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# SIMULTANEOUS SEPARATION OF ENANTIOMERS OF DIASTEREOMERS BY LIPASES

**H.Hönig*, P. Seufer-Wasserthal and H. Weber**
**Institute of Organic Chemistry, Graz University of Technology**
**A-8010 Graz, Stremayrgasse 16, Austria**

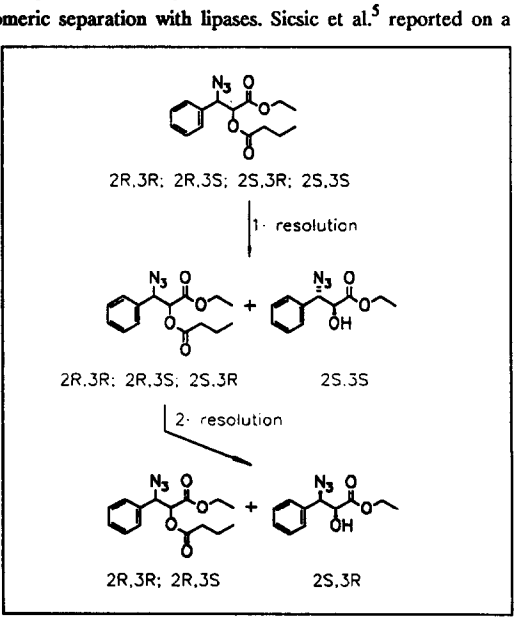
**Abstract:** Enantiomerically and diastereomerically pure 3-azido-2-hydroxy-3-phenyl propanoates are obtained from a mixture of racemic *threo*- and *erythro*-3-azido-2-butanoyloxy-3-phenyl propanoates by asymmetric hydrolysis with lipases.

Enzymatic resolution of diastereomerically pure educts is a well established procedure for the preparation of enantiomerically pure products.[1] In our continuing efforts to prepare rare amino acids in optical pure form by enzymatic methods, we chose 3-phenylserines and 3-phenylisoserines as model substrates for our approach to the class of hydroxy amino acids.[2] Recently we reported on the syntheses of enantiomerically pure 3-phenylisoserines by enzymatic hydrolyses of diastereomerically pure 3-azido-2-butanoyloxy-3-phenyl propanoates and subsequent hydrogenation of the products obtained.[3]

These syntheses involve tedious separations (distillations) of a mixture of *cis/trans* ethyl phenyl glycidates, which serve as starting materials for the preparation of 3-azido-2-hydroxy-3-phenyl propanoates, because mixtures of both *threo/erythro*--2-azido-3-hydroxy-3-phenyl propanoates and *threo/erythro*-3-azido-2-hydroxy-3-phenyl propanoates cannot be separated by column chromatography.

Here we want to describe the separation of (2S,3S)-3-azido-2-hydroxy-3-phenyl ethyl propanoate and (2*S*,3*R*)-3-azido -2-hydroxy-3-phenyl ethyl propanoate from a diastereomeric mixture of racemic *threo/erythro*-3-azido-2-butanoyloxy-3-phenyl propanoates[4] by enzymatic resolution with lipases from *Pseudomonas* sp. and *Candida cylindracea*.

Only few literature examples are available on the diastereomeric separation with lipases. Sicsic et al.[5] reported on a separation of diastereomeric norbornenyl esters by enzymatic resolution with pig liver esterase, but without enantiodifferentiation. Chenevert and Letourneau[6] found that *erythro*-N-acetyl p-nitrophenylserinates can be resolved by enzymatic resolution with $\alpha$-chymotrypsin, whereas *threo*-N-acetyl p-nitrophenylserinates were not hydrolyzed at all. In our case the *threo*-3-azido-2-butanoyloxy-3-phenylpropanoate worked in the enzymatic resolution with lipases as well, but about 20 times slower than the *erythro*-isomer. So it was possible to hydrolyze the (2*S*,3*S*)-isomer in the first resolution step together with only negligible amounts of the (2*S*,3*R*)-3-azido-2-hydroxy--3-phenyl ethyl propanoate. After chromatographic separation of the (2S,3S)-alcohol the remaining ester now containing the (2*R*,3*R*)-, the (2*R*,3*S*)- and the (2*S*,3*R*)-isomer was submitted to a further resolution. Thus the (2*S*,3*R*)-alcohol could be obtained after chromatographic purification. It was very important to determine the ratios of the isomers by e.g. $^1$H-NMR prior to hydrolysis in order to know when the hydrolysis had to be stopped. Longer reaction times in the first resolution step decreased the diastereomeric excess (*de*) and enantiomeric



