SYNTHESIS OF LINEAR AND BRANCHED REGIOISOMERIC CHITOOLIGOSACCHARIDES AS POTENTIAL MIMETICS OF NATURAL OLIGOSACCHARIDE LIGANDS OF NATURAL KILLER CELLS NKR-P1 AND CD69 LECTIN RECEPTORS

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Dedicated with due respect to Professor Miloslav Černý on the occasion of his 75th birthday in recognition of his outstanding contributions to saccharide chemistry.

Regioisomer of chitobiose **13** with β(1→3) glycosidic bond and branched analog of chitotriose **25** having β(1→4) and β(1→3) glycosidic bonds, were prepared and tested as potential mimetics of natural oligosaccharide ligands for activating lectin receptors NKR-P1A and CD69 of natural killer (NK) cells. The structural requirements of NKR-P1 lectin receptor on effective mimetics of its natural ligands has been discussed. A significant binding activity of the branched trisaccharide **25** to the receptor CD69 was observed.

Keywords: Carbohydrates; Oligosaccharides; Aminosugars; D-Glucosamine; Glycosylation; Glycosides; NK cells; NKR-P1 and CD69 lectin receptors; Lectins; Immunotherapeutics.

In connection with the recent increasing occurrence of pathological states characterized by immunodeficiency, considerable attention has been paid to the investigation of effective modulation of individual components of the immune system. Natural killer (NK) cells have been one of the targets. Their activity is modulated by signals transmitted by interaction of their surface receptors with appropriate natural ligands. A systematic study of the activating lectin receptor NKR-P1 showed that complex oligosaccharide structures on the surface of tumour cells exert natural binding affinity to this receptor[1] and thereby increase the cytotoxic activity of NK cells. These natural oligosaccharide ligands are too complex for practical

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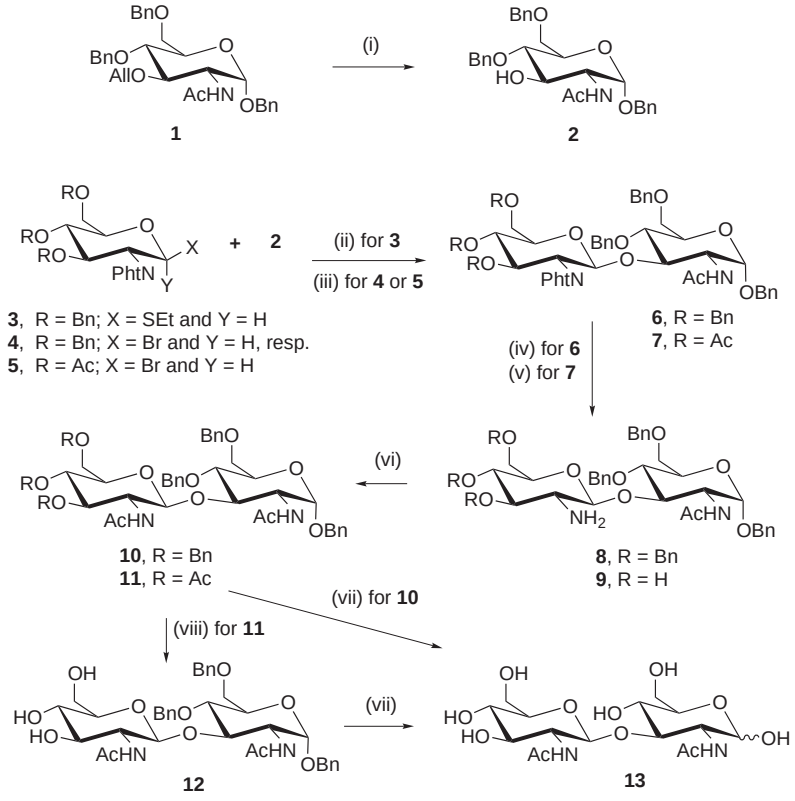
use as immunotherapeutics. This led us to the search for their simplified and hence better available mimetics. We have reported earlier that 2-acetamido-2-deoxy-D-hexoses also have a significant binding activity[2] and that in the series of chitooligomers consisting of β(1→4)-linked 2-acetamido-2-deoxy-D-glucopyranose units, this activity increases with elongation of the oligosaccharide chain to three sugar units[3]. Biological activity of oligosaccharides having other types of linkages has not been extensively tested, except for the disaccharides with β(1→6)-linked *N*-acetyl-D-hexosamine units, which were shown to be slightly less effective inhibitors[3]. With the aim to determine the steric requirements of NKR-P1 and CD69 receptors with respect to the character of glycosidic bonds and branching of oligosaccharide chain, we synthesized and tested for the binding activity 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucopyranose (**13**) and 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)- [2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-D-glucopyranose (**25**), wherein **13** represents the chitobiose analog with β(1→3) glycosidic bond (Scheme 1) and **25** represents the branched chitotriose analog containing β(1→4) and β(1→3) glycosidic bonds (Scheme 3).

In comparison with oligosaccharides consisting of 2-amino-2-deoxy-D-glucopyranose units linked with β(1→4β) or (1→6) glycosidic bonds, the synthesis of oligosaccharides having β(1→3) glycosidic bond has received less attention[4]. In their synthesis, common methods for the 1,2-*trans*-glycosidic linking of 2-amino-2-deoxyhexopyranose residue, i.e., the Koenigs–Knorr[5], oxazoline[6–8] and phthalimido[9,10] methods as well as the method controlled by 2,2,2-trichloroethyl carbamate group at C(2) of the glycosyl donor[10] were used. These oligosaccharides were also obtained by using glycosyl donors having at C(2) the nonparticipating azido or 2,5-dimethylpyrrole group and (trichloroacetimidoyl)oxy group at C(1) as leaving group[11,12]. The synthesis of branched oligosaccharides containing β(1→4) and β(1→3) glycosidic bonds has not been investigated so far.

RESULTS AND DISCUSSION

In the synthesis of the target branched trisaccharide β-D-GLc*p*NAc-(1→3)-[β-D-GLc*p*NAc-(1→4)]-D-GLc*p*NAc (**25**), we were confronted with a sterically hindered system. Therefore, the phthalimide method was applied to the glycosylation, because it is considered to be one of the most efficient 1,2-*trans*-stereoselective glycosylation processes[4] for low-reactive secondary OH groups. This glycosylation method was also used in the alternative synthesis of the disaccharide β-D-GLc*p*NAc-(1→3)-D-GLc*p*NAc (**13**; lit.[8]).

Benzyl 2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**2**) was used as a glycosyl acceptor in the preparation of target disaccharide **13** (Scheme 1). Compound **2** was obtained from benzyl 2-acetamido-3-*O*-allyl-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[13] (**1**) by splitting off the allyl group via its catalytic isomerization to prop-1-enyl group with Wilkinson catalyst [$RhCl(Ph_3P)_3$], followed by acid hydrolysis. Glycosylation of **2** with ethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[14] (**3**), promoted by a combination of methyl triflate and silver triflate, afforded benzyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**6**) in 59% yield. The unwanted *O*-methylation of the free OH group of glycosyl



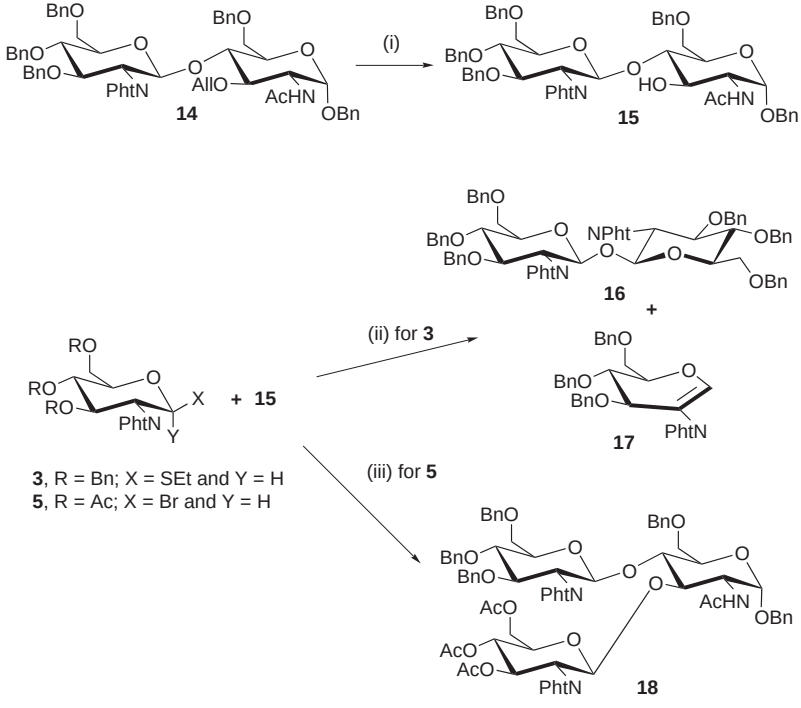
(i) $RhCl(Ph_3P)_3$in toluene, EtOH and H_2O, reflux and then HCOOH, reflux; (ii) MeOTf and AgOTf in CH_2Cl_2, r.t.; (iii) AgOTf in CH_2Cl_2,–45 °C; (iv) Na BH_4 in propan-2-ol and water, r.t. and then AcOH in toluene, 85 °C; (v) $BuNH_2$ in MeOH, 85 °C; (vi) Ac_2O in pyridine, r.t.; (vii) H_2 and Pd/C in AcOH, r.t.; (viii) MeONa in MeOH, r.t.

