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Synthesis of structural variants of *Staphylococcus aureus* lipoteichoic acid (LTA)

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Abstract—Based on 1,2-*O*-isopropylidene-*sn*-glycerol, which is readily available from D-mannitol, five chiral building blocks for the construction of structural variants of *Staphylococcus aureus* LTA designed and synthesized. Ligation of these building blocks led readily to the target molecules **1** and **2**. They demonstrated that the D-alanine residues at the glycerophosphate backbone are decisive for the activation of the immune system.

1. Introduction

The inflammatory response to Gram-negative and Gram-positive bacteria can hardly be distinguished. Most of the responses to Gram-negative bacteria could be attributed to lipopolysaccharides (LPS) and their lipid A anchor as general principles.[1,2] The response to Gram-positive bacteria could only recently be attributed clearly to lipoteichoic acid (LTA) for the *Staphylococcus aureus* LTA,[3] whose structure is shown in Scheme 1.[4] For unequivocal bioactivity assignment, besides an improved isolation procedure,[5] the chemical synthesis of the structurally closely related compound **A** was decisive.[6–8] **A** contains the hydrolytically labile D-alanine residues in the required ratio with other substituents at a hexameric glycerophosphate backbone; this compound exhibited the same biological activity in terms of initiation of cytokine release by human blood leukocytes as found for the natural product.

For the elucidation of the structural requirements for bioactivity two structural variants were designed, namely compounds **1** and **2** (Scheme 2). Compound **1** is closely related to **A**, however it lacks the phosphoryl-gentiobiose moiety. In compound **2** more rigorous changes are proposed: in addition to the gentiobiose residue the α-linked *N*-acetylglucosamine residue is omitted and the glycerol moieties are replaced by 2-aminopropane-1,3-diol residues with an enantiomeric configuration to which D-alanine residues are connected via amide linkages.

This way the hydrolytic lability of the D-alanine residues in *S. aureus* LTA and also in **A** and **1**, which is already fast at pH 8.5, can be overcome. Obviously it is of interest to determine the influence of the gentiobiose residue and of all the other structural entities on the biological activity. In both molecules the basic backbone and the D-alanine residues were retained, because their requirement for the activation of the immune system has already been demonstrated.[6]

2. Results and discussion

2.1. Synthesis of compound 1

The retrosynthesis of **1**, taking into consideration the hydrolytic lability of the D-alanine residues, led to building blocks **3**–**6** (Scheme 2) of which **3** and **6** are known compounds.[9,10] Building block **4** was readily obtained from 1,2-*O*-isopropylidene-*sn*-glycerol **8**[11] (Scheme 3). 3-*O*-Allylation with allyl bromide and sodium hydride (NaH) as base in DMF as solvent (→**9**), acid catalyzed *O*-deisopropylidenation (→**10**),[12] and then regioselective 1-*O*-silylation with *tert*-butyl-diphenylsilyl (TBDPS)

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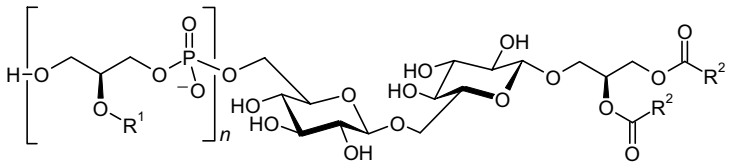


R^1 = Me (D-Ala) (~ 70%)

R^1 = H (~ 15%)

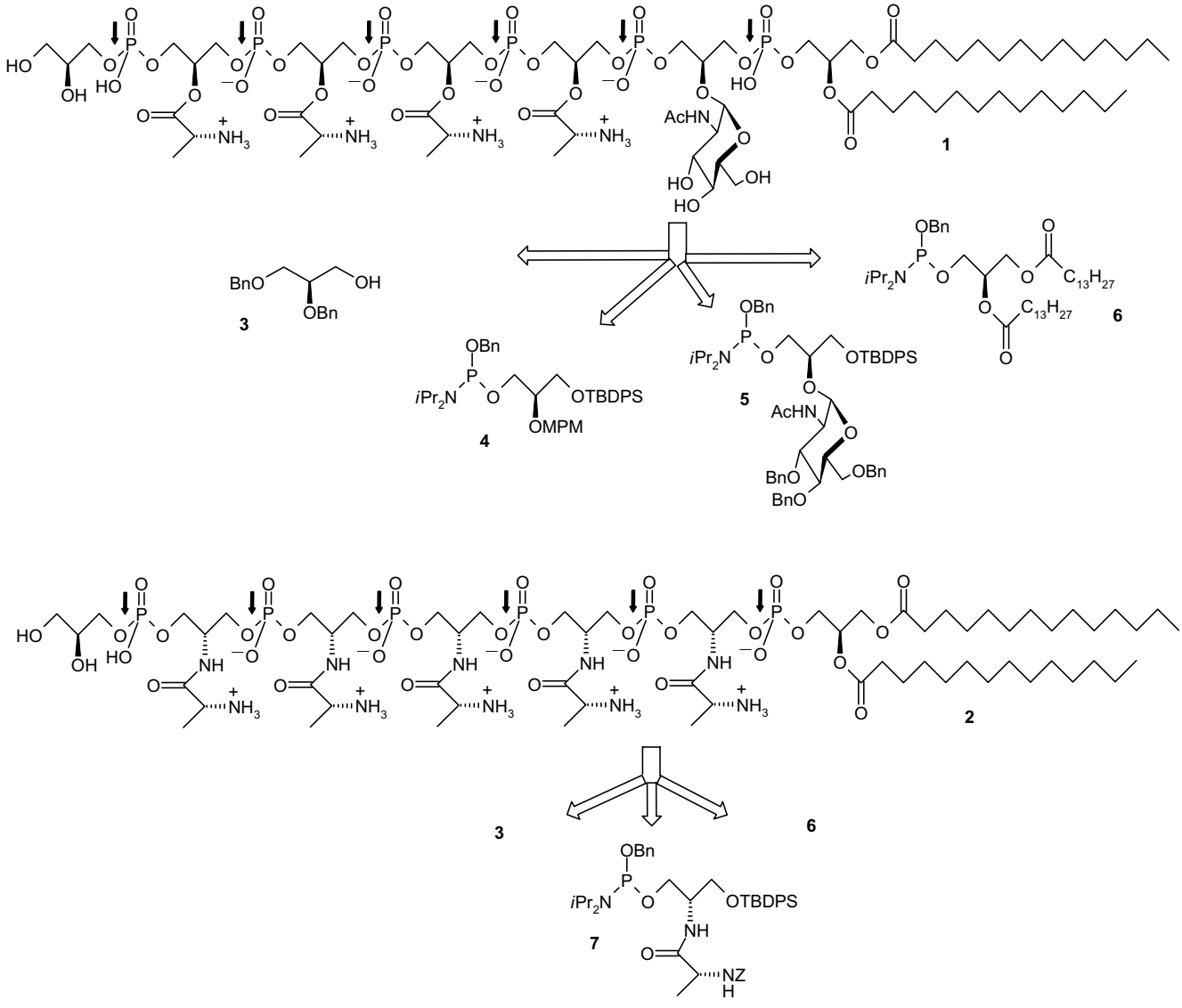
R^1 = (α-D-GlcNAc) (~ 15%)

R^2 = Alkyl and/or branched alkyl chain

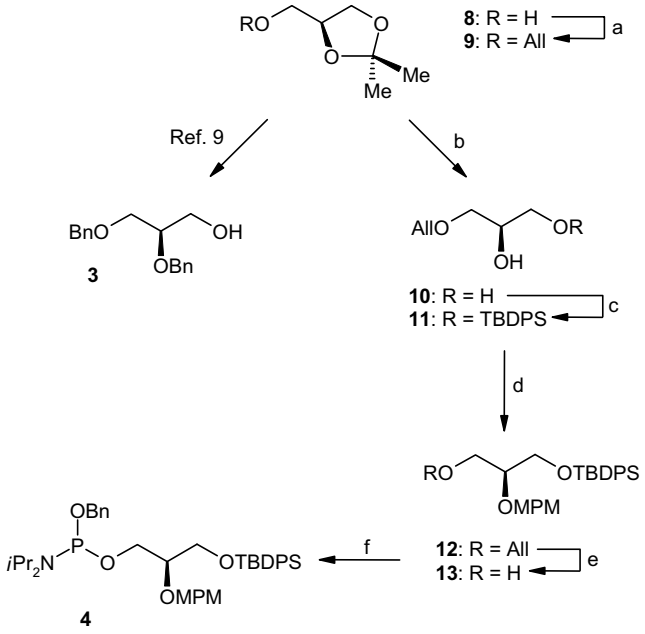
n ~ 40-50

A: n = 6; R^1 = 1 x H, 4 x D-Ala, 1 x α-D-GlcNAc; $R^2 = C_{13}H_{27}$

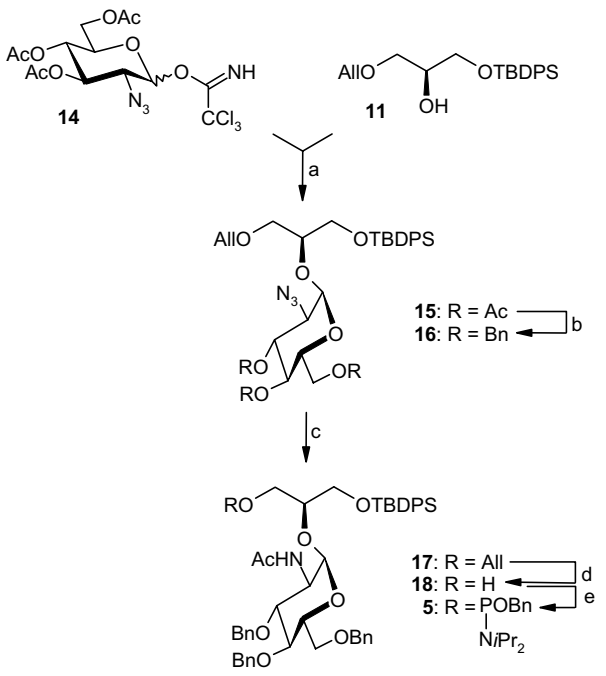
Scheme 1. General structure of lipoteichoic acid of *Staphylococcus aureus*.



Scheme 2. Structure of compounds **1** and **2** and their retrosynthesis.



Scheme 3. Synthesis of building blocks **3** and **4**. Reagents and conditions: (a) AllBr, NaH, DMF, rt (93%); (b) MeOH, Amberlyte 15 (H^+-form), 40 °C (88%); (c) TBDPS-Cl, imidazole, 0 °C, CH_2Cl_2 (75%); (d) MPM-Cl, NaH, DMF, rt (70%); (e) $(Ph_3P)_3RhCl$, DBU, EtOH, 90 °C; 1 M HCl, Me_2CO (71%); (f) tetrazole, CH_2Cl_2, rt (81%).

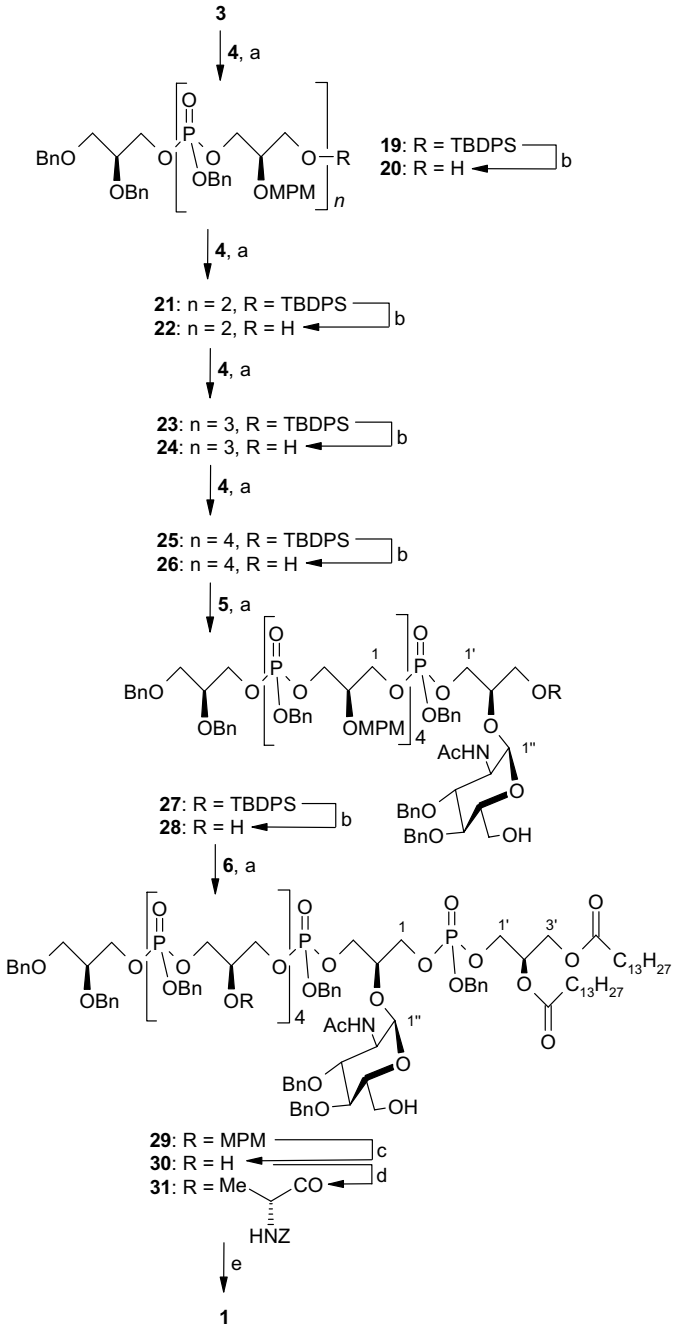


Scheme 4. Synthesis of building block **5**. Reagents and conditions: (a) TMSOTf (0.1 equiv), CH_2Cl_2, rt (84%, α:β = 9:1); (b) NaOMe, MeOH; BnBr, NaH, DMF, rt (74%); (c) CH_3COSH, 40 °C (83%); (d) $(Ph_3P)_3RhCl$, DBU, EtOH, 90 °C; 1 M HCl, Me_2CO (77%); (e) $(iPr_2N)_2P(OBn)$, tetrazole, CH_2Cl_2, rt (85%).

chloride in the presence of imidazole as base afforded 2-*O*-unprotected glycerol derivative **11**, which was also useful for the synthesis of building block **5**. The 4-methoxyphenylmethyl (MPM) group was selected as temporary protecting group for the 2-hydroxy group of **11**, because it can be selectively removed in the presence of *O*-benzyl protecting groups. To this end, **11** was reacted with MPM chloride and NaH as base in DMF to furnish orthogonally protected glycerol derivative **12**. 3-*O*-Deallylation with Wilkinson's catalyst[13] in the presence of DBU as base in ethanol furnished the 3-*O*-propenyl intermediate and then treatment with aqueous HCl in acetone afforded 3-*O*-unprotected **13**. Phosphitylation with benzyloxy-bis(diisopropylamino)phosphane[14] in the presence of tetrazole led to the desired phosphite derivative **4** in good yield.

