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A unique approach to the synthesis of a dengue vaccine and the novel tetrasaccharide that results

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This paper is dedicated to Professor George Fleet, who is an inspirational chemist, on the occasion of his 65th birthday

ABSTRACT

An approach to the development of a dengue vaccine by synthesizing the hexasaccharide epitope on the viral surface is examined. The stereochemical and structural challenges include the synthesis of a β-mannoside bond. Synthesis of this bond is approached via a trisaccharide analogue portion of the epitope. A novel tetrasaccharide with a mannose–mannose anomeric linkage results in the course of the synthetic attempts.

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1. Introduction

In the last 20 years, dengue fever, and its more severe counterpart, dengue haemorrhagic fever (DHF), have emerged as the most important arthropod-borne viral diseases of humans. It is estimated that there are of up to 100 million cases worldwide annually, greater than the combined cases of malaria and West Nile infections.[1] The dengue virus belongs to the Flaviviridae family[2] and there are at present four known antigenically distinct serotypes. Infection by one serotype guarantees lifelong immunity towards that serotype, but not towards the other three serotypes. In fact, infection by any one of three remaining serotypes increases the probability by at least 15-fold of the individual developing DHF. Consequently, for any vaccine to be considered adequate, it must of necessity be tetravalent in formulation.[3] There have been two general approaches to address these problems. Foremost of these have been the attempts aimed at controlling the *Aedes aegypti* mosquito vector.[4] The second approach has been focused on the preparation of synthetic vaccines.[5] Unfortunately, both approaches have had limited success; the former being plagued by a combination of social and political obstacles,[6] while the latter suffers from the various formulations not being tetravalent.[7]

It is known that the four serotypes share the same 3D structure of their envelope glycoprotein[8] (Fig. 1).

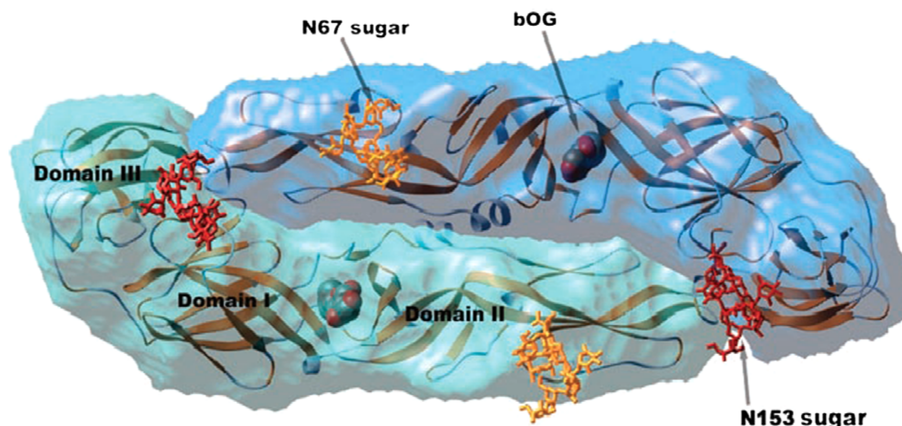

Figure 1. Semitransparent surface representation of the Dengue Virus E Protein. The β-*N*-octyl-glucoside molecule is added during crystallization of the structure to significantly improve the abundance and diffraction limit of the crystals. Copyright (2003) National Academy of Sciences, USA.

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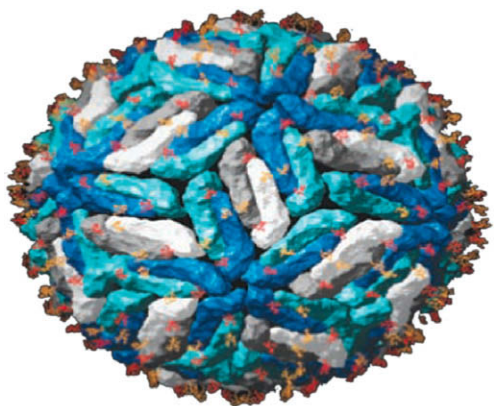


Figure 2. Carbohydrate distribution on the viral surface. Copyright (2003) National Academy of Sciences, USA.

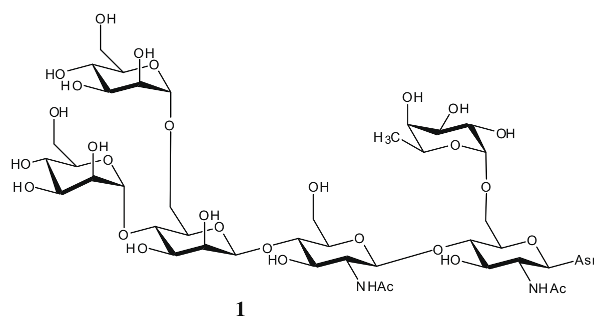
Furthermore, there are several glycosylation sites on the surface of the dengue virion[8] (Fig. 2). The conserved oligosaccharide at the Asn-153 glycosylation site has been elucidated and modelled as a hexasaccharide consisting of three mannose, two *N*-acetyl glucosamine and one fucose residues[9] (Fig. 3).

