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Glycosidase-Catalysed Synthesis of Glycosides by an Improved Procedure for Reverse Hydrolysis: Application to the Chemoenzymatic Synthesis of Galactopyranosyl-(1→4)-*O*-α-Galactopyranoside Derivatives.

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Abstract: β-Galactosidase from *Aspergillus oryzae*, α-galactosidase from *Aspergillus niger*, β-mannosidase from *Helix pomatia* and β-glucosidase from almond were used to synthesise different glycosides by reverse hydrolysis: Glcβ*O*$(CH_2)_6$OH **1** was obtained in 61% yield, β-D-Glc–*O*$(CH_2)_3CH{=}CH_2$ **2** in 50% yield, β-D-Glc–*O*$(CH_2)_2Si(Me)_3$ **3** in 11% yield, β-D-Gal–*O*$(CH_2)_6$OH **4** in 48% yield, β-D-Gal–*O*$(CH_2)_3CH{=}CH_2$ **5** in 22% yield, α-D-Gal–*O*$(CH_2)_6$OH **6** in 47% yield, α-D-Gal*O*$(CH_2)_3CH{=}CH_2$ **7** in 37% yield and β-D-Man*O*$(CH_2)_6$OH **8** in 12% yield. Using the appropriate glycosides methyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-1-*O*-α-D-galactopyranoside **11** and 6'-benzoylhexyl-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-1-*O*-β-D-galactopyranoside **15** were synthesised. This chemoenzymatic approach avoided at least four chemical steps that would have been necessary in a conventional synthesis.

INTRODUCTION

Although well developed the chemical synthesis of glycosidic structures is very time consuming when several steps of selective protection, activation and coupling are necessary.[1] However, over the last decade there has been a steadily growing interest in the use of enzymes in a complementary approach to glycoside and oligosaccharide synthesis using glycosidases.[2] Major advantages of glycosidase-catalysed synthesis are that protection-deprotection sequences are avoided and there is a complete control of the stereochemistry at the newly formed anomeric centre, controlled by the specificity of the enzyme. There are two basic ways of using glycosidases to synthesise glycosides: transglycosylation and reverse hydrolysis.

In transglycosylation a glycoside is used as glycosyl donor (e.g. *p*-nitrophenyl *O*-β-D-glycopyranosides) with respect to a nucleophile (e.g. alcohol) present in the reaction medium to generate a new glycosidic bond. The success of this kinetically controlled procedure depends on the rate of hydrolysis of the product being slower than hydrolysis of the glycosyl donor. This approach is the most commonly described in the literature and has been successfully applied to the synthesis of numerous glycosides.[3]

The reverse hydrolysis procedure is based on the shift of the reaction equilibrium, normally in favour of the hydrolysis of glycosidic linkage in aqueous medium, towards synthesis. Thermodynamic considerations indicate that this can be achieved by increasing substrate concentrations (hexose and nucleophile) and decreasing water activity. Relatively few results[4] have been reported using thermodynamic control, but we believe this

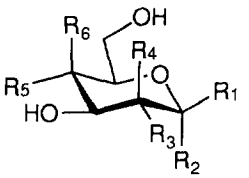
approach can provide an efficient synthesis of glycoside. Moreover, it constitutes very cost-effective and simple procedure which makes it very attractive for industrial scale-up.

In our previous reports[5] designed to understand the main features of this enzyme-catalysed synthesis we have shown that β-D-glucosides can be prepared with a high concentration of solvent [80 to 90% (v/v)] using almond β-D-glucosidase and with a maximum of 20% yield. More recently[6] we have shown that the yields could be considerably increased if the alcohol itself is used as solvent. We reported the synthesis of allyl β-D-*O*-glucopyranoside and benzyl β-D-*O*-glucopyranoside in 62% and 40% yields respectively. We now report an extended study illustrating the usefulness of this approach for glycoside synthesis (Scheme 1) on a preparative scale using a variety of biocatalysts (almond β-D-glucosidase, β-galactosidase from *Aspergillus oryzae*, α-galactosidase from *Aspergillus niger* and β-mannosidase from *Helix pomatia*) and various alcohols (hexane-1,6-diol, pent-4-en-1-ol and 2-(trimethylsilyl)ethanol). All the glycosides prepared can be used as building blocks in carbohydrate synthesis. In relation to current studies on the synthesis of glycosidic biological receptors, the application of this procedure is illustrated below in the synthesis of two α-D-galactopyranosyl-(1→4)-D-galactopyranoside derivatives which are potential ligands of the Shiga toxin,[7] verotoxin[8] and uropathogenic strains of *E. coli*[9]. These chemoenzymatic approaches make it possible to shorten significantly the synthesis of these disaccharides.

RESULTS AND DISCUSSION

Enzymatic synthesis of glycosides.

The enzymatic synthesis of glycosides reported here is very effective and straightforward. The procedure is very similar to a purely chemical one and therefore readily applicable by chemists. The hexose (galactose, glucose or mannose) is dissolved in the volume of water required, the alcohol is added and the reaction is initiated by adding the glycosidase. The reaction mixture is shaken at 50 °C and the bioconversion is followed by



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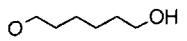
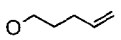
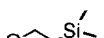
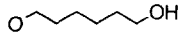
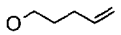
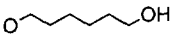
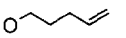
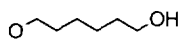
1		H	OH	H	OH	H
2		H	OH	H	OH	H
3		H	OH	H	OH	H
4		H	OH	H	H	OH
5		H	OH	H	H	OH
6	H		OH	H	H	OH
7	H		OH	H	H	OH
8		H	H	OH	OH	H

Scheme 1. Structures of enzymatically synthesised glycosides.

TLC. As the reaction is a thermodynamic control, the reaction time varied between 24 hours and 6 days. By using directly the alcohol as a solvent and by carrying out the reaction at high temperature (50 °C) glycosides were prepared in isolated yields significantly higher than any previously reported (Scheme 1 and Table 1).

