**Bacterial glycomimetics: synthesis and applications**
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# 5

# The development of a new tandem ring-closing metathesis linker system for solid phase synthesis

## Introduction

Since 1963, when R. B. Merrifield first introduced the concept of solid phase synthesis to generate short peptide fragments, (automated) solid phase synthesis has been widely explored for various applications.[1] The idea was simple (Figure 1), yet ingenious: the initial synthon is covalently bound to a solid support (an insoluble polymer which is stable towards solvents and reagents needed for the elongation process) and liquid phase containing the subsequent synthon together with reagents to promote the reaction between the first and second building block, is then added. After the reaction, the excess of reagents and activating can be washed away. An on-resin deprotection step then prepares the new terminal synthon for the next reaction. Because the reagents are easily removed a relatively large excess can be used to drive the reactions to completion and speed up the assembly process. Once the desired length of the target oligomer is reached the generated fragment can be cleaved off the solid support delivering the target product.[2, 3]

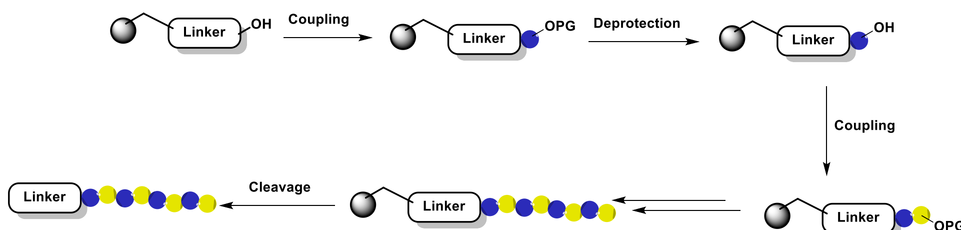



*Figure 1. Solid phase synthesis.*

At first, this technique was applied in peptide chemistry to streamline the synthesis of oligopeptides with the first commercial automatic peptide synthesizer launched in the

early 1980's. This was followed by the introduction of the first commercial oligonucleotide synthesizer in the late 80's and a prototype carbohydrate synthesizer in the 2000's.[4, 5, 6] The advent of these machines has made the production possible of molecules that were considered beyond reach, because of their large structure, opening many possibilities to investigate the activity and properties of these compounds.[7, 8]

Automated peptide and oligonucleotide synthesis technologies have been broadly implemented, with carbohydrate synthesis lagging behind, because of the complexity of the required building blocks as well as the intricacies associated with forging glycosidic linkages. However, research continues to further optimize yields and reduce the amount of building block waist. A big role is played by the linker anchoring the first synthon to the solid support. This entity, that can be regarded as an extra protecting group, has to be stable under all reaction conditions used yet be easily cleaved from polymeric support at the end of the synthesis. The most commonly used type of linkers in automated peptide and nucleotide synthesis are either acid or base labile. In the assembly of oligosaccharides most often base labile protecting groups are used in combination with (Lewis) acidic glycosylation conditions, precluding the use of base or acid labile linker systems. Therefore, other linker systems have been developed, including linker systems that are photo cleavable. Although these are typically stable under most reaction conditions, they require specific set-ups for efficient cleavage. Metathesis cleavable linkers have also been introduced as the alkene functionalities in these linkers are stable under most reaction conditions, but they can be addressed selectively by metathesis catalysts.[9, 10, 11, 12, 13]

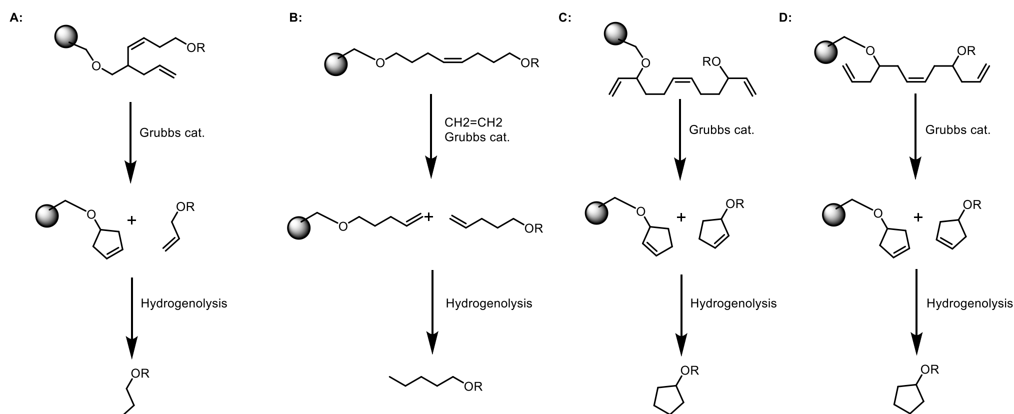



*Figure 2. Metathesis cleavable linkers in (automated) oligosaccharide synthesis.*

The first example of a metathesis labile-linker system that was applied in solid phase oligosaccharide synthesis was reported by the group of Schmidt (see Figure 2A), delivering the 1-*O*-allyl derivatives of the released glycans after treatment with Grubbs Catalyst. As the catalyst is bound to the cleaved substrate, cross metathesis with the next

resin bound substrate it challenging. Another linker system has been described by Seeberger and co-workers (Figure 2B), who foresaw the use of ethylene together with Grubbs catalyst to cleave the products from an octenediol functionalized resin, resulting in the release of pentenyl glycosides.[13, 14] Not long after two tandem ring-closing metathesis (RCM) linkers were developed (Figure 2C, D) by Timmer *et al.* and De Jong *et al.*. These systems release the unbound and active catalyst back in solution for the next cleavage reaction and deliver a the released glycans with an anomeric cyclopentene moiety. While the first RCM system provides the product with a chiral cyclopentenol, complicating characterisation and analysis, the latter provides the products with a symmetrical cyclopentenol group.[15, 16] A major drawback of these linkers is that the cycloalkene needs to be functionalized before global deprotection of the oligosaccharide as hydrogenolysis, typically used as one of the last synthetic steps to remove the protecting groups, also reduces the double bond in the anomeric functionality.

This chapter is focused on the design and development of a new tandem RCM linker system that can be used on different automated synthesizers, that is stable under most reaction conditions, that can be cleaved efficiently and chemoselectively and that delivers, after hydrogenolysis, a C6 amino functionalized linker (Figure 3). Thus, the linker that has been designed contains, besides the double RCM-cleavable tri-ene part developed in the preceding linker systems, a dual benzyl alcohol spacer, which connects the tri-ene part to the aminohexanol spacer through a carbamate linkage. The resulting benzyloxycarbamate may be regarded as a functionalized version of the often employed benzyloxycarbamate (the “Z” or “Cbz” group).

