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Hydroxy functional acrylate and methacrylate monomers prepared via lipase—catalyzed transacylation reactions

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ABSTRACT

Candida antarctica lipase B (CAL-B, Novozyme 435) catalyzes the transacylation of methyl acrylate and methyl methacrylate with diols and triols in 2-methyl-2-butanol at 50 °C. Under the experimental conditions, up to 70 mol% of the acyl donor methyl acrylate was converted. Methyl methacrylate is the less efficient acyl donor (up to 60 mol%) due to the higher sterical hindrance in the enzymatic transacylation. Under the reaction conditions high yields of the mono-acylated products are obtained, which contain minor amounts of bis(meth)acrylates. In addition it was observed that Novozyme 435 catalyzes regioselectively the acylation of the primary hydroxyl groups. In comparison with the chemical catalyzed route no selectivity was observed for unsubstituted diols. For substituted diols more mono-acylated product was formed in the lipase-catalyzed reaction than in the chemical catalyzed reaction.

1. Introduction

Hydroxy functional acrylates and methacrylates are interesting precursors for hydrophilic and water-soluble polymers which are promising functional polymers for biotechnological applications [1,2], including biomedical and pharmaceutical products such as contact lenses, dental materials, optical lenses, encapsulated cells, carriers for controlled drug delivery as well as hydrogels [3–6]. Hydroxy functional monomers are versatile and ideal co-monomers for cross-linking via the pendant hydroxy group; they can be used as precursor for further chemical modification leading to novel monomeric building blocks for polymer systems. Furthermore, hydroxy functional monomers can be used to tune the hydrophobic/hydrophilic balance of copolymers for different applications like thermoresponsive polymers [7,8]. Even though 2-hydroxyethyl acrylate and methacrylate (HEA, HEMA) are commercially available and well known, monomers like 3-hydroxypropyl acrylate and methacrylate (3HPA, 3HPMA), 4-hydroxybutyl acrylate and methacrylate (4HBA, 4HBMA), 2-hydroxypropyl acrylate and methacrylate (2HPA, 2HPMA), and glyceryl acrylate and methacrylate (GA, GMA) are either not commercially available, or only available at a very high price.

Chemical synthesis of hydroxy functional mono(meth)acrylates is based on the reaction of acrylic and methacrylic acid with different cyclic ethers like ethylene oxide and propylene oxide [9]. When cyclic oxides are not available or they are not reactive enough, it is necessary to use other procedures for the preparation of hydroxy functional monomers. A common method is the direct reaction of polyols with rather expensive (meth)acrylic acid chlorides or active esters of (meth)acrylic acids [10–12]. Chemical synthesis with non-activated (meth)acrylic alkyl esters, however, requires high temperatures, pressure, acidic catalysts, polymerization inhibitors and complex purification procedures to isolate the mono(meth)acrylates from the mixture of mono- and multifunctional monomers, leading to low overall yields.

In recent years, the employment of triacylglycerol lipases as biocatalysts for transesterification/transacylation reactions has emerged as a potential route to replace the conventional chemical methods. The main reason for this development is the hope for more efficient processes with higher chemo-, regio- and stereoselectivity [13–15] and with fewer environmental problems. The use of lipases as catalysts is a non-toxic and environmental friendly technology and requires mild operating conditions compared to chemical procedures [16–18]. Besides the advantage of mild operating conditions, the availability of lipases from different microbial sources possessing specificity of action and the fact that their catalytic activity can be easily regulated are some of the highlights that most of the chemical catalysts do not possess.

For lipase-catalyzed transacylation, a non-polar environment is required with low water content to shift the thermodynamic equilibrium in favour of esterification—disfavour of hydrolysis. Numerous reports have appeared in the literature on the lipase-catalyzed ester synthesis, with monoalcohols [19–22] as well as polyols demonstrating the regioselective acylation of primary alcohols in organic solvents [23–25]. Moreover, the use of

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Table 1
Lipase-catalyzed transacylation reactions performed for 120 h at 50 °C; starting materials.

No	Alcohol		Acyl donor		Novozyme 435 (mg)	Solvent (mL)
	Name	g (mmol)	Name	g (mmol)		
R1	1,4Bdiol	2.09 (23)	MA	1 (11.5)	100	3.09
R2	1,3Pdiol	1.76 (23)	MA	1 (11.5)	100	2.72
R3[a]	1,5Pdiol	2.42 (23)	MA	1 (11.5)	100	3.48
R4[a]	1,6Hdiol	2.74 (23)	MA	1 (11.5)	100	3.78
R5[a]	EG	1.44 (23)	MA	1 (11.5)	100	2.34
R6[a]	2Me1,3Pdiol	2.09 (23)	MA	1 (11.5)	100	3.1
R7	1,2Pdiol	1.76 (23)	MA	1 (11.5)	100	2.74
R8	Glycerol	3.2 (35)	MA	1 (11.5)	100	3.58
R9[a]	1,2,6Htriol	4.68 (35)	MA	1 (11.5)	100	5.26
R10[a]	NPG	2.42 (23)	MA	1 (11.5)	100	3.42
R11	1,4Bdiol	1.8 (20)	MMA	1 (10)	100	2.8
R12	1,3Pdiol	1.52 (20)	MMA	1 (10)	100	2.5
R13[a]	1,5Pdiol	2.42 (20)	MMA	1 (10)	100	3.48
R14[a]	1,6Hdiol	2.36 (20)	MMA	1 (10)	100	3.42
R15[a]	EG	1.24 (20)	MMA	1 (10)	100	2.17
R16[a]	2Me1,3Pdiol	1.77 (20)	MMA	1 (10)	100	2.83
R17	1,2Pdiol	1.52 (20)	MMA	1 (10)	100	2.53
R18	Glycerol	2.76 (30)	MMA	1 (10)	100	3.25
R19[a]	1,2,6Htriol	4.02 (30)	MMA	1 (10)	100	4.7
R20[a]	NPG	2.08 (20)	MMA	1 (10)	100	3.08

[a] No kinetic study was performed.

acrylic and methacrylic esters in transacylation reaction using lipases was investigated before: Hajjar et al. described the enzymatic transacylation of diols dissolved in ethyl acrylate using a commercial lipase from *Chromobacterium viscosum* [26]; Ikeda et al. reported the enzymatic transacylation of vinyl acrylate with alcohols using immobilized lipase from *Candida cylindracea* [27]; the synthesis of 2-carbamoyloxyethyl methacrylate from 2-hydroxyethylcarbamate and vinyl methacrylate in a solvent mixture of toluene/THF (3/1) catalyzed by lipase PS-30 *Pseudomonas* species was presented by Derango et al. [28]; Warwel et al. used Novozyme 435 as biocatalyst in the transacylation of methyl acrylate and methyl methacrylate with unsaturated fatty alcohols [29]; while Athawale et al. gave a comprehensive study of the reaction parameters governing the enzymatic synthesis of geranyl methacrylate using porcine pancreatic lipase and 2,3-butanedione mono-oxime methacrylate as acyl donor in diisopropyl ether as solvent [30]. Patents on this subject (as for example by Goldschmidt GmbH Essen, Germany) describe the preparation of surfactants from polyols and fatty acids showing the industrial relevance of this research [31].

In the present study we investigated the enzymatic transacylation of methyl acrylate and methacrylate with diols and triols, evaluating: (i) the possibility of acylation of one or both primary hydroxyl groups leading to either mono- or bis(meth)acrylates as well as (ii) the stereoselectivity and the possibility of acylation of secondary hydroxyl groups. For this purpose immobilized lipase B from *Candida antarctica* on a macroporous acrylic resin, known as Novozyme 435, which has exceptionally high activity and versatility towards transesterification reactions [21,32–36], was used. Since Novozyme 435 is commercially available, functional monomers can be prepared on larger scales and used without further purification, directly for copolymerization.

One of the main problems with the use of polyols lies in their hydrophilic nature, with the consequence that they do not dissolve in the commonly used non-polar solvents. To avoid the use of solvents like dimethyl formamide or pyridine in enzymatic acylations 2-methyl-2-butanol, a slightly polar solvent, was used in which CAL-B is stable and both reactants are soluble. This alcohol is not a substrate for the lipase being too sterically hindered to enter the active site [37,38]. However, the reactivity and selectivity of Novozyme 435 in this solvent has not yet been reported. Another bottleneck in enzymatic transacylation of methyl acrylate and methacrylate is the effect of methanol produced in the reaction on the activity of the lipase [39–42]. Nonetheless one can remove methanol either by distillation or by using molecular sieves.