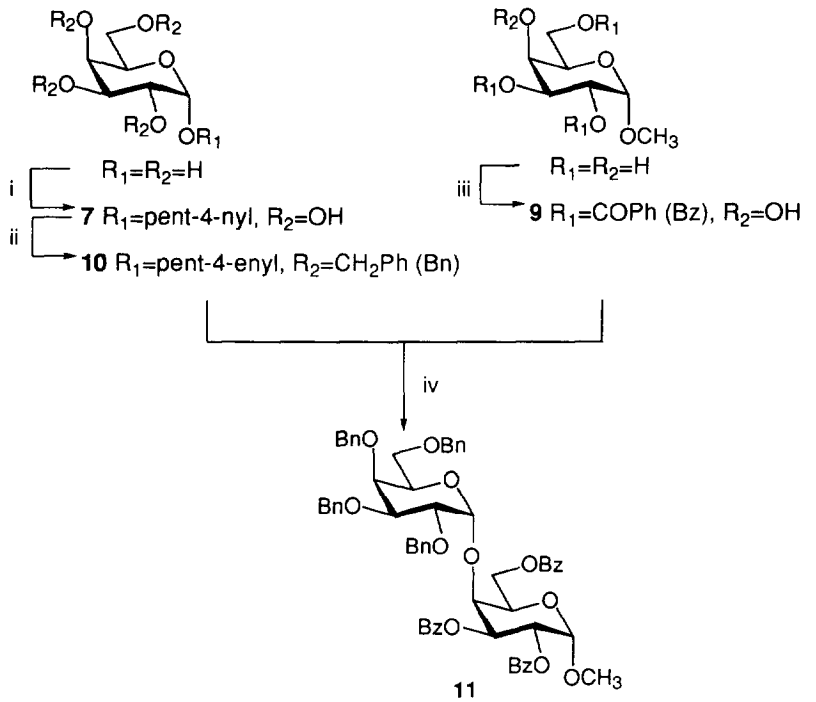
By contrast with lipase-catalysed ester formation,[10] with which the present procedure has some analogy, it was found that glycosidases need a higher percentage of water in the reaction medium to be active. After optimisation, between 8 and 20% (v/v) of water was used, depending on the enzyme and the alcohol. As shown in Table 1, almond β-D-glucosidase needs less water than the other glycosidases, but the optimum percentage of water depends also on the alcohol used. For example using almond β-D-glucosidase, 10% (v/v) of water was added with hexane-1-6-diol, 8% (v/v) with pent-4-en-1-ol and 5.6% (v/v) with 2-(trimethylsily)ethanol. The more hydrophilic the alcohol, the more water is needed in the medium to maintain the enzyme in an adequately hydrated state.

Table 1. Glycosidase-Catalysed Synthesis of Glycosides by Reverse Hydrolysis.

Glycosidase	Hexose	Alcohol	Alcohol/Water ratio (v : v)	Glycoside	Yield[a] (%)
β-Glucosidase (almond)	Glucose	hexane-1,6-diol	90 : 10	**1**	61
	Glucose	pent-4-en-1-ol	92 : 08	**2**	50
	Glucose	2(trimethylsilyl) ethanol	55 : 5.6[b]	**3**	11
β-Galactosidase (*Aspergillus oryzae*)	Galactose	hexane-1,6-diol	80 : 20	**4**	48
	Galactose	pent-4-en-1-ol	90 : 10	**5**	22
α-Galactosidase (*Aspergillus niger*)	Galactose	hexane-1,6-diol	80 : 20	**6**	47
	Galactose	pent-4-en-1-ol	90 : 10	**7**	37
β-mannosidase (*Helix pomatia*)	Mannose	hexane-1,6-diol	80 : 20	**8**	12

a: Isolated yields based on hexose added; b: 39.4% (v/v) of *tert*-butanol was added to the reaction mixture.

Using this reverse hydrolysis approach, 6'-hydroxyhexyl *O*-β-D-glucopyranoside **1** was prepared in 61% yield after 6 days and 6'-hydroxyhexyl *O*-β-D-galactopyranoside **4** and 6'-hydroxyhexyl *O*-α-D-galactopyranoside **6** in 48% and 47% yields respectively. These glycosidic derivatives with a spacer arm at the anomeric centre can be directly coupled to a solid phase as affinity supports[11] and immunoadsorbents.[12] Alternatively, they can be used in disaccharide glycoside synthesis as described below. Pent-4-enyl *O*-β-D-glucopyranoside **2**, pent-4-enyl *O*-β-D-galactopyranoside **5** and pent-4-enyl *O*-α-D-galactopyranoside **7** were also synthesised in 50%, 22% and 37% yields respectively. After suitable acylation or benzylation these derivatives can be directly used as glycosyl donors using Et_3SiOTf/NIS or TfOH/NIS as catalysts according to the methodology developed by Fraser-Reid and co-workers.[13] Finally the synthesis of 2-(trimethylsily)ethyl *O*-β-D-glucopyranoside **3** was achieved in 11% yield after addition of *tert*-butanol as co-solvent to aid dissolution of the glucose. The trimethylsilyl residue is a temporary protecting group for the anomeric centre and can be removed using $LiBF_4$[14] or directly transformed into the corresponding acetate using $BF_3.Et_2O/Ac_2O$.[15]

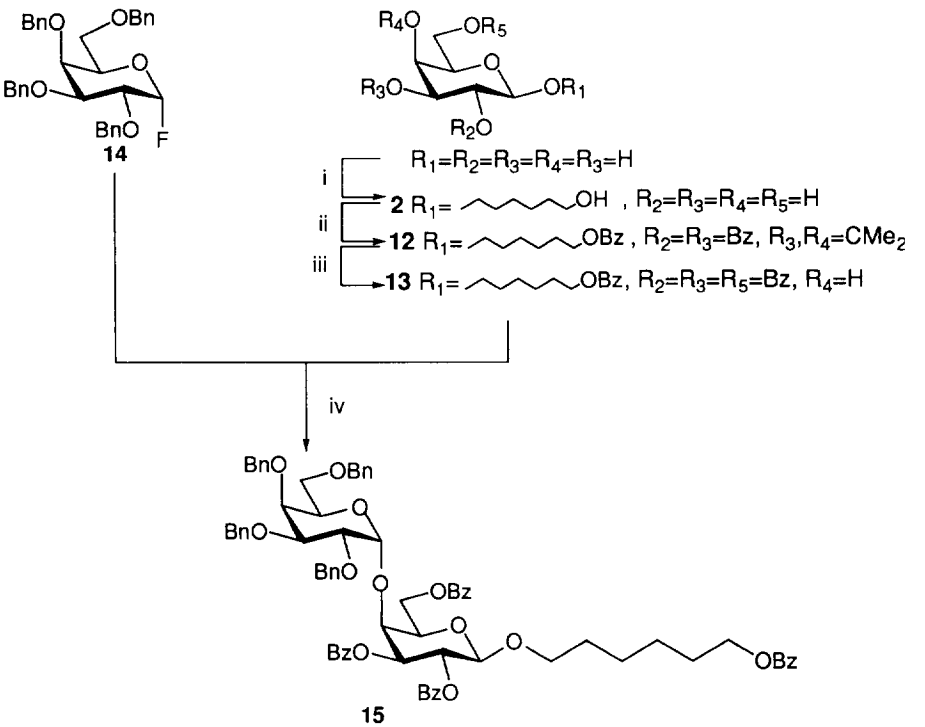


Scheme 2. Reagents: i, pent-4-en-1-ol [90% (v/v)], H_2O [10% (v/v)], α-galactosidase; ii $PhCH_2Br$, NaH, DMF; iii, PhCOCl, pyridine; iv, Et_3SiOTf, NIS, CH_2Cl_2.

