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Synthesis of monophosphoryl lipid A using 2-naphtylmethyl ethers as permanent protecting groups

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ABSTRACT

Lipid A, which is a conserved component of lipopolysaccharides of gram-negative bacteria, has attracted considerable interest for the development of immuno-adjuvants. Most approaches for lipid A synthesis rely on the use of benzyl ethers as permanent protecting groups. Due to the amphiphilic character of lipid A, these compounds aggregate during the hydrogenation step to remove benzyl ethers, resulting in a sluggish reaction and by-product formation. To address this problem, we have developed a synthetic approach based on the use of 2-naphtylmethyl ether (Nap) ethers as permanent protecting group for hydroxyls. At the end of a synthetic sequence, multiple of these protecting groups can readily be removed by oxidation with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ). Di-allyl *N,N*-diisopropylphosphoramidite was employed to install the phosphate ester and the resulting allyl esters were cleaved using palladium tetrakistriphenylphosphine. The synthetic strategy allows late stage introduction of different fatty acids at the amines of the target compound, which is facilitated by Troc and Fmoc as orthogonal amino-protecting groups.

1. Introduction

Adjuvants, which are molecules that can augment adaptive immune responses, can improve vaccine performance [1–3]. The use of an adjuvant is particularly important for subunit vaccines, such as proteins and polysaccharides, which often have reduced immunogenicity because of a lack of intrinsic immunostimulatory activity [4].

Advances in the understanding of innate immune responses has provided opportunities to design better adjuvants. The innate immune system senses microbes through pattern-recognition receptors, which include Toll-like receptors (TLRs) and C-type lectin-like receptors (CTRs) that are expressed by immune cells such as dendritic, neutrophiles and B-cells. Activation of these receptors leads to the production of cytokines that provide early defence during infection. Cytokines also regulate adaptive immunity by controlling the quantity and quality of B- and T-cell activation, which in turn results in protective immune responses [5]. Ligands for TLRs and CTRs are attractive compounds for the development of adjuvants. The challenge, however, is to discover compounds that can enhance immunogenicity without causing adverse effects.

Lipid A, which is a conserved component of lipopolysaccharides (LPS) of gram-negative bacteria, has attracted considerable interest for the development of adjuvants [4]. It is recognized by TLR4/MD2 complex of dendritic cells resulting in the activation of two intracellular signalling cascades, namely the MyD88 and TRIF pathways that result in the production of (pro)inflammatory mediators [5,6]. The lipid A moiety of *E. coli* consists of a hexaacylated bis-1,4′-phosphorylated glucosamine disaccharide that has (*R*)-3-hydroxymyristyl residues at C-2, C-2′, C-3, and C-3’. The (3)-hydroxyacyl chains of the distal glucosamine moiety are further modified by lauric and myristic acids (Fig. 1). Lipid A of *S. minnesota* has a different acylation pattern, it is hepta-acylated featuring an additional bi-antennary palmitic acid on the (3)-hydroxyacyl of the C-2 amine. The endotoxic activity of LPS and lipid A can result to overactivation of the immune system, which has precluded its use as an adjuvant.

Systematic studies by Ribi and co-workers uncovered that the immunostimulatory properties of lipid A can be separated from the endotoxic effects by hydrolytic treatment using acid and base, resulting in a major species having six fatty acids and only one phosphate moiety at the C-4’ position (Fig. 1) [7,8]. This preparation, which was named

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mono-phosphoryl lipid A (MPLA), is employed by GSK Biologicals for several vaccine formulations [9]. The favourable properties of MPLA probably arise from differences in gene expression compared to native LPS and lipid A, and it induces mainly cytokines associated with the TRIF-dependent pathway [10]. It has also been found that MPLA induces higher levels of IL-10, which has anti-inflammatory properties and may contribute to its low toxicity [11]. MPLA is structurally heterogeneous which may compromise consistent biological performance. To address this shortcoming, chemical approaches have been developed for the preparation of MPLA derivatives [12–18]. In particular, a derivative from the lipid A of *E. coli* (Fig. 1, MPL) has received considerable attention, and enhances immunological response against co-administered antigen epitopes [19–23]. Furthermore, higher T_h1 responses are seen which are linked to a longer lasting protection in vaccination [19,20,24]. MPLA has also been used for the development of self-adjuvating vaccines in which it is covalently attached to an antigen [15,16,25].

The chemical synthesis of lipid A is challenging, especially when an approach is needed that can readily provide various derivatives [26]. Most approaches for lipid A synthesis rely on the use of benzyl ethers as a permanent protecting group. Due to the amphiphilic character of lipid A, these compounds aggregate during the hydrogenation step to remove benzyl ethers, resulting in a sluggish reaction and by-product formation. In particular, we have observed that the benzyl ether at C-4 of the reducing GlcN moiety is particularly resistant to hydrogenation. To address these difficulties, we have employed allyloxycarbonates (Alloc) as permanent protecting groups for the C-3 and C-4 hydroxy groups of the acceptor for MPL synthesis [14]. This protecting group can easily be removed by treatment with $Pd(PPh_3)_4$ without effecting acyloxyacyl- or phosphate esters. MPLA has been synthesized by employing the *N*-2,2,2-trichloroethoxycarbonyl (Troc) protecting group for various hydroxyls and amino groups [12]. In both approaches, several alcohols were still protected as benzyl ether necessitating a hydrogenation step. Furthermore, Troc and Alloc carbonates are very base sensitive making compound handling difficult and restrict the range of chemical manipulations that can be employed. To address these difficulties, we report here a synthesis approach for monophosphoryl lipid A that is based on the use of 2-naphtylmethyl ether (Nap) ethers [27–31] as a permanent protecting group that can readily be cleaved by oxidation with 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) greatly simplifying the final deprotection step. The approach allows late stage modification of the amines thereby offering the possibility to easily prepare analogs having different fatty acid substitutions.

2. Results and discussion

We envisaged that *E. coli* monophosphoryl lipid A (**1**) can be synthesized from monosaccharide building blocks **2** and **3** and (*R*)-3-hydroxymyristic acids **4**, **5** and **6** (Fig. 2). The hydroxyls of the monosaccharides and fatty acid that require permanent protection are modified as Nap ethers. A number of our previous studies have shown that multiple of these protecting groups can be removed at a late stage of synthesis giving confidence in the approach [32,33]. The C-3 hydroxyl of **2** and **3** were already modified by a (*R*)-3-dodecanoyltetradecanoic and (*R*)-3-(2-naphthylmethoxy)tetradecanoic ester, respectively because previous studies had shown that late stage installation of these lipids is challenging and can lead to by-product formation [34]. The C-2 amine of **3** was protected as a Troc group because the carbamate can perform neighbouring group participation during glycosylations, thereby providing only 1,2-*trans*-glycosides. It can easily be removed by Zn in acetic acid to give a free amine that can then be acylated with **5**. The amine of acceptor **3** was protected as fluorenylmethyloxycarbamate (Fmoc), which can be cleaved under mild basic conditions and is fully orthogonal with the Troc protecting group allowing selective acylation of the amines. Finally, the phosphate was protected by allyl esters that can be cleaved by palladium tetrakistriphenylphosphine ($Pd(PPh_3)_4$), which was expected to be fully compatible with other functionalities of the target compound.

The syntheses of lipids **4**–**6** are described in the supporting information (Schemes S1 and S2). Glycosyl donor **2** and acceptor **3** were prepared from common intermediate **7** [35,36]. The preparation of donor **2** started with a Steglich acylation [14] of the C-3 hydroxyl of **7** using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) to give, after silica gel column chromatography, **8** in a yield of 78% (Scheme 1). The naphthylidene acetal of **8** was regioselectively opened using trifluoromethanesulfonic acid (TfOH) in the presence of triethylsilane (TES) [37] as a hydride donor to provide **9**, having a Nap ether at C-6 and a hydroxyl at C-4. The hydroxyl of the latter compound was phosphorylated using di-allyl *N,N*-diisopropylphosphoramidite in the presence of 1H-tetrazole as the activator, followed by *in-situ* of oxidation of the intermediate phosphite by *m*-chloroperbenzoic acid (mCPBA) to

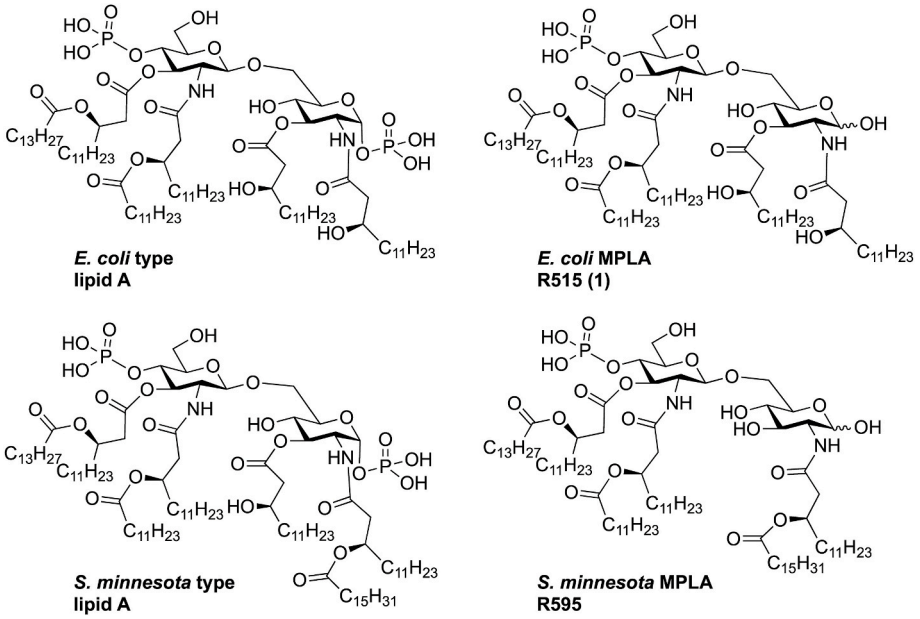


Fig. 1. Structures of *E. coli* and *S. minnesota* lipid A's and their relevant monophosphorylated derivatives.

