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Synthesis of a pyruvylated *N*-acetyl-β-D-mannosamine containing disaccharide repeating unit of a cell wall glycopolymer from *Paenibacillus alvei*

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Dedicated to Horst Kunz on the occasion of his 80th birthday

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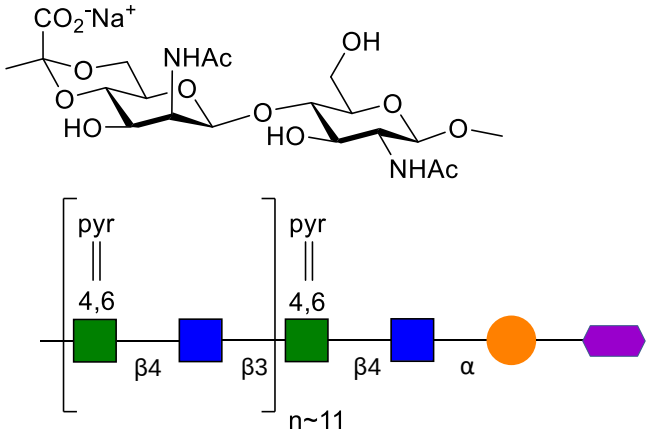
Abstract

A disaccharide implicated in anchoring of bacterial Surface-layer proteins to secondary cell wall glycopolymers has been prepared by glycosylation of a protected *N*-acetyl glucosamine acceptor with a glucopyranosyl donor to generate the β-(1→4)-linkage. Subsequent inversion of the configuration and azide introduction at position 2 with triflic anhydride, however, led to formation of a tetrazole derivative. Alternatively, displacement of a 2-*O*-mesylate by sodium azide, reduction and *N*-acetylation enabled the conversion into the distal *N*-acetyl-β-D-mannosamine residue. Pyruvylation of the latter unit and global deprotection afforded the disaccharide repeating unit from *Paenibacillus alvei* as a ligand for crystallographic and binding studies.



Keywords: Secondary cell wall polymer, pyruvate, glycosylation, surface layer protein, tetrazole

Introduction

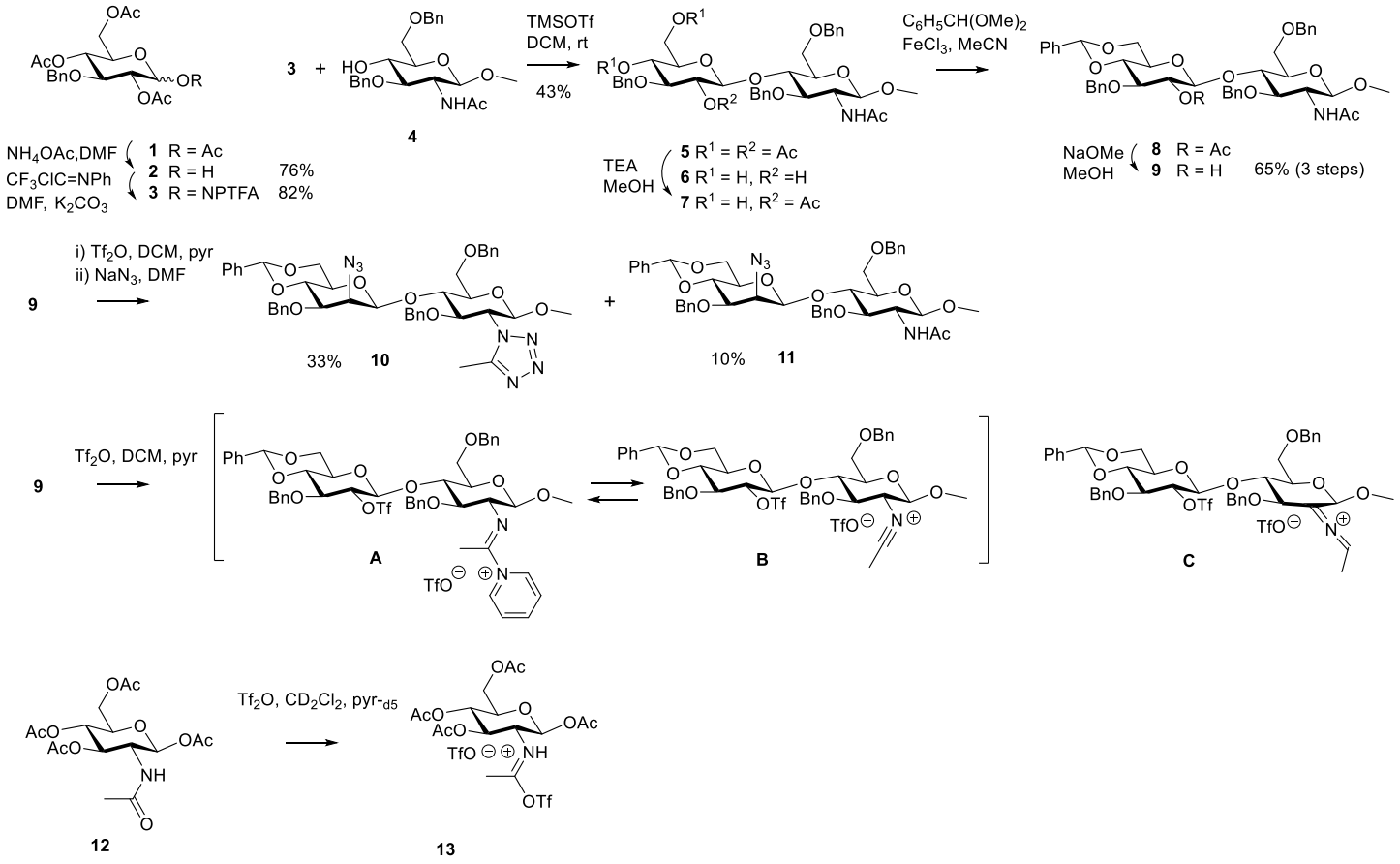
Pyruvic acid ketal substitutents attached to diol systems bridging positions 2,3; 3,4 as well as 4,6 on various glycans have increasingly been detected in the past few years.[1] Due to their negative charge, pyruvyl groups may exert a number of biologically relevant interactions such as contributing to antigenic properties in bacterial polysaccharides such as *Streptococcus pneumoniae* serotype 4, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Bacteroides fragilis* as examples.[2-7] In addition, in a few Gram-positive bacteria, pyruvylation of so-called non-classical secondary cell wall polymers (SCWP) has been implicated to exert non-covalent interactions with surface (S-) layer proteins.[8] S-layer proteins self-assemble into two-dimensional crystalline lattices that completely cover the bacterial surface.[9] A terminal 4,6-*O*-Pyr-β-D-ManNAc residue in the SCWP of *Bacillus anthracis* has been described to serve as the main mechanism to anchor the S-layer via N-terminal homology (SLH) domain.[10-12] A similar role may be relevant for SCWP-SLH interactions in *Bacillus cereus*.[13] We have been interested in studying the underlying molecular details of SCWP-SLH binding using the SCWP from *Paenibacillus alvei* as model system. This SCWP contains →3)-4,6-*O*-Pyr-β-D-ManNAc-(1→4)-β-D-GlcNAc-(1→ repeating units covalently attached to *N*-acetylmuramic residues of peptidoglycan.[14] A similar backbone structure has been found in an SCWP from *Lysinibacillus sphaericus* CCM 2177, albeit with only every second β-D-ManNAc unit being substituted by a pyruvyl ketal, probably due to hydrolysis occurring upon isolation from the peptidoglycan using hydrofluoric acid.[15] Recently we could show by using X-ray crystallography of a truncated variant of the *P. alvei* S-layer protein $SpaA_{SLH}$ - representing the three consecutive SLH domains - liganded to the monosaccharide 4,6-*O*-Pyr-β-D-ManNAcOMe and the disaccharide β-D-GlcNAc-(1→3)-4,6-*O*-Pyr-β-D-ManNAcOMe, that the 4,6(*S*)-*O*-pyruvylated *N*-acetyl-mannosamine residue was the main contributor to binding.[16] Main binding features included a π-stacking interaction of ManNAc with tryptophan 151 and ionic interactions of the pyruvyl carboxylic group with arginine 61. It should be noted that the synthetic mono- and disaccharide ligands were bound in two mutually exclusive different binding grooves of the protein allowing for dynamic properties during cell growth and division. Isothermal microcalorimetry data, however, indicated that the 4,6-*O*-Pyr-β-D-ManNAcOMe ligand was efficiently bound in solution (K_D ~29 nM), while the disaccharide fragment did not show any binding. The crystal structure of the complex with the latter ligand with a terminal *N*-acetylglucosamine residue also revealed disorder of the *N*-acetylglucosamine moiety and absence of any hydrogen bonding to the protein surface. In order to provide more insight into these interactions and to clarify the role of an "internal" *N*-acetyl glucosamine unit we have, thus, set out to prepare the alternate disaccharide mimicking the native situation in the SCWP repeat for crystallographic and binding studies. Herein we describe the synthesis of that disaccharide ligand and report on an unusual side reaction encountered during azide introduction of a triflate intermediate leading to formation of a tetrazole derivative.

Results and Discussion

The synthesis of the alternately connected repeating unit of the SCWP from *P. alvei* required the challenging 1,2-*cis*-connection of the ManNAc residue to position 4 of a suitable GlcNAc acceptor derivative. Previously, a 4,6-*O*-pyruvylated 2-azido-2-deoxy-mannopyranosyl phenylthioglycoside donor had been used for the assembly of a terminal trisaccharide fragment of the SCWP from *B. anthracis*, which, however, also led to formation of the unwanted α-anomer.[11] Reactions of 4,6-*O*-benzylidene protected 2-azido-2-deoxy-mannopyranosyl diphenyl phosphate as well as related phenylthioglycoside donors, however, were reported to proceed in high β-selectivity and in good yields.[17,18] Thus, we opted to use a 4,6-*O*-benzylidene group as a

temporary protecting group for a late stage introduction of the 4,6-*O*-pyruvyl ketal and follow the established route for β-mannosides by inverting the configuration of a *gluco*-precursor into the 2-deoxy-2-azido-*manno* derivative.[19-22]

The synthesis of the *gluco*-configured imidate donor - developed by R. Schmidt - commenced with the selective de-*O*-acetylation of the known (Ref 23) 3-*O*-benzyl substituted glucopyranose **1** to give the hemiacetal **2** in 76% yield (Scheme 1). Compound **2** was then converted into the *N*-phenyltrifluoroacetimidate donor **3** in 82% yield by reaction with *N*-phenyl trifluoroacetimidoyl chloride/K_2CO_3 in dichloromethane. As glycosyl acceptor, 3,6-di-*O*-benzyl methyl glycoside **4** was prepared according to literature.[24,25] The coupling step of **4** with donor **3** promoted by TMSO-triflate at room temperature led to complete consumption of the donor within 1 h and formation of a main product, which, however, was first identified as the orthoester intermediate. The structural assignment of the orthoester was based on the homonuclear coupling constant $J_{1',2'}$ (5 Hz), the upfield shifts of proton H-2' (4.46 ppm) and the orthoester methyl group (1.7 ppm), in agreement with literature data.[26] In order to allow for orthoester rearrangement into the glycoside, the reaction time was then increased to 3 days, when TLC controls revealed complete disappearance of the orthoester and formation of disaccharide product **5**, isolated in a 1:1 mixture with acceptor **4** in 43% yield (based on the respective ^{1}H NMR integration values of **4** and **5**). Separation of the residual glycosyl acceptor **4** - almost comigrating with disaccharide **5** - was attempted by de-*O*-acetylation with triethylamine.



