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Synthesis of mycobacterial phenolic glycolipids

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Chapter 2

Synthetic Phenolic Glycolipids for the Diagnosis of Leprosy

Part of this chapter has been published:

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Synthetic Phenolic Glycolipids for Application in Diagnostic Tests for Leprosy

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Introduction

Leprosy is a chronic disease caused by *Mycobacterium leprae* (*M. leprae*), a bacillus with tropism for skin and peripheral nerves.[1] Although the disease can be cured with multidrug therapy (MDT), it still is a harsh reality in 145 countries mostly affecting individuals in poor socio-economic circumstances. Its late- or misdiagnosis often leads to irreversible deformities and lifelong handicaps.[2] Leprosy´s featuring aspect is the plateauing global annual number of new cases of roughly 200,000 including 10% children, which indicates that transmission is ongoing.[3] Field-friendly diagnostic tests to facilitate identification of infected individuals, will enable timely prophylactic- and therapeutic treatment. Overall, this will help prevent permanent leprosy-associated disabilities and consequently support a higher quality of life, improved long-term outcomes and reduced economic burden.

Phenolic glycolipid-I (PGL-I, Figure 1) is a cell wall component of *M. leprae* which modulates the host immune response by limiting the production of nitric oxides synthase in a complement receptor 3 (CR3) dependent manner and inhibiting the TRIF-dependent TLR4 signaling pathway in macrophages.[4] The binding to CR3 also enhances the invasion of dendritic cells and polymorphonuclear neutrophils, which further dampens the immune response.[5] Furthermore, macrophages infected with *M. marinum* expressing PGL-I (as a proxy for live *M. leprae*) induce demyelination, a process leading to nerve damage in leprosy.[6] After infection a predominantly IgM antibody response can be generated against *M. leprae* PGL-I.[7] The level of anti-PGL-I antibodies strongly correlates with bacterial load,[8] allowing identification of those who are most infectious. Moreover, antibody levels in young children can be utilized to indicate recent transmission rates in endemic areas.[9] A low complexity, point-of-care (POC) lateral flow assay based on up-converting particles (UCP-LFA) has been developed that quantitatively measures anti-PGL-I IgM levels in serum and fingerstick blood.[10–12] This assay is currently applied in field trials in Africa, to identify and treat *M. leprae* infected individuals, and evaluated for surveillance purposes of *M. leprae*-infected red squirrels in the UK.[13]

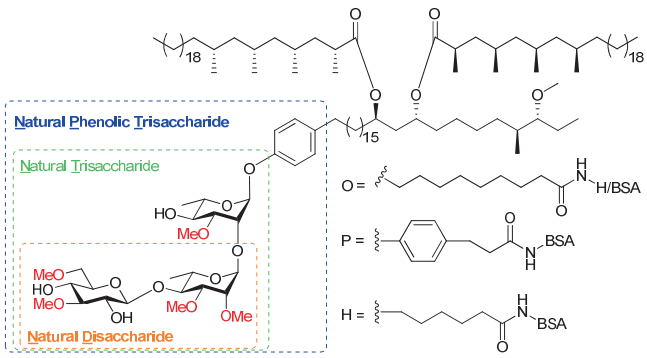


Figure 2. PGL-I and synthetic conjugates used for diagnostics.

For decades serological analyses for leprosy research have been performed using ND-O-HSA (Natural Disaccharide linked *via* an Octyl carboxylic acid linker to Human Serum Albumin, Figure 1),[7,14–16] a conjugate bearing a synthetic disaccharide antigen. This glycoconjugate contains the terminal two sugars of the naturally occurring *M. leprae* PGL-I, which has been synthesized to serve as a convenient water-soluble antigen for use in leprosy research.[17] The terminal 3,6-di-*O*-methylglucose was found to be the most

important structural determinant for antibody binding,[18] with disaccharides showing a higher sensitivity than monosaccharides.[19] Although in most sera of leprosy patients the disaccharide epitope was found to be effective for the detection of antibodies, it had been suggested that the structure of the native glycolipid needed to be more emulated to further improve the binding.[17] Therefore, the trisaccharide containing conjugate with the same octyl linker (Natural Trisaccharide linked *via* an Octyl carboxylic acid linker to Bovine Serum Albumin, NT-O-BSA) was probed by Brennan and co-workers.[20] While they found no discernable increase in sensitivity or specificity,[21] Izumi *et al*. found that a trisaccharide epitope with a phenol on the reducing end with a propyl linker (Natural Trisaccharide linked *via* an Phenol linker to Bovine Serum Albumin, NT-P-BSA) did improve the sensitivity, as well as the antigenic specificity, compared to their disaccharide (ND-P-BSA).[22] These findings were confirmed in a study, comparing ND-O-BSA to NT-O-BSA and NT-P-BSA, demonstrating that the latter provided the best sensitivity and specificity.[23] Combined, these results indicate that the phenol on the reducing end of the saccharides can increase specific antibody binding. Two additional phenolic glycolipids, PGL-II and PGL-III, differing from PGL-I in the methylation pattern of the trisaccharide, possibly because they are biosynthetic intermediates of PGL-I, are present in the cell wall of *M. leprae* as well, and can also modulate the host innate immune response against the mycobacterium.[24–26] Previous assessments by Lowary and coworkers who have described that the methylation pattern in related PGLs originating from *M. tuberculosis* and *M. kansasii* play an important role in shaping the immune response against these PGLs.[27–30]

Therefore, to investigate the binding of the *M. leprae* PGL-trisaccharides with the natural phenol anomeric appendage to human antibodies, this chapter describes the assembly of the Natural Phenolic Trisaccharides 1, 2 and 3 (NPT1, NPT2, NPT3, respectively), which are functionalized with a Hexanoic acid linker for conjugation to BSA, to provide the NPT1-H-BSA, NPT2-H-BSA and NPT3-H-BSA conjugates. These conjugates can then be evaluated using ELISAs to detect IgM antibodies against *M. leprae* in a cohort of leprosy patient sera. Since leprosy frequently occurs in remote, low resource areas the performance of these PGL conjugates will additionally be assessed in UCP-LFAs.

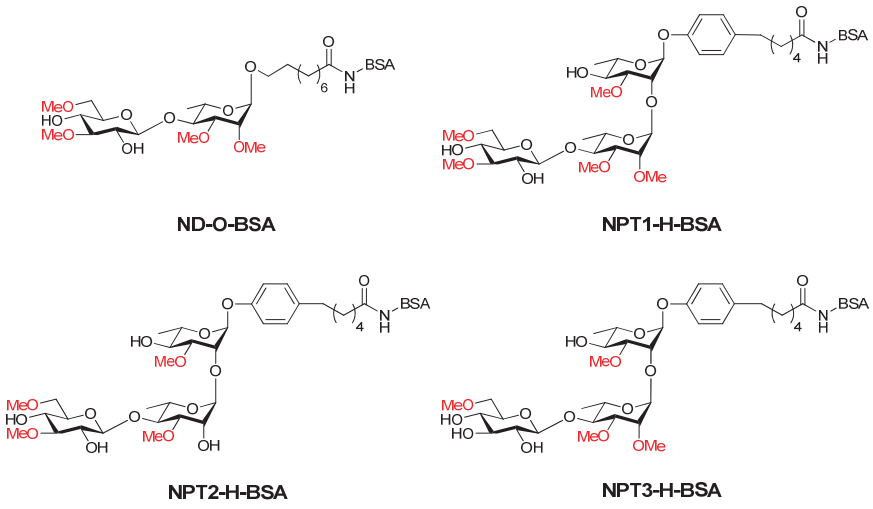


Figure 3. PGL-conjugates assembled in this chapter. Natural Disaccharide – Octyl carboxylic acid – BSA (ND-O-BSA), and Natural Phenolic Trisaccharide 1, 2 and 3 - hexyl - BSA (NPT1/2/3-H-BSA).

The strategy used for the synthesis of the trisaccharides (Figure 2) is based on an approach previously developed for the assembly of a *M. tuberculosis* phenolic glycolipid in which iodophenol glycosides were generated and subsequently functionalized with a lipid tail through a Sonogashira coupling (Figure 3).[31,32] The synthetic approach applied here can thus also be applied for the total synthesis of the natural PGL-I, PGL-II and PGL-III without any modifications. For comparison the synthesis of disaccharide epitope ND-O-BSA will also be described in this chapter.

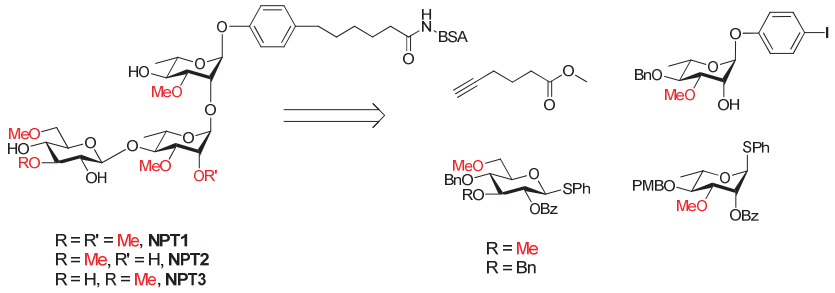
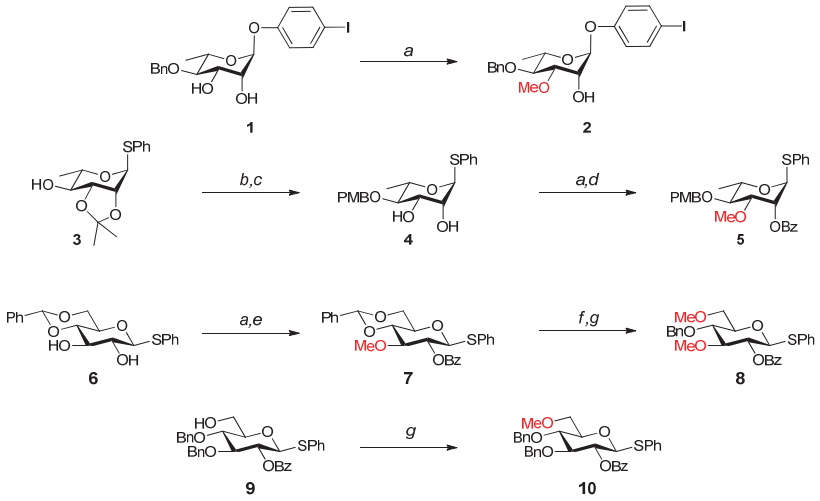


Figure 4. Retrosynthetic analysis of the desired trisaccharides.

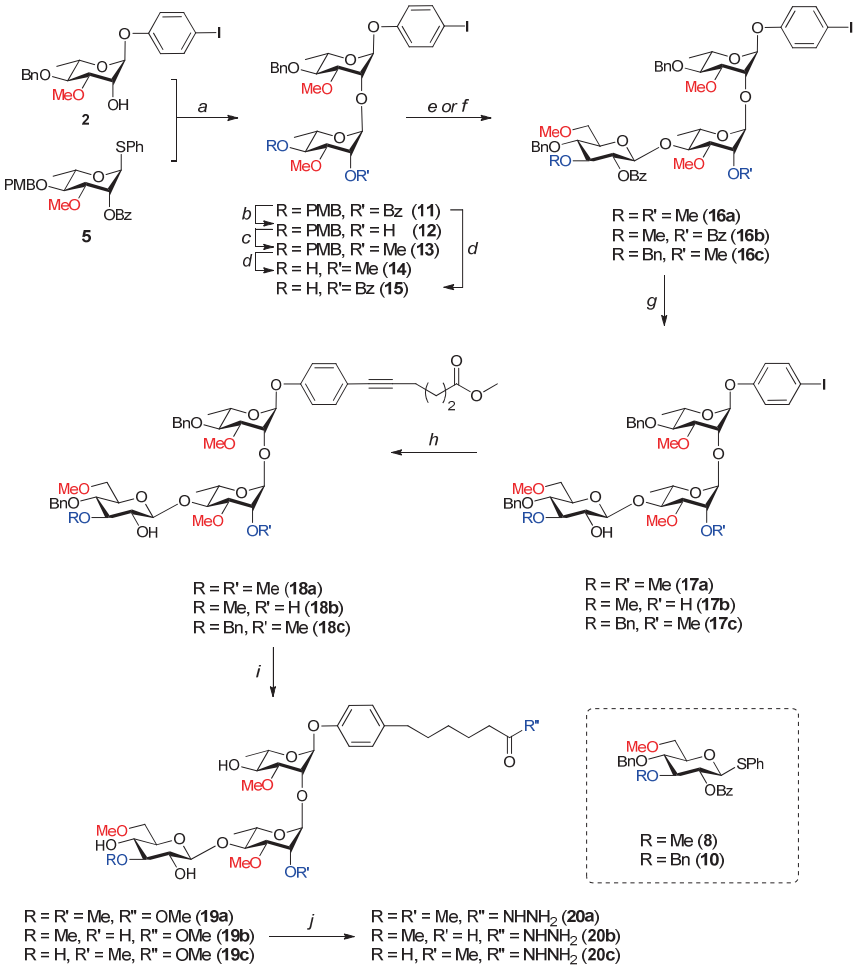
Results & Discussion

The requisite building blocks were prepared by the sequence of reactions depicted in Scheme 1. Iodoaryl acceptor **2** was synthesized by the regioselective methylation of the intermediate stannylidene complex of known[33] compound **1**. This method produced the acceptor in 91% yield as a 10:1 mixture of regioisomers. The purification of the product could be facilitated by the acetylation of the mixture, followed by separation with column chromatography and subsequent deacetylation. Donor **5** was synthesized by the protection of the C-4 position of intermediate **3**[33] with a *para*-methoxybenzyl ether, which was followed by a mild acidic hydrolysis of the isopropylidene ketal to give diol **4** in 86% yield over 2 steps. This diol was regioselectively methylated on the C-3 position as described above and subsequently benzoylated to give donor **5** in 87% yield over 2 steps. Glucose donor **8** was synthesized by the regioselective alkylation and subsequent benzoylation of known[34] benzylidene glucoside **6**. After the reductive opening of the benzylidene acetal, the newly liberated primary alcohol could be methylated to give donor **8** in 43% yield over 4 steps. Glucose **9**[35] was synthesized in a similar fashion and subsequently methylated to give donor **10** in 79% yield.



Scheme 1. Building block synthesis. Reagents and conditions: (a) 1. Bu_2SnO, toluene reflux, 2. MeI, CsF, DMF, 91% (**2**), 93% (**5**), 76% (**7**), (b) NaH, PMBCl, DMF, 0 °C -> RT, 98%, (c) $AcOH/H_2O$ (4:1), 45 °C, 88%, (d) BzCl, pyridine, DCM, 0 °C → RT, 94%, (e) BzCl, DMAP, pyridine, 0 °C → RT, 82% (f) $BH_3 \cdot THF$, TMSOTf, DCM, 96% (g) NaH, MeI, DMF, 0 °C → RT, 72% (**8**), 79% (**10**).

Scheme 2 depicts the synthesis of the required NPT-glycans. It was found that the condensation of acceptor **2** with rhamnosyl donor **5** under the agency of *N*-iodosuccinimide (NIS) and triflic acid (TfOH) led to partial iodination of the iodoaryl ring. Therefore the diphenylsulfoxide (Ph_2SO)-triflic anhydride (Tf_2O) reagent combination was used to activate the thioglycoside.[36] Subsequent addition of acceptor **2** to the activated donor provided dirhamnoside **11** in 66% yield. The C-2' benzoyl ester was then replaced for a methyl ether and subsequently the C-4' PMB ether was removed using a catalytic amount of HCl in HFIP,[37] resulting in disaccharide acceptor **14** in 86% yield over 3 steps. This acceptor was coupled with donor **8** using the Ph_2SO/Tf_2O activator delivering the fully protected trisaccharide **16a** in 93% yield. Similarly, the condensation of **14** and donor **10** delivered trisaccharide **16c** in quantitative yield. To synthesize trisaccharide **16b**, first disaccharide acceptor **15** was generated by removal of the PMB ether in **11** using a catalytic amount of HCl in HFIP to give the required alcohol in quantitative yield. Coupling of acceptor **15** to glucose donor **8** then gave trisaccharide **16b** in 88% yield.

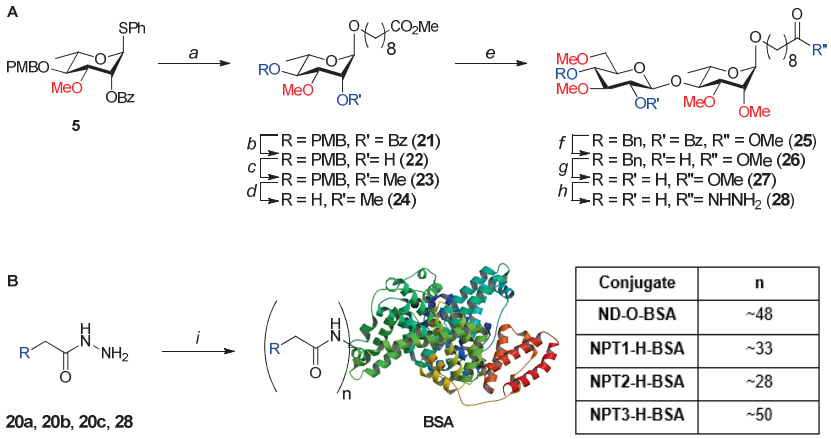


Scheme 2. Trisaccharide synthesis. Reagents and conditions: (a) Ph_2SO, Tf_2O, TTBP, DCM -60 °C, 66%, (b) Na, MeOH/THF, 97%, (c) NaH, MeI, DMF, 0 °C → RT, 95%, (d) HCl/HFIP, HFIP/DCM, 93% (**14**), 100% (**15**), (e) Donor **8**, Ph_2SO, Tf_2O, TTBP, DCM -60 °C, 93% (**16a**), 88% (**16b**), (f) Donor **10**, Ph_2SO, Tf_2O, TTBP, DCM -60 °C, 100% (**16c**), (g) Na, MeOH/THF, 99% (**17a**), 84% (**17b**), 97% (**17c**), (h) Methyl hex-5-ynoate, $Pd(PPh_3)_2Cl_2$, PPh_3, CuI, Et_3N, 99% (**18a**), 77% (**18b**), 82% (**18c**), (i) Pd/C, H_2, THF/MeOH, 100% (**19a**), 82% (**19b**), 90% (**19c**), (j) $N_2H_4 \cdot H_2O$, EtOH, 89% (**20a**), 100% (**20b**). 84% (**20c**).

After the benzoyl protecting groups were removed from the trisaccharides, the iodoaryl glycosides were coupled to methyl hex-5-ynoate using a Sonogashira cross-coupling. Global deprotection of the trisaccharides and reduction of the triple bond was

accomplished by a single hydrogenation reaction to provide the target trisaccharides having a methyl ester spacer, in excellent yields. The methyl esters could then be transformed into hydrazides **20a**, **20b** and **20c** which could be used for conjugation to BSA.

Scheme 3A depicts the assembly of the ND-O-disaccharide **28** which was required for comparison. The synthesis started with the coupling of rhamnose donor **5** with methyl 9-hydroxynonanoate[38] under the agency of Ph_2SO/Tf_2O, to give spacer equipped rhamnose **21** in good yield. After the C-2 benzoyl ester was removed and replaced with the required methyl ether, the C-4 PMB ether could be cleaved which provided acceptor **24** in excellent yield.



