Chemoenzymatic Synthesis of Neoglycopeptides: Application to an α-Gal-Terminated Neoglycopeptide†

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A novel methodology for the enzymatic preparation from suitably derivatized oligosaccharides of N-linked neoglycopeptides using the microbial glutaminyl-peptide γ-glutamyl transferase, transglutaminase (TGase), is described. *N*-Allyl glycosides of various oligosaccharides were photochemically coupled with cysteamine to yield amino-terminated thioether spacers, which were accepted by transglutaminase to transamidate the side-chain γ-carboxamide group in the dipeptide Z-Gln-Gly.

Introduction

The prominent importance of glycopeptides in intracellular processes is still not sufficiently paralleled by facile methods of preparation. In principle, there are two main alternatives, (i) the addition of glycosylated amino acids as building blocks in either solid- or solution-phase peptide synthesis or (ii) the blockwise addition of a preassembled saccharide moiety to a peptide. We were seeking for a solution to the latter problem, aiming at the chemoselective addition to the peptide of a functionalized oligosaccharide.

Microbial glutaminyl-peptide γ-glutamyl transferase (transglutaminase, TGase) cross-links proteins by catalyzing an acyl-transfer reaction. The enzyme is specific for the glutamine γ-carboxamide group, which acts as the acyl donor. Although it is conceivable that certain sequence requirements must exist for the enzyme to accept the substrate, research has hitherto not revealed any general rule for the peptide sequence.[1−10]

Nevertheless, glutamine residues in proteins which are accepted by TGase are mostly situated in flexible and solvent-accessible terminal polypeptide domains, or in surface loops.

This might be conceived as a limitation for the use of transglutaminase in synthetic applications;, however, as this study has shown, the enzyme accepts a wide range of primary amines. This allowed us to use the enzyme for the construction of a synthetic target, a neoglycopeptide, the oligosaccharide moiety of it would be furnished at the reducing end with an amino-terminated spacer.[11] To make the method applicable to a broader range of saccharide structures, a direct and simple way to functionalize the sugar backbone was sought (Scheme 1). The hemiacetal is N-glycosylated by using allylamine and is subsequently converted into the corresponding β-configured *N*-acetyl allyl glycoside. This anomeric protection allows for further manipulation of the glycoside (depicted as pathway b) prior to the assembly of the amino-terminated spacer.

All such synthesized compounds are substrates to transglutaminase-catalyzed reactions and they readily react with the model acceptor, Z-protected peptide Z-Gln-Gly, as depicted in Scheme 2.

The here-described structures will differ fundamentally from natural N-linked glycoproteins, in that they not only have a heteroatom linker region but also a glutamine attachment site. However, it is hoped that conformational effects by the saccharide on the protein folding can be studied and signaling functionality of the glycoprotein can be retained.

Results and Discussion

In the first entry, GlcNAc, maltose, cellobiose, and lactose (**1**−**4**) were chosen as model compounds (Scheme 3). The four hemiacetals were reacted with allylamine[12] and subsequently per-O-acetylated to yield the stable β-configured anomers **5**−**8**, which could be easily purified at this stage. Upon deacetylation, compounds **9**−**12** and cysteamine hydrochloride[13−16] were irradiated (254 nm),

† Dedicated to our friend Professor Joachim Thiem on the occasion of his 60th birthday.

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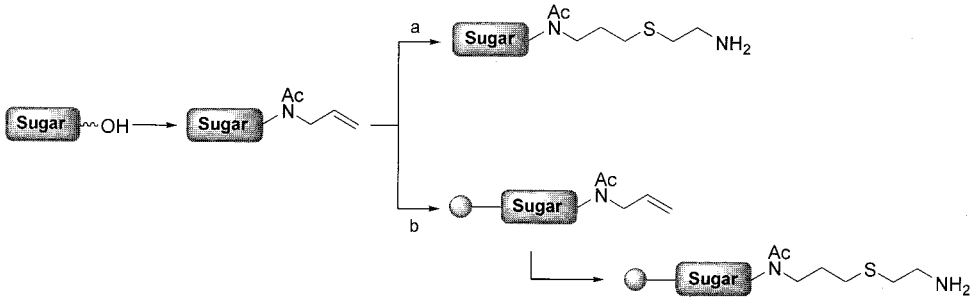
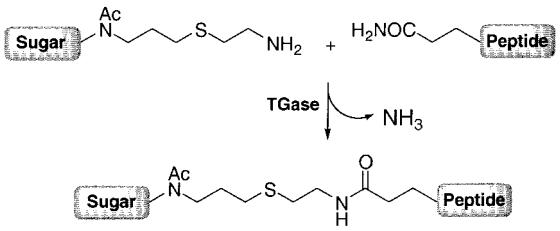
Scheme 1. Versatile Method for the Functionalization of the Sugar Backbone[a]

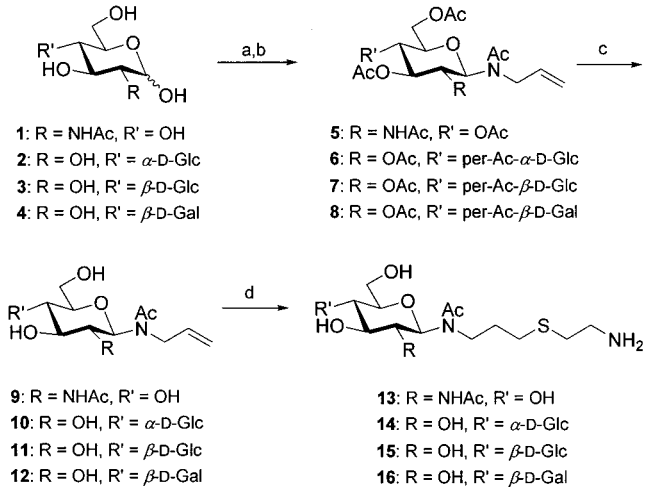


[a] Key: (a) direct activation by photochemical coupling with cysteamine; (b) modifcation on the sugar part by conventional glycoside synthesis prior to the photochemical coupling step.

Scheme 2. Enzymatic Cross-Linking of Amino-Functionalized Sugars and Glutamine-Containing Peptides



Scheme 3. General Scheme for the Synthesis of Spacer-Bridged Carbohydrates, Starting from GlcNAc (1), Maltose (2), Cellobiose (3), and Lactose (4)[a]



1: R = NHAc, R' = OH
2: R = OH, R' = α-D-Glc
3: R = OH, R' = β-D-Glc
4: R = OH, R' = β-D-Gal

5: R = NHAc, R' = OAc
6: R = OAc, R' = per-Ac-α-D-Glc
7: R = OAc, R' = per-Ac-β-D-Glc
8: R = OAc, R' = per-Ac-β-D-Gal

9: R = NHAc, R' = OH
10: R = OH, R' = α-D-Glc
11: R = OH, R' = β-D-Glc
12: R = OH, R' = β-D-Gal

13: R = NHAc, R' = OH
14: R = OH, R' = α-D-Glc
15: R = OH, R' = β-D-Glc
16: R = OH, R' = β-D-Gal

[a] Key: (a) allylamine, rt, 72 h; (b) Ac_2O/pyridine, DMAP (60% to quantitative yields); (c) MeONa/MeOH (90% to quantitative yields); (d) cysteamine hydrochloride (5 equiv), MeOH, *hν* (254 nm), argon, 8 h (70−80%).

yielding the amino-terminated thioethers **13**−**16** (70−80% yield). The basic concept of this two-step process, (i) the synthesis of the allylamine and (ii) the final activation toward the primary amine, was to have a stable intermediate at hand which could be further functionalized prior to the final coupling to a protein. To prove the feasibility of this methodology, lactoside **12** was used as the building block for the synthesis of the α-Gal trisaccharide derivative (**17**). Per-O-benzylated thioglycoside **19** was chosen as glycosyl donor. Lactoside **12** was subjected to regioselective monoalkylation at the C-3′ position with *p*-methoxybenzyl chloride (PMB-Cl). Compound **12** was therefore treated with dibutyltin oxide in methanol, involving the generation of a dibutylstannylene acetal as intermediate.[17−18] After concentration, the residue was suspended in dry benzene and reacted with tetrabutylammonium iodide (TBAI) and *p*-methoxybenzyl chloride to give **21** (30%) (Scheme 4). Acetylation of the remaining hydroxyl groups and oxidative cleavage of the *p*-methoxy benzyl group with cerium(IV) ammonium nitrate (CAN)[19] afforded compound **20** (90%). Coupling of thioglycoside **19**[20] with the lactoside **20** in dichloromethane at room temperature, using methyl trifluoromethanesulfonate as promotor,[21] gave the α-configured trisaccharide **18** in good yield (90%), with no β-anomer present.

Trisaccharide **18** was deacetylated and irradiated (254 nm) with cysteamine hydrochloride in methanol to yield the partially unprotected trisacchararide **24**. Attempts to remove the benzyl ether groups by either hydrogenation in the presence of Pd/C or reduction with Raney nickel[22] were unsuccessful, probably due to the presence of the thioether. Finally, radical reduction[23,24] employing sodium in liquid ammonia afforded the desired compound **25** in high purity. This fact was essential for the further process, since all attempted purification procedures failed for this compound. Thus, it was used without further purification as a starting material for the enzymatic coupling step.

The activity of TGase toward the artificial substrates **13**−**16** and **25** was measured and compared to the hydroxylamine standard (Scheme 5). The ammonia liberated during the enzymatic reaction, which is indirectly proportional to the amount of product, was estimated using the Sigma diagnostics method for plasma ammonia.

