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Synthesis of an α-kojibiosyl substituted glycerol teichoic acid hexamer

Wouter F. J. Hogendorf, Leendert J. Van den Bos, Herman S. Overkleeft, Jeroen D. C. Codée *, Gijsbert A. Van der Marel *

Leiden Institute of Chemistry, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands

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ABSTRACT

In this paper the synthesis of an *Enterococcus Faecalis* teichoic acid (TA) hexamer is presented. The key kojibiosyl-glycerol phosphoramidite building block was obtained by condensation of thioglucose donors, provided with various protecting groups at the C2 hydroxyl function with an orthogonally protected glycerol acceptor. After selective deprotection, the resulting 1,2-*cis*-linked pseudodisaccharide acceptor was coupled to an α-directing thioglucose donor, giving the corresponding pseudotrisaccharide, which is then transformed to a phosphoramidite synthon. The kojibiosyl-glycerol phosphoramidite in combination with a glycerolphosphoramidite, an aminohexylphosphoramidite and dibenzylglycerol were coupled to a fully protected glycerol TA hexamer, using chemistry that can be amended for future automated synthesis. Global deprotection afforded the target hexamer kojibiosyl-glycerol containing TA (**1**).

1. Introduction

Teichoic acids (TAs) are major constituents of the cell wall of most Gram-positive bacteria. TAs are polyanionic glycopolymers that can be divided in wall teichoic acids, which are covalently linked to the peptidoglycan layer, and lipoteichoic acids, which are anchored in the bacterial membrane.[1] The backbone of these anionic polymers is mostly composed of repeating alditol (glycerol, ribitol) phosphates. These monomers are often (randomly) decorated with D-alanine or carbohydrate residues.[2] Situated on the exterior of the bacterial cell wall, many teichoic acids are suspected or proven points of recognition for both the mammalian adaptive and the innate immune systems.[1–3]

In the elucidation of the molecular mode of action of TAs in effecting an immune response, pure and well-defined fragments would be valuable tools. We therefore set out to develop a synthetic methodology to efficiently assemble this type of glycopolymers. In this respect it is of interest to note that structure and activity studies of synthetic derivatives of *Staphylococcus aureus* lipoteichoic acid (LTA) fragments led to the discovery of ligands for the human TLR-2 receptor.[4] Our first attention was directed at the TA of *Enterococcus faecalis*, which is build up from 1,3-poly(glycerolphosphate) randomly decorated at the C-2 positions with D-alanine, kojibiose (α-D-glucopyranosyl-(1→2)-α-D-glucose), or 6,6′-di-alanyl-α-kojibiose as depicted in Figure 1.[5] *E. faecalis* is a commensal bacterium and generally of low virulence. However, with the advent of vancomycin-resistant strains an increasing amount of *E. faecalis* infections is being reported.[6]

Because of the repetitive nature of the TA structure, we elected to aim for a solution-phase synthetic strategy that in the future can be translated to an automated solid-phase synthesis.[7] We here

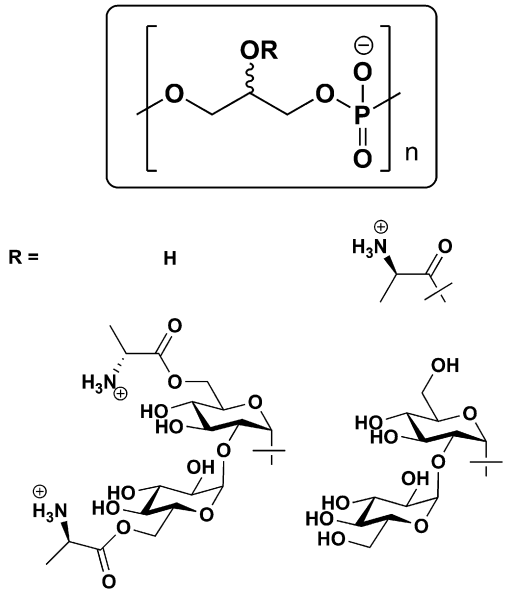

Figure 1. E. Faecalis TA general structure.

* Corresponding authors. Tel.: +31 71 527 4275; fax: +31 71 527 4307 (J.D.C.C.).
E-mail addresses: jcodee@chem.leidenuniv.nl (J.D.C. Codée), marel_g@chem.leidenuniv.nl (Gijsbert A. Van der Marel).

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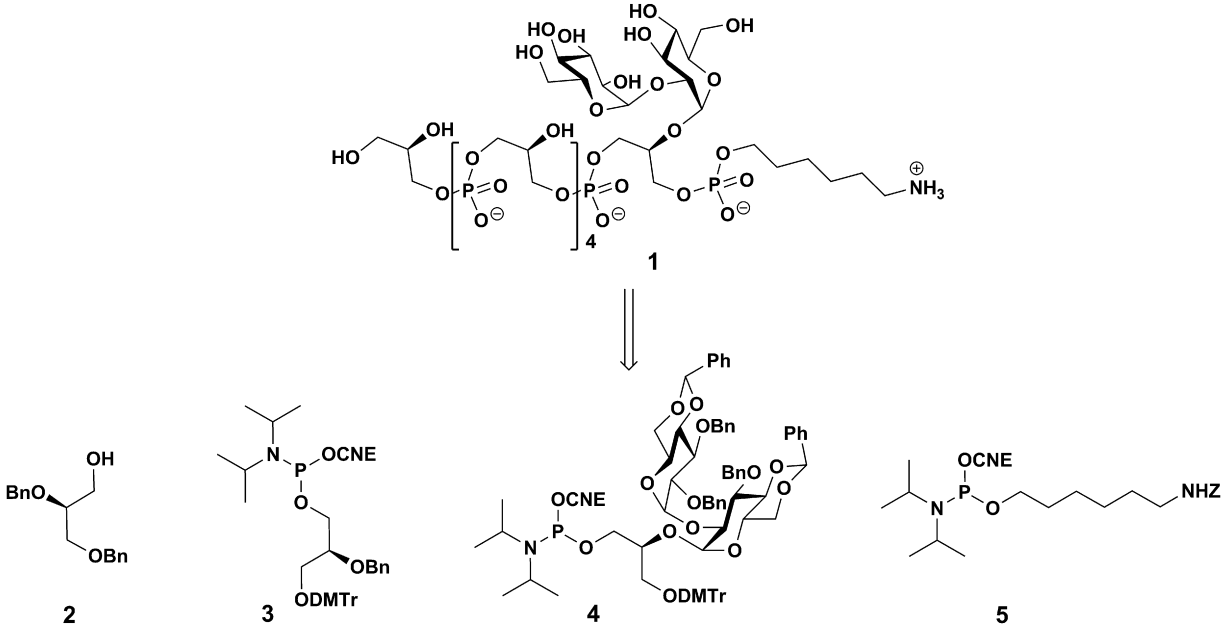
present the construction of an *E. faecalis* kojibiosyl-TA hexamer utilising synthons that are coupled with phosphoramidite chemistry.

2. Results and discussion

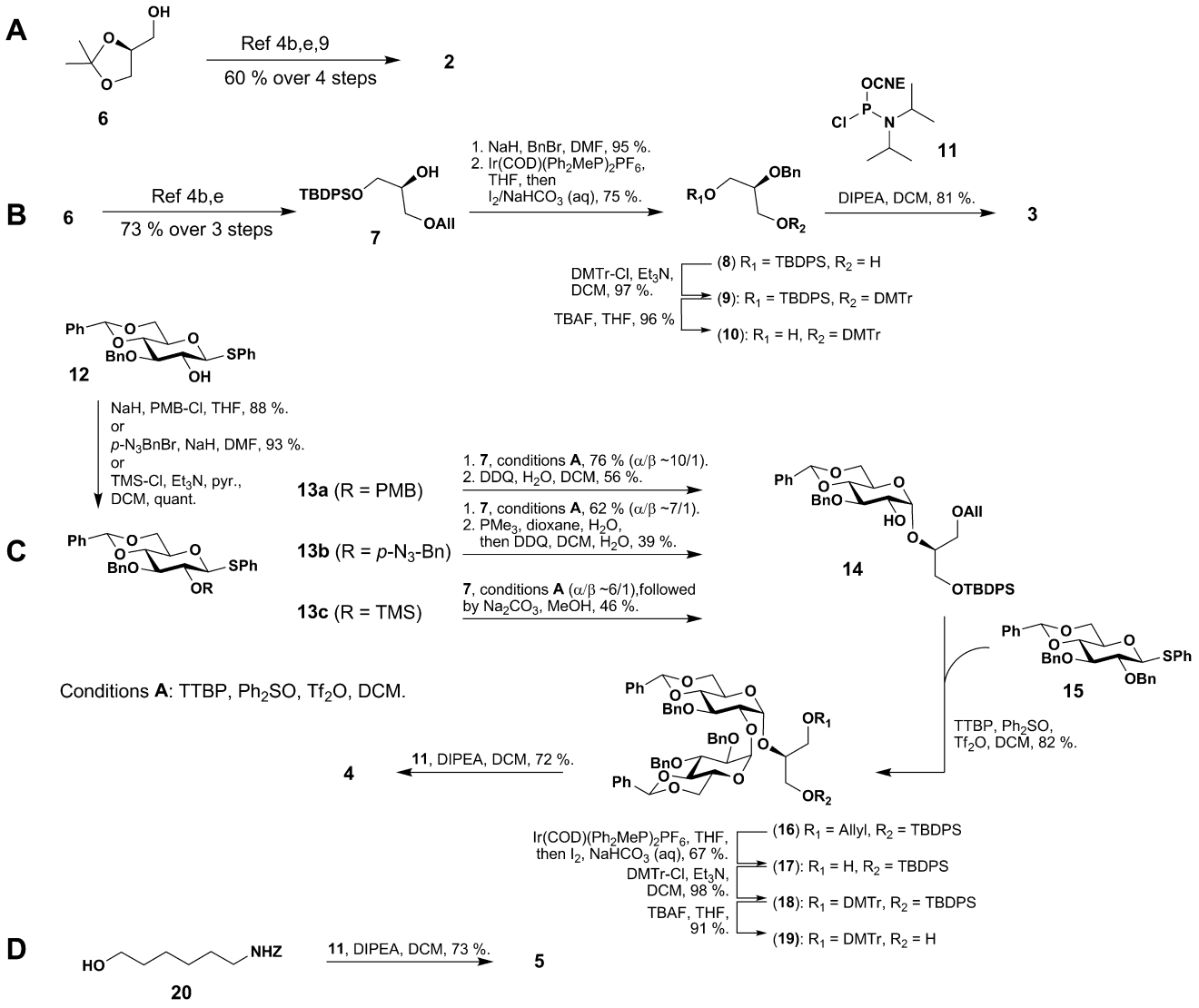
As retrosynthetically depicted in Scheme 1, TA hexamer **1** can be assembled from four synthons: dibenzyl glycerol **2**, which will be the starting building block; glycerol phosphoramidite **3** and kojibiosyl-glycerol phosphoramidite **4**, which will be used for chain elongation and aminohexylphosphoramidite **5**, which will be used as the terminal building block. The primary amino group of the hexyl spacer presents a chemoselective ligation handle for attachment of visualization tags, (micro)array slides or carrier proteins. The hydroxyl functions and the amino group of the aminohexyl spacer are masked with benzyl type protecting groups, which facilitates global deprotection in the final stage of the hexamer assembly by hydrogenolysis. As a temporary protecting group we selected the dimethoxytrityl (DMTr) group, which is commonly employed in contemporary DNA and RNA synthesis protocols and can be cleaved under mildly acidic conditions.[8]

The synthesis of the four building blocks is depicted in Scheme 2. Dibenzylglycerol **2** was synthesized according to literature procedures from solketal **6** (Scheme 2A).[4b,e,9] Solketal **6** also served as starting compound in the synthesis of glycerolphosphoramidite **3**. Allylation of the free hydroxyl function, acidic hydrolysis of the isopropylidene and subsequent selective silylation of the primary alcohol yielded partially protected glycerol **7** (Scheme 2B).[4b] The remaining hydroxyl in compound **7** was benzylated to provide the fully protected glycerol in excellent yield. The product was contaminated with a minor amount (~3%) of 1-*O*-benzyl-2-*O*-*tert*-butyldiphenylsilyl-3-*O*-allyl-*sn*-glycerol, which resulted from silyl migration during the basic etherification and could not be removed by silica gel chromatography. To liberate the C3 hydroxyl, the allyl ether was isomerized using $Ir(COD)(Ph_2MeP)_2PF_6$.[10] Subsequent enol ether cleavage using iodine and aqueous $NaHCO_3$ afforded glycerol **8** in 71% over two steps. Dimethoxytritylation of the primary alcohol furnished glycerol **9**, which was desilylated to provide alcohol **10**. At this stage the product could be separated by silica gel chromatography from its regioisomeric impurity. Finally, phosphitylation using 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite **11** provided building block **3**.

