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Note

Synthesis of the NAG–NAM disaccharide via a versatile intermediate

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ABSTRACT

A simple strategy for the synthesis of a β-GlcNAc-(1→4)-MurNAc (NAG–NAM) moiety, crucial for the preparation of synthetic components of a bacterial peptidoglycan, was achieved. This strategy relies on the use of three O-protecting groups, 4,6-O-benzilidene acetal, benzyl, and acetyl group, which allows further regioselective manipulation at O-3, O-4 positions, and on the insertion of the peptide chain at the lactate moiety in an advanced and versatile intermediate. Overall, a simple route to achieve the biological relevant NAG–NAM is presented, which may serve as a conceptual framework in the designing of synthetic strategies of different natural and non-natural polysaccharides.

The increasing occurrence of drug-resistant infections has stimulated the scientific community to develop new antibiotics against vancomycin-resistant *Enterococci* and methicillin-resistant *Staphylococcus aureus*, as well as to identify novel drug targets, or alternative strategies, that may help fighting bacterial infections.[1,2]

Peptidoglycan (PGN), a major constituent of the bacterial cell wall, has been well-known as a strong immunopotentiator.[3] PGN is recognized by invertebrate and vertebrate innate immune system (IIS) and is capable of inducing an innate immune response. PGN is able to induce many types of mediators, such as prostaglandins, platelet activation factor, NO, and cytokines that stimulate the immune system. Thus, PGN constitutes an excellent target to study recognition mechanisms and pathways by the IIS and novel strategies to modulate an IIS response. PGN is composed of polysaccharide chains linked through a peptide network that together assembles the rigid three-dimensional structure. The polysaccharide chain is constituted of repeating units of *N*-acetyl glucosamine (NAG) and N-acetylmuramic acid (NAM) linked by a β(1-4) glycosidic bond, which is crosslinked to a peptide network via the carboxy group of the NAM moiety (Fig. 1).[4] Unfortunately the study of PGN recognition has been hampered by the lack of pure and homogeneous fragments of PGN. Isolation of substantial amounts of these structures from natural sources is difficult, while the assembly of this complex structure has been extremely challenging from the synthetic point of view.

Excellent synthetic strategies for general PGN fragments have been reported.[5] In order to control the regioselective and enantioselective glycosylation, most of the synthetic sequences developed to date involve long multi-step sequences (up to 37 steps) with many protection–deprotection steps. In fact glycosylation of the 4-hydroxyl group of NAG derivatives is notoriously difficult, due to the well-known lack of reactivity of this hydroxyl group which is due to a combination of steric hindrance and to the involvement of the *N*-acetyl group in a hydrogen-bonded network.[6] The glycosylation of complex aglycones with glycosyl donors bearing a 2-acetamido-2-deoxy functionality is usually unfeasible, due to the formation of a rather stable 1,2-O,N-oxazoline intermediate during glycosylation, which significantly decreases the rate of glycosylation and yields.[7]

Properly functionalized glucosamine disaccharides constitute key scaffolds in the synthesis of complex and biologically important oligosaccharides, such as the *S. aureus* bacterial PGN (Fig. 1).

Recently we have explored a one-pot regioselective protection of glucosamine moieties (both donor and acceptor).[8] However, the glycosylation failed to occur under one-pot conditions. A synthetic strategy was also explored to prepare O-3-hydroxyl free *N*-acetyl glucosamine disaccharide.[9] Although, the introduction of the lactate moiety after glycosylation turned to be more difficult than anticipated and lower yields were obtained when compared to the introduction on the monomeric residue. The use of a fully acetylated glucosamine moiety limited a straightforward functionalization at this unit.

Pursuing our program directed toward the chemical synthesis of glucosamine building blocks, which is required for the synthesis of monomeric and dimeric muropeptides[8–10] (the smallest components of the PGN macromolecule), we report herein an improved and versatile preparation of β-GlcNAc-(1→4)-MurNAc (NAG–NAM) building blocks (Scheme 1). A suitable and selective protection/deprotection strategy involves as few functional group

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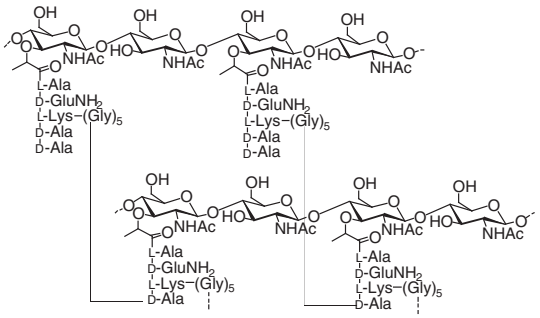
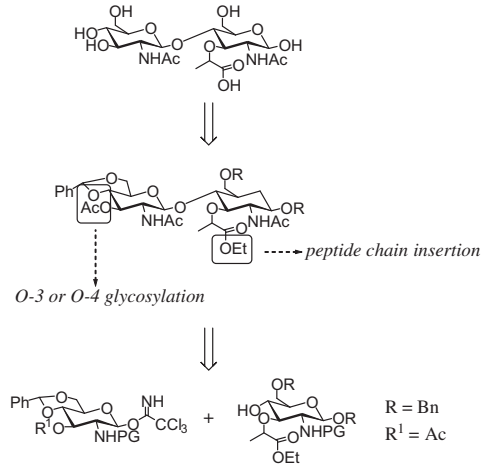


Figure 1. Structure of the *S. aureus* peptidoglycan (a Lys-type PGN).



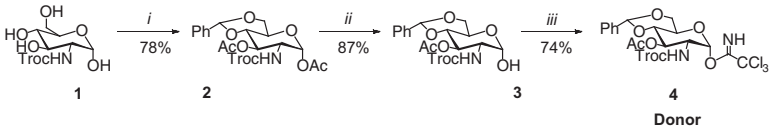
Scheme 1. Proposed approach toward NAG–NAM.

manipulations as possible, though affording regioselective control over further functionalization. We envisaged that introduction of an acetyl group at O-3 of the donor moiety would lead to a versatile NAG–NAM derivative disaccharide (after glycosylation with a NAM based acceptor).