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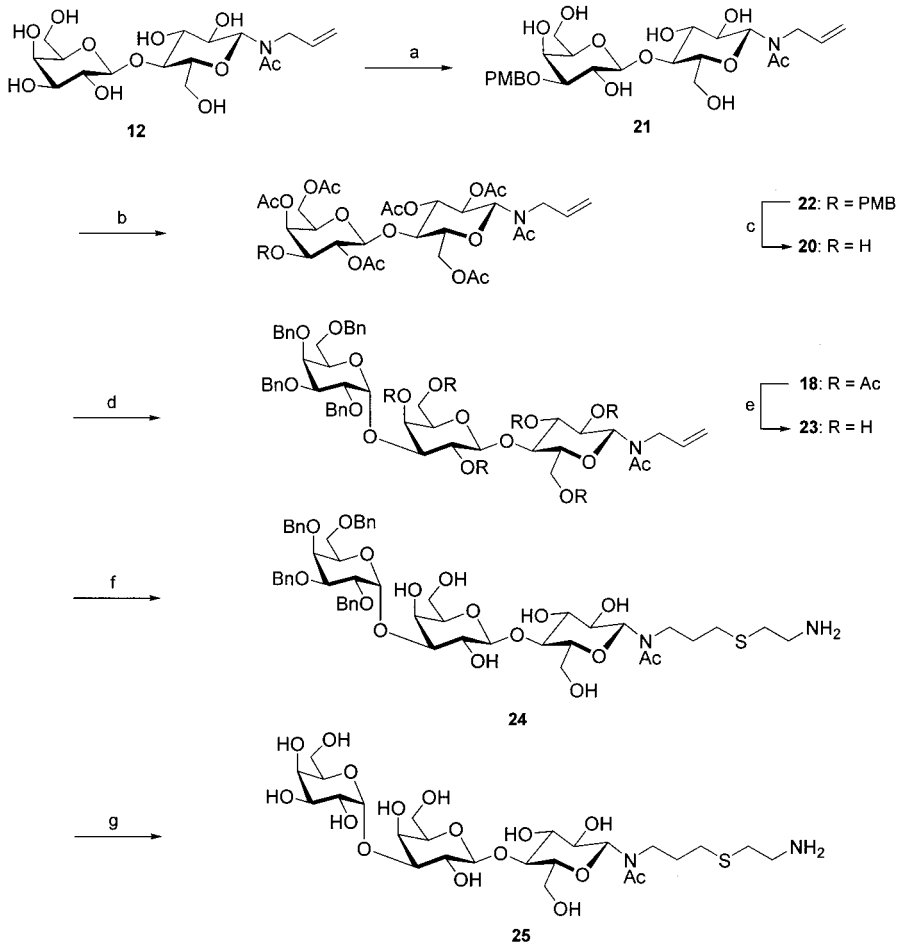
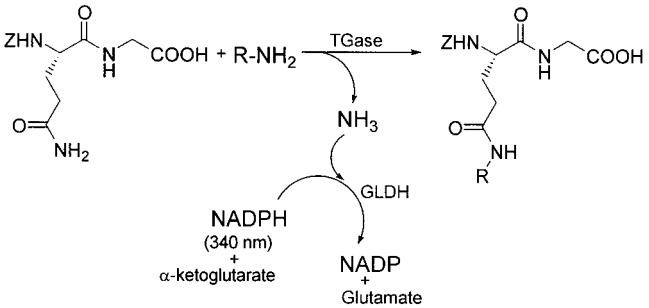
Scheme 4. Synthesis of a Derivative of an α-Galactosyl Trisaccharide[a]



[a] (a) (i) Bu_2SnO (1.1 equiv), MeOH (anhyd), molecular sieves 4 Å, argon, reflux, 20 h; (ii) TBAI (1 equiv), PMB-Cl (1.1 equiv), benzene (anhyd), molecular sieves 4 Å, argon, reflux, 4−5 h; (b) Ac_2O/pyridine, DMAP (22% from **12**); (c) CAN (3 equiv), acetonitrile/water (9/1), O°C → rt (88%); (d) thiophenyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside **19** (1.6 equiv), CH_2Cl_2 (anhyd), pulverized molecular sieves 4 Å, methyl trifluoromethanesulfonate (5 equiv), argon, rt (90%); (e) MeONa/MeOH (94%); (f) cysteamine hydrochloride, MeOH, *hν* (254 nm), 8 h (75%); (g) Na/NH_3, −78 °C (quantitative yields).

Scheme 5. Principle of the Determination of Ammonia in the Assay



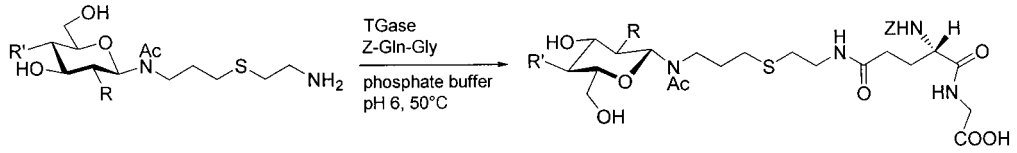
The enzyme (TGase, 10 g/L), peptide (Z-Gln-Gly, 15 mM) and substrate (NH_2OH, **13**−**16** and **25**, 15 mM) were mixed in phosphate buffer (NaH_2PO_4/Na_2HPO_4, 0.2 M, pH 6) and incubated at 50 °C for 5 min (Scheme 6). The enzyme was thermally inactivated, and the level of ammonia was measured for each substrate. Kinetic studies were also conducted using different concentrations of peptide and substrate at a constant ratio. The results are depicted in Table 1. For unknown reasons, no K_m or V_{max} values could be determined for trisaccharide **25**. Nevertheless, its relative activity was found to be two to four times higher than for the other substrates. No major difference was observed between the three disaccharide derivatives (**14**−**16**), whereas the GlcNAc derivative yielded very low values for V_{max} and K_m, indicating a good binding of **13** to transglutaminase and a slow coupling reaction. Further investigation on the structure−function relationship is necessary to rationalize all these observations.

For the transacylations on a preparative scale, compounds **13**−**16** and **25** were allowed to react with the dipeptide benzyloxycarbonyl-glutamine-glycine (Z-Gln-Gly) and TGase in a buffered solution (pH 6), at 50 °C with shaking. Except for the GlcNAc derivative (**13**), all products could be isolated and characterized as the result of a covalent cross-linking between the saccharide and the peptide. Yields of the coupling reactions varied from one substrate to the other (50 to 70%), mainly due to the laborious separation of the products from maltodextrins, which are added to the enzyme preparation by the manufacturer for stability reasons.

Conclusions/Summary

The above-described method is able to transfer a preassembled oligosaccharide block onto a peptide. Unlike the biosynthesis of natural glycopeptides, it does not

Scheme 6. Enzymatic Synthesis of Neoglycopeptides



13: R = NHAc, R' = OH
14: R = OH, R' = α-D-Glc
15: R = OH, R' = β-D-Glc
16: R = OH, R' = β-D-Gal
25: R = OH, R' = α-D-Gal-(1→3)-β-D-Gal

26: R = NHAc, R' = OH
27: R = OH, R' = α-D-Glc
28: R = OH, R' = β-D-Glc
29: R = OH, R' = β-D-Gal
17: R = OH, R' = α-D-Gal-(1→3)-β-D-Gal

Table 1. Results of the Kinetic Measurements for the Artificial Substrates (NH_2OH, 13−16 and 25)[a]

substrate	V_{max} (nmol/s)	K_m (10^{-3} M)	V_{max}/K_m (10^{-9})	activity (μmol/s/g enz)	rel activity (%)
NH_2OH	1.310	6.96	188	0.885	100
13	0.064	3.99	16	0.050	6
14	0.194	14.90	13	0.109	12
15	0.194	10.02	19	0.127	14
16	0.216	13.82	16	0.112	13
25				0.222	25

[a] All the assays were conducted with a concentration of enzyme of 10 g/L. The relative activities ($V_0^{obsd}/V_0^{NH_2OH}$) were estimated against hydroxylamine (standard 100%).

require dolichyl pyrophosphate activation; however, it differs in the chemoselectivity of the biosynthetic process, since it attaches the glycoside to glutamine instead of asparagine.

Research is presently under way to screen for a broader range of acceptors.

Experimental Section

General Procedures. Reactions were monitored by TLC on silica plates (Merck Kieselgel 60 F_{254}) and detected by UV absorption and charring with naphthoresorcin (200 mg) in sulfuric acid (2 N, 100 mL) and ethanol (100 mL) or molybdato phosphoric acid (25 g) and Ce(IV) sulfate (10 g) in water (940 mL) containing concentrated sulfuric acid (60 mL). Free amine was detected by spraying with a solution of ninhydrine (2% in butanol, 95 mL, containing acetic acid 10%, 5 mL). Column chromatography was performed on Kieselgel (Merck, 230−400 mesh, 0.063−0.200 μm). Medium-pressure liquid chromatography (MPLC, pressure 3−5 bar) was performed on Kieselgel (Merck, 230−400 mesh, 0.040−0.063 μm).

Anhydrous solvents (CH_2Cl_2, MeOH, $CHCl_3$) were prepared by passing solvents (analytical grade, p.a.) through molecular sieves (4 Å) under argon.

^1H and ^{13}C NMR spectra were recorded with a Bruker WM 300 (^1H, 300.1 MHz; ^{13}C, 75.5 MHz) or with a Bruker AMX400 (^1H, 400 MHz; ^{13}C, 100.6 MHz). Two-dimensional correlation spectra (GCOSY and GHSQC) were measured with a Varian Unity plus 600 (^1H, 599.2 MHz; ^{13}C, 150.7 MHz). Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si, $CDCl_3$ (^1H, 7.24; ^{13}C, 77.0), DMSO (d_6) (^1H, 2.49 (quintet); ^{13}C, 39.7), MeOH (d_4) (^1H, 4.7; ^{13}C, 49.3), and D_2O (^1H, 4.78).

Indications for methylene groups in compounds **16**, **17**, **24**, and **27**−**29** is as follows: R-NAc-CH_2(a)-CH_2(b)-CH_2(c)-S-CH_2(d)-CH_2(e)-NH_2 and R-NAc-CH_2(a)-CH_2(b)-CH_2(c)-S-CH_2(d)-CH_2(e)-NH-CO-CH_2(f)-CH_2(g)-CH(Gln)-CO-NH-CH_2(h)-CO-OH. In all following assignments, "m_c" stands for "multiplet centered".

Optical rotations were measured at 20 °C at 589 nm (Na) with a Perkin-Elmer 241 polarimeter using a 10 cm/1 mL cell. Mass spectra and exact mass were measured by ESI with a Micromass Quattro LC-Z spectrometer.

Transglutaminase was a generous gift from Ajinomoto Gmbh, Hamburg, Germany. The enzyme supplied was stabilized in maltodextrines, the concentration of which is unknown.

