Synthetic Polyprenol-Pyrophosphate Linked Oligosaccharides Are Efficient Substrates for Mycobacterial Galactan Biosynthetic Enzymes

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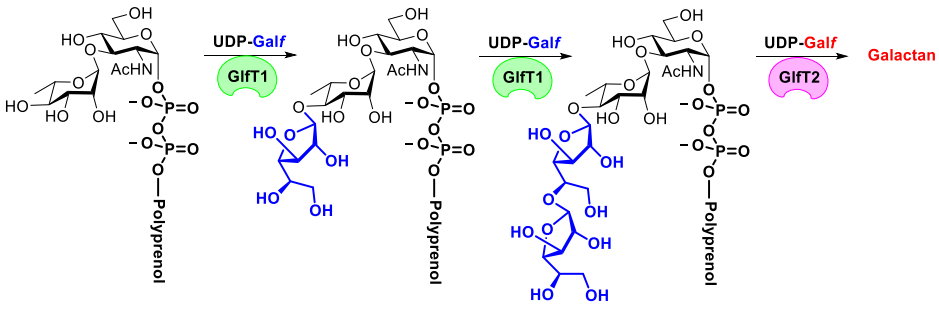

Abstract

Mycobacteria, including the human pathogen *Mycobacterium tuberculosis*, produce a complex cell wall that is critical for their survival. The largest structural component of the cell wall, the mycolyl–arabinogalactan–peptidoglycan complex, has at its core a galactan domain composed of D-galactofuranose residues. Mycobacterial galactan biosynthesis has been proposed to involve two glycosyltransferases, GlfT1 and GlfT2, which elongate polyprenol-pyrophosphate linked glycosyl acceptor substrates using UDP-galactofuranose as the donor substrate. We here report the first chemical synthesis of GlfT1 and GlfT2 acceptor substrates containing pyrophosphate and polyprenol moieties (compounds **3**, **4**, **22** and **23**). The approach involves chemical synthesis of an oligosaccharide, subsequent phosphorylation at the reducing end and coupling to a polyprenol phosphate. These compounds were shown to be substrates for either GlfT1 (**22** and **23**) or GlfT2 (**3** and **4**) and all were substantially more active than the corresponding alkyl glycoside substrates reported previously. Mass spectrometric analysis of the products formed from the reaction of **3**, **4**, **22** and **23** with the respective cognate enzyme and UDP-galactofuranose provide additional evidence for the galactan biosynthetic model in which GlfT1 adds the first two galactofuranose residues with the remainder being installed via GlfT2. Overall, these results highlight the importance of the pyrophosphate motif in recognition of acceptor substrates by both enzymes and demonstrate a straightforward route for the preparation of such compounds. The work also provides additional support for the process by which this important glycan is biosynthesized using, for the first time, close structural analogs to the natural substrates.

Introduction

The five-membered ring form of D-galactose, D-galactofuranose (Gal*f*), is found in many microbial glycoconjugates but not those produced by mammals.[1–3] Gal*f*-containing glycoconjugates are frequently essential for microbial pathogenesis[4,5] and survival.[6] Although glycoconjugates incorporating Gal*f* residues are widespread, a particularly impressive example is the arabinogalactan (AG) that is produced by *Mycobacterium tuberculosis*. Infections by *M. tuberculosis* lead to the disease tuberculosis and the AG is a critical component of the mycolyl–arabinogalactan–peptidoglycan complex, the major structural component of the protective mycobacterial cell wall.[7] At the core of the AG is a galactan with 30–40 Gal*f* residues connected through alternating β-(1→5) and β-(1→6) linkages attached to the peptidoglycan via a 'linker disaccharide' of rhamnose and *N*-acetylglucosamine (Figure 1).

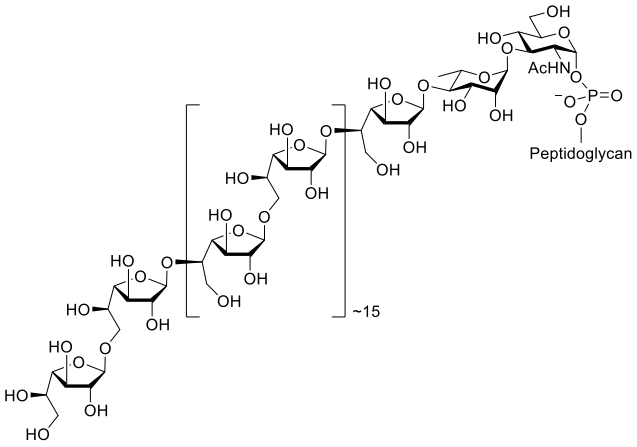

Figure 1. Structure of mycobacterial galactan attached via the linker disaccharide to peptidoglycan.

The ability of mycobacteria to produce the galactan is required for growth;[6] therefore, the enzymes involved in its assembly have become a focus for drug development.[8] Mycobacterial galactan biosynthesis involves two bifunctional galactofuranosyltransferases, GlfT1 and GlfT2, which add Gal*f*

residues to polyprenol-linked glycosyl pyrophosphate acceptor substrates (Figure 2). In the proposed biosynthetic model, GlfT1 introduces the first two Gal*f* residues to the linker disaccharide to provide a substrate for GlfT2, a polymerase that adds the remaining Gal*f* residues. The bifunctional nature of both GlfT1 and GlfT2 has sparked interest in exploring their mechanism and specificity. To date, both GlfT1 and GlfT2 have been recombinantly expressed[9–11] and a crystal structure of GlfT2 has been reported.[12] Spectrophotometric assays for both enzymes have been developed, employing acceptor substrates that contain hydrophobic alkyl/aryl aglycones lacking the native pyrophosphate moiety.[13–15] Phosphonophosphate derivatives have also been investigated as substrates for GlfT1.[9]

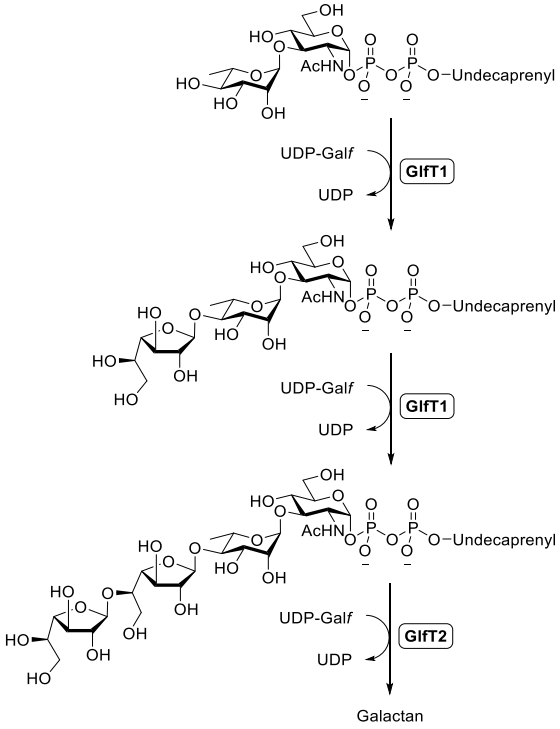

Figure 2. Mycobacterial galactan biosynthesis via the glycosyltransferases GlfT1 and GlfT2.

The acceptor analogs for GlfT1 and GlfT2 used to date have been effective biochemical tools. However, increasing evidence has suggested that answering some questions regarding the specificity

and mechanism of these enzymes requires substrates that contain a pyrophosphate moiety. For example, to date, we have been unable to obtain X-ray structures of GlfT2 bound to any of the acceptor substrates we have prepared, including those with as many as 12 monosaccharide Gal*f* residues.[16] Moreover, the crystal structure of GlfT2[12] shows a number of positively charged residues positioned to interact with a phospholipid headgroup; hence, such molecules may prove to be more effective crystallographic ligands. In addition, alkyl glycoside acceptors are very poor substrates for GlfT1, which prompted Kiessling and coworkers to prepare phosphono derivative **1** (Figure 3), which is efficiently recognized by the enzyme.[9] Finally, it is of interest to compare the activity of native pyrophosphate containing structures with those containing other linkers. However, isolating the native lipid-linked acceptor substrates from bacterial cultures is very tedious[17] and yields amounts of material too small for detailed biochemical work or to use as ligands for crystallographic investigations. In this paper, we describe the synthesis of pyrophosphate-containing acceptor substrates for GlfT1 and GlfT2, demonstrate their recognition by the enzymes, and compare their kinetic properties with previously reported substrates lacking this structural motif.

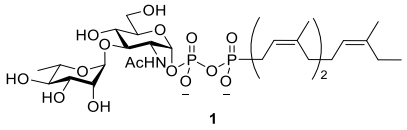

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Figure 3. Phosphono derivative **1** previously used as a substrate for GlfT1.[9]

Results and Discussion

Preparation and evaluation of substrates for GlfT2.

We focused initially on substrates for GlfT2, which has been subjected to substantially more study than GlfT1. Tetrasaccharide **2**[18] (Figure 4), an analog of the minimum natural substrate for the enzyme, is a

competent acceptor for the enzyme and our goals were to answer two questions: 1) What is the activity of the natural substrate for the enzyme, the undecaprenyl pyrophosphate tetrasaccharide **3**, compared to **2**?; and 2) Does the length of polyprenol chain substantially influence the recognition by the enzyme? To answer these questions, we targeted the synthesis of **3** and **4**. The latter differs from the former only by the replacement of the undecaprenyl moiety with a farnesyl chain.

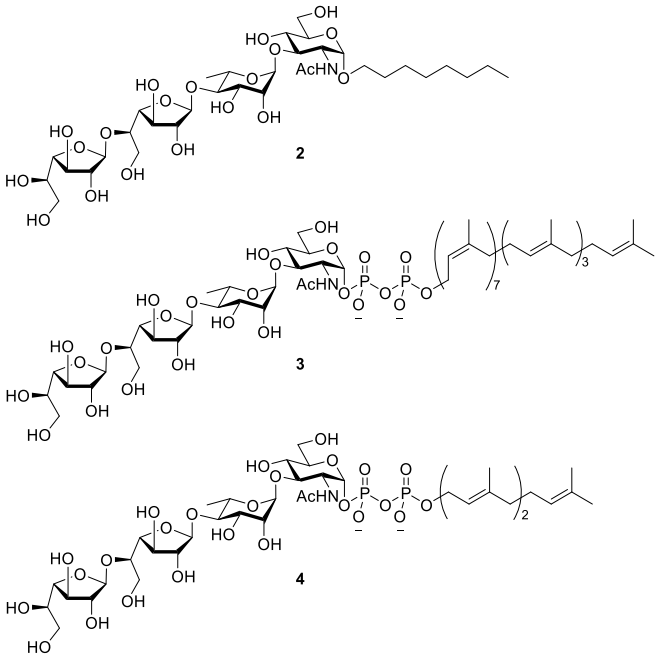
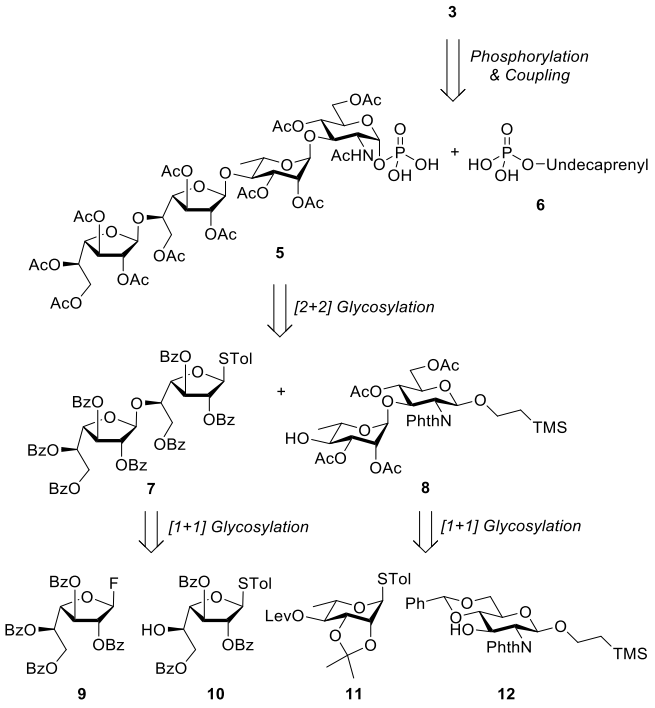


Figure 4. Tetrasaccharide **2**, an acceptor substrate for GlfT2, the natural substrate for the enzyme (**3**) and a pyrophosphate-containing derivative (**4**) in which the undecaprenol moiety is replaced with a farnesyl group.

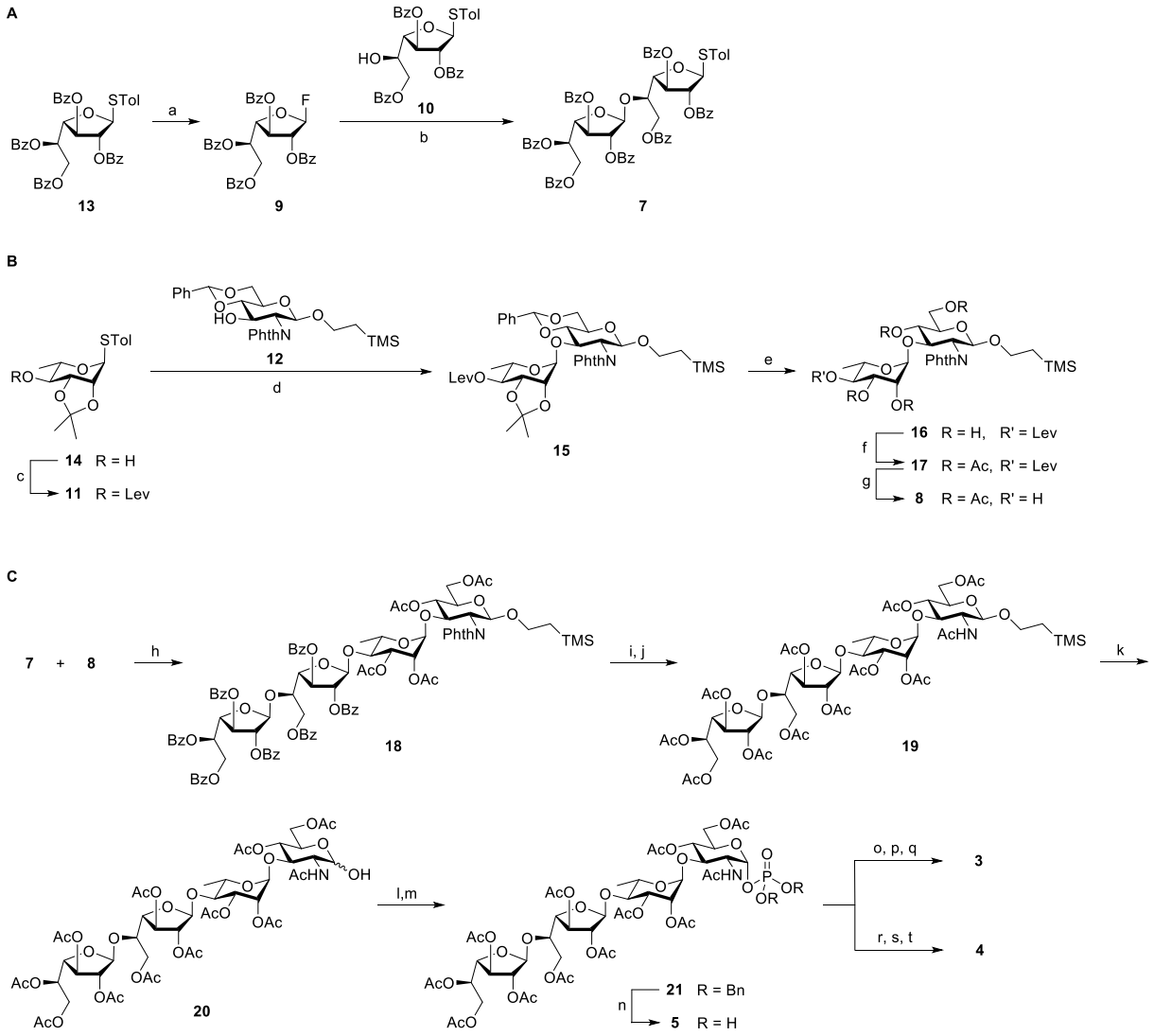
The retrosynthetic analysis of **3** (Scheme 1) illustrates the general strategy to all of the compounds reported in this paper. The pyrophosphate moiety could be introduced via phosphorylated tetrasaccharide **5** and undecaprenol phosphate (**6**), the latter obtained by phosphorylation of undecaprenol isolated from bay leaves.[19] Tetrasaccharide **5** could be obtained from disaccharide units **7** and **8**, which themselves could arise from monosaccharide precursors **9**–**12**. A key feature of the design was the stability of the thioglycoside moiety in **10** to allow glycosylation with fluorosugar **9** leading to

7. However, once incorporated into disaccharide **7** the thioglycoside could be induced to undergo a [2+2] glycosylation reaction with **8**. The disaccharide acceptor **8** would be generated from glycosylation between thioglycoside **11** and 2-(trimethylsilyl)ethyl glycoside **12**.



Scheme 1. Retrosynthetic analysis of tetrasaccharide **3**

The synthesis of **3** and **4** is shown in Scheme 2. First, disaccharide donor **7** was produced from monosaccharides **9** and **10** (Scheme 2A). Under the combined action of (dimethylamino)sulfur trifluoride and *N*-bromosuccinimide,[20,21] thioglycoside **13**[18] was converted smoothly to glycosyl fluoride **9** in 89% yield. Glycosylation of **10** with **9**[18] upon treatment with bis(cyclopentadienyl)zirconium(IV) dichloride and silver triflate,[22,23] provided disaccharide donor **7**. Next, to obtain the disaccharide acceptor **8** (Scheme 2B), rhammopyranoside **14**[24] was coupled with levulinic acid in the presence of *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride and



Scheme 2. Synthesis of **3** and **4**. Reagents and Conditions: (a) DAST, NBS, CH_2Cl_2, 0 °C, 2 h, 89%; (b) Cp_2ZrCl_2, AgOTf, 4Å MS, CH_2Cl_2, 0 °C, 2 h, 87%; (c) LevOH, EDC·HCl, DMAP, CH_2Cl_2, rt, overnight, 98%; (d) NIS, AgOTf, 4Å MS, CH_2Cl_2, −15 °C, 30 min, 79%; (e) *p*-TsOH·H_2O, CH_3CN–CH_3OH (10:1, v/v), rt, 5 h, 87%; (f) Ac_2O, Pyridine, rt, 24 h, 91%; (g) H_2NNH_2·HOAc, EtOH–toluene (1:1, v/v), rt, 6 h, 99%; (h) NIS, AgOTf, 4Å MS, CH_2Cl_2, rt, 30 min, 94%; (i) ethylenediamine, EtOH, reflux, 24 h; (j) Ac_2O, pyridine, rt, 48 h, 88% over two steps; (k) TFA, CH_2Cl_2, rt, 30 min, quant.; (l) *i*-$Pr_2NP(OBn)_2$, 1,2,4-triazole, CH_2Cl_2, rt, 5 h; (m) *m*-CPBA, CH_2Cl_2, −78 °C to rt, 30 min, 71% over two steps; (n) H_2, Pd/C, THF, rt, overnight, 90%; (o) CDI, CH_2Cl_2, rt, 2 h; (p) **6**, DMF, rt, four weeks; (q) CH_3ONa, CH_3OH, rt, 48 h, 15% over three steps. (r) CDI, CH_2Cl_2, rt, 2 h; (s) farnesyl-phosphate, DMF, rt, two weeks; (t) CH_3ONa, CH_3OH, rt, 48 h, 53% over three steps.

4-(dimethylamino)pyridine affording **11** in 98% yield. Subsequently, glycosylation of 2-(trimethylsilyl)ethyl glucoside **12**[25] with **11** produced disaccharide **15** in 79% yield with complete

α-selectivity as detected by TLC. The $^{1}J_{C\text{-}1,H\text{-}1}$ of the rhamnose residue in **15** is 167.2 Hz, which is consistent with the α-stereochemistry.[26] Acid hydrolysis of benzylidene acetal and isopropylidene ketal in **15** then gave tetraol **16**, which could be acetylated by acetic anhydride in pyridine to generate **17** in 79% yield over two steps. Exposure of **17** to hydrazine acetate in ethanol and toluene gave disaccharide acceptor **8** in 99% yield.