Conjugate	n
ND-O-BSA	~48
NPT1-H-BSA	~33
NPT2-H-BSA	~28
NPT3-H-BSA	~50

Scheme 3. A: Disaccharide synthesis. Reagents and conditions: (a) Methyl 9-hydroxynonanoate, Ph_2SO, Tf_2O, TTBP, DCM, -60 °C, 79%, (b), Na, MeOH/THF, 97%, (c), NaH, MeI, DMF, 91%, 0 °C → RT, (d) HCl/HFIP, HFIP/DCM, 95%, (e) Donor **8**, Ph_2SO, Tf_2O, TTBP, DCM, -60 °C, 84%, (f), Na, MeOH/THF, 82% (g) Pd/C, H_2, THF, 95%, (h) $N_2H_4 \cdot H_2O$, EtOH, 100%, **B: Conjugation of hydrazides to BSA**. Reagents and conditions: (i) 1. HCl/dioxane, *t*-BuONO, DMF, -30 °C, 2. BSA, $Na_2B_4O_7$, $NaHCO_3$, (pH = 9.2), H_2O, 0 °C.

This acceptor could be coupled to glucose donor **8**, using Ph_2SO/Tf_2O, after which the benzoate ester was cleaved by treatment with NaOMe and the benzyl ether removed with hydrogenation. Finally, the methyl ester of the linker was transformed into the corresponding hydrazide to provide the required ND-O-disaccharide **28** in quantitative yield.

The conjugation of the saccharides to BSA is depicted in Scheme 3B. First the hydrazides were transformed into the corresponding acyl azides in DMF using *tert*-butyl nitrite and HCl in dioxane at -30 °C. After complete conversion was observed, the reaction was quenched and the resulting mixture was transferred to an ice-cooled solution of BSA in aqueous $Na_2B_4O_7$ and $NaHCO_3$ (pH = 9.2). After desalting and purification by means of gel filtration the conjugates NPT1-H-BSA, NPT2-H BSA and NPT3-H-BSA were obtained, bearing 33, 28 and 50 trisaccharides per BSA, respectively and the control conjugate ND-O-BSA functionalized with 48 copies of the disaccharide on each protein, as revealed by SDS-PAGE and MALDI-TOF analyses. The synthetic route described in this chapter has also been applied in the gram scale production of NPT1-H-BSA.[39]

All newly synthesized conjugates were assessed in ELISA and UCP-LFA alongside the earlier described ND-O-HSA. ND-O-BSA, NPT1-H-BSA and NPT2-H-BSA showed a high correlation with ND-O-HSA in both ELISA and UCP-LFA, however considerably lower levels of IgM were binding to NPT3-H-BSA (Figure 4). This result shows the importance of the methylation pattern, and especially that of the C-3'' methyl, and indicates that human anti-PGL IgM binds to NPT3-H-BSA to a lesser extent.

To assess the stability of the different PGL conjugates in the UCP-LFA format, the stability was tested at seven different time points ranging from two months to thirteen months after production. Little variation was observed between the time points for test sera. Furthermore, analysis of the negative control serum sample at all time points indicated that no background signal developed in aging strips.

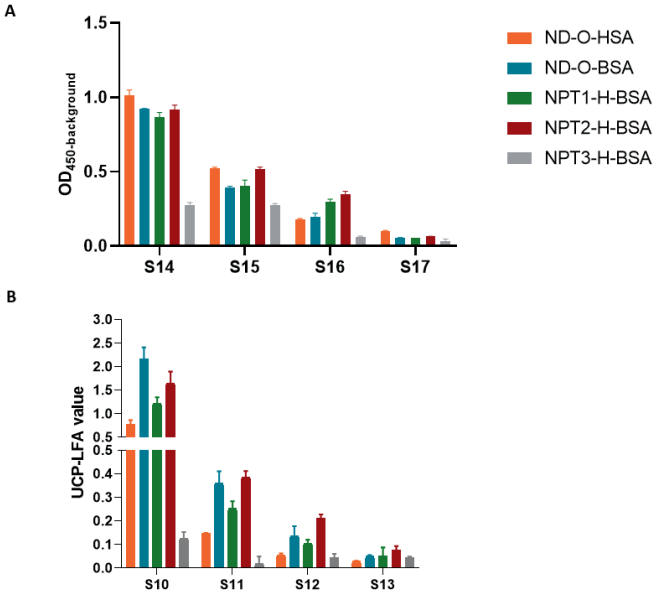


Figure 4. Comparison of IgM responses to five different synthetic PGLs in ELISA and UCP-LFA.[40] IgM responses to ND-O-HSA (orange), ND-O-BSA (blue), NPT1-H-BSA (green), NPT2-H-BSA (red) and NPT3-H-BSA were evaluated in ELISA (A) and UCP-LFA (B) using patient samples. (A) OD_{450}-background (y-axis) represents the IgM antibody levels detected by ELISA against the five different synthetic PGLs. (B) The UCP-LFA values (y-axis; arbitrary units) as determined using the UCP-LFA indicate the IgM antibody levels directed against the five different synthetic PGLs.

Conclusion

This chapter describes the synthesis of a set of BSA conjugates of *M. leprae* phenolic glycolipid trisaccharides connected to the protein via a hexanoic acid linker attached to the anomeric phenol on the reducing end, and the use of these for detection of anti-PGL IgM antibodies. The conjugates were evaluated as coating antigen both in ELISA and lateral flow format (UCP-LFA) in order to investigate the influence of the phenol on the reducing end as well as the methylation pattern of the three glycoforms. The required glycans were successfully synthesized using a route based on thioglycosides and an iodophenol bearing rhamnoside, which allowed for the functionalization with a linker through a Sonogashira coupling, a strategy which will also be applied to the total synthesis of complete PGLs. In ELISA the conjugates showed a high correlation with results obtained

with ND-O-HSA as coating antigen. Based on this data the conjugates were incorporated into the UCP-LFA format suitable for POC testing in the field. A high correlation with the results of ND-O-HSA was found here as well and therefore the conjugates are thought to be well applicable to the format. The stability of NPT1-H-BSA in the UCP-LFA format was also assessed yielding consistent results over a period of 13 months, without development of any background signal within this time frame. This is advantageous for the application of this format in low resource areas where a cold chain is not always available. When the three trisaccharides NPT1, NPT2 and NPT3 were compared, significantly lower levels of IgM were found to bind to the latter trisaccharide. This is in line with previous assessments which determined the C-3 methyl ether of the terminal glucose to be a highly important structural determinant for antibody binding.[18] In summary, the data obtained here indicates that trisaccharide conjugates represent robust targets for the detection of anti-PGL-I antibodies in point-of-care (POC) tests. The conjugates developed here can be used in future field studies in leprosy endemic areas.

Experimental

General procedures

All reactions were carried out in oven-dried glassware (80 °C). Prior to reactions, traces of water and solvent were removed by co-evaporation with toluene where appropriate. Reactions sensitive to air or moisture were carried out under N_2 atmosphere (balloon). Commercially available reagents and solvents (Aldrich Chemistry, Honeywell, Merck, Ficher Scientific, Biosolve, Fluka, VWR Chemicals, Acros Organics, Fluorochem, Brunschwig, Carbosynth) were used as received unless stated otherwise.

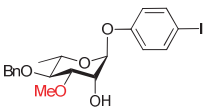
Solvents for reactions were reagent grade and dried by storage over flame dried 4 Å molecular sieves when needed. Tf_2O used in glycosylations was dried by distillation over P_2O_5 and stored under N_2 atmosphere in a Schlenk flask at -20 °C. Et_2O used for column chromatography was distilled before use and stored over iron filings. EtOAc used for column chromatography was distilled before use. NEt_3 used for Sonogashira couplings was distilled from KOH, degassed with N_2, and stored over KOH for a maximum of 24 hours.

Reaction progress was monitored using aluminium-supported silica gel TLC plates (Merck, Kieselgel 60, F254); visualization was carried out by irradiation with UV light (254 nm), and spraying with 20% H_2SO_4 in EtOH (w/v) or $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% H_2SO_4, followed by charring. Additional analysis with TLC-MS was used when needed.

Column chromatography was carried out using silica gel (Fluka, 40-63 µm mesh). The column was prepared using the apolar component mentioned in the corresponding experimental. If the apolar component was pentane the product was brought up in toluene. If the apolar component was DCM the product was brought up in DCM, possibly with a few drops of methanol if needed. Colum chromatography was performed using a gradient ranging from 0% polar component up to the ratio mentioned in the corresponding experimental in 2 to 5 steps depending on the ease of separation.

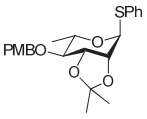
NMR spectra were recorded at ambient temperature on a Bruker AV-400LIQ spectrometer. Samples were prepared in $CDCl_3$ unless stated otherwise. Chemical shifts (δ) in $CDCl_3$ are reported in ppm relative to Me_4Si (δ: 0.00 ppm) for ^{1}H-NMR and $CDCl_3$ (δ: 77.16 ppm) for ^{13}C-NMR. Chemical shifts in CD_3OD are reported in ppm relative to H_2O (δ: 4.87 ppm) for ^{1}H-NMR and CD_3OD (δ: 49.00 ppm) for ^{13}C-NMR. ^{13}C-APT spectra are ^{1}H decoupled and structural assignment was achieved using HH-COSY and HSQC 2D experiments. Coupling constants (J) are given in Hz. Coupling constants of anomeric carbon atoms ($J_{H1,C1}$) were determined using HMBC-GATED experiments. MALDI measurements were carried out with a Bruker **A**utoflex Speed™ LRF. Optical rotations were measured on an Anton Paar Modular Circular Polarimeter MCP 100/150. High resolution mass spectra were recorded on a Synapt G2-Si or a Q Exactive HF Orbitrap equipped with an electron spray ion source positive mode. Infrared spectra were recorded on a Perkin Elmer Spectrum 2 FT-IR.

4-iodophenyl 3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (2)



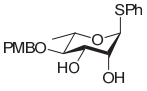
Compound **1**[33] (4.56 g, 10 mmol, 1.0 eq) was dissolved in toluene (500 mL, 0.02 M) and Bu_2SnO (2.74 g, 11 mmol, 1.1 eq) was added to the solution. The mixture was refluxed for 2 hours and then concentrated *in vacuo*. The mixture was then dissolved in dry DMF (100 mL, 0.1 M) and CsF (1.82 g, 12 mmol, 1.2 eq) and MeI (0.81 mL, 13 mmol, 1.3 eq) were added. The reaction was allowed to stir for 22 hours after which it was quenched by addition of H_2O and extracted with Et_2O (3x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:1) gave the title compound (4.28 g, 9.1 mmol, 91%, 10:1 mixture of regioisomers) as a clear oil. $[\alpha]_D^{25}$ -79.9 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.57-7.54 (m, 2H, C*H*$_{arom}$); 7.36-7.24 (m, 5H, C*H*$_{arom}$); 6.84-6.80 (m, 2H, C*H*$_{arom}$); 5.51 (d, 1H, *J* = 1.6 Hz, H-1); 4.86 (d, 1H, *J* = 10.8 Hz, PhC*H*H); 4.63 (d, 1H, *J* = 10.8 Hz, PhCH*H*); 4.21 (dd, 1H, *J* = 2.0, 3.2 Hz, H-2); 3.76-3.71 (m, 2H, H-3, H-5); 3.56 (s, 3H, OC*H*$_3$); 3.44 (t, 1H, *J* = 5.2 Hz, H-4); 2.74 (bs, 1H, 2-O*H*); 1.24 (d, 3H, *J* = 6.4 Hz, H-6); ^{13}C-APT NMR (101 MHz) δ: 156.0 ($C_{q,arom}$); 138.5 (*C*H$_{arom}$); 138.4 ($C_{q,arom}$); 128.5, 128.1, 127.9, 118.6 (*C*H$_{arom}$); 97.1 (C-1); 84.8 (*C*I$_{arom}$); 81.5 (C-3); 79.7 (C-4); 75.4 (*C*H$_{2.Bn}$); 68.2 (C-5); 67.8 (C-2); 57.8 (O*C*H$_3$); 18.0 (C-6). IR (thin film, cm^{-1}): 1026, 1095, 1133, 1177, 1233, 1452, 1484, 2927, 3408. HRMS calculated for $C_{20}H_{23}IO_5Na$ 493.0488 [M+Na]$^+$; found 493.0479.

Phenyl 2,3-*O*-isopropylidene-4-*O*-(4-methoxybenzyl)-1-thio-α-L-rhamnopyranoside (29)



Compound **3**[33] (7.45 g, 25 mmol, 1.0 eq) was dissolved in dry DMF (250 mL, 0.1 M) and PMBCl (4.8 mL, 35 mmol, 1.4 eq) was added to the solution. The mixture was cooled to 0 °C and NaH (60%, 1.40 g, 35 mmol, 1.4 eq) was then added. The reaction mixture was warmed to rt while stirring for 3 hours. The reaction was quenched by addition of H_2O and extracted with Et_2O (3x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 9:1) gave the title compound (10.2 g, 24.5 mmol, 98%) as a pale oil. $[\alpha]_D^{25}$ -148.8 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.46-7.43 (m, 2H, C*H*$_{arom}$); 7.29-7.19 (m, 5H, C*H*$_{arom}$); 6.87-6.84 (m, 2H, C*H*$_{arom}$); 5.74 (s, 1H, H-1); 4.84 (d, 1H, *J* = 10.8 Hz, PhC*H*H); 4.56 (d, 1H, *J* = 10.8 Hz, PhCH*H*); 4.35-4.28 (m, 2H, H-2, H-3); 4.16-4.11 (m, 1H, H-5); 3.73 (s, 3H, C*H*$_{3,PMB}$); 3.28 (dd, 1H, *J* = 7.0, 9.8 Hz, H-4); 1.51 (s, 3H, C(C*H*$_3$)$_2$); 1.36 (s, 3H, C(C*H*$_3$)$_2$); 1.21 (d, 3H, *J* = 6.4 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 159.3, 133.7 ($C_{q,arom}$); 131.8 (*C*H$_{arom}$); 130.4 ($C_{q,arom}$); 130.3, 129.7, 129.1, 127.6, 113.8 (*C*H$_{arom}$); 109.5 (*C*(CH$_3$)$_2$); 83.8 (C-1); 81.0 (C-4); 78.4 (C-3); 76.7 (C-2); 72.7 (Ph*C*H$_2$); 55.1 (*C*H$_{3,PMB}$); 28.0, 26.5 (C(*C*H$_3$)$_2$); 17.7 (C-6). IR (thin film, cm^{-1}): 1035, 1057, 1086, 1108, 1220, 1248, 1513. HRMS calculated for $C_{23}H_{28}O_5SNa$ 439.1555 [M+Na]$^+$; found 439.1553.

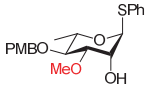
Phenyl 4-*O*-(4-methoxybenzyl)-1-thio-α-L-rhamnopyranoside (4)



Compound **29** (9.04 g, 21.7 mmol, 1.0 eq) was dissolved in AcOH/H_2O (4:1, 500 mL, 0.04 M) and stirred at 45 °C for 3h. The solvent was evaporated until a thick oil was formed. The oil was co-evaporated three times with toluene to give the title compound (8.16 g, 21.7 mmol, 100%) as a slightly yellow oil. $[\alpha]_D^{25}$ -70.8 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.45-7.42 (m, 2H, C*H*$_{arom}$); 7.31-7.22 (m, 5H, C*H*$_{arom}$); 6.91-6.88 (m, 2H, C*H*$_{arom}$); 5.46 (d, 1H, *J* = 1.6 Hz, H-1); 4.71-4.63 (m, 2H, C*H*$_{2,PMB}$); 4.23-4.16 (m, 2H, H-2, H-5); 3.89 (dd, 1H, *J* = 3.2, 9.2 Hz, H-3); 3.80 (s, 3H, C*H*$_{3,PMB}$);

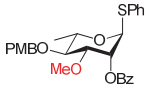
3.41 (t, 1H, *J* = 9.2 Hz, H-4); 1.34 (d, 3H, *J* = 6.0 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 159.6, 134.3 ($C_{q,arom}$); 131.4 (CH_{arom}); 130.3 ($C_{q,arom}$); 129.8, 129.3, 127.5, 114.2 (CH_{arom}); 87.5 (C-1); 81.5 (C-4); 74.9 (PhCH_2); 72.7 (C-2); 72.0 (C-3); 68.8 (C-5); 55.4 ($CH_{3,PMB}$); 18.0 (C-6). IR (thin film, cm^{-1}): 1036, 1065, 1097, 1250, 1513, 3328. HRMS calculated for $C_{20}H_{24}O_5SNa$ 399.1242 $[M+Na]^+$; found 399.1244.

Phenyl 3-*O*-methyl-4-*O*-(4-methoxybenzyl)-1-thio-α-L-rhamnopyranoside (30)



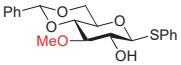
Compound **4** (8.16 g, 21.7 mmol, 1.0 eq) was dissolved in toluene (500 mL, 0.04 M) and Bu_2SnO (5.94 g, 23.9 mmol, 1.1 eq) was added to the solution. The mixture was refluxed for 2 hours and then concentrated *in vacuo*. The mixture was then dissolved in dry DMF (220 mL, 0.1 M) and CsF (3.96 g, 26 mmol, 1.2 eq) and MeI (1.8 mL, 28.2 mmol, 1.3 eq) were added. The reaction was allowed to stir for 20 hours after which it was quenched by addition of H_2O and extracted with Et_2O (3x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:1) gave the title compound (7.88 g, 20.2 mmol, 93%, 10:1 mixture of regioisomers) as a pale oil. $[\alpha]_D^{25}$ = -113.8 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.47-7.43 (m, 2H, CH_{arom}); 7.32-7.22 (m, 5H, CH_{arom}); 6.90-6.87 (m, 2H, CH_{arom}); 5.53 (d, 1H, *J* = 1.6 Hz, H-1); 4.78 (d, 1H, *J* = 10.4 Hz, PhC*H*H); 4.56 (d, 1H, *J* = 10.4 Hz, PhCH*H*); 4.29 (dd, 1H, *J* = 1.6, 3.6 Hz, H-2); 4.18-4.14 (m, 1H, H-5); 3.80 (s, 3H, $CH_{3,PMB}$); 3.56 (dd, 1H, *J* = 3.6, 9.2 Hz, H-3); 3.52 (s, 3H, OCH_3); 3.43 (t, 1H, *J* = 9.2 Hz, H-4); 2.74 (d, 1H, *J* = 4.8 Hz, 2-O*H*); 1.28 (d, 3H, *J* = 6.0 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 159.4, 134.3 ($C_{q,arom}$); 131.4 (CH_{arom}); 130.6 ($C_{q,arom}$); 129.8, 129.1, 127.4, 114.0 (CH_{arom}); 87.2 (C-1); 82.1 (C-3); 79.7 (C-4); 75.0 (PhCH_2); 69.5 (C-2); 68.7 (C-5); 57.6 (OCH_3); 55.4 ($CH_{3,PMB}$); 17.9 (C-6). IR (thin film, cm^{-1}): 1035, 1083, 1097, 1249, 1513, 3450. HRMS calculated for $C_{21}H_{26}O_5SNa$ 413.1394 $[M+Na]^+$; found 413.1399.