The α-D-Gal-(1→4)−D-Gal disaccharide moiety has a fundamental role in biological recognition as a stage-specific embryogenic antigen,[16] as a tumor antigen in Burkit Lymphoma,[17] as a receptor for Shiga toxin[7,8] and as a receptor for uropathogenic *Escherichia coli*.[9] Using a combination of enzymatic and chemical methods, methyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-1-*O*-α-D-galactopyranoside **11** was prepared in four steps and the 6'-benzoylhexyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-1-*O*-β-D-galactopyranoside **15** in 10 steps.

The synthetic approach to disaccharide **11** was as follows (Scheme 2): pent-4-enyl-*O*-α-D-galactopyranoside **7** was enzymatically synthesised in 37% yield using the α-galactosidase from *Aspergillus niger* and benzylated for coupling to methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside **9** which was prepared by selective benzoylation of methyl α-D-galactopyranoside according to the procedure of Reist *et al.*[20] coupling of **9** and **10** was carried out using triethylsilyl triflate and NIS to give disaccharide **11** in 40% yield. As expected from the presence of a benzyl group at C-2 of the pentenyl galactoside **10**, the newly generated glycosidic bond in **11** had the desired α configuration as confirmed by 2D ^1H-^1H homocorrelation and 2D ^1H-^{13}C heterocorrelation experiments (400-100MHz, CDCl3). The two anomeric protons gave signals at δ 4.94 (d, 1H, *J* 3.4 Hz, H-1') and 5.24 (d, 1H, *J* 3.3 Hz, H-1). The first coupling constant established the α configuration of the terminal saccharide residue. The two anomeric carbons gave signals at at δ 97.3 (C-1) and 100.6 (C-1').

The synthesis of disaccharide **15** was carried out as follows (Scheme 3): 6'-hydroxyhexyl *O*-β-galactopyranoside **4** was enzymatically synthesised in 48% yield using the β-galactosidase from *Aspergillus oryzae* and selectively transformed into its terminal 4,6-isopropylidene acetal. The crude product was benzoylated using benzoyl chloride in pyridine at room temperature to afford the tribenzoate **12** in 40% overall yield. Deprotection of **12** by acid hydrolysis (CF_3COOH) followed by selective C-6 benzoylation using benzoyl cyanide at -20 °C led to the desired galactosyl acceptor **13** in 60% overall yield. Glycoside **13** was coupled to the *per-O*-benzylgalactopyranosyl fluoride **14**, which was prepared in six steps according to the procedure of Nicolaou and co-workers[18], using silver triflate and stannous chloride in Et_2O giving disaccharide **15** in 50% yield. The α configuration of the newly generated glycosidic bond was also confirmed by NMR experiments. The anomeric proton of the terminal saccharide residue gave signals at δ 5.24 (d, 1H, *J* 3.3 Hz, H-1'). The two anomeric carbon atoms gave signals at δ 94.2 (C-1') and 101.7 (C-1).



Scheme 3. Reagents: i, 1,6-hexanediol [80% (v/v)], H_2O [20% (v/v)], β-galactosidase; ii, $Me_2C(OMe)_2$, cat. TSOH, DMF; iii, PhCOCl, pyridine; iv, AgOTf, $SnCl_2$, 4Å mol. sieves, Et_2O.

EXPERIMENTAL

Enzymes: β-D-glucosidase (EC.3.2.1.21 from almond, G-0395) and β-D-galactosidase (EC 3.2.1.23) from *Aspergillus oryzae*, G-7138) were purchased from Sigma Chemical Co. α-D-Galactosidase (EC 3.2.1.22) from *Aspergillus niger*, Gal 600L) was a gift from Novo Nordisk Bioindustries, Ltd., UK. β-D-Mannosidase (EC

3.2.1.25 from *Helix pomatia*) was a gift from Dr V. Kren, Institute of Microbiology of the Academy of Sciences of the Czech Republic, Prague.

General: ^{1}H NMR spectra were determined at either 250 MHz or 400 MHz using either Bruker AC 250 or Bruker AC-400 spectrometers respectively. ^{13}C NMR spectra were determined at either 62.89 MHz or 100.62 MHz using the same instruments. The structures of the enzymatically synthesised glycosides were determined by proton-proton shift correlation, proton-carbon shift correlation and DEPT experiments. Mass spectra were recorded with a Kratos MS 80 mass spectrometer or by the SERC mass spectrometry service centre at Swansea in the FAB mode. Optical rotations were determined using an Optical Activity Ltd. AA-1000 polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. Melting points were determined with a Gallenkamp melting point apparatus and are uncorrected. All non-enzymatic reactions were carried out under a nitrogen atmosphere with dry freshly distilled solvents. TLC was conducted on Merck Kieselgel 60 F_{254} 0.2 mm precoated plates with detection by spraying with 10% aq. H_2SO_4 and heating. Flash silica chromatography was performed on Merck silica gel (60, particle size 0.040-0.063 mm).

General enzymatic glycosylation procedure. The hexose (0.9 g, 5 mmol.) was dissolved in H_2O (2 to 5 ml) in a sealed flask and the alcohol was added to a final volume of 25 ml. The reaction was started by adding the enzyme and the mixture was shaken (110 rpm) at 50 °C. At the end of the incubation time the enzyme is removed by filtration, H_2O was added (50 ml) and the excess of alcohol was extracted four times with EtOAc or Et_2O (50 ml). The aqueous layer was concentrated under vacuum and the product was purified by flash chromatography [CH_2Cl_2-MeOH-H_2O (40 : 10 : 1)] to give the corresponding glycoside.

