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Synthesis of Diaminopimelic Acid Containing Peptidoglycan Fragments and Tracheal Cytotoxin (TCT) and Investigation of Their Biological Functions

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Abstract: Bacterial cell wall peptidoglycan (PGN) is a potent immunostimulator and immune adjuvant. The PGN of Gram-negative bacteria and some Gram-positive bacteria contain *meso*-diaminopimelic acid (*meso*-DAP), and we have recently shown that the intracellular protein Nod1 is a PGN receptor and recognizes DAP-containing peptides. In this study, we achieved the synthesis of DAP-containing PGN fragments, including the first chemical synthesis of tracheal cytotoxin (TCT), GlcNAc-(β1–4)-(anhydro)MurNAc-L-Ala-γ-D-Glu-*meso*-DAP-D-Ala, and a repeating-unit of DAP-type PGN, GlcNAc-(β1–4)-MurNAc-L-Ala-γ-D-Glu-*meso*-DAP-D-Ala. For the synthesis of PGN fragments, we first established a new synthetic method for an orthogonally protected *meso*-DAP derivative, and then we constructed the glycopeptide structures. The ability of these fragments to stimulate human Nod1, as well as differences in Nod1 recognition of the variety of synthesized ligand structures were examined. The results showed that the substitution of the N terminus of iE-DAP is necessary for stronger Nod1 recognition, but the structure of the substituent seems not to be strictly recognized. The importance of the carboxyl group at the 2-position of DAP for human Nod1 stimulation was also shown.

Keywords: carbohydrates · immunochemistry · peptides · tracheal cytotoxin

Introduction

Innate immunity is the first line of defense against microorganisms, and is activated when pathogen-recognizing receptors (PRRs) recognize particular molecules that are commonly found in microbes.[1] PPRs include membrane-bound Toll-like receptors (TLRs), cytosolic Nod-like receptors (NLRs), and RIG-I family proteins. Common microbial components recognized by PRRs include bacterial peptidoglycan (PGN), lipopolysaccharide (LPS), lipoproteins, flagellin, DNA, RNA, and also viral RNA. PGN is a component of the bacterial cell wall, and consists of polysaccharide chains linked to peptides. The polysaccharide is a β(1–4) glycan composed of alternating *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc); the carboxyl group of MurNAc is the point of linkage to the peptide. At the branched position of the peptide, there is usually a diaminocarboxylic acid, such as L-Lys (in Gram-positive bacteria) or *meso*-diaminopimelic acid (*meso*-DAP, in Gram-negative bacteria and some Gram-positive bacteria). PGN has been known as a potent immunostimulator and an immune adjuvant. Recently, Inohara et al. and Philpott et al independently showed that the intracellular protein Nod1, which is the founding member of the NLR protein family, is a receptor against PGN fragments (muropeptides).[2,3] Philpott et al. reported that Nod1 senses DAP-containing muropeptides, such as GlcNAc-MurNAc-L-Ala-γ-D-Glu-*meso*-DAP,[2] whereas we showed a DAP-containing smaller peptide, iE-DAP (γ-D-glutamyl diaminopimelic acid), activates Nod1 by using PGN synthetic peptide fragments.[3] It was also shown that *meso*-DAP itself activates Nod1 of human

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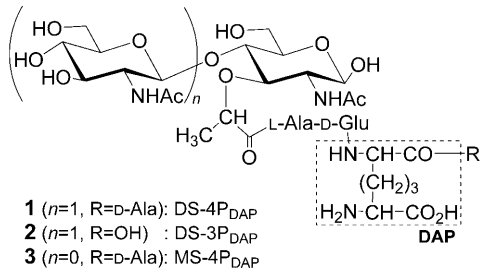
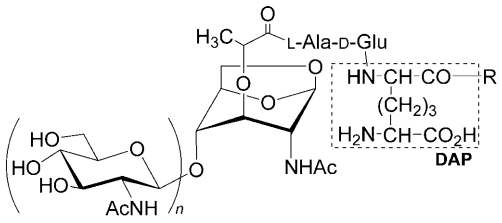
epithelial cells from various tissues.[4] Another intracellular protein, Nod2, recognizes PGN with a muramyl dipeptide (MDP) structure.[5,6]

Our recent findings showed that Gram-negative bacteria and certain Gram-positive bacteria that have DAP-type PGN secrete Nod1 ligands to the environment,[7] and the immune system seems to be regulated by the level of these ligands. Genetic studies have linked polymorphisms in the human Nod1 gene with susceptibility to several diseases, including allergic diseases, such as asthma,[8–10] Crohn's disease,[11] and sarcoidosis.[12] However, the function and immunostimulatory mechanism of Nod1 are not yet well understood. Although we found potent synthetic Nod1 ligands including *N*-myristoyl-iE-DAP (KF-1B) for in vivo analysis, which enabled the investigation of the Nod1 function,[13,14] it is also essential to understand the natural Nod1 ligands considered to be released into the environment.

It is often difficult to purify bacterial glycoconjugates, such as PGN fragments, from natural sources, because they are usually heterogeneous. In addition, the possibility of contamination of other immunostimulatory compounds cannot be eliminated even after extensive purification. We have thus synthesized a series of the PGN fragments, including Lys-type fragments found normally in Gram-positive bacteria, to study the key molecules in immune-system activation, and to determine the functions of ligands and receptors.[15,16] The repeating unit of DAP-type PGN, GlcNAc-(β1–4)-MurNAc-L-Ala-γ-D-Glu-*meso*-DAP-D-Ala **1**, however, has not been chemically synthesized, although the synthesis of monosaccharide DAP-type fragments has been performed.[17,18]



1 (*n*=1, R=D-Ala): DS-4P$_{DAP}$
2 (*n*=1, R=OH) : DS-3P$_{DAP}$
3 (*n*=0, R=D-Ala): MS-4P$_{DAP}$
4 (*n*=0, R=OH) : MS-3P$_{DAP}$



5 (*n*=1, R=D-Ala): tracheal cytotoxin (TCT)
6 (*n*=1, R=OH) : DS(anh)-3P$_{DAP}$
7 (*n*=0, R=D-Ala) : MS(anh)-4P$_{DAP}$
8 (*n*=0, R=OH) : MS(anh)-3P$_{DAP}$

Tracheal cytotoxin (TCT) **5** was originally found as a secretion of *Bordetella pertussis*, the causative agent of pertussis (whooping cough).[19] The structure of TCT was then determined as identical with GlcNAc-(anhydro)MurNAc-L-Ala-γ-D-Glu-*meso*-DAP-D-Ala.[20] It is a naturally occurring DAP-containing fragment of Gram-negative peptidoglycan, and considered as a potent stimulator of the innate immune response. TCT shows a variety of immunological activities, including immune stimulation in hamster tracheal epithelial cells,[21] activation of peptidoglycan recognition protein LC (PGRP-LC) in *Drosophila* to trigger an innate immune response,[22,23] and stimulation of the murine (but scarcely in human) immune system by Nod1.[24] TCT used in biological studies has been derived only from natural sources, and it has not been chemically synthesized.

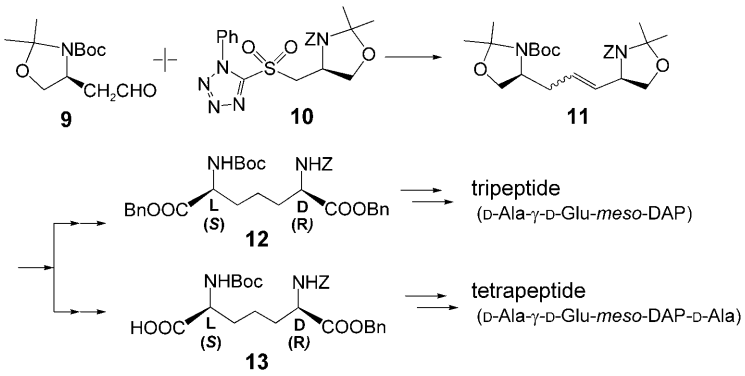
In this study, we synthesized DAP-type PGN repeating unit **1**, its fragments **2**, **3**, and **4**, and tracheal cytotoxin (TCT) **5** and its fragments **6**, **7**, and **8** to investigate their biological functions, including stimulation of NLR. To achieve the synthesis of these DAP-containing PGN fragments, a differently protected *meso*-DAP is necessary. The syntheses of some alternatively protected *meso*-DAPs have been reported by several groups,[18,25–37] as reviewed by Dzierzbicka.[38] In this study, two chiral fragments were coupled to prepare the DAP backbone. We have previously studied the biological activities of DAP isomers, which include the three DAP isomers: *meso*-, (2*S*,6*S*)-, and (2*R*,6*R*)-DAP[4] as well as four iE-DAP isomers: (2*R*,6*R*)-, (2*R*,6*S*)-, (2*S*,6*R*)-, and (2*S*,6*S*)-DAP.[13] The peptide, which containes the natural (2*S*,6*R*)-*meso*-DAP structure, showed the most potent Nod1 stimulatory activity. Establishing the efficient synthesis methods for these PGN fragments would lead to the new analogues with potent agonistic or antagonistic activities, and new molecular probes for the elucidation of biological function.

Results and Discussion

We synthesized *meso*-DAP-containing PGN fragments, including the repeating-unit of DAP-type PGN **1** and tracheal cytotoxin (TCT) **5**. We have previously synthesized Lys-type PGN fragments containing muramyl dipeptide (MDP; MurNAc-L-Ala-D-isoGln), which is recognized by intracellular receptor Nod2. In this study, we focused on DAP-containing PGN fragments and their Nod1 stimulatory activities. The difference between saccharide moieties MurNAc and (anhydro)MurNAc is also of interest due to their effect on the receptor recognition. For the synthesis of these fragments, we first established a synthetic method for the orthogonally protected *meso*-DAP.

To prepare the *meso*-DAP backbone, we coupled two α-amino acid derivatives, maintaining both chiral centers. We first attempted to use the Wittig reaction with several combinations of the intermediates (eg. Garner's aldehyde[39] and the corresponding bromide), but most of these reactions yielded an epimeric mixture of the compounds. We then

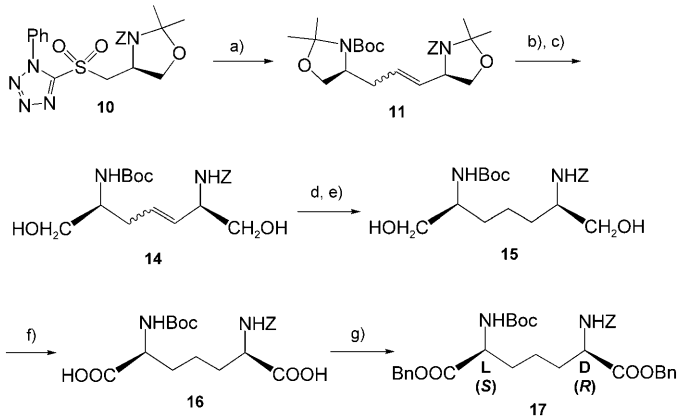
used Kocienski-modified Julia olefination (Scheme 1),[40] utilizing an aldehyde **9**[41] and a sulfone **10**,[42] both of which were derived from D-serine. The reaction proceeded without



Scheme 1. Synthesis of orthogonally protected *meso*-DAP.

epimerization to give the key intermediate **11**, which was converted to the protected *meso*-DAP **12** and **13**. Compound **12** was then used for the preparation of the tripeptide (L-Ala-γ-D-Glu-*meso*-DAP), and **13** was used for the tetrapeptide (L-Ala-γ-D-Glu-*meso*-DAP-D-Ala).

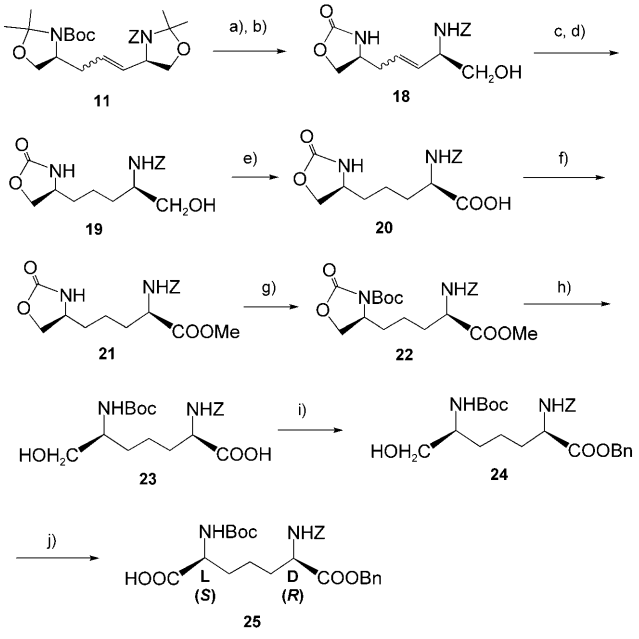
The synthesis of the protected *meso*-DAP **17** for the preparation of the tripeptide is shown in Scheme 2. The Julia–Kocienski reaction of sulfone **10** with aldehyde **9**, carried out with sodium hexamethyldisilazide (NaHMDS) in THF at −70 °C, gave the key intermediate **11** in 71 % yield. Once compound **17** had been formed, we used chiral HPLC analysis (CHIRALPAK AD-H, Daicel Chemical) to confirm that no epimerization had occurred in comparison with other epimers.[4] For the synthesis of the *meso*-DAP derivative for tripeptide preparation, two carboxylic acids can be protected with the same benzyl esters as in compound **17**. Hence, after cleavage of two isopropylidene aminals of **11** with *p*-toluenesulfonic acid (*p*TsOH), reprotection of the cleaved



Scheme 2. Synthesis of *meso*-DAP derivative **17**: a) NaHMDS, **9**, THF, −70 °C, 71 %; b) *p*TsOH·H_2O; c) Boc_2O, sat. $NaHCO_3$ aq., MeOH, 92 %; d) Pd/C, H_2, THF; e) ZCl, Et_3N, 58 % (for two steps); f) PDC, DMF; g) BnBr, Cs_2CO_3, DMF, 61 % (for two steps).

