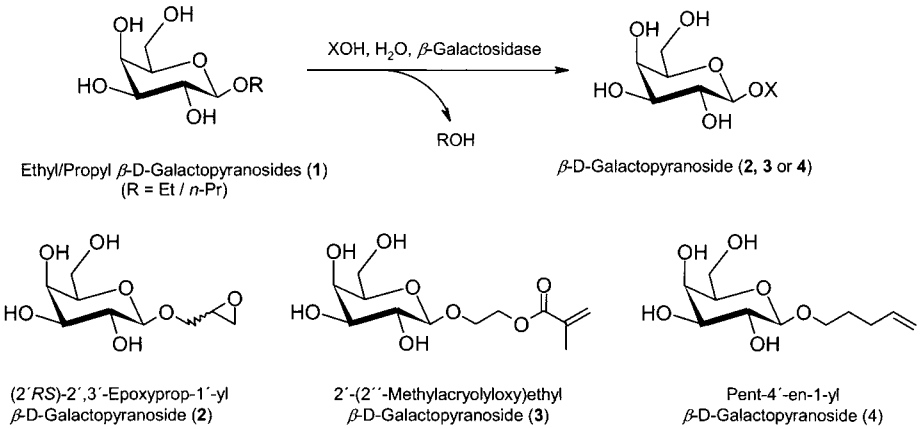

Figure 1. **Galactosidase-mediated syntheses of 1-O-β-D-galactopyranosides (2−4).**

issues, we disclosed the use of super-saturated sugar solutions and, more recently, plasticized glass phases as novel media for the glycosidase-mediated syntheses.[33−35] The latter approach appears especially useful as it permits *O*-glycosylation in highly concentrated media and dispenses with bulk solvents. The method relies upon the ability of alkyl glycosides to form low-melting point glasses, which can be liquefied (plasticized) by small amounts of water, alcohols, and liquid or solid aglycons to form homogeneous or heterogeneous systems which sustain glycosidase catalysis.[34,35] This allows one to achieve high sugar concentrations of 0.9−2.0 M (20−40% w/w) and acceptor contents of 0.9−5.4 M (20−60% w/w), while supporting the optimal water content of 5−10% for glycosidases. This permits the *O*-glycosylation of hydrophilic and hydrophobic acceptors in yields of 39−74%, provides 0.25−0.65 kg of product per kg of reaction mixture, and productivities 9−36 times higher than those of conventional methods.[35]

We now report the kilogram-scale enzymatic production of *O*-glycosides in plasticized glass phases, and demonstrate the scalability of the method. A mixture of ethyl and *n*-propyl 1-*O*-β-D-galactopyranosides was prepared and used to synthesize (2′*RS*)-2′,3′-epoxyprop-1′-yl (**2**), 2′-(2″-methylacrylylloxy)ethyl (**3**), and pent-4′-en-1-yl (**4**) 1-*O*-β-D-galactopyranosides, using commercial β-galactosidase catalysts (Figure 1). The β-D-galactosides were required for the synthesis/modification of acrylate, sol−gel, and silicone polymers for use as catalyst supports and molecularly imprinted materials for separations.

Results and Discussion

We previously reported that the β-D-galactosides (**2**−**4**) could be synthesized in homogeneous plasticized glass phases consisting of the acceptors (40−45% w/w), the glass-forming mixture of galactose and ethyl/*n*-propyl β-D-galactosides (40−50% w/w) and water (10%), using a variety of bacterial and fungal β-galactosidases as catalysts. Solutions showing 76−83% conversion to alkyl β-D-galactosides were readily prepared by the gradual addition of ethanol or *n*-propanol or both to β-galactosidases dissolved in concentrated solutions of D-galactose. Upon evaporation, these furnished hydrous glasses with glass transition temperatures (T_g's) of 15−24 °C, which were readily plasticized with the required amounts of water and acceptor substrates, and enabled the required transglycosylation reactions.[34,35]

In scaling-up the syntheses of the β-D-galactosides, we sought to address several concerns, namely whether crude β-galactosidases could be used as disposable catalysts, how the reactions would perform upon scale-up, and which reactor types could be employed for the viscous plasticized glass reaction mixtures. The production of ethyl/*n*-propyl β-D-galactoside substrates was studied optimised and scaled-up using standard batch reactors using the commercial food-grade *Aspergillus* and *Kluyveromyces* spp. β-galactosidases Lactozym 3000 and Lactase 17P (Table 1). The optimum protocol used Lactase 17P to catalyse the reaction of a galactose solution of 65% w/w concentration held at 50 °C, with 6−7 vols of alcohol added over a period of ca. 18 h, with a total reaction time of 50−60 h. A 3:2 v/v mixture of *n*-propanol−ethanol was used as the acceptor as it furnished a low glass transition (T_g) galactoside glass that could be plasticized with large amounts (40−50% w/w) of the requisite glycidol, 2-hydroxyethyl methacrylate (HEMA) and pent-1-en-4-ol acceptors without phase separation (data not shown). Although the use of higher *n*-propanol:ethanol ratios provided glasses with lower T_g's that could accommodate slightly larger amounts of acceptors, the corresponding syntheses required much more careful control of stirring rate, temperature and alcohol addition to avoid crystallization of galactose and phase separation of the reaction mixtures. In contrast, the optimal protocol was readily scaled up to 102 mol (18.4 kg) to produce 21.7 kg of 70% w/w β-D-galactoside glass with a productivity of 402 kg-product kg-enzyme^{-1} and a space−time yield of ca. 0.05 kg kg^{-1} d^{-1}, using a 425-L stirred reactor.

(29) Nakagawa, H.; Yoshiyama, M.; Shimura, S.; Kirimura, K.; Usami, S. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 1914−1915.
(30) Vic, G.; Thomas, D.; Crout, D. H. G. *Enzyme Microb. Technol.* **1997**, *20*, 597−603.
(31) Kren, V.; Auge, C.; Sedmera, P.; Havlicek, V. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2481−2484.
(32) Scheckermann, C.; Wagner, F.; Fischer, L. *Enzyme Microb. Technol.* **1997**, *20*, 629−634.
(33) Millqvist-Fureby, A.; Gill, I. S.; Vulfson, E. N. *Biotechnol. Bioeng.* **1998**, *60*, 190−196.
(34) Gill, I.; Valivety, R. *Angew. Chem., Int. Ed.* **2000**, *39*, 3801−3804.
(35) Gill, I.; Valivety, R. *Angew. Chem., Int. Ed.* **2000**, *39*, 3804−3808.