Scheme 1. Synthesis of disaccharide **9** and attempted azide introduction via triflate activation

After 40 h reaction time, the disaccharide product was obtained as 3:1 mixture of the 2,4,6-triol **6** and diol **7** containing a 2-*O*-acetyl group. The latter ester group was surprisingly stable and would have needed forcing

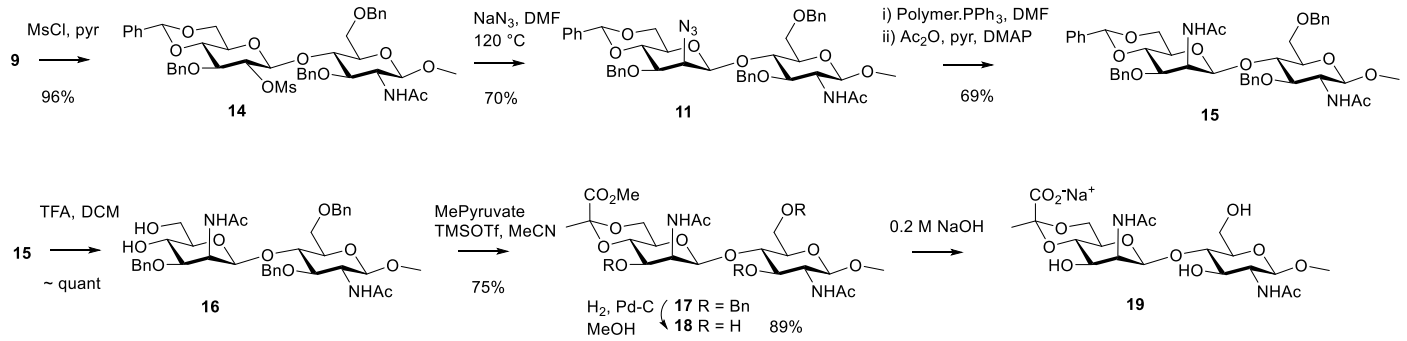
conditions for complete removal. Hence, the mixture was carried out through the ensuing introduction of the 4,6-*O*-benzylidene group using benzaldehyde dimethylacetal and $FeCl_3$ as catalyst[27] to give a mixture of **8** and **9** followed by a subsequent Zemplén transesterification - again with prolonged reaction time - to eventually provide the alcohol **9** in an overall isolated yield of 65% (for 3 steps).

The introduction of the 2-azido group with inversion of configuration was first attempted via nucleophilic triflic anhydride in pyridine resulted in the formation of a highly polar spot as observed on a TLC plate. After work-up of the reaction mixture the crude residue was dissolved in DMF and treated with sodium azide at 70 °C for 2 h. Chromatography of the reaction mixture afforded a major apolar product **10** (33%) and a poor yield of the expected azido derivative **11** (10 %). The NMR spectra of **10** showed a significant downfield proton shift of one methyl group (2.44 ppm), connected to an upfield shifted carbon at 8.8 ppm as observed in an HSQC experiment.[28] In addition, the N-H signal was absent, and a quaternary carbon was seen at 153.7 ppm, in a range expected for an N-C=N moiety. The upfield shifted methyl group showed an HMBC correlation to this quaternary carbon and a NOESY interaction with H-2 of the glucosamine residue. This data thus indicated the presence of a 1,2,3,4-tetrazole unit at position 2 of the glucosamine unit. The structure of **10** was eventually confirmed by HR-ESI-TOF MS data (*m/z* = 806.349) being consistent with the formula $C_{43}H_{47}N_7O_9$. Formation of the tetrazole ring may be rationalized by reaction of pyridine with trifluoromethanesulfonic anhydride to produce a cationic pyridinium ion intermediate **A** or a direct reaction of the acetamido group with triflic anhydride to produce an iminium triflate that is in equilibrium with the nitrilium ion **B**.[29-31] A subsequent formal 3+2 cycloaddition with the azide anion would then generate the 2'-tetrazolyl product **10**. The occurrence of a planar keteniminium intermediate **C** was considered to be less likely, since the *gluco*-configuration of the reducing unit in product **10** remained unchanged throughout the reaction. A non-charged triflate imidate was also ruled out, since a highly polar product was observed by TLC upon activation of **9** with triflic anhydride and pyridine. In order to gain more insight into the amide activation, a model reaction was carried out in an in-situ NMR experiment using the per-*O*-acetylated *N*-acetyl-glucosamine **12** in a 2:1 mixture of deuterated dichloromethane / pyridine with 2 equivalents of triflic anhydride at 27 °C. The NMR spectra of the resulting product **13** formed within one hour only showed broad lines of non-deuterated pyridine signals at 8.65, 7.61 and 7.21 ppm for H-2/6, H-4 and H-3/5, respectively, indicating the presence of pyridinium-hydrotriflate.[29] In addition, ^{13}C NMR signals showed only pyridine solvent signals at 149.11, 135.40 and 123.11 ppm for C-2/6, C-4 and C-3/5. Signals of pyridinium species were absent (see supporting information). Notably, a downfield ^1H NMR shift of one methyl group (2.88 ppm) connected to a carbon signal at 15.51 ppm and an HMBC correlation to a quaternary carbon signal at 158.66 ppm was observed. In addition, H-2 of the glucosamine unit also showed an HMBC correlation to the latter quaternary signal and an HSQC connectivity to an unusually downfield-shifted ^{13}C NMR signal at 64.23 ppm, in glucosamine residue present in a 2-acetimidoyl linkage had previously been observed.[32] Thus, the *in-situ* NMR experiment indicated the presence of the triflyl-imidate **13** as the reactive intermediate. Upon aqueous extraction - as used for the ensuing azidation reaction - the NMR spectrum of the crude mixture, however, indicated the presence of cationic pyridinium species as seen from characteristic ^1H and ^{13}C NMR shifts of H-4/C-4 (8.66/148.82 ppm) and C-3/C-5 (8.15/128.01 ppm), respectively.

To the best of our knowledge a similar tetrazole carbohydrate byproduct has rarely been described in the literature, but this side reaction could be a reason for reduced yields of triflation/azidation reactions when involving sterically hindered alcohols in the presence of an acetamido sugar.[33,34] On the other hand, this facile amide activation would merit to be explored in the future for versatile modifications of acetamido sugars.

Next, replacement of pyridine by less nucleophilic bases (*sym*-collidine, triethylamine) was tried but was not successful. Also, introduction of the 2-amino moiety via oxidation of the secondary alcohol, oxime formation

and reduction were explored, but proved to be inefficient. Eventually, reactivity of the leaving group was modified by replacing the triflyl group by a mesyl group in order to prevent amide activation. Reaction of **9** with methanesulfonyl chloride for 2 days at room temperature afforded the crude mesylate **14** which was directly subjected to reaction with sodium azide in DMF (Scheme 2). Reaction conditions had to be optimized to prevent degradation but still to enable progress of the reaction. Heating to 120 °C and a prolonged reaction time of 5 days was necessary to eventually give the azido-derivative **11** in 70% yield. The structure of the resulting 2-azido-2-deoxy-mannosyl fragment was evident from the small value of the homonuclear coupling constant $J_{1',2'}$ being characteristic of a *manno*-glycoside (1.4 Hz) and by the value of the heteronuclear coupling constant ($J_{C1',H1'}$ = 160 Hz).



Scheme 2. Synthesis of target disaccharide **19**: Azide introduction, pyruvylation and global deprotection

Reduction of the azido group with ensuing *N*-acetylation was accomplished via a Staudinger reaction of **11** using polymer-bound triphenylphosphine as described previously.[16] The resin had to be extracted thoroughly with aqueous MeOH to recover the intermediate free amine, which was subsequently *N*-acetylated under standard conditions to furnish the acetamido derivative **15** in 69% yield. For the introduction of the pyruvyl unit, the benzylidene group was cleaved by the action of trifluoroacetic acid to give diol **16** in near theoretical yield. Installation of the pyruvyl moiety on the β-D-ManNAc residue was achieved by reaction of **16** with methyl pyruvate - promoted by TMSO-triflate in acetonitrile - to generate the (*S*)-isomer **17** in a good yield of 75%.[35,36] The stereochemical assignment of the pyruvyl ketal was based on literature-known chemical shift features.[37] The ^{13}C NMR chemical shift of the methyl carbon in *S*-configured 4,6-*O*-pyruvyl ketals in hexopyranoses was observed significantly downfield (**17**: 25.55 ppm) compared to the *R*-configured counterparts.[38] In addition, diagnostic ROESY correlations were observed between the geminal H-6 protons of the ManNAc unit and the methyl ester group. Deprotection of **17** was carried out by hydrogenolysis of the benzyl protecting groups with 10% Pd-carbon in methanol to afford **18**, followed by saponification of the methyl ester with aqueous NaOH to furnish disaccharide **19** as the sodium salt. NMR data of **19** were in full agreement with the structural assignments and the NMR features of the native glycan.[14] Binding and crystallographic data with the $SpaA_{SLH}$ protein from *P. alvei* in the presence of disaccharide **19** will be published in due course.

