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Chemical synthesis of fragments of streptococcal cell wall polysaccharides

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Citation
Wang, Z. (2020, October 8). *Chemical synthesis of fragments of streptococcal cell wall polysaccharides*. Retrieved from https://hdl.handle.net/1887/137445

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Author: Wang, Z.
Title: Chemical synthesis of fragments of streptococcal cell wall polysaccharides
Issue Date: 2020-10-08

Chapter 3

The First Total Synthesis of Repeating Units of Glycerol Phosphate Modified Capsular Polysaccharides from Group A *Streptococcus*

Introduction

Group A *Streptococcus* (GAS), also known as *Streptococcus pyogenes*, a β-hemolytic Gram-positive bacterium, has been recognized as a remarkable human pathogen, ranking among the top ten causes of infectious disease induced mortality and morbidity.[1] It is responsible for streptococcal pharyngitis, acute rheumatic fever (ARF) and infective endocarditis complicating rheumatic heart disease (RHD), inflicting school-age children and people with a weakened immune system, such as pregnant woman on a global scale, but especially in poor and overcrowded regions.[2]

Despite the use of effective antimicrobials, annually, at least 517,000 people die and 18.1 million suffer from GAS infected disease.[1b] To improve this situation, the development of a safe and effective vaccine is urgent. Bacterial cell-surface carbohydrates play a crucial role in host–bacteria interactions, including immunological recognition events.[3] In last century, a variety of GAS proteins and group A carbohydrate (GAC) molecules[4] have been evaluated as vaccine candidates. Because of the diversity of serotypes, it is difficult to design and synthesize a versatile oligosaccharide vaccine,[5] and until now, no safe and effective vaccine is available to prevent infections, even although human vaccination trial was launched 100 years ago.[6]

The GAC was first characterized by the group of Kindt in 1975, and is built up from a polyrhamnose backbone, containing alternating α-(1,2)- and α-(1,3)-linked residues, having an *N*-acetyl glucosamine (GlcNAc) attached to the rhamnosyl O-3 of the backbone (Fig. 1a).[7] This polysaccharide adopts a helical conformation with the polyrhamnose forming the core of the helix and the immunodominant *N*-acetylglucosamine residue displaying on the periphery.[4d, 8] Various GAC fragments, ranging from disaccharide to nonasaccharide structures, have been generated by different groups, including the groups of Bundle[4a], Pinto[9], costantino[4b] and Gu[4c]. Several conjugates were synthesized to explore carbohydrate-based GAS vaccines, which were successfully used for the induction of antibodies and raise of immune responses, showing protective efficacy comparable to the GAS polysaccharide conjugate.[4b, c] It has been speculated that the GlcNAc residues play an important role in evading the innate immune system, because strains which lack GlcNAc transferase, responsible for the decoration of the polyrhamnose backbone with these residues, show attenuated virulence.[8c]

Recently, a glycerol phosphate (GroP) modification on the GAC (GroP GAC) was discovered by Van Sorge and Korotkova and co-workers (Fig. 1b).[10] They revealed that the biosynthetic cluster, gacABCDEFGHIJKL, not only encodes for enzymes responsible for the synthesis of the polyrhamnose backbone and the installation of the GlcNAc sidechain, but also for a GroP transferase enzyme, that can transfer an *sn*-Gro-1-P moiety to the C-6 of the GAC-GlcNAc residues using phosphatidylglycerol as donor. Based on NMR analysis, approximately 25% of the GAC sidechain GlcNAcs are functionalized by GroP at its O-6 position.

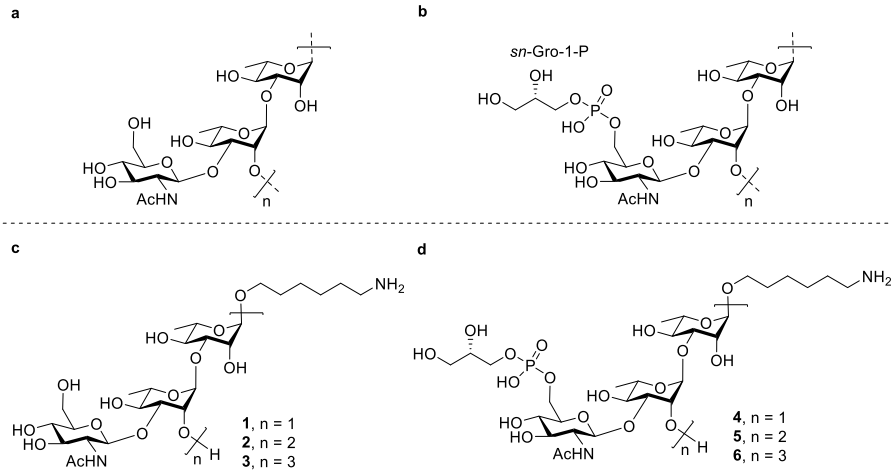


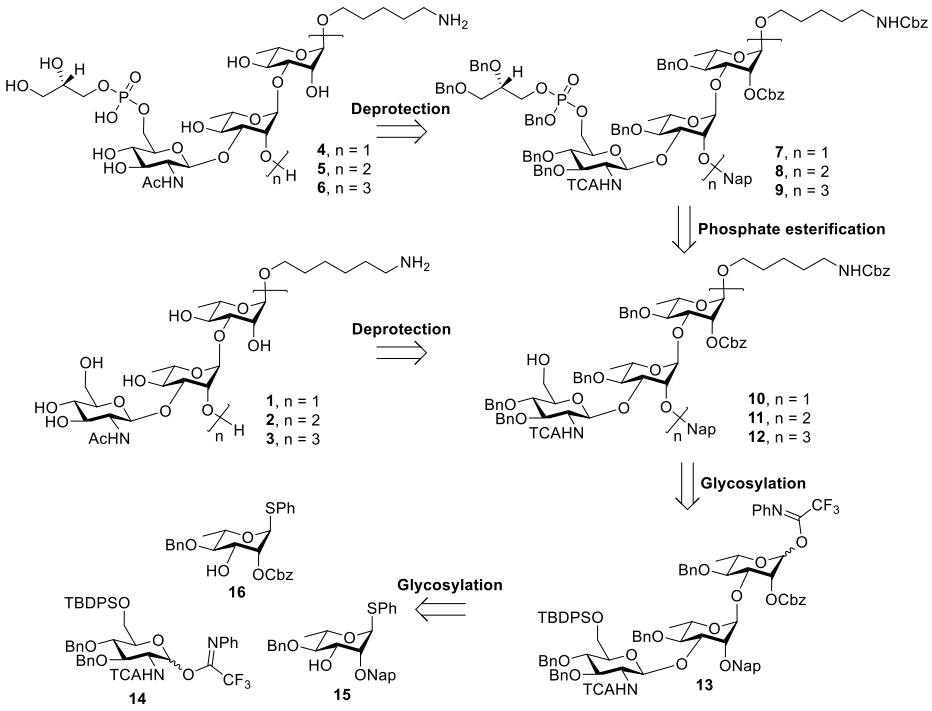
Figure 1. **a**, the structure of GAC. **b**, the structure of glycerol phosphate modified GAC. **c**, the designed fragments of GAC **1**-**3**. **d**, the designed fragments of GroP GAC **4**-**6**.

Fragments of the newly discovered *sn*-GroP modified GAC may be promising candidates to function as antigens in the development of a novel GAS vaccine. Therefore, this Chapter reports on the development of a synthetic methodology to generate GroP-GAC fragments of different length. Six fragments **1** - **6**, ranging from trisaccharides to nonasaccharides, with and without the *sn*-GroP-modification at each GlcNAc residue were designed as shown in Fig. 1c and 1d. For future conjugation purposes with proteins or other molecules, a spacer was required that can be chemoselectively addressed. To this and an aminohexanol spacer was included in the structures.

Results and discussion

Considering a late stage introduction of the glycerol phosphate groups and the required deprotection steps, the retrosynthetic analysis, shown in Scheme 1 was drafted. The spacer amine was protected with a benzyloxycarbonyl (Cbz) group. Commonly, primary amines are protected with both a Cbz and a benzyl group to prevent side reactions of the carbamate with electrophilic species generated during the glycosylation reactions. Removal of the benzyl group from the amine however can make the final deprotection step significantly more cumbersome. In addition, double protection of the amine leads to the formation of rotamers that complicate NMR analysis of the synthetic intermediates. Therefore, it was decided to solely protect the amine of the spacer with a Cbz group. To facilitate the global deprotection of the molecules, benzyl groups were planned to be used to mask the phosphotriesters and the C2-OH of the rhamnosides were blocked with benzyl carbonates. The latter groups can

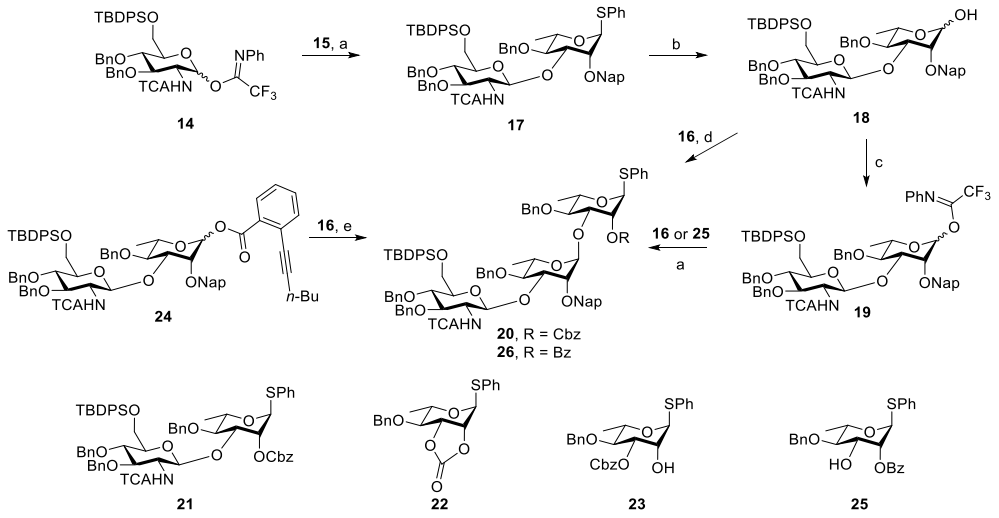
provide neighboring group participation during the construction of the desired α-rhamnosyl linkages. The 2-naphthylmethyl (Nap) group was chosen as temporary protecting group to mask the rhamnosyl C-2-OH to be elongated. This way, the most complex GroP modified nonasaccharide **6** could be obtained after only one hydrogenation step from the fully protected intermediate **9**, which in turn can be constructed by a coupling between a glycerol phosphoramidite and nonasaccharide **12**. The latter nonasaccharide, that can also be used to generate structures without a GroP appendage, could be obtained using three glycosylations with the key trisaccharide imidate donor **13**. It was envisioned that this key trisaccharide could be obtained from monosaccharides **14**-**16**.



Scheme 1. Retrosynthetic analysis of the GroP GAC-fragments. The orthogonal GlcNAc C-6-O-protecting group allows for the generation of fragments with and without a GroP groups.

Monosaccharide building blocks **14**-**16** were readily synthesized from D-glucosamine and L-rhamnose, and the detailed procedures are described in Scheme I – II in the Experiential Section. After all required building blocks were prepared, the glycosylation reactions outlined in Scheme 2 were undertaken. As expected, the TBSOTf mediated glycosylation between donor **14** and acceptor **15** produced disaccharide **17** in a good yield. To glycosylate the thioglycoside acceptor **16**, donor **17** was transformed into the corresponding imidate **19** in

two steps, including hydrolysis the thiophenyl acetal to the hemiacetal using NIS in a mixture of acetone/H_2O and installation of the imidate moiety. However, when the glycosylation conditions, used for the successful condensation of **14** and **15**, were employed to couple **19** and acceptor **16**, the desired trisaccharide **20** was obtained in a very poor yield (Table 1, entry 1). Three major side-products **21** - **23** were isolated and fully characterized with the aid of NMR-spectroscopy and HRMS-analysis. The last two byproducts were generated because of the acidic reaction conditions.[11] The transformation to provide disaccharide **21** would require cleavage of the glycosidic linkage in the donor disaccharide and subsequent addition of the acceptor. Why this has taken place is unclear at present but it is clear that the acid is infaust for the [2+1] glycosylation. To optimize the glycosylation, a dehydrative glycosylation was performed utilizing lactol **18** as donor. But even after significant optimization, the yield of **20** did not surpass 52% (Table 1, entry 2 and 3). Lastly, donor **24** was explored in combination with mild gold catalyzed activation conditions.[12] Unexpectedly, this glycosylation procedure produced an α/β-mixture in moderate yield (Table 1, entry 4 and 5). Based on these results, the acid labile Cbz group in **16** was replaced by a more stable benzoyl group and the use of monosaccharide **25** was explored. The glycosylation between imidate **19** and **25** using TBSOTf as promotor provided the key trisaccharide **26** in 73% as a single anomer (Table 1, entry 6).



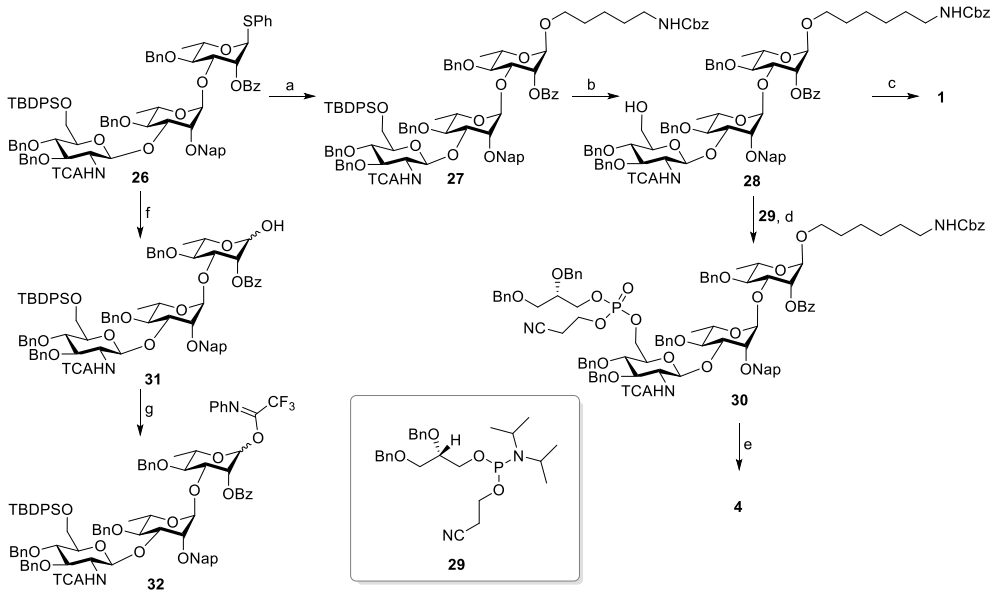
Scheme 2. The assembly of the key intermediate trisaccharide.

Reagents and conditions: a) TBSOTf, 4Å MS, DCM, 0 °C, **17**, 87%; **20**: 16%; **26**: 73%. b) NIS, acetone, water, 0 °C, 81%. c) Cs_2CO_3, acetone, *N*-phenyltrifluoroacetimidoyl chloride, 92%. d) Ph_2SO, DTBMP, DCM, 4Å MS, - 60 °C, then Tf_2O, 52%. e) PPh_3AuCl, $AgNTf_2$, DCM, 4Å MS, 0 °C, α/β mixture 55%.

Table 1. The optimization of [2+1] glycosylation

entry	donor	acceptor	condition	temperature	yield	α/β
1	**19**	**16**	TBSOTf	0 °C	16%	α only
2	**18**	**16**	Ph_2O, Tf_2O	- 40 °C to 0 °C	52%	α only
3	**18**	**16**	Ph_2O, Tf_2O	- 40 °C to -20 °C	28%	α only
4	**24**	**16**	PPh_3AuNTf_2	0 °C	55%	1.5/1
5	**24**	**16**	PPh_3AuNTf_2	- 78 °C	45%	1.1/1
6	**19**	**25**	TBSOTf	0 °C	73%	α only

With the key intermediate trisaccharide in hand, the synthesis of the first two target trisaccharides **1** and **4** were undertaken as depicted in Scheme 3. The glycosylation between the Cbz-protected aminohexanol spacer and thio-donor **26** was carried out under the promotion of NIS-TBSOTf in dry DCM to give the compound **27** in an excellent yield. Notably, treatment of **27** with HF/Pyridine or TBAF did not lead to the removal of the TBDPS group. The silyl ether could be selectively deprotected using TBAF and AcOH in THF at 50 °C for 5h.[13] The first target **1** was obtained from trisaccharide **28** after the removal of benzoyl group using NaOMe in methanol and global deprotection by hydrogenation, in 71% yield. The glycerol phosphate moiety was installed to the C-6 hydroxyl of the glucosamine residue utilizing glycerol phosphoramidite **29** that was activated by dicyanoimidazole, followed by in situ oxidation of the P(III) to P(V) using CSO. This way, phosphate **30** was assembled in 89% yield from **28**. In contrast to the described retrosynthetic pathway (Scheme 1), a cyanoethyl protecting group was used to mask the phosphate **30**, because the presence of the benzoate at the rhamnosyl C-2-hydroxyl required a deprotection step using basic conditions. The first GroP modified target trisaccharide **4** was obtained in 72% yield after a three step deprotection sequence, involving the subsequent removal the cyanoethyl and benzoyl groups using basic conditions and global hydrogenation using $Pd(OH)_2$ in *tert*-butanol/water.

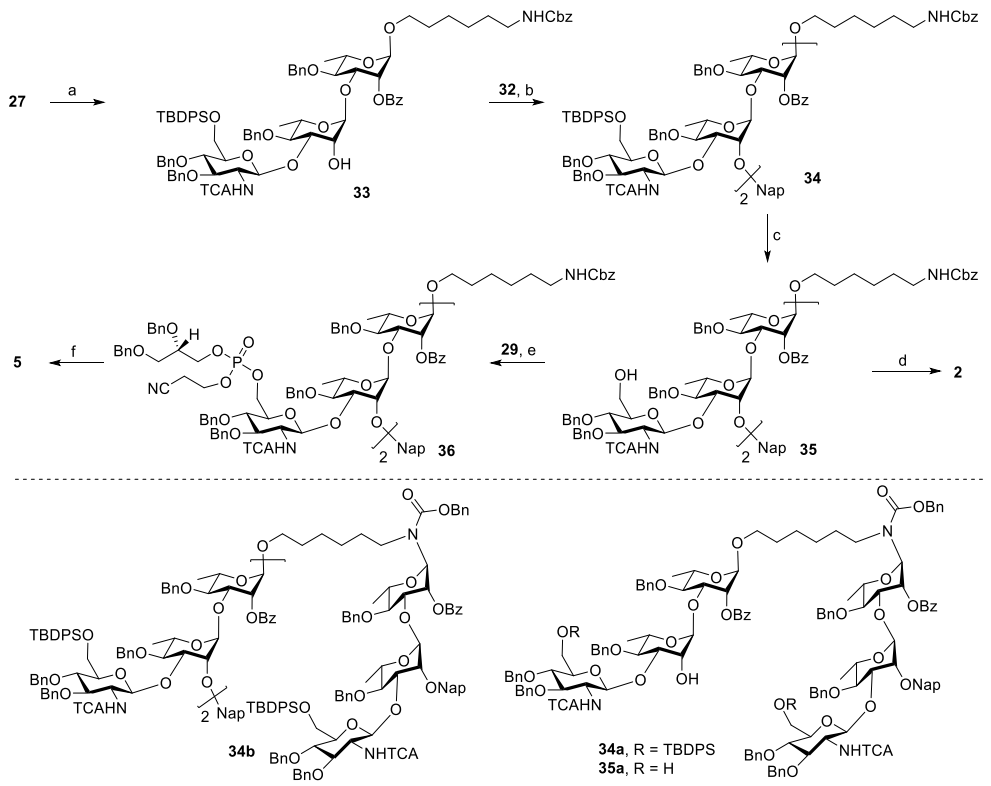


Scheme 3. The assembly of the target trisaccharides **1** and **4**.

Reagents and conditions: a) benzyl (6-hydroxyhexyl)carbamate, NIS, TBSOTf, 4Å MS, DCM, 0 °C, 95%. b) TBAF, AcOH, THF, 50 °C, 97%. c) i, NaOMe, MeOH/1,4-diaxane; ii, $Pd(OH)_2/C$, H_2, AcOH, *t*-BuOH/H_2O, 3 days, 71%. d) DCI, MeCN, 3Å MS, then CSO, 89%. e) i, ammonium hydroxide, 1,4-dioxane; ii, NaOMe, MeOH/1,4-diaxane; iii, $Pd(OH)_2/C$, H_2, AcOH, *t*-BuOH/H_2O, 3 days, 72%. f) NIS/TFA, DCM, 0 °C, 90%. g) *N*-phenyltrifluoroacetimidoyl chloride, Cs_2CO_3, acetone, 86%.

