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Chemical synthesis of trisaccharide epitope of Phenolic glycolipid-1 surface antigen from *Mycobacterium leprae*

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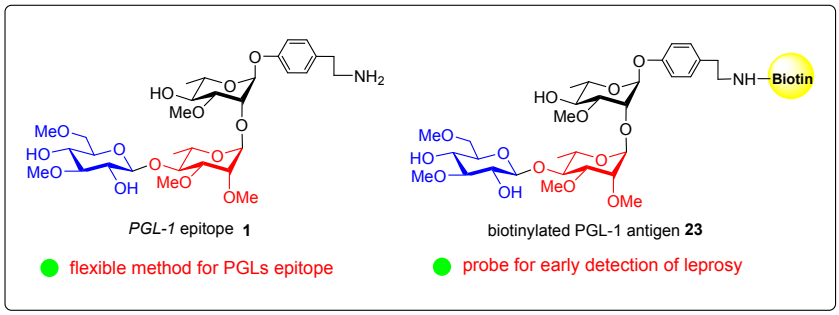


ABSTRACT: PGL-1 epitope **1** bearing a *p*-aminoethylphenol group was efficiently synthesized by using linear synthetic routes. A method for efficient synthesis of oligosaccharides containing rhamnose rings was developed. The chemistry is flexible and could be used for the synthesis of other PGLs antigens. A biotinylated PGL-1 antigen **23** was synthesized and could be used as a probe for early detection of leprosy.

Phenolic glycolipids (PGLs) are constituents of the mycobacterial cell wall and are polyketide-derived virulence factors produced by some mycobacterial species in particular the three main human mycobacterial pathogens: *M. tuberculosis, M. ulcerans* and *M. leprae.*[1] Phenolic glycolipid-1 (PGL-1) is the major PGLs from *M.leprae*, and occurs on the cell surface of *M. leprae* in copious amounts and was first isolated from the *M. leprae*-infected armadillos liver by Brennan *et al* [2] in 1981. As shown in **Fig.1**, natural PGL-1 consist of a lipid core formed by a long-chain β-diol (called phthiocerol), which occurs naturally as a diester of polymethyl-branched fatty acids (called mycocerosic acids). This core is terminated by a phenol ring (called PDIM) that is glycosylated by a species-specific oligosaccharide.[3] The oligosaccharide part of PGL-1 is composed of 3,6-dimethyl-D-glucose (β1→4) linked 2,3-di-*O*-methyl-L-rhamnopyranose, and further (α1→2) linked 3-*O*-methyl-L-rhamnopyranose.[4] PGL-1 can induce macrophages to produce excess nitric oxide and some of the synthetic PGL-1 antigens were used for detecting antibodies in patients with leprosy.[5] The trisaccharide moiety of PGL-1 from *M. leprae* can bind the lectin domain of complement receptor 3 (CR3) for efficient invasion of human macrophages.[6] It was claimed that the oligosaccharide unit of PGL-1 plays a crucial role in the pathogenesis of mycobacteria and could be developed as potential vaccine candidates.(**Fig.1**) [7]

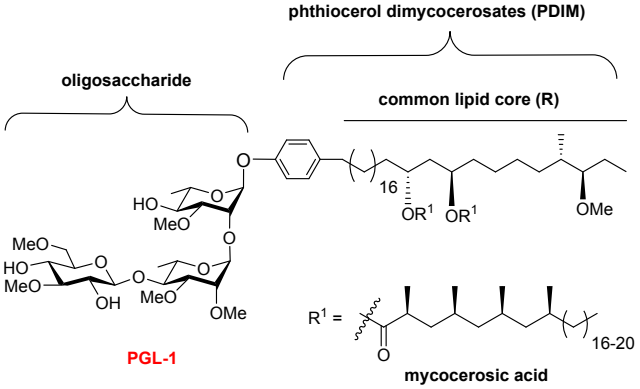
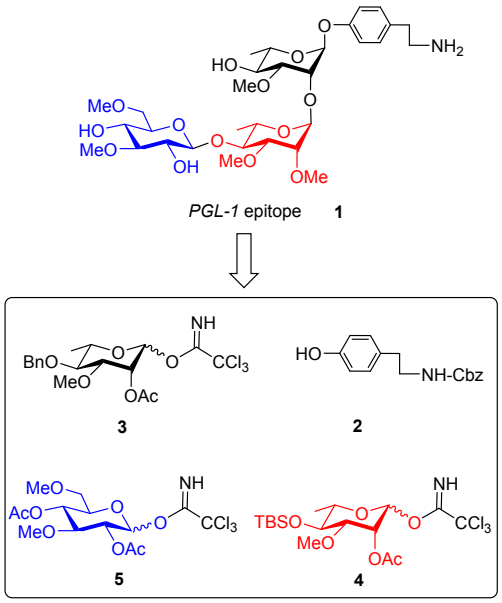


Fig. 1 Structure of natural PGL-1

Several methods were reported because of interest to the antigenic activity of the trisaccharide moiety of PGL-1.[8-9] The first attempt at synthesizing the trisaccharide unit of PGL-1 was reported by the Brennan group in 1984 with a total yield of only 2%.[10-11] Later, Lipták et al[12] synthesized a terminal phenyl substituted PGL-1 analogue in a yield of 21%. Recently, Lowary et al [13] synthesized several analogues of PGL-1 and evaluated their ability to stimulate cytokine release by activated THP-1 cells. The results showed that only the trisaccharide of PGL-1 could modulate the cytokine release.[6] So far, the methods for efficient synthesis of 1,2-*cis*-L-rhamnopyranosidic linkages with high stereoselectivity were limited.[14] Hence, we herein present a convenient and practical method for the

synthesis of the trisaccharide of PGL-1 (PGL-1 epitope **1**) and its conjugate with NHS-biotin **23**.

The retrosynthetic analysis of the targeted PGL-1 epitope **1** was shown in **Scheme 1**. Trisaccharide **1** was envisioned to be assembled by three glycosylation reactions. Firstly, glycosylation of **2** with **3** will form the monosaccharide, which will be converted to a glycosyl acceptor by removal of the *O*-2 acetyl group. The latter will be glycosylated with glycosyl donor **4** to provide the disaccharide, followed by deacetylation and methylation at the *O*-2 position of the second rhamnose unit. The resulting disaccharide will be converted to glycosyl acceptor by removal of the *O*-4 TBS group. The resulting disaccharide acceptor will be glycosylated with **5** and the resulting product will finally be converted to the targeted *PGL-1* epitope **1** by a sequence of conventional deprotection methods.