The synthesis of a NAG–NAM disaccharide started with the selection of suitable protecting groups for the monomeric building blocks (donor and acceptor). The strategy for the preparation of valuable *N*-acetyl glucosamine disaccharide involved the synthesis of a glycosyl trichloro-acetamidate donor **4** (Scheme 2) with an acetate group at the O-3 position and a glucosamine acceptor **10** (Scheme 3). Troc (2,2,2-trichloro-ethoxycarbonyl) group was chosen as N-protecting group for both donor and acceptor due to the higher reactivity and β-selectivity at glycosylation when compared to *N*-acetyl-, *N*-Phth-glucosamines, and other N-protecting groups as well can be removed under mild conditions.[7] Thus, starting from D-glucosamine hydrochloride, protection of amino group was performed using $NaHCO_3$ and TrocCl for the donor moiety **1** and with (Allyl chloroformate) AllocCl for the acceptor moiety **5**.[5c]

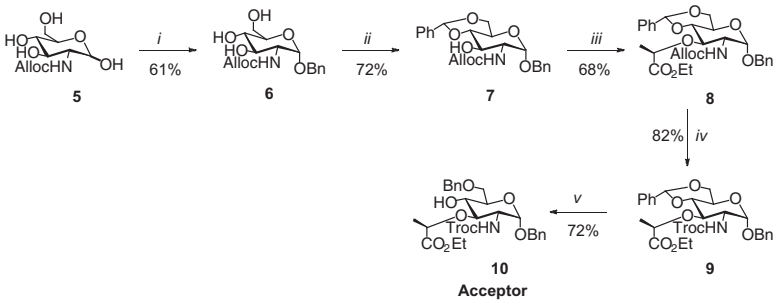
For the preparation of the donor moiety **4** (Scheme 2), after N-protection, the benzylidene acetal formation was achieved with zinc chloride and benzaldehyde followed by a classical acetylation to give **2** with 78% yield after two steps.[11] Selective removal of the anomeric acetyl group was performed by treatment with morpholine,[12] and **3** was obtained after column chromatography purification in 87% yield. Treatment of 3 with CCl_3CN and Cs_2CO3 afforded the desired glycosyl trichloro-acetamidate **4** that was used in the glycosylation step without further purification.

In order to prepare the acceptor moiety, *N*-alloc glucosamine **5** was treated with acetyl chloride in benzyl alcohol at 0 °C, followed by benzylidene acetal formation to give **7** (Scheme 3). While allyl ether group has been often used as anomeric protecting group, the benzyl group was selected to avoid the use of expensive metal catalysts frequently employed in deallylation procedures,[13] in addition to a full disaccharide deprotection in just one step (hydrogenation) in a late stage of the synthesis. Lactate moiety insertion at O-3 required the preparation of (−)-ethyl (*S*)-2-trifluromethanesulfonyl propionate. Thus, compound **7** was treated with NaH and then the freshly prepared OTf-ester was added drop-wise to afford **8** in 68% yield.[5c] The next step consisted on the replacement of *N*-Alloc group by *N*-Troc, via addiction of



i) a) PhCHO, $ZnCl_2$, 3 A MS, RT, overnight; b) Ac_2O, Pyridine, RT, overnight; *ii*) Morpholine, EtOAc, RT, overnight; *iii*) CCl_3CN, CS_2CO_3, DCM, RT, 2h.

Scheme 2. Synthesis of the glucosamine donor (NAG).



i) BnOH, AcCl, 80°C, 3h; *ii*) PhCHO, $ZnCl_2$, overnight; *iii*) Ethyl L-(*S*)-2- trifluoromethanesulphonyloxy-propionate, NaH, DCM, RT, 3h; *iv*) $Pd(PPh_3)_4$, AcOH, TrocCl, RT; *v*) BH_3Me_2N, BF_3OEt_2, RT, 3h.

Scheme 3. Synthesis of the glucosamine acceptor (NAM).

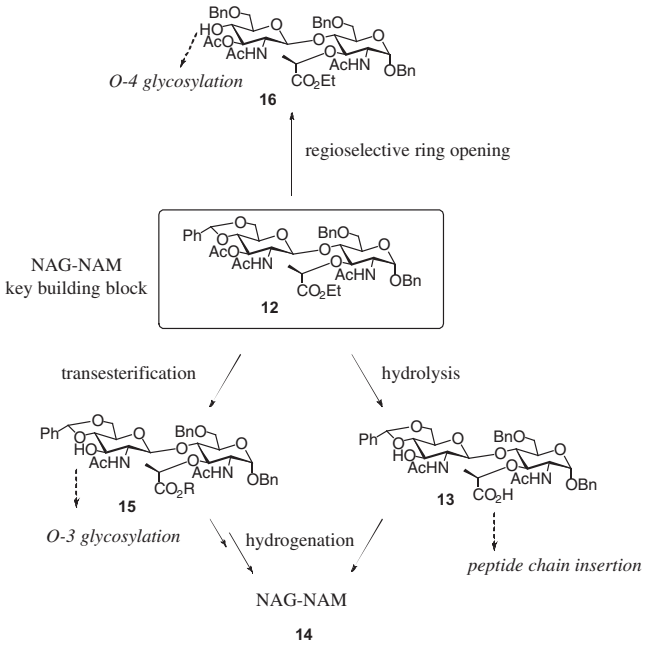
$Pd(PPh_3)_4$ to remove alloc group followed by in situ N-protection with the TrocCl to afford **9** in 82% yield. The acceptor **10** was obtained by regioselective benzylidene acetal ring opening under reductive conditions, by treatment of **9** with BH_3NMe_2 and $BF_3{\cdot}OEt_2$.

Glycosylation of donor **4** and acceptor **10** was achieved by treatment with TMSOTf at −15 °C giving the desired β(1-4)-linked disaccharide **11** in 60% yield (Scheme 4).

Exchange of both trichloroethyl carbamate groups by the natural *N*-acetyl group was performed after the glycosylation. Thus, Troc groups were removed by addiction of freshly prepared Zn–Cu couple[14] in AcOH/Ac_2O/THF, followed by N-acetylation to obtain **12** in 63% yield. Hydrolysis of **12** was performed by treatment with LiOH, which simultaneously led to the removal of the O-3 acetyl group and lactic acid formation, giving disaccharide **13** in 69% yield. The last step of the NAG–NAM synthesis consisted on the removal of the benzyl groups at O-1, O-6 of the muramyl residue and the benzylidene ring at O-6 and O-4 of the NAG unit by hydrogenation using $Pd(OH)_2$ on charcoal. The NAG–NAM disaccharide (**14**) was isolated in quantitative yield.[15]

Disaccharide **12**, a NAG–NAM precursor, constitutes a valuable intermediate for the assembly of peptidoglycan fragments and its non-natural derivatives. This is a highly useful strategy to achieve scaffolds possessing different substitution patterns on behalf of exploring key interactions with various biological targets and for molecular recognition studies to understand the innate immune response to bacterial infections, an emergent area of research.[16–18] In this sense, **12** is a key intermediate in the synthesis of NAG–NAM derivatives since it allows selective removal of O-3 protecting group (acetyl) by a transesterification reaction to give **15**, opening the possibility of a regioselective glycosylation at this position to obtain trisaccharides (Scheme 5). Additionally, **12** can be fully hydrolyzed to **13** to which a peptide chain can be coupled at the lactate group giving a straightforward access to a glycopeptide scaffold (unpublished results). Regioselective ring opening of **12** furnishes **16** with a O-4 free position for further glycosylation, according to a reported procedure.[5a]

