Glucose-Based AB_2-Building Blocks for the Construction of Branched Glycopeptidomimetics

Christoffer Kieburg,[a] Kashinath Sadalapure,[a] and Thisbe K. Lindhorst*[a]

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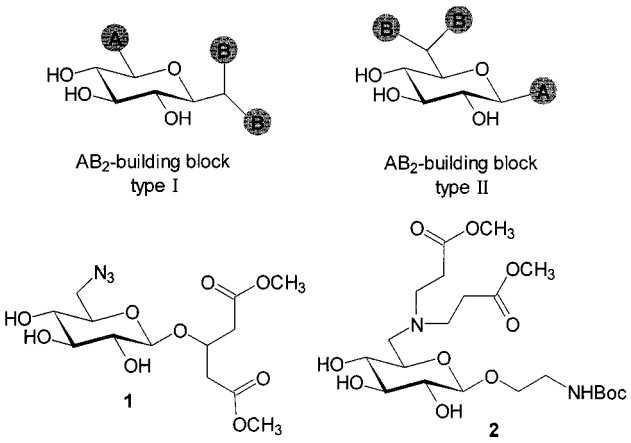
Glycopeptido derivatives are attractive oligosaccharide mimetics which allow the use of peptide-coupling reactions for the assembly of carbohydrate units instead of the more difficult glycosylation procedures. Moreover, glycopeptidomimetics are attractive as synthetic ligands for carbohydrate-specific molecular recognition. With regard to the multiantennary nature of the naturally occurring *N*-glycans, we synthesized the orthogonally protected glucose-based AB_2-building blocks **1** and **2**, which allow the assembly of branched glycopeptidomimetics by peptide-coupling reactions. This was exemplified by the synthesis of multivalent glycopeptidomimetics **14** and **15** derived therefrom.

Introduction

The formation of noncovalent complexes between carbohydrate ligands and protein receptors is a crucial event in numerous processes in cell biology,[1] including immune response, signal transduction, inflammatory processes and metastasis. Therefore, the investigation of the molecular details of the molecular recognition between lectins[2] and glycoconjugates is one of the most intensively studied areas in glycobiology. Synthetic derivatives of the natural carbohydrate ligands are named glycomimetics and have been widely evaluated as tools for the better understanding of structure-activity relationships in carbohydrate-protein interactions. This was exemplified with sialyl-Lewis-X glycomimetics which were used as ligands for selectins.[3]

Glycomimetics are often designed with additional functional groups which can contribute to lectin binding and recognition. Quite frequently, peptide linkages have been incorporated into glycomimetics, both to improve receptor binding by additional interactions between the glycopeptidomimetic and the lectin and to allow an easy access to a greater variety of structures. Thus, peptide linkages were, for example, used in the search for a simple synthetic access to analogues of naturally occurring glycolipids.[4] Moreover, amide linkages were used relatively early on for the assembly of disaccharide mimetics[5] and later for the synthesis of amide-linked tetrasaccharide analogues.[6] In one of these approaches peptide linkages were used as substitutes for interglycosidic bonds.[7] The latter contributions have in common that they start from AB-type building blocks which lead to linear oligosaccharide mimetics. However, carbohydrate ligands occur in vivo as multiple copies in multiantennary glycoconjugates, for example, in order to allow multivalent binding to the multiple carbohydrate recognition domains of lectins. The multivalency of carbohydrate−protein interactions was shown to be of functional importance in cell biology.[8] With regard to the multivalency principle operating in carbohydrate−protein interactions, our goal was to synthesize branched glycopeptidomimetics to allow the assembly of multivalent neoglycoconjugates. Consequently, we present here the synthesis of two glucose-based AB_2-type building blocks carrying a branching unit B_2 and exemplify their functionalization to the first multivalent glycopeptidomimetics.

Monosaccharides offer a straightforward entry into the construction of AB_2 building blocks as they carry many functional groups which can be further modified. Among them the anomeric lactol and the primary hydroxyl group in a chosen monosaccharide can be easily distinguished from the rest at an early stage of a synthetic sequence due to their different relative reactivities. Consequently, we targeted two complementary designed glucose derivatives of type I and type II (Scheme 1), which represent AB_2 building blocks, carrying one and two orthogonally protected func-



Scheme 1. Glucose-based AB_2-building blocks were designed for the construction of branched glycopeptidomimetics; the type I building block was realized as the 6-azido-6-deoxy-glucoside **1** and type II as the Boc-protected 2-aminoethyl glucoside **2**

[a] Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany
Fax: (internat.) + 49-40/42838-5592
E-mail: tklind@chemie.uni-hamburg.de

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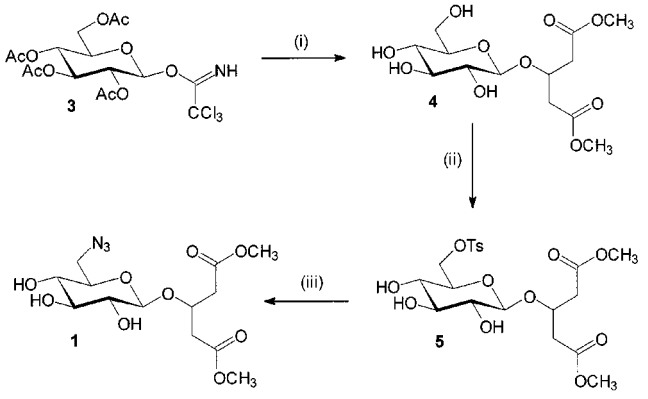
tions A and B, respectively, which are suited for peptide coupling reactions.

Type I was realized as the glucoside **1** carrying a branching unit B_2 as the aglycon part; type II was established as glucoside **2** with the branching unit connected to the 6-position of the monosaccharide. In **1** the azide group serves as a masked amino function, whereas in **2** the amino group is Boc (*tert*-butyloxycarbonyl)-protected. Both glycosides represent core molecules for the construction of branched glycopeptidomimetics and, moreover, offer the possibility for their assembly to larger, hyperbranched constructs in the sense of dendrimer chemistry.