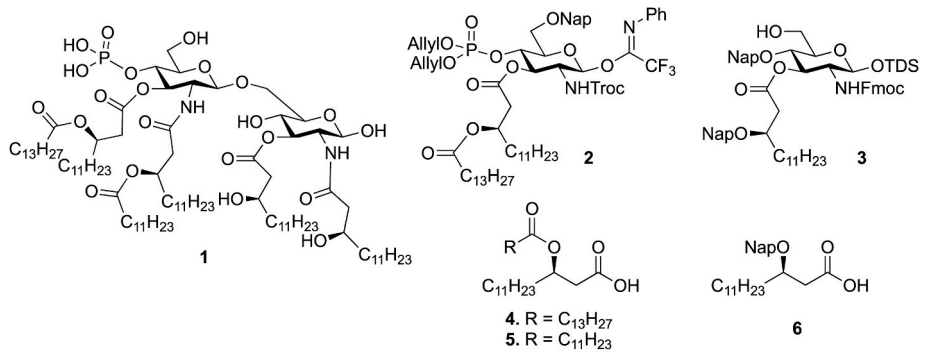
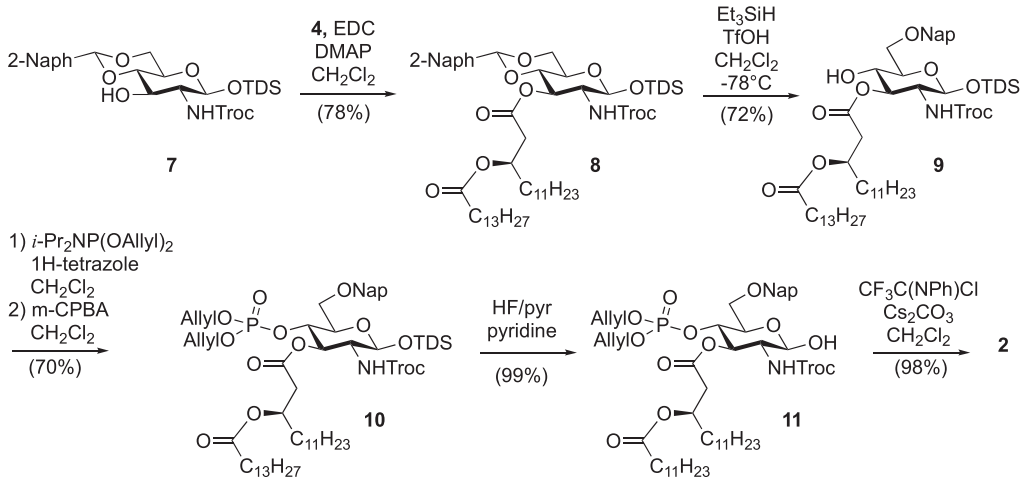


Fig. 2. Monophosphoryl lipid A (**1**) and building blocks (**2**–**6**) for chemical synthesis.

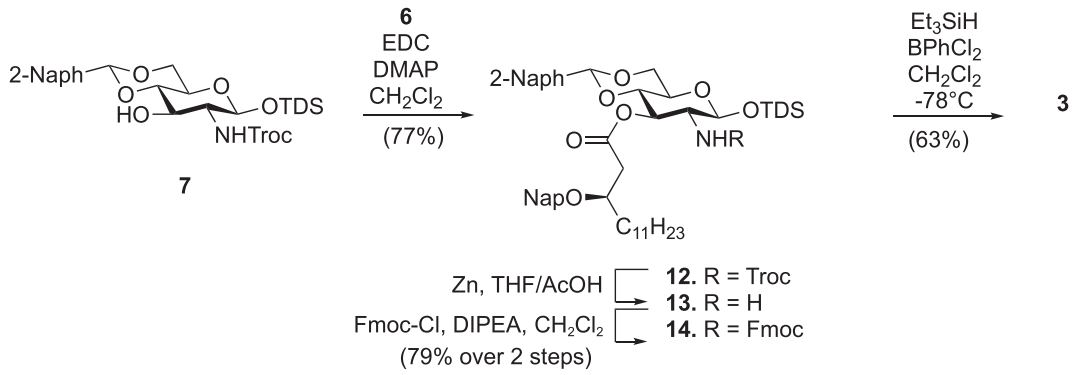


Scheme 1. Preparation of glycosyl donor **2.**

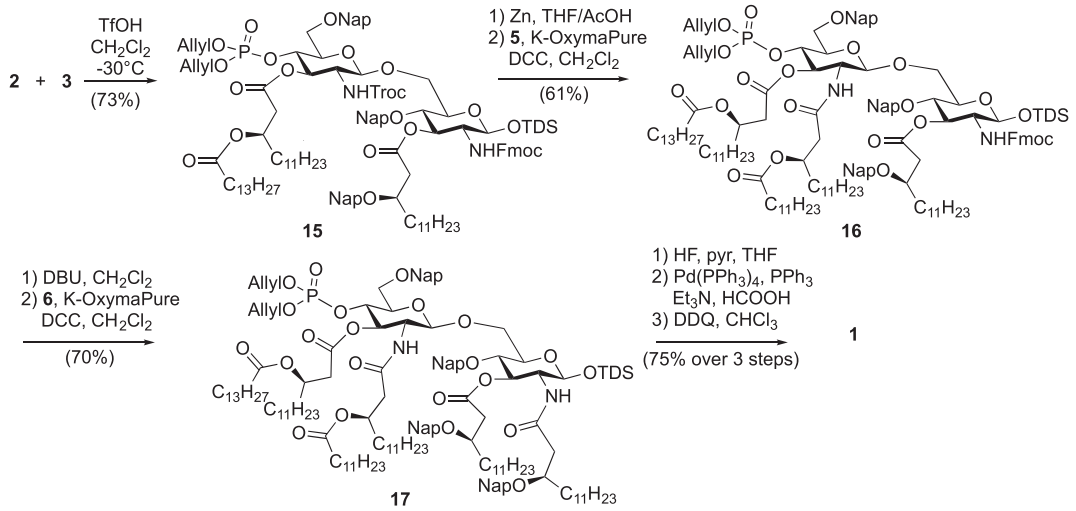
obtain phosphotriester **10**. The anomeric dimethylthexylsilyl (TDS) ether of **10** was removed in near quantitative yield by treatment with HF in pyridine to obtain hemiacetal **11**, which was converted into 2,2,2-trifluoro-*N*-phenylacetimidate **2** by reaction with the corresponding chloride in presence of cesium carbonate [26].

The preparation of glycosyl acceptor **3** started with a Steglich acylation [14] of common intermediate **7** using 2-naphthylmethylether protected lipid **6** to provide compound **12** (Scheme 2). The Troc protecting group of **12** was replaced by an Fmoc group involving a two-step procedure entailing reductive removal of the Troc group, followed by coupling of 9-fluorenylmethyloxycarbonyl chloride to the resulting amine to give **14** in a yield of 79%. Glycosyl acceptor **3** was obtained after selective opening of the naphthylidene acetal of **14** using dichlorophenylborane in the presence of TES [38,39].