We suggest that an alternative approach to the development of a dengue vaccine would be to synthesize an analogue of the conserved hexasaccharide residue.[10] This concept is not without precedent, as the literature is replete with examples of the success of this approach.[11] The third mannoside residue of the hexasaccharide has a β-mannoside bond, widely acknowledged as the most challenging glycoside bond in carbohydrate chemistry.[12]

Numerous approaches to the stereoselective synthesis of a β-mannoside bond have been reported over the years. Of particular interest to us is the use of a constraining 4,6-*O*-benzylidene ring proposed and developed by Crich.[13] The benzylidene ring has been shown to impart both torsional and electronic effects on stereoselectivity.[14] Another methodology is that employing the use of glycosyl phosphates as the anomeric leaving group, namely the propane-1,3-diyl phosphate coupling protocol developed by Singh.[15] The appeal of this approach is that the use of phosphates is biomimetic as per the Leloir biosynthetic pathway for the construction of glycoside bonds.[16]

It is well known that a major part of oligosaccharides involved in biological interactions are made up of hexopyranoses and their overall conformation (and hence interactions) can be described by bonds to the exocyclic groups, specifically the 6-OH and *N*-acetyl groups.[17] In addition, it has been shown that fucosyl residues present in an oligosaccharide significantly increase its overall immunogenicity.[18]

We therefore proposed to synthesize the structural analogue **1** of the native molecule. This molecule maintains all the critical and interaction determining linkages as the original, as well as the immunogenic fucosyl residue. We postulate that any sterically constraining and electron-withdrawing groups at the 4- and 6-positions of the donor mannose molecule should have a similar effect on β-mannoside selectivity as the benzylidene ring. This analogue should therefore also be synthetically simpler, as it allows the introduction of a β-mannoside bond as part of a trisaccharide.



To test this hypothesis, we set out to construct trisaccharide **2** (Scheme 1), a glycosyl phosphate donor.

Herein we report our approach to the synthesis of donor **2**, and the results of our endeavours.

2. Results and discussion

2.1. Attempts at synthesis involving a glycosyl acceptor having a free anomeric hydroxyl

We envisioned the synthesis of trisaccharide **2** through the coupling of monosaccharide derivatives **3** and **5** in a one-pot process, followed by subsequent phosphorylation of the anomeric centre.

The partially protected synthon **5** was obtained in higher overall yield than that obtained through the reported literature synthesis[19] by adopting the modified simpler process (Scheme 1). Our synthesis utilized the readily available methyl-α-D-mannopyranoside **6**. Protection of the C-4 and C-6 hydroxyls was accomplished with α,α-dimethoxytoluene and afforded the mono-benzylidene-protected monosacccharide **7**. The remaining C-2 and C-3 hydroxyl groups were protected as benzyl ethers to afford **8**. Subsequent treatment with TFA in acetonitrile and water gave the desired partially protected mannoside **5** in an improved overall yield (Scheme 2).

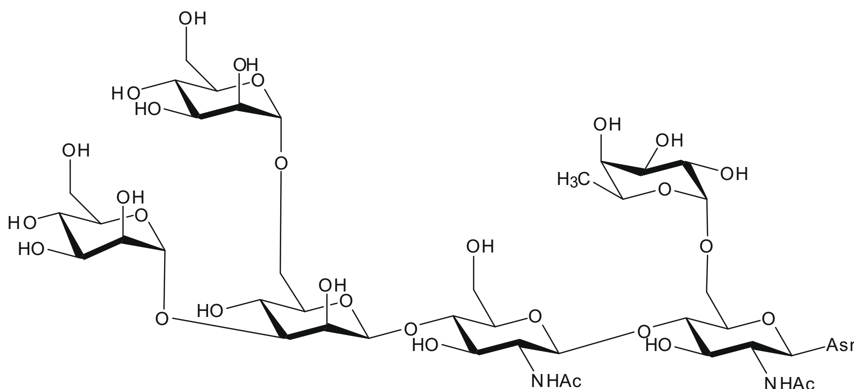
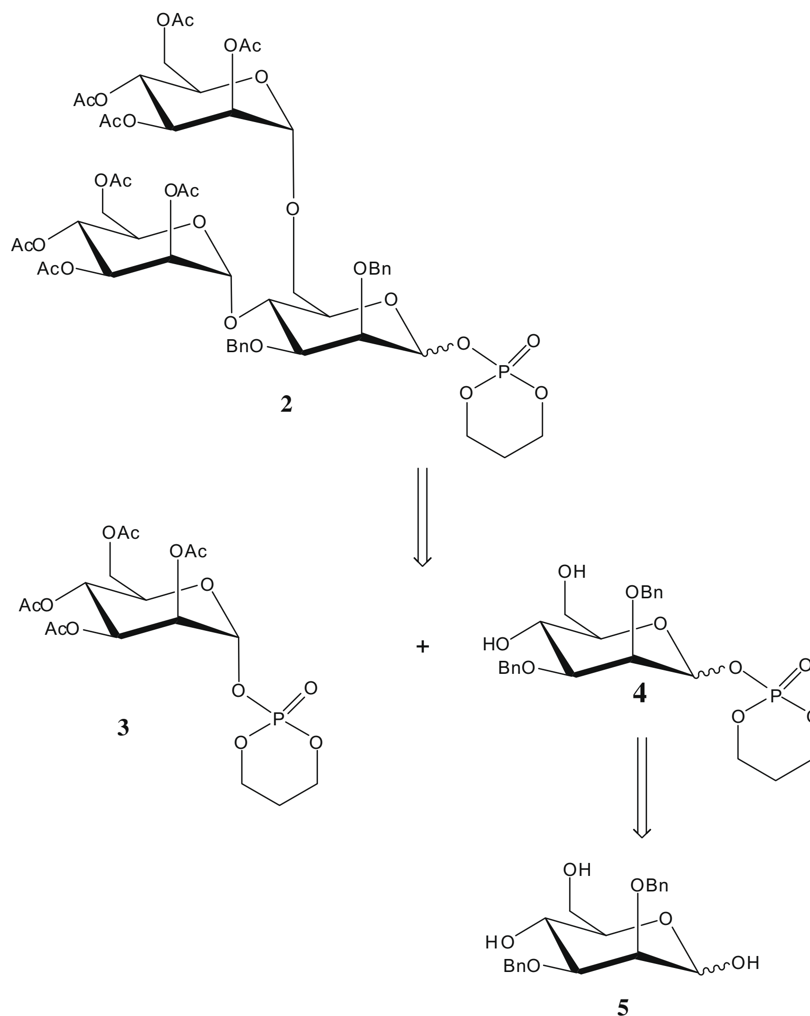


Figure 3. Hexasaccharide attached to Asn-153 of the Dengue E Glycoprotein



Scheme 1. Retrosynthetic analysis of trisaccharide mannosyl donor.