SCHEME 1

acceptor with methyl triflate[13] was suppressed by silver triflate. During the reaction of glycosyl bromide[14] **4**, obtained from **3** by its reaction with bromine, with glycosyl acceptor **2** in the presence of silver triflate as glycosylation promotor, no significant increase in the yield was observed. This reaction was carried without base at –45 °C (lit.[15]), because the base is known to inhibit glycosylation of less reactive hydroxy groups[16,17]. The reaction of glycosyl bromide **5**, protected by less bulky and electronegative *O*-acetyl groups, with glycosyl acceptor **2** under the last mentioned conditions gave disaccharide **13** in a yield of 72%. The disaccharide **6** was reductively dephthaloylated ($NaBH_4$), with respect to the presence of alkali-stable benzyl group on the vicinal OH-3 group[14]. The efficient splitting of the phthalimido group with commonly used butylamine in boiling methanol requires participation of free neighboring OH group in the dephthaloylation step; the alkali-labile groups were preferentially split off by the action of butylamine. The free OH group can hence participate in the dephthaloylation process[14]. The obtained crude benzyl 2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**8**) was subjected, without purification, to *N*-acetylation with acetic anhydride in pyridine to obtain benzyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**10**). Subsequent hydrogenolysis of its benzyl groups on Pd/C catalyst afforded the target disaccharide **13**. In the case of partially *O*-acetylated disaccharide **7**, the alkali-labile groups were removed with butylamine and the formed benzyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**9**) was converted, without purification, to benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**11**) by treatment with acetic anhydride in pyridine. The Zemplén *O*-deacetylation of **11** followed by hydrogenolysis of the obtained benzyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**12**) gave compound **13**. The presented syntheses of **13**, based on the use of fully *O*-benzyl-protected glycosyl donors **3** or **4** and glycosyl acceptor **2**, minimize the number of reaction steps which are necessary to obtain the target compound in comparison with the approaches mentioned above. The efficiency of the latter of the presented alternative syntheses of **13**, namely, the one employing *O*-acetyl-protected glycosyl donor **5**, is comparable with the approach described in lit.[8]

In the case of synthesis of the branched trisaccharide **25** (Scheme 3), benzyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-

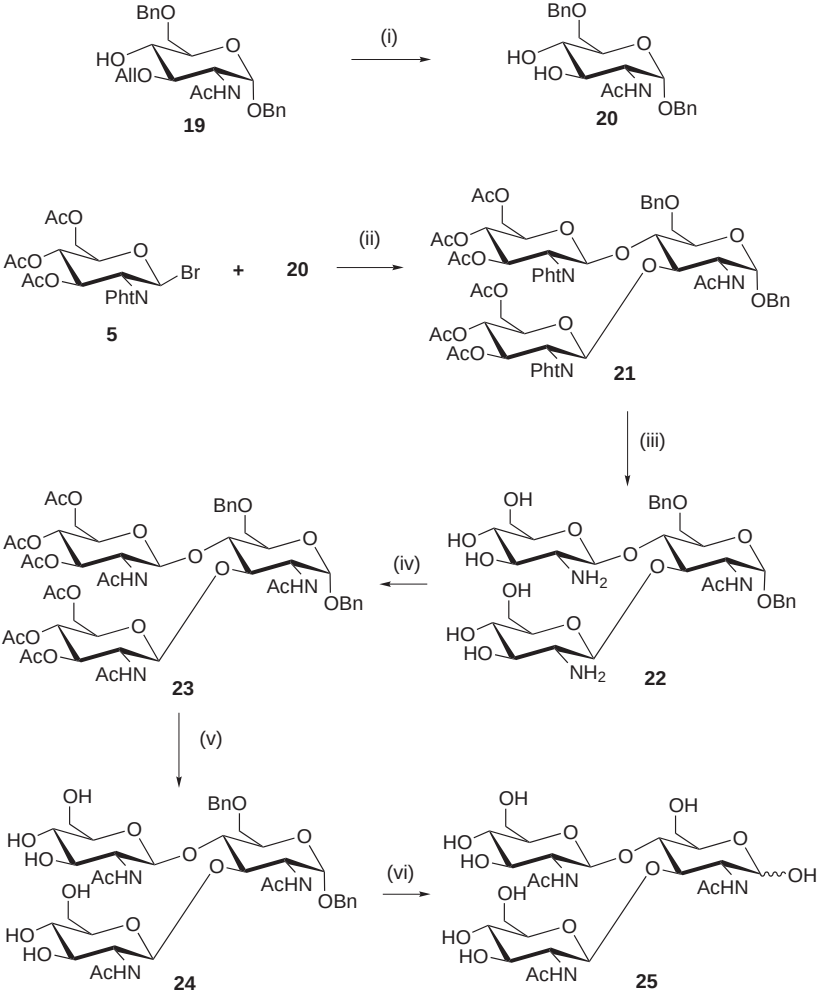
2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**15**) was used as the glycosyl acceptor at first (Scheme 2). The choice of the glycosyl acceptor **15** bearing phthalimido group in position C-2 of the second saccharide unit instead of analogous disaccharide with acetamido group[15] in the same position was aimed at decreasing of the negative influence of intra- and intermolecular H-bridges between OH group and the NH group of amide function in the glycosylation process[18–20]. Compound **15** was obtained from benzyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside[14] (**14**) by deallylation using the above mentioned procedure. The glycosylation of **15** promoted by methyl triflate in the presence of silver triflate with *O*-benzyl-protected ethyl 1-thioglycoside **3** gave a complex mixture of reaction products (Scheme 2). 3,4,6-Tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-gluco-



(i) $RhCl(Ph_3P)_3$ in toluene, EtOH and H_2O, reflux and then HCOOH, reflux; (ii) MeOTf and AgOTf in CH_2Cl_2, r.t.; (iii) AgOTf in CH_2Cl_2, –45 °C

SCHEME 2

pyranoside (**16**) and 1,5-anhydro-tri-*O*-benzyl-2-deoxy-2-phthalimido-D-*arabino*-hex-1-enitol (**17**) were isolated as the main reaction products, i.e., the product of cross-reaction of two molecules of the glycosyl donor and the product of its elimination. The formation of symmetrical disaccharide with the β,β(1↔1) glycosidic bond, similar to β,β-trehalose and glycal from



(i) $RhCl(Ph_3P)_3$ in toluene, EtOH and H_2O, reflux and then HCOOH, reflux; (ii) AgOTf in CH_2Cl_2, –45 °C; (iii) $BuNH_2$in MeOH, 85 °C; (iv) Ac_2O in pyridine, r.t.; (v) MeONa in MeOH, r.t.; (vi) H_2 and Pd/C in AcOH, r.t.

SCHEME 3

glycosyl donors derived from 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranose in the case of glycosylation of less reactive glycosyl acceptors is known from the literature[21,22]. Furthermore, the compound by its MS spectrum referred to as 3-*O*-methyl derivative of glycosyl acceptor **15**, was isolated in trace amounts. The use of glycosyl donor **5** protected by the less bulky and electronegative *O*-acetyl groups gave the required branched benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**18**) in low yield (11%), along with the unreacted glycosyl acceptor and unidentified products. The problem of effective synthesis of target branched trisaccharide **25** was solved by application of the approach based on simultaneous glycosylation of monosaccharide glycosyl acceptor with free 3-OH and 4-OH groups with a less bulky glycosyl donor (Scheme 3). The suitable glycosyl acceptor, benzyl 2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**20**), was prepared from benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside[15] (**19**) by its deallylation using the procedure mentioned above. Silver triflate-promoted glycosylation of **20** with *O*-acetyl protected glycosyl bromide **5** afforded the desired benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**21**) in good yield (64%). The alkali-labile protecting groups of trisaccharide **21** were removed by treatment with butylamine, and the obtained crude diamine **22** was peracetylated to give benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**23**). It was then *O*-deacetylated to give benzyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**24**). Subsequent debenzylation (H_2/Pd-C) finally afforded the unprotected branched trisaccharide **25**.

Biological Activity

Oligosaccharides **13** and **25** were tested as inhibitors of the binding of the soluble dimeric rat NKR-P1A receptors to the high-affinity ligand, neoglycoprotein GLc*p*NAc$_{17}$BSA. NKR-P1A receptor demonstrates strong interaction with monosaccharides such as GLc*p*NAc (IC_{50} = 10^{-7} M; Fig. 1), and this interaction is further increased with the elongation of linear

chitooligomer-type sequences such as chitobiose (IC_{50} = 3 × 10^{-8} M; Fig. 1). However, the β(1→3)-linked disaccharide β-D-GLc*p*NAc-(1→3)-D-GLc*p*NAc (**13**) is a much worse inhibitor compared with β(1→4) liked chitobiose β-D-GLc*p*NAc-(1→4)-D-GLc*p*NAc. These results indicate that the binding groove for linear oligosaccharides occurring in NKR-P1A receptor[23] accommodates well only the oligosaccharides with (1→4) glycosidic linkages. While the greater flexibility of the oligosaccharide chain of the above mentioned (1→6)-linked disaccharides still makes it possible to bind such structures[3], it is not possible to bind (1→3)-linked linear structures. Similarly, oligosaccharide structures that are branched and not sufficiently flexible are very poor ligands for rat NKR-P1A receptor, such as trisaccharide β-D-GLc*p*NAc-(1→3)-[β-D-GLc*p*NAc-(1→4)]-D-GLc*p*NAc (**25**) tested here (IC_{50} = 2 × 10^{-4} M; Fig. 1).

Both compounds were also tested as potential mimetics of the natural ligands of activating receptor of the haemopoietic cells CD69 where the branched trisaccharide **25** exerted a significant binding activity. Thus, in the standard inhibition assay, the trisaccharide **25** was very potent inhibitor with IC_{50} = 10^{-7} M, thus successfully competing with natural oligosaccharides of much greater complexity, such as the triantennary octasaccharide N3. The details of this study will be published separately.