In order to explore the insertion of a peptide chain at the lactate moiety of **13**, a pentapeptide containing the sequence of the Lys-type PGN was prepared via Fmoc (9-fluorenylmethoxycarbonyl) solid-phase peptide synthesis (SPPS) (Scheme 6). Thus HMPB-AM resin was used and the Fmoc-protected aminoacids sequentially

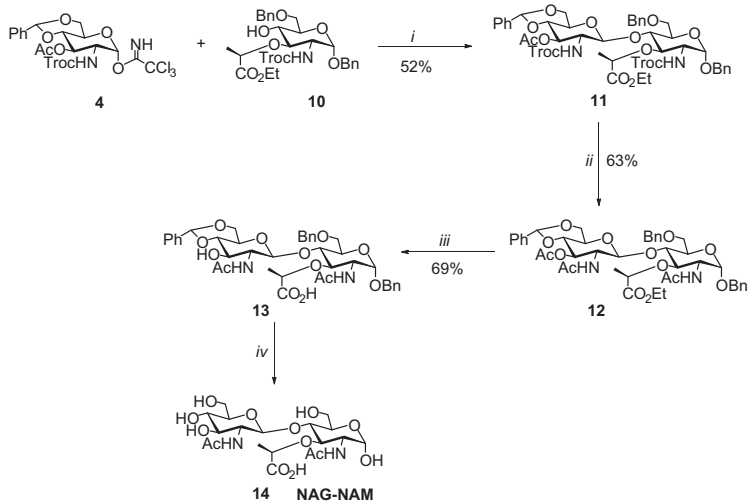


Scheme 5. Versatility of the NAG–NAM building block **12**.

added by traditional SPPS activation protocols, as well as disaccharide **13**. The reaction was monitored by high-resolution magic angle spinning NMR (HR-MAS NMR).[10] The glycopeptide **17** was isolated after cleavage from the resin with a TFA solution.

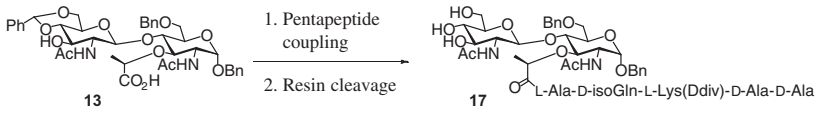
Despite the admirable synthetic approaches reported so far toward PGN fragments,[5] the orthogonal protecting group approach is still a puzzling strategy. In the synthesis of PGN there are some intricate steps, in particular the lactate insertion at O-3 and the establishment of a β(1-4) glycosidic bond for which a proper N-protecting group in both donor and acceptor is demanding.

In conclusion, the current work represents a simple strategy toward NAG–NAM unit which relies on the different protection of hydroxyl groups, using benzyl, benzylidene acetal, and acetyl group, allowing further regioselective manipulation at O-3, O-4 positions and also at the lactate moiety of a versatile intermediate



i) TMSOTf, DCM, 3A MS, -15 ºC; *ii*) Zn-Cu, THF: AcOH: Ac_2O (1:1:1), then Ac_2O/pyridine; *iii*) LiOH, THF:1,4-dioxane:H_2O; *iv*) $Pd(OH)_2$, H_2, AcOH, RT.

Scheme 4. Synthesis of the NAG–NAM disaccharide.



Scheme 6. Insertion of a pentapetide to **13** via Fmoc SPPS.

12. The conjugation of these protecting groups reduces the number of synthetic steps while allowing a complete protecting group removal in a late stage of the synthesis. Overall, this is a useful route to achieve the biologically relevant NAG–NAM disaccharide moiety crucial for the preparation of bacterial peptidoglycan as well as non-natural derivatives.

1. Experimental

1.1. General

Melting points were determined with a Reichert–Thermovar hot stage apparatus and are uncorrected. Optical rotation was measured for solutions in a 1 cm cell with a Perkin-Elmer 241 MC polarimeter. Matrix-assisted laser desorption ionization-time of flight spectra were recorded on a Voyager DE PRO Biospectrometry Workstation. High resolution mass spectra were recorded on an AutoSpeQ spectrometer. ^{1}H NMR spectra (400 MHz and 600 MHz) and ^{13}C NMR spectra (100.63 MHz and 150 MHz) were recorded on a Bruker ARX 400 spectrometer and on a Bruker Ultrashield Plus 600, respectively. NMR spectra were calibrated using solvent signals ($CDCl_3$: δ ^{1}H 7.26 and δ ^{13}C 77.00). Chemical shifts reported are relative to tetramethylsilane (TMS) as the internal reference (^{1}H 0.00) for ^{1}H NMR spectra and are expressed in parts per million (ppm), downfield from TMS ($\delta = 0$). All reagents and solvents were purified and dried by standard methods[19] before use. After classical work-up organic extracts were dried over anhydrous sodium sulfate or magnesium sulfate, filtered, and concentrated under reduced pressure (rotary evaporator and vacuum pump). Analytical thin-layer chromatography and preparative TLC (PTLC) were performed on E. Merck Kieselgel 60, F254 silica gel (0.2 mm thick) and 0.5, 1, or 2 mm thick plates (20 × 20 cm), respectively. Column chromatography was performed on E. Merck Kieselgel 60 (240–400 mm) normal silica gel. 'rt' denotes room temperature. (−)-Ethyl (*S*)-2-hydroxypropionate and Fmoc-aminoacids were purchased from Sigma–Aldrich.

1.2. 1,3-di-*O*-acetyl 2-deoxy-4,6-O-benzylidene-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (2)