Phenyl 2-*O*-benzoyl-3-*O*-methyl-4-*O*-(4-methoxybenzyl)-1-thio-α-L-rhamnopyranoside (5)



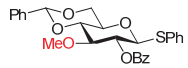
Compound **30** (0.15 g, 0.37 mmol, 1.0 eq) was dissolved in pyridine (1.9 mL, 0.2 M) and BzCl (86 µL, 0.74 mmol, 2.0 eq) was added to the solution. A catalytic amount of DMAP was added and the mixture was allowed to stir for 2 hours. The reaction was quenched by addition of MeOH and concentrated *in vacuo*. The resulting oil was dissolved in Et_2O and washed with 1M HCl, sat. aq. $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 9:1) gave the title compound (7.88 g, 20.2 mmol, 93%) as a pale oil. $[\alpha]_D^{25}$ -120.2 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.07-8.03 (m, 2H, CH_{arom}); 7.58-7.54 (m, 1H, CH_{arom}); 7.48-7.42 (m, 4H, CH_{arom}); 7.32-7.21 (m, 5H, CH_{arom}); 6.89 (d, 2H, *J* = 8.4 Hz, CH_{arom}); 5.82 (d, 1H, *J* = 1.6 Hz, H-2); 5.55 (s, 1H, H-1); 4.74 (dd, 2H, *J* = 10.6, 95.8 Hz, PhCH_2); 4.30-4.26 (m, 1H, H-5); 3.78-3.74 (m, 4H, H-3, $CH_{3,PMB}$); 3.57 (t, 1H, *J* = 9.4 Hz, H-4); 3.50 (s, 3H, OCH_3); 1.37 (d, 3H, *J* = 6.0 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 165.7 (CO_{Bz}); 159.4, 134.1 ($C_{q,arom}$); 133.4, 131.8 (CH_{arom}); 130.6 ($C_{q,arom}$); 130.0 (CH_{arom}); 129.9 ($C_{q,arom}$); 129.2, 128.5, 127.7, 113.9 (CH_{arom}); 86.2 (C-1); 80.8 (C-3); 79.8 (C-4); 75.1 (PhCH_2); 70.8 (C-2); 69.1 (C-5); 57.6 (OCH_3); 55.3 ($CH_{3,PMB}$); 18.1 (C-6). IR (thin film, cm^{-1}): 1070, 1093, 1109, 1251, 1267, 1513, 1722. HRMS calculated for $C_{28}H_{30}O_6SNa$ 517.1661 $[M+Na]^+$; found 517.1663.

Phenyl 3-*O*-methyl-4,6-*O*-benzylidene-1-thio-ß-D-glucopyranoside (31)



After co-evaporation with toluene, compound **6**[41] (3.60 g, 10 mmol, 1.0 eq) was dissolved in toluene (500 mL, 0.02 M). To the solution was added Bu_2SnO (2.70 g, 11 mmol, 1.1 eq) and refluxed for 2 hours after which it was concentrated *in vacuo*. The residue was dissolved in dry DMF (100 mL, 0.1 M) and MeI (0.8 mL, 13 mmol, 1.3 eq) along with CsF (1.82 g, 12 mmol, 1.2 eq) were added. The reaction mixture was stirred overnight after which it was quenched by addition of H_2O. The aqueous phase was extracted with Et_2O (3x) after which the combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-EtOAc, 7:3) gave the title compound (2.86 g, 7.64 mmol, 76%) as a white solid. ^{1}H-NMR (400 MHz) δ: 7.56-7.52 (m, 2H, C*H*$_{arom}$); 7.49-7.46 (m, 2H, C*H*$_{arom}$); 7.39-7.26 (m, 6H, C*H*$_{arom}$); 5.54 (s, 1H, PhC*H*); 4.66-4.63 (m, 1H, H-1); 4.38 (dd, 1H, *J* = 4.8, 10.4 Hz, H-6); 3.78 (t, 1H, *J* = 10.2 Hz, H-6); 3.67 (s, 3H, OC*H*3); 3.61-3.44 (m, 4H, H-2, H-3, H-4, H-5); 2.68 (s, 1H, 2-O*H*). ^{13}C NMR (100 MHz) δ: 137.2 (*C*$_{q,arom}$); 133.3 (*C*H$_{arom}$); 131.4 (*C*$_{q,arom}$); 129.2, 129.2, 128.6, 128.4, 126.1 (*C*H$_{arom}$); 101.4 (Ph*C*H); 88.6 (C-1); 83.7 (C-3); 81.2 (C-4); 72.3 (C-2); 70.8 (C-5); 68.7 (C-6); 61.2 (O*C*H$_3$). Spectroscopic data were in accordance with those previously reported in the literature[42].

Phenyl 2-*O*-benzoyl-3-*O*-methyl-4,6-*O*-benzylidene-1-thio-ß-D-glucopyranoside (7)



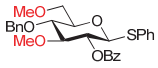
To a solution of compound **31** (2.30 g, 6.14 mmol, 1.0 eq) in pyridine (15.3 mL, 0.4 M), BzCl (1.4 mL, 12.3 mmol, 2.0 eq) was added dropwise after which it was stirred for 4.5 h. The reaction was quenched with H_2O and the aqueous phase was extracted with Et_2O (3x). The combined organic layers were washed with 1M HCl, sat. aq. $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. Recrystallization of the residue from EtOH afforded the title compound (2.42 g, 5.06 mmol, 82%) as a white solid. $[\alpha]_D^{25}$ 13.4 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.11-8.09 (m, 2H, C*H*$_{arom}$); 7.61 (t, 1H, *J* = 7.4 Hz, C*H*$_{arom}$); 7.50-7.43 (m, 8H, C*H*$_{arom}$); 7.40-7.35 (m, 2H, C*H*$_{arom}$); 7.31-7.25 (m, 2H, C*H*$_{arom}$); 5.59 (s, 1H, PhC*H*); 5.23 (dd, 1H, *J* = 8.4, 9.2 Hz, H-2); 4.89 (d, 1H, *J* = 10.0 Hz, H-1); 4.44-4.40 (m, 1H, H-6); 3.84 (t, 1H, *J* = 10.2 Hz, H-6); 3.77-3.67 (m, 2H, H-3, H-4); 3.61-3.55 (m, 1H, H-5); 3.51 (s, 3H, OC*H*$_3$). ^{13}C-APT NMR (101 MHz) δ: 165.3 (*C*O$_{Bz}$); 137.2 (C$_{q,arom}$); 133.4, 132.9 (*C*H$_{arom}$); 132.5 (C$_{q,arom}$); 130.0 (*C*H$_{arom}$); 129.9 (C$_{q,arom}$); 129.2, 129.1, 128.6, 128.4, 128.3, 126.2 (*C*H$_{arom}$); 101.1 (Ph*C*H); 87.2 (C-1); 82.4 (C-3); 81.1 (C-4); 72.3 (C-2); 70.8 (C-5); 68.7 (C-6); 60.9 (O*C*H$_3$). IR (thin film, cm^{-1}): 1026, 1069, 1093, 1178, 1268, 1727, 3451. HRMS calculated for $C_{27}H_{26}O_6SNa$ 501.1348 $[M+Na]^+$; found 501.1342.

Phenyl 2-*O*-benzoyl-3-*O*-methyl-4-*O*-benzyl-1-thio-ß-D-glucopyranoside (32)

Compound **7** (2.35 g, 4.91 mmol, 1.0 eq) was co-evaporated with toluene (3x) under N_2 atmosphere before it was dissolved in dry DCM (24.6 mL, 0.2 M). A 1M solution of BH_3·THF (24.6 mL, 24.6 mmol, 5 eq) in THF was added dropwise to the solution after which TMSOTf (0.13 mL, 0.74 mmol, 0.15 eq) was added to the mixture. The reaction mixture was stirred for 5 h and slowly quenched with NEt_3 (2.8 mL) followed by MeOH, which was added until the formation of H_2 ceased. The mixture was concentrated *in vacuo* and co-evaporated with MeOH (2x). Purification by means of column chromatography (*n*-pentane-Et_2O 7:3) gave the title compound (2.08 g, 4.33 mmol, 88%) as a white solid. $[\alpha]_D^{25}$ 32 (c = 0.4, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.12-8.10 (m, 2H, C*H*$_{arom}$); 7.62-7.58 (m, 1H, C*H*$_{arom}$); 7.50-

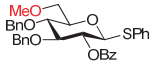
7.46 (m, 2H, C*H*$_{arom}$); 7.43-7.41 (m, 2H, C*H*$_{arom}$); 7.37-7.25 (m, 8H, C*H*$_{arom}$); 5.22-5.17 (m, 1H, H-2); 4.87-4.81 (m, 2H, H-1, PhC*H*H); 4.66 (d, 1H, *J* = 11.2 Hz, PhCH*H*); 3.92-3.88 (m, 1H, H-6); 3.75-3.68 (m, 1H, H-6); 3.62-3.59 (m, 2H, H-3, H-4); 3.51 (s, 3H, OC*H*$_3$); 3.50-3.47 (m, 1H, H-5); 1.98 (bs, 1H, 6-O*H*). ^{13}C-APT NMR (101 MHz) δ: 165.4 (*C*O$_{Bz}$); 138.0 (C$_{q,arom}$); 133.5 (*C*H$_{arom}$); 132.9 (C$_{q,arom}$) 132.4, 132.0, 130.1 (*C*H$_{arom}$); 129.9 (C$_{q,arom}$); 129.2, 128.7, 128.5, 128.4, 128.2, 128.1, 127.8 (*C*H$_{arom}$); 86.6 (C-3); 86.3 (C-1); 79.7 (C-5); 77.2 (C-4); 75.2 (Ph*C*H$_2$); 72.8 (C-2); 62.3 (C-6); 61.1 (O*C*H$_3$). IR (thin film, cm^{-1}): 1027, 1070, 1092, 1178, 1266, 1452, 1727, 3470. HRMS calculated for $C_{27}H_{28}O_6SNa$ 503.1504 [M+Na]$^+$; found 503.1499.

Phenyl 2-*O*-benzoyl-3,6-di-*O*-methyl-4-*O*-benzyl-1-thio-ß-D-glucopyranoside (8)



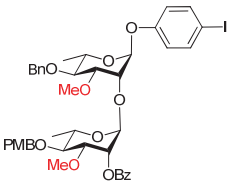
Compound **32** (0.60 g, 1.25 mmol, 1.0 eq) was dried by co-evaporation with toluene and dissolved in dry DMF (12.5 mL, 0.1 M). The solution was cooled to 0 °C after which MeI (0.16 mL, 2.51 mmol, 2.0 eq) was added. The reaction mixture was stirred for 5 minutes before NaH (60%, 84 mg, 2.51 mmol, 2.0 eq) was added and it was stirred for 5.5 hours while warming to rt. The reaction was quenched with H_2O and the aqueous phase was extracted with Et_2O (3x). The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O, 17:3) gave the title compound (0.45 g, 0.91 mmol, 72%) as a white solid. $[\alpha]_D^{25}$ 33.6 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.11-8.09 (m, 2H, C*H*$_{arom}$); 7.59-7.55 (m, 1H, C*H*$_{arom}$); 7.47-7.43 (m, 4H, C*H*$_{arom}$); 7.35-7.21 (m, 8H, C*H*$_{arom}$); 5.23 (t, 1H, *J* = 9.4 Hz, H-2); 4.85 (d, 1H, *J* = 10.8 Hz, PhC*H*H); 4.78 (d, 1H, *J* = 10.0 Hz, H-1); 4.64 (d, 1H, *J* = 10.8 Hz, PhCH*H*); 3.70-3.49 (m, 7H, H-3, H-4, H-5, H-6, OC*H*$_3$); 3.38 (s, 3H, OC*H*$_3$). ^{13}C-APT NMR (101 MHz) δ: 165.2 (*C*O$_{Bz}$); 138.1, 133.5 (C$_{q,arom}$); 133.3, 132.1, 129.9, 128.9, 128.5, 128.5, 128.1, 127.9, 127.7 (*C*H$_{arom}$); 86.6 (C-1); 86.5 (C-4); 79.2 (C-3); 77.5 (C-5); 75.0 (Ph*C*H$_2$); 72.6 (C-2); 71.3 (C-6); 60.9, 59.5 (O*C*H$_3$). IR (thin film, cm^{-1}): 1027, 1070, 1093, 1143, 1178, 1265, 1452, 1730. HRMS calculated for $C_{28}H_{30}O_6SNa$ 517.1661 [M+Na]$^+$; found 517.1655.

Phenyl 2-*O*-benzoyl-3-*O*-methyl-3,4-di-*O*-benzyl-1-thio-ß-D-glucopyranoside (10)



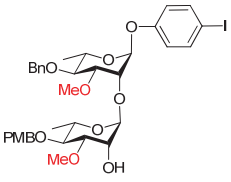
Compound **9**[35] (2.48 g, 4.31 mmol, 1.0 eq) was dried by co-evaporation with toluene and dissolved in dry DMF (43.1 mL, 0.1 M). The solution was cooled to 0 °C after which MeI (0.54 mL, 8.62 mmol, 2.0 eq) was added. The reaction mixture was stirred for 5 minutes before NaH (60%, 0.29 g, 8.62 mmol, 2.0 eq) was added and it was stirred for 5 hours while warming to rt. The reaction was quenched by addition of H_2O and the aqueous phase was extracted with Et_2O (3x). the organic layers washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O, 4:1) gave the title compound (1.94 g, 3.39 mmol, 79%) as a white solid. $[\alpha]_D^{25}$ 41.3 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.02 (d, 2H, *J* = 8.0 Hz, C*H*$_{arom}$); 7.57 (t, 1H, *J* = 7.4 Hz, C*H*$_{arom}$); 7.46-7.42 (m, 4H, C*H*$_{arom}$); 7.35-7.23 (m, 8H, C*H*$_{arom}$); 7.11 (s, 5H, C*H*$_{arom}$); 5.29 (t, 1H, *J* = 9.4 Hz, H-2); 4.84 (d, 1H, *J* = 10.8 Hz, PhC*H*H); 4.79-4.72 (m, 2H, H-1, PhC*H*H); 4.66-4.63 (m, 2H, PhCH*H*, PhCH*H*); 3.84 (t, 1H, *J* = 9.4 Hz, H-3); 3.77-3.63 (m, 3H, H-4, H-6); 3.57-3.54 (m, 1H, H-5); 3.39 (s, 3H, OC*H*$_3$). ^{13}C-APT NMR (101 MHz) δ: 165.3 (*C*O$_{Bz}$); 138.1, 137.8, 133.4 (C$_{q,arom}$); 133.3, 132.4, 130.0, 128.9, 128.6, 128.5, 128.3, 128.1, 128.0, 127.8, 127.8 (*C*H$_{arom}$); 86.6 (C-1); 84.3 (C-3); 79.4 (C-5); 77.8 (C-4); 75.4, 75.2 (Ph*C*H$_2$); 72.6 (C-2); 71.3 (C-6); 59.6 (O*C*H$_3$). IR (thin film, cm^{-1}): 1000, 1026, 1069, 1090, 1140, 1178, 1205, 1264, 1315, 1452, 1482, 1727. HRMS calculated for $C_{34}H_{34}O_6SNa$ 593.1974 [M+Na]$^+$; found 593.1968.

4-iodophenyl 2-*O*-(2-*O*-benzoyl-3-*O*-methyl-4-*O*-(4-methoxybenzyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (11)



Donor **5** (396 mg, 0.80 mmol, 1.0 eq), Ph_2SO (210 mg, 1.04 mmol, 1.3 eq) and TTBP (497 mg, 2.0 mmol, 2.5 eq) were dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges. The mixture was then dissolved in DCM (16 mL, 0.05 M) and flame-dried 3Å molecular sieves were added. The solution was then cooled to -70 °C after which Tf_2O (175 µL, 1.04 mmol, 1.3 eq) was added to the solution. After stirring for 30 minutes, acceptor **2** (752 mg, 1.60 mmol, 2.0 eq), which was also dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges, was dissolved in DCM (4 mL, 0.4 M) and added to the solution at -65 °C. After stirring for 2.5 hours the reaction reached -40 °C and was quenched by addition of NEt_3 (1 mL). The reaction mixture was then diluted with DCM, filtered over celite, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 4:1) gave the title compound (451 mg, 0.53 mmol, 66%) as a clear oil. $[\alpha]_D^{25}$ -33.2 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.10-8.08 (m, 2H, C*H*$_{arom}$); 7.60-7.54 (m, 3H, C*H*$_{arom}$); 7.50-7.46 (m, 2H, C*H*$_{arom}$); 7.38-7.25 (m, 7H, C*H*$_{arom}$); 6.89 (dd, 2H, *J* = 2.0, 6.8 Hz, C*H*$_{arom}$); 6.79 (dd, 2H, *J* = 2.0, 6.8 Hz, C*H*$_{arom}$); 5.70 (dd, 1H, *J* = 1.8, 3.0 Hz, H-2'); 5.45 (d, 1H, *J* = 2.0 Hz, H-1); 5.17 (d, 1H, *J* = 1.6 Hz, H-1'); 4.92 (d, 1H, *J* = 11.2 Hz, PhC*H*H); 4.84 (d, 1H, *J* = 10.4 Hz, PhC*H*H); 4.66 (d, 1H, *J* = 11.2 Hz, PhCH*H*); 4.59 (d, 1H, *J* = 10.4 Hz, PhCH*H*); 4.20 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2); 3.89-3.75 (m, 6H, H-3, H-3', H-5', C*H*$_{3,PMB}$); 3.71-3.67 (m, 1H, H-5); 3.53-3.45 (m, 8H, H-4, H-4', OC*H*$_3$); 1.34 (d, 3H, *J* = 6.0 Hz, H-6'); 1.24 (d, 3H, *J* = 6.4 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 165.8 (*C*O$_{Bz}$); 159.5, 156.1, 138.5 (C$_{q,arom}$); 138.5, 133.3 (*C*H$_{arom}$); 130.6 (C$_{q,arom}$); 130.1, 130.0, 128.6, 128.5, 128.2, 127.8, 118.6, 113.9 (*C*H$_{arom}$); 99.4 (C-1'); 96.9 (C-1); 84.8 (*C*I$_{arom}$); 81.4 (C-3'); 80.1 (C-3); 79.9 (C-4'); 79.7 (C-4); 75.4, 75.2 (Ph*C*H$_2$); 73.5 (C-2); 69.2 (C-2'); 68.8 (C-5); 68.5 (C-5'); 58.1, 57.8 (O*C*H$_3$); 55.4 (*C*H$_{3,PMB}$); 18.3 (C-6'); 18.1 (C-6). IR (thin film, cm^{-1}): 1042, 1072, 1098, 1118, 1233, 1268, 1452, 1484, 1514, 1724. HRMS calculated for $C_{42}H_{47}IO_{11}Na$ 877.2061 $[M+Na]^+$; found 877.2055.

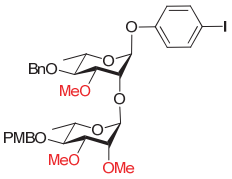
4-iodophenyl 2-*O*-(3-*O*-methyl-4-*O*-(4-methoxybenzyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (12)



Compound **11** (451 mg, 0.53 mmol, 1.0 eq) was dissolved in THF (2.6 mL, 0.2 M). A small piece of sodium was dissolved in MeOH and 2.6 mL of this solution was added. The reaction was stirred for 2 hours after which it was quenched with sat. aq. NH_4Cl and extracted with Et_2O (3x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:4) gave the title compound (384 mg, 0.51 mmol, 97%) as a pale oil. $[\alpha]_D^{25}$ -63.7 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.57-7.53 (m, 2H, C*H*$_{arom}$); 7.34-7.25 (m, 7H, C*H*$_{arom}$); 6.91-6.87 (m, 2H, C*H*$_{arom}$); 6.80-6.76 (m, 2H, C*H*$_{arom}$); 5.42 (d, 1H, *J* = 2.0 Hz, H-1); 5.13 (d, 1H, *J* = 1.6 Hz, H-1'); 4.88 (d, 1H, *J* = 10.8 Hz, PhC*H*H); 4.78 (d, 1H, *J* = 10.4 Hz, PhC*H*H); 4.61 (d, 1H, *J* = 10.8 Hz, PhCH*H*); 4.56 (d, 1H, *J* = 10.4 Hz, PhCH*H*); 4.21-4.19(m, 2H, H-2, H-2'); 3.81 (s, 3H, C*H*$_{3,PMB}$); 3.79-3.74 (m, 2H, H-3, H-5); 3.70-3.66 (m, 1H, H-5'); 3.60 (dd, 1H, *J* = 3.4, 9.0 Hz, H-3'); 3.57 (s, 3H, OC*H*$_3$); 3.53 (s, 3H, OC*H*$_3$); 3.43-3.34 (m, 2H, H-4, H-4'); 1.27 (d, 3H, *J* = 6.0 Hz, H-6'); 1.22 (d, 3H, *J* = 6.4 Hz, H-6).