Method 1. Per-O-acetylated β-NAc-allyl Glycosides. The sugar was dissolved in allylamine (0.1 M solution) and stirred at room temperature for ca. 72 h. To monitor the reaction, an aliquot of the reaction mixture was extracted and per-O-acetylated. Upon completion, the allylamine was evaporated under vacuum and co-distilled with toluene. The crude residue was dissolved in pyridine, a catalytic amount of DMAP was added, and at 0 °C, acetanhydride, pyridine/Ac_2O, (2:1, v/v) was added slowly. The reaction mixture was then allowed to reach rt and further stirred upon complete per-O-acetylation as judged by TLC (∼24 h). The reaction mixture was poured on ice−water and extracted with dichloromethane. The organic layer was successively washed with hydrochloric acid (1 N) and water. The extract was dried over $MgSO_4$, filtered, concentrated under vacuum, and co-distilled with toluene.

Method 2. De-O-acetylation. The per-O-acetylated compound was dissolved in methanol, and sodium methoxide (30 wt % solution in methanol) was added until pH 10 was reached. The mixture was stirred for 1 h, and then Dowex resin (ion exchanger H^+ form, 50Wx8) was added to neutralize the solution, and the suspension was further stirred for 1 h. The mixture was filtered, and methanol was concentrated under vacuum and then co-distilled with toluene.

Method 3. Addition of Cysteamine. The respective β-NAc-allyl glycoside and cysteamine (5 equiv) were dissolved in a minimum amount of methanol and transferred to a photochemical cell. The mixture was stirred at room temperature, under argon, and irradiated (254 nm) during 8 h. Upon completion, as judged by TLC, the reaction mixture was concentrated.

Method 4. Preparative Transacylation with Transglutaminase. The sugar derivative (30 mM) was shaken with Z-Gln-Gly (30 mM) and TGase (20 g/L) in a phosphate buffer (Na_2HPO_4/NaH_2PO_4 0.2 M, pH 6) at 50 °C. The reaction was followed by TLC (EtOAc/AcOH/H_2O, 3:1:1). The enzyme was deactivated at 100 °C (2 min), and the resulting reaction mixture was lyophilized. The residue was first applied to SiO_2 chromatography (MPLC) then to Sephadex G-10 chromatography. Appropriate fractions were pooled and lyophilized to yield the product.

Method 5. Estimation of the Ammonia and Determination of the Activity. This estimation was made using the Sigma diagnostics "Ammonia detection kit" ([No 171-A]).

Equal volumes of peptide (Z-Gln-Gly, 15 mM), substrate (NH_2OH, **13**−**16** and **25**, 15 mM) and transglutaminase (10 g/l) in solution in buffer (NaH_2PO_4/Na_2HPO_4, 0.2 M, pH 6) were mixed, incubated (50 °C), and shaken. Aliquots of the mixture (200 μL) were extracted at different times, and poured into boiling water (1 min), to denature the enzyme and stop the reaction. The latter extracts (100 μL) were then added to the ammonia reagent (1 mL) into the cuvette which was gently mixed. The absorbance A1 was measured after 3 min, at 340 nm, against the blank (buffer replaces the transglutaminase). l-GLDH (l-glutamate dehydrogenase, 10 μL) was then added, the cuvette was gently mixed and the absorbance A2 was read after 5 min at 340 nm, against the blank.

The activity, defined as the number of micromoles of NH_3 liberated in 1 s per gram of enzyme, was calculated as follows:

$$[\Delta A/\text{min}] = (A_2 - A_1)/t \ (t \text{ in min})$$

$$[NH_3]_{\text{produced}} = ([\Delta A/\text{min}]/\epsilon)(1.11/0.1) \text{ mol/L/min}$$

$(\epsilon = 6200\ (1000\ \text{cm}^2/\text{mol}) =$ molar absorptivity of NADPH at 340 nm)

$$n_{NH_3} = [([\Delta A/\text{min}] \times 10^6 \times 10^{-4})/\epsilon](1.11/0.1)\ \mu\text{mol/min}$$

$$= [([\Delta A/\text{min}] \times 10^2)/(\epsilon \times 60)](1.11/0.1)\ \mu\text{mol/s}$$

$$= [([\Delta A/\text{min}] \times 10^2)/(6200 \times 60)](1.11/0.1)\ \mu\text{mol/s}$$

$$= [[\Delta A/\text{min}]/(62 \times 60)](1.11/0.1)\ \mu\text{mol/s}$$

For a concentration of enzyme of 10 g/L, the extract of 100 μL contains 1 mg of enzyme and the activity per gram of enzyme is then expressed by the following formula:

$$\text{activity} = [([\Delta A/\text{min}] \times 1000)/(62 \times 60)](1.11/0.1)\ \mu\text{mol/s/g of enzyme}$$

***N*-Acetyl-*N*-allyl-2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-*β*-D-glucopyranosylamine (5).** 2-Acetamido-2-deoxy-D-glucose (5 g, 22.6 mmol) was reacted with allylamine (150 mL) according to method 1. After concentration, the residue was per-O-acetylated (TLC EtOAc/MeOH, 20:1). The residue was recrystallized from ethyl acetate to yield pure compound **5** (5.80 g, 13.5 mmol, 60%) as a white powder. $[\alpha]^{20}_D = -2$ (c = 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.34 (d, H-1), 5.80 (d, −N*H*), 5.76 (dddd−oct, −C*H*=CH_2), 5.20−5.08 (m, 3H, −CH=CH_2 and H-3 or H-4), 5.02 (dd−t, H-3 or H-4), 4.26 (ddd, H-2), 4.15−4.10 (m, 2H, H-6), 3.98−3.77 (m, 2H, −CH_2CH=CH_2), 3.74 (ddd, H-5), 2.05, 2.02, 1.99, 1.98, and 1.82 (5*s, 15H, 5*-CH_3 (Ac)); $J_{1,2}$ = 9.7, $J_{2,3}$ = 9.7, $J_{2,NH}$ = 9.7, $J_{3,4}$ = 9.7, $J_{5,6a}$ = 2.8, $J_{5,6b}$ = 4.3, Hz. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.70, 170.68, 170.36, 170.18, and 169.20 (5*-*C*=O), 134.95 (−*C*H=CH_2), 116.94 (−CH=*C*H_2), 81.08 (C-1), 74.21, and 73.40 (C-4 and C-3), 68.42 (C-5), 62.04 (C-6), 51.13 (C-2), 46.57 (−CH_2-CH=CH_2), 22.82−20.44 (5*-CH_3(Ac)) ppm. MS (ESI): m/z 429 $[M + H]^+$, 451 $[M + Na]^+$.

***N*-Acetyl-*N*-allyl-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosylamine (6).** According to the general method 1, maltose (5 g, 14.6 mmol) was reacted with allylamine (150 mL). After concentration, the residue was per-*O*-acetylated (TLC: toluene/acetone, 7:3). The crude product was applied to a SiO_2-chromatography and eluted with a linear gradient of toluene/acetone from 5:1 to 3:1. Appropriate fractions were combined, concentrated and **6** (9.85 g, 13.7 mmol, 94%) was isolated as a colorless foam. $[\alpha]^{20}_D$ = 71 (c = 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.86 (d, H-1), 5.74 (dddd−oct., −C*H*=CH_2), 5.36 (d, H-1′), 5.34 (dd−t, H-3), 5.31 (dd, H-3′), 5.10 (d, 2H, −CH=CH_2), 5.01 (dd−t, H-4′), 4.87 (dd−t, H-2), 4.82 (dd, H-2′), 4.40 (dd, H-6′a), 4.20 (dd, H-6′b), 4.15 (dd, H-6a), 4.03 (dd, H-6b), 4.00−3.85 (m, 2H, H-4 and H-5′), 3.83−3.68 (m, 3H, H-5 and −CH_2CH=CH_2), 2.09−1.90 (8*s, 24H, 8*-CH_3 (Ac)); $J_{1,2}$ = 9.5, $J_{2,3}$ = 9, $J_{3,4}$ = 9, $J_{5,6a}$ = 4, $J_{5,6b}$ = 2.5, $J_{6a,6b}$ = 12.4, $J_{1',2'}$ = 4, $J_{2',3'}$ = 10.4, $J_{3',4'}$ = 9.6, $J_{4',5'}$ = 10, $J_{5',6'a}$ = 2.5, $J_{5',6'b}$ = 4, $J_{6'a,6'b}$ = 12.3 Hz. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 171.00−170.00 (8*-*C*=O), 135.17 (−*C*H=CH_2), 117.43 (−CH=*C*H_2), 96.00 (C-1′), 80.34 (C-1), 76.27 (C-3), 74.98 (C-5), 73.04 (C-4), 71.32 (C-2), 70.49 (C-2′), 69.80 (C-3′), 69.03 (C-4′), 68.55 (C-5′), 62.99 (C-6), 61.95 (C-6′), 46.62 (−*C*H_2CH=CH_2), 22.00−20.92 (8*-CH_3 (Ac)) ppm. MS (ESI) m/z: 740 $[M + Na]^+$. Exact mass calcd for $C_{31}H_{43}NO_{18}Na^+$: 740.2378, found 740.2406.

***N*-Acetyl-*N*-allyl-2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosylamine (7).** Cellobiose (25 g, 73 mmol) was reacted with allylamine (450 mL). After concentration, the residue was per-O-acetylated (TLC toluene/acetone, 7:3). The crude product was applied to SiO_2 chromatography and eluted with a linear toluene/acetone gradient from 5:1 to 3:1, and appropriate fractions were pooled and concentrated, from which **7** (52.30 g, 72.8 mmol, quantitative yields) was isolated as a colorless foam. $[\alpha]^{20}_D = -13$ (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.89 (d, H-1), 5.73 (dddd−oct, −C*H*=CH_2), 5.28 (dd−t, H-3), 5.19−5.11 (m, 3H, −CH=CH_2 and H-3′), 5.07 (dd−t, H-4′), 4.99 (dd−t, H-2), 4.93 (dd, H-2′), 4.54 (d, H-1′), 4.53 (dd, H-6a), 4.37 (dd, H-6′a), 4.09 (dd, H-6b), 4.06 (dd, H-6′b), 3.93−3.63 (m, 5H, −CH_2CH=CH_2, H-4, H-5 and H-5′), 2.12−1.97 (8*s, 24H, 8*-CH_3 (Ac)); $J_{1,2}$ = 9.5, $J_{2,3}$ = 9, $J_{3,4}$ = 9, $J_{5,6a}$ = 1.5, $J_{5,6b}$ = 4.6, $J_{6a,6b}$ = 12, $J_{1',2'}$ = 8.5, $J_{2',3'}$ = 9, $J_{3',4'}$ = 9.5, $J_{5',6'a}$ = 4.5, $J_{5',6'b}$ = 2.3, $J_{6'a,6'b}$ = 12.3 Hz. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 172.09−168.73 (8*-*C*=O), 134.75 (-*C*H=CH_2), 116.81 (−CH=*C*H_2), 100.44 (C-1′), 80.04 (C-1), 74.96 (C-4), 72.80 (C-3 and C-3′), 71.95 (C-5′ and C-5′), 71.55 (C-2′), 68.87 (C-2), 67.83 (C-4′), 61.52 (C-6 and C-6′), 46.24 (−CH_2CH=CH_2), 21.96−20.33 (8*-CH_3 (Ac)) ppm. MS (ESI): m/z 718 $[M + H]^+$, 740 $[M + Na]^+$. Exact mass calcd for $C_{31}H_{43}NO_{18}Na^+$: 740.2378, found 740.2388.

