Tetrahedron Letters, Vol. 34, No. 18, pp. 2923-2926, 1993
Printed in Great Britain

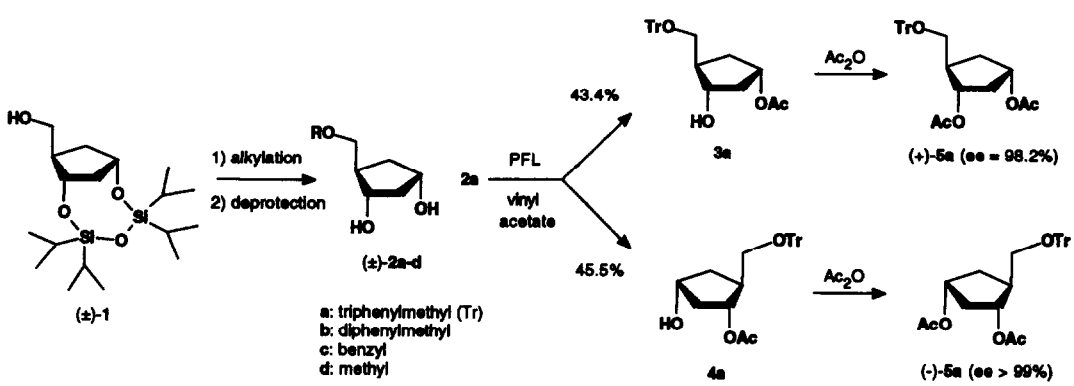
0040-4039/93 $6.00 + .00
Pergamon Press Ltd

The Influence of Protecting Groups on Lipase Catalyzed Transesterifications: Enzymatic Resolution of Racemic *cis*-1,3-Cyclopentanediol Derivatives

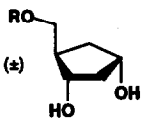
R. Henly[I], C.J.J. Elie[I], H.P. Buser[II], G. Ramos[II] and H.E. Moser[I]*
Central Research Laboratories[I] and Plant Protection Division[II]
Ciba-Geigy Ltd
CH-4002, Basle, Switzerland

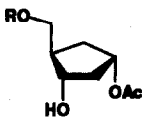
Abstract: *The enzyme catalyzed acetylation of racemic cis-1,3-cyclopentanediol derivatives (±)-2a-d in vinyl acetate is greatly influenced by the size of protecting groups at the additional trans-4-hydroxymethyl function. The highest regio- and enantioselectivities were obtained using the diol substrate protected with the bulky triphenylmethyl (trityl) group.*

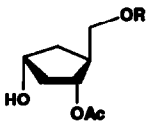
The demand for enantiomerically pure compounds is steadily increasing in fields like medicinal chemistry, agrochemistry or material sciences. To fulfill these requirements, methods like syntheses based on the chiral pool, enantioselective syntheses or resolution of racemic compounds become more and more important. For the latter procedure especially hydrolytic enzymes such as lipases, esterases or proteases have been shown to be useful in catalyzing the enantioselective transformation of racemic molecules[1]. In particular lipases were proven to be powerful catalysts for enantio- or regioselective acylations in organic solvents using enol esters as acyl donors[2].



As part of a program directed towards the total synthesis of enantiomerically pure, carbocyclic 2'-deoxynucleosides as building blocks for oligonucleotide analogs[3], the resolution of racemic trityl diol (±)-**2a** by enzymatic acyl transfer was investigated. The silyl-protected precursor (±)-**1**, readily available from *cis*-4-cyclopentene-1,3-diol[4] via protection, hydroformylation and reduction of the aldehyde, was protected at the primary hydroxy function with tritylchloride to give after desilylation the corresponding diol (±)-**2a**.







Racemic Diol	Lipase (time, h)	Monoacetate (Yield; %-ee)[a]	Monoacetate (Yield; %-ee)[a]
2a (R=C(C_6H_5)$_3$)	PFL (60)	**3a** (43%; *ee = 98*)	**4a** (46%; *ee* ≥ *99*)
2a (R=C(C_6H_5)$_3$)	CVL (32)	**3a** (49%; *ee* ≥ *99*)	**4a** (26%; *ee* ≥ *99*)
2b (R=CH(C_6H_5)$_2$)	PFL (16)	**3b** (47%; *ee = 89*)[b]	**4b** (25%; *ee = 98*)
2b (R=CH(C_6H_5)$_2$)	CVL (5)	**3b** (47%; *ee = 91*)[c]	**4b** (20%; *ee* ≥ *99*)
2c (R=$CH_2C_6H_5$)	PFL (22)	**3c** (39%; *ee = 73*)[d]	**4c** (21%)
2c (R=$CH_2C_6H_5$)	CVL (5)	**3c** (39%; *ee = 79*)[e]	**4c** (13%)
2d (R=CH_3)	PFL (48)	**3d** (22%; *ee = 15*)[f]	**4d** (22%)
2d (R=CH_3)	CVL (24)	**3d** (35%; *ee = 56*)[g]	**4d** (13%)

