Towards the development of synthetic vaccines against tuberculosis

Marino, L.

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Design and synthesis of stabilized mannosyl phosphomycoketide analogues

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Abstract

Mannose phosphomycoketide (MPM) is a glycolipid expressed on the cell wall of *Mycobacterium tuberculosis*, which has been shown to be presented by the CD1c protein to human interferon-γ (IFN-γ) producing T-cells. Although the ability of T-cells to respond to peptides presented by the major histocompatibility complex (MHC) protein is more widely known than their ability to respond to lipids presented by MHC class I-like CD1 proteins, the CD1c non-polymorphic nature makes it a very interesting target for the development of vaccines against tuberculosis (TB). Currently, two structurally related mycoketides have been shown to be antigenic to T-cells via CD1c: the MPM glycolipid and the phosphomycoketide (PM) lipid. Processing of MPM to PM by antigen-presenting cells like dendritic cells can reduce the availability of MPM during *in vitro* and *in vivo* experiments. In order to improve the stability of the glycosidic linkage between mannose and the phosphomycoketide moiety, which is the most likely point of degradation of MPM to PM, three synthetic analogues of MPM were designed and synthesized. These analogues, comprising a carba-mannose, a methyl *C*-glycoside and a difluoro-*C*-glycoside, were used to stimulate human monocyte-derived dendritic cells in the presence of either T-cells, that specifically recognize MPM (the CD8-1 T-cell) or PM (the DN6 cell). The difluoro-*C*-glycoside was shown to induce activation of the CD8-1 but not the DN6 T-cell line, indicating that this analogue did not give rise to PM in an antigen presentation assay nor did it cross-react with the DN6 T-cell receptor. Because of its desirable characteristics, the difluoro-*C*-mannoside was used to expand antigen-specific T-cells, which were then shown to cross-react to the natural MPM. IFN-γ dose-response curves obtained from the novel T-cell clone indicated that the stabilized difluoro-*C*-mannoside was more antigenic than MPM.

Introduction

Mannose phosphomycoketide (MPM, See Figure 1a), a naturally occurring glycolipid expressed on the cell wall of *Mycobacterium tuberculosis* (*Mtb*), is a known antigen presented by dendritic cells (DCs) to T-cells via cluster of differentiation 1c (CD1c).[1–4] Given its non-polymorphic nature and its ability to present *Mtb*-derived glycolipids to T-cells, CD1c represents an interesting target to exploit for development of vaccines against tuberculosis (TB).[5] CD1c is a protein expressed with high density on subsets of human dendritic cells and B cells. It resembles the major histocompatibility complex I (MHC-I) in its ability to present foreign and self-molecules to T-cells.[6–9] Unlike the MHC-I, which forms complexes with peptides, CD1c presents foreign and self-lipids to T-cells.[10] As shown in Figure 1, different classes of lipids have been identified as ligands for this receptor, including cholesteryl esters (Figure 1c) and phosphatidylcholines (Figure 1d),[1,11–13] in addition to phosphomycoketides (Figure 1a-b).[1,2] Although many lipids and glycolipids are presented by multiple CD1 receptors, phosphomycoketides are not recognized by the other members of the CD1 family. T-cells reactive to CD1c in complex with phosphomycoketides from *Mtb* are thought to have important effector functions, given their ability to produce IFN-γ, a cytokine involved in killing of intracellular mycobacteria through stimulation of endosomal maturation.[14–16]

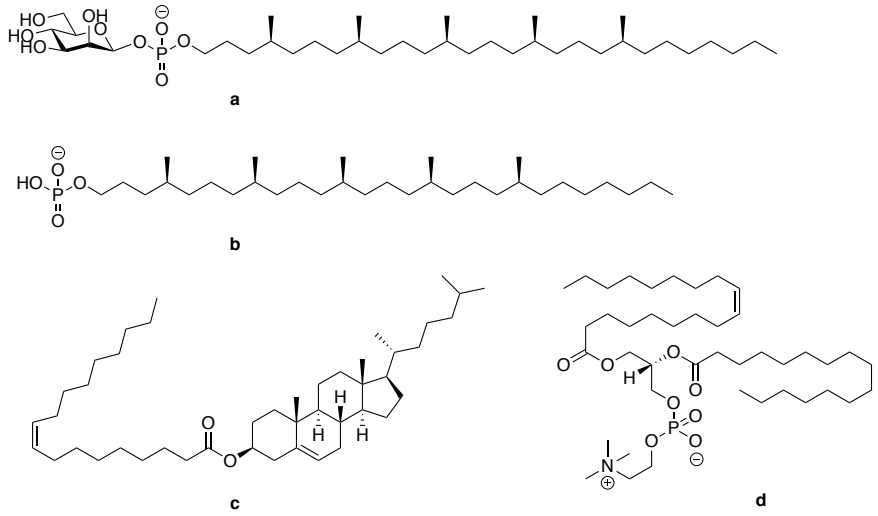


Figure 1 – Chemical structures of lipids that have been shown to interact with the CD1c receptor. a) β-D-mannosyl phosphomycoketide; b); Phosphomycoketide; c) cholesteryl esters; d) phosphatidylcholines (here PC(16:0/18:1) is shown).

The first example of CD1c presentation of MPM to T-cells dates back to 2000, when Moody and co-workers used a DC-mediated antigen presentation assay to prove that this glycolipid was presented to the CD1c restricted T-cell clone, CD8-1.[1] Thirteen years later, Ly *et al.* were able to show that another CD1c restricted T-cell line, DN6, was responding to dendritic cells stimulated by two lipids: MPM and phosphomycoketide (Figure 1a-b) (PM), a phospholipid differing from MPM only in the absence of the carbohydrate moiety.[2] Interestingly, DN6 T-cells did not respond to plate bound CD1c in the presence of MPM, but only in the presence of PM. This result led to the hypothesis that the mannose group is hydrolyzed by antigen-presenting cells via mannosidase enzymes or in the acidic endosomal compartment, yielding PM as a neo-epitope. The crystal structure later revealed PM to be the real epitope for DN6 T-cells.[4]

MPM has been suggested as a promising antigen for the development of anti-TB vaccines. However, natural processing of MPM to PM reduces the levels of the antigen, which is present in the mycobacterial cell wall in low quantities. To further probe MPM as an antigen, stabilized analogues of the glycolipid, which are not (as readily) transformed into PM, could be attractive tools. To this end, this Chapter reports the design and synthesis of three novel MPM analogues. These synthetic analogues, whose structures are shown in Figure 2, comprise a carba-mannose, where the ring oxygen is replaced by a methylene group (compound **1**), and two *C*-mannosides, where the anomeric oxygen is replaced by a methylene group or a difluoro methylene moiety (compounds **2** and **3**, respectively). These modifications render the molecules (more) stable towards chemical and enzymatic hydrolysis making these MPM-analogues ideal candidates to explore their antigenic potential in the context of TB vaccination and further understand the immune function of CD1c receptor.

The stability of the three analogues towards mannosidase enzymes can be explained on the basis of the mechanism of action of the glycosidases. During glycosidase-induced hydrolysis an oxocarbenium ion forms,[17] whose formation is not possible once the carbohydrate hemiacetal is replaced by the ether linkage in the carba- and *C*-glycosides here described. It is not possible to exclude occurrence of enzymatic hydrolysis induced by phosphatases during processing by antigen-presentation dendritic cells. However, in analogues **2** and **3** this would lead to formation of lipid **4** (Figure 2), which cannot be bound to the CD1c receptor. One challenge associated to the use of glycomimetics is related to the changes in conformation of the carbohydrate ring. Altering the structure of the mannose moiety of MPM to generate the more stable glycomimetic can result in conformational changes of the key exo-cyclic phosphate substituent. The exo-anomeric effect that determines the orientation of the exo-cyclic substituent is missing in all three stabilized analogues. The overall dipole of the molecules will be different as well as hydrogen bonding and accepting properties. Finally, the pKa of the phosphate in the analogies will differ

from the parent compound. These effects should be minimized in *C*-mannoside **3**, since the difluoro methylene has most similar electronic properties compared to those of the anomeric oxygen, present in the naturally occurring MPM.[18,19]

Results and discussion

Previously, van Summeren *et al.* have shown the importance of the stereochemistry of the lipid moiety in phosphomycoketides for T-cell response with the first stereoselective total synthesis of a β-D-mannosyl phosphomycoketide.[20] The same lipid moiety, compound **4** (Figure 2), was used to generate the three stabilized MPM-analogues described in the present work.

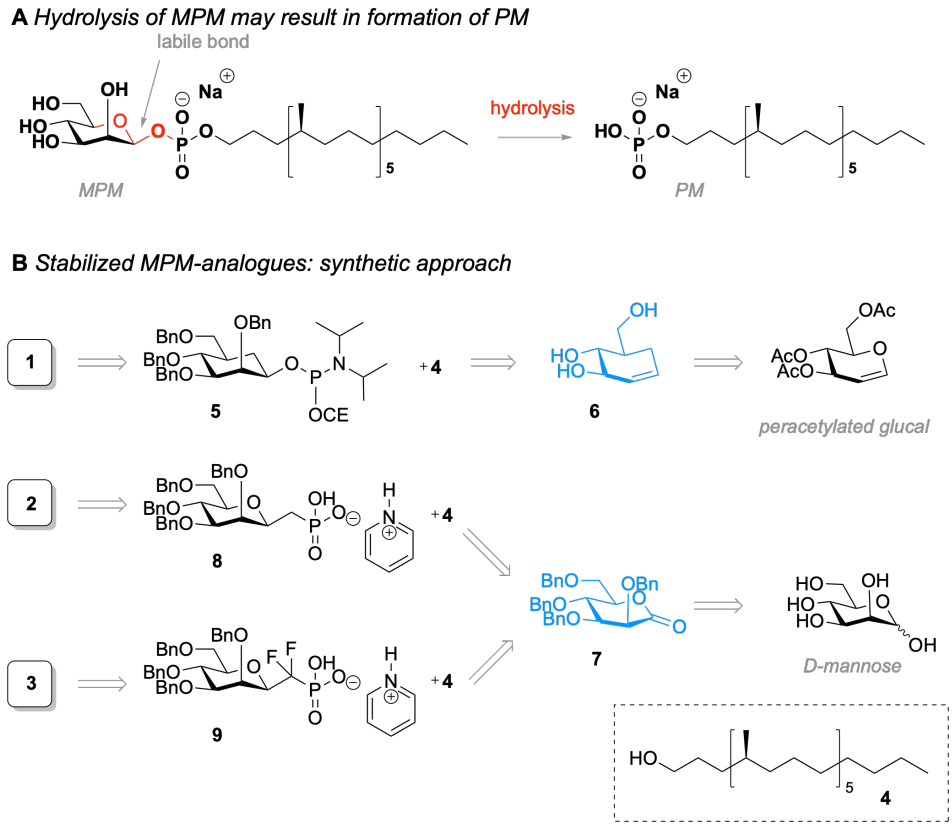


Figure 2 – (A) Chemical structures of β-D-mannosyl phosphomycoketide (MPM) and its synthetic analogues: carba-mannose **1** (MPM-1), and *C*-mannosides **2** (MPM-2) and **3** (MPM-3). Structural modifications, responsible for improved stability towards chemical and enzymatic hydrolysis, are highlighted in green. **(B)** Retrosynthetic approach to generation of carba-mannose (compound **1**) and two *C*-mannosides (compounds **2** and **3**), glycomimetics of MPM. Key intermediates, pseudo-glucal **6** and manno-lactone **6**, are highlighted in blue.

As represented in the retrosynthetic scheme of Figure 2, to generate the carba-analogue **1**, a phosphoramidite chemistry approach was chosen in coupling lipid **4** to β-carba-mannopyranoside **5**. Multiple synthetic strategies have been devised to generate carba-sugars, including those based on carbohydrate precursors as the prime source of chirality.[21–23] A route towards carba mannose **5** originating from pseudo-glucal **6** is shown in this chapter. This pseudo-glucal can be readily obtained from D-glucal. The two *C*-glycosides **2** and **3** can be obtained from D-mannose via lactone intermediate **7**, which can be converted to phosphonates **8** and **9**. Coupling between phosphonate **8** or **9** and lipid **4** then yields the desired analogues **2** and **3**. Importantly, chemical hydrolysis of the two *C*-mannopyranoside analogues **2** and **3** can never lead to formation of PM, as lipid **4** would be the hydrolysis product.

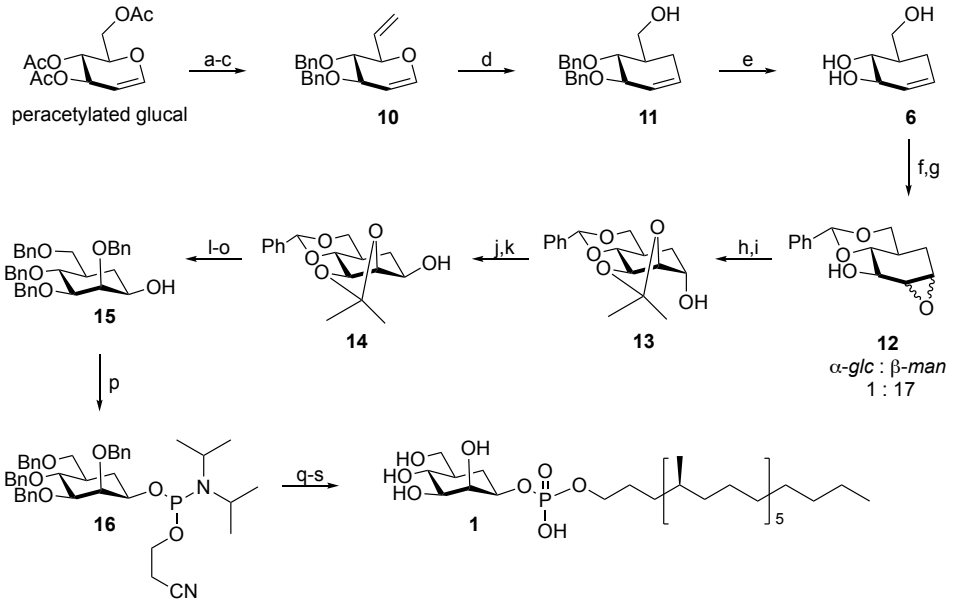


Figure 3 - Synthetic scheme for the generation of carba-mannose (compound 1). a) i. K_2CO_3, MeOH, ii. TDSCl, imidazole, DMF, -20°C, 82%, b) i. BnBr, NaH, TBAI, THF, ii. TBAF, THF, 69%, c) i. IBX, EtOAc, reflux, ii. PPh_3CH_3I, KHMDS, THF, -78°C to RT, 88%, d) i. *o*-dichlorobenzene, 230°C, ii. $NaBH_4$, EtOH/THF, 94%, e) Li-naphthalenide, THF, -20°C, 81%, f) $PhCH(OMe)_2$, *p*TsOH, DMF, 60°C, 67%, g) *m*-CPBA, PBS buffer, DCM, α 5%, β 86%, h) KOH, dioxane, H_2O, 90°C, quant., i) 2,2-dimethoxypropane, pTsOH, DMF, 90%, j) IBX, EtOAc, reflux, 82%, k) $NaBH_4$, DCM/MeOH, 0°C, 77%, l) NapBr, NaH, TBAI, THF/DMF, 88%, m) pTsOH, MeOH, 92%, n) BnBr, NaH, TBAI, DMF, 86%, o) DDQ, DCM/H_2O, 73%, p) (CEO)PCl(N-*i*Pr_2), DIPEA, DCM, 68%, q) i. compound **4,** DCI, CH_3CN, ii. CSO, CH_3CN, 70%, r) Et_3N, CH_3CN, 74%, s) Pd/C, H_2, $CHCl_3$:MeOH (1:1 v/v), 47%.

The synthesis of MPM-1 is depicted in Figure 3. First, pseudo-glucal **11** was synthesized from the commercially available peracetylated glucal, following the steps of Gao *et al.* with a key Claisen rearrangement reaction on substrate **10** to replace the ring oxygen atom with a methylene group.[24] The removal of the benzyl groups from compound **11** proved challenging, as most oxidative and reductive methods are not compatible with the presence of alkenes. Attempts of using the strong Lewis acids $FeCl_3$, BBr_3 and BCl_3 were unsuccessful (Table 1). Deprotection using $TiCl_4$, however, was successful as confirmed by NMR analysis. The downside of this Lewis acid was the formation of an unknown side-product, which proved difficult to remove by silica gel chromatography. Finally, a modified Birch-type reduction using lithium naphthalenide (LN) yielded compound **6** with reproducible results, independent on the reaction scale.

