Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 2193–2196

TETRAHEDRON: *ASYMMETRY*

Lipase-catalyzed resolution of stereogenic centers in steroid side chains by transesterification in organic solvents: the case of a 26-hydroxycholesterol

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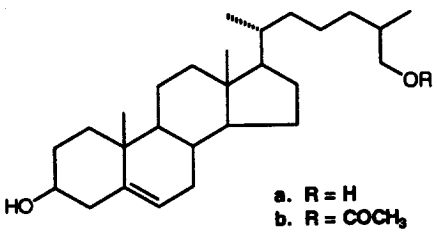
Received 24 March 1998; accepted 29 May 1998

Abstract

The *Pseudomonas cepacia* (PCL) lipase selectively catalyzes the acylation of the (25S)-isomer of the (25R,S)-26-hydroxycholesterol **1a** when the transesterification is irreversibly carried out with vinyl acetate in a mixture of organic solvents (chloroform/tetrahydrofuran).

The lipase-catalyzed transesterification in organic solvents of hydroxylated substrates is now a well established method extensively applied to the synthesis of enantiomerically pure compounds[1] and seems especially useful when applied to sterols that are highly insoluble in water. A few lipases have already shown the capability of catalyzing the regioselective acylation of hydroxy groups in the steroid rings and deacylation of the corresponding esters has already been described.[2,3] We have recently reported[4] that the *Pseudomonas cepacia* lipase[5] (PCL or PFL from the previous name *Pseudomonas fluorescens*) catalyzes the stereoselective acylation of a hydroxy group in the steroid side chain, under the conditions of irreversible transesterification in an organic solvent,[6] a method that we have used for the enantioselective resolution of a variety of 2-substituted alkanols.[7] We have now extended the above observation to another primary alcohol in the steroid side chain bearing the stereogenic center at position 25, namely 26-hydroxycholesterol **1a**.



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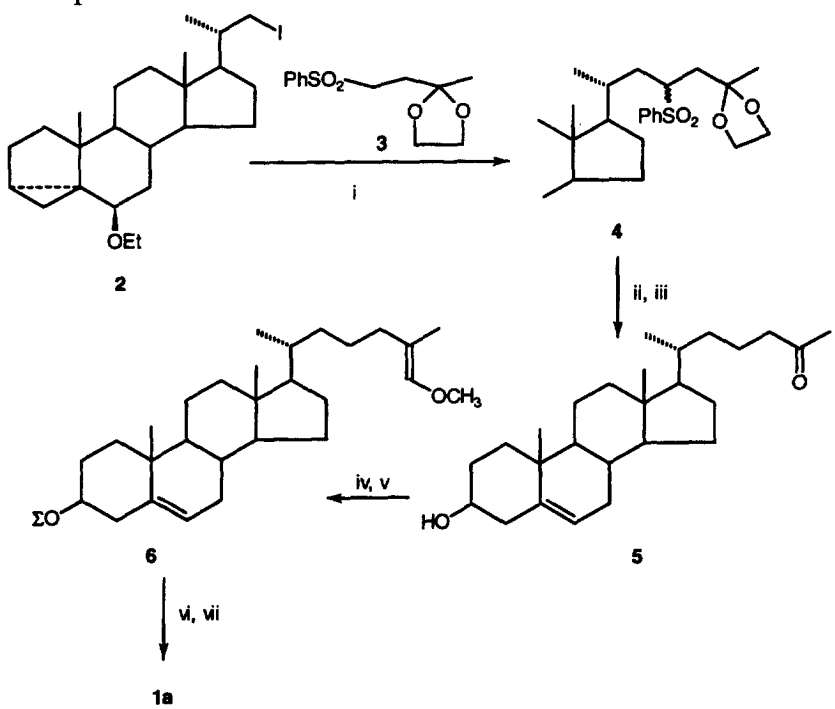
0957-4166/98/$ - see front matter
PII: S0957-4166(98)00222-5

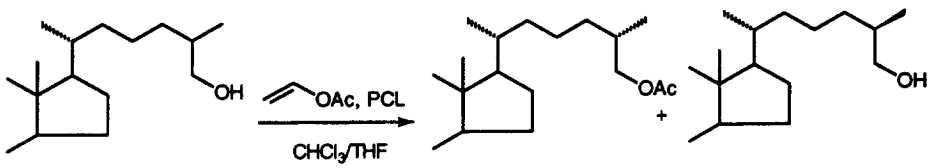
We had already prepared the (25S)-epimer of **1a** by a different biocatalytic approach, i.e. preparing a C-5 chiral synthon necessary for the construction of the side chain by baker's yeast-mediated bioreduction.[8] An alternative approach could be constituted by the use of other enantiomerically pure chiral building blocks such as phenylsulfones prepared by PFL-catalyzed resolution.[9]