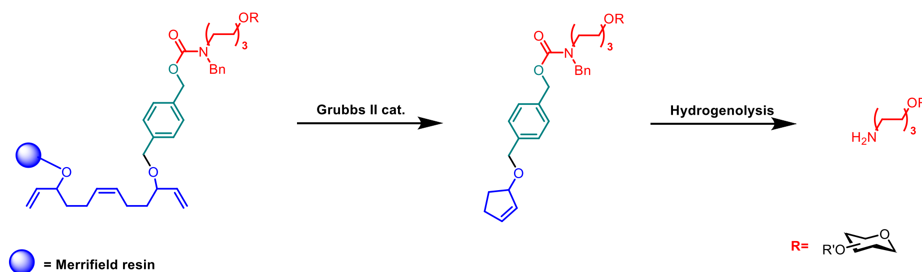


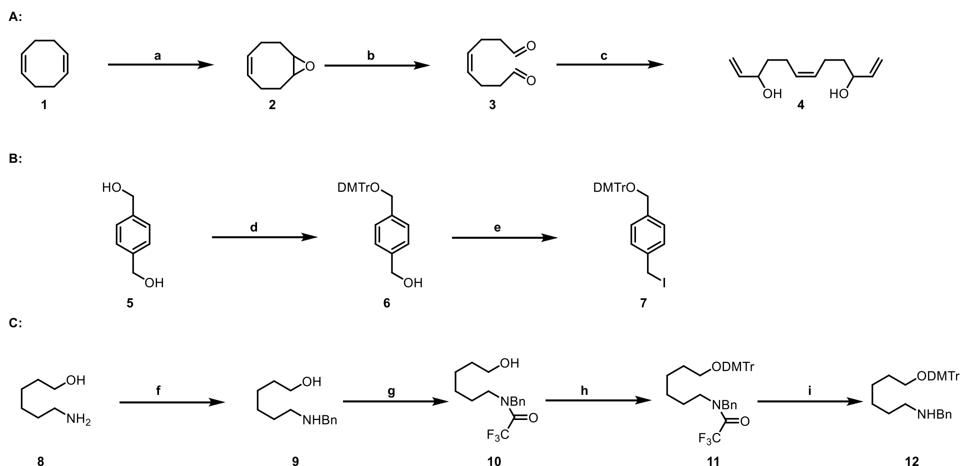

*Figure 3. The Structure and cleavage procedure of the new ring-closing metathesis linker system.*

## Results and discussion

The preparation of the third generation tandem RCM linker started with the synthesis of the key components **4**, **7** and **12** (Scheme 1). Triene **4** was obtained from cycloocta-1,5-diene (**1**) following a well-established route, comprising the selective oxidation to provide the monoepoxide **2**, oxidative opening of the epoxide to form the di-aldehyde **3** and finally a double Grignard reaction to give diol **4** in 30% over three steps.[15] Compound **7** was generated in two steps by treating a large excess of diol **5** with DMTrCl to attain

mono-protected alcohol **6**, followed by iodination using $I_2$, triphenylphosphine and imidazole in DCM to yield **7** in 80% yield. Lastly, spacer building block **12** was generated from 6-amino-1-hexanol **8** starting with double protection of the amine, with a benzyl group through a reductive amination using benzaldehyde and $NaBH_4$ (95% yield) and subsequently, with a trifluoroacetyl group using trifluoroacetic anhydride (TFAA) and triethyl amine (TEA) yielding **10** in 65%. The alcohol in **10** was tritylated using DMTrCl in presence of TEA quantitively forming **11**, which was subjected to basic conditions to give spacer **12** in 86% yield. [17]

The assembly of the linker **16** started by reacting tri-ene **4** with a large excess of **7** in presence of 1 eq of

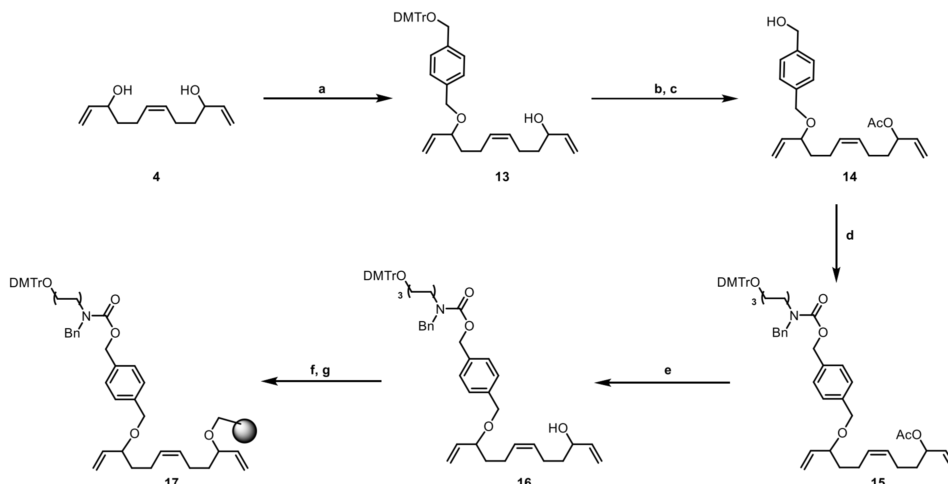



*Scheme 1. A synthesis of building block **4**: a) $K_2HPO_4$, m-CPBA, DCM/$H_2O$, 0°C-rt b) Periodic acid 0.8M in $H_2O$, Dioxane 0°C-rt c) VinylMgBr 1M sol. in $Et_2O$, THF, 30% over 3 steps. B synthesis of building block 7: d) DMTrCl, DCM/Pyr, quant. e) Imidazole, $I_2$, DCM, 80% C synthesis of building block **12**: f) Benzaldehyde, $NaBH_4$, MeOH, 95% g) TEA, TFAA, DCM 65%, h) TEA, DMTrCl, DCM, 0°C-rt, quant. i) $K_2CO_3$, MeOH, $H_2O$, 86%*

sodium hydride (NaH) to minimize the formation of the double substituted product, delivering **13** in 45% yield (Scheme 2). Alcohol **13** was acetylated and treated with a 3% TCA solution in DCM to remove the DMTr group yielding **14** in 71% over 2 steps.

To install the carbamate moiety, alcohol **14** was treated with *p*-nitrophenyl chloroformate in presence of TEA and the labile intermediate carbonate was subsequently reacted with amine **12**, yielding **15** in 72%. The selective removal of the acetyl group in **15** was achieved with a catalytic amount of NaOMe followed by neutralization using dry ice giving the linker **16** in quantitative yield.

Merrifield resin (loading 1.0 mmol/g) was functionalized with alcohol **16** by reaction of the chloromethyl groups on the resin using KOtBu, 18-crown-6 and tetrabutylammonium triflate (TBAOTf) in THF, while the mixture was slowly swirled for 24h at 40°C.