Table 1 – Optimization reaction conditions for debenzylation of substrate 11.

entry #	mmol 11	reagent (eq)	solvent	temperature (°C)	time (h)	yield (%)
1	0.39	$TiCl_4$ (2.5)	DCM	-78	1	N/A*
2	0.40	$TiCl_4$ (4.5)	DCM	-78	1.5	53
3	0.33	BCl_3 (20)	DCM	-78	16	N/A
4	0.33	BBr_3 (8.5)	DCM	-78	5	N/A
5	0.46	$FeCl_3$ (7)	DCM	0	2	N/A
6	0.42	LN (1.1)	THF	-78 to 0	16	18
7	0.32	LN (5)	THF	-78 to -20	1	30
8	0.30	LN (7.5)	THF	-78 to -20	16	79
9	27.8	LN (7.5)	THF	78 to -20	46	81
***50% monobenzylated product found**						

Pseudo-glucal **6** was then converted to α-carba-mannopyranoside **13** via epoxidation of the cyclic olefin, followed by opening of the epoxide in basic conditions and subsequent protection. Noteworthy is the stereo-convergence of epoxide formation and opening events, where both the "α-gluco" and "β-manno" configured epoxides (formed in a 1:17 ratio, isolated and reacted separately) lead to formation of the same α-carba-mannopyranoside. The opening of the epoxides follows a reaction trajectory that is in accordance with the Fürst-Plattner rule. Inversion of the pseudo-anomeric alcohol using an oxidation/reduction sequence, yielded the desired β-carba-mannopyranoside configuration of compound **14**. As expected, the intermediate ketone was reduced in an axial fashion by $NaBH_4$ and the small amount of the axial alcohol was readily removed by column chromatography. The free hydroxyl group of compound **14** was protected with a naphthyl ether (Nap)

before acidic removal of the two acetal protecting groups. Benzyl groups were introduced on the freed hydroxyls after which the Nap group was cleaved oxidatively with 1 equivalent of DDQ yielding intermediate **15**. Formation of a side product due to oxidative removal of benzyl protecting group from the C-3 position (mannose numbering) was observed when using DDQ in excess, as confirmed by NMR analysis. Compound **15** was then converted to the phosphoramidite **16** by treatment with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite under influence of *N,N*-diisopropylethylamine (DIPEA). Intermediate **16** was coupled to lipid **4** using DCI as activator, followed by oxidation of the intermediate phosphite with *(1S)*(+)-(10-camphorsulfonyl)oxaziridine (CSO) to give the corresponding phosphotriester. After removal of cyanoethyl protecting group and dehydrogenation MPM-analogue **1** was isolated in an overall yield of 1.4% over 19 steps starting from D-glucal.

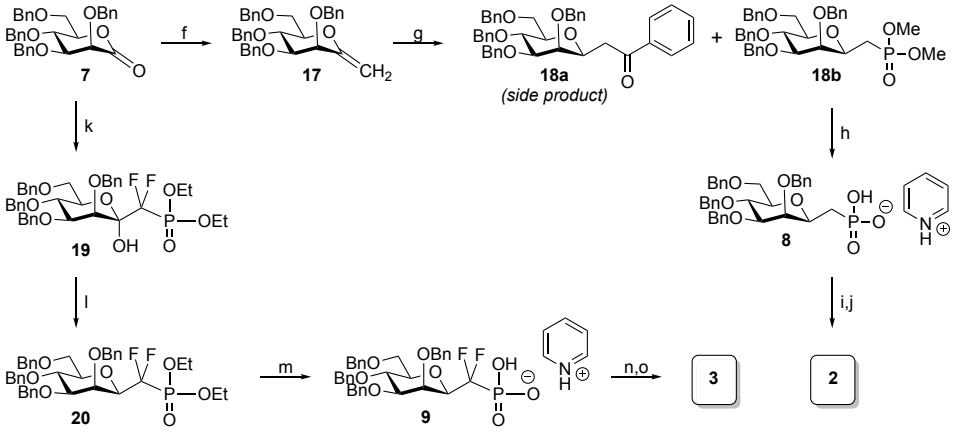


Figure 4 - Synthetic scheme for the generation of the two C-mannosides (compounds 2 and 3). a) Cp_2TiMe_2, toluene, 60°C, 79%, b) $(MeO)_2P(=O)H$, DPAP, neat, hν = 375 nm, y **18a**: 14%, y **18b**: 59%, c) TMSBr, pyridine, CH_3CN, 85%, d) compound **4**, *i*Pr_3PhSO_2Cl, pyridine, 50°C, 50%, e) Pd/C, H_2, THF/H_2O (1:1, v/v), 80%, f) LDA, $(EtO)_2P(=O)CHF_2$, THF, 98%, g) i. $MeO_2CC(=O)Cl$, DCM, ii. AIBN, Bu_3SnH, toluene, 30%, h) TMSBr, pyridine, CH_3CN, quant., i) compound **4**, *i*Pr_3PhSO_2Cl, toluene:DMF:pyridine (1.25:1:0.5, v/v/v), 50°C, 64%, j) Pd/C, H_2, THF:H_2O (2:1, v/v), 82%.

Next, the synthesis of *C*-mannosides **2** and **3** was undertaken. *C*-Glycosides are commonly synthesized by generating the anomeric C-C bond starting from carbohydrate substrates, such as glycosyl halides, sugar lactols or lactones, 1,2-anhydrosugars, thioglycosides/sulfoxides/sulfones or glycosyl imidates/phosphonates.[25] For the synthesis of the *C*-mannoside analogues **2** and **3**,

two separate reaction routes were developed, both starting from manno-lactone **7**, as shown in Figure 4. The approach chosen for the generation of compound **2** consisted of the conversion of the lactone into exo-glycal **17** using the Petasis reagent, followed by radical hydrophosphonylation to obtain the desired β-stereoisomer. This less common approach was inspired by the work of Dondoni *et al.*, where a similar hydrophosphonylation reaction was performed on peracetylated exo-glucal.[26] A plausible reaction mechanism, explaining the observed stereochemistry is shown in Figure 5. According to this mechanism, a phosphonate radical forms after hydrogen abstraction by radical fragments of the DPAP initiator. The phosphonate radical, in turn, adds to the exo-cyclic olefin, resulting in the generation of an anomeric radical. Abstraction of a hydrogen from dimethyl-H-phosphonate by this radical preferentially occurs from the more accessible bottom face, leading to the formation of the sterically more favorable equatorial *C*-phosphonate.

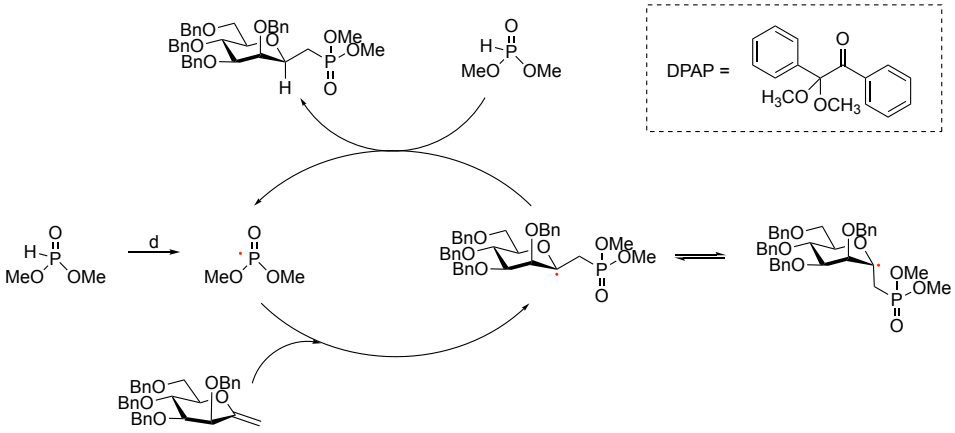


Figure 5 – Proposed reaction mechanism to explain stereochemical outcome of hydrophosphonylation reaction.

The reaction yield for the hydrophosphonylation was 43% when this reaction was performed under unfocused sunlight and it increased to 59% when using focused UV light (375 nm). Side-product **18a** formed by the undesired reaction of compound **17** with the radical initiator, was isolated in 14% yield and identified via H-NMR.[1] The

[1] Nucleophilic addition of methyl diethyl phosphonate carbanion to manno-lactone was considered as an alternative method to hydrophosphonylation, but not pursued due to the additional reaction steps and formation of an exo-cyclic olefin upon dehydroxylation of the newly formed lactol.

β-mannosyl phosphonate **18b** was demethylated to obtain free phosphonic acid **8** using TMSBr in the presence of pyridine to quench the forming acidic species.[2] The stereochemistry of the *C*-phosphonate was confirmed at this stage using Nuclear Overhauser Enhancement Spectroscopy (NOESY) experiments. The correlation between the H-1 and H-5 protons, and between H-1 and H-3, as shown in Figure 6, confirms the formation of the desired β-stereoisomer. Phosphonate **8** was finally coupled to lipid **4** by activation of the acid using 2,4,6-tri-*iso*-propylbenzenesulfonyl chloride, after which the protected glycolipid was deprotected via hydrogenation, yielding the desired MPM analogue **2** in an overall yield of 6.7% over 10 steps starting from D-mannose.

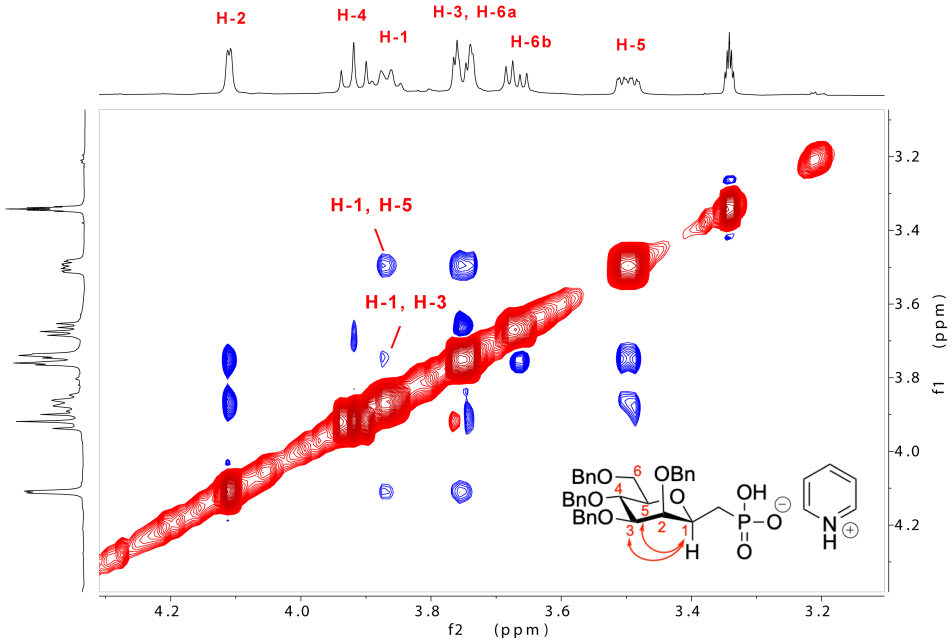


Figure 6 – NOESY spectrum of compound 8. The key NOE interaction can be found between H-1 and H-5, H-1 and H-3.

Although several synthetic strategies have been devised for the synthesis of anomeric phosphates/phosphonates,[27,28] as well as for the synthesis of fluorinated phosphonates,[29] the literature describing synthetic approaches to the generation of anomeric difluoro-*C*-phosphonates is relatively scarce. Examples of synthetic

[2] Key for obtaining good resolution NMR measurements has been the use of ultrapure silica for purification purposes, followed by treatment of the product with a Chelex® 100 resin. The use of glass-covered stirring rods in place of the classical Teflon-covered rods is recommended.

approaches to this class of compounds include the use of sugar-lactones or 1,2-anhydrosugars for a nucleophilic addition reaction of lithiated (difluoromethyl)phosphonates,[30,31] or the use of gem-difluoro exo-glycal intermediates in a phosphonyl radical addition.[32] In this work, a route involving a nucleophilic addition to the lactone was employed.

To assemble the MPM difluoro-*C*-mannoside analogue **3**, lactone **7** was treated with the carbanion formed from reaction of diethyl (difluoromethyl)phosphonate with LDA to provide lactol **19** in near quantitative yield (Figure 4). Removal of the newly formed anomeric alcohol, however, proved challenging. A Barton McCombie approach led to formation of a difluoro exo-glycal, as determined by NMR analysis. Treatment of the alcohol with catalytic quantities of Et_3SiH did not result in any product formation, while using an excess of silane exclusively led to the formation of the TES-protected hydroxyl. Reduction of the lactol eventually succeeded via the formation of the intermediate methyloxalate using monomethyl oxalyl chloride, followed by treatment with Bu_3SnH. In line with the synthesis of *C*-mannoside **2**, the phosphonate was converted into phosphonic acid **9**. NOESY-NMR confirmed the β-stereochemistry (see Figure 7).

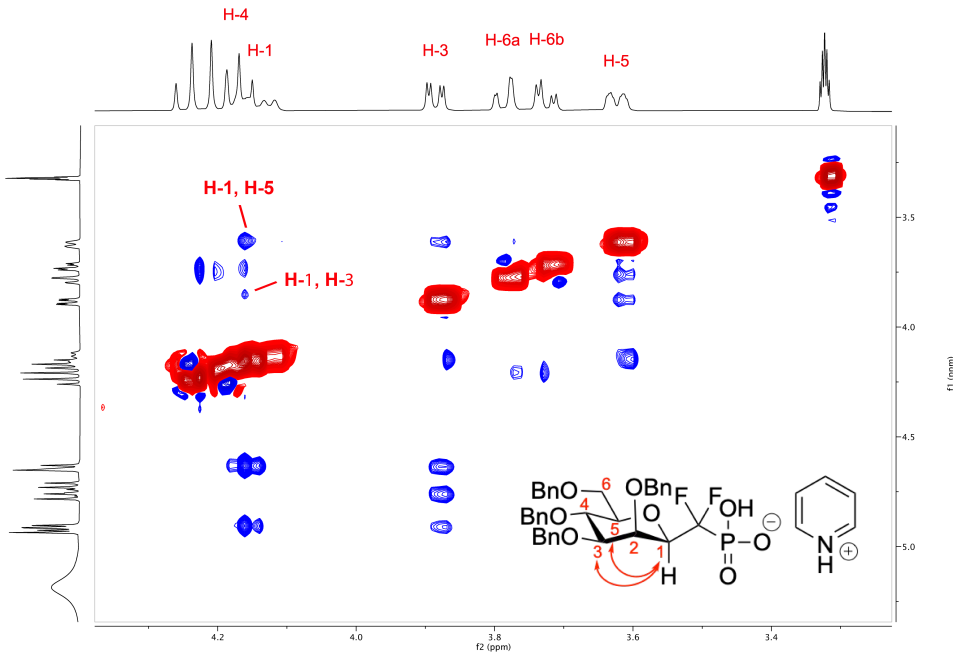


Figure 7 – NOESY spectrum of compound 9. The key NOE interaction can be found between H-1 and H-5, and H-1 and H-3.

Coupling of lipid **4** to difluoro-*C*-mannosyl phosphonic acid **9** was accomplished using *i*Pr$_3$PhSO$_2$Cl as condensing agent in a mixture of DMF/toluene/pyridine, which was required to solubilize all reagents. It was observed that an excess of lipid **4** was required for the coupling reaction to prevent formation of the pyrophosphonate side product (the identity of which was proven via NMR and LC-MS analysis). Finally, a hydrogenation reaction was performed to obtain the desired MPM difluoro-*C*-mannoside **3** which was obtained in 15 % overall yield starting from manno-lactone **7**.

With the stabilized MPM-analogues in hand, the consequences of the structural modifications on antigen presentation were assessed (see Figure 8A for a visual depiction of the chosen approach). The stabilized analogues were used to stimulate human monocyte-derived dendritic cells in the presence of either DN6 or CD8-1 T-cells.

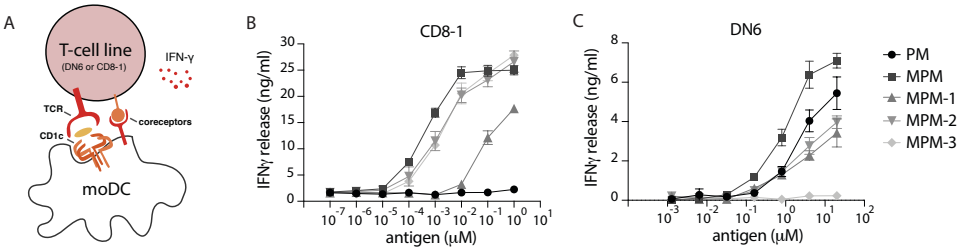


Figure 8 - MPM-3 withstands hydrolysis and cross-reacts with natural MPM. (A) Visual depiction of antigen presentation assay performed to compare IFN-γ production by CD8-1 or DN6 T-cells in the presence of lipid antigen. **(B)** IFN-γ production by MPM-specific CD8-1 T-cells co-cultured with moDCs in the presence of natural MPM or MPM analogues. **(C)** IFN-γ production by PM-specific DN6 T-cells co-cultured with moDCs in the presence of natural MPM and MPM analogues. Data are mean ± SD of triplicate measurements representative of three experiments.

Figure 8B shows that the three analogues were recognized by the CD8-1 T-cell receptor (TCR), which is known to interact with MPM but not with PM. Previously, the DN6 TCR has been shown to be specific for PM, formed from MPM after cellular processing. Unexpectedly, as determined by quantification of the IFN-γ response, carba-mannose **1** and *C*-mannoside **2** were able to activate the DN6 T-cell line, although to a lower extent than natural MPM (Figure 8C).[3] This could indicate that MPM-1 is hydrolyzed under the assay conditions to provide PM. Hydrolysis of *C*-mannoside **2** however would lead to the formation of lipid **4**, which cannot be

[3] As cross-contamination or hydrolysis during storage was unlikely and the samples were tested for the presence of traces amounts of PM using HPLC-MS (see supporting figure), an alternative possible explanation for the unexpected result is that the DN6 TCR recognizes the intact analogues in a cross-reactive manner.

presented by CD1c. Alternatively, it may be speculated that **1** and **2** bind to CD1c in such a manner that it exposes the PM moiety while folding away the mannoside. In contrast, the difluoro-*C*-mannoside **3** did not induce activation of the DN6 T-cell line, indicating that this analogue does not give rise to PM in an antigen presentation assay and that it does not cross-react with the DN6 TCR.

Because of its desirable characteristics, difluoro-*C*-mannoside **3** was used to generate a T-cell line named HD56-MPM-3, which was shown to be cross-reactive to natural MPM (see Figure 9A for a visual depiction of the chosen approach).