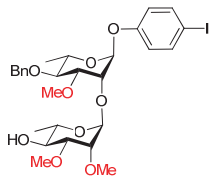
^{13}C-APT NMR (101 MHz) δ: 159.5, 156.1, 138.5 ($C_{q,arom}$); 138.5 (CH_{arom}); 130.6 ($C_{q,arom}$); 129.9, 128.5, 128.1, 127.9, 118.6, 114.0 (CH_{arom}); 100.9 (C-1'); 96.9 (C-1); 84.8 (CI_{arom}); 81.5 (C-3); 81.4 (C-3'); 80.1 (C-4); 79.6 (C-4'); 75.4, 75.1 (PhCH_2); 73.4 (C-2); 68.7 (C-5'); 68.1 (C-2'); 68.0 (C-5); 58.1, 57.7 (OCH_3); 55.4 ($CH_{3,PMB}$); 18.1 (C-6'); 18.0 (C-6). IR (thin film, cm^{-1}): 1045, 1070, 1113, 1139, 1233, 1249, 1484, 1513, 3484. HRMS calculated for $C_{36}H_{43}IO_{10}Na$ 773.1799 [M+Na]$^+$; found 773.1809.

4-iodophenyl 2-*O*-(2,3-di-*O*-methyl-4-*O*-(4-methoxybenzyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (13)



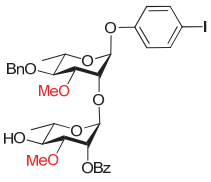
Compound **12** (196 mg, 0.26 mmol, 1.0 eq) was dissolved in dry DMF (2.6 mL, 0.1 M) and MeI (32 µL, 0.52 mmol, 2.0 eq) was added to the solution. The mixture was cooled to 0 °C and NaH (60%, 16 mg, 0.39 mmol, 1.5 eq) was then added. The reaction mixture was warmed to rt while stirring for 3 hours. The reaction was quenched by addition of H_2O and extracted with Et_2O (3x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:1) gave the title compound (207 mg, 0.26 mmol, 100%) as a pale oil. $[\alpha]_D^{25}$ -70.7 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.57-7.53 (m, 2H, CH_{arom}); 7.33-7.26 (m, 7H, CH_{arom}); 6.90-6.86 (m, 2H, CH_{arom}); 6.80-6.76 (m, 2H, CH_{arom}); 5.39 (d, 1H, *J* = 1.6 Hz, H-1); 5.14 (d, 1H, *J* = 1.6 Hz, H-1'); 4.88 (d, 1H, *J* = 11.2 Hz, PhC*H*H); 4.83 (d, 1H, *J* = 10.4 Hz, PhC*H*H); 4.64 (d, 1H, *J* = 10.8 Hz, PhCH*H*); 4.54 (d, 1H, *J* = 10.4 Hz, PhCH*H*); 4.19 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2); 3.80 (s, 3H, C$H_{3,PMB}$); 3.77-3.55 (m, 14H, H-2', H-3, H-3', H-5, H-5', OCH_3); 3.46-3.38 (m, 2H, H-4, H-4'); 1.27 (d, 3H, *J* = 6.0 Hz, H-6'); 1.22 (d, 1H, *J* = 6.0 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 159.4, 156.1 ($C_{q,arom}$); 138.5 (CH_{arom}); 130.8 ($C_{q,arom}$); 129.9, 128.5, 128.1, 127.9, 118.6, 114.0 (CH_{arom}); 98.9 (C-1'); 97.0 (C-1); 84.8 (CI_{arom}); 81.7 (C-3); 81.2 (C-3'); 80.2 (C-4); 80.0 (C-4'); 77.6 (C-2'); 75.3, 75.2 (PhCH_2); 68.8 (C-5); 68.6 (C-5'); 59.2, 58.2, 58.1 (OCH_3); 55.4 ($CH_{3,PMB}$); 18.1 (C-6); 18.1 (C-6'). IR (thin film, cm^{-1}): 1035, 1052, 1072, 1093, 1120, 1173, 1233, 1248, 1484, 1514. HRMS calculated for $C_{36}H_{45}IO_{10}Na$ 787.1955 [M+Na]$^+$; found 787.1945.

4-iodophenyl 2-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (14)



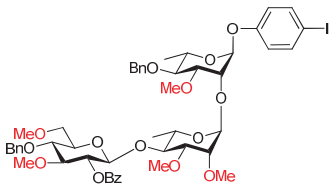
Compound **13** (199 mg, 0.26 mmol, 1.0 eq) was dissolved in a mixture of DCM and HFIP (1:1, 2.6 mL, 0.1 M) after which a solution of HCl in HFIP (0.13 mL, 0.2 M, 0.1 eq) was added. After complete conversion of the starting material, indicated by a dark purple colour (~2 minutes), the reaction was quenched by addition of sat. aq. $NaHCO_3$. The mixture was diluted with DCM, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:4) gave the title compound (155 mg, 0.24 mmol, 93%) as a pale oil. $[\alpha]_D^{25}$ -75.0 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.59-7.55 (m, 2H, C*H*$_{arom}$); 7.35-7.28 (m, 5H, C*H*$_{arom}$); 6.83-6.79 (m, 2H, C*H*$_{arom}$); 5.44 (d, 1H, *J* = 1.6 Hz, H-1); 5.18 (d, 1H, *J* = 1.6 Hz, H-1'); 4.77 (dd, 2H, *J* = 10.8, 65.6 Hz, PhC*H*$_2$); 4.22 (dd, 1H, *J* = 2.4, 2.8 Hz, H-2); 3.79-3.70 (m, 4H, H-2', H-3, H-5, H-5'); 3.62-3.40 (m, 12H, H-3', H-4, H-4', OC*H*$_3$); 2.34 (bs, 4'-OH); 1.30 (d, 3H, *J* = 6.4 Hz, H-6'); 1.25 (d, 3H, *J* = 6.0 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 156.0 (C$_{q,arom}$); 138.5 (*C*H$_{arom}$); 138.4 (C$_{q,arom}$); 128.5, 128.1, 127.9, 118.6 (*C*H$_{arom}$); 99.1 (C-1'); 97.0 (C-1); 84.9 (*C*I$_{arom}$); 81.6 (C-3); 80.8 (C-3'); 80.0 (C-4); 75.9 (C-2'); 75.2 (Ph*C*H$_2$); 73.5 (C-2); 71.7 (C-4'); 68.9 (C-5); 68.8 (C-5'); 59.1, 58.2, 57.1 (O*C*H$_3$); 18.2 (C-6'); 17.9 (C-6). IR (thin film, cm^{-1}): 1013, 1017, 1032, 1050, 1073, 1089, 1122, 1139, 1233, 1262, 1484, 2909, 2929, 3481. HRMS calculated for $C_{28}H_{37}IO_9Na$ 667.1380 $[M+Na]^+$; found 667.1374.

4-iodophenyl 2-*O*-(2-*O*-benzoyl-3-*O*-methyl-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (15)



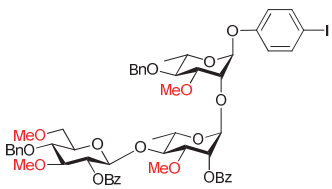
Compound **11** (88 mg, 0.10 mmol, 1.0 eq) was dissolved in a mixture of DCM and HFIP (1:1, 1 mL, 0.1 M) after which a solution of HCl in HFIP (50 μL, 0.2 M, 0.1 eq) was added. After complete conversion of the starting material, indicated by a dark purple colour (~2 minutes), the reaction was quenched by addition of sat. aq. $NaHCO_3$. The mixture was diluted with DCM, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:1) gave the title compound (80 mg, 0.10 mmol, 100%) as a pale oil. $[\alpha]_D^{25}$ -57.1 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.09-8.06 (m, 2H, C*H*$_{arom}$); 7.60-7.55 (m, 3H, C*H*$_{arom}$); 7.48-7.44 (m, 2H, C*H*$_{arom}$); 7.39-7.25 (m, 5H, C*H*$_{arom}$); 6.83-6.80 (m, 2H, C*H*$_{arom}$); 5.70 (dd, 1H, *J* = 2.0, 2.4 Hz, H-2'); 5.50 (d, 1H, *J* = 1.6 Hz, H-1); 5.22 (d, 1H, *J* = 1.6 Hz, H-1'); 4.80 (dd, 2H, *J* = 11.0, 103.4 Hz, PhC*H*$_2$); 4.24 (dd, 1H, *J* = 2.4, 2.8 Hz, H-2); 3.90-3.86 (m, 1H, H-5'); 3.79 (dd, 1H, *J* = 2.8, 9.2 Hz, H-3); 3.75-3.62 (m, 3H, H-3', H-4', H-5); 3.56-3.47 (m, 7H, H-4, OC*H*$_3$); 2.60 (bs, 1H, 4'-O*H*); 1.37 (d, 3H, *J* = 6.0 Hz, H-6'); 1.27 (d, 3H, *J* = 6.0 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 165.7 (*C*O$_{Bz}$); 156.1, 138.5 (C$_{q,arom}$); 138.5, 133.4, 130.0 (*C*H$_{arom}$); 129.8 (C$_{q,arom}$); 128.6, 128.5, 128.2, 127.9, 118.6 (*C*H$_{arom}$); 99.6 (C-1'); 97.0 (C-1); 84.9 (*C*I$_{arom}$); 81.4 (C-3); 79.9 (C-4); 79.6 (C-3'); 75.4 (C-2); 73.6 (C-4'); 68.9 (C-5); 68.9 (C-5'); 68.0 (C-2'); 58.2, 57.5 (O*C*H$_3$); 18.2 (C-6); 18.1 (C-6'). IR (thin film, cm^{-1}): 1040, 1073, 1096, 1119, 1176, 1202, 1232, 1271, 1316, 1385, 1452, 1484, 1585, 1724, 2931, 3446. HRMS calculated for $C_{34}H_{39}IO_{10}Na$ 757.1486 $[M+Na]^+$; found 757.1480.

4-iodophenyl 2-*O*-(2,3-di-*O*-methyl-4-*O*-(2-*O*-benzoyl-3,6-di-*O*-methyl-4-*O*-benzyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (16a)



Donor **8** (103 mg, 0.21 mmol, 1.5 eq), Ph_2SO (50 mg, 0.23 mmol, 1.7 eq) and TTBP (103 mg, 0.42 mmol, 3.0 eq) were dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges. The mixture was then dissolved in DCM (2.8 mL, 0.08 M) and flame-dried 3Å molecular sieves were added. The solution was then cooled to -60 °C after which Tf_2O (35 µL, 0.23 mmol, 1.7 eq) was added to the solution. After stirring for 30 minutes, acceptor **14** (90 mg, 0.14 mmol, 1.0 eq), which was also dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges, was dissolved in DCM (0.4 mL, 0.4 M) and added to the solution. After stirring for 1.5 hours the reaction was quenched by addition of NEt_3 (0.14 mL). The reaction mixture was then diluted with DCM, filtered over celite, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:4) gave the title compound (140 mg, 0.14 mmol, 98%) as a slightly yellow oil. $[\alpha]_D^{25}$ -74.9 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.16-8.14 (m, 2H, C*H*$_{arom}$); 7.58-7.56 (m, 3H, C*H*$_{arom}$); 7.47 (t, 1H, *J* = 7.8 Hz, C*H*$_{arom}$); 7.36-7.26 (m, 11H, C*H*$_{arom}$); 6.82-6.80 (m, 2H, C*H*$_{arom}$); 5.40 (d, 1H, *J* = 1.6 Hz, H-1); 5.17-5.12 (m, 2H, H-1', H-2''); 4.85-4.80 (m, 3H, H-1'', PhC*H*H, PhC*H*H); 4.66 (d, 1H, *J* = 11.2 Hz), PhCH*H*); 4.58 (d, 1H, *J* = 10.8 Hz, PhCH*H*); 4.17 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2); 3.74-3.39 (m, 19H, H-2', H-3, H-3', H-3'', H-4'', H-5, H-5', H-5'', H-6'', OC*H*$_3$); 3.35-3.30 (m, 2H, H-4, H-4'); 3.13 (s, 3H, OC*H*$_3$); 1.29 (d, 3H, *J* = 6.0 Hz, H-6''); 1.20 (d, 3H, *J* = 6.4 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 165.3 (*C*O$_{Bz}$); 156.0 (C$_{q,arom}$); 138.5 (*C*H$_{arom}$); 138.5, 138.4 (C$_{q,arom}$); 133.2 (*C*H$_{arom}$); 130.3 (C$_{q,arom}$); 130.0, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 118.6 (*C*H$_{arom}$); 101.4 (C-1''); 98.4 (C-1'); 96.8 (C-1''); 85.4 (C-3''); 84.8 (*C*I$_{arom}$); 81.8 (C-3); 80.5 (C-4'); 80.0 (C-4); 77.7 (C-3'); 77.5 (C-4''); 76.6 (C-2'); 75.2, 75.0 (Ph*C*H$_2$); 74.9 (C-5''); 74.3 (C-2''); 73.1 (C-2); 71.2 (C-6''); 68.8 (C-5'); 68.0 (C-5); 60.8, 59.8, 59.0, 58.3, 57.2 (O*C*H$_3$); 18.1 (C-6 and C-6'). IR (thin film, cm^{-1}): 1027, 1055, 1072, 1092, 1119, 1140, 1233, 1268, 1484, 1734, 2928. HRMS calculated for $C_{50}H_{61}IO_{15}Na$ 1051.2953 $[M+Na]^+$; found 1051.2947.

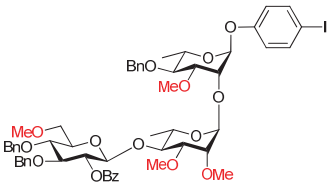
4-iodophenyl 2-*O*-(2-*O*-benzoyl-3-*O*-methyl-4-*O*-(2-*O*-benzoyl-3,6-di-*O*-methyl-4-*O*-benzyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (16b)



Donor **8** (69 g, 0.14 mmol, 1.5 eq), Ph_2SO (37 mg, 0.18 mmol, 2.0 eq) and TTBP (86 mg, 0.35 mmol, 3.8 eq) were dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges. The mixture was then dissolved in DCM (2.8 mL, 0.05 M) and flame-dried 3Å molecular sieves were added. The solution was then cooled to -60 °C after which Tf_2O (30 µL, 0.18 mmol, 2.0 eq) was added to the solution. After stirring for 30 minutes, acceptor **15** (68 mg, 93 µmol, 1.0 eq), which was also dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges, was dissolved in DCM (0.3 mL, 0.3 M) and added to the solution. After stirring for 2 hours the reaction was quenched by addition of NEt_3 (0.1 mL). The reaction mixture was then diluted with DCM, filtered over celite, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-

pentane-Et_2O 3:7) gave the title compound (92 mg, 82 µmol, 88%) as a pale oil. $[\alpha]_D^{25}$ -42.0 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.14-8.12 (m, 2H, C*H*$_{arom}$); 8.07-8.05 (m, 2H, C*H*$_{arom}$); 7.59-7.54 (m, 4H, C*H*$_{arom}$); 7.49-7.42 (m, 4H, C*H*$_{arom}$); 7.35-7.25 (m, 10H, C*H*$_{arom}$); 6.81 (dd, 2H, *J* = 2.0, 6.8 Hz, C*H*$_{arom}$); 5.45 (d, 1H, *J* = 2.0 Hz, H-1); 5.16-5.14 (m, 2H, H-1', H-2''); 4.87-4.83 (m, 3H, H-1'', PhC*H*H, PhC*H*H); 4.65 (d, 1H, *J* = 11.2 Hz, PhCH*H*); 4.59 (d, 1H, *J* = 10.8 Hz, PhCH*H*); 4.18 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2); 3.72-3.53 (m, 7H, H-3, H-4', H-4'', H-5, H-5', H-6''); 3.52-3.40 (m, 13H, H-3', H-3'', H-4, H-5'', OC*H*$_3$); 3.10 (s, 3H, OC*H*$_3$); 1.38 (d, 3H, *J* = 6.4 Hz, H-6'); 1.23 (d, 3H, *J* = 6.4 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 165.7, 165.3 (*C*O$_{Bz}$); 138.5 (*C*H$_{arom}$); 138.3 (C$_{q,arom}$); 133.4, 133.3 (*C*H$_{arom}$); 130.2 (C$_{q,arom}$); 130.1, 130.0, 128.6, 128.5, 128.2, 128.1, 128.0, 127.8, 118.7 (*C*H$_{arom}$); 101.5 (C-1''); 99.1 (C-1'); 96.8 (C-1); 85.4 (C-3''); 84.9 (*C*I$_{arom}$); 81.5 (C-3); 79.9 (C-4); 79.4 (C-3'); 78.2 (C-4'); 77.6 (C-4''); 75.3, 75.0 (Ph*C*H$_2$); 74.9 (C-5''); 74.2 (C-2''); 73.0 (C-2); 71.5 (C-6''); 68.9 (C-5); 68.4 (C-2'); 68.0 (C-5'); 60.8, 59.8, 58.2, 57.4 (O*C*H$_3$); 18.3 (C-6'); 18.1 (C-6). IR (thin film, cm^{-1}): 1000, 1027, 1070, 1096, 1112, 1140, 1178, 1233, 1268, 1452, 1484, 1723, 2931. HRMS calculated for $C_{56}H_{63}IO_{16}Na$ 1141.3059 $[M+Na]^+$; found 1141.3064.

