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Synthesis of the Core Structure of the Lipoteichoic Acid of *Streptococcus pneumoniae*

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Abstract: *Streptococcus pneumoniae* LTA is a highly complex glycophospholipid that consists of nine carbohydrate residues: three glucose, two galactosamine and two 2-acetamino-4-amino-2,4,6-trideoxygalactose (AATDgal) residues that are each differently linked, one ribitol and one diacylated glycerol (DAG) residue. Suitable building blocks for the glucose and the AATDgal residues were designed and their synthesis is described in this paper. These building blocks permitted the successful synthesis of the core structure Glcβ(1-3)AATDgalβ(1-3)Glcα(1-*O*)DAG in a suitably protected form for further chain extension (**1b**, **1c**) and as unprotected glycolipid (**1a**) that was employed in biological studies. These studies revealed that **1a** as well as **1** lead to interleukin-8 release, however not via TLR2 or TLR4 as receptor.

Keywords: carbohydrates • glycolipids • glycosidation • receptor recognition • total synthesis

Introduction

Streptococcus pneumoniae, one of the most common Gram-positive pathogens, colonizes the upper respiratory tract where it causes severe infections; life-threatening diseases like pneumonia, bacteremia, and meningitis. When it gains access to the lower respiratory tract or the bloodstream,[1] high mortality rates are often observed.[2,3]

As for all Gram-positive bacteria, the cell wall of *S. pneumoniae* consists of several layers of peptidoglycan, covalently linked to teichoic acid and of lipoteichoic acid (LTA) that is anchored in the cell membrane. Structural analysis of pneumococcal LTA of the R6 strain revealed that it contains phosphodiester interlinked pseudopentasaccharide repeating units carrying each two phosphocholine residues and a glycolipidic core structure comprising a trisaccharide linked to diacylglycerol (Scheme 1, **1**).[4] This structural analysis was confirmed by our recent total synthesis of compound **1** with $R{=}H$, $X{=}NH_3^+$ and $n{=}1$.[5] The synthesis of the glycolipid core structure **1a** and particularly of the required building blocks as well as further biological studies are reported in the present paper.

Ideal precursors for the construction of pneumococcal LTA **1** are the *O*-benzyl and *N*-benzyloxycarbonyl (Z) protected derivatives of **1a**, namely **1b** and **1c** (Scheme 1). Retrosynthesis of these compounds leads to commercially available 1,2-*O*-cyclohexylidene-*sn*-glycerol, to two glucosyl donors for constituents **A** and **C**, as for instance **2** and **4** having *O*-allyl as temporary protecting groups, and to a 2-acetamino-4-amino-2,4,6-trideoxygalactose (AATDgal) derivative for constituent **B**, as for instance **3** having temporary orthogonal protecting groups at 1-*O* (4-methoxyphenyl = MP) and at 3-*O* (allyloxycarbonyl = Alloc) to permit its use as donor and acceptor. In addition, for **3** Z-protection at the 4-amino group and trichloroethoxycarbonyl (Troc) protection at the 2-amino group are proposed; thus through anchimeric assistance β-linkage in the glycosylation step and finally chemoselective Troc group removal and *N*-acetylation without affecting the Z-protected amino group at C-4 are ensured. Other orthogonal temporary protecting group patterns for the AATDgal residue **B** were probed as well in

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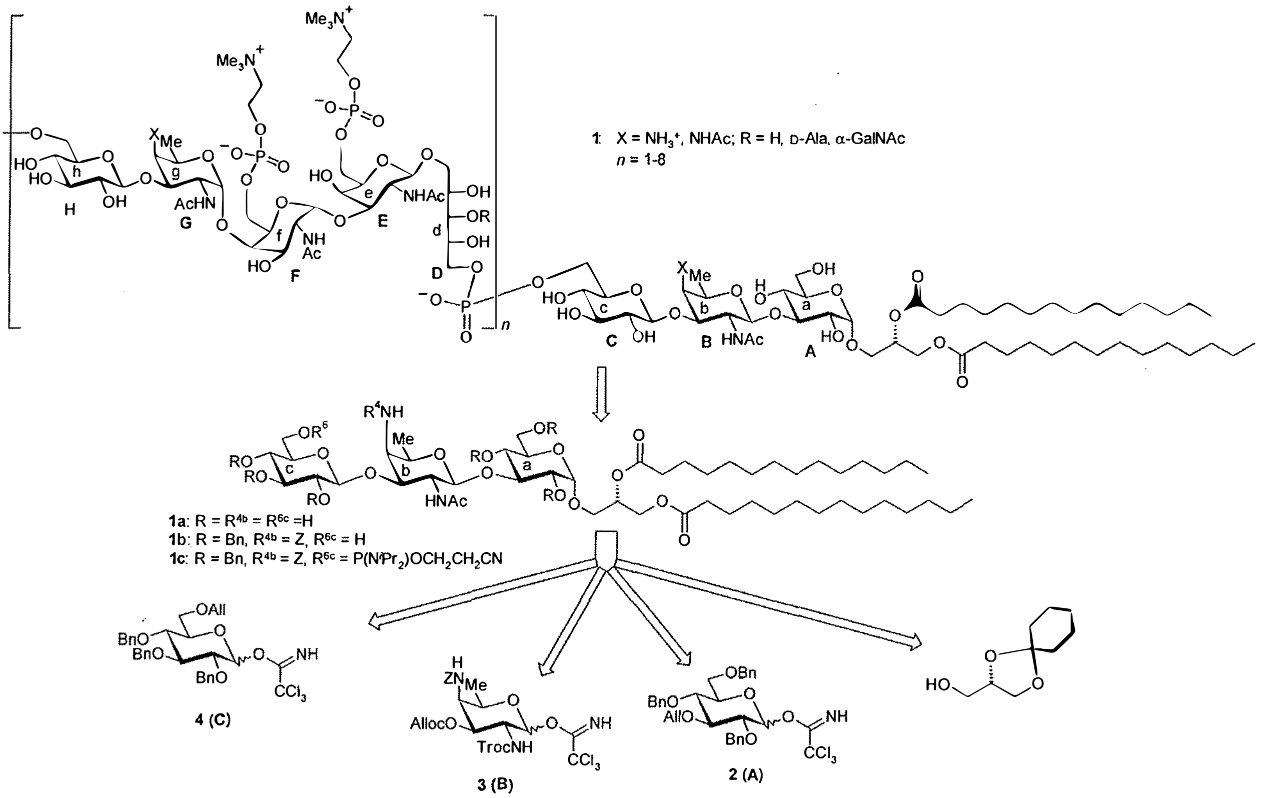


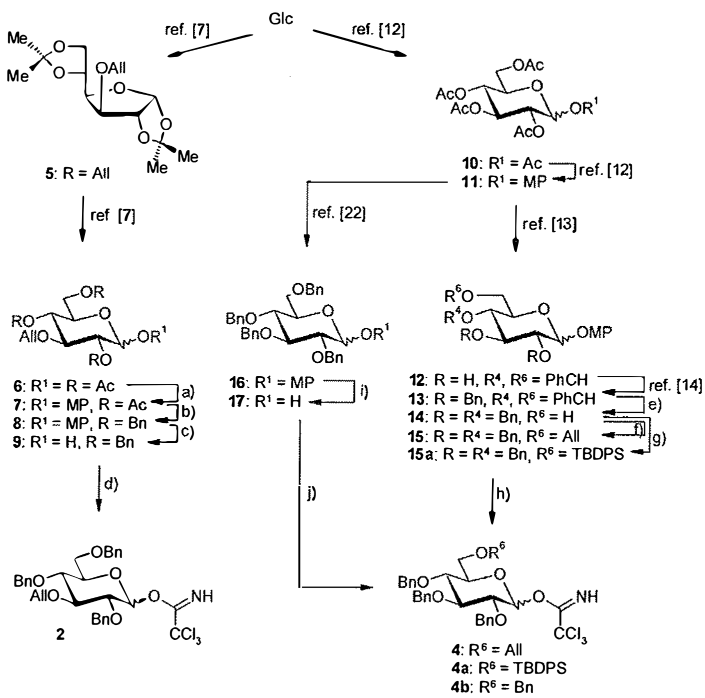
Scheme 1. Structure of the LTA of *Streptococcus pneumoniae* (**1**), the derived core structure (**1a**) and protected forms (**1b**, **1c**) for repeating unit attachment. A retrosynthetic Scheme for the synthesis of **1a–c**.

this demanding synthetic endeavour.[6] A further aim of this work was to provide building blocks for constituents **G** and **H** of the **DEFGH** repeating unit,[5] that take into account the linkage differences, particularly between **B** and **G**.

Results and Discussion

Synthesis of 1a–c: The synthesis of glucose derived building blocks **2** and **4** followed essentially published procedures (Scheme 2). Hence, glucose was transformed into 3-*O*-allyl protected furanoside **5** and then into pyranoside **6**.[7] Modifications of the procedures for the introduction of the 4-methoxyphenyl (MP) group (→ **7**),[8] *O*-deacetylation and per-*O*-benzylation (→ **8**),[9] then MP cleavage (→ **9**)[10] and finally reaction with trichloroacetonitrile in the presence of DBU as base led to trichloroacetimidate **2**[11] in excellent yield. For the synthesis of **4**, per-*O*-acetylated glucopyranose **10** was transformed into MP glucoside **11**;[12] *O*-deacetylation and 4,6-*O*-benzylidenation afforded 2,3-*O*-unprotected glucoside **12**[13] that was *O*-benzylated to give fully protected derivative **13**.[14] Reductive opening of the benzylidene ring with $H_3B{\cdot}THF$ complex in the presence of dibutylborane trifluoromethanesulfonate (triflate) as activator[15] furnished 6-*O*-unprotected compound **14**.[16] 6-*O*-Allylation with allyl bromide or silylation with *tert*-butyldiphenylsilyl (TBDPS) chloride afforded compounds **15** and **15a**,[17] respectively. Cleavage of the MP group by treatment first with *N*-bromosuccinimide (NBS) in acetone and then with aqueous $NaHCO_3$ furnished the 1-*O*-unprotected intermediates that were immediately transformed with CCl_3-CN and DBU as base into trichloroacetimidates **4**[18] and **4a**;[18–20] the 1-*O*-unprotected 6-*O*-allyl-2,3,4-tri-*O*-benzyl-D-glucopyranose intermediate could also be obtained form the corresponding methyl glucoside.[21] Compounds **4** and **4a** are useful building blocks for constituents **A** in the core structure and **H** in the repeating unit. Intermediate **11** was also transformed via per-*O*-benzylation (→ **16**),[22] MP-cleavage (→ **17**)[23] into known trichloroacetimidate **4b**[24] that is useful as building block for constituent **H** in repeating unit termination.

2-Acetamino-4-amino-2,4,6-trideoxy-D-galactopyranose (AATDgal), that is a constituent of LTA **1**, is present on the cell surface of a number of bacterial saccharides.[4,25,26] Hence, several reports on the synthesis of derivatives of this compound appeared.[27–33] However, due to a specific protecting group pattern required for the building blocks of constituents **B** and **G** of **1** a different route was designed. As discussed above, for constituent **B** glycosyl donor **3** might serve all the demands; however, for constituent **G** due to α-linkage an azido group in 2-position and a phthalimido group in 4-position will be ideal. Hence, in this synthetic endeavour a versatile intermediate was designed that serves



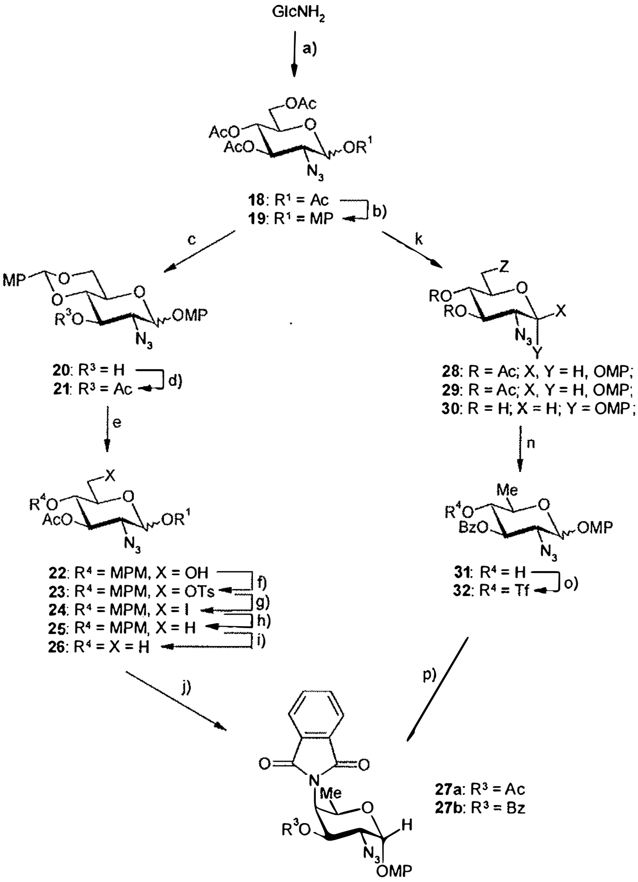
Scheme 2. Synthesis of glucosyl donors **2** and **4**, **4a**, **4b**. a) 4-Methoxyphenol, TfOH, CH_2Cl_2, 0°C (89%); b) NaOMe, MeOH; NaH, BnBr, DMF (71%); c) CAN, $MeCN/H_2O$ (69%); d) CCl_3CN, DBU, CH_2Cl_2 (87%); e) BH_3·THF, Bu_2BOTf (75%); f) NaH, All-Br, DMF (85%); g) TBDPS-Cl, imidazole, DMF (92%); h) NBS, Me_2CO, −15°C, aq. $NaHCO_3$; CCl_3CN, DBU, CH_2Cl_2 (**4**: 86%; **4a**: 98%); i) CAN, $MeCN/H_2O$ (79%); j) CCl_3CN, DBU, CH_2Cl_2 (89%).

both demands. To this end, as shown in Scheme 3, inexpensive glucosamine was selected as precursor. Transformation of glucosamine into per-*O*-acetylated azido derivative **18** followed known procedures.[34] Fischer-type glycosylation of MP-OH afforded 4-methoxyphenyl glucoside **19** as anomeric mixture (α/β ~4.5:1), that could be separated; in the following reactions mainly the α-anomer was used. Cleavage of the *O*-acetyl groups under Zemplén conditions and then treatment with 4-methoxybenzylidene dimethyl acetal in the presence of *p*-toluenesulfonic acid (*p*-TsOH) as catalyst afforded 4,6-*O*-methoxybenzylidene derivative **20**. 3-*O*-Acetylation with acetic anhydride in pyridine (→ **21**) and then reductive opening of the arylidene group with H_3B·THF as reducing agent and Bu_2BOTf as activator of the α-anomer of **21** afforded 6-*O*-unprotected 4-*O*-(4-methoxyphenylmethyl) (MPM)-protected derivative **22**. 6-*O*-Tosylation with Ts-Cl in pyridine (→ **23**), tosylate/iodide exchange with tetrabutylammonium iodide (TBAI) (→ **24**), and then treatment with sodium cyanoborohydride in DMPU as solvent at 95°C led to 6-deoxy derivative **25** in very good overall yield. Selective cleavage of the MPM group with 2,3-dichloro-5,6-dicyanoquinone (DDQ) in CH_2Cl_2/H_2O furnished 4-*O*-unprotected derivative **26** that was transformed under Mitsunobu conditions with phthalimide into the versatile 2,4-diamino-2,4,6-trideoxy-D-galactose intermediate **27a**. Alternatively and more straightforward *O*-deacetylated 2-azidoglucoside **19** could be regioselectively tosylated at 6-*O*, thus providing after reacetylation derivative **28** that gave on treatment with TBAI in refluxing acetonitrile 6-iodo derivative **29**. Following reductive deiodination as described above and then *O*-deacetylation afforded compound **30**. Regioselective 3-*O*-benzoylation with benzoyl chloride in pyridine at −30°C led to 4-*O*-unprotected **31** that was transformed with trifluoromethanesulfonyl (Tf) anhydride in pyridine into triflate intermediate **32**; following reaction with potassium phthalimide in DMF at room temperature led to **27b** in good overall yield. Thus, versatile intermediates were available for the construction of the designed building blocks for constituents **B** and **G** of **1**.

For the synthesis of the desired building block for constituent **G** cleavage of the MP group in **27a,b** was required (Scheme 4). To this end, two procedures were probed. The standard procedure, that is, treatment of **27a** with ceric(IV) ammonium nitrate (CAN) in acetonitrile at −15°C led to **33a** in good yield. Alternatively, treatment of **27b** with bis(trifluoracetoxy)iodobenzene[35] and BF_3·OEt_2 in CH_2Cl_2 was investigated, thus affording after hydrolysis **33b** in even better yield. Both compounds were readily transformed into the desired glycosyl donors **34a,b**, that were successfully employed in the total synthesis of **1**.[5]

For the synthesis of building block **3** the phthaloyl group was cleaved from **27a** or **27b** by treatment with ethylenediamine in butanol at 95°C,[36] thus liberating the amino group; then treatment with benzyloxycarbonyl chloride in the presence of $NaHCO_3$ in THF/H_2O afforded Z-protected **35**. Hydrogenation with Raney-nickel as catalyst liberated from the azido group the latent amino group; following reaction with 2,2,2-trichloroethoxycarbonyl (Troc) chloride with $NaHCO_3$ as acid scavenger gave *N*-Troc protected **38**. 3-*O*-Allyloxycarbonyl (Alloc) protection was performed with Alloc-Cl in pyridine affording compound **39**. Treatment of **39** with CAN in $MeCN/H_2O$ led to 1-*O*-unprotected derivative **40** that gave with trichloroacetonitrile in the presence of DBU as base the desired trichloroacetimidate **3** as glycosyl donor. 3-*O*-Acetylation of **35** and then MP cleavage under standard conditions provided 1-*O*-unprotected derivative **36** that was transformed into trichloroacetimidate **37**. This glycosyl donor was also probed in the construction of **CBA**-DAG intermediate **1b**.

