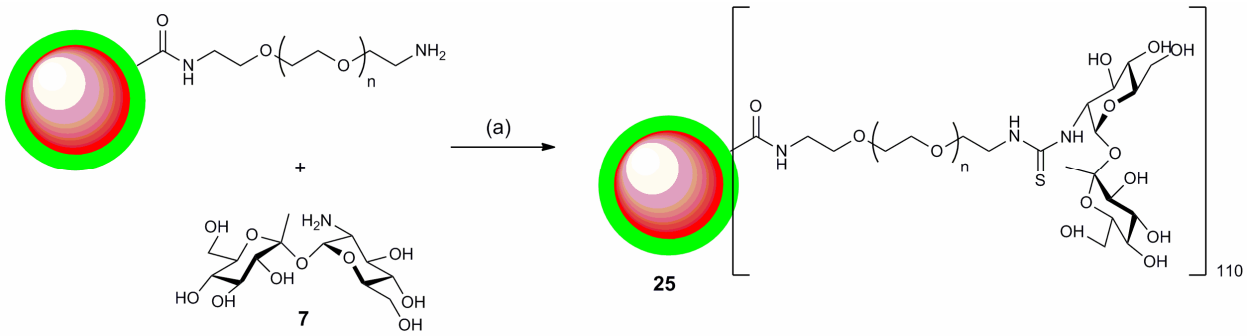
63 (5 mg, 12 µmol) was dissolved in water and pH was adjusted to 8.0 with 1M NaOH. 3 Units of alkaline phosphatase (Sigma) was added to initiate the reaction. The reaction mixture was incubated at 37 °C and the reaction progress was monitored by ESI MS. Typically, reaction was completed within 2 hours. After confirming the reaction was completed, the mixture was filtered using the centrifugal concentrator Viva spin 500 (Sartorius) of nominal molecular weight cutoff 10,000 to remove proteins. The filtrate was loaded onto a column packed with anion exchange resin DEAE cellulose (Sigma) and the fraction eluted with water was collected and concentrated under reduced pressure. The resulting syrup was loaded onto the Phenomenex Luna NH2 HPLC column (250 x 21.2 mm, 5 µm) on Dionex UltiMate 3000 system. Eluants were detected with an evaporative light scattering detector (ELSD). The product was eluted at 7.8 minutes of rentention time by an isocratic elution with 30/70 water/acetonitrile at a flow rate of 18.0 mL/min. Fractions containing the product was pooled and concentrated under reduced pressure, yielding a colorless wax (4.0 mg, 99%). ^{1}H NMR (400 MHz, D_2O): δ 3.36 (1H, at, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4'), 3.42 (1H, at, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 3.56 (1H, dd, $J_{1,2} = 3.7$ Hz, $J_{2,3} = 9.9$ Hz, H-2'), 3.58 – 3.81 (7H, m, H-5, H-6a, H-6b, H-3', H-5', H-6a', H-6b'), 3.97 (1H, dt, $J_{3,F} = 13.2$ Hz, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3), 4.36 (1H, ddd, $J_{2,F} = 48.7$ Hz, $J_{1,2} = 4.0$ Hz, $J_{2,3} = 9.6$ Hz, H-2), 5.07 (1H, d, $J_{1,2} = 3.7$ Hz, H-1'), 5.29 (1H, d, $J_{1,2} = 4.0$ Hz, H-1); ^{13}C NMR (126 MHz, D_2O): δ 60.3 (C-6 or C-6'), 60.5 (C-6 or C-6'), 69.0 (C-4), 69.1 (C-4'), 69.6 (C-3'), 70.9 (C-2'), 72.2 (C-5 or C-5'), 72.6 (C5 or C-5'), 71.1 (d, $J_{C-3,F} = 15.8$ Hz, C-3), 89.5 (d, $J_{C-2,F} = 188.1$ Hz, C-2), 91.2 (d, $J_{F2,C1} = 22.0$ Hz, C-1), 94.0 (C-1'); ^{19}F{^{1}H} NMR (376 MHz, D_2O): δ -201.2; HRMS (m/z): $[M-H]^-$ calcd. for $C_{12}H_{20}FO_{10}^-$,343.1046;found, 343.1040.



Supplementary Figure 31. *Preparative HPLC trace for the purification of* **22***. Conditions: the Phenomenex Luna NH2 HPLC column (250 x 21.2 mm, 5 µm) on Dionex UltiMate 3000 system. Eluants were detected with an evaporative light scattering detector (ELSD). The product* **22** *(2F-trehalose) was eluted at 7.8 minutes of rentention time by an isocratic elution with 30/70 water/acetonitrile at a flow rate of 18.0 mL/min*

Nature Chemical Biology: doi:10.1038/nchembio.539

Synthesis of Trehalose-modified Quantum dots.



Supplementary Scheme 14. *(a) i, thiophosgene,* **7**, *75mM $NaHCO_3$ pH 9, RT, 14 h; ii,Quantum dots, 75mM $NaHCO_3$ pH 9, 4 °C, 4 h.*

***Trehalose Quantum Dots* (25)**

7 (2.5 mg, 0.007 mmol, 1 eq) was dissolved in 75 mM $NaHCO_3$ buffer pH 9 (200 µl). To this was added thiophosgene as a solution (20 µl of thiophosgene into 1 mL of chloroform). 100 µl of thiophosgene solution was added to reaction (3 mg, 0.26, 4.2 eq). The resulting biphasic mixture was stirred at room temperature for 3 hours upon which TLC (5 ethanol : 3 NH_4OH : 1 water) showed complete consumption of starting material (R_f 0.2) and conversion to a single product (R_f 0.65). Excess thiophosgene and chloroform were removed *in vacuo* and crude thiocyanate was used without further purification. An 8 µM solution of CdSe-ZnS (50 µl) core-shell quantum dots (emission λ_{max} 655nm) in borate buffer at pH 8 (Invitrogen) was buffer exchanged into water by repeated (x5) centrifugal filtration through a 10 kDa cutoff spin filter. Dots were then resuspended in water. 50 µl of this 8 µM quantum dot solution was then added to the solution of crude thiocyanate, and the total volume made up to 1.0 mL with 75 mM $NaHCO_3$ buffer at pH 9.0 (pH electrode). Reaction mixture was shaken at 4 °C for 14 hours. Excess sugar and salt was removed from the reaction mixture by size exclusion chromatography (PD 10 column, Amersham) with water as the mobile phase. The quantum dot solution was concentrated to 1 mL, using 10 Kda spin filter, and the concentration

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determined using previously reported procedures[30] to be 0.44 μM (ε_{350} = 3880000 $M^{-1}cm^{-1}$). The modification of the quantum dots was confirmed by agarose gel electrophoresis.

Supplementary Figure 32. *Quantum dots run on an agarose gel, 100 Volts, 2 h. (i) modified (ii) unmodified (iii) DNA ladder*

The carbohydrate loading on the quantum dots was determined using the phenol sulphuric acid method. An aliquot of the quantum dot solution (50 μl) was treated with concentrated sulphuric acid (75 μl) and aqueous phenol (5% w/w, 10 μl) and heated to 90 °C. After 5 minutes the sample was cooled to room temperature and A_{490} measured, referenced to a solution of carbohydrate modified quantum dots and acid. The concentration of trehalose was determined by comparison to a standardised curve (Supplementary Figure 33). The carbohydrate content per dot was calculated from the ratio of trehalose concentration to the concentration of ZnS-CdSe quantum dots and was found to be ~110 sugars/dot.

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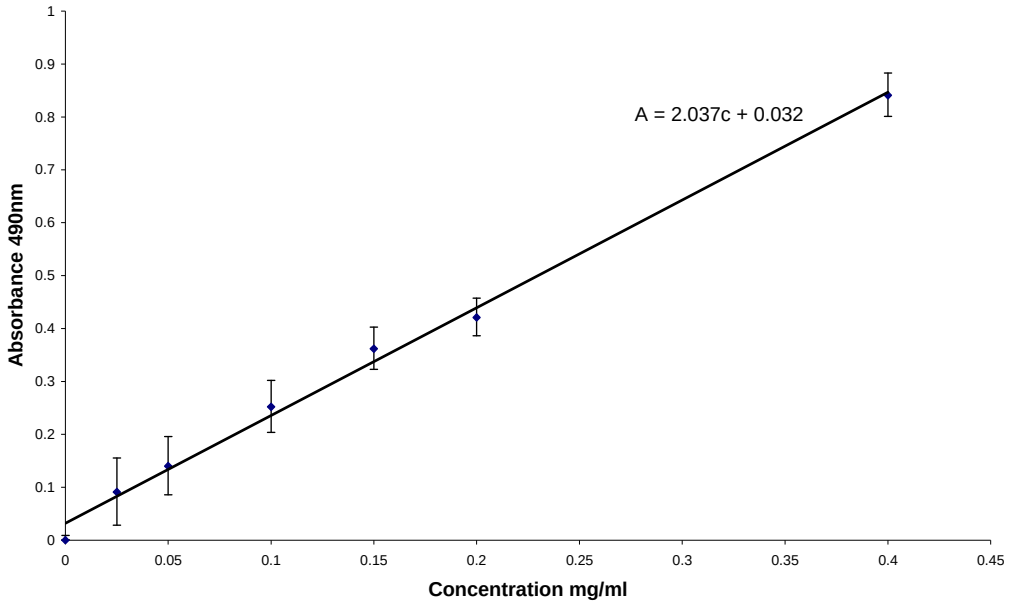

Supplementary Figure 33. *Standardised curve for determining trehalose concentration on modified quantum dots using the phenol-sulphuric acid method. Shown is the absorbance at 490nm after reaction with phenol and sulphuric acid against standardized concentrations of trehalose.*

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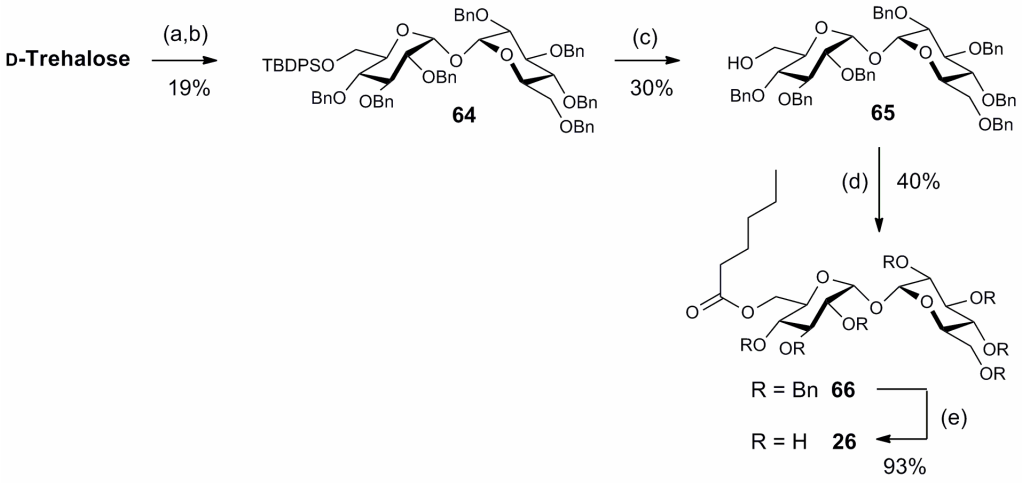
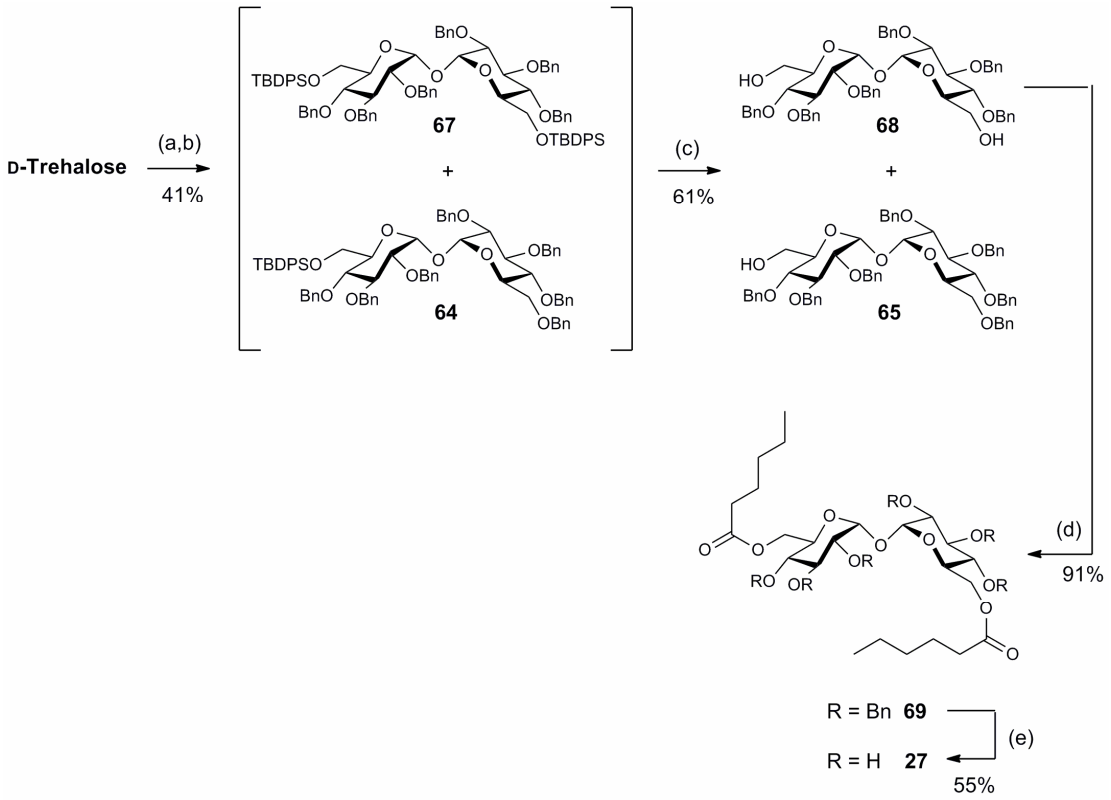
Synthesis of compounds 26 and 27.



Supplementary Scheme 15. *Reagents and conditions. (a) TBDPSCl, imidazole, RT, 36 h (b) BnBr, NaH, RT, 24 h (c) acetyl chloride, anhydrous MeOH, RT, 48 h (d) DCM, pyridine, hexanoyl chloride, RT, 16 h (e) 10%Pd/C cartridge, H-Cube™, 80 bar, 45 ˚C, 3 h*



Supplementary Scheme 16 . (a) TBDPSCl, imidazole, RT, 24 h, 55% (b) BnBr, NaH, RT, 18 h. (c) TBAF, 60 ˚C, 3 h, yields for individual compounds based on (2:1) ratios of starting materials (d) Pyridine, hexanoyl chloride, RT, 30 mins, 91% (e) H_2, Pd/C, ethanol, RT, 16 h, 55%.

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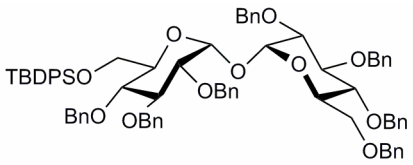
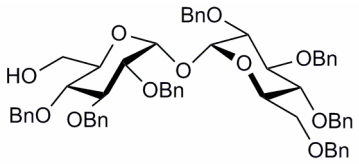
***6-O-tert-butyldiphenylsilyl-2,3,4,2',3',4',6'-hepta-O-benzyl-α,α-D-trehalose* (64)**



D-Trehalose (7.4 g, 21.75 mmol, 1 eq) was dissolved in anhydrous DMF (40 mL). To this was added tert-butyldiphenylchlorosilane (TBDPS-Cl) (5 mL, 18 mmol, 0.9 eq) and imidazole (1.4g, 21 mmol, 0.95 eq). Solution was stirred at RT under Ar atmosphere for 18 h. TLC (2:1 petrol/ethyl acetate) indicated primarily starting material (R_f 0.1) and a small amount of the mono-TBDPS-trehalose. Additional TBDPS-Cl was added (2.5 mL, 9 mmol, 0.45 eq) and reaction was left for a further 18 hours. NaH (8 g, 339 mmol, 15 eq) and benzyl bromide (25 mL, 145 mmol, 7 eq) were added *in situ*. Reaction was stirred for a further 24 hours under argon, until the desired product could be detected by TLC (5:1 petrol/ethyl acetate) (R_f 0.85). Column chromatography yielded the desired product as a slightly yellow oil. (4.83 g, 19% over two steps). TLC (petrol:EtOAc, 5:1, v/v): R_f=0.85; $[\alpha]_D^{25}$ + 16.13 (c = 0.88 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (9 H, s, 3 x C(C$\underline{H}_3$)$_3$), 3.47 (1 H, dd, $J_{6a,6b}$ = 10.2 Hz, $J_{5,6b}$ = 1.8 Hz, H-6b'), 3.60 (1 H, dd, J $J_{6a,6b}$ = 10.2 Hz, $J_{5,6a}$ = 2.7 Hz, H-6a'), 3.64 (1 H, dd, $J_{2,3}$ = 7.7 Hz, $J_{1,2}$ = 4.2 Hz, H-2'), 3.67 (1 H, dd, $J_{2,3}$ = 7.8 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 3.72 (1 H, dd, $J_{6a,6b}$ = 9.7 Hz, $J_{5,6a}$ = 3.7 Hz, H-6a), 3.78 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4'), 3.87 (1 H, dd, $J_{6a,6b}$ = 10.4 Hz, $J_{5,6b}$ = 2.3 Hz, H-6b), 3.97 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 4.13-4.17 (3 H, m, H-5, H-3, H-3') 4.27 (1 H, ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 2.8, $J_{5,6b}$ = 2.7 Hz, H-5'), 4.47 (1 H, d, J = 11.9 Hz, 1 x OCH_2Ph), 4.52 (1 H, d, J = 10.6 Hz, 1 x OCH_2Ph), 4.57 (1 H, d, J = 12.6 Hz, 1 x OCH_2Ph), 4.58 (1 H, d, J = 12.1 Hz, 1 x OCH_2Ph), 4.87 (1 H, d, J = 12.1 Hz, 1 x OCH_2Ph), 4.90 (1 H, d, J = 11.1 Hz, 1 x OCH_2Ph), 4.94 (1 H, d, J = 9.3 Hz, 1 x OCH_2Ph), 4.96 (1 H, d, J = 11.6 Hz, 1 x OCH_2Ph), 4.99 (1 H, d, J = 9.6 Hz, 1 x OCH_2Ph), 5.01 (1 H, d, J = 9.6 Hz, 1 x OCH_2Ph), 5.04 (1 H, d, J = 12.2 Hz, 1 x

OCH_2Ph), 5.07 (1 H, d, J = 11.0 Hz, 1 x OCH_2Ph), 5.09 (1 H, d, J =10.6 Hz, 1 x OCH_2Ph), 5.28 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1'), 5.37 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1), 7.00 - 7.61 (45 H, m, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 20.7 (3 x $C(C\underline{H}_3)_3$), 28.1 (1 x $\underline{C}(CH_3)_3$), 62.2 (C-6), 68.2 (C-6'), 70.6 (C-5'), 71.69 (C-5), 72.8 (OCH_2Ph), 72.9 (OCH_2Ph), 73.6 (OCH_2Ph), 75.1 (OCH_2Ph), 75.3 (OCH_2Ph), 75.7 (OCH_2Ph), 75.9 (OCH_2Ph), 77.70 (C-4 or C-4'), 77.7 (C-4 or C-4'), 79.5 (C-2'), 80.1 (C-2), 81.9 (C-3 or C-3'), 81.9 (C-3 or C-3'), 94.2 (2 C, C-1, C-1'), 126.0, 126.3, 126.4, 127,1 127.2, 127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.0, 128.1, 128.2, 128.4, 128.4, 128.5, 128.6, 128.9, 129.1, 129.5, 129.7 (7 x OCH_2Ph), 138.0, 138.4, 138.5, 138.6, 138.9, 139.0, 139.6 (7 x 1 C, 7 x OCH_2Ph); IR (thin film): 2925, 2875 (C=CH), 1454, 1444 (C=C), 698 cm^{-1}; MS (m/z): $[M+NH_4]^+$ calcd. for $C_{77}H_{86}NO_{11}Si^+$,1228.59;found, 1228.6; isotopic distribution: Species observed $[M+Na]^+$, peaks observed 1233.53 (100%), 1234.53 (86.6%), 1235.53 (43.1%), 1236.53 (14.5%), 1237.53 (4.0%), 1238.54 (1.1%) peaks calculated 1233.55 (100.0%), 1234.55 (89.7%), 1235.55 (45.2%), 1236.56 (16.0%), 1237.56 (4.2%), 1238.56 (0.6%)

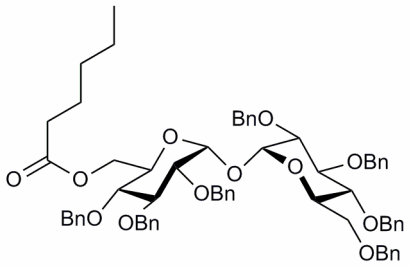
***2,3,4,2',3',4',6'-hepta-O-benzyl-α,α-D-trehalose* (65)[31]**



64 (4 g, 3.3 mmol, 1 eq) was dried under reduced pressure for 1 hour and dissolved in anhydrous methanol (20 mL). To this was added acetyl chloride (5 ml), generating acetic acid *in situ*. Reaction was stirred in acid for 48 hours, upon which time TLC (2:1 petrol/ethyl acetate) indicated product (R_f 0.4) and complete consumption of starting material (R_f 0.95). Reaction was quenched with water and poured into a satd. $NaHCO_3$ soln. When gas ceased to evolve, reaction was extracted into DCM and concentrated *in vacuo.* Column

chromatography (10:1 to 1:1 petrol/ethyl acetate) yielded the desired product as a colourless oil (1.1 g, 30%). TLC (petrol:EtOAc, 2:1, v/v): R_f=0.40; $[\alpha]_D^{25}$ + 54.3 (c = 0.77 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.38 (1 H, dd, $J_{6a,6b}$ = 10.7, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.51 (1 H, dd, $J_{6a,6b}$ = 10.4 Hz, $J_{5,6a}$ = 3.2 Hz, H-6a), 3.52 (1H, dd, $J_{6a,6b}$ = 9.6 Hz, $J_{5,6a}$ = 3.6 Hz, H-6a') 3.57 - 3.61 (4 H, m, H-2', H-2, H-4, H-6b'), 3.68 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4'), 4.03 (1 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 4.06 (1 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3'), 4.05 - 4.09 (1 H, m, H-5'), 4.15 (1 H, ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 3.5 Hz, $J_{5,6b}$ = 2.4 Hz, H-5), 4.38 (1 H, d, J = 12.0 Hz, 1 x OCH_2Ph), 4.46 (1 H, d, J = 11.0 Hz, 1 x OCH_2Ph), 4.54 (1 H, d, J = 12.0 Hz, 1 x OCH_2Ph), 4.65 (1 H, d, J = 11.0 Hz, 1 x OCH_2Ph), 4.69 (2 H, d, J = 10.0 Hz, 2 x OCH_2Ph), 4.71 (2 H, d, J = *2* x OCH_2Ph), 4.82 (1 H, d, J = 10.7 Hz, 1 x OCH_2Ph), 4.87 (1 H, d, J =11.0 Hz, 1 x OCH_2Ph), 4.88 (2 H, d, J = 10.7 Hz, 2 x OCH_2Ph), 4.99 (1 H, d, J = 11.0 Hz, 1 x OCH_2Ph), 5.00 (1 H, d, J = 10.7 Hz, 1 x OCH_2Ph), 5.18 (1 H, d, $J_{1,2}$ = 3.5 Hz, H-1), 5.19 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1'), 7.25-2.40 (35 H, m, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 61.5 (C-6), 68.12 (C-6'), 70.6 (C-5), 71.1 (C-5'), 72.1 (OCH_2Ph), 72.8 (OCH_2Ph), 72.8 (OCH_2Ph), 73.5 (OCH_2Ph), 75.0 (OCH_2Ph), 75.5 (2 C, 2 x OCH_2Ph), 77.0 (C-4'), 77.6 (C-4), 79.3 (C-2 or C-2'), 79.5 (C-2 or C-2'), 81.5 (C-3), 81.7 (C-3'), 94.1 (C-1 or C-1'), 94.3 (C-1 or C-1'), 127.4, 127.5, 127.6, 127.6, 127.7, 127.8, 127.9, 128.1, 128.3, 128.3, 128.4, 128.4 (7x OCH_2Ph), 134.6, 137.7, 138.1, 138.1, 138.3, 138.7, 138.8 (7 x 1 C, 7 x OCH_2Ph); MS (m/z): $[M+NH_4]^+$ calcd. for $C_{61}H_{68}NO_{11}^+$,990.48;found, 990.4.

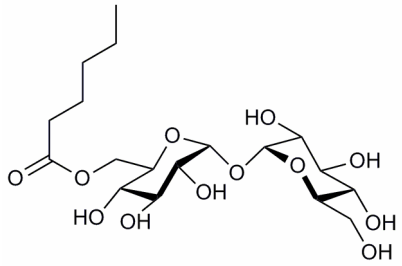
***6-O-hexanoyl-2,3,4,2',3',4,6'-hepta-O-benzyl-α,α-D-trehalose* (66)**



65 (116 mg, 0.119 mmol, 1 eq) was dissolved in anhydrous DCM (10 mL) with anhydrous pyridine (2 mL) and to this was added hexanoyl chloride (0.02 mL, 0.15 mmol, 1.25 eq), which, upon addition caused the reaction mixture to turn yellow. Reaction was stirred for 16 h at RT upon which time TLC (2:1 petrol/ethyl acetate) indicated incomplete conversion from starting material (R_f 0.1) to product (R_f 0.6). An additional portion of hexanoyl chloride (0.05 mL, 0.375 mmol, 3.125 eq) was added and reaction was left at RT for an additional 24 h. Reaction was washed with satd $NaHCO_3$ solution and concentrated *in vacuo*. Column chromatography (4:1 to 2:1 petrol/ethyl acetate) yielded the desired product as a clear oil (52.1 mg, 40%). TLC (petrol:EtOAc, 2:1, v/v): R_f=0.60; $[\alpha]_D^{25}$ + 30.4 (c = 0.56 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (3 H, t, *J* = 6.8 Hz, $OCO(CH_2)_4C\underline{H_3}$), 1.20 - 1.30 (2 H, m $OCO(CH_2)_3C\underline{H_2}CH_3$), 2.16 - 2.31 (4 H, m, $OCOCH_2(C\underline{H_2})_2CH_2CH_3$), 2.24 (2 H, t, *J* = 7.3 Hz, OCO $OCOC\underline{H_2}(CH_2)_3CH_3$), 3.38 (1 H, dd, $J_{6a,6b}$ = 10.7 Hz, $J_{5,6b}$ = 1.6 Hz, H-6b), 3.52 (1 H, dd, $J_{6a,6b}$ = 10.8 Hz, $J_{5,6a}$ = 3.7 Hz, H-6a), 3.53 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4), 3.56 (1 H, dd, $J_{2,3}$ = 9.4 Hz, $J_{1,2}$ = 3.5 Hz, H-2), 3.60 (1 H, dd, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 3.8 Hz, H-2'), 3.68 (1 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4'), 4.03 (1 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), 4.06 (1 H, at, $J_{2,3}$= $J_{3,4}$ = 8.8 Hz, H-3'), 4.06 (1 H, dd, $J_{6a,6b}$ = 10.8 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b'), 4.11 - 4.18 (2 H, m, H-6a', H-5'), 4.25 (1 H, ddd, $J_{4,5}$ = 9.9 Hz, $J_{5,6b}$ = 3.4 Hz, $J_{5,6a}$ = 2.2 Hz, H-5), 4.38 (1 H, d, *J* = 12.3 Hz, 1 x OCH_2Ph), 4.46 (1 H, d, *J* = 10.7 Hz, 1 x OCH_2Ph), 4.52 (1 H, d, *J* = 11.0 Hz, 1 x OCH_2Ph), 4.54 (1 H, d, *J* = 12.0 Hz, 1 x OCH_2Ph), 4.68 (2 H, d, *J* = 11.7 Hz, 2 x OCH_2Ph), 4.72 (2 H, d, *J* = 12.0 Hz, 2 x OCH_2Ph), 4.81 (1 H, d, *J* = 10.7 Hz 1 x

OCH_2Ph), 4.86 (3 H, d, J = 11.1 Hz, 3 x OCH_2Ph), 4.99 (1 H, d, J = 10.8 Hz, 1 x OCH_2Ph), 5.01 (1 H, d, J = 10.7 Hz, 1 x OCH_2Ph), 5.20 (1 H, d, $J_{1,2}$ =3.3 Hz, H-1), 5.21 (1 H, d, $J_{1,2}$ = 3.2 Hz, H-1'), 7.21 - 7.35 (35 H, m, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 13.9 ($OCO(CH_2)_4\underline{C}H_3$), 22.2 (1 C, 1 x $OCO(CH_2)_3\underline{C}H_2CH_3$), 24.5 (1 C, 1 x $OCO(CH_2)_2\underline{C}H_2CH_2CH_3$), 31.2 (1 C, $OCOCH_2\underline{C}H_2(CH_2)_2CH_3$), 34.0 (1 C, $OCO\underline{C}H_2(CH_2)_3CH_3$), 62.5 (C-6'), 68.1 (C-6), 69.0 (C-5), 70.7 (C-5'), 72.7 (OCH_2Ph), 72.9 (OCH_2Ph), 73.5 (OCH_2Ph), 75.1 (OCH_2Ph), 75.1 (OCH_2Ph), 75.6 (OCH_2Ph), 75.7 (OCH_2Ph), 77.5 (C-4 or C-4'), 77.6 (C-4 or C-4'), 79.2 (C-2 or C-2'), 79.4 (C-2 or C-2'), 81.6 (C-3 or C-3'), 81.8 (C-3 or C-3'), 94.0 (C-1 or C-1'), 94.4 (C-1 or C-1'), 127.3, 127.5, 127.5, 127.6, 127.6, 127.7, 127.8, 127.9, 127.9, 127.9, 128.1, 128.3, 128.4, 128.4, 128.5 (7 x OCH_2Ph), 137.7, 137.9 (2 C), 138.0, 138.3, 138.6, 138.8, (7 x 1 C = 7 x OCH_2Ph) 173.5 (C=O); IR (thin film): 3063, 3031, 2925, 2855 (C=CH), 1735 (C=O), 1586, 1496, 1454, 1360 (C=C), 1262, 1219, 1156, 1100, 772 cm^{-1}; MS (m/z): $[M+Na]^+$ calcd. for $C_{67}H_{74}O_{12}Na^+$,1093.51;found, 1093.4; isotopic distribution: species observed $[M+Na]^+$, peaks observed 1093.47 (100%), 1094.48 (73.7 %) 1095.49 (27.4 %), 1096.50 (6.4%) peaks calculated 1093.51 (100%), 1094.51 (73.8%), 1095.51(28.3%), 1096.52 (8.2%)

