Bacterial glycomimetics: synthesis and applications

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Synthesis of stabilized *Neisseria meningitidis* serotype A analogues

Introduction

Neisseria meningitidis is a Gram-negative bacterium that causes bacterial meningitis, especially in children and young adults. It has been observed that 6 out of 13 serogroups (A, B, C, Y, W135 and X31) are responsible for the large majority of the infections.[1, 2] For this reason, the development of new vaccination therapies has mainly been directed to these strains. In sub-Saharan Africa, in what is known as the meningitis belt, meningococcal infections threaten millions of people, with serogroup type A (MenA) being the major infectious agent. Recently a glycoconjugate vaccine has been introduced, leading to a significant reduction of MenA infections. The natural capsular polysaccharide (CPS) of MenA is composed of α-(1,6)-linked *N*-acetyl-D-mannosamine-1-phosphate repeating units (Figure 1), with acetyl decorations on the mannosamine C-3-OH (50-60%) and, to a lesser extent, on the C-4-OH (25-30%).[3] This polysaccharide is relatively unstable in aqueous media due to elimination of the anomeric phosphate group, the expulsion of which is assisted by the endocyclic oxygen and the axial C-2 acetamide of the mannosamine.[4] To overcome this stability issue Lay and co-workers suggested the replacement of the endocyclic oxygen with a methylene group forming so-called carba-analogues of this CPS. [5, 6, 7, 4, 8]

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Recently the groups of Lay, Codée and Adamo have shown how relatively short fragments (up to 8 repeating units) of non-acetylated carba-analogues conjugated to CRM_{197} could be used to raise specific IgG antibodies in mice that could recognize the synthetic structures and the non-acetylated natural CPS but to a lesser extent the native acetylated CPS.[9] The synthetic octamer, Carba ManA DP8, was acetylated in a random manner. The newly obtained acetylated carba MenA fragments, conjugated to CRM_{197} were used to raise antibodies in mice and it was shown that protective antibodies could be generated providing protection to the same level as the benchmark vaccine. These results have shown that the replacement of the endocyclic oxygen does not seem to affect the overall activity of the CPS fragments and that the acetyl decorations are important to raise a protective immune response.

This Chapter describes the development of a synthetic route towards carba MenA fragments with a predetermined acetylation pattern to unravel which acetyl decorations trigger an optimal immune response. An effective synthetic route towards carba-*N*-acetylmannosamine building block **19** bearing an acetyl substituent at the C-3-OH and its incorporation into longer fragments is presented to deliver a small library of C-3-OAc Carba MenA oligosaccharides, ranging from dimer **1** to decamer **6** (Figure 2). These target compounds only bear acetyl decorations at the C-3-OH, as acetylation at this position prevails in the natural MenA CPS.

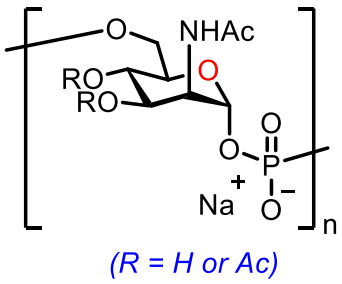


Figure 1. Repeating unit of MenA CPS.

Results and Discussion

The assembly of the non-acetylated carba MenA oligomers was executed using stepwise elongations with a monomeric phosphoramidite building block, having its primary alcohol function temporarily protected as dimethoxytrityl (DMTr) ether.[8] To follow a similar approach to the target acetylated carba MenA oligomers **1**-**6**, a synthetic route to

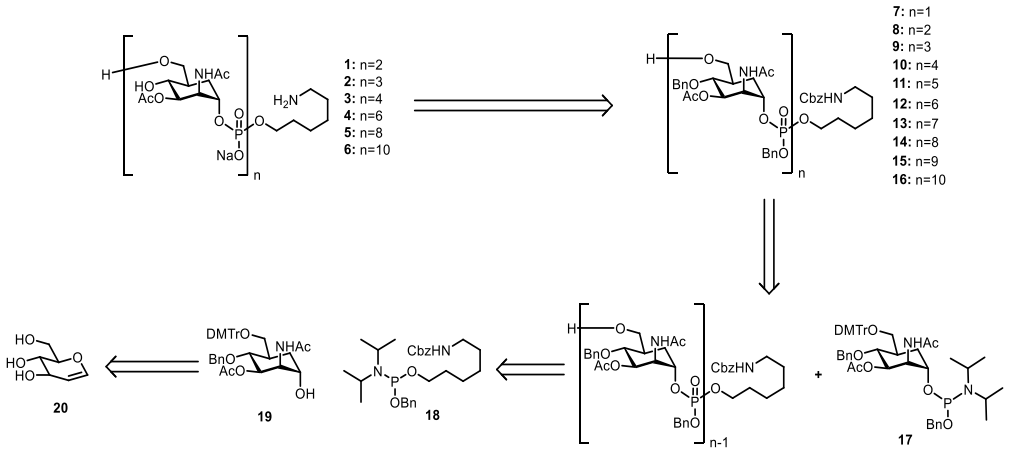
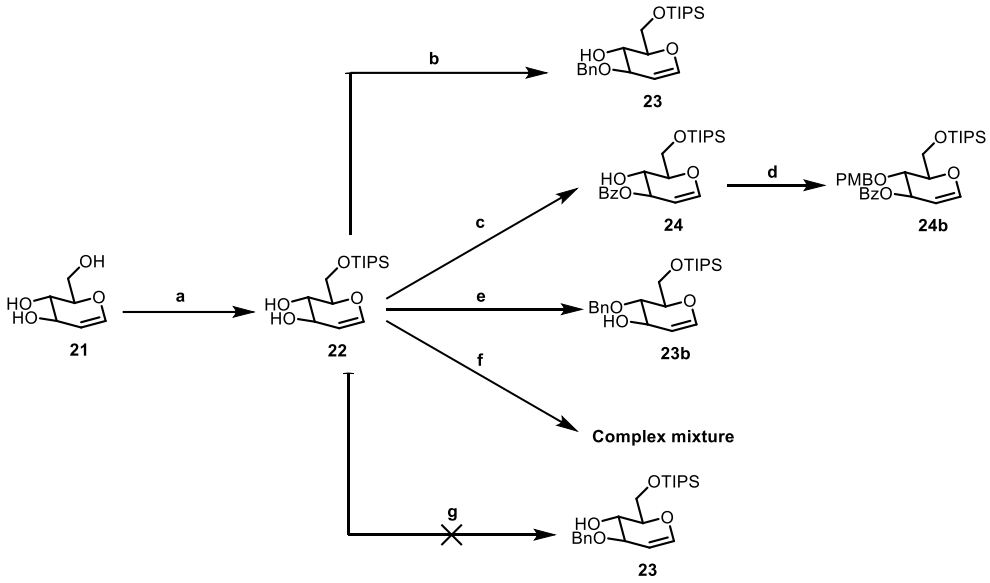


Figure 2. Retrosynthetic approach.

the acetylated carba-*N*-acetylmannosamine building block **19** had to be developed. Based on the previously reported route to the non-acetylated building block, glucal **21** was chosen as starting compound (see Scheme 1). [5] With the objective to differentiate the protection of the C-3-OH and C-4-OH at the glucal stage, several synthetic possibilities were investigated. As the introduction of the acetyl group at an early stage is impossible because the ester would be too labile to endure all the synthetic steps, the use of a temporary *p*-methoxybenzyl ether (PMB) benzyl ether was explored. In a first attempt, the *p*-methoxybenzylidene acetal was chosen to protect the C-4 and C-6-alcohols in **2**. The acidic conditions required for the introduction (catalytic *p*-TsOH, $ZnCl_2$ or $Cu(OTf)_2$) unfortunately proved incompatible with the acid-sensitive enol ether and therefore this strategy was abandoned. Therefore, a strategy was explored in which the expected higher reactivity of the glucal C-3-OH in comparison to the C-4-OH could be exploited to attain the regioselective protection of the former alcohol. First, the regioselective silylation of the primary alcohol in **21** provided diol **22**, which was subjected to various methods for the regioselective protection of diols. Compound **22** was converted into the corresponding dibutyltin acetal by treatment with dibutyltin oxide in boiling toluene, and then treated with BnBr. This unfortunately did not lead to any product formation. As an alternative, **22** was treated with 2-aminoethyldiphenylborinate in the presence of benzyl bromide (BnBr), potassium iodide (KI) and potassium carbonate (K_2CO_3) in ACN yielded compound **23** in 31%, which was considered too low to be used for scale-up.

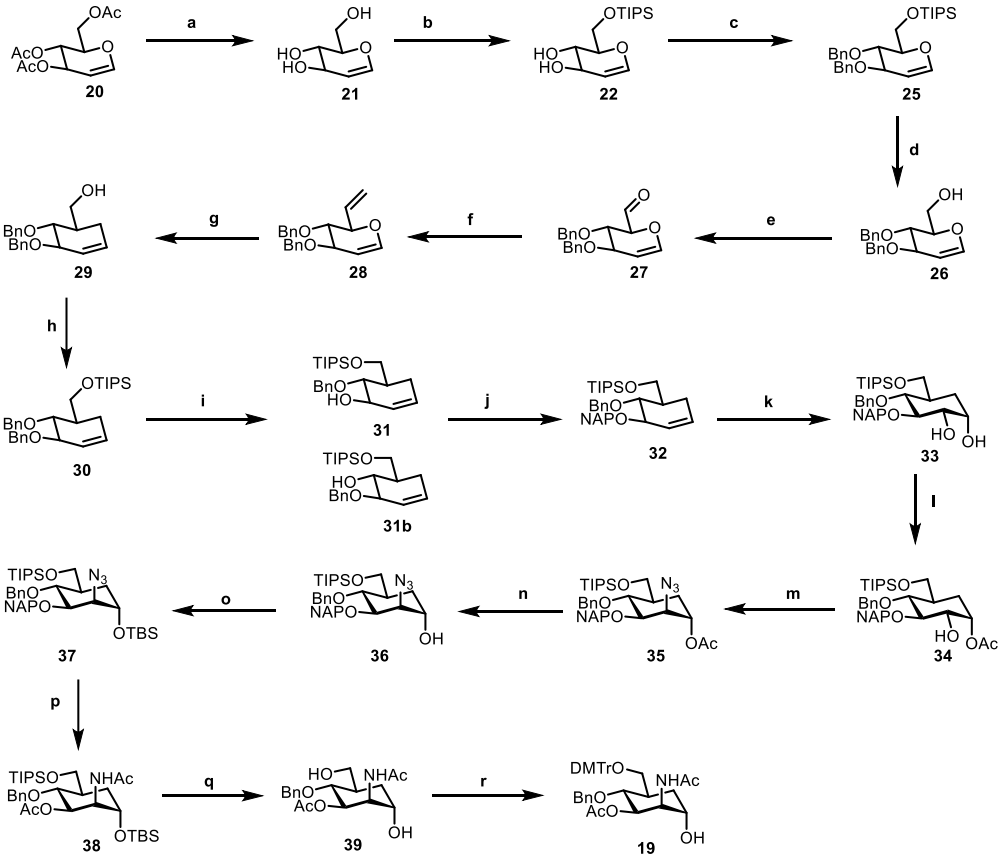
Subsequently **22** was treated with 2-aminoethyldiphenylborinate in the presence of benzoylchloride (BzCl), K_2CO_3, KI in ACN to give the expected product **24** in 80% yield. However, during the subsequent introduction of the PMB-ether at the C-4-OH partial migration of the Bz to this alcohol was observed, promoted by the basic environment, resulting in a complex mixture of compounds, from which the desired product could only be isolated in a poor 8% yield. Interestingly, when compound **22** was treated with stochiometric amounts of BnBr (1eq) and sodium hydride (NaH, 1eq) in DMF the product with the benzyl ether at C-4 was unexpectedly predominantly formed. Unfortunately, similar conditions using PMBCl (1eq) and NaH (1eq), to regioselectively install the PMB ether at this position, led to a complex mixture, from which the desired compound could not be purified.