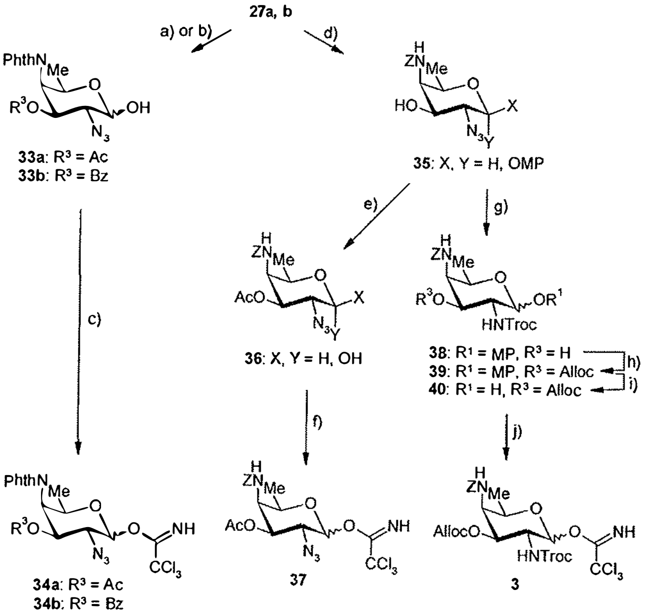
With these building blocks in hand the synthesis of the target molecule **1b** could be undertaken (Scheme 5). Glycosylation of 1,2-*O*-cyclohexylidene-*sn*-glycerol with glucosyl donor **2** in the presence of TMSOTf as catalyst at 0°C afforded a 3.2:1 anomeric mixture from which the α-anomer **41** was separated (^{1}H NMR: $J_{1,2} = 3.6$ Hz; ^{13}C NMR: δ C-1 = 97.45 ppm). Cleavage of the cyclohexylidene group with aqueous acetic acid at 80°C furnished **42** that gave with myristic acid and dicyclohexylcarbodiimide (DCC) as condensing agent diacyl glycerol (DAG) derivative **43**. 3-*O*-Deallylation with $PdCl_2$, NaOAc and aqueous acetic acid[37] afforded acceptor **44** that was available for further chain extension. Preliminary experiments on a convergent approach for the synthesis of **1b** via the disaccharide **X** obtained from **4** and



Scheme 3. Synthesis of 2,4-diamino-2,4,6-trideoxy-D-galactose intermediates **27a,b**. a) TfN$_3$; Ac$_2$O, Pyr (73%); b) 4-methoxyphenol, TfOH, CH$_2$Cl$_2$, 0°C (84%); c) NaOMe, MeOH; MeOC$_6$H$_4$-CH(OMe)$_2$, *p*-TsOH, DMF, 40°C (76%); d) Ac$_2$O, Pyr (95%); e) BH$_3$·THF, Bu$_2$BOTf, CH$_2$Cl$_2$, 0°C (76%); f) Ts-Cl, Pyr (79%); g) TBAI, MeCN, reflux (85%); h) NaBH$_3$CN, DMPU, 95°C (86%); i) DDQ, CH$_2$Cl$_2$/H$_2$O (88%); j) PPh$_3$, PhthNH, DIAD, Tol, 50°C (74%); k) NaOMe, MeOH; Ts-Cl, Pyr, Ac$_2$O, DMAP (95%); l) TBAI, MeCN, reflux (98%); m) NaBH$_3$CN, DMPU, 95%; MaOMe, MeOH (86%); n) BzCl, Pyr, −30°C (61%); o) Tf$_2$O, Pyr (crude prod.); p) PhthNK, DMF, room temperature (84%, two steps).

the α-anomer of **35** and the derived donor **Y** failed due to problems with the solubility of the starting materials and due to insufficient anomeric selectivity. Therefore, a linear synthesis of **1b** was envisaged. To this end, **44** was glycosylated first with donor **37**; this azide route furnished the desired β-linked disaccharide **45a** (^{13}C NMR: δ C-1a = 96.6; C-1b = 101.1 ppm) in acceptable yield. 3b-*O*-Deacetylation (→ **46a**) and then glycosylation with glycosyl donor **4a** in MeCN at −40°C furnished due to the nitrile effect[38] β-linkage affording trisaccharide **47a** (^{13}C NMR: δ C-1a = 96.4; C-1b = 101.1; C-1c = 104.2 ppm). Treatment of **47a** with pure thioacetic acid at 40°C led to azide reduction and liberation of the amino group and concomitant *N*-acetylation, thus affording the decisive intermediate **48a**. Alternatively, the Troc route was investigated, that is, glycosylation of acceptor **44** with glucosyl donor **3** that furnished under TMSOTf catalysis the desired β-linkage in disaccharide **45b** in almost quantitative yield (^{13}C NMR: δ = C-1a 96.8; C-1b = 101. ppm). The Alloc group in **45b** was selectively cleaved with Pd(PPh$_3$)$_4$ and sodium toluenesulfinate as nucleophile to afford acceptor **46b**. Glycosylation with glucosyl donor **4** by taking again advantage of the nitrile effect[38] at −40°C furnished mainly the desired β-anomer **47b** (α/β ~1:4.5) in high yield (^{13}C NMR: δ C-1a = 96.7; C-1b = 101.9; C-1c = 103.9 ppm). Treatment of **47b** with zinc in acetic anhydride[39] led to replacement of the Troc group by the acetyl group affording the desired intermediate **48b**. 6c-*O*-Deallylation under standard conditions provided target molecule **1b** that is a decisive intermediate in the total synthesis of **1**.[5] Hence, **1b** was transformed by treatment with bis-diisopropylaminocyanoethoxyphosphine in the presence of diisopropyl ammonium tetrazolide[40] into the phosphite derivative **1c** that was required for the chain extension in LTA total synthesis.[5] **1b** is also available for global hydrogenolytic deprotection with Pearlman's catalyst affording **CBA**-DAG **1a** that is of interest for biological studies.

The positive ion ESI FT-ICR mass spectrum of the purified glycolipid **1a** revealed abundant molecular and adduct ion peaks being in perfect agreement with the masses calculated of the target compound ($C_{51}H_{94}O_{18}N_2$), measured $[M+H]^+ =$ 1023.654 Da (calcd 1023.658 Da), measured $[M+Na]^+ =$ 1045.635 Da (calcd 1045.640 Da). For NMR spectroscopy methanol as solvent gave the best spectral resolution of **1a**. The assignment of the individual resonances was in good agreement with that obtained for **1**.[5] As **1**, **1a** showed high coupling constants ($^3J_{1,2}$ 8.5 and 7.7 Hz) of the anomeric 1^b-H and 1^c-H resonances of sugar residues **B** and **C** (β-configuration), whereas $^3J_{1,2}$ = 3.7 Hz for 1^a-H of sugar residue **A** indicated α-anomeric linkage to DAG (Table 1). In the HMQC experiment all protons could be correlated to the carbon signals allowing the determination of the substitution pattern. It is noteworthy that some, especially the anomeric signals of the ^{1}H and ^{13}C resonances showed slightly different chemical shifts when compared with **1**.[5] This can be explained by the use of two different solvent systems (methanol for **1a** and methanol/water for **1**). However, this solvent change was required in order to improve spectral resolution and to assign all signals unambiguously.



Scheme 4. Synthesis of 2,4-diamino-2,4,6-trideoxygalactose derived donors **34a,b**, **37** and **3**. a) CAN, MeCN, H_2O −15°C (75%); b) PhI-$(O_2C\text{-}CF_3)_2$, $BF_3\cdot OEt_2$, CH_2Cl_2, H_2O (87%); c) CCl_3-CN, DBU, CH_2Cl_2 (**34a**: 90%; **34b**: 72%); d) $H_2N\text{-}CH_2\text{-}CH_2\text{-}NH_2$, BuOH, 95°C (68%); Z-Cl, $NaHCO_3$, THF/H_2O (88%); e) Ac_2O, Pyr; CAN, $MeCN/H_2O$, −15°C (84%); f) CCl_3-CN, DBU, CH_2Cl_2 (87%); g) Raney-Ni, H_2, EtOH; $NaHCO_3$, Troc-Cl (91 %); h) Alloc-Cl, Pyr, (85%); i) CAN, $MeCN/H_2O$ (crude prod.); j) CCl_3-CN, DBU, CH_2Cl_2 (89%, two steps).

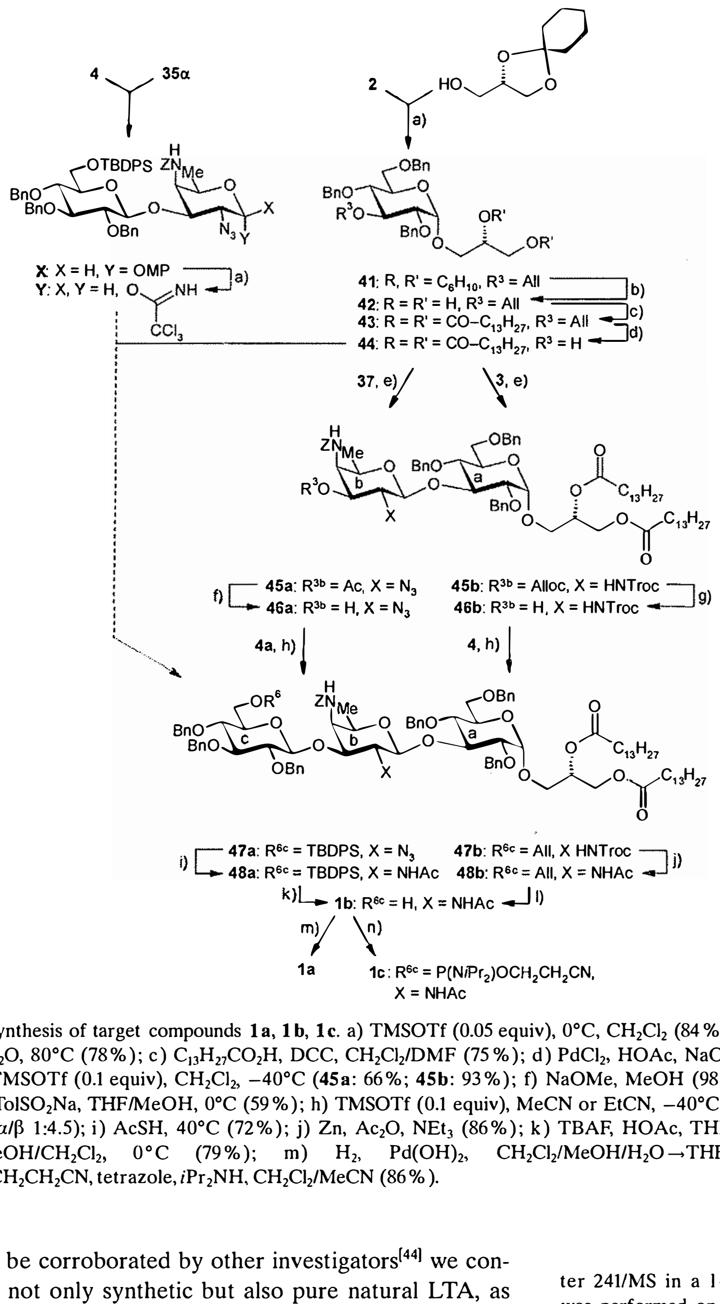
Table 1. ^1H, ^{13}C NMR chemical shift assignment for **1a**.[a]

Assignment	**C** β-Glc δ [ppm]	 J [Hz]	**B** β-AATGalNAc δ [ppm]	 J [Hz]	**A** α-Glc δ [ppm]	 J [Hz]
1-H	4.820	$J_{1,2}$ 8.5	4.387	$J_{1,2}$ 7.7	4.816	$J_{1,2}$ 3.7
2-H	3.241	$J_{2,3}$ 10.3	3.219	$J_{2,3}$ 9.3	3.529	$J_{2,3}$ 9.6
3-H	3.28[b]		3.83[b]		3.390	
4-H	3.28[b]		3.378	$J_{4,5}$ ~1.2	3.352	$J_{4,5}$ 9.7
5-H	3.678		4.00	$J_{5,6}$ 6.8	3.61	
6-H	3.67–3.63		1.32		3.71–3.69	
C-1	101.3		106.6		102.2	
C-2	75.6		55.8		73.3	
C-3	78.8		63.3		70.6	
C-4	72.1		50.5		78.3	
C-5	72.3		70.3		74.2	
C-6	63.5		22.5		63.2	
Gro				Fatty acids (14:0)		
1-H	4.241	$J_{1,2}$ 3.2, $J_{1,1'}$ 11.7		2-H	2.37	
1'-H	4.497	$J_{1',2}$ 6.5		3-H	1.64	
2-H	5.290			4-H	1.46	
3-H	3.68[b]			5- … 13-H	1.37 … 1.31	
3'-H	3.903	$J_{2,3'}$ 5.4, $J_{3,3'}$ 10.4		14-H	0.93	
C-1	64.5			C-1	n. d.	
C-2	73.6			C-2	35.7	
C-3	67.9			C-3	26.6	
				C-4	33.6	
				C-5 … -12	32 … 30.5	
				C-13	24.5	
				C-14	15.2	
NAc (C=O)	^{13}C	n.d.[c]				
NAc (CH_3)	^1H: 2.014	^{13}C: 24.92				

[a] 700.75 MHz. Homo- (^1H) and ^1H,^{13}C-heteromuclear NMR spectra (HMQC) were recorded at 300 K in [D$_4$]MeOH ($\delta_H = 3.34$ ppm, $\delta_C = 49.86$ ppm). For further details see Experimental Section. [b] Non-resolved multiplets. [c] n.d. = not determined.

Biological studies: The induction of innate immune responses by **1a** was tested in human peripheral blood cells using a whole blood assay (data not given)) and stimulation of isolated human mononuclear cells (MNCs) (Figure 2). Both tests revealed that **1a** stimulates interleukin-8 (IL-8) release. Hence, it is assumed that monocytes are the source of IL-8 producing cells because these cells are the prominent innate immune cells within the MNC preparation that are able to produce IL-8. The lipophilic part of **1a** is suggested to be responsible for the biological activity. However, the nature of the cells as well as the receptors remain to be investigated. In comparison to lipopolysaccharide (LPS) and lipopeptide (Pam$_3$C-SK$_4$) the concentration required for cell stimulation was rather high (10 μgmL^{-1}). Therefore, the presence of these bacterial cell wall products was excluded, as previously reported.[5] It was found that **1a** did not sense toll-like receptor2 (TLR2) as well as TLR4/MD2/CD14 indicating that the preparations were free of contaminating bacterial LPS and lipopeptide, respectively (Figure 1). In addition, these results clearly prove that neither TLR2 nor TLR4 are the signalling receptors of the lipophilic part of **1a**.

Although it has often been postulated that so called "highly purified" natural LTA activates pattern recognition receptors (PRRs) of the innate immune system via TLR2,[41,42] we could not obtain such TLR2-mediated activity with synthetic *Streptococcus pneumoniae* LTA **1**.[5] In addition, **1** did not show any TLR4-related activity, as investigated with transfected HEK 293-TLR4/MD-2/CD14 cells. Therefore, it was postulated that the immune-stimulatory activity of **1** is mediated by other, so far unidentified receptor(s). In the present work it is shown that also the glycolipid core structure **1a** consisting of a trisaccharide bound to the lipid anchor exhibits qualitatively and quantitatively the same biological profile as **1** and activates the release of IL-8 in MNCs (Figures 1 and 2). These results indicate that the lipid anchor and part of the attached oligosaccharide mediate the biological activities of LTA observed in cells. For **1** as well as for **1a** it was found that this activity is neither correlated to TLR2 nor to TLR4 indicating that other receptors of the innate immune system, such as the lectin pathway of the complement system, might be the most likely PRR for **1** and **1a**. Based on the biological activities observed for these two compounds it is speculated that this biological profile is identical also for bacterial 'highly purified' natural LTA preparations, free of lipopeptide contaminations, as has been isolated from lgt-mutant bacteria.[43–46] The TLR2-related activation, originally assigned to natural LTA, is rather due to minor contaminations of highly active lipoproteins of *Staphylococcus aureus* strains.[47] Since these observations



Scheme 5. Synthesis of target compounds **1a**, **1b**, **1c**. a) TMSOTf (0.05 equiv), 0°C, CH_2Cl_2 (84%, α/β 3.2:1); b) $HOAc/H_2O$, 80°C (78%); c) $C_{13}H_{27}CO_2H$, DCC, CH_2Cl_2/DMF (75%); d) $PdCl_2$, HOAc, NaOAc, AcOEt (68%); e) TMSOTf (0.1 equiv), CH_2Cl_2, −40°C (**45a**: 66%; **45b**: 93%); f) NaOMe, MeOH (98%); g) $[Pd(PPh_3)_4]$, p-$TolSO_2Na$, THF/MeOH, 0°C (59%); h) TMSOTf (0.1 equiv), MeCN or EtCN, −40°C (**47a**: 79%; **47b**: 83%, α/β 1:4.5); i) AcSH, 40°C (72%); j) Zn, Ac_2O, NEt_3 (86%); k) TBAF, HOAc, THF (79%); l) $PdCl_2$, $MeOH/CH_2Cl_2$, 0°C (79%); m) H_2, $Pd(OH)_2$, $CH_2Cl_2/MeOH/H_2O \rightarrow THF/H_2O$; n) $(iPr_2N)_2POCH_2CH_2CN$, tetrazole, iPr_2NH, CH_2Cl_2/MeCN (86%).

could also be corroborated by other investigators[44] we conclude that not only synthetic but also pure natural LTA, as can be isolated from lgt-mutant bacteria, does not activate the immune system via TLR2.

Conclusion

The core trisaccharide α-glycosidically linked to DAG **1a** was successfully synthesized. Starting from glucose the required glucose building blocks and from glucosamine versatile AATDgal building blocks were obtained. The anomeric stereocontrol in the construction of the target molecule was based on the nitrile effect for the Glcβ(1-3)AATDgal linkage, on anchimeric assistance for the AATDgalβ(1-3)Glc linkage, and on the anomeric effect for the Glcα(1-O)DAG linkage. Via **1b** the 6c-*O*-phosphitylated intermediate **1c** required for the total synthesis of **1**[5] and the totally unprotected core trisaccharide α-linked to DAG, respectively, are readily available. The biological studies with **1a** and comparison of the results with those obtained for **1** exhibited that both compounds stimulate IL-8 release, however not via TLR2. Hence, the results strongly support the view that previous reports on natural LTA-based signalling via TLR2 are due to lipopeptide contaminations.[43]

Experimental Section

General: Solvents were dried according to standard procedures. NMR spectroscopic measurements were performed at 22°C with Bruker DRX600, Bruker Avance 600 cryo, Bruker 400 Avance, Varian Mercury 300 and Bruker AC250 instruments. TMS or the resonances of the deuterated solvents were used as an internal standard. $CDCl_3$ (δ = 7.24 ppm) was used as an external standard; 85% of phosphoric acid was used as an external standard for ^{31}P spectra. MALDI mass spectra were recorded with a Kratos Kompact Maldi II spectrometer; 2,5-dihydroxybenzoic acid (DHB) or *p*-nitroaniline and NaI were used as matrices for positive measurements, and trihydroxyacetophenone (THAP) was used as the matrix for negative mode measurements. HRMS spectra were recorded with a Bruker ES-MS spectrometer. Optical rotations were measured with a Perkin–Elmer polarimeter 241/MS in a 1-dm cell at 22°C. Thin-layer chromatography (TLC) was performed on E. Merck Silica Gel 60 F_{254} plastic plates. The compounds were visualized by a treatment with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% H_2SO_4 (400 mL). Flash silica gel column chromatography was performed on J. T. Baker Silica Gel 60 (0.040–0.063 mm) at a pressure of 0.3 bar. The deprotected synthetic LTA trisaccharide **1a** (~10 mg) was purified in two steps first by hydrophobic interaction chromatography (HIC) as described.[4,5] Since TLC and NMR analysis revealed impurities still to be present after HIC, **1a** was further purified by preparative liquid chromatography (PLC) on TLC plates (silica gel 60 F_{254}, 0.25 mm, MERCK) developed with chloroform/methanol/water (100:100:30, v/v/v). The target compound **1a** (R_f = 0.55) was scrapped off and eluted from the plate to give 0.46 mg of pure **1a** suitable for NMR, MS and biological experiments.