With the first two building blocks in hand we directed our attention to the synthesis of the α-kojibiosyl-glycerol synthon (**4**), containing two 1,2-*cis* glucosidic linkages. Although tremendous progress has been made in the stereoselective construction of glycosidic bonds, the instalment of two 1,2-*cis*-glucosyl linkages still presents a significant challenge. Recently Boons and co-workers revealed an elegant approach to this long standing problem, by installing α-directing *S*-ethylmandelate or (1*S*)-phenyl(thiophenyl)ethyl ethers on the 2′ hydroxyl of glucose donors.[11] Unfortunately, the assembly of the kojibiose-glycerol pseudotri saccharide requires the glycosylation of the first glucose residue at the C-2 hydroxyl and the latter ether protecting groups cannot be removed selectively while keeping the other benzyl ethers intact. Alternative methods which have been used to construct 1,2-*cis* glucosidic linkages include Lemieux's in situ anomerization protocol[12] and the employment of large substituents such as the DMTr group at the glucosyl C-6 hydroxyl.[13] Given the relatively low reactivity of the secondary alcohol function which has to be glucosylated we deemed the former approach unsuitable. We dismissed the latter approach because this would exclude the use of trityl groups in the glycerol part of the pseudotrisaccharide, which was a prerequisite in the design of our protecting group strategy (vide supra). An alternative approach to the formation of 1,2-*cis* glucosidic linkages has been described by Crich et al. using benzylidene protected thioglucosyl donors.[14] We explored three 4,6-*O*-benzylidene functionalized glucosyl donors, having either a C2 *para*-methoxybenzyl (PMB, **13a**), a C2 *para*-azidobenzyl (PAB, **13b**) or a C2 trimethylsilyl (TMS, **13c**) protecting group (Scheme 2C). The installment of the *para*-methoxylbenzyl group in phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-thioglucose 12[15] led to benzylidene glucoside **13a** in 88%. This thioglycoside was then condensed with glycerol acceptor **7** using the Ph_2SO/Tf_2O activator system[16] to provide the intermediate pseudodisaccharide in good yield and excellent selectivity (α/β = 10/1). Subsequent treatment of the fully protected adduct with DDQ gave alcohol **14** in moderate yield (56%), resulting in a 37% total yield of α-glucosyl glycerol **14** from thioglucoside **12**. A similar series of reactions was employed to prepare pseudodisaccharide **14**



Scheme 1. Structures of TA hexamer **1** and (phosphoramidite) synthons **2**, **3**, **4** and **5**.

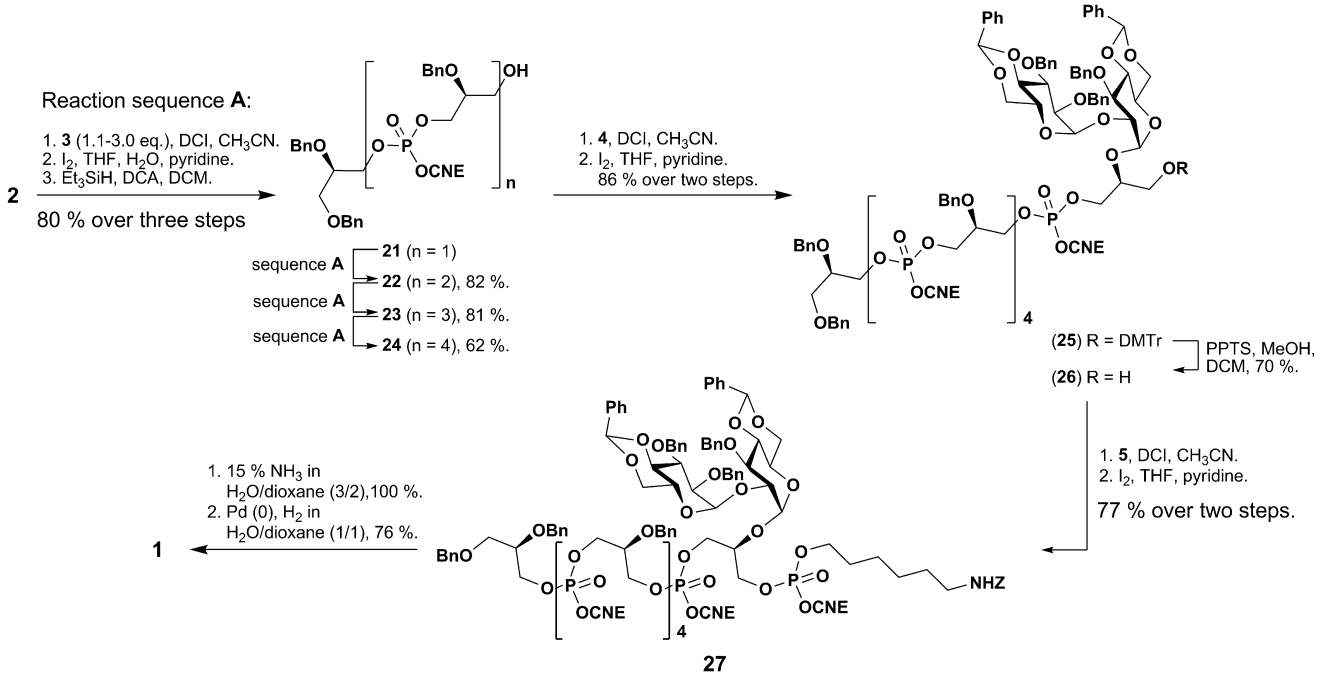


Scheme 2. Synthesis of A: dibenzylglycerol **2**, B: glycerol phosphoramidite **3**, C: kojibiosyl-glycerol synthon **4**, and D: aminohexyl building block **5**.

with the use of the *para*-azido benzyl ether[17] instead of the *para*-methoxy benzyl group as a non-participating group at C-2. In this case (**13b**), the benzylation reaction proceeded uneventfully and the ensuing glycosylation reaction provided the *cis*-coupled product in good selectivity (62%, $\alpha/\beta = 7/1$). Unfortunately, liberation of the C2 hydroxyl via a reduction–oxidation sequence provided target alcohol **14** in only 39%, to complete the four step sequence in a total yield of 22% of **14** from building block **12**. The last non-participating group we tried was the trimethylsilyl ether (TMS). Donor **13c** was obtained quantitatively from **12**, and used in the subsequent glycosylation reaction without purification. The coupling of TMS protected thioglucose **13c** and glycerol **7** was immediately followed by treatment of the resulting pseudodisaccharide (~6:1 mixture of α and β-anomers) with sodium carbonate in methanol to yield **14** in 46% over the three steps, using a single chromatographical purification step, making this the most efficient route.

With the first 1,2-*cis* glycosidic linkage in place, we continued our synthesis with the condensation of dibenzyl benzylidene thioglucoside **15** and alcohol **14**. Gratifyingly, the pseudotrisaccharide (**16**) was obtained as a single diastereoisomer in 82% yield. This trisaccharide was transformed into the phosphoramidite building block **4** following the same sequence of reactions as described for the transformation of 3-*O*-allyl-2-*O*-benzyl-1-*O*-TBDPS-*sn*-glycerol into phosphoramidite **3**. Briefly, the allyl ether in **16** was removed by iridium catalyzed isomerisation and iodine mediated cleavage of the intermediate enol ether. The DMTr group was installed, after which the TBDPS ether was removed by fluoride treatment and the primary alcohol was phosphitylated to complete the synthesis of the kojibiosyl-glycerol **4**. Finally, benzyloxycarbonyl protected aminohexanol **20** was treated with DIPEA and chlorophosporamidite **11** to complete the set of target synthons with phosphoramidite **5** (Scheme 2D).

With all necessary building blocks in hand the assembly of the hexamer was undertaken (Scheme 3). In the first step dibenzyl-glycerol **2** and glycerol phosphoramidite **3** were coupled with the use of 4,5-dicyanoimidazole (DCI) in acetonitrile. After oxidation of the intermediate phosphite (I_2 in THF/pyridine 4/1), the crude intermediate was detritylated using dichloroacetic acid and triethylsilane in dry DCM, giving dimer **21** in 80% yield. Repetition of this reaction sequence led to the consecutive formation of trimer **22** in 82% from **21**, tetramer **23** in 81% from **22** and pentamer **24** in 62% from **23**. Next, kojibiosyl-glycerol phosphoramidite **4** was coupled to the glycerolphosphate chain and after iodine mediated oxidation hexamer **25** was obtained in excellent yield (86%). This indicates that the relatively bulky kojibiosyl substituent did not have



Scheme 3. Elongation towards fully protected hexamer **27** and subsequent deprotection yielding TA **1**.

an adverse effect on the formation of the mixed phosphotriester. To avoid unwanted acidolysis of the benzylidene functionalities in **25** we used the mild pyridinium *para*-toluenesulphonate (PPTS)/MeOH cocktail for the cleavage of the DMTr group.[18] Alcohol **26** was obtained in 70% yield when 1 mg/ml PPTS in MeOH/DCM (9/1) was employed. Subsequent coupling of spacer phosphoramidite **5** and oxidation gave the fully protected target compound **27** in 77% yield.

The deprotection sequence started with ammonolysis of the cyanoethyl groups in **27**, giving the hexameric phosphodiester quantitatively. Finally, all benzyl ethers, the two benzylidene acetals and the benzyloxycarbamate were removed using H_2/Pd(0) in water/dioxane/AcOH. After size-exclusion chromatography, repeated lyophilization, and ion exchange the target *E. faecalis* TA hexamer (**1**) was obtained as the per sodium salt in 76% yield.

3. Conclusion

In summary, we have described the synthesis of a kojibiose containing *Enterococcus faecalis* teichoic acid hexamer. The teichoic acid fragment was constructed by coupling of suitably protected glycerol and α-kojibiosyl substituted glycerol phosphoramidites. The key kojibiosyl synthon was obtained via a sequence of reactions involving two successive stereoselective α-glucosylations. The use of a benzylidene thioglucose donor, temporarily protected at the C2-hydroxyl with a TMS-group proved to be the most efficient in the synthesis of the kojibiosyl-glycerol pseudotrisaccharide. The chemistry used for the assembly of the teichoic acid hexamer is compatible with an automated solid-phase approach, and we are currently studying the efficiency of this in the solid-phase synthesis of differentially substituted TAs.

4. Experimental

4.1. General

All chemicals (Acros, Fluka, Merck, Schleicher & Schuell, Sigma–Aldrich, Genscript) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Tf_2O was distilled over P_2O_5 using flame-dried glass-ware. Column chromatography was performed on Screening Devices Silica Gel 60 (0.040–0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, Silica Gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 25 g/l and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ 10 g/l, in 10% aqueous H_2SO_4 followed by charring at ±140 °C. Some unsaturated compounds were visualized by spraying with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in water. Optical rotation measurements ($[\alpha]_D^{20}$) were performed on a Propol automated polarimeter (Sodium D-line, $\lambda = 589$ nm) with a concentration of 10 mg/ml (*c* 1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300. ^{31}P, ^{1}H, and ^{13}C NMR spectra were recorded with a Bruker AV 400 (161.7, 400 and 100 MHz, respectively) or a Bruker DMX 600 (600 and 150 MHz, respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane, unless stated otherwise. High resolution mass spectra were recorded by direct injection (2 μl of a 2 μM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution $R = 60{,}000$ at m/z 400 (mass range $m/z = 150$–2000) and dioctylphthalate ($m/z = 391.28428$) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). TLC-MS (ESI) spectra of phosphoramidites **3**, **4** and **5** were obtained by analysis of TLC-plates (Schleicher & Schuell, treated with Et_3N before applying the phosphoramidites) with a CAMAG TLC-MS interface coupled to a Perkin Elmer Sciex API 165 mass instrument.

