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CHEMO-ENZYMATIC SYNTHESIS OF ALL ISOMERIC 3-PHENYLSERINES AND -ISOSERINES

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

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Abstract: The synthesis of all isomers of 3-phenylserines and 3-phenylisoserines in enantiomerically pure form is presented. Diastereomerically pure educts (*threo/erythro*-2-azido-3-butanoyloxy--3-phenyl-propionic esters, *threo/erythro*-3-azido-2-butanoyloxy-3-phenylpropionic esters, *threo*--2-butanoylamino-3-butanoyloxy-3-phenylpropionic ester, *erythro*-3-butanoylamino-2-butanoyloxy-3-phenyl-propionamide) were prepared from cinnamic acid derivatives or via aldol condensations of benzaldehyde and suitable enolates in few steps. These racemates were resolved with lipases from *Candida cylindracea* (CC) and *Pseudomonas fluorescens* (P) and the obtained products were hydrogenated to 3-phenylserines and -isoserines. The influence of the acyl group in the enzymatic resolution of *erythro*-3-azido-2-acyloxy-3-phenylpropionic esters was investigated.

INTRODUCTION

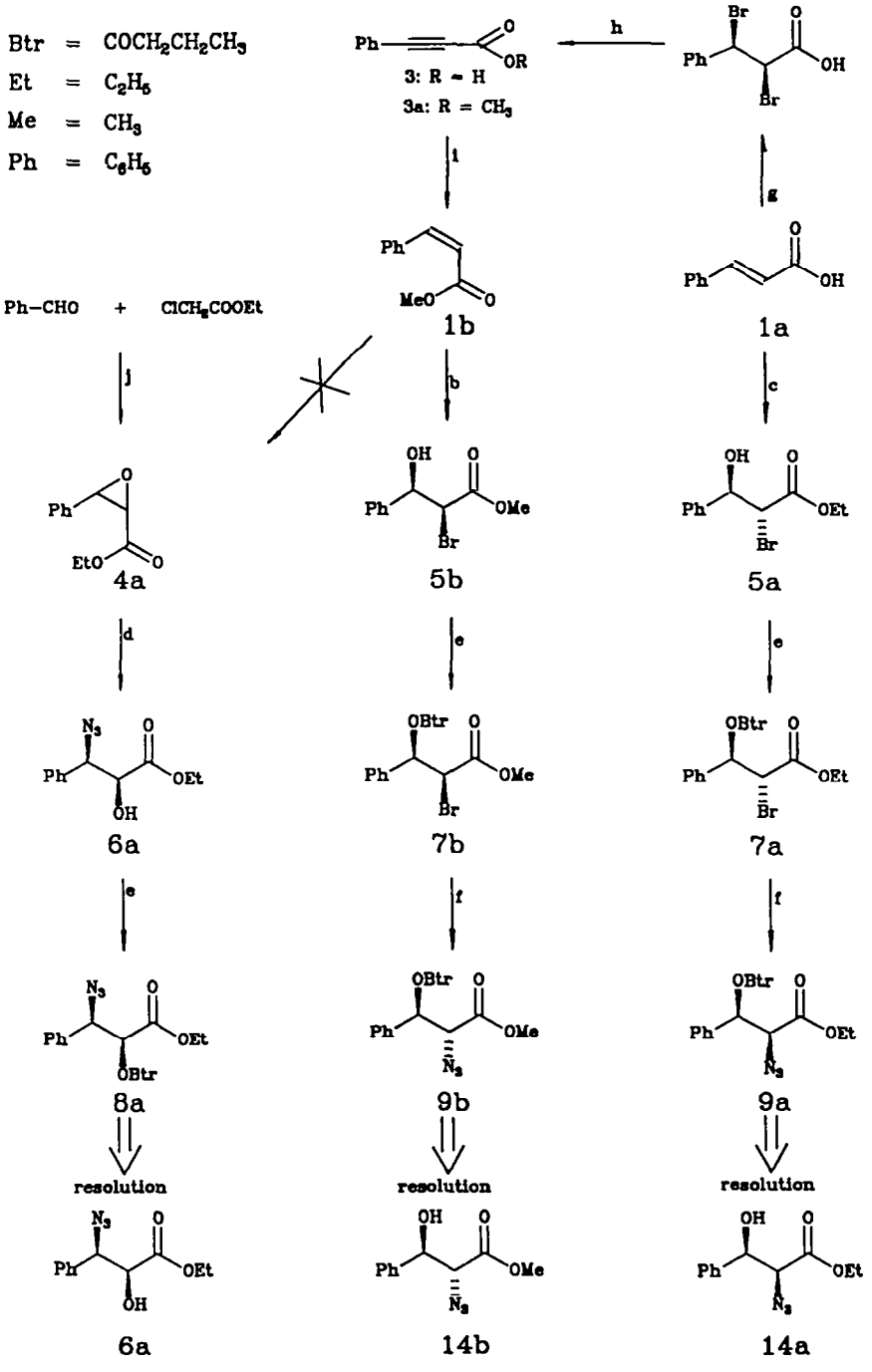
In recent years enzymatic hydrolysis has increasingly been used for the optical resolution of several functionalized chiral molecules such as amino acids, lactones, diesters and hydroxy acids[1]. In the cyclic[2] and acyclic series[3] of simple azido alcohols, which are suitable precursors for amino alcohols, we have shown, that enzymatic hydrolyses of the respective butyrates proceed with acceptable conversion rates and in excellent optical yields. Since it is known, that introduction of a carboxylic acid functionality in molecules - which otherwise give moderate optical yields - often improves the resolution[4], the application of our methodology to the preparation of hydroxy amino acids via azido alcohol precursors should be very promising.