ESI-MS analysis and NMR spectra of compound 1a: High-resolution electrospray ionization Fourier transform ion cyclotron mass spectrometry (ESI FT-ICR MS) was performed in the positive ion mode on a 7 T

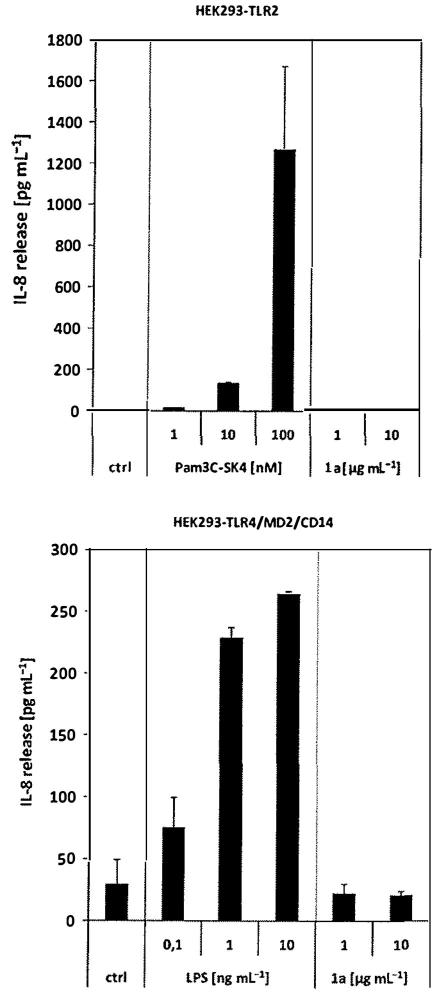


Figure 1. Induction of IL-8 release in TLR2 or TLR4/MD2/CD14 transient transfected HEK 293 cells by synthetic **1a**. The negative response in both cases shows that the **1a** preparation was free of contaminating lipopeptide and LPS, respectively.

APEX Qe (Bruker Daltonics, Billerica, USA). Samples preparation and instrumental settings were the same as described previously.[5] One- and two-dimensional homo- and heteronuclear NMR spectra were obtained with an Avance III 700 MHz spectrometer (Bruker, Rheinstetten, Germany) equipped with quadrupol-resonance cryogenic probe QXI. Purified **1a** (0.42 mg) was dissolved in 250 μL methanol ([D$_4$]MeOD, 99.96 %, Eurisotope) and transferred to a 3 mm NMR Tube (Deutero GmbH, Kastellaun). ^{1}H and ^{13}C Chemical shifts were referenced to internal methanol (δ_H 3.34, δ_C 49.86 ppm). For homo- and heteronuclear correlated 2D experiments (COSY, TOCSY, HMQC) Bruker standard software TOPSPIN (Version 2.1.1) was used to acquire and process all data.

4-Methoxyphenyl 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α,β-D-glucopyranoside (7): To a solution of **6**[7] (17.73 g, 45.65 mmol) and 4-methoxyphenol (11.33 g, 2 equiv) triflic acid (0.95 mL, 0.17 equiv) was added dropwise at 0 °C. The reaction mixture was stirred at this temperature for 3 h. Et$_3$N was added to neutralize the reaction and the solvent was removed in vacuo. Flash chromatography (petroleum ether/EtOAc 2:1) yielded **7** (15.00 g, 73 %). TLC (petroleum ether/EtOAc 2:1): R_f = 0.35. The physical data are in accordance with those reported.[8]

4-Methoxyphenyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-α,β-D-glucopyranoside (8): **7** (15.19 g, 33.57 mmol) was dissolved in MeOH and a freshly prepared NaOMe solution was added until pH 9 was reached; the reaction mixture was stirred for 2 h and then amberlite IR120 acid resin was added until neutralization. The mixture was filtered and the solvent evaporated in vacuo. The product was dried in vacuo for 2 h and the product redissolved in DMF, then BnBr (8.9 mL, 3.9 equiv) was added and the mixture cooled to 0°C. Then NaH 60% (3.5 g, 4.5 equiv) was added portionwise and the reaction mixture stirred overnight. MeOH was added to quench the reaction, the mixture was poured into water and extracted with EtOAc; the organic phase was washed with water and brine, dried over sodium sulfate and the solvent was removed in vacuo. Flash chromatography (petroleum ether/EtOAc 8:1) yielded **8** (10.97 g, 95 %). The physical data are in accordance with those reported.[9]

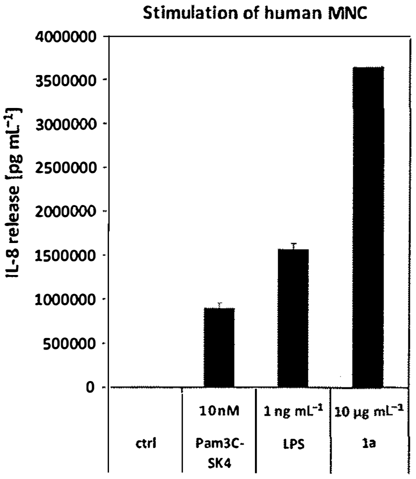


Figure 2. Induction of IL-8 release in human MNC by synthetic **1a**. After incubation for 16 h the release of IL-8 into the culture supernatant was determined by ELISA. Each result represents the mean ± SD of duplicate cultures.

3-*O*-Allyl-2,4,6-tri-*O*-benzyl-α,β-D-glucopyranose (9): To a −10°C cooled solution of **8** (10.95 g, 10.35 mmol) in acetonitrile (270 mL), CAN (20.1 g, 2.00 equiv, dissolved in 90 mL of water) was added dropwise. The reaction mixture was stirred at that temperature for 2 h and the reaction was neutralized with saturated NaHCO$_3$ solution: The mixture was extracted with EtOAc three times, the organic phase dried over sodium sulfate and the solvent removed in vacuo. Flash chromatography (petroleum ether/EtOAc 5:1) yielded **9** (6.65 g, 74 %). The physical data are in accordance with those reported.[10]

***O*-(3-*O*-Allyl-2,4,6-tri-*O*-benzyl-α,β-D-glucopyranosyl) trichloroacetimidate (2)**: To a solution of **9** (6.08 g, 12.4 mmol) in CH$_2$Cl$_2$ (220 mL), Cl$_3$CCN (24.85 mL, 20 equiv) and DBU (0.18 mL, 0.1 equiv) were added. The reaction mixture was stirred for 2 h and the solvent evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 6:1) yielded **2** (6.8 g, 88 %). The physical data are in accordance with those reported.[11]

4-Methoxyphenyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (14): A solution of **13**[14] (2.5 g, 5.08 mmol) in CH$_2$Cl$_2$ (20 mL) was cooled to 0°C and BH$_3$·THF complex (50.75 mL, 10 equiv, 1 m solution) was added dropwise and the reaction mixture stirred at 0°C for 2 h. The reaction was neutralized with Et$_3$N, then MeOH was added to quench the excess of borane. The solvent was evaporated in vacuo and the residue coevaporated several times with MeOH. Flash chromatography (petroleum ether/EtOAc 5:1) yielded **14** (1.89 g, 75 %). The physical data are in accordance with those reported.[16]

4-Methoxyphenyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (15a): To a solution of **14** (1.89 g, 3.82 mmol) in CH$_2$Cl$_2$ (15 mL), imidazole (0.39 g, 1.5 equiv) and TBDPSCl (1.09 mL, 1.1 equiv) were added and the reaction stirred for 1 h. After this time the mixture was diluted with CH$_2$Cl$_2$ and washed with water and brine; the organic phase was dried over sodium sulfate and the solvent evaporated in vacuo.

Flash chromatography (petroleum ether/EtOAc 10:1) yielded **15a** (2.57 g, 92 %). The physical data are in accordance with those reported.[17]

***O*-(6-*O*-Allyl-2,3,4-tri-*O*-benzyl-α,β-D-glucopyranosyl) trichloroacetimidate (4)**: To a cooled solution of 6-*O*-allyl-2,3,4-tri-*O*-benzyl-D-glucopyranose[17] (370 mg, 0.75 mmol) in CH_2Cl_2 (5 mL) and trichloroacetonitrile (0.4 mL, 5 equiv) was added DBU (23 mg, 0.2 equiv) and the reaction was stirred at room temperature over night. The reaction was concentrated on celite and purified by flash chromatography (EtOAc/petroleum ether, 1:10 to 1:5 + 1% Et_3N) to give **4** as colourless syrup (460 mg, 98 %). $[\alpha]_D = +86.5$ ($c = 1$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 8.38 (s, 1 H, NH), 7.50–7.28 (m, 15 H, Ar), 6.62 (d, $J = 3.5$ Hz, 1 H, 1-H), 5.95 (m, 1 H, All), 5.33 (m, 1 H, All), 5.23 (m, 1 H, All), 5.05 (d, $J = 11.0$ Hz, 1 H, Bn), 4.99 (d, $J = 10.7$ Hz, 1 H, Bn), 4.92 (d, $J = 11.0$ Hz, 1 H, Bn), 4.80 (d, $J = 11.7$ Hz, 1 H, Bn), 4.74 (d, $J = 11.7$ Hz, 1 H, Bn), 4.73 (d, $J = 10.7$ Hz, 1 H, Bn), 4.18–3.78 (m, 7H, 2-H, 3-H, 4-H, 5-H, 6-H, $-CH_2CHCH_2$), 3.71 ppm (dd, $J = 1.8$, 10.8 Hz, 1 H, 6′-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.3 (C=N), 138.7, 138.3, 138.0 (Ar), 134.5 (All), 128.5–127.6 (15 C, Ar), 117.4 (All), 94.4 (C-1), 91.4 ($-CCl_3$), 81.4, 79.4, 76.6 (C-2, C-3, C-4), 75.6 (Bn), 75.4 (Bn), 73.1 (C-5), 72.9 (Bn), 72.4 (CH_2CHCH_2), 68.1 ppm (C-6). elemental analysis calcd (%) for $C_{32}H_{34}Cl_3NO_6$ (700.2 g mol^{-1}): C 60.53, H 5.40, N 2.21; found: C 60.85, H 5.36, N 2.01.

***O*-(2,3,4-Tri-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α,β-D-glucopyranosyl) trichloroacetimidate (4a)**: To a solution of **15a** (2.5 g, 3.41 mmol) in acetone (60 mL) cooled to −15°C NBS (0.85 g, 1.4 equiv) was added and the reaction stirred in the darkness for 2 h. The reaction mixture was neutralized with saturated $NaHCO_3$ solution and extracted twice with CH_2Cl_2; the organic phase was dried over sodium sulfate and the solvent evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 5:1) yielded the pyranose (2.01 g, 86 %). The physical data are in accordance with those reported.[19] This compound was transformed into **4a** as previously described.[20]

***O*-(2,3,4,6-Tetra-*O*-benzyl-α,β-D-glucopyranosyl) trichloroacetimidate (4b)**: To a −15°C cooled solution of **16**[22] (10.0 g, 15.4 mmol) in acetonitrile (270 mL), CAN (20.34 g, 2.4 equiv dissolved in 70 mL H_2O) was added dropwise. The reaction mixture was stirred at −15°C for 3 h and the reaction quenched with saturated $NaHCO_3$ solution. The mixture was extracted three times with CH_2Cl_2, the organic phase dried over sodium sulfate and the solvent evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 2:1) yielded **17** (6.2 g, 74 %). The physical data are in accordance with those reported.[23] This compound was transformed into **4b** as previously described.[22]

4-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α,β-D-glucopyranoside (19): Compound **18**[34] (16.50 g, 44.20 mmol) was dissolved in CH_2Cl_2 (160 mL) and *p*-methoxyphenol (11.0 g, 2 equiv) was added; the mixture was cooled to 0°C and TfOH (0.92 mL, 0.17 equiv) was added dropwise; the reaction mixture was stirred for 3 h at 0 °C and another 3 h at room temperature. After this time a saturated solution of $NaHCO_3$ was added and the phases separated. The organic phase was washed with water and dried with sodium sulfate, the solvent evaporated in vacuo and the crude product purified by flash chromatography (petroleum ether/EtOAc 3:1) to yield **19** (16.2 g, 84 %). TLC (toluene/EtOAc 5:1): $R_f = 0.25$; $[\alpha]_D = +15.3$ ($c = 1$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) α-isomer: δ 7.40–6.90 (m, 4 H, Ar), 5.70 (dd, 1 H, $J_{3,2} = 10.5$, $J_{3,4} = 10$ Hz, 3-H), 5.50 (d, 1 H, $J_{1,2} = 3.5$ Hz, 1-H), 5.15 (t, 1 H, $J_{4,3} = J_{4,5} = 10$ Hz, 4-H), 4.30 (dd, 1 H, $J_{6a,6b} = 12.2$, $J_{6b,5} = 4.5$ Hz, 6a-H), 4.20 (m, 1 H, 5-H), 4.10 (dd, 1 H, $J_{6a,6b} = 12.2$, $J_{6a,5} = 2$ Hz, 6a-H′), 3.80 (s, 3 H, CH_3O), 3.50 (dd, 1 H, $J_{2,1} = 3.5$, $J_{2,3} = 10.6$ Hz, 2-H), 2.00 ppm (m, 9H, 3 × CH_3-Ac); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 460.1; found: 460.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{19}H_{23}N_3O_9$ (437.4 g mol^{-1}): C 52.17, H 5.30, N 9.61; found: C 52.03, H 5.45, N 9.76.

4-Methoxyphenyl 2-azido-2-deoxy-4,6-(4-methoxybenzylidene)-α,β-D-glucopyranoside (20): To a solution of **19** (16.1 g, 36.81 mmol) in MeOH (100 mL) NaOMe was added until pH 9. The mixture was stirred for 2 h and the amberlite IR 120 (H^+ form) was added until neutralization. The mixture was filtered and concentrated in vacuo. The crude residue was dissolved in DMF (200 mL) and anisaldehyde dimethylacetal (9.4 mL, 1.5 equiv) and *p*-TsOH (0.63 g, 0.1 equiv) were added. The reaction mixture was stirred overnight at 40°C and the mixture was poured into water and extracted twice with EtOAc. The organic phase was washed with water and brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 3:1) yielded **20** (12.05 g, 76 %).TLC (toluene/EtOAc 5:1); $R_f = 0.25$; $[\alpha]_D = +15.3$ ($c = 1$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) β-isomer: δ = 7.40–6.90 (m, 8H, Ar), 5.50 (m, 2H, 3-H, CHAr), 5.00 (d, 1 H, $J_{1,2} = 8.0$ Hz, 1-H), 4.40 (dd, 1 H, $J_{6a,6b} = 10.5$, $J_{6a,5} = 4.9$ Hz, 6a-H), 3.80 ppm (m, 11 H, 2-H. 5-H, 4-H, 6a-H, 2 × CH_3O); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 452.1; found: 452.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{21}H_{23}N_3O_7$ (429.42 gmol^{-1}): C 58.74, H 5.40, N 9.79; found: C 58.73, H 5.48, N 9.77.

4-Methoxyphenyl 3-*O*-acetyl-2-azido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α,β-D-glucopyranoside (21): Compound **20** (17.2 g, 40.1 mmol was dissolved in pyridine (180 mL) and Ac_2O (120 mL) was added. The reaction mixture was stirred for 5 h, the solvent was removed in vacuo and the residue coevaporated several times with toluene. Flash chromatography (petroleum ether/EtOAc 3:1) yielded **21** (20.7 g, 97 %). TLC (toluene/acetone 6:1): $R_f = 0.56$; $[\alpha]_D = +5.3$ ($c = 1$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) β-isomer: δ = 7.40–6.90 (m, 8 H, Ar), 5.50 (m, 2 H, 3-H, CHPh), 5.00 (d, 1 H, $J_{1.2} = 8.0$ Hz, 1-H), 4.40 (dd, 1 H, $J_{6a,6b} = 10.5$, $J_{6a,5} = 4.9$ Hz, 6a-H), 3.80 ppm (m, 11 H, 2-H, 5-H, 4-H, 6a-H, 2 × CH_3O); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 494.2; found: 494.3 $[M+Na]^+$; elemental analysis calcd (%) for $C_{23}H_{25}N_3O_8$ (471.46 gmol^{-1}): C 58.59, H 5.34, N 8.91; found: C 58.83, H 5.49, N 8.77.

4-Methoxyphenyl 3-*O*-acetyl-2-azido-2-deoxy-4-*O*-(4-methoxybenzyl)-α,β-D-glucopyranoside (22): To a solution of **21** (19.6 g, 36.7 mmol) in CH_2Cl_2 (150 mL) borane tetrahydrofuran (184 mL, 5 equiv, 1 m solution in THF) was added. The reaction mixture was cooled with an ice bath and Bu_2BOTf (36.7 mL, 1 equiv, 1 m solution in THF) was added dropwise. The reaction mixture was stirred at 0°C for 1 h and then neutralized with Et_3N. MeOH was added to quench excess borane and the solvent was evaporated in vacuo; the residue was coevaporated several times with MeOH. Flash chromatography on silica gel (petroleum ether/EtOAc 3:1) yielded **22** (14.95 g, 76 %). TLC (toluene/EtOAc 2:1): $R_f = 0.41$; $[\alpha]_D = +9.5$ ($c = 1$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ = 7.20–6.60 (m, 8 H, Ar), 6.00 (dd, 1 H, $J_{3,2} = 10.5$, $J_{3,4} = 9.1$ Hz, 3-H), 5.60 (d, 1 H, $J_{1,2} = 3.5$ Hz, 1-H), 4.60 (s, 2 H, CH_2Ar), 3.95 (m, 2 H, 5-H, 6b-H), 3.85 (m, 2H, 6a-H, 4-H), 3.80 (s, 3 H, CH_3O), 3.70 (s, 3 H, CH_3O), 3.35 (dd, 1 H, $J_{2,3} = 10.5$, $J_{1,2} = 3.5$ Hz, 2-H), 2.00 ppm (s, 3 H, CH_3CO); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 496.2; found: 496.3 $[M+Na]^+$, 512.2 $[M+K]^+$; elemental analysis calcd (%) for $C_{23}H_{27}N_3O_8$ (473.48 gmol^{-1}): C 58.34, H 5.75, N 8.87; found: C 58.41, H 5.92, N 8.77.