In order to study the resolution of the stereogenic center present in the side chain, the synthesis of (25R,S)-**1a** was required and we started from the 22-iodo derivative **2** obtained as previously described.[8] The phenylsulfone **3** was easily prepared from the corresponding phenylsulfonyl ketone[10] and reacted with the intermediate compound **2**.[11] The conversion of the steroidal phenylsulfone **4** to the desired **1a** required the removal of the phenylsulfonyl moiety followed by the deprotection to the 25-ketosteroid **5**.[12] The 3β-hydroxy group of this intermediate was silylated and the 25-keto group transformed into the corresponding methyl enol ether (compound **6**).[13] The enol ether was hydrolyzed to the corresponding aldehyde with simultaneous removal of the silyl protection and reduction of the intermediate 25-aldehyde afforded the final compound **1a**.[14]



i. LDA, -78 °C, 5h (87%); ii. Hg/Na, EtOH, 25 °C, 4h (quant.); iii. H_2SO_4, H_2O/THF (1/1), 4h (95%); iv. $tBuMe_2SiCl$ (ΣCl) / imidazole,THF, 25 °C, 12h (89%); v. Ph_3P^+-CH=OCH_3 Cl^-, LDA, -78 °C; THF/$PhCH_3$, 5h (85%); vi. $HClO_4$, Et_2O, 25 °C, 3h, (73%); vii. $NaBH_4$, MeOH, 25 °C, 3h, (79%).

The (2R,S)-3,26-diol **1a** prepared as above underwent a reaction with the lipase and vinyl acetate in chloroform/tetrahydrofuran[15] and after 1 h, 30% of the 26-acetate **1b** was formed (as established by GLC analysis)[16] thus showing that the enzymatic reaction was highly regioselective (no trace of the 3-acetate was observed).[17] A 70% conversion to **1b** was reached in 3 h and the 500 MHz ^{1}H-NMR of the 26-MTPA esters of the unreacted **1a** (the enzymatic product at 70% conversion) and of the alcohol from the acetate **1b** (at 30% conversion) showed that the enzymatic reaction may be carried out to produce pure epimers.[18]



(25R,S)-1a (25S)-1b (25R)-1a

The configuration of the enzymatic products was assigned by comparison of the published resonances[19] and from the results it was clear that the 25S-acetate **1b** is produced by the enzymatic transesterification. The fact that the 25S-alcohol **1a** is the substrate accepted by the enzyme in the conditions of the transesterification reaction to yield the 25S-acetate **1b** confirms the configurational outcome of the enzymatic reaction when 2-methyl alkanols are the substrates[20] and include the side chain of **1a** in this class of compounds.[21] This reaction is faster than the formation of the (20S)-acetate from the (20R,S)-22-hydroxy steroid reported by us[4] (30 h for a 30% conversion). However, it should be remembered that, due to different steric hindrance, the C-26 alcohol is more accessible than the C-22 analogue. In conclusion, this result offers an additional example of the regio- and enantioselective control of the enzymatic reaction on a polyfunctional steroid as a substrate and from this and our previous work[4] a new approach is opened to the stereoselective construction of steroid side chains.

Acknowledgements

This work has been financially supported by Ministero dell'Università e della Ricerca Scientifica (MURST, fondi 60%).