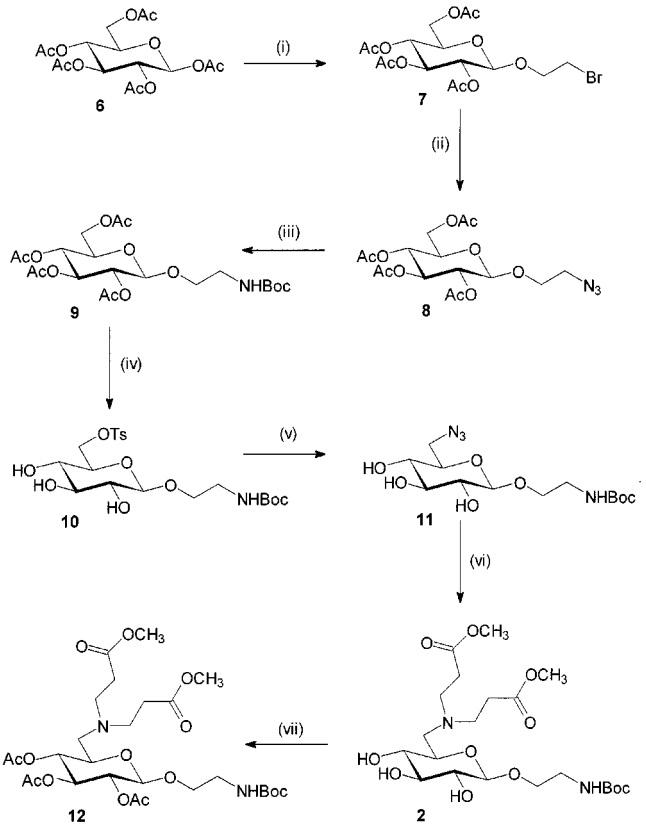
Results and Discussion

For the synthesis of type I AB_2 building block **1**, the branching unit was established as aglycon in glucoside **4** by glucosylation of 3-hydroxydimethyl glutarate with acetylated glucosyl trichloroacetimidate **3** (Scheme 2). Purification of the product was performed after Zemplén deprotection of the acetylated glycosylation product. In the next step the primary 6-hydroxyl group of **4** was selectively activated as its *p*-toluenesulfonyl ester to give **5**, which was subjected to a nucleophilic displacement reaction with sodium azide to yield the target AB_2-type glucoside **1**, in which the two protected functionalities B are represented by the bisecting diester moiety of the aglycon part and the 6-azido group serves as the masked amino function A. Characteristic for the ^1H NMR spectra of glucosides **4**, **5**, and **1** is a striking dddd multiplet around 4.4 ppm, accounting for the hydrogen atom at the prostereogenic carbon atom of the glutarate aglycon.



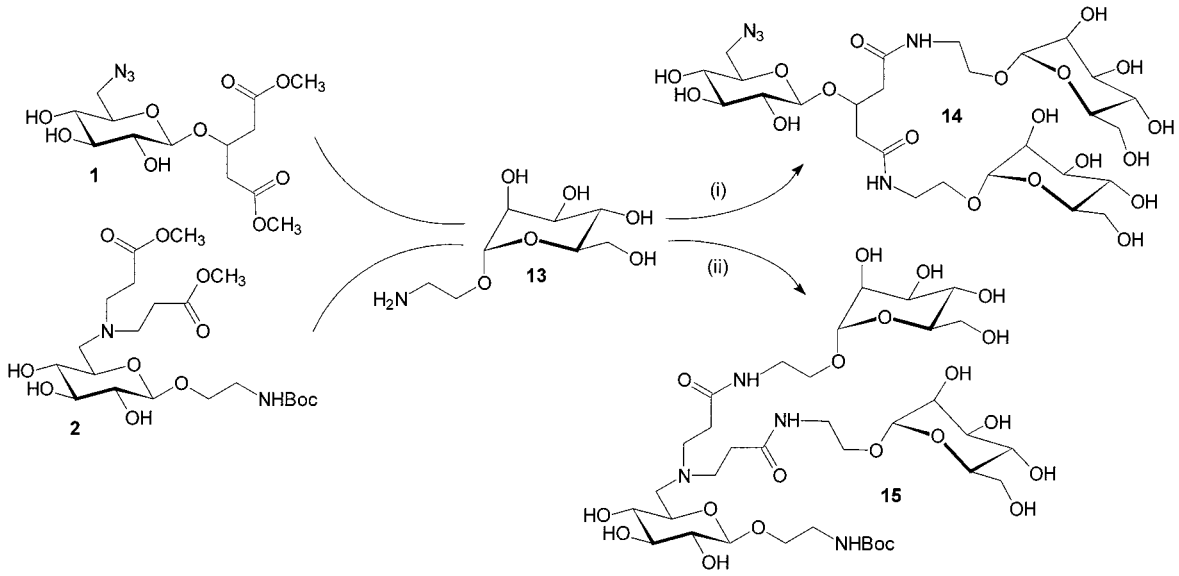
Scheme 2. (i) 3-hydroxydimethyl glutarate, TMSOTf, CH_2Cl_2, then NaOMe/MeOH, 82%; (ii) TsCl, pyridine, 68%; (iii) NaN_3, DMF, 65%

The synthesis of the type II AB_2 building block **2** utilized the known (2-azidoethyl) glucoside **8**,[9] which was obtained in two steps from glucose pentaacetate (**6**).[10] 2-Bromoethanol and not 2-azidoethanol was used for the initial Lewis acid catalyzed glycosylation step because 2-azidoethanol has been reported to be potentially explosive.[11] To obtain glucoside **9**, azide **8** was subjected to a palladium-catalyzed hydrogenation reaction in the presence of di-*tert*-butyldicarbonate. This allowed Boc-protection of the intermediate amine in situ and completely prevented *O*→*N* acetyl group migration. In glucoside **9** the function A is established at the terminus of the aglycon moiety as a Boc-protected amino group. As the Boc protecting group is stable against bases, deacetylation of **9** proceeded without problems allowing regioselective tosylation at the 6-position in the following step. This led to **10**, which was further converted in a tetra-*n*-butyl ammonium iodide-catalyzed nucleophilic displacement reaction to give the 6-azido-functionalized glucoside **11**. The glycoside **11** can be regarded as an orthogonally protected diamine, as the 6-azido group could be reduced to the amino group, leaving the aglycon amino group Boc-protected (Scheme 3).