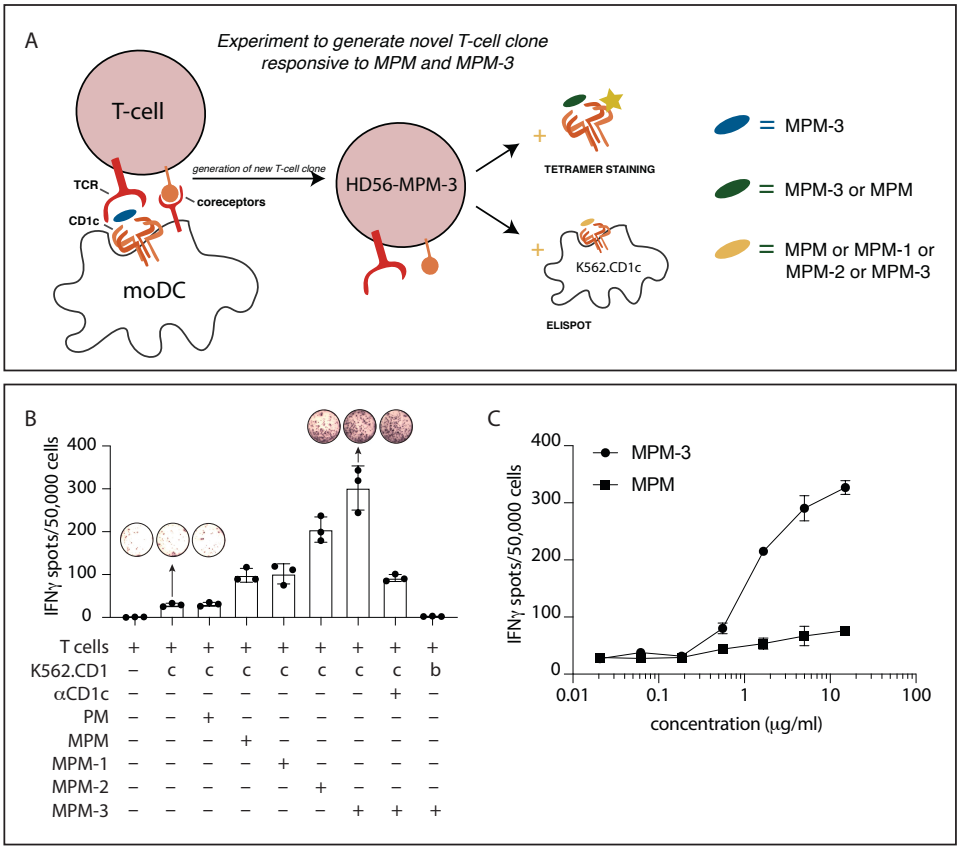


Figure 9 - HD56-MPM-3 T-cells produce IFN-γ after stimulation with MPM-3 and natural MPM. (A) Visual depiction of experimental approach to the generation and characterization of novel T-cell clone: the difluoro-C-mannoside **3** was used to generate T-cell clone HD56-MPM-3, which was shown to recognize both MPM and MPM-3 by flow cytometry using CD1c-tetramers and by ELISPOT using the myelogenous leukemia cell line K562 transduced with CD1c (K562.CD1c). **(B)** IFN-γ ELISPOT the HD56 cell line stimulated with K562.CD1c or K562.CD1b cells in the presence of the indicated lipid antigens. Error bars represent the SEM of triplicate wells. One representative experiment of three is shown. **(C)** Dose response curve of HD56-MPM-3 stimulated with K562.CD1c and lipid antigens.

The cross-reactivity was demonstrated by tetramer staining using CD1c tetramers loaded with either MPM or the difluoro-*C*-mannoside (data not shown). To further characterize the response of the HD56-MPM-3 cell line to MPM and its synthetic analogues, an antigen presentation assay using the myelogenous leukemia cell line K562 transduced with CD1c (K562.CD1c) was performed. The detected ELISPOT responses indicate that difluoro-*C*-mannoside **3** and MPM are both recognized by the HD56-MPM-3 cell line, with the synthetic analogue eliciting a stronger IFN-γ response (Figure 9B). Additionally, also the other synthetic analogues carba-mannoside **1** and *C*-mannoside **2** induced a low to intermediate response, while the amount of IFN-γ detected upon stimulation with PM is comparable to the background level of IFN-γ from T-cells stimulated without antigen.

Furthermore, no IFN-γ is detected in the presence of difluoro-*C*-mannoside **3** when K562.CD1c cells are absent, indicating that processing by the K562.CD1c cells is required. The requirement for processing by the K562.CD1c cell line with presentation via CD1c is shown by the decreased responses in the presence of anti-CD1c antibodies (α-CD1c) as compared to the difluoro-*C*-mannoside **3** stimulation condition. To further evaluate the responsiveness of the HD56-MPM-3 cell line to MPM and difluoro-*C*-mannoside analogue **3**, IFN-γ dose response curves were compared (Figure 9C), demonstrating that MPM is less antigenic than the stabilized difluoro-analogue **3**. This finding suggests that the hydrolysis of MPM to PM reduces the amount of available antigen, while the difluoro-*C*-mannoside **3** is not sensitive to hydrolysis.

Conclusions

The CD1c receptor expressed on antigen-presenting cells represents an attractive target for the development of vaccines against tuberculosis, given its non-polymorphic nature and its ability to present *Mtb*-specific glycolipids to T-cell. MPM is one of such glycolipids and its ability to induce strong T cell responses makes it a good candidate for subunit vaccine applications. However, the processing of MPM to PM by antigen presenting cells like dendritic cells reduces the availability of MPM *in vivo*.

In this chapter, the synthesis of three MPM-analogues, a carba-analogue and two *C*-mannosides, is presented. Characteristic of these analogues is their improved stability towards chemical and enzymatic hydrolysis. For the generation of the carba-analogue, a phosphoramidite chemistry approach was chosen to couple lipid alcohol to β-carba-mannopyranoside, which was obtained from a pseudo-glucal intermediate. The two *C*-glycosides were synthesized starting from D-mannose via a lactone intermediate, which was subsequently converted to the phosphonates using two different synthetic strategies. The coupling reaction between these phosphonates and lipid alcohol yielded the desired analogues in satisfactory yields.

The synthetic analogues were used to stimulate human monocyte-derived dendritic cells in the presence of two well-established T-cell clones, that can recognize CD1c presented MPM and PM analogues, respectively. The difluoro-*C*-mannoside **3** was shown to induce activation of the CD8-1 but not the DN6 T-cell line, indicating that this analogue did not give rise to PM in an antigen presentation assay and did not cross-react with the DN6 TCR.

Because of its desirable characteristics, difluoro-*C*-mannoside **3** was used to expand antigen-specific T-cells, which were further shown to cross-react to the natural MPM. IFN-γ dose-response curves obtained from the novel T-cell clone indicated that the stabilized difluoro-analogue **3** was more antigenic than MPM. Further studies, including experiments to assess recognition of the novel analogue by T-cells of individuals infected with *Mtb* and *in vivo* experiments, will be performed to provide more information regarding the potential of the difluoro-*C*-mannoside analogue for the development of TB vaccines.

Materials and methods

General methods

All reagents were of commercial grade and used as received unless stated otherwise. Ethyl acetate and toluene were distilled before use. Glassware was oven dried at 80°C. Anhydrous solvents used for water-sensitive reactions were stored on activated molecular sieves 3 Å for at least 24 hours before use. Molecular sieves 3 Å were flame dried under reduced pressure.

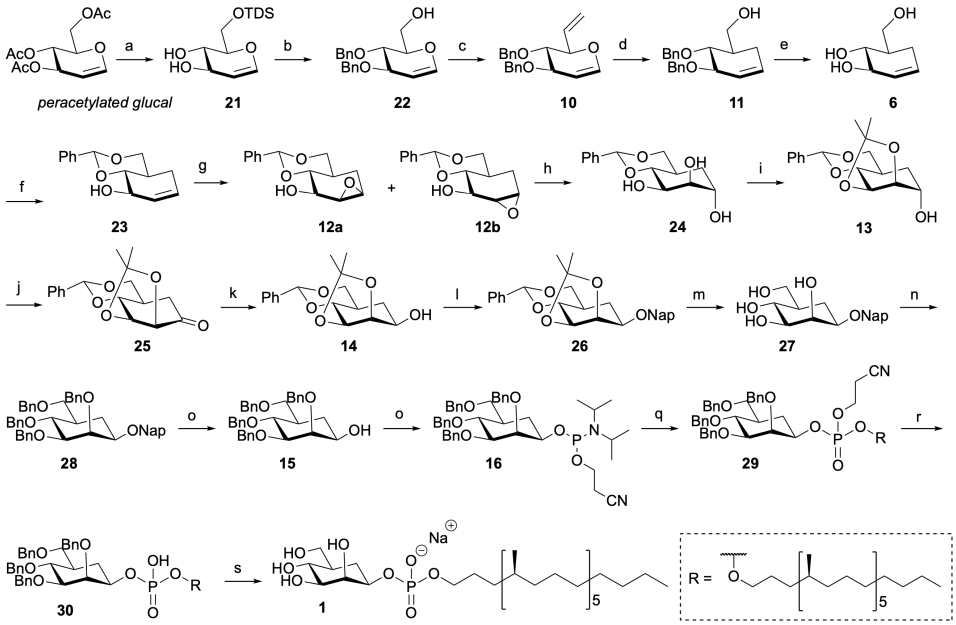
Reactions that required anhydrous conditions were co-evaporated as described in the experimental procedure to remove traces of water, and the reactions were performed under argon or nitrogen atmosphere. Reactions that required a microwave irradiation were performed using a Biotage initiator 2.5. Reactions that required UV irradiation (375 nm; 200 mW; 350 mA; 3.7 V) were performed in the dark using a high-power single chip led (H2A1 series, Roithner LaserTechnik), and the led was fastened to a plastic adapter (for flasks NS 14/23) with distance from the reaction mixture of 5 to 7 cm.

Flash chromatography was performed on Screening Devices silica gel 60 (0.040 - 0.063 mm). Size exclusion chromatography was performed on Sephadex LH-20 gel. The progress of each reaction was followed via TLC-analysis, conducted on DC-alufolien (Merck, Kieselgel 60, F245) with detection by UV-absorption (254 nm) for UV-active compounds and by spraying with one of the following TLC stain solutions: 20% H_2SO_4 in ethanol; 5% anisaldehyde and 5% H_2SO_4 in ethanol; $(NH_4)_6Mo_7O_{24}.4H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_4.2H_2O$ (10 g/L), 10% H_2SO_4 in H_2O; $KMnO_4$ (10 g/L) and K_2CO_3 (67 g/L) in H_2O; the staining was followed by charring at 150°C.

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AV 300 (300/75/121 MHz), Bruker DMX-400 (400/101 MHz), a Bruker AV 400 (400/101/126 MHz), a Bruker AV 500 (500/126/202 MHz) or a Bruker AV 600 (600/151 MHz) spectrometer. ^{19}F NMR spectra were recorded on a Bruker AV 500 (471 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to the residual solvent peak or tetramethylsilane as internal standard. Coupling constants (J) are given in Hz. All given ^{13}C, ^{31}P and ^{19}F spectra are proton decoupled unless stated otherwise.

High-resolution mass spectrometry (HRMS) was performed on a Thermo Finnigan LTQ Orbitrap mass spectrometer equipped with an electrospray ion source in positive-ion mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275 °C) with resolution R = 60.000 at m/z 400 (mass range of 150-4000) and dioctylphtalate (m/z=391.28428) as lock mass, or on a Waters Synapt G2-Si (TOF) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV) and LeuEnk (m/z = 556.2771) as internal lock mass.

C-mannosyl-1-phosphomycoketide



S1 Figure - Synthetic scheme for the generation of carba-mannose (compound 1). a) i. K_2CO_3, MeOH, ii. TDSCl, imidazole, DMF, -20°C, 82%, b) i. BnBr, NaH, TBAI, THF, ii. TBAF, THF, 69%, c) i. IBX, EtOAc, reflux, ii. PPh_3CH_3I, KHMDS, THF, -78°C to RT, 88%, d) i. *o*-dichlorobenzene, 230°C, ii. $NaBH_4$, EtOH/THF, 94%, e) Li-naphthalenide, THF, -20°C, 81%, f) $PhCH(OMe)_2$, pTsOH, DMF, 60°C, 67%, g) *m*-CPBA, PBS buffer, DCM, α 5%, β 86%, h) KOH, dioxane, H_2O, 90°C, quant., i) 2,2-dimethoxypropane, pTsOH, DMF, 90%, j) IBX, EtOAc, reflux, 82%, k) $NaBH_4$, DCM/MeOH, 0°C, 77%, l) NapBr, NaH, TBAI, THF/DMF, 88%, m) pTsOH, MeOH, 92%, n) BnBr, NaH, TBAI, DMF, 86%, o) DDQ, DCM/H_2O, 73%, p) (CEO)PCl(N-*i*Pr_2), DIPEA, DCM, 68%, q) i. compound **4,** DCI, CH_3CN, ii. CSO, CH_3CN, 70%, r) Et_3N, CH_3CN, 74%, s) Pd/C, H_2, $CHCl_3$:MeOH (1:1 v/v), 47%.

6-O-dimethylthexylsilyl-D-glucal (21).

3,4,6-O-acetyl-D-glucal (81.6 g, 300 mmol, 1.0 eq) was dissolved in MeOH (500 ml, 0.6M). To the solution K_2CO_3 (4.15 g, 30 mmol, 0.10 eq) was added and the reaction mixture was stirred for 1 hour at room temperature. Volatiles were then removed *in vacuo* and the crude was co-evaporated (1x) with toluene before being dissolved in dry DMF (500 ml, 0.6M). To this solution imidazole (68.1 g, 900 mmol, 3.0 eq) was added and the mixture was cooled to -20°C. TDSCl (65 ml, 330 mmol, 1.1 eq) was added dropwise via cannula and the reaction mixture was stirred overnight at -20°C. After warming to room temperature the reaction mixture was concentrated *in vacuo*, dissolved in EtOAc and transferred to a separatory funnel. The organic layer was washed with water (4x) and with brine (2x). The combined water layers were extracted (1x) with DCM and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **21** was obtained after silicagel chromatography

(Pentane/EtOAc 4:1→1:1; DCM loading of crude) as a yellow oil (71 g, 246 mmol, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.31 (dd, J = 6.1, 1.8 Hz, 1H, H-1), 4.72 (dd, J= 6.1, 2.2 Hz, 1H, H-2), 4.34 – 4.20 (m, 1H, H-3), 4.02 – 3.93 (m, 1H, H-6a), 3.93 – 3.84 (m, 1H, H-6b), 3.82 – 3.74 (m, 2H, H-4, H-5), 3.27 (bs, 1H, OH-3), 2.59 (bs, 1H, OH-2), 1.70 – 1.54 (m, 1H, CH-TDS), 0.90 – 0.84 (m, 12H, CH_3-TDS), 0.16 – 0.10 (m, 6H, CH_3-Si). ^{13}C-APT NMR (75 MHz, $CDCl_3$) δ: 144.3 (C-1), 102.6 (C-2), 76.7 (C-5), 72.7 (C-4), 69.5 (C-3), 63.8 (C-6), 34.2 (CH-TDS), 20.4 (CH_3-TDS), 18.6 (CH_3-Si).

3,4-di-O-benzyl-D-glucal (22).

Compound **21** (1.45 g, 5.0 mmol, 1 eq) was co-evaporated (3x) with toluene and dissolved in dry THF (10 mL). The solution was cooled to 0°C and TBAI (190 mg, 0.5 mmol, 0.1 eq), BnBr (2.4 mL, 20 mmol, 4 eq) and a 60% suspension in mineral oil of NaH (505 mg, 13 mmol, 2.6 eq) were added sequentially. The reaction mixture was stirred at RT overnight. Upon completion, the reaction mixture was quenched with the addition of MeOH, diluted in EtOAc and transfer- red to a separatory funnel. The organic layer was washed (3x) with water and (1x) with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting crude fully protected glucal was dissolved in dry THF (10 mL) and cooled to 0°C. Then a 0.1 M solution of TBAF (70.5 mL, 7.5 mmol, 1.5 eq) in THF was added dropwise to the reaction mixture via cannula. Upon complete addition, the reaction mixture was stirred at RT overnight and subsequently quenched with the addition of a saturated solution of NH_4Cl(aq). The reaction mixture was then diluted in DCM and transferred to a separatory funnel. The organic layer was washed (1x) with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **22** was obtained after silicagel chromatography (Pentane/Et_2O 9:1→1:1; DCM loading of crude) as a yellow syrup (1.13 g, 3.5 mmol, 69%). NMR analysis confirmed purity of the product, whose ^{1}H NMR and ^{13}C NMR spectra were in agreement with published literature.[33]

3,4-di-O-benzyl-6,7-ene-D-glucal (10).