Scheme 1. Initial attempts for 3/4OH differentiation: a) TIPSCl, Imidazole, DMF, -30°C, b) 2-aminoethyl diphenylborinate, BnBr, K_2CO_3, KI, ACN, 96h, 31% c) 2-aminoethyl diphenylborinate, BzCl, K_2CO_3, KI, ACN, 18h, 80% d) PMBCl, NaH, DMF, 8% e) BnBr, NaH, DMF, 50% f) PMBCl, NaH, DMF g) BnBr, $(Bu)_2SnO$, Toluene, 110°C, no reaction.

Based on these results, it was decided to postpone the differentiation of the C-3 and C-4 alcohols after the Claisen rearrangement at the carba-glucal stage (see Scheme 2). To this end, the previously published synthetic route was optimized to allow scale-up to 500mmol.[5] Thus, commercially available peracetylated glucal (**20**, Scheme 2) was transformed into compound **26** in 4 steps (deacetylation, regioselective silylation, benzylation of the remaining secondary alcohols and unmasking the primary alcohol), using only one silica gel chromatography purification delivering the desired compound in

75% yield, substantially cutting time and costs of the synthesis. Oxidation of alcohol **26** using IBX was then followed by Wittig olefination to give alkene **28**, amenable for the Claisen rearrangement. The rearrangement was carried out using microwave-assisted heating in batches of 16 mmol, and it was immediately followed by reduction of the newly formed aldehyde using $NaBH_4$ to provide alcohol **29** in 80% yield over 2 steps.



*Scheme 2. Synthetic route towards key building block **19**: a) K_2CO_3, MeOH, b) TIPSCl, Imidazole, DMF, -30°C c) BnBr, NaH, DMF, 0°C d) TBAF, THF, 75% over 4 steps e) IBX, AcOEt, quant. f) PPh_3CH_3I, KHMDS, THF, -78°C to rt, 50-67% g) i. 1,3-dichlorobenzene, 230°C, ii. $NaBH_4$, EtOH/THF, 80% over 2 steps h) TIPSCl, Imidazole, DMF, 90% i) $TiCl_4$, DCM/Toluene 2:8 ratio, 50 % **31**, 20% **31b** j) NAPCl, NaH, DMF, 0°C, 65-75% k) Me_3NO, Acetone/H_2O 3:1, OsO_4, 90% l) $(MeO)_3CMe$, PTSA, ACN, 90% m) i. Tf_2O, DCM/Pyridine 5:1 ratio, 0°C to rt, ii. NaN_3, DMF/H_2O 19:1 ratio, 40°C, 70% n) NaOMe, MeOH, o) TBSOTf, -10 to 70°C, Pyridine, DMAP, 77% over 2 steps p) i. Pd/C H_2, AcOH, ii. Ac_2O, Pyridine, 81% over 2 steps q) HF.Pyr, Pyridine, quant. r) DMTrCl, Pyridine, 0°C, 73%.*

Protection of **29** by treatment with triisopropylsilyl chloride and imidazole in DMF gave compound **30** ready for removal of the benzyl groups. Initially it was projected to simultaneously

*Table 1. Optimization conditions for selective benzyl removal on compound **30**.*

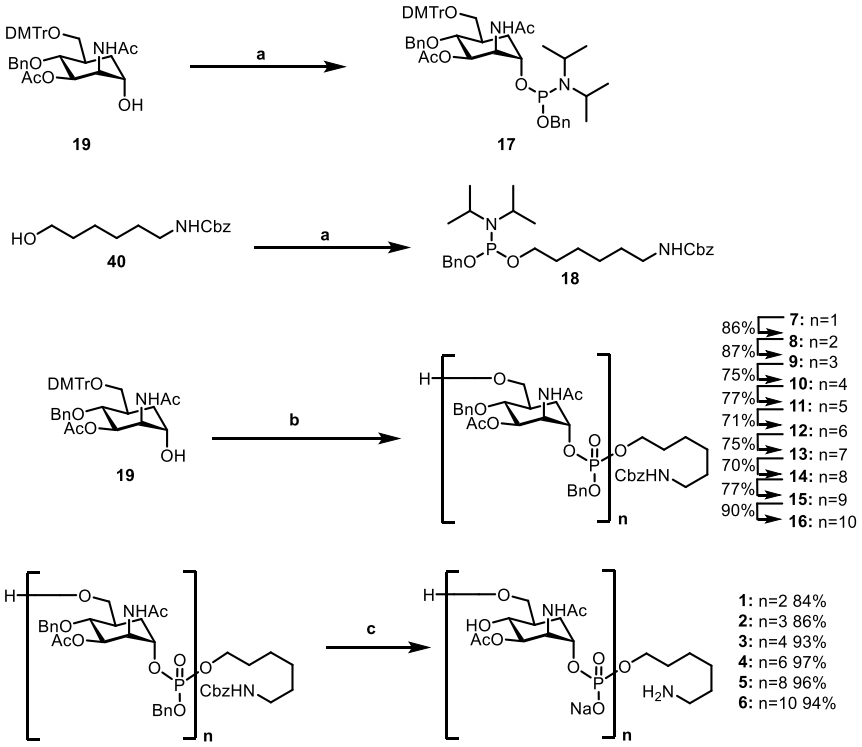
Entry	Scale (mmol)	Reagent	Solvent	T (°C)	Time (h)	Recovered Sm (%)	Yield product (%)	Yield Byproduct (%)
1	0.2	2.5eq $TiCl_4$	DCM	-75	2.5	-	68	-
2	2	2.5eq $TiCl_4$	DCM	-75	2	-	56	12
3	9	2.5eq $TiCl_4$	DCM	-78	5.25	53	17	7
4	5	2.5eq $TiCl_4$	DCM	-80 to -60	4	-	40	40
5	5	2.5eq $TiCl_4$	DCM	-75 to -50	4.5	-	41	15
6	1.5	2.5eq $TiCl_4$,	DCM	-75 to -30	2	37	34	27
7	3	2.5eq $TiCl_4$	DCM	-65	1.5	-	38	-
8	2	3.5eq $TiCl_4$	DCM	-75	3	-	27	35
9	1	2eq $TiCl_4$	DCM	-78	4	No reaction		
10	5	2.5eq $TiCl_4$	Toluene	-70	2	-	51	27

remove both benzyl ethers, but upon treatment of **30** with titanium tetrachloride ($TiCl_4$, 0.2 mmol scale), the formation of product **31**, bearing a benzyl ether only on at the C-4 position, was observed. Unfortunately, it proved difficult to scale up the reaction and significant optimization was required. Table 1 summarizes all attempts in which the temperature, equivalents of the Lewis acid as well as the solvent were varied. As can be seen, the best result was achieved, by performing the reaction on 5 mmol scale using 2.5 eq of $TiCl_4$ at -78°C in toluene (entry 10) to give compound **31** in 51% yield, alongside its regioisomer **31b**, which was isolated in 27%. Building on this selective removal of the benzyl ether, the obtained alcohol **31** was easily transformed into fully protected alkene **32** that was subsequently treated with osmium tetroxide (OsO_4) and TMANO as co-

oxidant to stereoselectively give the syn diol **33** as a single diastereoisomer. The axial alcohol in 5a-carba-α-D-glucopyranose **33** was selectively protected with an acetyl group and this was followed by substitution of the C-2-OH with an azide to provide the 5a-carba-α-D-mannosazide **35** in 70% over 2 steps. At this point the acetyl was removed and replaced by a silyl group arriving at compound **37**. In order to maximize the efficiency in terms of number of synthetic steps, an unconventional approach was then explored to reduce the azide and remove the NAP ether simultaneously with the subsequent installation of an acetyl moiety on both the newly formed amine and alcohol.

To do so, compound **37** was treated with a catalytic amount of Pd/C and acetic acid in a mixture of EtOH and THF. This way, first the azide was reduced and subsequently the NAP ether was cleaved while the benzyl ether stayed intact, even when the reaction was run for more than 72h. The chemoselectivity in this reaction can be explained by poisoning of the catalyst by the released 2-methylnaphthalene as proposed by Spencer *et al.* and Antus *et al.*.[10, 11] Upon completion of the reaction, the mixture was filtered to remove the catalyst and after concentration, the crude product was treated with acetic anhydride and pyridine to install the acetyl groups, giving compound **38** in 81% over two steps. Key building block **19** was then obtained by removing both silyl groups using HF pyridine and introduction of the dimethoxytrityl group on the C-6 alcohol. The overall yield of **19** from compound **20** is 3% over 21 steps, with only 10 purifications, providing a solid route of synthesis, that could be performed starting with >500 mmol triacetyl glucal **20**.

Having the building block **19** available, the assembly of the carba MenA analogues fragments using the well-established phosphoramidite chemistry was undertaken.[12] Because the lability of the acetyl groups, the commonly employed cyanoethyl phosphotriester protecting group could not be used and a benzyl group was selected instead, as this can be readily removed under mild conditions.[13] Thus, **19** was transformed into the required phosphoramidite **17** by treatment with benzyl



*Scheme 3. Elongation and global deprotection strategy towards final compounds: a) Tetrazole salt, P(N(i-*Pr)$_2$)$_2$OBn, *DCM, rt,* ***17*** *92%, 81%* ***18*** *b) i. 3Å ms, DCI,* ***17*** *or* ***18****, ACN, ii. CSO, DCM, iii. TCA, DCM, H_2O, c) i. H_2, Pd black, MilliQ water, ii. 0.01% AcOH in MilliQ water, 2:1 MilliQ/dioxane.*

phosphordiamidite using diisopropylammonium tetrazol-2-ide. The assembly of the oligomers started by introduction of the aminohexanol spacer. To this end spacer phosphoramidite **18**, generated in a similar manner as building block **17** from benzyloxycarbonyl protected aminohexanol **40**, and **19** were condensed using dicyanoimidazole (DCI) as activating agent. After oxidation with (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) and subsequent removal of the DMTr group using trichloroacetic acid monomer **7** was obtained in 79% yield. To further elongate **7** building block, repetitive elongations cycles comprising the phosphoramidite coupling with building block **17**, oxidation and DMTr removal, were performed to deliver protected

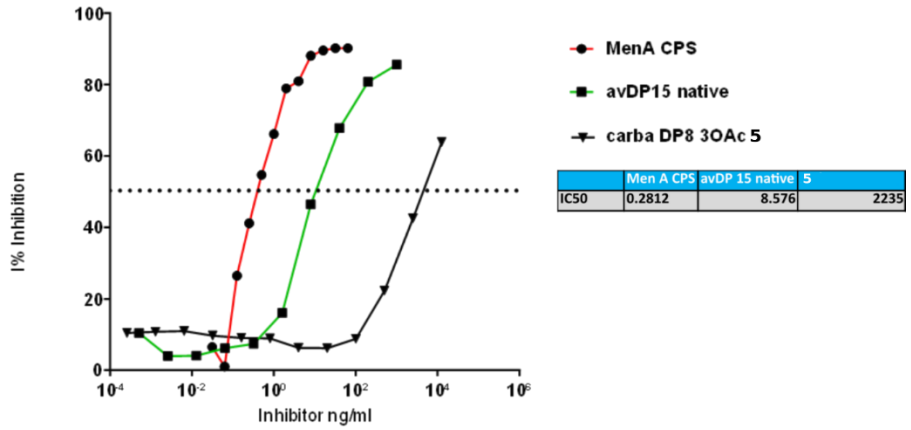
oligomers **8**-**16**. All elongation cycles proceeded uneventful to deliver the target products in good to excellent yield.

All oligomers were deprotected by hydrogenolysis reactions, which were performed in MilliQ water under H_2 atmosphere, using Pd black as catalyst in a 0.01% v/v AcOH/H_2O solution. Once the reaction was complete, the solution was filtered to remove the catalyst and concentrated *in vacuo* and subsequently dissolved in MilliQ water and passed through a sodium exchange resin to provide the phosphate sodium salts. The final products were purified by size exclusion chromatography (Toyopearl HW40, NH_4OAc buffer) and lyophilized. Unfortunately, it was observed that part of the acetyl moieties was cleaved or had migrated to neighboring alcohols. It was reasoned that these undesired side reactions could have occurred during either the ion exchange step (the resin, even if extensively washed, may remain basic enough to induce migration of the acetyls), the purification steps (to remove the NH_4OAc a large number of co-evaporations are needed) or the evaporation of water (which was performed at slightly elevated temperature). Therefore, another purification protocol was developed in which the ion exchange step was replaced by performing the size exclusion chromatography (Toyopearl HW40) with a NaCl solution, which also generates the phosphate sodium salts, and a subsequent size exclusion chromatography step on Sephadex G10 using MilliQ water to remove the NaCl. All water was removed during these steps by lyophilization. Following this procedure, the target compounds **1**-**6** were isolated in good yields. Analysis of the products by NMR revealed that the acetyl groups were left untouched during these steps.