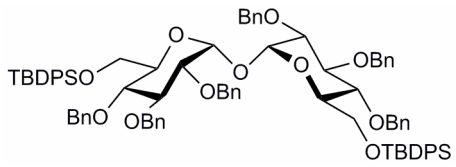
***6-O-hexanoyl-α,α-D-trehalose* (26)**



66 (6.2 mg, 0.019 mmol) was dissolved in 20 mL ethanol and circulated through a 10% Pd/C cartridge in the H-Cube™ at 80 bar, 45 ˚C and 1 mL/min. Reaction was monitored by mass

spec and after 3 hours TLC (2:1 ethyl acetate/methanol) indicated complete conversion from starting material (R_f 1) to product (R_f 0.4). Reaction was concentrated *in vacuo* and purified via column chromatography (3:1 ethyl acetate:methanol) to give **26** as a white crystalline solid (2.6 mg, 93%). M.p. = 135-137 ^{o}C; TLC (EtOAc:MeOH, 2:1, v/v): R_f=0.34; $[\alpha]_D^{25}$ = 111.3 (c = 1.0, MeOH). ^{1}H NMR (500 MHz, D_2O): δ 0.79 (3 H, t, *J* = 6.9 Hz, $OCO(CH_2)_4C\underline{H}_3$), 1.17 - 1.26 (4 H, m, $OCOCH_2CH_2(C\underline{H}_2)_2CH_3$), 1.54 (2 H, quin, *J* = 7.3 Hz, $OCOCH_2C\underline{H}_2(CH_2)_2CH_3$), 2.35 (2 H, t, *J* = 7.4 Hz, $OCOC\underline{H}_2CH_2(CH_2)_2CH_3$), 3.36 (1 H, at, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.41 (1 H, at, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4'), 3.55 (1 H, dd, $J_{2,3}$ = 9.9 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 3.57 (1 H, dd, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.8 Hz, H-2'), 3.66 - 3.82 (5 H, m, H-6a, H-6b, H-5, H-3, H-3'), 3.94 (2 H, ddd, $J_{4,5}$ = 10.2 Hz, $J_{5,6a}$ = 4.8 Hz, $J_{5,6b}$ = 2.0 Hz, H-5'), 4.22 (2 H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 5.0 Hz, H-6a'), 4.35 (2 H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b'), 5.07 (1 H, d, $J_{1,2}$ = 3.9 Hz, H-1), 5.10 (1 H, d, $J_{1,2}$ = 3.8 Hz, H-1'); ^{13}C NMR (126 MHz, D_2O): δ 13.1 (1 C, $OCO(CH_2)_4\underline{C}H_3$), 21.6 (1 C, $OCO(CH_2)_3\underline{C}H_2CH_3$), 24.0 (1 C, $OCO(CH_2)_3\underline{C}H_2CH_2CH_3$), 30.5 (1 C, $OCOCH_2\underline{C}H_2(CH_2)_2CH_3$), 33.7 (1 C, $OCO\underline{C}H_2(CH_2)_3CH_3$), 60.5 (C-6), 62.9 (C-6'), 69.6 (C-5'), 69.6 (C-4 or C-4'), 69.9 (1 C-4 or C-4'), 70.9 (C-2 or C-2'), 70.9 (C-2 or C-2'), 72.2 (C-5), 72.3 (C-3 or C-3'), 72.6 (C-3 or C-3'), 93.2 (C-1'), 93.4 (C-1), 176.8 (C=O); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{18}H_{32}O_{12}Na^+$,463.1786;found, 463.1793.

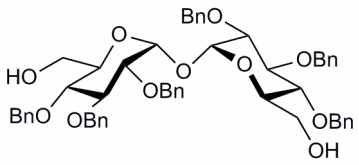
***6,6'-di-O-tert-butyldiphenylsilyl-2,3,4,2',3',4'-hexa-O-benzyl-α,α-D-trehalose* (67)**



To a stirred suspension of D-trehalose (2 g, 5.85 mmol, 1 eq) and imidazole (0.39 g, 5.29 mmol, 0.9 eq) in dry DMF (10 mL) was added *tert*-butyl diphenylchlorosilane (TBDPS-Cl) (3 mL, 11.5 mmol, 2.0 eq) at RT. After stirring for 36 hours, TLC (2:1 ethyl acetate/methanol), indicated the complete consumption of starting materials and the formation of the di TBDPS-trehalose (R_f 0.35) as well as the mono TBDPS-trehalose (R_f 0.2). The reaction mixture was concentrated *in vacuo* and purification attained via silica gel chromatography (2:1, ethyl acetate/isopropanol) to give the desired product as a mixture of mono and di-TBDPS protected compounds as a white solid. (2.3 g, 55%), which were used without further purification. This mixture was dissolved in anhydrous DMF (25 mL), and sodium hydride (60% dispersed in mineral oil) (700 mg, 29.1 mmol) was added portionwise for a period of 10 min at 0 ºC. Benzyl bromide (2 mL, 11.6 mmol, 6 eq) was then added dropwise and the mixture left to stir under an atmosphere of argon at room temperature. After an 18 h period, TLC (5:1 petrol/ethyl acetate) indicated the formation of product (R_f 0.9) with complete consumption of the starting material (R_f 0). The reaction mixture was quenched by the slow addition of methanol (150 mL) and stirred for 30 min, at which point the resulting solution was concentrated *in vacuo*. The residue was dissolved in DCM (800 mL), washed with water and brine, filtered and concentrated *in vacuo*. Purification by column column chromatography (petrol/ethyl acetate, 10:1) afforded **67** and **64** as a 2:1 mixture of products (by NMR), which were not fully separated (2.987 g, 41% over two steps) as a viscous clear oil. Further column chromatography yielded pure **67** (300 mg, 5% over two steps). Data for compound **67**; TLC (petrol:EtOAc, 5:1, v/v): R_f=0.90; $[\alpha]_D^{25}$ + 23.6 (c = 1.0 in

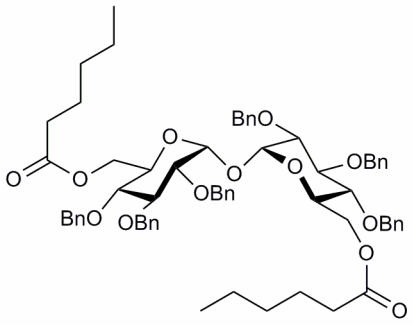
$CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.28 (18 H, s, 2 x C (CH_3)), 3.75 (2 H, dd, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 3.8 Hz, H-2, H-2'), 3.79 (2 H, dd, $J_{6a,6b}$ = 10.5 Hz, $J_{5,6b}$ = 1.8 Hz, H-6b, H-6b'), 3.92 (2 H, dd, $J_{6a,6b}$ = 10.6 Hz, $J_{5,6a}$ = 2.8 Hz, H-6a, H-6a'), 4.03 (2 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4, H-4'), 4.18 - 4.28 (4 H, m, H-5, H-5', H-3,H-3'), 4.72 (2 H, d, J = 11.8 Hz, 2 x OCH_2Ph), 4.80 (2 H, d, J = 11.9 Hz, 2 x OCH_2Ph), 4.89 (2 H, d, J = 10.9 Hz, 2 x OCH_2Ph), 5.04 (2 H, d, J = 10.6 Hz, 2 x OCH_2Ph), 5.10 (2 H, d, J = 10.6 Hz, 2 x OCH_2Ph), 5.15 (2 H, d, J = 10.6 Hz, 2 x OCH_2Ph), 5.39 (2 H, d, $J_{1,2}$ = 3.5 Hz, H-1, H-1'), 7.13 - 7.63 (40 H, m, Ar-H) 7.79 - 7.93 (10 H, m, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 19.5 (6 C, s, 3 x $C(\underline{C}H_3)_3$), 27.0 (2 C, s, 2 x $\underline{C}(CH_3)_3$), 65.6 (2 C, C-6, C-6'), 71.7 (2 C, C-5, C-5'), 72.9 (2 C, OCH_2Ph), 75.3 (2 C, OCH_2Ph), 76.0 (2 C, OCH_2Ph), 77.7 (2 C, C-4, C-4'), 80.3 (2 C, C-2, C-2'), 82.1 (2 C, C-3, C-3'), 94.2 (2 C, C-1, C-1'), 126.1, 127.0, 127.2, 127.4, 127.7, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.3, 128.6, 128.9, 129.2, 129.8, 129.9, 133.5, 133.6, 133.7, 135.6, 135.8, (6 x OCH_2Ph, 4 x OTBDPS), 137.9, 138.2, 138.4, 138.7, 138.9, (10 C, 6 x 1 C, OCH_2Ph, 4 x 1 C, OTBDPS); IR (thin film): 3069, 2930, 2856 (C=CH), 1454, 1428, (C=C), 1219, 111, 1069, 1027, 824, 773 cm^{-1}; MS (m/z): $[M+ NH_4]^+$ calcd. for $C_{86}H_{98}NO_{11}Si^+$,1376.67; found,1376.6; isotopic distribution: Species observed $[M+Na]^+$, peaks observed 1381.59 (91.5%), 1382.59 (100%), 1383.59 (48.3%), 1384.59 (17.8%), 1385.60 (5.8%), 1386.59 (1.7%) peaks calculated 1381.62 (95.5%), 1382.62 (100%), 1383.62 (59.8%), 1384.62 (25.3%), 1385.63 (8.0%).

***2,3,4,2',3',4'-hexa-O-benzyl-α,α-D-trehalose* (68)**[31]



The 2:1 mixture of **67** and **64** (1.01g, 0.83 mmol) was dissolved in DMF (10mL) and to this was added TBAF dropwise (70% in H_2O) (1 mL, 2.87 mmol, 3.5 eq). Reaction was stirred for 3 h at 60 ˚C. TLC (2:1 petrol/ethyl acetate) indicated products (R_f 0.4) and (R_f 0.05) and complete consumption of starting material (R_f 1.0). Crude product was washed with satd brine and $NaHCO_3$, extracted into dichloromethane and concentrated *in vacuo.* Purification by column chromatography (2:1 petrol/ethyl acetate followed by 1:1 petrol/ethyl acetate and ethyl acetate) yielded **68** (202.5 mg, 36%, based on 2:1 ratio of starting material) as a clear oil and **65** (156 mg, 58% based on 2:1 ratio of starting material) and recovered starting material (130 mg, 7%). Combined yield of both products was 61%. Data for compound **68**; TLC (petrol:EtOAc, 2:1, v/v): R_f=0.40; $[\alpha]_D^{25}$ + 88.1 (c = 1.0 in $CHCl_3$) [Lit. $[\alpha]_D^{25}$ +104 (c = 1.6 in $CHCl_3$)][31]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.55 (2 H, dd, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 3.1 Hz, H-2, H-2'), 3.59-3.64 (6 H, m, H-6a, H-6a', H-6b, H-6b'), 3.61 (2 H, at, $J_{3,4}$ = $J_{4,5}$ = 10.1 Hz, H-4, H-4'), 4.05 - 4.13 (4 H, m, H-3, H-3', H-5, H-5'), 4.67 (2 H, d, J = 10.9 Hz, 2 x OCH_2Ph), 4.68 (2 H, d, J = 11.9 Hz, 2 x OCH_2Ph), 4.73 (2 H, d, J =11.9 Hz, 2 x OCH_2Ph), 4.90 (2H, d, J =10.9 Hz, 2 x OCH_2Ph), 4.91 (2 H, d, J =11.4 Hz, 2 x OCH_2Ph), 5.02 (2 H, d, J =10.8 Hz, 2 x OCH_2Ph), 5.16 (2 H, d, $J_{1,2}$ = 3.8 Hz, H-1, H-1), 7.15 - 7.52 (30 H, m, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 61.4 (2 C, C-6, C-6'), 71.4 (2 C, C-5, C-5'), 72.9 (2 C, 2 x OCH_2Ph), 75.0 (2 C, 2 x OCH_2Ph), 75.6 (1 C, 2 x OCH_2Ph), 76.7 (2 C, s, C-4, C-4'), 79.4 (2 C, C-2, C-2'), 81.6 (2 C, C-3, C-3'), 93.9 (2 C, C-1, C-1'), 123.8, 127.5, 127.6, 127.7, 127.9, 128.1, 128.4, 128.5 (6 x OCH_2Ph), 138.0, 138.2, 138.7 (6 x 1 C, 6 x OCH_2Ph); MS (m/z): $[M+NH_4]^+$ calcd. for $C_{54}H_{62}NO_{11}^+$,900.07;found, 900.4.

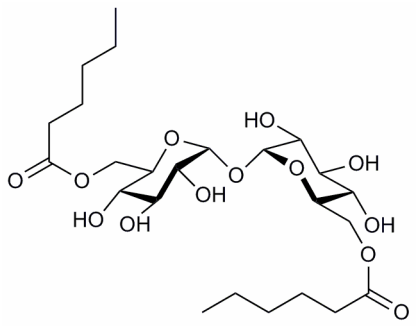
***6,6'-di-O-hexanoyl-2,3,4,2',3',4'-hexa-O-benzyl-α,α-D-trehalose* (69)**



68 (1 g, 1.13 mmol, 1 eq) was dissolved in anhydrous pyridine (2 mL) and to this was added hexanoyl chloride (0.5 mL, 3.8 mmol, 3.3 eq). Reaction was stirred for 30 min at RT upon which time TLC (2:1 petrol/ethyl acetate) indicated complete conversion from starting material (R_f 0.05) to product (R_f 0.9). Reaction was washed with satd. $NaHCO_3$ solution and concentrated *in vacuo*. Column chromatography (10:1 petrol/ ethyl acetate, 1% triethylamine) yielded the desired product as a clear oil (1.12g, 91%). TLC (petrol:EtOAc, 2:1, v/v): R_f=0.90; $[\alpha]_D^{25}$ + 75.6 (c = 0.39 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (6 H, t, J = 6.9 Hz, 2 x $OCO(CH_2)_4C\underline{H}_3$), 1.23 - 1.29 (8 H, m, 2 x $OCO(CH_2)_2(C\underline{H}_2)_2CH_3$), 1.54 - 1.65 (8 H, m, 2 x $OCO(C\underline{H}_2)_2(CH_2)_2CH_3$), 3.54 (4 H, at, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4, H-4'), 3.57 (4 H, dd, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.5 Hz, H-2, H-2'), 4.06 (4 H, at, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3, H-3'), 4.06 (4 H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 2.0 Hz, H-6a, H-6a'), 4.16 (2 H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 3.3 Hz H-6b, H-6b'), 4.24 (2 H, ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6b}$ = 3.4 Hz, $J_{5,6a}$ = 2.0 Hz, H-5, H-5'), 4.53 (2 H, d, J = 10.6 Hz, OCH_2Ph), 4.69 (2 H, d, J = 12.1 Hz, OCH_2Ph), 4.73 (2 H, d, J = 11.9 Hz, OCH_2Ph), 4.88 (4 H, d, J = 10.6 Hz, OCH_2Ph), 4.88 (3 H, d, J = 10.9 Hz, OCH_2Ph), 5.02 (2 H, d, J = 10.6 Hz, OCH_2Ph), 5.18 (2 H, d, $J_{1,2}$ = 3.5 Hz, H-1, H-1'), 7.04 - 7.51 (40 H, m, Ar-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 13.9 (2 C, $OCO(CH_2)_4\underline{C}H_3$), 22.3 (2 C, $OCO(CH_2)_4\underline{C}H_2CH_3$), 24.5 (2 C, $OCO(CH_2)_4\underline{C}H_2CH_2CH_3$), 31.3 (2 C, $OCOCH_2\underline{C}H_2(CH_2)_2CH_3$), 34.0 (2 C, $OCO\underline{C}H_2(CH_2)_3CH_3$), 62.5 (2 C, C-6, C-6'), 69.1 (2 C, C-5, C-5'), 72.9 (2 C, OCH_2Ph), 75.2 (4 C, 2 x OCH_2Ph), 75.7 (2 C, OCH_2Ph), 77.4 (2 C, C-4, C-4'),

79.2 (2 C, C-2, C-2'), 81.6 (2 C, C-3, C-3'), 94.0 (2 C, C-1, C-1'), 127.5, 127.7, 127.8, 127.9, 128.1, 128.4, 128.4 (6 x OCH_2Ph); 137.8, 137.8, 138.5 (6 x 1 C = 6 x OCH_2Ph); 173.5 (2 C, 2 x C=O); IR (thin film): 3419 br (OH), 2956, 2925, 2854 (C=CH), 1733 (C=O), 1637, 1456 (C=C), 1219, 1177, 1150, 1101, 1078, 1053, 1026, 990, 772 cm^{-1}; MS (m/z): $[M+NH_4]^+$ calcd. for $C_{66}H_{82}NO_{13}^+$,1098.58;found, 1096.5; isotopic distribution: species observed $[M+Na]^+$, peaks observed 1101.53 (100%), 1102.53 (72.8%), 1103.54 (28.8%), 1104.54 (8.1%), 1105.54 (1.8%), peaks calculated 1101.52 (100%), 1102.52 (74.4%), 1103.53 (30.0%), 1104.53 (8.3%), 1105.53 (1.9%), 1105.49 (1.4%).

***6,6'-di-O-hexanoyl-α,α-D-trehalose* (27)**

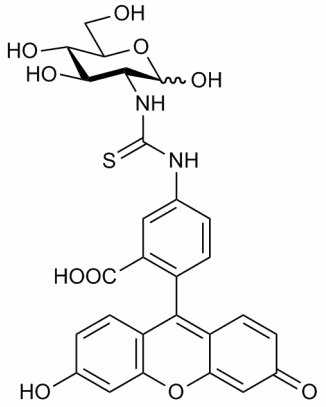


69 (474 mg, 0.44 mmol) was dissolved in 20 mL ethanol and the solvent was degassed under alternating reduced pressure and argon. 10% Pd/C (300 mg) was added to the solution, which was then activated through repeated vacuum, flush cycles with 2 hydrogen balloons. Reaction was stirred at RT for 16 h, upon which time ESI mass spec showed complete conversion to the desired product. TLC (2:1 ethyl acetate/methanol) indicated complete conversion from starting material (R_f 1) to product (R_f 0.6). Reaction was concentrated *in vacuo* to produce the desired product as a crystalline white solid (120.0 mg, 55%). M.p. = 157.7-159.0 ^oC; TLC (EtOAc:MeOH, 2:1, v/v): R_f=0.60; $[\alpha]_D^{25}$ + 152.3 (c = 0.13 in H_2O); ^1H NMR (500 MHz, D_2O): δ 0.78 (6 H, t, *J*= 6.9 Hz, 2 x $OCO(CH_2)_4C\underline{H}_3$), 1.11 - 1.29 (8 H, m, 2 x

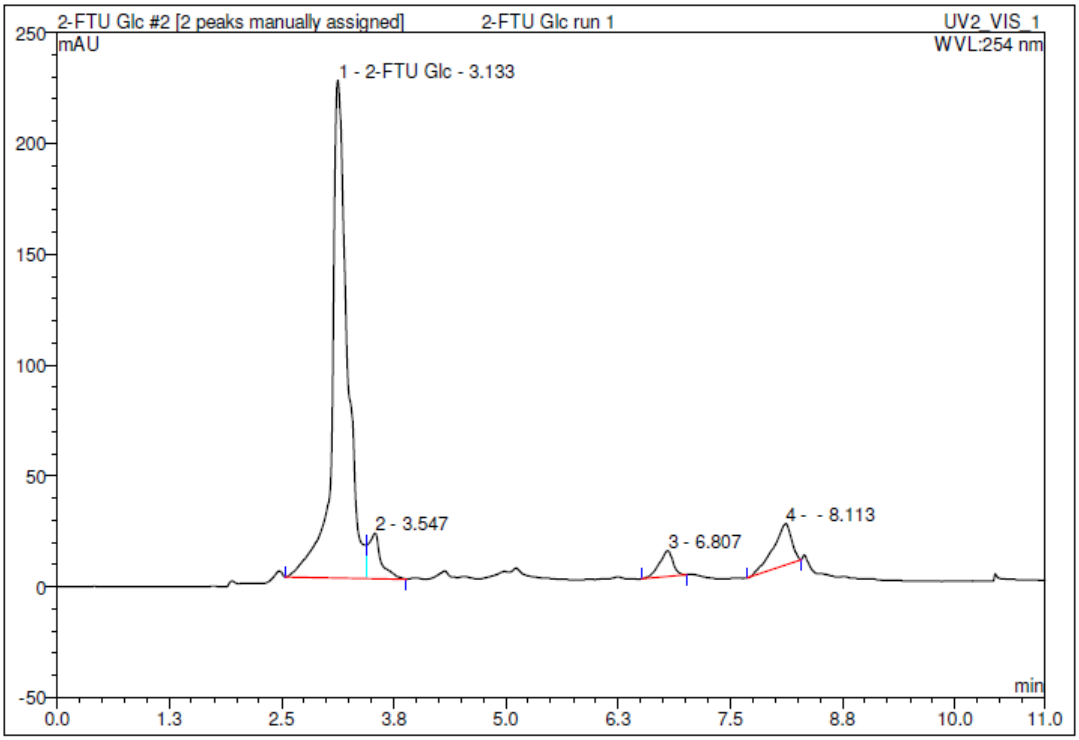
$OCO(CH_2)_2(C\underline{H}_2)_2CH_3$)1.53 (4 H, quin, J = 7.3 Hz, 2 x $OCOCH_2(C\underline{H}_2)(CH_2)_2CH_3$), 2.33 (4 H, t, J = 7.3 Hz, 2 x $OCOC\underline{H}_2(CH_2)_4CH_3$), 3.39 (2 H, at, $J_{3,4} = J_{4,5}$ = 9.6 Hz H-4, H-4'), 3.54 (2 H, dd, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$= 3.8 Hz, H-2, H-2'), 3.76 (2 H, at, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3, H-3'), 3.92 (2 H, ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.2 Hz, H-5, H-5'), 4.21 (2 H, dd, $J_{6a,6b}$ =12.3, $J_{5,6a}$ = 5.4 Hz, H-6a), 4.33 (2 H, dd, $J_{6a,6b}$ =12.3 Hz, $J_{5,6b}$ 2.2 Hz, H-6b), 5.05 (2 H, d, $J_{1,2}$ = 3.8 Hz, H-1, H-1'); ^{13}C NMR (126 MHz, D_2O): δ 13.1 (2 C, 2 x $OCO(CH_2)_4\underline{C}H_3$), 21.6 (2 C, 2 x $OCO(CH_2)_3\underline{C}H_2CH_3$), 24.0 (2 C, 2 x $OCO(CH_2)_2\underline{C}H_2CH_2CH_3$), 30.5 (2 C, 2 x OCO $CH_2\underline{C}H_2(CH_2)_2CH_3$), 33.8 (2 C, 2 x $OCO\underline{CH}_2(CH_2)_4CH_3$), 62.9 (2 C, C-6, C-6'), 69.7 (2 C, C-5, C-5'), 70.0 (2 C, C-4, C-4'), 70.92 (2 C, C-2, C-2'), 72.4 (2 C, C-3, C-3'), 93.3 (2 C, C-1, C-1'), 176.8 (2 C, 2 x C=O); HRMS (m/z): $[M+Na]^+$ calcd. for $C_{24}H_{42}O_{13}Na^+$,561.2518;found, 561.2513; analysis (calcd., found for $C_{24}H_{42}O_{13}$): C (53.52,53.56), H (7.86,7.84).

Synthesis of compound 28.

***2-deoxy-2-(N'-(Fluorescein-5-yl)-thioureido) -D-glucose* (28)**



Glucosamine hydrochloride (21.5 mg, 0.1 mmol) and fluorescein isothiocyanate (FITC, 58.4 mg, 0.15 mmol) were dissolved in a mixture of 75 mM solution of $NaHCO_3$ (3mL) and of acetonitrile (1.5 mL) . The mixture was heated at +55°C for 2 hours until TLC (1:2:8 water:isopropanol:ethyl acetate) indicated consumption of starting materials and formtion of two products α (R_f 0.64) and β (R_f 0.52). The mixture was concentrated under reduced pressure and purified by silica column chromatography (ethyl acetate then water:isopropanol:ethyl acetatate 1:2:8) to give the title compound **28** (29.7 mg, 0.052 mmol, 52%) as an orange solid. TLC (H_2O:*i*-PrOH:EtOAc,1:4:8, v/v/v): **28α** R_f=0.64; **28β** R_f=0.52; $[\alpha]_D^{25}$ = +32.4 (c=0.25 in CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6): δ **28α** 3.2-3.6 (3H, m, H-3α, H-4, H-6b), 3.62 (1H, m, H-6a), 3.72-3.82 (2H, m, H-5, H-2), 5.72 (1H, bs, H-1), 6.60 (4H, m, H-10' and H-12'), 6.70 (2H, m, H-9'), 7.29 (1H, d, $J_{7'-8'}$=8.3 Hz, H-7'), 8.03 (1H, dd, $J_{8'-7'}$=8.3 Hz, $J_{8'-2'}$=2.0Hz, H-8'), 8.19 (1H, d, $J_{2'-8'}$=2.0Hz, H-2'), 8.80 (1H, s, NHCS attached to C-2); **28β** 3.2-3.6 (H-3α, H-4, H-6b), 3.62 (1H, m, H-6a), 3.72-3.82 (3H, m, H-5, H-2, H-3), 4.19 (1H, d, J_{4-3}=2.4 Hz, H-4), 4.25 (1H, d, J_{2-1}=6.3 Hz, H-2), 6.16 (1H, d, J_{1-2}=6.3 Hz, H-1), 6.60 (4H, m, H-10' and H-12'), 6.70 (2H, m, H-9'), 7.28 (1H, d, $J_{7'-8'}$=8.3 Hz, H-7'), 8.02 (1H, dd, $J_{8'-7'}$=8.3 Hz, $J_{8'-2'}$=2.0Hz, H-8'), 8.17 (1H, d, $J_{2'-8'}$=2.0Hz, H-2'), 9.46 (1H, s, NHCS attached to C-2); ^{13}C NMR (126 MHz, DMSO-d6): δ **28α** 63.2 (C-6), 65.2 (C-2), 68.9 (C-5),



Supplementary Figure 34. *Preparative HPLC trace for the purification of* **28**. *Conditions: Phenomenex Synergi Hydro C18 column (150 mm x 21.2 mm, 4 µm), eluting with water with 0.1 % TFA (A) and acetonitrile (B) eluting at a flow rate of 20mL/min; Gradient: 0 min B=%0, 1 min B=20%, 5.5 min B=40%, 6 min B=100%, 8 min B=0%; Compound* **28** *elutes at 3.1 min (labeled 2-FTU Glc) while starting materials FITC elute at 6.8 and 8.1 min.*

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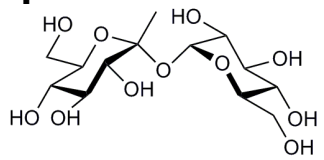

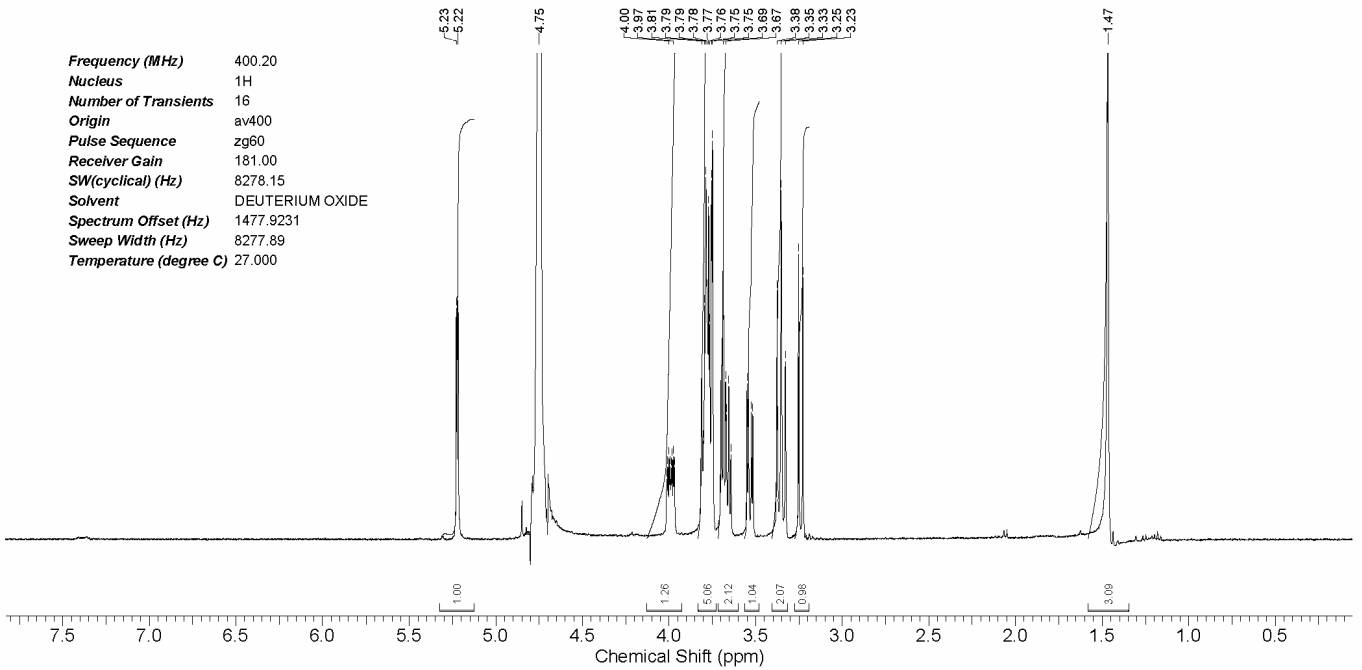

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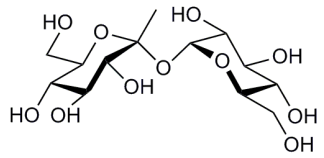