4.2. Experimental procedures

4.2.1. 1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-benzyl-sn-glycerol (8)

Silyl ether **7** (22.8 g, 61.5 mmol) was dissolved in DMF (200 ml) and cooled to 0 °C. After the addition of benzyl bromide (11.1 ml,

92.5 mmol) and heptane flushed NaH (60% dispersion in mineral oil, 3.70 g, 92.5 mmol), the mixture was allowed to stir for 1.5 h. H_2O (50 ml) was added, and after addition of Et_2O (1.0 l), the mixture was washed with H_2O (2 × 200 ml) and brine (200 ml). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Repeated co-evaporation of the residue with H_2O (5 × 100 ml) and toluene (5 × 100 ml), followed by column chromatography (toluene/PE) resulted in the benzylated adduct (28.3 g, 61.4 mmol, 100%, containing ~3% 1-*O*-benzyl-2-*O*-*tert*-butyldiphenylsilyl-3-*O*-allyl-*sn*-glycerol) as a colourless oil. (analytical data: $[\alpha]_D^{20}$ ($CHCl_3$): −10.4; IR (neat): 737, 924, 1103, 1427, 2856 cm^{-1}; ^1H NMR (400 MHz): δ = 1.05 (s, 9H, 3 × CH_3 TBDPS), 3.55–3.71 (m, 3H, CH glycerol, 2 × C*H*H glycerol), 3.77–3.80 (m, 2H, 2 × C*H*H glycerol), 3.99 (dt, 2H, *J* = 1.5 Hz, 1.5 Hz, 5.5 Hz, OCH_2–CH = CH_2), 4.64 (s, 2H, CH_2 Bn), 5.16 (ddd, 1H, *J* = 1.4 Hz, 3.1 Hz, 10.4 Hz, –CH=C*H*H), 5.26 (ddd, 1H, 1,7 Hz, 3.4 Hz, 17.3 Hz, –CH=C*H*H), 5.86–5.93 (m, 1H, –*CH*=CH_2), 7.18–7.43 (m, 11H, H_{arom}), 7.66–7.71 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2 (C_q *t*-butyl), 26.8 (3 × CH_3 TBDPS), 63.5 (CH_2 glycerol), 70.2 (CH_2 glycerol), 72.2, 72.3 (OCH_2–CH=CH_2, CH_2 Bn), 78.8 (CH glycerol), 116.8 (–CH=CH_2), 127.4, 127.7, 128.2, 129.6 (CH_{arom}), 133.5 (2 × C_q phenyl), 134.8 (–*CH*=CH_2), 135.6 (CH_{arom}), 138.8 (C_q Bn)). A solution of the fully protected intermediate (9.21 g, 20.0 mmol) in freshly distilled THF (125 ml) was stirred under argon atmosphere for 30 min. After addition of (1,5-cyclooctadiene)-*bis*-(methyldiphenylphosphine) iridium(I) hexafluorophosphate (423 mg, 2.5 mol %), the solution turned red. The solution was then purged with H_2 (g), until the colour disappeared (~1.5 min), and was allowed to stir for 2 h under argon atmosphere. The solution was diluted with THF (250 ml) and, after addition of satd aq $NaHCO_3$ (300 ml) and I_2 (7.61 g, 30.0 mmol), the mixture was allowed to stir overnight. The mixture was diluted with EtOAc (1.5 l) and washed with sat. aq. NaS_2O_3 (300 and 200 ml) and brine (200 ml). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue, using column chromatography (EtOAc/PE), yielded **8** (6.26 g, 14.9 mmol, 75%, containing ~3% 1-*O*-benzyl-2-*O*-*tert*-butyldiphenylsilyl-*sn*-glycerol) as slightly yellow oil. $[\alpha]_D^{20}$ ($CHCl_3$): −22.0; IR (neat): 739, 824, 1113, 1427, 2361, 2858 cm^{-1}; ^1H NMR (400 MHz): δ = 1.06 (s, 9H, 3 × CH_3 TBDPS), 2.05 (t, 1H, *J* = 6.2 Hz, OH), 3.60–3.82 (m, 5H, CH glycerol, 2 × CH_2 glycerol), 4.51 (d, 1H *J* = 11.7 Hz, C*H*H Bn), 4.63 (d, 1H, *J* = 11.7 Hz, C*H*H Bn), 7.25–7.45 (m, 11H, H_{arom}), 7.66–7.68 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2 (C_q *t*-butyl), 26.8 (3 × CH_3 TBDPS), 62.8 (CH_2 glycerol), 63.5 (CH_2 glycerol), 72.1 (CH_2 Bn), 79.6 (CH glycerol), 127.7, 128.4, 129.8 (CH_{arom}), 133.1, 133.2 (2 × C_q phenyl), 135.6 (CH_{arom}), 138.3 (C_q Bn); HRMS: $C_{26}H_{32}O_3Si+Na^+$ requires 443.2013, found 443.2013.

4.2.2. 1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-benzyl-3-*O*-(4,4′-dimethoxytrityl)-sn-glycerol (9)

Alcohol **8** (6.18 g, 14.7 mmol) was dissolved in DCM (100 ml) and cooled to 0 °C. Added were Et_3N (3.05 ml, 22.0 mmol) and 4,4'-dimethoxytrityl chloride (DMTr–Cl, 5.47 g, 16.2 mmol), respectively, and the mixture was allowed to stir overnight at room temperature. The reaction was quenched by the addition of H_2O (3 ml), and stirred for 15 min. After washing with H_2O (30 ml) and brine (30 ml), the organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting oil was further purified by column chromatography (EtOAc/PE/Et_3N), giving **9** (10.3 g, 14.2 mmol, 97%, containing ~3% 1-*O*-benzyl-2-*O*-*tert*-butyldiphenylsilyl-3-*O*-*(4,4'-dimethoxytrityl)-sn*-glycerol) as colourless oil. $[\alpha]_D^{20}$ (MeOH): +2.0; IR (neat): 827, 1036, 1113, 1250, 1508, 1609, 2359, 2930 cm^{-1}; ^1H NMR (400 MHz): δ = 0.97 (s, 9H, 3 × CH_3 TBDPS), 3.23–3.31 (m, 2H, 2 × C*H*H glycerol), 3.71–3.78 (m, 9H, 2 × OMe, 2 × C*H*H glycerol, CH glycerol), 4.66 (add, 2H, *J* = 12.2 Hz, 13.4 Hz, CH_2 Bn), 6.74–6.83 (m, 4H, H_{arom}), 7.00–7.45 (m, 20H, H_{arom}), 7.60–7.62 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.1 (C_q *t*-butyl), 26.8 (3 × CH_3 TBDPS), 55.1 (2 × OMe) 63.7 (CH_2 glycerol), 63.9 (CH_2 glycerol), 72.3 (CH_2 Bn), 79.3 (CH glycerol), 86.0 (C_q DMTr), 113.0, 113.6 (CH_{arom}), 126.1–130.3 (CH_{arom}), 133.4, 133.5 (2 × C_q phenyl), 136.3 (CH_{arom}), 136.4, 138.9, 145.1, 158.3 (C_q Bn, 5 × C_q DMTr); HRMS: $C_{47}H_{50}O_5Si+Na^+$ requires 745.3320, found 745.3322.

4.2.3. 2-*O*-Benzyl-3-*O*-(4,4′-dimethoxytrityl)-sn-glycerol (10)

To a solution of glycerol derivative **9** (25.5 g, 35.3 mmol) in THF (200 ml), was added TBAF (1.0 M solution in THF, 53 ml). After stirring for 3 h at room temperature, the solvent was removed in vacuo. The residue was purified by column chromatography (EtOAc/PE/Et_3N), affording primary alcohol **10** (16.4 g, 33.8 mmol, 96%) as colourless oil. $[\alpha]_D^{20}$ (MeOH): +10.6; IR (neat): 829, 1034, 1177, 1250, 1508, 1607, 2359 cm^{-1}; ^1H NMR (400 MHz): δ = 2.02 (br s, 1H, OH), 3.21–3.31 (m, 2H, 2 × C*H*H glycerol), 3.63–3.67 (m, 2H, CH glycerol, C*H*H glycerol), 3.70–3.74 (m, 7H, 2 × OMe, C*H*H glycerol), 4.53 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 4.66 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 6.80 (ad, 4H, *J* = 8.8 Hz, H_{arom}), 7.16–7.38 (m, 12H, H_{arom}), 7.44 (d, 2H, *J* = 7.6, H_{arom}); ^{13}C NMR (100 MHz): δ = 55.0 (2 × OMe), 63.0 (CH_2 glycerol), 63.3 (CH_2 glycerol), 72.0 (CH_2 Bn), 78.9 (CH glycerol), 86.4 (C_q DMTr), 113.2 (CH_{arom}),126.7–129.9 (CH_{arom}), 135.9, 138.2, 144.7, 158.4 (C_q Bn, 5 × C_q DMTr); HRMS: $C_{31}H_{32}O_5+Na^+$ requires 507.3142, found 507.3135.

4.2.4. 1-([*N*,*N*-Diisopropylamino]-2-cyanoethylphosphite)-2-*O*-benzyl-3-*O*-(4,4′-dimethoxytrityl)-sn-glycerol (3)

To a solution of **10** (3.19 g, 6.59 mmol) and DIPEA (1.72 ml, 9.88 mmol) in DCM (65 ml) were added activated MS 3 Å and (*N*,*N*-diisopropylamino)-2-cyanoethyl-chlorophosphite (**11**, 1.82 g, 7.90 mmol). After stirring for 2 h, the reaction was quenched with H_2O (5.0 ml) and washed with H_2O (50 ml) and brine (50 ml), respectively. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Column chromatography (EtOAc/heptane/Et_3N) gave phosphoramidite **3** (3.74 g, 5.34 mmol, 81%, mixture of diastereoisomers) as colourless oil. ^{31}P NMR (161.7 MHz, CD_3CN): δ = 149.2; ^1H NMR (400 MHz, CD_3CN): δ = 1.08 (t, 6H, *J* = 6.5 Hz, 2 × CH_3 isopropylamino), 1.14 (dd, 6H, *J* = 1.8 Hz, 6.8 Hz, 2 × CH_3 isopropylamino), 2.51–2.56 (m, 2H, CH_2 cyanoethoxy), 3.17–3.24 (m, 2H, 2 × CH isopropylamino), 3.50–3.58 (m, 2H, 2 × C*H*H glycerol), 3.64–3.81 (m, 11H, CH glycerol, 2 × C*H*H glycerol, CH_2 cyanoethoxy, 2 × OMe), 4.61–4.68 (m, 2H, CH_2 Bn), 6.83 (d, 4H, *J* = 8.9 Hz, H_{arom}), 7.19–7.39 (m, 12H, H_{arom}), 7.45–7.47 (m, 2H, H_{arom}); TLC-MS: $C_{40}H_{49}N_2O_6P+H^+$ requires 685.34, found 685.5.

4.2.5. Phenyl 2-*O*-(4-methoxybenzyl)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (13a)

To a cooled (0 °C) solution of thioglucose **12** (18.3 g, 40.5 mmol) in THF (250 ml) were added, respectively, *p*-methoxybenzyl chloride (6.03 ml, 44.5 mmol) and NaH (60% dispersion in mineral oil, 1.78 g, 44.5 mmol). After stirring for 5 h, the mixture was quenched with MeOH (5.0 ml), diluted with EtOAc (1.0 l) and washed with, satd aq $NaHCO_3$ (400 ml) and brine (400 ml). The organic layer was dried with $MgSO_4$ and concentrated in vacuo, after which crystallization (EtOAc/PE) yielded fully protected thioglucose derivative **13a** (20.4 g, 35.8 mmol, 88%) as white solid. Mp: 146–147 °C; $[\alpha]_D^{20}$ ($CHCl_3$): −11.2; IR (neat): 748, 818, 1030, 1088, 1250, 1516 cm^{-1}; ^1H NMR (400 MHz): δ = 3.45 (m, 1H, H-5), 3.50 (dd, 1H, *J* = 8.4 Hz, 9.7 Hz, H-2), 3.69 (at, 1H, *J* = 9.4 Hz, H4), 3.76–3.84 (m, 5H, OMe, H-3, H-6), 4.37 (dd, 1H, *J* = 5.0 Hz, 10.5 Hz, H-6), 4.73–4.81 (m, 4H, 3 × C*H*H Bn, H-1), 4.94 (d, 1H, *J* = 11.2 Hz, C*H*H Bn), 5.57 (s, 1H, CH benzylidene), 6.85–6.88 (m, 2H, H_{arom}), 7.26–7.39 (m, 13H, H_{arom}), 7.46–7.54 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 55.3 (OMe), 68.7 (C-6), 70.2 (C-5), 75.3, 75.5 (CH_2 Bn, CH_2 PMB), 80.1 (C-2), 81.4 (C-4), 83.0 (C-3), 88.3 (C-1), 101.1

(CH benzylidene), 113.8 (CH_{arom}), 126.0–129.9 (CH_{arom}), 130.2 (C_q *S*-phenyl), 132.2 (CH_{arom}), 133.2, 137.2 138.3 (C_q Bn, C_q PMB, C_q benzylidene), 159.4 (C_q PMB); HRMS: $C_{34}H_{34}O_6S+H^+$ requires 571.2149, found 571.2147.