With the two disaccharides in hand, coupling between **7** and **8** (Scheme 2C) generated tetrasaccharide **18** in 94% yield. The phthalamide group was converted to the corresponding acetamide **19** in 88% overall yield by reaction first with ethylenediamine in hot ethanol overnight[27] and then with acetic anhydride in pyridine. Next, the desired hemiacetal **20** was produced in quantitative yield upon treatment of **19** with trifluoroacetic acid in anhydrous CH_2Cl_2. Phosphorylation of **20** was achieved using a one-pot two-step protocol[28,29] involving first reaction with dibenzyl *N*,*N*′-diisopropylphosphoramidite and 1,2,4-triazole to give a phosphite intermediate that was oxidized with 3-chloroperbenzoic acid resulting in a 71% yield of dibenzyl phosphate **21**. Hydrogenation of **21** over palladium on carbon in THF gave **5** in 90% yield. Phosphate **5** was then activated with 1,1′-carbonyldiimidazole (CDI) in CH_2Cl_2,[30,31] leading to the imidazolide formation in two hours, as shown by a significant upfield shift in ^{31}P NMR spectrum (from −1.18 ppm to −12.51 ppm, $CDCl_3$). Excess CDI was quenched by the addition of methanol, undecaprenyl phosphate (**6**)[19] was added and the reaction was monitored by ^{31}P NMR spectroscopy in DMF-d_7. After four weeks, deprotection with sodium methoxide yielded the desired tetrasaccharide acceptor **3** in 15% yield over three steps.

The conversion of **5** into **4** was achieved using a similar series of transformations – activation with CDI, coupling with (2*E*,6*E*)-farnesyl phosphate[32] and deprotection – to yield the product in 53% overall

yield. In this case, however, the coupling reaction between **5** and the farnesyl phosphate was complete in two weeks.

Having synthesized **3** and **4**, we first carried out kinetic investigations with GlfT2 and compared the data with that obtained with octyl glycoside **2** (Table 1). The introduction of the pyrophosphate into this tetrasaccharide had a significant impact on its potency as a substrate for GlfT2. Comparing first octyl glycoside **2** and undecaprenyl pyrophosphate derivative **3**, the latter has a nearly 900-fold greater k_{cat}/K_m than the former, resulting both from a significant decrease in the K_m and a significant increase in the k_{cat}. When comparing the undecaprenol derivative **3** with the farnesyl derivative **4**, the latter is a slightly less effective substrate, with a k_{cat}/K_m of ~0.7-times that of the former, but still more than 600-fold better than **2**. This data underscores the clear preference of GlfT2 for pyrophosphate-containing substrates compared to simple glycosides. Moreover, these data suggest that the nature of the lipid is comparatively unimportant as both **3** and **4**, which contain polyprenols of different length and stereochemistry, have similar kinetic properties with GlfT2. This latter finding is perhaps not surprising as GlfT2 is believed to be a membrane-associated enzyme[12] and the natural undecaprenol moiety would be expected to be substantially embedded in the membrane, leaving little available to interact with the protein.

Table 1. Apparent K_m and k_{cat} values of **2**–**4** as substrates for GlfT2.

substrate	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m ($M^{-1}\cdot s^{-1}$)
2	1400 ± 112	11.3 ± 1.24	135
3	22.7 ± 2.3	163.5 ± 16.6	1.20×10^5
4	29.6 ± 0.8	148 ± 1.03	8.34×10^4

We next studied the enzymatic reactions by carrying out incubations of **2** and **4** with UDP-Gal*f* (5 eq.) at 37 °C. The crude enzymatic reaction mixtures were centrifuged, purified on a C18 Sep-pak cartridge, the eluent was lyophilized, and the resulting products were analyzed by electrospray ionization mass spectrometry. As shown in Figure 5, the octyl glycoside **2** gives a product corresponding to the addition of a single sugar residue. On the other hand, the pyrophosphate substrate **4** gives longer products (extended by adding as many as 44 Gal*f* residues), which have a comparable length to the natural galactan. These data again point to the superiority of the pyrophosphate-containing molecules as acceptor substrates for GlfT2 compared to simple glycoside analogs.

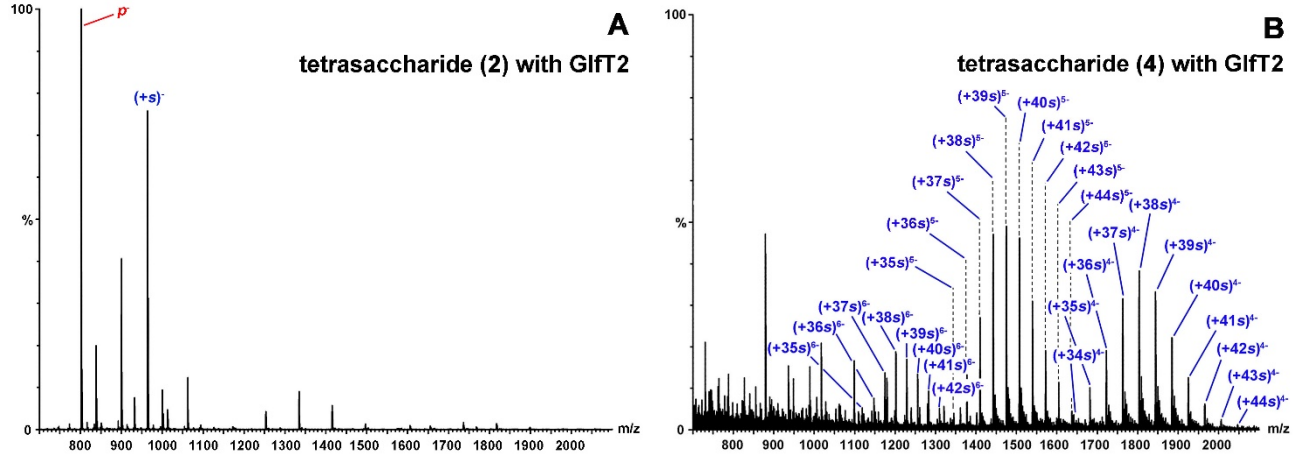


Figure 5. Negative ion mode ESI mass spectra of GlfT2-catalyzed polymerization reactions with acceptors **2** (Panel A) and **4** (Panel B). Peaks are labeled with the number of additional Gal*f* residues (s) in product and the charge state of the ion (superscripted number). The peaks labelled p correspond to the starting material (**2** or **4**).

Preparation and evaluation of substrates for GlfT1.

Once we demonstrated that molecules with a pyrophosphate moiety were superior GlfT2 substrates to those with simple alkyl aglycones, we turned our attention to GlfT1. Given that both **3** and **4** were of comparable activity for GlfT2, we focused only on preparing the farnesyl pyrophosphate derivatives for GlfT1. This was done as the species with the shorter polyprenol chain are easier to handle and the

formation of the pyrophosphate bond is appreciably faster (see above). We thus selected both possible acceptors for this enzyme, disaccharide **22** and trisaccharide **23** (Figure 6) linked to a farnesyl pyrophosphate motif, as targets. In accessing these compounds, the general retrosynthetic approach outlined in Scheme 1 was employed.

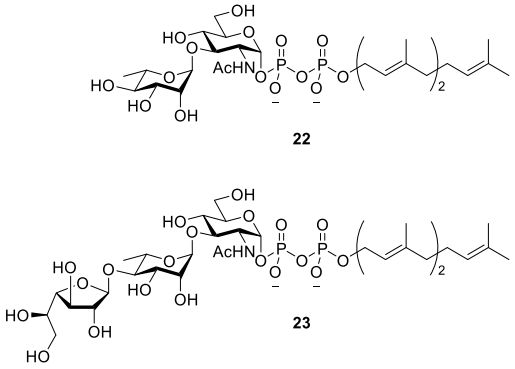
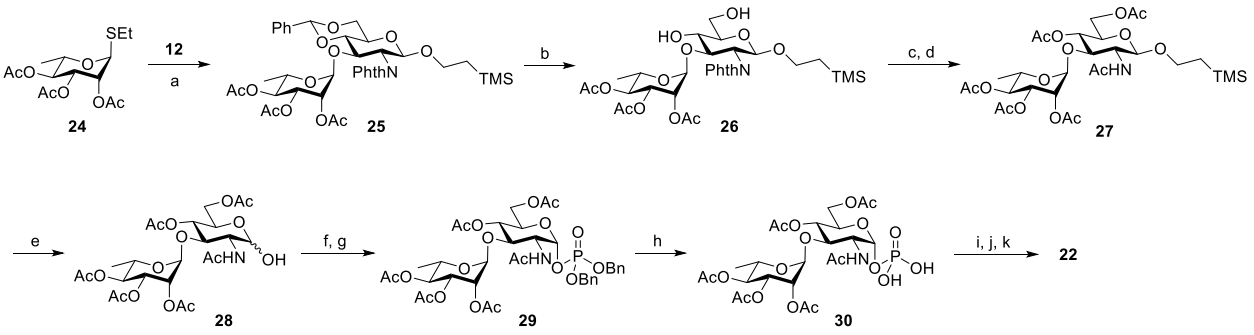


Figure 6. Disaccharide **22** and trisaccharide **23**, putative acceptor substrates for GlfT1.

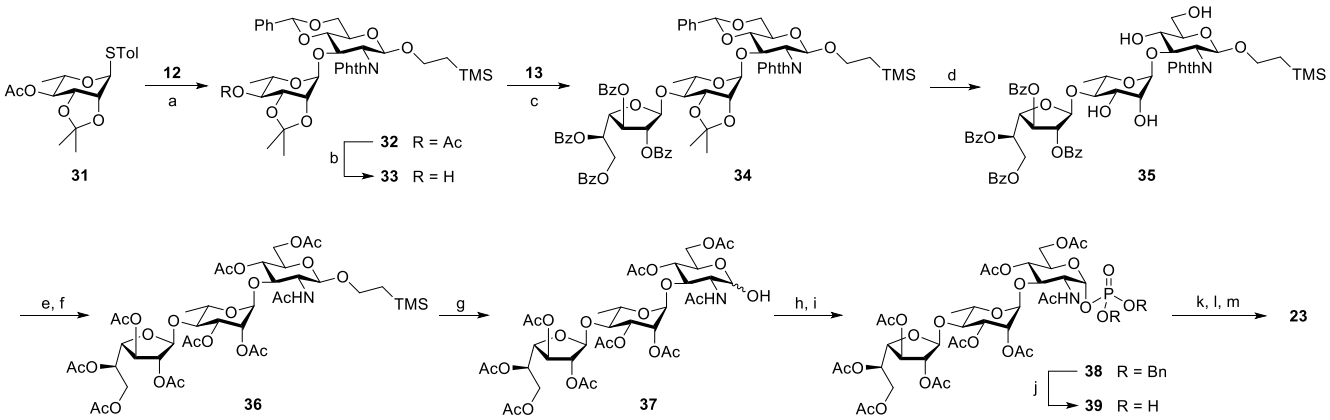
The synthesis of **22** is illustrated in Scheme 3. Exposure of thiorhamnoside **24**[33] and acceptor **12** to *N*-iodosuccinimide (NIS) and silver triflate, generated disaccharide **25** in quantitative yield. The $^{1}J_{C\text{-}1,H\text{-}1}$ of the rhamnose residue was 172.4 Hz, as expected based for the α-stereochemistry.[26] Methanolysis of the benzylidene acetal upon treatment with toluenesulfonic acid monohydrate in a mixture of CH_3CN and CH_3OH led to an 83% yield of diol **26**. Next, the phthalimide group was transformed into an acetamide and the free hydroxyl groups were acetylated upon reaction with ethylenediamine followed by acetylation. The resulting product, **27**, was obtained in 90% yield over two steps. Subjecting **27** to trifluoracetic acid led to cleavage of the 2-(trimethylsilyl)ethyl glycoside providing a quantitative yield of **28**. Conversion of **28** into the phosphate **29** was done as done for the tetrasaccharide – generation of a dibenzyl phosphite intermediate that was oxidized to the corresponding phosphate. Using this two-step approach, **29** was obtained in 52% yield from **28**. In turn,

hydrogenation of **29** gave the required phosphate **30** in 92% yield. Phosphate **30** was then converted into disaccharide acceptor **22** in 41% yield over three steps by reactions similar to those discussed above.



Scheme 3. Synthesis of disaccharide **22**. Reagents and Conditions: (a) NIS, AgOTf, 4Å MS, CH_2Cl_2, 0 °C, 30 min, quant.; (b) *p*-TsOH·H_2O, CH_3CN–CH_3OH (10:1, v/v), rt, 4 h, 83%; (c) ethylenediamine, EtOH, reflux, overnight; (d) Ac_2O, pyridine, rt, 48 h, 90% over two steps; (e) TFA, CH_2Cl_2, rt, 30 min, quant.; (f) *i*-$Pr_2NP(OBn)_2$, 1,2,4-triazole, CH_2Cl_2, rt, 3 h; (g) *m*-CPBA, CH_2Cl_2, –78 °C to rt, 30 min, 52% over two steps; (h) H_2, Pd/C, THF, rt, overnight, 92%; (i) CDI, CH_2Cl_2, rt, 2 h; (j) farnesyl-phosphate, DMF, rt, 10 days; (k) CH_3ONa, CH_3OH, rt, 48 h, 41% over three steps.

Our route to trisaccharide **23** (Scheme 4) started with thioglycoside **31**,[34] which was coupled to acceptor **12** using NIS and lanthanum triflate to give disaccharide **32** in 83% yield. Base-catalyzed methanolysis of the acetate ester converted **32** into **33**, which was glycosylated with thioglycoside **13**, giving a 97% yield of trisaccharide **34**. To effect benzylidene acetal and isopropylidene ketal cleavage, trisaccharide **34** was heated in a 4:1 solution of acetic acid and water; tetraol **35** was generated in 83% yield. Afterwards, using a series of reaction similar to those described above for installation of the phosphate and coupling of the lipid in **3**, **4** and **22**, the synthesis of trisaccharide **23** was achieved in 36% overall yield over nine steps.



Scheme 4. Synthesis of trisaccharide **23,** Reagents and Conditions: (a) NIS, La(OTf)$_3$, 4Å MS, CH_2Cl_2, 0 °C, 45 min, 83%; (b) CH_3ONa, CH_3OH, rt, 2 h, 96%; (c) NIS, AgOTf, 4Å MS, CH_2Cl_2, 0 °C, 30 min, 97%; (d) AcOH–H_2O (4:1, v/v), 80 °C, 2 h, 83%; (e) ethylenediamine, EtOH, reflux, overnight; (f) Ac_2O, Pyridine, rt, 48 h, 89% over two steps; (g) TFA, CH_2Cl_2, rt, 30 min, 97%; (h) *i*$Pr_2NP(OBn)_2$, 1,2,4-triazole, CH_2Cl_2, rt, 3 h; (i) *m*-CPBA, CH_2Cl_2, –78 °C to rt, 30 min, 60% over two steps; (j) H_2, Pd/C, THF, rt, overnight, 93%; (k) CDI, CH_2Cl_2, rt, 2 h; (l) farnesyl-phosphate, DMF, rt, two weeks; (m) CH_3ONa, CH_3OH, rt, 48 h, 74% over three steps.

Evaluation of **22** and **23** as substrates for GlfT1 was done next. In previous work, recombinant GlfT1 was expressed from non-pathogenic *M. smegmatis* (Ms-GlfT1).[9] To compare this protein with the enzyme from *M. tuberculosis*, the corresponding gene (*Rv3782*) was cloned from *M. tuberculosis* genomic DNA and expressed to give Tb-GlfT1 (see details in Supporting Information). With both recombinant Ms-GlfT1 and Tb-GlfT1 in hand, we carried out kinetic investigations with both enzymes (Table 2). As can be seen from the data, although there are small differences between the kinetic properties of the two enzymes, both recognize **22** and **23** with comparable efficiency. With regard to the relative efficiency as substrates for GlfT1, both **22** and **23** have similar k_{cat}/K_m values. However, trisaccharide **23** has an approximately two-fold smaller apparent K_m than disaccharide **22**, and the k_{cat} for the latter is 1.5-fold higher. As reported previously, simple alkyl glycosides are very poor substrates for GlfT1.[9] Therefore, obtaining kinetic data on such compounds to enable a comparison similar to that

done above for GlfT2 was not possible. However, the fact that kinetic data can be obtained for **22** and **23** underscores the beneficial effect of the pyrophosphate on activity.

Table 2. Apparent K_m and k_{cat} values of **22** and **23** as substrates for GlfT1.

substrate	enzyme	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m ($M^{-1}\cdot s^{-1}$)
22	Ms-GlfT1	864 ± 122	4.26 ± 0.08	82
	Tb-GlfT1	853 ± 48.4	3.54 ± 0.04	69
23	Ms-GlfT1	489 ± 34.4	2.98 ± 0.05	102
	Tb-GlfT1	480 ± 48.6	2.13 ± 0.03	74

As was done for the GlfT2 substrates, **22** and **23** were incubated with UDP-Gal*f* overnight at 37 °C together with GlfT1 and the products were characterized by mass spectrometry. With disaccharide **22**, the major products formed using Ms-GlfT1 (Figure 7A) were those resulting from extension by 2–3 Gal*f* residues. Trace amounts of the +1 product, as well longer oligomers (up to five additional Gal*f* residues) were seen. In contrast, with Tb-GlfT1 a broader range of products was formed (Figure 7B). The major product was a pentasaccharide, resulting from the addition of three Gal*f*-residues; smaller amounts of longer oligomers were also produced (the addition of up to eight Gal*f* residues). When trisaccharide phospholipid **23** was evaluated as a substrate for Ms-GlfT1, the +2 product (a pentasaccharide) was the major species formed, although smaller amounts of longer oligomers were also produced (Figure 7C). For the Tb-GlfT1 catalyzed reaction with **23**, the +1 (tetrasaccharide) and +2 (pentasaccharide) species were the major products (Figure 7D).

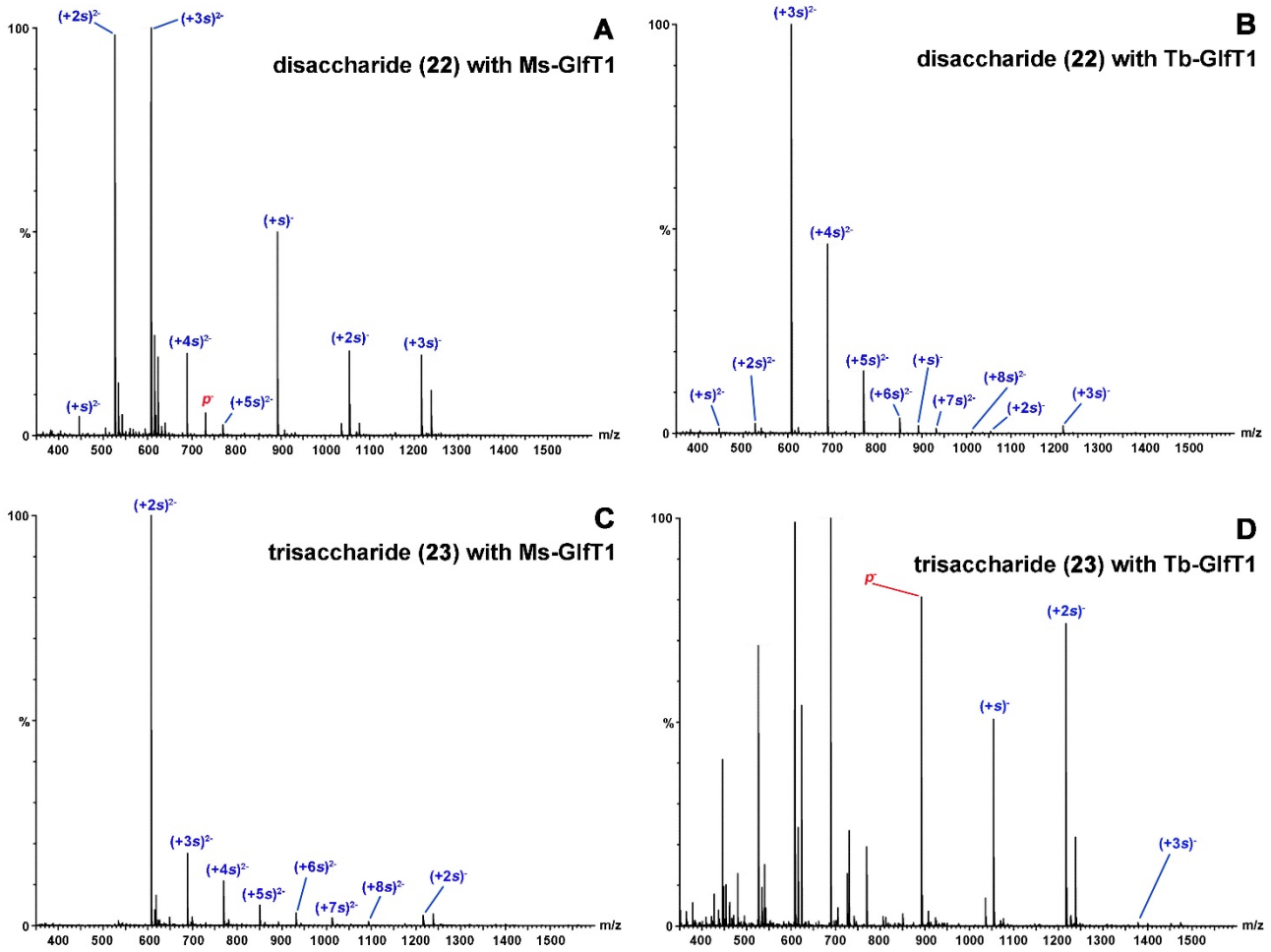


Figure 7. Negative ion mode ESI mass spectra of GlfT1-catalyzed elongation reactions with **22** and **23**. Panels A and B are the products of **22** and Tb-GlfT1 and Ms-GlfT1, respectively. Panels C and D are the products of **23** and Tb-GlfT1 and Ms-GlfT1, respectively. Peaks are labeled with the number of additional Gal*f* residues (s) in the product and the charge state of the ion (superscripted number). The peaks labelled p correspond to the starting material (**22** or **23**).