In Scheme 3 we detail the synthesis of the mannosyl phosphate donor **3**. We reasoned that its use in glycoside bond formation would yield the α-mannoside exclusively and in high yield due to the anchimeric participation of the C-2 acetate group.

The peracetylated mannose derivative **10** was obtained through iodine-promoted Lewis acid-catalyzed acetylation of D-mannose, **9**.[20] The monosaccharide **11** was obtained by the selective removal of the anomeric acetate group that resulted in the sole formation of the α-anomer.[21] This was then reacted with propane-1,3-diyldioxyphosphoryl chloride to yield the novel monosaccharide donor **3** in excellent overall yield.

With synthons **3** and **5** available, we coupled 1.0 equiv of **5** with 3.0 equiv of **3** (Scheme 4). We anticipated that such a reaction would result in the formation of a trisaccharide analogue of **2**. The outcome of this reaction was somewhat surprising in that we obtained tetrasaccharide **12** containing an α,α-anomeric–anomeric linkage. Support for the formation of this structure was evidenced by its ^{1}H and ^{13}C NMR data. In its ^{13}C spectrum, resonances at 91.01, 93.84, 99.03 and 100.75 were observed for the anomeric carbons. In addition, the MS gave a $(M+Na)^+$ of 1373.3901. Such a linkage is known in trehalose (a well-known glucose disaccharide), and has been reported for mannose disaccharides.[22]

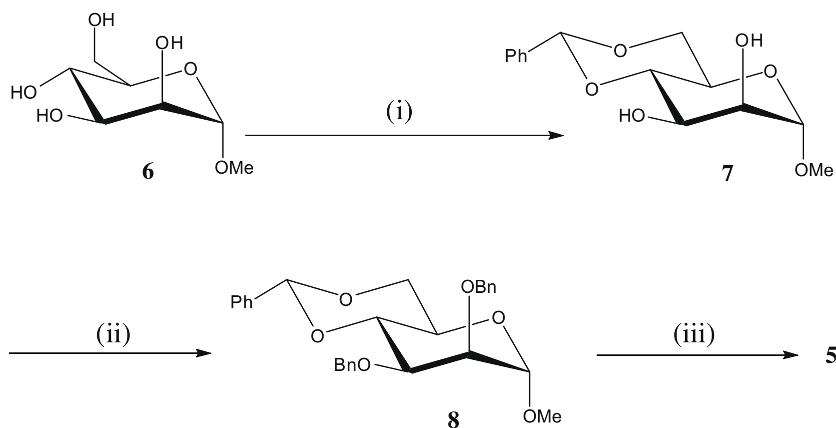
Coupling of **5** with 2.0 equiv of **3** produced an inconsistent mixture of disaccharides, varying with each repetition when carried out on a gram scale. In hindsight, these results are not surprising owing to the typically greater nucleophilicity of the anomeric hydroxyl over the remaining secondary hydroxyls of a monosaccharide.

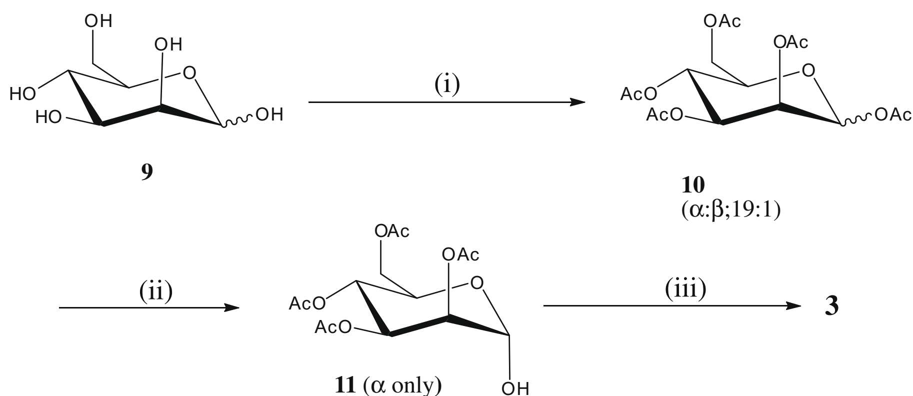
2.2. Synthesis of a trisaccharide donor via selective anomeric benzyl group deprotection

We were therefore still left with the issue of how to efficiently synthesize our target trisaccharide **2**. The use of ammonium formate in catalytic transfer hydrogenation (CTH) for the deprotection of benzyl groups is well documented in the literature.[23] However, there appears to be only one example of this method being applied to the regioselective removal of an anomeric benzyl group from a perbenzylated carbohydrate.[24] This methodology had been applied successfully to prepare monosaccharides and disaccharides.

Intrigued by this, we decided to investigate the utility of this approach for the preparation of the desired trisaccharide derivative bearing an anomeric benzyl group which on selective removal would provide a free anomeric hydroxyl. To this end, an acceptor bearing free hydroxyls at the 4- and 6-positions only was required (Scheme 5).

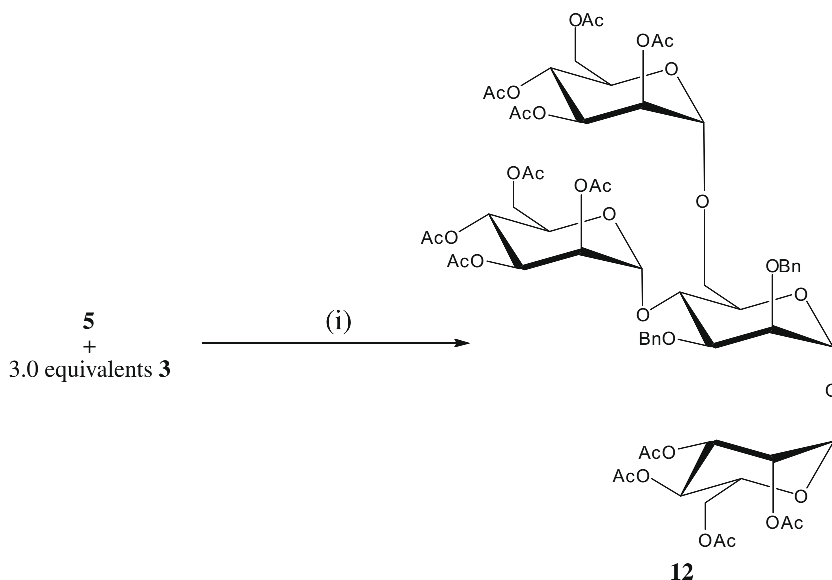
Mannose was mono-benzylideneated to afford the hitherto unknown mannosides **13**, the low yield being a reflection of the ready formation of the thermodynamic product: the 2,3:4,6-*O*-dibenzylidene derivative; in addition to the unreacted mannoses which were isolated. During the course of the protection reaction of the