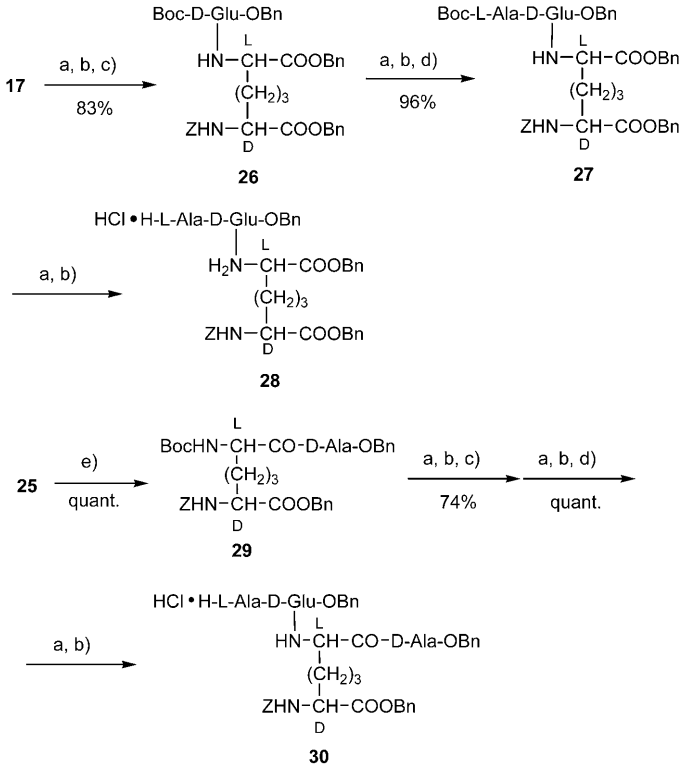
amino group with a *tert*-butoxycarbonyl (Boc) group, and reduction of the double bond with H_2 under Pd/C, both hydroxy groups of **15** were oxidized with pyridinium dichromate (PDC) to carboxyl groups to give **16**. Selective reduction of the double bond in the presence of the benzyloxycarbonyl (Z) group in **14** was attempted with Pd/Fib,[43] but the result was not satisfactory. Thus, the deprotected amino group was reprotected with benzyl chloroformate (ZCl). Esterification of **16** with benzyl bromide (BnBr) and Cs_2CO_3 in DMF gave fully protected *meso*-DAP **17**.

The synthesis of the protected *meso*-DAP **25** for the preparation of the tetrapeptide is shown in Scheme 3. The iso-



Scheme 3. Synthesis of *meso*-DAP derivative **25**: a) 1 N HCl in MeOH; b) triphosgene, Et_3N, CH_2Cl_2, 22 % (for two steps); c) Pd/C, H_2, MeOH; d) ZCl, $NaHCO_3$, 1,4-dioxane/H_2O 1:1, 82 % (for two steps); e) $RuCl_3$·*n*H_2O, $NaIO_4$, acetone/H_2O 1:1, 66 %; f) AcCl, MeOH, 89 %; g) Boc_2O, DMAP, Et_3N, THF, 86 %; h) LiOH, THF/H_2O 3:1; i) 1) Cs_2CO_3, MeOH; 2) BnBr, DMF, 83 % from **22**; j) PDC, DMF, 86 %.

propylidene groups and the Boc group of **11** were cleaved with 1 M HCl in MeOH, and monocarbamate formation at the liberated amino group with triphosgene and triethylamine (Et_3N) in CH_2Cl_2 gave **18**. Subsequent reduction and reprotection of the amino group with the Z group yielded compound **19**. The hydroxy group of **19** was oxidized to carboxylic acid **20** with $RuCl_3$·*n*H_2O and $NaIO_4$ in acetone/H_2O 1:1, and esterification by using acetyl chloride (AcCl) in methanol under acidic conditions gave compound **21**. The Boc group was introduced to the NH group of the carbamate by using di-*tert*-butyl dicarbonate (Boc_2O), *N*,*N*-dimethylaminopyridine (DMAP), and Et_3N, and the carbamate and ester were cleaved with LiOH to give **23**. For parts of the molecule in which the carbamate was protected with a Z group or there was no protecting group at the carbamate

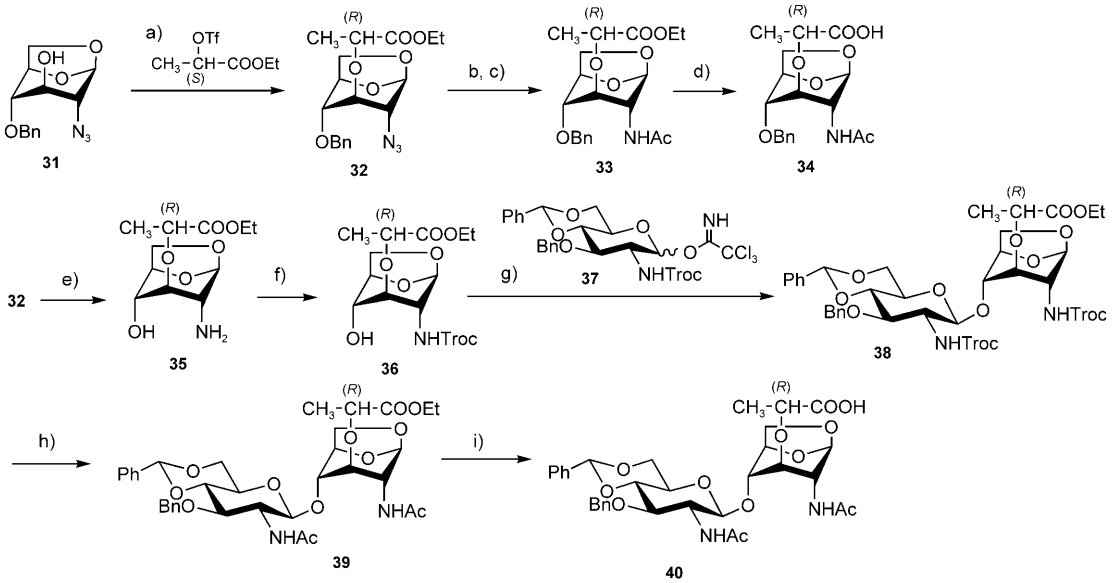


Scheme 4. Synthesis of the tripeptide **28** and tetrapeptide **30**: a) TFA; b) HCl in Et_2O; c) Boc-D-Glu-OBn, WSCD·HCl, HOBt, Et_3N, THF; d) Boc-L-Ala-OH, WSCD·HCl, HOBt, Et_3N, THF; e) HCl·H-D-Ala-OBn, WSCD·HCl, HOBt, Et_3N, THF.

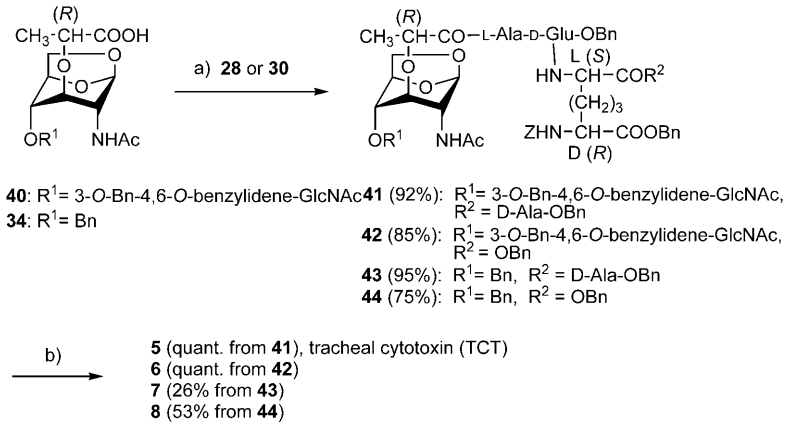
ring, the ring-opening reaction under basic conditions did not proceed, and cleavage of the Z group occurred. The liberated carboxyl group of **23** was derivatized to a benzyl ester to give **24**, and oxidation of the hydroxy group of **24** afforded the alternatively protected *meso*-DAP **25**.

The peptide moieties, tripeptide **28** and tetrapeptide **30**, were synthesized as shown in Scheme 4. For the preparation of tripeptide **28**, the Boc group of protected *meso*-DAP **17** was cleaved with trifluoroacetic acid (TFA), and the deprotected peptide was coupled to the side-chain carboxyl group of Boc-D-Glu-OBn by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (WSCI·HCl), 1-hydroxybenzotriazole (HOBt), and triethylamine in THF to give **26**. L-Alanine was introduced in a similar manner, and deprotection of the *N*-terminal Boc group gave tripeptide **28**. Tetrapeptide **30** was synthesized in a similar manner by starting from **25**. With these DAP-containing peptides in hand, we proceeded to the synthesis of the glycan (Scheme 5) and assembly of the peptidoglycan fragments (Scheme 6).

The anhydro-saccharides **34** and **40** were synthesized as shown in Scheme 5. Treatment of 1,6-anhydro sugar **31**[44,45] with NaH followed by addition of trifluoromethanesulfonyl-(*S*)-2-propionic acid ethyl ester afforded Mur(anh) derivative **32**. The azide group of **32** was reduced with Zn in THF/AcOH, and subsequent acetylation gave compound **33**. Cleavage of the ethyl ester gave 1,6-anhydro-muramic acid derivative **34**. For disaccharide **40**, the benzyl and azide groups of **32** were reduced by hydrogenation under H_2 (15 kg cm^{-2}) with $Pd(OH)_2$ in THF to give **35**. The liberated amino group was then protected with a 2,2,2-trichloroethoxycarbonyl (Troc) group. The resulting compound **36** was utilized for the glycosylation with imidate **37** by using trimethylsilyl trifluoromethanesulfonate (TMSOTf) and molecular sieves (MS, 4 Å) in CH_2Cl_2, at −15 °C, and gave the disaccharide **38**. When the N-acetylted acceptor was used for the glycosylation in place of **36**, the yield of glycosylation



Scheme 5. Synthesis of anhydro-saccharides **34** and **40**: a) NaH, CH_2Cl_2, 78%; b) Zn, THF/AcOH 1:1; c) Ac_2O/pyridine 1:1, 79% for two steps; d) LiOH, THF/1,4-dioxane/H_2O 4:2:1, 44%; e) H_2 (15 kg cm^{-2}), $Pd(OH)_2$, THF; f) TrocCl, Et_3N, CH_2Cl_2, 77% for two steps; g) TMSOTf, MS (4 Å), CH_2Cl_2, −15 °C, 30 min, 65%; h) Zn/Cu, AcOH/THF/Ac_2O 1:1:1, 81%; i) LiOH, THF/1,4-dioxane/H_2O 4:2:1, quant.



Scheme 6. The synthesis of tracheal cytotoxin (TCT) and other fragments: a) WSCD·HCl, HOBt, Et_3N, DMF; b) $Pd(OH)_2$, H_2 (20 kg cm^{-2}), THF.

was 37%. The obtained compound **38** was then treated with Zn/Cu in THF/acetic acid/acetic anhydride to cleave the *N*-Troc groups and acetylate the liberated amino groups. Subsequent deprotection of the ester group afforded disacchride **40**.

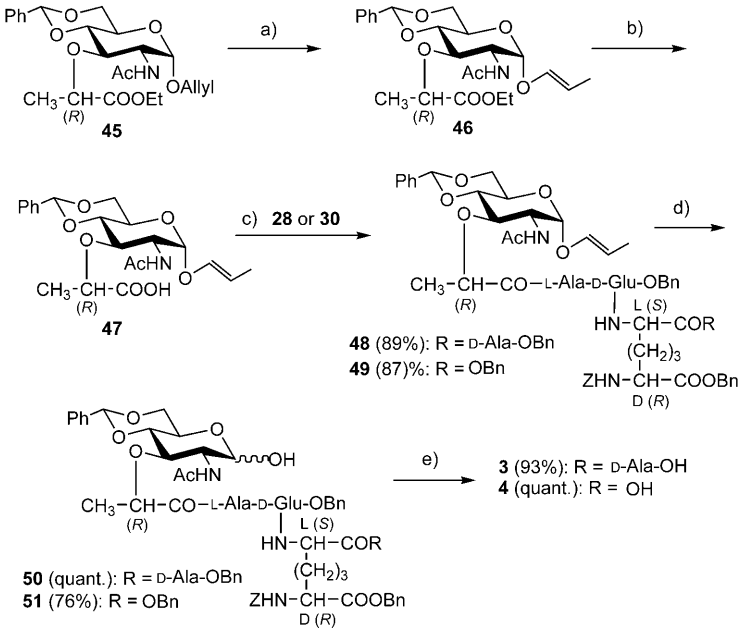
The syntheses of **5** (TCT), **6**, **7**, and **8**, which contain anhydro-muramic acid are shown in Scheme 6. Monosaccharide **34** and disaccharide **40** were condensed with tripeptide **28** and tetrapeptide **30** independently, by using WSCI·HCl, HOBt, and triethylamine to give protected disaccharide tetrapeptide **41** (92%), disaccharide tripeptide **42** (85%), monosaccharide teterapeptide **43** (95%), and monosaccharide tripeptide **44** (75%). All benzyl and benzyloxycarbonyl groups of **41**, **42**, **43**, and **44** were removed by catalytic hydrogenation with $Pd(OH)_2$ and H_2 to give **5** (TCT), **6**, **7**, and **8**, respectively. We thus achieved the first chemical synthesis of TCT.

The series of DAP-type peptidoglycan fragments were also synthesized as shown in Schemes 7 and 8. The monosaccharide fragments **3** and **4** were synthesized from **45**[15] (Scheme 7). The isomerization of the allyl group of **45** to a vinyl group was performed with H_2-activated [Ir(cod)-($MePh_2P$)$_2$]PF_6 (cod = 1,5-cyclooctadiene) to give compound **46**.[15,46] The ethyl ester of **46** was cleaved with LiOH in 1,4-dioxane/THF/H_2O 2:4:1, and tripeptide **28** or tetrapeptide **30** was subsequently introduced to the liberated carboxylic acid by using WSCD·HCl, HOBt, and triethylamine, to give **48** and **49**, respectively. The cleavage of the vinyl group with iodine and water gave **50** and **51**.[47] All remaining protecting groups, the benzyl groups in the peptide moiety, and the benzylidene group at the 4,6-position of the glycan, were cleaved by hydrogenation using $Pd(OH)_2$ catalysis to give compounds **3** and **4**.