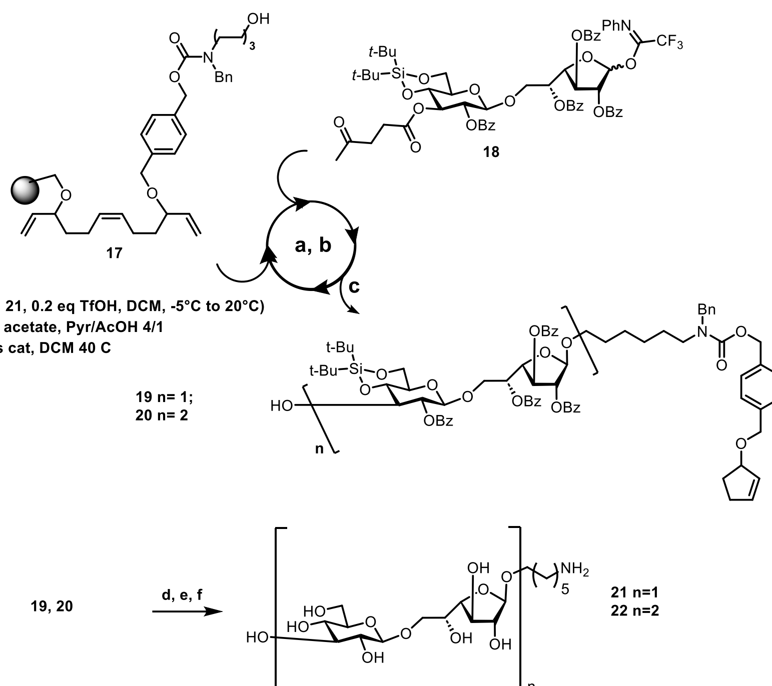



*Scheme 2. a) 7, NaH, THF/DMF, 0°C-rt, 45%, b) $Ac_2O$, pyr, c) 3% TCA in DCM, DCM 71% over 2 steps, d) p-Nitrophenylchloroformate, TEA, **12**, 45°C, DCE, 72%, e) NaOMe, MeOH/DCM, Dry ice, quant. f) Merrifield resin 1 mmol/g loading, KOtBu, 18-crown-6, TBAOTf, THF, 40°C, g) KOMe 0.31 mmol/g loading*

Next the unreacted chloromethyl groups were capped using a large excess potassium methoxide (KOMe). The loading of resin **17** was determined by quantification of the absorbance of the DMTr cation after treatment with a 3% TCA solution in DCM resulting indicating an average loading of 0.31 mmol/g.

At this stage the appropriateness of functionalized resin **17** for carbohydrate solid phase synthesis was explored by the assembly of two diheteroglycan (DHG) saccharides, mimicking the native DHG structure of *E. faecalis*. Chapter 4 describes not only the solution phase synthesis but also a solid phase synthesis, using a polystyrene resin that is functionalized with a base labile linker. With the aid of the fully automated Glyconeer® oligosaccharide synthesizer, the same *N*-phenyl trifluoroacetimidate dimer **18** was applied as donor together with the same elongation cycle entailing; (i) three times repetition of the coupling step, each time using 3 eq donor **18** with 0.2 eq triflic acid, during 1h at -5 to 20℃, (ii) three times repetition of treatment of the resin with 9 eq of hydrazine acetate at 40℃ for 15 min to remove the Lev protecting group. Excecution of one elongation cycle gave immobilized dimer and two cycles led to the immobilized tetramer. Finally, both resin**s** were removed from the Glyconeer machine and suspended in DCE, after which a catlytic amount of the second generation Grubbs catalyst was added to promote the tandem RCM cleavage. The suspensions were swirled overnight at 40℃ to give, after filtration, purification by flash column chromatography DHG dimer **19** and tetramer **20** in 72% and 51% yield, respectively, based on the initial loading of the resin.

Removal of the remaining protecting groups in dimer **19** and tetramer **20** was achieved by the following sequence of reactions; desilylation using $Et_3N{\bullet}3HF$, transesterification of benzoates with a solution of NaOMe in MeOH and final hydrogenolysis to obtain target DHG dimer **21** and tetramer **22**, provided with an amine for further functionalization in 83% and 43% yield, respectively.




*Scheme 3. Solid-phase synthesis of Dimer **21** and tetramer **22**. a) Coupling, 3 x (3 eq **18**, 0.2 eq TfOH, DCM, -5°C to r.t.; b) Deblock, $H_2NNH_2$ acetate, Pyr/AcOH (4:1), 40°C; c) Second Generation Grubbs Cat, DCE, 40°C, 74% (**19**) 51% (**20**), d) HF•Pyr, Pyr, e) NaOMe, MeOH, f) Pd black, $H_2$, $H_2O$, AcOH, 83% (**21**), 43% (**22**).*

## Conclusion

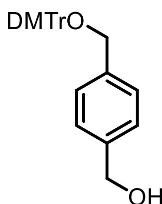
This Chapter deals with the design and synthesis of a new tandem-RCM cleavable linker, amenable for solid phase synthesis. The viability of this linker is illustrated by the synthesis of a DHG dimer and tetramer using an automated oligosaccharide synthesizer. The RCM mediated cleavage to furnish the protected dimeric and tetrameric precursors, as the first event of the isolation procedure, proved to be efficient, while the subsequent purification and NMR analysis showed the quality of these protected oligosaccharides having an unaffected linker, thereby showing the desired stability towards reaction conditions of the solid phase synthesis. Removal of the remaining protecting groups proceeded uneventful indicating that this new linker system is a valuable asset in further streamlining automated synthesis as not only the purification procedure is facilitated but

also oligosaccharides provided with an amine for further functionalization are obtained. Further application of the linker system will be required to illustrate its utility in the (automated) solid phase synthesis of various biopolymers, including oligosaccharides and teichoic acids.

## Experimental part

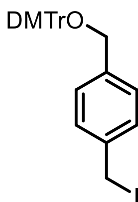
**General procedures and materials:** All chemicals (Acros, Biosolve, Sigma-Aldrich, TCI, etc) were used as received and all reactions were effectuated under an argon atmosphere, at ambient temperature (22°C), unless stated otherwise. For the TLC analysis were used aluminium sheets (Merck, TLC silica gel 60 $F_{254}$), sprayed with a solution of $H_2SO_4$ (20%) in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}$•$4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4$•$2H_2O$ (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 ($^1$H, $^{13}$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 (source 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.