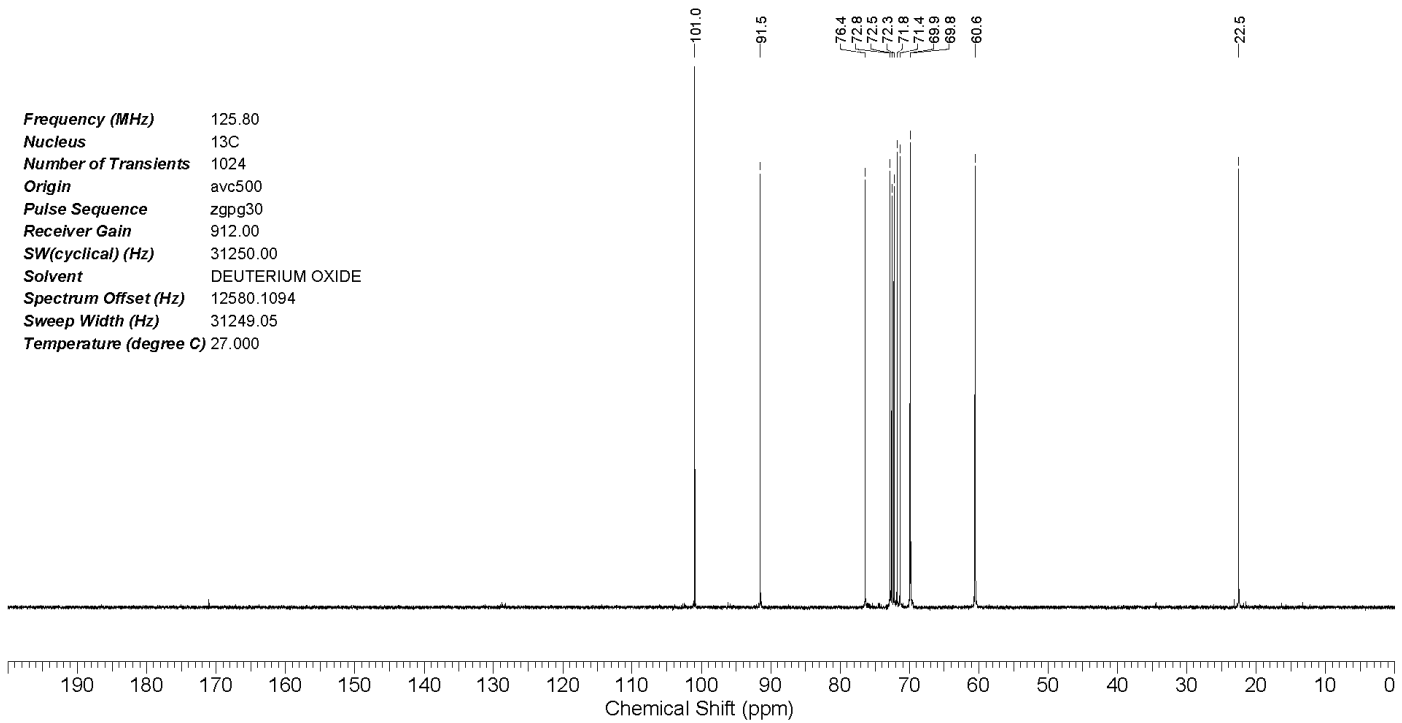

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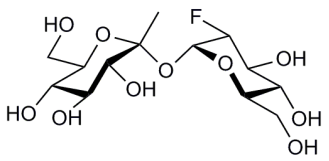

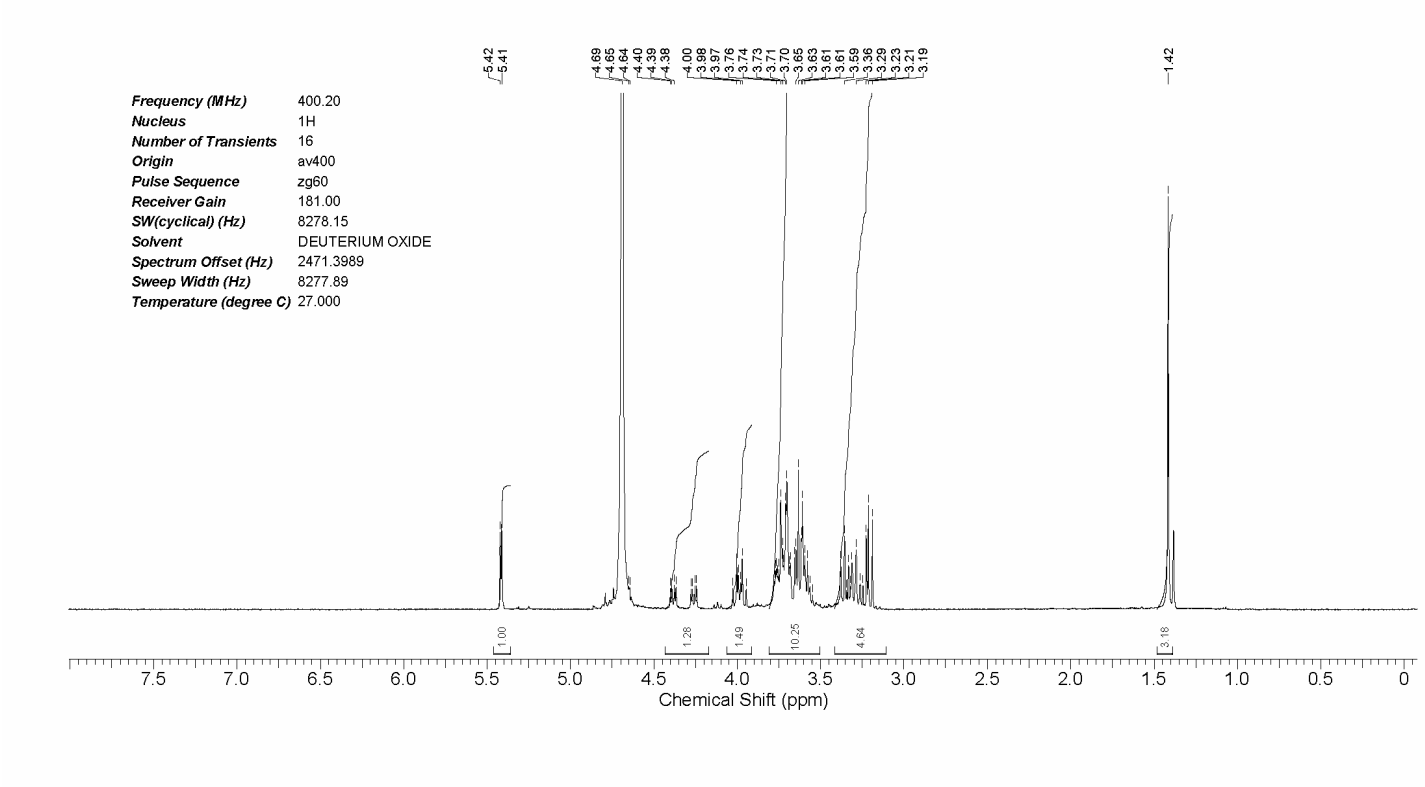

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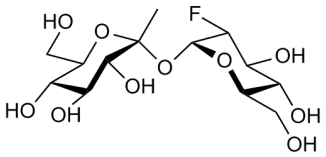

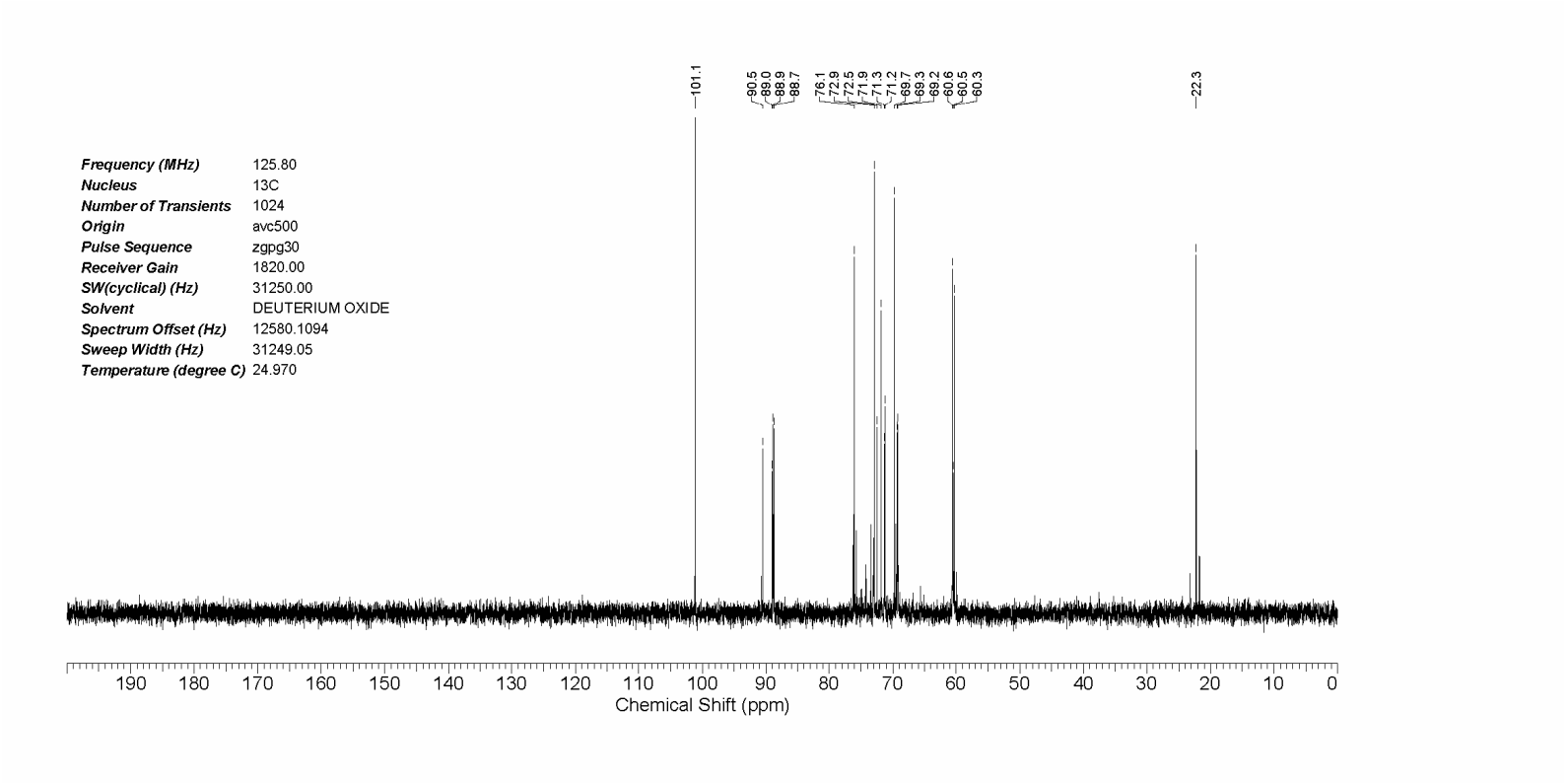

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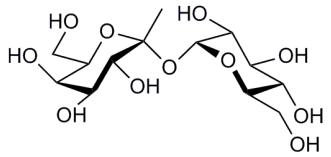

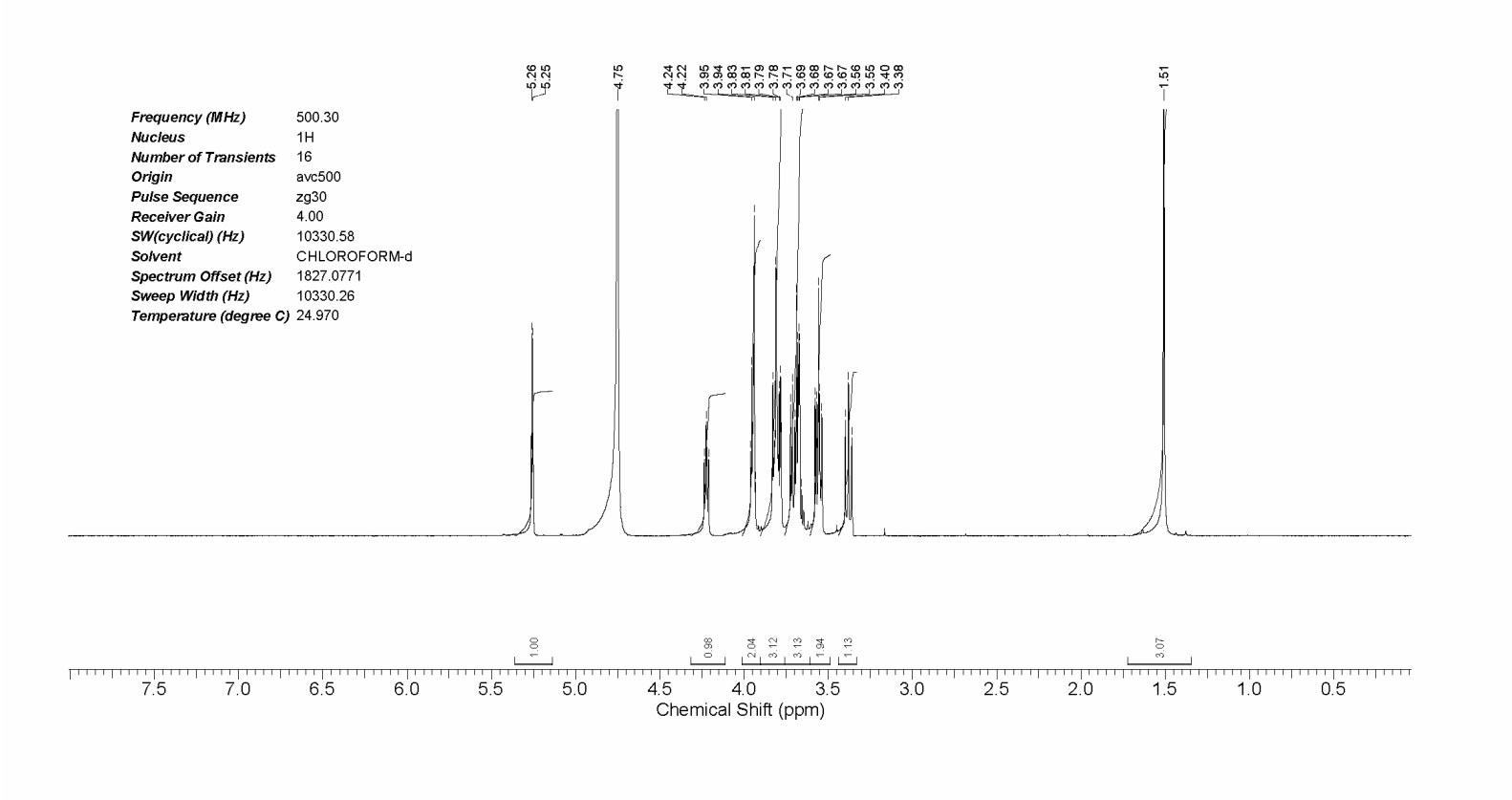

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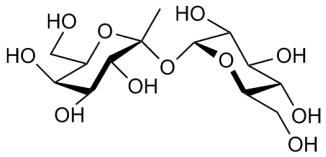

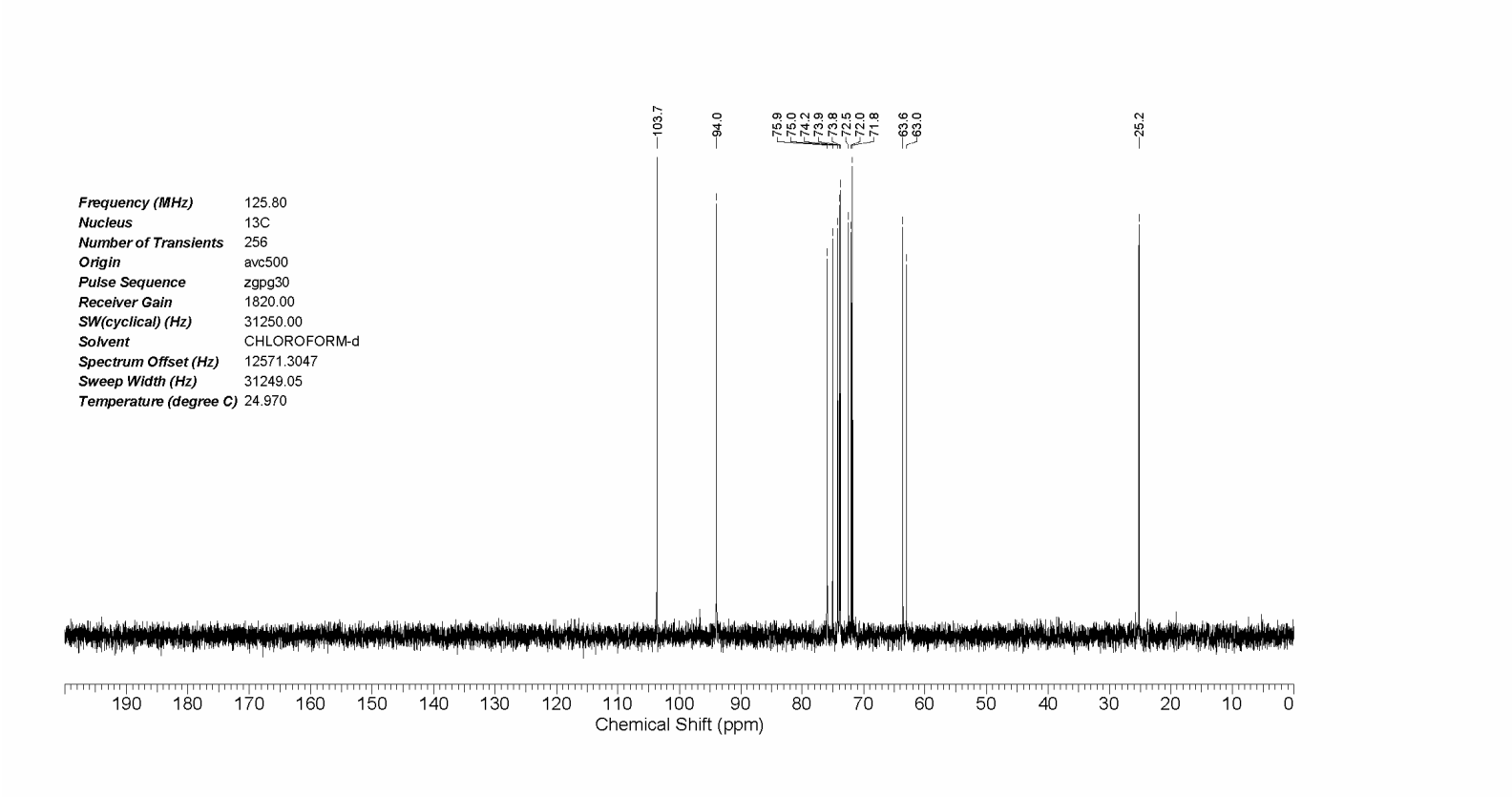

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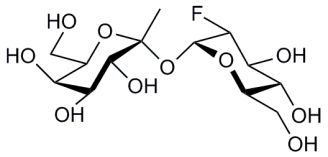

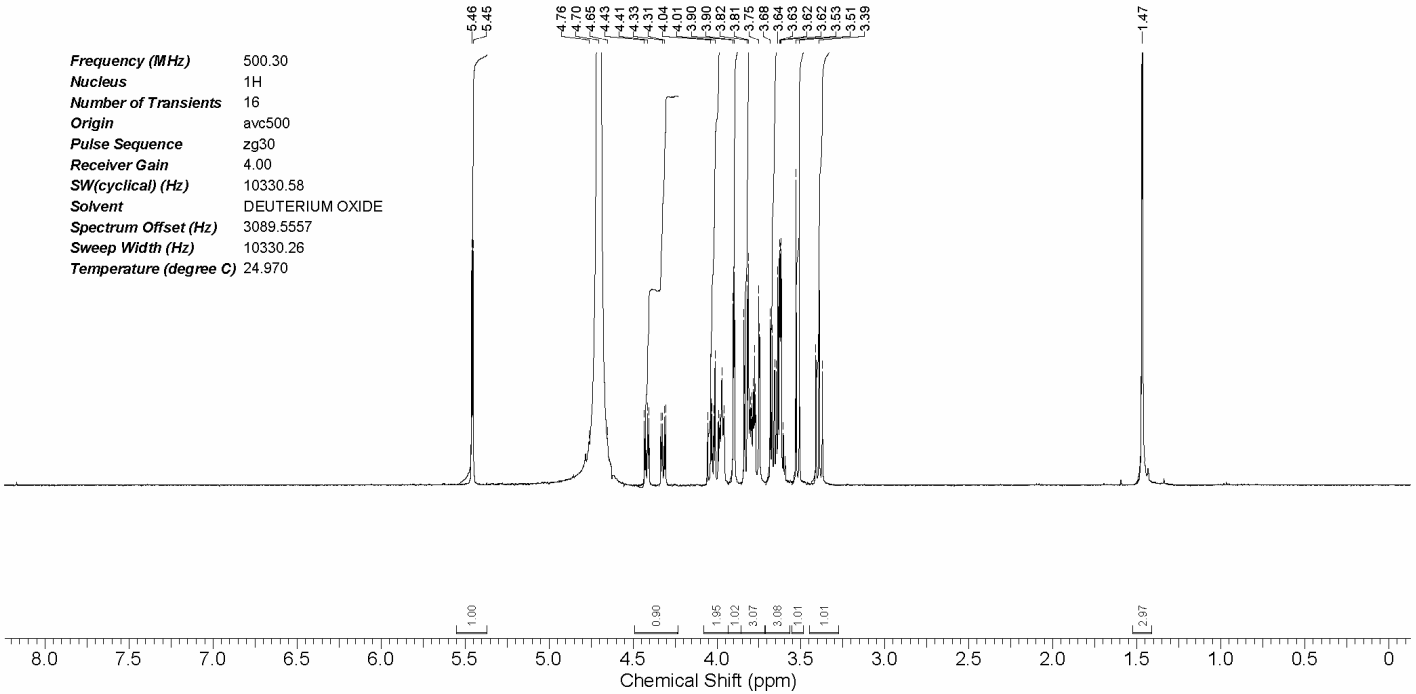

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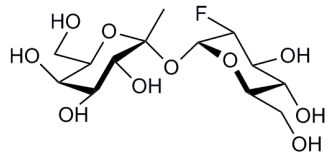

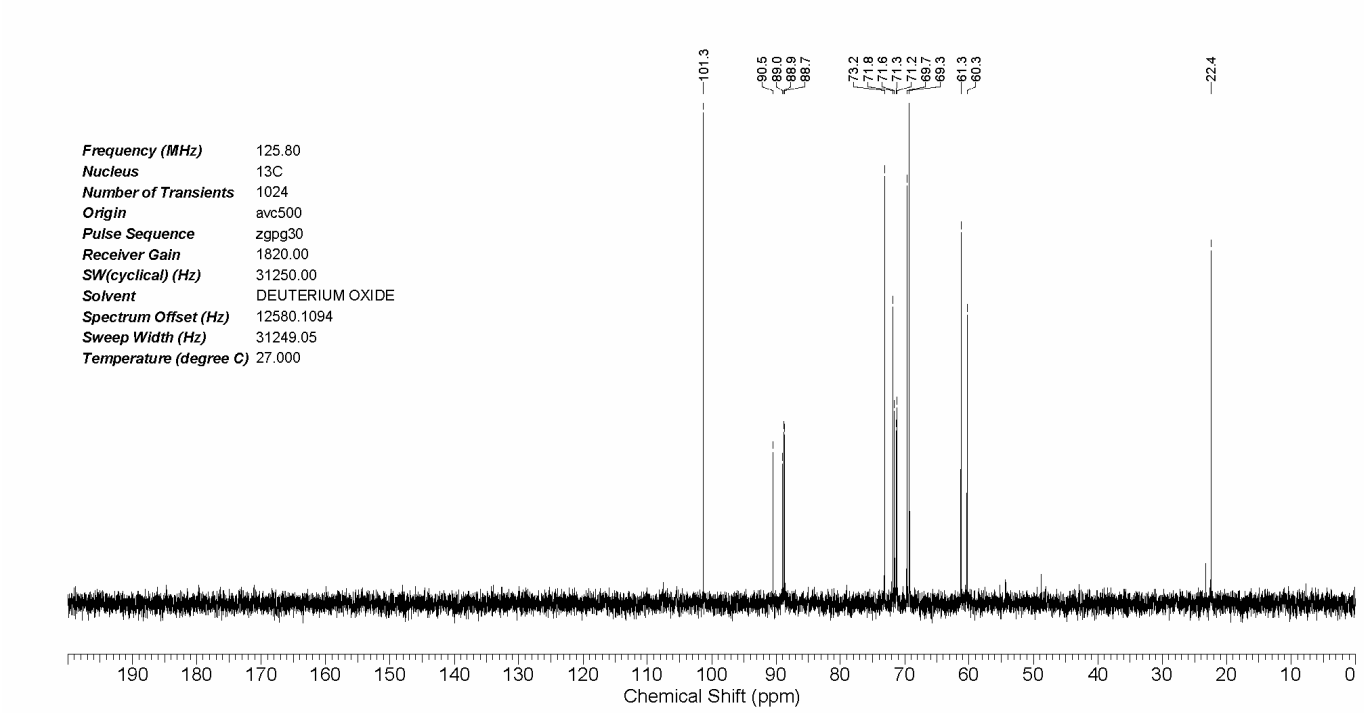

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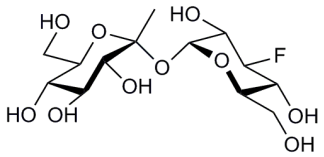

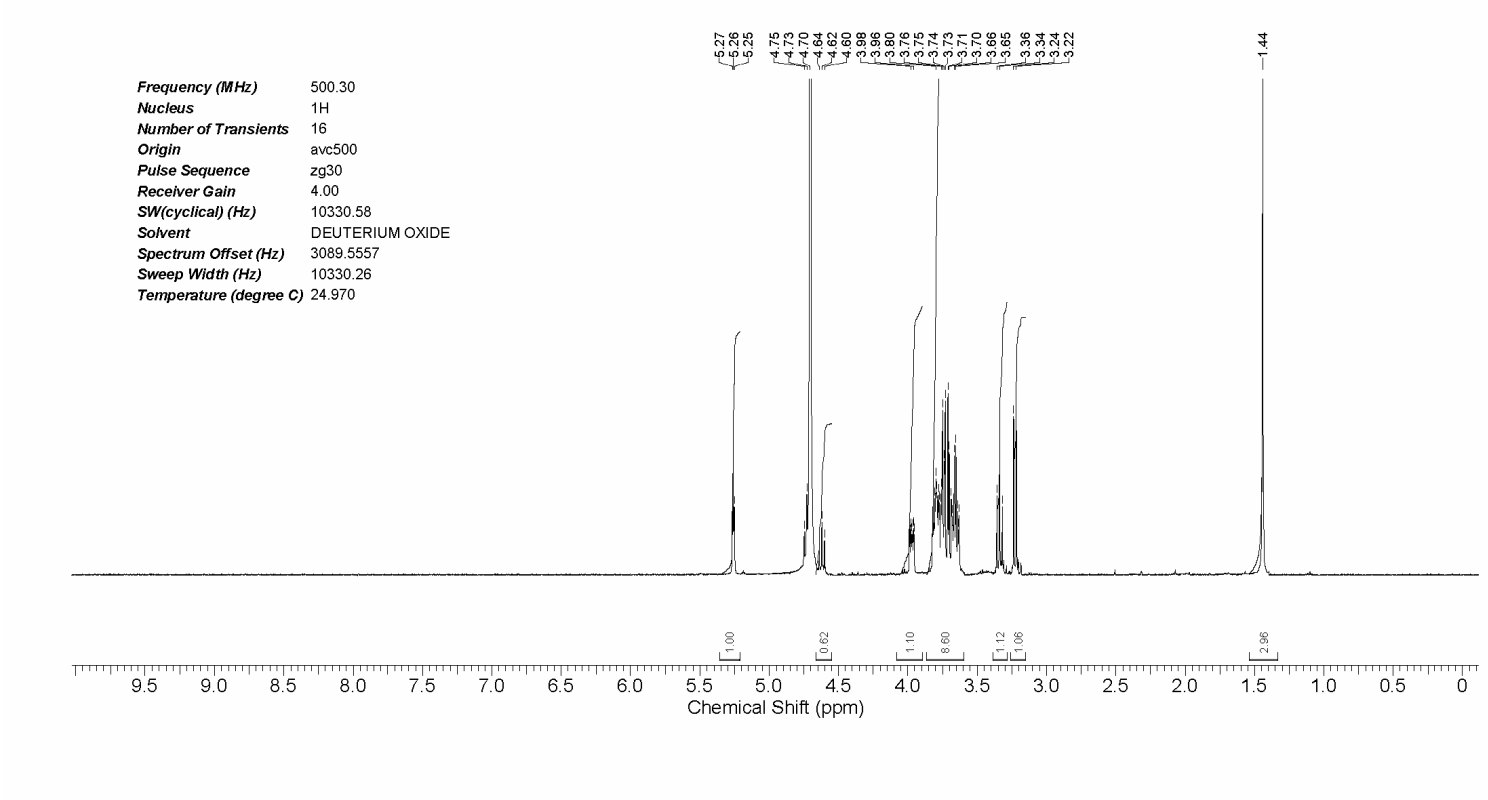

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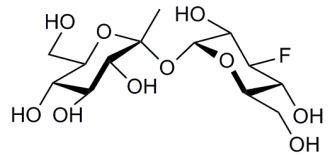