Experimental Section

General. All purchased chemicals were used without further purification unless stated otherwise. Solvents were dried over activated 4 Å (CH_2Cl_2, DMF) and 3 Å (CH_3CN) molecular sieves. Cation exchange resin DOWEX

50 H^+ was regenerated by consecutive washing with HCl (3 M), water and dry MeOH. Aqueous solutions of salts were saturated unless stated otherwise. Concentration of organic solutions was performed under reduced pressure < 40 °C. Optical rotations were measured with an Anton Paar MCP100 Polarimeter. Thin layer chromatography was performed on Merck precoated plates: generally, on 5 x 10 cm, layer thickness 0.25 mm, Silica Gel $60F_{254}$; alternatively, on HPTLC plates with 2.5 cm concentration zone (Merck). Spots were detected by staining with a dipping reagent (anisaldehyde-H_2SO_4) and heating. For column chromatography silica gel (0.040 – 0.063 mm) was used. HP-column chromatography was performed on pre-packed columns (YMC-Pack SIL-06, 0.005 mm, 25 x 1 cm and 25 x 2 cm). NMR spectra were recorded with a Bruker Avance III 600 instrument (600.22 MHz for ^{1}H, 150.93 MHz for ^{13}C, 564.77 MHz for ^{19}F) using standard Bruker NMR software. ^{1}H spectra were referenced to 7.26 ($CDCl_3$), 3.34 (MeOD) and 0.00 (D_2O, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) ppm unless stated otherwise. ^{13}C spectra were referenced to 77.16 ($CDCl_3$), 53.52 (CD_2Cl_2), 49.00 (CD_3OD) and 67.40 (D_2O, external calibration to 1,4-dioxane) ppm. Assignments were based on COSY, HSQC, HMBC and TOCSY data. ESI-MS data were obtained on a Micromass Q-TOF Ultima Global instrument.

2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-D-glucopyranose (2). A solution of **1** (3.8 g, 8.67 mmol) and NH_4OAc (2.67 g, 34.7 mmol) in dry DMF (35 mL) was stirred for 5 days at rt under Ar when TLC showed complete conversion. The solution was concentrated *in vacuo* and the residue purified by chromatography on silica gel (toluene-EtOAc 4:1 → 1:1) to give **2** (2.62 g, 76%) as colorless syrup; R_f = 0.46 (toluene-EtOAc 4:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.34-7.22 (m, 5 H, Ar-H), 5.45 (dd, 1 H, $^3J_{1,OH}$ = 3.7 Hz, $^3J_{1,2}$= 3.7 Hz, H-1$_\alpha$), 5.09 (t, 1 H, $^3J_{4,5}$ = $^3J_{4,3}$= 9.7 Hz, H-4$_\beta$), 5.09 (t, 1 H, $^3J_{4,5}$ = $^3J_{4,3}$ = 9.7 Hz, H-4$_\alpha$), 4.87 (dd, 1 H, $^3J_{2,3}$ = 9.2, $^3J_{2,1}$ = 7.9 Hz, H-2$_\beta$), 4.87 (dd, 1 H, $^3J_{2,3}$ = 9.7, $^3J_{2,1}$ = 3.9 Hz, H-2$_\alpha$), 4.71 (d, 1 H, ^2J = 12.1 Hz, CH_2Ar_α), 4.66 (d, 1 H, ^2J = 11.6 Hz, CH_2Ar_β), 4.62 (d, 1 H, ^2J = 11.9 Hz, CH_2Ar_α), 4.61 (d, 1 H, ^2J = 11.8 Hz, CH_2Ar_β), 4.61 (d, 1 H, $^3J_{1,2}$ = 8.2 Hz, H-1$_\beta$), 4.19 (dd, 1 H, $^2J_{6a,6b}$ = 12.4, $^3J_{6a,5}$ = 5.2 Hz, H-6a$_\beta$), 4.18 -4.08 (m, 4 H, H-6b$_\beta$, H-6a$_\alpha$, H-6b$_\alpha$, H-5$_\alpha$), 4.04 (t, 1 H, $^3J_{3,4}$ = $^3J_{3,2}$ = 9.6 Hz, H-3$_\alpha$), 3.79 (d, 1 H, $^3J_{OH,1}$ = 9.6 Hz, OH$_\beta$), 3.72 (t, 1 H, $^3J_{3,4}$ = $^3J_{3,2}$ = 9.3 Hz, H-3$_\beta$), 3.63 (ddd, 1 H, $^3J_{5,4}$ = 10.1, $^3J_{5,6a}$ = 5.2, $^3J_{5,6b}$ = 2.4 Hz, H-5$_\beta$), 3.33 (d, 1 H, $^3J_{OH,1}$ = 3.3 Hz, OH$_\alpha$), 2.08 (s, 3 H, CH_3CO), 2.06 (s, 3 H, CH_3CO_α), 2.05 (s, 3 H, CH_3CO_β), 1.97 (s, 3 H, CH_3CO_β), 1.95 (s, 3 H, CH_3CO_α); ^{13}C NMR for α-anomer (150 MHz, $CDCl_3$): δ = 170.90, 170.10 and 169.48 (C=O), 138.12 (Cq, Ar-*C*), 128.39 (2 C, Ar-*C*), 127.71 (Ar-*C*), 127.54 (2 C, Ar-*C*), 90.31 (C-1), 76.86 (C-3), 74.88 (*C*H_2Ar), 73.41 (C-2), 69.79 (C-4), 67.73 (C-5), 62.27 (C-6), 20.82, 20.74 and 20.70 (*C*H_3CO); ^{13}C NMR for β-anomer (150 MHz, $CDCl_3$): δ = 171.19, 170.85 and 169.39 (C=O), 137.68 (Cq, Ar-*C*), 128.46 (2 C, Ar-*C*), 127.90 (Ar-*C*), 127.67 (2 C, Ar-*C*), 95.84 (C-1), 79.59 (C-3), 75.39 (C-2), 74.46 (*C*H_2Ar), 72.30 (C-5), 69.66 (C-4), , 62.27 (C-6), 20.82, 20.74 and 20.70 (*C*H_3CO) ppm. NMR data were in agreement with the literature.[39]

3-*O*-Benzyl-2,4,6-tri-*O*-acetyl-D-glucopyranosyl *N*-phenyl-trifluoroacetimidate (3). K_2CO_3 (660 mg, 4.773 mmol) was added to a solution of **2** (860 mg, 2.170 mmol) in dry DCM (30 mL) under Ar followed by dropwise addition of *N*-phenyltrifluoroacetimidoyl chloride (NPTFI-Cl, 690 µL, 4.34 mmol) at room temperature. The suspension was stirred for 7 d, then filtered over Celite® and rinsed with DCM. The organic phase was concentrated *in vacuo* to give a yellowish, waxy product. Purification of the residue by column chromatography (toluene-EtOAc 4:1 containing 0.2% TEA) afforded 1.008 g (82%) of **3** as off-white waxy solid; R_f = 0.27 (toluene-EtOAc 4:1); $[\alpha]_D^{20}$ +29 (*c* 1.3 $CHCl_3$); ^{1}H NMR: (600 MHz, $CDCl_3$) δ = 7.36-7.32 (m, 3 H, Ar-H), 7.32-7.21 (m, 2 H, Ar-H), 7.25-7.23 (m, 2 H, Ar-H), 7.14-7.11 (m, 1 H, Ar-H), 6.86-6.80 (m, 2 H, Ar-H), 5.78-5.60 (br s, 1 H, H-1), 5.30 (t, 1 H, $^3J_{4,3}$ = $^3J_{4,5}$ = 8.3 Hz, H-4), 5.19 (t, 1 H, $^3J_{2,3}$ = $^3J_{2,1}$ = 9.3 Hz, H-2), 4.64 (d, 1 H, ^2J = 11.7 Hz, CH_2Ar), 4.61 (d, 1 H, ^2J = 11.8 Hz, CH_2Ar), 4.21 (dd, 1 H, $^3J_{6a,5}$ = 5.3, $^2J_{6a,6b}$ = 12.6 Hz, H-6a), 4.16-4.09 (m, 1 H, H-6b), 3.77-3.58 (m, 1 H, H-5), 3.75 (dd, 1 H, $^3J_{3,4}$ = 8.0 Hz, $^3J_{3,2}$ = 9.2 Hz, H-3), 2.06, 2.03 and 1.98 (3 x s, each 3 H, CH_3CO); ^{13}C NMR: (150 MHz, $CDCl_3$): δ = 128.82, 128.49, 127.97, 127.83, 124.57 and 119.24 (Ar-C), 79.55 (C-

3), 73.89 (*C*H_2Ar), 73.05 (C-5), 71.21 (C-4), 68.99 (C-2), 61.84 (C-6), 20.68 and 20.59 (*C*H_3CO) ppm. HRMS ($^+$ESI-TOF) *m/z* $[M+Na]^+$ calcd for $C_{27}H_{28}F_3NNaO_9$, found 590.1598.

Methyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (6). A suspension of **4** (490 mg, 1.18 mmol), donor **3** (880 mg, 1.55 mmol) and acid-washed molecular sieves 4 Å (1.37 g) in DCM (30 mL) was stirred at rt under Ar for 30 min, then cooled with an external NaCl/ice-bath for further 40 min. TMSOTf (21 µL, 0.12 mmol) was added to the mixture stirred overnight at rt. TLC showed complete consumption of the donor, intermediate orthoester (R_f = 0.63, EtOAc-DCM 4:1) and unreacted acceptor. The reaction mixture was stirred for additional 24 h, followed by quenching with TEA (150 µL) and filtration over Celite®. The filtrate was concentrated *in vacuo* and the residue subjected to column chromatography (toluene-EtOAc 3:2 →2:3→0:1) to give a 1:1 mixture of 620 mg of the desired disaccharide **5** (43 %) as well as recovered acceptor **4** (43 %); R_f = 0.39 (EtOAc-DCM 4:1). An analytical aliquot of the mixture was purified by HILIC-HPLC using a gradient H_2O→MeCN; $[\alpha]_D^{20}$ -19 (*c* 0.2, $CHCl_3$); ^{1}H NMR: (600 MHz, $CDCl_3$): δ = 7.37-7.20 (m, 15 H, Ar-H), 5.91 (d, 1 H, $^3J_{NH,2}$ = 8.3 Hz, NH), 5.08 (t, 1 H, $^3J_{4',3'}$ = $^3J_{4',5'}$ = 9.7 Hz, H-4'), 5.00 (dd, 1 H, $^3J_{2',3'}$ = 9.6, $^3J_{2',1'}$ = 8.2 Hz, H-2'), 4.72 (d, 1 H, ^2J = 11.9 Hz, CH_2Ar), 4.65 (d, 1 H, ^2J = 12.4 Hz, CH_2Ar), 4.63 (d, 1 H, ^2J = 12.2 Hz, CH_2Ar), 4.59 (d, 1 H, ^2J = 12.2 Hz, CH_2Ar), 4.57 (d, 1 H, $^3J_{1,2}$ = 8.0 Hz, H-1), 4.57 (d, 1 H, ^2J = 12.5 Hz, CH_2Ar), 4.49 (d, 1 H, ^2J = 12.0 Hz, CH_2Ar), 4.44 (d, 1 H, $^3J_{1',2'}$ = 8.0 Hz, H-1'), 4.18 (dd, 1 H, $^3J_{6a',5'}$ = 4.8, $^2J_{6a',6b'}$ = 12.3 Hz, H-6a'), 3.99 (dd, 1 H, $^3J_{6b',5'}$ = 2.7, $^2J_{6b',6a'}$ = 12.6 Hz, H-6b'), 3.97 (dd, 1 H, H-4), 3.84 (dd, 1 H, $^3J_{6a,5}$ = 5.2, $^2J_{6a,6b}$ = 10.2 Hz, H-6a), 3.81 (dd, 1 H, H-3), 3.79 (dd, 1 H, $^3J_{6b,5}$ = 4.7, $^2J_{6b,6a}$ = 10.4 Hz, H-6b), 3.64 (ddd, 1 H, $^3J_{5,6b}$ = 4.7, $^3J_{5,4}$ = 10.0, $^3J_{5,6a}$ = 5.2 Hz, H-5), 3.57 (t, 1 H, $^3J_{3',2'}$ = $^3J_{3',4'}$ = 9.5 Hz, H-3'), 3.43 (s, 3 H, OCH_3), 3.40 (ddd, 1 H, $^3J_{5',6b'}$ = 2.5, $^3J_{5',6a'}$ = 4.9, $^3J_{5',4'}$ = 10.1 Hz, H-5'), 1.99 (s, 3 H, CH_3CO), 1.98 (s, 3 H, CH_3CO), 1.97 (s, 3 H, CH_3CO), 1.92 (s, 3 H, CH_3CO); ^{13}C NMR: (150 MHz, $CDCl_3$): δ = 170.71 (C=O), 170.25 (C=O), 169.62 (C=O), 169.28 (C=O), 138.45 (Cq, Ar-C), 138.08 (Cq, Ar-C), 137.72 (Cq, Ar-C), 129.57 (Ar-C), 128.47 (2 C, Ar-C), 128.45 (2 C, Ar-C), 128.29 (2 C, Ar-C), 127.90 (2 C, Ar-C), 127.88 (2 C, Ar-C), 127.84 (2 C, Ar-C), 127.66 (2 C, Ar-C), 127.56 (1 C, Ar-C), 101.30 (C-1), 99.67 (C-1'), 79.86 (C-3'), 76.74 (C-3), 74.97 (C-4), 74.36 (C-5), 74.04 (*C*H_2Ar), 73.55 (*C*H_2Ar), 72.94 (C-2'), 72.89 (*C*H_2Ar), 72.04 (C-5'), 69.53 (C-4'), 69.12 (C-6), 61.98 (C-6'), 56.50 (OCH_3), 52.05 (C-2), 23.28 (HNCO*C*H_3), 20.88 (*C*H_3CO), 20.72 (*C*H_3CO), 20.68 (*C*H_3CO) ppm. HRMS ($^+$ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{42}H_{51}NO_{14}$ 794.3382; found 794.3384.

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (8). The mixture of **4** and **5** was dissolved in MeOH (8 mL), water (1 mL) and treated with triethylamine (1 mL) for 40 h at rt. The solution was then concentrated, and the residue was subjected to chromatography on silica gel (EtOAc) to remove monosaccharide acceptor **4** and to give a 3:1 mixture of **6** and the 2-O-acetyl derivative **7** (245 mg). Data for **6**: R_f = 0.23 (EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.37-7.27 (m, 15 H, Ar-H), 5.64 (d, 1 H, $^3J_{NH,2}$ = 7.8 Hz, N-H), 4.95 (d, 1 H, ^2J = 11.6 Hz, CH_2Ar), 4.87 (d, 1 H, ^2J = 11.6 Hz, CH_2Ar), 4.74 (d, 1 H, $^3J_{1,2}$ = 7.8 Hz, H-1), 4.73 (d, 1 H, ^2J = 11.6 Hz, CH_2Ar), 4.70 (d, 1 H, ^2J = 12.0 Hz, CH_2Ar), 4.62 (d, 1 H, ^2J = 11.9 Hz, CH_2Ar), 4.56 (d, 1 H, ^2J = 12.0 Hz, CH_2Ar), 4.51 (d, 1 H, $^3J_{1',2'}$ = 7.8 Hz, H-1'), 4.12 (dd, 1 H, $^3J_{3,4}$ = 8.9, $^3J_{3,2}$ = 8.9 Hz, H-3), 3.97 (dd, 1 H, $^3J_{4,3}$ = 8.9, $^3J_{4,5}$ = 8.9 Hz, H-4), 3.92 (dd, 1 H, $^3J_{6a,5}$ = 3.6, $^2J_{6a,6b}$ = 11.2 Hz, H-6a), 3.80 (dd, 1 H, $^3J_{6b,5}$ = 2.9, $^2J_{6b,6a}$ = 11.4 Hz, H-6b), 3.67 (dd, 1 H, $^3J_{6a',5'}$ = 3.5, $^2J_{6a',6b'}$ = 11.9 Hz, H-6a'), 3.59 (ddd, 1 H, $^3J_{5,6b}$ = 2.9, $^3J_{5,6a}$ = 3.4, $^3J_{5,4}$ = 9.1 Hz, H-5), 3.50 (dd, 1 H, $^3J_{6b',5'}$ = 5.3, $^2J_{6b',6a'}$ = 11.9 Hz, H-6b'), 3.47 (dd, 1 H, $^3J_{4',3'}$ = 9.3, $^3J_{4',5'}$ = 9.3 Hz, H-4'), 3.47 (s, 3 H, OMe), 3.44 (dd, 1 H, $^3J_{2',3'}$ = 9.3, $^3J_{2',1'}$ = 7.7 Hz, H-2'), 3.37 (ddd, 1 H, $^3J_{2,3}$ = 8.6, $^3J_{2,1}$ = 7.7, $^3J_{2,N\text{-}H}$ = 7.7 Hz, H-2), 3.26 (dd, 1 H, $^3J_{3',2'}$ = $^3J_{3',4'}$ = 9.1 Hz, H-3'), 3.11 (ddd, 1 H, $^3J_{5',6a'}$ = 3.5, $^3J_{5',6b'}$ = 5.4, $^3J_{5',4'}$ = 9.6 Hz, H-5') and 1.86 (s, 3H, NAc); ^{13}C NMR: (150 MHz, $CDCl_3$): δ = 170.60 (C=O), 138.60 (2 C, Cq, Ar-C), 137.60 (Cq, Ar-C), 128.62 (2 C, Ar-C), 128.47 (2 C, Ar-C), 128.09 (2 C, Ar-C), 127.93 (3 C, Ar-C), 127.70 (1 C, Ar-C), 127.37 (2 C, Ar-C), 102.56 (C-1'), 100.79 (C-1), 83.82 (C-3'), 78.71 (C-3), 77.53 (C-4), 75.28 (C-5'), 75.13 (C-2'), 74.68 (*C*H_2Ar), 74.46 (C-5), 74.20 (*C*H_2Ar), 73.69 (*C*H_2Ar), 70.24 (C-4'), 68.74 (C-6), 62.42 (C-

6'), 56.98 (C-2), 56.68 (OCH_3) and 23.57 ($NHCOCH_3$) ppm. ESI-QTOFMS: *m/z* calcd for $C_{36}H_{45}NO_{11}$: $[M+H^+]^+$ 668.3065; found 668.3066.