### (4-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)phenyl)methanol 6




Compound **5** (150 mmol, 20.72 g) was dissolved in a 1:1 mixture of pyridine/DCM (300.0 mL). To the reaction was added DMTrCl (30.0 mmol, 10.16 g). After the DMTrCl was fully converted, the mixture was diluted with DCM (100.0 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/Pentane), providing **6** (29.5 mmol, 13.0 g) in 98% yield. **$^1$H NMR** (400 MHz, $CD_3CN$) δ = 7.56 – 7.18 (m, 13H, aromatics), 6.89 – 6.83 (m, 4H, aromatics), 4.57 (s, 2H, $CH_2Bn$), 4.09 (s, 2H, $CH_2Bn$), 3.75 (s, 6H, 2x OMe), 3.21 (s, 1H, OH). **$^{13}$C NMR** (126 MHz, $CD_3CN$) δ = 159.6, 159.4, 146.2, 136.7, 130.9, 129.9, 128.9, 128.8, 128.5, 128.1, 127.7, 127.6, 114.0, 113.8, 66.2, 64.4, 55.8. **HRMS** *m/z*: $[M+Na]^+$ Calcd 463.18798; found 463.18798.

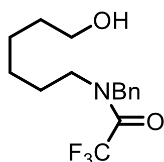
### 4,4'-(((4-(iodomethyl)benzyl)oxy)(phenyl)methylene)bis(methoxybenzene) 7




Compound **6** (0.49 mmol, 0.217 g) was co-evaporated 3 times with toluene and dissolved in dry DCM. The solution was cooled to 0°C. To the reaction mixture was added imidazole (0.98 mmol, 0.066 g), $PPh_3$ (0.49 mmol, 0.128 g) and $I_2$ (0.49 mmol, 0.124 g). After 1 h the reaction was diluted with DCM, washed with a sat. solution of $Na_2S_2O_3$ and once with brine.; The organic layer was dried over $Na_2SO_4$, filtered and

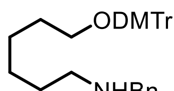
concentrated in *vacuo*. The crude was used in the following step without further purification.

**_N_-benzyl-2,2,2-trifluoro-_N_-(6-hydroxyhexyl)acetamide 10**



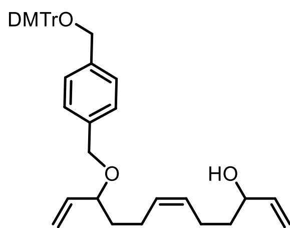

Compound **9** (18.29 mmol, 3.79 g) was dissolved in DCM (180,0 mL). To the mixture was added TEA (54.87 mmol, 7.65 mL). The reaction was cooled to 0 °C and TFAA (18.29 mmol, 2.55 mL) was added. The mixture was allowed to warm up to r.t. and was stirred until complete conversion of the starting material. The reaction was diluted with DCM (50.0 mL), 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 **10** (11.88 mmol, 3.60 g) in 65% yield. **$^1$H NMR** (400 MHz, $CDCl_3$) δ = 7.43 – 7.12 (m, 5H, aromatics), 4.64 (m, 2H, $CH_2$Bn), 3.62 (m, 2H, $OCH_2$), 3.30 (m, 2H, $NCH_2$), 1.70 – 1.45 (m, 4H, 2x $CH_2$), 1.41 – 1.19 (m, 4H, 2x $CH_2$). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 135.7, 135.1, 129.1, 129.0, 128.4, 128.4, 128.1, 128.1, 127.4, 77.5, 77.2, 76.8, 62.8, 62.8, 50.7, 50.7, 49.3, 46.8, 46.3, 32.6, 28.3, 26.5, 26.5, 26.4, 25.5, 25.4. **HRMS** *m/z*: $[M+H]^+$ Calcd 304.15189; found 304.15189.

**_N_-benzyl-6-(bis(4-methoxyphenyl)(phenyl)methoxy)hexan-1-amine 12**




Compound **10** (9.14 mmol, 2.77 g) was dissolved in DCM (100.0 mL). The solution was cooled to 0 °C. To the reaction was added DMTrCl (30.0 mmol, 10.16 g) and TEA (13.71 mmol, 1.83 mL). After the starting material was fully converted, to the mixture was added $H_2O$ (≃1.0 mL). The solution 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 9:1 mixture of MeOH/DCM (100.0 mL). To the reaction was added $K_2CO_3$ (27.42 mmol, 3.78 g) and $H_2O$ (14.0 mL). The mixture was left stirring overnight at 40°C. After the starting material was fully converted the mixture was concentrated at reduced pressure, dissolved in EtOAc and washed with $H_2O$. The water layer was extracted 3 times with EtOAc. The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated in *vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane), providing **11** (7.86 mmol, 4.0 g) in 86% yield. **$^1$H NMR** (400 MHz, $CD_3CN$) δ = 7.48 – 7.11 (m, 14H, aromatics), 6.90 – 6.80 (m, 4H, aromatics), 3.73 (m, 8H, 2x OMe, $CH_2$Bn), 2.99 (t, *J* = 6.5 Hz, 2H, $NHCH_2$), 2.50 (t, *J* = 7.1 Hz, 2H, $OCH_2$), 1.55 (m, 2H, $CH_2$), 1.49 – 1.17 (m, 6H, 3x $CH_2$). **$^{13}$C NMR** (101 MHz, $CD_3CN$) δ = 159.5, 146.6, 142.4, 137.6, 130.8, 129.9, 129.2, 129.1, 128.9, 128.9, 128.7, 127.6, 127.4, 126.2, 118.3, 113.9, 86.4, 64.0, 55.8, 54.4, 49.9, 30.7, 30.6, 27.8, 26.9. **HRMS** *m/z*: $[M+H]^+$ Calcd 510.30027; found 510.30027.

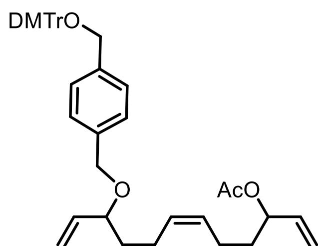
**(*Z*)-10-((4-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)benzyl)oxy)dodeca-1,6,11-trien-3-ol 13**



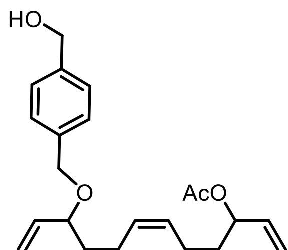

Starting diol **4** (0.5 mmol, 0.098 g) was co-evaporated 3 times with toluene, dissolved in dry DMF (5.0 mL) and cooled to 0°C. To the mixture was added NaH (0.588 mmol, 0.023 g) and a 0.2 M solution of **7** (2.5 mL) in THF dropwise. After full conversion of **4**, to the reaction mixture was added $H_2O$ (1.0 mL) and $Et_2O$ (25.0 mL). The mixture was washed 5 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 **13** (0.196 mmol, 0.121 g) in 45% yield over 2 steps. **$^1$H NMR** (400 MHz, $CD_3CN$) δ = 7.52 – 7.44 (m, 2H, aromatics), 7.41 – 7.14 (m, 11H, aromatics), 6.90 – 6.80 (m, 4H, aromatics), 5.89 – 5.69 (m, 2H, H2, H11), 5.43 – 5.32 (m, 2H, H6, H7), 5.27 – 4.97 (m, 4H, H1, H12), 4.53 (m, 1H, H13), 4.31 (m, 1H, H13), 4.10 (s, 2H, H14), 4.01 – 3.91 (m, 1H, H10), 3.75 (s, 7H, 2x OMe, H3), 2.14 – 2.01 (m, 4H, H5, H8), 1.70 – 1.38 (m, 4H, H4, H9). **$^{13}$C NMR** (101 MHz, $CD_3CN$) δ = 159.6, 146.3, 143.0, 140.1, 139.1, 139.1, 137.1, 130.9, 130.6, 130.4, 128.9, 128.8, 128.7, 128.6, 128.0, 127.8, 117.4, 114.1, 114.0, 87.2, 80.8, 72.5, 70.5, 66.2, 55.8, 37.9, 36.2, 23.9, 23.8. **HRMS** *m/z*: $[M+Na]^+$ Calcd 641.32375; found 641.32375.