There are several rare natural hydroxy amino acids of biological interest, most of them occurring as part of molecules with distinct biological effects. Bestatin, N-[(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine and analogs thereof are known to exhibit strong aminopeptidase B inhibitory effects and act as useful immunomodulators in cancer treatment[5]. Statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid is part of the natural peptide pepstatin (Iva-Val-Val-Sta-Ala-Sta). Incorporation of statine and analogs thereof into appropriate peptide sequences has led to the discovery of potent human renin inhibitors[6]. Moreover the (3*S*,4*R*)-diastereomer has received considerable attention as a key component of the didemnins, compounds with significant antitumor and antiviral activity[7]. Pepstatin and statine itself can act as inhibitors of the human immune deficiency virus-1 protease[8]. ß-Hydroxyhistidine as part of the bleomycins is essential for the complexation of iron and copper ions, important for the activation of oxygen, which in turn leads to their useful activity against squamous cell carcinomas and malignant lymphomas, including Hodgkin's disease[9].

We have chosen precursors of ß-phenylserine and ß-phenylisoserine to test our methodology for the enzymatic resolution of hydroxy azido esters. ß-Phenylserine and -isoserine themselves exhibit some distinct biological effects, like inhibition of growth of influenza viruses[10] as well as reducing the death rate of rats infected by rabies[11]. Moreover the precursors for these products can be prepared very easily, and therefore are especially well suited as a test series for our synthetic approach to the class of hydroxy amino acids.

Many syntheses of ß-phenylserine and -isoserine have been published, which give the acids in high diastereomerical purity[12]. There are fewer reports on the synthesis of enantiomerically pure ß-phenylserine and -isoserine, among them only one on the resolution of racemates with enzymes. *Fones* resolved racemic *erythro*- and racemic *threo*-phenylserine by the hydrolysis of the