Glycosylation of donor **2** with acceptor **3** using TfOH as the promoter, afforded β-linked disaccharide **15** in 73% yield (Scheme 3). The Troc-protecting group was removed using standard conditions. Next, several attempts were made to introduce acylated lipid **5**, which turned out to be challenging, and low yields were obtained by for example using asymmetric anhydride and HATU/DIPEA mediated coupling reaction conditions (≤25%). Under these reaction conditions, lipid migration and residual acetyl coupling was observed. Gratifyingly, **16** was obtained in a yield of 61% by pre-activation of lipid **5** with dicyclohexylcarbodiimide (DCC) and K-OxymaPure [40], followed by addition of the primary amine. Under these conditions, no migratory by-products were observed. Next, the Fmoc protecting group of **16** was removed by treatment with DBU and the resulting amine was efficiently acylated with **6** that was pre-activated with DCC and K-OxymaPure, to give fully



Scheme 2. Preparation of glycosyl acceptor **3.**



Scheme 3. Assembly and deprotecting of MPLA (**1**).

protected MPL **17** in 70% yield. Deprotection of **17** was performed by a three-step procedure entailing cleavage of the anomeric TDS group using HF-pyridine complex in THF followed by removal of the allyl groups using $Pd(PPh_3)_4$. Finally, the Nap ethers were cleaved by oxidation with DDQ in chloroform. DDQ mediated oxidation is usually performed in a mixture of CH_2Cl_2 and aqueous buffer, however chloroform gave a cleaner reaction profile and resulted in a facile purification. MPL (**1**) was obtained in 75% yield over the three deprotection steps after purification by using size exclusion chromatography over Sephadex LH20. The interpretation of ^1H and gHSQC spectra shows a clear indication that target compound 1 was obtained, this was confirmed by high resolution MS. ^{31}P NMR confirmed the presence of only one phosphate group.

3. Conclusion

In conclusion, we have developed an efficient synthetic approach for the preparation of monophosphoryl lipid A derived from *E.coli*. A key feature of the approach is the use of Nap ethers as permanent protecting group for hydroxyls that could readily be cleaved at the final step of deprotection by oxidation with DDQ. The use of di-allyl *N,N*-diisopropylphosphoramidite for installation of the phosphate ester ensured that no hydrogenation step was required for deprotection. The synthetic strategy is convergent and allows at a late stage of synthesis the introduction of different fatty acids at the amines of the target compound. The latter is facilitated by the selection of Troc and Fmoc as orthogonal amino-protecting groups.

4. Experimental section

General synthetic methods. Unless stated otherwise, all reagents were purchased from Sigma-Aldrich and Fischer Scientific. Carbohydrates were purchased from Carbosynth Limited (UK). Petroleum ether (boiling range 40–60 °C) was purchased from Biosolve BV (The Netherlands). Organic solvents for reactions were dried for at least 2 days over molecular sieves (3 or 4 Å). ^1H and ^{13}C NMR spectra were recorded on either an Agilent 400 instrument (400 and 101 MHz) or a Bruker Avance Neo 600 spectrometer (600 and 125 MHz). Chemical shifts are reported in parts per million (ppm) relative to TMS (0.00 ppm for ^1H NMR), MeOD (3.31 ppm for ^1H NMR, 49.2 ppm for ^{13}C NMR) or $CDCl_3$ (77.0 ppm for ^{13}C NMR) as the internal standard. NMR data are presented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, 2d = 2 doublets, t = triplet, dd = doublet of doublet, sept = septet, m = multiplet and/or multiple resonances); coupling constants are reported in Hertz (Hz). All NMR signals were assigned on the basis of ^1H NMR, COSY and HSQC experiments. Signals marked with L1 and L2 are of the bi-antennary lipids of the C-3′ and C-2′ respectively, the signals marked as L1′ and L2’ are of their corresponding lipid side chain. Signals marked with L3 and L4 are of the mono-antennary lipids attached to the C-3 and C-2 respectively. High resolution mass spectra were recorded on an Agilent technologies 6560 Ion mobility Q-TOF spectrometer. Sephadex LH20 (Sigma Aldrich) column chromatography was performed using a mixture of CH_2Cl_2 and MeOH (1:1, v/v) as the eluent. Silica column chromatography was performed using silica gel SiliaFlash P60 (SiliCycle, Canada, 40–63 μm, 239–400 mesh). TLC analysis was conducted on SiliaPlate TLC Aluminium Backed TLC F254 (SiliCycle) with examination under UV light (254 nm) where applicable, and with 5% sulfuric acid in ethanol or an aqueous solution of Ce $(NH_4)_2(NO_3)_6$ and $(NH4)_6Mo_7O_{24}{\cdot}4H_2O$ (20 g/L and 48 g/L, respectively) with 5% sulfuric acid, followed by heating. All reactions were carried out under nitrogen gas atmosphere unless when water was present in the reaction. All reactions were carried out at room temperature (RT) in glassware with magnetic stirring, unless when stated otherwise.

Dimethylthexylsilyl 2-deoxy-4,6-naphthylidene-3-*O*-(*R*)-(tetradecanoyloxy)tetradecanoate-2-(2,2,2-trichloroethoxycarbonylamino)-β-*D*-glucopyranoside (8). To a solution of **7** (3.29 g, 5.06 mmol) and **4** (2.96 g, 4.60 mmol) in CH_2Cl_2 (50 mL) were added EDC (1.42 g, 7.40 mmol) and catalytic DMAP (20 mg, 0.2 mmol) and the reaction was stirred for 16 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: PE/Et_2O, 7.5/2.5) to give **8** as a clear colorless oil (3.85 g, 3.59 mmol, 78%) $R_f = 0.8$ (PE/EtOAc, 4/1). ^1H NMR (400 MHz, $CDCl_3$): δ 7.83, 7.49 (2 m, 7H, aromatic (Nap)), 5.65 (s, 1H, CH (Nap)), 5.38 (t, 1H, $J = 10.0$ Hz, H-3), 5.23 (d, 1H, $J = 9.0$ Hz, N–H), 5.18 (m, 1H, C*H*CH_2COOC (L1)) 4.92 (d, 1H, $J = 7.8$ Hz, H-1), 4.77 (d, 1H, $J = 12.0$ Hz, CH_{2a}-CCl_3), 4.60 (d, 1H, $J = 12.0$ Hz, CH_{2b}-CCl_3), 4.35 (dd, 1H, $J_{6a,6b} = 10.5$ Hz, $J_{6a,5} = 5.0$ Hz, H-6_a), 3.85 (t, 1H, $J_{6b,6a} = 10.5$ Hz, $J_{6b,5} = 10.1$ Hz, H-6_b), 3.76 (t, 1H, $J = 9.4$, H-4), 3.57 (m, 2H, H-2, H-5), 2.60 (dd, 1H, $J_{gem} = 15.2$ Hz, $J = 7.2$ Hz, CH_{2a}COOC (L1)), 2.52 (dd, 1H, $J_{gem} = 15.2$ Hz, $J = 5.5$ Hz, CH_{2b}COOC (L1)), 2.12 (t, 2H, $J = 7.5$ Hz, CH_2COOC (L1′)) 1.64 (m, 1H, CH (TDS)) 1.49 (m, 4H, CH_2CHCH_2COO (L1), CH_2CH_2COOC(L1′)), 1.20 (m, 38H, 19x CH_2), 0.86 (m, 18H, 2x CH_3, 4x CH_3 (TDS)), 0.17, 0.14 (2s, 6H, 2x CH_3 (TDS)). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.4, 170.1 (2x C═O), 154.1 (*C*OCH_2CCl_3), 134.3, 134.3, 133.7, 133.6, 132.8, 132.8, 128.3, 128.1, 128.0, 127.6, 126.4, 126.1, 125.8, 125.6, 123.7, 123.6 (aromatic (Nap)), 101.7 (CH (Nap)), 96.6 (C-1), 95.3 (CCl_3), 79.1 (C-4), 74.7 (*C*H_2CCl_3), 71.2 (C-3), 70.0 (*C*HCH_2COOC (L1)), 68.7 (C-6), 66.4 (C-5), 59.1 (C-2), 39.2 (*C*H_2COOC (L1)), 34.3 (*C*H_2COOC (L1’)), 33.9

(CH_2CHCH_2COO (L1), 33.9 (CH (TDS)), 31.9, 29.7, 29.7.29.6, 29.6, 29.4, 29.3, 29.3, 29.3, 29.2, 29.0, 29.0, 25.0, 25.0, 24.9, 24.8, 24.7, 22.7 (20x CH_2), 19.9, 19.9, 18.5, 14.1 (4x CH_3 (TDS) + CH_2CH_3), −2.0, −3.4 (2x CH_3 (TDS)). HR MS (*m/z*) calcd for $C_{56}H_{90}Cl_3NNaO_{10}Si$ $[M + Na]^+$, 1092.5297; found, 1092.5382.

