J. prakt. Chem. 335 (1993) 628–632

Journal für praktische Chemie
Chemiker-Zeitung

Synthesis of Alkyl (±)-2,3-Di-O-acylglycerates and Attempts Directed to their Conversion into Alkyl (R)-2,3-Di-O-acylglycerates by Enzyme-catalyzed Enantioselective Deacylation Reactions [1]

H. Schick, E. Schrötter and M. Szymanowski

Berlin-Adlershof, Centre of Selective Organic Synthesis, Federal Republic of Germany

A. Knoll[1)]

Berlin, Research Centre of Biotechnology, Federal Republic of Germany

Received August 28th, resp. November 19th, 1992

Abstract. By screening several lipases and proteases as catalysts for an enantioselective partial deacylation of methyl (±)-2,3-di-O-acylglycerates, it was found that methyl (R)-2,3-di-O-butanoylglycerate can be obtained with an enantiomeric excess of >96% and a yield of 20% related to the racemic starting material. This was prepared from methyl acrylate through dihydroxylation with potassium permanganate at -30°C and a subsequent bisacylation.

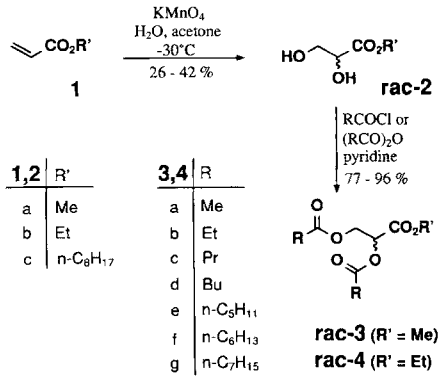
Introduction

Polyfunctional homochiral C_3 units have gained great interest as building blocks for the synthesis of various natural products and biologically active compounds [2–5]. For instance, methyl (R)-(+)-1,2-O-isopropylideneglycerate, an O-protected (R)-glyceric acid derivative, serves as a versatile intermediate for the synthesis of chiral lipids [6,7], vitamin D_3 metabolites [8,9], terpenes [10], and sterols with modified side chains [11]. It is available by a multistep transformation of D-mannitol [9,12] or from D-serine [6]. In connection with our interest in the use of enzymes for enantioselective acylations [13] and deacylations [14] we investigated the possibilities for converting the alkyl (±)-2,3-di-O-acylglycerates *rac*-**3** into homochiral glyceric acid derivatives suited for the synthesis of the above mentioned compounds.

Results and Discussion

Since methyl (±)-glycerate (*rac*-**2a**) is not commercially available, it was prepared by dihydroxylation of methyl acrylate by potassium permanganate in aqueous acetone. In order to suppress the undesired oxidative cleavage of the double bond, the reaction was performed at –30°C. Thus *rac*-**2a** was obtained in a yield of 42% in a one-step procedure. The homologues *rac*-**2b** and *rac*-**2c** were prepared analogously from the corresponding acrylates **1b** and **1c**. Despite the modest yield, this route seems to be superior to procedures which use peroxytrifluoroacetic acid [15] or sodium hypochlorite and osmium (VIII) oxide [16] for the dihydroxylation of **1**.

As substrates for the enzymatic screening the 2,3-di-O-acyl derivatives *rac*-**3a-g** and *rac*-**4a-e** were prepared in high yields by acylation of the alkyl glycerates *rac*-**2a** and



Scheme 1 Synthesis of the alkyl (±)-glycerates *rac*-**2** and the alkyl (±)-2,3-di-O-acylglycerates *rac*-**3** and *rac*-**4**

[1)]Present address: BioGenes Gesellschaft für Biopolymere mbH, Köpenicker Straße 325, 10997 Berlin, Federal Republic of Germany

