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Synthesis of a Fragment of Bacterial Cell Wall

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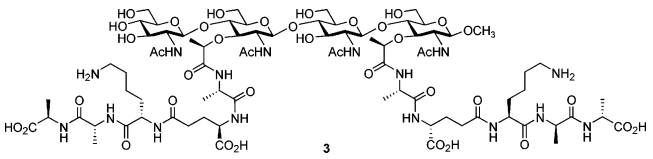
Cell wall is indispensable for survival of bacteria. This large molecular "mesh" encases the entire cytoplasm of bacteria, and it is comprised of repeating backbone units of *N*-acetyl-glucosamine (NAG)-*N*-acetyl-muramic acid (NAM). A pentapeptide is attached to each of the lactyl units of the *N*-acetyl-muramic acid. The cell wall has both cross-linked and non-cross-linked components. In the present paper, we have devised a synthetic route for the preparation of a fragment of the cell wall comprised of a tetrasaccharide (NAG-NAM-NAG-NAM), along with the two appended peptides. We also report the syntheses of three glycosyl donors (compounds **5**, **7**, and **9**) and three glycosyl acceptors (compounds **4**, **6**, and **8**) based on the D-glucosamine structure as a building unit. The synthetic strategy that is disclosed is generally useful in construction of other natural products containing the D-glucosamine as a building block.

Bacteria are not capable of maintaining their internal osmotic pressure. Therefore, they have developed the cell wall (also known as the cross-linked peptidoglycan or murein) as a rigid "molecular mesh", which encloses the cell and maintains the structural integrity of bacteria. This single macromolecule that encases the cytoplasm is also referred to as the sacculus (the "sack"), an entity that supports and controls the turgor pressure (of an ever-expanding cytoplasm). The polysaccharide part of the peptidoglycan is a repeating disaccharide unit of *N*-acetyl-glucosamine (NAG)-*N*-acetyl-muramic acid (NAM). A uniquely bacterial pentapeptide is attached to the D-lactyl moiety of NAM (**1**). The peptides from two separate strands are cross-linked (**2**) by DD-transpeptidases to give the rigid cell wall.[1]

A variety of techniques have been applied over the past 20−30 years to study the nature of the cell wall, resulting in four hypotheses for the formation of the sacculus.[2,3] Despite these efforts, there is no detailed knowledge of what the three-dimensional structure of sacculus looks like at the present. The major difficulty with the earlier efforts on the studies of the peptidoglycan had been the nonhomogeneous nature of the samples. These studies have invariably used samples from bacteria, which were mixtures of bits and pieces of different sizes. We have undertaken a series of studies to elucidate the structural aspects of the bacterial cell wall. In this vein, a glimpse into the cross-linked peptidoglycan was provided recently by the publication of a high-resolution X-ray structure for a DD-transpeptidase that had two mimics of the peptidoglycan sequestered in its active site, en route to the cross-linked species.[4] We are also interested in visualizing the solution structure of the non-cross-linked peptidoglycan. Toward this goal, we have devised a versatile synthetic route that allows for the assembly of sizable fragments of the bacterial cell wall. The synthetic work for the preparation of a fragment of cell wall that encompasses a tetrasaccharide made up of two repeats of NAG-NAM along with the two requisite appended peptides that are found in many Gram-positives (compound **3**) is disclosed herein. Insofar as lysine may serve



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as a simplified surrogate for diaminopimelate, the structure may also represent the situation seen in many Gram-negative organisms as well.[5] We hasten to add that 2-amino-2-deoxyglucose (i.e., glucosamine) is found in many natural products,[6,7] hence the synthetic approaches that we have developed should be of use for a much broader purpose.

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(3) Dmitriev, B. A.; Ehlers, S.; Rietchel, E. T. Layered Murein Revisited: a Fundamentally New Concept of Bacterial Cell Wall Structure, Biogenesis and Function. *Med. Microbiol. Immunol.* **1999**, *187*, 173−181.

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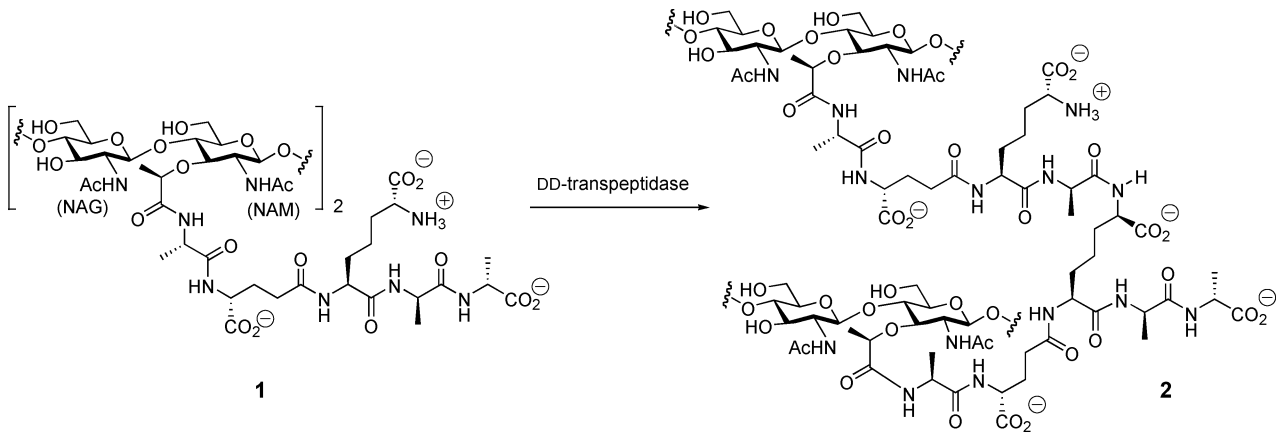
SCHEME 1



Results and Discussion

There is a paucity of precedent for construction of repeating units of the sugar backbone of peptidoglycan (PGN),[8] although some literature exists for construction of the NAG-NAM-containing derivatives of lipid II, a biosynthetic precursor for the bacterial cell wall.[9,10] Most of the effort has been focused on coupling of orthogonally protected monosaccharide building blocks derived from NAG and NAM, which already include the lactyl ether attached at the 3-*O*-position of D-Glc*N*Ac. The presence of the (*R*)-lactate moieties creates difficulties in assembling building blocks into complex structures, such as intramolecular lactonization at the 4-hydroxy position,[10] and more importantly racemization of the lactate residue. Therefore, we decided to introduce the lactate residue at a later stage in the synthesis and develop synthetic routes involving orthogonal protective groups that differentiate the hydroxyl groups in NAM from that in the NAG unit.

While the choices for *N*-protection of D-glucosamine are many,[11] we chose the recently developed *N*-dimethylmaleoyl protective group that participates in stereoselective β-(1−4)-glycoside bond formation.[12,13] We anticipated that this protective group would result in high coupling yields and complete removal of the DMM after coupling and would allow us to avoid potential solubility complications. Trichloroacetimidate was used as the leaving group and trifluoromethanesulfonic acid (TfOH) as the main promoter for the stereoselective β-(1−4)-glycosyl bond formation reaction.[7]

With these requirements in mind, commercially available D-glucosamine hydrochloride was used to design glycosyl building blocks and develop a synthetic protocol involving protection−deprotection motifs. The choice of commonly used protecting groups in sugar chemistry to mask free hydroxyl groups (e.g., *O*-benzylidene-, *O*-benzyl-, *O*-acetyl-, and *O*-*tert*-butyldimethylsilyl) allowed us to selectively introduce our desired functionality prior to coupling. Furthermore, the desired synthetic route should demonstrate an excellent regioselectivity and/or stereoselectivity, and be amenable to a single deprotection step of the final glycopeptide units for practical reasons. The linkages among the aminosugars are all β-(1−4). Hence, the terminal sugar should bear a β-methoxy group at the anomeric C-1 position of the terminal NAM residue (see **3**). Considering how the inherent properties of the individual glycosyl donor/acceptor building blocks could lead to the formation of several different derivatives as possible reaction intermediates, we synthesized a focused library of monosaccharide glycosyl donors and glycosyl acceptors that would serve the purpose (Figure 1). The syntheses of these building blocks (the number of synthetic steps and the overall reaction yields were calculated starting from D-glucosamine), leading to glycosyl acceptors **4** (7 steps, 16%), **6** (5 steps, 21%), and **8** (7 steps, 18%), and glycosyl donors **5** (3 steps, 32%), **7** (9 steps, 9%) and **9** (9 steps, 10%), are outlined in Scheme 2.

A systematic analysis of assembly of suitable libraries of donors and acceptors en route to cell wall fragments is absent from the literature. Five possible patterns for the syntheses of the NAG-NAM-NAG-NAM(β-OMe) sugar sequence were considered (Figure 1).