Scheme 3. (i) 2-bromoethanol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 60%; (ii) NaN_3, *n*-Bu_4NI, DMF, 87%; (iii) Pd-C, H_2, Boc_2O, EtOAc, 90%; (iv) NaOMe, MeOH; then TsCl, pyridine, 70%; (v) NaN_3, Bu_4NI, DMF, 81%; (vi) Pd-C, H_2, MeOH; then methyl acrylate, MeOH, 90%; (vii) Ac_2O, pyridine, CH_2Cl_2, quant.

Without purification the 6-amine resulting from hydrogenation of **11** was treated with an excess of methyl acrylate in methanol. This reaction is in analogy to the exhaustive Michael addition which is used for build-up of polyamidoamine (PAMAM) dendrimers.[12] When the 6-amine was stirred with methyl acrylate for 48 hours the desired branched diester **2** was the only product of the Michael addition. However, although the reaction was carried out in the dark to avoid polymeric side-products arising from photochemical reactions with methyl acrylate, **2** was found to be contaminated by methyl acrylate polymerisates which could not be completely separated by flash chromatography.



Scheme 4. (i) LiOH, MeOH/H_2O (3:1), then TBTU, DIPEA, 1-HOBT, DMF, 73%; (ii) LiOH, MeOH/H_2O (2:1); then EEDQ, DMAc, 43%

Therefore, the unprotected glucoside **2** was treated with acetic anhydride in pyridine to afford the 2,3,4-tri-*O*-acetylated derivative **12**. This could be obtained in pure form after chromatography on silica gel, and then Zemplén deacetylation gave rise to pure **2**. Furthermore, an NMR spectroscopic analysis of **2** was facilitated by the spectroscopic data obtained for its protected analogue **12**.

In order to demonstrate the usefulness of the two glucose-based AB_2-type building blocks **l** and **2** as scaffolds for the synthesis of glycopeptidomimetics, they were subjected to a peptide-coupling reaction (Scheme 4). Because we are especially interested in the synthesis of glycomimetics which can serve as antiadhesives in mannose-sensitive bacterial adhesion,[13] 2-aminoethyl α-D-mannoside (**13**)[14] was employed as the amine component. First the two ester functions B in glucoside **1** or **2** were saponified using LiOH in aqueous methanol.[15] After neutralisation with dilute HCl, the reaction mixture was freeze-dried and then subjected to the peptide-coupling reaction with amino-functionalized mannoside **13**. In the case of the diacid derived from **1**, tetramethyluronium salt-based TBTU was used as a coupling reagent together with an excess of DIPEA to provide the glycopeptide **14** in 73% yield. Compound **14** was readily purified by gel permeation chromatography. The ^{1}H NMR spectrum of **14** revealed one set of signals for the glucose moiety and one signal set for both mannosyl residues, displaying the anomeric glucose proton as a doublet at 4.44 ppm and the anomeric mannose protons at 4.85 ppm.

An excess of base was required when TBTU was used as the peptide-coupling reagent. As base has been shown to catalyze the retro-Michael reaction, a coupling reagent was sought for the reaction of the diacid derived from **2** with amine **13** which does not require any base. Therefore, EEDQ was chosen, which at the same time allowed us to use OH-unprotected carbohydrate coupling partners. Similarly to the NMR spectra of **14**, the glucose moiety and both mannosyl residues of **15** were detected as one signal set each in the ^{1}H NMR spectrum. The anomeric glucose proton was observed at 4.30 ppm and the anomeric mannose protons at 4.73 ppm.

Conclusion

In summary, we have demonstrated the synthesis of the two complementary AB_2-carbohydrate building blocks, **1** and **2**, using a minimum of protection groups and a peptide-coupling strategy. Both **1** and **2** allow a wide array of possible further modifications at the orthogonal functional groups A and B, respectively. This includes coupling to a solid phase, bio-labelling, or synthesis of branched glycopeptidomimetics such as **14** and **15**. These can be regarded as "inverse" molecular wedges, which could be utilized for the assembly of larger, mulivalent glycomimetics in a convergent manner, for example. Building blocks **1** and **2** also bear the potential for generationwise growth to glycopeptido dendrons. This approach is currently under investigation in our laboratory.

Experimental Section

General Methods: For flash chromatography Merck silica gel 60 (0.040−0.063 mm, 230−400 mesh) was used. TLC was performed on Kieselgel 60 F_{254} plates from Merck. Detection was carried out under UV light or by spraying with 20% ethanolic sulfuric acid or 20% ethanolic sulfuric acid containing 5% α-naphthol, the latter two followed by heating. Size-exclusion chromatography was performed on Sephadex G-10 and G-15 from Pharmacia. NMR spectra were measured with a Bruker AMX 400 (400 MHz for ^{1}H and 100.62 MHz for ^{13}C NMR); chemical shifts are in ppm, relative to internal TMS (0.00 ppm for ^{1}H and ^{13}C NMR). When NMR spectra were recorded in [D_6]acetone a few drops of [D_4]methanol were added to exchange protic hydrogen atoms. Wherever necessary, two-dimensional ^{1}H-^{1}H or ^{1}H-^{13}C COSY experiments were carried

out for complete signal assignments. Optical rotation values were obtained using a Perkin−Elmer polarimeter 341 or 243 (Na-D line, 589 nm, cell length 10 cm). Mass spectra were measured with a VG Analytical 70−250S (FAB MS) or Finnigan MAT 95 (ESI MS) instrument. Elemental analyses were measured in the microanalytical laboratory of the Institute of Organic Chemistry of the University Hamburg, Germany.

