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Supporting Information



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# Combined Inhibitor Free-Energy Landscape and Structural Analysis Reports on the Mannosidase Conformational Coordinate**

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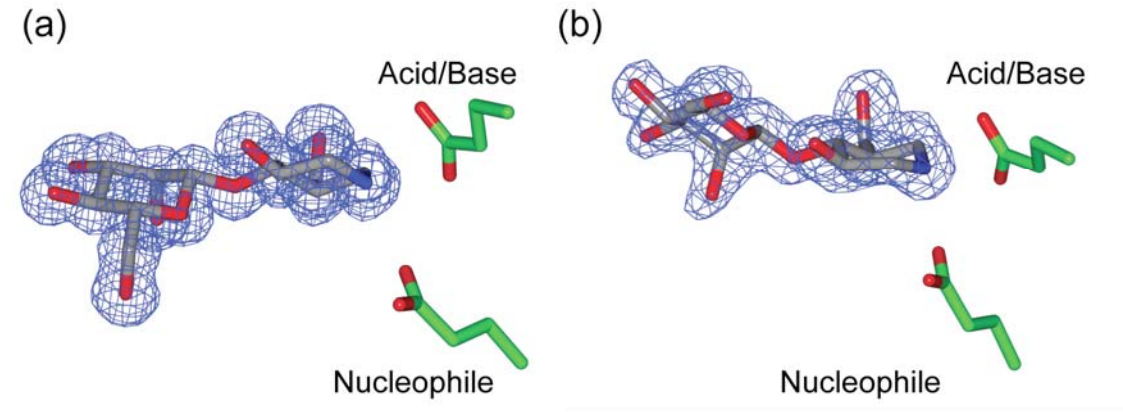



**Figure S1: X-ray structures of ManIFG bound to GH26 and GH113 β-mannanases.**

Binary complex of ManIFG **3** bound to (a) *Cj*Man26C and (b) GH113 *Aa*ManA. Depicted electron density is a REFMAC maximum-likelihood/$\sigma_A$ weighted $2F_o-F_c$ synthesis contoured at 0.41 electrons per Å$^3$.

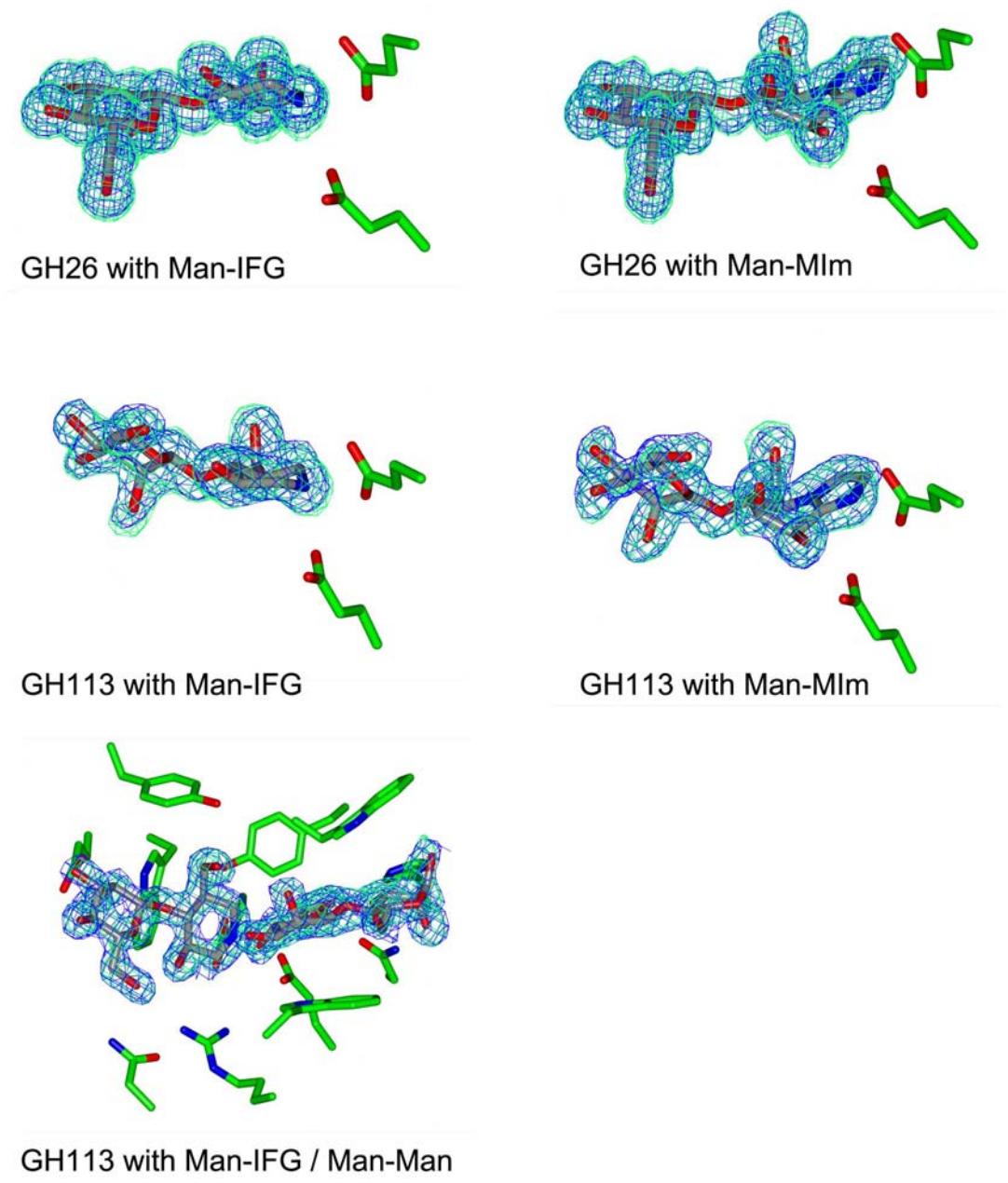



**Figure S2: GH26 and GH113 β-mannanases in complex with Man-IFG and Man-MIm (including a single ternary complex with Man-IFG and Man-Man).**

Figures show identical views to those depicted in Figures 2 and S1, with identical REFMAC maximum-likelihood/$\sigma_A$ weighted $2F_o-F_c$ syntheses, each contoured at 1.0σ (blue density). Additionally, in order to confirm binding, unbiased $F_o-F_c$ maps with phases calculated *prior* to the incorporation of any ligand in refinement are also shown (green density). Difference maps are REFMAC maximum-likelihood/$\sigma_A$ weighted $F_o-F_c$ syntheses contoured at 3.0σ.

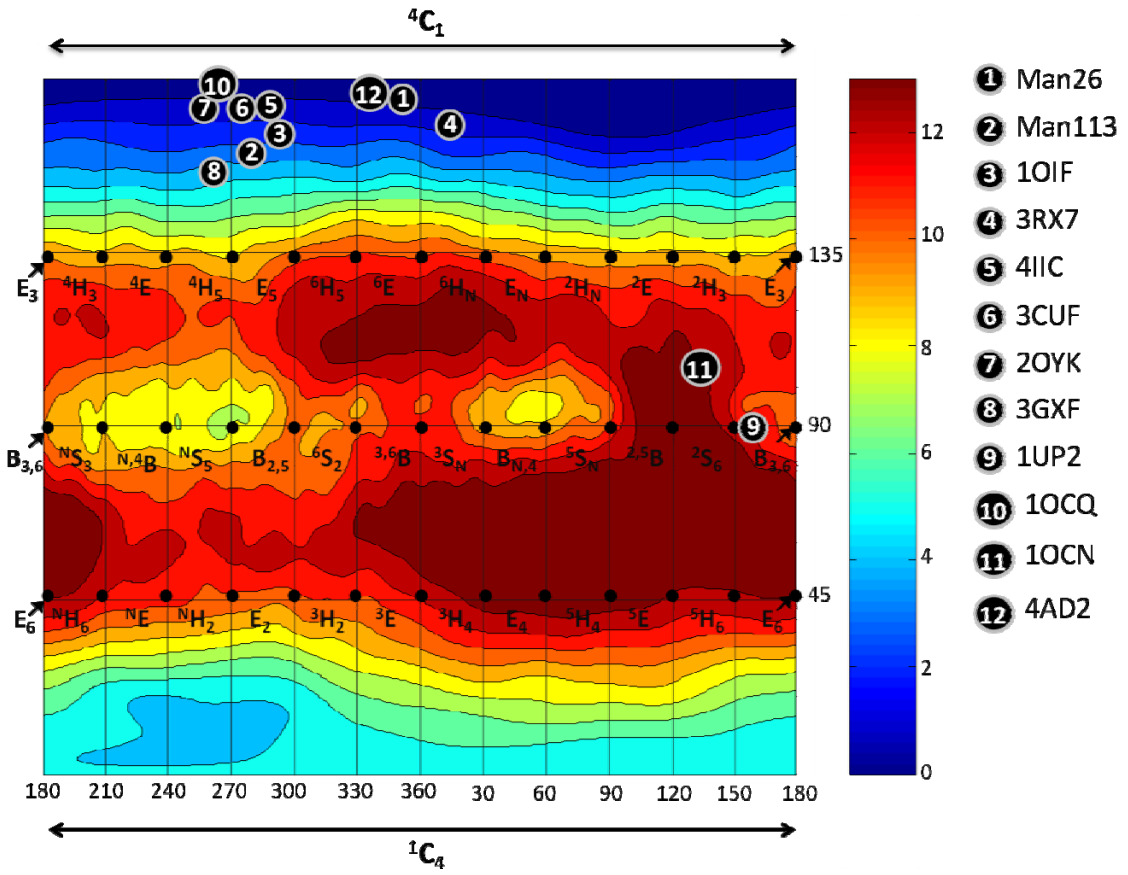



**Figure S3: Conformational free energy landscape (Mercator projection) of isolated isofagomine 1 annotated with the conformations of isofagomine-type inhibitors that have been observed on-enzyme for mannosidases and glucosidases.**

1: **3** bound to GH26 *Cj*Man26C (this work, PDB code 4CD4); 2: **3** bound to GH113 *Aa*ManA (this work, PDB code 4CD6); 3: **1** bound to GH1 *Thermotoga maritima* b-glucosidase (PDB code 1OIF);[1] 5: **1** bound to GH3 *Aspergillus aculeatus* b-glucosidase (PDB code 4IIC);[2] 6: β-Glc-1,4-isofagomine bound to GH10 *Cellulomonas fimi* xylanase/cellulase (PDB code 3CUF); 7: β-Glc-1,4-isofagomine bound to GH5 *Rhodococcus sp.* endoglycoceramidase II (PDB code 2OYK);[3]8: **1** bound to GH30 *Homo sapiens* acid β-glucosidase (PDB code 3GXF);[4] 9: β-Glc-1,4-isofagomine bound to GH6 *Mycobacterium tuberculosis* cellulase (PDB code 1UP2);[5] 10: β-Glc-1,4-isofagomine bound to GH5 *Bacillus agaradhaerans* Cel5A (PDB code 1OCQ);[6] 11: β-Glc-1,4-isofagomine bound to GH6 *Humicola insolens* Cel6A (PDB code 1OCN);[7] 12,13: α-Glc-1,3-isofagomine bound to *Bx*GH99 (PDB code 4AD2, 4AD4);[8] FEL contoured at 1 kcal $mol^{-1}$.

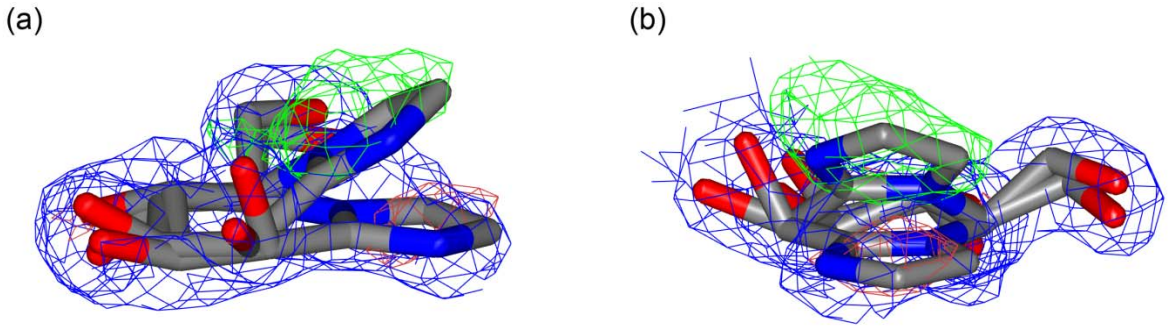



**Figure S4: Remodelled conformations for DmGManII GH38-bound mannoimidazole.**

**(a)** "Side" and **(b)** "front" view of the published $^{4}E$[9] and remodelled near-$B_{2,5}$ conformations. Electron density shown is a $2F_o$-$F_c$ synthesis contoured at about 1σ (0.4 electrons/Å$^3$ in blue) with the difference $F_o$-$F_c$ map contoured at approximately 2.3σ (0.15 / -0.15 electrons/Å$^3$ in green/red respectively). Phases used for maps are calculated from the deposited coordinates, prior to the incorporation of the second modelled conformation in refinement.

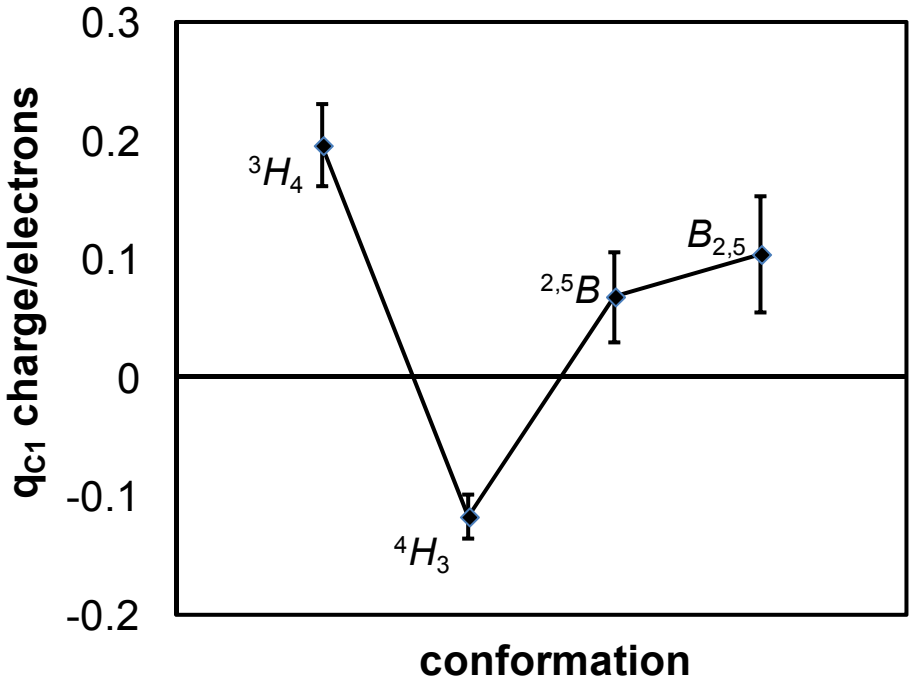



**Figure S5: Plot of atomic charge for protonated mannoimidazole 2 at C1.**
ESP charge computed from the electronic density.

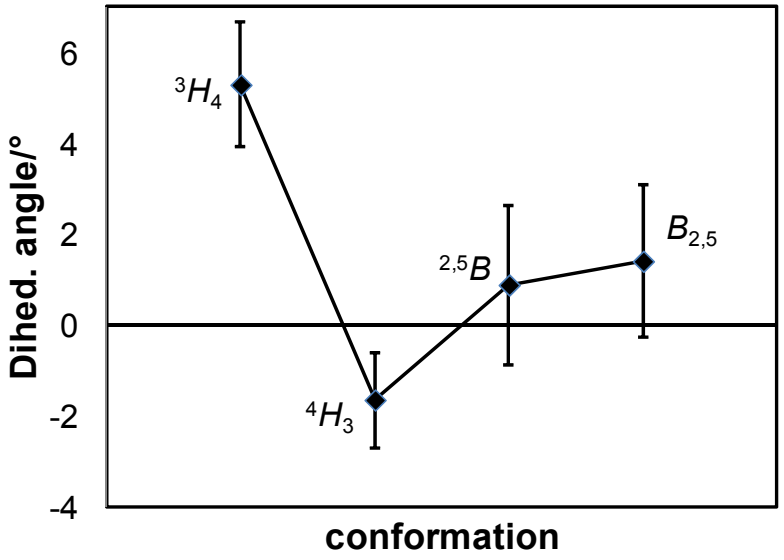



**Figure S6: C5-O5-C1-C2 dihedral angle for each relevant conformation of protonated mannoimidazole 2.**

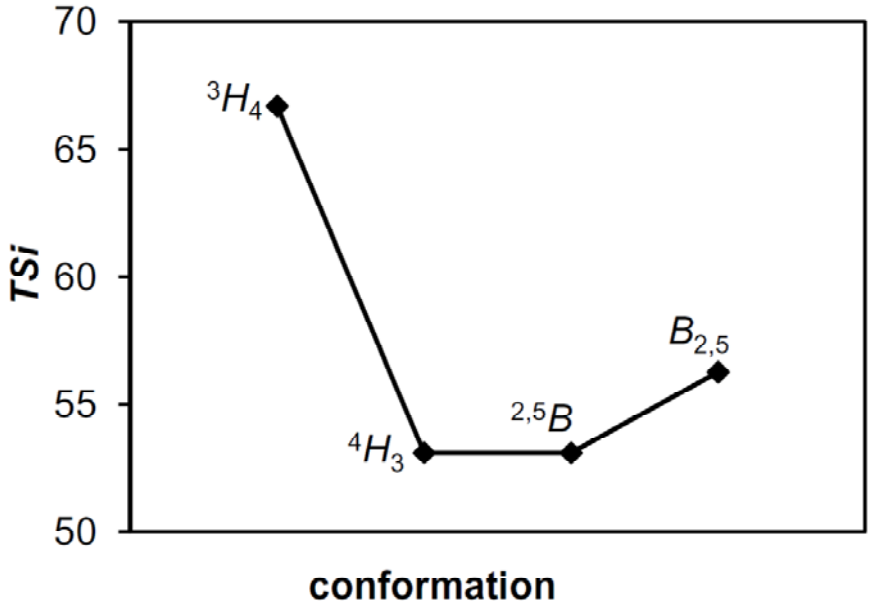



**Figure S7. Computed TS index (TSi) corresponding to the four relevant protonated mannoimidazole 2 conformations.**

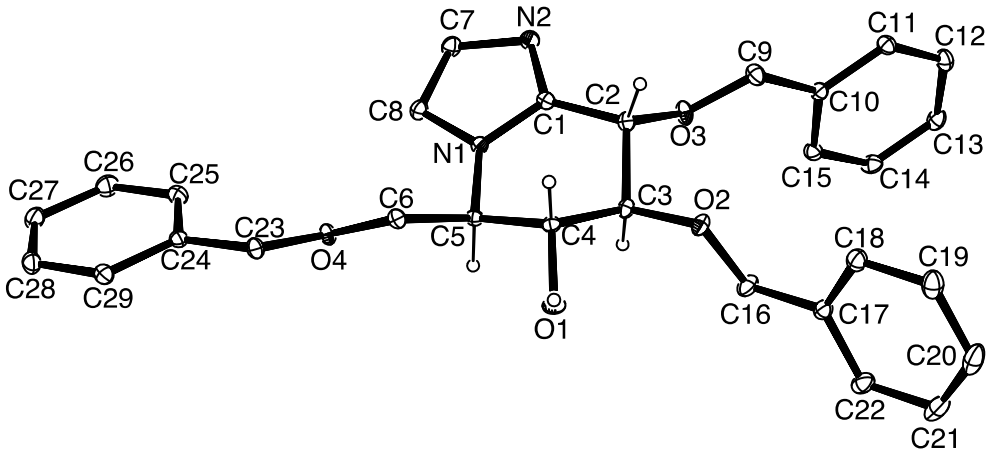



**Figure S8: X-ray structure for D-*gluco* isomer of 15.**

Plot shows thermal ellipsoid plot (at 20% probability level) for one of the two independent molecules of (5*R*,6*R*,7*S*,8*S*)-7,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-6-hydroxy-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine.

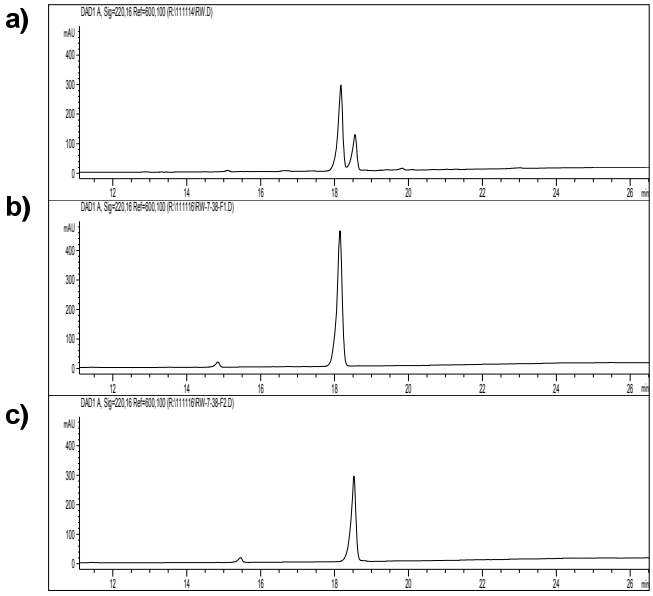



**Figure S9: HPLC purification of 17.**

**a)** Analytical HPLC trace of anomeric mixture of glycosylation prior to HPLC purification; **b)** analytical HPLC trace of **17** after preparative HPLC purification; **c)** analytical HPLC trace of (5*R*,6*R*,7*S*,8*R*)-6-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyloxy)-7,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine minor product after preparative HPLC purification. Minor impurities eluting at approximately $R_t$ = 15 min are attributed to low levels of hydrolysis of the benzylidene acetal under the acidic conditions employed. The impurities were not detectable by NMR.

## Table S1: X-ray data and structure refinement statistics.

| | *Cj*Man26C-ManIFG | *Cj*Man26C-ManMIm | *Aa*ManA-ManIFG | *Aa*ManA-ManIFG mannobiose | *Aa*ManA-ManMIm |
|---|---|---|---|---|---|
| Space Group | $P6_122$ | $P6_122$ | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ |
| Resolution (Å) | 1.20 (1.22)[a] | 1.10 (1.12) | 1.64 (1.69)[a] | 1.65 (1.68) | 1.48 (1.52) |
| $R_{merge}$ | 0.062 (0.694) | 0.097 (2.17) | 0.066 (0.61) | 0.156 (0.63) | 0.062 (0.68) |
| *Mean* ($I/\sigma I$) | 21.9 (0.8) | 11.7 (1.0) | 12.7 (2.5) | 6.7 (1.8) | 14.1 (2.7) |
| Completeness (%) | 88.8 (20.8) | 100.0 (100.0) | 98.0 (98.1) | 97.9 (78.3) | 98.8 (98.2) |
| $R_{cryst}/R_{free}$ (%) | 11.1/13.4 | 14.2/16.4 | 14.4/20.8 | 15.1/19.6 | 15.2/20.1 |
| r.m.s.d bonds (Å) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| r.m.s.d angles (°) | 1.52 | 1.30 | 1.36 | 1.41 | 1.43 |
| PDB code | 4CD4 | 4CD5 | 4CD6 | 4CD7 | 4CD8 |

[a] Values in parentheses denote highest resolution shell.

**Table S2: Calculated values of $q_{C1}$, the C5-O5-C1-C2 dihedral angle, $\Delta G_{rel}$ and *TSi* for the four relevant canonical conformations.**

Values of the different properties of interest, along with its score (in grey) and the resulting preactivation index (*TSi*), associated to each canonical conformation. ESP charges are given in electrons, the dihedral angle in degrees and free energy ($\Delta G_{rel}$) in kcal $mol^{-1}$.

| | $q_{C1}$ | dihed. angle | $\Delta G_{rel}$ | *TSi* |
|---|---|---|---|---|
| $^{3}H_{4}$ | 0.20 | 5.29 | 0.00 | 67 |
| | 100.00 | 0.00 | 100.00 | |
| $^{4}H_{3}$ | -0.12 | -1.65 | 1.36 | 53 |
| | 0.00 | 82.74 | 76.52 | |
| $^{2,5}B$ | 0.07 | 0.89 | 5.78 | 53 |
| | 59.25 | 100.00 | 0.00 | |
| $B_{2,5}$ | 0.10 | 1.41 | 5.21 | 56 |
| | 70.69 | 88.16 | 9.97 | |

**Table S3: Crystal data and structure refinement for (5*R*,6*R*,7*S*,8*S*)-7,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-6-hydroxy-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine.**

| | | |
|---|---|---|
| Identification code | CCDC 924306 | |
| Empirical formula | $C_{29}H_{30}N_2O_4$ | |
| Formula weight | 470.55 | |
| Temperature | 130.0(1) K | |
| Wavelength | 1.5418 Å | |
| Crystal system | Triclinic | |
| Space group | P 1 | |
| Unit cell dimensions | a = 6.2702(2) Å | $\alpha$ = 104.692(2)°. |
| | b = 13.9470(4) Å | $\beta$ = 90.398(2)°. |
| | c = 14.0503(4) Å | $\gamma$ = 93.268(2)°. |
| Volume | 1186.31(6) $Å^3$ | |
| Z | 2 | |
| Density (calculated) | 1.317 $Mg/m^3$ | |
| Absorption coefficient | 0.706 $mm^{-1}$ | |
| F(000) | 500 | |
| Crystal size | 0.55 x 0.13 x 0.08 $mm^3$ | |
| Theta range for data collection | 3.25 to 74.08°. | |
| Index ranges | -7<=h<=7, -17<=k<=17, -16<=l<=17 | |
| Reflections collected | 18067 | |
| Independent reflections | 8830 [R(int) = 0.0276] | |
| Completeness to theta = 74.08∞ | 98.5 % | |
| Absorption correction | Semi-empirical from equivalents | |
| Max. and min. transmission | 1.00000 and 0.84722 | |
| Refinement method | Full-matrix least-squares on $F^2$ | |
| Data / restraints / parameters | 8830 / 3 / 639 | |
| Goodness-of-fit on $F^2$ | 1.055 | |
| Final R indices [I>2sigma(I)] | R1 = 0.0301, wR2 = 0.0767 | |
| R indices (all data) | R1 = 0.0311, wR2 = 0.0777 | |
| Absolute structure parameter | 0.01(9) | |
| Largest diff. peak and hole | 0.170 and -0.209 $e.Å^{-3}$ | |

**Computational Chemistry.** Quantum mechanical calculations were performed using Density Functional Theory-based molecular dynamics (MD), according to the Car-Parrinello method.[10] The system analyzed consists of a single protonated mannoimidazole unit (27 atoms) enclosed in an isolated orthorhombic box of size 14.0 Å × 14.0 Å × 13.0 Å. The Kohn-Sham orbitals were expanded in a plane wave (PW) basis set with a kinetic energy cutoff of 70 Ry. Ab initio pseudopotentials generated within the Troullier-Martins scheme were employed.[11] The Perdew, Burke and Ernzerhoff generalized gradient-corrected approximation (PBE)[12] was selected in view of its good performance in previous work on isolated sugars,[13] glycosidases[14] and glycosyltransferases.[15] A fictitious electron mass of 850 au and a time step of 0.12 fs were used. The metadynamics algorithm[16] was used to explore the conformational free energy landscape of mannoimidazole, taking as collective variables two of the puckering coordinates of Cremer and Pople[17] (θ, φ), in the spirit of the pioneering work by Dowd, French and Reilly.[18] Initially, the height of these Gaussian terms was set at 0.19 kcal·mol$^{-1}$ and once the free-energy surface was fully explored it was reduced to 0.13 kcal·mol$^{-1}$, to ensure sufficient accuracy for the reconstruction of the free energy surface. The width of the Gaussian terms was set to 0.10 Å according to the oscillations of the selected collective variables observed in a free dynamics. At the beginning of the metadynamics simulation, a new Gaussian-like potential was added every 400 MD steps, which was increased up to 1000 MD steps towards the end of the simulation. The simulation was stopped after having added 2490 Gaussians. In terms of simulation time this corresponds to ≈ 1,5 x 10$^6$ MD steps (≈ 180 ps). The FEL of isofagomine was computed using the same setup as for mannoimizadole. The isofagomine molecule was considered as the neutral species to avoid spurious interactions of the hydroxymethyl and the $NH_2^+$ that take place in the absence of the enzyme environment and heavily affect the shape of the FEL. In the case of mannoimidazole, the planarity of the imidazole ring avoids such interactions and the FEL is not affected by protonation of imidazole nitrogen.

**Cloning, gene expression and protein purification.** The *C. japonicus* mannobiohydrolase gene (*man26C*) was cloned and expressed as described earlier.[19] The *A. acidocaldarius* mannanase gene (*man113A*) was optimized for expression in *Escherichia coli* and synthesized by GenScript, then cloned into pET28a (Novagen) and expressed in *E. coli* BL21 (DE3). *Aa*ManA-6x-His was purified using a $Ni^{2+}$ agarose column (His TrapTM FF, Amersham Biosciences) and then further purified by gel filtration using a HiLoad 16/60 Superdex 200 prep grade column and eluted with 50 mM HEPES, pH 7.5, 150 mM NaCl.

**Collection of structural data, processing and structure solution.** Pure *Cj*Man26C was crystallized as described before,[19] with 10 mM ManIFG or 10 mM ManMIm present in the protein solution. Purified *Aa*ManA was crystallized in 0.1 M sodium acetate pH 4.6 and 4% PEG 4000. Crystals of the ManIFG, ManIFG+mannobiose and ManMIm complexes were obtained by soaking $P2_12_12_1$ *Aa*ManA crystals for 10 min in mother liquor supplemented with 10mM ManIFG, 10 mM ManIFG and 10 mM mannobiose, or 10 mM ManMIm, respectively. X-ray data for both mannanase complexes were collected at the Diamond Light Source and processed using XIA2[13c] implementations of XDS[20] or MOSFLM.[21] Both structures were solved by molecular replacement using the CCP4 implementation[22] of the MOLREP program.[23] The E338A *Cj*Man26C-mannobiose (PDB 2VX7) and the native *A. acidocaldarius Aa*ManA (PDB 3CIV) were used as search models. The structures, each with one molecule in the asymmetric unit, were refined using numerous cycles of REFMAC[24] and manual corrections using COOT.[25] Data and structure quality statistics are in Table 1, Further details on structure solution and refinement are included in the PDB headers and the Supporting Information. Structural figures were drawn with CCP4MG.[26] The staff at the Diamond Light Source is thanked for provision of beamline facilities.