Strategy 1: Propagation of the sugar units one-by-one starting from a terminal glycosyl acceptor **4**, which represents ring A, seemed most logical at first glace. One advantage is the better freedom to choose the reaction conditions throughout the synthesis, because a desired β-OCH_3 substituent at C-1 is generally stable (in contrast to the *O*-silyl group). The second advantage is the chemical properties of the substrates (A, A−B, or A−B−C) being introduced into the next reaction steps that serve as glycosyl acceptors, without the added difficulty of employing acid-sensitive silyl-protected groups. However, this approach is complicated since glycosylation reactions with donors **7** and **9** (each made in 9 steps) are required, followed by regioselective reduction of 4,6-*O*-benzylidene protective groups, which dramatically would

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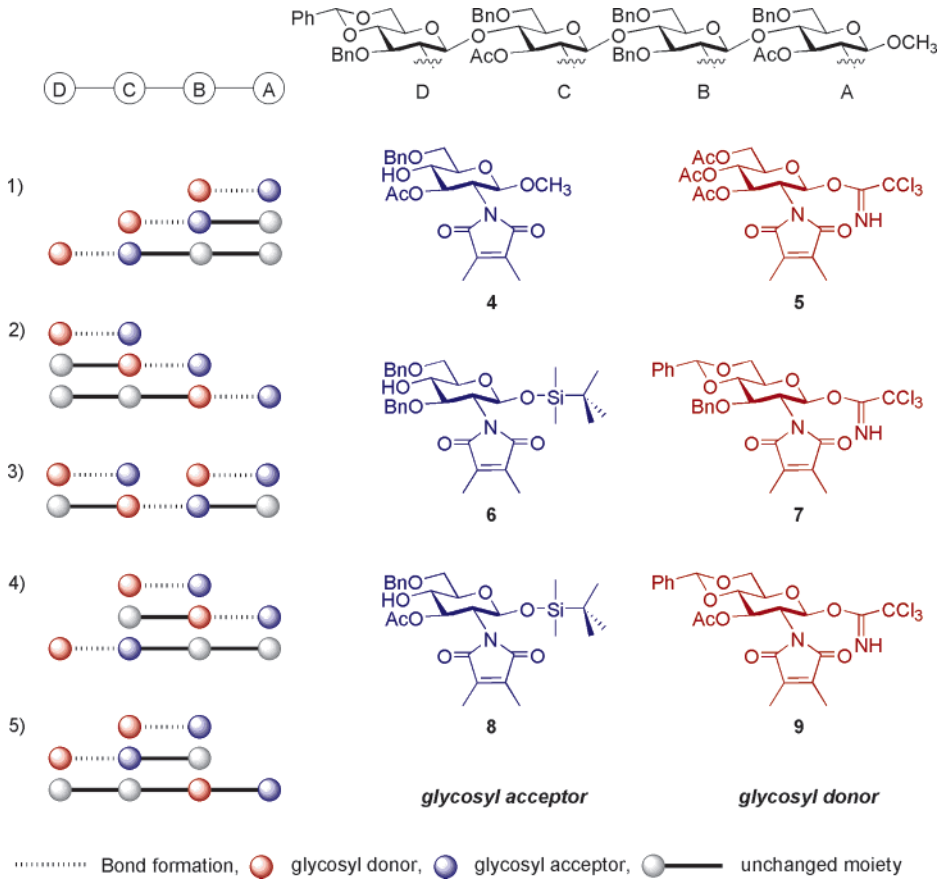
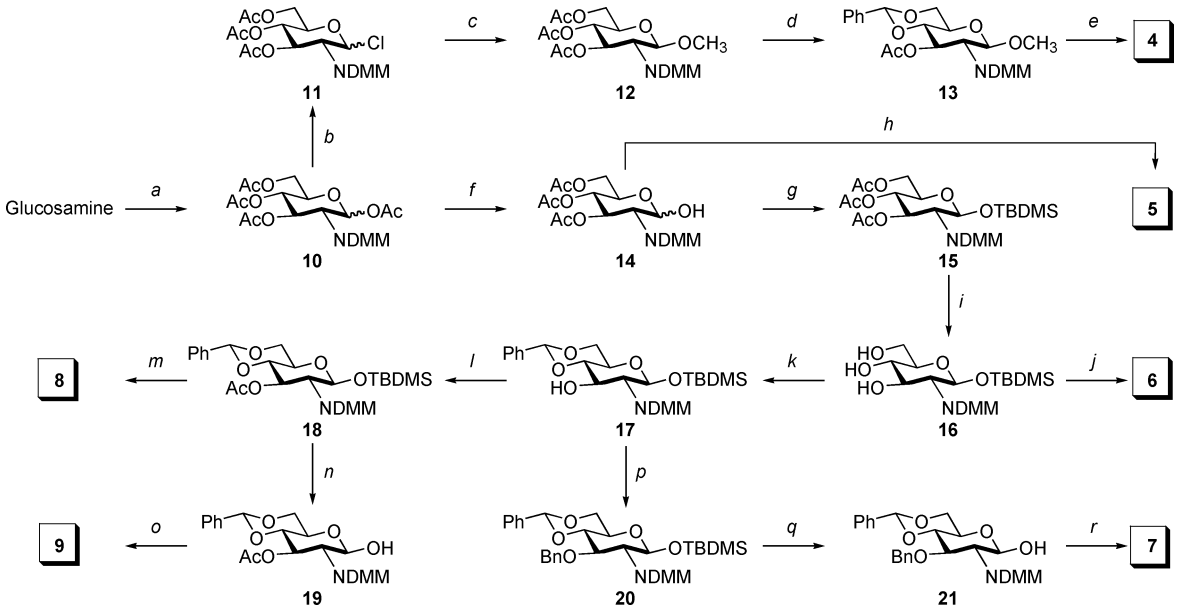


FIGURE 1. Synthetic strategy for construction of the tetrasaccharide made of repeating D-glucosamine units. The structures of the six glycosyl acceptors and donors employed in this study are depicted.

SCHEME 2[a]



[a] Reagents and conditions: (a) (i) 2,3-dimethylmaleic anhydride, (ii) Ac_2O, 55%; (b) $TiCl_4$, 76%; (c) HgO, $HgCl_2$, MeOH, 81%; (d) (i) NaOMe, Amberlite IR-120 (H^+), (ii) benzaldehyde dimethylacetal, *p*-TsOH, (iii) Ac_2O, 67%; (e) BH_3·NMe_3, BF_3·OEt_2, 76%; (f) N_2H_4·AcOH, 68%; (g) TBDMS−Cl, imidazole, 78%; (h) Cl_3CCN, DBU, 85%; (i) NaOMe, 73%; (j) Bu_2SnO, BnBr, 73%; (k) benzaldehyde dimethylacetal, *p*-TsOH; 91%; (l) Ac_2O, 85%; (m) BH_3·$NHMe_2$, BF_3·OEt_2, 90%; (n) *n*-Bu_4NF, 55%; (o) Cl_3CCN, DBU, 85%; (p) $Cl_3CC{=}NHOBn$, TfOH, 67%; (q) *n*-Bu_4NF, 72%; (r) Cl_3CCN, DBU, 80%.

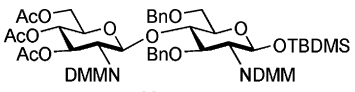
decrease the overall reaction yield. Therefore, this strategy would appear to be less economical.

Strategy 2: This strategy builds sugar units one-by-one, starting from glycosyl donor **7** (ring D). After many

attempts to couple the building blocks using strategy 2, we concluded that this route was the least effective of the methods proposed herein. During the coupling reactions, the purity of the coupling products was poor and they had to be resubjected to further purification with flash-column chromatography due to the similar nonpolar nature of product and starting materials. As a result, the major product typically had a maximum yield of 40% for each consecutive coupling step.

Strategy 3: This approach required the formation of each of the individual monoglycosyl entities on a large scale, which is a major disadvantage, followed by construction of the two separate disaccharide units to give the coupled intermediates **7**−**8** (D−C) and **7**−**4** (B−A). Subsequently, the dimers would be converted into their corresponding donor−acceptor counterparts, followed by the activation of the donor component, resulting in their linkage to give the tetrasaccharide moiety.

Strategies 4 and 5: To construct the disaccharide core C−B from our library first appeared to be the most favorable when acceptor B was coupled with donor C in the presence of a catalytic amount of the promoter. The C−B disaccharide could be prepared by stereoselective glycosylation of either trichloracetimidate glycosyl donor **5** or **9** with the corresponding acceptor **6**, resulting in disaccharides **5**−**6** (compound **22**) and **9**−**6** (compound **23**), respectively, in good overall yield in both cases. However, the different nature of disaccharide products formed would be distinguished. The synthesis of a disaccharide intermediate similar to **5**−**6** was recently described by Schmidt et al.[13] However, further transformations (three additional steps) should be implemented to obtain **23**.



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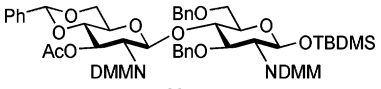
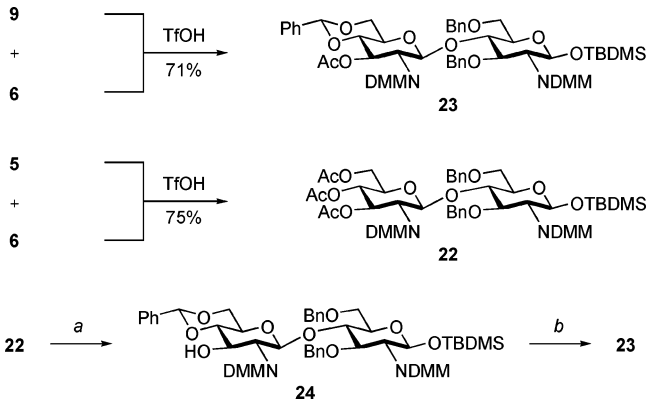


23

Mainly, one needs to convert 3,4,6-*O*-triacethyl protective groups in **22** into the corresponding 3-*O*-acetyl-4,6-*O*-benzylidene derivatives **23** to obtain the suitable disaccharide building blocks needed for sugar expansion. The efficient transformation of the glucoside C ring having the tri-*O*-acetyl protected group into the corresponding 4,6-*O*-benzylidene derivative was performed via a short route with standard reaction conditions. However, these transformations are carried out using the disaccharide segment that would decrease the overall yield for this strategy. Alternatively, a glycosylation reaction between the donor **9** and acceptor **6** affords disaccharide **23** in a facile way. To generate the donor **9**, it was necessary to remove the TBDMS protective group at the anomeric carbon of the corresponding intermediate (**18**). This particular desilylation step appears to be unusually

SCHEME 3[a]



[a] Reagents and conditions: (a) (i) NaOMe, Amberlite IR-120 (H^+), (ii) benzaldehyde dimethylacetal, *p*-TsOH, 60% in two steps; (b) Ac_2O, 85%.

sensitive to the reaction conditions employed (e.g., TBAF/acetic acid, prolonged exposure 1 h; temperature over 10 °C), resulting in the formation of undesirable side products. However, this strategy readily offers the highly desirable glycosyl dimer **23**, without further manipulation.

