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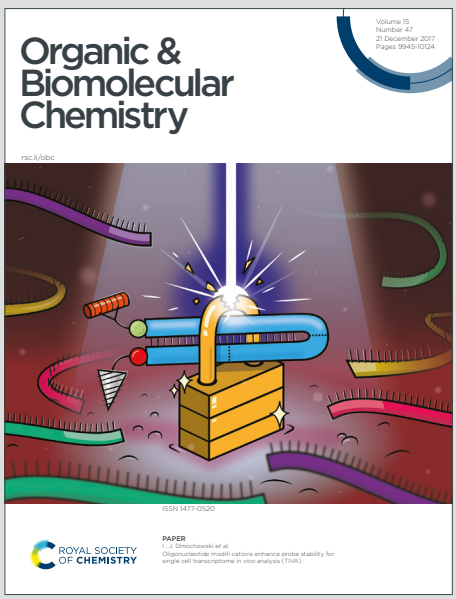

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Melioidosis Patient Serum-Reactive Synthetic Tetrasaccharides Bearing the Predominant Epitopes of *Burkholderia pseudomallei* and *Burkholderia mallei* O-Antigens

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ABSTRACT:

Melioidosis and glanders, respectively caused by the Gram-negative bacteria *Burkholderia pseudomallei* (*Bp*) and *Burkholderia mallei* (*Bm*), are considered as urgent public health issues in developing countries and potential bioterrorism agents. *Bp* and *Bm* lipopolysaccharides (LPS) have been identified as attractive vaccine candidates for the development of prophylactic measures against melioidosis and glanders. *Bp* and *Bm* express structurally similar LPSs wherein the O-antigen (OAg) portion consists of a heteropolymer whose repeating unit is a disaccharide composed of D-glucose and 6-deoxy-L-talose residues, the latter being diversely acetylated and methylated. Herein we report the synthesis of two tetrasaccharides mimicking the main substitution epitopes of *Bp* and *Bm* LPS OAgs. The assembly of the tetrasaccharides was achieved using a sequential glycosylation strategy while relying on the late-stage epimerization of the inner rhamnose into a 6-deoxy-L-talose residue. We show that these synthetic compounds strongly react with culture-confirmed Thai melioidosis patient serum and closely mimic the antigenicity of native *Bp* OAg. Our results suggest that these tetrasaccharides could be suitable candidates for the development of vaccines and/or diagnostic tools against melioidosis and glanders.

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INTRODUCTION

Burkholderia pseudomallei (*Bp*) and *Burkholderia mallei* (*Bm*), two closely related facultative intracellular Gram-negative bacteria (GNB), are the respective etiologic agents of melioidosis and glanders. Endemic in tropical and sub-tropical regions and sporadically found worldwide,[1, 2] these two neglected bacteria are highly virulent and, if left untreated, are fatal in up to 50% of infected patients.[3-5] Notably, the urgent public health issues that these diseases represent is highlighted by the fact that melioidosis is considered the third most common cause of infectious diseases-related deaths in Northeast Thailand.[6] The protean manifestations of melioidosis and glanders can hinder diagnosis, and treatment of infected patients is challenging due to the intracellular nature of these pathogens as well as their intrinsic resistance to commonly used antibiotics such as aminoglycosides, polymyxins, and β-lactams.[7] Importantly, in addition to the public health threat that they represent, *Bp* and *Bm* are considered potential biological weapons by the U. S. Centers for Disease Control and Prevention, as they have been classified as "Tier 1" biological select agents.[8]

Notwithstanding the efforts put towards the development of prophylactic measures against melioidosis and glanders, there are currently no licensed vaccines against these diseases. In light of the many attempts to develop protective vaccines,[9] recent studies underlined the potential of using lipopolysaccharides (LPS) as subunit vaccines owing to their stimulating effect on the adaptive immune system.[10] LPSs are surface-exposed antigenic and virulence-associated polysaccharides anchored in the outer membrane of GNB such as *Bp* and *Bm*.[11] It was shown that LPS-specific monoclonal antibodies (mAbs) have the ability to passively protect animal models from infection,[12-17] and that immunization of mice with LPS conjugates induces significant protection against lethal challenges of *Bp*.[10, 18]

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The predominant serotype A (97%) O-antigens (OAgs) of *Bp* and *Bm* LPSs[19] are of similar composition and consist of linear heteropolymers whose repeating unit is a disaccharide of the following structure: [→3)-6-deoxy-α-L-talopyranosyl-(1→3)-β-D-glucopyranosyl-(1→] (Figure 1). One atypical characteristic of these OAgs lies in the non-stoichiometric methylation and acetylation patterns of the 6-deoxy-L-talose residues, which slightly vary between species. It has been revealed that the predominant inner disaccharide unit of both *Bp* and *Bm* is acetylated at *O*-2. In addition, the terminal residue of *Bm* is 3-*O*-methylated and 2-*O*-acetylated whereas *Bp*'s is also acetylated at *O*-4.[20-25]

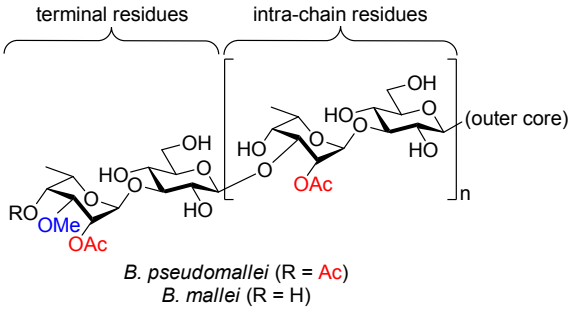

Figure 1. Structures of *B. pseudomallei* and *B. mallei* LPS O-antigens showing the predominant terminal and intra-chain epitopes.

We recently described the synthesis of seven di- and trisaccharides (**1**–**7**) as the minimal structures mimicking all of the reported substitution patterns of *Bp* and *Bm* OAgs (Figure 2A).[26] Enzyme-linked immunosorbent assay, saturation transfer difference-nuclear magnetic resonance, and surface plasmon resonance assays were jointly used to assess the molecular interactions of the synthetic oligosaccharides with LPS-specific mAbs. These biophysical studies revealed the crucial role of the capping 3-*O*-methylated-6-deoxy-L-talose residues in the binding process. Moreover, we showed that high-titer antibody responses can be raised in mice injected with a CRM197:*Bm*-

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like disaccharide **6** construct and that these responses were cross-reactive with *Bm*-like LPS. In contrast, a CRM197:*Bp*-like disaccharide **7** construct was not able to induce a strong antibody response in both BALB/c and C57BL/6 mice suggesting a gap in the mice B cell repertoire against the OAg motif bearing an additional 4-*O*-acetyl group. We also showed that Thai melioidosis patient serum samples can react with *Bp*-like disaccharide **7**, which indicates that, unlike mice, humans can generate antibody responses against this unique OAg epitope.

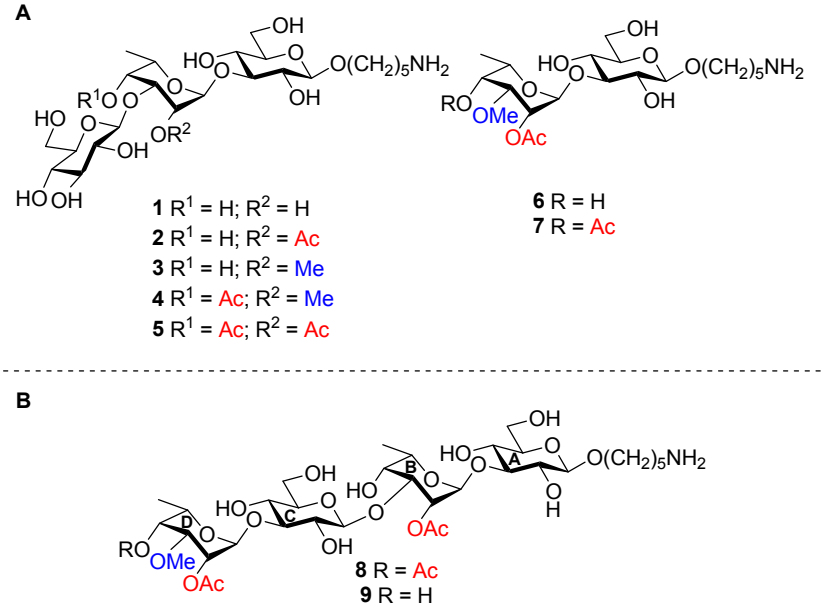

Figure 2. (**A**) Structures of previously synthesized di- and trisaccharides (**1**–**7**); and (**B**) target synthetic tetrasaccharides **8** and **9** related to *Bp* and *Bm* LPS OAgs, respectively.

We now show that longer oligosaccharidic fragments, such as tetrasaccharides **8** and **9** (Figure 2B), bearing both internal and terminal epitopes of *Bp* and *Bm* OAgs are suitable candidates for the development of diagnostics and subunit vaccines against melioidosis and glanders. We herein describe a synthetic approach enabling the preparation of tetrasaccharides related to *Bp* and *Bm* LPS OAg featuring the main intrachain and terminal acetylation and methylation patterns. The reactivity profiles of synthetic oligosaccharides **1**–**9** were assessed against Thai melioidosis patient

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serum samples highlighting that tetrasaccharides **8** and **9** act as exquisite mimics of the whole *Bp* and *Bm* LPS OAgs.

RESULTS AND DISCUSSION

Retrosynthetic approach

The presence of acetyl groups in target tetrasaccharides **8** and **9** posed a substantial challenge in the development of the synthetic route, which had to be designed so that their migration or cleavage would be avoided. Retrosynthetic analysis of these oligosaccharides, as depicted in Figure 3, led to the identification of synthons **13**, **14**,[26] **15**, and **16**[26] as the most readily accessible functionalized monosaccharides that could enable their assembly. Our synthetic approach would be based on a convergent [2 + 2] glycosylation strategy in which disaccharides **11** and **12**, assembled from building blocks **13–16**, would be coupled into fully protected tetrasaccharide **3**, the latter being a precursor of both *Bp*- and *Bm*-like oligosaccharides **8** and **9**, respectively. We plan to introduce *O*-acetyl and *O*-methyl groups in synthons **13** and **14** prior to the synthesis of the tetrasaccharides. These building blocks would have to be designed in order to preserve orthogonality among the temporary protecting groups and acetates. The acetyl groups at *O*-2 on rhamnosides **13** and **14** as well as the (2-azidomethyl)benzoyl (AZMB)[27] group of glucoside **15** would act as neighbouring participating groups (NPG) to ensure the stereoselective formation of 1,2-*trans* glycosidic linkages in the target compounds.[28] Moreover, assisted cleavage of the AZMB group, which involves the reduction of the primary azide into the corresponding amine followed by *in situ* intramolecular cyclization into an isoindolinone, thereby releasing the C2 hydroxyl group,[27-29] would be achieved during the final hydrogenolysis step. The anomeric position of monosaccharides **13** and **14** would be activated with a trichloroacetimidate (TCA) leaving group in an attempt to achieve high-yielding

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couplings, as previously reported for structurally similar L-rhamnose donors.[30] In contrast, glucoside **15** would be thiolated at the anomeric position to ensure its orthogonal glycosylation with respect to donor **14**. The methylphenylthio (STol) group is known to be highly stable under a broad variety of conditions while being readily accessible from peracetylated sugars,[31] and thioglucoside **15** would be converted into other donors in the event that the coupling proves ineffective.

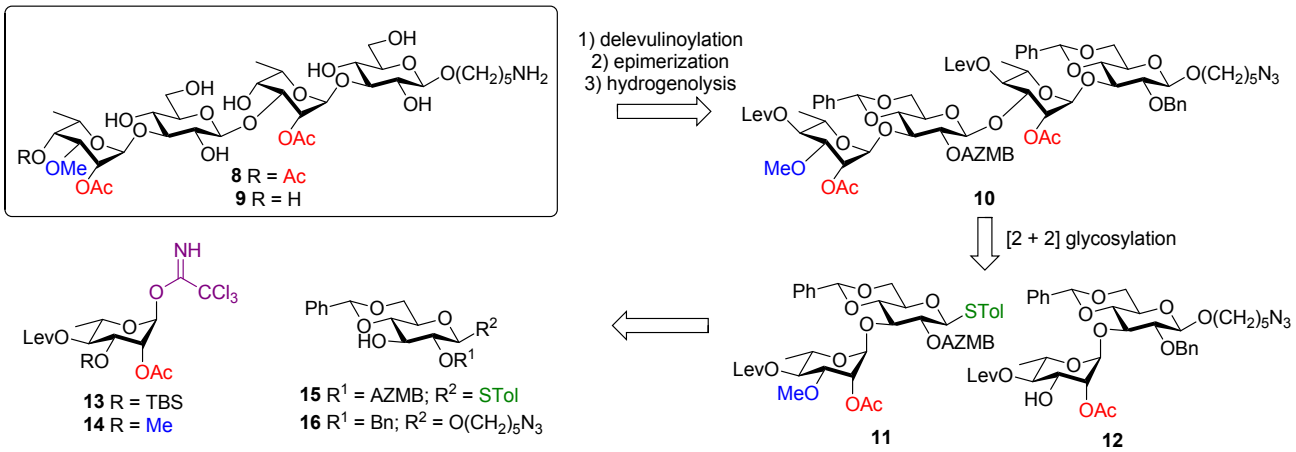

Figure 3. Retrosynthesis analysis of target tetrasaccharides **8** and **9**.

As 6-deoxy-L-talose derivatives are not commercially available, rhamnosides **13** and **14** would instead be employed. We envisioned to conduct both C4 epimerization simultaneously at a later stage of the synthetic route *via* a two-step oxidation/reduction sequence.[32] To reach *Bp*-like tetrasaccharide **8**, we intended to take advantage of the steric hindrance surrounding the inner 6-deoxy-L-talose residue to acetylate regioselectivity the *O*-4 position of the non-reducing end residue. Finally, acceptor **16** would be equipped with an azidolinker at C1, which upon reduction would allow the coupling of the fully assembled, unprotected oligosaccharides to activated ELISA plates enabling antigenicity assays with serum samples and/or covalent coupling with carrier proteins.

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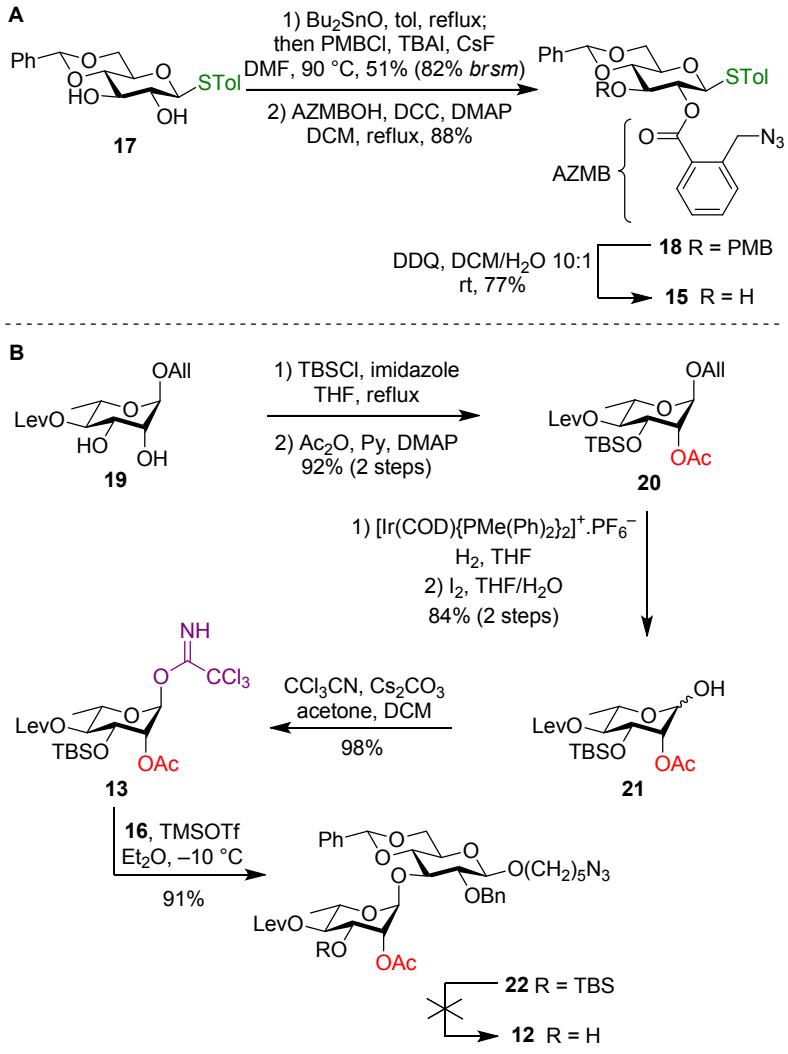
First generation synthesis of tetrasaccharides

As depicted in Scheme 1, the synthesis of glucoside acceptor **15** was performed in three steps from known diol **17**.[33] A *para*-methoxybenzyl (PMB) group was selectively introduced at *O*-3 through the formation of a stannylene acetal.[34] This step was followed by esterification of the resulting alcohol[35] with 2-(azidomethyl)benzoic acid (AZMBOH), which was prepared using Steglich conditions.[30,36] The PMB group finally underwent oxidative cleavage using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), furnishing acceptor **15**.



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Scheme 1. (**A**) Synthesis of 2-*O*-AZMB-containing glucose derivative **15**; and (**B**) failed attempts to cleave the TBS group in disaccharide **22**.

Allyl rhamnoside **19**[26] was used as starting material for the preparation of TCA **13**. Imidazole was used as catalyst for the selective protection of the C3 hydroxyl with a *tert*-butyldimethylsilyl (TBS) group and the remaining free OH was acetylated under standard conditions, yielding rhamnoside **20** in 92% yield over two steps. Allyl isomerization into the corresponding 1-propenyl ether was accomplished using an iridium-based catalyst[37, 38] and was followed by its hydrolysis promoted by iodine[39] in a mixture of THF and water. Resulting hemiacetal **21** was successfully converted into TCA **13** (~9:1 *α/β* mixture) through a standard procedure[40] involving the use of trichloroacetonitrile and cesium carbonate. The latter was coupled with acceptor **16** under the promotion of trimethylsilyl trifluoromethanesulfonate (TMSOTf),[41-44] providing disaccharide **22** in an excellent 91% yield as the sole α-anomer. In order to lengthen the disaccharide, deprotection of the TBS group was attempted using $Et_3N{\cdot}3HF$, but acetyl cleavage was unfortunately observed. Tetra-*n*-butylammonium fluoride (TBAF)-mediated cleavage, with and without acetic acid buffer, also led to acetyl deprotection.

Due to the poor selectivity of the silyl ether deprotection, we prepared thiorhamnoside **25**, bearing an orthogonal PMB group at *O*-3 instead of a TBS group as previously reported by us (Scheme 2).[26] Alcohol **23**[45] was therefore levulinoylated under the action of dicyclohexylcarbodiimide (DCC) and catalytic DMAP and the isopropylidene group was cleaved in acidic media, furnishing diol **24** in excellent yield. Tin acetal chemistry was taken advantage of for the selective alkylation of the *O*-3 position and the remaining hydroxyl group was acetylated into donor **25**. *N*-Iodosuccinimide (NIS)/silver triflate (AgOTf)-promoted glycosidation[46, 47] of the latter with

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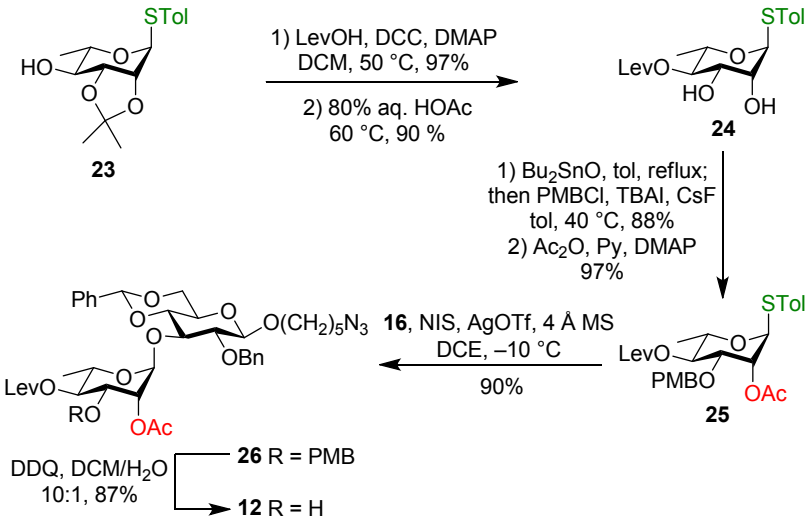
acceptor **16** at –10 °C in DCE provided disaccharide **26** in 90% yield. Satisfyingly, only the α-anomer was formed, which was then successfully transformed into acceptor **12** following PMB deprotection without noticeable acetyl migration.



Scheme 2. Synthesis of disaccharide acceptor **12**.

Planning on employing a [2 + 2] glycosylation strategy (Scheme 3), disaccharide **11** was then prepared through TMSOTf-promoted glycosylation of TCA **7**[26] and acceptor **15**. However, complete conversion could not be achieved and disaccharide **11** was contaminated with donor **14** even upon extensive column chromatography purifications. Synthesis of fully protected tetrasaccharide **10** was nonetheless attempted using NIS/AgOTf but proved unsuccessful, as NMR and HRMS analyses showed no sign of the expected glycosidic bond.

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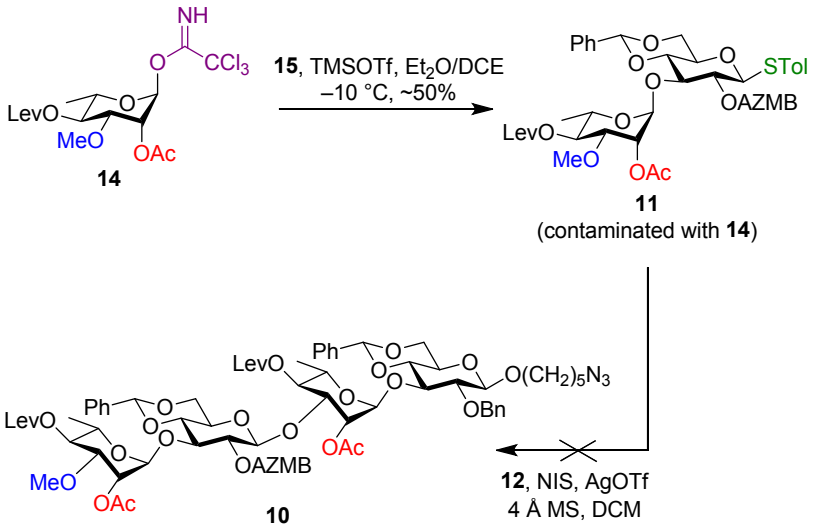

Scheme 3. Attempt to synthesize tetrasaccharide **10** *via* a [2 + 2] glycosylation.

Second generation synthesis of tetrasaccharides

As our previously envisioned methodology appeared ineffective, we chose to focus our attention on an alternative sequential [1 + 1 + 1 + 1] glycosylation pathway (Scheme 4). First, disaccharide acceptor **12** was glycosylated with donor **18** using the aforementioned conditions, yielding trisaccharide **27**. The AZMB group appeared to be an efficient NPG, as the β-anomer was exclusively formed. DDQ-mediated dealkylation of trisaccharide **27** enabled its coupling with thiorhamnoside **29**, which was prepared from intermediate **24** through a methodology similar as the one used for donor **25**. In the course of this reaction sequence, O-methylation of diol **24** partially occurred at *O*-2, resulting in a mixture of regioisomers. Upon glycosylation, tetrasaccharide **10** was produced in a 65% yield, along with an inseparable unknown impurity. To achieve the desired 6dTal*p* configuration, the levulinoyl groups in compound **10** were selectively cleaved under the action of hydrazine and epimerization of both free hydroxyl groups in resulting tetrasaccharide **30** was attempted.

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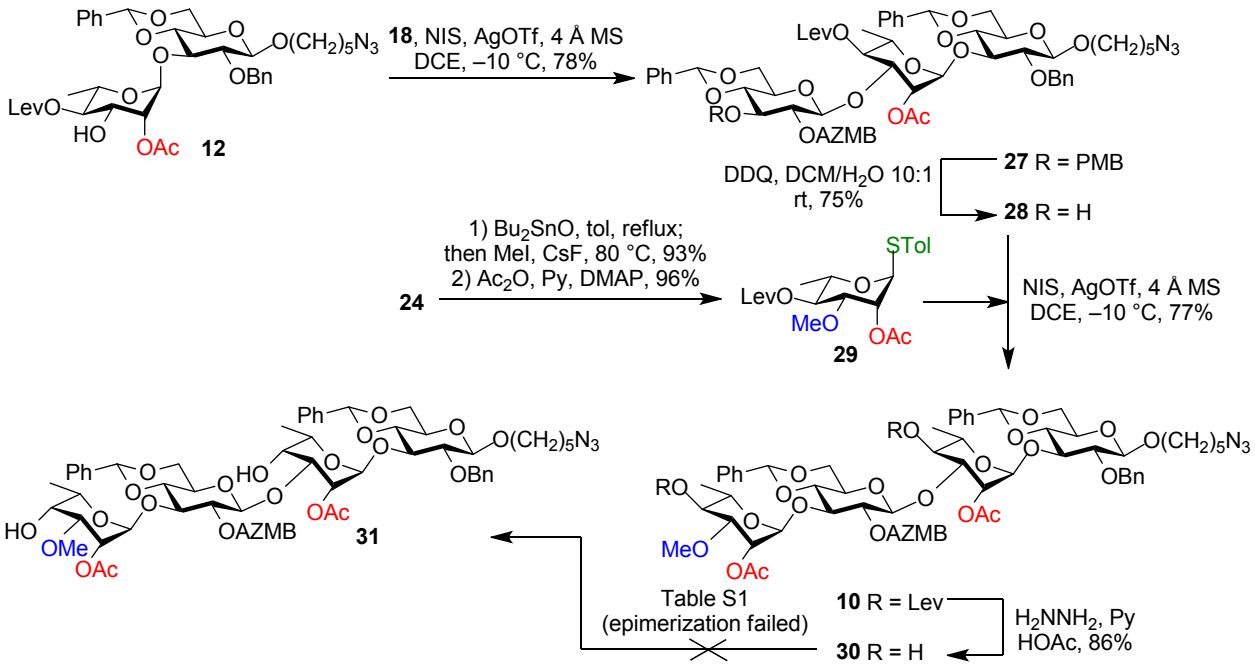

Scheme 4. Synthesis of rhamnose-containing tetrasaccharide **10** *via* a [1 + 1 + 1 + 1] glycosylation sequence.