4.2.6. Phenyl 2-*O*-(4-azidobenzyl)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (13b)

To a cooled (0 °C) solution of thioglucose **12** (895 mg, 1.99 mmol) in DMF (20 ml) were added *p*-azidobenzyl bromide (634 mg, 2.99 mmol) and NaH (60% dispersion in mineral oil, 127 mg, 3.18 mmol), respectively. After stirring for 1.5 h, MeOH (2.0 ml) was added before the mixture was diluted with Et_2O (80 ml) and washed with H_2O (20 ml) and brine (20 ml). The organic layer was dried with $MgSO_4$ and concentrated in vacuo. The residue was taken up in EtOAc (5 ml) and crystallized upon addition of PE, yielding **13b** (1.07 g, 1.84 mmol, 93%) as ochreous solid. Mp: 150–155 °C (decomp.); $[\alpha]_D^{20}$ ($CHCl_3$): +3.0; IR (neat): 991, 1088, 1508, 2112 cm^{-1}; ^1H NMR (400 MHz): δ = 3.44–3.51 (m, 2H, H-2, H-5), 3.70 (at, 1H, *J* = 9.4 Hz, H4), 3.78–3.84 (m, 2H, H-3, H-6), 4.39 (dd, 1H, *J* = 5.2 Hz, 10.4 Hz, H-6), 4.73–4.82 (m, 4H, 3 × C*H*H Bn, H-1), 4.95 (d, 1H, *J* = 11.2 Hz, C*H*H Bn), 5.59 (s, 1H, CH benzylidene), 6.96–7.00 (m, 2H, H_{arom}), 7.26–7.40 (m, 13H, H_{arom}), 7.45–7.53 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 68.7 (C-6), 70.2 (C-5), 75.1, 75.3 (CH_2 Bn, CH_2 *p*-N_3Bn), 80.3 (C-2), 81.5 (C-4), 82.9 (C-3), 88.2 (C-1), 101.1 (CH benzylidene), 118.9 (CH_{arom}), 126.0–132.2 (CH_{arom}), 133.0, 134.8, 137.2, 138.2, 139.5 (C_q *S*-phenyl, C_q Bn, 2 × C_q *p*-N_3Bn, C_q benzylidene); HRMS: $C_{34}H_{31}N_3O_5S+H^+$ requires 582.2057, found 582.2056.

4.2.7. 1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-allyl-sn-glycerol (14)

From **13a**: A solution of donor **13a** (2.85 g, 4.99 mmol), TTBP (2.86 g, 11.5 mmol) and Ph_2SO (1.11 g, 5.50 mmol) in freshly distilled DCM (100 ml), together with activated MS 3 Å, was stirred under argon at rt for 30 min. Subsequently, the mixture was cooled to −75 °C and stirred for another 15 min. After the addition of Tf_2O (0.93 ml, 5.5 mmol), the mixture was stirred for 45 min at −75 °C and, subsequently, glycerol acceptor **7** (2.22 g, 5.99 mmol) in DCM (10 ml) was added. After stirring for 60 min at −75 °C, the mixture was allowed to, gradually, warm to room temperature (∼3 h). The reaction was quenched upon addition of Et_3N (2.0 ml, 14 mmol) and stirred for 30 min. After washing the mixture with satd aq $NaHCO_3$ (30 ml) and brine (30 ml), the organic layer was dried over $MgSO_4$ and concentrated in vacuo. The resulting oil was dissolved in pyridine (30 ml) and, after the addition of Ac_2O (7 ml), stirred for 2 h. After removal of the solvents in vacuo, the residue was dissolved in EtOAc (200 ml) and washed with satd aq $NaHCO_3$ (2 × 60 ml) and brine (60 ml). The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification of the resulting oil by column chromatography (EtOAc/PE) gave the fully protected product (3.16 g, 3.80 mmol, 76%, α/β mixture, ∼10/1), as colourless oil. IR (neat): 737, 1088, 1369, 1454, 1751, 2855, 2924 cm^{-1}; ^1H NMR α-product (400 MHz): δ = 1.05 (s, 9H, 3 × CH_3 TBDPS), 3.51–3.65 (m, 4H, CH glycerol, C*H*H glycerol, H-2, H-6), 3.69–3.80 (m, 6H, OMe, 3 × C*H*H glycerol), 3.89 (m, 1H, H-5), 3.96–4.03 (m, 5H, OC*H*$_2$–CH=CH_2, H-3, H-4, H-6), 4.66 (d, 1H, *J* = 11.7 Hz, C*H*H Bn), 4.71 (d, 1H, *J* = 11.7 Hz, C*H*H Bn), 4.79 (d, 1H, *J* = 11.2 Hz, C*H*H Bn), 4.85 (d, 1H, *J* = 11.3 Hz, C*H*H Bn), 5.17 (dd, 1H, *J* = 1.6 Hz, 10.4 Hz, –CH=C*H*H), 5.20 (d, 1H, *J* = 3.8 Hz, H-1), 5.26 (dd, 1H, *J* = 1.7 Hz, 17.2 Hz, –CH=C*H*H), 5.49 (s, 1H, CH benzylidene), 5.89 (ddd, 1H, *J* = 5.5 Hz, 10.7 Hz, 22.7 Hz, –C*H*=CH_2), 6.83 (d, 2H, *J* = 8.6 Hz, H_{arom}), 7.24–7.47 (m, 18H, H_{arom}), 7.65–7.68 (m, 4H, H_{arom}); ^{13}C NMR α-product (100 MHz): δ = 19.2 (C_q *t*-butyl), 26.8 (3 × CH_3 TBDPS), 55.2 (OMe), 62.4 (C-5), 63.8 (CH_2 glycerol), 68.9 (C-6), 70.7 (CH_2 glycerol), 72.3 (OCH_2–CH=CH_2), 72.3, 75.2 (CH_2 Bn, CH_2 PMB), 76.6, 78.2 (C-3, C-4), 78.6 (CH glycerol), 82.1 (C-2), 97.2 (C-1), 101.2 (CH benzylidene), 113.7 (CH_{arom}), 116.9 (–CH=CH_2), 126.1–129.7 (CH_{arom}),130.4, 133.2, 133.2, 134.7, 135.5, 137.5, 138.9 (CH_{arom}, –C*H*=CH_2, 2 × C_q phenyl, C_q PMB, C_q Bn, C_q benzylidene), 159.2 (C_q PMB); HRMS: $C_{50}H_{58}O_9Si+Na^+$ requires 853.3742, found 853.3745). A combination of batches of the fully protected pesudodisaccharide (8.81 g, 10.6 mmol) was dissolved in DCM (100 ml) and cooled to 0 °C. After adding H_2O (5.0 ml) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 2.88 g, 12.7 mmol), respectively, the mixture was stirred vigorously for 4 h, before it was quenched by the addition of satd aq $NaHCO_3$ (25 ml). The mixture was diluted with EtOAc (500 ml) and washed with satd aq $NaHCO_3$ (3 × 200 ml) and brine, respectively, (3 × 150 ml) in order to remove the bulk of $DDQH_2$. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography (EtOAc/PE). Alcohol **14** (4.23 g, 5.95 mmol, 56%) was obtained as pale yellow oil.

From **13b**: solution of donor **13b** (145 mg, 0.250 mmol), TTBP (155 mg, 0.625 mmol) and Ph_2SO (60.7 mg, 0.300 mmol) in freshly distilled DCM (8.0 ml), together with activated MS 3 Å, was stirred under argon at rt for 30 min. Subsequently, the mixture was cooled to −78 °C and stirred for another 15 min. After the addition of Tf_2O (50.5 μl, 0.300 mmol), the mixture was stirred for 45 min at −50 °C before it was cooled down to −78 °C. After the addition of a solution of glycerol acceptor **7** (111 mg, 0.300 mmol) in DCM (2.5 ml), the mixture was stirred at −75 °C for 1 h, before it was allowed to gradually (∼3 h) warm to room temperature. The reaction was quenched by the addition of Et_3N (0.2 ml, 1.4 mmol) and stirred for 30 min. The mixture was diluted with DCM (30 ml) and after washing with satd aq $NaHCO_3$ (15 ml) and brine (15 ml), the organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification of the resulting oil by size-exclusion chromatography (sephadex LH-20, MeOH/DCM 1/1), followed by column chromatography (EtOAc/PE) gave the fully protected intermediate (131 mg, 0.156 mmol, 62%, α/β mixture, ∼7/1), as pale yellow oil. Analytical data: ^1H NMR α-product (400 MHz): δ = 1.06 (s, 9H, 3 × CH_3 TBDPS), 3.49–4.04 (m, 13H, CH glycerol, 2 × CH_2 glycerol, H-2, H-3, H-4, H-5, 2 × H-6, OCH_2–CH=CH_2), 4.68 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.74 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.80 (d, 1H, *J* = 12.0 Hz, C*H*H Bn), 4.88 (d, 1H, *J* = 11.6 Hz, C*H*H Bn), 5.17 (dd, 1H, *J* = 1.2 Hz, 10.4 Hz, –CH=C*H*H), 5.23–5.28 (m, 2H, H-1, –CH=C*H*H), 5.50 (s, 1H, CH benzylidene), 5.88 (ddd, 1H, *J* = 5.6 Hz, 10.6 Hz, 22.8 Hz, –C*H*=CH_2), 6.93 (d, 2H, *J* = 8.4 Hz, H_{arom}), 7.22–7.46 (m, 18H, H_{arom}), 7.64–7.68 (m, 4H, H_{arom}); ^{13}C NMR α-product (100 MHz): δ = 19.2 (C_q *t*-butyl, 26.8 (3 × CH_3 TBDPS), 62.4 (C-5), 63.9 (CH_2 glycerol), 68.8 (C-6), 70.9 (CH_2 glycerol), 71.8, 72.3, 75.2 (OCH_2–CH=CH_2, CH_2 *p*-N_3Bn, CH_2 Bn), 76.7, 78.2 (C-3, C-4), 79.0 (CH glycerol), 82.2 (C-2), 97.2 (C-1), 101.2 (CH benzylidene), 116.9 (–CH=CH_2), 118.8 (CH_{arom}) 126.1–129.7 (CH_{arom}), 133.1, 133.2 (2 × C_q phenyl), 134.6 (–*u*H=CH_2), 135.1 (C_q *p*-N_3Bn), 135.5 (CH_{arom}), 137.5, 138.8, 139.3 (C_q Bn, C_q benzylidene C_q *p*-N_3Bn)). A part of the intermediate (58 mg, 0.069 mmol) was dissolved in a mixture of dioxane/water (9/1, 10 ml) and treated with PMe_3 (1 M solution in toluene, 0.34 ml). After stirring for 1 h, the mixture was concentrated in vacuo and coevaporated three times with dioxane (portions of 5 ml) before the mixture was redissolved in moist DCM (10 ml) and DDQ (24 mg, 0.10 mmol) was added. After stirring vigorously for 4 h, the reaction was quenched by the addition of satd aq $NaHCO_3$ (2.5 ml). The mixture was then diluted with EtOAc (40 ml) and washed with, respectively, satd aq $NaHCO_3$ (3 × 15 ml) and brine (3 × 15 ml) in order to remove the bulk of $DDQH_2$. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography (EtOAc/PE), giving alcohol **14** (19.0 mg, 26.7 μmol, 39%) as pale yellow oil.