Zinc chloride (763 mg, 5.6 mmol) was added to a solution of prepared *N*-Troc glucosamine[5c] (2 g, 5.6 mmol) in benzaldehyde (10 ml) and 3 Å MS (200 mg). After stirring overnight at rt saturated aq $NaHCO_3$ (10 mL) and ether (25 mL) were added and the reaction mixture was stirred for 10 min. The precipitate was filtered, washed with water, then ether, and dried. The residue was dissolved in pyridine (4.5 mL) cooled to 0 °C, treated with Ac_2O (2.24 mL, 24 mmol) and stirred overnight at rt. The residue was concentrated with toluene, cooled to 0 °C and extracted with $CHCl_3$ and saturated aq $NaHCO_3$ (3×), then brine. The combined organic layers were dried, concentrated, and the crude was purified by column chromatography (Hexane/EtOAc (1:1)) to give **2** as a white solid (2.3 g, 78%). Mp 98–101 °C; $[\delta]_D^{23}$ +44.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C): δ_H 7.46–7.36 (m, 5H, ArH), 6.21 (d, 1H, *J* 3.6 Hz, H-1), 5.55 (s, 1H, CHPh), 5.39 (t, 1H, *J* 10.2 Hz, H-3), 5.25 (d, 1H, *J* 9.6, NH), 4.80 (d, 1H, *J* 12 Hz, CH_2CCl_3), 4.65 (d, 1H, *J* 12 Hz, CH_2CCl_3), 4.32 (dd, 1H, *J* 10.4 Hz, *J* 4.8 Hz, H-6b), 4.19 (td, 1H, *J* 3.6 Hz, *J* 3.6 Hz, *J* 3.2 Hz, H-2), 3.94 (td, 1H, *J* 4.8 Hz, H-5), 3.81-3.75 (m, 2H, H-4, H-6a), 2.21 (s, 3H,$COCH_3$), 2.11 (s, 3H,$COCH_3$).^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ_c 171.3 ($COCH_3$), 168.9 ($COCH_3$), 154.2 ($COCH_2CCl_3$), 136.6 (Ar*C*), 129.2 (Ar*C*), 128.2 (Ar*C*), 126.0 (Ar*C*), 101.6 (*C*HPh), 95.2 (CCl_3), 90.92 (C-1), 78.51 (C-4), 74.5 (CH_2CCl_3), 69.3 (C-3), 68.4 (C-6), 64.8 (C-5), 53.8 (C-2), 20.7 (2 × $COCH_3$). MALDI-TOF: $C_{20}H_{22}Cl_3NO_9$+Na: 548.0257. Found: 548.044.

1.3. 2-Deoxy-3-*O*-acetyl-4,6-O-benzylidene-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (3)

Morpholine (200 μL, 2.3 mmol) was added to a solution of compound **2** (500 mg, 0.95 mmol) in dry ethyl acetate (1.5 mL). After stirring overnight at rt the reaction mixture was quenched with a 3 N HCl solution (0.7 mL) and then stirred for 20 min. Extracted with EtOAc and washed with water, saturated aq $NaHCO_3$ and brine, dried, and concentrated. The crude was purified by column chromatography (Toluene/EtOAc (10:2)) to give **3** as a white solid (401 mg, 87%). Mp 148–151 °C (decompose), $[\delta]_D^{23}$ −11.2 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C): δ_H 7.46–7.43 (m, 2H, ArH), 7.38–7.35 (m, 3H, ArH), 5.53 (s, 1H, CHPh), 5.43 (t, 1H, *J* 9.8 Hz, H-3), 5.33 (t, 1H, *J* 3.6 Hz, H-1), 4.80 (d, 1H, *J* 12 Hz, CH_2CCl_3), 4.67 (d, 1H, *J* 12 Hz, CH_2CCl_3), 4.29 (dd, 1H, *J* 10.4 Hz, *J* 4.8 Hz, H-6b), 4.17 (td, 1H, *J* 4.8 Hz, H-5), 4.05 (td, *J* 2.8 Hz, H-2), 3.80-3.69 (m, 2H, H-6a, H-4), 2.06 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ171.1 ($COCH_3$), 154.5 ($COCH_2CCl_3$), 137.0 (ArC), 129.3 (ArC), 128.4 (ArC), 126.3 (ArC), 101.7 (*C*HPh), 95.5 (CCl_3), 92.7 (C-1), 79.31 (C-4), 74.7 (CH_2CCl_3), 69.9 (C-3), 69.0 (C-6), 62.9 (C-5), 55.0 (C-2), 21.0 ($COCH_3$). HR-MS (FI): *m*/*z*: $C_{18}H_{20}Cl_{13}NO_8$: 483.0254. Found: 483.0255.

1.4. 1-(2,2,2-Trichloroacetimine) 2-deoxy-3-*O*-acetyl-4,6-*O*-benzylidene-2-(2,2,2-tri-chloroethoxycarbonylamino)-α-D-glucopyranoside (4)

To a solution of **3** (260 mg, 0.54 mmol) in dry DCM (5.2 ml), Cs_2CO_3 (82.3 mg, 0.25 mmol) and CCl_3CN (520 μL, 5.18 mmol) were added. The reaction mixture was stirred for 2 h at rt, then filtered over celite and concentrated to give donor **4** as a light yellow solid (248 mg, 74%). Compound **4** was directly used for next reaction step (synthesis of compound **11**) without further purification. $[\delta]_D^{23}$ +22.3 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C): δ_H 8.76 (s, 1H, $NHCCl_3$), 7.47–7.36 (m, 5H, ArH), 6.40 (d, 1H, *J* 3.6 Hz, H-1), 5.57 (s, 1H, CHPh), 5.45 (t, 1H, *J* 10.2 Hz, H-3), 5.31 (d, 1H, *J* 9.2 Hz, NH), 4.72 (q, 2H, *J* 12 Hz, CH_2CCl_3), 4.36 (dd, 1H, *J* 10.4 Hz, *J* 4.8 Hz, H-6b), 4.27 (td, 1H, *J* 4 Hz, *J* 3.6 Hz, *J* 3.6 Hz, H-2), 4.08-4.03 (m, 1H, H-5), 3.86-3.78 (m, 2H, H-4, H-6a), 2.11 (s, 3H, CH_3CO).^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ 171.2 ($COCH_3$), 160.8 (C=NH) 154.4 ($COCH_2CCl_3$), 136.7 (ArC), 129.3 (Ar*C*), 128.4 (Ar*C*), 126.2 (Ar*C*), 101.7 (*C*HPh), 95.3 (CCl_3) 95.2 (C-1),78.5 (C-4), 74.7 (CH_2CCl_3), 69.3 (C-3), 68.6 (C-6), 65.4 (C-5), 54.7 (C-2) 21.0 ($COCH_3$).