Compound **22** (19.6 g, 60.3 mmol, 1 eq) was dissolved in dry EtOAc (1.2 L) after which IBX (84.6 g, 302 mmol, 5 eq) was added. The reaction mixture was stirred under reflux for 6 hours and then cooled to RT, filtered over celite and concentrated *in vacuo* to give the crude aldehyde. The crude aldehyde was co-evaporated (2x) with toluene and dissolved in dry THF (60 mL). A phosphonium ylide solution was then prepared by suspending PPh_3CH_3Br (43.0 g, 120.5 mmol, 2 eq) in dry THF (300 mL), cooling the suspension to -78°C and adding a 0.5 M solution of KHMDS in toluene (241 mL, 120.5 mmol, 2 eq) dropwise to the suspension via cannula. The solution was stirred for 30 minutes at -78°C and left to warm up to -50°C to obtain an intensely yellow colored solution. The phosphonium ylide solution was then cooled to -78°C and the crude aldehyde solution was added dropwise. The reaction mixture was then stirred at RT overnight. Upon completion, the reaction was quenched with the addition of a saturated solution of NH_4Cl(aq) (150 mL), then diluted in DCM and transferred to a separatory funnel. The organic layer was washed (1x) with brine and the water layer was extracted (1x) with DCM. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **10** was obtained after silicagel chromatography (Pentane/ Et_2O 39:1→3:2; DCM loading of crude) as a light brown syrup (17.1 g, 53.1 mmol, 88%). NMR analysis confirmed purity of the product, whose ^{1}H NMR and ^{13}C NMR spectra were in agreement with published literature.[34]

3,4-di-O-benzyl-pseudo-D-glucal (11).

Compound **10** (645 mg, 2 mmol, 1 eq) was co-evaporated (3x) with toluene, transferred to a 5 mL microwave vial purged with N_2 and dissolved in dry *o*-dichlorobenzene (5 mL). The microwave vial was purged once more with N_2 and stirred under microwave irradiation for 20 minutes at 230°C. The intermediate aldehyde was then reduced by pouring the reaction mixture in a solution on of $NaBH_4$ (113 mg, 3 mmol, 1.5 eq) in THF/EtOH 2:1 (5 mL). This mixture was stirred for 15 minutes and quenched with the addition of water before being transferred to a separatory funnel. The water layer was extracted (3x) with DCM. The combined organic layers were washed (1x) with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **11** was obtained after silicagel chromatography (Pentane/Et_2O 9:1→1:9; DCM loading of crude) as a yellow syrup (610 mg, 1.88 mmol, 94%). NMR analysis confirmed purity of the product, whose ^1H NMR and ^{13}C NMR spectra were in agreement with published literature.[34]

pseudo-D-glucal (6).

Naphthalene (26.8 g, 209 mmol, 7.5 eq) and freshly cut lithium pieces (10 eq) were suspended in freshly distilled dry THF (320 mL) under an argon atmosphere. The solution was then sonicated for 30 seconds, resulting in a dark green solution of naphthalenide radicals. Dibenzylated **11** (9.02 g, 27.8 mmol, 1 eq) was co-evaporated with toluene under argon and dissolved in freshly distilled dry THF (140 mL). The resulting solution was then added dropwise via a cannula to the lithium naphthalenide solution at -78°C and stirred for 2 days at -20°C. Upon completion, non-distilled THF was added and the reaction mixture was diluted with MeOH until the dark green color disappeared. The strongly basic solution was then neutralized using Amberlite H^+, resulting in a clear solution that was filtered and concentrated *in vacuo*. Compound **6** was obtained after silicagel chromatography (EtOAc/MeOH 1:0→4:1; EtOAc loading of crude) as white crystals (3.25 g, 22.5 mmol, 81%). $[a]_D^{20}$= - 13.7 °(c = 0.010, MeOH). ^1H NMR (400 MHz, MeOD) δ: 5.79 – 5.67 (m, 1H, H-1), 5.61 – 5.49 (m, 1H, H-2), 4.11 – 4.01 (m, 1H, H-3), 3.81 (dd, J = 10.9, 4.4 Hz, 1H, H-6a), 3.68 (dd, J = 10.8, 6.1 Hz, 1H, H-6b), 3.44 (dd, J = 11.1, 7.7 Hz, 1H, H-4), 2.33 – 2.21 (m, 1H, H-7a), 2.08 – 1.96 (m, 1H, H-7b), 1.93 – 1.79 (m, 1H, H-5). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 130.2 (C-2), 128.3 (C-1), 76.0 (C-4), 74.7 (C-3), 64.5 (C-6), 42.5 (C-5), 29.5 (C-7). HRMS $[M+Na]^+$: 167.06818 found, 167.06787 calculated.

4,6-O-benzylidene-pseudo-D-glucal (23).

Fully deprotected pseudo-glucal **6** (3.0 g, 20.8 mmol, 1 eq) was dissolved in dry DMF (42 mL). Benzaldehyde dimethyl acetal (4.65 mL, 31.2 mmol, 1.5 eq) and pTsOH (363 mg, 0.2 mmol, 0.1 eq) were added and the flask containing the reaction mixture was spun on a rotary evaporator under reduced pressure at 60°C. After 1 hour a saturated solution of $NaHCO_3$(aq) was added and the reaction mixture was diluted in water and Et_2O and transferred to a separatory funnel. The water layer was extracted (3x) with Et_2O. The combined organic layers were washed (1x) with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **23** was obtained after crystallization from EtOH as white crystals (3.24 g, 14 mmol, 67%). $[a]_D^{20}$= - 11.6 °(c = 0.010, DCM). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.58 – 7.48 (m, 2H, H-arom), 7.44 – 7.33 (m, 3H, H-arom), 5.74 (ddd, J = 8.9, 4.5, 2.2 Hz, 1H, H-1), 5.69 – 5.59 (m, 2H, H-2, CH-Ph), 4.48 – 4.38 (m, 1H, H-3), 4.22 (dd, J = 11.2, 4.8 Hz, 1H, H-6a), 3.75 – 3.62 (m, 2H, H-4, H-6b), 2.24 – 2.04 (m,

2H, H-5, H-7a), 1.84 – 1.77 (m, 1H, H-7b). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 138.2 (C-arom), 129.2 (C-arom), 128.6 (C-arom), 128.5 (C-2), 126.8 (C-1), 126.3 (C-arom), 101.8 (CH-Ph), 83.5 (C-4), 71.3 (C-6), 70.5 (C-3), 34.0 (C-5), 26.6 (C-7). HRMS $[M+Na]^+$: 255.09920 found, 255.09917 calculated.

1,2-oxirane-4,6-O-benzylidene-pseudo-D-pyranoside (12a & 12b).

Benzylidene protected pseudo-glucal **23** (2.5 g, 8.7 mmol, 1 eq) was dissolved in an emulsion of DCM (29 mL) and PBS (10.5 mL). Then *m*CPBA (4.0 g, 17.4 mmol, 2 eq) was added in various portions to the emulsion. After stirring for 2 hours, the reaction mixture was diluted in DCM and transferred to a separatory funnel. The organic layer was washed (1x) with water, (1x) with a saturated solution of $Na_2S_2O_3$(aq), (1x) with water and (1x) with brine. The organic layer was then dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **12a** was obtained after silicagel chromatography (Pentane/ Et_2O 7:3→0:1; DCM loading of crude) as a white solid (1.9 g, 7.5 mmol, 86%).

2

β-manno: $[a]_D^{20}$= - 0.4 °(c = 0.010, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51 – 7.44 (m, 2H, H-arom), 7.42 – 7.32 (m, 3H, H-arom), 5.49 (s, 1H, CH-Ph), 4.19 – 4.07 (m, 2H, H-6a, H-3), 3.72 (dd, J = 10.6, 8.4 Hz, 1H, H-4), 3.52 (pt, J = 10.9 Hz, 1H, H-6b), 3.41 (dd, J = 4.0, 2.0 Hz, 1H, H-2), 3.34 (dd, J = 4.8, 3.9 Hz, 1H, H-1), 2.55 (s, 1H, OH), 2.05 – 1.85 (m, 2H, H-7a, H-5), 1.63 – 1.52 (m, 2H, H-7b). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 138.0 (C-arom), 129.3 (C- arom), 128.5 (C-arom), 126.3 (C-arom), 101.7 (CH-Ph), 79.9 (C-4), 71.6 (C-3), 70.8 (C-6), 56.1 (C-2), 52.9 (C-1), 34.2 (C-5), 24.8 (C-7). HRMS $[M+Na]^+$: 271.09394 found, 271.09408 calculated.

The α-epoxide **12b** was also isolated as a white solid (108 mg, 0.44 mmol, 5%).

α-gluco: $[a]_D^{20}$= + 1.6 °(c = 0.010, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.50 – 7.43 (m, 2H, H-arom), 7.40 – 7.31 (m, 3H, H-arom), 5.45 (s, 1H, CH-Ph), 4.12 (dd, J = 11.2, 4.8 Hz, 1H, H-6a), 3.88 (dd, J = 8.2, 0.7 Hz, 1H, H-3), 3.47 (pt, J = 11.2 Hz, 1H, H-6b), 3.35 (dd, J = 10.9, 8.2 Hz, 1H, H-4), 3.18 (dt, J = 3.7, 1.9 Hz, 1H, H-1), 3.04 (pd, J = 3.5 Hz, 1H, H-2), 2.01 (ddd, J = 14.7, 4.6, 2.0 Hz, 1H, H-7a), 1.91 – 1.76 (m, 1H, H-5), 1.38 (ddd, J = 14.8, 11.9, 1.7 Hz, 1H, H-7b). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 137.8 (C-arom), 129.2 (C-arom), 128.4 (C-arom), 126.3 (C-arom), 101.6 (CH-Ph), 82.7 (C-4), 71.2 (C-6), 69.3 (C-3), 55.7 (C-2), 52.0 (C-1), 27.4 (C-5), 25.5 (C-7). HRMS $[M+Na]^+$: 271.09443 found, 271.09408 calculated.

4,6-O-benzylidene-7-carba-α-D-mannopyranoside (24).

A mixture of the epoxides **12a** and **12b** (745 mg, 3.0 mmol, 1 eq) was dissolved in dioxane (6 mL) and a 5M solution of KOH (54 mL) was added. The reaction mixture was stirred at 90°C for 2 hours and 30 minutes and upon completion cooled to 0°C, diluted in water and transferred to a separatory funnel. The water layer was extracted (3x) with EtOAc and the combined organic layers were washed (1x) with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **24** was obtained as a white solid (796 mg, 3 mmol, quant.) without any further purification. $[a]_D^{20}$= - 44.7 °(c = 0.010, MeOH). ^{1}H NMR (400 MHz, MeOD) δ: 7.62 – 7.48 (m, 2H, H-arom), 7.48 – 7.30 (m, 3H, H-arom), 5.64 (s, 1H, CH-Ph), 4.10 (dd, J = 10.9, 4.4 Hz, 1H, H-6a), 4.02 – 3.96 (m, 2H, H-1, H-2), 3.94 (dd, J = 9.6, 2.8 Hz, 1H, H-3), 3.86 (pt, J = 9.8 Hz, 1H, H-4), 3.70 (t, J = 11.0 Hz, 1H, H-6b), 2.32 – 2.11 (m, 1H, H-5), 1.62 (td, J = 13.4, 2.4 Hz, 1H, H-7a), 1.55 – 1.41 (m, 1H, H-7b). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 140.1 (C-arom), 129.7 (C-arom), 129.0 (C-arom), 127.5 (C-arom), 103.3 (CH-Ph), 82.2 (C-4), 74.8 (C-3),

72.4 (C-6), 71.1 (C-2), 70.7 (C-1), 34.1 (C-5), 28.4 (C-7). HRMS $[M+Na]^+$: 289.10451 found, 289.10464 calculated.

2,3-O-isopropylidene-4,6-O-benzylidene-7-carba-α-D- mannopyranoside (13).

Benzylidene protected pseudo-mannoside **24** (591 mg, 2.2 mmol, 1 eq) was dissolved in dry DMF (22 mL). The solution was then cooled to 0°C and 2,2-dimethoxypropane (1.1 mL, 8.8 mmol, 4 eq) and pTsOH (42 mg, 0.22 mmol, 0.1 eq) were added. The reaction mixture was left to stir at RT overnight. Then the reaction was quenched with Et_3N, diluted in water and transferred to a separatory funnel. The water layer was extracted (3x) with Et_2O. The combined organic layers were washed (1x) with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **13** was obtained after silicagel chromatography (Pentane/Et_2O 9:1→0:1; DCM loading of crude; silica was neutralized with Et_3N) as a white solid (606 mg, 1.98 mmol, 90%). Note: this compound readily degrades in non-neutralized $CDCl_3$. $[\alpha]_D^{20}$= -51.6 °(c = 0.010, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.56 – 7.45 (m, 2H, H-arom), 7.42 – 7.28 (m, 3H, H-arom), 5.55 (s, 1H, CH-Ph), 4.32 (dd, J = 7.8, 5.3 Hz, 1H, H-3), 4.24 – 4.15 (m, 3H, H-1, H-2, H-6a), 3.69 (dd, J = 11.2, 7.7 Hz, 1H, H-4), 3.61 (pt, J = 11.0 Hz, 1H, H-6b), 2.26 – 2.11 (m, 1H, H-5), 1.87 (s, 1H, OH), 1.64 – 1.41 (m, 5H, H-7, CH_3-isopr), 1.38 (s, 3H, CH_3-isopr). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 138.1 (C-arom), 129.0 (C-arom), 128.3 (C-arom), 126.5 (C-arom), 109.6 (C-isopr), 101.9 (CH-Ph), 82.3 (C-4), 78.8 (C-2), 76.9 (C-3), 71.5 (C-6), 67.2 (C-1), 30.3 (C-5), 28.6 (C-7), 28.3 (CH_3-isopr), 26.0 (CH_3-isopr). HRMS $[M+Na]^+$: 329.13530 found, 329.13594 calculated.

1-one-2,3-O-isopropylidene-4,6-O-benzylidene-7-carba-D- mannopyranoside (25).

Acetal protected compound **13** (319 mg, 1.0 mmol, 1 eq) was dissolved in dry EtOAc (20 mL). IBX (1.4 g, 5.0 mmol, 5 eq) was added to the solution and the reaction mixture was refluxed overnight. The reaction mixture was then cooled to RT, filtered over celite and concentrated *in vacuo*. Compound **25** was obtained after silicagel chromatography (Pentane/EtOAc 9:1→1:1; DCM loading of crude; silica was neutralized with Et_3N) as a white solid (250 mg, 0.82 mmol, 82%). Note: this compound readily degrades in non-neutralized $CDCl_3$. $[\alpha]_D^{20}$= -3.2 °(c = 0.010, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.56 – 7.49 (m, 2H, H-arom), 7.42 – 7.31 (m, 3H, H-arom), 5.57 (s, 1H, CH-Ph), 4.73 (dd, J = 8.5, 6.7 Hz, 1H, H-2), 4.63 (d, J = 8.6 Hz, 1H, H-3), 4.33 (dd, J = 11.3, 4.8 Hz, 1H, H-6a), 3.76 (dd, J = 11.3, 6.8 Hz, 1H, H-4), 3.61 (dd, J = 11.4, 10.4 Hz, 1H, H-6b), 2.56 (ddd, J = 17.6, 6.9, 1.0 Hz, 1H, H-7a), 2.52 – 2.40 (m, 1H, H-5), 2.03 (dd, J = 17.5, 11.0 Hz, 1H, H-7b), 1.54 (s, 3H, CH_3-isopr), 1.40 (s, 3H, CH_3-isopr). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 204.7 (C-1), 137.5 (C-arom), 129.4 (C-arom), 128.4 (C-arom), 126.4 (C-arom), 111.9 (C-isopr), 101.6 (CH-Ph), 80.7 (C-4), 79.3 (C-3), 78.5 (C-2), 71.3 (C-6), 36.7 (C-7), 30.8 (C-5), 27.2 (CH_3-isopr), 25.3 (CH_3-isopr). HRMS $[M+Na]^+$: 327.12039 found, 327.12029 calculated.

2,3-O-isopropylidene-4,6-O-benzylidene-7-carba-β-D-mannopyranoside (14).

Ketone **25** (250 mg, 0.82 mmol, 1 eq) was dissolved in a mixture of DCM/MeOH 20:1 (16.5 mL). The solution was cooled to 0°C and $NaBH_4$ (155 mg, 4.1 mmol, 5 eq) was added in portions. After stirring for 30 minutes the reaction was quenched with the addition of water and transferred to a separatory funnel. The water layer was extracted (3x) with DCM and the combined organic layers were washed (1x) with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **14** was obtained after silicagel chromatography

(Pentane/EtOAc 7:3→3:7; DCM loading of crude; silica was neutralized with Et_3N) as a white solid (193 mg, 0.63 mmol, 77%) with an axial to equatorial ratio of 1:32. Note: this compound readily degrades in non-neutralized $CDCl_3$. $[\alpha]_D^{20}$= -70.7 °(c = 0.010, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54 – 7.46 (m, 2H, H-arom), 7.38 – 7.30 (m, 3H, H-arom), 5.55 (s, 1H, CH-Ph), 4.39 (dd, J = 6.0, 3.8 Hz, 1H, H-2), 4.25 – 4.15 (m, 2H, H-3, H-6a), 4.06 – 3.96 (m, 1H, H-1), 3.84 (dd, J = 10.7, 7.6 Hz, 1H, H-4), 3.65 (pt, J = 10.8 Hz, 1H, H-6b), 2.29 (d, J = 6.6 Hz, 1H, OH), 1.81 – 1.66 (m, 2H, H-7a, H-5), 1.59 (s, 3H, CH_3-isopr), 1.46 – 1.34 (m, 4H, CH_3-isopr, H-7b). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 138.1 (C-arom), 129.0 (C-arom), 128.3 (C-arom), 126.4 (C-arom), 109.8 (C-isopr), 101.9 (CH-Ph), 81.9 (C-4), 77.9 (C-3), 76.6 (C-2), 71.4 (C-6), 67.7 (C-1), 32.5 (C-5), 28.6 (C-7), 28.0 (CH_3-isopr), 25.7 (CH_3-isopr). HRMS $[M+Na]^+$: 329.13596 found, 329.13594 calculated.

