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Synthesis of Deuterated sugar nucleotide donor Guanosine diphosphate mannose to investigate the mechanism of mannosyltranferases enzyme

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الملخص

تصنيع مركب ما نوز – نيكليوتيد -1- C ديتوريم

تعتبر النيوكليوتيدات السكرية ومشتقاتها من أهم المركبات الطبيعية والمصنعة وتحظى باهتمام كثير من العلماء والباحثين لأهميتها البيولوجية ولصعوبة تحضيرها. فهذه المركبات تدخل في تخليق السكريات بجميع أنواعها وألية عمل وتثبيط الأنزيمات وفي تركيب الجليكوبروتينات وفي كثير من العمليات الحيوية.

تتكون النيوكليوتيدات السكرية كيميائيا من سكر أحادي مرتبط مع نيوكليوتيد(النيوكليوتيد يتكون من قاعدة نيتروجينية وسكر خماسي ومجموعة أو مجموعتين فوسفات) وتتميز هذه المركبات بنشاطها الكيميائي لذلك تكون غير ثابته في درجات الحرارة العادية.

يوجد هناك الكثير من الأنزيمات تسمى ناقلات السكر(glycosyltransferases) التي تستخدم النيوكليوتيدات السكرية لتحضير سلاسل السكريات المتعددة التي ترتبط مع أكسجين أو نيتروجين الجليكوبروتين، وجلوكوز أمين جليكان والسكريات المعقدة التي تدخل في تركيب جدار الخلية والسكريات الدهنية المعقدة(LPS). هذه الأنزيمات تقوم بنقل جزئ سكر أحادي(منشط) من النيوكليوتيدات السكرية إلى مستقبلات مختلفة. أحد هذه الأنزيمات المهمة أنزيم يسمى ناقل المانوز فهو ينقل سكر المانوز من المانح(عادة ما يكون في شكل سكر- نيوكليوتيد)إلى مستقبل معين (اما على جدار الخلية أو الجليكوبروتين أو غيره). أي خلل في نقل سكر المانوز يؤدي الى خلل صحي في الأنسان. أضافة إلى ذلك أنزيمات ناقلة المانوز تعتبر ذات قيمة كبيرة في الصناعات الدوائية والتكنولوجيا الحيوية.

في هذا البحث تم تحضير مركب نيوكليوتيد– المانوز والذي يحتوي على نظير الديوتيريم (**D**) عند ذرة الكربون الأنومارية بطريقة جديدة و بكفاءة عالية في معظم الخطوات. بدأنا بحماية مجموعات الهيدروكسيل في مركب 2 وترك هيدروكسيل ذرة الكربون الأنومارية ثم أكسدتها بمركب بيريدنيم كلوروكرومات(PCC) لكيتون ثم اختزال الكيتون الى كحول بمركب صوديوم

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بوروديوتيريد($NaBD_4$)**.** بعد ذلك تم تحفيز الكحول بوضع مجموعة الفوسفات ثم إزالة الحماية عن باقي مجموعات الهيدوكسيل وتفاعل الناتج 8 مع النيوكليوتيد**(**GMP-morpholidate) ليعطي مركب1(GDP-{D-1}-Mannose)**.**

Abstract:

Synthesis of Deuterated sugar nucleotide donor, Guanosine diphosphate mannose(D-1-GDP-man) **1**is decribed in this work using improved synthetic route goes through oxidation of the anomeric hydroxyl to ketone and then reduction of the ketone using sodium borodeuteried to insert the deuterium. Finally the mannose monophosphate was coupled with nucleotide to give GDP-mannose label C-1 with deuterium. This sugar nucleotide is intended to be used to investigate the mechanism of mannosyltransferases enzymes.

Introduction:

Naturally occurring sugar-nucleotides, as well as synthesized analogues, are of immense interest as substrates for enzymatic reactions in carbohydrate synthesis, as enzyme inhibitors and mechanism, as tools for assay development and for the study of glycoconjugate biosynthesis [Varki, 1993,Varki et.al 2009]. So, Sugar-nucleotides are essential intermediates in carbohydrate metabolism and glycoconjugate biosynthesis. Structurally, sugar-nucleotides are composed of a sugar or sugar derivative and a nucleotide mono- or diphosphate. In nature, there is great number of sugars and nucleotides which can form different sugar – nucleotides such as (ADP-Glc, TDP-Glc, UDP-Glc, UDP-GlcNAc and GDP-Man, CMP-sialic acid and CMP-Kdo). However, only nine different sugar -nucleotides have so far been identified in mammalian cells, this number is much greater in other organisms [Werz et. al 2007].

It is known that, different types of enzymes can be used in a glycol-synthesis [Palcic, 1999], one of these types is glycosyltransferases family which generally afford the greatest control of region and stereo-chemistry

[Chokhawala and Chen 2007]. Glycosyltransferases require activated sugar donor species, typically in the form of sugar nucleotides, or occasionally as lipid-linked phosphosugars [Wagner et. al. 2009]. Important example of sugar nucleotides is mannose containing nucleotides (GDP-mannose) which works as substrate for mannosyltransferase enzyme, one of glycosyltranferases family [Flint et al. 2005].

Mannose sugar is of particular importance since oligo, polysaccharides and glycoconjugates containing mannose form an essential part of the bacterial and fungal cell wall [Flint et al. 2005]. In higher organisms, both O- and N-linked oligosaccharides of glycosylated proteins contain mannose, and therefore defects in mannosyl transfer lead to a number of different human congenital disorders [Flint et al. 2005, Parodi 2000]. Due to the diverse and key role of mannose in complex bioactive molecules [Finkelstein, 2007], there is a great interest in mannose nucleotides and in the enzymes-catalyzed transfer of the sugar from its activated donor moiety to different acceptors **(Figure 1)**.

