

Synthesis of a 3-hydroxyl- free *N*-acetyl glucosamine disaccharide

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Abstract

A simple and alternative route to a versatile *N*-acetyl glucosamine disaccharide building block was developed, possessing a free 3-hydroxyl group. In this strategy, the 2,2,2-trichloro-ethoxy carbonyl (Troc) group was used as an amino- and 3-hydroxyl- protecting group.

Keywords: *N*-acetyl glucosamine, glycosylation, trichloro-ethoxycarbonyl group, glucosamine

Introduction

Carbohydrates are the most abundant class of organic compounds in nature and have specific biological roles in living organisms.¹ The vast majority of carbohydrates exist as polysaccharides, glycoconjugates or glycosides linked to other carbohydrate units or to aglycones *via* O-glycosidic bonds. The most important classes of oligoconjugates and naturally occurring oligosaccharides contain 2-amino-2-deoxysugar moieties, which are connected to other residues, commonly *via* a 1,2-*trans*-glycosidic linkage.² Specifically, 2-*N*-acetamido-2-deoxyglycosides, most frequently of D-glucose and D-galactose sequences, are found abundantly as glycoconjugates in living organisms. These 2-*N*-acetamido-2-deoxyglycosides contain glucosamine units which can be glycosylated through O-3, O-4, and O-6 positions.³

2-Amino- sugars also play an important role on cell surfaces,¹ and consequently are attractive targets for medicinal research. To investigate the biological activities associated with these oligosaccharides, isolation and purification of natural materials, in pure form, and in significant amount are required. Owing to the increasing interest in 2-amino- sugar oligosaccharides, special efforts have been dedicated to searches for efficient synthetic approaches to such complex molecules involving efficient, simple, region- and stereo- selective methods.² To date, one of the most efficient strategies to prepare oligosaccharides consists in the preparation of key building blocks of di-, tri-, and higher- oligosaccharides, that can be further used to assemble larger molecules.³

Our group has focused on the development of straightforward routes to glucosamine disaccharides.⁴ Properly functionalized glucosamine disaccharides constitute key scaffolds in the synthesis of complex and biologically important oligosaccharides, such as the bacterial peptidoglycan unit (A),⁵ or for the preparation of branched tetrasaccharides (B),⁶ which are useful for the development of new anti-tumor therapies (Figure 1).

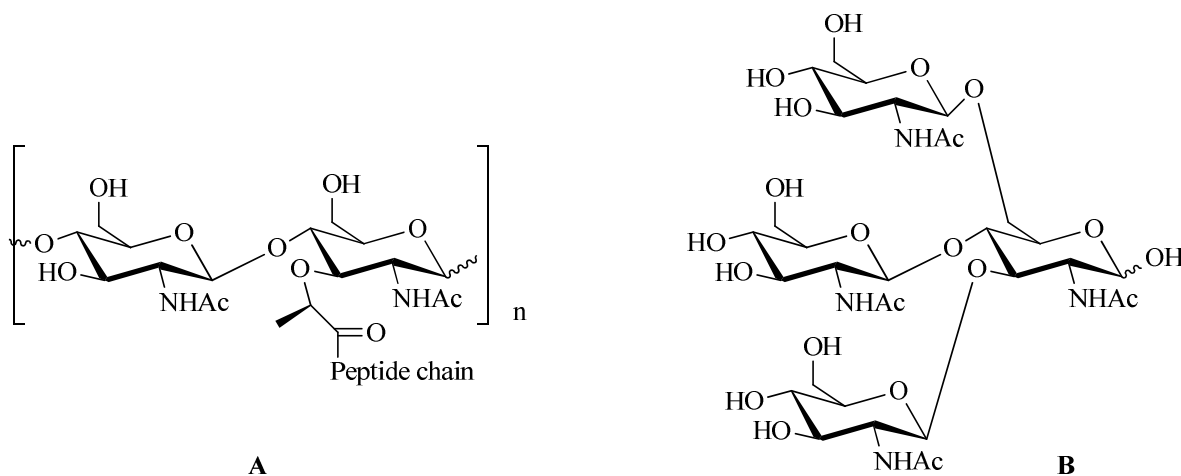


Figure 1. Examples of biologically important oligosaccharides.

Recently, we have explored a new synthetic strategy towards O-3- hydroxyl- free *N*-acetyl glucosamine disaccharides. We envisaged that a properly functionalized glucosamine disaccharide would allow further functionalization at O-3, such as regioselective glycosylation or lactate insertion. Also, the chosen substitution pattern would chemically differentiate the two glucosamine units, allowing the preparation of branched oligosaccharides as shown in Figure 2.

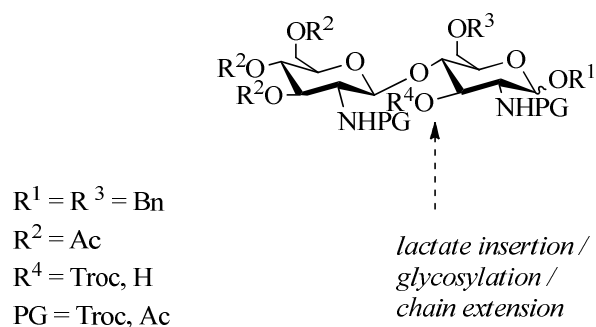


Figure 2. Proposed disaccharide substitution pattern.

