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Solid-Phase Modular Synthesis of Park Nucleotide and Lipids I and II Analogues

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A solid-phase synthesis of Park nucleotide as well as lipids I and II analogues, which is applicable to the synthesis of a range of analogues, is described in this work. This technique allows highly functionalized macromolecules to be modularly labeled. Multiple steps are used in a short time (4 d) with a single purification step to synthesize the molecules by solid-phase synthesis.

Key words peptidoglycan; natural product; solid-phase synthesis

Peptidoglycan is a component of bacterial cell walls that consists of repeating β-1-4-*N*-acetylglucosaminyl-*N*-acetylmuraminic acid (β-1-4-GlcNAc-MurNAc) units, which are further cross-linked with polypeptides. Peptidoglycan plays a role as an extracellular skeleton to prevent bacterial cells from lysis by intracellular high pressure and is a primary defense from a variety of physiological, chemical and biological attacks outside the cells. Peptidoglycan biosynthesis consists of several stages as shown in Fig. 1.[1,2)]

Uridine-5′-diphospho-MurNAc-pentapeptide (UDP-MurNAc-pentapeptide, **1**), which is also called as Park nucleotide, is synthesized by a series of MurA-F enzymes in cytoplasm. Phospho-MurNAc-pentapeptide transferase (MraY) catalyzes the first reaction step of the lipid-linked cycle, where Park nucleotide is attacked by undecaprenyl monophosphate in the bacterial cell membrane providing lipid I. Lipid I is further glycosylated by *N*-acetylglucosamine transferase (MurG) to afford lipid II. Lipid II in cytoplasm is then flipped out by a flipase called MurJ, which was recently identified.[3,4)] The lipid II in periplasm is then polymerized by a transglycosylase and a transpeptidase to form peptidoglycan. The biosynthesis of peptidoglycan is one of the major targets for antibacterial drug discovery and many drugs including β-lactams and vancomycin are currently clinically used drugs. In addition to the fact that the analogues of Park nucleotide, lipids I and II, which are precursors to peptidoglycan, have the potential to be inhibitors of peptidoglycan biosynthesis,[5,6)] they are used as biological tools to elucidate the biology of bacterial cell walls. For example, dansylated Park nucleotide has been used as a fluorescent substrate in an assay screening of MraY inhibitor,[7)] and lipids I and II analogues with short lipid tails, such as a neryl group instead of a undecaprenyl group, have been used in mechanistic studies of MurY and MurG[8,9)] (Fig. 2, **2** and **3**). As a result, these molecules constitute an important class of molecules in drug discovery and microbiology. The chemical synthesis of Park nucleotide, lipids I, II and their analogues have previously been accomplished by solution-phase synthesis.[6,7,10–20)] However, these molecules consist of amino acids, sugars, and diphosphates attached to uridine or the lipid chain and their amphiphilic properties as well as their molecular size occasionally face difficulty in purification during synthesis. Solid-phase syntheses have been extensively developed especially in the synthesis of biomacromolecules. Since synthetic intermediates remain immobilized on the solid support, it is easy to handle a molecule with the abovementioned properties. Although Kurosu's group reported the solid-phase synthesis of peptide fragments of Park nucleotide,[20)] the introduction of the sugar domain and construction of the diphosphate moiety on a solid-phase remains a challenge. Herein, a modular solid-phase synthesis of Park nucleotide (**1**), lipid I analogue **2** and lipid II analogue **3**, is described.

Results and Discussion

The retrosynthetic analysis is illustrated in Chart 1. The *C*-terminus of these molecules is immobilized onto a solid support with suitable protection of the functional groups to give **4**–**6**. Considering the lability of the diphosphate under acidic conditions, base-removable protecting groups were chosen for all the functional groups. Accordingly, cleavage from the solid-phase was used under basic conditions. In addition, the hydrophilicity of the target molecules upon simultaneous deprotection under basic aqueous conditions was considered. These considerations initiated the use of a 4-(hydroxymethyl)-bezoylamidyl polyethyleneglycol (HMBA-PEG) resin, which swells extensively in a wide range of solvents, including water.[21)] The diphosphate moieties of **4**–**6** are disconnected to give pentapeptidylglycosyl phosphates **7** and **8** as well as the corresponding uridine 5′-*O*-phosphate and neryl phosphate, respectively. Regarding the disconnection of **7** and **8**, it was reported by Ducho's group that coupling of a pentapeptide with a MurNAc derivative resulted in the formation of a diketopiperazine consisting of L-Ala and D-Glu and that it is better to connect a tetrapeptide and a MurNAc derivative condensed with L-Ala (MurNAc-Ala).[7)] This observation was modeled for the solid-phase synthesis to disconnect **7** and **8** between the L-Ala and D-Glu residues to give tetrapeptide **9**, MurNAc-Ala **10** and GlcNAc-MurNAc-Ala **11**.

Both **10** and **11** were easily prepared from GlcNAc and

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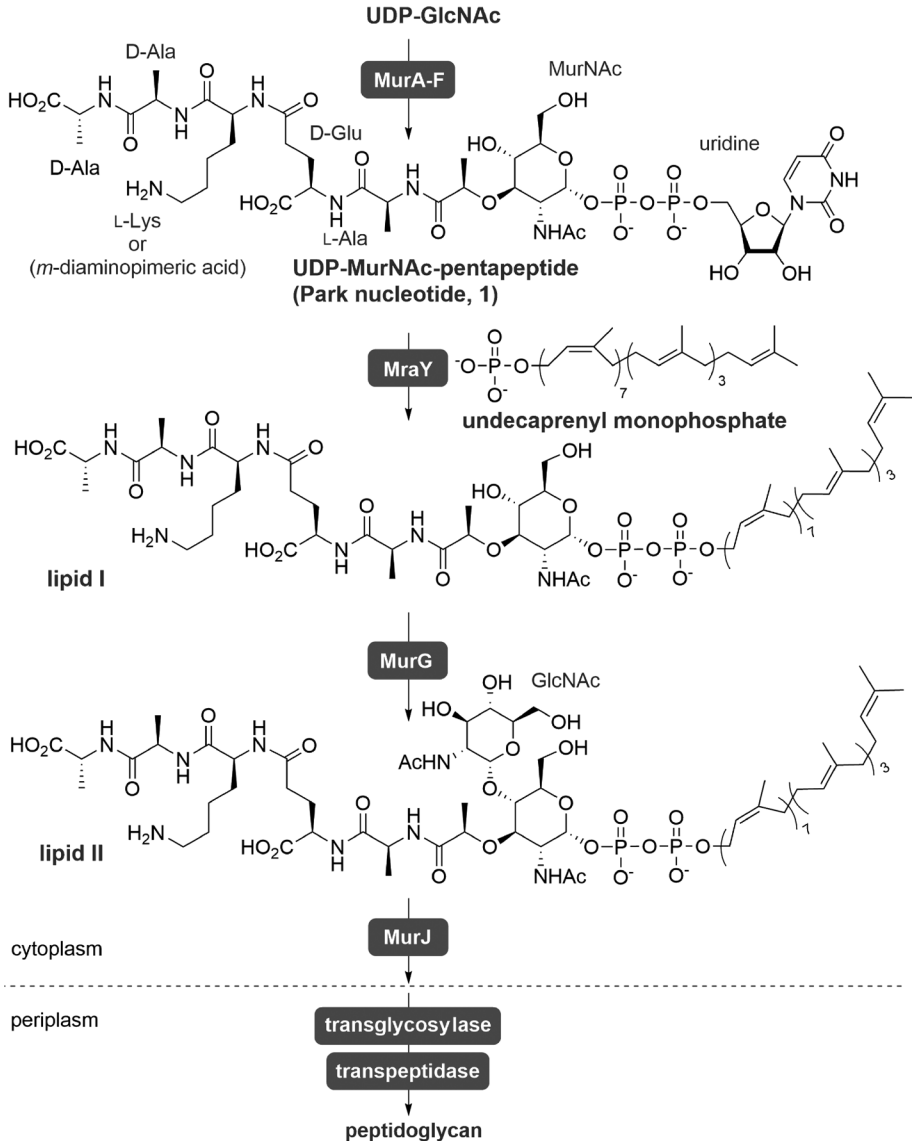