Furthermore, enzymes that catalyze the mannose transfer have industrial importance in addition to their therapeutic importance as mannose-containing polymers have many biotechnological application and used in drug design[Jmoudiak and Futerman2005].Therefore, we have great interest in studying the mechanism of mannosytranferases enzymes(Figure 1).

Because of the importance of sugar nucleotides, scientists have invested tremendous time and efforts in synthesis chemically and enzymatically of such nucleotides.

It becomes very clear that sugar nucleotide in general are non-trivial and very difficult to prepare due to: its activity, low solubility of in organic solvents, the presence of several polar or charged functional groups, and the susceptibility of the glycosidic and pyrophosphate bonds to hydrolytic cleavage [Wagner *et al*. 2009, Thibodeaux *et al.* 2008].

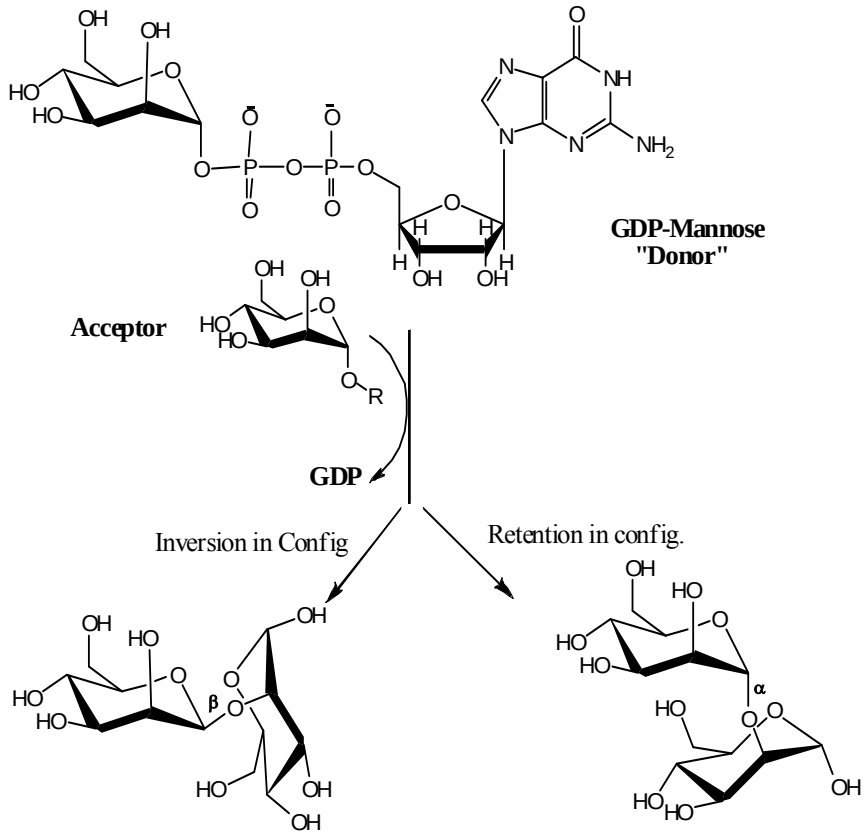


Figure 1: Mannose transfer from GDP-Mannose donor to the acceptor [Lee *et. al.* 2011]

General approaches for sugar nucleotides synthesis

Our study focus on mannosytranferases enzyme in which the substrate is GDP-mannose which transfer mannosyl moiety from the activated donor to the acceptor in plant, fungal and bacterial cell-walls.

So far, the literature has reported many different strategies for synthesis of sugar nucleotides [Wagner *et al.* 2009, Thibodeaux *et al.* 2008]. These strategies can be grouped into two classes,
nucleoside diphosphate sugars (NDP-sugars) which is the most naturally occurring sugar nucleotides and nucleoside monophosphate sugars (NMP-sugars, **Figure 2**) less common but biologically important in nature such as CMP-sialic acid and CMP-Kdo [Wagner *et al.* 2009;Varki *et. al.* 2009].
Most synthetic strategies in literature for the preparation of NDP-sugars

from smaller building blocks uses one of two approaches for the central pyrophosphate linkage **(Figure 2)**[*Wagner et al.* 2009]. In the most common approach, the pyrophosphate bond is formed from two monophosphate precursors, i.e. a nucleoside monophosphate and a glycosyl phosphate (Fig. 2, Path A).
One of the two reaction partners has to be activated prior to the formation of the pyrophosphate bond, and the identification of a suitable ‘‘activated monophosphate’’ species is critical for the success of this approach.

Alternatively, sugar-nucleotides can also be built from a nucleoside diphosphate and a monosaccharide species (Fig. 2, Path B). this approach is less famous since you need to find good ‘‘sugar electrophile’’ as a glycosylating reagent for the nucleoside diphosphate to couple with [Wagner *et al.* 2009].

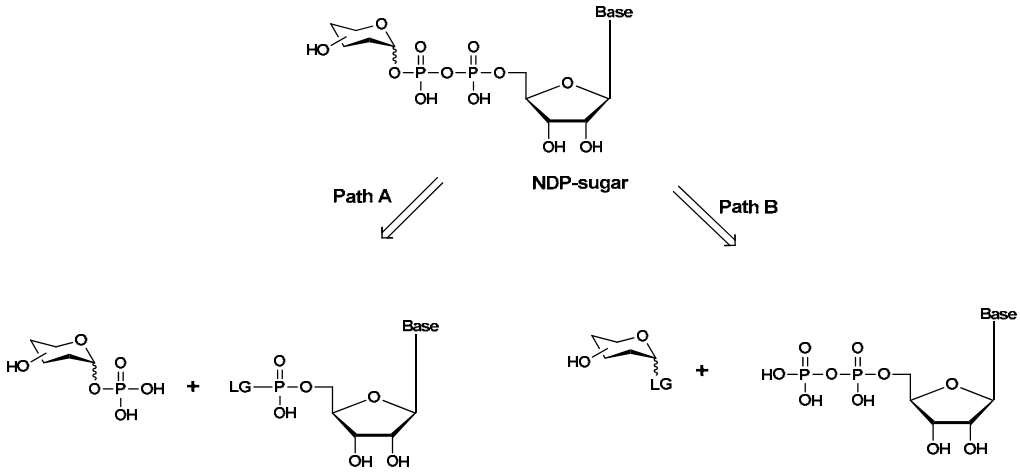


Figure 2: General synthetic strategies for NDP-sugars. (NDP: nucleoside diphosphate;LG: leaving group)