Dimethylthexylsilyl 2-deoxy-6-*O*-naphthalen-2-ylmethoxy-3-*O*-(*R*)-(tetradecanoyloxy)tetradecanoate-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (9). To a cooled (−78 °C) solution of compound **8** (2.82 g, 2.63 mmol) in CH_2Cl_2 (10 mL) was added triethylsilane (2.5 mL, 15.8 mmol). Next, TfOH (1.2 mL, 13.2 mmol) was added dropwise and the resulting reaction mixture was stirred at −78 °C for 0.5 h. The reaction was quenched with Et_3N and MeOH. The reaction mixture was concentrated in vacuo followed by partitioning in EtOAc and saturated aqueous sodium bicarbonate. The organic layer was dried with $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (PE/Et_2O, 7.5/2.5) to give compound **9** (2.05 g, 1.91 mmol, 72%) as a clear oil. $R_f = 0.5$ (PE/EtOAc, 4/1) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82, 7.48 (2 m, 7H, aromatic (Nap)), 5.14 (m, 2H, C*H*CH_2COOC (L1), NH), 5.01 (t, 1H $J = 9.8$ Hz, H-3), 4.74 (m, 4H, H-1, CH_{2a}-CCl_3 CH_2 (Nap)), 4.58 (d, 1H, $J = 11.8$ Hz, CH_{2b}-CCl_3), 3.81 (m, 2H, CH_2-6), 3.70 (m, 1H, H-4), 3.57 (m, 2H, H-2, H-5), 3.35 (d, 1H, $J = 2.5$, OH), 2.54 (m, 2H, CH_2COOC (L1)), 2.28 (t, 2H, $J = 7.5$ Hz, CH_2COOC (L1′)), 1.60 (m, 5H, CH (TDS), CH_2CHCH$_2$COO (L1), CH_2CH$_2$COOC (L1′)), 1.27 (m, 38H, 19x CH_2), 0.86 (m, 18H, 2xCH_3, 4x CH_3 (TDS)), 0.19, 0.14 (2s, 6H, 2x CH_3 (TDS)). ^{13}C NMR (101 MHz, $CDCl_3$): δ 174.3, 171.7 (2x C═O) 154.2 (*C*OCH_2CCl_3), 135.5, 135.2, 133.2, 133.2, 133.0, 133.0, 128.2, 128.1, 127.8, 127.7, 127.7126.4, 126.2, 126.1, 126.1, 125.9, 125.8, 125.5 (aromatic (Nap)), 96.3 (C-1), 95.4 (CCl_3), 76.0 (C-3), 74.9, 74.7, 74.6, 74.6, 74.2 (*C*H_2CCl_3 + C-5), 73.8, 73.7 (CH_2 (Nap)), 70.9 (*C*HCH$_2$COOC (L1)), 70.4 (C-4), 70.1, 70.0 (C-6), 57.8 (C-2), 40.0 (*C*H_2COOC (L1)), 34.5 (*C*H_2COOC (L1')), 33.9 (CH (TDS)), 34.3, 31.9, 31.9, 29.7, 29.7.29.6, 29.6, 29.6, 29.4, 29.3, 29.3, 29.3, 29.2, 29.1, 29.0, 25.1, 24.9, 24.8, 22.7 (20x CH_2), 20.0, 18.5, 14.1 (4x CH_3 (TDS) + CH_2*C*H_3), −1.8, −3.4 (2x CH_3 (TDS)). HR MS (*m/z*) calcd for $C_{56}H_{92}Cl_3NNaO_{10}Si$ $[M + Na]^+$, 1094.5454; found, 1094.5486.

Dimethylthexylsilyl 2-deoxy-4-*O*-di-*O*-allylphosphate-6-*O*-(naphthalen-2-ylmethyl)-3-*O*-(R)-(tetradecanoyloxy)tetradecanoate-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (10). 1H-tetrazole (669 mg, 9.55 mmol) was added to a solution of **9** (2.05 g, 1.91 mmol) under an atmosphere argon. Di-allyl diisopropylphosphoramidite (1.08 mL, 4.08 mmol) was added dropwise and the resulting reaction mixture was stirred at RT for 16 h. The reaction mixture was cooled (−40 °C) and m-CPBA (396 mg, 2.29 mmol) was added. After the addition, the reaction mixture was allowed to warm up to RT and stirring was continued for 1 h. The reaction mixture was concentrated and the crude was purified by silica gel column chromatography (PE/Et_2O, 7/3) to give **10** as a clear colorless oil (1.65 g, 1.34 mmol, 70%) $R_f = 0.6$ (PE/EtOAc, 4/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81, 7.47 (2 m, 7H, aromatic (Nap)), 5.82 (m, 2H, 2x CH_2═C*H*), 5.38 (t, 1H, $J = 9.8$ Hz, H-3), 5.24 (m, 6H, C*H*CH_2COOC (L1), NH, 2x CH_2=CH), 4.97 (d, 1H, $J = 7.9$ Hz, H-1), 4.74 (m, 3H, CH_{2a}-CCl_3, CH_2 (Nap)), 4.62 (d, 1H, $J = 12.0$ Hz, CH_{2b}-CCl_3), 4.41 (m, 5H, 2x CH_2–CH═CH_2, H-4), 3.84 (dd, 1H, $J_{6a,6b} = 10.9$ Hz, $J = 1.6$ Hz, H-6_a), 3.71 (m, 2H, H-6_b, H-5), 3.44 (m, 1H, H-2), 2.61 (m, 2H, CH_2COOC (L1)), 2.28 (t, 2H, $J = 7.5$ Hz, CH_2COOC (L1′)), 1.62 (m, 5H, CH (TDS), CH_2CHCH$_2$COO (L1), CH_2CH$_2$COOC (L1′)), 1.27 (m, 38H, 19x CH_2), 0.86 (m, 18H, 2x CH_3, 4x CH_3 (TDS)), 0.20, 0.14 (2s, 6H, 2x CH_3 (TDS)). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.5, 170.3 (2x C═O) 153.9 (*C*OCH_2CCl_3), 135.7, 133.2, 133.0, 132.3, 132.2, 132.0, 128.1, 127.8, 127.7, 126.2, 126.0, 125.8, 125.6 (aromatic (Nap) + *C*H═CH_2), 118.5, 118.4 (2x *C*H_2═CH), 95.5 (C-1), 95.3 (CCl_3), 74.6 (*C*H_2CCl_3), 74.1 (C-4), 74.1 (C-5), 73.6 (CH_2 (Nap)), 72.5 (C-3), 70.0 (*C*HCH$_2$COOC (L1)), 68.7, 68.6 (C-6), 68.4, 68.4 (*C*H_2–CH═CH_2), 58.6 (C-2), 39.6 (*C*H_2COOC (L1)), 34.5 (*C*H_2COOC (L1')), 34.2 (*C*H_2CHCH$_2$COO (L1) 34.0 (CH (TDS)), 31.9, 29.6, 29.6.29.5, 29.3, 29.2, 25.2, 25.0, 24.8, 22.7 (20x CH_2), 20.0, 20.0, 18.5, 14.1 (4x CH_3 (TDS) + CH_2*C*H_3), −1.9, −3.4 (2x CH_3 (TDS)). ^{31}P NMR (162 MHz, $CDCl_3$): δ −2.0. HR MS (*m/z*) calcd for $C_{62}H_{105}Cl_3N_2O_{13}PSi$ $[M + NH_4]^+$, 1249.6189; found, 1249.6182.

2-deoxy-4-*O*-di-O-allylphosphate-6-*O*-(naphthalen-2-ylmethyl)-3-*O*-(R) (tetradecanoyloxy)tetradecanoate-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranose (11). To a solution of compound **10** (800 mg, 648 μmol) in pyridine (16 mL) was added HF-pyridine (1.15 mL, 45.6 mmol). The reaction mixture was stirred for 16 h, after it was poured in water (200 mL) and stirring was continued for 2 h. The resulting precipitate was filtered off and dried in vacuo to give **11** as a white amorphous solid (697 mg, 639 μmol, 99%) $R_f = 0.5$ (PE/EtOAc, 7/3). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78, 7.46 (2 m, 7H, aromatic (Nap)), 5.80 (m, 2H, 2x CH_2═C*H*), 5.65 (d, 1H, $J = 9.1$ Hz, NH), 5.24 (m, 7H, H-3, C*H*CH_2COOC (L1), H-1, 2x CH_2=CH), 4.80 (d, 1H, $J = 12.2$ Hz, CH_{2a} (Nap)), 4.67 (m, 3H, CH_2–CCl_3, CH_{2b} (Nap)), 4.37 (m, 5H, 2x CH_2–CH═CH_2, H-4), 4.22 (m, 1H, H-5), 4.04 (bs, 1H, OH), 3.97 (m, 1H, H-2), 3.76 (m, 2H, CH_2-6), 2.64 (dd, 1H, $J_{gem} = 16.1$ Hz, CH_{2a}COOC (L1)), 2.55 (dd, 1H, $J_{gem} = 16.1$ Hz, CH_{2b}COOC (L1)), 2.24 (t, 2H, $J = 7.6$ Hz, CH_2COOC (L1′)), 1.56 (m, 4H, CH_2CHCH$_2$COO (L1), CH_2CH$_2$COOC(L1′)), 1.27 (m, 38H, 19x CH_2), 0.88 (t, 6H, $J = 6.8$ Hz, 2x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.4, 170.7 (2x C═O) 154.3 (*C*OCH_2CCl_3), 135.1, 133.2, 133.0, 132.2, 132.2, 132.0, 132.0, 129.4, 128.2, 127.9, 127.7, 126.7126.3, 126.1, 125.9, 125.8, 120.5 (aromatic (Nap) + *C*H═CH_2), 118.7, 118.4 (2x *C*H_2═CH), 95.3 (CCl_3), 91.5 (C-1), 74.6 (*C*H_2CCl_3), 73.8 (C-4), 73.6 (CH_2 (Nap)), 70.8 (C-3), 69.9 (*C*HCH$_2$COOC (L1)), 69.5 (C-5), 68.7, 68.6, 68.5, 68.4, 68.3 (C-6, *C*H_2–CH═CH_2), 54.3 (C-2), 39.1 (*C*H_2COOC (L1)), 34.4 (*C*H_2COOC (L1')), 34.1 (*C*H_2CHCH$_2$COO (L1) 31.9, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 29.3 29.3, 29.2, 25.2, 25.0, 22.7 (20x CH_2), 14.1 (CH_2*C*H_3). ^{31}P NMR (162 MHz, $CDCl_3$): δ −1.9. HR MS (*m/z*) calcd for $C_{54}H_{87}Cl_3N_2O_{13}P$ $[M + NH_4]^+$, 1107.5011; found, 1107.5003.

