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Full Papers

Selective lipase-catalyzed 6-*O*-acylation of alkyl α-D-glucopyranosides using functionalized ethyl esters

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Abstract. Alkyl α-D-glucopyranosides were selectively converted into their 6-*O*-acyl esters by lipase-catalyzed transesterification with ethyl acrylate or ethyl 4-chlorobutanoate. Comparison of six lipase preparations showed large differences in activity and selectivity. The addition of zeolite CaA for selective adsorption of ethanol and water, exerted profound effects on conversion and regioselectivity of the transesterification. A quantitative conversion with high selectivity was achieved using lipases from *C. antarctica* in the presence of zeolite CaA.

Introduction

Alkyl and acyl glucosides are of special interest because they show excellent surfactant properties[1], low toxicity, good biodegradability[2], and potential liquid-crystalline behaviour[3]. Moreover, they are easily synthesized from two major renewable natural feedstocks, namely carbohydrates and triglycerides.

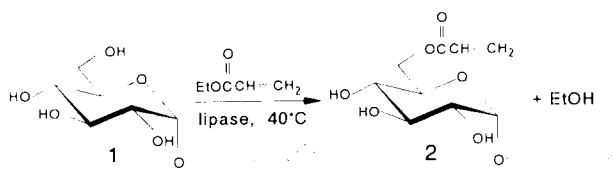
Recently, lipase-catalyzed transesterifications of alkyl glucopyranosides have been investigated for the preparation of surface-active or liquid-crystalline compounds[4–9]. Fatty acids and derivatives have generally been used as acylating agents; these compounds also provide the lipase catalysts with a natural substrate.

We have extended the investigation of the lipase-catalyzed transesterification of alkyl α-D-glucopyranosides with two functionalized esters, ethyl 2-propenoate (acrylate) and ethyl 4-chlorobutanoate, because their reaction products have potential for further syntheses. The alkyl glucopyranoside acrylates especially may serve to prepare polymeric products with liquid-crystalline character, polymeric surfactants or water-soluble polymers[10,11]. For optimal polymerization, it is important that the monomer is free from the diester by-products which are often formed in this type of reaction. We report here our results on the selective 6-*O*-acylation of alkyl glucopyranosides in the presence of various lipases. The application of zeolite CaA as a selective adsorbent of water and ethanol, proved to have interesting effects on the conversion and regioselectivity of the reaction.

Results and discussion

Selection of lipases and the reaction with ethyl acrylate

In order to select the optimal conditions for the synthesis of alkyl 6-*O*-acryl-α-D-glucopyranosides, initial experiments were performed with octyl α-D-glucopyranoside (**1**) (Scheme 1) for the preparation of which a convenient and effective procedure was developed[12]. Six lipases were tested in neat ethyl acrylate at 40°C; the progress of the reaction was monitored by HPLC.



Scheme 1. The reaction of octyl α-D-glucopyranoside with ethyl 2-propenoate.

The lipases tested differed greatly in performance (Table 1). As would be expected from published results[5,6,9], the reaction proceeded rapidly in the presence of *C. antarctica* lipase. Two by-products were formed. NMR data indicate these to be the 2-*O*-acryl and 2,6-*O*-diacryl esters of **1**. This is in agreement with earlier findings[4,5]. The progress of the reaction with *C. antarctica* lipase is depicted in Figure 1, indicating initial monoester formation followed by slow diester formation.

Lipase SP 435 has the same catalytic activity as *C. antarctica* lipase from which it has been derived[13], but with enhanced selectivity. These properties make SP 435 the most appropriate enzyme for this reaction. It should be

Table 1 Performance of lipases in the reaction [a] of **1** *with ethyl acrylate.*

Lipase	Reaction time (h) [b]	Conversion of **1** (%)	Selectivity to **2** (%)
Candida antarctica	24	93	89
SP 435	7	95	99
Mucor miehei	45	43	73
Porcine pancreas	240	51	92
Amano Ps	240	62	97
Candida cylindracea	240	0	

[a] Reactions were conducted by shaking **1** (40 mg), lipase (40 mg) and ethyl acrylate (4 ml) at 40°C in an incubator. [b] Reaction time is time for optimal conversion into **2**.