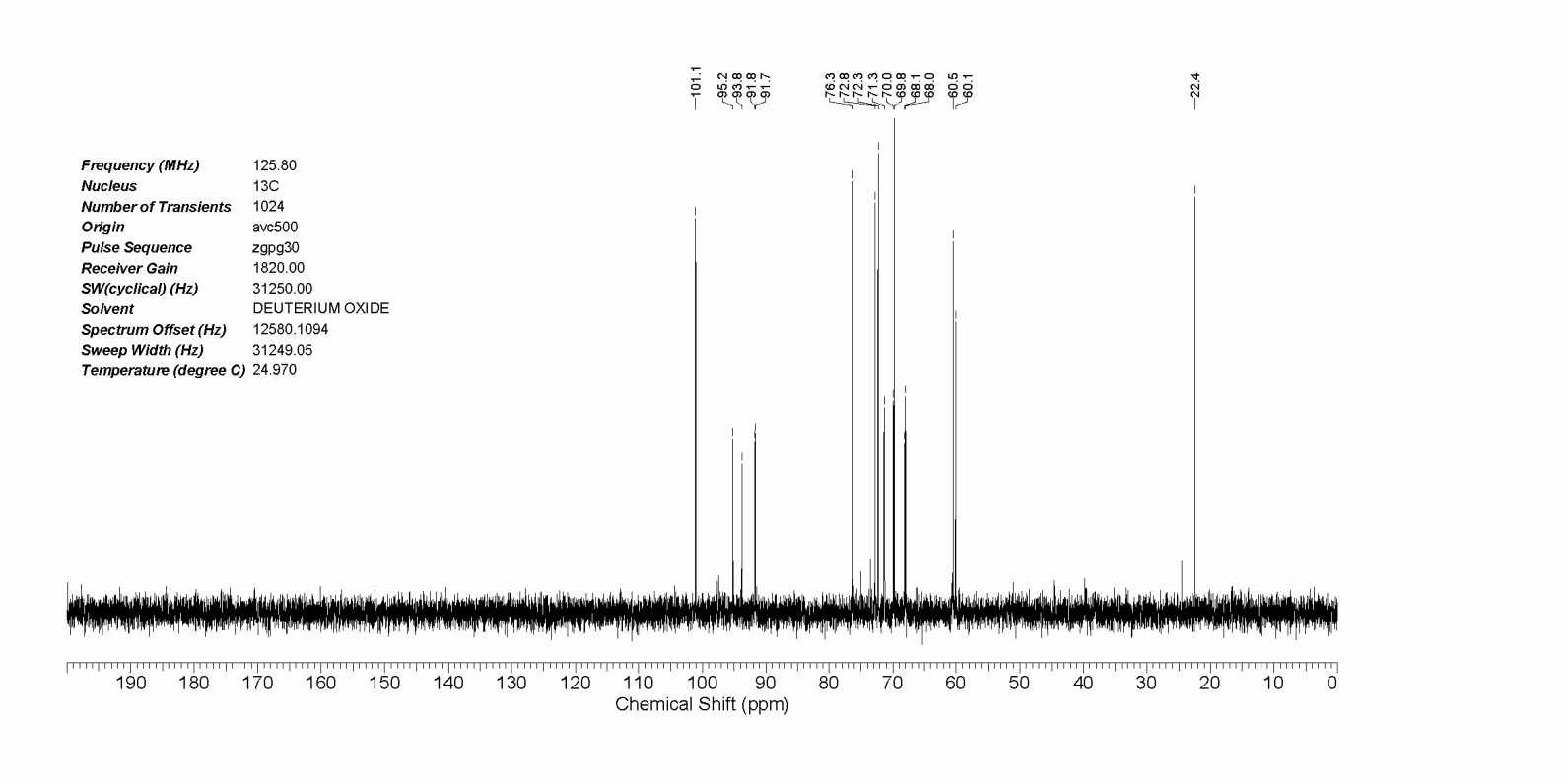

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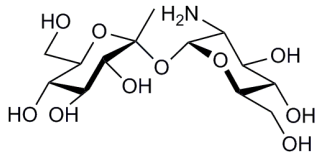

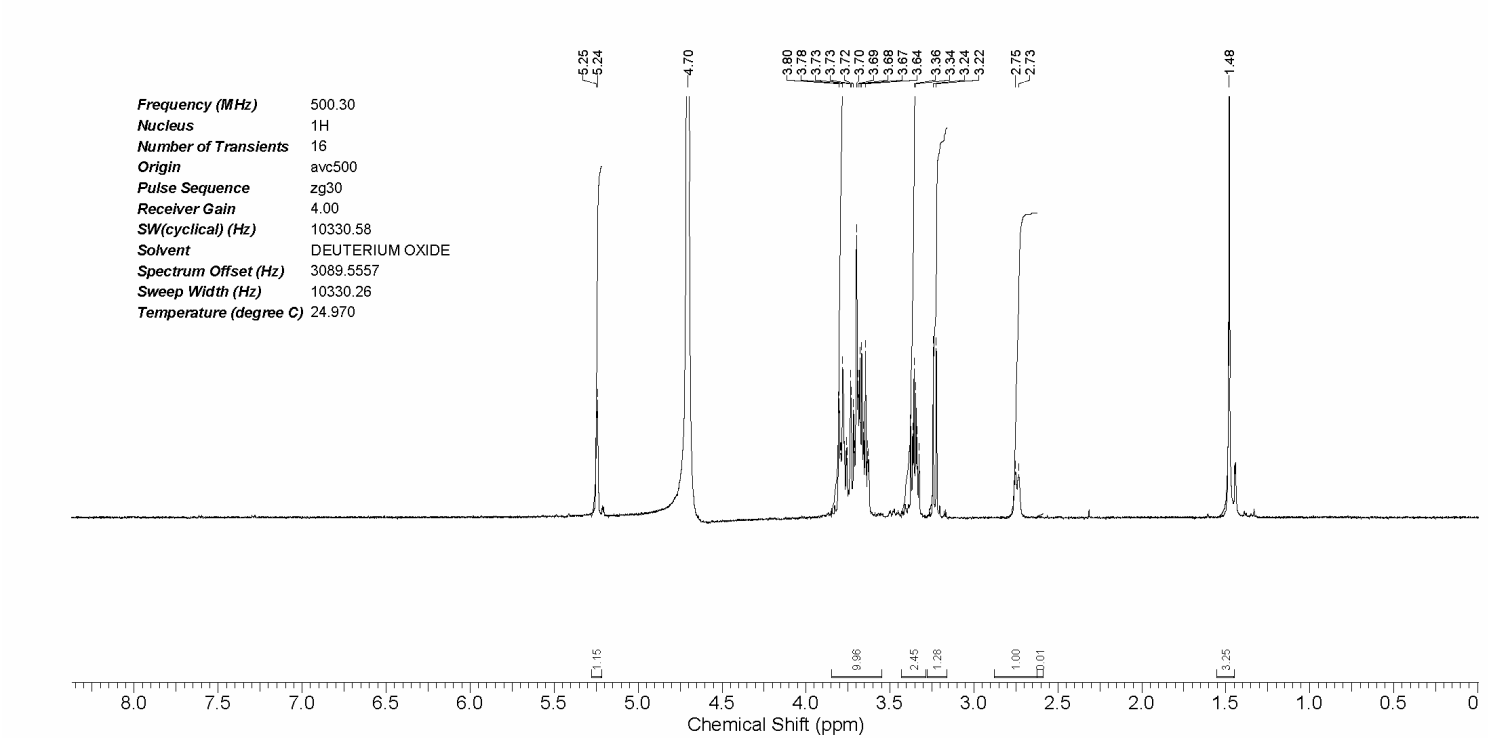

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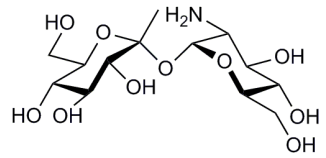

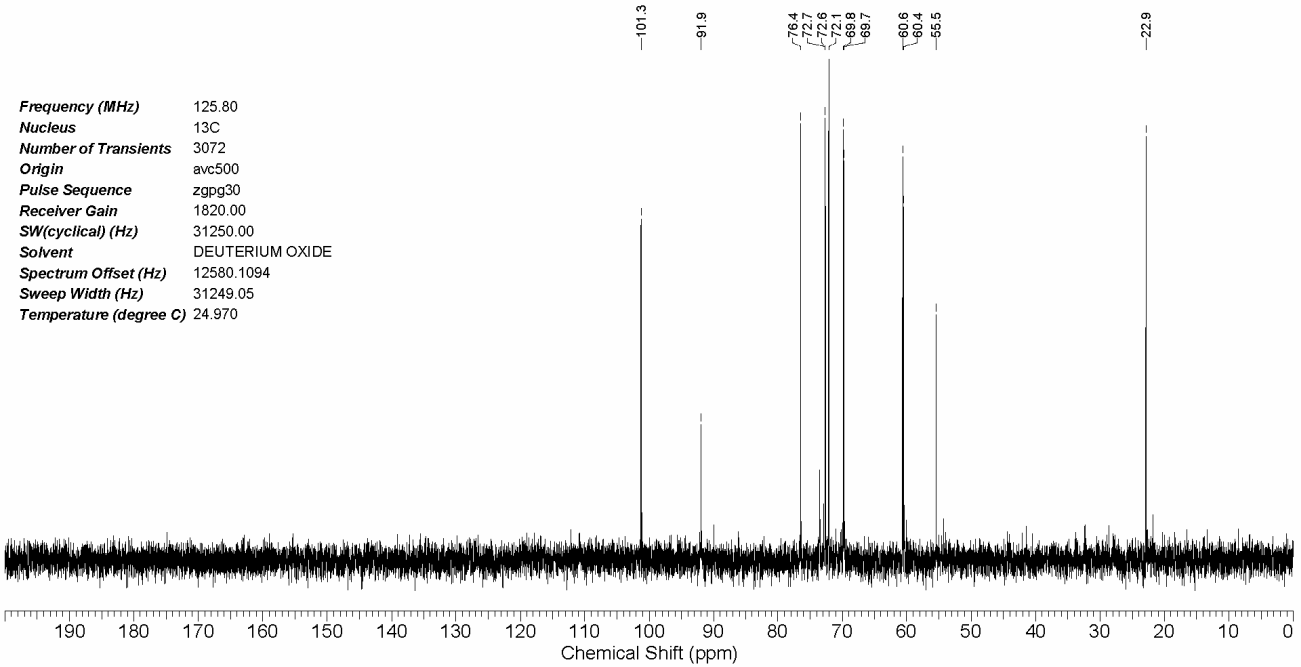

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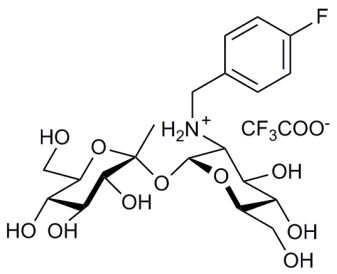

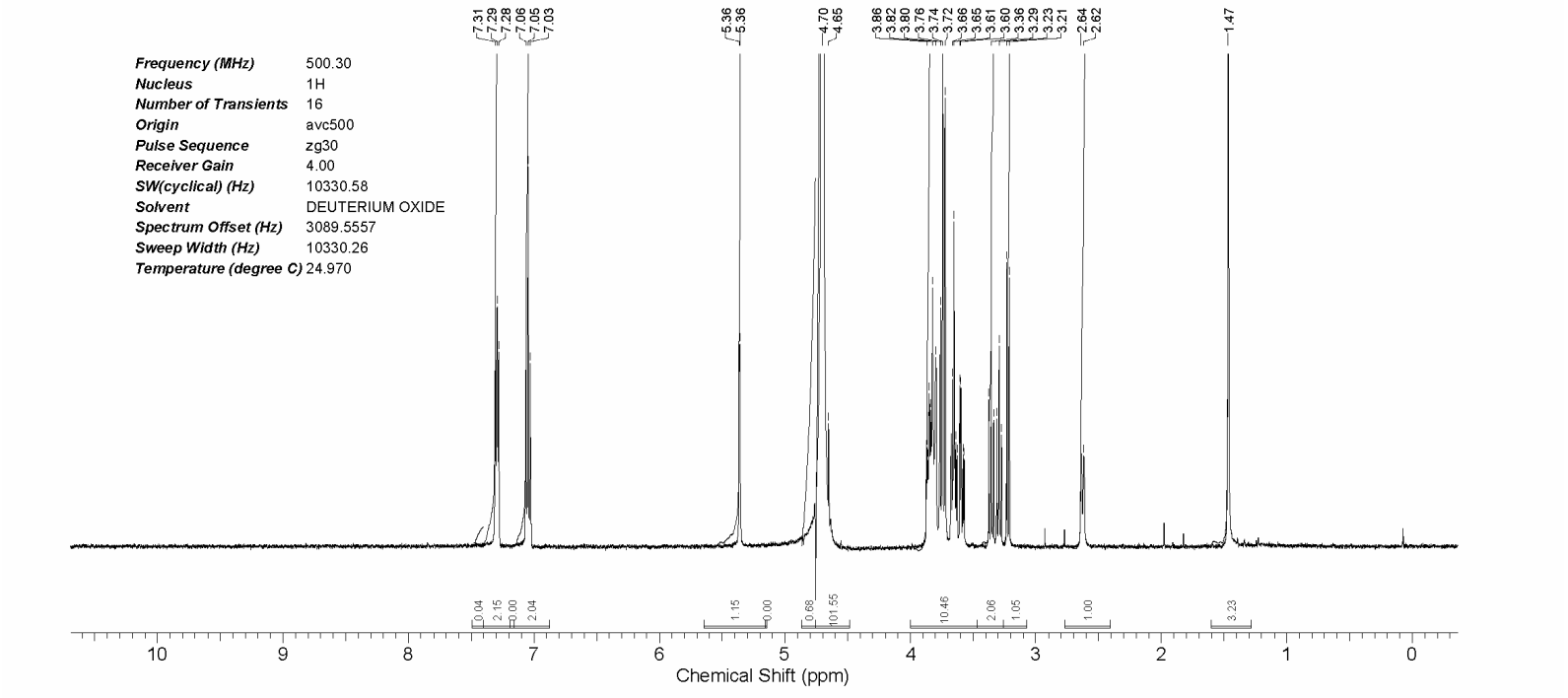

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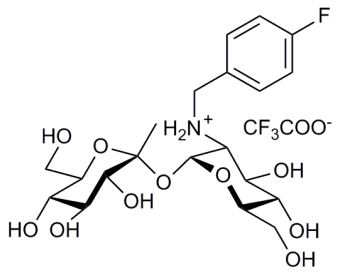

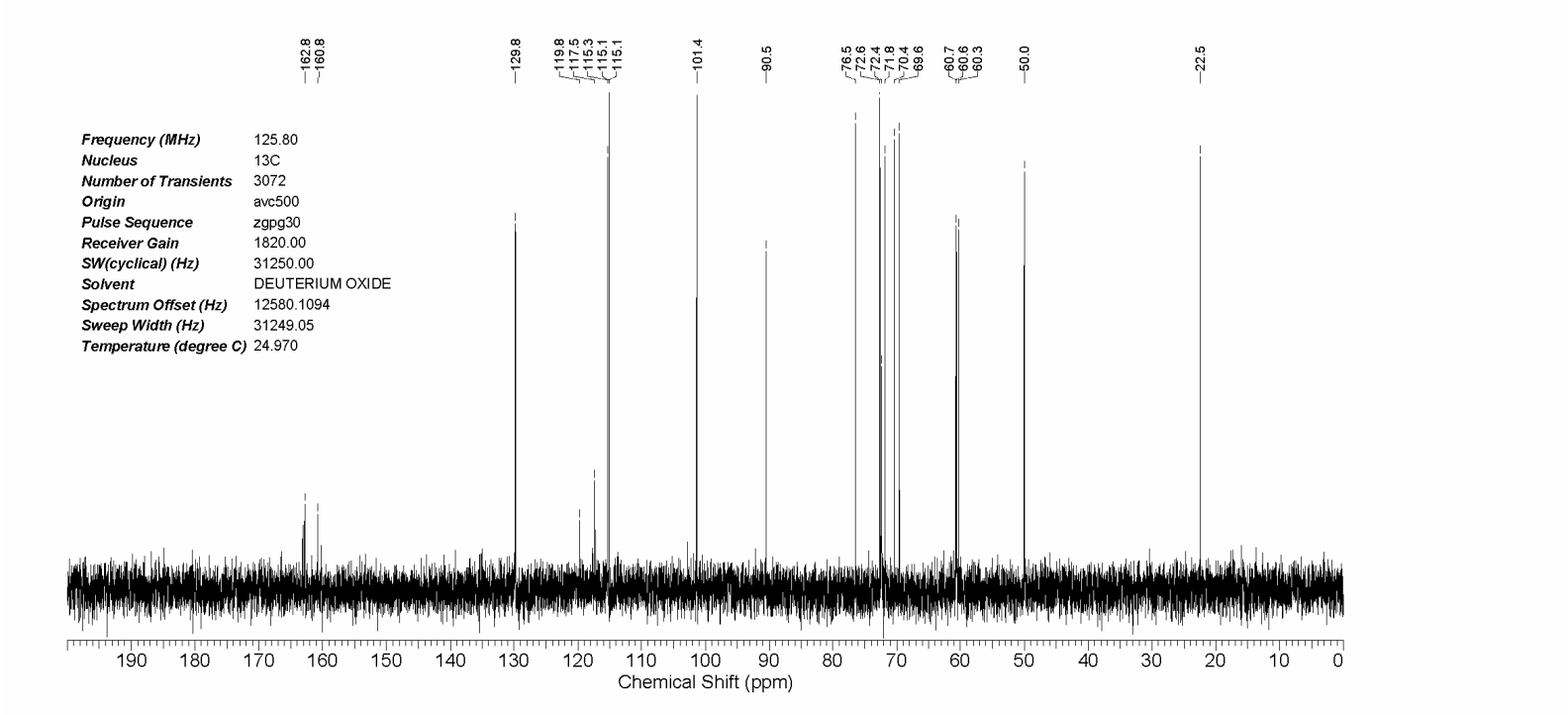

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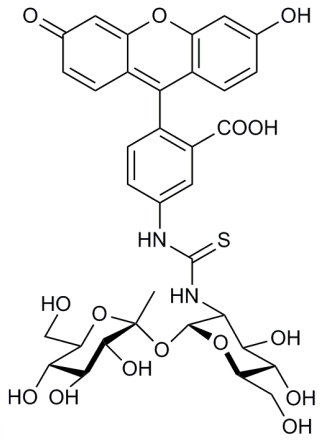

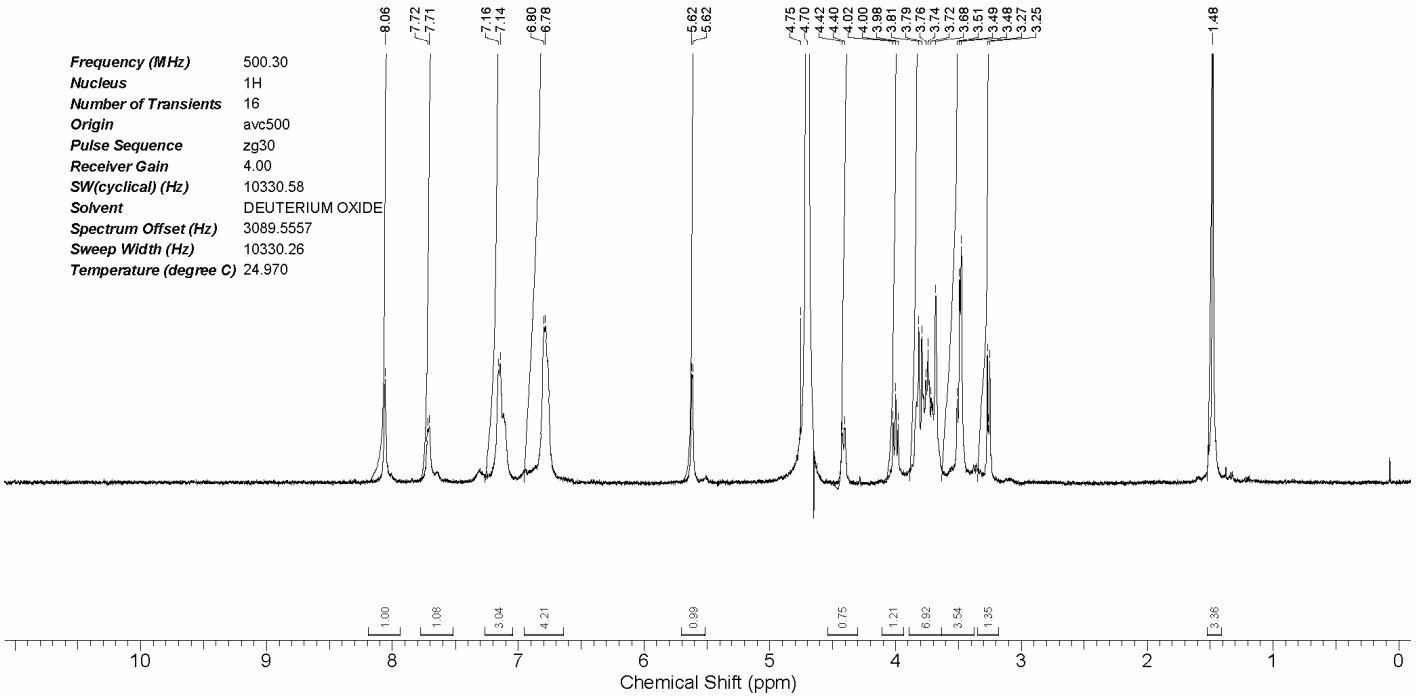

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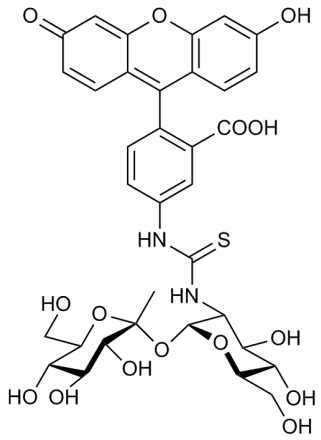

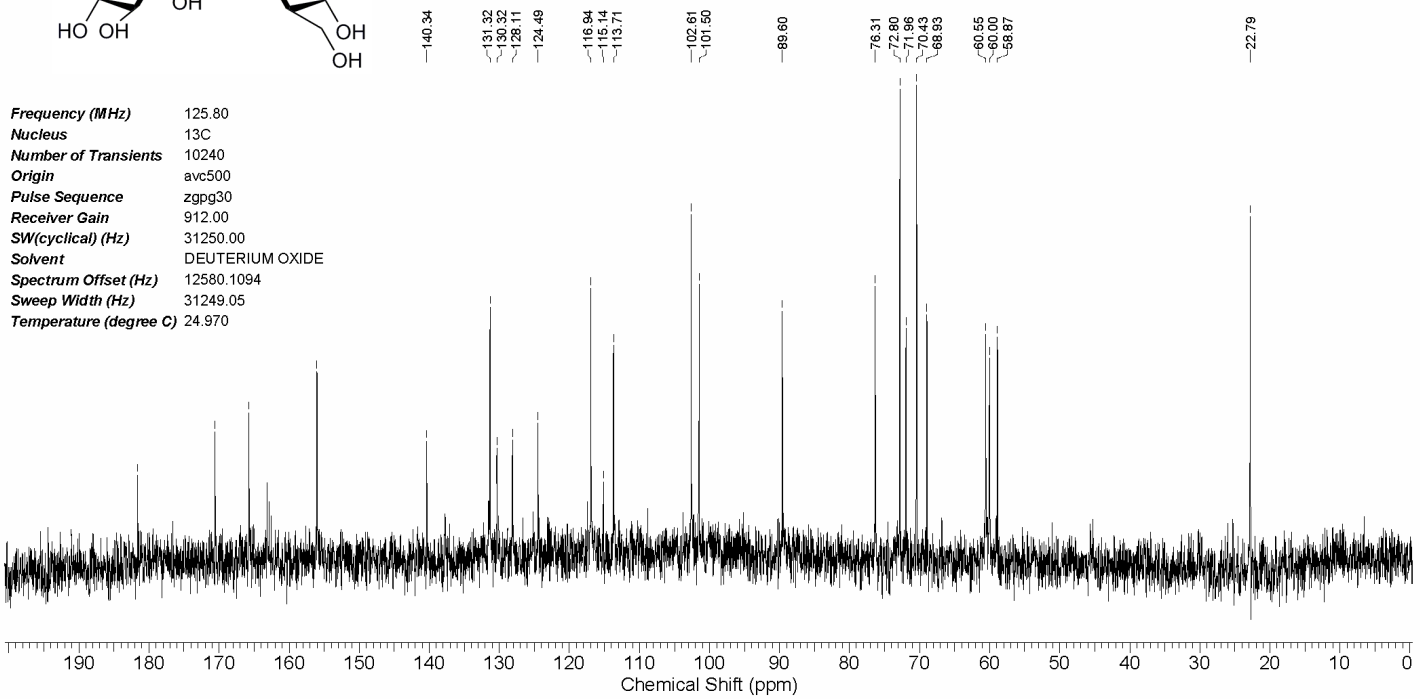

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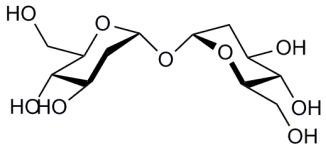

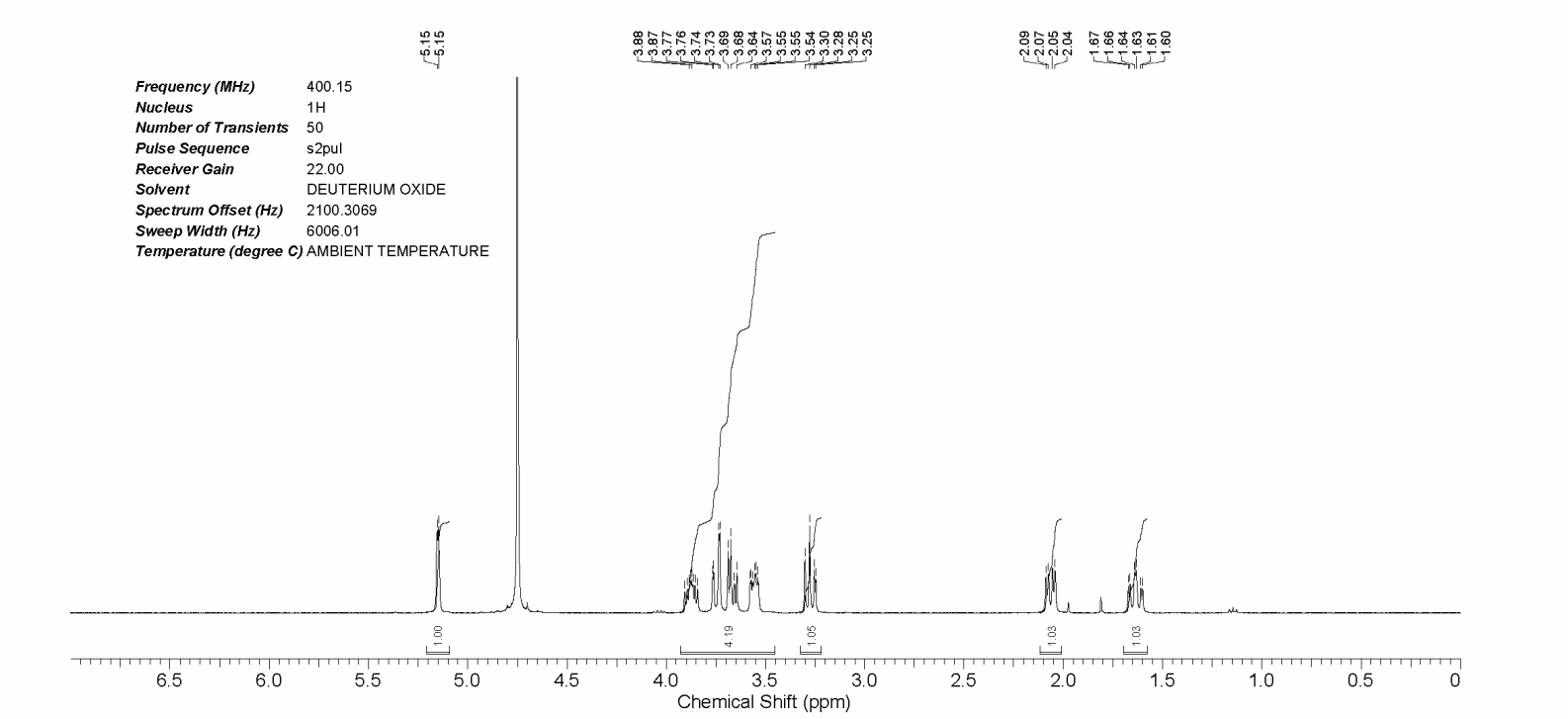

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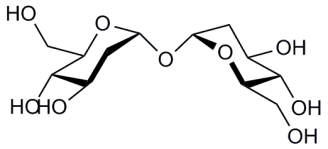