1-O-naphthyl-2,3-O-isopropylidene-4,6-O-benzylidene-7-carba-β-D-mannopyranoside (26).

Acetal protected pseudo-mannoside **14** (2.23 g, 7.28 mmol, 1 eq) was co-evaporated with toluene and dissolved in dry DMF (38 mL). The solution was cooled to 0°C and TBAI (269 mg, 0.728 mmol, 0.1 eq) and naphthyl bromide (3.22 g, 14.6 mmol, 2 eq) were added. Then a 60% suspension in mineral oil of NaH (43.7 mg, 10.9 mmol, 1.5 eq) was added in multiple portions to the reaction mixture. After the addition of NaH was complete, the solution was stirred at RT for 2 hours and then quenched with the addition of MeOH. The reaction mixture was then diluted in Et_2O and water and transferred to a separatory funnel. The water layer was extracted (3x) with Et_2O and the combined organic layers were washed (1x) with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **26** was obtained after silicagel chromatography (Pentane/EtOAc 4:1→0:1; DCM loading of crude; silica was neutralized with Et_3N) as a white solid (2.85 g, 6.41 mmol, 88%). Note: this compound readily degrades in non-neutralized $CDCl_3$. $[\alpha]_D^{20}$= -35.5 °(c = 0.010, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.89 – 7.77 (m, 4H, H-arom), 7.56 – 7.43 (m, 5H, H-arom), 7.36 – 7.26 (m, 3H, H-arom), 5.52 (s, 1H, CH-Ph), 4.92 – 4.78 (m, 2H, CH_2-Nap), 4.51 – 4.42 (m, 1H, H-2), 4.15 (dd, J = 11.0, 4.4 Hz, 1H, H-6a), 4.08 (dd, J = 7.8, 5.0 Hz, 1H, H-3), 3.81 – 3.72 (m, 1H, H-1), 3.72 – 3.59 (m, 2H, H-4, H-6b), 1.77 – 1.67 (m, 1H, H-7), 1.67 – 1.45 (m, 5H, CH_3-isopr, H-5, H-7), 1.41 (s, 3H, CH_3-isopr). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 138.0 (C-arom), 135.6 (C-arom), 133.3 (C-arom), 133.2 (C-arom), 129.0 (C-arom), 128.5 (C-arom), 128.2 (C-arom), 128.0 (C-arom), 127.9 (C-arom), 126.8 (C-arom), 126.4 (C-arom), 126.4 (C-arom), 126.2 (C-arom), 125.9 (C-arom), 110.1 (C-isopr), 102.0 (CH-Ph), 82.8 (C-4), 78.2 (C-3), 75.3 (C-2), 74.0 (C-1), 71.3 (CH_2-Nap), 71.1 (C-6), 33.3 (C-5), 28.7 (CH_3-isopr), 26.4 (CH_3-isopr), 26.0 (C-7). HRMS $[M+Na]^+$: 469.19828 found, 469.19855 calculated.

1-O-naphthyl-7-carba-β-D-mannopyranoside (27).

The naphthyl protected **26** (89.3 mg, 0.2 mmol, 1 eq) was dissolved in DCM/MeOH 1:1 (5 mL) and pTsOH (11.4 mg, 0.06 mmol, 0.3 eq) was added. The reaction mixture was stirred at RT for 2 hours, then quenched with the ad- dition of Et_3N and concentrated *in vacuo*. Compound **27** was obtained after silicagel chromatography (EtOAc/MeOH 1:0→4:1; EtOAc loading of crude) as a white solid (58.5 mg, 0.184 mmol, 92%). $[\alpha]_D^{20}$= + 20.5 °(c = 0.010, MeOH). ^{1}H NMR (400 MHz, MeOD) δ: 7.91 – 7.82 (m, 4H, H-arom), 7.58 – 7.44 (m, 3H, H-arom), 4.86 – 4.72 (m, 2H, CH_2-Nap), 4.30 – 4.19 (m, 1H, H-2), 3.84 (dd, J = 10.7, 4.3 Hz, 1H, H-6a), 3.64 – 3.50 (m, 3H, H-1, H-6, H-4), 3.30 (dd, J = 9.4, 2.8 Hz, 1H, H-3), 2.03 – 1.87 (m, 1H, H-7a), 1.73 (pq, J = 12.5

Hz, 1H, H-7b), 1.53 – 1.38 (m, 1H, H-5). ^{13}C-APT NMR (101 MHz, MeOD) δ: 137.5 (C-arom), 134.8 (C-arom), 134.5 (C-arom), 129.1 (C-arom), 128.9 (C-arom), 128.7 (C-arom), 127.5 (C-arom), 127.1 (C-arom), 126.9 (C-arom), 126.9 (C-arom), 78.1 (C-4), 76.4 (C-3), 72.3 (C-1), 72.0 (C-2), 71.4 (CH_2-Nap), 64.8 (C-6), 42.5 (C-5), 28.3 (C-7). HRMS $[M+Na]^+$: 341.13583 found, 341.13594 calculated.

1-O-naphthyl-2,3,4,6-tetra-O-benzyl-7-carba-β-D- mannopyranoside (28).

Naphthyl protected carba-mannoside **27** (726.4 mg, 2.28, 1 eq) was co-evaporated with toluene and dissolved in dry DMF (25 mL). The solution was cooled to 0°C and benzyl bromide (2.171 mL, 18.25 mmol, 8 eq) and TBAI (84 mg, 0.228 mmol, 0.1 eq) were added. Then a 60% suspension in mineral oil of NaH (456 mg, 11.41 mmol, 5 eq) was added in multiple portions to the reaction mixture. After the addition of NaH was complete, the solution was stirred at RT overnight and then quenched with the addition of MeOH. The reaction mixture was then diluted in Et_2O and water and transferred to a separatory funnel. The water layer was extracted (3x) with Et_2O and the combined organic layers were washed (1x) with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **28** was obtained after silicagel chromatography (Pentane/Et_2O 20:1→4:1; DCM loading of crude) as a pale syrup (1.36 g, 1.96 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86 – 7.72 (m, 4H, H-arom), 7.52 – 7.39 (m, 5H, H-arom), 7.37 – 7.17 (m, 19H, H-arom), 4.96 – 4.88 (m, 3H, CH_2-Nap, CH_2-Bn), 4.70 (d, J = 12.2 Hz, 1H, CH_2-Bn), 4.66 – 4.59 (m, 3H, CH_2-Bn), 4.53 – 4.46 (m, 3H, CH_2-Bn), 4.21 – 4.14 (m, 1H, H-2), 3.83 (dd, J = 10.6, 9.3 Hz, 1H, H-4), 3.64 (dd, J = 8.8, 3.0 Hz, 1H, H-6a), 3.48 (dd, J = 8.8, 6.9 Hz, 1H, H-6b), 3.43 (ddd, J = 11.2, 5.1, 2.1 Hz, 1H, H-1), 3.37 (dd, J = 9.4, 2.4 Hz, 1H, H-3), 2.15 – 1.99 (m, 2H, H-7), 1.74 – 1.61 (m, 1H, H-5). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 139.7 (C-arom), 139.0 (C-arom), 138.8 (C-arom), 138.7 (C-arom), 136.3 (C-arom), 133.4 (C-arom), 133.0 (C-arom), 128.5 (C-arom), 128.4 (C-arom), 128.2 (C-arom), 128.2 (C-arom), 128.0 (C-arom), 127.9 (C-arom), 127.8 (C-arom), 127.7 (C-arom), 127.7 (C-arom), 127.6 (C-arom), 127.6 (C-arom), 127.4 (C-arom), 127.3 (C-arom), 126.2 (C-arom), 126.0 (C-arom), 125.9 (C-arom), 125.6 (C-arom), 84.5 (C-3), 78.4 (C-4), 78.2 (C-1), 75.6 (C-2), 75.4 (CH_2-Bn), 73.8 (CH_2-Bn), 73.2 (CH_2-Bn), 72.3 (CH_2-Bn), 71.1 (C-6), 70.9 (CH_2-Nap), 39.9 (C-5), 28.4 (C-7). HRMS $[M+Na]^+$: 701.32288 found, 701.32375 calculated.

2,3,4,6-tetra-O-benzyl-7-carba-β-D-mannopyranoside (15).

Fully protected carba-mannoside **28** (505 mg, 0.744 mmol, 1 eq) was dissolved in an emulsion of DCM/water 9:1 (7.5 mL). The solution was stirred in the dark and then DDQ (169 mg, 0.744 mmol, 1 eq) was added. After 2 hours the reaction mixture was diluted with DCM and transferred to a separatory funnel. The organic layer was washed (1x) with (1x) a saturated solution of $NaHCO_3$(aq), (1x) with a saturated solution of $Na_2S_2O_3$(aq), and again (1x) a saturated solution of $NaHCO_3$(aq) until both the organic and the water layer turned clear. The organic layer was then washed (1x) with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **15** was obtained after silicagel chro- matography (Pentane/Et_2O 4:1→3:7; DCM loading of crude) as a light-yellow solid (292 mg, 0.542 mmol, 73%). $[\alpha]_D^{20}$= + 10.2 °(c = 0.010, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 – 7.19 (m, 20H, H-arom), 5.16 (d, J = 11.7 Hz, 1H, CH_2-Bn), 4.89 (d, J = 10.8 Hz, 1H, CH_2-Bn), 4.75 (s, 2H, CH_2-Bn), 4.65 (d, J = 11.6 Hz, 1H, CH_2-Ph), 4.52 (d, J = 10.9 Hz, 1H, CH_2-Ph), 4.47 (d, J = 3.1 Hz, 2H, CH_2-Ph), 4.05 – 3.98 (m, 1H, H-2), 3.82 (pt, J = 9.8 Hz, 1H, H-4), 3.62 – 3.50 (m, 3H, H-1, H-6), 3.47 (dd, J = 9.4, 2.3 Hz, 1H, H-3), 2.02 (s, 1H, OH), 1.94 – 1.84 (m, 1H, H-7a), 1.78 (pq, J = 12.1 Hz, 1H, H-7b), 1.72

– 1.61 (m, 1H, H-5). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 139.2 (C-arom), 138.9 (C-arom), 138.7 (C-arom), 138.6 (C-arom), 128.6 (C-arom), 128.6 (C-arom), 128.5 (C-arom), 128.2 (C-arom), 128.0 (C-arom), 127.8 (C-arom), 127.8 (C-arom), 127.7 (C-arom), 127.7 (C-arom), 127.6 (C-arom), 127.6 (C-arom), 85.0 (C-3), 79.4 (C-2), 78.0 (C-4), 75.3 (CH_2-Bn), 74.6 (CH_2-Bn), 73.1 (CH_2-Bn), 73.0 (CH_2-Bn), 70.6 (C-6), 69.6 (C-1), 39.5 (C-5), 32.2 (C-7). HRMS $[M+Na]^+$: 561.26071 found, 561.26115 calculated.

1-O-([N,N-diisopropylamino]-2-O-benzyl-phosphite)-2,3,4,6-tetra-O-benzyl-7-carba-β-D-mannopyranoside (16).

Carba-mannoside **15** (182.4 mg, 0.339 mmol, 1eq) was co-evaporated (2x) with toluene, dissolved in dry DCM (2 ml). DIPEA (0.089 mL, 0.51 mmol, 1.5 eq) and activated 4Å molecular sieves were added and the solution was stirred for 15 minutes under an Argon atmosphere. Then 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.406 mmol, 0.091 mL, 1.2 eq) was added to the solution. After stirring for 45 minutes, the reaction was quenched with the addition of water. The following workup and purification were performed as quickly as possible: the reaction mixture was diluted in DCM and transferred to a separatory funnel. The organic layer was washed (1x) with a mixture containing a saturated solution of $NaHCO_3$(aq) and brine 1:1. The organic layer was then dried over Na_2SO_4, filtered and concentrated *in vacuo*. Compound **16** was obtained after silicagel chromatography (Pentane/Et_2O 9:1→1:1; DCM loading of crude; silica was neutralized with Et_3N) as a colorless syrup (170 mg, 0.229 mmol, 68%). ^{1}H NMR (500 MHz, CD_3CN) δ: 7.59 – 7.07 (m, 20H, H-arom), 4.99 – 4.79 (m, 3H, CH_2-Bn), 4.78 – 4.68 (m, 1H, CH_2-Bn), 4.67 – 4.59 (m, 1H, CH_2-Bn), 4.53 – 4.41 (m, 3H, CH_2-Bn), 4.20 (pd, J = 70.87 Hz, 1H, H-2), 4.01 – 3.87 (m, 1H, H-1), 3.87 – 3.59 (m, 5H, CH-N, H-4, OCH_2), 3.59 – 3.48 (m, 3H, H-3, H-6), 2.73 – 2.59 (m, 2H, CH_2CN), 2.05 (pq, J = 12.5 Hz, 1H, H-7a), 1.99 – 1.81 (m, 1H, H-7b), 1.73 – 1.61 (m, 1H, H-5), 1.28 – 1.11 (m, 12H, CH_3). ^{31}P-NMR (202 MHz, CD_3CN) δ: 147.49, 148.66.

2,3,4,6-tetra-O-benzyl-7-carba-β-D-mannopyranosyl-1-(2-O-cyanoethylphosphate)-(4S,8S,12S,16S,20S)-4,8,12,16,20- pentamethylheptacosyl (29).

Lipid **4** (46.3 mg, 0.099 mmol, 1 eq) was co-evaporated (3x) with toluene and then dissolved in a 0.25 M solution of DCI in acetonitrile (0.60 mL, 0.15 mmol, 1.5 eq). Then an additional portion of dry acetonitrile (0.6 mL) was added to dissolve the lipid. 4Å Molecular sieves were added and the solution was stirred for 15 minutes under an Argon atmosphere. A 0.1 M solution of phosphoramidite **16** (2.2 mL, 0.22 mmol, 2.2 eq) in dry acetonitrile was then added slowly to the reaction mixture. The reaction mixture was stirred for 3 hours and upon complete coupling, a 0.25 M solution of CSO (1.2 mL, 0.31 mmol, 3 eq) in dry acetonitrile was added to the reaction mixture. After stirring for 15 minutes, water was added and the reaction mixture was diluted in EtOAc. The organic layer was washed with with a mixture containing a saturated solution of $NaHCO_3$(aq) and brine 1:1 and the water layer was extracted (2x) with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Compound **29** was obtained after silicagel chromatography (Pentane/EtOAc 5:1→1:1; DCM loading of crude; silica was neutralized with Et_3N) followed by size exclusion (LH-20, DCM/MeOH, 1/1, v/v) as a colorless syrup (78 mg, 0.070 mmol, 70%). $[\alpha]_D^{20}$ = + 1.2 °(c = 0.005, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.45 – 7.38 (m, 2H, H-arom), 7.36 – 7.25 (m, 16H, H-arom), 7.22 – 7.16 (m, 2H, H-arom), 5.03 – 4.92 (m, 1H, CH_2-Bn), 4.92 – 4.84 (m, 1H, CH_2-Bn), 4.80 (d, J = 12.1 Hz, 1H, CH_2-Bn), 4.74 – 4.61 (m, 2H, CH_2-Bn), 4.53 – 4.42 (m, 3H, CH_2-Bn), 4.42 – 4.33

(m, 1H, H-1), 4.26 – 4.18 (m, 1H, H-2), 4.18 – 4.10 (m, 1H, OCH_2-lipid), 4.10 – 3.95 (m, 3H, OCH_2-lipid, CH-N), 3.90 – 3.78 (m, 1H, H-4), 3.61 – 3.50 (m, 2H, H-6), 3.49 – 3.41 (m, 1H, H-3), 2.67 (t, J = 6.3 Hz, 1H, CH_2CN), 2.61 (td, J = 6.3, 2.7 Hz, 1H, CH_2CN), 2.29 – 2.14 (m, 1H, H-7a), 2.07 – 1.95 (m, 1H, H-7b), 1.76 – 1.55 (m, 3H, H-5, CH_2-lipid), 1.44 – 0.97 (m, 45H, CH_2-lipid, CH-lipid), 0.92 – 0.78 (m, 18H, CH_3-lipid). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 139.3 (C-arom), 139.2 (C-arom), 138.8 (C-arom), 138.6 (C-arom), 138.5 (C-arom), 138.5 (C-arom), 128.5 (C-arom), 128.4 (C-arom), 128.3 (C-arom), 128.2 (C-arom), 127.7 (C-arom), 127.7 (C-arom), 127.7 (C-arom), 127.6 (C-arom), 127.6 (C-arom), 127.6 (C-arom), 127.5 (C-arom), 127.5 (C-arom), 127.4 (C-arom), 127.4 (C-arom), 116.5 (CN), 116.4 (CN'), 84.0 (C-3), 77.6, 77.6, 77.5, 77.5, 77.4, 77.4, 77.3, 77.2, 77.1, 75.4 (CH_2-Bn), 74.6 (CH_2-Bn), 74.5 (CH_2-Bn'), 73.1 (CH_2-Bn), 72.6 (CH_2-Bn), 72.6 (CH_2-Bn'), 70.3 (C-6), 69.1 (d, J = 2.69 Hz, OCH_2-lipid), 69.0 (d, J = 2.61 Hz, OCH_2'-lipid), 61.7 (pt, J = 5.56 Hz, OCH_2-cyanoethyl), 39.2 (CH_2-lipid), 37.6, 37.5, 37.4, 37.2, 32.9 (CH-lipid), 32.9 (CH-lipid), 32.9 (CH-lipid), 32.7, 32.6 (CH-lipid), 32.6 (CH-lipid), 32.1, 30.1, 29.6, 29.5, 28.01 (d, J = 3.50 Hz, C-10, one diastereoisomer), 27.9 (d, J = 3.16 Hz, C-10, one diastereoisomer), 27.2, 24.6, 24.6, 22.8, 19.9 (CH_3-lipid), 19.9 (CH_3-lipid), 19.9 (CH_3-lipid), 19.7 (d, J = 1.15 Hz, CH_2CN), 19.7 (d, J = 1.36 Hz, CH_2CN'), 19.6 (CH_3-lipid), 19.6 (CH_3-lipid), 14.3 (CH_3-lipid). ^{31}P-NMR (162 MHz, $CDCl_3$) δ: -1.70, -1.65. HRMS $[M+Na]^+$: 1142.75454 found, 1142.75483 calculated.