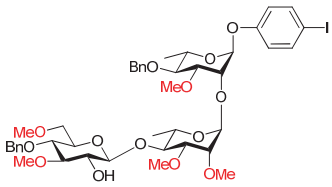
4-iodophenyl 2-*O*-(2,3-di-*O*-methyl-4-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-methyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (16c)



Donor **10** (204 mg, 0.41 mmol, 1.5 eq), Ph_2SO (92 mg, 0.45 mmol, 1.7 eq) and TTBP (205 mg, 0.83 mmol, 3.0 eq) were dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges. The mixture was then dissolved in DCM (5.5 mL, 0.07 M) and flame-dried 3Å molecular sieves were added. The solution was then cooled to -70 °C after which Tf_2O (76 µL, 0.45 mmol, 1.7 eq) was added to the solution. After stirring for 20 minutes, acceptor **14** (177 mg, 0.27 mmol, 1.0 eq), which was also dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges, was dissolved in DCM (0.6 mL, 0.4 M) and added to the solution. After stirring for 1 hour the reaction was quenched by addition of pyridine (0.28 mL). The reaction mixture was then diluted with DCM, filtered over celite, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:4) gave the title compound (284 g, 0.26 mmol, 93%) as a slightly yellow oil. $[\alpha]_D^{25}$ -76.0 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.12-8.10 (m, 2H, C*H*$_{arom}$); 7.62-7.58 (m, 3H, C*H*$_{arom}$); 7.49-7.45 (m, 2H, C*H*$_{arom}$); 7.37-7.32 (m, 10H, C*H*$_{arom}$); 7.16-7.13 (m, 5H, C*H*$_{arom}$); 6.85-6.83 (m, 2H, C*H*$_{arom}$); 5.43 (d, 1H, *J* = 2.0 Hz, H-1); 5.27 (t, 1H, *J* = 8.4 Hz, H-2''); 5.16 (d, 1H, *J* = 2.0 Hz, H-1'); 4.88-4.84 (m, 3H, H-1'', PhC*H*H, PhC*H*H); 4.79 (d, 1H, *J* = 11.2 Hz, PhC*H*H); 4.72-4.67 (m, 2H, PhCH*H*, PhCH*H*); 4.61 (d, 1H, *J* = 10.8 Hz, PhCH*H*); 4.21 (dd, 1H, *J* = 2.0, 3.2 Hz, H-2); 3.83-3.62 (m, 9H, H-2', H-3, H-3', H-3'', H-4'', H-5, H-5', H-6''); 3.55 (s, 3H, OC*H*$_3$); 3.50 (s, 4H, H-5'', OC*H*$_3$); 3.43 (s, OC*H*$_3$); 3.38-3.30 (m, 2H, H-4, H-4'); 3.15 (s, 3H, OC*H*$_3$); 1.33 (d, 3H, *J* = 5.6 Hz, H-6'); 1.24 (d, 3H, *J* = 6.0 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 165.2 (*C*O$_{Bz}$); 156.0 (C$_{q,arom}$); 138.5 (*C*H$_{arom}$); 138.3, 138.1 (C$_{q,arom}$); 133.1 (*C*H$_{arom}$); 130.3 (C$_{q,arom}$); 130.0, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 118.6 (*C*H$_{arom}$); 101.5 (C-1''); 98.4 (C-1'); 96.8 (C-1); 84.8 (*C*I$_{arom}$); 83.1 (C-3''); 81.8 (C-4''); 80.5 (C-4); 80.0 (C-4'); 78.1 (C-3); 77.7 (C-3'); 76.6 (C-2'); 75.2, 75.2, 75.1 (Ph*C*H$_2$); 75.0 (C-5''); 74.3 (C-2''); 73.1 (C-2); 71.2 (C-6''); 68.8 (C-5'); 68.0 (C-5); 59.9, 59.0, 58.2, 57.2 (O*C*H$_3$); 18.1 (C-6'); 18.1 (C-

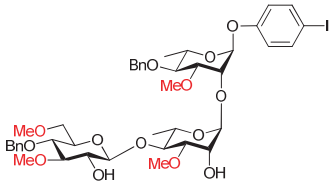
6). IR (thin film, cm^{-1}): 1000, 1027, 1055, 1072, 1095, 1120, 1140, 1233, 1268, 1452, 1484, 1731, 2931. HRMS calculated for $C_{56}H_{65}IO_{15}Na$ 1127.3266 $[M+Na]^+$; found 1127.3260.

4-iodophenyl 2-*O*-(2,3-di-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-4-*O*-benzyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (17a)



Compound **16a** (102 mg, 0.1 mmol, 1.0 eq) was dissolved in THF (0.49 mL, 0.2 M) and the solution was diluted with MeOH (0.49 mL). A small piece of sodium was added to the solution and the reaction was stirred for 2 hours. The reaction was then quenched with sat. aq. NH_4Cl and extracted with Et_2O (3x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:4) gave the title compound (92 mg, 0.1 mmol, 100%) as a pale oil. $[\alpha]_D^{25}$ -27.4 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.58-7.56 (m, 2H, C*H*$_{arom}$); 7.36-7.26 (m, 10H, C*H*$_{arom}$); 6.82-6.79 (m, 2H, C*H*$_{arom}$); 5.40 (d, 1H, *J* = 1.6 Hz, H-1); 5.14 (d, 1H, *J* = 1.6 Hz, H-1'); 4.89-4.83 (m, 2H, PhC*H*H, PhC*H*H); 4.66-4.60 (m, 2H, PhCH*H*, PhCH*H*); 4.37 (d, 1H, *J* = 8.0 Hz, H-1''); 4.18 (dd, 1H, *J* = 2.0, 3.2 Hz, H-2); 3.90 (bs, 1H, 2''-O*H*); 3.79-3.75 (m, 3H, H-2', H-3, H-5); 3.71-3.58 (m, 6H, H-3', H-5', H-5'', OC*H*$_3$); 3.56-3.50 (m, 11H, H-4'', H-6'', OC*H*$_3$); 3.46-3.36 (m, 6H, H-2'', H-4, H-4', OC*H*$_3$); 3.29 (t, 1H, *J* = 8.8 Hz, H-3''); 1.35 (d, 3H, *J* = 6.4 Hz, H-6'); 1.25 (d, 3H, *J* = 6.4 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 156.0 (C$_{q,arom}$); 138.5 (*C*H$_{arom}$); 138.4 (C$_{q,arom}$); 128.6, 128.5, 128.1, 128.1, 127.9, 118.6 (*C*H$_{arom}$); 105.8 (C-1''); 98.8 (C-1'); 96.9 (C-1); 86.4 (C-3''); 84.9 (*C*I$_{arom}$); 81.8 (C-5''); 81.6 (C-3); 80.3 (C-3'); 80.0 (C-4); 77.3 (C-4''); 75.8 (C-2'); 75.6 (C-2''); 75.3 (Ph*C*H$_2$); 75.2 (C-4'); 75.0 (Ph*C*H$_2$); 71.3 (C-6''); 68.7 (C-5'); 68.4 (C-5); 61.0, 59.5, 59.1, 58.3, 56.7 (O*C*H$_3$); 18.2 (C-6'); 17.7 (C-6). IR (thin film, cm^{-1}): 1000, 1002, 1030, 1032, 1052, 1071, 1120, 1233, 1455, 1485, 2896, 2923, 3445. HRMS calculated for $C_{43}H_{57}IO_{14}Na$ 947.2691 $[M+Na]^+$; found 947.2709.

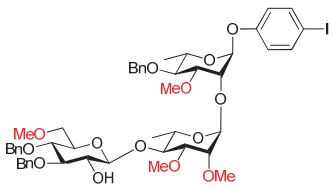
4-iodophenyl 2-*O*-(3-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-4-*O*-benzyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (17b)



Compound **16b** (90 mg, 80 µmol, 1.0 eq) was dissolved in THF (0.4 mL, 0.2 M). A small piece of sodium was dissolved in MeOH and 0.4 mL of this solution was added. The reaction was stirred for 4 hours after which it was quenched with sat. aq. NH_4Cl and extracted with Et_2O (3x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (DCM-EtOAc 7:3) gave the title compound (61 mg, 67 µmol, 84%) as a pale oil. $[\alpha]_D^{25}$ -65.2 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.57 (dd, 2H, *J* = 2.0, 6.8 Hz, C*H*$_{arom}$); 7.36-7.28 (m, 10H, C*H*$_{arom}$); 6.82-6.79 (m, 2H, C*H*$_{arom}$); 5.41 (d, 1H, *J* = 1.6 Hz, H-1); 5.14 (d, 1H, *J* = 1.6 Hz, H-1'); 4.89-4.86 (m, 2H, PhC*H*H, PhC*H*H); 4.63-4.61 (m, 2H, PhCH*H*, PhCH*H*); 4.35 (d, 1H, *J* = 7.6 Hz, H-1''); 4.25 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2'); 4.19 (dd, 1H, *J* = 2.4, 2.8 Hz, H-2); 3.85-3.79 (m, 1H, H-5'); 3.78-3.59 (m, 9H, H-3, H-3', H-4', H-5, H-6'', OC*H*$_3$); 3.55-3.50 (m, 7H, H-4'', OC*H*$_3$); 3.47-3.36 (m, 8H, H-2'', H-5'', OC*H*$_3$); 3.30 (t, 1H, *J* = 9.2 Hz, H-3''); 1.35 (d, 3H, *J* = 6.4 Hz, H-6'); 1.24 (d, 3H, *J* = 6.4 Hz, H-6). ^{13}C-APT NMR (101

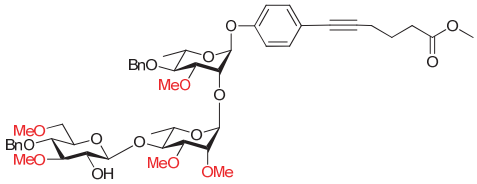
MHz) δ: 156.0 ($C_{q,arom}$); 138.5 (CH_{arom}); 138.5, 138.4 ($C_{q,arom}$); 128.6, 128.5, 128.1, 128.1, 127.9, 127.9, 118.6 (CH_{arom}); 105.7 (C-1''); 100.8 (C-1'); 96.9 (C-1); 86.2 (C-3''); 84.9 (CI_{arom}); 81.5 (C-3); 81.2 (C-4'); 80.6 (C-3'); 80.1 (C-4); 77.3 (C-4''); 75.6 (C-2''); 75.3 (PhCH_2); 75.3 (C-5''); 75.0 (PhCH_2); 73.6 (C-2); 71.3 (C-6''); 68.7 (C-5); 67.9 (C-5'); 66.9 (C-2'); 61.1, 59.5, 58.2, 56.9 (OCH_3); 18.1 (C-6); 17.6 (C-6'). IR (thin film, cm^{-1}): 1069, 1116, 1232, 1454, 1484, 2928, 3451. HRMS calculated for $C_{42}H_{55}IO_{14}Na$ 933.2534 $[M+Na]^+$; found 933.2529.

4-iodophenyl 2-*O*-(2,3-di-*O*-methyl-4-*O*-(3,4-di-*O*-benzyl-6-*O*-methyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranoside (17c)



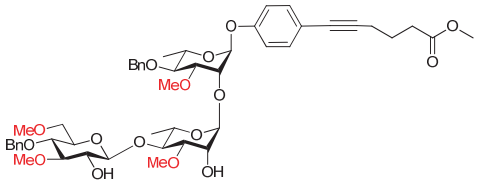
Compound **16c** (95 mg, 86 µmol, 1.0 eq) was dissolved in THF (0.8 mL, 0.1 M) and the solution was diluted with MeOH (0.8 mL). A small piece of sodium was added to the solution and the reaction was stirred for 2 hours. The reaction was then quenched with sat. aq. NH_4Cl and extracted with Et_2O (3x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:4) gave the title compound (85 mg, 85 µmol, 99%) as a pale oil. $[\alpha]_D^{25}$ -41.4 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.58-7.55 (m, 2H, CH_{arom}); 7.42-7.40 (m, 1H, CH_{arom}); 7.37-7.25 (m, 14H, CH_{arom}); 6.82-6.79 (m, 2H, CH_{arom}); 5.41 (d, 1H, *J* = 2.0 Hz, H-1); 5.15 (d, 1H, *J* = 1.6 Hz, H-1'); 5.02 (d, 1H, *J* = 11.6 Hz, PhC*H*H); 4.89-4.82 (m, 3H, PhC*H*H, PhC*H*H, PhCH*H*); 4.66-4.59 (m, 2H, PhCH*H*, PhCH*H*); 4.40 (d, 1H, *J* = 6.8 Hz, H-1''); 4.19 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2); 3.79-3.72 (m, 4H, H-2', H-3, H-5', 2''-O*H*); 3.70-3.51 (m, 17H, H-2'', H-3', H-3'', H-4'', H-5, H-5'', H-6'', OCH_3); 3.42-3.38 (m, 2H, H-4, H-4'); 3.35 (s, 3H, OCH_3); 1.36 (d, 3H, *J* = 6.4 Hz, H-6'); 1.25 (d, 3H, *J* = 6.4 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 156.0, 139.0 ($C_{q,arom}$); 138.4 (CH_{arom}); 138.4 ($C_{q,arom}$); 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.6, 118.6 (CH_{arom}); 105.7 (C-1''); 98.8 (C-1'); 96.9 (C-1); 84.8 (CI_{arom}); 84.8 (C-4''); 81.7 (C-3''); 81.6 (C-3); 80.3 (C-3'); 80.0 (C-4'); 77.1 (C-2''); 76.2 (C-4); 75.8 (C-2'); 75.2 (C-5''); 75.2, 75.2, 75.0 (PhCH_2); 73.7 (C-2); 71.3 (C-6''); 68.7 (C-5'); 68.4 (C-5); 59.5, 59.1, 58.3, 56.7 (OCH_3); 18.2 (C-6'); 17.7 (C-6). IR (thin film, cm^{-1}): 1053, 1070, 1089, 1119, 1232, 1454, 1484, 2928, 3454. HRMS calculated for $C_{49}H_{61}IO_{14}Na$ 1023.3004 $[M+Na]^+$; found 1023.2998.

Methyl 6-(4-(2-*O*-(2,3-di-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-4-*O*-benzyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranosyl)phenylhex-5-ynoate (18a)



Compound **17a** (67 mg, 72 µmol, 1.0 eq) was dissolved in freshly distilled NEt_3 (2 mL, 0.04 M) together with methyl hex-5-ynoate (28 µL, 0.22 mmol, 3.0 eq). A cocktail of $Pd(PPh_3)_2Cl_2$ (28 mg), PPh_3 (22 mg) and CuI (15 mg) in freshly distilled NEt_3 was stirred for 15 minutes at 40 °C . Of this cocktail 0.18 mL was added to the reaction mixture, amounting to 0.1 eq $Pd(PPh_3)_2Cl_2$, 0.2 eq PPh_3 and 0.2 eq CuI. The reaction was left to stir overnight at 40 °C after which it was diluted with Et_2O and washed with 1 M HCl, sat. aq. $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (DCM-EtOAc 6:4) gave the title compound (66 mg, 72 µmol, 99%) as a yellow oil. $[\alpha]_D^{25}$ -56.0 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.36-7.26 (m, 12H, C*H*$_{arom}$); 6.95-6.92 (m, 2H, C*H*$_{arom}$); 5.44 (d, 1H, *J* = 1.6 Hz, H-1); 5.15 (d, 1H, *J* = 1.6 Hz, H-1'); 4.90-4.83 (m, 2H, PhC*H*H, PhC*H*H); 4.66-4.60 (m, 2H, PhCH*H*, PhCH*H*); 4.38 (d, 1H, *J* = 7.6 Hz, H-1''); 4.19 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2); 3.90 (bs, 1H, 2''-O*H*); 3.79-3.70 (m, 3H, H-2', H-3, H-5); 3.69-3.66 (m, 9H, H-3', H-5', H-5'', OC*H*$_3$, COOC*H*$_3$); 3.64-3.59 (m, 2H, H-6''); 3.56-3.50 (m, 10H, H-4'', OC*H*$_3$); 3.46-3.37 (m, 6H, H-2'', H-4, H-4', OC*H*$_3$); 3.36 (s, 3H, OC*H*$_3$); 3.29 (t, 1H, *J* = 8.6 Hz, H-3''); 2.53-2.45 (m, 4H, C*H*$_{2,linker}$, C*H*$_{2,linker}$); 1.92 (quint, 2H, *J* = 7.2 Hz, C*H*$_{2,linker}$); 1.36 (d, 3H, *J* = 6.0 Hz, H-6'); 1.24 (d, 3H, *J* = 6.4 Hz, H-6). ^{13}C-APT NMR (101 Mz) δ: 173.8 (*C*OOCH$_3$); 155.6, 138.4, 138.4 (C$_{q,arom}$); 133.0, 128.7, 128.5, 128.5, 128.1, 128.1, 127.9 (*C*H$_{arom}$); 117.6 (C$_{q,arom}$); 116.1 (*C*H$_{arom}$); 105.8 (C-1''); 98.8 (C-1'); 87.8 (C$_{q,alkyne}$); 86.4 (C-3''); 81.8 (C-3); 81.6 (C-5''); 81.0 (C$_{q,alkyne}$); 80.3 (C-3'); 80.1 (C-2''); 77.3 (C-4''); 75.8 (C-2'); 75.6 (C-4); 75.2 (C-4'); 75.2, 75.0 (Ph*C*H$_2$); 73.7 (C-2); 71.3 (C-6''); 68.7 (C-5'); 68.4 (C-5); 61.0, 59.5, 59.1, 58.3, 56.7 (O*C*H$_3$); 51.7 (COO*C*H$_3$); 33.0, 24.0, 19.0 (*C*H$_{2,linker}$); 18.1 (C-6'); 17.7 (C-6). IR (thin film, cm^{-1}): 1005, 1016, 1030, 1053, 1070, 1088, 1120, 1140, 1233, 1507, 1739, 2930, 3420. HRMS calculated for $C_{50}H_{66}O_{16}Na$ 945.4249 $[M+Na]^+$; found 945.4244.

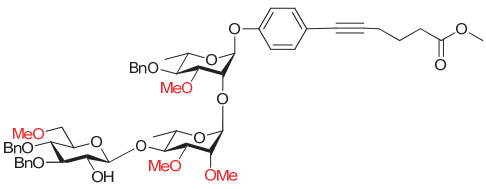
Methyl 6-(4-(2-*O*-(3-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-4-*O*-benzyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranosyl)phenylhex-5-ynoate (18b)



Compound **17b** (61 mg, 67 µmol, 1.0 eq) was dissolved in freshly distilled NEt_3 (1 mL, 0.07 M) together with methyl hex-5-ynoate (28 µL, 0.20 mmol, 3.0 eq). A cocktail of $Pd(PPh_3)_2Cl_2$ (14 mg), PPh_3 (11 mg) and CuI (7 mg) in freshly distilled NEt_3 was stirred for 15 minutes at 40 °C . Of this cocktail 0.34 mL was added to the reaction mixture, amounting to 0.1 eq $Pd(PPh_3)_2Cl_2$, 0.2 eq PPh_3 and 0.2 eq CuI. The reaction was left to stir overnight at 40 °C after which it was diluted with Et_2O, filtered over celite and washed with 1 M HCl, sat. aq. $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (DCM-EtOAc 7:3) gave the title compound (47 mg, 52 µmol, 77%) as a yellow oil. $[\alpha]_D^{25}$ -60.5 (c = 1.0, $CHCl_3$). ^{1}H-

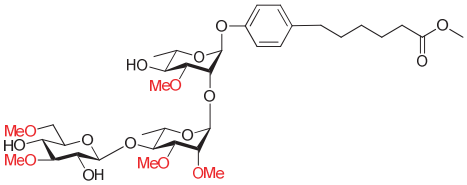
NMR (400 MHz) δ: 7.36-7.26 (m, 12H, C*H*$_{arom}$); 6.93 (dd, 2H, *J* = 2.2, 7.0 Hz, C*H*$_{arom}$); 5.45 (d, 1H, *J* = 2.0 Hz, H-1); 5.14 (d, 1H, *J* = 1.6 Hz, H-1'); 4.89-4.84 (m, 2H, PhC*H*H, PhC*H*H); 4.64-4.61 (m, 2H, PhCH*H*, PhCH*H*); 4.35 (d, 1H, *J* = 7.6 Hz, H-1''); 4.25 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2'); 4.20 (dd, 1H, *J* = 2.4, 2.8 Hz, H-2); 3.88-3.80 (m, 1H, H-5'); 3.78 (dd, 1H, *J* = 3.0, 9.4 Hz, H-3); 3.71-3.60 (m, 11H, H-3', H-4', H-5, H-6'', COOC*H*$_3$, OC*H*$_3$); 3.54-3.50 (m, 7H, H-4'', OC*H*$_3$); 3.47-3.36 (m, 6H, H-2'', H-4, H-5'', OC*H*$_3$); 3.30 (t, 1H, *J* = 9.0 Hz, H-3''); 2.51 (t, 2H, *J* = 7.4 Hz, C*H*$_{2,linker}$); 2.47 (t, 2H, *J* = 6.8 Hz, C*H*$_{2,linker}$); 1.92 (quint, 2H, *J* = 7.2 Hz, C*H*$_{2,linker}$); 1.35 (d, 3H, *J* = 6.0 Hz, H-6'); 1.24 (d, 3H, *J* = 6.4 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 173.8 (*C*OOCH$_3$); 155.6, 138.5, 138.4 (C$_{q,arom}$); 133.1, 128.6, 128.5, 128.1, 128.1, 128.0, 127.9 (*C*H$_{arom}$); 117.6 (C$_{q,arom}$); 116.2 (*C*H$_{arom}$); 105.7 (C-1''); 100.8 (C-1'); 96.8 (C-1); 87.9 (C$_{q,alkyne}$); 86.3 (C-3''); 81.6 (C-3); 81.2 (C-4'); 81.1 (C$_{q,alkyne}$); 80.7 (C-3'); 80.1 (C-4); 77.4 (C-4''); 75.6 (C-2''); 75.3 (C-5''); 75.0, 75.0 (Ph*C*H$_2$); 73.6 (C-2); 71.3 (C-6''); 68.7 (C-5); 67.9 (C-5'); 66.9 (C-2'); 61.1, 59.5, 58.2, 57.0 (O*C*H$_3$); 51.8 (COO*C*H$_3$); 33.1, 24.1, 19.0 (*C*H$_{2,linker}$); 18.2 (C-6); 17.6 (C-6'). IR (thin film, cm^{-1}): 1049, 1069, 1139, 1233, 1454, 1508, 1560, 1736, 2923, 3464. HRMS calculated for $C_{49}H_{64}O_{16}Na$ 931.4092 [M+Na]$^+$; found 931.4087.