6'-Hydroxyhexyl*O*-β-D-glucopyranoside 1. Glucose (0.9 g, 5 mmol), water (2.5 ml), hexane-1,6-diol (22.5 ml) and almond β-D-glucosidase (125 mg) were incubated together as described in the general procedure to give after 6 days the glucoside **1** (0.85 g, 61%) as an oil. $[\alpha]_D^{27}$ -31.6 (c 0.28, H_2O); ^{1}H-NMR (400 MHz; H_2O) δ 1.21-1.31 (m, 4 H, O(CH_2)2C*H2*C*H2*-), 1.50-1.64 (m, 4 H, OCH_2C*H2*(CH_2)2C*H2*-), 3.21 (dd, 1 H, *J* 8.0, 9.3 Hz, H-2), 3.31-3.47 (m, 3 H, H-3, H-4, H-5), 3.57 (t, 2 H, *J* 6.6 Hz, O(CH_2)5C*H2*OH), 3.61-3.65 (m, 1 H, OC*H2*-), 3.68 (dd, 1 H, *J* 5.8, 12.3 Hz, H-6_a), 3.89 (m, 2 H, H-6_b, OCH_2), 4.41 (d, 1 H, *J* 7.9 Hz, H-1); ^{13}C-NMR (100 MHz; H_2O) δ 25.4 (O(CH_2)2CH_2-), 25.4 (O(CH_2)3CH_2-), 29.3 (OCH_2CH_2-), 31.8 (O(CH_2)4CH_2-), 61.4 (C-6), 62.4 (O(CH_2)5CH_2-), 70.3, 71.2, 73.8, 76.5, 76.5, 102.8 (C-1); m/z found: ($M+Na^+$) 303.1415. $C_{12}H_{24}O_7$.Na requires 303.1419.

Pent-4-enyl *O*-β-D-glucopyranoside 2. Glucose (0.9 g, 5 mmol), water (2 ml), pent-4-en-1-ol (23 ml) and almond β-D-glucosidase (125 mg) were incubated together together as described in the general procedure to give after 28 hours the glucoside **2** (0.62 g, 50%) as an oil. In the work-up the solvent for the extraction was Et_2O instead of EtOAc. $[\alpha]_D^{28}$ -31.2 (c 0.28, MeOH). ^{1}H-NMR (400 MHz; H_2O) δ 1.62-1.69 (m, 2 H, O(CH_2)C*H2*-), 2.05-2.15 (m, 2 H, O(CH_2)2C*H2*-), 3.19 (dd, 1 H, *J* 8.0, 9.2 Hz, H-2), 3.30 (dd, 1 H, *J* 9.0, 9.5 Hz, H-4), 3.35-3.40 (m, 1 H, H-5), 3.41 (dd, 1 H, *J* 8.9, 9.1 Hz, H-3), 3.59-3.67 (m, 2 H, H-6_a, OC*H2*), 3.82-3.88 (m, 2 H, H-6_b, O<u>CH_2</u>-), 4.38 (d, 1 H, *J* 8.0 Hz, H-1), 4.94-5.05 (m, 2 H, O(CH_2)3CH=C*H2*), 5.79-5.89 (m, 1 H, O(CH_2)3C*H*=); ^{13}C-(100 MHz; H_2O) δ 28.6 (OCH_2CH_2-), 30.0 (O(CH_2)2CH_2-), 61.4 (C-6), 70.3 (C-4), 70.5 (OCH_2-), 73.7 (C-2), 76.4 (C-3 or C-5), 76.5 (C-5 or C-3),

102.8 (C-1), 115.4 ($O(CH_2)_3CH=CH_2$), 139.4 ($O(CH_2)_3CH=$), m/z found (for the peracetate): ($M+Na^+$) 439.1554. $C_{19}H_{28}O_{10}$.Na requires 439.1580.

2-(Trimethylsilyl)ethyl *O*-β-D-glucopyranoside 3. Glucose (0.9 g, 5 mmol), water (1.3 ml), 2-(trimethylsilyl)ethanol (12.6 ml), *tert*-butanol (9 ml) and almond β-D-glucosidase (135 mg) were incubated together as described in the general procedure. After 8 days the the reaction mixture was filtered through a pad of cotton wool, evaporated under reduced pressure and purified by flash chromatography to afford the glucoside **3** (86.2 mg, 11%) as an oil. $[\alpha]_D^{24}$ -29.5 (c 0.20, MeOH). ^{1}H-NMR (400 MHz; H_2O) δ 0.88-1.05 (m, 2 H, OCH_2CH_2-), 3.17 (dd, 1 H, *J* 8.0, 9.2 Hz, H-2), 3.29-3.43 (m, 3 H, H-3, H-4, H-5), 3.65 (dd, 1 H, *J* 5.5, 12.1 Hz, H-6_a), 3.69-3.73 (m, 1 H, OCH_2-), 3.84 (dd, 1 H, *J* 2.0, 12.1 Hz, H-6_b), 3.94-4.01 (m, 1 H, OCH_2-), 4.40 (d, 1 H, *J* 8.0 Hz, H-1); ^{13}C-(100 MHz; H_2O) δ -1.8 (3x-CH_3), 18.2 (OCH_2CH_2-), 61.3 (C-6), 69.0 (OCH_2-), 70.2 (C-4), 73.8 (C-2), 76.5 (C-3 or C-5), 76.5 (C-5 or C-3), 102.1 (C-1); m/z found (for the peracetate): ($M+Na^+$) 471.1678. $C_{19}H_{32}O_{10}Si$.Na requires 471.1662.

6'-Hydroxyhexyl *O*-β-D-galactopyranoside 4. Galactose (0.9 g, 5 mmol), water (5 ml), hexane-1,6-diol (20 ml) and β-D-galactosidase from *Aspergillus oryzae* (248 mg) were incubated together as described in the general procedure to give after 6 days the galactoside **2** (0.67 g, 48%) as an oil. $[\alpha]_D^{30}$ -4.5 (c 0.66, H_2O); ^{1}H-NMR (400 MHz; H_2O) δ 1.25-1.36 (m, 4H, $O(CH_2)_2CH_2CH_2$-), 1.46-1.61 (m, 4 H, $OCH_2CH_2(CH_2)_2CH_2$-), 3.43 (dd, 1 H, *J* 7.9, 9.9 Hz, H-2), 3.53 (t, 2 H, *J* 6.6 Hz, $O(CH_2)_5CH_2OH$), 3.56-3.72 (m, 5 H, H-3, H-5, H-$6_{a,b}$, OCH_2-), 3.85 (d, 1 H, *J* 2.1 Hz, H-4), 3.86-3.89 (m, 1 H, OCH_2-), 4.32 (d, 1 H, *J* 7.9 Hz, H-1); ^{13}C-(100 MHz; H_2O) δ 25.4 ($O(CH_2)_2CH_2$-), 25.4 ($O(CH_2)_3CH_2$-), 29.3 (OCH_2CH_2-), 31.8 ($O(CH_2)_4CH_2$-), 61.5 (C-6), 62.4 ($O(CH_2)_5CH_2$-), 69.2 (C-4), 71.1 (OCH_2-), 71.4 (C-2), 73.4 (C-3), 75.7 (C-5), 103.4 (C-1); m/z found: ($M+Na^+$) 303.1414. $C_{12}H_{24}O_7$.Na requires 303.1419.