Table 1. **Optimisation and scale-up of β-galactosidase-mediated synthesis of ethyl/*n*-propyl β-D-galactosides**

galactose[a] solution (% w/w)	alcohol added[b]/ alcohol/vol/addn time	scale (mol)	catalyst	temp (°C)	time[c] (h)	product[d] composition (% w/w) Gal:EtGal:PrGal	T_g[e] (°C)
50	3:2/PrOH−EtOH/6 vols/14 h	0.11	Lactase 17P	40	60	37:26:36	59−60
55	3:2/PrOH−EtOH/6 vols/14 h	0.11	Lactase 17P	40	60	32:30:38	56−58
60	3:2/PrOH−EtOH/6 vols/14 h	0.11	Lactase 17P	40	60	28:32:40	53−55
65	3:2/PrOH−EtOH/6 vols/14 h	0.11	Lactase 17P	40	60	25:33:42	52−53
70	3:2/PrOH−EtOH/6 vols/14 h	0.11	Lactase 17P	40	60	48:26:26	66−68
65	1:1/PrOH−EtOH/6 vols/14 h	0.11	Lactase 17P	40	60	22:42:36	53−54
65	2:3/PrOH−EtOH/6 vols/14 h	0.11	Lactase 17P	40	60	26:41:33	57−59
65	2:1/PrOH−EtOH/6 vols/14 h	0.11	Lactase 17P	40	60	26:29:45	55−56
65	3:1/PrOH−EtOH/6 vols/14 h	0.11	Lactase 17P	40	60	29:28:43	53−56
65	4:1/PrOH−EtOH/6 vols/14 h	0.11	Lactase 17P	40	60	30:21:49	54−56
65	1:0/PrOH−EtOH/6 vols/14 h	0.11	Lactase 17P	40	60	27:0:73	50−51
65	3:2/PrOH−EtOH/5 vols/14 h	0.11	Lactozym	40	60	28:32:40	54−56
65	3:2/PrOH−EtOH/6 vols/16 h	0.11	Lactozym	40	60	25:34:41	53−54
65	3:2/PrOH−EtOH/7 vols/18 h	0.11	Lactozym	40	60	22:34:44	49−50
65	3:2/PrOH−EtOH/8 vols/22 h	0.11	Lactozym	40	60	27:33:40	54−56
65	3:2/PrOH−EtOH/5 vols/14 h	0.11	Lactase 17P	40	60	25:31:44	51−53
65	3:2/PrOH−EtOH/6 vols/16 h	0.11	Lactase 17P	40	60	21:34:45	47−48
65	3:2/PrOH−EtOH/7 vols/18 h	0.11	Lactase 17P	40	60	19:37:44	45−47
65	3:2/PrOH−EtOH/8 vols/22 h	0.11	Lactase 17P	40	60	22:36:42	47−49
65	3:2/PrOH−EtOH/7 vols/18 h	0.11	Lactozym	45	52	22:33:45	45−46
65	3:2/PrOH−EtOH/7 vols/18 h	0.11	Lactozym	50	52	24:33:43	47−49
65	3:2/PrOH−EtOH/7 vols/18 h	0.11	Lactozym	55	52	26:32:42	53−56
65	3:2/PrOH−EtOH/7 vols/18 h	0.11	Lactase 17P	45	52	18:36:46	45−46
65	3:2/PrOH−EtOH/7 vols/18 h	0.11	Lactase 17P	50	52	17:38:45	43−45
65	3:2/PrOH−EtOH/7 vols/18 h	0.11	Lactase 17P	55	52	23:35:42	48−50
65	3:2/PrOH−EtOH/7 vols/18 h	5.0	Lactozym	45	60	22:37:41	48−50
65	3:2/PrOH−EtOH/7 vols/18 h	10.0	Lactozym	45	60	23:37:40	52−53
65	3:2/PrOH−EtOH/7 vols/18 h	10.3	Lactase 17P	50	60	19:38:43	46−48
65	3:2/PrOH−EtOH/7 vols/18 h	20.1	Lactase 17P	50	60	16:40:44	42−43
65	3:2/PrOH−EtOH/7 vols/18 h	102	Lactase 17P	50	57	17:37:46	44−46

[a] Concentration of the galactose syrup used in the reaction. [b] Composition of the alcohol mix added, the volume (relative to the galactose syrup) added, and the time over which it was added. [c] Total reaction time. [d] Composition of the glass obtained after evaporation, discounting any alcohol or water present (generally between 1 and 3% and 5−8% w/w respectively). [e] Glass transition temperature recorded for the anhydrous glass. Reactions were conducted by adding Lactase 17P (3 g per kg of galactose, added as a 200 g L^{-1} suspension in 20 mM acetate buffer, pH 5.5, containing 5 mM calcium and magnesium chlorides and 5 mM DTT) or Lactozym (30 mL per kg of galactose, solution adjusted to pH 6.5) to the galactose solution (in 20 mM sodium acetate buffer, pH 5.5, or 20 mM sodium phosphate buffer, 6.5) stirred at ca. 230−400 rpm, followed by the addition of the alcohol mix over the required time period. Reactions at 0.11, 5, 10, and 20 mol scale were conducted in 0.3-, 20-, 50-, and 75-L stirred glass vessels, and the 102 mol reaction in a 425-L stainless steel reactor, all fitted with reflux heads. Upon completion of the reactions, the mixtures were filtered through Celite, concentrated by rotary evaporation at 70 °C, and the syrup obtained was exhaustively dried under vacuum over phosphorus pentoxide and calcium chloride at rt for 2−3 d. Reactions were followed by HPLC.