The data presented in Figure 7 generally supports the proposed biosynthetic model in which GlfT1 uses either disaccharide or trisaccharide acceptor substrates (*e.g.,* **22** or **23**) to generate products with one or two additional Gal*f* residues, respectively. However, the data also suggest that the enzyme can extend the chain past a tetrasaccharide; similar results were seen previously with the phosphonophosphate derivative **1** (See Figure 3 for structure).[9] The significance of the formation of

products longer than a tetrasaccharide is unclear, although it should be noted that the *in vitro* conditions leading to the products shown in Figure 7 are likely more forcing than *in vivo*. Moreover, these reactions were not carried out in the presence of GlfT2. In the absence of the second polymerizing enzyme (GlfT2) *in vitro* it is plausible that GlfT1 will extend the galactan chain beyond the length it would *in vivo*, where the process is likely coordinated.

Evaluation of 22 and 23 as substrates for GlfT2.

The work above suggests that, at least under *in vitro* conditions, GlfT1 has some promiscuity with regard to the reactions it catalyzes. To evaluate the ability of **22** and **23** to act as acceptors for GlfT2, both compounds were incubated with the enzyme and UDP-Gal*f* under the conditions used with **2** and **4**. Under these conditions, no significant extension reaction was seen, except for trace amounts of the +1 and +2 products (Figure S1). This result is consistent with the proposed biosynthetic model and previous work demonstrating that substrates incorporating the linker disaccharide alone, or with a single Gal*f* residue are not competent GlfT2 substrates.[35] It is interesting to note that previous studies have shown that single Gal*f* residue linked to 12-phenoxydodec-2-enyl aglycone does act as a substrate for GlfT2, leading to polymer.[36] This observation, and the small amount of chain-extended product formed from **22** and **23**, is likely the result of the artificial nature of the conditions used in these *in vitro* reactions, as discussed above.

Conclusion

In summary, we describe here the synthesis of four polyprenol-containing pyrophosphate linked oligosaccharide acceptors (**3, 4, 22** and **23**) for GlfT1 and GlfT2, the two galactofuranosyltransferases responsible for the assembly of mycobacterial galactan. The route developed involves the preparation of an oligosaccharide as a 2-(trimethylsilyl)ethyl glycoside, its conversion to the corresponding reducing sugar, phosphorylation and finally introduction of the lipid. This work represents the first synthesis of GlfT1 and GlfT2 acceptor substrates containing the natural pyrophosphate linkage. Tetrasaccharide **3**, which has both the pyrophosphate and an undecaprenyl moiety, is the minimal natural acceptor substrate for GlT2. The ability of GlfT2 to recognize **3** and **4** was compared to a previously synthesized octyl glycoside with the same glycan sequence (**2**), revealing that incorporation of the pyrophosphate had a substantial beneficial effect on activity. In contrast, the difference in activity between **3** and **4**, which differ in the identity of the lipids, was modest. Disaccharide phospholipid **22** and trisaccharide phospholipid **23** were shown to be substrates for GlfT1 (both the previously reported *M. smegmatis* enzyme and the *M. tuberculosis* enzyme expressed here for the first time). These compounds were substantially more active than previously-described alkyl glycoside GlfT1 acceptor substrates. However, direct comparison was not possible as the glycoside derivatives are such poor GlfT1 substrates that measuring their kinetic properties is difficult to impossible. These results provide further support for the proposed mechanism of galactan biosynthesis, which involves two glycosyltransfearses GlfT1 and GlfT2. In addition, this work clearly demonstrates that polyprenol-pyrophosphate linked glycans, which can be straightforwardly prepared from commercial,

or readily-isolated polyprenols, are preferred substrates for these enzymes compared to alkyl glycoside acceptors.

Experimental

General Methods

All reagents were purchased from commercial sources and were used without further purification unless noted. All reactions were monitored by TLC on silica gel 60-F_{254} (0.25 mm). Visualization of the reaction components was achieved using UV fluorescence (254 nm) and/or by charring with acidified anisaldehyde solution in ethanol. Organic solvents were evaporated under reduced pressure, and the products were purified by chromatography on silica gel (230−400 mesh), reverse-phase chromatography (C_{18}) or size exclusion column chromatography (Sephadex LH-20). Optical rotations were measured in a microcell (1 cm, 1 mL) at ambient temperature and are in units of degree·mL/(g·dm). ^{1}H NMR spectra were recorded at 400 MHz, 500 MHz, 600 MHz or 700 MHz, and chemical shifts are referenced to residual $CHCl_3$ (7.26 ppm, $CDCl_3$), CHD_2OD (3.30 ppm, CD_3OD), HDO (4.78 ppm, D_2O) ^{13}C NMR spectra were recorded at 125 MHz or 175 MHz, and chemical shifts are referenced to $CDCl_3$ (77.0 ppm) or CD_3OD (49.3 ppm). Reported splitting patterns are abbreviated as s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Assignments of NMR spectra were based on two-dimensional experiments (^{1}H−^{1}H COSY, HSQC and HMBC), and stereochemistry of the anomeric centers of the pyranose rings was confirmed by measuring $^{1}J_{C\text{-}1,H\text{-}1}$ via coupled HSQC experiments. ESI/TOF-HRMS spectra of synthetic samples were recorded on samples suspended in THF or CH_3OH and added NaCl.

P^1-β-D-Galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl P^2-undecaprenyl diphosphate (3): 1,1′-Carbonyldiimidazole (377 mg, 2.33 mmol) was dissolved in CH_2Cl_2 (3 mL), a solution of phosphate **5** (103.6 mg, 77.6 μmol) in CH_2Cl_2 (10 mL) was added dropwise, the resulting mixture was stirred at rt for a further 2 h. Then a solution of methanol (7.93 mL, 5% v/v in CH_2Cl_2) was added dropwise to quench the excess 1,1′-carbonyldiimidazole and the mixture was stirred at rt for another 30 min. After removal of the solvent under vacuum, the residue was dissolved in DMF (5 mL) and undecaprenyl phosphate (32.9 mg, 38.8 μmol) was added in one portion. After stirring at rt for 4 weeks and removal of the solvent under vacuum, the crude residue was then dissolved in a solution of sodium methoxide (20 mL, 5 mM in methanol) and the mixture was stirred at rt for 48 h. Then, Amberlite IR-120 resin (NH_4^+ form), was added to neutralize the solution. The mixture was filtered and the filtrate was concentrated under vacuum before the residue was purified by reverse-phase chromatography (C_{18}, CH_3OH–H_2O, 50:1) to afford glycolipid **3** (9.39 mg, 15%) as a white amorphous solid after lyophilization in water: R_f = 0.19 (H_2O–isopropranol-EtOAc, 1:2:4); $[\alpha]_D^{25}$ = +6.7 (c = 0.30, CH_2Cl_2–CH_3OH, 1:1); ^{1}H NMR (700 MHz, CD_3OD) δ 5.52 (br s, 1H), 5.44 (t, 1H, J = 6.2 Hz), 5.26 (d, 1H, J = 1.7 Hz), 5.17–5.08 (m, 11H), 4.54 (br s, 2H), 4.11 (dd, 1H, J = 5.9, 2.5 Hz), 4.07 (dd, 1H, J = 6.7, 4.1 Hz), 4.04–3.98 (m, 4H), 3.95 (br s, 1H), 3.91–3.89 (m, 1H), 3.85–3.61 (m, 11H), 3.52 (t, 1H, J = 9.5 Hz), 3.42 (t, 1H, J = 9.4 Hz), 2.13–1.96 (m, 43H), 1.73 (s, 3H), 1.68 (s, 15H), 1.67 (s, 6H), 1.61 (s, 3H), 1.60 (s, 9H), 1.25 (d, 3H, J = 6.2 Hz) ppm; ^{31}P NMR (162 MHz, CD_3OD) δ –10.42, –13.09 ppm; HRMS (ESI): m/z Calcd for $C_{81}H_{133}NO_{26}P_2$ $[M–2H]^{2–}$ 798.9301. Found: 798.9316.

***P*^{1}-β-D-Galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl *P*^{2}-(2*E*,6*E*)-3,7,11-trimethyl-2,6,10-dodecatrien-1-yl diphosphate (4)**: Compound **5** (78.5 mg, 63.6 μmol) and 1,1′-carbonyldiimidazole (103 mg, 636 μmol) were dissolved in CH_2Cl_2 (5 mL) and stirred at rt for 2 h. Then a solution of methanol (1.03 mL, 10% v/v in CH_2Cl_2) was added dropwise to quench the excess 1,1′-carbonyldiimidazole and the mixture stirred at rt for another 30 min. After removal of the solvent under vacuum, the residue was dissolved in DMF (3 mL), (2*E*,6*E*)-farnesyl monophosphate (57.5 mg, 191 μmol) was added in one portion. After stirring at rt for 2 weeks, removal of the solvent under vacuum, the crude residue was then dissolved in a solution of sodium methoxide (20 mL, 5 mM in methanol) and stirred at rt for 48 h. Then quenched with Amberlite IR-120 resin (NH_4^+ form), the mixture was filtered and the filtrate was concentrated under vacuum, the residue was purified by reverse-phase chromatography (C_{18}, CH_3OH–H_2O, 1:4) and size exclusion column chromatography (Sephadex LH-20, CH_2Cl_2–CH_3OH, 1:1) to afford glycolipid **4** (35.6 mg, 53%) as a white solid: R_f = 0.35 (RP-18; CH_3OH–H_2O, 1:4); $[\alpha]_D^{25}$ = –38.8 (*c* = 1.00, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.51 (dd, 1H, *J* = 6.9, 3.1 Hz, H-1), 5.47 (t, 1H, *J* = 7.0 Hz, C=C*H*CH_2O), 5.29 (s, 1H, H-1‴), 5.25–5.20 (m, 3H, H-1″, C=C*H*CH_2 × 2), 4.88(s, 1H, H-1′), 4.54–4.51 (m, 2H, C=CHC*H*$_2$O), 4.18 (br s, 1H, H-2″), 4.15–4.06 (m, 7H), 4.00–3.95 (m, 2H), 3.93–3.68 (m, 10H), 3.62 (t, 1H, *J* = 9.8 Hz, H-4), 3.57 (t, 1H, *J* = 9.7 Hz, H-4′), 2.22–2.05 (m, 11H, C*H*$_3$CONH, C*H*$_2$C*H*$_2$CH=C × 2), 1.75 (s, 3H, C*H*$_3$), 1.72 (s, 3H, C*H*$_3$), 1.66 (s, 6H, C*H*$_3$ × 2), 1.28 (d, 3H, *J* = 6.3 Hz, H-6′) ppm; ^{13}C NMR (125 MHz, D_2O) δ 175.0, 143.7, 137.3, 134.1, 125.1, 124.9, 120.0, 109.0 (*C*-1″), 107.9 (*C*-1‴), 102.0 (*C*-1′), 95.3 (d, *J* = 6.2 Hz, *C*-1), 83.2, 82.2, 81.9, 80.2, 78.9, 77.1, 77.0, 76.3, 73.9, 71.6, 71.3, 71.1, 68.8, 68.0, 63.8 (d, *J* = 5.7 Hz), 63.5, 62.0, 61.0, 54.0 (d, *J* = 8.5 Hz), 39.4, 26.4, 26.2, 25.5,

17.64, 17.59, 16.3, 15.9 ppm; ^{31}P NMR (162 MHz, D_2O) δ –10.65 (d, J = 21.2 Hz), –13.26 (d, J = 21.7 Hz) ppm; HRMS (ESI): m/z Calcd for $C_{41}H_{70}NO_{26}P_2$ $[M–H]^-$ 1054.3667. Found: 1054.3675.

2,3,5,6-Tetra-*O*-acetyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-acetyl-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl 1-phosphate triethylammonium salt (5): Phosphate **21** (107.4 mg, 75.9 μmol) was dissolved in dry THF (5 mL) and palladium on carbon (50 mg, dry, 5%) was added in one portion. The flask was evacuated under vacuum and re-charged with hydrogen three times while cooling on dry ice. After stirring at rt overnight, the reaction mixture was filtered with Celite and the filtrate was neutralized with triethylamine, then the solvent was removed under vacuum to give compound **5** (91.2 mg, 90%) as a white amorphous solid: R_f = 0.41 (EtOAc–2-propanol–H_2O, 2:2:1); $[\alpha]_D^{25}$ = –21.1 (c = 1.00, $CHCl_3$–CH_3OH, 1:3); ^{1}H NMR (700 MHz, CD_3OD) δ 5.45 (br s, 1H), 5.36 (dt, 1H, J = 7.2, 3.9 Hz), 5.33 (s, 1H), 5.24–5.23 (m, 2H), 5.13 (br t, 1H, J = 2.7 Hz), 5.12–5.06 (m, 3H), 5.03 (dd, 1H, J = 5.6, 2.0 Hz), 4.94 (d, 1H, J = 1.1 Hz), 4.92 (s, 1H), 4.46 (dd, 1H, J = 5.7, 3.5 Hz), 4.36 (ddd, 1H, J = 10.5, 5.6, 1.3 Hz), 4.31–4.16 (m, 8H), 4.10–4.03 (m, 2H), 3.78 (dq, 1H, J = 9.5, 6.2 Hz), 3.64 (t, 1H, J = 9.7 Hz), 3.14 (q, 6H, J = 7.2 Hz), 2.11 (s, 6H), 2.103 (s, 6H), 2.101 (s, 3H), 2.09 (s, 6H), 2.08 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.30 (t, 9H, J = 7.2 Hz), 1.28 (d, 3H, J = 6.3 Hz) ppm; ^{13}C NMR (175 MHz, CD_3OD) δ 174.0, 172.7, 172.3, 172.2, 171.9, 171.8, 171.7, 171.6, 171.5, 171.4, 171.3, 171.2, 108.0, 106.5, 100.6, 95.6 (d, J = 5.4 Hz), 83.4, 82.9, 82.8, 81.9, 79.2, 78.0, 77.8, 77.0, 74.3, 72.9, 71.3, 71.2, 70.7, 70.1, 69.0, 64.4, 64.0, 63.1, 54.4, 22.9, 21.4, 21.0, 20.8, 20.72, 20.67, 20.66, 20.6, 18.5 ppm; ^{31}P NMR (162 MHz, CD_3OD) δ –1.18 ppm; HRMS (ESI): m/z Calcd for $C_{48}H_{68}NNaO_{34}P$ $[M+Na]^+$ 1256.3253. Found: 1256.3236.

***p*-Tolyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (7)**: A mixture of *p*-tolyl 2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (**10**) (162.8 mg, 272 μmol), **9** (195.1 mg, 326 μmol) and molecular sieves (1.3 g, 4Å, powder) in CH_2Cl_2 (13 mL) was stirred for 30 min at rt under an argon atmosphere, then cooled to 0 °C, before bis(cyclopentadienyl)zirconium(IV) dichloride (114.6 mg, 392 μmol) and silver trifluoromethanesulfonate (201 mg, 783 μmol) were added successively. After stirring for 2 h at 0 °C, triethylamine was added and the resulting mixture was filtered through Celite. The filtrate was diluted with CH_2Cl_2 (30 mL), washed with saturated $NaHCO_3$ solution, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (15 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered, concentrated and the residue was purified by chromatography (Hexane–EtOAc, 4:1) to afford **7** (297.3 mg, 87%) as a white foam: R_f = 0.27 (Hexane–EtOAc, 4:1); $[\alpha]_D^{25}$ = –54.7 (*c* = 0.80, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.04–7.99 (m, 6H, Ar), 7.97–7.96 (m, 2H, Ar), 7.91–7.90 (m, 2H, Ar), 7.87–7.86 (m, 2H, Ar), 7.83–7.82 (m, 2H, Ar), 7.57–7.55 (m, 1H, Ar), 7.52–7.50 (m, 1H, Ar), 7.47–7.45 (m, 3H, Ar), 7.43–7.40 (m, 6H, Ar), 7.36–7.33 (m, 2H, Ar), 7.31–7.29 (m, 2H, Ar), 7.27–7.24 (m, 6H, Ar), 7.21–7.19 (m, 2H, Ar), 7.05–7.04 (m, 2H, Ar), 6.02–6.00 (m, 1H, H-5′), 5.90 (dd, 1H, *J* = 4.8, 1.6 Hz, H-3), 5.77 (s, 1H, H-1′), 5.71 (d, 1H, *J* = 1.7 Hz, H-1), 5.69 (t, 1H, *J* = 1.7 Hz, H-2), 5.66 (s, 1H, H-2′), 5.64 (d, 1H, *J* = 5.1 Hz, H-3′), 5.00 (t, 1H, *J* = 5.1 Hz, H-4′), 4.78 (dd, 1H, *J* = 4.8, 3.5 Hz, H-4), 4.74–7.69 (m, 3H, H-5, H-6a, H-6b), 4.66 (dd, 1H, *J* = 12.0, 4.2 Hz, H-6′a), 4.63 (dd, 1H, *J* = 12.0, 7.3 Hz, H-6′b), 2.28 (s, 3H, ArCH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2 (Ph*C*O), 166.1 (Ph*C*O), 165.8 (Ph*C*O), 165.7 (Ph*C*O), 165.4 (Ph*C*O), 165.3 (Ph*C*O), 138.3 (Ar), 133.7 (Ar), 133.6 (Ar), 133.4 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 130.12 (Ar), 130.08 (Ar), 130.0

(Ar), 129.89 (Ar), 129.86 (Ar), 129.82 (Ar), 129.79 (Ar), 129.75 (Ar), 129.74 (Ar), 129.72 (Ar), 129.6 (Ar), 129.04 (Ar), 129.01 (Ar), 128.92 (Ar), 128.91 (Ar), 128.7 (Ar), 128.6 (Ar), 128.47 (Ar), 128.45 (Ar), 128.44 (Ar), 128.37 (Ar), 105.6 (*C*-1′), 91.3 (*C*-1), 82.5 (*C*-4), 82.3 (*C*-2′), 82.3 (*C*-4′), 81.6 (*C*-2), 77.9 (*C*-3′), 77.4 (*C*-3), 73.6 (*C*-5), 70.6 (*C*-5′), 64.5 (*C*-6′), 63.9 (*C*-6), 21.2 (Ar*C*H$_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{68}H_{60}NO_{17}S$ $[M+NH_4]^+$ 1194.3576. Found: 1194.3601; $C_{68}H_{56}NaO_{17}S$ $[M+Na]^+$ 1199.3130. Found: 1199.3142.

