Tetrahedron: Asymmetry Vol. 3, No. 1, pp. 137-142, 1992
Printed in Great Britain

0957-4166/92 $3.00+.00
Pergamon Press plc

Asymmetric Enzymatic Hydrolysis of Prochiral 2-O-Allylglycerol Ester Derivatives

Beat Wirz*[a], Rudolf Schmid[b] and Joseph Foricher[b]

Pharma Division, Microbiology Department[a] and Preclinical Research[b],
F. Hoffmann-La Roche AG, CH-4002 Basle, Switzerland

(*Received* 26 *November* 1991)

Abstract: The generation of optically active glycerol derivatives via enzymatic ester hydrolysis of prochiral 1,3-diacyl derivatives of 2-O-allyl protected glycerol has been investigated. Lipase M-AP, under optimum conditions, afforded asymmetric inductions of 94-96 % ee. The obtained (R)-monoacyl derivative was converted to (S)-configurated tosylglycerol compounds.

Optically active glycerol synthons play an important role in the syntheses of natural and unnatural glycerides as well as glyco-, phospho- and etherlipids. Moreover, they are key intermediates in the synthesis of β-blockers and natural compounds[1]. The asymmetric enzymatic monoesterification of 2-O-benzylglycerol[2] and the stereoselective monohydrolysis of the corresponding 1,3-diacylates[3] has been demonstrated by several authors. Additionally, several kinetic resolutions of racemic glycerol derivatives using enzymes have been described[4]. In the present work we wish to report on the asymmetric hydrolysis of diacylated 2-O-allylglycerol **1** to generate the corresponding (R)-monoester **2** (Scheme 1). Employing an allyl instead of a benzyl protecting group may have any of the following potential advantages: i) it allows deprotection under non-hydrogenating conditions useful e.g. for olefinic derivatives of **2**, ii) the allyl group can be selectively removed in the presence of a benzyl ether protecting group[5] hence increasing synthetic flexibility, iii) **2** can be converted to an acetal of type **6**.

2-O-allylglycerol **1** was readily synthesized[6] on the mol-scale from glycerol via the 1,3-O-benzylidene intermediate and subsequently acylated to the substrates **1a-e** by standard methods. Various commercial lipases and esterases were tested for asymmetric hydrolysis of the diacetate and dibutyrate[7]. Generally, the dibutyrate **1c** was hydrolyzed much more rapidly than the diacetate **1a**. The highest ee value for the

Scheme 1: Chemoenzymatic approach to chiral 2-O-allylglycerol derivatives.

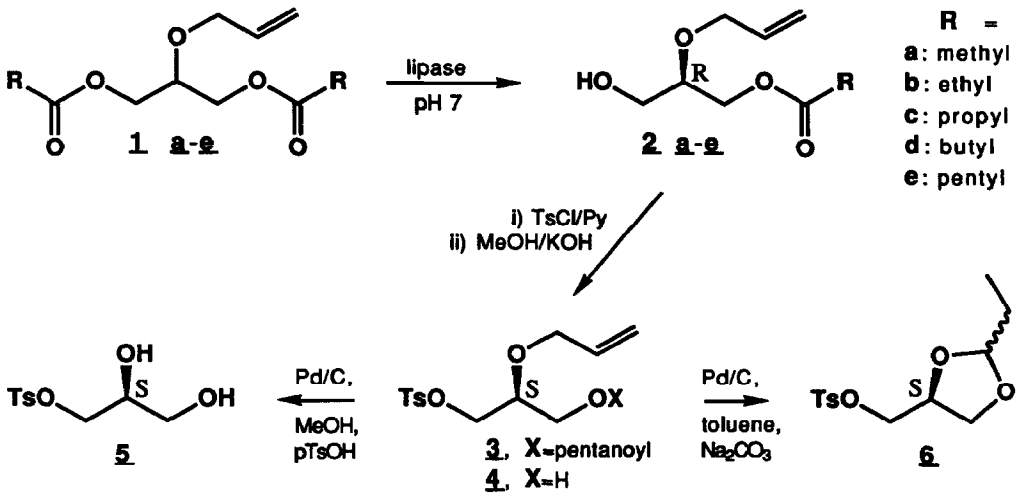

Table 1: Hydrolysis of **1d** by lipase M-AP 10 under various conditions.