Results and discussion:

Our approach was preparing first the monophosphorylated mannose where we insert the deuterium on C-1 since we need this compound in our mechanistic studies of the mannosyltranferase and then, couple this active precursors with the nucleoside monophosphate guanosine 5`-monophosphomorpholidate (Scheme 1).

we started from the readily available starting material D-mannose **9** which was protected by acetylation in acetic anhydride and pyridine to give the percetylated product **10 (Scheme 2)** [Read *et. al.* 2004;Yamamoto and Davis 2012]. This followed by anomeric de-acetylation using ammonium carbonate in DMF to get **11** as described in the literature [Read *et. al.* 2004; Ribic *et. at.* 2010]. The next step was to oxidize the anomeric hydroxyl group to ketone **12** . We tried most of literate procedure including Swern oxidation and failed completely. We could not know the exact reason. It could be the anomeric effect but not sure.

So, failing to obtain the ketone **12,** we went through changing the protecting groups to benzyl group (**Scheme 1**). Benzyl ethers are widely used protecting groups in carbohydrate chemistry because they are particularly stable, resistant to strong basic or acidic conditions [Ribic *et. al.* 2010; Kotto *et. al.* 1980; Girard *et. al.* 2002]. Starting with α–methoxy D-mannose **2** treated with benzylbromide and using the sodium hydride base in DMF at room temperature gave the completely benzylated product **3** in 28% after purification. Obviously, this is not acceptable result since we need good amount to the benzylated compound **3**. Although the synthesis of compound **3** using sodium hydride base has been achieved by several groups none of them reported better than 28% for this reaction [Girard *et. al.* 2002].

The problem was solved by using potassium hydroxide as base [Kotto *et. al.* 1980; Lichtenthaler and Metz 2003] in DMSO solvent. The reaction was done by careful addition of the base to avoid any release of heat and solidification of the D-mannose which can cause problems. The yield was 87% which is excellent comparing with the previous results.

The benzylated product **3** was then treated with acetic acid and 3M sulphuric acid and heat to 90°C for half an hour. This deprotected the mannose anomeric position to get tetra-benzylated compound **4** as α/ β mixture in moderate yield after column chromatography. To improve the yield of **4** we tried to change the OMe group in **3** to acetate then hydrolyse the acetate **14** to hydroxyl group **(Scheme 3)**. We tried same condition, using sulphuric acid and acetic anhydride in acetic acid at -10°C

but we got the diacetate at the anomeric position and 6 position. Therefore, controlling the temperature in very important.
Oxidation of the anomeric hydroxy group was achieved using pyridinium chlorochromate (PCC) in 3.5 molar ratio in dichloromethane (DCM) gave ketone **5**. The reaction was smooth and after purification using ethyl acetate and pet ether (1:1) ratio the ketone crystallized to give white crystals of compound **5** 82% as needles.
To insert deuterium on C-1, mannolactone **5** was treated with 1 equivalence of Sodium Borodeuteride in methanol and water mixture (9:1), stir for four hours at room temperature. This gave mixture of deuterated tetrabenzylated mannose **6** in 95% α/ β anomers. However, this step was successful it was hard to separate both α and β anomers, α was the predominant product as shown by ^{1}H NMR spectra.

The next step was monophosphorylation at the anomeric position. Although, there is many phosphorylating reagents used in the literate [Zamyatina *et. al.* 2003; Burgess and Cook 2000; Moffatt and Khorana 1958; J. Moffatt and Khorana 1961; Roseman *et. al.* 1961] this step is hard to achieve in good yield. The reaction carried under relatively low temperature, minimizing degradation and suppressing the formation of undesired homodimers or cyclization with the phosphate group.

Initial attempts were carried using dibenzyldiethylphosphoramidite $Et_2NP(OBn)_2$ and triazole in DCM then followed by oxidation of the intermediate formed by hydrogen peroxide [Zamyatina *et. al.* 2003; Stanetty *et. al.* 2014]. However, this attempt was not successful. The phosphorylating reagent was changed to diphenylchlorophosphate $(C_6H_5O)_2POCl$ in pyridine and catalytic amount of 4-Dimethylaminopyridine (DMAP) but did not give the required product.

