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Analogues of the Mycobacterial Arabinogalactan Linkage Disaccharide as Cell Wall Biosynthesis Inhibitors

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Abstract—The mycobacterial arabinogalactan linkage disaccharide [α-L-Rha-(1→3)-α-D-GlcNAc] provides a basis for the design of new antitubercular drugs, since it supports a key skeletal structure in the bacterial cell wall. A series of analogues of the linker was synthesized by coupling appropriate thiorhamnosyl donors modified at their 4-positions, with an *N*-acetyl glucosamine acceptor. In a cell-free enzyme inhibition assay, three analogues inhibited the activity of the galactosyltransferase that adds a Gal*f* residue to the linkage disaccharide. Although the compounds were modest inhibitors, these data confirm the viability of this approach to anti-mycobacterial agents. It is especially significant that the three effective compounds are modified at the site of the acceptor atom in the natural substrate.

Introduction

Mycobacterium tuberculosis is responsible for over 2 million deaths worldwide annually, largely in the developing countries. Multidrug resistance and the problem of dual HIV and *M. tuberculosis* infections are causes for growing global concern.[1] There is therefore a clear need for new therapeutic strategies. The search for such strategies is facilitated by the detailed information about mycobacterial biochemistry that has become available in recent years.

The cell wall of *Mycobacterium* spp. is composed of a covalently linked complex of mycolic acids, arabinan, and galactan attached to the underlying peptidoglycan layer.[2] The arabinogalactan (AG) is a central supporting structure of the cell wall. Disruption of AG biosynthesis is known to be lethal to mycobacteria, and some front-line drugs interfere with aspects of this key process. In the quest for new anti-mycobacterial agents, considerable effort has been directed to the synthesis of arabinofuranose derivatives and other compounds related to the AG.[3]

In 1990, Brennan et al. reported that the AG is linked to the underlying peptidoglycan via a unique pyranosidic disaccharide: α-L-Rha-(1→3)-α-D-GlcNAc (Fig. 1).[4] In the biosynthesis of the mycobacterial cell wall,[5] the disaccharide linker is formed first, as a diphosphoprenol ester. The poly(galactofuranose) chain of the AG is then constructed from the C-4′ hydroxyl of the linker, after which the arabinofuranosyl and mycolate components are added. It is not clear whether the mycolic acids are attached to AG before or after ligation to peptidoglycan. However, recent experiments using whole cell labeling suggest that mycolylation of the arabinan termini follows ligation of AG to peptidoglycan.[6] The linkage disaccharide is thus a key structure in the overall biosynthesis of the cell wall. Although this fact has not yet been widely exploited in the quest for anti-mycobacterial agents,[7,8] the McNeil laboratory has identified the biosynthesis of dTDP-Rha as a potential source of drug targets.[9]

We report here our preparation of a set of α-L-Rha-(1→3)-α-D-GlcNAc analogues in which the C-4′ position

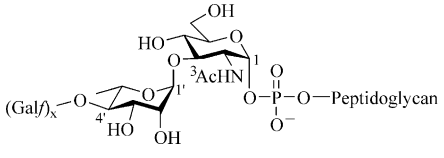

Figure 1. The AG linkage disaccharide.

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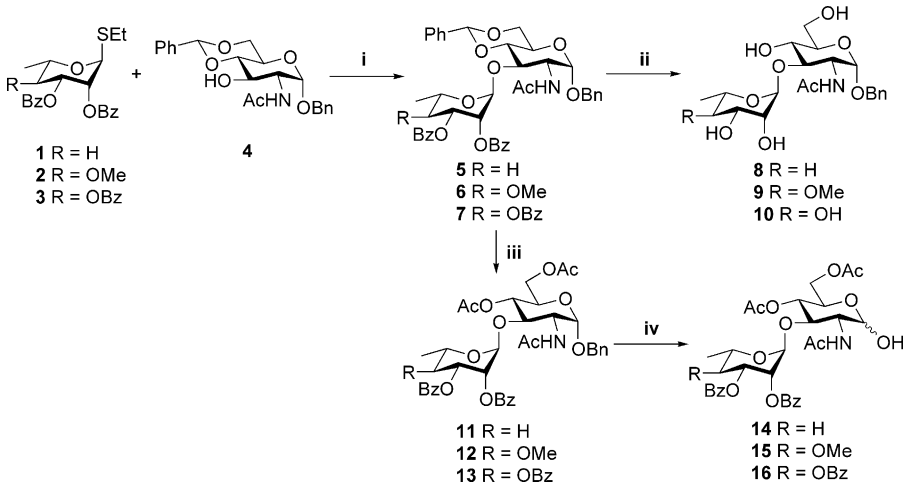


Scheme 1. Reagents and conditions: (i) NIS, TMSOTf, CH_2Cl_2, 4 Å molecular sieves, rt; (ii) (a) 80% HOAc, 70 °C; (b) NaOMe/MeOH, rt; (iii) (a) 80% HOAc, 70 °C; (2) Ac_2O, pyridine, DMAP, rt; (iv) Pd/C, H_2, HOAc, rt.

of the disaccharide has been modified to hinder the formation of the glycosidic bond to the AG polymer. We show that some of these linkage region analogues are modest inhibitors of the biosynthesis of mycobacterial cell wall components.

Results and Discussion

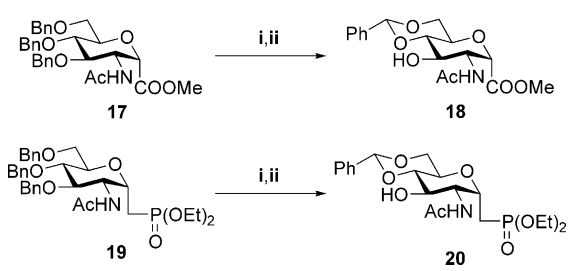
We elected to use the thioglycoside approach[10,11] to prepare our disaccharides. Thus, we obtained thioglycosyl donors **1**, **2** and **3** using literature methods.[8,12] The acceptor, benzyl *N*-acetyl-2-amino-2-deoxy-glucoside **4**,[13] was likewise a known compound. Preliminary experiments showed that NIS/TfOH was a less effective glycosylation promoter than NIS/TMSOTf. Using the latter reagent, **5**–**7** were obtained in 92–95% yields (Scheme 1). The α anomer was the exclusive product in each case, which was confirmed by the absence of NOE among H-1′, H-3′ and H-5′.

Deprotection of **5**–**7** was achieved in two steps. Initial treatment with 80% acetic acid cleaved the 4,6-*O*-benzylidene acetals, after which the benzoyl protecting groups on the rhamnosyl moieties were removed using NaOMe. Products **8**–**10** retaining the benzyl glycoside group were thus obtained in 90–93% yields. Performing these deprotection steps in the opposite order rendered the removal of the benzylidene group extremely difficult.

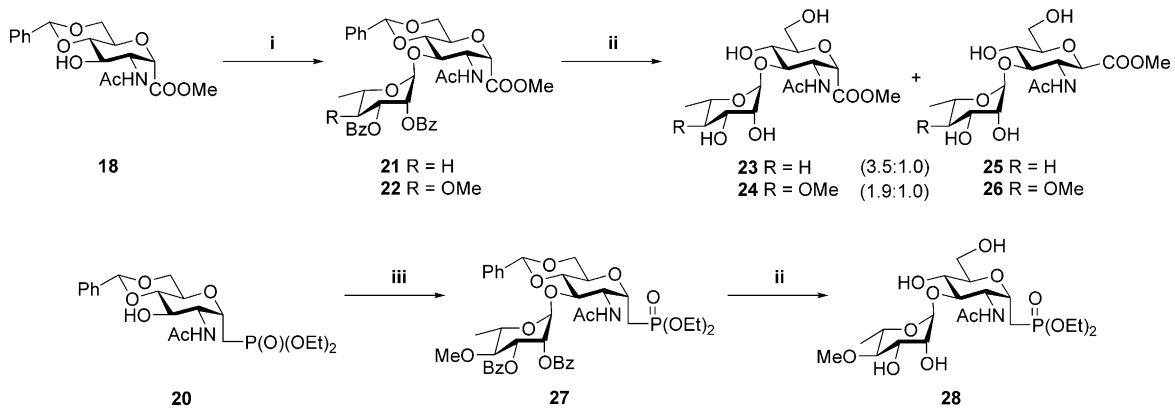
Pathak et al. had found that octyl D-Gal*f*-α-L-Rha compounds blocked by acetyl groups on the Gal*f* residue, or by a 2,3-*O*-isopropylidene group on rhamnose inhibited the growth of mycobacteria in a whole cell assay.[7] We therefore also prepared derivatives in which the non-anomeric hydroxyls were blocked by ester groups, which might be removed by nonspecific esterases present in the cell wall environment. Removal of the benzylidene protecting group followed by acetylation gave **11**–**13** in good yields. Hydrogenolysis of **11**–**13** afforded the reducing disaccharides **14**–**16**. These reactions were quite sluggish and proceeded only in moderate yields, even when performed in acetic acid solvent.

