Chemical synthesis of UDP-Glc-2,3-diNAcA, a key intermediate in cell surface polysaccharide biosynthesis in the human respiratory pathogens *B. pertussis* and *P. aeruginosa*†

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In connection with studies on lipopolysaccharide biosynthesis in respiratory pathogens we had a need to access potential biosynthetic intermediate sugar nucleotides. Herein we report the chemical synthesis of uridine 5′-diphospho 2,3-diacetamido-2,3-dideoxy-α-D-glucuronic acid (UDP-Glc-2,3-diNAcA) (**1**) from *N*-acetyl-D-glucosamine in 17 steps and ~9% overall yield. This compound has proved invaluable in the elucidation of biosynthetic pathways leading to the formation of 2,3-diacetamido-2,3-dideoxy-D-mannuronic acid-containing polysaccharides.

Introduction

Bordetella pertussis is an important human respiratory pathogen that causes whooping cough (pertussis).[1] Despite high levels of vaccine coverage in recent years, there has been a worldwide resurgence of *B. pertussis* infection—up *ca.* 15–20 fold compared to 20 years ago. With pertussis continuing to pose a serious threat to infants, and also affecting adolescents and adults, there remains a need for improved diagnosis and treatment.[2,3] Vaccination represents the most practical way to deal with whooping cough, so alternatives to the established lysed cell and acellular vaccines may offer opportunities to address this matter. Analysis of genome sequences for three *Bordetella* spp. gives clues to the differences in host range and the severity of the diseases that they cause.[4] Such top-down analyses may eventually underpin the identification of new vaccine candidates. However, an alternative, bottom-up approach based on knowledge of bacterial cell surface composition is also feasible. Bacterial lipopolysaccharides (LPS)[5] are important bacterial surface components. Immunologically, the carbohydrate component of LPS can give rise to a highly specific immune response, rendering it suitable for use in glycoconjugate vaccines, as is the case for bacterial capsular polysaccharides.[6] However, the reducing terminal lipid A component of LPS is an endotoxin that is capable of causing septic shock.[7] Hence, a source of LPS lacking this core is key to therapeutic utility. In the case of *B. pertussis*, the glycan in question is the so-called Band A trisaccharide, which contains two highly functionalised monosaccharides that are not found in humans (Fig. 1).[8]

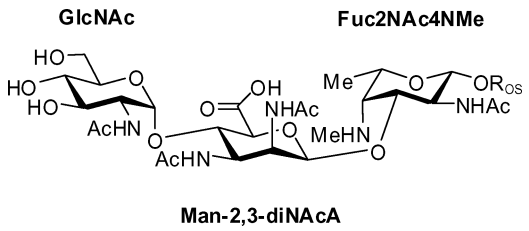

Fig. 1 Band A trisaccharide consisting of *N*-acetylglucosamine (GlcNAc), 2,3-diacetamido-2,3-dideoxy-D-mannuronic acid (Man-2,3-diNAcA) and 2,4-dideoxy-2-*N*-acetyl-4-*N*-methylfucos-2,4-diamine (Fuc2NAc4NMe). R_{os} designates a connection to an oligosaccharide.

The central 2,3-diacetamido-2,3-dideoxy-D-mannuronic acid (Man-2,3-diNAcA) unit of this repeating trisaccharide is also found in the LPS-associated O antigen of *Pseudomonas aeruginosa*,[9,10] an opportunistic pathogen that frequently infects the airways of cystic fibrosis patients.[11] It is thought that *B. pertussis* Band A trisaccharide may make a good vaccine component because it is highly immunogenic, highly conserved, and passive transfer of anti-band A antibodies was protective in a mouse challenge model.[12] Whilst the research scale chemical synthesis of a GlcNAc-Man-2,3-diNAcA disaccharide fragment of Band A trisaccharide has been reported,[13] scale-up would be tortuous. We were drawn to consider biotransformation routes instead, which led us to investigate the biosynthesis of Band A trisaccharide. It has been shown that the biosynthesis of this glycan is dependent on the *Wlb* gene locus,[14] which potentially provides the full repertoire of genes/enzymes necessary to synthesise the key trisaccharide by *in vitro* biotransformation, or with a genetically engineered heterologous expression host.[15] However, review of the initially

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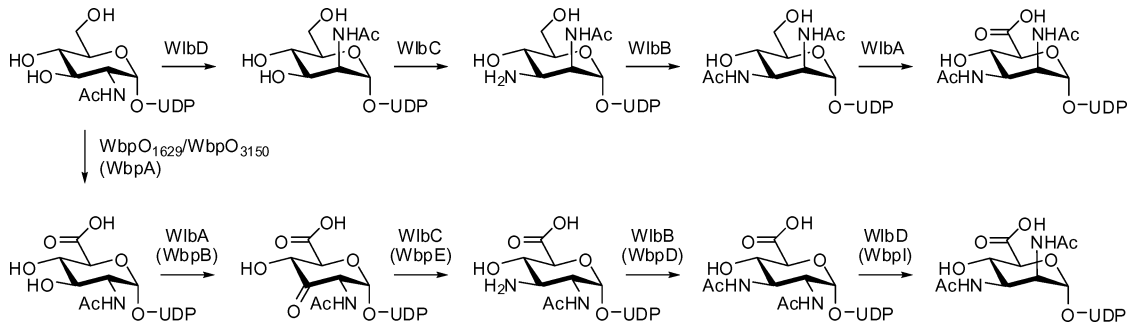


Fig. 2 Biosynthetic pathway for UDP-Man-2,3-diNAcA in *B. pertussis* and *P. aeruginosa* (previously proposed version in upper line and revised version in bottom line). Protein names are indicated in the following order: *B. pertussis* (*P. aeruginosa*).

proposed biosynthetic pathway for uridine 5′-diphospho 2,3-diacetamido-2,3-dideoxy-α-D-mannosuronic acid (UDP-Man-2,3-diNAcA) formation[14] (Fig. 2) alerted us to potential issues arising from the assignment of Wlb gene function based on weak sequence homology to proteins in the Protein Data Bank.