Biological results

As first step to evaluate the immunogenicity of the synthesized C-3-OAc octamer **5**, a competitive SPR experiment was performed (Figure 3). Results showed that acetylated structures are better inhibitors of the binding between anti-MenA mAb and MenA CPS immobilized on the chip compared to non-acetylated structures, confirming the importance of the acetyl decorations for the recognition.

Encouraged by these preliminary results, a carba MenA octamer fragment glycoconjugate was prepared in order to test it in an immunization experiment in mice

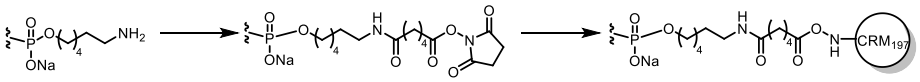


	Men A CPS	avDP 15 native	5
IC50	0.2812	8.576	2235

Figure 3. Competitive SPR.

(Scheme 4). To this end, the amine on the linker was used to functionalize the oligomer with an hydroxysuccinimide ester by treatment with an excess of di-N-hydroxysuccinimidyl adipate in the presence of triethylamine (TEA) in DMSO. The isolated activated esters (70 equivalents) were incubated with the carrier protein CRM_{197} in 1M NaPi buffer at pH 7.2. The glycoconjugates were purified by filtration against sodium phosphate buffer and characterized by SDS-PAGE to estimate the carbohydrate/protein ratio and microBCA to quantify the protein content.

The generated CRM_{197} glycoconjugate will be used in *in vivo* immunization experiments to check the real potential of these synthetic structures.



Scheme 4. Fragments activation and conjugation to carrier protein CRM_{197}.

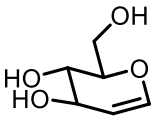
Conclusion

In this chapter a new reliable synthetic route towards well-defined C-3-OAc carba MenA analogues has been designed and used successfully to deliver target structures **1-6**. Octamer **5** has been selected for a competitive SPR experiment, showing far better IC_{50} value compared to its non-acetylated counterpart. **5** has been also conjugated to CRM_{197} as carrier protein to generate a glyconjugate which will be evaluated for its ability to stimulate production of anti-ManA Ab in mice.

Experimental part

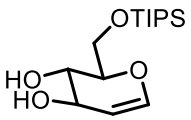
General procedures and materials: All chemicals (Acros, Biosolve, Sigma-Aldrich, TCI, etc) were used as received and all reactions were effectuated under an argon atmosphere, at ambient temperature (22°C), unless stated otherwise. For the TLC analysis were used aluminium sheets (Merck , TLC silica gel 60 F_{254}), sprayed with a solution of H_2SO_4 (20%) in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}\bullet 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\bullet 2H_2O$ (10g/L) in 10% aqueous H_2SO_4 or with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in H_2O and then heated at ≈ 140°C. For the column chromatography was used 40-63 µm 60Å silica gel (SD Screening Devices). NMR spectra (^1H, ^{13}C and ^{31}P) were recorded with a Bruker AV-400liq or a Bruker DMX-400solid or a Bruker AV-500 or a Bruker AV-600. High resolution mass spectra were recorded by direct injection on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (soure voltage 3.5 kV, sheath gas flow 10, capillary temperature 250°C) with resolution R= 60000 at m/z 400 (mass range m/z= 150-2000) and dioctylphthalate (m/z= 391.28428)as a lock mass.

D-glucal 21



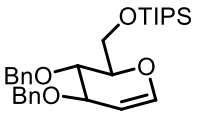
3,4,6-tri-*O*-acetyl-D-glucal (301.7 mmol, 82,14 g) was dissolved in MeOH (2000 mL). To the solution was added K_2CO_3 (5.09 gram, 36.8 mmol) and was stirred overnight at room temperature. The reaction was concentrated *in vacuo* and co-evaporated three times with toluene. The crude was used directly in the following step without further purification.

6-*O-triisopropylsilyl* -D-glucal 22



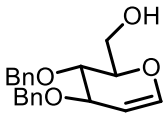
Crude D-glucal **21** was dissolved in DMF (865 mL), Imidazole (402 mmol, 27.34 g) was added and the resulting solution was cooled to –30 °C. TIPSCl (141 mmol, 30.3 mL) was added dropwise while keeping the reaction mixture between –30 °C and –20°C. The reaction mixture was allowed to reach room temperature and was stirred overnight. The reaction mixture was cooled to –30°C and TIPSCl (32.7 mmol, 7.0 mL) was added dropwise in order to have a full conversion of starting material. The mixture was concentrated *in vacuo* to remove the DMF and EtOAc and H_2O were added. The product was extracted twice with EtOAc and the combined organic layers were washed twice with H_2O and once with brine. The crude was concentrated *in vacuo* to obtain a yellow oil. The crude was co-evaporated with toluene three times and was used directly in the subsequent step without further purification.

3,4-di-*O*-benzyl-6-*O*-*triisopropylsilyl* -D-glucal 25



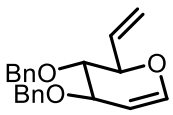
Crude diol **22** was dissolved in DMF (1000 mL) and cooled to 0°C. BnBr (288 mmol, 34.0 mL) was added. NaH (60% dispersed in mineral oil, 301.2 mmol, 12.05 g) was added to the solution in portions. The mixture was allowed to reach room temperature and stirred overnight. To the reaction was added MeOH at 0 °C. The mixture was diluted with Et_2O and H_2O and washed 3 times with H_2O and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo.* The crude was used directly in the following step without further purification.

3,4-di-*O*-benzyl-D-glucal 26



Crude compound **25** was dissolved in THF (600 mL). To the resulting solution, a 1M TBAF solution in THF (200 mmol, 200 mL) was added slowly and the reaction was stirred overnight. After the starting material was fully converted, to the reaction was added a saturated NH_4Cl solution at 0 °C and the product was extracted three times with EtOAc. The combined organic layers were washed twice with H_2O and once with brine. The crude was purified by flash chromatography (EtOAc/Pentane) providing **26** (211 mmol, 68.9 g) in 70% yield over 4 steps. **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.39 – 7.28 (m, 10H, CH Bn), 6.41 (dd, *J* = 6.1, 1.2 Hz, 1H, CH-1), 4.90 (dd, *J* = 6.2, 2.7 Hz, 1H, CH-2), 4.87 (d, *J* = 11.5 Hz, 1H, ½ CH_2 Bn), 4.73 (d, *J* = 11.5 Hz, 1H, ½ CH_2 Bn), 4.68 (d, *J* = 11.6 Hz, 1H, ½ CH_2 Bn), 4.57 (d, *J* = 11.6 Hz, 1H, ½ CH_2 Bn), 4.27 – 4.21 (m, 1H, CH-3), 3.95 (dt, *J* = 8.4, 4.1 Hz, 1H, CH-5), 3.87 (dd, *J* = 6.7, 4.2 Hz, 2H, CH_2-6), 3.81 (dd, *J* = 8.5, 6.2 Hz, 1H, CH-4). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 144.7 (CH 1), 138.3, 138.1 (2 C_q Bn), 128.7, 128.6, 128.2, 128.1, 127.9, 127.1 (10 CH Bn), 100.3 (CH 2), 77.4 (CH 5), 75.7 (CH 3), 74.6 (CH 4), 73.9, 70.8 (CH_2 Bn), 61.9 (CH_2 6).

1,5-Anhydro-di-*O*-benzyl-2,6,7-trideoxy-D-arabino-hept-1,6-dienitol 28



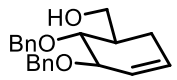
Alcohol **26** (25 mmol, 8.16 g) was co-evaporated with toluene 3 times, dissolved in dry EtOAc (500 mL) and the resulting solution was purged with N_2. Subsequently, freshly prepared IBX (150 mmol, 42 g) was added and the resulting suspension was heated to 80 °C and stirred for 5 h. After the starting material was fully converted the mixture was filtered over Celite® and washed with EtOAc. The resulting solution was concentrated at reduced pressure at 30 °C. The resulting oil was co-evaporated with toluene once and set under nitrogen atmosphere.

About 2 h before the full conversion of the aldehyde, the ylide was prepared, a 1 M NaHMDS solution (37.5 mL) was added dropwise to a suspension of PPh_3MeBr (37.5 mmol, 13.4 g) in dry THF (114 mL) at –78°C. The resulting yellow suspension was allowed

to warm up to 0 °C for 2 h. Before the aldehyde was added, the mixture was cooled to –78 °C again.

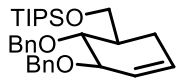
The aldehyde was dissolved in dry THF (30 mL), added dropwise to the yellow suspension at –78 °C and stirred overnight After the starting material was fully converted to the reaction was added a saturate solution of NH_4Cl (70 mL) and extracted with DCM. The organic layer was washed 2 times with H_2O and once with brine. The aqueous layers were extracted once with DCM. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane, silica neutralized with Et_3N) providing **28** (14.0 mmol, 4.5 g) in 56% yield over 3 steps. by column chromatography in the following manner. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ = 7.41 – 7.28 (m, 10H, CH Bn), 6.43 (dd, *J* = 6.1, 1.4 Hz, 1H, CH-1), 6.06 (ddd, *J* = 17.2, 10.5, 6.5 Hz, 1H, CH-6), 5.44 (dt, *J* = 17.3, 1.4 Hz, 1H, ½ CH=C*H*$_2$), 5.31 (dt, *J* = 10.6, 1.3 Hz, 1H, ½ CH=C*H*$_2$), 4.89 (dd, *J* = 6.2, 2.7 Hz, 1H, CH-2), 4.83 – 4.67 (m, 2H, CH_2 Bn), 4.62 (q, *J* = 11.7 Hz, 2H, CH_2 Bn), 4.40 – 4.28 (m, 1H, CH-5), 4.21 (dd, *J* = 6.8, 3.3 Hz, 1H, CH-3), 3.61 (dd, *J* = 8.5, 6.1 Hz, 1H, CH-4). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ = 144.6 (CH 1), 138.5, 138.2 (2 C_q Bn), 134.4 (CH 6), 128.5, 128.1, 127.9, 127.8, 127.8 (CH Bn), 118.4 (CH=*CH*$_2$), 100.4 (CH 2), 78.4 (CH 4), 78.1 (CH 5), 75.5 (CH 3), 73.9, 70.8 (2 CH_2 Bn).

3-4-di-*O*-benzyl-5a-carba-D-glucal 29



Alkene **28** (16.0 mmol, 5.15 g) was co-evaporated 3 times with toluene and dissolved in *o*-dichlorobenzene (45 mL) and put under a nitrogen atmosphere. The resulting solution was divided over 10 microwave vials and capped under a nitrogen atmosphere. After being pre-stirred for 10 min at rt and heated in a microwave at 230°C for 20 min, the resulting orange solution was added dropwise to a suspension of $NaBH_4$ (17.6 mmol, 0.67 g) in a 4:1 THF/EtOH mixture (70 mL). After 1 h, the reaction mixture was quenched with MeOH and the mixture was extracted with EtOAc, washed 2 times with H_2O and once with brine. The combined aqueous layers were extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was used directly in the following step without further purification.