Fig. 1. Biosynthesis of Peptidoglycan

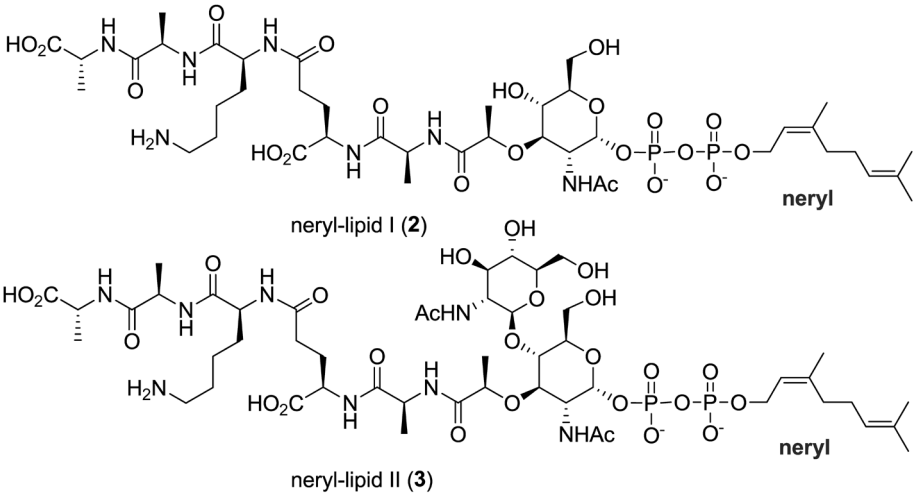


Fig. 2. Structures of Neryl Analogues of Lipids I and II

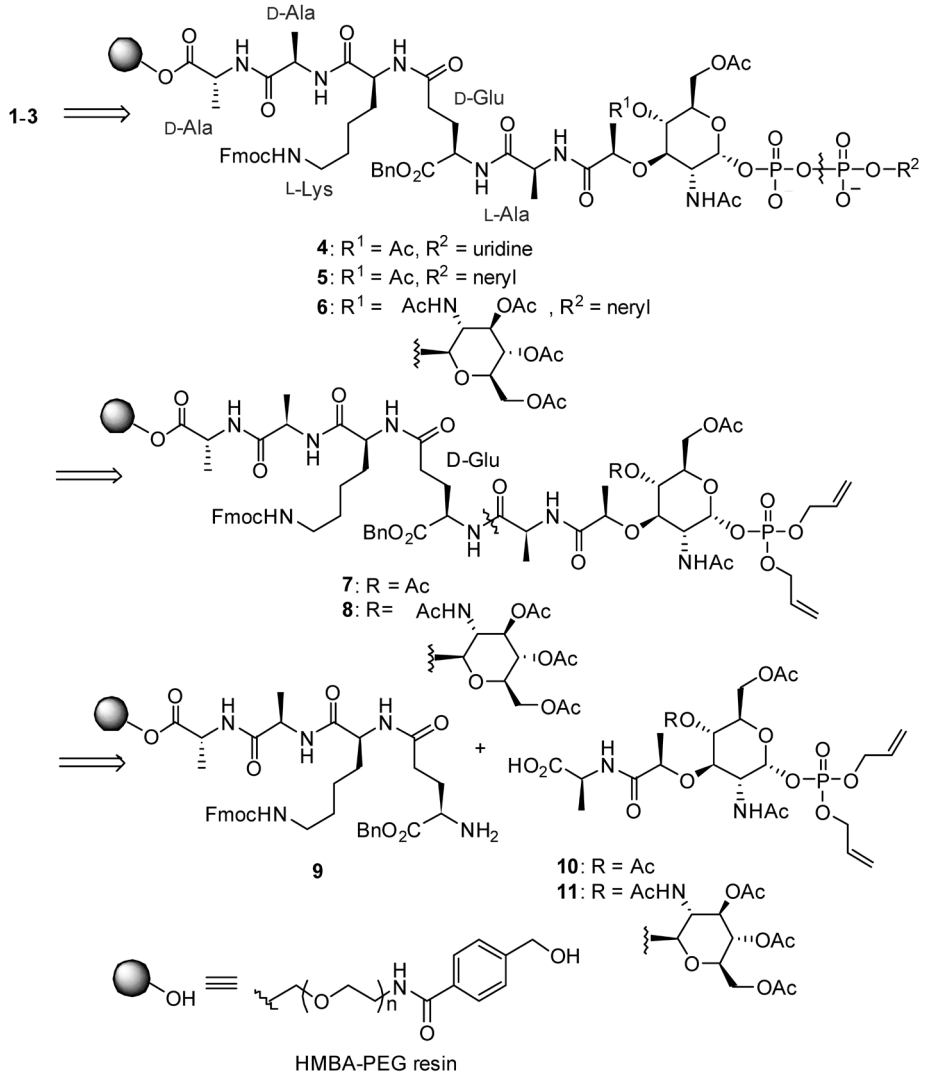


Chart 1. Retrosynthetic Analysis of Target Molecules

D-glucosamine ($GlcNH_2$) *via* known compounds **12** and **15**, respectively (Chart 2).[22] Namely, the 4,6-*O*-benzylidene protecting group of **12**, which was obtained from D-GlcNAc over four steps, was converted to acetyl groups suitable for the following synthetic route. The benzyl group at the *O*-1-position of the resulting **13** was removed by hydrogenolysis and the liberated alcohol was phosphorylated by two steps to give *O*-1-*α*-diallylphosphate **14** in 39% yield over three steps. The phenylsulfonylethyl group at the Ala residue was removed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford the carboxylic acid **10**. The glycosyl phosphates **11** was prepared by the same procedure for the synthesis of **10**.

The synthesis on the solid-phase was then investigated (Chart 3). First, Fmoc-D-Ala was immobilized onto the HMBA-PEG resin (0.39 mmol/g). Then, Fmoc-peptide synthesis was applied to give tripeptide **19**. The protecting group of *N*-terminal peptide **19** was then switched to an Alloc group. After coupling of **19** with Alloc-D-Glu-OBn and the removal of the Alloc group of **20**, the resulting amine was coupled with **10** to afford glycosyl peptapeptide **7**. It should be noted that considerable epimerization occurred during the coupling with **10** using 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU) and $^{i}Pr_2NEt$. After extensive investigations, it was found that the conditions using (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP), 1-hydroxy-7-azabenzotriazole (HOAt) and acridine in *N*,*N*-dimethylformamide (DMF)/CH_2Cl_2 completely suppressed the epimerization. A single coupling resulted in incomplete reaction and therefore a double-coupling was conducted to completely consume the amine. Deprotection of the allyl groups on the phosphate was conducted by the conditions using $Pd(PPh_3)_4$ and $PhSiH_3$ to cleanly provide the phosphate **21**. Instead of **10**, coupling of the disaccharide **11** to the tetrapeptide amine gave **22**.

With **21** and **22** in hand, the key diphosphate formation reaction on the solid-phase was investigated. Generally, the diphosphate moiety is classically constructed by the condensation of a phosphate monoester with a phosphoroamidate upon activation by an activator, such as 1*H*-tetrazole (**26**).[23] The reaction rate, however, was very slow even in the solution-phase synthesis and remained to be improved when applied to solid-phase synthesis. Thus, the reaction conditions were optimized using model substrates **23** and **24**[24] in the solution-phase, and the results are summarized in Table 1. Treatment of **24** with uridine-5′-phosphorylmorphoridate **23** in the pres-

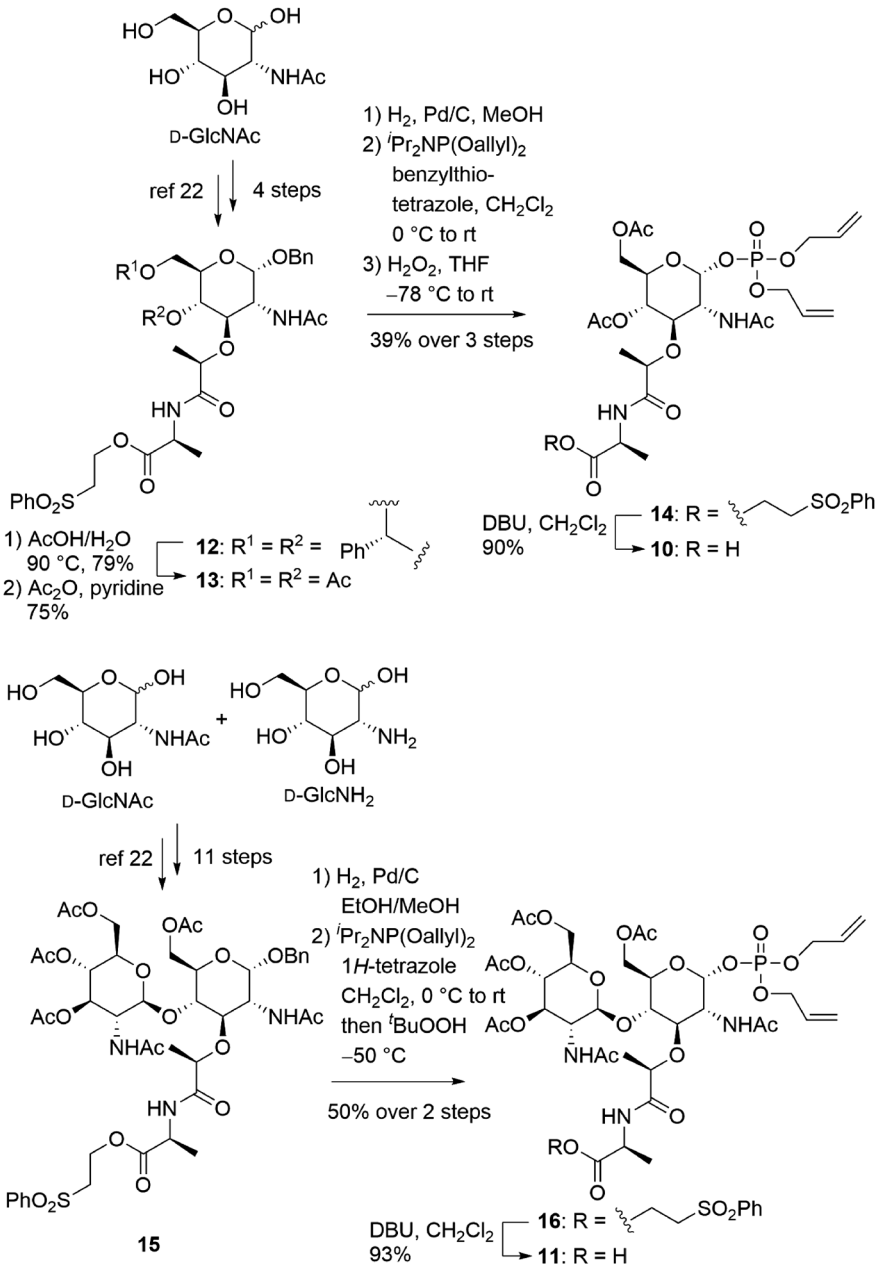


Chart 2. Preparation of **10** and **11**

ence of **26** as an activator, which is conventionally used in diphosphate coupling, gave desired **25** in 18% yield after 24 h and 42% yield after 72 h (Fig. 3). The activation ability of the phosphorylamidate correlates to the acidity of the activators. The activator acidity improves the chemical yields of **25**, and the use of **29**[25] gave **25** in 58% yield.

The conditions were then applied to the coupling of **21** and **23** to complete the total synthesis of **1** (Chart 4a). Although the reaction catalyzed by **29** resulted in no reaction at room temperature, elevating the temperature to 50 °C accelerated the reaction rate to produce **4**. Finally, cleavage from the resin as well as global deprotection by treating **4** with piperidine followed by aq. NaOH successfully afforded Park nucleotide (**1**) in 44% yield over 12 steps from **17** after purification by HPLC. In a manner similar to the synthesis of **1**, neryl-lipid I (**2**), where the undecaprenyl group of lipid I was replaced with a short lipid (a neryl group) was synthesized in 20% yield over 12 steps from **17** (Chart 4b). Using **22** instead of **21**, neryl-lipid II (**3**) was also synthesized (Chart 4c). This strategy allows for the modular labeling of highly functionalized macromolecules, which were obtained in multiple steps in a short time (4 d) using a single purification step by virtue of solid-phase synthesis.

Conclusion

In conclusion, a solid-phase modular synthesis has been established for Park nucleotide and lipids I and II analogues. These analogues could be useful as chemical probes for discovering novel antibacterial agents and elucidating detailed mechanistic studies on peptidoglycan biosynthesis.