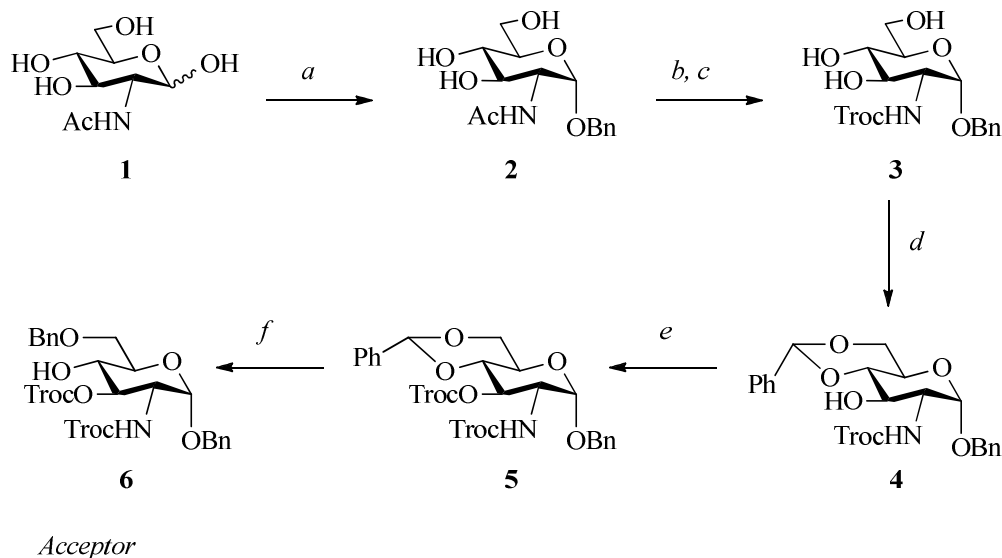
The preparation of monomeric building blocks, donors and acceptors, commonly requires the manipulation of several protecting groups. Despite the stereoselective formation of the glycosidic bond, special attention has been given to the selective protection- and deprotection-

strategy and to the use of suitable protecting groups.⁷ In general, a standard strategy demands the use of robust protecting groups that survive various reaction conditions in multi-step sequences. The deprotection steps must occur under mild conditions and be performed in the presence of other functional groups. Therefore, it is highly desirable to develop new synthetic routes involving as few functional- group manipulations as possible.

Results and Discussion

The first step relied on the choice of the nitrogen protecting group for donor- and acceptor-moieties. It is well known that *N*-Troc (2,2,2-trichloro-ethoxy carbonyl)- glucosamine donors- and acceptors are more reactive than the corresponding *N*-Phth glucosamines.⁸ The Troc group also gives higher β -selectivities than other groups.⁹ Also, the Troc group as an *N*-protecting group enhances glucosamine-4-hydroxy- acceptor reactivity when compared to other *N*-protecting groups,¹⁰ and can be removed under mild conditions.¹¹ On the other hand, a limited number of *O*-protecting groups has been reported in approaches developed towards *N*-Troc glucosamine units possessing an *O*-3 hydroxyl- group masked: Fmoc (9-fluorenylmethyl carbonate),¹² Cbz (carbobenzyloxy),¹³ Ac (acetyl)¹⁴ and Troc.¹⁵ However, the Troc group has been scarcely used as an *O*-protecting group, and has only recently been reported in *N*-Troc glucosamine^{15a} glycosyl units.

We therefore selected Troc as an amine- protecting group for both an acceptor and donor. Particularly, in the case of the acceptor, the Troc group was also chosen to temporarily mask the *O*-3. This would allow protecting group removal under mild conditions, in a more advanced stage of the synthesis, in order to afford, after selective *N*-acetylation, a free hydroxyl group at *O*-3 at the final *N*-acetyl glucosamine disaccharide.