2-(Trimethylsilyl)ethyl 2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (8): To a stirred solution of **17** (34 mg, 41.4 μmol) in a mixture of EtOH (2 mL) and toluene (1 mL) was added hydrazine acetate (6.5 mg, 70.3 μmol) in one portion at rt. The resulting reaction mixture was left stirring at rt for 6 h, then the solvent was removed under vacuum, and the resulting crude residue was purified by chromatography (Hexane–EtOAc, 2:3) to afford alcohol **8** (29.7 mg, 99%) as a white foam: R_f = 0.53 (Hexane–EtOAc, 1:2); $[\alpha]_D^{25}$ = +2.8 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.86 (br s, 2H, Ar), 7.73–7.69 (m, 2H, Ar), 5.14 (d, 1H, *J* = 8.5 Hz, H-1), 5.12 (dd, 1H, *J* = 10.0, 9.0 Hz, H-4), 4.83 (dd, 1H, *J* = 10.1, 3.3 Hz, H-3′), 4.61 (d, 1H, *J* = 2.0 Hz, H-1′), 4.56 (dd, 1H, *J* = 10.7, 8.8 Hz, H-3), 4.53 (dd, 1H, *J* = 3.3, 2.0 Hz, H-2′), 4.30 (dd, 1H, *J* = 10.7, 8.5 Hz, H-2), 4.25 (dd, 1H, *J* = 12.2, 4.9 Hz, H-6a), 4.13 (dd, 1H, *J* = 12.2, 2.6 Hz, H-6b), 3.89 (ddd, 1H, *J* = 10.5, 9.8, 5.6 Hz, OC*H*$_2$CH$_2$TMS), 3.72–3.66 (m, 2H, H-5, H-5′), 3.49 (dt, 1H, *J* = 10.0, 6.5 Hz, OC*H*$_2$CH$_2$TMS), 3.41 (t, 1H, *J* = 9.4 Hz, H-4′), 2.10 (s, 3H, C*H*$_3$CO), 2.08 (s, 3H, C*H*$_3$CO), 1.88 (s, 3H, C*H*$_3$CO), 1.84 (s, 3H, C*H*$_3$CO), 1.23 (d, 3H, *J* = 6.2 Hz, H-6′), 0.83–0.77 (m, 1H, OCH$_2$C*H*$_2$TMS), 0.75–0.69 (m, 1H, OCH$_2$C*H*$_2$TMS), –0.13 (s, 9H, Si(C*H*$_3$)$_3$) ppm; ^{13}C NMR (175 MHz, $CDCl_3$) δ 171.0 (CH_3*C*O), 170.3 (CH_3*C*O), 169.7 (CH_3*C*O), 169.5 (CH_3*C*O), 134.1 (Ar), 131.8

(Ar), 99.8 (*C*-1′), 97.9 (*C*-1), 78.9 (*C*-3), 72.1 (*C*-5), 71.5 (*C*-3′), 71.0 (*C*-4′), 70.6 (*C*-4), 70.3 (*C*-2′), 70.1 (*C*-5′), 67.3 (O*C*$H_2$$CH_2$TMS), 62.4 (*C*-6), 55.6 (*C*-2), 21.3 (*C*H_3CO), 20.9 (*C*H_3CO), 20.71 (*C*H_3CO), 20.70 (*C*H_3CO), 17.9 (OCH$_2$*C*H_2TMS), 17.5 (*C*-6′), –1.4 (Si(*C*H_3)$_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{33}H_{45}NNaO_{15}Si$ $[M+Na]^+$ 746.2451. Found: 746.2444.

2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl fluoride (9): To the solution of *p*-tolyl 2,3,5,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (**13**) (286.5 mg, 408 μmol) in CH_2Cl_2 (20 mL) was added *N*,*N*-diethylaminosulphur trifluoride (815 μL, 815 μmol, 1 M in CH_2Cl_2) at 0 °C followed by *N*-bromosuccinimide (145 mg, 815 μmol). The reaction mixture was kept stirring at 0 °C for 2 h and methanol was added. The reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with saturated $NaHCO_3$ solution, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered, concentrated, and the resulting residue was purified by chromatography (Hexane–EtOAc, 6:1) to afford **9** (217 mg, 89%) as a white foam: R_f = 0.41 (Hexane–EtOAc, 4:1); $[\alpha]_D^{25}$ = +17.9 (*c* = 2.10, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07–8.05 (m, 4H, Ar), 7.96–7.94 (m, 2H, Ar), 7.89–7.88 (m, 2H, Ar), 7.61–7.58 (m, 1H, Ar), 7.54–7.49 (m, 3H, Ar), 7.46–7.43 (m, 2H, Ar), 7.37–7.27 (m, 6H, Ar), 6.12–6.09 (m, 1H, H-5), 6.05 (d, 1H, *J* = 58.3 Hz, H-1), 5.69 (d, 1H, *J* = 5.8 Hz, H-3), 5.67 (d, 1H, *J* = 6.6 Hz, H-2), 4.92 (td, 1H, *J* = 3.8, 1.2 Hz, H-4), 4.81 (dd, 1H, *J* = 12.0, 4.4 Hz, H-6a), 4.74 (dd, 1H, *J* = 12.0, 7.0 Hz, H-6b) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2 (Ph*C*O), 165.8 (Ph*C*O), 165.7 (Ph*C*O), 165.3 (Ph*C*O), 133.9 (Ar), 133.8 (Ar), 133.5 (Ar), 133.3 (Ar), 130.1 (Ar), 130.0 (Ar), 129.8 (Ar), 129.6 (Ar), 129.4 (Ar), 128.8 (Ar), 128.7 (Ar), 128.64 (Ar), 128.61 (Ar), 128.54 (Ar), 128.47 (Ar), 112.5 (d, $J_{C\text{-}F}$ = 225.1 Hz, *C*-1), 84.9 (*C*-4), 81.1 (d, $J_{C\text{-}F}$ = 40.3 Hz, *C*-2), 76.7 (*C*-3), 70.4 (*C*-5), 63.5 (*C*-6) ppm; HRMS (ESI): *m*/*z*

Calcd for $C_{34}H_{31}FNO_9$ $[M+NH_4]^+$ 616.1977. Found: 616.1986; $C_{34}H_{27}FNaO_9$ $[M+Na]^+$ 621.1531. Found: 621.1520.

***p*-Tolyl 2,3-*O*-isopropylidene-4-*O*-levulinoyl-1-thio-α-L-rhamnopyranoside (11)**: To a stirred solution of *p*-tolyl 2,3-*O*-isopropylidene-1-thio-α-L-rhammopyranoside (**14**) (1.295 g, 4.17 mmol) in CH_2Cl_2 (20 mL) were added levulinic acid (727 mg, 6.26 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (1.2 g, 6.26 mmol) and 4-dimethylaminopyridine (51 mg, 417 μmol) successively at rt, After stirring overnight, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with water. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (30 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered, concentrated, the and residue was then purified by chromatography (Hexane–EtOAc, 3:1) to give **11** (1.678 g, 98%) as a white foam: R_f = 0.32 (Hexane–EtOAc, 3:1); $[\alpha]_D^{25}$ = –166.9 (*c* = 0.95, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.35 (m, 2H, Ar), 7.14–7.12 (m, 2H, Ar), 5.68 (s, 1H, H-1), 4.92 (dd, 1H, *J* = 10.0, 7.9 Hz, H-4), 4.35 (d, 1H, *J* = 5.4 Hz, H-2), 4.23–4.17 (m, 2H, H-3, H-5), 2.91–2.84 (m, 1H, $CH_3COCH_2CH_2CO$), 2.73–2.65 (m, 2H, $CH_3COCH_2CH_2CO$), 2.61–2.55 (m, 1H, $CH_3COCH_2CH_2CO$), 2.33 (s, 3H, $ArCH_3$), 2.19 (s, 3H, $CH_3COCH_2CH_2CO$), 1.55 (s, 3H, CH_3C), 1.35 (s, 3H, CH_3C), 1.14 (d, 3H, *J* = 6.3 Hz, H-6) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5 ($CH_3COCH_2CH_2CO$), 172.2 ($CH_3COCH_2CH_2CO$), 138.2 (Ar), 132.7 (Ar), 130.0 (Ar), 129.5 (Ar), 110.1 ($C(CH_3)_2$), 84.2 (*C*-1, $J_{C\text{-}H}$ = 169.2 Hz), 76.6 (*C*-2), 75.6 (*C*-3), 75.1 (*C*-4), 65.7 (*C*-5), 38.1 ($CH_3COCH_2CH_2CO$), 29.9 ($CH_3COCH_2CH_2CO$), 28.1 ($CH_3COCH_2CH_2CO$), 27.9 (CH_3C), 26.7 (CH_3C), 21.3 ($ArCH_3$), 17.0 (*C*-6) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{21}H_{32}NO_6S$ $[M+NH_4]^+$ 426.1945. Found: 426.1938; $C_{21}H_{28}NaO_6S$ $[M+Na]^+$ 431.1499. Found: 431.1499.

2-(Trimethylsilyl)ethyl 2,3-*O*-isopropylidene-4-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (15): A mixture of 2-(trimethylsilyl)ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**12**) (1.34 g, 2.69 mmol), **11** (1.21 g, 2.96 mmol) and molecular sieves (7.2 g, 4Å, powder) in CH_2Cl_2 (72 mL) was stirred under an argon atmosphere for 30 min at rt. The mixture was then cooled to –15 °C and *N*-iodosuccinimide (909 mg, 4.04 mmol) and silver trifluoromethanesulfonate (138.4 mg, 538.6 μmol) were added successively. After stirring for 30 min at –15 °C, triethylamine was added. The mixture was filtered through Celite and the filtrate was washed with a mixture of saturated $Na_2S_2O_3$ (40 mL) and saturated $NaHCO_3$ (40 mL) solution. The aqueous layer was extracted with CH_2Cl_2 (30 mL × 3) and the combined organic layer was then washed with brine, dried over Na_2SO_4, filtered, concentrated, the residue was purified by chromatography (Hexane–EtOAc, 2:1) to afford **15** (1.658 g, 79%) as a white foam: R_f = 0.27 (Hexane–EtOAc, 2:1); $[\alpha]_D^{25}$ = –30.8 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87–7.84 (m, 2H, Ar), 7.76–7.73 (m, 2H, Ar), 7.49–7.47 (m, 2H, Ar), 7.37–7.32 (m, 3H, Ar), 5.54 (s, 1H, PhC*H*), 5.31 (d, 1H, *J* = 8.5 Hz, H-1), 4.72 (s, 1H, H-1′), 4.62 (dd, 1H, *J* = 10.2, 8.9 Hz, H-3), 4.58 (dd, 1H, *J* = 10.2, 7.8 Hz, H-4′), 4.42–4.39 (m, 1H, H-6a), 4.26 (dd, 1H, *J* = 10.2, 8.5 Hz, H-2), 3.98 (dd, 1H, *J* = 7.8, 5.4 Hz, H-3′), 3.91 (ddd, 1H, *J* = 10.5, 9.8, 5.4 Hz, OC*H*$_2$CH$_2$TMS), 3.84–3.77 (m, 3H, H-6b, H-2′, H-5′), 3.68–3.66 (m, 2H, H-4, H-5), 3.50 (td, 1H, *J* = 10.0, 6.5 Hz, OC*H*$_2$CH$_2$TMS), 2.77–2.72 (m, 1H, CH$_3$COC*H*$_2$CH$_2$CO), 2.64–2.46 (m, 3H, CH$_3$COC*H*$_2$C*H*$_2$CO), 2.14 (s, 3H, C*H*$_3$COCH$_2$CH$_2$CO), 1.30 (s, 3H, C*H*$_3$C), 1.02 (s, 3H, C*H*$_3$C), 0.81 (ddd, 1H, *J* = 14.0, 10.7, 6.6 Hz, OCH$_2$C*H*$_2$TMS), 0.72 (ddd, 1H, *J* = 14.0, 10.3, 5.5 Hz, OCH$_2$C*H*$_2$TMS), 0.59 (d, 3H, *J* = 6.3 Hz, H-6′), –0.14 (s, 9H, Si(C*H*$_3$)$_3$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.2 (CH$_3$*C*OCH$_2$CH$_2$CO), 171.9

($CH_3COCH_2CH_2CO$), 137.1 (Ar), 134.5 (Ar), 131.5 (Ar), 129.3 (Ar), 128.3 (Ar), 126.5 (Ar), 123.7 (Ar), 109.5 ($C(CH_3)_2$), 102.2 (C*H*Ph), 98.3 (*C*-1), 97.6 (*C*-1′, $J_{C\text{-}H}$ = 167.2 Hz), 80.8 (*C*-5), 75.9 (*C*-2′), 75.5 (*C*-3′), 74.6 (*C*-3), 74.4 (*C*-4′), 68.9 (*C*-6), 67.6 (OCH_2CH_2TMS), 66.5 (*C*-4), 64.7 (*C*-5′), 57.0 (*C*-2), 37.9 ($CH_3COCH_2CH_2CO$), 29.9 ($CH_3COCH_2CH_2CO$), 28.0 ($CH_3COCH_2CH_2CO$), 27.5 (CH_3C), 26.1 (CH_3C), 18.0 (OCH_2CH_2TMS), 16.2 (*C*-6′), –1.5 ($Si(CH_3)_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{40}H_{51}NNaO_{13}Si$ $[M+Na]^+$ 804.3022. Found: 804.3010.

2-(Trimethylsilyl)ethyl 4-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-2-deoxy-2-phthalimido-β-D-glucopyranoside (16): To a solution of **15** (282.4 mg, 361 μmol) in a mixture of CH_3CN (10 mL) and CH_3OH (1 mL) was added *p*-toluenesulfonic acid monohydrate (206 mg, 1.08 mmol) in one portion at rt. After stirring for 5 h, triethylamine was added and then the solution was concentrated. The crude residue was purified by chromatography (CH_2Cl_2–CH_3OH, 25:1) to afford **16** (205 mg, 87%) as a white foam: R_f = 0.40 (CH_2Cl_2–CH_3OH, 25:1); $[\alpha]_D^{25}$ = +14.9 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85–7.82 (m, 2H, Ar), 7.76–7.72 (m, 2H, Ar), 5.19 (d, 1H, *J* = 8.6 Hz, H-1), 4.84 (t, 1H, *J* = 9.4 Hz, H-4′), 4.79 (d, 1H, *J* = 1.8 Hz, H-1′), 4.40 (d, 1H, *J* = 1.8 Hz, O*H*), 4.29 (dd, 1H, *J* = 10.8, 8.0 Hz, H-3), 4.14 (dd, 1H, *J* = 10.8, 8.6 Hz, H-2), 4.00–3.89 (m, 3H, H-6a, H-5′, OCH_2CH_2TMS), 3.87–3.83 (m, 1H, H-6b), 3.78 (dt, 1H, *J* = 9.2, 3.2 Hz, H-3′), 3.59 (td, 1H, *J* = 8.0, 1.5 Hz, H-4), 3.53–3.46 (m, 3H, H-5, H-2′, OCH_2CH_2TMS), 3.39 (d, 1H, *J* = 3.3 Hz, O*H*), 2.80–2.76 (m, 2H, $CH_3COCH_2CH_2CO$), 2.54–2.50 (m, 3H, $CH_3COCH_2CH_2CO$, O*H*), 2.16 (s, 3H, $CH_3COCH_2CH_2CO$), 2.14 (br s, 1H, O*H*) 1.19 (d, 3H, *J* = 6.3 Hz, H-6′), 0.82 (ddd, 1H, *J* = 14.1, 10.5, 6.7 Hz, OCH_2CH_2TMS), 0.73 (ddd, 1H, *J* = 14.1, 10.0, 5.6 Hz, OCH_2CH_2TMS), –0.16 (s, 9H, $Si(CH_3)_3$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 207.8 ($CH_3COCH_2CH_2CO$), 173.1 ($CH_3COCH_2CH_2CO$), 134.5 (Ar),

131.5 (Ar), 123.8 (Ar), 101.1 (C-1′), 97.9 (*C*-1), 83.2(*C*-3), 75.3 (*C*-5), 74.6 (*C*-4′), 71.2 (*C*-4), 71.0 (*C*-2′), 69.3 (*C*-3′), 67.5 (*C*-5′), 67.3 (OCH_2CH_2TMS), 62.6 (*C*-6), 55.0 (*C*-2), 38.3 ($CH_3CO\mathit{C}H_2CH_2CO$), 29.9 ($\mathit{C}H_3COCH_2CH_2CO$), 28.2 ($CH_3COCH_2\mathit{C}H_2CO$), 17.9 (*C*-6′), 17.4 ($OCH_2\mathit{C}H_2TMS$), –1.4 ($Si(\mathit{C}H_3)_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{30}H_{43}NNaO_{13}Si$ $[M+Na]^+$ 676.2396. Found: 676.2387.

2-(Trimethylsilyl)ethyl 2,3-di-*O*-acetyl-4-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (17): Alcohol **16** (205 mg, 314 μmol) was dissolved in pyridine (10 mL), acetic anhydride (5 mL) was added dropwise at 0 °C and the resulting solution was allowed to warm to rt and stirred for 24 h. After excess acetic anhydride was quenched with CH_3OH (5 mL) at 0 °C, the mixture was concentrated. The residue was dissolved in CH_2Cl_2 (30 mL), washed with HCl (1 M) solution. The aqueous layer was extracted with CH_2Cl_2 (20 mL × 3) and the combined organic layer was washed with saturated $NaHCO_3$ (aq.) solution, dried over Na_2SO_4, filtered, concentrated and the resulting residue was purified by chromatography (Hexane–EtOAc, 2:3) to give **17** (234 mg, 91%) as a white foam: R_f = 0.54 (Hexane–EtOAc, 1:2); $[\alpha]_D^{25}$ = +5.9 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.86 (br s, 2H, Ar), 7.71–7.69 (m, 2H, Ar), 5.13 (d, 1H, *J* = 8.4 Hz, H-1), 5.12 (dd, 1H, *J* = 10.8, 8.8 Hz, H-4), 4.99 (dd, 1H, *J* = 10.3, 3.4 Hz, H-3′), 4.87 (t, 1H, *J* = 10.0 Hz, H-4′), 4.62 (d, 1H, *J* = 2.0 Hz, H-1′), 4.57 (dd, 1H, *J* = 10.7, 8.9 Hz, H-3), 4.52 (dd, 1H, *J* = 3.3, 2.0 Hz, H-2′), 4.31 (dd, 1H, *J* = 10.8, 8.5 Hz, H-2), 4.24 (dd, 1H, *J* = 12.2, 5.0 Hz, H-6a), 4.12 (dd, 1H, *J* = 12.2, 2.5 Hz, H-6b), 3.89 (ddd, 1H, *J* = 10.5, 9.8, 5.5 Hz, $OC\mathit{H}_2CH_2TMS$), 3.82 (dq, 1H, *J* = 9.7, 6.1 Hz, H-5′), 3.69 (ddd, 1H, *J* = 10.0, 4.8, 2.5 Hz, H-5), 3.49 (dt, 1H, *J* = 10.1, 6.1 Hz, $OC\mathit{H}_2CH_2TMS$), 2.69–2.66 (m, 2H, $CH_3COC\mathit{H}_2CH_2CO$), 2.46–2.44 (m, 2H, $CH_3COCH_2C\mathit{H}_2CO$), 2.13

(s, 3H, C*H*$_3$CO), 2.09 (s, 3H, C*H*$_3$CO), 2.07 (s, 3H, C*H*$_3$CO), 1.90 (s, 3H, C*H*$_3$CO), 1.77 (s, 3H, C*H*$_3$CO), 1.09 (d, 3H, *J* = 6.2 Hz, H-6′), 0.83–0.77 (m, 1H, OCH$_2$C*H*$_2$TMS), 0.75–0.69 (m, 1H, OCH$_2$C*H*$_2$TMS), –0.13 (s, 9H, Si(C*H*$_3$)$_3$) ppm; ^{13}C NMR (175 MHz, $CDCl_3$) δ 206.1 (CH$_3$*C*OCH$_2$CH$_2$CO), 171.8 (CH$_3$*C*O), 170.9 (CH$_3$*C*O), 169.7 (CH$_3$*C*O), 169.6 (CH$_3$*C*O), 169.2 (CH$_3$*C*O), 134.1 (Ar), 131.8 (Ar), 100.0 (*C*-1′), 97.9 (*C*-1), 79.5 (*C*-3), 72.1 (*C*-5), 70.8 (*C*-4′), 70.6 (*C*-4), 70.0 (*C*-2′), 68.2 (*C*-3′), 67.8 (*C*-5′), 67.3 (O*C*H$_2$CH$_2$TMS), 62.4 (*C*-6), 55.4 (*C*-2), 37.7 (CH$_3$CO*C*H$_2$CH$_2$CO), 29.8 (*C*H$_3$CO), 28.0 (CH$_3$COCH$_2$*C*H$_2$CO), 21.3 (*C*H$_3$CO), 20.9 (*C*H$_3$CO), 20.7 (*C*H$_3$CO), 20.5 (*C*H$_3$CO), 18.9 (OCH$_2$*C*H$_2$TMS), 17.2 (*C*-6′), –1.4 (Si(*C*H$_3$)$_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{38}H_{51}NNaO_{17}Si$ $[M+Na]^+$ 844.2818. Found: 844.2808.