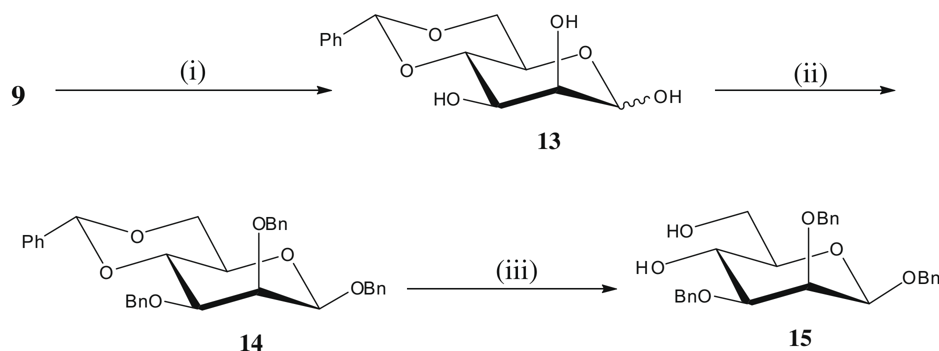
Scheme 2. Reagents and conditions: (i) α,α-dimethoxytoluene, *p*TSA, DMF, 4 h, 50–70 °C (78%); (ii) BnBr, NaH, TBAI, DMF, 24 h, 0 °C to rt (78%); (i) H_2O: CH_3CN: TFA (4:3:3), 48 h, 98 °C (74%).

Scheme 3. Reagents and conditions: (i) I_2, Ac_2O, 1 h, rt (100%); (ii) NH_3, CH_3CN, 6 h, rt (98%); (iii) *N*-MeIm, propane-1,3-diyldioxyphosphoryl chloride, DCM, 0 °C to rt, 24 h (93%).



Scheme 4. Reagents and conditions: (i) 1.5 equiv TMSOTf, DCM, 3 h, −78 °C to rt (93%).

free hydroxyl functions as their benzyl ethers, it was interesting to note that the β-anomer was obtained exclusively affording **14** in 81% yield. Given that the anomeric mixture **13** was predominately the α-anomer, the fact that derivatization of the anomeric centre gives only the β-product lends further support to the directing effect of the 4,6-*O*-benzylidene ring. Subsequent benzylation fur-



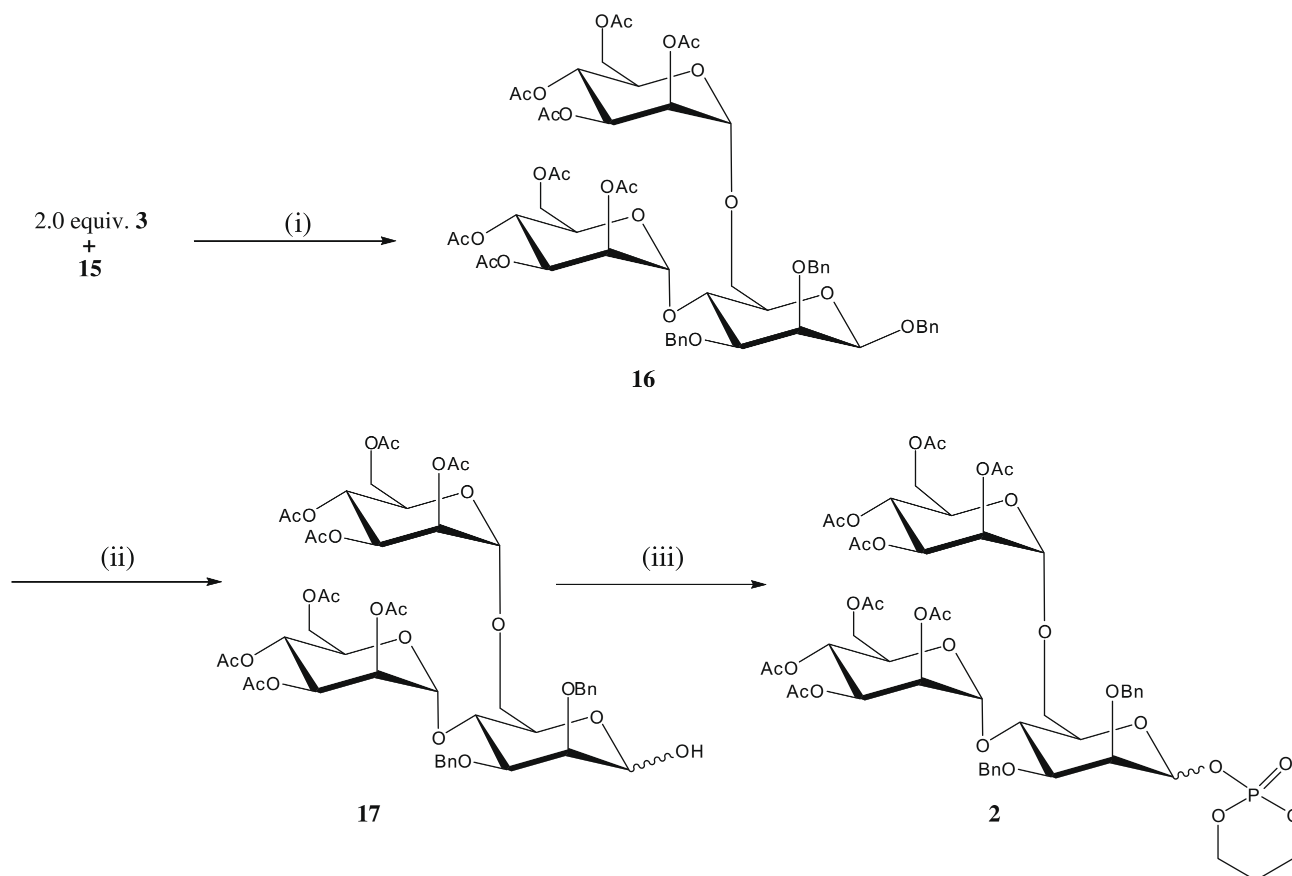
Scheme 5. Reagents and conditions: (i) α,α-dimethoxytoluene, *p*TSA, DMF, 4 h, 50–70 °C (53%); (ii) BnBr, NaH, TBAI, DMF, 24 h, 0 °C to rt (81%); (iii) 80% AcOH, 2 h, 80 °C (90%).