For the synthesis of building block **5** known glycosyl donor **14**[15,16] was reacted with 2-*O*-unprotected glycerol derivative **11** as acceptor (Scheme 4); as expected based on previous experiments,[16] with 0.1 equiv of TMSOTf as catalyst the desired α-glycoside **15** was obtained in high yield (81%, α:β = 9:1). *O*-Deacetylation with NaOMe in methanol and then *O*-benzylation with benzyl bromide and NaH as base in DMF furnished tri-*O*-benzyl derivative **16**. Following known procedures,[17,18] treatment of **16** with thioacetic acid transformed the azido group into the acetylamino group to give **17**; the same result was obtained with 1,3-propane-dithiol as reducing agent and then *N*-acetylation. Following the above described procedures, 3-*O*-deallylation (→**18**) and then phosphitylation afforded the desired building block **5** again in very good yield.

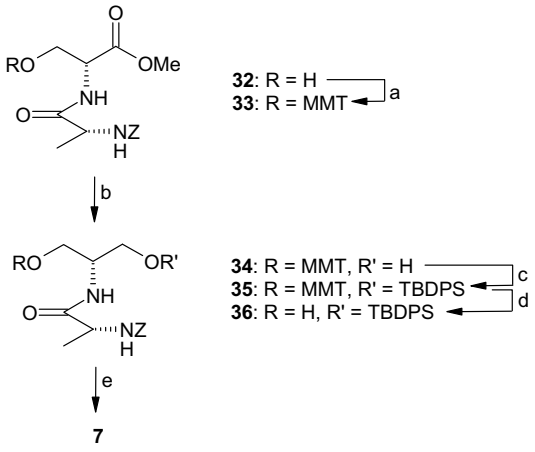
The construction of target molecule **1** from building blocks **3**–**6** was performed stepwise in solution starting with di-*O*-benzylglycerol **3** (Scheme 5). Phosphitylation with **4** in the presence of tetrazole and then oxidation of the phosphite moiety with *tert*-butyl hydroperoxide afforded phosphate intermediate **19**, which was desilylated with tetrabutylammonium fluoride (TBAF) in THF as solvent to furnish *O*-unprotected **20** as acceptor for the next reaction cycle for chain extension. Thus, this phosphitylation/oxidation and deprotection sequence was repeated three times with **4** (→**21**–**26**), then with **5** (→**27**, **28**) and finally the chain extension was terminated by phosphitylation with **6** and then oxidation affording **29** in very good yield; **29** contains the desired glycerophosphate backbone. For the attachment of the D-alanine residues, the four MPM groups were selectively cleaved with ceric ammonium nitrate (CAN)[19] in an acetonitrile–toluene–water mixture as solvent affording compound **30** in 70% yield. D-Alanylation was performed with the triethylammonium salt of *N*-benzyloxycarbonyl (Z) protected D-alanine in the presence of *N*-methylimidazole and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) as condensing agent furnishing **31** in 70% yield. Hydrogenolytic *O*-debenzylation (i.e., cleavage of 15 *O*-benzyl groups) was performed with Pearlman's catalyst[20] in a dichloromethane–methanol–water mixture as solvent; the crude product was purified by hydrophobic interaction chromatography (HIC) to afford target molecule **1** in 41% yield. **1** was structurally ascertained as all intermediates by NMR and MS data and most intermediates also by elemental analyses.



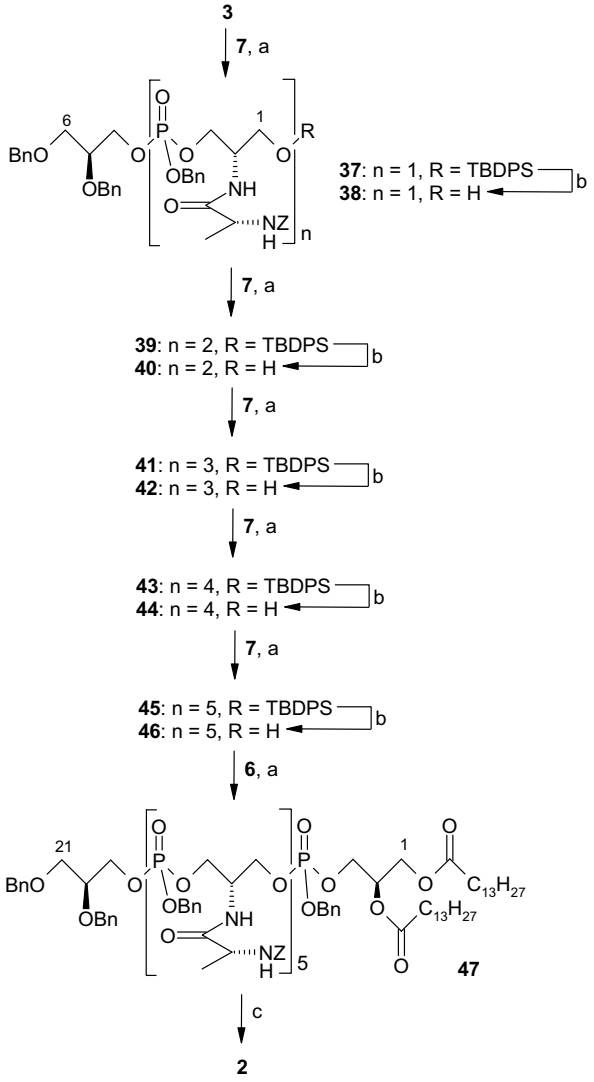
Scheme 5. Synthesis of target molecule **1**. Reagents and conditions: (a) tetrazole, CH_2Cl_2, rt; *t*-BuO_2H (**19**: 76%; **21**: 95%; **23**: 93%; **25**: 90%; **27**: 89%; **29**: 82%); (b) TBAF, THF, rt (**20**: 87%; **22**: 80%; **24**: 92%; **26**: 93%; **28**: 80%); (c) CAN, MeCN/toluene/H_2O; rt (70%); (d) (D)-$MeCH(NHZ)CO_2^-HNEt_3^+$, PyBOP, *N*-methylimidazole, rt (70%); (e) $Pd(OH)_2$/C, H_2, CH_2Cl_2/MeOH/H_2, rt; HI chromatography (41%).

2.2. Synthesis of compound 2

The retrosynthesis of target molecule **2** led to building blocks **3**, **6** and **7** (Scheme 2). Hence, because of the stability of the amide linked D-alanyl residues the protecting group pattern for the synthesis of **2** required only *O*-benzyl and *N*-Z for permanent protection and *O*-silyl for temporary protection. The synthesis of building block **7** was initiated with commercially available D-serine methyl ester and Z-protected D-alanine, which were connected with *N*-hydroxybenzotriazole (HOBt), dicyclohexylcarbodiimide (DCC) in the presence of Hünig's base (DIPEA) to afford dipeptide **32** (Scheme 6). In order to distinguish the primary hydroxy groups after ester group reduction in **32**, first *O*-tritylation with monomethoxytrityl (MMT) chloride in pyridine (→**33**) and then reduction with $NaBH_4$ in ethanol as solvent was performed leading to 2-aminopropane-1,3-diol derivative **34**. *O*-Silylation with TBDPS-Cl and imidazole (→**35**) and then *O*-detritylation with camphorsulfonic acid (CSA) as catalyst in dichloromethane and methanol as nucleophile afforded intermediate **36**. Phosphitylation as described above led to the desired building block **7** in very good yield.



Scheme 6. Synthesis of building block **7**. Reagents and conditions: (a) MMT-Cl, pyr, rt (92%); (b) $NaBH_4$, EtOH (78%); (c) TBDPS-Cl, imidazole, rt (85%); (d) CSA, CH_2Cl_2/MeOH, rt (86%); (e) $(i\mathrm{Pr}_2\mathrm{N})_2$-P(OBn), tetrazole, CH_2Cl_2; t-BuO_2H, CH_2Cl_2 (82%).



Scheme 7. Synthesis of target molecule **2**. Reagents and conditions: (a) tetrazole; CH_2Cl_2; $^tBuO_2H$, CH_2Cl_2 (**37**: 97%; **39**: 95%; **41**: 77%; **43**: 91%; **45**: 81%; **47**: 82%); (b) TBAF, THF, rt (**38**: 82%, **40**: 78%; **42**: 72%; **44**: 72%; **46**: 78%); (c) $Pd(OH)_2$, H_2; CH_2Cl_2/MeOH/H_2O (35%).

The construction of target molecule **2** from building blocks **3**, **6** and **7** was also performed stepwise starting from di-*O*-benzylglycerol **3** (Scheme 7). Phosphitylation with **7** and oxidation to phosphate and then desilylation was repeated five times (→**37**–**46**); this reaction sequence for chain extension was terminated by phosphitylation of **46** with **6** and then oxidation with *tert*-butyl hydroperoxide affording **47**, which contained the target molecule in protected form. Hydrogenolytic *O*-debenzylation and HIC-purification as described for the isolation of **1** furnished target molecule **2** in 35% yield. Again, structural assignments were based on NMR and MS data and on the elemental analyses.

The evaluation of the biological activity of **1** and **2** revealed that initiation of cytokine release by human blood leukocytes is practically the same as observed for **A** and for natural LTA.[21] Hence, it seems that neither the gentiobiose or the α-linked *N*-acetylglucosamine residues, nor the configuration or the type of functional group at the glycerol moiety of the glycerophosphate backbone are required for an immune response. Obviously, this biological activity is essentially dependent on the presence of D-alanine residues in a specific alignment. Hence, quite a few questions as to the importance of the other groups and functionalities of LTA remain to be elucidated.

3. Conclusion

In conclusion, with the help of synthetic structural variants of *S. aureus* LTA the prerequisites for the stimulation of the immune system could be further elucidated: the most important constituents are D-alanine residues. The synthesis design considers the variable substitution pattern and the hydrolytic lability of ester bound D-alanyl residues. Hence, based on a small number of versatile building blocks, the synthesis design also opens ready access to a multitude of structural variants of glycerophosphate backbone containing LTAs.

4. Experimental

4.1. General

Solvents were dried according to the standard procedures. NMR spectroscopic measurements were performed at 22 °C with Bruker DRX600 and Bruker AC250 Cryospec instruments. TMS or the resonances of the deuterated solvents were used as internal standard. $CDCl_3$ (δ = 7.24 ppm) was used as external standard; 85% of phosphoric acid was used as external standard for ^{31}P spectra. MALDI mass spectra were recorded with a Kratos Kompact Maldi II spectrometer; 2,5-dihydroxybenzoic acid (DHB) or *p*-nitroaniline and NaI were used as matrices for positive mode measurements, and trihydroxyacetophenone (THAP) was used as matrix for negative mode measurements. Optical rotations were measured with a Perkin Elmer polarimeter 241/MS in a 1-dm cell at 22 °C. Thin layer chromatography (TLC) was performed on Merck silica gel 60

F_{254} plastic plates. Compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% sulfuric acid (400 mL). Flash chromatography was performed on J. T. Baker silica gel 60 (0.040–0.063 mm) at a pressure of 0.3 bar. Target molecules were purified by Hydrophobic Interaction Chromatography on octyl-sepharose as stationary phase and as elution phase was used as a gradient of propanol (15–60%) in 0.1 M ammonium acetate buffer (pH = 4.8).