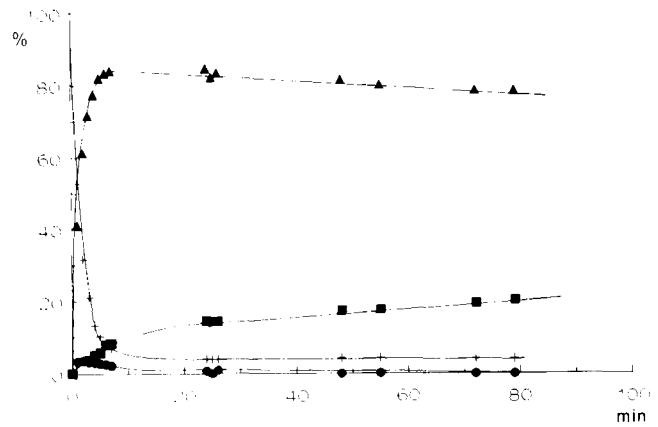


Fig. 1. Transesterification of **1** *with ethyl acrylate and C. antarctica as monitored by HPLC. Reaction mixture composition (%) vs. time (min).* + *1,* ▲ *2,* • *2-O-acryl monoester,* ■ *2,6-O-diacryl ester.*

noted that *C. antarctica* lipase consists of two enzymes, namely types A and B[14], whereas SP 435 consists exclusively of type B, which is the more selective and active catalyst [a] for this kind of transesterification. The catalytic activity of the lipases from porcine pancreas and Amano Ps was relatively low. Lipase from *C. cylindracea* was inactive under the standard conditions but the reaction proceeded sluggishly with low selectivity upon the addition of a small amount of water. Ethyl propanoate similarly failed to react with **1** in the presence of *C. cylindracea* lipase; apparently, this lipase is in need of a higher water activity and is inactive with the two short esters under the conditions applied. This is rather surprising, because transesterification of alkyl glucopyranosides at the 6-*O*-position is reported to be catalyzed by this lipase[4]. The lipase from *Mucor miehei* showed a useful activity, but the selectivity was low.

We assume that the differences in conversion given in Table I can be at least partly attributed to the influence of the water content, apart from the activity of the enzyme. The water activity may vary due to water introduced together with the lipase, with unknown effect on the activity and selectivity of the enzyme. Water, besides being considered as an essential compound of working enzymes, also engages in hydrolysis of the esters, which reaction is also lipase-catalyzed. Partial hydrolysis would lead to the formation of acrylic acid. Deactivation of lipases by short-chain carboxylic acids has been reported[15]. In this way, the presence of water contributes to inactivation of the enzyme, which might stop the reaction before equilibrium is reached.

Optimization

Under the usual conditions for enzyme-catalyzed transesterification, the composition at chemical equilibrium is often far from optimal, which constitutes a trivial problem. Complete conversion can be achieved by physical or chemical means:

- Adsorption of the alcohol formed by a zeolite. This is a well-known technique for driving transesterification reactions towards completion[16]. It has been used in lipase-catalyzed esterifications[17,18], but not always with success[19].
- Removal of the alcohol or water formed by applying vacuum[20] or azeotropic distillation. This practice precludes the use of volatile esters and heat-susceptible groups.
- Use of enol esters and removal of the carbonyl compound by distillation. The reaction is rendered irreversible in this way[21], but the limited accessibility of the enol esters and the possible formation of by-products diminishes the practical value of this method.
- Use of oxime esters, which give high selectivity for this type of reaction[8]. The value of this method is still under discussion[22].

[a] Experiments performed with a second batch of *C. antarctica* lipase immobilized on a different carrier showed somewhat higher selectivities than the first batch, therefore carrier-induced effects might also play a role.

Table II Loss of water, in % of total weight, upon drying of the enzymes used [a].

Lipase	Water loss over CaA (%)	Water loss over P_2O_5 (%)
Candida antarctica	≪ 1 (≪ 1 [b])	2 (1 [b])
SP 435	≪ 1	3
Mucor miehei	8	10
Porcine pancreas	1	2
Amano Ps	1	2
Candida cylindracea	2.5	5

[a] Enzymes were kept at 15 mm Hg and 20°C over P_2O_5 or CaA for 72 h. [b] A second batch of *C. antarctica* lipase was used for the experiments with ethyl 4-chlorobutanoate.