Methyl 6-(4-(2-*O*-(2,3-di-*O*-methyl-4-*O*-(3,4-di-*O*-benzyl-6-*O*-methyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-4-*O*-benzyl-α-L-rhamnopyranosyl)phenylhex-5-ynoate (18c)



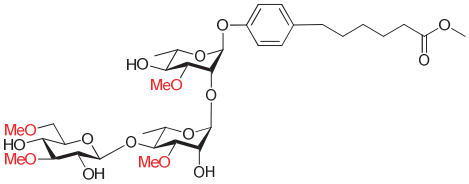
Compound **17c** (85 mg, 85 µmol, 1.0 eq) was dissolved in freshly distilled NEt$_3$ (1.79 mL, 0.05 M) together with methyl hex-5-ynoate (33 µL, 0.26 mmol, 3.0 eq). A cocktail of Pd(PPh$_3$)$_2$Cl$_2$ (28 mg), PPh$_3$ (22 mg) and CuI (15 mg) in freshly distilled NEt$_3$ was stirred for 15 minutes at 40 °C . Of this cocktail 0.21 mL was added to the reaction mixture, amounting to 0.1 eq Pd(PPh$_3$)$_2$Cl$_2$, 0.2 eq PPh$_3$ and 0.2 eq CuI. The reaction was left to stir overnight at 40 °C after which it was diluted with Et$_2$O and washed with 1 M HCl, sat. aq. NaHCO$_3$ and brine. The organic layer was dried with MgSO$_4$ and concentrated *in vacuo*. Purification by means of column chromatography (DCM-EtOAc 7:3) gave the title compound (70 mg, 70 µmol, 82%) as a yellow oil. [α]$_D^{25}$ -67.5 (c = 1.0, CHCl$_3$). ^{1}H-NMR (400 MHz) δ: 7.42-7.26 (m, 17H, C*H*$_{arom}$); 6.93 (dd, 2H, *J* = 2.0, 6.8 Hz, C*H*$_{arom}$); 5.44 (d, 1H, *J* = 2.0 Hz, H-1); 5.16 (d, 1H, *J* = 1.6 Hz, H-1'); 5.02 (d, 1H, *J* = 11.6 Hz, PhC*H*H); 4.90-4.82 (m, 3H, PhCH*H*, PhC*H*H, PhC*H*H); 4.66-4.59 (m, 2H, PhCH*H*, PhCH*H*); 4.40 (d, 1H, *J* = 6.8 Hz, H-1''); 4.19 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2); 3.83-3.65 (m, 4H, H-2', H-3, H-5', 2''-O*H*); 3.62-3.53 (m, 20H, H-2'', H-3', H-3'', H-4, H-4'', H-5, H-6'', OC*H*$_3$); 3.42-3.38 (m, 2H, H-4', H-5''); 3.35 (s, 3H, OC*H*$_3$); 2.53-2.45 (m, 4H, C*H*$_{2,Linker}$); 1.95-1.90 (m, 2H, C*H*$_{2,Linker}$); 1.36 (d, 3H, *J* = 6.0 Hz, H-6'); 1.25 (d, 3H, *J* = 6.4 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 173.8 (*C*OOCH$_3$); 155.6, 139.1, 138.5, 138.4 (C$_{q,arom}$); 133.1, 128.5, 128.4, 128.1, 128.0, 127.9, 127.9, 127.6 (*C*H$_{arom}$); 117.6 (C$_{q,arom}$); 116.1 (*C*H$_{arom}$); 105.7 (C-1''); 96.8 (C-1'); 96.8 (C-1); 87.9 (C$_{q,alkyne}$); 84.8 (C-4''); 81.7 (C-3); 81.6 (C-3'); 81.1 (C$_{q,alkyne}$); 80.4 (C-3''); 80.1 (C-4'); 77.3 (C-2''); 76.2 (C-4); 75.9 (C-2'); 75.3 (C-5''); 75.2, 75.2, 75.0 (Ph*C*H$_2$); 73.8 (C-2); 71.4 (C-6''); 68.7 (C-5); 68.4 (C-5'); 59.5, 59.1, 58.3, 56.7 (O*C*H$_3$); 51.7 (COO*C*H$_3$); 33.0, 24.1, 19.0 (*C*H$_{2,Linker}$); 18.2 (C-6); 17.8 (C-6'). IR (thin film, cm^{-1}): 1000, 1055, 1070, 1120, 1203, 1233, 1286, 1454, 1507, 1605, 1736, 2932, 3453. HRMS calculated for $C_{56}H_{70}O_{16}Na$ 1021.4562 [M+Na]$^+$; found 1021.4556.

Methyl 6-(4-(2-*O*-(2,3-di-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-α-L-rhamnopyranosyl)phenylhexanoate (19a)



Compound **18a** (66 mg, 72 µmol, 1.0 eq) was dissolved in a mixture of THF and MeOH (1:1, 3 mL, 0.03 M) and the solution was purged with N_2. Palladium on carbon (10%, 15 mg, 14 µmol, 0.2 eq) was added to the solution. The solution was then purged with H_2 and stirred for 40 hours under H_2 atmosphere. The mixture was then purged with N_2, diluted with EtOAc, filtered over celite and concentrated *in vacuo*. Purification by means of column chromatography (MeOH-DCM 1:19) gave the title compound (53 mg, 72 µmol, 100%) as a pale oil. $[\alpha]_D^{25}$ -46.6 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.09 (d, 2H, *J* = 8.4 Hz, C*H*$_{arom}$); 6.97-6.94 (m, 2H, C*H*$_{arom}$); 5.43 (d, 1H, *J* = 1.6 Hz, H-1); 5.10 (d, 1H, *J* = 1.6 Hz, H-1'); 4.41 (d, 1H, *J* = 7.6 Hz, H-1''); 4.23 (dd, 1H, *J* = 2.0, 2.4 Hz, H-2); 3.90 (bs, 1H, 2''-O*H*); 3.79-3.72 (m, 3H, H-2', H-5, H-5'); 3.68-3.60 (m, 11H, H-3, H-3', H-4', H-6'', OC*H*$_3$, COOC*H*$_3$); 3.58-3.49 (m, 11H, H-4, H-4'', OC*H*$_3$); 3.48-3.38 (m, 5H, H-2'', H-5'', OC*H*$_3$); 3.17 (t, 1H, *J* = 9.0 Hz, H-3''); 2.56 (t, 2H, *J* = 7.8 Hz, C*H*$_{2,linker}$); 2.31 (t, 2H, *J* = 7.6 Hz, C*H*$_{2,linker}$); 1.70-1.57 (m, 4H, C*H*$_{2,linker}$, C*H*$_{2,linker}$); 1.40-1.27 (m, 5H, C*H*$_{2,linker}$, H-6'); 1.25 (d, 3H, *J* = 6.0 Hz, H-6). ^{13}C-APT NMR (101 MHz) δ: 174.4 (*C*OOCH$_3$); 154.3, 136.5 (C$_{q,arom}$); 129.5, 116.2 (*C*H$_{arom}$); 105.7 (C-1''); 98.5 (C-1'); 97.4 (C-1); 85.6 (C-3''); 81.7, 81.5 (C-3 and C-3'); 80.3 (C-4'); 75.8 (C-2'); 75.1, 74.2 (C-2'' and C-5''); 72.9 (C-6''); 72.2 (C-2); 71.9 (C-4); 71.2 (C-4''); 69.1, 68.4 (C-5 and C-5'); 60.7, 59.7, 59.2, 57.8, 56.7 (O*C*H$_3$); 51.6 (COO*C*H$_3$); 35.0, 34.1, 31.3, 28.8, 24.9 (*C*H$_{2,linker}$); 17.9 (C-6); 17.7 (C-6'). IR (thin film, cm^{-1}): 1009, 1067, 1120, 1201, 1228, 1454 1510, 1736, 1933, 3436. HRMS calculated for $C_{36}H_{58}O_{16}Na$ 769.3623 $[M+Na]^+$; found 769.3617.

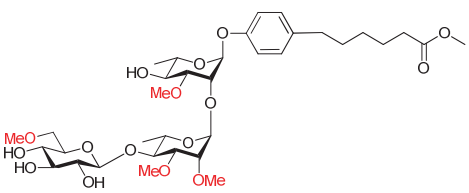
Methyl (6-(4-(2-*O*-(3-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-L-rhamnopyranosyl)phenylhexanoate (19b)



Compound **18b** (35 mg, 38 µmol, 1.0 eq) was dissolved in a mixture of THF and MeOH (1:1, 3.8 mL, 0.01 M) and the solution was purged with N_2. Palladium on carbon (10%, 8 mg, 8 µmol, 0.2 eq) was added to the solution. The solution was then purged with H_2 and stirred overnight under H_2 atmosphere. The mixture was then purged with N_2, diluted with EtOAc, filtered over celite and concentrated *in vacuo*. Purification by means of column chromatography (MeOH-DCM 3:17) gave the title compound (23 mg, 31 µmol, 82%) as a pale oil. $[\alpha]_D^{25}$ -63.0 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.09 (d, 2H, *J* = 8.8 Hz, C*H*$_{arom}$); 6.94 (dd, 2H, *J* = 2.2, 6.6 Hz, C*H*$_{arom}$); 5.45 (d, 1H, *J* = 1.6 Hz, H-1); 5.08 (d, 1H, *J* = 1.6 Hz, H-1'); 4.39 (d, 1H, *J* = 8.0 Hz, H-1''); 4.22-4.19 (m, 2H, H-2, H-2'); 3.83-3.72 (m, 2H, H-5, H-5'); 3.69-3.53 (m, 13H, H-3, H-3', H-4, H-4', H-4'', H-6'', OC*H*$_3$, COOC*H*$_3$); 3.51 (s, 6H, OC*H*$_3$); 3.45-3.39 (m, 5H, H-2'', H-5'', OC*H*$_3$); 3.18 (t, 1H, *J* = 9.0 Hz, H-3''); 2.91 (bs, 1H, O*H*); 2.56 (t, 2H, *J* = 7.8 Hz, C*H*$_{2,linker}$); 2.42 (bs, 1H, O*H*); 2.31 (t, 2H, *J* = 7.6 Hz, C*H*$_{2,lnker}$); 1.70-1.57 (m, 4H, C*H*$_{2,linker}$, C*H*$_{2,linker}$); 1.40-1.32 (m, 5H, H-6, C*H*$_{2,linker}$); 1.27 (d, 3H, *J* = 6.4 Hz, H-6'). ^{13}C-APT NMR (101 MHz) δ: 174.4 (*C*OOCH$_3$); 154.4, 136.5 (C$_{q,arom}$); 129.5, 116.2

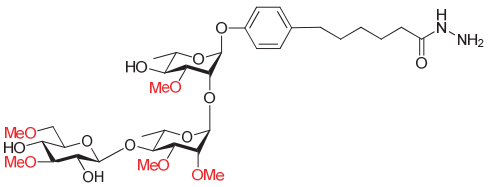
(*C*H$_{arom}$); 105.6 (C-1''); 100.7 (C-1'); 97.4 (C-1''); 85.5 (C-3''); 81.3, 81.1 (C-3 and C-3'); 75.2, 74.3 (C-2'' and C-5''); 72.9 (C-6''); 72.5 (C-2); 71.8 (C-4); 71.3 (C-4''); 69.1 (C-5); 67.9 (C-5'); 66.9 (C-2'); 60.7, 59.8, 57.7, 56.9 (O*C*H$_3$); 51.6 (COO*C*H$_3$); 35.0, 34.1, 31.4, 28.9, 24.9 (*C*H$_{2,linker}$); 17.9 (C-6); 17.6 (C-6'). IR (thin film, cm^{-1}): 1013, 1065, 1122, 1202, 1228, 1261, 1455, 1510, 1736, 2858, 2931, 3426. HRMS calculated for $C_{35}H_{56}O_{16}Na$ 755.3466 [M+Na]$^+$; found 755.3457.

Methyl 6-(4-(2-*O*-(2,3-di-*O*-methyl-4-*O*-(6-*O*-methyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-α-L-rhamnopyranosyl))phenylhexanoate (19c)



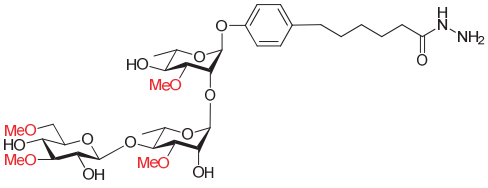
Compound **18c** (70 mg, 70 µmol, 1.0 eq) was dissolved in a mixture of THF and MeOH (1:1, 7 mL, 0.01 M) and the solution was purged with N_2. Palladium on carbon (10%, 15 mg, 14 µmol, 0.2 eq) was added to the solution. The solution was then purged with H_2 and stirred overnight under H_2 atmosphere. The mixture was then purged with N_2, diluted with EtOAc, filtered over celite and concentrated *in vacuo*. Purification by means of column chromatography (MeOH-DCM 3:17) gave the title compound (46 mg, 63 µmol, 90%) as a pale oil. $[\alpha]_D^{25}$ -51.6 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.10 (d, 2H, *J* = 8.4 Hz, C*H*$_{arom}$); 6.96-6.94 (m, 2H, C*H*$_{arom}$); 5.44 (d, 1H, *J* = 1.6 Hz, H-1); 5.10 (d, 1H, *J* = 1.6 Hz, H-1'); 4.45 (d, 1H, *J* = 7.6 Hz, H-1''); 4.22 (dd, 1H, *J* = 2.0, 2.4 Hz, H-2); 3.79-3.72 (m, 3H, H-2', H-5, H-5'); 3.70-3.61 (m, 8H, H-3, H-3', H-4, H-6'', COOC*H*$_3$); 3.58-3.48 (m, 12H, H-3'', H-4', H-5'', OC*H*$_3$); 3.45-3.34 (m, 5H, H-2'', H-4'', OC*H*$_3$); 2.56 (t, 2H, *J* = 7.8 Hz, C*H*$_{2,linker}$); 2.31 (t, 2H, *J* = 7.6 Hz, C*H*$_{2,linker}$); 1.70-1.57 (m, 4H, C*H*$_{2,linker}$, C*H*$_{2,linker}$); 1.40-1.32 (m, 5H, H-6, C*H*$_{2,linker}$); 1.27 (d, 3H, *J* = 6.0 Hz, H-6'). ^{13}C-APT NMR (101 MHz) δ: 174.4 (*C*OOCH$_3$); 154.3, 136.5 (C$_{q,arom}$); 129.5, 116.2 (*C*H$_{arom}$); 105.2 (C-1''); 98.5 (C-1'); 97.4 (C-1); 81.5, 81.3 (C-3 and C-4'); 80.2 (C-3'); 76.6 (C-3''); 75.9 (C-2'); 74.8 (C-2''); 74.3 (C-4''); 72.9 (C-6''); 72.3 (C-2); 71.9 (C-4); 71.5 (C-5''); 69.1, 68.3 (C-5 and C-5'); 59.8, 59.1, 57.8, 56.7 (O*C*H$_3$); 51.6 (COO*C*H$_3$); 35.0, 34.1, 31.3, 28.8, 24.9 (*C*H$_{2,linker}$); 17.9, 17.7 (C-6 and C-6'). IR (thin film, cm^{-1}): 1007, 1067, 1118, 1201, 1229, 1457, 1508, 1736, 2931, 3413. HRMS calculated for $C_{35}H_{56}O_{16}Na$ 755.3466 [M+Na]$^+$; found 755.3461.

6-(4-(2-*O*-(2,3-di-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-α-L-rhamnopyranosyl)phenylhexanohydrazide (20a)



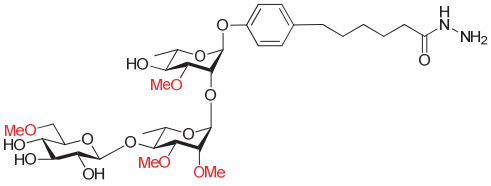
Compound **19a** (51 mg, 68 µmol, 1.0 eq) was dissolved in a mixture of EtOH and $N_2H_4 \cdot H_2O$ (1:2, 3 mL, 0.02 M) and stirred for 3 hours after which it was concentrated *in vacuo*. Purification by means of column chromatography (MeOH-DCM 1:9) gave the title compound (45 mg, 60 µmol, 82%) as a pale oil. $[\alpha]_D^{25}$ -64.5 (c = 1.0, MeOH). ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.10 (d, 2H, *J* = 8.8 Hz, C*H*$_{arom}$); 6.94-6.92 (m, 2H, C*H*$_{arom}$); 5.49 (d, 1H, *J* = 2.0 Hz, H-1); 5.09 (d, 1H, *J* = 2.0 Hz, H-1'); 4.54 (d, 1H, *J* = 8.0 Hz, H-1''); 4.22 (dd, 1H, *J* = 2.4, 2.8 Hz, H-2); 3.78-3.74 (m, 2H, H-2', H-5'); 3.68-3.54 (m, 12H, H-3, H-3', H-4', H-5, H-6'', OC*H*$_3$, COOC*H*$_3$); 3.49-3.44 (m, 7H, H-4, OC*H*$_3$); 3.37 (s, 3H, OC*H*$_3$); 3.34-3.29 (m, 2H, H-4'', H-5''); 3.19 (t, 1H, *J* = 7.6 Hz, H-2''); 3.09 (t, 1H, *J* = 8.4 Hz, H-3''); 2.55 (t, 2H, *J* = 7.6 Hz, C*H*$_{2,linker}$); 2.13 (t, 2H, *J* = 7.4 Hz, C*H*$_{2,linker}$); 1.62-1.58 (m, 4H, C*H*$_{2,linker}$); 1.32-1.27 (m, 4H, C*H*$_{2,linker}$); 1.24-1.21 (m, 6H, H-6, H-6'). ^{13}C-APT NMR (101 MHz) δ: 175.3 (*C*ONHNH$_2$); 155.9, 137.8 (C$_{q,arom}$); 130.5, 117.4 (*C*H$_{arom}$); 104.8 (C-1''); 100.4 (C-1'); 98.9 (C-1); 87.6 (C-3''); 82.1, 81.9 (C-3 and C-3'); 79.1 (C-4'); 77.7 (C-2'); 76.7 (C-5''); 76.0 (C-2); 75.4 (C-2''); 73.3 (C-4); 73.0 (C-6''); 71.2 (C-4''); 70.7 (C-5); 69.1 (C-5'); 61.0, 59.8, 59.1, 58.5, 57.5 (O*C*H$_3$); 35.8, 34.9, 32.4, 29.7, 26.7 (*C*H$_{2,linker}$); 18.3, 18.2 (C-6 and C-6'). IR (thin film, cm^{-1}): 1068, 1119, 1201, 1228, 1294, 1387, 1452, 1510, 2931, 3398. HRMS calculated for $C_{35}H_{58}N_2O_{15}Na$ 769.3735 $[M+Na]^+$; found 769.3729.