To assemble the hexa- and nonasaccharides, the trisaccharide donor **26** was transformed into imidate donor **32** using an NIS/TFA-mediated hydrolysis to provide the hemiacetal,[14] which was reacted with the *N*-phenyltrifluoroacetimidoyl chloride in the presence of Cs_2CO_3 to deliver **32** (Scheme 3). To elongate trisaccharide **27**, the Nap protecting group was removed oxidatively using DDQ in DCM and neutral water to provide the trisaccharide alcohol **33** in 91% yield as shown in Scheme 4. The [3 + 3] glycosylation represents a crucial but difficult step because of the low reactivity of the axial hydroxyl in L-rhamnose, the steric hindrance of the adjacent glucosamine residue and the possibility of *N*-glycosylation of the linker. The glycosylation between **33** and thio-donor **26** was tested employing NIS and TBSOTf as promotor to give the desired hexasaccharide **34** in 56% yield, alongside with 23% of the recovered acceptor **33** (Table 2, entry 1). Additionally, a significant amount of side-product nonasaccharide **34b** was isolated (21%), whose structure was verified by NMR. Another side product, **34a** was also obtained and its structure was determined after desilylation, giving product **35a**, by NMR and HRMS. The spacer *N*-glycosylation was not

observed in the generation of trisaccharide **27**, indicating that the lower reactivity of the rhamnosyl C-2-OH in **33**, opens up the way for this type of side reaction. To prevent the *N*-glycosylation, the imidate donor **32** was used and the reaction of this donor with **33** was optimized as shown in Table 2. It can be seen that a lower temperature was favorable for the construction of the desired product, and that the use of more donor leads to more side product **34b** (Table 2, entry 5). Finally, the use of 1.3 equivalents of the donor at – 20 °C was found to be optimal delivering the desired product in 78% yield (Table 2, entry 4). Subsequently, the two TBDPS ethers were removed by overnight treatment with TBAF/AcOH, to provide hexasaccharide **35** in 82% yield. To complete the synthesis of target hexasaccharide **2**, the compound **35** was deprotected by subsequent basic hydrolysis and hydrogenation using $Pd(OH)_2$ in *tert*-butanol and water to give compound **2** in 60% yield (5.5 mg). The conjugation of hexasaccharide **35** with phosphoramidite **29** using dicyanoimidazole as activator and in-situ oxidation by CSO proceeded smoothly to furnish the glycerol phosphate modified **36** in excellent yield. Hydrolysis of the cyanoethyl groups and subsequent removal of the benzoates was followed by global hydrogenation using $Pd(OH)_2$ in *tert*-butanol and water to afford the desired hexasaccharide **5**. Notably, the benzoyls of the two hexasaccharides were difficult to remove, and after the hydrogenation it appeared that they were in part still present. Therefore, the final product was treated with NaOH in water after the hydrogenation, to effectively remove the remaining benzoates and deliver the pure target compound, which was isolated in 65% yield (15 mg).



Scheme 4. The assembly of the target hexasaccharide of **2** and **5**

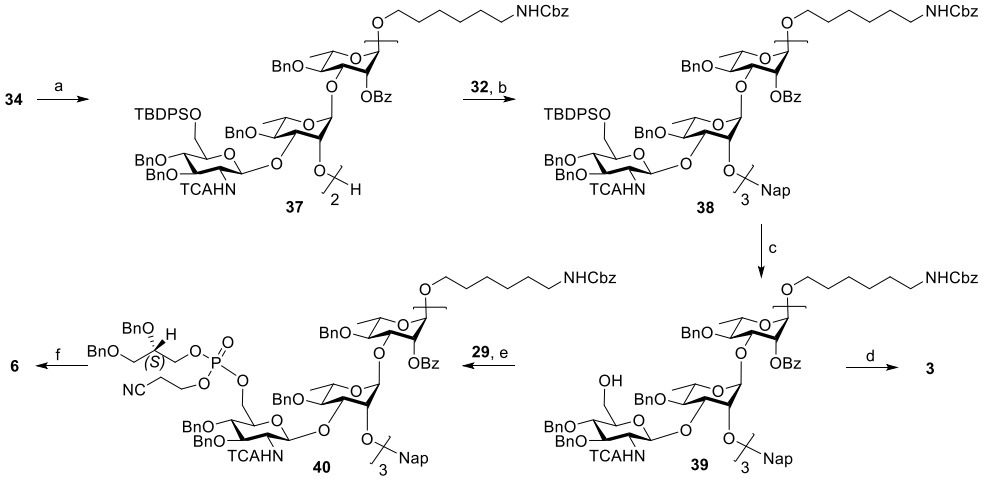
Reagents and conditions: a) DDQ, DCM, pH 7 phosphate buffer in water, 0 °C, 91%. b) TBSOTf, DCM, - 20 °C, 78%. c) TBAF, AcOH, THF, 50 °C, 82%. d) i, NaOMe, MeOH/1,4-diaxane; ii, $Pd(OH)_2/C$, H_2, AcOH, *t*-BuOH/H_2O, 3 days, 60% (over three steps). e) DCI, MeCN, 3Å MS, then CSO, 95%. f) i, ammonium hydroxide, 1,4-dioxane; ii, NaOMe, MeOH/1,4-diaxane; iii, $Pd(OH)_2/C$, H_2, AcOH, *t*-BuOH/H_2O, 3 days; iv, 1M NaOH, water, 65% (over four steps).

Table 2. Optimization of the **[3+3]** glycosylation

entry	donor	equivalent	temperature	**33**/%	**34**/%	**34b**/%
1	**26**	2.0	0 °C	23	56	21
2	**32**	1.2	0 °C	-	51	-
3	**32**	1.1	-20 °C	35	58	4
4	**32**	1.3	-20 °C	14	78	8
5	**32**	1.7	-20 °C	-	65	18

Based on the established procedure, described above, the two nonasaccharide targets **3** and **6** were synthesized via a [3 + 6] glycosylation as described in Scheme 5. First, and according

to the synthesis of **2** and **5**, removal of the Nap protecting group in hexasaccharide **34** through an oxidation by DDQ in a mixture of DCM and a neutral phosphate buffer, provided hexasaccharide **37** in 77%. Then, this hexasaccharide **37** was glycosylated with 1.3 equivalents of the trisaccharide imidate donor **32** under the promotion of TBSOTf at -20 °C to furnish the nonasaccharide **38** in 49% yield. The *N*-glycosylated side-product **38a** was formed in 21% under these conditions. Thereafter, the three TBDPS protecting groups were unmasked using TBAF/AcOH at 50 °C to generate the triol **39** in 65% yield. Because of the difficult deprotection of the benzoyl protecting groups, the sequence of the deprotection was reversed, performing the hydrogenation prior to the removal of the benzoyls. By doing so, the nonasaccharide **3** was generated in 54% yield (9.4 mg). The glycerol phosphate modified nonasaccharide was assembled from triol **39**. First the three protected glycerol phosphate triesters were installed using 9 equivalents of glycerol phosphoramidite **29** in combination with an excess DCI and oxidation of the so-formed phosphites by CSO. Then, the glycerol phosphate GAS nonasaccharide was deprotected by the removal of the cyanoethyl groups using ammonium hydroxide in dioxane, hydrogenation with $Pd(OH)_2$ and finally saponification of the benzoates to deliver the target nonasaccharide **6** in 65% yield (18 mg).



Scheme 5. The assembly of the two nonasaccharide targets **3** and **6**

Reagents and conditions: a) DDQ, DCM, pH 7 phosphate buffer in water, 0 °C, 77%. b) TBSOTf, DCM, - 20 °C, 49%. c) TBAF, AcOH, THF, 50 °C, 65%. d) i, $Pd(OH)_2$/C, H_2, AcOH, *t*-BuOH/H_2O, 3 days; ii, NaOH, water, 54%. e) DCI, MeCN, 3Å MS, then CSO, 91%. f) i, ammonium hydroxide, 1,4-dioxane; ii, $Pd(OH)_2$/C, H_2, AcOH, *t*-BuOH/H_2O, 3 days,; iii, NaOH, water, 65%.

Conclusion

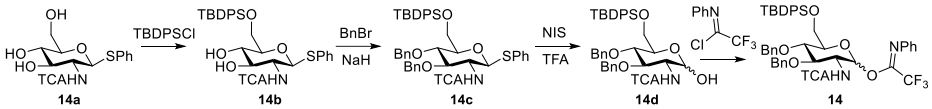
This Chapter describes the first total synthesis of glycerol phosphate modified fragments of the *Streptococcus* group A carbohydrate ranging in length from one to three repeating units. Also, the corresponding GAC-fragments lacking the glycerol phosphate appendages were synthesized. All six oligosaccharides were functionalized with a free amine terminated six-carbon spacer for further modification. A properly protected trisaccharide was used to assemble the two tri-, two hexa- and two nonasaccharides, employing a highly convergent strategy via [3 + 3] and [3 + 6] glycosylations. The glycosylation results showed that the reactivity of Cbz protected amines can lead to the formation of *N*-glycosylated side products, which become more prevalent upon decreasing reactivity of the acceptor alcohol. Nonetheless the desired tri-, hexa- and nonasaccharides were obtained in sufficient yields to complete the syntheses and deliver the target compounds in multi-milligram amounts. The set of compounds will be tested for their antigenic activity. The chemistry developed here can be readily adapted to generate GAC-fragments, that are substituted in a no-stoichiometric manner with glycerol phosphate groups. The generation of a set of substituted GAC-fragments will be valuable for more detailed structure-activity relationship studies.

Experimental section

General experimental procedures

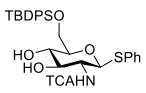
All reagents were of commercial grade and used as received. All moisture sensitive reactions were performed under an argon atmosphere. DCM used in the glycosylation reactions was dried with flamed 4Å molecular sieves before being used. Reactions were monitored by TLC analysis with detection by UV (254 nm) and where applicable by spraying with 20% sulfuric acid in EtOH 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% sulfuric acid (aq.) followed by charring at ~150 °C. Flash column chromatography was performed on silica gel (40-63μm). ^1H and ^{13}C spectra were recorded on a Bruker AV 400 or Bruker AV 500 or Bruker AV 600 and Bruker AV 850 in $CDCl_3$ or D_2O. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard (^1H NMR in $CDCl_3$) or the residual signal of the deuterated solvent. Coupling constants (*J*) are given in Hz. All ^{13}C spectra are proton decoupled. NMR peak assignments were made using COSY and HSQC experiments, where applicable Clean TOCSY, HMBC and GATED experiments were used to further elucidate the structure. The anomeric product ratios were analyzed through integration of proton NMR signals.

Experimental Procedures and Characterization Data of Products



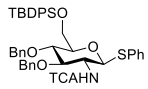
Scheme I. The synthesis of building block **14**.

Phenyl 2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-glucopyranoside (14b)



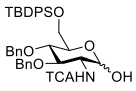
The known compound **14a**[15] (17.2 g, 41.2 mmol, 1.0 eq) was dissolved in DMF (140 mL) and cooled to 0 °C. *tert*-Butyl(chloro)diphenylsilane (TBDPSCl) (16 mL, 61.8 mmol, 1.5 eq) and imidazole (5.6 g, 82 mmol, 2 eq) were added at 0 °C. It was stirred at RT 4 hours and checked by TLC. After completed consumption of the starting material, diluted with EtOAc and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 8:1:1 - 2:1:1) to yield compound **14b** (24.8 g, 38 mmol, 92%). ^1H NMR (400 MHz, Chloroform-*d*) δ 7.77 – 7.64 (m, 4H), 7.54 – 7.45 (m, 2H), 7.45 – 7.30 (m, 6H), 7.27 – 7.17 (m, 3H), 7.13 (d, *J* = 7.8 Hz, 1H, NH), 4.93 (d, *J* = 10.2 Hz, 1H, H-1), 4.01 – 3.79 (m, 4H, H-6, H-3), 3.60 – 3.37 (m, 4H, H-2, H-4, H-5), 1.05 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.53 (NHTCA), 135.75, 135.71, 133.02, 132.92, 132.88, 132.05, 129.99, 129.16, 128.32, 127.94, 92.48 (TCA), 85.24 (C-1), 79.55 (C-5), 75.07 (C-3), 72.08 (C-4), 64.15 (C-6), 56.76 (C-2), 26.92 (*t*-Bu), 19.33 (*t*-Bu). HR-MS: Calculated for $C_{30}H_{34}Cl_3NO_5SSi$ $[M+Na]^+$: 676.08847, found: 676.08855. $[\alpha]^{25}_D$ = - 15.9° (c = 1, $CHCl_3$). TLC: Rf = 0.15 (PE/EA = 2/1, v/v).

Phenyl 3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-glucopyranoside (14c)



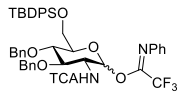
Diol **14b** (3.92 g, 6.0 mmol, 1 eq) was dissolved in DMF (3 mL) and THF (30 mL), then cooled to 0 °C. Sodium hydride (1.44 g, 36 mmol, 6 eq) was added, then after stirred 30 min, benzyl bromide (4.3 mL, 36 mmol, 6 eq) was added dropwise, the reaction was stirred for overnight. After analysis by TLC showed complete consumption of the starting material, quenched by MeOH, extracted with Et_2O and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 14:1 – 12/1) to yield compound **14c** (3.85 g, 4.62 mmol, 77%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.82 – 7.75 (m, 2H), 7.73 – 7.67 (m, 2H), 7.60 – 7.55 (m, 2H), 7.44 – 7.38 (m, 2H), 7.38 – 7.18 (m, 15H), 7.18 – 7.12 (m, 2H), 6.88 (d, *J* = 8.0 Hz, 1H, NH), 5.15 (d, *J* = 10.1 Hz, 1H, H-1), 4.89 – 4.79 (m, 2H), 4.74 – 4.65 (m, 2H), 4.16 – 4.09 (m, 1H, H-3), 4.04 (dd, *J* = 11.4, 2.1 Hz, 1H, H-6), 3.96 (dd, *J* = 11.4, 3.5 Hz, 1H, H-6), 3.84 (t, *J* = 9.1 Hz, 1H, H-4), 3.67 – 3.57 (m, 1H, H-2), 3.57 – 3.48 (m, 1H, H-5), 1.09 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.55 (TCA), 137.97, 137.68, 135.98, 135.73, 133.47, 133.18, 132.91, 132.24, 129.87, 129.85, 129.20, 128.73, 128.61, 128.32, 128.22, 128.18, 127.97, 127.93, 127.86, 127.85, 92.65 (TCA), 84.61 (C-1), 81.65 (C-3), 80.34 (C-5), 78.17 (C-4), 75.72 (Bn), 75.02 (Bn), 62.60 (C-6), 56.98 (C-2), 26.97 (*t*-Bu), 19.42 (*t*-Bu). HR-MS: Calculated for $C_{44}H_{46}Cl_3NO_5SSi$ $[M+Na]^+$: 856.18237, found: 856.18220. $[\alpha]^{25}_D$ = - 11.3° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA = 10/1, v/v).

3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-α/β-D-glucopyranoside (14d)

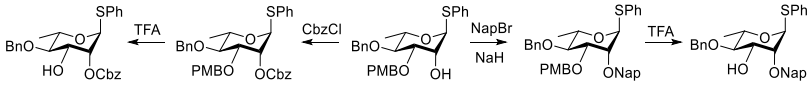


Compound **14c** (847 mg, 1.02 mmol, 1 eq) was dissolved in DCM (10 mL) and reduced to 0 °C. NIS (343 g, 1.52 mmol, 1.5 eq) and TFA (94 µL, 1.22 mmol, 1.2 eq) were added and the solution stirred for 1 hour. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with triethyl amine and saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). The organic phase was dried with $MgSO_4$, filtered and concentrated in *vacuo*. The compound was purified by flash chromatography (PE/EA 15:1 - 8:1) to yield compound **14d** (645 mg, 0.87 mmol, 85%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.75 – 7.62 (m, 4H), 7.46 – 7.22 (m, 14H), 7.21 – 7.13 (m, 2H), 6.91 (d, *J* = 9.2 Hz, 1H, NH), 5.27 (t, *J* = 3.4 Hz, 1H, H-1), 4.92 – 4.78 (m, 2H, Bn), 4.78 – 4.67 (m, 2H, Bn), 4.27 – 4.14 (m, 1H, H-2), 4.03 – 3.79 (m, 5H, H-6, H-5, H-4, H-3), 3.04 (d, *J* = 3.1 Hz, 1H, OH), 1.06 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.92 (TCA), 138.09, 137.86, 136.00, 135.72, 133.71, 133.14, 129.84, 128.67, 128.60, 128.17, 128.00, 127.95, 127.86, 127.73, 92.67 (TCA), 91.52 (C-1), 79.72 (C-3), 78.14 (C-4), 75.57 (Bn), 75.18 (Bn), 72.33 (C-5), 62.62 (C-6), 55.19 (C-2), 26.99 (*t*-Bu), 19.45 (*t*-Bu). HR-MS: Calculated for $C_{38}H_{42}Cl_3NO_6Si$ $[M+NH_4]^+$: 759.21852, found: 759.21809. TLC: Rf = 0.15 (PE/EA = 9/1, v/v).

***N*-Phenyl-trifluoroacetimidate 3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-α/β-D-glucopyranoside (14)**

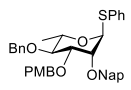


Hemiacetal **14d** (3.57 g, 4.80 mmol, 1.0 eq) was dissolved in acetone (50 mL) and cooled to 0 ℃. Cesium carbonate (1.9 g, 5.83 mmol, 1.2 eq) was added. After 15 min, N-phenyl trifluoroacetimidoyl chloride (1.5 g, 7.23 mmol, 1.5 eq) was added, and then the reaction was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by Et_3N, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/Et_2O 10:1 – 7/1) to yield compound **14** (3.76 g, 4.11 mmol, 86%). ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.57 – 8.41 (m, 1H), 7.84 – 7.70 (m, 4H), 7.51 – 7.20 (m, 18H), 7.18 – 7.09 (m, 1H), 6.91 – 6.79 (m, 2H), 6.62 – 6.52 (m, 1H), 5.07 – 4.93 (m, 3H), 4.86 – 4.78 (m, 1H), 4.78 – 4.71 (m, 1H), 4.52 – 4.40 (m, 2H), 4.06 – 4.01 (m, 4H), 1.13 (s, 9H). ^{13}C NMR (126 MHz, Acetone) δ 162.95, 144.27, 139.40, 139.00, 136.45, 136.25, 134.11, 133.67, 130.58, 129.64, 129.09, 129.00, 128.59, 128.51, 128.49, 128.46, 128.41, 128.30, 125.24, 120.04, 105.51, 93.43, 78.77, 75.68, 75.43, 73.37, 71.78, 66.34, 63.27, 55.80, 27.19, 19.79. HR-MS: Calculated for $C_{46}H_{46}Cl_3F_3N_2O_6Si$ [M-[O(C=NPh)CF_3]+OH+NH_4]$^+$: 759.21852, found: 759.21811. TLC: Rf = 0.3 (PE/ Et_2O = 9/1, v/v).



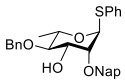
Scheme II. The synthesis of acceptors **15** and **16**

Phenyl 4-*O*-benzyl-3-*O*-*para*-methoxybenzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside (15b)



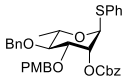
The known alcohol **15a**[11] (6.1 g, 13.07 mmol, 1 eq) was dissolved in DMF (40 mL), then cooled to 0 ℃. Sodium hydride (1.1 g, 26.2 mmol, 2 eq) was added, then 2-naphthylmethyl bromide (3.8 g, 17 mmol, 1.3 eq) was added, the reaction was stirred for 6h. After analysis by TLC showed complete consumption of the starting material, quenched by MeOH, extracted with Et_2O and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 20:1 – 10/1) to yield compound **15b** (7.59 g, 12.5 mmol, 96%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.85 – 7.69 (m, 4H), 7.55 – 7.39 (m, 3H), 7.38 – 7.13 (m, 12H), 6.84 – 6.77 (m, 2H), 5.48 (d, *J* = 1.8 Hz, 1H, H-1), 4.98 (d, *J* = 10.8 Hz, 1H, Bn), 4.82 (q, *J* = 12.7 Hz, 2H, Nap), 4.65 (d, *J* = 10.9 Hz, 1H, Bn), 4.60 – 4.48 (m, 2H, PMB), 4.20 – 4.07 (m, 1H, H-5), 4.01 – 3.94 (m, 1H, H-2), 3.84 (dd, *J* = 9.4, 3.1 Hz, 1H, H-3), 3.76 (s, 3H, PMB), 3.70 (t, *J* = 9.4 Hz, 1H, H-4), 1.36 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.29 (PMB), 138.64, 135.43, 134.66, 133.25, 133.12, 131.45, 130.38, 129.55, 129.03, 128.45, 128.31, 128.06, 128.01, 127.77, 127.73, 127.32, 126.98, 126.18, 126.16, 126.04, 113.86, 86.06 (C-1), 80.56 (C-4), 79.73 (C-3), 76.63 (C-2), 75.49 (Bn), 72.37 (Nap), 72.02 (PMB), 69.54 (C-5), 55.29 (PMB), 18.03 (C-6). HR-MS: Calculated for $C_{38}H_{38}O_5S$ [M+Na]$^+$: 629.23322, found: 629.23345. $[\alpha]^{25}_D$ = - 45.9° (c = 1, $CHCl_3$). TLC: Rf = 0.5 (PE/EA = 9/1, v/v).