Experimental

General Experimental Methods All reactions except that carried out in aqueous phase were performed under argon

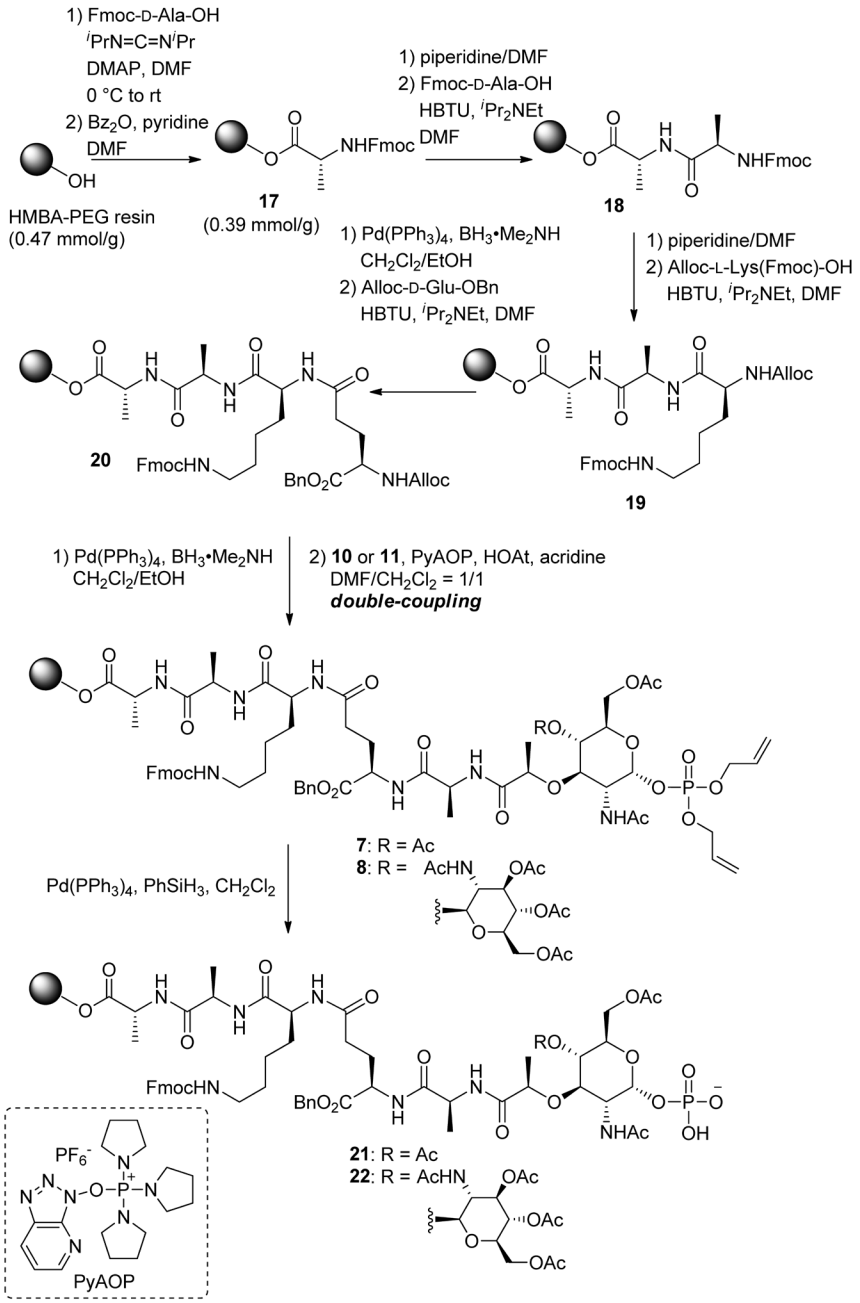
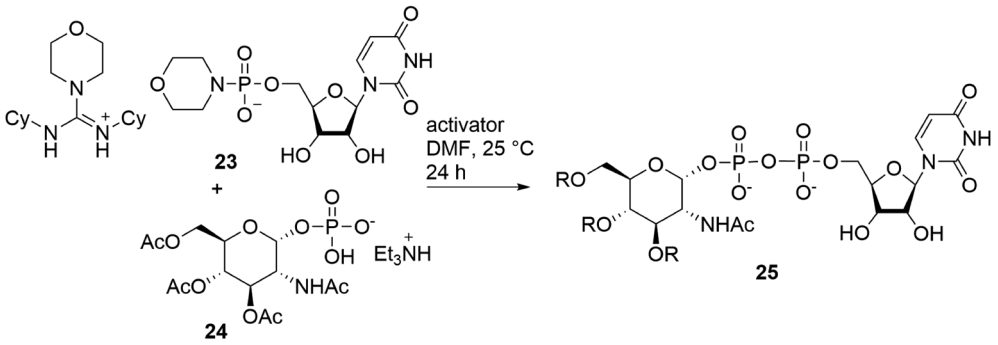


Chart 3. Solid-Phase Synthesis of **21** and **22**

atmosphere, unless otherwise noted. Isolated yields were calculated by weighing products. The weight of the starting materials and the products were not calibrated. Materials were purchased from commercial suppliers and used without further purification, unless otherwise noted. Solvents are distilled according to the standard protocol. Analytical TLC was performed on Merck silica gel 60F254 plates. Normal-phase column chromatography was performed on Merck silica gel 5715 or Kanto Chemical silica gel 60N (neutral). High-flash column chromatography was performed on Fuji Sylysia silica gel PSQ 60B. ^{1}H-NMR were measured in $CDCl_3$, dimethyl sulfoxide (DMSO)-d_6, or D_2O solution, and referenced to TMS (0.00 ppm) using JEOL ECA 500 (500 MHz), JEOL ECS 400 (400 MHz) or JEOL ECX 400P (400 MHz) spectrophotometers, unless otherwise noted. ^{13}C-NMR were measured in $CDCl_3$, DMSO-d_6, or D_2O solution, and referenced to residual solvent peaks using JEOL ECA 500 (125 MHz), JEOL ECS 400 (100 MHz) or JEOL ECX 400P (100 MHz) spectrophotometers. ^{31}P-NMR were measured in $CDCl_3$, DMSO-d_6, or D_2O solution, and referenced to H_3PO_4 (0.00 ppm) using JEOL ECA 500 (202 MHz), JEOL ECS 400 (162 MHz) or JEOL ECX 400P (162 MHz) spectrophotometers. Abbreviations of multiplicity were as follows; s: singlet, d: doublet, t: triplet, q: quartet, sept: septet, m: multiplet, br: broad. Data were presented as follows; chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC) and heteronuclear multiple quantum coherence (HMQC) NMR spectra. Optical rotations were determined on JASCO P-1010-GT. Mass spectra were recorded on Thermo Scientific Exactive. The mass analyzer type used for the high resolution (HR)-MS measurements was time-of-flight (TOF).

Table 1. Optimization of Diphosphate Formation



entry	activator	yield (%)[a]	entry	activator	yield (%)[a]
1	none	no reaction			
2	1*H*-tetrazole (**26**) pK_a 4.8	18	4	**28** pK_a 3.7	47
3	**27** pK_a 4.2	48	5	**29** pK_a 2.9	58 (31)[b]

[a]Yields were determined by ^{31}P NMR. [b]Isolated yield.

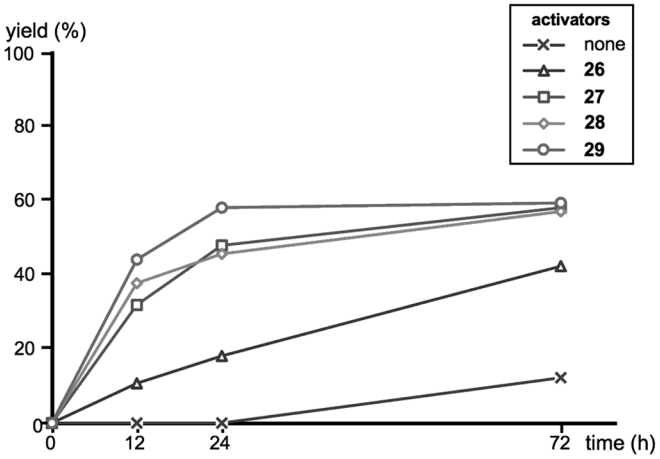


Fig. 3. Time Course of Diphosphate Formation

Benzyl-*N*-acetyl-4,6-diacetylmuramyl-L-alanine Phenylsulfonylethyl Ester (**13**)

Compound **12** (1.75 g, 2.46 mmol) was treated with 60% AcOH/H_2O (60 mL) at 90°C for 1 h. The mixture was concentrated *in vacuo*. The residue was crystalized by $CHCl_3$/Et_2O to afford benzyl-*N*-acetyl-muramyl-L-alanine phenylsulfonylethyl ester (1.21 g, 1.94 mmol, 79%) as a white solid.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.91 (d, 2H, *o*-$PhSO_2$, $J_{o,m}$=7.3 Hz), 7.69 (t, 1H, *p*-$PhSO_2$, $J_{p,m}$=7.3 Hz), 7.59 (dd, 2H, *m*-$PhSO_2$, $J_{m,o}$=$J_{m,p}$=7.3 Hz), 7.40–7.29 (m, 5H, Ph), 6.92 (d, 1H, Ala-N*H*, $J_{\text{Ala-N}H,\text{Ala-}\alpha\text{-}CH}$=7.3 Hz), 5.04 (d, 1H, 2-N*H*, $J_{2\text{-N}H,2}$=9.6 Hz), 4.91 (d, 1H, H-1, $J_{1,2}$=3.6 Hz), 4.71 (d, 1H, Bn, *J*=11.9 Hz), 4.47 (d, 1H, Bn, *J*=11.9 Hz), 4.50–4.37 (m, 2H, $PhSO_2CH_2CH_2$), 4.29 (dq, 1H, Ala-*α*-C*H*, $J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-N}H}$=$J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-}\beta\text{-}CH}$=7.3 Hz), 4.23 (ddd, 1H, H-2, $J_{2,1}$=3.6, $J_{2,2\text{-N}H}$=9.6, $J_{2,3}$=10.1 Hz), 4.16 (q, 1H, Lac-*α*-C*H*, $J_{\text{Lac-}\alpha\text{-}CH,\text{Lac-}\beta\text{-}CH}$=6.9 Hz), 3.83 (d, 2H, H-6, $J_{6,5}$=3.2 Hz), 3.76–3.65 (m, 2H, H-5, H-4), 3.58 (dd, 1H, H-3, $J_{3,2}$=$J_{3,4}$=10.1 Hz), 3.47–3.33 (m, 2H, $PhSO_2CH_2CH_2$), 1.92 (s, 3H, NAc), 1.42 (d, 3H, Lac-*β*-C*H*, $J_{\text{Lac-}\beta\text{-}CH,\text{Lac-}\alpha\text{-}CH}$=6.9 Hz), 1.33 (d, 3H, Ala-*β*-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=7.3 Hz). This is a known compound reported in ref. 14).