We also prepared some disaccharide analogues containing modified GlcNAc units (Scheme 2). *C*-Glycosyl GlcNAc derivatives **18** and **20** were chosen as containing sterically conservative replacements for the labile 1-phosphate group in the mycobacterial linkage disaccharide. They were prepared from known precursors **17**[14] and **19**[15] as shown in Scheme 2. Glycosidation reactions of **18** and **20** with rhamnosyl donors **1** and **2** (Scheme 3) proceeded smoothly, affording heptonic acid disaccharides **21** (61%) and **22** (83%), and phosphonate **27** (75%). Deprotection of **27** was routine, giving the free disaccharide analogue **28** in 89% yield. However, all attempts to remove the benzoyl groups from the rhamnosyl moieties in **21** and **22** led to some degree of epimerization of the methyl ester. The desired axial *C*-glycosides **23** and **24** predominated, as expected based on the known anomeric effect of *C*-linked carboxylic ester groups,[16] but we were unable to suppress this base-catalyzed process. Nevertheless, the axially- and equatorially-substituted *C*-glycosyl disaccharide analogues were separable by chromatography, and reasonable yields of **23**–**26** were obtained.

The effectiveness of our compounds as inhibitors of mycobacterial AG biosynthesis was assayed in a cell-free model system. A membrane and cell wall enriched fraction isolated from *Mycobacterium smegmatis*[17] is able to catalyze the transfer of *N*-acetyl glucosamine 1-phosphate (GlcNAc-1-P) and L-rhamnose (Rha) from their respective nucleotide donors to endogenous polyprenyl phosphate (Pol-P), yielding Pol-P-P-GlcNAc



Scheme 2. Reagents and conditions: (i) H_2, Pd/C, HOAc, rt; (ii) $C_6H_5CH(OMe)_2$, *p*-TsOH, DMF, 40 °C.



Scheme 3. Reagents and conditions: (i) **1** or **2**, NIS/TMSOTf, CH_2Cl_2, 4 Å molecular sieves, rt; (ii) (a) 80% HOAc, 70 °C; (b) NaOMe/MeOH, rt; (iii) **2**, NIS/TMSOTf, CH_2Cl_2, 4 Å molecular sieves, rt.

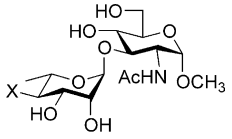


Figure 2.

(GL-1) and Pol-P-P-GlcNAc-Rha (GL-2). Galactosyl transferase-1 (Galtase-1) mediates the transfer of a galactofuranosyl (Gal*f*) residue from UDP-Gal*f* to GL-2, giving rise to Pol-P-P-GlcNAc-Rha-Gal*f* (GL-3). Our compounds were designed to disrupt the conversion of GL-2 to GL-3.

The inhibition of Galtase-1-mediated Gal*f* transfer was assayed in mixtures containing radiolabeled UDP-Gal, UDP-Gal*p* mutase,[18] dTDP-Rha, UDP-GlcNAc, and the cell-wall-enriched fraction (as the source of Galtase-1 and Pol-P) essentially as previously described.[5] The disaccharides **5**–**16**, **23**–**26** and **28** prepared in this work, as well as analogues **29** and **30** (Fig. 2) available from previous work,[8] were assayed. The identities of the radiolabeled products were confirmed by TLC.

Of the 19 analogues tested, three (**14**, **15** and **16**) inhibited the galactosyl transferase activity with IC_{50} values shown in Table 1. In order to confirm that formation of GL-3 was inhibited and not an earlier step, the activity of *N*-acetylglucosamine 1-phospate transferase was also assayed in the presence of **14**, **15** and **16** at 300 μg/mL. Assays were done essentially as described for the galactosyl transferase except the unlabelled UDP-GlcNAc was replaced with 1 μCi of UDP-[6-^{3}H(N)]GlcNAc (60 Ci/mmol, American Radiolabeled Chemicals Inc., St. Louis, MO, USA), UDP-Gal and dTDP-Rha were omitted and the reaction was incubated for 15 min to measure formation of GL-1. The compounds tended to reduce the enzymatic activity, but not significantly (Fig. 3). In other experiments, only the UDP-Gal was omitted from the reaction mixture, assaying the formation of GL-2, with similar results to those of Figure 3 (data not shown). These data strongly suggest that we are indeed blocking the biosynthetic conversion of GL-2 to GL-3, as planned.

Table 1. Gal*f* transferase inhibition by disaccharide analogues

Compd	IC_{50} (μg/mL)
14	410
15	370
16	240

The value of the disaccharide linker between the mycobacterial peptidoglycan and arabinogalactan as a drug target has been demonstrated by the observations that the dTDP-Rha synthase (dTDP-Rha is the Rha donor in the biosynthesis of the linker),[19] the rhamnosyl transferase that forms the linker[20] and a subsequent Gal*f* transferase (Rv3808c)[21] are all essential enzymes in mycobacteria. The inhibition of the Galtase enzyme in vitro by **14**–**16** clearly demonstrates the validity of designing inhibitors with the purpose of disrupting the processing of the linker between the mycobacterial peptidoglycan and arabinogalactan.

It is surprising that the protected compounds **14**, **15** and **16** were the most active inhibitors. This may be due to their lipophilic nature, which likely gives the inhibitors physical/solubility properties similar to the native lipid-linked substrates. Notably, each of these compounds is modified at the rhamnosyl 4-OH position, which is the acceptor site for the GL-2 to GL-3 conversion. This is highly unusual, as very few compounds modified at the acceptor atom have proven to be effective inhibitors of

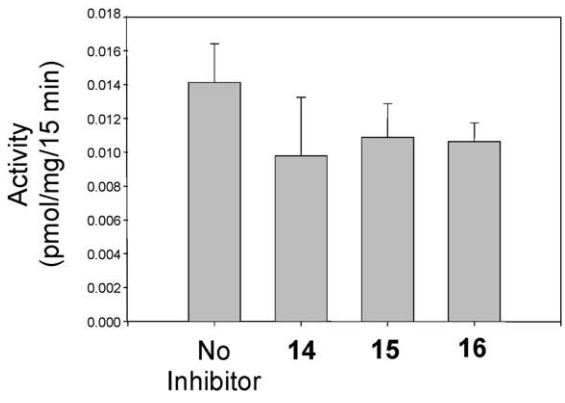


Figure 3. Effect of disaccharide inhibitors on the formation of GL-1.

glycosyltransferases,[22] let alone analogues bearing groups as large as a benzoyl.

Although the synthesized compounds are not 'drug-like' in the traditional sense, interest in carbohydrate structures as leads or drug candidates is growing.[23] In the near term, inhibitors **14–16** are much-needed tools for the study of the enzymes involved in the early stages of mycobacterial galactan synthesis. We will use them as leads for the development of better inhibitors and affinity ligands, which will greatly aid identification of the Galtase that adds the first Gal*f* residue to the disaccharide inkage unit.

Experimental

Reactions were performed under a nitrogen atmosphere in oven-dried glassware except as noted. Reagents were purchased from the Aldrich Chemical Co. and were used as received. Solvents were purified or dried according to standard procedures.[24] Flash column chromatography was performed on silica gel 60 (230–400 mesh). NMR spectra were recorded at 300 MHz for ^{1}H, 75.5 MHz for ^{13}C and 121.5 MHz for ^{31}P in the deuterated solvents except as noted. Melting points were determined on a capillary apparatus and are uncorrected. Optical rotations were measured at ambient temperature using a Rudolph Research Autopol III polarimeter. Mass spectra were obtained using electrospray ionization (ES-MS) on a Micromass Quattro LC instrument.

3-Acetamido-2, 6-anhydro-5,7-benzylidene-3-deoxy-D-*glycero*-D-*ido*-heptonic acid methyl ester 18. A vigorously stirred mixture of 4,5,7-tri-*O*-benzyl-protected ester **17**[14] (740 mg, 1.4 mmol), Pd/C catalyst (10% w/w, 500 mg), and AcOH (10 mL) was degassed under vacuum and then placed under an atmosphere of H_2 gas using a balloon. The suspension was stirred overnight at rt, before being filtered through Celite and concentrated. The residue was dissolved in DMF (5 mL) and *p*-TsOH·H_2O (20 mg) and benzaldehyde dimethyl acetal (0.25 mL, 1.7 mmol) were added. After stirring at 40 °C for 2 h, the reaction was quenched with Et_3N, and the mixture was concentrated. Crystallization of the residue in MeOH gave **18** as white crystals (310 mg, 64%).