Product[a]	Yield (%)	IR (film) ν (cm^{-1})	^{1}H-NMR δ (ppm)	^{13}C-NMR δ (ppm)	MS (m/e, %)
rac-**4b**	91	1730, 1170	1.09 (2t,6H,2CH_3), 1.21 (t,3H,CH_3), 2.33 (m,4H,2CH_2), 4.16 (q,2H,CH_2), 4.39 (dd,2H,CH_2), 5.24 (dd,1H,CH)	8.97 (q,2CH_3), 14.13 (q,CH_3), 27.30, (t,2CH_2), 61.80, 62.59 (2t,2CH_2), 70.47 (d,OCH), 167.34, 173.37, 173.62 (3s,3CO)	201 [(M–OEt)$^+$,1], 173 (3), 160 (3), 117 (3), 100 (1), 57 (100
rac-**4c**	83	1730, 1160	0.90 (2t,6H,2CH_3), 1.23 (t,3H,CH_3), 1.60 (m,4H,2CH_2), 2.29 (m,4H,2CH_2), 4.16 (q,2H,CH_2), 4.39 (dd,2H,CH_2), 5.25 (dd,1H,CH)	13.56 (q,2CH_3), 14.11 (q,CH_3), 18.28 (t,2CH_2), 35.75, 35.90 (2t,2CH_2), 61.85, 62.48 (2t,2OCH_2), 70.33 (d,OCH), 167.33, 172.64, 172.93 (3s,3CO)	229 [(M–OEt)$^+$,1], 201 (7), 187 (4), 131 (5), 71 (100
rac-**4d**	94	1740, 1160	0.86 (2t,6H,2CH_3), 1.22 (t,3H,CH_3), 1.38 (m,8H,4CH_2), 2.28 (m,4H,2CH_2), 4.15 (q,2H,CH_2), 4.38 (dd,2H,CH_2), 5.24 (dd,1H,CH)	13.56 (q,2CH_3), 14.08 (q,CH_3), 22.18, 26.92 (2t,4CH_2), 33.61, 33.72 (2t,2CH_2), 61.80, 62.47 (2t,2OCH_2), 70.29 (d,OCH), 167.32, 172.76, 173.06 (3s,3CO)	257 [(M–OEt)$^+$,1], 229 (2), 201 (2), 145 (2), 85 (100
rac-**4e**	92	1740, 1160	0.84 (2t,6H,2CH_3), 1.23 (t,3H,CH_3), 1.41 (m,12H, 6CH_2), 2.32 (m,4H,2CH_2), 4.15 (q,2H,CH_2), 4.38 (dd, 2H,CH_2), 5.24 (dd,1H,CH)	13.87 (q,2CH_3), 14.10 (q,CH_3), 22.34, 24.57, 31.25 (3t,6CH_2), 33.89, 33.99 (2t,2CH_2), 61.81, 62.50 (2t,2OCH_2), 70.32 (d,OCH), 167.30, 172.78, 173.04 (3s,3CO)	285 [(M–OEt)$^+$,1] 257 (3), 215 (6), 159 (3), 99 (100)

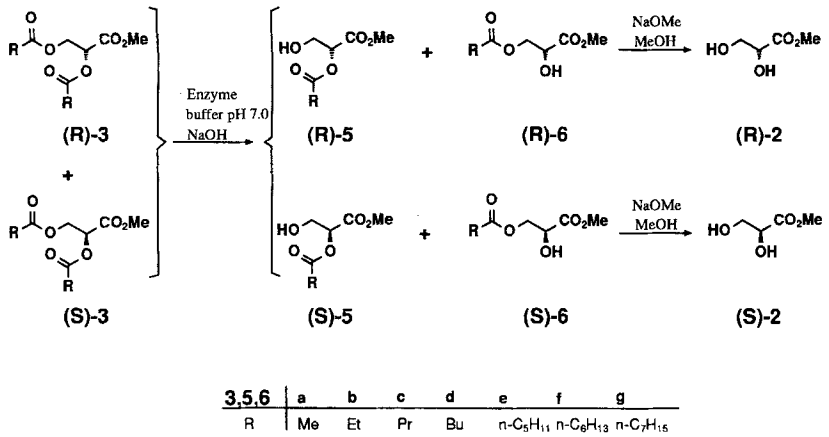
[a] Satisfying microanalyses were obtained for all products: C ± 0.30%, H ± 0.28%.

rac-**2b** by using the corresponding acid chlorides or acid anhydrides in pyridine/trichloromethane.