2. Experimental part

2.1. Materials

Methyl methacrylate (MMA, 99+%, Fluka), methyl acrylate (MA, 99%, Acros Organics), ethylene glycol (EG, 99.8%, Riedel-de Haën), 1,3-propanediol (1,3Pdiol, 99.6+%, Aldrich), 1,4-butanediol (Bdiol, 99+%, Acros Organics), 1,5-pentanediol (1,5Pdiol, 96%, Fluka), 1,6-hexanediol (1,6Hdiol, 97%, Fluka), neopentyl glycol (NPG, 99%, Aldrich), 2-methyl-1,3-propanediol (2M1,3Pdiol, 99%, Aldrich), 1,2-propanediol (1,2Pdiol, 99.5+%, Sigma–Aldrich), 1,2,6-hexanetriol (Htriol, 97%, Acros Organics), glycerol (99.5+%, Sigma–Aldrich), 2-methyl-2-butanol (2Me2BuOH, 96+%, Fluka) and all solvents were used as received. A commercial lipase, Novozyme 435 (Lipase B from *C. antarctica* immobilized on a macroporous acrylic resin, 10,000 U g^{-1} Novo Nordisk) was dried in vacuum at room temperature for 24 h and stored under nitrogen before it was used as a biocatalyst for the transacylation reactions.

All reactions were carried out under nitrogen atmosphere. Nitrogen (Linde, 5.0) was passed over molecular sieves (4 Å) and finely distributed potassium on aluminium oxide before use.

2.2. Measurements

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT-NMR spectrometer at 400 MHz and 101 MHz, respectively, using deuterated dimethyl sulfoxide (DMSO-d_6) as solvent. Gas chromatography measurements with chiral columns were recorded on a Sichromat 1-4 apparatus. A precolumn (L = 3 m) and an Ivadex 1 column (L = 25 m) were applied. GC–MS analysis was performed on a Shimadzu GC-MS-QP5000. All spectra were measured with a column temperature program from 80 to 300 °C (25 °C/min) and 3 min hold time at 300 °C. The injection temperature was 300 °C and the detector temperature 250 °C. Identification of the compounds was performed using the mass/charge ratio (m/z) from the MS detector.

Table 2
Lipase-catalyzed transacylation reactions performed for 120 h at 50 °C; conversion of the acyl donor and the concentration of products.

No	Acyl donor conversion		Product concentration	
	Name	Conversion (%)	Mono-acylated (mol%)	Di-acylated (mol%)
R1	MA	76	88	12
R2	MA	74	91	9
R3	MA	78	88	12
R4	MA	79	88	12
R5	MA	14	99	1
R6	MA	74	94	6
R7	MA	57	77 and 21	2
R8	MA	73	94 and 3	3
R9	MA	75	72 and 14 and 8	4 and 2
R10	MA	64	95	5
R11	MMA	60	87	13
R12	MMA	82	92	8
R13	MMA	70	87	13
R14	MMA	73	88	12
R15	MMA	0	0	0
R16	MMA	66	92	8
R17	MMA	57	80 and 20	0
R18	MMA	50	92 and 6	2
R19	MMA	73	71 and 19 and 5	4 and 1
R20	MMA	50	91	9

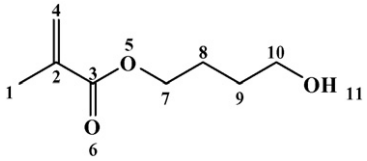
3. Syntheses

3.1. Representative procedure for lipase-catalyzed synthesis of hydroxy (meth)acrylates

1,4-Butanediol (2.09 g, 23 mmol) was dissolved in 2-methyl-2-butanol (3.09 mL, 50 vol% of the reaction mixture). To this mixture, methyl acrylate (1 g, 11.5 mmol) and Novozyme 435 (100 mg, 10 wt% with respect to MA), were added and stirred for 120 h at 50 °C. The reaction progress was monitored by ^{1}H NMR spectroscopy and GC–MS of samples taken at different reaction times: 0.36, 0.75, 1.5, 3, 6, 12, 24, 72, 96 and 120 h. One to two drops of sample were directly dissolved in deuterated dimethyl sulfoxide (DMSO-d_6) and 50 μL was transferred in GC–MS vials. The molar ratio of the products was determined by ^{1}H NMR spectroscopy as well as by GC–MS assuming similar sensitivity for the various compounds. For **R1** the following concentrations of 4-hydroxybutyl acrylate were determined at the times mentioned above: 11.6%, 17.8%, 25.6%, 35.8%, 47.2%, 55.1%, 63.3%, 66.8%, 67.8% and 68%. All other reactions were performed according to this procedure (Tables 1 and 2).

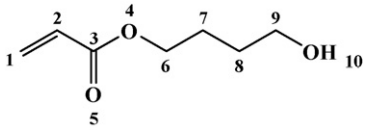
The enzyme was removed by filtration and M(M)A by distillation in vacuo at 40 °C from the reactions **R1**, **R2**, **R7**, **R8**, **R11**, **R12**, **R17**, and **R18**. The following monomers were isolated by this procedure and analysed by ^{1}H NMR and ^{13}C NMR spectroscopy.

3.1.1. 4-Hydroxybutyl methacrylate (4HBMA)



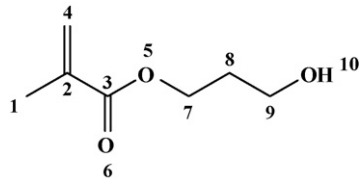
^{1}H NMR (DMSO): δ = 1.48 (m, H^9), 1.65 (m, H^8), 1.88 (s, H^1), 3.43 (m, H^{10}), 4.10 (t, J = 5.11 Hz, H^7), 4.46 (t, J = 5.10 Hz, H^{11}), 5.5 (s, H^4), 6.02 (s, H$^{4'}$).
^{13}C NMR (DMSO): δ = 17.8 (C^1), 28.51 (C^8), 28.83 (C^9), 60.23 (C^{10}), 64.17 (C^7), 125.3 (C^4), 136 (C^2), 166.4 (C^3).

3.1.2. 4-Hydroxybutyl acrylate (4HBA)



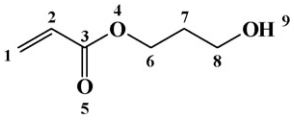
^{1}H NMR (DMSO): δ = 1.47 (m, H^8), 1.65 (m, H^7), 3.43 (m, H^9), 4.11 (t, J = 5.11 Hz, H^6), 4.46 (t, J = 5.46 Hz, H^{10}), 5.91 (d, H^1), 6.17 (m, H^2), 6.3 (d, H^1).
^{13}C NMR (DMSO): δ = 28.51 (C^7), 28.83 (C^8), 60.23 (C^9), 64.17 (C^6), 128.3 (C^2), 131.2 (C^1), 165.5 (C^3).

3.1.3. 3-Hydroxypropyl methacrylate (3HPMA)



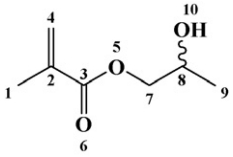
^{1}H NMR (DMSO): δ = 1.76 (m, H^8), 1.87 (s, H^1), 3.46 (m, H^9), 4.15 (t, J = 6.49 Hz, H^5), 4.56 (t, J = 5.17 Hz, H^{10}), 5.65 (s, H^4), 6.01 (s, H^4).
^{13}C NMR (DMSO): δ = 17.88 (C^1), 35.68 (C^8), 57.17 (C^9), 61.54 (C^7), 125.32 (C^4), 135.96 (C^2), 166.51 (C^3).

3.1.4. 3-Hydroxypropyl acrylate (3HPA)



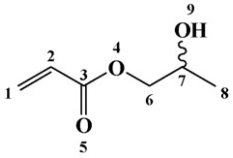
^{1}H NMR (DMSO): δ = 1.75 (m, H^7), 3.48 (m, H^8), 4.16 (t, J = 6.56 Hz, H^6), 4.55 (t, J = 5.17 Hz, H^9), 5.92 (d, H^1), 6.17 (m, H^2), 6.31 (d, H^1).
^{13}C NMR (DMSO): δ = 31.4 (C^7), 57.1 (C^8), 61.4 (C^6), 128.3 (C^2), 131.2 (C^1), 165.5 (C^3).

