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ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 18 (2007) 1299–1307

Synthesis of UDP-GlcNAc derivatives modified at OH-4 as potential chain-terminators of chitin biosynthesis

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Received 28 March 2007; accepted 29 May 2007

Abstract—A series of UDP-GlcNAc derivatives and precursors that have been modified at the 4-position were synthesised from *N*-acetyl glucosamine as potential chain terminators of chitin biosynthesis. None of the UDP-derivatives or the precursors tested displayed any significant anti-fungal activity in cell adhesion or germination assays on the dermatophyte *Trichophyton rubrum*.

1. Introduction

As bacterial, viral and fungal drug resistance to currently administered treatments increases, the need for the development of new therapeutic strategies against infective agents becomes more urgent. Crucial to the survival of many pathogens are carbohydrate structures, which are either themselves structurally unique or specific to non-mammalian organisms. Indeed, inhibition or interference with the correct biosynthesis of oligosaccharide materials represents an attractive and potentially highly selective strategy for the development of new classes of therapeutic agents.[1]

In recent years, substantial effort has been expended in the search for inhibitors of particular glycosyl transferase and glycosidase enzymes as a means of disrupting specific biosynthetic pathways.[2] The design of such inhibitors can be problematic, since in many cases little is known about the precise enzymes involved. However, an alternative strategy, and one that does not rely on precise structural information about specific enzymes in particular biochemical pathways, is to exploit the structural analysis of the key targeted oligosaccharide structure. This strategy is to invoke chain-termination of oligosaccharide biosynthesis.

Chain-termination of oligonucleotide synthesis was originally developed by Sanger[3] as a means of DNA sequencing. Subsequently, chain-termination strategies have found routine and widespread use as a means of interfering with oligonucleotide synthesis, and, moreover, have become the molecular basis of anti-viral therapies in clinical use, perhaps most pre-eminently in the case of AZT.[4] Although chain-termination processes have been implicated in the biological affects of some monosaccharide derivatives on mammalian glycoconjugate[5] and glycosoaminoglycan biosynthesis,[6] it is curious that a chain-termination approach has not been widely promulgated as a strategy for the development of new classes of inhibitors of the biosynthesis of pathogenic oligosaccharides.

Although there are potential pitfalls in this approach, it is our considered opinion that this research route merits further investigation. For example, in cases where multiple repeat units of carbohydrate structures are essential for pathogenic survival (e.g., in cell wall formation), the statistical chances of incorporation leading to chain-termination would be more favourable. Moreover, there is now good evidence that glycosyl transferases do process activated donor substrates that are modified in a minimal way at a single hydroxyl group.[7] Although some of these compounds have been shown to act as enzyme inhibitors, many are good substrates that are readily processed.[8] Indeed, as alluded to above, there is also a literature precedent that chain-terminating modified carbohydrates have already been incorporated into mammalian glycoprotein oligosaccharides[5] and glycosaminoglycans,[6] with the net results of inhibition of their biosynthesis.

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doi:10.1016/j.tetasy.2007.05.032

As part of a program aimed at investigating the potential opportunities that chain-termination of oligosaccharide biosynthesis offers to disease control, we became interested in the rational design of novel anti-fungal agents. Fungal infections represent a serious hazard to human and animal health,[9] and, just as for bacterial infection, drug resistance to current therapies is increasing.[10] The fungal cell wall[11] consists of large sections of oligosaccharide materials including chitin, a polymer of β(1–4)*N*-acetylglucosamine (GlcNAc), and also β-glucan. The biosynthesis of both of these non-mammalian oligosaccharides could be potentially targeted[12] using a chain-termination approach.

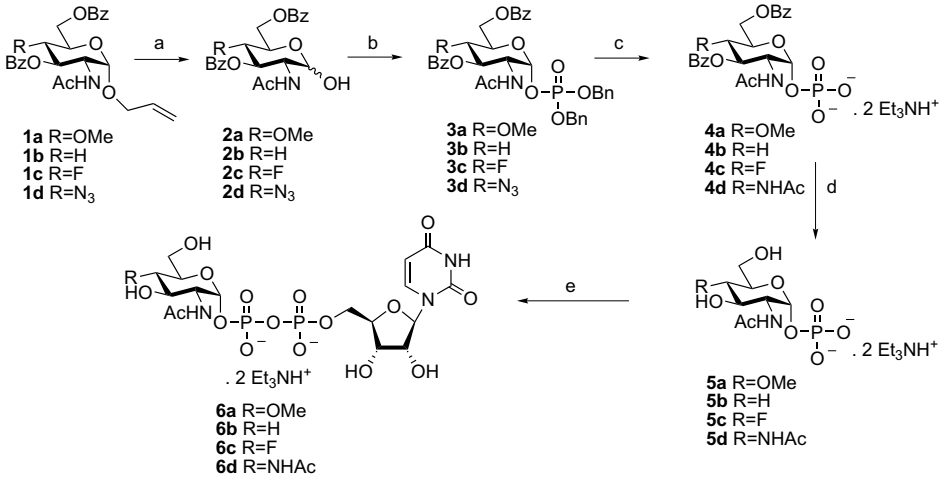
The present study focuses on the attempted inhibition of chitin biosynthesis. Chitin is assembled stepwise by the enzyme chitin synthase,[13] which transfers single GlcNAc residues to a growing oligomeric chain; the donor substrate for the enzyme being UDP-GlcNAc. Potential chain terminators of this process are therefore GlcNAc residues in which the 4-hydroxyl has been modified.[14] If such materials are processed by chitin synthase, then their transfer to the terminus of the growing chitin chain results in a chain-termination step since the required 4-hydroxyl at which subsequent units would be added, will now be lacking. The most obvious approach was that of synthesising and testing the modified UDP-donors themselves, since these are the actual substrates processed by the chitin synthase. Alternatively potential pro-drug molecules could be synthesised, which may then be converted to the active UDP-donor once inside the cell.[15] Herein, we report the synthesis and investigation of a variety of glycosyl phosphate and UDP-derivatives of GlcNAc which have been modified at the 4-position.

2. Results and discussion

As a first foray into this field, a decision was taken to access compounds where the normal 4-hydroxyl of GlcNAc was replaced by –H, –OMe, –F and –NHAc. We recently disclosed[15] the synthesis of GlcNAc derivatives **1a**–**d**, in which OH-4 had been modified, and these were used as a starting point for the synthesis of the corresponding glycosyl phosphates and UDP compounds. Removal of the allyl protection for methoxy-, deoxy- and fluoro-compounds **1a**–**c** was readily achieved by heating with tetrakistriphenylphosphine palladium in acetic acid. However, this procedure was found to also reduce the azide functionality in compound **1d**. A variety of alternative[16] deprotection conditions were investigated until a successful procedure, involving treatment with $PdCl_2$ in methanol, was arrived upon.

Subsequent conversion to the desired α-glycosyl phosphates **3a**–**d** was achieved by treatment of the hemiacetals with LDA and tetrabenzyl pyrophosphate in THF at −78 °C. Removal of the benzyl protecting groups by catalytic hydrogenation in the presence of Pearlman's catalyst, allowed the formation of the corresponding glycosyl phosphates **4a**–**c** as their triethylamine salts, and which were used immediately in the next step after filtering over Celite®. Alternative deprotection conditions were investigated in the case of azido compound **3d** which would not cause simultaneous reduction of the azido group, but all of the conditions investigated were found to be unsatisfactory. Therefore, in this case, the catalytic hydrogenation was run in an MeOH/acetic anhydride solvent mixture, which led directly to the formation of the corresponding 4-NHAc derivative[17] **4d**. Removal of the benzoyl protection of the 3- and 6-hydroxyls then allowed access to the corresponding, completely de-protected glycosyl phosphates **5a**–**d**, which were purified by anion exchange chromatography. Finally the protracted reaction of the de-protected glycosyl phosphates **5a**–**d** with UMP-morpholidate led to the formation of the corresponding UDP-derivatives **6a**–**d**, which were also purified by anion exchange chromatography (Scheme 1).