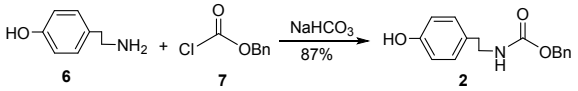


Scheme 1. Retrosynthetic analysis of PGL-1 epitope **1**

Synthesis of the building blocks

Synthesis of Compound 2

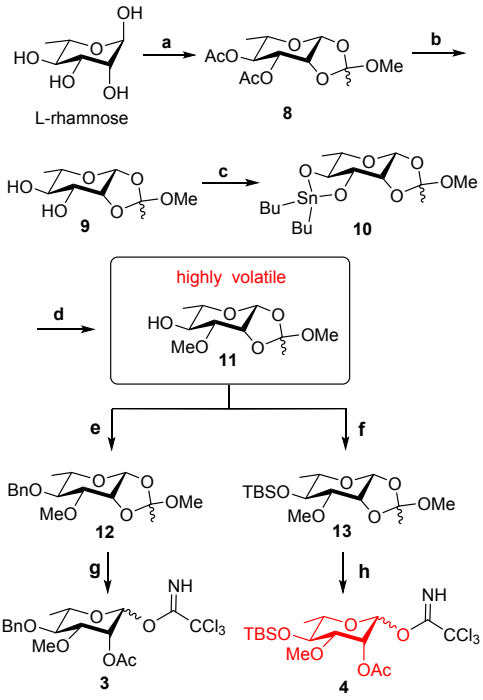
At first, we used benzyl chloroformate **7** (Cbz-Cl) [15] to protect the amino group of tyramine **6** in the presence of $NaHCO_3$, affording **2** in 87 % yield (**Scheme 2**). The benzyloxycarbonyl group (Cbz) will be removed by hydrogenolysis together with the benzyl groups in the final step of the synthesis.



Scheme 2. Synthesis of N-protected phenolic compound **2**

Synthesis of rhamnose units 3 and 4

The strategy we utilized here for the preparation of the key intermediate **9** was reported by Martine et al[16] and Furstner et al.[17] As shown in **Scheme 3**, peracetylation of L-rhamnose, followed by the formation of rhamnosyl bromide and subsequently refluxed in methanol with *sym*-collidine as an acid scavenger to provide orthoester **8** in 50% yield over three steps. Removal of the acetyl groups afforded the key intermediate **9** in quantitative yield. Then **9** was transformed to the stannylene acetal **10** by using dibutyltin oxide in refluxing toluene for 8 hours.[18] The solvent toluene was removed under vacuum and the resulting **10** was used in the next step without purification. Regioselective methylation of the 3-OH of **10** afforded **11** as a single product. However, compound **11** was highly volatile and was lost under vaccum. To overcome this problem, the reaction mixture containing **11** was filtrated through silica, eluted with THF containing 5% Et_3N and concentrated in *vacuo* at low temperature. The resulting solution of **11** in DMF was directly reacted with TBDMSCl in the presence of imidazole to afford **13** in 68% isolated yield over two steps. Similarly, compound **12** was obtained in 56% yield over two steps by treating with benzyl bromide. Hydrolysis of compounds **12** and **13** with (±)10-camphorsulfonic acid (CSA) and then followed by treatment with trichloroacetonitrile (CCl_3CN) and 1,8-dizabicyclo[5,4,0] undec-7-ene (DBU) lead to the trichloroacetimidates **3** and **4**, respectively. [19]

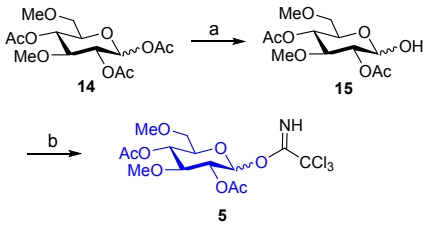


Scheme 3. Synthesis of rhamnose units **3** and **4**

Reagents and conditions: (a) i)Ac_2O, Py; ii) HBr, AcOH; iii) lutidine, MeOH, 50%, 3 steps; (b) NaOMe, MeOH, quant. (c) Bu_2SnO, quant, (d) MeI, TBAB; (e) NaH, BnBr, 56%, 2 steps; (g) i) CSA, THF/H_2O; ii) DBU, CCl_3CN, 55%, 2 steps; (f) TBDMSCl, imidazole, 68%, 2 steps; (h) i) CSA, THF/H_2O; ii) DBU, CCl_3CN, 62%, 2 steps

Synthesis of glycosyl imidate donor 5

As shown in **Scheme 4,** compound **14** has been employed as a glycosyl donor to provide oligosaccharide of PGL-1 in the presence of Lewis acid,[20-21] but the yield was not satisfactory. Hence, we intend to introduce a more active group instead of the acetyl group and trichloroacetimidate **5** was chosen. Compound **14** was synthesized from commercially available *D*-glurono-6,3-lactone according to the literature procedure[22]. Then the 1-OAc group of **14** was selectively removed to afford **15** in 84% yield as a mixture of α and β anomers. Subsequently, **15** was converted to the trichloroacetimidate **5** in 75% yield. [18]

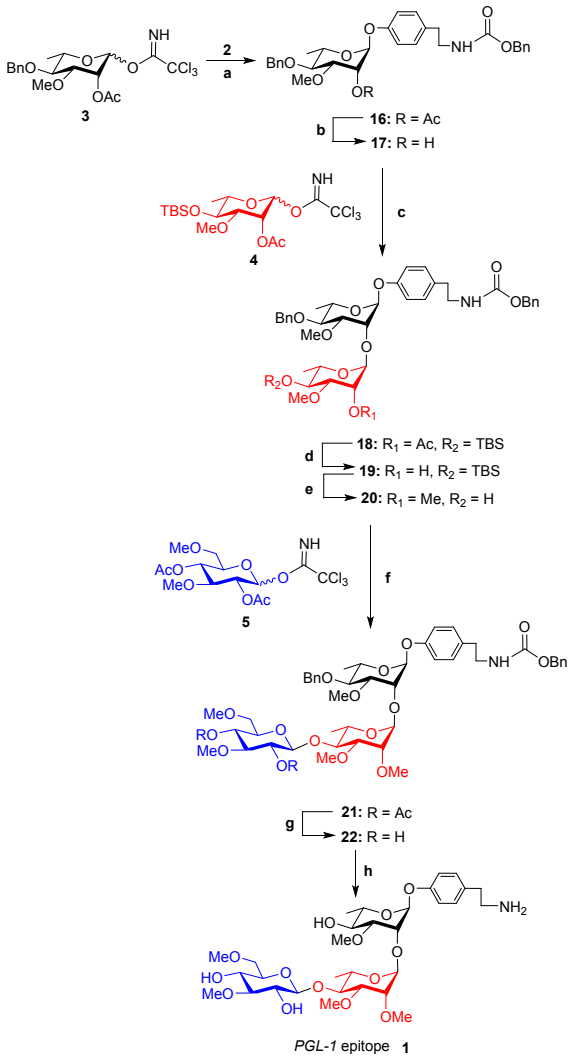


Scheme 4. Synthesis of glycosyl imidate donor **5**

Reagents and conditions: (a) $MeNH_2$, EtOH, 84%; (b) CCl_3CN, DBU, 75%.