**Enzyme kinetics.** The activity of *Cj*Man26C (5 nM) was determined at 37 $^{o}$C using mannotriose as the substrate at 500 μM, which is $<<K_M$ estimated at 5 mM. The reactions were carried out in the absence or presence of inhibitor at concentrations ranging from 50 to 500 nM. The reaction product mannose was determined continuously, using the mannose/glucose/fructose detection kit supplied by Megazyme International, in 50 mM Na-HEPES buffer containing 2 mM $MgCl_2$. The activity of *Aa*ManA-6x-His at 20 nM was determined using 600 μM of the substrate mannotetraose ($<<K_M$ of 7 mM) in 50 mM potassium phosphate/12 mM citrate buffer pH 6.5 at 50 $^{o}$C. The inhibitor, when incorporated into the assay, was at 3 mM for ManMIm and from 0.1 to 2 mM for ManIFG. In this discontinuous assay the rate of substrate depletion was measured using Dionex high performance anion-exchange chromatography as described previously.[27] The reaction rates for both enzymes gave a direct read out of $k_{cat}/K_M$ in the presence of a range of [I] for *Cj*Man26C (single [I] for *Aa*ManA-6x-His), enabling $K_I$ to be calculated from equation (1):

$$\frac{v_0}{v_i} = \frac{1}{K_I} \times [I] + 1 \qquad (1)$$

where $v_0$ and $v_i$ are the rates of the reaction in the absence and presence of inhibitor, respectively. Under conditions where [S]>> $K_M$ the fractional decrease in rate thus yields the $K_I$ for a competitive inhibitor.[28]

**Calculation of transition state inhibition index (*TSi*).** For configurationally-matched inhibitors, design features that mimic the transition state of glycosidase-catalyzed hydrolysis reactions include: a) a suitably positioned positive charge that can interact with the catalytic carboxylate groups; and b) chemical modifications that enforce planarity of the 'carbohydrate' ring. The charge development at the anomeric carbon ($q_{C1}$) and the value of the dihedral angle (C2-C1-O5-C5) of protonated mannoimidazole **1** were calculated in order to assess which conformations are the most 'pre-activated' for inhibition. A set of approximately 100 structures (approx 25 structures for each one of the conformations of interest) were selected from the metadynamics simulation and submitted to geometry optimization. The optimized structures were clustered to each one of the four possible transition state conformations according to the values of their θ and φ puckering coordinates.[17] Atomic charges (ESP), C2-C1-O5-C5 dihedral angle and free energy were extracted for each structure within the group and average values were computed. Plots of atomic charge and dihedral angle for each conformation are available as supporting information (Figure S5 and S6).

Both parameters ($q_{C1}$ and dihedral angle) were combined, along with the relative free energy ($\Delta G_{rel}$), into an index that reflects the likelihood for a given conformation of the inhibitor to be adopted on-enzyme. This was done by assigning for each conformation, j, a score for each parameter, $x_i$, using the equations (2) and (3):[13a]

$$\text{score}\left(x_{i,j}\right) = \frac{x_{i,j} - x_{i,j}^{\min}}{x_{i,j}^{\max} - x_{i,j}^{\min}} \times 100 \quad \text{for } x_i = q_{C1} \tag{2}$$

$$\text{score}\left(x_{i,j}\right) = \frac{x_{i,j}^{\max} - x_{i,j}}{x_{i,j}^{\max} - x_{i,j}^{\min}} \times 100 \quad \text{for } x_i = \Delta G_{rel}, \left|Dihed.angle_{C2-C1-O5-C5}\right| \tag{3}$$

The values of the parameters and the corresponding scores are given in Table S2. Since the score for each parameter is normalized, they can be directly compared. The transition state index, $TSi_j$, is defined as the average of the scores for the n parameters (n = 3) for a given conformation j (equation (4)):

$$TSi_j = \sum_i score\left(x_{i,j}\right) / n \tag{4}$$

The values are plotted in Figure S7.

**Re-refinement of GH38 mannoimidazole complex.** The counter-intuitive observation of a $^4E$ conformation for mannoimidazole in the deposited *Dm*GManII GH38 complex (PDB: 3D4Y) led us to inspect the coordinates.[9] It is clearly apparent from the density that although the deposited $^4E$ conformation is a major conformer, that an unmodelled second conformer exists, reflected in positive $F_o$-$F_c$ difference density "above" the imidazole ring (Supplementary Figure 1, coloured green) and negative $F_o$-$F_c$ difference density on the imidazole ring, as modelled. In order to investigate the nature and relative occupancy of such an additional mannoimidazole conformer, we refined the original deposited data (structure factors that accompany PDB 3D4Y) both against dual occupancy ligands inserted into both the MIm-bound model (3D4Y) and, in order to remove any possible bias, against an earlier entirely ligand-free model (PDB 3BUB). Manual modelling was carried out using the real-space refinement options of COOT,[29] followed by several refinement rounds of maximum likelihood refinement with REFMAC.[30] The second conformer indeed refines to a near $B_{2,5}$ conformation for the mannoimidazole, however, it is difficult to judge the relative occupancies of the two conformations. Manual manipulation of the occupancies of the two respective conformers, such that they yield the same refined average temperature factor (about 24 Å$^2$) post-refinement, suggests an approximate relative occupancy of 0.65:0.35 (envelope:boat) for structures refined using either starting model.

**Small molecule crystallographic methods.** Crystals of (5*R*,6*R*,7*S*,8*S*)-7,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-6-hydroxy-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine were slowly grown in ethyl acetate and mounted in low temperature oil then flash cooled to 130 K using an Oxford low temperature device. Intensity data were collected at 130 K with an Oxford SuperNova X-ray diffractometer with CCD detector using Cu-Kα radiation (λ = 1.54184 Å). Data were reduced and corrected for absorption.[22] The structures were solved by direct methods and difference Fourier synthesis using the SHELX suite of programs[31] as implemented within the WINGX software.[32] Thermal ellipsoid plots were generated using the program ORTEP-3 (Figure S8).

**Crystallographic data.** Crystal data for (5*R*,6*R*,7*S*,8*S*)-7,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-6-hydroxy-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine**:** $C_{29}H_{30}N_2O_4$ $M$ = 470.55, $T$ = 130.0(1) K, λ = 1.54180, triclinic, space group P1 $a$ = 6.2702(2) $b$ = 13.9470(4)*,* $c$ = 14.0503(4) *Å,* $V$ = 1186.31(6) *Å*$^3$, Z = 2, $D_c$ = 1.317 Mg M$^{-3}$ μ(Cu-Kα) 0.706 mm$^{-1}$, F(000) = 500, crystal size 0.55 x 0.13 x 0.08 mm. 18067 reflections measured, 8830

independent reflections ($R_{int}$ = 0.0276), the final R was 0.0301 [$I > 2\sigma(I)$] and $wR(F^2)$ (all data) was 0.0777. This data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif under the code CCDC 924306.

**Synthetic overview.** ManIFG **3** was prepared by a β-selective mannosylation using Crich's pre-activation protocol[33] on the protected acceptor alcohol **7**, followed by global deprotection (Scheme 1A). The synthesis of ManMIm **4** commenced through annulation of the imidazole ring onto the amidine **8** utilizing the approach developed by Vasella and co-workers (Scheme 1B).[34] Cyclization of **14** using TsOH resulted in simultaneous removal of the PMB group and afforded a mixture of D-*gluco*- and D-*manno*-configured alcohols whose assignment on the basis of $^{3}J_{H,H}$ coupling constants was ambiguous. Fortunately, the D-*gluco*-diastereomer of **15** was crystalline, allowing its characterization by X-ray crystallography (Figure S7). Efforts to β-mannosylate the D-*manno*-alcohol **15** utilizing donor **16** under Crich's pre-activation protocol were unsuccessful. The weakly β-selective mannosylation conditions reported by Shin and co-workers[35] using the same donor **16** afforded *pseudo*-disaccharide **17** in 40% yield, with a modest β-stereoselectivity of 2:1. Purification of **17** was achieved by HPLC (Figure S9). Global deprotection afforded **4**, in 17 linear steps (from D-glucose).

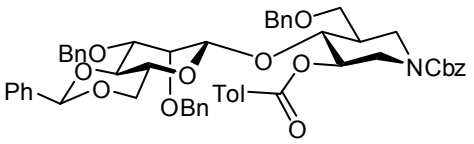
**General synthetic methods.** Thin layer chromatography (TLC) was performed using aluminium-backed plates of Silica Gel 60 $F_{254}$. Detection was by visualisation in UV light and/or by charring in a mixture of 5% $H_2SO_4$/MeOH, 10% (w/v) phosphomolybic acid/EtOH or a solution of ceric ammonium molybdate (made from 1:5:10:90 $Ce(SO)_4/(NH_4)_6Mo_7O_{24}\cdot 4H_2O/H_2SO_4/H_2O$). $^{1}H$, $^{13}C$ and 2-D NMR spectra were collected on a nominal 500 MHz instrument (499.7 MHz for $^{1}H$ and 125.8 MHz for $^{13}C$) at 25.0 ºC. Spectra are referenced to the following solvent peaks: $CDCl_3$ (δ 7.27 ppm for $^{1}H$; 77.16 ppm for $^{13}C$) or $d_4$-methanol (δ 3.34 ppm for $^{1}H$; 49.0 ppm for $^{13}C$). Superscript$^{A,B}$ specifying carbohydrate rings starting from the non-reducing end for *pseudo*-disaccharides. Flash chromatography was performed according to the method of Still *et al.* with Silica Gel 60.[36] Pyridine was distilled over KOH. Acetonitrile was distilled over $P_2O_5$. Toluene was distilled over $K_2CO_3$. Dimethylformamide and dimethylsulfoxide were dried using 4 Å molecular sieves. $CH_2Cl_2$ was dried using a solvent purification apparatus (Glass Contour, USA) as described by Pangborn *et al.*[37] Solvents were evaporated under reduced pressure using a rotary evaporator. $[\alpha]_D$ values are given in $deg.dm^{-1}\ cm^3\ g^{-1}$. Melting points were obtained using a hot stage melting point apparatus and are corrected. High resolution mass spectra (HRMS) were acquired on a Finnegan FT-ICR-MS.

**4,5'-*O*-Benzylidene-3-*O*-*p*-toluoyl-*N*-benzyloxycarbonyl-isofagomine (6).** 4-Dimethylaminopyridine (0.0130 g, 0.109 mmol) was added to a solution of 4,5'-*O*-benzylidene-*N*-benzyloxycarbonyl-isofagomine **5**[38] (0.202 g, 0.546 mmol), *p*-toluoyl chloride (0.144 ml, 1.09 mmol), distilled pyridine (0.44 ml, 5.46 mmol) and dry dichloromethane (5.0 ml) under $N_2$. After 3 h TLC analysis (1:1 EtOAc/pet. spirits with 1% $Et_3N$) indicated complete consumption of starting material. The mixture was diluted with dichloromethane and washed with sat. aq. $NaHCO_3$ (3 × 5 ml) and brine. The organic extracts were dried ($MgSO_4$), the solvent evaporated under reduced pressure and the resulting residue purified by flash chromatography (30:70 EtOAc/pet. spirits with 1% $Et_3N$) to afford the protected carbamate **6** (0.234 g, 88%) as a colourless oil that crystallised. A small portion was recrystallised from EtOAc/pet. spirits, 149-150 °C; $[\alpha]_D^{24}$ -47 (c 0.9, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 2.10-2.19 (1H, m, H5), 2.39 (3H, s, $CH_3$), 2.50-2.61, 2.86-2.97 (2H, 2m, $H2_{ax}$, $H6_{ax}$), 3.67 (1H, dd, $J_{5,5'}$ 10.5, $J_{5',5'}$ 11.0 Hz, C$H_2$(C5)), 3.87 (1H, $J_{3,4}$ 9.5, $J_{4,5}$ 10.0 Hz, H4), 4.00-4.11 (1H, m, H6eq), 4.15-4.22 (1H, m, C$H_2$(C5)), 4.60-4.74 (1H, m, $H2_{eq}$), 5.11-5.29 (3H, m, H3,PhC$H_2$), 5.61 (1H, s, PhC*H*), 7.22 (2H, app d, Ar), 7.29-7.45 (10H, m, Ar), 7.93 (2H, app d, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$) 21.77 (1C, $CH_3$), 37.61 (1C, C5), 43.37, 46.20 (2C, C2,6), 67.79, 68.33, 69.86, 81.84 (4C, C3,4,5',Ph$CH_2$), 101.58 (1C, Ph*C*H), 126.12, 127.27, 128.12, 128.28, 128.68, 128.97, 129.17, 129.87, 136.39, 137.77, 143.95 (18C, Ar), 155.11 (1C, NC=O), 165.72 (1C, OC=O); HRMS *m/z* ($ESI^+$) 510.1887 ($C_{29}H_{29}NNaO_6$ $[M + Na]^+$ requires 510.1887).

**5'-*O*-Benzyl-3-*O*-*p*-toluoyl-*N*-benzyloxycarbonyl-isofagomine (7).** Trifluoroacetic acid (0.027 ml, 0.368 mmol) was added to a mixture of protected carbamate **6** (0.036 g, 0.074 mmol), triethylsilane (0.058 ml, 0.368 mmol), freshly-activated 4 Å molecular sieves and dry dichloromethane (0.50 ml). After 5.5 h under $N_2$ TLC analysis (40:60 EtOAc/toluene with 1% $Et_3N$) indicated that significant amounts of starting material remained and further trifluoroacetic acid (0.027 ml, 0.368 mmol) and triethylsilane (0.058 ml, 0.368 mmol) were added. After 20 h TLC analysis (40:60 EtOAc/toluene with 1% $Et_3N$) indicated complete consumption of starting material and the mixture was filtered through Celite, diluted with dichloromethane and washed with sat. aq. $NaHCO_3$ (3 × 5 ml) and brine. The organic extracts were dried ($MgSO_4$), the solvent removed under reduced pressure and the resulting residue subjected to flash chromatography (15:85 EtOAc/toluene with 1% $Et_3N$) to afford the alcohol **7** (0.034 g, 93%) as a colourless oil. $[\alpha]_D^{23}$ +10 (c 0.77, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 1.92-1.99 (1H, m, H5), 2.42 (3H, s, $CH_3$), 2.77-2.89 (1H, m, $H6_{ax}$), 2.91 (1H, $J_{2,2}$ 13.0, $J_{2,3}$

10.0 Hz, $H2_{ax}$), 3.06 (1H, bs, 4-OH), 3.61 (1H, dd, $J_{5,5'}$ 5.0, $J_{5',5'}$ 9.5 Hz, C$H_2$(C5)), 3.65-3.77 (1H, m, C$H_2$(C5)), 3.81-3.94, 4.14-4.25 (2H, 2m, $H4,6_{eq}$), 4.41-4.49 (1H, m, $H2_{eq}$), 4.52 (2H, s, PhC$H_2$), 4.92-4.99 (1H, m, H3), 5.09-5.23 (2H, m, PhC$H_2$), 7.24-7.38 (12H, m, Ar), 7.93 (2H, app d, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$) 21.84 (1C, $CH_3$), 42.20 (1C, C5), 44.71, 45.51 (2C, C2,6), 67.63, 69.56, 73.34, 73.60, 73.68 (5C, C3,4,5', 2 × PhC$H_2$), 127.01, 127.75, 127.91, 128.08, 128.21, 128.58, 128.66, 129.27, 129.95, 136.59, 137.95, 144.22 (18C, Ar), 155.25 (1C, NC=O), 166.49 (1C, OC=O); HRMS *m/z* ($ESI^+$) 512.2044 ($C_{29}H_{31}NNaO_6$ $[M + Na]^+$ requires 512.2044).

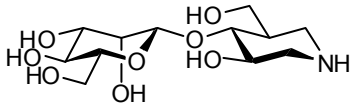
**5'-*O*-Benzyl-4-*O*-(2,3-di-O-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-3-*O*-*p*-toluoyl-*N*-benzyloxycarbonyl-isofagomine (8).**




Triflic anhydride (0.059 ml, 0.351 mmol) was added to a solution of 4-methylphenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside[39] **16** (0.140 g, 0.251 mmol), diphenylsulfoxide (0.142 g, 0.703 mmol) and 2,4,6-tri-*tert*-butylpyrimidine (0.187 g, 0.753 mmol) in dry dichloromethane (4 ml) at -65 °C under $N_2$. After 10 min a solution of alcohol **7** (0.090 g, 0.183 mmol) in dry dichloromethane (2 ml) was cannulated into the sulfoxide solution. The mixture was stirred at -65 °C for 1 h then was slowly warmed to -40 °C. This temperature was maintained for 30 min and then the mixture was allowed to slowly warm to rt. TLC analysis (30:70 EtOAc/pet. spirits with 1% $Et_3N$) indicated consumption of acceptor **7** at approximately -40 °C. The reaction was quenched with sat. aq. $NaHCO_3$, diluted with dichloromethane and washed with sat. aq. $NaHCO_3$ (3 × 5 ml) and brine. The organic extracts were dried ($MgSO_4$), the solvent removed under reduced pressure and the resulting residue subjected to flash chromatography (25:75 EtOAc/pet. spirits with 1% $Et_3N$) to afford the *pseudo*-disaccharide **8** (0.101 g, 60%) as a colourless oil. $[\alpha]_D^{24}$ -37 (c 0.62, $CHCl_3$); $\delta_H$ (499.7 MHz, $(CD_3)_2SO$, 60.0 °C) 2.03-2.10 (1H, m, $H5^B$), 2.40 (3H, s, $CH_3$), 3.19 (1H, ddd, $J_{4,5}$ 10.0, $J_{5,6}$ 10.0, $J_{5,6}$ 4.5 Hz, $H5^A$), 3.37 (1H, dd, $J_{5,6}$ 8.0, $J_{6,6}$ 13.5 Hz, $H6_{ax}{}^B$), 3.41 (1H, bdd, $J_{2,2}$ 13.5, $J_{2,3}$ 7.0 Hz, $H2_{ax}{}^B$), 3.53-3.60 (3H, m, $H6^A$, 2 × C$H_2$(C5)$^B$), 3.67 (1H, dd, $J_{2,3}$ 3.0, $J_{3,4}$ 10.0 Hz, $H3^A$), 3.76-3.81 (1H, m, $H6_{eq}{}^B$), 3.78 (1H, dd, $J_{6,6}$ 10.0 Hz, $H6^A$), 3.89 (1H, dd, $H4^A$), 3.92 (1H, bdd, $J_{2,3}$ 4.5 Hz, $H2_{eq}{}^B$), 3.97 (1H, app d, $H2^A$), 4.01 (1H, dd, $J_{3,4}$ 7.0, $J_{4,5}$ 7.0 Hz, $H4^B$), 4.41-4.46 (2H, m, 2 × PhC$H_2$), 4.63 (2H, s, 2 × PhC$H_2$), 4.64 (1H, *J* 11.5 Hz, PhC$H_2$), 4.74 (1H, *J* 11.5 Hz, PhC$H_2$), 4.77 (1H, app s, $H1^A$), 4.96 (1H, ddd, $H3^B$), 5.06-5.13 (2H, m, PhC$H_2$), 5.58 (1H, s, PhC*H*), 7.22-7.40 (27H, m, Ar), 7.86 (2H, app d, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$, 25.0 °C) 21.84 (1C, $CH_3$), 29.84 (1C, $C5^B$), 41.05, 42.98, 43.26, 44.82 (2C,

(C2,6)$^{B}$), 66.00, 67.48, 67.73, 68.44, 68.58, 70.52, 72.71, 73.52, 74.85, 76.58, 78.22, 78.68 (12C, (C2,3,4,5,6)$^{A}$, (C3,4,5')$^{B}$, 4 × Ph*C*H$_2$), 101.50 ($J_{C,H}$ 161.1 Hz), 102.45 ($J_{C,H}$ 155.8 Hz) (2C, C1$^{A}$, Ph*C*H), 126.21, 127.43, 127.67, 127.74, 127.94, 127.98, 128.13, 128.28, 128.31, 128.49, 128.57, 128.62, 128.99, 129.23, 129.84, 137.73, 137.98, 138.53, 143.97 (36C, Ar), 155.75 (1C, NC=O), 165.41 (1C, OC=O); HRMS *m/z* (ESI$^{+}$) 942.3823 ($C_{56}H_{57}NNaO_{11}$ [M + Na]$^{+}$ requires 942.3824).

**(3*R*,4*R*,5*R*)-3-Hydroxy-5-(hydroxymethyl)-4-(β-D-mannopyranosyloxy)piperidine**



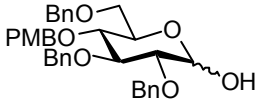

**(ManIFG; 3).** NaOMe (1.0 M, 0.060 ml) was added to a solution of the protected *pseudo*-disaccharide **8** (0.029 g, 0.030 mmol) in methanol (2.0 ml) and dichloromethane (1.0 ml). After 15 h TLC analysis (30:70 EtOAc/pet. spirits with 1% $Et_3N$) indicated complete consumption of starting material. Amberlite IR-120 resin ($H^{+}$ form) was added to neutralise the solution and, following filtration, the solvent was removed under reduced pressure. $Pd(OH)_2$ (30 mg, 20% w/w) was added to a solution of the crude filtrate in AcOH/$H_2O$/THF (4:2:1, 1.75 ml). The reaction vessel was filled with $H_2$ (6 bar) and agitated for 16 h. At this point TLC analysis (5:95 MeOH/$H_2O$ upon $NH_3$-neutralised TLC plate) indicated formation of a highly polar product. The suspension was filtered through Celite then was concentrated under reduced pressure. The residue was purified by ion-exchange chromatography [i) Dowex 1X-8 ($OH^{-}$ form), eluted with water; ii) Dowex-50W-X2 ($H^{+}$ form), eluted with water then 6 M aq. $NH_3$] followed by $C_{18}$ reversed-phase chromatography (5:95 MeOH/$H_2O$) to afford **3** (0.070 g, 80%) as a colourless glass. $[\alpha]_D^{24}$ -24 (c 0.36, $H_2O$); $\delta_H$ (499.7 MHz, $D_2O$) 1.86 (1H, m, H5$^{B}$), 2.50 (1H, app bt, $J_{2,2}$ 12.5 Hz, H2$_{ax}^{B}$), 2.57 (1H, app bt, *J* 13.5 Hz, H6$_{ax}^{B}$), 3.18 (1H, dd, $J_{5,6}$ 4.0, $J_{6,6}$ 12.5 Hz, H6$_{eq}^{B}$), 3.27 (1H, dd, $J_{2,2}$ 12.5, $J_{2,3}$ 5.0 Hz, H2$_{eq}^{B}$), 3.44 (1H, ddd, $J_{4,5}$ 9.0, $J_{5,6}$ 2.0, $J_{5,6}$ 6.5 Hz, H5$^{A}$), 3.57-3.61 (2H, m, H3$^{A}$,4$^{A}$), 3.66-3.79 (5H, m, H6$^{A}$,3$^{B}$,4$^{B}$, 2 × C*H*$_2$(C5)$^{B}$), 3.94 (1H, dd, $J_{5,6}$ 2.0, $J_{6,6}$ 12.0 Hz, H6$^{A}$), 4.11 (1H, app d, $J_{2,3}$ 3.5 Hz, H2$^{A}$), 4.75 (1H, app s, H1$^{A}$); $\delta_C$ (125.8 MHz, $D_2O$) 42.91 (1C, C5$^{B}$), 45.86, 48.30 (2C, (C2,6)$^{B}$), 59.61, 60.85, 66.61, 70.05, 70.36, 72.80, 76.29, 83.53 (8C, (C2,3,4,5,6)$^{A}$, (C3,4,5')$^{B}$), 100.47 (1C, C1$^{A}$); HRMS *m/z* (ESI$^{+}$) 310.1507 ($C_{12}H_{24}NO_8$ [M + H]$^{+}$ requires 310.1496).

**4-Methylphenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (9).** Trifluoroacetic acid (3.34 ml, 45.1 mmol) was slowly added to a solution of 4-methylphenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[40] (5.00 g, 9.01 mmol) and triethylsilane (7.20 ml, 45.1 mmol) in $CH_2Cl_2$ (50 ml) at 0 ºC under $N_2$. After 90 min TLC analysis (30:70 EtOAc/pet. spirits) of the mixture showed complete consumption of the starting material. The mixture was diluted with $CH_2Cl_2$ and quenched with ice then was washed with sat. aq. $NaHCO_3$ (× 3) and brine. The organic extracts were dried ($MgSO_4$), the solvent removed under reduced pressure and the resulting residue subjected to flash chromatography (20:80 EtOAc/pet. spirits) to afford the alcohol **9** (4.47 g, 89%) as a colourless oil; $[\alpha]_D^{24}$ -32 (c 1.0, $CHCl_3$ lit.[41] -30); $\delta_H$ (499.7 MHz, $CDCl_3$) 2.31 (3H, s, $CH_3$), 2.54 (1H, d, $J_{4,OH}$ 2.5 Hz, OH), 3.45 (1H, dd, $J_{1,2}$ 9.5, $J_{2,3}$ 9.0 Hz, H2), 3.46 (1H, m, H5), 3.50 (1H, dd, $J_{3,4}$ 9.0 Hz, H3), 3.64 (1H, ddd, H4), 3.73-3.80 (2H, m, H6,6), 4.54 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.58 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.62 (1H, d, H1), 4.73 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.77 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.90 (1H, d, *J* 11.5 Hz, PhC$H_2$), 4.92 (1H, d, *J* 10.5 Hz, PhC$H_2$), 7.04 (2H, app d, Ar), 7.26-7.27 (17H, m, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$) 21.25 (1C, $CH_3$), 70.61, 71.94, 73.82, 75.49, 75.64, 78.18, 80.63, 86.36, 88.13 (9C, C1,2,3,4,5,6, 3 × *C*$H_2$Ph), 127.85, 128.03, 128.07, 128.10, 128.42, 128.56, 128.58, 128.75, 129.82, 129.94, 132.78, 137.92, 138.20, 138.64 (24C, Ar).

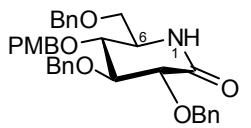
**4-Methylphenyl 2,3,6-tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-1-thio-β-D-glucopyranoside (10).** Sodium hydride (60% dispersion in mineral oil, 0.832 g, 20.8 mmol) was added to alcohol **9** (5.79 g, 10.4 mmol) in dry DMF (40 ml) under $N_2$. After ten minutes *para*-methoxybenzyl chloride (2.12 ml, 15.6 mmol) was added dropwise at 0 ºC. The mixture was slowly allowed to attain room temperature and left to stand overnight. At this time TLC analysis (10:60:30 EtOAc/toluene/pet. spirits) showed complete consumption of the starting material. The reaction was diluted with EtOAc then was quenched with ice and washed with $H_2O$ (× 2) and brine. The organic extracts were dried ($MgSO_4$), the solvent removed under reduced pressure and the resulting residue subjected to flash chromatography (5:60:35 EtOAc/toluene/pet. spirits with 1% $Et_3N$) to afford the thioglycoside **10** (6.62 g, 94%) as a colourless oil; $[\alpha]_D^{24}$ -5.5 (c 0.7, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 2.30 (3H, s, ArC$H_3$), 3.45 (1H, m, H5), 3.47 (1H, dd, $J_{1,2}$ 9.5, $J_{2,3}$ 9.0 Hz, H2), 3.60 (1H, dd, $J_{3,4}$ 9.0, $J_{4,5}$ 9.5 Hz, H4), 3.67 (1H, dd, H3), 3.70 (1H, dd, $J_{5,6}$ 4.5, $J_{6,6}$ 10.5 Hz, H6), 3.76 (1H, dd, $J_{5,6}$ 2.0 Hz, H6), 3.77 (3H, s, OC$H_3$), 4.51

(1H, d, *J* 11.0 Hz, ArC$H_2$), 4.53 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.59 (1H, d, H1), 4.60 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.71 (1H, d, *J* 10.5 Hz, ArC$H_2$), 4.73 (1H, d, *J* 10.5 Hz, ArC$H_2$), 4.86 (1H, d, *J* 10.5 Hz, ArC$H_2$), 4.89 (2H, d, *J* 11.0 Hz, 2 × ArC$H_2$), 6.79 (2H, app d, Ar), 7.02 (2H, app d, Ar), 7.10 (2H, app d, Ar), 7.23-7.49 (17H, m, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$) 21.24 (1C, Ar*C*$H_3$), 55.42 (1C, *C*$H_3$OAr), 69.21, 71.72, 73.56, 74.84, 75.51, 75.92, 77.69, 79.25, 80.96. 86.94, 87.81 (11 C, C1,2,3,4,5,6, 5 × *C*$H_2$Ar), 113.98, 127.66, 127.82, 127.89, 127.97, 128.37, 128.48, 128.54, 128.55, 128.59, 129.55, 129.79, 129.92, 130.38, 132.83, 137.81, 138.26, 138.52, 138.65, 159.46 (30 C, Ar); HRMS *m/z* ($ESI^+$) 699.2748 ($C_{42}H_{44}NaO_6S$ [M + Na]$^+$ requires 699.2751).

**2,3,6-Tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-D-glucopyranose.** *N*-Iodosuccinimide (1.15 g, 5.13 mmol) was added to a solution of the thioglycoside **10** (2.32 g, 3.42 mmol) in acetone (1% aq., 60 ml) at 0 ºC. After 30 min TLC analysis (30:70 EtOAc/pet. spirits) of the mixture showed complete consumption of the starting material. The solution was quenched with 0.5 M aq. $Na_2S_2O_3$ and diluted with EtOAc (100 ml) before being washed with 0.5 M aq. $Na_2S_2O_3$ (50 ml), $NaHCO_3$ (3 × 50 ml) and brine (50 ml). The organic extracts were dried ($MgSO_4$), the solvent removed under reduced pressure and the resulting residue recrystallised from EtOAc/pet. spirits to afford 2,3,6-tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-D-glucopyranose (1.48 g, 78%) as a white powder in a mixture of anomers (α/β ratio 25:1); α-anomer signals: $\delta_H$ (499.7 MHz, $CDCl_3$) 3.57 (1H, dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.0 Hz, H2), 3.61 (1H, dd, $J_{3,4}$ 9.5, $J_{4,5}$ 10.0 Hz, H4), 3.64 (1H, dd, $J_{5,6}$ 2.5, $J_{6,6}$ 11.0 Hz, H6), 3.70 (1H, dd, $J_{5,6}$ 4.0 Hz, H6), 3.79 (3H, s, $OCH_3$), 3.95 (1H, dd, H3), 4.02 (1H, ddd, H5), 4.43 (1H, d, *J* 10.5 Hz, ArC$H_2$), 4.50 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.61 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.69 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.74 (1H, d, *J* 10.5 Hz, ArC$H_2$), 4.77 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.86 (1H, d, *J* 11.0 Hz, ArC$H_2$), 4.95 (1H, d, *J* 10.5 Hz, ArC$H_2$), 5.23 (1H, d, H1), 6.80 (2H, app d, Ar), 7.05 (2H, app d, Ar), 7.26-7.36 (15H, m, Ar).



