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Esterification of streptol — a cyclitol derivative — by *Candida rugosa* lipase: influence of the acyl donor on regioselectivity

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Abstract

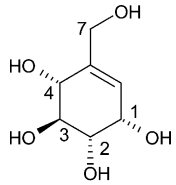
The influence of the nature of acyl donors on the regioselectivity of *Candida rugosa* lipase for the esterification of streptol — a cyclitol derivative — was investigated. Excellent regioselectivity for the formation of 3,7-disubstituted derivatives was observed for vinyl butyrate (100% 3,7-derivative, 68% yield) and vinyl propionate (100% 3,7-derivative, 46% yield) as acyl donors. In contrast, for vinyl methacrylate as acyl donor, a mixture of 71% 3,7-derivative and 29% 1,7-derivative was obtained. Varying the chain length, a certain dependency of regioselectivity on the acyl donor was observed, however, no logical correlation satisfying all cases was found. Mono-substituted streptol derivatives were obtained by employing Novozym 243.

Keywords: *Candida rugosa* lipase; Regioselectivity; Esterification; Streptol; Biocatalysis

1. Introduction

Selective acylation of a given primary or secondary hydroxy group of a sugar requires elaborate synthetic control via blocking and deblocking methodologies [1–3]. A highly selective tool to circumvent the additional blocking and deblocking steps is the usage of enzymes, which are capable of regioselectively acylating sugars [4–7]. Enzymes can differentiate on the basis of chemical functionality (chemoselectivity), chirality (enantioselectivity) and position of the functional group within the molecule (regioselectivity). In contrast to enantioselectivity, where the enzyme has to distinguish between two possibilities (*R*, *S*), regioselectivity can involve multiple possibilities, particularly in complex molecules, for instance, for the enzymatic acylation of glucose the enzyme has to differentiate between one primary and four secondary hydroxy groups [8]. In the presence of a suitable anhydrous organic solvent (e.g., pyridine, THF, DMF, etc.) sugars are enzymatically acylated at primary [9–14] and secondary sites [15,16]. Using this methodology, selectively blocked sugar derivatives have been prepared for the use in the synthesis of sweeteners [17], linear polymers [18–20] and hydrogels [21,22].

The cyclitol derivative streptol (**1**) [23,24] is a plant growth regulator and has the same absolute configuration at C1 to C4 as α-D-glucose. We wished to investigate how the formal replacement of the C5–O bond of the glucose ring by a C=C double bond as in streptol (**1**) influences the regioselectivity of an acylating enzyme. We also investigated the reactivity of the hydroxy moieties and checked the possible influence of different acyl donors on the regioselectivity of an enzyme, especially in view of the formation of diesters.



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2. Experimental

2.1. General

Reactions were monitored by TLC (Merck Silica Gel 60 F_{254}) and visualized after dipping in a vanillin solution (6 g/l vanillin, 500 ml/l EtOH, 600 ml/l H_2O, 40 ml concentrated H_2SO_4) by heating. NMR spectra were measured on a Varian Unity 500 instrument.

2.2. Materials

Enzymes were obtained from the following suppliers: Novozym 243 (batch PM 20018, *Bacillus licheniformis* protease) from Novo Nordisk, *Candida rugosa* lipase (batch 03689) and *Chromobacterium viscosum* lipase (batch 33190) from Biocatalysts Ltd. Vinyl chloroacetate, vinyl capronate, vinyl acrylate were purchased from TCI, vinyl methacrylate from Lancaster, vinyl butyrate from Fluka, and vinyl 2-ethylhexanoate, vinyl decanoate, vinyl 4-^{t}butylbenzoate, vinyl benzoate, vinyl crotonate, vinyl propionate and vinyl pivalate were obtained from Aldrich. Streptol was isolated and purified from *Streptomyces xanthochromogenes* ID-40174 by the natural products group of Syngenta Crop Protection AG.

2.3. Mono-substituted streptols (7-O-butanoate as representative example)

Novozym 243 (300 mg) was suspended in toluene (450 μl) in an Eppendorf tube (1.5 ml). A solution of streptol (30 mg, 0.17 mmol) in DMF (225 μl) and vinyl butyrate (15.6 μl, 0.12 mmol) were added. The tubes were shaken horizontally at 36 °C and 180 rpm and the reaction was followed by TLC. The reaction was stopped after 4 days by removing the catalyst by centrifugation (5 min, 12,000 rpm). The supernatant was decanted and the catalyst was washed with toluene (600 μl). The combined organic phases were evaporated under reduced pressure, and the residue was purified by column chromatography (CH_2Cl_2/MeOH = 4:1) to give 7-*O*-butanoyl streptol (20.6 mg, 76% based on vinyl butyrate). All structures were confirmed by 2D NMR.