3.1.5. 2-Hydroxypropyl methacrylate (2HPMA)



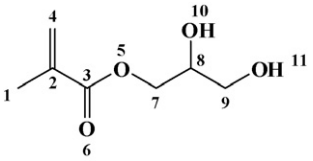
^{1}H NMR (DMSO): δ = 1.07 (d, J = 6.25 Hz, H^9), 1.88 (s, H^1), 3.85 (m, H^7), 3.91 (m, H^8, H^7), 4.86 (d, J = 4.86 Hz, H^{10}), 5.67 (s, H^4), 6.07 (s, H^4).
^{13}C NMR (DMSO): δ = 17.94 (C^1), 19.77 (C^9), 63.92 (C^8), 68.88 (C^7), 125.64 (C^4), 135.87 (C^2), 166.45 (C^3).

3.1.6. 2-Hydroxypropyl acrylate (2HPA)



^{1}H NMR (DMSO): δ = 1.08 (d, J = 6.31, H^8), 3.84 (m, H^6), 3.94 (m, H^6, H^7), 4.46 (d, J = 4.88, H^4), 4.86 (d, J = 4.86 Hz, H^9), 5.9 (d, H^1), 6.19 (m, H^2), 6.34 (d, H^1).
^{13}C NMR (DMSO): δ = 19.71 (C^8), 63.9 (C^7), 68.88 (C^6), 128.31 (C^2), 131.34 (C^1), 165.39 (C^3).

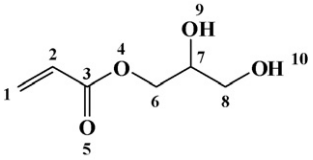
3.1.7. Glyceryl methacrylate (GMA)



^{1}H NMR (DMSO): δ = 1.87 (s, H^1), 3.44 (m, H^9), 3.7 (m, H^8), 3.98 (m, H^7), 4.08 (m, H^7), 4.69 (t, J = 5.67 Hz, H^{11}), 4.95 (d, J = 5.27, Hz H^{10}), 5.65 (s, H^4), 6.04 (s, H^4).

^{13}C NMR (DMSO): δ = 17.9 (C^1), 62.47 (C^9), 65.82 (C^7), 69.1 (C^8), 125.7 (C^4), 135.85 (C^2), 166.6 (C^3).

3.1.8. Glyceryl acrylate (GA)



^{1}H NMR (DMSO): δ = 3.42 (m, H^8), 3.69 (m, H^7), 4 (m, H^6), 4.13 (m, H^6), 4.67 (t, J = 5.69 Hz, H^{10}), 4.93 (d, J = 5.26 Hz, H^9), 5.92 (d, H^1), 6.2 (m, H^2),6.32 (d, H^1).

^{13}C NMR (DMSO): δ = 62.47 (C^8), 65.05 (C^6), 69.17 (C^7), 128 (C^2), 131.4 (C^1), 165.5 (C^3).

4. Results and discussion

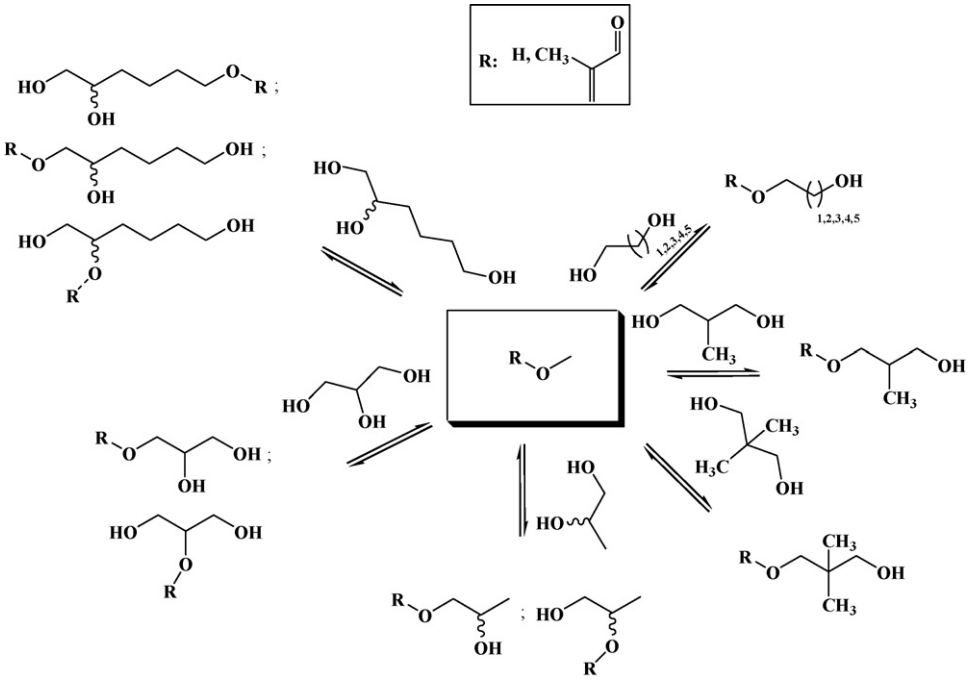
In our previous work, we developed a simple route for preparing functional acrylates and methacrylates via an enzymatic transacylation of methyl acrylate and methyl methacrylate as substrates with different monoalcohols [21]. Hydrophilic, hydrophobic as well as tertiary amine functionalized (meth)acrylates were prepared and could be readily used in a further step for polymerization. Thus, different poly[(meth)acrylate]s were synthesized via a cascade reaction of enzymatic transacylation and radical polymerization. In the present work, our previous approach was expanded to the more demanding syntheses of hydroxy functional acrylates and methacrylates via an enzymatic transacylation of methyl acrylate and methacrylate with different diols and triols (Scheme 1). In this way, monofunctional (meth)acrylate monomers, which are difficult to synthesize via conventional methods or are hardly acquired and are even not commercially available could be obtained. As will be shown in a forthcoming paper, the obtained monomers can be used without further purification to prepare copolymers via free radical and controlled radical polymerization.

To investigate the effect of the alcohol structure used as substrate on the formation of hydroxy functional mono(meth)acrylate monomers, symmetrical diols (EG; 1,3Pdiol; 1,4Bdiol; 1,5Pdiol; 1,6Hdiol), substituted symmetrical diols (2M1,3Pdiol; NPG), an asymmetrical diol (1,2Pdiol), a symmetrical triol (glycerol) as well as an asymmetrical triol (1,2,6Htriol) were selected. The enzymatic transacylation is reversible and, therefore, mono-, di- and tri-functional (meth)acrylates can be obtained. Nonetheless, sterical hindrance at the active site of the enzyme is expected to suppress multiple acylation as well as acylation of secondary alcohol groups. In addition enantioselectivity studies are performed on the transacylation of the asymmetrical diol (1,2Pdiol) and triol (1,2,6Htriol) of which racemic mixtures were used. Furthermore, it should be noted that the reaction rate and product distribution depend on the ratio of the alcohol to M(M)A [10–12], the specificity of Novozyme 435 and the thermodynamic water activity [43]. During the current work the molar ratio of the acyl donor/polyol was set to 1/number of OH groups in the polyol (this means M(M)A/diol = 1/2 and M(M)A/triol = 1/3).

4.1. Kinetic studies

4.1.1. Conversion/time dependence

To gain a better understanding of the mechanism of the lipase-catalyzed transacylation we conducted kinetic studies using 1,3Pdiol, 1,4Bdiol, 1,2Pdiol as well as glycerol as polyols and MA and MMA as acyl donors. These alcohols were used in order to synthesize hydroxy functional acrylates and methacrylates that may be suitable for a wide variety of applications [1–6]. Novozyme 435 was selected for this study being a very active and stable commer-



Scheme 1. Transacylation reaction of MA and MMA with different symmetrical and asymmetrical diols and triols. Conditions: T = 50 °C; Novozyme 435; 2Me2BuOH was used as solvent. [M(M)A]:[OH] = 1:2 for diols and 1:3 for triol.