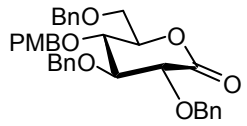

**(3*R*,4*S*,5*S*,6*S*)-3,4,-Bis(benzyloxy)-6-[(benzyloxy)methyl]-5-(4-methoxybenzyloxy)piperidin-2-one (12).** Acetic anhydride (8.30 ml) and dry dimethylsulfoxide (13.9 ml) were added to 2,3,6-tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-D-glucopyranose (2.90 g, 5.09 mmol) and the solution was stirred under $N_2$ for 18 h. At this time TLC analysis (30:70 EtOAc/pet. spirits) showed complete consumption of the starting material. The reaction was diluted with EtOAc then was quenched with ice and washed with



$H_2O$ (× 5) and brine. The organic extracts were dried ($MgSO_4$) and evaporated. Azeotropic evaporation using toluene was performed to remove residual AcOH. The crude residue was employed directly in the next step. A dry-ice/acetone cold finger cooling trap was employed to condense ammonia (6 ml) into a solution of crude lactone **11** in dry $Et_2O$ (65 ml) at -78 °C. The solution was then allowed to reflux at 0 °C for 2 h. The solution was evaporated to dryness and the crude residue was employed directly in the next step. Acetic anhydride (8.30 ml) and dry dimethylsulfoxide (13.9 ml) were added to the crude 2,3,6-tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-D-gluconamide and the mixture was stirred under $N_2$ for 18 h. At this time TLC analysis (70:30 EtOAc/pet. spirits) showed complete consumption of the starting material. The reaction was diluted with EtOAc then was quenched with ice and washed with $H_2O$ (× 4) and brine. The organic extract was dried ($MgSO_4$) and evaporated. The crude residue was dissolved in dry acetonitrile (70 ml) and formic acid (18.8 mL) then sodium cyanoborohydride (1.64 g, 26.2 mmol) were added. After 2 h stirring under $N_2$ TLC analysis (40:60 EtOAc/toluene) of the solution indicated complete consumption of the starting material. The reaction was diluted with EtOAc then was washed with sat. aq. $NaHCO_3$ (× 3) and brine. The aqueous extract was treated with aq. sodium hypochlorite prior to disposal. The organic extracts were dried ($MgSO_4$), the solvent removed under reduced pressure and the resulting residue was subjected to flash chromatography (30:70 EtOAc/pet. spirits with 1% $Et_3N$) to afford the lactam **12** (2.13 g, 74% over four steps) as a colourless oil that crystallised upon standing. A small portion was recrystallised from EtOAc/pet. spirits, mp 100-102 °C; $[\alpha]_D^{22}$ +96 (c 1.0, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 3.22 (1H, dd, $J_{6,6'}$ 8.0, $J_{6',6}$ 9.0 Hz, C$H_2$(C6)), 3.50 (1H, dd, $J_{4,5}$ 8.5, $J_{5,6}$ 9.0 Hz, H5), 3.56 (1H, m, H6), 3.58 (1H, dd, $J_{6,6'}$ 2.5 Hz, C$H_2$(C6)), 3.81 (3H, s, $OCH_3$), 3.90 (1H, dd, $J_{3,4}$ 8.0 Hz, H4), 3.99 (1H, d, H3), 4.42 (1H, d, *J* 10.5 Hz, ArC$H_2$), 4.44 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.47 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.74 (1H, d, *J* 11.0 Hz, ArC$H_2$), 4.76 (1H, d, *J* 10.5 Hz, ArC$H_2$), 4.77 (1H, d, *J* 11.0 Hz, ArC$H_2$), 4.86 (1H, d, *J* 11.0 Hz, ArC$H_2$), 5.17 (1H, d, *J* 11.5 Hz, ArC$H_2$), 5.87 (1H, broad s, NH), 6.82 (2H, m, Ar), 7.10 (2H, m, Ar), 7.28-7.43 (15H, m, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$) 53.93, 55.39, 70.12, 73.44, 74.33, 74.71, 74.80, 76.78, 78.91, 82.50 (10C, C3,4,5,6,6', 4 × Ar$CH_2$, $OCH_3$), 113.98, 127.88, 127.98, 128.07, 128.11, 128.46, 128.51, 128.65, 129.83, 129.95, 137.44, 137.99, 138.23, 159.60 (24C, Ar), 170.64 (1C, C2); HRMS *m/z* ($ESI^+$) 590.2509 ($C_{35}H_{37}NNaO_6$ $[M + Na]^+$ requires 590.2513).

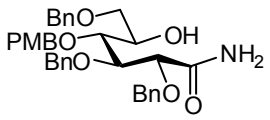
Intermediates in this sequence were purified and characterised in parallel experiments as described below:

**2,3,6-Tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-D-gluconolactone (11).** A portion of the




lactone **11**, resulting from a parallel experiment, was purified by flash chromatography (10:90 EtOAc/ pet. spirits) to afford a colourless oil; $[\alpha]_D^{22}$ +76 (c 1.2, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 3.67 (1H, dd, $J_{5,6}$ 3.5, $J_{6,6}$ 11.0 Hz, H6), 3.72 (1H, dd, $J_{5,6}$ 2.0 Hz, H6), 3.79 (3H, s, $OCH_3$), 3.91 (dd, $J_{2,3}$ 6.5, $J_{3,4}$ 6.5 Hz, H3), 3.95 (dd, $J_{4,5}$ 8.0 Hz, H4), 4.13 (1H, d, H2), 4.44 (1H, m, H5), 4.46 (1H, d, *J* 11.0 Hz, ArC$H_2$), 4.49 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.58 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.61 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.65 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.74 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.99 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.82 (2H, m, Ar), 7.10 (2H, m, Ar), 7.26-7.40 (15H, m, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$) 55.39, 68.41, 73.70, 73.84, 73.86, 75.79, 77.55, 78.35, 81.17 (10C, C2,3,4,5,6, 4 × Ar*C*$H_2$, $OCH_3$), 113.98, 127.94, 128.04, 128.10, 128.22, 128.52, 128.57, 128.60, 129.74, 129.84, 137.10, 137.73, 137.77, 159.59 (24C, Ar), 169.48 (1C, C=O); HRMS *m/z* ($ESI^+$) 591.2351 ($C_{35}H_{36}NaO_7$ $[M + Na]^+$ requires 591.2353).

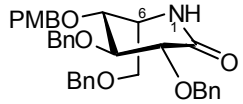
**2,3,6-Tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-D-gluconamide.** A portion of 2,3,6-tri-*O*-




benzyl-4-*O*-(4-methoxybenzyl)-D-gluconamide, resulting from a parallel experiment, was purified by flash chromatography (60:40 EtOAc/ pet. spirits) to afford a colourless oil; $[\alpha]_D^{23}$ +24 (c 1.1, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 2.85 (1H, bs, OH), 3.58 (1H, $J_{5,6}$ 5.0, $J_{6,6}$ 9.5 Hz, H6), 3.65 (1H, $J_{5,6}$ 3.0 Hz, H6), 3.80 (3H, s, $OCH_3$), 3.85 (1H, dd, $J_{3,4}$ 6.0, $J_{4,5}$ 8.0 Hz, H4), 3.91 (1H, m, H5), 4.07 (1H, dd, $J_{2,3}$ 3.0 Hz, H3), 4.26 (1H, d, H2), 4.45 (1H, d, *J* 10.5 Hz, ArC$H_2$), 4.51 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.57 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.59 (1H, d, *J* 11.0 Hz, ArC$H_2$), 4.60 (1H, d, *J* 12.5 Hz, ArC$H_2$), 4.64 (1H, d, *J* 11.0 Hz, ArC$H_2$), 4.65 (1H, d, *J* 11.0 Hz, ArC$H_2$), 4.71 (1H, d, *J* 11.0 Hz, ArC$H_2$), 4.59 (1H, bs, NH), 6.60 (1H, bs, NH), 6.82 (2H, m, Ar), 7.15 (2H, m, Ar), 7.28-7.36 (15H, m, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$) 55.41, 71.27, 71.51, 73.56, 73.90, 73.92, 75.32, 77.31, 79.80, 80.74 (10C, C2,3,4,5,6, 4 × Ar*C*$H_2$, $OCH_3$), 113.90, 127.84, 127.99, 128.01, 128.39, 128.45, 128.47, 128.49, 128.54, 128.57, 128.77, 129.90, 130.45, 136.94, 137.93, 138.26, 159.40 (24C, Ar), 174.16 (1C, C=O); HRMS *m/z* ($ESI^+$) 608.2615 ($C_{35}H_{39}NNaO_7$ $[M + Na]^+$ requires 608.2619).

**(3*R*,4*S*,5*S*,6*R*)-3,4,-Bis(benzyloxy)-6-[(benzyloxy)methyl]-5-(4-**

**methoxybenzyloxy)piperidin-2-one.** A portion of (3*R*,4*S*,5*S*,6*R*)-




3,4,-Bis(benzyloxy)-6-[(benzyloxy)methyl]-5-(4-methoxybenzyloxy)piperidin-2-one resulting from a parallel experiment, was purified by flash chromatography (30:70 EtOAc/

pet. Spirits with 1% $Et_3N$) to afford a colourless oil (0.013 g, 10%). The diastereomeric lactam **12** (0.103 g, 74%) was also isolated in this experiment. $[\alpha]_D^{25}$ +22 (c 0.2, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 3.54 (1H, dd, $J_{6,6'}$ 3.5, $J_{6',6'}$ 9.0 Hz, C$H_2$(C6)), 3.61 (1H, dd, $J_{6,6'}$ 9.0 Hz, C$H_2$(C6)), 3.68 (1H, dd, $J_{4,5}$ 5.0, $J_{5,6}$ 3.5 Hz, H5), 3.75 (1H, m, H6), 3.81 (3H, s, $OCH_3$), 3.92 (1H, dd, $J_{3,4}$ 6.0 Hz, H4), 4.00 (1H, d, H3), 4.43 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.47 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.52 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.56 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.57 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.65 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.75 (1H, d, *J* 11.5 Hz, ArC$H_2$), 5.11 (1H, d, *J* 11.5 Hz, ArC$H_2$), 5.88 (1H, broad s, NH), 6.82 (2H, m, Ar), 7.12 (2H, m, Ar), 7.25-7.43 (15H, m, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$) 52.21, 55.42, 69.87, 71.73, 73.34, 73.63, 74.30, 74.98, 78.35, 79.02 (10C, C3,4,5,6,6', 4 × Ar*C*$H_2$, $OCH_3$), 113.98, 127.89, 127.93, 127.99, 128.02, 128.07, 128.49, 128.50, 128.57, 128.66, 129.64, 129.76, 137.62, 138.01, 138.15, 159.58 (24C, Ar), 170.68 (1C, C2); HRMS *m/z* ($ESI^+$) 590.2511 ($C_{35}H_{37}NNaO_6$ $[M + Na]^+$ requires 590.2513).

**(3*R*,4*S*,5*S*,6*S*)-3,4-Bis(benzyloxy)-6-[(benzyloxy)methyl]-5-(4-methoxybenzyloxy)piperidin-2-thione (13).** Lawesson's reagent (0.40 g, 0.99 mmol) was added to a mixture containing the lactam **12** (0.28 g, 0.493 mmol), pyridine (20 μl, 0.246 mmol), freshly activated 4 Å molecular sieves and dry toluene (20 ml). The reaction mixture was stirred for 20 h when TLC analysis (40:60 EtOAc/toluene) indicated complete consumption of the starting material. The mixture was filtered, stirred with MeOH (5 ml) for 2 h and the solvent removed under reduced pressure. The residue was subjected to flash chromatography (1:1 EtOAc/pet. spirits) to afford the thiolactam **13** (0.284 g, 99%) as a colourless oil; $[\alpha]_D^{23}$ +123 (c 0.9, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 3.34 (1H, dd, $J_{6,6'}$ 7.5, $J_{6',6'}$ 9.5 Hz, C$H_2$(C6)), 3.53 (1H, dd, $J_{4,5}$ 4.5, $J_{5,6}$ 9.0 Hz, H5), 3.61 (1H, dd, $J_{6,6'}$ 3.5 Hz, C$H_2$(C6)), 3.78 (3H, s, $OCH_3$), 3.84 (1H, m, H6), 3.89 (1H, dd, $J_{3,4}$ 4.5 Hz, H4), 4.28 (1H, d, *J* 11.0 Hz, ArC$H_2$), 4.44 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.45 (1H, d, H3), 4.46 (1H, d, *J* 11.5 Hz, ArC$H_2$), 4.47 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.52 (1H, d, *J* 11.0 Hz, ArC$H_2$), 4.66 (1H, d, *J* 12.0 Hz, ArC$H_2$), 4.73 (1H, d, *J* 12.5 Hz, ArC$H_2$), 5.01 (1H, d, *J* 11.5 Hz, ArC$H_2$), 6.79-6.81 (2H, m, Ar), 7.05-7.07 (2H, m, Ar), 7.26-7.42 (15H, m, Ar), 8.10 (1H, broad s, NH); $\delta_C$ (125.8 MHz, ($CDCl_3$) 55.44, 56.06, 68.44, 72.54, 72.67, 72.74, 73.54, 78.03, 81.57, 82.63 (10C, C3,4,5,6,6', 4 × Ar*C*$H_2$, $OCH_3$), 113.97, 128.02, 128.13, 128.25, 128.33, 128.46, 128.53, 128.60, 128.73, 128.89, 128.95, 137.25, 137.58, 137.67, 159.61 (24C, Ar), 200.58 (1C, C2); HRMS *m/z* ($ESI^+$) 606.2284 ($C_{35}H_{37}NNaO_5S$ $[M + Na]^+$ requires 606.2284).

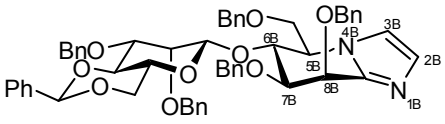
**Glycoimidazole formation.** Thiolactam **13** (0.144 g, 0.247 mmol) was dissolved in aminoacetaldehyde dimethyl acetal (0.40 ml, 3.71 mmol) and stirred under $N_2$ for 18 h. At this time TLC analysis (30:70 EtOAc/pet. spirits) indicated complete consumption of the starting material. The solution was evaporated to dryness and *p*-toluenesulfonic acid (0.085 g, 0.494 mmol) was added to a solution of the crude amidine **14** in toluene (1.1 ml) and water (0.05 ml). The solution was stirred at 50 °C for 5 d. At this time the reaction was diluted with EtOAc then was washed with $NaHCO_3$ (× 3) and brine. The organic extracts were dried ($MgSO_4$), the solvent removed under reduced pressure and the residue was subjected to flash chromatography (40:60 EtOAc /pet. spirits) to afford the glycoimidazoles **15** and (5*R*,6*R*,7*S*,8*S*)-7,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-6-hydroxy-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine (0.077 g, 65% over two steps) as separable diastereomers (D-Man/D-Glc ratio 4:3).

**i) (5*R*,6*R*,7*S*,8*R*)-7,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-6-hydroxy-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (15).** $[\alpha]_D^{24}$ -61 (c 0.8, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 3.06 (1H, broad s, 6-OH), 3.66 (1H, dd, $J_{6,7}$ 10.0, $J_{7,8}$ 3.0 Hz, H7), 3.79 (1H, dd, $J_{5,5'}$ 7.0, $J_{5',5'}$ 10.0 Hz, C$H_2$(C5)), 4.05 (1H, dd, $J_{5,5'}$ 2.5 Hz, C$H_2$(C5)), 4.08 (1H, ddd, $J_{5,6}$ 8.0 Hz, H5), 4.41 (1H, dd, H6), 4.45 (1H, d, *J* 11.5 Hz, PhC$H_2$), 4.57 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.61 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.65 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.68 (1H, d, *J* 12.5 Hz, PhC$H_2$), 4.75 (1H, d, *J* 12.5 Hz, PhC$H_2$), 4.85 (1H, d, H8), 7.10 (1H, d, $J_{2,3}$ 1.0 Hz, H3), 7.27-7.42 (16H, m, H2, Ph); $\delta_C$ (125.8 MHz, $CDCl_3$) 60.13, 65.81, 67.15, 70.65, 71.29, 71.57, 73.57, 78.94 (8C, C5,5',6,7,8, 3 × Ph*C*$H_2$), 119.55 (1C, C3), 127.76, 127.78, 127.90, 127.99, 128.14, 128.23, 128.33, 128.43, 128.62, 128.69, 129.16, 137.34, 137.66, 138.07 (19C, C2, Ph), 142.75 (1C, C8'); HRMS *m/z* ($ESI^+$) 471.2278 ($C_{29}H_{31}N_2O_4$ $[M + H]^+$ requires 471.2278).

**ii) (5*R*,6*R*,7*S*,8*S*)-7,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-6-hydroxy-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine.** mp 92-93 °C; $[\alpha]_D^{24}$ +57 (c 0.9, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 3.69 (broad d, $J_{6,OH}$ 6.0 Hz, 6-OH), 3.74 (dd, $J_{5,5'}$ 7.5, $J_{5',5'}$ 10.5 Hz, C$H_2$(C5)), 3.86 (dd, $J_{5,5'}$ 4.0 Hz, C$H_2$(C5)), 4.04 (1H, dd, $J_{6,7}$ 6.5, $J_{7,8}$ 5.0 Hz, H7), 4.09 (1H, broad ddd, $J_{5,6}$ 6.5 Hz, H6), 4.36 (1H, ddd, H5), 4.51 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.55 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.57 (1H, d, *J* 11.5 Hz, PhC$H_2$), 4.77 (1H, d, *J* 11.5 Hz, PhC$H_2$), 4.78 (1H, d, H8), 4.84 (1H, d, *J* 11.5 Hz, PhC$H_2$), 5.10 (1H, d, *J* 12.0 Hz, PhC$H_2$), 7.15 (1H, d, $J_{2,3}$ 1.0 Hz, H3), 7.19 (1H, d, H2), 7.22-7.39 (15H, m, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$) 61.07, 68.12, 71.12, 72.38, 72.69,

73.45, 73.50, 78.45 (8C, C5,5',6,7,8, 3 × Ph*C*H$_2$), 119.05 (1C, C3), 127.86, 127.94, 127.99, 128.02, 128.21, 128.30, 128.60, 128.62, 128.69, 129.16, 137.47, 137.58, 137.64 (19C, C2, Ph), 142.06 (1C, C8'); HRMS *m/z* (ESI$^+$) 471.2278 ($C_{29}H_{31}N_2O_4$ [M + H]$^+$ requires 471.2278).

**Glycosylation of 15.** A mixture of alcohol **15** (66 mg, 0.137 mmol), 4-methylphenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside[39] **16** (154 mg, 0.277 mmol) and freshly activated 4 Å molecular sieves in dichloromethane (5 ml) was stirred for 30 min at room temperature then was cooled to 0 °C. NIS (77 mg, 0.345 mmol) followed by TfOH (13 μL, 0.151 mmol) were added to the cooled mixture. After 20 min the reaction mixture was filtered through a Celite pad before being diluted with dichloromethane and washed successively with aq. 0.5 M $Na_2SO_3$, sat. aq. $NaHCO_3$ (× 3) and brine. The organic extracts were dried ($MgSO_4$), the solvent removed under reduced pressure and the resulting residue subjected to flash chromatography to afford a mixture of disaccharides (47.4 mg, 39%) as a colourless oil (α/β ratio of 1:2). The diastereomers were separated by HPLC (5 micron 100A Axia Pac 50 ×21 mm column, flow rate 8 ml/min, 30/70 MeCN:$H_2O$ in 1% TFA with a 1%/min solvent gradient). Compound **17** eluted at $R_t$ 46.5 min and, after dissolving in $CH_2Cl_2$ and washing with sat. aq. $NaHCO_3$ to convert to the free-base, was obtained as a colourless oil (14.9 mg, 12%). (5*R*,6*R*,7*S*,8*R*)-6-(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyloxy)-7,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine eluted at $R_t$ 49.5 min and, after dissolving in $CH_2Cl_2$ and washing with $NaHCO_3$ to obtain the free-base, was obtained as a colourless oil (6.5 mg, 5%).

**i) (5*R*,6*R*,7*S*,8*R*)-6-(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyloxy)-7,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (17).** 

$[\alpha]_D^{25}$ -67 (c 0.7, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 3.09 (1H, ddd, $J_{4,5}$ 10.0, $J_{5,6}$ 5.0, $J_{5,6}$ 10.0 Hz, H5$^A$), 3.46 (1H, dd, $J_{2,3}$ 3.0, $J_{3,4}$ 10.0 Hz, H3$^A$), 3.63 (1H, dd, $J_{5,5'}$ 7.0, $J_{5',5'}$ 9.5 Hz, C*H*$_2$(C5)$^B$), 3.72 (1H, dd, $J_{5,5'}$ 6.0 Hz, C*H*$_2$(C5)$^B$), 3.80 (1H, dd, $J_{6,6}$ 10.0 Hz, H6$^A$), 3.80 (1H, app d, H2$^A$), 3.97 (1H, $J_{6,7}$ 7.5, $J_{7,8}$ 3.5 Hz, H7$^B$), 4.11 (1H, dd, H6$^A$), 4.14 (1H, dd, H4$^A$), 4.18 (1H, m, H5$^B$), 4.42 (2H, s, PhC*H*$_2$), 4.46 (1H, dd, $J_{5,6}$ 3.5 Hz, H6$^B$), 4.59 (1H, d, *J* 12.5 Hz, PhC*H*$_2$), 4.63 (1H, app s, H1$^A$), 4.64 (2H, broad d, *J* 12.0 Hz, 2 × PhC*H*$_2$), 4.67 (1H, d, *J* 12.0 Hz, PhC*H*$_2$), 4.72 (1H, d, *J* 12.0 Hz, PhC*H*$_2$), 4.75 (1H, d, *J* 12.0 Hz, PhC*H*$_2$), 4.79 (1H, d, *J* 12.0 Hz, PhC*H*$_2$), 4.81 (1H, d, H8$^B$), 4.83 (1H, d, *J* 11.5 Hz, PhC*H*$_2$), 5.57 (1H, s, PhC*H*), 7.05 (1H, s, H3$^B$), 7.12 (1H, s, H2$^B$), 7.23-7.42 (28H, m, Ar),

7.49-7.51 (2H, m, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$) 59.98, 67.60, 68.63, 69.70, 71.50, 71.96, 72.69, 73.59, 75.01, 75.82, 76.75, 77.75, 78.20, 78.71 (15C, $(C2,3,4,5,6)^A$, $(C5,5',6,7,8)^B$, 5 × Ph*C*$H_2$), 100.79 (1C, $H1^A$), 101.54 (1C, Ph*C*H), 119.27 (1C, $C3^B$), 126.20, 127.62, 127.71, 127.73, 127.75, 127.91, 127.96, 128.23, 128.25, 128.33, 128.43, 128.49, 128.70, 129.03, 129.26, 137.48, 137.68, 138.09, 138.14, 138.43, 138.50 (37C, $C2^B$, Ph), 143.22 (1C, $C8'^B$); HRMS *m/z* ($ESI^+$) 901.4060 ($C_{56}H_{45}N_2O_9$ $[M + H]^+$ requires 901.4059).

**ii) (5*R*,6*R*,7*S*,8*R*)-6-(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyloxy)-7,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine.** $[\alpha]_D^{25}$ -23 (c 0.3, $CHCl_3$); $\delta_H$ (499.7 MHz, $CDCl_3$) 3.62 (1H, dd, $J_{5,5'}$ 8.0, $J_{5',5'}$ 10.0 Hz, $CH_2(C5)^B$), 3.74 (1H, dd, $J_{6,7}$ 9.5, $J_{7,8}$ 3.0 Hz, $H7^B$), 3.82-3.92 (5H, m, $(H2,3,5,6)^A$, $CH_2(C5)^B$), 4.06 (1H, dd, $J_{5,6}$ 3.5, $J_{6,6}$ 9.0 Hz, $H6^A$), 4.18 (1H, ddd, $J_{5,6}$ 6.0, $J_{5,5'}$ 2.5 Hz, $H5^B$), 4.27 (1H, dd, $J_{3,4}$ 9.5, $J_{4,5}$ 9.5 Hz, $H4^A$), 4.31 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.36 (1H, dd, $H6^B$), 4.37 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.44 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.51 (2H, s, 2 × PhC$H_2$), 4.52 (1H, d, *J* 11.5 Hz, PhC$H_2$), 4.55 (1H, d, *J* 12.0 Hz, PhC$H_2$), 4.64 (2H, d, *J* 12.0 Hz, PhC$H_2$), 4.79 (1H, d, $H8^B$), 4.82 (1H, d, *J* 12.5 Hz, PhC$H_2$), 5.29 (1H, d, $J_{1,2}$ 2.0 Hz, $H1^A$), 5.64 (1H, s, Ph*C*H), 7.08 (1H, s, $H3^B$), 7.20 (1H, s, $H2^B$), 7.23-7.42 (28H, m, Ar), 7.52-7.54 (2H, m, Ar); $\delta_C$ (125.8 MHz, $CDCl_3$) 59.91, 65.29, 67.16, 68.71, 70.37, 71.13, 71.84, 73.08, 73.16, 73.78, 75.10, 76.17, 77.05, 79.04, 79.82 (15C, $(C2,3,4,5,6)^A$, $(C5,5',6,7,8)^B$, 5 × Ph*C*$H_2$), 101.55 (2C, $H1^A$, Ph*C*H), 120.12 (1C, $C3^B$), 126.21, 127.57, 127.65, 127.79, 127.82, 128.05, 128.18, 128.32, 128.35, 128.42, 128.45, 128.66, 128.67, 128.99, 129.32, 137.50, 137.56, 137.72, 137.91, 138.35, 138.82, (37C, $C2^B$, Ph), 142.46 (1C, $C8'^B$); HRMS *m/z* ($ESI^+$) 901.4060 ($C_{56}H_{45}N_2O_9$ $[M + H]^+$ requires 901.4059).

**(5*R*,6*R*,7*S*,8*R*)-7,8-Dihydroxy-5-[(hydroxy)methyl]-6-(β-D-mannopyranosyloxy)-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-7,8-diol (ManMIm; 4).** $Pd(OH)_2$ (22 mg, 20% w/w) was added to a solution of EtOAc/MeOH/$H_2O$ (5:17:3, 1.80 ml), AcOH (0.40 ml) and **17** (14.8 mg, 16.5 μmol). The reaction vessel was filled with $H_2$ (6 bar) and agitated for 5 d. At this point TLC analysis (7:3:2 EtOAc/MeOH/$H_2O$) indicated complete conversion to a single species. The suspension was filtered through Celite then was purified by flash chromatography (7:3:2 EtOAc/MeOH/$H_2O$). Trace silica was removed by dissolving the product in a small quantity of methanol and filtering through

cotton wool to afford **4** (3.5 mg, 58%) as a colourless glass; $[\alpha]_D^{25}$ -51 (c 0.2, $H_2O$); $\delta_H$ (499.7 MHz, $CDCl_3$) 3.51 (1H, ddd, $J_{4,5}$ 9.5, $J_{5,6}$ 2.0, $J_{5,6}$ 6.5 Hz, $H5^A$), 3.64 (1H, dd, $J_{3,4}$ 9.5 Hz, $H4^A$), 3.72 (1H, dd, $J_{2,3}$ 3.0 Hz, $H3^A$), 3.80 (1H, dd, $J_{6,6}$ 12.5 Hz, $H6^A$), 4.00 (1H, dd, $H6^A$), 4.03 (1H, dd, $J_{5,5'}$ 3.0, $J_{5',5'}$ 12.0 Hz, $CH_2(C5)^B$), 4.14 (1H, app d, $H2^A$), 4.21 (1H, dd, $J_{6,7}$ 8.0, $J_{7,8}$ 4.0 Hz, $H7^B$), 4.24 (1H, dd, $J_{5,5'}$ 3.5, $CH_2(C5)^B$), 4.27 (1H, m, $H5^B$), 4.53 (1H, dd, $J_{5,6}$ 6.5 Hz, $H6^B$), 4.95 (1H, app s, $H1^A$), 5.03 (1H, d, $H8^B$), 7.15 (1H, s, $H3^B$), 7.32 (1H, s, $H2^B$); $\delta_C$ (125.8 MHz, $CDCl_3$) 59.97, 60.09, 60.96, 63.48, 66.65, 69.73, 70.64, 72.77, 75.39, 76.49 (10C $(C2,3,4,5,6)^A$, $(C5,5',6,7,8)^B$), 100.17 (1C, $H1^A$), 118.49 (1C, $C3^B$), 128.73 (1C, $C2^B$), 144.81 (1C, $C8'^B$); HRMS *m/z* ($ESI^+$) 363.1397 ($C_{14}H_{22}N_2O_9$ $[M + H]^+$ requires 363.1398).