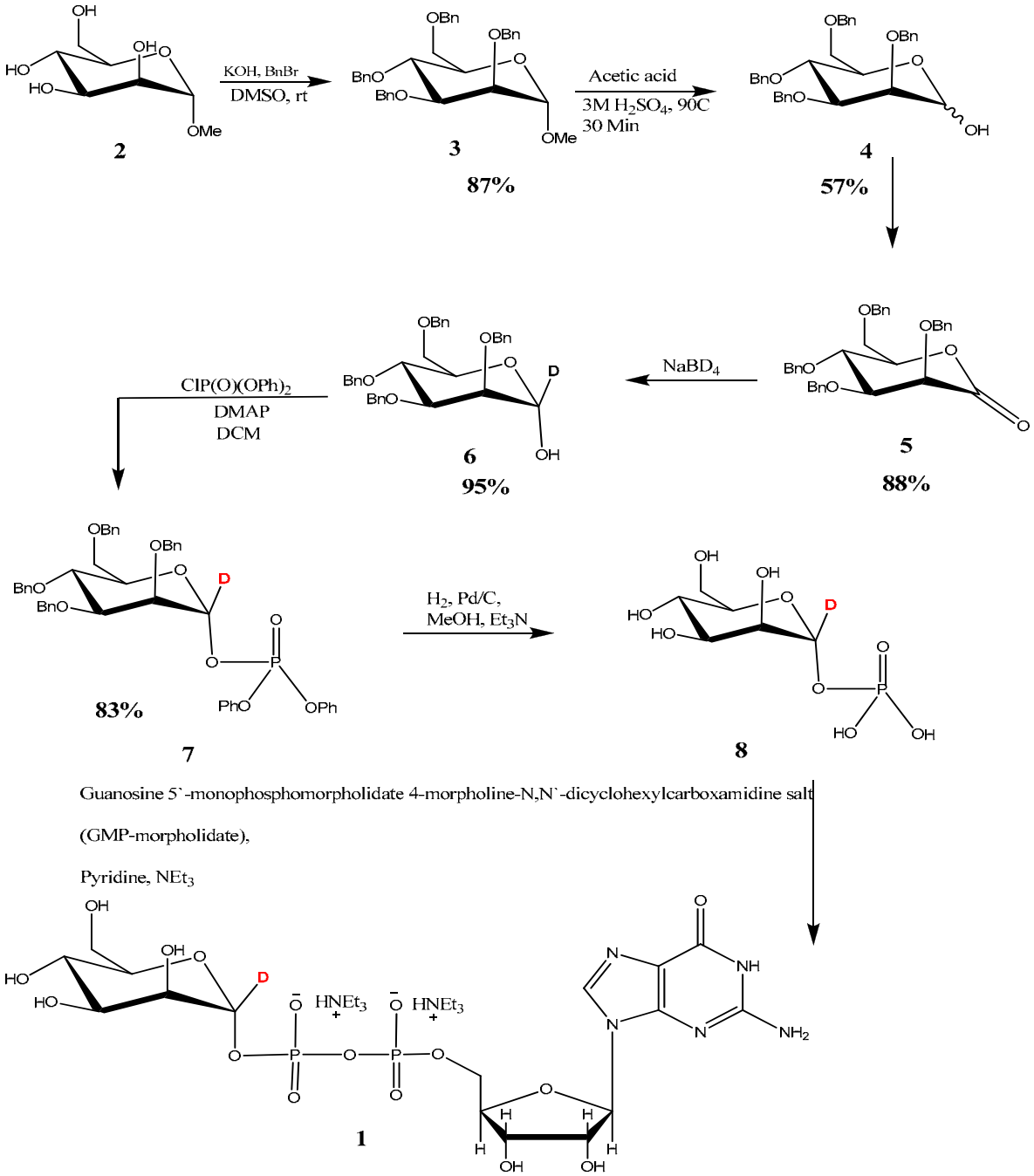
However, changing the reaction conditions and using diphenylchlorophosphate 1.5 eq. at 0C then room temperature in DCM, gave product **7** in good yield 83% (α/ β anomers) and the product was mainly α -anomer. To remove of the diphenyl groups selectively, we used platinum oxide PtO_2 in methanol with traces of acetic acid or triethylamine the reaction was not very successful. The reaction was not clean and seems

to have very little product judged by TLC and ^{1}H NMR. Changing the catalyst to Pd/C and Palladium hydroxide $Pd(OH)_2$ was not helpful.

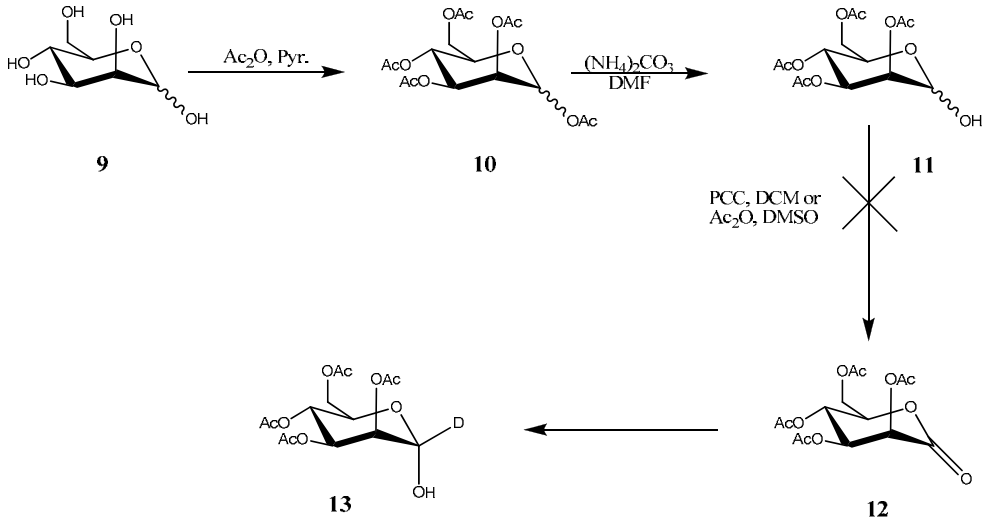
In attempt to solve the problem, we changed the procedure by reacting the deuterated mannose **6** with phosphorus oxychloride $POCl_3$ in pyridine to form the dichloro phosphate (**scheme 4)** then hydrolyse *in situ* with water to get the dihydroxy product **15.** This was successful however, the yield was about 20 %.

Having these problems, we decided to do the deprotection followed by coupling with the nucleotide monophosphate direct without purification. Global deprotection was achieved by using Pd/C in MeOH and trace amount of triethylamine. Completion of the reaction was determined by ^{1}H NMR spectroscopy which showed disappearance of the aromatic signals. This crude product was carried forward to the next reaction without any further purification.
The reaction was followed by mass spectroscopy until the reaction is completed. The catalyst was filtered through a bed of celite and the solvents were evaporated.