4.2. General procedures

4.2.1. General procedure for phosphate formation. The alcohol and the phosphane (1.2 equiv) were coevaporated with dry CH_2Cl_2 and dried in high vacuum for 1 h. The mixture was dissolved in dry CH_2Cl_2 and tetrazole (2.5 equiv, previously dried 1 h in high vacuum) was added. The reaction mixture was stirred for 1.5 h at room temperature (TLC control), and after this time *t*-BuO_2H (1.3 equiv of 5.5 M solution in decane) was added. The reaction mixture was stirred for another 30–45 min and was diluted with EtOAc, washed with saturated $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, and evaporated in vacuo. Purification by flash chromatography in silica gel gave the desired product.

4.2.2. General procedure for the removal of *tert*-butyldiphenylsilyl protecting group (B). The silylated compound was dissolved in THF (p.a. quality) and treated with TBAF (1.2 equiv of 1 M solution in THF). The reaction mixture was stirred for 30–45 min at room temperature (monitoring by TLC). After this time the reaction mixture was diluted with EtOAc and washed with saturated NH_4Cl solution and water. The organic phase was dried over $MgSO_4$ and the solvent was evaporated in vacuo. Flash chromatography in silica gel gave the desired compound.

4.3. Preparation of 4

4.3.1. 3-*O*-Allyl-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol 11. To a solution of compound **10**[12] (22.9 g, 0.17 mol) and imidazole (17.7 g, 0.26 mol) in dry CH_2Cl_2 (450 mL), 46 mL of TBDPS-Cl were added at 0 °C and the reaction mixture was stirred for 10 min. The solution was washed with water; the organic phase was dried over $MgSO_4$ and then evaporated in vacuo. Purification by flash silica gel (petroleum ether/EtOAc 10:1) yielded compound **11** (48 g, 75%) as a colourless syrup. TLC (petroleum ether/EtOAc 5:1): $R_f = 0.9$. $[\alpha]_D = -3.6$ (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.0$ (s, 9H, *t*Bu), 1.49 (d, 1H, $J = 5.1$ Hz, OH), 3.47–3.60 (m, 2H, CH_2–glycerol), 3.71 (d, 2H, $J = 5.5$ Hz, CH_2–glycerol), 3.85–3.94 (m, 1H, CH–glycerol), 3.98–4.02 (m, 2H, CH_2CHCH_2), 5.15–5.30 (m, 2H, CH_2CHCH_2), 5.81–5.97 (m, 1H, CH_2CHCH_2), 7.35–7.47, 7.64–7.69 (m, 10H, Ph).

4.3.2. 3-*O*-Allyl-2-*O*-(4-methoxybenzyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol 12. To a solution of compound **11** (26 g, 0.07 mol) and *p*-methoxybenzyl chloride (15 mL, 1.5 equiv) in dry DMF (250 mL), NaH (2.5 g, 0.1 mol, 1.5 equiv) was added portionwise and the reaction mixture was stirred for 1 h at room temp. The DMF was removed in vacuo; the rest was redissolved in EtOAc and washed with a saturated NH_4Cl solution; the organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. After flash chromatography (petroleum ether/EtOAc 30:1) compound **12** (24 g, 70%) was obtained as colourless syrup. TLC (petroleum ether/EtOAc 7:1): $R_f = 0.59$. $[\alpha]_D = -11.8$ (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.05$ (s, 9H, *t*Bu), 3.50–3.82 (m, 8H, OMe, 1-H, 2-H, 3-H) 3.96–4.02 (m, 2H, CH_2CHCH_2), 4.58 (s, 2H, CH_2Ph_{PMB}), 5.12–5.30 (m, 2H, CH_2CHCH_2), 5.80–5.99 (m, 1H, CH_2CHCH_2), 6.80–6.89 (m, 2H, Ph_{PMB}), 7.19–7.28 (m, 2H, Ph_{PMB}), 7.30–7.47, 7.61–7.74 (m, 10H, Ph). $C_{30}H_{38}O_4Si{\cdot}1/4H_2O$ (495.2). Anal. Calcd: C: 72.76 H: 7.84. Found: C: 72.62, H: 7.91.

4.3.3. 2-*O*-(4-Methoxybenzyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol 13. Compound **12** (16 g, 32.6 mmol) was dissolved in dry EtOH (200 mL), DBU (0.48 mL) and $(Ph_3P)_3RhCl$ (0.61 g, 0.02 equiv) were added and the reaction mixture was stirred at 90 °C for 15 min. The solvent was removed in vacuo and the isomerized product ($R_f = 0.57$, petroleum ether/EtOAc 7:1) was redissolved in 200 mL of 1 M HCl/acetone (1:9) solution. The reaction mixture was stirred at 70–80 °C for 10 min and then neutralized with Et_3N, diluted with EtOAc and washed with saturated $NaHCO_3$. The organic phase was dried with $MgSO_4$ and the solvent removed in vacuo. Flash chromatography (petroleum ether/EtOAc petroleum ether/EtOAc 4:1) gave **13** (10.5 g, 71%) as colourless syrup. TLC (petroleum ether/EtOAc 7:1): $R_f = 0.16$. $[\alpha]_D = -25.4$ (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.06$ (s, 9H, *t*Bu), 2.02 (br s, 1H, OH), 3.57–3.85 (m, 5H, 1-H, 2-H, 3-H) 3.80 (s, 3H, OMe) 4.44, 4.57 (2d, 2H, $J = 11.4$ Hz, CH_2Ph), 6.81–6.90 (m, 2H, Ph_{PMB}), 7.17–7.25 (m, 2H, Ph_{MPM}), 7.33–7.48 (m, 6H, Ph_{TBDPS}), 7.63–7.71 (m, 4H, Ph_{TBDPS}). $C_{27}H_{35}O_4Si$ (451.7). Anal. Calcd: C: 71.80, H: 7.81. Found: C: 71.67, H: 7.65.

4.3.4. [Benzyloxy]-[diisopropylamino]-[1-*O*-*tert*-butyldiphenylsilyl-2-*O*-(4-methoxybenzyl)-*sn*-glycero] phosphane 4. Tetrazole (517 mg, 0.6 equiv) and compound **13** (5.55 g, 12.32 mmol) were dried separately for 1 h in high vacuum. After unification of both compounds, benzyloxybis(diisopropylamino)phosphane[14] (4.8 g, 1.2 equiv) was added (dissolved in 100 mL of dry CH_2Cl_2). After 30 min the reaction mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo below 30 °C. Fast purification by flash silica gel (petroleum ether/EtOAc 15:1, 1% Et_3N) yielded **4** (6.86 g, 81%) as colourless oil. TLC (petroleum ether/EtOAc 7:1, 1% NEt_3): $R_f = 0.84$. $[\alpha]_D = -4.7$ (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.05$ (s, 9H, *t*Bu), 1.10–1.30 (m, 12H, $CH(CH_3)_2$), 3.52–3.90 (m, 10H, OMe, $CH(CH_3)_2$, 1-H, 2-H, 3-H), 4.54–4.79 (m, 4H, $POCH_2Ph$, CH_2Ph_{PMB}), 6.78–6.88 (m, 2H, Ph_{PMB}), 7.19–7.45, 7.62–7.73 (m, 12H, Ph). $C_{40}H_{54}NO_5PSi$

(687.9). Anal. Calcd: C: 69.84, H: 7.91, N: 2.04. Found: C: 69.69, H: 7.91, N: 2.02.

4.4. Preparation of 5

4.4.1. 3-*O*-Allyl-2-*O*-(2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-1-*O*-*tert*-butyldiphenylsilyl-*sn*-glycerol 15. To a solution of acceptor **11** (8.1 g, 21.86 mmol) and donor **14**[15,16] (9.58 g, 20.18 mmol) in dry CH_2Cl_2 (150 mL) was added TMSOTf (0.37 mL, 0.1 equiv) at room temp. After 2 min the reaction mixture was neutralized with Et_3N; the solvent was removed in vacuo. Flash chromatography (petroleum ether/EtOAc 6:1) yielded compound **15** (11.4 g, 76%). β-Product ($R_f = 0.34$). TLC (petroleum ether/EtOAc 3:1): $R_f = 0.42$. $[\alpha]_D = +107$ (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.06$ (s, 9H, *t*Bu), 1.95 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.09 (s, 3H, Ac), 3.22 (dd, 1H, $J_{2,1} = 3.6$ Hz, $J_{2,3} = 10.7$ Hz, 2-H), 3.56–3.66 (m, 2H, CH_2–glycerol), 3.66–3.73 (m, 1H, CH_2–glycerol), 3.75–3.86 (m, 2H, 6-H, CH_2–glycerol), 3.93–4.12 (m, 4H, 6-H, 2′-H, CH_2CHCH_2–H), 4.19–4.27 (m, 1H, 5-H), 5.04 (t, 1H, $J_{4,3} = J_{4,5} = 9.7$ Hz, 4-H), 5.13–5.20, 5.21–5.28 (m, 2H, CH_2CHCH_2), 5.40 (d, 1H, $J_{1,2} = 3.6$ Hz, 1-H), 5.54 (dd, 1H, $J = 9.4$ Hz, $J = 10.4$ Hz, 3-H), 5.83–5.92 (m, 1H, CH_2CHCH_2). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 19.15$ (1C, $C(CH_3)_3$), 20.53, 20.66, 20.72 (3C, CH_3CO), 26.84 (3C, C(CH_3)$_3$), 60.79 (1C, C-2), 61.60 (1C, C-6), 64.04 (1C, C–CH_2–glycerol), 67.36 (1C, C-5), 68.35 (1C, C-4), 70.19 (1C, C–CH_2–glycerol), 70.23 (1C, C-3), 72.39 (1C, CH_2CHCH_2), 77.3 (1C, CH), 97.06 (1C, C-1), 117.1 (1C, CH_2CHCH_2), 127.86–135.54 (12C, Ph), 134.39 (1C, CH_2CHCH_2), 169.58, 169.97, 170.57 (3C, CH_3CO). $C_{34}H_{45}N_3O_{10}\cdot 1/2H_2O$ (692.8). Anal. Calcd: C: 58.94, H: 6.69, N: 6.07. Found: C: 58.72, H: 6.50, N: 5.86.

4.4.2. 3-*O*-Allyl-2-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-1-*O*-*tert*-butyldiphenylsilyl-*sn*-glycerol 16. Compound **15** (9.8 g, 14.33 mmol) was dissolved in dry MeOH (200 mL) and 4 mL of 0.1 M NaOMe solution (in MeOH) were added (pH 9). The reaction mixture was stirred overnight and neutralized with ion exchange resin IR120 (H^+-Form); the solvent was removed in vacuo. This intermediate (7.4 g, 13.28 mmol $R_f = 0.15$, toluene/acetone 2:1) was dried in high vacuum and dissolved in dry DMF (150 mL), BnBr (8 mL, 66.67 mmol) was added and NaH (1.28 g, 53.33 mmol) was added portionwise. After 24 h the solvent was removed in vacuo, redissolved in EtOAc and washed with a saturated solution of NH_4Cl. The organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. Purification by flash chromatography (petroleum ether/EtOAc 9:1) yielded **16** (8.17 g, 74%) as colourless syrup. TLC (petroleum ether/EtOAc 5:1): $R_f = 0.53$. $[\alpha]_D = +63$ (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.04$ (s, 9H, *t*Bu), 3.01 (dd, 1H, $J_{2,1} = 3.6$ Hz, $J_{2,3} = 10.3$ Hz, 2-H), 3.36 (dd, 1H, $J_{vic} = 2.0$ Hz, $J_{gem} = 10.9$ Hz, 6-H), 3.55 (dd, 1H, $J_{vic} = 3.0$ Hz, $J_{gem} = 10.9$ Hz, 6-H), 3.61 (dd, 1H, $J_{vic} = 6.3$ Hz, $J_{gem} = 10.3$ Hz, CH_2–glycerol), 3.71–3.73 (m, 3H, 4-H, CH_2–glycerol), 3.77 (dd, 1H, $J_{vic} = 5.3$ Hz, $J_{gem} = 10.6$ Hz, CH_2–glycerol), 3.86–3.89 (m, 1H, 5-H), 3.96–4.04 (m, 4H, CHglycerol, CH_2CHCH_2–H), 4.36, 4.54 (dd, 2H, $J = 12.1$ Hz, CH_2–Ph), 4.47, 4.77 (dd, 2H, $J = 11.0$ Hz, CH_2–Ph), 4.83 (dd, 2H, $J = 10.7$ Hz, CH_2–Ph), 5.16–5.30 (m, 2H, CH_2CHCH_2), 5.25 (d, 1H, $J_{1,2} = 3.7$ Hz, 1-H), 5.85–5.95 (m, 1H, CH_2CHCH_2), 7.05–7.15, 7.16–7.42, 7.57–7.69 (m, 25H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 19.21$ (1C, $C(CH_3)_3$), 26.84 (3C, C(CH_3)$_3$), 63.3 (1C, C-2), 63.87 (1C, C–CH_2–glycerol), 67.97 (1C, C-6), 70.67 (1C, C-5), 72.35 (1C, CH_2CHCH_2), 73.47–76.79 (3C, CH_2–Ph), 77.21 (1C, C–CH_2–glycerol), 78.15 (1C, C-4), 79.89 (1C, C-3), 97.49 (1C, C-1), 116.87 (1C, CH_2CHCH_2), 127.51–138.26 (30C, Ph), 134.67 (1C, CH_2CHCH_2). $C_{49}H_{57}N_3O_7$ (828.1). Anal. Calcd: C: 71.07, H: 6.94, N: 5.07. Found: C: 70.90, H: 6.96, N: 4.97.