The dimer **23** can be converted to either a glycosyl acceptor, ready for coupling with the donor **4**, or to a glycosyl donor for coupling with the acceptor **7**; one or the other approach makes the difference between strategies 4 and 5. The more extensive synthetic demand for preparation of acceptor **7** (over donor **4**), as well as the need for purification via strategy 5, makes strategy 4 more favorable for the preparation of compound **3**.

After a small modification of disaccharide **23**, it was employed in the construction of the trisaccharide in which acceptor **4** was used (Strategy 4). This step was optimized and was scaled up in our synthesis. It is noteworthy that dimer **23** is a useful intermediate, because it can be converted to both a glycosyl acceptor and a donor, which has the potential desired application for construction of oligosaccharide by a repeat of the coupling procedures.

The route that was ultimately chosen was based on a combination of the donors and acceptors depicted in Scheme 3. The donor **9** and acceptor **6** were converted to disaccharide β-(1→4) **23** in a TfOH-mediated reaction at low temperature (approximately −10 °C, 71%) in a single step. The use of scrupulously dry conditions prevented the formation of any byproducts. However, when TMSOTf was used as the promoter, it was noted that on longer reaction time decomposition occurred to give undesired products, along with the formation of a bissilylated version of **6**, with TMS−ether attached at the 4-position. Alternatively, the identical disaccharide **23** was obtained more circuitously when derivative **22** was converted to **24**. This product was obtained after hydrolysis of the acetyl groups without affecting dimethylmaleoyl (DMM) and treatment with benzaldehyde dimethyl acetal in the presence of *p*-TsOH as a catalyst. Subsequently, acetylation of the remaining free 4′-hydroxyl group in the product **24** afforded **23** in a slightly lower overall reaction yield in comparison to that described above.

Elaboration of the substrate **23** into the required glycosyl donor **26** (Scheme 4) was performed by applying

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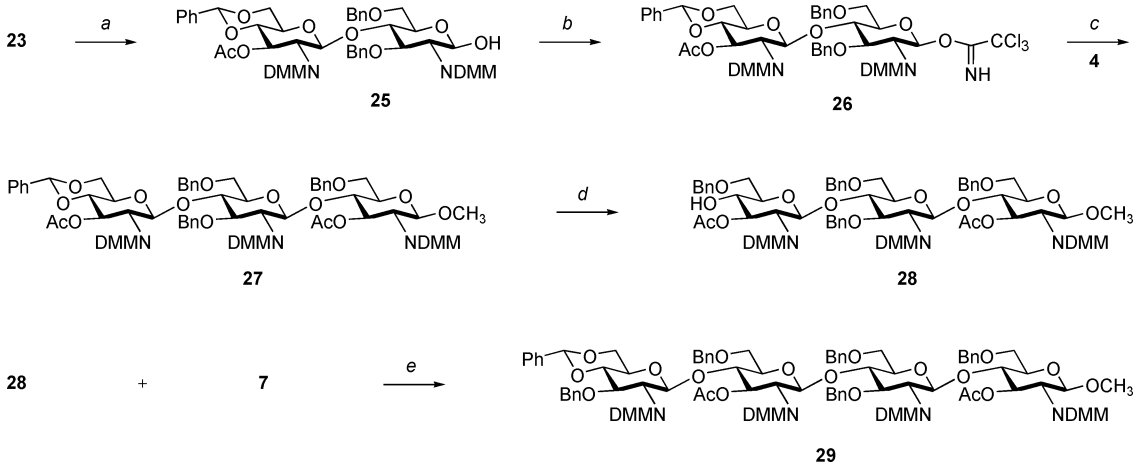
SCHEME 4[a]



[a] Reagents and conditions: (a) *n*-Bu_4NF, 75%; (b) Cl_3CCN, DBU; (c) **4**, TfOH, 55% in two steps; (d) $BH_3{\cdot}NMe_3$, $BF_3{\cdot}OEt_2$, 62%; (e) TfOH, 59−68%.

standard procedures and needs little comment, apart from the fact that removal of the TBDMS ether at the C-1 position of disaccharide **23** was accomplished on treatment with a 1 M solution of tetrabutylammonium fluoride (TBAF) in ethyl acetate, furnishing **25** in 75% yield. However, a prolonged reaction time led to a substantial decrease in yield due to mainly undesired hydrolysis of the 3-*O*-acetyl group by the trace of moisture in the reagent. Stereoselective β-glycosylation between acceptor **4** and trichloracetimidate **26** in the presence of TfOH gave the corresponding trisaccharide **27** in good yield (62%). Regioselective reductive ring opening of the 4,6-*O*-benzylidene group in **27** was carried out by treatment with the borane−trimethylamine complex ($BH_3{\cdot}NMe_3$) and $BF_3{\cdot}OEt_2$, utilizing a modification of a published procedure for similar derivatives.[14] This reaction resulted in the formation of the trisaccharide acceptor **28** with an unmasked 4-hydroxyl functionality typically in 59−68%. To obtain the desired tetrasaccharide **29**, the reaction sequence was put through an additional *O*-glycosyl trichloracetimidate method with **7** to provide exclusively the tetramer of the β-glycosyl linkage.

Deprotection of the DMM groups in **29** was carried out by a careful acid/base treatment (NaOH, 6 h; HCl, pH 3.0, 24 h), essentially by the kind of procedure reported earlier.[12] The attendant *N*-acetylation of **29** gave **30** (Scheme 5). The overall yield for the two steps was 48%.

Whereas the outlined strategy is seemingly laborious, the assembly of the building blocks into the larger oligosaccharides without the lactate functionality appears to be a practical route for the synthesis of derivatives with repeating NAG-NAM sequence. Due to limited solubility, the in situ generated compound **31**, possessing free 3-*O*-hydroxy groups, was committed to the next step without further purification. The formation of fragment **32** by the reaction of **31** in the presence of an excess of (*S*)-2-chloropropionic acid was a relatively simple transformation (Scheme 5). The lactate moieties in **32** were coupled with the pentapeptide in the next reaction. The desired pentapeptide (**33**, L-Ala-D-Glu-α-OBn-γ-*N*-ϵ-Cbz-L-Lys-D-Ala-D-Ala-OBn) was synthesized by a fragment condensation approach, using standard Boc chemistry (Scheme 6). The two carboxylic acids and one amino group in this peptide chain were protected by benzyl and benzyloxycarbonyl (Cbz) groups, respectively. The *p*-nitrophenylester (**36**) of the known dipeptide (Boc-L-Ala-D-Glu-α-OBn-γ-OH)[15] was prepared from Boc-L-Ala and Boc-D-Glu-α-OBn by the active ester method. The tripeptide (**38**, *N*-ϵ-Cbz-L-Lys-D-Ala-D-Ala-OBn) was readily made by stepwise condensation using the water-soluble coupling agent EDCI. The active ester method was applied again in the final assembly step to obtain the clean protected pentapeptide **39**. The Boc group in **39** was readily deprotected by trifluoroacetic acid to afford **33** as a TFA salt.

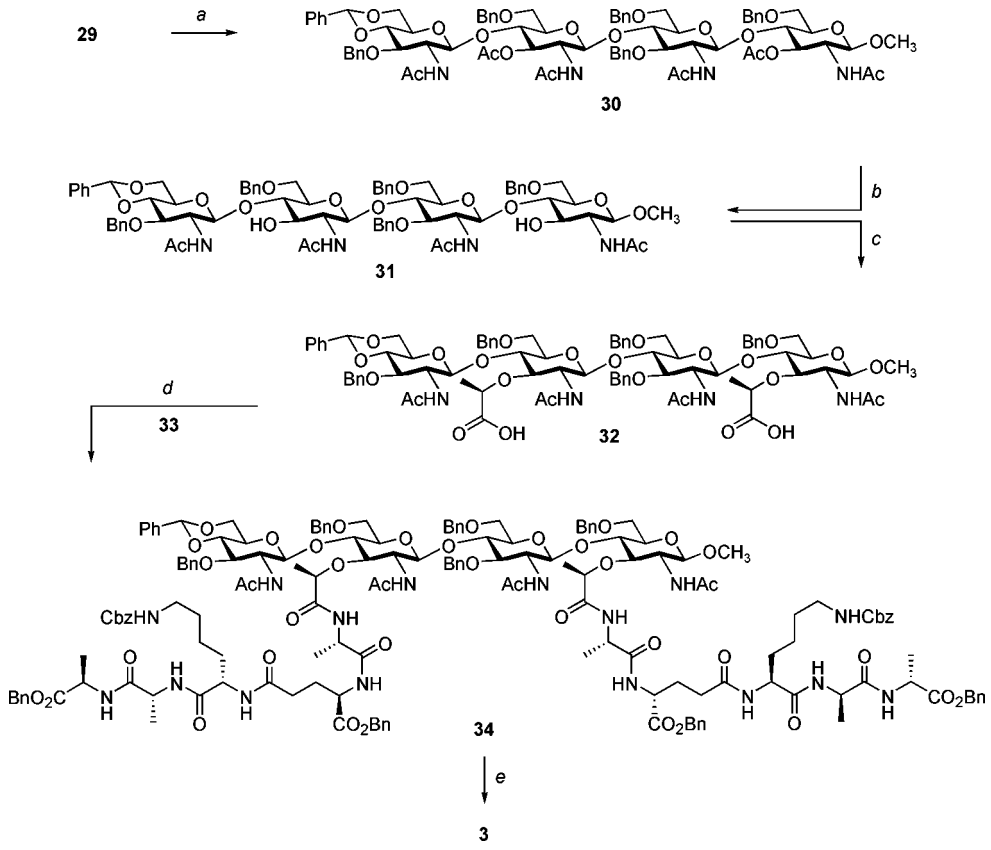
Subsequently, the lactate moieties in **32** were activated to the corresponding *p*-nitrophenol ester derivatives by treatment with *p*-nitrophenyl trifluoroacetate. Although the use of *p*-nitrophenyl ester is not common for activation of carboxylates in sugar chemistry, it is noteworthy that the introduction of the chromophore to the sugar molecules made it facile to follow the reaction. Furthermore, the *p*-nitrophenyl ester that was formed was stable and could be kept at room temperature in a protic solvent such as methanol without racemization or anomerization. The active ester was converted to the protected form of the peptidoglycan tetrasaccharide **34** by coupling to the requisite pentapeptide **33**. The last step of the preparation was 4,6-*O*-benzylidene deprotection in 60% acetic acid, which was allowed to proceed at 70 °C for 1 h. The global deprotection of the benzyl esters, benzyl ethers, and benzyloxycarbonyl functionalities was accomplished by hydrogenolysis over 10% Pd/C in methanol as the final step in the synthesis to give the crude **3**.

