Ionic liquid-coated immobilized lipase for the synthesis of methylglucose fatty acid esters

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A homologous series of biosurfactants has been synthesized by a novel sustainable biotransformation technique and compared with three other enzymatic processes. 6-O-Alkanoyl-methyl-α-D-glucopyranosides were obtained by lipase mediated esterification of methyl-α-D-glucopyranoside with capric acid $C_{10:0}$, lauric acid $C_{12:0}$, myristic acid $C_{14:0}$, palmitic acid $C_{16:0}$, and oleic acid $C_{18:1}$. Solvent free transformations were compared with the use of ionic liquids and organic solvents. The lipase from *Candida antarctica* B, immobilized on macroporous acrylic acid beads (Novozyme 435), was employed either untreated or coated with small amounts of ionic liquids. This resulted in superior efficiencies (80%) with 1-butyl-4-methylpyridine hexafluorophosphate [4bmpy][PF_6] and broader substrate tolerance in comparison to solvent free transformation. The results show a positive correlation with increasing polarity of the ionic liquids used as liquid film-coating, which was in opposition to the use of the same ionic liquid as solvent. The analysis of the ionic liquid film coated catalyst carriers was performed by optical and scanning electron microscopy (SEM).

Introduction

Non-ionic surfactants from renewable resources are in general composed of one or two alkyl chains forming the hydrophobic part and a hydrophilic head such as a monosaccharide.

Chemical esterification yields a product mixture, as is easily shown by APCI mass spectroscopy of commercial products. Enzymatic catalysis in ionic liquids offers regio-selectivity and delivers therefore rather uniform products with low diester content.[1] α/β-Alkyl-glycopyranoside can be used as polar head, it is a non-reducing sugar and inert toward Maillard type reactions. In addition methylglucose fatty acid monoesters such as the investigated **3a–e** have been found to prevent microbial activity in salad dressing.[2] These kinds of surfactants like lauryl sucrose esters prove useful for a less toxic drug delivery of antifungal Amphotericin B.[3]

The enzymatic synthesis of 6-O-alkanoyl-methyl-α-D-glucopyranosides **3a–e** (Scheme 1) has been studied in order to produce glycolipid type biosurfactants[4] in a sustainable process. The use of renewable resources and the application of enzymes as catalysts is a timely concept in the development of a sustainable chemical production. In the case of performance chemicals, and in particular non-ionic surfactants, enzyme-based industrial processes tend to be more expensive than standard chemical manufacturing.[5] Therefore biotransformations are mainly used for speciality chemicals, where either small amounts of molecules are needed, such as aspartame, a dipeptide sweetener, or sold in specific markets such as natural cosmetics. Lipases are frequently used in industrial biotransformations and will become even more important as their application is more and more often

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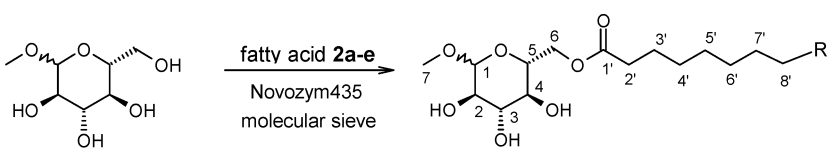

1 **3a-e**

R = -CH_2CH_3 (**a**), -$(CH_2)_3CH_3$ (**b**), -$(CH_2)_5CH_3$ (**c**), -$(CH_2)_7CH_3$ (**d**), -CH=CH$(CH_2)_7CH_3$ (**e**)

Scheme 1 Regioselective O-6 acylation of methyl-α-D-glycopyranoside **1** with fatty acids **2a–e** by *Candida antarctica B*. Fatty acids are: capric acid **2a** ($C_{10:0}$), lauric acid **2b** ($C_{12:0}$), myristic acid **2c** ($C_{14:0}$), palmitic acid **2d** ($C_{16:0}$), and oleic acid **2e** ($C_{18:1}$).

considered by manufacturers.[4d,6] In current lipase catalysis organic solvents are usually required, which cause elevated down-stream processing costs.

In order to find an ideal solvent for lipase mediated transformations one can look first at a natural solvent appropriate for lipases, such as an aqueous solution with co-solutes. However, efficient esterification is effected under minimal water conditions (0.01%) and therefore organic solvents are well suited and known to perform as needed.[7] The use of other solvents such as ionic liquids[8] can be considered as an option since they dissolve polar compounds and as non-protic solvents prevent product hydrolysis. The polar monosaccharide **1** and the amphiphilic fatty acids **2a–e** do not dissolve equally well in most liquid and therefore a solvent with an appropriate polarity is ideal.

Lipase catalysis works the more efficient the longer the alkyl glucoside substituents[9] are because they improve solubility during enzymatic reaction. Long alkyl chains turn the acylated glycoside into a surfactant with emulgator properties. Therefore the ideal surfactant contains only a methoxy substituent to provide the preferred stable acetal function. The challenge is to obtain high yields with methyl-α-D-glucopyranoside **1** although it does not dissolve well in organic solvents. To overcome this difficulty one can use supersaturated mixtures of glucose and ionic liquids to obtain high conversions in non-stoichiometric transformations.[10] However, high solvent polarity can have an adverse effect on lipase activity and ionic liquids (ILs) appear as unsuitable solvents in view of their often high polarity. Therefore less polar ILs appear appropriate for lipase catalysis. A closer look at the polarity data shows that in general the E_T^N values of ionic liquids are between 0.4 and 0.8 on the Reichardt polarity scale.[11a,b] Apart from the polarity effect it was also found that a high water binding capacity can be an indicator for unsuitable ionic liquids.[12] But fortunately there are polar ionic liquids, which are immiscible with water and in which lipase activity is better maintained than in polar organic solvents[13] or water miscible ionic liquids. In the here described investigations variants of typical ionic liquids[14] with $[PF_6]$ and $[BF_4]$ anions have been used, for which polarity data are not always available. In these cases polarity (E_T^N) was determined by UV-Vis spectroscopy with the Reichardt's dye, a betaine (2,6-diphenyl-4-(2,4,6-triphenylpyridinium-1-yl)phenolate). Stoichiometric transformations in ionic liquids with polarities as found for typical organic solvents yielded in the lower range (30–54%) and based on this result the focus was directed to solvent free conditions.