4.4.3. 2-*O*-(2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-3-*O*-allyl-1-*O*-*tert*-butyldiphenylsilyl-*sn*-glycerol 17. Compound **16** (7.2 g, 8.7 mmol) was dissolved in pyridine/H_2O (5:1, 150 mL), 0.7 mL of Et_3N and 1,3-propanedithiol (4.4 mL, 5 equiv) were added. The reaction mixture was stirred overnight and the solvent was removed in vacuo. The product was coevaporated twice with toluene and redissolved in pyridine/Ac_2O (2:1, 150 mL). The reaction mixture was stirred for 2 h. The solvent was evaporated in vacuo and the product was coevaporated with toluene. Purification by flash chromatography (toluene/acetone 8:1) yielded compound **17** (5.82 g, 85%) as colourless foam. TLC (toluene/acetone 2:1): $R_f = 0.31$. $[\alpha]_D = +42$ (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.04$ (s, 9H, *t*Bu), 1.84 (s, 3H, NHAc), 3.39 (dd, 1H, $J_{vic} < 1$ Hz, $J_{gem} = 10.6$ Hz, 6-H), 3.52 (dd, 1H, $J_{vic} = 6.7$ Hz, $J_{gem} = 10.3$ Hz, 1′-H), 3.61 (dd, 1H, $J_{vic} = 2.9$ Hz, $J_{gem} = 10.8$ Hz, 6-H), 3.62–3.69 (m, 3H, 3′-H, 1′-H, 3-H), 3.72–3.82 (m, 3H, 3′-H, 5-H, 4-H), 3.84 (m, 1H, 2′-H), 3.91–4.01 (m, 2H, CH_2CHCH_2–H), 4.26 (ddd, 1H, $J = 3.4$ Hz, 2-H), 4.37/4.54 (dd, 1H, $J = 12.2$ Hz, CH_2–Ph), 4.48/4.78 (dd, 2H, $J = 10.9$ Hz, CH_2–Ph), 4.63/4.79 (dd, 2H, $J = 11.5$ Hz, CH_2–Ph), 4.89 (d, 1H, $J_{1,2} = 3.5$ Hz, 1-H), 5.15–5.28 (m, 2H, CH_2CHCH_2), 5.79–5.87 (m, 1H, CH_2CHCH_2), 7.09–7.58, 7.57–7.67 (m, 25H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 19.20$ (1C, $C(CH_3)_3$), 23.43 (1C, NHAc), 26.81 (3C, C(CH_3)$_3$), 52.77 (1C, C-2), 63.47 (1C, C-3′), 68.18 (1C, C-6), 69.65 (1C, C-1′), 71.36 (1C, C-5), 72.28 (1C, CH_2CHCH_2), 73.32–74.90 (3C, CH_2–Ph), 77.97 (1C, C-4), 78.09 (1C, C-2′), 80.91 (1C, C-3), 98.33 (1C, C-1), 117.39 (1C, CH_2CHCH_2), 127.56–138.53 (31C, Ph, CH_2CHCH_2), 169.81 (1C, $COCH_3$). $C_{51}H_{51}NO_8Si\cdot 1/2H_2O$ (853.1). Anal. Calcd: C: 71.80, H: 7.33, N: 1.64. Found: C: 71.84, H: 7.27, N: 1.42.

4.4.4. 2-*O*-(2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-1-*O*-*tert*-butyldiphenylsilyl-*sn*-glycerol 18. Compound **17** (2.7 g, 3.43 mmol) was dissolved in dry EtOH (40 mL), DBU (77 μL, 0.15 equiv) and $(Ph_3P)_3RhCl$ (1 g, 0.3 equiv) were added and the reaction mixture was stirred at 90 °C for 15 min. The solvent was removed in vacuo, and the reaction intermediate

(R_f = 0.76, toluene/acetone 1.5:1) was redissolved in 50 mL of a 1 M HCl/acetone solution (1:9). The reaction mixture was stirred at 70 °C for 15 min. The reaction mixture was neutralized with Et_3N, diluted with EtOAc and washed with saturated $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, and the solvent was removed in vacuo. Flash chromatography (toluene/acetone 2:1) yielded **18** (1.98 g, 77%) as light brown foam. TLC (toluene/acetone 1.5:1): R_f = 0.55. $[\alpha]_D$ = +62.6 (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.05 (s, 9H, *t*Bu), 1.81 (s, 3H, NHAc), 2.52 (s, 1H, OH), 3.42 (dd, 1H, J_{vic} = 1.3 Hz, J_{gem} = 10.7 Hz, 3′-H), 3.60 (dd, 1H, J_{vic} = 3.7 Hz, J_{gem} = 10.8 Hz, 3′-H), 3.74–3.84 (m, 8H, 1′-H, 6-H, 2′-H, 3-H, 4-H, 5-H), 4.19 (m, 1H, 2-H), 4.34/4.54 (dd, 2H, J = 12.2 Hz, CH_2–Ph), 4.51/4.80 (dd, 2H, J = 10.9 Hz, CH_2–Ph), 4.63/4.81 (dd, 2H, J = 11.6 Hz, CH_2–Ph), 5.0 (d, 1H, $J_{1,2}$ = 3.6 Hz, 1-H), 5.91 (d, 1H, J = 8.6 Hz, NH), 7.10–7.46, 7.60–7.77 (m, 25H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 19.12 (1C, *C*(CH_3)$_3$), 23.24 (1C, NHAc), 26.80 (3C, C(*C*H_3)$_3$), 52.93 (1C, C-2), 62.29 (1C, C-1′), 64.94 (1C, C-6), 68.26 (1C, C-3′), 71.36 (1C, C-2′), 73.33–74.83 (3C, *C*H_2–Ph), 78.01–80.39 (3C, C-3, C-4, C-5), 97.28 (1C, C-1), 127.58–138.78 (30C, Ph, CH_2*C*HCH_2–C), 170.25 (1C, *C*OCH_3). $C_{48}H_{57}NO_8Si$ (804.1). Anal. Calcd: C: 71.70, H: 7.15, N: 1.74. Found: C: 71.45, H: 7.20, N: 1.36.

4.4.5. [Benzyloxy]-[diisopropylamino]-[2-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-1-*O*-*tert*-butyldiphenylsilyl-*sn*-glycero] phosphane 5. Compound **18** (1.92 g, 2.57 mmol) was dried for 1 h in high vacuum together with tetrazole (108 mg, 0.6 equiv). Under argon atmosphere benzyloxybis(diisopropylamino)phosphane (1 g, 1.2 equiv) was added (dissolved in 30 mL of dry CH_2Cl_2). After 15 min the reaction mixture was diluted with CH_2Cl_2 and washed with a saturated $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo below 30 °C. Fast purification by flash silica gel (toluene/EtOAc 4:1, 1% Et_3N) yielded compound **5** (2.27 g, 85%) as colourless oil. TLC (toluene/acetone 1.5:1, 1% NEt_3): R_f = 0.71. $[\alpha]_D$ = +31.2 (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, DMSO-d_6): δ = 0.96 (s, 9H, *t*Bu), 1.0–1.17 (m, 12H, C(CH_3)$_2$), 1.80/1.81 (2s, 3H, NHAc), 3.36–3.41 (m, 1H, 3′-H), 3.44–3.49 (m, 1H, 3′-H), 3.49–3.59 (m, 3H, CH, 4-H), 3.59–3.65 (m, 1H, 1′-H), 3.67–3.82 (m, 4H, 1′-H, 3-H, 6-H), 3.87–3.98 (m, 3H, 2′-H, 2-H, 5-H), 4.33–4.39, 4.42–4.51, 4.55–4.74 (m, 8H, CH_2–Ph, POCH_2–Ph), 4.92 (m, 1H, 1-H), 7.07–7.43, 7.55–7.65, 7.70–7.82 (m, 30H, Ph). ^{13}C NMR (150.9 MHz, DMSO-d_6): δ = 18.72 (1C, *C*(CH_3)$_3$), 22.53 (1C, NHAc), 24.32 (4C, C(*C*H_3)$_2$), 26.62 (3C, C(*C*H_3)$_3$), 42.40 (2C, *C*(CH_3)$_2$), 52.63 (1C, C-2), 60.70 (1C, C-1′), 61.30/63.78 (1C, C-6), 64.55/64.67 (1C, PO*C*H_2–Ph), 68.33 (1C, C-3′), 70.39 (1C, C-5), 73.85–73.99 (3C, *C*H_2–Ph), 76.43 (1C, C-2′), 78.10 (1C, C-4), 79.68 (1C, C-3), 96.17/96.34 (1C, C-1), 125.30–139.11 (36C, Ph), 169.26 (1C, *C*OCH_3). ^{31}P NMR (242.9 MHz, DMSO-d_6): δ = 148.38 (s), 148.63 (s). $C_{61}H_{77}N_2O_9PSi$ (1041.3). Anal. Calcd: C: 70.36, H: 7.45, N: 2.69. Found: C: 70.39, H: 7.64, N: 2.54.

4.5. Preparation of target molecule 1

4.5.1. Benzyl[(2*S*)-1,2-di-benzyloxy-propan-3-yl]-[(2*R*)-1-*tert*-butyldiphenylsilyloxy-2-(4-methoxybenzyloxy)-propan-3-yl] phosphate 19. Procedure A. Compound **3** (1.44 g, 5.29 mmol), **4** (3.64 g, 1 equiv). TLC before oxidation (petroleum ether/EtOAc 2:1, 1% Et_3N) R_f = 0.74. Flash chromatography (petroleum ether/EtOAc 3:1) yielded phosphate **19** (3.5 g, 76%) as colourless syrup. TLC (petroleum ether/EtOAc 2:1): R_f = 0.41. $[\alpha]_D$ = −6 (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.03 (s, 9H, *t*Bu), 3.46–3.56 (m, 2H, 6-H), 3.59–3.80 (m, 7H, 1-H, 2-H, 5-H, OMe), 4.07–4.30 (m, 4H, 3-H, 4-H), 4.41–4.65 (m, 6H, CH_2–Ph, CH_2–PhOMe), 4.97–5.04 (m, 2H, POCH_2–Ph), 6.75–6.83, 7.12–7.20 (m, 4H, Ph_{PMB}), 7.20–7.44, 7.58–7.68 (m, 25H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 18.68 (1C, *C*(CH_3)$_3$), 26.29 (3C, C(*C*H_3)$_3$), 54.73 (1C, OMe), 62.18 (1C, C-1), 66.42, 66.51, 66.55 (2C, C-3, C-4), 68.72 (2C, PO*C*H_2–Ph, C-6), 71.34, 71.73, 72.91 (3C, *C*H_2–Ph), 76.1 (1C, C-5), 77.27 (1C, C-2), 113.20 (2C, C_{PMB}), 127.09–137.66 (33C, Ph), 158.63 (1C, C–OMe). ^{31}P NMR (242.9 MHz, $CDCl_3$): δ = 0.39, 0.40 (2s, 1P). FAB-MS (positive mode, Matrix NBOH+NaI, THF): $[M+Na]^+$, *m*/*z* = 897; found: *m*/*z* = 897.

4.5.2. Benzyl[(2*S*)-1,2-di-benzyloxy-propan-3-yl]-[(2*R*)-1-hydroxy-2-(4-methoxybenzyloxy)-propan-3-yl] phosphate 20. Procedure B. Phosphate **19** (2.79 g, 3.19 mmol) Purification by flash silica gel (petroleum ether/EtOAc 1:1) yielded compound **20** (1.75 g, 87%) as colourless syrup. TLC (petroleum ether/EtOAc 1:1): R_f = 0.10; (toluene/acetone 1:1): R_f = 0.63. $[\alpha]_D$ = +8.6 (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.15–2.38 (br s, 1H, OH), 3.46–3.73 (m, 5H, 1-H, 2-H, 6-H), 3.73–4.11 (m, 4H, 5-H, OMe), 4.03–4.29 (m, 4H, 3-H, 4-H), 4.41–4.71 (m, 6H, CH_2–Ph, CH_2–PhOMe), 5.00–5.12 (m, 2H, POCH_2–Ph), 6.79–6.92, 7.12–7.20 (m, 2H, Ph_{PMB}), 7.14–7.41 (m, 20H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$,): δ = 55.2 (1C, OMe), 61.0 (1C, C-1), 65.5–67.5 (2C, C-3, C-4), 69.0 (1C, C-6), 69.4 (1C, PO*C*H_2–Ph), 71.6–73.8 (3C, *C*H_2–Ph), 76.7 (1C, C-5), 77.1 (1C, C-2). ^{31}P NMR (242.9 MHz, $CDCl_3$): δ = −1.55, −1.58 (2s, 1P). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, *m*/*z* = 659.7; found: *m*/*z* = 659.5, $[M+K]^+$, *m*/*z* = 675.8; found: *m*/*z* = 675.8. $C_{35}H_{41}O_9P$ (636.7). Anal. Calcd: C: 66.03, H: 6.49. Found: C: 65.74, H: 6.50.