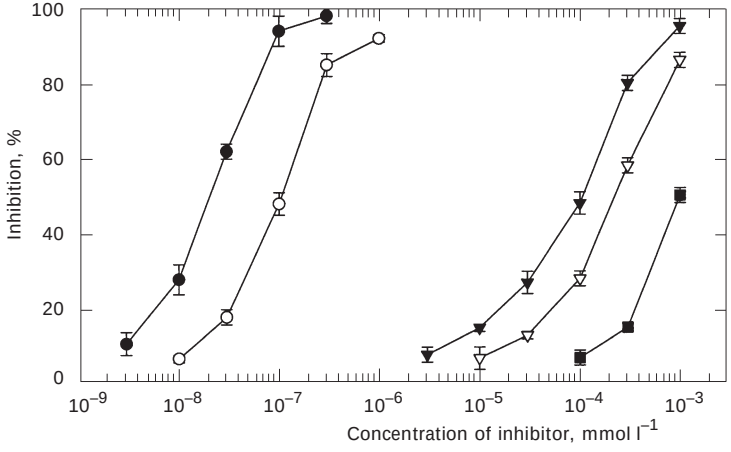


FIG. 1

Plate inhibition assay for the evaluation of biological activity of the synthesized oligosaccharides: ● β-D-GLc*p*NAc-(1→4)-D-GLc*p*NAc, ○ D-GLc*p*NAc, ▼ β-D-GLc*p*NAc-(1→3)-D-GLc*p*NAc, ▽ β-D-GLc*p*NAc-(1→3)-[β-D-GLc*p*NAc-(1→4)]-D-GLc*p*NAc, ■ D-Man*p*

In summary, this paper provides new findings about the structural requirements of NKR-P1 lectin receptor for effective mimetics of its natural ligands, and, in the case of branched trisaccharide **25**, the first efficient mimetic of natural oligosaccharide ligands of CD69 lectin receptor. These results, therefore, open the way to a more exact design of new effective ligands of both named lectin receptors and hence of potential immunotherapeutics.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin–Elmer 141 polarimeter at 25 °C and are given in 10^{-1} deg cm^2 g^{-1}. The IR spectra were recorded on a Bruker IFS 88 (FTIR) spectrometer, wavenumbers are given in cm^{-1}. NMR spectra were recorded using a Bruker Avance spectrometer in the FT mode at 500.1 MHz (^{1}H) and at 125.8 MHz (^{13}C) in deuteriochloroform, using tetramethylsilane as internal standard for ^{1}H NMR spectra and deuteriochloroform (δ 77.0) as standard for ^{13}C NMR spectra. Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. For unambiguous assignment of signals in ^{13}C NMR spectra of compounds **6**, **7**, **10**, **11**, **15**, **21**, and **24**, the heterocorrelated 2D NMR spectra were measured by the HSQC technique using the standard pulse sequence delivered by the manufacturer of the spectrometer. The following set of characteristic parameters was used: spectral width in both *f*1 and *f*2 dimensions 4500 and 17 000 Hz, respectively, number of scans 32, number of increments in *f*1 dimension 256, recycle delay 1 s, acquisition time 0.2 s, data matrix for processing 2048 × 2048 datapoints. For processing sine squared weighting function was used. NMR data (if not given in the text) are summarized in Tables I–VI. Positive-ion FAB mass spectra were measured on a BEqG geometry mass spectrometer ZAB-EQ (VG Analytical, Manchester, U.K.), using an M-Scan FAB gun (Xe, energy 8 keV) at an accelerating voltage of 8 kV. Samples were dissolved in chloroform or methanol and the mixture glycerol–thioglycerol or 3-nitrobenzyl alcohol was used as matrix. For complex mixtures, an A P 4000 pump with SCM 1000 vacuum degasser (all Finnigan, Inc., U.S.A.) were used for pumping the flow. The mobile phase consisted of 90% MeOH, the flow rate was 0.7 ml min^{-1}. An amount of 5 μl of analyte solution was directly introduced to an LCQ mass spectrometric detector (Finnigan, Inc., U.S.A.) equipped with an ion-trap analyzer. +APCI ionization was used for recording spectra. The spectrometer worked in a full-scan mode with the *m/z* 50–2000 Dalton range of analyzed ions. The capillary was heated at 200 °C, the ion source was heated to 475 °C, discharge current was set to 4.5 μA, 24 V on capillary. Thin-layer chromatography (TLC) was performed on Silufol UV_{254} sheets (Kavalier, Votice, Czech Republic) or DC-Alufolien Kieselgel 60 F_{254} (Merck, Darmstadt, Germany), and column chromatography on silica gel Fluka Silica gel 60 (40–63 μm; Fluka, Neu-Ulm, Switzerland). Analytical RP HPLC was performed with a Waters Alliance HPLC System (PDA 996 detector, software Millennium^{32} Chromatography Manager, Waters, Milford, Massachusetts, U.S.A.) equipped with a column (150.0 × 3.9 mm) filled with Nova-Pak C18 (4 μm; Waters). Preparative RP HPLC was performed with a Knauer apparatus (Bad Homburg, Germany) equipped with a column (250 × 25 mm) filled with LiChrosorb RP-18 (5 μm; Merck, Darmstadt, Germany). Solutions were evaporated on a rotatory evaporator. Analytical samples were dried at 6.5 Pa and at 25 °C

for 8 h. Dichloromethane was distilled from phosphorus pentoxide and stored over molecular sieve 4A. Silver trifluoromethanesulfonate was recrystallized from toluene.

Preparation and Radioiodination of Soluble Dimeric Rat NKR-P1A Receptor

Soluble dimeric rat NKR-P1A receptor (NKR358) was expressed in *Escherichia coli*, refolded, cleaved with enterokinase, and purified essentially as described previously[3]. This protein was radioiodinated as reported[2], with carrier-free $Na^{125}I$ (Amersham) to a specific activity of 10^7 cpm per μg protein.

Plate Inhibition Assays

Binding and inhibition assays were performed as described previously[2] with minor modifications. Briefly, 96-well poly(vinyl chloride) microplates (Titertek Immuno Assay-Plate, ICN Flow, Irvine, U.K.) were coated overnight at 4 °C with 50 μl of GLc*p*NAc_{17}BSA (10 μg/ml, Sigma) in TBS+C buffer (10 mM Tris-HCl pH 8.0 with 150 mM NaCl, 1 mM $CaCl_2$, and 1 mM NaN_3). Plates were blocked with 1% BSA (Sigma) in TBS+C at 4 °C for 2 h, and incubated with ^{125}I-NKR358 corresponding to half of the saturation amount and the indicated dilutions of the tested compounds in a total reaction volume of 100 μl. Plates were washed three times with TBS+C, drained, dried, and 100 μl of a scintillation solution was added to each well. Radioactivity in wells was determined in a β-counter Microbeta (Wallac). All experiments were performed in duplicate. The degree of inhibition was calculated relative to wells without any inhibitor (addition of water).

Microtitre plates were coated with a high-affinity ligand, and the binding of the radiolabeled NKR-P1A protein to this ligand was inhibited by serial dilutions of the tested oligosaccharides as described in Experimental. D-GLc*p*NAc and D-Man*p* were used as a positive and negative control, respectively. Chitobiose (β-D-GLc*p*NAc-(1→4)-D-GLc*p*NAc) was used as the high-affinity ligand. The results are average values from duplicate experiments within the range indicated by error bars.

Benzyl 2-Acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**2**)

Benzyl 2-acetamido-3-*O*-allyl-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[13] (**1**; 1.035 g, 2 mmol) and tris(triphenylphosphine)rhodium(I) chloride (233 mg, 0.25 mmol) were refluxed in a mixture of ethanol–toluene–water (7:3:2; 60 ml) under stirring for 4 h. Formic acid (2 ml) was added and reflux was continued for another 3 h. The mixture was evaporated in vacuo and the residue was chromatographed on a silica gel column (40 ml) in toluene–ethyl acetate (2:1) to give 698 mg (71%) of compound **2**. An analytical sample was prepared by crystallization from ethyl acetate–petroleum ether, m.p. 134–135 °C, $[\alpha]_D$ +69 (*c* 0.4, chloroform); lit.[24] $[\alpha]_D$ +65 (*c* 1.1, chloroform). ^{1}H NMR spectrum agrees with the data for the authentic sample prepared by procedure described in lit.[24] For **2** ($C_{29}H_{33}NO_6$) calculated: relative molecular mass 491.6, monoisotopic mass 491.2. FAB MS, *m/z*: 492 $[M + H]^+$. For $C_{29}H_{33}NO_6$ (491.6) calculated: 70.86% C, 6.77% H, 2.85% N; found: 70.77% C, 6.82% H, 2.78% N.

Benzyl 3,4,6-Tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**6**)

Method A: Ethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[14] (**3**; 624 mg, 1 mmol), compound **2** (246 mg, 0.5 mmol), silver trifluoromethanesulfonate (129 mg, 0.5 mmol) and crushed molecular sieve 4A (1 g) were dried in an apparatus equipped with a septum at room temperature and 1.32 Pa for 10 h and then the apparatus was flushed with argon (2×). Dry dichloromethane (5 ml) was added through the septum under stirring and the mixture was stirred at room temperature for 1 h. Then methyl trifluoromethanesulfonate (113 μl, 1 mmol) was added through the septum and stirring was continued at room temperature for 12 h. The addition of the same amount of methyl trifluoromethanesulfonate followed by 12-h stirring was repeated twice more. After cooling to –20 °C, dry pyridine (1 ml) was added through the septum and the mixture was stirred at –15 °C for 30 min and then at room temperature for 1 h. The mixture was diluted with chloroform (150 ml) and filtered through Celite. The filtrate was washed with 10% aqueous $NaHSO_4$, saturated aqueous $NaHCO_3$, and 5% aqueous NaCl (30 ml each). The organic phase was dried over anhydrous $MgSO_4$ and evaporated in vacuo. Chromatography of the solid residue on a silica gel column (70 ml) in toluene–ethyl acetate (2:1) followed by HPLC separation on a silica gel column C18 in the solvent system water–methanol (linear gradient 75→90%/70 min) afforded 310 mg (59%) of solid compound **6**.