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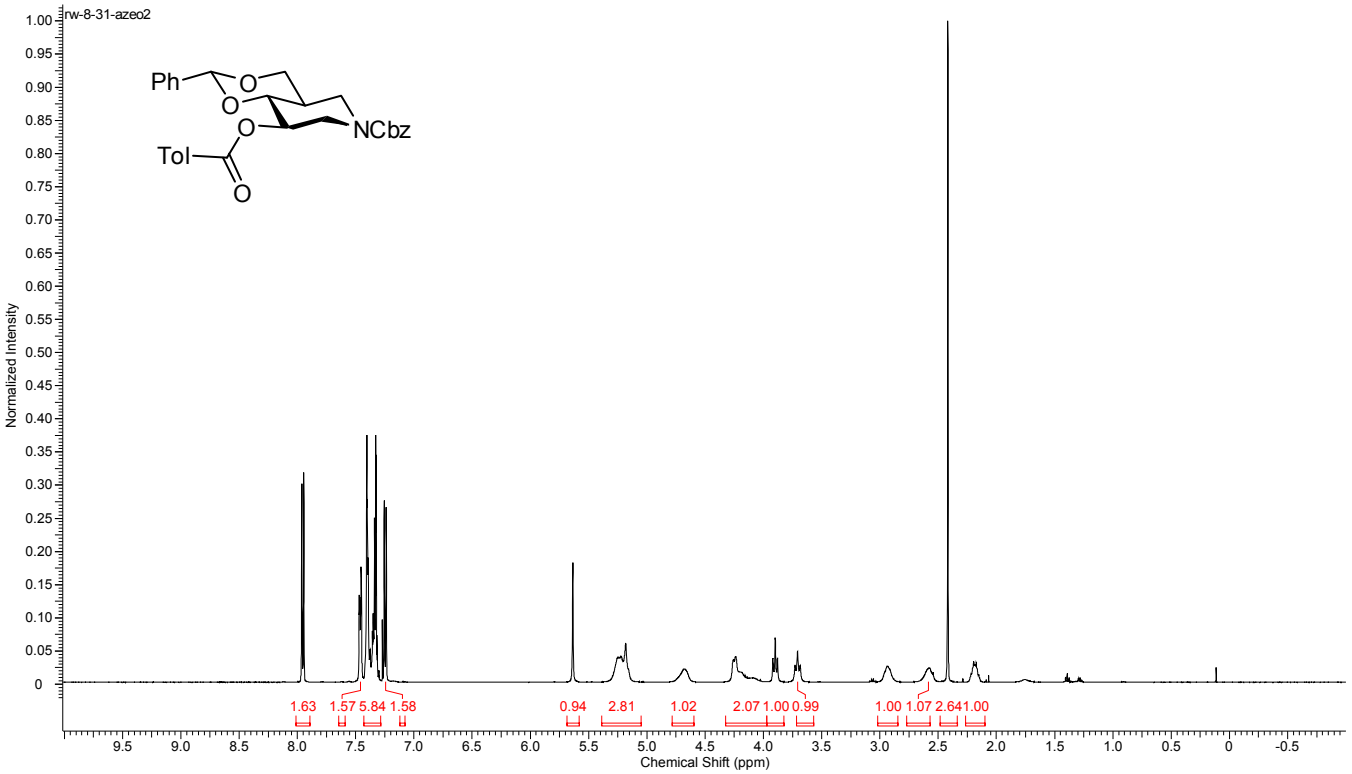
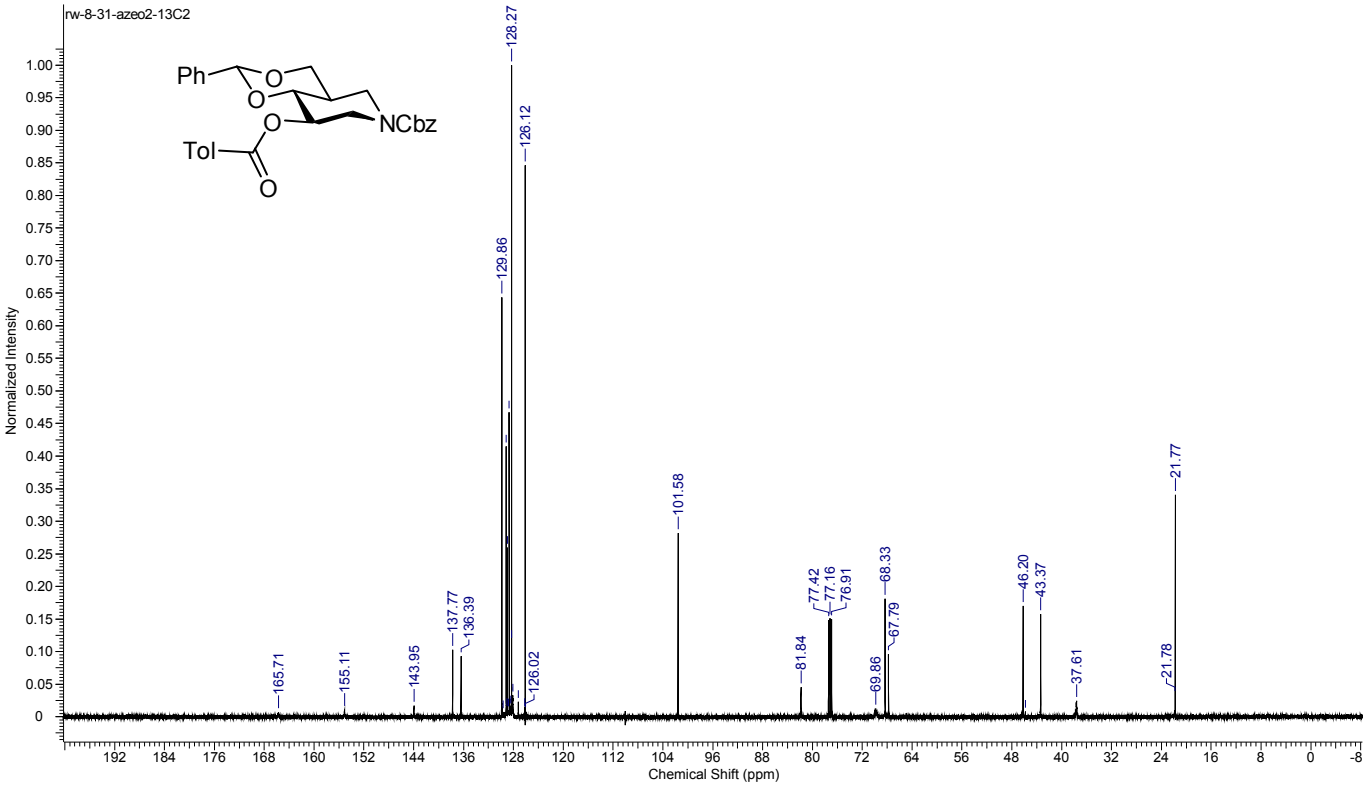
## NMR Spectra

### 4,5'-*O*-Benzylidene-3-*O*-*p*-toluoyl-*N*-benzyloxycarbonyl-isofagomine (6)



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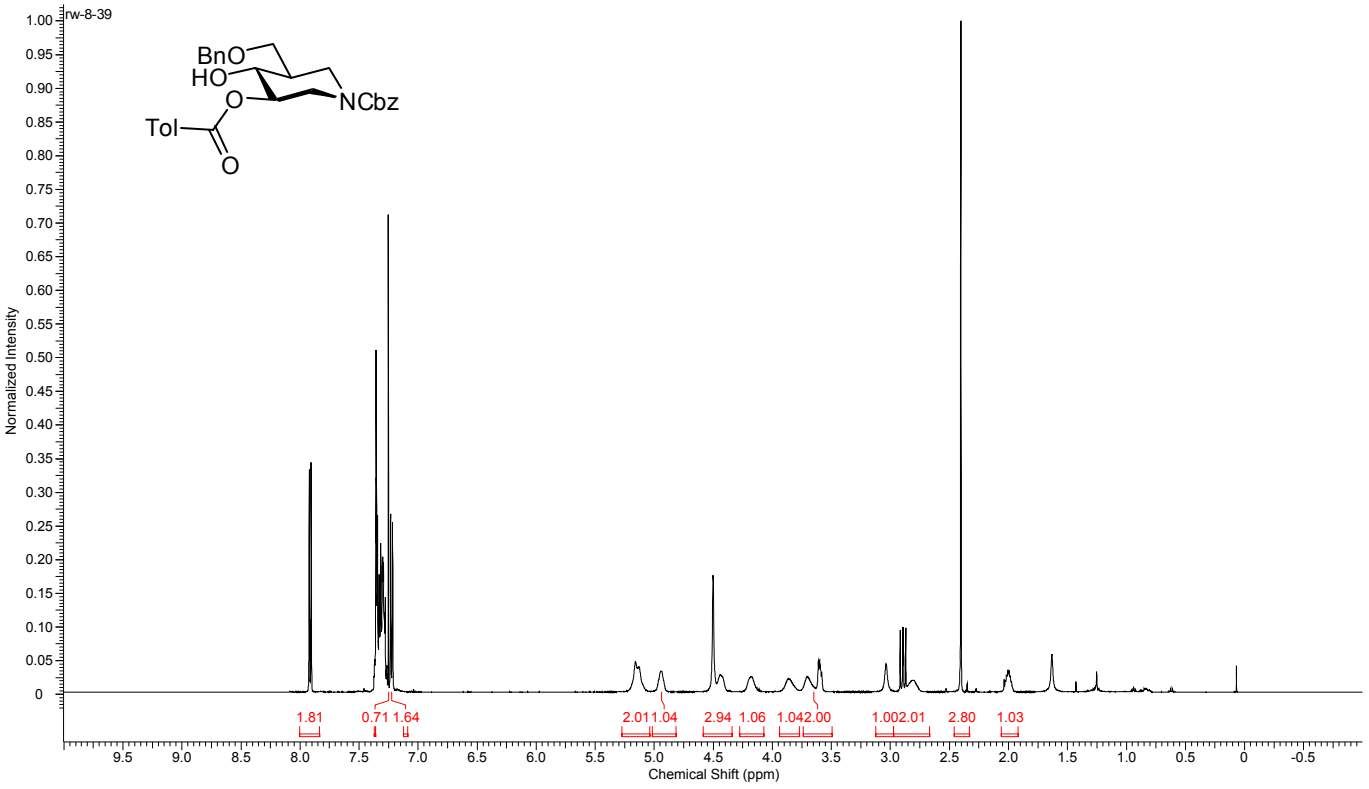


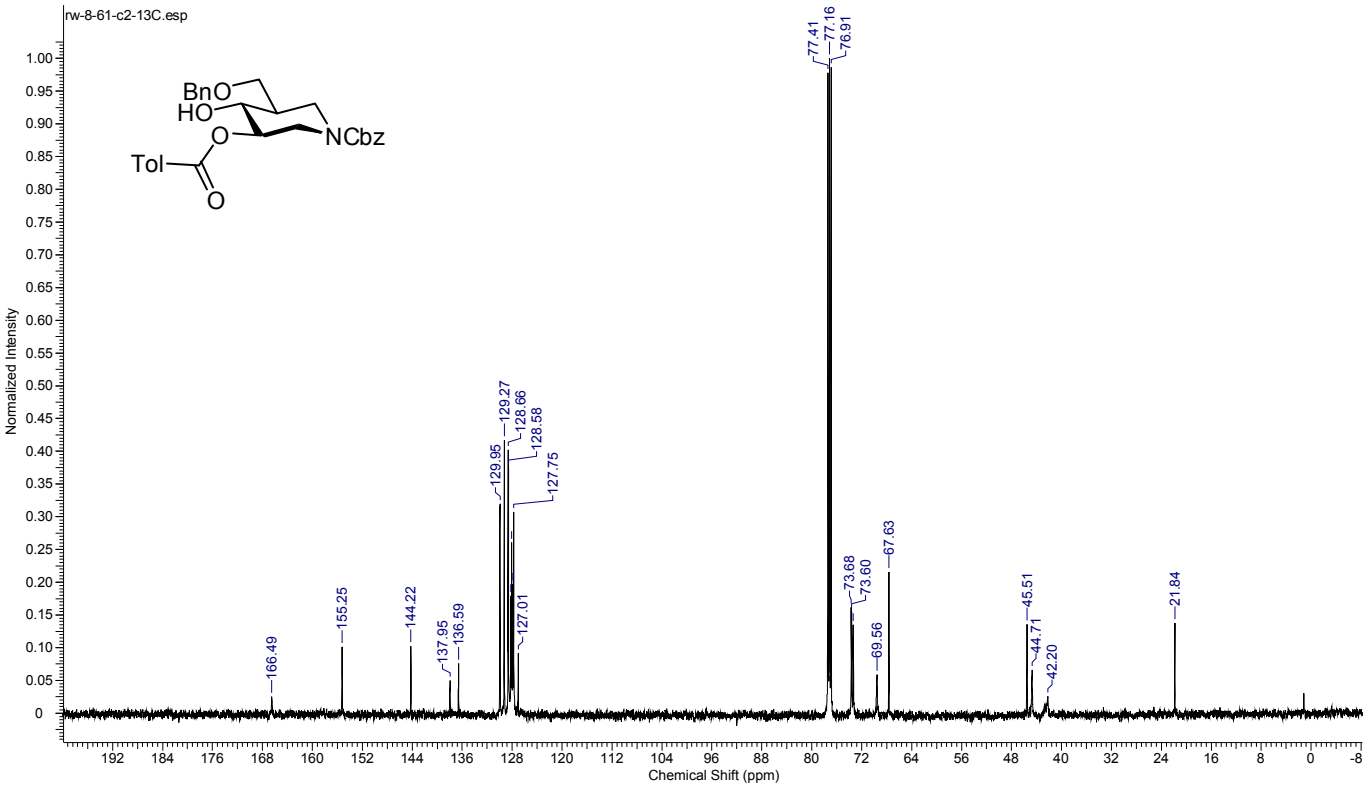

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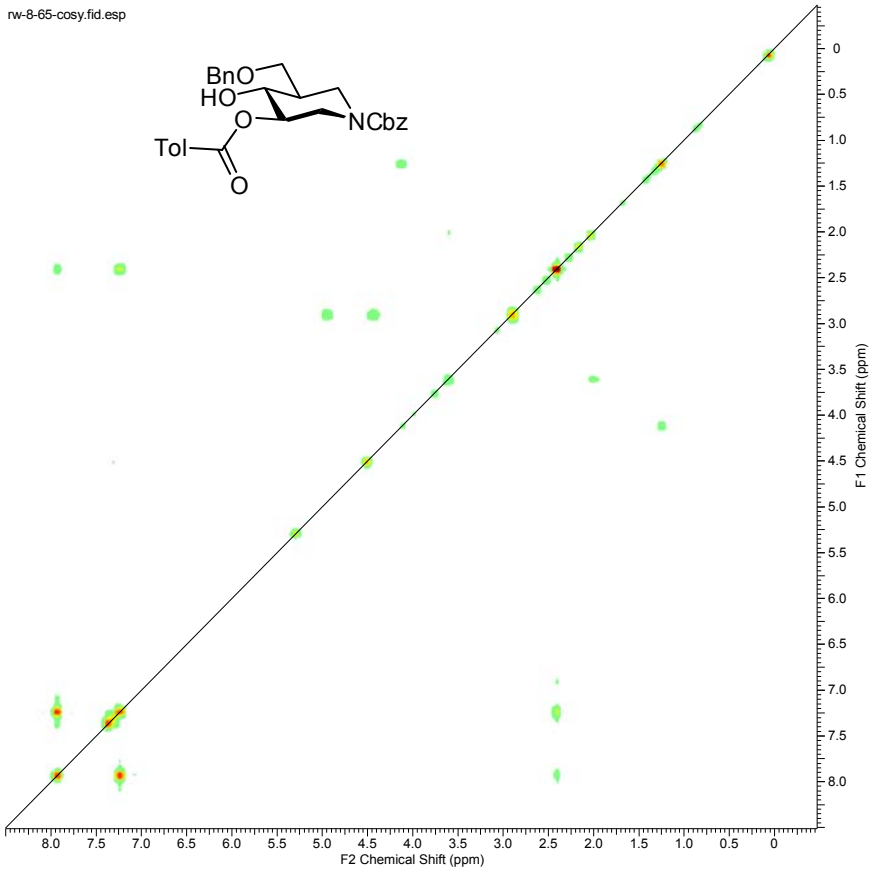
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BnO
HO
O
NCbz
Tol
O
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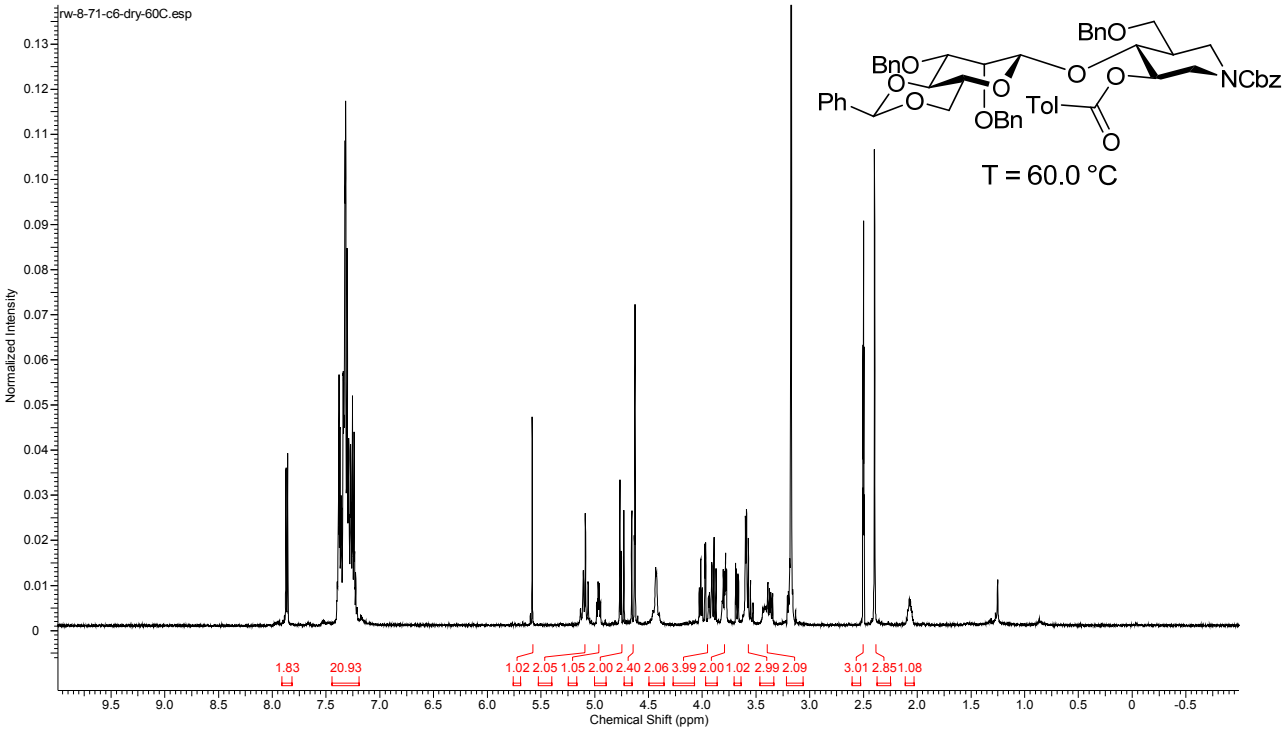
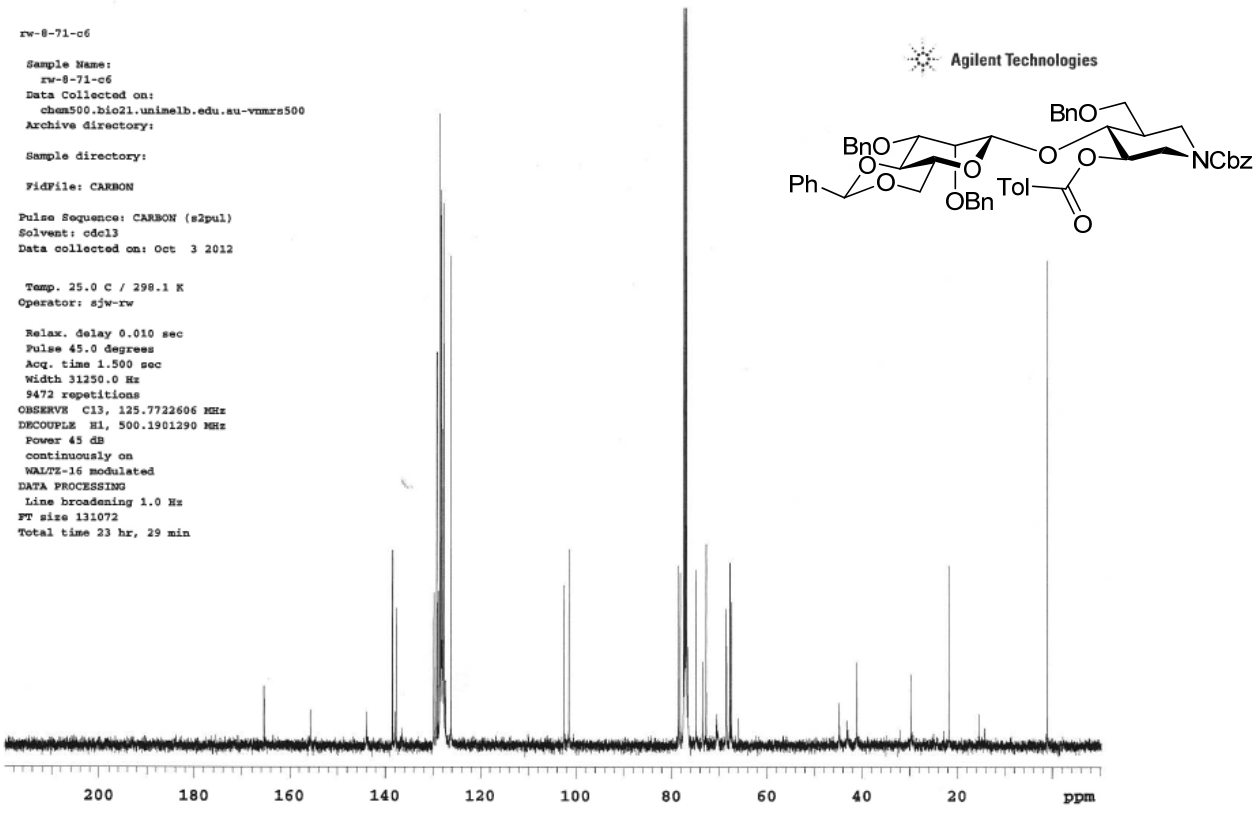
F2 Chemical Shift (ppm)

**5'-*O*-Benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-3-*O*-*p*-toluoyl-*N*-benzyloxycarbonyl-isofagomine (8)**




$^{13}C$ decoupled with $^{1}H$



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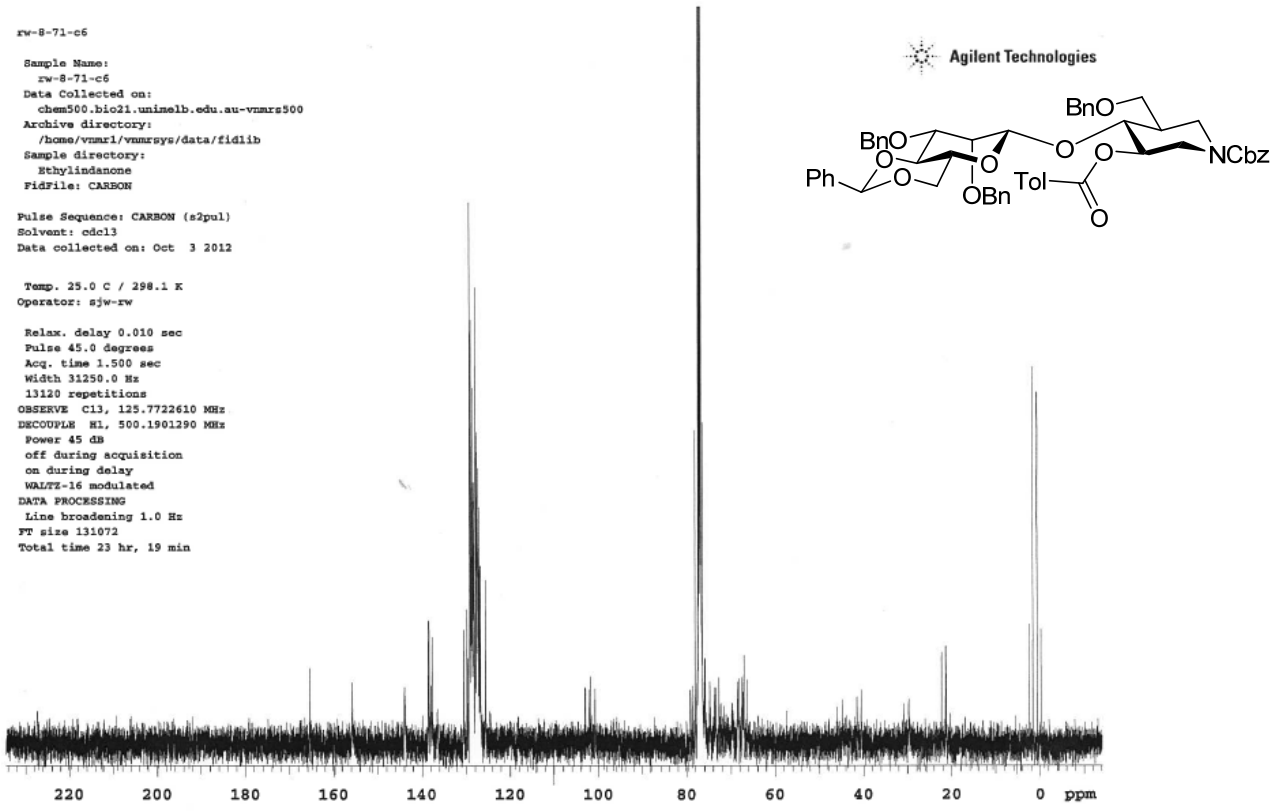


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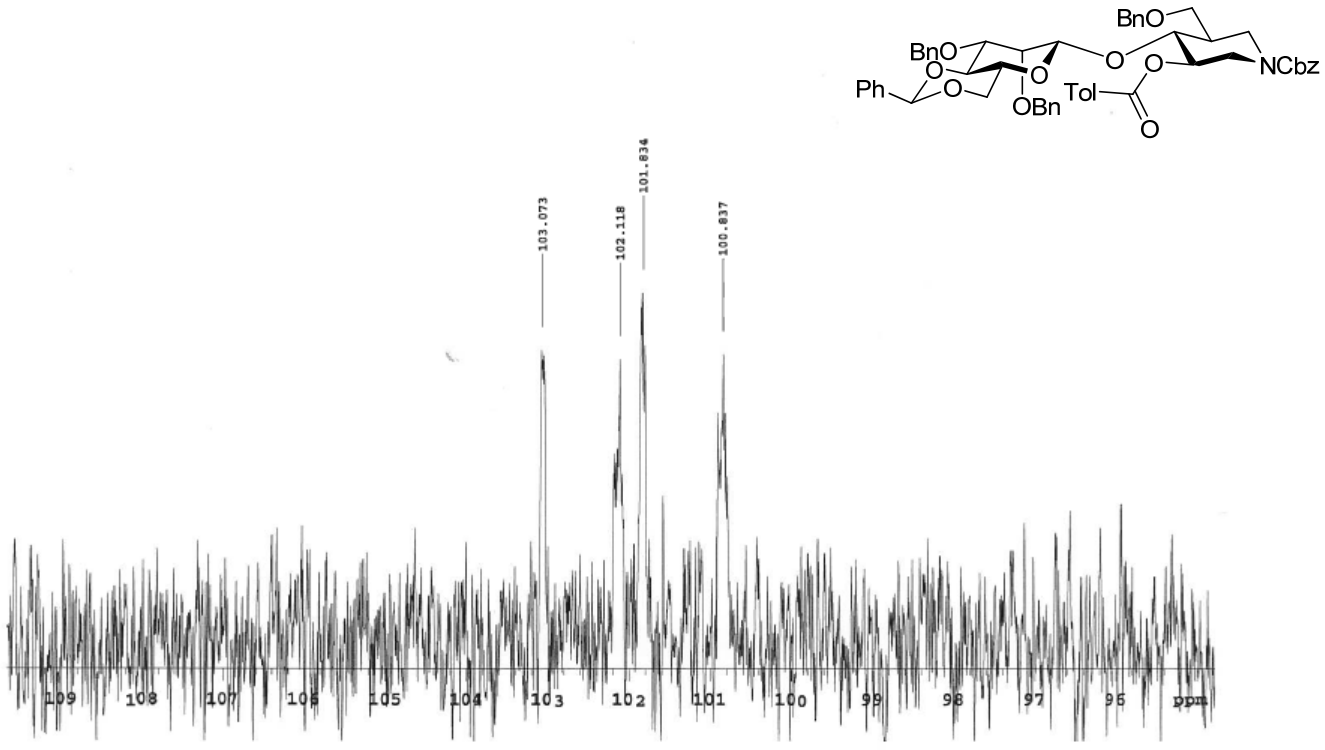

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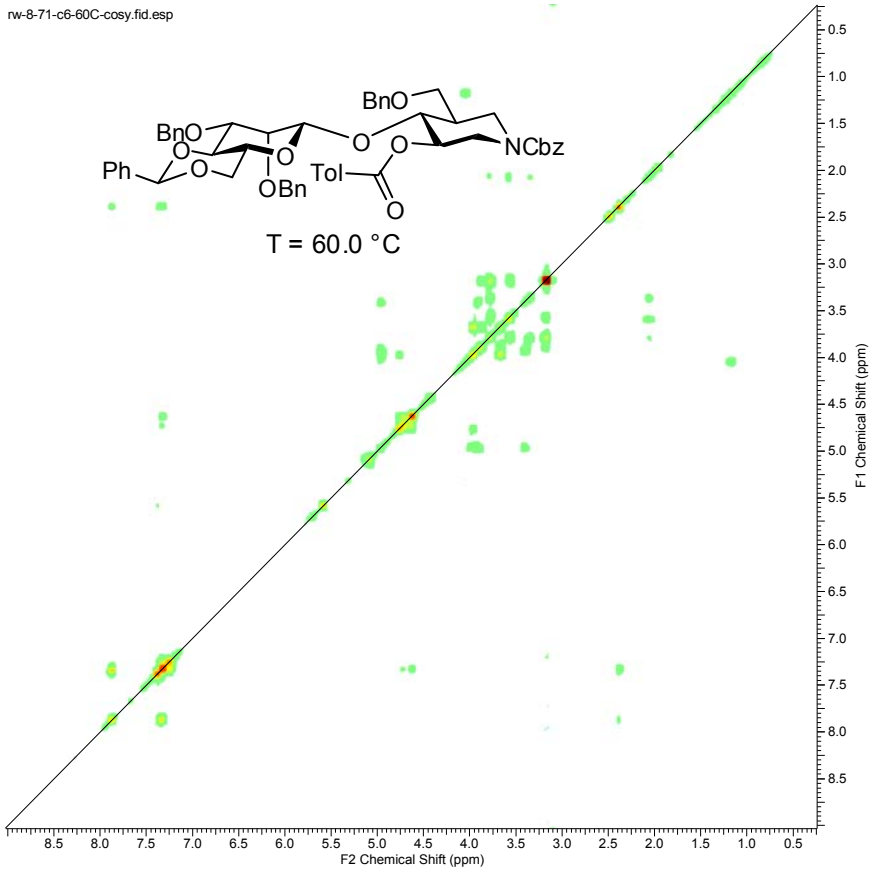
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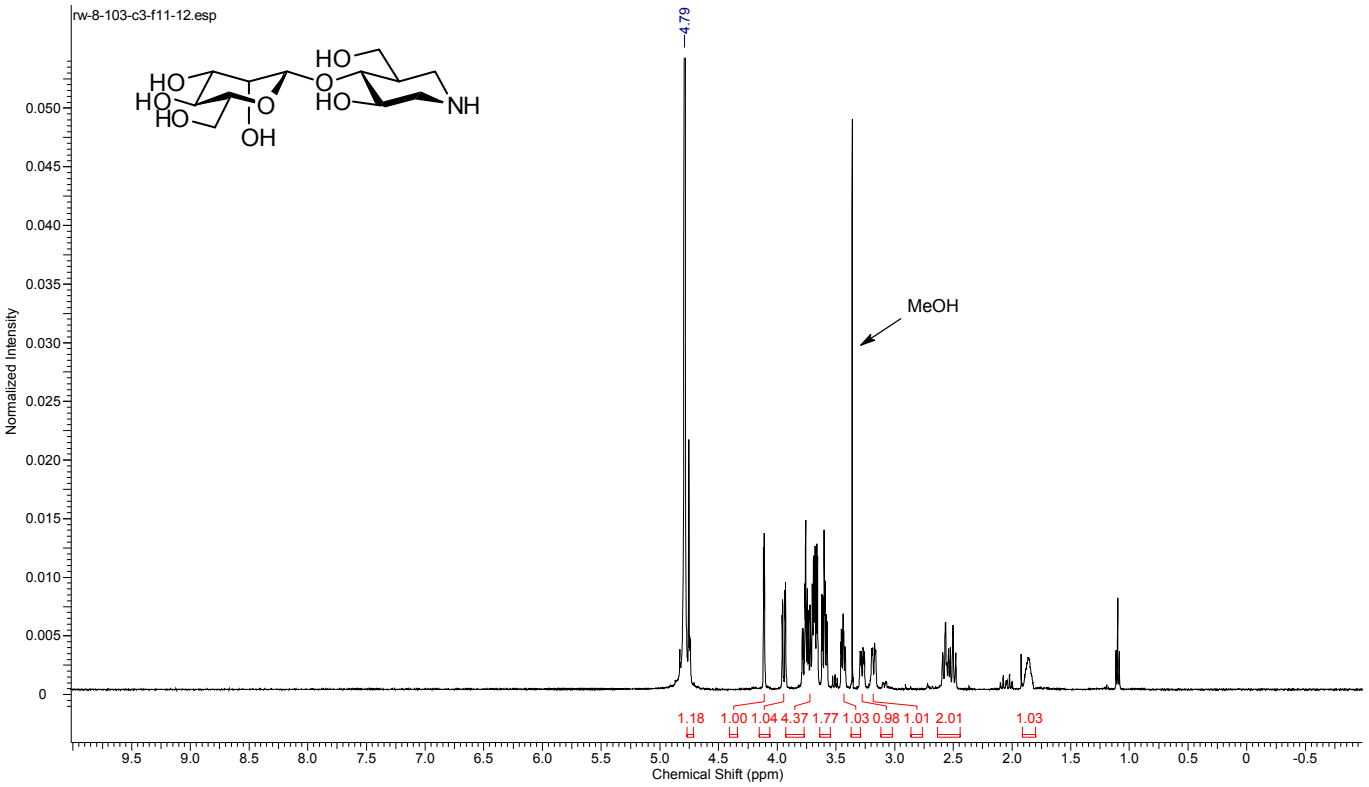
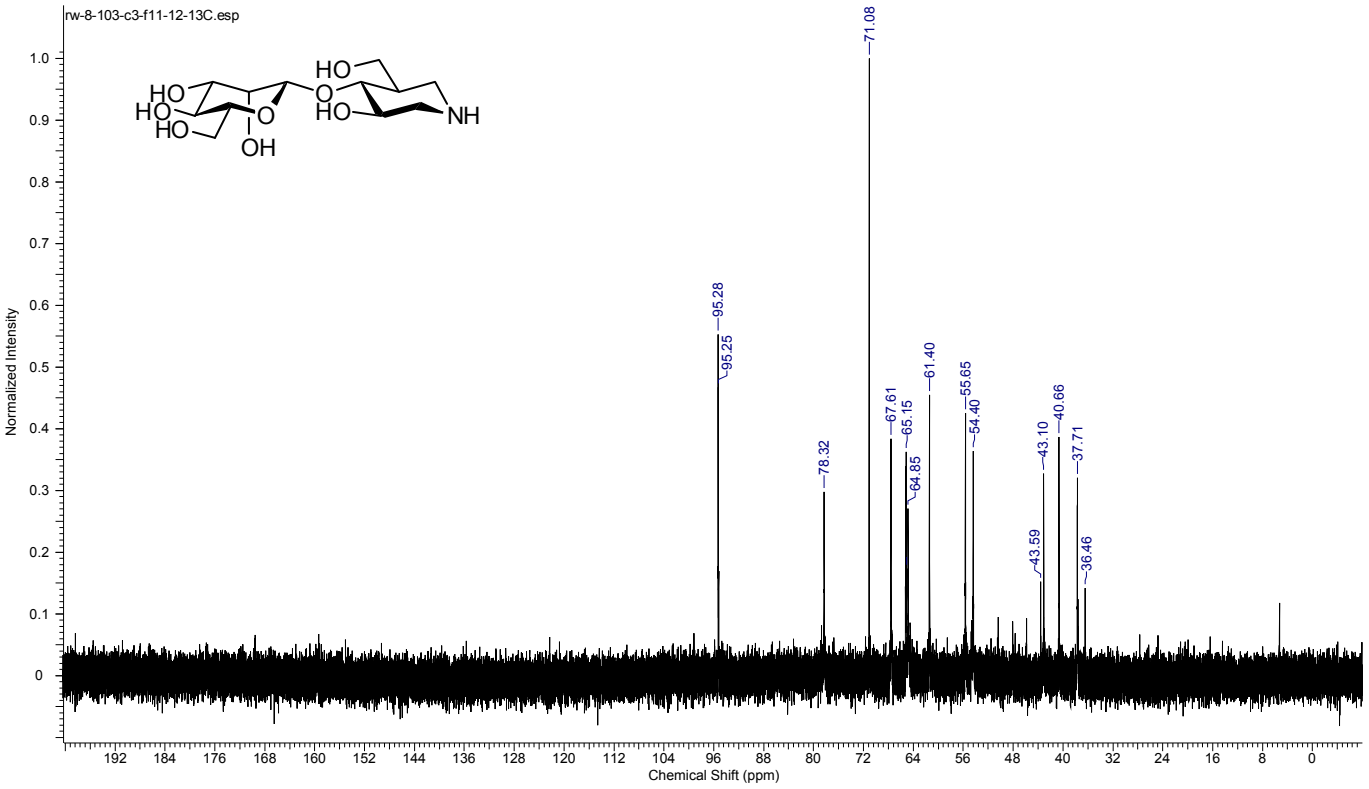
O
O
O
O
Ph
OBn
Tol
O
O
NCbz
T = 60.0 °C
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