A mixture of benzyl-*N*-acetyl-muramyl-L-alanine phenylsulfonylethyl ester (1.12 g, 1.80 mmol) in pyridine (20 mL) was treated with Ac_2O (406 μL, 4.32 mmol) at room temperature for 1 d. Ac_2O (102 μL, 1.08 mmol) was added to the mixture, which was stirred for 24 h. The reaction was quenched with MeOH, then the resulting mixture was concentrated *in vacuo*. The residue was partitioned between AcOEt and *sat. aq.* $NaHCO_3$, and the organic phase was washed with 1 M *aq.* HCl and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (50–85–100% AcOEt/hexane) to afford **13** (955 mg, 1.35 mmol, 75%) as a colorless foam.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.92 (d, 2H, *o*-$PhSO_2$, $J_{o,m}$=6.9 Hz), 7.68 (t, 1H, *p*-$PhSO_2$, $J_{p,m}$=7.5 Hz), 7.59 (dd, 2H, *m*-$PhSO_2$, $J_{m,o}$=6.9, $J_{m,p}$=7.5 Hz), 7.41–7.56 (m, 5H, Ph), 6.85 (d, 1H, Ala-N*H*, $J_{\text{Ala-N}H,\text{Ala-}\alpha\text{-}CH}$=6.9 Hz), 5.81 (d, 1H, 2-N*H*, $J_{2\text{-N}H,2}$=9.2 Hz), 5.07 (dd, 1H, H-4, $J_{4,3}$=$J_{4,5}$=9.8 Hz), 4.88 (d, 1H, H-1, $J_{1,2}$=4.0 Hz), 4.69 (d, 1H, Bn, *J*=11.5 Hz), 4.50 (d, 1H,

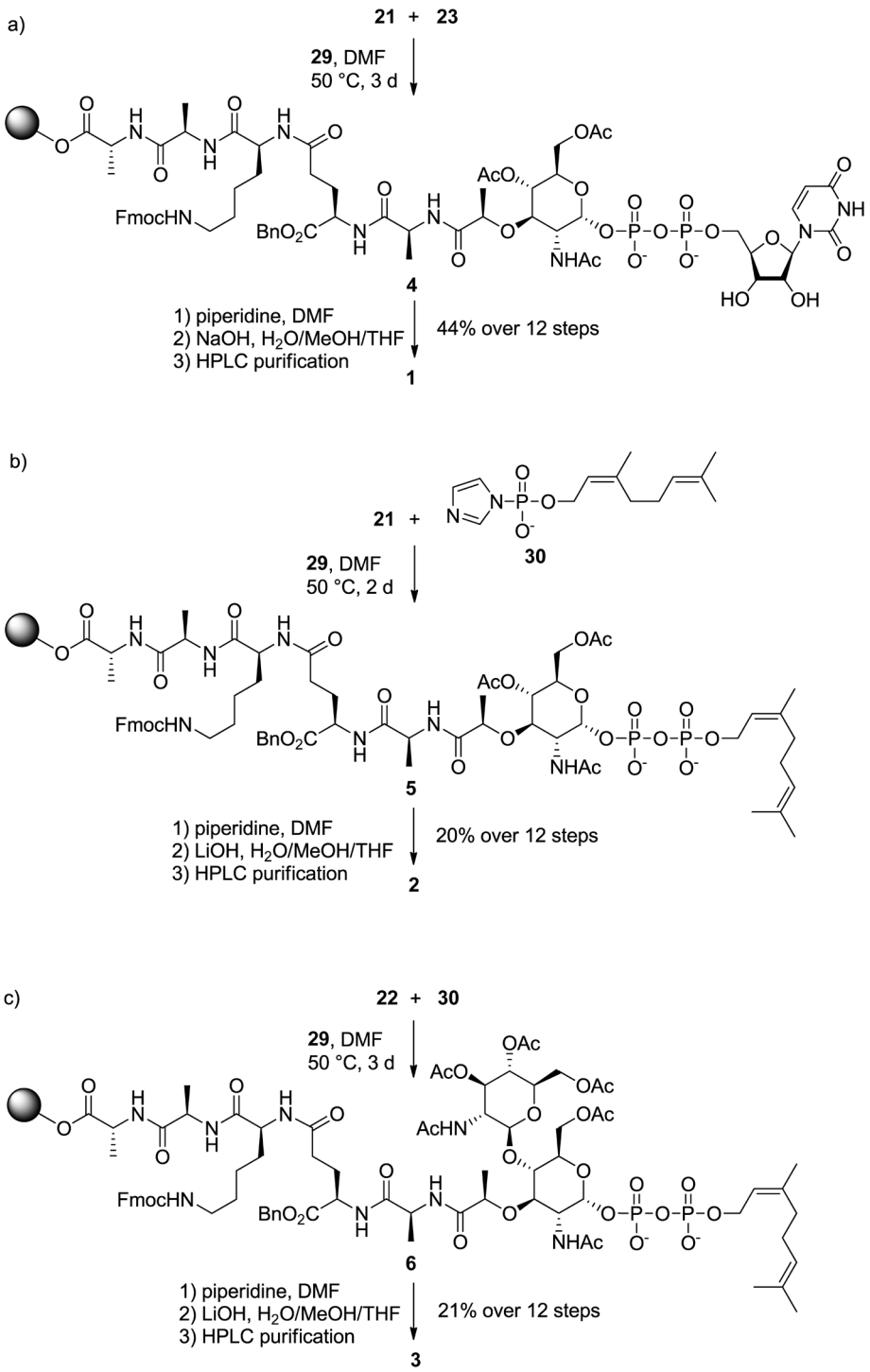


Chart 4. Completion of the Synthesis of **1**–**3**

Bn, J=11.5 Hz), 4.48–4.40 (m, 2H, $PhSO_2CH_2CH_2$), 4.38 (ddd, 1H, H-2, $J_{2,1}$=4.0, $J_{2,2\text{-}NH}$=9.2, $J_{2,3}$=9.8 Hz), 4.20 (dd, 1H, H-6, $J_{6,6}$=12.0, $J_{6,5}$=4.6 Hz), 4.13 (dq, 1H, Ala-α-CH, $J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-N}H}$= $J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-}\beta\text{-}CH}$=6.9 Hz), 4.03 (dd, 1H, H-6, $J_{6,6}$=12.0, $J_{6,5}$=2.3 Hz), 3.95 (q, 1H, Lac-α-CH, $J_{\text{Lac-}\alpha\text{-}CH,\text{Lac-}\beta\text{-}CH}$=6.3 Hz), 3.91 (ddd, 1H, H-5, $J_{5,6}$=2.3, $J_{5,6}$=4.6, $J_{5,4}$=9.8 Hz), 3.64 (dd, 1H, H-3, $J_{3,2}$=$J_{3,4}$=9.8 Hz), 3.50–3.38 (m, 2H, $PhSO_2CH_2CH_2$), 2.11 (s, 3H, OAc), 2.07 (s, 3H, OAc), 1.88 (s, 3H, NAc), 1.30 (d, 3H, Lac-β-CH, $J_{\text{Lac-}\beta\text{-}CH,\text{Lac-}\alpha\text{-}CH}$=6.3 Hz), 1.29 (d, 3H, Ala-β-CH, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=6.9 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 172.2, 171.8, 170.9, 170.4, 169.5, 139.3, 136.7, 134.2, 129.6, 128.9, 128.7, 128.5, 128.3, 97.2, 78.9, 78.6, 70.4, 69.5, 68.7, 62.3, 58.2, 55.1, 53.1, 48.1, 23.5, 21.0, 20.9, 18.7, 17.0; electrospray ionization (ESI)-MS-low resolution (LR) m/z 729.23 [$(M+Na)^+$]; ESI-MS-HR Calcd for $C_{33}H_{42}O_{13}N_2NaS$ 729.2230. Found 729.2299; $[\alpha]_D^{20}$ +68.36 (c 0.23, $CHCl_3$).

Glycosyl Phosphate **14**

A mixture of **13** (424 mg, 0.60 mmol) and 10% Pd/C (600 mg) in MeOH (6 mL) was vigorously stirred under H_2 atmosphere at room temperature for 2.5 h. 10% Pd/C (600 mg)