2,2,2-trifluoro-N-phenylacetimidoyl 2-deoxy-4-*O*-di-O-allylphosphate-6-*O*-(naphthalen-2-ylmethyl)-3-*O*-(R)-(tetradecanoyloxy)tetradecanoate-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (2). To a solution of **11** (670 mg, 614 μmol) in CH_2Cl_2 (10 mL) were added 2,2,2-trifluoro-*N*-phenyl acetimidoyl chloride (0.6 mL, 4.40 mmol) and cesium carbonate (400 mg, 1.23 mmol). After stirring at RT for 1 h, the reaction mixture was directly loaded on a silica gel column (PE/Et_2O, 9/1–4/1) to give **2** as a clear colorless oil (756 mg, 599 μmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81, 7.48 (2 m, 7H, aromatic (Nap)), 7.15, 6.74 (m, d, 5H, aromatic (NPh)), 5.83 (m, 2H, 2x CH_2═C*H*), 5.67 (d, 1H, $J_{NH,2} = 8.2$ Hz, NH), 5.26 (m, 6H, H-3, C*H*CH_2COOC (L1), 2x CH_2=CH), 4.70 (m, 5H, CH_2–CCl_3, CH_2 (Nap), H-4), 4.43 (m, 4H, 2x CH_2–CH═CH_2), 4.19 (m, 1H, H-2), 4.02 (m, 1H, H-5), 3.76 (m, 2H, CH_2-6), 2.62 (m, 2H, CH_2COOC (L1)), 2.27 (m, 2H, $J = 7.6$ Hz, CH_2COOC (L1′)), 1.61 (m, 4H, CH_2CHCH$_2$COO (L1), CH_2CH$_2$COOC(L1′)), 1.25 (m, 38H, 19x CH_2), 0.88 (t, 6H, $J = 6.2$ Hz, 2x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.3, 170.0 (2x C═O) 154.3 (*C*OCH_2CCl_3), 135.3, 133.2, 133.0, 128.7, 128.1, 127.9, 127.6, 126.5, 126.4, 126.1, 125.9, 125.7, (aromatic (Nap)), 132.1 (*C*H═CH_2), 118.6 (CH═*C*H_2), 74.7 (*C*H_2CCl_3), 73.6 (*C*H_2 (Nap)), 72.8 (C-4), 72.3 (C-5), 70.3 (C-3), 69.9 (*C*HCH$_2$COOC (L1)), 68.8, 68.7, 68.6, 68.5 (*C*H_2–CH═CH_2) 67.7 (C-6), 53.7 (C-2), 39.2 (*C*H_2COOC (L1)), 34.4 (*C*H_2COOC (L1')) 34.2 (*C*H_2CHCH$_2$COO (L1), 31.9, 29.6, 29.6, 29.3, 29.2, 25.2, 25.0, 25.0, 22.7 (20x CH_2), 14.1 (CH_2*C*H_3). ^{19}F NMR (376 MHz, $CDCl_3$): δ 65.5. The anomeric carbon and proton could not be assigned due to signal overlap.

Dimethylthexylsilyl 2-deoxy-4,6-naphthylidene-3-*O*-(R)-((naphthalen-2-ylmethyl))tetradecanoate-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (12). To a solution of **7** (5.19 g, 8.00 mmol) and **6** (2.80 g, 7.28 mmol) in CH_2Cl_2 (50 mL) were added EDC (2.51 g, 13.1 mmol) and catalytic DMAP (20 mg, 0.2 mmol) and the reaction was stirred for 16 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: PE/Et_2O, 7.5/2.5) to give **13** as a clear colorless oil (5.60 g, 5.59 mmol, 77%) $R_f = 0.8$ (PE/EtOAc,

4/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81, 7.69, 7.39 (s, m, m, 14H, aromatic (2x Nap)), 5.52 (s, 1H, CH (Nap)), 5.40 (t, 1H J = 9.9 Hz, H-3), 5.07 (d, 1H, J = 9.3 Hz, N–H), 4.88 (d, 1H, J = 7.9 Hz, H-1), 4.62 (m, 4H, CH_2–CCl_3, CH_2 (Nap)), 4.33 (dd, 1H, $J_{6a,6b}$ = 10.4 Hz, $J_{6a,5}$ = 5.0 Hz, H-6_a), 3.82 (m, 2H, H-6_b, H-4), 3.72 (t, 1H, J = 9.2 Hz, C$H$$CH_2$COOC (L3)), 3.64 (m, 1H, H-2), 3.55 (m, 1H, H-5), 2.69 (dd, 1H, J_{gem} = 14.9 Hz, J = 6.2 Hz, CH_{2a}COOC (L3)), 2.51 (dd, 1H, J_{gem} = 14.9 Hz, J = 5.8 Hz, CH_{2b}COOC (L3)), 1.61 (m, 1H, CH (TDS)) 1.53 (m, 2H, C$H_2$$CH_2$COO (L3)), 1.21 (m, 18H, 9x CH_2), 0.86 (m, 15H, CH_3, 4x CH_3 (TDS)), 0.17, 0.14 (2s, 6H, 2x CH_3 (TDS)). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.5 (C═O), 154.0 (C$O$$CH_2CCl_3$), 135.9, 134.2, 133.6, 133.2, 132.9, 132.8, 128.3, 128.0, 128.0, 127.9, 127.6, 126.3, 126.3, 126.1, 126.0, 125.8, 125.7, 125.7, 123.6 (aromatic (Nap)), 101.7 (CH (Nap)), 96.8 (C-1), 95.3 (CCl_3), 79.0 ($C$$CH_2$COOC (L3)), 75.5 (C-4), 74.7 (CH_2CCl_3), 71.2 (CH_2 (Nap)), 71.1 (C-3), 68.6 (C-6), 66.6 (C-5), 59.1 (C-2), 39.5 (CH_2COOC (L3)) 34.5 ($CH_2$$CH_2$COO (L3)) 33.9 (CH (TDS)), 31.9, 29.6, 29.6, 29.3, 25.2, 24.8, 22.7 (9x CH_2), 19.9, 18.5, 14.1 (4x CH_3 (TDS) + CH_2CH_3), −1.9, −3.4 (2x CH_3 (TDS)). HR MS (*m/z*) calcd for $C_{53}H_{72}Cl_3NNaO_9Si$ $[M + Na]^+$, 1022.3940; found, 1022.3967.

Dimethylthexylsilyl 2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-4,6-naphthylidene-3-*O*-(R)-(naphthalen-2-ylmethoxy)tetradecanoate-β-D-glucopyranoside (14). To a solution of **13** (5.60 g, 5.59 mmol) in THF/AcOH 4/1 (50 mL) was added Zn (5.46 g, 84.0 mmol) and was stirred for 2 h. After sonication the Zn was filtered off over a Celite pad and afforded after evaporation the crude free amine **14**. To a solution of crude **14** in CH_2Cl_2 (50 mL) were added 9-fluorenylmethyloxycarbonyl chloride (1.74 g, 6.73 mmol) and *N,N*-diisopropylethylamine (2.3 mL, 13.4 mmol). The reaction mixture was stirred for 16 h, and the resulting product was washed with 1 M HCl (aq. 2x 50 mL). The organic layer was dried with $MgSO_4$, filtered and concentrated in vacuo. Column chromatography (PE/EtOAc, 95/5–9/1) resulted in formation of compound **15** over two steps as a pale yellow oil (2.25 g, 2.15 mmol, 38%) R_f = 0.7 (PE/EtOAc, 4/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (m, 22H, aromatic (2x Nap, Fmoc)), 5.53 (s, 1H, CH (Nap)), 5.43 (t, 1H J = 9.7 Hz, H-3), 4.92 (d, 2H, J = 5.3 Hz, H-1, NH), 4.63 (d, 1H, J = 11.9 Hz, CH_{2a} (Nap)), 4.50 (d, 1H, J = 11.9 Hz, CH_{2b} (Nap)), 4.30 (m, 3H, H-6_a, CH_2 (Fmoc)), 4.18 (m, 1H, CH (Fmoc)), 3.82 (m, 2H, H-6_b, H-4), 3.74 (t, 1H, J = 10.0 Hz, C$H$$CH_2$COOC (L3)), 3.64 (m, 1H, H-2), 3.58 (m, 1H, H-5), 2.69 (dd, 1H, J_{gem} = 14.9 Hz, J = 6.4 Hz, CH_{2a}COOC (L3)), 2.49 (dd, 1H, J_{gem} = 14.9 Hz, J = 5.7 Hz, CH_{2b}COOC (L3)), 1.59 (m, 1H, CH (TDS)), 1.46 (m, 2H, C$H_2$$CH_2$COO (L3)), 1.20 (m, 18H, 9x CH_2), 0.87 (t, 3H, J = 7.0 Hz, CH_3), 0.82 (m, 12H, 4x CH_3 (TDS)), 0.15, 0.11 (2s, 6H, 2x CH_3 (TDS)). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.7 (C═O) 154.0 (C═O (Fmoc)), 143.8, 141.2, 135.9, 134.2, 134.2, 133.6, 133.1, 133.1, 132.8, 132.7, 132.7, 129.0, 128.3, 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 127.0, 126.3, 126.2, 126.0, 125.9, 125.8, 125.6, 125.1, 123.6, 119.9 (aromatic (Nap, Fmoc)), 101.6 (CH (Nap)), 97.0 (C-1), 79.0 ($C$$CH_2$COOC (L3)), 75.6 (C-4), 71.3 (CH_2 (Nap)), 71.2 (C-3), 68.7 (C-6), 67.2 (CH_2 (Fmoc)), 66.5 (C-5), 58.9 (C-2), 47.0 (CH (Fmoc)) 39.7 (CH_2COOC (L3)) 34.5 ($CH_2$$CH_2$COO (L3)) 33.9 (CH (TDS)), 31.9, 29.6, 29.6, 29.5, 29.3, 25.1, 24.7, 22.7 (9x CH_2), 19.9, 19.8, 18.5, 14.1 (4x CH_3 (TDS) + CH_2CH_3), −1.9, −3.4 (2x CH_3 (TDS)). HR MS (*m/z*) calcd for $C_{65}H_{81}NNaO_9Si$ $[M + Na]^+$, 1070.5578; found, 1070.5570.