Having optimized the preparation of the galactoside donor substrate **1**, we then turned our attention to the enzyme-mediated transglycosylation reactions leading to **2**, **3**, and **4**, and initiated a screening program, using Lactozym 3000 and Lactase 17P as catalysts (Table 2). An ethyl/*n*-propyl galactoside glass of composition 42:34:16:2:1:5 (*n*-Pr-β-D-Gal:Et-β-D-Gal:Gal:PrOH:EtOH:H_2O) was used as the sugar donor, as this substrate supported the inclusion of up to 55% of glycidol, HEMA, and pent-4-en-1-ol without macroscopic phase separation. The glass was liquefied by heating and plasticized with buffer and acceptor alcohol to furnish a mobile liquid reaction medium, and the requisite reaction was initiated by the addition of enzyme stock. As with previous studies,[35] a water content of about 10% w/w was found to provide the best synthetic rates and yields. Lower water contents significantly suppressed transglycosylation rates and yields, while higher amounts, although increasing the fluidity of the reaction media and in some cases improving initial rates, invariably led to increased hydrolysis of the donor/product and thus reduced final yields. The addition of ethanol or propanol, despite reducing viscosity and elevating initial rates in the range of 5−10% w/w, had deleterious effects on the final yields, probably due to inactivation of the enzymes. Interestingly, it was observed that the reactions progressed with a notable decrease in viscosity between 0.5 and 3 h of reaction, presumably due to the release of ethanol/*n*-propanol promoting greater plasticization of the reaction media. This behaviour is rather similar to that observed for protease-mediated peptide synthesis in eutectoids.[36−38]

Lactozym 3000 was found to be the best catalyst for the synthesis of **2**, while Lactase 17P was most efficient for obtaining **3** and **4**. Ethyl/*n*-propyl galactoside substrate concentrations of 50, 40, and 45% w/w and acceptor alcohol contents of 40, 50, and 45% w/w were found to be optimal for the synthesis of **2**, **3**, and **4**, respectively. Under these conditions, the reaction mixtures were observed to be monophasic **2** or microemulsions (**3** and **4**), and provided substrate concentrations of 2.21, 1.77, and 1.99 M (Table

(36) Gill, I.; Vulfson, E. N. *Trends Biotechnol.* **1994**, *12*, 118−122.
(37) López-Fandino, R.; Gill, I.; Vulfson, E. N. *Biotechnol. Bioeng.* **1994**, *43*, 1016−1023.
(38) López-Fandino, R.; Gill, I.; Vulfson, E. N. *Biotechnol. Bioeng.* **1994**, *43*, 1024−1030.

Table 2. **Galactosidase-mediated syntheses of 1-*O*-*β*-D-galactopyranosides 2, 3, and 4**

product	reaction mixture composition (% w/w) Et/Pr-O-Gal:acceptor:EtOH:H_2O	system	concn[a] (M)	biocatalyst	initial rate[b] (mol kg^{-2} h^{-1})	[prod.][c] (g g^{-1})	yield (%) (60 h)
2	60:34:0:6	monophasic	2.65	Lactozym	0.68	0.18	21
	60:34:0:6	monophasic	2.65	Lactase 17P	0.35	0.12	13
	60:32:0:8	monophasic	2.65	Lactozym	1.33	0.49	55
	60:32:0:8	monophasic	2.65	Lactase 17P	0.87	0.26	27
	60:30:0:10	monophasic	2.65	Lactozym	1.81	0.52	61
	60:30:0:10	monophasic	2.65	Lactase 17P	1.19	0.37	42
	60:28:0:12	monophasic	2.65	Lactozym	2.07	0.48	55
	60:28:0:12	monophasic	2.65	Lactase 17P	1.33	0.36	40
	55:35:0:10	monophasic	2.43	Lactozym	2.21	0.46	65
	50:40:0:10	monophasic	2.21	Lactozym	2.13	0.49	72
	45:45:0:10	monophasic	1.99	Lactozym	1.98	0.43	69
	40:50:0:10	microemulsion	1.77	Lactozym	1.70	0.33	60
	40:45:5:10	microemulsion	1.77	Lactozym	1.86	0.35	63
	40:40:10:10	monophasic	1.77	Lactozym	1.53	0.31	58
3	60:32:0:8	monophasic	2.46	Lactozym	4.15	0.26	28
	60:32:0:8	monophasic	2.46	Lactase 17P	3.88	0.31	32
	60:30:0:10	monophasic	2.30	Lactozym	4.32	0.35	39
	60:30:0:10	monophasic	2.30	Lactase 17P	5.72	0.44	51
	60:28:0:12	monophasic	2.15	Lactozym	4.60	0.30	37
	60:28:0:12	monophasic	2.15	Lactase 17P	5.18	0.39	46
	55:35:0:10	monophasic	2.43	Lactase 17P	5.67	0.48	52
	50:40:0:10	monophasic	2.21	Lactase 17P	6.07	0.48	58
	45:45:0:10	microemulsion	1.99	Lactase 17P	6.22	0.51	65
	40:50:0:10	microemulsion	1.77	Lactase 17P	6.76	0.49	70
	35:55:0:10	microemulsion	1.55	Lactase 17P	6.44	0.39	63
4	60:32:0:8	monophasic	2.65	Lactozym	2.46	0.18	21
	60:32:0:8	monophasic	2.65	Lactase 17P	3.72	0.43	49
	60:30:0:10	monophasic	2.65	Lactozym	3.58	0.28	35
	60:30:0:10	monophasic	2.65	Lactase 17P	4.23	0.49	56
	60:28:0:12	monophasic	2.65	Lactozym	2.80	0.20	24
	60:28:0:12	monophasic	2.65	Lactase 17P	4.05	0.40	47
	55:35:0:10	monophasic	2.43	Lactase 17P	4.51	0.49	63
	50:40:0:10	monophasic	2.21	Lactase 17P	4.37	0.45	64
	45:45:0:10	microemulsion	1.99	Lactase 17P	4.49	0.42	67
	40:50:0:10	microemulsion	1.77	Lactase 17P	4.33	0.36	61
	35:55:0:10	macroemulsion	1.55	Lactase 17P	4.30	0.29	53