Pent-4-enyl-*O*-β-D-galactopyranoside 5. Galactose (0.9 g, 5 mmol), water (2.5 ml), pent-4-en-1-ol (22.5 ml) and β-D-galactosidase from *Aspergillus oryzae* (266 mg) were incubated together as described in the general procedure to give after 2 days the galactoside **5** (0.27 g, 22%) as an oil. In the work-up the solvent for the extraction was Et_2O instead of EtOAc. $[\alpha]_D^{22}$ -18.0 (c 0.30, MeOH); ^{1}H-NMR (400 MHz; H_2O) δ 1.64-1.72 (m, 2 H, $O(CH_2)CH_2$-), 2.07-2.13 (m, 2 H, $O(CH_2)_2CH_2$-), 3.45 (dd, 1 H, *J* 8.1, 9.5 Hz, H-2), 3.57-3.76 (m, 5 H, H-3, H-5, H-$6_{a,b}$, OCH_2-), 3.87-3.92 (m, 2 H, H-4, OCH_2-), 4.34 (d, 1 H, *J* 7.9 Hz, H-1), 4.96-5.07 (m, 2 H, $O(CH_2)_3CH=CH_2$), 5.82-5.92 (m, 1H, $O(CH_2)_3CH=$); ^{13}C-(100 MHz; H_2O) δ 28.6 (OCH_2CH_2-), 30.0 ($O(CH_2)_2CH_2$-), 61.5 (C-6), 69.3 (C-4), 70.5 (OCH_2-), 71.4 (C-2), 73.4 (C-3), 75.7 (C-5), 103.4 (C-1), 115.4 ($O(CH_2)_3CH=CH_2$), 139.5 ($O(CH_2)_3CH=$); m/z found (for the peracetate): ($M+Na^+$) 439.1560. $C_{19}H_{28}O_{10}$.Na requires 439.1580.

6'-Hydroxyhexyl *O*-α-D-galactopyranoside 6. Galactose (0.9 g, 5 mmol), water (2.5 ml), hexane-1,6-diol (20 ml) and α-D-galactosidase from *Aspergillus niger* (2.5 ml) were incubated together as described in the general procedure to give after 2 days the galactoside **6** (0.65 g, 47%) as an oil. $[\alpha]_D^{24}$ +106.2 (c 0.59, MeOH); ^{1}H-NMR (400 MHz; H_2O) δ 1.29-1.38 (m, 4 H, $O(CH_2)_2CH_2CH_2$-), 1.48-1.62 (m, 4 H, $OCH_2CH_2(CH_2)_2CH_2$-), 3.45-3.51 (1 H, m, OCH_2-), 3.55 (t, 2 H, *J* 6.6 Hz, $O(CH_2)_2CH_2OH$), 3.65-3.71 (m, 3 H, H-$6_{a,b}$, OCH_2-), 3.88 (t, 1 H, *J* 6.1 Hz, H-5), 3.92 (d, 1 H, *J* 3.0 Hz, H-4), 4.89 (d, 1 H, *J* 3.4 Hz,

H-1); ^{13}C-(100 MHz; H_2O) δ 25.4 ($O(CH_2)_2CH_2$-), 25.8 ($O(CH_2)_3CH_2$-), 29.1 (OCH_2CH_2-), 31.8 ($O(CH_2)_4CH_2$-), 61.8 (C-6), 62.4 ($O(CH_2)_5CH_2$-), 68.9 (C-2 or OCH_2-), 68.9 (OCH_2- or C-2), 69.9 (C-4), 70.2 (C-3), 71.5 (C-5), 98.9 (C-1); m/z found: ($M+Na^+$) 303.1416. $C_{12}H_{24}O_7$.Na requires 303.1419.

Pent-4-enyl-*O*-α-D-galactopyranoside 7. Galactose (0.9 g, 5 mmol), water (2.5 ml), pent-4-en-1-ol (22.5 ml) and freeze dried α-D-galactosidase from *Aspergillus niger* (500 mg) were incubated together as described in the general procedure to give after 24 hours the galactoside **7** (0.45 g, 37%) as an oil. In the work-up the solvent for the extraction was Et_2O instead of EtOAc.$[\alpha]_D^{22}$ +145.8 (c 0.26, MeOH); ^{1}H-NMR (400 MHz; H_2O) δ 1.66-1.74 (m, 2 H, $O(CH_2)CH_2$-), 2.09-2.15 (m, 2 H, $O(CH_2)_2CH_2$-), 3.47-3.53 (m, 1 H, OCH_2-), 3.68-3.72 (m, 3 H, H-$6_{a,b}$, OCH_2-), 3.74-3.83 (m, 2 H, H-2, H-3), 3.89-3.92 (m, 1 H, H-5), 3.94 (d, 1 H, *J* 2.9 Hz, H-4), 4.90 (d, 1 H, *J* 3.6 Hz, H-1), 4.97-5.09 (m, 2 H, $O(CH_2)_3CH=CH_2$), 5.84-5.94 (m, 1 H, $O(CH_2)_3CH$=); ^{13}C-(100 MHz; H_2O) δ 28.6 (OCH_2CH_2-), 30.0 ($O(CH_2)_2CH_2$-), 61.5 (C-6), 68.3 (OCH_2-), 68.9 (C-2), 69.9 (C-4), 70.2 (C-3), 71.5 (C-5), 98.9 (C-1), 115.3 ($O(CH_2)_3CH=CH_2$), 139.7 ($O(CH_2)_3CH$=), m/z found (for the peracetate): ($M+Na^+$) 439.1562. $C_{19}H_{28}O_{10}$.Na requires 439.1580.