In Gram-negative enterobacteria, such as *Escherichia coli*, ManNAcA is found in the cell-surface enterobacterial common antigen (ECA),[16] where it is derived from uridine 5′-diphospho 2-acetamido-2-deoxy-α-D-mannuronic acid (UDP-ManNAcA). This uronic acid is generated from uridine 5′-diphospho 2-acetamido-2-deoxy-α-D-glucose (UDP-GlcNAc) by the sequential action of UDP-GlcNAc 2-epimerase and UDP-ManNAc 6-dehydrogenase.[17] Hence it seemed reasonable to anticipate that *B. pertussis* would employ a similar order of events to produce *manno*-configured uronic acids (*i.e.* epimerisation preceding oxidation). *B. pertussis* WlbD, with 32% amino acid sequence identity to the *E. coli* UDP-GlcNAc 2-epimerase RffE,[18] seemed a logical choice for the requisite 2-epimerase involved in UDP-Man-2,3-diNAcA biosynthesis. However, despite having established UDP-GlcNAc 2-epimerase assays and validated them with authentic *E. coli* RffE,[18] we were unable to detect epimerase activity with recombinant WlbD,[19] which caused us to review the proposed biosynthetic pathway leading to UDP-Man-2,3-diNAcA. On reflection, we were struck by the proposed replacement of a hydroxyl group with an amino group at the C3 position of UDP-GlcNAc—a proposed single step (bio)transformation that is without precedent, although the 2-step conversion (oxidation plus transamination) of UDP-GlcNAc to uridine 5′-diphospho 2-acetamido-3-amino-2,3-dideoxy-α-D-glucose (UDP-3-NH_2-GlcNAc) has been demonstrated in *Acidithiobacillus ferrooxidans* lipid A biosynthesis.[20] Parallel studies in *P. aeruginosa* led to the biochemical characterization of WbpA, demonstrating it to be an NAD-dependent UDP-*N*-acetyl-D-glucosamine 6-dehydrogenase that generates uridine 5′-diphospho 2-acetamido-2-deoxy-α-D-glucuronic acid (UDP-GlcNAcA).[21] This suggested that, in contrast to ECA biosynthesis, the first committed step in UDP-Man-2,3-diNAcA biosynthesis is the oxidation of UDP-GlcNAc to the corresponding uronic acid, UDP-GlcNAcA. This notion was further supported by studies on the *B. pertussis* WlbA oxidase,‡ which was shown to require a 6-carboxyl group on GlcNAc in order to effect oxidation of the sugar ring.[22] Drawing all of these points together, a revised pathway for the biosynthesis of UDP-Man-2,3-diNAcA from UDP-GlcNAc has been proposed (Fig. 2).[23]

An important intermediate in the new pathway is represented by uridine 5′-diphospho 2,3-diacetamido-2,3-dideoxy-α-D-glucuronic acid (UDP-Glc-2,3-diNAcA) (**1**) (Fig. 3). This sugar nucleotide is not readily available.

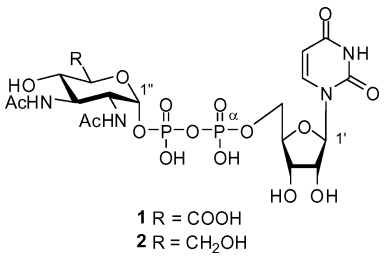


Fig. 3 Uridine 5′-diphospho 2,3-diacetamido-2,3-dideoxy-α-D-glucuronic acid (UDP-Glc-2,3-diNAcA) (**1**) and the precursor sugar nucleotide uridine 5′-diphospho 2,3-diacetamido-2,3-dideoxy-α-D-glucose (UDP-Glc-2,3-diNAc) (**2**). Numbering scheme for NMR purposes.

We were therefore minded to chemically synthesise authentic UDP-Glc-2,3-diNAcA in order to assess its ability to act as a substrate for *B. pertussis* WlbD, and the orthologous *P. aeruginosa* WbpI. A positive result in such experiments would add weight to, and evidence for, the proposed revised biosynthetic pathway leading from UDP-GlcNAc to UDP-Man-2,3-diNAcA. Herein we report the chemical synthesis and spectroscopic characterisation of the key target molecule UDP-Glc-2,3-diNAcA (**1**) and the precursor sugar nucleotide uridine 5′-diphospho 2,3-diacetamido-2,3-dideoxy-α-D-glucose (UDP-Glc-2,3-diNAc) (**2**).

Results and discussion

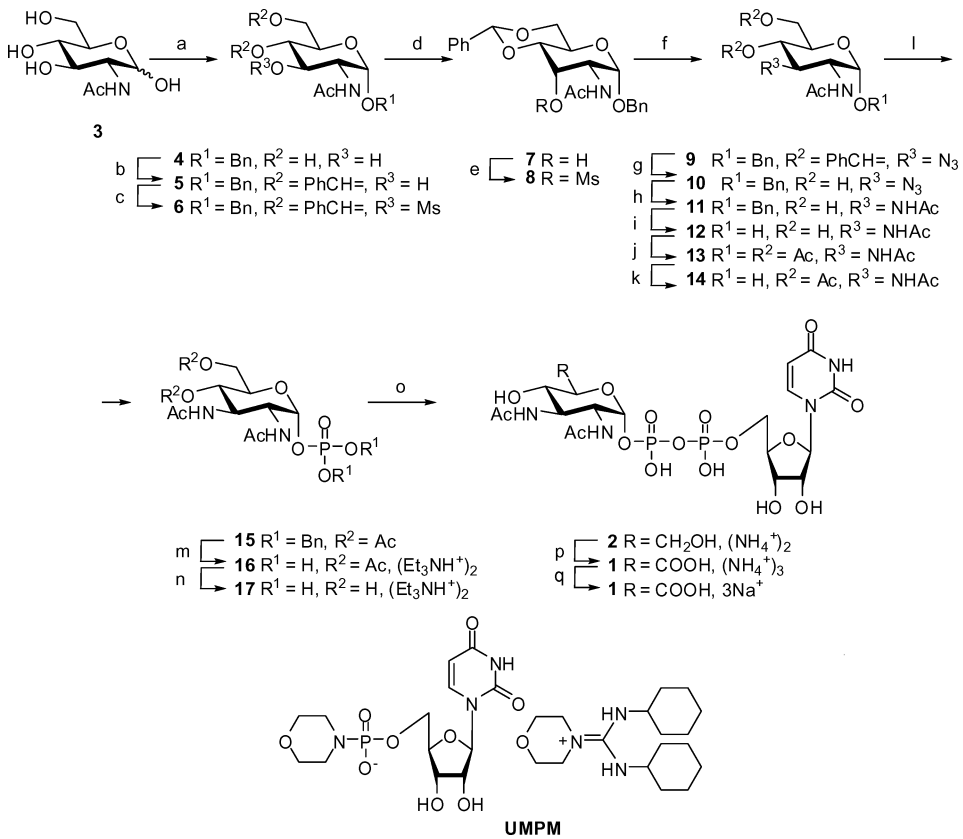
N-Acylated versions of the naturally occurring 2,3-diamino-2,3-dideoxy-D-glucose are found in the lipid A of some *Rhodospirillaceae*.[24] This sugar has previously been synthesised by approaches that involve a double inversion at C-3 of a *gluco*-configured sugar building block, such as methyl 2-benzamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside.[25] Another approach to this sugar has used methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-3-nitro-β-D-glucopyranoside, which was converted by the action of ammonia in aqueous tetrahydrofuran into a mixture of two stereoisomeric nitroamines and separated by fractional crystallisation.[26]

‡ WlbA was originally proposed (Fig. 1)[14] to be required for the generation of the 6-carboxyl group, but this assignment was suspicious, based on the lack of sequence homology to established UDP-sugar 6-dehydrogenases.