#	**1d** (g); (%)	aqueous solution[a]	temp. (°C)	lipase (mg)	conv. (%)[c]	time (min)	% ee[7] of **2d**
1	0.2; 0.8	0.1 M NaCl	20	7	50	85	92
2	0.2; 0.8	0.1 M NaCl	20	7	55	138	93
3	0.5; 1.9	0.1 M NaCl	20	14	50	117	90
4	0.5; 1.9	0.1 M NaCl	1	28	50	83	96
5	0.5; 1.9	0.1 M guanidine·HCl	20	14	50	117	95
6	0.5; 1.9	0.1 M LiSCN	20	14	50	100	95
7	0.5; 1.9	0.1 M Na_2SO_4	20	14	50	119	96
8	0.5; 1.9	0.1 M Na citrate pH 7.0	20	14	50	107	96
9	0.5; 1.9	0.1 M $CaCl_2$	20	14	50	89	93
10	1.0; 3.7	0.1 M $CaCl_2$[b]	4	28	50	183	95

a: additionally containing 4 mM sodium phosphate buffer pH 7.0.
b: imidazole buffer (pH 7.0) instead of phosphate buffer was used.
c: with respect to ester equivalents.

butyrate (R)-**2c** under standard conditions[7] was 78 % ee obtained with Lipase D-20 (Amano); Lipase M-AP (Amano) afforded (R)-**2c** in 57 % ee. Surprisingly, the stereoselectivity of Lipase M-AP towards **1c** was strongly dependent on the substrate concentration, attaining 88 % ee when a concentration of 2 % w/v was employed and decreasing to 70 % ee at 3.7 % concentration. Studying the influence of the acyl group (**1a-e**) on the stereoselectivity of Lipase M-AP revealed the divalerate **1d** to be the most suitable substrate under standard conditions (92 % ee). Aiming at a procedure of higher preparative value physical and chemical parameters were optimized using **1d** as substrate (Table 1): Hydrolysis beyond 50 % conversion slightly increased, while higher substrate concentration slightly decreased the ee value of **2d** (entry 2 and 3). As observed previously[8], lower temperatures enhanced the stereoselectivity of enzymatic hydrolysis (entry 4). Salting-in salts (entry 5 and 6), salting-out salts (entry 7 and 8) and calcium ions (entry 9) enhanced stereoselectivity and, partially, specific activity of the enzyme. At higher substrate concentration (3.7 %, entry 10) an ee value of 95 % was achieved by combination of several favourable parameters. This value decreased by ~1 % when the experiment was carried out on a larger scale (60 g)[9].

2d of 94 % ee was converted to **4** by means of tosylation and methanolysis without loss of enantiomeric purity[10,11] (75 % yield with respect to **1d**). Removing the allyl protecting group by treating **4** with Pd/C in the presence of p-TsOH in $MeOH/H_2O$[5] afforded (S)-**5** in 85 % yield and retained enantiomeric purity[12]. At this stage the absolute configuration could be assigned by comparing the specific rotation of **5** to reference values from literature[13]. Recrystallization of **5** from Et_2O raised the enantiomeric excess to 96 %. Treatment of **4** with Pd/C in nonprotic solvents (THF or toluene) under neutral conditions produced the dioxolane **6** via double bond isomerization and cyclization (50-79 % yield[14]). According to NMR an epimeric ratio at C(2) of 1:1 to 10:1 was obtained depending on the reaction conditions.

We have also briefly investigated the enzymatic hydrolysis of a 2-O-benzyl protected diglyceride using an optimized low-temperature system: 1,3-di-O-acetyl-2-O-benzylglycerol was asymmetrically hydrolyzed using lipase P (Amano) providing [(R)-2-(benzyloxy)-3-hydroxypropyl] acetate (**7**)[3] in a maximum of 95 % ee at 50 % conversion. At a higher substrate concentration (3.7 % w/v) and on a larger scale (25 g) **7** was obtained in 93 % ee and 87 % yield[15]. With respect to enantiomeric purity and chemical yield this compares favourably to the results obtained by other investigators[3] in the 2-O-benzyl protected series.

Acknowledgement:
The authors are grateful to F. Vazquez and T. Gysin for skillful technical assistance and to their colleagues of the analytical service department, especially to W. Walther for carrying out numerous ee determinations.