The crude compound **3** was purified by preparative thin-layer chromatography. The sample was pure by HPLC analysis and produced the desired mass spectrometric data. The compound was subsequently studied by a series of sophisticated high-field NMR analyses for determination of the solution structure, which will be the

(14) Oikawa, M.; Liu, W.-C.; Nakai, Y.; Koshida, S.; Fukase, K. *Synlett* **1996**, 1179−1180.

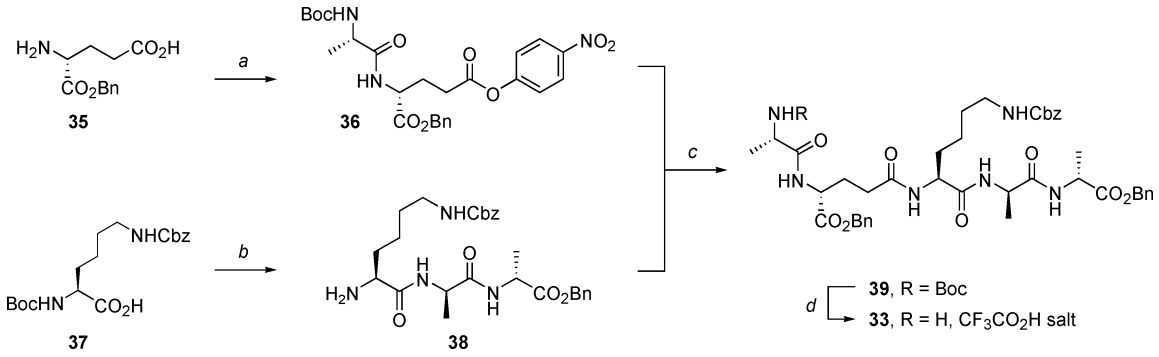
(15) Takeno, H.; Okada, S.; Hemmi, K.; Aratani, M.; Kitaura, Y.; Hashimoto, M. *Chem. Pharm. Bull.* **1984**, *32*, 2925−2931.

SCHEME 5[a]



[a] Reagents and conditions: (a) (i) NaOH, dioxane/water (4:1); HCl, pH 3.0, (ii) Ac_2O, 48%; (b) NaOMe, 74%; (c) NaH, (*S*)-2-chloropropionic acid, 46%; (d) 4-nitrophenyl trifluoroacetate, pyridine; **33**, 41%; (e) 60% AcOH; H_2, Pd/C, 76%.

SCHEME 6[a]



[a] Reagents and conditions: (a) (i) L-alanine *p*-nitrophenyl ester, 70%, (ii) *p*-nitrophenol, DCC, 75%; (b) (i) D-Ala-D-Ala-OBn, EDCI, HOBt, 32%, (ii) TFA, 93%; (c) HOBt, 48%; (d) TFA, 96%.

subject of a subsequent paper that concerns the structural biological aspects of our interest in this synthetic fragment of bacterial cell wall.

Methods

Compounds **5**, **6**, **10**, **14**−**17**,[12] **7**, **20**, **21**,[16] and **22**[13] were prepared by published literature methods. TfOH (trifluoromethanesulfonic acid) was used as a 1 M solution in CH_2Cl_2, which was kept over 4-Å molecular sieves. Procedures for syntheses of the monomer compounds in Scheme 2 and the pentapeptide synthesis of Scheme 6 are given in the Supporting Information.

***tert*-Butyldimethylsilyl 3-*O*-Acetyl-4,6-*O*-benzylidene-2-deoxy-2-dimethylmaleimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-*β*-D-glucopyranoside (23).** Compound **23** was obtained by two methods. One was by coupling of **9** with **6** and the other was conversion from **22**. In the former method, a mixture of compound **9** (5.6 g, 10 mmol) and compound **6** (6.4 g, 11 mmol) was dissolved in dry CH_2C1_2 (100 mL, distilled over CaH_2 and then over 4-Å molecular sieves), 4-Å molecular sieves (5 g) was added, and the suspension was stirred under argon for 2 h. The mixture was then diluted with cyclohexane (50 mL) and cooled to −10 °C. The catalyst TfOH (1.5 mL, 1 M solution in CH_2C1_2) was added in two portions, and the solution was

(16) Hesek, D.; Suvorov, M.; Morio, K.; Lee, M.; Brown, S.; Vakulenko, S. B.; Mobashery, S. *J. Org. Chem.* **2004**, *69*, 778−784.

stirred at −10 °C for 1 h, and then at room temperature for an additional 1 h, after which the reaction was quenched by adding triethylamine (0.21 mL) and filtered through a layer of Celite, and the organic solvents were removed in vacuo. The residue was purified by flash chromatography (*n*-hexane/EtOAc, 3.2:1) to give **23** (7.0 g) in 71% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.10, 0.11, 0.72 (3s, 15H), 1.79 (br s, 6H), 1.93, 1.96 (2s, 9H), 3.37−3.51 (m, 3H, H-5^{I}, H-6^{I}, H-6′ ^{I}), 3.53 (dd, 1H, J = 6.9, 10.1 Hz, H-6^{II}), 3.61 (dd, 1H, J = 8.9, 9.7 Hz, H-4^{II}), 3.85 (dd, 1H, J = 8.1, 10.5 Hz, H-2^{I}), 4.03 (dd, 1H, J = 8.5, 10.1 Hz, H-2^{II}), 4.09−4.13 (m, 2H, H-3^{I}, H-4^{I}), 4.18 (dd, 1H, J = 4.5, 10.1 Hz, H-6′ ^{II}), 4.61 (2d, 2H, J = 12.2 Hz, CH_2-Ph), 4.46, 4.81 (2d, 2H, J = 12.2 Hz, CH_2Ph), 5.08 (d, 1H, J = 8.1 Hz, H-1^{I}), 5.41 (s, 1H, C*H*Ph), 5.46 (d, 1H, J = 8.1 Hz, H-1^{II}), 5.69 (t, 1H, J = 9.7 Hz, H-3^{II}), 7.18−7.41 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3, −4.0, 8.8, 9.1, 17.8, 20.9, 25.6, 56.2 (C-2^{II}), 57.8 (C-2^{I}), 66.0 (C-5^{II}), 68.2 (C-6^{I}), 68.9 (C-6^{II}), 70.1 (C-3^{II}), 73.2, 74.3 (2*C*H$_2$Ph), 74.8 (C-5^{I}), 76.7 (C-3^{I}), 77.5 (C-4^{I}), 79.5 (C-4^{II}), 93.6 (C-1^{I}), 97.9 (C-1^{II}), 101.8 (*C*HPh), 126.5, 127.3, 127.6, 127.8, 128.1, 128.4, 128.5, 128.6, 129.4, 137.0, 137.2, 138.6, 139.3, 170.4, 171.8; MS (ESI) *m*/*z* 1003.41 [M + Na]$^+$.