Mp 260.5–261.0 °C. $[\alpha]_D = +74.9°$ (*c* 0.35, DMSO). ^{1}H NMR ($CDCl_3$): δ 2.04 (s, 3H, C*H*$_3$C=O), 2.73 (d, 1H, *J* = 3.0 Hz, O*H*), 3.53 (ddd, 1H, *J* = 9.7, 9.3 and 4.2 Hz, H-6), 3.59 (dd, 1H, *J* = 9.3 and 9.3 Hz, H-5), 3.74 (dd, 1H, *J* = 9.7 and 9.7 Hz, H-7), 3.83 (s, 3H, OC*H*$_3$), 4.05 (ddd, 1H, *J* = 9.3, 9.3 and 3.0 Hz, H-4), 4.33 (dd, 1H, *J* = 9.7 and 4.2 Hz, H-7), 4.48 (ddd, 1H, *J* = 9.3, 8.7 and 5.7 Hz, H-3), 4.60 (d, 1H, *J* = 5.7 Hz, H-2), 5.55 (s, 1H, PhC*H*), 6.62 (d, 1H, *J* = 8.7 Hz, N*H*Ac), 7.36–7.49 (m, 5H, *Ph*). ^{13}C NMR ($CDCl_3$): δ 23.36 (*C*H$_3$C=O), 51.60 (C-3), 52.71 (O*C*H$_3$), 67.98 (C-6), 68.66 (C-7), 70.08 (C-4), 74.49 (C-2), 82.25 (C-5), 101.96 (Ph*C*H), 170.87, 171.04. Anal. calcd for $C_{17}H_{21}NO_7$: C, 58.11; H, 6.02; N, 3.99. Found: C, 58.34; H, 6.20;N, 4.11.

Diethyl *C*-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-α-D-glucopyranosyl) methanephosphonate 20. A suspension of 3,4,6-*O*-benzyl-protected phosphonate **19**[15] (449 mg, 0.72 mmol), Pd/C catalyst (10% w/w, 400 mg), and acetic acid (1.5 mL) was degassed and flushed with H_2 gas as described above. The mixture was vigorously stirred for 3 h at rt, and then filtered through Celite and concentrated. The residue was dissolved in DMF (1 mL) and treated with *p*-TsOH·H_2O (20 mg) and benzaldehyde dimethyl acetal (0.25 mL, 1.7 mmol) at 40 °C for 1 h. The reaction was quenched with Et_3N and concentrated. Chromatography of the residue (EtOAc/CH_2Cl_2/MeOH 1/1/0.05–1/1/0.2) afforded **20** as a white solid (184 mg, 58%).

Mp 188.5–189.2 °C. $[\alpha]_D = +3.8°$ (*c* 1.0, $CHCl_3$). ^{1}H NMR (acetone-d_6, 500 MHz): δ 1.28 (t, 6H, *J* = 7.1 Hz, 2 × C*H*$_3$CH$_2$), 1.92 (s, 3H, C*H*$_3$C=O), 2.09–2.13 (m, 1H, PC*H*$_2$), 2.43 (ddd, 1H, *J* = 16.1, 16.1 and 11.3 Hz, PC*H*$_2$), 3.55 (dd, 1H, *J* = 9.1 and 9.1 Hz, H-4), 3.66–3.80 (m, 2H, H-5, 6), 3.90 (ddd, 1H, *J* = 10.5, 9.1 and 4.6 Hz, H-3), 4.04–4.10 (m, 4H, 2×OC*H*$_2$CH$_3$), 3.98–4.13 (m, 1H, H-2), 4.16–4.20 (m, 1H, H-6), 4.53–4.61 (m, 1H, H-1), 5.62 (s, 1H, PhC*H*), 7.30–7.54 (m, 6H, *Ph* and N*H*Ac). ^{13}C NMR (acetone-d_6, 125.8 MHz): δ 16.86 (d, *J* = 3.3 Hz, *C*H$_3$CH$_2$), 16.90 (d, *J* = 3.1 Hz, *C*H$_3$CH$_2$), 23.03 (*C*H$_3$C=O), 24.49 (d, *J* = 142.9 Hz, P*C*H$_2$), 55.56 (d, *J* = 12.9 Hz, C-2), 61.90 (d, *J* = 5.9 Hz, CH$_3$*C*H$_2$), 62.37 (d, *J* = 5.9 Hz, CH$_3$*C*H$_2$), 65.26 (C-5), 68.85 (C-3), 69.76 (C-6), 71.81 (d, *J* = 4.8 Hz, C-1), 84.26 (C-4), 102.37 (Ph*C*H), 170.82. ^{31}P NMR ($CDCl_3$): δ 29.20. Anal. calcd for $C_{20}H_{32}NO_8P$: C, 53.93; H, 7.24; N, 3.15. Found: C, 54.34; H, 7.31; 3.20.

General procedure for glycoside synthesis

The thiorhamnosyl donor **1**, **2**, or **3** (0.50 mmol), *N*-acetyl glucosamine acceptor **4**, **18** or **20** (0.60 mmol) and freshly activated, powdered 4 Å molecular sieves (500 mg) were dissolved in dry CH_2Cl_2 (4 mL). The mixture was vigorously stirred for 1 h, after which *N*-iodosuccinimide (0.65 mmol) and TMSOTf (10 μL) were added. The reaction was allowed to proceed at rt until TLC indicated the complete consumption of the donor. The reaction mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed successively with saturated aqueous solutions of $Na_2S_2O_3$ and $NaHCO_3$, water and brine, then dried and concentrated. The residue was purified by flash chromatography, eluting with a mixture of hexane and EtOAc, to obtain **5–7**, **21**, **22** or **27**.

Benzyl 2′,3′-di-*O*-benzoyl-4′,6′-dideoxy-α-L-*lyxo*-hexopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside 5. White solid, 95%. Mp 232–233 °C. $[\alpha]_D = +68.5°$ (*c* 1.03, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 0.77 (d, 3H, *J* = 6.2 Hz, H-6′), 1.75–1.90 (m, 2H, H-4′), 2.10 (s, 3H, C*H*$_3$C=O), 3.70 (dd, 1H, *J* = 9.5 and 9.5 Hz, H-4), 3.79 (dd, 1H, *J* = 10.2 and 10.2 Hz, H-6), 3.93 (ddd, 1H, *J* = 10.2, 9.5 and 4.7 Hz, H-5), 3.98

(dd, 1H, $J=9.5$ and 9.5 Hz, H-3), 4.25 (dd, 1H, $J=10.2$ and 4.7 Hz, H-6), 4.27 (m, 1H, H-5′), 4.45 (ddd, 1H, $J=9.9$, 9.5 and 3.7 Hz, H-2), 4.49 (d, 1H, $J=11.8$ Hz, PhCH_2), 4.74 (d, 1H, $J=11.8$ Hz, PhCH_2), 4.87 (d, 1H, $J=3.7$ Hz, H-1), 5.17 (m, 2H, H-2′, 1′), 5.51–5.54 (m, 1H, H-3′), 5.57 (s, 1H, PhC*H*), 5.69 (d, 1H, $J=9.9$ Hz, N*H*Ac), 7.30–8.10 (m, 20H, 4×*Ph*). ^{13}C NMR ($CDCl_3$): δ 20.35 (C-6′), 23.42 (*C*H_3C=O), 23.72 (C-4′), 53.12 (C-2), 63.50 (C-5), 64.28 (C-5′), 67.75 (C-3′), 68.95 (C-6), 69.47 (C-2′), 70.03 (Ph*C*H_2), 76.58 (C-3), 80.36 (C-4), 97.81 (C-1), 99.09 (C-1′), 102.19 (Ph*C*H), 165.45 (Ph*C*=O), 165.70 (Ph*C*=O), 170.61 (CH_3*C*=O). Anal. calcd for $C_{42}H_{43}NO_{11}$: C, 68.37; H, 5.87; N, 1.90. Found: C, 68.54; H, 6.05; N, 1.94.