In order to determine the enantio- and regioselectivity of a partial deacylation, in a first series of experiments the diacyl compounds *rac*-**3a-g** were suspended in a phosphate buffer of pH 7.0 with porcine pancreatic lipase (PPL). The pH was kept at this value by automatic titration with 0.1N sodium hydroxide solution. After consumption of 0.50 – 0.55 equivalents of sodium hydroxide per mol of substrate, the enzymatic deacylation was stopped by extraction of the reaction mixture with ethyl acetate. The extract was concentrated under reduced pressure and the residue separated by flash chromatography on silica gel into a fraction containing the unaffected diacyl compounds (*R*-**3**/(*S*)-**3**, and a fraction containing a mixture of the monoacyl compounds (*R*)-**5**/(*S*)-**5** and (*R*)-**6**/(*S*)-**6**. The enantiomeric excess of these fractions was determined by polarimetry after conversion into a mixture of (*R*)-**2**/(*S*)-**2** by a complete nonenzymatic deacylation with sodium methanolate in methanol. The results are compiled in Table 1, entries 1 – 7.

In a second series of experiments the most promising substrate, *rac*-**3c**, was partially deacylated with pancreatin, a yeast lipase, and a Rhizopus lipase under the aforementioned conditions. The results are given in Table 2, entries 8 – 10. Finally, the alkaline protease aP 41 and subtilisin DY were used instead of the lipases, giving rise to the results reported in Table 2 as entries 11 – 13.

All of the investigated lipases exclusively catalyze the hydrolysis of the acyl groups of the diacyl compounds *rac*-**3**, thus giving rise to the formation of a mixture of (*R*)-**3**/(*S*)-**3** and the monoacyl derivatives (*R*)-**5**/(*S*)-**5**



Scheme 2 Enzyme-catalyzed enantioselective deacylation of the methyl (±)-2,3-di-O-acylglycerates *rac*-**3**

Table 1 Yields and physical data of the alkyl (±)-glycerates *rac*-**2** and the alkyl (±)-2,3-di-O-acylglycerates *rac*-**3** and *rac*-**4**

