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Enzymatic preparation of homochiral 2-isobutyl succinic acid derivatives

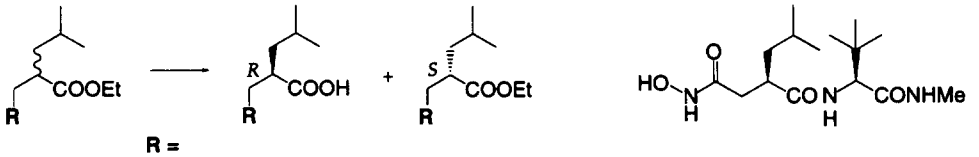
Beat Wirz [a,*] and Milan Soukup [b]

[a] Hoffmann-La Roche Ltd., Pharmaceutical Division, Preclinical Research, Dept. of Biotechnology, Basel, Switzerland

[b] Hoffmann-La Roche Ltd., Pharmaceutical Division, Preclinical Research, Dept. of Process Research, Basel, Switzerland

Abstract: An efficient enzymatic procedure for the enantio- and regioselective monohydrolysis of diethyl 2-isobutyl succinate **3** using subtilisin Carlsberg has been developed. The product (R)-2-isobutyl succinic acid 4-ethyl ester **4**, a collagenase inhibitor building block, was obtained in high enantiomeric excess (>99%) and yield (>45%). Similarly, (R)-2-isobutyl succinic acid 4-nitrile **2** was produced in 98% ee from its ethyl ester **1**.

In connection with a project devoted to the development of a practical synthesis of collagenase inhibitor **5**[1–3], a facile and inexpensive access to the (R)-acid building blocks **2** or **4** was expected to be provided by enzymatic racemic resolution of their esters. The enantioselectivity of enzymes towards 2-substituted succinic acid ester substrates has been reported to be strongly dependent upon the nature of the substituent and the ester group[4–9].



R =
1 -CN 2
3 -COOEt 4

5

A preliminary synthetic approach towards inhibitor **5** was based upon (R)-succinic acid mononitrile **2** as a chiral key intermediate[3]. In an enzyme screening for the enantioselective hydrolysis of the ethyl ester **1**, subtilisin Carlsberg showed reasonable activity and good enantioselectivity (Table 1, entry 1). To improve further the initial result, the influence of a few parameters on the reaction rate and enantioselectivity of the enzyme was studied. Among several cosolvents and salt additives tested, DMSO (Table 1, entry 2) and guanidinium chloride (Table 1, entry 3), respectively, had the most beneficial effects on both reactivity and selectivity. As DMSO was expected to interfere with work up, the use of guanidinium chloride was favoured. Low temperature (Table 1, entry 4) also improved enantioselectivity but at the expense of the reaction rate. A combination of guanidinium chloride (exhibiting its positive influence already at a concentration of 10 mM) at 0°C was finally chosen as a suitable system (Table 1, entry 5). At higher, technically more favoured substrate concentrations (10–16%)[10], however, the reaction slowed down considerably after 35–40% conversion.

A more straight-forward synthetic route towards **5** was later designed *via* (R)-2-isobutyl succinic acid 4-ethyl ester **4** which was obtained by enantio- and regioselective monohydrolysis of diester **3**. The substrate diester is readily accessible from the extremely cheap bulk agents isobutylene and maleic anhydride[2,3]. Again subtilisin Carlsberg showed excellent enantioselection and also the required positional preference for the sterically more hindered ester group. An experiment at 9% substrate

* Corresponding author. Email: beat.wirz@roche.com

Table 1. Influence of reaction conditions on the reaction rate and enantioselectivity of subtilisin Carlsberg[a]

#	cosolvent	salt (used instead of 0.1 M NaCl)	°C	time elapsed for 40% conv.	% ee of **2**
1	-	-	20	20 min	94
2	5% (v/v) DMSO	-	20	13 min	96
3	-	0.1 M guanidine HCl	20	14 min	96
4	-	-	1-3	29 min	98
5	-	10 mM guanidine HCl	0	21 min	98

a: **1** (100 mg) emulsified in 0.1 M NaCl, 4 mM phosphate buffer pH 7.5 (15.6 ml) was hydrolyzed by Alcalase 2.5 L (200 µl; Novo Nordisk). The pH was maintained by adding 0.1 N NaOH (pH-stat).

concentration[11,13] afforded halfester **4** in 99% ee and 47% yield. Less than 1% of the undesired diacid was formed, and the alternative monoacid could be, if at all, observed only in $\ll$0.2% (GC, silylated). Extending the substrate concentration to 20% did not affect the stereoselectivity of the enzyme but the reaction again became rather slow, especially after about 35% conversion. The *dimethyl* ester was also tested but gave inferior results with respect to reaction rate and stereoselectivity. The reaction products were separated by means of extraction[11,13]. After some minor modification, the reaction was carried out on the multi-kg-scale.