Benzyl 2′,3′- di-*O*-benzoyl-4′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside 6. White solid, 93%. Mp 216–217 °C. $[\alpha]_D=+114°$ (*c* 1.07, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 0.95 (d, 3H, $J=6.2$ Hz, H-6′), 2.09 (s, 3H, C*H*$_3$C=O), 3.39 (s, 3H, OC*H*$_3$), 3.40 (dd, 1H, $J=9.8$ and 9.8 Hz, H-4′), 3.77 (dd, 1H, $J=9.8$ and 9.6 Hz, H-4), 3.83 (d, 1H, $J=10.1$ Hz, H-6), 3.94 (dd, 1H, $J=9.8$ and 4.6 Hz, H-5), 4.01 (dd, 1H, $J=9.6$ and 9.6 Hz, H-3), 4.14 (dq, 1H, $J=9.8$ and 6.2 Hz, H-5′), 4.28 (dd, 1H, $J=10.1$ and 4.6 Hz, H-6), 4.51 (ddd, 1H, $J=9.8$, 9.6 and 3.7 Hz, H-2), 4.52 (d, 1H, $J=11.8$ Hz, PhCH_2), 4.76 (d, 1H, $J=11.8$ Hz, PhCH_2), 4.90 (d, 1H, $J=3.7$ Hz, H-1), 5.14 (d, 1H, $J=1.7$ Hz, H-1′), 5.37 (dd, 1H, $J=3.4$ and 1.7 Hz, H-2′), 5.60 (s, 1H, PhC*H*), 5.67 (dd, 1H, $J=9.8$ and 3.4 Hz, H-3′), 5.74 (d, 1H, $J=9.8$ Hz, N*H*Ac), 7.28–8.06 (m, 20H, 4×*Ph*). ^{13}C NMR ($CDCl_3$): δ 17.18 (C-6′), 23.28 (*C*H_3C=O), 53.02 (C-2), 59.89 (O*C*H_3), 63.39 (C-5), 67.56 (C-5′), 68.88 (C-6), 69.99 (Ph*C*H_2), 71.05 (C-3′), 71.97 (C-2′), 75.71 (C-3), 80.33 (C-4), 80.77 (C-4′), 97.73 (C-1), 97.87 (C-1′), 101.91 (Ph*C*H), 165.10 (Ph*C*=O), 165.42 (Ph*C*=O), 170.38 (CH_3*C*=O). Anal. calcd for $C_{43}H_{45}NO_{12}$: C, 67.26; H, 5.91; N, 1.82. Found: C, 66.91; H, 6.04; N, 1.89.

Benzyl 2′,3′,4′-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4, 6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside 7. Glassy solid, 92%. $[\alpha]_D=+100°$ (*c* 1.03, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.86 (d, 3H, $J=6.2$ Hz, H-6′), 2.12 (s, 3H, C*H*$_3$C=O), 3.83 (dd, 1H, $J=10.0$ and 10.0 Hz, H-6), 3.84 (dd, 1H, $J=10.0$ and 10.0 Hz, H-4), 3.99 (ddd, 1H, $J=10.0$, 10.0 and 4.6 Hz, H-5), 4.09 (dd, 1H, $J=10.0$ and 10.0 Hz, H-3), 4.32 (dd, 1H, $J=10.0$ and 4.6 Hz, H-6), 4.48 (dq, 1H, $J=10.0$ and 6.2 Hz, H-5′), 4.53 (d, 1H, $J=11.8$ Hz, PhCH_2), 4.60 (ddd, 1H, $J=10.4$, 10.0 and 3.7 Hz, H-2), 4.78 (d, 1H, $J=11.8$ Hz, PhCH_2), 4.92 (d, 1H, $J=3.7$ Hz, H-1), 5.27 (d, 1H, $J=1.6$ Hz, H-1′), 5.45 (dd, 1H, $J=3.4$ and 1.6 Hz, H-2′), 5.60 (dd, 1H, $J=10.0$ and 10.0 Hz, H-4′), 5.67 (s, 1H, PhC*H*), 5.85 (d, 1H, $J=10.4$ Hz, N*H*Ac), 5.90 (dd, 1H, $J=10.0$ and 3.4 Hz, H-3′), 7.20–8.15 (m, 20H, 4×*Ph*). ^{13}C NMR ($CDCl_3$): δ 16.74 (C-6′), 23.14 (*C*H_3C=O), 53.20 (C-2), 63.58 (C-5), 66.82 (C-5′), 69.12 (C-6), 69.70 (C-3′), 70.39 (Ph*C*H_2), 71.89 (C-2′), 72.12 (C-4′), 75.70 (C-3), 80.55 (C-4), 98.20 (C-1′), 98.43 (C-1), 102.70 (Ph*C*H). Anal. calcd for $C_{49}H_{47}NO_{13}$: C, 68.60; H, 5.52; N, 1.63. Found: C, 68.24; H, 5.29; N, 2.07.

2′,3′-Di-*O*-benzoyl-4′,6′-dideoxy-α-L-*lyxo*-hexopyranosyl-(1→4)-3-acetamido-2,6-anhydro-5,7-benzylidene-3-deoxy-D-*glycero*-D-*ido*-heptonic acid methyl ester 21. Crystals, 61%, mp 242–243 °C. $[\alpha]_D=+22.9°$ (*c* 1.25, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 0.78 (d, 3H, $J=6.2$ Hz, H-6′), 1.84–2.04 (m, 2H, H-4′), 2.16 (s, 3H, C*H*$_3$C=O), 3.51 (ddd, 1H, $J=9.7$, 9.7 and 4.8 Hz, H-6), 3.71 (dd, 1H, $J=9.7$ and 9.7 Hz, H-5), 3.76 (dd, 1H, $J=10.0$ and 9.7 Hz, H-7), 3.85 (s, 3H, OC*H*$_3$), 4.10 (dd, 1H, $J=10.0$ and 9.7 Hz, H-4), 4.25 (m, 1H, H-5′), 4.34 (dd, 1H, $J=10.0$ and 4.8 Hz, H-7), 4.51 (d, 1H, $J=5.6$ Hz, H-2), 4.69 (ddd, 1H, $J=10.0$, 9.9 and 5.6 Hz, H-3), 5.21–5.24 (m, 2H, H-2′, 1′), 5.50–5.58 (m, 1H, H-3′), 5.57 (s, 1H, PhC*H*), 6.39 (d, 1H, $J=9.9$ Hz, N*H*Ac), 7.33–8.10 (m, 15H, 3×*Ph*). ^{13}C NMR ($CDCl_3$): δ 20.35 (C-6′), 23.45 (*C*H_3C=O), 33.70 (C-4′), 51.07 (C-3), 52.78 (O*C*H_3), 64.39 (C-5′), 67.72 (C-3′), 68.72 (C-2′), 68.78 (C-6), 69.29 (C-7), 75.03 (C-2), 75.93 (C-4), 80.51 (C-5), 99.13 (C-1′), 102.19 (Ph*C*H), 165.47 (Ph*C*=O), 165.75 (Ph*C*=O), 170.79 (R*C*=O), 170.96 (R*C*=O). Anal. calcd for $C_{37}H_{39}NO_{12}$: C, 64.43; H, 5.70; N, 2.03. Found: C, 64.15; H, 5.67; N, 2.15.

2′,3′-Di-*O*-benzoyl-4′-*O*-methyl-α-L-rhamnopyranosyl-(1→4)-3-acetamido-2,6-anhydro-5,7-benzylidene-3-deoxy-D-*glycero*-D-*ido*-heptonic acid methyl ester 22. Amorphous glassy solid, 83%, $[\alpha]_D=+91.3°$ (*c* 1.05, EtOAc). ^{1}H NMR ($CDCl_3$): δ 0.92 (d, 3H, $J=6.2$ Hz, H-6′), 2.12 (s, 3H, C*H*$_3$C=O), 3.39 (s, 3H, OC*H*$_3$), 3.42 (dd, 1H, $J=9.8$ and 9.8 Hz, H-4′), 3.51 (ddd, 1H, $J=9.8$, 9.8 and 4.8 Hz, H-6), 3.77 (dd, 1H, $J=9.8$ and 9.8 Hz, H-5), 3.77 (ddd, 1H, $J=10.6$, 9.8 and 4.8 Hz, H-7), 3.84 (s, 3H, OC*H*$_3$), 4.10 (dd, 1H, $J=10.0$ and 9.8 Hz, H-4), 4.10 (dq, 1H, $J=9.8$ and 6.2 Hz, H-5′), 4.34 (dd, 1H, $J=10.6$ and 4.8 Hz, H-7), 4.53 (d, 1H, $J=5.6$ Hz, H-2), 4.70 (ddd, 1H, $J=10.0$, 9.7 and 5.6 Hz, H-3), 5.17 (d, 1H, $J=1.6$ Hz, H-1′), 5.37 (dd, 1H, $J=3.4$ and 1.6 Hz, H-2′), 5.57 (s, 1H, PhC*H*), 5.64 (dd, 1H, $J=9.8$ and 3.4 Hz, H-3′), 6.36 (d, 1H, $J=9.7$ Hz, N*H*Ac), 7.32–8.04 (m, 15H, 3×*Ph*). ^{13}C NMR ($CDCl_3$): δ 17.20 (C-6′), 23.35 (*C*H_3C=O), 51.05 (C-3), 52.74 (O*C*H_3), 60.12 (O*C*H_3), 67.76 (C-5′), 68.57 (C-6), 68.75 (C-7), 71.22 (C-3′), 71.81 (C-2′), 74.93 (C-2), 75.65 (C-4), 80.49 (C-5), 80.79 (C-4′), 97.95 (C-1′), 101.96 (Ph*C*H), 165.23 (Ph*C*=O), 165.53 (Ph*C*=O), 170.70 (R*C*=O), 170.87 (R*C*=O). Anal. calcd for $C_{38}H_{41}NO_{13}$: C, 63.41; H, 5.74; N, 1.95. Found: C, 63.01; H, 5.78; N, 1.95.