2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (18): A mixture of **7** (88.8 mg, 123 μmol), **8** (159 mg, 135 μmol) and molecular sieves (600 mg, 4Å, powder) in CH_2Cl_2 (6 mL) was stirred under an argon atmosphere for 30 min at rt, then *N*-iodosuccinimide (45.6 mg, 203 μmol) and silver trifluoromethanesulfonate (7.88 mg, 30.7 μmol) were added successively. After stirring for 30 min at rt, triethylamine was added and then the mixture was filtered through Celite. The filtrate was diluted with CH_2Cl_2 (20 mL) and washed with a mixture of saturated $Na_2S_2O_3$ (20 mL) and saturated $NaHCO_3$ (20 mL) solution. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (15 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered, concentrated and the residue was purified by chromatography (Hexane–EtOAc, 3:2) to afford tetrasaccharide **18** (205.2 mg, 94%) as a white foam: R_f = 0.31 (Hexane–EtOAc, 3:2); $[\alpha]_D^{25}$ = –4.8 (*c* = 0.26, CH_2Cl_2); ^{1}H NMR (700

MHz, $CDCl_3$) δ 8.04–8.03 (m, 2H), 7.99–7.98 (m, 4H), 7.92–7.90 (m, 5H), 7.82–7.81 (m, 2H), 7.75–7.74 (m, 2H), 7.71 (br s, 2H), 7.60–7.58 (m, 1H), 7.48–7.41 (m, 8H), 7.37–7.32 (m, 5H), 7.26–7.23 (m, 6H), 7.13–7.11 (m, 2H), 6.05 (br s, 1H), 5.86 (d, 1H, J = 3.3 Hz), 5.78 (s, 1H), 5.70 (s, 1H), 5.61 (d, 1H, J = 4.1 Hz), 5.36 (s, 1H), 5.34 (s, 1H), 5.13–5.10 (m, 3H), 5.05 (d, 1H, J = 8.3 Hz), 4.78–4.76 (m, 2H), 4.71–4.67 (m, 3H), 4.62–4.60 (m, 3H), 4.50 (br s, 1H), 4.35 (t, 1H, J = 9.1 Hz), 4.25 (dd, 1H, J = 11.9, 4.4 Hz), 4.14 (d, 1H, J = 11.9 Hz), 3.93–3.89 (m, 1H), 3.81–3.79 (m, 1H), 3.69 (br s, 1H), 3.61 (t, 1H, J = 9.7 Hz), 3.53–3.49 (m, 1H), 2.11 (s, 3H), 1.97 (s, 3H), 1.89 (s, 3H), 1.70 (s, 3H), 1.15 (d, 3H, J = 5.7 Hz), 0.85–0.80 (m, 1H), 0.77–0.73 (m, 1H), − 0.12 (s, 9H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 169.7, 169.6, 169.0, 166.2, 166.1, 166.0, 165.8, 165.7, 165.4, 165.1, 133.9, 133.7, 133.5, 133.4, 133.33, 133.31, 133.2, 133.1, 130.08, 130.05, 130.0, 129.83, 129.81, 129.76, 129.74, 129.73, 129.71, 129.68, 129.03, 128.97, 128.84, 128.82, 128.81, 128.7, 128.51, 128.46, 128.4, 128.2, 106.9, 105.1, 100.3, 98.0, 83.9, 82.3, 82.1, 81.8, 79.5, 78.1, 75.2, 72.13, 72.06, 71.2, 70.7, 70.6, 70.3, 68.5, 67.3, 64.3, 63.9, 62.5, 55.3, 21.3, 21.0, 20.8, 20.6, 17.9, 17.8, –1.4 ppm; HRMS (ESI): *m*/*z* $C_{94}H_{93}NNaO_{32}Si$ $[M+Na]^+$ 1798.5342. Found: 1798.5387.

2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-acetyl-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (19): A solution of **18** (293.5 mg, 165 μmol) in a mixture of ethylenediamine (3 mL) and ethanol (12 mL) was heated at reflux for 24 h. Then the reaction mixture was cooled to rt and co-concentrated with dry toluene three times. The residue was dissolved in pyridine (8 mL), acetic anhydride (4 mL) was added dropwise at 0 °C and the resulting solution was allowed to warm to rt and stirred for 48 h. Excess acetic anhydride was quenched by the addition of

CH_3OH (4 mL) at 0 °C and the mixture was concentrated. The residue was dissolved in CH_2Cl_2 (30 mL), washed with HCl (1 M) solution and the aqueous layer was extracted with CH_2Cl_2 (20 mL × 3). The combined organic layer was washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4, filtered, concentrated and purified by chromatography (Hexane–EtOAc, 1:4) to give **19** (182.2 mg, 88%) as a white foam: R_f = 0.31 (Hexane–EtOAc, 1:4); $[\alpha]_D^{25}$ = –28.7 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.14 (d, 1H, *J* = 6.8 Hz), 5.35 (dt, 1H, *J* = 7.4, 3.8 Hz), 5.29 (s, 1H), 5.26 (s, 1H), 5.21–5.16 (m, 4H), 5.12 (dd, 1H, *J* = 2.5, 0.9 Hz), 5.02 (dd, 1H, *J* = 6.1, 2.4 Hz), 4.95–4.91 (m, 2H), 4.65 (d, 1H, *J* = 1.3 Hz), 4.53 (dd, 1H, *J* = 10.2, 9.0 Hz), 4.38 (dd, 1H, *J* = 6.0, 3.6 Hz), 4.33–4.28 (m, 2H), 4.24–4.13 (m, 5H), 4.04 (dd, 1H, *J* = 12.1, 2.4 Hz), 3.93 (ddd, 1H, *J* = 10.9, 9.8, 5.4 Hz), 3.81 (dq, 1H, *J* = 9.6, 6.2 Hz), 3.66–3.60 (m, 2H), 3.51 (dt, 1H, *J* = 10.5, 6.5 Hz), 2.89 (ddd, 1H, *J* = 10.3, 8.1, 6.8 Hz), 2.10 (s, 3H), 2.08 (s, 6H), 2.071 (s, 3H), 2.068 (s, 3H), 2.054 (s, 3H), 2.051 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.21 (d, 3H, *J* = 6.2 Hz), 0.97–0.92 (m, 1H), 0.90–0.84 (m, 1H), –0.01 (s, 9H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 170.8, 170.5, 170.4, 170.2, 170.1, 170.0, 169.8, 169.7, 169.5, 169.4, 106.4, 104.9, 100.5, 98.3, 81.9, 81.7, 81.4, 80.5, 80.2, 76.3, 74.7, 72.2, 72.1, 71.4, 70.6, 69.7, 69.1, 68.4, 67.4, 63.1, 62.8, 62.4, 59.3, 23.5, 21.2, 20.83, 20.81, 20.7, 20.58, 20.55, 20.5, 18.0, 17.7, –1.4 ppm; HRMS (ESI): *m*/*z* Calcd for $C_{53}H_{79}NNaO_{31}Si$ $[M+Na]^+$ 1276.4298. Found: 1276.4278.

2,3,5,6-Tetra-*O*-acetyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-acetyl-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranose (20): A solution of **19** (182.2 mg, 145.3 μmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C and then trifluoroacetic acid (5.0 mL) was added dropwise. The solution was allowed to warm to rt and stirred for 30 min. Then the mixture was concentrated under vacuum and the residue was purified by

chromatography (Hexane–Acetone, 2:3) to afford the desired hemiacetal **20** (167.6 mg, quant.) as a white amorphous solid: R_f = 0.47 (Hexane–Acetone, 1:2); $[\alpha]_D^{25}$ = –23.5 (c = 1.00, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.11 (d, 1H, J = 8.9 Hz), 5.36 (dt, 1H, J = 7.5, 3.7 Hz), 5.30 (s, 1H), 5.272–5.266 (m, 2H), 5.19 (dd, 1H, J = 5.3, 1.6 Hz), 5.14–5.02 (m, 5H), 4.93 (d, 1H, J = 1.9 Hz), 4.74 (d, 1H, J = 1.7 Hz), 4.65 (s, 1H), 4.39 (dd, 1H, J = 6.0, 3.7 Hz), 4.34–4.29 (m, 2H), 4.21–4.01 (m, 9H), 3.83 (dq, 1H, J = 9.4, 6.3 Hz), 3.63 (t, 1H, J = 9.6 Hz), 2.12 (s, 3H), 2.103 (s, 6H), 2.096 (s, 3H), 2.09 (s, 3H), 2.083 (s, 3H), 2.081 (s, 3H), 2.064 (s, 3H), 2.057 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.21 (d, 3H, J = 6.2 Hz) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 171.0, 170.6, 170.5, 170.4, 170.2, 170.0, 169.9, 169.7, 169.6, 169.4, 106.6, 105.1, 100.0, 92.5, 81.91, 81.85, 81.6, 80.6, 79.2, 76.5, 75.3, 72.3, 71.9, 70.6, 70.3, 69.3, 68.3, 67.9, 63.3, 62.9, 62.3, 53.9, 23.3, 21.3, 20.0, 20.91, 20.87, 20.79, 20.75, 20.72, 20.71, 17.7 ppm; HRMS (ESI): m/z Calcd for $C_{48}H_{67}NNaO_{31}$ $[M+Na]^+$ 1176.3589. Found: 1176.3576.

2,3,5,6-Tetra-*O*-acetyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-acetyl-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl 1-dibenzylphosphate (21): Hemiacetal **20** (13.6 mg, 11.8 μmol) was dissolved in dry CH_2Cl_2 (2 mL), 1,2,4-triazole (12.2 mg, 176.8 μmol) was added in one portion, and the solution was cooled to 0 °C. Dibenzyl *N*,*N*-diisopropylphosphoramidite (39.6 μL, 117.8 μmol) was added dropwise and the mixture was stirred at rt for a further 5 h. Then, the mixture was cooled to –78 °C and 3-chloroperbenzoic acid (55.5 mg, 176.8 μmol, 55%) was added in one portion. The reaction mixture was then allowed to warm to rt and stirred for 30 min, before a mixture of saturated $Na_2S_2O_3$ (3 mL) and saturated $NaHCO_3$ (3 mL) solution was added before the organic layer was separated. The

aqueous layer was extracted with CH_2Cl_2 (5 mL × 3) and EtOAc (10 mL) and the combined organic layer was then dried over Na_2SO_4, filtered, concentrated and purified by chromatography (EtOAc) to afford **21** (11.8 mg, 71%) as a colorless syrup: R_f = 0.34 (CH_2Cl_2–CH_3OH, 40:1); $[\alpha]_D^{25}$ = –19.7 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.40–7.32 (m, 10H), 5.61 (dd, 1H, *J* = 5.7, 3.1 Hz), 5.48 (d, 1H), 5.36 (dt, 1H, *J* = 7.2, 4.0 Hz), 5.30 (s, 1H), 5.26 (s, 1H), 5.18 (dd, 1H, *J* = 5.1, 1.5 Hz), 5.13 (dd, 1H, *J* = 2.5, 0.9 Hz), 5.10–5.01 (m, 8H), 4.92 (d, 1H, *J* = 1.6 Hz), 4.70 (d, 1H, *J* = 1.8 Hz), 4.43–4.38 (m, 2H), 4.34–4.28 (m, 2H), 4.21–4.15 (m, 4H), 4.04 (dd, 1H, *J* = 12.6, 4.5 Hz), 3.91–3.86 (m, 2H), 3.78 (dq, 1H, *J* = 9.5, 6.2 Hz), 3.66–3.60 (m, 2H), 2.13 (s, 3H), 2.11 (s, 3H), 2.09 (s, 6H), 2.07 (s, 3H), 2.06 (s, 3H), 2.051 (s, 3H), 2.047 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.87 (s, 3H), 1.20 (d, 3H, *J* = 6.2 Hz) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.8, 170.60, 170.59, 170.4, 170.3, 170.1, 169.9, 169.6, 169.5, 169.4, 169.3, 135.63, 135.61, 135.41, 135.37, 129.1, 128.87, 128.85, 128.3, 128.2, 106.6, 105.0, 99.7, 97.0 (d, *J* = 6.8 Hz), 81.9, 81.7, 81.5, 80.5, 78.6, 76.44, 76.41, 75.3, 72.3, 71.6, 70.2, 70.1, 70.03, 70.02, 69.5, 69.3, 68.3, 63.3, 62.9, 61.6, 51.8 (d, *J* = 7.5 Hz), 23.0, 21.2, 21.0, 20.9, 20.84, 20.81, 20.78, 20.75, 20.7, 20.6, 17.7 ppm; ^{31}P NMR (162 MHz, $CDCl_3$) δ –2.38 ppm; HRMS (ESI): *m*/*z* Calcd for $C_{62}H_{80}NNaO_{34}P$ $[M+Na]^+$ 1436.4192. Found: 1436.4169.

***P*^{1}-α-L-Rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl *P*^{2}-(2*E*,6*E*)-3,7,11-trimethyl-2,6,10-dodecatrien-1-yl diphosphate (22)**: Compound **30** (26.0 mg, 39.5 μmol) and 1,1′-carbonyldiimidazole (25.6 mg, 158 μmol) were dissolved in CH_2Cl_2 (3 mL) and the solution was stirred at rt for 2 h. Then a solution of methanol (1.28 mL, 2% v/v in CH_2Cl_2) was added dropwise to quench the excess 1,1′-carbonyldiimidazole and the mixture stirred at rt for another 30 min. After removal of the solvent under vacuum, the residue was dissolved in DMF (2 mL) and (2*E*,6*E*)-farnesyl

monophosphate (35.8 mg, 118.5 μmol) was added in one portion. After stirring at rt for 10 days and removal of the solvent under vacuum, the crude residue was dissolved in a solution of sodium methoxide (10 mL, 5 mM in methanol) and the solution was stirred at rt for 48 h. Amberlite IR-120 resin (NH_4^+ form) was then added and the mixture was filtered. The filtrate was concentrated under vacuum and the resulting residue was purified by reverse-phase chromatography (C_{18}, CH_3OH–H_2O, 1:1) to afford **22** (10.3 mg, 41%) as a white solid: R_f = 0.30 (RP-18; CH_3OH–H_2O, 3:1); $[\alpha]_D^{25}$ = +21.4 (*c* = 0.60, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 5.55 (br s, 1H, H-1), 5.43 (br s, 1H, C=C*H*CH_2O), 5.14–5.08 (m, 2H, C=C*H*CH_2 × 2), 4.87 (s, 1H, H-1′), 4.58 (br s, 2H, C=CHC*H*$_2$O), 4.13 (br d, 1H, *J* = 10.3 Hz, H-2), 3.97–3.89 (m, 3H, H-5, H-6a, H-5′), 3.81 (br t, 1H, *J* = 9.3 Hz, H-3), 3.75–3.72 (m, 2H, H-6b, H-2′), 3.64 (dd, 1H, *J* = 9.5, 3.4 Hz, H-3′), 3.45–3.43 (m, 1H, H-4), 3.37 (t, 1H, *J* = 9.5, H-3), 2.15–1.96 (m, 11H, C*H*$_2$C*H*$_2$CH=C × 2, C*H*$_3$CONH), 1.72 (s, 3H, C*H*$_3$), 1.67 (s, 3H, C*H*$_3$), 1.61 (s, 3H, C*H*$_3$), 1.60 (s, 3H, C*H*$_3$), 1.24 (d, 3H, *J* = 6.3 Hz, H-6′) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 173.9, 141.7, 136.3, 132.1, 125.4, 125.2, 121.8, 103.6 (d, *J* = 9.5 Hz, *C*-1′), 96.7 (d, *J* = 5.9 Hz, *C*-1), 81.7 (d, *J* = 17.9 Hz), 75.2 (d, *J* = 9.5 Hz), 73.9, 72.6, 72.1, 70.6 (d, *J* = 9.6 Hz), 70.3, 64.4, 62.5, 54.5, 40.9, 40.6, 27.8, 27.4, 25.9, 22.9 (d, *J* = 8.6 Hz), 17.9 (d, *J* = 3.0 Hz), 17.7, 16.6, 16.1 ppm; ^{31}P NMR (162 MHz, CD_3OD) –9.23, –11.74 ppm; HRMS (ESI): *m*/*z* Calcd for $C_{29}H_{50}NO_{16}P_2$ $[M–H]^-$ 730.2610. Found: 730.2602.

***P*^{1}-β-D-Galactofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl *P*^{2}-(2*E*,6*E*)-3,7,11-trimethyl-2,6,10-dodecatrien-1-yl diphosphate (23)**: Compound **39** (114.7 mg, 110 μmol) and 1,1′-carbonyldiimidazole (178 mg, 1.10 mmol) were dissolved in CH_2Cl_2 (10 mL) and the mixture stirred at rt for 2 h. A solution of methanol (1.95 mL, 10% v/v in CH_2Cl_2) was

then added dropwise to quench the excess 1,1′-carbonyldiimidazole and the mixture stirred at rt for another 30 min. After removal of the solvent under vacuum, the residue was dissolved in DMF (5 mL) and (2*E*,6*E*)-farnesyl monophosphate (66 mg, 219 μmol) was added in one portion. After stirring at rt for 15 days, the solvent was removed under vacuum. The crude residue was then dissolved in a solution of sodium methoxide (20 mL, 5 mM in methanol) and the mixture was stirred at rt for 48 h. Amberlite IR-120 resin (NH_4^+ form was added, the mixture was filtered, and the filtrate was concentrated under vacuum to give a residue that was purified by reverse-phase chromatography (C_{18}, CH_3OH–H_2O, 3:2) and then size exclusion column chromatography (Sephadex LH-20, CH_2Cl_2–CH_3OH, 1:1) to afford **23** (72.2 mg, 74%) as a white solid: R_f = 0.35 (RP-18; CH_3OH–H_2O, 3:2); $[\alpha]_D^{25}$ = –16.7 (*c* = 1.15, CH_3OH); ^{1}H NMR (700 MHz, CD_3OD) δ 5.53 (br s, 1H, H-1), 5.43 (br s, 1H, C=C*H*CH_2O), 5.28 (d, 1H, *J* = 1.5 Hz, H-1″), 5.13 (t, 1H, *J* = 6.9 Hz, C=C*H*CH_2), 5.09 (tt, 1H, *J* = 7.1, 1.3 Hz, C=C*H*CH_2), 4.86 (s, 1H, H-1′), 4.54 (s, 2H, C=CHC*H*$_2$O), 4.13 (d, 1H, *J* = 9.9 Hz, H-2), 4.03–3.99 (m, 4H, H-5′, H-2″, H-3″, H-5), 3.95 (dd, 1H, *J* = 6.4, 2.8 Hz, H-4″), 3.90 (br s, 1H, H-6a), 3.82–3.80 (m, 2H, H-3′, H-3), 3.73 (d, 1H, *J* = 2.2 Hz, H-2′), 3.71 (dd, 1H, *J* = 6.2, 2.8 Hz, H-5″), 3.69 (dd, 1H, *J* = 10.9, 5.0 Hz, H-6b), 3.63 (dd, 1H, *J* = 11.0, 6.8 Hz, H-6″a), 3.61 (dd, 1H, *J* = 11.0, 5.9 Hz, H-6″b), 3.55 (t, 1H, *J* = 9.5 Hz, H-4′), 3.41 (t, 1H, *J* = 9.3 Hz, H-4), 2.13–1.96 (m, 11H, C*H*$_2$C*H*$_2$CH=C × 2, C*H*$_3$CONH), 1.71 (s, 3H, C*H*$_3$), 1.67 (s, 3H, C*H*$_3$), 1.61 (s, 3H, C*H*$_3$), 1.60 (s, 3H, C*H*$_3$), 1.26 (d, 3H, *J* = 6.2 Hz, H-6′) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 174.0 (CH_3*C*ONH), 141.2, 136.2, 132.1, 125.4, 125.2, 122.2, 110.2 (*C*-1″), 103.3 (*C*-1′), 96.4 (*C*-1), 84.3, 83.5, 81.5, 79.1, 78.5, 75.1, 72.8, 72.6, 72.3, 70.8, 68.9, 64.8, 64.0, 62.8, 54.6, 40.9, 40.7, 27.8, 27.5, 25.9, 23.0, 18.4, 17.8, 16.6, 16.1 ppm; ^{31}P NMR (202

MHz, CD_3OD) δ –10.41, –12.83 ppm; HRMS (ESI): *m*/*z* Calcd for $C_{35}H_{60}NO_{21}P_2$ $[M–H]^-$ 892.3139. Found: 892.3150.