3-*O*-(β-D-Glucopyranosyl)dimethyl Glutarate (4): Glucosyl trichloroacetimidate **3** (2.5 g, 5.07 mmol) and 3-hydroxydimethyl glutarate (0.80 g, 4.54 mmol) were dissolved in dry CH_2Cl_2 (100 mL) and treated with a solution of TMSOTf in CH_2Cl_2 (0.02 M, 2 mL). The reaction mixture was stirred at room temperature for 1 h, then triethylamine (1 mL) was added, the solution was concentrated in vacuo and the residue was purified by flash chromatography (silica gel, toluene/ethyl acetate, 2:1) to yield the acetylated glucoside (2.7 g, 4.47 mmol). The glucoside was then dissolved in dry methanol (50 mL) and treated with sodium methoxide (1 M in methanol, 1 mL) until the deprotection reaction was complete. In the subsequent steps, the reaction mixture was neutralized with ion exchange resin (Amberlite IR 120, H^+), filtered and the filtrate evaporated in vacuo to yield **4** (1.26 g, 3.72 mmol, 82%) as colourless syrup. − $[\alpha]_D^{20} = -14.4$ (c = 1.0 in MeOH). − ^{1}H NMR (400 MHz, D_2O): δ = 2.54−2.71 (m, 4 H, 2 CH_2), 3.02 (dd, $J_{3,4}$ = 9.3, $J_{4,5}$ = 9.6 Hz, 1 H, 4-H), 3.30 (m, 3 H, 2-H, 3-H, 5-H), 3.55 (m, 7 H, 6-H, 2 OCH_3), 3.69 (dd, $J_{5,6'}$ = 5.2, $J_{6,6'}$ = 12.2 Hz, 1 H, 6′-H), 4.37 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1-H), 4.46 [dddd≈m, 1 H, CH(CH_2)$_2$]. − ^{13}C NMR (100.62 MHz, D_2O): δ = 40.3, 40.6 (2 CH_2), 52.7 (2 OCH_3), 60.9 (C-6), 69.8, 73.3, 74.1, 75.9, 76.1 [C-2, C-3, C-4, C-5, CH(CH_2)$_2$], 102.7 (C-1), 173.9, 174.0 (2 C=O). − $C_{13}H_{22}O_{10}$ (338.3): calcd. C 46.14, H 6.55; found C 46.49, H 6.65.

3-*O*-(6-*O*-Tosyl-β-D-glucopyranosyl)dimethyl Glutarate (5): To a solution of dimethyl ester **4** (2.0 g, 5.91 mmol) in dry pyridine (40 mL) was added 4-toluenesulfonyl chloride (1.37 g, 7.21 mmol) at 0 °C. Then, the reaction mixture was stirred at room temperature for ca. 12 h, pyridine was removed in vacuo and the residue was purified by flash chromatography (ethyl acetate/MeOH, 10:1) to yield the desired tosylate **5** (1.99 g, 4.04 mmol, 68%) as colourless syrup. − $[\alpha]_D^{20} = -6.6$ (c = 1.0 in MeOH). − ^{1}H NMR (400 MHz, [D_4]MeOH): δ = 2.50 (s, 3 H, Ts-CH_3), 2.59−2.80 (m, 4 H, 2 CH_2), 2.94 (dd, $J_{2,3}$ = 9.2 Hz, 1 H, 2-H), 3.10 (dd≈t, $J_{4,5}$ = 9.6 Hz, 1 H, 4-H), 3.17 (dd≈t, $J_{3,4}$ = 9.3 Hz, 1 H, 3-H), 3.28 (ddd, $J_{4,5}$ = 9.6, $J_{5,6}$ = 1.6, $J_{5,6'}$ = 5.6 Hz, 1 H, 5-H), 3.56, 3.58 (each s, each 3 H, 2 OCH_3), 4.02 (dd, $J_{6,6'}$ = 10.7 Hz, 6-H), 4.19 (dd, $J_{6,6'}$ = 10.7 Hz, 1 H, 6′-H), 4.24 (d, $J_{1,2}$ = 7.6 Hz, 1 H, 1-H), 4.32 [dddd≈m, 1 H, CH(CH_2)$_2$], 7.35 (d, $J_{TsCHa,TsCHb}$ = 8.1 Hz, 2 H, Ts-H,), 7.70 (d, 2 H, Ts-H). − ^{13}C NMR (100.62 MHz, [D_4]MeOH): δ = 20.6 (Ts-CH_3), 39.3, 40.2 (2C, 2 CH_2), 51.2 (2 OCH_3), 69.6 (C-6), 69.9 (C-4), 73.7 (C-2), 73.8 (C-5, OCH(CH_2)$_2$), 76.6 (C-3), 103.6 (C-1), 128.1 (2 aryl-CH), 130.1 (2 aryl-CH), 133.4 (aryl-C), 145.5 (aryl-C), 171.9, 172.2 (2 C=O). − FAB-MS: m/z = 493.4 $[M + H]^+$ (492.1 calcd. for $C_{20}H_{28}O_{12}S$).

3-*O*-(6-Azido-6-deoxy-β-D-glucopyranosyl)dimethyl Glutarate (1): Tosylate **5** (1.0 g, 2.03 mmol) was dissolved in dry DMF (20 mL) in a round-bottomed flask, which was equipped with a condenser and the solution was stirred with sodium azide (0.66 g, 10.15 mmol) at 60 °C until the displacement reaction was complete. Then, DMF was removed under high vacuum, the residue was dissolved in ethyl acetate (50 mL) and washed twice with water (20 mL each). The organic phase was coevaporated with toluene and the resulting syrup was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to yield the desired AB_2 building block **1** (0.48 g, 1.3 mmol, 65%) as a colourless syrup. − $[\alpha]_D^{20} = -14.3$ (c = 1.0 in MeOH). − ^{1}H NMR (400 MHz, [D_4]MeOH): δ = 2.60−2.69 (m, 3 H, CH_aH$_b$, CH_2), 2.79 (dd, $J_{CHa,CH}$ = 5.6, J_{gem} = 16.3 Hz, 1 H, CH$_a$$H_b$), 3.10 (dd, $J_{2,3}$ = 9.2 Hz, 1 H, 2-H), 3.23 (dd≈t, $J_{4,5}$ = 9.6 Hz, 1 H, 4-H), 3.29 (dd≈t, $J_{3,4}$ = 9.3 Hz, 1 H, 3-H), 3.29−3.35 (m, 2 H, 5-H, 6-H), 3.48 (m, 1 H, 6′-H), 3.64, 3.67 (each s, each 3 H, 2 OCH_3), 4.43 (d, $J_{1,2}$ = 7.6 Hz, 1 H, 1-H), 4.49 (dddd≈m, 1 H, CH(CH_2)$_2$). − ^{13}C NMR (100.62 MHz, [D_4]MeOH): δ = 39.3, 40.2 (2 CH_2), 51.2, 51.3 (2 OCH_3), 51.6 (C-6), 71.1 (C-4), 73.4 (CH(CH_2)$_2$), 73.9 (C-2), 75.6 (C-5), 76.5 (C-3), 103.6 (C-1), 171.9, 172.3 (2 C=O). − $C_{13}H_{21}N_3O_9$ (363.3): calcd. C 42.98, H 5.83; found C 43.12, H 5.89.