4.5.3. Diphosphate 21. Procedure A. Compound **20** (1.67 g, 2.62 mmol) TLC before oxidation (petroleum ether/EtOAc 1:1, 1% Et_3N, R_f = 0.62). Purification by flash chromatography (petroleum ether/EtOAc 2:1→1:1) yielded compound **21** (3.1 g, 95%) as colourless syrup. TLC (petroleum ether/EtOAc 1:1): R_f = 0.38. $[\alpha]_D$ = −4.7 (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.02 (s, 9H, *t*Bu), 3.46–3.55 (m, 2H, 9-H), 3.59–3.77 (m, 11H, 1-H, 2-H, 5-H, 8-H, OMe), 3.94–4.30 (m, 8H, 3-H, 4-H, 6-H, 7-H), 4.38–4.62 (m, 8H, CH_2–Ph, CH_2–PhOMe), 4.92–5.03 (m, 4H, POCH_2–Ph), 6.70–6.80 (m, 4H, Ph_{PMB}), 7.12–7.43, 7.58–7.66 (m, 38H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 19.16 (1C, *C*(CH_3)$_3$), 26.77 (3C, C(*C*H_3)$_3$),

55.20 (1C, OMe), 62.63 (1C, C-1), 65.81 (2C, C-4, C-6), 67.08 (2C, C-3, C-7), 69.09 (1C, C-9), 69.28 (2C, PO*C*H$_2$–Ph), 71.81–73.39 (4C, *C*H$_2$–Ph), 75.42 (1C, C-5), 76.53 (1C, C-8), 77.71 (1C, C-2), 113.69, 113.74 (4C, *C*$_2$COMe), 127.58–138.11 (42C, Ph), 159.13, 159.25 (2C, C–OMe). ^{31}P NMR (600 MHz, $CDCl_3$): $\delta = 0.069$–0.041 (m, 2P). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 1262.4$; found: $m/z = 1261.8$, $[M+K]^+$, $m/z = 1278.5$; found: $m/z = 1277.9$. $C_{69}H_{80}O_{15}P_2Si$ (1239.4). Anal. Calcd: C: 66.87, H: 6.51. Found: C: 65.60, H: 6.53.

4.5.4. Diphosphate 22. Procedure B. Compound **21** (3.08 g, 2.49 mmol) Purification by flash chromatography (toluene/acetone 2:1) yielded product **22** (2.0 g, 80%) as colourless syrup. TLC (toluene/acetone 1:1): $R_f = 0.38$. $[\alpha]_D = +1.5$ (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 3.47$–3.80 (m, 13H, 1-H, 2-H, 5-H, 8-H, 9-H, OMe), 3.91–4.22 (m, 8H, 3-H, 4-H, 6-H, 7-H), 4.41–4.65 (m, 8H, C*H*$_2$–Ph, C*H*$_2$–PhOMe), 4.95–5.06 (m, 4H, POC*H*$_2$–Ph), 6.76–6.88 (m, 4H, Ph$_{PMB}$), 7.12–7.36 (m, 26H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 55.24$ (2C, OMe), 60.9 (1C, C-1), 65.30–66.60 (3C, C-3, C-4, C-6), 67.13 (1C, C-7), 69.00 (1C, C-9), 69.42–6953 (2C, PO*C*H$_2$–Ph), 71.70–73.42 (4C, *C*H$_2$–Ph), 76.46 (1C, C-5), 76.79 (1C, C-8), 77.30 (1C, C-2), 113.80, 113.86 (4C, C$_2$COMe), 127.61–137.93 (30C, Ph), 159.35 (2C, C–OMe). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 1024.0$; found: $m/z = 1022.8$, $[M+K]^+$, $m/z = 1040.1$; found: $m/z = 1039.0$. $C_{53}H_{62}O_{15}P_2 \cdot 1/4H_2O$ (1005.5). Anal. Calcd: C: 63.31, H: 6.27. Found: C: 63.17, H: 6.14.

4.5.5. Triphosphate 23. Following procedure A. Compound **22** (2.21 g, 2.21 mmol). TLC before oxidation (toluene/acetone 2:1, 1% Et_3N, $R_f = 0.66$). Purification in (toluene/acetone 4:1→3:1) yielded **23** (3.3 g, 93%) as colourless syrup. TLC (toluene/acetone 3:1): $R_f = 0.20$. $[\alpha]_D = -3.5$ (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.02$ (s, 9H, *t*Bu), 3.48–3.55 (m, 2H, 12-H), 3.59–3.80 (m, 15H, 1-H, 2-H, 5-H, 8-H, 11-H, OMe), 3.90–4.29 (m, 12H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H), 4.39–4.51, 4.57–4.62 (m, 10H, C*H*$_2$–Ph, C*H*$_2$–PhOMe), 4.91–5.03 (m, 6H, POC*H*$_2$–Ph), 6.72–6.83 (m, 6H, Ph$_{PMB}$), 7.11–7.44 (m, 37H, Ph), 7.58–7.68 (m, 4H, Ph$_{TBDPS}$). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 1626.7$; found: $m/z = 1627.1$. $C_{87}H_{101}O_{21}P_3Si \cdot 3/4\ H_2O$ (1617.2). Anal. Calcd: C: 64.61 H: 6.39. Found: C: 64.63 H: 6.51.

4.5.6. Triphosphate 24. Procedure B. Compound **23** (3.32 g, 2.07 mmol). Purification in (toluene/acetone 2:1→1:1) yielded **24** (2.6 g, 92%) as colourless syrup. TLC (toluene/acetone 1:1): $R_f = 0.22$. $[\alpha]_D = +0.6$ (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 2.88$ (br s, 1H, OH), 3.47–3.79 (m, 17H, 1-H, 2-H, 5-H, 8-H, 11-H, 12-H, OMe), 3.91–4.25 (m, 12H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H), 4.41–4.62 (m, 10H, C*H*$_2$–Ph, C*H*$_2$–PhOMe), 4.93–5.07 (m, 6H, POC*H*$_2$–Ph), 6.72–6.88 (m, 6H, Ph$_{PMB}$), 7.10–7.38 (m, 31H, Ph). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 1388.3$; found: $m/z = 1388.2$. $C_{71}H_{83}O_{21}P_3 \cdot H_2O$ (1383.3). Anal. Calcd: C: 61.65, H: 6.19. Found: C: 61.68, H: 6.42.

4.5.7. Tetraphosphate 25. Procedure A. Compound **24** (2.37 g, 1.74 mmol). TLC before oxidation (toluene/acetone 1:1, 1% Et_3N, $R_f = 0.74$). After purification in silica gel (toluene/acetone 3:1) compound **25** (3.1 g, 90%) was obtained as colourless syrup. TLC (toluene/acetone 1:1): $R_f = 0.70$. $[\alpha]_D = -3.6$ (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.02$ (s, 9H, *t*Bu), 3.46–3.53 (m, 2H, 15-H), 3.59–3.77 (m, 19H, 1-H, 2-H, 5-H, 8-H, 11-H, 14-H, OMe), 3.90–4.28 (m, 16H, 3-H, 4-H, 6-H, 7-H, 8-H, 10-H, 12-H, 13-H), 4.38–4.50, 4.52–4.63 (m, 12H, C*H*$_2$–Ph, C*H*$_2$–PhOMe), 4.92–5.03 (m, 8H, POC*H*$_2$–Ph), 6.71–6.81 (m, 8H, Ph$_{PMB}$), 7.10–7.43 (m, 44H, Ph), 7.59–7.67 (m, 4H, Ph$_{TBDPS}$). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 26.77$ (3C, C(*C*H$_3$)$_3$), 55.17 (4C, OMe), 62.6 (1C, C-1), 65.79–67.1 (3C, C-3, C-4, C-6, C-7, C-8, C-10, C-12, C-13), 69.06 (1C, C-15), 69.41 (4C, PO*C*H$_2$–Ph), 71.86–73.39 (6C, *C*H$_2$–Ph), 75.36 (3C, C-5, C-8, C-11), 76.79 (1C, C-14), 77.7 (1C, C-2), 113.76 (8C, *C*$_2$COMe), 127.59–135.57 (Ph). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 1991.1$; found: $m/z = 1989.9$. $C_{105}H_{122}O_{27}P_4Si$ (1968.1). Anal. Calcd: C: 64.08, H: 6.25. Found: C: 64.18, H: 6.23.

4.5.8. Tetraphosphate 26. Procedure B. Compound **25** (3.05 g, 1.55 mmol) Purification by flash chromatography (toluene/acetone 2:1→1:1) yielded compound **26** (2.5 g, 93%) as colourless syrup. TLC (toluene/acetone 1:1): $R_f = 0.64$. $[\alpha]_D = -0.4$ (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 3.47$–3.82 (m, 21H, 1, 2, 5, 8, 11, 14, 15-H, OMe), 3.91–4.25 (m, 16H, 3, 4, 6, 7, 9, 10, 12, 13-H), 4.40–4.64 (m, 12H, C*H*$_2$–Ph, C*H*$_2$–PhOMe), 4.92–5.09 (m, 8H, POC*H*$_2$–Ph), 6.71–6.89 (m, 8H, Ph$_{PMB}$), 7.11–7.41 (m, 38H, Ph). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 1752.7$; found: $m/z = 1750.2$. $C_{89}H_{104}O_{27}P_4$ (1729.66). Anal. Calcd: C: 61.80, H: 6.06. Found: C: 61.71, H: 6.16.

4.5.9. Pentaphosphate 27. Procedure A. Compound **26** (2.08 g, 1.20 mmol), compound **5** (1.5 g, 1.2 eq). TLC before oxidation (toluene/acetone 1:1, 1% Et_3N, $R_f = 0.64$). Purification in silica gel (toluene/acetone 2:1→1:3) gave compound **27** (2.9 g, 89%) as colourless syrup. TLC (toluene/acetone 1:1): $R_f = 0.62$; 0.58. $[\alpha]_D = +9.2$ (*c* 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 1.01$ (s, 9H, *t*Bu), 1.88–2.02 (m, 3H, NHCOC*H*$_3$), 3.13–3.21 (m, 1H, 6″-H), 3.44–3.55 (m, 4H, 5″-H, 6″-H, 18-H), 3.55–3.81 (m, 22H, 1-H, 2-H, 5-H, 8-H, 11-H, 14-H, 17-H, 3″-H, 4″-H, OMe), 3.91–4.14, 4.15–4.22 (m, 18H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H, 16-H), 4.22–4.35, 4.36–4.52, 5.55–4.67, 4.67–4.77 (m, 22H, 1″-H, 2″-H, 3-H, C*H*$_2$–Ph, C*H*$_2$–PhOMe), 4.84–5.02 (m, 10H, POC*H*$_2$–Ph), 6.68–6.82, 7.01–7.43, 7.52–7.63 (m, 76H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$,): $\delta = 52.7$ (2″-C), 55.2 (OMe), 61.6 (C-1), 65.9 (C-4, C-6, C-7, C-9, C-10, C-12, C-13, C-15), 67.1 (3, C-16), 68.0 (C-6″), 69.1 (C-18), 69.3 (PO*C*H$_2$–Ph), 71.1–75.3 (*C*H$_2$–Ph), 75.5–77.0 (C-2, C-5, C-8, C-11, C-14, C-17), 77.8–81.1 (C-3″, C-4″), 100.2 (C-1″).

MALDI-MS (positive mode, Matrix DHB, THF): $[M+H_3O^+]^+$, $m/z = 2704.8$; found: $m/z = 2704.7$. $C_{144}H_{166}NO_{37}P_5Si{\cdot}H_2O$ (2703.8). Anal. Calcd: C: 63.97, H: 6.26, N: 0.52. Found: C: 64.01, H: 6.40, N: 0.52.

4.5.10. Pentaphosphate 28. Procedure B. Compound **27** (2.87 g, 1.07 mmol) Purification by flash chromatography (toluene/acetone 2:1→1:1) yielded compound **28** (2.1 g, 80%) as colourless syrup. TLC (toluene/acetone 1:1): $R_f = 0.4$, 0.51, 0.57. $[\alpha]_D = +15.2$ (*c* 1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.84$–2.02 (m, 3H, NHCOCH_3), 3.38–3.80, 3.88–4.21 (m, 39H, 3″-H, 4″-H, 5″-H, 6″-H, OMe, 1–18-H), 4.21–4.82 (m, 19.5H, 1″-H, 2″-H, CH_2–Ph, CH_2–PhOMe), 4.88–5.06 (m, 10.5H, 1″-H, POCH_2–Ph), 6.69–6.82 (m, 8H, Ph), 7.05–7.36 (m, 58H, Ph). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 2470.4$; gef.: $m/z = 2469.9$. $C_{128}H_{148}NO_{37}P_5$ (2447.4). Anal. Calcd: C: 62.82, H: 6.10, N: 0.57. Found: C: 62.82, H: 6.33, N: 0.58.