2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (25): A mixture of 2-(trimethylsilyl)ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**12**) (440 mg, 884 μmol), ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**24**) (325 mg, 972 μmol) and molecular sieves (2.0 g, 4Å, powder) in dry CH_2Cl_2 (20 mL) was stirred at rt for 30 min under argon. The mixture was cooled to 0 °C and then *N*-iodosuccinimide (298 mg, 1.33 mmol) and silver trifluoromethanesulfonate (212 mg, 361 μmol) were added successively. The resulting mixture was stirred at 0 °C for 30 min when TLC (Hexane–EtOAc, 2:1) showed complete conversion of the starting materials. Triethylamine was then added and the solution was filtered through a pad of Celite. The filtrate was washed with a mixture of saturated $Na_2S_2O_3$ (50 mL) and saturated $NaHCO_3$ (50 mL) solution and the aqueous layer was extracted with CH_2Cl_2 (30 mL × 3). The combined organic layer was then washed with brine (100 mL), dried over Na_2SO_4, filtered, concentrated, and the residue was purified by chromatography (Hexane–CH_2Cl_2–EtOAc, 4:1:1) to afford disaccharide **25** (680 mg, quant.) as a white amorphous solid: R_f = 0.33 (Hexane–EtOAc, 2:1); $[\alpha]_D^{25}$ = –28.5 (*c* = 0.80, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (br s, 2H, Ar), 7.75–7.71 (m, 2H, Ar), 7.51–7.48 (m, 2H, Ar), 7.34–7.33 (m, 3H, Ar), 5.58 (s, 1H, PhC*H*), 5.25 (d, 1H, *J* = 8.4 Hz, H-1), 5.23 (dd, 1H, *J* = 10.1, 3.6 Hz, H-3′), 4.81 (t, 1H, *J* = 10.1 Hz, H-4′), 4.71 (dd, 1H, *J* = 3.6, 1.5 Hz, H-2′), 4.61 (dd, 1H, *J* = 10.3, 9.0 Hz, H-3), 4.52 (d, 1H, *J* = 1.5 Hz, H-1′), 4.42 (dd, 1H, *J* = 10.6, 4.9 Hz, H-6a), 4.30 (dd, 1H, *J* = 10.3, 8.4 Hz, H-2), 4.01 (dq, 1H, *J* = 10.0, 6.3 Hz, H-5′), 3.90 (ddd, 1H, *J* = 10.7, 9.8, 5.5 Hz, OC*H*$_2$CH$_2$TMS), 3.85 (t, 1H, *J* = 10.3 Hz,

H-6b), 3.73 (t, 1H, J = 9.3 Hz, H-4), 3.67 (td, 1H, J = 9.7, 4.7 Hz, H-5), 3.48 (td, 1H, J = 10.1, 6.6 Hz, OCH_2CH$_2$TMS), 1.94 (s, 3H, CH_3CO), 1.90 (s, 3H, CH_3CO), 1.78 (s, 3H, CH_3CO), 0.79 (ddd, 1H, J = 13.9, 10.8, 6.5 Hz, OCH$_2$CH_2TMS), 0.70 (ddd, 1H, J = 13.9, 10.4, 5.5 Hz, OCH$_2$CH_2TMS), 0.59 (d, 3H, J = 6.3 Hz, H-6′), –0.14 (s, 9H, Si(CH_3)$_3$) ppm; ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.1 (CH$_3$*C*O), 169.8 (CH$_3$*C*O), 169.5 (CH$_3$*C*O), 137.3 (Ar), 134.2 (Ar), 131.8 (Ar), 129.3 (Ar), 128.3 (Ar), 126.6 (Ar), 123.8 (Ar), 102.2 (Ph*C*H), 98.4 (*C*-1), 97.5 (*C*-1′, $J_{C\text{-}H}$ = 172.4 Hz), 80.6 (*C*-4), 74.8 (*C*-3), 71.4 (*C*-4′), 70.4 (*C*-2′), 69.0 (*C*-6), 68.5 (*C*-3′), 67.6 (O*C*H$_2$CH$_2$TMS), 66.6 (*C*-5), 66.5 (*C*-5′), 56.6 (*C*-2), 20.9 (*C*H$_3$CO), 20.8 (*C*H$_3$CO), 20.7 (*C*H$_3$CO), 18.0 (OCH$_2$*C*H$_2$TMS), 16.6 (*C*-6′), –1.4 (Si(*C*H$_3$)$_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{38}H_{51}N_2O_{14}Si$ $[M+NH_4]^+$ 787.3104. Found: 787.3110; $C_{38}H_{47}NNaO_{14}Si$ $[M+Na]^+$ 792.2658. Found: 792.2670.

2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-deoxy-2-phthal-imido-β-D-glucopyranoside (26): To a stirred solution of **25** (627 mg, 815 μmol) in a mixture of CH$_3$CN (30 mL) and CH$_3$OH (3 mL) was added *p*-toluenesulfonic acid monohydrate (465 mg, 2.44 mmol) in one portion at rt. After stirring for 4 h, triethylamine was added and then the solution was concentrated. The resulting crude residue was purified by chromatography (Hexane–EtOAc, 2:3) to afford disaccharide **26** (460 mg, 83%) as a white foam: R_f = 0.39 (Hexane–EtOAc, 2:3); $[\alpha]_D^{25}$ = +13.0 (c = 0.73, CH$_2$Cl$_2$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.88 (br s, 2H, Ar), 7.75–7.72 (m, 2H, Ar), 5.22 (d, 1H, J = 8.4 Hz, H-1), 5.08 (dd, 1H, J = 9.8, 3.3 Hz, H-3′), 4.99 (t, 1H, J = 9.7 Hz, H-4′), 4.73 (d, 1H, J = 2.2 Hz, H-1′), 4.70 (t, 1H, J = 2.4 Hz, H-2′), 4.33 (dd, 1H, J = 10.8, 8.3 Hz, H-3), 4.22 (dd, 1H, J = 10.8, 8.4 Hz, H-2), 4.10 (dq, 1H, J = 8.9, 6.3 Hz, H-5′), 3.98 (dt, 1H, J = 11.8, 4.3 Hz, H-6a), 3.92 (td, 1H, J = 10.3, 5.5 Hz, OCH_2CH$_2$TMS), 3.87 (ddd, 1H, J = 11.8, 7.4, 4.6 Hz, H-6b), 3.81 (d, 1H, J = 2.1

Hz, 4-O*H*), 3.65 (td, 1H, *J* = 8.9, 2.1 Hz, H-4), 3.54–3.49 (m, 2H, H-5, OC*H*$_2$CH$_2$TMS), 2.10 (t, 1H, *J* = 7.2 Hz, 6-O*H*), 2.03 (s, 3H, C*H*$_3$CO), 1.93 (s, 3H, C*H*$_3$CO), 1.84 (s, 3H, C*H*$_3$CO), 1.23 (d, 3H, *J* = 6.3 Hz, H-6′), 0.83 (ddd, 1H, *J* = 14.1, 10.6, 6.7 Hz, OCH$_2$C*H*$_2$TMS), 0.74 (ddd, 1H, *J* = 14.1, 10.2, 5.6 Hz, OCH$_2$C*H*$_2$TMS), –0.13 (s, 9H, Si(C*H*$_3$)$_3$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0 (CH$_3$*C*O), 169.7 (CH$_3$*C*O), 169.3 (CH$_3$*C*O), 134.2 (Ar), 131.7 (Ar), 123.8 (Ar), 99.2 (*C*-1′), 98.0 (*C*-1), 83.3 (*C*-3), 75.3 (*C*-5), 71.2 (*C*-4), 70.7 (*C*-4′), 69.7 (*C*-2′), 68.5 (*C*-3′), 68.2 (*C*-5′), 67.4 (O*C*H$_2$CH$_2$TMS), 62.7 (*C*-6), 54.9 (*C*-2), 20.9 (*C*H$_3$CO), 20.7 (*C*H$_3$CO), 20.6 (*C*H$_3$CO), 18.0 (OCH$_2$*C*H$_2$TMS), 17.6 (*C*-6′), –1.4 (Si(*C*H$_3$)$_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{31}H_{47}N_2O_{14}Si$ $[M+NH_4]^+$ 699.2791. Found: 699.2794; $C_{31}H_{43}NNaO_{14}Si$ $[M+Na]^+$ 704.2345. Found: 704.2344.

2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (27): A solution of **26** (460 mg, 675 μmol) in a mixture of ethylenediamine (6.75 mL) and ethanol (27 mL) was heated at reflux overnight. The reaction mixture was cooled to rt and co-concentrated with dry toluene three times. The residue was dissolved in pyridine (10 mL), acetic anhydride (5 mL) was added dropwise at 0 °C and the resulting solution was allowed to warm to rt and stirred for 48 h. After the excess acetic anhydride was quenched by the addition of CH_3OH (5 mL) at 0 °C, the mixture was concentrated. The residue was dissolved in CH_2Cl_2 (50 mL), washed with HCl (1 M) solution and then the aqueous layer was extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was washed with saturated $NaHCO_3$ (aq.) solution, dried over Na_2SO_4, filtered, concentrated and the resulting residue was purified by chromatography (Hexane–EtOAc, 2:3) to give disaccharide **27** (413 mg, 90%) as a white foam: R_f = 0.36 (Hexane–EtOAc, 2:3); $[\alpha]_D^{25}$ = +7.7 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.09 (d, 1H, *J* =

6.8 Hz, N*H*), 5.20–5.14 (m, 3H, H-1, H-2′, H-3′), 5.02 (t, 1H, *J* = 10.0 Hz, H-4′), 4.96 (dd, 1H, *J* = 10.1, 9.0 Hz, H-4), 4.72 (d, 1H, *J* = 2.0 Hz, H-1′), 4.55 (dd, 1H, *J* = 10.1, 9.1 Hz, H-3), 4.24 (dd, 1H, *J* = 12.4, 5.1 Hz, H-6a), 4.06 (dd, 1H, *J* = 12.4, 2.2 Hz, H-6b), 3.94 (ddd, 1H, *J* = 10.9, 5.4, 4.2 Hz, OC*H*$_2$CH$_2$TMS), 3.87 (dq, 1H, *J* = 9.8, 6.1 Hz, H-5′), 3.63 (ddd, 1H, *J* = 10.2, 5.1, 2.5 Hz, H-5), 3.56 (ddd, 1H, *J* = 10.6, 9.7, 6.4 Hz, OC*H*$_2$CH$_2$TMS), 2.94 (ddd, 1H, *J* = 10.1, 8.2, 6.8 Hz, H-2), 2.10 (s, 3H, C*H*$_3$CO), 2.062 (s, 3H, C*H*$_3$CO), 2.057 (s, 3H, C*H*$_3$CO), 2.02 (s, 3H, C*H*$_3$CO), 1.99 (s, 3H, C*H*$_3$CO), 1.96 (s, 3H, C*H*$_3$CO), 1.13 (d, 3H, *J* = 6.2 Hz, H-6′), 0.99–0.93 (m, 1H, OCH$_2$C*H*$_2$TMS), 0.91–0.79 (m, 1H, OCH$_2$C*H*$_2$TMS), 0.01 (s, 9H, Si(C*H*$_3$)$_3$) ppm; ^{13}C NMR (175 MHz, CDCl$_3$) δ 171.8 (CH$_3$*C*O), 170.9 (CH$_3$*C*O), 170.6 (CH$_3$*C*O), 170.3 (CH$_3$*C*O), 170.0 (CH$_3$*C*O), 169.8 (CH$_3$*C*O), 100.5 (*C*-1), 98.4 (*C*-1′), 80.4 (*C*-3), 71.5 (*C*-5), 70.6 (*C*-4), 70.4 (*C*-4′), 69.6 (*C*-3′), 69.5 (*C*-2′), 68.0 (*C*-5′), 67.5 (O*C*H$_2$CH$_2$TMS), 62.5 (*C*-6), 59.4 (*C*-2), 23.6 (*C*H$_3$CO), 21.3 (*C*H$_3$CO), 21.0 (*C*H$_3$CO), 20.9 (*C*H$_3$CO), 18.1 (*C*-6′), 17.5 (OCH$_2$*C*H$_2$TMS), –1.4 (Si(*C*H$_3$)$_3$) ppm; HRMS (ESI): *m*/*z* Calcd for C$_{29}$H$_{47}$NNaO$_{15}$Si [M+Na]$^+$ 700.2600. Found: 700.2607.

2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-D-glucopyranose (28): To a solution of **27** (412 mg, 608 μmol) in CH$_2$Cl$_2$ (10 mL), at 0 °C was added trifluoroacetic acid (5.0 mL) dropwise. The solution was allowed to warm to rt and the mixture was stirred for 30 min. The mixture was concentrated under vacuum and the residue was purified by chromatography (EtOAc) to afford the desired hemiacetal **28** (351 mg, quant.) as a white amorphous solid: R_f = 0.16 (EtOAc); $[\alpha]_D^{25}$ = +26.3 (*c* = 1.00, CHCl$_3$); α-anomer (major): ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.03 (d, 1H, *J* = 9.3 Hz, N*H*), 5.21 (br s, 1H, H-1), 5.12–5.05 (m, 3H, H-4, H-2′, H-3′), 4.99 (t, 1H, *J* = 9.8 Hz, H-4′), 4.79 (d, 1H, *J* = 1.2 Hz, H-1′), 4.23 (dt, 1H, *J* = 9.5, 3.5 Hz, H-2), 4.15 (dd, 1H, *J*

= 12.0, 3.9 Hz, H-6a), 4.11–4.04 (m, 2H, H-5, H-6b), 3.94 (dd, 1H, *J* = 10.5, 9.1 Hz, H-3), 3.87 (dq, 1H, *J* = 9.8, 6.3 Hz, H-5′), 2.11 (s, 3H, C*H*$_3$CO), 2.07 (s, 3H, C*H*$_3$CO), 2.061 (s, 3H, C*H*$_3$CO), 2.059 (s, 3H, C*H*$_3$CO), 2.01 (s, 3H, C*H*$_3$CO), 1.93 (s, 3H, C*H*$_3$CO), 1.12 (d, 3H, *J* = 6.3 Hz, H-6′) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7 (CH_3CO), 171.2 (CH_3CO), 170.6 (CH_3CO), 170.2 (CH_3CO), 169.9 (CH_3CO), 169.7 (CH_3CO), 99.9 (*C*-1′), 92.0 (*C*-1), 79.9 (*C*-3), 70.7 (*C*-4′), 70.3 (*C*-2′), 70.2 (*C*-4), 69.1 (*C*-3′), 67.8 (*C*-5), 67.6 (*C*-5′), 62.4 (*C*-6), 53.2 (*C*-2), 23.3 (CH_3CO), 21.3 (CH_3CO), 21.0 (CH_3CO), 20.90 (CH_3CO), 20.88 (CH_3CO), 20.76 (CH_3CO), 17.3 (*C*-6′) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{24}H_{35}NNaO_{15}$ $[M+Na]^+$ 600.1897. Found: 600.1899.

2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranose 1-dibenzylphosphate (29): Hemiacetal **28** (99 mg, 171 μmol) was dissolved in dry CH_2Cl_2 (10 mL), 1,2,4-triazole (177 mg, 2.57 mmol) was added in one portion, and the solution was cooled to 0 °C. Dibenzyl *N*,*N*-diisopropylphosphoramidite (576 μL, 1.71 mmol) was added dropwise and the mixture was stirred at rt for a further 3 h. Then, the mixture was cooled to –78 °C and 3-chloroperbenzoic acid (1.08 g, 3.43 mmol, 55%) was added in one portion. The reaction mixture was then allowed to warm to rt and stirred for 30 min, before the addition of a mixture of saturated $Na_2S_2O_3$ (10 mL) and saturated $NaHCO_3$ (10 mL) solution. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 3) and EtOAc (15 mL), The combined organic layer was then dried over Na_2SO_4, filtered, concentrated, and the resulting residue was purified by chromatography (EtOAc) to afford **29** (74 mg, 52%) as a colorless syrup: R_f = 0.45 (EtOAc); $[\alpha]_D^{25}$ = +26.2 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.31 (m, 10H, Ar), 5.59 (dd, 1H, *J* = 5.7, 3.3 Hz, H-1), 5.56 (d, 1H, *J* = 9.7 Hz, N*H*), 5.12–4.99 (m, 7H, H-4, H-2′, H-3′, PhC*H*$_2$O × 2), 4.99 (t,

1H, J = 9.6 Hz, H-4′), 4.79 (d, 1H, J = 1.6 Hz, H-1′), 4.42 (tt, 1H, J = 9.7, 3.2 Hz, H-2), 4.05 (dd, 1H, J = 12.5, 4.2 Hz, H-6a), 3.90–3.86 (m, 2H, H-5, H-6b), 3.87 (dq, 1H, J = 9.5, 6.2 Hz, H-5′), 3.94 (dd, 1H, J = 10.5, 9.2 Hz, H-3), 2.13 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO), 1.88 (s, 3H, CH_3CO), 1.12 (d, 3H, J = 6.2 Hz, H-6′) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8 (CH$_3$*C*O), 170.7 (CH$_3$*C*O), 170.4 (CH$_3$*C*O), 170.1 (CH$_3$*C*O), 169.6 (CH$_3$*C*O), 169.4 (CH$_3$*C*O), 129.1 (Ar), 128.94 (Ar), 128.92 (Ar), 128.3 (Ar), 128.2 (Ar), 99.7 (*C*-1′), 97.0 (d, J = 7.0 Hz, *C*-1), 79.0 (*C*-3), 70.6 (*C*-5), 70.2 (*C*-4′), 70.1 (Ph*C*H$_2$O), 70.04 (Ph*C*H$_2$O), 70.01 (*C*-3′), 69.2 (*C*-4), 68.9 (*C*-2′), 67.7 (*C*-5′), 61.5 (*C*-6), 51.9 (d, J = 7.5 Hz, *C*-2), 23.0 (*C*H$_3$CO), 21.1 (*C*H$_3$CO), 21.0 (*C*H$_3$CO), 20.9 (*C*H$_3$CO), 20.74 (*C*H$_3$CO), 20.71 (*C*H$_3$CO), 17.3 (*C*-6′) ppm; ^{31}P NMR (162 MHz, $CDCl_3$) δ –2.30 ppm; HRMS (ESI): m/z Calcd for $C_{38}H_{48}NNaO_{18}P$ $[M+Na]^+$ 860.2501. Found: 860.2502.