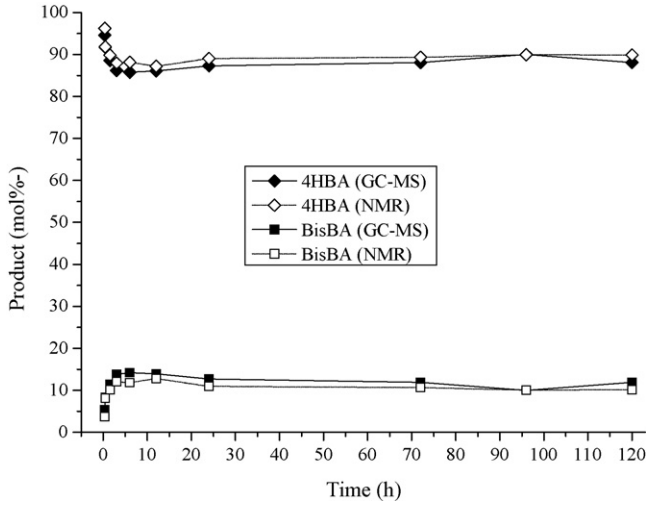


Fig. 1. Concentration of mono- and bisacrylates from the reaction of MA with 1,4Bdiol vs. time. Comparison between data obtained from ^{1}H NMR and GC–MS analysis (MA:1,4Bdiol = 1:2 M; T = 50 °C in 2Me2BuOH).

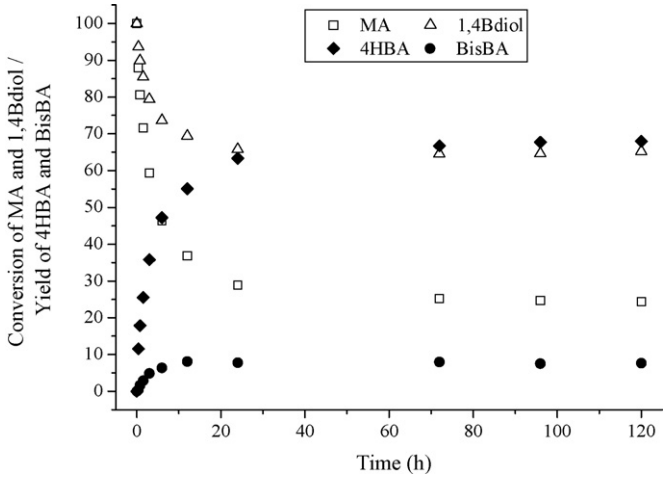


Fig. 3. Time-conversion plot for the enzymatic transacylation of MA and 1,4Bdiol as determined via ^{1}H NMR analysis. MA:1,4Bdiol = 1:2 molar ratio; T = 50 °C; 2Me2BuOH as solvent.

cially available lipase. 2Me2BuOH was used as the solvent because of its polarity allowing the dissolution of the polar polyols while the enzyme retains its catalytically active conformation [36,37]. The reaction temperature was set to 50 °C, which is within the optimum temperature range for enzyme stability (40–60 °C) [44]. In order to decrease the formation of bis(meth)acrylates, the molar ratio of the acyl donor/polyol was set to 1/number of OH groups in the alcohol according to a theoretical model presented in the literature [10–12]. Probes were taken at different reaction times and were analysed by ^{1}H NMR spectroscopy and GC–MS to determine the conversion and ratio of the products. The results from both methods were compared with respect to the formation of monoacrylate and bisacrylate (Fig. 1) for the transacylation of MA as acyl donor and 1,4Bdiol as alcohol in a molar ratio of 1/2. Fig. 1 shows that the evolution of the ratio of the new monomers, 4-hydroxybutyl acrylate (4HBA) and bisacrylate (BisBA), in time is approximately the same from ^{1}H NMR spectroscopy (dotted line) and from GC–MS (continues line).

The small differences are within experimental error and are due to overlapping signals in the ^{1}H NMR spectrum. Fig. 2 shows the ^{1}H NMR spectra at the beginning of the reaction (t_0) and after 24 h (t_{24}): characteristic for the progress of the reaction are signals of the methyl ester group (signal 3) at δ = 3.68 ppm and protons at the

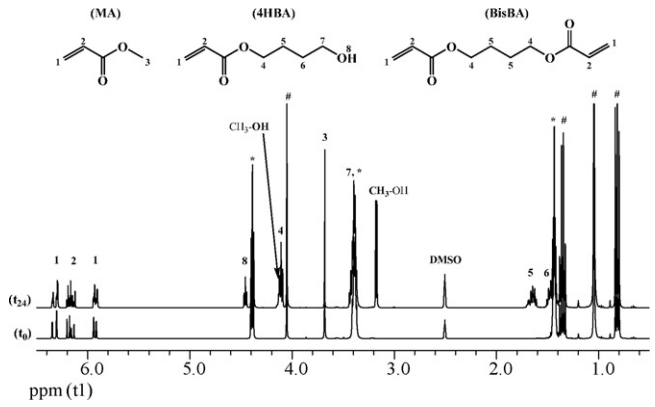


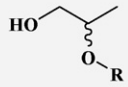
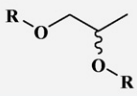
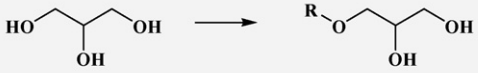
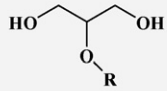
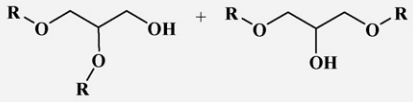
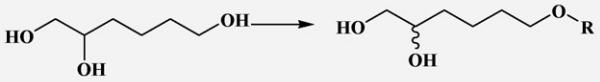
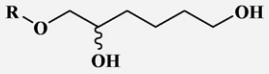
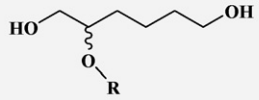
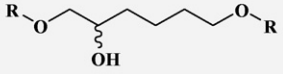
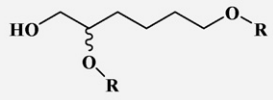
Fig. 2. ^{1}H NMR spectra of the enzymatic transacylation reaction between MA and 1,4Bdiol at time 0 h (t_0) and after 24 h (t_{24}). MA:1.4Bdiol = 1:2 molar ratio, T = 50 °C in 2Me2BuOH. (#) Signals corresponding to 2Me2BuOH and (*) signals corresponding to 1,4Bdiol.

sp^2 hybridised C-atoms at (signals 1 and 2) δ = 5.9–6.3 ppm. After 24 h (t_{24}) the formation of 4HBA is proven by the appearance of signal 8 at δ = 4.46 ppm as a triplet, corresponding to the OH group (in some cases, the coupling interaction between the CH_2 and the OH protons may be observed in DMSO [45]), the methylene group 4 at δ = 4.11 ppm as a triplet, the methylene groups 5 and 6 at δ = 1.65 and 1.5 ppm as multiplets as well as the methylene group 7 at δ = 3.42 ppm. The appearance of methanol signals at δ = 3.17 ppm for the methyl group and at δ = 4.11 ppm for the hydroxyl group further indicates that the transacylation was successful. From the ^{1}H NMR spectrum the concentration of the products can be calculated using the integral of the methyl groups from the solvent, 2Me2BuOH, at δ = 1.05 ppm as reference. The concentration of 4HBA was calculated according to the integrals of signal 8 at δ = 4.46 ppm and signal 3 corresponding to the methoxy group of MA at δ = 3.68 ppm. In order to determine the concentration of BisBA formed, integration of the double bonds region was taken into consideration.

Fig. 3 shows time conversion plots of the reaction between MA and 1,4Bdiol as determined by ^{1}H NMR spectroscopy. The formation of mono- and bisacrylates follows the statistical model [10] for the first 24 h, where both the monoacrylate and bisacrylate are formed in a ratio of 89 mol% of 4HBA and 11 mol% of BisBA. This ratio remained constant from 24 h to 120 h showing that the reaction reached the equilibrium after 24 h. Similarly GC–MS revealed 88 mol% 4HBA and 12 mol% BisBA after 24 h and for 120 h. This result corresponds to the calculated model and reveals that for the 1,4Bdiol no regiospecificity is observed in the lipase-catalyzed reaction [10].

