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Chemical synthesis of N-acetylglucosamine derivatives and their use as glycosyl acceptors by the *Mesorhizobium loti* chitin oligosaccharide synthase NodC

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Abstract

Rhizobial bacteria synthesize lipo-chitin oligosaccharide signal molecules (Nod factors) that are essential for the formation of symbiotic organs on the roots of host plants, a process known as nodulation. Biosynthesis of the chitin oligosaccharide moiety in Nod factors is carried out by the rhizobial N-acetylglucosaminyltransferase NodC. The initial acceptor or primer used for the synthesis of chitin oligosaccharides in vivo is unknown. To investigate the acceptor specificity of NodC, we have synthesized derivatives of N-acetylglucosamine (GlcNAc) with different aglycones and tested whether they are acceptors for NodC in vitro using a membrane preparation of an *Escherichia coli* strain expressing the *Mesorhizobium loti* chitin oligosaccharide synthase NodC. Analysis of reaction products using thin-layer chromatography shows that GlcNAc derivatives containing simple alkyl chains or other hydrophobic groups linked to C-1 are acceptors for NodC. The enzyme appears to be specific for acceptors in which the aglycone is β-linked. GlcNAc derivatives in which the methyl group of the N-acetyl moiety of GlcNAc is replaced by an allyloxy or benzyloxy group are still used as acceptors by NodC. The original methyl group at this position therefore does not appear to be essential for the interaction between NodC and GlcNAc. A NodC-dependent reaction product that is more hydrophobic than GlcNAc was detected in reaction mixtures containing 5% methanol but lacking an exogenously added acceptor. This may be due to the presence of a natural hydrophobic glycosyl acceptor for NodC in the membranes of *E. coli*, but the structure of this reaction product remains to be investigated.

Keywords: Nodulation; Polysaccharides; *N*-Acetylglucosamine; Glycosyl acceptors

1. Introduction

Soil bacteria belonging to the genera *Rhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Bradyrhizobium*, *Azorhizobium* and *Allorhizobium* (collectively called rhizobia) are able to establish a symbiotic relationship with leguminous plants such as soybean, pea, alfalfa and clover [1–3]. This process is called nodulation and involves the induction of a new, nodular organ on the roots of the host plant in which the bacteria convert atmospheric nitrogen into

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ammonia. Bacterial signal molecules, known as Nod factors, are essential for nodule formation [4]. Purified Nod factors induce many of the plant root processes that normally occur during nodulation, sometimes even leading to the formation of complete nodules [5]. Nod factors consist of linear oligomers of mostly four or five β-(1→4)-linked N-acetylglucosamine (GlcNAc) residues, in which the N-acetyl group of the nonreducing-terminal residue is replaced with a fatty acid. Biosynthesis of Nod factors requires a series of enzymes, encoded by the rhizobial nodulation genes [6,7].

The nodulation protein NodC is a rhizobial N-acetylglucosaminyltransferase [8], which synthesizes chitin oligosaccharides [9,10]. We have recently shown that the synthesis of chitin oligosaccharides by NodC proceeds by the addition of GlcNAc residues from the donor UDP-GlcNAc to O-4 of the nonreducing-terminal residue of the growing chain [11]. The enzyme is able to use free GlcNAc and the artificial glycoside GlcNAc-1-*O*-*p*-nitrophenyl as acceptors. The use of hydrophobic sugar derivatives as artifical glycosyl acceptors greatly simplifies quantification of enzyme activity, since the reaction products can be readily purified using reversed-phase cartridges [12]. Artificial acceptors are not only used to assay glycosyltransferase activities but also to characterize substrate specificity. This approach has been used for many glycosyltransferases involved in protein glycosylation. Mammalian β-(1→4)-galactosyltransferases, for instance, were shown to have a broad acceptor specificity [13,14], whereas a related N-acetylglucosaminyltransferase from the snail *Lymnea stagnalis* shows a clear preference for acceptors containing a terminal GlcNAc residue in β-(1→6) linkage to galactose or N-acetylgalactosamine [15]. These enzymes are so-called nonprocessive glycosyltransferases; they transfer only a single monosaccharide to an acceptor. NodC, however, belongs to the processive glycosyltransferases, which produce linear oligosaccharides or polysaccharides by repetitively adding monosaccharide residues to a growing chain. We herein describe the synthesis of several derivatives of GlcNAc and their ability to act as acceptors for *M. loti* NodC.

2. Results

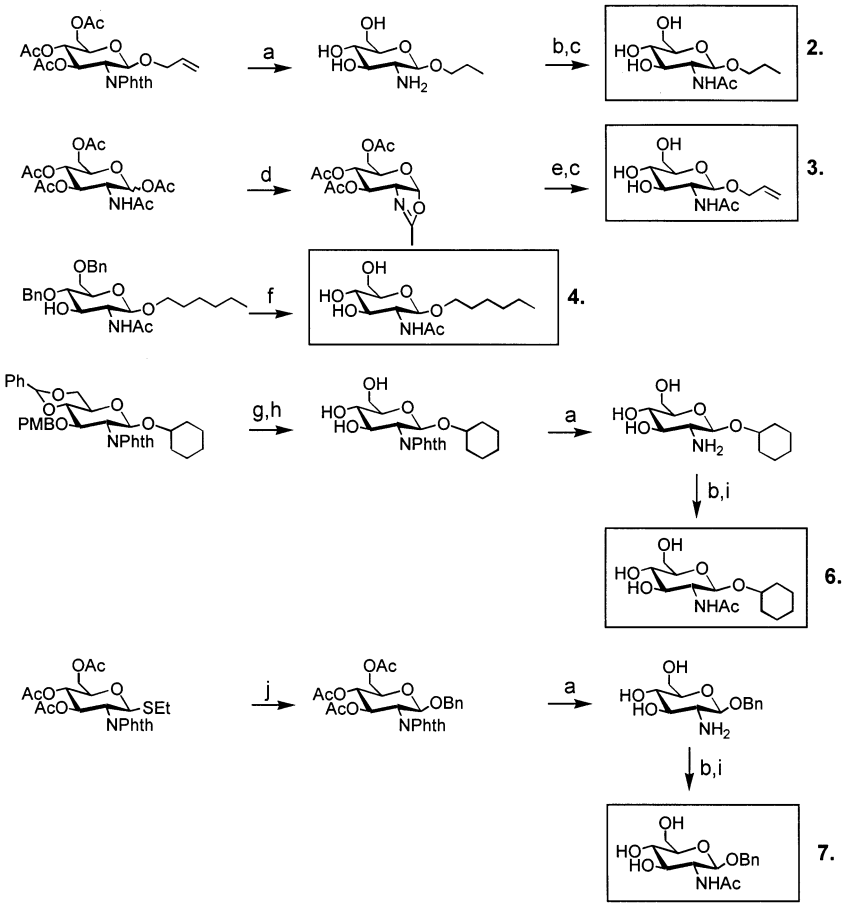
Chemical synthesis of GlcNAc derivatives.— In order to investigate the substrate specificity of NodC we have synthesized GlcNAc derivatives with a range of aglycones (**2**–**4**, **6**–**13**), and in which the N-linked substituent on C-2 is also varied (**14**, **15**). The structures of the resulting glycosides were verified using NMR spectroscopy and mass spectrometry. The spectra obtained demonstrated formation of the expected reaction products and the absence of starting materials.

The synthetic routes to compounds **2**–**4**, **6**, and **7** are depicted in Scheme 1. β-Propyl-containing monosaccharide **2** was prepared by hydrolysis of the phthaloyl group of allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (a gift of R. Lagas, Leiden University, Leiden, The Netherlands) followed by acetylation and selective deacetylation. Glycosylation of allyl alcohol with oxazoline [16] under the influence of Me_3SiOTf resulted, after deacetylation, in the β-allyl-containing derivative **3**. Hydrogenolysis of hexyl 2-acetamido-2-deoxy-4,6-di-*O*-benzyl-β-D-glucopyranoside (a gift of R. Lagas, Leiden University, Leiden, The Netherlands) under standard conditions gave **4**. Hexyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimodo-β-D-glucopyranoside was submitted to DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) and acid hydrolysis to give a triol, the phthaloyl of which was hydrolyzed. The resulting compound was completely acetylated and subsequently O-deacetylated to give the desired hexyl derivative **6**. The synthesis of compound **7** was achieved by N-iodosuccinomide (NIS)-assisted glycosylation of benzyl alcohol [17] followed by hydrolysis of the phthaloyl group, acetylation, and selective deacetylation. The synthesis of compound **8** (Scheme 2) started with the condensation of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranoside with a spacer molecule to give, after deacetylation, compound **11**. Hydrogenolysis of triol **11** resulted in the free amine-containing derivative **8**, which was N-substituted with a photo-affinity label yielding compound **10**. Derivative **9** was acquired following the same sequence of reactions starting with an N-protected tyramine derivative.