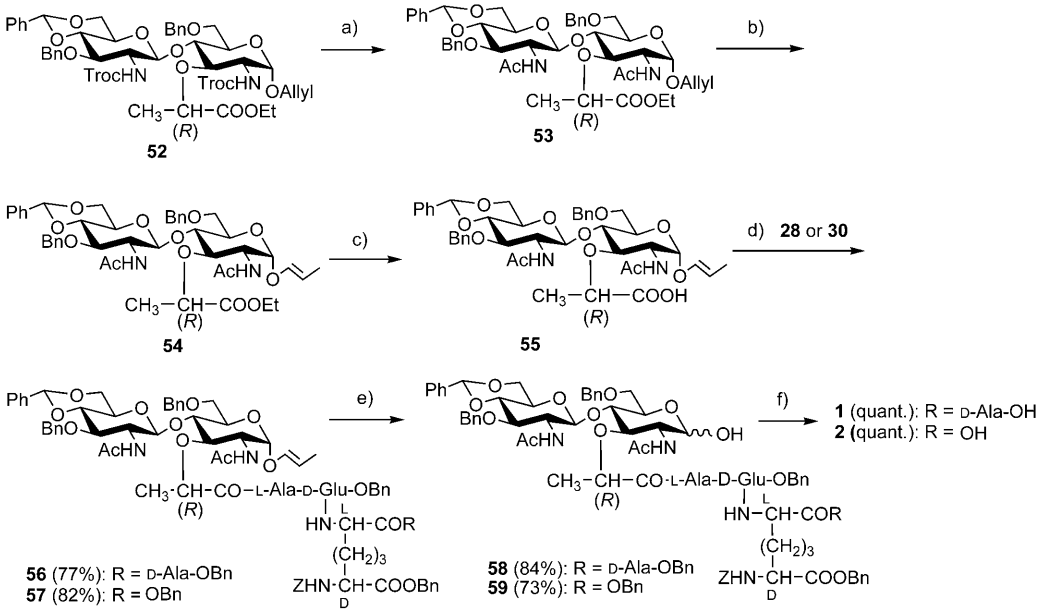
Disaccharide tetrapeptide **1** and disaccharide tripeptide **2** were then synthesized from **52**[15,16] (Scheme 8), in a similar manner as used for the monosaccharides **3** and **4**. First, **53** was obtained by deprotection of the *N*-Troc groups of **52** and subsequent one-pot acetylation of the liberated amino groups with Zn/Cu in THF/AcOH/Ac_2O. Isomerization of the allyl group with Ir complex, and hydrolysis of ethyl ester gave **55**. Peptide coupling with tripeptide **28** or tetrapeptide **30**, cleavage of their vinyl groups with I_2 and H_2O, and final deprotection afforded disaccharide with tripeptide **1** and disaccharide with tetrapeptide **2**, respectively. This synthesis of **1** is the first chemical synthesis of a repeating unit of DAP-type PGN.

The human Nod1 stimulating activity of each synthetic peptidoglycan fragment was then evaluated by HEK293T bioassay as previously described[3] (Figures 1 and 2). First, the anhydro-muramic acid containing compounds **5** (TCT) and **6** (DS(anh)-3P_{DAP}) were examined in comparison with shorter Nod1 ligands, A-iE-DAP (L-Alanyl-γ-D-glutaminyl-diaminopimelic acid)[14] as shown in Figure 1.

In these compounds, **5** (TCT) showed only very weak human-Nod1 stimulatory activity. These data are consistent with a report that utilized TCT from a natural source.[24] On the other hand, **6** (DS(anh)-3P_{DAP}) showed approximately 10-fold higher activity than that of A-iE-DAP. This demonstrated that the difference of one D-alanine residue attached to DAP has a strong affect on recognition by human Nod1.



Scheme 7. Synthesis of monosaccharide-tetrapeptide **3** and -tripeptide **4**: a) [Ir(cod)(H)($MePh_2P$)$_2$]PF_6, THF, 91%; b) LiOH, THF/1,4-dioxane/H_2O 4:2:1, quant.; c) WSCD·HCl, HOBt, Et_3N, DMF; d) I_2, THF/H_2O 1:1; e) $Pd(OH)_2$, H_2 (20 kg cm^{-2}), AcOH.



Scheme 8. Synthesis of a DAP-type PGN repeating unit **1** and the fragments **2**: a) Zn/Cu, THF/AcOH/Ac_2O 1:1:1, 46%; b) $[Ir(cod)(H)(MePh_2P)_2]PF_6$, THF, 80%; c) LiOH, THF/1,4-dioxane/H_2O 4:2:1; d) WSCD·HCl, HOBt, Et_3N, DMF; e) I_2, THF/H_2O 1:1; f) H_2 (20 kg cm^{-2}), $Pd(OH)_2$, AcOH.

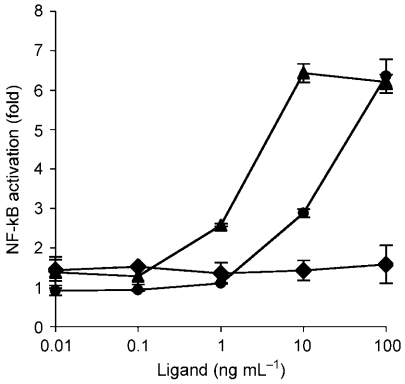


Figure 1. Stimulation of Nod1 by PG fragments, A-iE-DAP (L-Alanyl-γ-D-glutamyl-diaminopimelic acid), **5** (TCT), and **6** (DS(anh)-3P_{DAP}). HEK293T cells were transfected with human-Nod1, and the indicated amount of each compound was added to the cells and the ability of each compound to activate NF-κB was determined by luciferase reporter assay.[7] ▲: **6**, ◆: TCT (**5**) ●: A-iE-DAP.

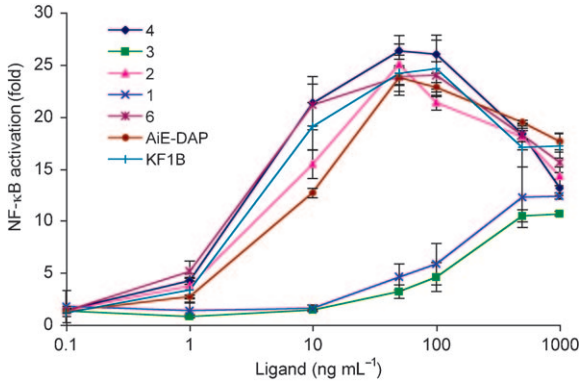


Figure 2. Stimulation of Nod1 by PG fragments, A-iE-DAP(L-Alanyl-γ-D-glutamyl-diaminopimelic acid), C14-iE-DAP (KF1B; *N*- myristoyl-iE-DAP), **1** (DS-4P_{DAP}), **2** (DS-3P_{DAP}), **3** (MS-4P_{DAP}), **4** (MS-3P_{DAP}), and **6** (DS(anh)-3P_{DAP}). HEK293T cells were transfected with human-Nod1, and the indicated amount of each compound was added to the cells and the ability of each compound to activate NF-κB was determined by luciferase reporter assay.[7]

A free carboxyl group at the 2-position of DAP is, therefore, favorable for the receptor recognition.

We then examined the Nod1 stimulatory activity of the PG fragments, **1** (DS-4P_{DAP}), **2** (DS-3P_{DAP}), **3** (MS-4P_{DAP}), and **4** (MS-3P_{DAP}), in comparison with the anhydro-MurNAc-containing **6** (DS(anh)-3P_{DAP}), A-iE-DAP, and a known potent ligand, C14-iE-DAP (KF1B; *N*-myristoyl-iE-DAP;[14] Figure 2). This also showed similar tendencies to the activity determined by peptide structure, with tripeptide compounds (**2** and **4**) being stronger human Nod1 stimulators than tetrapeptide compounds (**1** and **3**). Among these fragments, **4** (MS-3P_{DAP}) showed the most potent activity, which was similar to that of **6** (DS(anh)-3P_{DAP}) and C14-iE-DAP (KF1B). These results suggested that the substitution of the N-terminus of iE-DAP is necessary for stronger Nod1 recognition, but the structure of the substituent seems not to be strictly recognized. The importance of the carboxyl group at the 2-position of DAP for the Nod1 stimulation is also shown by these results. It has previously been observed that **3** (MS-4P_{DAP}) and **4** (MS-3P_{DAP}) showed similar activities in the human Nod1 stimulation at a higher concentration (5 μM).[48] Although we also observed that **3** (MS-4P_{DAP}) and **4** (MS-3P_{DAP}) showed similar stimulatory activities at a higher concentrations, such as 1000 ng mL^{-1} (1.4–1.6 μM), de-

tailed Nod1 stimulatory activities were observed at relatively lower doses from 0.1 to 100 ng mL^{-1} in this study.

Conclusion

As described, we have developed a new method for the preparation of orthogonally protected *meso*-DAP, and achieved the first chemical synthesis of TCT and the repeating-unit of DAP-type PGN, and various other DAP-type PGN partial structures, to investigate the PGN-receptor recognition. We determined human Nod1 stimulatory activities with these synthetic fragments. The results demonstrated that the free carboxyl group at the 2-position of DAP is important for human Nod1 stimulation, and that substitution of the N terminus of iE-DAP is recognized by Nod1, although this seems not to be a strict recognition. TCT only weakly stimulates human Nod1, but it has been reported that TCT has a key role in innate immune systems of other species.[22,23] One of these examples is the activation of PGRP-LC in *Drosophila*,[22,23] which is a trigger for its innate immune response against Gram-negative bacteria. It was also recently shown that recognition of DAP-type PGN by PGRP-LE in *Drosophila* was crucial for the induction of autophagy, which prevented the intracellular growth of *Listeria monocytogenes* and promoted host survival after the infection.[49] The structurally defined synthesized PGN fragments should contribute to further understanding of the mechanism of PGN action in the immune system.

Experimental Section

General procedures: ^{1}H NMR spectra were recorded at 400 MHz by using a JEOL JMN-GSX 400 spectrometer and at 500 MHz by using a JEOL JNM-LA 500 spectrometer. The chemical shifts in $CDCl_3$ are given in δ values from tetramethylsilane as an internal standard. For the measurement in D_2O, the HDO signal ($\delta = 4.718$ ppm at 30 °C) was used as a reference. ESI-TOF mass spectrometry was carried out by using an Applied Biosystem Mariner Biospectrometry Workstation. ESI-QTOF mass spectrometry was carried out by using a Waters-Micromass Q-Tof micro. Elemental analyses were performed with Yanaco CHN corder MT-6. Silica-gel column chromatography was carried out by using Kieselgel 60 (Merck, 0.040–0.063 mm) at medium pressure (2–4 kg cm^{-2}). HPLC analysis was carried out with CHROMATOPAC C-R7A plus and LCsolution by SHIMADZU CORPORATION. Anhydrous CH_2Cl_2 was prepared by distillation from calcium hydride. Molecular sieves 4 Å were activated in vacuo at 250 °C for 3 h before use. All other reagents and solvents used were purchased from commercial sources.

1-[(4*R*)-2,2-Dimethyl-3-*N*-benzyloxycarbonyloxazolidin-4-yl]-3-[(4*S*)-2,2-dimethyl-3-*N*-*tert*-butyloxycarbonyloxazolidin-4-yl]-1-propene (11): NaHMDS (7.7 mL, 1.0 M in THF) was added dropwise to a solution of **10** (2.7 g, 5.90 mmol) in dry THF (50 mL) at −70 °C. Then aldehyde **9** (1.4 g, 5.90 mmol) in dry THF (10 mL) was added, and the resulting mixture was stirred for 2 h at RT. After this time, the reaction was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (150 g, toluene/AcOEt 15:1) to give **11** (2.1 g, 75 %). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.40$–7.28 (5H, m; C_6H_5−), 5.56–5.38 (2H, m; −C*H*=C*H*−), 5.09 (2H, brs; −CH_2−Ph), 4.11–4.05 (1H, m; C≡C−C*H*−), 3.90–3.57 (5H, m; −C*H*−CH_2−C≡C-, −O−CH_2−CH− ×2), 2.53–1.96 (2H, m; −CH_2−C≡C−), 1.63–1.48 ppm (7H, m; CH_3− ×7); elemental analysis calcd (%) for $C_{26}H_{38}N_2O_6 \cdot 0.9H_2O$: C 63.63, H 8.17, N 5.71; found: C 63.28, H 7.91, N 6.18; ESI-TOF-MS (positive): *m*/*z*: 475.1 $[M+H]^+$.

(2*R*,6*S*)-2-(Benzyloxycarbonylamino)-6-(*tert*-butyloxycarbonylamino)-hept-3-ene-1,7-diol (14): *p*TsOH·H_2O (2 g, 0.011 mol) was added to a solution of **11** (2.4 g, 5.05 mmol) in MeOH, and the resulting mixture was stirred for 3 h. After this time, saturated $NaHCO_3$ aq. was added to bring the mixture to pH 8 and then Boc_2O (1.2 mL, 5.05 mmol) was added. After the mixture had been stirred for 5 h, it was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (150 g, $CHCl_3$/acetone 5:1) to give **14** (1.7 g, 89 %). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.37$–7.29 (5H, m; C_6H_5−), 5.54 (1H, td, $J = 10$, 5 Hz; −CH_2−C*H*=CH−, *cis*), 5.40 (1H, t, $J = 10$ Hz; −CH_2−CH=C*H*−, *cis*), 5.11 (1H, d, $J = 12$ Hz; C_6H_5−CH_2−), 5.05 (1H, d, $J = 12$ Hz; C_6H_5−CH_2−), 4.54 (1H, brs; −HN−C*H*−CH_2−), 3.67–3.59 (4H, m; −CH_2OH ×2), 3.55–3.53 (1H, m; =CH−C*H*−), 2.29–2.24 (2H, m; −CH_2−CH=), 2.14–1.62 (2H, m; −CH_2O*H* ×2), 1.44 ppm (9H, s; (CH_3)$_3$C−); elemental analysis calcd (%) for $C_{20}H_{30}N_2O_6$: C 60.90, H 7.67, N 7.10; found: C 63.28, H 7.91, N 6.18; ESI-TOF-MS (positive): *m*/*z*: 395.2 $[M+H]^+$.