Phenyl 4-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside (15)



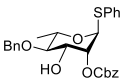
Compound **15b** (613 mg, 1.01 mmol, 1.0 eq) was dissolved in DCM (10 mL) and thiophenol (0.12 mL, 1.21 mmol, 1.2 eq), then TFA (1.0 mL) was added dropwise. The solution was stirred for 4 h at RT. After TLC showed complete consumption of the starting material, the reaction was quenched by saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 10:1 - 8:1) to yield compound **15** (456 mg, 0.94 mmol, 93%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.89 – 7.78 (m, 3H), 7.78 – 7.72 (m, 1H), 7.54 – 7.43 (m, 3H), 7.42 – 7.31 (m, 6H), 7.31 – 7.19 (m, 4H), 5.55 (d, *J* = 1.5 Hz, 1H, H-1), 4.90 (t, *J* = 11.5 Hz, 2H, CH_2), 4.68 (t, *J* = 11.2 Hz, 2H, CH_2), 4.23 – 4.10 (m, 1H, H-5), 4.08 – 4.02 (m, 1H, H-2), 4.01 – 3.92 (m, 1H, H-3), 3.43 (t, *J* = 9.2 Hz, 1H, H-4), 2.44 (s, 1H, 3-OH), 1.36 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.51, 134.83, 134.45, 133.30, 133.26, 131.67, 129.14, 128.68, 128.56, 128.08, 127.90, 127.87, 127.52, 127.24, 126.44, 126.33, 125.98, 85.31 (C-1), 82.52 (C-4), 80.09 (C-2), 75.28 (*C*H_2), 72.75 (*C*H_2), 72.19 (C-3), 68.77 (C-5), 18.06 (C-6). HR-MS: Calculated for $C_{30}H_{30}O_4S$ $[M+NH_4]^+$: 504.22031, found: 504.22046. $[\alpha]^{25}_D$ = - 86.8° (c = 1, $CHCl_3$). TLC: Rf = 0.6 (PE/EA = 3/1, v/v).

Phenyl 4-*O*-benzyl-2-*O*-benzyloxycarbonyl-3-*O*-*para*-methoxybenzyl-1-thio-α-L-rhamnopyranoside (16a)



Compound **15a** (4.04 g, 8.66 mmol, 1.0 eq) was dissolved in DCM (70 mL), then cooled to 0 °C and 4-dimethylaminopyridine (DMAP) (3.2 g, 26.19 mmol, 3 eq) were added, benzyloxycarbonyl chloride (CbzCl) (3.7 mL, 26.03 mmol, 3 eq) was added dropwise. The solution was stirred for overnight at RT. After TLC showed complete consumption of the starting material, the reaction was quenched by saturated aqueous sodium bicarbonate and diluted with EtOAc. The solution was washed with water (2x) and brine. The aqueous layer was extracted with EtOAc (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/Et_2O 20:1 - 10:1) to yield compound **16a** (4.6 g, 7.66 mmol, 88%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 – 7.41 (m, 2H), 7.40 – 7.21 (m, 15H), 6.87 – 6.79 (m, 2H), 5.50 (d, *J* = 1.6 Hz, 1H, H-1), 5.43 – 5.37 (m, 1H, H-2), 5.17 (s, 2H, Cbz), 4.90 (d, *J* = 10.8 Hz, 1H, Bn), 4.68 (d, *J* = 11.0 Hz, 1H, PMB), 4.59 (d, *J* = 10.9 Hz, 1H, Bn), 4.52 (d, *J* = 11.0 Hz, 1H, PMB), 4.25 – 4.14 (m, 1H, H-5), 3.89 (dd, *J* = 9.3, 3.1 Hz, 1H, H-3), 3.79 (s, 3H, PMB), 3.52 (t, *J* = 9.4 Hz, 1H, H-4), 1.32 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.43 (PMB), 154.82 (Cbz), 138.50, 135.11, 133.93, 131.86, 129.93, 129.86, 129.21, 128.70, 128.68, 128.51, 128.50, 128.09, 127.84, 127.78, 113.93, 85.95 (C-1), 80.04 (C-4), 77.97 (C-3), 75.66, 74.77 (C-2), 71.67, 70.09, 69.31 (C-5), 55.37 (PMB), 17.86 (C-6). HR-MS: Calculated for $C_{35}H_{36}O_7S$ $[M+NH_4]^+$: 618.25200, found: 618.25195. $[\alpha]^{25}_D$ = - 70.0° (c = 1, $CHCl_3$). TLC: Rf = 0.6 (PE/Et_2O = 9/1, v/v).

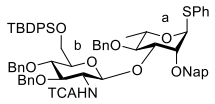
Phenyl 4-*O*-benzyl-2-*O*-benzyloxycarbonyl-1-thio-α-L-rhamnopyranoside (16)



Compound **16a** (316 mg, 0.53 mmol, 1.0 eq) was dissolved in DCM (5 mL), and *p*-thiocresol (78 mg, 0.63 mmol, 1.2 eq) were added, then TFA (0.5 mL) was added dropwise. The solution was stirred for 4 h at RT. After TLC showed complete consumption of the starting material, the reaction

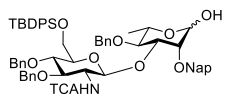
was quenched by saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 10:1 - 8:1) to yield compound **16** (217 mg, 0.45 mmol, 86%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 – 7.39 (m, 2H), 7.39 – 7.19 (m, 13H), 5.54 (d, *J* = 1.5 Hz, 1H, H-1), 5.25 – 5.19 (m, 1H, H-2), 5.14 (s, 2H, Cbz), 4.81 (d, *J* = 11.1 Hz, 1H, Bn), 4.66 (d, *J* = 11.1 Hz, 1H, Bn), 4.26 – 4.15 (m, 1H, H-5), 4.10 – 4.01 (m, 1H, H-3), 3.44 (t, *J* = 9.4 Hz, 1H, H-4), 2.58 (d, *J* = 5.8 Hz, 1H, 3-OH), 1.33 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.74 (Cbz), 138.16, 134.74, 133.79, 131.79, 129.13, 128.75, 128.68, 128.61, 128.59, 127.99, 127.91, 127.71, 85.56 (C-1), 81.78 (C-4), 78.15 (C-2), 75.39 (Bn), 70.77 (C-3), 70.27 (Cbz), 68.90 (C-5), 17.87 (C-6). HR-MS: Calculated for $C_{27}H_{28}O_6S$ $[M+NH_4]^+$: 498.19448, found: 498.19450. $[\alpha]^{25}_D$ = - 126.8° (c = 1, $CHCl_3$). TLC: Rf = 0.15 (PE/EA = 9/1, v/v).

Phenyl 4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamnopyranoside (17)



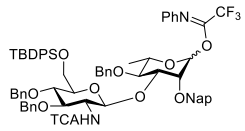
Donor **14** (7.6 g, 8.31 mmol, 2.0 eq) and acceptor **15** (2.03 g, 4.17 mmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (40 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (191 µL, 0.83 mmol, 0.2 eq) was added. The solution was stirred for 2.5 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 20:1 - 14:1) to yield compound **17** (4.4 g, 3.63 mmol, 87%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.83 – 7.74 (m, 4H), 7.74 – 7.65 (m, 4H), 7.59 – 7.53 (m, 1H), 7.44 – 7.37 (m, 2H), 7.37 – 7.12 (m, 24H), 7.10 – 7.02 (m, 2H), 6.72 (d, *J* = 8.9 Hz, 1H, NH), 5.50 (s, 1H, H-1a), 5.08 (d, *J* = 7.7 Hz, 1H, H-1b), 4.98 (d, *J* = 12.0 Hz, 1H, CH_2), 4.88 – 4.62 (m, 7H, CH_2), 4.54 (d, *J* = 10.8 Hz, 1H, CH_2), 4.28 – 4.13 (m, 3H, H-2a, H-3a, H-5a), 4.10 – 3.97 (m, 2H, H-2b, H-6b), 3.89 (dd, *J* = 11.2, 4.4 Hz, 1H, H-6b), 3.80 – 3.70 (m, 3H, H-4a, H-4b, H-3a), 3.62 – 3.52 (m, 1H, H-5b), 1.26 (d, *J* = 6.3 Hz, 3H, H-6a), 1.10 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.76 (TCA), 138.31, 137.67, 137.62, 136.02, 135.75, 135.63, 134.83, 133.36, 133.26, 133.09, 133.03, 130.94, 129.76, 128.92, 128.53, 128.50, 128.44, 128.06, 127.83, 127.80, 127.76, 127.68, 127.38, 127.04, 126.79, 126.43, 125.91, 125.72, 100.59 (C-1b), 92.52 (TCA), 86.65 (C-1a), 81.23 (C-4a), 80.43 (C-3b), 80.19 (C-2a), 78.01 (C-3a), 77.69 (C-4b), 76.41 (C-5b), 74.63 (*C*H_2), 74.53 (*C*H_2), 74.43 (*C*H_2), 73.76 (*C*H_2), 69.16 (C-5a), 63.04 (C-6b), 57.51 (C-2b), 27.07 (TBDPS), 19.39 (TBDPS), 17.97 (C-6a). HR-MS: Calculated for $C_{68}H_{70}Cl_3NO_9SSi$ $[M+NH_4]^+$: 1227.39444, found: 1227.39434. $[\alpha]^{25}_D$ = - 47.2° (c = 1, $CHCl_3$). TLC: Rf = 0.6 (PE/EA = 17/3, v/v).

4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α/β-L-rhamnopyranoside (18)



Compound **17** (4.40 g, 3.63 mmol, 1.0 eq) was dissolved in acetone (40 mL) and water (4 mL), then reduced to 0 °C. NIS (1.63 g, 7.24 mmol, 2.0 eq) was added and the solution stirred for 1 hour. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). The aqueous layer was extracted with DCM (3x), dried with MgSO4, filtered and concentrated in vacuo. The compound was purified by flash chromatography (PE/EA/DCM 8:1:1 – 6:1:1) to yield compound **18** (3.28 g, 2.93 mmol, 81%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.86 – 7.73 (m, 4H), 7.70 – 7.61 (m, 4H), 7.53 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.48 – 7.22 (m, 21H), 7.17 – 7.06 (m, 2H), 6.61 (d, *J* = 9.0 Hz, 0.6H), 6.54 (d, *J* = 9.1 Hz, 0.4H), 5.24 (d, *J* = 11.6 Hz, 0.4H), 5.16 – 5.09 (m, 0.6H), 5.07 – 4.96 (m, 1.6H), 4.85 – 4.70 (m, 3.5H), 4.70 – 4.54 (m, 3H), 4.33 (dd, *J* = 9.5, 3.1 Hz, 0.6H), 4.07 – 3.60 (m, 7.5H), 3.58 – 3.44 (m, 1.5H), 3.29 (dq, *J* = 9.2, 6.1 Hz, 0.4H), 2.60 (d, *J* = 3.4 Hz, 0.6H), 1.28 – 1.18 (m, 3H), 1.12 – 1.02 (m, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.89, 161.86, 138.48, 138.24, 137.79, 137.77, 137.65, 137.61, 136.43, 135.86, 135.78, 135.75, 135.71, 135.65, 133.56, 133.43, 133.37, 133.29, 133.20, 133.16, 133.10, 133.04, 130.10, 129.99, 129.80, 128.69, 128.67, 128.65, 128.64, 128.62, 128.58, 128.46, 128.18, 128.13, 128.10, 128.08, 128.06, 127.99, 127.96, 127.94, 127.93, 127.86, 127.84, 127.82, 127.75, 127.54, 127.40, 127.36, 126.89, 126.55, 126.16, 126.03, 125.95, 125.74, 100.83, 100.69, 93.85, 93.28, 92.62, 92.55, 81.47, 80.77, 80.71, 80.66, 79.88, 79.85, 78.77, 78.17, 78.06, 76.39, 76.29, 75.74, 74.90, 74.84, 74.82, 74.75, 74.73, 74.57, 74.21, 71.54, 68.16, 62.77, 57.86, 57.75, 26.99, 19.48, 19.42, 18.20, 18.03. HR-MS: Calculated for $C_{62}H_{66}Cl_3NO_{10}Si$ $[M+NH_4]^+$: 1135.38598, found: 1135.38586. TLC: Rf = 0.20 (PE/Actone = 8/1, v/v).

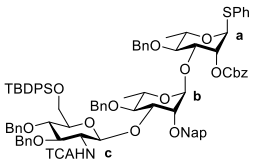
***N*-phenyl-trifluoroacetimidate 4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α/β-L-rhamnopyranoside (19)**



The hemiacetal **18** (3.28 g, 2.93 mmol, 1.0 eq) was dissolved in acetone (30 mL) and cooled to 0 °C. Cesium carbonate (1.24 g, 3.81 mmol, 1.3 eq) was added. After 15 min, *N*-phenyl trifluoroacetimidoyl chloride (800 mg, 3.81 mmol, 1.3 eq) was added, and then the reaction was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by Et_3N, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 10:1 – 5/1) to yield compound **19** (3.49 g, 2.7 mmol, 92%). ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.65 (d, *J* = 9.4 Hz, 1H), 8.07 (s, 1H), 8.03 – 7.96 (m, 2H), 7.96 – 7.88 (m, 1H), 7.86 – 7.79 (m, 1H), 7.76 – 7.65 (m, 4H), 7.53 – 7.21 (m, 24H), 7.14 – 7.03 (m, 3H), 6.75 – 6.67 (m, 1H), 6.00 – 5.65 (m, 1H), 5.35 – 5.17 (m, 2H), 5.17 – 5.00 (m, 2H), 4.94 – 4.79 (m, 3H), 4.79 – 4.69 (m, 1H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.51 – 4.41 (m, 2H), 4.41 – 4.27 (m, 1H), 4.23 – 3.93 (m, 3H), 3.81 – 3.73 (m, 1H), 3.69 – 3.56 (m, 2H), 3.54 – 3.41 (m, 1H), 1.17 – 0.99 (m, 12H). ^{13}C NMR (126 MHz, Acetone) δ 162.46, 144.02, 139.29, 139.02, 138.63, 137.26, 135.94, 135.87, 133.92, 133.71, 133.69, 133.61, 130.36, 129.82, 129.17, 128.70, 128.64, 128.61, 128.53, 128.48, 128.37, 128.35, 128.28, 128.23, 128.16, 128.04, 127.95, 127.90, 127.88, 127.76, 127.73, 127.56, 126.36,

126.19, 124.72, 119.71, 119.38, 101.93, 93.52, 82.17, 81.19, 79.82, 79.10, 78.61, 76.84, 75.45, 75.29, 75.11, 74.94, 72.70, 63.75, 58.80, 26.89, 19.44, 17.73. HR-MS: Calculated for $C_{70}H_{70}Cl_3F_3N_2O_{10}Si$ [M-[O(C=NPh)CF_3]+OH+NH_4]$^+$: 1135.38598, found: 1135.38765. TLC: Rf = 0.15 (PE/ EA = 10/1, v/v).

Phenyl 4-*O*-benzyl-3-*O*-(4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-2-*O*-benzyloxycarbonyl-1-thio-α-L-rhamnopyranoside (20)



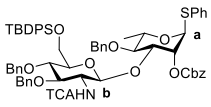
Imidate condition: Donor **19** (128.4 mg, 0.1 mmol, 1.0 eq) and acceptor **16** (66.0 mg, 0.14 mmol, 1.4 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (2 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (4.6 µL, 0.02 mmol, 0.2 eq) was added. The solution was stirred for 2.5 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 15:1:1 - 13:1:1) to yield compound **20** (25 mg, 16 µmol, 16%) and side product **21** (16.5 mg, 14 µmol, 14%).

Tf_2O/Ph_2SO mediated pre-activation condition: Donor **18** (52 mg, 46 µmol, 1.0 eq), Ph_2SO (21 mg, 104 µmol, 2.2 eq.) and DTBMP (24 mg, 117 µmol, 2.5 eq.) were co-evaporated with dry toluene three times under nitrogen. Then they were dissolved in DCM (2 mL) and activated 4Å molecular sieves and the reaction mixture stirred for 20 min at room temperature. The solution was cooled to -60°C and Tf_2O (8.5 µL, 50 µmol, 1.1 eq.) was slowly added. The reaction mixture was allowed to warm to -40°C in approximately 60 min, then added the acceptor **16** (45 mg, 94 µmol, 2 eq.) in DCM. The reaction mixture was allowed to warm slowly to 0 °C and stirred 5 h. After TLC showed complete consumption of the starting material, the reaction was quenched with Et_3N and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 15:1:1 - 13:1:1) to yield compound **20** (38 mg, 24 µmol, 52%).

Gold catalyzed condition: Donor **24** (42.2 mg, 32 µmol, 1.0 eq) synthesized following the reported procedure[12] and acceptor **16** (31.1 mg, 65 µmol, 2.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (2 mL) and 4Å molecular sieves were added and the solution stirred for 30 minutes at RT. The reaction was then cooled to 0 °C and a freshly prepared DCM solution of PPh_3AuNTf_2 (prepared by stirring 1:1 PPh_3AuCl (5.7 mg, 6.4 µmol, 0.2 eq) and $AgNTf_2$ (4.5 mg, 6.4 µmol, 0.2 eq) in DCM for 30 minutes). The solution was stirred for 2.5 hours. After TLC showed complete consumption of the starting material, the reaction was filtered and concentrated *in vacuo*. The compound was firstly purified by size-exclusion chromatography (Sephadex LH-20, DCM/MeOH, 1:1 v/v) gave the α:β ratio, then purified by preparative TLC plates (Macherey-Nagel, pre-coated TLC plates SIL G-100 UV254) (PE/EA/DCM 8:1:1) to yield compound **20** (28 mg, 17.7 µmol, 55%) as a mixture of anomers.

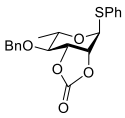
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.78 – 7.69 (m, 3H), 7.64 (s, 1H), 7.63 – 7.58 (m, 2H), 7.58 – 7.54 (m, 2H), 7.48 – 7.44 (m, 2H), 7.43 – 7.38 (m, 3H), 7.38 – 7.20 (m, 27H), 7.16 – 7.06 (m, 7H), 6.77 (d, *J* = 8.5 Hz, 1H, NHTCA), 5.54 (d, *J* = 1.7 Hz, 1H, H-1a), 5.25 (dd, *J* = 3.2, 1.8 Hz, 1H, H-2a), 5.11 – 5.02 (m, 4H, Cbz, H-1c, H-1b), 4.89 (d, *J* = 11.8 Hz, 1H, C*H*$_2$), 4.79 – 4.49 (m, 8H, C*H*$_2$), 4.35 – 4.25 (m, 2H, C*H*$_2$, H-3b), 4.20 – 4.03 (m, 2H, H-5a, H-3a), 3.94 – 3.62 (m, 8H, H-6c, H-2b, H-2c, H-5b, H-5c, H-4b), 3.53 – 3.41 (m, 2H, H-4a), 1.24 (d, *J* = 6.4 Hz, 3H, H-6a), 1.19 (d, *J* = 6.2 Hz, 3H, H-6b), 0.99 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.89 (NHTCA), 154.73 (Cbz), 138.45, 138.12, 137.91, 137.85, 136.08, 135.80, 135.58, 134.79, 133.91, 133.59, 133.34, 133.07, 133.00, 131.92, 129.97, 129.87, 129.21, 128.94, 128.70, 128.69, 128.66, 128.60, 128.54, 128.53, 128.48, 128.15, 128.05, 127.97, 127.94, 127.91, 127.80, 127.78, 127.76, 127.60, 127.57, 126.67, 126.32, 125.91, 125.72, 101.47 (C-1b), 99.81 (C-1c), 92.57 (NHTCA), 85.46 (C-1a), 81.35 (C-4b), 80.16, 80.08 (C-4a), 78.65, 77.84 (C-3a), 77.68 (C-2a), 76.52 (C-3b), 75.29, 74.50, 74.30, 74.17, 73.86, 70.17 (Cbz), 69.35 (C-5a), 68.76 (C-5b), 62.92 (C-6c), 57.96 (C-2c), 26.96 (TBDPS), 19.39, 18.11, 17.87. HR-MS: Calculated for $C_{89}H_{92}Cl_3NO_{15}SSi$ [M+NH_4^+]: 1597.53607, found: 1597.53592. $[\alpha]^{25}_D$ = - 37.1° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA/DCM = 8/1/1, v/v).