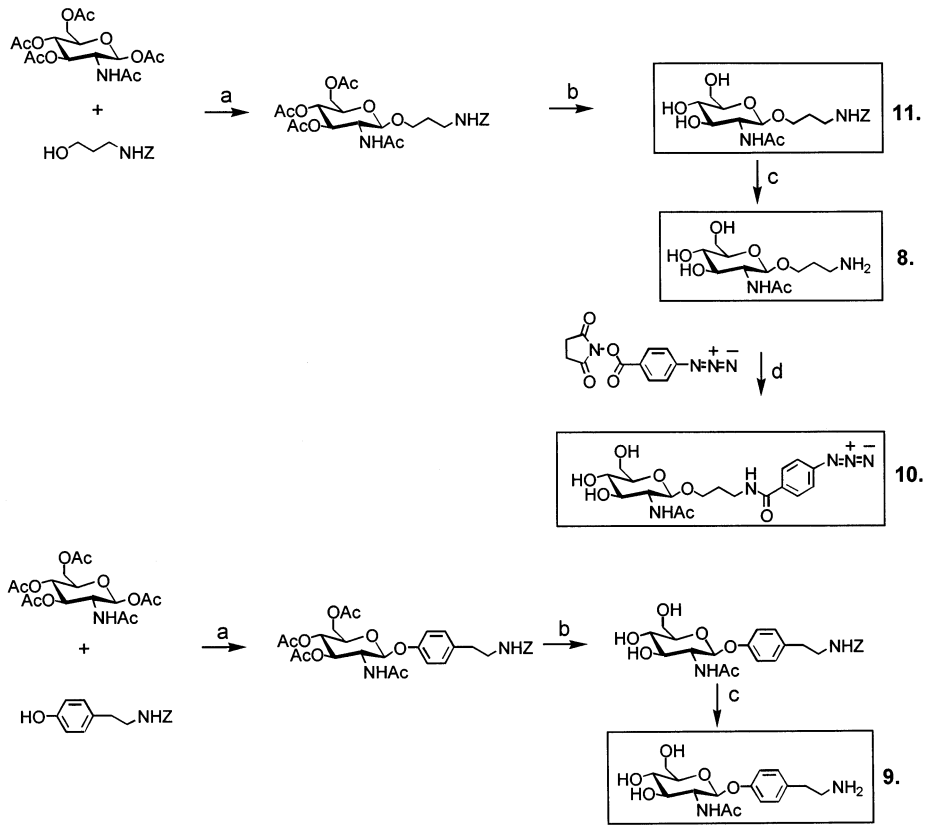
Synthesis of GlcNAc derivatives containing α-linked aglycones corresponding to compounds **2** and **3** is depicted in Scheme 3. Propyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**12**) was prepared by standard Fischer-glycosylation of *N*-benzyloxycarbonylamino-D-glucopyranoside (**15**) with allyl alcohol, followed by hydrogenolysis and N-acetylation. The α-allyl derivative **13** was obtained by Fischer-glycosylation of GlcNAc with allyl alcohol followed by acetylation, column chromatography, and subsequent selective deacetylation.

The influence of the substituent at the amino group of GlcNAc for recognition by NodC was investigated with compounds **14** and **15** (Scheme 3) having an allyloxy and a benzyloxy function, respectively. To obtain these compounds, glucosamine was treated with either allyl- or benzylchloroformate, followed by acetylation, purification, and selective deacetylation.

Use of chemically synthesized GlcNAc derivatives as acceptors by NodC.—GlcNAc derivatives were added to reaction mixtures containing membranes from an *E. coli* strain expressing the *M. loti* chitin oligosaccharide synthase NodC [18]. After incubation of these mixtures with UDP-[^{14}C]GlcNAc, reaction products were analyzed using thin-layer chromatography (TLC). Two factors indicate whether NodC uses the GlcNAc derivative as an acceptor: (i) the appearance of NodC-dependent spots that are more hydrophobic than free chitin oligosaccharides, and (ii) the absence of these reaction products when GlcNAc derivatives are not added to the reaction mixture, or when control preparations lacking NodC are used. A reduction in the formation of chitinpentaose, the natural major reaction



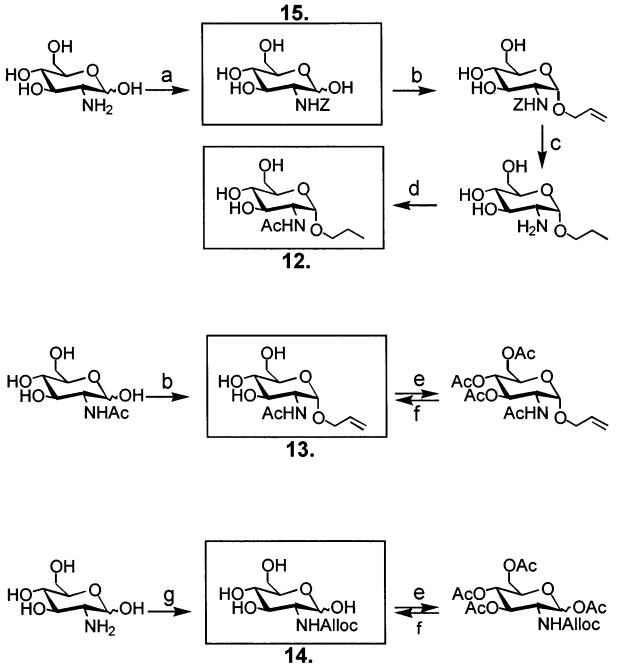
Scheme 1. Chemical synthesis of GlcNAc derivatives containing β-glycosidically linked aglycones. Reagents used were: (a) EtOH, $H_2NNH_2{\cdot}H_2O$ Δ; (b) pyridine, Ac_2O; (c) MeOH, KO*t*-Bu; (d) DCE, Me_3SiOTf, 50 °C; (e) DCE, AllOH, MS 4 Å, Me_3SiOTf; (f) 5:2:0.5 *i*PrOH–H_2O–AcOH, N_2, Pd/C, H_2; (g) 8:1 CH_2Cl_2–H_2O, DDQ; (h) AcCl, NEt_3; (i) 1:1 MeOH–THF, KO*t*-Bu; (j) DCE, THF, BnOH, MS 4 Å, NIS, Me_3SiOTf.



Scheme 2. Chemical synthesis of GlcNAc derivatives containing β-glycosidically linked nitrogen-containing aglycones. Reagents used were: (a) CH_3CN, MS 4 Å, $SnCl_4$ Δ; (b) MeOH, KO*t*-Bu; (c) 1:1 *i*PrOH–H_2O, N_2, Pd/C, H_2; (d) DMF, DIPEA.

product of *M. loti* NodC [18], relative to the amount of chitinpentaose formed in a corresponding control reaction lacking an exogenously added acceptor indicates that the GlcNAc derivative is bound by NodC, regardless of whether or not it acts as an acceptor. In control reactions, either water, methanol or dimethylsulfoxide (DMSO) was added since these are the solvents used for dissolving the GlcNAc derivatives. The results of this analysis are shown in Fig. 1 and summarized in Table 1. In the absence of exogenously added acceptors, the major product of *M. loti* NodC is chitinpentaose (Fig. 1, lanes labelled H_2O, MeOH, and DMSO). Surprisingly, the presence of methanol resulted in the appearance of a NodC-dependent spot (Fig. 1, lane MeOH) that is more hydrophobic and therefore migrates faster than GlcNAc and that was not detected in a standard reaction mixture lacking methanol (Fig. 1, lane H_2O).