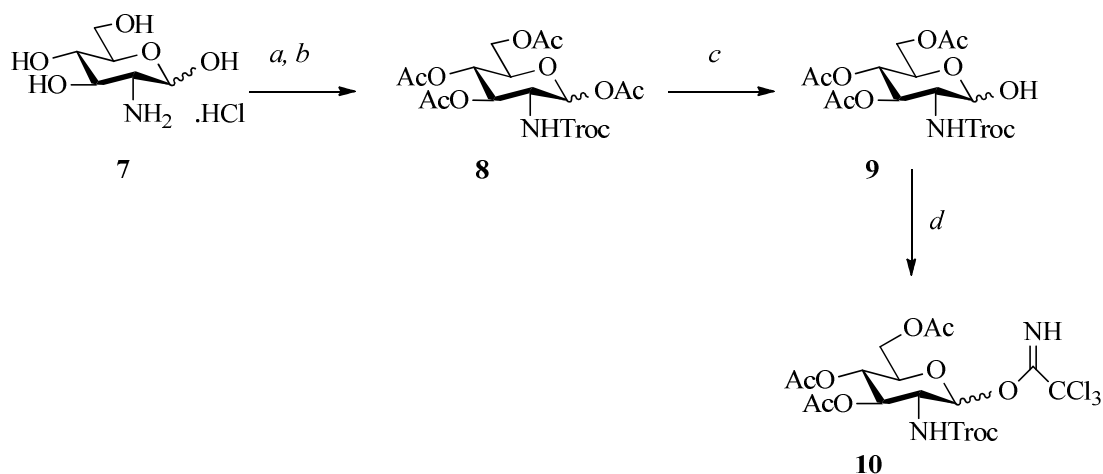
a) BnOH, *p*-TsOH, toluene, reflux, 5 h; b) KOH, EtOH, reflux; c) TrocCl, NaHCO₃, H₂O, RT; d) PhCH(OMe)₂, CSA, MeCN, RT, 24 h; e) TrocCl, pyridine, RT, 12 h; f) Et₃SiH, BF₃·OEt₂, CH₂Cl₂, RT, 3 h.

Scheme 1. Synthesis of the glucosamine acceptor.

The synthesis started with the acceptor preparation (Scheme 1). Although the allyl ether has been frequently used as an anomeric protecting group, the benzyl group was chosen as an anomeric protecting group to avoid the use of expensive metal catalysts frequently employed in deallylation procedures.¹⁶ Thus, benzylation of *N*-Acetyl glucosamine (**1**) afforded **2**,¹⁷ and subsequent replacement of the *N*-acetyl group by the *N*-Troc group gave **3** in 69% yield. The next step consisted in the arylidene-acetal formation to give **4** in 62% yield, followed by protection of the O-3 position with a Troc group, to afford **5** in 61% yield. Although **4** has already been prepared by a different route,¹⁸ our protocol allows an easier manipulation of the sequence intermediates, while avoiding the use of excess benzaldehyde and HCl.

The selective benzylidene acetal ring opening was achieved by reductive ring-opening using triethylsilane and BF₃·OEt₂, and the acceptor **6** was isolated in 63% yield.

The preparation of the donor moiety was carried out using a simple sequence starting from *D*-glucosamine hydrochloride (**7**) (Scheme 2). Thus, *N*-Troc-1,3,4,6-*O*-tetra-acetyl glucosamine (**8**) was isolated in 85% yield after two steps.¹⁹ Selective removal of the anomeric acetyl group was performed by using morpholine, and after an acidic work-up and column chromatography⁹ was isolated in 79% yield.¹⁹ Treatment of **9** with CCl₃CN and Cs₂CO₃ afforded the desired glycosyl trichloro-acetamidate **10**²⁰ in quantitative yield.

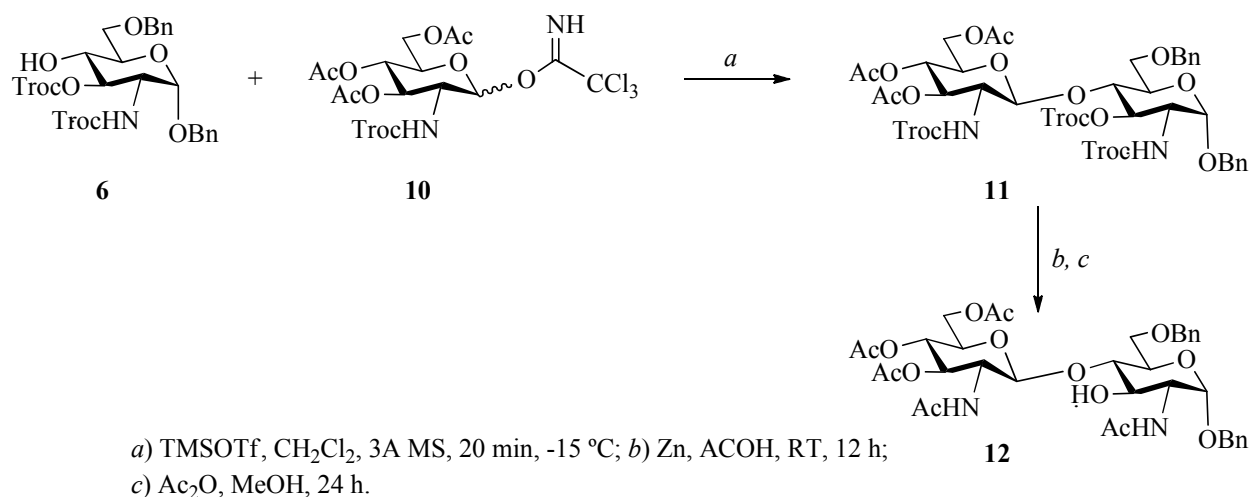


a) TrocCl, H₂O, NaHCO₃, 0 °C to RT, 12 h; *b)* Ac₂O, Pyridine, RT, 12 h; *c)* morpholine, EtOAc, RT, 8 h; *d)* Cs₂CO₃, CH₂Cl₂, RT, 1.5 h.

