Optimized Conditions for the Palladium-Catalyzed Hydrogenolysis of Benzyl and Naphthylmethyl Ethers: Preventing Saturation of Aromatic Protecting Groups

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Abstract

Whilst carrying out palladium catalysed hydrogenolysis to deprotect synthetic oligosaccharides, saturation of the benzyl and naphthylmethyl ether groups to their corresponding ether was observed. In order to suppress this unwanted hydrogenation, we report a scalable practical approach using a catalyst pre-treatment strategy, which is effective under batch or continuous flow conditions. This suppressed the unwanted hydrogenation side-products and created a selective catalyst for hydrogenolysis of benzyl and naphthylmethyl ethers. We demonstrate the efficient deprotection of a set of structurally diverse oligosaccharides (5 examples, >73%).

Introduction

Organic synthesis of both natural and unnatural products requires the use of protecting groups, therefore great interest in the development of new protecting groups, and methods for their selective deprotection is sought.[1] Total synthesis of complex natural products is challenging, requiring multistep protecting group manipulations, with often only small quantities of pure synthetic structures available. This requires the development of high yielding, chemoselective deprotection methodologies of protecting groups. Hydroxyl groups are ubiquitous in natural products, e.g., in steroids, opoids, tetracyclins, prostaglandins, and carbohydrates.[2] Carbohydrates play key roles in biology, and access to pure synthetic glycans is an essential component to further our understanding of their roles in biology.[3–6] A popular protecting group for hydroxyl groups is the benzyl ether, in part due to their ease of formation, high stability to a variety of reaction conditions, and a variety of methods for deprotection.[1] One of the most common methods for deprotection of benzyl groups is metal-catalyzed hydrogenolysis. Palladium is often preferred over ruthenium or platinum due to its lower propensity to cause the saturation of aromatics.[7] Thus, the mild nature of palladium-catalyzed hydrogenolysis has led to its popularity in global deprotections of, e.g., peptides and oligosaccharides, even allowing for the presence of biologically interesting functionalization of hydroxyl group such as acetates (*Cryptococcus neoformans*) and cyclic phosphates (*Vibrio cholerae*).[8–12] Despite the wide spread use of catalytic palladium hydrogenolysis, deprotections can sometimes be mired with poor yields, and are difficult to optimize, due their late-stage in a total synthesis.

Results & Discussion

In some projects in our laboratory we encountered poor results in the global hydrogenolysis (de-benzylation) reactions including; long reaction times, poor yields, and loss of 6-*O*-acetylation pattern (*C. neoformans*) and fucose residues (Lewis b) in target structures. However, the primary problem encountered was the saturation of the

naphthylmethyl and benzyl ethers to their corresponding ether. This caused major difficulty in purification of the target structures from their saturated side-products, further reducing the yield of the deprotection step. In literature, we could find few examples of this unexpected reactivity of the palladium catalysts being described. Ley *et al.* found competitive hydrogenation of benzyl ethers in the synthesis of a high mannose type nonasaccharide but were unable to eliminate this side reaction and ultimately had to accept a 35% yield in the final step.[13] While Ellervik *et al.* described partial and total saturation of naphthoxylosides during hydrogenolysis in their efforts towards developing anti-tumor drugs, ultimately using solvent effects to tune the selectivity of the catalyst.[14]

In our lab, this unwanted hydrogenation was observed in projects based on the synthesis of the pellicle repeating unit of *Lactococcus lactis* (**10**) and the synthesises of the capsular polysaccharides of *Neisseria meningitidis* and *Cryptococcus neoformans* (**9**, **13**) (Figure 2, Supporting Information). It is worthy to note that the hydrogenation side-products occurred to varying extents on these synthetic structures. The oligosaccharides based on *C. neoformans* glucuronoxylomannan (GXM) and *Lactococcus lactis* pellicle repeating unit showed a higher propensity for this unwanted hydrogenation. In GXM related oligosaccharides the hydrogenation side-products began to occur on trisaccharide structures and was present on all larger structures (up to a hexadecasaccaride). Initial efforts to understand this side reaction were challenging, ^{1}H NMR showed peaks appearing as three 'humps' with the chemical shift of δ 0.8 – 1.9 ppm (Figure 1). The peaks observed were broad and poorly defined, suggesting a heterogeneous nature of the side-products. Mass spectrometry analysis was diagnostic, confirming the presence of structures that were +96 and +150 *m/z*, which corresponded to the saturation of benzyl (+96) and naphthylmethyl (+150) ethers to the corresponding saturated side-products. Using size-exclusion chromatography it was possible to separate the saturated naphthylmethyl side-product from a combined fraction of the desired compound and cyclohexyl ether side-product. However, separation of the cyclohexyl and desired product was not possible under these conditions or others attempted (C_{18} column).