Table 1 (panel A) shows the series of compounds bearing acyl chains of various lengths and hydrophobicity β-glycosidically linked to C-1 of GlcNAc. Compounds **8** and **9** both bear an aglycone terminating with a free amino group and do not seem to be used as primers by NodC. Judging from spot intensities (Fig. 1, lanes 8, 9, and H_2O) and the quantification of the radioactive counts using the PhosphorImager (data not shown), the level of chitinpentaose synthesized by NodC is not substantially altered by the presence of these GlcNAc derivatives, which indicates that they are probably not even bound by NodC. The other compounds in panel A all function as acceptors. Differences in hydrophobicity between these acceptors correlate with the differences in migration of their respective reaction products (Fig. 1). Due to the intense spot of GlcNAc it not always possible to see all expected reaction products (e.g. lanes 6, 7, and 10). The intense spots migrating between those for chitinpentaose and GlcNAc observed with compounds **1**, **2**, and **3** (Fig. 1) are likely to represent oligosaccharides synthesized using these GlcNAc derivatives as starter units, since these spots do not migrate with exactly



Scheme 3. Chemical synthesis of GlcNAc derivatives containing α-glycosides, or amide-bound substituents. Reagents used were: (a) H_2O, $NaHCO_3$, Z-Cl; (b) AllOH, AcCl; (c) 1:1 *i*PrOH–H_2O, Pd/C, H_2; (d) MeOH, Ac_2O; (e) pyridine, Ac_2O; (f) MeOH, KO*t*-Bu; (g) H_2O, $NaHCO_3$, AllocCl.

the same R_f values as free chitin oligosaccharides, and are absent when the GlcNAc derivatives are omitted from the reaction mixtures. The largest amount of reaction product was obtained with compounds **4**, **5**, **6**, **7**, **10**, and **11**. In all cases, the formation of free chitinpentaose was reduced in comparison with control reactions to which only solvent was added, particularly strongly with compounds **4**, **5**, **10**, and **11**. This indicates that these GlcNAc derivatives, which are the most hydrophobic, are more efficiently bound by NodC than the other compounds tested.

Next, we investigated whether NodC specifically uses acceptors with β-linked aglycones since the GlcNAc residues in chitin oligosaccharides are β-(1→4) glycosidically linked. The two hydrophobic aglycones that are β-glycosidically linked in glycosides **2** and **3** (Table 1, panel A) were therefore coupled in α-linkage to C1 of GlcNAc, resulting in compounds **12** and **13** (panel B). Elongation of the α-linked derivatives by NodC was not detectable (Fig. 1). The only apparent NodC-dependent spot in these samples is the methanol-dependent spot discussed above.

Table 1
Acceptor activity of NodC [a]

GlcNAc derivative		Use as acceptor by NodC
A		
1.	β–O–CH_3	+
2.	β–O–CH_2CH_3	+
3.	β–O–$CH_2CH{=}CH_2$	+
4.	β–O–$(CH_2)_5CH_3$	+ *
5.	β–O–$(CH_2)_8C(=O)OCH_3$	+ *
6.	β–O–cyclohexyl	+
7.	β–O–CH_2–phenyl	+
8.	β–O–$(CH_2)_2NH_2$	–
9.	β–O–phenyl–CH_2NH_2	–
10.	β–O–$(CH_2)_2$–N(C=O)–phenyl–$N^{\oplus}N^{\ominus}{\equiv}N$	+ *
11.	β–O–$(CH_2)_2$–N(C=O)–O–CH_2–phenyl	+ *
B		
12.	α–O–CH_2CH_3	–
13.	α–O–$CH_2CH{=}CH_2$	–
C		
14.	N(C=O)–O–$CH_2CH{=}CH_2$	+
15.	N(C=O)–O–CH_2–phenyl	±

[a] The scores are based on the TLC analysis shown in Fig. 1. The compounds are divided into categories A, B and C on the basis of the difference in conformational or positional linkage of the indicated substitions. With compounds that scored negatively (–) no detectable incorporation could be detected. The compounds scoring positively (+) as acceptor lead to the production of new metabolites. +/– indicates a marginal incorporation. Asterisks indicate compounds that are used as acceptors by NodC and in addition lead to a reduction in chitinpentaose formation by more than 90%, compared with control reactions to which only solvents were added. Compounds were tested at a concentration of 1 mM in the presence of 10 mM UDP-GlcNAc.

However, the intensity of the spot representing chitinpentaose in these cases is less than that in the control reaction (Fig. 1, lanes 12, 13, and MeOH), suggesting that compounds **12** and **13** may be bound by NodC even though they do not apparently function as primers. In contrast, the β-linked analogues **2** and **3** are clearly used as substrates by NodC

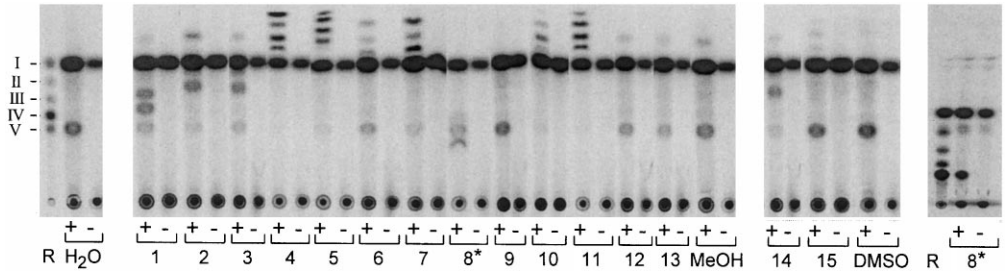


Fig. 1. NH_2-TLC analysis of NodC reaction products synthesized in the presence of hydrophobic GlcNAc derivatives as acceptors. Samples were taken from reaction mixtures with membranes containing NodC protein (+ lanes) or control membranes lacking NodC (− lanes). Control reactions were performed with NodC reaction mixtures to which the following solvents had been added: H_2O (solvent for compounds **8** and **9**), DMSO (solvent for compound **15**), or methanol (MeOH, solvent for the remaining GlcNAc derivatives). The numbers beneath the lanes correspond to the numbers of the GlcNAc derivatives listed in Table 1. Reactions were performed in the presence of 10 mM UDP-[^{14}C]GlcNAc. GlcNAc derivatives were added to a final concentration of 1 mM and the final solvent concentration was 5%. Lane R contains a standard mixture of purified, radiolabelled chitin-oligosaccharides prepared as described [18]. Roman numerals indicate the migration positions of GlcNAc (I) and chitin oligosaccharides ranging from chitinbiose (II) to chitinpentaose (V). The top of each chromatogram shown corresponds to the front of the mobile phase. *: The appearance of a spot that migrates slower than chitinpentaose in the initial experiment shown in the second panel from the left probably represents an artefact since it was not observed in later experiments, of which an example is shown in the right-hand panel.

(Fig. 1). NodC therefore appears to be specific for β-glycosides.

The chemical synthesis of two GlcNAc analogs in which the N-acetyl is replaced is shown in Scheme 3. In these derivatives **14** and **15** (Table 1, panel C) an allyloxy or benzyloxy moiety replaces the acetyl-CH_3 group. Both compounds are used as acceptors by NodC (Fig. 1), showing that the CH_3 of the N-acetyl group of GlcNAc is not essential for interaction with NodC. Glycoside **15** is clearly less efficiently bound by NodC than is compound **14**. This suggests that in order to be bound to NodC, the N-linked group on C-2 should not be very large.

3. Discussion

NodC is a bacterial chitin oligosaccharide synthase that is capable of using free GlcNAc and GlcNAc-1-*O*-*p*-nitrophenyl as acceptors [11]. It is, however, not known how chitin oligosaccharide synthesis is initiated in vivo. Previous results have indicated that prenyl-pyrophosphate carriers are not required [10,11]. In addition, NodC-dependent hydrophobic reaction products were not detected in vivo or in vitro in reaction mixtures following extraction with organic solvents [11]. The detection of a hydrophobic NodC-dependent product in reaction mixtures containing 5% methanol described in our present report is therefore an unexpected finding but can potentially be explained in three ways. First, methanol could release membrane components, which may then be used as glycosyl acceptor by NodC. A second possibility is that this spot corresponds to a compound that is also produced in the standard reaction mixture lacking methanol, but is now rendered soluble in the presence of methanol. The third possible explanation is that the presence of methanol somehow stimulates or reveals a modification of the reaction products of NodC by an endogenous *E. coli* enzyme. Structural analysis of this reaction product is required in order to distinguish between these possibilities.