2.4. Higher substituted streptols (3,7-di-O-butanoate as representative example)

Lipase from *Candida rugosa* (100 mg) was suspended in toluene (400 μl) in an Eppendorf tube (1.5 ml). A solution of streptol (**1**) (22 mg, 0.13 mmol) in 200 μl DMF and vinyl butyrate (550 μl, 5.3 mmol) were added. The tubes were shaken horizontally at 36 °C and 180 rpm and the reaction was followed by TLC. The reaction was stopped by removing the catalyst by centrifugation (5 min, 12,000 rpm) after 4 days. The supernatant was decanted and the catalyst washed with toluene (600 μl). The combined organic phases were evaporated under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2/MeOH = 10:1 to 4:1). The fractions containing streptol derivatives were pooled and the composition determined by 2D NMR. 3,7-Di-*O*-butanoyl streptol was obtained in 68% yield.

2.4.1. Streptol (**1**)

^{13}C NMR (CD_3OD): δ 144.1 (C5), 122.9 (C6), 74.1 (C3), 73.8 (C4), 72.6 (C2), 67.5 (C1) and 62.8 (C7).

^{1}H NMR (CD_3OD): δ 5.82 (1H, m, H-C6), 4.14–4.23 (3H, m, H_2-C7, H-C1), 3.98 (1H, d, 7 Hz, H-C4), 3.73 (1H, dd, 7 and 10 Hz, H-C3) and 3.46 (1H, dd, 4 and 10 Hz, H-C2).

2.4.2. 7-O-butanoyl streptol (**2a**)

^{1}H NMR (CD_3OD): δ 5.83 (1H, m, H-C6), 4.78 (1H, d, 13 Hz, H_A-C7), 4.61 (1H, d, 13 Hz, H_B-C7), 4.19 (1H, br t, 5 Hz, H-C1), 3.95 (1H, d, 7 Hz, H-C4), 3.73 (1H, dd, 7 and 10 Hz, H-C3), 3.50 (1H, dd, 4 and 10 Hz, H-C2), 2.35 (2H, t, 7 Hz, $C(O)CH_2$), 1.66 (2H, sext, 7 Hz, $CH_2CH_2CH_3$) and 0.96 (3H, t, 7 Hz, CH_3).

2.4.3. 7-O-acetyl streptol (**2b**)

^{13}C NMR (CD_3OD): δ 172.4 (CO), 139.5 (C5), 125.8 (C6), 73.7 (C3), 73.1 (C4), 72.2 (C2), 67.4 (C1), 64.8 (C7), 20.2 (CH_3-acetyl).

^{1}H NMR (CD_3OD): δ 5.82 (1H, m, H-C6), 4.77 (1H, d, 13 Hz, H_A-C7), 4.59 (1H, d, 13 Hz, H_B-C7), 4.19 (1H, br t, 5 Hz, H-C1), 3.95 (1H, br d, 7 Hz, H-C4), 3.73 (1H, dd, 7 and 10 Hz, H-C3), 3.47 (1H, dd, 4 and 10 Hz, H-C2) and 2.07 (3H, s, CH_3).

2.4.4. 7-O-(2,2-dimethylpropanoyl) streptol (**2c**)

^{1}H NMR (CD_3OD): δ 5.82 (1H, m, H-C6), 4.77 (1H, d, 13 Hz, H_A-C7), 4.61 (1H, d, 13 Hz, H_B-C7), 4.21 (1H, t, 4 Hz, H-C1), 3.96 (1H, br d, 7 Hz, H-C4), 3.74 (1H, dd, 7 and 10 Hz, H-C3), 3.47 (1H, dd, 4 and 10 Hz, H-C2), 1.22 (9H, s, 3× CH_3).

2.4.5. 7-O-propenoyl streptol (**2d**)

^{1}H NMR (CD_3OD): δ 6.42 (1H, dd, 17 Hz, 1 Hz, $=CH_2(Z)$), 6.20 (1H, dd, 17 Hz, 11 Hz, OC–CH=), 5.91 (1H, dd, 11 Hz, 1 Hz, $=CH_2(E)$), 5.84 (1H, m, H-C6), 4.87 (1H, d, 13 Hz, H_A-C7), 4.70 (1H, d, 13 Hz, H_B-C7), 4.20 (1H, t, 4 Hz, H-C1), 3.97 (1H, br d, 7 Hz, H-C4), 3.74 (1H, dd, 7 and 10 Hz, H-C3) and 3.47 (1H, dd, 4 and 10 Hz, H-C2).