In order to facilitate faster, higher yielding and more selective global deprotections we sought first to optimize the de-benzylation reactions on *C. neoformans* GXM based oligosaccharides, and then hoped to demonstrate the wider utility of our strategy on multiple oligosaccharide substrates. Model compound hexasaccharide **8**, was chosen as:

1) It contained two acetyl groups which stability we could monitor, to ensure our methodology is amenable to acetyl groups.

2) Compound **8** contained all the carbohydrate residues present in the repeating motifs of the *C. neoformans*: glucuronic acid, xylose and mannose so we could ensure that the saturation was not 'saccharide-dependent'.

3) We also hoped that this branched hexasaccharide **8** would likely assume similar conformations in solution to that of the larger structures.[8,15] This was attractive so that on larger oligosaccharides, no conformational related issues arise due to sterically hindered access to certain benzyl groups.

Identification of high-quality commercial catalyst

In order to optimize our hydrogenolysis reaction we first sought to identify a high-quality commercial catalyst. Initial screening studies in our attempt to find an efficient catalyst focused on screening of different catalyst types: palladium on activated carbon (Pd/C), pallidum hydroxide [$Pd(OH)_2$], Degussa catalyst and

palladium black from different suppliers: Sigma-Aldrich, AlfaAesar and Strem chemicals. We found all catalysts resulted in a degree of hydrogenation of the benzyl and naphthylmethyl groups but varying isolated yields and reaction times were observed (Table 1). The most efficient catalyst identified was a 5% Pd/C (50-70% wetted powder) Evonik Noblyst® from Strem Chemicals (P1090), which gave the greatest level of recovered material, lowest levels of hydrogenation and the shortest reaction times (Table 1). While the Evonik Noblyst® gave low levels of saturation impurities, it was still difficult to separate these compounds. Thus, we further sought to optimize the reaction to totally inhibit the presence of the saturation side-products. Next, we investigated carrying out the reaction in a continuous flow system, hypothesizing it would lead to greater activity and selectivity of the palladium catalyst. Using a commercially available flow reactor (H-Cube®, Thales nano) and two different catalysts cartridges (Pd/C and $[Pd(OH)_2]$) we found reaction times were greatly reduced and isolated yields were high (68-88%).

Notably, the saturation side-products were still observed under continuous flow conditions, accounting for on average 25% of the product of the reaction (integrated by ^{1}H NMR). Furthermore, great variability in the activity of different catalyst cartridges from the same supplier (Sigma-Aldrich) was observed, this ultimately affected the reproducibility of the reaction times and yields, overall making the H-Cube® unattractive for the deprotection of target structures. Next, we explored the role of pressure in the side-product formation by varying the pressure of hydrogen in the hydrogenolysis reaction by using a balloon or a high pressure reactor (10, 20, 40 bar), finding no significant difference in the level of saturation side-products but increased pressure led to faster reaction times. Changing the source of hydrogen from hydrogen gas to ammonia formate (i.e. hydrogen transfer reaction) led to no saturation of aromatic groups, as reported by Kozioł *et al.* (Table 2, entry 3).[16] However, these conditions also led to loss of the acetylation pattern on our substrate, making this methodology not suitable for *C. neoformans* based oligosaccharides.