The data reported here show that NodC does not display a strict specificity towards the structure of the aglycone in the GlcNAc derivatives that were used. Only those compounds that contain a free amino group in the aglycone are not acceptors. Their inability to act as acceptors is probably due to a low affinity of NodC for these compounds, rather than a reduction in the reaction velocity, since these GlcNAc derivatives do not inhibit the synthesis of free chitin oligosaccharides by NodC. The most hydrophobic GlcNAc derivatives were found to be used most efficiently as acceptors. This could be due to a higher affinity of NodC for these acceptors but could also be caused by an increased

accumulation of these compounds in membranes, leading to higher local concentrations in the vicinity of NodC.

There is a remarkable difference in the ratios of the oligosaccharides of different chain length that are produced in the presence of various GlcNAc derivatives. The most obvious explanation for this phenomenon is that the nature of the modifications in the GlcNAc analogue influence the binding affinity of NodC for the oligosaccharide intermediates and therefore leads to a different ratio between the kinetics of release of the end products and elongation with additional GlcNAc units. The fact that in the case of the incorporation of pNP-GlcNAc [11] there is no effect of varying concentrations of this compound on the relative quantities of the products with different chain lengths shows that this phenomenon cannot be explained by assuming a mechanism in which the GlcNAc analogs are incorporated as chain terminators. The simple rationale behind this is that, in such an alternative mechanism, an increasing concentration of chain terminators should lead to a relative increase in the production of shorter chain length products, which was not observed [11].

Compound **10** contains an azido group on the phenyl moiety linked to the reducing terminus of GlcNAc. Upon exposure to UV radiation, this azido group is known to become highly reactive towards compounds bearing the R–NH–R moiety, such as is found in the protein backbone, and results in the formation of a covalent bond. Since compound **10** is clearly used as an acceptor by NodC, it is expected to be a valuable tool for identifying the acceptor-binding site in this enzyme.

Until recently, synthetic glycosides have only been used to study non-processive glycosyltransferases. The results described here, however, clearly show that synthetic glycosides are also useful for assaying enzyme activity and investigating the substrate specificity of glycosyltransferases that act in a processive manner.

4. Experimental

General methods.—Toluene, 1,2-dichloroethane, and CH_2Cl_2 were distilled from P_2O_5. Methanol was dried by refluxing with magnesium methoxide, and subsequently distilled. Pyridine was refluxed for 18 h in the presence of calcium hydride and then distilled. Diethyl ether was distilled from $LiAlH_4$. Acetonitrile (p.a., Rathburne) was dried over 4 Å molecular sieves (Aldrich). Tetrahydrofuran (THF, p.a., E. Merck) was stored over 4 Å molecular sieves before use. Toluene and diethyl ether were stored over sodium wire. Methanol was stored over 3 Å molecular sieves. Dichloromethane and 1,2-dichloroethane were stored over 4 Å molecular sieves. Solvents used for column chromatography were of technical grade and distilled before use. Reactions were performed under anhydrous conditions at room temperature (rt), unless stated otherwise. Solvents were evaporated under reduced pressure at 40 °C. TLC analyses were conducted on Schleier & Schüll DC Fertigfolien (F 1500 LS 254). Compounds were visualized using UV light and charring with 1:4 H_2SO_4–ethanol. Those compounds that contain free amines were stained using a ninhydrin solution (0.3 g dissolved in a mixture of 3 mL AcOH and 100 mL EtOH). Column chromatography was performed on columns of silica gel (Baker, 0.063–0.200 nm). Petroleum ether used for elution of the columns was low-boiling (40–60 °C). Gel-filtration was performed on Sephadex LH20 (Pharmacia).

Structural analysis of synthetic GlcNAc derivatives.—^{13}C and ^{1}H NMR spectra were recorded using a Bruker DPX-300 spectrometer at 75 and 300 MHz, respectively. Chemical shifts are given in ppm using Me_4Si in $CDCl_3$ as internal reference. In addition, final reaction products were analyzed using mass spectrometry. Spectra were recorded on a Finnigan MAT TSQ-70 equipped with a custom-made electrospray interface (ESI).

Chemical synthesis of GlcNAc derivatives.—Compounds **1** and **5** were kind gifts from S.H. van Leeuwen (Leiden University, Leiden, The Netherlands) and M. Palcic (University of Alberta, Edmonton, Canada), respectively.

*Propyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**2**).—Starting material was allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside, a gift from R. Lagas (Leiden University, Leiden, The Netherlands). ^{13}C NMR ($CDCl_3$): δ 20.0, 20.2, 20.3 (CH_3 Ac), 54.2 (C-2), 61.6 (C-6), 68.6, 70.4, 71.4, (C-3, C-4, C-5), 69.8

(CH_2 Allyl), 96.7 (C-1), 117.5 (CH_2 Allyl), 123.2 (CH Phth), 130.9 (qC Phth), 132.9, 134.1 (CH Phth, CH Allyl), 169.2, 169.8, 170.4 (C=O Ac). Hydrazine monohydrate (0.5 mL, 10.3 mmol) was added to a solution of the glucopyranoside derivative (0.50 g, 1.1 mmol) in EtOH (96%, 30 mL). The reaction mixture was stirred under reflux for 17 h and subsequently cooled. The solids were filtered and the filtrate was concentrated. The crude amine-containing derivative (propyl 2-amino-2-deoxy-β-D-glucopyranoside) was dried by evaporation with toluene and dissolved in pyridine (5 mL). Acetic anhydride (2.0 mL, 21.3 mmol) was added to the solution and the reaction mixture was stirred for 20 h at rt. The reaction mixture was concentrated and the residue was taken up in EtOAc (15 mL). The resulting solution was washed with HCl (3%, 10 mL), water (10 mL), and $NaHCO_3$ (10%, 10 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated. The crude compound was purified by column chromatography (20–100% EtOAc in petroleum ether) to give the pure compound propyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (87%). ^{13}C NMR characterisation showed that the allyl group was converted to the propyl function. ^{13}C NMR ($CDCl_3$): δ 9.4 (CH_3 Propyl), 19.7 (CH_3 Ac), 21.8 (CH_3 NHAc), 22.0 (CH_2 Propyl), 53.7 (C-2), 61.7 (C-6), 68.5, 70.8, 72.2 (C-3, C-4, C-5), 70.9 (CH_2 Propyl), 100.1 (C-1), 169.2, 170.0, 170.4, 171.0 (C=O Ac). Potassium *tert*-butanolate (4 mg, 0.04 mmol) was added to a solution of this saccharide (354 mg, 0.91 mmol) in MeOH (4 mL). After stirring for 5 h, the reaction mixture was neutralized with Dowex 50W × 4 and subsequently filtered. The filtrate was concentrated and the pure product **2** was obtained in 86% yield after column chromatography (80% petroleum ether to 30% MeOH in EtOAc).

^{13}C NMR (CD_3OD): δ 10.7 (CH_3 Propyl), 23.0 (CH_3 NHAc), 23.4 (CH_2 Propyl), 56.9 (C-2), 62.3 (C-6), 71.6, 75.5, 77.2 (C-3, C-4, C-5), 71.6 (CH_2 Propyl), 102.2 (C-1), 173.7 (C=O NHAc). ^1H NMR (CD_3OD): δ 0.91 (t, 3 H, CH_3 Propyl, $J_{H,H}$ 7.3 Hz), 1.51–1.61 (m, 2 H, CH_2 Propyl), 1.97 (s, 3 H, CH_3 NHAc), 3.22–3.91 (m, 8 H, H-2, H-3, H-4, H-5, H-6, CH_2 Propyl), 4.39 (d, 1 H, H-1, $J_{1,2}$ 8.04 Hz). MS: $[M + Na]^+$ for $C_{11}H_{21}NO_6$: m/z 286.0.

Allyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**3**).—The fresh oxazoline, prepared as described [16] (937 mg, 3.0 mmol) was dried by evaporation with toluene and subsequently dissolved in dichloroethane (15 mL). According to Refs. [19,20], allyl alcohol (0.61 mL, 9.0 mmol) and 4 Å molecular sieves were added and the resulting mixture was stirred at rt for 30 min. Trimethylsilyl trifluoromethanesulfonate (0.115 mL, 0.59 mmol) was added and the reaction mixture was stirred for 19 h. The reaction mixture was neutralized with Et_3N. The solids were filtered and the filtrate was concentrated. The reaction product was a 2:1 mixture of oxazoline and product. The pure product allyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside was obtained in 21% yield after column chromatography (50–100% EtOAc in petroleum ether). ^{13}C NMR ($CDCl_3$): δ 20.2 (CH_3 Ac), 22.7 (CH_3 NHAc), 53.9 (C-2), 62.0 (C-6), 68.7, 71.1, 72.2, (C-3, C-4, C-5), 69.6 (CH_2 allyl), 99.4 (C-1), 117.0 (CH_2 allyl), 133.4 (CH allyl), 169.1, 170.2, 170.3, 170.6 (C=O Ac). The acetylated sugar (245 mg, 0.63 mmol) was dissolved in MeOH (6.0 mL) and potassium *tert*-butanolate (7 mg, 0.06 mmol) was added. The solution was stirred for 5 h. The reaction mixture was neutralized with Dowex 50W × 4 and the solids were filtered. The filtrate was concentrated and the residue was purified by column chromatography (0–30% MeOH in EtOAc) to give the pure product **3** in 74% yield.

^{13}C NMR (CD_3OD): δ 23.0 (CH_3 NHAc), 56.8 (C-2), 62.4 (C-6), 70.5 (CH_2 allyl), 71.7, 75.6, 77.3, (C-3, C-4, C-5), 101.4 (C-1), 117.1 (CH_2 allyl), 135.0 (CH allyl), 173.5 (C=O NHAc). ^1H NMR (CD_3OD): δ 1.97 (s, 3 H, CH_3 NHAc), 3.24–4.39 (m, 8 H, H-2, H-3, H-4, H-5, H-6, CH_2 Allyl), 4.43 (d, 1 H, H-1, $J_{1,2}$ 8.0 Hz), 5.04–5.31 (m, 2 H, CH_2 Allyl), 5.79–5.98 (m, 1 H, CH Allyl). MS: $[M + Na]^+$ for $C_{11}H_{19}NO_6$: m/z 284.1

Hexyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**4**).—Starting material was hexyl 2-acetamido-2-deoxy-4,6-di-*O*-benzyl-β-D-glucopyranoside, a gift from R. Lagas (Leiden University, Leiden, The Netherlands). ^{13}C

NMR ($CDCl_3$): δ 13.9 (CH_3 Hexyl), 23.4 (CH_3 NHAc), 22.5, 25.6, 29.4, 31.5 ($4 \times CH_2$ Hexyl), 58.1 (C-2), 69.0, 69.5 (C-6, CH_2 Hexyl), 74.9, 75.6, 78.3 (C-3, C-4, C-5), 100.2 (C-1), 127.5, 127.7, 128.0, 128.2 (CH Bn), 138.1, 138.3 (qC Bn), 172.2 (C=O NHAc). The benzylated glycoside (200 mg, 0.41 mmol) was dissolved in a 5:2:0.5 mixture of isopropanol–water–AcOH (5 mL). After stirring for 30 min under bubbling nitrogen, palladium on charcoal was added and hydrogen was bubbled through for 3 h. The hydrogen was removed by bubbling nitrogen and subsequently, the palladium was filtered over a bed of hyflo. The filtrate was concentrated and the residue was purified by silica gel chromatography (0–40% MeOH in EtOAc). The final product **4** was obtained in 76% yield.

^{13}C NMR ($CDCl_3$): δ 14.3 (C-6 hexyl), 23.0 (CH_3 NHAc), 23.4, 26.4, 30.3, 32.4 ($4 \times CH_2$ hexyl), 56.9 (C-2), 62.4 (C-6), 70.5 (C-1 hexyl), 71.7, 75.6, 77.3 (C-3, C-4, C-5), 102.3 (C-1), 173.5 (C=O NHAc).

*Cyclohexyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**6**).—Starting material was cyclohexyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranoside, a gift from R. Lagas (Leiden University, Leiden, The Netherlands). ^{13}C NMR ($CDCl_3$): δ 23.3, 23.6, 25.2, 31.3, 33.0 ($5 \times CH_2$ cyclohexyl), 54.7, 56.0 (C-2, CH_3 PMB), 68.7 (C-6), 65.9, 74.1, 77.1, 82.9 (C-3, C-4, C-5, CH cyclohexyl), 97.1, 100.6 (C-1, CH CHPh), 113.2 (CH PMB), 122.9 (CH Phth), 125.9, 128.1, 128.5, 129.6 (CH Ar), 130.0 (qC PMB), 131.4 (qC Phth), 133.6 (CH Phth), 137.3 (qC Ar), 158.7 (qC PMB). The protected saccharide (0.83 g, 1.38 mmol) was dissolved in an 8:1 mixture of CH_2Cl_2–water (7.0 mL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.47 g, 2.1 mmol) was added at rt [21]. The reaction mixture was stirred for 1 h, then the solids were filtered over a path of hyflo. The filtrate was diluted with CH_2Cl_2 (10 mL). The organic solution was washed with water (10 mL) and subsequently with $NaHCO_3$ (10%, 10 mL, twice), dried ($MgSO_4$), filtered, and concentrated. The crude product cyclohexyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside was used without further purification. ^{13}C NMR ($CDCl_3$): δ 23.2, 23.5, 25.1, 31.2, 33.0 ($5 \times CH_2$ cyclohexyl), 56.9 (C-2), 68.6 (C-6), 66.0, 68.4, 77.1, 82.0 (C-3, C-4, C-5, CH cyclohexyl), 97.1, 101.6 (C-1, CH CHPh), 123.2 (CH Phth), 126.2, 128.1, 129.0 (CH Ar), 131.4 (qC Phth), 133.9 (CH Phth), 137.0 (qC CHPh). According to [22], acetyl chloride (0.11 mL, 1.5 mmol) was added to a solution of this crude monosaccharide in a 1:1 mixture of MeOH–CH_2Cl_2 (14 mL) at 0 °C. The ice-bath was removed and the reaction was stirred for 1.5 h at rt. The reaction mixture was neutralized with Et_3N and the solvents were evaporated. The residue was purified by column chromatography (30–100% EtOAc in petroleum ether) to give the pure cyclohexyl 2-deoxy-2-phthalimido-β-D-glucopyranoside in 71% yield over two steps. ^{13}C NMR (CD_3OD): δ 24.1, 24.4, 26.4, 32.2, 34.0 ($5 \times CH_2$ cyclohexyl), 58.6 (C-2), 62.6 (C-6), 72.5, (d.i.), 77.3, 78.0 (C-3, C-4, C-5, CH cyclohexyl), 97.6 (C-1), 124.1 (CH Phth), 132.8 (qC Phth), 135.5 (CH Phth). This triol (370 mg, 0.98 mmol) was dissolved in EtOH (96%, 30 mL) and heated at reflux temperature for 18 h in the presence of hydrazine monohydrate (0.5 mL, 10.3 mmol) as described [23,24]. The solution was cooled to rt and a white precipitate was formed. The phthaloyl salts were removed by filtration and washed with EtOH. The filtrate was concentrated to give the crude amine, which was dried by evaporation with toluene. The dry residue was dissolved in pyridine (5 mL) and Ac_2O (1.0 mL, 10.6 mmol) was added. After stirring for 18 h, the solution was concentrated and once rotory-evaporated with toluene. The residue was redissolved in EtOAc (10 mL) and washed with HCl (3%, 10 mL), water (10 mL), and $NaHCO_3$ (10%, 10 mL). The organic solution was dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by column chromatography (10–80% EtOAc in petroleum ether). Concentration of the appropriate fractions gave the desired derivative in 81% yield. ^{13}C NMR ($CDCl_3$): δ 20.3 (CH_3 Ac), 22.7 (CH_3 NHAc), 23.4, 23.4, 25.1, 31.2, 32.8 ($5 \times CH_2$ cyclohexyl), 54.6 (C-2), 62.1 (C-6), 69.0, 71.0, 72.2, 77.2 (C-3, C-4, C-5, CH cyclohexyl), 98.7 (C-1), 169.1, 170.1, 170.3 (C=O Ac). To a solution of the fully acetylated sugar (328 mg, 0.79

mmol) in a 1:1 mixture of MeOH–THF (8 mL) was added potassium *tert*-butanolate (18 mg, 0.16 mmol). The mixture was stirred for 18 h, neutralized with Dowex 50W × 4, filtered, and concentrated. Purification of the residue was achieved by silica gel column chromatography. The column was eluted with a gradient of 0–30% MeOH in EtOAc to yield the product **6** in 59% yield.