References and Notes:

[1] H.-J. Altenbach, *Nachr. Chem. Tech. Lab.* **1988**, *36*, 33.

[2] Y.-F. Wang, C.-H. Wong, *J. Org. Chem.* **1988**, *53*, 3128.
Y.-F. Wang, J.J. Lalonde, M. Momongan, D.E. Bergbreiter, C.-H. Wong, *J. Am. Chem. Soc.* **1988**, *110*, 7200.
Y. Terao, M. Murata, K. Achiwa, *Tetrahedron Lett.* **1988**, *29*, 5173.

[3] H. Suemune, Y. Mizuhara, H. Akita, K. Sakai, *Chem. Pharm. Bull.* **1986**, *34*, 3440.
W. Kreiser, V. Kerscher, *Offenlegungsschrift* DE 36'15'657 (09. 05. **1986**)
D. Breitgoff, K. Laumen, M.P. Schneider, *J. Chem. Soc., Chem. Commun.* **1986**, 1523.
V. Kerscher, W. Kreiser, *Tetrahedron Lett.* **1987**, *28*, 531.

[4] S. Iriuchijima, N. Kojima, *Agric. Biol. Chem.* **1982**, *46*, 1153.
S. Iriuchijima, A. Keiyn, N. Kojima, *Agric. Biol. Chem.* **1982**, *46*, 1593.
B. Cambou, A.M. Klibanov, *J. Am. Chem. Soc.* **1984**, *106*, 2687.
W.E. Ladner, G.M. Whitesides, *J. Am. Chem. Soc.* **1984**, *106*, 7250.
S. Hamaguchi, T. Ohashi, K. Watanabe, *Agric. Biol. Chem.* **1986**, *50*, 375.
M. Kloosterman, V.H.M. Elferink, J. van Iersel, J.-H. Roskam, E.M. Meijer, L.A. Hulshof, R.A. Sheldon, *TIBTECH* **1988**, *6*, 251.
P. Sonnett, E. Antonian, *J. Agric. Food Chem.* **1988**, *36*, 856.
C. Fuganti, P. Grasselli, S. Servi, A. Lazzarini, P. Casati, *Tetrahedron* **1988**, *44*, 2575.

[5] R. Boss, R. Scheffold, *Angew. Chem.* **1976**, 578.

[6] R.M. Evans, L.N. Owen, *J. Chem. Soc.* **1949**, 244.

[7] Standard conditions: **1** (0.8 % w/v) was emulsified in 0.1 M NaCl, 4 mM sodium phosphate, pH 7.0. Hydrolysis was started by adding the enzyme and the pH was maintained at 7.0 using 0.1 N NaOH. After ~50 % conversion the emulsion was extracted with CH_2Cl_2. The organic phase was dried ($MgSO_4$) and evaporated and residual **2** was subjected to ee-determination (by GLC on a permethylated β-cyclodextrin column (**2a**, **2c**) or, after derivatization with (R)-Trolox methyl ether[16], on an SP 2340 capillary column (**2b**, **2d**, **2e**)).

[8] B. Wirz, R. Schmid, W. Walther, *Biocatalysis* **1990**, *3*, 159.

[9] Enzymatic hydrolysis: 60 g **1d** emulsified in 1.6 l 0.1 M $CaCl_2$, 6 mM imidazole, pH 7.0 at 1-2 °C was hydrolyzed with 1.2 g Lipase M-AP 10 keeping the pH constant at 7.0 by the addition of 1.0 N NaOH. After 50.3 % conversion (6 h) the reaction mixture was extracted with CH_2Cl_2 (filtering the emulsion through Dicalite Speedex for faster phase separation), the organic phase dried ($MgSO_4$) and evaporated to provide 40.6 g of crude **2d** of 94 % ee as a colourless oil; $[\alpha]_D$= -6.6 (1% in EtOH), $[\alpha]_D$= +18.6 (1% in $CHCl_3$).