2.4.6. 7-O-crotonyl streptol (**2e**)

^{1}H NMR (CD_3OD): δ 7.04 (1H, dq, 16 and 7 Hz, $=CH–CH_3$), 5.92 (1H, d, 16 Hz, OC–CH=), 5.82 (1H, d, 4 Hz, H-C6), 4.73 (1H, d, 13 Hz, H_A-C7), 4.66 (1H, d, 13 Hz, H_B-C7), 4.20 (1H, m, H-C1), 3.97 (1H, d, 7 Hz, H-C4), 3.74 (1H, dd, 7 and 10 Hz, H-C3), 3.47 (1H, dd, 4 and 10 Hz, H-C2) and 1.90 (3H, d, 7 Hz, CH_3).

2.4.7. 7-O-(2-ethyl hexanoyl) streptol (**2f**)

^{1}H NMR (CD_3OD): δ 5.86 (1H, d, 4 Hz, H-C6), 4.78 (1H, d, 13 Hz, H_A-C7), 4.65 (1H, d, 13 Hz, H_B-C7), 4.20 (1H, br t, 4 Hz, H-C1), 3.96 (1H, br d, 7 Hz, H-C4), 3.74 (1H, dd, 7

and 10 Hz, H-C3), 3.48 (1H, dd, 4 and 10 Hz, H-C2), 2.33 (1H, m, C(O)CH), 1.22–1.68 (8H, m, 4× CH_2) and 0.94 (6H, d, 7 Hz, 2× CH_3).

*2.4.8. 7-O-decanoyl streptol (**2g**)*

^{1}H NMR (CD_3OD): δ 5.82 (1H, m, H-C6), 4.78 (1H, d, 13 Hz, H_A-C7), 4.62 (1H, d, 13 Hz, H_B-C7), 4.19 (1H, t, 4 Hz, H-C1), 3.95 (1H, br d, 7 Hz, H-C4), 3.73 (1H, dd, 7 and 10 Hz, H-C3), 3.47 (1H, dd, 4 and 10 Hz, H-C2), 2.36 (2H, t, 7 Hz, $C(O)CH_2$), 1.63 (2H, quint, 7 Hz, $C(O)CH_2CH_2$), 1.32 (12H, m, CH_2-chain) and 0.90 (3H, d, 7 Hz, CH_3).

*2.4.9. 7-O-benzoyl streptol (**2h**)*

^{1}H NMR (CD_3OD): δ 8.06 (2H, dd, 8 Hz, 1 Hz, *o*-Ph-H), 7.62 (1H, tt, 7 Hz, 1 Hz, *p*-Ph-H), 7.49 (2H, br t, 8 Hz, Ph-H), 5.94 (1H, m, H-C6), 5.03 (1H, d, 13 Hz, H_A-C7), 4.87 (1H, d, 13 Hz, H_B-C7), 4.23 (1H, t, 4 Hz, H-C1), 4.06 (1H, br d, 7 Hz, H-C4), 3.78 (1H, dd, 7 and 10 Hz, H-C3) and 3.51 (1H, dd, 4 and 10 Hz, H-C2).

*2.4.10. 3,7-Di-O-butanoyl streptol (**3a**)*

^{1}H NMR ($CDCl_3$): δ 5.87 (1H, m, H-C6), 5.22 (1H, dd, 9 Hz, 6 Hz, H-C3), 4.81 (1H, d, 13 Hz, H_A-C7), 4.67 (1H, d, 13 Hz, H_B-C7), 4.32 (1H, t, 4 Hz, H-C1), 4.14 (1H, br d, 7 Hz, H-C4), 3.79 (1H, dd, 4 and 9 Hz, H-C2), 2.39 (2H, t, 7 Hz, $C(O)CH_2$), 2.34 (2H, t, 7 Hz, $C(O)CH_2$), 1.68 (4H, m, 2× $CH_2CH_2CH_3$), 0.98 (3H, t, 7 Hz, CH_3) and 0.96 (3H, t, 7 Hz, CH_3).

*2.4.11. 3,7-Di-O-propenoyl streptol (**3d**)*

^{1}H NMR ($CDCl_3$): δ 6.47 and 6.46 (1H each, dd, 17 Hz, 1 Hz, $=CH_2$(*Z*)), 6.19 and 6.16 (1H each, dd, 17 and 11 Hz, COCH=), 5.92 and 5.90 (1H each, dd, 11 and 1 Hz, $=CH_2$(*E*)), 5.91 (1H, m, H-C6), 5.32 (1H, dd, 9 Hz, 6 Hz, H-C3), 4.89 (1H, d, 13 Hz, H_A-C7), 4.78 (1H, d, 13 Hz, H_B-C7), 4.34 (1H, t, 4 Hz, H-C1), 4.22 (1H, d, 6 Hz, H-C4) and 3.83 (1H, dd, 4 and 9 Hz, H-C2).