**(3*R*,4*R*,5*R*)-3-Hydroxy-5-(hydroxymethyl)-4-(β-D-mannopyranosyloxy)piperidine (ManIFG; 3)**

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71.08
67.61
65.15
64.85
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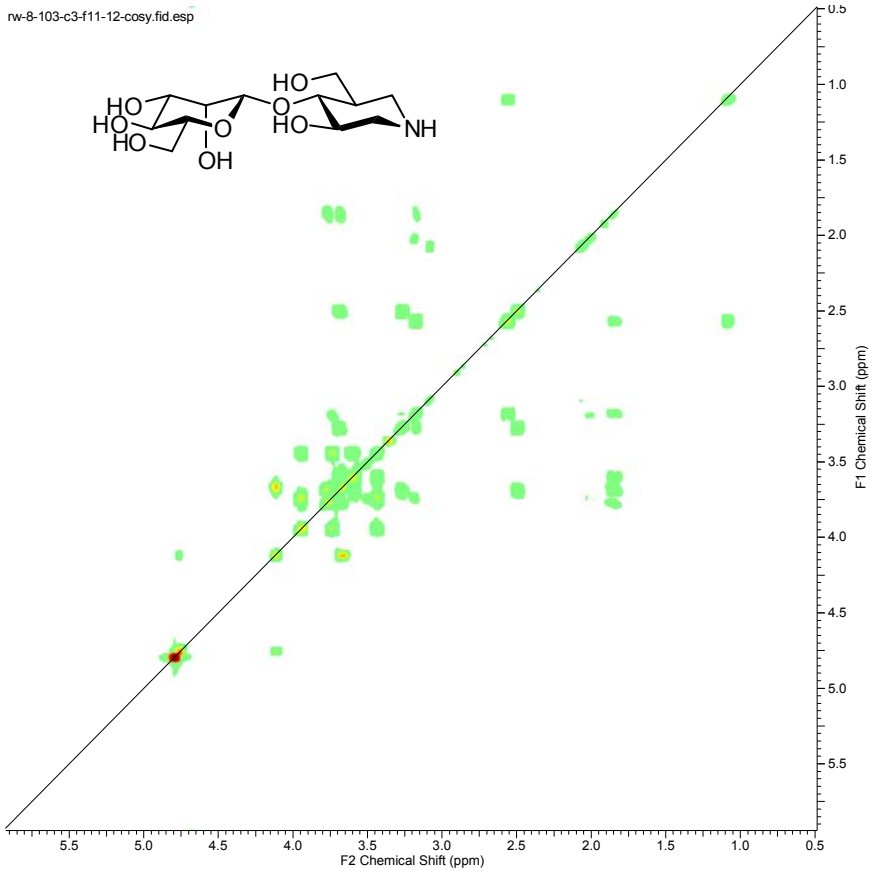
36.46
Chemical Shift (ppm)

$^{1}$H-$^{1}$H COSY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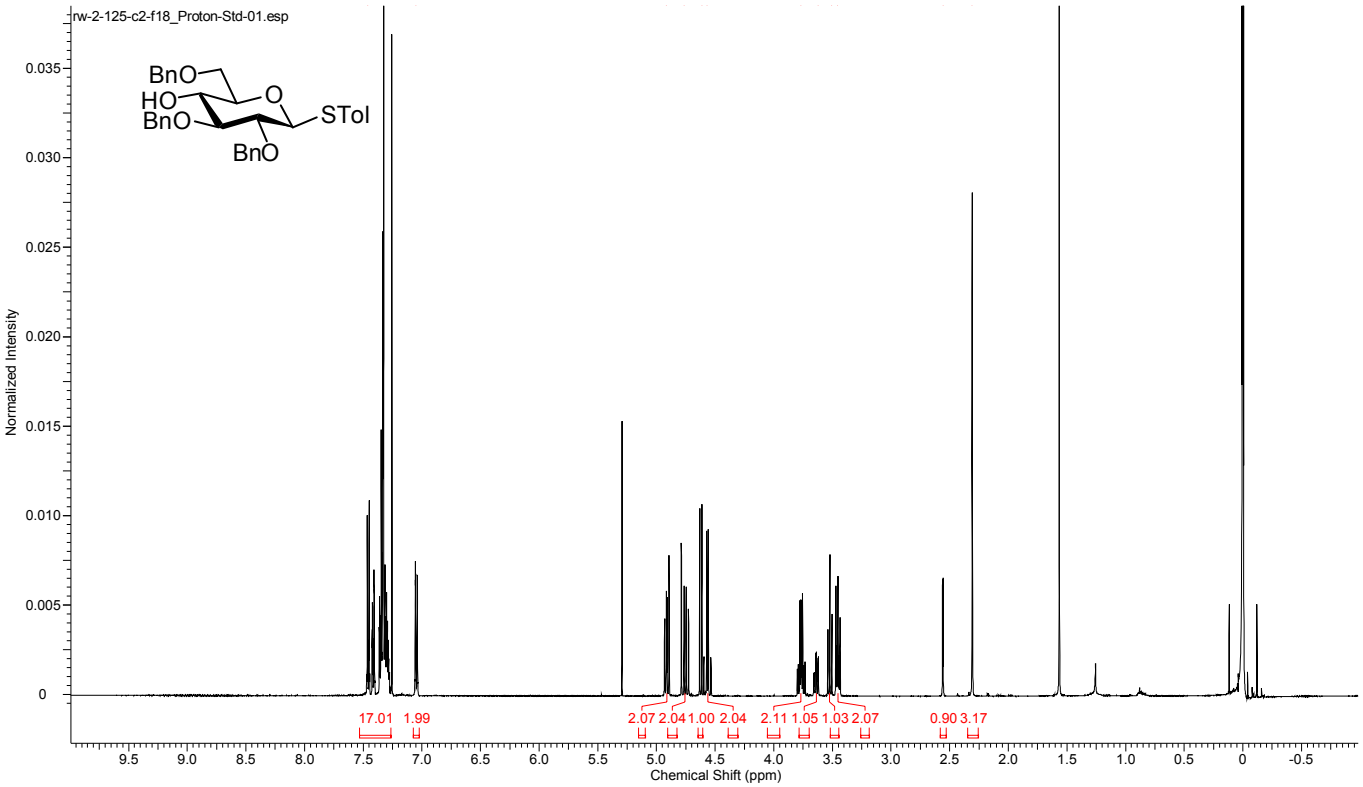
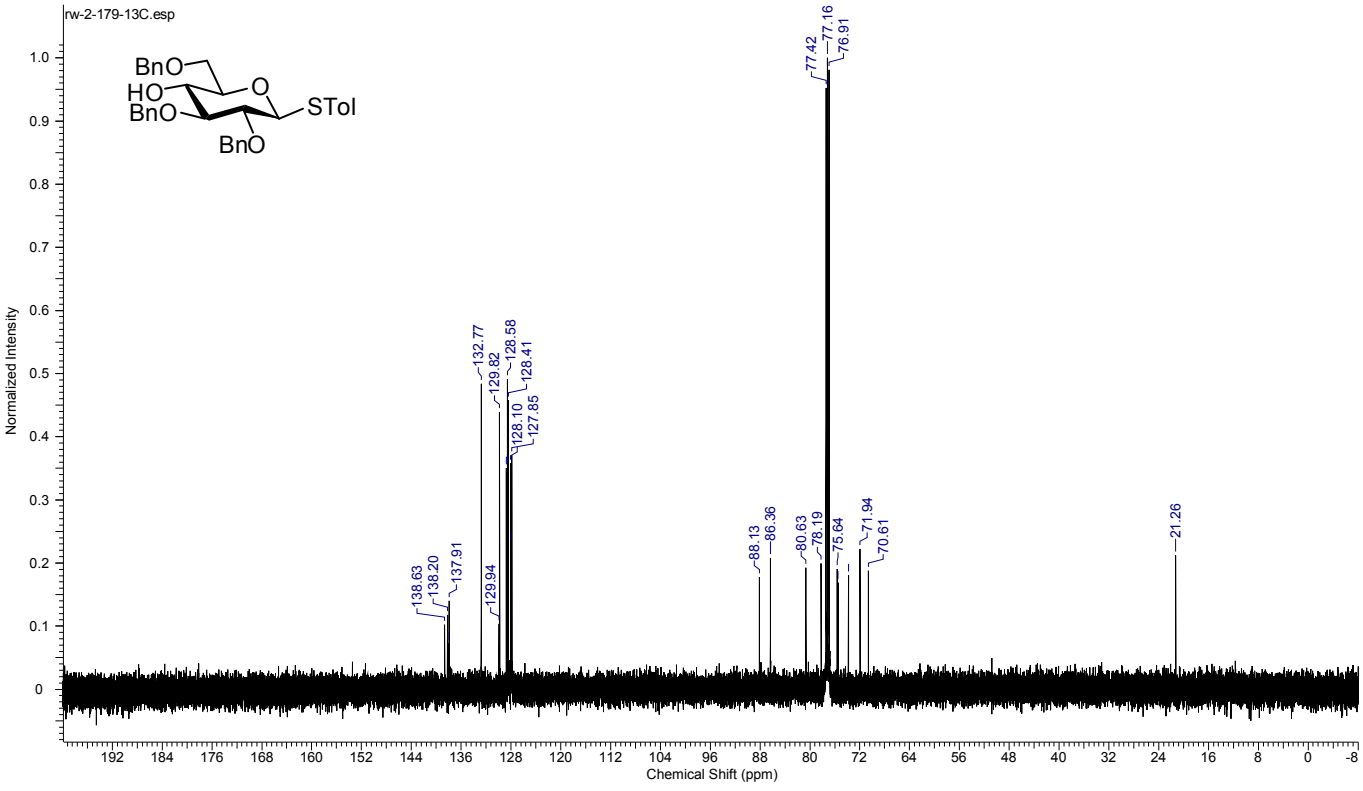
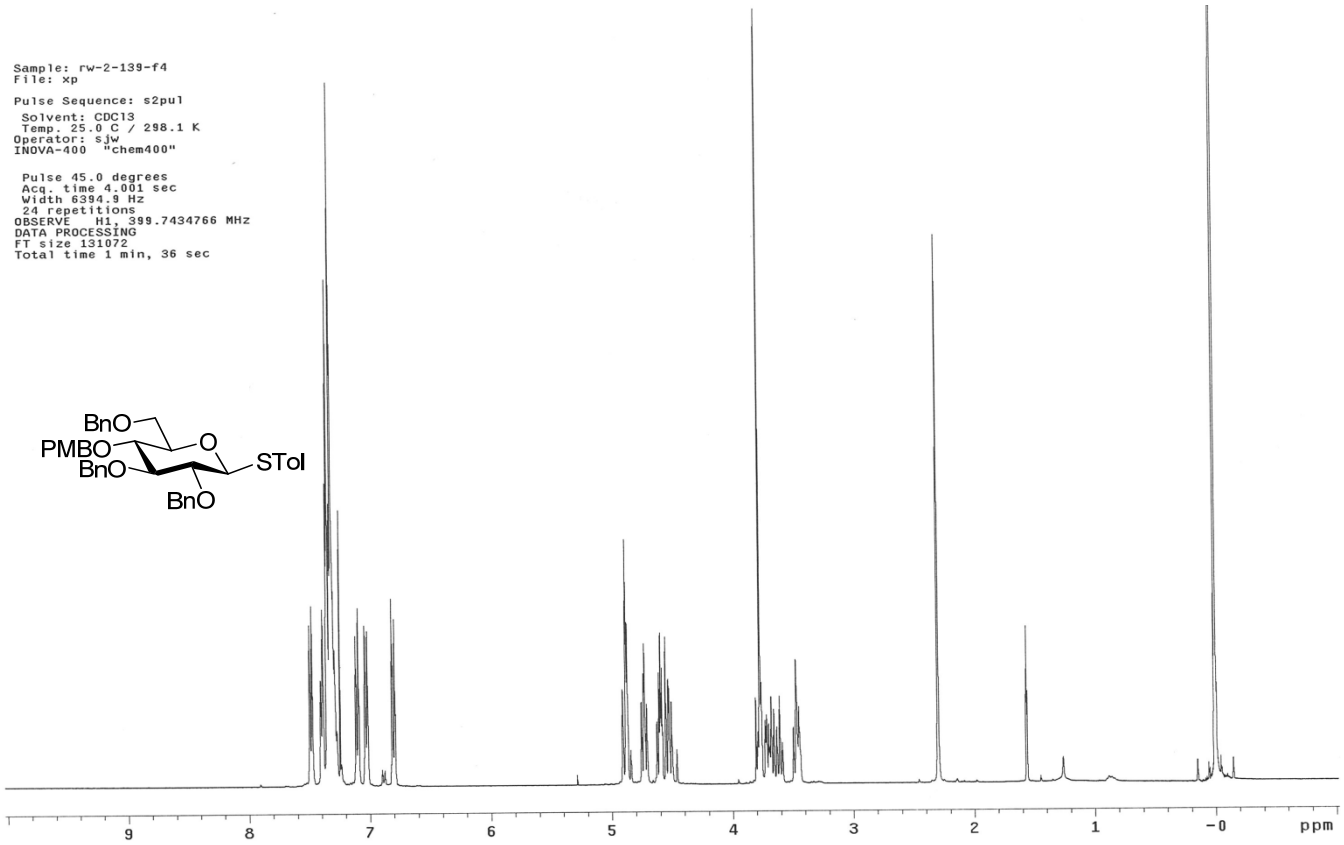
HO
HO
HO
O
OH
HO
O
HO
NH
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

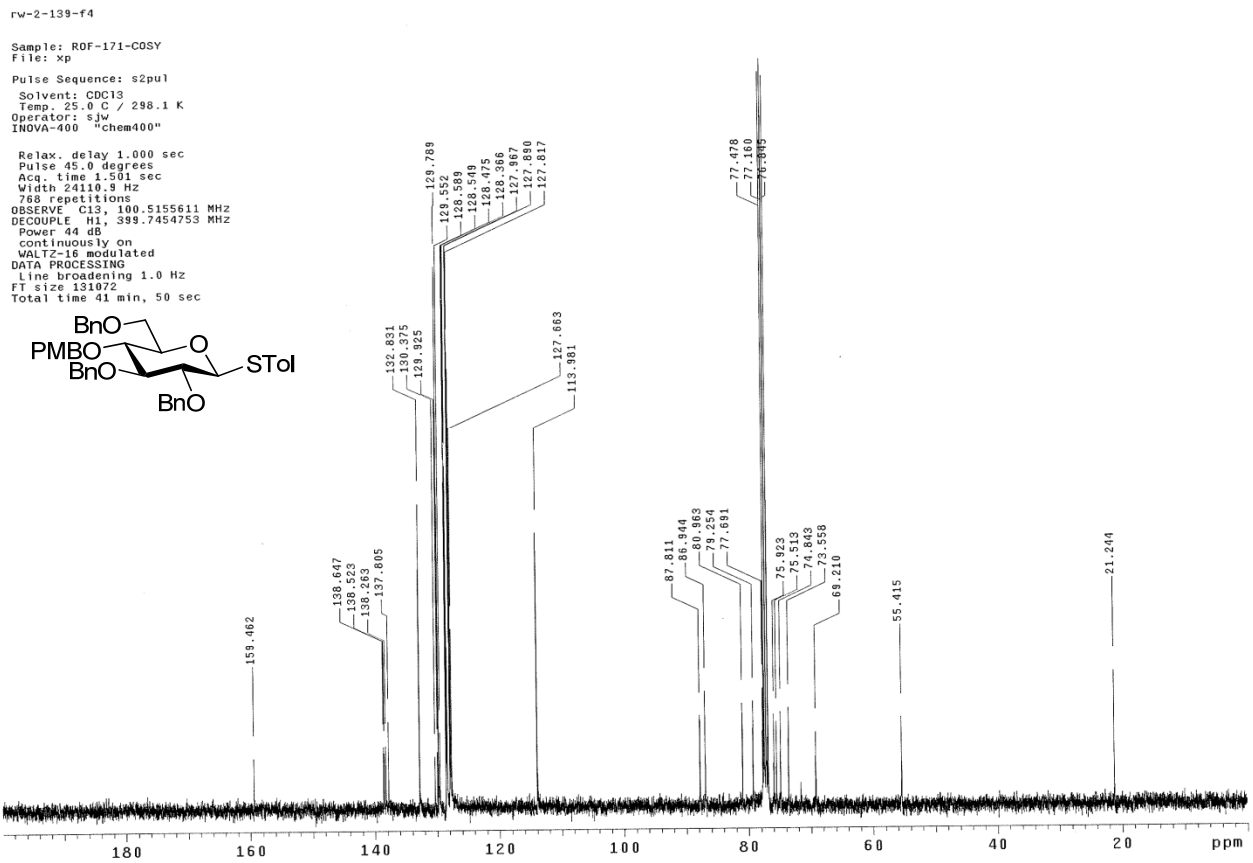
## 4-Methylphenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (9)