Assembly of the targeted PGL-1 epitope 1

With the glycoside donors **3**, **4** and **5** in hand, the assembly of the trisaccharide PGL-1 epitope **1** was performed as shown in **Scheme 5**. First, glycosylation of imidate **3** and **2** gave the monosaccharide **16** in 64% yield as a pure α-anomer. Removal of the 2-OAc group of **16** provided **17** in 92% yield. The latter was glycosylated with **4** to produce **18** in 54% yield as a pure α-anomer. The 2-OAc group of **18** was removed to afford **19**. The resulting disaccharide **19** was methylated and then removal of the 4-OTBS group gave **20** in 84% yield over two steps. The glycosyl donor **5** was glycosylated with the disaccharide acceptor **20** to provide the β-linked trisaccharide **21** in 77% yield. Removal of the acetyl groups of **21** gave **22** in 92% yield. Finally, deprotection of **22** via hydrogenolysis over palladium hydroxide on activated charcoal lead to the targeted *PGL-1* epitope **1** in 66% yield. The total yield of compound **1** based on **3** was 12%.

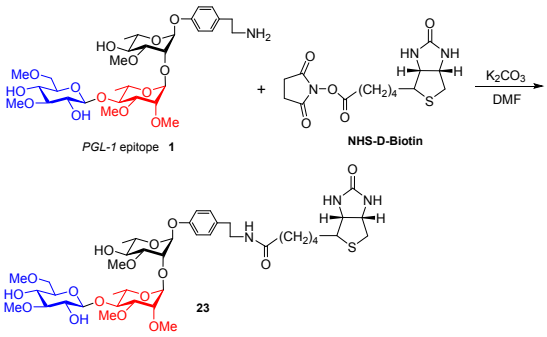


Scheme 5. Synthesis of *PGL-1* epitope **1**

Reagents and conditions: (a) TMSOTf, -20 °C, 64%; (b) NaOMe, MeOH, 92%; (c) TMSOTf, -20 °C, 54%; (d) NaOMe, MeOH, 100%; (e) (i) MeI,NaH, (ii) TBAF, 84%, 2 steps. (f) TMSOTf, -20 °C, 77%; (g) NaOMe, MeOH, 92%; (h) $Pd(OH)_2/C$, H_2, 66%.

Synthesis of a biotinylated PGL-1 antigen 23

NHS-D-Biotin (*N*-hydroxysuccinimido biotin) can be used for protein labeling and intracellular labeling.[23-24] In order to better understand the role of PGL-1 in the pathogenesis of *Mycobacterium leprae*, we synthesized biotinylated PGL-1 antigen **23** for sequential monitoring of serum antibody levels of leprosy patients in future**.** As shown in **Scheme 6,** PGL-1 epitope **1** was treated with NHS-D-Biotin in the presence of K_2CO_3 in DMF at room temperature for 24 hours to afford compound **23** in 75% yield.[25]



Scheme 6. Synthesis of the biotinylated PGL-1 antigen **23**

In conclusion, PGL-1 epitope **1** which bears a *p*-aminoethylphenol group was efficiently synthesized using linear synthetic routes in a good overall yield. The rhamnose units (**3** and **4**) of PGL-1 can be easily prepared from the key orthoester **11**. What's more, the orthoester protection of the rhamnose unit **11** could be used for syntheses of other types of the PGL epitopes. In addition, the amino group on the 4-substituted phenol ring made the targeted PGL-1 epitope **1** easily functionalized with NHS-biotin to obtain a biotinylated PGL-1 antigen **23**, which could be used as a probe for early detection of leprosy.

Experimental Section

General Methods. All air sensitive reactions were run in anhydrous solvents under a dry argon atmosphere. Solvents were distilled before use. Reactions were monitored by thin layer chromatography (TLC) on silica gel 60F254 sheets with dection by UV (254 nm) or 5% H_2SO_4/EtOH or phosphomolybdic acid. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ^1H, ^{13}C NMR spectra were recorded on Bruker AV400. Chromatographic purifications (flash chromatography) were done using silica 60 (35-70µm) from SDS (Peypin France) or on an automated PuriflashTM apparatus (Interchim, France) using 50µm spherical silica. High-resolution mass spectra (HRMS) were obtained by electrospray ionization (ESI) and Q-TOF in a Bruker micrOTOF-Q II spectrometer.

4-*O*-benzyl-3-*O*-methyl-1,2-*O*-(1-methoxyethylidene)-β-L-rhamnopyranose (12). A mixture of **10** (2.40 g, 5.33 mmol), TBAB (1.71 g, 5.33 mmol) and MeI (662 µL, 10.66 mmol) in DMF (15 mL) were stirred at 40 °C overnight. Then the reaction mixture containing **11** was filtrated on silica, eluted with THF containing 5% Et_3N and concentrated in *vacuo* at low temperature. Sodium hydride (60% dispersion in mineral oil, washed two times with petroleum ether) (426 mg, 10.66 mmol) was added in portions to a solution of crude **11** in DMF (5 mL) at 0 °C under argon. The resulting suspension was stirred for 30 minutes, and benzyl bromide (887 µL, 7.46 mmol) in dry DMF (2 mL) was added dropwise. The mixture was stirred at r.t overnight and quenched with methanol. Then the reaction mixture was diluted with water and extracted with dichloromethane. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (Petroleum ether /EtOAc = 7:3) to give compound **12** as a colorless oil (0.96 g, 56% yield). [6] Rf = 0.46 (Petroleum ether /EtOAc = 2:1).^1H NMR (300 MHz, $CDCl_3$): δ 5.39 (d, 1H, J =2.5 Hz), 4.93 (d, J = 12.0 Hz, 1H), 4.67 (d, J = 12.0 Hz, 1H), 4.62 (dd, 1H, J = 4.0 Hz, J = 2.5 Hz), 3.60 (s, 3H), 3.55-3.36 (m, 3H), 3.35 (s, 3H), 1.75 (s, 3H), 1.34 (d, 3H, J = 6.0 Hz).

2-*O*-acetyl-4-*O*-benzyl-3-*O*-methyl-α,β-L-rhamnopyranosyl trichloroacetimidate (3). A mixture of compouds **12** (0.2 g, 0.6 mmol) and CSA (5 mg) in mixed solvent THF and H_2O (1:1, 8 mL) was stirred at r.t for 2 hours. The reaction mixture was diluted with water and extracted with dichloromethane. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (Petroleum ether /EtOAc = 2:1) and used in the next step. Followed by added trichloroacetonitrile (0.3 mL, 3.0 mmol) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (0.03 mL, 0.20 mmol) at 0 °C under argon. The reaction mixture was stirred overnight, then quenched with a saturated NH_4Cl solution, extracted with dichloromethane. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The product was chromatographed on silica gel (Petroleum ether /EtOAc = 6:1) to give compound **3** as a colorless oil (0.248 g, 55% yield). [19] Date for one isomer:Rf = 0.67 (Petroleum ether /EtOAc = 2:1).^1H NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 1H), 6.21 (d, 1H, J = 2.0 Hz), 5.50 (dd, 1H, J = 3.5 Hz, J = 2.0 Hz), 4.95 (d, J = 12.0 Hz, 1H), 4.68 (d, J = 12.0 Hz, 1H), 4.01-3.92(m, 1H), 3.75 (dd, 1H, J = 9.0 Hz, J = 3.5 Hz), 3.50 (s, 3H), 3.48 (dd,1H, J = 9.5 Hz, J = 9.5 Hz), 2.21 (s, 3H), 1.37 (d, 3H, J = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 160.2, 95.1, 79.8, 79.4, 75.5, 70.5, 67.1, 57.8, 21.0, 18.0.