An emerging biotransformation technique is the employment of coated lipase beads, where the coating is an ionic liquid, and to use them with co-solvents such as $scCO_2$[15] or in solvent free conditions,[16] thus obtaining good to excellent yields. The application of ionic liquid coated beads in solvent free conditions results in improved conversions of **1** (Fig. 1) in comparison to the use of ionic liquids as solvents and it was observed in a trend study with myristic acid **2c** that this technique has the potential to outperform organic solvent conditions (Fig. 3).

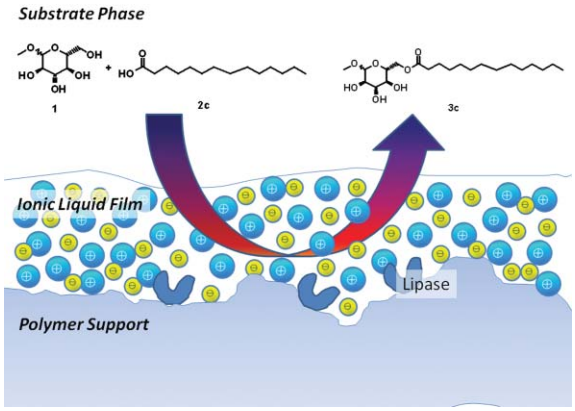

Fig. 1 Lipase catalysis with ionic liquid film coated macroporous polyacrylate carriers.

The esterification liberates condensing water, which is removed to force the reaction equilibrium to the product side. The water content influences lipase activity, which reaches an optimum at a minimal amount of water.[17] The water removal is either effected under reduced pressure[18] or in the presence of molecular sieves 3Å as used in this work.

Materials and methods

Material

Methyl-α-D-glucopyranoside and oleic acid ((9Z)-octadec-9-enoic acid) were offered by Schärer + Schläpfer Ltd (Rothrist AG, Switzerland). Methyl-β-D-glucopyranoside and capric acid (decanoic acid) [Sigma-Aldrich, Germany], myristic acid (tetradecanoic acid) 97% [Fluka AG, Switzerland], lauric acid (dodecanoic acid) 98% and palmitic acid

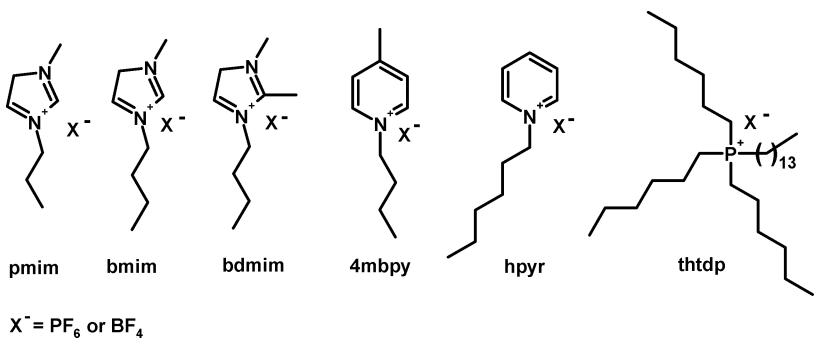

Fig. 2 Ionic liquids; [pmim] 1-propyl-3-methylimidazol, [bmim] 1-butyl-3-methylimidazol, [bdmim] 1-butyl-3,4-dimethylimidazol, [hpyr] 1-hexylpyridin, [4mbpy] 1-butyl-4-methylpyridin, [thtdp] trihexyl(tetradecyl)phosphonium. X^- corresponds to the anions $[PF_6^-]$ hexafluorophosphate and $[BF_4^-]$ tetrafluoroborate.