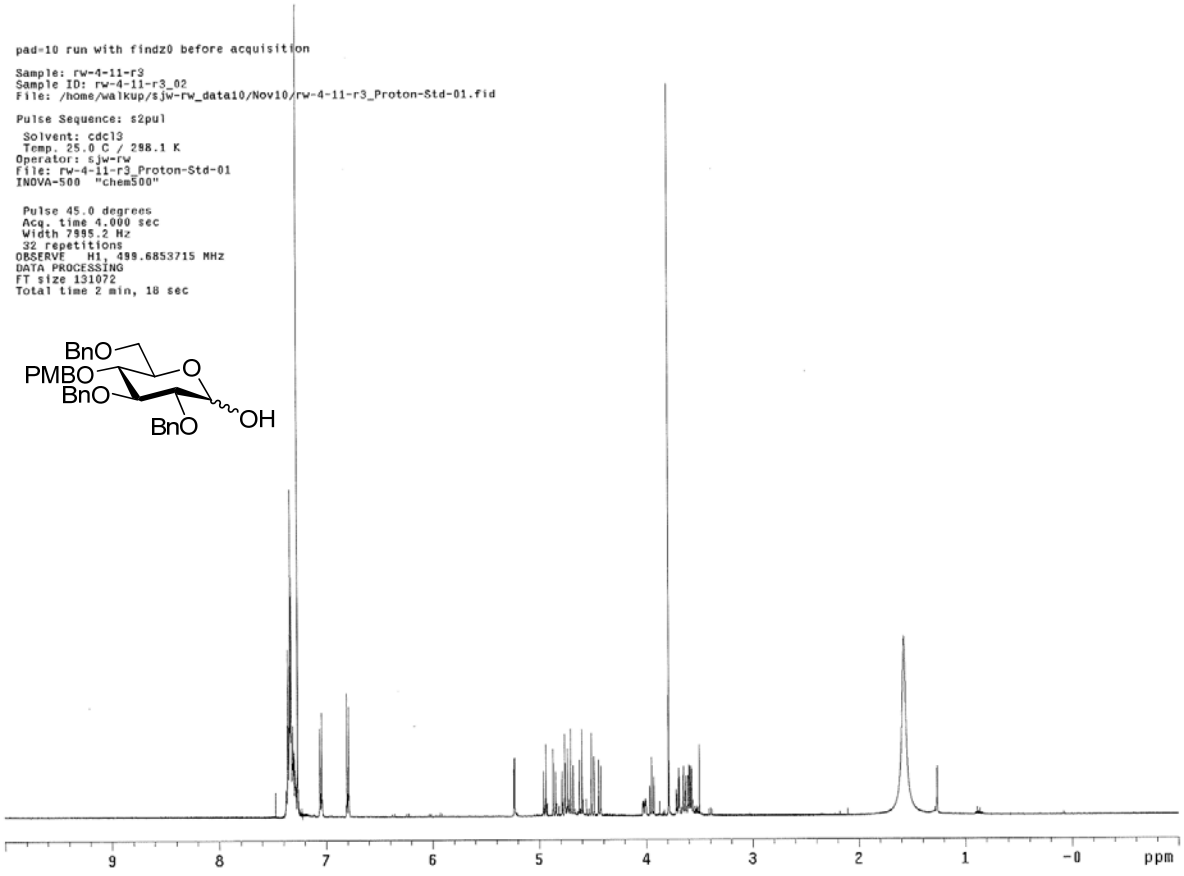



**4-Methylphenyl 2,3,6-tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-1-thio-β-D-glucopyranoside (10)**

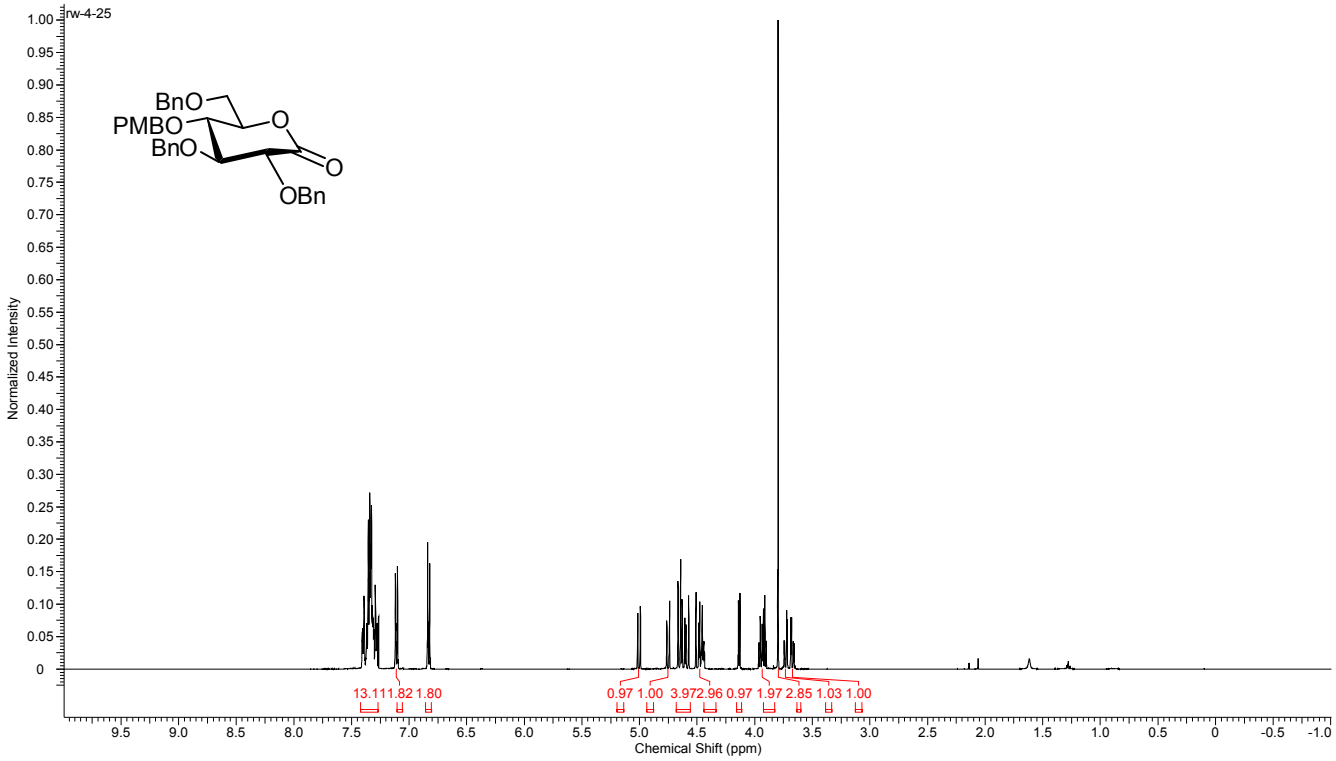


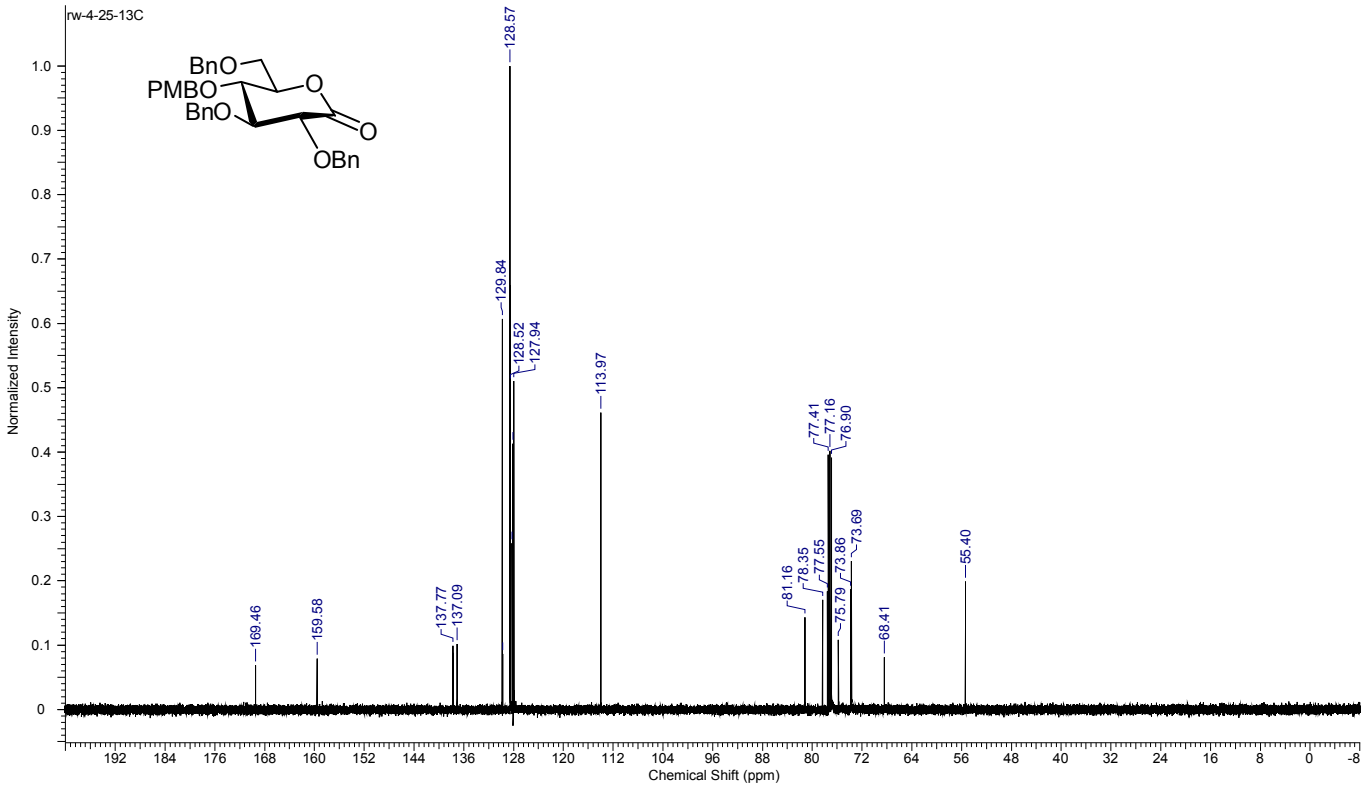



## 2,3,6-Tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-D-glucopyranose

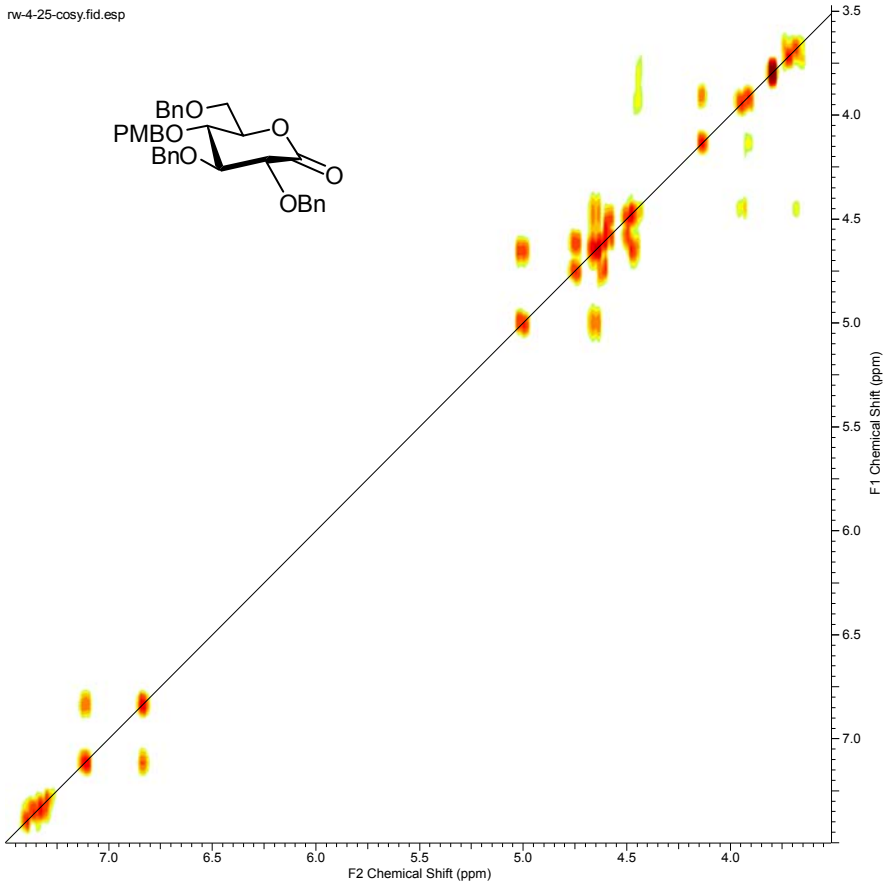


## 2,3,6-Tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-D-gluconolactone (11)

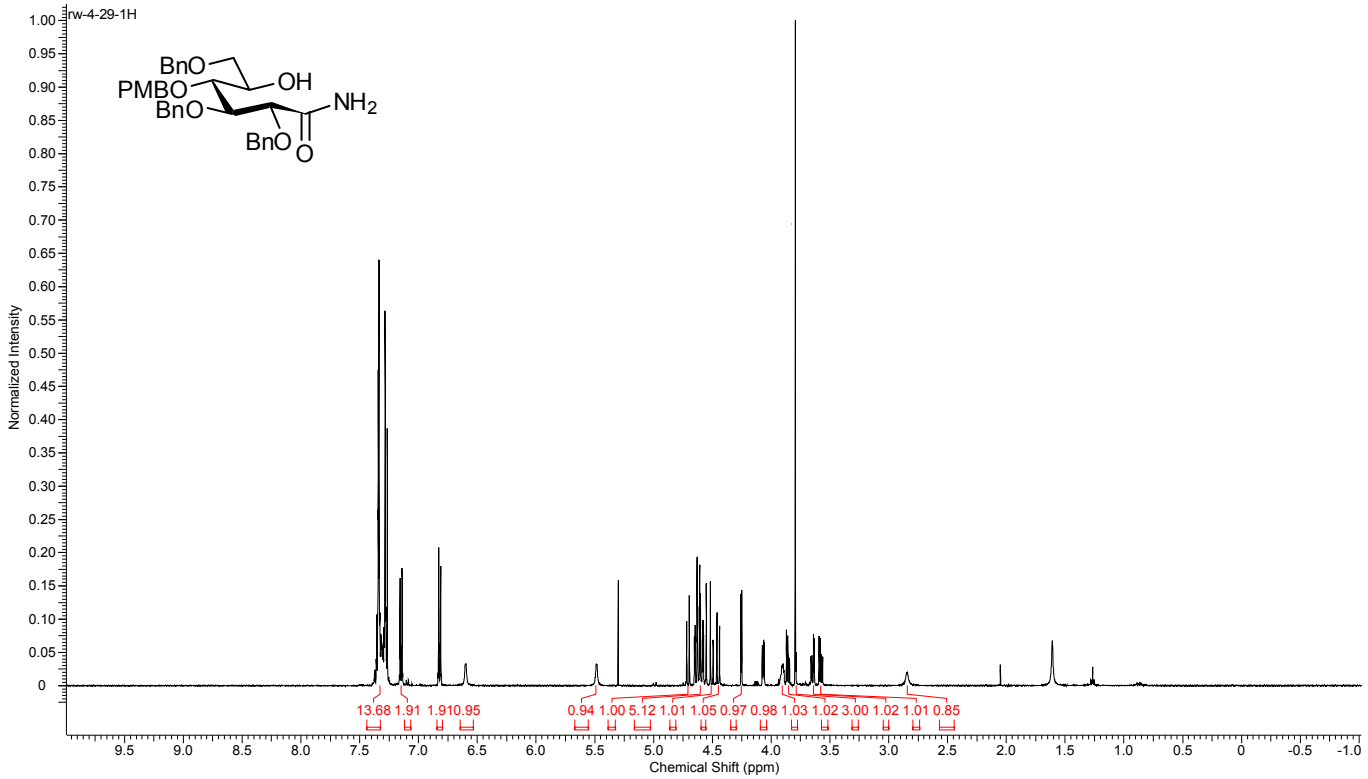


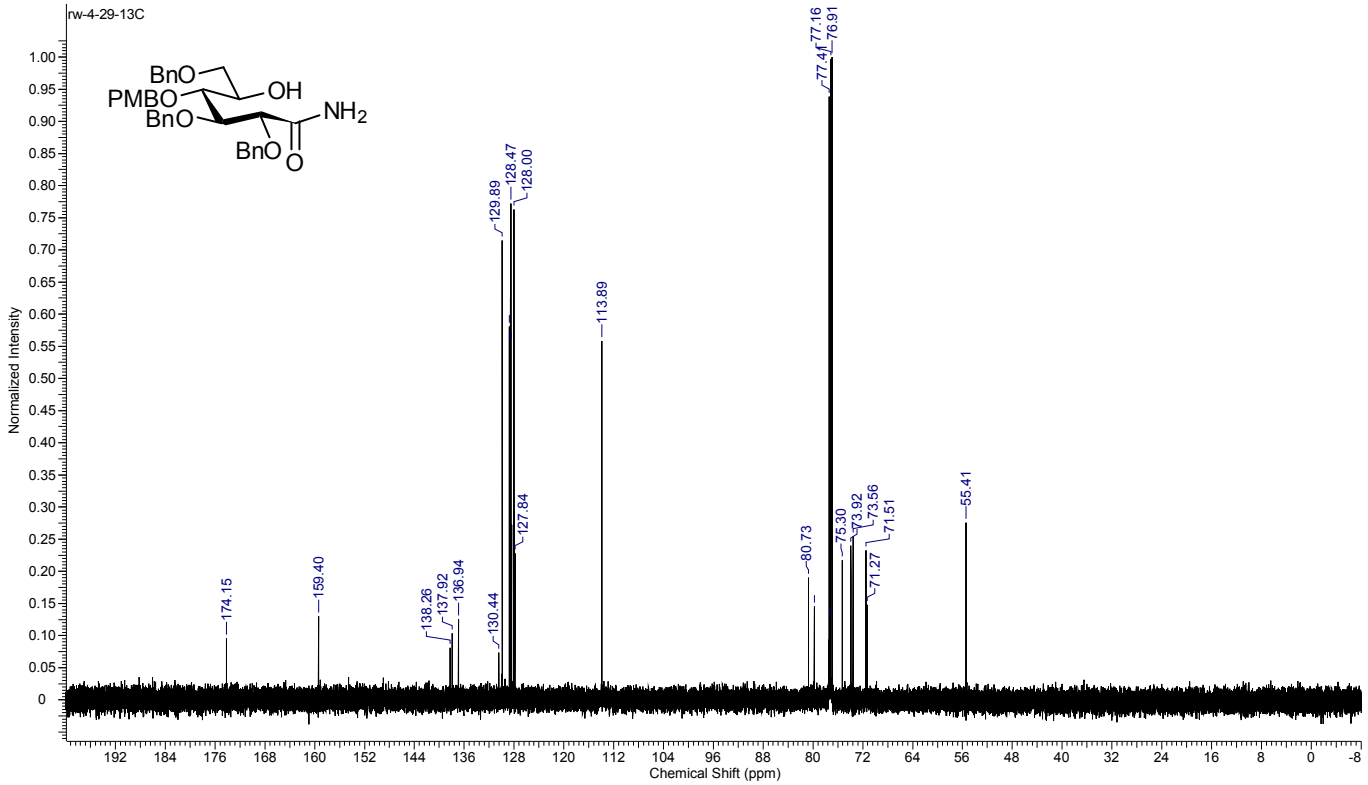



$^{1}$H-$^{1}$H COSY

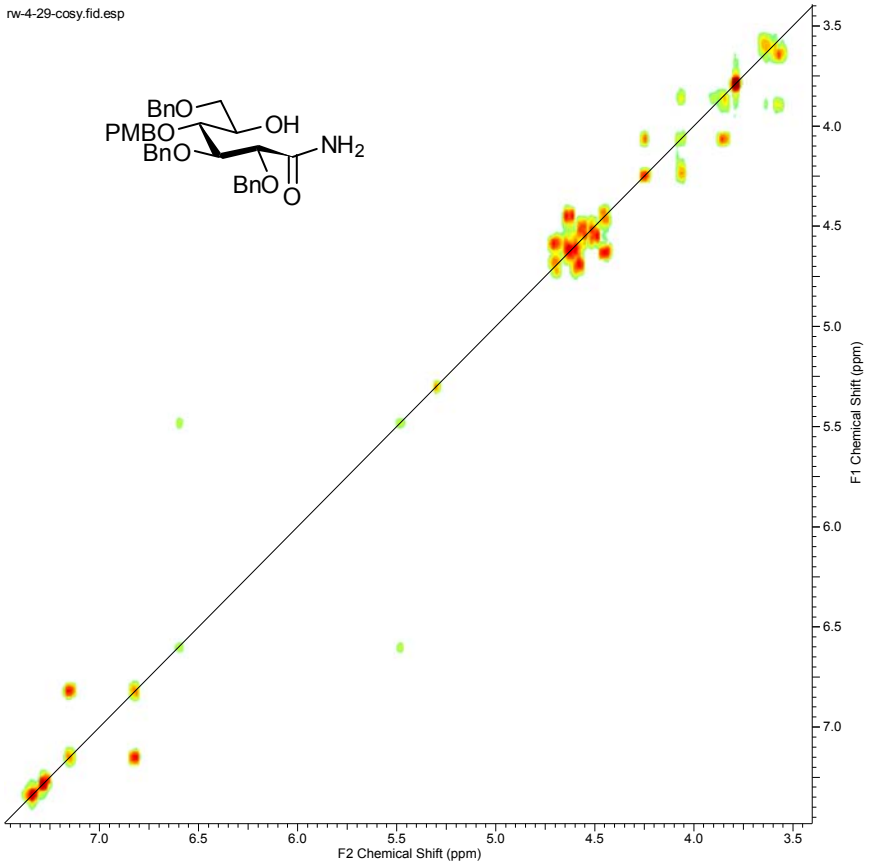

rw-4-25-cosy.fid.esp
BnO
PMBO
BnO
O
O
OBn
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
F1 Chemical Shift (ppm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
F2 Chemical Shift (ppm)

## 2,3,6-Tri-*O*-benzyl-4-*O*-(4-methoxybenzyl)-D-gluconamide

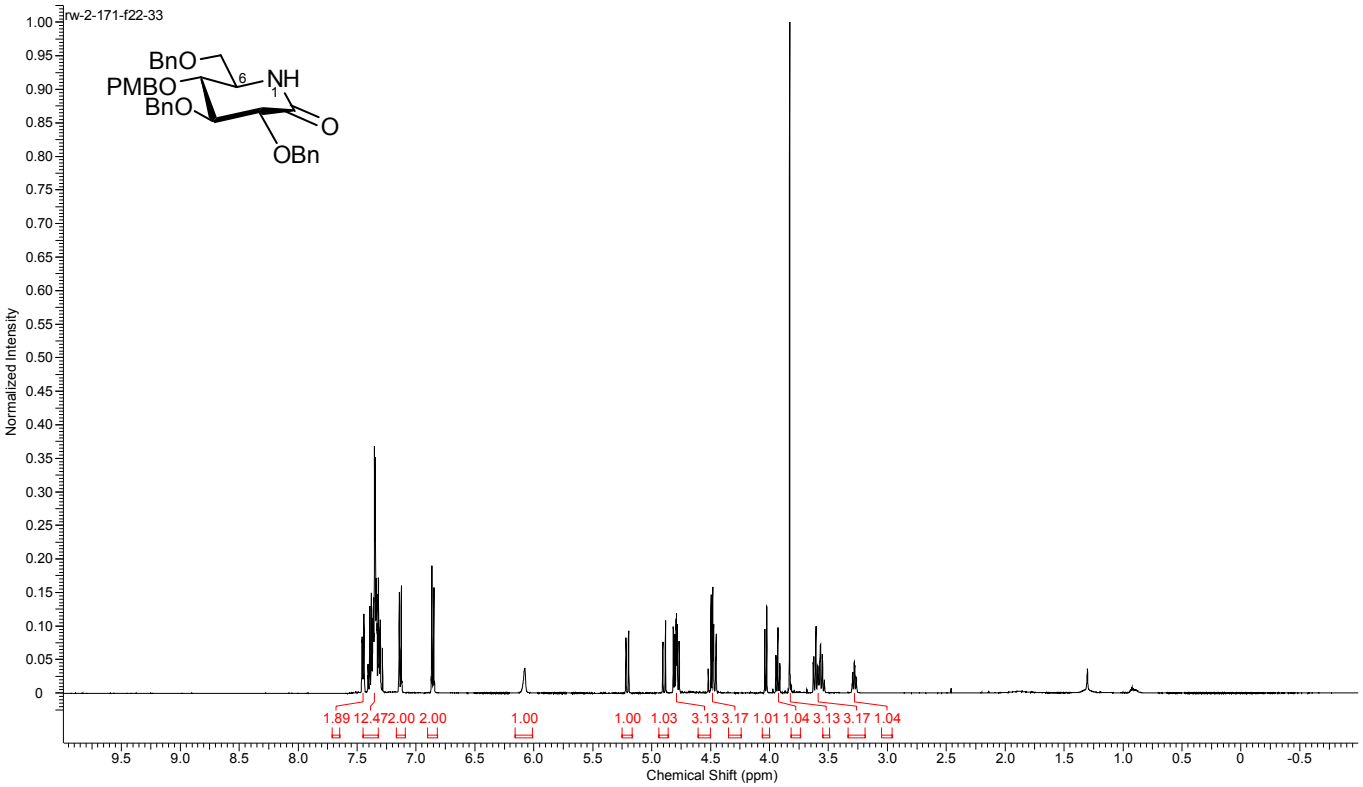


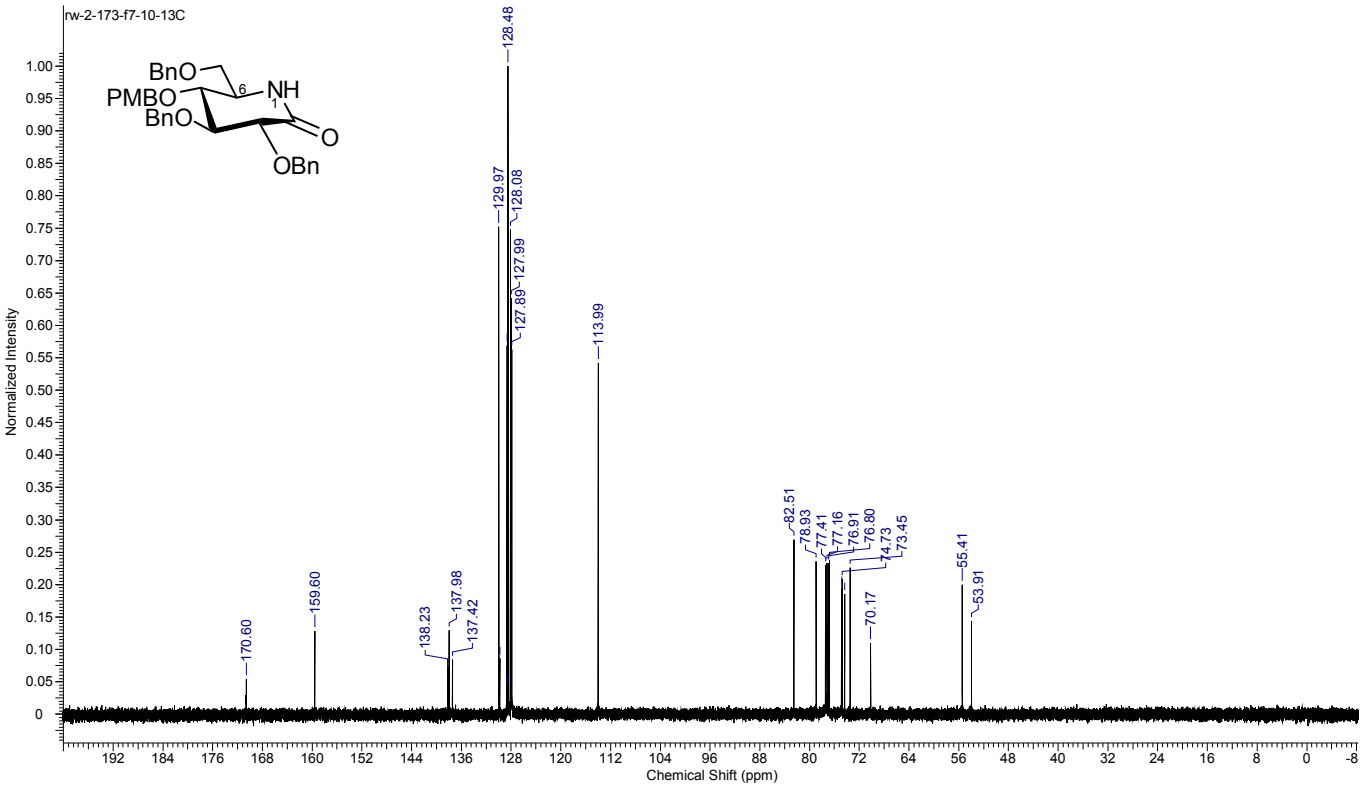



$^{1}$H-$^{1}$H COSY

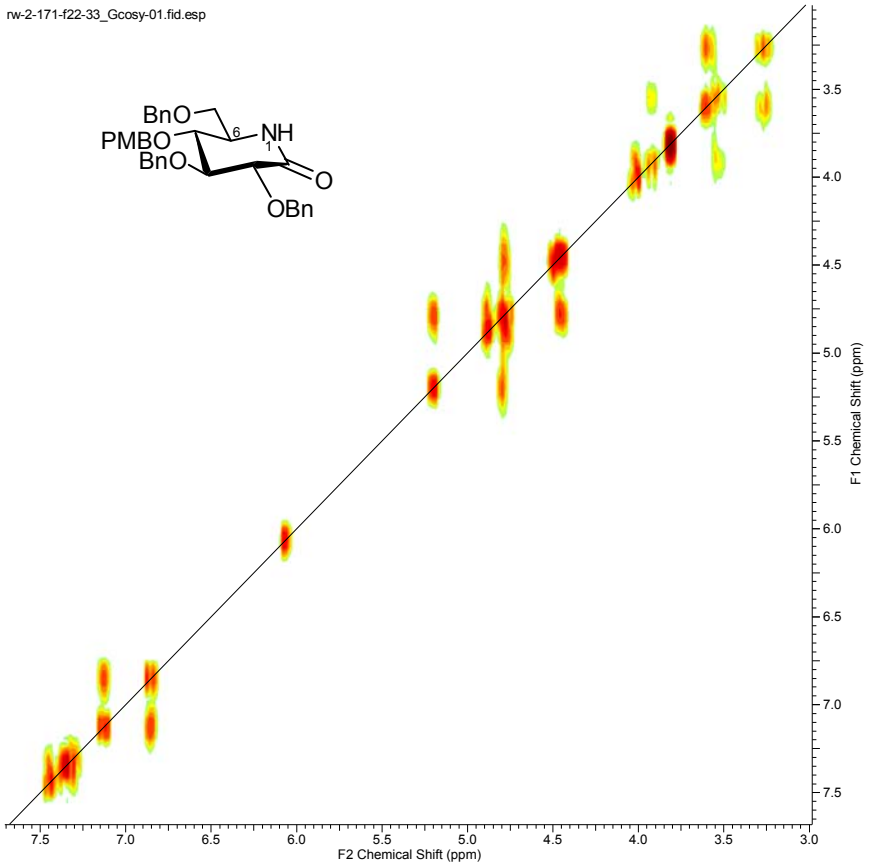

rw-4-29-cosy.fid.esp
BnO
PMBO
BnO
BnO
OH
NH2
O
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5

**(3*R*,4*S*,5*S*,6*S*)-3,4,-Bis(benzyloxy)-6-[(benzyloxy)methyl]-5-(4-methoxybenzyloxy)piperidin-2-one (12)**

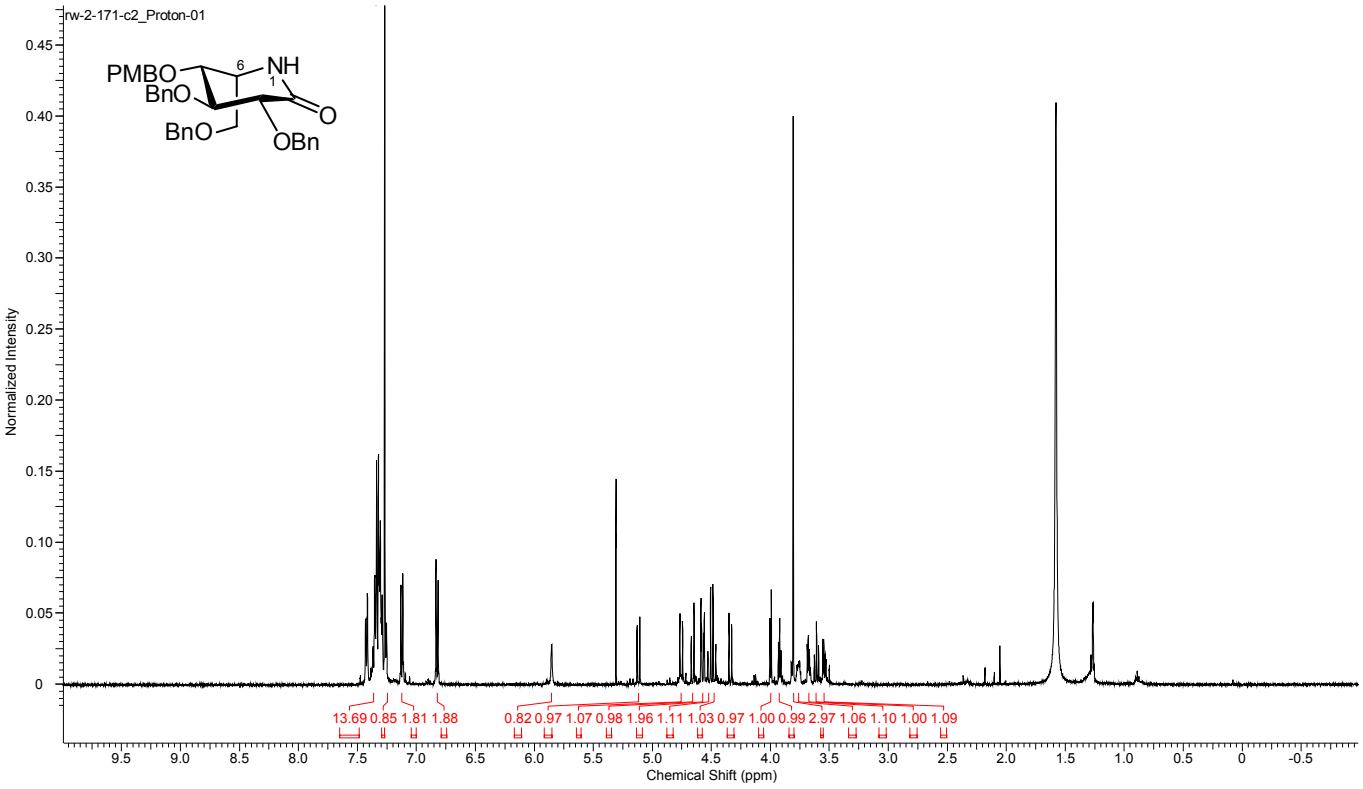


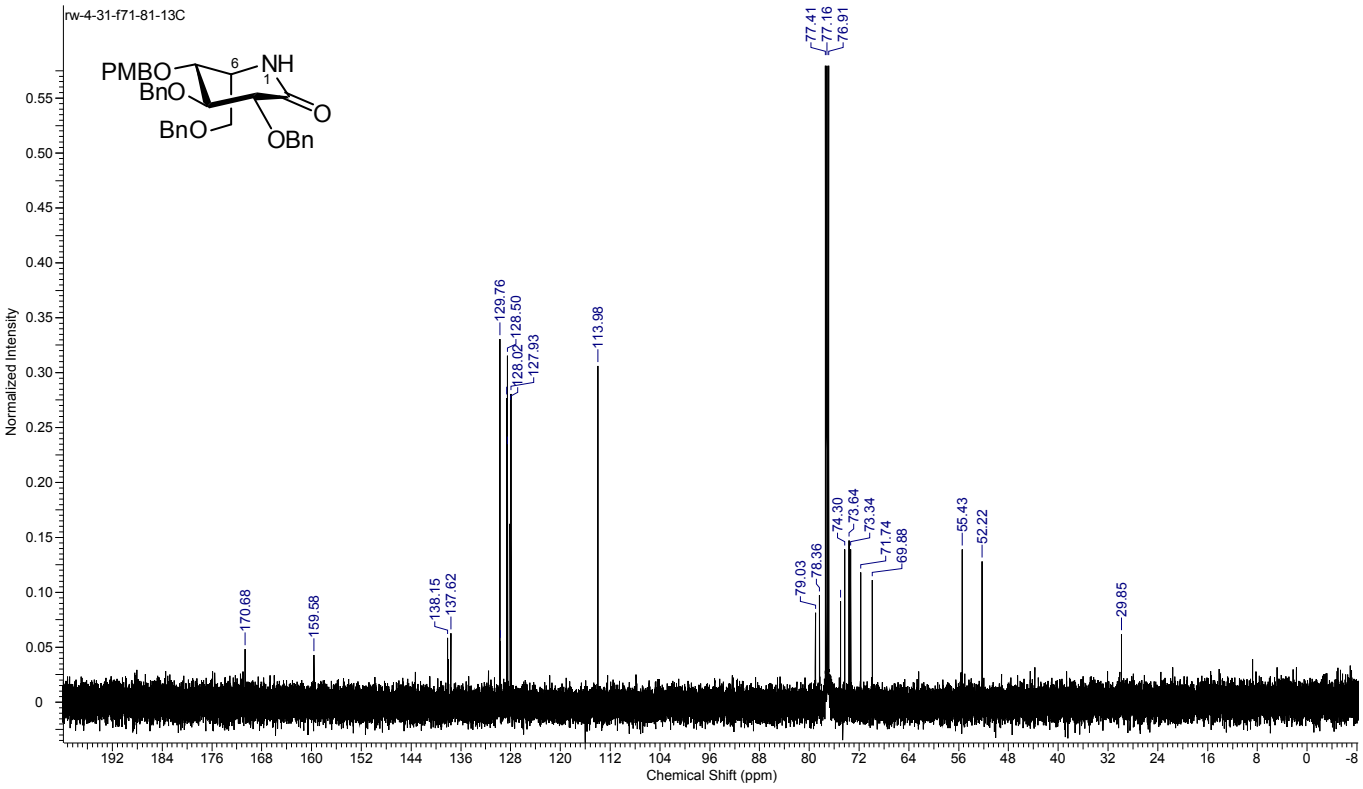



$^{1}H$-$^{1}H$ COSY

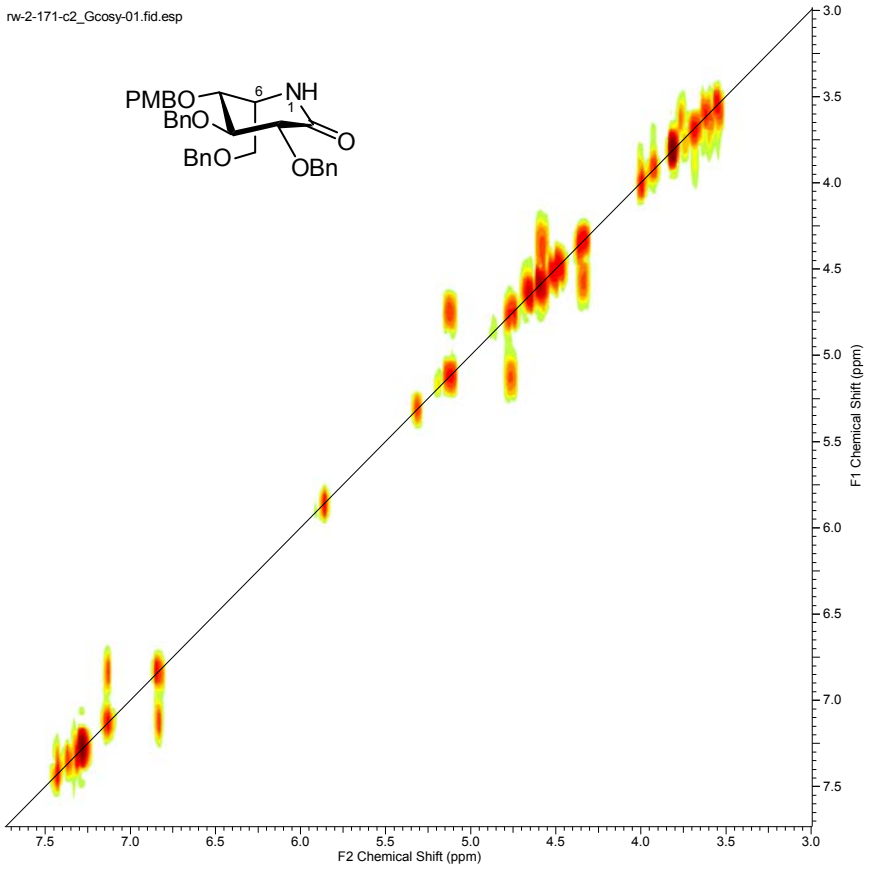

rw-2-171-f22-33_Gcosy-01.fid.esp
BnO
PMBO
BnO
6
NH
1
O
OBn
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