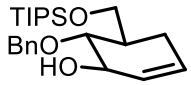
3-4-di-*O*-benzyl-6-*O*-triisopropylsilyl-5a-carba-D-glucal 30



Crude compound **29** (12.6 mmol, 3.94 g) was dissolved in DMF (120.0 mL). To the mixture was added imidazole (61.9 mmol, 4.2 g) and TIPSCl (15.5 mmol, 7.0 mL). After the starting material was fully converted, to the reaction was added H_2O. The mixture was diluted with Et_2O (360.0 mL) and washed 3 times with H_2O and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo.* The crude was purified by flash chromatography (EtOAc/Pentane) providing **30** (9.71 mmol, 4.67 g) in 80% yield over 3 steps. **H NMR** (400 MHz, $CDCl_3$) δ: 7.51 – 7.12 (m, 10H, CH_{arom}), 5.89 – 5.75 (m, 1H, H-1),

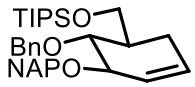
5.71 – 5.57 (m, 1H, H-2), 4.92 (d, *J* = 11.1 Hz, 1H, C*H*H Bn), 4.78 – 4.58 (m, 3H, C*H*H Bn, CH_2 Bn), 4.30 – 4.10 (m, 1H, H-3), 3.98 – 3.78 (m, 2H, H-6), 3.67 (m, 1H, H-4), 2.29 – 2.11 (m, 2H, H-5a), 2.01 – 1.91 (m, 1H, H-5), 1.05 (m, 21H, TIPS). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 128.9, 128.5, 127.9, 127.6, 126.1, 81.7, 79.5, 76.9, 74.5, 71.7, 63.6, 41.6, 28.7, 18.3, 12.2. **HRMS** *m/z*: $[M+H]^+$ Calcd for 481.31328; found 481.31325

4-*O*-benzyl-6-*O*-triisopropylsilyl-5a-carba-D-glucal 31



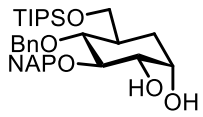
Compound **30** (5.1 mmol, 2.88 g) was co-evaporated 3 times with toluene and dissolved in dry toluene (51.0 mL). The solution was cooled to -70°C and a 1M $TiCl_4$ solution in DCM (12.7 mmol, 12.7 mL) was added. The reaction mixture was stirred for 2.5h and TEA (35.0 mmol, 5.0 mL) was added at-70°C. The solution was poured into an erlenmeyer containing a sat. solution of $NaHCO_3$ and filtered over Celite®. The water layer was extracted 2 times with EtOAc and the combined organic layers were washed 2 times with H_2O and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo.* The crude was purified by flash chromatography (EtOAc/Pentane), providing **31** (2.62 mmol, 1.02 g) in 51% yield. **H NMR** (400 MHz, $CDCl_3$) *δ*: 7.40 – 7.27 (m, 5H, CH_{arom}), 5.74 (m, 1H, H-1), 5.54 (d, *J* = 10.0 Hz, 1H, H-2), 4.89 – 4.68 (m, 2H, CH_2 Bn), 4.36 – 4.19 (m, 1H, H-3), 3.99 (m, 1H, H-6), 3.83 (m, 1H, H-6), 3.49 (dd, *J* = 10.7, 7.4 Hz, 1H, H-4), 2.36 – 2.20 (m, 2H, H-5a), 2.08 (bs, 1H, OH), 1.94 (m, 1H, H-5), 1.13 – 1.01 (m, 21H, TIPS). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 139.1, 128.7, 128.4, 128.2, 127.9, 127.9, 82.2, 74.4, 73.4, 63.4, 41.3, 28.9, 18.2, 12.2. **HRMS** *m/z*: $[M+H]^+$ Calcd for 391.26630; found 391.26630

4-*O*-benzyl-3-*O*-naphthyl-6-*O*-triisopropylsilyl-5a-carba-D-glucal 32



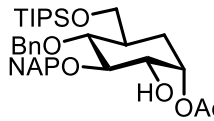
Compound **31** (15.4 mmol, 6.02 g) was co-evaporated 3 times with toluene and dissolved in DMF (150.0 mL) and cooled to 0°C. To the mixture was added NaH (60% dispersion in mineral oil, 19.4 mmol, 0.78 g), TBAI (1.54 mmol, 0.48 g) and NapBr (20.9 mmol, 4.61 g). After the starting material was fully converted, to the reaction was added MeOH. The mixture was diluted with Et_2O (260 mL) and washed 3 times with H_2O and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo.* The crude was purified by flash chromatography (EtOAc/Pentane), providing **32** (12.6 mmol, 6.68 g) in 82% yield. **H NMR** (400 MHz, $CDCl_3$) *δ*: 7.88 – 7.73 (m, 4H, CH_{arom}), 7.51 – 7.41 (m, 3H, CH_{arom}), 7.36 – 7.20 (m, 5H, CH_{arom}), 5.85 – 5.73 (m, 1H, H-1), 5.70 (d, *J* = 10.1 Hz, 1H, H-2), 4.95 – 4.73 (m, 4H, CH_2 Bn, CH_2 Nap), 4.28 – 4.21 (m, 1H, H-3), 3.91 – 3.75(m, 2H, H-6), 3.70 (m, 1H, H-4), 2.31 – 2.14 (m, 2H, H-5a), 1.97 (m, 1H, H-5), 1.17 – 0.87 (m, 21H, TIPS). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 139.3, 136.3, 133.5, 133.1, 128.9, 128.5, 128.2, 128.1, 127.9, 127.8, 127.6, 126.6, 126.1, 125.9, 81.7, 79.6, 74.5, 71.8, 63.5, 41.7, 28.7, 18.3, 12.2. **HRMS** *m/z*: $[M+Na]^+$ Calcd for 553.31084; found 553.30970

4-*O*-benzyl-3-*O*-naphthyl-6-*O*-triisopropylsilyl-5a-carba-α-D-glucopyranose 33



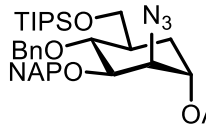
Compound **232** (12.7 mmol, 6.63 g) was dissolved in a mixture of Acetone/H_2O (63.5 mL, 4:1 ratio). To the mixture was added Me_3NO (27.9 mmol, 3.10 g) and an OsO_4 (CAUTION!) 0.22 M solution in H_2O (0.63 mmol, 2.9 mL) The reaction was stirred for 3 days. After the starting material was fully converted, to the reaction was added a sat. solution of $Na_2S_2O_3$ and stirred for 30 min. The organic layer was washed twice with H_2O and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo.* The crude was purified by flash chromatography (EtOAc/Pentane), providing **33** (11.1 mmol, 6.3 g) in 88% yield. **H NMR** (400 MHz, $CDCl_3$) δ: 7.78 (m, 4H, CH_{arom}), 7.51 – 7.39 (m, 3H, CH_{arom}), 7.38 – 7.18 (m, 5H, CH_{arom}), 5.14 – 4.74 (m, 4H, CH_2 Bn, CH_2 Nap), 4.05 (m, 2H, H-1, C*H*H-6), 3.79 (m, 1H, H-2), 3.66 (m, 1H, H-3), 3.53 (m, 2H, H-4, C*H*H-6), 2.66 (bs, 2H, OHx2), 2.11 (t, *J* = 9.6 Hz, 1H, H-5), 1.94 – 1.53 (m, 2H, H-5a), 1.15 – 0.90 (m, 21H, TIPS). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 138.9, 136.2, 133.5, 133.1, 128.6, 128.5, 128.1, 127.8, 127.8, 127.7, 127.6, 126.8, 126.3, 126.1, 125.9, 83.8, 80.9, 75.5, 74.9, 74.7, 68.5, 62.91 39.3, 30.5, 18.2, 12.1. **HRMS** *m/z*: $[M+H]^+$ Calcd for 565.33438; found 565.33374

1-*O*-Acetyl-4-*O*-benzyl-3-*O*-naphthyl-6-*O*-triisopropylsilyl-5a-carba-α-D-glucopyranose 34



Compound **33** (8.67 mmol, 4.90 g) was co-evaporated 3 times with toluene and dissolved in ACN (86.0 mL). To the solution was added $(MeO)_3CCH_3$ (28.6 mmol, 3.6 mL) and PTSA•H_2O (0.92 mmol, 0.175 g). After the starting material was fully converted, to the reaction was added a mixture of AcOH/H_2O (100.0 mL, 4:1 ratio). The organic layer was diluted with DCM and washed once with H_2O, twice with a sat. solution of $NaHCO_3$ and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo* and the crude was used in the subsequent step without further purification.

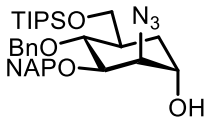
1-*O*-Acetyl-2-azido-4-*O*-benzyl-3-*O*-naphthyl-2-deoxy-6-*O*-triisopropylsilyl-5a-carba-α-D-mannopyranose 35



Compound **34** (6.95 mmol, 4.22 g) was co-evaporated 3 times with toluene and dissolved in a mixture of DCM/pyridine (140.0 mL, 5:1 ratio). The solution was cooled to -10°C and stirred for 10 min. To the reaction mixture was added Tf_2O (34.8 mmol, 5.83 mL) dropwise. After the starting material was fully converted, to the reaction was added a sat. solution of $NaHCO_3$ (50.0 mL) and was washed once with H_2O and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was dissolved in a mixture of DMF/H_2O (35mL, 19:1 ratio). To the mixture was added NaN_3 (46.1 mmol, 3.0 g) and 15-crown-5 (1.4 mmol, 0.3 mL) and stirred overnight at 40°C. After the starting material was fully converted, to the reaction was added a sat. solution of $NaHCO_3$ (50.0 mL). The water layer was extracted 3 times with DCM and the combined

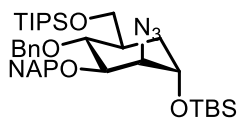
organic layers were washed once with H_2O and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane), providing **35** (5.10 mmol, 3.75 g) in 73% yield over 2 steps. **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.87 – 7.71 (m, 4H, CH_{arom}), 7.54 – 7.42 (m, 3H, CH_{arom}), 7.35 – 7.24 (m, 5H, CH_{arom}), 5.00 – 4.60 (m, 5H, H-1, CH_2 Bn, CH_2 Nap), 4.00 – 3.62 (m, 5H, H-2, H-3, H-4, H-6), 1.89 (m, 5H, OAc, H-5, C*H*H-5a), 1.73 (m, 1H, C*H*H-5a), 1.13 – 0.90 (m, 21H, TIPS). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 138.8, 135.4, 133.3, 133.2, 128.5, 128.5, 128.0, 127.9, 127.8, 127.1, 126.3, 126.2, 126.1, 81.3, 76.8, 75.3, 73.4, 70.5, 63.1, 61.5, 40.2, 27.2, 18.2, 12.1. **HRMS** *m/z*: $[M+H]^+$ Calcd for 632.35142; found 632.35095

2-Azido-4-*O*-benzyl-3-*O*-naphthyl-2-deoxy-6-*O*-triisopropylsilyl-5a-carba-α-D-mannopyranose 36



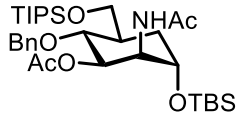
Compound **35** (8.91 mmol, 5.41 g) was and dissolved in a mixture of MeOH/THF (90.0 mL, 4.6:1 ratio). To the solution was added a 5.4 M NaOMe solution in MeOH (2.4 mmol, 0.44 mL). After the starting material was fully converted, to the reaction was added AcOH until neutral pH was reached. The reaction was concentrated in *vacuo* and the crude was used in the subsequent step without further purification.