[a] Concentration of the limiting reactant. [b] Initial rate of product formation, determined at 20 min, and quoted as mol per kg of reaction mixture per kg of biocatalyst per h. [c] Product concentration attained at the end of the reaction, as gram of product per gram of reaction mixture. Reactions were conducted on a 10-g scale in open vials (50 mL) incubated at 40 °C, 150 rpm. Ethyl/*n*-propyl galactoside of composition 42:34:16:2:1:5 (*n*-Pr-*β*-D-Gal:Et-*β*-D-Gal:Gal: PrOH:EtOH:H_2O) was heated to 50 °C until liquefied, and phosphate acetate or phosphate buffer (25 mM, pH 5.5 or 6.5, with 5 mM calcium and magnesium chlorides) was added. The liquid was cooled to 40 °C, and the acceptor alcohol was added, followed by Lactozym 3000 or Lactase 17P (neat solution and 0.1 g mL^{-1} stock respectively, both containing 5 mM DTT). Lactozym was used at 25 g per kg of reaction mixture, and Lactase 17P at 3 g kg^{-1} (**2** and **3**) or 4 g kg^{-1} **4**. The mixture was shaken vigorously (400 rpm), and the vial was sealed and incubated at 40 °C, 130−150 rpm for 1 h. The vial was then opened, and the reaction was allowed to continue for 60 h. Weighed samples were taken as required and quenched with pure methanol for HPLC analysis.

2). These conditions furnished product yields of 72, 70, and 67% respectively, and resulted in corresponding product concentrations of 0.49, 0.49, and 0.42 kg per kg of reaction mixture (Figure 3). The high substrate concentrations achieved in the reaction mixtures and the correspondingly elevated product concentrations are notable—these are a factor of 9−12-fold higher than those obtained with conventional solvent-based media.[17,19−32]

Once optimal conditions had been identified for the small scale reactions, the reactions were up-scaled into stirred reactors to evaluate the suitability of plasticized glass phases for large-scale syntheses. Although three reactor configurations were considered (i.e., shaken open kettles, rotating-drum reactors and wiped-blade reactors) the first two types were discounted since insufficient mixing of the viscous plasticized glass reaction mixtures was achieved in these when scaling-up (>1 kg) syntheses. The wiped-blade configuration provided efficient mixing, and syntheses were accordingly transferred to reactors fitted with these stirrers and connected to reflux/distillation heads (Figure 2 and Table 3). Reaction mixtures with compositions of 50:40:10, 40:50:10 and 45:45:10 (galactose donor:acceptor:water) were chosen for **2**, **3**, and **4** respectively, and scale-up of the reactions was examined from 0.01 to 1.0 kg. Under atmospheric pressure, and without removal of the formed volatiles, significant reductions in initial rates of synthesis and product yields were observed (Figure 3). This appeared to be due to the increased build-up of ethanol/*n*-propanol during the progress of transglycosylation in the larger-scale reactions, resulting in the more rapid establishment of the reaction equilibrium together with increased inactivation of the biocatalysts. To overcome this, vacuum was applied to the reaction mixtures and the released alcohols distilled off. Application of a vacuum of 300 mbar from the beginning of the reaction until its completion provided little improvement, while delaying its application to 1 h significantly

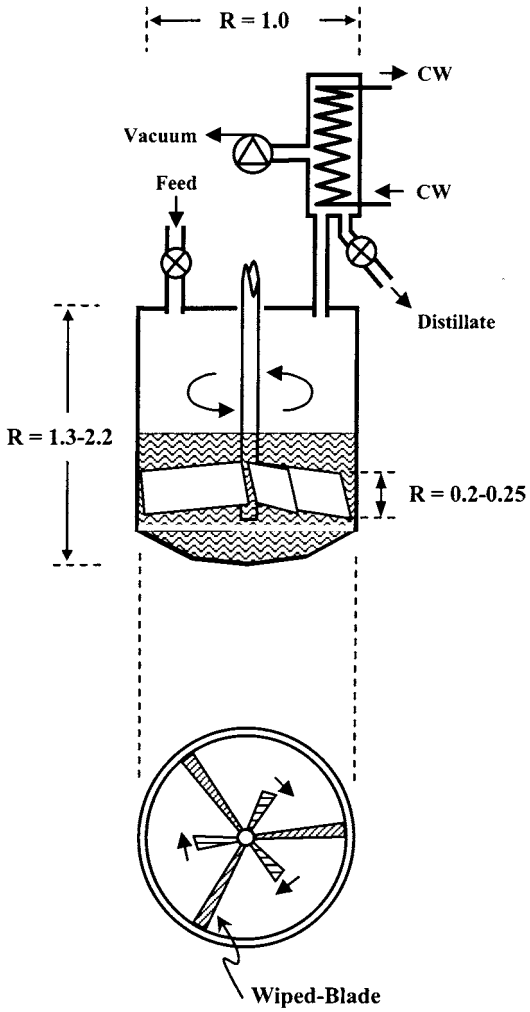

Figure 2. **Schematic of the wiped-blade reactor used for scale-up.**

elevated product yields. With vacuum applied at $t = 0$ h, the fluidization of the reaction mixtures observed at 0.5 h was substantially reduced, while delaying the initiation of vacuum to 1 h did not appear to appreciably affect the course of viscosity reduction. It is proposed that this initial plasticization of the reaction medium is critical to maintenance of catalysis, and that its reduction results in a significantly reduced rate of transglycosylation. It would appear that by 1 h, sufficient plasticization has taken place to sustain efficient catalysis, and that application of a 300 mbar vacuum at this point does not have appreciable deleterious effects. In addition, it can also be argued that premature application of vacuum strips off sufficient water from the reaction mixture to reduce catalytic function. Increasing the vacuum to 120 mbar elevated yields, while a 60 mbar vacuum had significant adverse effects, probably due to excessive stripping of ethanol/*n*-propanol and water. On the other hand, the application of 120 mbar at 1 h, followed by 60 mbar at 3 h, further improved yields (Figure 3), again suggesting the importance of promoting fluidization of the reaction mixtures for optimal catalysis whilst also enabling controlled removal of alcohols in order to shift the reaction equilibrium. These results can be contrasted with protease-mediated peptide synthesis in eutectic media, where in general, the application