A further approach to derivatives of 2,3-diamino-2,3-dideoxy-D-glucose was based on ring opening of the aziridine in methyl 2,3-benzoylepimino-4,6-*O*-benzylidene-2,3-dideoxy-α-D-allopyranoside by sodium azide[27] to form methyl 3-amino-2-benzamido-2,3-dideoxy-α-D-glucopyranoside. An improved version of this approach was employed later[28] in a glycophospholipid synthesis. The 2,3-diacetamido-mannuronate unit has also been synthesised from D-glucose *via* azide opening of a 2,3-epoxide, followed by azide introduction at C-3 by means of double inversion chemistry.[13] In our work, we chose to use an approach based on double inversion at C-3 of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside, involving neighbouring group participation of the C-2 acetamido function.[31] This approach offers full control of the stereochemical outcome of the C-3 double inversion, purification of intermediates is straightforward, as is scale-up to multigram quantities. Overall, our strategy was to prepare the *gluco*-configured 2,3-diacetamido-2,3-dideoxy-hexose as its anomeric phosphate, couple this material to give the corresponding UDP-adduct, and finally effect a selective oxidation of the hexose primary alcohol to the desired uronic acid.

Commercially available *N*-acetyl-D-glucosamine (**3**) was chosen as a starting material. This material was converted to the corresponding benzyl glycoside **4** following a literature procedure[29] and the C-4/6 hydroxyl groups were protected,[30] giving benzylidene derivative **5** (Scheme 1). The C-3 hydroxyl group of **5** was converted into an acetamido derivative **12** with the required *gluco*-configuration by a double inversion at C-3. This involved initial mesylation, followed by acetate displacement with *in situ* de-*O*-acetylation to form the *allo*-configured intermediate **7**, installation of the C-3 azide **9** *via* a mesylate, benzylidene deprotection, azide reduction followed by acetylation, and finally hydrogenation to cleave the benzyl glycoside to yield **12**. These transformations followed a literature procedure[31] with some modifications: tetrabutylammonium hydrogen sulfate (TBAHS)-assistance[32] was required for the efficient formation of azide **9**, and hydrogenation of benzyl glycoside **11** to give the free sugar **12** was more effective when using Pearlman's catalyst than with palladium on charcoal. An attempt to selectively deprotonate the anomeric hydroxyl group of the free sugar **12** using LDA in a mixture of THF and DMF and to react this species *in situ* with tetrabenzyl pyrophosphate[33,34] resulted in a low yield of the desired dibenzyl sugar-1-phosphate, along with a number of unidentified side products.

Therefore the deprotected sugar **12** was first peracetylated, to give **13**, followed by selective deacetylation of the anomeric position using hydrazine acetate in DMF[35] to give hemiacetal **14** in 88% yield. This time, deprotonation of the anomeric hydroxyl group in **14** with LDA in THF followed by treatment



Scheme 1 *Reagents and conditions:* (a) BnOH, $HCl_{(g)}$; (b) PhCHO, $ZnCl_2$, 89% over 2 steps; (c) MsCl, pyridine; (d) NaOAc, methyl cellosolve, H_2O, 89% over 2 steps; (e) MsCl, pyridine; (f) NaN_3, DMF, TBAHS, 82% over 2 steps; (g) 80% AcOH, reflux; (h) H_2, Pd-C then Ac_2O, 84% over 2 steps; (i) H_2, $Pd(OH)_2$, MeOH, 92%; (j) Ac_2O, pyridine, DMAP, 71%; (k) NH_2-NH_2–AcOH, DMF, rt to 60 °C, 88%; (l) LDA, THF then $[(BnO)_2PO]_2O$, 92%; (m) Pd-C, H_2, MeOH then Et_3N, 88%; (n) MeOH–H_2O–Et_3N 7 : 3 : 1, 4 days, quantitative; (o) uridine 5′-monophosphomorpholidate 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt (UMPM), pyridine, 4 °C, 14 days then SAX HPLC (gradient NH_4HCO_3), 49%; (p) Pt, O_2, 100 °C, $NaHCO_3$ then SAX HPLC, 69%; (q) Dowex 50Wx8-200 (Na^+ form), quantitative.

of the resulting lithium salt with tetrabenzyl pyrophosphate, gave smoothly the desired sugar-1-phosphate **15** in 92% yield after chromatography on silica gel. This material proved to be solely the α-anomer, as judged by ^{1}H NMR spectroscopy (H1, dd, $^3J_{1,2}$ = 3.2 Hz, $^3J_{1,P}$ = 5.6 Hz). Removal of the benzyl protecting groups, followed by addition of Et_3N yielded a bistriethylammonium salt of **16** in 88% yield. Selective *O*-deacetylation gave the deprotected sugar-1-phosphate **17** in quantitative yield.

With the sugar-1-phosphate **17** in hand, the next stage of the preparation required installation of the sugar nucleotide pyrophosphate bridge. Initially the enzymatic formation of the pyrophosphate bond in **2** was attempted using the galactose-1-phosphate uridyltransferase (Gal-1-PUT) protocol reported by Errey *et al.*[36] Although Gal-1-PUT has been shown to possess a remarkably relaxed substrate specificity, on this occasion 2,3-diacetamido sugar phosphate **17** proved not to be a substrate for this enzyme. The formation of the pyrophosphate bond in **2** was therefore achieved by chemical means. The bistriethylammonium salt§ of **17** was treated with uridine 5′-*O*-monophosphomorpholidate 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt in dry pyridine at 4 °C,[37,38] the reaction being monitored by strong anion exchange HPLC. After 2 weeks the conversion reached ~82%. The sugar nucleotide was then isolated in 49% yield¶ by strong anion exchange chromatography, as an ammonium salt of **2** and characterised by NMR (see also Table 1 and 2 in ESI†). ^{31}P NMR spectrum confirmed successful formation of the pyrophosphate moiety (two doublets at δ −7.60 (P_β, $J_{P\alpha,P\beta}$ = 20.5 Hz) and δ −9.38 (P_α, $J_{P\alpha,P\beta}$ = 20.5 Hz)).