2-Azido-4-*O*-benzyl-3-*O*-naphthyl-1-*O*-tertbutyldimethylsilyl-2-deoxy-6-*O*-triisopropylsilyl-5a-carba-α-D-mannopyranose 37



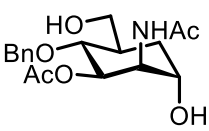
Crude **36** was co-evaporated 3 times with toluene and dissolved in pyridine (45.0 mL). To the solution was added DMAP (1.9 mmol, 0.24 g). The reaction mixture was cooled to 0°C and TBSOTf (20.0 mmol, 4.6 mL) was added dropwise. After 20 min the reaction was heated to 70°C and stirred overnight. After the starting material was fully converted, the reaction was cooled to r.t. and was added MeOH. The mixture was diluted with Et_2O (70.0 mL) and washed twice with a 1M HCl solution, once with a sat. solution of $NaHCO_3$ and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (Et_2O/Pentane), providing **37** (6.86 mmol, 4.83 g) in 77% yield over 2 steps. **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 8.14 – 7.97 (m, 4H, CH_{arom}), 7.89 – 7.67 (m, 3H, CH_{arom}), 7.64 – 7.50 (m, 5H, CH_{arom}), 5.28 – 4.86 (m, 4H, CH_2 Bn, CH_2 Nap), 4.29 (dd, *J* = 9.2, 3.4 Hz, 1H, H-3), 4.16 (d, *J* = 3.4 Hz, 1H, H-1), 4.12 – 3.93 (m, 4H, H-4, H-2, H-6), 2.33 – 2.21 (m, 1H, H-5), 2.05 – 1.84 (m, 2H, H-5a), 1.40 – 1.23 (m, 21H, TIPS), 1.02 (d, *J* = 2.4 Hz, 9H, TBS), 0.27 – 0.13 (m, 6H, TBS). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 138.9, 135.9, 133.4, 133.2, 128.5, 128.4, 128.3, 128.1, 127.8, 127.7, 127.1, 126.2, 126.2, 126.0, 81.4, 78.1, 75.5, 73.4, 69.1, 65.3, 63.8, 39.6, 30.6, 25.7, 18.2, 12.1, -4.8, -5.2. **HRMS** *m/z*: $[M+Na]^+$ Calcd for 726.40928; found 726.40981

2-Acetamido-3-*O*-acetyl-4-*O*-benzyl-1-*O*-tertbutyldimethylsilyl-2-deoxy-6-*O*-triisopropylsilyl-5a-carba-α-D-mannopyranose 38



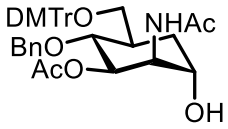
Compound **37** (3.9 mmol, 2.74 g) was dissolved in a mixture of THF/EtOH (70.0 mL, 3:1 ratio). To the mixture was added AcOH (17.5 mmol, 1.0 mL) and the solution was purged with argon for 20 min. To the reaction was added Pd/C (10 wt. % loading, 0.690 mmol, 0.744 g) and the solution was purged with argon for 20 min. The reaction mixture was purged with H_2 for 3 min and left stirring for 3 days under H_2 atmosphere. To the solution was added pyridine and the reaction mixture was filtrated over Celite® and concentrated in *vacuo*. The crude was dissolved in pyridine (39.0 mL) and Ac_2O (39 mmol, 3.7 mL). The reaction was stirred overnight and MeOH was added. The mixture was diluted with EtOAc (100 mL) and washed twice with a 1M HCl solution, once with H_2O, once with a sat. solution of $NaHCO_3$ and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane), providing **38** (2.6 mmol, 1.6 g) in 66% yield over 2 steps. **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.37 – 7.27 (m, 5H, CH_{arom}), 5.49 – 5.43 (m, 1H, H-3), 5.31 (d, *J* = 7.4 Hz, 1H, NH), 4.76 – 4.58 (m, 2H, CH_2 Bn), 4.35 – 4.27 (m, 1H, H-2), 4.12 (d, *J* = 3.2 Hz, 1H, H-2), 4.06 (dd, *J* = 9.8, 3.8 Hz, 1H, C*H*H-6), 3.74 – 3.58 (m, 2H, C*H*H-6, H-4), 2.20 – 2.08 (m, 1H, H-5), 2.00 (s, 3H, OAc), 1.98 (s, 3H, NHAc), 1.64 – 1.53 (m, 2H, H-5a), 1.13 – 1.02 (m, 21H, TIPS), 0.88 (s, 9H, TBS), 0.15 (s, 3H, TBS), 0.07 (s, 3H, TBS). **^{13}C NMR** (126 MHz, $CDCl_3$) δ: 169.9, 169.7, 138.6, 137.9, 129.1, 128.5, 128.3, 127.7, 127.5, 125.4, 77.4, 77.1, 76.9, 75.9, 74.7, 73.2, 68.0, 63.0, 53.9, 39.1, 30.5, 25.8, 23.6, 21.2, 18.2, 18.2, 12.1, -4.9, -5.0. **HRMS** *m/z*: $[M+H]^+$ Calcd for 622.39537; found 622.39537.

2-Acetamido-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranose 39



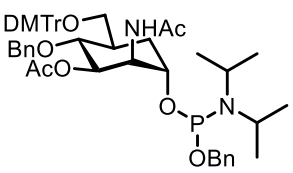
Compound **38** (3.4 mmol, 2.14 g) was dissolved in THF (9.0 mL). The mixture was cooled to 0°C. To the reaction was added a 70% HF solution in pyridine (6.95 mL). After the starting material was fully converted, the mixture was diluted with EtOAc (30.0 mL). The solution was washed once with a 1M HCl solution, once with a sat. solution of $NaHCO_3$ and once with brine. The combined water layers were extracted 7 times with a 4:1 mixture of $CHCl_3$/*i*-PrOH. The combined organic layers were dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/EtOH), providing **39** (3.03 mmol, 1.06 g) in 89% yield. **^{1}H NMR** (400 MHz, MeOD) δ: 7.39 – 7.21 (m, 5H, CH_{arom}), 5.38 – 5.25 (m, 1H, H-3), 4.66 (dd, *J* = 11.4 Hz, 2H, CH_2 Bn), 4.44 (t, *J* = 4.8 Hz, 1H, H-2), 3.89 – 3.81 (m, 1H, H-1), 3.79 – 3.62 (m, 3H, H-6, H-4), 2.16 – 2.07 (m, 1H, H-5), 2.00 (s, 3H, OAc), 1.96 (s, 3H, NHAc), 1.82 (dd, *J* = 8.1, 4.0 Hz, 2H, H-5a). **^{13}C NMR** (101 MHz, MeOD) δ: 173.6, 171.9, 139.9, 129.3, 128.7, 128.6, 77.2, 74.9, 74.6, 68.2, 63.1, 53.9, 40.6, 30.9, 22.5, 21.1. **HRMS** *m/z*: $[M+H]^+$ Calcd for 352.17546; found 352.17546

2-Azido-3-*O*-acetyl-4-*O*-benzyl-6-*O*-4,4'dimethoxytrityl-2-deoxy-5a-carba-α-D-mannopyranose 19



Compound **29** (6.60 mmol, 2.32 g) was in pyridine (33.0 mL). To the reaction was added DMTrCl (9.90 mmol, 3.35 g). After the starting material was fully converted, the mixture was diluted with DCM (20.0 mL). The solution was washed once with a 1M HCl solution, once with a sat. solution of $NaHCO_3$ and once with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/EtOH), providing **19** (6.14 mmol, 4.01 g) in 93% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ: 7.51 – 7.05 (m, 14H, CH_{arom}), 6.92 – 6.80 (m, 4H, CH_{arom}), 6.41 (d, *J* = 8.6 Hz, 1H, NH), 5.16 (dd, *J* = 8.3, 4.3 Hz, 1H, H-3), 4.55 (d, *J* = 11.2 Hz, 1H, C*H*H Bn), 4.37 – 4.27 (m, 2H, C*H*H Bn, H-2), 3.86 – 3.80 (m, 1H, H-1), 3.76 (d, *J* = 1.3 Hz, 6H, OMex2), 3.63 (t, *J* = 8.4 Hz, 1H, H-4), 3.36 (bs, 1H, OH), 3.30 (dd, *J* = 8.9, 4.0 Hz, 1H, C*H*H-6), 3.10 (dd, *J* = 8.8, 7.3 Hz, 1H, C*H*H-6), 2.25 (s, 1H, H-5), 2.04 – 1.95 (m, 2H, H-5a), 1.94 (s, 3H, OAc), 1.89 (s. 3H, NHAc). **^{13}C NMR** (126 MHz, CD_3CN) δ: 171.3, 171.0, 159.6, 146.5, 139.6, 138.9, 137.3, 137.1, 131.1, 129.9, 129.2, 129.2, 129.1, 128.8, 128.7, 128.4, 127.7, 126.3, 114.0, 113.9, 86.7, 76.9, 74.3, 74.1, 67.9, 64.5, 55.9, 53.6, 38.7, 31.7, 23.1, 21.4. **HRMS** *m/z*: $[M+Na]^+$ Calcd for 676.28809; found 676.28809.

2-Acetimido-3-*O*-acetyl-4-*O*-benzyl-6-*O*-4,4'dimethoxytrityl-1-*O*-((*N*,*N*-Diisopropylamino)-*O*-2-benzyl-phosphoramidite))-2-deoxy-5a-carba-α-D-mannopyranose 17



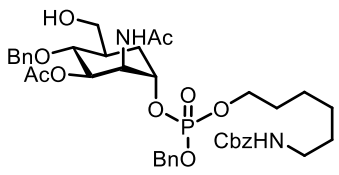
Compound **19** (3.04 mmol, 1.98 g) was co-evaporated 3 times with ACN, and dissolved in dry DCM (30.0 mL). To the mixture were added freshly activated MS3Å and diisopropylammoniumtetrazol-2-ide (2.43 mmol, 0.42 g). To the mixture was added a 0.2M 1-(benzyloxy)-1-chloro-*N*,*N*-diisopropylphosphanamine solution in DCM (3.34 mmol, 16.7 mL). After the starting material was fully converted, to the mixture was added H_2O and diluted with DCM. The reaction was washed once with a 1:1 solution of brine/$NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated in *vacuo.* The crude was purified by flash chromatography (pentane/EtOAc) leading to product **17** (2.81 mmol, 2.50 g) in 92% yield (mixture of diastereoisomers). **^{1}H NMR** (500 MHz, CD_3CN) δ: 7.51 – 7.17 (m, 18H), 7.12 – 6.93 (m, 2H), 6.92 – 6.75 (m, 4H), 6.44 (dd, *J* = 11.7, 8.5 Hz, 1H), 5.21 (ddd, *J* = 17.3, 9.4, 4.5 Hz, 1H), 4.84 – 4.64 (m, 2H), 4.59 – 4.38 (m, 2H), 4.27 (dd, *J* = 16.1, 11.0 Hz, 1H), 4.18 – 4.06 (m, 1H), 3.81 – 3.55 (m, 9H), 3.34 (ddd, *J* = 21.4, 8.7, 3.4 Hz, 1H), 3.03 (dd, *J* = 8.7, 7.2 Hz, 1H), 2.34 – 2.05 (m, 2H), 2.03 – 1.81 (m, 11H), 1.35 – 1.13 (m, 12H). **^{13}C NMR** (126 MHz, CD_3CN) δ: 170.9, 170.8, 170.7, 159.5, 159.5, 146.4, 146.4, 139.5, 139.4, 137.1, 137.1, 137.0, 131.0, 131.0, 129.2, 129.1, 129.1, 129.0, 129.0, 128.7, 128.7, 128.4, 128.4, 128.2, 128.0, 127.9, 127.7, 127.7, 113.9, 113.9, 86.5, 77.0, 74.5, 74.4, 74.3, 70.9, 70.8, 66.0, 65.9, 65.9, 65.8, 64.4, 64.2, 55.8, 53.1, 53.0, 52.8,

44.1, 44.0, 44.0, 43.9, 39.0, 38.8, 30.9, 25.1, 25.0, 24.9, 24.9, 24.9, 24.9, 23.1, 21.3. **^{31}P NMR** (202 MHz, CD_3CN) δ: 147.5, 146.7.

General procedure A: Phosphoramidite coupling, oxidation and detritylation on a typical scale (0.07–0.9 mmol)

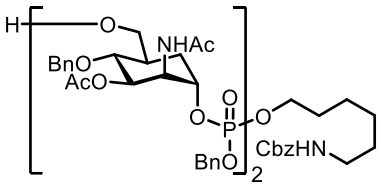
Starting alcohol was co-evaporated 3 times with ACN and was added freshly activated MS3Å and a 0.25M DCI solution in ACN (1.5 eq). The solution was stirred for 15 min. To the mixture was added phosphoramidite reagent (0.2 M solution in ACN, 1.3–3 eq) and stirred until the total conversion of the starting material (≈4 hours). Subsequently CSO (0.5M solution in ACN, 2eq) was added to the reaction mixture and stirred for 15 min. The mixture was diluted with DCM and washed with a 1:1 solution of brine/$NaHCO_3$. The water layer was extracted 3 times with DCM. The combined organic layers were dried over Na_2SO_4 and concentrated in *vacuo.* The crude was treated with a 0.18M TCA solution in DCM (5 eq) a stirred for an hour. The reaction mixture was washed with a 1:1 solution of brine/$NaHCO_3$. The water layer was extracted 4 times with DCM. The combined organic layers were dried over Na_2SO_4 and concentrated in *vacuo.* The crude was purified by flash chromatography (DCM/Acetone) and/or by size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1).