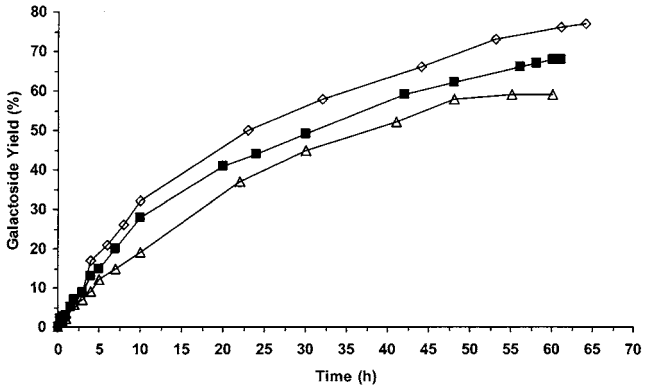

Figure 3. **Effect of scale-up and vacuum on Lactozym-mediated synthesis of 2. (■) Reaction conducted on a 0.01 kg (0.02 mol) scale in an open 0.1-L wiped-blade reactor without vacuum. (△) Reaction conducted on a 1.13 kg (2.49 mol) scale in an open 10-L wiped-blade reactor without vacuum. (◇) Reaction conducted on a 4.61 kg (10.19 mol) scale in a closed 20-L wiped-blade reactor with the application of 120 mbar vacuum at 1−3 h, then 60 mbar vacuum at 3−64 h. The reaction mixtures comprised 50:40:10 (% w/w) ethyl/*n*-propyl galactoside glass:glycidol:water, and contained 25 g of Lactozym per kg of reaction mixture. Reactions were carried out at 40 °C, with stirring at 110−120 rpm. Reactions were followed by HPLC analysis.**

of a vacuum is deleterious to the reactions due to its promotion of premature precipitation of the product and ensuing rapid solidification of the reaction mixture.[36−38] In this context, an important difference between peptide eutectics and glycoside glasses should be noted, namely that the latter are by definition glasses which are readily plasticized and typically resistant to crystallization/solidification, while the former, although prone to supercooling, often exhibit product precipitation-induced solidification.

Using the optimized conditions (Table 3), the syntheses of **2**, **3**, and **4** were efficiently scaled up to 9.4, 16.8, and 11.9 kg (20.9, 29.6, and 23.6 mol), respectively. The required products were synthesized with respective yields of 72, 68, and 69% (isolated yields of 66, 61, and 62%), and with product concentrations of 0.48, 0.46, and 0.45 kg kg^{-1}. These corresponded to productivities of ca. 96, 115, and 118 kg-product kg-enzyme^{-1}, which are well within economic margins. In addition, initial experiments with the galactosidases immobilized in hydrophobic sol−gel polymers indicate that the enzymes can be reused 5−7 times before the activities decay to 40% of their initial values, and that immobilization would enable productivities of 267, 365, and 377 kg kg^{-1} respectively. Also, the corresponding space time yields were 0.41, 0.21, and 0.19 kg kg^{-1} d^{-1}, well above the threshold of 0.08 kg kg^{-1} d^{-1} that was necessary for industrial scale production of the galactosides.

Conclusions

Glycosidase-mediated synthesis of β-D-galactosides in plasticized glass phases can be efficiently scaled up to produce kilogram quantities of 2′,3′-epoxyprop-1′-yl (**2**), 2′-(2″-methylacryolyloxy)ethyl (**3**), and pent-4′-en-1-yl (**4**) 1-*O*-β-D-galactopyranosides with high yields and productivities. Syntheses are readily implemented using the cheap, com-

Table 3. **Scale-up of galactosidase-mediated syntheses of 1-*O*-*β*-D-galactopyranosides 2, 3, and 4**

product	reaction mixture (% w/w) Et/Pr-O-Gal:ROH:H_2O	scale (kg)	mol	vacuum applied.[a] (mbar)	initial rate[b] (mol kg^{-2} h^{-1})	[prod.][c] (g g^{-1})	yield (%) (60 h)
2	50:40:10	0.01	0.02	none	2.08	0.45	70
	50:40:10	0.10	0.22	none	1.83	0.44	66
	50:40:10	1.13	2.49	none	1.58	0.40	59
	50:40:10	0.10	0.22	300 (0−60 h)	1.77	0.43	65
	50:40:10	0.10	0.22	300 (1−60 h)	1.91	0.44	69
	50:40:10	0.10	0.22	300 (2−60 h)	1.95	0.42	63
	50:40:10	0.10	0.22	300 (3−60 h)	1.80	0.41	62
	50:40:10	0.10	0.22	120 (1−60 h)	1.78	0.47	70
	50:40:10	0.10	0.22	60 (1−60 h)	1.85	0.38	56
	50:40:10	0.10	0.22	120 (1−3 h); 60 (3−60 h)	1.83	0.48	72
	50:40:10	1.02	2.25	120 (1−3 h); 60 (3−60 h)	1.72	0.44	69
	50:40:10	4.61	10.19	120 (1−3 h); 60 (3−60 h)	1.75	0.50	76
	50:40:10	9.43	20.86	120 (1−3 h); 60 (3−60 h)	1.63	0.48	72
3	40:50:10	0.01	0.02	none	5.82	0.44	67
	40:50:10	0.11	0.19	none	5.60	0.42	62
	40:50:10	1.06	1.87	none	5.34	0.38	58
	40:50:10	0.10	0.02	120 (1−60 h)	5.58	0.43	65
	40:50:10	0.10	0.02	60 (1−60 h)	5.70	0.36	53
	40:50:10	0.10	0.02	120 (1−3 h); 60 (3−60 h)	5.33	0.44	65
	40:50:10	2.28	4.03	120 (1−3 h); 60 (3−60 h)	5.33	0.45	66
	40:50:10	4.10	7.25	120 (1−3 h); 60 (3−60 h)	5.38	0.43	63
	40:50:10	16.76	29.64	120 (1−3 h); 60 (3−60 h)	5.57	0.46	68
4	45:45:10	0.10	0.20	none	4.41	0.44	67
	45:45:10	0.10	0.20	none	4.28	0.39	59
	45:45:10	0.93	0.20	none	4.07	0.34	53
	45:45:10	0.10	0.20	120 (1−60 h)	4.33	0.39	60
	45:45:10	0.10	0.20	60 (1−60 h)	4.25	0.32	51
	45:45:10	0.10	0.20	120 (1−3 h); 60 (3−60 h)	4.30	0.41	63
	45:45:10	2.03	4.04	120 (1−3 h); 60 (3−60 h)	3.95	0.41	63
	45:45:10	3.88	7.72	120 (1−3 h); 60 (3−60 h)	4.10	0.47	72
	45:45:10	11.88	23.63	120 (1−3 h); 60 (3−60 h)	3.86	0.45	69