(2-*tert*-Butoxycarbonylamidoethyl) 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (9): A suspension of azide **8** (10.5 g, 25.16 mmol), di-*tert*-butyldicarbonate (8.18 g, 37.48 mmol) and activated palladium on charcoal (Pd-C, 10%, 0.1 g) in ethyl acetate was stirred under hydrogen atmosphere (1 bar) for 6 h at room temperature. The reaction mixture was filtered through a celite bed (1.0 g) and the filtrate was washed subsequently with water and saturated aqueous NaCl solution and then dried with Na_2SO_4. Filtration followed by concentration of the organic layer in a rotary evaporator afforded the crude product, which was purified by flash column chromatography (ethyl acetate/light petroleum ether, 2:1) to yield the desired protected amine **9** (11.1 g, 22.58 mmol, 90%) as a colourless glass. − $[\alpha]_D^{20} = -14$ (c = 1.0 in $CHCl_3$). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.44 [s, 9 H, C(CH_3)$_3$], 2.01, 2.03, 2.06, 2.09 (each s, each 3 H, 4 OAc), 3.31 (m_c, 2 H, CH_2NHBoc), 3.65 (m_c, 1 H, OCH_aH$_b$), 3.71 (ddd, $J_{4,5}$ = 9.67, $J_{5,6}$ = 4.5, $J_{5,6'}$ = 2.04 Hz, 1 H, 5-H), 3.86 (m_c, 1 H, OCH$_a$$H_b$), 4.14 (dd, $J_{5,6}$ = 4.5, $J_{6,6'}$ = 12.21 Hz, 1 H, 6-H), 4.26 (dd, $J_{5,6'}$ = 2.04, $J_{6,6'}$ = 12.21 Hz, 1 H, 6′-H), 4.51 (d, $J_{1,2}$ = 8.14 Hz, 1 H, 1-H), 4.94 (br. s, 1 H, NH), 4.99 (dd, $J_{2,3}$ = 8.14 Hz, 1 H, 2-H), 5.08 (dd≈t, $J_{4,5}$ = 9.67 Hz, 1 H, 4-H), 5.21 (dd≈t, $J_{3,4}$ = 9.16 Hz, 1 H, 3-H). − ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 20.5, 20.6, 20.7 (4 COCH_3), 28.4 [C(CH_3)$_3$], 40.3 (CH_2NH), 61.9 (C-6), 68.3 (C-4), 69.8 (OCH$_2$), 71.3 (C-2), 71.9 (C-5), 72.7 (C-3), 79.4 [C(CH$_3$)$_3$], 101.1 (C-1), 155.8 (NHCO), 169.4, 169.5, 170.2, 170.6 (4 COCH$_3$). − $C_{21}H_{33}NO_{12}$ (491.5): calcd. C 51.32, H 6.77, N 2.85; found C 49.86, H 6.78, N 2.42.

(2-*tert*-Butoxycarbonylamidoethyl) 6-*O*-Tosyl-β-D-glucopyranoside (10): An ice-cold solution of the acetylated glucoside **9** (10.0 g, 20.34 mmol) in dry MeOH (80 mL) was treated with freshly prepared sodium methoxide solution (2 N, 10 mL). The reaction mixture was stirred at 0 °C until the reaction was complete (after approx. 30 min), then it was neutralized with ion exchange resin (IR 120, H^+). The resin was filtered off and the filtrate was concentrated in vacuo to obtain (2-*tert*-butoxycarbonylamidoethyl) β-D-glucopyranoside as a white foam. This was dissolved in pyridine (60 mL), without further purification, and treated with 4-toluenesulfonyl chloride (5.74 g, 30.1 mmol) at 0 °C. The reaction mixture was allowed to attain room temperature and was stirred for another 12 h. The excess 4-toluenesulfonyl chloride was quenched by the addition of methanol at 0 °C and then solvents were removed on a rotary evaporator with the water bath temperature not exceeding 30 °C. The crude product so obtained was purified by flash column chromatography (chloroform/methanol, 10:1) to furnish the unprotected title compound **10** (6.8 g, 14.24 mmol, 70%) as a white foam. − $[\alpha]_D^{20} = -5$ (c = 1.0 in CH_3OH). − ^{1}H NMR (400 MHz, [D_6]acetone): δ = 1.42 [s, 9 H, C(CH_3)$_3$], 2.46 (s, 3 H, TsCH_3), 3.11 (dd, $J_{2,3}$ = 8.1 Hz, 1 H, 2-H), 3.18 (m_c, 1 H, NCH_aH$_b$), 3.24 (ddd, $J_{4,5}$ = 9.2, $J_{5,6}$ = 6.6, $J_{5,6'}$ = 3.1 Hz, 1 H, 5-H), 3.31 (m_c, 1 H, NCH$_a$$H_b$), 3.33 (dd, $J_{3,4}$ = 9.2 Hz, 1 H, 3-H),

3.48 (m_c, 1 H, OCH_aCH$_b$), 3.54 (dd≈t, $J_{4,5}$ = 9.2 Hz, 1 H, 4-H), 3.76 (m_c, 1 H, OCH$_a H_b$), 4.16 (dd, $J_{6,6'}$ = 10.7 Hz, 1 H, 6-H), 4.25 (d, $J_{1,2}$ = 7.6 Hz, 1 H, 1-H), 4.36 (dd, $J_{6,6'}$ = 10.7 Hz, 1 H, 6′-H), 7.49 (d, $J_{TsCHa,\ TsCHb}$ = 8.1 Hz, 2 H, 2 Ts-H), 7.8 (d, 2 H, 2 Ts-H). − ^{13}C NMR (100.62 MHz, [D$_6$]acetone): δ = 21.5 (Ts-CH$_3$), 28.6 [C(*C*H$_3$)$_3$], 41.1 (*C*H$_2$NH), 69.7 (OCH$_2$), 70.6 (C-5, C-6), 74.5 (C-2, C-4), 77.4 (C-3), 79.2 [*C*(CH$_3$)$_3$], 104.1 (C-1), 128.8 (2 TsCH), 131.0 (2 TsCH), 134.2 (TsC_q), 146.0 (TsC_q), 158.0 (BocC=O). − FAB-MS: *m*/*z* = 478.9 [M + H]$^+$ (477.5 calcd. for $C_{20}H_{31}NO_{10}S$). − $C_{20}H_{31}NO_{10}S$ (477.5): calcd. C 50.31, H 6.54, N 2.93, S 6.71; found C 49.54, H 6.67, N 2.95, S 6.61.