[10] [(S)-2-(Allyloxy)trimethylene] valerate p-toluenesulfonate (3): To a solution of 35 g of crude 2d (94 % ee) in 26.8 ml pyridine and 150 ml CH_2Cl_2 at -10°C was added in portions 38.2 g p-toluenesulfonyl chloride. The reaction mixture was stirred for 16 h at RT and washed several times with H_2O, sat. $NaHCO_3$ and sat. NaCl solution. The organic phase was dried ($MgSO_4$) and evaporated to yield crude 3 which was employed without further purification in the next step[11]. For analytical purposes a small fraction of crude 3 was chromatographed on silica gel 60 (hexane/CH_2Cl_2 25→100 %) to yield a colourless oil: $[\alpha]_D$= -4.6 (1 % in $CHCl_3$); IR (neat): 1739 (C=O), 1365 (-SO_2-), 1177 (C-O), 931 (-CH=CH_2); NMR (250 MHz, $CDCl_3$): 7.80 (d, J=8, 2 arom. H), 7.35 (d, J=8, 2 arom. H), 5.9-5.7 (m, 1H, -CH=CH_2), 5.3-5.15 (m, 2H, -CH=CH_2), 4.2-4.0 (m, 6H, 3 -CH_2O-), 3.76 (quint., J=5, H-C(2)), 2.46 (s, arom. CH_3), 2.27 (t, J=7, 2H, -$COCH_2CH_2CH_2CH_3$), 1.55 (m, 2H, -$COCH_2CH_2CH_2CH_3$), 1.32 (m, 2H, -$COCH_2CH_2CH_2CH_3$), 0.90 (t, J=7, 3H, -$COCH_2CH_2CH_2CH_3$); MS: 313 (16, M^+-OCH_2CH=CH_2), 185 (44), 155 (26), 85 (63), 41 (100); $C_{18}H_{26}O_6S$, calc. C 58.36, H 7.07, found C 58.96, H 7.38.

[11] [(S)-2-(Allyloxy)-3-hydroxypropyl] p-toluenesulfonate (4): Crude 3[10] was dissolved in 200 ml MeOH and 100 ml 2 M KOH (at 0°C). After stirring for 1 h 1 l of sat. $NaHCO_3$ and 200 ml H_2O were added and the solution was extracted with 5x400 ml EtOAc. The combined organic phases were dried (MgSO4), evaporated and the residue chromatographed on silica gel 60 (500 g; CH_2Cl_2/EtOAc 5→10%) to yield 37 g of 4 as a colourless oil in ~80 % yield (94 % ee[12]): $[\alpha]_D$= -29.5 (1 % in $CHCl_3$); IR (neat): 3446 (-OH), 1360, 1175 (-SO_2-), 982 (-CH=CH_2); NMR (250 MHz, $CDCl_3$): 7.79 (d, J=8, 2 arom. H), 7.35 (d, J=8, 2 arom. H), 5.95-5.75 (m, 1H, -CH=), 5.3-5.15 (m, 2H, =CH_2), 4.15-4.0 (m, 4H, 2 -CH_2O-), 3.75-3.5 (m, 3H), 2.46 (s, 1 arom. CH_3), 1.89 (bs, -OH); MS: 255 (2, M^+-CH_2OH), 155 (33), 41 (100); $C_{13}H_{18}O_5S$, calc. C 54.53, H 6.34, found C 54.94, H 6.21.

[12] [(S)-2,3-Dihydroxypropyl] p-toluenesulfonate (5): A mixture of 2.86 g 4, 0.3 g 10 % Pd/C and 0.3 g p-toluenesulfonic acid in 30 ml MeOH and 6 ml H_2O was refluxed for 18 h. After evaporation to dryness the residue was taken up in CH_2Cl_2, the solution dried ($MgSO_4$), filtered and concentrated. Chromatography on silica gel 60 (100 g; CH_2Cl_2/EtOAc 0→50 %) afforded 2.1 g (85 %) of 5 of 94 % ee[17] as a white powder. Recrystallization from Et_2O gave a product of 96 % ee[17]: $[\alpha]_D$= +9.7 (5.0 % in MeOH), $[\alpha]_D$= +11.8 (1.0 % in EtOH); m.p. 59.5-60.5 °C; IR (KBr): 3316 (-OH), 1356, 1182 (-SO_2-), 988 (-OH); NMR (250 MHz, $CDCl_3$): 7.80 (d, J=8, 2 arom. H), 7.37 (d, J=8, 2 arom. H), 4.1-3.9 (m, 3 H), 3.75-3.55 (m, 2 H), 2.85 (d, J=5, 1 OH), 2.46 (s, 1 arom. CH_3), 2.28 (t, J=5, 1 -OH); MS: 216 (11), 215 (9, M^+-CH_2OH), 173 (30), 155 (47), 91 (100); $C_{10}H_{14}O_5S$, calc. C 48.77, H 5.73, found C 48.81, H 5.75.