Product[a)]	Yield (%)	IR (film) ν (cm^{-1})	^{1}H-NMR δ (ppm)	^{13}C-NMR δ (ppm)	MS (m/e, %)
rac-**2a**	42	1720	3.72 (s,3H,Ch_3, 3.76 (dd,2H,CH_2), 4.22 (m,1H, CH),	52.52 (q,OCH_3), 64.18 (t,$HOCH_2$), 72.10 (d,HOCH), 173.48(s,CO)	121 [(M+1)$^+$,42], 103 (3), 90 (58), 61(100)
rac-**2b**	39	1730	1.24 (t,3H,CH_3), 3.81 (m,2H,CH_2), 4.23 (q,2H,CH_2), 4.26 (m,1H,CH), 4.44 (s,2H,2OH)	14.11 (q,CH_3), 61.68 (t,OCH_2Me), 64.23 (t,$HOCH_2$), 72.12 (d, HOCH), 173.0 (s, CO)	135 [(M+1)$^+$,5], 104 (100), 89 (6), 61(90)
rac-**2c**	26	1730	0.81 (t,3H,CH_3), 1.36 (m,12H,6CH_2), 3.78 (d,2H,CH_2), 4.12 (2t,3H,CH,CH_2)	14.06 (q,CH_3), 22.68, 25.89, 28.62, 29.26, 31.87 (5t,5CH_2), 64.29, 65.82, (2t,2OCH_2), 72.17 (d,HOCH), 173.11 (s,CO).	219 [(M+1)$^+$,15], 188 (3), 157 (2), 129 (1), 89 (2), 61 (100)
rac-**3a**	95	1750, 1210	2.02 and 2.11 (2s,6H,2CH_3), 3.72 (s,3H,OCH_3), 4.36 (dd,2H,CH_2), 5.24 (dd,1H,CH)	20.54(q,2CH_3), 52.70 (q,OCH_3), 62.59(t,OCH_2), 70.30 (d,OCH), 167.70, 169.94, 170.31 (3s,3CO)	205 [(M+1)$^+$,1], 174 (10), 145 (30),132 (58), 90 (100), 73 (8), 59 (18)
rac-**3b**	94	1740, 1170	1.08 (2t,6H,2CH_3), 2.29 (m,4H,2CH_2), 3.68 (s,3H, OCH_3), 4.35 (dd,2H,CH_2), 5.21 (dd,1H,CH)	8.89, 9.00 (2q,2CH_3), 27.25, 27.38 (2t,2CH_2), 52.58 (q,OCH_3), 62.53 (t,OCH_2),70.34 (d,OCH), 167.87, 173.43, 173.74(3s,3CO)	233[(M+1)$^+$,0.5], 202 (1), 173 (3), 159 (8), 146 (4), 57 (100)
rac-**3c**	90	1740, 1160	0.90(2t,6H,2CH_3), 1.59 (m,4H,2CH_2), 2.28 (m,4H, 2CH_2), 3.67 (s,3H,OCH_3), 4.34 (dd,2H,CH_2), 5.20 (dd,1H,CH)	13.51 (q,2CH_3), 18.38 (t,2CH_2), 35.74, 35.91 (2t,2CH_2), 52.52 (q,OCH_3), 62.40 (t,OCH_2), 70.28 (d,OCH), 167.87, 172.51, 172.81 (3s,3CO)	261 [(M+1)$^+$,0.1], 230 (0.6), 201 (1.2), 173 (3), 160 (4), 71 (100)
rac-**3d**	77	1730, 1150	0.86 (2t,6H,2CH_3), 1.40 (m,8H,4CH_2), 2.29 (m,4H, 2CH_2), 3.68 (s,3H,OCH_3), 4.34 (dd,2H,CH_2), 5.21 (dd, 1H,CH)	13.64 (q,2CH_3), 22.20, 26.98 (2t,4CH_2), 33.61, 33.73 (2t,2CH_2), 55.51 (q,OCH_3), 62.42 (t,OCH_2), 70.28 (d,OCH), 167.83, 172.64, 172.95 (3s,3CO)	258 [(M+1–OMe)$^+$,0.3], 229 (1.2), 187 (4), 174 (4), 85 (100)
rac-**3e**	87	1740, 1150	0.82 (2t,6H,2CH_3), 1.24 (m,8H,4CH_2), 2.29 (m,4H, 2CH_2), 3.68 (s,3H,OCH_3), 4.34 (dd,2H,CH_2), 5.21 (dd,1H,CH)	13.82 (q,2CH_3), 22.29 (t,2CH_2), 24.45, 24.55 (2t,2CH_2), 31.21 (t,2CH_2), 33.84, 33.96 (2t,2CH_2), 52.57 (q,OCH_3), 62.40 (t,OCH_2), 70.16 (d,OCH), 167.81, 172.78, 173.12 (3s,3CO)	317 [(M+1)$^+$,0.9], 286 (0.5), 257 (2), 201 (21), 188 (4), 99 (100)
rac-**3f**	96	1740, 1150	0.81 (2t,6H,2CH_3), 1.22 (m,12H,6CH_2), 1.56 (m,4H, 2CH_2), 2.30 (m,4H,2CH_2), 3.68 (s,3H,OCH_3), 4.35 (dd,2H,CH_2), 5.21 (dd,1H,CH)	13.95 (q,2CH_3), 22.33, 22.48, 24.44, 24.77 (4t,4CH_2), 28.71, 31.45 (2t,4CH_2), 33.89, 34.02 (2t,2CH_2), 52.53 (q,OCH_3), 62.41 (t,OCH_2), 70.16 (d,OCH), 167.81, 172.76, 173.11 (3s,3CO)	345[(M+1)$^+$,1.6], 313 (0.2), 285 (1.2), 215 (48), 201 (8),113 (100)
rac-**3g**	94	1740, 1150	0.80 (2t,6H,2CH_3), 1.24 (m,16H,8CH_2), 1.56 (m,4H, 2CH_2), 2.29 (m,4H,2CH_2), 3.68 (s,3H,OCH_3), 4.35 (dd,2H,CH_2), 5.21 (dd,1H,CH)	14.07 (q,2CH_3), 22.74 (t,2CH_2), 24.45, 24.98 (2t,2CH_2), 29.12 (m,4CH_2), 31.86 (t,2CH_2), 33.91, 34.02 (2t,2CH_2), 52.40 (q,OCH_3), 62.46 (t,OCH_2), 70.35 (d, OCH), 167.81, 172.51, 172.81 (3s,3CO)	373 [(M+1)$^+$,0.6], 342 (0.2), 313 (0.8), 229 (20), 216 (2.4), 127 (75), 57 (100)
rac-**4a**	83	1740, 1200	1.22 (t,3H,CH_3), 2.02 and 2.11 (2s,6H,$COCH_3$), 4.18 (q,2H,CH_2), 4.39 (dd,2H,CH_2), 5.23 (dd,1H,CH)	14.07 (q,CH_3), 20.50 (q,2CH_3), 61.87, 62.67 (2t,2OCH_2), 70.47 (d,OCH), 167.21, 169.94, 170.26 (3s,3CO)	218 (M$^+$,1), 173 (5), 145 (37), 103 (100)