Compound 23 (obtained via conversion of 22). A solution of compound **22** (1.8 g, 1.9 mmol) and NaOMe (0.05 g) in anhydrous MeOH (15 mL) was stirred at room temperature. The mixture was stirred at room temperature until starting material was consumed (1 h). The reaction was quenched by the addition of an excess amount of Amberlite IR-120 (H$^+$). After an additional 20 min of stirring, the resin was filtered and the resulting solution was concentrated to dryness. The residue was dissolved in acetonitrile and re-evaporated to dryness, and the residue was kept under vacuum for 1 h. The residue was dissolved in anhydrous acetonitrile (15 mL) and the resulting solution was treated with benzaldehyde dimethylacetal (0.8 mL, 5.2 mmol) and *p*-toluenesulfonic acid monohydrate (0.06 g). After being stirred at room temperature for 3 h, the reaction was neutralized by the addition of a few drops of saturated solution of $NaHCO_3$ and stirring was continued for an additional 0.5 h. The reaction mixture was concentrated to dryness and the residue was chromatographed on silica gel (*n*-hexane to *n*-hexane/EtOAc, 2:1) to give a white solid (**24**, 1.1 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.10, 0.02, 0.73, (3s, 15H), 1.79 (br s, 6H), 1.96 (s, 6H), 3.33 (dd, 1H, J = 4.9, 9.7 Hz, 1H, H-5^{II}), 3.37−3.47 (m, 2H, H-6^{I}, H-6′ ^{I}), 3.39 (m, 1H, H-5^{I}), 3.43 (t, 1H, J = 8.9 Hz, H-4^{II}), 3.53 (dd, 1H, J = 9.7, 12.2 Hz, H-6^{II}), 3.84 (dd, 1H, J = 8.5, 10.1 Hz, H-2^{I}), 3.98 (dd, 1H, J = 8.1, 10.5 Hz, H-2^{II}), 4.09−4.11 (m, 2H, H-3^{I}, H-4^{I}), 4.18 (dd, 1H, J = 4.9, 9.7 Hz, H-6′ ^{II}), 4.65 (t, 1H, J = 8.9 Hz, H-3^{II}), 4.56, 4.62 (2d, 2H, J = 12.2 Hz, CH_2Ph), 4.48, 4.81 (2d, 2H, J = 12.2 Hz, CH_2Ph), 5.08 (d, 1H, J = 8.1 Hz, H-1^{I}), 5.26 (d, 1H, J = 8.1 Hz, H-1^{II}), 5.44 (s, 1H, C*H*Ph), 7.17−7.46 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.3, −4.0, 8.8, 9.1, 17.8, 20.9, 25.6, 57.2 (C-2^{II}), 57.8 (C-2^{I}), 66.0 (C-5^{II}), 68.3 (C-6^{II}), 68.6 (C-3^{II}), 68.9 (C-6^{I}), 73.2, 74.3 (2*C*H$_2$Ph), 74.9 (C-5^{I}), 76.8 (C-3^{I}), 77.3 (C-4^{I}), 82.4 (C-4^{II}), 93.5 (C-1^{I}), 98.2 (C-1^{II}), 102.1 (*C*HPh), 126.6, 127.3, 127.6, 127.8, 128.0, 128.1, 128.4, 128.6, 129.6, 137.0, 137.3, 137.6, 138.6, 139.3, 171.9; MS (ESI) *m*/*z* 961.35 [M + Na]$^+$.

Compound **24** obtained above (1.1 g, 1.2 mmol) was mixed with Ac_2O (3.1 mL) and pyridine (2.7 mL) and the resultant solution was stirred at room temperature for 3 h. Evaporation of the solvent gave the crude product, which was taken up in a mixture of CH_2Cl_2 and water. Layers were separated and the organic layer was washed with water (3×). The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to afford compound **23**, which was used for the next step without further purification (1.5 g, 85%).

3-*O*-Acetyl-4,6-*O*-benzylidene-2-deoxy-2-dimethylmaleimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-*β*-D-glucopyranose (25). Compound **23** (6.0 g, 6.1 mmol) in ethyl acetate (30 mL) was treated by the dropwise addition of tetrabutylammonium fluoride (8.0 mL, 1.0 M in THF) at −20 °C. The mixture was stirred at the same temperature until the starting material was consumed (1 h). After the addition of a few drops of sat. $NaHCO_3$, the solution was quickly filtered through a small layer of silica gel and the silica pad was washed with cold ethyl acetate. The collected filtrate was concentrated in vacuo and the residue was chromatographed (*n*-hexane/EtOAc, 2:1 to 1.2:1) to give the title compound (4.0 g, 75%) as a single isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.76, 1.93, 1.97 (3s, 15H), 3.35 (m, 1H, H-5^{II}), 3.41−3.45 (m, 3H, H-5^{I}, H-6^{I}, H-6′ ^{I}), 3.46 (t, 1H, J = 9.7 Hz, H-6^{II}), 3.58 (dd, 1H, J = 9.7, 11.4 Hz, H-4^{II}), 3.80 (dd, 1H, J = 8.1, 10.5 Hz, H-2^{I}), 4.00 (dd, 1H, J = 8.1, 10.5 Hz, H-2^{II}), 4.12−4.16 (m, 2H, H-3^{I}, H-4^{I}), 4.19 (dd, 1H, J = 5.3, 10.9 Hz, H-6′ ^{II}), 4.43, 4.82 (2d, 2H, J = 12.2 Hz, CH_2Ph), 4.57 (s, 2H, CH_2Ph), 5.07 (d, 1H, J = 8.1 Hz, H-1^{I}), 5.39 (s, 1H, C*H*Ph), 5.40 (d, 1H, J = 8.1 Hz, H-1^{II}), 5.65 (dd, 1H, J = 8.9, 10.5 Hz, H-3^{II}), 7.18−7.41 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.9, 20.9, 56.1 (C-2^{II}), 57.4 (C-2^{I}), 66.0 (C-5^{II}), 68.4 (C-6^{II}), 68.8 (C-6^{I}), 70.0 (C-3^{II}), 73.2, 74.5 (2*C*H$_2$Ph), 74.7 (C-5^{I}), 76.1 (C-3^{I}), 77.3 (C-4^{I}), 79.4 (C-4^{II}), 93.1 (C-1^{I}), 97.6 (C-1^{II}), 101.8 (*C*HPh), 126.5, 127.4, 127.7, 127.8, 128.0, 128.1, 128.4, 128.5, 128.6, 129.4, 137.2, 138.2, 139.1, 170.4, 172.0; MS (ESI) *m*/*z* 889.30 [M + Na]$^+$.

3-*O*-Acetyl-4,6-*O*-benzylidene-2-deoxy-2-dimethylmaleimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-*β*-D-glucopyranosyl Trichloroacetimidate (26). Compound **25** (2.9 g, 3.3 mmol) in anhydrous CH_2Cl_2 (15 mL) was treated with 2,2,2-trichloroacetonitrile (1.8 mL, 18.0 mmol) and a catalytic amount of DBU and the resultant solution was stirred at ice-water temperature. When the starting material was consumed completely (∼1 h), the reaction mixture was filtered through a small layer of silica gel. The filtrate was concentrated under scrupulously dry condition and the residue was kept under high vacuum for 0.5 h. This material was used without further purification because of adequate purity for the glycosidation step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.77, 1.94, 1.98 (3s, 15H), 3.35 (m, 1H, H-5^{II}), 3.47 (dd, 1H, J = 3.2, 11.4 Hz, H-6^{I}), 3.52 (t, 1H, J = 10.1 Hz, H-6^{II}), 3.55−3.62 (m, 2H, H-5^{I}, H-4^{II}), 3.68 (d, 1H, J = 11.3 Hz, H-6′ ^{I}), 4.02 (dd, 1H, J = 8.3, 10.3 Hz, H-2^{II}), 4.15−4.20 (m, 3H, H-6′ ^{II}, H-4^{I}, H-2^{I}), 4.28 (m, 1H, H-3^{I}), 4.47, 4.84 (2d, 2H, J = 12.2 Hz, CH_2Ph), 4.59, 4.63 (2d, 2H, J = 12.2 Hz, CH_2Ph), 5.40 (s, 1H, C*H*Ph), 5.44 (d, 1H, J = 8.1 Hz, H-1^{II}), 5.68 (t, 1H, J = 9.7 Hz, H-3^{II}), 6.15 (d, 1H, J = 8.9 Hz, H-1^{I}), 7.17−7.42 (m, 15H), 8.51 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.9, 20.9, 54.5 (C-2^{I}), 56.1 (C-2^{II}), 66.0 (C-5^{II}), 67.9 (C-6^{I}), 68.8 (C-6^{II}), 70.0 (C-3^{II}), 73.1, 74.6 (2*C*H$_2$Ph), 75.7 (C-5^{I}, C-3^{I}), 77.2 (C-4^{I}), 79.5 (C-4^{II}), 94.2 (C-1^{I}), 97.4 (C-1^{II}), 101.8 (*C*HPh), 126.5, 127.5, 127.8, 127.9, 128.2, 128.4, 128.5, 128.6, 129.4, 137.2, 138.3, 139.0, 161.1, 170.4, 171.4.

Methyl 3-*O*-Acetyl-4,6-*O*-benzylidene-2-deoxy-2-dimethylmaleimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-*β*-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-*β*-D-glucopyranoside (27). Compound **26** (3.0 g, 3.0 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) and was treated with compound **4** (1.6 g, 3.7 mmol) and 4-Å molecular sieves (3 g). After being stirred for 0.5 h at room temperature, the mixture was cooled to −5 °C and was treated with a catalytic amount of TfOH (0.56 mL, 1 M solution in CH_2C1_2). The reaction was monitored by TLC (R_f 0.71 for **26**, R_f 0.36 for **4**, R_f 0.50 for **27** in *n*-hexane/EtOAc, 1:2), as well as HPLC. Reverse-phase HPLC analysis was performed on a Waters Delta 600 liquid chromatography system, vydac protein/peptide C-18 column (5 μm, 4.6 × 250 mm), and monitored with a Waters 2996 photodiode array spectrometer equipped with the Millenium software. Isocratic condition (80% MeOH) with a flow rate of 0.8 mL/min was used. The unreacted glycosyl donor (**4**, t_R = 3.8 min) and the product (**27**, t_R = 7.7 min) were readily detected by this procedure. When the starting material was consumed completely (∼1−2 h), a few