The anti-fungal action of the putative chain-termination compounds **6a**–**d** was assessed using two assays on micro-



Scheme 1. Reagents and conditions: (a) $(Ph_3P)_4Pd$, H_2O, AcOH, 80 °C; **2a**, 83%; **2b**, 81%; **2c**, 79%; or $PdCl_2$, MeOH, rt; **2d**, 73%; (b) LDA, $[(BnO)_2P(O)]_2O$, THF, −78 to 0 °C; **3a**, 62%; **3b**, 65%; **3c**, 89%; **3d**, 73%; (c) H_2, $Pd(OH)_2$, MeOH then Et_3N; **4a**, 86%; **4b**, 93%; **4c**, 89%; or MeOH/Ac_2O, rt then Et_3N; **4d**, 70%; (d) cyclohexylamine, MeOH, 70 °C; **5a**, 84%; **5b**, 78%; **5c**, 86%; **5d**, 65%; (e) UMP-morpholidate, tetrazole, pyridine, rt; **6a**, 65%; **6b**, 65%; **6c**, 47%; **6d**, 67%.

conidia and germlings of the dermatophyte[18] *Trichophyton rubrum*, a fungal pathogen that causes superficial skin diseases in humans and animals. These two assays measured the effects of the compounds both on adhesion and germination of fungal spores/germlings. However, none of the completely de-protected putative chain terminators **6a–d** displayed significant biological activity in the 1–1000 μM range that was examined. Moreover the de-protected glycosyl phosphates **5a–d** were also investigated for any anti-fungal activity, but again none of these compounds displayed any significant anti-fungal effects in the range tested.

These results are in contrast to the biological test data obtained[15] for a series of protected GlcNAc derivatives, which had been modified at the 4-position. Moreover the UDP-methoxy derivative **6a** has recently been synthesised by Finney, and has been reported to be a substrate for chitin synthase.[14] One rationalisation for the lack of activity of the UDP derivatives, in line with previous hypotheses,[5d] is the poor compound polarity profile[19] which could mean that these UDP derivatives are unable to effectively penetrate intracellularly.

3. Conclusions

A series of UDP-GlcNAc derivatives that have been modified at the 4-position have been synthesised as potential chain terminators of chitin biosynthesis. However, none of the UDP-derivatives displayed any significant anti-fungal activity in either cell adhesion or germination assays on *T. rubrum*. It is possible that this lack of biological activity is due to poor intracellular penetration of these highly polar compounds, particularly since one had been previously synthesised and in that study was demonstrated to be a substrate for a fungal chitin synthase. Further investigations into potential uses of carbohydrate chain terminators as the basis for novel strategies against a variety of other infective agents are currently in progress, and the results will be reported in due course.

4. Experimental

4.1. General

Melting points were recorded on a Kofler hot block and are uncorrected. Proton and carbon nuclear magnetic resonance (δ_H, δ_C) spectra were recorded on Bruker DPX 250 (250 MHz), Bruker DPX 400 (400 MHz), Bruker DQX 400 (400 MHz), Bruker AVC 500 (500 MHz) or Bruker AMX 500 (500 MHz) spectrometers. All chemical shifts are quoted on the δ-scale in ppm using residual solvent as an internal standard. Low resolution mass spectra were recorded on a Micromass Platform 1 spectrometer using electrospray ionisation in either positive or negative polarity (ES^+ or ES^-), or using a VG Micromass spectrometer. High resolution mass spectra were recorded on a Walters 2790-Micromass LCT electrospray ionisation mass spectrometer, using either electrospray ionisation (NH_3, Cl) techniques as stated. *m*/*z* values are reported in Daltons and are followed by their percentage abundance in parentheses. Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 mL. Microanalyses were performed by the Inorganic Chemistry Laboratory Elemental Analysis service, Oxford University, UK. Thin Layer Chromatography (TLC) was carried out on Merck Kieselgel $60F_{254}$ precoated glass-backed plates. Visualisation of the plates was achieved using a UV lamp ($\lambda_{max} = 254$ or 365 nm), and/or ammonium molybdate (5% in 2 M sulphuric acid), or sulphuric acid (5% in ethanol). Flash column chromatography was carried out using Sorbsil C60 40/60 silica. Dichloromethane was distilled from calcium hydride, or dried on an alumina column. Anhydrous THF, DMF, pyridine, methanol and toluene were purchased from Fluka over molecular sieves. 'Petrol' refers to the fraction of light petroleum ether boiling in the range of 40–60 °C. CMAW (chloroform/methanol/acetic acid/water) used as eluant was prepared in the following ratio ($CHCl_3$/MeOH/AcOH/H_2O, 60:30:3:5).

A BioRad AG 50 W-X2 cation exchange column (NEt_3H^+-form, typically 2 × 20 cm) was used for the formation of the triethylamine salts. The NEt_3H^+-form of the resin was prepared by passing 1 L of a 0.1 M aqueous solution of NEt_3 through the column and then a minimum 1 L of water until pH ~ 7 was reached; the product was eluted with water and collected in 1–2 mL fractions (monitoring by TLC, eluting with CMAW). After the column, the H^+-form of the resin was regenerated by passing 1 L of 1 M HCl and then 1 L (minimum) of water until pH ~ 7 was again reached; the resin could be used several times but must be stored in the H^+-form. General procedure for the purification of UDP-derivatives:[20] after co-evaporating several times with water, all the crude materials were submitted to a DEAE Sephadex A25 anion exchange column (typical size 2 × 20 cm) using a triethylammonium bicarbonate buffer (TEAB, gradient 0.35–0.5 M). The TEAB was obtained by preparing a 2 M aqueous solution of NEt_3 (not miscible), and by adding dry ice until a clear solution was obtained (this stock solution could be kept in the fridge for several days, if it turned cloudy, dry ice could be added until a clear solution was obtained again). The clean resin was kept in 5 mM TEAB in the fridge. The column was prepared by first eluting with 500 mL of a 350 mM solution of TEAB under light pressure (~1 drop per second). The crude reaction product was then applied and the fraction collected whilst eluting consecutively with 100 mL of 350, 400, 450 mM TEAB and finally with 500 mM TEAB until compound elution was complete. In cases where it was difficult to visualise the product by TLC (CMAW) MS (ES^-) was useful to locate material in the different factions (fraction size ~1–2 mL). It should be noted that the resin can be regenerated up to 20 times by heating it for up to 2 h at 50 °C in 2 M TEAB followed by washing with water until pH ~ 7 is reached, and it can then be kept in 5 mM TEAB in a fridge.

4.2. 2-Acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-methyl-D-glucopyranose 2a

Tetrakis(triphenylphosphine)palladium (3.96 g, 3.44 mmol, 0.5 equiv) was added to a solution of allyl glycoside **1a**

(3.32 g, 6.87 mmol, 1 equiv) in AcOH (40 mL) and the resulting mixture was heated at 80 °C. After 4 h, the mixture was evaporated to dryness. The residue was then purified by flash column chromatography (CH_2Cl_2/MeOH, 95:5) to afford hemiacetal **2a** (2.53 g, 83%) as a white solid and as a mixture of anomers, α/β-ratio being approximately 10:1 (by integration over selected parts of the spectrum); ν_{max} (KBr) 1722 (br s, C=O), 1658 (br w, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.85 (6H, s, OCCH_3), 3.43 (6H, s, CH_3), 3.64 (1H, at, *J* 9.6 Hz, H-4α), 3.88 (1H, at, *J* 9.6 Hz, H-4β), 4.25 (1H, dt, *J* 2.8 Hz, *J* 10.0 Hz, H-5α), 4.37–4.43 (2H, m, H-2), 4.54–5.58 (4H, m, H-6, H-6′), 5.26 (1H, d, $J_{1,2}$ 3.6 Hz, H-1α), 5.53 (1H, dd, *J* 9.2 Hz, *J* 10.8 Hz, H-3β), 5.64 (1H, dd, *J* 9.2 Hz, *J* 10.8 Hz, H-3α), 6.22 (1H, d, $J_{2,NH}$ 9.6 Hz, N*H*α), 6.33 (1H, d, *J* 9.6 Hz, H-1β), 6.67 (1H, d, *J* 7.2 Hz, N*H*β), 7.41–7.50 (8H, m, 8× Ar-H), 7.55–7.65 (4H, m, 4× Ar-H), 7.99–8.08 (8H, m, 8× Ar-H); δ_c (100.6 MHz, $CHCl_3$) 23.1 (2× C=O*C*H_3), 52.6 (2× C-2), 60.7 (2× *C*H_3), 63.2 (2× C-6), 68.9 (2× C-5), 73.9 (2× C-3), 78.1 (2× C-4), 91.7 (C-1α), 97.8 (C-1β), 128.5, 128.6, 128.7, 129.5, 129.7, 129.8 (12× Ar-CH, 4× Ar-C), 132.1, 133.2 (4× Ar-CH), 166.4, 166.9 (4× Ph*C*=O), 170.5 (2× CH_3C=O); *m*/*z* (ES^+) 502 (M + MeCN/NH_4^+, 8), 336 (100%); HRMS (ES^+): (MNa^+) calcd for $C_{23}H_{25}NO_8Na$, 466.1483; found, 466.1467. ($C_{23}H_{25}NO_8$ requires C, 62.30; H, 5.68; N, 3.16. Found: C, 62.42; H, 5.97; N, 3.38.)