(2-*tert*-Butoxycarbonylamidoethyl) 6-Azido-6-deoxy-β-D-glucopyranoside (11): A mixture of tosylate **10** (6.5 g, 13.61 mmol), sodium azide (2.65 g, 40.76 mmol) and a catalytic amount of tetra-*n*-butylammonium iodide (100 mg) in dry DMF (40 mL) was stirred at 50 °C. After the reaction was complete (after approx. 30 min, TLC in chloroform/methanol, 4:1), DMF was removed under high vacuum and the residual solid was stirred with methanol (50 mL) for 10 min at ambient temperature. The resulting suspension was filtered through a bed of celite to remove inorganic impurities. The resulting filtrate was concentrated and the crude product was purified by flash column chromatography (chloroform-methanol, 4:1) to yield the title azide **11** (3.84 g, 11.02 mmol, 81%) as a white foam. − $[\alpha]_D^{20}$ = −13 (*c* = 1.0 in CH$_3$OH). − ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 1.40 (s, 9 H, C(CH$_3$)$_3$), 3.21 (m_c, 1 H, CH_aH$_b$NH), 3.25 (dd, $J_{2,3}$ = 9.2 Hz, 1 H, 2-H), 3.30 (ddd, $J_{4,5}$ = 7.7, $J_{5,6}$ = 9.1, $J_{5,6'}$ = 2.0 Hz, 1 H, 5-H), 3.34 (m_c, 1 H, CH$_a H_b$NH), 3.42 (dd, $J_{3,4}$ = 7.7 Hz, 1 H, 3-H), 3.45 (dd, $J_{6,6'}$ = 13.2 Hz, 1 H, 6-H), 3.50 (dd, $J_{4,5}$ = 7.7 Hz, 1 H, 4-H), 3.53 (dd, $J_{6,6'}$ = 13.2 Hz, 1 H, 6′-H), 3.63 (m_c, 1 H, OCH_aH$_b$), 3.87 (m_c, 1 H, OCH$_a H_b$), 4.38 (d, $J_{1,2}$ = 7.6 Hz, 1 H, 1-H). − ^{13}C NMR (100.62 MHz, [D$_6$]acetone): δ = 28.6 [C(*C*H$_3$)$_3$], 41.1 (CH$_2$NH), 52.4 (C-6), 69.7 (OCH$_2$), 71.9 (C-5), 74.6 (C-2), 76.5 (C-4), 77.2 (C-3), 79.2 [*C*(CH$_3$)$_3$], 103.9 (C-1), 161.7 (NHCO). − $C_{13}H_{24}N_4O_7$ (348.4): calcd. C 44.82, H 6.94, N 16.08; found C 44.80, H 7.17, N 16.45.

2-(*tert*-Butoxycarbonylamidoethyl) 6-Deoxy-6-[bis(methoxycarbonylethyl)]amino-β-D-glucopyranoside (2): A mixture of a catalytic amount of activated palladium on charcoal (10%, 50 mg) and azide **11** (3.7 g, 10.62 mmol) in methanol (30 mL) was hydrogenated (1 bar H$_2$) for 6 h at room temperature. The reaction mixture was filtered through a bed of celite and the resulting filtrate was concentrated to quantitatively afford (2-*tert*-butoxycarbonylamidoethyl) 6-deoxy-6-amino-β-D-glucopyranoside as a white foam. This was then dissolved in dry methanol (10 mL) and further treated with freshly distilled methyl acrylate (9.55 mL, 106 mmol). The reaction mixture was stirred at room temperature for 48 h in the dark, concentrated in a rotary evaporator and the residue purified by flash chromatography (chloroform/methanol, silica gel, 10:1) to provide the AB$_2$ building block **2** (4.75 g) which was still contaminated with traces of polymeric methyl acrylate side products. Therefore, this product was dissolved in a mixture of dry pyridine (25 mL) and dry dichloromethane (25 mL) and treated with acetic anhydride (9 mL) at 0 °C. The reaction mixture was allowed to attain room temperature and was then stirred for a further 6 h. The excess of acetic anhydride was quenched by the addition of methanol at 0 °C and then the mixture was coevaporated with toluene in the rotary evaporator. Purification of the crude product by flash column chromatography (light petroleum ether/ethyl acetate, 4:1) afforded (2-*tert*-butoxycarbonylamidoethyl) 2,3,4-tri-*O*-acetyl-6-deoxy-6-[bis(methoxycarbonylethyl)]-amino-β-D-glucopyranoside (**12**, 5.94 g) as a thick colourless syrup. This was deacetylated in dry MeOH by addition of a catalytic amount of 2 N sodium methoxide solution. When the reaction was complete, it was neutralized with ion exchange resin (Amberlite IR 120, H$^+$), the resin was filtered off and the filtrate was concentrated in vacuo to obtain the title compound **2** in pure form (4.73 g, 9.56 mmol, 90%) as a colourless thick syrup. − $[\alpha]_D^{20}$ = −7 (*c* = 1.0 in CH$_3$OH). − ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 1.41 [s, 9 H, C(CH$_3$)$_3$], 2.50 (ddd, $J_{CH,NCHa}$ = 1.5, $J_{CH,NCHb}$ = 7.1, J_{gem} = 13.2 Hz, 4 H, 2 CH_2CO$_2$Me), 2.66 (dd, $J_{6,6'}$ = 14.2 Hz, 1 H, 6-H), 2.88 (ddd, 4 H, 2 NCH$_2$), 2.97 (dd, $J_{6,6'}$ = 14.2 Hz, 1 H, 6′-H), 3.18 (m_c, 3 H, 2-H, 4-H, CH_aH$_b$NH), 3.31 (m_c, 1 H, CH$_a H_b$NH), 3.36 (dd≈t, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, 1 H, 3-H), 3.42 (ddd, $J_{4,5}$ = 7.2, $J_{5,6}$ = 3.1, $J_{5,6'}$ = 2.0 Hz, 1 H, 5-H),), 3.60 (m_c, 1 H, OCH_aH$_b$), 3.63 (s, 6 H, 2 CO$_2$CH$_3$), 3.83 (m_c, 1 H, OCH$_a H_b$), 4.27 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1-H). − ^{13}C NMR (100.62 MHz, [D$_6$]acetone): δ = 28.6 [C(*C*H$_3$)$_3$], 33.1 (2 CH$_2$CO), 41.3 (CH$_2$NH), 51.1 (2 NCH$_2$), 51.6 (2 CO$_2$*C*H$_3$), 55.9 (C-6), 69.6 (OCH$_2$), 73.6 (C-4), 74.8 (C-2), 75.1 (C-5), 77.8 (C-3), 78.8 [CO$_2$*C*(CH$_3$)$_3$], 104.0 (C-1), 158.5 (Boc-C=O), 173.3 (2 C=O). − $C_{21}H_{38}N_2O_{11}\cdot H_2O$ (512.6): C 49.19, H 7.87, N 5.47; found C 48.88, H 7.50, N 5.43.