Several approaches have been developed for the epimerization of rhamnose glycosides. For instance, Kovác reported the synthesis of the C-2 rhamnose epimer L-quinovose through the formation of 1,2-*O*-stannylene acetals.[48] Chloral/DCC-induced inversion, which requires the presence of a 2,3,4-triol, resulted in C-3 epimerization of rhamnose.[49] The Mitsunobu reaction[50,51] has been shown to lead to an elimination reaction in rhamnosides due to the axial H-5.[32] Intramolecular displacement of an *O*-4 triflate has also been reported for the synthesis of 6-deoxy-taloside from rhamnose.[52,53] However, this approach has been shown to be less reliable compared to the oxidation-reduction sequence in which the stereochemical outcome can be consistently predicted using the Cram chelate model.[32, 54-56]

Therefore, our strategy was based on an oxidation step (see Table S1) followed by a stereoselective reduction using $NaBH_4$ in a mixture of MeOH and DCM. Pfitzner-Moffatt oxidation[57] (entry 1)

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was first tried as we previously showed that this condition was high-yielding for similar substrates,[26] nevertheless complete degradation of the starting material was observed. The same reaction was conducted at –78 °C (entry 2) with the hope of stabilizing the reactive intermediate formed between phenyl dichlorophosphate (PDCP) and DMSO, but without success. Substituting the PDCP reagent with oxalyl chloride[32] also gave no conversion (entry 3). Other oxidation reactions were carried out using DMSO and Ac_2O at room temperature[58] (entry 4) or Dess-Martin periodinane in refluxing DCE[26] (entry 5), but both failed to provide clean oxidation products.

Third generation synthesis of tetrasaccharides

As the previous double epimerization step could not be carried out successfully, we hypothesized that the simultaneous presence of two hydroxyl groups could lead to macromolecular interactions preventing the oxidation step to occur. Therefore, we envisioned that the introduction of a terminal 6-deoxy-talose residue would restrict these interactions and consequently enable the epimerization of the inner rhamnose unit. As depicted in Scheme 5, derivatives **34** and **35** were prepared from building block **29**. Delevulinoylation of the latter was performed using standard conditions. Pfitzner-Moffatt oxidation followed by reduction of the crude ketone with $NaBH_4$ furnished thiotaloside **33** in 65% yield over three steps. Acetylation of the remaining hydroxyl group using Ac_2O and catalytic DMAP in refluxing EtOAc led to building block **34**, which mimics *Bp* LPS OAg terminal residue. As for *Bm*-like LPS OAg terminal residue, a chloroacetyl group was used as a temporary protecting group, which could be further transformed into an acetyl group following its orthogonal deprotection. This reaction was performed using chloroacetic anhydride and catalytic DMAP in refluxing EtOAc providing building block **35** in an almost quantitative yield.

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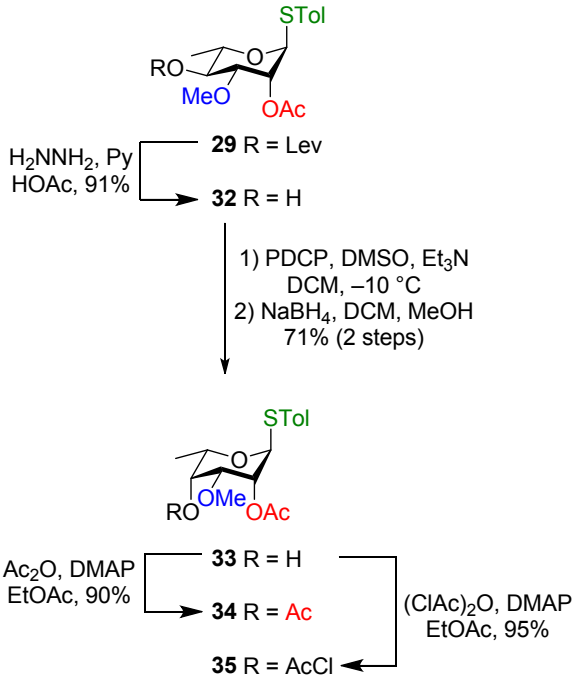

Scheme 5. Synthesis of 6-deoxy-L-talopyranoside derivatives **34** and **35**.

To prepare *Bp* and *Bm* LPS OAg-like tetrasaccharides, trisaccharide acceptor **28** was coupled with thiotaloside **34** and **35**, respectively, under previously mentioned conditions, affording tetrasaccharides **36** and **37** exclusively as the α-anomers (Scheme 6). Upon delevulinoylation of these latter, either Dess-Martin periodinane reagent or Pfitzner-Moffatt conditions were successfully used for their oxidations. In both cases, the resulting ketone was filtered over silica gel and then reduced with complete stereoselectivity into its talose configuration under the action of $NaBH_4$. This two-step sequence enabled the isolation of tetrasaccharide **40** in a moderate 52% yield due to its partial degradation during the oxidation step. Derivative **31** was ultimately reached in a 58% yield over three steps following chloroacetyl cleavage using thiourea in a mixture of pyridine and methanol.

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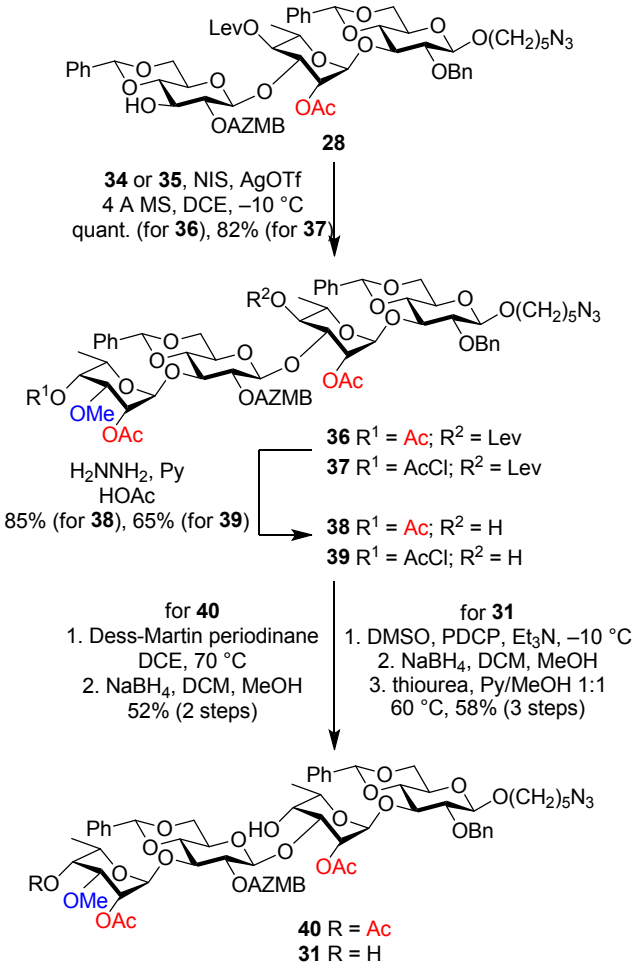

Scheme 6. Final approach for the synthesis of protected tetrasaccharides **31** and **40**.

Global deprotection of tetrasaccharides

Two alternative pathways were studied for the global deprotection of tetrasaccharides **40** and **31** in order to reach target compounds **8** and **9**, respectively. The first pathway (route A) consisted in the selective reduction of both azides followed by hydrogenolysis of the remaining benzyl and benzylidene groups, whereas the second route (route B) only involved the hydrogenolysis step. We therefore investigated a series of conditions for the selective reduction of both azides *via* the Staudinger reaction, which would result in the assisted cleavage of the AZMB group. We first planned to use trisaccharide **27** as a model compound, as we hypothesized that if difficulties had

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to arise during AZMB deprotection, it would be due to the electrophilic acetyl group at the inner talose residue. Intramolecular rearrangement of iminophosphorane intermediates during the Staudinger reduction can indeed occur in compounds containing a neighbouring ester.[28] Preliminary reduction tests were first conducted with trisaccharide **27**, but we noticed that the reduction of the azidolinker reduction required harsher conditions than the AZMB group itself. Our hypothesis was that the formation of the isoindolinone by-product acted as the driving force for the AZMB cleavage. Our attention therefore shifted toward disaccharide **26** and conditions were optimized for the sole reduction of the azidolinker (Table 1). Triphenylphosphine was first used in a mixture of THF/H_2O in the presence of SiO_2 (entry 1).[30] After 20 h, the starting material was totally consumed but, as shown by TLC, the intermediates were not fully hydrolysed. Following work-up with aqueous $NaHCO_3$ and purification, target amine **41** was isolated in low yield (21%). We then attempted to conduct the reaction through a two-step procedure, *i.e.*, 1) formation of the iminophosphorane and 2) its hydrolysis, while heating the reaction mixture to 60 °C, but a lower yield of amine **41** was obtained (entry 2). Switching the solvent for DMF and avoiding the work-up procedure allowed to improve the yield to 38% (entry 3). Satisfyingly, using the same reaction conditions than in entry 3 while avoiding the work-up provided amine **41** in excellent yield (88%, entry 4). Tris(4-methoxyphenyl)phosphine [$P(PMP)_3$] was also tested but without yield improvement (entry 5). The optimized reduction conditions were then applied to trisaccharide **27** bearing both the azidolinker and the AZMB group, providing alcohol **42** in good yield (76%, entry 6). These conditions finally successfully furnished tetrasaccharide **43** from diol **31** (63%, entry 7), which was then deprotected into target compound **9** through a Pd-catalyzed hydrogenolysis, as shown in Scheme 7.

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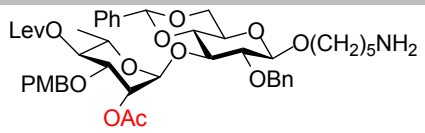
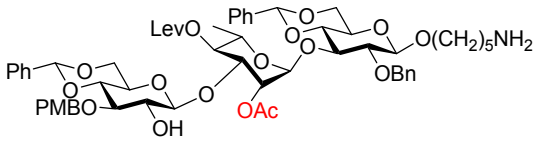
Table 1. Optimization of the Staudinger reaction for azide reduction

entry	substrate	conditions	work-up	product	yield (%)[a]
1	**26**	PPh_3, SiO_2 THF/H_2O, rt 20 h	$NaHCO_3$ H_2O, DCM	 **41**	21
2	**26**	PPh_3, THF 60 °C, 2 h then H_2O, 60 °C, 4 h	$NaHCO_3$ H_2O, DCM	**41**	13
3	**26**	PPh_3, DMF, 0 °C to rt 2 h, then H_2O rt, 26 h	toluene co-concentration	**41**	38
4	**26**	PPh_3, THF, 60 °C 2 h then H_2O 60 °C, 4 h	concentration	**41**	88
5	**26**	$P(PMP)_3$ THF 60 °C, 2 h then H_2O, 60 °C, 4 h	concentration	**41**	63
6	**27**	PPh_3, THF, 60 °C 2 h then H_2O 60 °C, 4 h	concentration	 **42**	76
7	**31**	PPh_3, THF, 60 °C 2 h then H_2O, 60 °C, 4 h	concentration	**43**	63

[a]Isolated yield.

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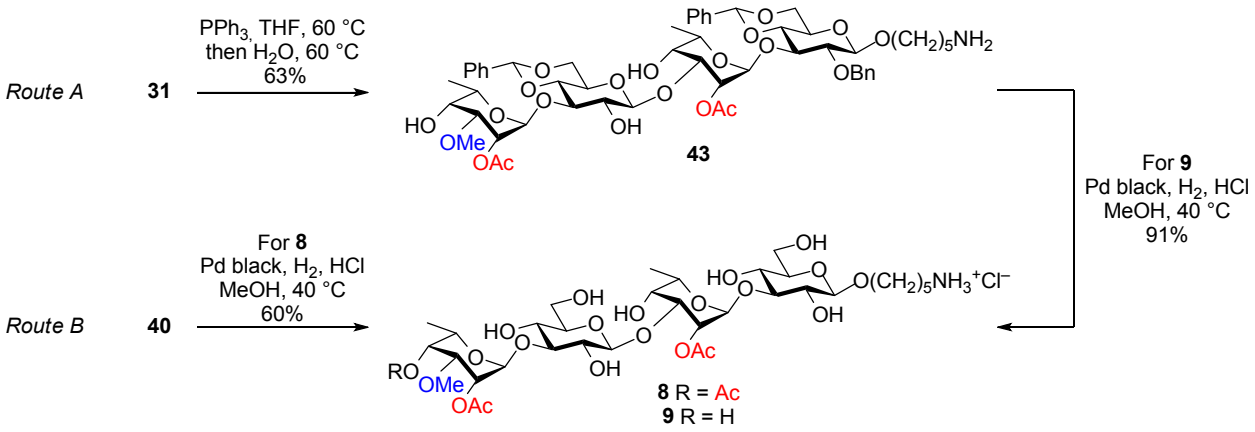

Scheme 7. Alternative pathways for the final deprotection of tetrasaccharides **31** and **40**.

Alternatively, reduction of the azides into the corresponding amines and cleavage of the permanent protecting groups were carried out *via* a one-step hydrogenolysis procedure, enabling the conversion of compound **40** into *Bp*-like tetrasaccharide **8**. Noteworthy, the presence of the azidolinker required the addition of concentrated HCl (1.0 equiv) in the reaction mixture in order to protonate the amine formed upon its reduction, as primary amines are known to poison transition metal catalysts.[26] Partial protonation of the 2-(aminomethyl)benzoyl group therefore also occurred prior to completion of the intramolecular cyclization, preventing the complete release of the corresponding hydroxyl group. The partial cleavage of the AZMB group not only diminished the isolated yield, but also complicated the purification of the target compound. First, exclusion size chromatography using LH-20 resin was employed to purify the tetrasaccharides from the isoindolinone, which was released following AZMB cleavage. Then, reverse phase chromatography was required to isolate tetrasaccharide **8** from the derivative still bearing the protonated 2-(aminomethyl)benzoyl group. Despite this, direct hydrogenolysis of compound **40** enabled the isolation of pure tetrasaccharide **8** in good yield (60%), as shown in Scheme 7.

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Reactivity of the synthetic oligosaccharides with *Bm* LPS-specific mAbs.

Studies from our laboratories and others have shown that LPS-specific mAbs differentially recognize *Bm* and *Bp* OAgs as well as disaccharides **6** and **7**.[23, 26, 59] To determine whether tetrasaccharides **8** and **9** synthesized in this study (oligosaccharides **8** and **9**) were recognized by *Bm* LPS-specific mAbs 3D11 and 9C1-2, ELISAs were conducted using oligosaccharides **6-9** along with *Bp* LPS controls. As shown in Figure 4, mAbs 3D11 and 9C1-2 reacted strongly with RR4744 LPS (4-*O*-acetyl mutant), oligosaccharides **6** and **9**, but only weakly with RR2808 LPS (wild type), and oligosaccharides **7** and **8**. These findings are consistent with our previous work and also show that mAb 3D11 appears to recognize the newly synthesized tetrasaccharide (oligosaccharide **9**), which represents both intrachain and terminal residues associated with *Bm* OAg, more strongly than the previously synthesized disaccharide (oligosaccharide **6**). The reactivity levels of mAb 9C1-2 with RR2808 LPS and oligosaccharides **6** and **9** were similar to one another. Taken together, these results suggest that the epitopes on *Bm* OAg that are recognized by these two mAbs are slightly different from one another. Importantly, since 9C1-2 has been shown to be passively protective in a mouse model of glanders,[15] these data support the potential use of oligosaccharide **9** in the development of novel glanders vaccine candidates.

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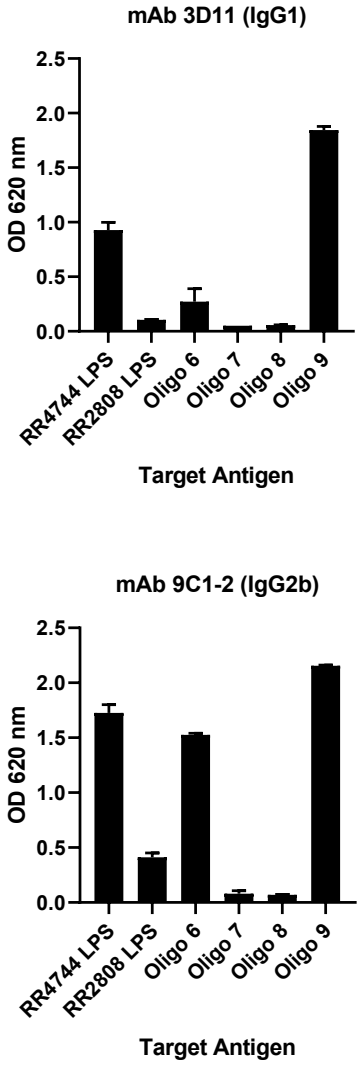

Figure 4. Interactions of *Bm* LPS-specific mAbs with *Bp* LPS antigens and synthetic oligosaccharides. Reactivity profiles of mAbs 3D11 and 9C1-2 with LPS antigens purified from *Bp* strains RR2808 (wild type) and RR4744 (4-*O*-acetyl mutant) and synthetic oligosaccharides **6-9** as determined by ELISA. Values represent the means ± SD of assays conducted in triplicate.

Reactivity of the synthetic oligosaccharides with melioidosis patient serum.

We have previously demonstrated that both *Bp* OAg and oligosaccharide **7** are recognized more strongly by serum from culture-confirmed Thai melioidosis patients compared to serum from healthy donors.[26] Extending upon these findings, ELISAs[59] were used to assess the reactivity of an

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expanded set of culture-confirmed Thai melioidosis patient and Thai healthy donor serum samples with oligosaccharides **1-9** along with a *Bp* LPS control. Results demonstrated strong reactivity of immune serum samples with *Bp* LPS, oligosaccharides **8** and **9**, moderate reactivity with oligosaccharide **2**, and low to moderate reactivity with oligosaccharides **1**, **3**, **4**, **5**, **6** and **7** (based upon mean reactivities; Figure 5). In contrast, the healthy donor serum samples showed relatively low reactivity with all of the tested target antigens (based upon mean reactivities; Figure 5). These results agree with our previous studies and indicate that oligosaccharides **8** and **9**, which consist of a combination of epitopes associated with oligosaccharides [**2** + **7**] and [**2** + **6**], respectively, closely mimic the antigenicity of native *Bp* OAg. Additionally, these results demonstrate that the lack of the C4 *O*-acetyl group in *Bm*-like tetrasaccharide **9** does not significantly impact its recognition by melioidosis patient serum. This is not entirely surprising since human immune responses against OAgs following infection with *Bp* would be expected to be polyclonal and thus recognize multiple epitopes at the non-reducing end of the polysaccharide. Based on these observations, we anticipate that glycoconjugates synthesized using either oligosaccharide **8** or **9** will be able to stimulate the production of carbohydrate-specific polyclonal responses capable of recognizing both native *Bp* and *Bm* OAgs. Collectively, our findings suggest that oligosaccharides **8** and **9** may be valuable tools for developing countermeasures to combat diseases caused by *Bm* and *Bp*.

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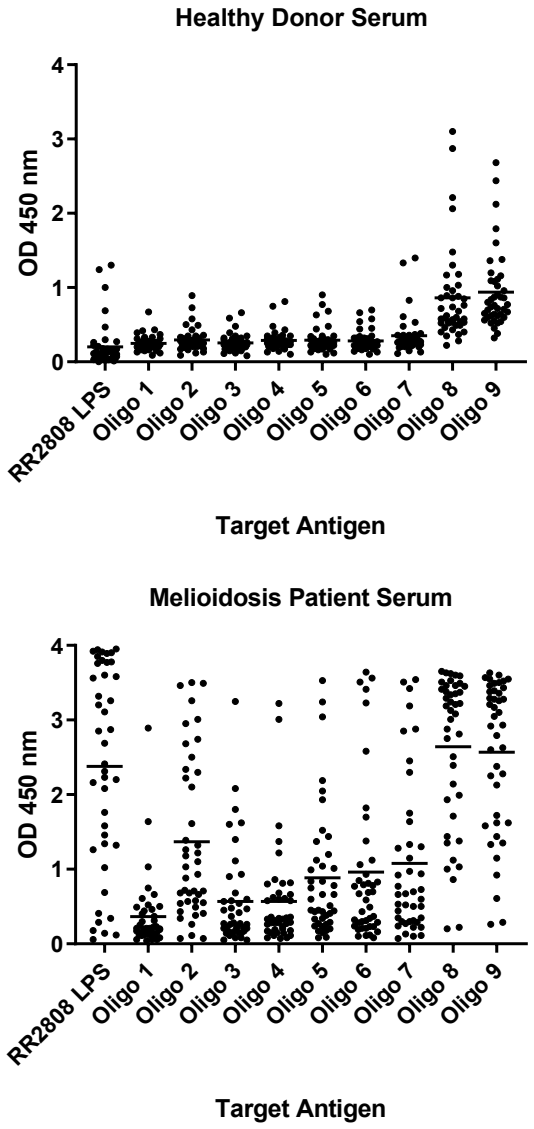

Figure 5. Reactivity of human serum samples with *Bp* LPS and synthetic oligosaccharides. Serum samples from culture confirmed Thai melioidosis patients (n = 42) and Thai healthy donors (n = 42) were assayed for reactivity with *Bp* RR2808 LPS and synthetic oligosaccharides **1-9** using single-dilution ELISAs (1:2,000). Black dots represent the means of assays conducted in duplicate for individual samples. Black bars represent mean reactivity for each target antigen.

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EXPERIMENTAL SECTION

General methods

All starting materials and reagents were purchased from commercial sources and used as received without further purification. Air and water sensitive reactions were performed in oven-dried glassware under Ar atmosphere. Moisture sensitive reagents were introduced *via* a dry syringe. Anhydrous solvents were either prepared from commercial solvents and dried over heat-gun activated 4 Å molecular sieves or supplied over molecular sieves and used as received. Powdered 4 Å molecular sieves were activated before use by heating with a heat gun for ~5 min under high vacuum. Reactions were monitored by thin-layer chromatography (TLC) with silica gel 60 F_{254} 0.25 mm pre-coated aluminium foil plates. Compounds were visualized by using UV_{254} and/or orcinol (1 mg·mL^{-1}) in 10% aq H_2SO_4 solution with heating. Normal-phase flash column chromatography was performed on silica gel 60 Å (15-40 μm). Reversed-phase flash column chromatography was performed on C_{18} silica gel (fully capped, 25-40 μm). Size exclusion chromatography was performed on GE Healthcare Sephadex LH-20 resin (70 μm). NMR spectra were recorded at 297 K in the indicated solvent ($CDCl_3$, py-d_5 or D_2O) with a 600 MHz instrument, employing standard softwares given by the manufacturer. ^{1}H and ^{13}C NMR spectra were referenced to tetramethylsilane (TMS, $\delta_H = \delta_C = 0.00$ ppm) as internal reference for spectra in $CDCl_3$ and py-d_5, or to internal acetone ($\delta_H = 2.218$ ppm; $\delta_C = 30.9$ ppm) for spectra in D_2O. Assignments were based on ^{1}H, ^{13}C, COSY, HSQC and undecoupled HSQC experiments. Interchangeable assignments are marked with an asterisk (*). Sugar moieties are identified from the reducing end (sugar A) to the non-reducing end (sugar D). High-resolution mass spectra (HRMS) were recorded on an ESI-Q-TOF mass spectrometer. Optical rotations $[\alpha]^{20}_D$ were measured on an Anton Paar polarimeter. The retention factors (R_f) were calculated from silica gel 60 F_{254} 0.25 mm pre-coated

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glass TLC plates. Di- and trisaccharides **1-7** have been synthesized according to our previous work.[26]

ELISAs

Reactivity with Bm LPS-specific mAbs. Reactivity of the *Bm* LPS-specific mAbs (3D11 and 9C1-2) with synthetic oligosaccharides **6-9** and *Bp* LPS antigens was assessed by ELISA essentially as previously described.[26] In brief, maleic anhydride 96-well plates (Pierce) were coated overnight at 4 °C with oligosaccharides **6-9** (5 μg/mL) or purified LPS antigens (10 μg/mL) solubilized in carbonate buffer (pH 9.6). The LPS antigens used in this study were purified from *Bp* strains RR2808 (Δ*wcbB*; *Bp* LPS) and RR4744 (Δ*wcbB*Δ*oacA*; *Bm*-like LPS) as previously described.[60] The coated plates were blocked at room temperature for 30 min with StartingBlock T20 (TBS) Blocking Buffer (SB; Pierce), washed with Tris-buffered saline + 0.05% Tween 20 (TBS-T), incubated for 1 h at 37 °C with the mAbs diluted 1/2000 in TBS-T + 10% SB, washed with TBS-T, and then incubated for 1 h at 37 °C with a 1/2000 dilution of goat anti-mouse IgG-horse radish peroxidase (HRP) conjugate (Southern Biotech). Following a final wash with TBS-T, the plates were developed with TMB substrate (KPL) and read at 620 nm. The data were plotted and analyzed using GraphPad Prism 8 (GraphPad Software Inc.).

Reactivity with serum samples from Thai melioidosis patients and Thai healthy donors. Reactivity of serum samples from culture-confirmed Thai melioidosis patients ($n = 42$) and Thai healthy donors ($n = 42$) with oligosaccharides **1-9** and *Bp* LPS was assessed by ELISA. Plates were coated with the oligosaccharides and LPS as described above and ELISAs were performed essentially as previously described.[59] In brief, the coated plates were blocked at 37 °C for 2 h with TBS-T + 5% skim milk, washed with TBS-T, incubated for 30 min at 37 °C with human serum samples diluted 1/2000 in TBS-T + 1% bovine serum albumin (BSA), washed with TBS-T, and then incubated for 30 min at 37 °C with a 1/2000 dilution of rabbit anti-human IgG- HRP conjugate

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(Dako). Following a final wash with TBS-T, the plates were developed with TMB substrate (Invitrogen), after 15 min 1 N aq HCl was added to stop the reaction and plates were read at 450 nm. The study was approved by the Ethics Committee of Faculty of Tropical Medicine, Mahidol University (approval number MUTM 2014-079-02). Written informed consent was obtained from all subjects.