Dimethylthexylsilyl 2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-4-*O*-(naphthalen-2-ylmethyl)-3-*O*-(R)-(naphthalen-2-ylmethoxy)tetradecanoate-β-D-glucopyranoside (3). To a cooled (−78 °C) solution of compound **14** (2.25 g, 2.15 mmol) in dry CH_2Cl_2 (20 mL) containing 4 Å molecular sieves were added triethylsilane (1.0 mL, 6.26 mmol) and dichlorophenylborane (1.1 mL, 8.12 mmol) The reaction mixture was stirred at −78 °C for 0.5 h. The reaction was quenched by addition of MeOH and Et_3N, and pure glycosyl acceptor **3** was obtained as a pale yellow oil after column chromatography (PE/Et_2O, 7/3) (780 mg, 743 μmol, 33%) R_f = 0.4 (PE/EtOAc, 4/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (m, 22H, aromatic (2x Nap, Fmoc)), 5.32 (t, 1H J = 9.8 Hz, H-3), 4.89 (d, 1H, $J_{NH,2}$ = 9.1 Hz, NH), 4.80 (d, 1H, J = 7.5 Hz, H-1), 4.72 (q, 2H, J_{gem} = 11.7 Hz, CH_2 (Nap)), 4.57 (q, 2H, J_{gem} = 11.8 Hz, CH_2 (Nap)), 4.25 (m, 2H, CH_2 (Fmoc)), 4.15 (m, 1H, CH (Fmoc)), 3.84 (m, 2H, H-6_a, C$H$$CH_2$COOC (L3)), 3.70 (m, 2H, H-6_b, H-4), 3.60 (m, 1H, H-2), 3.50 (m, 1H, H-5), 2.54 (dd, 1H, J_{gem} = 15.5 Hz, J = 7.1 Hz, CH_{2a}COOC (L3)), 2.39 (dd, 1H, J_{gem} = 15.5 Hz, J = 4.9 Hz, CH_{2b}COOC (L3)), 1.85 (t, 1H, J = 6.2 Hz, OH), 1.54 (m, 1H, CH (TDS)) 1.44 (m, 2H, C$H_2$$CH_2$COO (L3)), 1.21 (m, 18H, 9x CH_2), 0.88 (t, 3H, J = 7.0 Hz, CH_3), 0.80 (m, 12H, 4x CH_3 (TDS)), 0.14, 0.09 (2s, 6H, 2x CH_3 (TDS)). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.1 (C═O) 155.7 (C═O (Fmoc)), 143.8, 141.2, 135.9, 135.0, 133.2, 133.1, 132.9, 132.9, 128.2, 128.0, 127.9, 127.8, 127.6, 127.6, 127.0, 126.6, 126.2, 126.1, 125.9, 125.9125.8, 125.7, 125.2, 119.9 (aromatic (Nap, Fmoc)), 96.4 (C-1), 75.7 ($C$$HCH_2$COOC (L3)), 75.7 (C-4), 75.2 (C-5), 74.7 (C-3), 74.5 (CH_2 (Nap)), 71.4 (CH_2 (Nap)), 67.2 (CH_2 (Fmoc)), 62.0 (C-6), 58.4 (C-2), 47.0 (CH (Fmoc)) 39.7 (CH_2COOC (L3)) 34.1 ($CH_2$$CH_2$COO (L3)), 33.9 (CH (TDS)), 31.9, 29.6, 29.6, 29.6, 29.5, 29.3, 25.1, 24.8, 22.7, 22.6, 22.3 (9x CH_2), 19.9, 18.4 (4x CH_3 (TDS)), 14.1 (CH_2CH_3), −1.8, −3.4 (2x CH_3 (TDS)). HR MS (*m/z*) calcd for $C_{65}H_{83}NNaO_9Si$ $[M + Na]^+$, 1072.5735; found, 1072.5729.

Dimethylthexylsilyl [2-deoxy-4-*O*-di-O-allylphosphate-6-*O*-(naphthalen-2-ylmethyl)-3-*O*-(R)-(tetradecanoyloxy)tetradecanoate-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-(1 → 6)-2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-4-*O*-(naphthalen-2-ylmethyl)-3-*O*-(R)-(naphthalen-2-ylmethoxy)tetradecanoate-β-D-glucopyranoside (15). To a cooled (−30 °C) solution of N-phenyl-trifluoroacetimidate **2** (1.88 g; 1.49 mmol) and acceptor **3** (1.09 g; 1.03 mmol) in dry CH_2Cl_2 (25 mL) was added acid washed molecular sieves 4 Å (1.09 g). Next, a 100-fold diluted solution of TfOH in dry CH_2Cl_2 (1.4 mL, 155 μmol) was added dropwise and the resulting reaction mixture was stirred at −30 °C for 0.5 h. The reaction mixture was quenched with Et_3N. After column chromatography (PE/EtOAc, 1/0–8/2) disaccharide **15** was obtained as a clear yellow oil (1.59 g; 749 μmol; 73%) R_f = 0.3 (PE/EtOAc, 4/1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.53 (m, 29H, aromatic (3x Nap, Fmoc)), 5.78 (m, 2H, 2x CH_2═CH), 5.45 (d, 1H, J = 7.0 Hz, NHTroc), 5.38 (t, 1H, J = 9.8 Hz, H-3′), 5.22 (m, 6H, C$H$$CH_2$COOC (L1), H3, 2x C$H_2$═CH), 4.81 (m, 2H, N$H$Fmoc, H-1′), 4.69 (m, 7H, CH_2–CCl_3, 2x CH_2 (Nap), H1), 4.57 (m, 3H, CH_2 (Nap), H-4′), 4.57 (m, 5H, 2x CH_2–CH═CH_2, H-5′), 4.24 (m, 2H, CH_2 (Fmoc)), 4.14 (d, 1H, J = 7.3 Hz, CH (Fmoc)), 4.06 (d, 1H, J = 10.4 Hz, H_{6a}), 3.76 (m, 5H, H-6_b, CH_2-6′, H-4, C$H$$CH_2$COOC (L3)), 3.60 (m, 2H, H-2, H-5′), 3.40 (dd, 1H, $J_{2',NH}$ = 8.8 Hz, H-2′), 2.60 (m, 2H, CH_2COOC (L1)), 2.49 (dd, 1H, J_{gem} = 15.7 Hz, J = 7.1 Hz, CH_{2a}COOC (L3)), 2.34 (dd, 1H, J_{gem} = 15.7 Hz, J = 5.2 Hz, CH_{2b}COOC (L3)), 2.28 (t, 2H, J = 7.5 Hz, CH_2COOC (L1′)), 1.57 (m, 7H, CH (TDS), C$H_2$$CH_2$COO (L1), C$H_2$$CH_2$COOC(L1′), C$H_2$$CH_2$COO (L3)), 1.27 (m, 56H, 28x CH_2), 0.87 (t, 9H, J = 6.9 Hz, 3x CH_3), 0.79 (m, 12H, 4x CH_3 (TDS)), 0.17, 0.12 (2s, 6H, 2x CH_3 (TDS)). ^{13}C NMR (151 MHz, $CDCl_3$): δ 173.7, 171.8, 170.3 (3x C═O) 155.7, 154.0 (2x NCO), 143.8, 141.2, 135.9, 135.6, 135.3, 135.0, 133.2, 133.2, 133.1, 132.9, 132.7, 132.2, 132.1, 132.1, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.6, 127.6, 127.6, 127.0, 126.2, 126.0, 125.9, 125.9, 125.9, 125.8, 125.7, 125.6, 125.2, 119.9 (aromatic (Nap, Fmoc) + CH═CH_2), 118.5, 118.3 (2x CH_2═CH), 100.1 (C-1′), 96.4 (C-1), 95.4 (CCl_3), 76.1 ($CHCH_2$COOC (L3)), 75.7 (C-4), 75.2, 74.8, 74.6, 74.4, 74.3, 74.1, 74.1, 74.0, 73.9, 73.6, 73.6 (CH_2CCl_3, 2x CH_2 (Nap), C-3, C-4, C-5, C-4′), 72.4 (C-3′), 71.4 (CH_2 (Nap)), 70.1 ($CHCH_2$COOC (L1)), 68.6, 68.5, 68.4, 68.3 (C-6, C-6′, CH_2–CH═CH_2), 67.2 (CH_2 (Fmoc)), 58.4 (C-2), 56.8 (C-2′), 47.0 (CH (Fmoc), 39.7 (CH_2COOC (L1), CH_2COOC (L3)), 34.5 (CH_2COOC (L1′)), 34.4 ($CH_2$$CH_2$COO (L1), 34.1 ($CH_2$$CH_2$COO (L3)), 33.9 (CH (TDS)),