***N*-Acetyl-*N*-allyl-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosylamine (8).** Lactose (5.06 g, 14.8 mmol) was subjected to the general methods to react with allylamine (150 mL). After concentration, the residue was per-O-acetylated (TLC toluene/acetone, 7:3). The crude product was applied to a SiO_2 chromatography and eluted by toluene/acetone 4:1. The product **8** (10.57 g, 14.73 mmol, quantitative yields) was yielded as a white foam. $[\alpha]^{20}_D$: −3 (c = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.90 (d, H-1), 5.73 (dddd−oct, −C*H*=CH_2), 5.35 (d, H-4′), 5.30 (dd−t, H-3), 5.14 (m_c, 2H, −CH=CH_2), 5.11 (dd, H-2′), 4.97 (m_c, 2H, H-2 and H-3′), 4.51 (2*d, 2H, H-1′ and H-6a), 4.20−4.06 (m, 3H, H-6b, H-6′a, and H-6′b), 3.96−3.66 (m, 5H, H-5′, H-4, H-5, and −CH_2-CH=CH_2), 2.24−1.96 (m, 24H, 8*-CH_3 (Ac)); $J_{1,2}$ = 9.5, $J_{2,3}$ = 10, $J_{3,4}$ = 8, $J_{5,6a}$ = 1.8, $J_{5,6b}$ = 6.4, $J_{6a,6b}$ = 11.2, $J_{1',2'}$ = 8, $J_{2',3'}$ = 10.5, $J_{3',4'}$ = 3.3, $J_{4',5'}$ = 0.9, $J_{5',6'a}$ = 5, $J_{5',6'b}$ = 7 $J_{6'a,6'b}$ = 11.5 Hz. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.14−169.82 (8*-*C*=O), 134.72 (-*C*H=CH_2), 116.80 (−CH=*C*H_2), 100.73 (C-1′), 79.99 (C-1), 75.88 (C-5), 74.94 (C-4), 73.06 (C-3), 70.85 and 70.69 (C-5′ and C-3′), 69.08 and 68.96 (C-2′ and C-2), 66.59 (C-4′), 61.64 (C-6), 60.75 (C-6′), 46.23 (−*C*H_2-CH=CH_2), 21.98−20.29 (8*-CH_3 (Ac)) ppm. MS (ESI): m/z 718 $[M + H]^+$, 740 $[M + Na]^+$.

***N*-Acetyl-*N*-allyl-2-acetamido-2-deoxy-*β*-D-glucopyranosylamine (9).** Compound **5** (5.17 g, 12.06 mmol) was deacetylated according to the general method. The residue was recrystallized from EtOAc/MeOH 3:1 to yield compound **9** (3.28 g, 10.8 mmol, 90%) as a colorless powder. $[\alpha]^{20}_D$ = 14 (c = 1.0, MeOH). MS (ESI): m/z 325 $[M + Na]^+$.

***N*-Acetyl-*N*-allyl-α-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosylamine (10).** Compound **6** (5 g, 6.9 mmol) was deacetylated according to the general method. The residue was purified by MPLC and eluted with EtOAc/MeOH/AcOH, 60:15:5 (TLC EtOAc/*i*-PrOH/H_2O, 9:4:2) yielding compound **10** (2.9 g, 6.8 mmol, quantitative yields) as a white foamy compound. $[\alpha]^{20}_D$ = 77 (c = 1.0, CH_3OH). MS (ESI): m/z: 446 $[M + Na]^+$, 284 $[(M - Glc) + Na]^+$ (daughter ion of 446). Exact mass calcd for $C_{17}H_{29}NO_{11}Na^+$ 446.1638, found 446.1634.

***N*-Acetyl-*N*-allyl*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosylamine (11).** Compound **7** (10 g, 13.93 mmol) was deacetylated according to the general method. The residue was purified by MPLC and eluted with EtOAc/MeOH/AcOH, 60:15:5 (TLC EtOAc/^{i}PrOH/H_2O, 9:4:2) and recrystallized from EtOAc/MeOH, yielding compound **11** (5.85 g, 13.8 mmol, quantitative yields) as a white foamy compound. $[\alpha]^{20}_D$ = 9 (c 1.0, CH_3OH). ^{1}H NMR (400 MHz, D_2O): δ = 5.89 (dddd−oct, −C*H*=CH_2), 5.51 (d, H-1), 5.39−5.00 (m, 2H, −CH=CH_2), 4.52 (d, H-1′), 4.06 (m_c, 2H, −CH_2CH=CH_2), 3.92 (m_c, 2H, H-6′), 3.84 (m_c, 2H, H-6), 3.71 (m_c, 4H, H-2, H-3, H-4, and H-5), 3.56−3.30 (m, 4H, H-3′, H-5′, H-4′, and H-2′), 2.26 and 2.20 (2*s, 3H, −CH_3 (Ac)); $J_{1,2}$ = 8.4, $J_{6a,6b}$ = 11.9, $J_{1',2'}$ = 7.9, $J_{2',3'}$ = 9.5, $J_{3',4'}$ = 9.3, $J_{6'a,6'b}$ = 12.7 Hz. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 177.08 and 175.61 (−*C*=O), 134.54 (−*C*H=CH_2), 117.16 (−CH=*C*H_2), 103.01 (C-1′), 82.95 (C-1), 78.52, 77.27, 76.56, 76.07, and 75.62 (C-2, C-3, C-3′, C-5, and C-5′), 73.71 (C-2′), 70.53 and 70.04 (C-4 and C-4′), 61.19 (C-6′), 60.55 (C-6), 44.32

(−CH_2CH=CH_2), 22.26 and 21.78 (−CH_3 (Ac)) ppm. MS (ESI): *m/z* 424 $[M + H]^+$, 446 $[M + Na]^+$. Exact mass calcd for $C_{17}H_{29}NO_{11}Na^+$ 446.1638, found 446.1661. Anal. Calcd for $C_{17}H_{29}NO_{11}$ + CH_3OH (1 mol): C, 47.47; H, 7.30; N, 3.08. Found: C, 47.33; H, 7.42; N, 3.08.

***N*-Acetyl-*N*-allyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosylamine (12).** Compound **8** (3.8 g, 5.3 mmol) was deacetylated according to the general method (TLC EtOAc/^{i}PrOH/H_2O, 9:4:2). The residue was recrystallized from EtOAc/MeOH, 3:1 and washed with ethyl acetate and diethyl ether yielding compound **12** (2.15 g, 5.08 mmol, 96%) as a white powder. $[\alpha]^{20}_D$ = −6 (*c* 1.0, CH_3OH). ^{1}H NMR (400 MHz, D_2O): δ = 6.05 (dddd−oct, −CH=CH_2), 5.67 (d, H-1), 5.53−5.29 (m, 2H, −CH=CH_2), 5.23 (d, H-4′), 4.62 (d, H-1′), 4.32−4.15 (m, 2H, −CH_2CH=CH_2), 4.14−4.04 (m, 2H, H-6a and H-3), 4.03−3.78 (m, 8H, H-6b, H-6′a, H-6′b, H-4, H-3′, H-2, H-5, H-5′), 3.72 (dd−t, H-2′), 2.41 and 2.36 (2*s, 3H, −CH_3); $J_{1,2}$ = 8.3, $J_{2,3}$ = 9, $J_{1',2'}$ = 8, $J_{2',3'}$ = 10.5, $J_{3',4'}$ = 8 Hz. ^{13}C NMR (100.6 MHz, D_2O): δ = 177.12 and 175.66 (−*C*=O), 134.59 (−*C*H=CH_2), 117.93 (−CH=*C*H_2), 103.43 (C-1′), 87.47 (C-4′), 83.00 (C-1), 78.28, 77.45, 75.49, and 73.11 (C-2, C-3, C-5, and C-5′), 71.52 (C-2′), 70.47 (C-3′), 69.15 (C-4), 61.62 (C-6), 60.59 (C-6′), 44.33 (−*C*H_2CH=CH_2), 22.27−21.79 (−*C*H_3(Ac)) ppm. MS (ESI): *m/z* 424 $[M + H]^+$, 446 $[M + Na]^+$. Anal. Calcd for $C_{17}H_{29}NO_{11}$: C, 48.22; H, 6.90; N, 3.31. Found: C, 48.14; H, 6.86; N, 3.29.

***N*-Acetyl-*N*-allyl-(3-*O*-(4-methoxybenzyl)-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosylamine (21).** A suspension of NAc-allyl lactoside **12** (3 g, 7.09 mmol) and dibutyltin oxide (1.94 g, 7.8 mmol, 1.1 equiv) in anhyd methanol (100 mL) was refluxed in a Soxhlet apparatus containing molecular sieves (4 Å) under exclusion of moisture. After 20 h, the reaction mixture was concentrated, and the residue was codistilled three times with toluene. The residue was dried in a vacuum, suspended in dry benzene containing molecular sieves (4 Å) and tetrabutylammonium iodide (2.62 g, 7.09 mmol, 1 equiv). *p*-Methoxybenzyl chloride (1.06 mL, 7.8 mmol, 1.1 equiv) was added at once under argon atmosphere, and the reaction mixture was refluxed in a Soxhlet apparatus filled with molecular sieves (4 Å). Upon completion as judged by TLC (EtOAc/*i*-PrOH/H_2O, 9:4:2, few hours), the mixture was cooled to room temperature, filtered through Celite, and concentrated. The residue was applied to a silica gel chromatography (CH_2Cl_2/MeOH, 20:1−10:1) to yield **21** as a colorless powder, which was immediately reacted further. $[\alpha]^{20}_D$ = 17 (*c* = 1.0, MeOH). MS (ESI): *m/z* 544 $[M + H]^+$, 566 $[M + Na]^+$. Exact mass calcd for $C_{25}H_{37}NO_{12}Na^+$: 566.2214, found 566.2239.