a) All reactions were run on a 100 mg scale of diol **2**. The ee-values of **3a**, **3b**, **4a**, and **4b** were determined by HPLC on Chiralcel OD after conversion to the diacetyl derivatives **5**. The optical purity of **3c** was determined without derivatization on the same column. **3d** and **4d** could not be separated and were analyzed as a mixture by 500 MHz ^{1}H-NMR. This mixture was converted to the dinitrobenzoates which were separated on a Pirkle column. As two of the four compounds comigrated, only the ee-values of **3d** could be determined. b) Besides traces of unreacted diol **2b**, 3% of diacetate **5b** were isolated. c) Isolation of 11% **5b**. d) Isolation of 13% diacetate **5c** along with 16% unresolved monoacetates **3c** and **4c**. e) Isolation of 21% **5c** along with approx. 10% unresolved monoacetates **3c** and **4c**. f) Isolation of 26% unreacted diol **2d** and 1% diacetate **5d**. g) Isolation of 18% diacetate **5d**.

Pseudomonas fluorescens lipase (PFL) in vinyl acetate catalyzes the acetylation of (±)-**2a** to give in high yield roughly equal amounts of two compounds which were identified by 500 MHz ^{1}H-NMR COSY spectra to be the regioisomeric monoacetates **3a** and **4a**[5]. Both compounds were separately converted by a standard acetylation procedure into the corresponding enantiomers of diacetate **5a** which proved to be well-suited for determination of the enantiomeric excess (ee) by HPLC on a

commercially available Chiralcel OD column. Whereas in the diacetate (+)-**5a**, prepared from the monoacetate **3a** and corresponding to the "unnatural" *L*-enantiomer of deoxyribose[6], the other enantiomer was not detectable (ee ≥ 99%), the diacetate (-)-**5a**, the acetylation product of **4a**, contained 0.9% of the opposite enantiomer (ee = 98%). This rather unexpected high enantioselectivity in the lipase catalyzed acetylation of a racemic diol to its regioisomeric monoacetyl derivatives gave rise to the assumption that the size of the bulky trityl group played an essential role in the stereodifferentiation step.

To verify this hypothesis we not only examined a series of other enzymes on the substrate (±)-**2a**, but also replaced the bulky trityl moiety by smaller protecting groups. In addition to PFL, commercially available lipases from *Candida cylindracea, Chromobacterium viscosum* (CVL), *Mucor miehei, Rhizopus japonicus, Rhizopus javanicus, LPL 'Amano' III, Aspergillus niger, Porcine pancreas* and the esterase from *Pig liver* were investigated for catalyzing the acetylation of the racemic diol (±)-**2a**. Only the reaction with CVL gave monoacetates **3a** and **4a** in comparable yields and optical purities and was therefore also used to examine the influence of different protecting groups on the primary hydroxy function of diols (±)-**2** which are available according to the trityl derivative (±)-**2a** from (±)-**1**. As shown in the table, CVL catalyzes the transesterification to give the corresponding monoacetates with comparable enantioselectivities. However, the formation of monoacetates **3** proceeds faster and the enzyme shows an increased tendency to catalyze the further acetylation of acetates **4** to give substantial amounts of diacetates **5**.

PFL or CVL catalyzed transesterification of diols (±)-**2b-d** in vinyl acetate at room temperature followed by separation on silicagel afforded the regioisomeric monoacetates as main products. The enantiomeric purities of monoacetates **3a-d** clearly reflect the importance of the protecting group: the change trityl → diphenylmethyl → benzyl → methyl causes a remarkable decrease of the enantiomeric excess (98% → 87% → 73% → 15%). An accurate determination of the enantiomeric purity for regioisomers **4** was achieved only with the trityl and diphenylmethyl derivative **4a** and **4b**. Both compounds were shown to be >98% optically pure.