***para*-Methylphenyl 2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-3-*O*-*para*-methoxybenzyl-1-thio-β-D-glucopyranoside (18).** Bu_2SnO (1902 mg, 7.640 mmol, 1.1 equiv) was added to a solution of diol **17**[33] (2411 mg, 6.946 mmol, 1.0 equiv) in toluene (83 mL). The mixture was refluxed with a Dean-Stark trap for 3 h, after which the solvents were evaporated under reduced pressure. The residue was solubilized in anhydrous DMF (83 mL) and CsF (1076 mg, 7.085 mmol, 1.02 equiv), TBAI (2617 mg, 7.085 mmol, 1.02 equiv), and PMBCl (1.13 mL, 8.34 mmol, 1.2 equiv) were successively added. The mixture was stirred at 90 °C for 16 h and the resulting suspension was cooled at 0 °C and filtered over Celite. The solution was co-evaporated with toluene and the residue was purified by silica gel flash chromatography (Hex/EtOAc 9:1 to 3:7) to give *para*-methylphenyl 4,6-*O*-benzylidene-3-*O*-*para*-methoxybenzyl-1-thio-β-D-glucopyranoside[35] (1738 mg, 51%, 82% brsm) as a white amorphous solid. DMAP (144 mg, 1.18 mmol, 1.0 equiv), DCC (486 mg, 2.36 mmol, 2.0 equiv), and AZMBOH (313 mg, 1.77 mmol, 1.5 equiv) were successively added to a solution of the previously prepared alcohol (582 mg, 1.18 mmol, 1.0 equiv) in anhydrous DCM (12 mL). The mixture was refluxed for 4 h under Ar and the resulting suspension was filtered over Celite. The solvent was evaporated under reduced pressure and the residue was purified by silica gel flash chromatography (Hex/EtOAc 9:1 to 2:8), furnishing glucoside **18** (751 mg, 98%) as a white amorphous solid: R_f 0.6 (Hex/EtOAc 7:3); $[\alpha]_D^{20}$ +36 (*c* 0.7, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.87–7.86 (m, 1H, CH_{AZMB}), 7.61–7.59 (m, 1H, CH_{Ar}), 7.56–7.55 (m, 1H, CH_{Ar}), 7.50–7.49 (m, 2H, 2 × CH_{Ar}), 7.41–7.37 (m, 4H, 4 × CH_{Ar}), 7.35–

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7.33 (m, 2H, 2 × CH_{STol}), 7.10–7.09 (m, 2H, 2 × CH_{STol}), 7.05–7.03 (m, 2H, 2 × CH_{PMB}), 6.60–6.59 (m, 2H, 2 × CH_{PMB}), 5.60 (s, 1H, H-7), 5.20 (dd, J = 9.9 Hz, J = 9.0 Hz, 1H, H-2), 4.81–4.72 (m, 4H, H-1, CH_{2AZMB}, CHH$_{PMB}$), 4.57 (d, J = 11.6 Hz, 1H, CHH_{PMB}), 4.41 (dd, $J_{6a\text{-}6b}$ = 10.5 Hz, $J_{6a\text{-}5}$ = 5.0 Hz, 1H, H-6a), 3.87–3.82 (m, 2H, H-3 and H-6b), 3.77 (t, J = 9.3 Hz, 1H, H-4), 3.69 (s, 3H, CH_{3PMB}), 3.55 (td, $J_{5\text{-}4}$ = 9.8 Hz, $J_{5\text{-}6a}$ = 5.0 Hz, 1H, H-5), 2.33 (s, 3H, CH_{3STol}); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 165.0 (COOR$_{AZMB}$), 159.3 (C$_{Ar}$), 138.8 (C$_{Ar}$), 137.8 (C$_{Ar}$), 137.3 (C$_{Ar}$), 133.8 (2C, 2 × CH$_{STol}$), 133.0 (CH$_{Ar}$), 131.2 (CH$_{AZMB}$), 130.0 (C$_{Ar}$), 129.9, 129.8 (4C, 2 × CH$_{STol}$, 2 × CH$_{PMB}$), 129.5 (CH$_{Ar}$), 129.2 (C$_{Ar}$), 128.6 (CH$_{Ar}$), 128.4 (3C, 3 × CH$_{Ar}$), 128.0 (C$_{Ar}$), 126.1 (2C, 2 × CH$_{Ar}$), 113.7 (2C, 2 × CH$_{PMB}$), 101.4 (C-7), 87.1 (C-1), 81.7 (C-4), 79.2 (C-3), 74.1 (CH$_{2PMB}$), 72.1 (C-2), 70.7 (C-5), 68.7 (C-6), 55.2 (CH$_{3PMB}$), 53.0 (CH$_{2AZMB}$), 21.3 (CH$_{3STol}$); HRMS (ESI-TOF) *m/z* [M + Na]$^+$ calcd for $C_{36}H_{35}N_3NaO_7S$ 676.2088; found 676.2067.

***para*-Methylphenyl 2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (15).** DDQ (378 mg, 1.66 mmol, 2.0 equiv) was added to a solution of glucoside **18** (544 mg, 0.832 mmol, 1.0 equiv) in DCM (18 mL) and H_2O (1.7 mL) and the mixture was stirred at rt for 2 h. Saturated $NaHCO_3$(aq) (20 mL) was added to quench the solution, which was then diluted in EtOAc (30 mL) and transferred into a separatory funnel. The organic layer was washed with saturated $NaHCO_3$(aq) (30 mL) and brine (30 mL), dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 90:10 to 85:15) to give alcohol **15** (343 mg, 77%) as a white amorphous solid: R_f 0.5 (Hex/EtOAc 7:3); $[\alpha]_D^{20}$ –26 (*c* 0.7, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 8.05–8.04 (m, 1H, CH_{AZMB}), 7.60–7.58 (m, 1H, CH_{Ar}), 7.52–7.51 (m, 1H, CH_{Ar}), 7.49–7.47 (m, 2H, 2 × CH_{Ar}), 7.46–7.43 (m, 1H, CH_{Ar}), 7.37–7.36 (m, 5H, 2 × CH_{STol}, 3 × CH_{Ar}), 7.12–7.11 (m, 2H, 2 × CH_{STol}), 5.56 (s, 1H, H-7), 5.15 (dd, $J_{2\text{-}1}$ = 9.9 Hz, $J_{2\text{-}3}$ = 8.9 Hz, 1H, H-2), 4.85–4.76 (m, 3H, H-1, CH_{2AZMB}),

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4.41 (dd, $J_{6a\text{-}6b}$ = 10.5 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6a), 4.05 (t, J = 9.0 Hz, 1H, H-3), 3.82 (t, J = 10.2 Hz, 1H, H-6b), 3.61 (t, J = 9.3 Hz, 1H, H-4), 3.55 (ddd, $J_{5\text{-}6b}$ = 9.7 Hz, $J_{5\text{-}4}$ = 9.2 Hz, $J_{5\text{-}6a}$ = 4.9 Hz, 1H, H-5), 2.35 (s, 3H, C$H_{3\text{STol}}$); ^{13}C NMR (150 MHz, $CDCl_3$) : δ (ppm) 166.0 (COOR$_{\text{AZMB}}$), 138.9 (C$_{\text{Ar}}$), 137.3 (C$_{\text{Ar}}$), 136.9 (C$_{\text{Ar}}$), 133.7 (2C, 2 × CH$_{\text{STol}}$), 133.1 (CH$_{\text{Ar}}$), 131.3 (CH$_{\text{AZMB}}$), 130.0–126.4 (11C, 2 × C$_{\text{Ar}}$, 9 × CH$_{\text{Ar}}$), 102.1 (C-7), 86.7 (C-1), 80.6 (C-4), 73.7 (C-3), 73.4 (C-2), 70.6 (C-5), 68.6 (C-6), 53.3 (CH$_{\text{2AZMB}}$), 21.3 (CH$_{\text{3STol}}$); HRMS (ESI-TOF) *m/z* [M + Na]$^+$ calcd for $C_{28}H_{27}N_3NaO_6S$ 556.15128; found 556.15109; *m/z* [M+NH$_4$]$^+$ calcd for $C_{28}H_{31}N_4O_6S$ 551.19588; found 551.19581.

Allyl 2-*O*-acetyl-3-*O*-*tert*-butyldimethylsilyl-4-*O*-levulinoyl-α-L-rhamnopyranoside (20). TBSCl (1989 mg, 13.23 mmol, 4.0 equiv), imidazole (674 mg, 9.92 mmol, 3.0 equiv), and DMAP (81 mg, 0.7 mmol, 0.2 equiv) were successively added to a solution of diol **19**[26] (1000 mg, 3.307 mmol, 1.0 equiv) in anhydrous THF (80 mL). The mixture was refluxed for 16 h under Ar. The suspension was filtered over Celite, rinsed, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc) to give allyl 3-*O*-*tert*-butyldimethylsilyl-4-*O*-levulinoyl-α-L-rhamnopyranoside (1375 mg, quantitative) as a yellow oil: R_f 0.4 (Hex/EtOAc 7:3); $[\alpha]_D^{20}$ –40 (*c* 1.1, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ (ppm) 5.91 (dddd, $J_{2\text{-}3a}$ = 17.2 Hz, $J_{2\text{-}3b}$ = 10.4 Hz, $J_{2\text{-}1a}$ = 6.1 Hz, $J_{2\text{-}1b}$ = 5.1 Hz, 1H, H-2$_{\text{Allyl}}$), 5.29 (ddt, J = 17.2 Hz, 5.4 Hz, 1.6 Hz, 1H, H-3a$_{\text{Allyl}}$), 5.21 (ddt, J = 10.4 Hz, 4.8 Hz, 1.3 Hz, 1H, H-3b$_{\text{Allyl}}$), 4.97 (t, J = 9.6 Hz, 1H, H-4), 4.87 (d, J = 1.4 Hz, 1H, H-1), 4.17 (ddt, J = 13.1 Hz, 5.1 Hz, 1.5 Hz, 1H, H-1a$_{\text{Allyl}}$), 4.04–3.98 (m, 2H, H-3, H-1b$_{\text{Allyl}}$), 3.83 (dd, $J_{2\text{-}3}$ = 3.6 Hz, $J_{2\text{-}1}$ = 1.5 Hz, 1H, H-2), 3.76 (dq, $J_{5\text{-}4}$ = 9.8 Hz, $J_{5\text{-}6}$ = 6.3 Hz, 1H, H-5), 2.89–2.81 (m, 1H, CHH$_{\text{Lev}}$), 2.74–2.67 (m, 1H, CHH_{Lev}), 2.64 (m, 1H, CHH$_{\text{Lev}}$), 2.54–2.47 (m, 1H, CHH_{Lev}), 2.20 (s, 3H, C$H_{3\text{Lev}}$), 1.19 (d, J = 6.3 Hz, 3H, H-6), 0.88 (s, 9H, C(CH_3)$_{3\text{TBS}}$), 0.11 (s, 3H, C$H_{3\text{TBS}}$), 0.08 (s, 3H, C$H_{3\text{TBS}}$); ^{13}C NMR (176 MHz,

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$CDCl_3$) δ (ppm) 206.5 (CO_{Lev}), 172.1 ($COOR_{Lev}$), 133.9 (C-2_{Allyl}), 117.6 (C-3_{Allyl}), 98.0 (C-1), 74.3 (C-4), 71.8 (C-2), 70.6 (C-3), 68.2 (C-1_{Allyl}), 66.3 (C-5), 38.0 (CH_{2Lev}), 30.0 (CH_{3Lev}), 28.2 (CH_{2Lev}), 25.7 (3C, $C(CH_3)_{3TBS}$), 18.0 ($C(CH_3)_{3TBS}$), 17.5 (C-6), –4.5 (CH_{3TBS}), –4.7 (CH_{3TBS}); HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{20}H_{36}NaO_7Si$ 439.21225; found 439.21102. The latter alcohol (1000 mg, 2.400 mmol, 1.0 equiv) was dissolved in anhydrous pyridine (40 mL), and Ac_2O (40 mL) and DMAP (59 mg, 0.48 mmol, 0.2 equiv) were added. The mixture was stirred at rt for 16 h under Ar. The solution was diluted in toluene and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc) to give glycoside **20** (1013 mg, 92%) as a colorless oil: R_f 0.6 (Hex/EtOAc 7:3); $[\alpha]_D^{20}$ –23 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 5.90 (dddd, $J_{2\text{-}3a}$ = 16.6 Hz, $J_{2\text{-}3b}$ = 10.5 Hz, $J_{2\text{-}1a}$ = 6.0 Hz, $J_{2\text{-}1b}$ = 5.3 Hz, 1H, H-2_{Allyl}), 5.29 (ddt, *J* = 17.1 Hz, 3.6 Hz, 1.5 Hz, 1H, H-$3a_{Allyl}$), 5.22 (ddt, *J* = 10.4 Hz, 3.2 Hz, 1.3 Hz, 1H, H-$3b_{Allyl}$), 5.10 (dd, $J_{2\text{-}3}$ = 3.6 Hz, $J_{2\text{-}1}$ = 1.8 Hz, 1H, H-2), 4.98 (t, *J* = 9.7 Hz, 1H, H-4), 4.73 (d, *J* = 1.6 Hz, 1H, H-1), 4.16 (ddt, *J* = 13.0 Hz, 5.2 Hz, 1.4 Hz, 1H, H-$1a_{Allyl}$), 4.08 (dd, $J_{3\text{-}4}$ = 9.5 Hz, $J_{3\text{-}2}$ = 3.6 Hz, 1H, H-3), 3.99 (ddt, *J* = 13.0 Hz, 6.1 Hz, 1.3 Hz, 1H, H-$1b_{Allyl}$), 3.78 (dq, $J_{5\text{-}4}$ = 9.9 Hz, $J_{5\text{-}6}$ = 6.4 Hz, 1H, H-5), 2.88–2.82 (m, 1H, C*H*H_{Lev}), 2.70–2.62 (m, 2H, CH*H*$_{Lev}$, C*H*H_{Lev}), 2.52–2.47 (m, 1H, CH*H*$_{Lev}$), 2.20 (s, 3H, CH_{3Lev}), 2.11 (s, 3H, CH_{3Ac}), 1.20 (d, *J* = 6.3 Hz, 3H, H-6), 0.82 (s, 9H, $C(CH_3)_{3TBS}$), 0.07 (s, 3H, CH_{3TBS}), 0.05 (s, 3H, CH_{3TBS}); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.5 (CO_{Lev}), 172.0 ($COOR_{Lev}$), 170.5 ($COOR_{Ac}$), 133.7 (C-2_{Allyl}), 117.8 (C-3_{Allyl}), 96.9 (C-1), 74.5 (C-4), 72.3 (C-2), 68.4, 68.3 (2C, C-1_{Allyl}, C-3), 66.7 (C-5), 38.0 (CH_{2Lev}), 30.0 (CH_{3Lev}), 28.2 (CH_{2Lev}), 25.6 (3C, $C(CH_3)_{3TBS}$), 21.1 (CH_{3Ac}), 17.9, 17.6 (2C, C-6, $C(CH_3)_{3TBS}$), –4.7 (CH_{3TBS}), –4.9 (CH_{3TBS}); HRMS (ESI-TOF) *m/z* $[M + NH_4]^+$ calcd for $C_{22}H_{42}NO_8Si$ 476.26742; found 476.26647; *m/z* $[M + Na]^+$ calcd for $C_{22}H_{38}NaO_8Si$ 481.22282; found 481.22179.

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2-*O*-Acetyl-3-*O*-*tert*-butyldimethylsilyl-4-*O*-levulinoyl-L-rhamnopyranose (21). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)-iridium(I) hexafluorophosphate (132 mg, 0.156 mmol, 0.05 equiv) was dissolved in anhydrous THF (15 mL) and the red solution was degassed under Ar. Hydrogen was bubbled through the solution for 5 min and the resulting yellow solution was once again degassed under argon. A solution of rhamnoside **20** (1435 mg, 3.129 mmol, 1.0 equiv) in anhydrous THF (15 mL) was added and the reaction mixture was stirred for 2 h at rt under Ar. Then, a solution of iodine (1588 mg, 6.258 mmol, 2.0 equiv) in THF/H_2O (4:1, 18 mL) was added to the mixture, which was stirred for 1 h at rt. The excess of iodine was quenched by adding a freshly prepared 10% $Na_2S_2O_3$(aq) solution and stirred until the color turned bright yellow. THF was evaporated under reduced pressure and the resulting aqueous phase was extracted using EtOAc (3 × 25 mL). The combined organic layers were washed with saturated $NaHCO_3$(aq) (50 mL) and brine (50 mL). The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc) to give hemiacetal **21** (1100 mg, 84%, ratio α/β ~ 85:15) as a brown oil: R_f 0.6 (Tol/EtOAc 1:1); $[\alpha]_D^{20}$ –14 (*c* 1.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, α-anomer) δ (ppm) 5.11 (m, 2H, H-1, H-2), 4.98 (t, J = 9.6 Hz, 1H, H-4), 4.14 (dd, $J_{3\text{-}4}$ = 9.4 Hz, $J_{3\text{-}2}$ = 3.0 Hz, 1H, H-3), 4.01 (dq, $J_{5\text{-}4}$ = 9.7 Hz, $J_{5\text{-}6}$ = 6.3 Hz, 1H, H-5), 3.14 (br s, 1H, OH), 2.88–2.82 (m, 1H, C*H*H_{Lev}), 2.69–2.64 (m, 2H, CH*H*$_{Lev}$, C*H*H_{Lev}), 2.54–2.49 (m, 1H, CH*H*$_{Lev}$), 2.20 (s, 3H, C*H*$_{3Lev}$), 2.12 (s, 3H, C*H*$_{3Ac}$), 1.20 (d, J = 6.3 Hz, 3H, H-6), 0.83 (s, 9H, (C(C*H*$_3$)$_{3TBS}$), 0.08 (s, 3H, C*H*$_{3TBS}$), 0.06 (s, 3H, C*H*$_{3TBS}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.6 (*C*O_{Lev}), 172.1 (*C*OOR_{Lev}), 170.7 (*C*OOR_{Ac}), 92.4 (C-1), 74.5 (C-4), 72.6 (C-2), 67.9 (C-3), 66.9 (C-5), 38.0 (*C*H_{2Lev}), 30.0 (*C*H_{3Lev}), 28.2 (*C*H_{2Lev}), 25.6 (3C, C(*C*H_3)$_{3TBS}$), 21.1 (*C*H_{3Ac}), 17.9, 17.7 (2C, C-6, *C*$(CH_3)_{3TBS}$), –4.7 (*C*H_{3TBS}), –4.9 (*C*H_{3TBS}); HRMS

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(ESI-TOF) *m/z* $[M + NH_4]^+$ calcd for $C_{19}H_{38}NO_8Si$ 436.23612; found 436.2351; *m/z* $[M + Na]^+$ calcd for $C_{19}H_{34}NaO_8Si$ 441.19152; found 441.19063.

2-*O*-Acetyl-3-*O*-*tert*-butyldimethylsilyl-4-*O*-levulinoyl-α-L-rhamnopyranosyl 2,2,2-trichloroacetimidate (13). To a cooled (0 °C) solution of hemiacetal **21** (383 mg, 0.915 mmol, 1.0 equiv) in acetone (2.3 mL) and DCM (11.5 mL) were added Cs_2CO_3 (59 mg, 0.18 mmol, 0.2 equiv) and CCl_3CN (0.46 mL, 4.6 mmol, 5.0 equiv). The mixture was stirred for 1 h at rt, then the suspension was filtered over Celite and rinsed with DCM. The solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Tol/EtOAc + 1% Et_3N 95:5 to 9:1) to give trichloroacetimidate **13** (504 mg, 98%, $\alpha/\beta \sim 9{:}1$) as a yellow oil: R_f 0.3 (Tol/EtOAc 9:1 + 1% Et_3N); R_f 0.5 (Tol/AcOET 8:2 + 1% Et_3N); ^{1}H NMR (600 MHz, py-d_5, α-anomer) δ (ppm) 6.71 (br s, 1H, H-1), 5.75 (br s, 1H, H-2), 5.58 (t, J = 9.7 Hz, 1H, H-4), 4.60 (dd, $J_{3\text{-}4}$ = 9.6 Hz, $J_{3\text{-}2}$ = 3.5 Hz, 1H, H-3), 4.40 (dq, $J_{5\text{-}4}$ = 12.2 Hz, $J_{5\text{-}6}$ = 6.0 Hz, 1H, H-5), 2.96-2.73 (m, 4H, 2 × CH_{2Lev}), 2.08 (s, 3H, CH_{3Lev}), 2.03 (s, 3H, CH_{3Ac}), 1.48 (d, J = 6.2 Hz, 3H, H-6), 0.96 (s, 9H, C(CH_3)$_{3TBS}$), 0.26 (s, 3H, CH_{3TBS}), 0.24 (s, 3H, CH_{3TBS}); ^{13}C NMR (150 MHz, py-d_5) δ (ppm) 206.7 (CO$_{Lev}$), 172.9 (COOR$_{Lev}$), 170.4 (COOR$_{Ac}$), 159.3 (OCN), 95.8 (C-1), 91.8 (CCl$_3$), 74.3 (C-4), 71.5 (C-2), 70.7 (C-5), 69.5 (C-3), 38.4 (CH$_{2Lev}$), 30.0 (CH$_{3Lev}$), 29.1 (CH$_{2Lev}$), 26.2 (3C, C(CH$_3$)$_{3TBS}$), 21.0 (CH$_{3Ac}$), 18.6, 18.4 (C-6, C(CH$_3$)$_{3TBS}$), –4.3 (CH$_{3TBS}$), –4.4 (CH$_{3TBS}$); HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{21}H_{34}NaO_8Si$ 584.10115; found 584.10006.

(5-Azido-1-pentyl) 2-*O*-acetyl-3-*O*-*tert*-butyldimethylsilyl-4-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (22). To solution of trichloroacetimidate **13** (449 mg, 0.798 mmol, 1.5 equiv) in anhydrous Et_2O (7 mL) cooled at −10 °C were successively added glucoside **16** (250 mg, 0.532 mmol, 1.0 equiv) and TMSOTf (1.9 μL, 11 μmol, 0.02 equiv). The mixture was stirred under Ar at −10 °C for 15 min

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after which the solution was quenched with Et_3N (0.07 mL, 0.5 mmol, 1.0 equiv). The solvents were evaporated under reduced pressure and co-evaporated with toluene. The residue was purified by silica gel flash chromatography (Tol/EtOAc 95:5 to 80:20) to give disaccharide **22** (421 mg, 91%) as a white amorphous solid: R_f 0.7 (Tol/EtOAc 7:3); $[\alpha]_D^{20}$ –51 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.48–7.46 (m, 2H, 2 × CH_{Ar}), 7.38–7.34 (m, 3H, 3 × CH_{Ar}), 7.32–7.30 (m, 4H, 4 × CH_{Ar}), 7.28–7.27 (m, 1H, CH_{Ar}), 5.56 (s, 1H, H-7A), 5.20 (dd, $J_{2\text{-}3}$ = 3.6 Hz, $J_{2\text{-}1}$ = 1.6 Hz, 1H, H-2B), 5.13 (d, J = 1.3 Hz, 1H, H-1B), 4.87-4.84 (m, 2H, CHH$_{Bn}$, H-4B), 4.72 (d, J = 10.8 Hz, 1H, CHH_{Bn}), 4.49 (d, J = 7.8 Hz, 1H, H-1A), 4.36 (dd, $J_{6a\text{-}6b}$ = 10.5 Hz, $J_{6a\text{-}5}$ = 5.0 Hz, 1H, H6aA), 4.04 (dq, $J_{5\text{-}4}$ = 9.7 Hz, $J_{5\text{-}6}$ = 6.2 Hz, 1H, H-5B), 3.99 (dd, $J_{3\text{-}4}$ = 9.5 Hz, $J_{3\text{-}2}$ = 3.6 Hz, 1H, H-3B), 3.94–3.89 (m, 2H, H-1a$_{linker}$, H-3A), 3.78 (t, J = 10.3 Hz, 1H, H-6bA), 3.59–3.54 (m, 2H, H-1b$_{linker}$, H-4A), 3.46 (dd, J = 8.7 Hz, J = 7.9 Hz, 1H, H-2A), 3.42 (dt, J = 9.8 Hz, J = 5.0 Hz, 1H, H-5A), 3.22 (t, J = 6.9 Hz, 2H, H-5$_{linker}$), 2.78–2.73 (m, 1H, CHH$_{Lev}$), 2.64–2.59 (m, 1H, CHH_{Lev}), 2.54–2.49 (m, 1H, CHH$_{Lev}$), 2.47–2.42 (m, 1H, CHH_{Lev}), 2.18 (s, 3H, CH_{3Lev}), 2.04 (s, 3H, CH_{3Ac}), 1.69–1.63 (m, 2H, H-2$_{linker}$), 1.62–1.59 (m, 2H, H-4$_{linker}$), 1.50–1.41 (m, 2H, H-3$_{linker}$), 0.81 (s, 9H, C(CH_3)$_{3TBS}$), 0.78 (d, J = 6.3 Hz, 3H, H-6B), 0.08 (s, 3H, CH_{3TBS}), 0.04 (s, 3H, CH_{3TBS}); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.3 (*C*O$_{Lev}$), 171.9 (*C*OOR$_{Lev}$), 170.1 (*C*OOR$_{Ac}$), 138.0 (C$_{Ar}$), 137.3 (C$_{Ar}$), 129.3–126.3 (10C, 10 × *C*H$_{Ar}$), 104.3 (C-1A), 101.8 (C-7A), 98.2 (C-1B), 82.6 (C-2A), 79.3 (C-4A), 76.2 (C-3A), 74.9 (*C*H$_{2Bn}$), 74.5 (C-4B), 72.0 (C-2B), 70.2 (C-1$_{linker}$), 69.0 (C-6A), 68.3 (C-3B), 66.43, 66.37 (2C, C-5A, C-5B), 51.4 (C-5$_{linker}$), 37.9 (*C*H$_{2Lev}$), 30.1 (*C*H$_{3Lev}$), 29.5 (C-2$_{linker}$), 28.8 (C-4$_{linker}$), 28.2 (*C*H$_{2Lev}$), 25.6 (3C, C(*C*H$_3$)$_{3TBS}$), 23.5 (C-3$_{linker}$), 21.0 (*C*H$_{3Ac}$), 17.9 (*C*(CH$_3$)$_{3TBS}$), 17.0 (C-6B), –4.6 (*C*H$_{3TBS}$), –4.9 (*C*H$_{3TBS}$); HRMS (ESI-TOF) *m/z* [M + NH$_4$]$^+$ calcd for $C_{44}H_{67}N_4O_{13}Si$ 887.44684; found 887.44683; *m/z* [M + Na]$^+$ calcd for $C_{44}H_{63}NaO_{13}Si$ 892.4022; found 892.4035.