drops of Et_3N was added and the solution was stirred at room temperature for 0.5 h. The reaction mixture was filtered through a small layer of silica gel, and the silica layer was washed with CH_2Cl_2. The filtrate was concentrated to dryness and the resultant residue was chromatographed (*n*-hexane/EtOAc, 1:2) to give compound **27** (2.3 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.83, 1.91, 1.92, 2.01 (4s, 18H), 1.76 (br s, 6H), 3.37 (s, 3H, OCH_3), 3.11 (2m, 1H, H-5^{II}), 3.20 (m, 1H, H-5^{III}), 3.32 (m, 1H, H-6^{II}), 3.36−3.43 (m, 2H, H-6^{I}, H-6′ ^{I}), 3.41 (m, 1H, H-6^{III}), 3.47 (m, 1H, H-5^{I}), 3.52 (dd, 1H, J = 8.9, 9.7 Hz, H-4^{III}), 3.59 (dd, 1H, J = 6.5, 10.6 Hz, H-6′ ^{II}), 3.78 (dd, 1H, J = 8.5, 10.9 Hz, H-2^{II}), 3.91−3.94 (m, 2H, H-3^{II}, H-4^{I}), 3.91 (t, 1H, J = 7.3 Hz, H-2^{I}), 3.97 (t, 1H, J = 8.1 Hz, H-2^{III}), 4.13 (dd, 1H, J = 5.3, 10.1 Hz, H-6′ ^{III}), 4.20 (dd, 1H, J = 8.9, 9.7 Hz, H-4^{II}), 4.38, 4.80 (2d, 2H, J = 12.2 Hz, CH_2Ph), 4.53 (s, 2H, CH_2Ph), 4.49, 4.60 (2d, 2H, J = 11.4 Hz, CH_2Ph), 4.94 (d, 1H, J = 8.1 Hz, H-1^{II}), 5.01 (d, 1H, J = 8.1 Hz, H-1^{I}), 5.35 (s, 1H, C*H*Ph), 5.36 (d, 1H, J = 8.1 Hz, H-1^{III}), 5.43 (dd, 1H, J = 9.7 10.5 Hz, H-3^{I}), 5.61 (t, 1H, J = 9.7 Hz, H-3^{III}), 7.12−7.39 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.0, 20.8, 20.9, 54.9 (C-2^{I}), 55.0 (C-2^{III}), 56.1 (C-2^{II}), 56.9 (OCH_3), 65.9 (C-5^{III}), 68.1 (C-6^{I}, C-6^{II}), 68.8 (C-6^{III}), 70.1 (C-3^{III}), 71.1 (C-3^{I}), 72.7, 73.1 (2CH_2Ph), 73.8 (C-3^{II}), 74.7 (C-5^{II}), 74.5 (CH_2Ph), 74.7 (C-5^{I}), 75.3 (C-4^{II}), 77.4 (C-4^{I}), 79.4 (C-4^{III}), 97.1 (C-1^{II}, C-1^{III}), 99.1 (C-1^{I}), 101.7 (*C*HPh), 126.5, 127.4, 127.5, 127.7, 127.9, 128.0, 128.1, 128.4, 128.6, 129.4, 137.2, 138.3, 138.7, 139.2, 170.4, 170.5; MS (ESI) *m*/*z* 1304.41 [M + Na]$^+$.

Methyl 3-*O*-Acetyl-6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (28). Compound **27** (1.3 g, 1.0 mmol) was dissolved in CH_3CN (10 mL) and the solution was cooled in an ice−water bath. Borane−trimethylamine complex (BH_3·NMe_3, 0.15 g, 2.0 mmol) and BF_3·OEt_2 (0.25 mL, 2.0 mmol) were added dropwise to the reaction mixture in the respective order. The flask was removed from the bath after 1 h and the solution was stirred at room temperature for 0.5 h, at which time TLC (*n*-hexane/EtOAc, 2:3) indicated complete conversion of a faster moving material. $NaHCO_3$ (0.14 g) was added to this solution and the solution was evaporated to dryness. The crude product was dissolved in ethyl acetate and washed with aq NaCl. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness to afford the crude product. Purification by chromatography on silica gel (*n*-hexane/EtOAc, 2:1 to 1.5:1) gave the desired compound, followed by crystallization from diethyl ether (0.80 g, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.75 (br s, 2CH_3), 1.80, 1.95 (2s, 2CH_3CO), 1.90, 2.00 (2s, 4CH_3), 3.11 (dd, 1H, J = 1.5, 9.6 Hz, H-5^{II}), 3.15 (dd, 1H, J = 1.5, 9.6 Hz, H-5^{III}), 3.36 (s, OCH_3), 3.37, 3.55 (m, 2H, H-6^{I}, H-6′ ^{I}), 3.39, 3.61 (m, 2H, H-6^{II}, H-6′ ^{II}), 3.47 (m, 1H, H-5^{I}), 3.47, 3.58 (m, 2H, H-6^{III}, H-6′ ^{III}), 3.68 (t, 1H, J = 9.1 Hz, H-4^{III}), 3.77 (dd, 1H, J = 8.6, 10.6 Hz, H-2^{II}), 3.88−3.95 (overlapping dd, 4H, H-2^{I}, H-2^{III}, H-3^{II}, H-4^{I}), 4.19 (dd, 1H, J = 8.6, 10.1 Hz, H-4^{II}), 4.35, 4.78 (2d, 2H, J = 12.2 Hz, CH_2Ph), 4.45 (s, 2H, CH_2Ph), 4.49, 4.61 (2d, 2H, J = 11.6 Hz, CH_2Ph), 4.52 (d, 2H, J = 2.0 Hz, CH_2Ph), 4.92 (d, 1H, J = 8.1 Hz, H-1^{II}), 5.00 (d, 1H, J = 8.6 Hz, H-1^{I}), 5.25 (d, 1H, J = 8.6 Hz, H-1^{III}), 5.39 (dd, 1H, J = 8.6, 10.6 Hz, H-3^{III}), 5.42 (dd, 1H, J = 9.6, 10.6 Hz, H-3^{I}), 7.05−7.35 (m, 20H, 4Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.0 (CH_3), 20.8, 20.9 (2CH_3CO), 54.9 (C-2^{I}), 55.4 (C-2^{III}), 55.9 (C-2^{II}), 56.9 (OCH_3), 68.0 (C-6^{I}), 68.2 (C-6^{II}), 70.3 (C-6^{III}), 71.1 (C-3^{III}), 72.0 (C-4^{III}), 72.5 (CH_2Ph), 73.1 (CH_2Ph), 73.1 (C-5^{III}), 73.6 (C-3^{I}), 73.9 (CH_2Ph), 73.9 (C-3^{II}), 74.3 (C-5^{II}), 74.5 (CH_2Ph), 74.7 (C-5^{I}), 74.9 (C-4^{II}), 77.3 (C-4^{I}), 96.3 (C-1^{III}), 97.2 (C-1^{II}), 99.1 (C-1^{I}), 127.2, 127.3, 127.5, 127.6, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 128.7, 137.1, 137.8, 138.4, 138.7, 139.2, 170.5, 171.1, 171.7; MS (ESI) *m*/*z* 1306.49 [M + Na]$^+$.

Methyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (29). Compound **28** (0.45 g, 0.35 mmol) and compound **7** (0.35 g, 0.57 mmol) were added to a suspension of activated 4-Å molecular sieves (0.5 g) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for 1 h under an argon atmosphere. The reaction mixture was cooled in an ice−water bath and *n*-hexane (3 mL) was added with vigorous stirring, followed by the addition of TfOH (52 μL, 1 M solution in CH_2C1_2) dropwise over a period of 15 min. Stirring was continued at the same temperature for 2 h, the reaction was quenched by the addition of a few drops of Et_3N, and the solution was filtered through a pad of Celite. The volatiles were removed in vacuo and the residue was purified by flash chromatography (*n*-hexane/EtOAc, 1.2:1) to give **29** in yields ranging from 59 to 68%. ^{1}H NMR (500 MHz, acetone-d_6) δ 1.78, 1.87, 1.94, 2.00, 2.01 (overlapping s, 30H, CH_3 and CH_3CO), 3.06 (dd, 1H, J = 1.5, 9.6 Hz, H-5^{III}), 3.14 (dd, 1H, J = 1.5, 9.6 Hz, C-5^{II}), 3.21 (dd, 1H, J = 3.0, 11.1 Hz, C-6^{III}), 3.32 (s, OCH_3), 3.43 (m, 1H, H-5^{IV}), 3.42 (dd, 1H, J = 4.2, 11.0 Hz, H-6^{I}), 3.47 (dd, 1H, J = 2.0, 9.1 Hz, H-6′ ^{III}), 3.48 (dd, 1H, J = 4.3, 10.1 Hz, H-5^{I}), 3.49 (dd, 1H, J = 3.9, 11.0 Hz, H-6^{II}), 3.58 (d, 1H, J = 9.9 Hz, H-6′ ^{I}), 3.66 (d, 1H, J = 10.6 Hz, H-6′ ^{II}), 3.71 (dd, 1H, J = 8.6, 10.6 Hz, H-2^{II}), 3.76 (t, 1H, J = 9.1 Hz, H-6^{IV}), 3.80 (dd, 1H, J = 8.7, 10.5 Hz, H-2^{I}), 3.81 (dd, 1H, J = 8.9, 9.9 Hz, H-2^{IV}), 3.81 (dd, 1H, J = 9.1, 10.2 Hz, H-4^{IV}), 3.94 (dd, 1H, J = 8.7, 10.5 Hz, H-2^{III}), 3.94 (t, 1H, J = 9.5 Hz, H-4^{I}), 3.95 (t, 1H, J = 9.4 Hz, H-4^{III}), 3.97 (dd, 1H, J = 9.1, 10.4 Hz, H-3^{II}), 4.19 (dd, 1H, J = 8.8, 10.0 Hz, H-4^{II}), 4.24 (dd, 1H, J = 8.6, 10.6 Hz, H-3^{IV}), 4.28 (dd, 1H, J = 4.8, 10.4 Hz, H-6′ ^{IV}), 4.42−4.55 (overlapping ds, 7H, CH_2Ph), 4.62 (d, 1H, J = 11.6 Hz, CH_2Ph), 4.75 (d, 1H, J = 12.2 Hz, CH_2Ph), 4.84 (d, 1H, J = 12.2 Hz, CH_2Ph), 4.98 (d, 1H, J = 8.6 Hz, H-1^{II}), 4.99 (d, 1H, J = 8.4 Hz, H-1^{I}), 5.14 (d, 1H, J = 8.6 Hz, H-1^{IV}), 5.29 (d, 1H, J = 8.6 Hz, H-1^{III}), 5.41 (dd, 1H, J = 9.1, 10.6 Hz, H-3^{I}), 5.50 (dd, 1H, J = 8.9, 10.4 Hz, H-3^{III}), 5.72 (s, 1H, C*H*Ph), 7.03−7.54 (m, 30H, 6Ph); ^{13}C NMR (125 MHz, acetone-d_6) δ 8.1, 8.2 (CH_3), 20.1, 20.3 (2CH_3CO), 54.9 (C-2^{IV}), 55.8 (C-2^{III}), 56.0 (C-2^{II}, C-2^{I}), 56.1 (OCH_3), 66.2 (C-5^{IV}), 67.3 (C-6^{III}), 68.2 (C-6^{I}, C-6^{II}), 68.5 (C-6^{IV}), 71.0 (C-3^{I}), 71.5 (C-3^{III}), 72.1 (CH_2Ph), 72.5 (CH_2Ph), 72.7 (CH_2Ph), 73.9 (CH_2Ph), 74.2 (C-5^{II}), 74.3 (C-3^{II}), 74.5 (CH_2Ph), 74.6 (C-5^{I}), 74.7 (C-5^{III}), 75.0 (C-4^{II}), 75.1 (C-3^{IV}), 75.5 (C-4^{I}), 77.2 (C-4^{III}), 82.7 (C-4^{IV}), 96.3 (C-1^{III}), 97.6 (C-1^{II}), 98.9 (C-1^{IV}), 99.1 (C-1^{I}), 101.2 (*C*HPh), 126.4, 127.4, 127.5, 127.6, 127.7, 128.1, 128.3, 128.4, 128.5, 128.8, 129.0, 137.1, 137.3, 138.0, 138.2, 138.7, 138.8, 138.9, 139.1, 139.2, 169.4, 169.7, 171.2, 172.1; MS (ESI) *m*/*z* 1304.41 [M + Na]$^+$.