1.5. Benzyl 2-deoxy-2-(allyloxycarbonylamino)-α-D-glucopyranoside (6)

Prepared *N*-Alloc glucosamine[5c] (9.5 g, 36.3 mmol) was dissolved in BnOH (57 mL) and acetyl chloride (9.8 mL, 137 mmol)

was added dropwise at 0 °C. After stirring for 3 h at 80 °C the reaction mixture was quenched with cold saturated aq $NaHCO_3$ and stirred for 30 min. Cold water and ether were added and the reaction mixture was stirred for 30 min. The ppt was filtered and washed several times with cold ether (until no traces of BnOH). Product **6** was obtained as a white solid (7.86 g, 61% calculated yield) and it was directly used for next reaction step without further purification. The acquired spectroscopic data is in accordance with the literature.[20] Mp 120–122°C; $[\delta]_D^{22}$ +161.4 (*c* 0.5, MeOH). ^1H NMR (400 MHz, DMSO-d_6, 23 °C): δ_H 7.35–7.25 (m, 5H, ArH), 7.09 (d, 1H, *J* 8.4 Hz, NH), 5.91–5.84 (m, 1H, C*H*=CH_2), 5.26 (dd, 1H, *J* 17.2 Hz, *J* 1.2 Hz, CH=C*H*$_2$), 5.18–5.15 (m, 1H, OH, CH=C*H*$_2$), 4.97 (d, 1H, *J* 5.6 Hz, OH), 4.78 (t, 1H, *J* 5.6 Hz, OH), 4.73 (d, 1H, *J* 3.4 Hz, H-1), 4.64 (d, 1H, *J* 12.5 Hz, CH_2Ph), 4.47–4.39 (m, 3H, CH_2-Ph, —C*H*$_2$—CH=CH_2), 3.70–3.34 (m, 5H+H_2O-DMSO-d_6, H-6b, H-3, H-6a, H-5, H-2), 3.21–3.12 (m, 1H, H-4). ^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ_c 156.7 (NH*C*O), 138.2 (Ar*C*), 134.0 (*C*H=CH_2), 128.7 (Ar*C*), 128.0 (Ar*C*), 117.5 (CH=*C*H$_2$), 96.4 (C-1), 73.3 (C-5), 71.1 (C-4), 70.9 (C-3), 68.2 (*C*H$_2$Ph), 64.9 (*C*H$_2$CH=CH_2), 61.3 (C-6), 56.2 (C-2). HR-MS (FI): $C_{17}H_{23}NO_7$: 353.1474. Found: 353.1455.

1.6. Benzyl 2-deoxy-4,6-*O*-benzylidene-2-(allyloxycarbonylamino)-α-D-glucopyranoside (7)

Zinc chloride (313 mg, 2.3 mmol) was added to a solution of **6** (802 mg, 2.3 mmol) in benzaldehyde (4 mL) and 3 Å MS (325 mg). After stirring for 12 h at rt the reaction mixture was treated with saturated aq $NaHCO_3$ (5 mL), petroleum ether (30 mL), and stirred for 10 min. The ppt was filtered, washed with petroleum ether and dissolved in $CHCl_3$. The organic solution was extracted with saturated aq $NaHCO_3$, water, and brine, dried and concentrated. The crude was purified by column chromatography (10:1→10:2→10:3 $CHCl_3$/EtOAc) to give **7** as a white solid (733 mg, 72%). Mp 182–184 °C; $[\delta]_D^{25}$ +92 (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.51–7.32 (m, 5H, ArH), 5.95–5.87 (m, 1H, C*H*=CH_2), 5.56 (s, 1H, CHPh), 5.31 (dd, 1H, *J* 17.2 Hz, *J* 1.4 Hz, CH=C*H*$_2$), 5.23 (d, 1H, *J* 10.4 Hz, CH=C*H*$_2$), 5.10 (d, 1H, *J* 7.0 Hz, NH), 4.96 (d, 1H, *J* 2.4 Hz, H-1), 4.74 (d, 1H, *J* 11.7 Hz, CH_2Ph), 4.58 (d, 1H, *J* 5.6 Hz, C*H*$_2$—CH=CH_2), 4.51 (d, 1H, *J* 11.7 Hz, CH_2Ph), 4.24 (dd, 1H, *J* 10.1 Hz, *J* 4.7 Hz, H-6a), 3.95–3.84 (m, 3H, H-2, H-4, H-6b), 3.76 (t, 1H, *J* 10.2 Hz, H-3), 3.59 (t, 1H, *J* 8.9 Hz, H-5). ^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ_c 156.9 (NH*C*O), 134.6 (Ar*C*), 132.6 (*C*H=CH_2), 129.0 (Ar*C*), 128.4 (Ar*C*), 128.1 (Ar*C*), 127.9 (Ar*C*), 126.4 (Ar*C*), 117.6 (CH=*C*H$_2$), 101.9 (*C*HPh), 97.4 (C-1), 82.0 (C-4), 69.8 (*C*H$_2$Ph), 69.2 (C-3), 68.3 (C-6), 62.6 (C-5), 65.7 (O*C*H$_2$-CH=CH_3), 55.9 (C-2). MALDI-TOF: $C_{24}H_{27}NO_7$+Na: 464.1685. Found: 464.266.

1.7. Benzyl 2-deoxy-3-*O*-((*R*)-1′-ethoxycarbonylethyl)-4,6-*O*-benzylidene-2-(allyloxy-carbonylamino)-α-D-glucopyranoside (8)

Preparation of (−)-ethyl (*S*)-2-trifluromethanesulfonyl propionate: To a solution of (−)-ethyl (*S*)-2-hydroxypropionate (0.23 mL, 2 mmol) in dry DCM (0.56 mL) was added 2,6-lutidine (236 μL, 2 mmol). The reaction mixture was kept at −78 °C and Tf_2O (344 μL, 2 mmol) was added dropwise, stirred for 40 min, warmed up to rt and stirred for 1 h. The mixture was diluted with dry DCM: Hexane ((1:1) 0.7 mL) and filtered through a short silica gel column (approx. pack 1 cm of silica gel pad). Then the reaction mixture was washed with 0.7 mL of dry DCM: Hexane (1:1) and concentrated to give (−)-ethyl (*S*)-2-trifluromethanesulfonyl propionate quantitatively.