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***para*-Methylphenyl 4-*O*-levulinoyl-1-thio-α-L-rhamnopyranoside (24).** Levulinic acid (1.0 mL, 9.5 mmol, 1.15 equiv), DCC (1952 mg, 9.462 mmol, 1.15 equiv), and DMAP (101 mg, 0.823 mmol, 0.1 equiv) were added in a solution of alcohol **23**[45] (2554 mg, 8.228 mmol, 1 equiv) in anhydrous DCM (100 mL). The mixture was stirred under Ar at 50 °C for 2 h. The suspension was cooled to 0 °C, filtered over Celite, and rinsed with cold DCM. The solvents were evaporated under reduced pressure and co-evaporated with toluene. The residue was purified by silica gel flash chromatography (Tol/EtOAc 8:2) to give *para*-methylphenyl 4-*O*-levulinoyl-2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (3254 mg, 97%) as a white amorphous solid: R_f 0.5 (Tol/EtOAc 7:3); $[\alpha]_D^{20}$ –158 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.37–7.35 (m, 2H, 2 × CH_{STol}), 7.14–7.12 (m, 2H, 2 × CH_{STol}), 5.68 (s, 1H, H-1), 4.92 (dd, J = 9.9 Hz, J = 7.9 Hz, 1H, H-4), 4.35 (d, J = 5.3 Hz, 1H, H-2), 4.24–4.18 (m, 2H, H-3, H-5), 2.90–2.85 (m, 1H, CHH$_{Lev}$), 2.72–2.69 (m, 1H, CHH_{Lev}), 2.68–2.66 (m, 1H, CHH$_{Lev}$), 2.61–2.56 (m, 1H, CHH_{Lev}), 2.33 (s, 3H, CH_{3STol}), 2.19 (s, 3H, CH_{3Lev}), 1.55 (s, 3H, CH_{3iso}), 1.35 (s, 3H, CH_{3iso}), 1.15 (d, J = 6.3 Hz, 3H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.5 (CO$_{Lev}$), 172.1 (COOR$_{Lev}$), 138.2 (C_{STol}), 132.7 (2C, 2 × CH$_{STol}$), 130.0 (2C, 2 × CH$_{STol}$), 129.4 (C_{STol}), 110.1 (C_{iso}), 84.1 (C-1), 76.6 (C-2), 75.6 (C-3), 75.1 (C-4), 65.6 (C-5), 38.0 (CH$_{2Lev}$), 29.9 (CH$_{3Lev}$), 28.1 (CH$_{2Lev}$), 27.8 (CH$_{3iso}$), 26.7 (CH$_{3iso}$), 21.3 (CH$_{3STol}$), 16.9 (C-6); HRMS (ESI-TOF) *m/z* [M + NH$_4$]$^+$ calcd for $C_{21}H_{32}NO_6S$ 426.1945; found 426.1957; *m/z* [M + Na]$^+$ calcd for $C_{21}H_{28}NaO_6S$ 431.1499; found 431.1512. The latter thiorhamnoside (3239 mg, 7.930 mmol, 1.0 equiv) was dissolved in 80% AcOH(aq) (99 mL) and the solution was stirred at 60 °C for 2 h. The solvents were evaporated under reduced pressure and co-evaporated with toluene. The residue was purified by silica gel flash chromatography (Hex/EtOAc 1:1 to 2:8) to give diol **24** (2615 mg, 90%) as a white amorphous solid: R_f 0.5 (DCM/MeOH 95:5); $[\alpha]_D^{20}$ –230 (*c* 0.7, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.36–7.34

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(m, 2H, 2 × CH_{STol}), 7.13–7.11 (m, 2H, 2 × CH_{STol}), 5.46 (d, J = 0.6 Hz, 1H, H-1), 4.95 (t, J = 9.6 Hz, 1H, H-4), 4.29 (dq, $J_{5\text{-}4}$ = 9.8 Hz, $J_{5\text{-}6}$ = 6.2 Hz, 1H, H-5), 4.22 (br s, 1H, H-2), 3.95–3.94 (m, 1H, H-3), 3.48 (d, J = 4.4 Hz, 1H, O$H_{C\text{-}3}$), 3.06 (br s, 1H, O$H_{C\text{-}2}$), 2.88–2.79 (m, 2H, CH_{2Lev}), 2.65–2.56 (m, 2H, CH_{2Lev}), 2.33 (s, 3H, CH_{3STol}), 2.20 (s, 3H, CH_{3Lev}), 1.22 (d, J = 6.3 Hz, 3H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 207.7 (CO$_{Lev}$), 173.6 (COOR$_{Lev}$), 137.9 (C_{STol}), 132.1 (2C, 2 × CH$_{STol}$), 130.2 (C_{STol}), 130.0 (2C, 2 × CH$_{STol}$), 87.8 (C-1), 75.8 (C-4), 72.4 (C-2), 70.7 (C-3), 67.0 (C-5), 38.5 (CH$_{2Lev}$), 29.9 (CH$_{3Lev}$), 28.4 (CH$_{2Lev}$), 21.3 (CH$_{3STol}$), 17.4 (C-6); HRMS (ESI-TOF) *m/z* [M + NH_4]$^+$ calcd for $C_{18}H_{28}NO_6S$ 386.1632; found 386.1641; *m/z* [M + Na]$^+$ calcd for $C_{18}H_{24}NaO_6S$ 391.1186; found 391.1197.

***para*-Methylphenyl 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-*para*-methoxybenzyl-1-thio-α-L-rhamnopyranoside (25).** Bu_2SnO (1536 mg, 6.170 mmol, 1.1 equiv) was added to a solution of compound **24** (2067 mg, 5.609 mmol, 1.0 equiv) in toluene (67 mL) and the mixture was refluxed with a Dean-Stark trap for 2 h. The solution was cooled to rt and CsF (895 mg, 5.89 mmol, 1.05 equiv), TBAI (2175 mg, 5.889 mmol, 1.05 equiv), and PMBCl (0.91 mL, 5.9 mmol, 1.2 equiv) were successively added. The mixture was stirred under Ar at 40 °C for 16 h. The suspension was cooled at 0 °C, filtered over Celite, and rinsed with DCM. The solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 9:1 to 7:3) to give *para*-methylphenyl 4-*O*-levulinoyl-3-*O*-*para*-methoxybenzyl-1-thio-α-L-rhamnopyranoside (2412 mg, 88%) as a yellow oil: R_f 0.6 (Hex/EtOAc 4:6); $[\alpha]_D^{20}$ –140 (*c* 1.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.33–7.32 (m, 2H, 2 × CH_{STol}), 7.27–7.26 (m, 2H, 2 × CH_{PMB}), 7.12–7.10 (m, 2H, 2 × CH_{STol}), 6.91–6.89 (m, 2H, 2 × CH_{PMB}), 5.46 (d, J = 1.2 Hz, 1H, H-1), 5.08 (t, J = 9.6 Hz, 1H, H-4), 4.59 (d, J = 11.7 Hz, 1H, CHH_{PMB}), 4.53 (d, J = 11.8 Hz, 1H, CHH$_{PMB}$), 4.22 (dq, $J_{5\text{-}4}$ = 9.9 Hz, $J_{5\text{-}6}$ = 6.2 Hz, 1H, H-5), 4.18 (dd, $J_{2\text{-}3}$ = 3.0 Hz, $J_{2\text{-}1}$ = 1.5 Hz,

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1H, H-2), 3.81 (s, 3H, CH_{3PMB}), 3.75 (dd, $J_{3\text{-}4}$ = 9.4 Hz, $J_{3\text{-}2}$ = 3.3 Hz, 1H, H-3), 2.77–2.72 (m, 2H, CH_{2Lev}), 2.59–2.49 (m, 2H, CH_{2Lev}), 2.32 (s, 3H, CH_{3STol}), 2.19 (s, 3H, CH_{3Lev}), 1.18 (d, J = 6.3 Hz, 3H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.5 (CO$_{Lev}$), 172.1 (COOR$_{Lev}$), 159.6 (C$_{Ar}$), 137.8 (C$_{Ar}$), 132.1 (2C, 2 × CH$_{STol}$), 130.0, 129.7 (5C, C$_{Ar}$, 2 × CH$_{STol}$, 2 × CH$_{PMB}$), 129.6 (C$_{Ar}$), 114.1 (2C, 2 × CH$_{PMB}$), 87.3 (C-1), 76.7 (C-3), 73.0 (C-4), 71.7 (CH$_{2PMB}$), 69.9 (C-2), 67.6 (C-5), 55.4 (CH$_{3PMB}$), 37.9 (CH$_{2Lev}$), 30.0 (CH$_{3Lev}$), 28.1 (CH$_{2Lev}$), 21.2 (CH$_{3STol}$), 17.4 (C-6); HRMS (ESI-TOF) *m/z* [M + NH$_4$]$^+$ calcd for $C_{26}H_{36}NO_7S$ 506.2207; found 506.22041; *m/z* [M + Na]$^+$ calcd for $C_{26}H_{32}NaO_7S$ 511.1761; found 511.17561. The latter alcohol (2249 mg, 4.603 mmol, 1.0 equiv) was dissolved in anhydrous pyridine (23 mL) and Ac_2O (23 mL), and DMAP (6 mg, 0.05 mmol, 0.01 equiv) was added. The solution was stirred under Ar at rt for 16 h, then the solvents were evaporated under reduced pressure and co-evaporated with toluene. The residue was purified by silica gel flash chromatography (Hex/EtOAc 9:1 to 7:3) to give compound **25** (2378 mg, 97%) as a white amorphous solid: R_f 0.6 (Hex/EtOAc 1:1); $[\alpha]_D^{20}$ –37 (*c* 1.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.33–7.32 (m, 2H, 2 × CH_{STol}), 7.24–7.23 (m, 2H, 2 × CH_{PMB}), 7.12–7.11 (m, 2H, 2 × CH_{STol}), 6.90–6.88 (m, 2H, 2 × CH_{PMB}), 5.56 (dd, $J_{2\text{-}3}$ = 3.2 Hz, $J_{2\text{-}1}$ = 1.6 Hz, 1H, H-2), 5.34 (d, J = 1.4 Hz, 1H, H-1), 5.05 (t, J = 9.8 Hz, 1H, H-4), 4.58 (d, J = 11.9 Hz, 1H, CHH$_{PMB}$), 4.38 (d, J = 11.9 Hz, 1H, CHH_{PMB}), 4.24 (dq, $J_{5\text{-}4}$ = 9.9 Hz, $J_{5\text{-}6}$ = 6.2 Hz, 1H, H-5), 3.81 (s, 3H, CH_{3PMB}), 3.77 (dd, $J_{3\text{-}4}$ = 9.7 Hz, $J_{3\text{-}2}$ = 3.2 Hz, 1H, H-3), 2.80–2.75 (m, 1H, CHH$_{Lev}$), 2.70–2.65 (m, 1H, CHH_{Lev}), 2.60–2.55 (m, 1H, CHH$_{Lev}$), 2.52–2.47 (m, 1H, CHH_{Lev}), 2.33 (s, 3H, CH_{3STol}), 2.18 (s, 3H, CH_{3Lev}), 2.12 (s, 3H, CH_{3Ac}), 1.21 (d, J = 6.3 Hz, 1H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.5 (CO$_{Lev}$), 172.1 (COOR$_{Lev}$), 170.4 (COOR$_{Ac}$), 159.5 (C$_{Ar}$), 138.2 (C$_{Ar}$), 132.4 (2C, 2 × CH$_{STol}$), 130.1, 129.8 (5C, C$_{Ar}$, 2 × CH$_{STol}$, 2 × CH$_{PMB}$), 129.7 (C$_{Ar}$), 113.9 (2C, 2 x CH$_{PMB}$), 86.6 (C-1), 74.2 (C-3), 72.9 (C-4), 71.1 (CH$_{2PMB}$), 70.2 (C-2), 68.0 (C-5), 55.4 (CH$_{3PMB}$),

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38.0 (CH_{2Lev}), 30.0 (CH_{3Lev}), 28.1 (CH_{2Lev}), 21.3 (CH_3), 21.2 (CH_3), 17.5 (C-6); HRMS (ESI-TOF) *m/z* $[M + NH_4]^+$ calcd for $C_{28}H_{38}NO_8S$ 548.23216; found 548.23176; *m/z* $[M + Na]^+$ calcd for $C_{28}H_{34}NaO_8S$ 553.18666; found 553.1874.

(5-Azido-1-pentyl) 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-*para*-methoxybenzyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (26). Alcohol **16**[26] (702 mg, 1.50 mmol, 1.0 equiv), donor **25** (952 mg, 1.79 mmol, 1.2 equiv), and NIS (505 mg, 2.24 mmol, 1.5 equiv) were dried together under high vacuum for 1 h. Activated ground molecular sieves (4 Å, 2808 mg) and anhydrous DCM (30 mL) were successively added and the mixture was stirred under Ar for 1 h. The reaction flask was cooled to −10 °C and protected from light using aluminum foil. AgOTf (38 mg, 0.15 mmol, 0.1 equiv) was added and the mixture was stirred under Ar for 1 h while being gradually warmed to 0 °C. Et_3N (0.21 mL, 1.5 mmol, 1.0 equiv) was added, the yellow suspension was filtered over Celite, and the solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 8:2 to 6:4) to give disaccharide **26** (1177 mg, 90%) as a white amorphous solid: R_f 0.6 (Hex/EtOAc 1:1); $[\alpha]_D^{20}$ –31 (*c* 0.6, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.45–7.43 (m, 2H, 2 × CH_{Ar}), 7.35-7.28 (m, 8H, 8 × CH_{Ar}), 7.19–7.18 (m, 2H, 2 × CH_{PMB}), 6.85–6.83 (m, 2H, 2 × CH_{PMB}), 5.52 (s, 1H, H-7A), 5.43 (dd, $J_{2\text{-}3}$ =3.2 Hz, $J_{2\text{-}1}$ = 1.6 Hz, 1H, H-2B), 5.17 (d, *J* = 1.0 Hz, 1H, H-1B), 4.90–4.85 (m, 2H, H-4B, CHH$_{Ph}$), 4.69 (d, *J* = 10.8 Hz, 1H, CHH_{Ph}), 4.58 (d, *J* = 11.5 Hz, 1H, CHH$_{Ph}$), 4.50 (d, *J* = 7.8 Hz, 1H, H-1A), 4.35 (dd, $J_{6a\text{-}6b}$ = 10.5 Hz, $J_{6a\text{-}5}$ = 5.0 Hz, 1H, H-6aA), 4.33 (d, *J* = 11.6 Hz, 1H, CHH_{Ph}), 4.09–4.04 (m, 1H, H-5B), 3.96–3.92 (m, 1H, H-1a$_{linker}$), 3.90 (t, *J* = 9.2 Hz, 1H, H-3A), 3.79–3.75 (m, 5H, H-3B, H-6bA, CH_{3PMB}), 3.59–3.56 (m, 1H, H-1b$_{linker}$), 3.53 (t, *J* = 9.5 Hz, 1H, H-4A), 3.46 (t, *J* = 8.1 Hz, 1H, H-2A), 3.44–3.39 (m, 1H, H-5A), 3.23 (t, *J* = 6.9 Hz, 2H, H-5$_{linker}$), 2.71–2.66 (m, 1H, CHH$_{Lev}$), 2.62–2.57 (m, 1H, CHH_{Lev}), 2.48–2.43 (m, 1H, CHH$_{Lev}$),

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2.42–2.38 (m, 1H, CHH_{Lev}), 2.15 (s, 3H, CH_{3Lev}), 2.05 (s, 3H, CH_{3Ac}), 1.70–1.65 (m, 2H, H-2$_{linker}$), 1.64–1.59 (m, 2H, H-4$_{linker}$), 1.50–1.44 (m, 2H, H-3$_{linker}$), 0.78 (d, J = 6.2 Hz, 3H, H-6B); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.4 (CO_{Lev}), 172.0 ($COOR_{Lev}$), 170.2 ($COOR_{Ac}$), 159.3 (C_{Ar}), 137.9 (C_{Ar}), 137.2 (C_{Ar}), 130.4 (C_{Ar}), 129.5–126.3 (12C, 12 × CH_{Ar}), 113.8 (2C, 2 × CH_{PMB}), 104.3 (C-1A), 101.8 (C-7A), 98.4 (C-1B), 82.8 (C-2A), 79.3 (C-4A), 76.3 (C-3A), 75.0 (CH_{2Ph}), 74.6 (C-3B), 72.9 (C-4B), 71.1 (CH_{2Ph}), 70.2 (C-1$_{linker}$), 68.9 (C-6A), 68.5 (C-2B), 66.5 (2C, C-5A, C-5B), 55.4 (CH_{3PMB}), 51.4 (C-5$_{linker}$), 37.9 (CH_{2Lev}), 30.0 (CH_{3Lev}), 29.5 (C-2$_{linker}$), 28.8 (C-4$_{linker}$), 28.1 (CH_{2Lev}), 23.5 (C-3$_{linker}$), 21.1 (CH_{3Ac}), 17.0 (C-6B); HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{46}H_{57}N_3NaO_{14}$ 898.3733; found 898.3756; *m/z* $[M + NH_4]^+$ calcd for $C_{46}H_{61}N_4O_{14}$ 893.4179; found 893.4196.

(5-Azido-1-pentyl) 2-*O*-acetyl-4-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (12). DDQ (609 mg, 2.68 mmol, 2.0 equiv) was added to a solution of disaccharide **26** (1175 mg, 1.342 mmol, 1.0 equiv) in DCM (27 mL) and H_2O (2.7 mL), and the mixture was stirred at rt for 2 h. Saturated $NaHCO_3$(aq) (40 mL) was added to quench the solution, which was then diluted in EtOAc (30 mL) and transferred into a separatory funnel. The organic layer was washed with saturated $NaHCO_3$(aq) (40 mL) and brine (40 mL), dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 9:1 to 1:1) to give alcohol **12** (878 mg, 87%) as a white amorphous solid: R_f 0.3 (Hex/EtOAc 4:6); $[\alpha]_D^{20}$ –43 (*c* 0.7, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.47–7.46 (m, 2H, 2 × CH_{Ar}), 7.35–7.34 (m, 3H, 3 × CH_{Ar}), 7.32–7.31 (m, 4H, 4 × CH_{Ar}), 7.28–7.27 (m, 1H, CH_{Ar}), 5.52 (s, 1H, H-7A), 5.22–5.21 (m, 2H, H-1B, H-2B), 4.87 (d, J = 10.8 Hz, 1H, CHH$_{Bn}$), 4.79–4.73 (m, 2H, H-4B, CHH_{Bn}), 4.49 (d, J = 7.8 Hz, 1H, H-1A), 4.35 (dd, $J_{6a\text{-}6b}$ = 10.5 Hz, $J_{6a\text{-}5}$ = 5.0 Hz, 1H, H-6aA), 4.13 (dq, $J_{5\text{-}4}$ = 10.1 Hz, $J_{5\text{-}6}$ = 6.2 Hz, 1H, H-5B), 4.04

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(dd, $J_{3\text{-}4}$ = 9.8 Hz, $J_{3\text{-}2}$ = 3.2 Hz, 1H, H-3B), 3.95-3.90 (m, 2H, H-3A, H-1a$_{linker}$), 3.77 (t, $J_{6b\text{-}6a}$ = 10.3 Hz, 1H, H-6bA), 3.57-3.53 (m, 2H, H-4A, H-1b$_{linker}$), 3.47–3.40 (m, 2H, H-2A, H-5A), 3.22 (t, J = 6.9 Hz, 2H, H-5$_{linker}$), 2.77–2.69 (m, 2H, CH_{2Lev}), 2.51–2.49 (m, 2H, CH_{2Lev}), 2.16 (s, 3H, CH_{3Lev}), 2.07 (s, 3H, CH_{3Ac}), 1.68–1.63 (m, 2H, H-2$_{linker}$), 1.62–1.58 (m, 2H, H-4$_{linker}$), 1.48–1.41 (m, 2H, H-3$_{linker}$), 0.82 (d, J = 6.2 Hz, 3H, H-6B); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 207.0 (CO_{Lev}), 173.2 (COOR$_{Lev}$), 170.5 (COOR$_{Ac}$), 138.1 (C$_{Ar}$), 137.3 (C$_{Ar}$), 129.2 (CH$_{Ar}$), 128.4 (2C, 2 × CH$_{Ar}$), 128.3 (2C, 2 × CH$_{Ar}$), 128.3 (2C, 2 × CH$_{Ar}$), 127.9 (CH$_{Ar}$), 126.4 (2C, 2 × CH$_{Ar}$), 104.3 (C-1A), 101.8 (C-7A), 97.8 (C-1B), 82.9 (C-2A), 79.3 (C-4A), 76.1 (C-3A), 75.2, 75.0 (2C, C-4B, CH$_{2Bn}$), 72.3 (C-2B), 70.2 (C-1$_{linker}$), 68.9 (C-6A), 68.6 (C-3B), 66.4 (C-5A), 65.8 (C-5B), 51.4 (C-5$_{linker}$), 38.2 (CH$_{2Lev}$), 29.9 (CH$_{3Lev}$), 29.4 (C-2$_{linker}$), 28.7 (C-4$_{linker}$), 28.2 (CH$_{2Lev}$), 23.5 (C-3$_{linker}$), 21.1 (CH$_{3Ac}$), 16.9 (C-6B); HRMS (ESI-TOF) *m/z* [M + Na]$^+$ calcd for $C_{38}H_{49}N_3NaO_{13}$ 778.3158; found 778.3159; *m/z* [M + K]$^+$ calcd for $C_{38}H_{49}KN_3O_{13}$ 794.2897; found 794.292.

(5-Azido-1-pentyl) 2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-3-*O*-*para*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (27). Disaccharide **12** (583 mg, 0.772 mmol, 1.0 equiv), thioglucoside **18** (757 mg, 1.16 mmol, 1.5 equiv), and NIS (260 mg, 1.16 mmol, 1.5 equiv) were dried together under high vacuum for 1 h. Activated ground molecular sieves (4 Å, 2333 mg) and anhydrous DCM (15 mL) were successively added and the mixture was stirred under Ar for 1 h. The reaction flask was cooled to −10 °C and protected from light using aluminum foil. AgOTf (20 mg, 0.077 mmol, 0.1 equiv) was added and the mixture was stirred under Ar for 2 h while being gradually warmed to 0 °C. Et_3N (0.11 mL, 0.77 mmol, 1.0 equiv) was added, the yellow suspension was filtered over Celite, and the solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 8:2 to

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7:3) to give trisaccharide **27** (773 mg, 78%) as a white solid foam along with an inseparable unknown impurity (10%): R_f 0.7 (Hex/EtOAc 6:4); $[\alpha]_D^{20}$ –28 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.71–7.69 (m, 1H, CH_{AZMB}), 7.56–7.55 (m, 1H, CH_{Ar}), 7.51–7.50 (m, 2H, 2 × CH_{Ar}), 7.41–7.25 (m, 15H, 15 × CH_{Ar}), 7.07–7.05 (m, 2H, 2 × CH_{PMB}), 6.63–6.61 (m, 2H, 2 × CH_{PMB}), 5.60 (s, 1H, H-7C), 5.51 (s, 1H, H-7A), 5.35 (dd, $J_{2\text{-}3}$ = 3.4 Hz, $J_{2\text{-}1}$ = 1.5 Hz, 1H, H-2B), 5.19 (d, J = 1.3 Hz, 1H, H-1B), 5.13 (dd, $J_{2\text{-}3}$ = 8.5 Hz, $J_{2\text{-}1}$ = 7.6 Hz, 1H, H-2C), 4.89 (t, J = 10.0 Hz, 1H, H-4B), 4.86 (d, J = 10.7 Hz, 1H, CH_{Bn}), 4.78–4.75 (m, 2H, CH_{Bn}, CH_{PMB}), 4.72 (d, J = 15.1 Hz, 1H, CH_{AZMB}), 4.71 (d, J = 7.5 Hz, 1H, H-1C), 4.63 (d, J = 15.1 Hz, 1H, CH_{AZMB}), 4.59 (d, J = 11.7 Hz, 1H, CH_{PMB}), 4.49 (d, J = 7.8 Hz, 1H, H-1A), 4.37 (dd, $J_{6a\text{-}6b}$ = 10.5 Hz, $J_{6a\text{-}5}$ = 5.0 Hz, 1H, H-6aC), 4.34 (dd, $J_{6b\text{-}6a}$ = 10.5 Hz, $J_{6b\text{-}5}$ = 4.9 Hz, 1H, H-6aA), 4.06 (dq, $J_{5\text{-}4}$ = 10.0 Hz, $J_{5\text{-}6}$ = 6.1 Hz, 1H, H-5B), 4.02 (dd, $J_{3\text{-}4}$ = 9.8 Hz, $J_{3\text{-}2}$ = 3.5 Hz, 1H, H-3B), 3.93–3.90 (m, 2H, H-3A, H-1a$_{linker}$), 3.83–3.81 (m, 2H, H-3C, H-4C), 3.80–3.75 (m, 2H, H-6bA, H-6bC), 3.70 (s, 3H, CH_{3PMB}), 3.58–3.53 (m, 2H, H-1b$_{linker}$, H-4A), 3.51–3.46 (m, 2H, H-2A, H-5C), 3.40 (ddd, $J_{5\text{-}4,\ 5\text{-}6b}$ = 9.6 Hz, $J_{5\text{-}6a}$= 5.0 Hz, 1H, H-5A), 3.21 (t, J = 6.9 Hz, 2H, H-5$_{linker}$), 2.30–2.24 (m, 1H, CH_{Lev}), 2.13–2.09 (m, 1H, CH_{Lev}), 2.07 (s, 3H, CH_{3Ac}), 2.04–2.02 (m, 1H, CH_{Lev}), 2.01 (s, 3H, CH_{3Lev}), 1.82–1.77 (m, 1H, CH_{Lev}), 1.67–1.63 (m, 2H, H-2$_{linker}$), 1.62–1.57 (m, 2H, H-4$_{linker}$), 1.49–1.41 (m, 2H, H-3$_{linker}$), 0.75 (d, J = 6.2 Hz, 3H, H-6B); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.1 (CO_{Lev}), 171.4 ($COOR_{Lev}$), 170.0 ($COOR_{Ac}$), 164.7 ($COOR_{AZMB}$), 159.2 (C_{Ar}), 138.2 (C_{Ar}), 138.0 (C_{Ar}), 137.3 (C_{Ar}), 137.1 (C_{Ar}), 132.7 (CH_{AZMB}), 130.7 (CH_{Ar}), 130.1 (C_{Ar}), 129.7 (2C, 2 × CH_{PMB}), 129.2 (CH_{Ar}), 129.1–126.1 (17C, C_{Ar}, 16 × CH_{Ar}), 113.6 (2C, 2 × CH_{PMB}), 104.2 (C-1A), 101.7 (C-7A), 101.3 (C-7C), 101.1 (C-1C), 97.8 (C-1B), 82.7 (C-2A), 81.6 (C-4C), 79.2 (C-4A), 77.6 (C-3C), 76.0 (C-3A), 74.9 (2C, C-3B, CH_{2Bn}), 73.6, 73.5 (2C, C-2C, CH_{2PMB}), 72.5 (C-4B), 71.0 (C-2B), 70.2 (C-1$_{linker}$), 68.9, 68.7 (2C, C-6A, C-6C), 66.3, 66.17, 66.15 (3C, C-5A, C-5B, C-5C),

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55.2 (CH_{3PMB}), 52.9 (CH_{2AZMB}), 51.3 (C-5_{linker}), 37.5 (CH_{2Lev}), 29.7 (CH_{3Lev}), 29.4 (C-2_{linker}), 28.7 (C-4_{linker}), 27.4 (CH_{2Lev}), 23.5 (C-3_{linker}), 21.0 (CH_{3Ac}), 16.7 (C-6B); HRMS (ESI-TOF) *m/z* $[M + NH_4]^+$ calcd for $C_{67}H_{80}N_7O_{20}$ 1302.5453; found 1302.5453; *m/z* $[M + Na]^+$ calcd for $C_{67}H_{76}N_6NaO_{20}$ 1307.5007; found 1307.5003.