**(3*R*,4*S*,5*S*,6*R*)-3,4,-Bis(benzyloxy)-6-[(benzyloxy)methyl]-5-(4-methoxybenzyloxy)piperidin-2-one**

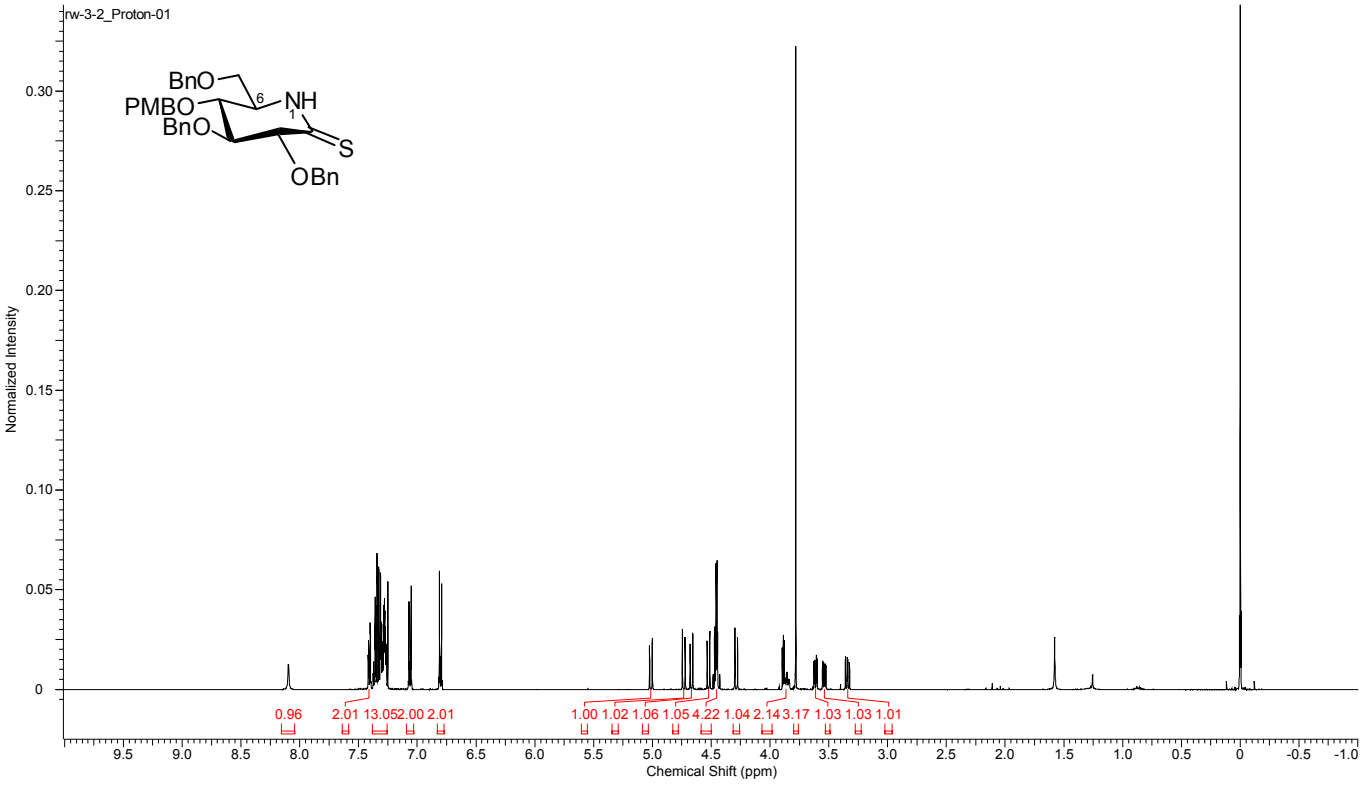


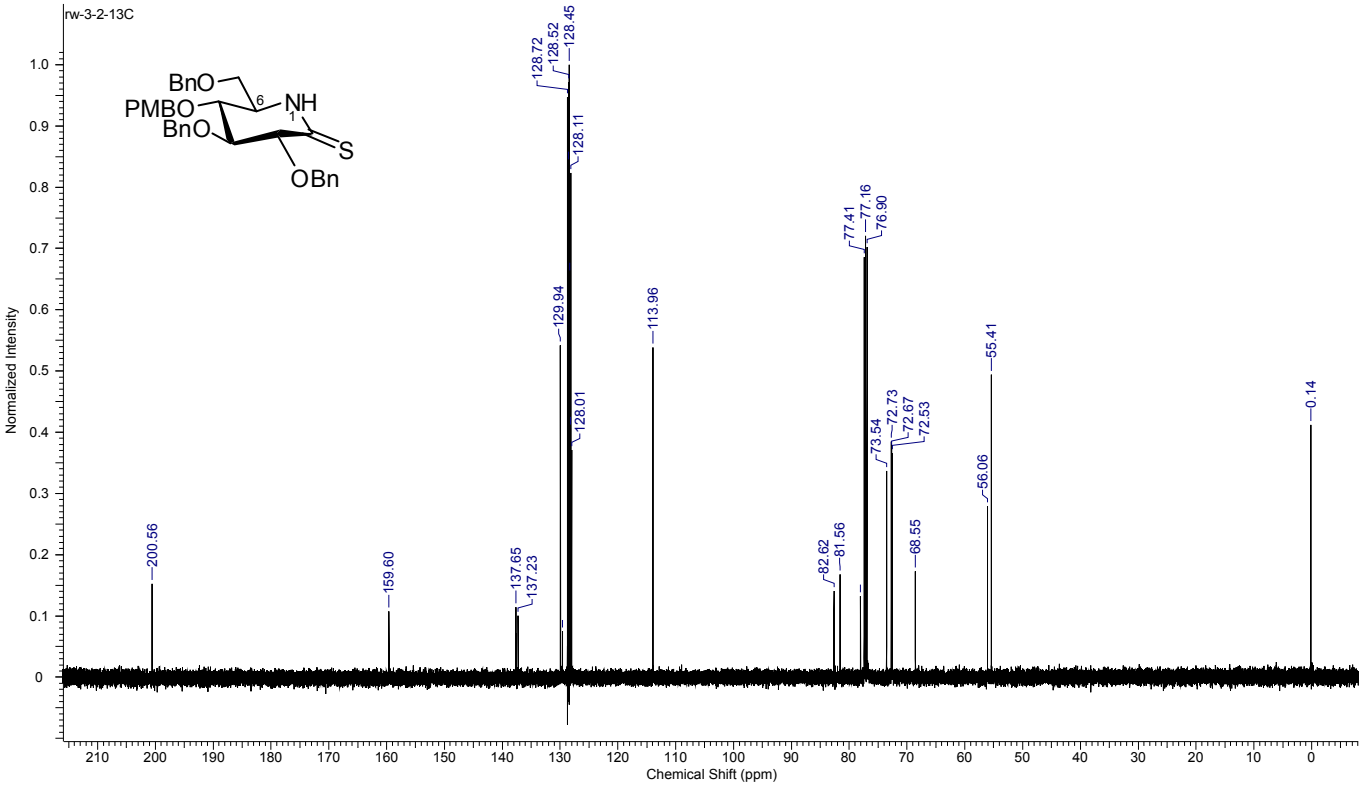



$^{1}$H-$^{1}$H COSY

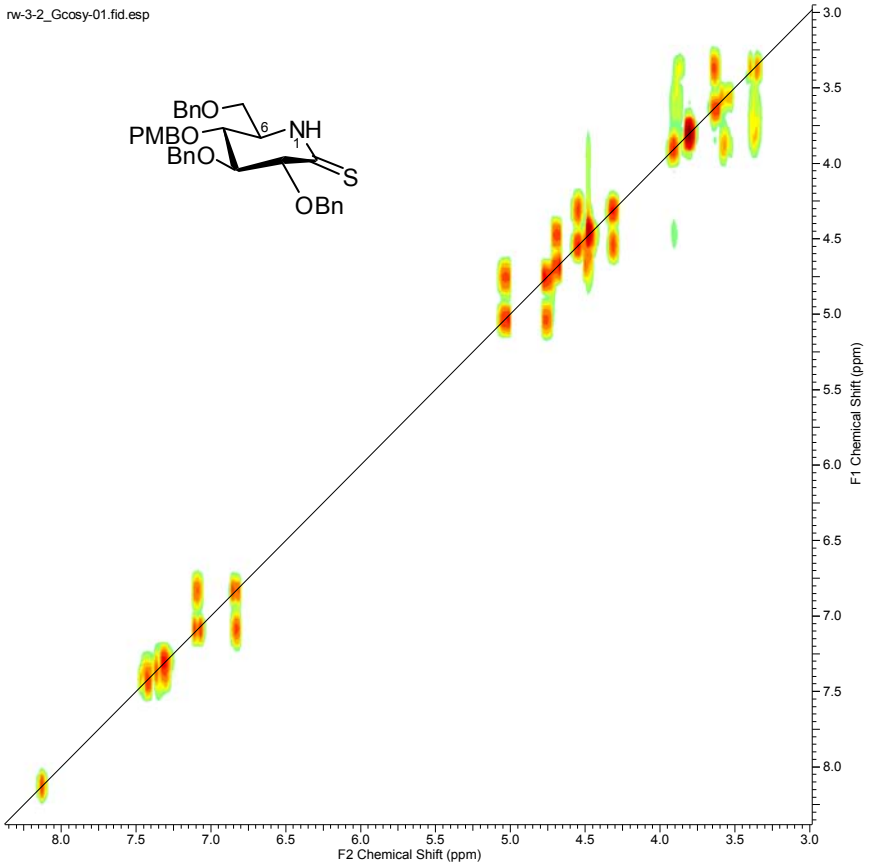

rw-2-171-c2_Gcosy-01.fid.esp
PMBO
BnO
BnO
OBn
NH
O
6
1
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0

**(3*R*,4*S*,5*S*,6*S*)-3,4,-Bis(benzyloxy)-6-[(benzyloxy)methyl]-5-(4-methoxybenzyloxy)piperidin-2-thione (13)**

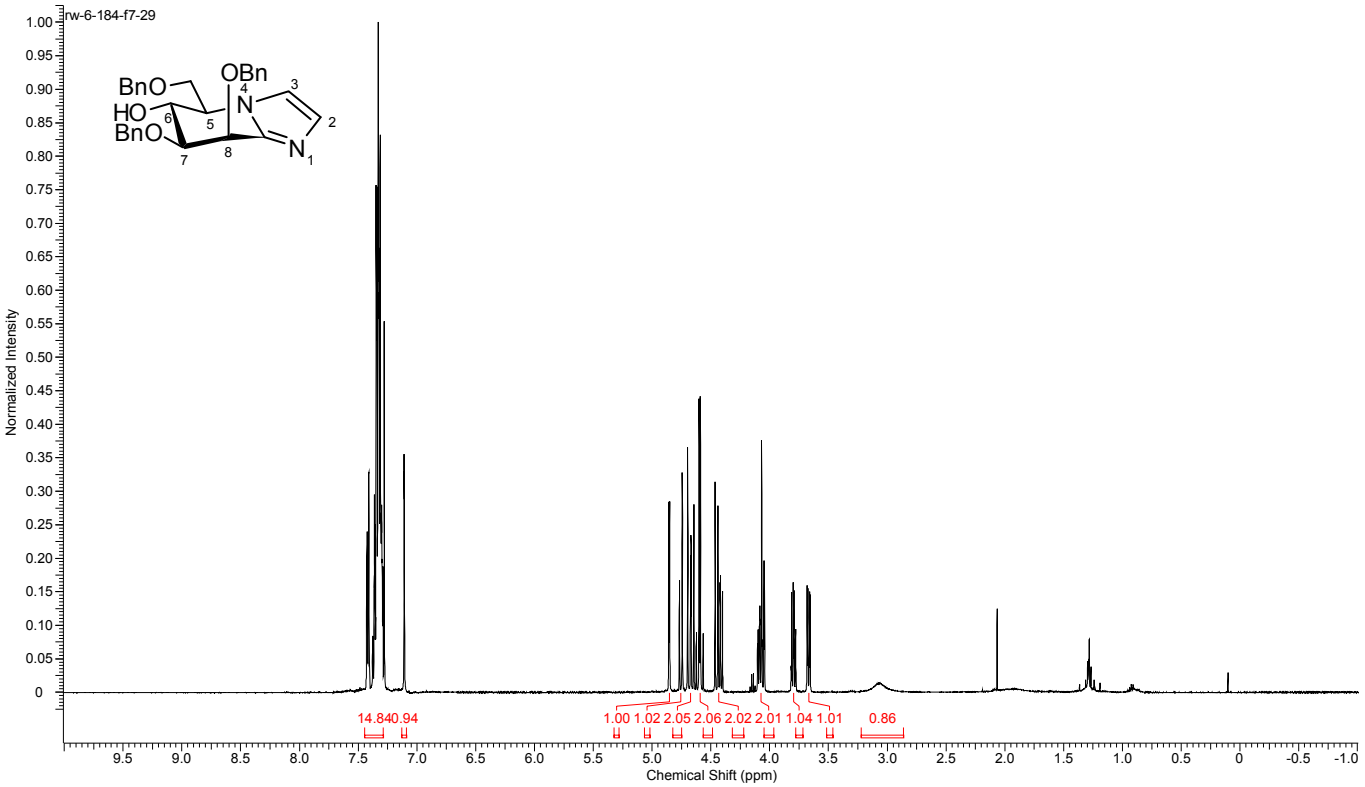


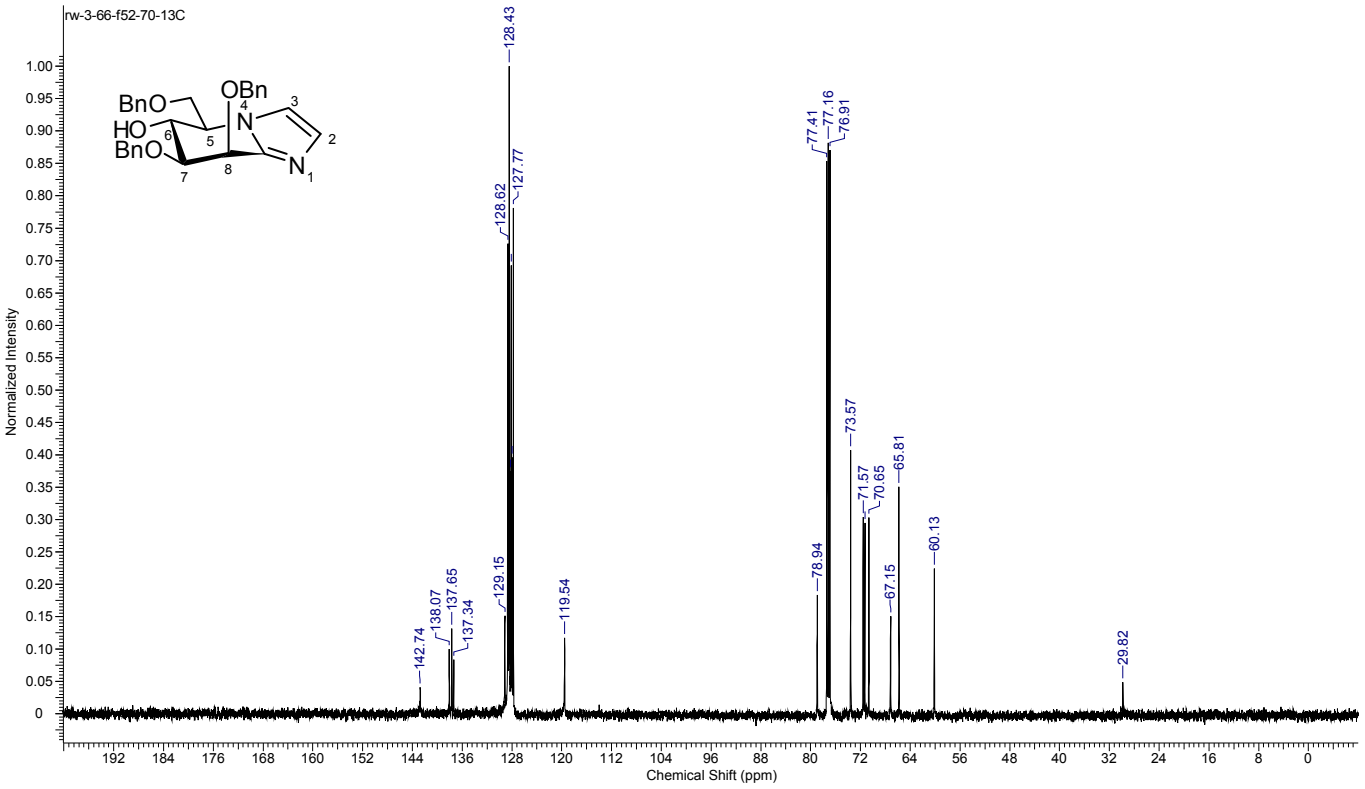



$^{1}$H-$^{1}$H COSY

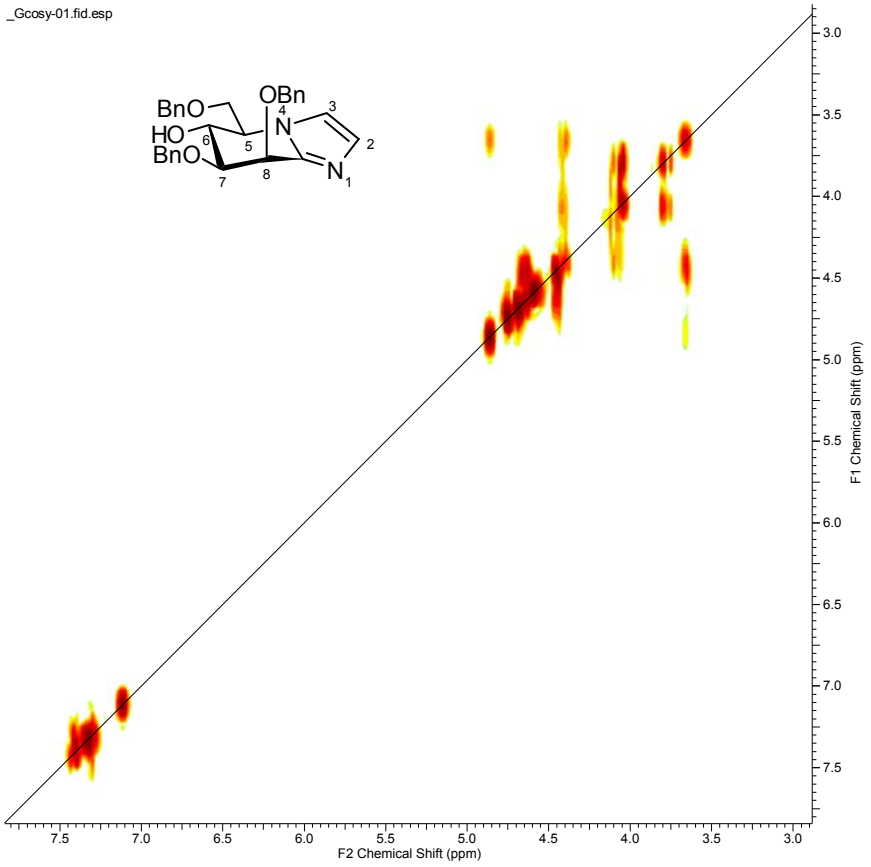

rw-3-2_Gcosy-01.fid.esp
BnO
PMBO
BnO
6
NH
1
S
OBn
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

**(5*R*,6*R*,7*S*,8*R*)-7,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-6-hydroxy-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (15)**

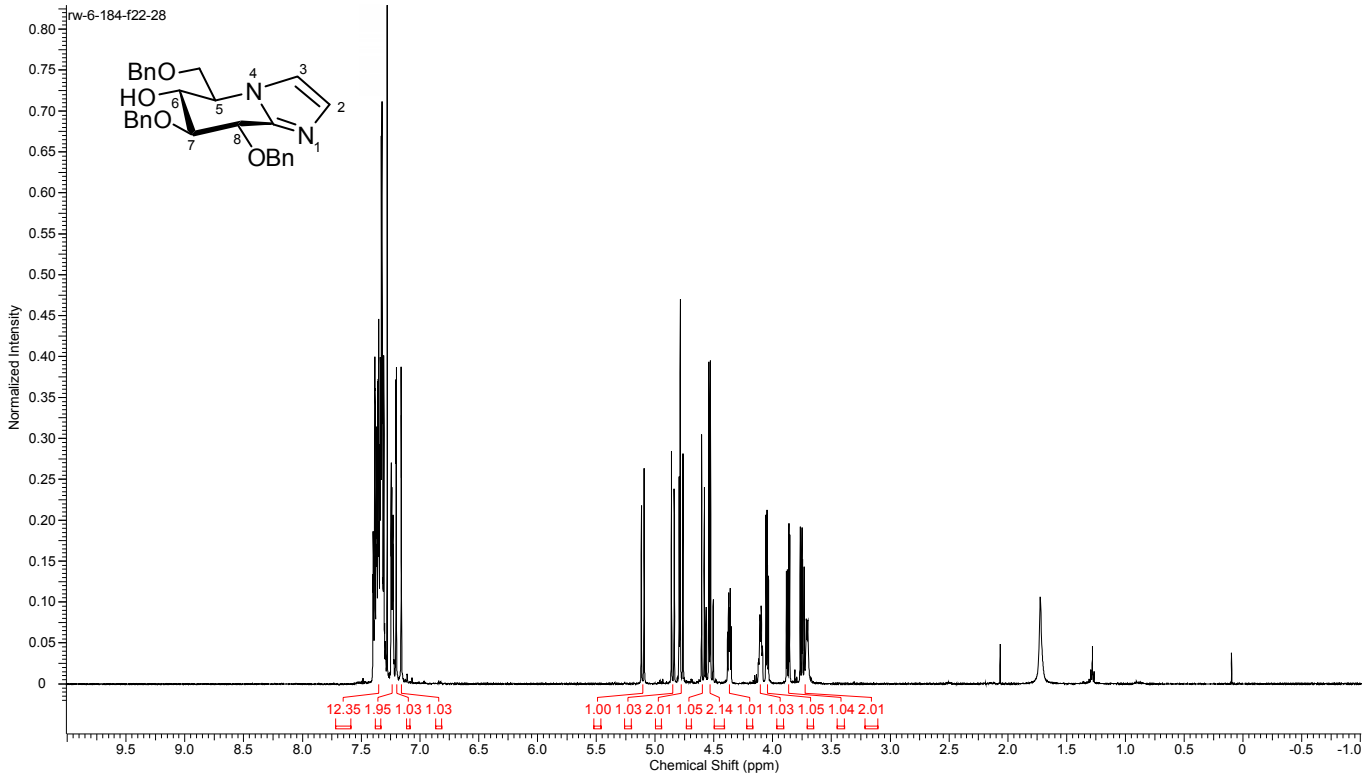


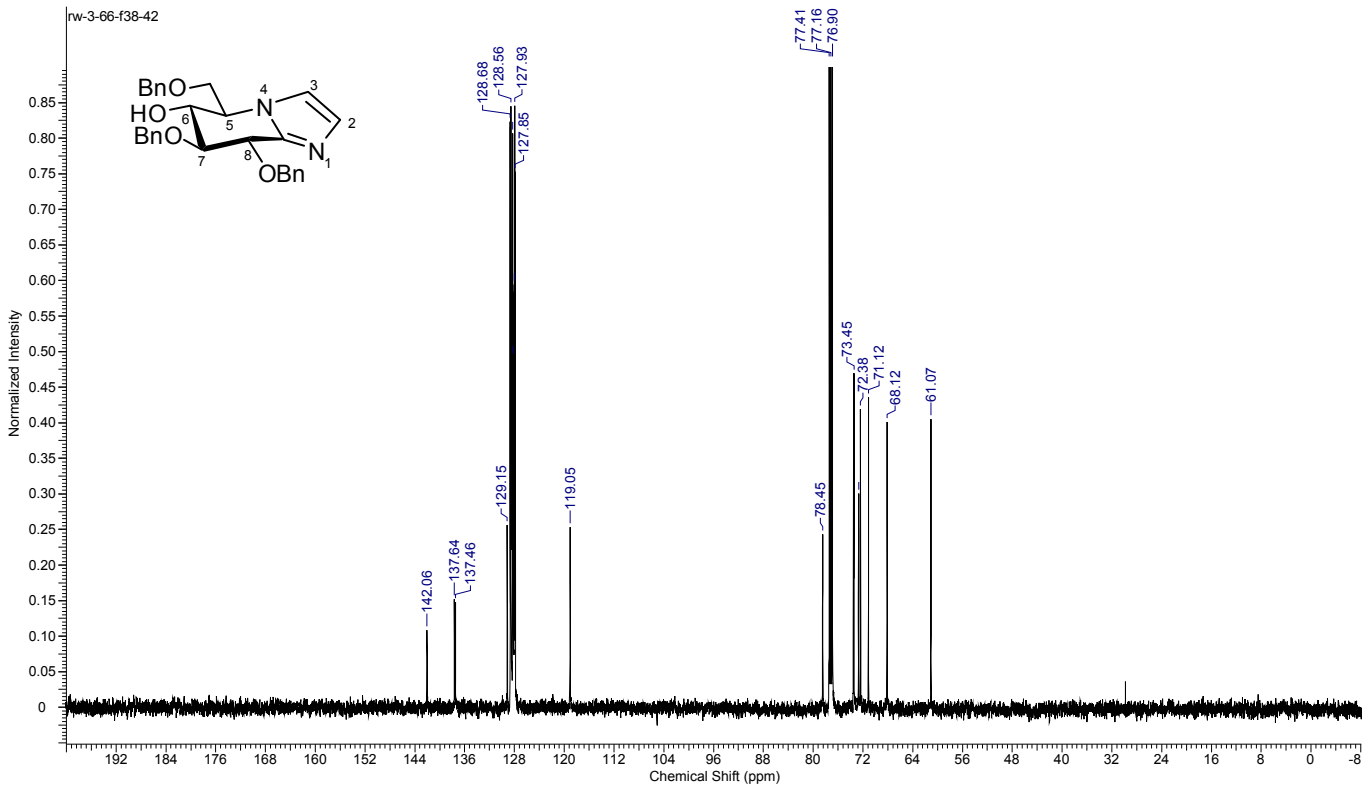



$^{1}$H-$^{1}$H COSY

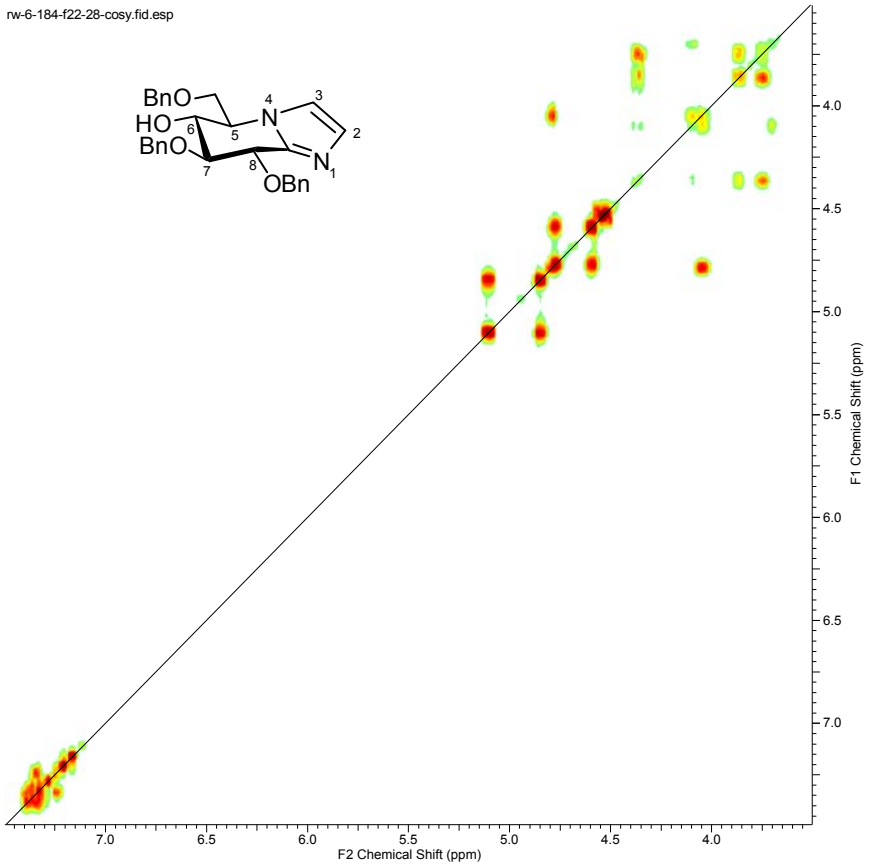

_Gcosy-01.fid.esp
BnO
HO
BnO
OBn
N
N
1
2
3
4
5
6
7
8
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

**(5*R*,6*R*,7*S*,8*S*)-7,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-6-hydroxy-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine**