**(*Z*)-10-((4-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)benzyl)oxy)dodeca-1,6,11-trien-3-yl acetate 13a**

Compound **13** (0.54 mmol, 0.334 g) was dissolved in pyridine (2.0 mL). To the solution was added $Ac_2O$ (5.4 mmol, 0.51 mL). After complete conversion of the starting material $H_2O$ was added and the reaction mixture was diluted with DCM and washed with a sat. solution of $CuSO_4$ (3x), 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 **13a** (0.54 mmol, 0.357 g) in 98% yield. **$^1$H NMR** (500 MHz, $CD_3CN$) δ = 7.52 – 7.17 (m, 13H, aromatics), 6.92 – 6.82 (m, 4H, aromatics), 5.84 – 5.69 (m, 2H, H2, H11), 5.45 – 5.29 (m, 2H, H6, H7), 5.28 – 5.07 (m, 5H, H1, H12, H3), 4.53 (m, 1H, H13), 4.31 (m, 1H, H13), 4.11 (s, 2H, H14), 3.76 (s, 7H, 2x OMe, H10), 2.13 – 2.01 (m, 4H, H5, H8), 1.97 – 1.95 (m, 3H, OAc), 1.69 – 1.45 (m, 4H, H4, H9). **$^{13}$C NMR** (126 MHz, $CD_3CN$) δ = 170.9, 159.6, 146.3, 140.1, 140.1, 139.2, 139.1, 137.9, 137.9, 137.1, 131.0, 131.0, 130.9, 129.8, 128.9, 128.8, 128.7, 128.7, 128.0, 127.8, 117.5, 117.4, 116.6, 116.5, 114.0, 87.2, 80.7, 74.7, 74.6, 70.6, 70.5, 66.2, 55.8, 36.2, 34.8, 34.8, 23.8, 23.8, 23.6, 23.6, 21.3. **HRMS** *m/z*: $[M+Na]^+$ Calcd 683.33431; found 683.33431.

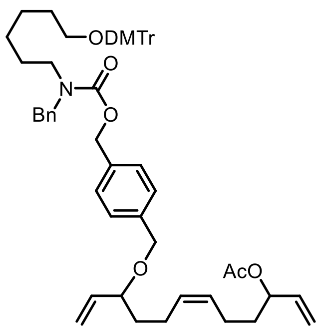


**(*Z*)-10-((4-(hydroxymethyl)benzyl)oxy)dodeca-1,6,11-trien-3-yl acetate 14**




Compound **13a** (4.84 mmol, 3.19 g) was dissolved in DCM (14.0 mL). To the mixture was added a 3% w/v solution of TCA in DCM (24.21 mmol, 134.5 mL). After complete conversion of the starting material a 1:1 mixture of MeOH/$H_2O$ (20.0 mL) was added. The organic layer was washed 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 **14** (3.48 mmol, 1.25 g) in 72% yield. **$^1$H NMR** (400 MHz, $CDCl_3$) δ = 7.34 (s, 4H, aromatics), 5.82 – 5.67 (m, 2H, H2, H11), 5.43 – 5.28 (m, 2H, H6, H7), 5.28 – 5.10 (m, 5H, H1, H12, H3), 4.68 (s, 2H, H14), 4.59 (m, 1H, H13), 4.33 (m, 1H, H13), 3.73 (m, 1H, H10), 2.16 – 1.94 (m, 7H, H5, H8, OAc), 1.89 – 1.47 (m, 4H, H4, H9). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 170.5, 140.3, 139.0, 138.4, 136.5, 130.3, 130.2, 129.0, 128.9, 128.0, 128.0, 127.2, 117.4, 116.9, 116.9, 80.2, 80.2, 74.5, 70.0, 65.3, 35.5, 34.2, 34.2, 23.3, 23.1, 23.1, 21.4. **HRMS** *m/z*: $[M+Na]^+$ Calcd 381.20363; found 381.20363.

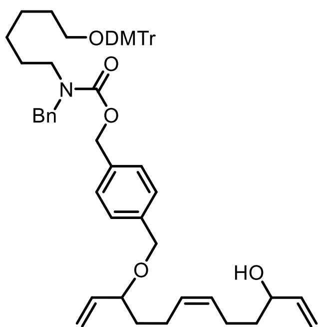
**(*Z*)-10-((4-(((benzyl(6-(bis(4-methoxyphenyl)(phenyl)methoxy)hexyl)carbamoyl)oxy)methyl)benzyl)oxy)dodeca-1,6,11-trien-3-yl acetate 15**




Compound **14** (3.09 mmol, 1.11 g) was co-evaporated 3 times with toluene and dissolved in dry DCE (31.0 mL). To the solution was added TEA (12.36 mmol, 1.72 mL). The reaction mixture was cooled to 0°C and *p*-nitrophenylchloroformate (4.63 mmol, 0.934 g) was added. The reaction left stirring overnight and after complete conversion of the starting alcohol a 0.3 M DCE solution of compound **12** (4.02 mmol, 13.4 mL) was added dropwise. The reaction mixture was heated up to 47°C and stirred for 72h. To the mixture was added MeOH (5.0 mL) and concentrated *in vacuo*. The crude was purified by flash chromatography (EtOAc/Pentane) providing **15** (2.19 mmol, 1.96 g) in 71% yield. **$^1$H NMR** (400 MHz, $CD_3CN$) δ = 7.47 – 7.08 (m, 18H, aromatics), 6.90 – 6.79 (m, 5H, aromatics), 5.85 – 5.67 (m, 2H, H2, H11), 5.45 – 5.28 (m, 2H, H6, H7), 5.28 – 5.05 (m, 7H, H1, H12, H3, $CH_2Bn$), 4.57 – 4.47 (m, 1H, H13), 4.44 (m, 2H, H14), 4.28 (m, 1H, H13), 3.74 (s, 7H, 2x OMe, H10), 3.18 (s, 2H, H20), 2.95 (s, 2H, H15), 2.12 – 2.00 (m, 4H, H5, H8), 1.97 (s, 3H, OAc), 1.71 – 1.06 (m, 12H, H4, H9, H16, H17, H18, H19). **$^{13}$C NMR** (101 MHz, $CD_3CN$) δ 159.5, 146.6, 140.1, 139.6, 137.9, 137.5, 131.0, 130.8, 129.9, 129.8, 129.4, 129.2, 129.1,