(5-Azido-1-pentyl) 2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (28). Trisaccharide **27** (99 mg, 0.077 mmol, 1.0 equiv) was dissolved in DCM (1.6 mL) and H_2O (0.2 mL) and DDQ (35 mg, 0.16 mmol, 2.0 equiv) was added. The mixture was stirred at rt for 2 h, then quenched with saturated $NaHCO_3$(aq) (2 mL). The solution was transferred into a separatory funnel, DCM (10 mL) was added, and the organic and aqueous layers were separated. The aqueous phase was extracted with DCM (2 × 10 mL). The combined organic phases were washed with saturated $NaHCO_3$(aq) (20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 8:2 to 4:6) to give alcohol **28** (68 mg, 75%) as a white solid foam: R_f 0.6 (Hex/EtOAc 1:1); $[\alpha]_D^{20}$ –62 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.89–7.88 (m, 1H, CH_{AZMB}), 7.56–7.54 (m, 1H, CH_{Ar}), 7.51–7.49 (m, 3H, 3 × CH_{Ar}), 7.44–7.43 (m, 2H, 2 × CH_{Ar}), 7.39–7.28 (m, 12H, 12 × CH_{Ar}), 5.54 (s, 1H, H-7C), 5.53 (s, 1H, H-7A), 5.36 (dd, $J_{2\text{-}3}$ = 1.9 Hz, $J_{2\text{-}1}$ = 1.0 Hz, 1H, H-2B), 5.20 (br s, 1H, H-1B), 5.08 (dd, $J_{2\text{-}3}$ = 8.6 Hz, $J_{2\text{-}1}$ = 7.8 Hz, 1H, H-2C), 4.92 (t, *J* = 9.9 Hz, 1H, H-4B), 4.86 (d, *J* = 10.7 Hz, 1H, CHH$_{Bn}$), 4.77 (d, *J* = 10.7 Hz, 1H, CHH_{Bn}), 4.75 (d, *J* = 14.8 Hz, 1H, CHH$_{AZMB}$), 4.74 (d, *J* = 7.4 Hz, 1H, H-1C), 4.69 (d, *J* = 14.7 Hz, 1H, CHH_{AZMB}), 4.48 (d, *J* = 7.7 Hz, 1H, H-1A), 4.37–4.33 (m, 2H, H-6aA, H-6aC), 4.09 (dq, $J_{5\text{-}4}$ = 10.1 Hz, $J_{5\text{-}6}$ = 5.8 Hz, 1H, H-5B), 4.05 (dd, $J_{3\text{-}4}$ = 9.8 Hz, $J_{3\text{-}2}$ = 3.5 Hz, 1H, H-3B), 3.99 (t, *J* = 9.0 Hz, 1H, H-3C), 3.94–3.89 (m, 2H, H-1a$_{linker}$, H-3A), 3.76 (t, *J* = 10.2 Hz,

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2H, H-6bA, H-6bC), 3.62 (t, J = 9.3 Hz, 1H, H-4C), 3.58–3.53 (m, 2H, H-1b$_{linker}$, H-4A), 3.51–3.46 (m, 2H, H-2A, H-5C), 3.40 (td, $J_{5\text{-}4,\ 5\text{-}6b}$ = 9.7 Hz, $J_{5\text{-}6a}$ = 5.0 Hz, 1H, H-5A), 3.21 (t, J = 6.9 Hz, 2H, H-5$_{linker}$), 2.87 (br s, 1H, O*H*), 2.28–2.22 (m, 1H, C*H*H$_{Lev}$), 2.17–2.12 (m, 1H, C*H*H$_{Lev}$), 2.09 (s, 3H, C*H*$_{3Ac}$), 2.07–2.03 (m, 1H, CH*H*$_{Lev}$), 2.01 (s, 3H, C*H*$_{3Lev}$), 1.88–1.84 (m, 1H, CH*H*$_{Lev}$), 1.65 (quint, J = 7.1 Hz, 2H, H-2$_{linker}$), 1.59 (quint, J = 7.3 Hz, 2H, H-4$_{linker}$), 1.48–1.41 (m, 2H, H-3$_{linker}$), 0.78 (d, J = 6.2 Hz, 3H, H-6B); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.2 (*C*O$_{Lev}$), 171.5 (*C*OOR$_{Lev}$), 170.0 (*C*OOR$_{Ac}$), 165.6 (*C*OOR$_{AZMB}$), 138.0 (C$_{Ar}$), 137.5 (C$_{Ar}$), 137.1 (C$_{Ar}$), 137.0 (C$_{Ar}$), 132.7–126.2 (20C, C$_{Ar}$, 19 × *C*H$_{Ar}$), 104.2 (C-1A), 101.9, 101.7 (2C, C-7A, C-7C), 101.0 (C-1C), 97.7 (C-1B), 82.6 (C-2A), 80.7 (C-4C), 79.2 (C-4A), 76.1 (C-3A), 75.0, 74.9, 74.8 (3C, *C*H$_{2Bn}$, C-3B, C-2C), 72.7 (C-4B), 72.2 (C-3C), 71.1 (C-2B), 70.2 (C-1$_{linker}$), 68.9, 68.6 (2C, C-6A, C-6C), 66.3, 66.2 (3C, C-5A, C-5B, C-5C), 53.0 (*C*H$_{2AZMB}$), 51.3 (C-5$_{linker}$), 37.4 (*C*H$_{2Lev}$), 29.7 (*C*H$_{3Lev}$), 29.4 (C-2$_{linker}$), 28.7 (C-4$_{linker}$), 27.5 (*C*H$_{2Lev}$), 23.4 (C-3$_{linker}$), 21.0 (*C*H$_{3Ac}$), 16.8 (C-6B); HRMS (ESI-TOF) *m/z* [M + Na]$^+$ calcd for $C_{59}H_{68}N_6NaO_{19}$ 1187.4431; found 1187.4412.

***para*-Methylphenyl 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-methyl-1-thio-α-L-rhamnopyranoside (29).** Bu_2SnO (1557 mg, 6.254 mmol, 1.1 equiv) was added to a solution of diol **24** (2095 mg, 5.686 mmol, 1.0 equiv) in toluene (23 mL) and the mixture was refluxed with a Dean-Stark trap for 5 h. The solution was cooled to rt, and CsF (881 mg, 5.80 mmol, 1.02 equiv) and MeI (17.7 mL, 284 mmol, 50.0 equiv) were successively added. The mixture was stirred under Ar at 80 °C for 16 h. The suspension was cooled at 0 °C, filtered over Celite, and rinsed with DCM. The solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 1:1) to give *para*-methylphenyl 4-*O*-levulinoyl-3-*O*-methyl-1-thio-α-L-rhamnopyranoside (2019 mg, 93%, ~83:17 mixture with its inseparable 2-*O*-methyl anomer) as a colorless oil: R_f 0.3 (Hex/EtOAc 4:6); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.36–7.35 (m, 2H,

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2 × CH_{STol}), 7.13–7.11 (m, 2H, 2 × CH_{STol}), 5.49 (d, J = 1.4 Hz, 1H, H-1), 5.05 (t, J = 9.5 Hz, 1H, H-4), 4.31 (dd, J_{2-3} = 3.2 Hz, J_{2-1} = 1.6 Hz, 1H, H-2), 4.27 (dq, J_{5-4} = 9.8 Hz, J_{5-6} = 6.2 Hz, 1H, H-5), 3.53 (dd, J_{3-4} = 9.4 Hz, J_{3-2} = 3.3 Hz, 1H, H-3), 3.45 (s, 3H, CH_{3OMe}), 2.82–2.77 (m, 2H, CH_{2Lev}), 2.63–2.60 (m, 2H, CH_{2Lev}), 2.33 (s, 3H, CH_{3STol}), 2.20 (s, 3H, CH_{3Lev}), 1.20 (d, J = 6.3 Hz, 3H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.6 (CO$_{Lev}$), 172.2 (COOR$_{Lev}$), 137.8 (C$_{STol}$), 132.1 (2C, 2 × CH$_{STol}$), 130.02 (C$_{STol}$), 129.97 (2C, 2 × CH$_{STol}$), 87.4 (C-1), 79.2 (C-3), 73.0 (C-4), 69.2 (C-2), 67.4 (C-5), 57.8 (CH$_{3OMe}$), 38.0 (CH$_{2Lev}$), 29.9 (CH$_{3Lev}$), 28.1 (CH$_{2Lev}$), 21.2 (CH$_{3STol}$), 17.3 (C-6); HRMS (ESI-TOF) m/z [M + NH_4]$^+$ calcd for $C_{19}H_{30}NO_6S$ 400.1788; found 400.1806; m/z [M + Na]$^+$ calcd for $C_{19}H_{26}NaO_6S$ 405.1342; found 405.1362. The latter alcohol (1678 mg, 4.387 mmol, 1.0 equiv) was dissolved in anhydrous pyridine (22 mL) and Ac_2O (22 mL), and DMAP (5 mg, 0.04 mmol, 0.01 equiv) were added. The reaction mixture was stirred at rt for 16 h under Ar. The solution was evaporated under reduced pressure and co-evaporated with toluene (3×). The residue was purified by silica gel flash chromatography (Hex/EtOAc 5:5 to 4:6) to give thiorhamnoside **29** (1786 mg, 96%, ~9:1 mixture with its inseparable 3-*O*-acetyl-2-*O*-methyl isomer) as a white amorphous solid: R_f 0.6 (Hex/EtOAc 4:6); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.36–7.35 (m, 2H, 2 × CH_{STol}), 7.13–7.12 (m, 2H, 2 × CH_{STol}), 5.57 (dd, J_{2-3} = 3.2 Hz, J_{2-1} = 1.6 Hz, 1H, H-2), 5.36 (d, J_{1-2} = 1.3 Hz, 1H, H-1), 5.04 (t, J = 9.8 Hz, 1H, H-4), 4.30 (dq, J_{5-4} = 9.8 Hz, J_{5-6} = 6.2 Hz, 1H, H-5), 3.59 (dd, J_{3-4} = 9.8 Hz, J_{3-2} = 3.3 Hz, 1H, H-3), 3.37 (s, 3H, CH_{3OMe}), 2.87–2.84 (m, 1H, CHH$_{Lev}$), 2.76–2.71 (m, 1H, CHH_{Lev}), 2.69–2.63 (m, 1H, CHH$_{Lev}$), 2.61–2.57 (m, 1H, CHH_{Lev}), 2.33 (s, 3H, CH_{3STol}), 2.20 (s, 3H, CH_{3Lev}), 2.11 (s, 3H, CH_{3Ac}), 1.23 (d, J = 6.3 Hz, 3H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.5 (CO$_{Lev}$), 172.2 (COOR$_{Lev}$), 170.4 (COOR$_{Ac}$), 138.2 (C$_{STol}$), 132.4 (2C, 2 × CH$_{STol}$), 130.1 (2C, 2 × CH$_{STol}$), 129.9 (C$_{STol}$), 86.6 (C-1), 77.6 (C-3), 73.0 (C-4), 69.7 (C-2), 67.8 (C-5), 57.9 (CH$_{3OMe}$), 38.0 (CH$_{2Lev}$), 30.0 (CH$_{3Lev}$),

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28.1 (CH_{2Lev}), 21.2 (CH_3), 21.1 (CH_3), 17.4 (C-6); HRMS (ESI-TOF) *m/z* $[M + NH_4]^+$ calcd for $C_{21}H_{32}NO_7S$ 442.1894; found 442.19005; *m/z* $[M + Na]^+$ calcd for $C_{21}H_{28}NaO_7S$ 447.1448; found 447.1453.

(5-Azido-1-pentyl) 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (10). Trisaccharide **28** (300 mg, 0.257 mmol, 1.0 equiv), thiorhamnoside **29** (164 mg, 0.386 mmol, 1.5 equiv) and NIS (87 mg, 0.39 mmol, 1.5 equiv) were dried together under high vacuum for 1 h. 4 Å activated ground molecular sieves (1200 mg) and anhydrous DCM (5 mL) were successively added and the mixture was stirred under Ar for 1 h. The reaction flask was cooled at −10 °C and protected from light using aluminum foil. AgOTf (7 mg, 0.03 mmol, 0.1 equiv) was added and the mixture was stirred under Ar for 2 h while being gradually warmed to 0 °C. Et_3N (0.04 mL, 0.3 mmol, 1.0 equiv) was added, the yellow suspension was filtered over Celite, and the solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 6:4 to 5:5) to give tetrasaccharide **10** (246 mg, 65%) as a white amorphous solid along with an inseparable unknown impurity (12%): R_f 0.5 (Hex/EtOAc 4:6); $[\alpha]_D^{20}$ –62 (*c* 0.4, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.87–7.84 (m, 1H, CH_{AZMB}), 7.59–7.55 (m, 2H, 2 × CH_{Ar}), 7.50–7.47 (m, 2H, 2 × CH_{Ar}), 7.43–7.42 (m, 2H, 2 × CH_{Ar}), 7.38–7.29 (m, 12H, 12 × CH_{Ar}), 5.57 (s, 1H, H-7C), 5.53 (s, 1H, H-7A), 5.36 (d, J = 1.7 Hz, 1H, H-2B), 5.21–5.19 (m, 2H, H-1B, H-2C), 5.07 (d, J = 1.1 Hz, H-2D), 4.91–4.87 (m, 2H, CHH$_{Bn}$, H-4B), 4.85–4.76 (m, 6H, CHH_{Bn}, H-1C, H-1D, CH_{2AZMB}, H-4D), 4.49 (d, J = 7.7 Hz, 1H, H-1A), 4.39 (dd, $J_{6a\text{-}6b}$ = 10.5 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6aC), 4.35 (dd, $J_{6a\text{-}6b}$ = 10.2 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6aA), 4.10–4.03 (m, 3H, H-3B, H-3C, H-5B), 4.01 (dq, $J_{5\text{-}4}$ = 10.0 Hz, $J_{5\text{-}6}$ = 5.6 Hz, 1H, H-5D), 3.94–3.90 (m, 2H, H-1a$_{linker}$, H-

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3A), 3.82–3.75 (m, 2H, H-6bA, H-6bC), 3.72 (t, J = 9.4 Hz, 1H, H-4C), 3.59–3.52 (m, 3H, H-1b$_{linker}$, H-4A, H-5C), 3.50–3.47 (m, 2H, H-2A, H-3D), 3.41 (td, $J_{5\text{-}4}$ = 9.6 Hz, $J_{5\text{-}6}$ = 4.9 Hz, 1H, H-5A), 3.22 (t, J = 14.2 Hz, 2H, H-5$_{linker}$), 3.21 (s, 3H, CH_{3OMe}), 2.78–2.73 (m, 1H, CHH$_{Lev}$), 2.67–2.62 (m, 1H, CHH_{Lev}), 2.56–2.47 (m, 2H, CH_{2Lev}), 2.32–2.29 (m, 1H, CHH$_{Lev}$), 2.16 (s, 3H, CH_{3Lev}), 2.13–2.11 (m, 2H, CH_{2Lev}), 2.08 (s, 3H, CH_{3Ac}), 2.04 (s, 3H, CH_{3Lev}), 1.87–1.80 (m, 4H, CH_{3Ac}, CHH_{Lev}), 1.68–1.63 (m, 2H, H-2$_{linker}$), 1.62–1.58 (m, 2H, H-4$_{linker}$), 1.48–1.42 (m, 2H, H-3$_{linker}$), 0.77–0.76 (m, 6H, H-6B, H-6D); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.4 (CO$_{Lev}$), 206.1 (CO$_{Lev}$), 172.0 (COOR$_{Lev}$), 171.4 (COOR$_{Lev}$), 170.0 (COOR$_{Ac}$), 169.5 (COOR$_{Ac}$), 164.4 (COOR$_{AZMB}$), 139.2 (C$_{Ar}$), 138.0 (C$_{Ar}$), 137.1 (C$_{Ar}$), 137.0 (C$_{Ar}$), 133.1–126.2 (20C, C$_{Ar}$, 19 × CH$_{Ar}$), 104.2 (C-1A), 101.8, 101.7 (2C, C-7A, C-7C), 100.9 (C-1C), 98.2 (C-1D), 97.7 (C-1B), 82.7 (C-2A), 79.2 (C-4A), 79.0 (C-4C), 76.7 (C-3D), 76.6 (C-3C), 76.1 (C-3A) 74.9, 74.7, 74.5 (3C, C-3B, CH$_{2Bn}$, C-2C), 72.6 (2C, C-4B, C-4D), 71.0 (C-2B), 70.2 (C-1$_{linker}$), 68.9, 68.7 (2C, C-6A, C-6C), 67.74 (C-2D), 66.71, 66.4, 66.3, 66.1 (4C, C-5A, C-5B, C-5C, C-5D), 57.6 (CH$_{3OMe}$), 53.0 (CH$_{2AZMB}$), 51.3 (C-5$_{linker}$), 37.9 (CH$_{2Lev}$), 37.4 (CH$_{2Lev}$), 29.9 (CH$_{3Lev}$), 29.7 (CH$_{3Lev}$), 29.4 (C-2$_{linker}$), 28.7 (C-4$_{linker}$), 28.0 (CH$_{2Lev}$), 27.4 (CH$_{2Lev}$), 23.4 (C-3$_{linker}$), 21.0, 20.6 (2C, 2 x CH$_{3Ac}$), 16.8, 16.7 (2C, C-6B, C-6D); HRMS (ESI-TOF) m/z [M + NH$_4$]$^+$ calcd for $C_{73}H_{92}N_7O_{26}$ 1482.6087; found 1482.6047; m/z [M + Na]$^+$ calcd for $C_{73}H_{88}N_6NaO_{26}$ 1487.564; found 1487.5617.

(5-Azido-1-pentyl) 2-*O*-acetyl-3-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (30). A solution of tetrasaccharide **10** (214 mg, 0.146 mmol, 1.0 equiv) in anhydrous pyridine (1 mL) was cooled at 0 °C. Acetic acid (0.6 mL) and hydrazine monohydrate (0.07 mL, 1 mmol, 10.0 equiv) were successively slowly added. The mixture was stirred for 16 h under Ar while gradually being

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warmed to rt. The solution was evaporated under reduced pressure and co-evaporated with toluene (3×). The residue was purified by silica gel flash chromatography (Tol/EtOAc 9:1 to 7:3) to give diol **30** (160 mg, 86%) as a white amorphous solid: R_f 0.5 (Tol/EtOAc 1:1); $[\alpha]_D^{20}$ –40 (*c* 1.0, AcOEt); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.97–7.95 (m, 1H, C*H*$_{AZMB}$), 7.55–7.52 (m, 1H, C*H*$_{Ar}$), 7.50–7.47 (m, 3H, 3 × C*H*$_{Ar}$), 7.40–7.39 (m, 2H, 2 × C*H*$_{Ar}$), 7.36–7.31 (m, 8H, 8 × C*H*$_{Ar}$), 7.29–7.27 (m, 2H, 2 × C*H*$_{Ar}$), 7.25–7.23 (m, 2H, 2 × C*H*$_{Ar}$), 5.55 (s, 1H, H-7A), 5.49 (s, 1H, H-7C), 5.32 (dd, $J_{2\text{-}3}$ = 3.5 Hz, $J_{2\text{-}1}$ = 1.6 Hz, 1H, H-2B), 5.28 (dd, $J_{2\text{-}3}$ = 8.6 Hz, $J_{2\text{-}1}$ = 7.8 Hz, 1H, H-2C), 5.14 (d, J = 1.2 Hz, 1H, H-1B), 5.08 (dd, $J_{2\text{-}3}$ = 3.2 Hz, $J_{2\text{-}1}$ = 1.7 Hz, 1H, H-2D), 4.91 (d, J = 14.7 Hz, 1H, C*H*H$_{AZMB}$), 4.86 (d, J = 10.7 Hz, 1H, C*H*H$_{Bn}$), 4.85 (d, J = 7.6 Hz, 1H, H-1C), 4.82 (d, J = 1.3 Hz, 1H, H-1D), 4.79 (d, J = 10.7 Hz, 1H, CH*H*$_{Bn}$), 4.76 (d, J = 14.7 Hz, 1H, CH*H*$_{AZMB}$), 4.48 (d, J = 7.8 Hz, 1H, H-1A), 4.37 (dd, $J_{6a\text{-}6b}$ = 10.6 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6aC), 4.33 (dd, $J_{6a\text{-}6b}$ = 10.4 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6aA), 4.07 (t, J = 9.0 Hz, 1H, H-3C), 3.97–3.89 (m, 4H, H-1a$_{linker}$, H-5B, H-5D, H-3A), 3.87 (dd, $J_{3\text{-}4}$ = 9.4 Hz, $J_{3\text{-}2}$ = 3.6 Hz, 1H, H-3B), 3.77–3.69 (m, 3H, H-6bA, H-6bC, H-4C), 3.57–3.51 (m, 3H, H-1b$_{linker}$, H-5C, H-4A), 3.48–3.44 (m, 2H, H-2A, H-4B), 3.40–3.38 (m, 2H, H-3D, H-5A), 3.35–3.31 (m, 1H, H-4D), 3.29 (s, 3H, C*H*$_{3OMe}$), 3.20 (t, J = 6.9 Hz, 2H, H-5$_{linker}$), 2.41 (s, 1H, O*H*), 2.25 (br s, 1H, O*H*), 2.04 (s, 3H, C*H*$_{3Ac}$), 1.80 (s, 3H, C*H*$_{3Ac}$), 1.66–1.61 (m, 2H, H-2$_{linker}$), 1.61–1.56 (m, 2H, H-4$_{linker}$), 1.47–1.40 (m, 2H, H-3$_{linker}$), 0.90 (d, J = 6.2 Hz, 3H, H-6B*), 0.86 (d, J = 6.2 Hz, 3H, H-6D*); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 169.9 (*C*OOR$_{Ac}$), 169.6 (*C*OOR$_{Ac}$), 165.2 (*C*OOR$_{AZMB}$), 138.6 (C$_{Ar}$), 138.2 (C$_{Ar}$), 137.1 (C$_{Ar}$), 137.0 (C$_{Ar}$), 133.3-126.2 (20C, C$_{Ar}$, 19 × *C*H$_{Ar}$), 104.2 (C-1A), 101.8, 101.5 (2C, C-7A, C-7C), 101.4 (C-1C), 98.5 (C-1D), 98.1 (C-B), 82.8 (C-2A), 79.3, 79.2 (2C, C-4A, C-3D), 78.2 (2C, C-3B, C-4C), 76.6, 76.5 (2C, C-3A, C-3C), 75.1, 74.9 (2C, *C*H$_{2Bn}$, C-2C), 71.7, 71.5 (2C, C-4B, C-4D), 70.7 (C-2B), 70.2 (C-1$_{linker}$), 68.8, 68.6 (2C, C-6A, C-6C), 68.5, 67.8 (2C, C-5B, C-5D), 67.3

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(C-2D), 66.5 (C-5C), 66.3 (C-5A), 57.3 (CH_{3OMe}), 53.3 (CH_{2AZMB}), 51.3 ($C\text{-}5_{linker}$), 29.4 ($C\text{-}2_{linker}$), 28.7 ($C\text{-}4_{linker}$), 23.4 ($C\text{-}3_{linker}$), 21.0 (CH_{3Ac}), 20.6 (CH_{3Ac}), 17.14, 17.07 (2C, C-6B, C-6D); HRMS (ESI-TOF) *m/z* $[M + NH_4]^+$ calcd for $C_{63}H_{80}N_7O_{22}$ 1286.5351; found 1286.5342; *m/z* $[M + Na]^+$ calcd for $C_{63}H_{76}N_6NaO_{22}$ 1291.4905; found 1291.4892.

***para*-Methylphenyl 2-*O*-acetyl-3-*O*-methyl-1-thio-α-L-rhamnopyranoside (32).** A solution of thiorhamnoside **29** (876 mg, 2.06 mmol, 1.0 equiv) in anhydrous pyridine (13 mL) was cooled at 0 °C. Acetic acid (8.5 mL) and hydrazine monohydrate (0.5 mL, 10 mmol, 5.0 equiv) were successively added dropwise. The mixture was stirred for 16 h under Ar while gradually being warmed to rt. The solution was evaporated under reduced pressure and co-evaporated with toluene (3×). The residue was purified by silica gel flash chromatography (Hex/EtOAc 7:3 to 6:4) to give alcohol **32** (616 mg, 91%, ~83:17 mixture with its inseparable 3-*O*-acetyl-2-*O*-methyl isomer) as a colorless oil: R_f 0.5 (Hex/EtOAc 4:6); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.36–7.35 (m, 2H, 2 × CH_{STol}), 7.13–7.12 (m, 2H, 2 × CH_{STol}), 5.56 (dd, $J_{2\text{-}3}$ = 3.1 Hz, $J_{2\text{-}1}$ = 1.5 Hz, 1H, H-2), 5.35 (d, J = 1.2 Hz, 1H, H-1), 4.21 (dq, $J_{5\text{-}4}$ = 9.3 Hz, $J_{5\text{-}6}$ = 6.2 Hz, 1H, H-5), 3.58 (td, $J_{4\text{-}3,4\text{-}5}$ = 9.4 Hz, $J_{4\text{-}OH}$ = 2.0 Hz, 1H, H-4), 3.47 (dd, $J_{3\text{-}4}$ = 9.4 Hz, $J_{3\text{-}2}$ = 3.1 Hz, 1H, H-3), 3.43 (s, 3H, CH_{3OMe}), 2.50 (d, J = 2.2 Hz, 1H, OH), 2.33 (s, 3H, CH_{3STol}), 2.11 (s, 3H, CH_{3Ac}), 1.36 (d, J = 6.2 Hz, 3H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 170.3 ($COOR_{Ac}$), 138.1 (C_{STol}), 132.4 (2C, 2 × CH_{STol}), 130.1 (C_{STol}), 130.0 (2C, 2 × CH_{STol}), 86.8 (C-1), 80.1 (C-3), 72.1 (C-4), 69.4, 69.3 (2C, C-2, C-5), 57.5 (CH_{3OMel}), 21.3 (CH_3), 21.1 (CH_3), 17.7 (C-6); HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{16}H_{22}NaO_5S$ 349.1080; found 349.1078.

***para*-Methylphenyl 2-*O*-acetyl-6-deoxy-3-*O*-methyl-1-thio-α-L-talopyranoside (33).** A solution of anhydrous DMSO (0.57 mL, 8.0 mmol, 5.0 equiv) and anhydrous DCM (16 mL) was cooled to −10 °C and PDCP (0.72 mL, 4.8 mmol, 3.0 equiv) and Et_3N (1.11 mL, 7.98 mmol, 5.0

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equiv) were successively added. A solution of alcohol **32** (521 mg, 1.60 mmol, 1.0 equiv) in anhydrous DCM (8 mL) was added dropwise during 1 h. The mixture was stirred at −10 °C for 10 min under Ar, and for an additional 30 min while gradually being warmed to rt. Water (50 mL) was added to the solution, which was then transferred into a separatory funnel. The organic and aqueous layers were separated, and the aqueous phase was extracted with DCM (3 × 30 mL). The combined organic layers were washed with brine (50 mL), then dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The resulting ketone was solubilized in MeOH (16 mL) and cooled at −10 °C. $NaBH_4$ (109 mg, 2.87 mmol, 1.8 equiv) was slowly added and the mixture was stirred for 1 h while gradually being warmed to 0 °C. The solution was diluted with DCM (50 mL), transferred into a separatory funnel, and washed with water (30 mL). The aqueous phase was extracted with DCM (3 × 30 mL), the combined organic phases were washed with brine (60 mL) and dried over anhydrous $MgSO_4$. The solution was evaporated under reduced pressure and the residue was purified by silica gel flash chromatography (Tol/EtOAc 95:5 to 85:15) to give taloside **33** (371 mg, 71%, 2 steps) as a colorless oil: R_f 0.4 (Tol/EtOAc 1:1); $[\alpha]_D^{20}$ +268 (*c* 0.4, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.37–7.35 (m, 2H, 2 × CH_{STol}), 7.14–7.12 (m, 2H, 2 × CH_{STol}), 5.46 (m, 1H, H-2), 5.41 (d, J = 1.1 Hz, 1H, H-1), 4.40 (qd, $J_{5\text{-}6}$ = 6.5 Hz, $J_{5\text{-}4}$ = 0.7 Hz, 1H, H-5), 3.83 (br dd, $J_{4\text{-}OH}$ = 9.1 Hz, $J_{4\text{-}3}$ = 3.2 Hz, 1H, H-4), 3.55 (t, J = 3.5 Hz, 1H, H-3), 3.46 (s, 3H, CH_{3OMe}), 2.46 (d, J = 9.3 Hz, 1H, O*H*), 2.33 (s, 3H, CH_{3STol}), 2.14 (s, 3H, CH_{3Ac}), 1.36 (d, J = 6.5 Hz, 3H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 169.6 (*C*OOR_{Ac}), 138.3 (C_{STol}), 132.5 (2C, 2 × *C*H$_{STol}$), 130.1 (2C, 2 × *C*H$_{STol}$), 129.8 (C_{STol}), 86.9 (C-1), 75.0 (C-3), 70.2 (C-2), 69.7 (C-4), 68.4 (C-5), 56.5 (*C*H_{3OMe}), 21.3 (2C, 2 × *C*H_3), 16.5 (C-6); HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{16}H_{22}NaO_5S$ 349.10802; found 349.10839.