Method B: Ethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[14] (**3**; 624 mg, 1 mmol) was dried in an apparatus equipped with septum at room temperature and 1.32 Pa for 10 h. The apparatus was flushed with argon (2×) and dry dichloromethane (2.3 ml) was added through the septum. After dissolution, the mixture was cooled to 0 °C and 1 M solution of bromine in dry dichloromethane (1.1 ml) was added through the septum under stirring. Then the mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h. In the same apparatus the solvents were evaporated in vacuo (water pump) with exclusion of moisture. The residue was co-evaporated with toluene (3 × 2 ml) at 20 Pa, added through the septum and the residue was dissolved in dry dichloromethane (1.3 ml). The solution of glycosyl bromide[14] **4** was used for the condenzation with compound **2**.

Compound **2** (246 mg, 0.5 mmol) and silver trifluoromethanesulfonate (129 mg, 0.5 mmol) were dried in an apparatus equipped with septum at room temperature and 1.32 Pa for 10 h. The apparatus was washed with argon (2×) and dry dichloromethane (1.3 ml) was added through the septum. After dissolution, the mixture was cooled to –45 °C and a solution of glycosyl bromide **4** was added under stirring through the septum during 1 h. The mixture was stirred at –45 °C for another 30 min and then at –20 °C for 1 h. Pyridine (0.4 ml) was added through the septum and the mixture was stirred at –20 °C for 20 min. After warming to room temperature, the mixture was diluted with chloroform (100 ml), filtered and the filtrate was washed with saturated aqueous $NaHCO_3$ (25 ml) and water (3 × 25 ml). The organic phase was separated, dried over anhydrous $MgSO_4$, evaporated in vacuo and the residue was co-distilled with toluene. The residue was worked up using the same procedure as given in method *A* to afford 331 mg (63%) of solid compound **6**, m.p. 150 °C, $[\alpha]_D$ +41 (*c* 0.3, chloroform). For **6** ($C_{64}H_{64}N_2O_{12}$) calculated: relative molecular mass 1053.2, monoisotopic mass 1052.5. FAB MS, *m/z*: 1053.4 $[M + H]^+$, 1075.3 $[M + Na]^+$. For $C_{64}H_{64}N_2O_{12}$ (1053.2) calculated: 72.99% C, 6.12% H, 2.66% N; found: 72.71% C, 6.29% H, 2.48% N.

TABLE I
^{1}H NMR parameters of compounds **6**, **7**, **10**–**12**

Parameter	**6**	**7**	**10**	**11**	**12**
δ(H-1)	4.63 d	4.64 d	4.78 d	4.79 d	4.81 d
δ(H-2)	4.01 ddd	4.04–4.07 m	4.24 dt	4.25 ddd	4.31 ddd
δ(H-3)	4.15 dd	4.04–4.07 m	4.14 dd	4.06 dd	4.04 dd
δ(H-4)	3.44 dd	3.51 dd	3.58 dd	3.59 dd	3.66 dd
δ(H-5)	3.80 dt	3.80 ddd	3.85 ddd	3.84 ddd	3.84 ddd
δ(H-6a)	3.60 d	3.58 dd	3.65 dd	3.63 dd	3.66 dd
δ(H-6b)	3.60 d	3.62 dd	3.72 dd	3.70 dd	3.78 dd
δ(H-1′)	5.29 d	5.47 d	4.68 d	4.67 d	4.39 d
δ(H-2′)	4.16 dd	4.28 dd	3.72 dd	3.95 dt	3.47 dd
δ(H-3′)	4.44–4.46 m	5.86 dd	3.61 dd	5.01 dd	3.37 ddd
δ(H-4′)	3.62–3.65 m	5.12 dd	3.72 dd	5.09 t	3.41 ddd
δ(H-5′)	3.62–3.65 m	3.83 ddd	3.46 ddd	3.60 ddd	3.27 ddd
δ(H-6a′)	3.51–3.54 m	4.05 dd	3.55 dd	4.04 dd	3.46 dd
δ(H-6b′)	3.78 dd	4.30 dd	3.75 dd	4.25 dd	3.76 dd
J(1,2)	3.8	3.8	4.0	4.1	4.1
J(2,3)	10.5	[a]	9.9	9.8	9.9
J(3,4)	8.5	8.4	8.4	8.4	8.7
J(4,5)	10.1	10.1	10.2	10.1	10.1
J(5,6a)	3.3	2.2	2.1	2.1	1.9
J(5,6b)	3.3	4.2	4.3	4.3	3.8
J(6a,6b)	[a]	10.6	10.7	10.7	10.6
J(1′,2′)	8.3	8.3	8.3	8.4	8.1
J(2′,3′)	10.7	10.7	9.5	10.4	9.5
J(3′,4′)	[a]	9.0	8.5	9.3	8.4
J(4′,5′)	[a]	10.2	9.5	9.8	9.2
J(5′,6a′)	[a]	2.6	2.2	2.7	3.4
J(5′,6b′)	1.3	4.5	5.5	4.4	6.4
J(6a′,6b′)	10.5	12.2	10.6	12.2	11.9

[a] Value not determined. Additional NMR parameters and parameters of substituents: **6** – arom. H: 6.86–7.33 m, N**H**Ac: 5.58 d (1 H, *J* = 9.8), $OCH_2C_6H_5$: 5.01 d (1 H, *J* = 11.1), 4.81 d (1 H, *J* = 11.0), 4.76 d (1 H, *J* = 11.9), 4.60 d (1 H, *J* = 11.8), 4.60 d (1 H, *J* = 11.8), 4.50 d (1 H, *J* = 12.2), 4.49 d (1 H, *J* = 11.6), 4.48 d (1 H, *J* = 11.1), 4.42 d (1 H, *J* = 11.6), 4.41 d (1 H, *J* = 12.2), 4.40 d (1 H, *J* = 11.9), 4.34 d (1 H, *J* = 11.8), NH**Ac**: 1.85 s (3 H); **7** – arom. H: 7.24–7.89 m, N**H**Ac: 5.45 d (1 H, *J* = 9.8), $OCH_2C_6H_5$: 5.02 d (1 H, *J* = 11.1), 4.64 d (1 H, *J* = 11.6), 4.52 d (1 H, *J* = 12.1), 4.48 d (1 H, *J* = 11.1), 4.46 d (1 H, *J* = 12.1), 4.35 d (1 H, *J* = 11.6), OAc: 2.00 s (3 H), 1.93 s (3 H), 1.92 s (3 H), NH**Ac**: 1.84 s (3 H); **10** – arom. H: 7.12–7.35 m, N**H**Ac: 6.07 d (1 H, *J* = 7.8), 5.58 d (1 H, *J* = 9.2), $OCH_2C_6H_5$: 4.99 d (1 H, **J** = 10.9), 4.77 d (1 H, *J* = 10.9), 4.76 d (1 H, *J* = 11.0), 4.70 d (1 H, *J* = 11.0), 4.69 d (1 H, *J* = 11.7), 4.59 d (1 H, *J* = 12.1), 4.53 d (1 H, *J* = 10.9), 4.52 d (1 H, *J* = 12.1), 4.46 d (1 H, *J* = 12.1), 4.44 d (1 H, *J* = 10.9), 4.43 d (1 H, *J* = 11.7), 4.41 d (1 H, *J* = 12.1), NH**Ac**: 1.97 s (3 H), 1.90 s (3 H); **11** – arom. H: 7.20–7.39 m, N**H**Ac: 6.22 d (1 H, *J* = 8.2), 5.94 d (1 H, *J* = 9.3), $OCH_2C_6H_5$: 4.97 d (1 H, *J* = 10.7), 4.70 d (1 H, *J* = 11.6), 4.58 d (1 H, *J* = 12.1), 4.51 d (1 H, *J* = 12.1), 4.43 d (1 H, *J* = 11.6), 4.41 d (1 H, *J* = 10.7), OAc: 2.01 s (3 H), 2.00 s (3 H), 1.99 s (3 H), NH**Ac**: 1.98 s (3 H), 1.93 s (3 H); **12** – arom. H: 7.18–7.40 m, 3′-OH: 6.42 d (1 H, *J* = 2.1), N**H**Ac: 6.11 d (1 H, *J* = 9.3), $OCH_2C_6H_5$: 4.84 d (1 H, *J* = 10.4), 4.72 d (1 H, *J* = 11.7), 4.64 d (1 H, *J* = 12.1), 4.53 d (1 H, *J* = 12.1), 4.47 d (1 H, *J* = 11.7), 4.43 d (1 H, *J* = 10.4), 4′-OH: 3.00 d (1 H, *J* = 2.0), NH**Ac**: 2.17 s (3 H), 1.96 s (3 H).

Benzyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**7**)

Compound **2** (492 mg, 1 mmol) and silver trifluoromethanesulfonate (520 mg, 2.02 mmol) were dried in an apparatus equipped with septum at room temperature and 1.32 Pa for 10 h. The apparatus was flushed with argon (2×) and then dry dichloromethane (2 ml) was added through the septum. After dissolution, the mixture was cooled to –45 °C and a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide[15] (**5**; 996 mg, 2 mmol) in dry dichloromethane (2 ml) was added under stirring through the septum during 1 h. Then the mixture was stirred at –45 °C for another 30 min and at –20 °C for 1 h. Pyridine (0.4 ml) was added through the septum and the mixture was stirred at –20 °C for 20 min. After warming to room temperature, the mixture was diluted with chloroform (250 ml), filtered and the filtrate was washed with saturated aqueous $NaHCO_3$ (50 ml) and water (3 × 50 ml). The organic phase was separated, dried over anhydrous $MgSO_4$, evaporated in vacuo and the residue was co-distilled with toluene. Chromatography of the solid residue on a silica gel column (60 ml) in toluene–ethyl acetate (1:1) afforded 657 mg (72%) of compound **7** as a solid foam, $[\alpha]_D$ +11 (*c* 0.2, chloroform). For **7** ($C_{49}H_{52}N_2O_{15}$) calculated: relative molec-

TABLE II
^{13}C NMR chemical shifts of compounds **6**, **7**, **10**–**12** [a]

Carbon	**6**	**7**	**10**	**11**	**12**
1	96.73	96.89	96.78	96.78	96.63
2	52.76	52.63	53.31	53.30	53.08
3	76.47	77.75	77.66	79.08	79.20
4	76.17	76.17	76.09	72.02	72.72
5	70.70	70.70	70.49	70.21	70.43
6	68.90	68.74	68.73	68.40	68.30
1′	97.11	97.64	99.12	99.55	99.60
2′	56.76	55.14	56.62	54.23	58.27
3′	79.21	70.23	78.56	73.49	77.72
4′	80.00	69.27	82.49	68.61	75.52
5′	75.08	71.39	75.67	75.60	75.43
6′	69.11	62.17	69.46	62.08	62.59

[a] Chemical shifts for aromatic carbons from protecting groups are not given. Additional ^{13}C NMR chemical shifts of substituents: **6** – $OCH_2C_6H_5$: 75.11 t, 74.92 t, 74.92 t, 73.47 t, 73.33 t, 69.46 t, NHAc: 169.82 s, 23.32 q; **7** – $OCH_2C_6H_5$: 75.14 t, 73.39 t, 69.67 t, NHAc: 169.54 s, 23.46 q, OAc: 170.67 s, 170.14 s, 170.00 s, 20.60 q, 20.60 q, 20.40 q; **10** – $OCH_2C_6H_5$: 75.11 t, 74.92 t, 74.81 t, 73.52 t, 73.52 t, 69.65 t, NHAc: 171.18 s, 170.49 s, 23.63 q, 23.52 q; **11** – $OCH_2C_6H_5$: 75.21 t, 73.56 t, 69.79 t, NHAc: 169.27 s, 170.74 s, 23.85 q, 23.15 q, OAc: 171.41 s, 170.74 s, 170.74 s, 20.69 q, 20.61 q, 20.58 q; **12** – $OCH_2C_6H_5$: 75.30 t, 73.61 t, 69.87 t, NHAc: 173.29 s, 171.43 s, 23.68 q, 22.65 q.

ular mass 908.9, monoisotopic mass 908.3. FAB MS, *m/z*: 909 $[M + H]^+$, 932 $[M + Na]^+$. For $C_{49}H_{52}N_2O_{15}$ (908.9) calculated: 64.75% C, 5.77% H, 3.08% N; found: 64.59% C, 5.87% H, 2.95% N.

Benzyl 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**10**)

Sodium borohydride (287 mg, 7.6 mmol) was gradually added during 20 min to a stirred suspension of compound **6** (480 mg, 0.46 mmol) in a mixture of propan-2-ol–water (6:1; 20 ml) at room temperature and the mixture was stirred for 6 h. Then another portion of sodium borohydride (28.7 mg, 0.76 mmol) was added and the stirring was continued for another 6 h. The solvents were evaporated in vacuo and the residue was taken between chloroform (50 ml) and 5% aqueous NaCl (5 ml). The organic layer was separated, washed with 5% aqueous NaCl (2 × 5 ml), dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was co-distilled with toluene (2 × 5 ml) and dissolved in a mixture of toluene–acetic acid (6:1; 7 ml) and the solution was heated at 70 °C for 6 h. The solvents were evaporated in vacuo and the residue was co-distilled with toluene (2 × 5 ml) to give crude benzyl 2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**8**), which was used without further purification for the preparation of compound **10**.

The obtained compound **8** was dissolved in a mixture of dichloromethane–pyridine (10:1; 5 ml). To the stirred solution at room temperature, acetic anhydride (0.2 ml) was slowly added and stirring was continued for 2 h. The solvents were evaporated in vacuo and the residue was co-distilled with toluene (2 × 5 ml). Chromatography of the residue on a silica gel column (50 g) in toluene–ethyl acetate (1:1) gave 270 mg (61%) of solid compound **10**. An analytical sample was prepared by crystallization from toluene–petroleum ether, m.p. 164–166 °C, $[\alpha]_D$ +41 (*c* 0.4, chloroform). For **10** ($C_{58}H_{64}N_2O_{11}$) calculated: relative molecular mass 965.1, monoisotopic 964.5. FAB MS, *m/z*: 965.0 $[M + H]^+$, 987 $[M + Na]^+$. For $C_{58}H_{64}N_2O_{11}$ (965.1) calculated: 72.18% C, 6.68% H, 2.90% N; found: 72.27% C, 6.76% H, 2.79% N.

Benzyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**11**)

A solution of compound **7** (650 mg, 0.72 mmol) in a mixture of dry methanol–butylamine (4:1; 15 ml) was heated in a pressure bottle at 85 °C for 10 h. After cooling, the mixture was evaporated and the solid residue was extracted with ether (3 × 50 ml). The insoluble residue was dissolved in a mixture of methanol–water (9:1; 5 ml), the solution was adjusted to pH 4 with formic acid and poured onto a column of ion-exchange resin Dowex 50 (in H^+ form) (150 ml). The column was washed with a mixture of methanol–water (9:1; 250 ml) and the product was eluted with a mixture of methanol–25% aqueous ammonia (7:1; 500 ml). Evaporation of the eluate in vacuo yielded 241 mg (51.3%) of syrupy crude benzyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**9**), which was used without any further purification for the preparation of compound **11**. For **9** ($C_{35}H_{45}N_2O_{10}$) calculated: relative molecular mass 652.7, monoisotopic mass 652.3. FAB MS, *m/z*: 653 $[M + H]^+$.

A solution of compound **9** (210 mg, 0.322 mmol) in a mixture of pyridine–acetic anhydride (2:1; 5 ml) was kept at room temperature for 20 h. Methanol (5 ml) was added

under stirring at 0 °C to decompose excess of acetic anhydride. After 20 min standing at room temperature, the mixture was evaporated in vacuo and the residue was co-distilled with toluene (3 × 10 ml). Chromatography of the residue on a silica gel column (60 ml) in chloroform–ethyl acetate (3:2) followed by crystallization from ethyl acetate and chloroform afforded 180 mg (68%) of compound **11**, m.p. 165–167 °C, $[\alpha]_D$ +52 (*c* 1, methanol). For **11** ($C_{43}H_{52}N_2O_{14}$) calculated: relative molecular mass 820.9, monoisotopic mass 820.3. FAB MS, *m/z*: 821 $[M + H]^+$, 843 $[M + Na]^+$, 713 $[M - OBn + H]^+$. For $C_{43}H_{52}N_2O_{14}$ (820.9) calculated: 62.92% C, 6.38% H, 3.41% N; found: 62.78% C, 6.45% H, 3.30% N.

Benzyl 2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**12**)

A suspension of compound **11** (130 mg, 0.158 mmol) in 0.01 M CH_3ONa in methanol (4 ml) was stirred at room temperature for 2 h and the obtained solution kept at –3 °C overnight. The mixture was neutralized by addition of Dowex 50 (pyridinium form), the ion exchanger was filtered off, washed with methanol (20 ml) and the filtrate was evaporated in vacuo. The HPLC separation of the residue on a silica gel column C18 in the solvent system water–methanol (linear gradient 55→75%/60 min) afforded 109 mg (99%) of solid compound **12**, $[\alpha]_D$ +73 (*c* 0.4, methanol). For **12** ($C_{37}H_{46}N_2O_{11}$) calculated: relative molecular mass 694.8, monoisotopic mass 694.3. FAB MS, *m/z*: 695 $[M + H]^+$. For $C_{37}H_{46}N_2O_{11}$ (694.8) calculated: 63.96% C, 6.67% H, 4.03% N; found: 64.08% C, 6.73% H, 3.88% N.