4-Methoxyphenyl 3-*O*-acetyl-2-azido-2-deoxy-4-*O*-(4-methoxybenzyl)-6-*O*-(4-methylbenolsulfonyl)-α,β-D-glucopyranoside (23): To a solution of **22** (13.6 g, 25.4 mmol) in pyridine (100 mL) cooled to 0°C, TsCl (9.68 g, 2 equiv dissolved in 50 mL of pyridine) was added dropwise. The reaction mixture was stirred overnight and the solvent was evaporated in vacuo. The crude material was dissolved in EtOAc and washed with 1 n HCl solution and water. The product was purified by flash chromatography (petroleum ether/EtOAc 3:1) to yield **23** (12.8 g, 79 %). TLC (toluene/EtOAc 3:1): $R_f = 0.34$; $[\alpha]_D = +7.5$ ($c = 1$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ = 7.20–6.60 (m, 12H, Ar), 6.00 (dd, 1 H, $J_{3,2} = 10.5$, $J_{3,4} = 9.1$ Hz, 3-H), 5.50 (d, 1 H, $J_{1,2} = 3.5$ Hz, 1-H), 4.50 (s, 2 H, CH_2Ar), 4.35 (m, 1 H, 6b-H), 4.20 (m, 1 H, 6a-H), 4.10 (m, 1 H, 5-H), 3.90 (t, 1 H, $J_{3,4} = 10.5$ Hz, 4-H), 3.80 (s, 3H, CH_3O), 3.70 (s, 3 H, CH_3O), 3.35 (dd, 1 H, $J_{3,2} = 10.5$, $J_{1,2} = 3.5$ Hz, 2-H), 2.50 (s, 3H, CH_3(Ts)), 2.00 ppm (s, 3H, CH_3CO); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 650.2; found: 650.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{30}H_{33}N_3O_{10}S$ (627.66 gmol^{-1}): C 57.41, H 5.30, N 6.69; found: C 57.28, H 5.12, N 6.89.

4-Methoxyphenyl 3-*O*-acetyl-2-azido-2,6-dideoxy-6-iodo-4-*O*-(4-methoxybenzyl-α,β-D-glucopyranoside (24): To a solution of **23** (12.6 g, 20.1 mmol) in acetonitrile (300 mL) TBAI (9.64 g, 1.3 equiv) was added and the reaction mixture was refluxed overnight. The solvent was evaporated in vacuo and the residue dissolved in EtOAc and washed with water twice. The organic phase was dried over sodium sulfate and the solvent evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 7:1) yielded **24** (9.93 g, 85%). TLC (toluene/EtOAc 5:1): R_f= 0.41; $[\alpha]_D$= +11.5 (c = 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.20–6.90 (m, 8H, Ar), 6.10 (dd, 1H, $J_{3,2}$=10.5, $J_{3,4}$=9.1 Hz, 3-H), 5.60 (d, 1H, $J_{1,2}$=3.5 Hz, 1-H), 4.60 (s, 2H, CH_2Ar), 3.75 (m, 8H, 2 × CH_3O, 5-H, 6b-H), 3.40 (m, 3H, 2-H, 4-H, 6a-H), 2.00 ppm (s, 3H, CH_3CO); MALDI-MS (positive mode, matrix DHB, THF): *m/z*: 606.1; found: 606.3 $[M+\mathrm{Na}]^+$; elemental analysis calcd (%) for $C_{23}H_{26}IN_3O_7$ (583.37 $\mathrm{g\,mol^{-1}}$): C 47.35, H 4.49, N 7.20; found: C 47.58, H 4.63, N 7.32.

4-Methoxyphenyl 3-*O*-acetyl-2-azido-2,6-dideoxy-4-*O*-(4-methoxybenzyl)-α,β-D-glucopyranoside (25): To a solution of **24** (9.90 g, 15.3 mmol) in DMPU (100 mL) sodium cyanoboronhydride (6.75 g, 7 equiv) was added and the reaction mixture stirred overnight at 95°C. The mixture was poured into water and extracted twice with EtOAc. The organic phase was washed with water, dried over sodium sulfate and the solvent removed in vacuo. Flash chromatography (petroleum ether/EtOAc 6:1) yielded **25** (6.85 g, 86%). TLC (toluene/EtOAc 5:1): R_f=0.38; $[\alpha]_D$= +11.1 (c = 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.20–6.90 (m, 8H, Ar), 6.00 (dd, 1H, $J_{3,2}$=10.5, $J_{3,4}$=9.1 Hz, 3-H), 5.50 (d, 1H, $J_{1,2}$= 3.5 Hz, 1-H), 4.60 (s, 2H, CH_2Ar), 4.10 (m, 1H, 5-H), 3.80 (s, 3H, CH_3O), 3.70 (s, 3H, CH_3O), 3.40 (m, 2H, 2-H, 4-H), 2.00 (s, 3H, CH_3CO), 1.30 ppm (d, 3H, $J_{6,5}$=6.6 Hz, 6-H); MALDI-MS (positive mode, matrix DHB, THF): *m/z*: 650.2; found: 650.2 $[M+\mathrm{Na}]^+$; elemental analysis calcd (%) for $C_{23}H_{27}N_3O_7$ (457.48 $\mathrm{g\,mol^{-1}}$): C 60.38, H 5.95, N 9.19; found: C 60.56, H 6.12, N 8.96.

4-Methoxyphenyl 3-*O*-acetyl-2-azido-2,6-dideoxy-α,β-D-glucopyranoside (26): To a solution of **25** (6.80 g, 14.9 mmol in CH_2Cl_2/water 20:1 (84 mL) DDQ (6.75 g, 2 equiv) was added. The reaction mixture was stirred for 2 h and saturated $NaHCO_3$ was added to quench the reaction. The organic phase was washed with water and dried over sodium sulfate. The solvent was removed in vacuo. Flash chromatography (petroleum ether/EtOAc 3:1) yielded **26** (4.4 g, 88%). TLC (toluene/EtOAc 3:1): R_f=0.29; $[\alpha]_D$= +19.2 (c= 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.20–6.90 (m, 4H, Ar), 5.70 (dd, 1H, $J_{3,2}$=10.5, $J_{3,4}$=9.1 Hz, 3-H), 5.50 (d, 1H, $J_{1,2}$=3.5 Hz,1-H), 4.10 (m, 1H, 5-H), 3.60 (s, 3H, CH_3O), 3.50 (m, 2H, 2-H, 4-H), 2.00 (s, 3H, CH_3CO), 1.20 ppm (d, 3H, $J_{6,5}$=6.6 Hz, 6-H); MALDI-MS (positive mode, matrix DHB, THF): *m/z*: 360.1; found: 360.2 $[M+\mathrm{Na}]^+$; elemental analysis calcd (%) for $C_{15}H_{19}N_3O_6$ (337.33 $\mathrm{g\,mol^{-1}}$): C 53.41, H 5.68, N 12.46; found: C 53.48, H 5.79, N 12.71.

4-Methoxyphenyl 3-*O*-acetyl-2-azido-2,4,6-trideoxy-4-phthalimido-α-D-galactopyranoside (27a): To a solution of **26** (4.3 g, 12.7 mmol), phthalimide (3.75 g, 25.4 mmol), triphenylphosphine (5.01 g, 19.1 mmol) in dry toluene (90 mL) DIAD was added and the reaction mixture stirred for 1.5 h at 60°C. The mixture was diluted with CH_2Cl_2 and washed with water. The organic phase was dried over sodium sulfate and the solvent removed in vacuo. Flash chromatography (petroleum ether/EtOAc 4:1) yielded **27a** (4.41 g, 74%). TLC (toluene/EtOAc 3:1): R_f=0.59; $[\alpha]_D$= +8.2 (c= 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.00–6.90 (m, 8H, Ar), 5.85 (dd, 1H, $J_{3,2}$=10.4, $J_{3,4}$=6.5 Hz, 3-H), 5.70 (d, 1H, $J_{1,2}$= 3.7 Hz, 1-H), 5.00 (dd, 1H, $J_{4,3}$=6.4, $J_{4,5}$=3.4 Hz, 4-H), 4.50 (m, 2H, 2-H, 5-H), 3.80 (s, 3H, CH_3O), 2.00 (s, 3H, CH_3CO), 1.10 ppm (d, 3H, $J_{6,5}$= 6.6 Hz, 6-H); MALDI-MS (positive mode, matrix DHB, THF): *m/z*: 489.1; found: 489.3 $[M+\mathrm{Na}]^+$; elemental analysis calcd (%) for $C_{23}H_{22}N_4O_7$ (466.44 $\mathrm{g\,mol^{-1}}$): C 59.22, H 4.75, N 12.01; found: C 59.46, H 5.02, N 11.83.

4-Methoxyphenyl 2-azido-3-*O*-benzoyl-2,4,6-trideoxy-4-phthalimido-α-D-galactopyranoside (27b): The mono-benzoylated sugar **31** (3.188 g, 7.98 mmol) was dissolved in CH_2Cl_2 (50 mL) containing pyridine (1 mL, 2 equiv) and cooled by and ice bath followed by addition of Tf_2O (2.0 mL, 1.5 equiv) by syringe. After 1 h the reaction was finished (TLC: petroleum ether/EtOAc 3:1) and the reaction was quenched with water and worked up by washing with HCl (1 m), water and brine followed by drying and concentration in vacuo to give the crude triflate **32** as a foam which was used directly in the next reaction without further purification. The triflate **32** was dissolved in DMF (20 mL), cooled by an ice bath followed by addition of potassium phthalimide (3.0 g, 2 equiv). The reaction mixture was allowed to reach room temperature and stirred overnight. The reaction was diluted with water and extracted with EtOAc. The organic phase was washed successively with water to remove DMF, HCl (1 m) and brine, dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography (petroleum ether/EtOAc 4:1 to 2:1) yielded **27b** (3.521 g, overall 84%) as a solid. M.p. 92–93°C; $[\alpha]_D$= +137.7 (c = 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.83 (d, 2H, J=7.5 Hz, Bz), 7.83 (m, 2H, Phth), 7.74 (brs, 1H, Phth), 7.46 (t, 1H, J=7.5 Hz, Bz), 7.28 (dd, 2H, J=7.8 Hz, Phth), 7.13 (d, 2H, J=9.2 Hz, MP), 6.87 (d, 2H, J= 9.2 Hz, MP), 6.06 (dd, 1H, J=6.4 Hz, 11.0 Hz, 3-H), 5.78 (d, 1H, J= 4.0 Hz, 1-H), 5.16 (dd, 1H, J=3.3 Hz, 6.4 Hz, 4-H), 4.79 (dd, 1H, J= 3.9 Hz, 11.0 Hz, 2-H), 4.62 (m, 1H, 5-H), 3.79 (s, 3H, OMe), 1.13 ppm (d, 3H, J=6.7 Hz, 6-H); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 168.8 (2 C, C=O), 165.2 (C=O), 155.4 (MP), 150.8 (MP), 134.6 (Ar), 133.5 (Ar), 129.8 (2 C, Ar), 129.0 (Ar), 128.5 (2 C, Ar), 123.7 (2 C, Ar), 117.8 (2 C, Ar), 114.9 (2 C, Ar, 98.2 (C-1), 68.5 (C-3), 64.7 (C-5), 58.6 (C-2), 55.8 (OMe), 52.4 (C-4), 16.6 ppm (C-6); HRMS: *m/z*: 551.1537; found: 551.1620 $[M+\mathrm{Na}]^+$; elemental analysis calcd (%) for $C_{28}H_{24}N_4O_7$ (528.5128): C 63.63, H 4.58, N 10.60; found: C 63.20, H 4.73, N 10.46.

4-Methoxyphenyl 3,4-di-*O*-acetyl-2-azido-2-deoxy-6-*O*-(4-methylbenzylsulfonyl)-α,β-D-glucopyranoside (28): To a solution of **19** (8.57 g, 19.6 mmol) in MeOH (100 mL) NaOMe was added until pH 9. The mixture was stirred for 2 h and then Amberlite IR120 (H^+ form) was added until neutralization. The mixture was filtered and concentrated in vacuo. The crude residue was dissolved in pyridine (100 mL) and cooled to 0°C, where TsCl (1.05 equiv, 20.6 mmol, 3.92 g) was added. The reaction mixture was allowed to reach room temperature and after reaction overnight Ac_2O (50 mL) was added together with DMAP (ca. 200 mg). After disappearing of intermediates from TLC the reaction was concentrated and purified by column chromatography (petroleum ether/EtOAc 5:1 to 3:1) to yield **28** (10.23 g, 95%, α/β 2:1). ^{1}H NMR (600 MHz, $CDCl_3$) α-isomer: δ = 7.67 (d, 2H, J=8.4 Hz, Ar), 7.25 (d, 2H, J=8.4 Hz, Ar), 6.91 (m, 2H, Ar), 6.75 (m, 2H, Ar), 5.55 (dd, 1H, J=9.4 Hz, 10.4 Hz, 3-H), 5.34 (d, 1H, J=3.5 Hz, 1-H), 4.99 (dd, 1H, J=9.7 Hz, 4-H), 4.98–4.12 (m, 3H, 5-H, 6-H), 3.71 (s, 3H, Me), 3.31 (dd, 1H, J=3.3 Hz, 10.6 Hz, 2-H), 2.38 (s, 3H, OMe), 2.04 (s, 3H, Ac), 1.91 ppm (s, 3H, Ac); β-isomer: 7.67 (d, 2H, J=8.4 Hz, Ts), 7.21 (d, 2H, J=8.4 Hz, Ts), 6.91 (m, 2H, MP), 6.75 (m, 2H, MP), 4.94 (dd, 1H, J=9.6 Hz, 9.6 Hz, 3-H), 4.86 (dd, 1H, J=9.7 Hz, 4-H), 4.69 (d, 1H, J=8.1 Hz, 1-H), 4.12–3.98 (m, 2H, 6-H), 3.75 (m, 1H, 5-H), 3.72 (s, 3H, Me), 3.61 (dd, 1H, J=8.1 Hz, 10.0 Hz, 2-H), 3.34 (s, 3H, OMe), 2.02 (s, 3H, Ac), 1.93 ppm (s, 3H, Ac); ^{13}C NMR (150.9 MHz, $CDCl_3$) α-isomer: δ 170.1, 169.4, 155.8, 150.1, 145.1, 132.5, 129.9, 128.1, 118.0, 114.80, 97.5, 70.2, 68.3, 67.9, 67.0, 60.6, 55.7, 21.7, 20.7, 20.5 ppm; β-isomer: δ = 169.9, 169.6, 156.0,.150.6, 145.2, 132.5, 129.9, 128.1, 118.8, 114.7, 72.1, 71.7, 68.4, 67.4, 63.4, 55.7, 21.7, 20.7, 20.5 ppm; HRMS: *m/z*: 572.1309 $[M+\mathrm{Na}]^+$; found: 572.1305; elemental analysis calcd (%) for $C_{24}H_{27}N_3O_{10}S$ (549.55 $\mathrm{g\,mol^{-1}}$): C 52.45, H 5.03, N 7.65; found: C 52.46, H 5.03, N 7.43.

4-Methoxyphenyl 3,4,di-*O*-acetyl-2-azido-2-deoxy-6-iodo-α,β-D-glucopyranoside (29): The tosylate **28** (12.24 g, 22.3 mmol) was dissolved in MeCN (50 mL) and TBAI (2.2 equiv, 52 mmol, 19.2 g) added. The reaction mixture was refluxed overnight, concentrated and purified by flash chromatography (EtOAc/petroleum ether 6:1 to 2:1) to give **29** (11.04 g, 98%). ^{1}H NMR (600 MHz, $CDCl_3$) α-isomer: δ = 7.12 (d, 2H, J= 9.1 Hz, C_6H_4), 6.86 (d, 2H, J=9.1 Hz, C_6H_4), 5.68 (dd, 1H, J= 10.7 Hz, 3-H), 5.50 (d, 1H, J=3.4 Hz, 1-H), 4.97 (dd, 1H, J=9.2, 9.7 Hz, 4-H), 3.93 (m, 1H, m, 5-H), 3.78 (s, 2H, OMe), 3.43 (dd, 1H, J=3.4, 10.7 Hz, 2-H), 3.29 (dd, 1H, J=2.9, 11.1 Hz, 6-H), 3.14 (dd, 1H, J=7.0, 11.1 Hz, 6-H), 2.11 (s, 3H, Ac), 2.07 ppm (s, 3H, Ac); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 169.6 (Ac), 169.1 (Ac), 155.7 (Ar), 150.1 (Ar), 118.0 (2 C, Ar), 114.7 (2 C, Ar), 97.7 (C-1), 72.7 (C-4), 69.8 (C-3), 69.1 (C-5), 60.9 (C-2), 55.7 (OMe), 20.7 (Ac), 20.6 (Ac), 3.68 ppm (C-6); β-isomer: ^{1}H NMR (600 MHz, $CDCl_3$) β: δ = 7.17 (d, 2H, J=9.0 Hz, C_6H_4), 6.86 (d, 2H, J= 9.0 Hz, C_6H_4), 504 (dd, 1H, J=9.2, 10.1 Hz, 3-H), 4.87 (dd, 1H, J=9.2, 9.7 Hz, 4-H), 4.84 (d, 1H, J=8.1 Hz, 1-H), 3.78 (dd, 1H, J=8.1, 10.1 Hz, 2-H), 3.78 (s, 3H, OMe), 3.63 (m, 1H, 5-H), 3.33 (dd, 1H, J=2.5, 11.1 Hz, 6-H), 3.16 (dd, 1H, J=8.8, 11.0 Hz, 6-H), 2.17 (s, 3H, Ac), 2.04 ppm (s, 3H, Ac); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 169.6 (2 C, Ac), 156.0 (Ar), 150.7 (Ar), 118.8 (2 C, Ar), 114.6 (2 C, Ar), 101.5 (C-1), 74.0 (C-5), 72.2 (C-4), 63.8 (C-2), 55.8 (OMe), 20.6 (2 C, Ac), 2.33 ppm

(C-6); elemental analysis calcd (%) for $C_{17}H_{20}IN_3O_7$: C 40.41, H 3.99, N 8.32; found: C 42.38, H 4.55, N 8.05.