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***para*-Methylphenyl 2,4-di-*O*-acetyl-6-deoxy-3-*O*-methyl-1-thio-α-L-talopyranoside (34).** Alcohol **33** (197 mg, 0.613 mmol, 1.0 equiv) was solubilized in EtOAc (6 mL) and Ac_2O (0.58 mL, 6.1 mmol, 10.0 equiv) and DMAP (8 mg, 0.06 mmol, 0.1 equiv) were added. The mixture was refluxed for 3 h under Ar. The solvents were evaporated under reduced pressure and co-evaporated with toluene (3×). The residue was purified by silica gel flash chromatography (Hex/EtOAc 9:1 to 7:3) to give compound **34** (200 mg, 90%) as a white amorphous solid: R_f 0.6 (Hex/EtOAc 1:1); $[\alpha]_D^{20}$ –137 (*c* 0.7, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.35–7.34 (m, 2H, 2 × CH_{STol}), 7.13–7.11 (m, 2H, 2 × CH_{STol}), 5.48 (s, 1H, H-1), 5.40 (d, J = 3.7 Hz, 1H, H-2), 5.31 (br d, J = 3.4 Hz, 1H, H-4), 4.52 (qd, $J_{5\text{-}6}$ = 6.3 Hz, $J_{5\text{-}4}$ = 0.8 Hz, 1H, H-5), 3.59 (t, J = 3.6 Hz, 1H, H-3), 3.40 (s, 3H, CH_{3OMe}), 2.33 (s, 3H, CH_{3STol}), 2.16 (s, 3H, CH_{3Ac}), 2.13 (s, 3H, CH_{3Ac}), 1.21 (d, J = 6.5 Hz, 3H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 170.8 (*C*OOR_{Ac}), 170.4 (*C*OOR_{Ac}), 138.0 (C_{STol}), 132.0 (2C, 2 × *C*H_{STol}), 130.0 (2C, 2 × *C*H_{STol}), 129.8 (C_{STol}), 86.9 (C-1), 74.5 (C-3), 68.6, 68.5 (2C, C-2, C-4), 66.5 (C-5), 57.4 (*C*H_{3OMe}), 21.3, 21.2 (2C, 2 × *C*H_3), 21.0 (*C*H_3), 16.4 (C-6); HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{18}H_{24}NaO_6S$ 391.11858; found 391.11991.

***para*-Methylphenyl 2-*O*-acetyl-4-*O*-chloroacetyl-6-deoxy-3-*O*-methyl-1-thio-α-L-talopyranoside (35).** Chloroacetic anhydride (268 mg, 1.57 mmol, 5.0 equiv) and DMAP (4 mg, 0.03 mmol, 0.1 equiv) were added to a solution of alcohol **33** (102 mg, 0.313 mmol, 1.0 equiv) in EtOAc (3 mL). The mixture was refluxed for 1 h under Ar, then diluted with EtOAc (3 mL). The solution was poured into a separatory funnel, washed with saturated $NaHCO_3$(aq) (3 × 5 mL) and brine (5 mL), and dried over anhydrous $MgSO_4$. The solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 8:2 to 7:3) to give compound **35** (120 mg, 95%) as a yellow oil: R_f 0.4 (Hex/EtOAc 7:3); $[\alpha]_D^{20}$ –107 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.35–7.33 (m, 2H, 2 × CH_{STol}), 7.13–7.12 (m, 2H, 2

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× CH_{STol}), 5.48 (d, J = 1.0 Hz, 1H, H-1), 5.41–5.38 (m, 1H, H-2), 5.37 (br d, J = 3.5 Hz, 1H, H-4), 4.56 (qd, $J_{5\text{-}6}$ = 6.5 Hz, $J_{5\text{-}4}$ = 1.1 Hz, 1H, H-5), 4.19 (d, J = 14.9 Hz, 1H, CHH$_{AcCl}$), 4.14 (d, J = 14.9 Hz, 1H, CHH_{AcCl}), 3.62 (t, J = 3.6 Hz, 1H, H-3), 3.40 (s, 3H, CH_{3OMe}), 2.33 (s, 3H, CH_{3STol}), 2.13 (s, 3H, CH$_{3Ac}$), 1.23 (d, J = 6.5 Hz, 3H, H-6); ^{13}C NMR (150 MHz, CDCl$_3$) δ (ppm) 170.4 (COOR$_{Ac}$), 167.3 (COOR$_{AcCl}$), 138.2 (C$_{Ar}$), 132.2 (2C, 2 × CH$_{STol}$), 130.1 (2C, 2 × CH$_{STol}$), 129.6 (C$_{Ar}$), 86.8 (C-1), 74.2 (C-3), 70.5 (C-4), 68.3 (C-2), 66.2 (C-5), 57.4 (CH$_{3OMe}$), 40.9 (CH$_{2AcCl}$), 21.3, 21.2 (2C, 2 × CH$_3$), 16.3 (C-6); HRMS (ESI-TOF) *m/z* [M + Na]$^+$ calcd for $C_{18}H_{23}ClNaO_6S$ 425.07961; found 425.07992.

(5-Azido-1-pentyl) 2,4-di-*O*-acetyl-6-deoxy-3-*O*-methyl-α-L-talopyranosyl-(1→3)-2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (36). Trisaccharide **29** (87 mg, 0.074 mmol, 1.0 equiv), thiotaloside **34** (41 mg, 0.11 mmol, 1.5 equiv) and NIS (25 mg, 0.11 mmol, 1.5 equiv) were dried together under high vacuum for 1 h. Activated ground molecular sieves (4 Å, 346 mg) and anhydrous DCM (1.5 mL) were successively added and the mixture was stirred under Ar for 1 h. The reaction flask was cooled to −10 °C and protected from light using aluminum foil. AgOTf (2 mg, 0.007 mmol, 0.1 equiv) was added and the mixture was stirred under Ar for 2 h while being gradually warmed to 0 °C. Et$_3$N (0.01 mL, 0.09 mmol, 1.0 equiv) was added, the yellow suspension was filtered over Celite, and the solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 8:2 to 5:5) to give tetrasaccharide **36** (105 mg, quant.) as a white amorphous solid: R_f 0.5 (Hex/EtOAc 4:6); $[\alpha]_D^{20}$ –54 (*c* 0.6, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ (ppm) 7.86–7.85 (m, 1H, CH_{Ar}), 7.61–7.59 (m, 1H, CH_{Ar}), 7.57–7.54 (m, 1H, CH_{Ar}), 7.45–7.41 (m, 4H, 4 × CH_{Ar}), 7.36–7.33 (m, 7H, 7 × CH_{Ar}), 7.32–7.27 (m, 5H, 5 × CH_{Ar}), 5.53 (s, 1H, H-7C),

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5.52 (s, 1H, H-7A), 5.35 (dd, $J_{2\text{-}3}$ = 3.5 Hz, $J_{2\text{-}1}$ = 1.6 Hz, 1H, H-2B), 5.21, (dd, $J_{2\text{-}3}$ = 8.7 Hz, $J_{2\text{-}1}$ = 7.8 Hz, 1H, H-2C), 5.18 (br s, 1H, H-1B), 5.02 (d, J = 3.1 Hz, 1H, H-2D), 4.96 (br d, J = 3.9 Hz, 1H, H-4D), 4.91–4.82 (m, 5H, CH_{2AZMB}, CHH$_{Bn}$, H-4B, H-1D), 4.77 (d, J = 10.6 Hz, 1H, CHH_{Bn}), 4.76 (d, J = 7.5 Hz, 1H, H-1C), 4.49 (d, J = 7.7 Hz, 1H, H-1A), 4.38 (dd, $J_{6a\text{-}6b}$ = 10.7 Hz, $J_{6a\text{-}5}$ = 5.0 Hz, 1H, H-6aC), 4.35 (dd, $J_{6a\text{-}6b}$ = 11.1 Hz, $J_{6a\text{-}5}$ = 5.6 Hz, 1H, H-6aA), 4.17 (qd, $J_{5\text{-}6}$ = 6.5 Hz, $J_{5\text{-}4}$ = 1.3 Hz, 1H, H-5D), 4.09 (t, J = 9.2 Hz, 1H, H-3C), 4.10–4.04 (m, 2H, H-3B, H-5B), 3.94–3.90 (m, 2H, H-1a$_{linker}$, H-3A), 3.79–3.75 (m, 2H, H-6bA, H-6bC), 3.67 (t, J = 9.4 Hz, 1H, H-4C), 3.59–3.52 (m, 3H, H-1b$_{linker}$, H-5C, H-4A), 3.50–3.45 (m, 2H, H-2A, H-3D), 3.41 (td, $J_{5\text{-}4,\ 5\text{-}6b}$ = 9.7 Hz, $J_{5\text{-}6a}$ = 5.0 Hz, H-5A), 3.24 (s, 3H, CH_{3OMe}), 3.22 (t, J = 6.9 Hz, 2H, H-5$_{linker}$), 2.36–2.30 (m, 1H, CHH$_{Lev}$), 2.17–2.10 (m, 2H, CHH_{Lev}, CHH$_{Lev}$), 2.08 (s, 3H, CH_{3Ac}), 2.05 (s, 3H, CH_{3Lev}), 2.04 (s, 3H, CH_{3Ac}), 1.81–1.77 (m, 1H, CHH_{Lev}), 1.77 (s, 3H, CH_{3Ac}), 1.67–1.64 (m, 2H, H-2$_{linker}$) 1.60–1.59 (m, 2H, H-4$_{linker}$), 1.49–1.41 (m, 2H, H-3$_{linker}$), 0.76 (d, J = 6.2 Hz, 3H, H-6B), 0.70 (d, J = 6.5 Hz, 3H, H-6D); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.2 (CO$_{Lev}$), 171.4 (COOR$_{Lev}$), 170.8 (COOR$_{Ac}$), 170.0 (COOR$_{Ac}$), 169.5 (COOR$_{Ac}$), 164.2 (COOR$_{AZMB}$), 138.7 (C$_{Ar}$), 138.0 (C$_{Ar}$), 137.14 (C$_{Ar}$), 137.07 (C$_{Ar}$), 133.1–126.2 (20C, C$_{Ar}$, 19 × CH$_{Ar}$), 104.2 (C-1A), 102.1, 101.7 (2C, C-7A, C-7C), 100.9 (C-1C), 99.1 (C-1D), 97.8 (C-1B), 82.7 (C-2A), 79.3 (C-4A), 79.0 (C-4C), 76.2, 76.1 (2C, C-3A, C-3C), 75.0, 74.9 (2C, C-3B, CH$_{2Bn}$), 74.6 (C-2C), 73.4 (C-3D), 72.6 (C-4B), 70.9 (C-2B), 70.3 (C-1$_{linker}$), 68.9, 68.7, 68.5 (3C, C-6A, C-6C, C-2D), 66.5, 66.3, 66.2 (4C, C-5A, C-5B, C-5C, C-4D), 65.5 (C-5D), 57.2 (CH$_{3OMe}$), 53.1 (CH$_{2AZMB}$), 51.4 (C-5$_{linker}$), 37.5 (CH$_{2Lev}$), 29.8 (CH$_{3Lev}$), 29.4 (C-2$_{linker}$), 28.8 (C-4$_{linker}$), 27.5 (CH$_{2Lev}$), 23.5 (C-3$_{linker}$), 21.1 (CH$_{3Ac}$), 21.0 (CH$_{3Ac}$), 20.8 (CH$_{3Ac}$), 16.8 (C-6B), 15.8 (C-6D); HRMS (ESI-TOF) m/z [M + NH$_4$]$^+$ calcd for $C_{70}H_{88}N_7O_{25}$ 1426.58244; found 1426.58819; m/z [M + Na]$^+$ calcd for $C_{70}H_{84}N_6NaO_{25}$ 1431.53873; found 1431.54367.

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(5-Azido-1-pentyl) 2-*O*-acetyl-4-*O*-chloroacetyl-6-deoxy-3-*O*-methyl-α-L-talopyranosyl-(1→3)-2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (37). Trisaccharide **29** (109 mg, 0.0933 mmol, 1.0 equiv), thiotaloside **35** (56 mg, 0.14 mmol, 1.5 equiv), and NIS (32 mg, 0.14 mmol, 1.5 equiv) were dried together under high vacuum for 1 h. Activated ground molecular sieves (4 Å, 435 mg) and anhydrous DCM (1.9 mL) were successively added and the mixture was stirred under Ar for 1 h. The reaction flask was cooled to −10 °C and protected from light using aluminum foil. AgOTf (2 mg, 0.009 mmol, 0.1 equiv) was added and the mixture was stirred under Ar for 2 h while being gradually warmed to 0 °C. Et_3N (0.01 mL, 0.09 mmol, 1.0 equiv) was added, the yellow suspension was filtered over Celite, and the solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 8:2 to 5:5) to give tetrasaccharide **37** (110 mg, 82%) as a white amorphous solid: R_f 0.4 (Tol/EtOAc 7:3); $[\alpha]_D^{20}$ –68 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.86–7.85 (m, 1H, CH_{AZMB}), 7.61–7.59 (m, 1H, CH_{Ar}), 7.57–7.55 (m, 1H, CH_{Ar}), 7.45–7.44 (m, 2H, 2 × CH_{Ar}), 7.42–7.41 (m, 2H, 2 × CH_{Ar}), 7.37–7.33 (m, 7H, 7 × CH_{Ar}), 7.31–7.28 (m, 5H, 5 × CH_{Ar}), 5.53 (s, 1H, H-7C), 5.52 (s, 1H, H-7A), 5.35 (dd, $J_{2\text{-}3}$ = 3.5 Hz, $J_{2\text{-}1}$ = 1.7 Hz, 1H, H-2B), 5.19 (dd, $J_{2\text{-}3}$ = 8.7 Hz, $J_{2\text{-}1}$ = 7.8 Hz, 1H, H-2C), 5.18 (d, J = 1.6 Hz, 1H, H-1B), 5.07 (d, J = 3.3 Hz, 1H, H-2D), 4.94 (br d, J = 3.9 Hz, 1H, H-4D), 4.90–4.81 (m, 5H, CH_{2AZMB}, CHH$_{Bn}$, H-1D, H-4B), 4.77 (d, J = 7.4 Hz, 1H, H-1C), 4.76 (d, J = 11.0 Hz, 1H, CHH_{Bn}), 4.49 (d, J = 7.7 Hz, 1H, H-1A), 4.38 (dd, $J_{6a\text{-}6b}$ = 10.6 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6aC), 4.35 (dd, $J_{6a\text{-}6b}$ = 10.4 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6aA), 4.20 (qd, $J_{5\text{-}6}$ = 6.2 Hz, $J_{5\text{-}4}$ = 1.1 Hz, 1H, H-5D), 4.10–4.05 (m, 3H, H-3B, H-5B, H-3C), 4.03 (d, J = 14.8 Hz, 1H, CHH$_{AcCl}$), 4.01 (d, J = 14.8 Hz, 1H, CHH_{AcCl}), 3.94–3.90 (m, 2H, H-1a$_{linker}$, H-3A), 3.77 (m, 2H, H-6bA, H-6bC), 3.67 (t, J = 9.4 Hz, 1H, H-4C), 3.59–3.52

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(m, 3H, H-1b$_{linker}$, H-5C, H-4A), 3.49–3.47 (m, 2H, H-2A, H-3D), 3.41 (td, $J_{5\text{-}4,\ 5\text{-}6b}$ = 9.8 Hz, $J_{5\text{-}6a}$ = 5.0 Hz, 1H, H-5A), 3.24 (s, 3H, CH_{3OMe}), 3.22 (t, J = 6.9 Hz, 2H, H-5$_{linker}$), 2.36–2.30 (m, 1H, CHH$_{Lev}$), 2.14–2.07 (m, 2H, CHH_{Lev}, CHH$_{Lev}$), 2.07 (s, 3H, CH_{3Ac}), 2.05 (s, 3H, CH_{3Lev}), 1.82–1.80 (m, 1H, CHH_{Lev}), 1.77 (s, 3H, CH_{3Ac}), 1.69–1.63 (m, 2H, H-2$_{linker}$), 1.61–1.60 (m, 2H, H-4$_{linker}$), 1.48–1.42 (m, 2H, H-3$_{linker}$), 0.76 (d, J = 6.2 Hz, 3H, H-6B), 0.70 (d, J = 6.5 Hz, 3H, H-6D); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 206.2 (CO$_{Lev}$), 171.4 (COOR$_{Lev}$), 170.0 (COOR$_{Ac}$), 169.4 (COOR$_{Ac}$), 167.3 (COOR$_{AcCl}$), 164.2 (COOR$_{AZMB}$), 139.7 (C$_{Ar}$), 138.0 (C$_{Ar}$), 137.2 (2C, 2 × C$_{Ar}$), 137.1–126.2 (20C, C$_{Ar}$, 19 × CH$_{Ar}$), 104.3 (C-1A), 102.2, 101.7 (2C, C-7A, C-7C), 100.9 (C-1C), 99.0 (C-1D), 97.8 (C-1B), 82.7 (C-2A), 79.3 (C-4A), 79.0 (C-4C), 76.4, 76.1 (2C, C-3A, C-3C), 75.0, 74.9 (2C, C-3B, CH$_{2Bn}$), 74.6 (C-2C), 73.1 (C-3D), 72.6 (C-4B), 71.0 (C-2B), 70.6 (C-2D), 70.3 (C-1$_{linker}$), 68.9, 68.7 (2C, C-6A, C-6C), 66.5, 66.3, 66.2, 66.1 (4C, C-5A, C-5B, C-5C, C-4D), 65.2 (C-5D), 57.3 (CH$_{3OMe}$), 53.1 (CH$_{2AZMB}$), 51.4 (C-5$_{linker}$), 40.9 (CH$_{2AcCl}$), 37.5 (CH$_{2Lev}$), 29.8 (CH$_{3Lev}$), 29.5 (C-2$_{linker}$), 28.8 (C-4$_{linker}$), 27.5 (CH$_{2Lev}$), 23.5 (C-3$_{linker}$), 21.1 (CH$_{3Ac}$), 20.8 (CH$_{3Ac}$), 16.8 (C-6B), 15.7 (C-6D); HRMS (ESI-TOF) *m/z* [M + NH_4]$^+$ calcd for $C_{70}H_{87}ClN_7O_{25}$ 1460.54347; found 1460.54641.

(5-Azido-1-pentyl) 2,4-di-*O*-acetyl-6-deoxy-3-*O*-methyl-α-L-talopyranosyl-(1→3)-2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (38). A solution of tetrasaccharide **36** (98 mg, 0.070 mmol, 1.0 equiv) in anhydrous pyridine (0.45 mL) was cooled to 0 °C. Acetic acid (0.3 mL) and hydrazine monohydrate (0.02 mL, 0.4 mmol, 5.0 equiv) were successively added dropwise. The mixture was stirred for 16 h under Ar while gradually being warmed to rt. The solution was evaporated under reduced pressure and co-evaporated with toluene (3×). The residue was purified by silica gel flash chromatography (Tol/EtOAc 9:1 to 7:3) to give

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alcohol **38** (78 mg, 85%) as a white amorphous solid: R_f 0.5 (Tol/EtOAc 6:4); $[\alpha]_D^{20}$ –59 (*c* 0.8, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.95 (m, 1H, CH_{AZMB}), 7.55–7.50 (m, 2H, 2 × CH_{Ar}), 7.45–7.43 (m, 2H, 2 × CH_{Ar}), 7.39–7.27 (m, 12H, 12 × CH_{Ar}), 7.24–7.22 (m, 2H, 2 × CH_{Ar}), 5.52 (s, 1H, H-7C), 5.48 (s, 1H, H-7A), 5.31 (dd, $J_{2\text{-}3}$ = 3.5 Hz, $J_{2\text{-}1}$ = 1.5 Hz, 1H, H-2B), 5.29 (dd, *J* = 8.7 Hz, *J* = 8.0 Hz, 1H, H-2C), 5.14 (s, 1H, H-1B), 5,03 (d, *J* = 3.1 Hz, 1H, H-2D), 4.98 (br d, *J* = 3.9 Hz, 1H, H-4D), 4.94 (d, *J* = 14.9 Hz, 1H, C*H*H$_{AZMB}$), 4.91 (s, 1H, H-1D), 4.86 (d, *J* = 10.7 Hz, 1H, C*H*H$_{Bn}$), 4.83 (d, *J* = 7.7 Hz, 1H, H-1C), 4.79–4.77 (m, 2H, CHH_{Bn}, CHH_{AZMB}), 4.48 (d, *J* = 7.7 Hz, 1H, H-1A), 4.36 (dd, $J_{6a\text{-}6b}$ = 10.6 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6aC), 4.34 (dd, $J_{6a\text{-}6b}$ = 10.4 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6aA), 4.18 (br q, *J* = 6.5 Hz, 1H, H-5D), 4.08 (t, *J* = 9.2 Hz, 1H, H-3C), 3.96–3.89 (m, 3H, H-1a$_{linker}$, H-5B, H-3A), 3.85 (dd, $J_{3\text{-}4}$ = 9.4 Hz, $J_{3\text{-}2}$ = 3.6 Hz, 1H, H-3B), 3.77–3.72 (m, 2H, H-6bA, H-6bC), 3.66 (t, *J* = 9.4 Hz, 1H, H-4C), 3.56–3.52 (m, 2H, H-1b$_{linker}$, H-4A), 3.50 (m, 1H, H-5C), 3.48–3.44 (m, 3H, H-3D, H-2A, H-4B), 3.39 (td, *J* = 9.7 Hz, *J* = 4.7 Hz, 1H, H-5A), 3.26 (s, 3H, CH_{3OMe}), 3.20 (t, *J* = 6.9 Hz, 2H, H-5$_{linker}$), 2.11 (br s, 1H, O*H*), 2.05 (s, 3H, CH_{3Ac}), 2.04 (s, 3H, CH_{3Ac}), 1.76 (s, 3H, CH_{3Ac}), 1.66–1.63 (m, 2H, H-2$_{linker}$), 1.60–1.56 (m, 2H, H-4$_{linker}$), 1.46–1.40 (m, 2H, H-3$_{linker}$), 0.85 (d, *J* = 6.2 Hz, 3H, H-6B), 0.72 (d, *J* = 6.5 Hz, 3H, H-6D); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 170.8 (*C*OOR$_{Ac}$), 169.9 (*C*OOR$_{Ac}$), 169.6 (*C*OOR$_{Ac}$), 165.0 (*C*OOR$_{AZMB}$), 139.0 (C$_{Ar}$), 138.2 (C$_{Ar}$), 137.1 (2C, 2 × C$_{Ar}$), 133.4–126.2 (20C, C$_{Ar}$, 19 × *C*H$_{Ar}$), 104.2 (C-1A), 102.1 (C-7C), 101.5 (2C, C-7A, C-1C), 99.0 (C-1D), 98.1 (C-1B), 82.8 (C-2A), 79.3, 79.1, 78.9 (3C, C-4A, C-4C, C-3B), 76.5 (C-3A), 75.9 (C-3C), 75.1, 75.0 (2C, C-2C, *C*H$_{2Bn}$), 73.4 (C-3D), 71.7 (C-4B), 70.5 (C-2B), 70.2 (C-1$_{linker}$), 68.9, 68.7, 68.5 (3C, C-6A, C-6C, C-2D), 67.8 (C-5B), 66.6, 66.4 (3C, C-4D, C-5A, C-5C), 65.5 (C-5D), 57.2 (*C*H$_{3OMe}$), 53.4 (*C*H$_{2AZMB}$), 51.4 (C-5$_{linker}$), 29.4 (C-2$_{linker}$), 28.7 (C-4$_{linker}$), 23.5 (C-3$_{linker}$), 21.1 (*C*H$_{3Ac}$), 21.0 (*C*H$_{3Ac}$), 20.8 (*C*H$_{3Ac}$), 17.1 (C-6B), 15.8 (C-6D); HRMS (ESI-TOF) *m/z* [M + NH_4]$^+$ calcd for

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$C_{65}H_{82}N_7O_{23}$ 1328.54566; found 1328.5449; *m/z* $[M + Na]^+$ calcd for $C_{65}H_{78}N_6NaO_{23}$ 1333.50105; found 1333.50103.