***N*-Acetyl-*N*-allyl-2,4,6-tri-*O*-acetyl-3-*O*-(4-methoxybenzyl)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosylamine (22).** Compound **21** was acetylated according to the general method. The residue was applied to silica gel chromatography (toluene/acetone, 5:1) to give **22** as a colorless powder (1.22 g, 1.53 mmol, 22% from the unprotected **13**). $[\alpha]^{20}_D$ = 36 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.12 and 6.83 (2d, 4 H-arom), 5.85 (d, H-1), 5.67 (dddd−oct, −C*H*=CH_2), 5.42 (d, H-4′), 5.25 (dd, H-3), 5.11 (m_c, 2H, −CH=CH_2), 4.97 (dd, H-2′), 4.93 (dd−t, H-2), 4.56 and 4.26 (each d, each 1H, −CH_2Ph), 4.42 (d, H-6a), 4.34 (d, H-1′), 4.10−4.03 (2*dd−d, 2H, H-6′a and H-6′b), 4.05 (d, H-6b), 3.73 (m_c, 4H, H-5, H-5′ and −CH_2CH=CH_2), 3.68 (dd−d, H-4), 3.44 (dd, H-3′), 2.32 (s, 3H, Ph-OCH_3), 2.16−1.92 (m, 21H, 7*-CH_3 (Ac)); $J_{1,2}$ = 9.5, $J_{2,3}$ = 9.5, $J_{3,4}$ = 5.7, $J_{6a,6b}$ = 12.1, $J_{1',2'}$ = 8, $J_{2',3'}$ = 10, $J_{3',4'}$ = 3.2, J_{CH2-Ph} = 11.8 Hz. ^{13}C NMR (150.7 MHz, $CDCl_3$): δ = 172.23−168.89 (7*-*C*=O), 159.33, 129.34, 128.99, and 128.17 (6*C-arom), 134.75 (−*C*H=CH_2), 116.93 (−CH=*C*H_2), 100.90 (C-1′), 79.95 (C-1), 76.30 (C-3′), 75.90 (C-4), 74.98 (C-5), 73.09 (C-3), 70.90 (C-5′), 70.58 (C-2′), 68.99 (C-2), 65.38 (C-4′), 61.89 (C-6), 61.46 (C-6′), 46.31 (−*C*H_2CH=CH_2), 22.14, 21.40, 20.76, 20.74, 20.72, 20.69, and 20.57 (7*-*C*H_3 (Ac)) ppm. MS (ESI): *m/z* 818 $[M + Na]^+$, 834 $[M + K]^+$. Exact mass calcd for $C_{37}H_{49}NO_{18}Na^+$: 818.2847, found 818.2847.

***N*-Acetyl-*N*-allyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosylamine (20).** Compound **22** (6.63 g, 8.34 mmol) was dissolved in a mixture of acetonitrile and water (9:1, 100 mL) and cooled to 0 °C. At this temperature, CAN (13.71 g, 25 mmol, 3 equiv) was added in four aliquots within 2 h. The reaction mixture was then allowed to reach rt and was further stirred for 0.5 h (TLC, toluene/acetone, 1:1). The reaction mixture was diluted with CH_2Cl_2 (300 mL) and washed with a saturated NH_4HCO_3 solution. The aqueous layer was extracted once with CH_2Cl_2, and the combined organic layers were then washed with water, dried over $MgSO_4$, filtered, and concentrated to yield a yellowish foam, which was subjected to SiO_2 chromatography (toluene/acetone, 7:2 to 2:1), to yield pure **20** as a white fluffy compound (4.95 g, 7.32 mmol, 88%). $[\alpha]^{20}_D$ = −2 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.90 (d, H-1), 5.73 (dddd−oct, −C*H*=CH_2), 5.31 (d, H-4′), 5.28 (m_c, H-3), 5.11 (m_c, 2H, −CH=CH_2), 4.98 (dd, H-2), 4.87 (dd−t, H-2′), 4.52 (d, H-6a), 4.44 (d, H-1′), 4.16 (d, H-6b), 4.09 (m_c, 2H, H-6′a and H-6′b), 3.94−3.66 (m, 6H, H-3′, H-5′, H-5, H-4, and −CH_2CH=CH_2), 2.22−1.96 (7*s, 21H, 7*-CH_3 (Ac)); $J_{1,2}$ = 9.1, $J_{2,3}$ = 9.1, $J_{6a,6b}$ = 11.7, $J_{1',2'}$ = 8.4, $J_{2',3'}$ = 10, $J_{3',4'}$ = 3.5 Hz. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.48−169.36 (7*-*C*=O), 134.70 (−*C*H=CH_2), 116.79 (−CH=*C*H_2), 100.45 (C-1′), 80.03 (C-1), 75.83 and 75.01 (C-4 and C-5), 72.83 (C-2′), 71.22 and 70.97 (C-3′ and C-5′), 69.20 and 68.92 (C-3, C-4′ and C-2), 61.81 (C-6), 61.40 (C-6′), 46.27 (−*C*H_2CH=CH_2), 21.93−20.37 (7*-*C*H_3 (Ac)) ppm. MS (ESI): *m/z* 676 $[M + H]^+$, 698 $[M + Na]^+$. Exact mass calcd for $C_{29}H_{41}NO_{17}Na^+$ 698.2272, found 698.2264.

Thiophenyl 2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranoside (19). 2,3,4,6-Tetra-*O*-acetyl-α-D-galactosyl bromide (4.75 g, 11.55 mmol, purchased from Fluka) was reacted as previously reported[20] to yield pure compound **19** (4.4 g, 6.96 mmol, 60%, white crystals). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40−7.12 (H-arom), 4.96 and 4.59 (each d, each 1H, −CH_2-Ph (on C-4)), 4.79 and 4.73 (each d, each 1H, −CH_2−Ph (on C-2)), 4.78 and 4.70 (each d, each 1H, −CH_2Ph (on C-3)), 4.64 (d, H-1), 4.47 and 4.41 (each d, each 1H, −CH_2Ph (on C-6)), 3.97 (dd−d, H-4), 3.93 (dd∼t, H-2), 3.65 (m_c, 2H, H-6a and H-6b), 3.60 (m_c, H-5), 3.59 (dd, H-3); $J_{1,2}$ = 9.7, $J_{2,3}$ = 9.4, $J_{3,4}$ = 2.6, J_{CH2-Ph} = 11.4, 10.0, 11.8, and 11.7 Hz.^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 132.21−127.68 (C arom), 88.46 (C-1), 84.95 (C-3), 78.08, and 78.06 (C-5 and C-2), 76.28, 75.17, 74.27 and 73.46 (4*-*C*H_2Ph), 74.46 (C-4), 69.53 (C-6) ppm. MS (ESI): *m/z* 655 $[M + Na]^+$, 671 $[M + K]^+$.

***N*-Acetyl-*N*-allyl-2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosylamine (18).** Acceptor **20** (3 g, 4.4 mmol) and donor **19** (4.42 g, 7 mmol, 1.6 equiv) were dissolved in anhyd CH_2Cl_2 (90 mL), pulverized molecular sieves (4 Å, 15 g) were added, and the suspension was stirred at room temperature under argon. After 5 min, methyl trifluoromethanesulfonate (2.4 mL, 22 mmol, 5 equiv) was added, and stirring was continued overnight (TLC, toluene/acetone, 2:1). Triethylamine (3 mL, 22 mmol, 5 equiv) was added, and stirring was continued for a further 30 min. The suspension was filtered through a Celite pad and concentrated. The resulting yellow syrup was applied to MPLC (toluene/acetone, linear gradient 10:1 to 8:1) to yield the trisaccharide **18** as a white foam (4.77 g, 3.99 mmol, 90%). $[\alpha]^{20}_D$ = 43 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.38−7.13 (H-arom), 5.89 (d, H-1), 5.71 (dddd−oct, −C*H*=CH_2), 5.43 (d, H-4′), 5.28 (dd−t, H-3), 5.14 (m_c, 2H, −CH=CH_2), 5.10 (dd, H-2″), 5.04 (d, H-1″), 4.96 (dd∼t, H-2), 4.91 and 4.49 (each d, each 1H, −CH_2Ph), 4.81 and 4.63 (each d, each 1H, −CH_2-Ph), 4.71 and 4.69 (each d, each 1H, −CH_2Ph), 4.48 (d, H-6a), 4.45 and 4.39 (each d, each 1H, −CH_2Ph), 4.34 (d, H-1′), 4.10−3.99 (m, 3H, H-6b, H-6′a, and H-6′b), 3.98 (dd, H-2″), 3.90−3.75 (m, 7H, −CH_2CH=CH_2, H-5′, H-5″, H-4″, H-3′ and H-3″), 3.71 (m_c, H-4), 3.66 (m_c, H-5), 3.50 (dd−d, 2H, H-6″a, and H-6″b), 2.35 (s, 3H, −CH_3 (Ac)), 2.10−1.80 (m, 18H, 6*-CH_3 (Ac)); $J_{1,2}$ = 9.1, $J_{2,3}$ = 9.1, $J_{3,4}$ = 9.1, $J_{6a,6b}$ = 12, $J_{1',2'}$ = 8.1, $J_{2',3'}$ = 10, $J_{3',4'}$ = 3.2, $J_{1'',2''}$ = 3.1, $J_{2'',3''}$ = 10, $J_{6''a,6''b}$ = 6.2, J_{CH_2Ph} = 11.8 Hz. ^{13}C NMR (150.7 MHz, $CDCl_3$): δ = 172.26−168.66 (7*-*C*=O), 134.78 (−*C*H=CH_2), 129.00−127.38 (C-arom), 116.96 (−CH=*C*H_2), 100.84 (C-1′), 95.08 (C-1″), 80.01 (C-1), 78.38 (C-3″), 75.68 (C-2″), 75.55 (C-5″), 75.05 (C-4),

74.78, 73.27 and 73.22 (4*-CH_2-Ph), 73.57 (C-3′), 73.06 (C-3), 73.02 (C-4″), 71.05 (C-5), 70.49 (C-2′), 69.84 (C-5′), 69.07 (C-2), 68.48 (C-6″), 64.81 (C-4′), 61.87 (C-6), 61.37 (C-6′), 46.37 (−CH_2CH=CH_2), 22.18, 21.44, 20.75, 20.71, 20.66, 20.61 and 20.40 (7*-CH_3 (Ac)) ppm. MS (ESI): *m/z* 1221 $[M + Na]^+$. Exact mass calcd for $C_{63}H_{75}NO_{22}Na^+$: 1220.4679, found 1220.4659.