SCHEME 1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Et = C2H5
Me = CH3
Ph = C6H5
3: R = H
3a: R = CH3
h
i
g
1b
1a
Ph–CHO + ClCH2COOEt
j
b
c
4a
5b
5a
d
e
e
6a
7b
7a
e
f
f
8a
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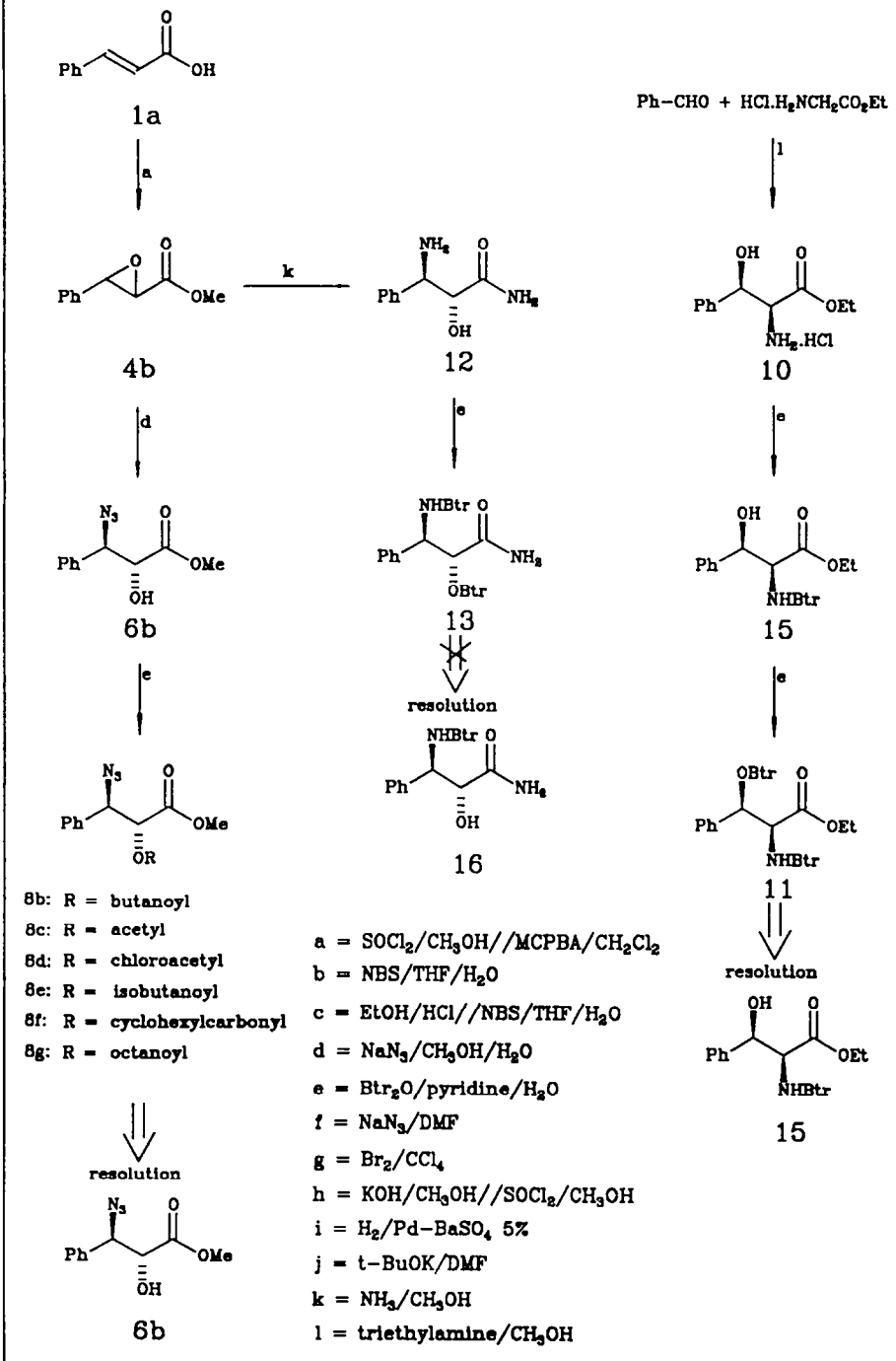
9a
resolution
resolution
resolution
6a
14b
14a

SCHEME 2


1a
4b
12
10
6b
13
15
16
11
15
6b
Ph–CHO + HCl.H2NCH2CO2Et
resolution
resolution
8b: R = butanoyl
8c: R = acetyl
8d: R = chloroacetyl
8e: R = isobutanoyl
8f: R = cyclohexylcarbonyl
8g: R = octanoyl
a = SOCl2/CH3OH//MCPBA/CH2Cl2
b = NBS/THF/H2O
c = EtOH/HCl//NBS/THF/H2O
d = NaN3/CH3OH/H2O
e = Btr2O/pyridine/H2O
f = NaN3/DMF
g = Br2/CCl4
h = KOH/CH3OH//SOCl2/CH3OH
i = H2/Pd–BaSO4 5%
j = t-BuOK/DMF
k = NH3/CH3OH
l = triethylamine/CH3OH

respective N-trifluoracetyl derivatives with proteases[13]. Other authors describe the syntheses of some of the enantiomerically pure acids via enantioselective aldol condensations[14], or openings of the respective enantiomerically pure epoxides[15].