4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-1,2-*O*-(1-methoxyethylidene)-β-L-rhamnopyranose (13). A mixture of **11** (400 mg, 0.886 mmol), TBAB (285 mg, 0.886 mmol) and MeI (110 µL, 1.77 mmol) in DMF (4 mL) were stirred at 40 °C overnight. Then the reaction mixture containing **11** was filtrated on silica, eluted with THF containing 5% Et_3N and concentrated in *vacuo* at low temperature. The resulting solution of **11** in DMF was treated with TBDMSCl (187 mg, 1.24 mmol) and imidazole (180 mg, 2.658 mmol) at room temperature for 12 hours. The mixture was quenched with water and extracted with dichloromethane. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on

silica gel (Petroleum ether /EtOAc = 20:1) to give compound **13** as a colorless oil (210 mg, 68% yield).[6] Rf = 0.76 (dichloromethane /methanol = 20:1).^{1}H NMR (300 MHz, $CDCl_3$): δ 5.37 (d, 1H, J =2.5 Hz), 4.67 (dd, 1H, J = 4.0 Hz, J = 2.5 Hz), 3.48 (s, 3H), 3.46-3.49 (m, 1H), 3.32 (s, 3H), 3.22-3.31 (m, 2H), 1.71 (s, 3H), 1.28 (d, 3H, J = 6.0 Hz), 0.90 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H).

2-*O*-acetyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-α,β-L-rhamnopyranosyl trichloroacetimidate (4). A mixture of compouds **13** (1.8 g, 5.17 mmol) and CSA (50 mg) in mixed solvent THF and H_2O (1:1, 20 mL) was stirred at r.t for 2 hours. The reaction mixture was diluted with water and extracted with dichloromethane. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (Petroleum ether /EtOAc = 3:1) and used in the next step. Followed by added trichloroacetonitrile (1.4 mL, 14.34 mmol) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (0.15 mL, 0.10 mmol) at 0 °C under argon. The reaction mixture was stirred overnight, then quenched with a saturated NH_4Cl solution, extracted with dichloromethane. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The product was chromatographed on silica gel (Petroleum ether /EtOAc = 5:1) to give compound **4** as a colorless oil (0.70 g, 62% yield).[26] Date for one isomer: Rf = 0.80 (Petroleum ether /EtOAc = 5:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (s, 1H), 6.19 (d, 1H, J = 2.0 Hz), 5.50 (dd, 1H, J = 3.0 Hz, J = 2.0 Hz), 3.90-3.82 (m, 1H), 3.58 (dd,1H, J = 9.0 Hz, J = 9.0 Hz), 3.43 (dd, 1H, J = 9.0 Hz, J = 3.5 Hz), 3.35 (s, 3H), 2.17 (s, 3H), 1.33 (d, 3H, J = 6.2 Hz), 0.91 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.2, 95.4, 90.9, 79.7, 72.5, 72.1, 70.0, 66.6, 57.2, 25.9, 25.7, 20.9, 18.3, 18.0, -4.0, -4.8.

2,4-di-*O*-acetyl-3,6-di-*O*-methyl-α,β-D-glucosyl trichloroacetimidate (5). To a stirred solution of **15** (0.16 g, 0.5 mmol) in dry dichloromethane (5 mL) was added trichloroacetonitrile (0.3 mL, 3.28 mmol) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (0.033 mL, 0.219 mmol) at 0 °C under argon. [22] The reaction mixture was stirred overnight, then quenched with a saturated NH_4Cl solution, extracted with dichloromethane. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The product was chromatographed on silica gel (Petroleum ether /EtOAc = 3:1) to give compound **5** as a colorless oil (0.178 g, 75% yield). [4] Data for α-anomer: Rf = 0.62 (Petroleum ether /EtOAc = 1:1).$[\alpha]_D^{25}$ = + 90.7 (*c* = 0.89, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (s, 1H), 6.54 (d,1H, J = 3.5 Hz), 5.12 (t,1H, J = 9.5 Hz), 5.03 (dd, 1H, J = 10.0 Hz, J = 3.5 Hz), 4.09-4.03 (m, 1H), 3.86 (t, 1H, J = 9.5 Hz), 3.55-3.42 (m, 2H), 3.50 (s, 3H), 3.35 (s, 3H), 2.14 (s, 3H), 2.07 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 169.5, 160.8, 93.5, 78.2, 71.7, 71.6, 71.2, 69.7, 60.0, 59.4, 20.9, 20.6.

4-(*N*-Benzyloxycarbonyl-2-aminoethyl)-phenyl-2-*O*-acetyl-4-*O*-benzyl-3-*O*-methyl-α-L-rhamnopyranoside (16). Compounds **2** (0.49 g, 1.07 mmol) and **3**[19] (0.232 g, 0.856 mmol) were coevaporated from dry dichloromethane and dried under high vacuum for 30 minutes and then flushed with argon. Anhydrous dichloromethane (10 mL) was added followed by 4 Å molecular sieves and the suspension was stirred at room temperature for 10 minutes under argon. The solution was cooled to -20 °C and TMSOTf (0.15 mL, 0.15 mmol) was added dropwise. After stirring at -20 °C for 2 hours, the mixture was allowed to warm to room temperature, the reaction was quenched with phosphate buffer (PH=7), and the mixture was filtered through a small column of Celite. The Celite was washed with dichloromethane, the organic phase was separated and the water phase was extracted three times with dichloromethane, then the combined organic phase was washed with water, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (Petroleum ether /EtOAc = 3:1) to give compound **16** as a colorless oil (0.36 g, 64% yield). Rf = 0.57 (Petroleum ether /EtOAc = 3:1).$[\alpha]_D^{25}$= - 64.0 (*c* = 1.0, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11 (d, 2H, J = 8.4 Hz), 6.98 (d, 2H, J = 8.4 Hz), 5.50 (dd, 1H, J = 3.5 Hz, J = 2.0 Hz), 5.45 (d, 1H, J = 2.0 Hz), 4.81 (brs, 1H), 3.90 (dd, 1H, J = 9.0 Hz, J = 3.5 Hz), 3.93-3.84 (m, 1H), 3.53 (s, 3H), 3.47 (dd,1H, J = 9.5 Hz, J = 9.5 Hz), 3.48-3.41 (m, 2H), 2.78 (t, 2H, J = 6.5 Hz), 2.22 (s, 3H), 1.33 (d, 3H, J = 6.0 Hz). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 170.4, 156.3, 154.8, 138.5, 129.8, 116.6, 95.9, 80.0, 79.7, 75.4, 68.5, 68.4, 66.7, 57.7, 42.3, 35.2, 21.1, 18.0. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{32}H_{38}NO_8$ 564.2597; Found 564.2585.