[13] J.J. Baldwin, A.W. Raab, K. Mensler, B.H. Arison, D.E. McClure, *J. Org. Chem.* **1978**, *43*, 4876: $[\alpha]_D$= -9.3 (5.0 % in MeOH) for (R)-5.
H.R. Pfaendler, F.K. Meier, *Liebigs Ann. Chem.* **1989**, 691: $[\alpha]_D$= +9.6 (5.0 % in MeOH) for (S)-5.

[14] (4S)-2-Ethyl-4-(tosyloxy)methyl-1,3-dioxolane (**6**): A mixture of 1.8 g 10 % Pd/C and 0.5 g Na_2CO_3 in 20 ml toluene was refluxed for 1 h. After addition of 6.0 g **4** the mixture was refluxed for another 2 h, then allowed to cool and filtered. The filtrate was evaporated and the residue chromatographed on silica gel 60 (hexane/EtOAc 1:1) to yield 4.0 g (67 %) of **6** (~1:1 at C(2)) as a colourless oil: $[\alpha]_D$= +2.8 (1.0 % in $CHCl_3$); IR (neat): 1363, 1177 (-SO_2-), 1097 (C-O-C); NMR (250 MHz, $CDCl_3$): 7.80 (d, J=8, 2 arom. H), 7.35 (d, J=8, 2 arom. H), 4.82 (2t≈q, J=5.5, H-C(2) of two C(2)-epimers), 4.3-3.6 (m, 5 H), 2.46 (s, 1 arom. CH_3), 1.61 and 1.59 (m, -C$\underline{H}_2$$CH_3$), 0.90 and 0.89 (2t, J=7.5, -CH_2C$\underline{H}_3$ of 2 epimers); MS: 285 (4, M^+-H), 257 (68), 155 (76), 101 (32), 91 (100); $C_{13}H_{18}O_5S$, calc. C 54.53, H 6.34, found C 54.00, H 6.39, H_2O 0.37.

[15] [(R)-2-(Benzyloxy)-3-hydroxypropyl] acetate (**7**): 25.0 g of [2-(benzyloxy)trimethylene] diacetate was emulsified in 625 ml 0.1 M NaCl, 25 ml 0.1 M sodium phosphate buffer pH 7.0 by stirring at 0 °C. The reaction was started by adding 5.0 g lipase P-30 and the pH kept constant by addition of 1.0 N NaOH. After 53.9 % conversion (3.5 h) the reaction mixture was extracted with 2x500 ml CH_2Cl_2, and the combined organic phases were dried ($MgSO_4$) and evaporated to give 18.4 g (87 %) **7** as a colourless oil: 96 % (GLC); 93 % ee (derivatization with (S)-Trolox methyl ether[16] and separation of the diastereoisomers on an OV-1 capillary column); $[\alpha]_D$= -14.7 (3 % in EtOH), $[\alpha]_D$= +19.1 (2 % in $CHCl_3$); IR (neat): 3456 (-OH), 1740, 1240 (ester), 1119 (C-O-C), 1051 (-OH), 741, 700 (monosubst. benzene); NMR (250 MHz, DMSO): 7.40-7.23 (m, 5 arom. H), 4.79 (t, 1H, -OH), 4.57/4.60 (AB, J_{AB}=12, 2H, -C$\underline{H}_2$Ph), 4.25-3.98 (m, 2H, -$COOCH_2$-), 3.56 (m, 1H, -CH-), 3.53-3.41 (m, 2H, -C$\underline{H}_2$OH), 2.01 (s, 3H, -$COCH_3$); MS: 193 (1, M-CH_2OH), 107 (18), 92 (12), 91 (100); $C_{12}H_{16}O_4$, calc. C 64.27, 7.19, found C 63.74, H 7.34.

[16] W.Walther, W. Vetter, M. Vecchi, H. Schneider, R.K. Müller, T. Netscher, *Chimia* **1991**, *45*, 121.

[17] Both hydroxy groups of **5** were esterified with "Mosher's reagent" and the two diastereoisomers analyzed by ^{1}H-NMR (400 MHz, $CDCl_3$).