(2*S*,6*R*)-6-(Benzyloxycarbonylamino)-2-(*tert*-butyloxycarbonylamino)-heptane-1,7-diol: Compound **14** (1.57 g, 3.99 mmol) was dissolved in THF (40 mL) and Pd/C (1.5 g) was added to the resulting solution. The reaction was stirred for 1 day under a H_2 atmosphere. After this time, Pd/C was removed by membrane filtration and the solution was concentrated in vacuo. The residue was dissolved in 1,4-dioxane (20 mL) at 0 °C, and then $NaHCO_3$ aq. (1.3 g, 0.016 mol, 20 mL) and ZCl (855 μL, 5.99 mol) were added. The mixture was stirred for 30 min at RT. After this time, the reaction mixture was extracted with AcOEt. The organic layer was washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was recrystallized by $CHCl_3$/hexane to give **15** (869 mg, 58 %). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.38$–7.29 (5H, m; C_6H_5−), 5.10 (2H, s; C_6H_5−CH_2−), 3.72–3.3.61 (4H, m; −CH_2OH ×2), 3.54–3.52 (2H, m; αH ×2), 2.03 (2H, m; −CH_2O*H* ×2), 1.64–1.56 (4H, m; −CH−CH_2−CH_2− ×2), 1.44 (9H, s; (CH_3)$_3$C−), 1.33–1.23 ppm (2H, m; −CH_2−CH_2−CH_2−); elemental analysis calcd (%) for $C_{20}H_{32}N_2O_6$: C 60.59, H 8.14, N 7.07; found: C 60.51, H 8.02, N 7.12; ESI-TOF-MS (positive): *m*/*z*: 397.3 $[M+H]^+$.

Dibenzyl (2*S*,6*R*)-6-(benzyloxycarbonylamino)-2-(*tert*-butyloxycarbonylamino)heptanedioate (17): Compound **15** (420 mg, 1.06 mmol), PDC (4 g, 0.011 mol), and Celite (4 g) were added to dry DMF (15 mL), and the resulting mixture was stirred for 1 day. After this time, the mixture was filtrated through Celite, and the filtrate was extracted with Et_2O. The organic layer was washed with 10 % citric acid solution, dried over Na_2SO_4, and then concentrated in vacuo. The residue and Cs_2CO_3 (363 mg, 1.11 mmol) were dissolved in MeOH (5 mL) and the solution was concentrated in vacuo. The residue was dissolved in dry DMF (10 mL) and then BnBr (265 μL, 2.23 mmol) was added under an Ar atmosphere. After the reaction had been stirred for 1 day, it was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (40 g, toluene/AcOEt 8:1) to give **17** (475 mg, 74 %). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.35$–7.28 (5H, m; C_6H_5−), 5.28 (1H, brs; NH), 5.17–5.07 (6H, m; −CH_2−Ph ×3), 4.98 (1H, brs; NH), 4.36 (1H, d, $J = 5$ Hz; αH), 4.28 (1H, d, $J = 4$ Hz; αH), 1.56–1.26 (6H, m; −(CH_2)$_3$−), 1.42 ppm (9H, s; −C(CH_3)$_3$); elemental analysis calcd (%) for $C_{34}H_{40}N_2O_8 \cdot 0.3H_2O$: C 66.94, H 6.71, N 4.59; found: C 66.95, H 6.60, N 4.45; HPLC analysis: (Daicel CHIRAL PAK AD-H, 0.46 × 25 cm, 1 mL min^{-1}, hexane/2-propanol 7:3): 254 nm; ESI-TOF-MS (positive): *m*/*z*: 605.3 $[M+H]^+$.

(4*S*)-4-[(4*R*)-4-(Benzyloxycarbonyamino)-5-hydroxy-2-pentenyl]oxazolidine-2-one (18): Compound **11** (700 mg, 1.48 mmol) was dissolved in MeOH (9 mL) and added to 4 N HCl in 1,4-dioxane (3 mL). After the reaction mixture had been stirred for 1.5 h, it was concentrated in vacuo. The residue was dissolved in dry CH_2Cl_2 (14 mL) at 0 °C, and then Et_3N (1 mL, 7.40 mmol) was added. A solution of triphosgene (158 mg, 0.53 mmol) in CH_2Cl_2 (7 mL) was added to the mixture, and it was stirred for a further 1 h. After this time, the reaction mixture was quenched by

saturated $NaHCO_3$ aq. The organic layer was washed with saturated $NaHCO_3$ aq., dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (50 g, $CHCl_3$/acetone 2:1) to give **18** (387 mg, 82%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 7.37–7.27 (5H, m; C_6H_5-), 5.61–5.51 (2H, m; $-CH=CH-$), 5.13–5.06 (2H, s; $-CH_2-$Ph), 4.59–4.44 (2H, m; $-C\equiv C-CH-$, $-C(O)O-CH_2-CH-$), 4.02–3.99 (1H, m; $-C(O)O-CH_2-CH-$), 3.89 (1H, brs; $C(O)O-CH_2-CH-$), 3.73–3.65 (2H, m; $-CH_2OH$), 2.24–2.16 ppm (2H, m; $-CH_2-C\equiv C$); elemental analysis calcd (%) for $C_{16}H_{20}N_2O_5\cdot 0.5$ 1,4-dioxane$\cdot 0.5\,H_2O$: C 57.90, H 6.75, N 7.50; found: C 57.87, H 6.62, N 7.43; ESI-TOF-MS (positive): m/z: 321.2 $[M+H]^+$.

(4*S*)-4-[(4*R*)-4-(Benzyloxycarbonyamino)-5-hydroxypentyl]oxazolidine-2-one (19): Compound **18** (123 mg, 0.384 mmol) was dissolved in MeOH (4 mL) and Pd/C (120 mg) was added to the resulting solution. The reaction mixture was stirred for 1 day under a H_2 atmosphere. Pd/C was removed by membrane filtration and the solution was concentrated in vacuo. The residue was dissolved in 1,4-dioxane (2 mL), and then ZCl (82 μL, 0.576 mmol) and $NaHCO_3$ aq. (129 mg, 1.54 mmol, l2 mL) were added. The reaction mixture was stirred for 1.5 h. After this time, the reaction was extracted with $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (7 g, $CHCl_3$/acetone 2:1) to give **19** (97 mg, 79%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$7.35–7.28 (5H, m; C_6H_5-), 5.09 (2H, s; $-CH_2-$Ph), 4.44 (1H, m; $-C(O)O-CH_2-CH-$), 3.96 (1H, m; $-C(O)O-CH_2-CH-$), 3.84 (1H, brs; $C(O)O-CH_2-CH-$), 3.65–3.63 (3H, m; $-CH-CH_2OH$), 1.60–1.25 ppm (6H, m; $-CH_2CH_2CH_2-$); elemental analysis calcd (%) for $C_{16}H_{22}N_2O_5\cdot 0.4$ 1,4-dioxane$\cdot 0.2\,H_2O$: C 58.52, H 7.14, N 7.76; found: C 58.50, H 7.14, N 7.83; ESI-TOF-MS (positive): m/z: 323.2 $[M+H]^+$.

(4*S*)-4-[(4*R*)-4-(Benzyloxycarbonyamino)-4-(carboxy)pentyl]oxazolidine-2-one (20): $RuCl_3\cdot n\,H_2O$ (56 mg, 0.27 mmol) and $NaIO_4$ (1.9 g, 8.98 mmol) were dissolved in acetone (3 mL) and H_2O (3 mL) at −18 °C. A solution of **19** (290 mg, 0.898 mmol) in acetone (3 mL) was then added dropwise and the reaction mixture was stirred for 20 min at −18 °C. After this time, 2-propanol (4 mL) was added to the reaction mixture and it was stirred for a further 1 h. Insoluble matter was removed by Celite filtration, and the filtrate was extracted with $CHCl_3$. The organic layer was washed with 10% citric acid aq. and brine, dried over Na_2SO_4, and then concentrated in vacuo to give crude carboxylic acid **20**.

(4*S*)-4-[(4*R*)-4-(Benzyloxycarbonyamino)-4-(methyloxycarbonyl)pentyl]-oxazolidine-2-one (21): AcCl (639 μL, 8.98 mmol) was added dropwise to MeOH (6 mL) at 0 °C. The solution was stirred for 30 min at RT. After this time, a solution of **20** (0.898 mmol) in MeOH (3 mL) was added and the reaction mixture was stirred for a further 2 h. After this time, the solution was concentrated in vacuo and the residue was purified by using a Biotage column (7 g, $CHCl_3$/acetone 10:1) to give **21** (284 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$7.35–7.28 (5H, m; C_6H_5-), 5.09 (2H, s; $-CH_2-$Ph), 4.44 (1H, m; $-C(O)O-CH_2-CH-$), 3.96 (1H, m; $-C(O)O-CH_2-CH-$), 3.84 (1H, brs; $C(O)O-CH_2-CH-$), 3.65–3.63 (3H, m; $-CH-CH_2OH$), 1.60–1.25 ppm (6H, m; $-CH_2CH_2CH_2-$); elemental analysis calcd (%) for $C_{17}H_{22}N_2O_6\cdot 0.5$ 1,4-dioxane$\cdot 0.5\,H_2O$: C 56.57, H 6.75, N 6.94; found: C 56.57, H 6.63, N 6.91; ESI-TOF-MS (positive): m/z: 351.19 $[M+H]^+$.

(4*S*)-4-[(4*R*)-4-(Benzyloxycarbonyamino)-4-(methyloxycarbonyl)pentyl]-3-*N*-*tert*-butoxycarbonyloxazolidine-2-one (22): Compound **21** (128 mg, 0.37 mmol) was dissolved in THF (4 mL). DMAP (13 mg, 0.11 mmol), triethylamine (102 μL, 0.73 mmol), and Boc_2O (119 μL, 0.55 mmol) were then added to the THF solution, and the reaction mixture was stirred for 1 h. After this time, the reaction was extracted with $CHCl_3$ and the resulting organic layer was washed with 10% citric acid and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (toluene/AcOEt: 3:1) to give **22** (142 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$7.38–7.26 (5H, m; C_6H_5-), 5.29 (1H, d, $J=$7.5 Hz; N*H*), 5.11 (2H, s; $-CH_2-$Ph), 4.39–4.38 (1H, m; $-CH-$COOMe), 4.28 (1H, t, $J=$8.3 Hz; $-CH-CH_2-O-$), 4.20–4.17 (1H, m; $-CH-CH_2-O-$), 3.97 (1H, d, $J=$6.5 Hz; $-N-CH-CH_2-$), 3.74 (3H, s; CH_3), 1.91–1.83 (2H, m; $-CH_2-$), 1.74–1.65 (2H, m; $-CH_2-$), 1.53 ppm (9H, s; $-C(CH_3)_3$); elemental analysis calcd (%) for $C_{22}H_{30}N_2O_8\cdot 0.5$ dioxane$\cdot 0.15\,H_2O$: C 57.97, H 6.95, N 5.63; found: C 57.94, H 6.85, N 6.22; ESI-TOF-MS (positive): m/z: 473.23 $[M+Na]^+$.

Benzyl (2*R*,6*S*)-2-(benzyloxycarbonylamino)-6-(*tert*-butoxycarbonylamino)-7-hydroxylheptanoate (24): $LiOH\cdot H_2O$ aq. (31 mg, 0.74 mmol, 2 mL) was added dropwise to a solution of **22** (341.6 mg, 0.758 mmol) in THF (6 mL), and the resulting mixture was stirred for 1 h. After this time, $LiOH\cdot H_2O$ aq. (31 mg, 0.74 mmol, 2 mL) was again added dropwise to the reaction mixture, and it was stirred for a further 1 h. Then, more $LiOH\cdot H_2O$ aq. (31 mg, 0.74 mmol, 2 mL) was added dropwise to the reaction mixture, and it was stirred for a final 1 h. The reaction mixture was then quenched by Dowex H^+. The Dowex H^+ was removed by filtration and the filtrate was concentrated in vacuo. The residue was freeze-dried with 1,4-dioxane to give crude **23**, which was then dissolved in MeOH (8 mL). Cs_2CO_3 (148 mg, 0.455 mmol) was added to the solution and it was concentrated in vacuo and then co-evaporated with toluene. The residue was dissolved in dry DMF (8 mL) and BnBr (108 μL, 0.910 mmol) was added to the solution. The resulting reaction mixture was stirred for 1 day under an Ar atmosphere. After this time, the reaction mixture was extracted with AcOEt and the resulting organic layer was washed with 10% citric acid aq. and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (toluene/AcOEt 2:1) to give **24** (313 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$7.38–7.29 (10H, m; $C_6H_5\times 2$), 5.40–5.39 (1H, brd; N*H*), 5.19 (1H, d, $J=$12 Hz; $-COOCH_2$Ph), 5.14 (1H, d, $J=$12 Hz; $-COOCH_2$Ph), 5.10 (2H, s; NH$-COOCH_2$Ph), 4.72 (1H, brs; N*H*), 4.45–4.41 (1H, m; $-CH-$COOBn), 3.57–3.46 (3H, m; $-CH-CH_2OH$), 1.87–1.30 (6H, m; $-(CH_2)_3-$), 1.43 ppm (9H, s; $-C(CH_3)_3$); elemental analysis calcd (%) for $C_{27}H_{36}N_2O_7\cdot 0.15\,H_2O$: C 64.43, H 7.27, N 5.57; found: C 64.43, H 7.27, N 5.57; ESI-TOF-MS (positive): m/z: 501.2 $[M+H]^+$.

7-Benzyl (2*R*,6*S*)-2-*N*-benzyloxycarbonyl-6-*N*-*tert*-butoxycarbonylamino-diaminopimelate (25): Compound **24** (301 mg, 0.60 mmol) and PDC (1.1 g, 3.0 mmol) were dissolved in dry DMF (6 mL) and the resulting mixture was stirred for 1 day under an Ar atmosphere. After this time, the reaction mixture was extracted with Et_2O and the resulting organic layer was washed with 10% citric acid aq. and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography ($CHCl_3$/MeOH 10:1 and $CHCl_3$/MeOH/AcOH 5:1:0.01) to give **25** (264 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 7.34–7.27 (10H, m; Ar*H*$\times 2$), 5.42 (1H, brs; NH), 5.19 (1H, d, $J=$12 Hz; $-COOCH_2$Ph), 5.14 (1H, d, $J=$12 Hz; $-COOCH_2$Ph), 5.10 (2H, s; NH$-COOCH_2$Ph), 4.40 (1H, brs; αH), 4.24 (1H, brs; αH), 1.91–1.31 (6H, m; $-(CH_2)_3-$), 1.44 ppm (9H, s; $C(CH_3)_3$); elemental analysis calcd (%) for $C_{27}H_{34}N_2O_8\cdot 0.4\,H_2O$: C 62.15, H 6.72, N 5.37; found: C 62.18, H 6.68, N 5.33; ESI-TOF-MS (positive): m/z: 515.2 $[M+H]^+$.