The mixture was dispersed in MeCN (5 mL) under Ar. Benzylidene dimethylacetal (0.079 mL, 0.526 mmol) and $FeCl_3$ (11 mg, 0.07 mmol) were then added and the dispersion was stirred for 3 h at rt. The mixture was concentrated, and the residue was dissolved in EtOAc (100 mL), washed with satd aq $NaHCO_3$ and water. The organic phase was dried and concentrated to afford a ~4:1 mixture of **8** and **9** as syrup (275 mg). A solution of the residue in dry MeOH (9 mL) was stirred with solid NaOMe (13 mg, 0.241 mmol) for 18 days at rt. The reaction was quenched by addition of Dowex 50 H^+ resin and filtered. The filtrate was concentrated and flash-chromatographed with dichloromethane to give **9** as colorless fluffy solid. Yield: 250 mg (0.33 mmol, 65% for 3 steps). R_f = 0.45 (EtOAc); $[\alpha]_D^{20}$ +5.6 (*c* 0.95, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.42-7.26 (m, 20 H, ArH), 5.56 (d, 1 H, $^3J_{NH,2}$ = 7.7 Hz, NH), 5.47 (s, 1 H, ArCH), 4.93 (d, 1 H, ^2J = 11.5 Hz, CH_2Ar), 4.88 (d, 1 H, ^2J = 11.5 Hz, CH_2Ar), 4.78 (d, 1 H, $^3J_{1,2}$ = 7.8 Hz, H-1), 4.76 (d, 1 H, ^2J = 11.7 Hz, CH_2Ar), 4.69 (d, 1 H, ^2J = 11.9 Hz, CH_2Ar), 4.59 (d, 1 H, ^2J = 11.9 Hz, CH_2Ar), 4.59 (d, 1 H, $^3J_{1',2'}$ = 7.0 Hz, H-1'), 4.54 (d, 1 H, ^2J = 12.2 Hz, CH_2Ar), 4.12 (t, 1 H, $^3J_{3,4}$ = $^3J_{3,2}$ = 9.0 Hz, H-3), 4.08 (dd, 1 H, $^3J_{6a',5'}$ = 5.0, $^2J_{6a',6b'}$ = 10.4 Hz, H-6a'), 4.00 (t, 1 H, $^3J_{4,3}$ = $^3J_{4,5}$ = 8.8 Hz, H-4), 3.98 (dd, 1 H, $^3J_{6a,5}$ = 3.3, $^2J_{6a,6b}$ = 11.0 Hz, H-6a), 3.80 (dd, 1 H, $^2J_{6b,6a}$ = 11.2,$^3J_{6b,5}$ = 2.3 Hz, H-6b), 3.58 (dd, 1 H,$^3J_{4',3'}$ = 8.9, $^3J_{4',5'}$ = 8.9 Hz, H-4'), 3.58 (m, 1 H, H-5), 3.53 (dd, 1 H,$^2J_{6b',6a'}$ = 10.4, $^3J_{6b',5'}$ = 10.4 Hz, H-6b'), 3.49 (t, 1 H, $^3J_{3',2'}$ = $^3J_{3',4'}$ = 8.4 Hz, H-3'), 3.47 (dd, 1 H,$^3J_{2',3'}$ = 7.7, $^3J_{2',1'}$ = 7.3 Hz, H-2'), 3.47 (s, 3 H, OCH_3), 3.31 (ddd, 1 H, $^3J_{2,3}$ = 9.4, $^3J_{2,1}$ = 7.8, $^3J_{2,NH}$ = 7.8 Hz, H-2), 2.87 (dt, 1 H, $^3J_{5',6a'}$ = 5.0, $^3J_{5',6b'}$ = $^3J_{5',4'}$ = 9.7 Hz, H-6b'), 1.86 (s, 3 H, $NHCOCH_3$); ^{13}C NMR: (150 MHz, $CDCl_3$): 170.41 (C=O), 138.79 (Cq, Ar-C), 138.44 (Cq, Ar-C), 137.81 (Cq, Ar-C), 137.31 (Cq, Ar-C), 128.96 (Ar-C), 128.42 (2 C, Ar-C), 128.37 (2 C, Ar-C), 128.29 (2 C, Ar-C), 128.22 (2 C- Ar-C), 128.02 (2 C, Ar-C), 127.97 (2 C, Ar-C), 127.86 (Ar-C), 127.76 (Ar-C), 127.60 (Ar-C), 127.44 (2 C, Ar-C), 126.03 (2 C, Ar-C), 103.21 (C-1), 101.20 (Ar*C*H), 100.77 (C-1), 81.31 (C-4'), 80.33 (C-3'), 78.33 (C-3), 77.91 (C-4), 75.03 (C-2'), 74.52 (CH_2Ar), 74.42 (C-5), 74.24 (CH_2Ar), 73.53 (CH_2Ar), 68.64 (C-6'), 68.49 (C-6), 66.28 (C-5'), 57.09 (C-2), 56.69 (OCH_3), 23.60 (CH_3CO). ESI-QTOFMS: *m/z* calcd for $C_{43}H_{49}NO_{11}$: $[M+H^+]^+$ 756.3378; found 756.3381.

Methyl 2-azido-3-*O*-benzyl-4,6-O-benzylidene-2-deoxy-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-(1*H*-5-methyl-1,2,3,4-tetrazol-1-yl)-β-D-glucopyranoside (10). Compound **9** (20 mg, 0.026 mmol) was dried on a high vacuum line and coevaporated with toluene twice. DCM (0.2 mL) and pyridine (0.1 mL) were added under Ar through a septum and the mixture was cooled with an external ice/NaCl-mixture. A 1 M Tf_2O-solution in DCM (63 µL) was added and the colour changed to dark violet/blue. The solution was stirred for 30 min, whereupon additional Tf_2O-solution (20 µL) was added and the reaction warmed up to room temperature and kept stirring for 70 min. The mixture was diluted with DCM (10 mL) and quenched with saturated $NaHCO_3$-solution (10 mL). The aqueous phase was washed with DCM (5 mL), the combined organic phases were dried over $MgSO_4$ and filtered. Concentration of the filtrate afforded the crude triflate (25 mg) as brown oil. The activated triflate was dissolved in dry DMF (0.3 mL) under Ar-atmosphere and NaN_3 (8.5 mg, 0.13 mmol) was added and the mixture was stirred for a6 h at rt. Thereafter a condenser was attached to the flask and the mixture was heated to 70 °C for 2 h, when TLC showed complete consumption of the starting material. The reaction mixture was diluted with EtOAc (10 mL) and washed with H_2O (10 mL) and brine (10 mL). The organic phase was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (toluene-EtOAc 5:1) giving **10** (7 mg, 33 %) and **11** (2 mg, 10 %) as syrup. Data for **10**: R_f = 0.88 (toluene-EtOAc 5:1); $[\alpha]_D^{20}$ -29.8 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.48-7.46 (m, 2 H, Ar-H), 7.41-7.31 (m, 13 H, Ar-H), 7.25-7.23 (m, 3 H, Ar-H), 6.94-6.92 (m, 2 H, Ar-H), 5.51 (s, 1 H, C*H*Ar), 4.92 (d, 1 H, ^2J = 10.6 Hz, CH_2Ar), 4.82 (d, 1 H, $^3J_{1,2}$ = 8.2 Hz, H-1), 4.81 (d, 1 H, ^2J = 12.3 Hz, CH_2Ar), 4.75 (d, 1 H, ^2J = 11.7 Hz, CH_2Ar), 4.66 (d, 1 H, ^2J = 12.4 Hz, CH_2Ar), 4.63 (d, 1 H, $^3J_{1',2'}$ = 1.3 Hz, H-1'), 4.44 (d, 1 H, ^2J = 12.0 Hz, CH_2Ar), 4.28 (dd, 1 H, $^3J_{3,4}$ = 9.2, $^3J_{3,2}$ = 10.8 Hz, H-3), 4.16 (dd, 1 H, $^3J_{4,3}$ = 8.9, $^3J_{4,5}$ = 9.9 Hz, H-4), 4.11 (dd, 1 H, $^3J_{6a',5'}$ = 5.0, $^2J_{6a',6b'}$ = 10.4 Hz, H-6a'),

4.10 (d, 1 H, ^{2}J = 10.7 Hz, CH_2Ar), 4.05 (dd, 1 H, $^{3}J_{2,1}$ = 8.1, $^{3}J_{2,3}$ = 10.4 Hz, H-2), 3.91 (t, 1 H, $^{3}J_{4',3'}$ = $^{3}J_{4',5'}$ = 9.5 Hz, H-4'), 3.80 (dd, 1 H, $^{3}J_{6a,5}$ = 2.3, $^{2}J_{6a,6b}$ = 11.2 Hz, H-6a), 3.80 (dd, 1 H, $^{3}J_{2',1'}$ = 1.2, $^{3}J_{2',3'}$ = 3.8 Hz, H-2'), 3.78 (dd, 1 H, $^{3}J_{6b,5}$ = 2.8, $^{2}J_{6b,6a}$ = 11.4 Hz, H-6b), 3.65 (ddd, 1 H, $^{3}J_{5,6a}$ = 2.5, $^{3}J_{5,6b}$ = 2.5, $^{3}J_{5,4}$ = 9.8 Hz, H-5), 3.56 (dd, 1 H, $^{3}J_{6b',5'}$ = 10.1, $^{2}J_{6b',6a'}$ = 10.5 Hz, H-6b'), 3.47 (dd, 1 H, $^{3}J_{3',4'}$ = 9.6, $^{3}J_{3',2'}$ = 3.9 Hz, H-3'), 3.37 (s, 3 H, OCH_3), 3.07 (dt, 1 H, $^{3}J_{5',6a'}$ = 4.8, $^{3}J_{5',6b'}$ = $^{3}J_{5',4'}$ = 9.8 Hz, H-5'), 2.44 (s, 3 H, CH_3C); ^{13}C NMR: (150 MHz, $CDCl_3$): δ = 153.69 (N-C=N), 137.91 (Cq, Ar-C), 137.52 (Cq, Ar-C), 137.35 (Cq, Ar-C), 137.25 (Cq, Ar-C), 129.04 (1 C, Ar-C), 128.70 (2 C, Ar-C), 128.50 (2 C, Ar-C), 128.35 (1 C, Ar-C), 128.27 (2 C, Ar-C), 128.25 (2 C, Ar-C), 128.12 (2 C, Ar-C), 127.88 (1 C, Ar-C), 127.84 (1 C, Ar-C), 127.79 (2 C, Ar-C), 127.49 (2 C, Ar-C), 126.03 (2 C, Ar-C), 101.72 (C-1), 101.57 (*C*HAr), 99.89 (C-1'), 80.03 (C-3), 78.41 (C-4'), 77.58 (C-4), 76.53 (C-3'), 75.39 (*C*H_2Ar), 74.40 (*C*H_2Ar), 73.84 (*C*H_2Ar), 72.84 (C-6'), 68.33 (C-6), 68.06 (C-5'), 67.29 (C-2'), 63.63 (C-2), 57.42 (OCH_3), 8.82 (*C*H_3C) ppm; HRMS ($^{+}$ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{43}H_{47}N_7O_{11}$, found 806.3490.

Reaction of 2-acetamido-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (12) with triflic anhydride and pyridine. Compound **12** (8 mg, 0.021 mmol) was dried in high vacuum overnight and dissolved in CD_2Cl_2 (0.4 mL) and pyridine-$_{d5}$ (0.2 mL) under Ar-atmosphere in an NMR tube. Tf_2O (0.045 mL of a 1 M solution in DCM) was added slowly at room temperature and NMR spectra recorded over several hours. Data for **13**: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.56 (br signal, 0.6 H, H-2,6-pyr), 7.61 (br signal, 0.53 H, H-4-pyr), 7.21 (br signal, H-3,5-pyr), 6.02 (dd, 1 H, $^{3}J_{1,2}$ = 8.1, *J* = 0.8 Hz, H-1), 5.57 (t, 1 H, $^{3}J_{3,2}$ = $^{3}J_{3,4}$ = 9.5 Hz, H-3), 5.28 (ddd, 1 H, $^{3}J_{4,3}$ = 9.5, $^{3}J_{4,5}$ = 10.2 Hz, H-4), 4.35 (dd, 1 H, $^{3}J_{6a,5}$ = 4.5, $^{2}J_{6a,6b}$ = 12.5 Hz, H-6a), 4.23 (ddd, 1 H, *J* = 0.7, $^{3}J_{2,1}$ = 8.1, $^{3}J_{2,3}$ = 9.4 Hz, H-2), 4.14 (dd, 1 H, $^{3}J_{6b,5}$ = 2.3, $^{2}J_{6b,5}$ = 12.5 Hz, H-6b), 4.06 (ddd, 1 H, $^{3}J_{5,6b}$ = 2.4, $^{3}J_{5,6a}$ = 4.4, $^{3}J_{5,4}$ = 10.2 Hz, H-5), 2.88, 2.01, 2.00, 1.99 and 1.97 (5 s, each 3 H, CH_3CO); ^{13}C NMR: (150 MHz, $CDCl_3$): δ = 170.12 (C=O), 169.99 (C=O), 169.39 (C=O), 168.70 (C=O), 158.66 (C=N), 149.12 (2 C, *J* = 27.4 Hz, C-2,6-pyr), 135.40 (*J* = 24.7 Hz, C-4-pyr), 123.11 (2 C, *J* = 24.8 Hz, C-3,5-pyr), 92.71 (C-1), 73.12 (C-3), 72.86 (C-5), 67.58 (C-4), 64.23 (C-2), 61.54 (C-6), 20.43 (CH_3CO), 20.31 (CH_3CO), 20.28 (CH_3CO), 20.22 (CH_3CO), 15.51 (*C*H_3C=N); ^{19}F NMR (564.7 MHz, $CDCl_3$): δ -78.41 ppm.

Methyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (11). A solution of **9** (160 mg, 0.212 mmol) in dry pyridine (2.5 mL) and DCM (5 mL) was cooled with an ice bath under Ar. Mesyl chloride (49 µL, 0.635 mmol), was then added through a septum with a syringe and the solution was stirred for 2 d at rt. The mixture was diluted with DCM (20 mL), washed with water (20 mL) and $NaHCO_3$-solution (20 mL). The aqueous phase was reextracted with DCM (3 x 10 mL). The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo* to obtain 170 mg (96 %) of **14** as a brownish amorphous solid; R_f = 0.58 (EtOAc); $[\alpha]_D^{20}$ -27.4 (*c* 0.23, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.40-7.27 (m, 20 H, Ar-H), 5.94 (d, 1 H, $^{3}J_{NH,2}$ = 8.3 Hz, NH), 5.51 (s, 1 H, ArCH), 4.99 (d, 1 H, ^{2}J = 11.2 Hz, CH_2Ar), 4.70 (s, 2 H, CH_2Ar), 4.68 (d, 1 H, $^{3}J_{1,2}$ = 6.5 Hz, H-1), 4.65 (d, 1 H, ^{2}J = 11.8 Hz, CH_2Ar), 4.64 (d, 1 H, ^{2}J = 11.1 Hz, CH_2Ar), 4.57 (d, 1 H, $^{3}J_{1',2'}$ = 7.9 Hz, H-1'), 4.50 (d, ^{2}J = 11.5 Hz, CH_2Ar), 4.37 (dd, 1 H, $^{3}J_{2',1'}$ = 8.4 Hz, $^{3}J_{2',3'}$ = 8.4 Hz, H-2'), 4.24 (dd, 1 H, $^{3}J_{6a',5'}$ = 4.9 Hz, $^{2}J_{6a',6b'}$ = 10.5 Hz, 1 H, H-6a'), 4.10 (dd, 1 H, $^{3}J_{3,4}$ = 5.7, $^{3}J_{3,2}$ = 5.7 Hz, H-3), 3.91 (dd, 1 H, $^{3}J_{6a,5}$ = 2.9, $^{2}J_{6a,6b}$ = 9.2 Hz, H-6a), 3.84-3.74 (m, 4 H, H-2, H-5, H-6b, H-5) 3.68 (t, 1 H,$^{3}J_{3',4'}$ = $^{3}J_{3',2'}$ = 8.4 Hz, H-3'), 3.66 (t, 1 H, $^{3}J_{4',3'}$ = $^{3}J_{4',5'}$ = 9.1 Hz, H-4'), 3.60 (dd, 1 H, $^{3}J_{6b',5'}$ = 10.5, $^{2}J_{6b',6a'}$ = 10.5 Hz, H-6b'), 3.46 (s, 3 H, OMe), 3.16 (ddd, 1 H, $^{3}J_{5',6b'}$ = 4.6, $^{3}J_{5',6a'}$ = 9.4, $^{3}J_{5',4'}$ = 9.4 Hz, H-5'), 2.84 (s, 3 H, CH_3SO_2), 1.90 (s, 3 H, NAc). HRMS ($^{+}$ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{44}H_{51}NO_{13}S$, found 834.3154.

A solution of **14** (36 mg, 0.043 mmol) and NaN_3 (44 mg, 0.68 mmol) in dry DMF (1 mL) was stirred under Ar at 140 °C for 16 d. The mixture was diluted with EtOAc and washed with H_2O (10 mL) and brine (10 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was purified by column chromatography (toluene-EtOAc 1:1) to give 23 mg (70 %) of **11** as colorless amorphous solid; R_f = 0.6 (EtOAc); $[\alpha]_D^{20}$ -28.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.47-7.27 (m, 20 H, Ar-H), 5.66 (d, 1 H, $^{3}J_{NH,2}$ = 7.8 Hz,

N-H), 4.88 (d, 1 H, ^{2}J = 11.6 Hz, CH_2Ar), 4.78 (d, 1 H, ^{2}J = 12.1 Hz, CH_2Ar), 4.75 (d, 1 H, $^{3}J_{1,2}$ = 7.1 Hz, H-1), 4.66 (d, 1 H, ^{2}J = 11.8 Hz, CH_2Ar), 4.65 (d, 1 H, ^{2}J = 12.4 Hz, CH_2Ar), 4.63 (d, 1 H, ^{2}J = 11.3 Hz, CH_2Ar), 4.62 (d, 1 H, $^{3}J_{1',2'}$ = 1.4 Hz, H-1'), 4.45 (d, 1 H, ^{2}J = 11.9 Hz, CH_2Ar), 4.09 (dd, 1 H, $^{3}J_{6a',5'}$ = 5.0, $^{2}J_{6a',6b'}$ = 10.6 Hz, H-6a'), 4.01 (t, 1 H, $^{3}J_{3,2}$ = $^{3}J_{3,4}$ = 8.0 Hz, H-3), 3.95 (t, 1 H, $^{3}J_{4,3}$ = $^{3}J_{4,5}$ = 7.9 Hz, H-4), 3.91 (t, $^{3}J_{4',3'}$ = $^{3}J_{4',5'}$ = 9.5 Hz, 1 H, H-4'), 3.84 (dd, 1 H, $^{3}J_{2',1'}$ = 1.1 Hz, $^{3}J_{2',3'}$ = 3.7 Hz, H-2'), 3.79 (dd, 1 H, $^{3}J_{6a,5}$ = 3.7, $^{3}J_{6a,6b}$ = 10.7 Hz, H-6a), 3.75 (dd, 1 H, $^{3}J_{6b,5}$ = 3.6, $^{2}J_{6b,6a}$ = 10.8 Hz, H-6b), 3.62 (ddd, 1 H, $^{3}J_{5,6a}$ = 3.8 , $^{3}J_{5,4}$ = 7.8, $^{3}J_{5,6b}$ = 3.8 Hz, H-5), 3.59 (t, 1 H, $^{3}J_{6b',5'}$ = 10.1, $^{2}J_{6a',6b'}$ = 10.1 Hz, H-6b'), 3.52 (dd, 1 H, $^{3}J_{3',2'}$ = 3.8, $^{3}J_{3',4'}$ = 9.6 Hz, H-3'), 3.48 (ddd, 1 H, $^{3}J_{2,1}$ = 7.7, $^{3}J_{2,3}$ = 8.0, $^{3}J_{2,NH}$ = 8.0 Hz, H-2), 3.47 (s, 3 H, OCH_3), 3.07 (ddd, 1 H, $^{3}J_{5',6a'}$ = 4.9, $^{3}J_{5',6b'}$ = 9.8, $^{3}J_{5',4'}$ = 9.8 Hz, H-5'), 1.89 (s, 3 H, NAc); ^{13}C NMR: (150 MHz, $CDCl_3$): δ = 170.24 (C=O), 138.69 (Cq, Ar-C), 137.83 (Cq, Ar-C), 137.82 (Cq, Ar-C), 137.30 (Cq, Ar-C), 128.99 (Ar-C), 128.54 (2 C, Ar-C), 128.47 (2 C, Ar-C), 128.35 (2 C, Ar-C), 128.22 (2 C, Ar-C), 128.01 (1 C, Ar-C), 127.92 (4 C, Ar-C), 127.86 (Ar-C), 127.65 (Ar-C), 127.54 (2 C, Ar-C), 126.04 (2 C, Ar-C), 101.55 (Ar*C*H), 101.00 (C-1), 99.95 (C-1'), 78.44 (C-4'), 77.94 (C-3), 77.77 (C-4), 76.68 (C-3'), 74.41 (C-5), 74.03 (*C*H_2Ar), 73.64 (*C*H_2Ar), 72.85 (*C*H_2Ar), 69.05 (C-6), 68.34 (C-6'), 67.21 (C-5'), 63.63 (C-2'), 56.67 (OCH_3), 55.59 (C-2), 23.51 (*C*H_3CO). HRMS ($^{+}$ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{43}H_{48}N_4O_{10}$ 781.3447; found 781.3443.

Methyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (15). A suspension of **14** (50 mg, 0.064 mmol) in DCM (4 mL) was stirred with polymer-bound PPh_3 (475 mg, 1.425 mmol) for 2 d at rt under Ar. H_2O (3 mL) was added followed by 5 minutes of vigorous stirring. Then, the polymer was filtered off with Celite® followed by washing with MeOH (10 mL) and H_2O (10 mL). Concentration of the filtrate afforded the amine intermediate (33 mg, 69%). The residue (33 mg, 0.044 mmol) and a catalytic amount of DMAP were dissolved in pyridine (0.5 mL) under Ar. Ac_2O (25 µL, 0.264 mmol) was added at ice-bath temperature and the solution warmed to rt and stirred for 1.5 h. The reaction was quenched by addition of MeOH (0.1 mL), concentrated and coevaporated with toluene three times *in vacuo* which gave **15** (29 mg, 83 %) as colorless amorphous solid. R_f = 0.4 (EtOAc); $[\alpha]_D^{20}$ -0.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.49-7.27 (m, 20 H, Ar-H), 5.67 (d, 1 H, $^{3}J_{NH,2}$ = 8.2 Hz, NH), 5.56 (d, 1 H, $^{3}J_{NH',2'}$ = 9.1 Hz, NH'), 5.48 (d, 1 H, ArCH), 4.82 (d, 1 H, ^{2}J = 11.8 Hz, CH_2Ar), 4.78 (d, 1 H, $^{3}J_{1,2}$ = 7.7 Hz, H-1), 4.71-4.67 (m, 2 H, H-2', H-1'), 4.70 (d, 1 H, ^{2}J = 12.2 Hz, CH_2Ar), 4.70 (d, 1 H, ^{2}J = 11.9 Hz, CH_2Ar), 4.63 (d, 1 H, ^{2}J = 11.6 Hz, CH_2Ar), 4.52 (d, 1 H, ^{2}J = 12.1 Hz, CH_2Ar), 4.47 (d, 1 H, ^{2}J = 12.1 Hz, CH_2Ar), 4.15 (dd, 1 H, $^{3}J_{6a',5'}$ = 4.8, $^{2}J_{6a',6b'}$ = 10.4 Hz, H-6a'), 4.08 (t, 1 H, $^{3}J_{3,2}$ = 8.5, $^{3}J_{3,4}$ = 8.5 Hz, H-3), 4.04 (dd, 1 H, $^{3}J_{4,3}$ = 8.3, $^{3}J_{4,5}$ = 8.3 Hz, H-4), 3.80 (dd, 1 H, $^{3}J_{6a,5}$ = 3.3, $^{2}J_{6a,6b}$ = 11.1 Hz, H-6a), 3.73 (dd, 1 H, $^{3}J_{6b,5}$ = 2.8, $^{2}J_{6b,5}$ = 10.9 Hz, H-6b), 3.61 (t, 1 H,$^{3}J_{6b',5'}$ = 10.2, $^{2}J_{6b',6a'}$ = 10.2 Hz, H-6b'), 3.57 (t, 1 H, $^{3}J_{4',3'}$ = 9.3, $^{3}J_{4',5'}$ = 9.3 Hz, H-4'), 3.55 (ddd, 1 H, $^{3}J_{5,6b}$ = 2.7, $^{3}J_{5,6a}$ = 3.2, $^{3}J_{5,4}$ = 8.4 Hz, H-5), 3.50-3.45 (m, 1 H, H-3'), 3.48 (s, 3 H, OMe), 3.36 (ddd, 1 H, $^{3}J_{2,1}$ = 8.1, $^{3}J_{2,3}$ = 8.1, $^{3}J_{2,NH}$ = 8.1 Hz, H-2), 3.15 (ddd, 1 H, $^{3}J_{5',6a'}$ = 5.0, $^{3}J_{5',6b'}$ = 9.7, $^{3}J_{5',a'}$ = 9.7 Hz, H-5'), 1.92 (s, 3 H, NAc), 1.90 (s, 3 H, NAc); ^{13}C NMR: (150 MHz, $CDCl_3$): δ = 170.51 (2 C, C=O), 137.89 (Cq, Ar-C), 129.03 (Ar-C), 128.56 (2 C, Ar-C), 128.43 (2 C, Ar-C), 128.39 (2 C, Ar-C), 128.23 (2 C, Ar-C), 128.00 (1 C, Ar-C), 127.90 (2 C, Ar-C), 127.72 (2 C, Ar-C), 127.7 (2 C, Ar-C), 126.08 (2 C, Ar-C), 101.68 (Ar*C*H), 100.77 (C-1), 99.61 (C-1'), 78.69 (C-4'), 78.58 (C-3), 76.70 (C-4)*, 75.64 (C-3'), 74.21 (C-5), 73.97 (*C*H_2Ar), 73.55 (*C*H_2Ar), 71.44 (*C*H_2Ar), 68.63 (C-6), 68.63 (C-6'), 67.03 (C-5'), 56.79 (2 C, C-2, OCH_3), 50.42 (C-2'), 23.56 (CH_3CO), 23.39 (CH_3CO) ppm. HRMS ($^{+}$ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{45}H_{52}N_2O_{11}$ 797.364; found 797.3645.

Methyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (16). Trifluoroacetic acid (290 µL) was added to a solution of **15** (26 mg, 0.033 mmol) in DCM (1150 µL) externally cooled with an ice/NaCl-bath below 0 °C under Ar and stirred for 25 min. The solution was diluted with DCM (5 mL), followed by addition of DOWEX anion-exchange resin (HCO_3^-form, 10 g). The resin was filtered off and washed with DCM (20 mL). The filtrate was concentrated and the residue was purified by column chromatography (EtOAc-MeOH 95:5) to give **16** (23 mg, 99%) as colorless crystals, m.p.

113-115°C; R_f = 0.25 (EtOAc-MeCN 4:1), $[\alpha]_D^{20}$ -45.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.35-7.26 (m, 15 H, Ar-H), 6.03 (d, 1 H, $^3J_{NH',2'}$ = 9.7 Hz, NH'), 5.66 (d, 1 H, $^3J_{NH,2}$ = 8.3 Hz, NH), 4.83 (d, 1 H, ^2J = 11.8 Hz, CH_2Ar), 4.76 (d, 1 H, ^2J = 11.2 Hz, CH_2Ar), 4.65 (ddd, 1 H, $^3J_{2',1'}$ = 1.4, $^3J_{2',NH'}$ = 9.8, $^3J_{2',3'}$ = 4.5 Hz, H-2'), 4.65 (d, 1 H, ^2J = 11.9 Hz, CH_2Ar), 4.61 (d, 1 H, ^2J = 11.9 Hz, CH_2Ar), 4.60 (d, 1 H, $^3J_{1',2'}$ = 1.4 Hz, H-1'), 4.60 (d, 1 H, $^3J_{1,2}$ = 7.0 Hz, H-1), 4.44 (d, 1 H, ^2J = 12.0 Hz, CH_2Ar), 4.29 (d, 1 H, ^2J = 11.4 Hz, CH_2Ar), 4.04 (t, 1 H, $^3J_{3,4}$ = 8.4, $^3J_{3,2}$ = 8.4 Hz, H-3), 3.90 (t, 1 H, $^3J_{4,5}$ = 8.6, $^3J_{4,5}$ = 8.6 Hz, H-4), 3.72 (dd, 1 H, $^3J_{6a',5'}$ = 2.8, $^2J_{6a',6b'}$ = 12.0 Hz, H-6a'), 3.71 (d, 1 H, $^3J_{6,5}$ = 3.8 Hz, H-6a), 3.71 (app d, 1 H, $^3J_{6,5}$ = 3.8 Hz, H-6b), 3.61 (dd, 1 H, $^3J_{6b',5'}$ = 4.8, $^2J_{6b',6a'}$ = 12.1 Hz, H-6b'), 3.57 (ddd, 1 H, $^3J_{2,1}$ = 7.5, $^3J_{2,3}$ = 8.4, $^3J_{2,NH}$ = 8.4 Hz, H-2), 3.54 (t, 1 H, $^3J_{4',3'}$ = 9.5, $^3J_{4',5'}$ = 9.5, H-4'), 3.53 (ddd, 1 H, $^3J_{5,6a}$ = 3.3, $^3J_{5,4}$ = 8.8, $^3J_{5,6b}$ = 3.3 Hz, H-5), 3.45 (s, 3 H, OCH_3), 3.21 (dd, 1 H, $^3J_{3',2'}$ = 4.2, $^3J_{3',4'}$ = 9.4 Hz, H-3'), 3.10 (ddd, 1 H, $^3J_{5',6a'}$ = 3.1, $^3J_{5',6b'}$ = 4.7, $^3J_{5',4'}$ = 9.8 Hz, H-5'), 1.93 (s, 3 H, CH_3CO), 1.82 (s, 3 H, CH_3CO); ^{13}C NMR: (150 MHz, $CDCl_3$): 170.68 (C=O), 170.51 (C=O), 138.50 (Cq, Ar), 137.78 (Cq, Ar), 137.53 (Cq, Ar-C), 128.52 (6 C, Ar-C), 128.22 (2 C, Ar-C), 127.97 (1 C, Ar-C), 127.95 (2 C, Ar-C), 127.87 (1 C, Ar-C), 127.82 (1 C, Ar-C), 127.62 (2 C, Ar-C), 101.10 (C-1), 98.80 (C-1'), 79.52 (C-3'), 78.71 (C-4), 76.35 (C-3), 76.16 (C-5'), 74.13 (C-5), 74.02 (*C*H_2Ar), 73.49 (*C*H_2Ar), 70.68 (*C*H_2Ar), 68.76 (C-6), 66.74 (C-4'), 61.83 (C-6'), 56.58 (OCH_3), 55.69 (C-2), 49.11 (C-2'), 23.48 (*C*H_3CO), 23.26 (*C*H_3CO) ppm. HRMS ($^+$ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{38}H_{48}N_2O_{11}$ 709.3331; found 709.3332.