From **12** (via **13c**): To a solution of thioglucose **12** (225 mg, 0.500 mmol) in DCM (5.0 ml) were added Et_3N (0.69 ml, 5.0 mmol),

TMS-Cl (0.32 ml, 2.50 mmol) and a drop of pyridine. After stirring for 2 h, the reaction was diluted with EtOAc (40 ml) and washed with H_2O (15 ml) and brine (15 ml), respectively. The organic layer was dried with $MgSO_4$ and concentrated in vacuo. Crude **13c**, together with Ph_2SO (121 mg, 0.600 mmol) and TTBP (323 mg, 1.30 mmol) were coevaporated with toluene (three times 5 ml) and together with activated MS 3 Å dissolved in dry DCM (10 ml). After stirring for 15 min, the mixture was cooled to −78 °C and stirred for 5 min. After the addition of Tf_2O (101 μl, 0.600 mmol), the mixture was allowed to slowly warm up to −60 °C (∼30 min), after which the mixture was cooled down to −78 °C and glycerol acceptor **7** (278 mg, 0.750 mmol, dissolved in 2.0 ml DCM) was added. After stirring for 1 h at −78 °C, the mixture was allowed to warm up to rt overnight. After the addition of a few drops of H_2O the mixture was diluted with MeOH (20 ml). K_2CO_3 (10 g) was subsequently added and, after stirring for 5 h, the mixture was diluted with EtOAc (100 ml). The organic layer was washed with H_2O (40 ml) and brine (40 ml) and dried with $MgSO_4$. After removal of the solvents in vacuo, the residue[19] was purified by column chromatography (EtOAc/PE), affording **14** (165 mg, 0.232 mmol, 46%, only α) as pale yellow oil. $[\alpha]_D^{20}$ ($CHCl_3$): +33.6; IR (neat): 741, 1040, 1072, 2343, 2361 cm^{-1}; ^1H NMR (400 MHz): δ = 1.06 (s, 9H, 3 × CH_3 TBDPS), 2.92 (br s, 1H, OH), 3.54–3.79 (m, 8H, 2 × CH_2 glycerol, H-2, H-3, H-4, H-6), 3.85–3.95 (m, 2H, H-5, CH glycerol), 4.00 (ad, 2H, *J* = 5.6 Hz, O*Cu*$_2$–CH=CH_2), 4.05 (dd, 1H, *J* = 4.9 Hz, 10.2 Hz, H-6), 4.85 (d, 1H, *J* = 11.8 Hz, C*u*H Bn), 4.91 (d, 1H, *J* = 11.8 Hz, C*H*H Bn), 5.06 (d, 1H, *J* = 3.8 Hz, H-1), 5.20 (dd, 1H, *J* = 1.1 Hz, 10.4 Hz, –CH=C*H*H), 5.28 (dd, 1H, *J* = 1.5 Hz, 17.2 Hz, –CH=C*H*H), 5.51 (s, 1H, CH benzylidene), 5.88 (ddd, 1H, *J* = 5.5 Hz, 10.8 Hz, 16.0 Hz, –*CH*=CH_2), 7.23–7.47 (m, 16H, H_{arom}), 7.64–7.68 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2 (C_q *t*-butyl, 26.8 (3 × CH_3 TBDPS), 63.0 (C-5), 63.8 (CH_2 glycerol), 68.8 (C-6), 69.8 (CH_2 glycerol), 72.3 (OCH_2–CH=CH_2), 73.1 (C-2), 74.6 (CH_2 Bn), 79.0 (CH glycerol), 79.1, 81.5 (C-3, C-4), 99.8 (C-1), 101.2 (CH benzylidene), 117.5 (–CH=CH_2), 126.1–129.8 (CH_{arom}), 133.0, 133.1 (2 × C_q phenyl), 134.2 (–*C*H=CH_2), 135.5 (CH_{arom}), 137.5, 138.8 (C_q Bn, C_q benzylidene); HRMS: $C_{42}H_{50}O_8Si+Na^+$ requires 734.3201, found 734.3203.

4.2.8. 1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(2-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl]-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-allyl-sn-glycerol (16)

A solution of glucose donor **15** (130 mg, 0.240 mmol), TTBP (149 mg, 0.600 mmol) and Ph_2SO (57 mg, 0.28 mmol) in freshly distilled DCM (5.0 ml), together with activated MS 3 Å, was stirred under argon at rt for 30 min. The mixture was cooled to −78 °C and stirred for another 15 min before Tf_2O (47 μl, 0.28 mmol) was added and the mixture stirred for another 45 min at −70 °C. Subsequently, alcohol **14** (142 mg, 0.200 mmol, dissolved in 2.5 ml DCM) was added and after stirring for 60 min at −75 °C, the mixture was allowed to, gradually, warm to room temperature (∼3 h). The reaction was quenched by the addition of Et_3N (0.14 ml, 1.0 mmol) and stirred for 30 min. The mixture was diluted with EtOAc (30 ml) and washed with satd aq $NaHCO_3$ (10 ml) and brine (10 ml). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The resulting oil was purified by size-exclusion chromatography (sephadex LH-20, MeOH/DCM 1/1) and, subsequently, column chromatography (EtOAc/pentane). Kojibiose derivative **16** (187 mg, 0.164 mmol, 82%, pure α) was obtained as white foam. $[\alpha]_D^{20}$ ($CHCl_3$): +34.4; IR (neat): 746, 997, 1028, 1074, 1369, 1454, 2860 cm^{-1}; ^1H NMR (400 MHz): δ = 1.05 (s, 9H, 3 × CH_3 TBDPS), 3.54 (dd, 1H, *J* = 5.9 Hz, 10.5 Hz, C*H*H glycerol), 3.57–3.69 (m, 7H, 2 × C*H*H glycerol, H-2, 2 × H-4, 2 × H-6), 3.74 (dd, 1H, *J* = 5.3 Hz, 10.4 Hz, C*H*H glycerol), 3.80 (dd, 1H, *J* = 3.7 Hz, 9.4 Hz, H-2), 3.88 (add, 2H, *J* = 1.1 Hz, 5.5 Hz, OCH_2–CH=CH_2), 3.91–4.00 (m, 2H, CH glycerol, H-5), 4.04–4.21 (m, 5H, 2 × H-3, H-5, 2 × H-6), 4.70 (d, 1H, *J* = 11.8 Hz, C*H*H Bn), 4.74–4.83 (m, 4H, CH_2 Bn), 4.90 (d, 1H, *J* = 11.8 Hz, C*H*H Bn), 5.12 (dd, 1H, *J* = 1.1 Hz, 10.4 Hz, –CH=C*H*H), 5.19 (dd, 1H, *J* = 1.6 Hz, 17.3 Hz, –CH=C*H*H), 5.27 (d, 1H, *J* = 3.5 Hz, H-1), 5.40 (d, 1H, *J* = 3.6 Hz, H-1), 5.52 (s, 1H, CH benzylidene), 5.54 (s, 1H, CH benzylidene), 5.82 (ddd, 1H, *J* = 5.6 Hz, 10.7 Hz, 22.7 Hz, –*CH*=CH_2), 7.06–7.11 (m, 2H, H_{arom}), 7.19–7.39 (m, 25H, H_{arom}), 7.44–7.48 (m, 4H, H_{arom}), 7.64–7.68 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.1 (C_q *t*-butyl), 26.8 (3 × CH_3 TBDPS), 62.5 (C-5), 62.8 (C-5), 63.5 (CH_2 glycerol), 68.8 (C-6), 68.9 (C-6), 69.7 (CH_2 glycerol), 72.1 (OCH_2–CH=CH_2), 72.4 (CH_2 Bn), 75.0 (CH_2 Bn), 75.3 (C-2), 75.3 (CH_2 Bn), 75.9 (CH glycerol), 76.7 (C-3), 78.1 (C-3), 79.0 (C-2), 82.2 (2 × C-4), 95.4 (C-1), 95.5 (C-1), 101.2 (2 × CH benzylidene), 117.0 (–CH=CH_2), 126.1–129.7 (CH_{arom}), 133.2 (2 × C_q phenyl), 134.5 (–*C*H=CH_2), 135.5 (CH_{arom}), 137.5, 137.6, 138.3, 138.6 (3 × C_q Bn, 2 × C_q benzylidene); HRMS: $C_{69}H_{76}O_{13}Si+Na^+$ requires 1163.4947, found 1163.4962.

4.2.9. 1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(2-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl]-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-sn-glycerol (17)

A solution of **16** (3.18 g, 2.79 mmol) and freshly activated MS 3 Å in freshly distilled THF (35 ml) was stirred under argon for 30 min. After the addition of $Ir(COD)(Ph_2MeP)_2PF_6$ (236 mg, 10 mol %) the solution turned red and the mixture was purged with H_2 (g) until the solution turned colourless again (∼1 min). After stirring under argon for 4 h, the solution was diluted with THF (30 ml) and satd aq $NaHCO_3$ (100 ml). After the addition of I_2 (1.06 g, 4.19 mmol), the mixture was allowed to stir overnight at room temperature. The mixture was diluted with EtOAc (250 ml) and washed with satd aq NaS_2O_3 (2 × 80 ml) and brine (80 ml), respectively. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Column chromatography (EtOAc/toluene) afforded **17** (2.05 g, 1.86 mmol, 67%) as pale yellow foam. $[\alpha]_D^{20}$ ($CHCl_3$): +29.4; IR (neat): 748, 997, 1028, 1074, 1371, 2360, 2858 cm^{-1}; ^1H NMR (400 MHz): δ = 1.07 (s, 9H, 3 × CH_3 TBDPS), 3.44 (dd, 1H, *J* = 5.3 Hz, 8.1 Hz, OH), 3.50–3.68 (m, 8H, 2 × C*H*H glycerol, 2 × H-2, 2 × H-4, 2 × H-6), 3.76–3.91 (m, 5H, 2 × C*H*H glycerol, CH glycerol, H-5, H-6), 3.96 (dd, 1H, *J* = 4.9 Hz, 10.2 Hz, H-6), 4.02 (at, 1H, *J* = 9.3 Hz, H-3), 4.16 (at, 1H, *J* = 9.3 Hz, H-3), 4.13–4.22 (m, 1H, H-5), 4.73–4.86 (m, 5H, H-1, 2 × CH_2 Bn), 4.94 (d, 1H, *J* = 11.1 Hz, C*H*H Bn), 4.97 (d, 1H, *J* = 10.7 Hz, C*H*H Bn), 5.09 (d, 1H, *J* = 3.6 Hz, H-1), 5.48 (s, 1H, CH benzylidene), 5.50 (s, 1H, CH benzylidene), 7.12–7.15 (m, 3H, H_{arom}), 7.27–7.47 (m, 28H, H_{arom}), 7.66–7.68 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.1 (C_q *t*-butyl), 26.8 (3 × CH_3 TBDPS), 62.4 (CH_2 glycerol), 62.5 (C-5), 62.8 (C-5), 63.7 (CH_2 glycerol), 68.7 (2 × C-6), 74.0 (CH_2 Bn), 75.1 (CH_2 Bn), 75.2 (CH_2 Bn), 76.4 (C-3), 77.2 (C-2), 77.7 (C-2), 78.8 (C-3), 80.7 (CH glycerol), 78.1 (C-3), 79.0 (C-2), 82.5 (C-4), 82.8 (C-4), 97.6 (C-1), 98.6 (C-1), 101.2 (CH benzylidene), 101.3 (CH benzylidene), 126.0–129.8 (CH_{arom}), 133.0, 133.1 (2 × C_q phenyl), 135.5 (CH_{arom}), 137.2, 137.5, 137.6, 138.0, 138.5 (3 × C_q Bn, 2 × C_q benzylidene); HRMS: $C_{66}H_{72}O_{13}Si+Na^+$ requires 1123.4634, found 1123.4648.