Solvent Screening

Inspired by the work of Morooka *et al.* and Ellervik *et al.*,[14,17] we explored the role of solvents in influencing palladiums selectivity, with an emphasis on our desire to eliminate the saturation of the benzyl and naphthylmethyl groups. Aqueous solvent mixtures containing tetrahydrofuran, acetone, and *N,N*-dimethylformamide (DMF) mixtures all reduced the level of saturation compared to ethyl acetate and 1,4-dioxane aqueous mixtures (organic:aqueous, 80:20, v/v). Ethyl acetate containing mixtures also led to the formation of large quantities of an insoluble material, which complicated the purification and made the recovery of product challenging. To attempt to improve these results we 'pre-conditioned' the catalyst (stirring with hydrogen and acid without substrate). We reasoned that a pre-conditioned catalyst could improve the selectivity of the reaction by tuning the catalyst to the 'correct' reactivity prior to being exposed to our substrate.[14] Pre-conditioned aqueous mixtures of tetrahydrofuran and acetone still led to quantities of the saturated ethers (confirmed by ^{1}H NMR). Acetone containing solvent systems also had to be abandoned due to the reaction of the amino group of **8** with the carbonyl carbon of the acetone. Pleasingly, the pre-conditioned aqueous DMF mixture gave no saturated side-products (as detected through ^{1}H NMR or MS). Therefore, our optimized procedure going forward used a preconditioned DMF aqueous mixture.

Catalyst pre-treatment strategy

Initially, we presumed the aqueous DMF solvent system suppressed the formation of saturation side-products due to solvent effects, e.g., intermolecular interactions or dielectric constants.[14,17,18] However, conflicting observations including the requirement of stoichiometric quantities of catalyst *in batch*, the loss of our acetylation pattern when samples were stored in crude mixtures, and the loss of activity of catalyst-cartridges *in flow* led us to investigate further. In literature, it is known that in acidic or basic aqueous systems DMF can undergo a gradual decomposition to dimethylamine and carbon monoxide.[19] The presence of amines in the hydrogenolysis reaction could explain the loss of activity of the catalyst overtime, while also explaining the increased 'selectivity' towards the hydrogenolysis reaction rather than hydrogenation (due to amine poisoning). To test this hypothesis, we performed an amine catalyst treatment using DMF as an *in situ* source of dimethylamine. This was achieved by stirring the Pd/C catalyst in an acidic aqueous DMF mixture for 15 minutes followed by filtration to recover the catalyst. The presence of dimethylamine was confirmed by ninhydrin staining, IR-spectroscopy, and seen as an adduct to target compounds in mass spectrometry. This pre-treated palladium on carbon catalyst was then tested on our model hexasaccharide **8** and was found to be a highly selective catalyst for the hydrogenolysis of benzyl and naphthylmethyl ethers (isolated yield 80%), with no saturation of aromatic protecting groups observed. Our model conditions used THF and ^{t}BuOH, and a buffered aqueous component (PBS, 100 mM, pH 5) to ensure the stability of the acetylation pattern upon deprotection. We then performed a substrate scope in order to test the generality of our findings on a set of five structurally diverse synthetic oligosaccharides (Scheme 2 and Table 3). Using the combination of our catalyst treatment strategy and the optimal palladium on carbon catalyst we experienced short reaction times (0.5-2 days) and high yields (73-86%) and most importantly no saturation of benzyl or naphthylmethyl ethers (Table 3, Entries 1-5). To further test the generality of our methodology for 'tuning' the palladium's selectivity towards hydrogenolysis, we performed the pre-treatment strategy on both a 10% Pd/C – flow cartridge and a 10% Pd/C Sigma-Aldrich catalyst, the latter of which was previously shown to led the highest quantity of saturation observed (Table 1, Entry 1). Using our pre-treatment strategy, we observed no saturation of the benzyl and naphthylmethyl protecting groups, demonstrating the generality of this approach under either batch or continuous flow conditions (Table 3, Entry 6 and 7)

In conclusion, a complementary approach of identifying both a high-quality source of palladium catalyst and using a pre-treated catalyst was effective to deprotect a variety of synthetic oligosaccharides. 'Tuning' of the palladium catalyst was found to inhibit saturation of benzyl and naphthylmethyl ethers and allowed the efficient deprotection of oligosaccharides, overall leading to a selective catalyst under hydrogenolysis conditions.