*2.4.12. 3,7-Di-O-(but-2-enoyl) streptol (**3e**)*

^{1}H NMR ($CDCl_3$): δ 7.02 (2H, m, 2× =C*H*–CH_3), 5.86 (1H, m, H-C6), 5.80 (2H, m, 2× COCH=), 5.28 (1H, dd, 9 Hz, 6 Hz, H-C3), 4.83 (1H, d, 13 Hz, H_A-C7), 4.73 (1H, d, 13 Hz, H_B-C7), 4.29 (1H, t, 4 Hz, H-C1), 4.16 (1H, br d, 6 Hz, H-C4), 3.77 (1H, dd, 4 and 9 Hz, H-C2) and 1.89 (6H, 2× d, 7 Hz, 2× CH_3).

*2.4.13. 3,7-Di-O-benzoyl streptol (**3h**)*

^{1}H NMR ($CDCl_3$): δ 8.06 (4H, m, Ph-H), 7.58 (2H, m, Ph-H), 7.43 (4H, m, Ph-H), 6.01 (1H, m, H-C6), 5.55 (1H, dd, 9 Hz, 7 Hz, H-C3), 5.06 (1H, d, 14 Hz, H_A-C7), 4.95 (1H, d, 14 Hz, H_B-C7), 4.41 (1H, m, H-C1), 4.12 (1H, d, 7 Hz, H-C4) and 3.98 (1H, dd, 4 and 9 Hz, H-C2).

*2.4.14. 3,7-Di-O-propanoyl streptol (**3i**)*

^{1}H NMR ($CDCl_3$): δ 5.84 (1H, m, H-C6), 5.24 (1H, dd, 10 Hz, 7 Hz, H-C3), 4.76 (1H, d, 13 Hz, H_A-C7), 4.70 (1H, d, 13 Hz, H_B-C7), 4.28 (1H, br t, 4 Hz, H-C1), 4.13 (1H, d, 7 Hz, H-C4), 3.72 (1H, dd, 4 and 9 Hz, H-C2), 2.44 (2H, q, 7 Hz, $C(O)CH_2$), 2.38 (2H, q, 7 Hz, $C(O)CH_2$) and 1.15 (2× 3H, 2× t, 7 Hz, CH_3).

*2.4.15. 3,7-Di-O-hexanoyl streptol (**3j**)*

^{1}H NMR ($CDCl_3$): δ 5.85 (1H, m, H-C6), 5.23 (1H, dd, 9 Hz, 6 Hz, H-C3), 4.78 (1H, d, 13 Hz, H_A-C7), 4.68 (1H, d, 13 Hz, H_B-C7), 4.29 (1H, t, 4 Hz, H-C1), 4.13 (1H, d, 7 Hz, H-C4), 3.74 (1H, dd, 4 and 9 Hz, H-C2), 2.40 (2H, t, 7 Hz, $C(O)CH_2$), 2.36 (2H, t, 7 Hz, $C(O)CH_2$), 1.63 (4H, m, 2× $C(O)CH_2CH_2$), 1.32 (8H, m, 2× $CH_2CH_2CH_3$) and 0.90 (6H, t, 7 Hz, 2× CH_3).

*2.4.16. 3,7-Di-O-(2-methylpropenoyl) streptol (**3k**)*

^{1}H NMR ($CDCl_3$): δ 6.16 (1H, m, =C*H*H), 5.87 (3H, m, H-C6), 5.62 (1H, m, =CH*H*), 5.31 (1H, dd, 9 Hz, 6 Hz, H-C3), 4.85 (1H, d, 13 Hz, H_A-C7), 4.78 (1H, d, 13 Hz, H_B-C7), 4.32 (1H, br t, 4 Hz, H-C1), 4.19 (1H, d, 7 Hz, H-C4), 3.83 (1H, dd, 4 and 9 Hz, H-C2) and 1.95 (3H, m, CH_3).

*2.4.17. 3,7-Di-O-^{t}butylbenzoyl streptol (**3l**)*

^{1}H NMR ($CDCl_3$): δ 8.00 (4H, m, Ph-H), 7.45 (4H, m, Ph-H), 6.01 (1H, m, H-C6), 5.52 (1H, dd, 9 Hz, 7 Hz, H-C3), 5.06 (1H, d, 14 Hz, H_A-C7), 4.94 (1H, d, 14 Hz, H_B-C7), 4.40 (1H, m, H-C1), 4.37 (1H, d, 7 Hz, H-C4), 3.98 (1H, dd, 4 and 9 Hz, H-C2) and 1.33 (18H, s, CH_3).