Diethyl 2′,3′-di-*O*-benzoyl-4′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-*C*-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-α-D-glucopyranosyl) methanephosphonate 27. Amorphous white solid, 75%, $[\alpha]_D=+25.0°$ (*c* 0.76, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.93 (d, 3H, $J=6.1$ Hz, H-6′), 1.23 (t, 3H, $J=7.0$ Hz, C*H*$_3$CH$_2$), 1.25 (t, 3H, $J=7.0$ Hz, C*H*$_3$CH$_2$), 2.10 (s, 3H, C*H*$_3$C=O), 2.10–2.30 (m, 2H, PCH_2), 3.37 (s, 3H, OC*H*$_3$), 3.40 (dd, 1H, $J=9.7$ and 9.7 Hz, H-4′), 3.71–3.74 (m, 3H, H-5, 4, 6), 3.94–4.12 (m, 6H, 2×OCH_2CH$_3$, H-3, 5′), 4.23 (m, 1H, H-6), 4.40–4.50 (m, 2H, H-2, 1), 5.24 (d, 1H, $J=1.4$ Hz, H-1′), 5.42 (dd, 1H, $J=3.4$ and 1.4 Hz, H-2′), 5.57 (s, 1H, PhC*H*), 5.62 (dd, 1H, $J=9.7$ and 3.4 Hz, H-3′), 7.32–8.02 (m, 16H, 3×*Ph*, N*H*Ac). ^{13}C NMR ($CDCl_3$): δ 16.29 (*C*H$_3$CH$_2$), 16.37 (*C*H$_3$CH$_2$), 17.31 (C-6′), 23.03

(CH_3C=O), 25.68 (d, J=143.4 Hz, PCH_2), 53.38 (d, J=13.5 Hz, C-2), 60.03 (OCH_3), 62.14 (d, J=6.5 Hz, CH_3CH_2), 62.32 (d, J=6.5 Hz, CH_3CH_2), 65.10 (C-5), 67.60 (C-5′), 69.29 (C-6), 70.75 (d, J=4.5 Hz, C-1), 71.30 (C-2′), 71.55 (C-3′), 74.68 (C-3), 80.85 (C-4), 81.00 (C-4′), 97.55 (C-1′), 101.92 (PhCH), 165.21 (PhC=O), 165.29 (PhC=O), 171.17 (CH_3C=O). ^{31}P NMR ($CDCl_3$): δ 28.50.

Preparation of 8–10, 23–26 and 28

The disaccharide **5**, **6** **7** or **27** (0.2 mmol) was treated with 80% aqueous acetic acid at 70 °C for 2 h. The reaction mixture was then evaporated to dryness and 0.1 M NaOMe in MeOH (5 mL) was added. After a further 1 h at rt, Amberlite IR-120(H^+) was added. The resin was removed by filtration, and the filtrate was concentrated. Column chromatography using mixtures of EtOAc, CH_2Cl_2 and MeOH as the eluant afforded the pure products **8**–**10** or **28**. The same procedure applied to **21** or **22** led to mixtures of stereoisomers **23** and **25**, or **24** and **26** respectively, which were separated chromatographically.

Benzyl 4′,6′-dideoxy-α-L-*lyxo*-hexopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside 8. Crystals, 93%. Mp 193–194 °C. $[\alpha]_D$ = +81.1° (*c* 0.35, CH_2Cl_2). ^{1}H NMR (D_2O): δ 1.15 (d, 3H, J=6.3 Hz, H-6′), 1.55 (ddd, 1H, J=11.9, 11.9 and 11.9 Hz, H-4′), 1.70 (ddd, 1H, J=11.9, 2.3 and 0.7 Hz, H-4′), 2.00 (s, 3H, CH_3C=O), 3.53 (dd, 1H, J=9.0 and 9.0 Hz, H-4), 3.59 (dd, 1H, J=1.7 and 1.7 Hz, H-2′), 3.72–3.86 (m, 4H, H-6, 5, 3), 3.96 (ddd, 1H, J=11.9, 1.7 and 0.7 Hz, H-3′), 4.02 (dd, 1H, J=10.4 and 3.6 Hz, H-2), 4.20 (ddd, 1H, J=11.9, 6.3 and 2.3 Hz, H-5′), 4.57 (d, 1H, J=11.8 Hz, PhCH_2), 4.78 (d, 1H, J=11.8 Hz, PhCH_2), 4.88 (d, 1H, J=1.7 Hz, H-1′), 4.90 (d, 1H, J=3.6 Hz, H-1), 7.40–7.46 (m, 5H, *Ph*). ^{13}C NMR (D_2O): δ 20.25 (C-6′), 22.23 (CH_3C=O), 34.76 (C-4′), 53.51 (C-2), 60.85 (C-6), 65.48 (C-3′), 66.01 (C-5′), 68.79 (C-4), 68.82 (C-2′), 69.95 (PhCH_2), 72.64 (C-5), 79.60 (C-3), 96.57 (C-1), 102.29 (C-1′), 174.49 (CH_3C=O). ESI-MS: $C_{21}H_{31}NO_9$, [M+H^+], 442.48; Found 442.59.

Benzyl 4′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside 9. Crystals, 90%, mp 161–163 °C. $[\alpha]_D$ = +61.9° (*c* 0.75, CH_3OH). ^{1}H NMR (D_2O): δ 1.25 (d, 3H, J=6.3 Hz, H-6′), 1.99 (s, 3H, CH_3C=O), 3.15 (dd, 1H, J=9.6 and 9.6 Hz, H-4′), 3.52 (s, 3H, OCH_3), 3.55 (dd 1H, J=10.0 and 10.0 Hz, H-4), 3.75–3.85 (m, 3H, H-6, 5), 3.76 (dd, 1H, J=2.9 and 1.4 Hz, H-2′), 3.77 (dd, 1H, J=10.0 and 10.0 Hz, H-3), 3.78 (dd, 1H, J=9.6 and 2.9 Hz, H-3′), 3.97 (dq, 1H, J=9.6 and 6.3 Hz, H-5′), 4.03 (dd, 1H, J=10.0 and 3.6 Hz, H-2), 4.56 (d, 1H, J=11.8 Hz, PhCH_2), 4.76 (d, 1H, J=11.8 Hz, PhCH_2), 4.80 (d, 1H, J=1.4 Hz, H-1′), 4.89 (d, 1H, J=3.6 Hz, H-1), 7.30–7.50 (m, 5H, *Ph*). ^{13}C NMR (D_2O): δ 17.02 (C-6′), 22.22 (CH_3C=O), 53.53 (C-2), 60.36 (OCH_3), 60.84 (C-6), 68.21 (C-5′), 68.69 (C-4), 69.94 (PhCH_2), 70.30 (C-3′), 71.23 (C-2′), 72.71 (C-5), 79.58 (C-3), 82.60 (C-4′), 96.53 (C-1), 101.40 (C-1′), 174.47 (CH_3C=O). Anal. calcd for $C_{22}H_{33}NO_{10}$: C, 56.04; H, 7.05; Found: C, 55.51; H, 6.58.

Benzyl α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside 10. Crystals, 93%, mp 211–212 °C. $[\alpha]_D$ = +69.0° (*c* 0.50, CH_3OH). ^{1}H NMR (D_2O): δ 1.21 (d, 3H, J=6.3 Hz, H-6′), 1.99 (s, 3H, CH_3C=O), 3.41 (dd, 1H, J=9.6 and 9.6 Hz, H-4′), 3.55 (dd, 1H, J=9.1 and 9.1 Hz, H-4), 3.75 (dd, 1H, J=9.6 and 3.3 Hz, H-3′), 3.75 (dd, 1H, J=3.3 and 1.2 Hz, H-2′), 3.75 (dd, 1H, J=9.1 and 9.1 Hz, H-3), 3.77–3.84 (m, 3H, H-6, 5), 3.96 (dq, 1H, J=9.6 and 6.3 Hz, H-5′), 4.04 (dd, 1H, J=9.1 and 3.6 Hz, H-2), 4.56 (d, 1H, J=11.8 Hz, PhCH_2), 4.77 (d, 1H, J=11.8 Hz, PhCH_2), 4.83 (d, 1H, J=1.2 Hz, H-1′), 4.89 (d, 1H, J=3.6 Hz, H-1), 7.30–7.50 (m, 5H, *Ph*). ^{13}C NMR (D_2O): δ 16.83 (C-6′), 22.24 (CH_3C=O), 53.54 (C-2), 60.86 (C-6), 68.72 (C-4), 69.24 (C-5′), 69.94 (PhCH_2), 70.58 (C-3′), 71.12 (C-2′), 72.27 (C-4′), 72.72 (C-3), 79.72 (C-5), 96.54 (C-1), 101.56 (C-1′), 174.47 (CH_3C=O). Anal. calcd for $C_{21}H_{31}NO_{10}$: C, 55.14; H, 6.83; Found: C, 54.76; H, 6.95.