Experimental section

General methods

Unless otherwise noted all reactions containing air- and moisture-sensitive reagents were carried out under an inert atmosphere of nitrogen in oven-dried glassware with magnetic stirring. N_2-flushed stainless cannulas or plastic syringes were used to transfer air- and moisture-sensitive reagents. All reactions were monitored by thin-

layer chromatography (TLC) on Merck DC-Alufolien plates precoated with silica gel 60 F254. Visualisation was performed with UV-light (254 nm) fluorescence quenching, and/or by staining with an 8% H_2SO_4 dip (stock solution: 8 mL conc. H_2SO_4, 92 mL EtOH), and/or ninhydrin dip (stock solution: 0.3 g ninhydrin, 3 mL AcOH, 100 mL EtOH). Evaporation *in vacuo*/under vacuum refers to the removal at 40 ° C, unless otherwise stated, of volatiles on a Buchi rotary evaporator with integrated vacuum pump.

Chromatography

Silica gel flash chromatography was carried out using *Davisil LC60A* (40-63 µm) silica gel or with automated flash chromatography systems, Buchi Reveleris® X2 (UV 200-500 nm and ELSD detection, Reveleris® silica cartiges 40 µm, BÜCHI Labortechnik AG) and Biotage® SP4 HPFC (UV 200-500 nm, Biotage® SNAP KP-Sil 50 µm irregular silica, Biotage AB). Size-exclusion chromatography was performed on Bio-Gel® P-2 (Bio-Rad Laboratories Inc.) using isocratic elution (H_2O:^{t}BuOH, 99:1, v/v). Instrumentation: peristaltic pump P-3 (Pharmacia Fine Chemicals), refractive index detector Iota 2 (Precision Instruments), PrepFC fraction collector (Gilson Inc.). Software: Trilution® LC (version 1.4, Gilson Inc.). Standard parameters for the purification of deprotected carbohydrates were 64 $\cdot 10^{-5}$ ΔR_i, speed of peristaltic pump: 3 to 4.5 rpm, fraction collection: 120 fractions over 1200 min. Reversed phase chromatography was performed on silica gel 100 C_{18}-reversed phase fully end-capped purchased by Fluka.

Materials

All chemicals for the synthesis were purchased from commercial suppliers (Acros, Carbosynth Ltd, Fisher Scientific Ltd, A/S, Merck, Sigma-Aldrich, VWR, Strem Chemicals and AlfaAesar) and used without purification. Dry Solvents were obtained from a PureSolv-EN^{TM} solvent purification system (Innovative Technology Inc.). All other anhydrous solvents were used as purchased from Sigma-Aldrich in AcroSeal® bottles.

Instrumentation

^{1}H NMR (400 or 500 MHz), ^{13}C NMR (101 MHZ or 125 MHz), spectra were recorded on Varian-inova spectrometers at 25 °C in chloroform-d1 ($CDCl_3$), methanol-d4 (CD_3OD), water-d2 (D_2O). ^{1}H NMR spectra were standardized against the residual solvent peak ($CDCl_3$, δ = 7.26 ppm; CD_3OD, δ = 3.31 ppm; D_2O, δ = 4.79 ppm; or internal trimethylsilane, δ = 0.00 ppm). ^{13}C NMR spectra were standardized against the residual solvent peak ($CDCl_3$, δ = 77.16 ppm). All ^{13}C NMR are ^{1}H decoupled. ^{13}C chemical shifts were reported with one digit after the decimal point, unless an additional digit was reported to distinguish overlapping peaks. Software for data processing: MestReNova, version 11.0.0-17609 (MestReLab Research S.L.). High-resolution mass spectrometry (HRMS) data were recorded on a Waters micromass LCT LC-Tof instrument using electrospray ionisation (ESI) in either positive or negative mode. Low-resolution mass spectrometry (LRMS) experiments were recorded on a Waters micromass Quattro Micro LC-MS/MS instrument using electrospray ionisation (ESI) in either positive or negative mode. Optical rotations were recorded on Perkin-Elmer polarimeter (Model 343) at the sodium D-line (589 nm) at 20 °C using a 1 dm cell. Samples were prepared at the concentration (g/mL) in the

solvent indicated. Deprotected sugars were lyophilised using a freeze-dryer Alpha 1-2 LDplus (Christ Ltd). Pressure: 0.035 mbar; ice condenser temperature: - 55 °C.