was added to the mixture and vigorously stirred for 8 h under H_2 atmosphere. 10% Pd/C (300 mg) was added to the mixture and vigorously stirred for 12 h under H_2 atmosphere. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to afford a crude lactol. A mixture of the lactol and 5-(benzylthio)-1*H*-tetrazole (208 mg, 1.08 mmol) in CH_2Cl_2 (6 mL) was treated with diallyl *N,N*-diisopropylphosphoramidite (238 μL, 0.90 mmol) at 0°C for 5 min. The mixture was warmed to room temperature and stirred for 1 h. 5-(Benzylthio)-1*H*-tetrazole (138 mg, 0.72 mmol) and diallyl *N,N*-diisopropylphosphoramidite (159 μL, 0.60 mmol) was added to the mixture and stirred for 1 h. The mixture was partitioned between CH_2Cl_2 and *sat. aq.* $NaHCO_3$, and the organic phase was washed with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford a crude phosphite. A mixture of the phosphite in tetrahydrofuran (THF) (6 mL) was treated with 30% H_2O_2 (600 μL) at −78°C for 5 min. The mixture was warmed to room temperature and stirred for 2.5 h. The reaction was quenched with *sat. aq.* $Na_2S_2O_3$ at 0°C, and the mixture was partitioned between AcOEt and *sat. aq.* $NaHCO_3$. The organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (50–100% AcOEt/hexane-0–1–2% MeOH/AcOEt) to afford **14** (180 mg, 0.23 mmol, 39% over 3 steps) as a colorless foam.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.93 (d, 2H, *o*-$PhSO_2$, $J_{o,m}$=7.4 Hz), 7.67 (t, 1H, *p*-$PhSO_2$, $J_{p,m}$=7.5 Hz), 7.60 (dd, 2H, *m*-$PhSO_2$, $J_{m,o}$=7.4, $J_{m,p}$=7.5 Hz), 6.76 (d, 1H, Ala-N*H*, $J_{\text{Ala-N}H,\text{Ala-}\alpha\text{-}CH}$=6.9 Hz), 6.55 (d, 1H, 2-N*H*, $J_{2\text{-N}H,2}$=8.6 Hz), 6.01–5.88 (m, 2H, Hc), 5.70 (dd, 1H, H-1, $J_{1,2}$=2.9, $J_{1,\text{P}}$=5.8 Hz), 5.40 (dd, 1H, H_a, $J_{\text{Ha,Hc}}$=17.2, $J_{\text{Ha},1'}$=1.2 Hz), 5.38 (dd, 1H, H_a, $J_{\text{Ha,Hc}}$=17.2, $J_{\text{Ha},1'}$=1.2 Hz), 5.31 (dd, 1H, H_b, $J_{\text{Hb,Hc}}$=10.3, $J_{\text{Ha},1'}$=1.2 Hz), 5.30 (dd, 1H, H_b, $J_{\text{Hb,Hc}}$=10.3, $J_{\text{Ha},1'}$=1.2 Hz), 5.14 (dd, 1H, H-4, $J_{4,3}$=10.3, $J_{4,5}$=9.8 Hz), 4.64–4.55 (m, 4H, H-1′), 4.53–4.44 (m, 2H, $PhSO_2CH_2CH_2$), 4.41–4.35 (m, 1H, H-2), 4.24 (dq, 1H, Ala-α-C*H*, 7.5, $J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-N}H}$=$J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-}\beta\text{-}CH}$=6.9 Hz), 4.20 (dd, 1H, H-6, $J_{6,6}$=12.0, $J_{6,5}$=4.0 Hz), 4.13–4.05 (m 2H, H-5, H-6), 4.03 (q, 1H, Lac-α-C*H*, $J_{\text{Lac-}\alpha\text{-}CH,\text{Lac-}\beta\text{-}CH}$=6.3 Hz), 3.69 (dd, 1H, H-3, $J_{3,2}$=$J_{3,4}$=10.3 Hz), 3.52–3.41 (m, 2H, $PhSO_2CH_2CH_2$), 2.09 (s, 3H, OAc), 2.08 (s, 3H, OAc), 1.96 (s, 3H, NAc), 1.34 (d, 3H, Lac-β-C*H*, $J_{\text{Lac-}\beta\text{-}CH,\text{Lac-}\alpha\text{-}CH}$=6.3 Hz), 1.33 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=7.5 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 172.3, 171.6, 170.9, 170.8, 169.3, 139.2, 134.3, 132.2, 132.2, 132.0, 132.0, 129.6, 128.2, 119.4, 119.3, 78.4, 77.0, 70.3, 69.1, 69.0, 69.0, 68.9, 61.8, 58.2, 55.0, 53.3, 53.3, 48.1, 25.1, 23.3, 20.9, 20.9, 18.9, 17.2; ^{31}P-NMR ($CDCl_3$, 202 MHz) δ −1.8; ESI-MS-LR *m/z* 799.21 [(M+Na)$^+$]; ESI-MS-HR Calcd for $C_{32}H_{45}O_{16}N_2NaPS$ 799.2120. Found 799.2125; $[\alpha]_D^{20}$ +49.99 (*c* 1.49, $CHCl_3$).

Glycosyl Phosphate **10**

A mixture of **14** (212 mg, 0.27 mmol) in CH_2Cl_2 (3 mL) was treated with DBU (44.8 μL, 0.30 mmol) at room temperature for 40 min. The mixture was partitioned between AcOEt and 1 M *aq.* HCl, and the aqueous phase was extracted with AcOEt (×2). Combined organic phase was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0–1–2% MeOH/CH_2Cl_2) to afford **10** (149 mg, 0.25 mmol, 90%) as a colorless foam.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.47 (d, 1H, 2-N*H*, $J_{2\text{-N}H,2}$=8.0 Hz), 6.86 (d, 1H, Ala-N*H*, $J_{\text{Ala-N}H,\text{Ala-}\alpha\text{-}CH}$=6.3 Hz), 5.98–5.88 (m, 2H, Hc), 5.72 (dd, 1H, H-1, $J_{1,2}$=3.4, $J_{1\text{P}}$=6.3 Hz), 5.39 (dd, 1H, H_a, $J_{\text{Ha,Hc}}$=16.6, $J_{\text{Ha},1'}$=1.2 Hz), 5.38 (dd, 1H, H_a, $J_{\text{Ha,Hc}}$=17.2, $J_{\text{Ha},1'}$=1.2 Hz), 5.30 (d, 2H, H_b, $J_{\text{Hb,Hc}}$=10.3 Hz), 5.12 (dd, 1H, H-4, $J_{4,3}$=$J_{4,5}$=9.2 Hz), 4.62–4.55 (m, 4H, H-1′), 4.37 (qd, 1H, Ala-α-C*H*, $J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-}\beta\text{-}CH}$=$J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-N}H}$=6.9 Hz), 4.31–4.25 (m, 1H, H-2), 4.22–4.15 (m, 2H, Lac-α-C*H*, H-6), 4.09–4.04 (m 2H, H-5, H-6), 3.75 (dd, 1H, H-3, $J_{3,2}$=$J_{3,4}$=9.7 Hz), 2.11 (s, 3H, OAc), 2.08 (s, 3H, OAc), 1.97 (s, 3H, NAc), 1.47 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=7.5 Hz), 1.33 (d, 3H, Lac-β-C*H*, $J_{\text{Lac-}\beta\text{-}CH,\text{Lac-}\alpha\text{-}CH}$=6.3 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 174.4, 173.4, 171.7, 170.7, 170.8, 169.4, 132.1, 132.0, 131.9, 131.8, 119.5, 119.2, 96.8, 96.8, 78.3, 76.7, 70.4, 69.3, 69.2, 69.0, 69.0, 61.9, 53.5, 53.4, 48.5, 23.0, 20.9, 20.8, 19.2, 17.5; ^{31}P-NMR ($CDCl_3$, 202 MHz) δ −2.7; ESI-MS-LR *m/z* 631.19 [(M+Na)$^+$]; ESI-MS-HR Calcd for $C_{24}H_{37}O_{14}N_2NaP$ 631.1875. Found 631.1882; $[\alpha]_D^{20}$ +63.23 (*c* 3.80, $CHCl_3$).

Glycosyl Phosphate **16**

A mixture of **15** (497 mg, 0.50 mmol) and 10% Pd/C (600 mg) in MeOH/EtOH=1/1 (10 mL) was vigorously stirred under H_2 atmosphere at room temperature for 24 h. The catalyst was filtered off through a Celite pad, and the filtrate was concentrated *in vacuo* to afford a crude lactol. A mixture of the lactol and 1*H*-tetrazole (70 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) was treated with diallyl *N,N*-diisopropylphosphoramidite (198 μL, 0.75 mmol) at 0°C for 5 min. The mixture was warmed to room temperature and stirred for 1.5 h. Diallyl *N,N*-diisopropylphosphoramidite (49.5 μL, 0.19 mmol) was added to the mixture and stirred for 10 min. The mixture was cooled to −50°C, and treated with 80% ^{t}BuOOH (1 mL) for 1 h. The reaction was quenched with *sat. aq.* $Na_2S_2O_3$, and the mixture was partitioned between AcOEt and *sat. aq.* $NaHCO_3$. The organic phase was washed with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0-1-2-5% MeOH/AcOEt) to afford **16** (271 mg, 0.25 mmol, 50% over 2 steps) as a colorless foam.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.93 (d, 2H, *o*-$PhSO_2$, $J_{o,m}$=7.5 Hz), 7.70 (t, 1H, *p*-$PhSO_2$, $J_{p,m}$=7.5 Hz), 7.66–7.58 (m, 3H, *m*-$PhSO_2$, 2-N*H*), 7.18 (d, 1H, Ala-N*H*, $J_{\text{Ala-N}H,\text{Ala-}\alpha\text{-}CH}$=8.1 Hz), 6.10 (d, 1H, 2′-N*H*, $J_{2'\text{-N}H,2'}$=8.6 Hz), 5.98–5.87 (m, 3H, Hc, H-1), 5.37 (dd, 1H, H_a, $J_{\text{Ha,Hc}}$=17.2, $J_{\text{Ha},1'}$=1.2 Hz), 5.35 (dd, 1H, H_a, $J_{\text{Ha,Hc}}$=17.2, $J_{\text{Ha},1'}$=1.2 Hz), 5.26 (d, 1H, H_b, $J_{\text{Hb,Hc}}$=10.3 Hz), 5.24 (d, 1H, H_b, $J_{\text{Hb,Hc}}$=10.3 Hz), 5.20 (dd, 1H, H-3′, $J_{3',4'}$=9.8, $J_{3',2'}$=10.9 Hz), 5.11 (dd, 1H, H-4′, $J_{4',3'}$=$J_{4',5'}$=10.9 Hz), 4.64 (q, 1H, Lac-α-C*H*, $J_{\text{Lac-}\alpha\text{-}CH,\text{Lac-}\beta\text{-}CH}$=6.3 Hz), 4.61–4.54 (m, 3H, H-1′, H-1″), 4.54–4.48 (m, 4H, $PhSO_2CH_2CH_2$, H-1″), 4.39 (dq, 1H, Ala-α-C*H*, $J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-N}H}$=8.1, $J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-}\beta\text{-}CH}$=7.5 Hz), 4.35–4.27 (m, 2H, H-6, H-6′), 4.23 (dd, 1H, H-6, $J_{\text{H-6,H-6}}$=12.6, $J_{6,5}$=3.4 Hz), 4.09 (dd, 1H, H-6′, $J_{6',6'}$=12.6, $J_{6',5'}$=2.3 Hz), 4.02–3.85 (m, 4H, H-2, H-2′, H-4, H-5), 3.68–3.63 (m, 1H, H-5′), 3.55 (dd, 1H, H-3, $J_{3,4}$=$J_{\text{H-3,H-2}}$=10.1 Hz), 3.51 (t, 2H, $PhSO_2CH_2CH_2$, $J_{\text{PhSO}_2CH_2CH_2,\text{PhSO}_2CH_2CH_2}$=5.7 Hz), 2.11 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.00 (s, 3H, NAc), 1.99 (s, 3H, NAc), 1.96 (s, 3H, NAc), 1.38 (d, 3H, Lac-β-C*H*, $J_{\text{Lac-}\beta\text{-}CH,\text{Lac-}\alpha\text{-}CH}$=6.9 Hz), 1.35 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=7.5 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 174.8, 171.8, 171.4, 171.3, 171.2, 171.0, 170.7, 169.5, 139.1, 134.3, 132.4, 129.6, 128.3, 118.8, 118.7, 99.8, 95.7, 95.7, 75.0, 74.0, 72.2, 72.2, 71.2, 68.7, 68.6, 68.6, 68.6, 68.4, 62.2, 61.9, 58.4,

55.1, 54.9, 53.9, 53.8, 48.1, 23.4, 23.1, 21.0, 20.8, 20.7, 18.9, 17.3; ^{31}P-NMR ($CDCl_3$, 162 MHz) δ −2.3; ESI-MS-LR *m/z* 1086.31 [(M+Na)$^+$]; ESI-MS-HR Calcd for $C_{44}H_{62}O_{23}N_3NaPS$ 1086.3125. Found 1086.3120; $[\alpha]_D^{20}$ +8.24 (*c* 1.24, $CHCl_3$).