Phenyl 4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-benzyloxycarbonyl-1-thio-α-L-rhamnopyranoside (21)



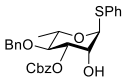
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.74 – 7.68 (m, 5H), 7.42 – 7.00 (m, 30H), 6.65 (d, *J* = 8.5 Hz, 1H), 5.50 (d, *J* = 1.6 Hz, 1H, H-1a), 5.38 (dd, *J* = 3.4, 1.6 Hz, 1H, H-2a), 5.14 (s, 2H, Cbz), 5.11 – 5.06 (m, 1H, H-1c), 4.82 – 4.51 (m, 6H, C*H*$_2$), 4.29 (dd, *J* = 9.5, 3.3 Hz, 1H, H-3a), 4.24 – 4.15 (m, 1H, H-5a), 4.03 (dd, *J* = 11.3, 2.8 Hz, 1H, H-6c), 3.95 (dd, *J* = 11.2, 4.4 Hz, 1H, H-6c), 3.90 – 3.72 (m, 3H, H-3c, H-4c, H-2c), 3.62 – 3.52 (m, 2H, H-4a, H-5c), 1.25 – 1.23 (m, 3H, H-6a), 1.13 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.69 (TCA), 154.44 (Cbz), 138.18, 137.93, 137.78, 135.97, 135.85, 135.34, 134.11, 133.62, 133.25, 131.63, 129.83, 129.81, 129.22, 129.14, 128.85, 128.70, 128.66, 128.61, 128.55, 128.53, 128.48, 128.16, 127.94, 127.92, 127.90, 127.88, 127.78, 127.70, 127.65, 127.58, 99.86 (C-1c), 92.60 (TCA), 85.62 (C-1a), 81.28 (C-4a), 80.01 (C-3c), 78.02 (C-2a), 77.86 (C-4c), 76.66 (C-5c), 75.01 (Bn), 74.77 (C-3a), 74.51 (Bn), 74.49 (Bn), 70.09 (Cbz), 69.07 (C-5a), 63.06 (C-6c), 57.91 (C-2c), 27.19 (TBDPS), 19.54 (TBDPS), 17.87 (C-6a). HR-MS: Calculated for $C_{65}H_{68}Cl_3NO_{11}SSi$ [M+NH_4]$^+$: 1221.36862, found: 1221.36830. $[\alpha]^{25}_D$ = - 44.1° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (PE/EA/DCM = 8/1/1, v/v/v).

Phenyl 4-*O*-benzyl-2,3-*O*-carbonyl-1-thio-α-L-rhamnopyranoside (22)



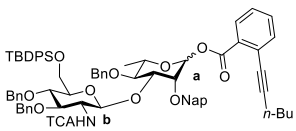
The analytical data were in full accord with the reported previously[16]. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.46 – 7.39 (m, 2H), 7.36 – 7.31 (m, 4H), 7.31 – 7.24 (m, 4H), 5.73 (d, *J* = 0.7 Hz, 1H, H-1), 4.85 – 4.74 (m, 3H, H-2, H-3, Bn), 4.57 (d, *J* = 11.3 Hz, 1H, Bn), 4.24 – 4.16 (m, 1H, H-5), 3.35 (dd, *J* = 9.7, 6.1 Hz, 1H, H-4), 1.23 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.14, 137.00, 132.47, 131.64, 129.24, 128.43, 128.30, 128.03, 82.05 (C-1), 80.14 (C-4), 78.93 (C-3), 77.47 (C-2), 73.38 (Bn), 65.49 (C-5), 17.53 (C-6). HR-MS: Calculated for $C_{20}H_{20}O_5S$ [M+NH_4]$^+$: 390.13697, found: 390.13702. TLC: Rf = 0.2 (PE/EA = 9/1, v/v).

Phenyl 4-*O*-benzyl-3-*O*-benzyloxycarbonyl-1-thio-α-L-rhamnopyranoside (23)



^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47 – 7.39 (m, 2H), 7.39 – 7.15 (m, 13H), 5.44 (d, *J* = 1.8 Hz, 1H, H-1), 5.21 – 5.04 (m, 3H, H-3, Cbz), 4.67 (d, *J* = 11.0 Hz, 1H, Bn), 4.57 (d, *J* = 11.0 Hz, 1H, Bn), 4.36 (s, 1H, H-2), 4.31 – 4.20 (m, 1H, H-5), 3.66 (t, *J* = 9.4 Hz, 1H, H-4), 3.05 (s, 1H, 2-OH), 1.31 (d, *J* = 6.2 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.32, 137.83, 134.95, 133.97, 131.42, 129.06, 128.69, 128.67, 128.52, 128.48, 128.40, 127.92, 127.82, 127.42, 87.44 (C-1), 78.74 (C-4), 78.43 (C-3), 75.20 (Bn), 70.82 (C-2), 70.05 (Cbz), 69.06 (C-5), 17.78 (C-6). HR-MS: Calculated for $C_{27}H_{28}O_6S$ $[M+NH_4]^+$: 498.19448, found: 498.19438. $[\alpha]^{25}_D$ = - 77.2° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (PE/EA = 9/1, v/v).

***ortho*-hexynylbenzoyl 4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α/β-L-rhamnopyranoside (24)**

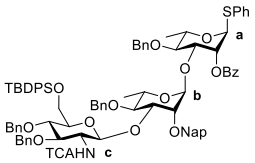


Hemiacetal **18** (258 mg, 213 µmol, 1.0 eq) was dissolved in DCM (3 mL). DMAP (52 mg, 426 µmol, 2.0 eq), DIPEA (148 µL, 852 µmol, 4.0 eq), EDCI·HCl (116 mg, 747 µmol, 3.5 eq) and freshly prepared *ortho*-hexynylbenzoic acid (129 mg, 639 µmol, 3 eq) were added and the mixture was stirred overnight. After analysis by TLC showed complete consumption of the starting material, diluted by DCM, the reaction was quenched with saturated aqueous sodium bicarbonate. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 20:1 - 7:1) to yield compound **24** (254 mg, 195 µmol, α:β 1:1.35, 92%). α-**24**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.86 – 7.76 (m, 4H), 7.73 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.59 – 7.45 (m, 6H), 7.45 – 7.36 (m, 3H), 7.36 – 7.04 (m, 22H), 6.82 (d, *J* = 8.7 Hz, 1H, NHTCA), 6.37 (d, *J* = 2.0 Hz, 1H, H-1a), 5.15 (d, *J* = 7.4 Hz, 1H, H-1b), 5.05 (d, *J* = 12.0 Hz, 1H, Nap), 4.89 – 4.59 (m, 7H, Bn, Nap), 4.36 (dd, *J* = 9.4, 3.1 Hz, 1H, H-3a), 4.04 – 3.81 (m, 7H, H-2a, H-2b, H-6b, H-5a, H-5b, H-3b), 3.77 (t, *J* = 9.5 Hz, 1H, H-4a), 3.61 – 3.53 (m, 1H, H-4b), 2.58 – 2.37 (m, 2H), 1.63 – 1.50 (m, 2H), 1.49 – 1.37 (m, 2H), 1.31 – 1.17 (m, 3H, H-6a), 0.97 – 0.85 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.19, 161.85 (NHTCA), 138.24, 137.76, 137.69, 136.07, 135.59, 135.53, 134.91, 133.38, 133.36, 133.11, 132.77, 131.97, 130.74, 130.71, 129.85, 129.83, 128.61, 128.57, 128.55, 128.12, 128.07, 127.99, 127.96, 127.90, 127.86, 127.80, 127.76, 127.73, 127.68, 127.65, 127.51, 127.12, 126.65, 126.23, 125.96, 125.76, 125.17, 100.61 (C-1b, J_{CH} = 163 Hz), 96.79, 92.87 (C-1a, J_{CH} = 176 Hz), 92.52 (NHTCA), 80.79 (C-4a), 79.92 (C-3b), 79.81, 77.96 (C-2a), 77.76 (C-3a), 77.46 (C-5b), 76.28 (C-4b), 75.04, 74.54, 74.44, 74.22, 70.77 (C-5a), 63.00 (C-6b), 57.37 (C-2b), 30.85, 26.87, 22.14, 19.69, 19.29, 18.13 (C-6a), 13.80. HR-MS: Calculated for $C_{75}H_{78}Cl_3NO_{11}Si$ $[M+Na]^+$: 1324.43019, found: 1324.43018.

β-**24**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.90 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.84 (d, *J* = 1.6 Hz, 1H), 7.81 – 7.73 (m, 2H), 7.73 – 7.67 (m, 1H), 7.67 – 7.57 (m, 5H), 7.53 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.46 – 7.37 (m, 3H), 7.36 – 7.22 (m, 18H), 7.21 (s, 1H), 7.16 (td, *J* = 7.7, 1.4 Hz, 1H), 7.13 – 7.05 (m, 2H), 6.64 (d, *J* = 8.8 Hz, 1H, NHTCA), 5.83 (d, *J* = 1.0 Hz, 1H, H-1a), 5.14 – 4.97 (m, 3H, CH_2, H-1b), 4.86 – 4.60 (m, 5H, CH_2), 4.52 (d, *J* = 10.8 Hz, 1H, CH_2), 4.16 (dd, *J* = 2.9, 1.1 Hz, 1H, H-2a), 4.01 – 3.82 (m, 3H, H-3a, H-6b, H-2b), 3.82 – 3.65 (m, 3H, H-6b, H-3b, H-4a), 3.61 – 3.43 (m, 3H, H-5b, H-5a, H-4b), 2.41 (t, *J* = 7.1 Hz, 2H), 1.63 – 1.52 (m, 2H), 1.49 – 1.38 (m, 2H), 1.28 (d,

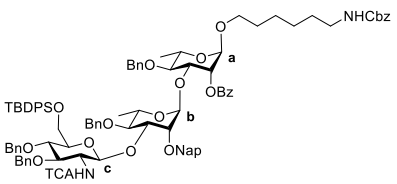
J = 6.1 Hz, 3H, H-6a), 1.02 (s, 9H, TBDPS), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.66, 161.83 (TCA), 138.16, 137.72, 137.62, 136.48, 135.67, 135.59, 134.50, 133.32, 133.23, 133.07, 132.94, 132.13, 130.74, 130.68, 129.97, 129.90, 128.63, 128.58, 128.56, 128.06, 128.01, 127.94, 127.91, 127.86, 127.80, 127.77, 127.63, 127.09, 127.05, 125.87, 125.71, 100.60 (C-1b, J_{CH} = 163 Hz), 97.01, 92.68 (C-1a, J_{CH} = 176 Hz), 92.54 (TCA), 80.45 (C-3b), 80.39 (C-4a), 79.99 (C-3a), 79.05, 78.44 (C-2a), 78.09 (C-5b), 76.37 (C-4b), 75.28, 74.97, 74.68, 74.65, 72.70 (C-5a), 62.88 (C-6b), 57.77 (C-2a), 30.73, 26.86, 22.16, 19.64, 19.30, 17.96 (C-6a), 13.77. TLC: Rf = 0.6-0.7 (PE/EA = 4/1, v/v).

Phenyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-1-thio-α-L-rhamnopyranoside (26)



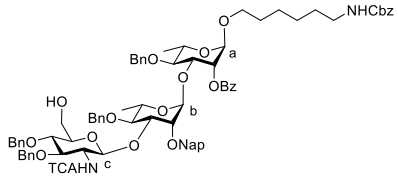
Donor **19** (703 mg, 0.54 mmol, 1.0 eq) and acceptor **25**[17] (497 mg, 1.1 mmol, 2 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (6 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (15 µL, 0.06 mmol, 0.1 eq) was added. The solution was stirred for 2.5 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 20:1:1 - 10:1:1) to yield compound **26** (610 mg, 0.39 mmol, 73%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.02 – 7.94 (m, 2H, Bz), 7.76 – 7.65 (m, 3H), 7.65 – 7.58 (m, 3H), 7.58 – 7.52 (m, 2H), 7.51 – 7.44 (m, 2H), 7.42 – 7.16 (m, 33H), 7.15 – 7.09 (m, 2H), 6.72 (d, *J* = 8.7 Hz, 1H, N*H*TCA), 5.73 – 5.65 (m, 1H, H-2a), 5.53 (d, *J* = 1.6 Hz, 1H, H-1a), 5.16 (d, *J* = 2.1 Hz, 1H, H-1b), 4.96 – 4.87 (m, 2H, CH_2, H-1c), 4.78 – 4.64 (m, 4H, CH_2), 4.64 – 4.49 (m, 5H, CH_2), 4.35 – 4.24 (m, 2H, H-5a, H-3a), 4.16 (dd, *J* = 9.0, 3.0 Hz, 1H, H-3b), 4.03 – 3.82 (m, 4H, H-2c, H-2b, H-5c, H-5b), 3.74 – 3.48 (m, 5H, H-3c, H-6c, H-4b, H-4a), 3.34 – 3.23 (m, 1H, H-4c), 1.35 (d, *J* = 6.2 Hz, 3H, H-6a), 1.20 (d, *J* = 6.2 Hz, 3H, H-6b), 1.00 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.71 (Bz), 161.89 (TCA), 138.41, 138.01, 137.94, 137.76, 135.99, 135.72, 135.51, 133.82, 133.54, 133.33, 133.27, 133.09, 133.00, 131.89, 129.78, 129.73, 129.71, 129.25, 129.17, 128.57, 128.54, 128.49, 128.10, 127.99, 127.92, 127.89, 127.84, 127.80, 127.75, 127.67, 127.65, 127.45, 127.22, 126.66, 126.31, 125.80, 125.63, 100.62 (C-1b, J_{CH} = 171 Hz), 100.46 (C-1c, J_{CH} = 162 Hz), 92.58 (TCA), 85.95 (C-1a, J_{CH} = 168 Hz), 80.92 (C-3c), 80.83 (C-4b), 80.78 (C-4a), 78.65 (C-2b), 77.43 (C-5c), 76.89 (C-3b), 76.34 (C-4c), 76.29 (C-3a), 74.95, 74.62, 74.36, 74.29 (C-2a), 74.07, 72.54, 69.25 (C-5a), 68.41 (C-5b), 62.67 (C-6c), 57.48 (C-2c), 26.98 (TBDPS), 19.43 (TBDPS), 18.10 (C-6a), 18.08 (C-6b). HR-MS: Calculated for $C_{88}H_{90}Cl_3NO_{14}SSi$ $[M+H]^+$: 1550.49896, found: 1550.49891. $[\alpha]^{25}_D$ = - 60.1° (c = 1, $CHCl_3$). TLC: Rf = 0.4 (PE/EA = 6/1, v/v).

***N*-benzyloxycarbonyl-6-aminohexanyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (27)**



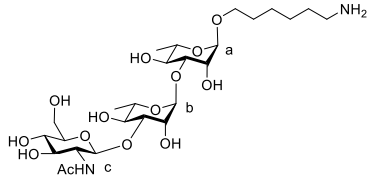
Donor **26** (201 mg, 0.13 mmol, 1.0 eq) and acceptor benzyl (6-hydroxyhexyl)carbamate (92 mg, 0.39 mmol, 3.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (4 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was then cooled to 0 °C and then *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (6 µL, 0.026 mmol, 0.2 eq) and NIS (58 mg, 0.26 mmol, 2.0 eq) were added. The solution was stirred for 3 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 8:1 - 4:1) to yield compound **27** (208 mg, 0.123 mmol, 95%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.03 – 7.96 (m, 2H, Bz), 7.76 – 7.65 (m, 3H), 7.65 – 7.58 (m, 3H), 7.58 – 7.52 (m, 2H), 7.41 – 7.15 (m, 35H), 7.14 – 7.07 (m, 2H), 6.77 (d, *J* = 8.8 Hz, 1H, N*H*TCA), 5.44 – 5.38 (m, 1H, H-2a), 5.15 (d, *J* = 2.0 Hz, 1H, H-1b), 5.14 – 5.03 (m, 2H, Cbz), 4.93 – 4.78 (m, 4H, H-1c, H-1a, N*H*Cbz, CH_2), 4.76 – 4.62 (m, 4H, CH_2), 4.62 – 4.47 (m, 5H, CH_2), 4.30 (dd, *J* = 9.4, 3.4 Hz, 1H, H-3a), 4.16 (dd, *J* = 9.2, 3.0 Hz, 1H, H-3b), 4.02 – 3.76 (m, 5H, H-2c, H-2b, H-4c, H-5b, H-5a), 3.72 – 3.62 (m, 3H, H-4b, H-6c), 3.62 – 3.47 (m, 3H, H-6c, H-4a, H-3c), 3.44 – 3.34 (m, 1H), 3.30 – 3.22 (m, 1H, H-5c), 3.21 – 3.06 (m, 2H), 1.64 – 1.44 (m, 4H), 1.41 – 1.23 (m, 7H, H-6a), 1.18 (d, *J* = 6.1 Hz, 3H, H-6b), 0.99 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.91 (Bz), 161.85 (TCA), 156.45 (Cbz), 138.41, 138.02, 137.77, 136.74, 136.03, 135.69, 135.62, 135.47, 133.54, 133.24, 133.20, 133.08, 132.96, 132.12, 129.86, 129.74, 129.70, 129.68, 129.07, 128.63, 128.52, 128.48, 128.47, 128.44, 128.11, 128.06, 127.96, 127.91, 127.87, 127.82, 127.79, 127.77, 127.76, 127.69, 127.62, 127.60, 127.46, 127.20, 126.55, 126.26, 125.74, 125.55, 125.26, 100.61 (C-1b), 100.45 (C-1c), 97.29 (C-1a), 92.59 (TCA), 80.88 (C-4b, C-4a), 80.83 (C-3c), 78.79 (C-2b), 77.39 (C-4c), 76.73 (C-3b), 76.28 (C-5c), 76.17 (C-3a), 74.93, 74.56, 74.30, 73.99, 72.95 (C-2a), 72.25, 68.12 (C-5b), 67.83, 67.70 (C-5a), 66.55 (Cbz), 62.69 (C-6c), 57.45 (C-2c), 41.04, 29.92, 29.29, 26.95 (TBDPS), 26.51, 25.86, 19.38 (TBDPS), 18.20 (C-6a), 18.01 (C-6b). HR-MS: Calculated for $C_{96}H_{105}Cl_3N_2O_{17}Si$ $[M+Na]^+$: 1713.61403, found: 1713.61377. $[\alpha]^{20}_D$ = - 24.5º (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA = 3/1, v/v).

_N_-benzyloxycarbonyl-6-aminohexanyl 2-_O_-benzoyl-4-_O_-benzyl-3-_O_-(4-_O_-benzyl-3-_O_-(3,4-di-_O_-benzyl-2-trichloroacetamido-2-deoxy-β-D-glucopyranosyl)-2-_O_-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (28)



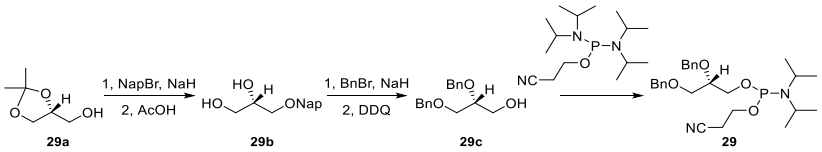
Protected trisaccharide **27** (91.8 mg, 54 µmol, 1.0 eq) was dissolved in anhydrous THF (3 mL) and AcOH (31 µL, 540 µmol, 10 eq). Then 1M TBAF in THF (540 µL, 540 µmol, 10 eq) was added in 0 °C. The reaction mixture was stirred at 50 °C for 5 h. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous ammonium chloride and diluted with EA. The solution was washed with water (2x) and brine. The aqueous layer was extracted with EA (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA/DCM 5:1:1 - 4:1:1) to yield compound **28** (76.4 mg, 52.5 mmol, 97%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.11 – 8.04 (m, 2H, Bz), 7.82 – 7.72 (m, 3H), 7.64 (d, *J* = 1.6 Hz, 1H), 7.62 – 7.55 (m, 1H), 7.51 – 7.38 (m, 5H), 7.37 – 7.20 (m, 23H), 7.20 – 7.15 (m, 2H), 6.63 (d, *J* = 8.7 Hz, 1H), 5.40 – 5.33 (m, 1H, H-2a), 5.19 – 5.03 (m, 3H, H-1b, Cbz), 4.85 – 4.77 (m, 2H, H-1a), 4.77 – 4.56 (m, 9H, H-1c, CH_2), 4.52 (d, *J* = 12.1 Hz, 1H, CH_2), 4.46 (d, *J* = 11.1 Hz, 1H, CH_2), 4.26 (dd, *J* = 9.4, 3.3 Hz, 1H, H-3a), 3.97 (dd, *J* = 9.3, 2.9 Hz, 1H, H-3b), 3.84 – 3.71 (m, 4H, H-2b, H-5a, H-2c, H-5b), 3.68 – 3.48 (m, 4H, H-4b, H-3c, H-4a), 3.45 – 3.23 (m, 4H, H-6c, H-4c), 3.22 – 3.09 (m, 2H), 3.01 – 2.93 (m, 1H, H-5c), 1.64 – 1.45 (m, 4H), 1.42 – 1.22 (m, 7H, H-6a), 1.14 (d, *J* = 6.2 Hz, 3H, H-6b). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.95 (Bz), 161.97 (TCA), 156.51 (Cbz), 138.51, 138.23, 137.82, 137.76, 136.78, 135.87, 133.43, 133.23, 133.06, 130.03, 129.94, 128.69, 128.63, 128.61, 128.56, 128.48, 128.20, 128.15, 128.11, 128.08, 128.01, 127.93, 127.91, 127.85, 127.81, 127.67, 127.45, 127.31, 126.78, 126.42, 126.12, 125.88, 100.54 (C-1c), 100.25 (C-1b), 97.26 (C-1a), 92.47 (TCA), 80.84 (C-4a), 80.57 (C-4b), 80.29 (C-3c), 78.16 (C-2b), 77.98 (C-4c), 77.86 (C-3b), 76.72 (C-3a), 75.27 (C-5c), 75.05, 74.75, 74.69, 73.63, 73.15, 73.11 (C-2a), 68.96 (C-5b), 67.94, 67.82 (C-5a), 66.66 (Cbz), 61.70 (C-6c), 57.77 (C-2c), 41.13, 29.99, 29.35, 26.59, 25.93, 18.17 (C-6a), 18.02 (C-6b). HR-MS: Calculated for $C_{80}H_{87}Cl_3N_2O_{17}$ $[M+Na]^+$: 1470.54086, found: 1470.54103. $[\alpha]^{20}_D$ = - 26.0º (c = 1, $CHCl_3$). TLC: Rf = 0.25 (PE/EA/DCM = 4/1/1, v/v/v).