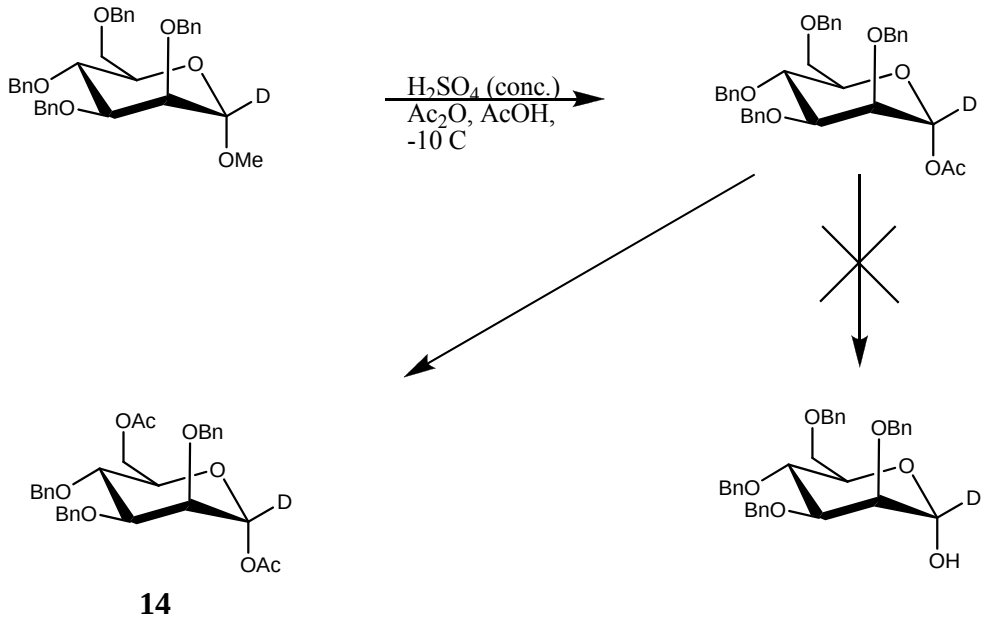
Product **8** was taken direct without further purification into the final step by chemically reacting with Guanosine 5`- monophosphomorpholidate 4-morpholine-N,N`-dicyclohexylcarboxamidine salt (GMP-morpholidate), in dry pyridine under strictly anhydrous conditions. After 3 days the reaction was complete and product **1** is formed judged by UV and ^{31}P NMR by the formation of the diphosphate peak. However, no purification has been done yet. These compound are proved to be extremely unstable and has to be lyophilized at -10 ºC to avoid decomposition.



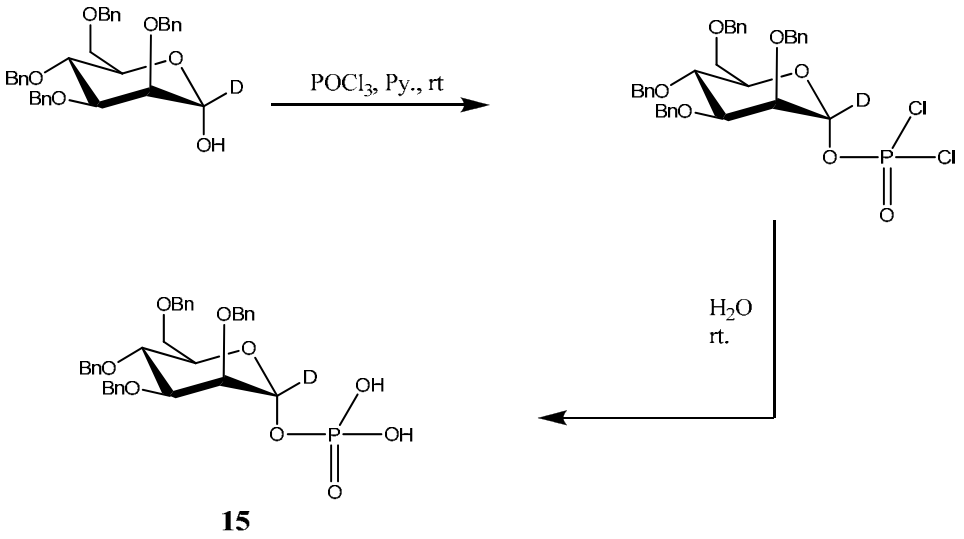
Scheme 1: Synthesis of Deuterated GDP-Mannose



Scheme 2: Synthesis of the ketone through the percetylated mannose



Scheme 3: Formation of the diacetate when chaging temperature



Scheme 4: Formation of the tetrabenzylated monophosphate using $POCl_3$ *in situ*

Experimental Section

Materials and General Methods:

Chemicals were purchased from Sigma-Aldrich and used as supplied, unless otherwise stated.

Anhydrous solvents were purchased from Fluka or Acros. Triethylamine was stored over NaOH pellets. All other solvents were used as supplied (analytical or HPLC grade) without prior purification. 'Petroleum ether' refers to the fraction of light petroleum ether boiling in the range of 40-60 ºC. All reactions using anhydrous conditions were performed using flame-dried apparatus under an atmosphere of argon or nitrogen.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AVII500 (500 MHz) or a Varian Mercury VX 400 (400 MHz) spectrometer, as indicated. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker AVII500 (125.8 MHz) or a Varian Mercury VX 400 (100.6 MHz) spectrometer, as Indicated and are proton Decoupled. Phosphorus nuclear magetic resonance (^{31}P NMR) spectra were recorded on a Bruker AVII500 (202 MHz) or a Varian Mercury VX 400 (162 MHz) spectrometer, as indicated.