4.3. 2-Acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-D-*xylo*-hexopyranose 2b

Tetrakis(triphenylphosphine)palladium (4.84 g, 4.19 mmol, 0.5 equiv) was added to a solution of allyl glycoside **1b** (3.8 g, 8.38 mmol, 1 equiv) in AcOH (30 mL) and the resulting mixture was heated at 80 °C. After 2 h, the mixture was evaporated to dryness. The residue was then purified by flash column chromatography (CH_2Cl_2/MeOH, 95:5) to afford hemiacetal **2b** (2.8 g, 81%) as a colourless solid as a mixture of anomers, α/β-ratio being approximately 10:1 (by integration over selected parts of the spectrum); ν_{max} (KBr) 1653 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.84–1.93 (2H, m, H-4), 1.90 (3H, s, CH_3α), 1.96 (3H, s, CH_3β), 2.26 (1H, ddd, J_{gem} 12.4 Hz, $J_{3,4'}$ 5.0 Hz, *J* 1.8 Hz, H-4′β), 2.30 (1H, ddd, J_{gem} 12.4 Hz, $J_{3,4'}$ 5.0 Hz, *J* 1.8 Hz, H-4′α), 3.92–3.99 (1H, m, H-2β), 4.33–4.42 (5H, m, H-2α, H-6, H-6′), 4.44–4.47 (1H, m, H-5β), 4.48–4.54 (1H, m, H-5α), 4.64 (1H, d, $J_{1,2}$ 8.4 Hz, H-1β), 5.25 (1H, td, *J* 5.4 Hz, *J* 10.8 Hz, H-3β), 5.36 (1H, d, $J_{1,2}$ 3.6 Hz, H-1α), 5.46 (1H, td, *J* 5.2 Hz, *J* 11.2 Hz, H-3α), 6.08 (1H, d, $J_{2,NH}$ 9.6 Hz, N*H*α), 6.64 (1H, d, $J_{2,NH}$ 6.8 Hz, N*H*α), 7.40–7.44 (8H, m, 8× Ar-H), 7.53–7.57 (4H, m, 4× Ar-H), 7.99–8.04 (8H, m, 8× Ar-H); δ_C (100.6 MHz, $CDCl_3$) 23.0 (*C*H_3β), 23.3 (*C*H_3α), 52.6 (C-2α), 58.2 (C-2β), 65.8 (C-5α), 65.9 (C-6β), 66.3 (C-6α), 69.3 (C-3α), 69.5 (C-3β), 92.6 (C-1α), 98.1 (C-1β), 128.4, 128.5, 128.6, 129.7, 129.8, 129.9 (12× Ar-CH, 4× Ar-C), 133.3, 133.9 (8× Ar-CH), 166.5, 166.8 (4× Ph*C*=O), 170.7 (2× CH_3C=O); *m*/*z* (ES^+) 332 ($M+H^+$, 3), 331 (M, 21), 246 ($M-H^+$, 100); HRMS (ES^+): (MNa^+) calcd for $C_{22}H_{23}NO_7Na$, 436.1372; found, 436.1367. ($C_{22}H_{23}NO_7$ requires C, 63.91; H, 5.61; N, 3.39. Found: C, 63.67; H, 5.63; N, 3.27.)

4.4. 2-Acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-fluoro-D-glucopyranose 2c

Tetrakis(triphenylphosphine)palladium (0.897 g, 0.78 mmol, 0.5 equiv) was added to a solution of allyl glycoside **1c** (0.73 g, 1.55 mmol, 1 equiv) in AcOH (12 mL) and the resulting mixture was heated at 80 °C. After 2 h, the mixture was evaporated to dryness. The residue was then purified by flash column chromatography (CMAW) to hemiacetal **2c** (0.528 g, 79%) as a colourless solid and as a mixture of anomers, α/β-ratio being approximately 25:1 (by integration over selected parts of the spectrum); ν_{max} (KBr) 1724 (br s, C=O), 1660 (w, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.83 (3H, s, CH_3), 4.36–4.41 (1H, m, H-5), 4.42–4.51 (3H, m, H-2, H-6, H-6′), 4.76 (1H, dt, $J_{4,F}$ 51.1 Hz, *J* 9.4 Hz, H-4), 5.24 (1H, t, *J* 3.6 Hz, H-1), 5.70–5.79 (1H, m, H-3), 6.16 (d, 1H, $J_{2,NH}$ 9.6 Hz), 7.41–7.49 (4H, m, 4× Ar-H), 7.54–7.57 (2H, m, 2× Ar-H), 7.62–7.67 (4H, m, 4× H); δ_C (100.6 MHz, $CDCl_3$) 23.1 (2× *C*H_3), 52.2 (d, $J_{2,F}$ 7.1 Hz, 2× C-2), 62.5 (2× C-6), 67.1 (d, $J_{C\text{-}5,F}$ 23.1 Hz, 2× C-5), 71.7 (d, $J_{C\text{-}3,F}$ 18.1 Hz, 2× C-3), 87.2 (d, $J_{C\text{-}4,F}$ 187.1 Hz, 2× C-4), 128.5, 128.7, 129.3, 129.7, 129.9 (12× Ar-CH, 4 Ar-C), 133.5, 133.4 (8× Ar-CH), 166.3, 166.8 (4× PhC=O), 170.4 (2× CH_3C=O); δ_F (376.6 MHz, $CDCl_3$) −196.7 (m, F). *m*/*z* (ES^+) 490 (M + MeCN/NH_4^+, 12), 337 (100%); HRMS (ES^+): (MNa^+) calcd for $C_{22}H_{22}FNO_7Na$, 454.1270; found, 454.1273. ($C_{22}H_{22}FNO_7$ requires C, 61.25; H, 5.14; N, 3.25. Found: C, 61.35; H, 5.15; N, 3.43.)