2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucopyranose (**13**)

Compound **10** (193 mg, 0.2 mmol) or compound **12** (139 mg, 0.2 mmol) was hydrogenolyzed in acetic acid (15 ml) over 10% palladium catalyst on carbon (200 mg) at room temperature for 20 h. After this time the vessel was flushed with argon, the catalyst was filtered off, washed with acetic acid (30 ml) and the filtrate was lyophilized. The solid residue was separated by HPLC on a silica gel column C18 in water followed by permeating chromatography on Toyopearl HW 40F (450 ml) in water. The homogenous fraction was evaporated in vacuo and the residue was lyophilized from water, to give an α/β-anomeric mixture of compound **13**. Yield 65 mg (76%) of α/β-anomeric mixture (5:3) from **10**. Yield 56 mg (66%) of α/β-anomeric mixture (5:3) from **12**. The ratio of anomers was determined by ^{1}H NMR spectra. $[\alpha]_D$ –17 (*c* 0.2, water); lit.[25] $[\alpha]_D$ +14.5 → +6.5 (water) and lit.[8] $[\alpha]_D$ +40 → +4 (water) . ^{1}H NMR (500 MHz, D_2O, 300 K): 5.20 d (1 H, *J* = 3.5, H-1); 4.74 d (1 H, *J* = 8.3, H-1); 4.67 d (1 H, *J* = 8.3, H-1′); 4.66 d (1 H, *J* = 8.3, H-1′); 4.06 m (1 H, H-2); 3.81 m (1 H, H-2); 3.78 m (2 H, H-2′); 3.53–4.02 m (10 H, H-3, H-4, H-5, H-6a, H-6b, H-3′, H-4′, H-5′, H-6′a, H-6′b); 2.31 s, 2.30 s, 2.21 s, 2.18 s (4 × 3 H, NH**Ac**). ^{13}C NMR (125 MHz, D_2O, 300 K): 174.50 s, 174.46 s, 173.80 s (2 C); 101.14 d (2 C, C-1′); 95.08 d (C-1); 90.94 d (C-1); 81.53 d, 79.02 d, 75.86 d, 75.79 d, 75.50 d, 73.37 d, 73.35 d, 71.19 d, 69.85 d (2 C); 68.61d, 68.60 d, 60.85 t (C-6); 60.71 t (2 C, C-6); 60.64 t (C-6); 55.81 d (C-2′); 55.78 d (C-2′); 55.68 d (C-2); 53.03 d (C-2); 22.39 q, 22.38 q, 22.33 q, 22.15 q. For **13** ($C_{16}H_{28}N_2O_{11}$) calculated: relative molecular mass 424.4, monoisotopic mass 424.2. FAB MS, *m/z*: 425 $[M + H]^+$. For $C_{16}H_{28}N_2O_{11}$ (424.4) calculated: 45.28% C, 6.65% H, 6.60% N; found: 45.13% C, 6.76% H, 6.48% N.

Benzyl 3,4,6-Tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**15**)

Benzyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside[14] (**14**; 1 g, 1 mmol) and tris(triphenylphosphine)rhodium(I) chloride (0.1 g, 0.11 mmol) were refluxed in a mixture of ethanol–toluene–water (7:3:2; 30 ml) under stirring for 5 h. Formic acid (0.7 ml) was added and the reflux was continued for another 2 h. The mixture was evaporated in vacuo and the residue was chromatographed on a silica gel column (120 ml) in toluene–ethyl acetate (10:4) to give 482 mg (50%) of compound **15** as a solid foam, $[\alpha]_D$ +99 (*c* 0.3, chloroform). For **15** ($C_{57}H_{58}N_2O_{12}$) calculated: relative molecular mass 963. 1, monoisotopic mass 962.4. FAB MS, *m/z*: 963.7 $[M + H]^+$. For $C_{57}H_{58}N_2O_{12}$ (963.1) calculated: 71.09% C, 6.07% H, 2.91% N; found: 70.90% C, 6.12% H, 3.02% N.

An Attempt of Preparation of Benzyl 3,4,6-Tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside

Ethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[14] (**3**; 262 mg, 0.42 mmol), silver trifluoromethanesulfonate (54 mg, 0.21 mmol) and crushed molecular sieve 4A (0.3 g) were dried in an apparatus equipped with a septum at room temperature and 1.32 Pa for 10 h and then the apparatus was twice flushed with argon. A solution of compound **15** (200 mg, 0.21 mmol) in dry dichloromethane (3 ml) was added through the septum under stirring and the mixture was stirred at room temperature for 1 h. Then methyl trifluoromethanesulfonate (0.05 ml, 0.42 mmol) was added through the septum and stirring was continued for 12 h. The addition of the same amount of methyl trifluoromethanesulfonate followed by 12-h stirring was repeated three more times. After cooling to –15 °C, dry pyridine (0.63 ml) was added through the septum and the mixture was stirred at –15 °C for 30 min and at room temperature for 1 h. The mixture was diluted with chloroform (80 ml) and filtered through a Celite pad. The filtrate was washed with 10% aqueous $NaHSO_4$ (30 ml), saturated aqueous $NaHCO_3$ (30 ml), 5% aqueous NaCl (30 ml). The organic phase was dried over anhydrous $MgSO_4$ and evaporated in vacuo. Chromatography of the residue on a silica gel column (600 ml) in toluene followed by HPLC separation on a silica gel column C18 in the solvent system water–methanol (linear gradient 75→90%/70 min) afforded 120 mg (59%) of glycosyl acceptor **15**, 82 mg (17%) of syrupy compound **16** and 33 mg (14%) of syrupy compound **17**.

3,4,6-Tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl
3,4,6-Tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**16**)

Syrup: $[\alpha]_D$ +30 (*c* 0.4, chloroform). For **16** ($C_{70}H_{64}N_2O_{13}$) calculated: relative molecular mass 1141.3, monoisotopic mass 1140.4. FAB MS, *m/z*: 1142 $[M + H]^+$, 1163.7 $[M + Na]^+$. For $C_{70}H_{64}N_2O_{13}$ (1141.3) calculated: 73.67% C, 5.65% H, 2.45% N; found: 73.55% C, 5.71% H, 2.38% N.

TABLE III
^{1}H NMR parameters of compounds **15**–**17**

Parameter	**15**	**16**	**17**
δ(H-1)	4.91 d	5.34 d	3.67 d
δ(H-2)	4.03 ddd	4.03 dd	–
δ(H-3)	3.78 dd	4.30 dd	4.55 dt
δ(H-4)	3.60 dd	3.63 dd	4.06 dd
δ(H-5)	3.64 ddd	3.47 ddd	4.38 bddd
δ(H-6a)	3.10 dd	3.37 dd	3.83 dd
δ(H-6b)	3.18 dd	3.65 dd	3.93 dd
δ(H-1′)	5.30 d	5.34 d	–
δ(H-2′)	4.20 dd	4.03 dd	–
δ(H-3′)	4.39 dd	4.30 dd	–
δ(H-4′)	3.70 dd	3.63 dd	–
δ(H-5′)	3.74–3.76 m	3.47 ddd	–
δ(H-6a′)	3.64–3.66 m	3.37 dd	–
δ(H-6b′)	3.74–3.76 m	3.65 dd	–
J(1,2)	3.7	8.7	–
J(2,3)	10.6	10.7	–
J(3,4)	7.9	8.7	5.3
J(4,5)	9.9	9.9	7.3
J(5,6a)	1.6	1.9	3.4
J(5,6b)	4.1	3.4	5.5
J(6a,6b)	11.0	11.2	10.8
J(1′,2′)	8.5	8.7	–
J(2′,3′)	10.7	10.7	–
J(3′,4′)	8.5	8.7	–
J(4′,5′)	9.6	9.9	–
J(5′,6a′)	[a]	1.9	–
J(5′,6b′)	[a]	3.4	–
J(6a′,6b′)	[a]	11.2	–

[a] Not determined. Additional NMR parameters and parameters of substituents: **15** – arom. H: 6.79–7.38 m, N**H**Ac: 5.53 d (1 H, *J* = 8.4), $OCH_2C_6H_5$: 4.81 d (1 H, *J* = 11.0), 4.77 d (1 H, *J* = 12.1), 4.59 d (1 H, *J* = 11.9), 4.59 d (1 H, *J* = 11.0), 4.57 d (1 H, *J* = 11.7), 4.50 d (1 H, *J* = 11.9), 4.40 d (1 H, *J* = 12.1), 4.33 d (1 H, *J* = 11.7), 3.94 d (1 H, *J* = 12.0), 3.88 d (1 H, *J* = 12.0), NH**Ac**: 1.91 s (3 H); **16** – arom. H: 6.80–7.32 m, $OCH_2C_6H_5$: 4.68 d (1 H, *J* = 12.3), 4.68 d (1 H, *J* = 10.9), 4.53 d (1 H, *J* = 10.9), 4.36 d (1 H, *J* = 12.3), 4.20 d (1 H, *J* = 11.8), 4.05 d (1 H, *J* = 11.8); **17** – arom. H: $J_{1,3}$ = 1.2, $J_{3,5}$ = 0.8, 6.92–7.80 m, $OCH_2C_6H_5$: 4.78 d (1 H, *J* = 11.5), 4.68 d (1 H, *J* = 11.5), 4.60 s (2 H), 4.57 d (1 H, *J* = 12.0), 4.31 d (1 H, *J* = 12.0).

1,5-Anhydrotri-*O*-benzyl-2-phthalimido-2-deoxy-D-*arabino*-hex-1-enitol (**17**)

Syrup: $[\alpha]_D$ +52 (*c* 0.4, chloroform). For **17** ($C_{35}H_{31}NO_6$) calculated: relative molecular mass 561.6, monoisotopic mass 561.2. FAB MS, *m/z*: 561 $[M + H]^+$, 584.6 $[M + Na]^+$. For $C_{35}H_{31}NO_6$ (561.6) calculated: 74.85% C, 5.56% H, 2.49% N; found: 75.11% C, 5.63% H, 2.43% N.

Benzyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**18**)

Compound **15** (200 mg, 0.21 mmol) and silver trifluoromethanesulfonate (126 mg, 0.5 mmol) were dried in an apparatus equipped with a septum at room temperature and 1.32 Pa for 20 h. Apparatus was flushed with argon (2×) and dry dichloromethane (1 ml) was added through the septum. After dissolution, the mixture was cooled to –45 °C and a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide[15] (**5**; 210 mg, 0.42 mmol) in dry dichloromethane (1 ml), was added through the septum under stirring during 2 h. Then the mixture was stirred at –45 °C for another 30 min and at –20 °C for 1 h. Dry pyridine (0.2 ml) was added through the septum at –20 °C and after warming to room temperature the mixture was diluted with chloroform (150 ml) and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ (40 ml) and water (2 × 40 ml), dried over

TABLE IV
^{13}C NMR chemical shifts of compounds **15**–**17**[a]

Carbon	**15**	**16**	**17**
1	96.46	96.88	146.50
2	53.03	55.49	107.96
3	70.53	78.83	74.13
4	81.37	78.93	77.65
5	69.58	75.29	74.26
6	68.34	68.62	67.93
1′	98.86	96.88	–
2′	55.92	55.49	–
3′	79.50	78.83	–
4′	78.93	78.93	–
5′	74.32	75.29	–
6′	68.76	68.62	–

[a] Chemical shifts for aromatic carbons from protecting groups are not given. Additional ^{13}C NMR chemical shifts of substituents: **15** – $OCH_2C_6H_5$: 75.00 t, 74.95 t, 73.45 t, 72.67 t, 69.60 t, NHAc: 170.10 s, 23.34 q; **16** – $OCH_2C_6H_5$: 74.77 t, 74.53 t, 73.54 t; **17** – $OCH_2C_6H_5$: 73.51 t, 73.27 t, 72.35 t.

anhydrous $MgSO_4$, evaporated in vacuo and the residue was co-distillated with toluene (2×). Chromatography of the residue on a silica gel column (80 ml) in toluene–ethyl acetate (10:1) followed by HPLC separation on a silica gel column C18 in the solvent system water–methanol (85% methanol/20 min and then linear gradient 85→90%/60 min) afforded 35 mg (11%) of solid compound **18**, m.p. 103–105 °C, $[\alpha]_D$ +44 (*c* 0.14, chloroform). For **18** ($C_{77}H_{77}N_3O_{21}$) calculated: relative molecular mass 1380.4, monoisotopic mass 1379.5. FAB MS, *m/z*: 1402.5 $[M + Na]^+$. For $C_{77}H_{77}N_3O_{21}$ (1380.4) calculated: 66.99% C, 5.62% H, 3.04% N; found: 66.79% C, 5.68% H, 2.96% N.

Benzyl 2-Acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**20**)

Benzyl 2-acetamido-3-*O*-allyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside[15] (**19**; 5 g, 11.33 mmol) and tris(triphenylphosphine)rhodium(I) chloride (1.2 g, 1.34 mmol) were refluxed in a mixture of ethanol–toluene–water (7:3:2; 300 ml) under stirring for 4 h. Formic acid (10 ml) was added and the reflux was continued for another 2 h. Then the mixture was evaporated in vacuo. Chromatography of the residue on a silica gel column (500 ml) in chloroform–methanol (30:1) followed by crystallization from ethanol gave 3.45 g (76%) of compound **20**, m.p. 187–188 °C, $[\alpha]_D$ +52 (*c* 0.3, methanol); lit.[26] m.p. 183 °C (methanol), $[\alpha]_D$ +45 (*c* 1.0, methanol). ^{1}H NMR spectrum agrees with the data for an authentic sample prepared by the procedure described in lit.[26] For **20** ($C_{22}H_{27}NO_6$) calculated: relative molecular mass 401.5, monoisotopic mass 401.2. FAB MS, *m/z*: 402 $[M + H]^+$. For $C_{22}H_{27}NO_6$ (401.5) calculated: 65.82% C, 6.78% H, 3.49% N; found: 65.70% C, 6.83% H, 3.54% N.

Benzyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**21**)

Compound **20** (100 mg, 0.25mmol) and silver trifluoromethanesulfonate (257 mg, 0.1 mmol) were dried in an apparatus equipped with a septum at room temperature and 1.32 Pa for 20 h. Apparatus was flushed with argon (2×) and dry dichloromethane (1 ml) was added through the septum. After dissolution, the mixture was cooled to –45 °C and the solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide[15] (**5**; 996 mg, 2 mmol) in dry dichloromethane (1 ml) was added under stirring through the septum during 1 h. Then the mixture was stirred at –45°C for another 30 min and at –20 °C for 1 h. Dry pyridine (0.4 ml) was added through the septum at –20 °C and, after warming to room temperature, the mixture was diluted with chloroform (150 ml) and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ (30 ml) and water (2 × 30 ml), dried over anhydrous $MgSO_4$, evaporated in vacuo and the residue was co-distilled with toluene (2 × 20 ml). Chromatography of the residue on a silica gel column (60 ml) in toluene–ethyl acetate (3:1) followed by lyophilization from benzene afforded 238 mg (64%) of compound **21**, $[\alpha]_D$ +12 (*c* 0.25, chloroform). For **21** ($C_{62}H_{65}N_3O_{24}$) calculated: relative molecular mass 1236.2, monoisotopic mass 1235.4. FAB MS, *m/z*: 1258.5 $[M + Na]^+$. For $C_{62}H_{65}N_3O_{24}$ (1236.2) calculated: 60.24% C, 5.30% H, 3.40% N; found: 60.19% C, 5.38% H, 3.33% N.

Benzyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**23**)

A solution of compound **21** (600 mg, 0.49 mmol) in a mixture of absolute methanol–butylamine (4:1; 10 ml) was heated in a pressure bottle at 85 °C for 7 h. After cooling, the mixture was evaporated and the solid residue was extracted with ether (3 × 30 ml). The insoluble residue was dissolved in a mixture of methanol–water (9:1; 5 ml), the solution was adjusted to pH 4 with formic acid and poured onto an ion-exchange resin Dowex 50 (in H^+ form) column (150 ml). The column was washed with a mixture of methanol–water (9:1; 500 ml) and the product was desorbed with a mixture of methanol–25% aqueous ammonia

TABLE V
^{1}H NMR parameters of compounds **18**, **21**, **23**, **24**

Parameter	**18**	**21**	**23**	**24**
δ(H-1)	4.54 d	4.58 d	4.82 d	4.77 d
δ(H-2)	3.97 ddd	3.97 ddd	4.20 dt	4.19 dd
δ(H-3)	3.80 dd	3.70 dd	3.92 dd	4.21 dd
δ(H-4)	3.89 dd	3.86 dd	3.85 dd	4.00 dd
δ(H-5)	3.53 ddd	3.47 ddd	3.76 ddd	3.91 ddd
δ(H-6a)	3.92 dd	3.38 dd	3.57 dd	3.74 dd
δ(H-6b)	4.05 dd	3.42 dd	3.65 dd	3.79 dd
δ(H-1′)	5.14 d	5.29 d	4.82 d	4.80 d
δ(H-2′)	4.40 dd	4.39 dd	3.60 ddd	3.63 dd
δ(H-3′)	5.82 dd	5.83 dd	5.34 dd	3.51 dd
δ(H-4′)	5.39 dd	5.33 dd	5.02 dd	3.27 dd
δ(H-5′)	3.81 ddd	3.56 ddd	3.63 ddd	3.24 ddd
δ(H-6a′)	4.34 dd	4.22 dd	3.96 dd	3.65 dd
δ(H-6b′)	4.72 dd	4.81 dd	4.53 dd	3.87 dd
δ(H-1″)	5.37 d	5.30 d	4.61 d	4.69 d
δ(H-2″)	4.44 dd	4.40 dd	3.69 ddd	3.63 dd
δ(H-3″)	4.25 dd	5.84 dd	5.15 dd	3.51 dd
δ(H-4″)	3.80 dd	5.35 dd	4.98 dd	3.40 dd
δ(H-5″)	3.49 dt	3.83 ddd	3.51 ddd	3.22 ddd
δ(H-6a″)	3.36–3.38 m	4.29 dd	4.05 dd	3.76 dd
δ(H-6b″)	3.36–3.38 m	4.87 dd	4.54 dd	3.82 dd

TABLE V
(*Continued*)

Parameter	**18**	**21**	**23**	**24**
J(1,2)	3.9	3.9	3.9	3.8
J(2,3)	9.7	10.5	9.7	9.7
J(3,4)	8.6	8.6	8.7	7.3
J(4,5)	9.8	10.1	9.5	8.9
J(5,6a)	3.9	3.5	2.1	2.3
J(5,6b)	1.8	1.6	3.4	4.5
J(6a,6b)	10.5	10.9	10.8	10.9
J(1′,2′)	8.2	7.3	8.1	8.3
J(2′,3′)	10.9	11.0	10.7	10.5
J(3′,4′)	9.2	9.2	9.2	9.3
J(4′,5′)	10.0	10.0	10.0	9.8
J(5′,6a′)	2.1	1.5	2.2	2.1
J(5′,6b′)	3.6	3.1	5.0	6.1
J(6a′,6b′)	12.3	12.6	12.3	12.1
J(1″,2″)	8.1	8.2	8.3	8.2
J(2″,3″)	10.8	10.9	10.7	10.4
J(3″,4″)	8.2	9.3	9.2	8.7
J(4″,5″)	9.0	10.0	10.1	9.7
J(5″,6a″)	3.4	1.6	2.4	2.3
J(5″,6b″)	3.4	3.1	5.5	4.6
J(6a″,6b″)	[a]	12.6	12.3	11.9