All chemical shifs are quoted on δ scale in ppm using TMS as the internal standard. Coupling constants (J) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quarter, app = apparent.
Low resolution mass spectra were recorded on a Micromass Platform 1 spectrometer using electrospray ionization (ES), or on a Bruker Daltronic MicroTOF spectrometrer. High resolution mass spectra (HRMS) were recorded on a Bruker Daltronic MicroTOF spectrometer. m/z values are reported in Daltons.
Thin Layer Chromatography (TLC) was carried out using Merck aluminium backed sheets coated with Kieselgel 60F254 silica gel. Visualization of the sheets was achieved using a UV lamp (λmax = 254 or 365 nm) and/or ammonium molybdate (5% in 2M H_2SO_4), or sulfuric acid (0.2M in 1 MeOH : 1 H_2O).

Methyl 2, 3, 4, 6-tetra-O-benzyl-α-D-mannopyranoside 3 [Girard *et. al.* 2002; Kondo *et. al.* 1994]

Methyl α-D-mannopyranoside (1 g, 5.6 mmol) was dissolved in DMSO (4 mL) and stirred under Ar. BnBr (4.8 mL, 40.3 mmol) was added direct to the solution and the mix. was cooled down to 0°C. Powdered KOH (3.5 g, 63.5 mmol) was added carefully portionwise over 20 minutes. The mix. left to stir untill it came to room temperature and stirred for further 4 h. Then it was diluted with diethyl ether (50mL) and saturated ammonium chloride (70 mL). The organic layer was collected and washed with saturated $NaHCO_3$ (20 mL × 2) and water (20 mL × 2), dried over anhydrous magnesium sulphate and evaporated. Purification using column chromatography eluting with pet. Ether and ethyl acetate (initially 100% pet ether to flash the BnBr, then 8:2 pet ether :EtOAc) gave product **3** (2.47 g, 87%); R_f (7:3 Pet. Ether:EtOAc) = 0.62; ^{1}H NMR (400 MHz, CDCl3): δ ppm = 7.40 – 7.20 (m, 20 H, 4 x C_6H_5), 5.18 (m, 1 H, H-1), 4.84-4.47 (m, 8 H, 4 x PhCH_2), 4.15 (m, 1 H, H-5), 3.89 (m, 2 H, H-2 and 3), 3.85 (app t, J = 9.61 Hz, J = 10.16 Hz, 1 H, H-4), 3.65 (m, 2 H, H-6a, H-6b), 3.51 (s, 3 H, CH_3).

3,4,5 –tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2H-pyran-2-ol

The product **3** (5.6 g 10.01 mmol) was suspended in a mixture of acetic acid (30 mL) and 3M H_2SO_4. The mixture was heated to 90 ^oC for 30 min. The mixture was then cooled to room temperature and ice-cooled water (100 mL) was added. The resulting mixture was extracted with dichloromethane (3 × 70 mL). The combined organic layers was washed again with water and brine. The product dried over $MgSO_4$ and evaporated. The crude product was purified by flash chromatography (2:8 EtOAc/Pet ether) to yield product **4** (3.1 g, 57%).^{1}H NMR (400 MHz, CDCl3): δ ppm = 7.44 – 7.22 (m, 20 H, 4 x C_6H_5), 5.26 (m, 1 H, H-1), 4.84-4.47 (m, 8 H, 4 x PhCH_2), 4.15 (m, 1 H, H-5), 3.89 (m, 2 H, H-2 and 3), 3.85 (m, 1 H, H-4), 3.65 (m, 2 H, H-6a, H-6b), 3.04 (br, 1H, OH).

2,3,4,6 -tetra-O-benzyl-D-mannolactone 5 [Bernardes 2008]

A solution of protected sugar **4** (1.44 g, 2.68 mmol) in dry dichloromethane (15 mL) was added under argon to a suspension of pyridinium chlorochromate(2 g, 9.3 mmol)and molecular sieves (1.5g)in dichloromethane (8 mL). The reaction was stirred at dark for 3 hours followed by TLC (till the starting material disappeared). The resultant mixture was diluted with dichloromethane (20 mL), filtered through a pad of Celite then through a small column of silica. Column chromatography was done using pet ether:EtOAc (10:1 to 1:1). The product **5** was crystallized from methanol to give white crystals 1.28 g (88 %); ^{1}H NMR (400 MHz, CDCl3): δ ppm 7.47-7.23 (m, 20 H, 4 × Ar), 4.91 (m, 6H, PhCH_2), 4.1 – 3.85 (m, 2H, H-4, H-5), 3.79–3.70 (m, 2H, H-2, H-3), 3.67 – 3.60 (m, 2H, H-6a, H-6b); ESI-MS *m/z* 561.22 $[M + Na]^+$

2,3,4,6-Tetra-O-benzyl-α-D-[1-^{2}H] mannopyranose 6

To a solution of 2,3,4,6-terta-O-benzyl-D-mannolactone **12** (250 mg, 0.47 mmol) in methanol and water (9:1, 10 mL) was added sodium borodeuteride (20 mg, 0.47 mmol) and the resulting mixture was stirred for 4 hours at room tepertaure. The mixture concentrated in vacuo and dissolved in ethyl acetate. The solution was neutralized with aqueous solution 1M of hydrochloric acid. The organic layer was washed with water (30 mL) and brine (3 × 20 mL) dried over $MgSO_4$ and evaporated. The resultant oil was purified by flash column chromatography eluting with pet