nished the saccharide **14**. Treatment of the latter with 80% acetic acid resulted in the removal of the benzylidene ring and afforded the target glycosyl acceptor **15**.

The partially protected monosaccharide **15** was then coupled with 2.0 equiv of phosphate **3** to give the protected trisaccharide **16** (Scheme 6). Extending the CTH principle, we were pleased when the anomeric benzyl group was selectively removed in good yield to afford **17**. In this process we did not observe any evidence of deprotection of the acetates or secondary benzyl groups. It is important to note that the methanol used was of HPLC grade and not dried methanol stored under molecular sieves. The reason being that the latter system deprotected the acetates, as has been previously reported.[25] Reaction of this compound with propane-1,3-diyldioxyphosphoryl chloride provided our target donor molecule **2**.

3. Conclusion

Whilst not our originally intended target, the synthesis of tetrasaccharide **12** provides further insight into the synthetic utility of glycosyl phosphates as anomeric leaving groups. Normally regarded as being mild coupling agents, these phosphates can be very active and efficient. Furthermore, the nature of this tetrasaccharide presents a cage-like structure which we believe opens up the possibility for encapsulation properties not unlike lipid vesicles; thereby conferring the ability to sequester and deliver small



Scheme 6. Reagents and conditions: (i) 1.5 equiv TMSOTf, DCM, 3 h, −78 °C to rt (77%); (ii) Pd/Al_2O_3, NH_4HCO_2, MeOH, 12 h, rt, (71%); (iii) *N*-MeIm, propane-1,3-diyldioxyphosphoryl chloride, DCM, 0 °C to rt, 24 h (71%).

molecules. Further work is currently being undertaken to examine its novel biological properties.

Additionally, our findings highlight that a benzyl group, not usually considered for protection of the anomeric centre, is indeed useful and is applicable for higher oligosaccharides without erosion of the regioselectivity of the catalytic transfer hydrogenation process. It allows one ready access to a free anomeric hydroxyl, and is compatible with commonly used protecting groups. Work is already underway to examine the β-mannoside selectivity of trisaccharide **2** and will be reported in due course.

4. Experimental

4.1. General experimental

All chemicals used were of reagent or HPLC grade and were used as supplied without prior purification unless otherwise stated. The solvents: DCM, diethyl ether and DMF were dried over calcium hydride for 24 h, distilled and stored over 4 Å molecular sieves. Flash column chromatography was carried out using forced flow of the indicated solvent on Merck (230–400 mesh) silica gel. TLC was performed using pre-coated Silica Gel 60 F_{254} plates; compounds were visualized using acidic ammonium molybdate solution (ammonium molybdate (VI) tetrahydrate (25 g) in 1 M H_2SO_4 (500 ml)). Anhydrous reactions were performed under argon in oven-dried apparatus; anhydrous transfers were done with standard syringe techniques.

^1H, ^{13}C and ^{31}P NMR spectra were recorded on Bruker 600, 400 or 300 MHz spectrometers in the deuterated solvent stated. IR spectra were recorded on a Perkin Elmer Spectrum RXI FT-IR spectrometer. High resolution mass data were obtained using a Bruker Daltonics micrOToF-Q instrument in the electron spray ionization mode. Optical rotations were measured on a Bellingham & Stanley ADP 220 polarimeter at 24 °C. Specific rotations are reported in 10^{-1} deg cm^2/g and concentrations in g/100 mL and for anomeric mixtures the equilibrated (eq), values are reported.

4.1.1. 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-1-*O*-propane-1,3-diyl phosphate 3

The *tetra*-acetylmannoside **11** (1.74 g, 5 mmol) was dissolved in DCM (30 ml) under an argon atmosphere. Propane-1,3-diyldioxyphosphoryl chloride (2.0 g, 12.5 mmol) was added at 0 °C with stirring and after 5 min, *N*-methylimidazole (1.0 ml, 12.5 mmol) was added at 0 °C. Stirring was continued at rt for 24 h under an argon atmosphere. The solvent was removed at 35 °C in vacuo. The residue was redissolved in DCM (30 ml) and washed with saturated $NaHCO_3$ (3 × 25 ml) and water (3 × 25 ml). The DCM layer was dried over Na_2SO_4, filtered and the solvent removed was at 35 °C in vacuo. The resultant residue was purified by flash chromatography (elution with 4:1 ethyl acetate/petroleum ether), Yield = 2.2 g, 93%, colourless thick oil, R_f = 0.27. $[\alpha]_D$ = +33.5 (*c* 1.0, $CHCl_3$). ^1H NMR (400 MHz), ($CDCl_3$): δ 1.86–1.90 (1H, m, J_{P-H} = 15.1 Hz, H_{ax}-5), 2.02 (3H, s, CH_3CO), 2.07 (3H, s, CH_3CO), 2.10 (3H, s, CH_3CO), 2.18 (3H, s, CH_3CO), 2.29–2.33 (1H, m, J_{P-H} = 15.1 Hz, H_{eq}-5), 4.09–4.24 (1H, m), 4.25–4.32 (1H, m), 4.30–4.44 (4H, m), 4.45–4.55 (2H, m), 5.22–5.40 (2H, m), 5.71 (1H, d, $J_{1,2}$ = 6.7 Hz, H-1). ^{13}C NMR (100 MHz), ($CDCl_3$): δ 20.43, 20.55, 20.58, 20.64 (4C, CH_3CO), 25.78 (1C, J_{P-C} = 7.6 Hz), 61.99, 65.36, 68.29, 68.70, 68.80, 69.25, 70.38, 94.80 (1C, C-1, J_{P-C} = 5.0 Hz), 169.49, 169.60, 169.94, 170.60 (4C, C=O). ^{31}P NMR ($CDCl_3$): δ −11.27. HRMS calcd for $C_{17}H_{25}O_{13}PNa$: 491.0930. Found: 491.0756 $(M+Na)^+$.