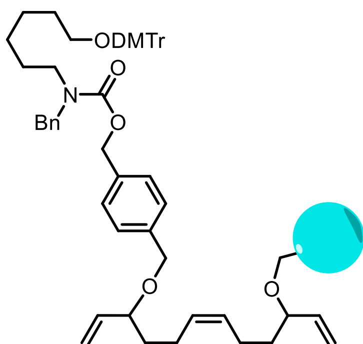
128.9, 128.7, 128.6, 128.4, 128.0, 127.6, 126.2, 117.5, 116.5, 113.9, 86.4, 80.8, 74.6, 70.4, 67.4, 63.8, 55.8, 53.3, 36.2, 34.8, 30.4, 27.1, 23.8, 23.6, 21.4. **HRMS** *m/z*: $[M+H]^+$ Calcd 894.49394; found 894.49394.

**(*Z*)-4-(((10-hydroxydodeca-1,6,11-trien-3-yl)oxy)methyl)benzyl benzyl(6-(bis(4-methoxyphenyl)(phenyl)methoxy)hexyl)carbamate 16**




Compound **15** (2.0 mmol, 1.79 g) was dissolved in a 1:1 mixture of DCM/MeOH (20.0 mL). A 5.4 M solution of NaOMe in MeOH (0.2 mmol, 0.037 mL) was added. After complete conversion of the starting material the reaction mixture was neutralized to pH 7 by adding dry ice. The mixture was concentrated *in vacuo* and purified by flash chromatography (EtOAc/Pentane), providing **16** (1.98 mmol, 1.68 g) in quant. yield. **$^1$H NMR** (500 MHz, $CD_3CN$) δ = 7.48 – 7.11 (m, 18H, aromatics), 6.88 – 6.78 (m, 4H, aromatics), 5.88 – 5.66 (m, 2H, H2, H11), 5.45 – 5.28 (m, 2H, H6, H7), 5.27 – 4.95 (m, 6H, H1, H12, $CH_2Bn$), 4.50 (m, 1H, H13), 4.44 (s, 2H, H14), 4.29 (m, 1H, H13), 3.96 (m, 1H, H10), 3.75 (s, 7H, H3, 2x OMe), 3.18 (m, 2H, H20), 2.94 (m, 2H, H15), 2.11 – 1.98 (m, 4H, H5, H8), 1.71 – 1.07 (m, 12H, H4, H9, H16, H17, H18, H19). **$^{13}$C NMR** (126 MHz, $CD_3CN$) δ = 159.4, 146.6, 142.9, 140.1, 139.5, 137.5, 130.8, 130.6, 130.4, 129.4, 128.9, 128.7, 128.0, 127.6, 118.3, 117.4, 114.0, 113.8, 80.8, 72.5, 70.4, 55.8, 37.9, 36.2, 23.9, 23.7. **HRMS** *m/z*: $[M+Na]^+$ Calcd 874.46532; found 874.46532.

**Functionalized Merrifield resin 17**




Copound **16** (2.64 mmol, 2.24 g) was co-evaporated 3 times with toluene, dissolved in dry THF (27.0 mL) and cooled to 0°C. To the solution K*t*BuO (2.70 mmol, 0.3 g) was added and the mixture was allowed to reach r.t. over 1h. To the reaction was added Merrifield resin (1.73 mmol, 1.73 g, loading: 1 mmol/g, pre-washed with THF (3x) and $Et_2O$ (1x)), TBAOTf (0.18 mmol, 0.070 g) and 18-crown-6 (0.18 mmol, 0.036 g). The mixture was rotated at the rotavap at 40°C for 24h under nitrogen atmosphere. To the mixture was added KOMe (17.3 mmol, 1.2 g) and rotated for other 24h at 40°C. To the reaction was added $H_2O$ (2.0 mL) and the resin was filtered in a fritted filter glass vessel. Subsequently the resin was washed with MeOH (2x), THF (2x), MeOH/THF 1:1 (2x), MeOH (2x), THF (2x), THF/*i*PrOH 1:1 (2x), DCM (2x) and $Et_2O$ (2x). Loading calculation: DMT functionalized resin **17** (5.1 mg) was added to a 10 mL volumetric flask and treated 10 mL of 3% TCA solution in DCM (w/v). A 1.0 mL aliquot was taken and diluted 100x with the 3% TCA solution. Subsequently the

absorbance at λ= 503 nm was registered and applied in the following equation for the loading calculation (performed in triplo).

$$\frac{[(A_{503})(100mL)]}{76} = mmol\ in\ final\ solution$$

$$\left(\frac{mmol\ in\ final\ solution}{volume\ aliquot}\right) * 10\ mL = mmol\ in\ intial\ solution$$

$$\frac{(0.121)(100\ mL)}{76\ mL/\mu mol} = 0.159\ \mu mol$$

$$\left(\frac{0.000159}{1\ mL}\right) * 10\ mL = 0.00159\ mmol$$

$$\boldsymbol{Loading} = \frac{0.00159}{0.0051} = 0.312\ {}^{mmol}/_{g}$$

A loading of 0.300-0.312 mmol/g was determined. After loading calculation the functionalized resin was treated with a 3% TCA solution in DCM (w/v) (3x) and subsequently washed with MeOH (2x), THF (2x), MeOH/THF 1:1 (2x), MeOH (2x), THF (2x), THF/*i*PrOH 1:1 (2x), DCM (2x) and $Et_2O$ (2x). The resin was dried under reduced pressure and stored at -20°C under inert atmosphere.

**Automated synthesis methods:**

All solvents used in the machine are pre-dried 24h before use on a 4Å ms and are HPLC grade. The activator and the deblock solutions are always freshly prepared before use and are made using pre-dried solvents.

**Method A: agitation of the resin during couplings and washes**

After addition of the appropriate solvent, an Ar flow is applied from the bottom of the reaction vessel, suspending the resin in solution. The flow duration is 15s for the washing steps while for the coupling steps it's of 10s every 30s.

**Method B: swelling of the resin**

Dry resin is inserted in the reaction vessel and washed with DCM (3x) and alternating THF (3x) and DCM(3x).