4.5.11. Hexaphosphate 29. Procedure A. Compound **28** (530 mg, 0.217 mmol), **6** (211.2 mg, 1.3 equiv). Purification by flash chromatography (toluene/acetone 2:1) gave **29** (550 mg, 82%) as colourless syrup. TLC (toluene/acetone 1:1): $R_f = 0.48$, $R_f = 0.56$. $[\alpha]_D = -16$ (*c* 0.25, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.80$–0.90 (t, 6H, Me), 1.15–1.32 (m, 40H, CH_2 chain), 1.48–1.63 (m, 4H, $COCH_2CH_2R$), 1.90–2.0 (m, 3H, NHAc), 2.19–2.29 (m, 4H, $COCH_2CH_2R$), 3.46–3.55 (m, 2H, 18-H), 3.62 (m, 1H, 6″-H), 3.65 (m, 4H, 5-H, 8-H, 11-H, 14-H), 3.68 (m, 1H, 6″-H), 3.69 (m, 13H, OMe, 3″-H), 3.71 (m, 1H, 4″-H), 3.73 (m, 2H, 17-H, 2-H), 3.78 (m, 1H, 5″-H), 3.98–4.07 (m, 22H, 1-H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H, 1′-H), 4.07 (m, 1H, 3′-H), 4.08 (m, 1H, 16-H), 4.18 (m, 1H, 16-H), 4.26 (m, 1H, 3′-H), 4.78 (m, 1H, 2″-H), 3.79–4.76 (m, 18H, CH_2Ph), 4.78 (m, 1H, 1″-H), 4.96 (m, 12H, POCH_2Ph), 5.16 (m, 1H, 2′-H), 6.73–6.82 (m, 8H, Ph_{MPM}), 7.08–7.38 (m, 63H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$,): $\delta = 52.9$ (1C, C-2″), 55.6 (4C, OMe), 62.1 (1C, C-3′), 66.2 (11C, C-1, C-3, C-4, C-6, C-7, C-9, C-10, C-12, C-13, C-15, C-1′), 66.8 (1C, C-16), 68.9 (1C, C-6″), 69.4 (1C, C-18), 69.8 (1C, C-2′), 70.0 (6C, POCH_2Ph), 72.0–76.0 (9C, CH_2Ph), 72.2 (1C, C-5″), 75.9 (4C, C-5, C-8, C-11, C-14), 77.0 (2C, C-2, C-17), 78.0 (1C, C-4″), 81.5 (1C, C-3″), 100.7 (1C, C-1″). MALDI-MS (positive mode, Matrix *p*-nitroaniline+NaI, THF): $[M+Na]^+$, $m/z = 3136.3$; found: $m/z = 3135.2$. $C_{166}H_{214}NO_{44}P_6$ (3113.3). Anal. Calcd: C: 64.04, H: 6.93, N: 0.45. Found: C: 63.97, H: 7.05, N: 0.41.

4.5.12. Hexaphosphate 30. Compound **29** (490 mg, 0.157 mmol) was dissolved in acetonitrile/toluene/water (60:3:4, 15 mL), the solution was cooled to 0 °C and $Ce(NH_4)_2(NO_3)_6$ (1.15 g, 20 equiv) was added portionwise. After 10 min the ice bath was removed and the reaction mixture was stirred for another 50–70 min (monitoring by TLC). The reaction mixture was diluted with EtOAc and washed with saturated $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$ and the solvent was evaporated in vacuo. A fast flash chromatography (toluene/acetone 1:2→acetone) gave **30** (290 mg, 70%) as colourless syrup, which was stored at –20 °C. TLC (toluene/acetone 1:2): $R_f = 0.47$, 0.58. $[\alpha]_D = +7$ (*c* 0.5, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.80$–0.92 (t, 6H, Me), 1.10–1.36 (m, 40H, CH_2 chain), 1.47–1.61 (m, 4H, $COCH_2CH_2R$), 1.88–1.99 (m, 3H, NHAc), 2.18–2.30 (m, 4H, $COCH_2CH_2R$), 3.52 (m, 2H, 18-H), 3.61, 3.66 (m, 2H, 6″-H), 3.68 (m, 1H, 4″-H), 3.69 (m, 1H, 3″-H), 3.73 (m, 2H, 17, 2-H), 3.80 (m, 1H, 5″-H), 3.98, 4.08 (m, 4H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H), 4.06 (m, 7H, 1′-H, 3′-H, 1-H, 3-H), 4.09, 4.19 (m, 2H, 16-H), 4.26 (m, 1H, 3′-H), 4.34 (m, 1H, 2″-H), 4.38–4.76 (m, 10H, CH_2Ph), 4.85/4.89 (m, 1H, 1″-H), 5.02 (m, 12H, POCH_2Ph), 5.08 (m, 1H, 2′-H), 7.05–7.45 (m, 55H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 14.1$ (2C, Me), 22.9 (1C, NHAc), 24.8 (2C, $COCH_2CH_2R$), 22.7–31.9 (20C, CH_2 chain), 34.0, 34.1 (2C, $COCH_2R$), 52.7 (1C, C-2″), 61.6 (1C, C-3′), 65.6 (3C, C-1, C-3, C-1′), 67.3 (1C, C-16), 68.2 (CH_2Glyc.), 68.5 (1C, C-6″), 69.0 (1C, C-18), 69.1 (1C, C-2′), 69.9 (6C, POCH_2Ph), 71.7 (1C, C-5″), 72.2–75.5 (5C, CH_2Ph), 76.5 (2C, C-2, C-17), 77.8 (1C, C-4″), 80.8 (1C, C-3″), 99.5/100.0 (1C, C-1″), 127.6–138.4 (C–Ph), 172.8, 173.2 (2C, COOR). MALDI-MS (positive mode, Matrix *p*-nitroaniline+NaI, THF): $[M+Na]^+$, $m/z = 2654.7$; found: $m/z = 2656.4$. $C_{134}H_{181}NO_{40}P_6$ (2631.7). Anal. Calcd: C: 61.16, H: 6.93, N: 0.53. Found: C: 60.94, H: 7.38, N: 0.47.

4.5.13. Compound 31. Compound **30** (250 mg, 0.095 mmol), PyBOP (992 mg, 20 equiv) and Z-D-alanine triethylammonium salt (619 mg, 20 equiv) were dried separately in high vacuum for 3 h. The mixture was dissolved in dry CH_2Cl_2 (20 mL), *N*-methylimidazole (305 μL, 40 equiv) was added dropwise and the reaction mixture was stirred for 2.5–3 h at room temp under argon atmosphere. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated NH_4Cl solution. The organic phase was dried over $MgSO_4$, and the solvent was removed in vacuo. Flash chromatography (toluene/acetone 3:1) and second column (toluene/acetone 1:1.5) gave **31** (230 mg, 70%) as colourless foam, which was stored at −20 °C. TLC (toluene/acetone 1:1.5): $R_f = 0.62$, 0.69. $[\alpha]_D = +14.95$ (*c* 0.58, acetone). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.79$–0.92 (t, 6H, Me), 1.07–1.32 (m, 52H, CH_2 chain, Ala–Me), 1.45–1.59 (m, 4H, $COCH_2CH_2R$), 1.86–2.01 (m, 3H, NHAc), 2.15–2.29 (m, 4H, $COCH_2CH_2R$), 3.49 (m, 2H, 18-H), 3.64 (m, 2H, 6″-H), 3.67 (m, 1H, 4″-H), 3.69 (m, 3H, 3″-H, 17-H, 2-H), 3.80 (m, 1H, 5″-H), 4.04 (m, 1H, 3′-H), 4.05 (m, 22H, 1′-H, 1-H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H), 4.06, 4.16 (m, 2H, 16-H), 4.23 (m, 1H, 3′-H), 4.30 (m, 1H, 2″-H), 4.31 (m, 4H, CHNHZ), 4.32–4.74 (m, 10H, CH_2Ph), 4.87/4.89 (m, 1H, 1″-H), 4.96 (m, 4H, CH_2-Z), 5.01 (m, 12H, POCH_2Ph), 5.06 (m, 4H, CH_2-Z), 5.12 (m, 4H, 5-H, 8-H, 11-H, 14-H), 5.14 (m, 1H, 2′-H), 5.45–6.02 (NH), 7.00–7.46 (m, 75H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 14.6$ (2C, Me), 18.3 (4C, Ala–Me), 23.2 (1C, NHAc), 23.1/29.9–32.3 (20C, CH_2 chain), 25.1 (2C, $COCH_2CH_2R$), 34.4 (2C, $COCH_2R$), 49.9 (4C, CH–

NHZ), 53.0 (1C, C-2″), 62.0 (1C, C-3′), 65.7 (11C, C-1′, C-1, C-3, C-4, C-6, C-7, C-9, C-10, C-12, C-13, C-15), 67.2 (4C, CH_2Z), 67.9 (1C, C-16), 68.5 (1C, C-6″), 69.1 (1C, C-18), 69.5 (1C, C-2′), 70.1 (6C, POCH_2Ph), 70.8 (4C, C-5, C-8, C-11, C-14), 71.9 (1C, C-5″), 72.2–76.0 (5C, CH_2Ph), 76.8 (2C, C-2, C-17), 78.0 (1C, C-4″), 81.1 (1C, C-3″), 100.2 (1C, C-1″). MALDI-MS (positive mode, Matrix *p*-nitroaniline+NaI, THF): $[M+Na]^+$, *m/z* = 3475.5; found: *m/z* = 3478.0. To produce the triethylammonium salt, the commercial Z-D-alanine was dissolved in methanol (1.1 equiv NEt_3) and coevaporated with toluene. The product was dried in high vacuum.

4.5.14. Target molecule 1. Compound **31** (105 mg, 0.03 mmol) was dissolved in CH_2Cl_2/MeOH/H_2O (5:5:1 5 mL) and Pearlman's catalyst was added (10.5 mg). The reaction was stirred overnight under H_2 atmosphere and after this time was filtered through Celite with the same solvent mixture. The solution was diluted with 0.1 M NH_4OAc buffer (pH 4.8), frozen and lyophilized. The crude white solid was purified by 'Hydrophobic Interaction Chromatography', and after lyophilization compound **1** (24 mg, 41%) was obtained as white powder. ^{1}H NMR (600 MHz, D_2O): δ = 0.85 (br s, 6H, Me), 1.09–1.42 (m, 40H, CH_2 chain), 1.47–1.67 (m, 16H, Ala–Me, $COCH_2CH_2$R), 2.03/2.07 (2s, 3H, NHAc), 2.22–2.44 (m, 4H, $COCH_2CH_2$R), 3.40–3.50 (m, 1H, 4c-H), 3.58, 3.65 (m, 2H, 18-H), 3.70–4.58 (m, 37H), 4.95–5.08 (m, 1H, 1″-H), 5.20–5.40 (m, 5H, CH-Ala, 2′-H). ^{13}C NMR (150.9 MHz, D_2O): δ = 16.5 (2C, Me), 18.0 (4C, Ala–Me), 24.8 (1C, NHAc), 27.5 (2C, $COCH_2CH_2$R), 25.3/33.6/34.7 (20C, CH_2 chain), 36.7, 36.8 (2C, $COCH_2CH_2$R), 51.6 (4C, $CHNH_3^+$), 56.3 (1C, C-2″), 63.3 (1C, C-6″), 64.7 (1C, C-18), 65.8 (1C, C-3′), 66.2, 67.9 (C–$CH_{2\text{-Glyc.}}$), 68.9 (1C, C-16), 73.0 (1C, C-4″), 73.2 (1C, C-2′), 73.4 (1C, C-17), 73.7 (1C, C-3″), 74.9 (1C, C-5″), 76.6 (4C, CH-Ala), 78.5 (1C, C–CH_{GlcNAc}), 99.7 (1C, C-1″). MALDI-MS (negative mode, Matrix THAP, CH_3CN/H_2O 3:2): $[M-H]^-$, *m/z* = 1923.7; found: *m/z* = 1921.5; $[(M-Ala)-H]^-$, *m/z* = 1852.6; found: *m/z* = 1850.3.

4.6. Preparation of 7

4.6.1. *N*-Benzyloxycarbonyl-D-alanyl-D-serine methyl ester 32. To a solution of D-serine-methyl ester (2 g, 12.8 mmol) and *N*-benzyloxycarbonyl-D-alanine (3.44 g, 1.2 equiv) in dry DMF, HOBt (2.08 g, 1.2 equiv), DIPEA (13.1 mL, 6 equiv) and DCC (3.18 g, 1.2 equiv) were added. After 3 h the reaction mixture was concentrated to half its volume and diluted with CH_2Cl_2, filtered, washed with water and brine. The organic layer was dried over $MgSO_4$ and evaporated. Purification by flash chromatography (toluene/acetone 1:1) yielded **32** (4.15 g, 77%) as white solid. TLC (toluene/acetone 1:1) = 0.29. $[\alpha]_D$ = +10.3 (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.4 (d, 3H, CH_3 D-Ala); 3.75 (s, 3H, $COOCH_3$); 3.95 (m, 1H, CH_2OH); 4.3 (q, 1H, CH D-Ala); 4.65 (m, 1H, CH D-Ser); 5.1 (dd, 2H, CH_2Ph); 5.7 (d, 1H, NH D-Ala); 7.25 (d, 1H, NH D-Ser); 7.35 (m, 5H, Ph). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, *m/z* = 347.1; found *m/z* = 346.7. $C_{15}H_{20}N_2O_6$ (324.3). Anal. Calcd: C: 55.55; H, 6.22; N, 8.64. Found: C: 55.48; H, 6.17; N, 8.53.