When zeolites are applied to transesterification with formation of methanol or ethanol, water is also adsorbed into the zeolite. The role of water in enzyme-catalyzed transesterifications has been studied extensively[23], but remains enigmatic. A small amount of water is essential for the enzymatic activity, whilst a low water concentration enhances the stability of the enzyme. Water also engages in unwanted side-reactions such as hydrolysis. Finally, water present in the active site inhibits the wanted reaction[24] by acting as a competitor for the enzyme domain. Commercial enzyme preparations contain up to 10% water. Addition of water is often recommended for optimal enzyme activity[6]. Addition of zeolite to a solvent results in a very low water concentration. In the present system zeolite and enzyme will compete for the water. This may have an effect on the activity and selectivity of the enzyme. The loss of water as a result of drying in the presence of an excess of phosphorus pentoxide or zeolite CaA of the enzymes used is given in Table II. The phosphorus pentoxide is assumed to bind the water irreversibly under the conditions applied. It may be noted that these enzyme preparations are very dry when received and that their water-binding properties are high.

In the transesterification of alkyl glucopyranosides zeolite CaA serves both to fix the water concentration at a low level and to shift the reaction equilibrium by adsorption of ethanol. The results of the reaction of **1** with ethyl acrylate are compiled in Table III.

The experiments show that *Mucor miehei* lipase becomes less active in the presence of the drying agent, which seems to indicate that the essential water is partly stripped from the enzyme. In contrast, the conversion of the other four active enzymes is enhanced; *C. antarctica* and SP 435 lipases reach quantitative conversion in a shorter time in

Table III Reaction of **1** *with ethyl acrylate in the presence of zeolite CaA* [a].

Lipase	Reaction time (h)	Conversion of **1** (%)	Selectivity to **2** (%)
Candida antarctica	5	99	99
SP 435	4	99	99
Mucor miehei	100	12	66
Porcine pancreas	72	80	80
Candida cylindracea	100	0	
Amano Ps	100	78	87

[a] Formulation as in Table I, but 0.4 g of zeolite CaA added.

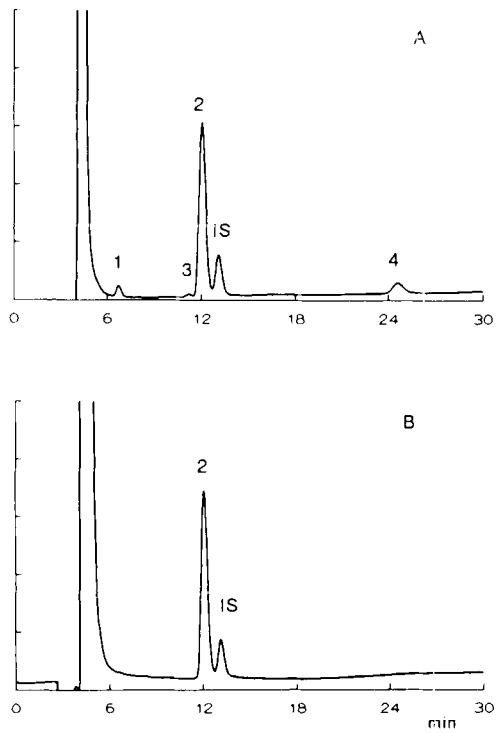


Fig. 2. Product analysis of C. antarctica *lipase-catalyzed transesterification of octyl α-D-glucopyranoside with ethyl acrylate using a Novapak C18 column and MeOH/H_2O 80/20 v/v as the eluent. Chromatogram A: 40 mg of* C. antarctica *lipase and 40 mg of octyl α-D-glucopyranoside in 4 ml of ethyl acrylate; Chromatogram B: As above but with 4 mg of* C. antarctica *lipase and 0.4 g of zeolite CaA. IS = Internal Standard (diethylene glycol dibutyl ether, 1 compound 1, 2 compound 2, 3 2-O-acryl ester, 4 2,6-O-diacryl ester.*

this essentially anhydrous medium. The amount of enzyme could be reduced by 90% (*i.e* to 4 mg) when zeolite CaA was added, without loss of conversion. Under these conditions preparative experiments for characterization were performed. Because the literature NMR data of alkyl 6-*O*-acyl-α-D-glucopyranosides are confusing[4,5,9] COSY and HETCOR NMR experiments were performed to prove the structure of **2**. Our assignments are in agreement with those of references 4 and 9.