4-Methoxyphenyl 2-azido-2,6-dideoxy-α-D-glucopyranoside (30): To a solution of **29** (23.34 g, 46.4 mmol) in DMPU (100 mL) sodium cyanoboronhydride (14 g, 4.8 equiv) was added and the reaction stirred overnight at 95 °C. The mixture was poured into water and extracted twice with EtOAc, the organic phase was washed with water 4 times, brine, dried over $MgSO_4$ and concentrated in vacuo to give the crude product which was deacetylated under standard Zemplén conditions. Flash chromatography (petroleum ether/EtOAc 1:1) yielded **30** (11.81 g, 86%) as a solid. M.p. 104–105°C); $[\alpha]_D = +113$ ($c = 1$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): $\delta = 7.03$ (d, 2H, $J=9$ Hz, MP), 6.84 (d, 2H, $J=9$ Hz, MP), 5.40 (d, 1H, $J=3.7$ Hz, 1-H), 4.15 (m, 1H, 3-H), 3.92 (m, 1H, 5-H), 3.78 (s, 3H, OMe), 3.30 (m, 2H, 2-H, 4-H), 1.30 ppm (d, 3H, $J=6.3$ Hz, 6-H); ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 155.4$ (MP), 150.6 (MP), 118.1 (2 C, MP), 114.7 (2 C, MP), 97.7 (C-1), 76.5 (C-4), 71.7 (C-3), 68.0 (C-5), 63.1 (C-2), 55.7 (OMe), 17.5 ppm (C-6); HRMS: m/z: 318.1060; found: 318.1057 $[M+Na]^+$.

Alternatively the acetylated product could be isolated by flash chromatography (EtOAc/petroleum ether 7:1 to 4:1). M.p. 94–95 °C; $[\alpha]_D = +190$ ($c = 1$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): $\delta = 7.05$ (d, 2H, $J=9$ Hz, MP), 6.84 (d, 2H, $J=9$ Hz, MP), 5.64 (dd, 1H, $J=9.4$ Hz, 10.4 Hz, 3-H), 5.44 (d, 1H, $J=3.4$ Hz, 1-H), 4.86 (dd, 1H, $J=9.6$ Hz, 9.6 Hz, 4-H), 4.08 (m, 1H, 5-H), 3.77 (s, 3H, OMe), 3.39 (dd, 1H, $J=3.5$ Hz, 10.4 Hz, 2-H), 2.11 (Ac), 2.05 (Ac), 1.18 ppm (d, 3H, $J=6.4$ Hz, 6-H); ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 170.2$ (Ac), 170.0 (Ac), 155.6 (MP), 150.4 (MP), 117.9 (MP), 114.9 (MP), 97.5 (C-1), 73.9 (C-4), 70.3 (C-3), 66.3 (C-5), 61.2 (C-2), 55.8 (OMe), 20.9 (Ac), 20.8 (Ac), 17.4 ppm (C-6); elemental analysis calcd (%) for $C_{24}H_{27}N_3O_{10}S$ (549.55 g mol^{-1}): C 53.82, H 5.58, N 11.08; found: C 53.75, H 5.47, N 10.84.

4-Methoxyphenyl 2-azido-3-*O*-benzoyl-2,6-dideoxy-α-D-glucopyranoside (31): The diol **30** (4.709 g, 15.9 mmol) was dissolved in dry pyridine (100 mL) containing 4 Å molecular sieves (2 g). The mixture was stirred for 2 h at room temperature and cooled to −30°C where BzCl (1.1 equiv, 15.5 mmol, 2.2 mL) was added slowly by syringe. The reaction was quenched after 1 h with water and concentrated in vacuo, dissolved in EtOAc, washed with water, HCl (1 m), $NaHCO_3$ (sat) and brine. The organic phase was dried over $MgSO_4$, concentrated in vacuo and purified by flash chromatography (EtOAc/petroleum ether 1:5 to 1:3) to give **31** (3.88 g, 61 %). M.p. 164–165°C. The side products and starting material were collected, debenzoylated and recycled. $[\alpha]_D = +230$ ($c = 1$, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): $\delta = 8.11$ (d, 2H, $J=8.0$ Hz, Bz), 7.62 (t, 1H, $J=7.5$ Hz, Bz), 7.48 (dd, 2H, $J=7.5$ Hz, 8.0 Hz, Bz), 7.08 (d, 2H, $J=8.8$ Hz, MP), 6.86 (d, 2H, $J=8.8$ Hz, MP), 5.70 (dd, 1H, $J=9.9$ Hz, 9.9 Hz, 3-H), 5.48 (d, 1H, $J=3.3$ Hz, 1-H), 4.03 (m, 1H, 5-H), 3.79 (s, 3H, OMe), 3.53 (m, 2H, 2-H, 4-H), 1.36 ppm (d, 3H, $J=6.1$ Hz, 6-H); ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 170.0$ (C=O), 167.4 (Ar), 150.4 (Ar), 133.6 (Ar), 130.1 (Ar), 129.2 (Ar), 128.5 (Ar), 118.0 (Ar), 114.7 (Ar), 97.7 (C-1), 75.2 (C-4), 74.3 (C-3), 68.7 (C-5), 61.3 (C-2), 55.5 (OMe), 17.5 ppm (Me); HRMS: m/z: 422.1313; found: 422.1313 $[M+Na]^+$.

3-*O*-Acetyl-2-azido-2,4,6-trideoxy-4-phthalimido-α,β-D-galactopyranose (33a): To a solution of **27a** (1.6 g, 3.43 mmol) in acetonitrile (50 mL) cooled to −15 °C, an aqueous solution of CAN (4.51 g, 2.4 equiv, in 10 mL H_2O) was added. The reaction mixture was stirred for 1.5 h and a saturated $NaHCO_3$ solution was added to quench the reaction. The mixture was extracted 3 times with CH_2Cl_2, the organic phase was dried over sodium sulfate and the solvent was removed in vacuo. Flash chromatography (toluene/acetone 5:1) yielded **33a** (0.93 g, 75%). TLC (toluene/acetone 5:1): $R_f=0.29$; $[\alpha]_D = +8.8$ ($c = 1$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 8.00$–7.40 (m, 4H, Ph), 5.85 (dd, 1H, $J_{3,2}=11.4$, $J_{3,4}=6.5$ Hz, 3-H), 5.50 (d, 1H, $J_{1,2}=3.7$ Hz, 1-H), 5.00 (dd, 1H, $J_{4,3}=6.4$, $J_{4,5}=3.4$ Hz, 4-H), 4.50 (m 1H, 5-H), 4.20 (dd, $J_{2,1}=3.6$, $J_{2,3}=10.7$ Hz, 2-H), 2.00 (s, 3H, CH_3CO), 1.10 ppm (d, 3H, $J_{6,5}=6.6$ Hz, 6-H). MALDI-MS (positive mode, matrix DHB, THF): m/z: 383.1; found: 383.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{16}H_{16}N_4O_6$ (360.32 g mol^{-1}): C 53.33, H 4.48, N 15.55; found: C 53.49, H 4.22, N 15.83.

2-Azido-3-*O*-benzoyl-2,4,6-trideoxy-4-phthalimido-α,β-D-galactopyranose (33b): The OMP-protected sugar **27b** (1.54 g, 2.9 mmol) and bis(trifluoroacetoxy)iodobenzene (2 equiv, 2.51 g) were dissolved in CH_2Cl_2 (50 mL) and water (10 mL) was added. The mixture was stirred vigorously and $BF_3 \cdot OEt_2$ (1.8 equiv, 0.7 mL) was added slowly by syringe. The reaction was quenched with Et_3N after 1 h, worked up by washing with water, brine and drying over $MgSO_4$. After concentration in vacuo the red crude product was purified by flash chromatography (EtOAc/petroleum ether 1:2) to give the desired reducing sugar **33b** containing some red impurities which could partly be removed by dissolving it in CH_2Cl_2 and stirring it with activated carbon overnight. After filtration and concentration a pink foam of **33b** (1.06 g, 87 %) was obtained consisting of an α/β mixture (1:1.7). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 8.00$–7.25 (m, 9H, Phth, Bz), 5.84 (dd, 1H, $J=6.5$ Hz, 11.4 Hz, 3α-H), 5.63 (d, 1H, $J=3.4$ Hz, 1α-H), 5.32 (dd, 1H, $J=6.9$ Hz, 10.0 Hz, 3β-H), 5.09 (dd, 1H, $J=3.7$ Hz, 6.8 Hz, 4α-H), 4.97 (dd, 1H, $J=3.0$ Hz, 7.0 Hz, 4β-H), 4.86–4.66 (m, 1H, 1β-H, 5α-H, 5β-H), 4.14-4.02 (m, 1H, 2α-H, 2β-H), 1.17 (d, 3H, $J=6.6$ Hz, 6β-H), 1.10 ppm (d, 3H, $J=6.6$ Hz, 6α-H).

***O*-(3-*O*-Acetyl-2-azido-2,4,6-trideoxy-4-phthalimido-α,β-D-galactopyranosyl) trichloroacetimidate (34a)**: To a solution of **33a** (0.90 g, 2.50 mmol) and trichloroacetonitrile (6.57 mL, 20 equiv) in CH_2Cl_2 (20 mL), DBU (49 μL, 0.1 equiv) was added. The reaction mixture was stirred for 1 h and the solvent evaporated in vacuo. A fast purification by flash chromatography (petroleum ether/EtOAc 5:1) yielded **34a** (1.13 g, 90%). TLC (toluene/EtOAc 3:1): $R_f=0.42$; $[\alpha]_D = +3.8$ ($c = 1$, $CHCl_3$); α-isomer: ^1H NMR (250 MHz, $CDCl_3$): $\delta = 8.80$ (s, 1H, NH), 8.00–7.40 (m, 4H, Ph), 6.60 (d, 1H, $J_{1,2}=3.7$ Hz, 1-H), 5.90 (dd, 1H, $J_{3,2}=11.4$, $J_{3,4}=6.5$ Hz, 3-H), 5.00 (dd, 1H, $J_{4,3}=6.4$, $J_{4,5}=3.4$ Hz, 4-H), 4.50 (m, 1H, 5-H); 4.25 (dd, 1H, $J_{2,1}=3.6$, $J_{2,3}=10.7$ Hz, 2-H), 2.00 (s, 3H, CH_3CO), 1.10 ppm (d, 3H, $J_{6,5}=6.6$ Hz, 6-H); MALDI-MS (positive mode, matrix DHB, THF): m/z: 526.0; found: 526.1 $[M+Na]^+$ and 383.1 (loss of imidate); elemental analysis calcd (%) for $C_{18}H_{16}Cl_3N_5O_6$ (504.71 g mol^{-1}): C 42.84, H 3.20, N 13.88; found: C 42.59, H 3.42, N 13.83.

***O*-(2-Azido-3-*O*-benzoyl-2,4,6-trideoxy-4-phthalimido-α,β-D-galactopyranosyl) trichloroacetimidate (34b)**: Crude **33b** (1.06 g) was dissolved in dry CH_2Cl_2 (15 mL) and Cl_3CCN (2.7 mL). DBU (0.1 equiv, 35 μL) was added and the red solution turned dark. After completion, estimated from TLC, the reaction was concentrated on silica gel and purified by flash chromatography (EtOAc/petroleum ether 1:4 + 1% Et_3N) to give **34b** (1.017 g, 72%) as a foam; α/β mixture (1:7). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.78$ (s, 1H, =NH), 7.93–7.23 (m, 9H, Ar), 6.73 (d, 1H, $J=3.8$ Hz, 1α-H), 5.90 (dd, $J=6.5$, 11.4 Hz, 3α-H), 5.84 (d, 1H, $J=8.3$ Hz, 1β-H), 5.42 (dd, 1H, $J=7.3$, 10.8 Hz, 5.25–5.02 (m, 3H, 4a-H, 4b-H, 2b-H), 4.67 (m, 1H, 5a-H), 4.19 (m, 1H, 5b-H); 1.22 (d, 3H, $J=6.4$ Hz, 6b-H), 1.15 ppm (d, 3H, $J=6.4$ Hz, 6a-H).

4-Methoxyphenyl 2-azido-4-benyloxycarbonylamino-2,4,6-trideoxy-α-D-galactopyranoside (35): To a solution of **27a** (2.59 g, 5.55 mmol) in *n*-butanol (300 mL) ethylendiamine (64 mL) was added and the reaction mixture stirred for 12 h at 95°C. The solvent was evaporated and the residue filtered through a pad of silica gel (toluene/acetone 2:1) to yield the amino intermediate (1.11 g, 68 %) which without any further purification was directly used in the next step. **27b** was transformed into this intermediate by the same procedure. The amine (1.18 g, 4.0 mmol) was dissolved in THF/H_2O (4:1, 50 mL) containing $NaHCO_3$ (1.0 g, 11.9 mmol) and cooled by an ice bath followed by addition of Z-Cl (0.90 mL, 6.3 mmol). After 20 min at 0°C no starting material was observed by TLC (EtOAc) and the reaction mixture was diluted with EtOAc, washed with water, NH_4Cl (aq, sat) and brine followed by drying ($MgSO_4$) and concentration in vacuo. The crude compound was purified by flash chromatography (petroleum ether/EtOAc 5:1 to 2:1) to give **35** (1.455 g, 85%). M.p. 46-47°C; $[\alpha]_D = +77.0$ ($c = 1$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.35$ (m, 5H, Ph), 7.02 (d, 2H, $J=8.9$ Hz, MP), 6.84 (d, 2H, $J=8.9$ Hz, MP), 5.46 (brd, 1H, $J=9.4$ Hz, NH), 5.31 (d, 1H, $J=3.5$ Hz, 1-H), 5.16 (d, 1H, $J=12.0$ Hz, Bn), 5.12 (d, 1H, $J=12.0$ Hz, Bn), 4.41 (m, 1H, 3-H), 4.29 (m, 1H, 5-H), 4.13 (brd, 1H, $J=3.2$, 9.0 Hz, 4-H), 3.82 (brd, 1H, $J=4.7$ Hz, OH), 3.77 (s, 3H, OMe), 3.34 (dd, 1H, $J=3.7$, 10.8 Hz, 2-H), 1.17 ppm (d, 3H, $J=6.5$ Hz, 6-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 157.1$, 155.4, 150.7, 136.04, 128.6, 128.4, 128.2, 118.2, 114.8, 98.1, 68.4, 67.6, 65.7, 60.2, 56.0, 16.6 ppm; HRMS (amine intermediate): *m*/*z*: 452.1588; found: 452.1676 $[M+Na]^+$.

3-*O*-Acetyl-2-azido-4-benzoyloxycarbonylamino-2,4,6-trideoxy-α,β-D-galactopyranose (36): *a) 3-O-Acetylation of* ***35***: Compound **35** (1.46 g, 3.40 mmol) was dissolved in pyridine (10 mL) and Ac_2O (7 mL) was added. The reaction mixture was stirred for 2 h at room temperature and the solvent evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 3:1) yielded **36** (1.40g, 88%). TLC (petroleum ether/EtOAc 3:1): $R_f=0.19$; $[\alpha]_D = +8.8$ ($c = 1$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.50$–6.80 (m, 9H, Ph), 5.45 (dd, 1H, $J_{3,2}=11.3$, $J_{3,4}=3.9$ Hz, 3-H), 5.40 (d, 1H, $J_{1,2}=3.7$ Hz, 1-H), 5.15 (m, 3H, CH_2Ar, NH), 4.40 (m, 1H, 5-H), 4.30 (m, 1H, 4-H), 3.75 (s, 3H, CH_3O), 3.50 (dd, 1H, $J_{1,2}=3.7$, $J_{2,3}=11.3$ Hz, 2-H), 2.00 (s, 3H, CH_3CO), 1.20 ppm (d, 3H, $J_{6,5}=6.6$ Hz, 6-H); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 493.2; found: 493.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{23}H_{26}N_4O_7$ (470.48 g mol^{-1}): C 58.72, H 5.57, N 11.91; found: C 58.97, H 5.68, N 11.99.

b) Cleavage of the MP group: To a solution of the 3-*O*-acetyl derivative (1.4 g, 2.98 mmol) in acetonitrile (42 mL, cooled to −15°C) a solution of CAN (3.92 g, 2.4 equiv in 14 mL of water) was added dropwise and the reaction mixture was stirred for 2 h. $NaHCO_3$ was added then to quench the reaction and the mixture was extracted with CH_2Cl_2; the organic phase was dried over sodium sulfate and the solvent evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 2:1) yielded **36** (0.91 g, 84%). TLC (toluene/EtOAc 2:1): $R_f=0.21$; $[\alpha]_D = +4.5$ ($c = 1$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 7.45$ (m, 5H, Ph), 5.40 (d, 1H, $J_{1,2}=10$ Hz, NH), 5.30–5.00 (m, CH_2Ph-Cbz, 1α-H, 3α-H), 4.80–4.60 (m, 5β-H, 3β-H, 1β-H), 4.50 (m, 1H, 5α-H), 4.22 (m, 1H, 4α-H), 4.20 (m, 1H, 4β-H), 3.75 (m, 2β-H), 3.50 (m, 1H, 2α-H), 2.00 (s, 3H, CH_3CO α and β), 1.20 ppm (d, $J_{6,5}=6.6$ Hz, 6α-H, 6β-H); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 387.1; found: 387.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{163}H_{20}N_4O_6$ (364.35 g mol^{-1}): C 52.74, H 5.53, N 15.38; found: C 52.79, H 5.58, N 15.59.

***O*-(3-*O*-Acetyl-2-azido-4-benzyloxycarbonylamino-2,4,6-trideoxy-α,β-D-galactopyranosyl) trichloroacetimidate (37)**: To a solution of **36** (0.85 g, 2.33 mmol) in CH_2Cl_2 (30 mL) Cl_3CCN (2.34 mL, 10 equiv) and DBU (35 μL, 0.1 equiv) were added and the reaction mixture stirred for 1 h at room temperature. The solvent was evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 4:1) yielded **37** (1.03 g, 88%). $[\alpha]_D = +12.3$ ($c = 1$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) α-isomer: $\delta = 8.70$ (s, 1H, NH-imidate), 7.45 (m, 5H, Ph), 6.30 (d, 1H, $J_{1,2}=3.9$ Hz, 1-H), 5.15 (m, 4H, CH_2Ph-Cbz, 3-H, NH), 4.35 (m, 2H, 4-H, 5-H), 3.80 (dd, $J_{2,1}=3.9$, $J_{2,3}=11.2$ Hz, 3-H), 2.00 (s, 3H, CH_3CO), 1.20 (d, $J_{6,5}=6.6$ Hz, 6-H); β-isomer: δ 8.70 (s, 1H, NH-imidate), 7.45 (m, 5H, Ph), 5.60 (d, 1H, $J_{1,2}=8.5$ Hz, 1-H), 5.20 (m, 3H, CH_2Ph-Cbz, NH), 4.80 (dd, $J_{2,1}=3.9$, $J_{2,3}=10.7$ Hz, 3-H), 4.20 (m, 1H, 4-H), 3.90 (m, 1H, 5-H), 3.75 (dd, 1H, $J_{2,1}=8.6$, $J_{2,3}=10.7$ Hz, 2-H), 2.00 (s, 3H, CH_3CO), 1.20 ppm (d, $J_{6,5}=6.6$ Hz, 6-H); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 530.1; found: 530.1 $[M+Na]^+$; elemental analysis calcd (%) for $C_{18}H_{20}Cl_3N_3O_6$ (508.74 g mol^{-1}): C 42.50, H 3.96, N 13.77; found: C 42.71, H 4.12, N 13.59.