Glycosyl Phosphate **11**

A mixture of **16** (127 mg, 0.12 mmol) in CH_2Cl_2 (1.5 mL) was treated with DBU (19.4 μL, 0.13 mmol) at room temperature for 40 min. The mixture was partitioned between AcOEt and 1 M *aq.* HCl, and the aqueous phase was extracted with AcOEt (×2). Combined organic phase was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by high-flash silica gel column chromatography (0–5–10% MeOH/CH_2Cl_2) to afford **11** (100 mg, 0.11 mmol, 93%) as a colorless foam.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.92 (d, 1H, 2-N*H*, $J_{2\text{-NH},2}$=4.0 Hz), 7.73 (d, 1H, Ala-N*H*, $J_{\text{Ala-NH,Ala-}\alpha\text{-CH}}$=7.5 Hz), 6.49 (d, 1H, 2′-N*H*, $J_{2'\text{-NH},2'}$=9.7 Hz), 5.95–5.84 (m, 3H, Hc, H-1), 5.37 (d, 2H, H_a, $J_{Ha,Hc}$=17.2 Hz), 5.25 (dd, 1H, H_b, $J_{Hb,Hc}$=10.3, $J_{Ha,1'}$=1.2 Hz), 5.24 (dd, 1H, H_b, $J_{Hb,Hc}$=10.3, $J_{Hb,1'}$=1.2 Hz), 5.12–5.05 (m, 2H, H-3′, H-4′), 4.71 (q, 1H, Lac-α-C*H*, $J_{\text{Lac-}\alpha\text{-CH,Lac-}\beta\text{-CH}}$=6.3 Hz), 4.59–4.52 (m, 3H, H-1′, H-1″), 4.52–4.45 (m, 2H, H-1″), 4.45 (dq, 1H, Ala-α-C*H*, $J_{\text{Ala-}\alpha\text{-CH,Ala-NH}}$=$J_{\text{Ala-}\alpha\text{-CH,Ala-}\beta\text{-CH}}$=7.5 Hz), 4.31 (dd, 1H, H-6, $J_{6,6}$=12.6, $J_{6,5}$=4.6 Hz), 4.22 (d, 1H, H-6, $J_{6,6}$=12.6, $J_{6,5}$=3.4 Hz), 4.17–4.09 (m, 1H, H-2′), 4.08 (dd, 1H, H-6, $J_{6,6}$=12.6, $J_{6,5}$=2.3 Hz), 3.97 (dd, 1H, H-4, $J_{4,5}$=$J_{4,3}$=9.7 Hz), 3.92–3.86 (m, 1H, H-2), 3.77–3.71 (m, 1H, H-5), 3.66–3.58 (m, 2H, H-5, H-3′), 2.09 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.01 (s, 3H, NAc), 2.00 (s, 3H, NAc), 1.96 (s, 3H, NAc), 1.48 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-CH,Ala-}\alpha\text{-CH}}$=7.5 Hz), 1.35 (d, 3H, Lac-β-C*H*, $J_{\text{Lac-}\beta\text{-CH,Lac-}\alpha\text{-CH}}$=6.3 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 175.7, 174.3, 171.8, 171.1, 171.1, 170.6, 169.5, 132.1, 132.0, 132.0, 131.9, 119.1, 118.9, 99.3, 95.6, 95.5, 73.7, 73.0, 72.3, 72.0, 71.7, 69.1, 69.0, 68.9, 68.8, 68.5, 62.5, 61.9, 54.7, 53.9, 53.9, 48.8, 23.3, 23.0, 20.9, 20.8, 20.7, 20.7, 18.7, 16.8; ^{31}P-NMR ($CDCl_3$, 202 MHz) δ: −3.2; ESI-MS-LR *m/z* 918.29 [(M+Na)$^+$]; ESI-MS-HR Calcd for $C_{36}H_{54}O_{21}N_3NaP$ 918.2880. Found 918.2885; $[\alpha]_D^{20}$ +7.00 (*c* 0.21, $CHCl_3$).

Diphosphate **25**

A solution of **23** (16.5 mg, 0.024 mmol) and **24** (12.7 mg, 0.024 mmol) in DMF (250 μL) was treated with **29** (5.3 mg, 0.024 mmol) at 25°C for 24 h. The mixture was concentrated *in vacuo*. The residue was purified by high-flash ODS column chromatography (0–10% MeCN/25 mM *aq.* AcOH·Et_3N). The product was further purified by reversed phase HPLC (YMC-Pack R&D ODS-A, 250×20 mm, 8% MeCN/25 mM *aq.* AcOH·t_3N) to afford **25** (6.9 mg, 0.0074 mmol, 31%) as a white powder, after freeze drying.

^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 9.53 (d, 1H, 2″-N*H*, $J_{2''\text{-NH},2''}$=9.2 Hz), 7.93 (d, 1H, H-6, $J_{6,5}$=6.3 Hz), 5.75 (s, 1H, H-1′), 5.56 (d, 1H, H-5, $J_{5,6}$=7.5 Hz), 5.37–5.32 (m, 1H, H-1″), 5.08 (dd, 1H, H-3, $J_{3,2}$=9.2, $J_{3,4}$=9.7 Hz), 4.93 (dd, 1H, H-4, $J_{4,3}$=$J_{4,5}$=9.5 Hz), 4.19–3.91 (m, 9H, H-2′, H-3′, H-4′, H-5′, H-2″, H-5″, H-6″), 3.11–3.07 (m, 2H, CH_3CH_2N), 2.01 (s, 3H, OAc), 1.94 (s 3H, OAc), 1.85 (s, 3H, OAc), 1.83 (s, 3H, NAc), 1.17 (t, 3H CH_3CH_2N); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 170.1, 170.1, 169.6, 169.3, 163.2, 150.8, 140.8, 139.5, 101.7, 93.9, 82.8, 71.6, 67.4, 67.7, 68.4, 67.7, 61.5, 50.9, 47.7, 45.5, 22.3, 20.5, 20.4, 19.0, 11.0, 8.6; ^{31}P-NMR (DMSO-d_6, 202 MHz) δ: −11.3 (d, $J_{P,P}$=26.3 Hz), −14.1 (d, $J_{P,P}$=26.3 Hz); ESI-MS-LR *m/z* 732.11 [(M−H)$^-$]; ESI-MS-HR Calcd for $C_{23}H_{32}O_{20}N_3P_2$ 732.1072. Found 732.1060; $[\alpha]_D^{20}$ +10.94 (*c* 0.60, DMSO-d_6).

Neryl Phosphoryl Imidazolide (**30**)

Neryl phosophate ammonium salt (2.9 mg, 0.012 mmol) was co-evaporated with Et_3N (20 μL) in pyridine (1 mL) twice. The residue was co-evaporated with toluene (1 mL) twice to afford neryl phosphate triethylamine salt. A mixture of the phosphate salt in DMF was treated with 1,1′-carbonyldiimidazole (9.5 mg, 0.059 mmol) at room temperature for 3 h. The reaction was quenched with MeOH (100 μL) and stirred for 30 min. The mixture was concentrated *in vacuo* and co-evaporated with toluene twice to afford **30**. This compound was used without further purification.

^{31}P-NMR (DMSO-d_6, 162 MHz) δ: −9.6.

Procedure for the Synthesis of **20**

Each HMBA-PEG resin (150 mg, 0.71 mmol) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. Each resin was agitated with CH_2Cl_2 (1.5 mL, 1 h), After removal of CH_2Cl_2, a solution of Fmoc-D-Ala-OH·H_2O (69 mg, 0.21 mmol) and *N,N′*-dissopropylcarbodiimide (33 μL, 0.21 mmol) in DMF (1 mL) was added at 0°C. Each mixture was agitated for 40 min. 4-Dimethylaminopyridine (2.5 mg, 0.021 mmol) was added to the mixture at 0°C, which was warmed to room temperature. After agitation for 1 h at room temperature, solvent and soluble reagents were removed by suction. All resins were subjected to the following washing treatment with DMF (2 mL×3), EtOH/CH_2Cl_2=1/1 (2 mL×3), CH_2Cl_2 (2 mL×3) and DMF (2 mL×3). The resins were treated with Bz_2O (48 mg, 0.021 mmol) in 20% pyridine/DMF (1 mL) at room temperature for 1 h, and the resins were washed with DMF (2 mL×3) and CH_2Cl_2 (2 mL×3) to afford **17**. The amount of loading on the resin was determined as follow. Dried **17** (6.0 mg) was agitated with DMF (2 mL) for 30 min, and DBU (40 μL) was added to the mixture. The mixture was agitated for 30 min. The supernatant was diluted with DMF and MeCN and subjected to UV measurement at 294 nm. The loading rate was determined to be 0.39 mmol/g from the observed absorbance (0.172). The resins **17** were treated with piperidine/DMF (1 : 4, 5 min, then 1 : 9, 15 min) to remove the Fmoc group, and the resins were washed with DMF (2 mL×3) and CH_2Cl_2 (2 mL×3). A solution of Fmoc-D-Ala-OH·H_2O (94 mg, 0.28 mmol), HBTU (105 mg, 0.28 mmol) and $^iPr_2NEt$ (97 μL, 0.57 mmol) in DMF (750 μL) was added to the resins, which were agitated for 2 h. All the resins were washed with DMF (2 mL×3) and CH_2Cl_2 (2 mL×3) to afford **18**. Kaiser test indicated the completion of the all coupling reactions. The loading rate was determined as described above. Namely, dried **18** (4.9 mg) was agitated with DMF (2 mL) for 30 min, DBU (40 μL) was added to the mixture. The mixture was agitated for 30 min. The supernatant was diluted with DMF and MeCN and subjected to UV measurement at 294 nm. The yield was determined to be quantitative from the observed absorbance (0.135). The resins **18** were treated with piperidine/DMF (1 : 4, 5 min, then 1 : 9, 15 min) to remove Fmoc group, and the resins were washed with DMF (2 mL×3) and CH_2Cl_2 (2 mL×3). A solution of Alloc-L-Lys(Fmoc)-OH (96 mg, 0.21 mmol), HBTU (68 mg, 0.21 mmol) and $^iPr_2NEt$ (72 μL, 0.43 mmol) in DMF (750 μL) was added to the resins, which were agitated for 2 h. All the resins were washed with DMF (2 mL×3) and CH_2Cl_2 (2 mL×3) to afford **19**. Kaiser test indicated the completion of the all coupling reactions. The amount of loading on the resin was determined as follow.