Scheme 2. Synthesis of the glucosamine donor.

With the glucosamine acceptor, **6**, and donor, **10**, in hand, the next stage consisted in the glycosylation reaction. Thus, several experiments were carried to improve the yield of the glycosylation reaction, and several donor/acceptor ratios were investigated. The best results were obtained, under standard glycosylation conditions, when a 2:1 donor/acceptor ratio was used, and the desired β(1-4) glycoside **11** could be isolated in 40% yield. However, when the donor molar ratio increased, undesired side products were formed.

The next step consisted in the removal of the three trichloro-ethyl carbamate protective groups, which was achieved by treatment of **11** with freshly activated zinc in acetic acid. Selective N-acetylation was performed using Ac₂O in methanol, and the *N*-acetyl glucosamine disaccharide **12**, possessing a free 3-OH, was isolated in 50% yield (Scheme 3).



Scheme 3. Glycosylation and Troc group removal.

The disaccharide **12** constitutes a valuable intermediate for peptidoglycan fragments assembly. It is well known that in peptidoglycan synthesis, manipulation of the muramic acid building blocks can occur with side-reactions on the (*R*)-lactyl moiety, such as racemization, or intramolecular lactonization at O-4.²¹ Thus, with our approach the lactate insertion can be performed in a later stage of the synthesis, with the *N*-acetyl group already installed. Moreover, some difficulties are reported regarding the low nucleophilicity associated with the O-4 position,²² and some methods have been reported to overcome this problem, such as the use of the oxazolidine group.⁹ The approach presented herein uses the Troc group as an amino group with the advantage of guaranteeing the β -stereoselectivity at the glycosylation step, as well as Troc as an O-3 protecting group due to its easy removal in a one-pot procedure, at an advanced stage of the synthesis.

Additionally, the disaccharide **12** can be suitable for the preparation of glycoconjugates that contain *N*-acetylglucosamine and that glycosylate at O-3 and O-4, and allows an easy access to regioselective glycosylation at O-3 with other carbohydrate units (glucose, galactosamine).⁶

Conclusions

In summary, the use of two different substitution patterns for acceptor- and donor-, benzyl- and acetyl- groups, respectively, permits manipulation of the two units of the disaccharide independently by simply protecting- group removal. The protecting groups used are simple to remove, and all the steps involved in this route were performed under mild conditions, and avoided expensive reagents. The disaccharide **12** is a key scaffold for the preparation of several *N*-acetyl glucosamine derivatives useful for medicinal research, such as cancer diseases. Overall,

this constitutes a simple and alternative route to a versatile glucosamine disaccharide building block.

Experimental Section

General. Melting points were recorded on a Reichert-Thermovar hot stage apparatus and are uncorrected. Ordinary mass spectra were recorded on a Fisons Trio or an AEI MS-9 spectrometer. High resolution mass spectra were recorded on an AutoSpeQ spectrometer. ^1H and ^{13}C NMR spectra were recorded in CDCl_3 on a Bruker ARX 400 spectrometer (400 MHz for ^1H and 100.63 MHz for ^{13}C). Chemical shifts reported are relative to tetramethylsilane as the internal reference (^1H 0.00) for ^1H NMR spectra and to CDCl_3 (^{13}C 77.00) for ^{13}C NMR spectra. Chemical shifts are expressed in parts per million downfield from TMS ($\delta=0$) or residual dichloromethane ($^1\text{H}=5.32$, $^{13}\text{C}=53.1$) as internal standards. IR spectra were run on Perkin-Elmer 683- and Spectrum 1000- instruments with absorption frequencies expressed in reciprocal centimeters. All reagents and solvents were purified and dried by standard methods²³ before use. The term “usual work-up” implies that organic extracts were washed with water and dried over anhydrous sodium sulfate or magnesium sulfate, filtered, and solvent removed from the filtrate under reduced pressure. Analytical thin-layer chromatography and preparative TLC (PTLC) were performed on E. Merck Kieselgel 60, F254 silica gel (0.2 mm thick), or 0.5-, 1- or 2- mm thick plates (20x20 cm), respectively. Column chromatography was performed on E. Merck Kieselgel 60 (240–400 mm) silica gel. “RT” denotes room temperature.