Methyl 2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-*O*-benzyl-3-*O*-((*R*)-1′-carboxyethyl)-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-6-*O*-benzyl-3-*O*-((*R*)-1′-carboxyethyl)-2-deoxy-β-D-glucopyranoside (30). NaOH (1.0 g of solid pellets, 25 mmol) was added to a stirred solution of **29** (1.7 g, 1 mmol) in a 1,4-dioxane−water mixture (4:1, 50 mL) at 10 °C under a nitrogen atmosphere; the reaction mixture was sonicated. After 15 min, the mixture was allowed to warm to room temperature and was stirred for an additional 6 h. The pH was adjusted to 3 by the addition of 0.5 N HCl, and the pH of the solution was kept at 3.0 for 24 h. Evaporation to dryness of the reaction mixture gave a yellow oily residue, which was treated with MeOH (60 mL). Precipitated inorganic material was filtered off and washed well with cold MeOH, and the filtrate was evaporated to dryness. The residue in pyridine (5 mL) was treated with an excess of acetic anhydride (7 mL) and the resultant solution was stirred at room temperature overnight, followed by evaporation to dryness. The product was purified with flash chromatography using EtOAc/CH_3CN (3:2) to give **30** (0.7 g, 48%) as a white powder. ^{1}H NMR (500 MHz, 5% CD_3OD in $CDCl_3$) δ 1.71, 1.72, 1.76, 1.91, 1.97, 1.98 (6s, 18H), 3.19 (2m, 1H, H-5^{III}), 3.25 (m, 1H, H-5^{IV}), 3.29 (m, 1H, H-5^{II}), 3.44 (t, 1H, J = 9.2 Hz, H-2^{IV}),

3.40−3.50 (m, 4H, H-3^{II}, H-6^{III}, H-5^{I}, H-6^{I}), 3.52−3.64 (m, 6H, H-6′ ^{I}, H-6^{II}, H-6′ ^{II}, H-6^{IV}, H-3^{IV}, H-4^{IV}), 3.65−3.73 (m, 2H, H-3^{IV}, H-6′ ^{III}), 3.71 (t, 1H, J = 7.9 Hz,H-2^{II}), 3.80 (t, 1H, J = 8.4 Hz, H-4^{I}), 3.81 (t, 1H, J = 9.0 Hz, H-4^{III}), 3.92 (t, 1H, J = 9.6 Hz, H-2^{III}), 3.92 (t, 1H, J = 7.3 Hz, H-4^{II}), 4.02 (t, 1H, J = 8.6 Hz, H-2^{I}), 4.25 (d, 1H, J = 7.6 Hz, H-1^{I}), 4.25 (d, 1H, J = 8.4 Hz, H-1^{III}), 4.28 (dd, 1H, J = 4.9, 10.5 Hz, H-6′ ^{IV}), 4.33 (d, 1H, J = 8.1 Hz, H-1^{II}), 4.49 (d, 1H, J = 7.1 Hz, H-1^{IV}), 4.75 (t, 1H, J = 9.6 Hz, H-3^{III}), 4.95 (t, 1H, J = 8.9 Hz, 1H, H-3^{I}), 5.52 (s, 1H, C*H*Ph), 7.15−7.47 (m, 30H, 6Ph); ^{1}H NMR (500 MHz, 5% $CDCl_3$ in CD_3CN) δ 1.90, 1.94, 1.96, 2.01, 2.11 (5s, 18H), 3.29 (2m, 1H, H-5^{III}), 3.35 (m, 1H, H-5^{II}), 3.39 (m, 1H, H-5^{IV}), 3.56 (dd, 1H, J = 4.9, 11.4 Hz, H-6^{III}), 3.58 (m, 1H, H-3^{II}), 3.62 (m, 2H, H-5^{I}, H-6^{I}), 3.64 (dd, 1H, J = 8.5, 9.7 Hz, H-2^{II}), 3.69−3.74 (m, 3H, H-2^{IV}, H-3^{IV}, H-4^{IV}), 3.74−3.72 (m, 2H, H-6^{II}, H-6′ ^{II}), 3.78 (dd, 1H, J = 10.9 Hz, H-6′ ^{I}), 3.84 (m, 1H, H-6^{III}), 3.85 (m, 1H, H-6^{IV}), 3.86 (t, 1H, J = 9.5 Hz, H-2^{III}), 3.86 (m, 1H, dd, 1H, J = 8.5, 9.3 Hz, H-4^{III}), 3.87 (t, 1H, J = 9.3 Hz, H-2^{I}), 3.87 (m, 1H, H-4^{I}), 4.07 (t, 1H, J = 8.9 Hz, H-4^{II}), 4.36 (dd, 1H, J = 4.9, 9.7 Hz, H-6′ ^{IV}), 4.46 (d, 1H, J = 8.1 Hz, H-1^{I}), 4.49 (d, 1H, J = 8.5 Hz, H-1^{II}), 4.57 (d, 1H, J = 7.3 Hz, H-1^{IV}), 4.68 (d, 1H, J = 8.1 Hz, H-1^{III}), 5.05 (t, 1H, J = 9.7 Hz, 1H, H-3^{I}), 5.06 (t, 1H, J = 9.7 Hz, H-3^{III}), 5.52 (s, 1H, C*H*Ph), 7.06−7.50 (m, 30H, 6Ph); ^{13}C NMR (125 MHz, 5% $CDCl_3$ in CD_3CN) δ 20.5, 20.8 (2*C*H_3CO), 22.4, 22.6, 22.8 (4*C*H_3CONH), 53.8 (C-2^{I}), 54.9 (C-2^{III}), 55.2 (C-2^{II}), 55.5 (C-2^{IV}), 56.5 (O*C*H_3), 66.1 (C-5^{IV}), 68.3 (C-6^{III}), 68.6 (C-6^{I}), 68.7 (C-6^{IV}), 68.9 (C-6^{II}), 72.6 (*C*H_2Ph), 73.0 (*C*H_2Ph), 73.1 (*C*H_2Ph), 73.3 (C-3^{I}), 73.5 (C-3^{III}), 73.9 (*C*H_2Ph), 74.2 (*C*H_2Ph), 74.7 (C-5^{II}), 74.8 (C-5^{I}), 74.7 (C-5^{III}), 75.1 (C-4^{II}), 75.2 (C-4^{I}), 76.2 (C-4^{III}), 78.7 (C-3^{IV}), 80.7 (C-3^{II}), 81.9 (C-4^{IV}), 99.6 (C-1^{III}), 101.1 (C-1^{II}), 101.2 (*C*HPh), 101.8 (C-1^{IV}), 102.0 (C-1^{I}), 126.4, 127.5, 127.8, 128.0, 128.1, 128.4, 128.6, 128.7, 128.8, 129.2, 138.2, 138.7, 139.0, 139.7, 170.4, 170.5, 170.7, 170.8; MS (ESI) *m*/*z* 1467.77 $[M + H]^+$, 1489.79 $[M + Na]^+$.