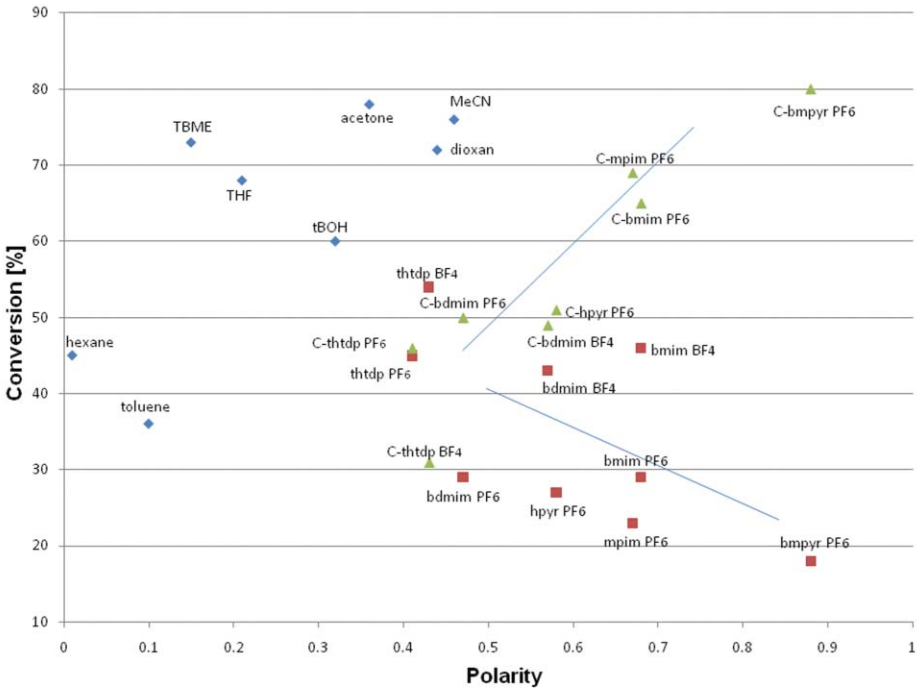

Fig. 3 Lipase mediated transformations of methyl-α-D-glucopyranoside **1** and myristic acid **2c**. Conversions of **1** against polarity on the Reichardt scale. ◆ Organic solvent conditions, ■ ionic liquids as solvent and ▲ solvent free conditions with ionic liquid coated Novozyme 435.

(hexadecanoic acid) 98% [Acros Organics, Belgium]. Ionic liquids (purum grade) 1-butyl-2,3-dimethylimidazolium hexafluorophosphate [bdmim][PF_6], the tetrafluoroborate derivative [bdmim] [BF_4], trihexyl(tetradecyl)phosphonium hexafluorophosphate [thtdp][PF_6] (CYPHOS® IL110), the tetrafluoroborate [thtdp][BF_4] (CYPHOS® IL111), 1-butyl-4-methylpyridin [4mbpy][PF_6], 1-hexylpyridinium hexafluorophosphate [hpyr][PF_6], and 1-methyl-3-propylimidazolium hexafluorophosphate [mpim][PF_6] were purchased from Fluka AG, Switzerland and IoLiTec (Ionic Liquids Technologies GmbH & Co.) KG Germany. The Reichardt dye 2,6-diphenyl-4-(2,4,6-triphenylpyridinium-1-yl)phenolate was used as bought [Fluka, AG, Switzerland] for $E_T(30)$ determination. Molecular sieves 3Å (10 meshes) [Metrohm, Switzerland] were heat activated. Acetone and *n*-hexane [Panreac Quimica, Spain] and dioxan [Fluka AG, Switzerland] were analytical grade. Demineralized water was obtained by double filtration through ion exchange columns, accompanied by reverse osmosis and UV irradiation. Novozym 435® made from methacrylate macroporous resin containing lipase *Candida Antarctica* B was offered by Novozymes A/S, Denmark or bought [Univar, Switzerland].

Analysis

HPLC. Methyl-α-D-glucopyranoside **1** conversions were determined by a HPLC setup with an Agilent 1100 series pump [Agilent technologies, USA], a differential refractometer [Knauer GmbH, Germany] and an Eppendorf CH-30 column heater coupled to a TC-50 controller [Eppendorf AG, Germany]. The separations were performed on a Bio-Rad Aminex® HPX-87H column (300 mm × 7.8 mm) heated at 55 °C under a 0.6 ml/min flow with a 4 mM H_2SO_4 solution. Data were analysed by Chrom-Card 32 bits software, v1.07 [ThermoQuest, Italy].

Sample preparation for conversion analysis. The work-up from organic solvent reaction mixtures was effected by adding 20 ml hexane and filtering on a fritted glass funnel (porosity 3), the extraction was repeated once. The retentate was dissolved in 15 ml H_2O and filtered through a syringe filter (0.45 µm), and a second time washed with 15 ml H_2O. The preparation from ionic liquid reaction mixtures followed the same procedure but it was in general implemented above the melting point (50 °C) of the ionic liquid in use.

Structure analysis: based on NMR, IR and mass spectroscopy. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer in CD_3OD and d_6-DMSO. IR spectra were obtained with a Nicolet 5700 FT-IR spectrometer [Thermo Electron Corporation] using KBr pellets. APCI Mass Spectroscopy was carried out with a Hewlett Packard Series 1100 MSD.

Melting point by DSC. The melting points were determined with a differential scanning calorimeter, DSC 821^e [Mettler Toledo].

Ionic liquid polarity determination. In cases where no polarity data were found in the literature, it was determined using Reichardt's dye.[11a] In this method a betaine (2,6-diphenyl-4-(2,4,6-triphenylpyridinio)-phenolate) is dissolved in the ionic liquid and a UV-λ_{max} was recorded. The absorption maximum of the betaine dye shifts depending on the polarity of the

ionic liquid. A spatula tip of betaine was usually added to a UV-cuvette containing the ionic liquid and after homogenization the UV-λ_{max}/50 °C was detected. An $E_{T(\text{ionic liquid})}$ value was calculated ($E_T = h \cdot C \cdot \nu_{max} \cdot N_a$) and transformed into an E_T^N value by $E_T^N = \{E_{T(\text{ionic liquid})} - 30.7\}/32.4$.

Microscopy of ionic liquid coated lipase carriers

Optical microscopy: a Zeiss AxioPlan with Objectif EC Plan-Neofluar with 25× magnification and an Olympus Europe Highlight 3000 lamp as light source were used. Data was analysed with AnalySIS [Soft Imaging System].

Scanning electron microscopy (SEM): a Zeiss type LEO 1525 FEM equipped with a GEMINI column and an "In-Lens" detector served in the submicron analysis; for elemental analysis an EDX-Link-ISIS Pentafet Super ATW detector was used. The samples were immobilized on a carbon patch fixed on an aluminium support and pretreated with gold plasma.