[a] A vacuum was applied at the indicated time to remove the produced ethanol/*n*-propanol. [b] Initial rate of product formation, determined at 20 min, and quoted as mol per kg of reaction mixture per kg of biocatalyst per h. [c] Product concentration attained at the end of the reaction, as gram of product per gram of reaction mixture. Reactions were conducted in 0.1-, 10-, or 20-L glass reactors or a 38-L stainless steel reaction kettle, each fitted with a 4/6-blade wiped-blade stirrer, a water-cooled reflux condenser. Ethyl/*n*-propyl galactoside glass of composition 42:34:16:8 (*n*-Pr-*β*-D-Gal:Et-*β*-D-Gal:Gal:H_2O) was heated to 50 °C until liquefied, and phosphate acetate or phosphate buffer (25 mM, pH 5.5 or 6.5, with 5 mM calcium and magnesium chlorides) was added with stirring (50−150 rpm). The liquid was cooled to 40 °C, and the acceptor alcohol was added, followed by Lactozym 3000 or Lactase 17P (neat solution and 0.1 g mL^{-1} stock respectively, both containing 5 mM DTT). Lactozym was used at 25 g kg^{-1} for **1**, and Lactase 17P at 4 g kg^{-1} for **3** and **4**. The mixture was stirred at 40 °C, and vacuum was applied as indicated. Weighed samples were taken as required and quenched with pure methanol for HPLC analysis.

mercially Lactase 17P and Lactozym 3000 biocatalysts in wiped-blade reactors to produce multikilogram quantities of the targets, and the results suggest that the approach can provide facile, highly productive and economic protocols for the industrial production of *O*-glycosides. We are examining the production of other glycosides in plasticized glasses, and further refining reaction protocols and reactor configurations for large-scale syntheses.

Experimental Section

Enzymes and Chemicals. Lactase 17P (*Aspergillus oryzae β*-D-galactosidase, ca. 65 kU g^{-1}) was obtained from Biocatalysts Ltd, England, and Lactozym 3000 (*Kluyveromyces fragilis β*-D-galactosidase, ca. 3 kU mL^{-1}) from Novo Nordisk AS, Denmark. Glycidol, 2-hydroxyethyl methacrylate (HEMA), pent-4-en-1-ol, dithiothreitol (DTT), and all solvents were obtained from Aldrich Chemical Co.

Analytical Methods. Reactions were analysed by HPLC, using an Agilent CM4000 1100 LC equipped with a diode array detector or a Varian 9012 LC connected to an Alltech Light Scattering detector, interfaced to HP Chemstation data collection and processing software. Samples were quenched in pure methanol, centrifuged (12 000 rpm, 10 min), and then analyzed on a Hichrom RPB5, 5 μm, 0.46 cm × 15 cm column, eluted with water−acetonitrile (10 to 50% v/v MeCN, 30 min), 30 °C, a Hypercarb, 5 μm, 0.46 cm × 5 cm column eluted with water−acetonitrile (10 to 60% v/v MeCN, 35 min) 35 °C, or a Bio-Rad Aminex HPX-87C, 10 μm, 0.46 cm × 15 cm column eluted with acetonitrile−water (0 to 40% MeCN, 25 min), 60 °C. Water contents were determined by Karl Fischer titration (Metrohm 758 KFD Titrino), samples being diluted in anhydrous methanol for analysis.

^{1}H, ^{13}C, DEPT, ^{1}H−^{1}H COSY and ^{1}H−^{13}C COSY spectra were recorded on a JEOL EX270 FT spectrometer, 20 °C, with D_2O as solvent. Proton spectra were recorded at 270.0 MHz and carbon-13 spectra at 67.8 MHz with broad-band proton decoupling, using a solvent field lock. FAB-MS spectra were recorded on a Kratos MS9/50TC spectrometer using a xenon gun operating at 5−7 kV, or on a VG AUTOSPEC spectrometer using a cesium gun operating at 30 kV. The samples were dissolved in 5% v/v acetic acid, 5 or 10% w/v sodium acetate, or UHQ water, and applied to a polished copper probe smeared with glycerol or thiogly-

cerol. Spectra were recorded at 100 or 0.1 mmu in positive or negative ionization mode, using polyglycerol ions as reference. Optical rotations were measured on a Autopol-IV digital polarimeter, at 589.3 nm, 20 °C, using a 0.5-cm cell, with methanol as solvent, and are given in units of 10^{-1} deg cm^2 g^{-1}. Melting points were measured on a MelTemp-3 or Stuart Scientific SMP-1 melting point apparatus, using sealed capillaries, and are uncorrected.

Preparative Column Chromatography. Preparative column chromatography was carried out using a jacketed 12 cm × 120 cm or 15 cm × 160 cm glass chromatography column connected to two Ismatec High Flow Gear Pumps and a flow splitter connected to a Waters RI detector fitted with a preparative flow cell. The columns were slurry packed with Kieselgel 60, 40−63 μm or Fluka Silica Gel C_8, 40−63 μm, maintained at 30 °C, and step-eluted with mixtures of ethyl acetate, methanol, and 9:1 water−methanol.

Scale-Up of Syntheses in Wiped-Blade Reactors. The syntheses were scaled-up in wiped-blade reactors, consisting of jacketed 0.1-, 10-, or 20-L glass reactors or a 38-L stainless steel reaction kettle, with aspect ratios of ca. 1.3:1−2.2:1 (height:diameter). The reactors were fitted with overhead stirrers carrying turbine consisting of four or six 40° inclined polyethylene blades, each with a width ca. 0.2−0.25 × reactor vessel height. Two/three of the blades were of length ca. 0.25 × vessel diameter, while the remaining blades were of length ca. 0.52 × vessel diameter and wiped the reactor walls. The reactors were also fitted with water-cooled reflux condensers and vacuum lines.