***N*-Acetyl-*N*-allyl-2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosylamine (23).** Compound **18** (3.67 g, 3.06 mmol) was deacetylated according to the general method. The residue was applied to SiO_2 chromatography (EtOAc/MeOH, 20:1 then 10:1) to yield trisaccharide **23** as a colorless foam (2.74 g, 2.89 mmol, 94%). $[\alpha]^{20}_D = 63$ (*c* = 1.0, CH_3OH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38−7.20 (H-arom), 5.84 (dddd−oct, −C*H*=CH_2), 5.54 (d, H-1), 5.12−4.95 (m, 2H, −CH=CH_2), 2.16 and 2.02 (2*s, 3H, −CH_3 (Ac)); $J_{1,2}$ = 9.1 Hz. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 175.52 (*C*=O), 128.47−127.30 (C-arom), 134.16 (−*C*H=CH_2), 116.69 (−CH=*C*H_2), 103.93 (C-1′), 95.80 (C-1″), 87.37 (C-1), 70.7−70.2 (4*-*C*H_2Ph), 22.26 (−*C*H_3 (Ac)) ppm. MS (ESI): *m/z* 946 $[M + H]^+$, 968 $[M + Na]^+$, 984 $[M + K]^+$. Exact mass calcd for $C_{51}H_{63}NO_{16}Na^+$: 968.4045, found 968.4036.

***N*-Acetyl-*N*-(3-thio-(2-aminoethyl))propyl-2-acetamido-2-deoxy-β-D-glucopyranosylamine (13).** Compound **9** (2 g, 6.6 mmol) and cysteamine (2.54 g, 33 mmol, 5 equiv) were dissolved in methanol and reacted according to method 3 (TLC EtOAc/AcOH/H_2O, 3:3:1). The residue was applied to a MPLC and eluted with EtOAc/AcOH/H_2O, 3:1:1 then 3:3:1 to yield compound **13** (2 g, 5.3 mmol, 80%). $[\alpha]^{20}_D = 14$ (*c* = 1.0, H_2O). ^{1}H NMR (300 MHz, D_2O): δ = 5.80 (d, −NH_2), 5.24 (d, H-1), 4.14 (dd−t, 1H, H-3 or H-4), 4.04 (dd, H-6a), 3.88 (dd, H-6b), 3.84−3.46 (m, 5H, H-2, H-3 or H-4, H-5, −CH_2), 3.41−3.33 (m, 2H, −CH_2), 3.06−2.97 (m, 2H, −CH_2), 2.82−2.69 (m, 2H, −CH_2), 2.35 and 2.30 (2*s, 3H, −CH_3 (Ac)), 2.12 and 2.10 (2*s, 3H, −CH_3 (Ac)), 2.09 and 1.88 (m, 2H, −CH_2); $J_{1,2}$ = 9.3, $J_{5,6a}$ = 3.2, $J_{5,6b}$ = 5.7, $J_{6a,6b}$ = 12.5 Hz. ^{13}C NMR (75 MHz, D_2O): δ = 177.76−174.68 (2*-*C*=O), 86.29 (C-1), 78.83 and 75.09 (C-4 and C-3), 70.22 (C-5), 61.51 (C-6), 53.48 (C-2), 41.50, 38.94, 38.39, 33.98, and 29.80 (5*-*C*H_2), 22.54, 21.86 and 21.43 (2*-*C*H_3 (Ac)) ppm. MS (ESI): *m/z* 380 $[M + H]^+$. Exact mass calcd for $C_{15}H_{29}N_3O_6SH^+$: 380.1855, found 380.1835.

***N*-Acetyl-*N*-(3-thio(2-aminoethyl))propyl-(α-D-glucopyranosyl)-(1→4)-β-D-glucopyranosylamine (14).** Compound **10** (2 g, 4,7 mmol) and cysteamine (1.82 g, 23.6 mmol, 5 equiv) were dissolved in methanol and processed according to method 3 (TLC EtOAc/AcOH/H_2O, 3:3:1). The residue was subjected to MPLC and eluted with EtOAc/AcOH/H_2O, 3:1:1 then 3:3:1 to yield compound **14** (1.88 g, 3.75 mmol, 80%). $[\alpha]^{20}_D = 77$ (*c* = 1.0, H_2O). MS (ESI): *m/z*: 501 $[M + H]^+$, 523 $[M + Na]^+$. Exact mass calcd for $C_{19}H_{36}N_2O_{11}SH^+$: 501.2118, found 501.2120.

***N*-Acetyl-*N*-(3-thio(2-aminoethyl))propyl(β-D-glucopyranosyl)-(1→4)-β-D-glucopyranosylamine (15).** Compound **11** (1.0 g, 2.4 mmol) and cysteamine (910 mg, 11.8 mmol, 5 equiv) were processed according to method 3 (TLC EtOAc/AcOH/H_2O, 3:3:1). The residue was subjected to MPLC and eluted with EtOAc/AcOH/H_2O, 3:1:1 then 3:3:1. Recrystallization from EtOAc/MeOH yielded pure compound **15** (845 mg, 1.7 mmol, 71%). $[\alpha]^{20}_D = 4$ (*c* 1.0, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 5.58 (d, −NH_2), 5.20 (d, H-1), 4.69 (d, H-1′), 4.14 (dd−d, H-6a), 4.10 (dd−d, H-6′a), 4.00 (dd, H-6b), 3.96−3.80 (m, 5H, H-6′b, H-5, H-2, H-3, and H-4), 3.74 (m_c, 1H, −CH_2), 3.67 (m_c, 2H, H-3′ and H-5′), 3.62−3.56 (m, H-4′), 3.54−3.47 (m, 2H, H-2′ and −CH_2), 3.43−3.37 (m, 2H, −CH_2), 3.08−3.01 (m, 2H, −CH_2), 2.85−2.75 (m, 2H, −CH_2), 2.41 and 2.39 (2*s, 3H, −CH_3 (Ac)), 2.24−1.96 (m, 2H, −CH_2); $J_{1,2}$ = 8, $J_{2,3}$ = 9, $J_{6a,6b}$ = 12, $J_{1',2'}$ = 7.2, $J_{2',3'}$ = 9.5, $J_{6'a,6'b}$ = 12.5 Hz. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 176.84 and 176.00 (-*C*=O), 103.12 (C-1′), 87.50 (C-1), 78.89 and 77.64 (C-2 and C-3), 76.66 and 76.18 (C-3′ and C-5′), 75.74 (C-5), 73.82 (C-2′), 70.58 (C-4), 70.16 (C-4′), 61.32 (C-6′), 60.80 (C-6), 39.05 (-*C*H_2), 29.75 (-*C*H_2), 28.95, 28.78, and 28.51 (3*-*C*H_2), 22.17 and 22.01 (-*C*H_3 (Ac)) ppm. MS (ESI): *m/z*: 501 $[M + H]^+$, 523 $[M + Na]^+$. Exact mass calcd for $C_{19}H_{36}N_2O_{11}SH^+$: 501.2118, found 501.2120.

***N*-Acetyl-*N*-(3-thio(2-aminoethyl))propyl (β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosylamine (16).** Compound **12** (1.55 g, 3.67 mmol) and cysteamine (1.41 g, 18.34 mmol, 5 equiv) were dissolved in methanol and processed according to method 3 (TLC EtOAc/AcOH/H_2O, 3:3:1). The residue was applied to MPLC and eluted with EtOAc/AcOH/H_2O, 3:1:1 then 3:3:1 to yield **16** (1.52 g, 3 mmol, 82%). $[\alpha]^{20}_D$ = 21 (c 1.0, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 5.50 (d, −NH_2), 5.11 (d, H-1), 4.56 (d, H-1′), 4.04 (m_c, H-3), 4.02 (d, H-4′), 3.95−3.72 (m, 9H, H-4, H-5, H-5′, H-6a, H-6b, H-6′a, H-6′b, H-2, H-3′), 3.65 (dd, H-2′), 3.50−3.38 (m, 2H, −CH_2-(a)), 3.35−3.29 (m, 2H, −CH_2(e)), 3.00−2.93 (m, 2H, −CH_2-(d)), 2.77−2.67 (m, 2H, −CH_2(c)), 2.34 and 2.32 (2*s, 3H, −CH_3 (Ac)), 2.18−1.86 (m, −CH_2(b)); $J_{1,2}$ = 8.7, $J_{1',2'}$ = 8.3, $J_{2',3'}$ = 10, $J_{3',4'}$ = 3.9 Hz. ^{13}C NMR (100.6 MHz, D_2O): δ = 176.03 (−*C*=O), 103.50 (C-1′), 83.61 (C-1), 78.63, 77.47, 76.00, 75.80, 73.19, 71.60, and 70.52 (C-2, C-3, C-4, C-5, C-2′, C-3′, and C-5′), 69.21 (C-4′), 61.68 (C-6), 60.81 (C-6′), 39.08 (−*C*H_2(e)), 28.94, 28.78, 28.73, and 28.53 (−*C*H_2(c), −*C*H_2(d), −*C*H_2(a), and −*C*H_2(b)), 22.23 and 21.99 (−*C*H_3 (Ac)) ppm. MS (ESI): *m/z*: 501 $[M + H]^+$, 523 $[M + Na]^+$. Exact mass calcd for $C_{19}H_{36}N_2O_{11}SH^+$: 501.2118, found 501.2135.

