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# Galactosylation with β-Galactosidase from Bovine Testes Employing Modified Acceptor Substrates†

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**Abstract**—In this study β1-3 linked analogues of the T-antigen determinant were synthesized in preparative scale by transgalactosylation using β-galactosidase from bovine testes to give synthetic antigens. Acceptors with modifications of the sugar residue such as α-glycosylated spacers, as well as GlcNAc-αOR- and 2dGal-αOR-substrates opened further possibilities for galactosylation. © 1997 Elsevier Science Ltd.

## Introduction

As the amazing diversity of carbohydrate structures is intimately related to their large variety of biological functions, the need for cheap, synthetically produced oligosaccharides has increased. Even though in recent years some elegant methods for glycosylation have been developed and previously existing methods have been improved, approaches towards such structures following classical organic synthetic means is often restrained by the complex protective group chemistry, leading to time-consuming multistep syntheses with low overall yields.[1-3]

As most of the complex hetero-oligosaccharides of interest are produced in nature by enzymes, it is entirely plausible to use the synthetic potential of enzymes in vitro as well. Instead of multistep chemical reactions, the synthetic process would be expected to work regio- and stereospecifically and the products would become available more easily.[4]

We have been concerned with the synthesis of analogues of the T-antigen determinant[5] in which 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranose is α-glycosidically linked to L-serine or L-threonine, using β-galactosidase from bovine testes. Our interest in this work has been focused on an extension with regard to acceptor modifications such as α-glycosylated spacers, as well as GlcNAc-αOR- and 2dGal-αOR-substrates which are of considerable interest in enzymatically catalyzed reactions. Information about the acceptor substrates can lead to a better understanding of the mechanism involved in the reaction, and for the preparative chemist it is of interest to enhance the number of novel compounds by applying new acceptors.

## Results and Discussion

Our investigations started with efficient syntheses of the acceptor substrates **4.1–4.5**, **4.6**,[6] **4.7**,[7] **4.8**,[8] **4.9**,[9] **4.10**,[10] and **4.11**[10] (Table 1).

The synthetic approach to the acceptor derivatives **4.1–4.3** is shown in Figure 1. Azidonitration conditions[11] were employed to tri-*O*-acetyl-galactal followed by trichloro-acetimidate chemistry.[12] The crucial glycosylation of 3-oxo-5-tosyl-1,5-pentanediol[13] was realized by using the β-imidate in the presence of trimethylsilyl triflate, to give the α-glycoside **1**. Reduction of the azide under hydrogenolysis conditions using palladium-carbon as a catalyst and subsequent acetylation of the product gave the acetamido derivative **2**. Further functionalization, as recently outlined,[14] led to the precursors **3.1–3.3**, and deprotection using methanolic sodium hydroxide afforded the target compounds **4.1–4.3** in a preparative scale.

The acceptor derivatives **4.4** and **4.5** were synthesized employing both classical chemistry as well as enzymatic condensation conditions.[15,16] Enzymatic synthesis was observed employing L-serine or L-threonine and *N*-acetyl-D-galactosamine by reverse hydrolysis with α-*N*-acetyl galactosaminidase isolated from beef liver (EC 3.2.1.49) (Fig. 2). In comparison the chemical method proved to be superior with regard to yield and expense of work.[15]

The synthesis of all β1-3 linked disaccharide derivatives **5.1–5.10** are based on the transgalactosylation of **4.1–**

†This paper is dedicated to the 75th anniversary of Professor Dr Hans Paulsen.

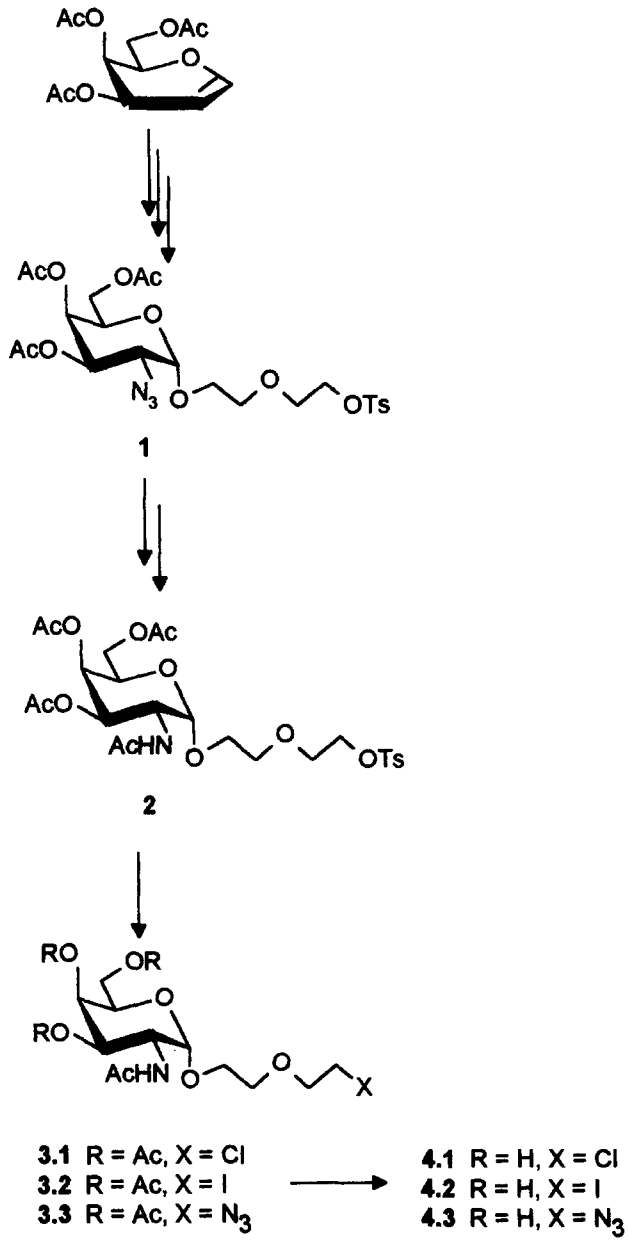



Figure 1.