Monomer 7



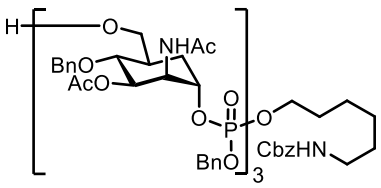
Alcohol **19** (0.9 mmol, 0.591 g), was coupled to phosphoramidite **18** (1.18 mmol, 5.93 mL 0.2 M in ACN), oxidized, detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/acetone) leading to product **7** (0.710 mmol, 0.536 g) in 79% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ= 7.46 – 7.13 (m, 15H, CH_{arom}), 6.62 (bs, 1H, NH), 5.78 (bs, 1H, NH), 5.17 – 4.96 (m, 5H, H-3, CH_2 Bn x2), 4.72 – 4.56 (m, 2H, CH_2 BnOP), 4.56 – 4.46 (m, 2H, H-1, H-2), 4.08 – 3.91 (m, 2H, CH_2 linker), 3.73 (td, *J* = 8.4, 3.1 Hz, 1H, H-4), 3.70 – 3.49 (m, 2H, H-6), 3.11 – 2.88(m, 3H, CH_2 linker, OH), 2.03 – 1.78 (m, 9H, NHAc, OAc, H-5, H-5a), 1.61 (q, *J* = 6.7 Hz, 2H, CH_2 linker), 1.43 (pd, *J* = 7.9, 7.5, 2.7 Hz, 2H, CH_2 linker), 1.37 – 1.20 (m, 4H, CH_2 linker x2). **^{13}C NMR** (101 MHz, CD_3CN) δ= 129.5, 129.3, 129.2, 128.8, 128.7, 128.6, 128.5, 76.2, 75.2, 74.5, 73.8, 70.0, 68.8, 66.5, 51.7, 41.3, 40.1, 30.7, 29.4, 26.7, 25.7, 21.2. **^{31}P NMR** (162 MHz, CD_3CN) δ: -0.42, -0.55. **HRMS** *m/z*: $[M+H]^+$ Calcd 755.33032, founded 755.33032.

Dimer 8



Alcohol **7** (0.71 mmol, 0.536 g), was coupled to phosphoramidite **17** (0.92 mmol, 4.6 mL 0.2 M in ACN), oxidized, detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/MeOH) and size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1) leading to product **8** (0.61 mmol, 0.770 g) in 86% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ: 7.52 – 7.23 (m, 25H, CH_{arom}), 7.24 – 7.00 (m, 1H, NH), 6.80 (dd, *J* = 18.0, 8.2 Hz, 1H, NH), 5.84 (d, *J* = 22.1 Hz, 1H, NH), 5.23 – 5.00 (m, 8H, CH_2 Cbz, CH_2 Bn x2, H-3 x2), 4.75 – 4.49 (m, 8H, H-1 x2, H-2 x2, CH_2 BnOP x2), 4.30 (m, 1H, C*H*H-6), 4.11 – 3.88 (m, 3H, CH_2 linker, C*H*H-6), 3.84 – 3.73 (m, 2H, H-4 x2), 3.73 – 3.55 (m, 2H, H-6), 3.19 – 2.97 (m, 3H, CH_2 linker, OH), 2.20 – 1.79 (m, 18H, OAc x2, NHAc x2, H-5 x2, H-5a x2), 1.62 (m, 2H, CH_2 linker), 1.51 – 1.40 (m, 2H, CH_2 linker), 1.40 – 1.22 (m, 4H, CH_2 linker x2). **^{13}C NMR** (101 MHz,CD_3CN) δ: 171.9, 171.7, 171.3, 171.2, 171.0, 171.0, 170.9, 139.8, 139.7, 139.6, 139.6, 139.6, 129.6, 129.5, 129.5, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 129.0, 128.9, 128.9, 128.8, 128.8, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 76.4, 76.4, 75.6, 75.6, 75.5, 75.4, 75.4, 75.2, 75.0, 74.8, 74.0, 73.9, 73.8, 73.7, 70.3, 70.2, 70.2, 70.2, 70.1, 70.1, 70.1, 70.0, 70.0, 68.9, 68.8, 68.0, 66.5, 62.3, 62.2, 52.0, 51.7, 51.6, 51.6, 41.3, 40.2, 40.0, 38.3, 30.7, 30.7, 30.3, 29.3, 26.7, 25.7, 25.7, 25.7, 23.1, 23.1, 23.0, 23.0, 21.2, 21.2, 21.2. **^{31}P NMR** (162 MHz, CD_3CN) δ: -1.40, -1.42, -1.44, -1.99, -2.01, -2.03, -2.06, -2.09, -2.16, -2.17, -2.19. **HRMS** *m/z*: $[M+H]^+$ Calcd 1258.50123, founded 1258.50123.

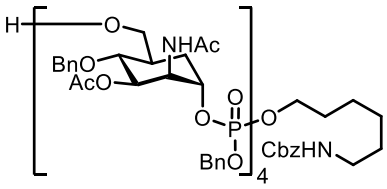
Trimer 9



Alcohol **8** (0.430 mmol, 0.545 g), was coupled to phosphoramidite **17** (2.8 mL 0.2 M in ACN, 0.56 mmol), oxidized, detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/acetone) and size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1) leading to product **9** (0.376 mmol, 0.662 g) in 86% yield. **^{1}H NMR** ^{1}H NMR (500 MHz, CD_3CN) δ: 7.50 – 7.20 (m, 35H, CH_{arom}), 7.17 – 7.08 (m, 1H, NH), 6.80 – 6.69 (m, 2H, NH), 5.89 – 5.79 (m, 1H, NH), 5.22 – 4.98 (m, 11H, H-3 x3, CH_2Bn x3, CH_2Cbz), 4.76 – 4.44 (m, 12H, H-1 x3, H-2 x3, , CH_2BnP x3), 4.36 – 4.22 (m, 2H, H-6), 4.13 – 3.88 (m, 4H, H-6, CH_2 Linker), 3.86 – 3.70 (m, 3H, H-4 x3), 3.68 – 3.51 (m, 2H, H-6), 3.15 – 2.92 (m, 3H, CH_2 Linker, OH), 2.12 – 1.78 (m, 27H, NHAc x3, OAc x3, H-5 x3, H-5a x3), 1.60 (m, 2H, CH_2 Linker), 1.42 (m, 2H, CH_2 Linker), 1.37 – 1.19 (m, 4H, CH_2 Linker x2). **^{13}C NMR** (126 MHz, CD_3CN) δ: 171.2, 171.0, 139.8, 139.6, 129.6, 129.6, 129.5, 129.5, 129.4, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.9, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 76.4, 75.7, 75.6, 75.2, 74.8, 73.8, 73.8, 70.3, 70.0, 68.9, 66.5, 62.4, 62.2, 55.8, 51.5,

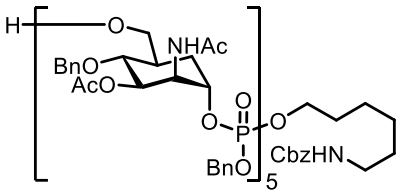
41.4, 40.0, 30.8, 30.7, 30.3, 26.8, 25.7, 23.2, 23.0, 21.2. **^{31}P NMR** (202 MHz, CD_3CN) δ: 0.07, -0.07, -0.54, -0.61, -0.65, -0.69, -0.71, -0.81, -0.84, -0.88, -0.91. **HRMS** *m/z*: $[M+H]^+$ Calcd 1783.65408, founded 1783.65407.

Tetramer 10



Alcohol **9** (0.290 mmol, 0.511 g), was coupled to phosphoramidite **17** (1.86 mL 0.2 M in ACN, 0.372 mmol), oxidized, detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/acetone) and size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1) leading to product **10** (0.210 mmol, 0.476 g) in 75% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ: 7.58 – 6.98 (m, 45H, CH_{arom}), 6.89 – 6.69 (m, 2H, NH), 5.89 – 5.79 (m, 1H, NH) 5.22 – 4.97 (m, 14H, H-3 x4, CH_2Bn x4, CH_2Cbz), 4.75 – 4.46 (m, 16H, H-1 x4, H-2 x4, CH_2BnP x4), 4.39 – 4.22 (m, 3H, H-6, C*H*H-6), 4.10 – 3.88 (m, 5H, H-6 x2, C*H*H-6), 3.88 – 3.53 (m, 6H, H-4 x4, H-6), 3.07 (m, 2H, CH_2 Linker), 2.24 – 1.72 (m, 36H, NHAc x4, OAc x4, H-5 x4, H-5a x4), 1.61 (m, 2H, CH_2 Linker), 1.50 – 1.38 (m, 2H, CH_2 Linker), 1.40 – 1.24 (m, 4H, CH_2 Linker x2). **^{13}C NMR** (126 MHz, CD_3CN) δ: 171.9, 171.3, 171.2, 171.0, 171.0, 170.9, 139.8, 139.6, 137.1, 131.1, 131.0, 129.6, 129.6, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.1, 129.0, 129.0, 128.9, 128.9, 128.8, 128.8, 128.8, 128.7, 128.7, 128.6, 128.5, 128.5, 127.7, 114.0, 113.9, 76.4, 75.7, 75.6, 75.2, 75.1, 74.8, 74.0, 73.8, 73.7, 70.3, 70.0, 68.9, 68.0, 66.5, 62.2, 55.8, 55.1, 51.7, 51.5, 41.3, 40.2, 40.0, 38.2, 30.7, 30.7, 30.6, 30.3, 29.7, 29.3, 26.7, 25.7, 23.2, 23.1, 23.0, 21.2, 21.2. **^{31}P NMR** (202 MHz, CD_3CN) δ: -1.28, -1.33, -1.36, -1.40, -1.47, -1.55, -1.71, -1.97, -2.05, -2.09, -2.11, -2.14, -2.24, -2.27, -2.30, -2.32, -2.34, -2.37. **HRMS** *m/z*: $[M+H]^+$ Calcd 2265.84639, founded 2265.84634.

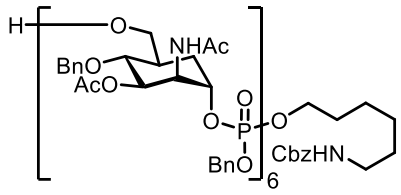
Petamer 11



Alcohol **10** (0.210 mmol, 0.470 g), was coupled to phosphoramidite **17** (1.35 mL 0.2 M in ACN, 0.27 mmol), oxidized, detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/acetone) and size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1) leading to product **11** (0.161 mmol, 0.446 g) in 77% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ: 7.49 – 7.21 (m, 55H, CH_{arom}), 7.14 (s, 1H, NH), 6.82 – 6.67 (m, 1H, NH), 5.82 (s, 1H, NH), 5.21 – 4.96 (m, 17H, H-3 x5, CH_2Bn x5, CH_2Cbz), 4.80 – 4.42 (m, 20H, H-1 x5, H-2 x5, CH_2BnP x5), 4.42 – 4.21 (m, 4H, H-6 x2), 4.13 – 3.53 (m, 13H, CH_2 Linker, H-4 x5, H-6 x3), 3.11 – 3.02 (m, 2H, CH_2 Linker), 2.16 – 1.71 (m, 45H, NHAc x5, OAc x5, H-5 x5, H-5a x5), 1.71 – 1.52 (m, 2H, CH_2 Linker), 1.44 (m, 2H, CH_2 Linker), 1.41 – 1.17 (m, 4H, CH_2 Linker x2). **^{13}C NMR** (126 MHz, CD_3CN) δ: 171.2, 171.1, 171.0, 170.9, 139.8, 139.6, 137.1, 129.6, 129.6, 129.6, 129.5, 129.4, 129.4, 129.4, 129.2, 129.2, 129.1, 129.0,

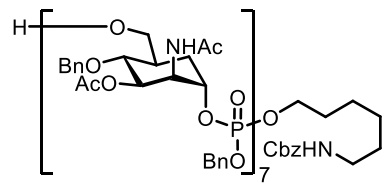
128.9, 128.8, 128.8, 128.8, 128.7, 128.7, 128.6, 128.5, 128.4, 76.4, 75.7, 75.5, 74.8, 73.8, 70.3, 70.1, 70.0, 68.8, 68.0, 66.5, 62.2, 51.7, 51.5, 41.3, 40.0, 38.2, 30.7, 30.7, 30.3, 29.2, 26.7, 25.7, 23.1, 23.0, 21.2, 21.2. **^{31}P NMR** (202 MHz, CD_3CN) δ: -1.30, -1.32, -1.35, -1.38, -1.40, -1.47, -1.54, -1.96, -2.05, -2.11, -2.14, -2.24, -2.27, -2.30, -2.32, -2.35. **HRMS** *m/z*: $[M+H]^{2+}$ Calcd 1385.01228, founded 1385.01226.