To a solution of 7 (175 mg, 0.4 mmol) in dry DCM (2.6 mL) was added NaH (35 mg, 1.5 mmol, 60% oil dispersion), stirred for 1.5 h, added more NaH (10 mg, 0.4 mmol) and stirred for 1 h at rt. The mixture was then treated with neat (−)-ethyl (*S*)-2-trifluromethanesulfonyl propionate dropwise and stirred for 3 h at rt. The reaction mixture was quenched by addition of ice and extracted with $CHCl_3$. The organic solution was washed with saturated aq $NaHCO_3$ and brine, dried and concentrated. The residue was purified by column chromatography ($CHCl_3$/EtOAc 7:0.5) to give **8** as a white solid (145 mg, 68%). Mp 130–132 °C; $[\delta]_D^{25}$ +122 (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, 23 °C): δ_H 7.46–7.33 (m, 10H, ArH), 6.63 (d, 1H, *J* 5.4 Hz, NH), 6.0–5.89 (m, 1H, CH=CH_2), 5.57 (s, 1H, CHPh), 5.35–5.26 (m, 2H, H-1, CH=C*H*$_2$), 5.20 (d, 1H, *J* 11 Hz, CH=C*H*$_2$), 4.69 (d, 1H, *J* 11.6 Hz, CH_2Ph), 4.57–4.46 (m, 4H, CH_2Ph, C*H*CH_3, C*H*$_2$CH=CH_2), 4.23–4.11 (m, 3H, OCH_2CH_3, H-6b), 3.89–3.85 (m, 2H, H-3, H-5), 3.78–3.68 (m, 3H, H-2, H-6a, H-4), 1.40 (d, 3H, *J* 7.0 Hz, CHCH_3), 1.27 (t, 3H, *J* 7.1 Hz, CH_2C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ_c 174.2 (*C*OCH_2CH_3), 156.3 (*C*OCH_2CH_3), 137.3 (Ar*C*), 137.2 (Ar*C*), 133.1 (Ar*C*), 128.9 (Ar*C*), 128.3 (Ar*C*), 127.7 (Ar*C*), 125.8 (Ar*C*), 117.0 (CH=*C*H$_2$), 101.2 (*C*HPh), 97.4 (C-1), 83.3 (C-4)75.1 (*C*HCH_3), 74.7 (C-3), 70.1 (CH_2-Ph), 68.9 (H-6), 65.8 (CH_2CH=CH_2), 62.8 (C-5), 61.2 (OCH_2CH_3), 55.3 (C-2), 18.6 (CHCH_3), 14.0 (OCH_2C*H*$_3$). MALDI-TOF: $C_{29}H_{35}$-NO_9+Na: 564.2209. Found: 564.244.

1.8. Benzyl 2-deoxy-3-*O*-((*R*)-1′-ethoxycarbonylethyl)-4,6-*O*-benzylidene-2-(2,2,2-tri-chloroethoxycarbonylamino)-α-D-glucopyranoside (9)

Tetrakis(triphenylphosphine) palladium (476 mg, 0.41 mmol) and AcOH (124 μL, 2.17 mmol) were added to a solution of **8** (764 mg, 1.4 mmol) in dry DCM (14 mL). The reaction mixture was stirred at rt for 15 min and then TrocCl (395 μL, 2.87 mmol) was added dropwise and stirred for 1 h at rt. The reaction mixture was quenched with saturated aq $NaHCO_3$, extracted with $CHCl_3$, washed with H_2O and brine. After being concentrated the residue was dissolved in ether and insoluble materials were filtered off (repeated until no insoluble materials were observed). The organic phase was dried, concentrated, and purified by column chromatography (Toluene:Acetone 10:1) to give **9** as a white solid (730 mg, 82%). Mp 121–123 °C, $[\delta]_D^{25}$ +125.2 (*c* 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, 23 °C): δ_H 7.46–7.29 (m, 10H, ArH), 6.85 (d, 1H, *J* 5.1 Hz, NH), 5.58 (s, 1H, C*H*Ph), 5.31 (d, 1H, *J* 3.6 Hz, H-1), 4.83 (d, 1H, *J* 12 Hz, C*H*$_2$CCl$_3$), 4.67 (dd, 2H, *J* 17.1 Hz, 12.0 Hz, C*H*$_2$Ph, C*H*$_2$CCl$_3$), 4.55–4.50 (m, 2H, C*H*$_2$Ph, C*H*CH_3), 4.26–4.11 (m, 3H, H-6b, OC*H*$_2$CH$_3$,) 3.94–3.85 (m, 2H, H-3, H-5), 3.81–3.68 (m, 3H, H-2, H-6a, H-4), 1.41 (d, 3H, *J* 7.0 Hz, CHC*H*$_3$), 1.28 (t, 3H, *J* 7.2 Hz, OCH$_2$C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ_c 174.3 (*C*OCH_2CH_3), 154.9 (*C*OCH_2CCl_3), 137.3 (Ar*C*), 129.1 (Ar*C*), 128.5 (Ar*C*), 128.4 (Ar*C*), 128.0 (Ar*C*), 126.0 (Ar*C*), 101.5 (*C*HPh), 97.3 (C-1), 95.9 (*C*Cl$_3$), 83.4 (C-4), 75.5 (*C*HCH_3), 74.8 (C-3), 74.6 (*C*H$_2$CCl$_3$), 70.4 (*C*H$_2$Ph), 69.1 (C-6), 63.1 (C-5), 61.5 (O*C*H$_2$CH$_3$), 55.7 (C-2), 18.8 (CH*C*H$_3$), 14.3 (COCH_2C*H*$_3$). MALDI-TOF: $C_{28}H_{32}Cl_3NO_9$+Na: 654.1040. Found: 654.106.

1.9. 1,6-Di-*O*-benzyl 2-deoxy-3-*O*-((*R*)-1′-ethoxycarbonylethyl)-2-(2,2,2-trichloroetho-xycarbonylamino)-α-D-glucopyranoside (10)

To a solution of **9** (218 mg, 0.34 mmol) in dry CH_3CN (3.4 mL) at 0 °C was added a solution of Me_3NBH_3 (30 mg, 0.41 mmol) in CH_3-CN (0.2 mL) and then a solution of $BF_3{\cdot}OEt_2$ (260 μL, 2.1 mmol) in CH_3CN (0.73 mL) was added dropwise. After stirring for 3 h at 0 °C, the mixture was quenched with cold saturated aq $NaHCO_3$ (1.8 mL), diluted with ethyl acetate, and washed with saturated aq $NaHCO_3$, 5% citric acid (4 × 4.4 mL), saturated aq $NaHCO_3$ (3.6 mL), and brine (2.2 mL). The organic layer was dried, concentrated, and the crude was purified by column chromatography (Toluene:Acetone, 10:1) to give **10** as a colorless foam (158 mg,

72%). Mp 105–108 °C, $[\delta]_D^{25}$ +91.6 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C): δ_H 7.39–7.27 (m, 10H, ArH), 6.77 (d, 1H, *J* 4.5 Hz, NH), 5.23 (d, 1H, *J* 2.8 Hz, H-1), 4.79 (d, 1H, *J* 12.0 Hz, CH_2CCl_3), 4.68–4.59 (m, 4H, ½ CH_2CCl_3, $CHCH_3$, CH_2Ph), 4.52 (dd, 2H, *J* 12.2, 4.7 Hz, CH_2Ph), 4.27–4.17 (m, 2H, OCH_2CH_3), 3.74–3.60 (m, 6H, H-6b,H-3, H-4,H-2, H-5, H-6a), 1.41 (d, 3H, *J* 6.9 Hz, $CHCH_3$), 1.28 (t, 3H, *J* 7.2 Hz, CH_2CH_3).^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ_c 175.0 ($COCH_2CH_3$), 154.8 ($COCH_2CCl_3$), 137.5 (ArC), 137.4, (ArC) 128.6 (ArC), 128.5 (ArC), 128.1(ArC), 128.0 (ArC), 127.9 (ArC), 96.6 (C-1), 95.9 (CCl_3),77.3 (C-3) 74.8 ($CHCH_3$), 74.5 (CH_2CCl_3), 74.6 (C-4), 73.9 (CH_2Ph), 71.1 (C-6), 70.1 (CH_2Ph), 69.4 (C-5), 61.5 (OCH_2CH_3), 54.4 (C-2), 19.1 ($CHCH_3$), 14.2 (OCH_2CH_3). MALDI-TOF: $C_{28}H_{34}Cl_3NO_9$+Na: 656.1196. Found: 656.029.