Methyl 2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-6-*O*-benzyl-3-*O*-((*R*)-1′-carboxyethyl)-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-6-*O*-benzyl-3-*O*-((*R*)-1′-carboxyethyl)-2-deoxy-β-D-glucopyranoside (32). A solution of NaOMe (0.05 g) in dry MeOH (1 mL) was added dropwise to a suspension of **30** (1 g, 0.68 mmol) in dry MeOH (15 mL). The reaction mixture was stirred at room temperature for 1 h under an atmosphere of nitrogen and then after neutralization with acetic acid the solution was evaporated to obtain a residue, which was washed with cold water, then filtered and dried under vacuum. The white solid material was dispersed in a minimal amount of dry CH_3CN, stirred under an atmosphere of nitrogen for 1 h, and then filtered to give a pure dihydroxy derivative **31** (0.7 g, 74%), which was used in the next reaction without further purification. MS (ES) *m*/*z* 1383.75 $[M + H]^+$, 1405.70 $[M + Na]^+$.

The residue **31** (0.7 g, 0.51 mmol) was dissolved in anhydrous DMF (15 mL) and the solution was stirred in the presence of activated 4-Å molecular sieves (3 g), followed by treatment with NaH (0.5 g, 12.5 mmol, 60% oil dispersion), and (*S*)-2-chloropropionic acid (1.6 g, 14.7 mmol), and the mixture was allowed to stir at 10 °C for 0.5 h. Additional NaH (0.8 g, 20 mmol) in two portions was added to the reaction mixture and the resultant solution was stirred at room temperature overnight. The pH of the reaction mixture was brought to pH 4−5 by addition of dilute HCl and the suspension was filtered through a layer of Celite, which was subsequently washed well with MeOH. The volatiles were evaporated to dryness and the residue was taken up in a mixture of water and chloroform. The layers were separated and the aqueous portion was washed with chloroform. The combined organic layer was concentrated in vacuo to obtain a solid residue, which was purified by chromatography on a small silica gel column, eluting with CH_2Cl_2/MeOH (5:1) to afford the title compound (0.49 g) in 46% yield. ^{1}H NMR (400 MHz, 5% CD_3OD in $CDCl_3$) δ 1.29, 1.32 (2d, 6H, J = 6.4 Hz, O−CH−CH_3), 1.76, 1.78, 1.94, 1.95 (4s, 12H, NHCOCH_3), 2.85 (m, 1H), 3.12 (m, 1H), 3.20 (m, 1H), 3.30 (m, 1H), 3.38 (s, 3H, OCH_3), 3.38−3.51 (m, 5H), 3.54−3.74 (m, 10H), 3.74−3.95 (m, 5H), 4.00−4.62 (m, 9H, 4CH_2Ph and H-1), 4.03 (d, 1H, J = 8.1 Hz, H-1), 4.27 (d, 1H, J = 8.1 Hz, H-1), 4.41 (d, 1H, J = 8.1 Hz, H-1), 4.54−4.66 (overlapping 2q, 2H, O−C*H*-CH_3), 4.79 (t, 2H, J = 12.2 Hz, CH_2Ph), 5.58 (s, 1H, C*H*Ph), 7.00−7.45 (m, 30H, 6Ph); ^{13}C NMR (100 MHz, 5% CD_3OD in $CDCl_3$) δ 18.4, 18.9, 21.8, 23.2, 23.3, 53.8, 54.3, 55.2, 55.6, 56.6, 65.9, 67.7, 68.6, 68.9, 73.2, 73.5, 73.6, 74.2, 74.5, 74.8, 75.2, 75.3, 75.8, 76.7, 77.1, 77.4, 77.5, 77.7, 78.0, 80.6, 82.6, 100.5, 100.7, 101.3, 101.4, 103.1, 126.1, 127.3, 127.6, 127.8, 128.1, 128.3, 128.6, 128.8, 129.0, 129.3, 137.2, 137.9, 138.0, 138.1, 138.4, 139.2, 171.1, 171.6, 172.8, 173.1, 177.1, 177.6; MS (ESI) *m*/*z* 1550.91 $[M + Na]^+$.

Compound 34. *p*-Nitrophenyl trifluoroacetate (780 mg, 3.3 mmol) was added to a solution of **32** (510 mg, 0.33 mmol) in pyridine (20 mL) and the mixture was stirred at room temperature for 18 h. Water and dichloromethane were added into the reaction mixture and layers were separated. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure to give the crude material. Subsequently, diethyl ether was added and the precipitate was filtered, washed with diethyl ether, and dried over P_2O_5 to give the *p*-nitrophenyl ester of **32** (420 mg, 72%) as a white solid.

Peptide **33** (510 mg, 0.56 mmol) in dry DMF (30 mL) was neutralized with Et_3N (78 μL, 0.56 mmol) and was stirred at room temperature for 15 min. The *p*-nitrophenyl ester of **32** (300 mg, 0.17 mmol) was added into this amine solution and the resultant mixture was stirred at room temperature for 18 h. The solvent was removed in vacuo and the residue was suspended in water and filtered. The residue was purified by short-path column chromatography ($CHCl_3$/MeOH, 5:1) to give the fully protected **34** (300 mg, 57%). ^{1}H NMR (400 MHz, acetone-d_6) δ 1.29, 1.32 (2d, 6H, J = 6.4 Hz, O−CH−CH_3), 1.76, 1.78, 1.94, 1.95 (4s, 12H, NHCOCH_3), 2.85 (m, 1H), 3.12 (m, 1H), 3.20 (m, 1H), 3.30 (m, 1H), 3.38 (s, 3H, OCH_3), 3.38−3.51 (m, 5H), 3.54−3.74 (m, 10H), 3.74−3.95 (m, 5H), 4.00−4.62 (m, 9H, 4CH_2Ph and H-1), 4.03 (d, 1H, J = 8.1 Hz, H-1), 4.27 (d, 1H, J = 8.1 Hz, H-1), 4.41 (d, 1H, J = 8.1 Hz, H-1), 4.54−4.66 (overlapping 2q, 2H, O−C*H*−CH_3), 4.79 (t, 2H, J = 12.2 Hz, CH_2Ph), 5.58 (s, 1H, C*H*Ph), 7.00−7.45 (m, 30H, 6Ph); ^{13}C NMR (100 MHz, acetone-d_6) δ 16.9, 17.1, 17.4, 17.6, 17.7, 18.1, 18.5, 19.0, 22.3, 22.8, 23.0, 23.1, 23.2, 27.0, 29.7, 29.8, 29.9, 30.1, 31.4, 40.7, 48.3, 48.5, 48.6, 48.8, 49.6, 51.5, 51.7, 52.6, 54.6, 54.9, 55.1, 55.9, 56.4, 65.8, 66.2, 66.4, 66.5, 66.6, 68.7, 69.8, 72.7, 72.9, 73.5, 74.0, 74.2, 74.6, 75.1, 75.4, 75.8, 76.6, 77.0, 78.4, 79.0, 80.7, 82.6, 99.8, 100.1, 100.9, 102.5, 126.5, 127.5, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.5, 128.6, 128.7, 136.3, 136.4, 136.5, 137.8, 138.4, 139.0, 139.1, 139.3, 139.9, 156.7, 169.5, 170.5, 170.9, 171.0, 171.6, 172.4, 172.5, 172.6, 172.9, 173.2, 173.8.

Compound 3. Compound **34** (110 mg, 36 μmol) was dissolved in 60% acetic acid (10 mL), and the solution was stirred at 70 °C for 1h. The solvent was removed under reduced pressure. The residue was dissolved in MeOH (20 mL), and the stirred mixture was cautiously treated with Pd/C (10%, 0.2 g) and then kept under an atmosphere of hydrogen with continued stirring at room temperature overnight. The mixture was diluted with MeOH (20 mL) and filtered through a layer of Celite, and the Celite pad was washed with MeOH. The volatiles were evaporated in vacuo. Purification by preparative thin-layer chromatography (^{n}BuOH/AcOH/H_2O, 3:2:1) afforded **3** (56 mg, 76%). The purity of the isolated product was analyzed by HPLC. Reverse-phase HPLC analysis was performed on a Waters Delta 600 liquid chromatography system, delta-pak C-18 column (5 μm, 3.9 × 150 mm), and monitored with a Waters 2414 refractive index detector equipped with the Millenium software. Isocratic condition (90% acetonitrile) with a flow rate of 0.75 mL/min was used. The retention time of

compound **3** was 4.97 min and the purity was 97%. The chromatogram of **3** is given in the Supporting Information. ^{1}H NMR (500 MHz, D_2O) δ 1.20−1.29 (overlapping d and m, 28H), 1.54 (t, 4H), 1.65 (m, 4H), 1.77−1.92 (overlapping s and m, 15H), 1.95 (m, 2H), 2.19 (m, 4H), 2.85 (t, 4H), 3.32 (s, 3H), 3.25−3.35 (m, 4H), 3.38−3.64 (m, 15H), 3.68−3.77 (m, 7H), 3.97−4.30 (m, 13H), 4.35−4.39 (overlapping d, 3H); ^{13}C NMR (125 MHz, D_2O) δ16.7, 17.1, 17.2, 17.5, 18.0, 18.2, 22.2, 22.3, 22.4, 26.4, 28.5, 30.6, 32.0, 39.3, 49.7, 50.0, 54.2, 54.3, 54.8, 55.5, 56.1, 57.2, 59.9, 60.0, 60.4, 61.2, 70.3, 72.3, 73.7, 74.9, 75.1, 75.2, 75.8, 76.2, 78.1, 78.4, 79.5, 79.6, 100.6, 101.9, 102.2, 174.2; HRMS (ESI) $[M + 2H]^{2+}$ calcd for $C_{79}H_{134}N_{16}O_{39}^{2+}$ 965.4492, found 965.4446.

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Supporting Information Available: Experimental procedures for syntheses of the monomer compounds in Scheme 2 and the pentapeptide synthesis of Scheme 6 and ^{1}H, ^{13}C NMR, H−H COSY, and HMQC spectra for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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