Hexamer 12



Alcohol **11** (0.154 mmol, 0.429 g), was coupled to phosphoramidite **17** (1.00 mL 0.2 M in ACN, 0.200 mmol), oxidized, detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/acetone) and size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1) leading to product **12** (0.109 mmol, 0.357 g) in 71% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ: 7.49 – 7.21 (m, 65H, CH_{arom}), 7.16 (bs, 1H, NH), 6.82 – 6.67 (m, 1H, NH), 5.83 (s, 1H, NH), 5.22 – 4.98 (m, 20H, H-3 x6, CH_2Bn x6, CH_2Cbz), 4.78 – 4.42 (m, 24H, H-1 x6, H-2 x6, CH_2BnP x6), 4.31 (m, 4H, H-6 x2), 4.12 – 3.52 (m, 16H, CH_2 Linker, H-4 x6, H-6 x4), 3.06 (m, 2H, CH_2 Linker), 2.97 – 2.85 (bs, 1H, OH), 2.23 – 1.69 (m, 54H, NHAc x6, OAc x6, H-5 x6, H-5a x6), 1.62 (m, 2H, CH_2 Linker), 1.44 (m, 2H, CH_2 Linker), 1.29 (s, 4H, CH_2 Linker x2). **^{13}C NMR** (126 MHz, CD_3CN) δ: 172.0, 171.1, 170.9, 139.6, 137.1, 129.6, 129.6, 129.6, 129.5, 129.4, 129.4, 129.4, 129.2, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 76.4, 75.7, 75.6, 75.2, 73.8, 70.3, 70.0, 68.0, 66.5, 62.2, 51.8, 41.3, 40.0, 38.2, 30.7, 30.3, 29.3, 26.7, 25.7, 23.2, 23.0, 21.2, 21.2. **^{31}P NMR** (202 MHz, CD_3CN) δ: 0.15, 0.11, 0.08, 0.05, -0.03, -0.09, -0.51, -0.60, -0.66, -0.69, -0.80, -0.82, -0.86, -0.90, -0.93. **HRMS** *m/z*: $[M+H]^{2+}$ Calcd 1636.59774, founded 1636.59771.

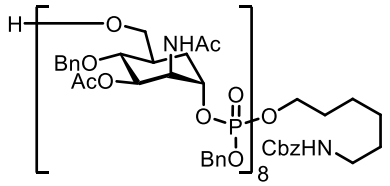
Heptamer 13



Alcohol **12** (0.097 mmol, 0.317 g), was coupled to phosphoramidite **17** (0.73 mL 0.2 M in ACN, 0.146 mmol), oxidized, detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/MeOH) and size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1) leading to product **13** (0.073 mmol, 0.276 g) in 75% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ: 7.59 – 7.19 (m, 75H, CH_{arom}), 6.76 (s, 1H, NH), 5.82 (s, 1H, NH), 5.26 – 4.90 (m, 23H, H-3 x7, CH_2Bn x7, CH_2Cbz), 4.81 – 4.45 (m, 28H, H-1 x7, H-2 x7, CH_2BnP x7), 4.29 (m, 6H, H-6 x3), 4.14 – 3.51 (m, 17H, CH_2 Linker, H-4 x7, H-6 x4), 3.11 – 3.03 (m, 2H, CH_2 Linker), 2.32 – 1.74 (m, 63H, NHAc x7, OAc x7, H-5 x7, H-5a x7), 1.62 (m, 2H, CH_2 Linker), 1.44 (m, 2H, CH_2 Linker), 1.29 (s, 4H, CH_2 Linker x2). **^{13}C NMR** (126 MHz, CD_3CN) δ: 172.0, 171.1, 170.9, 139.8, 139.6, 137.2, 129.6, 129.6, 129.6, 129.5, 129.5, 129.4, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.9, 128.9, 128.8, 128.8, 128.7, 128.7, 128.6,

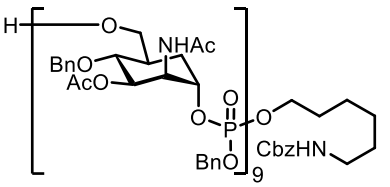
128.5, 128.5, 75.7, 75.6, 75.2, 75.1, 74.8, 73.9, 73.8, 70.3, 70.0, 68.9, 68.0, 66.5, 62.3, 51.8, 51.5, 41.4, 40.0, 38.2, 30.8, 30.7, 30.3, 29.2, 26.8, 25.7, 23.2, 23.0, 21.3, 21.2. **^{31}P NMR** (202 MHz, CD_3CN) δ: -1.31, -1.34, -1.38, -1.40, -1.49, -1.55, -1.96, -1.98, -2.05, -2.11, -2.14, -2.25, -2.27, -2.31, -2.35, -2.38. **HRMS** *m/z*: $[M+H]^{3+}$ Calcd 1259.45900, founded 1259.45893.

Octamer 14



Alcohol **13** (0.070 mmol, 0.264 g) was coupled to phosphoramidite **17** (0.70 mL 0.2 M in ACN, 0.140 mmol), oxidized, detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/MeOH) and size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1) leading to product **14** (0.049 mmol, 0.211 g) in 70% yield. **^{1}H NMR** (600 MHz, CD_3CN) δ: 7.51 – 7.01 (m, 85H, CH_{arom}), 6.72 (s, 1H, NH), 5.80 (s, 1H, NH), 5.22 – 4.95 (m, 26H, H-3 x8, CH_2Bn x8, CH_2Cbz), 4.82 – 4.40 (m, 32H, H-1 x8, H-2 x8, CH_2BnP x8), 4.40 – 4.15 (m, 7H, H-6 x3, C*H*H-6), 4.13 – 3.48 (m, 17H, CH_2 Linker, H-4 x8, H-6 x3, C*H*H-6), 3.07 – 3.00 (m, 2H, CH_2 Linker), 2.21 – 1.69 (m, 72H, NHAc x8, OAc x8, H-5 x8, H-5a x8), 1.69 – 1.52 (m, 2H, CH_2 Linker), 1.52 – 1.40 (m, 2H, CH_2 Linker), 1.40 – 1.14 (m, 4H, CH_2 Linker x2). **^{13}C NMR** (151 MHz, CD_3CN) δ: ^{13}C NMR (151 MHz, CD_3CN) δ 172.0, 171.9, 171.7, 171.2, 171.2, 171.1, 171.0, 171.0, 170.9, 170.9, 170.9, 157.3, 139.8, 139.7, 139.7, 139.6, 139.6, 138.6, 137.1, 137.1, 129.6, 129.6, 129.6, 129.5, 129.5, 129.5, 129.5, 129.5, 129.4, 129.4, 129.4, 129.4, 129.3, 129.3, 129.2, 129.2, 129.1, 129.1, 129.1, 129.0, 129.0, 128.9, 128.9, 128.9, 128.9, 128.8, 128.8, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 76.4, 75.7, 75.6, 75.3, 75.2, 75.1, 75.0, 74.8, 74.0, 73.9, 73.8, 73.7, 70.4, 70.3, 70.2, 70.1, 70.0, 70.0, 68.8, 68.0, 66.5, 62.4, 62.3, 62.2, 52.0, 51.8, 51.5, 51.5, 41.3, 40.2, 40.0, 38.2, 30.7, 30.7, 30.3, 30.3, 29.2, 26.7, 25.7, 25.7, 25.7, 23.2, 23.2, 23.0, 21.3, 21.2, 21.2, 21.2, 21.2. **^{31}P NMR** (202 MHz, CD_3CN) δ: -1.31, -1.34, -1.38, -1.40, -1.49, -1.55, -1.96, -1.98, -2.05, -2.11, -2.14, -2.25, -2.27, -2.31, -2.35, -2.38. **HRMS** *m/z*: $[M+H]^{3+}$ Calcd 1427.18264, founded 1427.18257.

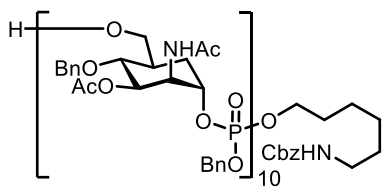
Nonamer 15



Alcohol **14** (0.014 mmol, 0.06 g), was coupled to phosphoramidite **17** (0.245 mL 0.2 M in ACN, 0.049 mmol), oxidized, detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/MeOH) and size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1) leading to product **15** (0.011 mmol, 0.052 g) in 77% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ: 7.57 – 7.05 (m, 95H, CH_{arom}), 6.90 (s, 1H, NH), 5.92 – 5.78 (m, 1H, NH), 5.18 – 4.92 (m, 29H, H-3 x9, CH_2Bn x9, CH_2Cbz), 4.80 – 4.41 (m, 36H, H-1 x9, H-2 x9,

CH_2BnP x9), 4.41 – 4.19 (m, 8H, H-6 x4), 4.12 – 3.51 (m, 21H, CH_2 Linker, H-4 x9, H-6 x5), 3.08 – 3.00 (m, 2H, CH_2 Linker), 2.19 – 1.64 (m, 81H, NHAc x9, OAc x9, H-5 x9, H-5a x9), 1.58 (m, 2H, CH_2 Linker), 1.48 – 1.35 (m, 2H, CH_2 Linker), 1.35 – 1.16 (m, 4H, CH_2 Linker x2). **^{13}C NMR** (126 MHz, CD_3CN) δ: 172.0, 171.9, 171.3, 171.3, 171.1, 171.0, 170.9, 170.9, 170.9, 139.8, 139.7, 139.6, 139.6, 137.1, 129.6, 129.6, 129.5, 129.5, 129.5, 129.4, 129.4, 129.3, 129.2, 129.2, 129.1, 129.1, 129.0, 128.9, 128.9, 128.8, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 76.3, 75.7, 75.5, 75.4, 75.2, 75.1, 75.0, 74.8, 73.9, 73.9, 73.8, 73.8, 70.4, 70.3, 70.3, 70.1, 70.0, 70.0, 68.9, 68.9, 68.0, 66.5, 62.2, 52.0, 51.7, 51.5, 51.5, 41.3, 40.0, 38.2, 30.7, 30.7, 30.3, 30.3, 29.2, 26.7, 25.7, 25.7, 25.7, 23.2, 23.2, 23.1, 23.0, 21.3, 21.2, 21.2, 21.2. **^{31}P NMR** (202 MHz, CD_3CN) δ: -1.32, -1.36, -1.40, -1.43, -1.47, -1.51, -1.57, -2.00, -2.07, -2.12, -2.16, -2.25, -2.28, -2.31, -2.34, -2.38. **HRMS** *m/z*: $[M+3H]^{3+}$ Calcd 1594.90627, founded 1594.90621.