4.1.2. 2,3-Di-*O*-benzyl-α,β-D-mannopyranoside 5

The mannoside **8** (5.0 g, 10.8 mmol) was added to a solvent mixture of H_2O (60 ml): CH_3CN (45 ml): TFA (45 ml). The system was heated at 98 °C for 48 h. The solvents were removed in vacuo and saturated $NaHCO_3$ was added until effervescence ceased. The mixture was extracted into ethyl acetate (100 ml) and washed with water (4 × 50 ml). It was then dried over Na_2SO_4, filtered and concentrated. The residue was subjected to column chromatography; pet ether/ethyl acetate (1:4) to give 102 (2.9 g, 74%), a colourless oil, as an α:β mixture in the ratio 3.7:1. R_f = 0.22. $[\alpha]_D$ (anomeric mixture, at equilibrium) = −27.2 (*c*, 1.03, $CHCl_3$). [lit.[19] $[\alpha]_D$ = −20.1, α-isomer only, (*c* 1.02, $CHCl_3$)]; ^1H NMR (600 MHz, $CDCl_3$): δ α-(major anomer): 5.20 (1H, s, H-1), 4.59–4.71 (3H, m), 4.56 (1H, d, *J* = 11.4 Hz, PhC*H*$_2$O–), 4.48 (1H, d, *J* = 11.4, Hz PhC*H*$_2$O–), 4.26–4.34 (1H, m), 3.89 (4H, dd, *J* = 9.0 Hz, *J* = 9.6 Hz), 3.72–3.78 (2H, m), 3.29 (1H, bs), 7.26–7.32 (10H, m, Ar); ^{13}C NMR (150 MHz, $CDCl_3$): δ α-: [92.83 (1C, J_{C1-H1} = 169.8 Hz, C-1), 62.79, 67.42, 71.85, 72.52, 72.86, 74.33, 79.27 (7C)]; β-: [94.06 (1C, J_{C1-H1} = 161.9 Hz, C-1), 62.27, 66.78, 72.43, 75.01, 75.59, 75.88, 82.49 (7C)]; α- and β-mixture: 127.82, 127.85, 127.89, 127.99, 128.13, 128.15, 128.22, 128.31, 128.42, 128.53, 128.60, 128.69 (20C, Ar), 137.59, 137.81, 137.99 (4C, Ar *quat*). HRMS (NH_3) calcd for $C_{20}H_{28}NO_6$: 378.1917. Found: 378.1904 $(M+NH_4)^+$.

4.1.3. 2′,3′,4′,6′-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1′→6)-(2″,3″,4″,6″-tetra-*O*-α-D-acetyl-mannopyranosyl-(1″→4))-(2‴,3‴,4‴,6‴-tetra-*O*-acetyl-α-D-mannopyranosyl-(1‴→1))-2,3-di-*O*-benzyl-α-D-mannopyranoside 12

The phosphate **3** (0.7 g, 1.5 mmol) was dissolved in DCM (10 ml) at −78 °C under an argon atmosphere. TMSOTf (0.14 ml, 0.75 mmol) was then added and after 5 min, a solution of the glycosyl acceptor **7** (0.18 g, 0.5 mmol) in DCM (10 ml) was added at −78 °C. The reaction mixture was stirred for 30 min at −78 °C, at 0 °C for a further 30 min and then for 2 h at rt. The solvent was removed in vacuo. The residue was redissolved in DCM (30 ml) and washed with saturated $NaHCO_3$ (3 × 25 ml) and then with water (3 × 25 ml). The DCM layer was dried over Na_2SO_4, filtered and the solvent was removed in vacuo to furnish crude **12**. The residue was purified by column chromatography (elution with 6:4 petroleum ether/ethyl acetate) to yield **12** (0.63 g, 93%) as a dark orange viscous oil, R_f = 0.20. $[\alpha]_D$ = +42.2 (*c* 10.6, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 2.015 (3H, s), 2.020 (3H, s), 2.052 (3H, s), 2.055 (3H, s), 2.060 (3H, s), 2.074 (3H, s), 2.089 (3H, s), 2.095 (3H, s), 2.102 (3H, s), 2.174 (3H, s), 2.177 (3H, s), 2.185 (3H, s), 3.58 (1H, add, *J* = 1.7 Hz, *J* = 5.0 Hz), 3.72 (1H, aq, *J* = 6.7 Hz), 3.79 (1H, add, *J* = 1.3 Hz, *J* = 5.4 Hz), 4.03 (3H, m), 4.10 (2H, dd, *J* = 2.2 Hz, *J* = 5.3 Hz), 4.13 (2H, dd, *J* = 2.3 Hz, *J* = 5.1 Hz), 4.18 (2H, m), 4.28 (4H, m), 4.53 (1H, add, *J* = 7.5 Hz, *J* = 12.2 Hz), 4.60 (1H, dd, *J* = 6.8 Hz), 4.65 (1H, m), 4.75 (1H, m), 5.16 (2H, m), 5.27 (4H, m), 5.34 (5H, td, *J* = 3.1 Hz, *J* = 6.37 Hz), 6.09 (1H, d, *J* = 1.8 Hz), 7.27–7.40 (10H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.140 (12C), 62.54, 62.99, 65.36, 65.93, 66.00, 66.03, 66.07, 68.74, 68.76, 69.11, 69.15, 69.24, 69.51, 69.65, 70.12, 70.15, 71.02, 71.04, 72.12, 74.49, 74.97, 79.30 (22C), 91.02, 93.84, 99.04, 100.75, 128.19, 128.23 (2C), 128.38, 128.71, 128.74, 128.84 (2C), 128.92, 128.94, 138.03, 138.24, 168.49, 169.95, 170.02, 170.12, 170.16, 170.23, 170.37, 170.41, 170.91, 171.01, 171.07, 171.13 (12C, C=O). *m/z* HRMS (EI) calcd for $C_{62}H_{78}O_{33}Na$: 1373.4323 Found: 1373.3870 $(M+Na)^+$.