General procedures for 6-O-alkanoyl-methyl-α/β-D-glucopyranosides 3a–e

Method A: Transformation in organic solvent. Typically, the reaction mixture consisted of equimolar amounts of methyl-α-D-glucopyranoside **1** (0.97 g, 5 mmol) and (5 mmol) fatty acid **2a–e** catalysed by Novozym® 435 (0.49 g, 50% w/w of sugar) dissolved in an organic solvent (10 ml). Thermally activated molecular sieves (800 mg, 3Å, 10 mesh) were used for the adsorption of condensing water. The reaction mixture was placed in a 20 ml sealed glass vial, and incubated for 1–3 days in a Shel lab 1004 hybridization oven (Sheldon MFG. Inc., United States) at 60–80 °C and a rotational speed of 7 rpm. Conversions were determined by HPLC according to the method described above. In order to obtain purified products **3a–e** dried reaction mixtures were dissolved in ethylacetate and eluted on a silica gel (60Å) column by ethylacetate.

Method B: Solvent free conditions. The fatty acids were heated above the melting point (60–70 °C) in order to function as substrate solvent. The other parameters correspond to *Method A*.

Method C: Ionic liquids as solvent. In comparison to *Method A* only 1 ml solvent in the form of ionic liquids was used.

Method D: Ionic liquid film coated lipase beads. Before the transformation a coating of Novozym 435 beads with ionic liquids was applied. 500 mg ionic liquid and 10 ml acetonitrile were mixed in a 100 ml flask and 485 mg Novozym 435 was added and mixed with the rotavapor for 20 minutes at atmospheric pressure. The acetonitrile was then removed at reduced pressure 2 kPa/RT. The coated carriers were stocked over night in a dessicator at room temperature until use the next day. The transformation effected with no additional solvents (Fig. 1) corresponds to *Method B*.

6-O-decanoyl-methyl-α-D-glucopyranoside 3a

δ_H(400 MHz, CD_3OD) 0.89 (3 H, t, $J_{10',9'} = 6.4$ Hz, CH_2CH_3), 1.3 (12 H, m, 6×CH_2), 1.61 (2 H, m, $CH_2CH_2CH_2$), 2.34 (2 H, t, $J_{2',3'} = 7.2$ Hz, CH_2CO), 3.26 (1 H, m, CHOH), 3.35 (1 H, m, CHOH), 3.38 (3 H, s, OCH_3), 3.60 (1 H, t, CHOH), 3.67 (1 H, m, $CHOCH_2$), 4.17 (1 H, dd, $J_{6b,5} = 12.0$, 6.2, CH_2OCO), 4.37 (1 H, dd, $J_{6a,5} = 12.0$, 2.2 Hz, CH_2OCO), 4.64 (1 H, d, $J_{1,2} = 3.6$ Hz, $CHOCH_3$), 4.86 (3 H, s, 3×OH).

δ_C(100 MHz, CD_3OD) 14.6 (p), 23.9 (s), 26.2 (s), 30.6 (4 × s), 33.2 (s), 35.2 (s), 55.7 (p), 64.9 (p), 71.2(s), 72.1(s), 73.6(t), 75.2(s), 101.4(t), 175.5(q).

FT-IR (KBr): 3379 b-w, 2918 w-m, 2850 w-m, 1728 s, 1464 m-s, 1417 m.

m/z (APCI-MS) 349.2 ($M\text{-}H^+$, $C_{17}H_{32}O_7$ requires 348.4404). Melting point (DSC): 51.40 °C.

6-O-dodecanoyl-methyl-α-D-glucopyranoside 3b

δ_H(400 MHz; d_6-DMSO) 0.84 (3 H, t, $J_{12',11'} = 6.9$ Hz, CH_2CH_3), 1.23 (16 H, m, 8×CH_2), 1.50 (2 H, m, $CH_2CH_2CH_2$), 2.27 (2 H, t, $J_{2',3'} = 7.3$, CH_2CO), 3.04 (1 H, m, CHOH), 3.18 (1 H, m, CHOH), 3.24 (3 H, s, OCH_3), 3.36 (1 H, m, CHOH), 3.50 (1 H, m, $CHOCH_2$), 4.00 (1 H, dd, $J_{6b,5} = 11.7$, 6.8 Hz, CH_2OCO), 4.28 (1 H, dd, $J_{6a,5} = 12.0$, 2.0 Hz, CH_2OCO), 4.51 (1 H, d, $J_{1,2} = 3.7$ Hz, $CHOCH_3$), 4.76 (1 H, d, $J_{OH,2} = 6.4$ Hz, OH), 4.85 (1 H, d, $J_{OH,3} = 4.9$ Hz, OH), 5.10 (1 H, d, $J_{OH,4} = 5.9$ Hz, OH).

δ_C(100 MHz; CD_3OD) 13.1(p), 22.4(s), 24.7(s), 6×29.0(s), 31.7(s), 33.6(s), 54.2(p), 63.4(p), 69.7(s), 70.5(s), 72.1(t), 73.6(s), 99.9(t), 174.0(q).

FT-IR (KBr): 3379 b-w, 2915 w-m, 2849 w-m, 1727 s, 1474 m-s, 1464 m.

m/z (APCI-MS) 377.2 ($M\text{-}H^+$, $C_{19}H_{36}O_7$ requires 376.4946). Melting point (DSC): 70.30 °C.