Decamer 16



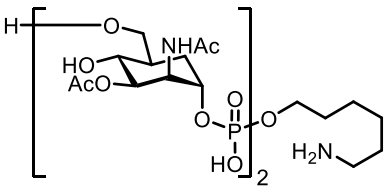
Alcohol **15** (0.010 mmol, 0.05 g), was coupled to phosphoramidite **17** (0.1 mL 0.2 M in ACN, 0.02 mmol), oxidized, detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/MeOH) and size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1) leading to product **16** (0.009 mmol, 0.051 g) in 90% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ: 7.59 – 7.04 (m, 105H, CH_{arom}), 6.86 (s, 1H, NH), 5.86 (s, 1H, NH), 5.20 – 4.93 (m, 32H, H-3 x10, CH_2Bn x10, CH_2Cbz), 4.80 – 4.40 (m, 40H, H-1 x10, H-2 x10, CH_2BnP x10), 4.40 – 4.18 (m, 8H, H-6 x4), 4.11 – 3.51 (m, 24H, CH_2 Linker, H-4 x10, H-6 x6), 2.21 – 1.64 (m, 90H, NHAc x10, OAc x10, H-5 x10, H-5a x10), 1.64 – 1.49 (m, 2H, CH_2 Linker), 1.42 (m, 2H, CH_2 Linker), 1.35 – 1.24 (m, 4H, CH_2 Linker x2). **^{13}C NMR** (126 MHz, CD_3CN) δ: 172.0, 171.9, 171.7, 171.3, 171.2, 171.1, 171.0, 170.9, 170.9, 170.9, 157.3, 139.8, 139.7, 139.6, 139.6, 138.5, 137.1, 129.6, 129.6, 129.6, 129.5, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 129.1, 129.0, 128.9, 128.9, 128.8, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 76.3, 75.7, 75.6, 75.4, 75.2, 75.1, 74.8, 74.0, 73.9, 73.8, 70.4, 70.3, 70.1, 70.0, 70.0, 68.9, 68.9, 68.0, 66.5, 62.4, 62.2, 52.0, 51.7, 51.5, 41.3, 40.2, 40.0, 38.2, 30.7, 30.7, 30.3, 30.3, 29.2, 26.7, 25.7, 25.7, 25.7, 23.2, 23.1, 23.0, 21.2, 21.2, 21.2. **^{31}P NMR** (202 MHz, CD_3CN) δ: -1.29, -1.34, -1.40, -1.48, -1.54, -1.97, -1.99, -2.07, -2.11, -2.15, -2.25, -2.27, -2.30, -2.34, -2.38. **HRMS** *m/z*: $[M+3H]^{3+}$ Calcd 1762.62991, founded 1762.62994.

General procedure B: hydrogenolysis on a typical scale of 2.9-20.0 µmol

Starting alcohol was dissolved in a 0.01% v/v AcOH solution in MilliQ water (2 mL per 10 µmol). Dioxane was added until the starting compound was fully dissolved. The reaction mixture was purged with Argon, followed by the catalytic addition of Pd black. The mixture was purged with Argon and subsequently purged with H_2 and left stirring in a H_2

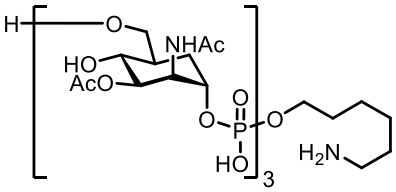
atmosphere for 3 days. The reaction mixture was filtrated over a Whatman® filter and concentrated in *vacuo*. The crude was purified over C-18 column (brand) and lyophilized.

Dimer 1



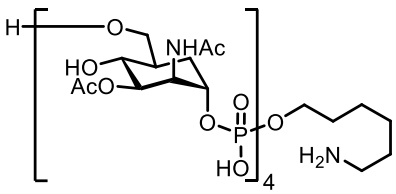
Alcohol **8** (18.0 µmol, 23.0 mg) was deprotected using the general procedure described above leading to product **1** (15.2 µmol, 11.6 mg) in 84% yield. **^{1}H NMR** (500 MHz, D_2O) δ: 5.05 (m, 2H, H-3 x2), 4.57 (s, 2H, H-2 x2), 4.35 (m, 2H, H-1 x2), 4.17 (m, 1H, C*H*H-6), 3.96 – 3.68 (m, 7H, H-6, C*H*H-6, H-4 x2), 2.98 (t, *J* = 7.4 Hz, 2H, CH_2 Linker), 2.15 – 1.69 (m, 18H, NHAc x2, OAc x2, H-5 x2, H-5a x2), 1.65 (q, *J* = 7.4, 6.4 Hz, 4H, CH_2 Linker x2), 1.40 (d, *J* = 5.3 Hz, 4H, CH_2 Linker x2). **^{13}C NMR** (126 MHz, D_2O) δ: ^{13}C NMR (126 MHz, D_2O) δ 174.5, 174.5, 173.5, 173.4, 73.8, 72.1, 72.0, 71.9, 71.9, 67.5, 66.5, 66.4, 66.4, 65.3, 62.5, 61.7, 51.0, 51.0, 50.9, 50.9, 39.4, 38.7, 37.7, 37.6, 29.5, 29.4, 28.1, 26.6, 25.1, 24.4, 21.9, 21.8, 20.4, 20.4. **^{31}P NMR** (202 MHz, D_2O) δ -0.43, -0.55. **MALDI-MS** *m/z*: $[M+Na]^+$ Calcd 830.2225, founded 830.2210.

Trimer 2



Alcohol **9** (16.0 µmol, 29.0 mg) was deprotected using the general procedure described above leading to product **2** (15.0 µmol, 18.0 mg) in 86% yield. **^{1}H NMR** (500 MHz, D_2O) δ: 5.05 (m, 3H, H-3 x3), 4.59 (m, 3H, H-2 x3), 4.37 (m, 3H, H-1 x3), 4.19 (m, 2H, H-6), 3.96 – 3.68 (m, 7H, H-6 x2, H-4 x3), 2.98 (t, *J* = 7.5 Hz, 2H, CH_2 Linker), 2.16 – 1.85 (m, 27H, NHAc x3, OAc x3, H-5 x3, H-5a x3), 1.69 – 1.60 (m, 4H, CH_2 Linker x2), 1.41 (dd, *J* = 7.2, 3.5 Hz, 4H, CH_2 Linker x2). **^{13}C NMR** (126 MHz, D_2O) δ: 174.5, 173.5, 173.4, 73.8, 73.8, 73.7, 72.3, 72.1, 67.5, 66.6, 66.5, 62.6, 61.7, 51.0, 39.4, 38.8, 37.7, 29.5, 29.4, 28.1, 26.6, 25.1, 24.4, 21.9, 21.9, 20.5. **^{31}P NMR** (202 MHz, D_2O) δ -0.54, -0.61, -0.73.

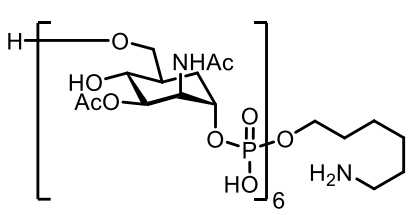
Tetramer 3



Alcohol **10** (11.1 µmol, 26.0 mg) was deprotected using the general procedure described above leading to product **3** (10.0 µmol, 15.8 mg) in 93% yield. **^{1}H NMR** (500 MHz, D_2O) δ: 5.01 – 4.92 (m, 4H, H-3 x4), 4.50 (m, 4H, H-2 x4), 4.28 (s, 4H, H-1 x4), 4.18 – 4.05 (m, 3H, H-6, C*H*H-6), 3.96 – 3.68 (m, 9H, H-6 x2, C*H*H-6, H-4 x4), 2.89 (t, *J* = 7.5 Hz, 2H, CH_2 Linker), 2.11 – 1.61 (m, 36H, NHAc x4, OAc x4, H-5 x4, H-5a x4), 1.57 (q, *J* = 6.8 Hz, 4H, CH_2 Linker x2), 1.37 – 1.29 (m, 4H, CH_2 Linker x2). **^{13}C**

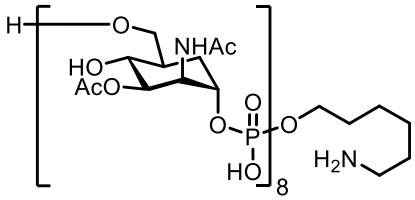
NMR (126 MHz, D_2O) δ: 174.5, 173.6, 173.4, 73.8, 73.7, 72.2, 72.1, 69.2, 67.5, 66.6, 66.6, 66.5, 65.4, 62.6, 62.4, 61.7, 51.0, 39.4, 38.8, 37.7, 29.5, 29.4, 28.1, 26.6, 25.1, 24.5, 21.9, 20.5, 20.4. **^{31}P NMR** (202 MHz, D_2O) δ -0.49, -0.56, -0.59, -0.69. **MALDI-MS** *m/z*: $[M+Na]^+$ Calcd 1520.3404, founded 1520.3399.

Hexamer 4



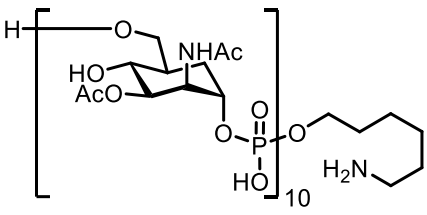
Alcohol **12** (2.9 µmol, 9.00 mg) was deprotected using the general procedure described above leading to product **4** (2.8 µmol, 6.0 mg) in 97% yield. **^{1}H NMR** (500 MHz, D_2O) δ: 4.98 (m, 6H, H-3 x6), 4.57 – 4.47 (m, 6H, H-2 x6), 4.44 – 4.23 (m, 6H, H-1 x6), 4.13 (m, 6H, H-6 x3), 3.92 – 3.52 (m, 24H, H-6 x3, H-4 x6), 2.91 (t, *J* = 7.5 Hz, 2H, CH_2 Linker), 2.14 – 1.64 (m, 54H, NHAc x6, OAc x6, H-5 x6, H-5a x6), 1.58 (q, *J* = 8.0, 7.0 Hz, 4H, CH_2 Linker x2), 1.33 (dd, *J* = 7.3, 4.0 Hz, 4H, CH_2 Linker x2). **^{13}C NMR** (126 MHz, D_2O) δ: 174.5, 173.4, 73.9, 71.6, 67.6, 66.6, 62.6, 61.7, 60.5, 39.5, 37.7, 29.4, 26.6, 25.2, 24.5, 21.9, 20.5. **^{31}P NMR** (202 MHz, D_2O) δ -0.42, -0.47, -0.51, -0.58. **MALDI-MS** *m/z*: $[M+Na]^+$ Calcd 2210.4582, founded 2210.4655.

Octamer 5



Alcohol **14** (2.9 µmol, 9.00 mg) was deprotected using the general procedure described above leading to product **5** (2.8 µmol, 6.0 mg) in 96% yield. **^{1}H NMR** (500 MHz, D_2O) δ: 5.06 (d, *J* = 14.1 Hz, 8H), 4.64 – 4.55 (m, 7H), 4.43 (d, *J* = 40.5 Hz, 12H), 4.22 (q, *J* = 5.1, 4.7 Hz, 8H), 3.94 (d, *J* = 9.0 Hz, 13H), 3.88 – 3.59 (m, 17H), 2.98 (t, *J* = 7.6 Hz, 3H), 2.22 – 1.71 (m, 82H), 1.66 (s, 5H), 1.45 – 1.37 (m, 5H). **^{13}C NMR** (126 MHz, D_2O) δ: 174.5, 173.4, 73.7, 69.1, 66.5, 65.6, 62.6, 50.9, 39.4, 37.6, 26.6, 25.1, 21.9, 20.5. **^{31}P NMR** (202 MHz, D_2O) δ: 0.85, 0.76, 0.71, 0.61. **MALDI-MS** *m/z*: $[M+Na]^+$ Calcd 2900.5762, founded 2900.5766.

Decamer 6



Alcohol **16** (9.4 µmol, 50.0 mg) was deprotected using the general procedure described above leading to product **6** (9.3 µmol, 15,6 mg) in 99% yield. **^{1}H NMR** (600 MHz, D_2O) δ: 5.15 – 4.96 (m, 10H), 4.62 – 4.39 (m, 10H), 4.39 – 4.07 (m, 21H), 4.00 – 3.77 (m, 21H), 3.77 – 3.49 (m, 5H), 2.97 (t, *J* = 7.5 Hz, 2H), 2.26 (s, 2H), 2.19 – 1.76 (m, 81H), 1.75 – 1.58 (m, 6H), 1.45 – 1.34 (m, 4H). **^{13}C NMR**

(151 MHz, D_2O) δ: 175.8, 175.5, 175.4, 174.5, 174.3, 74.8, 73.3, 72.9, 71.3, 71.2, 70.9, 69.9, 69.4, 67.4, 67.3, 67.1, 66.5, 66.3, 66.0, 54.5, 51.9, 49.8, 40.3, 38.5, 37.2, 36.7, 30.4, 29.2, 29.1, 27.5, 26.1, 25.4, 23.0, 22.8, 21.5, 21.4. **^{31}P NMR** (202 MHz, D_2O) δ: -0.19, -0.23, -0.26, -0.30, -0.34, -0.39, -0.44, -0.46, -0.64, -0.67, -0.72, -0.78, -0.98. **MALDI-MS** $[M+Na]^+$ Calcd 3371.8779, founded 3371.8247.

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