4′,6′-Dideoxy-α-L-*lyxo*-hexopyranosyl-(1→4)-3-acetamido-2, 6-anhydro-3-deoxy-D-*glycero*-D-*ido*-heptonic acid methyl ester 23. Amorphous white solid, 44%. $[\alpha]_D$ = +8.2° (*c* 0.69, H_2O). ^{1}H NMR (D_2O): δ 1.17 (d, 3H, J=6.3 Hz, H-6′), 1.58 (q, 1H, J=11.9 Hz, H-4′), 1.73 (ddd, 1H, J=11.9, 4.5 and 2.2 Hz, H-4′), 2.04 (s, 3H, CH_3C=O), 3.59 (t, 1H, J=7.8 Hz, H-5), 3.69 (dd, 1H, J=3.2 and 1.6 Hz, H-2′), 3.75 (m, 1H, H-6), 3.80 (m, 2H, H-7), 3.81 (s, 3H, OCH_3), 3.85 (dd, 1H, J=11.0 and 7.8 Hz, H-4), 4.00 (ddd, 1H, J=11.9, 4.5 and 3.2 Hz, H-3′), 4.16 (ddq, 1H, J=11.9, 6.3 and 2.2 Hz, H-5′), 4.30 (dd, 1H, J=9.1 and 5.2 Hz, H-3), 4.65 (d, 1H, J=5.2 Hz, H-2), 5.00 (d, 1H, J=1.6 Hz, H-1′). ^{13}C NMR (D_2O): δ 20.29, 22.32, 34.80, 50.47, 53.27, 60.47, 65.48, 66.19, 68.29, 68.68, 77.94, 78.00, 101.56, 171.53, 174.64.

4′,6′-Dideoxy-α-L-*lyxo*-hexopyranosyl-(1→4)-3-acetamido-2, 6-anhydro-3-deoxy-D-*glycero*-D-*gulo*-heptonic acid methyl ester 25. Amorphous white solid, 12%. $[\alpha]_D$ = −43.8° (*c* 0.37, H_2O). ^{1}H NMR (D_2O): δ 1.17 (d, 3H, J=6.3 Hz, H-6′), 1.56 (q, 1H, J=11.9 Hz, H-4′), 1.74 (ddd, 1H, J=11.9, 2.3 and 0.7 Hz, H-4′), 2.03 (s, 3H, CH_3C=O), 3.52 (ddd, 1H, J=9.8, 5.3 and 1.9 Hz, H-6), 3.54 (t, 1H, J=9.8 Hz, H-5), 3.65 (dd, 1H, J=5.3 and 1.5 Hz, H-2′), 3.68 (dd, 1H, J=10.6 and 9.8 Hz, H-4), 3.75 (s, 3H, OCH_3), 3.76 (dd, 1H, J=12.7 and 5.3 Hz, H-7), 3.91 (dd, 1H, J=12.7 and 1.9 Hz, H-7), 4.00 (ddd, 1H, J=11.9, 5.3 and 0.7 Hz, H-3′), 4.01 (t, 1H, J=10.6 Hz, H-3), 4.09 (d, 1H, J=10.6 Hz, H-2), 4.22 (ddq, 1H, J=11.9, 6.3 and 2.3 Hz, H-5′), 4.94 (d, 1H, J=1.5 Hz, H-1′). ^{13}C NMR (D_2O): δ 20.26 (C-6′), 22.22 (CH_3C=O), 34.77 (C-4′), 53.35 (C-3), 53.64 (OCH_3), 61.14 (C-7), 65.46 (C-3′), 66.13 (C-5′), 68.66 (C-5), 68.74 (C-2′), 76.45 (C-2), 80.10 (C-6), 81.92 (C-4), 102.41 (C-1′), 171.25 (C=O), 174.77 (C=O).

4′-*O*-Methyl-α-L-rhamnopyranosyl-(1→4)-3-acetamido-2, 6-anhydro-3-deoxy-D-*glycero*-D-*ido*-heptonic acid methyl ester 24. Amorphous white solid, 28%. $[\alpha]_D$ = −1.0° (*c* 1.23, H_2O). ^{1}H NMR (D_2O): δ 1.27 (d, 3H, J=6.3 Hz, H-6′), 2.04 (s, 3H, CH_3C=O), 3.18 (dd, 1H, J=9.6 and 9.6 Hz, H-4′), 3.54 (s, 3H, OCH_3), 3.59 (dd, 1H, J=8.0

and 8.0 Hz, H-5), 3.67–3.73 (m, 1H, H-6), 3.81 (s, 3H, OCH_3), 3.77–3.95 (m, 6H, H-7, 4, 5′, 3′, 2′), 4.29 (dd, 1H, J = 9.3 and 5.3 Hz, H-3), 4.64 (d, 1H, J = 5.3 Hz, H-2), 4.94 (d, 1H, J = 1.6 Hz, H-1′). ^{13}C NMR (D_2O): δ 17.09, 22.32, 50.60, 53.28, 60.44, 60.55, 68.26, 68.40, 70.33, 71.13, 72.71, 77.98, 78.20, 82.64, 100.79, 171.49, 174.63.

4′-*O*-Methyl-α-L-rhamnopyranosyl-(1→4)-3-acetamido-2, 6-anhydro-3-deoxy-D-*glycero*-D-*gulo*-heptonic acid methyl ester 26. Amorphous white solid, 15%. $[\alpha]_D = -40.0°$ (*c* 0.35, CH_2Cl_2). ^{1}H NMR (D_2O): δ 1.26 (d, 3H, J = 6.3 Hz, H-6′), 2.02 (s, 3H, CH_3C=O), 3.17 (dd, 1H, J = 7.8 and 7.8 Hz, H-4′), 3.53 (s, 3H, OCH_3), 3.48–3.57 (m, 2H, H-5, 3′), 3.67 (dd, 1H, J = 10.6 and 10.0 Hz, H-4), 3.71–3.81 (m, 3H, H-2′ 6, 7,), 3.93 (dd, 1H, J = 12.5 and 1.9 Hz, H-7), 3.99 (dd, 1H, J = 10.0 and 6.3 Hz, H-5′), 4.00 (dd, 1H, J = 10.6 and 10.6 Hz, H-3), 4.09 (d, 1H, J = 10.6 Hz, H-2), 4.87 (d, 1H, J = 1.4 Hz, H-1′). ^{13}C NMR (D_2O): δ 17.04, 22.22, 53.37, 53.64, 60.37, 61.12, 68.33, 68.54, 70.27, 71.18, 76.41, 80.17, 81.89, 82.60, 101.52, 171.21, 174.76.

Diethyl 4′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-*C*-(2-acetamido-2-deoxy-α-D-glucopyranosyl) methanephosphonate 28. Colorless thick oil, 89%. $[\alpha]_D = +11.0°$ (*c* 0.20, CH_2Cl_2). ^{1}H NMR (D_2O): δ 1.27 (d, 3H, J = 6.3 Hz, H-6′), 1.20–1.40 (m, 6H, 2×CH_3CH$_2$), 2.04 (s, 3H, CH_3C=O), 2.11 (ddd, 1H, J = 19.4, 16.0 and 3.5 Hz, PCH_2), 2.45 (ddd, 1H, J = 16.0, 16.0 and 11.0 Hz, H-1), 3.18 (dd, 1H, J = 9.5 and 9.5 Hz, H-4′), 3.54 (s, 3H, OCH_3), 3.64 (dd, 1H, J = 7.9 and 7.9 Hz, H-4), 3.66 (dd, 1H, J = 9.4 and 7.9 Hz, H-3), 3.71 (ddd, 1H, J = 7.9, 4.0 and 2.5 Hz, H-5), 3.77 (dd, 1H, J = 11.9 and 2.5 Hz, H-6), 3.81 (dd, 1H, J = 9.5 and 3.0 Hz, H-3′), 3.84 (dd, 1H, J = 3.0 and 1.5 Hz, H-2′), 3.88 (dd, 1H, J = 11.9 and 4.0 Hz, H-6), 3.92 (dq, 1H, J = 9.5 and 6.3 Hz, H-5′), 4.10–4.20 (m, 4H, CH$_3$CH_2), 4.33–4.43 (m, 1H, H-1), 4.89 (d, 1H, J = 1.5 Hz, H-1′). ^{13}C NMR (D_2O): δ 15.95 (*C*H$_3$CH$_2$), 16.03 (*C*H$_3$CH$_2$), 17.14 (C-6′), 22.33 (*C*H$_3$C=O), 23.51 (d, J = 142.6 Hz, P*C*H$_2$), 52.25 (d, J=13.5 Hz, C-2), 60.32 (C-6), 60.43 (O*C*H$_3$), 63.90 (d, J=6.7 Hz, CH$_3$*C*H$_2$), 64.05 (d, J = 6.7 Hz, CH$_3$*C*H$_2$), 68.22 (C-4), 68.47 (C-5′), 68.60 (d, J = 5.2 Hz, C-1), 70.32 (C-3′), 71.15 (C-2′), 74.87 (C-3), 77.97 (C-5), 82.64 (C-4′), 100.75 (C-1′), 174.55 (CH$_3$*C*=O). ^{31}P NMR (D_2O): δ 32.33. Anal. calcd for $C_{20}H_{38}NO_{12}P$: C, 46.60; H, 7.43; N, 2.72. Found: C, 46.26; H, 7.52; N, 2.80.