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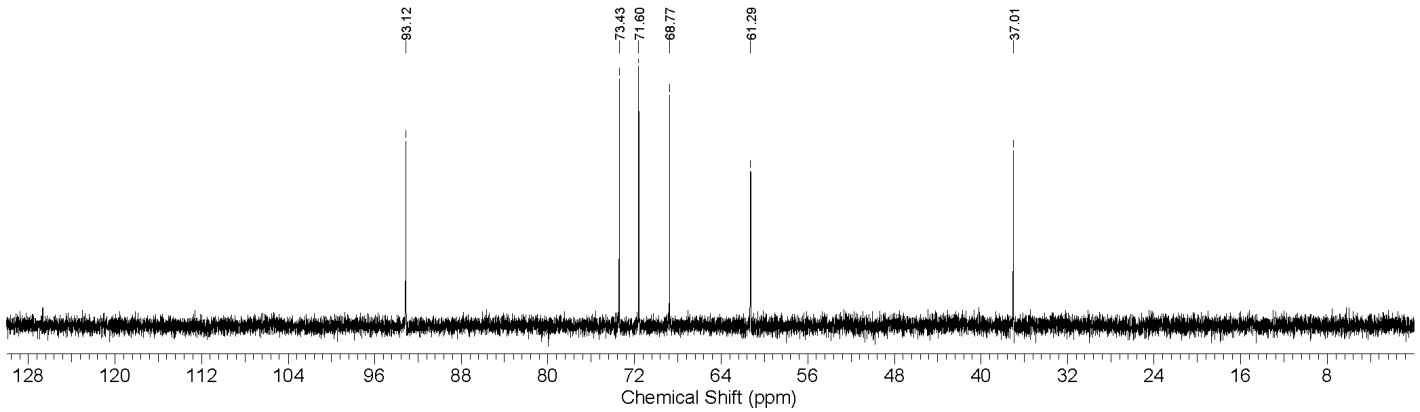

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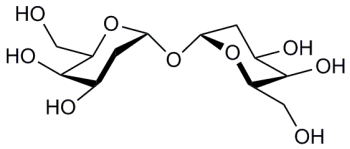

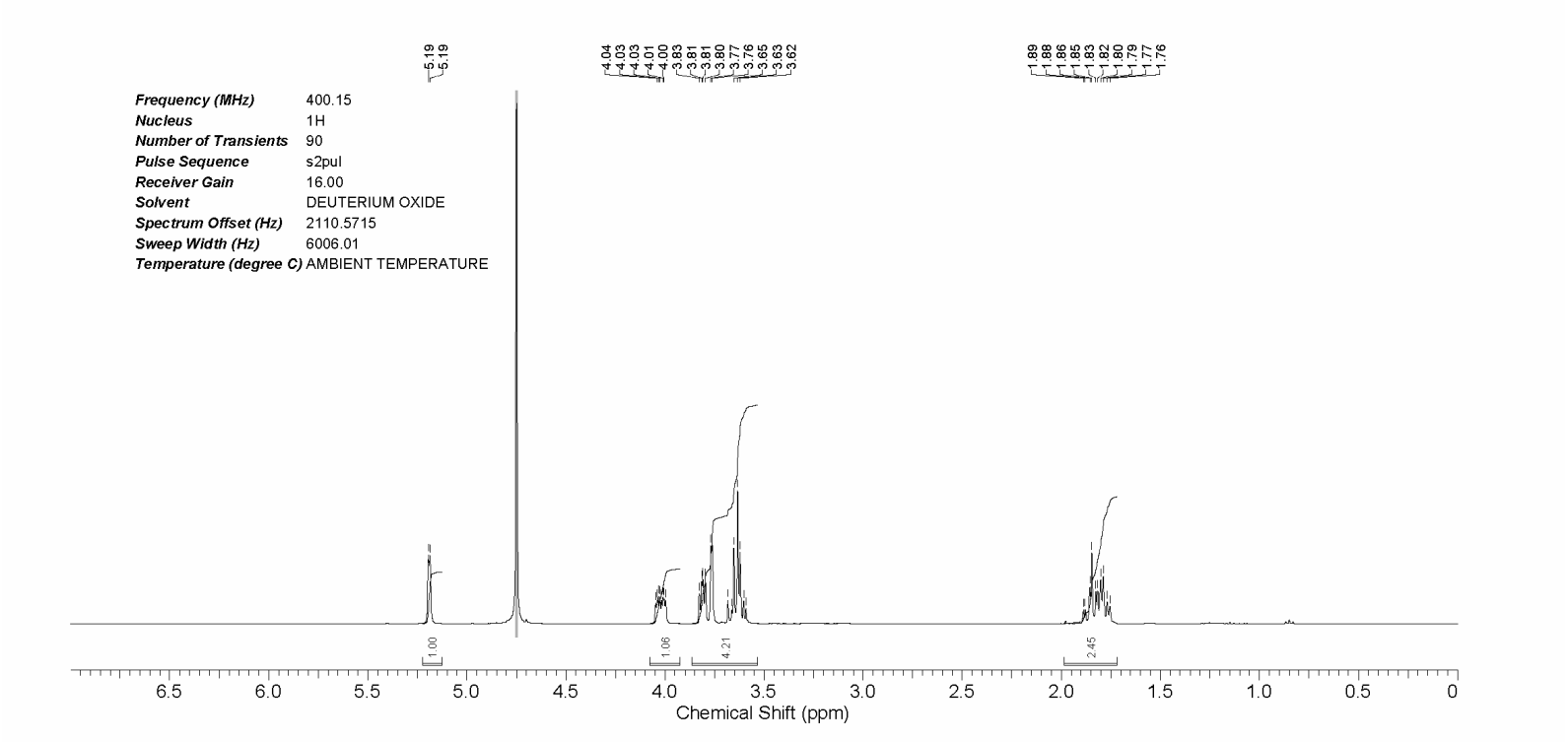

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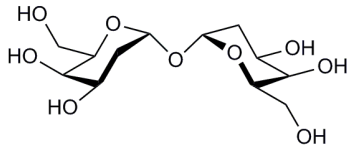

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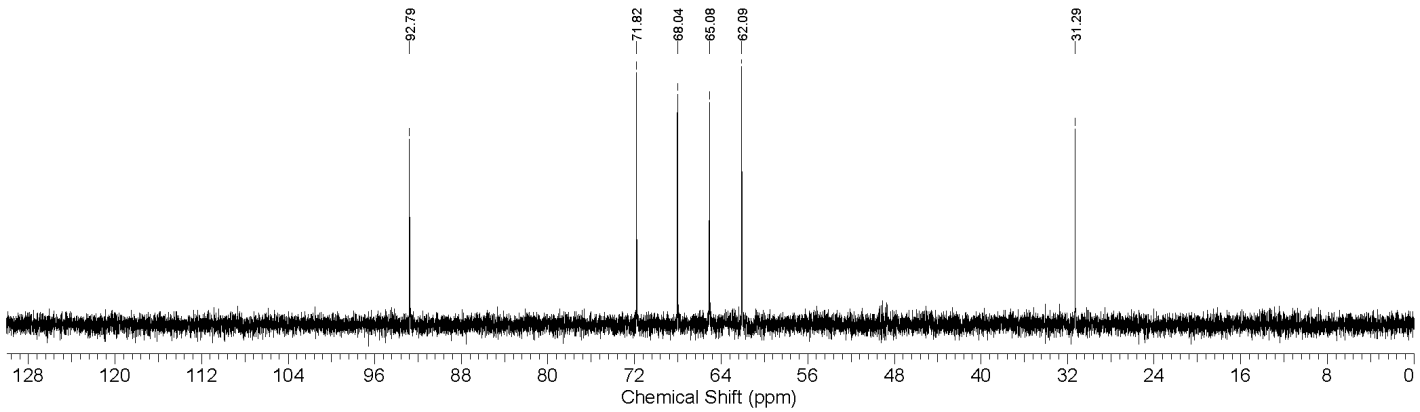

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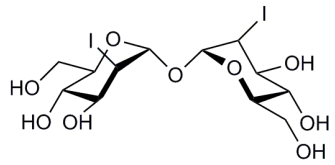

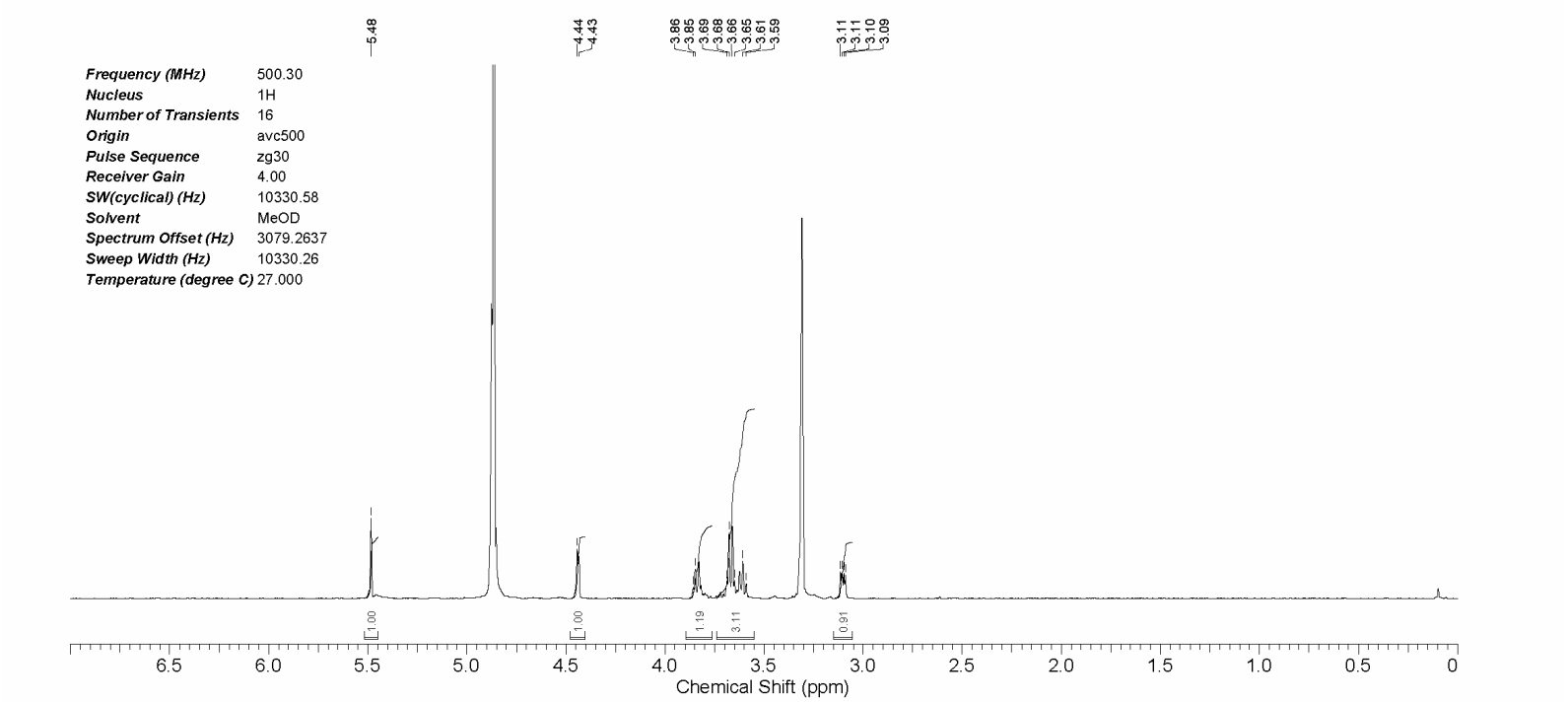

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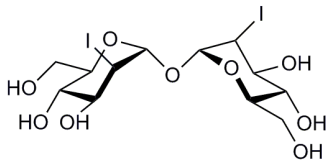

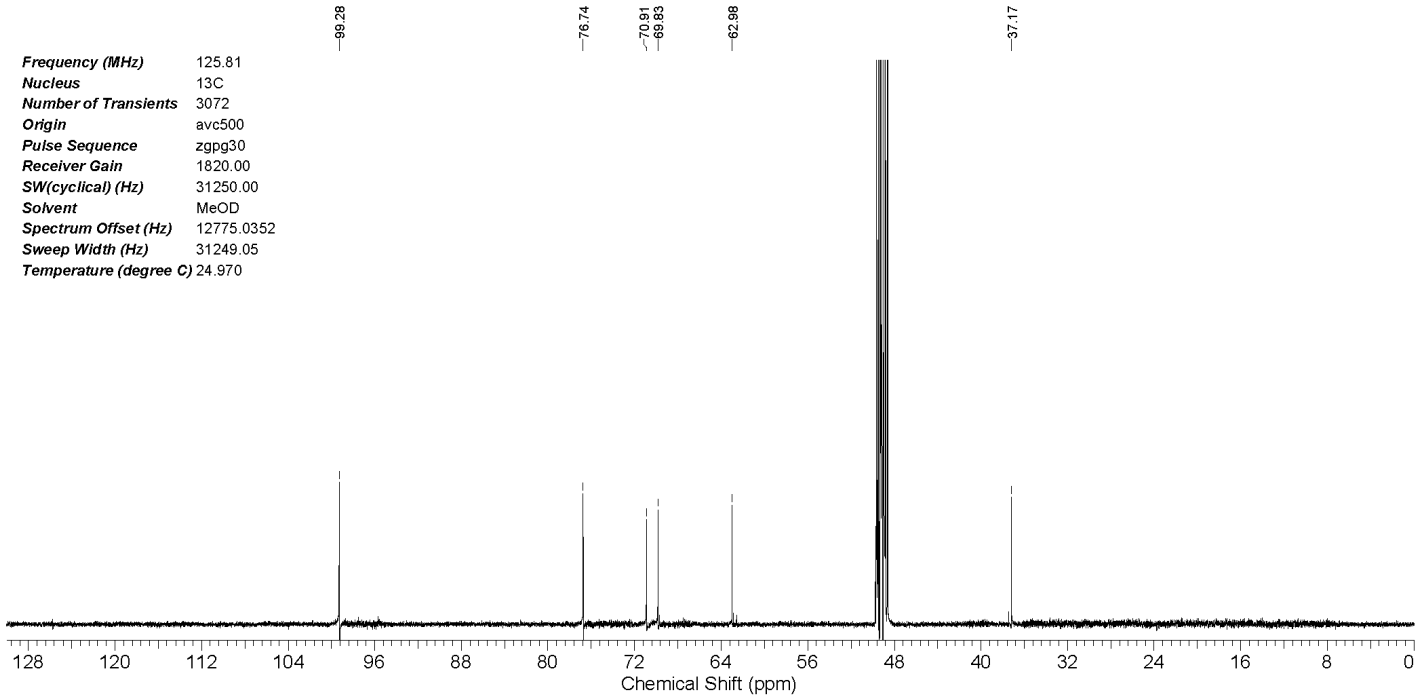

Nature Chemical Biology: doi:10.1038/nchembio.539

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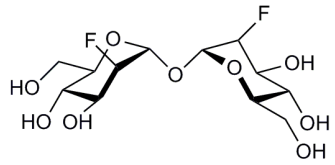

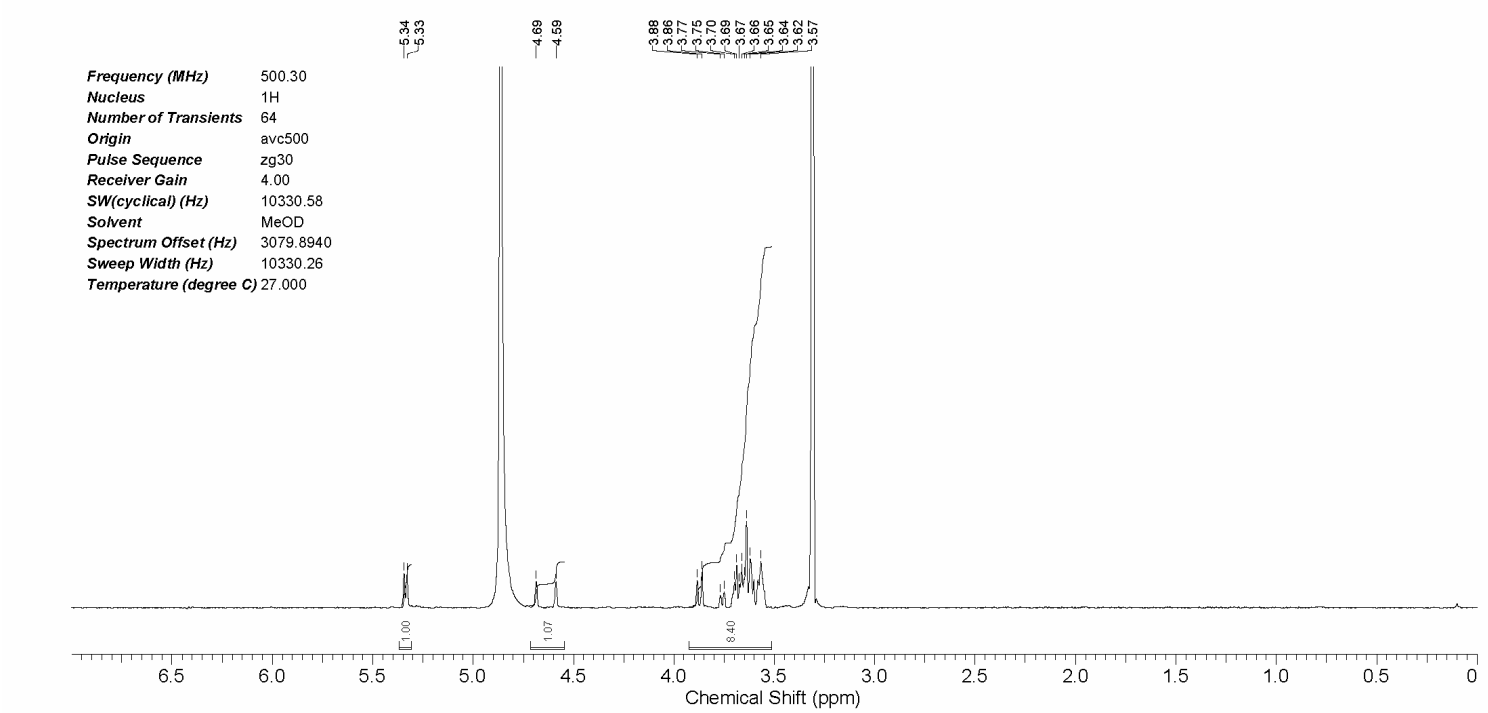

Nature Chemical Biology: doi:10.1038/nchembio.539

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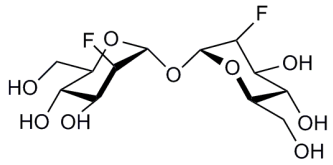
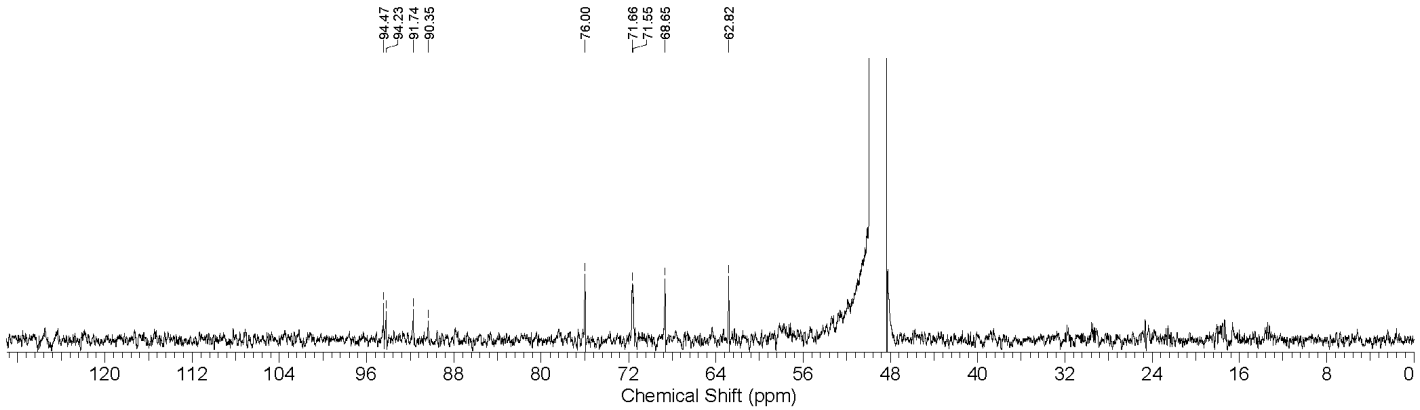


Frequency (MHz)	125.81
Nucleus	13C
Number of Transients	4096
Origin	avc500
Pulse Sequence	zgpg30
Receiver Gain	1820.00
SW(cyclical) (Hz)	31250.00
Solvent	MeOD
Spectrum Offset (Hz)	12775.9893
Sweep Width (Hz)	31249.05
Temperature (degree C)	24.970



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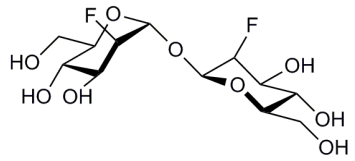

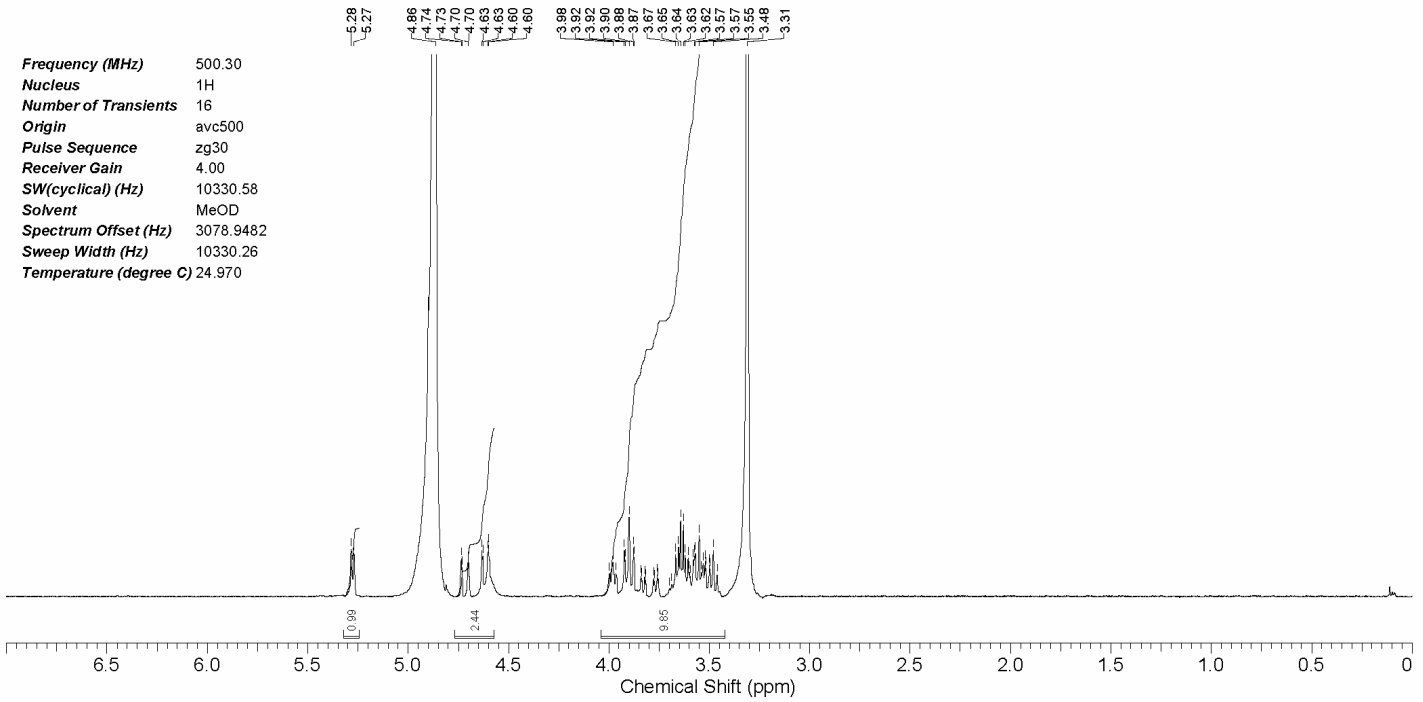

Nature Chemical Biology: doi:10.1038/nchembio.539

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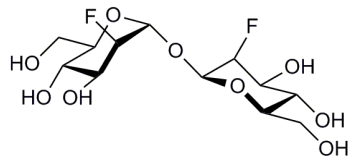

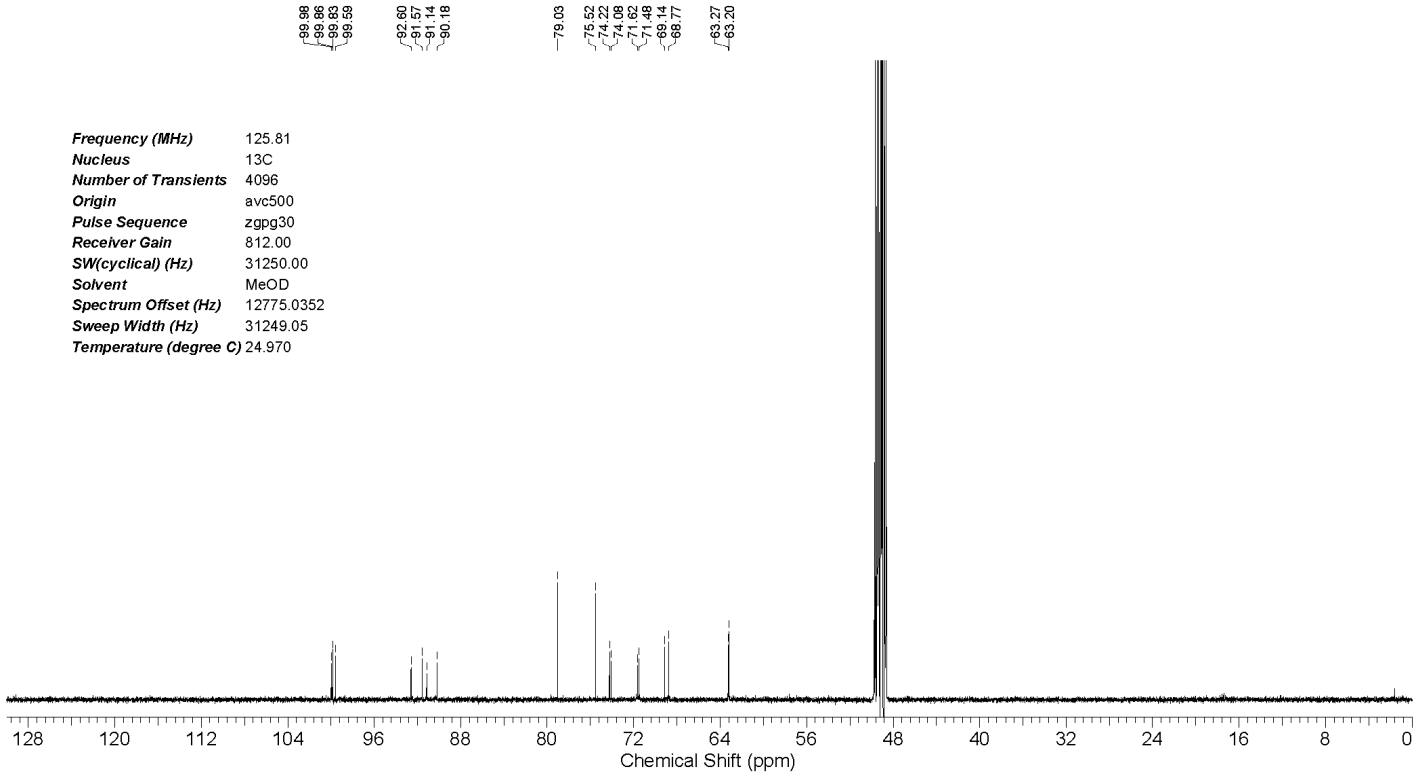

Nature Chemical Biology: doi:10.1038/nchembio.539

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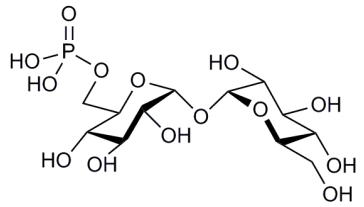