*2.4.18. 1,7-Di-O-crotonyl streptol (**4e**)*

^{1}H NMR ($CDCl_3$): δ 7.01 (2H, m, 2× =C*H*–CH_3), 5.90 (2H, m, COCH=), 5.85 (1H, m, H-C6), 5.49 (1H, m, H-C1), 4.91 (1H, d, 14 Hz, H_A-C7), 4.62 (1H, d, 14 Hz, H_B-C7), 4.11 (1H, d, 7 Hz, H-C4), 3.91 (1H, dd, 9 Hz, 7 Hz, H-C3), 3.76 (1H, dd, 4 and 9 Hz, H-C2), 1.80 (2× 3H, 2× s, 2× CH_3).

*2.4.19. 1,7-Di-O-benzoyl streptol (**4h**)*

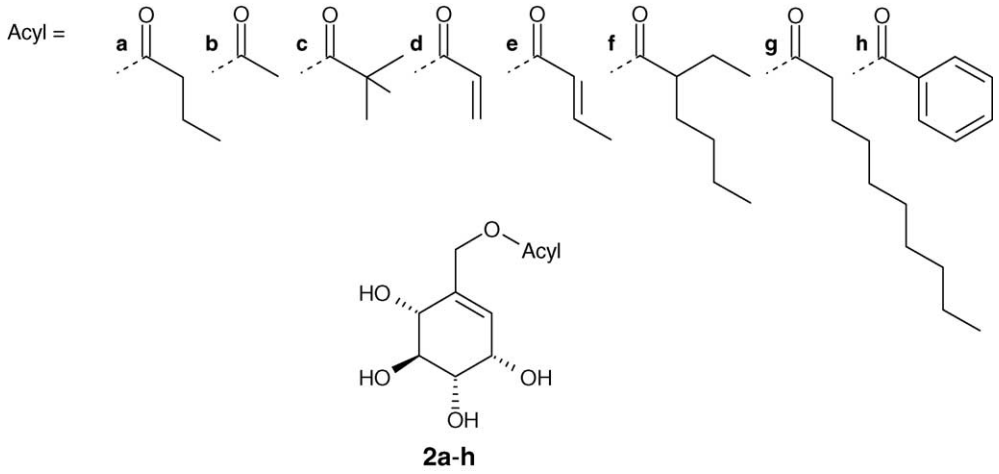
^{1}H NMR ($CDCl_3$): δ 8.06 (4H, m, Ph-H), 7.58 (2H, m, Ph-H), 7.43 (4H, m, Ph-H), 6.03 (1H, m, H-C6), 5.75 (1H, m, H-C1), 5.18 (1H, d, 14 Hz, H_A-C7), 4.82 (1H, d, 14 Hz, H_B-C7), 4.25 (1H, d, 7 Hz, H-C4), 4.10 (1H, dd, 9 Hz, 7 Hz, H-C3) and 3.90 (1H, dd, 4 and 9 Hz, H-C2).

*2.4.20. 1,7-Di-O-hexanoyl streptol (**4j**)*

^{1}H NMR ($CDCl_3$): δ 5.83 (1H, m, H-C6), 5.46 (1H, m, H-C1), 4.89 (1H, d, 14 Hz, H_A-C7), 4.56 (1H, d, 14 Hz, H_B-C7), 4.08 (1H, d, 7 Hz, H-C4), 3.87 (1H, dd, 9 Hz, 7 Hz, H-C3), 3.75 (1H, dd, 4 and 9 Hz, H-C2), 2.40 (2H, t, 7 Hz, $C(O)CH_2$), 2.34 (2H, t, 7 Hz, $C(O)CH_2$), 1.63 (4H, m, 2× $C(O)CH_2CH_2$), 1.32 (8H, m, 2× $CH_2CH_2CH_3$) and 0.90 (6H, t, 7 Hz, 2× CH_3).

*2.4.21. 1,7-Di-O-(2-methylpropenoyl) streptol (**4k**)*

^{1}H NMR ($CDCl_3$): δ 6.16 (1H, m, =C*H*H), 5.86 (3H, m, H-C6), 5.62 (1H, m, =CH*H*), 5.52 (1H, t, 4 Hz, H-C1), 4.94 (1H, d, 13 Hz, H_A-C7), 4.66 (1H, d, 13 Hz, H_B-C7), 4.14 (1H,



Scheme 1. Preparative synthesized 7-*O*-mono-substituted streptol derivatives using Novozym 243.

d, 7 Hz, H-C4), 3.93 (1H, dd, 9 Hz, 6 Hz, H-C3), 3.79 (1H, dd, 4 and 9 Hz, H-C2) and 1.93 (3H, m, CH_3).