^{13}C NMR (CD_3OD): δ 23.1 (CH_3 NHAc), 24.4, 24.5, 26.5, 32.3, 34.2 (5 × CH_2 cyclohexyl), 57.5 (C-2), 62.6 (C-6), 71.9, 75.6, 77.4, 77.7 (C-3, C-4, C-5, CH cyclohexyl), 100.6 (C-1), 173.4 (C=O NHAc). MS: $[M + Na]^+$ for $C_{14}H_{25}NO_5$: m/z 326.1.

*Benzyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**7**).—The thio-ethyl donor (480 mg, 1.0 mmol) [17] was dried by evaporation with toluene and subsequently dissolved in 1,2-dichloroethane (16 mL). Benzyl alcohol (0.21 mL, 2.0 mmol) was added and the mixture was stirred in the presence of 4 Å molecular sieves under a blanket of argon for 30 min. The mixture was cooled with an ice-bath and a solution of *N*-iodosuccinimide (270 mg, 1.2 mmol) and trimethylsilyl trifluoromethanesulfonate (33 mL, 0.17 mmol) in THF (8 mL) was added. The ice-bath was removed and the reaction mixture was stirred for 6 h at rt. The reaction mixture was neutralized with Et_3N (0.3 mL) and filtered over a path of hyflo. The filtrate was diluted with EtOAc (20 mL) and washed with $Na_2S_2O_3$ (1 M, 15 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. The pure product benzyl 2-phthalimido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside was obtained in 90% yield after column chromatography (0–40% EtOAc in petroleum ether).

^{13}C NMR ($CDCl_3$): δ 19.8, 20.0, 20.2 (CH_3 Ac), 54.1 (C-2), 61.5 (C-6), 68.5, 70.1, 71.4 (C-3, C-4, C-5), 71.3 (CH_2 Bn), 96.7 (C-1), 123.0 (CH Phth), 127.3, 127.7 (CH Bn), 130.8 (qC Phth), 133.8 (CH Phth), 136.2 (qC Bn), 166.9 (C=O Phth), 169.0, 169.5, 170.1 (C=O Ac).

^{1}H NMR ($CDCl_3$): δ 1.86, 2.03, 2.14 (3 × s, 3 H, CH_3 Ac), 3.40–3.55 (m, 1 H, H-2), 3.87 (ddd, 1 H, H-5, $J_{5,4}$ 10.2 Hz, $J_{5,6}$ 2.2 Hz, $J_{5,6}$ 4.4 Hz), 4.19 (dd, 1 H, H-6, $^2J_{6,6}$ 12.4 Hz, $J_{6,5}$ 2.2 Hz), 4.31–4.39 (m, 1 H, H-6), 4.69 (AB, CH_2 Bn), 5.19 (t, 1 H, H-3/H-4, $J_{H,H}$ 9.5 Hz), 5.38 (d, 1 H, H-1, $J_{1,2}$ 8.8 Hz), 5.79 (dd, 1 H, H-3/H-4, $J_{H,H}$ 9.1 Hz, $J_{H,H}$ 10.6 Hz), 7.06–7.13 (m, 5 H, CH Ar), 7.70–7.77 (m, 4 H, CH Ar). The acetylated saccharide (788 mg, 1.5 mmol) was dissolved in EtOH (96%, 45 mL) and hydrazine hydrate (0.73 mL, 15.0 mmol) was added. The resulting solution was heated under reflux for 18 h and subsequently cooled to rt. The solids were filtered and the filtrate was concentrated to dryness. The residue was roto-evaporated with toluene and dissolved in pyridine (8.0 mL) and acetic anhydride (4.0 mL, 42.3 mmol). The reaction was stirred at rt for 21 h and then concentrated. The residue was redissolved in EtOAc (20 mL) and washed with HCl (3%, 15 mL), water (15 mL), and $NaHCO_3$ (10%, 15 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by column chromatography (20–100% EtOAc in petroleum ether) to give the pure product benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside in 81% yield. ^{13}C NMR ($CDCl_3$): δ 20.3 (CH_3 Ac), 22.7 (CH_3 NHAc), 54.0 (C-2), 62.0 (C-6), 68.6, 71.4, 72.3 (C-3, C-4, C-5), 70.4 (CH_2 Bn), 99.4 (C-1), 127.6, 128.1 (CH Bn), 136.8 (qC Bn), 169.3, 170.5 (C=O Ac). To a solution of the acetylated derivative (262 mg, 0.5 mmol) dissolved in 1:1 THF–MeOH (5 mL) was added potassium *tert*-butanolate (6 mg, 0.05 mmol). After stirring for 4 h, the reaction mixture was neutralized with Dowex 50W × 4, filtered and the solvents were evaporated. The residue was purified by column chromatography (0–20% MeOH in EtOAc) to give the pure compound **7** (72%).

^{13}C NMR (CD_3OD): δ 23.0 (CH_3 NHAc), 57.3 (C-2), 62.9 (C-6), 71.5 (CH_2 Bn), 72.2, 77.9, 78.0 (C-3, C-4, C-5), 101.8 (C-1), 128.8, 129.3 (CH Bn). MS: $[M + H]^+$ for $C_{15}H_{21}NO_6$: m/z 334.1.

*3-Aminopropyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**8**).—The monosaccharide donor 1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranoside [25] (778 mg, 2.0 mmol) and 3-(benzyloxycarbonylamino)propanol [26] (1.0 g, 5.0 mmol) were dried by evaporation of toluene and dissolved in MeCN (20 mL) according to Ref. [27]. 4 Å molecular sieves were

added and the mixture was stirred for 30 min at rt under a blanket of argon. Tin tetrachloride (0.28 mL, 2.4 mmol) was added and the reaction mixture was heated at reflux temperature for 18 h. The reaction mixture was cooled, diluted with EtOAc (20 mL), and the solids were filtered over a path of hyflo. The filtrate was washed with KF (1 M, 15 mL) and water (15 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (30% petroleum ether → 10% MeOH in EtOAc) to yield the pure product 3-(benzyloxycarbonylamino)propyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside in 78% yield. ^{13}C NMR ($CDCl_3$): δ 20.3, 20.6 (CH_3 Ac), 22.7 (CH_3 NHAc), 29.3, 36.7 ($2 \times CH_2$ Spacer), 53.7 (C-2), 61.8 (C-6), 66.2, 66.4 (CH_2 Spacer, Z), 68.4, 71.4, 72.9 (C-3, C-4, C-5), 100.9 (C-1, $^1J_{C,H}$ 161.8 Hz), 127.3, 127.7, 128.2 (CH Z), 136.6 (qC Z), 156.6 (C=O Z), 169.0, 170.3, 170.6 (C=O Ac, NHAc). To a solution of this acetylated monosaccharide (839 mg, 1.6 mmol) in MeOH (16 mL), potassium *tert*-butanolate (18 mg, 0.16 mmol) was added. After stirring for 4 h, TLC analysis showed the reaction to be complete. The reaction mixture was neutralized with Dowex 50W × 4 and the solids were filtered. The filtrate was concentrated and the residue was purified by column chromatography (0–30% MeOH in EtOAc) to yield the desired compound 3-(benzyloxycarbonylamino)propyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**11**, Scheme 2) (76%). ^{13}C NMR (CD_3OD): δ 23.1 (CH_3 NHAc), 30.5, 38.5 ($2 \times CH_2$ Spacer), 57.0 (C-2), 62.5 (C-6), 66.9, 67.6 (CH_2 Spacer, Z), 71.7, 75.9, 77.4 (C-3, C-4, C-5), 102.3 (C-1), 128.5, 128.8, 129.3 (CH Z), 138.0 (qC Z), 158.5 (C=O Z), 173.8 (C=O NHAc). ^1H NMR (CD_3OD): δ 1.68–1.78 (m, 2 H, CH_2 spacer), 1.98 (s, 3 H, CH_3 NHAc), 3.15–3.95 (m, 10 H, H-2, H-3, H-4, H-5, H-6, $2 \times CH_2$ spacer), 4.33 (d, 1 H, $J_{1,2}$ 8.8 Hz), 5.06 (s, 2 H, CH_2 Z), 7.31–7.33 (m, 5 H, CH Z). The spacer-containing monosaccharide (507 mg, 1.23 mmol) was dissolved in a 1:1 mixture of isopropanol–water (10 mL). The solution was de-aired by nitrogen and subsequently palladium on charcoal was added. Hydrogen was bubbled through the reaction mixture for 2 h. Then, the reaction mixture was placed under nitrogen for 20 min and subsequently the solids were filtered over a path of hyflo. The filtrate was concentrated and purified by column chromatography (0–100% MeOH in EtOAc in the presence of 1% Et_3N) to give the unprotected product **8** (78%).