ether : EtOAc (1:1) to yield the anomeric mixture **6** as yellow oil 95 % (90:10 α/β). Spectral data are only given for the predominant α anomer. ^{1}H NMR (400 MHz, $CDCl_3$):): δ ppm 7.37-7.12 (m, 20 H, 4 × Ar), 4.83 (d, *J*gem =
11.21 Hz, 1H, CH*H*Ph), 4.74–4.52 (m, 6H, 3 CH_2Ph), 4.50 (d, *J*gem = 11.21 Hz, 1H, CH_2Ph), 4.24 (app t, *J* = 9.5 Hz, *J* = 9.5 Hz, 1H, H-4), 3.99 (dd, *J* = 2.91 Hz, *J* = 9.48 Hz, 1H, H-3), 3.85 (m, 1H, H-2), 3.66 (*J* = 10.8, 4.5 Hz, 1H, H-6a), 3.61 (dd, *J* = 10.8, 4.1 Hz, 1H, H-6b), 3.47 (m, 1H, H-5), 3, 32 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$): δ ppm: 174.69 (C=O), 138.52, 138.45, 138.42, 138.34 (*C*–Ar), 128.35–127.26 (*CH*–Ar), 97.88 (C-1), 80.01, 74.80, 74.73, 71.99 (C-2–5), 74.85, 73.26, 72.55, 72.16 (CH_2Ph), 69.20 (C-6); ESI-MS *m/z* 564.25 $[M + Na]^+$.

2,3,4,6-Tetra-O-benzyl-α-D-[1-^{2}H] mannopyranose-1-diphenylphosphate 7 [Nunez et. al. 1981]

Diphenylphosphoryl chloride (5 mL, 0.023 mmol)was added to a stirred of lactol **16** (10 mg, 0.018 mmol) and DMAP (6 mg, 0.045 mmol) in dichloromethane (200 L) at 0°C. After 30 minutes, the reaction was quenched with crushed ice, followed by stirring at room temperature for 15 minutes. The mixture was poured into a two-layer mixture of diethyl ether and saturated aqueous $NaHCO_3$ (8 mL) and the whole mix was extracted with ethyl acetate (15 mL). The organic extract was washed with brine (10 mL) and dried over anhydrous $MgSO_4$. Filtered and evaporated under vacuum. Flash chromatography using pet ether: EtOAc gave anomeric mixture as yellow oil 83% (92:8). Spectral data are only given for the predominant anomer. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.37-7.28 (m, 20 H, 4 × Ar), 7.22-7.17 (m, 10H, C_6H_5), 5.31 (s, 2H, $PhCH_2$), 4.86 (d, J_{gem} = 10.69 Hz, 1H, $PhCH_2$), 4.71 (s, 4H, $PhCH_2$), 4.64 (d, J_{gem} = 12.06 Hz, 1H, $PhCH_2$), 4.46 (app t, J = 10.78, J= 10.78 Hz, 1H, H-4), 4.10 (t, J = 9.7 Hz, 1H, H-5), 3.86(dd, J = 10.63 Hz, J = 3.0 Hz, 1 H, H-6a), 3.80 (dd, J = 9.5, J = 3.01 Hz, 1H, H-6b), 3.73 (dd, J = 12.5 Hz, J = 3.33 Hz, 1H, H-3), 3.53 (dd, J = 11.0 Hz, J = 1.13 Hz, 1H, H-2); ^{13}C NMR (125 MHz, CDCl3): δ ppm 130.01, 129.8, 129.7, 128.4, 128.3, 128.2, 127.9, 127.8, 127.6, 127.5 (Ar –C), 125.1, 125.4, 120.1, 120.0 119.7 (Ph-C), 94.1 (C-1), 78.8, 75.3, 75.2, 73.5, 72.3, 72.1 68.3; HRMS (ESI) m/z Calcd for

$C_{46}H_{44}DO_9PNa$ $[M + Na]^+$: 796.2762. Found: 796.2768.

Guanosine 5` (*α-D-[1-^{2}H] mannopyranose-1- diphosphate*) bis(triethylamine) salt (GDP-Man) 1

Tetrabenzylated mannose monophosphate **7** (80 mg, 0.103 mmol) was dissolved in methanol (15 mL), triethylamine (2 mL) and 10 % Pd/C (500 mg) was added. This mixture was then equilibrated to an atmosphere of hydrogen and stirred at rt for 24 h. The catalyst was filtered through a bed of celite, and the solvent was evaporated to dryness to give product **8**. The oily residue **8** was taken to the next step direct without any purification. Compound **8** was dissolved in dry pyridine (12mL) and evaporated. The process was repeated 3 times then the flask was flashed with Ar. Gas. Guanosine 5`-monophosphomorpholidate 4- morpholine-N,N`-dicyclohexylcarboxamidine salt (150 mg, 0.206 mmol) was dissolved in dry pyridine (10 mL × 3) and evaporated on the rotatory evaporator and then redissolved in dry pyridine (20 mL) and added to compound **8** under sealed Ar atmosphere. The mixture was concentrated under reduced pressure to 10 mL and kept under Ar to stir at room teperature for 3 days. The reaction was followed by UV spectrum and ^{31}P NMR. TLC using NH4OH:MeOH:$CHCl_3$ in 3:5:1 ratio. Currently the reaction mixture is under purification using ion exchange column.

References:

1. Burgess K. and Cook D. **2000**: Syntheses of nucleoside triphosphates, *Chem. Rev.* 100, 2047–2059.

2. Bernardes G. **2008**, Ph.D thesis**:** The synthesis of homogenously modified proteins, Oxford University, UK, p 110.

3. Chokhawala H. and Chen X. **2007**: Enzymatic Approaches to OGlycoside Introduction: Glycosyltransferases, pp 415–451, in Comprehensive Glycoscience, Volume 1, Eds Boons G.; Lee Y.; Suzuki A.; Taniguchi N. and Voragen A.; Elsevier, Oxford, UK.

4. Flint J.; Taylor E.; Yang M.; Bolam D.; Tailford L.; Martinez-Fleites C.; Dodson E.; Davis B.; Gilbert H. and Davies G. **2005**: Structural dissection and high-throughput screening of mannosylglycerate synthase, *Nat. Struct. & Mol. Biol.* **12,** 608-14.