*2.4.22. 1,7-Di-O-^{t}butylbenzoyl streptol (**4l**)*

^{1}H NMR ($CDCl_3$): δ 8.00 (4H, m, Ph-H), 7.45 (4H, m, Ph-H), 6.02 (1H, m, H-C6), 5.74 (1H, m, H-C1), 5.19 (1H, d, 14 Hz, H_A-C7), 4.81 (1H, d, 14 Hz, H_B-C7), 4.25 (1H, d, 7 Hz, H-C4), 4.11 (1H, dd, 9 Hz, 7 Hz, H-C3), 3.89 (1H, dd, 4 and 9 Hz, H-C2) and 1.33 (18H, s, CH_3).

3. Results and discussion

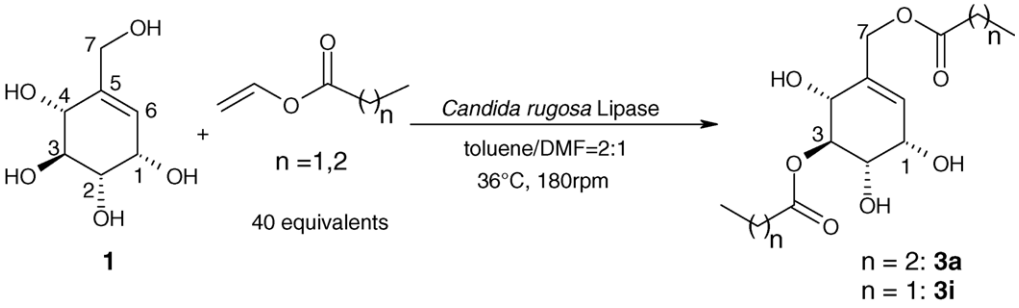
Transesterification of streptol (**1**) with vinyl butyrate employing commercially available protease Novozym 243 and vinyl butyrate (0.6 equiv.) as limiting acylation reagent in DMF/toluene afforded exclusively 7-*O*-butanoyl streptol (**2a**) without a trace of any other derivative. Using this method a number of 7-*O*-mono-substituted strepol esters (**2a**–**h**) (Scheme 1) were prepared in reasonable yields (41–80% yield).

In an enzyme screening with 40-fold excess of acyl donor involving 32 commercially available lipases and esterases, *Mucor javanicus* lipase was identified to catalyze exclusively the formation of the mono-substituted 7-*O*-butanoyl derivative **2a**, if vinyl butyrate was used as the acyl donor. However, this reaction was too slow to be used on a preparative scale.

Candida rugosa lipase (CRL) was found to catalyze exclusively the formation of 3,7-di-*O*-butanoyl streptol (**3a**) (Scheme 2), thus no other derivative was detected. All other investigated lipases and esterases led to a mixture of 1,7- and 3,7-di-*O*-substituted butanoyl derivatives, e.g. catalysis by *Chromobacterium viscosum* lipase yielded 65% 3,7-di-*O*-butanoyl (**3a**) and 35% 1,7-di-*O*-butanoyl streptol (**4a**).

Comparing the acylation of streptol (**1**) by *Candida rugosa* lipase, where the C3-hydroxyl group is preferentially acylated after C7 is acylated, with the acylation of a 6-protected glucose derivative [15], glucose is preferentially acylated at the C2-hydroxyl group by CRL. Shortening of the alkyl chain of the acyl donor from butyrate to propionate for the acylation of streptol with *Candida rugosa* lipase did not affect the absolute regioselectivity (Scheme 2, Table 1), however, employing other vinyl esters as acyl donors, resulted in a mixture of the 3,7-derivative **3** and the 1,7-derivative **4** (Scheme 3, Table 1).

Increasing the chain length and introducing C=C double bonds in the acyl donor caused a decrease in selectivity (acyl donors **d** and **e** in Table 1). Similar results were obtained by Rich et al. [16] for the esterification of sucrose when it was tried to elongate the alkyl chain of the acyl donor. They ex-