4.2.10. 1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(2-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl]-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-(4,4′-dimethoxytrityl)-sn-glycerol (18)

Alcohol **17** (480 mg, 0.436 mmol) was dissolved in DCM (20 ml) and cooled to 0 °C. After the addition of DIPEA (0.23 ml, 1.3 mmol) and DMTr–Cl (295 mg, 0.872 mmol), respectively, the mixture was allowed to stir at room temperature overnight. The reaction was quenched upon addition of H_2O (0.2 ml), and stirred for 15 min. The mixture was diluted with DCM (20 ml) and, after washing with H_2O (10 ml) and brine (10 ml), the organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting oil was purified by size-exclusion chromatography (sephadex LH-20, MeOH/DCM

1/1) and, subsequently, column chromatography (EtOAc/pentane/Et_3N), giving DMTr-ether **18** (601 mg, 0.428 mmol, 98%) as white foam. $[\alpha]_D^{20}$ ($CHCl_3$): +35.6; IR (neat): 752, 1030, 1074, 1250, 1508, 2361, 2858 cm^{-1}; ^1H NMR (400 MHz): δ = 0.97 (s, 9H, 3 × CH_3 TBDPS), 3.08 (dd, 1H, *J* = 5.7 Hz, 10.1 Hz, C*H*H glycerol), 3.44 (dd, 1H, *J* = 3.4 Hz, 9.2 Hz, H-2), 3.51 (dd, 1H, *J* = 2.9 Hz, 10.1 Hz, C*H*H glycerol), 3.55–3.71 (m, 12H, 2 × OMe, 2 × C*u*H glycerol, 2 × H-4, 2 × H-6), 3.77 (dd, 1H, *J* = 3.4 Hz, 9.4 Hz, H-2), 3.93–4.02 (m, 3H, CH glycerol, H-3, H-5), 4.07–4.17 (m, 4H, H-3, H-5, 2 × H-6), 4.30 (d, 1H, *J* = 11.5 Hz, C*u*H Bn), 4.36 (d, 1H, *J* = 11.4 Hz, C*u*H Bn), 4.56 (d, 1H, *J* = 11.4 Hz, C*u*H Bn), 4.72 (d, 1H, *J* = 11.4 Hz, C*H*H Bn), 4.78 (d, 1H, *J* = 11.0 Hz, C*H*H Bn), 4.88 (d, 1H, *J* = 11.0 Hz, C*H*H Bn), 4.98 (d, 1H, *J* = 3.4 Hz, H-1), 5.39 (d, 1H, *J* = 3.4 Hz, H-1), 5.53 (s, 1H, CH benzylidene), 5.55 (s, 1H, CH benzylidene), 6.74 (d, 4H, *J* = 8.7 Hz, H_{arom}), 7.08–7.48 (m, 40H, H_{arom}), 7.55 (d, 2H, *J* = 7.1 Hz, H_{arom}), 7.59 (d, 2H, *J* = 6.7 Hz, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.1 (C_q *t*-butyl), 26.8 (3 × CH_3 TBDPS), 55.1 (2 × OMe), 62.5 (C-5), 62.8 (C-5), 63.3 (CH_2 glycerol), 64.3 (CH_2 glycerol), 68.8 (C-6), 68.9 (C-6), 72.4 (CH_2 Bn), 75.0 (CH_2 Bn), 75.4 (CH_2 Bn), 75.7 (C-2), 76.8, 76.9 (C-3, CH glycerol), 78.1 (C-3), 79.4 (C-2), 82.1 (C-4), 82.3 (C-4), 86.4 (C_q DMTr), 95.2 (C-1), 95.6 (C-1), 101.2 (CH benzylidene), 101.3 (CH benzylidene), 113.1 (CH_{arom}), 126.1–130.0 (CH_{arom}), 133.1, 133.2 (2 × C_q phenyl), 135.5 (CH_{arom}), 137.5, 137.7, 138.1, 138.2, 138.7, 144.9, 158.4 (3 × C_q Bn, 2 × C_q benzylidene, 5 × C_q DMTr); HRMS: $C_{87}H_{90}O_{15}Si+Na^+$ requires 1425.5941, found 1425.5961.

4.2.11. 2-*O*-(2-[2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl]-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-(4,4′-dimethoxytrityl)-sn-glycerol (19)

To a solution of kojibiose derivative **18** (575 mg, 0.410 mmol) in THF (10 ml), was added TBAF (1.00 M solution in THF, 1.20 ml). After stirring for 48 h at rt, the solvent was removed in vacuo. The residue was purified by column chromatography (EtOAc/pentane/Et_3N), affording compound **19** (435 mg, 0.373 mmol, 91%) as white foam. $[\alpha]_D^{20}$ ($CHCl_3$): +38.0; IR (neat): 1030, 1074, 1508, 2341, 2361 cm^{-1}; ^1H NMR (400 MHz): δ = 2.52 (br s, 1H, OH), 3.02 (dd, 1H, *J* = 4.1 Hz, 9.9 Hz, C*H*H glycerol), 3.44–3.49 (m, 2H, C*H*H glycerol, H-2), 3.55–3.78 (m, 14H, 2 × OMe, 2 × C*H*H glycerol, CH glycerol, H-2, 2 × H-4, 2 × H-6), 3.96 (at, 1H, *J* = 9.3 Hz, H-3), 4.04–4.16 (m, 4H, H-3, 2 × H-5, H-6), 4.31 (dd, 1H, *J* = 4.8 Hz, 10.2 Hz, H-6), 4.49–4.55 (m, 3H, 3 × C*H*H Bn), 4.73 (d, 1H, *J* = 11.4 Hz, C*H*H Bn), 4.83 (d, 1H, *J* = 11.0 Hz, C*H*H Bn), 4.90 (d, 1H, *J* = 3.3 Hz, H-1), 4.93 (d, 1H, *J* = 11.0 Hz, C*H*H Bn), 5.17 (d, 1H, *J* = 3.5 Hz, H-1), 5.52 (s, 1H, CH benzylidene), 5.58 (s, 1H, CH benzylidene), 6.75 (d, 2H, *J* = 8.8 Hz, H_{arom}), 6.76 (d, 2H, *J* = 8.8 Hz, H_{arom}), 7.16–7.50 (m, 34H, H_{arom}); ^{13}C NMR (100 MHz): δ = 55.1 (2 × OMe), 63.0 (2 × C-5), 63.8 (CH_2 glycerol), 64.3 (CH_2 glycerol), 68.7 (C-6), 68.9 (C-6), 73.5 (CH_2 Bn), 75.0 (CH_2 Bn), 75.4 (CH_2 Bn), 76.9 (C-3), 77.0 (C-2), 78.4 (C-3), 79.0 (C-2), 79.8 (CH glycerol), 82.3 (C-4), 82.5 (C-4), 86.3 (C_q DMTr), 97.1 (C-1), 97.2 (C-1), 101.2 (CH benzylidene), 101.3 (CH benzylidene), 113.1 (CH_{arom}), 126.0–129.9 (CH_{arom}), 135.7, 135.9, 137.2, 137.6, 137.8, 138.1, 138.6, 144.7, 158.4 (3 × C_q Bn, 2 × C_q benzylidene, 5 × C_q DMTr); HRMS: $C_{71}H_{72}O_{15}+Na^+$ requires 1187.4763, found 1187.4782.

4.2.12. 1-([*N*,*N*-Diisopropylamino]-2-cyanoethyl-phosphite)-2-*O*-(2-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl]-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-(4,4′-dimethoxytrityl)-sn-glycerol (4)

To a cooled (0 °C) solution of alcohol **19** (1.37 g, 1.17 mmol) and DIPEA (0.31 ml, 1.75 mmol) in freshly distilled DCM (12 ml) was added chlorophosphite **11** (333 mg, 1.41 mmol). After stirring for 4 h, the reaction was quenched with H_2O (1.0 ml), diluted with DCM (50 ml) and washed with H_2O (20 ml) and brine (20 ml), respectively. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. After purification of the residue by column chromatography (EtOAc/pentane/Et_3N), phosphoramidite **4** (1.15 g, 0.842 mmol, 72%) was obtained as colourless oil. ^{31}P NMR (161.7 MHz, CD_3CN): δ = 148.5, 148.7 (diastereoisomers); ^1H NMR (400 MHz, CD_3CN): δ = 1.10–1.17 (m, 12H, CH_3 isopropylamino), 2.46–2.52 (m, 2H, CH_2 cyanoethoxy), 3.12–3.16 (m, 1H, CH isopropylamino), 3.32–3.39 (m, 1H, CH isopropylamino), 3.51–4.15 (m, 24H, 2 × OMe, 2 × CH_2 glycerol, CH glycerol, 2 × H-2, 2 × H-3, 2 × H-4, 2 × H-5, 3 × H-6, CH_2 cyanoethoxy), 4.29 (dd, 1H, *J* = 4.2 Hz, 10.0 Hz, H-6), 4.39 (d, 1H, *J* = 11.4 Hz, C*H*H Bn), 4.46 (d, 1H, *J* = 11.3 Hz, C*H*H Bn), 4.60–4.70 (m, 2H, 2 × C*H*H Bn), 4.81 (s, 2H, 2 × C*H*H Bn), 5.12 (d, 1H, *J* = 3.3 Hz, H-1), 5.30 (d, 0.5H, *J* = 3.3 Hz, H-1 diastereomer 1), 5.34 (d, 0.5H, *J* = 3.3 Hz, H-1 diastereomer 2), 5.60 (s, 1H, CH benzylidene), 5.66 (s, 1H, CH benzylidene), 6.82 (d, 4H, *J* = 8.6 Hz, H_{arom}), 7.16–7.50 (m, 34H, H_{arom}); TLC-MS: $C_{80}H_{89}N_2O_{16}P+H^+$ requires 1365.60, found 1365.6.

4.2.13. Benzyl 6-([*N*,*N*-diisopropylamino]-2-cyanoethyl-phosphite)-hexyl-1-carbamate (5)

To a cooled (0 °C) solution of 6-(benzyloxycarbonylamino)-1-hexanol[20] (3.02 g, 12.0 mmol) and DIPEA (2.51 ml, 14.4 mmol) in DCM (60 ml) was added chlorophosphite **11** (2.90 g, 12.2 mmol). After stirring for 4 h, the reaction was washed with H_2O (20 ml) and brine (20 ml), respectively. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. After purification of the residue by column chromatography (EtOAc/PE/Et_3N), phosphoramidite **5** (3.97 g, 8.79 mmol, 73%) was obtained as colourless oil. ^{31}P NMR (161.7 MHz, CD_3CN): δ = 148.2; ^1H NMR (400 MHz, CD_3CN): δ = 1.18 (d, 6H, *J* = 2.7 Hz, 2 × CH_3 isopropylamino), 1.19 (d, 6H, *J* = 2.7 Hz, 2 × CH_3 isopropylamino), 1.30–1.43 (m, 4H, 2 × CH_2), 1.48 (m, 2H, CH_2), 1.59 (m, 2H, CH_2), 2.65 (t, 2H, *J* = 6.0 Hz, CH_2 cyanoethoxy), 3.10 (dd, 2H, *J* = 6.7 Hz, 13.2 Hz, CH_2–N hexyl), 3.57–3.68 (m, 4H, CH_2–O hexyl), 2 × CH isopropylamino), 3.73–3.85 (m, 2H, CH_2 cyanoethoxy), 5.06 (br s, 2H, CH_2 benzylcarbamate), 5.64 (br s, 1H, NH), 7.31–7.42 (m, 5H, H_{arom}); TLC-MS: $C_{23}H_{38}N_3O_4P+H^+$ requires 452.27, found 452.1.

4.2.14. Glycerol phosphate dimer (21)

To a solution of dibenzylglycerol **2** (163 mg, 0.600 mmol) in acetonitrile (4.0 ml), containing freshly activated MS 3 Å, were added DCI (0.25 M in acetonitrile, 9.6 ml) and phosphoramidite **3** (0.2 M in acetonitrile, 3.9 ml). After stirring for 2 h, I_2 (0.2 M in THF/pyridine 4/1, 9.0 ml) and H_2O (1.0 ml) were added and the mixture was allowed to stir for 1 h. EtOAc (80 ml) was added and the mixture was washed with satd aq $Na_2S_2O_3$ (30 ml), aqueous $KHSO_4$ (0.5 M, 30 ml), satd aq $NaHCO_3$ (30 ml) and brine (30 ml), respectively. The organic layer was dried with Na_2SO_4 and concentrated in vacuo, after which the residue was redissolved in DCM (30 ml), containing freshly activated MS 3 Å. Et_3SiH (0.97 ml, 6.0 mmol) and dichloroacetic acid (0.49 ml, 6.0 mmol) were added and the mixture stirred until the orange colour disappeared (~1 h), after which the mixture was diluted with DCM (20 ml) and washed with satd aq $NaHCO_3$ (20 ml) and brine (20 ml), respectively. The organic layer was dried with Na_2SO_4, concentrated in vacuo and subsequently purified by column chromatography (MeOH/DCM), yielding dimer **21** (272 mg, 0.478 mmol, 80%, mixture of diastereoisomers) as colourless oil. ^{31}P NMR (161.7 MHz): δ = −0.6, −0.7; ^1H NMR (400 MHz): δ = 2.50 (t, 1H, *J* = 6.2 Hz, CH_2 cyanoethoxy), 2.54 (t, 1H, *J* = 6.2 Hz, CH_2 cyanoethoxy), 2.66 (br s, 1H, OH), 3.54–3.73 (m, 5H, CH glycerol, 2 × CH_2 glycerol), 3.77–3.82 (m, 1H, CH glycerol), 4.03–4.32 (m, 6H, CH_2 cyanoethoxy, 2 × CH_2 glycerol), 4.51–4.66 (m, 6H, 3 × CH_2 Bn), 7.25–7.35 (m, 15H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2, 19.3, 19.3 (CH_2 cyanoethoxy), 60.6, 60.7 (CH_2 glycerol), 61.9 (CH_2 cyanoethoxy), 66.3, 66.4 (CH_2 glycerol),

67.4, 67.5, 67.6 (CH_2 glycerol), 68.6 (CH_2 glycerol), 71.9, 72.0 (CH_2 Bn), 72.1 (CH_2 Bn), 73.4 (CH_2 Bn), 76.2, 76.3 (CH glycerol), 77.3, 77.4, 77.5 (CH glycerol), 116.4 (Cq cyanoethoxy), 127.6–128.4 (CH_{arom}), 137.7, 137.8, 137.8 (C_q Bn); HRMS: $C_{30}H_{37}NO_8P$+Na^+ requires 592.2071, found 592.2070.