(5-Azido-1-pentyl) 2-*O*-acetyl-4-*O*-chloroacetyl-6-deoxy-3-*O*-methyl-α-L-talopyranosyl-(1→3)-2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (39). A solution of tetrasaccharide **37** (476 mg, 0.330 mmol, 1.0 equiv) in anhydrous pyridine (2 mL) was cooled at 0 °C. Acetic acid (1.4 mL) and hydrazine monohydrate (0.08 mL, 2 mmol, 5.0 equiv) were successively added dropwise. The mixture was stirred for 3 h under Ar while gradually being warmed to rt. The solution was evaporated under reduced pressure and co-evaporated with toluene (3×). The residue was purified by silica gel flash chromatography (Tol/EtOAc 9:1 to 7:3) to give alcohol **39** (290 mg, 65%) as a white amorphous solid: R_f 0.7 (Tol/EtOAc 7:3); $[\alpha]_D^{20}$ –52 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.95–7.93 (m, 1H, C*H*$_{AZMB}$), 7.55–7.51 (m, 2H, 2 × C*H*$_{Ar}$), 7.45–7.43 (m, 2H, 2 × C*H*$_{Ar}$), 7.39–7.27 (m, 12H, 12 × C*H*$_{Ar}$), 7.24–7.22 (m, 2H, 2 × C*H*$_{Ar}$), 5.52 (s, 1H, H-7C), 5.49 (s, 1H, H-7A), 5.31 (dd, $J_{2\text{-}3}$ = 3.5 Hz, $J_{2\text{-}1}$ = 1.6 Hz, 1H, H-2B), 5.28 (dd, *J* = 8.8 Hz, *J* = 7.8 Hz, 1H, H-2C), 5.14 (d, *J* = 1.2 Hz, H-1B), 5.08 (br s, 1H, H-2D), 4.97 (br d, *J* = 3.9 Hz, 1H, H-4D), 4.94 (d, *J* = 14.9 Hz, 1H, C*H*H$_{AZMB}$), 4.90 (br s, 1H, H-1D), 4.86 (d, *J* = 10.7 Hz, 1H, C*H*H$_{Bn}$), 4.83 (d, *J* = 7.7 Hz, 1H, H-1C), 4.79-4.76 (m, 2H, CH*H*$_{AZMB}$, CH*H*$_{Bn}$), 4.48 (d, *J* = 7.7 Hz, 1H, H-1A), 4.36 (dd, $J_{6a\text{-}6b}$ = 10.8 Hz, $J_{6a\text{-}5}$ = 5.1 Hz, 1H, H-6aC), 4.34 (dd, $J_{6a\text{-}6b}$ = 10.6 Hz, $J_{6a\text{-}5}$ = 5.1 Hz, 1H, H-6aA), 4.20 (br q, *J* = 6.6 Hz, 1H, H-5D), 4.08–4.00 (m, 3H, C*H*$_{2AcCl}$, H-3C), 3.95–3.89 (m, 3H, H-1a$_{linker}$, H-5B, H-3A), 3.85 (dd, $J_{3\text{-}4}$ = 9.4 Hz, $J_{3\text{-}2}$ = 3.6 Hz, 1H, H-3B), 3.76 (br d, *J* = 10.4 Hz, 1H, H-6bA) 3.73 (br d, *J* = 10.5 Hz, 1H, H-6bC), 3.67 (t, *J* = 9.4 Hz, 1H, H-4C), 3.56–3.50 (m, 4H, H-1b$_{linker}$, H-3D, H-4A, H-5C), 3.48–3.43 (m, 2H, H-2A, H-4B), 3.39 (td, *J* = 9.7 Hz, *J* = 5.0 Hz, 1H, H-5A), 3.26 (s, 3H, C*H*$_{3OMe}$), 3.20 (t, *J* = 6.9 Hz,

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2H, H-5_{linker}), 2.08 (br s, 1H, O*H*), 2.03 (s, 3H, CH_{3Ac}), 1.76 (s, 3H, CH_{3Ac}), 1.66–1.62 (m, 2H, H-2_{linker}), 1.61–1.56 (m, 2H, H-4_{linker}), 1.47–1.40 (m, 2H, H-3_{linker}), 0.85 (d, J = 6.2 Hz, 3H, H-6B), 0.72 (d, J = 6.2 Hz, 3H, H-6D); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 169.9 (COOR$_{Ac}$), 169.5 (COOR$_{Ac}$), 167.3 (COOR$_{AcCl}$), 165.0 (COOR$_{AZMB}$), 139.0 (C$_{Ar}$), 138.2 (C$_{Ar}$), 137.1 (C$_{Ar}$), 137.0 (C$_{Ar}$), 133.4–126.2 (20C, C$_{Ar}$, 19 × CH$_{Ar}$), 104.2 (C-1A), 102.2, 101.59. 101.56 (3C, C-1C, C-7A, C-7C), 98.9 (C-1D), 98.1 (C-1B), 82.8 (C-2A), 79.4, 79.1, 78.9 (3C, C-4A, C-4C, C-3B), 76.5 (C-3A), 76.1 (C-3C), 75.1, 75.0 (2C, CH$_{2Bn}$, C-2C), 73.1 (C-3D), 71.8 (C-4B), 70.6, 70.5 (2C, C-2B, C-2D), 70.2 (C-1_{linker}), 68.9, 68.7 (2C, C-6A, C-6C), 67.8 (C-5B), 66.5 (C-5A), 66.4 (C-5C), 66.2 (C-4D), 65.2 (C-5D), 57.3 (CH$_{3OMe}$), 53.4 (CH$_{2AZMB}$), 51.4 (C-5_{linker}), 40.9 (CH$_{2AcCl}$), 29.4 (C-2_{linker}), 28.8 (C-4_{linker}), 23.5 (C-3_{linker}), 21.1 (CH$_{3Ac}$), 20.8 (CH$_{3Ac}$), 17.1 (C-6B), 15.8 (C-6D); HRMS (ESI-TOF) *m/z* [M + NH_4]$^+$ calcd for $C_{65}H_{81}N_7O_{23}$ 1362.50669; found 1362.51071; *m/z* [M + Na]$^+$ calcd for $C_{65}H_{77}ClN_6NaO_{23}$ 1367.46208; found 1367.46835.

(5-Azido-1-pentyl) 2,4-di-*O*-acetyl-6-deoxy-3-*O*-methyl-α-L-talopyranosyl-(1→3)-2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-6-deoxy-α-L-talopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (40). Dess-Martin periodinane (10 mg, 0.023 mmol, 2.2 equiv) was added to a solution of alcohol **38** (14 mg, 0.011 mmol, 1.0 equiv) in anhydrous DCE (0.16 mL). The mixture was refluxed under Ar for 1 h, then cooled to rt. The solution was diluted with DCM (1 mL) and quenched with 10% $Na_2S_2O_3$(aq) (1 mL). The solution was transferred into a separatory funnel and the organic and aqueous layers were separated. The organic phase was washed with brine (1 mL), dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Hex/EtOAc 9:1 to 6:4) to give the corresponding ketone as a white solid. The latter was dissolved in anhydrous DCM/MeOH (0.2 mL, 1:3) and the solution was cooled to –10 °C. $NaBH_4$ (2 mg,

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0.04 mmol, 4.0 equiv) was slowly added and the mixture was stirred under Ar for 1 h while gradually being warmed to 0 °C. The solution was diluted with DCM (1 mL) and washed with water (1 mL). The aqueous layer was extracted with DCM (3 × 1 mL). The combined organic layers were washed with brine (4 mL), dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Tol/EtOAc 9:1 to 7:3) to give alcohol **40** (7 mg, 52%) as a white amorphous solid: R_f 0.3 (Tol/EtOAc 7:3); $[\alpha]_D^{20}$ –96 (*c* 0.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 8.00-7.98 (m, 1H, C*H*$_{AZMB}$), 7.56-7.52 (m, 2H, 2 × C*H*$_{Ar}$), 7.45-7.42 (m, 4H, 4 × C*H*$_{Ar}$), 7.38-7.34 (m, 6H, 6 × C*H*$_{Ar}$), 7.31-7.27 (m, 5H, 5 × C*H*$_{Ar}$), 7.25-7.24 (m, 1H, C*H*$_{Ar}$), 5.54 (s, 1H, H-7C), 5.50 (s, 1H, H-7A), 5.31 (dd, $J_{2\text{-}3}$ = 8.9 Hz, $J_{2\text{-}1}$ = 7.9 Hz, 1H, H-2C), 5.22 (br d, *J* = 3.7 Hz, 1H, H-2B), 5.20 (s, 1H, H-1B), 5.03 (br d, *J* = 3.1 Hz, 1H, H-2D), 4.98 (br d, *J* = 3.9 Hz, 1H, H-4D), 4.93 (d, *J* = 7.7 Hz, 1H, H-1C), 4.89-4.84 (m, 4H, C*H*$_{2AZMB}$, C*H*H$_{Bn}$, H-1D), 4.71 (d, *J* = 10.8 Hz, 1H, CH*H*$_{Bn}$), 4.48 (d, *J* = 7.8 Hz, 1H, H-1A), 4.36 (dd, $J_{6a\text{-}6b}$ = 10.8 Hz, $J_{6a\text{-}5}$ = 5.1 Hz, 1H, H-6aC), 4.33 (dd, $J_{6a\text{-}6b}$ = 11.0 Hz, $J_{6a\text{-}5}$ = 5.4 Hz, 1H, H-6aA), 4.17 (br q, *J* = 5.4 Hz, 1H, H-5D), 4.12-4.08 (m, 2H, H-5B, H-3C), 4.07 (t, *J* = 3.6 Hz, 1H, H-3B), 3.94 (t, *J* = 8.2 Hz, 1H, H-3A), 3.93-3.88 (m, 1H, H-1a$_{linker}$), 3.80-3.74 (m, 2H, H-6bA, H-6bC), 3.69 (t, *J* = 9.4 Hz, 1H, H-4C), 3.57-3.50 (m, 4H, H-1b$_{linker}$, H-4A, H-4B, H-5C), 3.48 (t, *J* = 3.7 Hz, 1H, H-3D), 3.46-3.38 (m, 2H, H-2A, H-5A), 3.27 (s, 3H, C*H*$_{3OMe}$), 3.19 (t, *J* = 6.9 Hz, 2H, H-5$_{linker}$), 2.24 (br d, *J* = 9.7 Hz, 1H, O*H*), 2.04 (s, 3H, C*H*$_{3Ac}$), 1.76 (s, 3H, C*H*$_{3Ac}$), 1.67-1.61 (m, 2H, H-2$_{linker}$), 1.61 (s, 3H, C*H*$_{3Ac}$), 1.60-1.53 (m, 2H, H-4$_{linker}$), 1.46-1.39 (m, 2H, H-3$_{linker}$), 0.93 (d, *J* = 6.5 Hz, 3H, H-6B), 0.72 (d, *J* = 6.5 Hz, 3H, H-6D); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 170.9 (*C*OOR$_{Ac}$), 169.6 (*C*OOR$_{Ac}$), 168.8 (*C*OOR$_{Ac}$), 164.5 (*C*OOR$_{AZMB}$), 139.4 (C$_{Ar}$), 138.2 (C$_{Ar}$), 137.4 (C$_{Ar}$), 137.0 (C$_{Ar}$), 133.2-126.28 (20C, C$_{Ar}$, 19 × *C*H$_{Ar}$), 104.3 (C-1A), 102.2, 101.8 (2C, C-7A, C-7C), 99.0 (C-1D), 98.4 (C-1B), 97.2 (C-1C), 83.1 (C-2A), 79.23, 79.16 (2C,

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C-4A, C-4C), 76.1 (C-3A), 75.7 (C-3C), 74.9 (CH$_{2Bn}$), 74.5 (C-2C), 73.4 (C-3D), 70.4 (C-3B), 70.3 (C-1$_{linker}$), 69.4, 69.0, 68.8, 68.5, 68.0 (5C, C-2B, C-2D, C-6A, C-6C, C-4B), 66.9, 66.41, 66.39, 66.37 (4C, C-5A, C-5B, C-5C, C-4D), 65.4 (C-5D), 57.2 (CH$_{3OMe}$), 53.1 (CH$_{2AZMB}$), 51.4 (C-5$_{linker}$), 29.4 (C-2$_{linker}$), 28.7 (C-4$_{linker}$), 23.5 (C-3$_{linker}$), 21.0 (CH$_{3Ac}$), 20.8 (CH$_{3Ac}$), 20.3 (CH$_{3Ac}$), 16.1 (C-6B), 15.9 (C-6D); HRMS (ESI-TOF) *m/z* [M + NH$_4$]$^+$ calcd for $C_{65}H_{82}N_7O_{23}$ 1328.54566; found 1328.54556; *m/z* [M + Na]$^+$ calcd for $C_{65}H_{78}N_6NaO_{23}$ 1333.50105; found 1333.50256.

(5-Azido-1-pentyl) 2-*O*-acetyl-4-*O*-chloroacetyl-6-deoxy-3-*O*-methyl-α-L-talopyranosyl-(1→3)-2-*O*-*ortho*-(azidomethyl)benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-6-deoxy-α-L-talopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (31). A solution of anhydrous DMSO (0.06 mL, 0.8 mmol, 10.0 equiv) in anhydrous DCM (1.4 mL) was cooled at −10 °C and PDCP (0.07 mL, 0.5 mmol, 6.0 equiv) and Et_3N (0.11 mL, 0.80 mmol, 10.0 equiv) were successively added. A solution of alcohol **39** (108 mg, 0.0803 mmol, 1.0 equiv) in anhydrous DCM (0.4 mL) was added dropwise for 30 min. The mixture was stirred at −10 °C for 10 min under Ar, and for an additional 30 min while gradually being warmed to rt. Water (5 mL) was added to the solution, which was then transferred into a separatory funnel. The organic and aqueous layers were separated, and the aqueous phase was extracted with DCM (3 × 5 mL). The combined organic layers were washed with brine (10 mL), then dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Tol/EtOAc 9:1 to 8:2) to give the ketone as a white amorphous solid. The latter was solubilized in MeOH (1.1 mL) and DCM (0.4 mL) and cooled to −10 °C. $NaBH_4$ (12 mg, 0.32 mmol, 4.0 equiv) was slowly added and the mixture was stirred 1 h while gradually being warmed to 0 °C. The solution was diluted with DCM (5 mL), transferred in a separatory funnel, and washed with water (5 mL). The aqueous phase was extracted with DCM

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(3 × 5 mL), the combined organic phases were washed with brine (10 mL) and dried over anhydrous $MgSO_4$. The solution was evaporated under reduced pressure. The resulting crude alcohol (80.8 mg) was dissolved in anhydrous MeOH (2.3 mL) and anhydrous pyridine (2.3 mL). Thiourea (183 mg, 2.40 mmol, 30 eq.) was added and the solution was stirred under Ar for 3 h at 60 °C. The solvents were evaporated under reduced pressure and co-evaporated with toluene. The resulting white solid was dissolved in a 2:1 mixture of DCM/MeOH (10 mL) and washed with 1 N HCl(aq) (10 mL). The aqueous phase was extracted with DCM (3 × 10 mL) and the combined organic layers were washed with saturated $NaHCO_3$(aq) (30 mL) and brine (30 mL). The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Tol/EtOAc 9:1 to 7:3) to give alcohol **31** (58.9 mg, 58% over 3 steps) as a white amorphous solid: *Rf* 0.5 (Tol/EtOAc 1:1); $[\alpha]_D^{20}$ –79 (*c* 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 8.02–8.00 (m, 1H, CH_{AZMB}), 7.56–7.52 (m, 2H, 2 × CH_{Ar}), 7.47–7.45 (m, 2H, 2 × CH_{Ar}), 7.43–7.42 (m, 2H, 2 × CH_{Ar}), 7.37–7.28 (m, 11H, 11 × CH_{Ar}), 7.26–7.22 (m, 1H, CH_{Ar}), 5.54 (s, 1H, H-7C), 5.50 (s, 1H, H-7A), 5.30 (dd, $J_{2\text{-}3}$ = 9.0 Hz, $J_{2\text{-}1}$ = 7.8 Hz, 1H, H-2C), 5.22 (br d, *J* = 3.8 Hz, 1H, H-2B), 5.20 (br s, 1H, H-1B), 5.02 (br d, *J* = 3.7 Hz, 1H, H-2D), 4.92 (d, *J* = 7.8 Hz, 1H, H-1C), 4.88–4.85 (m, 4H, C*H*H$_{Bn}$, CH_{2AZMB}, H-1D), 4.71 (d, *J* = 10.8 Hz, 1H, CHH_{Bn}), 4.48 (d, *J* = 7.8 Hz, 1H, H-1A), 4.36 (dd, $J_{6a\text{-}6b}$ = 10.6 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6aC), 4.33 (dd, $J_{6a\text{-}6b}$ = 10.6 Hz, $J_{6a\text{-}5}$ = 5.0 Hz, 1H, H-6aA), 4.13 (t, *J* = 9.2 Hz, 1H, H-3C), 4.11–4.05 (m, 3H, H-3B, H-5B, H-5D), 3.94 (t, *J* = 9.2 Hz, 1H, H-3A), 3.91–3.89 (m, 1H, H-1a$_{linker}$), 3.78 (t, *J* = 10.2 Hz, 1H, H-6bA*), 3.76 (t, *J* = 10.3 Hz, 1H, H-6bC*), 3.68 (t, *J* = 9.4 Hz, 1H, H-4C), 3.58–3.50 (m, 5H, H-1b$_{linker}$, H-5C, H-4B, H-4D, H-4A), 3.45–3.39 (m, 3H, H-5A, H-2A, H-3D), 3.32 (s, 3H, CH_{3OMe}), 3.19 (t, *J* = 6.9 Hz, 2H, H-5$_{linker}$), 2.22 (d, *J* = 9.8 Hz, 1H, O*H*), 2.20 (d, *J* = 8.5 Hz, 1H, O*H*), 1.78 (s, 3H, CH_{3Ac}), 1.66–1.62 (m, 2H, H-2$_{linker}$), 1.60 (s, 3H, CH_{3Ac}), 1.59–1.56 (m,

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2H, H-4_{linker}), 1.45–1.40 (m, 2H, H-3_{linker}), 0.93 (d, J = 6.5 Hz, 3H, H-6B*), 0.90 (d, J = 6.5 Hz, 3H, H-6D*); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 168.9 (COOR$_{Ac}$), 168.8 (COOR$_{Ac}$), 164.6 (COOR$_{AZMB}$), 139.3 (C_{Ar}), 138.1 (C_{Ar}), 137.3 (C_{Ar}), 137.0 (C_{Ar}), 133.2–126.3 (20C, C_{Ar}, 19 × CH$_{Ar}$), 104.2 (C-1A), 102.1, 101.8 (2C, C-7A, C-7C), 98.6, 98.4 (2C, C-1B, C-1D), 97.2 (C-1C), 83.0 (C-2A), 79.2 (2C, C-4A, C-4C), 76.1 (C-3A), 75.8 (C-3C), 74.9 (CH$_{2Bn}$), 74.5 (C-2C), 73.9 (C-3D), 70.4 (C-3B), 70.2 (C-1_{linker}), 69.34, 69.26, 68.9, 68.8 (4C, C-6A, C-6C, C-2B, C-4D), 68.0, 67.7, 67.2 (3C, C-4B, C-2D, C-5D), 66.8 (C-5C), 66.4 (2C, C-5A, C-5B), 56.3 (CH$_{3OMe}$), 53.1 (CH$_{2AZMB}$), 51.4 (C-5_{linker}), 29.4 (C-2_{linker}), 28.7 (C-4_{linker}), 23.5 (C-3_{linker}), 20.7 (CH$_{3Ac}$), 20.3 (CH$_{3Ac}$), 16.1, 16.0 (2C, C-6B, C-6D); HRMS (ESI-TOF) *m/z* [M + NH_4]$^+$ calcd for $C_{63}H_{80}N_7O_{22}$ 1286.53509; found 1286.53314; *m/z* [M + Na]$^+$ calcd for $C_{63}H_{76}N_6NaO_{22}$ 1291.49049; found 1291;48805.

(5-Amino-1-pentyl) 2-*O*-acetyl-4-*O*-levulinoyl-3-*O*-*para*-methoxybenzyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (41). PPh_3 (3 mg, 0.01 mmol, 2.0 equiv) was added to a solution of disaccharide **26** (5 mg, 0.006 mmol, 1.0 equiv) in anhydrous THF (0.17 mL). The mixture was stirred at 60 °C under argon for 2 h, after which water (0.02 mL) was added. The solution was stirred for an additional 4 h at 60 °C and the solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (DCM/MeOH 95:5 to 8:2) to give disaccharide **41** (4 mg, 88%) as a white amorphous solid; R_f 0.5 (DCM/MeOH 8:2); $[\alpha]_D^{20}$ –41 (c 0.4, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.44–7.43 (m, 2H, 2 × CH-Ar), 7.35–7.30 (m, 8H, 8 × CH-Ar), 7.19 (m, 2H, 2 × CH_{PMB}), 6.85 (m, 2H, 2 × CH_{PMB}), 5.51 (s, 1H, H-7A), 5.43 (dd, $J_{2\text{-}3}$ = 3.2 Hz, $J_{2\text{-}1}$ = 1.7 Hz, 1H, H-2B), 5.17 (br s, 1H, H-1B), 4.89–4.83 (m, 2H, H-4B, CHHPh), 4.69 (d, J = 10.8 Hz, 1H, CHHPh), 4.58 (d, J = 10.6 Hz, 1H, CHHPh), 4.49 (d, J = 7.8 Hz, 1H, H-1A), 4.35 (dd, $J_{6a\text{-}6b}$ = 10.6 Hz, $J_{6a\text{-}5}$ = 5.0

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Hz, 1H, H-6aA), 4.32 (d, J = 11.6 Hz, 1H, CH*H*Ph), 4.06 (dq, $J_{5\text{-}4}$ = 10.0 Hz, $J_{5\text{-}6}$ = 6.2 Hz, 1H, H-5B), 3.94–3.88 (m, 2H, H-1a$_{linker}$, H-3A), 3.80–3.78 (m, 1H, H-3B), 3.77 (s, 3H, CH_{3PMB}), 3.75 (m, 1H, H-6bA), 3.58–3.51 (m, 2H, H-1b$_{linker}$, H-4A), 3.45–3.39 (m, 2H, H-2A, H-5A), 2.80 (br s, 2H, H-5$_{linker}$), 2.71–2.66 (m, 1H, C*H*H$_{Lev}$), 2.62–2.57 (m, 1H, CHH_{Lev}), 2.48–2.38 (m, 2H, CH_{2Lev}), 2.14 (s, 3H, CH_{3Lev}), 2.05 (s, 3H, CH_{3Ac}), 1.69–1.64 (m, 4H, H-2$_{linker}$, H-4$_{linker}$), 1.45–1.41 (m, 2H, H-3$_{linker}$), 0.78 (d, J = 6.2 Hz, 3H, H-6B). ^{13}C NMR (600 MHz, $CDCl_3$) δ (ppm) 206.4 (CO$_{Lev}$), 172.0 (COOR$_{Lev}$), 170.2 (COOR$_{Ac}$), 159.3 (C-Ar), 137.9 (C-Ar), 137.2 (C-Ar), 130.4 (C-Ar), 129.5–126.3 (12C, 12 × CH-Ar), 113.8 (2C, 2 × CH$_{PMB}$), 104.3 (C-1A), 101.8 (C-7A), 98.4 (C-1B), 82.7 (C-2A), 79.3 (C-4A), 76.3 (C-3A), 75.0 (CH$_2$Ph), 74.6 (C-3B), 72.9 (C-4B), 71.1 (CH$_2$Ph), 70.2 (C-1$_{linker}$), 68.9 (C-6A), 68.5 (C-2B), 66.5 (2C, C-5A, C-5B), 55.4 (CH$_{3PMB}$), 40.5 (C-5$_{linker}$), 38.0 (CH$_{2Lev}$), 30.0 (CH$_{3Lev}$), 29.9 (C-2$_{linker}$), 29.5 (C-4$_{linker}$), 28.1 (CH$_{2Lev}$), 23.4 (C-3$_{linker}$), 21.1 (CH$_{3Ac}$), 17.0 (C-6B); HRMS (ESI-TOF) *m/z* [M + H]$^+$ calcd for $C_{46}H_{60}NO_{14}$ 850.40083; found 850.40044; *m/z* [M + Na]$^+$ calcd for $C_{46}H_{59}NNaO_{14}$ 872.3828; found 872.3821.

(5-Amino-1-pentyl) 4,6-*O*-benzylidene-3-*O*-*para*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D glucopyranoside (42). PPh_3 (8 mg, 0.03 mmol, 4.0 equiv) was added to a solution of trisaccharide **27** (10 mg, 0.0078 mmol, 1.0 equiv) in anhydrous THF (0.23 mL). The mixture was stirred at 60 °C under argon for 2 h, after which water (0.02 mL) was added. The solution was stirred for an additional 4 h at 60 °C and the solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (DCM/MeOH 95:5 to 8:2) to give trisaccharide **42** (7 mg, 76%) as a white amorphous solid: R_f 0.4 (DCM/MeOH 8:2); $[\alpha]_D^{20}$ –42 (c 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.48–7.46 (m, 4H, 4 × C*H*-Ar), 7.37–7.30 (m, 13H, 13 × C*H*-

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Ar), 6.83–6.81 (m, 2H, 2 × C*H*-Ar), 5.54 (s, 1H, H-7C), 5.52 (s, 1H, H-7A), 5.30 (br d, J = 3.7, 1H, H-2B), 5.21 (br s, 1H, H-1B), 4.97 (t, J = 9.9 Hz, 1H, H-4B), 4.85–4.76 (m, 4H, CH_{2Bn}, CH_{2PMB}), 4.46 (d, J = 7.7 Hz, 1H, H-1A), 4.41 (d, J = 7.5 Hz, 1H, H-1C), 4.34 (dd, $J_{6a\text{-}6b}$ = 10.4 Hz, $J_{6a\text{-}5}$ = 4.7 Hz, 1H, H-6aC), 4.31 (dd, $J_{6a\text{-}6b}$ = 10.5 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6aA), 4.16 (dq, $J_{5\text{-}4}$ = 10.0 Hz, $J_{5\text{-}6}$ = 6.1 Hz, 1H, H-5B), 4.04 (dd, $J_{3\text{-}4}$ = 9.8 Hz, $J_{3\text{-}2}$ = 3.5 Hz, 1H, H-3B), 3.93 (t, J = 9.2 Hz, 1H, H-3A), 3.88-3.85 (m, 1H, H-1a$_{linker}$), 3.80–3.75 (m, 4H, CH_{3PMB}, H-6bC), 3.70–3.66 (m, 1H, H-6bA), 3.59–3.51 (m, 5H, H-1b$_{linker}$, H-2C, H-3C, H-4C, H-4A), 3.47 (t, J = 8.3 Hz, 1H, H-2A), 3.41 (t, J = 9.8 Hz, 1H, H-4A*), 3.40 (t, J = 9.6 Hz, 1H, H-5C*), 2.85–2.82 (m, 2H, H-5$_{linker}$), 2.81–2.78 (m, 1H, C*H*H$_{Lev}$), 2.65–2.53 (m, 2H, CHH_{Lev}, C*H*H$_{Lev}$), 2.38–2.34 (m, 1H, CHH_{Lev}), 2.18 (s, 3H, CH_{3Lev}), 2.04 (s, 3H, CH_{3Ac}), 1.70–1.68 (m, 2H, H-2$_{linker}$), 1.62–1.60 (m, 2H, H-4$_{linker}$), 1.40–1.36 (m, 2H, H-3$_{linker}$), 0.82 (d, J = 6.1 Hz, 3H, H-6B); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 207.7 (*C*O$_{Lev}$), 172.4 (*C*OOR$_{Lev}$), 170.0 (*C*OOR$_{Ac}$), 159.3 (C-Ar), 138.1 (C-Ar), 137.6 (C-Ar), 137.3 (C-Ar), 131.0-126.2 (18C, C-Ar, 17 x *C*H-Ar), 113.8 (2C, 2 x *C*H$_{PMB}$), 104.6, 104.2 (2C, C-1A, C-1C), 101.8, 101.3 (2C, C-7A, C-7C), 97.7 (C-1B), 82.7 (C-2A), 81.0, 79.3 (2C, C-4A, C-4C), 76.5, 76.0 (C-3A, C-3C*), 74.9 (2C, C-3B, *C*H$_{2Bn}$), 74.4, 74.3 (2C, *C*H$_{2PMB}$, C-2C*), 72.8 (C-4B), 71.4 (C-2B), 70.0 (C-1$_{linker}$), 68.9, 68.8 (2C, C-6A, C-6C), 66.44, 66.35, 65.9 (3C, C-5A, C-5B, C-5C), 55.4 (*C*H$_{3PMB}$), 39.9 (C-5$_{linker}$), 37.9 (*C*H$_{2Lev}$), 30.1 (*C*H$_{3Lev}$), 29.9 (C-2$_{linker}$), 29.2 (C-4$_{linker}$), 27.4 (*C*H$_{2Lev}$), 23.1 (C-3$_{linker}$), 21.2 (*C*H$_{3Ac}$), 16.9 (C-6B); HRMS (ESI-TOF) *m/z* [M + H]$^+$ calcd for $C_{59}H_{74}NO_{19}$ 1100.48496; found 1100.48469; *m/z* [M + Na]$^+$ calcd for $C_{59}H_{73}NNaO_{19}$ 1122.4669; found 1122.4666.