31.9, 29.6, 29.6, 29.3, 29.2, 25.2, 25.1, 25.0, 24.8, 24.7, 22.7 (28x CH_2), 20.0, 19.9, 18.5, 18.4 (4x CH_3 (TDS)), 14.1 (CH_2CH_3), −1.6, −1.8, −3.4, −3.5 (2x CH_3 (TDS)). ^{31}P NMR (162 MHz, $CDCl_3$): δ −2.0, −2.1. HR MS (*m/z*) calcd for $C_{119}H_{168}Cl_3N_3O_{21}PSi$ $[M + NH_4]^+$, 2139.0743; found, 2139.0697.

Dimethylthexylsilyl [2-deoxy-4-*O*-di-O-allylphosphate-2-N-(dodecanoyloxy)tetradecanoyl)-6-*O*-naphthalen-2-ylmethoxy-3-*O*-(R)-(tetradecanoyloxy)tetradecanoate-β-D-glucopyranosyl] -(1 → 6)-2-(9-fluorenylmethoxycarbonylamino)-2-deoxy-4-*O*-(naphthalen-2-ylmethyl)-3-*O*-(R)-(naphthalen-2-ylmethoxy)tetradecanoate-β-D-glucopyranoside (16). To a solution of **15** (1.59 g, 749 μmol) in THF/AcOH (7.5 mL, 4/1) was added Zn (3.00 g; 45.9 mmol). The reaction mixture was stirred for 2 h. After sonication the Zn was filtered off over a Celite pad. The THF/AcOH was evaporated and the crude amine was subsequently partitioned in saturated sodium bicarbonate (50 mL) and EtOAc (50 mL) to give the free amine. The organic layer was further washed with brine and dried with $MgSO_4$, filtered and concentrated in vacuo. To a solution of the crude amine in dry CH_2Cl_2 (10 mL) were added **5** (766 mg, 1.88 mmol), DCC (370 mg, 1.88 mmol) and K-OxymaPure (324 mg; 1.88 mmol). This mixture was stirred for 16 h. Compound **16** was obtained as a yellow oil after column chromatography (PE/EtOAc, 1/0–8/2) (1.07 g, 454 μmol, 61% over two steps) R_f = 0.4 (PE/EtOAc, 4/1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.49 (m, 29H, aromatic (3x Nap, Fmoc)), 6.17 (d, 1H, *J* = 7.6 Hz, NH′), 5.77 (m, 2H, 2x CH_2═C*H*), 5.44 (t, 1H, *J* = 9.4 Hz, H-3′), 5.19 (m, 8H, C*H*CH_2COOC (L1), C*H*CH_2COOC (L2), H-1′, H3, 2x C*H*$_2$═CH), 4.87 (d, 1H, *J* = 9.7 Hz, N*H*Fmoc), 4.71 (m, 5H, 2x CH_2 (Nap), H1), 4.50 (m, 3H, CH_2 (Nap), H-4′), 4.37 (m, 4H, 2x C*H*$_2$–CH═CH_2), 4.22 (m, 2H, CH_2 (Fmoc)), 4.12 (d, 1H, *J* = 7.5 Hz, CH (Fmoc)), 4.06 (d, 1H, *J* = 10.9 Hz, H_{6a}), 3.83 (m, 2H, $H6_b$, $H6_a$'), 3.76 (t, 1H, *J* = 6.0 Hz, C*H*CH_2COOC (L3), 3.67 (m, 5H, H-6_b', H-4, H-5′, H-2, H-5), 3.52 (dd, 1H, $J_{2',NH}$ = 8.5 Hz, H-2′), 2.59 (m, 2H, C*H*$_2$COOC (L1)), 2.46 (m, 1H, C*H*$_{2a}$COOC (L3)), 2.29 (m, 7H, C*H*$_2$COOC (L1′), C*H*$_2$COOC (L2′) C*H*$_{2b}$COOC (L3)), 1.55 (m, 11H, CH (TDS), C*H*$_2$CHCH$_2$COO (L1), C*H*$_2$CH$_2$COOC(L1′), C*H*$_2$CHCH$_2$COO (L2), C*H*$_2$CH$_2$COOC(L2′), C*H*$_2$CHCH$_2$COO (L3)), 1.14 (m, 90H, 45x CH_2), 0.87 (t, 15H, *J* = 6.8 Hz, 5x C*H*$_3$), 0.79 (m, 12H, 4x CH_3 (TDS)), 0.17, 0.11 (2s, 6H, 2x CH_3 (TDS)). ^{13}C NMR (151 MHz, $CDCl_3$): δ 173.7, 173.5, 170.1, 170.0 (5x C═O) 155.7 (*C*O (Fmoc)), 143.9, 141.2, 136.0, 135.7, 135.2, 133.2, 133.1, 132.9, 132.3, 128.1, 128.0, 127.9, 127.8, 127.6, 127.6, 127.0, 126.3, 126.2, 126.0, 125.9, 125.8, 125.7, 125.6, 125.2, 119.9 (aromatic (Nap, Fmoc) + *C*H═CH_2), 118.4, 118.2 (2x CH_2═CH), 100.0 (C-1′), 96.4 (C-1), 76.3 (*C*HCH_2COOC (L3)), 75.5 (C-4), 74.8, 74.4, 74.2, 74.1, 74.0, 74.0, 73.6 (2x CH_2 (Nap), C-3, C-4. C-5, C-4′), 72.9 (C-3′), 71.3 (CH_2 (Nap)), 70.5 (*C*HCH_2COOC (L2)), 70.3 (*C*HCH_2COOC (L1)), 68.6, 68.5, 68.5, 68.3, 68.2 (C-6, C-6′, *C*H_2–CH═CH_2), 67.1 (*C*H_2 (Fmoc)), 58.3 (C-2), 56.1 (C-2′), 47.0 (*C*H (Fmoc), 41.4, 39.8, 39.6 (*C*H_2COOC (L1), *C*H_2COOC (L2), *C*H_2COOC (L3)), 34.5, 34.5 (*C*H_2COOC (L1′), *C*H_2COOC (L2′)), 34.3, 34.2 (*C*H_2CHCH$_2$COO (L1), *C*H_2CHCH$_2$COO (L2), 34.1 (*C*H_2CHCH$_2$COO (L3)), 33.9 (CH (TDS)), 31.9, 30.9, 29.7, 29.6, 29.4, 29.4, 29.3, 25.2, 25.1, 25.0, 24.7, 22.7 (48x CH_2), 20.0, 18.5 (4x CH_3 (TDS)), 14.1 (CH_2CH_3), −1.5–3.4 (2x CH_3 (TDS)). ^{31}P NMR (162 MHz, $CDCl_3$): δ −2.1. HR MS (*m/z*) calcd for $C_{142}H_{211}N_2NaO_{22}PSi$ $[M + Na]^+$, 2378.4858; found, 2378.4764. C-1' was determined on basis of HSQC, because it was not clearly visible in the ^{13}C spectrum.