The final step in the synthesis required conversion of the hexose unit to the corresponding uronic acid. Using a procedure that we reported recently[39] for the synthesis of UDP-GlcNAcA from UDP-GlcNAc, the primary hydroxyl group in **2** was selectively oxidised using platinum-catalysed oxidation with molecular oxygen to give the desired uronic acid. Whilst the practical oxidation of 2-acetamido-2-deoxy-sugar glycosides to the corresponding uronic acids can be effected with TEMPO-mediated oxidation,[40] in our hands the oxidation of the corresponding sugar nucleotides proved problematic. However, this issue was resolved by resorting to the more forcing conditions of Pt/O_2 in boiling water.[39] Again, the course of the reaction was monitored by anion exchange HPLC. After 24 h the conversion typically reached ~35%. A new batch of the catalyst was then added and the reaction was continued for a further 24 h, when the conversion reached ~70%. The uronic acid was then isolated, by strong anion exchange chromatography, as the triammonium salt of **1** in 69% yield. In ^{13}C NMR spectra of **2** (diammonium salt) the C-6″ signal changes markedly (δ 60.9 ppm, t, becomes 176.7 ppm, s) on oxidation to **1**. Moreover, on oxidation to **1** (triammonium salt) the C-4″ signal in **2** (diammonium salt) sustained a downfield shift (from 67.9 to 70.6 ppm), a behaviour typical for the transformation of hexoses into the corresponding uronic acids.[41] Interestingly, the triammonium salt of uronic acid **1** exhibited limited water solubility and consequently some spectral analyses were difficult to perform (especially ^{13}C NMR). In addition ES-MS of the triammonium salt showed a very weak molecular ion and HR-MS failed altogether. For these reasons, the triammonium salt of **1** was converted into the much more water soluble trisodium salt by treatment with a cation exchange resin (Dowex 50Wx8-200, Na^+ form). The trisodium salt of **1** gave a stronger ^{13}C NMR spectrum and reliable MS data.

Synthetic UDP-Glc-2,3-diNAcA (**1**) has been subjected to a number of biochemical analyses, including its assessment in (*B. pertussis*) WlbD- and (*P. aeruginosa*) WbpI-mediated epimerisation to give UDP-Man-2,3-diNAcA, as shown by capillary electrophoresis and NMR spectroscopy.[23] Neither enzyme utilised UDP-GlcNAc, UDP-GlcNAcA or UDP-Glc-2,3-diNAc (**2**) as substrates. With these authentic sugar nucleotides in hand, we have also been able to demonstrate that the biosynthesis of the di-*N*-acetylated sugar in the lipopolysaccharides of both *B. pertussis* and *P. aeruginosa* occurs *via* an identical route, despite differences in the arrangement of the gene clusters concerned.[45] Further, we have been able to reconstitute the UDP-MandiNAcA biosynthetic pathway *in vitro*.[42] Beyond assessment of their direct physiological function, synthetic materials generated in this study have also been useful in studies aimed at predicting protein function from structure in the short chain dehydrogenase/reductase family[43] and for exploring the potential of *Pasteurella* heparosan synthases for the chemoenzymatic synthesis of distinct monodisperse polymers with unnatural structures.[44]

Conclusions

In conclusion, starting from *N*-acetyl-D-glucosamine the title compound UDP-Glc-2,3-diNAcA (**1**) was synthesised in 17 steps and in an overall yield of 9%. Using this compound we have been able to provide evidence that *B. pertussis* WlbD and *P. aeruginosa* WbpI are uridine diphosphate 2,3-diacetamido-2,3-dideoxy-α-D-glucuronic acid 2-epimerases.[23,45] This observation, together with evidence for several related sugar nucleotides not being substrates for these enzymes, has allowed us to confirm a revised biosynthetic pathway for the synthesis of UDP-Man-2,3-diNAcA.[23] The availability of authentic, synthetic materials has been key to these developments.

Experimental

General procedures

All chemicals were purchased as reagent grade and used without further purification. TLC were performed on precoated silica plates containing a fluorescence indicator. Silica gel (63 μm) was used for analytical TLC. Compounds were visualised under UV (254 nm) and by heating after dipping in a solution of 5% H_2SO_4 in EtOH.

HPLC was performed on a Biocad Sprint Perfusion Chromatography instrument. For analytical purposes or small scale separations the mixtures were applied on a Poros HQ 50 strong anion exchange column (10 × 50 mm, CV ~4 mL). The column was first equilibrated with 5 CV of 5 mM NH_4HCO_3 buffer, then the mixture was applied and eluted with linear gradient of NH_4HCO_3 from 5 mM to 250 mM in 15 CV, then with NH_4HCO_3 250 mM in

§ The triethylammonium counterion in **17** facilitates the solubility of the salt in pyridine and thus substantially improves the yield of the pyrophosphate formation. Attempts to perform this reaction with the sodium salt resulted in much lower yields.

¶ Some material was lost in the separation due to co-eluting impurities.

5 CV at a flow rate of 12 mL min^{-1} and detection with an on-line detector to monitor A_{265}. Finally, the column was washed with 3 CV of 1 M NH_4HCO_3 followed by 1 CV of 5 mM NH_4HCO_3 at the same flow rate after each run. In the case of large scale separations a Poros HQ 20 strong anion exchange column (16 × 100 mm, CV ~20 mL) was used under similar conditions (with a different flow rate of 30 mL min^{-1} and detection with an on-line detector to monitor A_{280}).

NMR spectra were recorded on a Varian Unity Plus spectrometer at 400 MHz (^{1}H) or 100.6 MHz (^{13}C), or on a Bruker Avance DPX-300 MHz NMR spectrometer at 121 MHz (^{31}P). ^{1}H NMR spectra were referenced to δ_H 7.26 for $CDCl_3$, δ_H 3.34 for CD_3OD, δ_H 4.63 ppm for D_2O, and ^{13}C NMR spectra were referenced to δ_C 77.36 for $CDCl_3$ and δ_C 49.86 for CD_3OD. ^{31}P NMR spectra were referenced to δ_P 0.0 for external 85% phosphoric acid. Chemical shifts of NMR signals recorded in D_2O are reported with respect to the methyl resonance of internal acetone at δ_H 2.22 ppm and δ_C 30.89 ppm. Assignments were made with the aid of gCOSY and gHSQC experiments. Coupling constants are reported in Hz. In the NMR spectra of anomeric mixtures, only those signals attributable to the minor anomer that are clearly discernable in 1D spectra are reported. Optical rotations were measured at ambient temperature on a Perkin-Elmer model 141 polarimeter using a sodium lamp. Accurate mass electrospray ionization mass spectra (ESI-MS) were obtained from the EPSRC National Mass Spectrometry Service Centre, Swansea using positive ionization mode on a Finnigan MAT 900 XLT mass spectrometer or from the John Innes Centre metabolite analysis service on a Thermo Finningan DecaXP^{plus} mass spectrometer. For full experimental data related to compounds **5**, **7**, **11** and **12** see ESI†.

Attention. Extreme care should be observed when catalytic hydrogenation is performed. Before any manipulation (addition of new catalyst or work-up), hydrogen was removed by a water aspirator and replaced by nitrogen (repeated 3 times). To prevent self-ignition, the catalyst was collected on a filter paper, wetted with a small amount of water and sealed in an air-tight container.