6-O-tetradecanoyl-methyl-α-D-glucopyranoside 3c

δ_H(400 MHz, CD_3OD) 0.90 (3 H, t, $J_{14',13'} = 6.8$ Hz, CH_2CH_3), 1.29 (20 H, m, 10×CH_2), 1.62 (2 H, m, $CH_2CH_2CH_2$), 2.34 (2 H, t, $J_{2',3'} = 7.6$ Hz, CH_2CO), 3.27 (1 H, m, CHOH), 3.37 (1 H, m, CHOH), 3.39 (3 H, s, OCH_3), 3.60 (1 H, m, CHOH), 3.68 (1 H, m, $CHOCH_2$), 4.18 (1 H, dd, $J_{6b,5} = 11.6$, 6.2 Hz, CH_2OCO), 4.37 (1 H, dd, $J_{6a,5} = 12.0$, 2.2 Hz CH_2OCO), 4.64 (1 H, d, $J_{1,2} = 4.0$ Hz, $CHOCH_3$), 4.86 (3 H, s, 3×OH).

δ_C(100 MHz; CD_3OD) 14.6(p), 23.9(s); 26.2(s), 8 × 30.9(s); 33.2(s); 35.2(s); 55.7(p); 64.9(s); 71.1(s), 72.0(s), 73.6(t), 75.1(s), 101.4(t); 175.5(q).

FT-IR (KBr): 3411 b-w, 2916 w-m, 2849 w-m, 1727 s, 1463 m-s.

m/z (APCI-MS) 405.2 ($M\text{-}H^+$, $C_{21}H_{40}O_7$ requires 404.5488). Melting point (DSC): 71.17 °C.

6-O-hexadecanoyl-methyl-α-D-glucopyranoside 3d

δ_H(400 MHz, CD_3OD) 0.91 (3 H, t, $J_{16',15'} = 7.2$ Hz, CH_2CH_3), 1.30 (24 H, m, 12xCH_2), 1.63 (2 H, m, $CH_2CH_2CH_2$), 2.35 (2 H, t, $J_{2',3'} = 7.2$ Hz, CH_2CO), 3.31 (1 H, m, CHOH), 3.37 (1 H, m, CHOH), 3.41 (3 H, s, OCH_3), 3.59 (1 H, m, CHOH), 3.68 (1 H, m, $CHOCH_2$), 4.21 (1 H, dd, $J_{6b,5} = 11.6$, 6.2 Hz, CH_2OCO), 4.37 (1 H, dd, $J_{6a,5} = 12.0$, 2.2 Hz, CH_2OCO), 4.65 (1 H, d, $J_{1,2} = 4.0$ Hz, $CHOCH_3$), 4.86 (3 H, s, 3xOH).

δ_C(100 MHz; CD_3OD) 13.1(p), 22.4(s), 24.7(s), 10 × 29.0(s), 31.7(s), 33.7(s), 54.2(p), 63.4(s), 69.7(s), 70.5(s), 72.09(t), 73.7(s), 99.9(t), 174.0(q).

FT-IR (KBr): 3405 b-w, 2916 w-m, 2849 w-m, 1727 s, 1473 m-s.

Melting point (DSC): 86.42 °C.

6-O-(9Z)-octadecenoyl-methyl-α-D-glucopyranoside 3e

δ_H(400 MHz; d_6-DMSO) 0.84 (3 H, t, $J_{18',17'} = 6.8$ Hz, CH_2CH_3), 1.22 (16 H, m, 10xCH_2), 1. (2 H, m, $CH_2CH_2CH_2$), 2.27 (2 H, t, $J_{2',3'} = 7.3$ Hz, CH_2CO), 3.04 (1 H, m, CHOH), 3.18 (1 H, m, CHOH), 3.24 (3 H, s, OCH_3), 3.37 (1 H, m, CHOH), 3.50 (1 H, m, $CHOCH_2$), 4.00 (1 H, m, CH_2OCO), 4.27 (1 H, dd, $J_{6a,5} = 11.7$, 1.9 Hz, CH_2OCO), 4.51 (1 H, d, $J_{1,2} = 3.6$ Hz, $CHOCH_3$), 4.77 (1 H, d, $J_{OH,2} = 6.4$ Hz, OH), 4.86 (1 H, d, $J_{OH,3} = 4.9$ Hz, OH), 5.11 (1 H, d, $J_{OH,4} = 5.8$ Hz, OH) 5.31 (2H, m, $CH_2CH{=}CHCH_2$).

δ_C(100 MHz; CD_3OD) 13.1(p), 22.4(s), 24.7(s), 10 × 29.0(s), 31.7(s), 33.6(s), 54.2(p), 63.4(s), 69.7(s), 70.5(s), 72.1(t), 73.6(s), 99.9(t), 129.3(t), 129.5(t), 173.9(q).

FT-IR (KBr): 3399 b-w, 2925 w-m, 2854 w-m, 1740 s, 1458 m-s.

m/z (APCI-MS) 459.2 ($M\text{-}H^+$, $C_{25}H_{46}O_7$ requires 458.6412). Transparent oil at room temperature.

Results and discussion

Transformation in organic solvents

Transformations in organic solvents (*Method A*) were productive as expected and served as benchmark for the other *Methods B–C*. The *Methods A–D* were evaluated by two different approaches: By the application of five fatty acids **2a–e** (Table 1) examining substrate selectivity and in a trend study (Fig. 3) comparing the *Methods A, C, D* in view of solvent polarity and productivity, which was realized with myristic acid **2c**.