and (*R*)-**6**/(*S*)-**6**. The proteases, however, additionally hydrolyse the methyl ester group forming the free acids of the monoacyl derivatives. Due to acyl group migration a separation of the formed primary and secondary monoacyl compounds and a determination of the regioselectivity of the deacylation reaction could not be performed.

In almost all of the investigated cases the (S)-2,3-di-O-acylglycerates (*S*)-**3** are deacylated faster than the corresponding (R)-enantiomers (*R*)-**3**. After partial enzyme-catalyzed deacylation the remaining diacyl compounds exhibit an excess of the (*R*)-enantiomer (*R*)-**3**, except in the case of the dioctanoyl compound *rac*-**3g** (Table 2, entry 7).

The rate of the deacylation depends significantly on the length of the acyl residues. With PPL the lowest rate is observed for the diacetyl compound *rac*-**3a** (entry 1), the highest rate for the dibutanoyl compound *rac*-**3c** (entry 3). A second rate minimum is found for the dihexanoyl compound *rac*-**3e** (entry 5).

In comparison with PPL, pancreatin increases the rate (entry 8), whereas the lipases from yeast and Rhizopus sp. decrease the rate of the deacylation of *rac*-**3c** (entries 9 and 10). The deacylation is also slower with alkaline protease aP 41 and subtilisin DY (entries 12 and 13).

The highest enantiomeric excess (e.e.) for a PPL-catalyzed deacylation was obtained with the dibutanoyl compound *rac*-**3c** (entry 3). However, with an e.e. of 43% the enantioselectivity is unsufficient for preparative purposes. An e.e. of 71% was obtained by use of a yeast lipase (entry 9). This value is sufficient for the preparation of enantiomerically pure (*R*)-2,3-dihydroxy-3-methylbutyl p-toluenesulfonate, a side-chain precursor for vitamin D_3 metabolites [9]. Finally, with an e.e. of >96% (*R*)-**3c** was obtained in a yield of 20 – 22% related to the starting rac-**3c** by use of the alkaline protease aP 41 or subtilisin DY (entries 12 and 13).