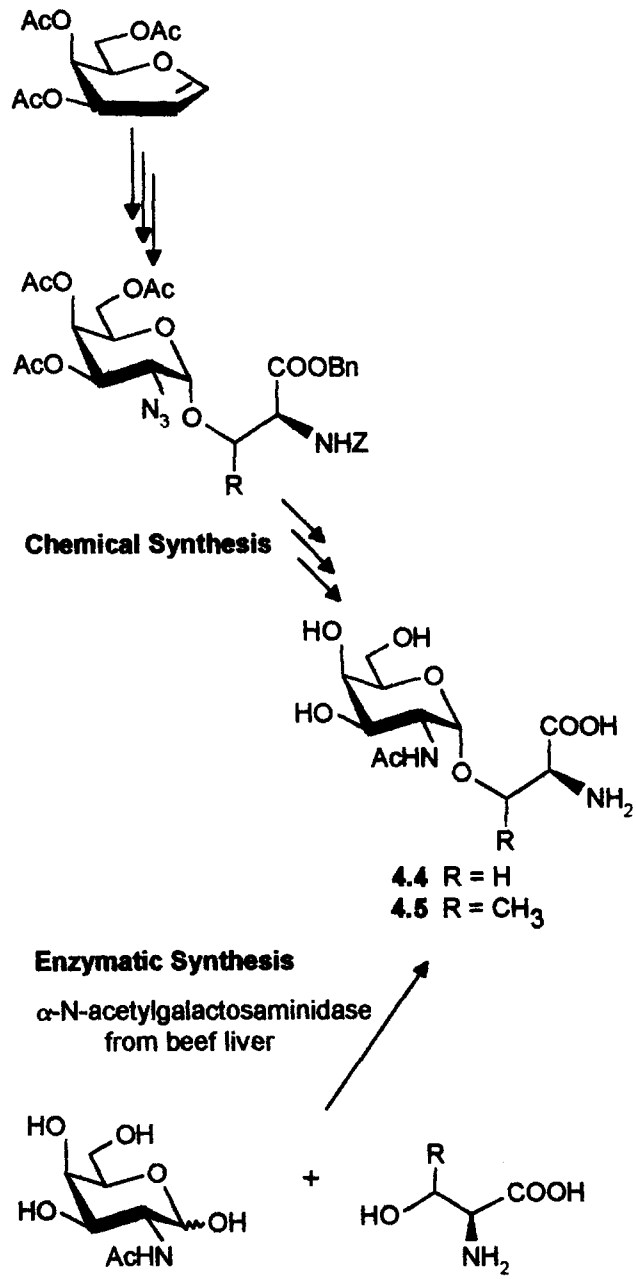


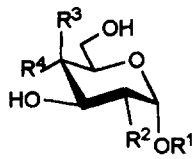

Figure 2.

**4.11** using β-galactosidase from bovine testes (EC 3.2.1.23) (Fig. 3).[15]

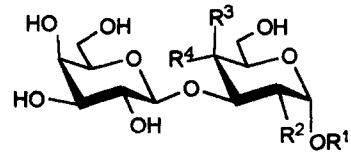
The bovine testes enzyme is commercially available but too expensive to be used in large-scale chemistry. A simplified purification procedure[17] was performed, which followed the initial steps described by Distler and Jourdian.[18] Working with crude enzyme preparation can prove to be problematic, for example, there is always a risk of destroying enzymes due to the presence of proteases, and there may be undesirable reactions due to other contaminant enzymes. Whereas in the crude enzyme mixture hydrolysis of GalNAcβ1-OR derivatives was observed due to contamination with β-hexosaminidase, no contamination with α-hexosaminidase could be detected after the simple purification procedure. Glucose, *N*-acetyl galactosamine, and *N*-acetyl glucosamine are known to be excellent acceptors whereas mannose, L-arabinose and L-fucose were only slightly active.[18]

These results were in accordance with our investigations of the various α-glycosylated *N*-acetyl galactosamine (**4.1–4.7**) and *N*-acetyl glucosamine analogues (**4.8** and **4.9**). A surprising result was observed when 2-deoxy galactose (2-deoxy-D-lyxo-hexose) derivatives were galactosylated: allyl 2-deoxy-α-D-galactopyranoside (**4.10**) turned out to be a good substrate for β-galactosidase whereas the anomeric mixture of 2-deoxy-D-galactopyranose (**4.11**) itself was not accepted by the enzyme. A plausible explanation for this specificity is presently not at hand.

Table 1.



4.1 - 4.11



5.1 - 5.10

| Substitution pattern | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| 1 | $-(CH_2)_2O(CH_2)_2Cl$ | NHAc | OH | H |
| 2 | $-(CH_2)_2O(CH_2)_2I$ | NHAc | OH | H |
| 3 | $-(CH_2)_2O(CH_2)_2N_3$ | NHAc | OH | H |
| 4 | $-CH_2CH(NH_2)COOH$ | NHAc | OH | H |
| 5 | $-CH(CH_3)CH(NH_2)COOH$ | NHAc | OH | H |
| 6 | $-p\text{-}NO_2\text{-}C_6H_4$ | NHAc | OH | H |
| 7 | $-CH_2CH=CH_2$ | NHAc | OH | H |
| 8 | $-CH_2CH=CH_2$ | NHAc | H | OH |
| 9 | $-CH_2\text{-}C_6H_5$ | NHAc | H | OH |
| 10 | $-CH_2CH=CH_2$ | H | OH | H |
| 11 | -H | H | OH | H |

It is known that β-galactosidase from bovine testes hydrolyses 1-3-, 1-4-, and 1-6-linkages of *N*-acetyl lactosamine derivatives and isomers. In the enzymatic synthesis of the disaccharides **5.1–5.10** (Table 1) the equilibrium was shifted to result in the formation of a 1-3-interglycosidic linkage by reversing the hydrolytic activity of this galactosidase; the corresponding yields are summarized in Table 2. For better NMR-characterization of the products **5.1–5.3**, **5.8**, and **5.9** the disaccharides were treated with pyridine and acetic anhydride following the general conditions to obtain the acetylated compounds **6.1–6.3**, **6.8**, and **6.9**.