Procedure for Catalyst Pre-treatment

500 mg Pd/C, was suspended in 1 mL DMF:H_2O mixture (80:20 v/v), and the solution was made acidic by the addition of 200 µL HCl (ACS Reagent, 37%, pH 2-3), with or without an atmosphere of hydrogen gas for ~20 minutes. The presence of dimethylamine was confirmed via ninhydrin staining. The treated Pd/C catalysts was re-isolated though filtration. The moistened catalyst was then be used directly in the hydrogenolysis reaction.

Synthesis of Model Hexasaccharide

Synthesis of the oligosaccharide **8**, used acceptor **1** and donors **2** and **7**, they were synthesized using published methods[9,20,21]. Briefly, disaccharide building blocks were coupled using thiophilic promotor dimethyl(methylthio)-sulfonium-trifluoromethansulfonate (DMTST) to yield tetrasaccharide **3** in 80% yield.[22] Selective removal of the 2-naphthyl methyl (NAP) ether was complete in an 85% yield to give **4**. Buffering of the aqueous phase with phosphate buffered saline helped to reduce the acidic cleavage of the benzyl ethers. Next acceptor **4** was coupled using glucuronic acid containing disaccharide **7** to complete the synthesis of the model compound hexasaccharide **8** in a 65% yield (Scheme 1).

DMTST Mediated Glycosylation[22]

A mixture of thioglycoside donor (1.5eq), acceptor (1eq) and crushed 4 Å molecular sieves in dry Et_2O (0.01M) was stirred at 20°C for 60 min. The reaction mixture was cooled to 0°C, DMTST (3eq) was added, and the reaction mixture was stirred at 0°C for 60 min. The reaction was allowed to rise to room temperature. If required, the reaction was pushed to completion by an additional amount of DMTST (3eq). Stirring was continued until the reaction was complete (ca. 4-8 h). The reaction quenched with Et_3N at 0°C. The solution was filtered through a pad of Celite®, and the filtrate was concentrated *in vacuo*. Purification by flash column chromatography.

2-Naphthylmethyl Removal

DDQ (2 eq) was added to a vigorously stirred solution of compound (1eq) in CH_2Cl_2/PBS (100mM, pH 7.5) (0.02M, 85:15) at 5-10°C, in the dark. The progress of the reaction was carefully monitored by TLC, and quenched upon completion, by adding 10% aq. $Na_2S_2O_3$-solution and $NaHCO_3$ (1:1, v/v). The resulting mixture was extracted CH_2Cl_2, and the organic layer was washed sequentially with sat. $NaHCO_3$-solution, brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography.

General Procedure for Hydrogenolysis Batch Reaction

The treated catalyst (0.2-0.5 eq. per benzyl group) was added to a solution of oligosaccharide (1eq) dissolved in THF:*tert*-butyl alcohol:PBS (100 mM, pH 4) (60:10:30, v/v/v). The reaction was placed in a high pressure reactor at 10 bar and was monitored *via* normal phase TLC (MeCN:H_2O mixtures). Once complete the reaction mixture was filtered through a plug of Ceilte® and then concentrated in vacuo. The residue was then re-dissolved in sterile water and purified with a Bio-gel P2 Column or a Sep-Pak C18 cartridge to yield the desired product.

General Procedure for Hydrogenolysis Using the H-Cube®

A solution of substrate was dissolved to the concentration of 1 mg/mL in an RBF flask and passed through an H-Cube® in ‘full mode’ using a HPLC pump (flow rate 0.4 ml/min). The solution was passed continuously through the H-Cube® until complete (monitored via MALDI-TOF and TLC).