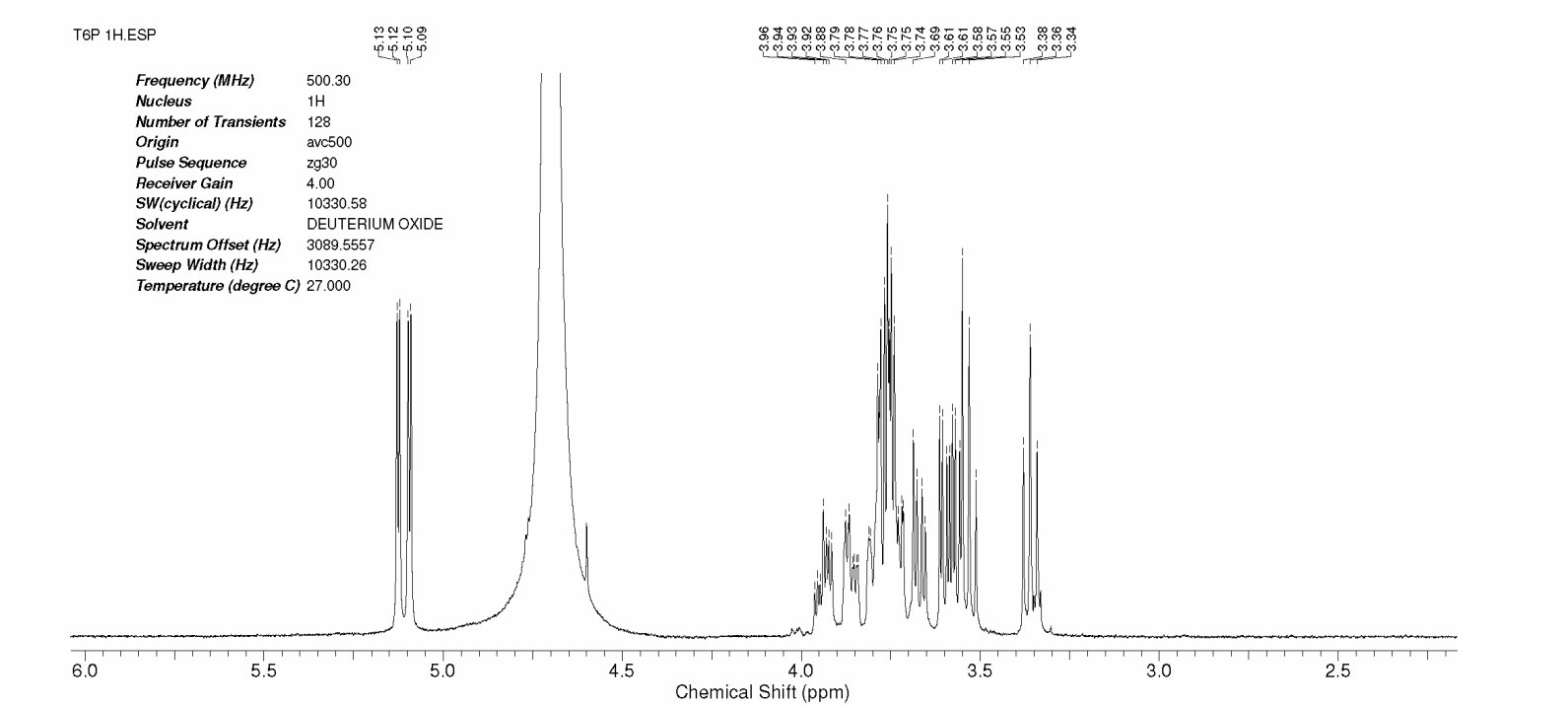

Nature Chemical Biology: doi:10.1038/nchembio.539

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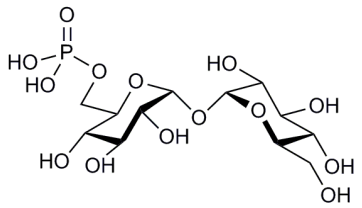

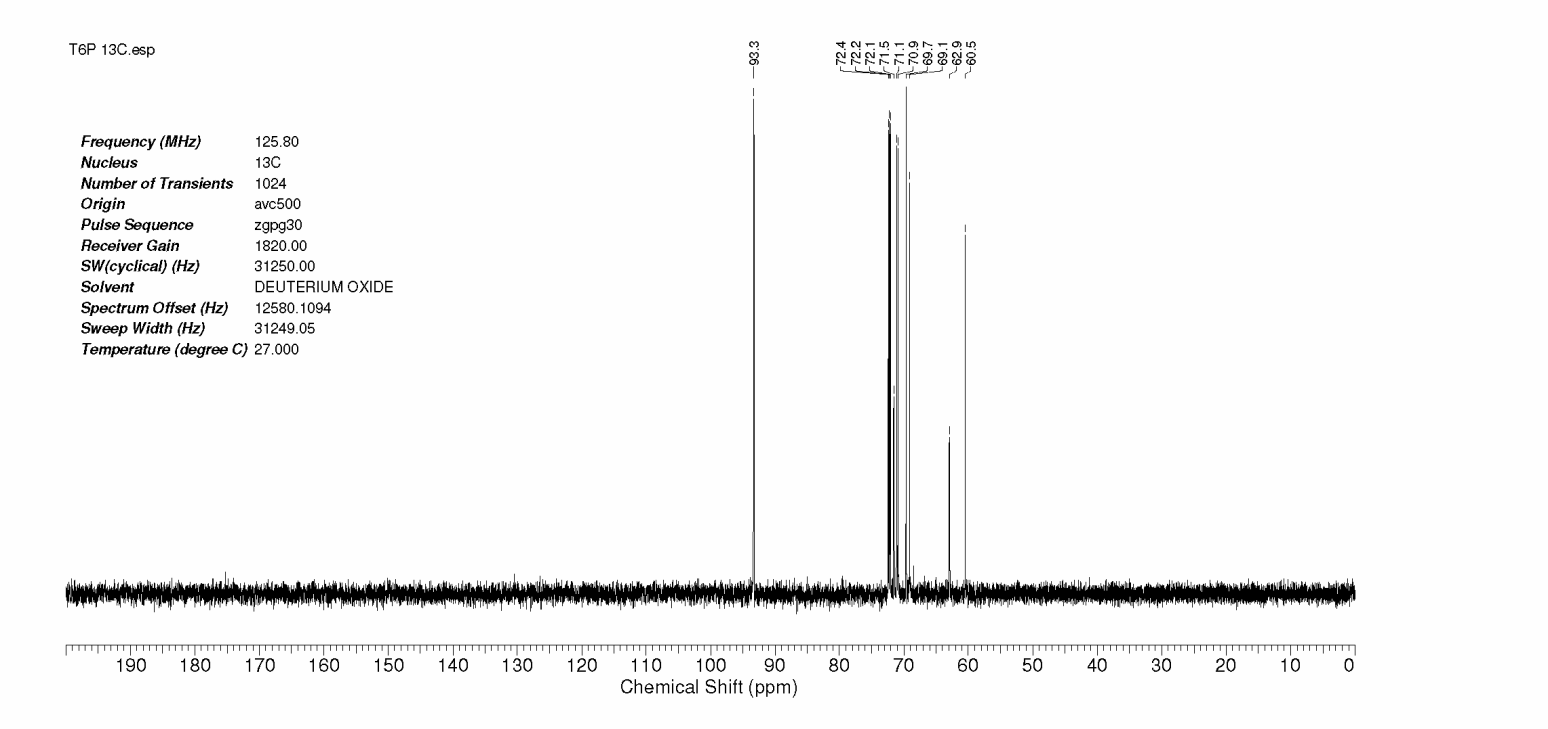

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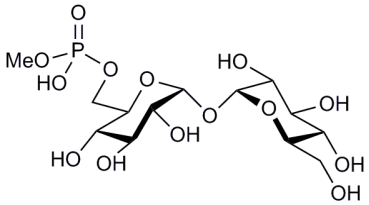

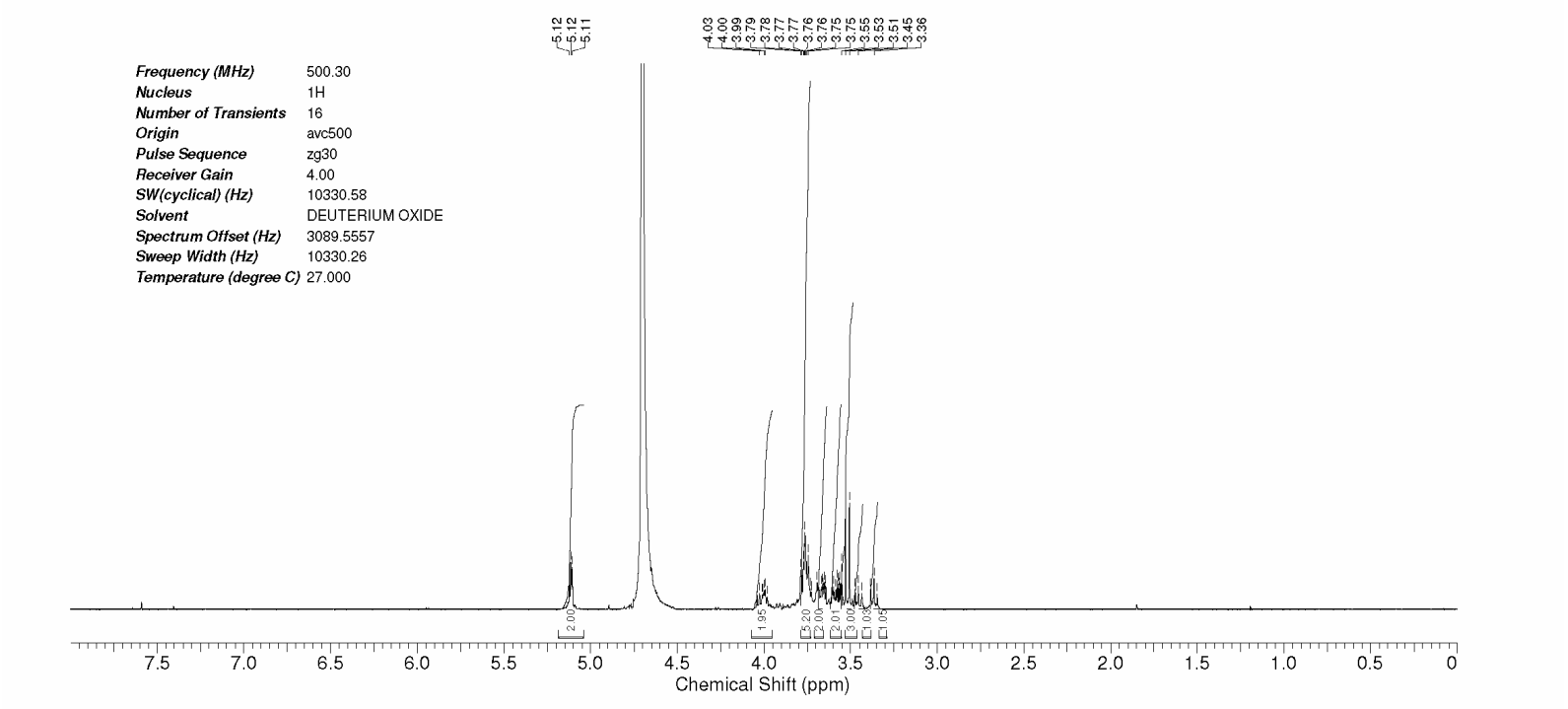

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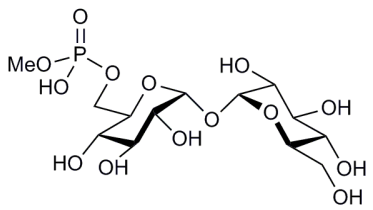

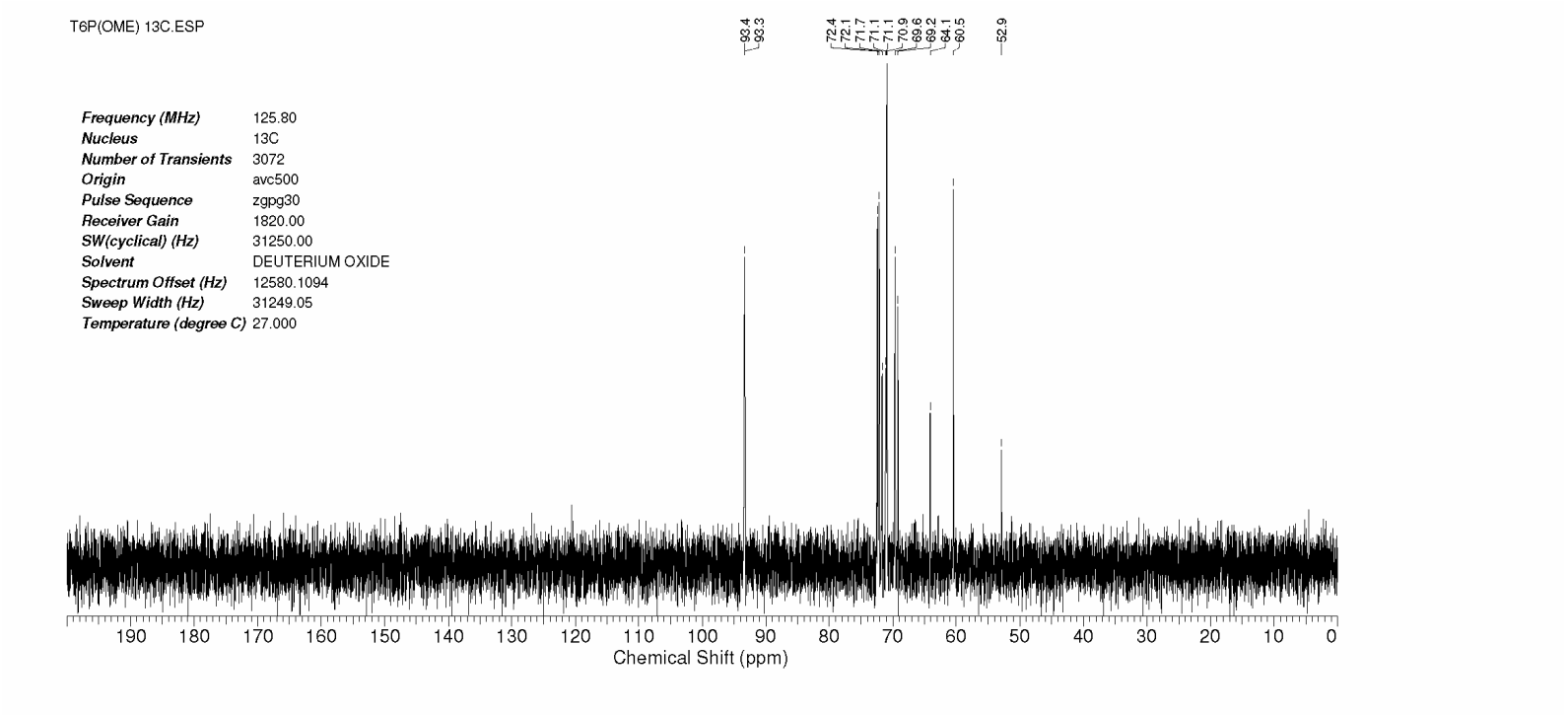

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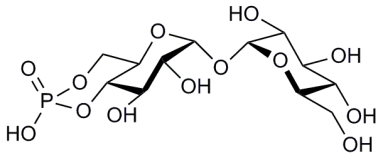

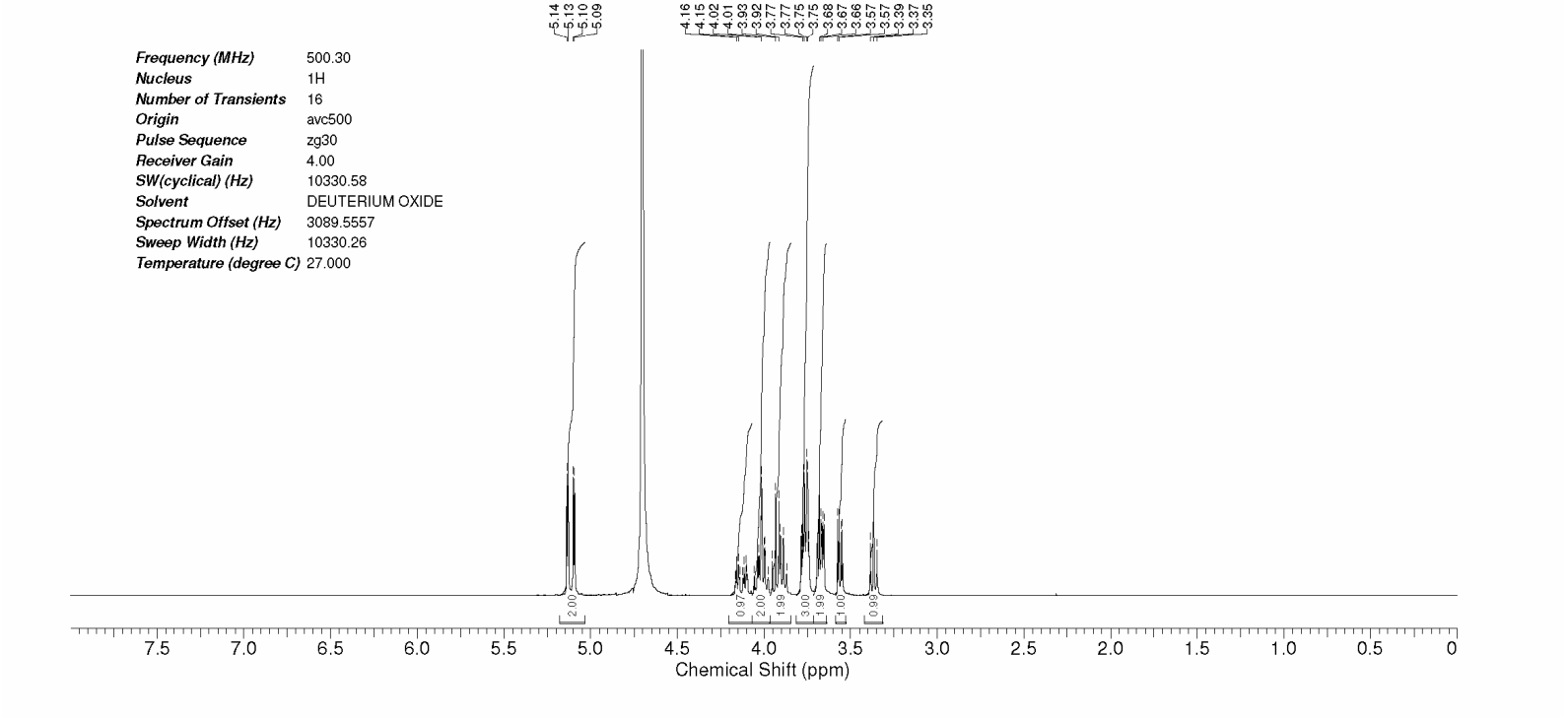

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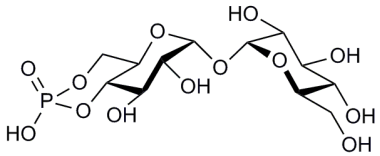

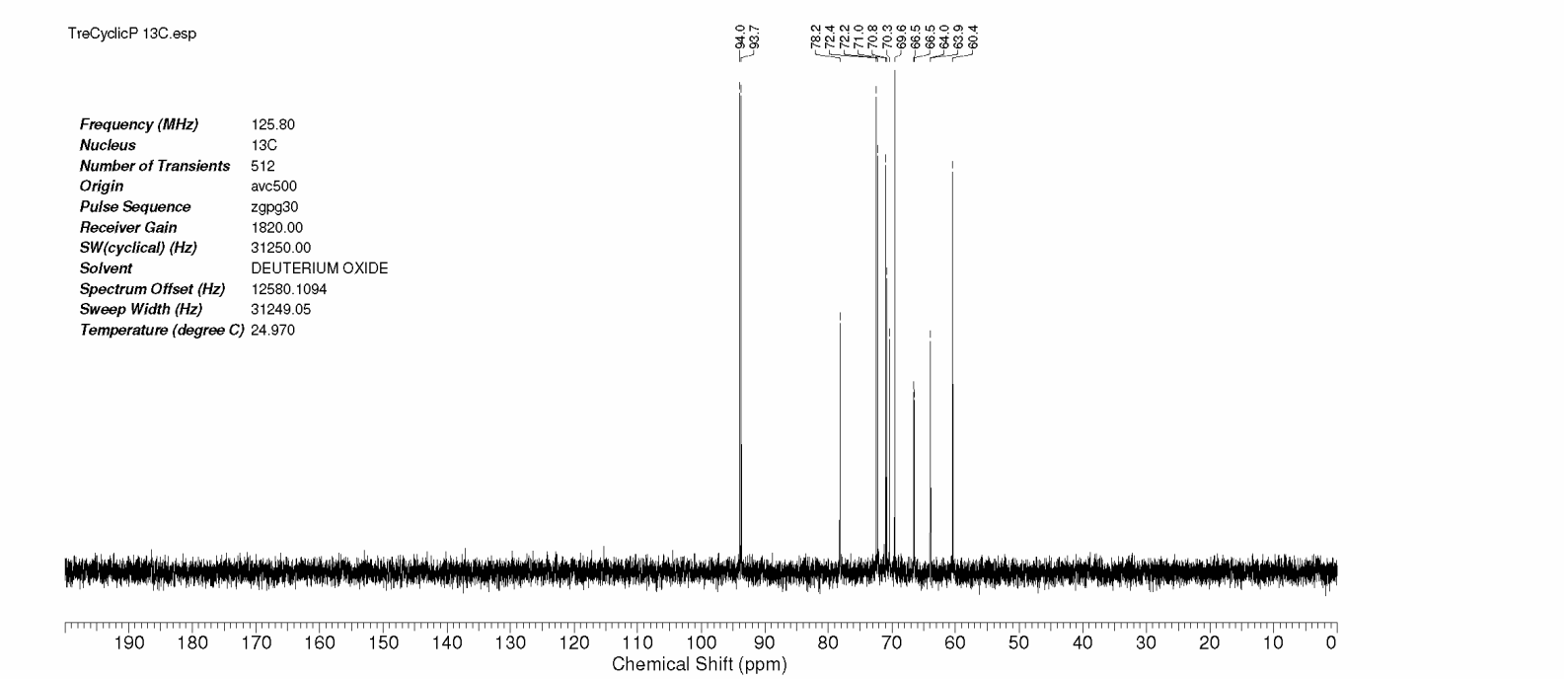

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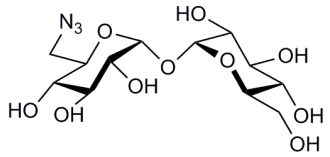

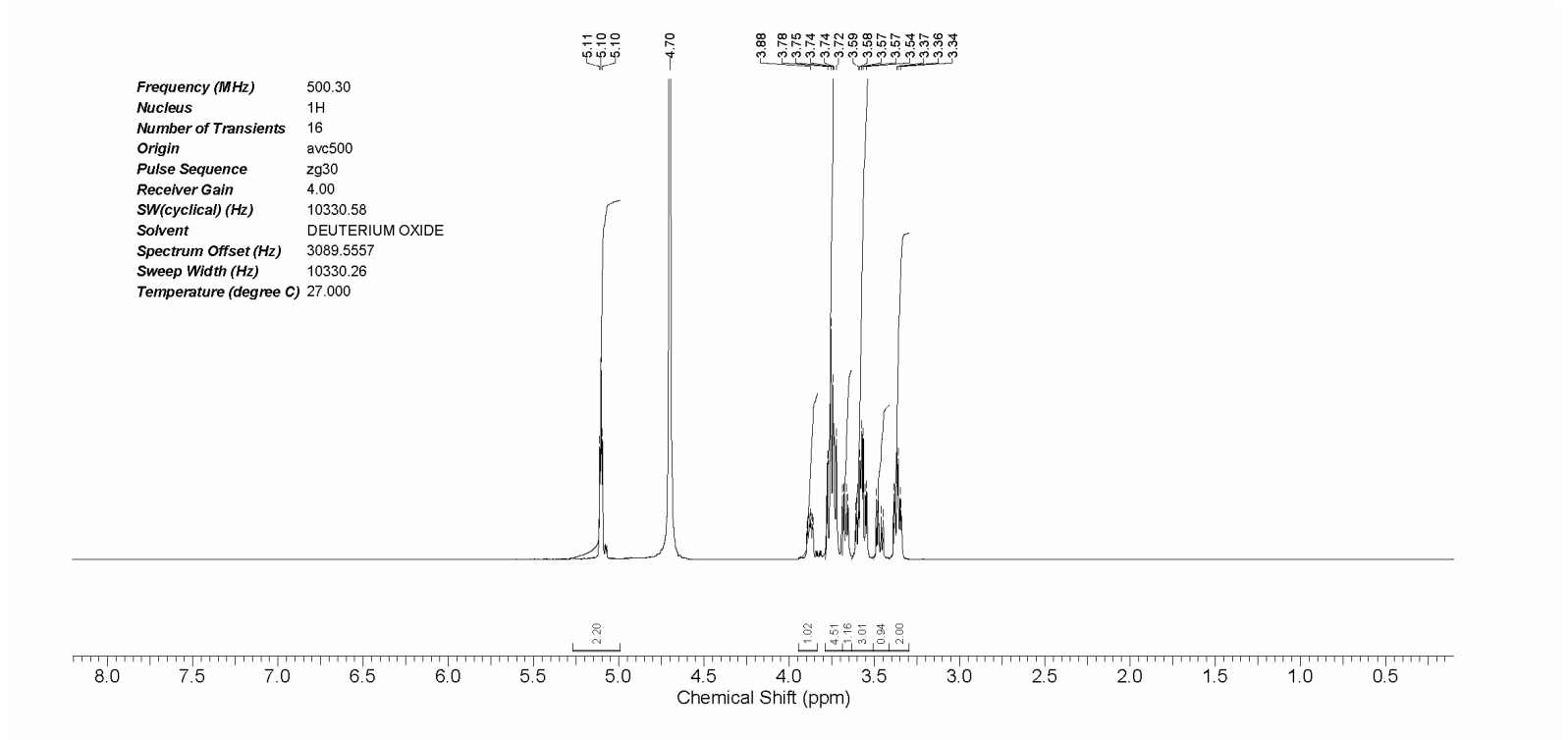

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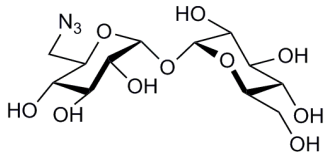

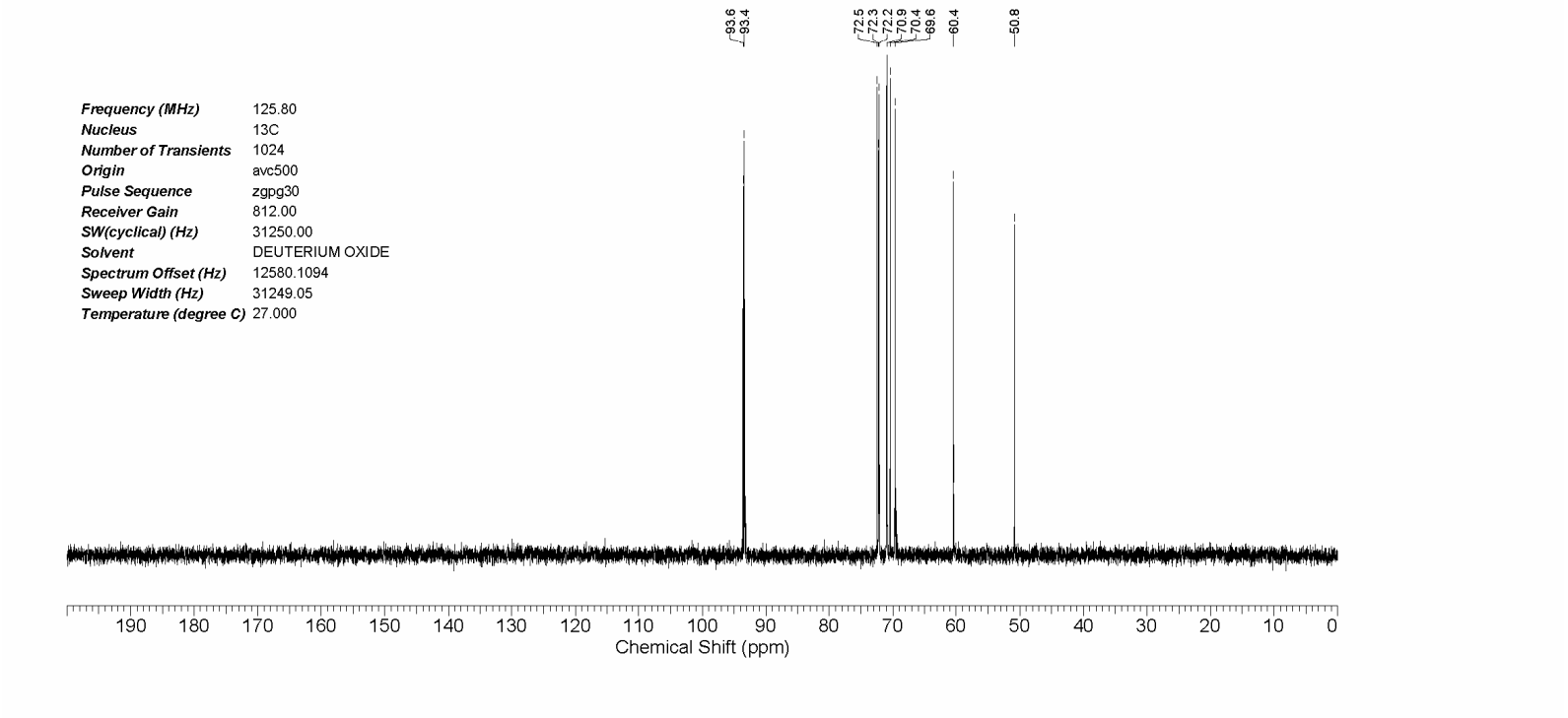

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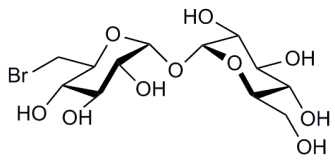