Benzyl 2-deoxy-2-(2,2,2-trichloro-ethoxycarbonylamino)- α -D-glucopyranoside (3).¹⁸ To a solution of *N*-Acetyl-glucosamine **1** (3 g, 13.5 mmol) and benzyl alcohol (22 mL) in toluene (36 mL), was added *p*-toluenesulfonic acid monohydrate (0.15 g, 1.15 mmol). The reaction mixture was refluxed in a Dean–Stark apparatus with water- removal by the azeotrope mixture. After 5 h the reaction mixture was cooled to RT and a saturated solution of sodium bicarbonate was added. Toluene was removed under reduced pressure and diethyl ether: *n*-hexane (2:1, 80 mL) was added and stirred vigorously for 3 h. The light brown- colored precipitate was filtered off, washed with ether, and the crude product was recrystallized from ethanol to give a light brown solid, **2**, in (2.1 g, 50% yield).¹⁷ The compound **2** was dissolved in 40 mL of ethanol, KOH (12.0 g) was added, and the mixture heated at reflux under N_2 overnight. The flask was cooled in an ice bath and the mixture neutralized with concentrated HCl. The precipitate formed was filtered off, washed with ethanol, and the ethanol layer concentrated. The residue obtained was dissolved in H_2O (20 mL), and NaHCO_3 (2 g) was added at 0 °C. TrocCl (1.2 mL, 9 mmol) was added dropwise and the mixture stirred for 2 h, warmed to RT, and stirred overnight. The crude material was neutralized with 1N HCl and the resulting white precipitate was filtered off, washed with water (2x10 mL) and ether (2x10 mL), and vacuum- dried. The compound obtained was recrystallized from ethanol and identified as **3**, a white solid (2.0 g) in 69% yield, m.p. 85-87 °C;

$[\delta]_{\text{D}}^{25} +100^{\circ}$ (c 1, MeOH). ^1H NMR (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, 23 $^{\circ}\text{C}$): δ_{H} 7.14 (5H, m, ArH), 5.88 (1H, d, $J = 6.7$ Hz, NH), 4.83 (1H, s, H-1), 4.61 (1H, d, $J = 12$ Hz, CH_2CCl_3), 4.54 (1H, d, $J = 11.8$ Hz, CH_2Ph), 4.43 (1H, d, $J = 12$ Hz, CH_2CCl_3), 4.28 (1H, d, $J = 11.8$ Hz, CH_2Ph), 3.48-3.27 (6H, m, H-2, H-4, H-5, H-3, H-6a, H-6b). ^{13}C NMR (100 MHz, $\text{CDCl}_3 + \text{DMSO}-d_6$, 23 $^{\circ}\text{C}$): δ_{C} 55.3, 61.3, 68.8, 70.5, 71.1, 71.6, 73.9, 95.2, 96.1, 127.3, 127.4, 127.9, 136.8, 154.2.

Benzyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloro-ethoxycarbonylamino)- α -D-glucopyranoside (4). To a solution of **3** (400 mg 0.90 mmol) in CH_3CN (1.3 mL), were added CSA (41 mg, 0.17 mmol) and benzaldehyde dimethylacetal (243 μL , 1.59 mmol) and the mixture stirred at RT for 24 h. Then Et_3N (0.5 mL) was added, the reaction mixture azeotroped with toluene, and the resulting crude material was kept under high vacuum for 2h. The residue was purified by silica-gel flash chromatography using chloroform: ethyl acetate (10:1). The product was identified as **4**, a white solid, 297 mg (62%) yield, m.p. 154-157 $^{\circ}\text{C}$.¹⁸ $[\delta]_{\text{D}}^{22} +68.7^{\circ}$ (c 1, CHCl_3). ^1H NMR (400 MHz, CDCl_3 , 23 $^{\circ}\text{C}$): δ_{H} 7.50-7.36 (10H, m, ArH), 5.54 (1H, s, CHPh), 5.31 (1H, d, $J = 7.9$ Hz, NH), 4.97 (1H, s, 1-H), 4.81 (1H, d, $J = 11.8$ Hz, CH_2Ph), 4.74 (1H, d, $J = 11.6$ Hz, CH_2CCl_3), 4.64 (1H, d, $J = 11.8$ Hz, CH_2Ph), 4.51 (1H, d, $J = 11.6$ Hz, CH_2CCl_3), 4.26-4.23 (1H, m, H-2), 3.93-3.72 (4H, m, H-5, H-3, H-6a, H-6b), 3.57 (1H, t, $J = 8.6$ Hz, H-4), 2.75 (1H, s, OH). ^{13}C NMR (100 MHz, CDCl_3 , 23 $^{\circ}\text{C}$): δ_{C} 55.6, 62.7, 68.7, 69.9, 74.7, 81.8, 95.3, 97.1, 101.9, 126.2, 128.1, 128.3, 128.6, 129.3, 136.5, 136.9, 154.7; HR-MS (FI): m/z 531.0607 (Calc. for $\text{C}_{23}\text{H}_{24}\text{Cl}_3\text{NO}_7$, 531.0618).