Preparation of Ethyl + *n*-Propyl 1-*O*-*β*-D-Galactopyranoside Mixture (1). D-Galactose (18.41 kg, 102.3 mol) was dissolved in hot sodium acetate buffer (8.62 L, 20 mM, pH 5.5, containing 5 mM calcium chloride and 10 mM magnesium chloride) in a jacketed, stirred, 425-L stainless steel reactor maintained at 90 °C, ca. 230 rpm. Once dissolution was complete, the solution was cooled to 50 °C, and Lactase 17P (39 g in 0.45 L of acetate buffer) was added, followed by ethanol (3.20 L, over 10 min); then a 2:3 mixture of ethanol + *n*-propanol (182 L) was added over a period of 18 h. A second portion of Lactase 17P (15 g in 200 mL acetate buffer) was added after 10 h, and the reaction continued for 57 h, by which time the conversion of galactose to alkyl galactosides had reached 79%. The suspension was drained, filtered through an in-line filter packed with polypropylene wool, filtered through a Celite filter bed, and then evaporated under vacuum at 70 °C in a continuous rotary evaporator to give a viscous yellow liquid (21.73 kg) containing 42, 34, 16, 7, and 1% w/w of *n*-propyl 1-*O*-*β*-D-galactopyranoside, ethyl 1-*O*-*β*-D-galactopyranoside, galactose, water and *n*-propanol, respectively. On cooling to room temperature, the syrup formed a sticky, plastic glass with a glass transition temperature of 15−17 °C.

Preparation of (*2′RS*)-2′,3′-Epoxyprop-1′-yl 1-*O*-*β*-D-Galactopyranoside (2). Ethyl/propyl galactoside mixture (**1**, 5.13 kg, 20.86 mol equivalent galactose) was heated to 50 °C in a jacketed, stirred 38-L stainless steel reaction kettle fitted with a wiped-blade stirrer and reflux/takeoff head, until the glass had liquefied. Sodium phosphate buffer (0.29 L, 25 mM, pH 6.5, containing 5 mM calcium and magnesium chlorides) was then added with stirring (ca. 80 rpm) to form a viscous liquid. The liquid was cooled to 40 °C, and (*2RS*)-glycidol (3.78 kg, 51.02 mol) was added over 20 min with stirring (ca. 110 rpm) to furnish a homogeneous viscous liquid. Lactozym 3000 (0.24 L, adjusted to pH 6.5, containing 5 mM DTT) was added and stirred (ca. 120 rpm); stirring continued under reflux. The composition of the starting reaction mixture (% w/w) was *n*-Pr-*β*-D-Gal (23), Et-*β*-D-Gal (19), D-Gal (8), H_2O (10), glycidol (40). After 1 h a vacuum of 120−130 mbar was applied, and the produced ethanol/*n*-propanol was distilled off at a rate of ca. 0.01−0.04 L h^{-1}. After 3 h the vacuum was increased to 60−70 mbar, and the distillate was collected at ca. 0.04−0.09 L h^{-1}. The reaction/distillation was continued for 28 h, by which time ca. 1.7 L of distillate (ethanol, *n*-propanol, glycidol and water) had been collected, and the product yield had reached 72%. The viscous suspension was diluted with methanol (ca. 7.2 L), and the extract was filtered through Celite and concentrated by rotary evaporation at 30 °C and then 60 °C to a yellow emulsion (6.41 kg). This was diluted with methanol (ca. 3.1 L), and purified by preparative column chromatography (Kieselgel 60, eluted with 90:9:1, then 70:18:2, then 50:45:5 v/v ethyl acetate−methanol−water) to give the product as an off-white solid: 3.26 kg; 66% yield; 96% purity (by HPLC); mp 125−127 °C; $[\alpha]_D^{20} = -25.1$ (c = 0.3 in methanol); ^{1}H NMR (270 MHz, D_2O, 20 °C): δ = 2.86−3.01 (m, 2H, *H*-3′), 3.29 (m, 1H, *H*-2′), 3.43 (dd, 1H, *J* 7.5 Hz, 10.0 Hz, *H*-2), 3.45−3.72 (m, 4H, *H*-3 + *H*-5 + *H*-6), 3.80−3.93 (m, 2H, *H*-4 + *H*-1′a), 4.17 (m, 1H, *H*-1′b), 4.46 (d, 1H, *J* 7.8 Hz, *H*-1) ppm; ^{13}C NMR (67.8 MHz, D_2O, 20 °C): δ = 45.87 (*C*-3′), 51.35 (*C*-2′), 61.53 (*C*-6), 68.75 (*C*-1′), 69.38 (*C*-4), 71.23 (*C*-2), 73.66 (*C*-3), 75.83 (*C*-5), 103.67 (*C*-1) ppm; FAB-MS (glycerol matrix): (M + H) calculated for $C_9H_{17}O_7$ 237.0974, observed 237.0961.

Preparation of 2′-(2″-Methylacryolyloxy)ethyl 1-*O*-*β*-D-Galactopyranoside (3). Ethyl/propyl galactoside mixture (**1**, 7.29 kg, 29.64 mol equivalent galactose) was liquefied by heating to 50 °C in a jacketed, stirred 38-L stainless steel reaction kettle fitted with a wiped-blade stirrer and reflux/takeoff head. Sodium acetate buffer (0.43 L, 25 mM, pH 5.5, containing 5 mM of calcium and magnesium chlorides) was then added with stirring (ca. 70 rpm) over 10 min to form a viscous liquid. After cooling to 37 °C, HEMA (8.38 kg, 64.49 mol) was added over a period of 20 min to give a microemulsion, and then Lactase 17P (67 g in 0.67 L of buffer containing 5 mM DTT) was added, and stirring (ca. 100 rpm) continued under reflux. The composition of the starting reaction mixture (% w/w) was *n*-Pr-*β*-D-Gal (19), Et-*β*-D-Gal (15), D-Gal (6), H_2O (10), HEMA (50). After 1 h a vacuum of 120−130 mbar was applied, and the produced ethanol/*n*-propanol was distilled off at a rate of ca. 0.01−0.03 L h^{-1}. At 3 h the vacuum was increased to 50−65 mbar, and distillate was collected at ca. 0.03−0.07 L h^{-1}. The reaction/distillation was continued for 53 h, by which time ca. 2.2 L of distillate (ethanol, *n*-propanol and water with traces of HEMA) had been collected, and the product yield had reached 68%. The viscous suspension was diluted with