4-(*N*-Benzyloxycarbonyl-2-aminoethyl)-phenyl-4-*O*-benzyl-3-*O*-methyl-α-L-rhamnopyranoside (17). A freshly prepared sodium methoxide solution (1 mL, 0.5 M in methanol) was added to a solution of **16** (0.36 g, 0.0.638 mmol) in methanol (10 mL), the reaction mixture was stirred at room temperature for 1 hour and monitored by TLC. The methanol was removed in vacuo and the residue was extracted by dichloromethane, the organic phase was washed with water, dried over $MgSO_4$, filtered and concentrated in vacuo. The product was chromatographed on silica gel (Petroleum ether /EtOAc = 2:1) to give compound **17** as a colorless oil (0.306 g, 92 % yield). Rf = 0.27 (Petroleum ether /EtOAc = 2:1).$[\alpha]_D^{25}$ = -88.9 (*c* = 1.02, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11 (d, 2H,

J = 8.4 Hz), 6.99 (d, 2H, J = 8.4 Hz), 5.55 (d, 1H, J = 1.0 Hz), 4.84 (brs, 1H), 4.28-4.24 (m, 1H), 3.89-3.82 (m, 1H), 3.79 (dd, 1H, J = 9.0 Hz, J = 3.5 Hz), 3.59 (s, 3H), 3.48 (dd,1H, J = 9.5 Hz, J = 9.5 Hz), 3.47-3.41 (m, 2H), 2.79 (t, 2H, J = 6.5 Hz), 2.74-2.68 (m, 1H), 1.29 (d, 3H, J = 6.0 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 156.3, 154.9, 138.5, 132.4, 116.5, 97.2, 81.5, 79.8, 75.3, 67.9, 67.8, 66.7, 57.6, 42.3, 35.2, 17.9. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{30}H_{36}NO_7$ 522.2492; Found 522.2494.

4-(*N*-Benzyloxycarbonyl-2-aminoethyl)-phenyl-2-*O*-[2-*O*-acetyl-4-*O*-(*tert*-butyldimethylsilyl)-3-*O*-methyl-α-L-rhamnopyranosyl]-4-*O*-benzyl-3-*O*-methyl-α-L-rhamnopyranoside (18). Compounds **17** (0.255 g, 0.489 mmol) and **4** [26] (0.328 g, 0.684 mmol) were coevaporated from dry dichloromethane and dried under high vacuum for 30 minutes and then flushed with argon. Anhydrous dichloromethane (10 mL) was added followed by 4 Å molecular sieves and the suspension was stirred at room temperature for 10 minutes under argon. The mixture was cooled to -20 °C and TMSOTf (0.10 mL, 0.10 mmol) was added dropwise. After stirring at -20 °C for 2 hours, the mixture was allowed to warm to room temperature, the reaction was quenched with phosphate buffer (PH=7), and the mixture was filtered through a small column of Celite. The Celite was washed with dichloromethane, a saturated $Na_2S_2O_3$ solution was added to the filtrate and it turned to colorless. The organic phase was separated and the water phase was extracted three times with dichloromethane, then the combined organic phase was washed with water, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (Petroleum ether /EtOAc = 3:1) to give compound **18** as a colorless oil (0.22 g, 54% yield). Rf = 0.37 (Petroleum ether /EtOAc = 3:1).$[\alpha]_D^{25}$ = -75.8 (*c* = 1.0, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12 (d, 2H, J = 8.4 Hz), 7.00 (d, 2H, J = 8.8 Hz), 5.50 (dd, 1H, J = 3.0 Hz, J = 1.5 Hz), 5.49 (d, 1H, J = 2.0 Hz), 5.11 (d, 1H, J = 1.5 Hz), 4.24 (dd, 1H, J = 2.5 Hz, J = 2.5 Hz), 3.86-3.71 (m, 3H), 3.56 (s, 3H), 3.39 (s, 3H), 3.55-3.40 (m, 5H), 2.79 (t, 2H, J = 6.5 Hz), 2.21 (s, 3H), 1.29 (d, 6H, J = 6.4 Hz), 0.93 (s, 9H). 0.13 (s, 3H), 0.11 (s, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 170.2, 156.3, 154.8, 138.5, 129.8, 116.5, 99.2, 97.1, 81.6, 79.8, 79.5, 75.1, 73.1, 72.9, 69.7, 68.7, 68.2, 66.7, 58.0, 57.0, 42.3, 35.2, 26.0, 21.1, 18.4, 18.2, 17.7, -3.9, -4.8. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{45}H_{64}NO_{12}Si$ 838.4198; Found 838.4196.

4-(*N*-Benzyloxycarbonyl-2-aminoethyl)-phenyl-4-*O*-benzyl-2-*O*-[4-*O*-(*tert*-butyldimethylsilyl)-3-*O*-methyl-α-L-rhamnopyranosyl]-3-*O*-methyl-α-L-rhamnopyranoside (19). A freshly prepared sodium methoxide solution (1 mL, 0.5 M in methanol) was added to a solution of **18** (0.18 g, 0.215 mmol) in methanol (10 mL), the reaction mixture was stirred at room temperature for 1 hour and monitored by TLC. The methanol was removed in vacuo and the residue was taken up in dichloromethane, washed with water, dried over $MgSO_4$, filtered and concentrated in vacuo. The product was chromatographed on silica gel (Petroleum ether /EtOAc = 2:1) to give compound **19** as a colorless oil (0.171 g, 100 % yield). Rf = 0.50 (Petroleum ether /EtOAc = 2:1). $[\alpha]_D^{25}$ = -77.6 (*c* = 1.01, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11 (d, 2H, J = 8.0 Hz), 6.99 (d, 2H, J = 8.4 Hz), 5.49 (d, 1H, J = 1.8 Hz), 5.20 (d, 1H, J = 1.0 Hz), 4.27 (d, 1H, J = 1.5 Hz), 4.25-4.22 (m,1H), 3.86-3.70 (m, 3H), 3.57 (s, 3H), 3.46 (s, 3H), 3.56-3.36 (m, 5H), 2.78 (t, 2H, J = 6.5 Hz), 1.30 (d, 3H, J = 6.2 Hz), 1.29 (d, 3H, J = 6.2 Hz), 0.93 (s, 9H), 0.13 (s, 3H), 0.10 (s, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 156.3, 154.9, 138.5, 129.8, 116.5, 100.7, 97.2, 81.7, 81.3, 80.0, 75.1, 73.0, 72.6, 69.0, 68.6, 67.2, 66.7, 58.0, 56.7, 42.3, 35.2, 26.0, 18.2, 18.1, -4.0, -4.7. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{43}H_{61}NO_{11}Si$ 796.4092; Found 796.4067.