6-aminohexanyl 3-_O_-(3-_O_-(2-acetamito-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (1)



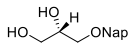
The protected trimer **28** (34.6 mg, 23.8 µmol, 1.0 eq) was dissolved in *tert*-butanol (7 mL) and 0.1% AcOH in water (3 mL). After $Pd(OH)_2$/C (60 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The crude was dissolved in sodium hydroxide (0.1 M, 5 mL), stirred overnight, quenched with acetic acid and then quenched the excess acid using ammonia solution. The compound was purified by gel filtration (HW-40, 0.15M, NH_4HCO_3 in H_2O) with a Shimadzu RID-10A refractive index detector and lyophilized to yield compound **1** (10.3 mg, 18.8 µmol, 71%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 4.98 (d, *J* =

1.8 Hz, 1H, H-1b), 4.72 (d, *J* = 1.8 Hz, 1H, H-1a), 4.67 (d, *J* = 8.5 Hz, 1H, H-1c), 4.24 (dd, *J* = 3.3, 1.8 Hz, 1H, H-2b), 3.96 (dd, *J* = 3.4, 1.8 Hz, 1H, H-2a), 3.91 – 3.85 (m, 2H, H-6c, H-3b), 3.81 – 3.64 (m, 6H, H-3a, H-5b, H-2c, H-6c, H-5a), 3.55 – 3.38 (m, 6H, H-3c, H-4a, H-4b, H-4a, H-5c), 2.95 (t, *J* = 7.6 Hz, 2H), 1.99 (s, 3H, NHAc), 1.68 – 1.53 (m, 4H), 1.44 – 1.32 (m, 4H), 1.28 – 1.20 (m, 6H, H-6a, H-6b). ^{13}C NMR (151 MHz, D_2O) δ 175.83 (NHAc), 103.60 (C-1c), 102.85 (C-1b), 100.53 (C-1a), 80.78 (C-3b), 79.06 (C-3a), 76.56 (C-5c), 74.57 (C-3c), 72.31 (C-4a), 71.79 (C-4b), 70.87 (C-2a), 70.75 (C-4c), 70.70 (C-3a), 70.19 (C-5b), 69.63 (C-5a), 68.68, 61.50 (C-6c), 56.65 (C-2c), 40.35, 29.19, 27.55, 26.28, 25.84, 23.09 (NHAc), 17.50, 17.48 (C-6a, C-6b). HR-MS: Calculated for $C_{26}H_{48}N_2O_{14}$ [M+H$^+$]: 613.31783, found: 613.31731.



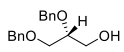
Scheme III. The synthesis of the space **29**.

1-*O*-(2-naphthylmethyl)-*sn*-glycerol (29b)



The commercially available reagent (R)-(−)-2,2-Dimethyl-1,3-dioxolane-4-methanol **29a** (6.52 g, 49.3 mmol, 1.0 eq) was dissolved in DMF (150 mL), then cooled to 0 °C. Sodium hydride (3.94 g, 98.6 mmol, 2 eq) was added, then 2-naphthylmethyl bromide (16.4 g, 74.0 mmol, 1.5 eq) was added, the reaction was stirred for 7h. After analysis by TLC showed complete consumption of the starting material, quenched by MeOH, extracted with Et_2O and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude compound was dissolved in AcOH (200 mL) and water (200 mL). The mixture was warmed to 50 °C under 300 mbar in rotary evaporator for 4h. After analysis by TLC showed complete consumption of the starting material, concentrated *in vacuo*. The crude was dissolved in EA and washed with brine (3x). The aqueous layer was extracted with EA (3x), dried with MgSO4, filtered and concentrated in vacuo. The compound was purified by column chromatography (DCM/Acetone 9:1 – 4/1) to yield compound **29b** (10.07 g, 43.4 mmol, 88%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.87 – 7.77 (m, 3H, Nap), 7.77 – 7.70 (m, 1H, Nap), 7.53 – 7.36 (m, 3H, Nap), 4.67 (s, 2H, Nap), 3.96 – 3.81 (m, 1H, H-2), 3.67 (dd, *J* = 11.5, 3.8 Hz, 1H, H-3), 3.63 – 3.49 (m, 3H, H-3, H-1), 3.17 – 2.97 (m, 1H, 2-OH), 2.63 (s, 1H, 3-OH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.29, 133.38, 133.21, 128.52, 128.05, 127.89, 126.85, 126.41, 126.22, 125.85, 73.83 (Nap), 71.90 (C-1), 70.90 (C-2), 64.21 (C-3). HR-MS: Calculated for $C_{14}H_{16}O_3$ [M+Na]$^+$: 255.09917, found: 255.09921. $[\alpha]^{25}_D$ = + 0.6° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (DCM/Acetone = 2/1, v/v).

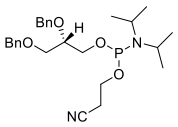
2,3-di-*O*-benzyl-*sn*-glycerol (29c)



Diol **29b** (10.0 g, 43.0 mmol, 1.0 eq) was dissolved in DMF (180 mL), then cooled to 0 °C. Benzyl bromide (16 mL, 129.0 mmol, 3.0 eq) was added and then sodium hydride (8.6 g, 215.0 mmol, 5 eq) was added slowly, the reaction was stirred for overnight. After analysis by TLC showed complete consumption of the starting material, quenched by MeOH, extracted with Et_2O and washed with water and brine. The organic

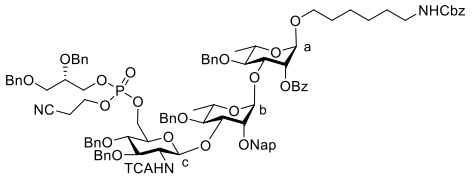
layer was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude compound was dissolved in DCM (500 mL) and water (50 mL). After cooled to 0 °C, 2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ) (12.0 g, 51.6 mmol, 1.2 eq) was added. The reaction was stirred at RT for 6 hours. After analysis by TLC showed complete consumption of the starting material, quenched by saturated aqueous sodium thiosulphate, extracted with DCM and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA 10:1 – 6/1) to yield compound **29c** (10.1 g, 37.1 mmol, 86%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.38 – 7.25 (m, 10H), 4.70 (d, *J* = 11.7 Hz, 1H, Bn), 4.61 (d, *J* = 11.8 Hz, 1H, Bn), 4.53 (d, *J* = 2.3 Hz, 2H, Bn), 3.80 – 3.56 (m, 5H, H-1, H-2, H-3), 2.24 – 2.17 (m, 1H, 1-OH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.35, 138.05, 128.56, 128.54, 127.91, 127.90, 127.84, 127.76, 78.16 (C-2), 73.62 (Bn), 72.25 (Bn), 70.27 (C-3), 62.93 (C-1). HR-MS: Calculated for $C_{17}H_{20}O_3$ $[M+NH_4]^+$: 290.17507, found: 290.17496. $[\alpha]^{25}_D$ = + 20.4° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (PE/EA = 6/1, v/v).

2,3-di-*O*-benzyl-1-*O*-([*N*,*N*-diisopropylamino]-2-cyanoethylphosphite)-*sn*-glycerol (29)



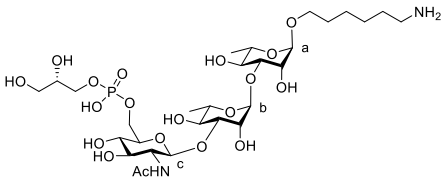
Alcohol **29c** (379 mg, 1.39 mmol, 1.0 eq) and diisopropylammonium tetrazolide (120 mg, 0.5 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (10 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. 0.3M Bis(diisopropylamino)(2-cyanoethoxy)phosphine in DCM (7.0 ml, 2.1 mmol, 1.5 eq) was added and the reaction mixture was stirred for 3 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with Et_3N and diluted with DCM. The solution was washed with saturated aqueous sodium bicarbonate and brine. The aqueous layer was extracted with DCM (1x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/EA 20:1 - 6:1) to yield compound **29** (462 mg, 0.98 mmol, 70%). ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 7.42 – 7.24 (m, 10H, Bn), 4.71 – 4.59 (m, 2H, 2-OBn), 4.52 (s, 2H, 3-OBn), 3.86 – 3.53 (m, 9H), 2.69 – 2.53 (m, 2H, OCH_2CH_2CN), 1.22 – 1.11 (m, 12H, *i*-Pr). ^{13}C NMR (101 MHz, CD_3CN) δ 140.03, 139.69, 129.26, 129.20, 128.65, 128.63, 128.58, 128.44, 128.38, 119.53 (CN), 78.94, 78.92, 78.86, 78.84 (C-2), (C-3), 73.79 (OBn), 72.52, 72.50 (OBn), 70.79, 70.72 (C-3), 63.93, 63.91, 63.77, 63.75 (C-1), 59.47, 59.42, 59.29, 59.24 (OCH_2CH_2CN), 43.89, 43.87, 43.77, 43.75 ($CH(CH_3)_2$), 24.98, 24.91, 24.85 ($CH(CH_3)_2$), 21.03, 20.96 (OCH_2CH_2CN). ^{31}P NMR (162 MHz, CD_3CN) δ 149.47, 149.41. HR-MS: Calculated for $C_{26}H_{37}N_2O_4P$ $[M-N(i\text{-}Pr)_2+OH+NH_4]^+$: 407.17303, found: 407.17259. TLC: Rf = 0.5 (PE/EA = 6/1, v/v).

***N*-benzyloxycarbonyl-6-aminohexanyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-(2,3-di-*O*-benzyl-1-*O*-(2-cyanoethylphosphate)-*sn*-glycerol)-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (30)**



Alcohol **28** (57 mg, 39 µmol, 1.0 eq) and 0.1M phosphite **29** in ACN (1.2 mL, 118 µmol, 3.0 eq) were co-evaporated with dry acetonitrile 3 times under nitrogen. The mixture was dissolved in dry acetonitrile (4 ml) and 3Å molecular sieves was added. The mixture was stirred for 15 mins under argon atmosphere. 4,5-dicyanoimidazole (DCI, 0.25M in acetonitrile) (470 µL, 0.12 mmol, 3.0 eq) was added and the reaction mixture was stirred for 6 hours. After analysis by TLC showed complete consumption of the starting material, (10-Camphorsulfonyl)-oxaziridine (CSO, 0.5M in acetonitrile) (314 µL, 0.16 mmol, 4.0 eq) was added. Stirred another 15 mins and diluted with EtOAc. The solution was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (PE/Acetone 5:1 - 3:1) yielded **30** (64 mg, 35 µmol, 89%). ^{1}H NMR (850 MHz, Chloroform-*d*) δ 8.11 (d, *J* = 7.7 Hz, 2H, Bz), 7.83 – 7.72 (m, 3H), 7.72 – 7.68 (m, 1H), 7.64 – 7.58 (m, 1H), 7.53 – 7.46 (m, 2H), 7.46 – 7.40 (m, 4H), 7.39 – 7.19 (m, 32H), 7.16 – 7.10 (m, 2H), 6.79 (s, 1H), 5.44 – 5.37 (m, 1H), 5.18 – 5.04 (m, 3H), 4.92 – 4.33 (m, 14H), 4.27 – 4.19 (m, 1H), 4.16 – 3.96 (m, 5H), 3.93 – 3.85 (m, 1H), 3.84 – 3.50 (m, 11H), 3.48 – 3.35 (m, 3H), 3.23 – 3.10 (m, 3H), 2.23 – 1.91 (m, 2H), 1.62 – 1.45 (m, 4H), 1.41 – 1.17 (m, 7H), 1.14 – 1.04 (m, 3H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 166.05, 166.04, 162.11, 162.04, 156.52, 138.69, 138.64, 138.52, 138.06, 137.98, 137.93, 137.88, 137.81, 137.71, 137.70, 136.78, 136.20, 136.15, 133.48, 133.31, 133.28, 133.03, 132.99, 130.09, 130.08, 129.98, 128.72, 128.66, 128.64, 128.64, 128.59, 128.58, 128.56, 128.53, 128.52, 128.51, 128.25, 128.19, 128.15, 128.13, 128.10, 128.07, 128.06, 128.04, 128.00, 127.98, 127.94, 127.89, 127.88, 127.86, 127.85, 127.83, 127.80, 127.78, 127.77, 127.74, 127.73, 127.71, 127.67, 127.53, 127.41, 127.39, 126.48, 126.28, 126.25, 126.13, 126.08, 126.05, 125.89, 125.83, 116.70, 116.59, 100.44, 97.20, 92.37, 92.33, 80.41, 80.38, 80.22, 80.10, 78.72, 78.58, 77.76, 76.51, 76.49, 76.47, 76.44, 75.16, 75.10, 75.06, 75.02, 74.66, 74.56, 73.70, 73.67, 73.57, 73.49, 73.41, 73.25, 73.16, 72.22, 72.16, 68.78, 68.73, 68.59, 67.94, 67.93, 67.80, 67.71, 67.68, 67.50, 67.47, 66.68, 66.18, 66.11, 61.80, 61.78, 61.76, 58.33, 58.27, 41.15, 30.01, 29.84, 29.38, 26.63, 25.96, 19.19, 19.16, 19.11, 19.08, 18.18, 18.07. ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.61, -0.82. HR-MS: Calculated for $C_{100}H_{109}Cl_3N_3O_{22}P$ $[M+NH_4]^+$: 1857.66442, found: 1857.66590. TLC: Rf = 0.35 (PE/Acetone = 3/1, v/v).

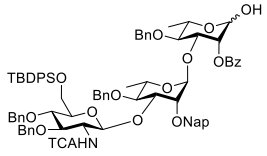
6-aminohexanyl 3-*O*-(3-*O*-(2-acetamito-2-deoxy-6-*O*-(1-*O*-phosphate-*sn*-glycerol)-β-D-glucopyranosyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (4)



Full protected trimer **30** (10 mg, 5.4 µmol, 1.0 eq) was dissolved in dioxane (4 mL) and ammonia solution (35%) (2 mL). The mixture was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, co-evaporated with toluene to remove the

solvent. The crude was dissolved in methanol (2 mL) and dioxane (2 mL). Sodium methoxide (25 wt. % in methanol) (0.1 mL, 0.44 mmol, 81 eq) was added. The reaction was stirred overnight. After analysis by TLC showed complete consumption of the starting material, quenched with acetic acid and then quenched the excess acid using ammonia solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The mixture was purified by flash size exclusion (LH-20) (DCM/MeOH 1:1). The compound was dissolved in *tert*-butanol (6 mL), water (3 mL) and 3 drops acetic acid. After $Pd(OH)_2/C$ (60 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector, transformed into its sodium salt by passing a short Dowex Na^+ column and lyophilized to yield compound **4** (3.0 mg, 3.9 µmol, 72%). ^{1}H NMR (850 MHz, Deuterium Oxide) δ 5.02 (d, *J* = 1.8 Hz, 1H, H-1b), 4.77 (d, *J* = 1.8 Hz, 1H, H-1c), 4.72 (d, *J* = 8.5 Hz, 1H, H-1c), 4.36 – 4.33 (m, 1H, H-2b), 4.22 – 4.16 (m, 1H, H-6c), 4.10 – 4.05 (m, 1H, H-6c), 4.03 – 3.99 (m, 1H, H-2a), 3.97 – 3.86 (m, 4H, H-3b), 3.86 – 3.81 (m, 1H, H-5b), 3.81 – 3.76 (m, 2H, H-3a, H-2c), 3.75 – 3.68 (m, 3H, H-5a), 3.63 (dd, *J* = 11.8, 6.1 Hz, 1H), 3.61 – 3.53 (m, 5H, H-5c, H-3c, H-4c, H-4a), 3.51 (t, *J* = 9.7 Hz, 1H, H-4b), 3.01 – 2.96 (m, 2H), 2.04 (s, 3H, NHAc), 1.70 – 1.58 (m, 4H), 1.48 – 1.38 (m, 4H), 1.31 (d, *J* = 6.3 Hz, 3H, H-6a), 1.29 (d, *J* = 6.3 Hz, 3H, H-6b). ^{13}C NMR (214 MHz, D_2O) δ 175.79 (NHAc), 103.76 (C-1c), 103.04 (C-1b), 100.52 (C-1a), 81.45 (C-3b), 79.32 (C-3a), 75.31, 75.27 (C-5c), 74.46 (C-3c), 72.12 (C-4a), 71.63 (C-4b), 71.59, 70.93 (C-2a), 70.63 (C-2b), 70.39 (C-4c), 70.11 (C-5b), 69.70 (C-5a), 68.64, 67.27, 67.25, 65.41, 65.39 (C-6c), 62.97, 56.62 (C-2c), 40.39, 29.19, 27.81, 26.29, 25.88, 23.08, 17.52, 17.49. ^{31}P NMR (162 MHz, D_2O) δ 1.44. HR-MS: Calculated for $C_{29}H_{55}N_2O_{19}P$ $[M+H]^+$: 767.32094, found: 767.32051.

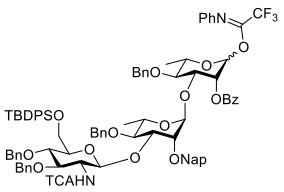
2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(4-*O*-benzyl-3-*O*-(3,4-di-*O*-benzyl-2-trichloroacetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2-*O*-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-α/β-L-rhamnopyranoside (31)



The compound **26** (3.22 g, 2.07 mmol, 1 eq) was dissolved in DCM (20 mL) and reduced to 0 °C. NIS (934 mg, 4.15 mmol, 2.0 eq) and TFA (207 µL, 2.69 mmol, 1.3 eq) were added and the solution stirred for 1 hour. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with triethyl amine and saturated aqueous sodium thiosulphate. The solution was diluted with DCM and washed with brine (3x). The organic phase was dried with $MgSO_4$, filtered and concentrated in *vacuo*. The compound was purified by flash chromatography (PE/Acetone 10:1 - 4:1) to yield compound **31** (2.72 g, 1.86 mmol, 90%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 – 7.94 (m, 2H), 7.77 – 7.49 (m, 10H), 7.42 – 7.06 (m, 30H), 6.75 – 6.64 (m, 1H), 5.46 – 5.39 (m, 1H), 5.23 (d, *J* = 2.0 Hz, 1H), 5.13 (d, *J* = 2.0 Hz, 1H), 4.90 – 4.82 (m, 2H), 4.77 – 4.43 (m, 9H), 4.35 (dd, *J* = 9.4, 3.4 Hz, 1H), 4.13 (dd, *J* = 9.1, 3.1 Hz, 1H), 4.09 – 3.79 (m, 5H), 3.74 – 3.44 (m, 5H), 3.28 – 3.19 (m, 1H), 3.06 – 2.93 (m, 1H), 1.45 – 1.13 (m, 6H), 0.97 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.93, 161.90, 138.43, 138.05, 137.79, 136.01, 135.73, 135.66, 135.52, 135.47, 133.58, 133.28, 133.11, 133.00, 129.89, 129.81, 129.78, 129.76, 129.73, 128.68, 128.66, 128.63, 128.58, 128.53, 128.50, 128.47, 128.10, 128.07, 127.96, 127.93, 127.87, 127.82, 127.79, 127.74, 127.66, 127.49, 127.44, 127.41, 127.26, 127.24, 126.66, 126.32, 125.76, 125.59,

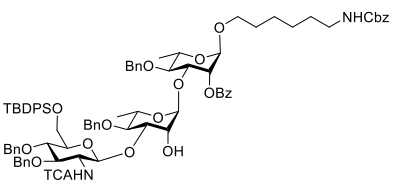
100.54, 92.58, 92.15, 80.88, 80.84, 78.74, 77.39, 76.30, 75.43, 74.78, 74.62, 74.35, 74.06, 73.10, 72.27, 68.14, 67.87, 62.68, 57.44, 29.80, 29.75, 26.96, 19.43, 18.24, 18.05. HR-MS: Calculated for $C_{82}H_{86}Cl_3NO_{15}Si$ [M+Na$^+$]: 1480.47245, found: 1480.47295. TLC: Rf = 0.6 (PE/Acetone = 3/1, v/v).