6-(4-(2-*O*-(3-*O*-methyl-4-*O*-(3,6-di-*O*-methyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-α-L-rhamnopyranosyl))phenylhexanohydrazide (20b)



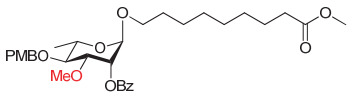
Compound **19b** (23 mg, 31 µmol, 1.0 eq) was dissolved in a mixture of EtOH and $N_2H_4 \cdot H_2O$ (1:2, 3 mL, 0.01 M) and stirred for 3 hours after which it was concentrated *in vacuo*. Purification by means of column chromatography (MeOH-DCM 1:4) gave the title compound (23 mg, 31 µmol, 100%) as a pale oil. $[\alpha]_D^{25}$ -52.9 (c = 1.0, MeOH). ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.10 (d, 2H, *J* = 8.4 Hz, C*H*$_{arom}$); 6.93 (d, 2H, *J* = 8.8 Hz, C*H*$_{arom}$); 5.50 (d, 1H, *J* = 1.6 Hz, H-1); 4.98 (d, 1H, *J* = 1.6 Hz, H-1'); 4.56 (d, 1H, *J* = 8.0 Hz, H-1''); 4.20 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2); 4.13 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2'); 3.80-3.76 (m, 1H, H-5'); 3.70-3.45 (m, 15H, H-3, H-3', H-4, H-5, H-6'', OC*H*$_3$); 3.37-3.29 (m, 5, H-4'', H-5'', OC*H*$_3$); 3.20 (dd, 1H, *J* = 7.8, 9.0 Hz, H-2''); 3.07 (t, 1H, *J* = 8.2 Hz, H-3''); 2.55 (t, 2H, *J* = 7.6 Hz, C*H*$_{2,linker}$); 2.13 (t, 2H, *J* = 7.4 Hz, C*H*$_{2,linker}$); 1.64-1.58 (m, 4H, C*H*$_{2,linker}$); 1.39-1.21 (m, 8H, H-6, H-6', C*H*$_{2,linker}$). ^{13}C-APT NMR (101 MHz) δ: 175.4 (*C*ONHNH$_2$); 155.9, 137.8 (C$_{q,arom}$); 130.5, 117.4 (*C*H$_{arom}$); 105.0 (C-1''); 103.5 (C-1'); 98.9 (C-1); 87.6 (C-3''); 82.1 (C-3'); 81.7 (C-3); 79.1 (C-4'); 76.8 (C-5''); 75.7 (C-2); 75.5 (C-2''); 73.2 (C-4); 73.0 (C-6''); 71.2 (C-4''); 70.7 (C-5); 69.1 (C-5'); 67.8 (C-2'); 60.9, 59.8, 58.3, 57.0 (O*C*H$_3$); 35.8, 34.9, 32.4, 29.7, 26.7 (*C*H$_{2,linker}$); 18.2 (C-6); 18.2 (C-6'). IR (thin film, cm^{-1}): 1017, 1063, 1116, 1228, 1248, 1268, 1454, 1510, 1637, 2926, 3386. HRMS calculated for $C_{34}H_{56}N_2O_{15}Na$ 755.3578 $[M+Na]^+$; found 733.3754.

6-(4-(2-*O*-(2,3-di-*O*-methyl-4-*O*-(6-*O*-methyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)-3-*O*-methyl-α-L-rhamnopyranosyl))phenylhexano-hydrazide (20c)



Compound **19c** (46 mg, 63 µmol, 1.0 eq) was dissolved in a mixture of EtOH and $N_2H_4 \cdot H_2O$ (1:2, 3 mL, 0.02 M) and stirred for 3 hours after which it was concentrated *in vacuo*. Purification by means of column chromatography (MeOH-DCM 1:4) gave the title compound (39 mg, 53 µmol, 84%) as a pale oil. $[\alpha]_D^{25}$ -42.9 (c = 1.0, MeOH). ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.10 (d, 2H, *J* = 8.8 Hz, C*H*$_{arom}$); 6.95-6.92 (m, 2H, C*H*$_{arom}$); 5.50 (d, 1H, *J* = 1.6 Hz, H-1); 5.10 (d, 1H, *J* = 1.6 Hz, H-1'); 4.54 (d, 1H, *J* = 7.6 Hz, H-1''); 4.22 (dd, 1H, *J* = 2.0, 2.8 Hz, H-2); 3.78-3.74 (m, 2H, H-2, H-5); 3.70-3.54 (m, 9H, H-3, H-3', H-4, H-5, H-6'', OC*H*$_3$); 3.49-3.44 (m, 7H, H-4', OC*H*$_3$); 3.37-3.24 (m, 6H, H-3'', H-4'', H-5'', OC*H*$_3$); 3.15 (dd, 1H, *J* = 8.0, 8.8 Hz, H-2''); 2.55 (t, 2H, *J* = 7.6 Hz, C*H*$_{2,linker}$); 2.13 (t, 2H, *J* = 7.4 Hz, C*H*$_{2,linker}$); 1.66-1.56 (m, 4H, C*H*$_{2,linker}$, C*H*$_{2,linker}$); 1.36-1.27 (m, 2H, C*H*$_{2,linker}$); 1.25-1.21 (m, 6H, H-6, H-6'); ^{13}C-APT NMR (101 MHz) δ: 175.3 (*C*OOCH$_3$); 155.9, 137.8 (C$_{q,arom}$); 130.5, 117.4 (*C*H$_{arom}$); 104.8 (C-1''); 100.4 (C-1'); 98.9 (C-1); 82.1, 81.9 (C-3 and C-3'); 79.0 (C-4); 77.9 (C-4''); 77.7 (C-2'); 76.9 (C-3''); 76.0 (C-2); 75.5 (C-2''); 73.3 (C-4'); 73.1(C-6''); 71.7 (C-5''); 70.7 (C-5'); 69.1 (C-5); 59.8, 59.1, 58.5, 57.5 (O*C*H$_3$); 35.8, 32.4, 29.7, 26.7 (*C*H$_{2,linker}$); 18.3 (C-6); 18.2 (C-6'). IR (thin film, cm^{-1}): 1012, 1066, 1116, 1201, 1228, 1387, 1457, 1510, 1656, 1731, 2932, 3380. HRMS calculated for $C_{34}H_{57}N_2O_{15}$ 733.3759 $[M+H]^+$; found 733.37462.

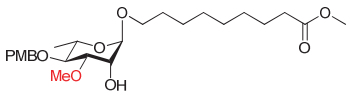
Methyl 9-(2-*O*-benzoyl-3-*O*-methyl-4-*O*-(4-methoxybenzyl)-α-L-rhamnopyranosyl)nonanoate (21)



Donor **5** (148 mg, 0.30 mmol, 1.0 eq), Ph_2SO (79 mg, 0.39 mmol, 1.3 eq) and TTBP (186 mg, 0.75 mmol, 2.5 eq) were dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges. The mixture was then dissolved in DCM (6 mL, 0.05 M) and flame-dried 3Å molecular sieves were added. The solution was then cooled to -60 °C after which Tf_2O (65 µL, 0.39 mmol, 1.3 eq) was added. After stirring for 30 minutes, methyl 9-hydroxylnonanoate[38] (282 mg, 1.5 mmol, 5.0 eq), which was also dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges, was dissolved in DCM (3.8 mL, 0.4 M) and added to the solution. After stirring for 1 hour the reaction was quenched by addition of NEt_3 (0.3 mL). The reaction mixture was then diluted with DCM, filtered over celite, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 4:1) gave the title compound (136 mg, 0.24 mmol, 79%) as a pale oil. $[\alpha]_D^{25}$ -5.6 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.11-8.09 (m, 2H, C*H*$_{arom}$); 7.58-7.56 (m, 1H, C*H*$_{arom}$); 7.48-7.44 (m, 2H, C*H*$_{arom}$); 7.31-7.26 (m, 2H, C*H*$_{arom}$); 6.90-6.86 (m, 2H, C*H*$_{arom}$); 5.52 (dd, 1H, *J* = 1.8, 3.4 Hz, H-2); 4.85-4.81 (m, 2H, H-1, PhC*H*H); 3.80-3.75 (m, 5H, H-3, H-5, OC*H*$_3$); 3.68-3.62 (m, 4H, C*H*H$_{linker}$, OC*H*$_3$); 3.48-3.37 (m, 5H, H-4, CH*H*$_{linker}$, OC*H*$_3$); 2.30 (t, 2H, *J* = 7.4 Hz, C*H*$_{2,linker}$); 1.64-1.53 (m, 4H, C*H*$_{2,linker}$); 1.35-1.26 (m, 12H, H-6, C*H*$_{2,linker}$). ^{13}C-APT NMR (101 MHz) δ: 174.4 (*C*OOCH$_3$); 165.9 (*C*O$_{Bz}$); 159.4 (C$_{q,arom}$); 133.3 (*C*H$_{arom}$); 130.8, 130.1 (C$_{q,arom}$); 130.0, 129.9, 128.5, 113.9 (*C*H$_{arom}$); 97.7 (C-1); 80.4 (C-4); 79.9 (C-3); 75.1 (Ph*C*H$_2$); 69.3 (C-2); 68.0 (O*C*H$_{2,linker}$); 67.6 (C-5); 57.5 (O*C*H$_3$); 55.4 (*C*H$_{3,PMB}$); 34.2, 29.5, 29.3, 29.2, 29.2, 26.2, 25.0 (*C*H$_{2,linker}$); 18.3 (C-6). IR (thin film, cm^{-1}): 1003, 1027, 1070, 1099,

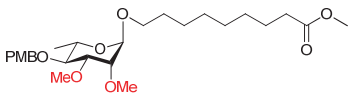
1112, 1173, 1193, 1251, 1269, 1319, 1364, 1452, 1514, 1724, 2855, 2925. HRMS calculated for $C_{32}H_{44}O_9Na$ 595.2883 $[M+Na]^+$; found 595.2879.

Methyl 9-(3-*O*-methyl-4-*O*-(4-methoxybenzyl)-α-L-rhamnopyranosyl)nonanoate (22)



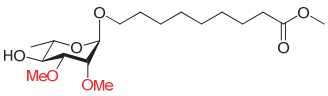
Compound **21** (264 mg, 0.46 mmol, 1.0 eq) was dissolved in THF (2.3 mL, 0.2 M). A small piece of sodium was dissolved in MeOH and 2.3 mL of this solution was added and the reaction was stirred for 2 hours. The reaction was then quenched with sat. aq. NH_4Cl and extracted with Et_2O (3x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (Et_2O) gave the title compound (210 mg, 0.45 mmol, 97%) as a pale oil. $[\alpha]_D^{25}$ -36.5 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.30-7.26 (m, 2H, C*H*$_{arom}$); 6.90-6.86 (m, 2H, C*H*$_{arom}$); 4.78 (s, 1H, H-1); 4.66 (dd, 2H, *J* = 10.6, 87.4 Hz, PhC*H*$_2$); 4.02 (dd, 1H, *J* = 1.6, 3.6 Hz, H-2); 3.81 (s, 3H, C*H*$_{3,PMB}$); 3.69-3.59 (m, 5H, H-5, OC*H*H$_{linker}$, COOC*H*$_3$); 3.56-3.51 (m, 4H, H-3, OC*H*$_3$); 3.41-3.31 (m, 2H, H-4, OCH*H*$_{linker}$); 2.41 (bs, 1H, 2-O*H*); 2.30 (t, 2H, *J* = 7.4 Hz, C*H*$_{2,linker}$); 1.61 (t, 2H, *J* = 7.2 Hz, C*H*$_{2,linker}$); 1.53 (t, 2H, *J* = 6.6 Hz, C*H*$_{2,linker}$); 1.29-1.27 (m, 11H, H-6, C*H*$_{2,linker}$). ^{13}C-APT NMR (101 MHz) δ: 174.5 (*C*OOCH$_3$); 159.4, 130.8 (C$_{q,arom}$); 129.8, 113.9 (*C*H$_{arom}$); 99.1 (C-1); 81.9 (C-3); 79.7 (C-4); 75.1 (Ph*C*H$_2$); 68.2 (C-2); 67.7 (O*C*H$_{2,linker}$); 67.2 (C-5); 57.6 (O*C*H$_3$); 55.4 (*C*H$_{3,PMB}$); 51.6 (COO*C*H$_3$); 34.2, 29.6, 29.3, 29.3, 29.2, 26.2, 25.1 (*C*H$_{2,linker}$); 18.0 (C-6). IR (thin film, cm^{-1}): 1073, 1079, 1083, 1109, 1113, 1251, 1457, 1514, 1734, 2916, 3490. HRMS calculated for $C_{25}H_{40}O_8Na$ 491.2621 $[M+Na]^+$; found 491.2615.

Methyl 9-(2,3-di-*O*-methyl-4-*O*-(4-methoxybenzyl)-α-L-rhamnopyranosyl)nonanoate (23)



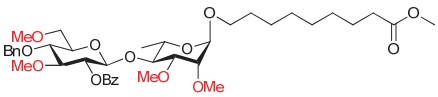
Compound **22** (105 mg, 0.22 mmol, 1.0 eq) was dissolved in dry DMF (1.5 mL, 0.15 M) and MeI (42 µL, 0.67 mmol, 3.0 eq) was added to the solution. The mixture was cooled to 0 °C and NaH (60%, 27 mg, 0.67 mmol, 3.0 eq) was then added. The reaction mixture was warmed to rt while stirring for 1 hour after which it was quenched by addition of MeOH and partitioned between water and Et_2O. The aqueous layer was extracted with Et_2O (3x) and the organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 4:6) gave the title compound (98 mg, 0.20 mmol, 91%) as a pale oil. $[\alpha]_D^{25}$ -41.5 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.30-7.27 (m, 2H, C*H*$_{arom}$); 6.89-6.86 (m, 2H, C*H*$_{arom}$); 4.83 (d, 1H, *J* = 10.4 Hz, PhC*H*H); 4.81 (s, 1H, H-1); 4.53 (d, 1H, *J* = 10.4 Hz, PhCH*H*); 3.80 (s, 3H, C*H*$_{3,PMB}$); 3.66-3.42 (m, 13H, H-2, H-3, H-5, OC*H*H$_{linker}$, COOC*H*$_3$, OC*H*$_3$); 3.40-3.34 (m, 2H, H-4, OCH*H*$_{linker}$); 2.30 (t, 2H, *J* = 7.6 Hz, C*H*$_{2,linker}$); 1.62 (t, 2H, *J* = 7.2 Hz, C*H*$_{2,linker}$); 1.54 (t, 2H, *J* = 6.4 Hz, C*H*$_{2,linker}$); 1.30-1.27 (m, 11H, H-6, C*H*$_{2,linker}$). ^{13}C-APT NMR (101 MHz) δ: 174.4 (*C*OOCH$_3$); 159.3, 131.0 (C$_{q,arom}$); 129.8, 113.9 (*C*H$_{arom}$); 96.9 (C-1); 81.7 (C-3); 80.3 (C-4); 77.7 (C-2); 75.1 (*C*H$_{3,PMB}$); 67.7 (O*C*H$_{2,linker}$); 67.7 (C-5); 59.2, 57.9 (O*C*H$_3$); 55.4 (*C*H$_{3,PMB}$); 51.6 (COO*C*H$_3$); 34.2, 29.6, 29.3, 29.3, 29.2, 26.2, 25.0 (*C*H$_{2,linker}$); 18.0 (C-6). IR (thin film, cm^{-1}): 1036, 1072, 1093, 1120, 1142, 1173, 1198, 1249, 1457, 1464, 1514, 1739, 2932. HRMS calculated for $C_{26}H_{42}O_8Na$ 505.2777 $[M+Na]^+$; found 505.2771.

Methyl 9-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)nonanoate[43] (24)



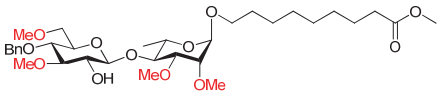
Compound **23** (98 mg, 0.20 mmol, 1.0 eq) was dissolved in a mixture of DCM and HFIP (1:1, 2 mL, 0.1 M) after which a solution of HCl in HFIP (0.1 mL, 0.2 M, 0.1 eq) was added. After complete conversion of the starting material, indicated by a dark purple colour (~2 minutes), the reaction was quenched by addition of sat. aq. $NaHCO_3$. The mixture was diluted with DCM, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 1:4) gave the title compound (70 mg, 0.19 mmol, 95%) as a pale oil. ^{1}H-NMR (400 MHz) δ: 4.85 (d, 1H, *J* = 1.2 Hz, H-1); 3.70-3.60 (m, 6H, H-2, H-5, OC*H*H$_{linker}$, COOC*H*$_3$); 3.56 (t, 1H, *J* = 9.4 Hz, H-4); 3.50 (s, 3H, OC*H*$_3$); 3.47 (s, 3H, OC*H*$_3$); 3.44-3.37 (m, 2H, H-3, OCH*H*$_{linker}$); 2.31 (t, 2H, *J* = 7.6 Hz, OC*H*$_{2,linker}$); 1.64-1.54 (m, 4H, C*H*$_{2,linker}$); 1.38-1.31 (m, 11H, H-6, C*H*$_{2,linker}$). ^{13}C-APT NMR (101 MHz) δ: 174.5 (*C*OOCH$_3$); 97.2 (C-1); 81.2 (C-3); 76.1 (C-2); 71.9 (C-4); 68.2 (C-5); 67.8 (O*C*H$_{2,linker}$); 59.1, 57.1 (O*C*H$_3$); 51.6 (COO*C*H$_3$); 34.2, 29.6, 29.4, 29.3, 29.2, 26.3, 25.1 (*C*H$_{2,linker}$); 17.8 (C-6).