4.1.4. 4,6-*O*-Benzylidene-1,2,3-tri-*O*-benzyl-β-D-mannopyranoside 14

The partially protected mannoside **13** (5.0 g, 18.6 mmol) was added to DMF (50 ml) and the mixture was stirred at 0 °C for 30 min. NaH in mineral oil (3.0 g, 74.6 mmol) was then added under argon in small portions over 30 min, with the temperature being maintained at 0 °C. Tetrabutyl ammonium iodide (1.72 g, 4.66 mmol) was then added and the mixture was stirred for a further 2 h at 0 °C. Benzyl bromide (9.0 ml, 74.6 mmol) was then added slowly. The reaction mixture was stirred at 0 °C for a further

30 min, and then was allowed to warm to room temperature and was stirred for 24 h. Methanol was added slowly to destroy the excess NaH and the solvents were then removed in vacuo. The residue was subjected to column chromatography; petroleum ether/ethyl acetate (3:2) to furnish **14** as a pale yellow oil (8.1 g, 81%). R_f = 0.64. $[\alpha]_D$ = −14.3 (*c* 0.5, $CHCl_3$) ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.33 (1H, appt.t, *J* = 4.8 Hz), 3.57 (1H, d, *J* = 9.4 Hz), 3.95 (2H, m), 4.24 (1H, t, *J* = 9.4 Hz), 4.35 (1H, pseudot, *J* = 4.8 Hz), 4.51 (1H, s, H-1), 4.58 (2H, d, *J* = 12.3 Hz, PhC*H*$_2$O–), 4.68 (1H, d, *J* = 12.4 Hz, PhCH*H*O–), 4.89 (1H, d, *J* = 12.4 Hz, PhCH*H*O–), 5.00 (2H, d, *J* = 12.6 Hz, PhC*H*$_2$O–), 5.63 (1H, s, PhC*H*O$_2$–), 7.23–7.50 (20H, m, Ar); ^{13}C NMR (150 MHz, $CDCl_3$): δ 67.63, 68.62, 71.09, 72.39, 74.77, 75.87, 76.82, 78.67 (8C), 100.93 (1C, J_{C1-H1} = 155.0 Hz, C-1), 101.43 (PhCHO$_2$–), 127.45, 127.51, 127.61, 127.72, 127.76, 127.80, 127.84, 127.95, 127.99, 128.02, 128.06, 128.10, 128.17, 128.22, 128.29, 128.32, 128.36, 128.42, 128.48, 128.53 (20C, Ar), 137.20, 137.59, 138.32, 138.39 (4C, Ar *quat*). HRMS calcd for $C_{34}H_{35}O_6$: 539.2434. Found: 539.2801 $(M+H)^+$.

4.1.5. 1,2,3-Tri-*O*-benzyl-β-D-mannopyranoside 15

The protected mannoside **14** (7.0 g, 13.0 mmol) was added to 80% AcOH (100 ml) and heated to 85 °C for 3 h. After the system was cooled, the solvent was removed in vacuo. The crude residue was subjected to column chromatography; petroleum ether/ethyl acetate (1:4) to furnish **15** as white crystals (5.3 g, 90%). $[\alpha]_D$ = −29.9 (*c* 0.34, $CHCl_3$) R_f = 0.41. mp = 132.5–133.5 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.26 (1H, br s, OH), 2.42 (1H, s, OH), 3.31 (2H, m), 3.85 (1H, m), 3.98 (3H, m), 4.26 (1H, d, *J* = 12.0 Hz, PhCH*H*O–), 4.47 (1H, d, *J* = 11.4 Hz, PhCH*H*O–), 4.53 (1H, s, H-1), 4.63 (1H, d, *J* = 12.0 Hz, PhCH*H*O–), 4.80 (1H, d, *J* = 12.0 Hz, PhCH*H*O–), 4.98 (2H, d, *J* = 12.6 Hz, PhC*H*$_2$O–), 7.24–7.41 (15H, m, Ar); ^{13}C NMR (150 MHz, $CDCl_3$): δ 63.10, 67.50, 71.03, 71.22, 73.14, 74.18, 75.85, 81.58 (8C), 100.64 (1C, J_{C1-H1} = 157.0 Hz, C-1), 127.62, 127.71, 127.83, 127.92, 127.99, 128.23, 128.38, 128.49, 128.56 (15C, Ar), 137.26, 137.49, 138.36 (3C, Ar *quat*). HRMS calcd for $C_{27}H_{30}O_6$: 450.2042. Found: 450.2043 (M^+).