It seems that the dehydration of the lipases has a two-fold effect: (*i*) The enzyme is deactivated by loss of part of its water resulting in a concomitant change in tertiary structure and (*ii*) the reaction rate increases because the concentration of water in the reaction cavity – which retards the reaction[24] – is reduced.

Apparently, in the case of *C. antarctica* and SP 435 the latter effect predominates. Because of the large excess of ethyl acrylate applied the adsorption of water is considered to be of much more importance than the removal of ethanol. In the case of a smaller excess of ester the influence of CaA zeolite on the conversion is more pronounced.

Effect of zeolite on the selectivity

The addition of zeolite CaA increases the selectivity of the conversion of **1** into **2** with *Candida antarctica* lipase to the level of SP 435. (*cf.* Figure 2) [b]. The other enzymes produced compound **2** less selectively when zeolite was present (Tables I and III).

It should be noted that without zeolite, the reaction proceeds, at least in principle, to the thermodynamic equilibrium. In nearly anhydrous medium, applying adsorption with zeolite, the reaction product reflects the kinetic selectivity of the catalyst because the backward reactions of the alkyl glucopyranoside esters with water or ethanol are essentially blocked.

We suppose that an anhydrous reaction medium influences *C. antarctica* lipase in a way that leads to increased selectivity. This result is not entirely unprecedented, because an effect of the water concentration on the selectivity of enzyme-catalyzed reactions has been reported previously[25]. In the present case the effect is quite large and very useful.

The reason why *C. antarctica* lipase becomes as selective as SP 435 is not clear. The A-type enzyme in *C. antarctica* lipase possibly becomes inactive in this medium, leaving only the B-type active. However, the A-type hardly shows any transesterification activity[5] and thus may not cause the high diester formation. Activation of the enzyme is also observed. This makes inactivation of one of the *C. antarctica* enzymes unlikely unless this inactivation is more than compensated for by the activation of the B type enzyme. Another explanation is that a minor loss of structural water from the enzyme leads to tightening of its structure resulting in a stronger structural preference and greater activity of its active site.

Water activity

The effect of water on the reaction was investigated in a number of experiments with decreasing water concentration. Four levels of water activity were obtained in the following ways:

(*A*) Reactants and enzyme were used without any pretreatment,

(*B*) Reactants were dried before reaction and the enzyme was used without pretreatment,

(*C*) Reactants and enzyme were dried for 24 hours before reaction,

(*D*) Reactants and enzyme were used without pretreatment, but activated zeolite was added to adsorb any water.

The results of these experiments are given in Table IV.

C. antarctica lipase appears to be much more sensitive to variation in water concentration than SP 435. The effect of *in-situ* zeolite drying and adsorption of ethanol is larger than any predrying. We assume that *in-situ* drying is the best guarantee for low water activity. To gain more insight in the actual water activity of the reaction mixture, water concentrations were measured by Karl–Fischer titration (Table V).

These measurements show the very low water activities applied during these experiments. Despite this, *C. antarctica* lipase absorbs water from the reaction mixture.

Table IV Reaction of **1** *and ethyl acrylate applying various different drying conditions* [a].

Lipase	Conditions [a]	Reaction time (h)	Conversion of **1** (%)	Selectivity to **2** (%)
Candida antarctica	(*A*)	28	25	88
SP 435	(*A*)	28	73	99
Candida antarctica	(*B*)	28	43	90
SP 435	(*B*)	28	87	99
Candida antarctica	(*C*)	28	43	95
SP 435	(*C*)	28	80	99
Candida antarctica	(*D*)	24	99	99
SP 435	(*D*)	24	99	99

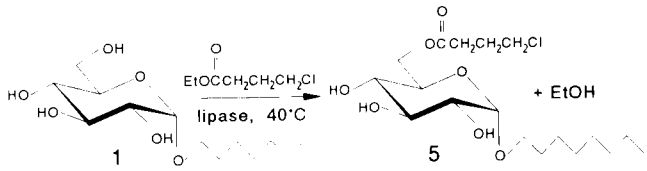
[a] (A): no drying; (B): anhydrous ethyl acrylate and **1**; (C): (B) and dried enzyme (P_2O_5); (D) *In situ* drying with zeolite CaA. Further reaction conditions as in Table III but using 4 mg of lipase.

[b] After prolonged reaction times SP 435 produces small amounts of diester in the presence of zeolite.