4.2.15. Glycerol phosphate trimer (22)

To a solution of dimer **21** (229 mg, 0.402 mmol) in acetonitrile (4.0 ml), containing freshly activated MS 3 Å, were added DCI (0.25 M in acetonitrile, 6.4 ml) and phosphoramidite **3** (0.2 M in acetonitrile, 2.6 ml). After stirring for 2 h, more **3** (0.2 M in acetonitrile, 0.8 ml) was added and the mixture was allowed to react for another 2 h. I_2 (0.2 M in THF/pyridine 4/1, 6.0 ml) and H_2O (1.0 ml) were added and the mixture was stirred for 1 h. EtOAc (50 ml) was added and the mixture was washed with satd aq $Na_2S_2O_3$ (20 ml), aqueous $KHSO_4$ (0.5 M, 20 ml), satd aq $NaHCO_3$ (20 ml) and brine (20 ml), respectively. The organic layer was dried with Na_2SO_4 and concentrated in vacuo, after which the residue was redissolved in DCM (20 ml), containing freshly activated MS 3 Å. Et_3SiH (0.65 ml, 4.0 mmol) and dichloroacetic acid (0.32 ml, 4.0 mmol) were added and the mixture stirred until the orange colour disappeared (~1.5 h), after which the mixture was diluted with DCM (30 ml) and washed with satd aq $NaHCO_3$ (20 ml) and brine (20 ml), respectively. The organic layer was dried with Na_2SO_4, concentrated in vacuo and the residue purified by column chromatography (MeOH/DCM), yielding trimer **22** (286 mg, 0.330 mmol, 82%) as colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.2, −1.2, −1.1, −1.0, −0.8, −0.8; ^1H NMR (400 MHz): δ = 2.47–2.58 (m, 4H, 2 × CH_2 cyanoethoxy), 2.97 (br s, 1H, OH), 3.53–3.80 (m, 7H, 3 × CH glycerol, 2 × CH_2 glycerol), 4.03–4.32 (m, 12H, 2 × CH_2 cyanoethoxy, 4 × CH_2 glycerol), 4.48–4.66 (m, 8H, 4 × CH_2 Bn), 7.25–7.33 (m, 20H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2–19.4 (CH_2 cyanoethoxy), 60.5, 60.6 (CH_2 glycerol), 61.8–62.1 (2 × CH_2 cyanoethoxy), 65.5–65.9 (2 × CH_2 glycerol), 66.5–66.7 (CH_2 glycerol), 67.4–67.7 (CH_2 glycerol), 68.6–68.7 (CH_2 glycerol), 71.9–72.1 (3 × CH_2 Bn), 73.4 (CH_2 Bn), 75.3–75.5 (CH glycerol), 76.3–76.4 (CH glycerol), 77.4–77.6 (CH glycerol), 116.4–116.5 (2 × Cq cyanoethoxy), 127.6–128.4 (CH_{arom}), 137.2, 137.7–137.8 (4 × C_q Bn); HRMS: $C_{43}H_{52}N_2O_{13}P_2$+Na^+ requires 889.2837, found 889.2843.

4.2.16. Glycerol phosphate tetramer (23)

To a solution of trimer **22** (280 mg, 0.323 mmol) in acetonitrile (3.0 ml), containing freshly activated MS 3 Å, were added DCI (0.25 M in acetonitrile, 5.2 ml) and phosphoramidite **3** (0.2 M in acetonitrile, 2.6 ml). After stirring for 2 h, more **3** (0.2 M in acetonitrile, 1.3 ml) was added and the mixture was allowed to react for another 2 h. I_2 (0.2 M in THF/pyridine 4/1, 6.5 ml) and H_2O (1.0 ml) were added and the mixture was stirred for 1 h. EtOAc (50 ml) was added and the mixture was washed with satd aq $Na_2S_2O_3$ (20 ml), aqueous $KHSO_4$ (0.5 M, 20 ml), satd aq $NaHCO_3$ (20 ml) and brine (20 ml), respectively. The organic layer was dried with Na_2SO_4 and concentrated in vacuo, after which the residue was redissolved in DCM (15 ml), containing freshly activated MS 3 Å. Et_3SiH (0.52 ml, 3.23 mmol) and dichloroacetic acid (0.27 ml, 3.23 mmol) were added and the mixture stirred until the orange colour disappeared (~2 h), after which the mixture was diluted with DCM (20 ml) and washed with satd aq $NaHCO_3$ (15 ml) and brine (15 ml), respectively. The organic layer was dried with Na_2SO_4, concentrated in vacuo and, subsequently, purified by column chromatography (MeOH/DCM), yielding tetramer **23** (305 mg, 0.262 mmol, 81%) as colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.3 to −0.8; ^1H NMR (400 MHz): δ = 2.47–2.61 (m, 6H, 3 × CH_2 cyanoethoxy), 3.20 (br s, 1H, OH), 3.56–3.58 (m, 2H, CH_2 glycerol) 3.62–3.71 (m, 3H, CH glycerol, CH_2 glycerol), 3.78–3.81 (m, 3H, 3 × CH glycerol), 4.03–4.30 (m, 18H, 3 × CH_2 cyanoethoxy, 6 × CH_2 glycerol), 4.50–4.65 (m, 10H, 5 × CH_2 Bn), 7.26–7.34 (m, 25H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2–19.4 (3 × CH_2 cyanoethoxy), 60.4, 60.5 (CH_2 glycerol), 61.8–62.2 (3 × CH_2 cyanoethoxy), 65.5–66.0 (4 × CH_2 glycerol), 66.5–66.7 (CH_2 glycerol), 67.4–67.7 (CH_2 glycerol), 68.6 (CH_2 glycerol), 71.9–72.1 (4 × CH_2 Bn), 73.4 (CH_2 Bn), 75.2–75.5 (2 × CH glycerol), 76.2–76.4 (CH glycerol), 77.4–77.6 (CH glycerol), 116.5 (3 × Cq cyanoethoxy), 127.6–128.4 (CH_{arom}), 137.2, 137.7–137.9 (5 × C_q Bn); HRMS: $C_{56}H_{68}N_3O_{18}P_3$+Na^+ requires 1186.3603, found 1186.3614.

4.2.17. Glycerol phosphate pentamer (24)

To a solution of tetramer **23** (283 mg, 0.243 mmol) in acetonitrile (3.0 ml), containing freshly activated MS 3 Å, were added DCI (0.25 M in acetonitrile, 4.0 ml) and phosphoramidite **3** (0.2 M in acetonitrile, 2.5 ml). After stirring for 4 h, more **3** (0.2 M in acetonitrile, 1.2 ml) was added and the mixture was allowed to react overnight. I_2 (0.2 M in THF/pyridine 4/1, 5.0 ml) and H_2O (1.0 ml) were added and the mixture was stirred for 1 h. EtOAc (30 ml) was added and the mixture was washed with satd aq $Na_2S_2O_3$ (15 ml), aqueous $KHSO_4$ (0.5 M, 15 ml), satd aq $NaHCO_3$ (15 ml) and brine (15 ml), respectively. The organic layer was dried with Na_2SO_4 and concentrated in vacuo, after which the residue was redissolved in DCM (15 ml), containing freshly activated MS 3 Å. Et_3SiH (0.39 ml, 2.4 mmol) and dichloroacetic acid (0.20 ml, 2.4 mmol) were added and the mixture stirred until the orange colour disappeared (~3 h), after which the mixture was diluted with DCM (20 ml) and washed with, respectively, satd aq $NaHCO_3$ (15 ml) and brine (15 ml). The organic layer was dried with Na_2SO_4, concentrated in vacuo and, subsequently, purified by column chromatography (MeOH/DCM), yielding pentamer **24** (221 mg, 0.151 mmol, 62%) as colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.3 to −0.8; ^1H NMR (400 MHz): δ = 1.67 (br s, 1H, OH), 2.45–2.64 (m, 8H, 4 × CH_2 cyanoethoxy), 3.55–3.58 (m, 2H, CH_2 glycerol) 3.62–3.71 (m, 3H, CH glycerol, CH_2 glycerol), 3.75–3.80 (m, 4H, 4 × CH glycerol), 4.04–4.28 (m, 24H, 4 × CH_2 cyanoethoxy, 8 × CH_2 glycerol), 4.49–4.51 (m, 2H, CH_2 Bn), 4.56–4.66 (m, 10H, 5 × CH_2 Bn), 7.26–7.35 (m, 30H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2–19.4 (4 × CH_2 cyanoethoxy), 60.2–60.4 (CH_2 glycerol), 62.0–62.5 (4 × CH_2 cyanoethoxy), 65.6–66.1 (6 × CH_2 glycerol), 66.7–66.9 (CH_2 glycerol), 67.6–67.9 (CH_2 glycerol), 68.5 (CH_2 glycerol), 71.9–72.2 (5 × CH_2 Bn), 73.4 (CH_2 Bn), 75.2–75.4 (3 × CH glycerol), 76.3–76.4 (CH glycerol), 77.4–77.5 (CH glycerol), 116.6–116.8 (4 × Cq cyanoethoxy), 127.6–128.5 (CH_{arom}), 137.2–137.3, 137.7–137.8 (6 × C_q Bn); HRMS: $C_{69}H_{84}N_4O_{23}P_4$+Na^+ requires 1483.4369, found 1483.4377.

4.2.18. Hexamer (25)

To a solution of pentamer **24** (150 mg, 0.103 mmol) and kojibiose-glycerol phosphoramidite **5** (350 mg, 0.257 mmol) in acetonitrile (3.0 ml), containing freshly activated MS 3 Å, was added DCI (0.25 M in acetonitrile, 2.0 ml). After stirring the mixture overnight at room temperature, I_2 (0.2 M in THF/pyridine 4/1, 2.5 ml) and H_2O (1.0 ml) were added and the mixture was stirred for 1 h. The mixture was diluted with EtOAc (30 ml) and subsequently washed with satd aq $Na_2S_2O_3$ (10 ml), aqueous $KHSO_4$ (0.5 M, 10 ml), satd aq $NaHCO_3$ (10 ml) and brine (10 ml). The organic layer was dried with Na_2SO_4 and concentrated in vacuo, after which the residue was purified by size-exclusion chromatography (sephadex LH-20, THF), followed by column chromatography (MeOH/DCM), yielding dimethoxytritylated intermediate **25** (243 mg, 88.6 μmol, 86%) as white foam. ^{31}P NMR (161.7 MHz): δ = −1.3 to −0.9; ^1H NMR (400 MHz): δ = 2.29–2.56 (m, 10H, 5 × CH_2 cyanoethoxy), 3.10–4.36 (m, 58H, 5 × CH_2 cyanoethoxy, 6 × CH glycerol, 12 × CH_2 glycerol,

2 × OMe, 2 × H-2, 2 × H-3, 2 × H-4, 2 × H-5, 4 × H-6), 4.45–4.89 (m, 18H, 9 × CH_2 Bn), 4.92–4.94 (m, 1H, H-1), 5.10–5.13 (m, 1H, H-1), 5.52 (s, 1H, CH benzylidene), 5.56 (s, 1H, CH benzylidene), 6.74 (d, 4H, J = 8.6 Hz, H_{arom} DMTr), 7.14–7.48 (m, 64H, H_{arom}); HRMS: $[C_{143}H_{158}N_5O_{40}P_5+Na]^{2+}$ requires 1392.9478, found 1392.9489.