(2-*tert*-Butoxycarbonylamidoethyl) 2,3,4-Tri-*O*-acetyl-6-deoxy-6-[bis(methoxy carbonylethyl)]amino-β-D-glucopyranoside (12): $[\alpha]_D^{20}$ = + 4.7 (*c* = 1.0 in CHCl$_3$). − ^{1}H NMR (400 MHz, CDCl$_3$): δ = 1.43 [s, 9 H, (C(CH$_3$)$_3$], 2.04, 2.05, 2.06 (each s, each 3 H, 3 OAc), 2.42 (ddd, $J_{CH,NCHa}$ = 2.0, $J_{CH,NCHb}$ = 7.12, J_{gem} = 11.2 Hz, 4 H, 2 CH$_2$CO), 2.57 (dd, $J_{5,6}$ = 2.0, $J_{6,6'}$ = 14.2 Hz, 1 H, 6-H), 2.62 (dd, $J_{5,6'}$ = 6.6, $J_{6,6'}$ = 14.2 Hz, 1 H, 6′-H), 2.82 (ddd, 4 H, 2 NCH$_2$), 3.30 (m_c, 2 H, CH_2NH), 3.63 (m_c, 2 H, OCH_aH$_b$, 5-H), 3.66 (s, 6 H, 2 CO$_2$CH_3), 3.83 (ddd, $J_{CH,NCHa}$ = 6.1, $J_{CH,NCHb}$ = 10.2, J_{gem}= 14.2 Hz, 1 H, OCH$_a H_b$), 4.49 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1-H), 4.86 (dd≈t, $J_{4,5}$ = 9.7 Hz, 1 H, 4-H), 4.95 (dd≈t, $J_{2,3}$ = 9.7 Hz, 1 H, 2-H), 5.01 (br. s, 1 H, NH), 5.18 (dd≈t, $J_{3,4}$ = 9.7 Hz, 1 H, 3-H). − ^{13}C NMR (125.77 MHz, CDCl$_3$): δ = 21.0, 21.4 (3 OCO*C*H$_3$), 28.8 [C(*C*H$_3$)$_3$], 32.9 (2 *C*H$_2$CO), 40.7 (CH$_2$NH), 50.4 (2 NCH$_2$), 51.9 (2 CO$_2$*C*H$_3$), 54.9 (C-6), 69.2 (OCH$_2$), 70.7 (C-4), 71.8 (C-2), 73.4 (C-3), 73.8 (C-5), 79.69 [*C*(CH$_3$)$_3$], 101.0 (C-1), 156.2 (NHCO), 169.9, 170.1, 170.7 (3 *C*OCH$_3$), 173.1 (2 *C*O$_2$CH$_3$). − $C_{27}H_{44}N_2O_{14}\cdot H_2O$ (638.8): C 50.76 H 7.26, N 4.39; found C 50.79, H 7.11, N 4.35.

3-*O*-(6-Azido-6-deoxy-β-D-glucopyranosyl)-*N*,*N*′-bis[(2-α-D-mannopyranosyloxy)ethyl]glutaric Acid Diamide (14): A solution of the AB$_2$ building block **1** (150 mg, 0.41 mmol) in MeOH (9 mL) and water (3 mL) was cooled to 0 °C and then treated with LiOH·H$_2$O (260 mg, 6 mmol). During 5 h of stirring the reaction temperature was slowly elevated to room temperature. After the reaction was complete, pH 4 was adjusted by adding 2 N HCl, and the mixture was freeze dried. The lyophilisate so obtained was dissolved in dry DMF (50 mL) and 2-aminoethyl α-D-mannopyranoside (**13**, 280 mg, 1.25 mmol), TBTU (321 mg, 1 mmol), DIPEA (260 mg, 2 mmol), and 1-HOBT (135 mg, 1 mmol) were added. The reddish reaction mixture was stirred for 1 d at 40 °C. Then, DMF was removed in vacuo and the residue was dissolved in water (30 mL) and passed over a short Sephadex G-10 column to remove inorganic impurities and coloured side products. The early eluting fractions were combined, reduced to a volume of 10 mL and purified by gel permeation chromatography on a 120 cm Sephadex G-15 column to yield the title compound (220 mg, 0.29 mmol, 73%) after lyophilisation. − $[\alpha]_D^{20}$ = +17.2 (*c* = 0.5 in H$_2$O). − ^{1}H NMR (400 MHz, D$_2$O): δ = 2.46−2.58 (m, 4 H, 2 NCH$_2$), 3.14 (dd, $J_{2,3}$ = 9.2 Hz, 1 H, 2-H$_{glc}$), 3.27 (dd≈t, $J_{4,5}$ = 9.7 Hz, 2 H, 2 4-H$_{man}$), 3.32−3.55 [m, 12 H, 2 3-H$_{man}$, 3-H$_{glc}$, 4-H$_{glc}$, 5-H$_{glc}$, 2 5-H$_{man}$, 2 CH$_2$C(O), manOCH_aH$_b$], 3.62 (dd, $J_{5,6}$ = 2.6, $J_{6,6'}$ = 11.9 Hz, 2 H, 2 6-H$_{man}$), 3.64−3.71 (m, 5 H, 6-H$_{glc}$, 6′-H$_{glc}$, manO-