Table V Water activities determined by Karl-Fischer titration.

Conditions	Water content (%)	Water activity
Ethyl acrylate	0.027	0.016
Ethyl acrylate saturated with water	1.68	1.0
Ethyl acrylate dried on CaA	0.0075	0.004
Reaction mixture without zeolite [a]	0.012	0.007

[a] 40 ml of ethyl acrylate, 0.4 g of **1** and 0.4 g of *C. antarctica* lipase.



Scheme 2. Reaction of octyl α-D-glucopyranoside with ethyl 4-chlorobutanoate.

Reaction with ethyl 4-chlorobutanoate

In order to prepare a compound with a spacer between the carbohydrate moiety and a functional group, octyl α-D-glucopyranoside was subjected to lipase-catalyzed transesterification with ethyl 4-chlorobutanoate.

Six enzyme preparations were tested for this reaction, each with and without zeolite CaA. Initially, reaction of **1** and commercial ethyl 4-chlorobutanoate in the presence of *C. antarctica* lipase resulted in low conversions; similar results were obtained with ethyl 4-chlorobutanoate prepared in our laboratory from 4-chlorobutanoyl chloride and ethanol. This problem vanished when we changed to ester prepared from 4-chlorobutanoic acid by Fischer esterification. Apparently, a small amount of acid chloride (which is often difficult to remove) had remained in the product and deactivated the catalyst. Thus, esters prepared from the acid chloride generally are suspect as reagents in enzyme-catalyzed reactions. Table VI shows that SP 435 and *C. antarctica* lipase are the more active catalysts in this reaction, but again a nearly anhydrous reaction medium is required for *C. antarctica* lipase to attain high selectivity. When comparing these results with those of Tables I and III, the activation of *Mucor miehei* lipase in the presence of zeolite is noteworthy. Apparently, with ethyl 4-chlorobutanoate, less water is needed to achieve sufficient water activity for the enzyme, than in the case of ethyl acrylate. *C. cylindracea* lipase shows some activity while it was inactive with ethyl acrylate. In the presence of zeolite *C. cylindracea* lipase is not active. This makes *C. cylindracea* lipase unsuitable for reactions under low water conditions. Porcine pancreas lipase is activated by addition of zeolite, but the selectivity to **5** drops substantially. The main product seems to be a different monoester, presumably the 2-*O* acyl ester.

Other glucosides

To establish the scope of the zeolite-adsorption method, we also performed transesterifications of methyl α-D-glucopyranoside and dodecyl α-D-glucopyranoside with ethyl acrylate. The results are given in Table VII. Quantitative conversion with 99% selectivity to the 6-*O* monoester is obtained. Similar results were found for transesterification of the methyl and dodecyl α-D-glucopyranosides with ethyl 4-chlorobutanoate and ethyl decanoate. The latter compound was used as an example for reactions with long-chain fatty acid esters. Even methyl α-D-glucopyranoside which, according to the literature, is hard to esterify and until now has only been transesterified successfully using oxime esters[8], can be easily transesterified with a variety of esters using this procedure. Here *tert*-butanol proved to be a suitable cosolvent. On the other hand, alkyl β-D-glucopyranosides behave differently under these conditions. This subject is currently under investigation and the results will be published later.

Conclusions

Transesterification of alkyl α-D-glucopyranosides using *C. antarctica* type lipase and zeolite for *in-situ* selective drying and adsorption of the alcohol formed, is superior to other methods such as vacuum distillation or use of enol esters. It is an easy and straightforward method, giving high conversions and selectivities towards the 6-*O*-monoacyl esters and it can be used in the presence of sensitive groups. When zeolite adsorption is applied to lipase-catalyzed (trans)esterifications, the choice of the lipase is important. *C. cylindracea* lipase, which is often used, is not suitable under these conditions. For other

Table VI Reaction of **1** *and 4-chlorobutanoate without* [a] *(A) and with* [b] *(B) zeolite CaA.*

Lipase	Reaction time (h)		Conversion of **1** (%)		Selectivity to **5** (%)	
	A	*B*	*A*	*B*	*A*	*B*
Candida antarctica [c]	6	6 [d]	95	99 [d]	60	99 [d]
SP 435	4	6 [d]	95	99 [d]	99	99 [dc]
Mucor miehei	48	48	62	90	98	91
Porcine pancreas	48	48	7	50	50	10
Candida cylindracea	48	48	5	0	30	
Amano Ps	48	48	82	95	96	96

[a] Formulation as in Table I. [b] Formulation as in Table III. [c] A different batch of *Candida antarctica* lipase was used. [d] 4 mg of lipase used (instead of 40 mg). [e] After prolonged reaction time some diester was detected.

