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Chemoenzymatic syntheses of (25*R*)- and (25*S*)-25-hydroxy-27-nor-cholesterol, a steroid bearing a secondary hydroxy group in the side chain

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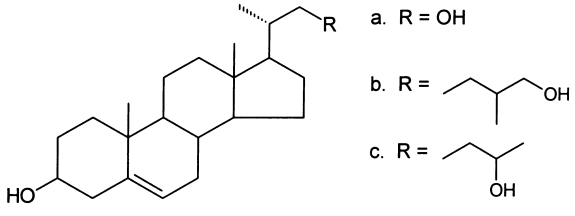
Abstract

(25*R*)- and (25*S*)-25-hydroxy-27-nor-cholesterol **1c** were prepared by enzymatic resolution of the stereogenic center in the side chain of compound **4a** or by synthesis of the side chain using, as a chiral synthon, the enantiomerically pure phenylsulfonyl alkanol **6a** prepared by different biocatalytic approaches.

We have recently reported that the *Pseudomonas cepacia* lipase (PCL) catalyzes the stereoselective acylation of the primary hydroxy group of a steroid side chain, as shown for the compound **1a**[1] and for 26-hydroxycholesterol **1b**.[2]



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We present here our results on the biocatalytic approach to the preparation of diastereomerically pure 25-hydroxy-27-nor-cholesterol **1c**, a cholesterol metabolite that has been used as the (25*RS*)-epimeric mixture in studies concerning the inhibitory effects of oxysterols on the hydroxymethylglutaryl coenzyme (HMGCoA) reductase activity.[3] The resolution of the C-25 stereogenic center was of special interest, because the stereochemical outcome of the process might be foreseen according to the model that has been proposed to explain the enantiopreference of a few lipases on secondary alcohols.[4] Furthermore, (25*R*)- and (25*S*)-**1c** could, in principle, be transformed into the corresponding stereoisomers

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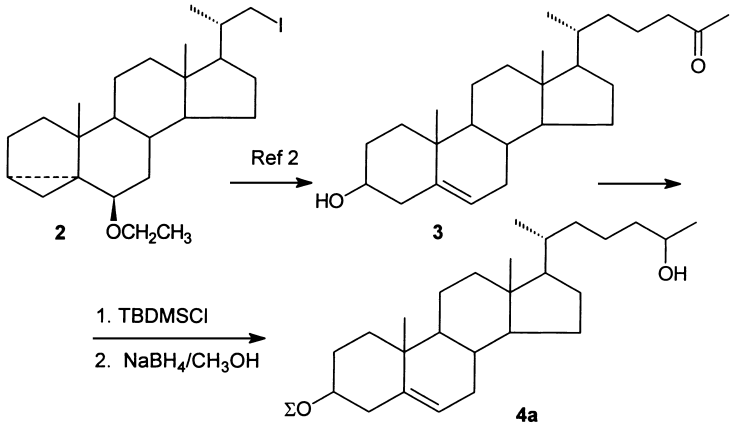
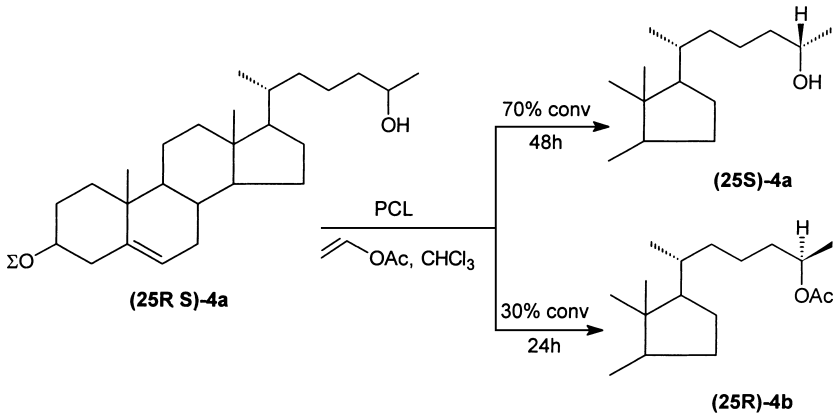
of 26-aminocholesterol,[5] that has been reported to interfere with myocite proliferation and cholesterol synthesis.[6]

In order to study the resolution of the C-25 stereogenic center, we prepared the 3β-*O*-silyl ether of (25*RS*)-**1c** (compound **4a**) as substrate of the enzymatic reaction[7] from the 25-ketosteroid **3**,[8] that was in turn obtained from the 22-iodo derivative **2**, an intermediate of the synthesis of 26-hydroxycholesterol **1b** (Scheme 1).[2]



Scheme 1.