Preparation of 11–13

Disaccharide **5**, **6** or **7** (0.2 mmol) was treated with 80% HOAc at 70 °C for 2 h. The solvent was then evaporated, and pyridine (2 mL), Ac_2O (1 mL) and DMAP (20 mg) were added. The reaction was stirred at rt until judged complete by TLC analysis. The mixture was diluted with CH_2Cl_2 and washed with 1 M HCl, satd $NaHCO_3$, water and brine. The organic layer was dried and concentrated. The residue was purified by chromatography, eluting with a mixture of hexane and EtOAc, to afford **11**, **12** or **13**.

Benzyl 2′,3′-di-*O*-benzoyl-4′,6′-dideoxy-α-L-*lyxo*-hexopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside 11. Amorphous white solid, 82%. $[\alpha]_D = +99.2°$ (*c* 0.65, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 1.24 (d, 3H, J = 6.1 Hz, H-6′), 1.92–2.00 (m, 2H, H-4′), 2.09 (s, 6H, 2×CH_3C=O), 2.10 (s, 3H, CH_3C=O), 3.89–3.95 (m, 2H, H-3, 5), 4.00–4.14 (m, 2H, H-6, 5′), 4.20 (dd, 1H, J = 12.3 and 4.6 Hz, H-6), 4.47 (ddd, 1H, J = 10.0, 9.7 and 3.6 Hz, H-2), 4.51 (d, 1H, J = 11.8 Hz, PhCH_2), 4.70 (d, 1H, J = 11.8 Hz, PhCH_2), 4.94 (d, 1H, J = 3.6 Hz, H-1), 5.10 (d, 1H, J = 1.6 Hz, H-1′), 5.15 (dd, 1H, J = 9.8 and 9.8 Hz, H-4), 5.28 (dd, 1H, J = 2.1 and 1.6 Hz, H-2′), 5.43–5.50 (m, 1H, H-3′), 5.91 (d, 1H, J = 9.7 Hz, N*H*Ac), 7.27–8.08 (m, 15H, 3×*Ph*). ^{13}C NMR ($CDCl_3$): δ 20.73 (C-6′), 21.10 (*C*H$_3$C=O), 23.26 (*C*H$_3$C=O), 33.67 (C-4′), 52.32 (C-2), 62.15 (C-6), 65.26 (C-5′), 67.38 (C-3′), 68.31 (C-5), 69.07 (C-2′), 69.93 (C-4), 70.01 (Ph*C*H$_2$), 79.98 (C-3), 96.92 (C-1), 100.73 (C-1′), 165.01 (Ph*C*=O), 165.94 (Ph*C*=O), 169.50 (CH$_3$*C*=O), 170.55 (CH$_3$*C*=O), 170.73 (CH$_3$*C*=O). ESI-MS: $C_{39}H_{43}NO_{13}$, [M + H$^+$], 734.77; Found 734.69.

Benzyl 2′, 3′-di-*O*-benzoyl-4′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4, 6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside 12. Amorphous white solid, 90%. $[\alpha]_D = +137°$ (*c* 1.07, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 1.33 (d, 3H, J = 6.1 Hz, H-6′), 2.07 (s, 3H, CH_3C=O), 2.09 (s, 6H, 2×CH_3C=O), 3.44 (dd, 1H, J = 9.3 and 9.3 Hz, H-4′), 3.48 (s, 3H, OCH_3), 3.90 (dq, 1H, J = 9.3 and 6.1 Hz, H-5′), 3.90 (ddd, 1H, J = 9.6, 4.6 and 2.2 Hz, H-5), 3.94 (dd, 1H, J = 9.6 and 9.6 Hz, H-3), 4.02 (dd, 1H, J = 12.3 and 2.2 Hz, H-6), 4.15 (dd, 1H, J = 12.3 and 4.6 Hz, H-6), 4.45 (dd, 1H, J = 9.8 and 3.6 Hz, H-2), 4.50 (d, 1H, J = 11.8 Hz, PhCH_2), 4.69 (d, 1H, J = 11.8 Hz, PhCH_2), 4.94 (d, 1H, J = 3.6 Hz, H-1), 5.02 (ws, 1H, H-1′), 5.15 (dd, 1H J = 9.6 and 9.6 Hz, H-4), 5.43–5.47 (m, 2H, H-2′, 3′), 5.82 (d, 1H, J = 9.6 Hz, N*H*Ac), 7.34–8.03 (m, 15H, 3×*Ph*). ^{13}C NMR ($CDCl_3$): δ 17.67 (C-6′), 20.62 (*C*H$_3$C=O), 20.94 (*C*H$_3$C=O), 23.09 (*C*H$_3$C=O), 52.08 (C-2), 60.27 (O*C*H$_3$), 62.02 (C-6), 68.20 (C-5), 68.44 (C-5′), 69.85 (C-4), 69.92 (Ph*C*H$_2$), 71.22 (C-3′), 71.67 (C-2′), 79.00 (C-3), 80.29 (C-4′), 96.82 (C-1), 99.18 (C-1′), 164.78 (Ph*C*=O), 165.60 (Ph*C*=O), 169.29 (CH$_3$*C*=O), 170.39 (CH$_3$*C*=O), 170.65 (CH$_3$*C*=O). Anal. calcd for $C_{40}H_{45}NO_{14}$: C, 62.90; H, 5.94; N, 1.83. Found: C, 63.29; H, 6.18; N, 1.84.

Benzyl 2′, 3′, 4′-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4, 6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside 13. Amorphous white solid, 88%. $[\alpha]_D = +184°$ (*c* 0.90, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.29 (d, 3H, J = 6.2 Hz, H-6′), 2.10 (s, 3H, CH_3C=O), 2.13 (s, 3H, *C*H$_3$C=O), 2.17 (s, 3H, *C*H$_3$C=O), 3.91–3.98 (m, 2H, H-3, 5), 4.01–4.15 (m, 1H, H-6), 4.21–4.26 (m, 2H, H-6, 5′), 4.54 (d, 1H, J = 11.8 Hz, PhCH_2), 4.55–4.63 (m, 1H, H-2), 4.73 (d, 1H, J = 11.8 Hz, PhCH_2), 4.99 (d, 1H, J = 3.6 Hz, H-1), 5.11 (d, 1H, J = 1.6 Hz, H-1′), 5.23 (dd, 1H, J = 9.6 and 9.6 Hz, H-4), 5.55 (m, 1H, H-2′), 5.62–5.71 (m, 2H, H-3′, 4′), 5.91 (d, 1H, J = 9.7 Hz, N*H*Ac), 7.21–8.09 (m, 20H, 4×*Ph*). ^{13}C NMR ($CDCl_3$): δ 17.26 (C-6′), 20.63 (*C*H$_3$C=O), 21.04 (*C*H$_3$C=O), 23.14 (*C*H$_3$C=O), 51.84 (C-2), 61.92 (C-6), 67.64 (C-5′), 68.20 (C-5), 69.72 (C-4′), 69.98 (C-4), 70.01 (Ph*C*H$_2$), 70.97 (C-3′), 71.18 (C-2′), 80.64 (C-3), 96.87 (C-1), 99.84 (C-1′), 164.85 (Ph*C*=O), 165.71 (Ph*C*=O), 165.82

(PhC=O), 169.43 (CH_3C=O), 170.55 (CH_3C=O), 170.62 (CH_3C=O). Anal. calcd for $C_{46}H_{47}NO_{15}$: C, 64.71; H, 5.55; N, 1.64. Found: C, 64.36; H, 5.76; N, 1.63.

Hydrogenolysis of benzyl glycosides 11, 12 and 13

The glycoside **11**, **12** or **13** (0.2 mmol) was suspended in glacial AcOH (2 mL) with Pd/C catalyst (10% w/w; 50 mg). The mixture was vigorously stirred under a H_2 atmosphere until TLC showed the completion of the reaction. The catalyst was removed by filtration through Celite, and the filtrate was then concentrated. The residue was purified by flash column chromatography using a hexane/EtOAc eluant, to afford the reducing disaccharide **14**, **15**, or **16**.