methanol (ca. 9.3 L), filtered through Celite, and concentrated by rotary evaporation at 30 °C to a viscous biphasic syrup (13.9 kg). The upper HEMA phase was decanted off and the product syrup diluted with methanol (ca. 3.8 L), then purified by preparative column chromatography (Fluka C_8 Silica, eluted with water, then 8:2, then 7:3 water−methanol) to give the product as a light yellow syrup: 5.27 kg; 61% yield; 97% purity (by HPLC); $[\alpha]_D^{20} = -46.0$ ($c = 0.3$ in methanol); ^{1}H NMR (270 MHz, D_2O, 20 °C): $\delta = 2.11$ (t, 3H, *J* 1.3 Hz, CH_3), 3.53 (dd, 1H, *J* 3.3 Hz, 9.5 Hz, *H*-3), 3.50 (dd, 1H, *J* 7.8 Hz, 9.7 Hz, *H*-2), 3.56−3.81 (m, 4H, *H*-3 + *H*-5 + *H*-6), 3.87 (m, 1H, *H*-4), 4.11 (t, 2H, *J* 5.3 Hz, *H*-2′), 4.29 (t, 2H, *J* 5.3 Hz, *H*-1′), 4.35 (d, 1H, *J* 7.8 Hz, *H*-1), 5.94 (t, 1H, *J* 1.7 Hz, *H*-3′a), 6.31 (d, 1H, *J* 1.1 Hz, *H*-3′b) ppm; ^{13}C NMR (67.8 MHz, MeOH-d_4, 20 °C): $\delta = 18.40$ (*C*H_3), 61.73 (*C*-6), 65.22 (*C*-2′), 68.64 (*C*-4), 69.44 (*C*-1′), 71.47 (*C*-2), 73.64 (*C*-3), 75.98 (*C*-5), 103.98 (*C*-1), 127.93 (*C*-3″), 136.59 (*C*-2″), 170.37 (*C*-1″) ppm; FAB-MS (thioglycerol matrix): (M − H) calcd for $C_{12}H_{19}O_8$ 291.1080, observed 291.1096.

Preparation of Pent-4′-en-1-yl 1-*O*-*β*-D-galactopyranoside (4). Ethyl/propyl galactoside mixture (**1**, 5.81 kg, 23.63 mol equivalent galactose) was liquefied by heating to 50 °C in a jacketed, stirred 38-L stainless steel reaction kettle fitted with a wiped-blade stirrer and reflux/takeoff head. A mixture of pent-4-en-1-ol (0.70 kg, 8.14 mol) and sodium acetate buffer (0.24 L, 25 mM, pH 5.5, containing 5 mM calcium and magnesium chlorides) was added with stirring (ca. 70 rpm) over 10 min to form a viscous liquid. After cooling to 40 °C, penten-4-en-1-ol (4.65 kg, 35.77 mol) was added over a period of 20 min to give a microemulsion, and then Lactase 17P (48 g in 0.48 L of buffer containing 5 mM DTT) was added, and stirring (ca. 120 rpm) continued under reflux. The composition of the starting reaction mixture (% w/w) was *n*-Pr-*β*-D-Gal (20), Et-*β*-D-Gal (17), D-Gal (8), H_2O (10), pent-4-en-1-ol (45). After 1 h a vacuum of 120−130 mbar was applied and the produced ethanol/*n*-propanol distilled off at a rate of ca. 0.01−0.03 L h^{-1}. At 3 h the vacuum was increased to 70−80 mbar, and distillate was collected at ca. 0.02−0.06 L h^{-1}. The reaction/distillation continued for 57 h, by which time ca. 2.4 L of distillate (ethanol, *n*-propanol, pent-4-en-1-ol and water) had been collected, and the product yield had reached 69%. The viscous suspension was diluted with methanol (ca. 8.3 L), filtered through Celite, and concentrated by rotary evaporation at 30 °C to a viscous biphasic mixture (9.6 kg). The upper pent-4-en-1-ol phase was decanted off, and the product syrup was diluted with methanol (ca. 3.4 L) and then purified by preparative column chromatography (Kieselgel 60, isocratic elution with 70:25:1 ethyl acetate−methanol−water) to give the product as a pale yellow syrup: 3.93 kg; 62% yield; 98% pure (by HPLC); $[\alpha]_D^{20} = -19.5$ ($c = 0.3$ in methanol); ^{1}H NMR (270 MHz, D_2O, 20 °C): $\delta = 1.71$−1.79 (m, 2H, *H*-2′), 2.09−2.15 (m, 2H, *H*-3′), 3.47 (dd, 1H, *J* 7.8 Hz, 9.5 Hz, *H*-2), 3.56−3.72 (m, 5H, *H*-3 + *H*-5 + *H*-6 + *H*-1′,a), 3.83−3.93 (m, 2H, *H*-4 + *H*-1′,b), 4.37 (d, 1H, *J* 7.8 Hz, *H*-1), 5.00−5.13 (m, 2H, *H*-5′), 5.82−5.89 (m, 1H, *H*-4′) ppm; ^{13}C NMR (67.8 MHz, D_2O, 20 °C): $\delta = 28.53$ (*C*-2′), 30.05 (*C*-3′), 61.55 (*C*-6), 69.39 (*C*-4), 70.57 (*C*-1′), 71.39 (*C*-2), 73.53 (*C*-3), 75.71 (*C*-5), 103.35 (*C*-1), 115.49 (*C*-5′), 139.47 (*C*-4′) ppm; FAB-MS (thioglycerol matrix): (M − H) calculated for $C_{11}H_{19}O_6$ 247.1182, observed 247.1175.

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