References

1. (a) Faber, K.; Riva, S. *Synthesis* **1992**, 895. (b) Santaniello, E.; Ferraboschi, P.; Grisenti, P. *Enzyme Microb. Technol.* **1993**, *15*, 367. (c) Fang, J.-M.; Wong, C.-H. *Synlett* **1994**, 393. (d) Carrea, G.; Ottolina, G.; Riva, S. *Trends Biotechnol.* **1995**, *13*, 63.
2. For a review, see: Riva, S. in *Applied Biocatalysis*, Blanch, H. W., Clark, D. S. Eds, M. Dekker, New York 1991, Vol. 1, 179–219.
3. (a) Njar, V. C. O.; Caspi, E. *Tetrahedron Lett.* **1987**, *28*, 6549. (b) Riva, S.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, *110*, 3291. For more recent examples, see: (c) Adamczyk, M.; Chen, Y.-Y.; Fishpaugh, J. R.; Gebler, J. C. *Tetrahedron: Asymmetry* **1993**, *4*, 1467. (d) Bertinotti, A.; Carrea, G.; Ottolina, G.; Riva, S. *Tetrahedron* **1994**, *50*, 13165. (e) Baldessari, A.; Maier, M. S.; Gros, E. G. *Tetrahedron Lett.* **1995**, *36*, 4349. (f) Baldessari, A.; Maier, M. S.; Gros, E. G. *Helv. Chim. Acta* **1997**, *36*, 4349.
4. Ferraboschi, P.; Molatore, A.; Verza, E.; Santaniello, E. *Tetrahedron: Asymmetry* **1996**, *7*, 1551
5. The lipase was a generous gift from the italian subsidiary of Amano (Japan) through the kind interest of Mr. I. Gaudio (Mitsubishi Italia, Milano).
6. (a) Degueil-Castaing, M.; De Jeso, B.; Drouillard, S.; Maillard, B. *Tetrahedron Lett.* **1987**, *28*, 953. (b) Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200.
7. Santaniello, E.; Ferraboschi, P.; Casati, S.; Manzocchi, *Tetrahedron: Asymmetry* **1995**, *6*, 1521 and references cited therein.
8. (a) Ferraboschi, P.; Grisenti, P.; Casati, R.; Fiecchi, A.; Santaniello, E. *J. Chem. Soc., Perkins Trans. 1* **1987**, 1743. (b) Ferraboschi, P.; Fiecchi, A.; Grisenti, P.; Santaniello, E. *ibidem 1* **1987**, 1749.
9. Santaniello, E.; Ferraboschi, P.; Fiecchi, A.; Grisenti, P.; Manzocchi, A. *J. Org. Chem.* **1987**, *117*, 701.
10. Reaction of sodium phenylsulfinate with methyl vinyl ketone quantitatively afforded 4-phenylsulfonyl-2-butanone (Julia, M.; Badet, B. *Bull. Soc. Chem.* **1975**, 1363). Ketalization (ethylene glycol in the presence of *p*-toluenesulfonic acid, 90% yield) afforded the required intermediate **3**.

11. The 22-iododerivative **2** prepared starting from stigmasterol as described in Ref. 8b (50% yield) was treated with the phenylsulfone **3** and LDA (from butyl lithium and diisopropylamine) affording the intermediate **4** (87%). The phenylsulfonyl moiety was quantitatively removed by reaction with sodium amalgam (see Ref. 8b).
12. Treatment of compound **4** with H_2SO_4 in water/tetrahydrofuran (1/1) hydrolyzed both protecting groups (the *i*-steroid moiety and the ketal function) affording the 25-keto-27 norcholesterol **5** (95%).
13. Compound **5** was silylated (89%) with *t*-butyldimethyl silyl chloride and imidazole (Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190) and the 25-keto group was reacted with methoxy methyl triphenyl phosphonium chloride and LDA to afford the intermediate **6** (85%).
14. Treatment of compound **6** with perchloric acid removed the silyl and the methyl enol ether groups affording the intermediate 25-aldehyde that was directly reduced to the required **1a** ($NaBH_4$ in methanol, 79%).
15. A solution of 25R,S-**1a** (0.4 g, 1 mmol) in chloroform/tetrahydrofuran 1/2 (5.5 ml) and vinyl acetate (0.32 ml, 3.46 mmol) was added to the solid lipase (14 mg, 31.5 U/mg) with stirring at room temperature.
16. GLC analysis (Hewlett Packard, mod. 5890/II, HP-5 capillary column, T 280°C) showed two peaks for the products at T_R 15.0 (alcohol) and 17.0 min (acetate).
17. The structure of the product from the enzymatic reaction was determined by ^{1}H-NMR (500 MHz): the proton at position 3 showed a multiplet centered at 3.50 ppm and the protons at positon 26 a multiplet centered at 3.87 ppm.
18. Although the enzymatic products were epimers, the optical purity could not be established directly by ^{1}H-NMR (500 MHz) analysis and the corresponding (R)-MTPA-esters were prepared. The derivative from (25R,S)-26-hydroxycholesterol showed a signal constituted by three groups of peaks: a pair of double doublets between 4.00–4.08 ppm and 4.18–4.25 ppm and a doublet at 4.13 ppm. In the case of MTPA ester of unreacted **1a** the doublet at 4.13 ppm was not detectable and the same derivative prepared from the alcohol obtained by the hydrolysis of acetate **1b** showed only the signal centered at 4.13 ppm.
19. Uomori, A.; Seo, S.; Sato, T.; Yoshimura, Y.; Takeda, K. *J. Chem. Soc., Perkins Trans. 1* **1987**, 1713.
20. Ferraboschi, P.; Casati, S.; De Grandi, S.; Grisenti, P.; Santaniello, E. *Biocatalysis* **1994**, *10*, 279.
21. A recent interpretation of the enantiopreference of *Pseudomonas cepacia* lipase toward primary alcohols has been proposed; see: Weissfloch, A. N. E.; Kazlauskas, R. J. *J. Org. Chem.* **1995**, *60*, 6959.