***N*-Acetyl-*N*-(3-thio(2-aminoethyl))propyl(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosylamine (24).** Compound **23** (2 g, 2.1 mmol) and cysteamine (1.2 g, 10.6 mmol, 5 equiv) were dissolved in methanol and processed according to method 3 (TLC EtOAc/MeOH/AcOH, 3:3:1). After concentration, the residue was dissolved in CH_2Cl_2 and successively washed with brine and water. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was applied to MPLC and eluted with EtOAc/MeOH 10:1 (to elute the starting material first) and then a linear gradient from 1:1 to 1:5 was applied to elute compound **24** (1.61 g, 1.57 mmol, 75%), which after concentration was yielded as a colorless foam. $[\alpha]^{20}_D$ = 63 (*c* = 1.0, CH_3OH). ^{1}H NMR (300 MHz, MeOH (d_4)): δ = 7.35 (m_c, H-arom), 5.55 (m_c, NH_2), 4.56 (dd−d, H-4′), 3.40 (m_c, −CH_2), 2.95, 2.75 and 2.63 (3*t, 6H, 3*-CH_2), 2.27 (s, 3H, −CH_3 (Ac)), 2.10−1.90 (m, 2H, −CH_2); $J_{3'-4'}$ = 3.8 Hz. ^{13}C NMR (75.5 MHz, MeOH (d_4)): δ = 174.65 (−*C*=O), 140.00−128.56 (C-arom), 105.02 (C-1′), 96.46 (C-1″), 88.73 (C-1), 80.85, 80.49, 80.07, 78.79, 77.55, 77.38, 76.58 and 76.42 (8*-*C*H), 75.98, 75.03, 74.12, and 73.58 (4*-*C*H_2-Ph), 71.67, 70.91, and 70.65 (3*-*C*H), 69.87 (C-6″), 66.78 (C-4′), 62.49 (C-6), 62.14 (C-6′), 41.66, 41.04, 34.05, 30.09, and 29.74 (5*-*C*H_2 (a−e)), 22.12 (−*C*H_3 (Ac)) ppm. MS (ESI): *m/z* 1023 $[M + H]^+$. Exact mass calcd for $C_{53}H_{70}N_2O_{16}SH^+$: 1023.4525, found 1023.4523.

***N*-Acetyl-*N*-(3-thio(2-aminoethyl))propyl(α-D-galactopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosylamine (25).** Ammonia (∼50 mL) was condensed by passing through a dry ice condenser (−78 °C) to which **24** (749 mg, 0.73 mmol) was added. To this suspension, stirred at −78 °C under argon, was added sodium in small aliquots, until the blue color of the solution persisted for at least 10 min. The completion of the reaction was checked by TLC (*i*-PrOH/CH_3COONH_4 (1 M), 2:1), after which ammonium chloride was added carefully until the blue color disappeared. The reaction mixture was concentrated to dryness by a stream of argon, yielding a slightly yellow residue. This was applied to Sephadex column chromatography (*d* = 25 mm, G-10) and eluted with water. After pooling the product fractions and lyophilization, **25** was obtained as a yellowish powder (480 mg, 0.72 mmol, quantitative yields) and could not be further purified; it was used as such in the next step. MS (ESI): *m/z* 663 $[M + H]^+$, 685 $[M + Na]^+$; daughters of 663: 501 [(M − Gal) + H]$^+$, 339 [(M − 2*Gal) + H]$^+$, 177 [(M − 2*Gal-Glu) + H]$^+$; daughters of 685: 523 [(M − Gal) + Na]$^+$, 361 [(M − 2*Gal) + Na]$^+$, 199 [(M − 2*Gal-Glu) + Na]$^+$. Exact mass calcd for $C_{25}H_{46}N_2O_{16}SH^+$: 663.2646, found 663.2603.

***N*-Acetyl-*N*-(3-thio-(2-acetamidoethyl))propyl(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→3)-(3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosylamine (25-Ac).** Compound **25** (130 mg, 0.19 mmol) was acetylated according to the general method (TLC: toluene/acetone, 1:1). The residue was applied to silica gel chromatography (toluene/acetone, 2:1 to 1:2) to give **25-Ac** as a colorless powder (180 mg, 0.16 mmol, 83%). ^{1}H NMR (600

MHz, $CDCl_3$): δ = 5.43 (dd, H-4″), 5.32 (dd∼m, H-4′), 5.23 (m_c, 3H, H-2, H-3, H-1″), 5.13 (dd−t, H-2′), 5.07 (m_c, 2H, H-2″ and H-3″), 4.97 (d, H-1), 4.45 (dd−d, H-6a), 4.43 (d, H-1′), 4.21−4.00 (m, 6H, H-5″, H-6b, H-6′a, H-6′b, H-6″a and H-6″b), 3.85−3.64 (m, 4H, H-3′, H-4, H-5, and H-5′), 3.58 (m_c, 1H, $-CH_2$), 3.39 (m_c, 2H, $-CH_2$), 3.26 (m_c, 1H, $-CH_2$), 2.63 (m_c, 2H, $-CH_2$), 2.47 (m_c, 2H, $-CH_2$), 2.16−1.90 (m, 36 H, 12*-CH_3 (Ac)), 1.74 (m_c, 2H, $-CH_2$); $J_{1,2}$ = 8.0, $J_{6a,6b}$ = 11.8, $J_{1',2'}$ = 8.5, $J_{2',3'}$ = 9.4, $J_{2'',3''}$ = 9.7, $J_{3'',4''}$ = 1.5 Hz. ^{13}C NMR (150.7 MHz, $CDCl_3$): δ = 170.67−168.55 (12*-*C*=O), 101.15 (C-1′), 93.40 (C-1″), 85.49 (C-1), 75.61 and 75.36 (C-5 and C-5′), 72.95 (C-3), 72.73 (C-3′), 70.73 (C-4), 69.71 (C-2′), 68.88 and 67.11 (C-2″ and C-3″), 67.60 (C-4″), 66.79 (C-5″), 66.42 (C-2), 64.52 (C-4′), 61.66 (C-6), 61.20 and 60.83 (C-6′ and C-6″), 43.09 ($-CH_2$), 38.43 ($-CH_2$), 30.23 ($-CH_2$), 28.89 ($-CH_2$), 28.37 ($-CH_2$), 23.19−20.45 (12*-CH_3 (Ac)) ppm. MS (ESI): *m/z* 1148 $[M + Na]^+$.

[*N*^{1}-(Benzyloxycarbonyl)-*N*^{4}-[*N*-acetyl-*N*-(3-thio(2-acetamidoethyl))propyl(2-acetamido-2-deoxy-β-D-glucopyranosylamine)]-L-glutamyl]-L-glycine (26). Compound **13** (300 mg, 0.79 mmol), Z-Gln-Gly (266 mg, 0.79 mmol), and TGase (526 mg) were dissolved in buffer (26 mL) and processed according to method 4. The coupling could only be detected by mass spectrometry analysis on the crude mixture, the product could not be isolated. MS (ESI): *m/z* 698 $[M - H]^-$.

[*N*^{1}-(Benzyloxycarbonyl)-*N*^{4}-[*N*-acetyl-*N*-(3-thio-(2-acetamidoethyl))propyl((α-D-glucopyranosyl)-(1→4)-β-D-glucopyranosylamine)]-L-glutamyl]-L-glycine (27). Compound **14** (300 mg, 0.6 mmol), Z-Gln-Gly (200 mg, 0.6 mmol), and TGase (400 mg) were reacted according to method 4. The buffered solution (20 mL) was shaken for few hours until no further cross-linking was detected on TLC (EtOAc/AcOH/H_2O, 3:1:1). After inactivation of the enzyme and lyophilization, the residue was applied to a MPLC and eluted with EtOAc/AcOH/H_2O, 6:2:1. The appropriate fractions were pooled and concentrated, and the resulting residue was further purified by Sephadex gel chromatography (G-10, eluted with water). Product-containing fractions were lyophilized to yield **27** (336 mg, 0.4 mmol, 68%). $[\alpha]^{20}_D$ = 83 (*c* = 1.0, H_2O). ^{1}H NMR (600 MHz, D_2O): δ = 7.45−7.37 (m, 5 H-arom), 5.41−5.36 (m, H-1′), 5.15 and 5.07 (each d, each 1 H, $-CH_2$Ph), 4.98 (d, H-1), 4.15−4.10 (m, $-CH$ (Gln)), 3.90−3.45 (m, 5* $-CH_2$: H-6, H-6′, H−f, H−g, H−h; and 7*-C*H*: H-2, H-2′, H-3, H-3′, H-4′, H-5, H-5′), 3.40 (dd−t, H-4), 3.35−3.25 (m, 2H,-CH_2(e)), 2.67−2.60 (m, 2H, $-CH_2$(d)), 2.57−2.43 (m, 2H, $-CH_2$(a)), 2.39−2.30 (m, 2H, $-CH_2$(c)), 2.19 (2*s−s, 3H, $-CH_3$ (Ac)), 2.13 and 1.87 (2*m_c, 2*1H, $-CH_2$(b)); $J_{1,2}$ = 8.5, $J_{3,4}$ or $J_{3',4'}$ = 9.5, $J_{4,5}$= 9.5, $J_{6a,6b}$ or $J_{6'a,6'b}$ = 12.0 Hz. ^{13}C NMR (150.7 MHz, D_2O): δ = 175.16−175.53 (5**C*=O), 136.12 (C-arom), 128.70−127.69 (5*-*C*H arom), 99.57 (C-1′), 86.73 (C-1), 77.19−69.20 (8**C*H: 2, 2′, 3, 3′, 4, 4′, 5, 5′; 2*-CH_2), 67.17 (-CH_2-Ph), 60.73 and 60.36 (C-6 and C-6′), 54.46 (−CH (Gln)), 43.33 ($-CH_2$(g)) 38.42 ($-CH_2$(e)), 31.87 ($-CH_2$(c)), 29.10 ($-CH_2$(d)), 27.85 ($-CH_2$(a)), 27.12 ($-CH_2$(b)), 21.41 and 21.25 ($-CH_3$ (Ac)) ppm. MS (ESI): *m/z* 865 $[M/Na + Na]^+$ (daughter 757 $[(M/Na - OCH_2Ph) + Na]^+$), 859 $[M + K]^+$ (daughter 751 $[(M - OCH_2$-Ph) + $K]^+$, 843 $[M + Na]^+$ (daughter 735 $[(M - OCH_2Ph) + Na]^+$, 819 $[M - H]^-$ (daughter 711 $[(M - OCH_2Ph) - H]^-$. Exact mass calcd for $C_{34}H_{52}N_4O_{17}SNa^+$ 843.2946, found 843.2984.