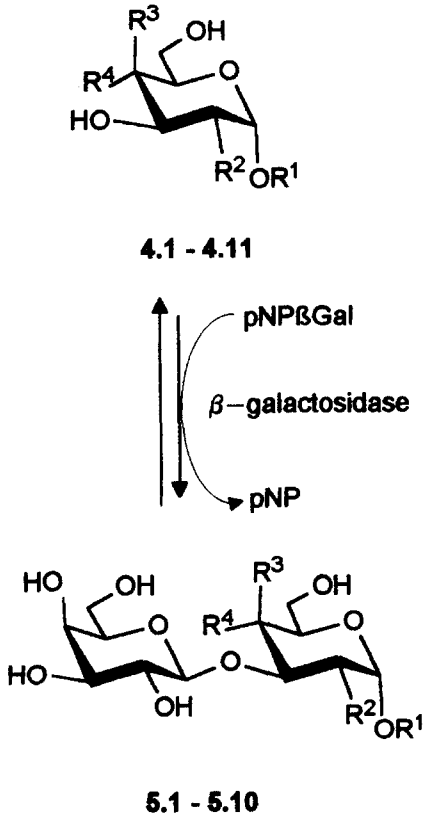



Figure 3.

Formation of 1-4- or 1-6-linkages was not observed under these conditions. Instead of lactose, *p*-nitrophenyl β-galactopyranoside (*p*NPβGal) was used as the donor to simplify the purification procedure, and furthermore the rate of galactose released could be raised.[19]

## Conclusion

In this study the efficient preparation of analogues (**5.1–5.10**) of the T-antigen determinants could be demonstrated combining the advantage of highly developed chemical synthesis with the selectivity of enzymatic transfer of carbohydrates.

Table 2

| Acceptor | Product | Yield |
|---|---|---|
| **4.1** | **5.1** | 40% |
| **4.2** | **5.2** | 35% |
| **4.3** | **5.3** | 35% |
| **4.4** | **5.4**[15,23] | 22% |
| **4.5** | **5.5**[15] | 28% |
| **4.6** | **5.6** | 21% |
| **4.7** | **5.7** | 22% |
| **4.8** | **5.8** | 13% |
| **4.9** | **5.9** | 32% |
| **4.10** | **5.10** | 15% |
| **4.11** | No product | — |

## Experimental

### Enzyme assay

The rate of hydrolysis of the substrate *p*-nitrophenyl β-D-galactopyranoside is determined by measuring the absorbance of the liberated *p*-nitrophenol in alkaline solutions described by Kuby and Lardy.[20] Incubation mixtures contained the following components in total volumes of 0.1 mL: sodium phosphate–citrate buffer, pH 4.3, as prepared by McIlvaine,[21] 25 μL; *p*-nitrophenyl β-Gal, 0.5 μmol; and 1–4 units of enzyme. Control tubes contained the same components but lacked either substrate or enzyme. Incubations were conducted for 30 min at 37 °C and were terminated by the addition of 1 mL of 0.25 M glycine buffer, pH 10. Absorbance was measured in cells with a 1-cm light path at 400 nm.

One Unit (U) is defined as the hydrolysis of 1 μmol of *p*NPβGal/min under the above conditions.

### Protein determination

Protein concentrations were determined using the method of Bradford.[22]

### Purification procedure

All manipulations were performed at 0–4 °C unless otherwise stated. Bovine testes (210 g) were obtained from a slaughterhouse and stored at −20 °C until used. The testes were thawed and homogenized with a blender. A 210 mL quantity of 0.1 M acetic acid was added, and the pH was adjusted to 4.0 by dropwise addition of 2 M HCl. The homogenate was stirred and centrifuged for 20 min at 10,000 *g*. Ammonium sulfate was added to the crude extract to 40% saturation and after stirring for 1 h, the precipitate was collected by centrifugation for 20 min at 10,000 *g* and dissolved in acetate buffer, pH 4.3. The solution was incubated at 50 °C for 15 min before centrifugation for 10 min at 20,000 *g*. The supernatant solution was dialysed against acetate buffer, pH 4.3 overnight and lyophilized. The specific activity was 30 mU/mg, and the total activity was 31 U.

### Chemical synthesis of the acceptor substrates

**Glycosylation. 3-Oxa-5-*O*-tosyl-pentyl-3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranoside (1).** To a mixture of *O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl)trichloro-acetimidate[12] (2.5 g, 5.2 mmol) and 3-oxa-5-tosyl-1,5-pentanediol (1.48 g, 5.7 mmol) in diethylether (50 mL) was added dropwise a 0.2 M solution of $Me_3SiOSO_2CF_3$ in diethylether (1.3 mL, 0.26 mmol) at −50 °C under argon. After stirring for 30 min at room temperature, the mixture was neutralized with sodium hydrogen carbonate, dried ($MgSO_4$) and evaporated in vacuo to give the α-glycoside **1** (4.0 g). The crude compound was used for the next reaction step.