Dimethylthexylsilyl 6-*O*-[2-N-(dodecanoyloxy)tetradecanoyl)-2-deoxy-4-*O*-di-O-allylphosphate-6-*O*-(naphthalen-2-ylmethyl)-3-*O*-(R)-(tetradecanoyloxy)tetradecanoate-β-D-glucopyranosyl] -2-N-(3-*O*-(R)-(naphthalen-2-ylmethoxy)tetradecanoyl)-2-deoxy-4-*O*-(naphthalen-2-ylmethyl)-3-*O*-(R)-(naphthalen-2-ylmethoxy)tetradecanoate-β-D-glucopyranoside (17). To a solution of **16** (203 mg, 86.1 μmol) in CH_2Cl_2 (2 mL) was added 1,8-diazabicyclo [5.4.0]undec-7-ene (26 μL, 172 μmol). The reaction mixture was stirred at RT for 1 h. To a solution of lipid **6** (50 mg, 129 μmol) in CH_2Cl_2 (2 mL) were added dicyclohexylcarbodiimide (27 mg, 129 μmol) and K-OxymaPure (23 mg, 129 μmol). The pre-activated lipid **6** was diluted with CH_2Cl_2 (2.0 mL) and added to Fmoc-cleaved **16** and the resulting mixture was stirred overnight at RT. The reaction mixture was directly loaded on a silica gel column and after eluting (PE/EtOAc, 95/5–8/2) **17** was obtained as a clear colorless oil (151 mg, 60.4 μmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51 (m, 28H, aromatic (4x Nap)), 6.16 (d, 2H, *J* = 8.7 Hz, NH, NH′), 5.76 (m, 2H, 2x CH_2═C*H*), 5.44 (t, 1H, *J* = 9.5 Hz, H-3′), 5.16 (m, 8H, C*H*CH_2COOC (L1), C*H*CH_2COOC (L2), H-1′, H3, 2x C*H*$_2$═CH), 4.66 (m, 6H, 3x CH_2 (Nap)), 4.49 (m, 3H, CH_2 (Nap), H-4′), 4.37 (m, 4H, 2x C*H*$_2$–CH═CH_2), 4.30 (d, 1H, $J_{H1\text{-}H2}$ = 7.6 Hz, H-1), 3.97 (d, 1H, *J* = 11.3 Hz, H_{6a}), 3.91 (m, 1H, H-2), 3.74 (m, 7H, H-6_b, CH_2-6′, H-4, C*H*CH_2COOC (L3), C*H*CH_2COOC (L4), H-5′), 3.46 (m, 1H, H-2′), 3.31 (m, 1H, H-5), 2.59 (m, 2H, C*H*$_2$COOC (L1)), 2.31 (m, 10H, C*H*$_2$COOC (L1′), C*H*$_2$COOC (L2), C*H*$_2$COOC (L2′), C*H*$_2$COOC (L3), C*H*$_2$COOC (L4)), 1.50 (m, 13H, CH (TDS), C*H*$_2$CHCH$_2$COO (L1), C*H*$_2$CH$_2$COOC(L1′), C*H*$_2$CHCH$_2$COO (L2), C*H*$_2$CH$_2$COOC(L2′), C*H*$_2$CHCH$_2$COO (L3), C*H*$_2$CHCH$_2$COO (L4)), 1.14 (m, 108H, 54x CH_2), 0.88 (t, 18H, *J* = 6.8 Hz, 6x C*H*$_3$), 0.79 (m, 12H, 4x CH_3 (TDS)), 0.09, −0.01 (2s, 6H, 2x CH_3 (TDS)). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.7, 173.5, 171.6, 170.7, 170.1, 170.0 (6x C═O), 136.1, 135.8, 135.3, 133.3, 133.2, 133.2, 133.1, 133.0132.9, 132.9, 132.8, 132.3, 132.2, 132.2, 132.1, 128.3, 128.0, 127.9, 127.9127.8, 127.7, 127.6, 127.6, 126.5, 126.4, 126.3, 126.2, 126.2, 126.0, 125.8, 125.8, 125.7, 125.6 (aromatic (Nap) + *C*H═CH_2), 118.4, 118.2 (2x *C*H_2═CH), 99.8 (C-1′), 96.3 (C-1), 76.2 (C-4), 75.7 (*C*HCH_2COOC (L3)), 75.5 (*C*HCH_2COOC (L4)), 74.9 (C-3), 74.4 (C-5), 74.2, 74.2, 74.0, 73.9 (2x CH_2 (Nap), C-5′, C-4′), 72.8 (C-3′), 71.3 (CH_2 (Nap)), 70.6 (CH_2 (Nap)), 70.4 (*C*HCH_2COOC (L2)), 70.3 (*C*HCH_2COOC (L1)), 68.6, 68.5, 68.5, 68.3, 68.2 (C-6, C-6′, *C*H_2–CH═CH_2), 56.3 (C-2′), 55.9 (C-2), 41.3, 41.2, 39.8, 39.6 (*C*H_2COOC (L1), *C*H_2COOC (L2), *C*H_2COOC (L3)), *C*H_2COOC (L4)), 34.6, 34.6, 34.5, 34.3, 34.3, 33.9 (*C*H_2COOC (L1′), *C*H_2COOC (L2')), (*C*H_2CHCH$_2$COO (L1), *C*H_2CHCH$_2$COO (L2), (*C*H_2CHCH$_2$COO (L3)), (*C*H_2CHCH$_2$COO (L4)), 33.7 (CH (TDS)), 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.3, 25.2, 25.1, 25.0, 24.6, 22.7 (58x CH_2), 20.0, 18.6 (4x CH_3 (TDS)), 14.1 (CH_2CH_3), −1.5–3.4 (2x CH_3 (TDS)). ^{31}P NMR (162 MHz, $CDCl_3$): δ −2.1.

[2-N-(dodecanoyloxy)tetradecanoyl)-2-deoxy-4-*O*- phosphate-3-*O*-(R)-(tetradecanoyloxy)tetradecanoate-β-D-glucopyranosyl] -(1–6)-2-*N*-(3-(R)- hydroxytetradecanoyl)-2-deoxy-3-(R)-hydroxytetradecanoate-β-D-glucopyranose (1). To a solution of **17** (49 mg, 19.6 μmol) in THF (10 mL) was added pyridine (3 mL, 37.2 mmol) and this mixture was cooled to −40 °C. Next, HF·pyridine (1 mL, 39.9 mmol, 70%) was added dropwise and the resulting mixture was stirred overnight at RT. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (2x 100 mL), the organic layer was dried with $MgSO_4$, filtered and concentrated. The residue was purified using Sephadex LH-20 column chromatography (CH_2Cl_2/MeOH, 1/1). To this pyranose (44 mg, 18.6 μmol) in THF (1.5 mL) were added triphenylphosphine (27 mg, 103 μmol), Et_3N (144 μL, 1.03 mmol) and formic acid (72 μL, 1.89 mmol). After stirring for 10 min at RT, tetrakis (triphenylphosphine)palladium (24 mg, 20.8 μmol) was added and stirring was continued for 4 h. Concentration in vacuo was followed by Sephadex LH-20 column chromatography (CH_2Cl_2/MeOH, 1/1), affording the free phosphate. To a solution of this phosphate (40 mg, 17.6 μmol) in $CHCl_3$ (8 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (40 mg, 176 μmol), and the mixture was stirred for 16 h at RT. The crude reaction mixture was diluted with MeOH (2 mL), and transferred to a Sephadex LH-20 column (CH_2Cl_2/MeOH, 1/1). Title compound **1** (25 mg, 14.5 μmol, 76% over three steps) was obtained as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$/MeOD 1/1): δ 5.15 (m, 4H, H-3, C*H*CH_2COOC (L1), C*H*CH_2COOC (L2), H-3′), 5.10 (d, 1H, *J* = 3.6 Hz, H-1), 4.62 (s, 1H, H-1′), 4.22 (m, 1H, H-4′), 4.09 (m, 3H, H-2, H-5, H_{6a}), 4.00 (m, 2H, $H6_a$', C*H*CH_2COOC (L3)), 3.89 (m, 1H, C*H*CH_2COOC (L4)), 3.83 (t, 1H, *J* = 9.5 Hz, H-2′), 3.73 (m, 2H, H_{6b}', H_{6b}), 3.46 (t, 1H, *J* = 9.8 Hz, H-4), 3.36 (m, 1H, H-5′), 2.65 (m, 2H, C*H*$_2$COOC (L1), 2.55–2.13 (m, 10H, C*H*$_2$COOC (L1′), C*H*$_2$COOC (L2), C*H*$_2$COOC (L2′), C*H*$_2$COOC (L3), C*H*$_2$COOC (L4)), 1.60 (m, 12H, C*H*$_2$CHCH$_2$COO (L1), C*H*$_2$CH$_2$COOC

(L1′), C*H*$_2$CHCH$_2$COO (L2), C*H*$_2$CH$_2$COOC(L2′), C*H*$_2$CHCH$_2$COO (L3), C*H*$_2$CHCH$_2$COO (L4)), 1.46, 1.31 (m, 108H, 54x CH_2), 0.89 (m, 18H, 6x C*H*$_3$). ^{31}P NMR (162 MHz, $CDCl_3$: MeOD 1:1): δ 4.4. The proton chemical shifts of CH-1 and CH-5' (4.62, 3.36) were determined based on the gHSQC spectrum. HR MS (*m/z*) calcd for $C_{94}H_{176}N_2O_{22}P$ $[M-H]^-$, 1716.2458; found, 1716.2357.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carres.2020.108152.

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