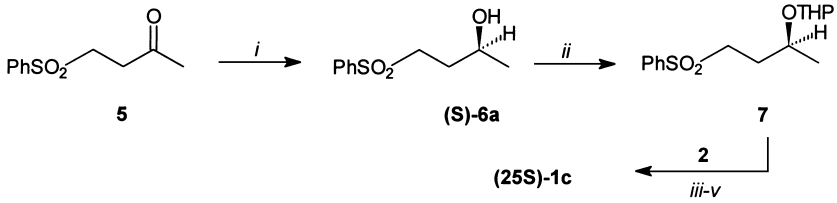
The compound (25*RS*)-**4a** was subjected to the enzymatic reaction (PCL/vinyl acetate)[9] in chloroform and the 25-acetate **4b** at 30% conversion (24 h) and the unreacted alcohol **4a** at 70% conversion (48 h) were isolated (Scheme 2).[10] The 500 MHz ^{1}H NMR spectra of the (*R*)-MTPA ester[11] of the alcohol **4a** and of the alcohol obtained from the acetate **4b** showed that the enzymatic reaction afforded the pure epimers[12] that could be prepared in nearly 30% yield after silica gel column chromatography.



Scheme 2.

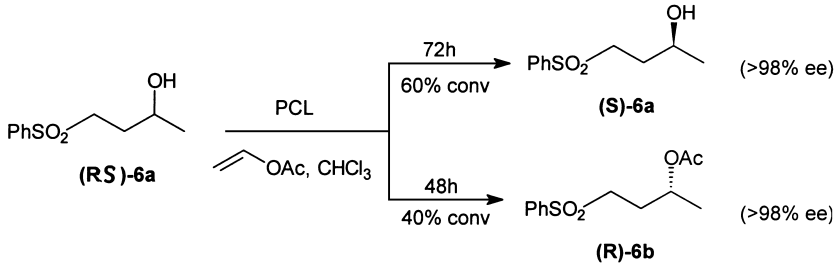
In order to assign the configuration of the enzymatic products, samples of the required (25*R*)- or (25*S*)-alcohol **4a** or deprotected **1c** could be prepared from the previous 22-iodo steroid **2** and a chiral phenylsulfonyl intermediate, by an approach that we had already applied to the synthesis of marine sterols[13] and 26-hydroxycholesterol.[14] (*S*)-4-Phenylsulfonyl-2-butanol **6a** was prepared in an enantiomerically pure form (>98% ee) by a baker's yeast-mediated reduction[15] of the known 4-phenylsulfonyl-2-butanone **5**.[16] The compound **6a** was converted to the corresponding THP ether **7** that

was coupled to the 22-iodo derivative **2** and, after a few additional steps, a sample of (25*S*)-compound **1c** was prepared in 58% yield (Scheme 3).



Scheme 3. (i) Baker's yeast, 30°C, 96 h (35%); (ii) DHP, pTSA, rt, 24 h (quant.); (iii) LDA, −78°C, 5 h (60%); (iv) Hg/Na, EtOH, 25°C, 6 h (quant); (v) H_2SO_4, H_2O/THF (1/1), 4 h (96%)

For a synthesis of both C-25 stereoisomers, the racemic phenylsulfonyl alcohol **6a** was also resolved by the PCL-catalyzed irreversible transesterification procedure[9] in chloroform, obtaining both (*S*)-alcohol **6a** and (*R*)-acetate **6b** enantiomerically pure (>98% ee, *E*=189) (Scheme 4).[17]



Scheme 4.

In any event, the ^{1}H NMR spectrum of the 3,25-diMTPA ester of 25*S*-**1c** prepared using (*S*)-phenylsulfonyl alcohol **6a** was identical to that obtained from the same diMTPA-ester of the desilylated product of the enzymatically prepared **4a**.[18] This result demonstrated that the stereochemical outcome of the enzymatic acylation of the alcohol **4a** is in agreement with the configurational preference of the enzyme when a secondary alcohol is the substrate.[4] Finally, the results presented here offer additional examples of the stereoselective control of the enzymatic reaction on a hydroxy group present in a steroid chain and propose a few biocatalytic approaches to the synthesis of chiral synthons such as phenylsulfonyl alcohols, useful intermediates for the construction of steroid side chains.