Compound Characterization

2-Azidoethyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(1→2)-6-*O*-acetyl-4-*O*-benzyl-3-*O*-(2-naphthylmethyl)-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→2)]-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl-(1→2)]-6-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranoside (8)

R_f (toluene-EtOAc, 9:1) = 0.25; $[\alpha]_D^{20} = -23.8$ (*c* 1.73, $CHCl_3$). **^{1}H NMR** (600 MHz, $CDCl_3$) *δ* 7.74-7.66 (m, 4H), 7.46-7.05 (m, 73H), 5.24 (d ≈ s, 1H), 5.16 (d ≈ s, 1H), 5.11-5.01 (m, 5H), 4.96 (d, 1H, J_{gem} = 10.8 Hz), 4.88 (d, 1H, J_{gem} = 11.4 Hz), 4.83-4.75 (m, 6H), 4.71-4.59 (m, 5H), 4.56-4.50 (m, 3H), 4.48-4.35 (m, 8H), 4.30-4.26 (m, 4H), 4.23-4.19 (m, 4H), 4.17-4.09 (m, 5H), 3.98-3.89 (m, 7H), 3.85-3.82 (m, 1H), 3.78-3.72 (m, 4H), 3.66 (dd ≈ d, 1H, *J* = 9.0 Hz), 3.61 (dd, 1H, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 10.8 Hz), 3.55-3.48 (m, 3H), 3.46-3.42 (m, 2H), 3.34-3.21 (m, 6H), 3.17 (dd ≈ t, 1H, *J* = 9.0 Hz), 3.06 (dd ≈ t, 1H, *J* = 10.8 Hz), 2.65-2.62 (m, 1H), 1.86 (s, 3H), 1.61 (s, 3H); **^{13}C NMR** (150 MHz, $CDCl_3$) *δ* 170.6, 168.0, 138.94, 138.92, 138.86, 138.8, 138.5, 138.42, 138.40, 138.3, 138.29, 138.1, 138.0, 135.5, 135.0, 133.2, 133.0, 129.2, 128.7, 128.72, 128.70, 128.6, 128.5, 128.4, 128.3, 128.28, 128.27, 128.25, 128.21, 128.20, 128.18, 128.15, 128.0, 127.96, 127.90, 127.8, 127.79, 127.7, 127.67, 127.66, 127.63, 127.58, 127.53, 127.48, 127.44, 127.40, 127.3, 126.9, 126.7, 125.9, 125.8, 104.3, 103.3, 103.0, 101.1, 99.5, 98.3, 83.8, 83.3, 83.3, 81.4, 81.3, 81.1, 79.4, 78.9, 78.7, 78.3, 77.9, 77.7, 77.6, 76.5, 75.4, 75.3, 75.2, 75.0, 74.9, 74.8, 74.6, 74.4, 74.3, 73.9, 73.7, 73.5, 73.0, 72.3, 71.7, 71.6, 70.4, 69.8, 69.6, 67.2, 66.6, 63.5, 63.3, 63.1, 63.0, 50.2, 20.7, 20.5. **Anal.** Calcd for $C_{149}H_{155}N_3O_{32}$: C, 71.59; H, 6.25; N, 1.68. Found: C, 71.23; H, 6.28; N, 1.72 %.

2-Aminoethyl [(β-D-glucopyranosyluronic acid)-(1→2)-6-*O*-acetyl-α-D-mannopyranosyl]-(1→3)-[(β-D-xylopyranosyl)-(1→2)-α-mannopyranosyl]-(1→3)-(β -D-xylopyranosyl)-(1→2)-6-*O*-acetyl-α-D-mannopyranoside (9)

R_f (MeCN:H_2O, 80:20) = 0.25 **^{1}H NMR** (400 MHz, Deuterium Oxide) δ 5.14 (s, 1H), 5.09 (s, 1H), 4.85 (d, J = 1.6 Hz, 1H), 4.39 – 3.05 (m, 39H), 2.07 (s, 3H), 2.02 (s, 3H). **^{13}C NMR** (151 MHz, Deuterium Oxide, as per HSQC) δ 100.0, 100.5, 98.1, 101.8, 103.5, 63.4, 103.4, 62.9, 77.7, 77.9, 70.5, 78.2, 74.7, 76.1, 73.2, 66.6, 70.0, 60.4, 66.3, 63.8, 75.9, 69.2, 71.7, 75.5, 75.7, 72.6, 72.6, 39.0, 65.2, 20.5, 20.4. **HRMS (ESI)** $[M + H]^+$ m/z Calc. for $C_{40}H_{66}NO_{32}$ 1072.3568 Found: 1072.3534.