4.5. 2-Acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-azido-D-glucopyranose 2d

Palladium(II) dichloride (0.007 g, 0.040 mmol, 0.4 equiv) was added to a solution of allyl glycoside **1d** (0.05 g, 0.101 mmol, 1 equiv) in MeOH (3 mL) under argon atmosphere and the resulting mixture was stirred at room temperature for 14 h. The mixture was filtered through Celite and concentrated in vacuo. The residue was then purified by flash column chromatography (CH_2Cl_2/MeOH, 95:5) to afford hemiacetal **2d** (0.033 g, 73%) as a white solid and as a mixture of anomers, α/β-ratio being approximately 6:1 (by integration over selected parts of the spectrum); ν_{max} (KBr) 3423 (br s, OH), 2111 (s, N_3), 1723 (br s, C=O), 1659 (br w, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.85 (3H, s, OCCH_3α), 1.95 (3H, s, OCCH_3β), 3.87–3.99 (2H, m, H-4), 4.12 (1H, m, H-5β), 4.20 (1H, adt, *J* 2.8 Hz, *J* 10.3 Hz, H-5α), 4.43 (2H, atd, *J* 3.3 Hz, *J* 10.0 Hz, H-2α), 4.50 (1H, m, H-2β), 4.55 (1H, dd, $J_{6,5}$ 3.6 Hz, $J_{6,6'}$ 12.3 Hz, H-6), 4.66 (1H, dd, $J_{6',5}$ 2.1 Hz, $J_{6',6}$ 12.3 Hz, H-6′), 5.26 (1H, d, $J_{1,2}$ 9.5 Hz, H-1β), 5.30 (1H, d, $J_{1,2}$ 3.1 Hz, H-1α), 5.48 (1H, m, H-3β), 5.62 (1H, at, *J* 10.2 Hz, H-3α), 6.22 (1H, d, $J_{2,NH}$ 9.4 Hz, N*H*α), 6.66 (1H, d, *J* 7.1 Hz, N*H*β), 7.44–7.51 (8H, m, 8× Ar-H), 7.58–7.62 (4H, m, 4× Ar-H), 8.04–8.11 (8H, m, 8× Ar-H); δ_c (100.6 MHz, $CHCl_3$) 23.1 (2× C=O*C*H_3), 52.5 (2× C-2), 60.9 (2× C-4), 63.3 (2× C-6), 68.3 (2× C-5), 72.3, 74.2 (C-3α, C-3β), 91.8 (C-1α), 97.8 (C-1β), 128.5, 128.6, 128.8, 129.7, 129.7, 129.8, 129.9130.1 (12× Ar-CH, 4× Ar-C), 133.4, 133.7, 134.3 (4× Ar-CH), 166.3, 166.9 (4× Ph*C*=O), 170.6 (2× CH_3C=O); *m*/*z* (ES^+) 513 (M + MeCN/NH_4^+, 100), 337 (42); HRMS (ES^+):

(MNa^+) calcd for $C_{22}H_{22}N_4O_7Na$, 477.1386; found, 477.1382. ($C_{22}H_{22}N_4O_7$ requires C, 58.15; H, 4.88; N, 12.33. Found: C, 57.95; H, 4.91; N, 11.93.)

4.6. Dibenzylphosphate-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-methyl-α-D-glucopyranoside 3a

LDA (0.218 mL, 1.8 M in THF–heptane–ethylbenzene, 0.38 mmol, 1.1 equiv) was added dropwise to a solution of hemiacetals **2a** (0.158 g, 0.36 mmol, 1 equiv) in dry THF (6 mL) which was cooled to −78 °C. After 15 min, a solution of tetrabenzyl pyrophosphate (0.25 g, 0.46 mmol, 1.3 equiv) in dry THF (6 mL) was slowly added and the mixture was then allowed to warm to 0 °C. After 3 h, a saturated solution of NH_4Cl (50 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc/petrol, 3:2) to afford α-glycosyl phosphate **3a** (0.155 g, 62%) as a white foam, $[\alpha]_D^{23} = +46.3$ (*c* 1.0, $CHCl_3$); ν_{max} (KBr) 1723 (br s, C=O), 1678 (br w, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.61 (3H, s, C=OC*H*$_3$), 3.41 (3H, s, C*H*$_3$), 3.64 (1H, at, *J* 9.6 Hz, H-4), 4.08–4.12 (1H, m, H-5), 4.40 (1H, dd, $J_{5,6}$ 2.0 Hz, $J_{6,6'}$ 12.4 Hz, H-6), 4.44–4.53 (2H, m, H-2, H-6′), 4.47 (1H, ttd, $J_{2,P}$ 1.2 Hz, $J_{1,2}$ 3.2 Hz, *J* 10.0 Hz, H-2), 5.02–5.14 (4H, m, 2× PhC*H*$_2$), 5.32 (1H, dd, *J* 9.2 Hz, *J* 10.8 Hz, H-3), 5.73 (1H, dd, $J_{1,2}$ 3.2 Hz, $J_{1,P}$ 6.0 Hz, H-1), 5.88 (1H, d, $J_{2,NH}$ 9.6 Hz, N*H*), 7.34–7.47 (14H, m, 14× Ar-H), 7.56–7.61 (2H, m, Ar-H), 8.01–8.10 (4H, m, Ar-H); δ_C (100.6 MHz, $CDCl_3$) 22.7 (C=O*C*H$_3$), 52.1 (d, $J_{C\text{-}2,P}$ 8.0 Hz, C-2), 60.9 (*C*H$_3$), 62.5 (C-6), 69.8 (d, $J_{C,P}$ 6.0 Hz, PhCH$_2$), 69.9 (d, $J_{C,P}$ 6.0 Hz, PhCH$_2$), 71.1 (C-5), 72.3 (C-3), 77.3 (C-4), 96.6 (d, $J_{C\text{-}1,P}$ 6.0 Hz, C-1), 128.0, 128.1, 128.2, 123.3, 128.5, 128.6, 128.8, 128.9, 129.1, 129.6, 129.7, 129.8 (16× Ar-CH, 2× Ar-C), 133.3, 133.6 (4× Ar-CH), 135.3, 135.4 (2× Ar-C), 166.1, 166.7 (2× PhC=O), 170.4 (CH_3C=O); δ_P (162 MHz, $CDCl_3$) −2.3 (^{1}H decoupled); *m*/*z* (ES^+) 762 ($M + MeCN/NH_4^+$, 100%); HRMS (ES^+): (MNa^+) calcd for $C_{37}H_{38}NO_{11}PNa$, 726.2075; found, 726.2054. ($C_{37}H_{38}NO_{11}P$ requires C, 63.15; H, 5.44; N, 1.99. Found: C, 63.10; H, 5.61; N, 1.89.)

4.7. Dibenzylphosphate-2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-α-D-*xylo*-hexopyranoside 3b

LDA (3.67 mL, 2.0 M in THF–heptane–ethylbenzene, 7.34 mmol, 1.1 equiv) was added dropwise to a solution of hemiacetals **2b** (2.76 g, 6.67 mmol, 1 equiv) in dry THF (30 mL) which had been cooled to −78 °C. After 15 min, a solution of tetrabenzyl pyrophosphate (4.67 g, 8.68 mmol, 1.3 equiv) in dry THF (20 mL) was slowly added and the mixture was then allowed to be warmed to 0 °C. After 3 h, a saturated solution of NH_4Cl (100 mL) was added and the aqueous phase extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was then purified by flash column chromatography (EtOAc/petrol, 3:2) to afford α-glycosyl phosphate **3b** (2.392 g, 65%) as a white foam, $[\alpha]_D^{23} = +58.5$ (*c* 1.0, $CHCl_3$); ν_{max} (KBr) 1653 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.68 (3H, s, C*H*$_3$), 1.86–1.91 (1H, m, H-4$_{ax}$), 2.32 (1H, ddd, J_{gem} 12.8 Hz, *J* 4.8 Hz, *J* 1.8 Hz, H-4$_{eq}$), 4.31–4.32 (2H, m, H-6, H-6′), 4.34–4.40 (1H, m, H-5), 4.47 (1H, ttd, $J_{2,P}$ 1.2 Hz, $J_{1,2}$ 3.2 Hz, *J* 10.0 Hz, H-2), 5.02–5.07 (4H, m, 2× PhC*H*$_2$), 5.32 (1H, td, H-3), 5.81 (1H, dd, $J_{1,P}$ 6.0 Hz, H-1), 5.95 (1H, d, N*H*), 7.33–7.44 (14H, m, 14× Ar-H), 7.51–7.58 (2H, m, Ar-H), 7.97–8.02 (4H, m, Ar-H); δ_C (100.6 MHz, $CDCl_3$) 22.9 (*C*H$_3$), 32.7 (C-4), 52.0 (d, $J_{C\text{-}2,P}$ 8.0 Hz, C-2), 65.5 (C-6), 68.1 (C-5), 68.2 (C-3), 69.8 (d, $J_{C,P}$ 6.0 Hz, PhCH$_2$), 69.9 (d, $J_{C,P}$ 6.0 Hz, PhCH$_2$), 97.9 (d, $J_{C\text{-}1,P}$ 7.0 Hz, C-1), 127.7, 128.1, 128.4, 128.5, 128.7, 128.8, 129.3, 129.5, 129.7, 129.8 (16× Ar-CH), 133.2, 133.4 (4× Ar-CH), 135.3, 135.4 (4× Ar-C), 166.1, 166.4 (2× PhC=O), 170.5 (CH_3C=O); δ_P (162 MHz, $CDCl_3$) −2.3 (^{1}H decoupled); *m*/*z* (ES^+) 732 ($M + MeCN/NH_4^+$, 100), 696 ($M+Na^+$, 3%); HRMS (ES^+): (MNa^+) calcd for $C_{36}H_{36}NO_{10}PNa$, 696.1969; found, 696.1966. ($C_{36}H_{36}NO_{10}P$ requires C, 64.19; H, 5.39; N, 2.08. Found: C, 64.33; H, 5.31; N, 2.24.)

