Tetrahedron Letters, Vol.31, No.21, pp 3011-3012, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

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

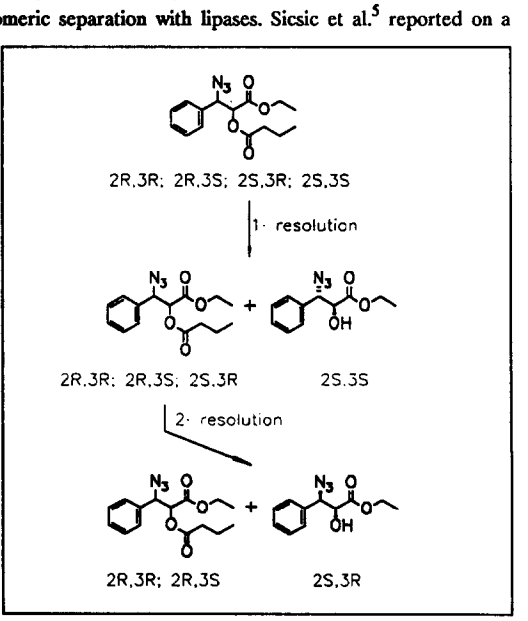
Abstract: Enantiomerically and diastereomerically pure 3-azido-2-hydroxy-3-phenyl propanoates are obtained from a mixture of racemic *threo*- and *erythro*-3-azido-2-butanoyloxy-3-phenyl propanoates by asymmetric hydrolysis with lipases.

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 α-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.

Literature and remarks:

1. (a) Porter R.; Clark S. (Eds), "*Enzymes in Organic Synthesis*", Pitman, London, 1985; (b) Tramper J.; van der Plas H.C.; Linko P. (Eds), "*Biocatalysts in Organic Synthesis*", Elsevier, Amsterdam, 1985; (c) Schneider M. (Ed), "*Enzymes as Catalysts in Organic Synthesis*", Reidel, Dortrecht, 1986; (d) Whitesides G.M.; Wong C.-H., *Angew. Chem.*, **1985**, *97*, 617; *Angew. Chem. Int. Ed. Eng.*, **1985**, *24*, 617; (e) Simon H.; Bader J.; Günther H.; Neumann S.; Thanos, J. *Angew. Chem.*, **1985**, *97*, 541; *Angew. Chem. Int. Ed. Eng.*, **1985**, *24*, 539; (f) Jones J.B., *Tetrahedron*, **1986**, *42*, 3351; (g) Wong C.-H. *Science*, **1989**, *244*, 1145; (h) Butt S.; Roberts S.M. *Chem. Br.*, **1987**, 127; (i) Klibanov A.M., *Chemtech*, **1986**, 354.
2. Part of the Ph.D.thesis of H.Weber.
3. (a)Hönig, H.; Seufer-Wasserthal, P.; Weber, H., in: "*Aminoacids: Chemistry, Biology and Medicine*", Lubec G.; Rosenthal G.A. (Eds), ESCOM Science Publishers B.V., Leiden, 1990, in press; (b)Hönig, H.; Seufer-Wasserthal, P.; Weber, H., *Tetrahedron*, **1990**, *46*, submitted.
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.
5. Sicsic, S.; Leroy, J,; Wakselman, C. *Synthesis* **1987**, 155-157.
6. Chenevert, R.; Letourneau, M., *Chem.Lett.* **1986**, 1151-1154.
7. Kamandi, E.; Frahm, A.W.; Zymalkowski, F., *Arch.Pharm.* **1974**,*307*,871.
8. Legters, J.; Thijs, L.; Zwanenburg, B., *Tetrahedron Lett.* **1989**,*30*,4881.
9. Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem.*, **1978**, *90*, 602; *Angew. Chem. Int. Ed. Eng.*, **1978**, *17*, 569.

(Received in Germany 7 March 1990)