4-Methoxyphenyl 4-benzyloxycarbonylamino-2-(2,2,2-trichloroethoxycarbonylamino)-2,4,6-trideoxy-α-D-galactopyranoside (38): The azide **35** (3.00 g, 7.00 mmol) was dissolved in EtOH (50 mL) and Raney nickel (approx. 2 g) was added. The reaction mixture was stirred under H_2 pressure until all starting material was consumed, followed by filtration through celite and concentration. The crude product was dissolved in CH_2Cl_2 and stirred vigorously with water containing $NaHCO_3$ (1.76 g, 1.5 equiv). TrocCl (1.4 mL, 1.5 equiv) was added slowly by syringe and the reaction was followed by TLC (petroleum ether/EtOAc 1:3). After complete reaction the phases were separated and the organic phase washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo to give a crude product that was purified by flash chromatography (petroleum ether/EtOAc 3:1 to 2:1) to give **38** (3.675 g, 91 %). M.p. 64°C; $[\alpha]_D = +82.8$ ($c= 1$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.40$–7.30 (m, 5H, Ph), 6.96 (d, 2H, $J=8.9$ Hz, MP), 6.82 (d, 2H, $J=8.9$ Hz, MP), 5.59 (d, 1H, $J=9.0$ Hz, NH), 5.49 (d, 1H, $J=9.3$ Hz, NH), 5.40 (d, 1H, $J=3.0$ Hz, 1-H), 5.20 (d, 1H, $J=12.2$ Hz, Bn), 5.09 (d, 1H, $J=12.2$ Hz, Bn), 4.78 (d 1H, $J=12.0$ Hz, Troc), 4.62 (d, 1H, $J=12.0$ Hz, Troc), 4.27 (brm, 1H, 3-H), 4.19–4.08 (brm, 1H, 4-H, 5-H), 3.97 (br m, 1H, 2-H), 3.77 (s, 3H, OMe), 3.36 (d, 1H, $J=6.4$ Hz, OH), 1.16 ppm (d, 1H, $J=6.4$ Hz, 6-H); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 158.2$, 155.5, 155.2, 150.5, 136.1, 128.7, 128.4, 128.3, 118.2, 114.8, 97.5, 95.4, 74.9, 69.1, 67.6, 66.0, 55.8, 55.7, 53.0, 16.7 ppm.

4-Methoxyphenyl 3-*O*-allyloxycarbonyl-4-benzyloxycarbonylamino-2,4,6-trideoxy-2-(2,2,2-trichloroethoxycarbonylamino-α-D-galactopyranoside (39): Compound **38** (1.00 g, 1.72 mmol) was dissolved in CH_2Cl_2 and cooled with an ice bath followed by addition of AllocCl (0.56 mL, 3 equiv) and pyridine (0.68 mL, 5 equiv). The reaction was allowed to reach room temperature over 1 h and stirred further for 1 h before it was diluted with EtOAc, washed with water, HCl (aq 1 M), water and brine, followed by drying ($MgSO_4$), concentration in vacuo on silica gel and purification by flash chromatography (petroleum ether/EtOAc 5:1 to 1:1) to give **39** (977 mg, 85%). $[\alpha]_D = +90.0$ ($c = 1$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.40$–7.28 (m, 5H, Ph), 6.96 (d, 2H, $J=9.1$ Hz, MP), 6.82 (d, 2H, $J=9.1$ Hz, MP), 5.91 (m, 1H, All), 5.48 (d, 1H, $J=9.5$ Hz, NH), 5.41 (d, 1H, $J=3.7$ Hz, 1-H), 5.36 (dd, 1H, $J=1.3$, 17.3 Hz, All), 5.29 (d, 1H, $J=9.3$ Hz, NH), 5.26 (brd, 1H, $J=10$ Hz, All), 5.15 (dd, 1H, $J=4.0$, 11.4 Hz, 3-H), 5.14 (d, 1H, $J=12.2$ Hz, Bn), 5.10 (d, 1H, $J=12.2$ Hz, Bn), 4.84 (d, 1H, $J=12.0$ Hz, Troc), 4.64 (brd, 2H, $J=5.6$ Hz, All), 4.36 (m, 2H, 4-H, 5-H), 4.22 (ddd, 1H, $J=3.7$, 9.8 Hz, 2-H), 3.77 (s, 3H, OMe), 1.16 ppm (d, 3H, $J=6.4$ Hz, 6-H); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 156.9$ (C=O), 155.6 (C=O), 154.8 (C=O), 154.5 (Ar), 150.3 (Ar), 136.3 (Ar), 131.5 (All), 128.7 (Ar), 128.3 (Ar), 128.2 (Ar), 119 L (All), 118.1 (Ar), 114.9 (Ar), 97.3 (C-1), 95.5 (CCl_3), 74.8 (CH_2-CCl_3), 73.7 (C-3), 69.1 ($CH_2CH{=}CH_2$), 67.3 (CH_2-Ph), 65.8 (C-5), 55.8 (OMe), 52.8 (C-4), 50.4 (C-2), 16.5 ppm (C-6); HRMS: *m*/*z*: 685.0909; found: 685.0910 $[M+Na]^+$.

***O*-(3-*O*-Allyloxycarbonyl)-4-benzyloxycarbonylamino-2,4,6-trideoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-galactopyranosyl) trichloroacetimidate (3)**: Compound **39** (977 mg, 1.48 mmol) was dissolved in MeCN (20 mL) containing water (8 mL) cooled with an ice bath and CAN (2.0 g, 2.5 equiv) dissolved in water (10 mL) was added slowly by syringe. After the addition no starting material was observed by TLC (EtOAc/petroleum ether 1:3) and the reaction was quenched by addition of $NaHCO_3$ (sat). The reaction mixture was diluted with EtOAc, washed with water and brine followed by drying ($MgSO_4$) and concentration in vacuo on silica gel. After a short path column the reddish product **40** was concentrated and dissolved in CH_2Cl_2 (10 mL) followed by addition of Cl_3CCN (1.5 mL, 10 equiv) and DBU (25 μL). After 1 h the reaction was concentrated on silica gel and purified by a short path column (petroleum ether/EtOAc 5:1 to 3:1 containing 1% Et_3N) to give **3** (917 mg, 89%) which was used directly in the following glycosylation. $[\alpha]_D = +78.1$ ($c= 1$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.74$ (m, 1H, NH), 7.40–7.28 (m, 5H, Ar), 6.38 (d, 1H, $J=3.6$ Hz, 1-H), 5.92 (m, 1H, All), 5.40–5.19 (m, 4H, All, 2×NH), 5.12 (s, 2H, CH_2Ar), 5.04 (dd, 1H, $J=4.0$, 11.7 Hz, 3-H), 4.71 (s, 2H, CH_2CCl_3), 4.65 (brd, 2H, $J=5.7$ Hz, All), 4.49–4.29 (m, 3H, 2-H, 4-H, 5-H), 1.24 ppm (d, 3H, $J=6.4$ Hz, 6-H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 160.7$, 156.9, 154.7, 154.4 (4× C=O), 136.2 (Ar), 131.4 (all), 128.7, 128.3, 128.2 (Ar), 119.2 (All), 95.4 ($CNCCl_3$), 95.2 (C-1), 90.9 (CH_2CCl_3), 74.8 (-CH_2CHCH_2), 73.3 (C-3), 69.2 (CH_2CCl_3), 67.9 (C-5), 67.4 (CH_2Ph), 52.4 (C-2), 49.9 (C-4), 16.6 ppm (C-6); elemental analysis calcd (%) for $C_{23}H_{25}Cl_6N_3O$, (700.2 gmol^{-1}): C 39.74, H 3.60, N 6.00; found: C 39.45, H 3.63, N 5.71.

3-*O*-(3-*O*-Allyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-1,2-*O*-cyclohexylidene-*sn*-glycerol (41): To a solution of 1,2-*O*-cyclohexylidene-*sn*-glycerol (1.72 g, 10 mmol) and donor **2** (7.6 g, 1.2 equiv) in CH_2Cl_2 (160 mL), TMSOTf (0.092 mL, 0.05 equiv) was added dropwise at 0°C and the cooling was removed. After 15 min the reaction mixture was neutralized with Et_3N and the solvent evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 4:1) yielded **41** (3.76 g, 58%). Overall yield 84%, α/β 3.2:1. TLC (petroleum ether/EtOAc 3:1): $R_f=0.42$; ^1H NMR (600 MHz, $CDCl_3$): $\delta = 7.40$–7.15 (m, 15H, Ph), 6.00 (m, 1H, CH-All), 5.25–5.15 (m, 2H, CH_2-All), 4.88 (d, $J_{1,2}=3.6$ Hz, 1-H), 4.85, 4.75, 4.65, 4.50 (6d, 6H, $J=12$ Hz, OCH_2Ph), 4.45 (m, 1H, OCH_2-All), 4.34 (m, 1H, 2-H), 4.25 (m, 1H, OCH_2-All), 4.00 (dd, 1H, $J_{gem}=8.4$, $J_{gem}=6.6$ Hz, 1_{Gro}-H), 3.80

(t, 1H, $J_{3,2}=J_{3,4}=9.0$ Hz, 3-H), 3.76–3.70 (m, 3H, 6-H, 1_{Gro}-H, 5-H), 3.63–3.56 (m, 4H, 4-H, 6′-H, 3_{Gro}-H), 3.49 (dd, 1H, $J_{2,1}=3.6$, $J_{2,3}=9.6$ Hz, 2-H), 1.55 (m, 8H, CH_2), 1.35 ppm (m, 2H, CH_2); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 116.6 (1 C, CH_2-All), 110.6 (1 C, C-Cy), 97.5 (1 C, C-1), 81.7 (1 C, C-3), 79.7 (1 C, C-2), 77.6 (1 C, C-4), 74.5 (1 C, CH_2-All), 74.3 (1 C, C-2), 75.1, 73.5, 73.0 (3 C, CH_2Ph), 70.3 (1 C, C-5), 69.0 (1 C, C-3_{Gro}), 68.5 (1 C, C-6), 66.7 (1 C, C-1_{Gro}), 36.5, 35.0, 25.2, 24.1, 23.9 ppm (5 C, CH_2); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 667.3; found: 667.0 $[M+Na]^+$; elemental analysis calcd (%) for $C_{39}H_{48}O_8$ (644.79 g mol^{-1}): C 63.75, H 7.50; found: C 63.81, H 7.68.

3-*O*-(3-*O*-Allyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-*sn*-glycerol (42): A solution of **41** (0.8 g, 1.24 mmol) in 80% aqueous acetic acid (15 mL) was stirred at 80°C for 3 h. After this time the solution was concentrated in vacuo and then diluted with toluene and concentrated to dryness in vacuo. Flash chromatography (petroleum ether/EtOAc 1.5:1) yielded **42** (0.55 g, 78%) as a colourless syrup. TLC (petroleum ether/EtOAc 3:1): $R_{f\alpha}=0.36$; $R_{f\beta}$ = 0.42; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.40–7.15 (m, 15H, Ph), 6.00 (m, 1H, CH-All), 5.25–5.15 (m, 2H, CH_2-All), 4.88 (d, $J_{1,2}=3.6$ Hz, 1-H), 4.85, 4.75, 4.65, 4.60, 4.50 (6d, 6H, $J=12$ Hz, OCH_2Ph), 4.45 (m, 1H, OCH_2-All), 4.25 (m, 1H, OCH_2-All), 4.00 (dd, 1H, $J_{gem}=8.4$, $J_{gem}=6.6$ Hz, 1_{Gro}-H), 3.76–3.70 (m, 5H, 6′-H, 1_{Gro}-H, 5-H, 3-H, 2_{Gro}-H), 3.63–3.56 (m, 4H, 4-H, 6-H, 3_{Gro}-H), 3.49 ppm (dd, 1H, $J_{2,1}=3.6$, $J_{2,3}=9.6$ Hz, 2-H); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 116.6 (1 C, CH_2-All), 97.5 (1 C, C-1), 81.7 (1 C, C-3), 79.7 (1 C, C-2), 77.6 (1 C, C-4), 74.5 (1 C, CH_2-All), 74.3 (1 C, C-2), 75.1, 73.5, 73.0 (3 C, CH_2Ph), 70.3 (1 C, C-5), 68.5 (1 C, C-6), 62.0 (1 C, C-1_{Gro}), 61.7 ppm (1 C, C-3_{Gro}); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 587.3; found: 587.3 $[M+Na]^+$; elemental analysis calcd (%) for $C_{33}H_{40}O_8$ (564.67 g mol^{-1}): C 70.19, H 7.14; found: C 70.38, H 7.38.

3-*O*-(3-*O*-Allyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-1,2-di-*O*-myristoyl-*sn*-glycerol (43): To a solution of **42** (0.55 g, 0.97 mmol) in CH_2Cl_2/DMF (15 mL, 6:1) DCC (0.52 g, 2.6 equiv), myristoic acid (0.56 g, 2.5 equiv dissolved in DMF) and DMAP (0.06 g, 0.5 equiv) were added. The reaction mixture was stirred overnight, filtered and washed with water and brine. The organic phase was dried over sodium sulfate and the solvent evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 8:1) yielded **43** (0.72 g, 75%) as a milky solid. TLC (petroleum ether/EtOAc 8:1): $R_f=0.36$; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.40–7.15 (m, 15H, Ph), 6.00 (m, 1H, CH-All), 5.25–5.15 (m, 2H, CH_2-All), 5.05 (m, 1H, 2_{Gro}-H), 4.88 (d, $J_{1,2}=3.6$ Hz, 1-H), 4.85, 4.75, 4.65, 4.60, 4.50 (6d, 6H, $J=12$ Hz, OCH_2Ph), 4.45 (m, 1H, OCH_2-All), 4.25 (m, 1H, OCH_2-All), 4.00 (dd, 1H, $J=8.4$, $J=6.6$ Hz, 1_{Gro}-H), 3.80 (t, 1H, $J_{3,2}=J_{3,4}=9.0$ Hz, 3-H), 3.76–3.70 (m, 3H, 6b-H, $1'_{\mathrm{Gro}}$-H, 5-H), 3.63–3.56 (m, 4H, 4-H, 6a-H, 3-H), 3.49 (dd, 1H, $J_{2,1}=3.6$, $J_{2,3}=9.6$ Hz, 2-H), 2.25 (t, 4H, $COCH_2R$), 1.55 (m, 4H, $COCH_2CH_2R$), 1.25 (m, 40H, CH_2-chain), 0.90 ppm (t, 6H, Me). MALDI-MS (positive mode, matrix DHB, THF):, *m*/*z*: 1007.7; found: 1007.8 $[M+Na]^+$; elemental analysis calcd (%) for $C_{61}H_{92}O_{10}$ (985.38 g mol^{-1}): C 74.35, H 9.41; found: C 74.38, H 9.63.

1,2-Di-*O*-myristoyl-3-*O*-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-*sn*-glycerol (44): To a solution of **43** (0.70 g, 0.71 mmol) in EtOAc (15 mL), NaOAc (0.35 g, 6 equiv), 90% HOAc (14 mL) and $PdCl_2$ (0.25 g, 1.5 equiv) were added. The reaction mixture was stirred overnight and filtered over celite. The filtrate was neutralized with solid $NaHCO_3$ and extracted several times with EtOAc, the organic phase was then washed with water and brine, dried over sodium sulfate and the solvent was evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 8:1) yielded **44** (2.10 g, 68%). TLC (petroleum ether/EtOAc 8:1): $R_f=0.27$; $[\alpha]_D$ = +11.8 (c = 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.40–7.15 (m, 15H, Ph), 5.05 (m, 1H, 2_{Gro}-H), 4.88 (d, $J_{1,2}=3.6$ Hz, 1-H), 4.85, 4.75, 4.65, 4.60, 4.50 (6d, 6H, $J=12$ Hz, OCH_2Ph), 4.00 (dd, 1H, $J_{gem}=8.4$, $J_{3,2}=6.6$ Hz, 1_{Gro} -H), 3.80 (t, 1H, $J_{3,2}=J_{3,4}=9.0$ Hz, 3-H), 3.76–3.70 (m, 3H, 6-H, $1'_{\mathrm{Gro}}$-H, 5-H), 3.63–3.56 (m, 4H, 4-H, 6′-H, 3_{Gro}-H), 3.49 (dd, 1H, $J_{2,1}=3.6$, $J_{2,3}=9.6$ Hz, 2-H), 2.25 (t, 4H, $COCH_2R$), 1.55 (m, 4H, $COCH_2CH_2R$), 1.25 (m, 40H, CH_2-chain), 0.90 ppm (t, 6H, Me). MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 967.6; found: 967.7 $[M+Na]^+$; elemental analysis calcd (%) for $C_{58}H_{88}O_{10}$ (945.31 g mol^{-1}): C 73.69, H 9.38; found: C 73.42, H 9.48.

3-*O*-[(3-*O*-Acetyl-2-azido-4-benzyloxycarbonylamino-2,4,6-trideoxy-β-D-galactopyranosyl)-(1–3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl]-1,2-di-*O*-myrisoyl-*sn*-glycerol (45a): To a solution of acceptor **44** (0.45 g, 0.48 mmol) and donor **37** (0.34 g, 1.4 equiv) in acetonitrile cooled to −40°C TMSOTf (8.6 µL, 0.1 equiv) was added and the reaction stirred for 30 min at this temperature. Et_3N was added to quench the reaction and the solvent was evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 7:1) yielded **45a** (0.405 g, 66%). TLC (petroleum ether/EtOAc 7:1): $R_f=0.21$; $[\alpha]_D$ = +21.3 (c = 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.40 (m, 20H, Ph), 5.05 (m, 5H, 3b-H, 1b-H, 4b-NH, CHPh-Cbz, 2_{Gro}-H), 4.90 (m, 2H, CHPh-Cbz, 1a-H), 4.78 (d, 1H, J = 10.2 Hz, CHPh), 4.66-4.48 (m, 5H, 2×CH_2Ph, CHPh), 4.26 (m, 2H, 4b-H, 3_{Gro}-H), 4.15 (dd, 1H, $J_{gem}=9.0$, $J_{3,2}=7.0$ Hz, 1_{Gro}-H), 3.80–3.58 (m, 6H, 6a-H, 4a-H, 2a-H, 5a-H, $1'_{\mathrm{Gro}}$-H), 3.55 (m, 2H, 3_{Gro}-H), 3.43 (m, 1H, 5b-H), 3.40 (dd, 1H, $J_{2,1}=9.0$, $J_{2,3}=10.8$ Hz, 2b-H), 2.25 (t, 4H, $COCH_2R$), 2.00 (s, 3H, CH_3-Ac), 1.55 (m, 4H, $COCH_2CH_2R$), 1.25 (m, 40H, CH_2-chain), 0.90 ppm (m, 9H, 2×CH_3, 6c); ^{13}C NMR (150.3 MHz, $CDCl_3$): δ = 172.2 (2×RCH_2CH_2CO), 165.0 (BnOC(O)N), 139.66–128.44 (Ph), 101.1 (C-1b), 96.6 (C-1a), 80.5 (C-2a), 80.0–66.3 (CH_2Ph-Cbz, C-4a, C-1_{Gro}, C-2_{Gro}, C-3_{Gro}, 3×CH_2Ph, C-3a, C-5a, C-6a, C-5b,C-3b), 60.6 (C-2b), 56.3 (C-4b), 37.3 (CH_3-Ac), 32.9–15.1 ppm (C-6b, CH_2-chain, 2×CH_3); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 1329.8; found: 1330.0 $[M+Na]^+$; elemental analysis calcd (%) for $C_{76}H_{110}N_2O_{16}$ (1307.69 g mol^{-1}): C 69.80, H 8.48, N 2.14; found: C 70.05, H 8.63, N 2.35.