Protected γ-D-Glu-*meso*-DAP (26): Compound **17** (500 mg, 0.83 mmol) was dissolved in 50% TFA in CH_2Cl_2 (4 mL) and the resulting reaction mixture was stirred for 10 min. After this time, the reaction mixture was concentrated and then co-evaporated in toluene. Addition of 1 M HCl ether solution gave a white solid. The supernatant was removed by decantation to give Boc-deprotected **17**. WSCD·HCl (168 mg, 1.08 mmol) and triethylamine (265 μL, 1.90 mmol) were added to a solution of the Boc-deprotected **17**, benzyl *tert*-butoxycarbonyl glutamate (279 mg, 0.83 mmol), and HOBt (146 mg, 1.08 mmol) in dry THF (8 mL). The reaction mixture was stirred for 1 day under an Ar atmosphere before being extracted with AcOEt. The organic layer was washed with 10% citric acid solution, saturated aq. $NaHCO_3$, and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (toluene/AcOEt 3:1) to give **26** (546 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$7.36–7.28 (20H, m; $-C_6H_5\times 4$), 6.57 (1H, d, $J=$7 Hz; NH), 5.28 (2H, brs; NH $\times 2$), 5.17–5.09 (8H, m; $-CH_2-$Ph $\times 4$), 4.54 (1H, td, $J=$6.88, 5.5 Hz; DAP 2-H), 4.40–4.34 (2H, m; Glu αH, DAP 6-H), 2.25–2.21 (2H, m; Glu-γ-CH_2), 2.18–2.12 (1H, m; Glu-βCH_2), 1.92–1.87 (1H, m; Glu-βCH_2), 1.83–1.58 (4H, m; DAP 3-CH_2, 5-CH_2), 1.41 (9H, s; $-C(CH_3)_3$), 1.39–1.23 ppm (2H, m; DAP 4-CH_2); ESI-TOF-MS (positive): m/z: 824.37 $[M+H]^+$.

Protected L-Ala-γ-D-Glu-*meso*-DAP 27: Compound **26** (304.6 mg, 0.37 mmol) was dissolved in TFA (3 mL) and stirred for 20 min. After

this time, the reaction mixture was concentrated in vacuo and 1 M HCl ether solution was added to the residue to a give white solid. The supernatant was removed by decantation to give Boc-deprotected **26**. WSCD·HCl (75 mg, 0.48 mmol) and triethylamine (129 μL, 0.93 mmol) were added to a solution of Boc-deprotected **26**, *tert*-butoxycarbonyl L-alanine (70 mg, 0.37 mmol), and HOBt (65 mg, 0.48 mmol) in dry THF (4 mL). The reaction was stirred for 3 h under an Ar atmosphere. After this time the reaction mixture was extracted with AcOEt and the resulting organic layer was washed with 10% citric acid solution, saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (toluene/AcOEt 1:1) to give **27** (319 mg, 96%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 7.34–7.27 (20H, m; $-C_6H_5 \times 4$), 7.16 (1H, d, $J = 5.5$ Hz; NH), 5.45 (1H, d, $J = 5$ Hz; NH), 5.16–5.06 (8H, m; $-CH_2-$Ph $\times 4$), 4.93 (1H, brs; NH), 4.53 (1H, dd, $J = 8$, 13 Hz; DAP 2-H), 4.48 (1H, brs; Glu-αH), 4.36 (1H, dd, $J = 8$, 13 Hz; DAP 6-H), 4.10–4.08 (1H, m; Ala-αH), 2.28–2.15 (3H, m; Glu-γH, Glu-βH), 2.04 (1H, brs; Glu-βH), 1.86–1.61 (4H, m; DAP 3-CH_2, 5-CH_2), 1.42 (9H, s; $C(CH_3)_3$, 1.40–1.32 (2H, m; DAP 4-CH_2), 1.26 ppm (3H, d, $J = 5.5$ Hz; Ala-βCH_3); ESI-TOF-MS (positive): m/z: 895.4 $[M+H]^+$.

Protected H-L-Ala-γ-D-Glu-*meso*-DAP 28: Compound **27** (46.5 mg, 0.052 mmol) was dissolved in TFA (400 μL) and the resulting mixture was stirred for 25 min. After this time, the reaction mixture was concentrated in vacuo and 1 M HCl ether solution was added to the residue to give a white solid. The supernatant was removed by decantation to give **28** (0.052 mmol). The compound was used without further purification.

Protected *meso*-DAP-D-Ala 29: WSCD·HCl (116 mg, 0.746 mmol) and triethylamine (200 μL, 1.44 mmol) were added to a solution of **25** (295 mg, 0.574 mmol), HCl·H-L-Ala-OBn (136 mg, 0.631 mmol), and HOBt (101 mg, 0.746 mmol) in dry THF (6 mL). The reaction mixture was stirred for 1 day under an Ar atmosphere. After this time, the reaction mixture was extracted with AcOEt and the resulting organic layer was washed with 10% citric acid solution, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash column chromatography ($CHCl_3$/acetone 20:1) to give **29** (347,7 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 7.36–7.28 (15H, m; $C_6H_5 \times 3$), 6.63 (1H, brs; NH), 5.39 (1H, d, $J =$ 7.5 Hz; NH), 5.19–5.07 (6H, m; $-CH_2-$Ph $\times 3$), 4.96 (1H, brs; NH), 4.58 (1H, m; DAP 2-H), 4.39 (1H, d, $J = 5.5$ Hz; DAP 6-H), 4.05 (1H, brs; Ala-αH), 1.89–1.53 (6H, m; DAP $-(CH_2)_3-$), 1.43 (9H, s; $-C(CH_3)_3$), 1.39 ppm (3H, d, $J = 7.5$ Hz; Ala-βCH_3); elemental analysis calcd (%) for $C_{37}H_{45}N_3O_9 \cdot 0.6\,H_2O$: C 64.73, H 6.78, N 6.12; found: C 64.33, H 6.42, N 6.39; ESI-TOF-MS (positive): m/z: 676.3 $[M+H]^+$.

Protected H-L-Ala-γ-D-Glu-*meso*-DAP-D-Ala 30: Compound **29** (328 mg, 0.486 mmol) was dissolved in TFA (4 mL) and the mixture was stirred for 1 h. After this time, the reaction mixture was concentrated and then co-evapolated in toluene (×2). 1 M HCl ether solution was added to the residue and the mixture was evaporated in vacuo. The residue was freeze-dried by using 1,4-dioxane to give an oil. WSCD·HCl (98 mg, 0.632 mmol) and triethylamine (169 μL, 1.22 mmol) were added to a solution of the oil, Boc-D-Glu-OBn (180 mg, 0.535 mmol), and HOBt (85 mg, 0.632 mmol) in dry THF (5 mL). The reaction mixture was stirred for 24 h under an Ar atmosphere, after which time, it was extracted with AcOEt. The organic layer was washed with 10% citric acid solution, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash column chromatography ($CHCl_3$/acetone 10:1) to give fully protected γ-D-Glu-*meso*-DAP-D-Ala (416.4 mg, 96%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 7.38–7.27 (20H, m; −Ph $\times 4$), 7.01 (1H, brs; NH), 6.22 (1H, brs; NH), 5.44 (1H, brs; NH), 5.31 (1H, brs; NH), 5.19–5.06 (8H, m; $-CH_2-$Ph $\times 4$), 4.56 (1H, m; DAP 2-H), 4.38–4.30 (3H, m; Ala-αH, Glu-αH, DAP 6-H), 2.28–2.15 (3H, m; Glu-γH, Glu-βH), 1.88–1.64 (5H, m; Glu-βH, DAP 3-CH_2, 5-CH_2), 1.40 (9H, s; $-C(CH_3)_3$), 1.35 (3H, d, $J = 7$ Hz; Ala-βCH_3), 1.27–1.23 ppm (2H, m; DAP-4-CH_2); elemental analysis calcd (%) for $C_{49}H_{58}N_4O_{12} \cdot H_2O$: C 64.46, H 6.62, N 6.14; found: C 64.66, H 6.54, N 6.31; ESI-TOF-MS (positive): m/z: 895.4 $[M+H]^+$.

The fully protected γ-D-Glu-*meso*-DAP-D-Ala (322 mg, 0.36 mmol) was dissolved in TFA (2 mL) and stirred for 40 min. The reaction mixture was concentrated and co-evaporated in toluene (×2). 1 M HCl ether solution was added to the residue to give white solid, and the supernatant was removed by decantation. WSCD·HCl (73 mg, 0.468 mmol) and triethylamine (125 μL, 0.900 mmol) were added to a solution of the residue, Boc-L-Ala-OBn (75 mg, 0.396 mmol), and HOBt (63 mg, 0.468 mmol) in dry DMF (4 mL). The reaction was stirred for 1 day under an Ar atmosphere. The reaction was extracted with AcOEt and the resulting organic layer was washed with 10% citric acid solution, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash column chromatography ($CHCl_3$/acetone 5:1) to give fully protected L-Ala-γ-D-Glu-*meso*-DAP-D-Ala (277 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.36$–7.28 (20H, m; $C_6H_5 \times 4$), 7.05 (1H, brs; NH), 7.05 (1H, brs; NH), 5.43 (1H, d, $J = 6$ Hz; NH), 5.18–5.05 (8H, m; $-CH_2-$Ph $\times 4$), 4.91 (1H, brs; NH), 4.57 (1H, m, $J = 7.5$ Hz; DAP 2-H), 4.51–4.36 (3H, m; Glu-αH, D-Ala-αH, DAP 6-H), 4.12–4.05 (1H, m; L-Ala-αH), 2.40 (1H, brs; NH), 2.25–2.15 (3H, m; Glu-γCH_2, Glu-βCH_2), 1.94–1.63 (5H, m; Glu-βCH_2, DAP 3-CH_2, 5-CH_2), 1.45–1.21 (2H, m; DAP 4-CH_2), 1.42 (9H, s; $C(CH_3)_3$), 1.37 (3H, d, $J = 7$ Hz; D-Ala-βCH_3), 1.27 (3H, d, $J = 7$ Hz; L-Ala-βH); elemental analysis calcd (%) for $C_{52}H_{63}N_5O_{13} \cdot H_2O$: C 63.46, H 6.66, N 7.12; found: C 63.55, H 6.52, N 7.33; ESI-TOF-MS (positive): m/z: 966.4 $[M+H]^+$.

The fully-protected L-Ala-γ-D-Glu-*meso*-DAP-D-Ala (28 mg, 0.0285 mmol) was dissolved in TFA (200 μL) and stirred for 20 min. The reaction mixture was concentrated in vacuo and 1 M HCl ether solution was added to the resulting residue to give a white solid. The supernatant was removed by decantation to give **30** (0.0285 mmol). The compound was used without further purification.

1,6-Anhydro-2-azide-4-*O*-benzyl-2-deoxy-3-*O*-[(1*R*)-1-(ethyoxycarbonyl)-ethyl]-β-D-glucopyranose (32): Tf_2O (5.1 mL, 0.027 mol) was added dropwise to a solution of (*S*)-LacOEt (3.2 g, 0.027 mol) and 2,6-lutidine (3 mL, 0.027 mol) in CH_2Cl_2 (50 mL) at −70 °C under an Ar atmosphere. The reaction mixture was stirred for 2 h at RT. Hexane (30 mL) was added to the solution, and the mixture was purified by silica-gel column chromatography (silica-gel 150 g, hexane/CH_2Cl_2 1:1) to give lactic triflate. NaH (1.3 g, 0.027 mol) was added to the CH_2Cl_2 solution of **31** (5 g, 0.018 mol) under an Ar atmosphere, and the mixture was stirred for 15 min. After this time, lactic triflate was added to the reaction mixture and it was stirred for a further 1 h. The reaction was quenched with ice and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography ($CHCl_3$/acetone 100:1) to give **32** (5.2 g, 78%); ESI-TOF-MS (positive): m/z: 777.32 $[2M+Na]^+$.

2-Acetylamino-1,6-anhydro-2-deoxy-4-*O*-benzyl-3-*O*-[(1*R*)-1-(ethyoxycarbonyl)ethyl]-β-D-glucopyranose (33): Zn powder was added to a solution of **32** (1 g, 2.65 mmol) in AcOH/THF 1:1 (4 mL) at 0 °C, and the resulting mixture was stirred for 10 min. After this time, the Zn powder was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in Ac_2O/pyr 1:1 (20 mL), and the reaction mixture was stirred for a further 45 min. The reaction mixture was evaporated in vacuo and the residue was extracted with AcOEt. The organic layer was washed with 1 M HCl aq. and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (toluene/AcOEt 2:1) to give **33** (786 mg, 79%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.39$–7.33 (5H, m; Ar*H*), 6.30 (1H, d, $J = 8.5$ Hz; NH), 5.35 (1H, s; H-1), 4.68 (1H, d, $J = 12$ Hz; PhCH_2-O−), 4.59 (1H, d, $J = 12$ Hz; PhCH_2-O−), 4.56 (1H, s; H-5), 4.24–4.18 (3H, m; Lac-αH, $-COOCH_2CH_3$), 3.72 (1H, dd, $J = 6.7$ Hz; H-6′), 3.48–3.46 (1H, m; H-3), 3.42 (1H, s; H-4), 1.94 (3H, s; NHAc), 1.38 (3H, d, $J = 7$ Hz; Lac-βCH_3), 1.29 ppm (3H, t, $J = 7$ Hz; $-COOCH_2CH_3$); ESI-TOF-MS (positive): m/z: 394.1 $[M+H]^+$.

2-Acetylamino-1,6-anhydro-2-deoxy-4-*O*-benzyl-3-*O*-[(1*R*)-1-(carboxyl)-ethyl]-β-D-glucopyranose (34): Compound **33** (75 mg, 0.19 mmol) was dissolved in THF/1,4-dioxane/H_2O 2:1:0.5 (2 mL). LiOH·H_2O (9 mg, 0.21 mmol) was added to the reaction mixture and it was stirred for 1.5 h. After this time, the reaction mixture was quenched with Dowex H^+, which was subsequently removed by filtration. The mixture was then concentrated in vacuo and the resulting residue was purified by silica-gel flash column chromatography ($CHCl_3$/MeOH 20:1) to give **34** (30.1 mg,

44%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.43–7.33 (5H, m; ArH), 6.14 (1H, d, J=5 Hz; NH), 5.45 (1H, s; H-1), 4.68 (1H, d, J=12 Hz; $PhCH_2$–O–), 4.66–4.64 (1H, m; H-5), 4.59 (1H, d, J=12 Hz; $PhCH_2$–O–), 4.26–4.21 (1H, m; Lac-αH), 4.18 (1H, d, J=7.5 Hz; H-6), 4.19–4.11 (1H, m; H-2), 3.80–3.76 (1H, m; H-6′), 3.55 (1H, s; H-3), 3.44 (1H, s; H-4), 1.95 (3H, s; NHAc), 1.39 ppm (3H, s; Lac-βCH_3); ESI-TOF-MS (positive): m/z: 364.1 $[M-H]^-$.