_N_-Phenyl-trifluoroacetimidate 2-_O_-benzoyl-4-_O_-benzyl-3-_O_-(4-_O_-benzyl-3-_O_-(3,4-di-_O_-benzyl-2-trichloroacetamido-2-deoxy-6-_O_-_tert_-butyldiphenylsilyl-β-D-glucopyranosyl)-2-_O_-(2-naphthylmethyl)-α-L-rhamnopyranosyl)-α/β-L-rhamnopyranoside (32)



Hemiacetal **31** (3.57 g, 4.80 mmol, 1.0 eq) was dissolved in acetone (50 mL) and cooled to 0 °C. Cesium carbonate (1.9 g, 5.83 mmol, 1.2 eq) was added. After 15 min, N-phenyl trifluoroacetimidoyl chloride (1.5 g, 7.23 mmol, 1.5 eq) was added, and then the reaction was allowed to stir for overnight at RT. After analysis by TLC showed complete consumption of the starting material, quenched by Et_3N, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/Et_2O 10:1 – 7/1) to yield compound **32** (3.76 g, 4.11 mmol, 86%). ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.53 (d, *J* = 9.2 Hz, 1H), 8.18 – 8.07 (m, 2H), 7.99 – 7.85 (m, 4H), 7.76 – 7.69 (m, 2H), 7.68 – 7.60 (m, 3H), 7.58 – 7.22 (m, 31H), 7.20 – 7.09 (m, 3H), 6.97 (d, *J* = 7.8 Hz, 2H), 6.44 (s, 1H), 5.86 (s, 1H), 5.42 – 5.34 (m, 1H), 5.29 – 5.17 (m, 2H), 5.07 (d, *J* = 12.4 Hz, 1H), 4.96 (d, *J* = 11.4 Hz, 1H), 4.92 – 4.61 (m, 7H), 4.61 – 4.51 (m, 1H), 4.46 – 4.28 (m, 2H), 4.27 – 4.20 (m, 1H), 4.18 – 4.04 (m, 2H), 4.04 – 3.85 (m, 3H), 3.81 – 3.59 (m, 3H), 3.44 – 3.35 (m, 1H), 1.47 (d, *J* = 6.2 Hz, 3H), 1.08 (s, 12H). ^{13}C NMR (126 MHz, Acetone) δ 165.74, 162.63, 144.26, 139.77, 139.20, 138.94, 137.41, 136.31, 136.13, 134.27, 134.13, 133.81, 133.77, 130.54, 130.47, 130.29, 130.19, 129.56, 129.38, 129.10, 129.08, 128.90, 128.87, 128.83, 128.71, 128.63, 128.54, 128.48, 128.45, 128.38, 128.34, 128.30, 128.26, 128.24, 128.16, 128.13, 128.05, 127.94, 127.29, 127.18, 126.53, 126.34, 125.16, 120.04, 102.11, 100.76, 93.77, 82.26, 80.84, 80.47, 80.12, 78.58, 76.65, 75.39, 75.25, 75.20, 74.77, 74.48, 74.34, 71.51, 71.34, 69.39, 63.47, 58.87, 27.40, 19.84, 18.42, 18.27. HR-MS: Calculated for $C_{90}H_{90}Cl_3F_3N_2O_{15}Si$ [M-[O(C=NPh)CF_3]+OH+NH_4^+]: 1480.47245, found: 1480.47442. TLC: Rf = 0.6 (PE/Acetone = 4/1, v/v).

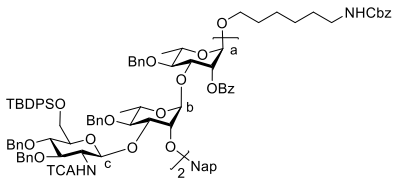
_N_-benzyloxycarbonyl-6-aminohexanyl 2-_O_-benzoyl-4-_O_-benzyl-3-_O_-(4-_O_-benzyl-3-_O_-(3,4-di-_O_-benzyl-2-trichloroacetamido-2-deoxy-6-_O_-_tert_-butyldiphenylsilyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (33)



The full protected trisaccharide **27** (643 mg, 0.38 mmol, 1.0 eq) was dissolved in DCM (5 mL) and pH 7 water buffer (0.50 mL). After cooled to 0 °C, 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (172 mg, 0.76 mmol, 2.0 eq) was added. The reaction was stirred at RT for 6 hours. After analysis by TLC showed complete consumption of the starting material, quenched by saturated aqueous sodium thiosulphate, extracted with DCM and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*, and the product purified by column chromatography (PE/EA/DCM 7:1:1 – 4:1:1) to yield compound **33** (539 mg,

0.35 mmol, 91%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.07 – 8.01 (m, 2H), 7.67 – 7.59 (m, 4H), 7.56 – 7.49 (m, 1H), 7.44 – 7.36 (m, 6H), 7.36 – 7.15 (m, 23H), 7.15 – 7.10 (m, 2H), 7.09 – 7.02 (m, 2H), 6.87 (d, *J* = 7.8 Hz, 1H), 5.43 – 5.34 (m, 1H), 5.13 – 5.02 (m, 3H), 4.90 – 4.78 (m, 4H), 4.78 – 4.71 (m, 2H), 4.66 (q, *J* = 11.8, 11.0 Hz, 3H), 4.56 (d, *J* = 11.0 Hz, 1H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.23 (dd, *J* = 9.4, 3.3 Hz, 1H), 4.06 – 4.00 (m, 1H), 3.95 (t, *J* = 9.2 Hz, 1H), 3.88 (dd, *J* = 8.6, 3.0 Hz, 1H), 3.85 – 3.76 (m, 1H), 3.76 – 3.53 (m, 6H), 3.47 (t, *J* = 9.0 Hz, 1H), 3.44 – 3.35 (m, 1H), 3.29 – 3.21 (m, 1H), 3.21 – 3.12 (m, 2H), 3.10 – 3.00 (m, 1H), 1.64 – 1.54 (m, 2H), 1.54 – 1.45 (m, 2H), 1.39 – 1.21 (m, 7H), 1.07 (d, *J* = 6.2 Hz, 3H), 1.05 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.98, 162.10, 156.47, 138.62, 138.28, 137.76, 137.66, 136.74, 135.76, 135.63, 133.25, 133.03, 132.68, 129.91, 129.88, 129.80, 129.10, 128.60, 128.56, 128.54, 128.53, 128.51, 128.46, 128.24, 128.13, 128.08, 128.04, 127.96, 127.91, 127.88, 127.84, 127.76, 127.68, 127.63, 127.61, 127.44, 127.38, 125.28, 101.49, 98.77, 97.06, 92.25, 81.13, 79.95, 79.81, 79.54, 78.54, 77.76, 76.08, 75.16, 75.13, 74.57, 73.85, 72.97, 69.81, 67.93, 67.88, 67.66, 66.57, 62.62, 58.47, 41.05, 29.92, 29.31, 26.92, 26.52, 25.88, 19.23, 18.16, 17.78. HR-MS: Calculated for $C_{85}H_{97}Cl_3N_2O_{17}Si$ $[M+NH_4]^+$: 1568.59603, found: 1568.59642. $[\alpha]^{20}_D$ = - 18.2° (c = 1, $CHCl_3$). TLC: Rf = 0.3 (PE/EA/DCM = 3/1/1, v/v/v).

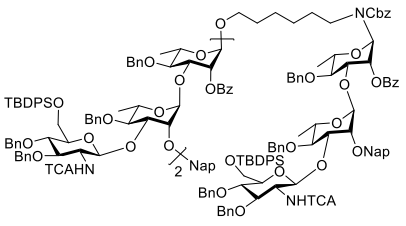
The hexasaccharide 34



Donor **32** (65 mg, 41.9 µmol, 1.3 eq) and acceptor **33** (50 mg, 32.2 µmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (2 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was cooled to 0 °C and then *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (1.5 µL, 8.4 µmol, 0.2 eq) was added. The solution was stirred for 3 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/Acetone 6:1 - 4:1) to yield compound **34** (75 mg, 25.0 µmol, 78%), recover acceptor **33** (7.8 mg, 5 µmol, 14%) and side product **34b** (12.3 mg, 2.8 µmol, 8%). ^{1}H NMR (850 MHz, Chloroform-*d*) δ 8.61 (d, *J* = 6.9 Hz, 1H, NHTCA-c), 8.00 – 7.93 (m, 4H, Bz), 7.77 – 7.73 (m, 1H), 7.73 – 7.67 (m, 2H), 7.63 – 7.56 (m, 4H), 7.52 (ddd, *J* = 8.1, 5.1, 1.5 Hz, 5H), 7.49 – 7.46 (m, 2H), 7.46 – 7.43 (m, 1H), 7.42 – 7.11 (m, 59H), 7.11 – 7.07 (m, 2H), 6.94 – 6.90 (m, 2H), 6.75 (d, *J* = 8.4 Hz, 1H, NHTCA-c'), 5.50 (dd, *J* = 3.4, 1.8 Hz, 1H, H-2a'), 5.41 – 5.36 (m, 2H, H-1a', H-2a), 5.34 (d, *J* = 8.0 Hz, 1H, H-1c), 5.11 – 5.04 (m, 3H, Cbz, H-1b'), 5.00 (d, *J* = 1.6 Hz, 1H, H-1b), 4.94 (d, *J* = 7.7 Hz, 1H, H-1c'), 4.89 (d, *J* = 11.7 Hz, 1H, CH_2), 4.83 (d, *J* = 1.9 Hz, 1H, H-1a), 4.80 – 4.44 (m, 16H, CH_2), 4.44 – 4.34 (m, 3H, CH_2), 4.33 – 4.28 (m, 2H, H-3b, H-3a'), 4.22 (dd, *J* = 9.4, 3.5 Hz, 1H, H-3a), 4.15 (dd, *J* = 3.2, 1.7 Hz, 1H, H-2b), 4.10 (dd, *J* = 9.1, 2.9 Hz, 1H, H-3b'), 3.92 – 3.36 (m, 19H), 3.32 – 3.27 (m, 1H, H-5c'), 3.27 – 3.22 (m, 1H, H-5c), 3.22 – 3.15 (m, 2H), 3.15 – 3.08 (m, 1H, H-2c), 1.63 – 1.56 (m, 2H), 1.54 – 1.44 (m, 2H), 1.41 – 1.21 (m, 7H), 1.07 – 0.97 (m, 18H), 0.94 (s, 9H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 167.18, 166.22, 161.95, 161.88, 156.53 (Cbz), 138.92, 138.38, 138.36, 138.35, 138.32, 138.25, 138.04, 137.84, 136.84, 136.21, 135.83, 135.81, 135.65, 135.60, 133.63, 133.62, 133.47, 133.35, 133.32, 133.26, 133.15, 133.05, 129.95,

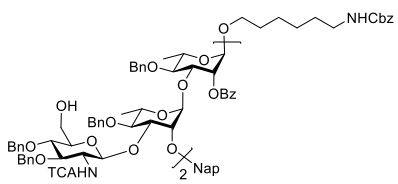
129.90, 129.80, 129.77, 129.69, 129.58, 128.64, 128.59, 128.55, 128.53, 128.52, 128.32, 128.31, 128.27, 128.12, 127.95, 127.94, 127.93, 127.88, 127.83, 127.75, 127.75, 127.66, 127.54, 127.48, 127.41, 127.38, 126.47, 126.21, 125.87, 125.66, 101.33 (C-1b, J_{CH} = 167 Hz), 100.46 (C-1b', J_{CH} = 172 Hz), 100.34 (C-1c', J_{CH} = 160 Hz), 98.38 (C-1a', J_{CH} = 175 Hz), 98.21 (C-1c, J_{CH} = 167 Hz), 97.16 (C-1a, J_{CH} = 166 Hz), 93.07 (TCA), 92.54 (TCA), 81.02, 80.82, 80.55, 80.47, 80.24 (C-3c'), 78.95 (C-4c), 78.76, 78.07 (C-3a, C-3b'), 77.67, 77.54, 77.31, 77.27 (C-2b), 76.98 (C-3b'), 76.41, 75.83 (C-5c), 75.44 (C-3b), 75.16, 74.98, 74.61, 74.48, 74.29, 74.25, 74.09, 73.99, 73.74, 73.46 (C-2d), 73.01 (C-2a), 68.83, 68.50, 68.37, 68.02, 67.55, 66.69 (Cbz), 62.94 (C-6c, C-6f), 61.17 (C-2c), 57.93 (C-2f), 41.16, 30.01, 29.84, 29.40, 27.12, 27.07, 26.61, 25.89, 19.45, 19.29, 18.29, 18.27, 18.12, 17.93. HR-MS: Calculated for $C_{167}H_{181}Cl_6N_3O_{31}Si_2$ $[(M+NH_4+NH_4)/2]^+$: 1513.05126, found: 1513.05116. $[\alpha]^{20}_D$ = - 31.5° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (PE/Acetone = 4/1, v/v).

The side-product nonasaccharide 34b



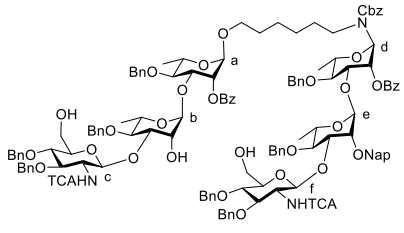
^{1}H NMR (850 MHz, Chloroform-*d*) δ 8.59 (d, *J* = 6.8 Hz, 1H), 8.11 – 8.04 (m, 1H), 8.00 – 7.94 (m, 4H), 7.94 – 7.84 (m, 2H), 7.80 – 7.76 (m, 1H), 7.76 – 7.73 (m, 1H), 7.72 – 7.67 (m, 3H), 7.67 – 7.62 (m, 2H), 7.63 – 7.56 (m, 8H), 7.55 – 7.50 (m, 6H), 7.49 – 7.46 (m, 3H), 7.45 – 7.41 (m, 3H), 7.41 – 7.11 (m, 82H), 7.10 – 7.06 (m, 3H), 6.94 – 6.87 (m, 2H), 6.75 (d, *J* = 8.5 Hz, 1H), 5.96 (d, *J* = 9.5 Hz, 1H), 5.52 – 5.49 (m, 1H), 5.41 – 5.34 (m, 3H), 5.32 (d, *J* = 8.0 Hz, 1H), 5.12 – 5.04 (m, 2H), 5.03 – 4.95 (m, 4H), 4.93 (d, *J* = 7.6 Hz, 1H), 4.88 (d, *J* = 11.7 Hz, 1H), 4.79 – 4.54 (m, 17H), 4.53 – 4.46 (m, 5H), 4.44 – 4.35 (m, 7H), 4.32 – 4.27 (m, 2H), 4.27 – 4.16 (m, 3H), 4.16 – 4.13 (m, 1H), 4.11 – 4.01 (m, 3H), 3.98 – 3.91 (m, 1H), 3.91 – 3.33 (m, 25H), 3.31 – 3.15 (m, 6H), 3.14 – 3.07 (m, 2H), 2.82 – 2.74 (m, 1H), 1.61 – 1.54 (m, 2H), 1.53 – 1.44 (m, 2H), 1.42 – 1.09 (m, 13H), 1.05 – 1.02 (m, 15H), 1.01 – 0.99 (m, 12H), 0.94 (s, 9H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 167.16, 166.10, 165.44, 161.93, 161.89, 161.87, 156.53, 138.93, 138.51, 138.39, 138.38, 138.36, 138.33, 138.29, 138.05, 137.84, 137.82, 137.77, 137.76, 136.22, 136.04, 136.02, 136.00, 135.84, 135.83, 135.80, 135.70, 135.64, 135.60, 133.62, 133.46, 133.35, 133.28, 133.20, 133.13, 133.12, 133.04, 129.98, 129.97, 129.94, 129.89, 129.87, 129.79, 129.77, 129.67, 129.58, 128.67, 128.59, 128.56, 128.54, 128.53, 128.52, 128.50, 128.35, 128.31, 128.30, 128.26, 128.12, 128.00, 127.99, 127.98, 127.96, 127.94, 127.92, 127.88, 127.87, 127.80, 127.74, 127.69, 127.68, 127.65, 127.62, 127.52, 127.48, 127.47, 127.39, 127.32, 126.60, 126.46, 126.21, 126.14, 126.13, 125.94, 125.85, 125.65, 101.17, 100.42, 100.29, 98.41, 98.28, 98.15, 97.30, 97.16, 93.05, 92.54, 80.96, 80.85, 80.81, 80.63, 80.56, 80.51, 80.27, 78.90, 78.79, 78.21, 78.18, 78.15, 77.75, 77.66, 77.53, 77.37, 76.95, 76.87, 76.38, 75.80, 75.57, 75.01, 74.91, 74.73, 74.61, 74.49, 74.45, 74.27, 74.25, 74.04, 73.99, 73.94, 73.75, 73.70, 73.44, 72.95, 72.68, 71.38, 68.80, 68.46, 68.34, 68.28, 68.15, 67.96, 67.88, 67.81, 67.40, 66.70, 63.14, 62.90, 62.87, 61.84, 61.11, 57.89, 56.37, 41.17, 29.84, 29.29, 27.12, 27.10, 27.06, 27.04, 26.99, 26.81, 25.55, 19.45, 19.44, 19.28, 18.28, 18.26, 18.24, 18.17, 18.10, 17.94, 17.45, 16.58. MALDI-FTICR: Calculated for $C_{249}H_{265}Cl_9N_4O_{45}Si_3$ $[M+Na]^+$: 4452.4968, found: 4452.4100. $[\alpha]^{25}_D$ = - 43.8° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (PE/Acetone = 4/1, v/v).

The hexasaccharide 35



Protected hexasaccharide **34** (54 mg, 18 µmol, 1.0 eq) was dissolved in anhydrous THF (4 mL) and AcOH (21 µL, 360 µmol, 20 eq). Then 1M TBAF in THF (360 µL, 360 µmol, 20 eq) was added in 0 °C. The reaction mixture was stirred at 50 °C for overnight. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous ammonium chloride and diluted with EA. The solution was washed with water (2x) and brine. The aqueous layer was extracted with EA (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/Acetone 4:1 - 2:1) to yield compound **35** (37.1 mg, 14.7 µmol, 82%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.15 (d, *J* = 6.8 Hz, 1H, NHTCA), 8.10 – 8.01 (m, 4H, Bz), 7.85 – 7.76 (m, 3H), 7.68 (s, 1H), 7.64 – 7.57 (m, 2H), 7.53 – 7.10 (m, 52H), 6.68 – 6.51 (m, 1H, NHTCA), 5.52 – 5.43 (m, 2H), 5.38 – 5.31 (m, 1H), 5.30 – 5.22 (m, 1H), 5.20 – 5.04 (m, 3H), 4.96 (d, *J* = 1.7 Hz, 1H), 4.91 – 4.37 (m, 21H), 4.31 (dd, *J* = 9.5, 3.3 Hz, 1H), 4.21 – 4.14 (m, 2H), 4.10 (dd, *J* = 9.3, 3.0 Hz, 1H), 3.99 (dd, *J* = 9.2, 2.9 Hz, 1H), 3.88 – 3.50 (m, 12H), 3.46 – 3.28 (m, 9H), 3.23 – 3.12 (m, 4H), 3.10 – 3.02 (m, 1H), 2.25 – 2.06 (m, 1H), 1.63 – 1.53 (m, 2H), 1.53 – 1.45 (m, 2H), 1.40 – 1.21 (m, 7H), 1.18 (d, *J* = 6.1 Hz, 3H), 1.06 (d, *J* = 6.1 Hz, 3H), 1.01 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.05, 166.09, 162.05, 161.97, 156.51, 138.65, 138.39, 138.22, 138.17, 138.11, 137.92, 137.79, 137.75, 136.80, 135.82, 133.81, 133.47, 133.27, 133.11, 130.03, 129.91, 129.56, 128.82, 128.80, 128.74, 128.68, 128.65, 128.63, 128.60, 128.51, 128.39, 128.31, 128.23, 128.18, 128.12, 128.09, 128.06, 127.99, 127.95, 127.88, 127.84, 127.74, 127.70, 127.62, 127.51, 127.48, 127.34, 126.79, 126.36, 126.18, 125.95, 101.12, 100.34, 98.79, 97.78, 96.98, 92.62, 92.43, 80.23, 79.85, 79.34, 78.77, 78.62, 78.05, 78.03, 77.65, 76.49, 75.35, 75.32, 75.13, 75.07, 74.79, 74.74, 74.67, 74.63, 74.19, 73.42, 73.26, 73.05, 69.20, 68.98, 68.55, 67.99, 67.67, 66.67, 61.74, 61.42, 60.93, 58.05, 41.13, 29.97, 29.82, 29.36, 26.58, 25.88, 18.14, 18.07, 17.83. HR-MS: Calculated for $C_{135}H_{145}Cl_6N_3O_{31}$ $[M+H]^+$: 2514.80660, found: 2514.81074. $[\alpha]^{20}_D$ = -57.4° (c = 1, $CHCl_3$). TLC: Rf = 0.2 (PE/Acetone = 3/1, v/v).