Acetyl 2,3-diacetamido-4,6-di-*O*-acetyl-2,3-dideoxy-α,β-D-glucopyranoside (13). To a suspension of 2,3-diacetamido-2,3-dideoxy-α,β-D-glucose (**12**) (565 mg, 2.2 mmol) in pyridine (20 mL), were added 4-dimethylaminopyridine (1 mg, 0.4 mol%) and acetic anhydride (2.0 mL, 21.5 mmol). The mixture was stirred at room temperature overnight. MeOH (1 mL) was added and the volatiles were evaporated under reduced pressure. Traces of pyridine were co-evaporated with toluene (2 × 5 mL). The residue was dissolved in ethyl acetate (10 mL), washed with 0.5 M HCl (5 mL), water (5 mL) and dried over $MgSO_4$. The aqueous layer was saturated with NaCl and extracted with dichloromethane (4 × 20 mL) and dried over $MgSO_4$. The combined organic extracts were evaporated under reduced pressure and the residue was purified using column chromatography on silica gel (dichloromethane–MeOH 10 : 1) to give pure **13** (593 mg, 71%) as an oil in a 8 : 1 α–β equilibrium (by ^{1}H NMR). R_f = 0.86 for β and 0.82 for α (dichloromethane–methanol 5 : 1); $[\alpha]_D^{23}$ +56.0 (*c* 1.0, $CHCl_3$); δ_H (400 MHz; $CDCl_3$; TMS) 6.75 (d, $^3J_{2,NH}$ = 9.2 Hz, 2$_\beta$–NH), 6.46 (d, $^3J_{3,NH}$ = 9.2 Hz, 3$_\beta$–NH), 6.37 (d, $^3J_{3,NH}$ = 8.4 Hz, 3$_\alpha$–NH), 6.28 (d, $^3J_{2,NH}$ = 8.4 Hz, 2$_\alpha$–NH), 6.10 (d, $^3J_{1,2}$ = 3.2 Hz, H1$_\alpha$), 5.61 (d, $^3J_{1,2}$ = 8.8 Hz, H1$_\beta$), 4.89 (t, $^3J_{4,5}$ = $^3J_{3,4}$ = 10.0 Hz, H4$_\alpha$), 4.43–4.35 (m, H3$_\alpha$), 4.31–4.21 (m, H2$_\alpha$, H6$_\alpha$), 4.19–4.13 (m, H2$_\beta$), 4.06–3.97 (m, H5$_\alpha$, H6$_\alpha$), 2.13, 2.01, 2.00, 1.88, 1.87 (s, 2 × C*H*$_3$CONH α anomer, 3 × C*H*$_3$(CO)O α anomer); δ_C (100 MHz; $CDCl_3$; TMS) 170.9, 169.9, 169.7, 169.6, 168.0 (s, 5C, 2 × CH$_3$*C*ONH α anomer, 3 × CH$_3$(*C*O)O α anomer), 92.0 (d, 1C, C1$_\beta$), 89.3 (d, 1C, C1$_\alpha$), 69.1 (d, 1C, C5$_\alpha$), 66.9 (d, 1C, C4$_\alpha$), 60.8 (t, 1C, C6$_\alpha$), 50.9 (d, 1C, C2$_\alpha$), 49.6 (d, 1C, C3$_\alpha$), 22.2, 21.9, 20.0, 19.7, 19.6 (q, 5C, 2 × *C*H$_3$CONH α anomer, 3 × *C*H$_3$(CO)O α anomer); *m*/*z* (CI^+) 406 ([M + NH_4]$^+$, 23.1%), 389 ([M + H]$^+$, 26.3), 329 (46.2), 77 (100); HR-MS calcd for $C_{16}H_{25}N_2O_9$ [M + H]$^+$ 389.1555, found 389.1559.

2,3-Diacetamido-4,6-di-*O*-acetyl-2,3-dideoxy-α,β-D-glucose (14). Acetyl 2,3-diacetamido-4,6-di-*O*-acetyl-2,3-dideoxy-α,β-D-glucopyranoside (**13**) (525 mg, 1.35 mmol) was dissolved in *N*,*N*-dimethylformamide (15 mL) and hydrazine hydrate (73 μL, 1.5 mmol) followed by glacial acetic acid (83 μL, 1.5 mmol) were added. The mixture was stirred for 20 h at room temperature but TLC showed incomplete conversion. For this reason, another aliquot of hydrazine hydrate (73 μL, 1.5 mmol) followed by glacial acetic acid (83 μL, 1.5 mmol) were added and the mixture was heated to 60 °C for 3 h when the conversion was complete (by TLC). The volatiles were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (dichloromethane–MeOH gradient from 20 : 1 to 5 : 1) to give pure **14** (413 mg, 88%) as an amorphous solid in a 7 : 1 α–β equilibrium (by ^{13}C NMR). R_f = 0.76 for α and 0.74 for β (dichloromethane–methanol 5 : 1); $[\alpha]_D^{23}$ +51.7 (*c* 1.0, MeOH); δ_H (400 MHz; MeOH-d_4; referenced to solvent residual peak at 3.34 ppm) 5.12 (d, $^3J_{1,2}$ = 3.2 Hz, H1$_\alpha$), 4.94–4.89 (m, H4$_\alpha$ overlapping with HDO signal), 4.74 (d, $^3J_{1,2}$ = 8.8 Hz, H1$_\beta$), 4.42–4.37 (m, H3$_\alpha$), 4.29–4.24 (m, H5$_\alpha$, H6$_{a\alpha}$), 4.11–4.06 (m, H2$_\alpha$, H6$_{b\alpha}$), 2.07, 2.04 (s, 2 × C*H*$_3$CONH α anomer), 1.97, 1.90 (s, 2 × C*H*$_3$(CO)O α anomer); δ_C (100 MHz; MeOH-d_4; referenced to solvent peak at 49.86 ppm) 174.8, 174.4, 173.3, 172.5 (s, 4C, 2 × CH$_3$*C*ONH α anomer, 2 × CH$_3$(*C*O)O α anomer), 98.2 (d, 1C, C1$_\beta$), 92.9 (d, 1C, C1$_\alpha$), 71.6 (d, 1C, C4$_\alpha$), 69.5 (d, 1C, C5$_\alpha$), 64.7 (t, 1C, C6$_\alpha$), 54.4 (d, 1C, C2$_\alpha$), 51.9 (d, 1C, C3$_\alpha$), 23.6, 23.3 (q, 2C, 2 × *C*H$_3$(CO)O α anomer), 21.5 (q, 2C, 2 × *C*H$_3$CONH α anomer); *m*/*z* (CI^+) 364 ([M + NH_4]$^+$, 3.4%), 347 ([M + H]$^+$, 10.6), 77 (100); HR-MS calcd for $C_{14}H_{23}N_2O_8$ [M + H]$^+$ 347.1449, found 347.1450.