Methyl-α-D-glucopyranoside **1** is a polar compound, while the fatty acids **2a–e** are amphiphilic and can be considered as non-polar compounds. The ideal organic solvent should therefore well dissolve both reaction partners (**1** and **2a–e**) at equal concentrations. The conversion of 1 : 1 mixtures in non-polar solvents such as n-hexane $E_T^N = 0.01$ and toluene $E_T^N = 0.10$[19] were low with 45/36%. With increasing solvent polarity methyl-α-D-glucopyranoside **1** conversions improved and in acetone $E_T^N = 0.36$, dioxan $E_T^N = 0.44$,[20] and acetonitrile $E_T^N = 0.46$ values of 72–88% were reached for **2c**,**d**. A conversion experiment employing only myristic acid **2c** indicates an increasing productivity with higher polar organic solvents (Fig. 3). This trend is probably based on an improved substrate (**1**) availability. The immobilized lipase *Candida antarctica* B showed under optimized organic solvent conditions good selectivity for myristic $C_{14:0}$ and palmitic acid $C_{16:0}$ but not for $C_{10:0}$ and $C_{18:1}$ (Table 1, Entries 1 and 2). Methylglucose **1** obtained from renewable resources like potato starch is a racemic mixture of α- and β-enantiomers. To examine a probable distant α/β stereo selectivity of Novozyme 435, methyl-β-D-glucopyranoside **1** was transformed and a very similar productivity, 75%, was measured (72% for the α-isomer).

Solvent free transformation

The solvent free transformation (*Method B*) with substrates obtained from natural resources is considered to be the ideal approach for a sustainable process. Also the work-up appears convenient and economic. However, the substrates need to be liquids or soluble in the second substrate. In addition, to push the esterification to completion, condensing water has to be removed, what might be possible at a high vacuum of less than 0.1 kPa.[21] The production plants in the surfactant industry provide the possibility to work at 4 kPa. Trials to remove H_2O at this reduced pressure did not appear to be productive at the laboratory scale. The second non-chemical option are molecular sieves, which are recyclable.

The initial solvent free transformations at 60 °C gave low yields or proved unproductive (<15%) because of the poor solubility of methyl-α-D-glucopyranoside **1** in the fatty acids **2a–e** (Table 1, Entries 3–5). To improve the solubility of **1**, the alkyl chain of the glycoside might be lengthened, but this would yield emulsifiers instead of surfactants.[22] The second option to

Table 1 Methyl-α-D-glucopyranoside **1** conversions with fatty acids **2a–e** under various catalytic conditions

Entry	Method[a]	Solvent	Catalyst coating[b]	Reaction temperature [°C]	Methyl-α-D-glucopyranoside **1** conversions [%] with fatty acids[c]				
					$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:1}$
1	A	Acetone	—	60	49	58	78	88	43
2	A	Dioxan	—	60	59	62	72	56	49
3	B	—	—	60	0	14	15	11	0
4	B	—	—	70	0	0	47	49	0
5	B	—	—	80	0	28	37	51	0
6	C	[bmim][PF_6]	—	60	13	3	29	5	0
7	C	[bdmim][PF_6]	—	60	31	30	29	27	26
8	C	[hpyr][PF_6]	—	60	30	23	27	22	22
9	C	[bmpy][PF_6]	—	60	19	23	18	12	18
10	D	—	[bmim][PF_6]	60	9	31	65	70	50
11	D	—	[bdmim][PF_6]	60	27	32	49	28	21
12	D	—	[bdmim][PF_6]	70	25	12	54	41	7
13	D	—	[hpyr][PF_6]	60	25	23	51	65	11
14	D	—	[hpyr][PF_6]	70	19	17	54	44	1
15	D	—	[hpyr][PF_6]	80	16	40	68	56	24
16	D	—	[mpim][PF_6]	60	25	44	69	35	28

[a] Four methods: *A* with organic solvent; *B* without solvent; *C* with ionic liquid and *D* with minimal amounts of ionic liquid as liquid film coating on the macroporous acrylate structure containing *Candida Antarctica* B. [b] Novozyme 435 used either untreated or coated with ionic liquids. [c] Fatty acids **2a–e** and methyl-α-D-glucopyranoside **1** used in a 1 : 1 stoichiometry.

effect a more productive catalysis is higher temperature. The conversions at 70 °C of **1** improved to 47% ($C_{14:0}$) and 49% ($C_{16:0}$) but $C_{10:0}$ and $C_{18:1}$ fatty acids were still not transformed (Table 1, Entry 4). These experiments led to trials at 80 and 90 °C that resulted in less productive reactions. Rising the process temperature above the limit of 45 °C is possible for hydrolases such as immobilized lipase from *Candida antarctica B* and according to conversion experiments the optimum temperature was found to be around 60 °C in acetone.

Transformations in ionic liquids

Ionic liquids (*Method C*) can replace organic solvents and simplify the development of sustainable down stream processing because of biphasic workup and ionic liquid (IL) recycling.[23] Initial tests with a range of ionic liquids showed that the anion is a decisive factor for the success of the transformations[24] and isolation procedure. The hexafluorophosphate anion [PF_6^-] permitted usually a clear phase separation with extracting solvents such as water and hexane. Ionic liquids with tetrafluoroborate anion [BF_4^-] delivered comparable conversion rates but dissolved partly in extracting aqueous solution[25] in the work up.