Methyl 9-(2,3-di-*O*-methyl-4-O-(2-*O*-benzoyl-3,6-di-*O*-methyl-4-*O*-benzyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)nonanoate (25)



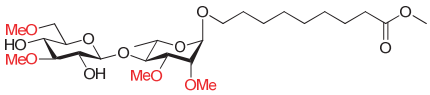
Donor **8** (230 mg, 0.47 mmol, 1.5 eq), Ph_2SO (123 mg, 0.61 mmol, 2.0 eq) and TTBP (291 mg, 1.17 mmol, 3.8 eq) were dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges. The mixture was then dissolved in DCM (9 mL, 0.05 M) and flame-dried 3Å molecular sieves were added. The solution was then cooled to -60 °C after which Tf_2O (102 µL, 0.61 mmol, 2.0 eq) was added to the solution. After stirring for 30 minutes, acceptor **24** (122 mg, 0.31 mmol, 1.0 eq), which was also dried by co-evaporation with toluene (3x) followed by 3 vacuum/nitrogen purges, was dissolved in DCM (0.75 mL, 0.4 M) and added to the solution. After stirring for 1.5 hours the reaction was quenched by addition of NEt_3 (0.3 mL). The reaction mixture was then diluted with DCM, filtered over celite, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 7:3) gave the title compound (194 mg, 0.26 mmol, 84%) as a pale oil. $[\alpha]_D^{25}$ -29.5 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 8.14-8.12 (m, 2H, C*H*$_{arom}$); 7.59-7.55 (m, 1H, C*H*$_{arom}$); 7.47-7.44 (m, 2H, C*H*$_{arom}$); 7.35-7.27 (m, 5H, C*H*$_{arom}$); 5.14 (dd, 1H, *J* = 8.0, 9.6 Hz, H-2'); 4.85-4.82 (m, 2H, H-1', PhC*H*H); 4.77 (d, 1H, *J* = 1.6 Hz, H-1); 4.66 (d, 1H, *J* = 10.8 Hz, PhCH*H*); 3.72-3.40 (m, 21H, H-2, H-3, H-3', H-4', H-5, H-5', H-6', OC*H*H$_{linker}$, COOC*H*$_3$, OC*H*$_3$); 3.34-3.27 (m, 2H, H-4, OCH*H*$_{linker}$); 3.08 (s, 3H, OC*H*$_3$); 2.30 (t, 2H, *J* = 7.4 Hz, C*H*$_{2,linker}$); 1.62 (t, 2H, *J* = 7.2 Hz, C*H*$_{2,linker}$); 1.52 (t, 2H, *J* = 6.6 Hz, C*H*$_{2,linker}$); 1.31-1.25 (m, 11H, H-6, C*H*$_{2,linker}$). ^{13}C-APT NMR (101 MHz) δ: 174.4 (*C*OOC*H*$_3$); 165.3 (*C*O$_{Bz}$); 138.4 (C$_{q,arom}$); 133.1 (*C*H$_{arom}$); 130.3 (C$_{q,arom}$); 129.9, 128.5, 128.5, 128.2, 127.9 (*C*H$_{arom}$); 101.4 (C-1'); 96.6 (C-1); 85.3 (C-3'); 80.9 (C-4); 77.8 (C-5'); 77.5 (C-3); 76.6 (C-2); 75.0 (Ph*C*H$_2$); 74.8 (C-4'); 74.3 (C-2'); 71.2 (C-6'); 67.8 (O*C*H$_{2,linker}$); 60.8, 59.8, 59.0, 56.7 (O*C*H$_3$); 51.6 (COO*C*H$_3$); 34.2, 29.8, 29.4, 29.3, 29.2, 29.2, 26.1, 25.0 (*C*H$_{2,linker}$); 18.0 (C-6). IR (thin film, cm^{-1}): 1029, 1057, 1072, 1090, 1116, 1143, 1268, 1733. HRMS calculated for $C_{40}H_{58}O_{13}Na$ 769.3775 $[M+Na]^+$; found 769.3770.

Methyl 9-(2,3-di-*O*-methyl-4-O-(3,6-di-*O*-methyl-4-*O*-benzyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)nonanoate (26)



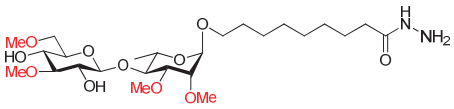
Compound **25** (116 mg, 0.155 mmol, 1.0 eq) was dissolved in THF (0.8 mL, 0.2 M). A small piece of sodium was dissolved in MeOH and 0.8 mL of this solution was added. The reaction was stirred for 2 hours after which it was quenched with sat. aq. NH_4Cl and extracted with Et_2O (3x). The organic layers were combined, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by means of column chromatography (*n*-pentane-Et_2O 3:7) gave the title compound (82 mg, 0.128 mmol, 82%) as a pale oil. $[\alpha]_D^{25}$ -21.7 (c = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz) δ: 7.34-7.27 (m, 5H, C*H*$_{arom}$); 4.86-4.82 (m, 2H, H-1, PhC*H*H); 4.61 (d, 1H, *J* = 10.8 Hz, PhCH*H*); 4.37 (d, 1H, *J* = 7.6 Hz, H-1'); 3.91 (bs, 1H, 2'-O*H*); 3.68-3.54 (m, 13H, H-2, H-3, H-4, H-5, H-6', OC*H*$_3$, COOC*H*$_3$); 3.51-3.35 (m, 12H, H-2', H-4', H-5', OC*H*$_3$); 3.28 (t, 1H, *J* = 9.0 Hz, H-3'); 2.31 (t, 2H, *J* = 7.6 Hz, C*H*$_{2,linker}$); 1.65-1.35 (m, 4H, C*H*$_{2,linker}$); 1.31-1.23 (m, 11H, H-6, C*H*$_{2,linker}$). ^{13}C-APT NMR (101 MHz) δ: 174.4 (*C*OOCH$_3$); 138.4 (C$_{q,arom}$); 128.5, 128.1, 127.9 (*C*H$_{arom}$); 105.8 (C-1'); 96.9 (C-1); 86.5 (C-3'); 82.0 (C-3); 80.7 (C-4); 77.3 (C-4'); 76.0 (C-2); 75.6 (C-2'); 75.2 (C-5'); 75.0 (Ph*C*H$_2$); 71.3 (C-6'); 67.9 (O*C*H$_{2,linker}$); 67.6 (C-5); 61.0, 59.5, 59.1 56.6 (O*C*H$_3$); 51.6 (COO*C*H$_3$); 34.2, 29.6, 29.3, 29.2, 29.2, 26.2, 25.0 (*C*H$_{2,linker}$); 17.7 (C-6). IR (thin film, cm^{-1}): 1029, 1070, 1118, 1143, 1192, 1251, 1269, 1454, 1736, 2856, 2928, 3476. HRMS calculated for $C_{33}H_{54}O_{12}Na$ 665.3513 [M+Na]$^+$; found 665.3500.

Methyl 9-(2,3-di-*O*-methyl-4-O-(3,6-di-*O*-methyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl)nonanoate[43] (27)



Compound **26** (49 mg, 76 µmol, 1.0 eq) was dissolved in THF (1.5 mL, 0.05 M) and the solution was purged with N_2. Palladium on carbon (10%, 8 mg, 7.6 µmol, 0.1 eq) was added to the solution. The solution was then purged with H_2 and stirred overnight under H_2 atmosphere. The mixture was then purged with N_2, diluted with EtOAc, filtered over celite and concentrated *in vacuo*. Purification by means of column chromatography (MeOH-DCM 1:19) gave the title compound (40 mg, 72 µmol, 95%) as a pale oil. ^{1}H NMR (400 MHz) δ: 4.82 (d, 1H, *J* = 1.2 Hz, H-1); 4.41 (d, 1H, *J* = 7.6 Hz, H-1'); 3.68-3.60 (m, 13 H, H-2, H-3, H-4, H'-5, H-6', OC*H*H$_{linker}$, OC*H*$_3$, COOC*H*$_3$); 3.55 (t, 1H, *J* = 9.2 Hz, H-4'); 3.49 (s, 3H, OC*H*$_3$); 3.47 (s, 3H, OC*H*$_3$); 3.45-3.35 (m, 6H, H-2', H-5', OCH*H*$_{linker}$); 3.17 (t, 1H, *J* = 9.0 Hz, H-3'); 2.13 (t, 2H, *J* = 7.6 Hz, C*H*$_{2,linker}$); 1.63 (t, 2H, *J* = 7.2 Hz, C*H*$_{2,linker}$); 1.55 (t, 2H, *J* = 6.4 Hz, C*H*$_{2,linker}$); 1.36-1.25 (m, 11H, H-6, C*H*$_{2,linker}$). ^{13}C-APT NMR (101 MHz) δ: 174.4 (*C*OOCH$_3$); 105.8 (C-1'); 96.9 (C-1); 85.7 (C-3'); 82.1 (C-3); 80.7 (C-4); 76.0 (C-2); 75.1, 74.2 (C-2'and C-5'); 72.9 (C-6'); 71.2 (C-4'); 67.9 (O*C*H$_{2,linker}$); 67.6 (C-5); 60.6, 59.7, 59.2, 56.5 (O*C*H$_3$); 51.6 (COO*C*H$_3$); 34.2, 29.6, 29.3, 29.2, 29.2, 26.2, 25.0 (*C*H$_{2,linker}$); 17.6 (C-6).

9-(2,3-di-*O*-methyl-4-O-(3,6-di-*O*-methyl-ß-D-glucopyranosyl)-α-L-rhamnopyranosyl nonanohydrazide[43] (28)



Compound **27** (17 mg, 31 µmol, 1.0 eq) was dissolved in a mixture of EtOH and $N_2H_4 \cdot H_2O$ (1:2, 1.5 mL, 0.02 M) and stirred for 3 hours after which it was concentrated *in vacuo*. Purification by means of column chromatography (MeOH-DCM 1:9) gave the title compound (17 mg, 31 µmol, 100%) as a pale oil. ^{1}H-NMR (400 MHz, CD_3OD) δ: 4.80 (d, 1H, *J* = 1.6 Hz, H-1); 4.53 (d, 1H, *J* = 7.6 Hz, H-1'); 3.68-3.54 (m, 9H, H-2, H-3, H-4, H-6', OC*H*H$_{linker}$, OC*H*$_3$); 3.44-3.40 (m, 7H, OCH*H*$_{linker}$, OC*H*$_3$, OC*H*$_3$); 3.37 (s, 3H, OC*H*$_3$); 3.35-3.20 (m, 2H, H-4', H-5'); 3.18 (dd, 1H, *J* = 8.0, 9.2 Hz, H-2''); 3.06 (dd, 1H, *J* = 8.4, 9.2 Hz, H-3'); 2.13 (t, 2H, *J* = 7.4 Hz, C*H*$_{2,linker}$); 1.63-1.54 (m, 4H, C*H*$_{2,linker}$); 1.39-1.21 (m, 11H, H-6, C*H*$_{2,linker}$). ^{13}C-APT NMR (101 MHz) δ: 175.3 (*C*ONHNH$_2$); 104.9 (C-1'); 98.3 (C-1); 87.6 (C-3'); 82.4 (C-3); 79.4 (C-4); 77.8 (C-2); 76.7 (C-5'); 75.5 (C-2'); 73.0 (C-6'); 71.2 (C-4'); 68.8 (O*C*H$_{2,linker}$); 68.5 (C-5); 61.0, 59.8, 59.1 (O*C*H$_3$); 35.0, 30.6, 30.3, 30.2, 29.3, 26.8 (*C*H$_{2,linker}$); 18.3 (C-6).

Study cohorts

HIV-negative, treated and untreated leprosy patients and controls were recruited on a voluntary basis at the Dept. Dermatology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands. This study was performed according to the Helsinki Declaration. All patients received treatment according to national guidelines. Ethical approval for the study was obtained from the ethical boards in The Netherlands (MEC-2012-589).

Conjugation[43]

Hydrazide (100 eq) was dissolved in DMF (0.05 M) and cooled to -30 °C (DCE bath, liquid N_2). a solution of *t*-BuONO in DMF (1:10, 400 eq) was then added, followed by HCl in dioxane (400 eq). The reaction was stirred until the starting material was converted to a higher running spot on TLC (MeOH-DCM 1:9) after which DIPEA (1000 eq) was added. The cold solution was then transferred to a 0 °C solution of BSA (1.0 eq) in borax buffer (0.1 mM, pH = 9.2) and stirred overnight while slowly warming to rt. The buffered solution was diluted (1:14) with miliQ and spun down using a 3kDa MWCO filter. The retentate was diluted to 4 mL with miliQ and transferred to a 10kDa MWCO filter. After spinning down the new retentate was purified by means of gel filtration with sephadex G-75 medium in miliQ. The product in the void volume was lyophilized and used without further purification.

Quality control for synthesized conjugates

The conjugates were analyzed with SDS-PAGE with BSA as a reference and stained with Coumassie brilliant blue to ensure no unconjugated protein was present. The amount of sugars per BSA was determined using MALDI-TOF analysis. The measurement procedure was as follows:
1 µL of sample solution (2 mg/mL in 7:3 MeCN:H_2O + 0.1% TFA) was mixed together with 1 µL of 3,5-dimethoxy-4-hydroxycinnamic acid and the dried-droplet sample preparation method was applied. Spectra were assembled from 2000 shots in the linear mode with a 1 kHz laser.

PGL-I ELISA

The PGL-I ELISA was performed as previously described.[8] Briefly, 200 ng synthetic PGL was coated per well in 50 µl in 0.1 M Na_2CO_3/$NaHCO_3$ buffer (pH 9.6) at 4 °C overnight. After blocking with 200 µl PBS/1%BSA/0.05% Tween-80 per well for 1 hour, 50 µl of 1:400 diluted sample was added and incubated for 2 hours at room temperature. Then, 50 µl per well of a 1:8000 dilution of anti-human IgM-HRP, (A6907, Sigma-Aldrich, St. Louis, Missouri, USA) in 0.05%Tween 20/PBS was incubated for 2 hours. In between each step the wells were washed 3 times with PBS/0,05% Tween-20. 50 µl of 3,3',5,5'-Tetramethylbenzidine (TMB) was added and the color reaction was stopped using H_2SO_4 after 10–15 minutes. Absorbance was determined at a wavelength of 450 nm.

ND-O-HSA

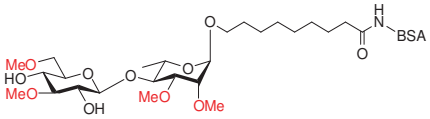
The disaccharide epitope (3,6-di-*O*-methyl-β-D-glucopyranosyl(1→4)2,3-di-*O*-methyl-α-L-rhamnopyranoside) coupled to human serum albumin (designated ND-O-HSA) was obtained through the Biodefense and Emerging Infections Research Resources Repository (https://www.beiresources.org/).

UCP-LFAs

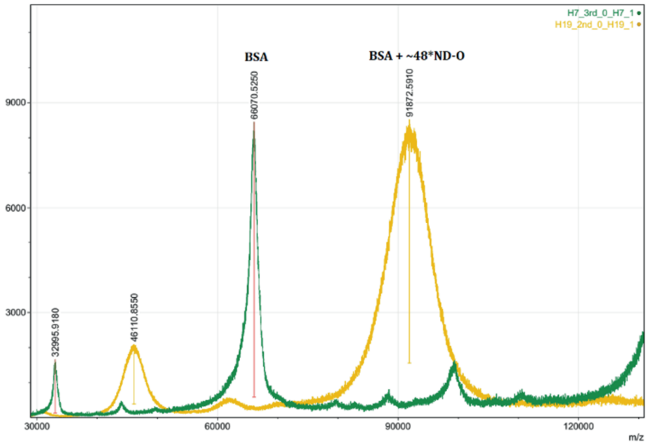
PGL-I lateral flow strips were produced as described earlier.[10] In short, the test line consists of 100 ng synthetic PGL-I and the flow control line of 100 ng Rabbit-anti-Goat (RαG; G4018, Sigma-Aldrich). Conjugates of UCP particles with goat anti-human IgM (I0759, Sigma-Aldrich, St. Louis, Missouri, USA) at a concentration of 50 µg antibody per mg UCP were applied to the sample/conjugate pad at a density of 400 ng. Samples were diluted 50-fold in high salt finger stick buffer supplemented with 1% (v/v) Triton X-100 (HSFS; 100mM Tris pH 8, 270mM NaCl, 1% (w/v) BSA). 50 µl of diluted sample was added to microtiterplate wells before LF strips were placed in the corresponding wells. Immunochromatography was allowed to continue for at least 30 min until dry. LF strips were analyzed using a UCP dedicated benchtop reader (UPCON; Labrox, Finland). Test results are displayed as an arbitrary value with Test signal normalized to the Flow-Control signal based on fluorescence units (RFUs) measured at the respective lines. Strips were stored in containers with silica dry packs at room temperature. Containers were sealed with parafilm.

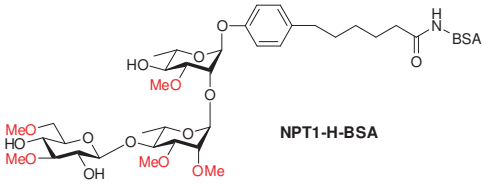
Statistical analysis

Graphpad Prism version 8.00 for Windows (GraphPad Software, San Diego CA, USA) was used to determine the correlation (R^2) between the different tests performed. The statistical significance level used was $p \leq 0.05$.

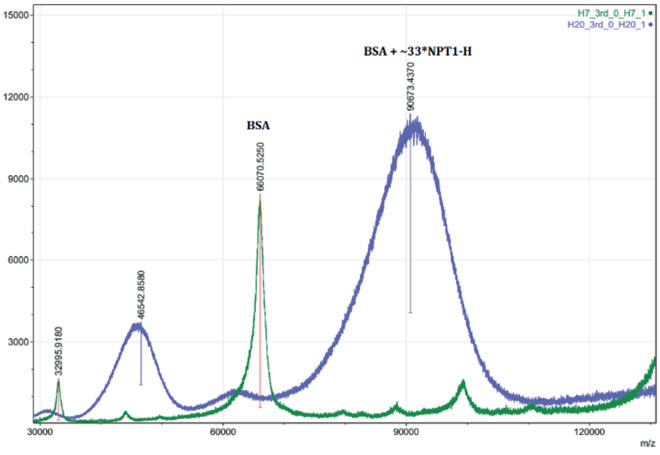


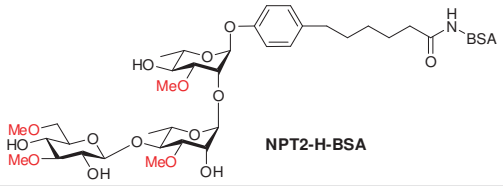
ND-O-BSA





NPT1-H-BSA

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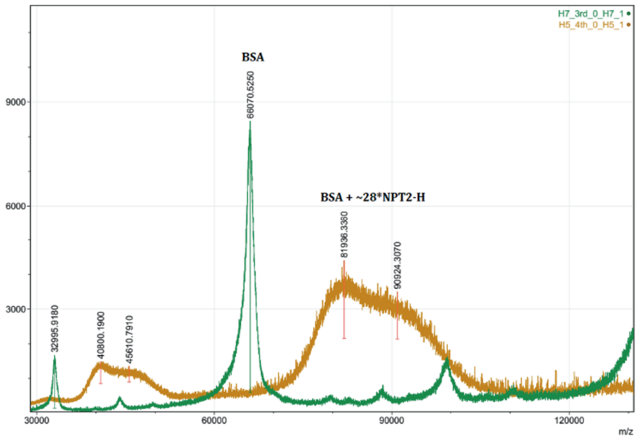
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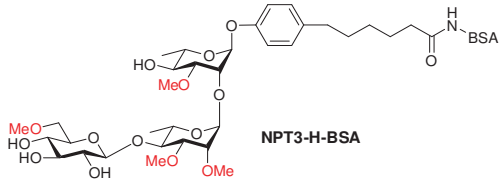
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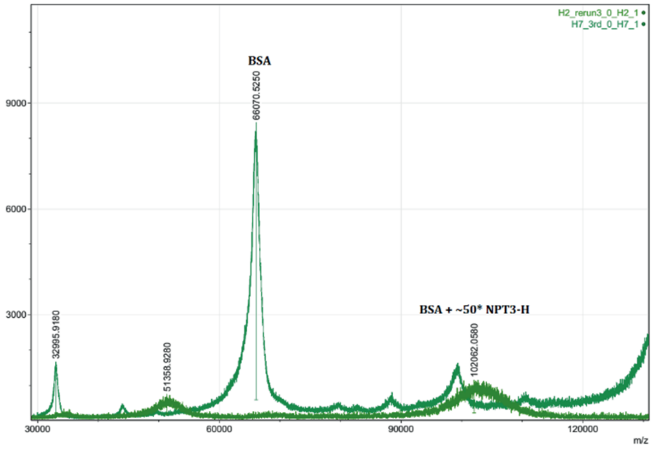
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