2,3-Diacetamido-4,6-di-*O*-acetyl-2,3-dideoxy-α-D-glucopyranose 1-dibenzylphosphate (15). 2,3-Diacetamido-4,6-di-*O*-acetyl-2,3-dideoxy-α,β-D-glucose (**14**) (698 mg, 2.02 mmol) was azeotropically dried with THF and cooled to −78 °C in absolute, freshly distilled THF (120 mL), and lithium diisopropylamide (1.455 mL, 2.93 mmol of a 2 M solution in heptane–THF–ethylbenzene) was added. After 10 min, tetrabenzyl pyrophosphate (1.52 g, 2.8 mmol) was added. The reaction was allowed to stir for 40 min and then warmed to 4 °C and allowed to stir for a further 12 h. The precipitated solid (lithium salt of dibenzyl phosphate) was filtered off, washed with ethyl acetate and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (20 mL) and washed with sat. aq. solution of $NaHCO_3$ (10 mL) and brine (10 mL), and then dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified using column chromatography on silica gel (dichloromethane–MeOH 20 : 1) to give pure **15** (1.13 g, 92%) as a pale yellow oil. R_f = 0.63 (dichloromethane–methanol 10 : 1); $[\alpha]_D^{23}$ +52.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz; $CDCl_3$; referenced to solvent residual peak at 7.26

ppm) 7.41–7.38 (m, 10H, aromatic), 6.11 (d, 1H, $^3J_{2,NH} = 8.8$ Hz, 2–NH), 5.86 (d, 1H, $^3J_{3,NH} = 8.8$ Hz, 3–NH), 5.69 (dd, 1H, $^3J_{1,2} = 3.2$ Hz, $^3J_{1,P} = 5.6$ Hz, H1), 5.16–5.04 (m, 4H, 2 × CH_2Ph), 4.88 (dd, 1H, $^3J_{4,5} = 10.4$ Hz, $^3J_{3,4} = 9.6$ Hz, H4), 4.39 (ddd, 1H, $^3J_{3,4} = 9.6$ Hz, $^3J_{2,3} = 10.0$ Hz, $^3J_{3,NH} = 8.8$ Hz, H3), 4.24–4.15 (m, 2H, H2, $H6_a$), 4.08–4.02 (m, 1H, H5), 4.91–3.87 (m, 1H, $H6_b$), 2.07, 2.02 (s, 6H, 2 × CH_3CONH), 1.92, 1.75 (s, 6H, 2 × CH_3(CO)O); δ_C (100 MHz; $CDCl_3$; referenced to solvent peak at 77.36 ppm) 171.2, 171.0, 170.7 (s, 4C, 2 × CH_3CONH, 2 × $CH_3(CO)O$), 135.5 (ds, 2C, $^3J_{C\ arom.,P} = 6.0$ Hz, aromatic), 129.2, 129.1, 129.0, 128.3, 128.3 (d, 10C, aromatic), 96.0 (dd, 1C, $^2J_{C1,P} = 6.0$ Hz, C1), 70.2 (dt, 2C, $^2J_{CH2,P} = 5.4$ Hz, 2 × CH_2Ph), 70.2 (d, 1C, C5), 67.8 (d, 1C, C4), 61.5 (t, 1C, C6), 52.9 (dd, 1C, $^3J_{C2,P} = 7.6$ Hz, C2), 50.7 (d, 1C, C3), 23.4, 23.0 (q, 2C, 2 × CH_3(CO)O), 20.9, 20.8 (q, 2C, 2 × CH_3CONH); *m/z* (ESI$^+$) 629 ([M + Na]$^+$, 43.2%), 607 ([M + H]$^+$, 6.8), 329 (91.9), 150 (100); HR-MS calcd for $C_{28}H_{36}N_2O_{11}P$ [M + H]$^+$ 607.2051, found 607.2053.

2,3-Diacetamido-4,6-di-*O*-acetyl-2,3-dideoxy-α-D-glucopyranose-1-phosphate, bistriethylammonium salt (16). 2,3-Diacetamido-4,6-di-*O*-acetyl-2,3-dideoxy-α-D-glucopyranose 1-dibenzylphosphate (**15**) (308 mg, 0.51 mmol) was dissolved in MeOH (10 mL) and palladium, 10% on carbon (20 mg) was added. The mixture was hydrogenated for 24 h at 1 atm pressure. The catalyst was removed by filtration through 2 filter papers and washed with MeOH. To the filtrate triethylamine (1.2 mL) was added and the volatiles were evaporated under reduced pressure to give pure **16** (281 mg, 88%) as a white amorphous solid. $R_f = 0.59$ (dichloromethane–methanol–H_2O 6 : 3 : 1); $[\alpha]_D^{23}$ +53.2 (*c* 1.0, MeOH); δ_H (400 MHz; CH_3OH-d_4; referenced to solvent residual peak at 3.34 ppm) 5.52 (dd, 1H, $^3J_{1,2} = 2.8$ Hz, $^3J_{1,P} = 7.2$ Hz, H1), 5.03 (dd, 1H, $^3J_{4,5} = 10.0$ Hz, $^3J_{3,4} = 10.0$ Hz, H4), 4.43 (dd, 1H, $^3J_{3,4} = 10.0$ Hz, $^3J_{2,3} = 10.0$ Hz, H3), 4.36 (dt, 1H, $^3J_{4,5} = 10.0$ Hz, $^3J_{5,6} = 2.6$ Hz, H5), 4.29 (dd, 1H, $^2J_{6a,6b} = 9.2$ Hz, $^3J_{5,6} = 2.6$ Hz, $H6_a$), 4.17–4.13 (m, 2H, H2, $H6_b$), 3.13 (q, 12H, $^3J_{CH2,CH3} = 7.2$ Hz, 2 × $(CH_3CH_2)_3NH^+$), 2.06, 2.03 (s, 6H, 2 × CH_3CONH), 1.97, 1.88 (s, 6H, 2 × CH_3(CO)O), 1.32 (t, 18H, $^3J_{CH3,CH2} = 7.2$ Hz, 2 × $(CH_3CH_2)_3NH^+$); δ_C (100 MHz; CH_3OH-d_4; referenced to solvent peak at 49.86 ppm) 174.6, 174.4, 173.2, 172.5 (s, 4C, 2 × CH_3CONH, 2 × $CH_3(CO)O$), 95.2 (dd, 1C, $^3J_{C1,P} = 5.3$ Hz, C1), 71.1 (d, 1C, C4), 70.5 (d, 1C, C5), 64.2 (t, 1C, C6), 54.6 (dd, 1C, $^3J_{C2,P} = 6.8$ Hz, C2), 53.2 (d, 1C, C3), 47.8 (t, 6C, 2 × $(CH_3CH_2)_3NH^+$), 23.6, 23.5 (q, 2C, 2 × $CH_3(CO)O$), 21.6 (q, 2C, 2 × CH_3CONH), 10.1 (q, 6C, 2 × $(CH_3CH_2)_3NH^+$); *m/z* (ESI$^+$) 629 ([M + H]$^+$, 10.1%), 528 ([M–Et_3NH + 2H]$^+$, 19.8), 102 (100); HR-MS calcd for $C_{26}H_{54}N_4O_{11}P$ [M + H]$^+$ 629.3521, found 629.3519.