Chosing an appropriate cation appeared less obvious and finally the investigations focused on three types of cations: the imidazol, pyridinium and phosphonium type (Fig. 2). They cover different polarity domains on the Reichardt scale.[11a,b] The phosphonium salts are known to be the least polar $E_T^N = 0.35$–0.45 and the imidazol type varies widely between $E_T^N = 0.5$–0.75 depending on the alkylation. This applies equally for pyridinium, with E_T^N values from 0.65 to 0.88, being the most polar. Polarity data can be determined by the Reichardt dye, betaine (2,6-diphenyl-4-(2,4,6-triphenylpyridinio)-phenolate).[11a] This analytical method is based on the determination of the UV_{max} absorption of the solvent, in which a betaine is dissolved. Due to the induced solvatochromic effect, the UV_{max} of the betaine shifts with solvent polarity. The combination of required lower temperature with anion and cation properties leads to the use of ionic liquids such as the typical room temperature ILs, [bmim][BF_4] and [bmim][PF_6], and others with a melting point between room temperature and 60 °C; (rt < [bdmim][BF_4] < [thtdp][BF_4] < [bdmim][PF_6] < [hp][PF_6] < [thtdp][PF_6] < [mpim][PF_6]). The obtained results in the trend analysis with myristic acid **2c** (Fig. 3) show that higher polarity of the ionic liquids correlates negatively to the conversions of methyl-α-D-glucopyranoside **1**. Therefore it seems obvious to test ionic liquids, whose polarities are close to a versatile organic solvent such as acetonitrile E_T^N =0.46. Phosphonium salts belong to the least polar ILs. Trihexyl(tetradecyl)phosphonium salts, [thtdp][BF_4] and [thtdp][PF_6], have polarities of $E_T^N = 0.41$ and 0.43, which are close to acetonitrile $E_T^N = 0.46$ and dioxan $E_T^N = 0.44$. The phosphonium cation [PR_4^+] with its four alkyl substituents (three C_6- and one C_{14} alkylchains) (Fig. 2) resembles the employed fatty acids **2a–e** and this is an advantage for the dissolution of substrates and products (Scheme 1). The conversions of **1** with C_{14} as substrate, for the trend study in Fig. 3, are still relatively low at 45–54% for the two phosphonium salts. This was a significantly lower productivity than with dioxan (72%) (Table 1, Entry 2). The imidazolium salt [bdmim][PF_6] has a similar polarity of $E_T^N = 0.47$ and furnishes even lower yields (29%). In view of obtained conversions the polarity is not the only reason for the reduced productivity in esterification[26] of fatty acids **2a–e** with methyl-α-D-glucopyranoside **1**. The influence of the ionic liquids on enzyme activity is a complex interplay of many factors.[27] It is noteworthy that the phosphonium type ionic liquid performed best and that there are to our knowledge no ILs with even lower polarity. The polarity determination by the betaine doesn't indicate the nature of the interaction on a molecular level between reaction partners. With respect to the molecular weight in relation to charge density, the phosphonium type has just half the number of charges per weight ($MW_{[thtdp][PF6]}$ = 628.82) compared with the classical room temperature ILs such as [bmim][PF_6] (MW = 284.18).

The transformations were carried out at 60–80 °C with ILs having a melting point below 60 °C. The screening with the fatty acids **2a–e** at elevated temperature >60 °C does not influence the productivity and in the case of [bdmim][PF_6] and [hpyr][PF_6] the average conversions dropped below 10% (Table 1, entries 6–9).

Solvent free catalysis with ionic liquid coated lipase carriers

Catalysis with ionic liquid coated macroporous polyacrylate lipase carriers (Novozyme 435) offers a feasible method (Fig. 1) for solvent free biotransformation of surfactants **3a–e** (*Method D*). Conversions of **1** up to 80% [mbpy] [PF_6] are in the same range or even higher than in organic solvents (72–78%) as the solvent polarity trend study with myristic acid **3c** shows (Fig. 3). The coating by ionic liquids allows a substantial solvent saving of these costly ionic liquids (only 1/20 is needed). Furthermore, this *Method D* permits a much simpler workup in comparision to ILs as solvents (*Method C*). The esterification of methyl-α-D-glucopyranoside **1** with the fatty acids **3b–d** by coated Novozyme 435 was on average ~4.5 times more efficient (Table 1, Entries 10–16) than under solvent free transformations (*Method B*) at 60 °C. The C_{10} (**3a**) and $C_{18:1}$ (**3e**) fatty acids were also transformed, which is not the case with untreated Novozyme 435 under solvent free conditions. In addition, the higher the polarity of the ionic liquid used as coating, the better the productivity of the transformation (Fig. 3). This trend is in opposition to the use of the same ionic liquids as solvent, where higher polarity yielded lower product quantities. The best conversion in the solvent trend analysis with myristic acid **2b** was obtained when using [bmpy] [PF_6] with a polarity of E_T^N = 0.88. In this case, the 80% conversion of **1** obtained contrasted with only 18% when the same ionic liquid was used as solvent *(Method C)*. The fair to good results with coated carriers by more polar ionic liquids are eventually related to the fact these ILs are better adsorbed on the polyacrylate beads. In addition the total mass of the ionic liquid is <20% of the reaction mixture and therefore not a dominating factor in the mixing process with substrate molecules.