2-Trichloroethoxycarbonylamino-1,6-anhydro-2-deoxy-4-*O*-[(1*R*)-1-(ethyoxycarbonyl)ethyl]-β-D-glucopyranose (36): $Pd(OH)_2$ (60 mg) was added to a solution of **32** (50.7 mg, 0.13 mmol) in THF (1 mL) and the reaction mixture was stirred for 2 days under a H_2 atmosphere (15 kg cm^{-2}). After this time, the $Pd(OH)_2$ was removed by membrane filtration, and the filtrate was concentrated in vacuo. The resulting residue, TrocCl (22 μL, 0.16 mmol), and triethylamine (28 μL, 0.20 mmol) were then dissolved in CH_2Cl_2 (1 mL) and stirred for 15 min. The reaction mixture was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (toluene/AcOEt 5:1) to give **36** (44.4 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.67 (1H, d, J=9 Hz; H-1), 5.44 (1H, s; N*H*), 4.73 (2H, dd, J=12, 21 Hz; $-CH_2-CCl_3$), 4.52 (1H, brs; H-5), 4.24–4.18 (4H, m; Lac-αH, $-COOCH_2CH_3$, H-6), 3.91 (1H, d, J=9.2 Hz; H-2), 3.80–3.76 (2H, m; H-6′, H-3), 3.50 ppm (1H, brs; H-4); ESI-TOF-MS (positive): m/z: 526.13 $[M+H]^+$.

1,6-Anhydro-4-*O*-[3′-*O*-benzyl-4′,6′-*O*-benzylidene-2′-deoxy-2′-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-3-*O*-[(*R*)-1-(ethoxycarbonyl)ethyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranoside (38): TMSOTf (5 mL, 0.030 mmol) was added to the solution of glucosamine imidate **37** (200 mg, 0.296 mmol), **36** (193.6 mg, 0.443 mmol), and MS (4 Å) in dry CH_2Cl_2 (3 mL) at −17 °C, and the mixture was stirred for 30 min under an Ar atmosphere. After this time, the reaction was quenched with saturated aqueous $NaHCO_3$ and the organic layer was washed with brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (toluene/AcOEt 7:1) to give **38** (182 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.52–7.18 (10H, m; Ar*H* ×2), 5.60 (1H, s; Ph–C*H*=O_2), 5.33 (1H, s; H_{anh}-1), 5.03 (1H, d, J=7.7 Hz; N*H*), 4.91 (1H, d, J=12 Hz; –O–CH_2–Ph), 4.82 (1H, d, J=8 Hz; H-1), 4.76 (2H, dd, J=12, 20 Hz; $-CH_2-CCl_3$), 4.7 (1H, d, J=12 Hz; –O–CH_2–Ph), 4.60 (1H, d, J=12 Hz; N*H*), 4.49 (1H, d, J=5.7 Hz; H_{anh}-5), 4.33 (1H, dd, J=5, 10 Hz; H-6), 4.22 (1H, q, J=3.6 Hz; Lac-αH), 4.20–4.14 (3H, m; $-CH_2CH_3$, H_{anh}-6′), 3.95 (1H, d, J=9.8 Hz; H_{anh}-2), 3.86–3.76 (3H, m; H-3, H-6, H-4), 3.74–3.72 (2H, m; H_{anh}-6′, H_{anh}-4), 3.58 (1H, brs; H_{anh}-3), 3.52–3.42 ppm (2H, m; H-2, H-5); elemental analysis calcd (%) for $C_{37}H_{42}Cl_6N_2O_{14}$: C 46.71, H 4.45, N 2.94; found: C 47.02, H 4.42, N 2.96; ESI-TOF-MS (positive): m/z: 949.17 $[M+H]^+$.

1,6-Anhydro-4-*O*-(3′-*O*-benzyl-4′,6′-*O*-benzylidene-2′-deoxy-2′-acetylamino-β-D-glucopyranosyl)-3-*O*-[(*R*)-1-(ethoxycarbonyl)ethyl-2-deoxy-2-acetylamino]-D-glucopyranoside (39): Compound **38** (40.6 mg, 0.0427 mmol) was dissolved in Ac_2O/AcOH/THF 1:1:1 (600 μL). Zn/Cu was added and the mixture was stirred for 1 h. After this time, the Zn/Cu was removed by filtration and the filtrate was concentrated in vacuo. The residue was extracted with AcOEt and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash column chromatography ($CHCl_3$/acetone 3:1) to give **39** (23.5 mg, 81%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.51–7.31 (10H, m; Ar*H* ×2), 6.77 (1H, d, J=9.5 Hz; NH), 5.61 (1H, s; Ph–C*H*=O_2), 5.25 (1H, s; H_{anh}-1), 5.07 (1H, d, J=8 Hz; NH), 4.91 (1H, d, J=12 Hz; –O–CH_2–Ph), 4.67 (1H, d, J=12 Hz; –O–CH_2–Ph), 4.54 (1H, d, J=8.5 Hz; H-1), 4.41 (1H, d, J=5 Hz; H_{anh}-5), 4.34 (1H, dd, J=5, 10.5 Hz; H-6), 4.24–4.16 (5H, m; H_{anh}-2, H_{anh}-6, Lac-αH, $-COOCH_2CH_3$), 3.92 (1H, dd, J=8.5, 13.5 Hz; H-2), 3.82 (1H, t, J=10.5 Hz; H-6′), 3.78 (1H, t, J=9.3 Hz; H-4), 3.72 (1H, s; H_{anh}-6′), 3.69 (1H, t; H_{anh}-4), 3.63 (1H, t, J=9.7 Hz; H-3), 3.46 (1H, s; H_{anh}-3), 3.42–3.37 (1H, m; H-5), 2.09 (3H, s; NHAc), 1.90 (3H, s; NHAc), 1.39 (3H, d, J=7 Hz; Lac-βCH_3), 1.27 (3H, t, J=3.8 Hz; $-COOCH_2CH_3$); elemental analysis calcd for $C_{35}H_{44}N_2O_{12}\cdot 0.4H_2O$: C 64.33, H 6.81, N 3.33; found: C 64.38, H 6.82, N 3.22; ESI-TOF-MS (positive): m/z: 685.35 $[M+H]^+$.

1,6-Anhydro-4-*O*-(3′-*O*-benzyl-4′,6′-*O*-benzylidene-2′-deoxy-2′-acetylamino-β-D-glucopyranosyl)-3-*O*-[(*R*)-1-(carbonyloxy)ethyl-2-deoxy-2-acetylamino]-D-glucopyranoside (40): Compound **39** (55.2 mg, 0.0806 mmol) was dissolved in THF/1,4-dioxane/H_2O 2:1:0.5. LiOH·H_2O (3.7 mg, 0.0887 mmol) was then added to the solution and the reaction mixture was stirred for 2 h. After this time, the reaction mixture was quenched with Dowex H^+, which was subsequently removed by filtration. The mixture was then concentrated in vacuo and the resulting residue was applied to an HP-20 column (2×8 cm). Organic and inorganic salts were removed by elution with H_2O, followed elution with MeOH to give **40** (45.5 mg, 88%).

Protected disaccharide(anh) tetrapeptide 41: WSCD·HCl (7 mg, 0.043 mmol) and triethylamine (14 μL, 0.098 mmol) were added to a solution of **40** (21.5 mg, 0.033 mmol), tetrapeptide **30**, and HOBt (6 mg, 0.043 mmol) in dry DMF (700 μL). The reaction mixture was stirred for 1 day under an Ar atmosphere. After this time, the reaction mixture was extracted with AcOEt and the organic layer was washed with 10% citric acid solution, saturated aq. $NaHCO_3$, and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash column chromatography ($CHCl_3$/acetone 3:2) to give **41** (45.3 mg, 92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.84 (1H, d, J=6 Hz; NH), 7.43–7.27 (30H, m; Ar*H*×6), 7.08 (1H, d, J=8 Hz; NH), 6.93 (1H, d, J=10.5 Hz; NH), 5.68 (1H, d, J=8 Hz; NH), 5.59 (1H, s; Ph–C*H*=O_2), 5.30 (1H, s; H_{anh}-1), 5.17–5.05 (10H, m; $-CH_2-Ph$), 4.91 (1H, d, J=12.5 Hz; $-OCH_2Ph$), 4.68 (1H, d, J=12 Hz; $-OCH_2Ph$), 4.53 (1H, m, J=7 Hz; D-Ala-αH), 4.45–4.42 (2H, m; H_{anh}-5, H-1), 4.38–4.28 (5H, m; H-6, Lac-αH, Glu-αH, DAP 2-H, DAP 6-H), 4.22 (1H, d, J=7 Hz; H_{anh}-6), 4.12–4.06 (2H, m; H_{anh}-2, L-Ala-αH), 4.03–3.98 (1H, m; H-2), 3.82–3.70 (4H, m; H-6′; H-4, H_{anh}-6′, H_{anh}-4), 3.55 (1H, t, J=9 Hz; H-3), 3.46 (1H, s; H_{anh}-3), 3.39–3.35 (1H, m; H-5), 2.41–2.34 (1H, m; Glu-γCH_2), 2.24–2.19 (1H, m; Glu-γCH_2), 2.17–2.11 (1H, m; Glu-βCH_2), 2.10 (3H, s; NHAc), 1.97–1.94 (1H, m; Glu-βCH_2), 1.91 (3H, s; NHAc), 1.88–1.61 (4H, m; DAP 3-CH_2, DAP 5-CH_2), 1.49–1.37 (2H, m; DAP 4-CH_2), 1.35 (6H, d, J=7 Hz; Lac-βCH_3, D-Ala-βCH_3), 1.30 ppm (3H, d, J=6.5 Hz; L-Ala-βCH_3); ESI-TOF-MS (positive): m/z: 1504.61 $[M+H]^+$; HRMS-ESI QTOF-MS (positive): m/z: calcd for $C_{80}H_{93}N_7O_{22}$: 1526.6272 $[M+Na]^+$; found: 1526.6249.

Protected disaccharide(anh) tripeptide 42: Compound **42** was synthesized from **40** and **28**, and monosaccharide(anh) tetrapeptides **43** and **44** were synthesized from **34** and **30** (for **43**) or **28** (for **44**), in a manner similar to the synthesis of **41**.

*Compound **42***: ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.82 (1H, d, J=6.5 Hz; NH), 7.42–7.27 (30H, m; Ar*H*×6), 6.94 (1H, d, J=9.5 Hz; NH), 5.72 (1H, d, J=8 Hz; NH), 5.59 (1H, s; Ph-C*H*=O_2), 5.30 (1H, s; H_{anh}-1), 5.15–5.05 (8H, m; $-COO-CH_2-Ph$ ×4), 4.91 (1H, d, J=12.5 Hz; –O–CH_2–Ph), 4.68 (1H, d, J=12.5 Hz; –O–CH_2–Ph), 4.47 (1H, td, J=8, 5 Hz; DAP 2-H), 4.43–4.40 (2H, m; H_{anh}-5, H-1), 4.35–4.25 (4H, m; H-6, Glu-αH, Lac-αH, DAP 6-H), 4.22 (1H, d, J=7.5 Hz; H_{anh}-6), 4.10–4.06 (2H, m; H_{anh}-2, Ala-αH), 4.00 (1H, q, J=9 Hz; H-2), 3.81–3.68 (4H, m; H-6′, H-4, H_{anh}-6′, H_{anh}-4), 3.54 (1H, t, J=9.5 Hz; H-3), 3.46 (1H, s; H_{anh}-3), 3.40–3.35 (1H, m; H-5), 2.30–2.12 (4H, m; Glu-γCH_2, Glu-βCH_2), 2.09 (3H, s; NHAc), 1.91 (3H, s; NHAc), 1.86–1.62 (4H, m; DAP 3-CH_2, DAP 5-CH_2), 1.44–1.39 (2H, m; DAP 4-CH_2), 1.33 (3H, d, J=7 Hz; Lac-βCH_3), 1.29 ppm (3H, d, J=6.5 Hz; Ala-βCH_3); ESI-TOF-MS (positive): m/z: 1434.11 $[M+H]^+$.

Disaccharide(anh) tetrapeptide (TCT, tracheal cytotoxin; 5): $Pd(OH)_2$ (20 mg) was added to a solution of **41** (5.3 mg, 3.52×10^{-6} mol) in THF (300 μL). The reaction mixture was stirred for 1 day under a H_2 atmosphere (20 kg cm^{-2}). After this time, the $Pd(OH)_2$ was removed by membrane filtration, and the filtrate was concentrated in vacuo to give **5** (3.2 mg, quant.). ESI-TOF-MS (positive): m/z: 920.4 $[M-H]^-$; HRMS-ESI QTOF-MS (positive): m/z: calcd for $C_{29}H_{48}N_6O_{16}$: 922.3893 $[M+H]^+$; found: 922.3898.

Disaccharide(anh) tripeptide 6 and monosaccharide(anh) tetrapeptides 7 and 8: Compounds **6**, **7**, and **8** were synthesized from **42**, **43**, and **44**, respectively, in a manner similar to the synthesis of **5**.