6'-Hydroxyhexyl *O*-β-D-mannopyranoside 8. Mannose (0.9 g, 5 mmol), water (5 ml), hexane-1,6-diol (20 ml) and β-D-mannosidase from *Helix pomatia* (475 mg) were incubated together as described in the general procedure to give after 6 days the galactoside **8** (0.16 g, 12%) as an oil. $[\alpha]_D^{27}$ -25.1 (c 0.68, H_2O) [Lit.[19] $[\alpha]_D^{20}$ -24.9 (c 0.70, H_2O)]; ^{1}H-NMR (400 MHz; H_2O) δ 1.26-1.35 (m, 4 H, $O(CH_2)_2CH_2CH_2$-), 1.46-1.59 (m, 4 H, $OCH_2CH_2(CH_2)_2CH_2$-), 3.27-3.32 (m, 1 H, H-5), 3.50 (dd, 1 H, *J* 9.4, 9.5 Hz, H-4), 3.53 (t, 2H, *J* 6.6 Hz, $O(CH_2)_5CH_2OH$), 3.56-3.63 (m, 2 H, H-3, OCH_2-), 3.66 (dd, 1 H, *J* 6.4, 12.2 Hz, H-6_a), 3.79-3.87 (m, 2 H, H-6_b, OCH_2-), 3.91 (d, 1 H, *J* 3.1 Hz, H-2), 4.60 (s, 1 H, H-1); ^{13}C-(100 MHz; H_2O) δ 25.4 ($O(CH_2)_2CH_2$-), 25.5 ($O(CH_2)_3CH_2$-), 29.2 (OCH_2CH_2-), 38.8 ($O(CH_2)_4CH_2$-), 61.7 (C-6), 62.3 ($O(CH_2)_5CH_2OH$), 67.5 (C-4), 70.5 (OCH_2-), 71.2 (C-2), 73.6 (C-3), 76.8 (C-5), 100.4 (C-1); m/z found: ($M+Na^+$) 303.1436. $C_{12}H_{24}O_7$.Na requires 303.1419.

Methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside 9. Methyl β-D-galactopyranoside (4.98 g, 25.69 mmol.) was dissolved in pyridine (51 ml) and treated with benzoyl chloride (10.31 ml, 88.74 mmol.) according to the procedure of Reist *et al.*[20] to give after flash chromatography (silica, 10% EtOAc in PhMe) the glucoside **9** (7.73 g, 59%). $[\alpha]_D^{25}$ +119.8 (c 1.01, $CHCl_3$); ^{1}H-NMR (400 MHz; $CDCl_3$) δ 3.44 (s, 3 H, OCH_3), 4.35 (t, 1 H, *J* 6.2 Hz, H-5), 4.42 (dd, 1 H, *J* 1.0, 2.7 Hz, H-4), 4.54-4.70 (m, 2 H, H-$6_{a,b}$), 5.21 (d, 1 H, *J* 3.1 Hz, H-1), 5.73 (dd, 2 H, *J* 2.6. 4.0 Hz, H-2, H-3), 7.30-7.59 (m, 9 H, aromat-H), 7.95-8.09 (m, 5 H, aromat-H); ^{13}C-(100 MHz; $CDCl_3$) δ 55.3 (OCH_3), 63.4 (C-6), 67.6 (C-3), 68.0 (C-2), 68;8 (C-4), 70.7 (C-5), 97.4 (C-1), 128.3, 128.3, 128.3, 129.2, 129.5, 129.6, 129.7, 129.7, 130.0 (aromat-CH), 133.1, 133.2, 133.4 (aromat-C), 165.7, 166.0, 166.4 (C=O); Found: C, 65.98; H, 5.19. $C_{28}H_{26}O_{19}$ requires C, 66.43; H, 5.13%.

Pent-4-enyl 2,3,4,6-tetra-*O*-benzyl-1-*O*-α-D-galactopyranoside 10. To a suspension of NaH (80%, 0.47 g, 15.7 mmol.) in DMF (15 ml) was added dropwise a solution of pent-4-enyl-*O*-α-D-galactopyranoside **7** (0.49 g, 2.0 mmol.) in DMF (6 ml) at 0 °C and the mixture was stirred for 30 min. To this mixture was added dropwise BnBr (1.90 ml, 16.05 mmol) in DMF (4 ml) and the mixture was stirred for 4 h at

10-20 °C. The excess of NaH was decomposed by dropwise addition of MeOH (1.5 ml). The reaction mixture was diluted with CH_2Cl_2 (100 ml), successively washed with a saturated aq. $NaHCO_3$ solution (2x50 ml) and twice with water (2x50 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash chromatography (silica, 30% Et_2O in light petroleum b.p. 40-60 °C) gave **10** (0.92 g, 76%) as an oil. $[\alpha]_D^{28}$ +27.2 (c 1.28, $CHCl_3$); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 1.67-1.78 (m, 2 H, $O(CH_2)C\underline{H_2}$-), 2.09-2.13 (m, 2 H, $O(CH_2)2CH_2$-), 3.41-3.50 (m, 1 H, OCH_2-), 3.52 (dd, 2 H, *J* 2.2, 6.4 Hz, H-$6_{a,b}$), 3.61-3.67 (m, 1 H, H-OCH_2-), 3.92-3.97 (m, 3 H, H-3, H-4, H-5), 4.03 (dd, 1 H, *J* 3.6, 9.6 Hz, H-2), 4.39 (d, 1 H, *J* 11.8 Hz, OCH_2Ph), 4.47 (d, 1 H, *J* 11.8 Hz, OCH_2Ph), 4.57 (d, 1 H, *J* 11.8 Hz, OCH_2Ph), 4.67 (d, 1 H, *J* 11.8 Hz, OCH_2Ph), 4.74 (d, 1 H, *J* 11.8 Hz, OCH_2Ph), 4.80 (d, 1 H, *J* 2.2 Hz, H-1), 4.81-4.86 (m, 2 H, OCH_2Ph), 4.94 (d, 2 H, J 11.5 Hz, $O(CH_2)3CH{=}CH_2$, OCH_2Ph), 4.98-5.28 (m, 1 H, $O(CH_2)3CH{=}CH_2$), 5.77-5.85 (m, 1 H, $O(CH_2)3CH{=}$), 7.23-7.40 (m, 20 H, aromat-H); ^{13}C-(100 MHz; $CDCl_3$) δ 28.4 (OCH_2CH_2-), 30.2 ($O(CH_2)2CH_2$-), 67.4 (C-1), 68.9 (OCH_2-), 69.1, 73.0, 73.1, 73.3, 74.6, 75.0, 76.5, 78.9 (C-2, C-3, C-4, C-5, 4xOCH_2Ph), 97.4 (C-1), 114.6 ($O(CH_2)3CH{=}CH_2$), 127.3, 127.4, 127.4, 127.5, 127.6, 127.8, 128.0, 128.1, 128.1, 128.1, 128.2, 128.2 (CH-aromat.), 137.9, 138.0, 138.6, 138.7 (C-aromat.), 138.7 ($O(CH_2)3CH{=}$), Found: C, 76.78; H, 7.20. $C_{39}H_{44}O_6$ requires C, 76.99; H, 7.23%.

Methyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-benzoyl-1-*O*-α-D-galactopyranoside 11. The pentenyl glycoside **10** (0.25 g, 0.40 mmol.) and the alcohol **9** (0.18 g, 0.35 mmol) were combined, co-evaporated twice with dry toluene and dried overnight under high vacuum. *N*-Iodosuccinimide (0.18 g, 0.79 mmol.), molecular sieves (4 Å, 0.7 g) and CH_2Cl_2 (8 ml) were added and the mixture was stirred for 15 min at 0 ° C followed by dropwise addition of Et_3SiOTf (124.3 µl, 0.55 mmol). After 5 h at 0° to 10° C the mixture was diluted with CH_2Cl_2 (5 ml) and washed with 10% $Na_2S_2O_3$ (7 ml), saturated aq. $NaHCO_3$ (7 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash chromatography (silica, 5% EtOAc in toluene) gave **11** (114 mg, 40%) as a white foam. $[\alpha]_D^{25}$ +106.9 (c 0.46, $CHCl_3$); ^{1}H-NMR (400 MHz; $CDCl_3$) δ 2.89-2.92 (m, 1 H, H-$6'_a$), 3.38 (t, 1 H, *J* 8.7 Hz, H-$6'_b$), 3.43 (s, 3 H, OCH_3), 3.98 (s, 2 H, OCH_2Ph), 4.09 (dd, 1 H, *J* 3.4, 10.2 Hz, H-2'), 4.12 (bs, 1 H, H-4'), 4.19 (dd, 1 H, *J* 2.5, 10.2 Hz, H-3'), 4.32-4.44 (m, 2 H, H-5, H-5'), 4.46 (s, 1 H, OCH_2Ph), 4.49 (bs, 1 H, H-4), 4.74-4.77 (m, 3 H, H-$6_{a,b}$, OCH_2Ph), 4.84-4.91 (m, 4 H, 2xOCH_2Ph), 4.94 (d, 1 H, *J* 3.4 Hz, H-1'), 5.24 (d, 1 H, *J* 3.3 Hz, H-1), 5.67 (dd, 1 H, *J* 2.6, 11.0 Hz, H-3), 5.73 (dd, 1 H, *J* 3.4, 11.0 Hz, H-2), 7.15-7.61 (m, 30 H, H-aromat.), 7.97-8.06 (m, 5 H, H-aromat.); ^{13}C-(100 MHz; $CDCl_3$) δ 55.2 (OCH_3), 62.7 (C-6), 67.4 (C-6'), 68.2 (C-5), 68.9 (C-2), 69.6 (C-5'), 70.7 (C-3), 72.3 (2xOCH_2Ph), 72.7 (OCH_2Ph), 74.0 (C-4), 74.5 (OCH_2Ph), 74.7 (C-2'), 75.6 (C-4'), 78.8 (C-3'), 97.3 (C-1), 100.6 (C-1'), 127.2, 127.2, 127.3, 127.4, 127.8, 127.9, 128.0, 128.1, 128.1, 128.2, 128.2, 128.3, 129.2, 129.3, 129.4, 129.6, 129.6, 129.7 (CH-aromat.), 132.9, 132.9, 133.0, 137.9, 138.0, 138.5, 138.6 (C-aromat.), 165.8, 166.2 (C=O); Found: C, 72.12; H, 5.75. $C_{62}H_{60}O_{14}$ requires C, 72.40; H, 5.83%.

6'-Benzoylhexyl *O*-(4,6-isopropylidene)-2,3-di-*O*-benzoyl-1-*O*-β-galactopyranoside 12. To a solution of **2** (0.76 g, 2.71 mmol.) in DMF (7 ml) *p*-toluenesulfonic acid (85 mg, 0.44 mmol.) and 2,2-dimethoxypropane (1.32 ml, 10.85 mmol.) were added. After 115 min at room temperature the reaction was quenched by addition of triethylamine (0.83 ml), concentrated under reduced pressure and dried overnight under high vacuum. The residue was re-dissolved in pyridine (9 ml) and benzoyl chloride (1.41 ml, 12.81 mmol.) was added dropwise at 0 °C. After 30 min the reaction mixture was allowed to warm-up at room temperature and

stirred for a further 4 h. The reaction medium was then diluted with ethyl acetate (40 ml) washed successively with H_2O (3x10 ml) and brine (10 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash chromatography (silica, 20% AcOEt in toluene) gave **12** (0.67 g, 40%) as an oil. $[\alpha]_D^{28}$ +18.7 (c 1.14, $CHCl_3$); ^{1}H-NMR (400 MHz; $CDCl_3$) δ 1.21-1.29 (m, 4 H, $O(CH_2)_2CH_2CH_2$-), 1.35 (s, 3 H, CH_3), 1.37-1.59 (m, 4 H, $OCH_2CH_2(CH_2)_2CH_2$-), 1.62 (s, 3 H, CH_3), 3.41-3.46 (m, 1 H, OCH_2-), 3.84-3.89 (m, 1 H, OCH_2-), 4.11 (t, 2H, *J* 6.6 Hz, $O(CH_2)_5CH_2$-), 4.18-4.22 (m, 1 H, H-5), 4.29 (dd, 1 H, *J* 2.2, 5.9 Hz, H-4), 4.37 (dd, 1 H, *J* 5.4, 7.3 Hz, H-3), 4.49 (d, 1 H, *J* 8.1 Hz, H-1), 4.61-4.74 (m, 2 H, H-$6_{a,b}$), 5.25 (t, 1 H, *J* 7.7 Hz, H-2), 7.33-7.57 (m, 10 H, H-aromat.), 7.98-8.07 (m, 5 H, H-aromat.); ^{13}C-(100.62 MHz; $CDCl_3$) δ 25.3, 25.3, 26.1, 27.5, 28.2, 29.0 ($OCH_2CH_2CH_2CH_2$-, 2xCH_3), 63.5 (C-6), 64.6 ($O(CH_2)_5CH_2$-), 69.4 (OCH_2-), 70.7 (C-5), 73.4 (C-2 or C-4), 73.4 (C-4 or C-2), 76.8 (C-3), 100.5 (C-1), 110.6 ($C(Me)_2$), 128.1, 128.2, 129.2, 129.4, 129.5, 129.5, 129.6, 129.7, 130.2, (CH-aromat.), 132.6, 132.8, 133.0 (C-aromat.), 165.1, 166.1, 166.3 (C=O); m/z found: ($M+Na^+$) 617.2519 $C_{36}H_{25}O_{10}$.Na requires 617.2625.