Methyl 2-acetamido-3-*O*-benzyl-2-deoxy-4,6-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (17). A solution of methyl pyruvate (100 µL of a stock solution containing 100 µL in 2.4 mL MeCN) was added to an ice-cold solution of **16** in MeCN (1150 µL) under Ar and stirred for 10 min. TMSOTf (19 µL, 0.105 mmol) was then added through a septum and the solution was stirred at ice-bath temperature for 4.5 h, when TLC showed consumption of the starting material. The solution was diluted with EtOAc and washed with satd aq $NaHCO_3$-solution. The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was purified by column chromatography (toluene-EtOAc 1:1) to give 10 mg (75 %) of **17** as syrup; R_f = 0.43 (EtOAc-MeCN 4:1); $[\alpha]_D^{20}$ -16 (*c* 0.15, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.32-7.20 (m, 15 H, Ar-H), 5.67 (br s, 1 H, NH), 5.50 (br s, 1 H, NH), 4.68 (d, 1 H, ^2J = 11.6 Hz, CH_2Ar), 4.65 (d, 1 H, $^3J_{1,2}$ = 7.4 Hz, H-1), 4.62 (d, 1 H, ^2J = 12.3 Hz, CH_2Ar), 4.58 (d, 1 H, ^2J = 12.1 Hz, CH_2Ar), 4.57 (d, 1 H, ^2J = 11.8 Hz, CH_2Ar), 4.553 (d, 1 H, $^3J_{1',2'}$ = 1.3 Hz, H-1'), 4.547 (ddd, 1 H, $^3J_{2',1'}$ = 1.4 , $^3J_{2',3'}$ = 5.0, $^3J_{2',NH'}$ = 9.9 Hz, H-2'), 4.51 (d, 1 H, ^2J = 11.6 Hz, CH_2Ar), 4.36 (d, 1 H, ^2J = 11.8 Hz, CH_2Ar), 3.93-3.88 (m, 2 H, H-3, H-4'), 3.81 (dd, 1 H, $^3J_{6a,5}$ = 5.0, $^2J_{6a,6b}$ = 10.6 Hz, H-6a), 3.75 (s, 3 H, CO_2CH_3), 3.73 (dd, 1 H, $^3J_{6a',5'}$ = 3.4, $^2J_{6a',6b'}$ = 10.9 Hz, H-6a'), 3.63 (dd, 1 H, $^3J_{6b',5'}$ = 3.1, $^2J_{6b',6a'}$ = 11.0 Hz, H-6b'), 3.49 (dd, 1 H, $^3J_{6b,5}$ = 10.3, $^2J_{6b,6a}$ = 10.7 Hz, H-6b), 3.47-3.40 (m, 2 H, H-5', H-4), 3.39 (s, 3 H, OCH_3), 3.36-3.30 (m, 2 H, H-2, H-3'), 2.97 (ddd,1 H, $^3J_{5,6a}$ = 4.8, $^3J_{5,6b}$ = 10.3, $^3J_{5,4}$ = 10.3 Hz, H-5), 1.81 (s, 6 H, 2 x CH_3CO), 1.48 (s, 3 H, CH_3); ^{13}C NMR: (150 MHz, $CDCl_3$): δ = 170.69 (2 x C=O), 170.10 (C=O, pyr), 138.63 (Cq, Ar-C), 138.21 (Cq, Ar-C 137.93 (Cq, Ar-C), 128.50 (2 C, Ar-C), 128.38 (2 C, Ar-C), 128.28 (2 C, Ar-C), 127.94 (1 C, Ar-C), 127.87 (2 C, Ar-C), 127.65 (1 C, Ar-C), 127.53 (3 C, Ar-C), 127.32 (2 C, Ar-C), 100.86 (C-1), 99.51 (C-1'), 99.11 (Cq, *C*CO_2CH_3), 78.67 (C-3), 76.47 (C-4'), 75.25 (C-4), 75.10 (C-3'), 74.26 (C-5'), 73.85 (*C*H_2Ar), 73.52 (*C*H_2Ar), 71.3 (*C*H_2Ar), 68.72 (C-6'), 66.43 (C-5), 65.18 (C-6), 56.72 (OCH_3), 56.24 (C-2), 52.76 (CO_2CH_3), 50.34 (C-2'), 25.54 (*C*$H_3CCO_2CH_3$), 23.36 (2 x CH_3CO) ppm. HRMS ($^+$ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{42}H_{52}N_2O_{13}$, 793.3542; found 793.3541.

Methyl 2-acetamido-2-deoxy-4,6-*O*-[1-(methoxycarbonyl)ethylidene]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (18). A suspension of **17** (5 mg, 0.007 mmol) in dry MeOH (1.5 mL) containing 10% Pd-carbon catalyst was evacuated and flushed with Ar four times. The atmosphere was switched to H_2 and the mixture was stirred for 5 h at rt, when TLC showed complete consumption of the starting material. The catalyst was filtered off over Celite® and washed with MeOH several times (10 mL in total). The filtrate was concentrated *in vacuo* to give **18** (3.2 mg, 89 %) as amorphous solid; R_f = 0.75 ($CHCl_3$-

MeOH-H_2O 3:2.5:0.5); ^{1}H NMR (600 MHz, MeOD): δ = 4.85 (d, 1 H, $^3J_{1',2'}$ = 1.7 Hz, H-1'), 4.61 (dd, 1 H,$^3J_{2',1'}$ = 1.6, $^3J_{2',3'}$ = 4.6 Hz, H-2'), 4.30 (d, 1 H, $^3J_{1,2}$ = 8.5 Hz, H-1), 4.00 (dd, 1 H, $^3J_{6a',5'}$ = 5.0, $^2J_{6a',6b'}$ = 10.5 Hz, H-6a'), 3.85 (dd, 1 H, $^3J_{3',2'}$ = 4.7, $^3J_{3',4'}$ = 9.8 Hz, H-3'), 3.82 (s, 3 H, CO_2CH_3), 3.81 (dd, 1 H, $^3J_{6a,5}$ = 2.1, $^2J_{6a,6b}$ = 12.3 Hz, H-6a), 3.77 (dd, 1 H, $^3J_{6b',5'}$ = 10.5, $^3J_{6b',6a'}$ = 10.5 Hz, H-6b'), 3.72 (dd, 1 H, $^3J_{6b,5}$ = 4.0, $^2J_{6b,6a}$ = 12.2 Hz, H-6b), 3.66 (dd, 1 H, $^3J_{2,3}$ = 10.3, $^3J_{2,1}$ = 8.5 Hz, H-2), 3.64 (dd, 1 H, $^3J_{4,5}$ = 9.2, $^3J_{4,3}$ = 9.2 Hz, H-4), 3.57 (dd, 1 H, $^3J_{4',5'}$ = 10.0, $^3J_{4',3'}$ = 10.0 Hz, H-4'), 3.53 (dd, 1 H, $^3J_{3,4}$ = 8.9, $^3J_{3,2}$ = 10.4 Hz, H-3), 3.44 (s, 3 H, OCH_3), 3.37 (dt, 1 H, $^3J_{5',6a'}$ = 5.0, $^3J_{5',6b'}$ = 10.0, $^3J_{5',4'}$ = 10.0 Hz, H-5'), 3.28 (ddd, 1 H, $^3J_{5,6a}$ = 2.0, $^3J_{5,6b}$ = 3.9, $^3J_{5,4}$ = 9.7 Hz, H-5), 2.01 (s, 3 H, CH_3CO), 1.96 (s, 3 H, CH_3CO), 1.48 (s, 3 H, CH_3); ^{13}C NMR: (150 MHz, MeOD): 174.72 (NHC=O), 173.63 (NHC=O), 171.96 (OC=O), 103.47 (C-1), 101.72 (C-1'), 100.72 (Cq, *C*CO_2CH_3), 80.64 (C-4), 76.34 (C-5), 75.96 (C-4'), 74.08 (C-3), 70.96 (C-3'), 68.38 (C-5'), 65.65 (C-6'),61.63 (C-6), 57.00 (OCH_3), 56.97 (C-2), 54.83 (C-2'), 53.17 (CO_2CH_3), 25.86 (CH_3), 22.89 (*C*H_3CO), 22.66 (*C*H_3CO) ppm. HRMS ($^+$ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{21}H_{34}N_2O_{13}$, 523.2134; found 523.2134.

Methyl 2-acetamido-2-deoxy-4,6-*O*-(1-carboxyethylidene)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside sodium salt (19). A solution of **18** (3.2 mg, 0.007 mmol) in MeOH (1.5 mL) and 0.2 M NaOH (530 µl) was stirred for 2 h at rt when TLC indicated complete consumption of the starting material. The pH-value was adjusted to neutral with Dowex cation exchange resin (H^+ form). The resin was filtered off and washed with water (10 mL). The solution was frozen and lyophilized to afford 2.5 mg (77 %) of product. Final purification was done by filtration on BioGel P-2 (5% EtOH in water) to give 2.2 mg (68 %) of **19** as colorless solid ; $[\alpha]_D^{20}$ -38.9 (*c* 0.1, H_2O); ^{1}H NMR (600 MHz, D_2O): δ = 4.94 (d, 1 H, $^3J_{1',2'}$ = 1.8 Hz, H-1'), 4.61 (dd, 1 H, $^3J_{2',1'}$ = 1.7, $^3J_{2',3'}$ = 4.7 Hz, H-2'), 4.43 (d, 1 H, $^3J_{1,2}$ = 8.0 Hz, H-1), 4.04 (dd, 1 H, $^3J_{6a',5'}$ = 5.3, $^2J_{6a',6b'}$ = 10.7 Hz, H-6a'), 3.99 (dd, 1 H, $^3J_{3',2'}$ = 4.7, $^3J_{3',4'}$ = 10.0 Hz, H-3'), 3.87 (dd, 1 H, $^3J_{6a,5}$ = 2.2, $^2J_{6a,6b}$ = 12.3 Hz, H-6a), 3.75 (t, 1 H, $^3J_{6b',5'}$ = 10.9, $^2J_{6b',6a'}$ = 10.9 Hz, H-6b'), 3.73 (dd, 1 H, $^3J_{6b,5}$ = 5.2, $^2J_{6b,6a}$ = 12.2 Hz, H-6b), 3.70 (dd, 1 H, $^3J_{2,1}$ = 8.1, $^3J_{2,3}$ = 10.2 Hz, H-2), 3.69 (t, 1 H, $^3J_{4,3}$ = 9.1, $^3J_{4,5}$ = 9.1 Hz, H-4), 3.65 (dd, 1 H, $^3J_{3,4}$ = 9.0, $^3J_{3,2}$ = 10.1 Hz, H-3), 3.63 (t, 1 H, $^3J_{4',5'}$ = 10.1, $^3J_{4',3'}$ = 10.1 Hz, H-4'), 3.50 (s, 3H, OCH_3), 3.49 (ddd, 1 H, $^3J_{5,6a}$ = 2.4, $^3J_{5,6b}$ = 5.0, $^3J_{5,4}$ = 9.2 Hz, H-4), 3.45 (ddd, 1 H, $^3J_{5',6a'}$ = 5.0, $^3J_{5',6b'}$ = 10.1, $^3J_{5',4'}$ = 10.1 Hz, H-5'), 2.08 (s, 3 H, CH_3CO), 2.04 (s, 3 H, CH_3CO), 1.47 (s, 3 H, CH_3); ^{13}C NMR: (150 MHz, D_2O): 176.30 (C=O), 176.25 (C=O), 175.50 (C=O), 102.69 (C-1), 102.67 (Cq, *C*CO_2), 100.65 (C-1'), 79.65 (C-4), 75.31 (C-5), 74.71 (C-4'), 73.11 (C-3), 70.16 (C-3'), 67.65 (C-5'), 64.87 (C-6'), 60.95 (C-6), 57.90 (OCH_3), 56.08 (C-2), 54.31 (C-2'), 25.49 (*C*H_3CCO_2Na), 22.97 (*C*H_3CO), 22.84 (*C*H_3CO) ppm. HRMS ($^+$ESI-TOF) *m/z* $[M+Na]^+$ calcd for $C_{20}H_{32}N_2NaO_{13}$ 531.1797, found 531.1798.

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Supplementary Material

^{1}H and ^{13}C NMR spectra of novel compounds can be found in the supporting information.

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