3-*O*-[(3-*O*-Allyloxycarbonyl-4-benzyloxycarbonylamino-2,4,6-trideoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl)-(1–3)-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)]-1,2-di-*O*-myristoyl-*sn*-glycerol (45b): The acceptor **44** (707 mg, 0.75 mmol) and the donor **3** (576 mg, 1.1 equiv) were coevaporated with toluene and dried under vacuum followed by addition of dry CH_2Cl_2 (9 mL) cooling to 0°C and addition of TMSOTf (7 µL in 1 mL CH_2Cl_2). After 10 min TLC (petroleum ether/EtOAc 5:1) showed disappearance of starting material and formation of one spot. The reaction was quenched with Et_3N, concentrated on silica gel and purified by flash chromatography to give **45b** (1.035 g, 93%) with a minor amount of trichloroacetamide. $[\alpha]_D$ = +30.4 (c = 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50–7.10 (m, 20H, Ar), 5.89 (m, 1H, All), 5.32 (dd, 1H, $J=17.3$, 1 Hz, All), 5.24 (d, 1H, $J=10.5$ Hz, All), 5.19 (m, 1H, 2_{Gro}-H), 5.11 (d, 1H, $J=12.0$ Hz, Z-CH_2), 5.04 (d, 1H, $J=12.0$ Hz, Z-CH_2), 5.00–4.30 (m, 10H, 3×$PhCH_2$, Cl_3CCH_2, C=C-CH_2), 4.74 (d, 1H, $J=3.4$ Hz, 1a-H), 4.72 (d, 1H, $J=8.6$ Hz, 1b-H), 4.48 (dd, 1H, $J=3.5$, 11 Hz, 3b-H), 4.35 (dd, 1H, $J=3.5$, 12 Hz, 1_{Gro}-H), 4.18 (brdd, $J=3.5$, 10 Hz), 4.15-4.07 (m, 2H, $1'_{\mathrm{Gro}}$-H, 4a-H), 3.74–3.63 (m, 5H, 3_{Gro}-H, 2b-H, 5b-H, 5a-H, 6′aH), 3.55 (m, 3H, 2a-H, 4a-H, 6″a-H), 3.39 (dd, 1H, $J=5.5$, 10.8 Hz, $3'_{\mathrm{Gro}}$-H), 2.25 (m, 4H, $COCH_2R$), 1.56 (m, 4H, $COCH_2CH_2R$), 1.24 (m, 40H, CH_2), 1.13 (d, 3H, $J=6.3$ Hz, 6b-H), 0.87 ppm (t, 6H, $J\approx 6.8$ Hz, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.5 (C=O acyl), 173.2 (C=O acyl), 156.9, 154.8, 154.4 (3×C–Alloc, Troc), 138.5 (Ar), 137.9 (Ar), 136.4 (Ar), 131.6 (Alloc), 129–127 (21 C, Ar), 118.9 (Alloc), 101.9 (C-1b), 96.8 (C-1a), 95.7 (Cl_3C-), 81.2 (C-2a), 79.5 (C-3a), 76.3 (C-3b), 75.6 (C-4a), 75.0 (CH_2), 74.8 (CH_2), 73.7 (CH_2), 72.6 (CH_2), 70.3, 69.9, 69.3, 69.0 (4×C, C-2_{Gro}, C-5a, C-5b, CH_2), 68.4 (C-6a), 67.2 (CH_2), 66.5 C-3_{Gro}), 62.6 (C-1_{Gro}), 53.4 (C-2b), 52.4 (C-4b), 34.4, 34.2, 32.0, 29.8, 29.8, 29.6, 29.5, 29.4, 29.3, 29.2, 25.0, 22.8 (CH_2-chain), 16.6 (C-6b), 14.3 ppm (2×CH_3); HRMS: *m*/*z*: 1505.6767; found: 1505.6790 $[M+Na]^+$.

3-*O*-[(2-Azido-4-benzyloxycarbonylamino-2,4,6-trideoxy-β-D-galactopyranosyl)-(1–3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)]-1,2-di-*O*-myristoyl-*sn*-glycerol (46a): To an ice bath cooled solution of **45a** (0.4 g, 0.31 mmol) in MeOH (10 mL) sodium methanolate (0.1 mL, 0.05 m) was added until neutralization. The mixture was filtered and the solvent evaporated in vacuo to give **46a** (0.38 g, 98%). TLC (petroleum ether/EtOAc 5:1): $R_f=0.26$; $[\alpha]_D$ = +22.5 (c = 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.40 (m, 20H, Ph), 5.05 (m, 5H, 3b-H, 1b-H, 4b-NH, CHPh-Cbz, 2_{Gro}-H), 4.90 (m, 2H, CHPh-Cbz, 1a-H), 4.78 (d, 1H, $J=10.2$ Hz, CHPh), 4.66–4.48 (m, 5H, 2×CH_2Ph, CHPh), 4.26 (m, 2H, 4b-H, 3a-H), 4.15 (dd, 1H, $J=9$, 7 Hz, 1_{Gro}-H), 3.8–3.58 (m, 7H, 3b-H, 6a-H, 4a-H, 2a-H, 5a-H, $1'_{\mathrm{Gro}}$-H, $3'_{\mathrm{Gro}}$-H), 3.55 (m, 2H, 3_{Gro}-H), 3.43 (m, 1H, 5b-H), 3.4 (dd, 1H, $J_{2,1}=9$, $J_{2,3}=10.8$ Hz, 2b-H), 2.25 (m, 4H, $COCH_2R$), 1.55 (m, 4H, $COCH_2CH_2R$), 1.25 (m, 40H, CH_2-chain), 0.90 ppm (m, 9H, 2×CH$_3$, 6b); ^{13}C NMR (150.9 MHz, CDCl$_3$): δ = 172.2 (2×RCH$_2$CH$_2$CO), 165.0 (BnOC(O)N), 139.7–128.4 (Ph), 104.2 (C-1c), 101.1 (C-1b), 96.4 (C-1a), 84.6 (C-4c), 81.9 (C-2c), 80.5 (C-2a), 80.0–65.3 (CH$_2$Ph, Cbz, C-4a, C-1$_{\mathrm{Gro}}$, C-2$_{\mathrm{Gro}}$, C-3$_{\mathrm{Gro}}$, 6×CH$_2$Ph, C-3a, C-5a, C-6a, C-5b, C-3b, C-4c, C-5c), 61.9 (C-6c), 60.6 (C-2b), 56.3 (C-4b), 32.9–15.1 ppm (C-6b, CH$_2$-chain); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 1287.8; found: 1287.8 [*M*+Na]$^+$, 1303.9 [*M*+K]$^+$; elemental analysis calcd (%) for $C_{74}H_{108}N_2O_{15}$ (1265.65 g mol^{-1}): C 70.22, H 8.60, N 2.21; found: C 70.28, H 8.65, N 2.32.

3-*O*-[(4-Benzyloxycarbonylamino-2,4,6-trideoxy-2-(2,2,2-trichloro-ethoxycarbonylamino)-β-D-galactopyranosyl)-(1–3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)]-1,2-di-*O*-myristoyl-*sn*-glycerol (46b): Disaccharide **45a** was dissolved in TMF/MeOH (2:1, 60 mL) and degassed with argon (g) for 15 min. [Pd(Ph$_3$)$_4$] (74 mg, 0.1 equiv) and sodium *p*-toluenesulfinate (114 mg, 1 equiv) were added at 0°C. TLC (petroleum ether/EtOAc 3:1) showed full conversion after 15 min and the reaction was concentrated in vacuo on silica gel followed by flash chromatography (petroleum ether/EtOAc 1:3 to 1:1) to give **46b** (530 mg, 59%). $[\alpha]_D$ = +34.1 (*c* = 1, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.45–7.10 (m, 20H, Ar), 5.16 (m, 2H, CH Z, 2$_{\mathrm{Gro}}$-H), 5.08 (m, 2H, CH Z, NH), 4.91 (m, 3H, NH, 2×CH$_2$), 4.83 (d, 1H, *J* = 3.4 Hz, 1a-H), 4.72–4.56 (m, 4H, 2×CH$_2$, CH$_2$), 4.59 (d, 1H, *J* = 8.4 Hz, 1b-H), 4.46 (d, 1H, *J* = 12 Hz, CH$_2$), 4.37 (d, 1H, *J* = 10.7 Hz, CH$_2$), 4.35 (dd, 1H, *J* = 3.5, 12 Hz, 1$_{\mathrm{Gro}}$-H), 4.13 (dd, 1H, *J* = 6, 12 Hz, 1$_{\mathrm{Gro}}$-H), 4.08 (t, 1H, *J* = 9.2 Hz, 3a-H), 3.95 (brd, 1H, *J* = 9 Hz, 4b-H), 3.72–3.62 (m, 4H, 5a-H, 5b-H, 6′a-H, 3$_{\mathrm{Gro}}$-H), 3.62–3.54 (m, 3H, 2a-H, 4a-H, 6″a-H), 3.50–3.35 (m, 3H, 2b-H, 3b-H, 3$_{\mathrm{Gro}}$-H), 3.29 (brs, 1H, OH), 2.23 (m, 4H, COCH$_2$R), 1.55 (m, 4H, COCH$_2$C*H*$_2$R), 1.24 (m, 40H, CH$_2$-chain), 1.13 (d, 3H, *J* = 6.3 Hz, 6b-H), 0.88 ppm (t, 6H, 2×CH$_3$); ^{13}C NMR (101 MHz, CDCl$_3$): δ = 173.5 (C=O acyl), 173.1 (C=O acyl), 157.9 (C=O, Z), 155.9 (C=O, Troc), 138.6 (Ar), 137.9 (Ar), 137.7 (Ar), 136.2 (Ar), 129.0–127.7 (20 C, Ar), 102.2 (C-1b), 96.6 (C-1a), 95.5 (Cl$_3$C-), 81.1 (C-2a), 80.3 (C-3a), 75.6 (C-4a), 75.1 (CH$_2$), 75.0 (CH$_2$), 73.7 (CH$_2$), 73.6 (C-4a), 72.1 (CH$_2$), 70.5, 69.9, 69.7 (C-2$_{\mathrm{Gro}}$), C-5a, C-5b), 68.3 (C-6a), 67.5 (CH$_2$, 66.5 (C-1$_{\mathrm{Gro}}$), 62.6 (C-3$_{\mathrm{Gro}}$), 56.7 (C-2b), 55.2 (C-4b), 34.4, 34.2, 32.1, 29.8, 29.8, 29.6, 29.5, 29.4, 29.3, 29.2, 25.0, 22.8 (CH$_2$-chain), 16.7 (C-6b), 14.3 ppm (2×CH$_3$); HRMS: *m*/*z*: 1421.6555; found: 1421.6593 [*M*+Na]$^+$.

3-*O*-[(6-*O*-*tert*-Butyldiphenylsilyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-(1–3)-(2-azido-4-benzyloxycarboylamino-2,4,6-trideoxy-β-D-galactopyranosyl)-(1–3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)]-1,2-di-*O*-myristoyl-*sn*-glycerol (47a): To a solution of acceptor **46a** (0.38 g, 0.304 mmol) and donor 4a (0.38 g, 1.5 equiv) in acetonitrile (15 mL) cooled to −40°C. TMSOTf (8.2 μL, 0.1 equiv) was added and the reaction mixture stirred for 30 min, then Et$_3$N was added to neutralize the reaction. The solvent was evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 10:1) yielded **47a** (0.46 g, 79%). TLC (petroleum ether/EtOAc 10:1): R_f = 0.19; $[\alpha]_D$ = +12.3 (*c* = 1, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$): δ = 7.40 (m, 45H, Ph), 5.05 (m, 4H, 1b-H, 4b-NH, CHPh-Cbz, 2$_{\mathrm{Gro}}$-H), 5.00–4.48 (m, 14H, 6×CH$_2$Ph, 1c-H, 1a-H), 4.15 (dd, 1H, *J* = 9.0, 7.0 Hz, 1$_{\mathrm{Gro}}$-H), 4.00–3.58 (m, 11 H, 6a-H, 4a-H, 2a-H, 5a-H, 1′$_{\mathrm{Gro}}$-H, 2c-H, 3c-H, 4c-H, 6c-H), 3.55–3.43 (m, 3H, 5b-H, 3$_{\mathrm{Gro}}$-H), 3.4 (m, 2H, 2c-H, 2b-H), 2.25 (m, 4H, COCH$_2$R), 1.55 (m, 4H, COCH$_2$C*H*$_2$R), 1.25 (m, 49H, CH$_2$-chain, C(CH$_3$)$_3$), 0.90 ppm (m, 9H, 2×CH$_3$, 6b-H); ^{13}C NMR (150.9 MHz, CDCl$_3$): δ = 172.2 (2×RCH$_2$CH$_2$CO), 165 (BnOC(O)N), 139.66–128.44 (Ph), 104.2 (C-1c), 101.1 (C-1b), 96.4 (C-1a), 84.6 (C-4c), 81.9 (C-2c), 80.5 (C-2a), 80.0–65.3 (CH$_2$Ph-Cbz, C-4a, C-1$_{\mathrm{Gro}}$, C-2$_{\mathrm{Gro}}$, C-3$_{\mathrm{Gro}}$, 6×CH$_2$Ph, C-3a, C-5a, C-6a, C-5b, C-3b, C-3c, C-5c), 61.9 (C-6c), 60.6 (C-2b), 50.4 (C-4b), 32.9–15.1 ppm (C-6b, CH$_2$-chain, 2×CH$_3$, C-(CH$_3$)$_3$); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 1942.1; found: 1942.3 [*M*+Na]$^+$, 1958.1 [*M*+K]$^+$; elemental analysis calcd (%) for $C_{115}H_{150}N_4O_{19}Si$ (1920.52 g mol^{-1}): C 71.92, H 7.87, N 2.92; found: C 72.13, H 8.05, N 3.02.

3-*O*-[(6-*O*-Allyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-(1–3)-(4-benzyloxycarbonylamino-2,4,6-trideoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl)-(1–3)-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-1,2-di-*O*-myristoyl-*sn*-glycerol (47b): Donor **4** (300 mg, 0.473 mmol) and acceptor **46b** (261 mg, 0.186 mmol and dried under vacuum. The mixture was dissolved in propionitrile (3 mL) and cooled to −40°C where TMSOTf (5 mL in propionitrile) was added slowly. TLC (petroleum ether/EtOAc 2:1) showed full conversion of the acceptor after 5 min and after additional 15 min the reaction was quenched by addition of Et$_3$N. The reaction mixture was concentrated in vacuo on silica gel and purified by column chromatography (petroleum ether/EtOAc 7:1 to 5:1) to give **47b** (295 mg, 83%, α/β 1:4.5). $[\alpha]_D$ = +39.7 (*c* = 1, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.36–7.05 (m, 35H), 5.83 (m, 1H, All), 5.22 (dd, 1H, *J* = 1.5, 17.2 Hz, All), 5.15–4.94 (m, 4H, 2$_{\mathrm{Gro}}$-H, CH$_2$, All), 4.90–4.35 (m, 15H, CH$_2$, 2×NH), 4.74 (d, *J* = 5 Hz, 1a-H), 4.60 (d, 1H, *J* ≈ 8.1 Hz, 1b-H), 4.30 (dd, 1H, *J* = 3.7, 11.9 Hz, 1$_{\mathrm{Gro}}$-H), 4.24 (d, 1H, *J* = 10.4 Hz, CH$_2$), 4.18 (d, 1H, *J* = 7.5 Hz, 1c-H), 4.10–3.90 (m, 5H, 4b-H, 1$_{\mathrm{Gro}}$-H, CH$_2$), 3.70–3.20 (m, 16H, 2a-H, 4a-H, 5a-H, 6′a-H, 6″a-H, 2b-H, 3b-H, 5b-H, 2c-H, 3c-H, 4c-H, 5c-H, 6′c-H, 6″c-H, 2′$_{\mathrm{Gro}}$-H, 2″$_{\mathrm{Gro}}$-H), 2.17 (m, 4H, COCH$_2$R), 1.48 (m, 4H, COCH$_2$C*H*$_2$R), 1.16 (m, 40H, CH$_2$-chain), 1.12 (d, 3H, *J* = 6.3 Hz, 6b-H), 0.81 ppm (t, 6H, 2× CH$_3$); ^{13}C NMR (101 MHz, CDCl$_3$): δ = 173.3 (C=O acyl), 173.0 (C=O acyl), 156.6 (C=O Z), 154.5 (C=O Troc), 139.0 (Ar), 138.7 (Ar), 138.4 (Ar), 138.2 (Ar), 138.1 (Ar), 137.0 (Ar), 136.6 (Ar), 135.0 (All), 128.9–127.3 (35 C, Ar), 116.6 (All), 103.9 (J_{C-H} = 163 Hz, C-1c), 101.9 (J_{C-H} = 165 Hz, C-1b), 96.7 (J_{C-H} = 172 Hz, C-1a), 95.6 (Cl$_3$C), 84.5 (C-4c), 82.4 (C-2a), 79.4 (C-3a), 78.1, 77.3, 75.5, 75.2, 75.1 (2 C), 74.4, 74.2, 73.6, 72.4, 71.9, 70.2, 69.8, 68.3, 66.7 (CH$_2$ Z, CH$_2$-Troc, C-4a, 6×Bn, C-5a, C-6a, C-5b, C-3b, C-5c, C-6c), 69.5 (C-2$_{\mathrm{Gro}}$), 66.4 (C-3$_{\mathrm{Gro}}$), 62.5 (C-1$_{\mathrm{Gro}}$), 55.4 (C-4b), 55.0 (C-2b), 34.3–22.7 (CH$_2$-chain), 16.6 (C-6b), 14.2 ppm (2×CH$_3$); HRMS: *m*/*z*: 1893.8807; found: 1893.8959 [*M*+Na]$^+$.

