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Enantio- and Regioselective Monohydrolysis of Diethyl 2-Ethoxysuccinate.

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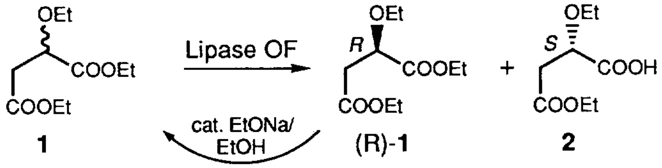
Abstract: A procedure for the synthesis of (S)-ethyl 2-ethoxy-4-succinate **2** in 95% e.e. using a lipase from *Candida rugosa* was developed. The enzymatic reaction was highly selective for the *secondary* ester group and provided the desired (S)-monoacid in 40% yield. The unchanged (R)-diester was readily racemized and recycled.

In connection with a project devoted to the development of an efficient synthesis of chiral pyrrolidines, a relatively facile and inexpensive access to suitable chiral precursors was expected to be provided by the enzymatic[1-3] resolution of the racemic diester **1**[4].



The enantioselectivity of the enzymatic hydrolysis of 2-substituted succinic acid diester substrates was reported to be strongly dependant on the nature of the substituent and the ester group[1]. In a preliminary screening of various commercial esterolytic enzymes for the enantioselective hydrolysis of rac-**1**, two enzymes were found to exhibit reasonable enantioselectivity and almost exclusive positional selectivity for the secondary ester: Lipase OF from *Candida rugosa* (formerly *C. cylindracea*)[5] cleaved predominantly the (S)-diester to form the acid **2** (68% e.e.; Table, entry 1) whereas α-chymotrypsin (α-CT) operated with opposite selectivity and provided the (S)-diester **1**. As the former lipase showed much higher activity and also tolerated higher substrate concentrations than α-CT (cf. ref. 6), it was selected for further optimization.

Enantioselectivity was improved upon dissolving the substrate in a water-immiscible cosolvent. With respect to both enantioselectivity *and* reaction rate, the most suitable solvent was clearly cyclohexane[7] (entry 2). At technically more favoured substrate concentrations (~10%), a significant deterioration in the enantioselectivity was noticed (entry 3). A concentration of ~5% was then chosen as an acceptable upper limit (entry 4). Employing LiSCN[8] rather than NaCl as buffer electrolyte enhanced the reaction rate only by a factor of 1.5. As is often observed, lowering the reaction temperature had a beneficial influence on the selectivity of the enzyme (entry 5) but was limited by the freezing point of the system (<3 °C). Intriguingly, both reaction rate and enantioselectivity decreased on raising the pH from 6.5 to 8.5. Similarly, the enzyme concentration played an important role and could not be taken lower than 1% w/w with respect to **1**. Presumably as the amount of enzyme is reduced (together with a concomittant extension of the reaction time), the process becomes increasingly thermodynamically controlled. In preparative experiments using 1-2% enzyme at temperatures of 3-5 °C and a conversion degree of ~40%, acid **2** was secured in 94-96% e.e. and 37-40% yield (entry 6)[9]. This enantiomeric excess was sufficiently high to be further enriched to 99.5% e.e. at a later stage of the subsequent reaction sequence. Since the undesired (R)-diester **1** could be easily racemized and recycled into the initial preparation of (rac)-**1**[4], the overall efficiency of the enzymatic step could be significantly improved.

Table: Hydrolysis of **1** with Lipase OF: optimization of reaction parameters[a].

entry	diester **1** overall conc. % (w/v)	ratio of c-hexane/buffer	pH	ratio of **1** / enzyme	temp. °C	conv. (%)	**2** % ee
1	0.4	0	7.0	20	20	50	68
2	0.4	0.12	7.0	20	20	50	90
3	7.4	1.0	7.0	20	20	45	77
4	4.8	1.0	7.0	20	20	45	86
5	4.8	1.0	7.0	20	1	45	90
6	4.8	1.0	6.5	100	3-4	40	94

a: reaction procedure according to ref. 9, except that 0.1 N NaOH was used in exp. 1 and 2.

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3. M. Soukup & B. Wirz, European Patent Application No. 94100852.6 (January 21, **1994**).
4. Diester **1** is readily prepared by base catalyzed conjugate addition of EtOH to diethyl maleate: M. Paci, V. Crescenzi & N. Supino, *Makromol. Chem.* **1982**, 183, 377-387.
5. Other lipases from *C. cylindracea* (Lipase MY from Meito Sangyo, Japan, and Lipase AY from Amano, Japan) exhibited similar specificities but showed much lower activity.
6. Diester **1** (35 g) emulsified in 20 mM $CaCl_2$, 4 mM sodium phosphate buffer pH 8.0 (3.5 l) and hydrolyzed with α-CT (3.0 g; Fluka) at 0 °C (1.0 N NaOH, 62% conversion, 41 h) gave (S)-**1** (13.0 g; 37%) in 94% e.e.
7. Other biphasic cosolvents tested were Et_2O, TBME, iPr_2O, toluene, n-pentane, n-hexane, n-heptane, i-octane, methylcyclohexane and decalin.
8. c.f. B. Wirz, R. Schmid & W. Walther, *Biocatalysis* **1990**, 3, 159-167.
9. A solution of **1** (100 g, 458 mmol) in c-hexane (1 l) was emulsified in 0.1 M NaCl, 10 mM sodium phosphate (1 l) by vigorous stirring. Hydrolysis was effected with Lipase OF 360 (2.0 g; Meito Sangyo) at pH 6.5 at 3-4 °C. After consumption of 90 ml 2.0 N NaOH (2.1 h; 39% conversion) the reaction was terminated by extracting **1** into EtOAc (3x1 l). The aqueous phase was acidified to pH 1.9 and extracted with EtOAc (5x1 l). From the combined organic phases was obtained acid **2** (33.7 g, 177 mmol; 39%) as an opalescent hygroscopic pale yellow oil in 99.7% purity (GC) and 95.8% e.e., $[\alpha]_D = -46.0$ (1% in EtOH). Usually **2** contained <0.2% of the corresponding undesired regioisomeric monoacid.

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