In preliminary screening experiments the ethyl esters *rac*-**4a-e** gave lower enantioselectivities than the corresponding methyl esters *rac*-**3a-e**.

Experimental

Thin-layer chromatography was carried out on plates precoated with silica gel 60 (E. Merck). Flash chromatography was performed on silica gel 60 (0.040 – 0.063 mm, E. Merck). Optical rotations were measured with the photoelectric polarimeter Polamat A (Carl Zeiss, Jena) at 546 and 578 nm and extrapolated to 589 nm. IR spectra were recorded on a Specord 75 IR spectrometer (Carl Zeiss, Jena). ^{1}H-NMR spectra were obtained at 100 MHz on a Tesla BS 567 and ^{13}C-NMR spectra at 20 MHz on a Varian CFT 20 spectrometer in $CDCl_3$ with hexamethyldisiloxane as internal standard. Electron impact mass spectra were obtained on the GC/MS Datensystem HP 5985 B. For

Table 2 Enzyme-catalyzed enantioselective deacylation of methyl (±)-2,3-di-O-acylglycerates *rac*-**3**

Entry	Substrate (2 mmol)	Enzyme[a]	NaOH (mmol)	Reaction time (min)	Recovered 2,3-di-O-acylglycerate (*R*)-**3**/(*S*)-**3**				2- and 3-O-acylglycerates (*R*)-**5**/(*S*)-**5** and (*R*)-**6**/(*S*)-**6**		
					Yield (%)	$[\alpha]_D^{20}$ [° (c in $CHCl_3$)]	e.e. (%)	Main enantiomer	yields (%)	e.e. (%)	Main enantiomer[b]
1	*rac*-**3a**	PPL	1.1	532	47	1.35 (14.4)	11	(*R*)-**3a**	13	7	(*S*)-**2a**
2	*rac*-**3b**	PPL	1	50	48	0.90 (11.1)	6	(*R*)-**3b**	41	13	(*S*)-**2a**
3	*rac*-**3c**	PPL	1	19	44	4.44 (17.1)	43	(*R*)-**3c**	56	25	(*S*)-**2a**
4	rac-**3d**	PPL	1	47	45	2.67 (11.0)	38	(*R*)-**3d**	51	27	(*S*)-**2a**
5	*rac*-**3e**	PPL	1	71	58	0.50 (18.3)	–	(*R*)-**3e**	42	–	(*S*)-**2a**
6	*rac*-**3f**	PPL	1	29	53	0.16 (19.4)	–	(*R*)-**3f**	47	12	(*S*)-**2a**
7	*rac*-**3g**	PPL	1	27	46	-0.12 (17.2)	18	(*S*)-**3g**	53	14	(*R*)-**2a**
8	*rac*-**3c**	Pan	1.2	13	28	4.50 (4.30)	44	(*R*)-**3c**	54	34	(*S*)-**2a**
9	*rac*-**3c**	YL	1.33	370	34	7.01 (15.6)	71	(*R*)-**3c**	28	33	(*S*)-**2a**
10	*rac*-**3c**	RhL	1.1	470	48	5.14 (19.2)	48	(*R*)-**3c**	11	58	(*S*)-**2a**
11	*rac*-**3c**	aP 41	2.0	85	34	8.71 (5.05)	88	(*R*)-**3c**			
12	*rac*-**3c**	aP 41	2.4	154	22	10.75 (4.40)	>96	(*R*)-**3c**			
13	*rac*-**3c**	Subt	2.4	335	20	10.74 (3.04)	>96	(*R*)-**3c**			

[a] Porcine pancreatic lipase (PPL), 20 mg, 35°C; pancreatin (Pan), 20 mg, 38.5°C; yeast lipase (YL), 5ml, 30°C; Rhizopus sp. lipase (RhL), 50 mg, 40°C; alkaline protease 41 P (aP), 20 mg, 31°C; 10 mg; subtilisin DY (Subt), 10 mg, 33.5°C.