4.1.6. 2′,3′,4′,6′-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1′→6)-(2″,3″,4″,6″-tetra-*O*-α-D-acetyl-mannopyranosyl-(1″→4))-1,2,3-tri-*O*-benzyl-β-D-mannopyranoside 16

Phosphate **3** (0.20 g, 0.44 mmol) was dissolved in DCM (10 ml) at −78 °C under an argon atmosphere. TMSOTf (0.06 ml, 0.33 mmol) was then added and after 5 min, a solution of the glycosyl acceptor **7** (0.1 g, 0.22 mmol) in DCM (5 ml) was added at −78 °C. The reaction mixture was stirred for 30 min at −78 °C, at 0 °C for a further 30 min and then for 2 h at rt. The solvent was removed in vacuo. The residue was redissolved in DCM (30 ml) and washed with saturated $NaHCO_3$ (3 × 25 ml) and then with water (3 × 25 ml). The DCM layer was dried over Na_2SO_4, filtered and the solvent was removed in vacuo to furnish crude **16**. The residue was purified by column chromatography (elution with 3:2 petroleum ether/ethyl acetate) to yield **16** (0.19 g, 77%) as a dark orange viscous oil, R_f = 0.16. $[\alpha]_D$ = +8.9 (*c*, 2.14, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.896 (3H, s, CH_3CO), 1.899 (3H, s, CH_3CO), 1.978 (3H, s, CH_3CO), 1.980 (3H, s, CH_3CO), 2.055 (3H, s, CH_3CO), 2.057 (3H, s, CH_3CO), 2.14 (3H, s, CH_3CO), 2.15 (3H, s, CH_3CO), 3.26 (1H, dd, *J* = 2.5 Hz, *J* = 9.3 Hz), 3.43 (1H, appt.dd, *J* = 8.4 Hz, *J* = 9.0 Hz), 3.82–3.97 (6H, m), 4.02–4.10 (1H, m), 4.16 (1H, appt.d, *J* = 12.0 Hz), 4.19–4.26 (4H, m), 4.44 (1H, d, *J* = 11.8 Hz), 4.49 (1H, m), 4.63 (1H, dd, *J* = 7.0 Hz, *J* = 11.9 Hz), 4.79 (1H, d, *J* = 12.4 Hz), 4.91–4.95 (1H, m), 4.97–5.02 (3H, m), 5.29–5.33 (3H, m), 5.41 (2H, dd, *J* = 3.6 Hz, *J* = 9.6 Hz), 7.19–7.42 (15H, Ar); ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.30, 20.46, 20.56, 20.67, 20.71, 20.76, 20.90, 20.93 (8C, *C*H$_3$CO), 62.30, 62.61, 65.86, 66.03, 66.06, 66.11, 66.23, 69.09, 69.17, 75.11, 74.61, 74.14, 73.53, 72.68, 71.21, 71.15, 70.69, 81.70 (18C), 100.40 (1C, J_{C1-H1} = 155.4 Hz, C-1, β), 97.13 (1C, J_{C1-H1} = 173.5 Hz, α), 92.04 (1C, J_{C1-H1} = 172.9 Hz, α), 127.48, 127.56, 127.64, 127.80, 127.87, 127.93, 127.99, 128.02, 128.15, 128.21, 128.35, 128.37, 128.49, 128.51, 128.54 (15C, Ar), 137.17, 137.24, 138.49 (3C, Ar *quat*), 169.52, 169.64, 169.81, 169.83, 169.98, 170.01, 170.06, 170.19 (8C, C=O). HRMS calcd for $C_{55}H_{66}O_{24}Na$: 1133.3842. Found: 1133.3492 $(M+Na)^+$.

4.1.7. 2′,3′,4′,6′-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1′→6)-(2″,3″,4″,6″-tetra-*O*-α-D-acetyl-mannopyranosyl-(1″→4))-2,3-tri-*O*-benzyl-α,β-D-mannopyranose 17

At first, Pd/Al_2O_3 (0.08 g) and ammonium formate (0.079 g, 1.25 mmol) were added to methanol (5 ml) and were stirred under an argon atmosphere for 5 min. Compound **16** (0.09 g, 0.08 mmol) was then added and the mixture was stirred at rt for 12 h. The system was then filtered through a pad of Celite, washed with methanol (20 ml) and the solvent was removed in vacuo. The residue was purified by column chromatography (elution with3:2 petroleum ether/ethyl acetate) to yield **17** (0.058 g, 71%) as a colourless oil, R_f = 0.05. $[\alpha]_D$ (at equilibrium) = +95.2 (*c* 0.01, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) for β-anomer: δ 1.97 (6H, s, 2CH_3CO), 2.03 (6H, s, 2CH_3CO), 2.07 (6H, s, 2CH_3CO), 2.14 (6H, s, 2CH_3CO), 3.78 (1H, *a*dd, *J* = 2.9 Hz, *J* = 9.2 Hz), 3.83–3.89 (3H, m), 3.92 (1H, *a*d, *J* = 2.9 Hz), 3.95–4.21 (6H, m), 4.23–4.34 (1H, m), 4.42–4.50 (1H, m), 4.53–4.63 (2H, m), 4.67 (1H, d, *J* = 2.7 Hz), 4.69–4.76 (1H, m), 4.89–4.97 (2H, m), 5.24 (1H, dd, *J* = 2.7 Hz, *J* = 9.5 Hz), 5.28–5.31 (3H, m), 5.33–5.38 (1H, m), 5.40 (1H, *appt*.dd, *J* = 1.6 Hz, *J* = 3.5 Hz), 7.27–7.40 (10H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.85, 20.70 (8C, *C*H$_3$CO), 62.38, 62.51, 65.96, 66.31, 66.83, 67.49, 68.02, 68.44, 68.76, 69.07, 69.20, 69.39, 69.65, 69.85, 71.37, 72.04, 79.34, 92.38, 94.19, 98.09 (20C), 127.71, 127.83, 127.88, 128.02, 128.10, 128.18, 128.38, 128.46, 128.54, 128.69 (10C, Ar), 137.89, 138.09 (2C, Ar *quat*), 169.80, 169.88, 170.01, 170.62, 170.85 (8C, C=O). HRMS calcd for $C_{48}H_{60}O_{24}Na$: 1043.3372. Found: 1043.2567 $(M+Na)^+$.

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