2-Aminoethyl [(β-D-xylopyranosyl)-(1→2)-α-mannopyranosyl]-(1→3)-(β-D-xylopyranosyl)-(1→2)-6-*O*-acetyl-α-D-mannopyranoside (13)

^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.24 (s, 1H), 5.00 (s, 1H), 4.47 – 4.36 (m, 4H), 4.23 – 4.14 (m, 2H), 4.12 – 3.60 (m, 21H), 3.49 – 3.09 (m, 11H), 2.16 (s, 3H) **^{13}C NMR** (151 MHz, Deuterium Oxide, as per HSQC) δ 100.0, 100.5, 98.1, 101.8, 103.5, 63.4, 103.4, 62.9, 77.7, 77.9, 70.5, 78.2, 74.7, 76.1, 73.2, 66.6, 70.0, 60.4, 66.3, 63.8, 75.9, 69.2, 71.7, 75.5, 75.7, 72.6, 72.6, 39.0, 65.2, 20.5, 20.4. **HRMS** (ESI) $[M + H]^+$ m/z Calcd for $C_{26}H_{46}NO_{20}$ 692.2613 Found: 692.2615.

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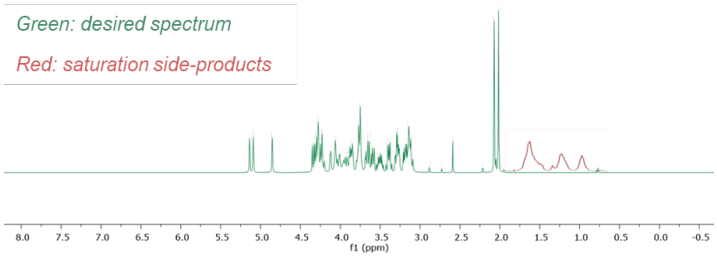
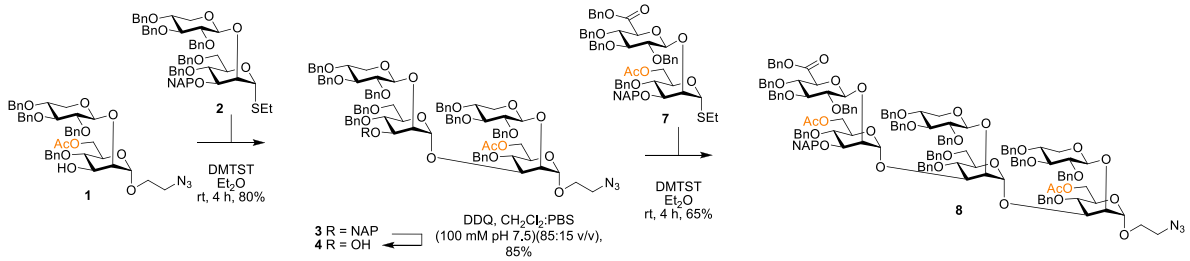


Figure 1. Representation of ^{1}H NMR of model hexasaccharide 1, containing side-products.



Scheme 1. Synthesis of Model Hexasaccharide 8.

Entry	Palladium Catalyst	Reaction time (days)	Saturation side-products (%)[a]	Total yield (%)[b]
1	10% Pd/C	4+	53	65
2	[Pd(OH)2]	2	39	59
3	Palladium Black	2	64	50
4	5% Pd/C Evonik Noblyst®	1	10	88
5	10% Pd/C Degussa	2	33	64
6	[Pd(OH)2] – Flow cartridge	0.5	26	68
7	10% Pd/C – Flow cartridge	0.5	24	88

Table 1. Screening of Different Palladium Catalysts Using Model Compound 8. Reactions were all carried out in the solvent system ethyl acetate:methanol:water:acetic acid (4:1:1:1) v/v/v/v, 10bar, ambient temperature to allow comparison. [a] Ratio of side-products was approximated by integration with ^{1}H NMR of anomeric mannose proton and was compared to an impurity peak of the cyclohexylether. [b] Combined yield of desired hexasaccharide and saturated side-products.