4-(Aminoethyl)phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**9**).—The monosaccharide 1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranoside (389 mg, 1.0 mmol) and the Z-protected tyramine [28] (542 mg, 2.0 mmol) were coupled according to Ref. [27]. Starting materials were dried by evaporation with toluene. The dry components were dissolved in MeCN (10 mL) and stirred in the presence of 4 Å molecular sieves under a blanket of argon for 30 min. Tin tetrachloride (0.14 mL, 1.2 mmol) was added and the resulting mixture was heated at reflux temperature for 18 h. The reaction mixture was cooled down and subsequently filtered over a path of hyflo. The filtrate was diluted with EtOAc (10 mL) and subsequently washed with KF (1 M, 10 mL) and water (10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. The pure product 4-[2-(benzyloxycarbonylamino)-ethyl]phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside was obtained in 51% yield after column chromatography (50–100% EtOAc in petroleum ether). ^{13}C NMR ($CHCl_3$): δ 20.3 (CH_3 Ac), 22.5 (CH_3 NHAc), 34.9, 42.1 ($2 \times CH_2$ Tyramine), 54.0 (C-2), 61.9 (C-6), 66.3 (CH_2 Z), 68.5, 71.5, 72.1 (C-3, C-4, C-5), 98.7 (C-1), 116.8, 127.8, 128.2, 128.3, 129.5 (CH Ar), 133.2 (qC Ar), 155.5 (C=O Z), 169.4, 170.5, 171.2 (C=O Ac, NHAc). ^1H NMR ($CHCl_3$): δ 2.00, 2.03, 2.04, 2.05 ($4 \times CH_3$ Ac, NHAc), 2.73 (t, 2 H, CH_2 Tyramine, $J_{H,H}$ 6.9 Hz), 3.38 (q, 2 H, CH_2 Tyramine, $J_{H,H} - J_{H,NH}$ 6.9 Hz), 3.77–3.85 (m, 1 H, H-5), 4.06–4.20 (m, 2 H, H-2, H-6), 4.27 (dd, 1 H, H-6, $^2J_{6,6}$ 12.4 Hz, $J_{6,5}$ 5.1 Hz), 5.07 (s, 2 H, CH_2 Z), 5.12 (t, 1 H, H-3/H-4, $J_{H,H}$ 9.9 Hz), 5.24 (d, 1 H, H-1, $J_{1,2}$ 8.8 Hz), 5.40 (t, 1 H, H-3/H-4, $J_{H,H}$ 9.9 Hz), 6.79–7.36 (m, 9 H, CH Ar). To a solution of this monosaccharide (306 mg, 0.51 mmol) in MeOH (5 mL) was added potassium *tert*-butanolate (6 mg, 0.05 mmol). After stirring for 4 h, TLC analysis showed the reaction to be complete. The

reaction mixture was neutralized with Dowex 50W × 4 and the solids were filtered. The filtrate was concentrated and the residue was redissolved in 1:1 isopropanol–water (10 mL). The solution was de-aired by nitrogen and palladium on charcoal was added. Hydrogen was bubbled through the reaction mixture for 2 h. The reaction mixture was placed under nitrogen for 20 min and the solids were subsequently filtered over a path of hyflo. The filtrate was concentrated and purified by column chromatography (0–100% MeOH and 1% Et_3N in EtOAc) to yield the tyramine-containing monosaccharide **9** in 65% yield.

^{13}C NMR (CD_3OD): δ 23.0 (CH_3 NHAc), 36.4, 43.1 (2 × CH_2 Tyramine), 57.2 (C-2), 62.4 (C-6), 71.7, 75.5, 78.1 (C-3, C-4, C-5), 101.0 (C-1), 117.9, 130.8 (CH Tyramine), 133.5 (qC Tyramine), 157.8 (qC Tyramine), 173.9 (C=O NHAc).

^1H NMR (CD_3OD): δ 1.98 (s, 3 H, CH_3 NHAc), 2.69–3.94 (m, 10 H, H-2, H-3, H-4, H-5, H-6, 2_ CH_2 Tyramine), 5.01 (d, 1 H, H-1, $J_{1,2}$ 8.4 Hz), 6.97 (d, 2 H, CH Tyramine, $J_{H,H}$ 8.8 Hz), 7.16 (d, 2 H, CH Tyramine, $J_{H,H}$ 8.8 Hz). MS: $[M+H]^+$ for $C_{16}H_{24}N_2O_6$: m/z 341.1.

1-O-*[*N-*(4-Azidobenzoyl)-3-amino]propyl-2-acetamido-2-deoxy-β*-D-*glucopyranoside* (**10**). —Compound **8** (5 mg, 18 mmol) was dissolved in DMF (0.5 mL) and subsequently the photoaffinity label succinimidyl 4-azidobenzoic acid (5 mg, 19 mmol) and di-*iso*-propylethylamine (6.3 mL, 36 mmol) were added. The reaction mixture was stirred for 55 h under exclusion of light. Then the reaction mixture was concentrated and the residue was rotory-evaporated with toluene and tested without further purification.

*Propyl 2-acetamido-2-deoxy-α-*D*-glucopyranoside* (**12**).—Glucosamine-hydrochloride (13.6 g, 63.1 mmol) was dissolved in water (100 mL) and $NaHCO_3$ (13.0 g, 155.0 mmol) was added. The solution was cooled in an ice-bath and benzyl chloroformate (15.0 mL, 106.0 mmol) was added in portions. The solution was stirred for 17 h at 4 °C until it became a suspension. The product (2-benzyloxycarbonylamino-2-deoxy-D-glucopyranoside, **15**, Scheme 3) was filtered, washed with EtOAc and dried (88%).^{13}C NMR (1:1 CD_3OD–DMSO): δ 57.6, 60.1 (C-2), 62.6 (C-6), 72.1, 72.3, 73.2, 75.7, 77.9 (C-3, C-4, C-5), 92.5, 97.0 (C-1), 129.1, 129.6 (CH Z), 138.5 (qC Z), 158.0 (C=O Z). A solution of this monosaccharide (1.57 g, 5.0 mmol) in allyl alcohol (40 mL) containing acetyl chloride (2%, 0.8 mL) was heated at reflux temperature for 43 h. The reaction mixture was neutralized with Et_3N and subsequently concentrated. The crude product allyl 2-benzyloxycarbonylamino-2-deoxy-D-glucopyranose was purified by column chromatography (80–100% EtOAc in petroleum ether) to give first the α anomer in 55% yield. Further elution of the column with 10% MeOH in EtOAc gave the β anomer in 6% yield. Allyl 2-acetamido-2-deoxy-α-D-glucopyranoside ^{13}C NMR (CD_3OD): δ 58.2 (C-2), 63.7 (C-6), 68.5 (CH_2 Z), 70.1 (CH_2 Allyl), 73.2, 73.9, 74.8 (C-3, C-4, C-5), 98.9 (C-1, $^1J_{C,H}$ 169.4 Hz), 118.5 (CH_2 Allyl), 129.8, 130.4 (CH Z), 136.4 (CH Allyl), 142.5 (qC Z), 159.8 (C=O Z). Allyl 2-benzyloxycarbonylamino-2-deoxy-β-D-glucopyranoside ^{13}C NMR (CD_3OD): δ 58.9 (C-2), 62.7 (C-6), 67.3 (CH_2 Z), 70.8 (CH_2 Allyl), 72.1, 75.8, 77.8 (C-3, C-4, C-5), 102.2 (C-1, $^1J_{C,H}$ 160.2), 117.0 (CH Allyl), 128.8, 129.4 (CH Z), 135.5 (CH_2 Allyl).