4.2.19. Hexamer (26)

Intermediate **25** (122 mg, 44.5 μmol) was dissolved in a mixture of DCM (2.5 ml) and MeOH (25 ml). After the addition of pyridinium *para*-toluenesulfonate (PPTS, 25 mg, 0.099 mmol), the solution was allowed to stir overnight. Toluene (50 ml) was added and the mixture was concentrated partially (leaving ~30 ml) under reduced pressure. Column chromatography of the solution (MeOH/DCM) yielded hexamer **26** (76.0 mg, 31.1 μmol, 70%) as colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.3 to −0.8; ^{1}H NMR (400 MHz): δ = 1.79 (br s, 1H, OH), 2.44–2.57 (m, 10H, 5 × CH_2 cyanoethoxy), 3.38–4.34 (m, 52H, 5 × CH_2 cyanoethoxy, 6 × CH glycerol, 12 × CH_2 glycerol, 2 × H-2, 2 × H-3, 2 × H-4, 2 × H-5, 4 × H-6), 4.51–4.65 (m, 12H, 6 × CH_2 Bn), 4.76–4.99 (m, 7H, 3 × CH_2 Bn, H-1), 5.11–5.13 (m, 1H, H-1), 5.50 (s, 1H, CH benzylidene), 5.55 (s, 1H, CH benzylidene), 7.15–7.17 (m, 3H, H_{arom}), 7.26–7.40 (m, 48H, H_{arom}), 7.42–7.47 (m, 4H, H_{arom}); HRMS: $[C_{122}H_{140}N_5O_{38}P_5+Na]^{2+}$ requires 1241.8824, found 1241.8837.

4.2.20. Hexamer-spacer (27)

To a solution of kojibiose substituted hexamer **26** (124 mg, 51.0 μmol) and *Z*-aminohexylphosphoramidite **5** (184 mg, 0.408 mmol) in acetonitrile (3.0 ml), containing freshly activated MS 3 Å, was added DCI (0.25 M in acetonitrile, 2.0 ml). After stirring the mixture for 4 h, acetonitrile (5.0 ml), I_2 (0.2 M in THF/pyridine 4/1, 3.1 ml) and H_2O (1.0 ml) were added and the mixture stirred for 1 h. The mixture was diluted with EtOAc (30 ml) and subsequently washed with satd aq $Na_2S_2O_3$ (10 ml), aqueous $KHSO_4$ (0.5 M, 10 ml), satd aq $NaHCO_3$ (10 ml) and brine (10 ml). The organic layer was dried with Na_2SO_4 and concentrated in vacuo, after which the residue was purified by size-exclusion chromatography (sephadex LH-20, MeOH/DCM), followed by column chromatography (MeOH/DCM), yielding compound **27** (111 mg, 39.5 μmol, 77%) as colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.3 to −0.9; ^{1}H NMR (400 MHz): δ = 1.18–1.32 (m, 4H, 2 × CH_2 hexylspacer), 1.35–1.44 (m, 2H, CH_2 hexylspacer), 1.53–1.62 (m, 2H, CH_2 hexylspacer), 2.44–2.58 (m, 12H, 6 × CH_2 cyanoethoxy), 3.05–3.13 (m, 2H, CH_2–N hexylspacer), 3.56–4.33 (m, 56H, 6 × CH_2 cyanoethoxy, 6 × CH glycerol, 12 × CH_2 glycerol, CH_2–O hexylspacer, 2 × H-2, 2 × H-3, 2 × H-4, 2 × H-5, 4 × H-6), 4.50–4.59 (m, 12H, 6 × CH_2 Bn), 4.74–5.15 (m, 10H, 4 × CH_2 Bn, 2 × H-1), 5.53 (s, 1H, CH benzylidene), 5.55 (s, 1H, CH benzylidene), 7.16–7.18 (m, 3H, H_{arom}), 7.26–7.39 (m, 53H, H_{arom}), 7.43–7.48 (m, 4H, H_{arom}); HRMS: $[C_{139}H_{163}N_7O_{43}P_6+Na]^{2+}$ requires 1424.9497 found 1424.9512.

4.2.21. Teichoic acid (1)

A solution of protected hexamer **27** (51.0 mg, 18.2 μmol) in dioxane (5.0 ml) was diluted with ammonia (25% in H_2O, 5.0 ml) and stirred overnight. The solution was concentrated in vacuo and the residue redissolved in H_2O (2.0 ml). The solution was then eluted through a small column containing Amberlite Na^+ resin. The volatiles were removed under reduced pressure and the residue lyophilized, yielding the partially protected intermediate (47.6 mg, 100%) as amorphous white solid. A part of the intermediate (23.6 mg, 9.01 μmol) was dissolved in a mixture of dioxane (2.0 ml), H_2O (2.0 ml) and AcOH (three drops). After the addition of Palladium black (~50 mg, 0.5 mmol), the mixture was allowed to stir for five days under a hydrogen atmosphere. The mixture was filtered, concentrated in vacuo, and purified by size-exclusion chromatography (Sephadex HW40, 0.15 M NH_4HCO_3). The purified residue was lyophilized twice before it was dissolved in H_2O (2.0 ml) and eluted through a small column containing Amberlite Na^+ resin. Cryodesiccation of the filtrate yielded deprotected teichoic acid **1** (10.6 mg, 6.81 μmol, 76%) as amorphous white solid. ^{31}P NMR (161.7 MHz, D_2O): δ = 0.9 (1P), 1.1 (1P), 1.2 (3P), 1.3 (1P); ^{1}H NMR (600 MHz, D_2O): δ = 1.36–1.40 (m, 4H, 2 × CH_2 hexylspacer), 1.59–1.66 (m, 4H, 2 × CH_2 hexylspacer), 2.95 (t, 2H, J = 7.5 Hz, CH_2–N hexylspacer), 3.37–3.41 (m, 2H, 2 × H-5), 3.51–3.56 (m, 2H, C*H*H glycerol, H-2), 3.61–3.64 (m, 2H, C*H*H glycerol, H-2), 3.70–4.01 (m, 37H, 5 × CH glycerol, 11 × CH_2 glycerol, CH_2–O hexylspacer, 2 × H-3, 2 × H-4, 4 × H-6), 4.11–4.14 (m, 1H, CH glycerol), 5.11 (d, 1H, J = 3.7 Hz, H-1), 5.39 (d, 1H, J = 3.5 Hz, H-1); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.1, 27.6 (3 × CH_2 hexylspacer), 30.4 (d, J = 6.7 Hz, CH_2 hexylspacer), 40.4 (CH_2–N hexylspacer), 61.4, 61.4 (2 × C-6), 63.0 (CH_2 glycerol), 65.5 (d, J = 5.1 Hz, CH_2 glycerol), 66.0 (d, J = 4.4 Hz, CH_2 glycerol), 67.0–67.3 (9 × CH_2 glycerol, CH_2–O hexylspacer), 70.4–70.6 (4 × CH glycerol, 2 × C-5), 71.7 (d, J = 7.8 Hz, CH glycerol), 72.1 (C-2, C-3), 72.7 (2 × C-4), 73.3 (C-3), 75.1 (C-2), 76.0 (t, J = 8.3 Hz, CH glycerol), 95.6 (C-1), 96.4 (C-1). HRMS: $C_{36}H_{77}NO_{41}P_6+H^+$ requires 1366.2469 found 1366.2483.

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Supplementary data

Supplementary data (^{1}H, ^{13}C, ^{31}P NMR spectra) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.03.071.

References and notes

1. (a) Weidenmaier, C.; Peschel, A. *Nat. Rev. Microbiol.* **2008**, *6*, 276; (b) Neuhaus, F. C.; Baddiley, J. *Microbiol. Mol. Rev.* **2003**, *67*, 686; (c) Fischer, W. *Adv. Microb. Physiol.* **1988**, *29*, 233.
2. (a) Naumova, I. B.; Shashkov, A. S.; Tul'skaya, E. M.; Streshinskaya, G. M.; Kozlova, Y. I.; Potekhina, N. V.; Evtushenko, L. I.; Stackebrandt, E. *FEMS Microbiol. Rev.* **2001**, *25*, 269; (b) Nikolaev, A. V.; Botvinko, I. V.; Ross, A. J. *Carbohydr. Res.* **2007**, *342*, 297.
3. Wicken, A. J.; Knox, K. W. *Science* **1975**, *187*, 1161.
4. (a) Morath, S.; Stadelmaier, A.; Geyer, A.; Schmidt, R. R.; Hartung, T. *J. Exp. Med.* **2002**, *195*, 1635; (b) Stadelmaier, A.; Morath, S.; Hartung, T.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **2003**, *42*, 916; (c) Deininger, S.; Stadelmaier, A.; von Aulock, S.; Morath, S.; Schmidt, R. R.; Hartung, T. *J. Immun.* **2003**, *170*, 4134; (d) Morath, S.; von Aulock, S.; Hartung, T. *J. Endotoxin Res.* **2005**, *6*, 348; (e) Figueroa-Perez, I.; Stadelmaier, A.; Morath, S.; Hartung, T.; Schmidt, R. R. *Tetrahedron: Asymmetry* **2005**, *16*, 493; (f) Figueroa-Perez, I.; Stadelmaier, A.; Deininger, S.; von Aulock, S.; Hartung, T.; Schmidt, R. R. *Carbohydr. Res.* **2006**, *341*, 2901; (g) Stadelmaier, A.; Figueroa-Perez, I.; Deininger, S.; von Aulock, S.; Hartung, T.; Schmidt, R. R. *Bioorg. Med. Chem.* **2006**, *14*, 6239.
5. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Fabretti, F.; Sange, T.; Holst, O.; Huebner, J. *Infect. Immun.* **2006**, *74*, 5703.
6. (a) Wang, Y.; Huebner, J.; Tzianabos, A. O.; Martirosian, G.; Kasper, D. L.; Pier, G. B. *Carbohydr. Res.* **1999**, *316*, 155; (b) Huebner, J.; Wang, Y.; Krueger, W. A.; Madoff, L. C.; Martirosian, G.; Boisot, S.; Goldmann, D. A.; Kasper, D. L.; Tzianabos, A. O.; Pier, G. B. *Infect. Immun.* **1999**, *67*, 1213.
7. (a) Westerduin, P.; Veeneman, G. H.; Pennings, Y.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1987**, *28*, 1557; (b) Elie, C. J. J.; Muntendam, H. J.; van den Elst, H.; van der Marel, G. A.; Hoogerhout, P.; van Boom, J. H. *Rec. Trav. Chim. Pays-Bas* **1989**, *108*, 219; (c) Veeneman, G. H.; Brugghe, H. F.; van den Elst, H.; van Boom, J. H. *Carbohydr. Res.* **1990**, *195*, C1.
8. (a) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223; (b) Smith, M.; Rammler, D. H.; Goldberg, I. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1962**, *84*, 430.
9. van Boeckel, C. A. A.; Visser, G. M.; van Boom, J. H. *Tetrahedron* **1985**, *41*, 4557.
10. Oltvoort, J. J.; van Boeckel, C. A. A.; De koning, J. H.; van Boom, J. H. *Synthesis* **1981**, 305.

11. (a) Kim, J.-H.; Yang, H.; Park, J.; Boons, G.-J. *J. Am. Chem. Soc.* **2005**, *127*, 2357; (b) Kim, J.-H.; Yang, H.; Boons, G.-J. *Angew. Chem., Int. Ed.* **2005**, *44*, 947; (c) Kim, J.-H.; Yang, H.; Khot, V.; Whitfield, D.; Boons, G.-J. *Eur. J. Org. Chem.* **2006**, 5007.
12. (a) Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056; (b) van Boeckel, C. A. A.; van Boom, J. H. *Chem. Lett.* **1981**, 581.
13. Adinolfi, M.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 6479.
14. Crich, D.; de la Mora, M.; Vinod, A. U. *J. Org. Chem.* **2003**, *68*, 8142.
15. Codée, J. D. C.; Stubba, B.; Schiattarella, M.; Overkleeft, H. S.; van Boeckel, C. A. A.; van Boom, J. H.; van der Marel, G. A. *J. Am. Chem. Soc.* **2005**, *127*, 3767.
16. Codée, J. D. C.; Litjens, R. E. J. N.; den Heeten, R.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1519.
17. Fukase, K.; Hashida, M.; Kusumoto, S. *Tetrahedron Lett.* **1991**, *32*, 3557.
18. Zheng, W.; DeMattei, J. A.; Wu, J.-P.; Duan, J. J.-W.; Cook, L. R.; Oinuma, H.; Kishi, Y. *J. Am. Chem. Soc.* **1996**, *118*, 7946.
19. Alternatively, the residue could be purified by size-exclusion chromatography (sephadex LH-20 DCM/MeOH 1/1). ^{1}H NMR analysis of the resulting mixture of pseudodisaccharides revealed an α/β ratio of $\sim$6/1, based on H-1 integrals.
20. Chipowsky, S.; Lee, Y. C. *Carbohydr. Res.* **1973**, *31*, 339.