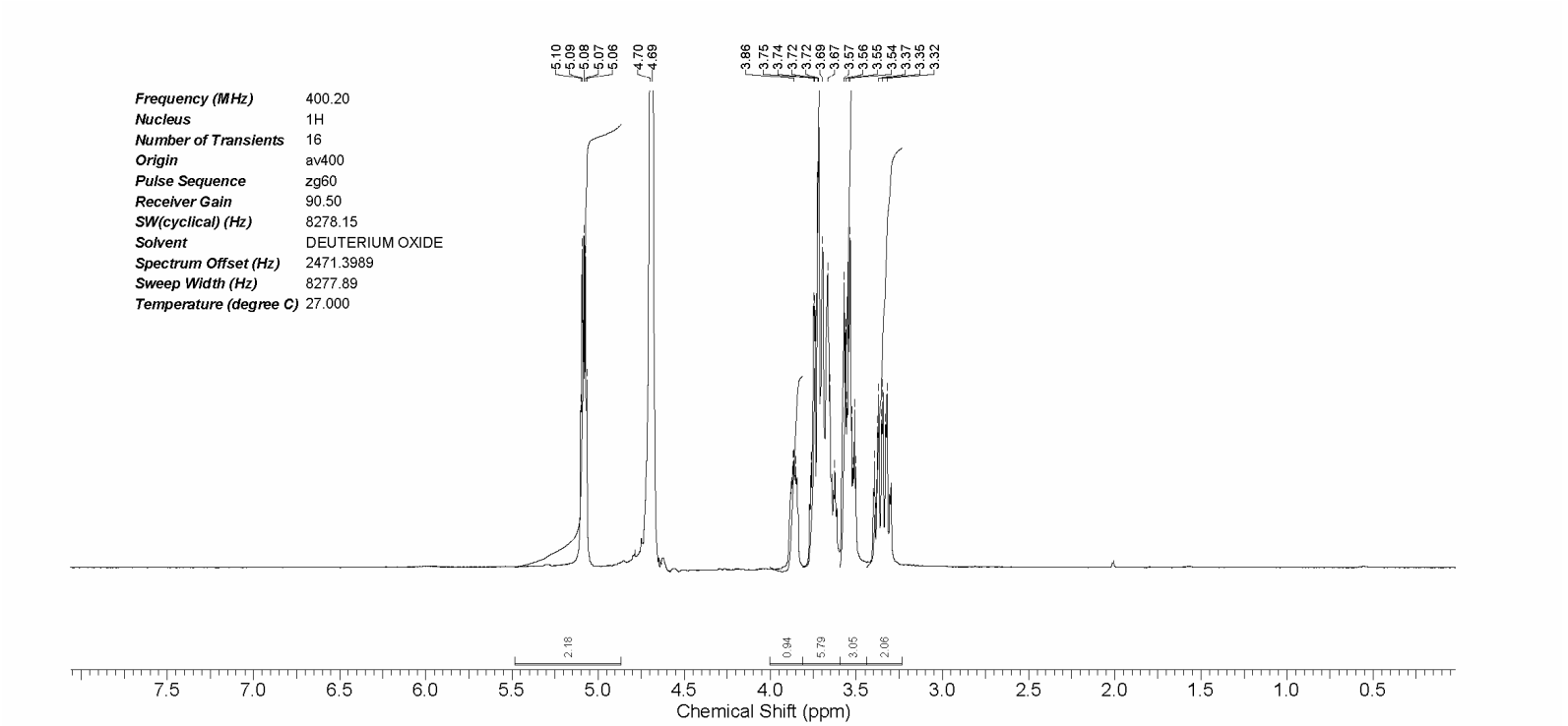

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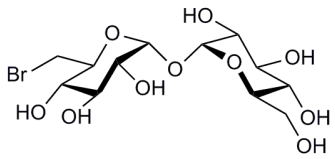

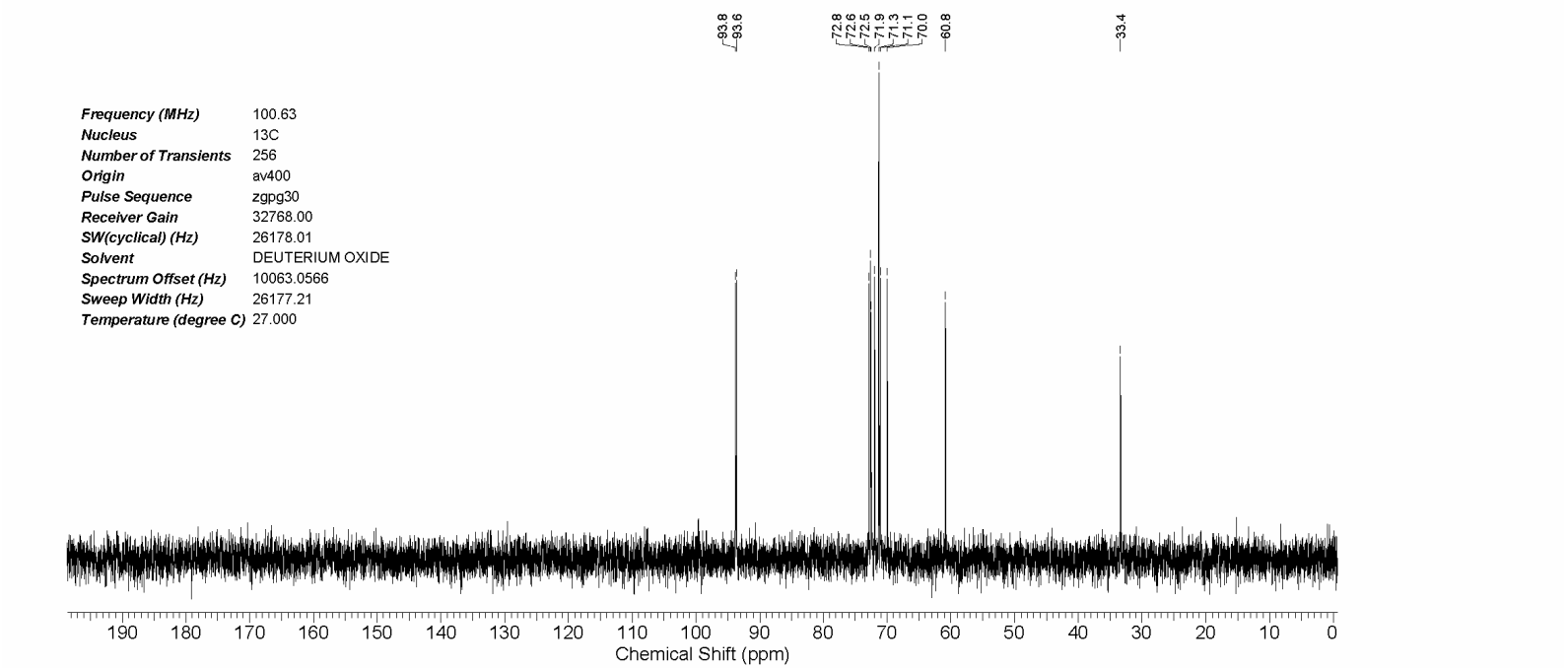

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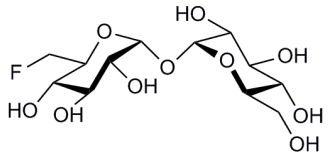

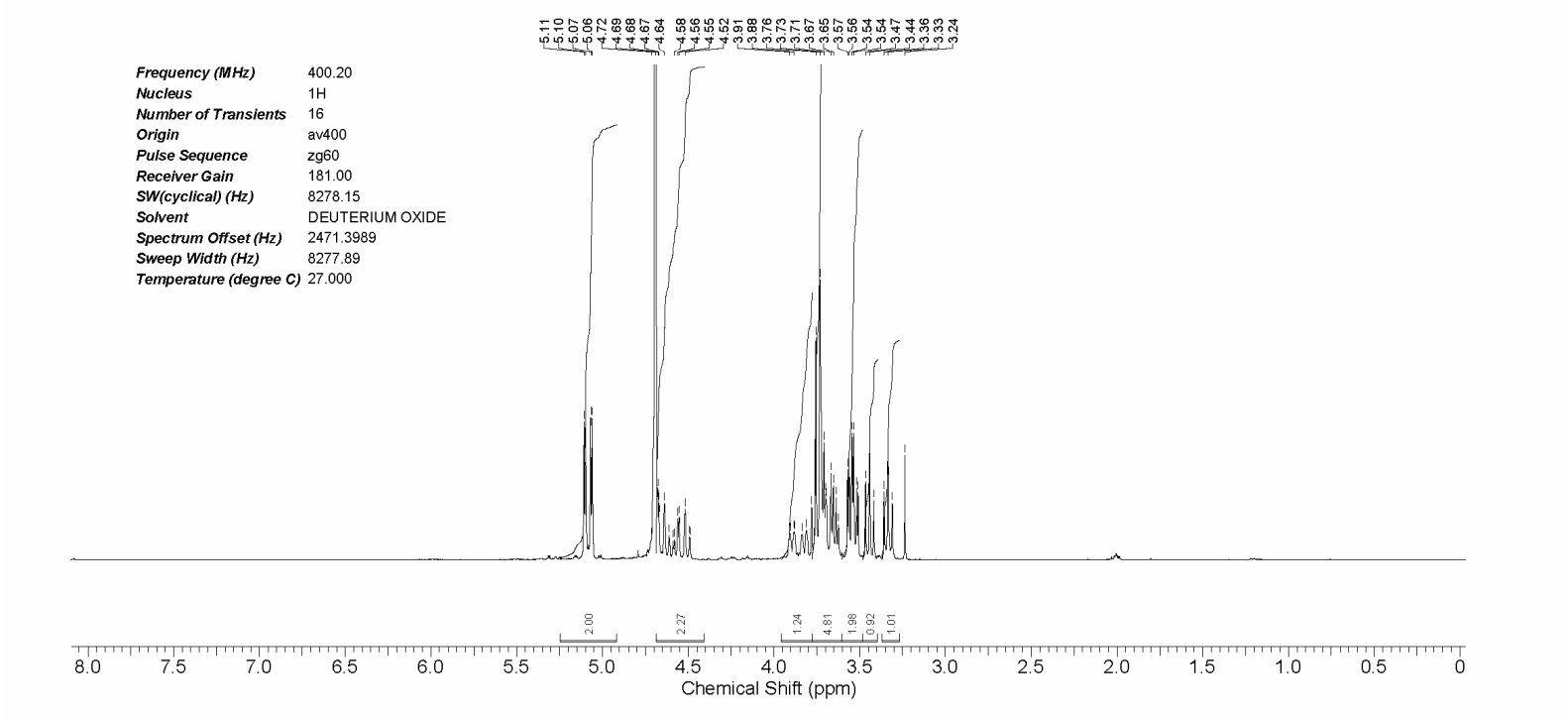

Nature Chemical Biology: doi:10.1038/nchembio.539

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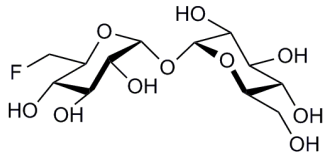

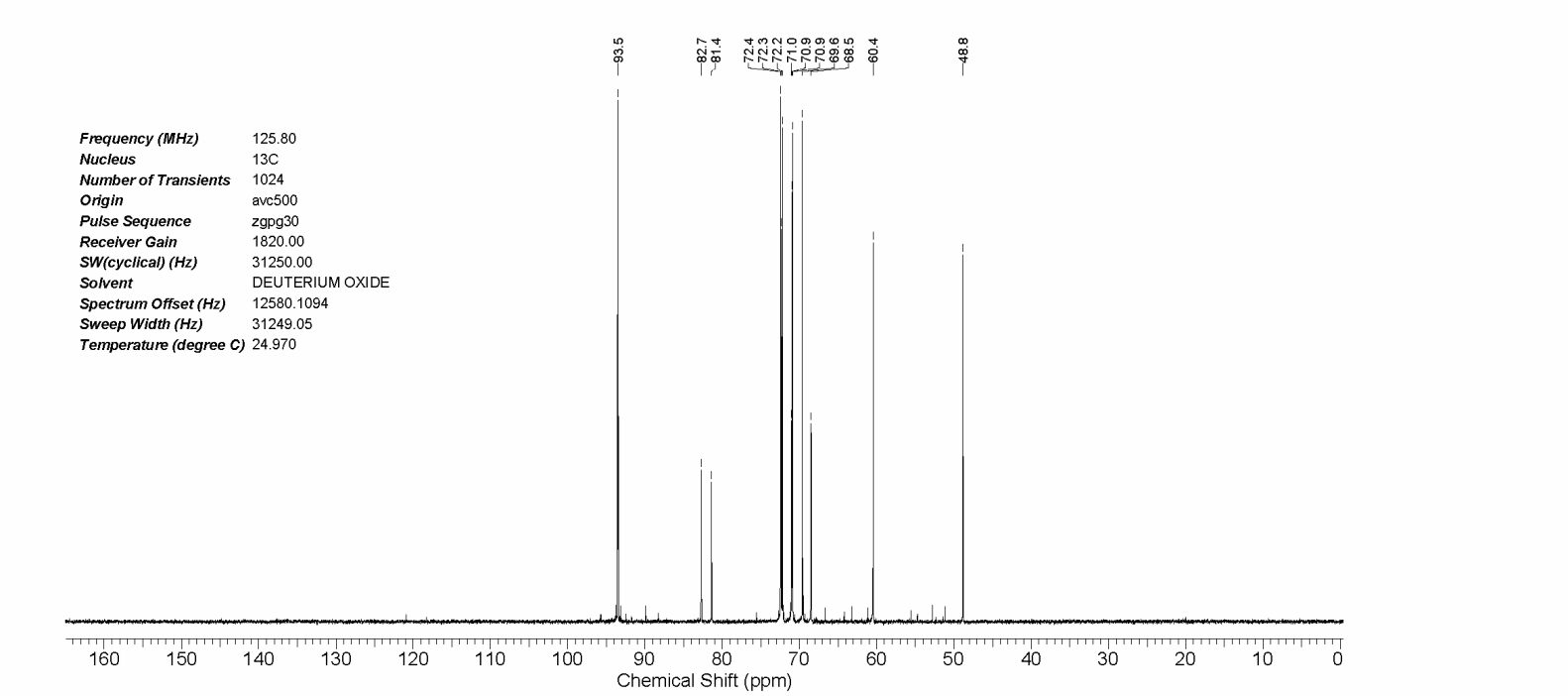

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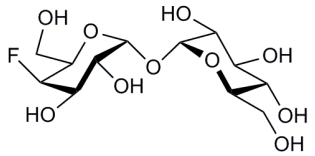

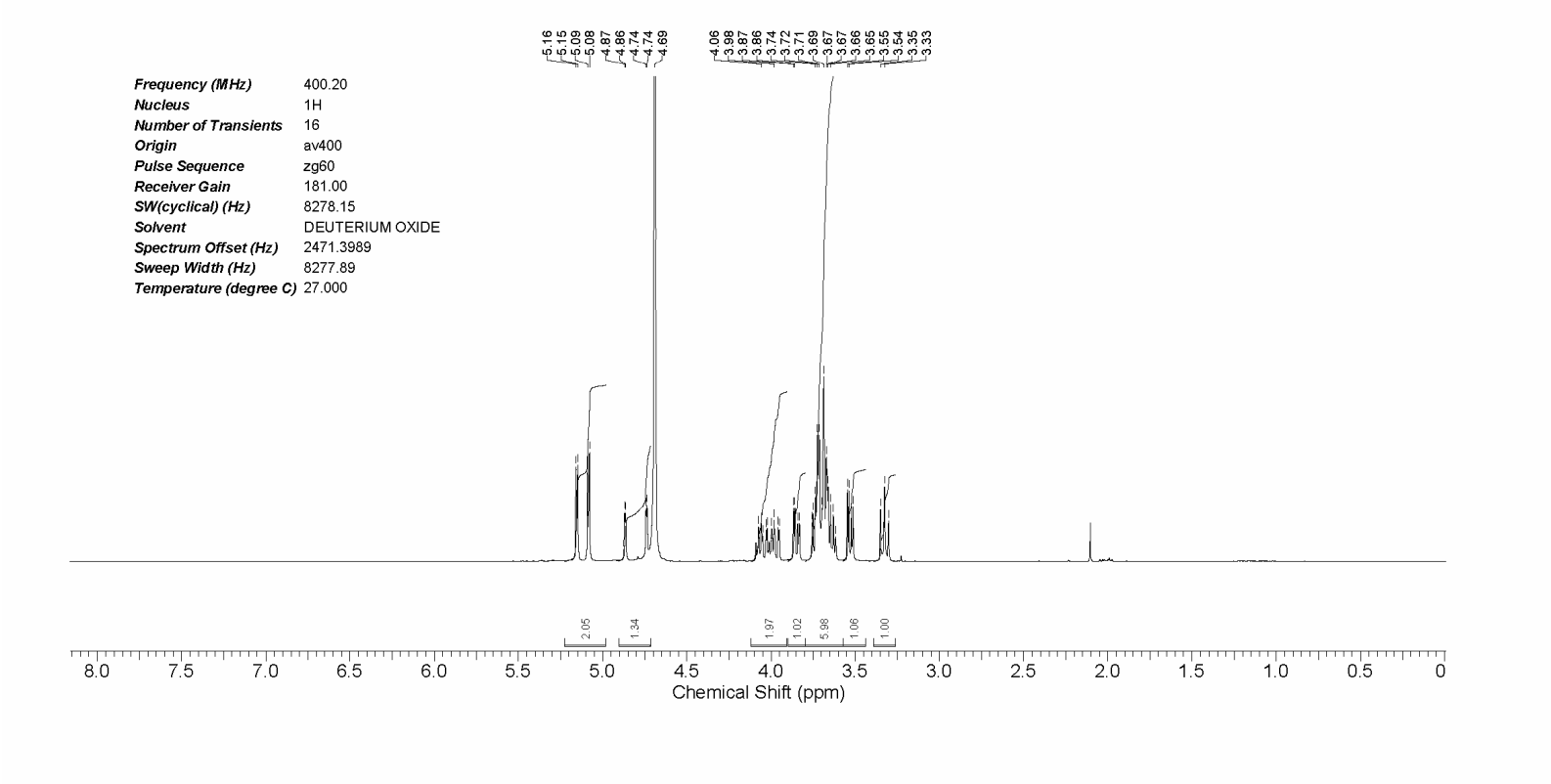

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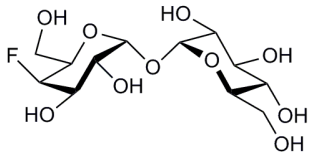

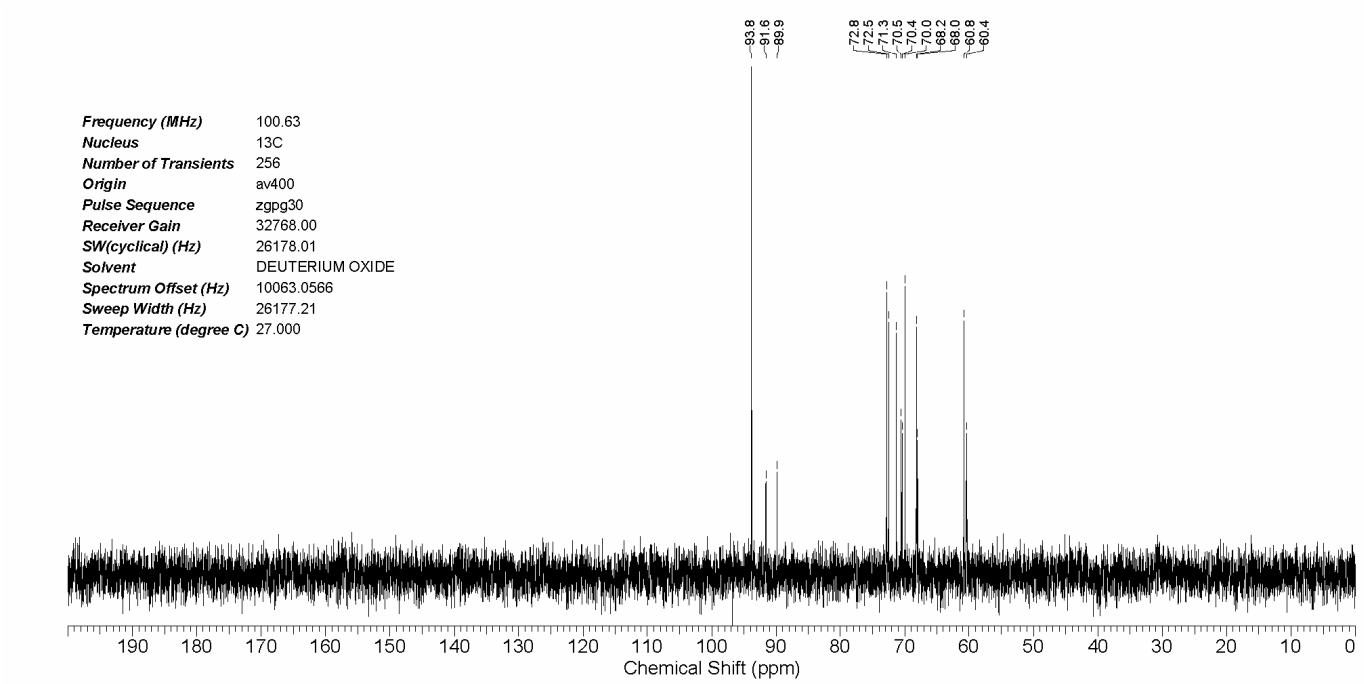

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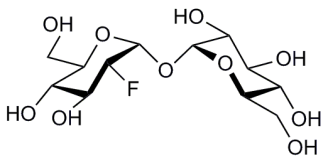

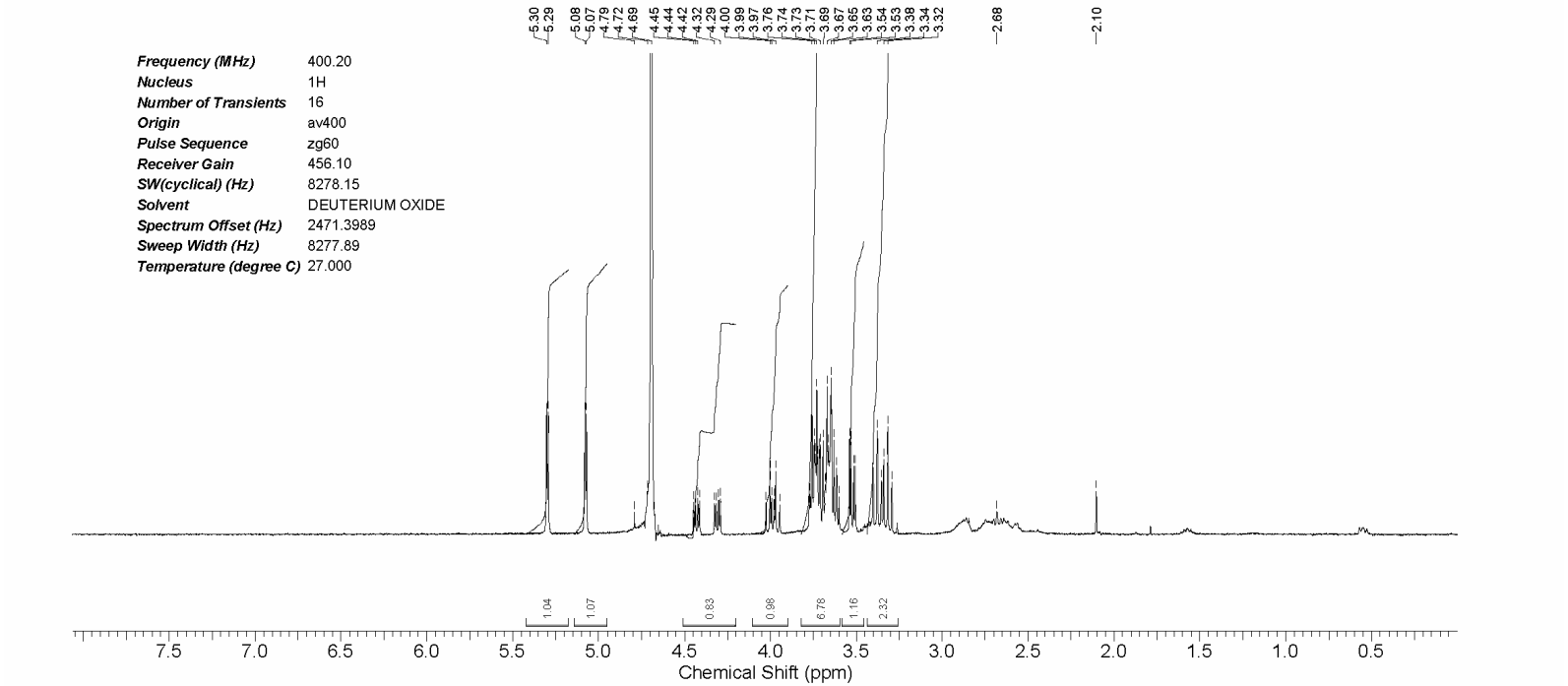

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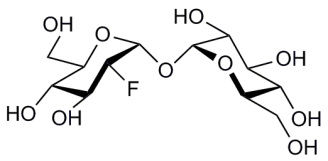

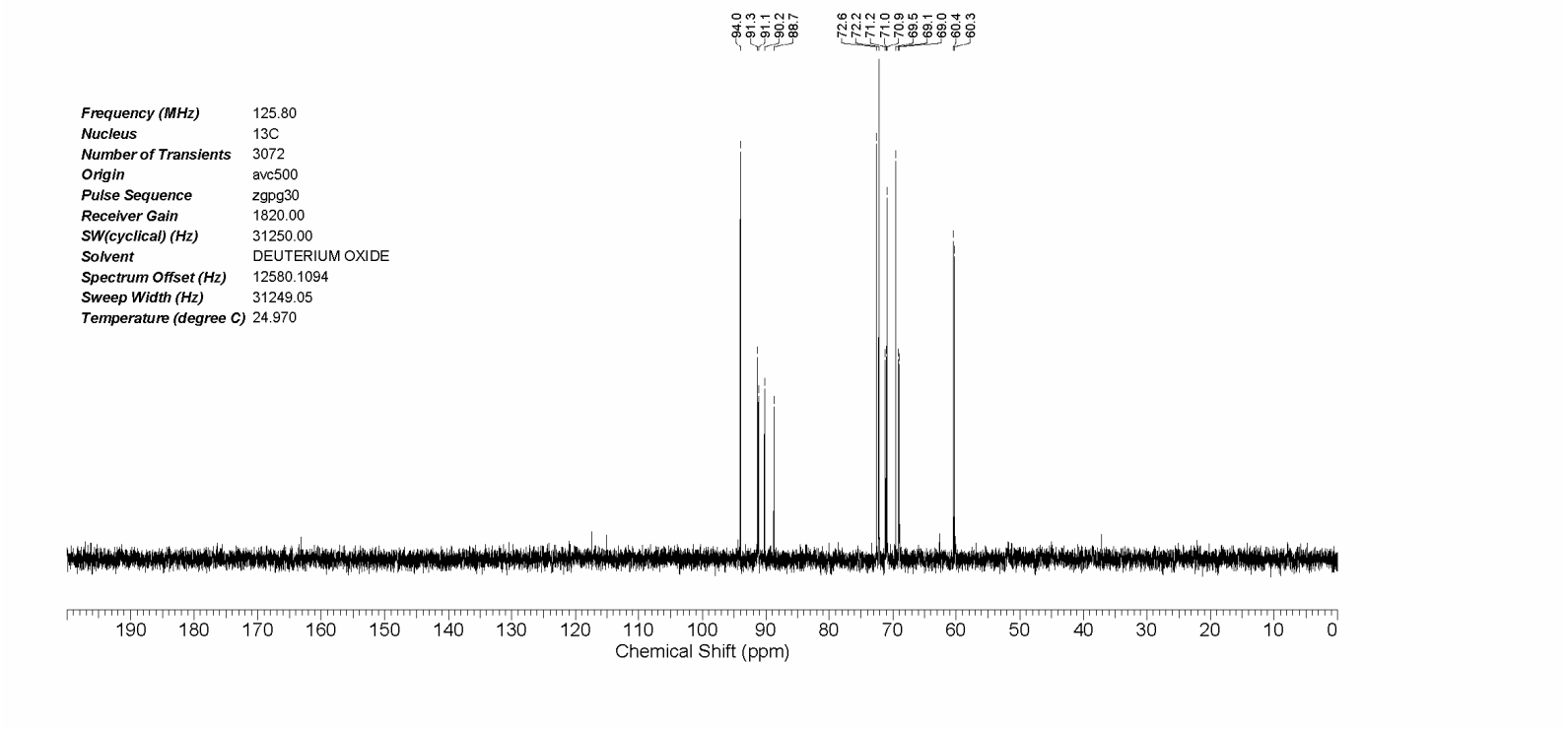

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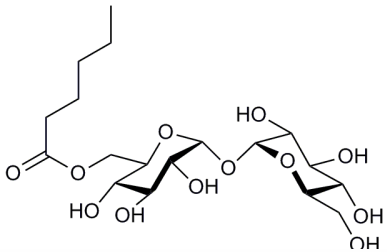
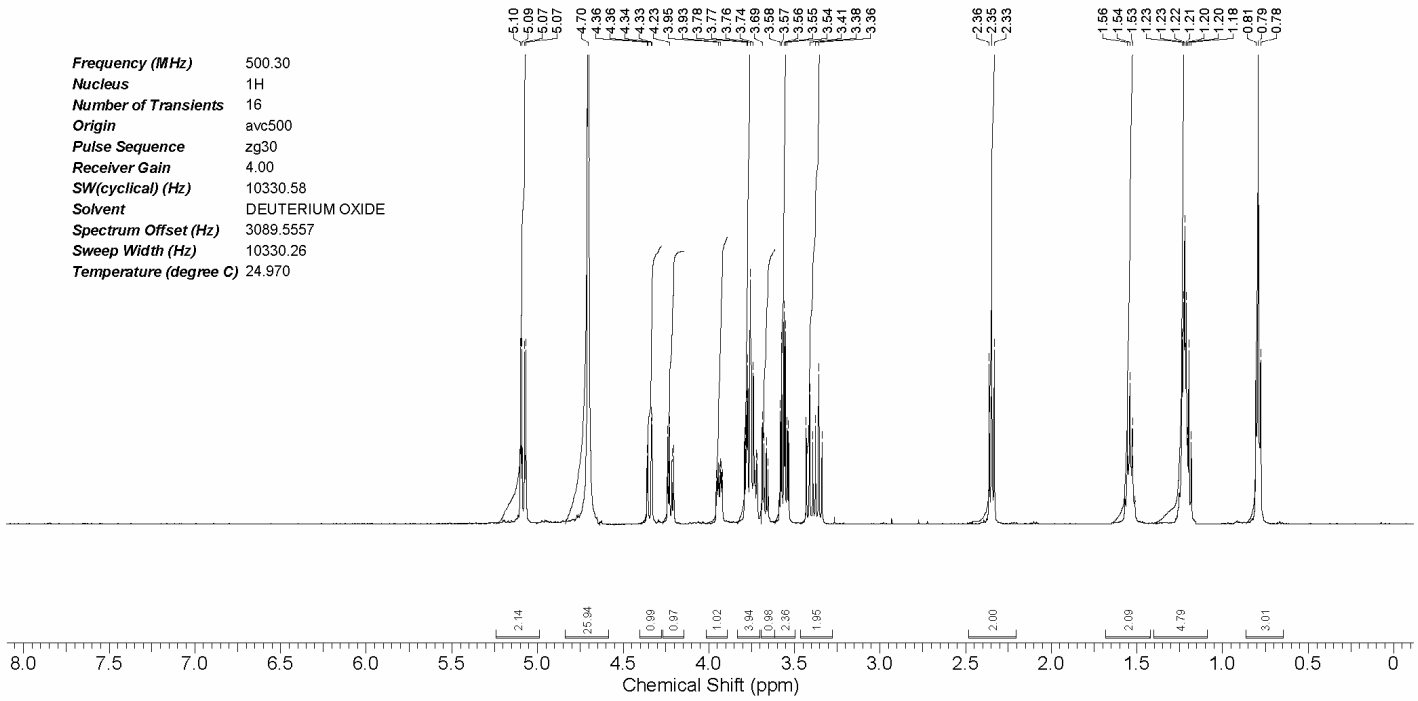