4.8. Dibenzylphosphate-2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-fluoro-α-D-glucopyranoside 3c

LDA (0.436 mL, 1.8 M in THF–heptane–ethylbenzene, 0.78 mmol, 1.1 equiv) was added dropwise to a solution of hemiacetals **2c** (0.308 g, 0.71 mmol, 1 equiv) in dry THF (12 mL) which had been cooled to −78 °C. After 15 min, a solution of tetrabenzyl pyrophosphate (0.5 g, 0.93 mmol, 1.3 equiv) in dry THF (12 mL) was slowly added and the mixture was allowed to warm to 0 °C. After 3 h, a saturated solution of NH_4Cl (50 mL) was added and the aqueous phase extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was then purified by flash column chromatography (EtOAc/petrol, 3:2) to afford α-glycosyl phosphate **3c** (0.44 g, 89%) as a white foam, $[\alpha]_D^{23} = +59.9$ (*c* 1.0, $CHCl_3$); ν_{max} (KBr) 1724 (br s, C=O), 1661 (br, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.69 (3H, s, C*H*$_3$), 4.28–4.32 (1H, m, H-5), 4.45–4.58 (3H, m, H-2, H-6, H-6′), 4.78 (1H, dt, $J_{4,F}$ 50.8 Hz, *J* 9.4 Hz, H-4), 5.06–5.18 (4H, m, 2× PhC*H*$_2$), 5.58–5.66 (1H, m, H-3), 5.72–5.73 (1H, m, H-1), 6.24 (1H, d, $J_{2,NH}$ 9.6 Hz, N*H*), 7.33–7.45 (14H, m, 14× Ar-H), 7.56–7.60 (2H, m, Ar-H), 8.01–8.06 (4H, m, Ar-H); δ_C (100.6 MHz, $CDCl_3$) 22.6 (*C*H$_3$), 52.1 (at, *J* 8.0 Hz, C-2), 61.8 (C-6), 69.3 (d, $J_{C\text{-}5,F}$ 24.1 Hz, C-5), 69.9 (d, $J_{C,P}$ 6.0 Hz, PhCH$_2$), 70.1 (d, $J_{C,P}$ 6.0 Hz, PhCH$_2$), 70.3 (d, $J_{C\text{-}3,P}$ 19.1 Hz, C-3), 86.3 (d, $J_{C\text{-}4,F}$ 188.1 Hz, C-4), 96.1 (d, $J_{C\text{-}1,P}$ 7.0 Hz, C-1), 128.1, 128.5, 128.6, 128.8, 128.9, 129.0, 129.3, 129.5, 129.7, 129.9 (16× Ar-CH, 2× Ar-C), 133.3, 133.7 (2× Ar-CH), 135.2, 135.3 (2× Ar-C), 166.1, 166.7 (2× PhC=O), 170.4 (CH_3C=O); δ_P (162 MHz, $CDCl_3$) −2.3 (^{1}H decoupled); δ_F (376.6 MHz, $CDCl_3$) −196.5 (m, F); *m*/*z* (ES^+) 750 ($M + MeCN/NH_4^+$, 100%); HRMS (ES^+): (MNa^+) calcd for $C_{36}H_{35}FNO_{10}PNa$, 714.1875; found, 714.1851. ($C_{36}H_{35}FNO_{10}P$ requires C, 62.52; H, 5.10; N, 2.03. Found: C, 62.54; H, 5.11; N, 1.99.)

4.9. Dibenzylphosphate-2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-azido-α-D-glucopyranoside 3d

LDA (0.12 mL, 2.0 M in THF–heptane–ethylbenzene, 0.39 mmol, 1.1 equiv) was added dropwise to a solution of

compound **2d** (0.10 g, 0.22 mmol, 1 equiv) in dry THF (11 mL) which had been cooled to −78 °C. After 15 min, a solution of tetrabenzyl pyrophosphate (0.15 g, 0.28 mmol, 1.3 equiv) in dry THF (5 mL) was slowly added and the mixture was allowed to warm to 0 °C. After 3 h, a saturated solution of NH_4Cl (50 mL) was added and the aqueous phase extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was then purified by flash column chromatography (EtOAc/petrol, 3:2) to afford α-glycosyl phosphate **3d** (0.114 g, 73%) as a white foam, $[\alpha]_D^{20} = +90.2$ (*c* 0.5, MeOH); ν_{max} (KBr) 2112 (s, N_3), 1725 (br s, C=O), 1676 (br w, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.52 (3H, s, C=OCH_3), 3.76–3.83 (2H, m, H-4, H-5), 4.29–4.42 (3H, m, H-6, H-6′, H-2), 4.90–5.04 (4H, m, 2× PhCH_2), 5.32 (1H, dd, *J* 9.4 Hz, *J* 10.8 Hz, H-3), 5.62 (1H, dd, $J_{1,2}$ 3.2 Hz, $J_{1,P}$ 6.0 Hz, H-1), 5.91 (1H, d, $J_{2,NH}$ 9.3 Hz, N*H*), 7.20–7.36 (14H, m, 14× Ar-H), 7.45–7.50 (2H, m, Ar-H), 7.90–7.96 (4H, m, Ar-H); δ_C (100.6 MHz, $CDCl_3$) 22.7 (C=O*C*H$_3$), 52.0 (d, $J_{C\text{-}2,P}$ 8.0 Hz, C-2), 60.2 (C-4), 62.5 (C-6), 69.9 (d, $J_{C,P}$ 6.0 Hz, Ph*C*H$_2$), 70.0 (d, $J_{C,P}$ 6.0 Hz, Ph*C*H$_2$), 70.4 (C-5), 71.5 (C-3), 96.5 (d, $J_{C\text{-}1,P}$ 7.0 Hz, C-1), 128.1, 128.5, 128.6, 128.8, 128.9, 129.7, 129.9 (16× Ar-CH, 2× Ar-C), 133.4, 133.8 (4× Ar-CH), 135.1, 135.2, 135.3 (4× Ar-C), 166.0, 166.6 (2× Ph*C*=O), 170.4 (CH_3C=O); δ_P (162 MHz, $CDCl_3$) −2.3 (^{1}H decoupled); *m*/*z* (ES^+) 773 (M + MeCN/NH_4^+, 100%); HRMS (ES^+): (MNa^+) calcd for $C_{36}H_{35}N_4O_{10}PNa$, 737.1988; found, 737.1985. ($C_{36}H_{35}N_4O_{10}P$ requires C, 60.50; H, 4.94; N, 7.84. Found: C, 60.22; H, 5.28; N, 7.29.)

4.10. Phosphate-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-methyl-α-D-glucopyranoside, ditriethylammonium salt 4a

$Pd(OH)_2$ 20% (0.108 g, 10% w/w) was added to a solution of dibenzylphosphate **3a** (1.08 g, 1.54 mmol, 1 equiv) in dry MeOH (15 mL) and the resulting solution stirred under an H_2-atmosphere. After 3 h, the solution was filtered over Celite, NEt_3 (0.453 mL, 3.23 mmol, 2.1 equiv) was added and the resulting mixture was concentrated in vacuo to afford triethylammonium salt **4a** (0.96 g, 86%) as a white solid, which was used in the next step without further purification.