Acknowledgements

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5. We have outlined the main steps to transform compound **4a** into 26-aminocholesterol (conversion of the 25-hydroxy into a 25-nitrile group, followed by lithium hydride reduction, 54% overall yields).
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7. We also prepared (25*RS*)-**1c** but it was insoluble in chloroform/tetrahydrofuran; we therefore prepared a less polar substrate such as the silyl derivative **4a**, so that we could carry on the enzymatic reaction in a solvent like chloroform or dichloromethane, that we have used for all our transesterifications.
8. The ketone **3** (83% from **2**) was silylated (89%) and reduced ($NaBH_4$/MeOH, 85%).
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10. A solution of (25*RS*)-**4a** (0.5 g, 1 mmol) in chloroform (3 mL) was treated with vinyl acetate and the lipase (14 mg) under stirring for the time required for the desired conversion. The enzymatic reactions were monitored by GLC analysis (Hewlett Packard, mod. 5890/II, HP-5 capillary column, *T* 280°C, T_R 21 min for the alcohol and 25 min for the acetate).
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12. In the spectrum of (25*RS*)-derivative the signal due to the C-26 methyl group showed two doublets at 1.24 and 1.32 ppm. The signal at 1.24 ppm was not detectable in the spectrum of the (*R*)-MTPA ester of the enzymatically prepared alcohol **4a**. In the spectrum of the (*R*)-MTPA ester of the alcohol obtained after removal ($LiAlH_4$ in THF) of the acetate **4b** only the signal at 1.24 ppm was present.
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15. Robin, S.; Huet, F.; Fauve, A.; Veschambre, H., *Tetrahedron: Asymmetry*, **1995**, *4*, 239. Using a 1/20 ratio (mmol substrate per gram yeast) instead of the described 1/6 ratio we reached a 75% conversion of the substrate and incubation time was lowered (from 192 h to 96 h). A 35% yield of the product was obtained after purification by silica gel chromatography.
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17. A solution of (*RS*)-**6a** (0.3 g, 1.4 mmol) in chloroform (2.6 mL) was treated with vinyl acetate and the lipase (19.5 mg) under stirring. The ees were determined by ^{1}H NMR analysis of the (*R*)-MTPA esters of the alcohol **6a** and the alcohol from the acetate **6b** after treatment with $LiAlH_4$ in THF. The signals at 1.24 and 1.32 ppm present in the derivative from (*RS*)-**6a** were absent respectively in the derivative from (*S*)- and (*R*)-**6a**. The value of *E* was calculated according to Sih and Wu (Sih, C. J.; Wu, S.-H. *Topics in Stereochemistry* **1989**, *19*, 63). The *S*-configuration of **6a** ($[\alpha]_D$ +19, *c* 1 in chloroform) was established by comparison with the specific rotation reported in the literature (Ref. 15).
18. ^{1}H NMR of 3β,25*S*-diMTPA ester of **1c**: δ 0.63 (s, 3H, CH_3-18), 0.80 (d, 3H, CH_3-21), 0.97 (s, 3H, CH_3-19), 1.30 (d, 3H, CH_3-26), 3.377 (s, 3H, OCH_3), 3.379 (s, 3H, OCH_3), 4.81–4.89 (m, 1H, CHOCO), 5.09–5.15 (m, 1H, CHOCO), 5.39–5.41 (m, 1H, CH=C). The significant signals for a stereochemical analysis were those due to the following methyl groups [from the ^{1}H NMR spectrum of (3β,25*RS*)-diMTPA ester of **1c**): 0.63 (s, 3H, CH_3-18), 0.65 (s, 3H, CH_3-18), 0.80 (d, 3H, CH_3-21), 0.87 (d, 3H, CH_3-21), 1.23 (d, 3H, CH_3-26), 1.30 (d, 3H, CH_3-26)]. The C-19 methyl group was a singlet at 0.97 ppm for both (25*R*)- and (25*S*)-isomers.