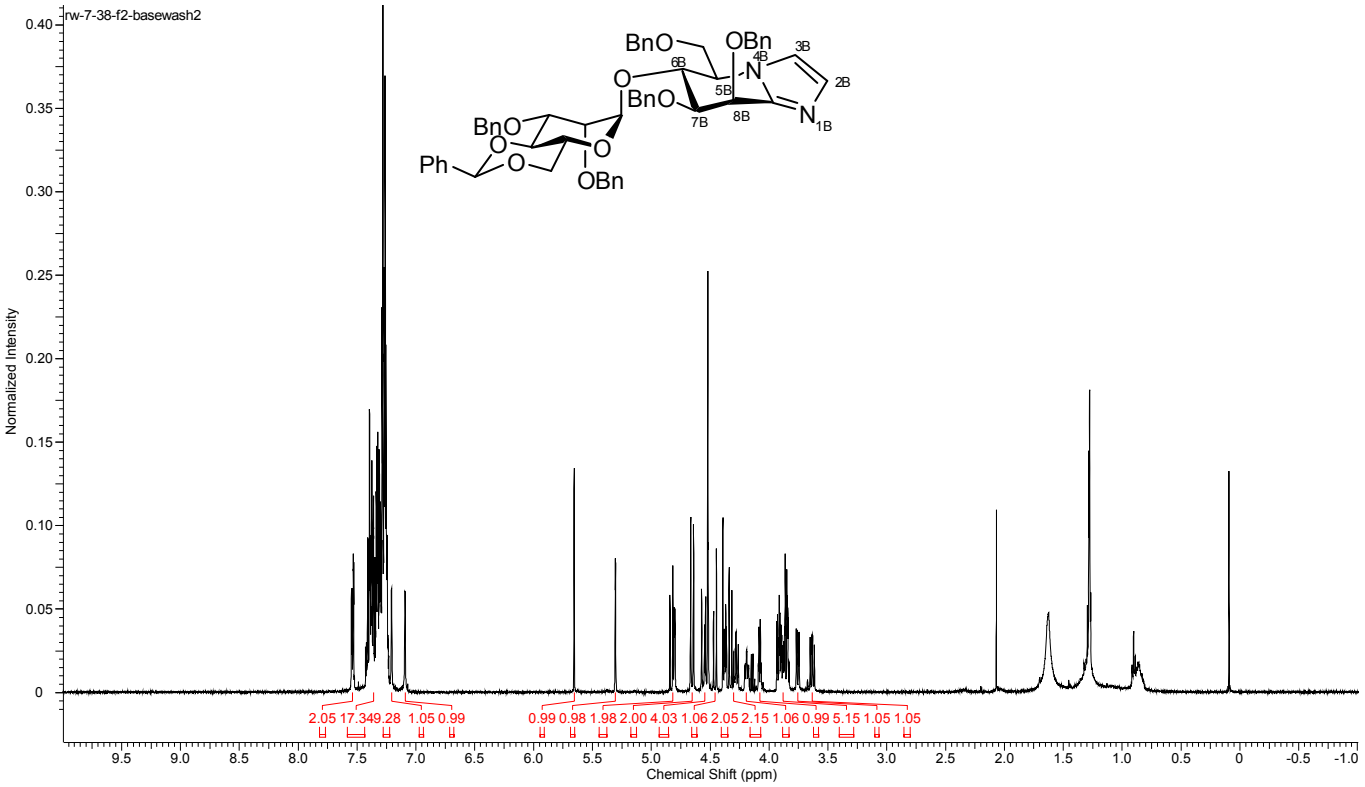


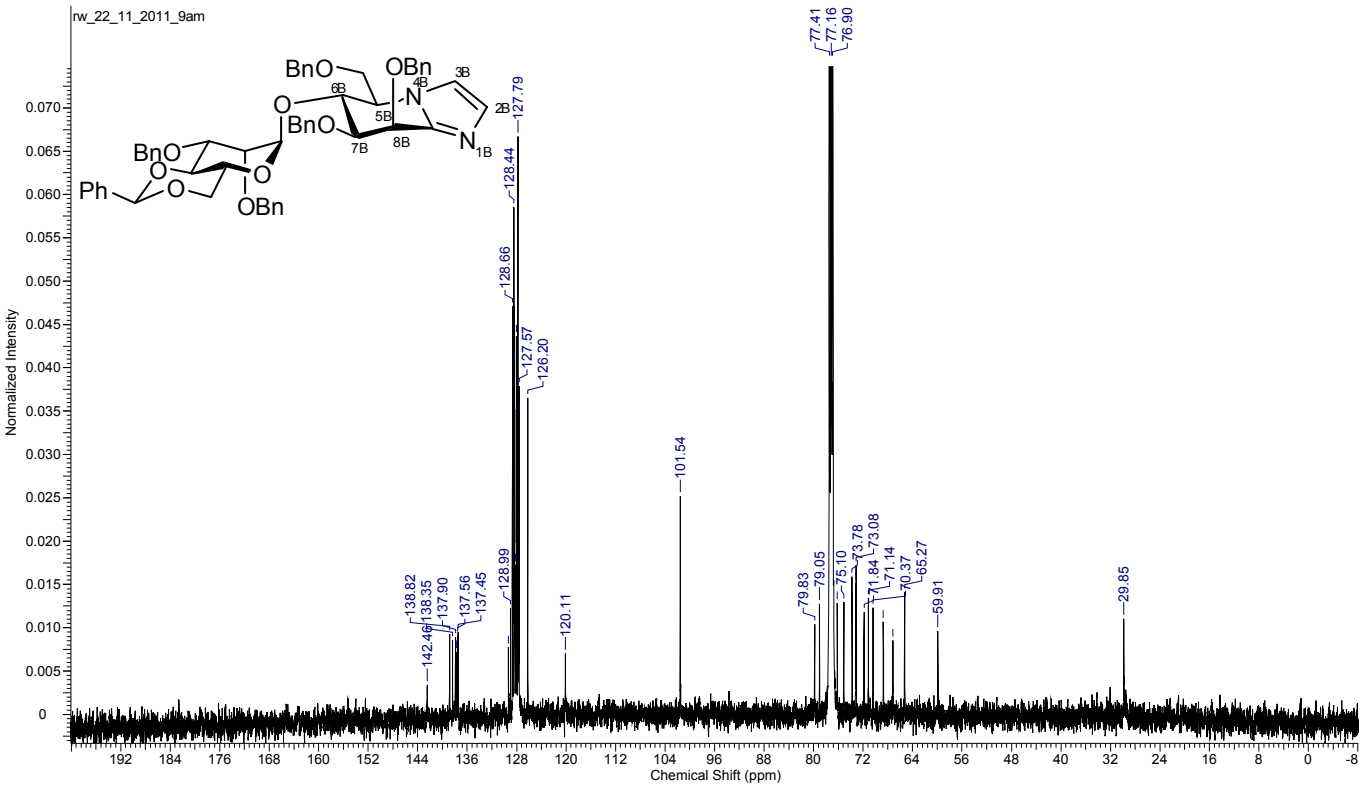



$^{1}H$-$^{1}H$ COSY

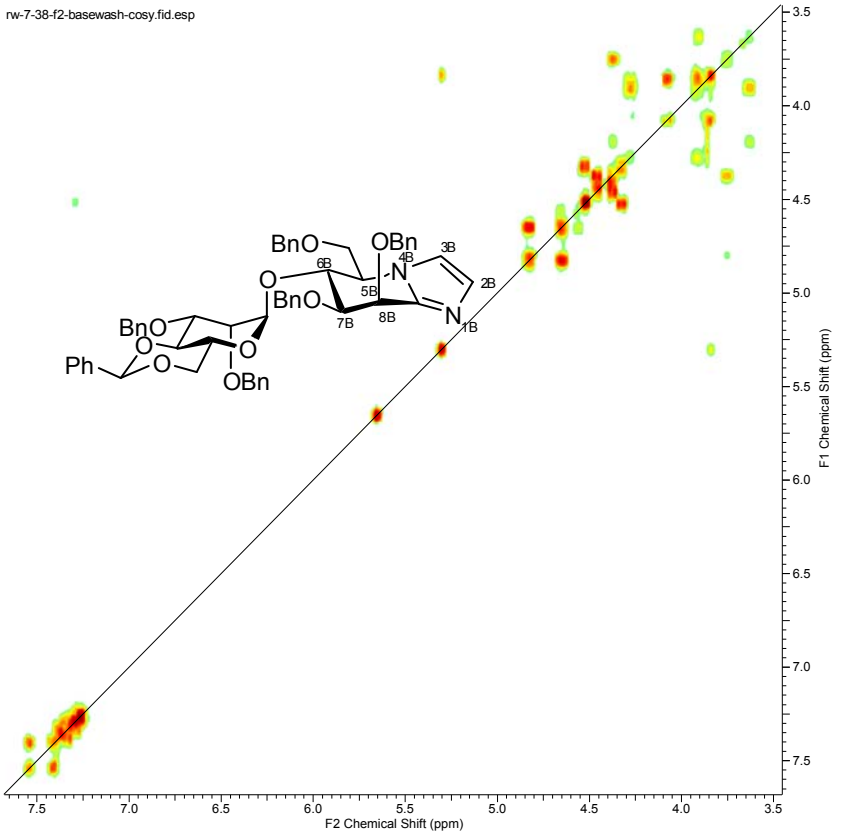

rw-6-184-f22-28-cosy.fid.esp
BnO
HO
BnO
OBn
N
N1
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)

**(5*R*,6*R*,7*S*,8*R*)-6-(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyloxy)-7,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine**

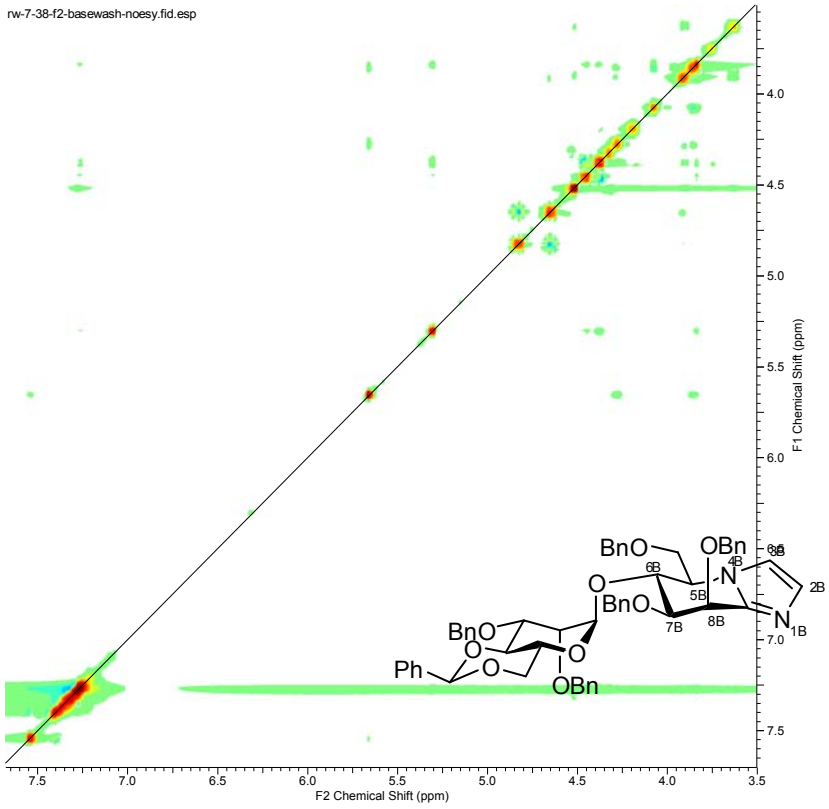


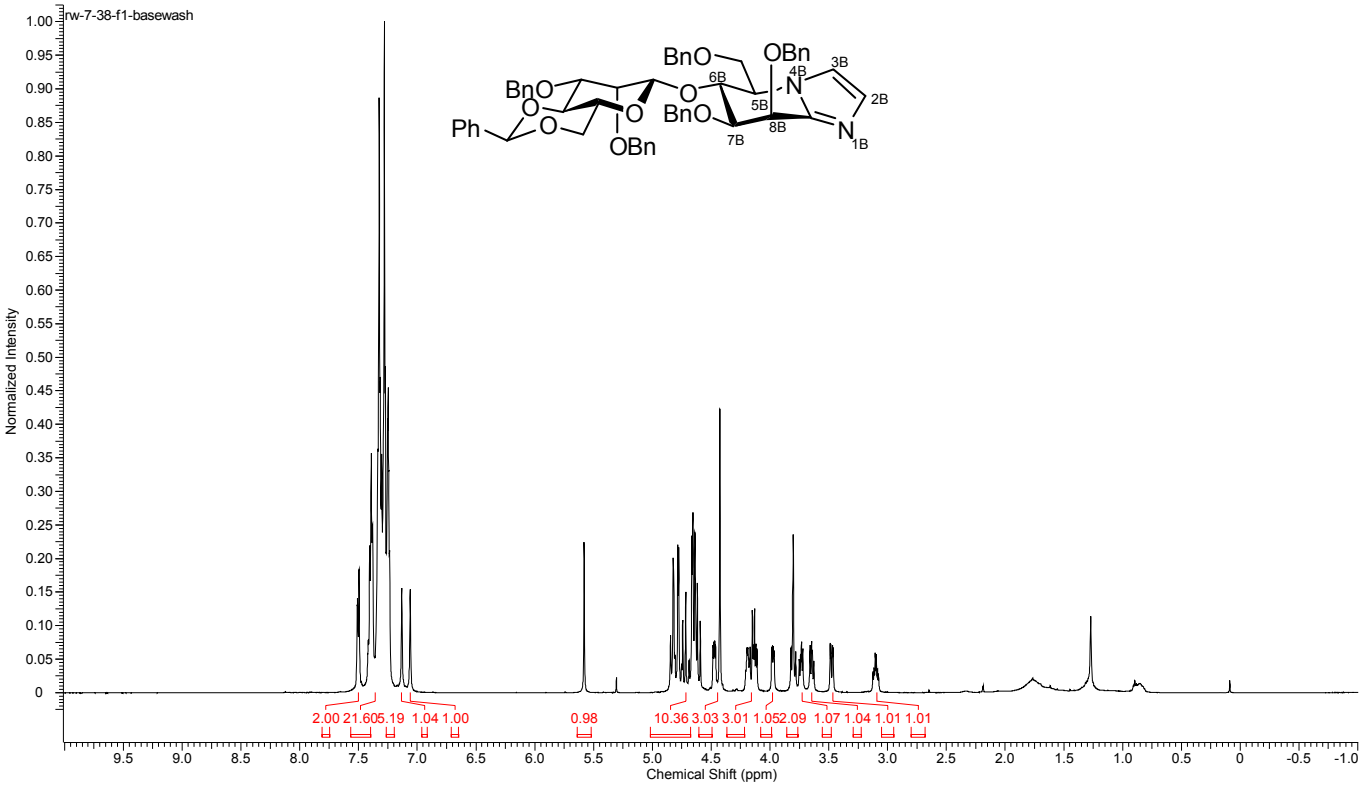
$^{1}$H-$^{1}$H COSY

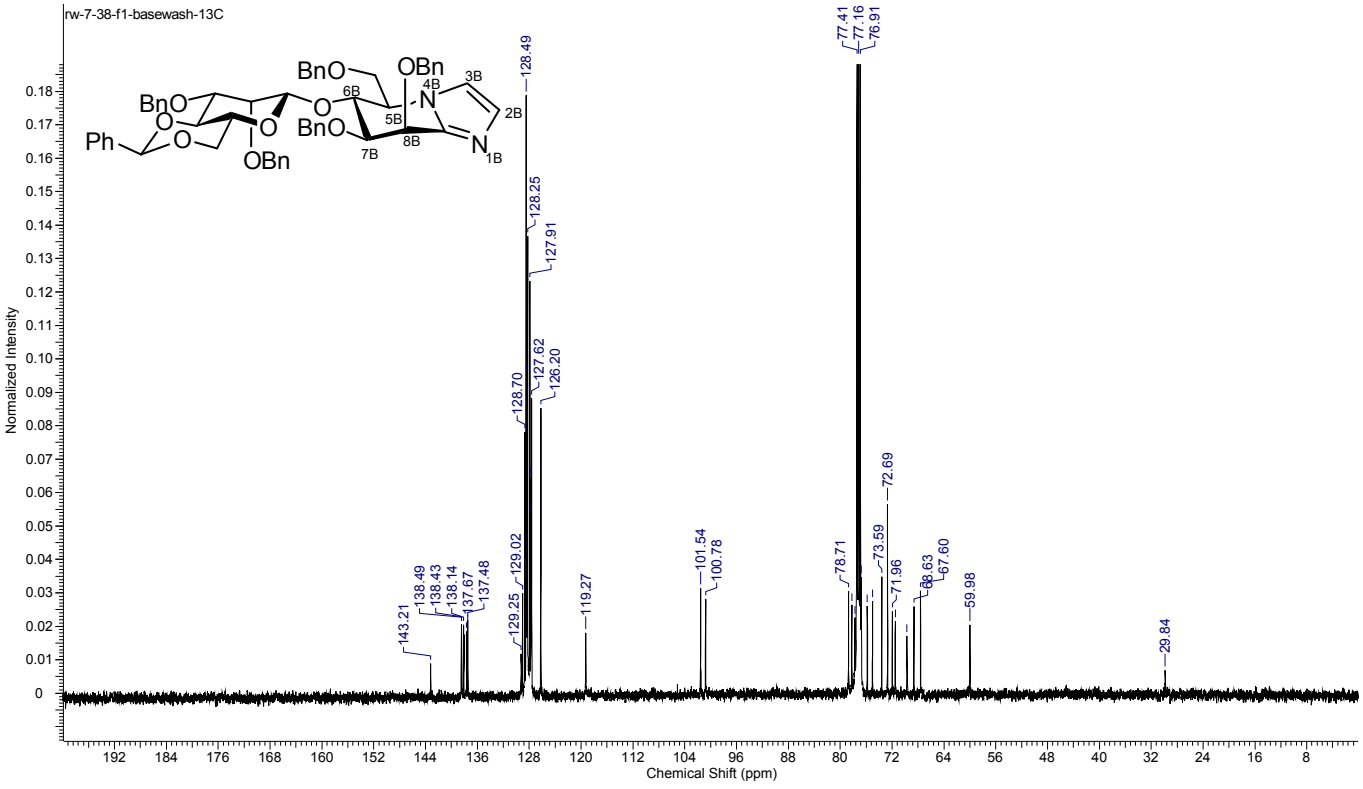



NOESY

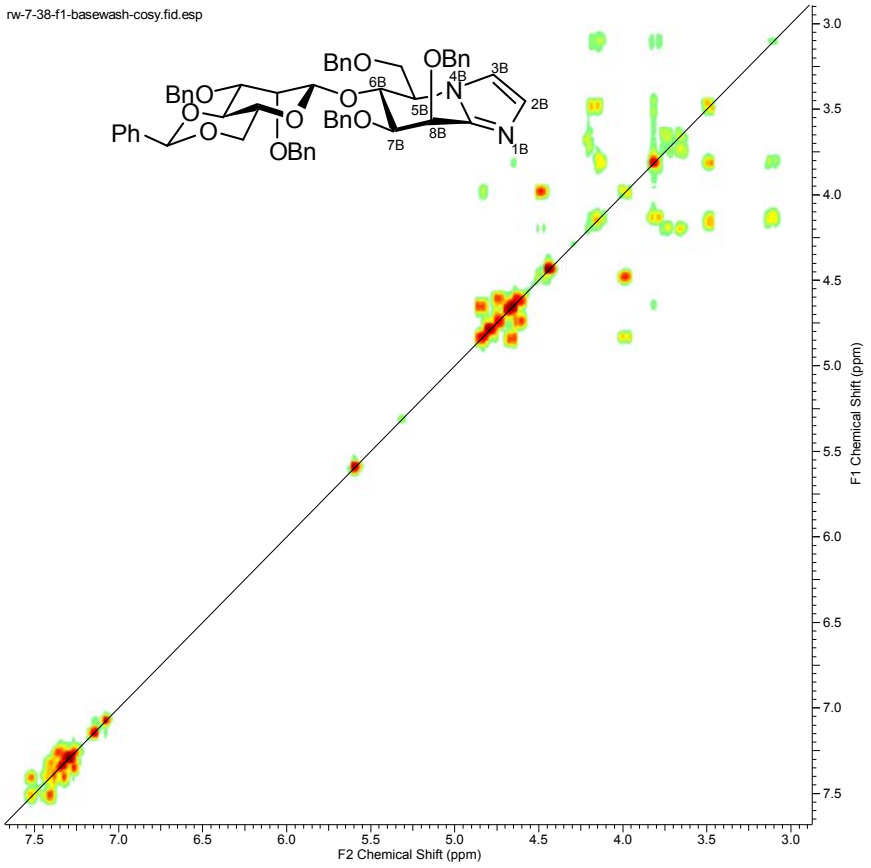


**(5*R*,6*R*,7*S*,8*R*)-6-(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyloxy)-7,8-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (17)**

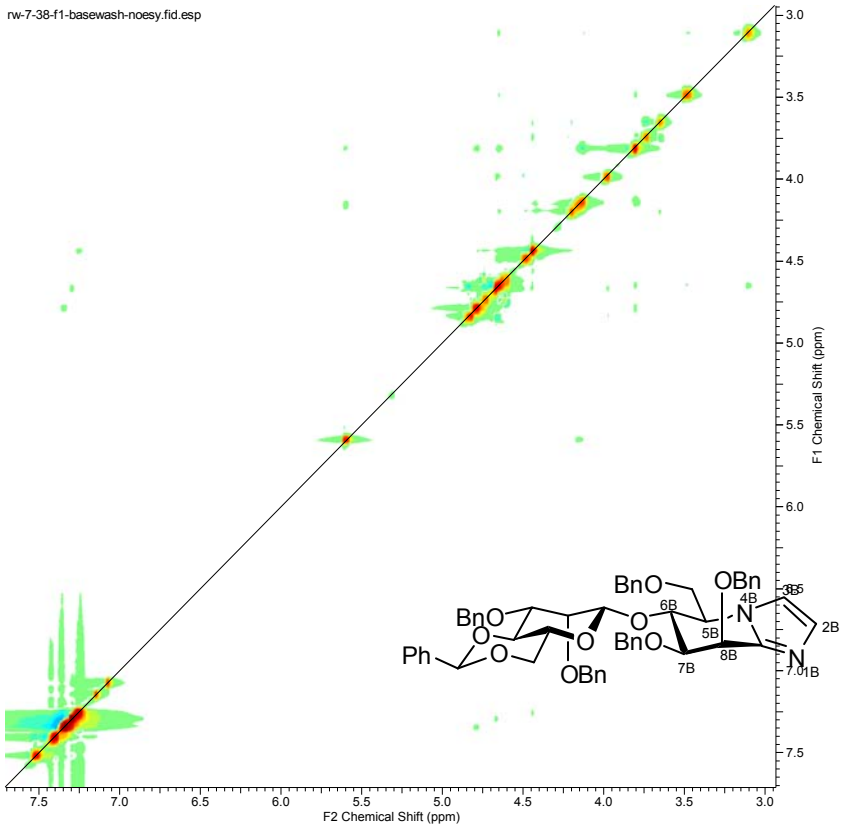


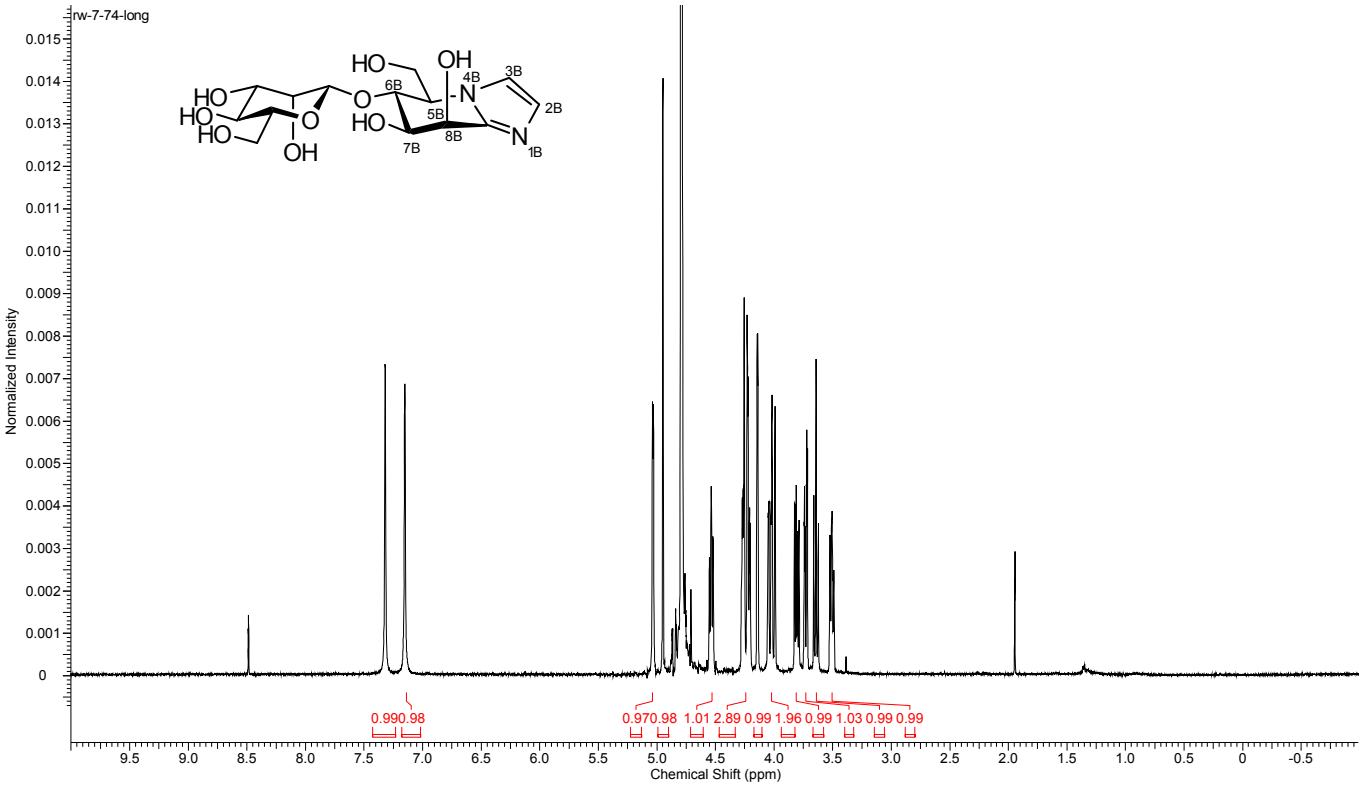
$^{1}$H-$^{1}$H COSY

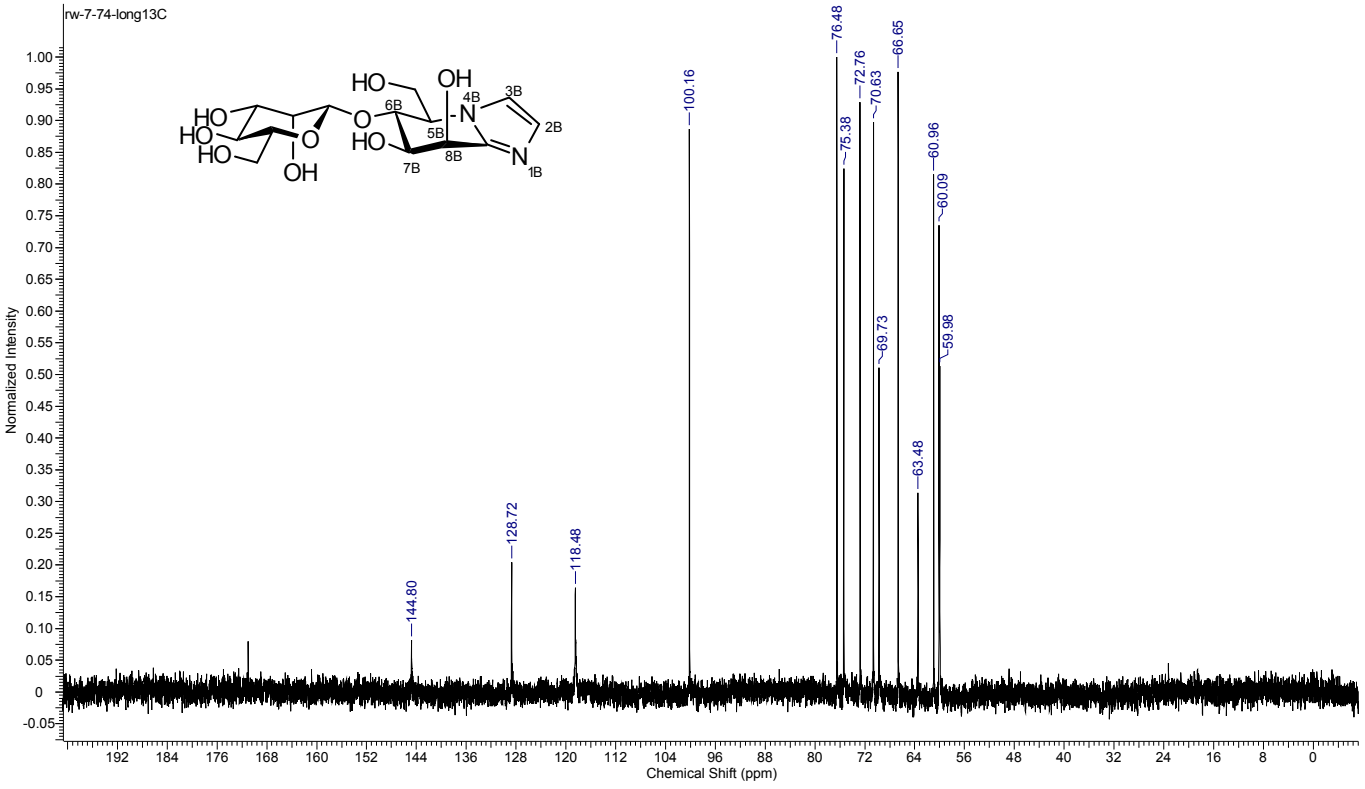



NOESY

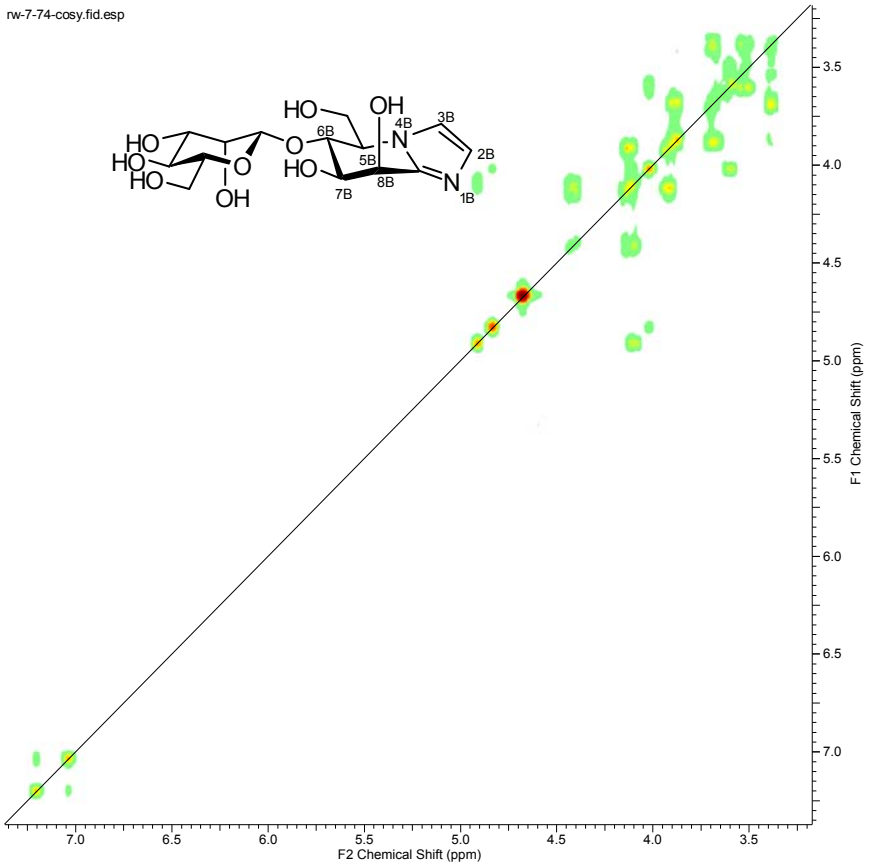


**(5*R*,6*R*,7*S*,8*R*)-7,8-Dihydroxy-5-[(hydroxy)methyl]-6-(β-D-mannopyranosyloxy)-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-7,8-diol (ManMIm; 4)**



$^{1}$H-$^{1}$H COSY


rw-7-74-cosy.fid.esp
HO
OH
6B
4B
3B
2B
5B
7B
8B
1B
HO
HO
HO
OH
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5