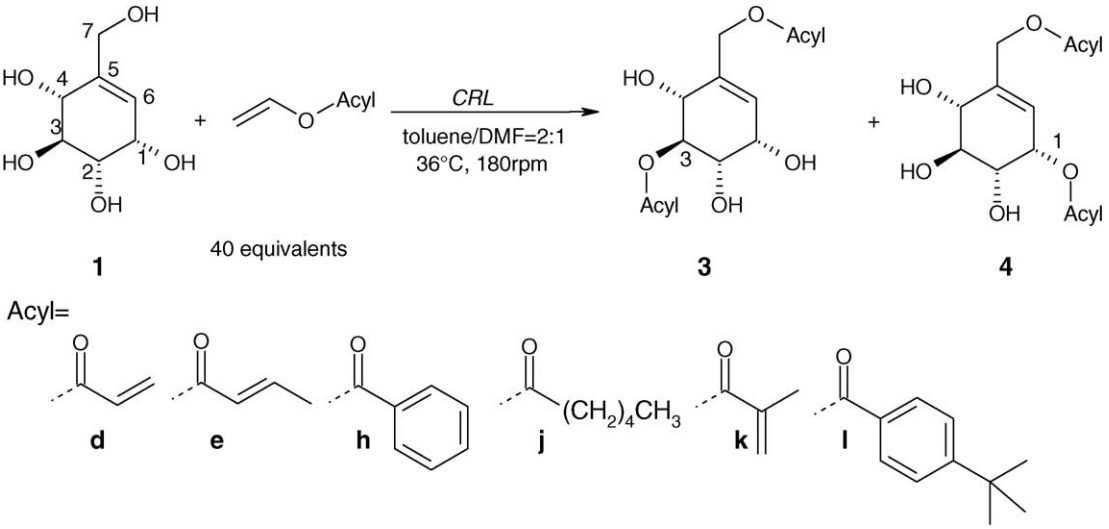


Scheme 2. Regioselective esterification of streptol (**1**) by *Candida rugosa* lipase.

Table 1
Results of the di-esterification of streptol with *Candida rugosa* lipase

Acyl group	3,7-*O*-derivative **3** (%)	1,7-*O*-derivative **4** (%)	Yield (%)	log *P*[a]	DM[a] (Debye)	MR[a]
a	100	0	68	1.084	1.52	30.333
i	100	0	46	1.481	1.554	25.732
d	90	6[b]	26	1.333	1.57	25.828
j	85	15	68	2.273	1.553	39.535
e	82	18	53	1.684	1.966	31.501
h	79	21	51	2.369	2.07	41.278
k	71	29	42	1.487	1.697	30.110
l	52	30[c]	39	3.996	2.538	59.945

[a] Values of the corresponding vinyl esters, DM (dipole moment) was calculated using Mopac93 computer program, MR (molar refractivity) and log *P* were calculated according to the methodology of Ghose and co-workers [25].
[b] +4% 1,3,7-*O*-derivative.
[c] The missing 18% result from undefined impurities.



Scheme 3. Esterification of streptol (**1**) with different acyl donors.

plained the phenomenon by a model, in which the bond angles between the resulting acyl-enzyme intermediate and sucrose affected the positioning of sucrose in the binding pocket. Another explanation was the different solvation of sucrose in different solvents. In our experiments we did not change the organic solvent, but only the acyl donor. The change in regioselectivity is therefore accounted to the acyl donor, since it was present in large excess and the polarity of the acyl donor affects the enzyme and the solvation of the substrate. If we take the log *P* and the dipole moment (DM) to describe the polarity of the vinyl esters and the molar refractivity (MR) for their molar volume and correlate it with the regioselectivity of the enzyme, a notable trend was observed. In the literature, such a correlation between log *P* of the solvent and the regioselectivity of the enzyme was only described for *Pseudomonas cepacia* [26] lipase. In the case of enantioselectivity, some authors claimed a correlation between enantioselectivity and the dielectric constant (ε) [27–29] and/or log *P* [27–30] or molar volume [31] of the solvent. Other experiments showed no correlation [32,33]. Carrea and co-workers demonstrated, that in no case any correlation with physico-chemical properties of the solvent (hydrophobicity, dielectric constant, dipole moment, normalized electron-pair-acceptance index and ability of the solvent to dissolve water or strip water from the enzymes) was present [29,34]. Other examples can be found in a review by Carrea et al. [35].

With some exceptions the log *P*, DM and MR increase in our experiments, while the regioselectivity decreases. In case of **3e** and **4e** the log *P* and MR have decreased from the previous row, but the DM has strongly increased. The same is true for **3d** and **4d**. However, the only exceptions are **3k** and **4k**, which do not fit in this concept. Nakamura et al. combined the molar volume and log *P* in a simple function to correlate to stereoselectivity [31]. Although a certain dependency in our experiments may be observed, no logic correlation satisfying all cases was found.

4. Conclusions

We have investigated the change of regioselectivity of *Candida rugosa* lipase for the esterification of streptol with

different acyl donors. No correlation between the regioselectivity and log *P*, DM and the molar volume of the acyl donor satisfying all cases was found. In general, a decrease of log *P*, DM and/or molar volume of the acyl donor resulted in an increase of the regioselectivity.