**Reduction and acetylation of the azide. 3-Oxa-5-*O*-tosyl-pentyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (2).** A solution of crude **1** (4.0 g) in acetic acid (25 mL) was hydrogenolyzed over 5% Pd/C for 8 h. The catalyst was collected and the filtrate was concentrated in vacuo. The residue was dissolved in pyridine (20 mL) and acetic anhydride (10 mL) was added. After 2 h at room temperature the volatile components were evaporated in vacuo. Toluene was distilled from the residue. Column chromatography on silica gel with petrolether:ethyl acetate (2:1) gave **2** (2.5 g) in 85% yield; $R_f$ 0.62 in 3:2 petrolether:ethyl acetate; $^1$H NMR (400 MHz, $CDCl_3$) δ 7.80–7.36 (m, 4 H, Ph), 5.89 (d, 1 H, $J_{CH,NH}$ 9.6, NH), 5.38 (d, 1 H, $J_{3,4}$ 3.5, H-4), 5.19 (dd, 1 H, $J_{2,3}$ 11.7, H-3), 4.88 (d, 1H, $J_{1,2}$ 3.5, H-1), 4.58 (ddd, 1 H, $J_{1,2}$ 3.5, H-2), 4.20–4.10 (m, 1 H, H-5), 4.08–4.01 (m, 1 H, H-6a), 3.52–3.47 (m, 1 H, H-6b), 2.40 (s, 3 H, $CH_3$Ph), 2.16 (s, 3 H, $CH_3$CONH), 2.03, 1.99, 1.95 (s, 3 H, $CH_3$CO); $^{13}$C NMR (250 MHz, $CDCl_3$) δ 97.7 (C-1), 69.9 ($CH_2$-OTs), 68.5, 67.2, 66.5 (C-3, C-4, C-5), 61.7 (C-6), 47.5 (C-2); $C_{23}H_{35}O_{13}NS$ (565.60).

**Functionalization.** To a solution of **2** (200 mg, 0.35 mmol) in butanone (10 mL) was added the corresponding sodium salt (NaX, X = Cl, I, $N_3$, 1.5 equiv) and a catalytic amount of dicyclohexano-18-crown-6. Each mixture was stirred until the starting material had completely disappeared, which was monitored by $^1$H NMR. The solvent was distilled off in vacuo, the residue dissolved in dichloromethane, extracted with water, dried ($MgSO_4$), and concentrated in vacuo.

**5-Chloro-3-oxa-pentyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (3.1).** (156 mg, 95%); $[\alpha]_D$ +80 (*c* 1.0, chloroform); $R_f$ 0.65 in 3:2 petrolether:ethyl acetate; $^1$H NMR (400 MHz, $CDCl_3$) δ 5.69 (d, 1 H, $J_{CH,NH}$ 9.1, NH), 5.30 (d, 1 H, $J_{3,4}$ 3.0, H-4), 5.13 (dd, 1 H, $J_{2,3}$ 11.1, H-3), 4.89 (d, 1 H, $J_{1,2}$ 3.5, H-1), 4.52 (ddd, 1 H, $J_{1,2}$ 3.5, H-2), 4.19 (ddd, 1 H, $J_{5,6}$ 7.1, H-5), 4.05–4.00 (m, 2 H, H-6a, H-6b), 3.80–3.76 (m, 1 H, H-Spacer), 3.70–3.67 (t, 2 H, H-Spacer), 3.63–3.56 (m, 5 H, H-Spacer), 2.09 (s, 3 H, $CH_3$CONH), 2.00, 1.92, 1.89 (s, 3 H, $CH_3$CO); $^{13}$C NMR (250 MHz, $CDCl_3$) δ 97.7 (C-1), 47.5 (C-2), 42.8 ($CH_2$-Cl); $C_{18}H_{28}O_{10}NCl$ (453.87).

**5-Iodo-3-oxa-pentyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (3.2).** (186 mg, 95%); $[\alpha]_D$ +58 (*c* 1.0, chloroform); $R_f$ 0.68 in 3:2 petrolether:ethyl acetate; $^1$H NMR (400 MHz, $CDCl_3$) δ 5.88 (d, 1 H, $J_{CH,NH}$ 9.6, NH), 5.38 (d, 1 H, $J_{3,4}$ 3.0, H-4), 5.21 (dd, 1 H, $J_{2,3}$ 11.1, H-3), 4.93 (d, 1 H, $J_{1,2}$ 3.5, H-1), 4.58 (ddd, 1 H, $J_{1,2}$ 3.5, H-2), 4.30 (t, 1 H, $J_{5,6}$ 6.6, H-5), 4.20–4.03 (m, 2 H, H-6a, H-6b), 3.80–3.70 (m, 1 H, H-Spacer), 3.68–3.66 (t, 2 H, H-Spacer), 3.63–3.55 (m, 5 H, H-Spacer), 2.10 (s, 3 H, $CH_3$CONH), 2.00, 1.98, 1.95 (s, 3 H, $CH_3$CO); $^{13}$C NMR (250 MHz, $CDCl_3$) δ 97.8 (C-1), 47.6 (C-2), 2.9 ($CH_2$-I); $C_{18}H_{28}O_{10}NI$ (545.32).

**5-Azido-3-oxa-pentyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (3.3).** (158 mg, 95%); $[\alpha]_D$ +86 (*c* 1.0, chloroform); $R_f$ 0.65 in 3:2 petrolether:ethyl acetate; $^1$H NMR (400 MHz, $CDCl_3$) δ 5.85 (d, 1 H,

$J_{CH,NH}$ 9.6, NH), 5.37 (d, 1 H, $J_{3,4}$ 3.0, H-4), 5.20 (dd, 1 H, $J_{2,3}$ 11.1, H-3), 4.91 (d, 1 H, $J_{1,2}$ 3.5, H-1), 4.60 (ddd, 1 H, $J_{1,2}$ 3.5, H-2), 4.20 (t, 1 H, $J_{5,6}$ 6.1, H-5), 4.15–4.00 (m, 2 H, H-6a, H-6b), 3.78–3.75 (m, 1 H, H-Spacer), 3.63–3.60 (m, 5 H, H-Spacer), 3.34–3.31 (t, 2 H, H-Spacer), 2.18 (s, 3 H, $CH_3CONH$), 2.10, 1.98, 1.90 (s, 3 H, $CH_3CO$); $^{13}C$ NMR (250 MHz, $CDCl_3$) δ 97.7 (C-1), 50.4 ($CH_2$-$N_3$), 47.8 (C-2); $C_{18}H_{28}O_{10}N_4$ (460.43).