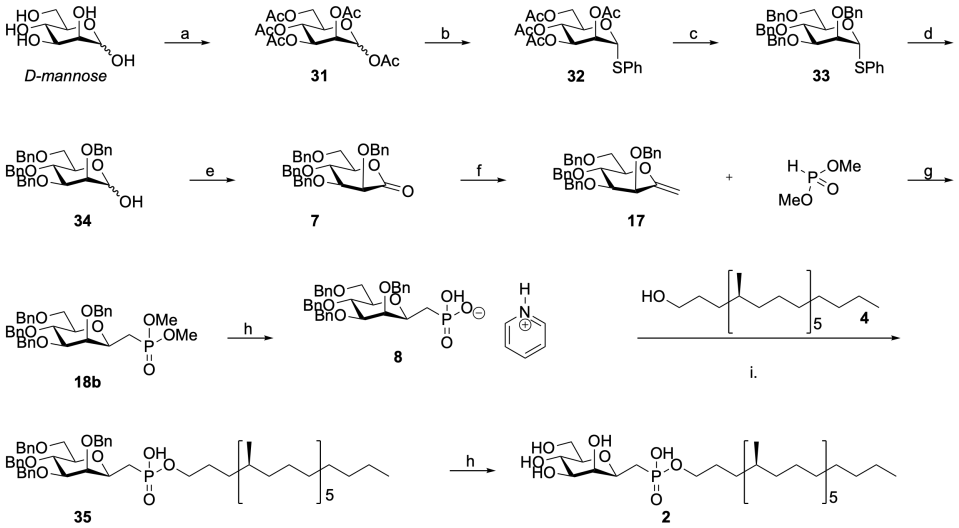
Sodium 2,3,4,6-tetra-O-benzyl-7-carba-β-D-mannopyranosyl-1-phosphoryl-(4S,8S,12S,16S,20S)-4,8,12,16,20-pentamethylheptacosyl (30).

Cyanoethyl protected **29** (36.1 mg, 0.031 mmol, 1 eq) was co-evaporated (2x) with toluene and dissolved in dry acetonitrile (3 mL). The reaction was cooled to 0°C, then Et_3N (0.36 mL, 2.6 mmol, 80 eq) was added and the reaction was stirred at RT for 5 days. Upon completion, the reaction mixture was diluted in dry distilled toluene and concentrated. Purification by size exclusion chromatography (LH-20, DCM/MeOH, 1/1, v/v) yielded the triethylammonium salt of the product. The triethylammonium salt was converted to the sodium salt by dissolving it in MeOH and passing through a small reaction syringe containing amberlite Na^+. Compound **30** was obtained after concentration of the eluate as a colorless oil (25,8 mg, 0.024 mmol, 74%). $[a]_D^{20}$= - 1.4 °(c = 0.008, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.47 – 7.37 (m, 2H, H-arom), 7.35 – 7.20 (m, 16H, H-arom), 7.20 – 7.13 (m, 2H, H-arom), 4.92 – 4.80 (m, 3H, CH_2-Bn), 4.58 (pq, J = 11.7 Hz, 2H, CH_2-Bn), 4.49 – 4.36 (m, 3H, CH_2-Bn), 4.34 – 4.25 (m, 1H, H-1), 4.25 – 4.19 (m, 1H, H-2), 3.93 (pq, J = 6.7 Hz, 2H, OCH_2-lipid), 3.87 – 3.75 (m, 1H, H-4), 3.58 – 3.45 (m, 2H, H-6), 3.40 (dd, J = 9.4, 2.4 Hz, 1H, H-3), 2.20 (pq, J = 12.6 Hz, 1H, H-7a), 2.04 (dt, J = 12.7, 4.5 Hz, 1H, H-7b), 1.73 – 1.48 (m, 3H, H-5, CH_2-lipid), 1.46 – 0.95 (m, 45H, CH_2-lipid, CH-lipid), 0.93 – 0.75 (m, 18H, CH_3-lipid). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 139.3 (C-arom), 138.9 (C-arom), 138.6 (C-arom), 138.6 (C-arom), 128.5 (C-arom), 128.5 (C-arom), 128.4 (C-arom), 128.3 (C-arom), 128.2 (C-arom), 127.7 (C-arom), 127.6 (C-arom), 127.6 (C-arom), 127.4 (C-arom), 84.0 (C-3), 77.2 (C-4), 77.2 (C-2), 76.9 (C-1), 75.3 (CH_2-Bn), 74.7 (CH_2- Bn), 73.1 (CH_2-Bn), 72.3 (CH_2-Bn), 70.4 (C-6), 68.3 (d, J = 5.46 Hz, OCH_2-lipid), 39.3 (C-5), 37.6 (CH_2-lipid), 37.6 (CH_2-lipid), 37.5 (CH_2-lipid), 37.2 (CH_2-lipid), 33.0 (CH-lipid), 33.0 (CH-lipid), 33.0 (CH-lipid), 32.9 (CH-lipid), 32.9 (CH_2-lipid), 32.7 (CH-lipid), 32.1 (CH_2-lipid), 30.2 (CH_2-lipid), 29.9 (CH_2-lipid), 29.6 (C-7), 28.0 (d, J = 7.42 Hz, CH_2-lipid), 27.2 (CH_2-lipid), 24.6 (CH_2-lipid), 24.6 (CH_2-lipid), 22.9 (CH_2-lipid), 20.0 (CH_3-lipid), 19.9 (CH_3-lipid), 19.9 (CH_3-lipid), 19.6 (CH_3-lipid), 14.3 (CH_3-lipid). ^{31}P-NMR (162 MHz, $CDCl_3$) δ: 1.04. HRMS $[M+Na]^+$: 1088.72064 found, 1088.72045 calculated.

Sodium 7-carba-β-D-mannopyranosyl-1-phosphoryl-(4S,8S,12S,16S,20S)-4,8,12,16,20-pentamethylheptacosyl (1).

Benzyl protected **30** (25.6 mg, 0.024 mol, 1 eq) was dissolved in a mixture of $CHCl_3$:MeOH (1:1, v:v, 2.4 mL) and the solution was purged and bubbled through with a flow of Argon. Then Pd/C (12 mg) was added and the solution was purged and bubbled through with Argon once more. The suspension was bubbled through with Hydrogen and subsequently stirred vigorously under a Hydrogen atmosphere. After 6 hours, the reaction mixture was purged of Hydrogen and filtered over a celite pad. The filtrate was concentrated and suspended in acetone. The suspension was filtered over another celite pad. The product was then eluted by washing the celite pad with a mixture of $CHCl_3$/MeOH/water 9.5:9.5:1 and the filtrate was concentrated *in vacuo*. Compound **1** was obtained after size exclusion chromatography (LH-20, DCM/MeOH, 1/1, v/v) as a white solid (8.0 mg, 0.024 mmol, 47%). $[a]_D^{20}$= + 6.0 °(c = 0.003, DCM). ^{1}H NMR (500 MHz, $CDCl_3$/MeOD/D_2O 95:95:10) δ: 4.26 – 4.10 (m, 2H, H-1, H-2), 3.92 – 3.78 (m, 2H, OCH_2-lipid), 3.77 – 3.65 (m, 2H, H-6), 3.65 – 3.56 (m, 1H, H-4), 3.45 – 3.38 (m, 1H, H-3), 1.88 – 1.70 (m, 2H, H-7), 1.70 – 1.57 (m, 2H, CH_2-lipid), 1.57 – 1.48 (m, 1H, H-5), 1.48 – 1.16 (m, 36H, CH_2-lipid, CH-lipid), 1.16 – 1.01 (m, 9H, CH_2-lipid, CH-lipid), 0.97 – 0.78 (m, 18H, CH_3-lipid). ^{13}C-APT NMR (126 MHz, $CDCl_3$/MeOD/D_2O 95:95:10) δ: 74.0 (C-3), 73.3 (d, J = 5.76 Hz, C-1), 72.0 (d, J = 3.12 Hz, C-2), 70.4 (C-4), 65.6 (d, J = 5.52 Hz, OCH_2-lipid), 63.1 (C-6), 40.1 (C-5), 36.9 (CH_2-lipid), 36.8 (CH_2-lipid), 36.8 (CH_2-lipid), 36.7 (CH_2-lipid), 36.4 (CH_2-lipid), 32.6 (CH_2-lipid), 32.3 (CH-lipid), 32.2 (CH-lipid), 32.2 (CH-lipid), 32.1 (CH-lipid), 31.4 (CH_2-lipid), 29.4 (CH_2-lipid), 29.0 (CH_2-lipid), 28.8 (CH_2-lipid), 27.9 (CH_2-lipid), 27.8 (C8), 27.6 (C-7), 26.5 (CH_2-lipid), 23.9 (CH_2-lipid), 23.9 (CH_2-lipid), 23.8 (CH_2-lipid), 22.1 (CH_2-lipid), 19.1 (CH_3-lipid), 19.0 (CH_3-lipid), 18.9 (CH_3-lipid), 18.7 (CH_3-lipid), 13.2 (CH_3-lipid). ^{31}P-Hdec NMR (202 MHz, $CDCl_3$/MeOD/D_2O 95:95:10) δ: 1.28. HRMS $[M+H]^+$: 707.55744 found, 707.55413 calculated.

Mannose-1-C-phosphonate mycoketide



S2 Figure - Synthetic scheme for the generation of C-mannoside 2. a) Ac_2O, pyridine, 77%, b) PhSH, $BF_3{\cdot}Et_2O$, DCM, 74%, c) i. NaOMe, MeOH, ii. BnBr, NaH, TBAI, DMF, 96%, d) NBS, acetone/H_2O 9:1, 30°C quant., e) DMSO, Ac_2O, 77%, f) Cp_2TiMe_2, toluene, 60°C, 79%, g) $(MeO)_2P(=O)H$, DPAP, neat, hν = 375 nm, 59%, h) TMSBr, pyridine, CH_3CN, 85%, i) iPr$_3$PhSO$_2$Cl, pyridine, 50°C, 50%, j) Pd/C, H_2, THF/H_2O (1:1, v/v), 80%.

1,2,3,4,6-penta-O-acetyl-D-mannopyranoside (31).

D(+)-mannose (27.02 g, 150 mmol, 1 eq) was dissolved in pyridine (250 ml). The solution was cooled to 0°C and then Ac_2O (102 ml, 1.08 mol, 7.2 eq) was added. The reaction mixture was stirred at RT overnight. The reaction was quenched with the addition of MeOH, diluted with Et_2O and transferred to a separatory funnel. The organic layer was washed (2x) with a 1 M HCl solution, (1x) with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **31** was obtained as a yellow syrup (45.38 g, 116.3 mmol, 77%) without any further purification. NMR analysis confirmed purity of the product, whose ^{1}H NMR and ^{13}C NMR spectra were in agreement with published literature.[35]

1-thiophenyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (32).

Compound **31** (45.3 g, 116 mmol, 1 eq) was co-evaporated (3x) with toluene and dissolved in dry DCM (130 ml). Then thiophenol (18 ml, 175 mmol, 1.5 eq) was added and the solution was cooled to 0°C. $BF_3{\cdot}Et_2O$ (72 ml, 581 mmol, 5 eq) was added via dropping funnel and the reaction mixture was stirred overnight at RT. Et_3N (80 ml, 581 mmol, 5 eq) and a saturated solution of $NaHCO_3$ were subsequently added before transferring the mixture to a separatory funnel. The water layer was extracted (2x) with DCM and the combined organic layers were dried with $MgSO_4$, filtered and concentrated *in vacuo*. Compound **32** was obtained after crystallization from Et_2O as a white crystal (37.87 g, 86 mmol, 74%). NMR analysis confirmed

purity of the product, whose ^{1}H NMR and ^{13}C NMR spectra were in agreement with published literature.[36]

1-thiophenyl-2,3,4,6-tetra-O-benzyl-α-D-mannopyranoside (33).

Compound **32** (22 g, 50 mmol, 1 eq) was dissolved in dry MeOH and cooled to 0°C. NaOMe (1.62 g, 30 mmol, 0.6 eq) was added to the solution, which was gradually warmed up to RT and stirred for 2 hours. The reaction mixture was then diluted with MeOH, quenched with amberlite H^+ until neutral pH, filtered and concentrated. The crude was dissolved in dry DMF (370 ml) and the solution was cooled to 0°C. After the careful addition of NaH (60% in oil) (12 g, 300 mmol, 6 eq), the reaction mixture was stirred for 2 hours. Benzyl bromide (35.7 ml, 300 mmol, 6 eq) and TBAI (1.55 g, 6.6 mmol, 0.13 eq) were added and the reaction mixture was stirred overnight at RT. The reaction was then quenched with MeOH and diluted with Et_2O and water and transferred to a separatory funnel. The water layer was extracted (4x) with Et_2O, the combined organic layers were washed (1x) with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **33** was obtained after silicagel chromatography (Pentane/EtOAc 20:1→9:1; DCM loading of crude) as a white solid (30.36 g, 48 mmol, 96%). NMR analysis confirmed purity of the product, whose ^{1}H NMR and ^{13}C NMR spectra were in agreement with published literature.[37]

2,3,4,6-tetra-O-benzyl-D-mannopyranoside (34).

Compound **33** (30.36 g, 48 mmol, 1 eq) was dissolved in a mixture of acetone/water 9:1 (800 ml) and cooled to 0°C. NBS (25 g, 140 mmol, 2.9 eq) was added and the reaction mixture was stirred for 4 hours at room temperature. The reaction mixture was diluted with water and Et_2O and transferred to a separatory funnel. The water layer was extracted (3x) with Et_2O and the combined organic layers were washed (1x) with a saturated solution of $NaHCO_3$ and (1x) with brine. The organic layer was then dried over Na_2SO_4, filtered and concentrated *in vacuo*. Compound **34** was obtained after silicagel chromatography (Petroleum ether/EtOAc 9:1→1:1; DCM loading of crude) as a colorless oil (26 g, 48 mmol, quant). NMR analysis confirmed purity of the product, whose ^{1}H NMR and ^{13}C NMR spectra were in agreement with published literature.[38]

1-one-2,3,4,6-tetra-O-benzyl-D-mannopyranoside (7).

Compound **34** (23.34 g, 43 mmol, 1 eq) was dissolved in dry DMSO (430 ml) and heated to 30°C. Then Ac_2O (81 ml, 860 mmol, 20 eq) was added and the reaction mixture was stirred overnight at 30°C. The reaction mixture was diluted with water and Et_2O and transferred to a separatory funnel. The water layer was extracted (4x) with Et_2O and the combined organic phases dried over dried over $MgSO_4$, filtered and concentrated *in vacuo*. Compound **7** was obtained after crystallization from Et_2O/pentane as a white crystal (17.85 g, 33 mmol, 77%). NMR analysis confirmed purity of the product, whose ^{1}H NMR and ^{13}C NMR spectra were in agreement with published literature.[39]

2,3,4,6-tetra-O-benzyl-1-deoxy-D-manno-hept-1-enitol (17).

Compound **7** (2.6 g, 4.8 mmol, 1 eq) was co-evaporated (2x) with toluene and dissolved in toluene (24 ml). After protecting the reaction vessel from light, a 5% solution of dimethyltitanocene (44 ml, 9.4 mmol, 1.95 eq) in THF/toluene was added, the reaction

mixture was heated to 60°C and stirred overnight. Since the reaction was not complete, an additional portion of dimethyltitanocene (20 ml, 4.6 mmol, 0.96 eq) was added and the reaction mixture stirred for an additional day. At this point the volatiles were removed *in vacuo*. Compound **17** was obtained after silicagel chromatography (Pentane/Et_2O 24:1→6:1; DCM loading of crude) as a colorless oil (2.03 g, 3.8 mmol, 79%). NMR analysis confirmed purity of the product, whose ^1H NMR and ^{13}C NMR spectra were in agreement with published literature.[40]

Dimethyl 2,3,4,6-tetra-O-benzyl-β-D-manno-heptulopyranose-1- phosphonate (18b).

Focused UV irradiation: A mixture of compound **17** (139 mg, 0.25 mmol, 1 eq), 2,2-dimethoxy-2-phenylacetophenone (27 mg, 0.13 mmol, 0.5 eq) and dimethyl phosphite (2.3 ml, 25 mmol, 100 eq) was stirred and irradiated for 3 hours and 30 minutes using UV light (375 nm). Volatiles were removed *in vacuo*. Compound **18b** was obtained after silicagel chromatography (DCM/acetone 15:1→8:2; DCM/acetone loading of crude) as a colorless oil (385 mg, 0.59 mmol, 59%).