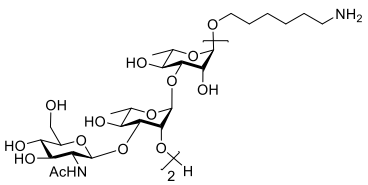
The side-product hexasaccharide 35a



^{1}H NMR (850 MHz, Chloroform-*d*) δ 8.08 – 8.03 (m, 3H), 7.96 (d, *J* = 7.6 Hz, 2H), 7.82 – 7.76 (m, 3H), 7.76 – 7.71 (m, 2H), 7.63 – 7.58 (m, 1H), 7.52 – 7.43 (m, 7H), 7.41 – 7.18 (m, 39H), 7.15 – 7.11 (m, 5H), 6.75 – 6.67 (m, 2H, NHTCA), 6.01 (d, *J* = 9.6 Hz, 1H, H-1d), 5.39 (dd, *J* = 9.7, 3.4 Hz, 1H, H-2d), 5.33 (dd, *J* = 3.4, 1.9 Hz, 1H, H-2a), 5.10 – 5.05 (m, 3H, H-1b, Cbz), 4.95 – 4.88 (m, 2H, H-1f), 4.88 – 4.83 (m, 2H, H-1c), 4.82 – 4.57 (m, 15H, H-1a, C*H*$_2$), 4.52 (s, 1H, H-1e), 4.50 – 4.42 (m, 5H, H-3d), 4.36 (d, *J* = 12.1 Hz, 1H), 4.20 (dd, *J* = 9.4, 3.3 Hz, 1H, H-3a), 4.06 – 3.95 (m, 3H, H-5d, H-2f, H-2b), 3.87 (dd, *J* = 8.7, 3.0 Hz, 1H, H-3b), 3.85 – 3.80 (m, 1H, H-3c), 3.80 – 3.69 (m, 4H, H-2e, H-5a, H-5b), 3.69 – 3.51 (m, 9H), 3.50 – 3.45 (m, 2H), 3.41 (t, *J* = 9.1 Hz, 2H, H-4b), 3.26 – 3.19 (m, 3H, H-5c), 3.14 – 3.06 (m, 2H), 2.99 – 2.91 (m, 1H, H-4d), 1.52 – 1.45 (m, 2H), 1.41 – 1.34 (m, 2H), 1.31 (d, *J* = 6.2 Hz, 3H, H-6b), 1.19 – 1.10 (m, 4H), 1.07

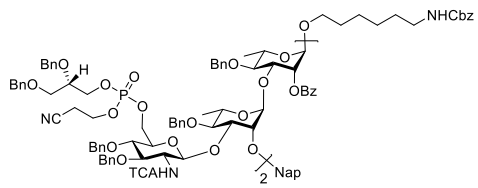
(d, *J* = 6.2 Hz, 3H, H-6a), 1.01 – 0.93 (m, 3H, H-6d), 0.67 (d, *J* = 6.0 Hz, 3H, H-6e). ^{13}C NMR (214 MHz, $CDCl_3$) δ 166.00, 165.38, 162.16 (NHTCA), 161.91 (NHTCA), 158.08 (Cbz), 138.57, 138.55, 138.31, 137.86, 137.80, 137.73, 137.72, 137.64, 135.84, 133.39, 133.37, 133.27, 133.17, 130.17, 129.97, 129.94, 129.35, 128.73, 128.72, 128.70, 128.69, 128.66, 128.65, 128.64, 128.62, 128.53, 128.42, 128.40, 128.32, 128.30, 128.21, 128.15, 128.13, 128.10, 128.06, 128.04, 128.03, 128.02, 127.96, 127.94, 127.89, 127.81, 127.75, 127.72, 127.69, 127.65, 127.52, 127.42, 126.74, 126.43, 126.22, 101.47 (C-1b), 100.73 (C-1f), 99.41 (C-1c), 97.18 (C-1a), 96.77 (C-1e), 92.63 (TCA), 92.28 (TCA), 80.57, 80.14, 80.03, 79.75 (C-3c), 78.15, 78.06, 77.79 (C-4c), 76.69 (C-2e), 75.90 (C-1d), 75.67, 75.47, 74.91, 74.71, 74.66, 74.55, 73.87, 73.15, 72.72, 71.47, 70.65, 68.82, 68.10, 68.08, 67.71 (C-2d), 67.70, 67.29 (Cbz), 61.80 (C-6c), 61.69 (C-6f), 58.07, 57.60, 45.99, 42.60, 29.85, 29.27, 26.84, 25.68, 18.20, 17.93, 17.29, 16.64. HR-MS: Calculated for $C_{135}H_{145}Cl_6N_3O_{31}$ $[M+H]^+$: 2514.80660, found: 2514.81265. $[\alpha]^{25}_D$ = - 67.0° (c = 0.2, $CHCl_3$). TLC: Rf = 0.1 (PE/Acetone = 3/1, v/v).

The target hexasaccharide 2



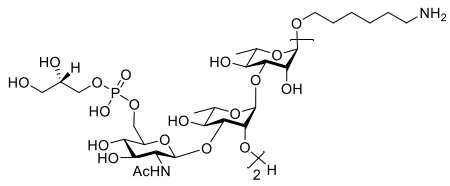
The hexamer **35** (20.6 mg, 8.2 µmol, 1.0 eq) was dissolved in methanol (3 mL) and dioxane (1 mL). Sodium methoxide (25 wt. % in methanol) (0.1 mL, 0.44 mmol, 53 eq) was added. The reaction was stirred overnight. Sodium methoxide (25 wt. % in methanol) (0.1 mL, 0.44 mmol, 53 eq) was added again. After analysis by TLC showed complete consumption of the starting material, quenched with acetic acid and then quenched the excess acid using ammonia solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The mixture was purified by flash size exclusion (LH-20) (DCM/MeOH 1:1). The crude was dissolved in *tert*-butanol (7 mL) and 0.1% AcOH in water (3 mL). After $Pd(OH)_2/C$ (70 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered, using ammonia solution quenched the AcOH and concentrated *in vacuo*. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector and lyophilized to yield compound **2** (5.5 mg, 5.0 µmol, 60%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.19 (d, *J* = 1.8 Hz, 1H), 5.09 (d, *J* = 1.8 Hz, 1H), 5.03 (d, *J* = 1.7 Hz, 1H), 4.76 (d, *J* = 1.7 Hz, 1H), 4.72 (d, *J* = 2.9 Hz, 1H), 4.71 (d, *J* = 3.0 Hz, 1H), 4.32 – 4.24 (m, 2H), 4.09 – 4.06 (m, 1H), 4.01 – 3.88 (m, 6H), 3.86 – 3.66 (m, 11H), 3.59 – 3.41 (m, 11H), 3.02 – 2.94 (m, 2H), 2.06 – 2.00 (m, 6H), 1.71 – 1.58 (m, 4H), 1.47 – 1.35 (m, 4H), 1.33 – 1.23 (m, 12H). ^{13}C NMR (126 MHz, D_2O) δ 174.97, 174.52, 102.79, 102.57, 101.76, 101.29, 101.02, 99.61, 79.91, 79.74, 77.46, 77.11, 76.00, 75.74, 75.66, 73.89, 73.69, 71.77, 71.51, 71.22, 70.92, 69.98, 69.89, 69.85, 69.79, 69.76, 69.41, 69.34, 68.70, 67.79, 60.84, 60.59, 55.89, 55.75, 39.46, 28.32, 26.68, 25.41, 24.96, 22.24, 22.20, 16.81, 16.66, 16.58, 16.50. HR-MS: Calculated for $C_{46}H_{81}N_3O_{27}$ $[M+2H^+]/2$: 554.76015, found: 554.75949.

The glycerol phosphate modified hexasaccharide 36



Alcohol **35** (37 mg, 14.7 µmol, 1.0 eq) and 0.1M phosphite **29** in ACN (588 µL, 58.8 µmol, 4.0 eq) were co-evaporated with dry acetonitrile 3 times under nitrogen. The mixture was dissolved in dry acetonitrile (2 ml) and 3Å molecular sieves was added. The mixture was stirred for 15 mins under argon atmosphere. 4,5-dicyanoimidazole (DCI, 0.25M in acetonitrile) (353 µL, 88 µmol, 6.0 eq) was added and the reaction mixture was stirred for 6 hours. After analysis by TLC showed complete consumption of the starting material, (10-Camphorsulfonyl)-oxaziridine (CSO, 0.5M in acetonitrile) (180 µL, 88 µmol, 6.0 eq) was added. Stirred another 15 mins and diluted with EtOAc. The solution was washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (PE/Acetone 3:1 - 2:1) yielded **36** (45.8 mg, 13.9 µmol, 95%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.80 – 8.62 (m, 1H), 8.16 – 7.99 (m, 4H), 7.86 – 7.67 (m, 4H), 7.66 – 7.02 (m, 64H), 6.80 (s, 1H), 5.55 – 5.42 (m, 1H), 5.38 – 5.24 (m, 3H), 5.19 – 5.04 (m, 3H), 5.04 – 3.24 (m, 80H), 3.23 – 2.99 (m, 4H), 2.26 – 1.94 (m, 4H), 1.64 – 1.44 (m, 4H), 1.40 – 1.30 (m, 4H), 1.29 – 0.93 (m, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.27, 166.15, 166.08, 162.11, 162.05, 161.99, 156.50, 138.73, 138.70, 138.66, 138.61, 138.58, 138.53, 138.42, 138.37, 138.10, 138.08, 138.05, 138.00, 137.97, 137.93, 137.83, 137.78, 137.72, 136.80, 136.14, 133.88, 133.50, 133.43, 133.31, 133.28, 133.02, 132.99, 130.00, 129.95, 128.91, 128.68, 128.64, 128.60, 128.57, 128.54, 128.51, 128.49, 128.47, 128.46, 128.41, 128.38, 128.35, 128.31, 128.29, 128.22, 128.14, 128.11, 128.03, 127.99, 127.97, 127.96, 127.93, 127.88, 127.82, 127.79, 127.76, 127.75, 127.70, 127.68, 127.65, 127.61, 127.53, 127.47, 127.44, 127.35, 126.23, 126.18, 126.16, 126.09, 126.02, 125.91, 125.86, 116.75, 116.60, 116.54, 116.49, 101.46, 101.09, 100.41, 100.16, 99.53, 98.40, 98.31, 98.01, 97.02, 96.96, 92.90, 92.29, 92.25, 80.48, 80.22, 79.97, 79.76, 79.62, 79.51, 79.17, 78.42, 78.27, 78.18, 78.00, 77.64, 77.49, 77.29, 76.53, 76.51, 76.48, 76.45, 76.43, 76.40, 75.82, 75.49, 75.35, 75.13, 75.03, 74.99, 74.62, 74.59, 74.53, 74.47, 74.42, 74.24, 74.17, 74.09, 73.64, 73.57, 73.46, 73.42, 73.40, 73.34, 73.30, 73.14, 72.19, 72.15, 72.13, 69.03, 68.93, 68.84, 68.78, 68.71, 68.59, 68.53, 68.38, 68.36, 67.94, 67.90, 67.78, 67.73, 67.71, 67.68, 67.63, 67.49, 67.44, 67.28, 67.23, 66.63, 66.35, 66.21, 66.09, 61.83, 61.79, 61.75, 61.70, 61.66, 61.09, 60.87, 58.90, 58.78, 41.12, 29.96, 29.35, 26.58, 25.86, 19.21, 19.16, 19.11, 19.04, 18.98, 18.25, 18.15, 18.12, 18.10, 18.06, 17.83, 17.80. ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.51, -0.56, -0.85, -1.01. HR-MS: Calculated for $C_{175}H_{189}Cl_6N_5O_{41}P_2$ $[(M+NH_4+NH_4)/2]^+$: 1662.05705, found: 1662.05788. TLC: Rf = 0.1 (PE/Acetone = 2.5/1, v/v).

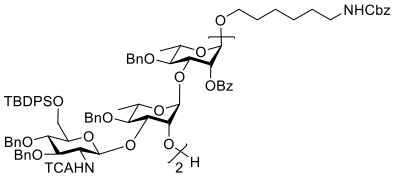
The glycerol phosphate modified hexasaccharide 5



Full protected trimer **36** (45 mg, 13.7 µmol, 1.0 eq) was dissolved in dioxane (6 mL) and ammonia solution (35%) (3 mL). The mixture was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, co-evaporated with toluene to remove the solvent. The crude was dissolved in methanol (3 mL) and dioxane (3 mL). Sodium methoxide (25 wt. % in methanol)

(0.1 mL, 0.44 mmol, 32 eq) was added. The reaction was stirred overnight. After analysis by TLC showed complete consumption of the starting material, quenched with acetic acid and then quenched the excess acid using ammonia solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The mixture was purified by flash size exclusion (LH-20) (DCM/MeOH 1:1). The compound was dissolved in *tert*-butanol (7 mL), water (3 mL) and 2 drops acetic acid. After $Pd(OH)_2/C$ (45 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The crude was dissolved in water (5 mL) and 1M NaOH in water (0.5 mL). After stirred overnight, the reaction was quenched with acetic acid and then quenched the excess acid using ammonia solution. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector, transformed into its sodium salt by passing a short Dowex Na^+ column and lyophilized to yield compound **5** (12.5 mg, 8.8 µmol, 65%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.17 – 5.15 (m, 1H), 5.06 – 5.01 (m, 2H), 4.77 – 4.73 (m, 2H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.35 – 4.32 (m, 1H), 4.29 – 4.26 (m, 1H), 4.22 – 4.13 (m, 2H), 4.11 – 4.02 (m, 3H), 4.00 – 3.97 (m, 1H), 3.97 – 3.46 (m, 30H), 3.01 – 2.96 (m, 2H), 2.05 – 2.00 (m, 6H), 1.72 – 1.57 (m, 4H), 1.47 – 1.37 (m, 4H), 1.34 – 1.24 (m, 12H). ^{13}C NMR (151 MHz, D_2O) δ 175.86, 175.43, 104.00, 103.16, 102.88, 102.83, 101.85, 100.52, 81.87, 80.51, 78.65, 78.38, 78.26, 75.56, 75.51, 75.27, 75.22, 74.74, 74.50, 72.48, 72.37, 72.03, 71.66, 71.61, 70.93, 70.73, 70.66, 70.47, 70.26, 70.18, 70.14, 69.66, 68.65, 67.30, 65.58, 65.28, 62.99, 62.98, 56.77, 56.62, 40.36, 29.28, 27.62, 26.36, 25.97, 23.15, 23.11, 17.86, 17.62, 17.45. ^{31}P NMR (202 MHz, D_2O) δ 1.52, 1.47. HR-MS: Calculated for $C_{52}H_{95}N_3O_{37}P_2$ [M+Na^+]: 1438.50118, found: 1438.50211.

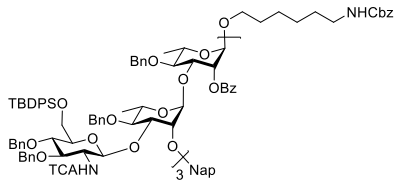
The hexasaccharide 37



The full protected hexamer **34** (597 mg, 0.2 mmol, 1.0 eq) was dissolved in DCM (3 mL) and pH 7 water buffer (0.3 mL). After cooled to 0 °C, 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (91 mg, 0.4 mmol, 2.0 eq) was added. The reaction was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, quenched by saturated aqueous sodium thiosulphate, extracted with DCM and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*, and the product purified by column chromatography (DCM/EA 30:1 – 20:1) to yield compound **37** (437 mg, 0.15 mmol, 77%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.75 (d, *J* = 6.8 Hz, 1H), 8.09 – 7.94 (m, 4H), 7.69 – 7.56 (m, 6H), 7.56 – 7.15 (m, 58H), 7.14 – 7.04 (m, 5H), 6.97 – 6.89 (m, 2H), 6.76 (d, *J* = 7.7 Hz, 1H), 5.53 – 5.47 (m, 1H), 5.44 – 5.35 (m, 3H), 5.12 – 5.02 (m, 3H), 5.01 – 4.96 (m, 1H), 4.89 (d, *J* = 11.5 Hz, 1H), 4.86 – 4.82 (m, 1H), 4.81 – 4.53 (m, 17H), 4.47 (d, *J* = 12.0 Hz, 1H), 4.45 – 4.32 (m, 4H), 4.26 (dd, *J* = 9.5, 3.3 Hz, 1H), 4.20 (dd, *J* = 9.4, 3.5 Hz, 1H), 4.17 – 4.13 (m, 1H), 4.02 – 3.92 (m, 2H), 3.88 – 3.35 (m, 15H), 3.31 – 3.24 (m, 1H), 3.22 – 3.10 (m, 4H), 3.01 (d, *J* = 3.1 Hz, 1H), 1.62 – 1.54 (m, 2H), 1.53 – 1.44 (m, 2H), 1.40 – 1.17 (m, 7H), 1.05 (d, *J* = 6.2 Hz, 15H), 0.96 (d, *J* = 6.0 Hz, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.15, 166.26, 162.06, 162.03, 156.51, 138.94, 138.68, 138.65, 138.37, 138.32, 138.21, 137.83, 137.72, 135.86, 135.80, 135.72, 135.59, 133.70, 133.60, 133.34, 133.25, 133.09, 132.72, 130.11, 130.01, 129.99, 129.85, 129.79, 129.76, 129.68, 129.56, 128.78, 128.69, 128.65, 128.63, 128.58, 128.54, 128.50, 128.47, 128.42, 128.33, 128.29, 128.28, 128.25, 128.18, 128.13, 128.07, 128.03, 127.97,

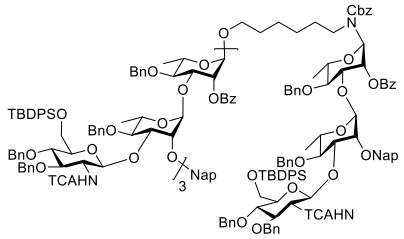
127.93, 127.89, 127.75, 127.71, 127.69, 127.64, 127.57, 127.50, 127.46, 127.42, 127.24, 101.48, 101.25, 98.67, 98.23, 97.98, 97.10, 93.09, 92.28, 81.97, 81.13, 80.47, 79.82, 79.67, 79.37, 79.03, 78.88, 78.44, 77.84, 77.62, 77.10, 76.10, 75.80, 75.30, 75.21, 75.09, 75.07, 74.58, 74.55, 74.53, 74.31, 74.16, 73.30, 73.04, 69.66, 68.52, 68.42, 68.30, 67.98, 67.51, 66.67, 63.02, 62.76, 61.33, 58.58, 41.14, 29.98, 29.84, 29.37, 27.09, 27.01, 26.59, 25.87, 19.34, 19.27, 18.27, 18.07, 18.00, 17.92. HR-MS: Calculated for $C_{156}H_{173}Cl_6N_3O_{31}Si_2$ [$(M+NH_4^+ + NH_4^+)/2$]: 1443.01997, found: 1443.01861. $[\alpha]^{20}_D$ = - 34.7° (c = 1, $CHCl_3$). TLC: Rf = 0.1 (DCM/EA = 40/1, v/v).

The nonasaccharide 38



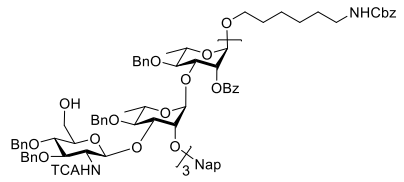
Donor **32** (47 mg, 29 µmol, 1.3 eq) and acceptor **37** (60 mg, 21 µmol, 1.0 eq) were co-evaporated with anhydrous toluene three times under nitrogen. Dry DCM (1 mL) and 4Å molecular sieves were added and the solution stirred for 20 minutes at RT. The reaction was cooled to -20 °C and then *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (1.0 µL, 4.4 µmol, 0.2 eq) was added. The solution was stirred for 5 hours. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous sodium bicarbonate and diluted with DCM. The solution was washed with water (2x) and brine. The aqueous layer was extracted with DCM (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/Acetone 5:1 - 3:1) to yield compound **38** (44 mg, 10.2 µmol, 49%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.77 (d, *J* = 6.8 Hz, 1H), 8.62 (d, *J* = 6.9 Hz, 1H), 8.02 – 7.90 (m, 5H), 7.77 – 7.03 (m, 109H), 6.95 – 6.84 (m, 3H), 6.76 (d, *J* = 8.2 Hz, 1H), 5.55 – 5.42 (m, 3H), 5.41 – 5.31 (m, 4H), 5.12 – 5.06 (m, 2H), 5.00 – 4.94 (m, 2H), 4.90 – 4.82 (m, 2H), 4.82 – 4.52 (m, 20H), 4.52 – 4.47 (m, 2H), 4.45 – 4.28 (m, 8H), 4.27 – 4.08 (m, 5H), 3.93 – 3.83 (m, 3H), 3.83 – 3.24 (m, 28H), 3.20 – 3.04 (m, 4H), 1.61 – 1.56 (m, 2H), 1.53 – 1.45 (m, 2H), 1.34 – 1.27 (m, 7H), 1.15 (d, *J* = 6.2 Hz, 3H), 1.08 – 0.92 (m, 39H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.46, 167.18, 166.31, 162.08, 161.97, 161.85, 156.50, 138.91, 138.85, 138.42, 138.35, 138.32, 138.28, 138.24, 138.21, 138.19, 138.01, 137.81, 136.18, 135.82, 135.79, 135.72, 135.68, 135.62, 135.60, 133.64, 133.55, 133.40, 133.36, 133.34, 133.32, 133.26, 133.10, 133.02, 129.95, 129.88, 129.85, 129.77, 129.68, 129.56, 129.34, 128.77, 128.70, 128.68, 128.66, 128.62, 128.58, 128.54, 128.51, 128.49, 128.43, 128.35, 128.32, 128.27, 128.25, 128.23, 128.20, 128.17, 128.15, 128.05, 128.01, 127.99, 127.97, 127.93, 127.91, 127.87, 127.84, 127.81, 127.78, 127.75, 127.73, 127.70, 127.68, 127.65, 127.61, 127.58, 127.56, 127.50, 127.47, 127.43, 127.40, 126.45, 126.19, 125.84, 125.64, 101.59, 101.21, 100.46, 98.61, 98.20, 98.15, 97.92, 97.08, 93.10, 93.00, 92.52, 81.12, 81.03, 80.79, 80.47, 80.42, 80.21, 80.15, 79.13, 78.99, 78.85, 78.77, 78.54, 77.69, 77.63, 77.56, 77.50, 76.60, 76.40, 75.99, 75.83, 75.70, 75.40, 75.30, 75.08, 75.05, 74.57, 74.51, 74.29, 74.25, 74.22, 74.09, 73.96, 73.68, 73.48, 73.43, 73.07, 68.78, 68.72, 68.59, 68.42, 68.09, 67.98, 67.51, 66.67, 63.13, 62.93, 61.41, 61.17, 57.81, 41.13, 29.97, 29.83, 29.37, 27.15, 27.12, 27.05, 26.57, 25.83, 19.44, 19.25, 19.23, 18.28, 18.14, 18.08, 17.83. MALDI-FTICR: Calculated for $C_{238}H_{257}Cl_9N_4O_{45}Si_3$ $[M+Na]^+$: 4312.4342, found: 4312.3813. $[\alpha]^{20}_D$ = - 40.0° (c = 0.1, $CHCl_3$). TLC: Rf = 0.2 (PE/Acetone = 3/1, v/v).