Benzyl 2-deoxy-4,6-*O*-benzylidene-3-*O*-(2,2,2-trichloro-ethoxycarbonyl)-2-(2,2,2-trichloro-ethoxycarbonylamino)- α -D-glucopyranoside (5). To compound **4** (400 mg, 0.75 mmol) in pyridine (1.5 mL), was added TrocCl (171 μL , 1.25 mmol), at 0 $^{\circ}\text{C}$, and the solution stirred at RT for 12 h. The reaction mixture was azeotroped with toluene, and the residue purified by silica gel column chromatography using *n*-hexane: ethyl acetate (10:2), and the product identified as **5**. This is a colorless solid, yield, 324mg (61%), m.p. 123-124 $^{\circ}\text{C}$; $[\delta]_{\text{D}}^{25} +35.2^{\circ}$ (c 0.2, CHCl_3). ^1H -NMR (400 MHz, CDCl_3 , 23 $^{\circ}\text{C}$): δ_{H} 7.43-7.32 (10H, m, ArH), 5.54 (1H, s, CHPh), 5.27 (1H, d, $J = 10$ Hz, NH), 5.25 (1H, t, $J = 10$ Hz, 3-H), 4.99 (1H, d, $J = 3.7$ Hz, 1-H), 4.80-4.70 (4H, m, CH_2Ph , CH_2CCl_3), 4.64 (1H, d, $J = 12$ Hz, CH_2CCl_3), 4.54 (1H, d, $J = 11.7$ Hz, CH_2Ph), 4.21 (1H, dd, $J = 4.8$ Hz, $J = 4.8$ Hz, H-6b), 4.13-4.00 (1H, m, H-2), 3.93-3.86 (1H, m, H-5), 3.75-3.70 (2H, m, H-4, H-6a). ^{13}C NMR (100 MHz, CDCl_3 , 23 $^{\circ}\text{C}$): δ_{C} 54.2, 63.0, 68.6, 70.1, 74.6, 75.2, 76.3, 78.8, 94.3, 95.2, 97.0, 101.5, 126.1, 128.1, 128.3, 128.4, 128.7, 129.1, 136.1, 136.6, 154.1; HR-MS (FI): m/z 704.9661 (Calc. for $\text{C}_{26}\text{H}_{25}\text{Cl}_6\text{NO}_9$, 704.9660).

1,6-Di-*O*-benzyl-2-deoxy-3-*O*-(2,2,2-trichloro-ethoxycarbonyl)-2-(2,2,2-trichloro-ethoxy-carbonylamino)- α -D-glucopyranoside (6). To a solution of **5** (800 mg, 1.12 mmol) in 13mL dry CH_2Cl_2 , was added triethylsilane (1mL, 6.2 mmol), then $\text{BF}_3\cdot\text{OEt}_2$ (148 μL , 1.17mmol) was slowly added. The reaction mixture was stirred for 3 h, until the reductive opening of the benzylidene ring was complete (confirmed by TLC). The reaction mixture was then diluted with

CH₂Cl₂. A saturated aqueous solution of NaHCO₃ was added, extracted with CH₂Cl₂, and washed with brine. The organic layer was dried over anhydrous MgSO₄ and the solvent removed under reduced pressure. The crude was purified by silica gel flash chromatography (ethyl acetate: *n*-hexane, 2:3) and identified as **6**, a viscous liquid (505 mg, yield 63%). [δ]_D²⁵ +35.2° (c 0.5, CHCl₃). ¹H NMR (400 MHz, CDCl₃, 23 °C): δ _H 7.36-7.33 (10H, m, ArH), 5.30 (1H, d, *J* = 9.4 Hz, NH), 5.06-4.96 (2H, m, H-1, H-3), 4.87-4.51 (8H, m, 2xCH₂CCl₃, 2xCH₂Ph), 4.11-4.06 (1H, m, H-2), 3.92-3.66 (4H, m, H-4, H-5, H-6), 2.75 (1H, s, OH). ¹³C NMR (100 MHz, CDCl₃, 23 °C): δ 53.6, 69.5, 69.7, 69.9, 70.0, 70.1, 73.7, 74.5, 76.9, 78.7, 94.2, 95.2, 96.5, 127.7, 127.7, 128.2, 128.5, 128.6, 136.4, 137.4, 154.1, 154.4; HR-MS (FD): *m/z* 706.9817 (Calcd for C₂₆H₂₇Cl₆NO₉, 706.9817).

Compounds 8, 9 and 10 were prepared according to the literature procedures;^{11a,19} however, instead of K₂CO₃, Cs₂CO₃ was used for the preparation of **10**.