[b] After complete deacylation with NaOMe/MeOH.

the deacylation reactions the following enzymes were used: porcine pancreatic lipase (PPL), Serva, lyophilized, 20 U/mg (triolein); pancreatin (Pan, Belger (Kleinmachnow), 6 x NF, 820 U/g (triolein); yeast lipase (YL), Institut für Biotechnologie Leipzig, culture fugate, 60 U/ml (p-nitrophenyl palmitate [17]); lipase from Rhizopus sp. (RhL), Serva, lyophilized, 60 U/mg (triolein); alkaline protease (aP 41) from Bacillus licheniformis 41P (ZIMET 10911), FZB Biotechnik GmbH Berlin, 21.5 U/mg (hemoglobin); subtilisin DY, Institute of Organic Chemistry of the Bulgarian Academy of Sciences Sofia, 417 U/g (casein).

Alkyl (±)-glycerates *rac*-**2**

General Procedure: Potassium permanganate (52.3g, 0.33 mol) was dissolved in a mixture of water (500 ml) and acetone (1000 ml) and cooled to -30°C. At this temperature a solution of an alkyl acrylate **1** (0.3 mol) in acetone (100 ml) was slowly added under stirring. After complete addition of the oxidant, the reaction mixture was allowed to warm up to 0°C. Manganese(IV) oxide was removed by filtration and washed with acetone (2 x 250 ml). The combined filtrates were concentrated under reduced pressure at a temperature below 40°C. Distillation of the residue afforded the alkyl glycerates *rac*-**2** as colourless liquids (*rac*-**2a**: b.p. 74°C/43 Pa; *rac*-**2b**: b.p. 76°C/66 Pa; *rac*-**2c**; b.p. 63–65°C/130 Pa). Yields and physical data are given in Table 1.

Alkyl (±)-2,3-di-O-acylglycerates *rac*-**3 and** *rac*-**4**

General Procedure: An acyl chloride or a carboxylic anhydride (22 mmol) was added at 0 – 5°C to a solution of an alkyl (±)-glycerate *rac*-**2** (10 mmol) and 4-dimethylaminopyridine (12.2 mg, 0.1 mmol) in pyridine (12 ml). The reaction mixture was stirred for 2 – 3 h at room temperature. Then the solvent was removed under reduced pressure by co-distillation with toluene. The residue was dissolved in dichloromethane (20 ml). The solution was washed with ice-cold diluted hydrochloric acid and saturated solutions of sodium hydrogen carbonate and sodium chloride (each 5 ml). The organic phase was dried with magnesium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel with hexane/ethyl acetate (5:1) as eluant. Yields and physical data of the diacyl compounds *rac*-**3** and *rac*-**4** obtained in this manner as colourless liquids are given in Table 1. When the preparation was performed on a scale of 100 mmol, the products were purified by distillation under reduced pressure (*rac*-**3c**: b.p. 112°C, 40 Pa; *rac*-**4a**: b.p. 92–94°C/133 Pa; *rac*-**4c**: b.p. 97°C/8 Pa).