Unfocused sunlight:

A mixture of compound **17** (47 mg, 0.08 mmol, 1 eq), 2,2-dimethoxy-2-phenylacetophenone (10 mg, 0.04 mmol, 0.5 eq) and dimethyl phosphite (0.7 ml, 8 mmol, 100 eq) was stirred for 1 day at RT in the absence of additional UV irradiation. Volatiles were removed *in vacuo*. Compound **18b** was obtained after silicagel chromatography (DCM/acetone 15:1→8:2; DCM/acetone loading of crude) as a colorless oil (22 mg, 0.034 mmol, 43%). ^1H NMR (300 MHz, $CDCl_3$) δ: 7.39 – 7.15 (m, 20H, H-arom), 5.07 (d, J = 11.5 Hz, 1H, CH_2-Bn), 4.87 (d, J = 10.8 Hz, 1H, CH_2-Bn), 4.84 – 4.71 (m, 2H, CH_2-Bn), 4.67 (d, J = 11.5 Hz, 1H, CH_2-Bn), 4.62 – 4.45 (m, 4H, CH_2-Bn), 3.95 – 3.84 (m, 2H, H-4, H-2), 3.84 – 3.71 (m, 2H, H-1, H-3), 3.71 – 3.56 (m, 8H, CH_3-O, H-6), 3.50 (ddd, J = 9.8, 5.1, 2.4 Hz, 1H, H-5), 2.21 (ddd, J = 18.1, 15.4, 6.9 Hz, 1H, CH_2-P), 1.98 (ddd, J = 18.3, 15.4, 6.1 Hz, 1H, CH_2-P). ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 138.7 (C-arom), 138.4 (C-arom), 128.6 (C-arom), 128.5 (C-arom), 128.4 (C-arom), 128.4 (C-arom), 128.3 (C-arom), 128.2 (C-arom), 128.0 (C-arom), 127.9 (C-arom), 127.8 (C-arom), 127.7 (C-arom), 127.7 (C-arom), 85.0 (C-3), 79.6 (C-5), 75.94 (d, J = 8.3 Hz, C-2), 75.3 (CH_2-Bn), 75.0 (C-4), 74.7 (CH_2-Bn), 73.5 (CH_2-Bn), 73.4 (C-1), 72.8 (CH_2-Bn), 69.6 (C-6), 52.57 (d, J = 74.0 Hz, CH_3-O), 27.76 (d, J = 140.8 Hz, CH_2-P). ^{31}P-Hdec NMR (121 MHz, $CDCl_3$) δ: 30.7. HRMS $[M+H]^+$: 647.2794 found, 647.2768 calculated.

2,3,4,6-tetra-O-benzyl-β-D-manno-heptulopyranose-1- benzyl ketone (18a).

^1H NMR (300 MHz, $CDCl_3$) δ: 7.88 – 7.78 (m, 2H, H-arom), 7.62 – 7.50 (m, 1H, H-arom), 7.47 – 7.02 (m, 22H, H-arom), 4.99 (d, J = 11.8 Hz, 1H, CH_2-Bn), 4.94 – 4.71 (m, 3H, CH_2-Bn), 4.63 – 4.46 (m, 4H, CH_2-Bn), 4.07 – 3.98 (m, 2H, H-3, H-1), 3.93 (t, J = 9.5 Hz, 1H, H-4), 3.80 – 3.73 (m, 1H, H-2), 3.73 – 3.62 (m, 2H, H-6), 3.51 (ddd, J = 9.7, 5.3, 2.1 Hz, 1H, H-5), 3.28 (dd, J = 6.3, 4.1 Hz, 2H, CH_2-C=O). ^{13}C-bbdec NMR (75 MHz, $CDCl_3$) δ: 138.5 (C-arom), 133.4 (C-arom), 128.6 (C-arom), 128.5 (C-arom), 128.5 (C-arom), 128.4 (C-arom), 128.4 (C-arom), 128.3 (C-arom), 128.2 (C-arom), 128.1 (C-arom), 127.8 (C-arom), 127.7 (C-arom), 127.6 (C-arom), 85.3, 79.8, 75.4, 74.6, 74.5, 74.5, 73.6, 72.7, 69.7 (C-6), 40.0 (CH_2-C=O).

Pyridinium 2,3,4,6-tetra-O-benzyl-β-D-manno-heptulopyranose-1-phosphonate (8).

Compound **18b** (385 mg, 0.59 mmol, 1 eq) was co-evaporated (3x) with toluene, dissolved in dry CH_3CN (39 ml) and cooled to 0°C. A glass stopper was used to seal the reaction vessel and a glass covered stirring rod was used to stir the reaction. Pyridine (0.55 ml, 6.8 mmol, 11.5 eq) and TMSBr (1.56 ml, 11.8 mmol, 20 eq) were added dropwise via syringe. The reaction was heated up to RT and stirred for 2 hours. Volatiles were removed *in vacuo*, with water bath temperature of 15°C under fume hood. Ice cold milli-q water and acetonitrile were added and after stirring the mixture for 20 minutes, the volatiles were removed *in vacuo*. Compound **8** was obtained as a white solid (349 mg, 0.5 mmol, 85%) without any further purification. ^{1}H NMR (400 MHz, MeOD/$CDCl_3$ 3:1) δ: 8.81 – 8.62 (m, 6H, H-pyr), 8.48 – 8.36 (m, 3H, H-pyr), 7.97 – 7.81 (m, 7H, H-pyr), 7.29 – 7.06 (m, 20H, H-arom), 4.91 (d, J = 10.6 Hz, 1H, CH_2-Bn), 4.76 (d, J = 11.3 Hz, 2H, CH_2-Bn), 4.61 (d, J = 11.1 Hz, 2H, CH_2-Bn), 4.48 (d, J = 10.9 Hz, 1H, CH_2-Bn), 4.35 – 4.26 (m, 2H, CH_2-Bn), 4.11 (d, J = 2.7 Hz, 1H, H-2), 3.90 – 3.77 (m, 2H, H-4, H-1), 3.68 (dd, J = 9.5, 2.8 Hz, 1H, H-3), 3.60 (dd (2X), J = 10.8, 3.1 Hz, 2H, H-6), 3.37 (ddd, J = 9.7, 4.3, 2.0 Hz, 1H, H-5), 2.09 (ddd (2X), J = 15.1, 11.0, 7.5 Hz, 2H, CH_2-P). ^{13}C-APT NMR (101 MHz, MeOD) δ: 148.1 (C-arom), 143.3 (C-arom), 140.3 (C-arom), 139.9 (C-arom), 139.9 (C-arom), 139.4 (C-arom), 130.8 (C-arom), 129.5 (C-arom), 129.5 (C-arom), 129.5 (C-arom), 129.4 (C-arom), 129.4 (C-arom), 129.4 (C-arom), 129.4 (C-arom), 129.3 (C-arom), 129.3 (C-arom), 129.2 (C-arom), 129.0 (C-arom), 128.9 (C-arom), 128.8 (C-arom), 128.8 (C-arom), 128.8 (C-arom), 128.8 (C-arom), 86.0 (C-3), 80.5 (C-5), 77.70 (d, J = 7.3 Hz, C-2), 76.3 (CH_2-Bn), 76.1 (CH_2-Bn), 75.9 (C-4), 75.5 (C-1), 74.5 (CH_2-Bn), 73.3 (CH_2-Bn), 70.6 (C-6), 31.00 (d, J = 137.6 Hz, CH_2-P). 31P-Hdec NMR (202 MHz, MeOD) δ: 26.5. HRMS $[M+H]^+$: 619.2466 found, 619.2455 calculated.

1-phosphoryl-(4S,8S,12S,16S,20S)-4,8,12,16,20-pentamethylheptacosyl-2,3,4,6-tetra-O-benzyl-β-D-manno- heptulopyranose (35).

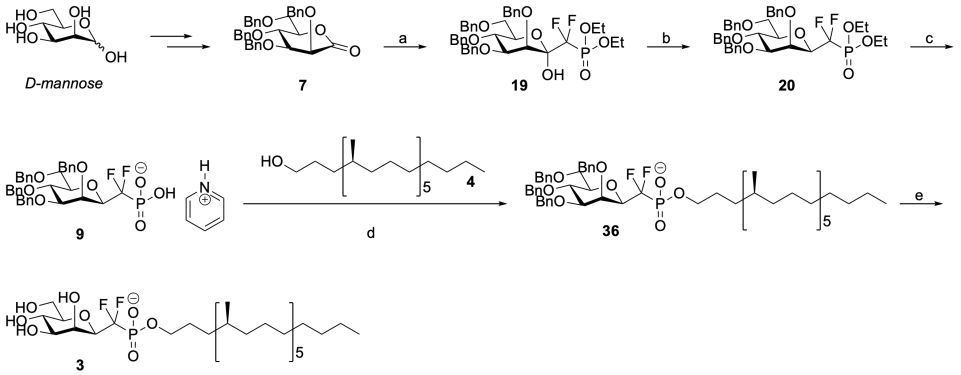
Compound **8** (100 mg, 0.1 mmol, 2 eq) and lipid 10 (20 mg, 0.04 mmol, 1 eq) were co-evaporated (2x) in toluene and dissolved in dry pyridine (1.4 ml). TIPPSCl (45 mg, 0.15 mmol, 3 eq) was added and the reaction mixture was stirred at 50°C overnight. The reaction was quenched with milli-q water and stirred for 2 hours, then EtOAc was added and the reaction mixture was transferred to a separatory funnel. The water layer was extracted (1x) with EtOAc and (2x) with DCM, dried over Na_2SO_4 and concentrated *in vacuo*. Compound **35** was obtained after silicagel chromatography (DCM/MeOH 20:1→1:1; DCM/MeOH loading of crude; ultrapure silica) and size exclusion (LH-20, DCM/MeOH, 1/1, v/v) as a colorless oil (20 mg, 0.02 mmol, 50%). The pyridinium salt was converted to the sodium salt by dissolving the product in MeOH and treating it with amberlite Na^+. ^{1}H NMR (600 MHz, $CDCl_3$/MeOD 9:1) δ: 7.38 – 7.21 (m, 18H, H-arom), 7.10 – 6.97 (m, 2H, H-arom), 4.98 (d, J = 11.1 Hz, 1H, CH_2-Bn), 4.83 – 4.72 (m, 2H, CH_2-Bn), 4.70 (d, J = 11.6 Hz, 1H, CH_2-Bn), 4.63 (d, J = 11.1 Hz, 1H, CH_2-Bn), 4.60 – 4.47 (m, 2H, CH_2-Bn), 4.36 (d, J = 10.8 Hz, 1H, CH_2-Bn), 3.85 – 3.77 (m, 2H, H-1, H-2), 3.77 – 3.67 (m, 3H, CH_2-O, H-6a), 3.66 – 3.55 (m, 2H, H-3, H-4), 3.55 – 3.45 (m, 2H, H-6b, H-5), 2.09 – 1.94 (m, 2H, CH_2-P), 1.65 – 1.47 (m, 3H, CH_2-lipid, CH-lipid), 1.37 – 1.17 (m, 33H, CH_2-lipid, CH-lipid), 1.10 – 1.01 (m, 9H, CH_2-lipid, CH-lipid), 0.88 (t, J = 7.0 Hz, 3H, CH_3-lipid), 0.86 – 0.76 (m, 15H, CH_3-lipid). ^{13}C-APT NMR (151 MHz, $CDCl_3$/MeOD 9:1) δ: 138.2 (C-arom), 138.1 (C-arom), 138.0 (C-arom), 137.9 (C-arom), 137.8 (C-arom), 137.0 (C-arom), 128.6 (C-arom), 128.5 (C-arom), 128.5 (C-arom), 128.4 (C-arom), 128.4 (C-arom), 128.4 (C-arom), 128.3 (C-arom), 128.3 (C-arom), 128.2 (C-arom), 128.1 (C-arom), 128.0 (C-arom), 128.0 (C-arom),

127.9 (C-arom), 127.9 (C-arom), 127.8 (C-arom), 127.8 (C-arom), 127.8 (C-arom), 127.7 (C-arom), 127.7 (C-arom), 84.5 (C-3), 78.1 (C-5), 77.3 (C-2), 75.2 (CH_2-Bn), 75.2 (CH_2-Bn), 75.0 (C-1), 74.8 (C-4), 72.9 (CH_2-Bn), 72.5 (CH_2-Bn), 68.6 (C-6), 64.8 (CH_2-O), 37.5 (CH_2-lipid), 37.5 (CH_2-lipid), 37.5 (CH_2-lipid), 37.5 (CH_2-lipid), 37.4 (CH_2-lipid), 37.4 (CH_2-lipid), 37.4 (CH_2-lipid), 37.1 (CH_2-lipid), 33.2 (CH_2-lipid), 32.9 (CH-lipid), 32.9 (CH-lipid), 32.8 (CH-lipid), 32.8 (CH-lipid), 32.8 (CH_3-lipid), 32.0 (CH_2-lipid), 30.0 (CH_2-P), 29.7 (CH_2-lipid), 29.5 (CH_2-lipid), 29.5 (CH_2-lipid), 29.4 (CH_2-P), 29.1 (CH_2-lipid), 28.9 (CH_2-lipid), 28.8 (CH_2-lipid), 28.7 (CH_2-lipid), 28.6 (CH_2-lipid), 28.2 (CH_2-lipid), 27.1 (CH_2-lipid), 24.6 (CH_2-lipid), 24.5 (CH_2-lipid), 24.5 (CH_2-lipid), 24.5 (CH_2-lipid), 22.7 (CH_2-lipid), 19.8 (CH_3-lipid), 19.8 (CH_3-lipid), 19.7 (CH_3-lipid), 19.5 (CH_3-lipid), 19.5 (CH_3-lipid), 14.1 (CH_3-lipid). ^{31}P-Hdec NMR (162 MHz, $CDCl_3$/MeOD 9:1) δ: 25.6, 24.9. HRMS $[M+H]^+$: 1067.74611 found, 1067.74633 calculated.

1-phosphoryl-(4S,8S,12S,16S,20S)-4,8,12,16,20-pentamethylheptacosyl-β-D-manno-heptulopyranose (2).

Compound **35** (20 mg, 0.02 mmol, 1 eq) was dissolved in a mixture of THF/H_2O 1:1 and the solution was purged and bubbled through with a flow of Argon. Then Pd/C (10 mg) was added and the solution was purged and bubbled through with Argon once more. The suspension was bubbled through with Hydrogen and subsequently stirred vigorously under a Hydrogen atmosphere. After 16 hours, the reaction mixture was purged of Hydrogen and filtered over a Whatman filter. Compound **2** was obtained after *in vacuo* removal of volatiles as an amorphous solid (12 mg, 0.016 mmol, 80%). ^{1}H NMR (850 MHz, $CDCl_3$/MeOD/D_2O 95:95:10) δ: 3.91 (d, J = 2.5 Hz, 1H, H-2), 3.88 – 3.78 (m, 4H, H-6a, CH_2-O, H-1), 3.71 (dd, J = 12.0, 5.6 Hz, 1H, H-6b), 3.60 – 3.53 (m, 2H, H-3, H-4), 3.28 (ddt, J = 8.0, 5.6, 2.3 Hz, 1H, H-5), 2.01 – 1.96 (m, 1H, CH_2-P), 1.95 – 1.92 (m, 1H, CH_2-P), 1.69 – 1.56 (m, 4H, CH_2-lipid), 1.43 – 1.15 (m, 43H, CH_2-lipid, CH-lipid), 1.12 – 1.04 (m, 8H, CH_2-lipid), 0.92 – 0.81 (m, 18H, CH_3-lipid). ^{13}C-APT NMR (214 MHz, $CDCl_3$/MeOD/D_2O 95:95:10) δ: 79.8 (C-5), 74.4 (C-1), 74.3 (C-3), 71.19 (d, J = 7.5 Hz, C-2), 66.7 (C-4), 64.32 (d, J = 5.6 Hz, CH_2-O), 60.9 (C-6), 37.0 (CH_2-lipid), 36.9 (CH_2-lipid), 36.9 (CH_2-lipid), 36.9 (CH_2-lipid), 36.9 (CH_2-lipid), 36.8 (CH_2-lipid), 36.8 (CH_2- lipid), 36.5 (CH_2-lipid), 32.7 (CH_2-lipid), 32.3 (CH-lipid), 32.3 (CH-lipid), 32.3 (CH-lipid), 32.2 (CH-lipid), 32.2 (CH-lipid), 31.4 (CH_2-lipid), 29.4 (CH_2-lipid), 29.2 (CH_2-lipid), 29.2 (CH_2-lipid), 28.9 (CH_2-lipid), 28.8 (CH_2-lipid), 28.2 (CH_2- lipid), 28.1 (CH_2-lipid), 26.5 (CH_2-lipid), 24.0 (CH_2-lipid), 24.0 (CH_2-lipid), 23.9 (CH_2-lipid), 23.9 (CH_2-lipid), 23.1 (CH_2-lipid), 22.2 (CH_2-lipid), 19.2 (CH_3-lipid), 19.1 (CH_3-lipid), 19.1 (CH_3-lipid), 19.0 (CH_3-lipid), 18.8 (CH_3-lipid), 13.3 (CH_3-lipid). ^{31}P-Hdec NMR (202 MHz, $CDCl_3$/MeOD/D_2O 95:95:10) δ: 23.0, 22.5. HRMS $[M+H]^+$: 707.5596 found, 707.5585 calculated.

Mannose-1-C-difluorophosphonate mycoketide



S3 Figure - Synthetic scheme for the generation of difluoro-C-mannoside 3. a) LDA, $(EtO)_2P(=O)CHF_2$, THF, 98%, b) i. $MeO_2CC(=O)Cl$, DCM, ii. AIBN, Bu_3SnH, toluene, 30%, c) TMSBr, pyridine, CH_3CN, quant., d) iPr$_3$PhSO$_2$Cl, toluene:DMF:pyridine (1.25:1:0.5, v/v/v), 50°C, 64%, 6) Pd/C, H_2, THF:H_2O (2:1, v/v), 82%.