1.10. Benzyl 6-*O*-benzyl-4-*O*-[2-deoxy-3-*O*-acetyl-4,6-O-benzylidene-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-2-deoxy-3-*O*-[(*R*)-1'-ethoxycarbonylethyl]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (11)

The donor **4** (235 mg, 0.37 mmol) and acceptor **10** (158 mg, 0.25 mmol), with 3 Å molecular sieves (27 mg) in dry DCM (7 mL) were treated with TMSOTf (27 μL, 0.15 mmol) at −15 °C. After stirring for 20 min, the mixture was quenched with a cold saturated aq of $NaHCO_3$ (1 mL) and extracted with $CHCl_3$ (6.7 mL). The organic layer was washed with saturated aq $NaHCO_3$ and brine, dried and concentrated. The residue was purified by column chromatography (Toluene/Acetone (10:2)) to give **11** as a white foam (164 mg, 60%). Mp 96–98 °C, $[\delta]_D^{25}$ +12 (*c* 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.60–7.27 (m, 16 H, NH, 15 ArH), 5.49 (s, 1H, $CHPh$), 5.29 (s, 1H, H-1), 4.95 (d, 1H, *J* 12.1 Hz, ½ CH_2-Ph), 4.78–4.73 (m, 3H, NH′, CH_2CCl_3), 4.67 (d, 1H, *J* 12.0 Hz, ½ CH_2-CCl_3), 4.61–4.56 (m, 3H, ½ CH_2Ph, ½ CH_2CCl_3, $CHCH_3$), 4.50 (d, 1H, *J* 12.0 Hz, ½ CH_2Ph), 4.43 (dd, 1H, *J* 10.3, 5.0 Hz, H-6b), 4.37–4.23 (m, 2H, ½ OCH_2CH_3, ½ CH_2Ph), 4.19–4.10 (m, 2H, ½ OCH_2CH_3, H-1′), 3.95–3.91 (m, 1H, H-6b′), 3.81–3.66 (m, 6H, H-2, H-4′, H-3′, H-3, H-5′, H-6a), 3.60–3.53 (m, 3H, H-2′, H-4, H-6a′), 3.42–3.29 (m, 1H, H-5), 2.03 (s, 3H, $COCH_3$), 1.35 (d, 3H, *J* 6.9 Hz, $CHCH_3$), 1.29 (t, 1H, *J* 7.1 Hz, CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ_c 174.8 ($COCH_2CH_3$), 169.8 ($COCH_3$), 154.8 ($COCH_2CCl_3$), 154.0 ($COCH_2CCl_3$), 101.3 ($CHPh$), 100.9 (C-1′), 95.9 (2 × CCl_3), 96.0 (C-1), 78.8 (C-4′), 78.1 (C-4), 75.2 (C-5′), 74.9 ($CHCH_3$), 74.6 (CH_2-CCl_3), 74.4 (CH_2Ph), 74.1 (CH_2CCl_3), 71.6 (C-3′), 70.3 (CH_2Ph), 70.1 (C-3), 68.8 (C-6′), 66.7 (C-6), 66.1 (C-5), 61.6 (OCH_2CH_3), 56.7 (C-2), 55.5 (C-2′), 20.6 ($COCH_3$), 18.9 ($CHCH_3$), 14.0 (OCH_2CH_3). MALDI-TOF: $C_{46}H_{52}Cl_6N_2O_{16}$+Na: 1121.1345. Found: 1121.099.

1.11. Benzyl 6-*O*-benzyl-4-*O*-[2-deoxy-3-*O*-acetyl-4,6-O-benzylidene-2-acetylamino-β-D-glucopyranosyl]-2-deoxy-3-*O*-[(*R*)-1′-ethoxycarbonylethyl]-2-acetylamino-α-D-glucopyranoside (12)

A solution of **11** (150 mg, 0.136 mmol) and freshly activated zinc–copper couple (390 mg) in AcOH/Ac_2O/THF 1:1:1 (1.5 mL) was stirred for 4 h at rt. The reaction mixture was filtered over celite, washed with ethyl acetate, and concentrated. Then the crude was dissolved in Pyridine: Ac_2O (2:1, 374 μL) and stirred overnight at rt. After completion of the reaction, the crude was concentrated and purified by column chromatography ($CHCl_3$/Acetone (7:1)) to give **12** as a white solid (71 mg, 63%). $[\delta]_D^{25}$ +31.4 (*c* 0.5, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.03 (d, 1H, *J* 4.4 Hz, NH), 7.56–7.27 (m, 15H, ArH), 5.50 (s, 1H, CHPh), 5.41 (d, 1H, *J* 3.4 Hz, H-1), 4.94 (d, 1H, *J* 12.1 Hz, CH_2Ph), 4.84 (t, 1H, *J* 9.9 Hz, H-3′), 4.61–4.57 (m, 2H, $CHCH_3$, CH_2Ph), 4.52 (d, 1H, *J* 12.3 Hz, CH_2Ph), 4.44 (dd, 1H, *J* 10.4 Hz, *J* 5.0 Hz, H-6′b), 4.40 (d, 1H, *J* 9.9 Hz, NH′), 4.29 (dd, 2H, *J* 10.3 Hz, *J* 7.7 Hz, H-1′, CH_2Ph), 4.24–4.21 (m, 1H, OCH_2CH_3), 4.14–4.11 (m, 1H, OCH_2CH_3), 3.97 (dd, 1H, *J* 18.6 Hz, *J* 10 Hz, H-2′), 3.90 (t, 1H, *J* 9.5 Hz, H-4), 3.79–3.74 (m, 2H, H-2, H-6′a), 3.64–3.58 (m, 2H, H-4′, H-3), 3.55–3.49 (m, 2H, H-5, H-6b), 3.35 (td, 1H, *J* 9.8 Hz, *J* 5.0 Hz, H-5′), 3.33 (dd, 1H, *J* 10.6 Hz, *J* 2.2 Hz, H-6a), 2.03 (s, 3H, $NHCOCH_3$), 1.98 (s, 3H, $COCH_3$), 1.68 (s, 3H, NH′$COCH_3$), 1.35 (d, 3H, *J* 7.0 Hz, $OCHCH_3$), 1.29 (t, 3H, *J* 7.2 Hz, OCH_2CH_3). ^{13}C NMR (150 MHz, $CDCl_3$, 23 °C): δ_c 175.9 ($COCH_2CH_3$), 170.7 ($COCH_3$, $NHCOCH_3$), 169.8 (NH′$COCH_3$), 137.8 (ArC), 137.3 (ArC), 136.8 (ArC), 129.6 (ArC), 129.5 (ArC),129.2 (ArC), 128.3 (ArC), 128.2 (ArC), 127.6 (ArC), 126.1 (ArC), 101.4 (CHPh), 101.0 (C-1′), 96.6 (C-1), 78.5 (C-4′), 78.1 (C-4),74.9 (C-3), 74.5 ($CHCH_3$), 74.1 (CH_2Ph),72.2 (C-3′), 70.4 (CH_2Ph), 70.1 (C-5), 68.7 (C-6′), 66.9 (C-6), 66.2 (C-5′), 61.4 (OCH_2CH_3), 54.4 (C-2), 54.2 (C-2′), 23.1 ($COCH_3$, $NHCOCH_3$), 20.8 (NH′$COCH_3$), 18.9 ($CHCH_3$), 14.1 (OCH_2CH_3),. MALDI-TOF: $C_{44}H_{54}N_2O_{14}$+Na: 857.3472. Found: 857.375.