The side-product dodecasaccharide 38a



^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.74 (d, *J* = 6.9 Hz, 1H), 8.61 (d, *J* = 6.8 Hz, 1H), 8.06 – 7.87 (m, 9H), 7.78 – 6.86 (m, 147H), 6.75 (d, *J* = 8.5 Hz, 1H), 5.97 (d, *J* = 9.5 Hz, 1H), 5.58 – 5.48 (m, 2H), 5.48 – 5.28 (m, 7H), 5.17 – 5.05 (m, 2H), 5.05 – 4.91 (m, 6H), 4.89 (d, *J* = 11.7 Hz, 1H), 4.83 – 4.02 (m, 53H), 3.99 – 3.35 (m, 36H), 3.35 – 3.06 (m, 9H), 2.79 (s, 1H), 1.53 – 1.38 (m, 4H), 1.31 – 0.73 (m, 57H), 0.55 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.42, 167.17, 166.17, 165.41, 162.05, 161.94, 161.83, 156.49, 156.10, 156.09, 138.91, 138.85, 138.50, 138.44, 138.35, 138.33, 138.30, 138.28, 138.24, 138.20, 138.19, 138.00, 137.80, 137.78, 137.75, 137.71, 136.18, 135.81, 135.79, 135.77, 135.71, 135.67, 135.59, 133.61, 133.56, 133.40, 133.34, 133.32, 133.17, 133.10, 133.01, 129.95, 129.91, 129.87, 129.84, 129.76, 129.72, 129.66, 129.58, 129.36, 128.73, 128.68, 128.64, 128.60, 128.56, 128.52, 128.50, 128.48, 128.46, 128.33, 128.30, 128.25, 128.24, 128.18, 128.15, 128.13, 128.11, 128.08, 128.03, 127.98, 127.96, 127.94, 127.91, 127.86, 127.82, 127.77, 127.73, 127.72, 127.70, 127.68, 127.66, 127.64, 127.62, 127.59, 127.55, 127.50, 127.48, 127.46, 127.44, 127.41, 127.38, 127.33, 127.29, 127.20, 126.57, 126.44, 126.18, 126.11, 125.92, 125.83, 125.62, 101.42, 101.12, 100.45, 98.62, 98.25, 98.21, 98.16, 97.99, 97.08, 93.09, 93.00, 92.53, 92.51, 81.08, 80.99, 80.79, 80.49, 80.21, 80.16, 79.08, 78.97, 78.79, 78.58, 78.41, 78.12, 77.74, 77.62, 77.56, 77.47, 77.37, 77.36, 77.02, 76.75, 76.56, 76.40, 75.98, 75.81, 75.24, 75.18, 75.04, 74.56, 74.53, 74.50, 74.44, 74.26, 74.22, 74.20, 74.04, 73.95, 73.91, 73.69, 73.64, 73.47, 73.37, 73.01, 72.97, 72.64, 71.36, 68.77, 68.70, 68.55, 68.41, 68.33, 68.27, 68.15, 68.05, 67.86, 67.39, 66.95, 66.64, 63.13, 63.07, 62.94, 61.35, 61.13, 57.77, 56.31, 42.62, 41.12, 29.25, 27.14, 27.12, 27.08, 27.04, 27.03, 26.75, 25.50, 19.42, 19.26, 19.24, 19.21, 18.31, 18.25, 18.13, 18.08, 17.85, 17.44, 16.55. MALDI-FTICR: Calculated for $C_{320}H_{341}C_{112}N_5O_{59}Si_4$ $[M+Na]^+$: 5751.9068, found: 5751.7993. $[\alpha]^{20}_D$ = - 33.9° (c = 1, $CHCl_3$). TLC: Rf = 0.25 (PE/Acetone = 3/1, v/v).

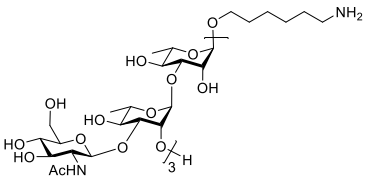
The nonasaccharide triol 39



Protected nonasaccharide **38** (10 mg, 2.3 µmol, 1.0 eq) was dissolved in anhydrous THF (1 mL) and AcOH (5.4 µL, 92 µmol, 40 eq). Then 1M TBAF in THF (92 µL, 92 µmol, 40 eq) was added in rt. The reaction mixture was stirred at 50 °C for overnight. After TLC showed complete consumption of the starting material, the reaction was quenched with saturated aqueous ammonium chloride and diluted with EA. The solution was washed with water (2x) and brine. The aqueous layer was extracted with EA (3x), dried with $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by flash chromatography (PE/Acetone/DCM 6:1:1 – 5:1:1) to yield compound **39** (5.4 mg, 1.5 µmol, 65%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.24 (d, *J* = 6.8 Hz, 1H), 8.15 – 8.00 (m, 7H), 7.82 – 7.73 (m, 3H), 7.67 (s, 1H), 7.64 – 7.56 (m, 3H), 7.53 – 7.07 (m, 74H), 6.64 – 6.54 (m, 1H), 5.57 (s, 1H), 5.53 – 5.41 (m, 3H), 5.36 – 5.24 (m, 3H), 5.18 (s, 1H), 5.08 (s, 2H), 5.00 – 4.36 (m, 32H), 4.33 (dd, *J* = 9.5, 3.3 Hz, 1H), 4.28 – 4.07 (m, 6H), 4.01 (dd, *J* = 9.2, 2.9 Hz, 1H), 3.94 – 3.49 (m, 14H), 3.49 – 3.25 (m, 13H),

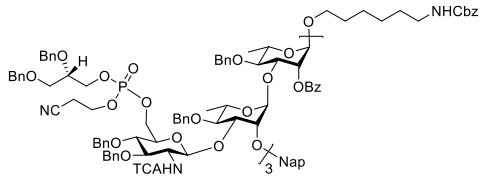
3.25 – 3.03 (m, 7H), 2.20 – 2.01 (m, 2H), 1.84 (s, 1H), 1.60 – 1.44 (m, 4H), 1.36 – 1.18 (m, 10H), 1.14 (d, *J* = 6.1 Hz, 3H), 1.05 (dd, *J* = 19.1, 6.2 Hz, 6H), 0.97 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.17, 167.02, 166.11, 162.09, 162.05, 161.93, 156.47, 138.68, 138.61, 138.36, 138.18, 138.12, 138.10, 137.94, 137.85, 137.75, 137.72, 136.77, 135.79, 133.81, 133.46, 133.23, 133.06, 130.03, 129.98, 129.53, 129.39, 128.85, 128.81, 128.78, 128.72, 128.68, 128.66, 128.63, 128.60, 128.58, 128.55, 128.50, 128.48, 128.43, 128.38, 128.35, 128.29, 128.26, 128.22, 128.19, 128.17, 128.13, 128.11, 128.09, 128.07, 128.02, 127.96, 127.94, 127.91, 127.85, 127.82, 127.80, 127.72, 127.67, 127.63, 127.61, 127.57, 127.49, 127.47, 127.41, 127.31, 126.74, 126.31, 126.15, 125.91, 101.26, 100.38, 98.75, 98.63, 97.85, 97.49, 96.90, 92.64, 92.58, 92.41, 80.27, 79.70, 79.53, 79.40, 79.26, 79.14, 78.83, 78.66, 78.56, 78.14, 78.00, 77.76, 77.59, 77.49, 77.10, 76.53, 75.93, 75.37, 75.32, 75.19, 75.06, 74.99, 74.80, 74.77, 74.73, 74.60, 74.16, 73.42, 73.37, 73.27, 73.04, 69.17, 69.10, 69.00, 68.62, 68.35, 67.93, 67.61, 66.64, 61.73, 61.41, 61.33, 61.06, 60.96, 58.01, 41.09, 29.93, 29.80, 29.33, 26.54, 25.82, 18.25, 18.16, 18.12, 18.04, 17.95, 17.76. HR-MS: Calculated for $C_{190}H_{203}Cl_9N_4O_{45}$ [M+2Na$^+$]/2: 1810.53502, found: 1810.55720. MALDI-FTICR: Calculated for $C_{190}H_{203}Cl_9N_4O_{45}$ [M+Na]$^+$: 3598.0808, found: 3597.9925. $[\alpha]^{20}_D$ = - 59.0 ° (c = 0.2, $CHCl_3$). TLC: Rf = 0.25 (PE/Acetone = 2/1, v/v).

The target nonasaccharide 3



The nonasaccharide **39** (38.8 mg, 10.8 µmol, 1.0 eq) was was dissolved in *tert*-butanol (7 mL) and 0.1% AcOH in water (3 mL). After $Pd(OH)_2$/C (80 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered, using ammonia solution quenched the AcOH and concentrated *in vacuo*. The crude was dissolved in water (5 mL). 1M Sodium hydroxide (0.5 mL) was added. The reaction was stirred overnight. The mixture was quenched with acetic acid and then quenched the excess acid using ammonia solution. Co-evaporated with toluene to remove all the solvent *in vacuo*. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector and lyophilized to yield compound **3** (9.4 mg, 5.9 µmol, 54%). ^{1}H NMR (850 MHz, Deuterium Oxide) δ 5.17 – 5.13 (m, 2H, H-1), 5.07 – 5.04 (m, 2H, H-1), 5.01 – 4.98 (m, 1H, H-1), 4.72 (d, *J* = 1.7 Hz, 1H, H-1), 4.69 – 4.66 (m, 3H, H-1), 4.27 – 4.22 (m, 3H), 4.06 – 4.02 (m, 2H), 3.97 – 3.91 (m, 3H), 3.91 – 3.85 (m, 4H), 3.83 – 3.64 (m, 16H), 3.54 – 3.45 (m, 10H), 3.44 – 3.39 (m, 6H), 2.81 – 2.77 (m, 2H), 2.01 – 1.95 (m, 9H), 1.64 – 1.51 (m, 4H), 1.41 – 1.31 (m, 4H), 1.29 – 1.20 (m, 18H). ^{13}C NMR (214 MHz, D_2O) δ 175.78, 175.34, 175.31, 103.62 (C-1), 103.45 (C-1), 103.38 (C-1), 102.57 (C-1), 102.19 (C-1), 102.09 (C-1), 101.80 (C-1), 101.75 (C-1), 100.42 (C-1), 80.74, 80.64, 80.45, 78.22, 77.94, 77.50, 76.94, 76.87, 76.56, 76.54, 76.46, 74.73, 74.70, 74.50, 72.61, 72.58, 72.36, 72.12, 72.02, 71.72, 70.78, 70.69, 70.65, 70.63, 70.59, 70.24, 70.22, 70.21, 70.19, 70.17, 69.51, 68.66, 61.64, 61.61, 61.39, 56.69, 56.69, 56.56, 40.66, 29.34, 29.17, 26.39, 25.89, 23.05, 23.01, 17.66, 17.62, 17.49, 17.40, 17.32, 17.30. HR-MS: Calculated for $C_{66}H_{114}N_4O_{40}$ [M+2H$^+$]/2: 802.35774, found: 802.35720.

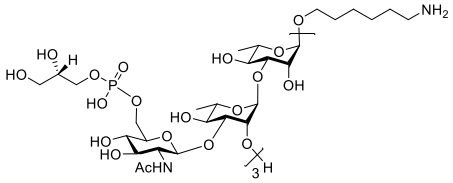
The glycerol phosphate modified nonasaccharide 40



Triol **39** (76 mg, 21.2 µmol, 1.0 eq) and 0.1M phosphite **29** in ACN (1.91 mL, 190.8 µmol, 9.0 eq) were co-evaporated with dry toluene 3 times under nitrogen. The mixture was dissolved in dry acetonitrile (4 ml) and 4Å molecular sieves was added. The mixture was stirred for 15 mins under argon atmosphere. 4,5-dicyanoimidazole (DCI, 0.25M in acetonitrile) (1.02 mL, 254.4 µmol, 12.0 eq) was added and the reaction mixture was stirred for 9 hours. After analysis by TLC showed complete consumption of the starting material, (10-Camphorsulfonyl)-oxaziridine (CSO, 0.5M in acetonitrile) (510 µL, 254.4 µmol, 12.0 eq) was added. Stirred another 1hour and diluted with EtOAc. The solution was washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Preparative TLC plate (Macherey-Nagel, pre-coated TLC plates SIL G-100 UV254) (DCM/Acetone 15:1) yielded compound **40** (91.3 mg, 19.2 µmol, 91%) as a mixture of anomers. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.84 – 8.63 (m, 2H), 8.14 – 8.00 (m, 5H), 7.83 – 7.67 (m, 4H), 7.66 – 6.96 (m, 109H), 6.83 – 6.71 (m, 1H), 5.53 – 5.45 (m, 1H), 5.44 – 5.25 (m, 5H), 5.18 – 5.03 (m, 3H), 5.01 – 3.28 (m, 102H), 3.28 – 3.03 (m, 5H), 2.24 – 1.95 (m, 6H), 1.58 – 1.44 (m, 4H), 1.43 – 0.80 (m, 22H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.47, 167.45, 167.43, 167.41, 167.28, 166.20, 166.11, 162.17, 162.13, 162.11, 162.08, 162.01, 161.96, 156.48, 138.65, 138.63, 138.58, 138.57, 138.53, 138.51, 138.41, 138.38, 138.37, 138.23, 138.20, 138.12, 138.09, 138.08, 138.05, 138.04, 138.03, 138.01, 137.99, 137.97, 137.95, 137.93, 137.86, 137.82, 137.77, 137.72, 136.82, 136.13, 133.96, 133.88, 133.47, 133.39, 133.30, 133.27, 133.01, 132.98, 130.11, 130.06, 130.01, 129.97, 129.93, 129.83, 129.51, 129.36, 129.34, 128.91, 128.86, 128.66, 128.65, 128.62, 128.61, 128.59, 128.58, 128.55, 128.51, 128.49, 128.47, 128.45, 128.43, 128.39, 128.38, 128.36, 128.34, 128.30, 128.27, 128.24, 128.18, 128.14, 128.12, 128.10, 128.09, 128.07, 128.05, 128.04, 128.01, 127.96, 127.94, 127.93, 127.90, 127.88, 127.86, 127.84, 127.83, 127.81, 127.80, 127.78, 127.75, 127.74, 127.69, 127.68, 127.66, 127.62, 127.59, 127.57, 127.55, 127.52, 127.50, 127.47, 127.45, 127.43, 127.35, 127.27, 126.40, 126.24, 126.22, 126.15, 126.12, 126.06, 126.00, 125.88, 125.83, 116.70, 116.56, 116.54, 116.51, 116.50, 116.45, 101.64, 101.59, 101.46, 101.19, 100.45, 100.22, 98.60, 98.54, 98.29, 98.21, 98.18, 98.13, 97.90, 97.85, 96.98, 96.91, 92.97, 92.95, 92.91, 92.88, 92.30, 92.26, 80.87, 80.67, 80.59, 80.40, 80.30, 80.08, 79.88, 79.65, 79.54, 78.94, 78.81, 78.55, 78.37, 78.26, 78.22, 78.14, 78.03, 77.83, 77.78, 77.66, 77.64, 77.61, 77.59, 77.29, 76.76, 76.55, 76.51, 76.47, 76.43, 76.42, 75.58, 75.39, 75.28, 75.23, 75.19, 75.09, 74.97, 74.94, 74.62, 74.59, 74.50, 74.48, 74.43, 74.39, 74.30, 74.27, 74.23, 74.19, 74.03, 73.79, 73.75, 73.68, 73.64, 73.57, 73.56, 73.53, 73.46, 73.44, 73.40, 73.33, 73.31, 73.28, 73.17, 72.32, 72.31, 72.19, 72.18, 72.13, 72.12, 68.99, 68.95, 68.94, 68.92, 68.88, 68.87, 68.81, 68.77, 68.72, 68.45, 68.41, 68.36, 68.29, 67.91, 67.86, 67.74, 67.71, 67.67, 67.65, 67.61, 67.56, 67.52, 67.49, 67.45, 67.36, 67.32, 67.28, 67.24, 66.59, 66.53, 66.50, 66.30, 66.21, 64.64, 61.85, 61.82, 61.80, 61.78, 61.74, 61.70, 61.66, 61.22, 61.15, 60.99, 60.93, 58.81, 58.72, 51.86, 41.10, 29.91, 29.78, 29.32, 26.53, 25.80, 19.42, 19.37, 19.22, 19.19, 19.14, 19.11, 19.09, 19.04, 18.99, 18.94,

18.94, 18.26, 18.17, 18.12, 18.08, 18.06, 18.06, 18.04, 17.98, 17.87, 17.83, 17.80, 17.77. ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.54, -0.57, -0.86, -1.08, -1.10. HR-MS: Calculated for $C_{250}H_{269}Cl_9N_7O_{60}P_3$ [(M+NH_4^++NH_4^+)/2]: 2386.26497, found: 2386.23688. MALDI-FTICR: Calculated for $C_{250}H_{269}Cl_9N_7O_{60}P_3$ $[M+Na]^+$: 4759.4515, found: 4759.2796. TLC: Rf = 0.2 (DCM/Acetone = 14/1, v/v).

The target nonasaccharide 6



Full protected nonasaccharide **40** (51.6 mg, 10.9 μmol, 1.0 eq) was dissolved in dioxane (6 mL) and ammonia solution (35%) (3 mL). The mixture was stirred at RT for overnight. After analysis by TLC showed complete consumption of the starting material, co-evaporated with toluene to remove the solvent. The mixture was purified by flash size exclusion (LH-20) (DCM/MeOH 1:1). The compound was dissolved in *tert*-butanol (7 mL) and 0.1% AcOH in water (3 mL). After $Pd(OH)_2/C$ (60 mg) was added, the reaction was stirred for 3 days under a H_2 atmosphere, filtered and concentrated *in vacuo*. The crude was dissolved in water (5 mL) and 1M NaOH in water (0.5 mL). After stirred overnight, the reaction was quenched with acetic acid and then quenched the excess acid using ammonia solution. The compound was purified by gel filtration (HW-40, 0.15M, NH_4OAc in H_2O) with a Shimadzu RID-10A refractive index detector, transformed into its sodium salt by passing a short Dowex Na^+ column and lyophilized to yield compound **6** (14.5 mg, 7.0 μmol, 65%). ^{1}H NMR (850 MHz, Deuterium Oxide) δ 5.21 – 5.18 (m, 1H, H-1), 5.15 (d, *J* = 1.8 Hz, 1H, H-1), 5.08 (d, *J* = 1.8 Hz, 1H, H-1), 5.05 (d, *J* = 1.9 Hz, 2H, H-1), 4.78 – 4.76 (m, 2H, H-1), 4.75 (d, *J* = 8.4 Hz, 1H, H-1), 4.72 (d, *J* = 8.5 Hz, 1H, H-1), 4.36 – 4.33 (m, 1H), 4.32 – 4.26 (m, 2H), 4.23 – 4.16 (m, 3H), 4.14 – 4.05 (m, 5H), 4.02 – 3.98 (m, 3H), 3.97 – 3.88 (m, 10H), 3.87 – 3.68 (m, 17H), 3.66 – 3.49 (m, 19H), 3.00 (t, *J* = 7.6 Hz, 2H), 2.07 – 2.01 (m, 8H), 1.74 – 1.59 (m, 4H), 1.49 – 1.39 (m, 4H), 1.36 – 1.25 (m, 18H). ^{13}C NMR (214 MHz, D_2O) δ 175.82, 175.44, 175.35, 103.94 (C-1), 103.30 (C-1), 103.06 (C-1), 102.95 (C-1), 102.80 (C-1), 102.61 (C-1), 101.90 (C-1), 101.64 (C-1), 100.49 (C-1), 81.85, 80.99, 80.15, 78.62, 78.46, 78.27, 77.90, 77.66, 75.54, 75.51, 75.45, 75.42, 75.25, 75.21, 74.76, 74.70, 74.49, 72.67, 72.45, 72.43, 72.26, 71.96, 71.68, 71.64, 71.63, 71.60, 71.59, 70.93, 70.72, 70.65, 70.47, 70.35, 70.23, 70.17, 70.16, 70.13, 70.06, 69.67, 68.62, 67.33, 67.32, 67.30, 67.27, 65.53, 65.43, 65.23, 65.21, 63.00, 62.98, 56.78, 56.73, 56.61, 40.35, 29.25, 27.57, 26.32, 25.93, 23.16, 23.15, 23.10, 17.90, 17.83, 17.60, 17.59, 17.52, 17.43. ^{31}P NMR (162 MHz, D_2O) δ 1.74, 1.69, 1.66. HR-MS: Calculated for $C_{75}H_{135}N_4O_{55}P_3$ [M+H^++Na^+]/2: 1044.35338, found: 1044.35484.

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