(5-Amino-1-pentyl) 2-*O*-acetyl-4-*O*-chloroacetyl-6-deoxy-3-*O*-methyl-α-L-talopyranosyl-(1→3)-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-6-deoxy-α-L-talopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (43). PPh_3 (3 mg, 0.01 mmol, 4.0

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equiv) was added to a solution of tetrasaccharide **31** (4 mg, 0.003 mmol, 1.0 equiv) in anhydrous THF (0.1 mL). The mixture was stirred at 60 °C under argon for 2 h, after which water (0.01 mL) was added. The solution was stirred for an additional 4 h at 60 °C, and the solvents were evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (DCM/MeOH 95:5 to 8:2) to give tetrasaccharide **43** (2.1 mg, 63%) as a white amorphous solid: R_f 0.5 (DCM/MeOH 8:2); $[\alpha]_D^{20}$ –75 (*c* 0.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.44–7.43 (m, 4H, 4 × C*H*-Ar), 7.37–7.29 (m, 11H, 11 × C*H*-Ar), 5.49 (2 × s, 2H, H-7A, H-7C), 5.31 (br s, 1H, H-1B*), 5.30 (br d, J = 2.7 Hz, 1H, H-2D), 5.28 (br d, J = 3.4 Hz, 1H, H-2B), 5.26 (s, 1H, H-1D*), 4.84 (d, J = 10.9 Hz, 1H, C*H*H$_{Bn}$), 4.76 (d, J = 10.9 Hz, 1H, CH*H*$_{Bn}$), 4.49 (d, J = 7.8 Hz, 1H, H-1A*), 4.48 (d, J = 7.7 Hz, 1H, H-1C*), 4.34 (dd, $J_{6a\text{-}6b}$ = 10.2 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, 1H, H-6aA*), 4.32 (dd, $J_{6a\text{-}6b}$ = 10.2 Hz, $J_{6a\text{-}5}$ = 4.7 Hz, 1H, H-6aC*), 4.19–4.14 (m, 2H, H-5B, H-5D), 4.03 (t, J = 3.5 Hz, 1H, H-3B), 3.95 (t, J = 9.2 Hz, 1H, H-3A), 3.91–3.86 (m, 2H, H-1a$_{linker}$, H-3C), 3.75 (t, J = 10.2 Hz, 2H, H-6bA, H-6bC), 3.60–3.58 (m, 2H, H-2C, H-4D), 3.54–3.50 (m, 5H, H-3D, H-4A, H-4B, H-4C, H-1b$_{linker}$), 3.46–3.42 (m, 3H, H-2A, H-5A, H-5C), 3.40 (s, 3H, C*H*$_{3Me}$), 2.85 (br s, 2H, H-5$_{linker}$), 2.11 (s, 3H, C*H*$_{3Ac}$), 2.05 (s, 3H, C*H*$_{3Ac}$), 1.72–1.70 (m, 2H, H-2$_{linker}$), 1.63–1.60 (m, 2H, H-4$_{linker}$), 1.41–1.38 (m, 2H, H-3$_{linker}$), 1.03 (d, J = 6.5 Hz, 3H, H-6B*), 0.92 (d, J = 6.4 Hz, 3H, H-6D*); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 169.8 (*C*OOR$_{Ac}$), 168.9 (*C*OOR$_{Ac}$), 138.2 (C-Ar), 137.2 (2C, 2 × C-Ar), 129.6–126.2 (15C, 15 × *C*H-Ar), 104.2 (C-1A*), 102.0, 101.8 (2C, C-7A, C-7C), 101.4 (C-1C*), 98.4, 98.2 (2C, C-1B, C-1D), 83.0 (C-2A), 79.2, 78.8 (2C, C-4A, C-4C), 76.4, 76.3 (C-3A, C-3C), 75.8 (C-2C), 74.9 (*C*H$_{2Bn}$), 74.3 (C-3D), 72.2 (C-3B), 70.0 (C-1$_{linker}$), 69.7–66.2 (10C, C-2B, C-2D, C-4B, C-4D, C-5A, C-5B, C-5C, C-5D, C-6A, C-6C), 56.4 (*C*H$_{3Me}$), 40.0 (C-5$_{linker}$), 29.2 (C-4$_{linker}$), 27.8 (C-2$_{linker}$), 23.2 (C-3$_{linker}$), 21.3 (*C*H$_{3Ac}$), 21.2 (*C*H$_{3Ac}$), 16.2,

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16.0 (2C, C-6B, C-6D); HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{55}H_{74}NO_{21}$ 1084.47478; found 1084.47259; *m/z* $[M + Na]^+$ calcd for $C_{55}H_{73}NNaO_{21}$ 1106.45673; found 1106.45461.

(5-Amino-1-pentyl) 2,4-di-*O*-acetyl-6-deoxy-3-*O*-methyl-α-L-talopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-6-deoxy-α-L-talopyranosyl-(1→3)-β-D-glucopyranoside hydrochloride (8). Alcohol **40** (37 mg, 0.028 mmol, 1.0 equiv) was dissolved in DCE (0.3 mL) and MeOH (7.2 mL). The solution was degassed with Ar and Pd black (37 mg, 1 mg/mg of alcohol **40**) and 12 N HCl(aq) (2.3 µL, 0.028 mmol, 1.0 equiv) were successively added. The suspension was stirred under an atmosphere of H_2 at 40 °C for 16 h. The mixture was filtered over Celite to remove the catalyst and the cake was rinsed with MeOH. The solution was evaporated under reduced pressure. The residue was purified by LH-20 resin (MeOH) followed by reverse phase chromatography (100% H_2O to 1:1 H_2O/MeOH) to give tetrasaccharide **8** (15 mg, 60%) as a white amorphous solid: R_f 0.3 ($CHCl_3$/MeOH/H_2O 10:10:3); $[\alpha]_D^{20}$ –34 (*c* 0.8, MeOH); ^{1}H NMR (600 MHz, D_2O) δ (ppm) 5.37 (d, J = 3.1 Hz, 1H, H-4D), 5.28 (br s, 2H, H-1B*, H-2D), 5.22–5.21 (m, 2H, H-1D*, H-2B), 4.63 (d, J = 8.0 Hz, 1H, H-1C*), 4.52 (br q, J = 6.4 Hz, 1H, H-5D), 4.45 (d, J = 8.1 Hz, 1H, H-1A), 4.36 (br q, J = 6.6 Hz, 1H, H-5B), 4.24 (t, J = 3.6 Hz, 1H, H-3B), 3.98 (t, J = 3.4 Hz, 1H, H-3D), 3.96–3.90 (m, 3H, H-1a$_{linker}$, H-6aA*, H-4B), 3.85 (dd, J = 12.3 Hz, J = 2.0 Hz, 1H, H-6aC*), 3.75–3.66 (m, 3H, H-1b$_{linker}$, H-6bA, H-6bC), 3.63–3.57 (m, 2H, H-4A, H-4C), 3.52–3.43 (m, 5H, H-2C*, H-3A, H-3C, H-5A, H-5C), 3.40 (s, 3H, CH_{3Me}), 3.37 (dd, J = 9.0 Hz, J = 8.3 Hz, 1H, H-2A*), 3.00 (t, J = 7.2 Hz, 2H, H-5$_{linker}$), 2.21 (s, 3H, CH_{3Ac}), 2.17 (s, 3H, CH_{3Ac}), 2.16 (s, 3H, CH_{3Ac}), 1.71–1.64 (m, 4H, H-2$_{linker}$, H-4$_{linker}$), 1.48–1.43 (m, 2H, H-3$_{linker}$), 1.24 (d, J = 6.6 Hz, 3H, H-6B), 1.14 (d, J = 6.6 Hz, 3H, H-6D); ^{13}C NMR (150 MHz, D_2O) δ (ppm) 174.4 (*C*OOR$_{Ac}$), 174.1 (*C*OOR$_{Ac}$), 173.7 (*C*OOR$_{Ac}$), 102.6, 102.4 (2C, $^1J_{C\text{-}H}$ = 163.9 Hz, $^1J_{C\text{-}H}$ = 162.1 Hz, C-1A, C-1C), 99.4, 99.3 (2C, $^1J_{C\text{-}H}$ = 173.4 Hz, $^1J_{C\text{-}H}$ = 176.5 Hz, C-1B, C-1D), 82.8, 82.6 (2C,

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C-4A, C-4C), 76.5, 76.4 (2C, C-3A, C-3C), 74.2, 74.1 (2C, C-2A, C-2C), 73.9, 73.6 (2C, C-3B, C-3D), 70.8, 70.7 (2C, C-1_{linker}, C-2B), 70.1, 69.2, 68.8, 68.3, 68.1, 67.8 (6C, C-2D, C-4B, C-4D, C-5A, C-5B, C-5C), 66.3 (C-5D), 61.4, 61.1 (2C, C-6A, C-6C), 57.3 (CH_{3Me}), 40.0 (C-5_{linker}), 28.8 (C-4_{linker}), 27.1 (C-2_{linker}), 22.7 (C-3_{linker}), 21.3 (CH_{3Ac}), 21.1 (CH_{3Ac}), 21.0 (CH_{3Ac}), 16.0, 15.8 (2C, C-6B, C-6D); HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{36}H_{62}NO_{22}$ 860.3758; found 860.3776.

(5-Amino-1-pentyl) 2-*O*-acetyl-6-deoxy-3-*O*-methyl-α-L-talopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-2-*O*-acetyl-6-deoxy-α-L-talopyranosyl-(1→3)-β-D-glucopyranoside hydrochloride (9). Tetrasaccharide **43** (2 mg, 0.002 mmol, 1.0 equiv) was dissolved in DCE (0.02 mL) and MeOH (0.5 mL). The solution was degassed with Ar and Pd black (2 mg, 1 mg/mg of tetrasaccharide **43**) and 12 N HCl(aq) (0.2 μL, 0.002 mmol, 1.0 equiv) were successively added. The suspension was stirred under an atmosphere of H_2 at 40 °C for 16 h. The mixture was filtered over Celite to remove the catalyst and the cake was rinsed with MeOH. The solution was evaporated under reduced pressure. The residue was purified on reverse phase chromatography (100% H_2O to 1:1 H_2O/MeOH) to give tetrasaccharide **9** (1.5 mg, 91%) as a white amorphous solid; R_f 0.2 ($CHCl_3$/MeOH/H_2O 10:10:3); $[\alpha]_D^{20}$ –25 (*c* 0.2, MeOH); ^{1}H NMR (600 MHz, D_2O) δ (ppm) 5.25 (br d, J = 3.8 Hz, 1H, H-2D), 5.24 (br s, 1H, H-1B*), 5.22–5.21 (m, 2H, H-1D*, H-2B), 4.63 (d, J = 8.1 Hz, 1H, H-1A*), 4.45 (d, J = 8.2 Hz, 1H, H-1C*), 4.38–4.33 (m, 2H, H-5B, H-5D), 4.24 (t, J = 3.6 Hz, 1H, H-3B), 3.96–3.95 (m, 2H, H-4B, H-4D), 3.94–3.90 (m, 2H, H-$1a_{linker}$, H-6aA*), 3.85 (dd, J = 12.3 Hz, J = 2.1 Hz, 1H, H-6aC*), 3.79 (t, J = 3.6 Hz, 1H, H-3D), 3.75–3.66 (m, 3H, H-$1b_{linker}$, H-6bA, H-6bC), 3.61–3.57 (m, 2H, H-4A, H-4C), 3.50–3.43 (m, 5H, H-2A*, H-3A, H-3C, H-5A, H-5C), 3.41 (s, 3H, CH_{3Me}), 3.38 (dd, J = 9.1 Hz, J = 8.4 Hz, 1H, H-2C*), 2.99 (t, J = 7.4 Hz, 2H, H-5_{linker}), 2.16 (s, 3H, CH_{3Ac}), 2.14 (s, 3H, CH_{3Ac}), 1.71-1.64 (m, 4H, H-2_{linker}, H-4_{linker}), 1.48–1.43 (m, 2H, H-3_{linker}), 1.24 (d, J = 6.6 Hz, 6H, H-6B, H-6D); ^{13}C NMR

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(150 MHz, D_2O) δ (ppm) 174.1 (*C*OOR_{Ac}), 173.9 (*C*OOR_{Ac}), 102.6, 102.4 (2C, C-1A, C-1C), 99.4, 99.3 (2C, C-1B, C-1D), 82.8, 82.4 (2C, C-4A, C-4C), 76.5, 76.4 (2C, C-3A, C-3C), 74.3, 74.2 (2C, C-2A, C-2C), 74.1, 73.9 (2C, C-3B, C-3D), 70.8, 70.7 (2C, C-1_{linker}, C-2B), 69.2, 68.8, 68.6, 68.3, 68.2, (5C, C-2D, C-4B, C-4D, C-5A, C-5C), 67.8, 67.7 (2C, C-5B, C-5D), 61.4, 61.1 (2C, C-6A, C-6C), 56.2 (*C*H_{3Me}), 40.0 (C-5_{linker}), 28.8 (C-4_{linker}), 27.1 (C-2_{linker}), 22.7 (C-3_{linker}), 21.3 (*C*H_{3Ac}), 21.2 (*C*H_{3Ac}), 16.04, 15.99 (2C, C-6B, C-6D); HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{34}H_{60}NO_{21}$ 818.36523; found 818.36495.

CONCLUSIONS

In summary, we have reported the synthesis of two tetrasaccharides mimicking the predominant epitopes of *Bp* and *Bm* LPS OAgs. We have shown that these compounds can be efficiently prepared using a sequential [1 + 1 + 1 + 1] glycosylation strategy. Oligosaccharide **9**, which mimics *Bm* LPS OAg, is strongly recognized by *Bm* LPS specific mAbs, highlighting the potential of using this compound as a vaccine candidate against glanders. Additionally, *Bp*- and *Bm*-related tetrasaccharides **8** and **9** exhibit high reactivity towards the serum of culture-confirmed Thai melioidosis patients. Therefore, we have shown that by including both the terminal and the major intra-chain epitopes of *Bp* and *Bm* LPS OAgs within the same compounds, their antigenicity is similar to that of native *Bp* OAg. These results suggest that synthetic oligosaccharides **8** and **9** represent exquisite mimics of the whole OAg epitope, which bodes well for their use as functional antigens in glycoconjugate vaccine preparations against melioidosis and glanders.

CONFLICTS OF INTEREST

A related provisional patent has been filed with the title “Tetrasaccharides for diagnosis, prevention, and treatment of melioidosis and glanders” by C. G., M. C. and the Institut national de

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la recherche scientifique (USPTO 62/857,346). The remaining authors have no conflicts of interest to declare.

AUTHORS CONTRIBUTION

C. G. designed and supervised the research; M.C., E. D., K. M., S. N., and R. R. H. performed the organic synthesis; M. C. analyzed the NMR data; M. N. B., P. J. B., T. K., and N. C. performed the antigenicity experiments; M. C., M. N. B., P. J. B, and C. G. wrote the manuscript.

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REFERENCES

1. D. Dance, *Clin. Microbiol. Rev.*, 1991, **4**, 52-60.
2. A. C. Cheng and B. J. Currie, *Clin. Microbiol. Rev.*, 2005, **18**, 383-416.
3. V. Keluangkhot, R. Pethsouvanh and M. Strobel, *Med. Maladies Infect.*, 2005, **35**, 469-475.
4. B. J. Currie, *Semin. Respir. Crit. Care Med.*, 2015, **36**, 111-125.
5. I. Khan, L. Wieler, F. Melzer, M. Elschner, G. Muhammad, S. Ali, L. Sprague, H. Neubauer and M. Saqib, *Transboud. Emerg. Dis.*, 2013, **60**, 204-221.

View Article Online
DOI: 10.1039/C9OB01711A

6. D. Limmathurotsakul, S. Wongratanacheewin, N. Teerawattanasook, G. Wongsuvan, S. Chaisuksant, P. Chetchotisakd, W. Chaowagul, N. P. Day and S. J. Peacock, *Am. J. Trop. Med. Hyg.*, 2010, **82**, 1113-1117.

7. W. J. Wiersinga, B. J. Currie and S. J. Peacock, *New Engl. J. Med.*, 2012, **367**, 1035-1044.

8. F. S. A. Program, *2017 Annual Report of the Federal Select Agent Program*, 2017.

9. R. W. Titball, M. N. Burtnick, G. J. Bancroft and P. Brett, *Vaccine*, 2017, **35**, 5981-5989.

10. L. A. Muruato, D. Tapia, C. L. Hatcher, M. Kalita, P. J. Brett, A. E. Gregory, J. E. Samuel, R. W. Titball and A. G. Torres, *Clin. Vaccine Immunol.*, 2017, **24**, e00206-00217.

11. M. Cloutier, K. Muru, G. Ravicoularamin and C. Gauthier, *Nat. Prod. Rep.*, 2018, **35**, 1251-1293.

12. L. E. Bryan, S. Wong, D. E. Woods, D. A. Dance and W. Chaowagul, *Can. J. Infect. Dis. Med.*, 1994, **5**, 170-178.

13. M. Ho, T. Schollaardt, M. D. Smith, M. B. Perry, P. J. Brett, W. Chaowagul and L. E. Bryan, *Infect. Immun.*, 1997, **65**, 3648-3653.

14. S. Jones, J. Ellis, P. Russell, K. Griffin and P. Oyston, *J. Med. Microbiol.*, 2002, **51**, 1055-1062.

15. S. R. Treviño, A. R. Permenter, M. J. England, N. Parthasarathy, P. H. Gibbs, D. M. Waag and T. C. Chanh, *Infect. Immun.*, 2006, **74**, 1958-1961.

16. S. Zhang, S.-H. Feng, B. Li, H.-Y. Kim, J. Rodriguez, S. Tsai and S.-C. Lo, *Clin. Vaccine Immunol.*, 2011, **18**, 825-834.

17. D. P. AuCoin, D. E. Reed, N. L. Marlenee, R. A. Bowen, P. Thorkildson, B. M. Judy, A. G. Torres and T. R. Kozel, *PLoS One*, 2012, **7**, e35386.

18. A. E. Scott, S. A. Ngugi, T. R. Laws, D. Corser, C. L. Lonsdale, R. V. D'Elia, R. W. Titball, E. D. Williamson, T. P. Atkins and J. L. Prior, *J. Immunol. Res.*, 2014, **2014**.

View Article Online
DOI: 10.1039/C9OB01711A

19. N. Anuntagool, V. Wuthiekanun, N. J. White, B. J. Currie, R. W. Sermswan, S. Wongratanacheewin, S. Taweechaisupapong, S. C. Chaiyaroj and S. Sirisinha, *Am. J. Trop. Med. Hyg.*, 2006, **74**, 348-352.

20. Y. A. Knirel, N. A. Paramonov, A. S. Shashkov, N. K. Kochetkov, R. G. Yarullin, S. M. Farber and V. I. Efremenko, *Carbohydr. Res.*, 1992, **233**, 185-193.

21. M. B. Perry, L. L. MacLean, T. Schollaardt, L. E. Bryan and M. Ho, *Infect. Immun.*, 1995, **63**, 3348-3352.

22. M. N. Burtnick, P. J. Brett and D. E. Woods, *J. Bacteriol.*, 2002, **184**, 849-852.

23. P. J. Brett, M. N. Burtnick and D. E. Woods, *FEMS Microbiol. Lett.*, 2003, **218**, 323-328.

24. C. Heiss, M. N. Burtnick, R. A. Roberts, I. Black, P. Azadi and P. J. Brett, *Carbohydr. Res.*, 2013, **381**, 6-11.

25. C. Heiss, M. N. Burtnick, I. Black, P. Azadi and P. J. Brett, *Carbohydr. Res.*, 2012, **363**, 23-28.

26. M. Tamigney Kenfack, M. Mazur, T. Nualnoi, T. L. Shaffer, A. Ngassimou, Y. Blériot, J. Marrot, R. Marchetti, K. Sintiprungrat, N. Chantratita, A. Silipo, A. Molinaro, D. P. AuCoin, M. N. Burtnick, P. J. Brett and C. Gauthier, *Nat. Commun.*, 2017, **8**, 115.

27. T. Wada, A. Ohkubo, A. Mochizuki and M. Sekine, *Tetrahedron Lett.*, 2001, **42**, 1069-1072.

28. K. R. Love, R. B. Andrade and P. H. Seeberger, *J. Org. Chem.*, 2001, **66**, 8165-8176.

29. A. Arasappan and P. Fuchs, *J. Am. Chem. Soc.*, 1995, **117**, 177-183.

30. C. Gauthier, P. Chassagne, F.-X. Theillet, C. Guerreiro, F. Thouron, F. Nato, M. Delepierre, P. J. Sansonetti, A. Phalipon and L. A. Mulard, *Org. Biomol. Chem.*, 2014, **12**, 4218-4232.

31. G. Lian, X. Zhang and B. Yu, *Carbohydr. Res.*, 2015, **403**, 13-22.

32. T. G. Frihed, C. M. Pedersen and M. Bols, *Eur. J. Org. Chem.*, 2014, **2014**, 7924-7939.

View Article Online
DOI: 10.1039/C9OB01711A

33. U. Ellervik, H. Grundberg and G. Magnusson, *J. Org. Chem.*, 1998, **63**, 9323-9338.

34. S. David and S. Hanessian, *Tetrahedron*, 1985, **41**, 643-663.

35. C.-C. Wang, J.-C. Lee, S.-Y. Luo, S. S. Kulkarni, Y.-W. Huang, C.-C. Lee, K.-L. Chang and S.-C. Hung, *Nature*, 2007, **446**, 896.

36. B. Neises and W. Steglich, *Angew. Chem. Int. Ed. Engl.*, 1978, **17**, 522-524.

37. D. Baudry, M. Ephritikhine and H. Felkin, *J. Chem. Soc., Chem. Commun.*, 1978, 694-695.

38. J. Oltvoort, C. Van Boeckel, J. De Koning and J. Van Boom, *Synthesis*, 1981, **1981**, 305-308.

39. M. A. Nashed and L. Anderson, *J. Chem. Soc., Chem. Commun.*, 1982, 1274-1276.

40. D. C. Baker, *J. Am. Chem. Soc.*, 1997, **119**, 12028-12029.

41. R. R. Schmidt and J. Michel, *Angew. Chem. Int. Ed. Engl.*, 1980, **19**, 731-732.

42. R. R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, 1986, **25**, 212-235.

43. R. R. Schmidt and W. Kinzy, In: *Advances in Carbohydrate Chemistry and Biochemistry*, Academic Press, 1994, vol. 50, pp. 21-123.

44. R. R. Schmidt and K.-H. Jung, *Prep. Carbohydr. Chem.*, 1997, **1**.

45. B. Mukhopadhyay and R. A. Field, *Carbohydr. Res.*, 2006, **341**, 1697-1701.

46. P. Konradsson, D. R. Mootoo, R. E. McDevitt and B. Fraser-Reid, *J. Chem. Soc., Chem. Commun.*, 1990, 270-272.

47. P. Konradsson, U. E. Udodong and B. Fraser-Reid, *Tetrahedron Lett.*, 1990, **31**, 4313-4316.

48. G. Hodosi and P. Kováč, *J. Carbohydr. Chem.*, 1998, **17**, 557-565.

49. R. Miethchen, *J. Carbohydr. Chem.*, 2003, **22**, 801-825.

50. O. Mitsunobu, *Synthesis*, 1981, **1981**, 1-28.

51. A. Banaszek, *J. Carbohydr. Chem.*, 1994, **13**, 285-291.

52. S. R. Sanapala and S. S. Kulkarni, *Org. Lett.*, 2016, **18**, 3790-3793.

View Article Online
DOI: 10.1039/C9OB01711A

53. I. E. Muskat, *J. Am. Chem. Soc.*, 1934, **56**, 2653-2656.

54. W. Song, J. Cai, X. Zou, X. Wang, J. Hu and J. Yin, *Chin. Chem. Lett.*, 2018, **29**, 27-34.

55. G. O. Aspinall, *Carbohydr. Res.*, 1983, **121**, 61-77.

56. V. Zsoldos-Mády and E. Zbiral, *Monatsh. Chem.* , 1986, **117**, 1325-1338.

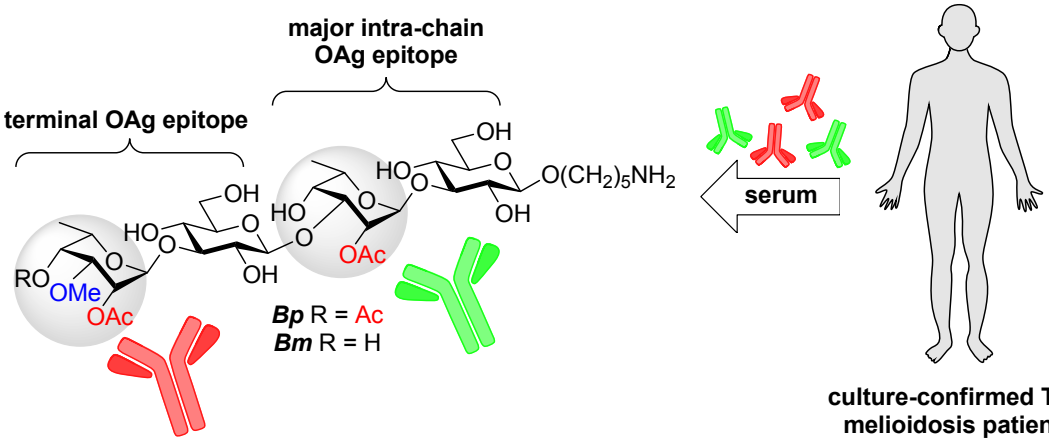
57. X. Cai, G. Zong, Y. Xu, J. Zhang, X. Liang and D. Wang, *Carbohydr. Res.*, 2010, **345**, 1230-1234.

58. K. Ohara, C.-C. Lin, P.-J. Yang, W.-T. Hung, W.-B. Yang, T.-J. R. Cheng, J.-M. Fang and C.-H. Wong, *J. Org. Chem.*, 2013, **78**, 6390-6411.

59. V. Suttisunhakul, V. Wuthiekanun, P. J. Brett, S. Khusmith, N. P. Day, M. N. Burtnick, D. Limmathurotsakul and N. Chantratita, *J. Clin. Microbiol.*, 2016, **54**, 1259-1268.

60. M. N. Burtnick, C. Heiss, A. Schuler, P. Azadi and P. J. Brett, *Front. Cell. Infect. Microbiol.*, 2012, **2**, 148.

View Article Online
DOI: 10.1039/C9OB01711A



Tetrasaccharides mimicking *Burkholderia pseudomallei* and *Burkholderia mallei* lipopolysaccharide O-antigens were synthesized and found to be highly reactive with Thai melioidosis patient serum, highlighting their potential as vaccine candidates.