For the reactions of 1,3Pdiol, 1,4Bdiol, 1,2Pdiol and glycerol with MA and MMA as acyl donor, the yield of the monoacrylates vs. time is shown in Fig. 4. The yield of bisacrylates after 120 h is shown in Figs. 5 and 6 and Table 3 for all the reactions. The first conclusion of the time/conversion plot (Fig. 4) is that acrylates react faster than methacrylates. One reason for this result could be the higher sterical demand of MMA compared to MA in the enzymatic transacylation reaction. In addition the higher chemical reactivity of MA than MMA could explain the experimental results. In the kinetically controlled regime of the reaction for MA, the highest rate of conversion is observed for glycerol followed by 1,3Pdiol, 1,4Bdiol, and 1,2Pdiol. In the case of MMA as acyl donor a similar behaviour is observed. However, the final conversion of glycerol and 1,2Pdiol is lower than that of 1,3Pdiol and 1,4Bdiol. This can be explained by the higher sterical demand of these substrates and by the increase of viscosity of the medium which makes difficult the diffusion to

Table 3
Molar ratios of the products obtained from the enzymatic transacylation of MA and MMA with 1,2Pdiol, glycerol and 1,2,6Htriol. M(M)A/1,2Pdiol = 1/2 M; M(M)A/glycerol (1,2,6Htriol) = 1/3 M; T = 50 °C; 2Me2BuOH as solvent.

Reaction	R=MA	R=MMA
	77 mol%	80 mol%
	21 mol%	20 mol%
	2 mol%	0 mol%
	94 mol%	92 mol%
	3 mol%	6 mol%
	3 mol%	2 mol%
	72 mol%	71 mol%
	14 mol%	19 mol%
	8 mol%	5 mol%
	4 mol%	4 mol%
	2 mol%	1 mol%

the active centre of the lipase [39]. To these arguments, the lower reactivity of secondary alcohol groups in glycerol and 1,2Pdiol can be added. As a consequence the unsubstituted α, ω diols (1,3Pdiol and 1,4Bdiol) reach higher equilibrium conversions than substituted polyols (glycerol and 1,2Pdiol). In addition the log$P$ of the mixture containing glycerol may play a significant role, resulting in a different water activity which results in a lower conversion.

4.1.2. Influence of the alcohol structure on the product ratio

4.1.2.1. Symmetrical, unsubstituted diols. To study the effect of the structure of the diol on the enzymatic transacylation, symmetrical diols (EG; 1,3Pdiol; 1,4Bdiol; 1,5Pdiol; 1,6Hdiol; 2Me1,3Pdiol; NPG), an asymmetrical diol (1,2Pdiol), a symmetrical triol (glycerol) and an asymmetrical triol (1,2,6Htriol) were analysed. All transacylation reactions studied were performed at 50 °C for 120 h in 2Me2BuOH as solvent with 10 wt% Novozyme 435 with respect to M(M)A. The molar ratio M(M)A/alcohol was set to 1/number of OH groups in the alcohol in order to decrease the probability of formation of bis(meth)acrylates. The concentration of products was determined as discussed above by ^{1}H NMR spectroscopy or GC–MS. In some cases both methods were used to obtain optimal results.

For all cases except 1,3Pdiol, the final conversion of MA is higher than that of MMA being in the range of 59–78 mol%, respectively, 50–73 mol% (Figs. 5 and 6). The conversion of both esters with EG is a special case due to the hydrophilicity of EG, which could trap the tightly bound water necessary for retaining the tertiary structure of the enzyme and thereby causing a rapid deactivation of the lipase with a consequence of no reaction respectively low conversion [43].

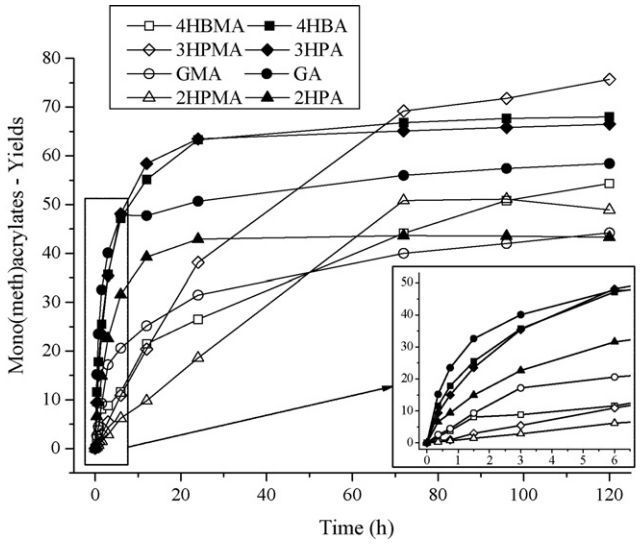


Fig. 4. Formation of mono(meth)acrylates in time as determined from ^{1}H NMR spectroscopy. M(M)A:diol = 1:2 molar ratio; M(M)A:glycerol = 1:3 molar ratio; T = 50 °C; 2Me2BuOH as solvent.

In the case of symmetrical diols (1,3Pdiol, 1,4Bdiol, 1,5Pdiol and 1,6Hdiol) the product distribution mostly follows the statistical rule as described in the literature [10,11] for chemical transacylation reactions, i.e., the enzyme does not have any selectivity for the formation of mono(meth)acrylate over bis(meth)acrylates. Nonetheless in the case of the reaction between MA and 1,3Pdiol or MMA and 1,3Pdiol the concentration of monosubstituted product is slightly higher than in the case of 1,4Bdiol, 1,5Pdiol and 1,6Hdiol and also higher than expected for the statistical product distribution (Fig. 5). This is most likely due to the chain length of the diol; the monosubstituted product formed in the reaction with 1,3Pdiol is more sterically hindered than longer diols. As a result the formation of the disubstituted compounds is suppressed. As expected, this effect is slightly stronger in the reaction with MMA where the sterical hindrance of the MMA plays an additional role (Fig. 6). However, the transacylation with EG, which is expected to have even more pronounced selectivity for the mono(meth)acrylate was not successful. In the case of the reaction between MA and EG a conversion of 14 mol% of products in respect with MA was achieved after 120 h corresponding to a concentration of mono- to bisacrylate of 99/1 as determined from GC–MS (Fig. 5) whereas in the case

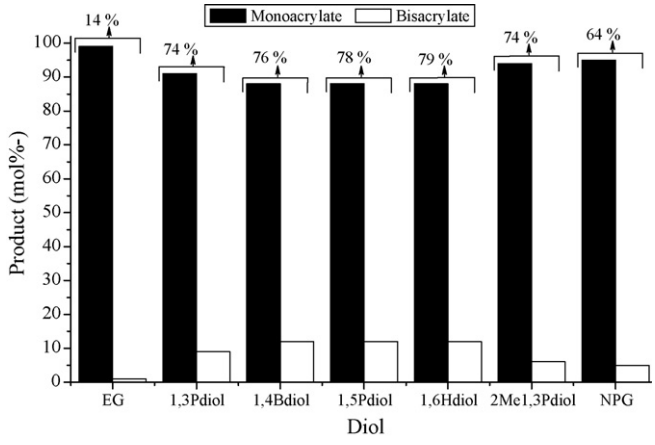


Fig. 5. Mol% of mono- and bisacrylates resulting from the transacylation of MA with different symmetrical diols after 120 h at 50 °C in 2Me2BuOH using a molar ratio MA:diol = 1:2.

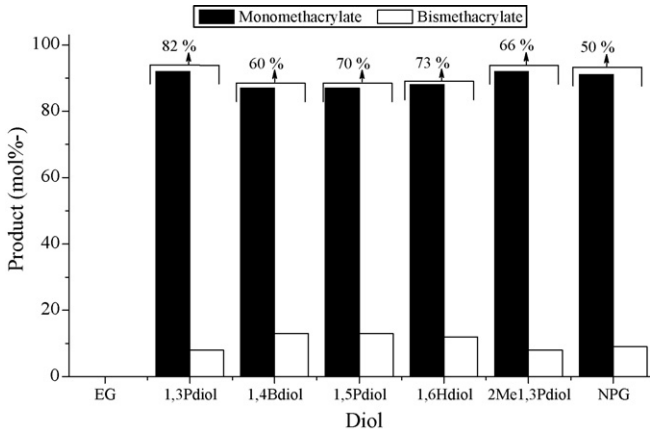


Fig. 6. Mol% of mono- and bismethacrylates resulting from the transacylation of MMA with different symmetrical diols after 120 h at 50 °C in 2Me2BuOH using a molar ratio MMA:diol = 1:2.

of MMA as acyl donor no reaction was observed at all after 120 h (Fig. 6).