Diethyl 1-hydroxy-2,3,4,6-tetra-O-benzyl-β-D-manno-heptulopyranose-1-(difluoro)phosphonate (19).

A solution of DIPA (1.6 ml, 11 mmol, 2 eq) in THF (30 ml) was cooled to -78°C. After the addition of n-BuLi (6.9 ml, 11 mmol, 2 eq) dropwise via syringe, the reaction mixture was rapidly brought to 0°C for 10 minutes and then cooled again to -78°C. A solution of diethyl (difluoromethyl)phosphonate (1.4 ml, 9.3 mmol, 1.7 eq) in THF (10 ml) was cooled to -78°C and added dropwise via cannula to the first solution. After 1 hour and 30 minutes, a solution of lactone **7** (2.96 g, 5.5 mmol, 1 eq) in THF (10 ml) was cooled to -78°C and added dropwise via cannula to the reaction mixture. After 10 minutes from the addition of the last drop of lactone the reaction was stirred for additional 10 minutes with a saturated solution of NH_4Cl(aq) and finally diluted with EtOAc and transferred to a separatory funnel. The water layer was extracted (3x) with EtOAc and the combined organic layers were washed (1x) with water and (1x) with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Compound **19** was obtained after silicagel chromatography (Pentane/EtOAc 10:1→2:1; DCM loading of crude) as a colorless syrup (3.95 g, 5.4 mmol, 98%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.40 – 7.17 (m, 20H, H-arom), 6.00 (s, 1H, -OH), 4.87 (d, J = 10.9 Hz, 1H, CH_2-Bn), 4.82 (d, J = 11.0 Hz, 1H, CH_2-Bn), 4.78 – 4.66 (m, 3H, CH_2-Bn), 4.60 – 4.51 (m, 2H, CH_2-Bn), 4.42 (d, J = 11.8 Hz, 1H, CH_2-Bn), 4.34 – 4.23 (m, 4H, CH_2-O), 4.20 (bs, 1H, H-2), 4.17 – 4.09 (m, 2H, H-3, H-5), 3.99 (t, J = 9.7 Hz, 1H, H-4), 3.79 (dd, J = 11.2, 5.9 Hz, 1H, H-6a), 3.69 (dd, J = 11.4, 1.7 Hz, 1H, H-6b), 1.38 (t, J = 7.1 Hz, 3H, CH_3), 1.17 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 138.6 (C-arom), 138.5 (C-arom), 138.4 (C-arom), 138.3 (C-arom), 128.5 (C-arom), 128.4 (C-arom), 128.4 (C-arom), 128.2 (C-arom), 128.1 (C-arom), 128.1 (C-arom), 127.8 (C-arom), 127.7 (C-arom), 127.6 (C-arom), 127.6 (C-arom), 127.6 (C-arom), 127.4 (C-arom), 116.56 (ddd, J = 286.9, 265.2, 194.0 Hz, CF2), 96.61 (ddd, J = 30.6, 19.1, 11.1 Hz, C-1), 81.08 (d, J = 2.6 Hz, C-3), 75.2 (C-2), 75.2

(CH_2-Bn), 74.7 (CH_2-Bn), 74.6 (C-4), 73.1 (CH_2-Bn), 73.1 (C-5), 72.4 (CH_2-Bn), 69.5 (C-6), 65.71 (dd, J = 50.5, 6.3 Hz, CH_2-O), 16.35 (dd, J = 15.2, 5.9 Hz, CH_3). ^{19}F NMR (471 MHz, $CDCl_3$) δ: -118.38 (dd, J = 304.5, 96.1 Hz), -119.83 (dd, J = 304.5, 100.1 Hz). ^{31}P NMR (202 MHz, $CDCl_3$) δ: 8.14 (dd, J = 100.0, 96.0 Hz). HRMS $[M+Na]^+$: 749.26594 found, 749.26615 calculated.

Diethyl 2,3,4,6-tetra-O-benzyl-β-D-manno-heptulopyranose-1-(difluoro)phosphonate (20).

Compound **19** (3.6 g, 5 mmol, 1 eq) was co-evaporated with toluene and dissolved in a dry mixture of DCM/pyridine 5:1 (25 ml) before being cooled to 0°C. Methyl oxalyl chloride (0.92 ml, 10 mmol, 2 eq) was dissolved in DCM (2.5 ml) and slowly added to the first solution via syringe before an extra portion of pyridine was added (2.5 ml). The reaction mixture was warmed up to RT and stirred for 10 minutes before quenching it with EtOH over 10 minutes. Then a saturated solution of $NaHCO_3$ and DCM were used to dilute the reaction mixture before it was transferred to a separatory funnel. The organic layer was concentrated *in vacuo* and the reaction intermediate was dissolved in dry toluene (250 ml) without further purification. This solution was purged and bubbled through with Argon before the addition of Bu_3SnH and AIBN. The reaction mixture was heated up to reflux and stirred overnight. After removal of volatiles *in vacuo*, the crude was purified via silicagel chromatography (Pentane/EtOAc 9:1→3:1), followed by treatment with a 1 M KF(aq) solution and silicagel/KCO_3 9:1 chromatography (Pentane/EtOAc 3:2). Compound **20** was obtained after silicagel chromatography as a colorless syrup (1.05 g, 1.48 mmol, 30% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.44 – 7.39 (m, 2H, H-arom), 7.35 – 7.23 (m, 16H, H-arom), 7.20 – 7.17 (m, 2H, H-arom), 4.91 – 4.84 (m, 2H, CH_2-Bn), 4.79 (d, J = 11.2 Hz, 1H, CH_2-Bn), 4.70 (d, J = 11.7 Hz, 1H, CH_2-Bn), 4.64 (d, J = 11.8 Hz, 1H, CH_2-Bn), 4.60 – 4.54 (m, 2H, CH_2-Bn), 4.47 (d, J = 11.8 Hz, 1H, CH_2-Bn), 4.30 – 4.18 (m, 5H, CH_2-O, H-2), 3.98 (t, J = 9.6 Hz, 1H, H-4), 3.91 (dt, J = 22.3, 3.4 Hz, 1H, H-1), 3.78 – 3.69 (m, 2H, H-6), 3.63 – 3.56 (m, 2H, H-3, H-5), 1.36 – 1.31 (m, 3H, CH_3), 1.21 – 1.16 (m, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 138.6 (C-arom), 138.3 (C-arom), 138.2 (C-arom), 138.1 (C-arom), 128.5 (C-arom), 128.5 (C-arom), 128.4 (C-arom), 128.3 (C-arom), 128.2 (C-arom), 128.2 (C-arom), 128.1 (C-arom), 127.9 (C-arom), 127.8 (C-arom), 127.8 (C-arom), 127.7 (C-arom), 127.6 (C-arom), 127.5 (C-arom), 117.40 (ddd, J = 280.4, 257.0, 209.8 Hz, CF2), 84.05 (d, J = 1.9 Hz, C-3), 80.6 (C-5), 75.74 (ddd, J = 31.4, 18.8, 13.6 Hz, C-1), 75.3 (CH_2-Bn), 74.7 (C-4), 74.4 (CH_2-Bn), 73.3 (CH_2-Bn), 72.34 (dd, J = 6.1, 2.1 Hz, C-2), 72.2 (CH_2-Bn), 69.5 (C-6), 64.96 (dd, J = 30.3, 6.5 Hz, CH_2-O), 16.43 (dd, J = 9.5, 5.7 Hz, CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ: 6.73 (dd, J = 101.2, 99.2 Hz). ^{19}F NMR (471 MHz, $CDCl_3$) δ: -116.13 (dd, J = 312.3, 100.9 Hz), -124.45 (ddd, J = 312.4, 99.0, 22.3 Hz). HRMS $[M+Na]^+$: 733.27157 found, 733.27123 calculated.

Pyridinium 2,3,4,6-tetra-O-benzyl-β-D-manno-heptulopyranose-1-(difluoro)phosphonate (9).

Compound **20** (71 mg, 0.1 mmol, 1 eq) was co- evaporated (3x) with toluene, dissolved in dry CH_3CN (6.7 ml) and cooled to 0°C. A glass stopper was used to seal the reaction vessel and a glass covered stirring rod was used to stir the reaction. Pyridine (0.1 ml, 1.2 mmol, 12 eq) and TMSBr (0.26 ml, 2 mmol, 20 eq) were added dropwise via syringe. The reaction was heated up to RT and stirred overnight. Volatiles were removed *in vacuo*, with water bath temperature of 20°C under fume hood. Ice cold milli-q water and acetonitrile were added and after stirring the mixture for 2 hours and 30 minutes, the volatiles were removed *in vacuo*. Compound **9** was obtained as a white solid (70 mg, 0.1 mmol, quant.) without any further purification. ^{1}H

NMR (500 MHz, MeOD) δ: 8.95 – 8.85 (m, 12H, H-pyr), 8.69 – 8.62 (m, 6H, H-pyr), 8.11 – 8.03 (m, 12H, H-pyr), 7.50 – 7.45 (m, 2H, H-arom), 7.39 – 7.24 (m, 15H, H-arom), 7.22 – 7.17 (m, 3H, H-arom), 7.11 – 7.06 (m, 2H, H-arom), 4.97 – 4.91 (m, 3H, CH_2-Bn), 4.79 – 4.74 (m, 2H, CH_2-Bn), 4.66 (d, J = 11.0 Hz, 2H, H-2, CH_2-Bn), 4.29 – 4.11 (m, 4H, CH_2-Bn, H-4, H-1), 3.89 (dd, J = 9.4, 2.7 Hz, 1H, H-3), 3.81 (dd, J = 11.0, 1.9 Hz, 1H, H-6a), 3.74 (dd, J = 11.0, 3.5 Hz, 1H, H-6b), 3.64 (ddd, J = 9.9, 3.6, 1.9 Hz, 1H, H-5). ^{13}C NMR (126 MHz, MeOD) δ: 148.5 (C-arom), 147.0 (C-arom), 143.0 (C-arom), 140.2 (C-arom), 140.0 (C-arom), 140.0 (C-arom), 139.2 (C-arom), 138.9 (C-arom), 129.9 (C-arom), 129.8 (C-arom), 129.8 (C-arom), 129.8 (C-arom), 129.7 (C-arom), 129.7 (C-arom), 129.7 (C-arom), 129.7 (C-arom), 129.6 (C-arom), 129.6 (C-arom), 129.5 (C-arom), 129.5 (C-arom), 129.4 (C-arom), 129.3 (C-arom), 129.2 (C-arom), 129.2 (C-arom), 129.1 (C-arom), 129.0 (C-arom), 129.0 (C-arom), 129.0 (C-arom), 128.9 (C-arom), 128.9 (C-arom), 85.7 (C-3), 81.4 (C-5), 78.12 (ddd, J = 27.8, 11.8, 4.7 Hz, C-1), 76.2 (CH_2-Bn), 76.1 (CH_2-Bn), 75.9 (C-4), 74.7 (C-2), 74.3 (CH_2-Bn), 73.4 (CH_2-Bn), 70.1 (C-6). ^{19}F NMR (471 MHz, MeOD) δ: -118.58 (ddd, J = 302.1, 88.8, 8.1 Hz), -123.20 (ddd, J = 302.2, 94.6, 19.9 Hz). ^{31}P NMR (202 MHz, MeOD) δ: 3.36 (dd, J = 94.7, 89.0 Hz). HRMS $[M+Na]^+$: 655.22681 found, 655.22669 calculated.

1-(difluoro)phosphoryl-(4S,8S,12S,16S,20S)-4,8,12,16,20-pentamethylheptacosyl-2,3,4,6-tetra-O-benzyl-β-D-manno- heptulopyranose (36).

Compound **9** (7 mg, 0.01 mmol, 1 eq) and lipid **4** (16 mg, 0.018 mmol, 3 eq) were co-evaporated (2x) in toluene and dissolved in a mixture of dry DMF/pyridine 2:1 (0.24 ml) and dry toluene (0.20 ml) respectively. This solution was heated up to 60°C before the addition of TIPPSCl (5.6 mg, 0.018 mmol, 1.8 eq). The reaction mixture was then stirred overnight at this temperature and then concentrated. Compound **36** was obtained after silicagel chromatography ($CHCl_3$/MeOH 40:1→9:1; $CHCl_3$/MeOH 40:1 loading of crude; ultrapure silica, neutralized with 1% Et_3N) as a colorless oil (7 mg, 0.0064 mmol, 64%). ^{1}H NMR (500 MHz, MeOD) δ: 7.64 – 6.96 (m, 20H, H-arom), 4.87 (d, J = 10.9 Hz, 2H, CH_2-Bn), 4.81 (d, J = 11.6 Hz, 1H, CH_2-Bn), 4.74 (d, J = 10.7 Hz, 1H, CH_2-Bn), 4.71 (d, J = 11.6 Hz, 1H, CH_2-Bn), 4.59 (d, J = 11.4 Hz, 2H, CH_2-Bn), 4.50 (d, J = 11.7 Hz, 1H, CH_2-Bn), 4.41 (bs, 1H, H-2), 4.06 – 3.93 (m, 2H, H-1, H-4), 3.93 – 3.81 (m, 6H, -OCH_3), 3.80 – 3.70 (m, 3H, H-3, H-6), 3.66 – 3.56 (m, 1H, H-5). ^{13}C NMR (126 MHz, MeOD) δ: 140.2 (C-arom), 140.0 (C-arom), 140.0 (C-arom), 139.8 (C-arom), 129.7 (C-arom), 129.6 (C-arom), 129.6 (C-arom), 129.5 (C-arom), 129.5 (C-arom), 129.4 (C-arom), 129.4 (C-arom), 129.2 (C-arom), 129.0 (C-arom), 129.0 (C-arom), 128.9 (C-arom), 128.8 (C-arom), 85.4 (d, J = 2.1 Hz, H-3), 81.8 (C-5), 77.6 – 77.0 (m, C-1), 76.4 (CH_2-Bn), 76.1 (C-4), 76.0 (CH_2-Bn), 74.5 (CH_2-Bn), 74.22 – 74.12 (m, C-2), 73.5 (CH_2-Bn), 70.7 (C-6), 56.3 (d, J = 6.4 Hz, -OCH_3), 56.0 (d, J = 6.7 Hz, -OCH_3). ^{31}P NMR (202 MHz, MeOD) δ: 9.85 (pt, J = 102.0 Hz). ^{19}F NMR (471 MHz, MeOD) δ: -114.97 (ddd, J = 313.6, 101.5, 4.3 Hz), -123.23 (ddd, J = 313.5, 102.7, 21.8 Hz). HRMS $[M+H]^+$: 1103.72789 found, 1103.72749 calculated.

1-(difluoro)phosphoryl-(4S,8S,12S,16S,20S)-4,8,12,16,20-pentamethylheptacosyl-β-D-manno-heptulopyranose (3).

Compound **36** (12 mg, 0.011 mmol, 1 eq) was dissolved in a mixture of THF/H_2O 2:1 (5 ml) and the solution was purged and bubbled through with a flow of Argon. Then Pd/C (10 mg) was added and the solution was purged and bubbled through with Argon once more. The suspension was bubbled through with Hydrogen and subsequently stirred vigorously under a Hydrogen atmosphere. After 16 hours, the reaction mixture was purged of Hydrogen and

filtered over celite. Compound **3** was obtained after *in vacuo* removal of volatiles as an amorphous solid (7 mg, 0.009 mmol, 82%). ^{1}H NMR (500 MHz, MeOD) δ: 4.28 (d, J = 3.1 Hz, 1H, H-2), 4.10 – 3.94 (m, 2H, OCH_2), 3.89 (pd, J = 12.3 Hz, 1H, H-6a), 3.87 – 3.61 (m, 4H, H-1, H-6b, H-4), 3.57 – 3.49 (m, 1H, H-3), 3.31 – 3.26 (m, 1H, H-5), 3.18 (q, J = 7.3 Hz, 4H, CH_2-triethylamonium), 1.77 – 1.54 (m, 4H, CH_2-lipid), 1.45 – 1.17 (m, 54H, CH_2-lipid, CH-lipid, CH_3-triethylamonium), 1.12 – 1.02 (m, 9H, CH_2-lipid, CH-lipid), 0.90 – 0.84 (m, 18H, CH_3-lipid). ^{13}C NMR (126 MHz, MeOD) δ: 81.0 (C-5), 79.0 (C-1), 74.2 (C-3), 67.3 (C-4), 67.0 (OCH_2), 66.4 (C-4), 60.8 (C-6), 46.2 (CH_2-triethylammonium), 36.9 (CH_2-lipid), 36.8 (CH_2-lipid), 36.7 (CH_2-lipid), 36.4 (CH_2-lipid), 32.4 (CH_2-lipid), 32.3 (CH-lipid), 32.2 (CH-lipid), 32.2 (CH-lipid), 31.4 (CH_2-lipid), 29.4 (CH_2-lipid), 29.1 (CH_2-lipid), 28.8 (CH_2-lipid), 26.5 (CH_2-lipid), 23.9 (CH_2-lipid), 23.9 (CH_2-lipid), 23.8 (CH_2-lipid), 22.1 (CH_2-lipid), 19.1 (CH_3-lipid), 19.1 (CH_3-lipid), 19.1 (CH_3-lipid), 19.0 (CH_3-lipid), 18.7 (CH_3-lipid), 13.3 (CH_3-lipid), 7.9 (CH_3-triethylammonium). ^{31}P NMR (202 MHz, MeOD) δ: 2.84 (t, J = 85.4 Hz). ^{19}F NMR (471 MHz, MeOD) δ: -115.15 (dd, J = 305.6, 86.7 Hz), -117.29 (dd, J = 305.5, 84.5 Hz). HRMS $[M+H]^+$: 683.25772 found, 683.25799 calculated.

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