1,6-Di-*O*-benzyl-4-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloro-ethoxycarbonylamino)- β -D-glucopyranosyl]-3-*O*-(2,2,2-trichloro-ethoxycarbonyl)-2-deoxy-2-(2,2,2-trichloro-ethoxycarbonylamino)- α -D-glucopyranoside (11**).** To a solution of the donor **10** (353 mg, 0.56 mmol), and acceptor **6** (200 mg, 0.28 mmol), with 3Å molecular sieves in dry CH₂Cl₂ (11 mL) at -15 °C, was added TMSOTf (15 μ L, 0.084 mmol). After stirring for 20 min, the reaction was quenched with a cold saturated aqueous solution of NaHCO₃ (1.5 mL), and extracted with CHCl₃ (10 mL). The organic layer was washed with saturated aqueous NaHCO₃ (4 mL) and brine (2 mL), dried over anhydrous Na₂SO₄, and the organic layer concentrated under reduced pressure. The residue was purified by silica-gel flash chromatography with toluene:acetone (10:1) and identified as **11**, a colorless solid (132mg), in 40% yield, m.p. 186-189 °C. [δ]_D²⁵ +35.2° (c 0.5, CHCl₃). ¹H NMR (400 MHz, CDCl₃, 23 °C): δ _H 7.54-7.25 (10H, m, ArH), 6.57 (1H, brs, NH), 5.75 (1H, brs, NH), 5.22-4.49 (13H, m, CH₂Ph, 3xCH₂CCl₃, H-1, H-2, H-2', H-1', H-3'), 4.34 (2H, d, *J* = 8 Hz, CH₂Ph), 4.09-3.92 (4H, m, H-3, H-6, H-5', H-4'), 3.75-3.44 (5H, m, H-6', H-6, H-4, H-5), 2.10 (3H, s, CH₃CO), 2.01 (3H, s, CH₃CO), 1.98 (3H, s, CH₃CO). ¹³C NMR (100 MHz, CDCl₃, 23 °C): δ 20.6, 20.8, 54.0, 55.6, 62.3, 66.4, 68.7, 69.9, 70, 70.1, 73.5, 74.5, 74.7, 76.5, 77.1, 91.7, 94.5, 95.5, 96.2, 97.5, 128.2, 128.3, 128.6, 129.1, 129.4, 129.6, 136.4, 137.4, 153.7, 154; MALDI-TOF: *m/z* 1195.142 (1194.970 calculated for C₄₁H₄₅Cl₉N₂NaO₁₈).

1,6-Di-*O*-benzyl-4-*O*-[3,4,6-tri-*O*-acetyl-2-acetamido- β -D-glucopyranosyl]-2-acetamido- α -D-glucopyranoside (12**).** To a solution of **11** (40 mg, 0.03 mmol) in freshly activated zinc dust (28 mg) and acetic acid (0.76 mL, 13 mmol), and the mixture was stirred overnight. The progress of the reaction was monitored by TLC using CHCl₃:MeOH (10:1). After completion of the reaction, the mixture was filtered over Celite, washed with ethyl acetate, azeotroped with toluene, and concentrated under reduced pressure. The residue was dissolved in MeOH (40 μ L), then Ac₂O (28 mg, 0.27 mmol) was added and the resulting mixture stirred overnight. The mixture was filtered off and washed with ethyl acetate, azeotroped with toluene, and concentrated under reduced pressure. The residue was purified by column chromatography using toluene:acetone (10:1) and identified as **12**, a viscous liquid (12 mg, 50% yield). ¹H NMR (400

MHz, CDCl₃, 23 °C): δ_H 7.41-7.25 (m, 10H), 6.38 (brs, 1H), 5.62 (d, 1H, *J* = 8.8 Hz), 4.98- 4.49 (m, 6H), 4.34-4.10 (m, 5H), 3.97-3.52 (m, 8H), 2.10-2.06 (m, 9H), 1.98 (s, 3H), 1.87 (s, 3H). MALDI-TOF: 751.337 [M-2] Calculated for C₃₆H₄₆N₂NaO₁₄ (753.284).

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References

1. Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357. (b) Helenius, A.; Aebi, M. *Science* **2001**, *291*, 2364. (c) Alper, J. *Science* **2001**, *291*, 2338. (d) Simon, P. M. *Drug Discov. Today* **1996**, 522. (e) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683. (h) Varki, A. *Glycobiology* **1993**, *3*, 97. (i) Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. *Chem. Rev.* **2000**, *100*, 4495. (j) Zhu, X.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 1900.
2. Bongat, A. F. G.; Demchenko, A. V. *Carbohydr. Res.* **2007**, *342*, 374.
3. Banoub, J.; Boullanger, P.; Lafont, D. *Chem. Rev.* **1992**, *92*, 1167. (b) Arya, P.; Ben, R. N. *Angew. Chem. Int. Ed.* **1997**, *36*, 1280. (c) Paulsen, H. *Angew. Chem. Int. Ed.* **1995**, *34*, 1432.
4. Enugala, R.; Carvalho, L. C.; Marques, M. M. B. *Synlett* **2010**, *18*, 2711. (b) Carvalho, L. C.; Enugala, R.; Corvo, M. C.; Cabrita, E. J.; Marques, M. M. B. *Magn. Reson. Chem.* **2010**, *48*, 323.
5. Swaminathan, C. P.; Brown, P. H.; Roychowdhury, A.; Wang, Q.; Guan, R.; Silverman, N.; Goldman, W. E.; Boons, G.-J.; Mariuzza R. A. *PNAS* **2006**, *103*, 684. (b) Inamura, S.; Fujimoto, Y.; Kawasaki, A.; Shiokawa, Z.; Woelk, E.; Heine, H.; Lindner, B.; Inohara, N.; Kusumoto, S.; Fukase, K. *Org. Biomol. Chem.* **2006**, *4*, 232. (c) Inamura, S.; Fukase, K.; Kusumoto, S. *Tetrahedron Lett.* **2001**, *42*, 7613.
6. Kovalová, A.; Ledvina, M.; Šaman, D.; Zyka, D.; Kubíčková, M.; Žídek, L.; Sklenář, V.; Pompach, P.; Kavan, D.; Bílý, J.; Vaněk, O.; Kubínková, Z.; Libigerová, M.; Ivanová, L.; Antolíková, M.; Mrázek, H.; Rozbeský, D.; Hofbauerová, K.; Křen, V.; Bezouška, K. *J. Med. Chem.* **2010**, *53*, 4050.
7. Matkins, W. M. *Science* **1966**, *152*, 172. (b) Vestweber, D.; Blanks, J. E. *Physiol. Rev.* **1999**, *79*, 181. (c) Strous, G. J.; Dekker, J. *Crit. Rev. Biochem. Mol. Biol.* **1992**, *27*, 57. (d) Mannori, G.; Crottet, P.; Cecconi, O.; Hanasaki, K.; Aruffo, A.; Nelson, R. M.; Varki, A.; Bevilacqua, M. P. *Cancer Res.* **1995**, *55*, 4425. (e) see also reference 1(e).
8. Micha, F.; Dmitry, S.; Shay, Y.; Timor, B. *Org. Lett.* **2002**, *4*, 281.