4.1.2.2. Symmetrical, substituted diols. The effect of sterical hindrance was further evaluated by the use of the substituted symmetrical diols 2Me1,3Pdiol and NPG. Not surprisingly the highest concentration of mono(meth)acrylates and the lowest of bis(meth)acrylates were obtained with these diols (Figs. 5 and 6). Again the sterical hindrance of the monosubstituted compound, which could react with the acyl donor to result in bis(meth)acrylates plays an important role. Iglesias et al. [10,11] demonstrated the formation of 73 ± 3 mol% monoacrylate and 80 mol% monomethacrylate for the reaction of NPG and acid chloride with a molar ratio of NPG to acid chloride of 7–3, while the calculated statistical amount of mono(meth)acrylate is 88 mol%. Comparing these results with results obtained by enzymatic transacylation having a similar NPG:M(M)A molar ratio of 2:1, one can notice the higher amount of the monosubstituted compounds (^{1}H NMR spectroscopy: 95 mol% for the acrylate and 92 mol% for the methacrylate), clearly proving the advantage of using Novozyme 435. One more important observation was made when evaluating the product ratio in time (Fig. 7). In fact, the conversion of monoacrylate formed in this reaction increases during the reaction from 39 mol% (t = 24 h) to 56 mol% (t = 120 h) in respect with the acyl donor MA, while the concentration of bisacrylate remains constant at 3 mol% up to 120 h. The same behaviour was observed for 2Me1,3Pdiol, where the concentration of the monoacrylate increases from 60 mol% to 67 mol% and

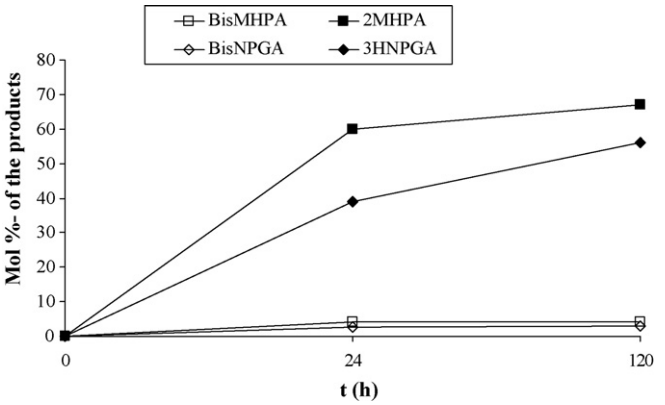


Fig. 7. Mol% of the products formed during the enzymatic transacylation of MA with NPG and 2Me1,3Pdiol at 50 °C in 2Me2BuOH.

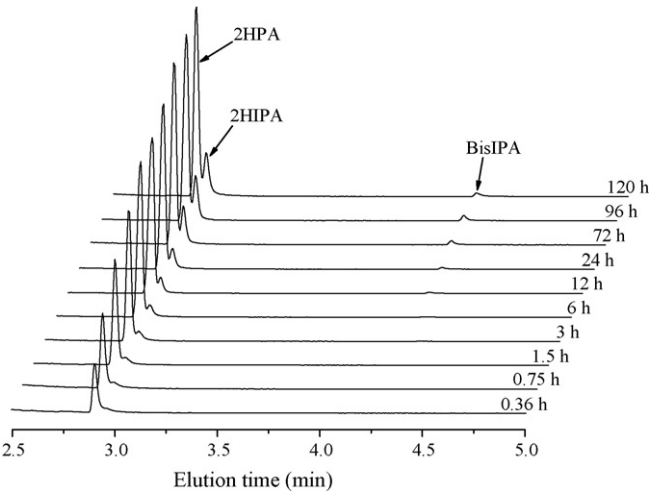


Fig. 8. Conversion scaled GC chromatograms for the reaction between methyl acrylate and 1,2-propanediol at different times (MA:1,2Pdiol = 1:2 molar; T = 50 °C in 2Me2BuOH).

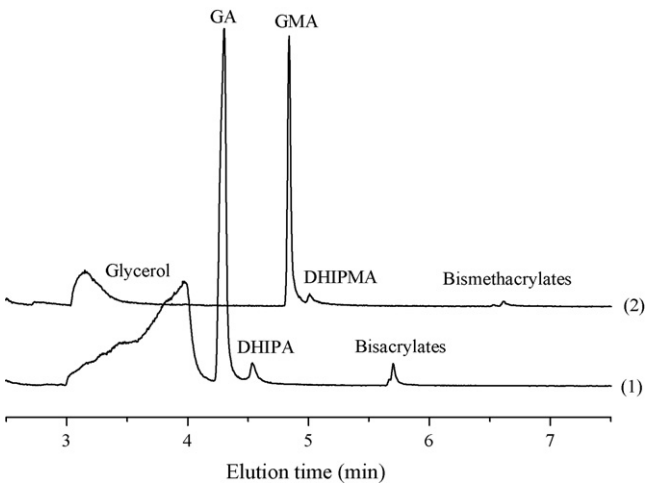


Fig. 9. Conversion scaled GC chromatogram corresponding to the reaction between (1) methyl acrylate and glycerol and (2) methyl methacrylates and glycerol after 120 h [M(M)A:glycerol = 1:3 M; T = 50 °C in 2Me2BuOH].

the concentration of bisacrylates remained constant. This result is a consequence of the sterical hindrance of the monoacrylates which cannot further react with the MA to form the bisacrylates, thus mostly monoacrylates are formed with remaining MA and the diol excess. A possible explanation for the higher concentration of 2MHPA than of 3HNPGA is again attributed to the sterical hindrance; NPG being more sterically hindered than 2Me1,3Pdiol due to the second methyl group at the C2 position shifts the equilibrium away back to the diol.

4.1.2.3. Diols with primary and secondary hydroxyl groups. When 1,2Pdiol an asymmetrical diol consisting of both a primary and a secondary hydroxyl group was used, a very small amount of bisacrylates (2 mol%) was observed while the formation of bismethacrylates could not be detected at all (Table 3). This suppression of bis(meth)acrylate formation is due to the lower reactivity of the secondary hydroxy group as well as the increased sterical hindrance caused by the vicinity of the two hydroxyl groups. However, the ratio of monosubstituted (meth)acrylates is 4:1 (77:21 mol% and 80:20 mol%), which is similar to the ratio observed for chemical synthesis of 2-hydroxypropyl acrylate from propylene oxide and acrylic acid where the minor isomer (2-hydroxy isopropylacrylate, 2HIPA) is formed in 25 mol% [9,46]. Nonetheless, the chemical route is more demanding and requires high purity reagents, CuCl as inhibitor, pyridine as solvent as well as a high temperature [46] in comparison with the enzymatic catalyzed reaction. In Fig. 8 the GC elugrams as a function of time of the reaction products of MA and 1,2Pdiol is shown. It is clearly observed that the appearance of the bisacrylate (BisIPA) starts after 6 h of reaction and a concentration of no more than 2 mol% is achieved, while the secondary hydroxyl group reacts from the beginning with a lower rate than the primary one, providing 2HIPA in 21 mol% and 2HPA in 77 mol%.

Being a racemic diol, the reaction with 1,2Pdiol can also be used to investigate the stereoselectivity of the lipase. It was found that both enantiomers reacted at the primary hydroxyl group to give 37 mol% of one isomer and 34 mol% of its enantiomer. In contrast to this result, it was previously reported for benzyl alcohol derivatives that Novozyme 435 discriminates between *R* and *S* enantiomers [47]. This difference might be an effect of the substrate and the polarity of the solvent used (2Me2BuOH), which apparently suppresses the selectivity of the Novozyme 435. In addition, the system can contain traces of water, which also has several effects on the enantioselectivity of *C. antarctica* lipase B (CAL-B) catalyzed

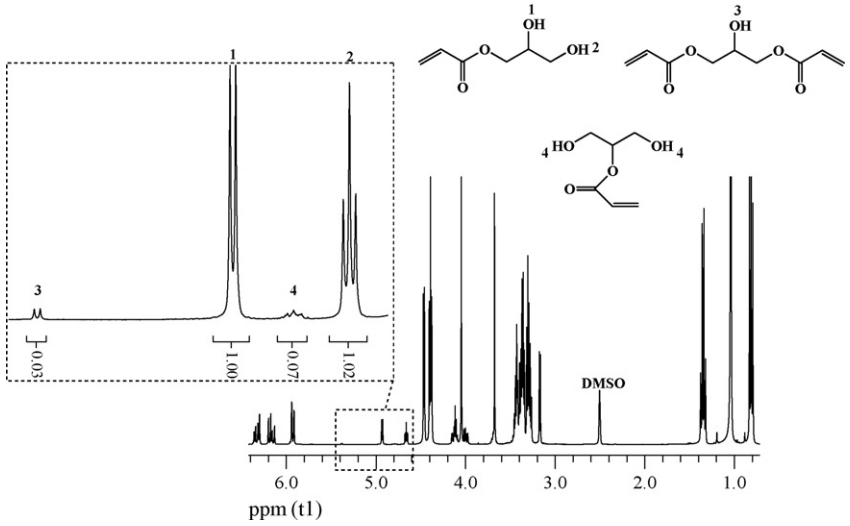


Fig. 10. ^{1}H NMR spectrum of the reaction between methyl acrylate and glycerol (MA:glycerol = 1:3 M; T = 50 °C in 2Me2BuOH).

reactions, since water could be simultaneously a competitive and enantioselective inhibitor, a competitive substrate and a lubricant [48,49].