4-(*N*-Benzyloxycarbonyl-2-aminoethyl)-phenyl-4-*O*-benzyl-3-*O*-methyl-2-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (20). Sodium hydride (60% dispersion in mineral oil, washed two times with petroleum ether) (0.015 g, 0.37 mmol) was added in portions to a solution of **19** (0.147 g, 0.185 mmol) in THF (5 mL) at 0 °C under argon. After stirring for 0.5 hour, methyl iodine (0.035 mL, 0.55 mmol) was added to the solution.The mixture was stirred at room temperature overnight, quenched with methanol and extracted with ethyl acetate. The organic phase was washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo to give yellow oil. Whithout purification, the yellow oil was diluted in anhydrous THF (3.0 mL) and was treated with tetrabutylammonium Fluoride (TBAF) (0.8 mL, 1.0 M in THF). The reaction mixture was sirred at 40 °C for 12 hours. The solvents were removed in vacuo and the residue was purified by column chromatography on silica gel (Petroleum ether /EtOAc = 2:3) to give **20** as a colorless oil (0.113 g, 84% yield). Rf = 0.18 (Petroleum ether /EtOAc = 1:1). $[\alpha]_D^{25}$ = -65.3 (*c* = 1.0, $CHCl_3$).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11 (d, 2H, J = 8.0 Hz), 6.97 (d, 2H, J = 8.4 Hz), 5.49 (d, 1H, J = 1.8 Hz), 5.21 (d, 1H, J = 1.5 Hz), 4.25 (d, 1H, J = 2.5 Hz), 3.86-3.74 (m, 4H), 3.65-3.59 (m, 1H), 3.58 (s, 3H), 3.55 (s, 3H), 3.54 (s, 3H), 3.53-3.42 (m, 4H), 2.78 (t, 2H, J = 6.5 Hz), 2.39 (s, 1H, OH), 1.32 (d, 3H, J = 6.0 Hz), 1.29 (d, 3H, J = 6.0 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 156.3, 154.9, 138.5, 129.8, 116.4, 98.9, 97.1, 81.7, 80.7, 80.0, 75.9, 75.0, 73.5, 71.6, 68.8, 68.5, 66.7, 58.9, 58.1, 57.0, 42.3, 35.2, 18.1, 17.8. HRMS

(ESI) m/z: $[M + H]^+$ Calcd for $C_{38}H_{50}NO_{11}$ 696.3384; Found 696.3382.

4-(*N*-Benzyloxycarbonyl-2-aminoethyl)-phenyl-2-*O*-[4-*O*-(2,4-di-*O*-acetyl-3,6-di-*O*-methyl-β-D-glucopyranosyl)-2,3-di-*O*-methyl-α-L-rhamnopyranosyl]-4-*O*-benzyl-3-*O*-methyl-α-L-rhamnopyranoside (21). Compounds **20** (0.096 g, 0.133 mmol) and **5** (0.081 g, 0.186 mmol) were coevaporated from dry dichloromethane and dried under high vacuum for 30 minutes and then flushed with argon. Anhydrous dichloromethane (10 mL) was added followed by 4 Å molecular sieves and the suspension was stirred at room temperature for 10 minutes under argon. The reaction mixture was cooled to -20 °C and TMSOTf (0.05 mL, 0.05 mmol) was added dropwise. After stirring at -20 °C for 2 hours, the mixture was allowed to warm to room temperature, the reaction was quenched with phosphate buffer (PH=7), and the mixture was filtered through a small column of Celite. The Celite was washed with dichloromethane, the organic phase was separated and the water phase was extracted three times with dichloromethane, then the combined organic phase was washed with water, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (Petroleum ether /EtOAc = 1:1) to give compound **21** as a colorless oil (0.101 g, 77% yield). Rf = 0.20 (Petroleum ether /EtOAc = 1:1). $[\alpha]_D^{25}$ = -50.4 (*c* = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.11 (d, 2H, J = 8.4 Hz), 6.98 (d, 2H, J = 8.8 Hz), 5.45 (d, 1H, J = 1.8 Hz), 5.22 (d, 1H, J = 1.8 Hz), 5.00 (dd, 1H, J = 9.5 Hz, J = 9.5 Hz), 4.93 (dd, 1H, J = 9.5 Hz, J = 9.5 Hz), 4.79 (d, 1H, J = 8.0 Hz), 4.25 (d, 1H, J = 2.5 Hz), 3.85-3.76 (m, 2H), 3.75-3.62 (m, 3H), 3.58 (s, 3H), 3.55 (s, 3H), 3.51 (s, 3H), 3.50-3.42 (m, 8H), 3.41 (s, 3H), 3.34 (s, 3H), 2.78 (t, 2H, J = 7.0 Hz), 2.16 (s, 3H), 2.10 (s, 3H), 1.31-1.25 (m, 6H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 169.7, 169.3, 156.3, 154.7, 138.5, 129.8, 116.4, 100.9, 98.2, 97.0, 81.9, 81.5, 80.8, 80.0, 77.5, 76.7, 75.0, 73.0, 72.9, 72.2, 72.1, 70.1, 68.5, 67.8, 66.6, 59.7, 58.9, 58.2, 58.1, 57.4, 42.3, 35.2, 18.1, 17.9. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{50}H_{68}NO_{18}$ 970.4436; Found 970.4420.

4-(*N*-Benzyloxycarbonyl-2-aminoethyl)-phenyl-4-*O*-benzyl-3-*O*-methyl-2-*O*-[2,3-di-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (22). A freshly prepared sodium methoxide solution (1 mL, 0.5 M in methanol) was added to a solution of **21** (97 mg 0.097 mmol) in methanol (5 mL), the reaction mixture was stirred at room temperature for 1 hour and monitored by TLC. The methanol was removed in vacuo and the residue was taken up in dichloromethane, washed with water, dried over $MgSO_4$, filtered and concentrated in vacuo. The product was chromatographed on silica gel (Petroleum ether /EtOAc = 1:3) to give compound **22** as a colorless oil (85 mg, 92% yield). Rf = 0.15 (Petroleum ether /EtOAc = 1:3). $[\alpha]_D^{25}$ = -57.3 (*c* = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.11 (d, 2H, J = 8.0 Hz), 6.96 (d, 2H, J = 8.8 Hz), 5.43 (s, 1H), 5.18 (s, 1H), 4.45 (d, 1H, J = 8.0 Hz), 4.22 (dd, 1H, J = 2.0 Hz, J = 2.0 Hz), 3.84-3.75 (m, 4H), 3.74-3.62 (m, 4H), 3.70 (s, 3H), 3.60-3.55 (m, 1H), 3.57 (s, 3H), 3.55 (s, 3H), 3.54 (s, 3H), 3.49-3.41 (m, 5H), 3.40 (s, 3H), 3.20 (dd, 1H, J = 9.0 Hz, J = 9.0 Hz), 2.78 (t, 2H, J = 7.0 Hz), 1.36 (d, 3H, J = 6.0 Hz), 1.27 (d, 3H, J = 6.0 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 156.3, 154.8, 138.4, 129.8, 116.4, 105.6, 98.6, 96.9, 85.5, 81.7, 81.6, 80.2, 80.1, 75.7, 75.1, 75.0, 74.1, 73.7, 72.8, 71.2, 68.5, 68.2, 66.6, 60.5, 59.6, 59.0, 58.2, 56.5, 42.3, 35.2, 18.1, 17.6. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{46}H_{64}NO_{16}$ 886.4225; Found 886.4207.