Table VII Transesterification [a] *of other alkyl α-D-glucopyranosides with ethyl acrylate in the presence of zeolite CaA.*

α-D-Glucopyranoside	Reaction time (h)		Conversion (%)		Selectivity (%)	
	C. ant	SP 435	*C. ant*	SP 435	*C. ant*	SP 435
Methyl α-D-glucopyranoside [b]	48	48	99	99	98 [c]	98 [c]
Dodecyl α-D-glucopyranoside	7	6	99	99	99	99

[a] Formulations as in Table III (4 mg of lipase used). [b] *tert*-Butanol 50 vol% was used as cosolvent. [c] After prolonged reaction times diester was formed.

lipases such as *Mucor miehei* results may vary and selectivities may dwindle.

Experimental

Enzyme preparations

Porcine pancreas lipase (Type II) 70000 U/g and *Candida cylindracea* 690000 U/g lipase (Type VII) were purchased from Sigma Chemical CO. *Mucor miehei* (lipozym IM 20) lipase 30 BIU/g (immobilized on a macroporous anion exchange resin), SP 435 7200 PLU/g and *Candida antarctica* 40 BIU/g lipase were donated by NOVO Industries. Amano Sp lipase 33.500 U/g was a gift of Andeno DSM. The commercial enzyme preparations were used directly without special treatment unless stated otherwise.

Chemicals

Ethyl 2-propenoate stabilized with 200 ppm 4-methoxyphenol was purchased from Merck. Solvents were used without further pretreatment unless stated otherwise. CaA (5A) zeolite was obtained from Uetikon and was activated at 400°C (24 h) before use.

Glucopyranosides

Methyl α-D-glucopyranoside was purchased from Aldrich.
Octyl α-D-glucopyranoside and dodecyl α-D-glucopyranoside were prepared according to a modified procedure of *Straathof* et al.[12] A mixture of D-glucose (21.0 g, 117 mmol), fatty alcohol (16 equivalents) and sulfuric acid (100 μl) was reacted at 115°C while passing dry air over the reaction mixture. The course of the reaction was monitored by HPLC. After 6 h (12 h for the dodecyl derivative) maximum conversion was obtained and the reaction was stopped. In the case of the octyl glucopyranoside 6 volumes of petroleum ether 40–60° were added. In 6 days octyl α-D-glucopyranoside slowly crystallised. Recrystallisation from ethyl acetate afforded the pure compound (8.0 g, 27 mmol, 23%).
For the dodecyl α-D-glucopyranoside two equivalents of diethyl ether were added and after 24 h at −8°C a mixture of α/β dodecyl glucopyranoside precipitated. Repeated crystallisation from ethyl acetate afforded the pure α-anomer (6.4 g, 18 mmol, 15%).

Analysis

HPLC analysis was carried out on a system equipped with a Waters M45 pump, a Millipore-Waters 8×100 mm 4μ Nova-Pak C18 cartridge contained in a Millipore-Waters 8×10 Radial Compression unit, a Shodex RI SE-61 refractive index detector and Shimadzu SPD-6A UV detector (220 nm). Methanol/water (70:30, v/v) at 1.0 ml/min was used as eluent. Peaks were integrated using a Spectra-Physics 4270 integrator.
GC analysis was performed on a Hewlett–Packard 5890 Series II gas chromatograph equipped with a 7673 auto-injector and a Chrompack 25 m×0.32 mm CP-Sil 5 CB, 0.12 μm column. Peaks were detected using FID and were integrated on a HP 3396A integrator. Samples were derivatized by reaction with a stock solution consisting of pyridine (104 ml), *N,N*-bis(trimethylsilyl)trifluoroacetamide (26 ml), and trimethylsilyl chloride (13 ml).
Coulometric Karl–Fischer titrations were performed using a Metrohm E 585 polarizer, a E 526 titrator, a E 524 coulostate, a E 525 integrator and a E 652 vessel using Hydranal Coulomat-C and Coulomat-A reagents.
NMR spectra were recorded in $CDCl_3$ solution with tetramethylsilane as internal standard using a 400 MHz Varian-VXR 400S spectrometer. The NMR spectra were analyzed with the aid of ^{1}H-homonuclear correlation spectroscopy (COSY) and ^{1}H–^{13}C chemical-shift correlation spectroscopy. Optical rotations were measured at 589 nm using a Perkin–Elmer 241 polarimeter.