3-*O*-[(6-*O*-*tert*-Butyldiphenylsilyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-(1–3)-(–2-acetamino-4-benzyloxycarboylamino-2,4–6-trideoxy-β-D-galactopyranosyl)-(1–3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)]-1,2-di-*O*-myristoyl-*sn*-glycerol (48a): Compound **47a** (0.4 g, 0.208 mmol) was dissolved in AcSH (3 mL, distilled until colourless) and the reaction mixture was stirred for 16 h at 40°C. The solvent was evaporated in vacuo and purified by flash chromatography (toluene/EtOAc 3:1) to give **48a** (0.29 g, 72%). TLC (toluene/EtOAc 3:1): R_f = 0.19; $[\alpha]_D$ = −9.3 (*c* = 1, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$): δ = 7.40 (m, 45H, Ph), 5.05 (m, 5H, 1b-H, 4b-NH, 2b-NH, CHPh-Cbz, 2$_{\mathrm{Gro}}$-H), 5.00–4.48 (m, 14H, 6×CH$_2$Ph, 1c-H, 1a-H), 4.15–4.40 (m, 5H, 1$_{\mathrm{Gro}}$-H, 4b-H, 3a-H, 3b-H, 2b-H), 4.00–3.43 (m, 13H, 3$_{\mathrm{Gro}}$-H, 5b-H, 6a-H, 4a-H, 2a-H, 5a-H, 1′$_{\mathrm{Gro}}$-H, 2c-H, 3c-H, 4c-H, 6c-H), 3.4 (m, 1H, 2c-H), 2.25 (m, 4H, COCH$_2$R), 1.55 (m, 7H, COCH$_2$C*H*$_2$R, CH$_3$C(O)N), 1.25 (m, 49H, CH$_2$-chain, C(CH$_3$)$_3$), 0.90 ppm (m, 9H, 2×CH$_3$, 6b-H); ^{13}C NMR (150.3 MHz, CDCl$_3$): δ = 172.2 (2×RCH$_2$CH$_2$CO), 165 (BnOC(O)N), 157.0 (CH$_3$CO), 139.7–128.4 (Ph), 103.9 (C-1c), 102.8 (C-1b), 96.4 (C-1a), 84.6 (C-4c), 81.9 (C-2c), 80.5 (C-2a), 80.0–65.3 (CH$_2$Ph-Cbz, C-4a, C-1$_{\mathrm{Gro}}$, C-2$_{\mathrm{Gro}}$, C-3$_{\mathrm{Gro}}$, 6×CH$_2$Ph, C-3a, C-5a, C-6a, C-5b, C-3b, C-3c, C-5c), 61.9 (C-6c), 58.3 (C-2b), 55.4 (C-4b), 32.9–15.1 ppm (C-6b, CH$_3$C(O)N, CH$_2$-chain, 2×CH$_3$, C-(CH$_3$)$_3$); MALDI-MS (positive mode, matrix DHB, THF): *m*/*z*: 1958.1; found: 1958.2 [*M*+Na]$^+$, 1974.1 [*M*+K]$^+$; elemental analysis calcd (%) for $C_{117}H_{154}N_2O_{20}Si$ (1936.56 g mol^{-1}): C 72.56, H 8.02, N 1.45; found: C 72.53, H 8.05, N 1.62.

3-*O*-[(6-*O*-Allyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-(1–3)-(2-acetylamino-4-benzyloxycarbonylamino-2,4,6-trideoxy-β-D-galactopyranosyl)-(1–3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)]-1,2-di-*O*-myristoyl-*sn*-glycerol (48b): Trisaccharide **47b** (108 mg, 57 mmol) was dissolved in Ac$_2$O (2 mL) containing Et$_3$N (10 μL, 2 equiv). Freshly activated Zn (100 mg) was added and the reaction mixture stirred for 14 h at room temperature followed by filtration through celite, concentration in vacuo on silica gel and purification by flash chromatography (petroleum ether/EtOAc 3:1 to 1:1) to give **48b** (86 mg, 86%). The reaction could be carried out in 45 min by using ultra sonic bath conditions. This however causes a slight decrease in yield. $[\alpha]_D$ = +38 (*c* = 1, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.36–7.05 (m, 35H), 5.83 (m, 1H, All), 5.22 (d, 1H, *J* = 17.2 Hz, All), 5.15–4.94 (m, 4H, 2$_{\mathrm{Gro}}$-H, CH$_2$, All), 4.90–4.35 (m, 13H, CH$_2$, 2×NH), 4.82 (d, 1H, *J* = 9.1 Hz, 1b-H), 4.69 (d, *J* ≈ 5 Hz, 1a-H), 4.28 (m, 1H, 1$_{\mathrm{Gro}}$-H), 4.24 (d, 1H, *J* = 10.4 Hz, CH$_2$), 4.24 (d, 1H, *J* ≈ 8.7 Hz, 1c-H), 4.10–3.90 (m, 5H, 3a-H, 4b-H, 1$_{\mathrm{Gro}}$-H, 3b-H, CH$_2$), 3.70–3.20 (m, 15H, 2a-H, 4a-H, 5a-H, 6′a-H, 6″a-H, 2b-H, 5b-H, 2c-H, 3c-H, 4c-H, 5c-H, 6′c-H, 6″c-H, 3′$_{\mathrm{Gro}}$-H, 3″$_{\mathrm{Gro}}$-H), 2.18 (m, 4H, COCH$_2$R), 1.53 (s, 3H, NAc), 1.50 (m, 4H, COCH$_2$C*H*$_2$R), 1.17 (m, 40H, CH$_2$-chain), 1.04 (brd, 3H, $J=6$ Hz, 6b-H), 0.80 ppm (t, 6H, $2\times\mathrm{CH_3}$); ^{13}C NMR (101 MHz, $\mathrm{CDCl_3}$): δ = 173.4 (C=O, aryl), 170.6 (NAc), 156.7 (C=O, Z), 138.9 (Ar), 138.9 (Ar), 138.6 (Ar), 138.3 (Ar), 138.1 (Ar), 137.9 (Ar), 136.7 (Ar), 135.1 (All), 129.0–127.5 (35 C, Ar), 116.7 (All), 104.1 ($J_{C-H}=$ 165 Hz, C-1c), 101.6 ($J_{C-H}=166$ Hz, C-1b), 97.0 ($J_{C-H}=170$ Hz, C-1a), 84.7 (C-4c), 82.3 (C-2c), 80.8 (C-2a), 79.1 (C-3a), 78.2, 77.4, 76.3, 76.0, 75.5, 75.2, 75.1, 74.9, 74.5, 73.7, 72.6, 70.4, 69.9, 68.3, 66.7 ($\mathrm{CH_2}$ Z, C-4a, $6\times$Bn, C-5a, C-6a, C-5b, C-3b. C-3c, C-5c, C-6c), 69.6 (C-2_{Gro}), 66.4 (C-3_{Gro}), 62.5 (C-1_{Gro}), 55.3 (C-4b), 54.6 (C-2b), 34.33–23.5 ($\mathrm{CH_2}$-chain), 22.8 (NAc), 16.8 (C-6b), 14.2 ppm ($2\times\mathrm{CH_3}$); HRMS: *m*/*z*: 1776.9665; found: 1777.0540 $[M+\mathrm{Na}]^+$.

3-*O*-[(2,3,4-Tri-*O*-benzyl-β-D-glucopyranosyl)-(1–3)-(2-acetylamino-4-benzyloxycarbonylamino-2,4,6-trideoxy-β-D-galactopyranosyl)-(1–3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)]-1,2-di-*O*-myristoyl-*sn*-glycerol (1b): *a) From* ***48a***: To a solution of **48a** (0.27 g, 0.139 mmol) in THF (5 mL) AcOH (9.5 µL, 1.2 equiv) and TBAF (0.167 mL, 1.2 equiv) were added and the reaction mixture stirred for 2 d. The mixture was diluted with $\mathrm{CH_2Cl_2}$ and washed with water, the aqueous phase was then extracted twice with $\mathrm{CH_2Cl_2}$, the unified organic phase was dried over sodium sulfate and the solvent evaporated in vacuo. Flash chromatography (toluene/EtOAc 1:1) yielded **1b** (0.186 g, 79%).

b) From ***48b***: Trisaccharide **48b** (120 mg, 69 µmol) was dissolved in MeOH/$\mathrm{CH_2Cl_2}$ (1:1, 3 mL) and $\mathrm{PdCl_2}$ (3 mg, 0.3 equiv) was added at 0°C. After 2 h additional catalyst (10 mg) was added and the reaction mixture was left for further 2 h. Then it was concentrated in vacuo on silica gel and purified by flash chromatography (petroleum ether/EtOAc 2:1) to give 1b (93 mg, 79%). $[\alpha]_D$ = +31.5 (c = 1, $\mathrm{CHCl_3}$); ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$): δ = 7.32–7.08 (m, 35H, Ar), 5.18 (d, 1H, $J=8.1$ Hz, NH), 5.11 (m, 1H, 2_{Gro}-H), 5.10 (d, 1H, $J\approx 12$ Hz, Bn Z), 5.02 (d, 1H, $J\approx 12$ Hz, Bn Z), 5.00 (d, $J=10.8$ Hz, NH), 4.88 (d, 1H, $J=10.7$ Hz, Bn), 4.83 (d, 1H, $J=8.2$ Hz, Ib-H), 4.79–4.61 (m, 5H, Bn), 4.73 (d, 1H, $J=$ 3.6 Hz, 1a-H), 4.56–4.46 (m, 4H, Bn), 4.43 (d, $J=11.7$ Hz, Bn), 4.38 (d, 1H, $J=12$ Hz, Bn), 4.37 (d, 1H, $J=7.6$ Hz, 1c-H), 4.30 (d, 1H, $J=$ 10.2 Hz, Bn), 4.28 (dd, 1H, $J=3.7$, 11.9 Hz, 1_{Gro}-H), 4.22 (bdd, 1H, $J=$ 4.3, 10.8 Hz (J_{N-H}), 4b-H), 4.16 (dd, 1H, $J=5.7$, 8.9 Hz, OH), 4.10–3.93 (m, 1H, 1_{Gro}-H, 3a-H, 3b-H), 3.75 (m, 1H, 3_{Gro}-H), 3.67–3.16 (m, 14H, 2a-H, 4a-H, 5a-H, 6′aH, 6″a-H, 2b-H, 5b-H, 2c-H, 3c-H, 4c-H, 5c-H, 6′c-H, 6″c-H, $3'_{Gro}$-H), 2.17 (m, 4H, $\mathrm{COCH_2R}$), 1.56 (s, 3H, NAc), 1.49 (m, 4H, $\mathrm{COCH_2C}H_2\mathrm{R}$), 1.17 (m, 40H, $\mathrm{CH_2}$-chain), 0.99 (d, 3H, $J=6.1$ Hz, 6b-H), 0.80 ppm (t, 6H, $2\times\mathrm{CH_3}$); ^{13}C NMR (101 MHz, $\mathrm{CDCl_3}$): δ = 173.4 (C=O, acyl), 171.0 (C=O, NAc), 157.5 (C=O, Z), 139.0 (Ar), 138.8 (Ar), 138.5 (Ar), 138.2 (Ar), 137.9 (Ar), 137.9 (Ar), 136.8 (Ar), 129.0–127.3 (35 C, Ar), 105.0 ($J_{C-H}=162$ Hz, C-1c), 101.5 ($J_{C-H}=161$ Hz, C-1b), 97.0 ($J_{C-H}=171$ Hz, C-1a), 84.4 (C-4c), 82.2 (C-2c), 80.6 (C-2a), 79.2 (C-3a), 78.1, 77.4, 76.6, 75.7, 75.4, 75.3, 74.8, 74.4, 73.7, 72.8, 70.5, 70.2, 68.3, 66.1, 66.5 ($\mathrm{CH_2}$ Z, C-4a, $6\times$Bn, C-5a, C-6a, C-5b, C-3b, C-3c, C-5c, C-6c), 69.9 (C-1_{Gro}), 62.4 (C-1_{Gro}), 61.4 (C-3_{Gro}), 55.6 (C-4b), 55.0 (C-2b), 34.4, 32.1, 29.8, 29.8, 29.7, 29.5, 29.4, 29.3, 29.2, 25.0, 22.8 ppm ($\mathrm{CH_2}$-chain), 23.4 (NAc), 16.6 (C-6b), 14.3 ($2\times\mathrm{CH_3}$); HRMS: *m*/*z*: 1720.9612; found: 1720.9566 $[M+\mathrm{Na}]^+$.

3-*O*-[(β-D-Glucopyranosyl)-(1–3)-(2-acetylamino-4-amino-2,4,6-trideoxy-β-D-galactopyranosyl)-(1–3)-α-D-glucopyranosyl)]-1,2-di-*O*-myristoyl-*sn*-glycerol (1a): Protected trisaccharide **1b** (15 mg, 8.8 µmol) was dissolved in a mixture of $\mathrm{CH_2Cl_2/MeOH/H_2O}$ (5:5:1) and Pearlman's catalyst [$\mathrm{Pd(OH)_2}$] was added together with $\mathrm{H_2}$ (1 atm). The reaction was followed by MS (MALDI-TOF) and due to incomplete reaction after 15 h the $\mathrm{Pd(OH)_2}$ was replaced by fresh catalyst and the solvent changed to THF/$\mathrm{H_2O}$ (2:1). After additional 20 h under $\mathrm{H_2}$ pressure (40 atm) only the product and some monobenzylated products were observed in the MS. The catalyst was again removed by filtration and the procedure repeated. When no further reaction took place the reaction mixture was filtered and concentrated to give the crude product which was purified by HIC chromatography (gradient from 15 to 60% *n*-propanol) to give 1.9 mg of deprotected trisaccharide **1a** containing very minor monobenzylated side products. Further purification was done by preparative TLC ($\mathrm{CHCl_3}$/MeOH 95:5). For NMR data see Table 1. HRMS ($\mathrm{C_{51}H_{94}N_2O_{18}}$, positive mode): *m*/*z*: 1023.6579; found: 1023.654 $[M+\mathrm{H}]^+$, 1045.635 $[M+\mathrm{Na}]^+$.

3-*O*-{[2,3,4-Tri-*O*-benzyl-6-*O*-(2-cyanoethoxydiisopropylaminophosphinyl)-β-D-glucopyranosyl]-(1–3)-(2-acetamino-4-benzyloxycarbonylamino-2,4,6-trideoxy-β-D-galactopyranosyl)-(1–3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)}-1,2-di-*O*-myristoyl-*sn*-glycerol (1c): Trisaccharide **1b** (65 mg, 38 µmol) was dissolved in $\mathrm{CH_2Cl_2}$/MeCN (1:1, 4 mL) together with 2-cyanoetyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (50 mL) and diisopropylammonium tetrazolide (10 mg, 1.7 equiv). After 3.5 h at room temperature the reaction was finished (TLC: EtOAc/toluene 1:2) and the mixture concentrated in vacuo on silica gel and purified by flash chromatography (toluene/EtOAc 1:0 to 3:1 + 1% $\mathrm{Et_3N}$) to give the phosphoramidite **1c** (72 mg, 86%) as a mixture of two diastereomers. ^{1}H NMR (600 MHz, $\mathrm{CDCl_3}$): δ = 7.35–7.05 (m, 35H, Ar), 5.64 (d, 0.25H, $J=$ 8.8 Hz, NH), 5.43 (d, 0.25H, $J=8.8$ Hz, NH), 5.11 (m, 1H, 2_{Gro}-H), 5.10–4.98 (m, 1.8H, $\mathrm{CH_2}$ Z, NH, Bn), 4.98–4.88 (m, 1.7H, 1b-H, Bn), 4.86–4.75 (m, 2.6H, Bn), 4.74–4.60 (m, 2.6H, 1a-H, Bn), 4.60–4.49 (m, 3H, Bn), 4.48–4.37 (m, 3.25H, 1c-H, Bn), 4.34–4.28 (m, 2.4H), 4.13–3.90 (m, 4H, 4b-H, 1_{Gro}-H, 3a-H, $\mathrm{C}H_2\mathrm{CH_2CN}$), 3.79 (m, 1H, 3_{Gro}-H), 3.72–3.24 (m, 16H, 2a-H, 4a-H, 5a-H, 6′a-H, 6a-H, 2b-H, 5b-H, 2c-H, 3c-H, 4c-H, 5c-H, 6′c-H, 6c-H, $3'_{Gro}$-H, $2\times\mathrm{C}H(\mathrm{CH_3})_2$), 2.50 (m, 2H, $\mathrm{CH_2C}H_2\mathrm{CN}$), 2.19 (m, 4H, $\mathrm{COCH_2R}$), 1.70, 1.65–1.46 (m, 7H, $\mathrm{COCH_2C}H_2\mathrm{R}$, NAc), 1.19 (m, 40H, $\mathrm{CH_2}$ chain), 1.17 ($4\times$s, 12H, $\mathrm{CH(C}H_3)_2$), 1.06 (m, 3H, 6b-H), 0.82 ppm (t, 6H, $2\times\mathrm{CH_3}$); ^{31}P NMR (162 MHz, $\mathrm{CDCl_3}$): δ = 149.7, 146.8 ppm; HRMS: *m*/*z*: 1932.0; found: 1932.6 $[M+\mathrm{Cl}]^-$; HRMS: *m*/*z*: 1999.4; found: 1999.2 $[M+(\mathrm{Me_2CH})_2\mathrm{NH_2}]^+$.

Transient transfected HEK293 cells with human TLR2: Transfection and culture of HEK293 cells were performed as described.[48] In short HEK293 cells were transiently transfected with either TLR2 or TLR4/MD2/CD14 using Polyfect (Quiagen, Hilden, Germany). After 6 h of transfection, cells were washed and stimulated for further 24 h with the ligands. IL-8 content in the culture supernatants were quantified using a commercial ELISA (Invitrogen GmbH, Karlsruhe, Germany).

Human mononuclear cells: Human mononuclear cells (MNC) were isolated from peripheral blood of healthy donors and stimulated as described.[49] Briefly, 100 µL of RPMI-1640 medium containing antibiotic, 10% heat-inactivated human serum, and the stimulus were added to 96-well U-form cell-culture microplates and then 1.5×10^4 MNC in 50 µL RPMI-1640 culture medium was added. After incubation for 16 h the cell-free supernatants were harvested and the IL-8 concentration was quantified using a commercial ELISA (Invitrogen GmbH, Karlsruhe, Germany).

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