Analysis of ionic liquid coated lipase carriers

The ionic liquid coating of the macroporous polyacrylate carriers (Novozyme 435) was analysed by optical and scanning electron microscopy (SEM). The initially opaque blue polyacrylate beads (Fig. 4a) became blue transparent (Fig. 4b) when coated. After three days of catalysis at 60 °C the colour

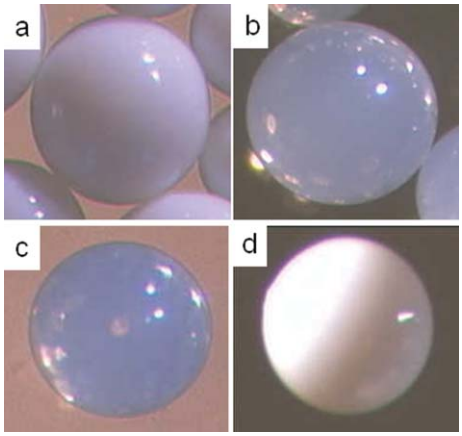

Fig. 4 25× magnification by optical microscopy of Novozyme 435 treated with ionic liquid [bdmim][PF_6]. (a) Before treatment, (b) coated with [bdmim][PF_6], (c) after three days of transformation, (d) containing pure acetonitrile used as auxiliary solvent in the coating process.

and transparency were maintained (Fig. 4c), which indicates an interaction between ionic liquid and the carrier polymer structure. The sorption behavior of Novozyme 435 is known;[28] it is based on the fact that the macroporous polyacrylate structure integrates a certain quantity of substrates and products; therefore also ionic liquid molecules can be adsorbed. To verify that the transparency was not an effect of CH_3CN serving as auxiliary solvent in the coating procedure, the beads were treated with pure CH_3CN; they became white and opaque (Fig. 4d).

Scanning electron microscopy allowed a closer examination of the surface structure of the beads. The obtained SEM images of the bead surface show dark spots, which are not present in untreated carriers (Fig. 5c). These zones contain no gold particles because an elemental scan by SEM of both areas revealed no significant difference in Phosphor and Fluor content (Fig. 5d) indicating that [PF_6] is evenly distributed on both kinds of surfaces. [bmim] [PF_6] is a room temperature ionic liquid and it is present as a liquid film in the polyacrylate carrier, therefore the gold particles on the surface can sink into the liquid film.

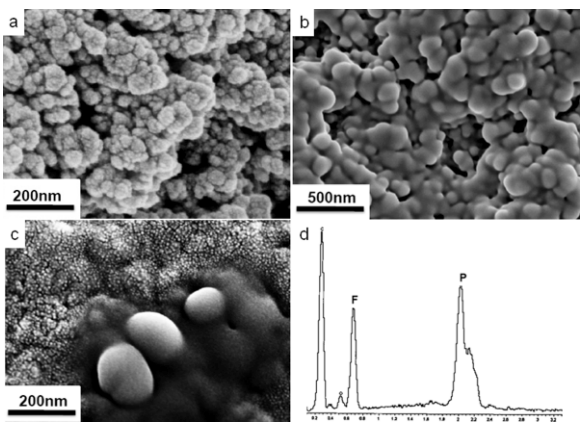

Fig. 5 Scanning electron microscopy of ionic liquid-coated Novozyme 435. (a) The inside of a non-treated carrier, (b) inside of a bead coated by [bmim][PF_6]. (c) Surface with gold particles and a dark area depleted in gold particles. (d) An elemental scan identifies [PF_6^-] evenly distributed on the surface.

For inside examinations the beads were mechanically crunched. The surfaces on the inside of the polymeric structures seemed smoother than in untreated beads (Fig. 5a,b). The ionic liquids are therefore covering the polymer structures as a liquid film, which is not strongly fixed, and allows for constant mixing with substrates (Fig. 1).

NMR of 6-O-Alkanoyl-methyl-α-D-glucopyranosides 3a–e

The structure elucidation of the surfactants **3a–e** can be based mainly on ^{1}H-NMR when d_6-DMSO is used. The hydroxyl groups C-OH(2,3,4) in the glycopyranoside are visible as the doublets ($J_{OH,C}$ = 4.9-5.9 Hz) at 4.76, 4.85 and 5.1 ppm (**3c**). It is noteworthy that with CD_3OD the hydroxyl groups appear as one broad singlet at 4.86 ppm. The ^{1}H-NMR recorded with d_6-DMSO shows therefore that the esterification is realized regioselectively on the C-OH(6) because no triplet is present and the integrals of C-OH(2,3,4) are found in 1 : 1 : 1 ratio. The presence of an ester linkage can be concluded from ^{13}C-NMR with the quarternary C(1′) at 174 ppm.

Conclusions

In order to obtain surfactants **3a–e** by a sustainable biotransformation from methyl-α-D-glucopyranoside **1** and fatty acids **2a–e** the comparison of four methods reveals that ionic liquid film-coated lipase beads are the preferred option. Organic solvents as reaction media serve as a benchmark for the various transformations. Solvent free conditions provide a simple work up. Ionic liquids were used in a series of transformations as green solvents, whereas ionic liquid-coated immobilized lipase beads were employed under solvent free conditions. The method based on coated lipase beads is quite promising, as it potentially outperforms the other methods considering productivity and sustainability at the same time.

Novozyme 435 was coated with minimal amounts of ionic liquids, which according to optical microscopy remained adsorbed throughout the biotransformation in the microporous polyacrylate beads, providing an enhanced esterification activity in the O-6 acylation of methyl-α-D-glucopyranoside **1** with fatty acids **2a–e**. Solvents of average polarity like acetone, acetonitrile and dioxan performed very well, whereas non-polar solvents such as n-hexane and toluene proved to be less productive. Ionic liquids applied as solvent were generally less efficient than organic solvents. This is also the case for ionic liquids, which provide a polarity in the range of efficient organic solvents. The conversion with ionic liquids as solvent decreased with increasing polarity. However, when the same ionic liquids were used as coating agent an improved productivity was observed. The more polar the ionic liquid applied as coating the better the yield, which improved for [bmpy][PF_6] from 18% under solvent conditions to 80%, when employing the coated catalyst.

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