**Deprotection**. Treatment of the acetylated monosaccharides **3.1–3.3** with sodium methoxide following the general method led to the deacetylated acceptor substrates **4.1–4.3**.

**5-Chloro-3-oxa-pentyl-2-acetamido-2-deoxy-α-D-galactopyranoside (4.1)**. $^1H$ NMR (400 MHz, $D_2O$) δ 4.75 (d, 1 H, $J_{1,2}$ 3.5, H-1), 3.80–3.50 (m, 12 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-Spacer), 3.21 (t, 2 H, $CH_2Cl$); $^{13}C$ NMR (250 MHz, $D_2O$) δ 99.6 (C-1), 51.0 (C-2), 45.6 ($CH_2$-Cl); $C_{12}H_{22}O_7NCl$ (327.76).

**5-Iodo-3-oxa-pentyl-2-acetamido-2-deoxy-α-D-galactopyranoside (4.2)**. $^1H$ NMR (400 MHz, $D_2O$) δ 4.85 (d, 1 H, $J_{1,2}$ 3.5, H-1), 3.94–3.60 (m, 12 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-Spacer), 3.20 (t, 2 H, $CH_2I$); $^{13}C$ NMR (250 MHz, $D_2O$) δ 99.7 (C-1), 52.3 (C-2), 6.2 ($CH_2$-I); $C_{12}H_{22}O_7NI$ (419.16).

**5-Azido-3-oxa-pentyl-2-acetamido-2-deoxy-α-D-galactopyranoside (4.3)**. $^1H$ NMR (400 MHz, $D_2O$) δ 4.89 (d, 1 H, $J_{1,2}$ 3.5, H-1), 3.85–3.50 (m, 12 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-Spacer), 3.20 (t, 2 H, $CH_2N_3$); $^{13}C$ NMR (250 MHz, $D_2O$) δ 97.8 (C-1), 48.8 (C-2), 50.6 ($CH_2$-$N_3$); $C_{12}H_{22}O_7N_4$ (334.32).

## Chemoenzymatic synthesis

**Transglycosylation**. The acceptor substrates (**4**) (1 equiv, 150 mM) and *p*-nitrophenyl β-D-galactopyranoside (1.5 equiv, 250 mM) were dissolved in 50 mM sodium phosphate–citrate buffer (pH 4.3). The reaction mixture was incubated with β-galactosidase from bovine testes (2 U/1 mmol donor) at 37 °C for 48 h. The reaction was terminated by heating to 90 °C for 5 min. The desired product was isolated from a Biogel P2 column with water.

**5-Chloro-3-oxa-pentyl-2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranoside (5.1)**. A quantity of 5-chloro-3-oxa-pentyl-2-acetamido-2-deoxy-α-D-galactopyranoside (**4.1**) (25 mg, 0.076 mmol) was incubated following the general transglycosylation method to give **5.1** (15 mg, 0.031 mmol) in 40% yield; $[\alpha]_D$ +134 (*c* 1.0, water); $R_f$ 0.35 in 2:1 ethyl acetate:methanol; $^1H$ NMR (400 MHz, $D_2O$) δ 4.83 (d, 1 H, $J_{1,2}$ 3.5, H-1), 4.36 (d, 1 H, $J_{1,2}$ 8.1, H-1′); $^{13}C$ NMR (250 MHz, $D_2O$) δ 107.1 (C-1′), 99.6 (C-1), 50.8 (C-2), 45.7 ($CH_2$-Cl); $C_{18}H_{32}O_{12}NCl$ (489.92).

**5-Iodo-3-oxa-pentyl-2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranoside (5.2)**. A quantity of 5-iodo-3-oxa-pentyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**4.2**) (25 mg, 0.059 mmol) was incubated following the general transglycosylation method to give **5.2** (12 mg, 0.021 mmol) in 35% yield; $[\alpha]_D$ +105 (*c* 1.0, water); $R_f$ 0.30 in 2:1 ethyl acetate:methanol; $^1H$ NMR (400 MHz, $D_2O$) δ 4.85 (d, 1 H, $J_{1,2}$ 3.5, H-1), 4.35 (d, 1 H, $J_{1,2}$ 8.1, H-1′); $^{13}C$ NMR (250 MHz, $D_2O$) δ 107.0 (C-1′), 99.7 (C-1), 50.7 (C-2), 6.2 ($CH_2$-I); $C_{18}H_{33}O_{12}NI$ (582.37).

**5-Azido-3-oxa-pentyl-2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranoside (5.3)**. A quantity of 5-azido-3-oxa-pentyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**4.3**) (25 mg, 0.074 mmol) was incubated following the general transglycosylation method to give **5.3** (13 mg, 0.026 mmol) in 35% yield; $[\alpha]_D$ +143 (*c* 1.0, water); $R_f$ 0.35 in 2:1 ethyl acetate:methanol; $^1H$ NMR (400 MHz, $D_2O$) δ 4.93 (d, 1 H, $J_{1,2}$ 3.5, H-1), 4.46 (d, 1 H, $J_{1,2}$ 8.1, H-1′); $^{13}C$ NMR (250 MHz, $D_2O$) δ 105.1 (C-1′), 97.8 (C-1), 50.6 ($CH_2$-$N_3$), 48.9 (C-2); $C_{18}H_{32}O_{12}N_4$ (496.48).