The importance of bulky protecting groups[7] such as phenyl, *tert.*-butyl or *tert.*-butyl-dimethylsilyl (TBDMS)[8] to obtain high enantioselectivities was demonstrated earlier in the case of lipase catalyzed acylations of 1,2- and 1,3-diols. Only recently, two examples were reported using trityl derivatives as substrates for lipase catalyzed acylations[9]; in agreement with our observations for both cases products were obtained with high enantioselectivities. The data reported here clearly indicate the importance of bulky protecting groups and especially of the trityl group. This particular protecting group is cheap and easy to introduce and remove on hydroxy functions, usually gives rise to crystalline derivatives, and - as shown above - supports high enantio- and regioselectivities for enzyme catalyzed transesterifications.

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5. Compounds **3a** and **4a** were prepared according to the following procedure: 6.22 g (16.6 mmol) **2a** were dissolved in 63 ml vinyl acetate and 1.24 g PFL (Biocatalyst) were added. The heterogenous reaction mixture was stirred at RT until TLC indicated complete conversion of **2a** and the formation of two major products (EtOAc/hexane 1:1, R_f = 0.33 and 0.48). After 50 h the reaction mixture was concentrated *in vacuo* and the residue chromatographed on silicagel (EtOAc/hexane 1:2) to give besides 250 mg (3.3%) diacetate, 3.00 g pure **3a** (43.4%) and 3.15 g pure **4a** (45.5%) as slightly yellow oils. The same reaction was also carried out on a 30-fold larger scale to give similar results. **3a**: ^{1}H-NMR (250 MHz, $CDCl_3$, δ in ppm vs. TMS = 0.00): 1.54 (*m*, H-C-(5)); 1.74 (m, H_2-C(2)); 1.89 (*m*, H-C(5)); 2.05 (*s*, (H_3CCOO); 2.40 (*m*, H-C(2) and H-C(4)); 2.52 (*d*, *J* = 4.0 Hz, HO-C(3)); 2.98 (*t*, *J* = 8.5 Hz, H-C(6)); 3.34 (*dd*, *J* = 9.0 and 5.0 Hz, H-C(6)); 3.95 (*dq*, *J* = 3.0 and 7.0 Hz, H-C(3)); 5.07 (*m*, H-C(1)); 7.21-7.35 (*m*, H-C(4') and H-C(3')); 7.39-7.45 (*m*, H-C(2')). HPLC (Chiralcel OD (Daicel), hexane/*i*-PrOH 9:1, flow: 1 ml/min): t_R = 11.1 min (99.1%) and t_R = 19.7 min (0.9%), ee = 98.2%. $[\alpha]_{589}$ = -18.0° (25°C, c = 2.21, CH_3Cl). **3a** was converted to the diacetate (+)-**5a** by acetylation with Ac_2O/Et_3N in CH_2Cl_2 followed by extractive workup: HPLC (Chiralcel OD, hexane/*i*-PrOH 98:2, flow: 0.5 ml/min): t_R = 23.9 min (99.1%) and t_R = 27.6 min (0.9%). The racemic diacetate (±)-**5a** could be baseline separated by this method (t_R = 23.5 min (49.9%) and t_R = 26.4 min (50.1%)). $[\alpha]_{589}$ = +25.6° (25°C, c = 2.19, CH_3Cl). **4a** : ^{1}H NMR (250 MHz, $CDCl_3$): 1.61-1.78 (*m*, H-C(2,5) and HO-C(1)); 1.95 (*m*, H-C(5)); 2.03 (*s*, CH_3CCOO); 2.30 (*ddd*, *J* = 14.5, 7.5 and 5.5 Hz, H-C(2)); 2.60 (*m*, H-C(4)); 3.12 (*ABM*-system, H_2-C(6)); 4.34 (*m*, H-C(1)); 5.05 (*m*, H-C(3)); 7.18-7.34 (*m*, H-C(4') and H-C(3')); 7.38-7.45 (*m*, H-C(2')). $[\alpha]_{589}$ = -22.1° (25°C, c = 2.31, CH_3Cl). The racemate of monoacetate **4a** was inseparable by HPLC on chiral columns and therefore **4a** was converted to the diacetate (-)-**5a**: HPLC (Chiralcel OD, hexane/*i*-PrOH 98:2, flow: 0.5 ml/min): t_R = 25.8 min (100%), ee ≥ 99%. $[\alpha]_{589}$ = -26.4° (25°C, c = 2.19, CH_3Cl).
6. To determine the absolute stereochemistry, compound **4a** was converted to the enantiomerically pure carbocyclic thymidine of known absolute stereochemistry: L. Ötvös, J. Béres, G. Sági, I. Tömösközi, L. Gruber, *Tetrahedron Lett.* **1987**, *28*, 6281.
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(Received in Germany 22 February 1993)