2′, 3′-Di-*O*-benzoyl-4′,6′-dideoxy-α-L-*lyxo*-hexopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside 14. Amorphous white solid, 63%. $[\alpha]_D = +78.7°$ (*c* 1.00, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 1.24 (d, 3H, *J* = 6.0 Hz, H-6′), 1.85–2.15 (m, 2H, H-4′), 2.05 (s, 3H, C*H*$_3$C=O), 2.09 (s, 3H, C*H*$_3$C=O), 2.10 (s, 3H, C*H*$_3$C=O), 4.01 (dd, 1H, *J* = 9.4 and 9.4 Hz, H-3), 4.01–4.23 (m, 4H, H-6, 5, 5′), 4.33 (ddd, 1H, *J* = 9.4, 9.4 and 3.2 Hz, H-2), 5.07 (bs, 1H, H-1′), 5.11 (dd, 1H, *J* = 9.4 and 9.4 Hz, H-4), 5.26 (bs, 1H, H-2′), 5.29 (d, 1H, *J* = 3.2 Hz, H-1), 5.50 (ddd, 1H, *J* = 10.7, 4.0 and 4.0 Hz, H-3′), 6.62 (d, 1H, *J* = 9.4 Hz, N*H*Ac), 7.26–8.06 (m, 10H, 2×*Ph*). ^{13}C NMR ($CDCl_3$): δ 20.77 (*C*H$_3$C=O), 21.13 (C-6′, *C*H$_3$C=O), 21.21 (*C*H$_3$C=O), 23.19 (C-4′), 53.23 (C-2), 62.37 (C-6), 65.33 (C-5′), 67.55 (C-3′), 67.69 (C-5), 69.18 (C-2′), 70.11 (C-4), 80.20 (C-3), 91.90 (C-1), 101.09 (C-1′), 165.32 (Ph*C*=O), 166.11 (Ph*C*=O), 169.76 (CH_3C=O), 171.09 (CH_3C=O), 171.69 (CH_3C=O). ESI-MS: $C_{32}H_{37}NO_{13}$, $[M+H^+]$, 644.65; Found 644.69. Anal. calcd for $C_{32}H_{37}NO_{13}$: C, 59.71; H, 5.79; N, 2.18. Found: C, 59.39; H, 5.72; N, 2.17.

2′,3′-Di-*O*-benzoyl-4′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside 15. Amorphous white solid, 77%. $[\alpha]_D = +136°$ (*c* 0.75, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 1.35 (d, 3H, *J* = 6.1 Hz, H-6′), 2.08 (s, 3H, C*H*$_3$C=O), 2.12 (s, 3H, C*H*$_3$C=O), 2.13 (s, 3H, C*H*$_3$C=O), 3.48 (s, 3H, OC*H*$_3$), 3.44 (dd, 1H, *J* = 9.6 and 9.6 Hz, H-4′), 3.96 (dq, 1H, *J* = 9.6 and 6.1 Hz, H-5′), 4.08 (dd, 1H, *J* = 10.0 and 9.4 Hz, H-3), 4.08–4.23 (m, 3H, H-6, 5), 4.33 (ddd, 1H, *J* = 10.0, 9.2 and 2.8 Hz, H-2), 5.02 (d, 1H, *J* = 1.3 Hz, H-1′), 5.14 (dd, 1H, *J* = 9.4 and 9.4 Hz, H-4), 5.30 (d, 1H, *J* = 2.8 Hz, H-1), 5.44 (dd, 1H, *J* = 3.2 and 1.3 Hz, H-2′), 5.51 (dd, 1H, *J* = 9.6 and 3.2 Hz, H-3′), 6.54 (d, 1H, *J* = 9.2 Hz, N*H*Ac), 7.30–8.10 (m, 10H, 2×*Ph*). ^{13}C NMR ($CDCl_3$): δ 18.15 (C-6′), 21.18 (*C*H$_3$C=O), 21.56 (*C*H$_3$C=O), 23.52 (*C*H$_3$C=O), 53.69 (C-2), 60.98 (OC*H*$_3$), 62.78 (C-6), 68.00 (C-5), 69.00 (C-5′), 70.64 (C-4), 71.84 (C-2′), 72.28 (C-3′), 79.78 (C-3), 81.10 (C-4′), 92.40 (C-1), 100.07 (C-1′), 165.52 (Ph*C*=O), 166.14 (Ph*C*=O), 170.20 (CH_3C=O), 171.47 (CH_3C=O), 172.15 (CH_3C=O). ESI-MS: $C_{33}H_{39}NO_{14}$, $[M+H^+]$, 674.67; Found 674.73. Anal. calcd for $C_{33}H_{39}NO_{14}$: C, 58.84; H, 5.84; N, 2.08. Found: C, 59.21; H, 6.21; N, 2.12.

2′, 3′, 4′-Tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4, 6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside 16. Amorphous white solid, 80%. $[\alpha]_D = +146°$ (*c* 0.50, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 1.28 (d, 3H, *J* = 6.1 Hz, H-6′), 2.09 (s, 3H, C*H*$_3$C=O), 2.10 (s, 3H, C*H*$_3$C=O), 2.19 (s, 3H, C*H*$_3$C=O), 4.06–4.22 (m, 2H, H-6), 4.12–4.28 (m, 2H, H-5,5′), 4.11 (dd, 1H, *J* = 10.1 Hz, H-3), 4.41 (ddd, 1H, *J* = 10.1 and 10.1, 9.4 and 3.3 Hz, H-2), 4.60 (d, 1H, *J* = 2.8 Hz, H-OH), 5.09 (d, 1H, *J* = 1.6 Hz, H-1′), 5.22 (dd, 1H, *J* = 9.2 and 9.2 Hz, H-4), 5.38 (dd, 1H, *J* = 3.3 and 2.8 Hz, H-1), 5.52–5.53 (m, 1H, H-2′), 5.63–5.68 (m, 2H, H-3′, 4′), 6.33 (d, 1H, *J* = 9.4 Hz, N*H*Ac), 7.20–8.10 (m, 15H, 3×*Ph*). ^{13}C NMR ($CDCl_3$): δ 17.41 (C-6′), 20.80 (*C*H$_3$C=O), 21.25 (*C*H$_3$C=O), 23.26 (*C*H$_3$C=O), 53.21 (C-2), 62.19 (C-6), 67.93 (C-5, C-5′), 69.92 (C-4), 70.26 (C-4′), 71.21 (C-2′), 71.24 (C-3′), 80.46 (C-3), 92.34 (C-1), 100.23 (C-1′), 165.26 (*C*=O), 165.87 (*C*=O), 166.26 (*C*=O), 169.68 (*C*=O), 170.95 (*C*=O), 171.62 (*C*=O). Anal. calcd for $C_{39}H_{41}NO_{15}$: C, 61.33; H, 5.41; N, 1.83. Found: C, 60.91; H, 5.56; N, 1.79.

Galactosyl transfer assays

Following the procedure described by Mikusova et al.,[5] solvent was removed from 1 μCi of UDP-[6-^{3}H]Gal*p* (American Radiolabeled Chemicals, Inc.; 60 Ci/mmol) under a stream of N_2. The radiolabeled compound was dissolved in 50 mM pH 8.0 MOPS buffer (22 μL) containing 5 mM 2-mercaptoethanol, and 10 mM $MgCl_2$ (buffer A). The mixture was incubated with 30 μL of UDP-Gal*p* mutase (100 μg of protein) at 37 °C for 30 min. Subsequently, reagents and buffer A were added to make a total volume of 320 μL, resulting in final concentrations of 20 μM UDP-GlcNAc, 20 μM dTDP-Rha, 60 μM ATP, 5.13 mg/mL protein, 1% DMSO and concentrations of analogues **5**–**6**, **23**–**26** or **28** up to 600 μg/mL. The reaction mixture was incubated for another 30 min at 37 °C, after which $CHCl_3/CH_3OH$ (2/1) was added. Protein was removed by centrifugation and the supernatant was transferred to a second tube. Deionized water was added to achieve a biphasic mixture, which was separated by centrifugation. The aqueous phase was removed and discarded; the organic phase was washed with $CHCl_3/CH_3OH/H_2O$ (3/47/48) after which the solvent was evaporated under a stream of N_2. The residue was dissolved in $CHCl_3/CH_3OH$ (2/1). The amounts of radiolabeled products formed were measured by liquid scintillation spectrometry. Their identities were confirmed by TLC analysis on silica gel G60 plates developed in $CHCl_3/CH_3OH/NH_4OH/H_2O$ (65/25/0.5/3.6, v/v).

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