[*N*^{1}-(Benzyloxycarbonyl)-*N*^{4}-[*N*-acetyl-*N*-(3-thio-(2-acetamidoethyl))propyl((β-D-glucopyranosyl)-(1→4)-β-D-glucopyranosylamine)]-L-glutamyl]-L-glycine (28). Compound **15** (300 mg, 0.6 mmol), Z-Gln-Gly (200 mg, 0.6 mmol), and TGase (400 mg) were reacted according to the general method. The buffered solution (20 mL) was shaken for few hours until no further cross-linking was detected on TLC (EtOAc/AcOH/H_2O, 3:1:1). After inactivation of the enzyme and lyophilization, the residue was applied to MPLC and eluted with EtOAc/AcOH/H_2O, 6:2:1. The residue was further purified by Sephadex G-10 gel chromatography (eluted with water). Appropriate fractions were lyophilized to yield colorless fluffy **28** (236 mg, 0.29 mmol, 48%). $[\alpha]^{20}_D$ = −8 (*c* = 1.0, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 7.59 (m_c, 5H arom), 5.55 (d, −N*H*), 5.33 and 5.25 (each d, each 1H, $-CH_2$Ph), 5.16 (d, H-1), 4.66 (d, H-1′), 4.33−4.23 (m, 1H, $-CH$ (Gln)), 4.11 (dd−d, H-6a), 4.07 (dd−d, H-6′a), 3.96 (dd, H-6b), 3.93−3.77 (m, 7H, H-6′b, H-4, H-5, $-CH_2$, H-2 and H-3), 3.70−3.45 (m, 8H, H-3′, H-5′, H-4′, 2*-CH_2 and H-2′), 2.85−2.78 (m, 2H, $-CH_2$), 2.75−2.60 (m, 2H, $-CH_2$), 2.54−2.46 (m, 2H, $-CH_2$), 2.37 and 2.36 (2*s, 3H, $-CH_3$ (Ac)), 2.30 (m_c, 1H, $-CH_2$), 2.16−2.00 (m, 3H, $-CH_2$ + $-CH_2$ (1H)); $J_{1,2}$ = 7.8, $J_{1',2'}$ = 8.4 $J_{6a,6b}$ = 12.0, J_{CH2Ph} = 12.0 Hz. ^{13}C NMR (100.6 MHz, D_2O): δ = 176.73−174.09 (5*-*C*=O), 136.24 (C arom), 129.43, 129.09 and 128.45 (5*-*C*H arom), 103.15 (C-1′), 87.54 (C-1), 78.98 and 77.51 (C-2 and C-3), 77.66 and 76.25 (C-5′ and C-3′), 75.75 (C-5), 73.82 (C-2′), 70.59 (C-4), 70,14 (C-4′), 67.84 (-CH_2-Ph), 61.25 (C-6), 60.88 (C-6′), 55.26 (−*C*H (Gln)), 43.98 ($-CH_2$(g)) 39.21 ($-CH_2$(e)), 32.55 ($-CH_2$(c)), 29.13 ($-CH_2$(d)), 28.71 ($-CH_2$(a)), 27.90 ($-CH_2$(b)), 22.18 and 21.97 ($-CH_3$ (Ac)) ppm. MS (ESI): *m/z*: 843 $[M + Na]^+$, 865 $[M + 2Na - H]^+$. Exact mass calcd for $C_{34}H_{52}N_4O_{17}SNa^+$ 843.2496, found 843.2922.

[*N*^{1}-(Benzyloxycarbonyl)-*N*^{4}-[*N*-acetyl-*N*-(3-thio-(2-acetamidoethyl))propyl((β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosylamine)]-L-glutamyl]-L-glycine (29). Compound **16** (300 mg, 0.6 mmol), Z-Gln-Gly (200 mg, 0.6 mmol), and TGase (400 mg) were reacted according to method 4. The buffered solution (20 mL) was shaken for few hours until no further cross-linking was detected by TLC (EtOAc/AcOH/H_2O, 3:1:1). After heat inactivation of the enzyme and lyophilization, the residue was applied to MPLC and eluted with EtOAc/AcOH/H_2O, 6:2:1. The product-containing fraction was further purified on Sephadex G-10 gel chromatography (eluted with water). Appropriate fractions were lyophilized to yield white fluffy **29** (438 mg, 0.53 mmol, 89%). $[\alpha]^{20}_D$ = 3 (*c* 1.0, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 7.50 (m_c, 5H arom), 5.50 (d, −NH), 5.27 and 5.19 (each d, each 1H, $-CH_2$Ph), 5.11 (d, H-1), 4.55 (d, H-1′), 4.30−4.20 (m, $-CH$ (Gln)), 4.09−3.99 (m, 2H, H-6), 3.98−3.55 (m, 15 H, H-6′, H−f, H−g, H−h, H-2, H-2′, H-3, H-3′, H-4, H-4′, H-5, and H-5′), 3.49−3.39 (m, 2H, $-CH_2$(e)), 2.80−2.71 (m, 2H, $-CH_2$(d)), 2.70−2.58 (m, 2H, $-CH_2$(a)), 2.50−2.42 (m, 2H, $-CH_2$(c)), 2.32 and 2.31 (2*s, 3H, $-CH_3$(Ac)), 2.23 and 2.00 (2*m_c, 2*1H, $-CH_2$(b)); $J_{1,2}$ = 8.3, $J_{6a,6b}$ = 12.0, $J_{1',2'}$ = 7.7, J_{CH_2Ph} = 12.4 Hz. ^{13}C NMR (150.7 MHz, D_2O): δ = 178.45−176.12 (5**C*=O), 137.27 (C arom), 131.09, 130.79, and 130.10 (5*-*C*H arom), 105.19 (C-1′), 89.16 (C-1), 80.45−70.88 (8*-*C*H: 2, 2′, 3, 3′, 4, 4′, 5, 5′; 2*-CH_2), 69.56 ($-CH_2$Ph), 63.31 and 62.62 (C-6 and C-6′), 56.34 (−CH (Gln)), 44.79 ($-CH_2$(g)) 40.84 ($-CH_2$(e)), 34.24 ($-CH_2$(c)), 32.77 ($-CH_2$(d)), 30.21 (CH_2-(a)), 29.53 (CH_2(b)), 23.82 and 23.69 ($-CH_3$(Ac)) ppm. MS (ESI): *m/z* 865 $[M + 2Na - H]^+$, 843 $[M + Na]^+$, 821 $[M + H]^+$, 819 $[M - H]^-$, 711 $[M - H - OBn]^-$ (daughter ion of 819). Exact mass calcd for $C_{34}H_{52}N_4O_{17}SNa^+$: 843.2946, found 843.2922.

[*N*^{1}-(Benzyloxycarbonyl)-*N*^{4}-[*N*-acetyl-*N*-(3-thio(2-acetamidoethyl))propyl((α-D-galactopyranosyl)-(1→3)-(β-D-galactopyranosylamine)-(1→4)-β-D-glucopyranosyl]-L-glutamyl]-L-glycine (17). Trisaccharide **25** (100 mg, 0.15 mmol), Z-Gln-Gly (51 mg, 0.15 mmol), and TGase (100 mg) were reacted according to method 4. The buffered solution (5 mL) was shaken for few hours until no further cross-linking was detected by TLC (EtOAc/AcOH/H_2O, 3:3:1). Upon heat inactivation of the enzyme and concentration, the residue was applied to silica gel chromatography (MPLC, EtOAc/AcOH/H_2O, 3:1:1). Product-containing fractions were pooled, concentrated and purified further by sephadex G-10 gel chromatography (eluted with water). The fractions containing the desired compound were lyophilized to yield **17** (93 mg, 0.095 mmol, 63%). ^{1}H NMR (600 MHz, D_2O): δ = 7.28 (m_c, 5H, H-arom), 5.01 and 4.94 (each d, each 1H, $-CH_2$Ph), 4.98 (d, H-1″), 4.86 (d, H-1), 4.35 (d, H-1′), 4.03 (dd−t, 1H), 4.01 (dd−d, H-4′), 3.97 (m_c, −*C*H (Gln)), 3.86 (d, 1H), 3.79 (dd, H-6a), 3.76 (m_c, 1H), 3.71 (dd, H-6b), 3.68−3.25 (m, 20H, 14*-*C*H and 3*-CH_2)), 3.17 (m_c, 2H, $-CH_2$(e)), 2.49 (m_c, 2H, $-CH_2$(d)), 2.37 (m_c, 2H, $-CH_2$-(a)), 2.20 (m_c, 2H, $-CH_2$(c)), 2.06 and 2.05 (2*s, 3H, $-CH_3$ (Ac)), 2.00 and 1.80 (2*m_c, 2*1H, $-CH_2$(b)); $J_{1,2}$ = 8.6, $J_{5,6a}$ = 3.0, $J_{5,6b}$ = 4.0, $J_{6a,6b}$ = 10.3, $J_{1',2'}$ = 7.9, $J_{3',4'}$ = 2.3, J_{CH_2Ph} = 12.4 Hz. ^{13}C NMR (150.7 MHz, $CDCl_3$): δ = 178.78−173.52 (5*-*C*=

O), 136.17 (C arom), 128.74, 128.40, and 127.75 (5*CH arom), 102.76 (C-1′), 95.36 (C-1″), 86.76 (C-1), 78.07, 77.12, 76.99, 76.80, 75.39, 75.11, 74.99, 70.74, 69.75, 69.47, 69.17, 69.06, and 68.08 (11*-*C*H and 2*-*C*H_2), 67.18 (−*C*H_2Ph), 64.74 (C-4′), 60.93, 60.84, and 60.16 (C-6, C-6′, and C-6″), 54.49 (−*C*H (Gln)), 43.23 (−*C*H_2(g)), 38.41 (−*C*H_2(e)), 30.17 (−*C*H_2(c)), 27.88 (−*C*H_2(a)), 27.16 (−*C*H_2(b)), 21.47 and 21.30 (−*C*H_3 (Ac)) ppm. MS (ESI): *m*/*z*: 981 $[M - H]^-$, 873 $[M - (BnO)]^-$ (daughter ion). Exact mass calcd for $[C_{40}H_{62}N_4O_{22}S - H]^-$ 981.3498, found 981.3531.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra of compounds **7**, **8**, **14**−**18**, **20**, **22**, **24**, **25-Ac**, and **27**−**29**. This material is available free of charge via the Internet at http://pubs.acs.org.

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