Namely, dried **19** (5.3 mg) was agitated with DMF (2 mL) for 30 min, DBU (40 μL) was added to the mixture. The mixture was agitated for 30 min. The supernatant was diluted with DMF and MeCN and subjected to UV measurement at 294 nm. The yield was determined to be 87% over 2 steps from the observed absorbance (0.107). The resins **19** were treated with a solution of $BH_3 \cdot Me_2NH$ (25 mg, 0.43 mmol) in EtOH (600 μL) for 5 min, then a solution of $Pd(PPh_3)_4$ (16 mg, 0.014 mmol) in CH_2Cl_2 (1 mL) was added to the mixture. The mixture was agitated for 15 min. All the resins were washed with 0.5% $^iPr_2NEt/CH_2Cl_2$ (2 mL×3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL×8), MeOH (2 mL×3) and CH_2Cl_2 (2 mL×3). A solution of Alloc-D-Glu-OBn (68 mg, 0.21 mmol), HBTU (68 mg, 0.21 mmol) and $^iPr_2NEt$ (72 μL, 0.43 mmol) in DMF (750 μL) was added to the resins, which were agitated for 2 h. All the resins were washed with DMF (2 mL×3) and CH_2Cl_2 (2 mL×3). Kaiser test indicated the completion of the all coupling reactions. The resin was dried *in vacuo* to afford **20** (194 mg, 0.055 mmol, 0.28 mmol/g, 89% over 2 steps). The yield was calculated by weighing resins.

Procedure for the Synthesis of **7**

Resin-bound peptide **20** (150 mg, 0.042 mmol) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with CH_2Cl_2 (1.5 mL, 1 h). After removal of CH_2Cl_2, the resin was treated with a solution of $BH_3 \cdot Me_2NH$ (13 mg, 0.22 mmol) in EtOH (600 μL) for 5 min, then a solution of $Pd(PPh_3)_4$ (8.4 mg, 7.3 μmol) in CH_2Cl_2 (1 mL) was added to the mixture. The mixture was agitated for 15 min. The resin was washed with 0.5% $^iPr_2NEt/CH_2Cl_2$ (2 mL×3), 0.5% (w/v) sodium diethyldithiocarbamate/DMF (1 mL×8), MeOH (2 mL×3) and DMF/CH_2Cl_2=1/1 (2 mL×3). A solution of **10** (50 mg, 0.082 mmol), PyAOP (57 mg, 0.11 mmol), HOAt (7.5 mg, 0.055 mmol), and acridine (30 mg, 0.16 mmol) in DMF/CH_2Cl_2 (700 μL) was added to the resin which was agitated for 3 h. The resin was washed with DMF (2 mL×3), CH_2Cl_2 (2 mL×3) and DMF/CH_2Cl_2=1/1 (2 mL×3). A solution of **10** (50 mg, 0.082 mmol), PyAOP (57 mg, 0.11 mmol), HOAt (7.5 mg, 0.055 mmol), and acridine (30 mg, 0.16 mmol) in DMF/CH_2Cl_2 (700 μL) was added to the resin which was agitated for 3 h. The resin was washed with DMF (2 mL×3), CH_2Cl_2 (2 mL×3). The resin was treated with a solution of Ac_2O (16 μL, 0.16 mmol) and $^iPr_2NEt$ (29 μL, 0.16 mmol) in DMF (800 μL) for 30 min. The resin was washed with DMF (2 mL×3), CH_2Cl_2 (2 mL×3). Kaiser test indicated the completion of the coupling reaction. The resin was dried *in vacuo* to afford **7** (189 mg, 0.042 mmol, 0.22 mmol/g, quantitative over 2 steps). The yield was calculated by weighing resin.

Procedure for the Synthesis of **8**

Resin-bound peptide **20** (100 mg, 0.028 mmol) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with CH_2Cl_2 (1.5 mL, 1 h). After removal of CH_2Cl_2, the resin was treated with a solution of $BH_3 \cdot Me_2NH$ (18 mg, 0.33 mmol) in EtOH (600 μL) for 5 min, then a solution of $Pd(PPh_3)_4$ (13 mg, 0.011 mmol) in CH_2Cl_2 (1 mL) was added to the mixture. The mixture was agitated for 15 min. The resin was washed with 0.5% $^iPr_2NEt/CH_2Cl_2$ (2 mL×3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL×8), MeOH (2 mL×3) and DMF/CH_2Cl_2=1/1 (2 mL×3). A solution of **11** (49 mg, 0.055 mmol), PyAOP (38 mg, 0.073 mmol), HOAt (5.0 mg, 0.037 mmol), and acridine (20 mg, 0.11 mmol) in DMF/CH_2Cl_2 (600 μL) was added to the resin which was agitated for 3 h. The resin was washed with DMF (2 mL×3), CH_2Cl_2 (2 mL×3) and DMF/CH_2Cl_2=1/1 (2 mL×3). A solution of **11** (49 mg, 0.055 mmol), PyAOP (38 mg, 0.073 mmol), HOAt (5.0 mg, 0.037 mmol), and acridine (20 mg, 0.11 mmol) in DMF/CH_2Cl_2 (600 μL) was added to the resin which was agitated for 3 h. The resin was washed with DMF (2 mL×3), CH_2Cl_2 (2 mL×3). The resin was treated with a solution of Ac_2O (10 μL, 0.11 mmol) and $^iPr_2NEt$ (19 μL, 0.11 mmol) in DMF (700 μL) for 30 min. The resin was washed with DMF (2 mL×3), CH_2Cl_2 (2 mL×3). Kaiser test indicated the completion of the coupling reaction. The resin was dried *in vacuo* to afford **8** (156 mg, 0.028 mmol, 0.18 mmol/g, quantitative over 2 steps).

Park Nucleotide (**1**)

Resin-bound peptide **7** (9.4 mg, 2.1 μmol) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with CH_2Cl_2 (1 mL, 1 h). After removal of CH_2Cl_2, the resin was treated with a solution of $Pd(PPh_3)_4$ (1.9 mg, 1.6 μmol) and $PhSiH_3$ (8.4 μL, 69 μmol) in CH_2Cl_2 (400 μL) for 2 h. The resin was washed with 0.5% $^iPr_2NEt/CH_2Cl_2$ (2 mL×3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL×8), MeOH (2 mL×3), CH_2Cl_2 (2 mL×3) and DMF (2 mL×3). The resin was treated with a solution of **23** (7.6 mg, 11 μmol) and **29** (2.4 mg, 11 μmol) in DMF (300 μL) at 50°C for 3 d. The resin was washed with DMF (2 mL×3), CH_2Cl_2 (2 mL×3) to afford **4**. The resin was treated with piperidine/DMF (1 : 4, 5 min, then 1 : 9, 15 min) to remove the Fmoc group, and the resin was washed with 0.5% $^iPr_2NEt/CH_2Cl_2$ (2 mL×3), DMF (2 mL×3), CH_2Cl_2 (2 mL×3) and THF (2 mL×3). The resin was treated with 2 M *aq*. NaOH/MeOH/THF=1/1/2 (400 μL) at 0°C, then warmed to room temperature and agitated for 2 h. The supernatant was neutralized by 1 M *aq*. HCl (220 μL) and purified with reversed phase HPLC (YMC-Pack R&D ODS-A, 250×20 mm, 1% MeCN/50 mM *aq*. NH_4HCO_3) to afford **1** (1.4 mg, 1.2 μmol, 44% over 12 steps) as a white powder, after freeze drying.

^{1}H-NMR (D_2O, 500 MHz) δ: 7.96 (d, 1H, H-6, $J_{6,5}$=8.0 Hz), 5.99 (d, 1H, H-1′, $J_{1',2'}$=5.2 Hz), 5.97 (d, 1H, H-5, $J_{5,6}$=8.0 Hz), 5.47 (dd, 1H, H-1″, $J_{1'',2''}$=2.9, $J_{1'',P}$=7.5 Hz), 4.39–4.30 (m, 3H, Ala-α-C*H*, H-2′, H-3′), 4.30–4.09 (m, 9H, H-4′, H-5′, H-2″, Ala-α-C*H*, Lac-α-C*H*, Lys-α-C*H*, D-Glu-α-C*H*), 3.98–3.94 (m, 1H, H-5″), 3.88 (dd, 1H, H-6″, $J_{6'',6''}$=12.6, $J_{6'',5''}$=2.3 Hz), 3.84 (dd, 1H, H-6″, $J_{6'',6''}$=12.6, $J_{6'',5''}$=4.0 Hz), 3.80 (dd, 1H, H-3″, $J_{3'',4''}$=$J_{3'',2''}$=10.3 Hz), 3.65 (dd, 1H, H-4″, $J_{4'',3''}$=10.3, $J_{4'',5''}$=9.7 Hz), 3.01 (t, 1H, Lys-ε-C*H*, $J_{\text{Lys-}\varepsilon\text{-}CH,\text{Lys-}\delta\text{-}CH}$=7.5 Hz), 2.31 (t, 2H, D-Glu-γ-C*H*, $J_{\text{D-Glu-}\gamma\text{-}CH,\text{D-Glu-}\beta\text{-}CH}$=8.0 Hz), 2.20–2.12 (m, 1H, D-Glu-β-C*H*), 2.02 (s, 3H, NAc), 1.92–1.85 (m, 1H, D-Glu-β-C*H*), 1.85–1.74 (m, 2H, Lys-β-C*H*), 1.74–1.65 (m, 2H, Lys-δ-C*H*), 1.52–1.41 (m, 2H, Lys-γ-C*H*), 1.45 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=7.5 Hz), 1.41 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=6.9 Hz), 1.37 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=6.9 Hz), 1.34 (d, 3H, Lac-β-C*H*, $J_{\text{Lac-}\beta\text{-}CH,\text{Lac-}\alpha\text{-}CH}$=6.9 Hz); ^{31}P-NMR ($D_2O$, 162 MHz) δ: −10.9 (d, $J_{P,P}$=21.7 Hz), −12.6 (d, $J_{P,P}$=21.7 Hz); ESI-MS-LR *m/z* 573.67 [$(M-2H)^{2-}$]; ESI-MS-HR Calcd for $C_{40}H_{63}O_{26}N_9P_2$ 573.6685. Found 573.6694; $[\alpha]_D^{20}$ +13.35 (*c* 0.08, MeOH).