9. Crich, D.; Vinod, A. U. *J. Org. Chem.* **2005**, *70*, 1291.
10. Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734.
11. Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296*, 135; (b) Ellervik, U.; Magnusson, G. *Carbohydr. Res.* **1996**, *280*, 251.
12. Tong, Z.; Boons, J.-T. *Tetrahedron: Asymmetry* **2000**, *11*, 199.
13. Luigi, C.; Emiliano, B.; Antonio, M.; Michelangelo, P. *Tetrahedron Lett.* **2010**, *51*, 1117.
14. Mong, T. K.-K.; Huang, C.-Y.; Wong, C.-H. *J. Org. Chem.* **2003**, *68*, 2135.
15. Miyajima, K.; Achiwa, K. *Chem. Pharm. Bull.* **1997**, *45*, 312. (b) Akihiro, I.; Hiromune, A.; Hideharu, I.; Makoto, K. *J. Org. Chem.* **2009**, *74*, 3009.
16. Nelson, G. S.; Bungard, C. J.; Wang, K. *J. Am. Chem. Soc.* **2003**, *125*, 13000. (b) Higashino, T.; Sakaguchi, S.; Ishii, Y. *Org. Lett.* **2000**, *2*, 4193. (c) Gigg, R. J. *Chem. Soc. Perkin Trans. I* **1980**, 738. (d) Boullanger, P.; Chateland, P.; Descotes, G.; Kloosterman, M.; Boom, V. H. J. *J. Carbohydr. Chem.* **1986**, *5*, 541. (e) Nishiguchi, T.; Tachi, K.; Fukuzumi, K. *J. Org. Chem.* **1975**, *40*, 237. (f) Mereyala, H. B.; Guntha, S. *Tetrahedron Lett.* **1993**, *34*, 6929. (g) Carless, H. A.; Haywood, D. J. *J. Chem. Soc., Chem. Commun.* **1980**, 980. (h) Lee, J.; Cha, J. K. *Tetrahedron Lett.* **1996**, *37*, 3663. (i) Honda, M.; Morita, H.; Nagagura, I. *J. Org. Chem.* **1997**, *62*, 8932. (j) Ito, H.; Taguchi, T.; Hanzawa, Y. *J. Org. Chem.* **1993**, *58*, 774. (k) Rao, G. V.; Reddy, D. S.; Mohan, G. H.; Iyengar, D. S. *Synth. Commun.* **2000**, *30*, 3565. (l) Bailey, W. F.; England, M. D.; Mealy, M. J.; Thongsornkleeb, C.; Teng, L. *Org. Lett.* **2000**, *4*, 489. (m) Diaz, R. R.; Melagatejo, C. R.; Espinosa, M. T. P. L.; Cubro, I. *J. Org. Chem.* **1994**, *59*, 7928.
17. Andrej, B.; Slavko, P. *Tetrahedron: Asymmetry* **2008**, *19*, 2265.
18. Gunter, S.; Thomas, Z. *Tetrahedron* **1996**, *52*, 2925.
19. Johnson, D. A.; Johnson, C. L.; Helene, B.-L. G.; Sowell, C.G. World Intellectual Property Organisation Patent W.O 005308, 2004; see also reference 11 (a).
20. Paulsen, H.; Helpap, B. *Carbohydr. Res.* **1991**, *216*, 289.
21. Saha, S. L.; Nieuwenhze, M. S. V.; Hornback, W. J.; Aikins, J. A.; Blaszcak, L. C. *Org. Lett.* **2001**, *3*, 3575. (b) Keglevic, D.; Kojić-Prodic, B.; Tomišić, Z. B.; Spek, A. L. *Carbohydr. Res.* **1998**, *313*, 1.
22. Lucas, R.; Hamza, D.; Lubineau, A.; Bonnaffé, D. *Eur. J. Org. Chem.* **2004**, *10*, 2107.
23. Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. In *Purification of Laboratory Chemicals*, 2nd Ed.; Pergamon: Oxford, 1980.