***p*-Nitrophenyl-2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranoside (5.6)**. *p*-Nitrophenyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**4.6**)[6] (20 mg, 0.058 mmol) was incubated following the general transglycosylation method to give **5.6** (5 mg, 0.0099 mmol) in addition to re-isolated **4.6** (4 mg, 0.012 mmol); based on the reacted monosaccharide the yield was 21%; $[\alpha]_D$ +46 (*c* 1.0, water); $R_f$ 0.41 in 7:3 1-propanol:water; $^1H$ NMR (400 MHz, $D_2O$) δ 7.93 (dd, 1 H, J 8.4, H-A), 7.65 (m, 1 H, H-A′), 7.44 (d, 1 H, J 8.6, H-B), 7.23 (m, 1 H, H-B′), 5.85 (d, 1 H, $J_{1,2}$ 3.6, H-1), 4.56 (d, 1 H, $J_{1,2}$ 7.6, H-1′), 4.53 (dd, 1 H, $J_{2,3}$ 11.2, H-2), 4.33 (d, 1 H, $J_{3,4}$ 3.1, H-4), 4.21 (d, 1 H, H-3), 4.08 (dd, 1 H, $J_{5,6}$ 6.6, H-5), 3.91 (d, 1 H, $J_{3,4}$ 3.6, H-4′), 3.76–3.68 (m, 6 H, H-3′, H-5′, H-6a, H-6b, H-6a′, H-6b′), 3.63 (dd, 1 H, $J_{2,3}$ 9.9, H-2′), 2.01 (s, 3 H, $CH_3CONH$); $^{13}C$ NMR (250 MHz, $D_2O$) δ 175.16 (C=O), 135.36, 126.04, 123.31, 118.50 (Ph), 105.04 (C-1′), 97.23 (C-1), 77.41 (C-3), 75.38 (C-5′), 72.91 (C-3′), 73.68 (C-5), 71.03 (C-2′), 68.95 (C-4), 68.76 (C-4′), 61.34 (C-6, C-6′), 48.73 (C-2), 22.35 ($NCOCH_3$), $C_{20}H_{28}O_{13}N_2$ (504.38).

**Allyl-2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranoside (5.7)**. Allyl-2-acetamido-2-deoxy-α-D-galactopyranoside (**4.7**)[7] (42 mg, 0.162 mmol) was incubated following the general transglycosylation method to give **5.7** (14 mg, 0.033 mmol) in 22% yield; $[\alpha]_D$ +54 (*c* 0.05, water); $R_f$ 0.38 in 7:3:1 ethyl acetate:methanol:water; $^1H$ NMR (400 MHz, $D_2O$) δ 6.10 (m, 1 H, CH=), 5.52 (dd, 2 H, $CH_2$=), 5.14 (d, 1 H, $J_{1,2}$ 3.5, H-1), 4.65 (d, 1 H, $J_{1,2}$ 8.1, H-1′), 4.56 (dd, 1 H, $J_{2,3}$ 11.2, H-2), 4.45 (d, 1 H, $J_{4,5}$ 0.5, H-4), 4.40 (d, 1 H, $CH_2$), 4.27–4.20 (m, 3 H, H-3, H-5, $CH_2$), 4.11 (d, 1 H, $J_{3,4}$ 3.6, H-4′), 3.96–3.93 (m, 4 H, H-6a, H-6b, H-6a′, H-6b′), 3.87–3.79 (m, 2 H, H-3′, H-5′), 3.72 (dd, 1 H, $J_{3,4}$ 10.2, H-2′), 2.20 (s, 3 H, $CH_3CONH$); $^{13}C$ NMR (250 MHz, $D_2O$) δ 175.01 (C=O), 134.13 (CH=), 118.30 ($CH_2$=), 105.12 (C-1′), 96.85 (C-1), 77.66 (C-3), 75.40 (C-5′), 72.96 (C-3′), 71.10 (C-2′), 71.05 (C-5), 69.17 (C-

4), 69.02 (C-4′), 68.86 ($CH_2$), 61.60 (C-6), 61.41 (C-6′), 49.04 (C-2), 22.41 ($CH_3$); $C_{17}H_{29}O_{11}N$ (423.41), *m/z* 424.

**Allyl-2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-glucopyranoside (5.8)**. Allyl-2-acetamido-2-deoxy-α-D-glucopyranoside (**4.8**)[8] (63 mg, 0.24 mmol) was incubated following the general transglycosylation method to give **5.8** (13 mg, 0.031 mmol) in 13% yield; $[\alpha]_D$ −55 (*c* 0.04, water); $R_f$ 0.51 in 7:3:1 ethyl acetate:methanol:water; $^1$H NMR (400 MHz, $D_2O$) δ 5.70 (m, 1 H, CH=), 5.05 (dd, 2 H, $CH_2$=), 4.62 (d, 1 H, $J_{1,2}$ 3.6, H-1), 4.18 (d, 1 H, $J_{1,2}$ 7.6, H-1′), 3.95 (dd, 1 H, $CH_2$), 3.83 (dd, 1 H, $J_{2,3}$ 11.2, H-2), 3.78 (dd, 1 H, $CH_2$), 1.78 (s, 3 H, $CH_3CONH$); $C_{17}H_{29}O_{11}N$ (423.41), *m/z* 424.

**Benzyl-2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-glucopyranoside (5.9)**. Benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside (**4.9**)[9] (25 mg, 0.080 mmol) was incubated following the general transglycosylation method to give **5.9** (12 mg, 0.025 mmol) in 32% yield; $[\alpha]_D$ +170 (*c* 1.0, water); $R_f$ 0.42 in 2:1 ethyl acetate:methanol; $^1$H NMR (400 MHz, $D_2O$) δ 4.94 (d, 1 H, $J_{1,2}$ 4.0, H-1), 4.44 (d, 1 H, $J_{1,2}$ 7.6, H-1′); $^{13}$C NMR (250 MHz, $D_2O$) δ 105.7 (C-1′), 98.4 (C-1), 72.0 ($CH_2$-Ph), 54.8 (C-2); $C_{21}H_{31}O_{11}N$ (473.48).