[a] Not determined. Additional NMR parameters and parameters of substituents: **18** – arom. H: 6.82–7.83 m, N**H**Ac: 5.36 d (1 H, *J* = 9.7), $OCH_2C_6H_5$: 5.01 d (1 H, *J* = 12.0), 4.73 d (1 H, *J* = 11.9), 4.66 d (1 H, *J* = 11.8), 4.64 d (1 H, *J* = 12.0), 4.50 d (1 H, *J* = 11.8), 4.41 d (1 H, *J* = 11.9), 4.38 d (1 H, *J* = 12.0), 4.38 d (1 H, *J* = 11.8), 4.38 d (1 H, *J* = 11.8), 4.20 d (1 H, *J* = 12.0), OAc: 2.17 s (3 H), 2.03 s (3 H), 1.66 s (3 H), NH**Ac**: 1.84 s (3 H); **21** – arom. H: 7.13–7.88 m, N**H**Ac: 5.30 d (1 H, *J* = 9.8), $OCH_2C_6H_5$: 4.63 d (1 H, *J* = 11.8), 4.52 d (1 H, *J* = 11.8), 4.48 d (1 H, *J* = 12.0), 4.27 d (1 H, *J* = 12.0), OAc: 2.31 s (3 H), 2.30 s (3 H), 2.02 s (3 H), 2.01 s (3 H), 1.81 s (3 H), 1.79 s (3 H), NH**Ac**: 1.87 s (3 H); **23** – arom. H: 7.23–7.48 m, N**H**Ac: 6.40 bd (1 H, *J* = 8.3), 2-N**H**Ac: 5.93 bd (1 H, *J* = 9.2), 2″-N**H**Ac: 5.55 bd (1 H, *J* = 8.9), $OCH_2C_6H_5$: 4.75 d (1 H, *J* = 12.0), 4.68 d (1 H, *J* = 11.8), 4.52 d (1 H, *J* = 12.0), 4.46 d (1 H, *J* = 11.8), OAc: 2.14 s (3 H), 2.07 s (3 H), 2.03 s (3 H), 2.00 s (3 × 3 H), NH**Ac**: 1.98 s (3 H), 1.93 s (3 H), 1.84 s (3 H); **24** – arom. H: 7.25–7.41 m, $OCH_2C_6H_5$: 4.68 d (1 H, *J* = 12.1), 4.64 d (1 H, *J* = 11.8), 4.60 d (1 H, *J* = 11.8), 4.49 d (1 H, *J* = 12.1), NH**Ac**: 1.98 s (3 H), 1.97 s (2 × 3 H).

(7:1; 500 ml). Evaporation of the eluate in vacuo yielded 271 mg (77%) of syrupy crude benzyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**22**), which was used without further purification for the preparation of compound **23**. For **22** ($C_{34}H_{49}N_3O_{14}$) calculated: relative molecular mass 723.8, monoisotopic mass 723.3. FAB MS, *m/z*: 724 $[M + H]^+$.

A solution of compound **22** (260 mg, 0.36 mmol) in a mixture of pyridine–acetic anhydride (2:1; 8 ml) was kept at room temperature for 20 h. Methanol (5 ml) was added under

TABLE VI
^{13}C NMR parameters of compounds **18**, **21**, **23**, **24**[a]

Parameter	**18**	**21**	**23**	**24**
δ(C-1)	95.98	96.51	96.60	96.97
δ(C-2)	52.24	52.43	52.64	54.36
δ(C-3)	75.66	75.75	73.64	74.75
δ(C-4)	73.23	72.85	73.39	74.10
δ(C-5)	70.86	70.34	70.58	73.02
δ(C-6)	68.31	68.00	67.74	70.03
δ(C-1′)	97.83	98.01	98.81	99.64
δ(C-2′)	54.79	54.37	54.78	57.81
δ(C-3′)	71.09	70.64	71.58	75.68
δ(C-4′)	68.76	68.26	68.93	72.20
δ(C-5′)	72.62	71.85	72.45	78.04
δ(C-6′)	61.95	61.28	61.83	62.67
δ(C-1″)	97.00	96.65	98.43	100.68
δ(C-2″)	56.32	54.66	55.01	57.92
δ(C-3″)	79.63	70.94	71.90	75.68
δ(C-4″)	79.61	68.43	68.89	71.74
δ(C-5″)	71.70	71.74	72.16	77.77
δ(C-6″)	68.96	61.52	61.91	62.04

[a] Chemical shifts for aromatic carbons from protecting groups are not given. Additional ^{13}C NMR chemical shifts of substituents: **18** – $OCH_2C_6H_5$: 75.11 t, 74.92 t, 74.49 t, 72.76 t, 69.45 t, NHAc: 170.02 s, 23.29 q, OAc: 171.33 s, 169.81 s, 169.38 s, 21.03 q, 20.69 q, 20.29 q; **21** – $OCH_2C_6H_5$: 72.64 t, 69.66 t, NHAc: 169.88 s, 23.35 q, OAc: 171.54 s, 171.47 s, 169.88 s, 169.81 s, 169.46 s, 164.41 s, 20.95 q, 20.95 q, 20.64 q, 20.63 q, 20.42 q, 20.40 q; **23** – $OCH_2C_6H_5$: 73.44 t, 69.90 t, NHAc: 170.16 s, 169.54 s, 169.45 s, 23.53 q, 23.31 q, 23.23 q, OAc: 171.22 s, 171.14 s, 170.91 s, 170.55 s, 170.51 s, 170.47 s, 20.84 q, 20.75 q, 20.67 q, 20.64 q, 20.64 q, 20.62 q; **24** – $OCH_2C_6H_5$: 74.35 t, 70.63 t, NHAc: 173.91 s, 173.78 s, 173.55 s, 23.42 q, 23.27 q, 22.82 q.

stirring at 0 °C to decompose excess acetic anhydride. After 20 min standing at room temperature, the mixture was evaporated in vacuo and the residue was co-distilled with toluene (3 × 20 ml). Chromatography of the residue on a silica gel column (60 ml) in toluene–acetone (2:1) followed by HPLC separation on a silica gel column C18 in solvent system water–methanol (linear gradient 63→70%/30 min, then 70% methanol/30 min and finally linear gradient 70→75%/30 min) afforded 250 mg (66%) of compound **23**, $[\alpha]_D$ +19 (*c* 0.7, methanol). For **23** ($C_{50}H_{65}N_3O_{22}$) calculated: relative molecular mass 1060.1, monoisotopic mass 1059.4. FAB MS, *m/z*: 1082.1 $[M + Na]^+$. For $C_{50}H_{65}N_3O_{22}$ (1060.1) calculated: 56.65% C, 6.18% H, 3.96% N; found: 56.78% C, 6.13% H, 3.88% N.

Benzyl 2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**24**)

A suspension of compound **23** (200 mg, 0.189 mmol; dried at a room temperature and 1.32 Pa for 10 h) in 0.01 M CH_3ONa in methanol (4 ml) was stirred at room temperature for 2 h and the solution was allowed to stand at –3 °C overnight. The mixture was neutralized by addition of Dowex 50 (pyridinium form). The resin was filtered off, washed with methanol (20 ml) and the filtrate was concentrated in vacuo. The HPLC separation of the residue on a silica gel column C18 in the solvent system water–methanol (linear gradient 50→60%/60 min) afforded 118 mg (77%) of syrupy compound **24**, $[\alpha]_D$ +34 (*c* 0.5, methanol). For **24** ($C_{38}H_{53}N_3O_{16}$) calculated: relative molecular mass 807.8, monoisotopic mass 807.3. FAB MS, *m/z*: 808 $[M + H]^+$, 830 $[M + Na]^+$. For $C_{38}H_{53}N_6O_{13}$ (807.8) calculated: 56.50% C, 6.61% H, 5.20% N; found: 56.48% C, 6.75% H, 5.28% N.

2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)]-2-acetamido-2-deoxy-D-glucopyranose (**25**)

Compound **24** (88 mg, 0.11 mmol) was hydrogenolyzed in acetic acid (10 ml) over 10% palladium catalyst on carbon (100 mg) at room temperature for 20 h. The vessel was than flushed with argon, the catalyst was filtered off, washed with acetic acid (30 ml) and the filtrate was lyophilized. The solid residue was separated by HPLC on a silica gel column C18 in water followed by permeating chromatography on Toyopearl HW 40F (450 ml) in water. The obtained homogenous fraction was evaporated in vacuo and the residue was lyophilized from water to give 46 mg (67%) of an α/β-anomeric mixture (3:2) of compound **25**, $[\alpha]_D$ –52 (*c* 0.7, water). The ratio of anomers was determined by ^{1}H NMR spectra. ^{1}H NMR (500 MHz, D_2O, 300 K): 5.15 d (1 H, *J* = 3.1, H-1); 4.78 d (1 H, *J* = 8.4); 4.738 d (1 H, *J* = 8.3); 4.733 d (1 H, *J* = 8.1); 4.717 d (1 H, *J* = 8.1); 4.704 d (1 H, *J* = 8.3, H-1′ and H-1″); 3.42–4.37 m (18 H, H-2, H-3, H-4, H-5, H-6, H-2′, H-3′, H-4′, H-5′, H-6′, H-2″, H-3″, H-4″, H-5″ and H-6″); 2.18 s, 2.15 s, 2.140 s, 2.136 s, 2.136 s, 2.126 s (6 × 3 H, NH**Ac**). ^{13}C NMR (125 MHz, D_2O, 300 K): 174.92 s, 174.76 s, 174.57 s, 174.54 s, 174.27, 173.84 s, 102.71 d, 101.34 d, 99.91 d, 99.91 d, 98.97 d, 94.76 d, 79.06 d, 76.95 d, 75.98 d, 75.92 d, 75.83 d, 75.83 d, 75.76 d, 75.73 d, 75.58 d, 74.25 d, 74.16 d, 73.88 d, 73.82 d, 73.73 d, 73.68 d, 73.63 d, 73.55 d, 73.40 d, 62.49 t, 60.83 t, 60.79 t, 60.71 t, 60.71 t, 60.59 t, 55.93 d, 55.90 d, 55.81 d, 55.80 d, 51.89 d, 51.79 d, 22.35 q, 22.32 q, 22.31 q, 22.30 q, 22.24 q, 22.21 q. For **25** ($C_{24}H_{41}N_3O_{16}$) calculated: relative molecular mass 627.6, monoisotopic mass 627.3. FAB MS, *m/z*: 628 $[M + H]^+$. For $C_{24}H_{41}N_3O_{16}$ (627.6) calculated: 45.93% C, 6.58% H, 6.70% N; found: 45.99% C, 6.53% H, 6.78% N.

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