The present resolution is particularly attractive because it allows easy racemization of the unwanted (S)-diester back to its racemic precursor **3** by distillation in the presence of a catalytic amount of NaOEt (1%) which does not represent an additional step. Furthermore, subtilisin Carlsberg is an extremely cheap bulk protease and therefore does not need to be immobilized for reuse. The configurations of the (R)-acids **2** and **4** were assigned by their conversion to the final product[3].

A more direct approach towards **5** would be the enzyme-catalyzed enantioselective aminolysis of the esters **1** or **3** with (S)-tLeu-NHMe or, assuming double enantioselection, even with (RS)-tLeu-NHMe. Orientating experiments carried out in eutectic systems[12] in the presence and absence of various additives using solid or liquid Alcalase (Novo Nordisk) were unsuccessful: in *anhydrous* systems, no activity was observed, whereas in aqueous systems aminolysis did occur to some minor extent but hydrolysis was the dominating pathway. Lipases also tested for aminolytic activity did not give positive results either.

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10. Crude ester **1** (100 g, 96% GC) was emulsified in 35 mM guanidinium chloride, 1 mM sodium phosphate pH 7.5 (700 ml) with vigorous stirring (pH readjusted). Hydrolysis was effected with 10 ml Alcalase 2.5 L DX (Novo, Denmark) at pH 7.5 (pH-stat) at 1–2°C. After consumption of 94 ml of 2 N NaOH (35% conversion), the reaction mixture was washed with ethyl acetate (3×1 l).

The aqueous phase was acidified to pH 2 and after extraction with ethyl acetate (2x1 l) provided 2 (28.8 g; ~35%) as a slightly brownish oil. Analysis: 97.3% GC; 98ee[13]; IR (neat): 2250, 1713 cm^{-1}; EI-MS: e/m 140 ($M-CH_3$); 250 Mhz-^{1}H-NMR ($CDCl_3$): δ 0.96 (t, 6H), 1.52 (m, 1H), 1.71 (m, 2H), 2.53–2.72 (ddd, 2H), 2.84 (m, 1H).

11. Diester **3** (256 g, 1.11 mol) was emulsified in 10 mM sodium phosphate pH 8.5 (2.5 l) with vigorous stirring (pH readjusted). Hydrolysis was carried out at r.t. and pH 8.5 (2 N NaOH) using 12 ml of Protease L-660 (Solvay Enzymes, Germany). After 48.7% conversion (47 h) the reaction mixture was washed with ethyl acetate (3×1.5 l), acidified to pH 2 (25% HCl) and extracted with ethyl acetate (2×1.5 l). Monoacid 4 (105.5 g, 522 mmol, 47%) was obtained as a colourless oil. Analysis: 98.5% GC (0.7% diacid; silylated); 99% ee[13]; $[\alpha]_d$=+19.1 (1%, EtOH), $[\alpha]_{365}$=+62.9 (1%, EtOH); IR (neat): 1738, 1710 cm^{-1}; EI-MS: e/m 184 ($M-H_2O$); 250 MHz-^{1}H-NMR ($CDCl_3$): δ 0.91 (d, 3H), 0.94 (d, 3H), 1.25 (t, 3H) interferred by 1.32 (m, 1H), 1.63 (m, 2H), 2.39–2.73 (ddd, 2H), 2.92 (m, 1H), 4.15 (q, 2H).
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13. The enantiomeric excess of monoacid **2** (methylated) and **4** was determined by means of a chiral-phase GC capillary column (OV-61/permethylated β-cyclodextrin; 25 m; H_2 at 50cm/sec; 80–130°C at 1°C.min^{-1} for **2** and 120–150°C at 0.5°C.min^{-1} for **4**; injector: 210°C).

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