Frequency (MHz)	500.30
Nucleus	1H
Number of Transients	16
Origin	avc500
Pulse Sequence	zg30
Receiver Gain	4.00
SW(cyclical) (Hz)	10330.58
Solvent	DEUTERIUM OXIDE
Spectrum Offset (Hz)	3089.5557
Sweep Width (Hz)	10330.26
Temperature (degree C)	24.970



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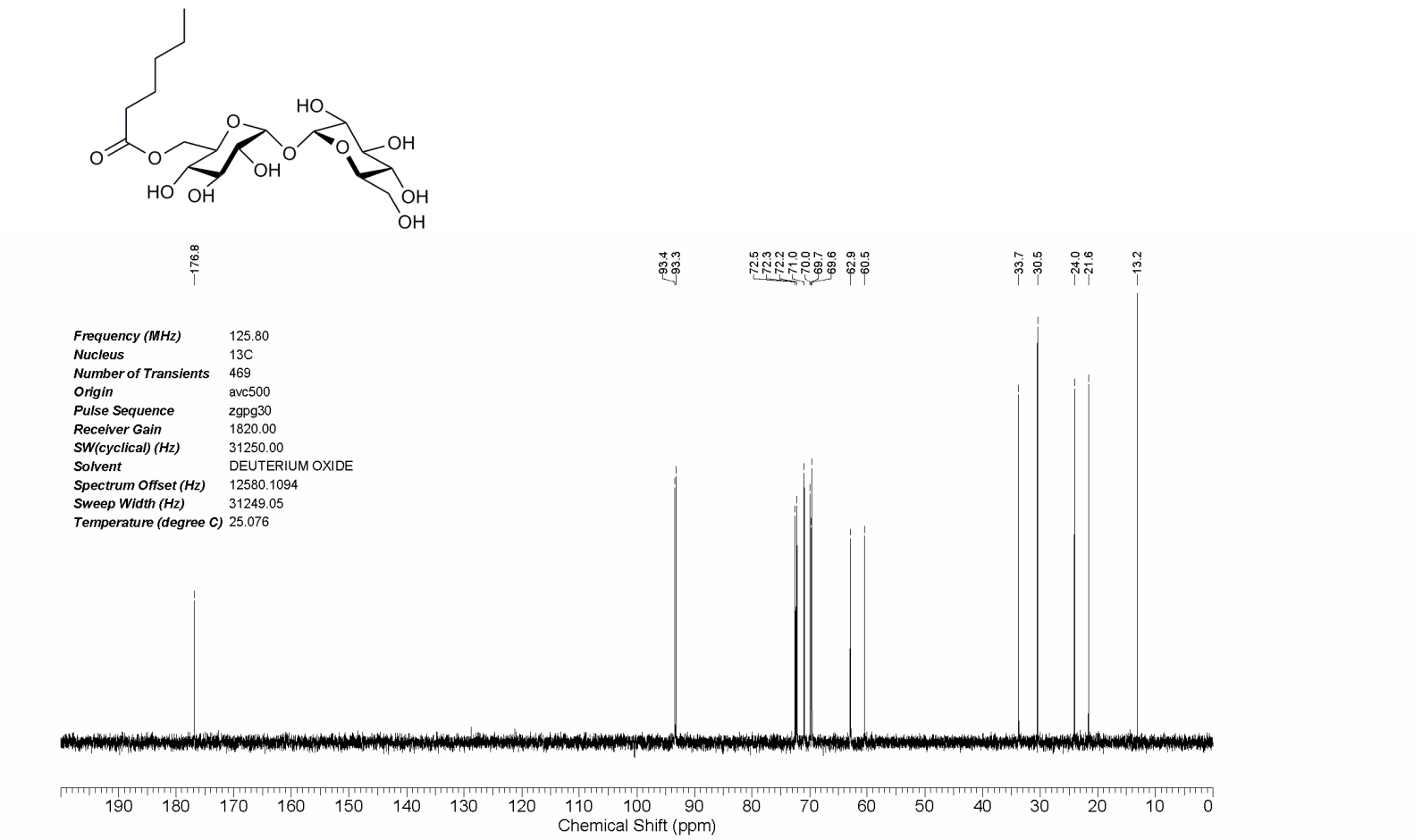

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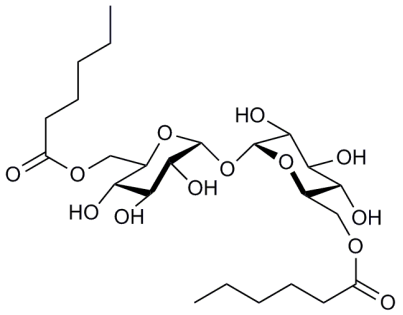

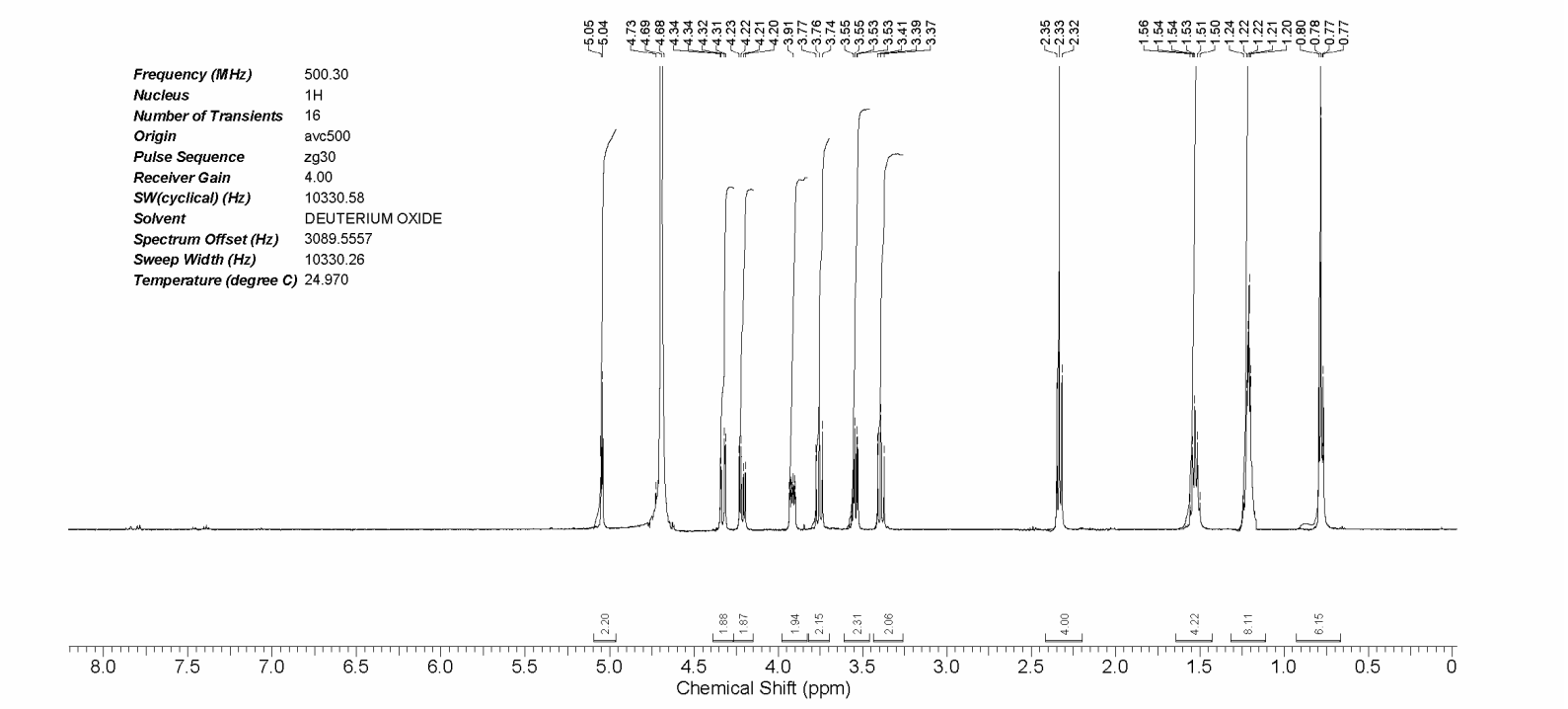

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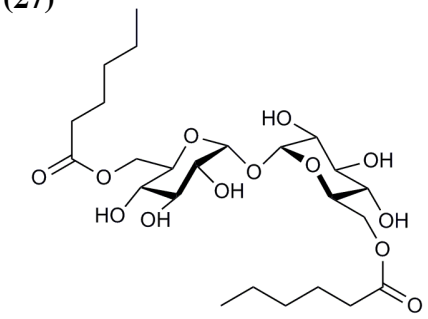

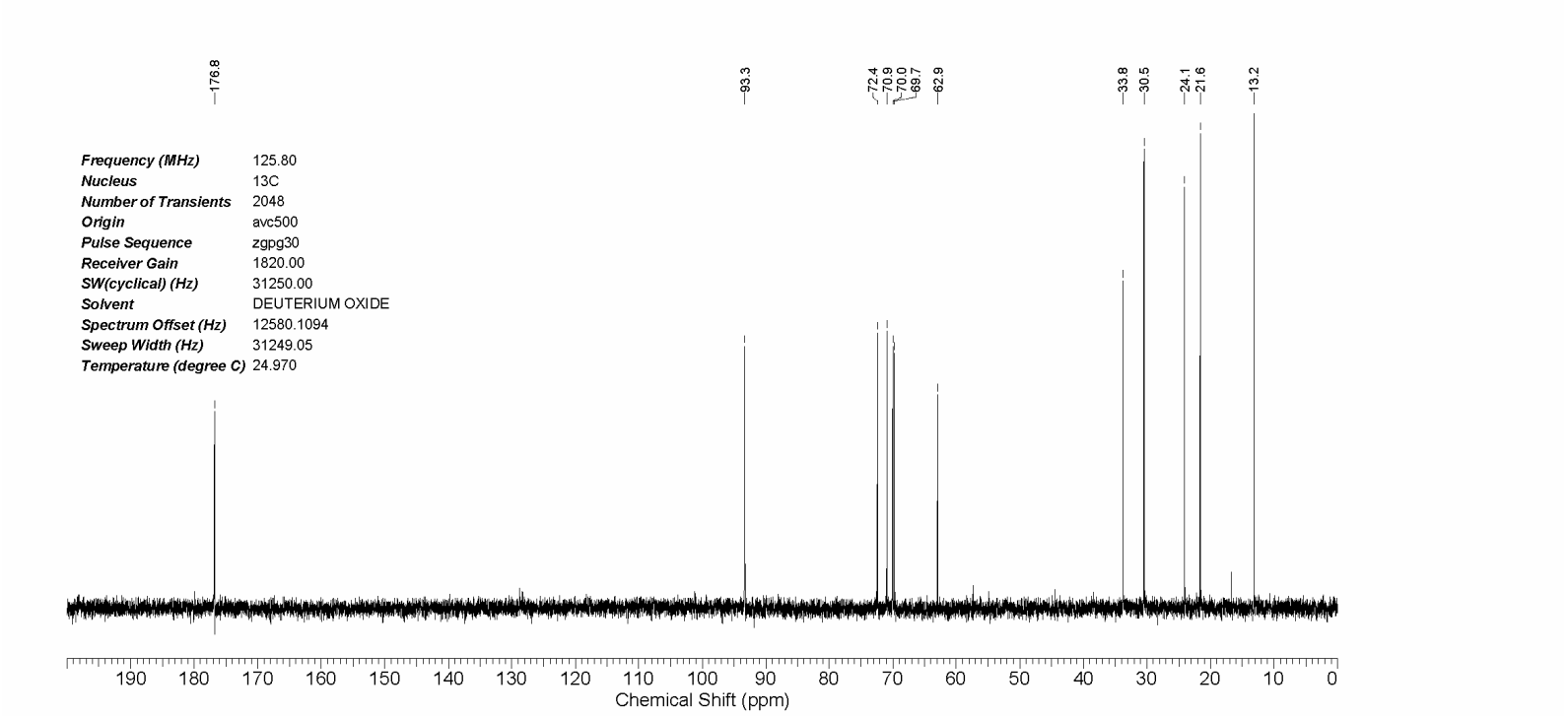

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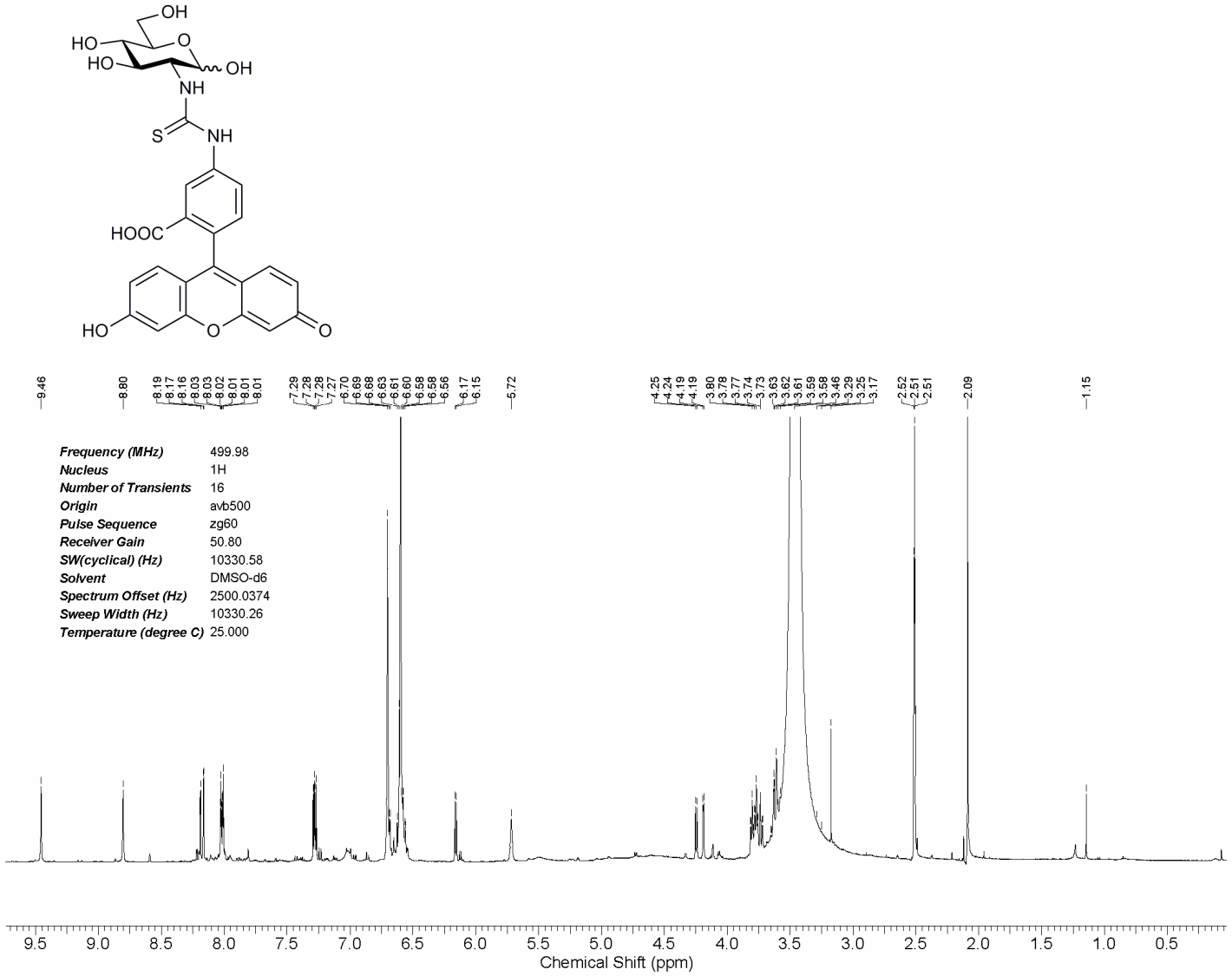

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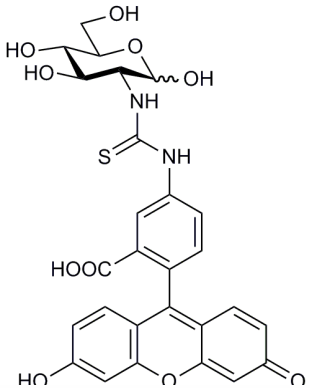

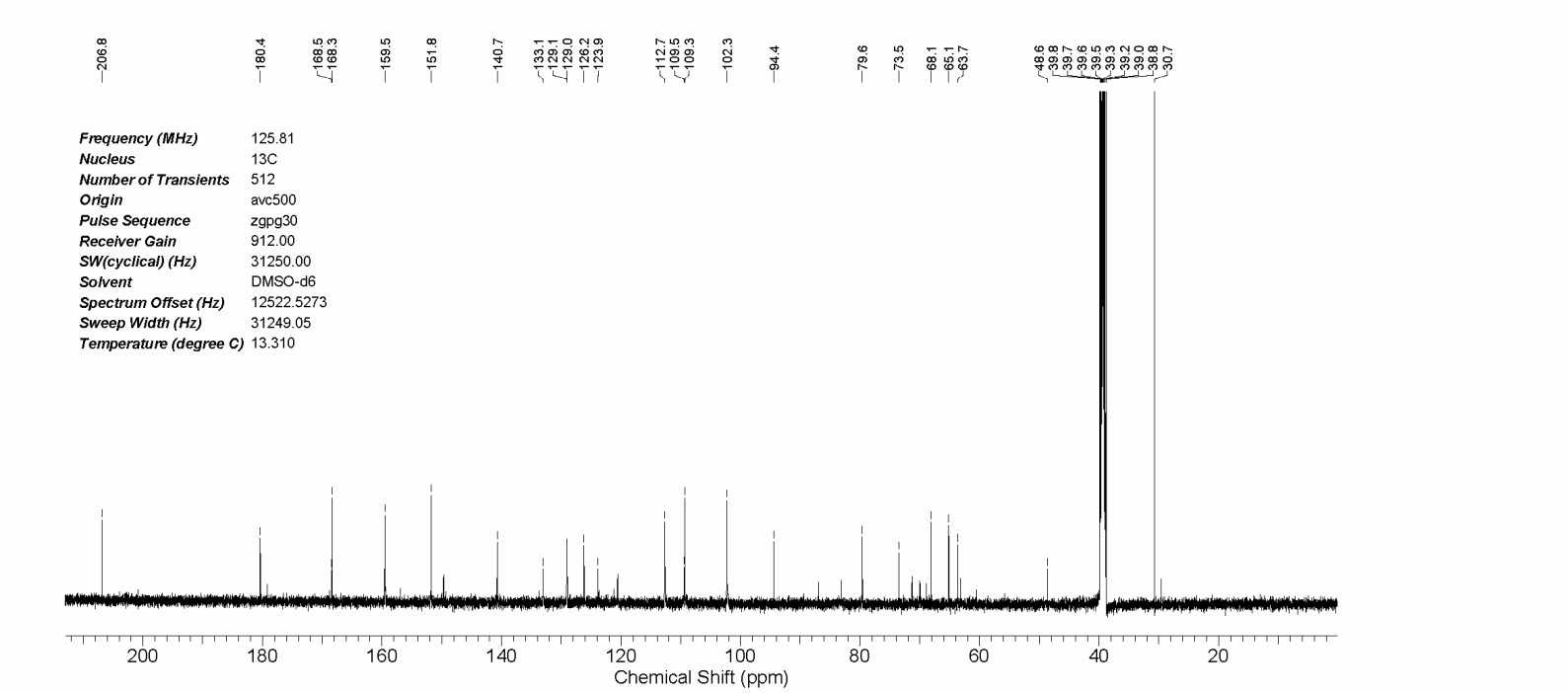

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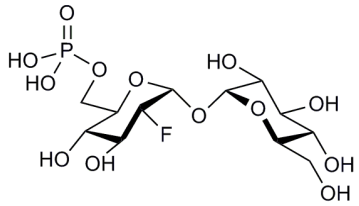

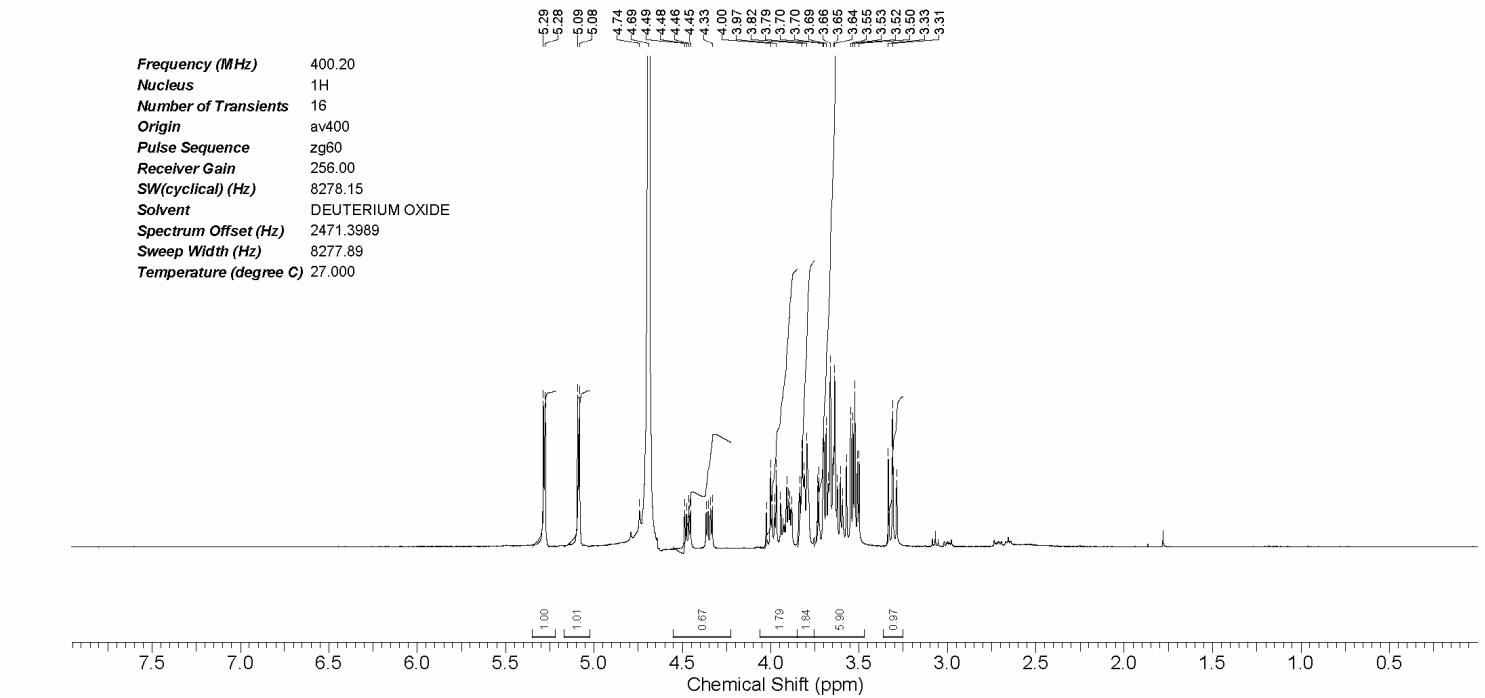

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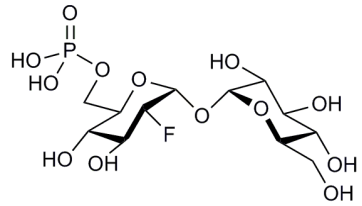

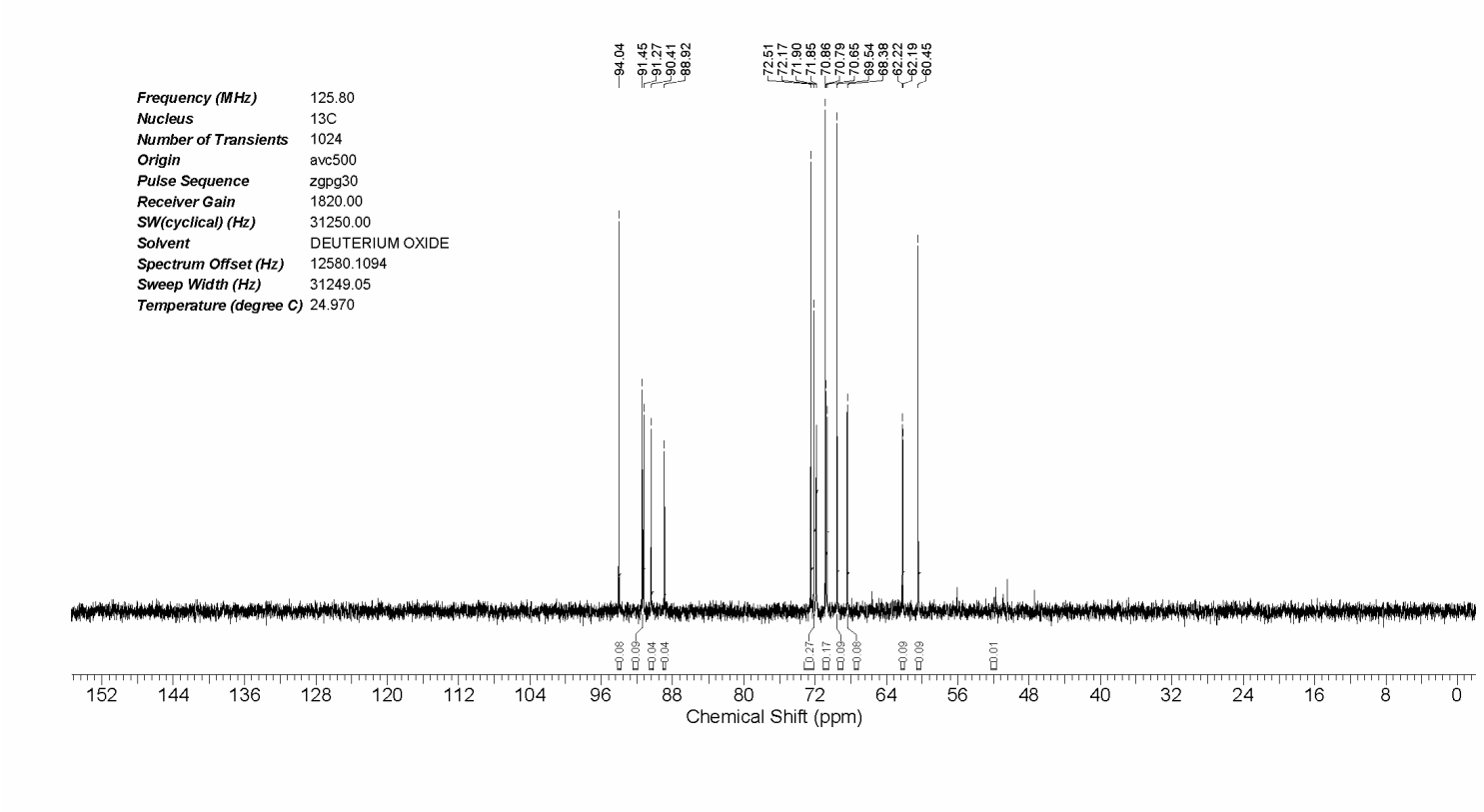

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