**Method C: coupling cycle**

The resin is suspended in DCM, the reaction vessel is emptied and the building block solution is added (1.0 mL) followed by flushing the building block line with DCM (0.5 mL) while being agitated. The temperature is set to 0°C while employing method A. A 10 min pause is started, after which the activator solution (0.1 M TfOH solution in DCE, 300 μL)

is added, keeping the temperature below 0°C. The delivery line is flushed with an additional DCM (0.5 mL) to the reaction vessel. Temperature is set to 20°C and method A is applied for 1h, after which the reaction vessel is emptied and the mixture is collected in the fraction collector. The resin is washed with DCM (3x 2.0 mL) and the washes are eluted to the fraction collector.

**Method E: deblock cycle**

The resin is washed with DMF (4x 3.0 mL). The deblock solution is added (0.08 M hydrazine acetate solution in pyridine/AcOH 4:1, 3.0 mL) and the temperature is set to 40°C, followed by a 5 minute incubation applying method A. The temperature is kept at 40°C, after which the solid support is incubated 10 minutes applying protocol A. Then the reaction vessel is emptied and the resin is washed with DMF (3x 3.0 mL) running method A.

**Method F: washing after coupling**

The temperature is set to 20°C. The resin is washed with MeOH (3x 2.0 mL), alternating THF (6x 2.0 mL) and DCM (5x 3.0 mL) while applying method A.

**Method G: washing after deblock**

The temperature is set to 20°C. The resin is washed with DMF (4x 3.0 mL), DCM (4x 3.0 mL), alternatingTHF (6x 3.0 mL), 0.01M AcOH in THF (6x 3.0 mL) and DCM (8x 5 mL).

**Method H: resin isolation**

To the dry resin is added DCM or MeOH (first 2 times DCM and last 2 times MeOH), after which the resin is agitated for 15s. The suspended resin is removed with a glass pipet.

**Method I: cleavage from the resin**

The isolated resin from the synthesizer is cherged in a 10 mL microwave glass vial and dry DCM is added (4.0 mL). The suspention is purged for 10 min with Ar. To the mixture is added Grubbs II cat. (5/10 mg) and the mixture is purged with Ar for other 10 min. The vial is subsequently closed with a cap with septum and wrapped in aluminium foil. The vial is agitated overnight at 40°C followed by removal, filtration and wash (DCM 3x , MeOH 3x) of the resin. The organic solution containing the crude is concentrated *in vacuo* and purified by flash chromatography.

| Method | # Cycles | Description | Time | Temperature | |
|---|---|---|---|---|---|
| **B** | 1 | Swelling of the resin | 30 min | 20°C | |
| **C** | 3 | Coupling: 3eq donor, 1eq TfOH | 60 min | 0→20°C | **Elongation block** |
| **F** | 3 | Wash after coupling | 10 min | 20°C | |
| **E** | 3 | Deblock: 9eq H2NNH2 AcOH | 15 min | 40°C | |
| **G** | 3 | Wash after deblock | 10 min | 20°C | |
| **H** | 1 | Resin isolation | 5 min | r.t. | |
| **I** | 1 | Cleavage from the resin | 12h | r.t. | |

**General procedure A: Automated synthesis of Diheteroglycan fragments**

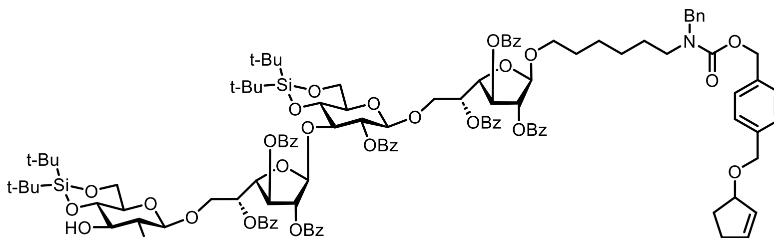
Functionalized resin **17** (0.03 mmol, 0.1 g) was introduced in the reaction vessel followed by method B. The elongation block was repeated ''n'' times where ''n'' is the number of repeating units to instal. Method H was used to isolate the resin and method I to cleave the fragments from the solid support.

**Dimer 19**

Functionalized resin **17** was treated using general procedure **A** providing compound **19** (0.022 mmol, 0.029 g) in 74% yield. **$^1$H NMR** (500 MHz, $CDCl_3$) δ = 8.03 – 7.78 (m, 9H), 7.58 – 7.07 (m, 34H), 6.02 (s, 1H), 5.88 (dq, *J* = 5.7, 2.2 Hz, 1H), 5.71 (ddd, *J* = 7.0, 5.6, 3.5 Hz, 1H), 5.50 – 5.39 (m, 1H), 5.34 (d, *J* = 1.3 Hz, 1H), 5.24 – 5.07 (m, 5H), 4.73 (d, *J* = 7.9 Hz, 1H), 4.65 (ddp, *J* = 7.3, 3.6, 1.2 Hz, 1H), 4.59 – 4.37 (m, 6H), 4.21 – 4.07 (m, 2H), 3.98 (dd, *J* = 10.6, 7.1 Hz, 1H), 3.90 – 3.58 (m, 4H), 3.45 (td, *J* = 9.7, 5.0 Hz, 2H), 3.30 – 3.08 (m, 2H), 2.71 (d, *J* = 46.3 Hz, 1H), 2.60 – 2.37 (m, 2H), 2.31 – 2.08 (m, 3H), 1.88 – 1.78 (m, 1H), 1.57 (d, *J* = 41.0 Hz, 7H), 1.26 (d, *J* = 1.3 Hz, 11H), 1.12 – 0.79 (m, 22H). **$^{13}$C NMR** (126 MHz, $CDCl_3$) δ = 165.7, 165.6, 138.1, 138.0, 135.9, 133.5, 133.4, 133.1, 133.0, 130.9, 130.0, 130.0, 129.9, 129.8, 129.7, 129.6, 129.3, 129.2, 128.7, 128.7, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.0, 127.3, 125.4, 105.4, 101.1, 84.6, 82.3, 80.7, 77.6, 77.4, 77.2, 77.2, 76.9, 75.4, 73.5, 71.1,

70.6, 70.4, 67.4, 67.2, 67.1, 66.2, 50.5, 50.2, 47.3, 46.3, 39.4, 32.1, 31.2, 29.9, 29.8, 29.4, 28.2, 27.8, 27.5, 27.4, 27.1, 27.0, 26.7, 26.0, 22.8, 22.8, 21.6, 20.1. **HRMS** *m/z*: $[M+H]^+$ Calcd 1318.57652; found 1318.57652.