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References

[1] T. Ziegler, in: G.-J. Boons (Ed.), Carbohydrate Chemistry, Blackie, London, 1998, p. 21.
[2] J.M. Ballard, L. Hough, S.P. Phadnis, A.C. Richardson, Carbohydr. Res. 83 (1980) 138.
[3] T. Otake, Bull. Chem. Soc. Jpn. 43 (1970) 3199.
[4] D. Kadereit, H. Waldmann, Chem. Rev. 101 (2001) 3367.
[5] G. Carrea, S. Riva, Angew. Chem., Int. Ed. Engl. 39 (2000) 2226.
[6] K.M. Koeller, C.-H. Wong, Chem. Rev. 100 (2000) 4465.
[7] N.B. Bashir, S.J. Phytian, A.J. Reason, S.M. Roberts, J. Chem. Soc. Perkin Trans. 1 (1995) 2203.
[8] B. Danieli, M. Luisetti, G. Sampognaro, G. Carrea, S. Riva, J. Mol. Catal. B: Enzym. 3 (1997) 193.
[9] I. Ikeda, A.M. Klibanov, Biotechnol. Bioeng. 42 (1993) 788.
[10] S. Riva, J. Chopineau, A.P.G. Kieboom, A.M. Klibanov, J. Am. Chem. Soc. 110 (1988) 584.
[11] M. Therisod, A.M. Klibanov, J. Am. Chem. Soc. 108 (1986) 5638.
[12] S. Shibatani, M. Kitagawa, Y. Tokiwa, Biotechnol. Lett. 19 (1997) 511.
[13] H.G. Park, H.N. Chang, J.S. Dordick, Ann. N.Y. Acad. Sci. 799 (1996) 595.
[14] L. Cao, U.T. Bornscheuer, R.D. Schmid, J. Mol. Catal. B. Enzym. 6 (1999) 279.
[15] M. Therisod, A.M. Klibanov, J. Am. Chem. Soc. 109 (1987) 3977.
[16] J.O. Rich, B.A. Bedell, J.S. Dordick, Biotechnol. Bioeng. 45 (1995) 426.
[17] C. Bennett, J.S. Dordick, A.J. Hacking, P.S.J. Cheetham, Biotechnol. Bioeng. 39 (1992) 211.
[18] X. Chen, A. Johnson, J.S. Dordick, D.G. Rethwisch, Macromol. Chem. Phys. 195 (1994) 3567.
[19] D.R. Patil, D.G. Rethwisch, J.S. Dordick, Biotechnol. Bioeng. 37 (1991) 639.
[20] D.R. Patil, D.G. Rethwisch, J.S. Dordick, Macromolecules 24 (1991) 3462.
[21] J.S. Dordick, R.J. Linhardt, D.G. Rethwisch, Chemtech (1994) 33.
[22] B.D. Martin, S.A. Ampofo, R.J. Lindhardt, J.S. Dordick, Macromolecules 25 (1992) 7081.
[23] A. Isogai, S. Sakuda, J. Nakayama, S. Watanabe, A. Suzuki, Agric. Biol. Chem. 51 (1987) 2277.
[24] A. Isogai, S. Sakuda, J. Nakayama, M. Washizu, K. Shindou, S. Watanabe, A. Suzuki, Proc. Plant Growth Regul. Soc. Am. 14 (1987) 250.
[25] V.N. Viswanadhan, A.K. Ghose, G.R. Revankar, R.K. Robins, J. Chem. Inf. Comput. Sci. 29 (1989) 163.
[26] E. Rubio, A. Fernandez-Mayorales, A.M. Klibanov, J. Am. Chem. Soc. 113 (1991) 695.
[27] P.A. Fitzpatrick, A.M. Klibanov, J. Am. Chem. Soc. 113 (1991) 3166.
[28] D. Bianchi, A. Bosetti, P. Cesti, P. Golini, Tetrahedron Lett. 33 (1992) 3231.
[29] R. Bovara, G. Carrea, L. Ferrara, S. Riva, Tetrahedron: Asymm. 2 (1991) 931.
[30] F. Terradas, M. Teston-Henry, P.A. Fitzpatrick, A.M. Klibanov, J. Am. Chem. Soc. 115 (1993) 390.
[31] K. Nakamura, M. Kinoshita, A. Ohno, Tetrahedron 16 (1994) 4681.
[32] H. Kitaguchi, P.A. Fitzpatrick, J.E. Huber, A.M. Klibanov, J. Am. Chem. Soc. 111 (1989) 3094.
[33] J.F. Martins, M.N. da Ponte, S. Barreiros, Biotechnol. Bioeng. 42 (1993) 465.
[34] C. Baldoli, S. Maiorana, G. Carrea, S. Riva, Tetrahedron: Asymm. 4 (1993) 767.
[35] G. Carrea, G. Ottolina, S. Riva, Trends Biotechnol. 13 (1995) 63.