4.1.2.4. Symmetrical triol. In the case of glycerol, a symmetrical triol, two equivalent primary hydroxyl groups and a secondary hydroxyl group of lower reactivity are involved in the reaction with the acyl donor. Garcia et al. studied the direct synthesis of monomers derived from glycerol and unsaturated acid chlorides in a stoichiometrical ratio obtaining five products: two mono-, two di- and one trisubstituted monomers [12]. Using Novozyme 435, and a 1:3 molar ratio of (meth)acrylate to glycerol only two mono(meth)acrylate products are formed and traces of bis(meth)acrylates (Fig. 9). The broad glycerol signal is due to the high polarity of the alcohol making it difficult to vaporise and causing column interactions. The major product obtained is the 1-glyceryl (meth)acrylate in a concentration of 92 mol% (for GMA) and 94 mol% (for GA) after 120 h whereas 2-glyceryl (meth)acrylate is obtained at a low extend (1,3-dihydroxyisopropyl acrylate (DHIPA) in a molar ratio of 3 mol% and 1,3-dihydroxyisopropyl methacrylate (DHIPMA) in 6 mol%). Only minor amounts of bis(meth)acrylates are formed, in maximum 3 mol% (Table 3). The different isomers of bis(meth)acrylates could not be determined due to very small amounts. In Fig. 9, in the bis(meth)acrylate region, two peaks are detected which were attributed to the two isomers obtained from both primary, respectively one primary and the secondary OH group.

In the ^{1}H NMR spectrum of the reaction between MA and glycerol after 120, Fig. 10, only three products are detected: two monosubstituted and a disubstituted glyceryl acrylate. The characteristic signals of the proton corresponding to the OH groups in all three products formed are present in the region between δ = 4.6–5.4 ppm. The proton signal characteristic to the OH group in glyceryl acrylate linked to the CH_2 group (signal 2) appears at δ = 4.66 ppm as a triplet while at δ = 4.92 ppm the proton signal corresponding to the secondary OH group is present as a doublet (signal 1). The doublet observed in the region δ = 5.4 ppm is assigned to the OH group of the bisacrylate (signal 3), whereas the triplet at d = 4.8 ppm is characteristic for the hydroxyl group of dihydroxyisopropyl acrylate (signal 4). The integration of these signals provides us the molar ratio of the products obtained as follows: GA:DHIPA:BisAc = 94:3:3 mol%, which is in accordance with the composition determined by GC–MS chromatography.

4.1.2.5. Asymmetrical triol. Transacylation of methyl acrylate and methyl methacrylate with 1,2,6Htriol using Novozyme 435 as catalyst was performed in 2Me2BuOH at 50 °C with a molar ratio of MA/1,2,6Htriol = 1/3. The product mixture was analysed via

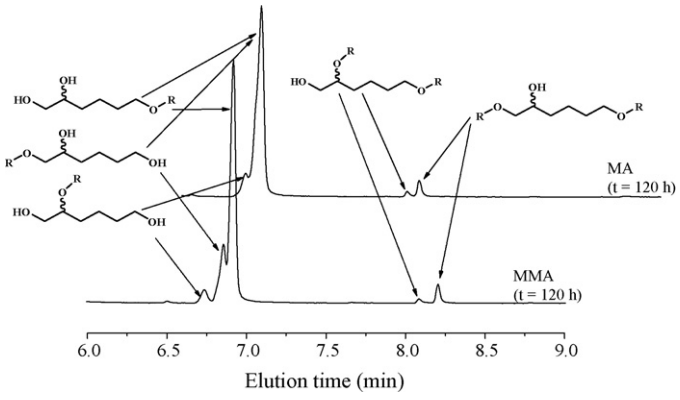


Fig. 11. GC–MS chromatograms for the reactions between MA and MMA with 1,2,6Htriol. MA:1,2,6Htriol = 1:3 molar ratio; T = 50 °C in 2Me2BuOH.

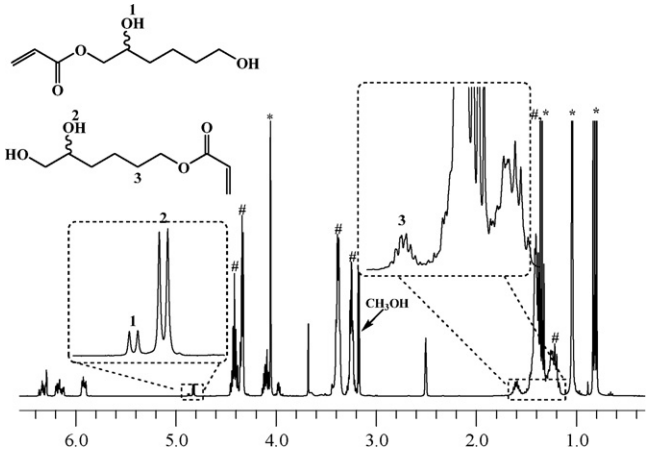


Fig. 12. ^{1}H NMR spectrum of the crude reaction product obtained via transacylation of methyl acrylate and 1,2,6-hexanetriol after 120 h (MA:1,2,6Htriol = 1:3 mol%; T = 50 °C in 2Me2BuOH). (*) Signals of 2Me2BuOH and (#) signals of 1,2,6Htriol.

GC–MS and ^{1}H NMR spectroscopy. From GC–MS analysis (Fig. 11) of the product mixture obtained with MMA as acyl donor five products were identified: three monosubstituted and two disubstituted hexanetriol derivates. Based on the assumption that the OH group in position 6 is the least hindered, 6-methacryloyl-1,2,6-hexanetriol was considered to be the major product (71 mol%, see Table 3) followed by 1-methacryloyl-1,2,6-hexantriol (19 mol%). 2-Methacryloyl-1,2,6-hexanetriol (5 mol%) was assigned to the peak of lower intensity based on the hypothesis that a secondary OH group is less reactive than a primary OH group. The two bismethacrylates were assigned to 1,6-dimethacryloyl-1,2,6-hexanetriol (4 mol%) and 2,6-dimethacryloyl-1,2,6-hexanetriol (1 mol%) again based on the reactivity hypothesis. It was assumed that 1,2-dimethacryloyl-1,2,6-hexanetriol is not formed due to sterical hindrance.

The same assumption can be made for MA as acyl donor. The ratio of the compounds corresponds to: 86 mol% of the two major products (6-acryloyl-1,2,6-hexanetriol and 1-acryloyl-1,2,6-hexanetriol), 8 mol% of 2-acryloyl-1,2,6-hexanetriol, 4 mol% of 1,6-diacryloyl-1,2,6-hexanetriol and 2 mol% of 2,6-diacryloyl-1,2,6-hexanetriol. However in this case the two major products are not well resolved by GC–MS; a shoulder can be observed.

For this reason ^{1}H NMR analysis was considered as additional analytical tool. For the transacylation using MMA as acyl donor the results obtained by GC–MS were confirmed. The results obtained using MA as acyl donor will be presented and discussed.

In Fig. 12 the ^{1}H NMR spectrum of a crude reaction mixture is shown, the main peaks (with high intensity) belong to 2Me2BuOH and 1,2,6Htriol. The formation of methanol is clearly observed proving the transacylation reaction. Since the sensitivity of the NMR spectroscopy is ±5 mol% only two major peaks can be assigned. For this the new signals assigned to methylene group 3 at d = 1.6 ppm and the signals at δ = 4.87 ppm and δ = 4.82 ppm assigned to the OH groups 1 and 2 were taken into consideration.