**Allyl-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-lyxo-hexopyranoside (5.10)**. Allyl-2-deoxy-α-D-lyxo-hexopyranoside (**4.10**)[10] (45 mg, 0.22 mmol) was incubated following the general transglycosylation method to give **5.10** (8 mg, 0.022 mmol) in addition to re-isolated **4.10** (15 mg, 0.073 mmol); based on the reacted monosaccharide the yield was 15%; $[\alpha]_D$ +12 (*c* 1.0, water); $R_f$ 0.53 in 7:3 1-propanol:water; $^1$H NMR (400 MHz, $D_2O$) δ 5.94 (m, 1 H, CH=), 5.28 (dd, 2 H, $CH_2$=), 5.09 (bs, 1 H, H-1), 4.47 (d, 1 H, $J_{1,2}$ 7.6, H-1′), 4.18–4.14 (m, 2 H, H-3, $CH_2$), 4.07 (d, 1 H, H-4), 4.01 (dd, 1 H, $CH_2$), 3.89–3.86 (m, 2 H, H-4′, H-5), 3.74–3.69 (m, 4 H, H-6a, H-6b, H-6a′, H-6b′), 3.68–3.64 (m, 1 H, H-5′), 3.61 (dd, 1 H, $J_{2,3}$ 10.2, H-3′), 3.49 (dd, 1 H, H-2′), 1.99–1.96 (m, 2 H, H-2ax, H-2eq); $^{13}$C NMR (250 MHz, $D_2O$) δ 133.3 (CH=), 118.2 ($CH_2$=), 101.7 (C-1′), 96.6 (C-1), 75.0 (C-3), 73.4 (C-3′), 72.5 (C-5′), 70.8 (C-5), 70.5 (C-2′), 68.4 (C-4), 67.8 (C-6), 67.2 (C-4′), 61.4 (C-6′), 60.8 ($CH_2$), 29.1 (C-2), $C_{15}H_{25}O_{10}$ (365.36), *m/z* 365.

**Peracetylation**. Treatment of the substrates **5.1–5.3**, **5.8**, and **5.9** with pyridine and acetic anhydride following the general method led to the peracetylated compounds **6.1–6.3**, **6.8**, and **6.9**.

**5-Chloro-3-oxa-pentyl-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (6.1)**. $^1$H NMR (400 MHz, $CDCl_3$) δ 5.83 (d, 1 H, $J_{CH,NH}$ 9.1, NH), 5.37 (1 H, $J_{3,4}$ 3.5, H-4), 5.35 (d, 1 H, $J_{3,4}$ 3.0, H-4′), 5.10 (dd, 1 H, $J_{1,2}$ 8.1, H-2′), 4.95 (dd, 1 H, $J_{2,3}$ 10.6, H-3′), 4.91 (d, 1 H, $J_{1,2}$ 3.5, H-1), 4.58 (d, 1 H, $J_{1,2}$ 8.1, H-1′), 4.55 (ddd, 1 H, $J_{1,2}$ 3.5, H-2), 4.25–4.19 (m, 1 H, H-5), 3.95 (dd, 1 H, $J_{2,3}$ 11.1, H-3), 3.93–3.83 (m, 1 H, H-5′), 3.80–3.60 (m, 8 H, H-Spacer), 2.18–1.81 (m, 21 H, 6 $CH_3CO$, $CH_3CONH$); $^{13}$C NMR (250 MHz, $CDCl_3$) δ 100.4 (C-1′), 97.6 (C-1), 72.6 (C-3), 70.3 (C-3′), 70.2 (C-5′), 68.5 (C-4), 68.2 (C-2′), 67.5 (C-5), 66.3 (C-4′), 62.2, 60.5 (C-6, C-6′), 48.4 (C-2), 42.8 ($CH_2$-Cl).

**5-Iodo-3-oxa-pentyl-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (6.2)**. $^1$H NMR (400 MHz, $CDCl_3$) δ 5.81 (d, 1 H, $J_{CH,NH}$ 9.1, NH), 5.35 (d, 1 H, $J_{3,4}$ 3.0, H-4′), 5.33 (1 H, $J_{3,4}$ 3.0, H-4), 5.10 (dd, 1 H, $J_{1,2}$ 8.1, H-2′), 4.95 (dd, 1 H, $J_{2,3}$ 10.6, H-3′), 4.90 (d, 1 H, $J_{1,2}$ 3.5, H-1), 4.58 (d, 1 H, $J_{1,2}$ 8.1, H-1′), 4.53 (ddd, 1 H, $J_{1,2}$ 3.5, H-2), 4.21–4.11 (m, 1 H, H-5), 3.91 (dd, 1 H, $J_{2,3}$ 11.1, H-3), 3.82–3.41 (m, 9 H, H-5′, H-Spacer), 2.18–1.81 (m, 21 H, 6 $CH_3CO$, $CH_3CONH$); $^{13}$C NMR (250 MHz, $CDCl_3$) δ 100.4 (C-1′), 97.5 (C-1), 72.5 (C-3), 70.3 (C-3′), 70.2 (C-5′), 68.6 (C-4), 66.9 (C-5), 68.2 (C-2′), 66.3 (C-4′), 62.2, 60.5 (C-6, C-6′), 50.1 ($CH_2$-$N_3$), 48.3 (C-2).

**5-Azido-3-oxa-pentyl-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (6.3)**. $^1$H NMR (400 MHz, $CDCl_3$) δ 5.73 (d, 1 H, $J_{CH,NH}$ 9.6, NH), 5.30 (1 H, $J_{3,4}$ 3.0, H-4), 5.28 (d, 1 H, $J_{3,4}$ 3.0, H-4′), 5.03 (dd, 1 H, $J_{1,2}$ 7.6, H-2′), 4.87 (dd, 1 H, $J_{2,3}$ 10.1, H-3′), 4.82 (d, 1 H, $J_{1,2}$ 3.5, H-1), 4.48 (d, 1 H, $J_{1,2}$ 7.6, H-1′), 4.43 (ddd, 1 H, $J_{1,2}$ 3.5, H-2), 4.11–4.01 (m, 1 H, H-5), 3.88 (dd, 1 H, $J_{2,3}$ 11.1, H-3), 3.72–3.68 (m, 1 H, H-5′), 3.66–3.59 (m, 6 H, H-Spacer), 3.30 (t, 2 H, H-Spacer), 2.22–1.81 (m, 21 H, 6 $CH_3CO$, $CH_3CONH$); $^{13}$C NMR (250 MHz, $CDCl_3$) δ 100.4 (C-1′), 97.8 (C-1), 72.5 (C-3), 70.3 (C-3′), 70.2 (C-5′), 68.4 (C-4), 68.2 (C-2′), 67.0 (C-5), 66.3 (C-4′), 62.2, 60.4 (C-6, C-6′), 48.6 (C-2), 2.9 ($CH_2$-I).