Compound **6**: ^{1}H NMR (500 MHz, CD_3OH): $\delta = 5.32$ (1H, s; H_{anh}-1), 4.62 (1H, d, $J = 5.5$ Hz; H-6), 4.51 (1H, d, $J = 8.5$ Hz; H-6′), 4.47–4.42 (1H, m; Lac-αH), 4.40–4.37 (2H, m; Glu-αH, DAP 2-H), 4.30 (1H, d, $J = 7$ Hz; H-1), 4.12 (1H, q, $J = 6.5$ Hz; Ala-αH), 3.98 (1H, s; H_{anh}-2), 3.91- 3.86 (2H, m; H_{anh}-4, H_{anh}-5), 3.79–3.72 (3H, m; H-2, H-5, DAP 6-CH_2), 3.71–3.68 (1H, m; H_{anh}-6), 3.55 (1H, brs; H_{anh}-3), 3.47–3.44 (1H, m; H-4), 3.36–3.34 (1H, m; H_{anh}-6), 3.32–3.30 (1H, m; H-3), 2.38–2.33 (2H, m; Glu-γCH_2), 2.23–2.15 (1H, m; Glu-βCH_2), 2.07 (3H, s; NHAc), 2.03 (3H, s; NHAc), 1.99–1.92 (1H, m; Glu-βCH_2), 1.87–1.71 (4H, m; DAP 3-CH_2, DAP 5-CH_2), 1.57–1.54 (2H, m; DAP 4-CH_2), 1.42–1.36 ppm (6H, m; Lac-βCH_3, Ala-βCH_3); HRMS-ESI QTOF-MS (positive): *m*/*z*: calcd for $C_{29}H_{48}N_6O_{16}$: 851.3522 $[M+H]^+$; found: 851.3531.

1-Propenyl-2-acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-1-(ethoxycarbonyl)ethyl]-α-D-glucopyranoside (46): H_2 activated [Ir(cod)-$(MePh_2P)_2]PF_6$ (38 mg, 0.0445 mmol) in dry THF (2 mL) was added to a solution of **45** (200 mg, 0.445 mmol) in dry THF (2 mL). After the reaction mixture had been stirred under an Ar atmosphere at RT for 45 min, it was extracted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica-gel flash column chromatography ($CHCl_3$/acetone 50:1) to give **46** (182 mg, 91%). ESI-TOF-MS (positive): *m*/*z*: 450.2 $[M+H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.47$–7.36 (5H, m; ArH), 6.10 (1H, dd, $J = 12$, 1.7 Hz; −O−C*H*=CH−), 5.59 (1H, s; Ph−C*H*=O_2), 5.52 (1H, d, $J = 3$ Hz; H-1), 5.14–5.07 (1H, m, $J = 7$ Hz; −O−CH=C*H*−), 4.51 (1H, q, $J = 7$ Hz; Lac-αH), 4.28–4.15 (4H, m; H-4, H-6, −COO$CH_2$$CH_3$), 3.89–3.68 (4H, m; H-2, H-3, H-5, H-6′), 2.05 (3H, s; NHAc), 1.54 (3H, dd, $J = 7$, 1.5 Hz; −CH=CH−CH_3), 1.43 (3H, d, $J = 7$ Hz; Lac-βCH_3), 1.29 (3H, t, $J = 7$ Hz; −COO$CH_2$$CH_3$).

1-Propenyl-2-acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*)-1-(carbonyloxy)ethyl]-α-D-glucopyranoside (47): Compound **46** (93.9 mg, 0.209 mmol) was dissolved in THF/1,4-dioxane/H_2O 2:1:0.5 (2.1 mL). LiOH·H_2O (10 mg) was added to the solution and the resulting reaction mixture was stirred for 1 h. After this time, the reaction was quenched with Dowex H^+, which was subsequently removed by filtration. The mixture was then concentrated in vacuo and the resulting residue was purified by silica-gel flash column chromatography ($CHCl_3$/MeOH 10:1) to give **47** (90 mg, quant.).

Protected monosaccharide tetrapeptide 48 and protected monosaccharide tripeptide 49: Compounds **48** and **49** were synthesized from **47** and **30** (for **48**) or **28** (for **49**), in a manner similar to the synthesis of **41**.

Compound **48**: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.46$–7.23 (10H, m; ArH), 5.56–5.54 (2H, brs; Ph−C*H*=O_2; H-1), 5.17–5.03 (9H, m; −O−CH=C*H*−, −CH_2−Ph ×4), 4.56–4.06 (9H, m; Glu-αH, Lac-αH, DAP 2-H, DAP 4-H, Ala-αH, D-Ala-αH, H-5, H-2, −O−C*H*=CH−), 3.87–3.60 (4H, m; H-4, H-3, H-6, H-6′), 2.17–1.81 (4H, m; Glu-βCH_2, Glu-γCH_2), 1.89 (3H, s; NHAc), 1.84–1.34 (11H, m; DAP 3-CH_2, DAP 5-CH_2, DAP 4-CH_2, Lac-βCH_3, Ala-βH, D-Ala-βH), 1.52 ppm (3H, dd, $J = 7$, 1.5 Hz; −O−C*H*=CH−CH_3); ESI-TOF-MS (positive): *m*/*z*: 1269.49 $[M+H]^+$; HRMS-ESI QTOF-MS (positive): *m*/*z*: calcd for $C_{68}H_{80}N_6O_{18}$: 1291.5427 $[M+Na]^+$; found: 1291.5427.

Compound **49**: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.37$–7.28 (10H, m; ArH), 5.56 (1H, s; Ph−C*H*=O_2), 5.51 (1H, d, $J = 8$ Hz; H-1), 5.12–5.08 (1H, m; −O−CH=C*H*−), 5.12–4.38 (4H, m; Glu-αH, Lac-αH, DAP 2-H, DAP 4-H), 4.34–4.14 (5H, m; H-6, H-2, H-4, −O−C*H*=CH−, Ala-αH), 3.84–3.69 (2H, m; H-5, H-6′), 2.28–2.06 (4H, m; Glu-βCH_2, Glu-γCH_2), 1.89 (3H, s; NHAc), 1.83–1.59 (4H, m; DAP 3-CH_2, DAP 5-CH_2), 1.52 (3H, dd, $J = 7$, 1.5 Hz; −O−CH=CH−CH_3), 1.40–1.38 (2H, m; DAP 4-CH_2), 1.38 (3H, d, $J = 7$ Hz; Lac-βCH_3), 1.35 (3H, d, $J = 6.5$ Hz; Ala-βH); ESI-TOF-MS (positive): *m*/*z*: 1198.53 $[M+H]^+$; HRMS-ESI QTOF-MS (positive): *m*/*z*: calcd for $C_{65}H_{75}N_5O_{17}$: 1220.5056 $[M+Na]^+$; found: 1220.5055.

1-*O*-Deprotected monosaccharide tetrapeptide 50: Compound **48** (4.6 mg, 3.62×10^{-6} mol) was suspended in THF/H_2O 2:1 (360 μL). I_2 (2 mg, 7.25×10^{-6} mol) was then added to the solution, and the reaction mixture was stirred for 30 min. The reaction was quenched by 10% $Na_2S_2O_4$ aq. and the mixture was extracted with AcOEt. The organic layer was washed with 10% $Na_2S_2O_4$ aq., saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography ($CHCl_3$/MeOH 30:1) to give **50** (4.6 mg, quant.). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.39$–7.27 (20H, m; ArH), 5.56 (1H, s; Ph−C*H*=O_2), 5.26 (1H, d, $J = 3$ Hz; H-1), 5.17–5.04 (8H, m; −COO−CH_2−Ph ×4), 4.51–4.19 (7H, m; Glu-αH, DAP 2-H, DAP 6-H, Lac-αH, Ala-αH, D-Ala-αH, H-4, H-2), 4.14–4.11 (1H, m; H-2), 3.79–3.63 (3H, m; H-6, H-6′, H-3), 3.41–3.40 (1H, m; H-5), 2.35–1.79 (4H, m; Glu-βCH_2, Glu-γ CH_2), 1.65–1.57 (4H, m; DAP 3-CH_2, DAP 5-CH_2), 1.95 (3H, s; NHAc), 1.39–1.32 ppm (8H, m; DAP 4-CH_2, Lac-βCH_3, Ala-βCH_3); HRMS-ESI QTOF-MS (positive): *m*/*z*: calcd for $C_{65}H_{76}N_6O_{18}$: 1251.5114 $[M+Na]^+$; found: 1251.5114.

1-*O*-Deprotected monosaccharide tripeptide 51: Compound **49** (5.6 mg, 4.67×10^{-6} mol) was suspended in THF/H_2O 2:1 (450 μL). I_2 (3 mg, 9.35×10^{-6} mol) was added to the solution, and the reaction mixture was stirred for 30 min. After this time, the reaction mixture was quenched by 10% $Na_2S_2O_4$ aq. and the mixture was extracted with AcOEt. The organic layer was washed with 10% $Na_2S_2O_4$ aq., saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography ($CHCl_3$/MeOH 30:1) to give **51** (4.1 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.35$–7.26 (20H, m; ArH), 5.56 (1H, s; Ph−C*H*=O_2), 5.34 (1H, s; H-1), 5.18–5.05 (8H, m; −COO−CH_2−Ph ×4), 4.51–4.15 (7H, m; Glu-αH, DAP 2-H, DAP 6-H, Lac-αH, Ala-αH, H-4, H-2), 3.82–3.61 (3H, m; H-6, H-6′, H-3), 3.44–3.41 (1H, m; H-5), 2.35–1.66 (8H, m; Glu-βCH_2, Glu-γ CH_2, DAP 3-CH_2, DAP 4-CH_2, DAP 5-CH_2), 1.95 (3H, s; NHAc), 1.38–1.32 ppm (6H, m; Lac-βCH_3, Ala-βCH_3); ESI-TOF-MS (positive): *m*/*z*: 1158.50 $[M+H]^+$; HRMS-ESI QTOF-MS (positive): *m*/*z*: calcd for $C_{62}H_{71}N_5O_{17}$: 1180.4743 $[M+Na]^+$; found: 1180.4746.

Monosaccharide tetrapeptide 3 and monosaccharide tripeptide 4: Compounds **3** and **4** were synthesized from **50** and **51**, respectively, in a manner similar to the synthesis of **5**.

Compound **3**: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 5.10$ (1H, d, $J = 4$ Hz; H-1), 4.27–4.19 (5H, m; Glu-αH, DAP 2-H, Ala-αH, D-Ala-αH, Lac-αH), 3.90 (1H, dd, $J = 3.5$, 11 Hz; H-2), 3.83–3.80 (1H, m; H-5), 3.77–3.67 (3H, m; DAP-αH, H-6, H-3), 3.53–3.45 (2H, m; H-4, H-6′), 2.30–2.27 (2H, m; Glu-γCH_2), 2.14–1.69 (8H, m; Glu-βCH_2, DAP 3-CH_2, 5-CH_2), 1.92 (3H, s; NHAc), 1.43–1.39 (2H, m; DAP 4-CH_2), 1.37 (3H, d, $J = 7$ Hz; Lac-βH), 1.34–1.30 ppm (6H, m; Ala-βH, D-Ala-βH); ESI-TOF-MS (negative): *m*/*z*: 735.30 $[M+H]^-$; HRMS-ESI QTOF-MS (positive): *m*/*z*: calcd for $C_{29}H_{48}N_6O_{16}$: 737.3205 $[M+Na]^+$; found: 737.3209.

Compound **4**: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 5.10$ (1H, d, $J = 3.5$ Hz; H-1), 4.28–4.21 (4H, m; Glu-αH, DAP-αH, Ala-αH, Lac-αH), 3.90 (1H, dd, $J = 3.5$, 10.5 Hz; H-2), 3.85–3.79 (1H, m; H-5), 3.77–3.70 (1H, m; DAP-αH, H-6), 3.63 (1H, d, $J = 10.5$ Hz; H-3), 3.53–3.45 (2H, m; H-4, H-6′), 2.32–2.21 (2H, m; Glu-γCH_2), 2.18–1.67 (6H, m; Glu-βCH_2, DAP 3-CH_2, 5-CH_2), 1.92 (3H, s; NHAc), 1.43–1.40 (2H, m; DAP 4-CH_2), 1.36 (3H, d, $J = 7.5$ Hz; Lac-βH), 1.32 ppm (3H, d, $J = 6.5$ Hz; Ala-βH); ESI-TOF-MS (negative): *m*/*z*: 664.26 $[M-H]^-$; HRMS-ESI QTOF-MS (positive): *m*/*z*: calcd for $C_{26}H_{43}N_5O_{15}$: 666.2834 $[M+Na]^+$; found: 666.2837.

1-Allyl 2-acetylamino-6-*O*-benzyl-4-*O*-(2′-acetylamino-3′-*O*-benzyl-4′,6′-*O*-benzylidene-2′-deoxy-β-D-glucopyranosyl)-2-deoxy-3-*O*-[(*R*)-1-(ethoxycarbonyl)ethyl]-α-D-glucopyranoside (53): Zn/Cu (three microspatula spoonfuls) was added to a solution of **52** (200 mg, 0.182 mmol) in THF/AcOH/Ac_2O 1:1:1 (2 mL), and the mixture was stirred at RT for 3.5 h. After this time, the insoluble materials were filtered off and the filtrate was concentrated in vacuo. The residue was extracted with AcOEt and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (toluene/AcOEt 2:1) to give **53** (69 mg, 46%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.44$–7.28 (15H, m; ArH), 5.88–5.81 (1H, m; −O−CH_2−C*H*=CH_2), 5.58 (1H, s; Ph−C*H*−O_2), 5.31–5.30 (1H, d, $J = 3$ Hz; H-1), 5.25–5.22 (1H, dd, $J = 1.5$, 17 Hz; −O−CH_2−CH=C$H_{trans}$$H_{cis}$), 5.16–5.13 (1H, dd, $J = 1.5$, 10 Hz; −O−CH_2−CH=$CH_{trans}$$H_{cis}$), 4.88 (1H, d, $J = 12$ Hz; Ph−CH_2−O−), 4.83 (1H, d, $J = 12$ Hz; Ph−CH_2−O−), 4.65 (1H, d, $J = 12$ Hz; Ph−CH_2−O−), 4.35 (1H, d, $J = 12$ Hz; Ph−CH_2−O−), 4.62–4.60 (1H, m; Lac-αH), 4.45–4.39 (2H, m; H-1′, H-6′), 4.26–4.11 (3H, m; −COO$CH_2$$CH_3$, H-6), 3.97–3.92 (2H, m; −O−CH_2−CH=CH_2), 3.78–3.74 (4H, m; H-2, H-2′, H-4′, H-6′), 3.69–3.44 (6H, m; H-4′, H-5, H-6, H-3, H-3′, H-4), 3.32–3.27 (1H, m; H-5′), 2.00

(3H, s; NHAc), 1.74 (3H, s; NHAc), 1.35 (3H, d, $J=7$ Hz; Lac-βCH$_3$), 1.30 (3H, t, $J=7$ Hz; −COOCH$_2$CH$_3$); ESI-TOF-MS (positive): m/z: 833.44 $[M+H]^+$.