4.6.2. *N*-Benzyloxycarbonyl-D-alanyl-(*O*-monomethoxytrityl)-D-serine methyl ester 33. To a solution of **32** (2.05 g, 6.3 mmol) in pyridine (20 mL), MMTrCl (2.33 g, 1.2 equiv) was added and the reaction mixture was stirred for 2 h at room temperature. The pyridine was evaporated, the material redissolved in EtOAc and washed with water. The organic phase was dried over $MgSO_4$, and the solvent was evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 3:1 to 1:1) yielded compound **33** (3.47 g, 92%). TLC (petroleum ether/EtOAc 1:1) = 0.48. $[\alpha]_D$ = +4.2 (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.4 (d, 3H, CH_3 D-Ala); 3.35 (dd, 1H, CH_2 D-Ser); 3.6 (dd, 1H, CH_2 D-Ser); 3.75 (s, 6H, $COOCH_3$, CH_3O); 4.3 (q, 1H, CH D-Ala); 4.65 (m, 1H, CH D-Ser); 5.1 (dd, 2H, CH_2Ph); 5.35 (d, 1H, NH D-Ala); 6.8 (d, 2H, *ortho*PhOMe); 7.25 (m, 18H, Ph, NH D-Ser). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, *m/z* = 619.7; found *m/z* = 619.8. $C_{35}H_{36}N_2O_7$ (596.7). Anal. Calcd: C: 70.45; H, 6.08; N, 4.69. Found: C: 70.52; H, 6.17; N, 4.56.

4.6.3. (2*S*)-2-*N*-(*N*-Benzyloxycarbonyl-D-alanyl)-2-amino-1-hydroxy-3-(monomethoxytrityloxy)propane 34. A solution of **33** (3.43 g, 5.75 mmol) in dry EtOH (130 mL) was cooled to 0 °C and $NaBH_4$ (870 mg, 4 equiv) was added. The reaction mixture was stirred for 30 min at 0 °C and overnight at room temp. The solvent was evaporated and the residue was redissolved in EtOAc, washed with water and brine. The organic phase was dried over $MgSO_4$ and the solvent evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 1:1 to 1:3) yielded **34** (2.55 g, 78%) as colourless syrup. TLC (petroleum ether/EtOAc 1:1) = 0.32. $[\alpha]_D$ = +7.8 (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.4 (d, 3H, CH_3 D-Ala); 3.35 (m, 2H, CH_2OH); 3.7 (m, 2H, CH_2OMMTr); 3.75 (s, 3H, CH_3O); 4,05 (m, 1H, CH D-Ala); 4.2 (m, 1H, CH D-Ser); 5.0 (dd, 2H, CH_2Ph); 5.45 (d, 1H, NH); 6.7 (d, NH D-Ser); 6.8 (d, 2H, *ortho-Ph*OMe); 7.25 (m, 17H, Ph). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, *m/z* = 591.3; found *m/z* = 591.4. $C_{34}H_{54}N_2O_6$ (568.7). Anal. Calcd: C: 71.81; H: 6.38; N: 4.93. Found: C: 71.69; H: 6.14; N: 4.86.

4.6.4. (2*R*)-2-*N*-(*N*-Benzyloxycarbonyl-D-alanyl)-2-amino-1-*tert*-butyldimethylsilyloxy-3-(monomethoxytrityloxy)propane 35. To a solution of **34** (2.5 g, 4.4 mmol) and imidazole (0.43 g, 1.5 equiv) in dry CH_2Cl_2 (30 mL) TBDPS-Cl (1.14 mL, 1.05 equiv) was added. The reaction mixture was stirred for 30 min, then diluted with CH_2Cl_2 and washed with water. The organic phase was dried over $MgSO_4$ and the solvent evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 3:1) yielded compound **35** (3.01 g, 85%) as colourless syrup. TLC (petroleum ether/EtOAc 2:1) = 0.47. $[\alpha]_D$ = +0.8 (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.95 (s, 9H, CCH_3); 1.3 (d, 3H, CH_3 D-Ala); 3.2 (m, 1H, CH_2OTBDPS) 3.45 (m, 1H,

CH_2OTBDPS); 3.75 (m, 4H, CH_2OMMTr, OCH_3); 3.9 (m, 1H, CH_2OMMTr); 4,15 (m, 2H, CH D-Ala, CH); 5.0 (dd, 2H, CH_2Ph); 5.2 (d, 1H, NH D-Ala); 6.2 (d, NH amide); 6.8 (d, 2H, *orthoPh*OMe); 7.25 (m, 23H, Ph); 7.6 (d, 4H, *orthoPh*OSi). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 829.4$; found $m/z = 829.4$. $C_{50}H_{54}N_2O_6Si$ (807.1). Anal. Calcd: C: 74.41; H: 6.74; N: 3.47. Found: C: 74.68; H: 6.64; N: 4.56.

4.6.5. (2*R*)-2-*N*-[(*N*-Benzyloxycarbonyl-D-alanyl)-2-amino]-1-*tert*-butyldimethylsiyloxy-3-hydroxypropane 36. To a solution of **35** (2.95 g, 3.66 mmol) in CH_2Cl_2/MeOH (30 mL), camphor-10-sulfonic acid (150 mg) was added. The reaction mixture was stirred for 20 min, then quenched with saturated solution of $NaHCO_3$ (15 mL). The organic phase was washed with water and dried over $MgSO_4$; the solvent was evaporated in vacuo. Flash chromatography (petroleum ether/EtOAc 2:1→1:1) yielded **36** (1.7 g, 87%) as colourless syrup. TLC (petroleum ether/EtOAc 1:1) = 0.53. $[\alpha]_D = +9.3$ (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.05$ (s, 9H, CCH_3); 1.35 (d, 3H, CH_3 D-Ala); 3.7 (m, 4H, CH_2OTBDPS, CH_2OH); 4.0 (m, 2H, CH D-Ala, CH); 5.0 (dd, 2H, CH_2Ph); 5.2 (d, 1H, NH D-Ala); 6.7 (d, NH amide); 7.4 (m, 11H, Ph); 7.65 (d, 4H, *orthoPh*OSi). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 557.2$; found $m/z = 557.0$. $C_{30}H_{38}N_2O_5Si$ (534.7). Anal. Calcd: C: 67.39; H: 7.16; N: 5.24. Found: C: 67.38; H: 7.24; N: 5.32.

4.6.6. Benzyloxy-[(2*R*)-2-*N*-(*N*-benzyloxycarbonyl-D-alanyl)-2-amino-1-*tert*-butyldimethylsilyloxy-propan-3-yloxy]-diisopropylaminophosphane 7. Compound **36** (1.65 g, 3.09 mmol) and tetrazole (130 mg, 0.6 equiv) were dried separately in high vacuum for 1 h. To the mixture of the two dried compounds a solution of benzyloxy(diisopropylamino)phosphane (1.2 g, 1.2 equiv) in dry CH_2Cl_2 (10 mL) was added and the reaction mixture was stirred for 45 min at room temp. The reaction was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ solution. The organic phase was dried over sodium sulfate and the solvent was removed in vacuo. Fast flash chromatography (petroleum ether/EtOAc 3:1 1% Et_3N) yielded **7** (2.26 g, 95%) as a colourless syrup. TLC (petroleum ether/EtOAc 1:1 1% Et_3N) = 0.82. TLC (petroleum ether/EtOAc 3:1 1% Et_3N) = 0.38. $[\alpha]_D = +0.9$ (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.05$ (s, 9H, CCH_3); 1.10–1.25 (m, 12H, CH(CH_3)$_2$); 1.35 (d, 3H, CH_3 D-Ala); 3.55 (m, 4H, H-1, H-2); 4,05 (m, 2H, CH D-Ala, CH); 5.1 (m, 5H, CH_2Ph, NH D-Ala, PhCH_2OP); 6.7 (d, NH amide); 7.4 (m, 16H, Ph); 7.65 (d, 4H, *orthoPh*OSi). $C_{43}H_{58}N_3O_6PSi$ (772.0). Anal. Calcd: C: 66.90; H: 7.57; N: 5.44. Found: C: 66.67; H: 7.35; N: 5.32.

4.7. Preparation of target molecule 2

4.7.1. Compound 37. Procedure A. Mixture of **3** (78 mg, 1.1 equiv) and **7** (240 mg, 0.26 mmol). TLC before oxidation (toluene/acetone 3:1, 1% Et_3N, $R_f = 0.74$). Flash chromatography (petroleum ether/EtOAc 2:1) yielded phosphate **36** (240 mg, 97%) as colourless syrup. TLC (toluene/acetone 3:1) = 0.37. $[\alpha]_D = -5.7$ (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.05$ (s, 9H, CCH_3); 1.35 (d, 3H, CH_3 D-Ala); 3.5 (d, 2H, 1-H); 3.6–3.8 (m, 3-H, 6-H, 5-H); 4.07–4.30 (m, 5H, 3-H, 4-H, CH D-Ala); 4.45 (s, 2H, CH_2Ph); 4.6 (m, 2H, CH_2Ph); 5.0 (m, 4H, POCH_2Ph, CH_2Z); 5.6 (d, 1H, NH D-Ala); 6.6 (d, NH amide); 7.3 (m, 26H, Ph); 7.65 (d, 4H, *orthoPh*OSi). ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 18.68$ (1C, *C* (CH$_3$)$_3$), 26.29 (3C, C(*C*H$_3$)$_3$), 48.96 (1C, *C*NHR), 50.58 (1C, *C*NHR), 62.18 (1C, C-1), 66.42, 66.51, 66.55 (2C, C-3, C-4), 68.72 (2C, PO*C*H$_2$–Ph, C-6), 71.34, 71.73, 72.91 (3C, *C*H$_2$–Ph), 76.1 (1C, C-5), 127.09–137.66 (30C, Ph), 173.09 (1C, *C*O), 178.66 (1C, *C*O). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 958.4$; found $m/z = 981.6$. $C_{54}H_{63}N_2O_{10}PSi$ (959.1). Anal. Calcd: C: 67.62; H: 6.62; N: 2.92. Found: C: 67.48; H: 6.44; N: 2.58.

4.7.2. Compound 38. Procedure B. Phosphate **37** (240 mg, 0.25 mmol). Purification by flash silica gel (petroleum ether/EtOAc 1:1) yielded compound **38** (130 mg, 82%) as colourless syrup. TLC (toluene/acetone 6:1) = 0.15. $[\alpha]_D = +8.8$ (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.35$ (d, 3H, CH_3 D-Ala); 1.8–2.0 (br s, 1H, OH); 3.5 (d, 2H, 1-H); 3.6–3.8 (m, 3H, 6-H, 5-H); 3.8–4.30 (m, 5H, 3-H, 4-H, CH D-Ala); 4.45 (m, 2H, CH_2Ph); 4.6 (s, 2H, CH_2Ph); 5.0 (m, 4H, POCH_2Ph, CH_2Z); 5.7 (d, 1H, NH D-Ala); 7.0 (d, NH amide); 7.3 (m, 20H, Ph). ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 48.96$ (1C, *C*NHR), 50.58 (1C, *C*NHR), 61.0 (1C, C-1), 65.5, 65.7 (2C, C-3, C-4), 68.7 (1C, PO*C*H$_2$–Ph), 69.3 (1C, C-6), 71.3–72.9 (3C, *C*H$_2$–Ph), 76.7 (1C, C-5), 127.09–137.66 (34C, Ph), 173.12 (1C, *C*O), 178.87 (1C, *C*O). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 743.3$; found $m/z = 743.4$. $C_{38}H_{45}N_2O_{10}P$ (720.8). Anal. Calcd: C: 63.32; H: 6.29; N: 3.89. Found: C: 63.44; H: 6.41; N: 3.57.

4.7.3. Diphosphate 39. Procedure A. Compound **38** (130 mg, 0.18 mmol). TLC before oxidation (toluene/acetone 2:1, 1% Et_3N, $R_f = 0.73$). Purification by flash chromatography (toluene/acetone 7:2) yielded compound **39** (240 mg, 95%) as colourless syrup. TLC (toluene/acetone 3:1) = 0.36, 0.28. $[\alpha]_D = -7.8$ (*c* 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): $\delta =$ 1.03 (s, 9H, CCH_3); 1.35 (m, 6H, CH_3 D-Ala); 3.45 (d, 2H, 1-H); 3.6–3.8 (m, 3H, 9-H, 8-H); 3.8–4.40 (m, 10H, 3-H, 4-H, 6-H, 7-H, CH D-Ala); 4.45 (s, 2H, CH_2Ph); 4.6 (m, 2H, CH_2Ph); 5.0 (m, 8H, POCH_2Ph, CH_2Z); 5.75 (d, 2H, NH D-Ala); 6.35 (d, NH amide); 7.3 (m, 36H, Ph); 7.65 (d, 4H, *orthoPh*OSi). ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 18.72$ (1C, *C*(CH$_3$)$_3$), 26.3 (3C, C(*C*H$_3$)$_3$), 48.9 (2C, *C*NHR), 50.62 (2C, *C*NHR), 62.18 (1C, C-1), 66.45, 66.50, 66.55 (4C, C-3, C-4, C-6, C-7), 68.69–69.7 (3C, PO*C*H$_2$–Ph, C-9), 71.30–72.57 (4C, *C*H$_2$–Ph), 76.15 (1C, C-8), 127.09–137.66 (40C, Ph), 173.09 (1C, *C*O), 177.68 (1C, *C*O). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 1431.5$; found: $m/z =$ 1431.6. $C_{75}H_{88}N_4O_{17}P_2Si$ (1407.6). Anal. Calcd: C: 64.00; H: 6.30; N: 3.98. Found: C: 63.67; H: 6.42; N: 4.16.