**Allyl-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (6.8.)**. $^1$H NMR (400 MHz, $CDCl_3$) δ 5.83 (m, 1 H, CH=), 5.51 (d, 1 H, NH), 5.28 (1 H, H-3), 5.26–5.19 (m, 1 H, $CH_2$=), 4.97–4.87 (m, 3 H, H-2, H-3, H-5′), 4.73 (d, 1 H, $J_{1,2}$ 3.8, H-1), 4.49 (d, 1 H, $J_{1,2}$ 7.6, H-1′), 4.36–4.31 (m, 1 H, H-2), 4.19–3.79 (m, 9 H, H-3, H-4, H-4′, H-6a, H-6b, H-6a′, H-6b′, $CH_2$), 2.05–1.89 (m, 21 H, 6 $CH_3CO$, $CH_3CONH$); $^{13}$C NMR (250 MHz, $CDCl_3$) δ 132.15 (CH=), 117.70 ($CH_2$=), 100.01 (C-1′), 95.81 (C-1), 75.20 (C-3), 69.94 (C-2′), 69.32 (C-4′), 67.99–67.08 (C-4, C-5, C-5′, $CH_2$), 65.81 (C-3′), 61.24 (C-6), 59.92 (C-6′), 51.17 (C-2′), 22.42–19.53 (6 $CH_3CO$, $CH_3CONH$).

**Benzyl-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (6.9)**. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.10–7.30 (m, 5 H, Ph), 5.46 (d, 1 H, $J_{CH,NH}$ 10.1, NH), 5.26 (d, 1 H, $J_{3,4}$ 3.0, H-4′), 4.92 (m, 2 H, $CH_2$Ph), 4.90 (t, 1 H, $J_{3,4}$ 10.1, H-4), 4.81–4.79 (m, 1 H, H-2), 4.78 (d, 1 H, $J_{1,2}$ 3.5, H-1), 4.46 (d, 1 H, $J_{1,2}$ 7.6, H-1′), 4.31 (dd, 1 H, $J_{2,3}$ 10.1, H-2), 3.92–3.86 (m, 1 H, H-5), 3.81 (t, 1 H, $J_{2,3}$ 10.1, H-3), 3.80–3.71 (m, 1 H, H-5′), 1.80–2.20 (m 21 H, 6 $CH_3CO$, $CH_3CONH$); $^{13}$C NMR (250 MHz, $CDCl_3$) δ 100.3 (C-1′), 96.9 (C-1), 71.0, 67.9, 68.1 (C-3, C-4, C-5), 70.2 ($CH_2$Ph), 70.1 (C-3′, C-5′), 68.O (C-2′), 66.4 (C-4′), 61.7, 60.5 (C-6, C-6′), 51.5 (C-2).

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**References**

1. Paulsen, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155.
2. Schmidt, R. R. *Angew. Chem. Int. Ed. Engl*, **1986**, *25*, 212.
3. Ogawa, T.; Yamamoto, H.; Nukada, T. *Pure Appl. Chem.* **1984**, *56*, 779.
4. Thiem, J. *FEMS Microbiol. Rev.* **1995**, *16*, 193.
5. Springer, G. F. *Science* **1984**, *224*, 1198.
6. Weissmann, B. *J. Org. Chem.* **1966**, *31*, 2505.
7. Farkas, E.; Lipták, A., manuscript in preparation.
8. Lee, R. T.; Lee, Y. C. *Carbohydr. Res.* **1974**, *37*, 193.
9. Hasegawa, A. *Carbohydr. Res.* **1973**, *29*, 209.
10. Sabesan, S.; Neira, S. *J. Org. Chem.* **1991**, *56*, 5468.
11. Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244.
12. Grundler, G.; Schmidt, R. R. *Liebigs Ann. Chem.* **1984**, 1826.
13. Gonzalez Lio, R.; Verez Bencomo, V. VIIth European Carbohydrate Symposium; Cracow, 1993, Abstract A168.
14. Fernandez Santana, V.; Gonzalez Lio, R.; Adan Padron, A.; Cabrera Blanco, O.; Aguiar Sanchez, A.; Verez Bencomo, V. XVIIIth International Carbohydrate Symposium; Milan, 1996, Abstract BP200.
15. Gambert, U.; Thiem, J. *Carbohydr. Res.* **1997**, *299*, 85.
16. Johansson, E.; Hedbys, L.; Larsson, P.-O. *Enzyme Microb. Technol.* **1991**, *13*, 781.
17. Hedbys, L.; Johansson, E.; Mosbach, K.; Larsson, P.-O. *Carbohydr. Res.* **1989**, *186*, 217.
18. Distler, J. J.; Jourdian, G. W. *J. Biol. Chem.* **1973**, *248*, 6772.
19. Kren, V.; Thiem, J. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 893.
20. Kuby, S. A.; Lardy, H. A. *J. Am. Chem. Soc.* **1953**, *75*, 890.
21. McIlvaine, I. *J. Biol. Chem.* **1921**, *49*, 183.
22. Bradford, M. *Anal. Biochem.* **1976**, *72*, 248.
23. Nilsson, K. G. I. *Biotechnol. Lett.* **1996**, *18*, 791.

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