Our synthetic approach is outlined in schemes 1 and 2.

RESULTS AND DISCUSSION

Synthesis of the azido alcohol derivatives as starting materials for enzymatic resolution:

3-Azido-2-butanoyloxy-3-phenyl-propionic acid esters **8a** and **8b** easily could be obtained by ring openings of the respective epoxides **4a** and **4b**[15c] with azide ion, and acylation of the resulting azido alcohols 6a and **6b** by standard procedures[16]. The *cis*-epoxide **4a** could not be synthesized in pure form by oxidation of the (*Z*)-methyl cinnamate **1b** with m-chloroperbenzoic acid analogous to the formation of the *trans*-epoxide **4b**, because partial isomerization to **4b** occurred in the oxidation step. Hence **4a** was prepared by condensation of benzaldehyde and ethyl chloroacetate with potassium *tert*-butoxide in DMF[12a], and subsequent purification by distillation. (*Z*)-methyl cinnamate **1b** was prepared via bromination of **1a** with bromine in tetrachloromethane[17], conversion of the resulting bromide **2** to phenylpropiolic acid **3** with KOH in methanol[18], esterification of **3** with thionyl chloride in methanol to the ester **3a**, and partial hydrogenation thereof. Many different catalysts were tested in the reduction to pure (*Z*)-methyl cinnamate and the system $Pd/BaSO_4$ (5%) with a small amount of pyridine[19] proved to be best. Other catalysts[20] (Lindlar catalyst, FLUKA; Pd/charcoal/$PbOAc_2$/quinoline[21], $Pd/BaSO_4$/quinoline) gave either high amounts of (*E*)-methyl cinnamate or very low reaction rates. Formation of methyl-3-phenylpropanoate was negligible.

Attempted preparation of the azidoalcohols **14a** and **14b** via aldol condensation of benzaldehyde and ethyl azidoacetate[22] led to a mixture of the two diastereomers, with a slight preponderance of the *threo*-isomer **14a**. Neither variations in temperature or solvent nor the use of different catalysis resulted in favorable *threo/erythro*-ratios. Only the formation of ethyl 2-azido cinnamate reported in ref.[22] was reduced by employing lower temperatures (-20°C) and very short reaction times (10'). Since the mixture thus obtained could not be resolved by chromatographic separation or by distillation, alternative strategies for the synthesis of **14a** and **14b** had to be found: As mentioned above, direct opening of the epoxides **4a** and **4b** leads to the 3-azido-2-hydroxy-3-phenyl-propionic acid esters **6a** and **6b**. Therefore, hydrobromination of the cinnamic acid esters **1a** and **1b** was used to prepare 2-bromo-3-hydroxy-3-phenyl-propionic esters **5a** and **5b** in high diastereomerical purity[23]. Contrary to the literature[23], **5a** and **5b** did not yield azidoalcohols **14a** and **14b** by treatment of the bromohydrins with NaN_3 in DMF, but the epoxides **4a** and **4b** together with the azidoalcohols **6a** and **6b**. The occurrence of **6a** and **6b** can be explained by the intermediate formation of epoxides **4a** and **4b**, and subsequent ring opening with azide anion. Other solvents (acetone, toluene/HMPTA, butanone) and LiN_3 instead of NaN_3 gave similiar results. Therefore, esterification of the bromohydrins **5a** or **5b** was done prior to azide exchange. This led to the diastereomerically pure 2-azido-3-butanoyloxy-3-phenyl-propionic acid esters **9a** and **9b** in high yields.

Synthesis of the protected phenylserine- and phenylisoserine-derivatives:

Preparation of **11** was attained by a modified condensation of benzaldehyde with H-Gly-OEt.HCl analogous to ref.[24] yielding the *threo*-phenylserine-derivative **10**, which on subsequent acylation[16] gave the hitherto unknown protected derivative **11**. The described condensation[25] of benzaldehyde with Z-Gly-OEt (Z = carboxybenzyl) to the Z-protected *threo*-2-amino -isomer of **10** could easily be reproduced, whereas the proposed synthetic strategy to the respective *erythro*-isomer via condensation of benzaldehyde and tris(trimethylsilyl)glycinate[25] in our hands did not work at all.