2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl 1-phosphate triethylammonium salt (30): Phosphate **29** (15.6 mg, 18.6 μmol) was dissolved in dry THF (2 mL) and palladium on carbon (20 mg, dry, 5%) was added in one portion. The flask was evacuated under vacuum and re-charged with hydrogen three times while cooling on dry ice. After stirring at rt overnight, the reaction mixture was filtered with Celite, the filtrate was neutralized with triethylamine, and then the solvent was removed under vacuum to give **30** (13 mg, 92%) as a white amorphous solid: R_f = 0.38 (EtOAc–2-propanol–H_2O, 2:2:1); $[\alpha]_D^{25}$ = +31.6 (c = 1.00, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 5.49 (br s, 1H, H-1), 5.18 (dd, 1H, J = 3.0, 2.1 Hz, H-2′), 5.13–5.08 (m, 2H, H-4, H-3′), 4.99–4.95 (m, 2H, H-1′, H-4′), 4.26–4.22 (m, 2H, H-2, H-6a), 4.16 (br d, 1H, J = 9.9, H-5), 4.10–4.03 (m, 2H, H-3, H-6b), 3.86 (dq, 1H, J = 9.8, 6.1 Hz, H-5′), 3.09 (q, 6H, J = 7.2 Hz,

N(CH_2CH_3)$_3$), 2.13 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO), 1.27 (t, 9H, J = 7.2 Hz, N(CH_2CH_3)$_3$), 1.14 (d, 3H, J = 6.1 Hz, H-6′) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 174.1 (CH_3CO), 172.6 (CH_3CO), 171.7 (CH_3CO), 171.6 (CH_3CO), 171.5 (CH_3CO), 171.2 (CH_3CO), 100.8 (*C*-1′), 95.8 (d, J = 3.3 Hz, *C*-1), 79.6 (*C*-3), 72.0 (*C*-4′), 71.1 (*C*-2′), 71.0 (*C*-4), 70.4 (*C*-3′), 70.3 (*C*-5), 68.6 (*C*-5′), 63.0 (*C*-6), 54.3 (d, J = 6.5 Hz, *C*-2), 47.4 (N(CH_2CH_3)$_3$), 22.9 (CH_3CO), 21.4 (CH_3CO), 20.72 (CH_3CO), 20.66 (CH_3CO), 20.64 (CH_3CO), 20.55 (CH_3CO), 17.7 (*C*-6′), 9.4 (N(CH_2CH_3)$_3$) ppm; ^{31}P NMR (162 MHz, CD_3OD) δ –0.43 ppm; HRMS (ESI): *m*/*z* Calcd for $C_{24}H_{36}NNaO_{18}P$ $[M+Na]^+$ 680.1562. Found: 680.1565.

2-(Trimethylsilyl)ethyl 4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (32): A mixture of 2-(trimethylsilyl)ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**12**) (598 mg, 1.2 mmol), *p*-tolyl 4-*O*-acetyl-2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (**31**) (508 mg, 1.44 mmol) and molecular sieves (1.2 g, 4Å, powder) in dry CH_2Cl_2 (12 mL) was stirred at rt for 30 min under argon. The solution was then cooled to 0 °C before *N*-iodosuccinimide (419 mg, 1.86 mmol) and lanthanum(III) trifluoromethanesulfonate (212 mg, 361 μmol) were added successively. The resulting mixture was stirred at 0 °C for 45 min when TLC (Hexane–EtOAc, 4:1) showed complete conversion of the starting materials. The solution was filtered through a pad of Celite and the filtrate was washed with a mixture of saturated $Na_2S_2O_3$ (20 mL) solution and saturated $NaHCO_3$ (20 mL) solution. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL), the combined organic layer was then washed with brine (100 mL), dried over Na_2SO_4, filtered, concentrated, the resulting residue was purified by chromatography (Hexane–EtOAc, 4:1) to afford disaccharide **32** (726 mg, 83%) as a white foam: R_f =

0.32 (Hexane–EtOAc, 4:1); $[\alpha]_D^{25}$ = –30.5 (*c* = 1.93, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90–7.85 (m, 2H, Ar), 7.78–7.73 (m, 2H, Ar), 7.51–7.47 (m, 2H, Ar), 7.34–7.32 (m, 3H, Ar), 5.54 (s, 1H, PhC*H*), 5.32 (d, 1H, *J* = 8.5 Hz, H-1), 4.72 (s, 1H, H-1′), 4.63 (dd, 1H, *J* = 10.2, 8.9 Hz, H-3), 4.60 (dd, 1H, *J* = 10.3, 7.9 Hz, H-4′), 4.42 (dd, 1H, *J* = 10.2, 3.9 Hz, H-6a), 4.27 (dd, 1H, *J* = 10.2, 8.5 Hz, H-2), 4.00 (dd, 1H, *J* = 7.8, 5.4 Hz, H-3′), 3.92 (ddd, 1H, *J* = 10.6, 9.8, 5.4 Hz, OC*H*$_2$CH$_2$TMS), 3.86–3.75 (m, 3H, H-6b, H-2′, H-5′), 3.71–3.64 (m, 2H, H-4, H-5), 3.51 (td, 1H, *J* = 10.0, 6.6 Hz, OC*H*$_2$CH$_2$TMS), 2.00 (s, 3H, C*H*$_3$CO), 1.33 (s, 3H, C*H*$_3$C), 1.05 (s, 3H, C*H*$_3$C), 0.81 (ddd, 1H, *J* = 14.0, 10.7, 6.6 Hz, OCH$_2$C*H*$_2$TMS), 0.72 (ddd, 1H, *J* = 14.0, 10.3, 5.6 Hz, OCH$_2$C*H*$_2$TMS), 0.56 (d, 3H, *J* = 6.2 Hz, H-6′), –0.13 (s, 9H, Si(C*H*$_3$)$_3$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0, 137.2, 134.5, 131.5, 129.3, 128.2, 126.6, 123.7, 109.5 (*C*(CH$_3$)$_2$), 102.2 (PhCH), 98.3 (*C*-1), 97.6 (*C*-1′, $J_{C\text{-}H}$ = 169.0 Hz), 80.8 (*C*-4), 75.9 (*C*-2′), 75.6 (*C*-3′), 74.3 (*C*-3), 74.2 (*C*-4′), 68.9 (*C*-6), 67.6 (OCH$_2$CH$_2$TMS), 66.5 (*C*-5), 64.6 (*C*-5′), 57.0 (*C*-2), 27.5 (*C*H$_3$C), 26.2 (*C*H$_3$C), 21.1 (*C*H$_3$CO), 18.0 (OCH$_2$*C*H$_2$TMS), 16.2 (*C*-6′), –1.4 (Si(*C*H$_3$)$_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{37}H_{51}N_2O_{12}Si$ $[M+NH_4]^+$ 743.3206. Found: 743.3215; $C_{37}H_{47}NNaO_{12}Si$ $[M+Na]^+$ 748.2760. Found: 748.2753.

2-(Trimethylsilyl)ethyl 2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (33): To a stirred solution of disaccharide **32** (719 mg, 991 μmol) in CH_3OH (10 mL) was added a solution of sodium methoxide (1 mL, 0.5 M in CH_3OH). The reaction was stirred at rt for 2 h and then neutralized by the addition of Amberlite IR-120 resin (H^+ form). The solution was filtered and the filtrate was concentrated to give alcohol **33** (650 mg, 96%) as a white foam, which was used in the next step without further purification: R_f = 0.45 (Hexane–EtOAc, 2:1); $[\alpha]_D^{25}$ = –40.8 (*c* = 1.46, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87–7.84 (m,

2H, Ar), 7.75–7.72 (m, 2H, Ar), 7.50–7.47 (m, 2H, Ar), 7.36–7.30 (m, 3H, Ar), 5.53 (s, 1H, PhC*H*), 5.32 (d, 1H, *J* = 8.6 Hz, H-1), 4.68 (s, 1H, H-1′), 4.60 (app dd, 1H, *J* = 10.2, 9.0 Hz, H-3), 4.41 (dd, 1H, *J* = 10.4, 4.1 Hz, H-6a), 4.26 (dd, 1H, *J* = 10.3, 8.6 Hz, H-2), 3.92 (td, 1H, *J* = 10.5, 5.4 Hz, OC*H*$_2$CH$_2$TMS), 3.88 (dd, 1H, *J* = 7.5, 5.8 Hz, H-3′), 3.83 (app t, 1H, *J* = 10.1 Hz, H-6b), 3.78 (d, 1H, *J* = 5.8 Hz, H-2′), 3.72–3.65 (m, 3H, H-4, H-5, H-5′), 3.50 (td, 1H, *J* = 10.5, 6.7 Hz, OC*H*$_2$CH$_2$TMS), 3.11 (ddd, 1H, *J* = 9.8, 7.5, 4.2 Hz, H-4′), 2.15 (d, 1H, *J* = 4.3 Hz, 4′-O*H*), 1.25 (s, 3H, C*H*$_3$C), 1.03 (s, 3H, C*H*$_3$C), 0.81 (ddd, 1H, *J* = 14.0, 10.7, 6.6 Hz, OCH$_2$C*H*$_2$TMS), 0.72 (ddd, 1H, *J* = 14.0, 10.2, 5.5 Hz, OCH$_2$C*H*$_2$TMS), 0.70 (d, 3H, *J* = 6.3 Hz, H-6′), – 0.14 (s, 9H, Si(C*H*$_3$)$_3$) ppm; ^{13}C NMR (125 MHz, CDCl$_3$) δ 137.2, 134.4, 131.6, 129.3, 128.3, 126.6, 123.7, 109.2 (*C*(CH$_3$)$_2$), 102.2 (PhCH), 98.3 (*C*-1), 98.0 (*C*-1′), 80.8 (*C*-4), 78.2 (*C*-3′), 75.9 (*C*-2′), 74.6 (*C*-3), 74.5 (*C*-4′), 68.9 (*C*-6), 67.5 (OCH$_2$CH$_2$TMS), 66.6 (*C*-5′), 66.4 (*C*-5), 57.0 (*C*-2), 27.8 (*C*H$_3$C), 25.9 (*C*H$_3$C), 18.0 (OCH$_2$*C*H$_2$TMS), 16.7 (*C*-6′), –1.4 (Si(*C*H$_3$)$_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{35}H_{46}NO_{11}Si$ $[M+H]^+$ 684.2835. Found: 684.2832; $C_{35}H_{49}N_2O_{11}Si$ $[M+NH_4]^+$ 701.3100. Found: 701.3109; $C_{35}H_{45}NNaO_{11}Si$ $[M+Na]^+$ 706.2654. Found: 706.2663.

2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (34): A mixture of disaccharide acceptor **33** (183 mg, 268 μmol), *p*-tolyl 2,3,5,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (**13**) (207 mg, 295 μmol) and molecular sieves (1.34 g, 4Å, powder) in dry CH_2Cl_2 (13.4 mL) were stirred at rt for 30 min under argon. The mixture was then cooled to 0 °C before *N*-iodosuccinimide (78.3 mg, 348 μmol) and silver trifluoromethanesulfonate (13.8 mg, 53.6 μmol) were added successively. The resulting mixture was

stirred at 0 °C for 30 min when TLC (Hexane–EtOAc, 2:1) showed complete conversion of the starting materials. Triethylamine was added and the solution was filtered through a pad of Celite. The filtrate was diluted with CH_2Cl_2 (20 mL), washed with a mixture of saturated $Na_2S_2O_3$ (25 mL) and saturated $NaHCO_3$ (25 mL) solution, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by chromatography (Hexane–EtOAc, 3:1) to afford trisaccharide **34** (328 mg, 97%) as a white foam: R_f = 0.60 (Hexane–EtOAc, 2:1); $[\alpha]_D^{25}$ = –20.6 (c = 1.50, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08–8.03 (m, 2H, Ar), 8.00–7.98 (m, 2H, Ar), 7.92–7.90 (m, 4H, Ar), 7.88–7.85 (m, 2H, Ar), 7.76–7.73 (m, 2H, Ar), 7.60–7.57 (m, 1H, Ar), 7.53–7.46 (m, 5H, Ar), 7.42–7.39 (m, 2H, Ar), 7.35–7.26 (m, 6H, Ar), 7.21–7.18 (m, 2H, Ar), 7.13–7.10 (m, 1H, Ar), 6.00 (app q, 1H, J = 5.0 Hz, H-5″), 5.69 (s, 1H, H-1″), 5.56 (s, 1H, PhC*H*), 5.55 (d, 1H, J = 6.5 Hz, H-3″), 5.47 (s, 1H, H-2″), 5.31 (d, 1H, J = 8.6 Hz, H-1), 4.74 (s, 1H, H-1′), 4.70–4.62 (m, 3H, H-6″a, H-6″b, H-3), 4.55 (t, 1H, J = 4.6 Hz, H-4″), 4.43 (dd, 1H, J = 11.1, 4.2 Hz, H-6a), 4.29 (dd, 1H, J = 10.2, 8.7 Hz, H-2), 4.15 (dd, 1H, J = 7.2, 5.9 Hz, H-3′), 3.93 (td, 1H, J = 10.2, 5.5 Hz, OC*H*$_2$CH$_2$TMS), 3.86–3.82 (m, 2H, H-6b, H-5′), 3.77 (d, 1H, J = 5.6 Hz, H-2′), 3.71–3.65 (m, 2H, H-4, H-5), 3.52 (td, 1H, J = 10.2, 6.7 Hz, OC*H*$_2$CH$_2$TMS), 3.44 (dd, 1H, J = 10.1, 7.6 Hz, H-4′), 1.27 (s, 3H, C*H*$_3$C), 0.98 (s, 3H, C*H*$_3$C), 0.83 (ddd, 1H, J = 14.0, 10.7, 6.7 Hz, OCH$_2$C*H*$_2$TMS), 0.79 (d, 3H, J = 6.1 Hz, H-6′), 0.73 (ddd, 1H, J = 14.0, 10.3, 5.5 Hz, OCH$_2$C*H*$_2$TMS), –0.12 (s, 9H, Si(C*H*$_3$)$_3$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1 (Ph*C*O), 165.8 (Ph*C*O × 2), 165.4 (Ph*C*O), 137.1 (Ar), 134.5 (Ar), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 131.5 (Ar), 130.04 (Ar), 130.01 (Ar), 130.0 (Ar), 129.8 (Ar), 129.60 (Ar), 129.58 (Ar), 129.2 (Ar), 129.15 (Ar), 129.13 (Ar), 128.6 (Ar), 128.48 (Ar), 128.47 (Ar), 128.45 (Ar), 128.2 (Ar),

126.4 (Ar), 123.7 (Ar), 109.3 (*C*(CH_3)$_2$), 104.0 (*C*-1″), 102.0 (Ph*C*H), 98.3 (*C*-1), 97.8 (*C*-1′), 82.3 (*C*-2″), 81.5 (*C*-4″), 80.8 (*C*-4), 78.2 (*C*-3″), 77.8 (*C*-3′), 76.3 (*C*-4′), 76.1 (*C*-2′), 74.5 (*C*-3), 70.4 (*C*-5″), 68.9 (*C*-6), 67.6 (O*C*$H_2$$CH_2$TMS), 66.6 (*C*-5), 64.8 (*C*-5′), 63.4 (*C*-6″), 57.0 (*C*-2), 27.7 (*C*H_3C), 26.1 (*C*H_3C), 18.0 (OCH$_2$*C*H_2TMS), 17.2 (*C*-6′), –1.4 (Si(*C*H_3)$_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{69}H_{75}N_2O_{20}Si$ $[M+NH_4]^+$ 1279.4677. Found: 1279.4684; $C_{69}H_{71}NNaO_{20}Si$ $[M+Na]^+$ 1284.4231. Found: 1284.4235.

2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-deoxy-2-phthalimido-β-D-glucopyranoside (35): Trisaccharide **34** (778 mg, 617 μmol) was dissolved in a solution of acetic acid (20 mL, 80% in water) and heated at 80 °C for 2 h. After cooling to rt, the reaction mixture was co-concentrated with dry toluene three times. The crude yellow solid was purified by chromatography (CH_2Cl_2–EtOAc, 3:1) to afford trisaccharide **35** (581 mg, 83%) as a white amorphous solid: R_f = 0.43 (CH_2Cl_2–EtOAc, 2:1); $[\alpha]_D^{25}$ = +5.6 (*c* = 1.32, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07–8.02 (m, 4H, Ar), 7.98–7.96 (m, 2H, Ar), 7.91–7.87 (m, 4H, Ar), 7.78–7.74 (m, 2H, Ar), 7.60–7.52 (m, 4H, Ar), 7.45–7.38 (m, 4H, Ar), 7.37–7.30 (m, 4H, Ar), 5.97 (dt, 1H, *J* = 6.5, 4.5 Hz, H-5″), 5.74 (dd, 1H, *J* = 6.4, 2.0 Hz, H-3″), 5.52 (s, 1H, H-1″), 5.32 (d, 1H, *J* = 2.1 Hz, H-2″), 5.22 (d, 1H, *J* = 8.5 Hz, H-1), 4.79 (d, 1H, *J* = 1.2 Hz, H-1′), 4.76 (dd, 1H, *J* = 6.4, 4.1 Hz, H-4″), 4.73 (dd, 1H, *J* = 12.0, 4.8 Hz, H-6″a), 4.67 (dd, 1H, *J* = 12.0, 6.6 Hz, H-6″b), 4.50 (d, 1H, *J* = 1.1 Hz, 4-O*H*), 4.29 (dd, 1H, *J* = 10.8, 7.9 Hz, H-3), 4.17 (dd, 1H, *J* = 10.8, 8.5 Hz, H-2), 4.01–3.86 (m, 5H, H-6a, H-5′, H-3′, OC*H*$_2$$CH_2$TMS, H-6b), 3.80 (d, 1H, *J* = 3.7 Hz, 3′-O*H*), 3.62–3.49 (m, 5H, H-4, H-4′, H-5, OC*H*$_2$$CH_2$TMS, H-2′), 2.38 (d, 1H, *J* = 1.5 Hz, 2′-O*H*), 2.16 (dd, 1H, *J* = 7.2, 6.0 Hz, 6-O*H*), 1.33 (d, 3H, *J* = 6.3 Hz, H-6′), 0.83 (ddd, 1H, *J* = 14.0, 10.5, 6.7 Hz, OCH$_2$C*H*$_2$TMS), 0.74 (ddd, 1H, *J*

= 14.0, 10.1, 5.6 Hz, OCH_2CH_2TMS), –0.13 (s, 9H, $Si(CH_3)_3$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.3 (Ph*C*O), 166.1 (Ph*C*O), 165.8 (Ph*C*O), 165.7 (Ph*C*O), 134.5 (Ar), 134.0 (Ar), 133.9 (Ar), 133.5 (Ar), 133.4 (Ar), 131.6 (Ar), 130.2 (Ar), 130.11 (Ar), 130.09 (Ar), 129.9 (Ar), 129.6 (Ar), 129.5 (Ar), 128.8 (Ar), 128.70 (Ar), 128.67 (Ar), 128.60 (Ar), 128.57 (Ar), 128.5 (Ar), 108.1 (*C*-1″), 101.3 (*C*-1′), 97.9 (*C*-1), 85.1 (*C*-2″), 83.7 (*C*-3), 81.0 (*C*-4′), 80.7 (*C*-4″), 77.0 (*C*-3″), 75.3 (*C*-5), 71.5 (*C*-2′), 71.4 (*C*-4), 70.9 (*C*-3′), 70.4 (*C*-5″), 68.1 (*C*-5′), 67.4 (OCH_2CH_2TMS), 63.2 (*C*-6″), 62.8 (*C*-6), 55.0 (*C*-2), 18.0 (OCH_2CH_2TMS), 17.8 (*C*-6′), –1.4 ($Si(CH_3)_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{59}H_{67}N_2O_{20}Si$ $[M+NH_4]^+$ 1151.4051. Found: 1151.4049; $C_{59}H_{63}NNaO_{20}Si$ $[M+Na]^+$ 1156.3605. Found: 1156.3604.