2,3-Diacetamido-2,3-dideoxy-α-D-glucopyranose-1-phosphate, bistriethylammonium salt (17). 2,3-Diacetamido-4,6-di-*O*-acetyl-2,3-dideoxy-α-D-glucopyranose-1-phosphate, bistriethylammonium salt (**16**) (281 mg, 0.45 mmol) was dissolved in a 7 : 3 : 1 mixture of MeOH–H_2O–Et_3N (11 mL) and stirred at room temperature. After 4 days the conversion was complete (by TLC). The volatiles were evaporated under reduced pressure to give **17** (243 mg, quantitative) as a pale yellow solid that was sufficiently pure for analysis. $R_f = 0.41$ (dichloromethane–methanol–H_2O 6 : 3 : 1); $[\alpha]_D^{23}$ +27.9 (*c* 1.0, MeOH); δ_H (400 MHz; CH_3OH-d_4; referenced to solvent residual peak at 3.34 ppm) 5.50 (dd, 1H, $^3J_{1,2} = 2.0$ Hz, $^3J_{1,P} = 6.0$ Hz, H1), 4.23 (dd, 1H, $^3J_{3,4} = 10.4$ Hz, $^3J_{2,3} = 11.2$ Hz, H3), 4.04 (td, 1H, $^3J_{2,3} = 11.2$ Hz, $^3J_{1,2} = 2.0$ Hz, H2), 3.97 (dd, 1H, $^3J_{4,5} = 9.4$ Hz, $^3J_{5,6} = 4.0$ Hz, H5), 3.86 (bd, 1H, $^2J_{6a,6b} = 11.8$ Hz, $^3J_{5,6b} \approx 0$ Hz, $H6_b$), 3.73 (dd, 1H, $^2J_{6a,6b} = 11.8$ Hz, $^3J_{5,6a} = 4.0$ Hz, $H6_a$), 3.50 (dd, 1H, $^3J_{4,5} = 9.4$ Hz, $^3J_{3,4} = 10.4$ Hz, H4), 3.19 (q, 12H, $^3J_{CH2,CH3} = 7.2$ Hz, 2 × $(CH_3CH_2)_3NH^+$), 1.97 (s, 6H, 2 × CH_3CONH), 1.33 (t, 18H, $^3J_{CH3,CH2} = 7.2$ Hz, 2 × $(CH_3CH_2)_3NH^+$); δ_C (100 MHz; CH_3OH-d_4; referenced to solvent peak at 49.86 ppm) 175.2, 174.5 (s, 2C, 2 × CH_3CONH), 95.7 (dd, 1C, $^3J_{C1,P} = 6.1$ Hz, C1), 75.8 (d, 1C, C5), 70.4 (d, 1C, C4), 63.4 (t, 1C, C6), 54.9 (d, 1C, C3), 54.8 (dd, 1C, $^3J_{C2,P} = 10.0$ Hz, C2), 48.3 (t, 6C, 2 × $(CH_3CH_2)_3NH^+$), 23.7, 23.5 (q, 2C, 2 × CH_3CONH), 10.0 (q, 6C, 2 × $(CH_3CH_2)_3NH^+$); *m/z* (ESI$^+$) 545 ([M + H]$^+$, 12.2%), 444 ([M–Et_3NH + 2H]$^+$, 63.5), 267 (100); HR-MS calcd for $C_{22}H_{50}N_4O_9P$ [M + H]$^+$ 545.3310, found 545.3313.

UDP-2,3-Diacetamido-2,3-dideoxy-α-D-glucose, diammonium salt (2). 2,3-Diacetamido-2,3-dideoxy-α-D-glucopyranose-1-phosphate, bistriethylammonium salt (**17**) (243 mg, 0.45 mmol) and uridine 5′-*O*-monophosphomorpholidate 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt (287 mg, 0.417 mmol) were separately dried by co-evaporation with absolute pyridine (2 × 5 mL). Both components were dissolved in absolute pyridine (7 mL each) and the solutions were combined. The resulting mixture was kept without stirring at 4 °C for 14 days. The conversion reached 82% (by SAX HPLC on Poros HQ50, for details see General procedures in the Experimental section). The mixture was concentrated to dryness under reduced pressure. The resulting syrup was dissolved in water (1 mL) and purified using SAX HPLC on Poros HQ20 as indicated in the General procedures in the Experimental section to give pure diammonium salt of **2** (138 mg, 49%). δ_H (400 MHz; D_2O; referenced to the methyl resonance of internal acetone at 2.22 ppm) 7.93 (d, 1H, $^3J_{5,6} = 8.2$ Hz, H6), 6.00 (d, 1H, $^3J_{1',2'} = 4.0$ Hz, H1′), 5.94 (d, 1H, $^3J_{5,6} = 8.2$ Hz, H5), 5.53 (dd, 1H, $^3J_{1'',P\beta} = 7.2$ Hz, $^3J_{1'',2''} = 3.2$ Hz, H1″), 4.38–4.34 (m, 2H, H2′, H3′), 4.29–4.27 (m, 1H, H4′), 4.25–4.23 (m, 1H, $H5'_a$), 4.22–4.19 (m, 1H, $H5'_b$), 4.12 (dd, 1H, $^3J_{3'',4''} = 9.6$ Hz, $^3J_{2'',3''} = 11.2$ Hz, H3″), 4.08 (dt, 1H, $^3J_{2'',3''} = 11.2$ Hz, $^3J_{1'',2''} = 3.2$ Hz, $^4J_{2'',P\beta} = 2.8$ Hz, H2″), 3.98 (ddd, 1H, $^3J_{4'',5''} = 10.0$ Hz, $^3J_{5'',6''a} = 4.0$ Hz, $^3J_{5'',6''b} = 2.4$ Hz, H5″), 3.87 (dd, 1H, $^2J_{6''a,6''b} = 12.6$ Hz, $^3J_{5'',6''b} = 2.4$ Hz, $H6''_b$), 3.81 (dd, 1H, $^2J_{6''a,6''b} = 12.6$ Hz, $^3J_{5'',6''a} = 4.0$ Hz, $H6''_a$), 3.62 (dd, 1H, $^3J_{3'',4''} = 9.6$ Hz, $^3J_{4'',5''} = 10.0$ Hz, H4″), 2.01, 1.98 (s, 6H, 2 × CH_3CONH); δ_C (100 MHz; D_2O; referenced to the methyl resonance of internal acetone at 30.89 ppm) 175.4, 175.1 (s, 2C, 2 × CH_3CONH), 168.1 (s, 1C, C4), 153.4 (s, 1C, C2), 142.1 (d, 1C, C6), 103.3 (d, 1C, C5), 94.6 (dd, 1C, $^2J_{C1'',P\beta} = 6.0$ Hz, C1″), 89.1 (d, 1C, C1′), 83.8 (dd, 1C, $^3J_{C4',P\alpha} = 9.9$ Hz, C4′), 74.4 (d, 1C, C2′), 73.8 (d, 1C, C5″), 70.3 (d, 1C, C3′), 67.9 (d, 1C, C4″), 65.7 (dt, 1C, $^2J_{C5',P\alpha} = 5.3$ Hz, C5′), 60.9 (t, 1C, C6″), 53.1 (d, 1C, C3″), 52.6 (dd, 1C, $^3J_{C2'',P\beta} = 9.2$ Hz, C2″), 22.7, 22.5 (q, 2C, 2 × CH_3CONH); δ_P (D_2O, 121 MHz, referenced to external 85% phosphoric acid at 0 ppm) –7.60 (d, P_β, $J_{P\alpha,P\beta} = 20.5$ Hz), –9.38 (d, P_α, $J_{P\alpha,P\beta} = 20.5$ Hz); *m/z* (ESI$^+$) for acid 649 ([M + H]$^+$, 1.0%), 294 (95.9), 245 (100); m/z (ESI$^-$) for acid 647 ([M – H]$^-$, 20.3%), 158 (40.5), 79 (100); HR-MS calcd $C_{19}H_{31}N_4O_{17}P_2$ [M + H]$^+$ 649.1154, found 649.1151.