General reaction procedure

All enzyme reactions were initiated by addition of the lipase to a mixture of the reactants. The mixture was shaken on an orbit shaker at 400 rpm at 40°C. Transesterifications were carried out with mixtures consisting of 40 mg of glucopyranoside, 40 mg of lipase, and either 4 ml of ester or a mixture of 2 ml of ester and 2 ml of cosolvent (*tert*-butanol). For the transesterification of **1** with ethyl acrylate, 20 μl of diethylene glycol dibutyl ether was added as internal standard before initiation. Periodically, aliquots of 20 μl were withdrawn and diluted with 200 μl of methanol, centrifuged to remove the enzyme, and analyzed by HPLC. For the other transesterifications 20 μl of dodecane was used as internal standard. Periodically, aliquots of 20 μl were withdrawn, derivatized with 500 μl of reagent stock solution, and analyzed on GC.

Methyl 6-O-acryl-α-D-glucopyranoside. A mixture of methyl α-D-glucopyranoside (5 g, 25.7 mmol), ethyl acrylate (125 ml), *tert*-butanol (125 ml), SP 435 lipase (500 mg), and zeolite CaA (10 g) were shaken in a stoppered flask at 500 rpm at 40°C. Progress of reaction was monitored by GC. After 72 h, a conversion of 99% was reached. Zeolite and enzyme were filtered off and washed with chloroform. The solvents were evaporated under reduced pressure. A colourless syrup was obtained which was purified by column chromatography to remove traces of ethyl acrylate and polymeric products. The column was first eluted with dichloromethane followed by methanol/dichloromethane (20:80, v/v). The yield was 5.93 g (23.9 mmol, 93%) of pure (according to GC and NMR) methyl 6-*O*-acryl-α-D-glucopyranoside as a syrup. The product was stabilized with 50 ppm of 4-methoxyphenol. $[\alpha]_D^{25}$ +94.8° (*c* 1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.38(t, 1H, H-4); 3.41(s, 3H, CH_3); 3.53(dd, 1H, H-2); 3.74(t, 1H, H-3); 3.75(m, 1H, H-5); 4.35(dd, 1H, H-6b); 4.48(dd, 1H, H-6a); 4.74(d, 1H, H-1); 5.88(dd, 1H, CH_2-cis); 6.16(dd, 1H, CH); 6.44(dd, 1H CH_2-trans). ^{13}C NMR ($CDCl_3$): δ 55.20(CH_3); 63.77(C-6); 69.65(C-5); 70.26(C-4); 71.86(C-2); 73.97(C-3); 99.45(C-1); 127.99(CH); 131.61(CH_2); 166.47(C=O).

Octyl 6-O-acryl-α-D-glucopyranoside. Octyl α-D-glucopyranoside (5 g, 17.1 mmol) was treated in the same way as above with ethyl acrylate (250 ml), *C. antarctica* lipase (500 mg), and zeolite CaA (10 g) now without *tert*-butanol. After 24 h a conversion of 99% was reached. Evaporation of the residue and purification on a silica-gel column, first eluting with dichloromethane followed by methanol/dichloromethane (5:95, v/v), yielded 5.57 g (16.1 mmol, 94%) of pure (according to GC and NMR) octyl 6-*O*-acryl-α-D-glucopyranoside as a syrup. The product was stabilized with 50 ppm of 4-methoxyphenol. $[\alpha]_D^{25}$ +63.9° (*c* 1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88(t, 3H, CH_3); 1.28(m, 10 H, 5×CH_2); 1.62(m, 2H, $CH_2\beta$); 3.37 [dt, 1H, H-4, *J*(H, OH) 4.2 Hz]; 3.47(dt, 1H, Hα′); 3.53(dd, 1H, H-2); 3.66(dt, 1H, Hα); 3.77(m, 1H, H-3); 3.80(m, 1H, H-5); 4.39(dd, 1H, H-6b); 4.53(dd, 1H, H-6a); 4.84(d, 1H, H-1); 5.85(dd, 1H, CH_2-cis); 6.16(dd, 1H, CH); 6.44(dd, 1H, CH_2-trans). ^{13}C NMR ($CDCl_3$): δ 14.10(CH_3); 22.65(CH_2); 26.06(CH_2); 29.29(CH_2); 29.42(CH_2); 29.44(CH_2); 31.87(CH_2); 63.91(C-6); 68.64(Cα); 69.82(C-5); 70.40(C-4); 72.01(C-2); 74.32(C-3); 98.35(C-1); 128.15(CH); 131.30(CH_2); 166.34(C=O).