**Tetramer 20**

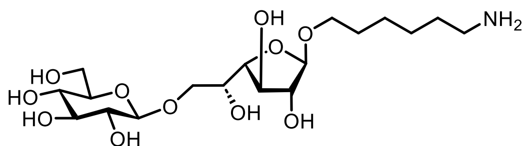



Functionalized resin **17** was treated using general procedure **A** providing compound **19** (0.014 mmol, 0.030 g) in 51% yield. **$^1$H NMR** (500 MHz, $CDCl_3$) δ = 8.12 – 7.58 (m, 9H), 7.58 – 6.89 (m, 40H), 6.02 (s, 1H), 5.88 (dq, *J* = 4.4, 2.1 Hz, 1H), 5.78 (dt, *J* = 7.6, 3.6 Hz, 1H), 5.69 (s, 1H), 5.48 – 5.18 (m, 5H), 5.18 – 5.01 (m, 5H), 4.72 – 4.36 (m, 9H), 4.25 – 3.99 (m, 4H), 3.99 – 3.55 (m, 9H), 3.42 (ddd, *J* = 32.5, 9.6, 5.0 Hz, 3H), 3.21 (dd, *J* = 41.3, 8.3 Hz, 3H), 2.76 – 2.38 (m, 3H), 2.32 – 1.79 (m, 6H), 1.79 – 0.63 (m, 36H). **$^{13}$C NMR** (126 MHz, $CDCl_3$) δ = 165.6, 165.6, 165.4, 164.8, 164.7, 136.0, 133.5, 133.4, 133.0, 132.9, 132.7, 132.6, 130.9, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.6, 129.5, 129.3, 129.2, 129.1, 128.7, 128.5, 128.4, 128.4, 128.2, 128.1, 128.1, 128.0, 127.3, 125.4, 106.4, 105.4, 101.3, 101.0, 84.6, 82.4, 82.2, 82.0, 80.8, 78.7, 77.6, 75.7, 75.4, 73.4, 73.3, 71.5, 71.1, 71.1, 70.5, 70.4, 68.6, 67.4, 67.1, 66.5, 66.2, 32.1, 31.6, 31.2, 30.3, 29.9, 29.8, 29.5, 29.4, 27.5, 27.3, 27.3, 27.1, 26.7, 22.8, 22.8, 22.6, 20.1, 20.0, 14.3. **HRMS** *m/z*: $[M+2H]^{2+}$ Calcd 1100.44989; found 1100.44977.

**General procedure B: Protecting groups removal**

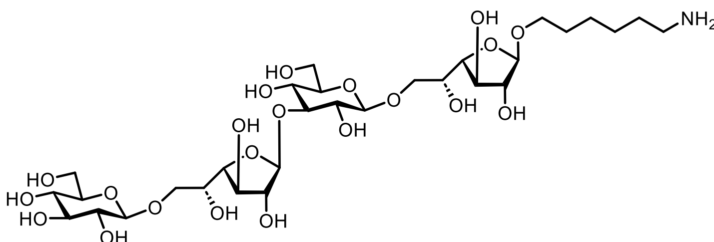
Starting compound was co-evaporated with toluene, cooled down to 0°C and a 0.1 M solution in THF of $Et_3N \bullet 3HF$ (2 eq. per silyl group) was added. The reaction mixture was stirred until full conversion of the starting material (≈ 6h). The solution was diluted with DCM and washed once with a sat. solution of $NaHCO_3$ and once with $H_2O$. The organic layer was dried over $Na_2SO_4$, filtered and concentrated in *vacuo*. The crude dissolved in MeOH (0.01-0.02 M) and a 0.54 M solution in MeOH of NaOMe (0.5 eq.) was added. The reaction mixture was stirred until full conversion of the starting material (≈24 h). To the mixture was added AcOH glacial until pH 7 is reached. The solution was concentrated in *vacuo*. The crude was dissolved in MilliQ $H_2O$ (2.0 mL). To the reaction mixture was added 4-5 drops of glacial AcOH. The mixture was purged with Ar. To the solution was added a scup of Pd black. The reaction mixture was purged with $H_2$ for a few seconds and stirred under $H_2$ atmosphere for ≈1 h. The solution was concentrated in *vacuo*. The crude was purified by size-exclusion chromatography (Sephadex LH-20, MeOH/MilliQ $H_2O$ 9:1 ratio).

**6-aminohexyl β-D-glucopyranosyl-(1→6)-β-D-galactofuranoside 21**




Compound **19** (0.012 mmol, 0.016 g) was deprotected using general procedure **B** providing **20** (0,012 mmol, 0.005 g) in 83% yield. $^{1}$**H NMR** (500 MHz, $D_2O$) δ = 4.97 (d, *J* = 2.3 Hz, 1H, H1 Galf), 4.48 (d, *J* = 7.9 Hz, 1H, H1 Glu), 4.10 – 3.85 (m, 6H), 3.77 – 3.65 (m, 4H), 3.65 – 3.59 (m, 1H), 3.57 – 3.39 (m, 3H), 3.39 – 3.26 (m, 2H), 3.06 – 2.88 (m, 2H, $CH_2NH_2$), 1.71 – 1.54 (m, 4H, $CH_2$Linker), 1.37 (m, 4H, $CH_2$Linker). $^{13}$**C NMR** (126 MHz, $D_2O$) δ = 107.0, 102.7, 82.5, 80.9, 76.4, 76.0, 75.6, 73.2, 71.6, 71.2, 69.7, 69.4, 68.4, 60.7, 60.4, 39.4, 28.4, 26.6, 25.3, 24.7, 23.3. **HRMS** *m/z*: [M+Na]$^+$ Calcd 464.21023; found 464.21023.

**6-aminohexyl β-D-glucopyranosyl-(1→6)-β-D-galactofuranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-galactofuranoside 22**




Compound **20** (0.014 mmol, 0.030 g) was deprotected using general procedure **B** providing **20** (0,006 mmol, 0.0053 g) in 43% yield. $^{1}$**H NMR** (400 MHz, D2O) δ= 5.30 (d, J = 1.8 Hz, 1H), 5.01 (d, J = 1.8 Hz, 1H), 4.53 (d, J = 7.9 Hz, 1H), 4.50 (dd, J = 8.0, 1.1 Hz, 1H), 4.16 (dt, J = 3.3, 1.4 Hz, 1H), 4.14 – 3.98 (m, 10H), 3.95 – 3.83 (m, 3H), 3.80 – 3.62 (m, 7H), 3.53 – 3.42 (m, 6H), 3.38 (ddd, J = 9.8, 8.9, 1.2 Hz, 1H), 3.31 (ddd, J = 9.3, 7.8, 1.2 Hz, 1H), 3.13 (t, J = 6.9 Hz, 2H), 2.03 – 1.94 (m, 4H), 1.90 (s, 4H). $^{13}$**C NMR** (126 MHz, D2O) δ= 108.2, 107.2, 102.8, 102.6, 83.4, 83.1, 82.1, 81.3, 80.7, 76.6, 76.5, 76.0, 75.8, 75.7, 73.3, 73.2, 71.4, 71.2, 69.0. **HRMS** *m/z*: [M+Na]$^+$ Calcd 892.34209; found 892.34211.

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