UDP-2,3-Diacetamido-2,3-dideoxy-α-D-glucuronic acid, triammonium and trisodium salts (1). Adam's catalyst (PtO_2; 10 mg) was hydrogenated in water (5 mL) under atmospheric pressure

for 2 h. The apparatus was evacuated and flushed with nitrogen 3 times. The freshly prepared high-surface platinum in water was added to a solution of UDP-2,3-diacetamido-2,3-dideoxy-α-D-glucose, diammonium salt (**2**) (63 mg, 92 μmol) and $NaHCO_3$ (40 mg, 480 μmol) in water (10 mL). The vigorously stirred mixture was heated to 100 °C and oxygen was passed into the mixture through a porosity 1 sinter. After 24 h the conversion reached 35% (by SAX HPLC on Poros HQ50, for details see the General procedures in the Experimental section). A new batch of the catalyst was added prepared by hydrogenation of Adam′s catalyst (10 mg) in water (5 mL) and the oxidation was continued overnight. After 48 h the conversion reached 71% (by SAX HPLC). The catalyst was removed by filtration through 2 filter papers and the filtrate was freeze-dried. The resulting solid was dissolved in water (1 mL) and the solution was filtered using a 0.45 μm Milipore Whatman filter. The filtrate was purified using SAX HPLC on Poros HQ20 as indicated in the General procedures in the Experimental section. Fractions containing the product were pooled and freeze-dried to give incompletely neutralised material (by ^{1}H NMR). The resulting solid was taken into water (1 mL) and aqueous ammonia (0.1 mL) was added. The solution was freeze-dried again to give the triammonium salt of **1** (46 mg, 69%) as a white solid in a purity of >98% (as judged by SAX HPLC). δ_H (400 MHz; D_2O; referenced to the methyl resonance of internal acetone at 2.22 ppm) 7.85 (d, 1H, $^3J_{5,6} = 8.0$ Hz, H6), 6.01 (d, 1H, $^3J_{1',2'} = 4.4$ Hz, H1′), 5.91 (d, 1H, $^3J_{5,6} = 8.0$ Hz, H5), 5.56 (dd, 1H, $^3J_{1'',P\beta} = 7.2$ Hz, $^3J_{1'',2''} = 2.4$ Hz, H1″), 4.37–4.33 (m, 2H, H2′, H3′), 4.28–4.20 (m, 3H, H4′, H5′$_b$, H5″), 4.19–4.16 (m, 2H, H5′$_a$, H3″), 4.16–4.12 (m, 1H, H2″), 3.65 (dd, 1H, $^3J_{3'',4''} = {}^3J_{4'',5''} = 9.6$ Hz, H4″), 2.00, 1.99 (s, 6H, 2 × CH_3CONH); δ_C (100 MHz; D_2O; referenced to the methyl resonance of internal acetone at 30.89 ppm) 176.7 (s, 1C, $C_{6''}OO^-$), 175.5, 175.1 (s, 2C, 2 × CH_3*C*ONH), 167.0 (s, 1C, C4), 159.8 (s, C4 or C2 enol form), 156.2 (s, 1C, C2), 141.6 (d, 1C, C6), 103.5 (d, 1C, C5), 94.3 (dd, 1C, $^2J_{C1'',P\beta} = 5.6$ Hz, C1″), 89.1 (d, 1C, C1′), 83.5 (dd, 1C, $^3J_{C4',P\alpha} = 9.3$ Hz, C4′), 74.3 (d, 1C, C2′), 74.0 (d, 1C, C5″), 70.6 (d, 1C, C4″), 70.3 (d, 1C, C3′), 65.8 (dt, 1C, $^2J_{C5',P\alpha} = 5.5$ Hz, C5′), 52.6 (d, 1C, C3″), 52.3 (dd, 1C, $^3J_{C2'',P\beta} = 8.8$ Hz, C2″), 22.7, 22.5 (q, 2C, 2 × *C*H_3CONH); δ_P (D_2O, 121 MHz, referenced to external 85% phosphoric acid at 0 ppm) –7.46 (d, P_β, $J_{P\alpha,P\beta} = 19.7$ Hz), –9.32 (d, P_α, $J_{P\alpha,P\beta} = 19.7$ Hz); *m/z* (ESI$^-$) for acid 683 ([M + Na – 2H]$^-$, very weak), 705 ([M + 2Na – 3H]$^-$, very weak); HR-MS failed to give a molecular ion. To a dispersion of the triammonium salt of **1** (46 mg, 64 μmol) in water (1 mL) was added a small amount of Dowex 50Wx8-200 (Na^+ form) to achieve complete dissolution of the sugar nucleotide. This mixture was applied on a column charged with Dowex 50Wx8-200 (Na^+ form; 5 g) and the column was eluted with water. Fractions containing the product were pooled and freeze-dried to give the trisodium salt of **1** (46 mg, quantitative). ^{1}H, ^{13}C and ^{31}P NMR spectra were identical to the triammonium salt of **1**. *m/z* (ESI$^+$) for acid 751 ([M + 4Na – 3H]$^+$, weak), 685 [M + Na]$^+$; *m/z* (ESI$^-$) for acid 749 ([M + 4Na – 5H]$^-$, weak), 705 ([M + 2Na – 3H]$^-$, weak); HR-MS calcd $C_{19}H_{25}N_4O_{18}P_2Na_4$ [M + 4Na – 3H]$^+$ 751.0224, found 751.0229.

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