4.11. Phosphate-2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-α-D-*xylo*-hexopyranoside, ditriethylammonium salt 4b

$Pd(OH)_2$ 20% (0.05 g, 10% w/w) was added to a solution of dibenzylphosphate **3b** (0.5 g, 0.74 mmol, 1 equiv) in dry MeOH (7 mL) and the resulting solution stirred under an H_2-atmosphere. After 3 h, the solution was filtered over Celite, NEt_3 (0.219 mL, 1.56 mmol, 2.1 equiv) was added and the resulting mixture was concentrated in vacuo to afford triethylammonium salt **4b** (0.48 g, 93%) as a white solid which was used in the next step without further purification.

4.12. Phosphate-2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-fluoro-α-D-glucopyranoside, ditriethylammonium salt 4c

$Pd(OH)_2$ 20% (0.108 g, 10% w/w) was added to a solution of dibenzylphosphate **3c** (0.678 g, 0.98 mmol, 1 equiv) in dry MeOH (8 mL) and the resulting solution stirred under an H_2-atmosphere. After 3 h, the solution was filtered over Celite, NEt_3 (0.289 mL, 2.05 mmol, 2.1 equiv) wad added and the resulting mixture was concentrated in vacuo to afford triethylammonium salt **4c** (0.538 g, 89%) as a white solid which was used in the next step without further purification.

4.13. Phosphate-2,4-diacetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-α-D-glucopyranoside, ditriethylammonium salt 4d

$Pd(OH)_2$ 20% (0.02 g, 20% w/w) and Ac_2O (0.66 mL, 6.95 mmol, 50 equiv) were added to a solution of dibenzylphosphate **3d** (0.10 g, 0.14 mmol, 1 equiv) in dry MeOH (3 mL) and the resulting solution stirred under an H_2-atmosphere. After 6 h, the solution was filtered over Celite, NEt_3 (0.04 mL, 0.29 mmol, 2.1 equiv) was added and the resulting mixture was concentrated in vacuo to afford triethylammonium salt **4d** (0.07 g, 70%) as a white solid which was used in the next step without further purification.

4.14. Phosphate-2-acetamido-2-deoxy-4-*O*-methyl-α-D-glucopyranoside, ditriethylammonium salt 5a

Cyclohexylamine (14.65 mL, 130.94 mmol, 100 equiv) was added to a solution of dibenzoate **4a** (0.95 g, 13.10 mmol, 1 equiv) in dry MeOH (20 mL) and the resulting solution heated at 70 °C. After 48 h, the solution was concentrated in vacuo, the residue dissolved in water (50 mL) and washed with $CHCl_3$ (3 × 50 mL) after which it was concentrated in vacuo by coevaporating MeOH. The residue was applied to a BioRad AG 50W-X2 cation exchange column (NEt_3H^+-form) to afford diol salt **5a** (0.57 g, 84%) as a white solid, δ_H (400 MHz, D_2O) 1.23 (18H, t, *J* 7.4 Hz, NCH_2CH_3), 2.01 (3H, s, C=OCH_3), 3.15 (12H, q, NCH_2), 3.54 (3H, s, CH_3), 3.71–3.75 (1H, m, H-6), 3.80–3.84 (3H, m, H-3, H-5, H-6′), 3.89 (1H, dt, $J_{1,2}$ 3.3 Hz, *J* 10.8 Hz, H-2), 5.34 (1H, dd, $J_{1,P}$ 7.3 Hz, H-1); δ_C (100.6 MHz, D_2O) 8.6 (NCH_2CH_3) 22.3 (C=O*C*H$_3$), 47.0 (N*C*H$_2$CH$_3$), 54.2 (d, $J_{C\text{-}2,P}$ 8.0 Hz, C-2), 70.9, 71.9 (C-3, C-5), 79.7 (*C*H$_3$), 93.5 (d, $J_{C\text{-}1,P}$ 6.0 Hz, C-1), 175.0 (CH_3C=O); δ_P (162 MHz, D_2O) −0.7 (^{1}H decoupled); *m*/*z* (ES^-) 314 (M−H^+, 100%); HRMS (ES^-): (M−H^+) calcd for $C_9H_{17}NO_9P$, 314.0635; found, 314.0640.

4.15. Phosphate-2-acetamido-2,4-dideoxy-α-D-*xylo*-hexopyranoside, ditriethylammonium salt 5b

Cyclohexylamine (2.54 mL, 22.7 mmol, 100 equiv) was added to a solution of dibenzoate **4b** (0.112 g, 0.23 mmol, 1 equiv) in dry MeOH (4 mL) and the resulting solution heated at 70 °C. After 48 h, the solution was concentrated in vacuo, the residue dissolved in water (50 mL) and washed with $CHCl_3$ (3 × 50 mL) after which it was concentrated in vacuo by coevaporating with MeOH. The residue was applied to a BioRad AG 50 W-X2 cation exchange column (NEt_3H^+-form) to afford diol triethylammonium salt **5b** (0.086 g, 78%) as a white solid, δ_H (400 MHz, D_2O) 1.13 (18H, t, *J* 7.4 Hz, NCH_2CH_3), 1.40 (1H, aq, *J* 12.2 Hz, H-4_{ax}), 1.89–1.94 (1H, m, H-4_{eq}), 1.93 (3H, s, C=OCH_3), 3.05 (12H, q, NCH_2), 3.46 (1H, dd, $J_{5,6}$

5.7 Hz, $J_{6,6'}$ 12.1 Hz, H-6), 3.55 (1H, dd, $J_{5,6'}$ 3.2 Hz, H-6′), 3.66 (1H, dt, $J_{1,2}$ 3.3 Hz, J 10.8 Hz, H-2), 3.89 (1H, td, J 4.7 Hz, J 11.0 Hz, H-3), 4.00–4.05 (1H, m, H-5), 5.32 (1H, dd, $J_{1,P}$ 7.3 Hz, H-1); δ_C (100.6 MHz, D_2O) 8.5 (NCH_2CH_3) 22.4 ($C{=}OCH_3$), 34.8 (C-4), 47.0 (NCH_2CH_3), 55.5 (d, $J_{C\text{-}2,P}$ 8.0 Hz, C-2), 64.0 (C-6), 65.5 (C-3), 69.9 (C-5), 94.5 (d, $J_{C\text{-}1,P}$ 6.3 Hz, C-1); δ_P (162 MHz, D_2O) −0.9 (^{1}H decoupled); m/z (ES^+) 344 ($M + MeCN/NH_4^+$, 100%); HRMS (ES^-): ($M-H^+$) calcd for $C_8H_{15}NO_8P$, 284.0530; found, 284.0520.

4.16. Phosphate-2-acetamido-2,4-dideoxy-4-fluoro-α-D-glucopyranoside, ditriethylammonium salt 5c

Cyclohexylamine (5.45 mL, 47.63 mmol, 100 equiv) was added to solution of dibenzoate **4c** (0.34 g, 0.48 mmol, 1 equiv) in dry MeOH (10 mL) and the resulting solution was heated at 70 °C. After 48 h, the solution was concentrated in vacuo, and the residue was dissolved in water (50 mL) and washed with $CHCl_3$ (3 × 50 mL) after which it was concentrated in vacuo by coevaporating with MeOH. The residue was applied to a BioRad AG 50 W-X2 cation exchange column (NEt_3H^+-form) to afford diol triethylammonium salt **5c** (0.209 g, 86%) as a white solid, δ_H (400 MHz, D_2O) 1.13 (18H, t, J 7.4 Hz, NCH_2CH_3), 1.99 (3H, s, $C{=}OCH_3$), 3.13 (12H, q, NCH_2), 3.73–3.82 (2H, m, H-6, H-6′), 3.90–3.96 (1H, m, H-5), 4.00–4.08 (2H, m, H-2, H-3) 4.38 (1H, dt, $J_{4,F}$ 50.8 Hz, J 9.2 Hz, H-4), 5.35–5.39 (1H, m, H-1); δ_C (100.6 MHz, D_2O) 8.6 (NCH_2CH_3), 22.2 ($C{=}OCH_3$), 46.9 (NCH_2CH_3), 53.7 (at, J 8.1 Hz, C-2), 60.0 (C-6), 69.2 (d, $J_{C\text{-}3,F}$ 19.1 Hz, C-3), 70.4 (d, $J_{C\text{-}5,F}$ 25.1 Hz, C-5), 89.4 (d, $J_{C\text{-}4,F}$ 180.1 Hz, C-4), 93.5 (d, $J_{C\text{-}1,P}$ 5.0 Hz, C-1); δ_P (162 MHz, D_2O) 0.1 (^{1}H decoupled); δ_F (376.6 MHz, D_2O) −197.9 (m, F); m/z (ES^-) 302 ($M-H^+$, 100%); HRMS (ES^-): ($M-H^+$) calcd for $C_8H_{14}FNO_8P$, 302.0436; found, 302.0436.