3-*O*-methyl-2-*O*-[2,3-di-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (1). 20% $Pd(OH)_2/C$ (0.5 mg) was added to a solution of **22** (87 mg, 0.091 mmol) in methanol and etyl acetate (1:1, 1 mL) under 1 atm H_2. The reaction mixture was stirred at 25 °C for 24 h and filtered through Celite. The Celite pad was washed with methanol and the filtrate was concentrated in vacuo. The residue was purified by chromatography on silica gel column (dichloromethane/MeOH = 4:1) to give **1** as a colorless oil (44 mg, 66 % yield). Rf = 0.32 (dichloromethane/MeOH = 3:1). $[\alpha]_D^{25}$ = -66.4 (*c* = 1.0, MeOH). ^1H NMR (400 MHz, MeOD): δ 7.20-7.10 (m, 2H), 7.02-6.95 (m, 2H), 5.44 (d, 1H, J = 1.5 Hz), 5.11 (d, 1H, J = 1.5 Hz), 4.43 (d, 1H, J = 7.8 Hz), 4.24 (dd, 1H, J = 2.0 Hz, J = 2.0 Hz), 3.80-3.71 (m, 3H), 3.69 (s, 3H), 3.68-3.60 (m, 5H), 3.55 (s, 3H), 3.54-3.53 (m, 1H), 3.51 (s, 3H), 3.50 (s, 3H), 3.46-3.40 (m, 2H), 3.39 (s, 3H), 3.17 (d, 1H, J = 9.0 Hz, J = 9.0 Hz), 2.97 (t, 2H, J = 7.0 Hz), 2.73 (t, 2H, J = 7.0 Hz), 1.35 (d, 3H, J = 6.0 Hz), 1.29 (d, 3H, J = 6.0 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, MeOD): δ 154.7, 133.4, 129.8, 116.3, 105.6, 98.5, 97.3, 85.6, 81.5, 81.4, 80.2, 75.8, 75.0, 74.3, 72.7, 72.2, 71.7, 70.9, 69.1, 68.3, 60.5, 59.6, 59.1, 57.7, 56.5, 42.3, 38.7, 17.8, 17.6. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{31}H_{52}NO_{14}$ 662.3388; Found 662.3387.

Synthesis of a biotinylated PGL-1 epitope (23). A mixture of **1** (10 mg, 0.0137 mmol), NHS-D-Biotin (7 mg, 0.0205 mmol) and K_2CO_3 (3.8 mg, 0.0274 mmol) in anhydrous DMF (1 mL) was stirred at room temperature for 2 hours. The excess K_2CO_3 was removed by filteration, the solvent were removed in vacuo and the residue was purified by silica gel column chromatography (DCM/MeOH = 10:1) to give **23** as a colorless oil (9.1 mg, 75% yield). Rf = 0.21 (dichloromethane /MeOH = 10:1). $[\alpha]_D^{25}$ = -21.5

(c = 0.94, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 7.21-7.16 (m, 2H), 7.03-6.98 (m, 2H), 5.54 (d, 1H, J = 1.5 Hz), 5.12 (d, 1H, J = 1.5 Hz), 4.57 (d, 1H, J = 7.8 Hz), 4.54-4.49 (m, 1H), 4.34-4.29 (m, 1H), 4.25 (dd, 1H, J = 2.5 Hz, J = 2.0 Hz), 3.65 (s, 3H), 3.82-3.59 (m, 9H), 3.58 (s, 3H), 3.50 (s, 3H), 3.49 (s, 3H), 3.54-3.47 (m, 1H), 3.45-3.36 (m, 3H), 3.40 (s, 3H), 3.26-3.18 (m, 2H), 3.10 (d, 1H, J = 8.5 Hz, J = 8.5 Hz), 2.99-2.93 (m, 1H), 2.78 (t, 2H, J = 7.0 Hz), 2.76-2.70 (m, 1H), 2.18 (t, 2H, J = 7.2 Hz), 1.79-1.54 (m, 4H), 1.46-1.38 (m, 2H), 1.27 (d, 3H, J = 6.0 Hz), 1.24 (d, 3H, J = 6.0 Hz). ^{13}C{^{1}H} NMR (100 MHz, MeOD): δ 174.6, 154.9, 133.2, 129.6, 116.2, 103.5, 99.0, 97.4, 80.7, 80.5, 77.8, 76.3, 75.3, 74.6, 74.0, 71.9, 71.6, 69.8, 69.3, 67.8, 61.9, 60.2, 59.5, 58.4, 57.7, 57.1, 56.1, 55.6, 40.5, 39.7, 35.4, 34.2, 28.3, 28.1, 25.5, 16.9, 16.7. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{41}H_{66}N_3O_{16}S$ 888.4164; Found 888.4167.