From the ratio of the two hydroxyl groups the ratio of 6-acryloyl-1,2,6-hexanetriol/1-acryloyl-1,2,6-hexanetriol was determined to be 83.57–16.42 mol%. Considering the results of the GC where the fraction of the two major compounds is 86 mol%, a final value of 72 mol% and 14 mol% were determined.

5. Conclusions

Because of the hydrophilic nature of diols and triols and their incompatibility with methyl acrylate and methyl methacrylate, the reactions presented in this paper were carried out in 2-

methyl-2-butanol, a tertiary alcohol, which is non-reactive in the lipase-catalyzed reaction but is a common solvent for both reagents: diols/triols and (meth)acrylates. In addition this work presents a detailed investigation of the enantio- and regioselectivity in the diols and triols as well as a detailed and elaborate analysis of the product distributions.

The transacylation of MA and MMA with diols and triols is carried out under mild conditions, is easy and rapid in processing, being suitable for the preparation of sensitive monomers, as can be depicted from this paper. The resulting monomers are ready for polymerization without further purification, as will be shown in an upcoming publication.

Acknowledgments

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References

[1] E.S. Gil, S.A. Hudson, Prog. Polym. Sci. 29 (2004) 1173–1222.
[2] B.R. Twaites, C.D. Alarcon, D. Cunliffe, M. Lavigne, S. Pennadam, J.R. Smith, D.C. Gorecki, C. Alexander, J. Control. Release 97 (2004) 551–566.
[3] J.F. Kunzler, J.A. McGee, Chem. Ind. (1995) 651–655.
[4] D. Schmaljohann, Adv. Drug Deliv. Rev. 58 (2006) 1655–1670.
[5] J.T. Zhang, Y.N. Xue, F.Z. Gao, S.W. Huang, R.X. Zhuo, J. Appl. Polym. Sci. 108 (2008) 3031–3037.
[6] M. Kurisawa, M. Yokoyama, T. Okano, J. Control. Release 69 (2000) 127–137.
[7] J.V.M. Weaver, I. Bannister, K.L. Robinson, X. Bories-Azeau, S.P. Armes, M. Smallridge, P. McKenna, Macromolecules 37 (2004) 2395–2403.
[8] T.M. Eggenhuisen, C.R. Becer, M.W.M. Fijten, R. Eckardt, R. Hoogenboom, U.S. Schubert, Macromolecules 41 (2008) 5132–5140.
[9] M. Save, J.V.M. Weaver, S.P. Armes, P. McKenna, Macromolecules 35 (2002) 1152–1159.
[10] M.T. Iglesias, J. Guzman, E. Riande, J. Polym. Sci. Polym. Chem. 32 (1994) 2565–2576.
[11] M.T. Iglesias, J. Guzman, E. Riande, J. Polym. Sci. Polym. Chem. 33 (1995) 2057–2067.
[12] F. Garcia, J.L. De la Pena, J.J. Delgado, N. Garcia, J. Guzman, E. Riande, P. Calle, J. Polym. Sci. Polym. Chem. 39 (2001) 1843–1853.
[13] M. Woundenberg-Van Oosterom, F. Van Rantwijk, R.A. Sheldon, Biotechnol. Bioeng. 49 (1996) 328–333.
[14] Q. Jing, R.J. Kaziauskas, Chirality 20 (2008) 724–735.
[15] P. Gadler, K. Faber, Trends Biotechnol. 25 (2007) 83–88.
[16] W. Du, Y.Y. Xu, D.H. Liu, Z.B. Li, J. Mol. Catal. B-Enzym. 37 (2005) 68–71.
[17] M. Iso, B.X. Chen, M. Eguchi, T. Kudo, S. Shrestha, J. Mol. Catal. B-Enzym. 16 (2001) 53–58.
[18] J.S. Yang, G.J. Jeon, B.K. Hur, J.W. Yang, J. Microbiol. Biotechnol. 15 (2005) 1183–1188.
[19] S. Bloomer, P. Adlercreutz, B. Mattiasson, Enzyme Microb. Technol. 14 (1992) 546–552.
[20] Z.Y. Li, O.P. Ward, J. Am. Oil Chem. Soc. 70 (1993) 745–748.
[21] D. Popescu, H. Keul, M. Moeller, Macromol. Chem. Phys. 210 (2009) 123–139.
[22] M.Y. Sen, J.E. Puskas, S. Ummadisetty, J.P. Kennedy, Macromol. Rapid Commun. 29 (2008) 1598–1602.
[23] S. Ramaswamy, B. Morgan, A.C. Oehlschlager, Tetrahedron Lett. 31 (1990) 3405–3408.
[24] X.F. Li, M.H. Zong, R.D. Yang, J. Mol. Catal. B-Enzym. 38 (2006) 48–53.
[25] A.W.-Y. Chan, B. Ganem, Biocatal. Biotransform. 8 (1993) 163–169.
[26] A.B. Hajjar, P.F. Nicks, C. Knowles, J. Biotechnol. Lett. 12 (1990) 825–830.
[27] I. Ikeda, J. Tanaka, K. Suzuki, Tetrahedron Lett. 32 (1991) 6865–6866.
[28] R. Derango, Y.F. Wang, R. Dowbenko, L.C. Chiang, Biotechnol. Lett. 16 (1994) 241–246.
[29] S. Warwel, G. Steinke, A. Rusch, M.R. Klaas, Biotechnol. Technol. 10 (1996) 283–286.
[30] V. Athawale, N. Manjrekar, M. Athawale, Tetrahedron Lett. 43 (2002) 4797–4800.
[31] G. Burghard, H. Geoffrey (invs.), Goldschmidt GmbH, EP 0965645B1, 1999.
[32] S. Kobayashi, H. Uyama, S. Kimura, Chem. Rev. 101 (2001) 3793–3818.
[33] R.A. Gross, A. Kumar, B. Kalra, Chem. Rev. 101 (2001) 2097–2124.
[34] F. Binns, P. Harffey, S.M. Roberts, A. Taylor, J. Chem. Soc.-Perkin Trans. 1 (1999) 2671–2676.
[35] E.M. Anderson, M. Karin, O. Kirk, Biocatal. Biotransform. 16 (1998) 181–204.
[36] M. Hans, H. Keul, A. Heise, M. Moeller, Macromolecules 40 (2007) 8872–8880.
[37] M. Ferrer, J. Soliveri, F.J. Plou, N. Lopez-Cortes, D. Reyes-Duarte, M. Christensen, J.L. Copa-Patino, A. Ballesteros, Enzyme Microb. Technol. 36 (2005) 391–398.
[38] F. Chamouleau, D. Coulon, M. Girardin, M. Ghoul, J. Mol. Catal. B-Enzym. 11 (2001) 949–954.
[39] Y. Watanabe, Y. Shimada, A. Sugihara, H. Noda, H. Fukuda, Y. Tominaga, J. Am. Oil Chem. Soc. 77 (2000) 355–360.
[40] M.M. Soumanou, U.T. Bornscheuer, Enzyme Microb. Technol. 33 (2003) 97–103.
[41] Y. Watanabe, Y. Shimada, A. Sugihara, Y. Tominaga, J. Am. Oil Chem. Soc. 78 (2001) 703–707.
[42] W. Du, L. Wang, D.H. Liu, Green Chem. 9 (2007) 173–176.
[43] S. Chand, P. Adlercreutz, B. Mattiasson, Enzyme Microb. Technol. 20 (1997) 102–106.
[44] J.E. Puskas, M.Y. Sen, J.R. Kasper, J. Polym. Sci. Polym. Chem. 46 (2008) 3024–3028.
[45] H.E. Gottlieb, V. Kotlyar, A. Nudelman, J. Org. Chem. 62 (1997) 7512–7515.
[46] K.L. Deng, H. Tian, P.F. Zhang, X.B. Ren, H.B. Zhong, Express. Polym. Lett. 3 (2009) 97–104.
[47] I. Hilker, G. Rabani, G.K.M. Verzijl, A.R.A. Palmans, A. Heise, Angew. Chem.-Int. Ed. 45 (2006) 2130–2132.
[48] V. Leonard, L. Fransson, S. Lamare, K. Hult, M. Graber, ChemBioChem 8 (2007) 662–667.
[49] V. Leonard-Nevers, Z. Marton, S. Lamare, K. Hult, M. Graber, J. Mol. Catal. B-Enzym. 59 (2009) 90–95.