4.17. Phosphate-2,4-diacetamido-2,4-dideoxy-α-D-glucopyranoside, ditriethylammonium salt 5d

Cyclohexylamine (1.37 mL, 11.95 mmol, 100 equiv) was added to a solution of dibenzoate **4d** (0.09 g, 0.11 mmol, 1 equiv) in dry MeOH (4 mL) and the resulting solution heated at 70 °C. After 48 h, the solution was concentrated in vacuo, the residue was dissolved in water (10 mL) and washed with $CHCl_3$ (3 × 10 mL) after which it was concentrated in vacuo by coevaporating with MeOH. The residue was applied to a DEAE Sephadex A25 anion exchange column using a triethylammonium bicarbonate buffer gradient (0.2–0.35 M) to afford diol triethylammonium salt **5d** (0.04 g, 65%) as a white solid, δ_H (400 MHz, D_2O) 1.16 (18H, t, J 7.3 Hz, NCH_2CH_3), 1.92 (3H, s, $C{=}OCH_3$), 1.93 (3H, s, $C{=}OCH_3$), 3.8 (12H, q, NCH_2, J 7.3 Hz), 3.50 (1H, dd, $J_{5,6}$ 4.1 Hz, $J_{6,6'}$ 12.4 Hz, H-6), 3.58 (1H, dd, $J_{5,6'}$ 1.7 Hz, $J_{6',6}$ 12.4 Hz, H-6′), 3.73–3.80 (3H, m, H-3, H-5, H-4), 3.86–3.91 (1H, dt, $J_{2,1}$ 3.2 Hz, J 10.0 Hz, H-2), 5.35 (1H, dd, $J_{1,P}$ 7.3 Hz, $J_{1,2}$ 3.2 Hz, H-1); δ_C (100.6 MHz, D_2O) 8.6 (NCH_2CH_3) 22.2, 22.3 (2× $C{=}OCH_3$), 47.0 (NCH_2CH_3), 51.9 (C-4), 54.5 (d, $J_{C\text{-}2,P}$ 9.0 Hz, C-2), 61.0 (C-6), 68.8 (C-5), 72.0 (C-3), 93.8 (d, $J_{C\text{-}1,P}$ 5.6 Hz, C-1), 174.9, 175.0 (2× $CH_3C{=}O$); δ_P (162 MHz, D_2O) −1.37 (^{1}H decoupled); m/z (ES^-) 341 ($M-H^+$, 100%); HRMS (ES^-): ($M-H^+$) calcd for $C_{10}H_{18}N_2O_9P$, 341.0749; found, 341.0744.

4.18. Uridinediphosphoryl-2-acetamido-2,4-dideoxy-4-*O*-methyl-α-D-glucopyranoside, ditriethylammonium salt 6a

4-Morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphormorpholidate (0.33 g, 0.49 mmol, 2 equiv) and tetrazole (0.052 g, 0.74 mmol, 3 equiv) were added to a solution of phosphate **5a** (0.128 g, 0.25 mmol, 1 equiv) in dry pyridine and the resulting solution stirred at rt. After 5 days, the solution was concentrated in vacuo. The residue was applied to a DEAE Sephadex A25 anion exchange column using a triethylammonium bicarbonate buffer gradient (0.35–0.5 M) to afford UDP derivative **6a** (0.133 g, 65%) as a white solid, $[\alpha]_D^{18} = +40.8$ (c 1.0, MeOH); δ_H (400 MHz, D_2O) 1.11 (18H, t, J 7.4 Hz, NCH_2CH_3), 1.94 (3H, s, $C{=}OCH_3$), 3.01 (12H, q, NCH_2), 3.46 (3H, s, CH_3), 3.66 (1H, dd, $J_{5,6}$ 4.0 Hz, $J_{6,6'}$ 12.6 Hz, glu: H-6), 3.77–3.83 (2H, m, glu: H-3, H-6′), 3.90 (1H, dt, $J_{1,2}$ 3.3 Hz, J 10.6 Hz, glu: H-2), 3.96–4.28 (6H, m, glu: H-5, rib: H-2, H-3, H-4, H-5, H-5′), 5.41 (1H, dd, $J_{1,P}$ 7.3 Hz, glu: H-1), 5.80 (1H, d, $J_{5,6}$ 8.0 Hz, U: H-5), 5.83–5.88 (1H, m, rib: H-1), 7.85 (1H, d, U: H-6); δ_C (100.6 MHz, D_2O) 8.5 (NCH_2CH_3) 22.4 ($C{=}OCH_3$), 46.9 (NCH_2CH_3), 54.1 (d, $J_{C\text{-}2,P}$ 8.0 Hz, glu: C-2), 60.4, 60.5 (glu: C-6, CH_3), 65.2 (d, $J_{C\text{-}5,P}$ 5.0 Hz, rib: C-5), 69.8 (glu: C-5), 71.1 (glu: C-3), 72.3 (rib: C-3), 73.3 (rib: C-2), 74.1 (glu: C-4), 83.3 (d, $J_{C\text{-}4,P}$ 9.0 Hz, rib: C-4), 88.8 (rib: C-1), 94.7 (d, $J_{C\text{-}1,P}$ 6.0 Hz, glu: C-1), 102.8 (U: C-5), 141.9 (U: C-6), 151.9 (U: C-4), 166.3 (U: C-2), 174.3 ($CH_3C{=}O$); δ_P (162 MHz, D_2O) −11.5, −13.2 (^{1}H decoupled); m/z (ES^-) 620 ($M-H^+$, 100%); HRMS (ES^-): ($M-H^+$) calcd for $C_{18}H_{28}N_3O_{17}P_2$, 620.0888; found, 620.0888.

4.19. Uridinediphosphoryl 2-acetamido-2,4-dideoxy-α-D-*xylo*-hexopyranoside, ditriethylammonium salt 6b

4-Morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphormorpholidate (0.18 g, 0.27 mmol, 2 equiv) and tetrazole (0.028 g, 0.41 mmol, 3 equiv) were added to a solution of phosphate **5b** (0.066 g, 0.13 mmol, 1 equiv) in dry pyridine and the resulting solution stirred at rt. After 5 days, the solution was concentrated in vacuo. The residue was applied to a DEAE Sephadex A25 anion exchange column using a triethylammonium bicarbonate buffer gradient (0.35–0.5 M) to afford UDP derivative **6b** (0.07 g, 65%) as a white solid, $[\alpha]_D^{18} = +40.2$ (c 1.0, MeOH); δ_H (400 MHz, D_2O) 1.24 (18H, t, J 7.4 Hz, NCH_2CH_3), 1.49–1.56 (1H, m, glu: H-4_{ax}), 1.87–1.94 (1H, m, glu: H-4_{eq}), 1.94 (3H, s, $C{=}OCH_3$), 3.11 (12H, q, NCH_2), 3.47 (1H, dd, $J_{5,6'}$ 5.5 Hz, $J_{6,6'}$ 12.4 Hz, glu: H-6), 3.67 (1H, dd, $J_{5,6'}$ 3.2 Hz, glu: H-6′), 3.82 (1H, dt, $J_{1,2}$ 3.3 Hz, J 10.6 Hz, glu: H-2), 3.97–4.35 (6H, m, glu: H-2, H-3, H-5, rib: H-2, H-5, H-5′), 5.52 (1H, dd, $J_{1,P}$ 7.3 Hz, glu: H-1), 5.91–5.96 (2H, m, rib: H-1, U: H-5), 7.93 (1H, d, $J_{5,6}$ 8.0 Hz, U: H-6); δ_C (100.6 MHz, D_2O) 8.6 (NCH_2CH_3) 22.5 ($C{=}OCH_3$), 34.6 (glu: C-4), 47.0 (NCH_2CH_3), 55.3 (d, $J_{C\text{-}2,P}$ 8.0 Hz, glu: C-2), 64.0 (glu: C-6), 65.3 (d, $J_{C\text{-}5,P}$ 5.0 Hz, rib: C-5), 65.8 (glu: C-3), 70.0 (glu: C-5), 70.3 (rib: C-3), 74.1 (rib: C-2), 83.5 (d, $J_{C\text{-}4,P}$ 9.5 Hz, rib: C-4),