The α-linked monosaccharide (200 mg, 0.57 mmol) was dissolved in a 1:1 mixture of isopropanol–water (10 mL). The solution was de-aired with nitrogen and subsequently palladium on charcoal was added. Hydrogen was bubbled trough the mixture for 30 min. The solution was satured with nitrogen and subsequently the solids were filtered through a bed of hyflo. The filtrate was concentrated and the residue was dried by evaporation with toluene. The crude product was redissolved in MeOH (29 mL) and the solution was cooled with an ice-bath. Acetic anhydride (0.91 mL, 9.6 mmol) was added, and the solution was stirred for 45 min at 0 °C. The reaction mixture was concentrated and purified by column chromatography. Elution of the column with 20% petroleum ether to 50% MeOH in EtOAc gave the pure propyl-containing product **12** in 84% yield.

^{13}C NMR (CD_3OD): δ 11.0 (CH_3 Propyl), 22.6 (CH_3 NHAc), 23.6 (CH_2 Propyl), 55.4 (C-2), 62.6 (C-6), 70.5 (CH_2 Propyl), 72.3,

72.7, 73.6 (C-3, C-4, C-5), 98.3 (C-1, $^1J_{C,H}$ 169.4 Hz), 173.6 (C=O NHAc). ^{1}H NMR (CD_3OD): δ 0.96 (t, 3 H, 7.3 Hz, CH_3 Propyl), 1.57–1.71 (m, 2 H, CH_2 Propyl), 1.98 (s, 3 H, CH_3 NHAc), 3.30–3.84 (m, 8 H, H-2, H-3, H-4, H-5, H-6, CH_2 Propyl), 4.78 (d, 1 H, H-1, $J_{1,2}$ 3.7 Hz, H-1). MS: $[M + Na]^+$ for $C_{11}H_{21}NO_6$: m/z 286.0.

*Allyl 2-acetamido-2-deoxy-α-*D*-glucopyranoside* (**13**).—GlcNAc (664 mg, 3.0 mmol) was dissolved in allyl alcohol (24 mL). Acetyl chloride (2%, 0.48 mL) was added and the resulting solution was stirred for 20 h at reflux temperature. Then, the reaction mixture was neutralized with Et_3N and concentrated. The crude product was dried by evaporation with toluene and dissolved in pyridine (10 mL) and Ac_2O (5 mL, 53.2 mmol). After stirring for 18 h the mixture was concentrated and evaporated with toluene. The residue was taken up in EtOAc (30 mL) and subsequently washed with HCl (3%, 25 mL), water (25 mL) and $NaHCO_3$ (10%, 25 mL). The resulting organic layer was dried ($MgSO_4$), filtered and concentrated. The pure product allyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside was obtained by column chromatography (10–50% EtOAc in petroleum ether) in 67% yield. ^{13}C NMR ($CDCl_3$): δ 20.0 (CH_3 Ac), 22.1 (CH_3 NHAc), 51.1 (C-2), 61.5 (C-6), 67.2, 67.8, 70.5, (C-3, C-4, C-5), 68.1 (CH_2 Allyl), 95.9 (C-1, $^1J_{C,H}$ 170.9 Hz), 117.6 (CH_2 Allyl), 132.8 (CH Allyl), 168.8, 170.3, 170.4, (C=O Ac), 173.3 (C=O NHAc). Potassium *tert*-butanolate (17 mg, 0.15 mmol) was added to a solution of the acetylated compound (580 mg, 1.5 mmol) in MeOH (15 mL). After stirring for 5 h, the reaction mixture was neutralized with Dowex 50W × 4 and the solids were filtered. The filtrate was concentrated and the crude product was purified by silica gel chromatography (0–40% MeOH in EtOAc) to give the pure α-glycoside **13** in 73% yield.

^{13}C NMR (CD_3OD): δ 22.6 (CH_3 NHAc), 55.2 (C-2), 62.5 (C-6), 69.0 (CH_2 Allyl), 70.6, 72.1, 72.6, (C-3, C-4, C-5), 97.5 (C-1), 117.5 (CH_2 Allyl), 135.3 (CH Allyl), 173.5 (C=O NHAc). ^{1}H NMR (CD_3OD): δ 1.98 (s, 3 H, CH_3 NHAc), 3.31–4.29 (m, 8 H, H-2, H-3, H-4, H-5, CH_2 Allyl), 4.83 (d, 1 H, H-1, $J_{1,2}$ 3.4 Hz), 5.01–5.36 (m, 2 H, CH_2 Allyl), 5.84–6.01 (m, 1 H, CH Allyl). MS: $[M + Na]^+$ for $C_{11}H_{19}NO_6$: m/z 284.1.

*2-Allyloxycarbonylamino-2-deoxy-*D*-glucopyranose* (**14**).—Allyl chloroformate (0.9 mL, 8.4 mmol) was added in portions to a cooled (0 °C) solution of glucosamine-hydrochloride (1.1 g, 5 mmol) and $NaHCO_3$ (420 mg, 12.3 mmol) in water (100 mL). The solution was stirred for 17 h at 4 °C. Then, the solution was concentrated and the residue was dried by evaporation with toluene. The crude product was dissolved in pyridine (15 mL) and Ac_2O (3.8 mL, 40 mmol) was added. After stirring for 19 h, the reaction mixture was concentrated and co-evaporated with toluene. The residue was taken up in EtOAc (50 mL) and washed with HCl (3%, 40 mL), water (40 mL), and $NaHCO_3$ (10%, 40 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by column chromatography (30–80% EtOAc in petroleum ether) to yield the acetylated product 1,3,4,6-tetra-*O*-acetyl-2-allyloxycarbonylamino-2-deoxy-D-glucopyranose in 72% yield. ^{13}C NMR ($CDCl_3$): δ 19.9, 20.1 (CH_3 Ac), 52.1, 54.0 (C-2), 61.0 (C-6), 65.1 (CH_2 Alloc), 67.4, 69.0, 70.0, 67.7, 71.8 (C-3, C-4, C-5), 90.1, 91.8 (C-1), 117.0 (CH Alloc), 132.1 (CH_2 Alloc), 155.3 (C=O Alloc), 168.3, 168.7, 169.9, 170.3 (C=O Ac).

^{1}H NMR ($CDCl_3$): δ showed that the major compound in this anomeric mixture was the α anomer. Potassium *tert*-butanolate (22 mg, 0.20 mmol) was added to a solution of this sugar (779 mg, 2.0 mmol) in MeOH (6 mL). After stirring for 6 h, the reaction mixture was neutralized with Dowex 50W × 4 and subsequently filtered. The filtrate was concentrated and the residue was purified by silica gel chromatography. The column was eluted with 20% petroleum ether to 30% MeOH in EtOAc. The pure compound **14** was obtained in 78% yield.

^{13}C NMR (CD_3OD): δ 57.3 (C-2), 62.6 (C-6), 66.5 (CH_2 Alloc), 72.1, 72.7 (d.i.), 75.8, 77.7 (C-3, C-4, C-5), 92.8, 97.0 (C-1), 117.6 (CH Alloc), 134.1 (CH_2 Alloc).

NodC enzyme preparation.—The *E. coli* strain BL21(DE3) containing a plasmid carrying the cloned *nodC* gene from *M. loti* strain E1R [18] was used as the source of NodC protein. Expression of *nodC* was induced in

exponentially growing cells by the addition of isopropylthiogalactopyranoside (IPTG), followed by disruption of the bacteria using sonication and isolation of membranes by differential centrifugation as described previously [18]. Membrane pellets were resuspended in buffer (100 mM Tris–HCl, 20 mM $MgCl_2$, pH 7.5) in 1/100 of the original culture volume and stored at −80 °C until use.

Enzyme reactions.—Reaction mixtures contained 1 mM acceptor, 10 mM UDP-[^{14}C]GlcNAc (0.05 μCi, 230 mCi/mmol; Amersham International, Amersham, UK), and 10 μL of membranes in a final reaction volume of 20 μL. After an incubation time of 30 min at 20 °C, reactions were stopped by boiling for 2 min, followed by centrifugation in a micro centrifuge for 5 min. Reaction products were then analyzed by loading 2 μL of the supernatant onto an NH_2-TLC plate which was developed using 60% MeCN. Spots were visualized using a PhosphorImager in combination with the ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

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