excess (*ee*) of the (2*S*,3*S*)-isomer, while increasing the *de* and the *ee* of the (2*S*,3*R*)-isomer, which is obtained in the second step (and *vice versa*). Results of the hydrolyses are shown in table 1 and 2.

| | | | *erythro*-alcohol | | | | | | remaining ester | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| experiment | starting ratio[b] | time | conv.[c] | yield[d] | *de*[b] | *ee*[e] | config. | $[\alpha]_D^{20}$ [f] | yield[d] | ratio[b] | isomers | $[\alpha]_D^{20}$ [f] |
| No. | *threo/erythro* | h | % | % | % | % | | | % | *threo/erythro* | | |
| 1 | 80:20[g] | 3 | 10 | 9 | 93 | 59 | 2S,3S | +50.3 | 86 | 87:13 | 2*R*,3*R*; 2*S*,3*R*;2*R*,3*S* | -6.8 |
| 2 | 34:66 | 3 | 32 | 29 | 98 | 98 | 2S,3S | +85.6 | 64 | 50:50 | 2*R*,3*R*; 2*S*,3*R*;2*R*,3*S* | -36.0 |

Table 1: Results of the first resolution[a]

| | | | *threo*-alcohol | | | | | | remaining ester | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| experiment | starting ratio[b] | time | conv.[c] | yield[d] | *de*[b] | *ee*[e] | config. | $[\alpha]_D^{20}$ [f] | yield[d] | ratio[b] | isomers | $[\alpha]_D^{20}$ [f] |
| No. | *threo/erythro* | h | % | % | % | % | | | % | *threo/erythro* | | |
| 1 | 87:13 | 64 | 39 | 27 | 97 | 82[h] | 2S,3R | -108.9 | 60 | 82:18 | 2*R*,3*R*; 2*R*,3*S* | +20.7 |
| 2 | 50:50 | 65 | 20 | 17 | 90 | 94 | 2S,3R | -124.5 | 75 | 44:56 | 2*R*,3*R*; 2*R*,3*S* | -29.0 |

Table 2: Results of the second resolution[a]

[a] All reactions carried out in 100 ml 0.1M phosphate buffer at pH = 7.00, 1.00g substrate, 0.30 g enzyme (Lipase P from Amano, except were indicated), titration with 1N NaOH; [b] determined by $^1$H-NMR (integration of the protons at C2 and C3); [c] measured by consumption of 1N NaOH; [d] isolated yield; [e] measured by $^1$H-NMR and $^{19}$F-NMR of the respective MTPA-esters; [f] c = 2,$CH_2Cl_2$; [g] prepared in the same way as described in ref.4, but the ethyl phenyl glycidate was distilled twice; [h] Candida cylindracea (CC) at pH 7.00 was used instead of Pseudomonas, CC gives less enantiodifferentiation in that case.

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4. This mixture is prepared as follows: Darzens reaction of benzaldehyde and ethyl chloroacetate in DMF with *t*-BuOK[7] results in a mixture of *cis/trans*-ethyl phenyl glycidates in ratios from *cis/trans* = 60:40 to 30:70 depending on the reaction time and the temperature applied. Epoxide opening with azide anion[8] and subsequent acylation[9] yields the mixture mentioned above, with the *threo/erythro*-ratios indicated in table 1 and 2. NMR-data and physical constants of all products were identical to those give in ref.3b.
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