1.12. Benzyl 6-*O*-benzyl-4-*O*-[2-deoxy -4,6-O-benzylidene-2-acetylamino-β-D-glucopyranosyl]-2-deoxy-3-*O*-[(*R*)-1′-ethoxycarbonylethyl]-2-acetylamino-α-D-glucopyranoside (13)

To a solution of 12 (91 mg, 0.109 mmol) in THF/1,4-dioxane/H_2O 4:2:1 (2.8 mL) was added $LiOH{\cdot}H_2O$ (56 mg, 1.34 mmol). After stirring for 2 h at rt, the reaction mixture was filtered over Dowex H^+ (freshly activated with 1 N HCl). The residue was purified by diaion HP-20 column chromatography (2 × 7 cm) previously washed with water and MeOH and then water. Column was eluted with H_2O (50 mL) and then eluted with MeOH (30 mL). The methanol fractions were concentrated to give 13 as a colorless solid (57.4 mg, 69%). Mp 102–105 °C, $[\delta]_D^{25}$ +40.1 (*c* 0.75, CH_3OH). ^{1}H NMR (600 MHz, CD_3OD) δ_H 7.51–7.27 (m, 15H, ArH), 5.59 (s, 1H, CHPh), 5.36 (d, *J* 3.1 Hz, H-1), 4.85 (m, (overlaped by solvent impurity) H_2O, H-1′, ½ CH_2Ph), 4.66–4.57 (m, 3H, *J* 12.1 Hz, $CHCH_3$, CH_2-Ph), 4.46 (d, 1H, *J* 12.2 Hz, ½ CH_2Ph), 4.29 (dd, 1H, *J* 10.3 Hz, *J* 5.0 Hz, H-6′b), 4.07 (t, 1H, *J* 9.6 Hz, H-3′), 3.95 (t, 1H, *J* 9.1 Hz, H-4), 3.82–3.77 (m, 2H, H-6b, H-3), 3.72–3.62 (m, 3H, H-6′a, H-5, H-6a), 3.55–3.40 (m, 3H, H-2′, H-4′,H-2), 3.30–3.27 (m, 1H, H-5′), 1.98 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 1.37 (d, *J* 6.9 Hz, 3H, $CHCH_3$). ^{13}C NMR (150 MHz, $CDCl_3$, 23 °C): δ_c 182.6 (COOH), 173.7 (2 × $NHCOCH_3$), 139.8 (ArC), 139.2 (ArC), 139.1 (ArC), 129.9 (ArC), 129.4 (ArC), 129.2 (ArC), 129.0 (ArC), 128.9 (ArC), 128.7 (ArC), 128.6 (ArC), 127.5 (ArC), 103.0 (CHPh), 101.5 (C-1), 97.0 (C-1), 83.1 (C-4′), 79.3 ($CHCH_3$), 78.3 (C-4), 75.8 (C-3), 74.1 (CH_2Ph), 72.6 (C-5), 71.0 (C-3′), 69.9 (C-6′), 69.3 (C-6), 67.6 (C-5′), 60.0 (C-2′), 56.3 (C-2), 23.1 ($NHCOCH_3$), 22.8 ($NHCOCH_3$), 20.2 ($CHCH_3$). MALDI-TOF: $C_{40}H_{48}N_2O_{13}$+Na: 787.3054. Found: 787.8830.

1.13. 4-*O*-[2-deoxy-2-acetylamino-β-D-glucopyranosyl]-2-deoxy-3-*O*-[(*R*)-1′-carboxyethyl]-2-acetylamino-α-D-glucopyranoside (14)

Compound **13** (20 mg, 0.026 mmol) was dissolved in acetic acid (8 mL), and then $Pd(OH)_2$/C (58 mg) was added. The mixture was stirred at rt for 6 h under H_2 atmosphere (3 balloons were used). The mixture was filtered over celite and concentrated. Compound **14** was isolated in quantitative yield. The data found was identical to that reported in the literature.[21]

1.14. Protected disaccharide pentapeptide (17)

The peptide chain (L-Ala-D-isoGln-L-Lys(Ddiv)-D-Ala-D-Ala) [Ddiv (1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl)] was assembled using Fmoc SPPS protocol[22] using HMPB-AM (NovaBiochem) resin. The peptide assembly was monitored by high-resolution magic angle spinning NMR (HR-MAS NMR).[10] The disaccharide was coupled under the conditions used for aminoacid

coupling and the resulting glycopeptide **17** was detached from the solid support by treatment with a trifluoroacetic acid solution (1% in dichloromethane). ^{1}H NMR (600 MHz, D_2O) δ_H 7.52–7.34 (m, 10H, ArH), 5.02 (s, 1H), 4.96–4.60 (overlaped by water peak, 5H), 4.37–4.10 (m, 9 H), 3.96–3.51 (m, 9H), 3.42–3.35 (m, 2H), 3.25–3.19 (m, 1H), 2.94 (d, *J* 6.8 Hz, 2H), 2.38 (s, 4H), 2.32–2.24 (m, 2H), 1.99 (s, 3H), 1.92 (s, 3H), 1.88–1.63 (m, 8H), 1.45–1.30 (m, 12H) , 0.99 (s, 6H), 0.91 (d, *J* 6.6 Hz, 6H). MALDI-TOF: $C_{53}H_{79}N_9O_{19}$+Na: 1168.5389. Found: 1168.4369.

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