1-Propenyl 2-acetylamino-6-*O*-benzyl-4-*O*-(2′-acetylamino-3′-*O*-benzyl-4′,6′-*O*-benzylidene-2′-deoxy-β-D-glucopyranosyl)-2-deoxy-3-*O*-[(*R*)-1-(ethoxycarbonyl)ethyl]-α-D-glucopyranoside (54): H_2 activated [Ir(cod)-(MePh$_2$P)$_2$]PF$_6$ (6.2 mg, 7.31×10^{-6} mol) in dry THF (500 μL) was added to a solution of **53** (60.9 mg, 0.0731 mmol) in dry THF (500 μL). After the reaction mixture had been stirred under an Ar atmosphere at RT for 2.5 h, the reaction mixture was extracted with AcOEt. The organic layer was washed with saturated aqueous NaHCO$_3$ and brine, dried over Na$_2$SO$_4$, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (CHCl$_3$/acetone 15:1) to give **54** (48.6 mg, 80%). ^{1}H NMR (500 MHz, CDCl$_3$): $\delta=7.44$–7.28 (15H, m; Ar*H*), 6.08 (1H, dd, $J=1.5$, 12 Hz; −O−C*H*=CH−CH$_3$), 5.59 (1H, s; −Ph−C*H*=O$_2$), 5.50 (1H, d, $J=3.5$ Hz; H-1), 5.10–5.04 (1H, m; −O−CH=C*H*−CH$_3$), 4.88 (1H, d, $J=12$ Hz; Ph−C*H*$_2$−O−), 4.83 (1H, d, $J=12$ Hz; Ph−C*H*$_2$−O−), 4.65 (1H, d, $J=12$ Hz; Ph−C*H*$_2$−O−), 4.64–4.60 (1H, m; Lac-αH), 4.44–4.40 (2H, m; H-1′, H-6′), 4.26–4.13 (3H, m; −COOC*H*$_2$CH$_3$), H-6), 3.96 (1H, t, $J=9$ Hz; H-2), 3.78–3.42 (8H, m; H-3, H-4, H-5, H-6, H-2′, H-3′, H-4′, H-6′), 3.32–3.27 (1H, m; H-5′), 1.20 (3H, s; NHAc), 1.73 (3H, s; NHAc), 1.54 (3H, s; −O−CH=CH−C*H*$_3$), 1.36 (3H, d, $J=7$ Hz; Lac-βCH$_3$), 1.30 (3H, t, $J=7$ Hz; −COOCH$_2$C*H*$_3$); ESI-TOF-MS (positive): m/z: 833.4 $[M+H]^+$.

1-Propenyl 2-acetylamino-6-*O*-benzyl-4-*O*-(2′-acetylamino-3′-*O*-benzyl-4′,6′-*O*-benzylidene-2′-deoxy-β-D-glucopyranosyl)-3-*O*-[(*R*)-1-(carbonyloxy)ethyl]-2-deoxy-α-D-glucopyranoside (55): Compound **54** (48.6 mg, 5.83×10^{-5} mol) was dissolved in THF/1,4-dioxane/H$_2$O 2:1:0.5 (700 μL). LiOH·H$_2$O (3 mg, 6.42×10^{-5} mol) was added to the solution and the reaction mixture was stirred for 3 h. After this time, the reaction was quenched with Dowex H$^+$, which was subsequently removed by filtration. The mixture was then concentrated in vacuo and the resulting residue was applied to an HP-20 column (2×7 cm). Organic and inorganic salts were removed by elution with H$_2$O, followed by elution with MeOH to give **55** (41.7 mg, 91%).

Protected disaccharide tetrapeptide 56 and protected disaccharide tripeptide 57: Compounds **56** and **57** were synthesized from **55** and **30** (for **56**) or **28** (for **57**), respectively, in a manner similar to the synthesis of **41**.

Compound ***56***: ^{1}H NMR (500 MHz, CDCl$_3$): $\delta=7.40$–7.24 (35H, m; Ar*H*), 6.06 (1H, d, $J=12.5$ Hz; −O−C*H*=CH−CH$_3$), 5.58 (1H, s; Ph−C*H*=O$_2$), 5.27 (1H, s; H-1), 5.15–5.02 (10H, m; −COO−C*H*$_2$−Ph ×4, −O−CH=C*H*−CH$_3$, H-1′), 4.86 (1H, d, $J=12$ Hz; −O−C*H*$_2$Ph), 4.75 (1H, d, $J=12$ Hz; −O−C*H*$_2$Ph), 4.65 (1H, d, $J=12$ Hz; −O−C*H*$_2$Ph), 4.58 (1H, brs; H-6), 4.52–4.30 (8H, m; Lac-αH, Ala-αH, D-Ala-αH, Glu-αH, DAP 2-H, DAP 6-H, H-6′×2), 3.98 (1H, t, $J=10$ Hz; H-3′), 3.92–3.90 (1H, m; H-2), 3.76 (1H, t, $J=10.5$ Hz; H-4), 3.66–3.58 (5H, m; H-4′, H-3, H-2′, H-5, H-6), 3.31–3.26 (1H, m; H-5), 2.31–2.11 (4H, m; Glu-βCH$_2$, Glu-γCH$_2$), 1.94 (3H, s; NHAc), 1.80 (3H, s; NHAc), 1.67–1.34 (6H, m; DAP 3-CH$_2$, DAP 4-CH$_2$, DAP 5-CH$_2$), 1.48 (3H, d, $J=6.5$ Hz; −O−CH=CH−C*H*$_3$), 1.38–1.34 ppm (6H, m; Lac-βCH$_3$, Ala-βCH$_3$); ESI-TOF-MS (positive): m/z: 1652.67 $[M+H]^+$.

Compound ***57***: ^{1}H NMR (500 MHz, CDCl$_3$): $\delta=7.42$–7.21 (35H, m; Ar*H*), 6.06 (1H, d, $J=12.5$ Hz; −O−C*H*=CH−CH$_3$), 5.58 (1H, s; Ph−C*H*=O$_2$), 5.28 (1H, d, $J=3.5$ Hz; H-1), 5.15–5.00 (9H, m; −COO−C*H*$_2$−Ph ×4, −O−CH=C*H*−CH$_3$), 4.86 (1H, d, $J=12$ Hz; −O−CH$_2$Ph), 4.73 (1H, d, $J=12$ Hz; −O−CH$_2$Ph), 4.64 (1H, d, $J=12$ Hz; −O−CH$_2$Ph), 4.61–4.29 (5H, m; Lac-αH, Ala-αH, Glu-αH, DAP 2-H, DAP 6-H), 3.97 (1H, t, $J=9.5$ Hz; H-5), 3.91 (1H, dd, $J=2.5$, 10.5 Hz; H-2), 3.76 (1H, t, $J=10.5$ Hz; H-6′), 3.70–3.60 (2H, m; H-4, H-4′), 3.59–3.53 (1H, m; H-3), 3.31–3.26 (1H, m; H-5′), 2.26–2.00 (4H, m; Glu-βCH$_2$, Glu-γCH$_2$), 1.94 (3H, s; NHAc), 1.79 (3H, s; NHAc), 1.82–1.57 (4H, m; DAP 3-CH$_2$, DAP 5-CH$_2$), 1.48 (3H, dd, $J=1.5$, 7 Hz; −O−CH=CH−C*H*$_3$), 1.40–1.34 (2H, m; DAP 4-CH$_2$), 1.33–1.34 (6H, m; Lac-βCH$_3$, Ala-βCH$_3$); ESI-TOF-MS (positive): m/z: 1581.73 $[M+H]^+$.

1-*O*-Deprotected disaccharide tetrapeptide 58 and 1-*O*-deprotected disaccharide tripeptide 59: Compounds **58** and **59** were synthesized from **56** and **57**, respectively, in a manner similar to the synthesis of **41**.

Compound ***58***: ^{1}H NMR (500 MHz, CDCl$_3$): $\delta=7.35$–7.24 (35H, m; ArH), 5.58 (2H, brs; Ph−C*H*=O$_2$, H-1), 5.15–5.06 (9H, m; −COO−C*H*$_2$−Ph ×4, H-1′), 4.87 (1H, d, $J=12$ Hz; −O−C*H*$_2$Ph), 4.75 (1H, d, $J=12$ Hz; −O−C*H*$_2$Ph), 4.64 (1H, d, $J=12$ Hz; −O−CH$_2$Ph), 4.52–4.36 (8H, m; Lac-αH, Ala-αH, D-Ala-αH, Glu-αH, DAP 2-H, DAP 6-H, H-6′, −O−C*H*$_2$Ph), 4.02–3.94 (2H, m; H-6, H-3′), 3.77–3.43 (7H, m; H-2, H-3, H-4, H-6, H-2′, H-4′, H-6′), 3.34–3.26 (2H, m; H-5, H-5′), 2.33–1.97 (4H, m; Glu-βCH$_2$, Glu-γCH$_2$), 1.97 (3H, s; NHAc), 1.72 (3H, s; NHAc), 1.85–1.36 (4H, m; DAP 3-CH$_2$, DAP 4-CH$_2$, DAP 5-CH$_2$), 1.38–1.31 ppm (6H, m; Lac-βCH$_3$, Ala-βCH$_3$); ESI-TOF-MS (positive): m/z: 1612.84 $[M+H]^+$.

Compound ***59***: ^{1}H NMR (500 MHz, CDCl$_3$): $\delta=7.40$–7.17 (35H, m; ArH), 5.58 (2H, brs; Ph−C*H*=O$_2$, H-1), 5.16–5.05 (9H, m; −COO−C*H*$_2$−Ph ×4, H-1′), 4.87 (1H, d, $J=12$ Hz; −O−C*H*$_2$Ph), 4.74 (1H, d, $J=12$ Hz; −O−C*H*$_2$Ph), 4.64 (1H, d, $J=12$ Hz; −O−C*H*$_2$Ph), 4.56–4.34 (9H, m; Lac-αH, Ala-αH, Glu-αH, DAP 2-H, DAP 6-H, H-6, H-6′, −O−C*H*$_2$Ph), 4.02–3.94 (2H, m; H-6, H-3′), 3.83–3.43 (7H, m; H-2, H-3, H-4, H-6, H-2′, H-4′, H-6′), 3.35–3.31 (1H, m; H-5), 3.29–3.24 (1H, m; H-5′), 2.31–1.98 (4H, m; Glu-βCH$_2$, Glu-γCH$_2$), 1.96 (3H, s; NHAc), 1.73 (3H, s; NHAc), 1.84–1.32 (6H, m; DAP 3-CH$_2$, DAP 4-CH$_2$, DAP 5-CH$_2$), 1.39–1.25 ppm (6H, m; Lac-βCH$_3$, Ala-βCH$_3$); ESI-TOF-MS (positive): m/z: 1541.79 $[M+H]^+$.

Disaccharide tetrapeptide 1 and disaccharide tripeptide 2: Compounds **1** and **2** were synthesized from **58** and **59**, respectively, in a manner similar to the synthesis of **5**.

Compound ***1***: ^{1}H NMR (500 MHz, D$_2$O): $\delta=5.07$ (1H, d, $J=3.5$ Hz; H-1), 4.41–4.37 (2H, m; Ala-αH, H-1′), 4.20–4.03 (5H, m; Lac-αH, D-Ala-αH, DAP 2-H, DAP 6-H, Glu-αH), 3.80–3.78 (2H, m; H-6′×2), 3.73–3.57 (5H, m; H-2, H-3, H-4, H-5, H-5′), 3.43–3.39 (1H, m; H-3′), 3.35–3.27 (3H, m; H-6×2, H-4′), 2.22–1.94 (4H, m; Glu-βCH$_2$, Glu-γCH$_2$), 1.90 (3H, s; NHAc), 1.80 (3H, s; NHAc), 1.83–1.57 (4H, m; DAP 3-CH$_2$, 5-CH$_2$), 1.33–1.25 (2H, m; DAP 4-CH$_2$), 1.29 (3H, d, $J=7.5$ Hz; Lac-βCH$_3$), 1.25 (3H, d, $J=6.5$ Hz; Ala-βCH$_3$), 1.20 (3H, d, $J=7$ Hz; D-Ala-βCH$_3$); ESI-TOF-MS (negative): m/z: 938.31 $[M-H]^-$; HRMS-ESI QTOF-MS (positive): m/z: calcd for $C_{37}H_{61}N_7O_{21}$: 940.3999 $[M+H]^+$; found: 940.4031.

Compound ***2***: ^{1}H NMR (500 MHz, CD$_3$OD): $\delta=5.20$ (1H, brs; H-1), 4.59–4.58 (2H, m; Ala-αH, H-1′), 4.43–4.42 (4H, m; Lac-αH, DAP 2-H, DAP 6-H, Glu-αH), 3.90–3.89 (2H, m; H-6′×2), 3.81–3.58 (5H, m; H-2, H-3, H-4, H-5, H-5′), 3.43–3.29 (4H, m; H-3′, H-6×2, H-4′), 2.32- 1.99 (4H, m; Glu-βCH$_2$, Glu-γCH$_2$), 1.97 (3H, s; NHAc), 1.95 (3H, s; NHAc), 1.80–1.53 (4H, m; DAP 3-CH$_2$, DAP 4-CH$_2$, DAP 5-CH$_2$), 1.40–1.28 ppm (6H, m; Lac-βCH$_3$, Ala-βCH$_3$); ESI-TOF-MS (negative): m/z: 867.37 $[M-H]^-$; HRMS-ESI QTOF-MS (positive) m/z: calcd for $C_{34}H_{56}N_6O_{20}$: 869.3627 $[M+H]^+$; found: 869.3622.

HEK293T Bioassay for Nod1 stimulation: Ligand dependent NF-κB activation was determined by using 0.5×10^5 HEK293T cells transfected with expression plasmids of Nod1 (0.17 ng of pCMV-SPORT6-Nod1), in the presence of reporter plasmids, NF-κB dependent pBxIV-luc, and control pEF1BOS-β-gal as described.[7] Briefly, HEK293T cells were transfected with expression plasmids by the calcium phosphate method, and 8 h after transfection, cells were treated with a medium containing various ligands. 24 h after transfection, ligand-dependent NF-κB activation was determined by the luciferase reporter assay.

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