6'-Benzoylhexyl 2,3,6-tri-*O*-benzoyl-1-*O*-β-D-galactopyranoside 13. To a solution of **12** (0.55 g, 0.87 mmol.) in THF (5 ml) at 0° C, H_2O (1.12 ml) and CF_3COOH (3.37 ml) were added. After 60 min the mixture was stirred at 50 °C for 4 hours and quenched by addition of saturated aq. $NaHCO_3$ (20 ml) and the product was extracted with EtOAc (2x30 ml). The combined organic layers were washed with brine (10 ml), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was co-evaporated twice with toluene and dried overnight under high vacuum. The total crude product was redissolved in DMF (7 ml) and cooled to -20 °C. Triethylamine (0.15 ml) and benzoylcyanide (0.11 ml) were added and the mixture was stirred for 15 min at -20 °C and for a further 15 min at 0 °C. The reaction mixture was quenched with MeOH (1 ml), diluted with EtOAc (25 ml), washed with H_2O (8 ml) and brine (8 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash chromatography (silica, 10% AcOEt in toluene) gave **13** (0.36 g, 60%) as an oil. $[\alpha]_D^{19}$ +7.2 (c 0.92, $CHCl_3$); ^{1}H-NMR (400 MHz; $CDCl_3$) δ 1.24-1.34 (m, 4 H, $O(CH_2)_2CH_2CH_2$-), 1.47-1.69 (m, 4 H, $OCH_2CH_2(CH_2)_2CH_2$-), 3.49-3.57 (m, 1 H, OCH_2-), 3.93-3.99 (m, 1 H, OCH_2-), 4.11-4.16 (m, 4 H, CH_2OBz, H-3, H-5), 4.40 (dd, 1 H, *J* 6.3, 11.3 Hz, H-6_a), 4.60 (dd, 1 H, *J* 6.8, 11.3 Hz, H-6_b), 4.68 (d, 1 H, *J* 7.8 Hz, H-1), 5.36 (dd, 1 H, *J* 7.8, 10.0 Hz, H-2), 5.76 (d, 1 H, *J* 3.1 Hz, H-4); ^{13}C (100 MHz; $CDCl_3$) δ 25.4, 25.5, 28.4, 29.2 ($OCH_2CH_2CH_2CH_2$-), 62.2 (C-6), 64.7 ($O(CH_2)_5CH_2$-), 70.1 (OCH_2-), 70.3, 71.2, 71.7, 73.5 (C-2, C-3, C-4, C-5), 101.2 (C-1), 128.0, 128.2, 128.3, 128.4, 128.9, 129.3, 129.4, 129.6, 129.7, 129.7, 130.0, 130.3 (CH-aromat.), 132.7, 133.1, 133.3, 133.5 (C-aromat.), 165.9, 166.1, 166.6, 167.1 (C=O); m/z found: ($M+2H+Na^+$) 719.2445. $C_{40}H_{40}O_{11}$.Na requires 719.2448.

6'-Benzoylhexyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-benzoyl-1-*O*-β-D-galactopyranoside 15. To **13** (0.25 g, 0.35 mmol.), silver triflate (0.25g, 0.99 mmol.), tin(II) chloride (0.14 g, 0.82 mmol.) and activated molecular sieves (4Å) were added. The mixture was dried for two hours under high vacuum, cooled to 0 °C and Et_2O (20 ml) was added. *O*-2,3,4,6-Tetra-benzyl-α-D-fluoro galactopyranoside[18] **14** (0.59 g, 1.08 mmol.) was dissolved in Et_2O (4 ml) and added dropwise to the reaction mixture. The mixture was stirred for 2 hours 30 min at 0 °C, EtOAc (40 ml) was added and the suspension was filtered through a pad of Celite. The organic layer was washed with a saturated aq. $NaHCO_3$ (3x40 ml) and brine (40 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash chromatography

(silica, 5% AcOEt in toluene) **gave 15** (0.21 g, 50%) as an oil. $[\alpha]_D^{28}$ +46.09 (c 1.23, $CHCl_3$); ^{1}H-NMR (400 MHz; $CDCl_3$) δ 1.24-1.30 (m, 4 H, 2xCH_2), 1.44-1.58 (m, 4 H, 2xCH_2), 3.21 (dd, 1 H, *J* 5.6, 9.4 Hz, H-6a), 3.28 (d, 1 H, *J* 1.8 Hz, H-4'), 3.37 (dd, 1 H, *J* 3.3, 9.4 Hz, H-2'), 3.50-3.66 (m, 2 H, H-3', OCH_2-), 3.85-4.01 (m, 4 H, H-2, H-5', H-5, OCH_2-), 4.11 (t, 2 H, J 6.6 Hz, -CH_2OBz), 4.21 (dd, 1 H, *J* 3.3, 10.2 Hz, H-6_b), 4.23-4.62 (m, 11 H), 5.24 (d, 1 H, *J* 3.4 Hz, H-1'), 5.67 (dd, 1 H, *J* 7.9, 10.2 Hz, H-2), 5.90 (d, 1 H, *J* 3.1 Hz, H-4), 7.08-7.59 (m, 35 H, H-aromat.), 8.00-8.10 (m, 5 H, H-aromat.); ^{13}C-(100 MHz; $CDCl_3$) δ 25.3, 25.4, 28.3, 29.1 ($OCH_2CH_2CH_2CH_2$-), 62.3 (C-6), 64.7 (-CH_2OBz), 66.1 (C-4), 69.2 (C-6'), 69.7 (C-2'), 69.9 (OCH_2-), 70.8 (C-2), 71.4 (C-5), 72.6 (CH_2Ph), 72.5 (C-3), 72.9 (CH_2Ph), 73.0 (CH_2Ph), 74.3 (CH_2Ph), 74.8 (C-4'), 75.0 (C-5'), 78.5 (C-3'), 94.2 (C-1'), 101.7 (C-1), 127.0, 127.2, 127.3, 127.4, 127.5, 127.6, 127.6, 127.7, 127.7, 127.8, 127.9, 128.0, 128.0, 128.2, 128.2, 128.3, 128.6, 129.3, 129.5, 129.6, 129.6, 130.0, 133.1, 132.6 (CH-aromat.), 137.7, 138.2, 138.3, 138.3, 138.5, 138.6, 138.6, 138.9 (C-aromat.), 164.7, 165.8, 165.9, 166.4 (C=O); m/z found: ($M+Na^+$) 1241.5240. $C_{74}H_{74}O_{16}$.Na requires 1241.5244.

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