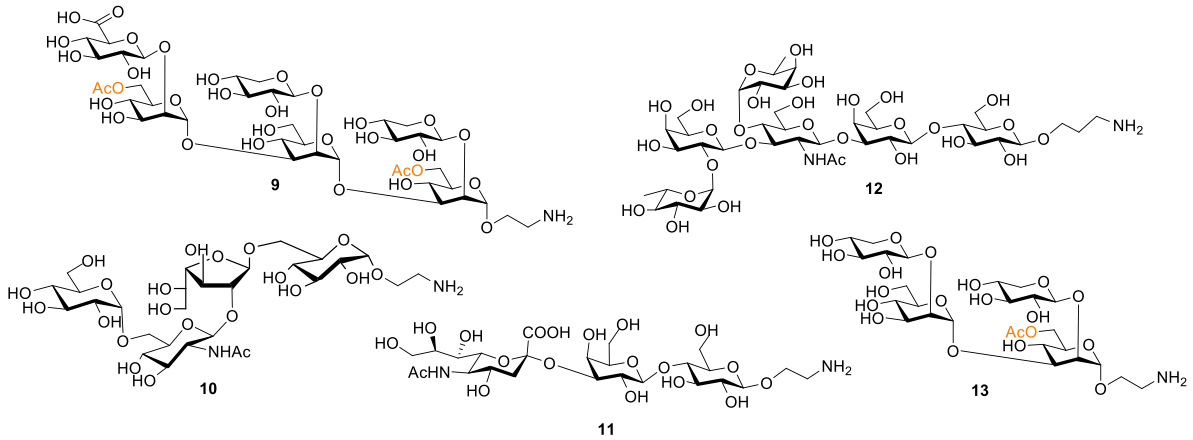


Figure 2. Synthetic Oligosaccharides Isolated Using Model Conditions.

Entry	Palladium Catalyst	Solvent system	Pre-conditioning	Source of Hydrogen	Saturation side-products?	Yield (%)
1	10% Pd/C Degussa or Pd Black or $[Pd(OH)_2]$	EtOAc:MeOH:H_2O:AcOH (55:15:15:15)	No	H_2	Yes	31-59
2	Pd Black	Dioxane:^{t}BuOH:H_2O (40:40:20)	No	H_2	Yes	45
3	$[Pd(OH)_2]$ or Pd Black	EtOAc:MeOH:H_2O (33:33:33)	No	HCO_2NH_4 or HCO_2K	No	65[a]
4	10% Pd/C Degussa	THF:H_2O (80:20)	Yes	H_2	Yes [b]	65
5	10% Pd/C Degussa	Acetone:H_2O (80:20)	Yes	H_2	Yes [b]	78
6	5% Pd/C Evonik Noblyst® or 10% Pd/C Degussa	DMF:H_2O (80:20)	Yes	H_2	No	77-80

Table 2. Screening the Effect of Solvent, Pre-conditioning and Hydrogen Source on Saturation Side-product Formation Using Model Compound 8. [a] Conditions caused deacetylation of hexasaccharide. [b] Lower-levels of side-product formation when using pre-conditioned catalysts.

Entry	Compound	Reaction Time (days)	Yield (%)
1	**9**	2	80 [a]
2	**10**[23]	1	73 [a]
3	**11**[24]	0.5	82 [a]
4	**12**[25]	1.5	76 [a]
5	**13**	1	84 [a]
6	**9**	2	74 [b]
7	**9**	0.2	86 [c]

Table 3. Optimized Hydrogenolysis Substrate Scope. Reaction conditions for hydrogenolysis reaction: pre-treated 5% Pd/C, H_2 (10 bar), THF:^{t}BuOH:PBS (100mM pH 5) (60:10:30 v/v/v). [a] 5% Pd/C Evonik Noblyst. [b] 10% Pd/C Sigma-Aldrich. [c] 10% Pd/C – Flow cartridge.

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