CH_aH_b, manOCH_2), 3.76−3.80 (dd≈d, $J_{6,6'}$ = 11.9 Hz, 2 H, 2 6′-H_{man}), 3.82 (d, $J_{2,3}$ = 3.0 Hz, 2 H, 2 2-H_{man}), 4.40 [dddd≈m, 1 H, O$CH(CH_2)_2$], 4.44 (d, $J_{1,2}$ = 8.2 Hz, 1 H, 1-H_{glc}), 4.85 (d, $J_{1,2}$ = 1.5 Hz, 2 H, 2 1-H_{man}). − ^{13}C NMR (100.62 MHz, D_2O): δ = 38.6 (2 CH_2CO), 40.0, 41.01 (2 NCH_2), 50.5 (C-6_{glc}), 60.3 (2 C-6_{man}), 65.3 (2 manOCH_2), 66.3 (2 C-4_{man}), 69.6 (2 C-2_{man}), 69.8 (C-4_{glc}), 70.1 (2 C-3_{man}), 72.6 (2 C-5_{man}), 72.7 (C-2_{glc}), 73.7 [O$CH(CH_2)_2$], 74.1 (C-5_{glc}), 75.0 (C-3_{glc}), 99.3 (2 C-1_{man}), 101.4 (C-1_{glc}), 172.1, 172.3 (2 C=O). − FAB-MS: *m/z* = 747 $[M + 2H]^+$. − ESI-MS: *m/z* = 746.9 $[M + 2H]^+$, 768.8 $[M + Na]^+$ (745.28 calcd. for $C_{27}H_{47}N_5O_{19}$).

(2-*tert*-Butoxycarbonylamidoethyl) 6-Deoxy-6-amino-*N*,*N*′-bis-{[(2-α-D-mannopyranosyloxy)ethyl]amidocarbonylethyl}-β-D-glucopyranoside (15): To a solution of the AB_2 building block **2** (40 mg, 0.08 mmol) in MeOH (2 mL) and water (2 mL) was added LiOH·H_2O (80 mg, 1.4 mmol) and the reaction mixture was stirred at room temperature for 5 h. Then pH 4 was adjusted by addition of 2 N HCl and the mixture was freeze dried. The lyophilisate so obtained was dissolved in dry DMF (10 mL), 2-aminoethyl α-D-mannopyranoside (13, 80 mg, 0.3 mmol) and EEDQ (80 mg, 0.3 mmol) were added and the reaction mixture was stirred for 4 d at 60 °C. Then, DMF was removed in vacuo and the residue dissolved in water (10 mL) and passed over a short Sephadex G-10 column to remove inorganic impurities and coloured side products. The early eluting fractions were combined, reduced to a volume of 10 mL and purified by gel permeation chromatography on a 120 cm Sephadex G-15 column to yield the title compound (30 mg, 0.03 mmol, 43%) after lyophilisation. − $[\alpha]_D^{20}$ = +16.3 (*c* = 0.8 in H_2O). − ^{1}H NMR (400 MHz, D_2O): δ = 1.29 [s, 9 H, C(CH_3)$_3$], 2.35 (t, 4 H, 2 CH_2CO), 2.60 (dd, $J_{5,6}$ = 1.5, $J_{6,6'}$ = 14.2 Hz, 1 H, 6-H_{glc}), 2.78 (t, 4 H, 2 NCH_2), 2.88 (dd, $J_{5,6'}$ = 8.2, $J_{6,6'}$ = 14.2 Hz, 1 H, 6′-H_{glc}), 3.10 (dd≈t, $J_{4,5}$ = 9.7 Hz, 1 H, 4-H_{glc}), 3.14 (dd≈t, $J_{3,4}$ = 9.7 Hz, 1 H, 3-H_{glc}), 3.15−3.68 (m, 22 H, 2 3-H_{man}, 2 4-H_{man}, 2 5-H_{man}, 2 6-H_{man}, 2-H_{glc}, 5-H_{glc}, 3 CH_2O, 3 NHCH_2), 3.74 (dd, $J_{5,6}$ = 2.0, $J_{6,6'}$ = 12.2 Hz, 2 H, 2 6′-H_{man}), 3.80 (dd, $J_{2,3}$ = 3.1 Hz, 2 H, 2 2-H_{man}), 4.30 (d, $J_{1,2}$ = 7.6 Hz, 1 H, 1-H_{glc}), 4.73 (d, $J_{1,2}$ = 1.6 Hz, 2 H, 2 1-H_{man}). − ^{13}C NMR (100.62 MHz, D_2O): δ = 28.1 [C(CH_3)$_3$], 33.0 (2 CH_2CO), 39.3 (2 CH_2NH), 40.3 (CH_2NH), 50.2 (2 CH_2N), 54.0 (C-6_{glc}), 61.3 (2 C-6_{man}), 66.2 (2 CH_2Oman), 67.1 (2 C-4_{man}), 69.3 (CH_2Oglc), 70.4 (2 C-2_{man}), 70.9 (2 C-3_{man}), 72.3 (C-5_{glc}), 73.3 (2 C-5_{man}), 73.5 (C-2_{glc}), 73.6 (C-4_{glc}), 75.9 (C-3_{glc}), 82.4 [C(CH_3)$_3$], 100.1 (2 C-1_{man}), 102.5 (C-1_{glc}), 158.5 (Boc-C=O), 175.1 (2 C=O). − ESI-MS: *m/z* = 877.0 $[M + H]^+$ (876.406 calcd. for $C_{35}H_{64}N_4O_{21}$).

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