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REFERENCES

1. Daffé, M.; Laneelle, M. A., Distribution of Phthiocerol Diester, Phenolic Mycosides and Related Compounds in Mycobacteria. *J. Microbiol.* **1988,** *134* (7), 2049-2055.
2. Hunter, S. W.; Brennan, P. J., A novel phenolic glycolipid from Mycobacterium leprae possibly involved in immunogenicity and pathogenicity. *J. Bacteriol.* **1981,** *147* (3), 728-735.
3. Nguyen, P. C.; Nguyen, V. S.; Martin, B. P.; Fourquet, P.; Camoin, L.; Spilling, C. D.; Cavalier, J.-F.; Cambillau, C.; Canaan, S., Biochemical and Structural Characterization of TesA, a Major Thioesterase Required for Outer-Envelope Lipid Biosynthesis in Mycobacterium tuberculosis. *J. Mol. Biol.* **2018,** *430* (24), 5120-5136.
4. Wu, X.; Mariño-Albernas, J.-R.; Auzanneau, F.-I.; Verez-Bencomo, V.; Pinto, B. M., Synthesis and NMR analysis of 13C-labeled oligosaccharides corresponding to the major glycolipid from Mycobacterium leprae. *Carbohyd. Res.* **1998,** *306* (4), 493-503.
5. Madigan, C. A.; Cambier, C. J.; Kelly-Scumpia, K. M.; Scumpia, P. O.; Cheng, T.-Y.; Zailaa, J.; Bloom, B. R.; Moody, D. B.; Smale, S. T.; Sagasti, A.; Modlin, R. L.; Ramakrishnan, L., A Macrophage Response to Mycobacterium leprae Phenolic Glycolipid Initiates Nerve Damage in Leprosy. *Cell* **2017,** *170* (5), 973-985.e10.
6. Arbués, A.; Malaga, W.; Constant, P.; Guilhot, C.; Prandi, J.; Astarie-Dequeker, C., Trisaccharides of Phenolic Glycolipids Confer Advantages to Pathogenic Mycobacteria through Manipulation of Host-Cell Pattern-Recognition Receptors. *ACS Chem. Biol.* **2016,** *11* (10), 2865-2875.
7. Tabouret, G.; Astarie-Dequeker, C.; Demangel, C.; Malaga, W.; Constant, P.; Ray, A.; Honoré, N.; Bello, N. F.; Perez, E.; Daffé, M.; Guilhot, C., Mycobacterium leprae Phenolglycolipid-1 Expressed by Engineered M. bovis BCG Modulates Early Interaction with Human Phagocytes. *PLoS Pathog.* **2010,** *6* (10), e1001159.
8. Yu, B.; Sun, J.; Yang, X., Assembly of Naturally Occurring Glycosides, Evolved Tactics, and Glycosylation Methods. *Accounts Chem. Res.* **2012,** *45* (8), 1227-1236.
9. Liptak, A.; Borbas, A.; Bajza, I., Synthesis of Carbohydrate-Containing Surface Antigens of Mycobacteria. *Med. Res. Rev.* **1994,** *14* (3), 307-352.
10. Fujiwara, T.; Hunter, S. W.; Cho, S. N.; Aspinall, G. O.; Brennan, P. J., Chemical synthesis and serology of disaccharides and trisaccharides of phenolic glycolipid antigens from the leprosy bacillus and preparation of a disaccharide protein conjugate for serodiagnosis of leprosy. *Infect.Immun.* **1984,** *43* (1), 245-252.
11. Fujiwara, T.; Aspinall, G. O.; Hunter, S. W.; Brennan, P. J., Chemical synthesis of the trisaccharide unit of the species-specific phenolic glycolipid from Mycobacterium leprae. *Carbohyd. Res.* **1987,** *163* (1), 41-52.
12. Borbás, A.; Lipták, A., A new approach to the chemical synthesis of the trisaccharide, and the terminal di- and mono-saccharide units of the major, serologically active glycoplipid from Mycobacterium leprae. *Carbohyd. Res.* **1993,** *241* (0), 99-116.
13. Elsaidi, H. R. H.; Barreda, D. R.; Cairo, C. W.; Lowary, T. L., Mycobacterial Phenolic Glycolipids with a Simplified Lipid Aglycone Modulate Cytokine Levels through Toll-Like Receptor 2. *ChemBioChem* **2013,** *14* (16), 2153-2159.
14. Lowary, T. L., Twenty Years of Mycobacterial Glycans: Furanosides and Beyond. *Accounts Chem. Res.* **2016,** *49* (7), 1379-1388.
15. Porcelli, L.; Gilardi, F.; Laghezza, A.; Piemontese, L.; Mitro, N.; Azzariti, A.; Altieri, F.; Cervoni, L.; Fracchiolla, G.; Giudici, M.; Guerrini, U.; Lavecchia, A.; Montanari, R.; Di Giovanni, C.; Paradiso, A.; Pochetti, G.; Simone, G. M.; Tortorella, P.; Crestani, M.; Loiodice, F., Synthesis, Characterization and Biological Evaluation of Ureidofibrate-Like Derivatives Endowed with Peroxisome Proliferator-Activated Receptor Activity. *J.Med. Chem.* **2011,** *55* (1), 37-54.
16. Heidelberg, T.; Martin, O. R., Synthesis of the Glycopeptidolipid of Mycobacterium avium Serovar 4: First Example of a Fully Synthetic C-Mycoside GPL. *J. Org. Chem.* **2004,** *69* (7), 2290-2301.
17. Fürstner, A.; Müller, T., Efficient Total Syntheses of Resin Glycosides and Analogues by Ring-Closing Olefin Metathesis. *J. Am. Chem. Soc.* **1999,** *121* (34), 7814-7821.
18. Gurjar, M. K.; Reddy, K. R., Synthesis of the oligosaccharide segment of a novel phenolic glycolipid antigen from Mycobacterium haemophilum. *J. Chem.Soc. Perk. T. 1* **1993,** (11), 1269-1272.
19. Gurjar, M. K.; Saha, U. K., Synthesis of the complete glycotetrapeptide core of structurally variant Mycobacterium fortuitum glycopeptidolipid. *Bioorg. Med. Chem. Lett.* **1993,** *3* (4), 697-702.
20. Barroso, S.; Geerdink, D.; ter Horst, B.; Casas-Arce, E.; Minnaard, A. J., Total Synthesis of the Phenolic Glycolipid Mycoside B and the Glycosylated p-Hydroxybenzoic Acid Methyl Ester HBAD-I, Virulence Markers of Mycobacterium tuberculosis. *Eur. J.Org. Chem.* **2013,** *2013* (21), 4642-4654.

21. Bourke, J.; Brereton, C. F.; Gordon, S. V.; Lavelle, E. C.; Scanlan, E. M., The synthesis and biological evaluation of mycobacterial p-hydroxybenzoic acid derivatives (p-HBADs). *Org. Biomol. Chem.* **2014,** *12* (7), 1114-1123.
22. Liav, A.; Goren, M. B., A facile synthesis of 3,6-di-O-methyl-d-glucose. *Carbohyd. Res.* **1986,** *149* (2), 13-16.
23. Chiu, N. H. L.; Christopoulos, T. K., Two-Site Expression Immunoassay Using a Firefly Luciferase-coding DNA Label. *Clin. Chem.* **1999,** *45* (11), 1954-1959.
24. Fouassier, L.; Yun, C. C.; Fitz, J. G.; Doctor, R. B., Evidence for Ezrin-Radixin-Moesin-binding Phosphoprotein 50 (EBP50) Self-association through PDZ-PDZ Interactions. *J. Biol. Chem.* **2000,** *275* (32), 25039-25045.
25. Bayer, E. A.; Wilchek, M., The Use of the Avidin-Biotin Complex as a Tool in Molecular Biology. In *Methods of Biochemical Analysis*, John Wiley & Sons, Inc.: 2006; pp 1-45.
26. Halcomb, R. L.; Boyer, S. H.; Wittman, M. D.; Olson, S. H.; Denhart, D. J.; Liu, K. K. C.; Danishefsky, S. J., Studies Related to the Carbohydrate Sectors of Esperamicin and Calicheamicin: Definition of the Stability Limits of the Esperamicin Domain and Fashioning of a Glycosyl Donor from the Calicheamicin Domain. *J.Am. Chem. Soc.* **1995,** *117* (21), 5720-5749.