88.2 (rib: C-1), 95.6 (d, $J_{C\text{-}1,P}$ 5.0 Hz, glu: C-1), 103.0 (U: C-5), 142.0 (U: C-6), 151.6 (U: C-4), 169.8 (U: C-2), 175.3 ($CH_3C{=}O$); δ_P (162 MHz, D_2O) −11.3, −12.8 (^1H decoupled); *m/z* (ES^-) 590 ($M-H^+$, 100%); HRMS (ES^-): ($M-H^+$) calcd for $C_{17}H_{26}N_3O_{16}P_2$, 590.0772; found, 590.0783.

4.20. Uridinediphosphoryl-2-acetamido-2,4-dideoxy-4-fluoro-α-D-glucopyranoside, ditriethylammonium salt 6c

4-Morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphormorpholidate (0.106 g, 0.16 mmol, 2 equiv) and tetrazole (0.016 g, 0.23 mmol, 3 equiv) were added to a solution of phosphate **5c** (0.04 g, 0.078 mmol, 1 equiv) in dry pyridine and the resulting solution stirred at rt. After 5 days, the solution was concentrated in vacuo. The residue was applied to a DEAE Sephadex A25 anion exchange column using a triethylammonium bicarbonate buffer gradient (0.35–0.5 M) to afford UDP derivative **6c** (0.03 g, 47%) as a white solid, $[\alpha]_D^{18} = +45.0$ (*c* 0.1, MeOH); δ_H (400 MHz, D_2O) 1.23 (18H, t, *J* 7.4 Hz, NCH_2CH_3), 2.04 (3H, s, $C{=}OCH_3$), 3.151 (12H, q, NCH_2), 3.74–4.38 (10, m, glu: H-2, H-3, H-5, H-6, H-6′, rib: H-2, H-3, H-4, H-5, H-5′), 5.46–5.48 (1H, m, glu: H-1), 5.89 (1H, d, $J_{5,6}$ 8.0 Hz, U: H-5), 5.94–5.97 (1H, m, rib: H-1), 7.87 (1H, d, U: H-6); δ_C (100.6 MHz, D_2O) 8.6 (NCH_2CH_3) 23.6 ($C{=}OCH_3$), 47.0 (NCH_2CH_3), 55.3 (at, *J* 8.1 Hz, glu: C-2), 59.3 (glu: C-6), 61.2 (d, $J_{C\text{-}5,P}$ 5.0 Hz, rib: C-5), 69.9 (d, $J_{C\text{-}3,F}$ 18.1 Hz, glu: C-3), 71.4 (d, $J_{C\text{-}5,F}$ 24 Hz, glu: C-5), 72.3 (rib: C-3), 72.3 (rib: C-2), 84.5 (d, $J_{C\text{-}4,P}$ 9.0 Hz, rib: C-4), 89.1 (rib: C-1), 89.4 (d, $J_{C\text{-}4,F}$ 83.4 Hz, glu: C-4), 94.5 (d, $J_{C\text{-}1,P}$ 6.0 Hz, glu: C-1), 103.1 (U: C-5), 141.6 (U: C-6), 151.7 (U: C-4), 168.3 (U: C-2), 175.1 ($CH_3C{=}O$); δ_P (162 MHz, D_2O) −11.3, −13.1 (^1H decoupled); δ_F (376.6 MHz, D_2O) −198.2 (m, F); *m/z* (ES^-) 608 ($M-H^+$, 100%); HRMS (ES^-): ($M-H^+$) calcd for $C_{17}H_{25}FN_3O_{16}P_2$, 608.0689; found, 608.0686.

4.21. Uridinediphosphoryl-2,4-diacetamido-2,4-dideoxy-α-D-glucopyranoside, ditriethylammonium salt 6d

4-Morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphormorpholidate (0.095 g, 0.14 mmol, 2 equiv) and tetrazole (0.015 g, 0.21 mmol, 3 equiv) were added to a solution of phosphate **5d** (0.039 g, 0.07 mmol, 1 equiv) in dry pyridine (3 mL) and the resulting solution stirred at rt. After 6 days, the solution was concentrated in vacuo. The residue was applied to a DEAE Sephadex A25 anion exchange column using a triethylammonium bicarbonate buffer gradient (0.35–0.5 M) to afford UDP derivative **6d** (0.040 g, 67%) as a white solid, $[\alpha]_D^{18} = +41.6$ (*c* 0.5, MeOH); δ_H (400 MHz, MeOD) 1.21 (18H, t, *J* 7.3 Hz, NCH_2CH_3), 2.02, 2.08 (2× 3H, 2× s, $C{=}OCH_3$), 2.94 (12H, q, *J* 7.3 Hz, NCH_2), 3.57 (1H, dd, $J_{6,5}$ 4.9 Hz, $J_{6,6'}$ 12.4 Hz, glu: H-6), 3.63 (1H, dd, $J_{6',5}$ 1.7 Hz, $J_{6',6}$ 12.4 Hz, glu: H-6′), 3.84 (2H, m, glu: H-4, H-5), 3.97 (1H, m, glu: H-3), 4.05 (1H, m, glu: H-2), 4.14 (1H, m, rib: H-4), 4.25–4.29 (3H, m, rib: H-2, H-5, H-5′), 4.35 (1H, at, *J* 4.8 Hz, rib: H-3), 5.59 (1H, dd, $J_{1,P}$ 7.4 Hz, $J_{1,2}$ 3.1 Hz, glu: H-1), 5.85 (1H, d, $J_{5,6}$ 8.1 Hz, U: H-5), 5.99 (1H, d, *J* 5.3 Hz, rib: H-1), 8.04 (1H, d, *J* 8.1 Hz, U: H-6); δ_C (100.6 MHz, MeOD) 9.0 (NCH_2CH_3), 21.8, 22.1 (2× $C{=}OCH_3$), 46.3 (NCH_2CH_3), 52.4 (glu: C-4), 55.0 (d, $J_{C\text{-}2,P}$ 8.0 Hz, glu: C-2), 62.0 (glu: C-6), 65.3 (d, $J_{C\text{-}5,P}$ 5.0 Hz, rib: C-5), 69.6 (glu: C-5), 70.4 (rib: C-3), 73.1 (glu: C-3), 74.6 (rib: C-2), 84.1 (d, $J_{C\text{-}4,P}$ 8.0 Hz, rib: C-4), 88.8 (rib: C-1), 95.4 (d, $J_{C\text{-}1,P}$ 6.0 Hz, glu: C-1), 102.3 (U: C-5), 141.8 (U: C-6), 151.9 (U: C-4), 165.1 (U: C-2), 173.2, 173.3 (2× $CH_3C{=}O$); δ_P (162 MHz, MeOD) −11.4, −13.1 (^1H decoupled); *m/z* (ES^-) 647 ($M-H^+$, 100%); HRMS (ES^-): ($M-H^+$) calcd for $C_{19}H_{29}N_4O_{17}P_2$, 647.1002; found, 647.1000.

Acknowledgments

The authors gratefully acknowledge the European Union (Marie Curie Intra-EU Fellowship to R.D.), the BBSRC and Stiefel Laboratories Inc. (CASE award to L.B.), and the EP Abraham Cephalosporin Fund for financial support.

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