5. Finkelstein J. **2007**: Glycochemistry and Glycobiology, *Nature,* 446, 999.

6. Girard C.; Miramon M-L.; Solminihac T. de and Herscovici J. **2002**: Synthesis of 3-C-(6-O-acetyl-2,3,4-tri-O-benzyl-α-D-mannopyranosyl)-1-propene, Carbohydr. Res. 337, 1769–1774.
7. Jmoudiak M. and Futerman AH. **2005**: Gaucher disease: pathological mechanisms and modern management, Br. J. Haematol.**129,** 178-88.
8. Kotto S.; Inada S.; Morishima N. and Zen S. **1980**: Some 2,3,4,6-tetra-O-(p-chlorobenzyl)-□-D-hexopyranoses, Carbohyrate Res. 87, 294-296.
9. Kondo H.; Aoki S.; Ichikawa Y.; Halcomb R.; Ritzen H.and Wong C. **1994**: Glycosyl Phosphites as Glycosylation Reagents: Scope and Mechanism, J. O. Chem. 59, 864.
10. Lichtenthaler F. and Metz T. **2003**: Efficient Generation of β-L-Rhamnosidic Linkages by the 2-Ulosyl Donor Approach: Synthesis of a Trisaccharide with a Central β-L-Rhamnose Unit, Eur. J. Org. Chem, 3081 – 3093.
11. Lee S.; Hong S.; Errey1 J.; Izumi A.; Davies G. and Davis B. **2011**: Mechanistic evidence for a front– side, S_Ni-type reaction in a retaining glycosyltransferase, Nature Chemical Biology, Vol. 7, 631-638.
12. Moffatt J. and Khorana H. **1958**: Nucleoside Polyphosphates. New and Improved Syntheses of Uridine Diphosphate Glucose and Flavin Adenine Dinucleotide Using Nucleoside-5' Phosphoramidates, J. Am. Chem. Soc. 80, 3756 – 3761.
13. Moffatt J. and Khorana H. **1961**: Nucleoside Polyphosphates. The Synthesis and Some Reactions of Nucleoside-5' Phosphoromorpholidates and Related Compounds. Improved Methods for the Preparation of Nucleoside-5' Polyphosphates, J. Am. Chem. Soc. 83, 649-658.
14. Nunez H.; O'Connor J. and Baker R. **1981**: The synthesis and characterization of □- and □-$_L$-fucopyranosyl phosphates and GDP fucose, Can. J. Chem. 59, 2086.
15. Parodi A. **2000**: Protein glucosylation and its role in protein folding, Ann. Rev Biochem. 69, 69-93.
16. Palcic M. **1999**: Biocatalytic synthesis of oligosaccharides, Curr. Opin. Biotechnol. 10, 616–624.
17. Read J.; Ahmed R.; Morrison J.; Coleman W. Jr. and Tanner M. **2004**: The mechanism of the reaction catalyzed by ADP-□-$_L$-glycero-$_D$-manno-heptose 6-epimerase, J. Am. Chem. Soc. 126, 8878-8879.
18. Ribić R.; Kovačević M.; Petrović-Peroković V.; Gruić-Sovulj I.; Rapić V. and Tomića S. **2010**: Synthesis and biological activity of mannose

conjugates with 1-adamantamine and ferrocene amines, *Croat. Chem. Acta* **83** (4) **,** 421–431.

19. Roseman S.; Distler J.; Moffatt J. and Khorana H. **1961**: Nucleoside Polyphosphates. An Improved General Method for the Synthesis of Nucleotide Coenzymes. Syntheses of Uridine-5', Cytidine-5' and Guanosine-5' Diphosphate Derivatives, *J. Am. Chem. Soc.* 83, 659–663.

20. Stanetty C.; Walter M. and Kosma P. **2014**: Convergent synthesis of 4-*O*-phosphorylated $_{L}$-glycero-$_{D}$-manno-heptosyl lipopolysaccharide core oligosaccharides based on regioselective cleavage of a 6,7-*O*-tetraisopropyldisiloxane-1, 3-diyl protecting group, J. *Org. Chem.* 79, 582−598.

21. Thibodeaux C.; Melancon C. and Liu H. **2008**: Natural-product sugar biosynthesis and enzymatic glycodiversification*, Angew. Chem., Int. Ed.* 47, 9814–9859.

22. Varki A. **1993**: Biological roles of oligosaccharides: all of the theories are correct, *Glycobiology*, 3, 97–130.

23. Varki A.; Cummings R.; Esko J.; Freeze H.; Stanley P.; Bertozzi C.; Hart G. and Etzler M. **2009**: Editors: *Essentials of Glycobiology*, 2nd edition, Cold Spring Harbor Laboratory Press, New York.

24. Werz D.; Ranzinger R.; Herget S.; Adibekian A.; Von der Lieth C. and Seeberger P. **2007**: Exploring the structural diversity of mammalian carbohydrates ("glycospace") by statistical databank analysis, *ACS Chem. Biol.* 2, 685– 691.

25. Wagner G.; Pesnota T. and Field R. **2009**: A survey of chemical methods for sugar-nucleotide synthesis, *Nat. Prod. Rep.* 26, 1172–1194.

26. Yamamoto K. and Davis B. **2012**: Creation of an □-mannosynthase from a broad glycosidase scaffold, *Angew. Chem. Int. Ed.* 51, 7449 – 7453.

27. Zamyatina A.; Gronow S.; Puchberger M.; Graziani A.; Hofinger A. and Kosma P. **2003**: Efficient chemical synthesis of both anomers of ADP $_{L}$-glycero- and $_{D}$-glycero-$_{D}$-manno- heptopyranose, *Carbohydrate Res.* Vol. 338, Issue 23, pp. 2571–2589.