Enzyme-catalyzed Partial Deacylation of Alkyl (±)-2,3-Di-O-acylglycerates *rac*-**3**

General Procedure: A diacyl compound *rac*-**3** was suspended in a 0.067M SÖRENSEN phosphate buffer (40 ml) at pH 7.0 and stirred with the enzyme under the conditions given in Table 2, footnote a. The pH was adjusted to 7.0 by an automatic titration with 0.1N sodium hydroxide. After the amount of alkali reported in Table 2 was consumed, the reaction was stopped by extraction of the mixture with ethyl acetate (4 x 40 ml). The combined extracts were washed with saturated aqueous solutions of sodium hydrogen carbonate (2 x 20 ml) and sodium chloride (2 x 20 ml), dried with magnesium sulfate, and concentrated under reduced pressure. The residue was separated into fractions of the diacyl compounds (*R*)-**3**/(*S*)-**3** and the monoacyl compounds (*R*)-**5**/(*S*)-**5** and (*R*-**6**/(*S*)-**6** by flash chromatography on silica gel with hexane/ethyl acetate (5:1) as eluant. Yields and e.e. are given in Table 2. When proteases were used as enzyme, only the fraction of the non-deacylated compounds was isolated.

For the determination of the e.e., in each case the separated diacyl and monoacyl compounds were dissolved in absolute methanol and treated with a methanolic 1N sodium methanolate solution. After complete deacylation (30–60 min, TLC-monitoring), the solution was neutralized by adding the basic ion-exchanging resin KPS, filtrated, and concentrated under reduced pressure. The remaining methyl (±)-glycerate (*R*)-**2a**/(*S*)-**2a** was purified by flash chromatography on silica gel with hexane/ethyl acetate (3:1) as eluant. The e.e. was determined by measuring the optical rotation {$[\alpha]_D^{20}$:5.3° (c 5.31, $CHCl_3$) for (*R*)-**2a** [18]}

References

[1] Enzymes in Organic Synthesis, 13. For part 12 see reference [19]
[2] M. Pottie, J. Van der Eycken, M. Vandewalle, J. M. Dewanckele, H. Röper, Tetrahedron Lett. **30** (1989) 5319
[3] J. Jurczak, S. Pikul, T. Bauer, Tetrahedron **42** (1986) 447
[4] S. Takano, Pure Appl. Chem. **59** (1987) 353
[5] U. Peters, W. Bankova, P. Welzel, Tetrahedron **43** (1987) 3803
[6] C. M. Lok, J. P. Ward, D. A. van Dorp. Chem. Phys. Lipids **16** (1976) 115
[7] S. K. Bhatia, J. Hajdu, J. Org. Chem. **53** (1988) 5034
[8] H. Takayama, M. Ohmori, S. Yamada, Tetrahedron Lett. **21** (1980) 5027
[9] E. Schrötter, A. Hauser, H.-J. Hamann, H. Schick, B. Schönecker, J. Prakt. Chem. **328** (1986) 705
[10] R. Dumont, H. Pfander, Helv. Chim. Acta **66** (1983) 814
[11] A. Burger, C. Hetru, B. Luu, Synthesis **1989**, 93
[12] E. Baer, J. M. Grosheintz, H. O. L. Fischer, J. Am. Chem. Soc. **61** (1939) 2607
[13] B. Henkel, A. Kunath, H. Schick, Liebigs Ann. Chem. **1992**, 809 and references cited therein
[14] F. Theil, H. Schick, P. Nedkov, M. Haupt, B. Häfner, S. Schwarz, J. Prakt. Chem. **330** (1988) 893
[15] W. D. Emmons, A. S. Pagano. J. P. Freeman, J. Am. Chem. Soc. **76** (1954) 3472
[16] FMC Corporation, Neth. Appl. 74 11150 (February 24, 1976) [Chem. Abstr. **86** (1977) 4925d]
[17] U. K. Winkler, M. Stuckmann, J. Bacteriol. **138** (1979) 663
[18] G. Hirth, W. Walter, Helv. Chim. Acta **68** (1985) 1863
[19] H. Schick, M. Szymanowski, J. Prakt. Chem. **335** (1993) 377

Address for correspondence:

Prof. Dr. Hans Schick
Zentrum für Selektive Organische Synthese
Rudower Chaussee 5
D-12498 Berlin
Bundesrepublik Deutschland