4.7.4. Diphosphate 40. Procedure B. Compound **39** (230 mg, 0.16 mmol). Purification by flash silica gel (toluene/acetone 3:1) yielded **40** (156 mg, 78%) as colourless syrup. TLC (toluene/acetone 2:1) = 0.36 and 0.40. $[\alpha]_D$ = +3.4 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 1.35 (d, 6H, C*H*$_3$ D-Ala); 1.9–2.2 (br s, 1H, OH); 3.5–3.8 (m, 5H, 1-H, 9-H, 8-H); 3.8–4.40 (m, 10H, 3-H, 4-H, 6-H, 7-H, C*H* D-Ala); 4.45 (m, 2H, C*H*$_2$Ph); 4.6 (m, 2H, C*H*$_2$Ph); 5.0 (m, 8H, POC*H*$_2$Ph, C*H*$_2$Z); 5.7 (br s, 2H, NH D-Ala); 7.0 (d, NH amide); 7.3 (m, 30H, Ph). ^{13}C NMR (63 MHz, $CDCl_3$): δ = 48.78 (2C, *C*NHR), 50.58 (2C, *C*NHR), 61.0 (1C, C-1), 65.51–66.93 (6C, C-3, C-4, C-6, C-7, *C*H$_2$Ph(Z)), 68.7 (2C, PO*C*H$_2$–Ph), 69.3 (1C, C-9); 71.3, 72.9 (2C, *C*H$_2$–Ph), 77.75 (1C, C-8), 127.09–137.66 (36C, Ph). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, *m*/*z* = 1191.4; found *m*/*z* = 1191.2. $C_{59}H_{70}N_4O_{17}P_2$ (1169.2). Anal. Calcd: C: 60.61; H: 6.03; N: 4.79. Found: C: 60.49; H: 6.24; N: 4.56.

4.7.5. Triphosphate 41. Procedure A. Compound **40** (156 mg, 0.133 mmol), TLC before oxidation (toluene/acetone 2:1, 1% Et_3N, R_f = 0.75). Purification by flash chromatography (toluene/acetone 3:1 to 2:1) yielded compound **41** (184 mg, 77%) as colourless syrup. TLC (toluene/acetone 2:1) = 0.42, 0.39. $[\alpha]_D$ = −6.5 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 1.03 (s, 9H, CC*H*$_3$); 1.35 (m, 9H, C*H*$_3$ D-Ala); 3.5–3.8 (m, 8H, 1-H, 2-H, 5-H, 8-H, 11-H, 12-H); 3.8–4.40 (m, 10H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, C*H* D-Ala); 4.45 (m, 2H, C*H*$_2$Ph); 4.6 (m, 2H, C*H*$_2$Ph); 5.0 (m, 12H, POC*H*$_2$Ph, C*H*$_2$Z); 5.75, 6.95 (m, 3H, NH D-Ala); 6.35 (m, NH amide); 7.3 (m, 46H, Ph); 7.65 (d, 4H, *ortho-Ph*OSi). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, *m*/*z* = 1854.7; found: *m*/*z* = 1877.6. $C_{96}H_{113}N_6O_{24}P_3Si$ (1856.0). Anal. Calcd: C: 62.13; H: 6.14; N: 4.53. Found: C: 62.05; H: 6.24; N: 4.59.

4.7.6. Triphosphate 42. Procedure B. Compound **41** (184 mg, 0.1 mmol). Purification by flash silica gel (toluene/acetone 1:1) yielded **42** (115 mg, 72%) as colourless syrup. TLC (toluene/acetone 1:1) = 0.29. $[\alpha]_D$ = +2.8 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 1.15–1.4 (m, 9H, C*H*$_3$ D-Ala); 3.45–3.80 (m, 8H, 1-H, 2-H, 5-H, 8-H, 11-H, 12-H); 3.85–4.40 (m, 15H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, C*H* D-Ala); 4.45 (m, 2H, C*H*$_2$Ph); 4.6 (m, 2H, C*H*$_2$Ph); 5.0 (m, 8H, POC*H*$_2$Ph, C*H*$_2$Z); 6.05 (br s, 3H, NH D-Ala); 7.3 (m, 40H, Ph). MALDI-MS (positive mode, Matrix DHB, THF): $[M+H]^+$, *m*/*z* = 1616.6; found: *m*/*z* = 1616.6. $C_{80}H_{95}N_6O_{24}P_3$ (1617.6). Anal. Calcd: C: 59.40; H: 5.92; N: 5.20. Found: C: 59.48; H: 5.64; N: 5.12.

4.7.7. Tetraphosphate 43. Procedure A. Compound **42** (115 mg, 0.071 mmol). TLC before oxidation (toluene/acetone 2:1, 1% Et_3N, R_f = 0.71). Purification by flash chromatography (toluene/acetone 2:1→1:2) yielded compound **43** (136 mg, 91%) as colourless syrup. $[\alpha]_D$ = −5.2 (*c* 1, $CHCl_3$). TLC (toluene/acetone 1:1) = 0.36 and 0.39. ^1H NMR (250 MHz, $CDCl_3$): δ = 1.03 (s, 9H, CC*H*$_3$); 1.25 (m, 12H, C*H*$_3$ D-Ala); 3.5–3.8 (m, 11H, 1-H, 2-H, 5-H, 8-H, 11-H, 14-H, 15-H); 3.8–4.40 (m, 20H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, C*H* D-Ala); 4.45 (s, 2H, C*H*$_2$Ph); 4.6 (s, 2H, C*H*$_2$Ph); 5.0 (m, 16H, POC*H*$_2$Ph, C*H*$_2$Z); 5.75–6.4 (m, 4H, NH); 7.3 (m, 56H, Ph); 7.65 (d, 4H, *ortho-Ph*OSi). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, *m*/*z* = 2325.8; found: *m*/*z* = 2325.7. $C_{117}H_{138}N_8O_{31}P_4Si$ (2304.4). Anal. Calcd: C: 60.98; H: 6.04; N: 4.86. Found: C: 60.53; H: 6.37; N: 4.52.

4.7.8. Tetraphosphate 44. Procedure B. Compound **43** (136 mg, 0.065 mmol). Purification by flash silica gel (toluene/acetone 1:3) yielded **44** (86 mg, 72%) as colourless syrup. TLC (toluene/acetone 1:1) = 0.15. $[\alpha]_D$ = +1.2 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 1.25 (m, 12H, C*H*$_3$ D-Ala); 2.35 (br s, 1H, OH); 3.5–3.8 (m, 11H, 1-H, 2-H, 5-H, 8-H, 11-H, 14-H, 15-H); 3.8–4.40 (m, 20H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, C*H* D-Ala); 4.45 (s, 2H, C*H*$_2$Ph); 4.6 (s, 2H, C*H*$_2$Ph); 5.0 (m, 16H, POC*H*$_2$Ph, C*H*$_2$Z); 6.05 (br s, 4H, NH); 7.3 (m, 50H, Ph). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, *m*/*z* = 2087.7; found: *m*/*z* = 2087.7. $C_{101}H_{120}N_8O_{31}P_4Si$ (2066.0). Anal. Calcd: C: 58.72; H: 5.85; N: 5.42. Found: C: 58.59; H: 5.71; N: 5.64.

4.7.9. Pentaphosphate 45. Procedure A. Compound **44** (65 mg, 0.031 mmol). TLC before oxidation (toluene/acetone 2:1, 1% Et_3N, R_f = 0.61). Purification by flash chromatography (toluene/acetone 2:1→1:2) yielded compound **45** (71 mg, 81%) as colourless syrup. $[\alpha]_D$ = −0.4 (*c* 1, $CHCl_3$). TLC (toluene/acetone 1:1) = 0.36 and 0.39. ^1H NMR (250 MHz, $CDCl_3$): δ = 1.03 (s, 9H, CC*H*$_3$); 1.25 (m, 15H, C*H*$_3$ D-Ala); 3.5–3.8 (m, 14H, 1-H, 2-H, 5-H, 8-H, 11-H, 14-H, 17-H, 18-H); 3.8–4.40 (m, 25H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H, 16-H, C*H* D-Ala); 4.45 (s, 2H, C*H*$_2$Ph); 4.6 (s, 2H, C*H*$_2$Ph); 5.0 (m, 20H, POC*H*$_2$Ph, C*H*$_2$Z); 5.75–6.4 (m, 5H, NH); 7.3 (m, 66H, Ph); 7.65 (d, 4H, *ortho-Ph*OSi). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, *m*/*z* = 2774.0; found: *m*/*z* = 2773.8. $C_{138}H_{163}N_{10}O_{38}P_5Si$ (2752.8). Anal. Calcd: C: 60.21; H: 5.97; N: 5.09. Found: C: 60.48; H: 6.14; N: 5.32.

4.7.10. Pentaphosphate 46. Procedure B. Compound **45** (56 mg, 0.02 mmol). Purification by flash silica gel (toluene/acetone 1:3) yielded **46** (40 mg, 78%) as colourless syrup. $[\alpha]_D$ = −3.1 (*c* 1, $CHCl_3$). TLC (toluene/acetone 1:1) = 0.13. ^1H NMR (250 MHz, $CDCl_3$): δ = 1.25 (m, 15H, C*H*$_3$ D-Ala); 3.5–3.8 (m, 14H, 1-H, 2-H, 5-H, 8-H, 11-H, 14-H, 17-H, 18-H); 3.8–4.40 (m, 25H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H, 16-H, C*H* D-Ala); 4.45 (s, 2H, C*H*$_2$Ph); 4.6 (s, 2H, C*H*$_2$Ph); 5.0 (m, 20H, POC*H*$_2$Ph, C*H*$_2$Z); 5.75–6.4 (m, 5H, NH); 7.3 (m, 66H, Ph). $C_{122}H_{145}N_{10}O_{38}P_5$ (2514.4). Anal. Calcd: C: 58.28; H: 5.81; N: 5.57. Found: C: 57.99; H: 5.84; N: 5.62.

4.7.11. Hexaphosphate 47. Procedure A. Compound **46** (40 mg, 0.016 mmol). TLC before oxidation (toluene/acetone 2:1, 1% Et_3N, R_f = 0.61). Purification by flash chromatography (toluene/acetone 1:1 to 1:2) yielded compound **47** (36 mg, 82%) as colourless syrup. $[\alpha]_D$ = +13.3 (*c* 1, $CHCl_3$). TLC (toluene/acetone 1:1) = 0.38 and 0.40. ^1H NMR (250 MHz, $CDCl_3$):

δ = 0.90 (t, 6H, Me); 1.25 (m, 55H, CH_3 D-Ala, CH_2 chain); 1.55 (m, 4H, COCH$_2$CH_2R); 2.25 (t, 4H, COCH_2R); 3.5 (m, 2H, H-21); 3.7 (m, 1H, H-20); 3.8–4.15 (22H, 4-H, 5-H, 6-H, 7-H, 8-H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H, 18-H, 19-H); 4.2–4.40 (m, 14H, 1-H, 2-H, 3-H, 4-H, 6-H, 16-H, 18-H, C*H* D-Ala); 4.45 (s, 2H, CH_2Ph); 4.6 (s, 2H, CH_2Ph); 5.0 (m, 22H, POCH_2Ph, CH_2Z); 5.75–6.4 (m, 5H, NH); 7.3 (m, 66H, Ph); 7.65 (d, 4H, *ortho*Ph*O*Si). MALDI-MS (positive mode, Matrix DHB, THF): $[M+Na]^+$, m/z = 3200.3; found: m/z = 3201.1. $C_{160}H_{210}N_{10}O_{45}P_6$ (3179.3). Anal. Calcd: C: 60.45; H: 6.66; N: 4.41. Found: C: 60.41; H: 6.74; N: 4.62.

4.7.12. Target molecule 2. Compound **47** (36 mg, 0.011 mmol) was dissolved in CH_2Cl_2/MeOH/H_2O (5:5:1 5 mL) and Pearlman's catalyst was added (4 mg). The reaction mixture was stirred overnight under H_2 atmosphere and after this time it was filtered through Celite using the same solvent mixture for washing. The solution was diluted with 0.1 M NH_4OAc buffer (pH 4.8), frozen and lyophilized. The crude white solid was purified by Hydrophobic Interaction Chromatography (HIC), and after lyophilization compound **2** (7 mg, 35%) was obtained. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.73 (m, 6H, Me); 1.15 (m, 55H, CH_3D-Ala, CH_2 chain); 1.45 (m, 4H, COCH$_2$CH_2R); 2.19 (m, 4H, COCH_2R); 3.40–3.55 (m, 3H, H-20, H-21); 3.65–4.25 (32H, 1-H, 2-H, 3-H, 4-H, 5-H, 6-H, 7-H, 8-H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H, 15-H, 16-H, 17-H, 18-H, 19-H); 5.5 (m, 5H, CHD-Ala). ^{31}P NMR (243 MHz, $CDCl_3$): δ = 2.077 (m, 6P, PO_4). MALDI-MS (negative mode, Matrix THAP, CH_3CN/H_2O 3:2): $[M-2H+Na]^-$, m/z = 1807.7; found: m/z = 1808.0. $C_{64}H_{132}N_{10}O_{35}P_6$ (1787.6).

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