The analytical data for synthetic **1** were in good agreement with the previously reported data.[6)]

Neryl-lipid I (**2**)

Resin-bound peptide **7** (9.4 mg, 2.1 μmol) was placed in a

5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with CH_2Cl_2 (1 mL, 1 h). After removal of CH_2Cl_2, the resin was treated with a solution of $Pd(PPh_3)_4$ (1.9 mg, 1.6 μmol) and $PhSiH_3$ (8.4 μL, 69 μmol) in CH_2Cl_2 (400 μL) for 2 h. The resin was washed with 0.5% $^iPr_2NEt/CH_2Cl_2$ (2 mL×3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL×8), MeOH (2 mL×3), CH_2Cl_2 (2 mL×3) and DMF (2 mL×3). The resin was treated with a solution of **30** (11 μmol) and **29** (2.4 mg, 11 μmol) in DMF (300 μL) at 50°C for 2 d. The resin was washed with DMF (2 mL×3), CH_2Cl_2 (2 mL×3) to afford **5**. The resin was treated with piperidine/DMF (1 : 4, 5 min, then 1 : 9, 15 min) to remove the Fmoc group, and the resin was washed with 0.5% $^iPr_2NEt/CH_2Cl_2$ (2 mL×3), DMF (2 mL×3), CH_2Cl_2 (2 mL×3) and THF (2 mL×3). The resin was treated with 2 M *aq*. LiOH/MeOH/THF=1/1/2 (400 μL) at 0°C, then warmed to room temperature and agitated for 2 h. The supernatant was neutralized by 1 M *aq*. HCl (210 μL) and purified with reversed phase HPLC (YMC-Pack R&D ODS-A, 250×20 mm, 15% MeCN/50 mM *aq*. NH_4HCO_3) to afford **2** (0.6 mg, 0.55 μmol, 20% over 12 steps) as a white powder after freeze drying.

^{1}H-NMR (D_2O, 500 MHz) δ: 5.47–5.42 (m, 2H, H-1, H-2′), 5.20 (t, 1H, H-6′, $J_{6',5'}$=6.9 Hz), 4.49–4.43 (m, 2H, H-1′), 4.33 (q, 1H, Ala-α-C*H*, $J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-}\beta\text{-}CH}$=6.9 Hz), 4.26 (q, 1H, Ala-α-C*H*, $J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-}\beta\text{-}CH}$=7.5 Hz), 4.23–4.18 (m, 2H, Ala-α-C*H*, Lys-α-C*H*), 4.17–4.08 (m, 3H, D-Glu-α-C*H*, Lac-α-C*H*, H-2), 3.98–3.93 (m, 1H, H-5), 3.91–3.86 (m, 1H, H-6), 3.84 (dd, 1H, H-6, $J_{6,6}$=12.6, $J_{6,5}$=4.0 Hz), 3.80 (dd, 1H, H-3, $J_{3,4}=J_{3,2}$=9.7 Hz), 3.64 (dd, 1H, H-4, $J_{4,3}$=9.7, $J_{4,5}$=10.3 Hz), 3.00 (t, 1H, Lys-ε-C*H*, $J_{\text{Lys-}\varepsilon\text{-}CH,\text{Lys-}\delta\text{-}CH}$=7.5 Hz), 2.31 (t, 2H, D-Glu-γ-C*H*, $J_{\text{D-Glu-}\gamma\text{-}CH,\text{D-Glu-}\beta\text{-}CH}$=8.0 Hz), 2.20–2.10 (m, 5H, D-Glu-β-C*H*, H-4′, H-5′), 2.00 (s, 3H, NAc), 1.93–1.85 (m, 1H, D-Glu-β-C*H*), 1.85–1.75 (m, 2H, Lys-β-C*H*), 1.77 (s, 3H, 3′-Me), 1.73–1.66 (m, 2H, Lys-δ-C*H*), 1.69 (s, 3H, 7′-Me), 1.62 (s, 3H, 7′-Me), 1.51–1.42 (m, 2H, Lys-γ-C*H*), 1.45 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=6.9 Hz), 1.41 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=6.9 Hz), 1.37 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=7.5 Hz), 1.33 (d, 3H, Lac-β-C*H*, $J_{\text{Lac-}\beta\text{-}CH,\text{Lac-}\alpha\text{-}CH}$=7.5 Hz); ^{31}P-NMR ($D_2O$, 202 MHz) δ: −10.2 (d, $J_{P,P}$=21.4 Hz), −12.7 (d, $J_{P,P}$=21.4 Hz); ESI-MS-LR *m/z* 528.70 [(M−2H)$^{2-}$]; ESI-MS-HR Calcd for $C_{41}H_{69}O_{21}N_7P_2$ 528.7016. Found 528.7025; $[\alpha]_D^{20}$ +20.42 (*c* 0.06, MeOH).

Neryl-lipid II (**3**)

Resin-bound peptide **8** (12.4 mg, 2.2 μmol) was placed in a 5 mL polypropylene syringe fitted with a polyethylene filter disc. The resin was agitated with CH_2Cl_2 (1 mL, 1 h). After removal of CH_2Cl_2, the resin was treated with a solution of $Pd(PPh_3)_4$ (2.0 mg, 1.7 μmol) and $PhSiH_3$ (8.9 μL, 73 μmol) in CH_2Cl_2 (300 μL) for 2 h. The resin was washed with 0.5% $^iPr_2NEt/CH_2Cl_2$ (2 mL×3), 0.5%(w/v) sodium diethyldithiocarbamate/DMF (1 mL×8), MeOH (2 mL×3), CH_2Cl_2 (2 mL×3) and DMF (2 mL×3). The resin was treated with a solution of **30** (12 μmol) and **29** (2.6 mg, 12 μmol) in DMF (300 μL) at 50°C for 3 d. The resin was washed with DMF (2 mL×3), CH_2Cl_2 (2 mL×3) to afford **6**. The resin was treated with piperidine/DMF (1 : 4, 5 min, then 1 : 9, 15 min) to remove the Fmoc group, and the resin was washed with 0.5% $^iPr_2NEt/CH_2Cl_2$ (2 mL×3), DMF (2 mL×3), CH_2Cl_2 (2 mL×3) and THF (2 mL×3). The resin was treated with 2 M *aq*. LiOH/MeOH/THF=1/1/2 (400 μL) at 0°C, then warmed to room temperature and agitated for 2 h. The supernatant was neutralized by 1 M *aq*. HCl (220 μL) and purified with reversed phase HPLC (YMC-Pack R&D ODS-A, 250×20 mm, 15% MeCN/50 mM *aq*. NH_4HCO_3) to afford **3** (0.8 mg, 0.64 μmol, 21% over 12 steps) as a white powder after freeze drying.

^{1}H-NMR (D_2O, 500 MHz) δ: 5.47–5.41 (m, 2H, H-1, H-2″), 5.22–5.17 (m, 1H, H-6″), 4.62 (d, 1H, H-1′, $J_{1',2'}$=8.6 Hz), 4.45 (dd, 2H, H-1″, $J_{1'',2''}=J_{1'',P}$=6.9 Hz), 4.33 (q, 1H, Ala-α-C*H*, $J_{\text{Ala-}\alpha\text{-}CH,\text{Ala-}\beta\text{-}CH}$=7.5 Hz), 4.28–4.12 (m, 5H, Ala-α-C*H*, Lys-α-C*H*, D-Glu-α-C*H*, H-2), 4.11 (q, 1H, Lac-α-C*H*, $J_{\text{Lac-}\alpha\text{-}CH,\text{Lac-}\beta\text{-}CH}$=7.5 Hz), 3.97–3.88 (m, 4H, H-4, H-6, H-6′), 3.81 (dd, 1H, H-3, $J_{3,4}=J_{3,2}$=9.7 Hz), 3.77–3.69 (m, 3H, H-5, H-2′, H-6′), 3.55 (dd, 1H, H-3′, $J_{3',4'}=J_{3',2'}$=8.6 Hz), 3.44–3.38 (m, 2H, H-4′, H-5′), 3.00 (t, 1H, Lys-ε-C*H*, $J_{\text{Lys-}\varepsilon\text{-}CH,\text{Lys-}\delta\text{-}CH}$=7.5 Hz), 2.31 (t, 2H, D-Glu-γ-C*H*, $J_{\text{D-Glu-}\gamma\text{-}CH,\text{D-Glu-}\beta\text{-}CH}$=8.6 Hz), 2.20–2.10 (m, 5H, D-Glu-β-C*H*, H-4″, H-5″), 2.05 (s, 3H, NAc), 1.99 (s, 3H, NAc), 1.93–1.85 (m, 1H, D-Glu-β-C*H*), 1.85–1.75 (m, 2H, Lys-β-C*H*), 1.77 (s, 3H, 3″-Me), 1.74–1.65 (m, 2H, Lys-δ-C*H*), 1.69 (s, 3H, 7″-Me), 1.62 (s, 3H, 7″-Me), 1.51–1.41 (m, 2H, Lys-γ-C*H*), 1.45 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=7.5 Hz), 1.44 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=6.9 Hz), 1.37 (d, 3H, Ala-β-C*H*, $J_{\text{Ala-}\beta\text{-}CH,\text{Ala-}\alpha\text{-}CH}$=7.5 Hz), 1.33 (d, 3H, Lac-β-C*H*, $J_{\text{Lac-}\beta\text{-}CH,\text{Lac-}\alpha\text{-}CH}$=6.9 Hz); ^{31}P-NMR ($D_2O$, 202 MHz) δ: −10.2 (d, $J_{P,P}$=21.4 Hz), −12.7 (d, $J_{P,P}$=21.4 Hz); ESI-MS-LR *m/z* 630.24 [(M−2H)$^{2-}$]; ESI-MS-HR Calcd for $C_{49}H_{82}O_{26}N_8P_2$ 630.2413. Found 630.2425; $[\alpha]_D^{20}$ +10.00 (*c* 0.08, MeOH).

The analytical data for synthetic **3** were in good agreement with the previously reported data.[14)]

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Supplementary Materials The online version of this article contains supplementary materials.

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