2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (36): A solution of **35** (521 mg, 460 μmol) in a mixture of ethylenediamine (5 mL) and ethanol (20 mL) was heated at reflux overnight. The reaction mixture was cooled to rt and co-concentrated with dry toluene several times. The residue was dissolved in dry pyridine (10 mL), acetic anhydride (5 mL) was added dropwise and the resulting solution was stirred at rt for 48 h. After the excess acetic anhydride quenched by the addition of CH_3OH (5 mL) at 0 °C, the mixture was concentrated. The resulting residue was dissolved in CH_2Cl_2 (50 mL), washed with HCl (1 M) solution and the aqueous layer was extracted with CH_2Cl_2 (25 mL × 3). The combined organic layer was washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography (Hexane–EtOAc, 1:1 → 1:2 → 1:3) to give **36** (395 mg, 89%) as a white foam: R_f = 0.35 (Hexane–EtOAc, 1:3); $[\alpha]_D^{25}$ = –12.5 (*c* = 0.90, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 6.18 (d, 1H, *J* = 6.8 Hz, N*H*), 5.37 (dt, 1H, *J* = 6.8, 4.8 Hz, H-5″), 5.27 (s, 1H, H-1″), 5.22 (d, 1H, *J* = 8.3 Hz,

H-1), 5.20–5.17 (m, 2H, H-3′, H-2′), 5.01 (dd, 1H, *J* = 5.6, 1.8 Hz, H-3″), 4.95 (t, 1H, *J* = 9.7 Hz, H-4), 4.92 (d, 1H, *J* = 1.8 Hz, H-2″), 4.68 (d, 1H, *J* = 1.6 Hz, H-1′), 4.55 (t, 1H, *J* = 10.0 Hz, H-3), 4.29–4.19 (m, 4H, H-4″, H-6″a, H-6″b, H-6a), 4.08 (dd, 1H, *J* = 12.3, 2.3 Hz, H-6b), 3.95 (ddd, 1H, *J* = 11.1, 9.8, 5.4 Hz, OC*H*$_2$CH$_2$TMS), 3.83 (dq, 1H, *J* = 9.6, 6.1 Hz, H-5′), 3.67–3.62 (m, 2H, H-4′, H-5), 3.57 (dt, 1H, *J* = 11.1, 6.5 Hz, OC*H*$_2$CH$_2$TMS), 2.91 (ddd, 1H, *J* = 10.2, 8.2, 8.1 Hz, H-2), 2.12 (s, 3H, C*H*$_3$CO), 2.095 (s, 3H, C*H*$_3$CO), 2.088 (s, 3H, C*H*$_3$CO), 2.073 (s, 3H, C*H*$_3$CO), 2.069 (s, 3H, C*H*$_3$CO), 2.067 (s, 3H, C*H*$_3$CO), 2.05 (s, 3H, C*H*$_3$CO), 2.00 (s, 3H, C*H*$_3$CONH), 1.98 (s, 3H, C*H*$_3$CO), 1.23 (d, 3H, *J* = 6.2 Hz, H-6′), 0.97 (ddd, 1H, *J* = 13.9, 10.9, 6.5 Hz, OCH$_2$C*H*$_2$TMS), 0.89 (ddd, 1H, *J* = 13.9, 10.5, 5.4 Hz, OCH$_2$C*H*$_2$TMS), 0.01 (s, 9H, Si(C*H*$_3$)$_3$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9 (CH$_3$*C*ONH), 170.9 (CH$_3$*C*O), 170.6 (CH$_3$*C*O), 170.5 (CH$_3$*C*O), 170.2 (CH$_3$*C*O × 2), 170.0 (CH$_3$*C*O), 169.8 (CH$_3$*C*O), 169.4 (CH$_3$*C*O), 106.6 (*C*-1″), 100.7 (*C*-1′), 98.4 (*C*-1), 81.6 (*C*-2″), 80.8 (*C*-4″), 80.4 (*C*-3), 76.4 (*C*-3″), 75.1 (*C*-4′), 72.2 (*C*-3′), 71.4 (*C*-5), 70.8 (*C*-4), 69.8 (*C*-2′), 69.4 (*C*-5″), 68.5 (*C*-5′), 67.6 (O*C*H$_2$CH$_2$TMS), 62.5 (*C*-6), 62.4 (*C*-6″), 59.5 (*C*-2), 23.6 (*C*H$_3$CONH), 21.3 (*C*H$_3$CO), 20.99 (*C*H$_3$CO), 20.97 (*C*H$_3$CO), 20.94 (*C*H$_3$CO), 20.93 (*C*H$_3$CO), 20.83 (*C*H$_3$CO), 20.81 (*C*H$_3$CO), 20.80 (*C*H$_3$CO), 18.1 (OCH$_2$*C*H$_2$TMS), 17.8 (*C*-6′), –1.3 (Si(*C*H$_3$)$_3$) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{41}H_{64}NO_{23}Si$ $[M+H]^+$ 966.3633. Found: 966.3638; $C_{41}H_{67}N_2O_{23}Si$ $[M+NH_4]^+$ 983.3898. Found: 983.3904; $C_{41}H_{63}NNaO_{23}Si$ $[M+Na]^+$ 988.3452. Found: 988.3443.

2,3,5,6-Tetra-*O*-acetyl-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranose (37): A solution of **36** (130 mg, 155 μmol) in dry CH_2Cl_2 (8.0 mL) was cooled to 0 °C before trifluoroacetic acid (4.0 mL) was added dropwise. The solution was allowed to warm to rt and stirred for 30 min. Then the mixture was

concentrated and the resulting crude residue was purified by chromatography (EtOAc) to afford the desired hemiacetal **37** (113.6 mg, 97%) as a white amorphous solid: R_f = 0.41 (CH_2Cl_2–CH_3OH, 20:1); $[\alpha]_D^{25}$ = +0.4 (*c* = 0.80, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.23 (d, 1H, *J* = 9.0 Hz, N*H*), 5.35 (dt, 1H, *J* = 5.8, 4.7 Hz, H-5″), 5.25 (s, 1H, H-1″), 5.23 (t, 1H, *J* = 3.1 Hz, H-1), 5.10 (t, 1H, *J* = 2.0 Hz, H-2′), 5.07 (dd, 1H, *J* = 9.5, 3.2 Hz, H-3′), 5.05 (t, 1H, *J* = 9.7 Hz, H-4), 4.98 (dd, 1H, *J* = 5.3, 1.4 Hz, H-3″), 4.96 (br s, 1H, 1-O*H*), 4.89 (app s, 1H, H-2″), 4.74 (app s, 1H, H-1′), 4.27–4.24 (m, 2H, H-6″a, H-4″), 4.21–4.03 (m, 5H, H-6″b, H-6a, H-2, H-5, H-6b), 3.99 (t, 1H, *J* = 9.2 Hz, H-3), 3.81 (dq, 1H, *J* = 9.5, 6.1 Hz, H-5′), 3.61 (t, 1H, *J* = 9.7 Hz, H-4′), 2.10 (s, 3H, C*H*$_3$CO), 2.09 (s, 6H, C*H*$_3$CO × 2), 2.07 (s, 3H, C*H*$_3$CONH), 2.06 (s, 3H, C*H*$_3$CO), 2.05 (s, 3H, C*H*$_3$CO), 2.04 (s, 3H, C*H*$_3$CO), 2.03 (s, 3H, C*H*$_3$CO), 1.95 (s, 3H, C*H*$_3$CO), 1.20 (d, 3H, *J* = 6.1 Hz, H-6′) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9 (CH_3*C*ONH), 171.1 (CH_3*C*O), 170.6 (CH_3*C*O), 170.5 (CH_3*C*O), 170.2 (CH_3*C*O), 170.1 (CH_3*C*O), 169.9 (CH_3*C*O), 169.7 (CH_3*C*O), 169.3 (CH_3*C*O), 106.6 (*C*-1″), 99.9 (*C*-1′), 92.2 (*C*-1), 81.4 (*C*-2″), 80.9 (*C*-4″), 79.3 (*C*-3), 76.4 (*C*-3″), 75.5 (*C*-4′), 71.8 (*C*-3′), 70.5 (*C*-4), 70.3 (*C*-2′), 69.4 (*C*-5″), 68.1 (*C*-5′), 67.8 (*C*-5), 62.4 (*C*-6″), 62.3 (*C*-6), 53.5 (*C*-2), 23.2 (*C*H_3CONH), 21.3 (*C*H_3CO), 21.0 (*C*H_3CO), 20.91 (*C*H_3CO), 20.89 (*C*H_3CO), 20.82 (*C*H_3CO), 20.79 (*C*H_3CO), 20.77 (*C*H_3CO), 20.7 (*C*H_3CO), 17.7 (*C*-6′) ppm; HRMS (ESI): *m*/*z* Calcd for $C_{36}H_{55}N_2O_{23}$ $[M+NH_4]^+$ 883.3190. Found: 883.3193; $C_{36}H_{51}NNaO_{23}$ $[M+Na]^+$ 888.2744. Found: 888.2749.

2,3,5,6-Tetra-*O*-acetyl-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranose 1-dibenzylphosphate (38): Hemiacetal **37** (103.5 mg, 120 μmol) was dissolved in dry CH_2Cl_2 (5 mL), 1,2,4-triazole (124 mg, 1.80 mmol) was added in one portion and the reaction mixture was cooled to 0 °C. After 5 min, dibenzyl

N,*N*-diisopropylphosphoramidite (403 μL, 1.20 mmol) was added dropwise and the mixture was stirred at rt for a further 3 h. The mixture was cooled to –78 °C and 3-chloroperbenzoic acid (565 mg, 1.80 mmol, 55%) was added in one portion. The reaction mixture was then allowed to warm to rt, After stirring for 30 min, quenched with a mixture of saturated $Na_2S_2O_3$ (5 mL) and saturated $NaHCO_3$ (5 mL) solution. The organic layer was separated, the aqueous layer was extracted with CH_2Cl_2 (10 mL × 3) and EtOAc (15 mL), the combined organic layer was then dried over Na_2SO_4, filtered, concentrated, the residue was purified by chromatography (EtOAc) to afford **38** (81 mg, 60%) as a white foam: R_f = 0.40 (EtOAc); $[\alpha]_D^{25}$ = +2.4 (*c* = 0.6, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.33 (m, 10H, Ar), 5.62 (dd, 1H, *J* = 5.6, 3.3 Hz, H-1), 5.51 (d, 1H, *J* = 9.4 Hz, N*H*), 5.37 (dt, 1H, *J* = 6.9, 4.7 Hz, H-5″), 5.26 (s, 1H, H-1″), 5.12–4.99 (m, 8H, H-2′, H-4, H-3′, H-3″, PhCH_2O × 2), 4.90 (d, 1H, *J* = 1.3 Hz, H-2″), 4.70 (d, 1H, *J* = 1.3 Hz, H-1′), 4.41 (tt, 1H, *J* = 10.2, 3.3 Hz, H-2), 4.29–4.24 (m, 2H, H-6″a, H-4″), 4.20 (dd, 1H, *J* = 11.7, 7.0 Hz, H-6″b), 4.05 (dd, 1H, *J* = 12.7, 4.2 Hz, H-6a), 3.91–3.87 (m, 2H, H-6b, H-5), 3.78 (dq, 1H, *J* = 12.3, 6.2 Hz, H-5′), 3.65 (t, 1H, *J* = 9.5 Hz, H-3), 3.61 (t, 1H, *J* = 9.8 Hz, H-4′), 2.13 (s, 3H, CH_3CO), 2.11 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO × 2), 2.00 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.89 (s, 3H, CH_3CONH), 1.21 (d, 3H, *J* = 6.2 Hz, H-6′) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9 (CH_3*C*ONH), 170.8 (CH_3*C*O), 170.6 (CH_3*C*O), 170.4 (CH_3*C*O), 170.2 (CH_3*C*O), 170.0 (CH_3*C*O), 169.6 (CH_3*C*O), 169.4 (CH_3*C*O), 169.2 (CH_3*C*O), 135.6 (d, *J* = 6.0 Hz, Ar), 135.4 (d, *J* = 6.5 Hz, Ar), 129.1 (Ar), 129.1 (Ar), 128.9 (Ar), 128.9 (Ar), 128.3 (Ar), 128.2 (Ar), 106.6 (*C*-1″), 99.7 (*C*-1′), 97.0 (d, *J* = 7.0 Hz, *C*-1), 81.3 (*C*-2″), 80.9 (*C*-4″), 78.7 (*C*-3), 76.4 (*C*-3″), 75.6 (*C*-4′), 71.5 (*C*-4), 70.2 (*C*-3′), 70.1 (d, *J* = 5.5 Hz, Ph*C*H_2O), 70.1 (*C*-2′), 70.0 (d, *J* = 5.5 Hz, Ph*C*H_2O), 69.5 (*C*-5), 69.4 (*C*-5″), 68.3 (*C*-5′), 62.5 (*C*-6″), 61.6 (*C*-6), 51.9

(d, J = 7.8 Hz, *C*-2), 23.0 (*C*H$_3$CONH), 21.2 (*C*H$_3$CO), 21.1 (*C*H$_3$CO), 20.9 (*C*H$_3$CO), 20.82 (*C*H$_3$CO), 20.80 (*C*H$_3$CO × 2), 20.78 (*C*H$_3$CO), 20.77 (*C*H$_3$CO), 17.6 (*C*-6′) ppm; ^{31}P NMR (202 MHz, CDCl$_3$) δ –2.42 ppm; HRMS (ESI): *m*/*z* Calcd for $C_{50}H_{65}NO_{26}P$ $[M+H]^+$ 1126.3527. Found: 1126.3510; $C_{50}H_{64}NNaO_{26}P$ $[M+Na]^+$ 1148.3346. Found: 1148.3341.

2,3,5,6-Tetra-*O*-acetyl-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl 1-phosphate triethylammonium salt (39): Phosphate **38** (22 mg) was dissolved in dry THF (2 mL) and palladium on carbon (20 mg, dry, 5%) was added in one portion. The flask was evacuated under vacuum and re-charged with hydrogen three times while cooling on dry ice. After stirring at rt overnight, the reaction mixture was filtered with Celite and the filtrate was neutralized with triethylamine (30 μL). The solvent was removed under vacuum to give **39** (19 mg, 93%) as a white solid: R_f = 0.53 (H$_2$O–2-propanol–EtOAc, 1:2:2); $[\alpha]_D^{25}$ = +9.1 (c = 0.80, CH$_2$Cl$_2$); ^{1}H NMR (700 MHz, CD$_3$OD) δ 5.43 (dd, 1H, J = 6.9, 3.1 Hz, H-1), 5.35 (dt, 1H, J = 6.9, 4.5 Hz, H-5″), 5.24 (s, 1H, H-1″), 5.14 (dd, 1H, J = 3.2, 2.0 Hz, H-2′), 5.10 (t, 1H, J = 9.6 Hz, H-4), 5.08 (dd, 1H, J = 9.9, 3.4 Hz, H-3′), 5.02 (dd, 1H, J = 4.9, 1.3 Hz, H-3″), 4.92 (d, 1H, J = 1.3 Hz, H-2″), 4.91 (d, 1H, J = 1.8 Hz, H-1′), 4.32 (t, 1H, J = 4.6 Hz, H-4″), 4.31 (dd, 1H, J = 11.9, 4.6 Hz, H-6″a), 4.26–4.20 (m, 4H, H-2, H-6a, H-6″b, H-5), 4.11 (dd, 1H, J = 12.4, 2.4 Hz, H-6b), 4.08 (t, 1H, J = 9.4 Hz, H-3), 3.78 (dq, 1H, J = 9.5, 6.2 Hz, H-5″), 3.63 (t, 1H, J = 9.8 Hz, H-4″), 2.95 (q, 6H, J = 7.2 Hz, (CH$_3$C*H*$_2$)$_3$N), 2.11 (s, 3H, C*H*$_3$CO), 2.10 (s, 3H, C*H*$_3$CO), 2.092 (s, 3H, C*H*$_3$CO), 2.085 (s, 3H, C*H*$_3$CO), 2.08 (s, 3H, C*H*$_3$CO), 2.06 (s, 3H, C*H*$_3$CO), 2.038 (s, 3H, C*H*$_3$CO), 2.035 (s, 3H, C*H*$_3$CO), 1.98 (s, 3H, C*H*$_3$CO), 1.27 (d, 3H, J = 6.2 Hz, H-6″), 1.22 (t, 9H, J = 7.2 Hz, (C*H*$_3$CH$_2$)$_3$N) ppm; ^{13}C NMR (125 MHz, CD$_3$OD) δ 174.0 (CH$_3$*C*ONH), 172.8 (CH$_3$*C*O), 172.2 (CH$_3$*C*O), 171.8 (CH$_3$*C*O),

171.7 (CH_3CO), 171.6 (CH_3CO), 171.5 (CH_3CO), 171.3 (CH_3CO), 171.1 (CH_3CO), 108.0 (*C*-1″), 100.4 (*C*-1′), 95.1 (d, *J* = 5.5 Hz, *C*-1), 82.7 (*C*-4″), 82.5 (*C*-2″), 79.3 (*C*-3), 77.9 (*C*-3″), 77.1 (*C*-4′), 72.9 (*C*-3′), 71.4 (*C*-2′), 71.3 (*C*-4), 71.0 (*C*-5″), 69.8 (*C*-5), 68.8 (*C*-5′), 63.6 (*C*-6″), 63.3 (*C*-6), 54.8 (d, *J* = 7.3 Hz, *C*-2), 47.1 (($CH_3CH_2)_3N$), 23.0 (CH_3CONH), 21.5 (CH_3CO), 20.9 (CH_3CO), 20.78 (CH_3CO), 20.76 (CH_3CO), 20.65 (CH_3CO), 20.60 (CH_3CO), 20.59 (CH_3CO), 20.58 (CH_3CO), 18.4 (*C*-6′), 9.8 (($CH_3CH_2)_3N$) ppm; ^{31}P NMR (202 MHz, CD_3OD) δ –1.01 ppm; HRMS (ESI): *m*/*z* Calcd for $C_{36}H_{51}NO_{26}P$ $[M–H]^-$ 944.2442. Found: 944.2447.

Enzyme Kinetics Measurements

Spectrophotometric assays and kinetics measuremend we carried out a described with minor modifications.[10,13] Briefly, seven different concentrations of acceptor (**3**, **4**, **22** or **23**) and donor (UDP-Gal*f*) were employed in the kinetics assay. Table 3 shows the donor and acceptor concentrations used in these determinations. The kinetic parameters k_{cat} and K_m were obtained by nonlinear regression analysis of the Michaelis–Menten equation with the Graph Pad PRISM 4.0 program (GraphPad Software, San Diego).

Table 3. Acceptor concentrations used in kinetic measurements of **2**–**4**, **22** and **23** with GlfT1 and GlfT2

Enzyme	Acceptor	[Acceptor] (μM)	[UDP-Gal*f*] (μM)
TB-GlfT1	**22**	4000, 2000, 1000, 500, 250, 125, 62.5	2000
MS-GlfT1	**22**	5000, 2500, 1250, 625, 312.5, 156.3, 78.1	5000
TB-GlfT1	**23**	4000, 2000, 1000, 500, 250, 125, 62.5	3000
MS-GlfT1	**23**	2000, 1000, 500, 250, 125, 62.5, 31.3	3000
GlfT2	**2**	6000, 4000, 2000, 1000, 500, 250, 125	3000
GlfT2	**3**	500, 250, 125, 62.5, 31.3, 15.6, 7.8	3000
GlfT2	**4**	100, 50, 25, 12.5, 6.3, 3.1, 1.6	3000

Large-scale enzymatic reaction catalyzed by GlfT1 or GlfT2

Following the spectrophotometric assays, UDP-Gal*f* (60 mmol) was added to the combined mixture. The mixture was incubated at 37 °C for 3 h and then an additional amount of UDP-Gal*f* (60 mmol) was added. After incubating at 37 °C overnight, the mixtures were centrifuged to remove precipitates and the supernatant was applied to the Sep-pak C18 cartridge. The cartridge was washed with Milli-Q water (50 mL) and the product was then eluted with methanol (3 mL). The eluates were concentrated under vacuum and the residue was lyophilized from Milli-Q water (1 mL) to give the products as white amorphous solids.

Mass Spectrometry of GlfT1 and GlfT2 reaction products.

Electrospray ionization mass spectrometry (ESI-MS) measurements were carried out in negative ion mode using a Synapt G2S quadrupole-ion mobility separation-time of flight (Q-IMS-TOF) mass spectrometer (Waters, Manchester, UK) equipped with a nanoflow ESI (nanoESI) source. The NanoESI

tips were produced from borosilicate capillaries (1.0 mm o.d., 0.68 mm i.d.) pulled to ~5 µm using a P-1000 micropipette puller (Sutter Instruments, Novato, CA). Each sample was diluted in Milli Q water (EMD Millipore, Billerica, MA) at room temperature and loaded into the nanoESI tip. To perform nanoESI, a platinum wire was inserted into the nanoESI tip and a capillary voltage of -0.8 kV was applied. The Source temperature was 60 °C. Cone, Trap and Transfer voltages of 5 V, 5V and 2V, respectively, were used; all other instrumental parameters were set at default values. Data acquisition and processing were performed using MassLynx software (version 4.1).

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