Dodecyl 6-O-acryl-α-D-glucopyranoside. Dodecyl α-D-glucopyranoside (1 g, 2.87 mmol) was treated in the same way as above with ethyl acrylate (100 ml), *C. antarctica* lipase (100 mg), and zeolite CaA (2 g). Evaporation of the residue and purification on a silica gel column gave 1.07 g (2.66 mmol, 93%) of pure (according to GC and NMR) dodecyl 6-*O*-acryl-α-D-glucopyranoside as a syrup. The product was stabilized with 50 ppm of 4-methoxyphenol. $[\alpha]_D^{25}$ +55.2° (*c* 1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86(t, 3H, CH_3); 1.24(m, 18H, 9×CH_2); 1.60(m, 1H, $CH_2\beta$); 3.38(t, 1H, H-4); 3.45(dt, 1H, Hα′); 3.46(dd, 1H, H-2); 3.70(m, 1H, Hα); 3.76(m, 1H, H-3); 3.79(m, 1H, H-5); 4.37(dd, 1H, H-6b); 4.45(dd, 1H, H-6a); 4.84(d, 1H, H-1); 5.85(d, 1H, CH_2-*cis*); 6.16(dd, 1H, CH); 6.44(d, 1H, CH_2-*trans*). ^{13}C NMR ($CDCl_3$): δ 14.12(CH_3); 22.70(CH_2); 26.06(CH_2); 29.39(CH_2); 29.43(CH_2); 29.49(CH_2); 29.69(3×CH_2); 29.72(CH_2); 31.94(CH_2); 63.69(C-6); 68.63(Cα); 69.78(C-5); 70.31(C-4); 71.97(C-2); 74.26(C-3); 98.32(C-1); 128.08(CH); 131.40(CH_2); 166.36(C=O).

Octyl 6-O-4-chlorobutanoyl-α-D-glucopyranoside. Octyl α-D-glucopyranoside (3 g, 10.3 mmol), SP 435 lipase (300 mg), ethyl 4-chlorobutanoate (100 ml), and zeolite CaA (6 g) were shaken at 500 rpm at 40°C. After 48 h the reaction was complete according to GC. Work-up as above yielded 3.88 g (9.78 mmol, 95%) pure (according to GC and NMR) octyl 6-*O*-4-chlorobutanoyl-α-D-glucopyranoside as a syrup. $[\alpha]_D^{25}$ +61.4° (*c*, 1 chloroform). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89(t, 3H, CH_3); 1.28(m, 10H, 5×CH_2); 1.61(m, 1H, $CH_2\beta$); 2.11(dt, 2H, CH_2-CH_2Cl); 2.57(t, 2H, CH_2-CH_2-CH_2Cl); 3.38(t, 1H, H-4); 3.46(m, 1H, Hα′); 3.48(m, 1H, H-2); 3.60(t, 2H, CH_2Cl); 3.69(m, 1H, Hα); 3.74(m, 1H, H-3); 3.77(m, 1H, H-5); 4.33(dd, 1H, H-6b); 4.41(dd, 1H, H-6a); 4.85(d, 1H, H-1). ^{13}C NMR ($CDCl_3$); δ 14.09(CH_3); 22.65(CH_2); 26.09(CH_2); 27.57(CH_2-CH_2Cl); 29.23($CH_2\beta$); 29.37(CH_2-CH_2-CH_2Cl); 29.42(CH_2); 31.07(CH_2); 31.82(CH_2); 49.94(CH_2Cl); 63.52(C-6); 68.62(Cα); 69.70(C5); 70.15(C4); 72.10(C2); 74.50(C3); 98.21(C1); 173.06(C=O).

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