The *erythro*-3-amino derivative **13** was prepared by ammonolysis[12b] of methyl phenyl glycidate **4b** to **12**, and subsequent acylation to the hitherto unknown **13**.

Enzymatic hydrolyses of the protected phenylserine- and phenylisoserine-derivatives:

The easily available N,O-diacylderivative **11** was hydrolyzed by lipase from *Candida cylindracea* (CC) only and showed only very small reaction rates. The very slow reaction could be explained by the insolubility of the substrate in the reaction medium. Better results were obtained, suspending the substrate in the buffer solution by applying ultrasound. An increase of the

enantiomeric excess (e.e.), together with a decrease of conversion rate was obtained, when the hydrolysis was performed in a buffer solution with 10 % DMF. Phenylisoserine derivative **13** was not hydrolyzed at all.

Attempted enzyme catalyzed esterification of phenylisoserinamide **12** and phenylserine derivative **15** with vinyl acetate in the presence of several different lipases gave no satisfactory results.

Enzymatic hydrolyses of azido alcohol derivatives:

To find a suitable acyl group to be hydrolyzed, esters **8b-g** were prepared and resolved with lipases from CC and P[26]. All of them were derivatives of azidoester **6b**. The results of the enzymatic resolutions are shown in table 1.

		Lipase CC				Lipase P			
substrate	acyl group	time,h[a]	c,%[b]	ee,%	E[c]	time,h[a]	c,%[b]	ee,%	E[c]
8b	butyrate	6	40	67	8	1	40	98	100
8c	acetate	50	25	85	16	1	40	97	100
8d	chloroacetate	1	50	2	1	1	40	86	13
8e	isobutyrate	200	24	84	15	100	40	98	100
8f	cyclohexylcarboxylate	200	27	72	8	100	27	93	39
8g	octanoate	3	40	36	3	1	40	85	12

[a] time for 40 % conversion; [b] conversion; [c] enantiomeric ratio[27]: $E = \ln[1-c(1+ee_p)]/\ln[1-c(1-ee_p)]$, p = product.

Table 1. Enzymatic resolution of derivatives of **6b** with different acyl groups.

Acetate **8c** and especially butyrate **8b** were found to be useful compounds with respect to activity and enantioselection. Chloroacetate **8d** gave very rapid conversions but chemical hydrolysis occurred to a high degree, which decreased the *e.e.* Isobutyrate **8e** and cyclohexylcarboxylate **8f** were resolved very slowly. Octanoate **8g** showed acceptable conversion rates but somewhat worse *e.e.*s than **8b** and **8c**. Therefore butyrates were used for all other enzymatic resolutions.

			Hydrolyzed Alcohol						Remaining Ester					
substrate[a]	enzyme	time,h	conv.,%	yield%[b]	$[\alpha]_D^{20}$ [c]	isomer[d]	ee,%[e]	E[f]	conv.,%	yield,%[b]	$[\alpha]_D^{20}$ [c]	isomer[d]	ee,%[g]	E[h]
8a	CC	36	36	34	-108.9	2*S*,3*R*	82	>100	57	41	+19.1	2*R*,3*S*	30	2
8a	P	68	35	26	-132.9	2*S*,3*R*	>98	>100	54	35	+63.1	2*R*,3*S*	98	>100
8b	CC	6	36	21	+86.5	2*S*,3*S*	68	8	54	36	-66.3	2*R*,3*R*	65	7
8b	P	3	41	39	+125.0	2*S*,3*S*	>98	>100	50	48	-102.5	2*R*,3*R*	>98	>100
9a	CC	12	38	23	+97.8	2*R*,3*S*	>98	>100	58	26	-56.0	2*S*,3*R*	>98	26
9b	CC	8	32	23	+98.6	2*S*,3*S*	>98	>100	52	42	-49.0	2*R*,3*R*	>98	32
11	CC	48	12	10	-69.0	2*R*,3*S*	68	6	--	85	+3.3	2*S*,3*R*	--	--
11	CC	96	13	10	-101.0	2*R*,3*S*	>98	>100	--	82	+6.5	2*S*,3*R*	--	--

[a] All reactions were performed in 0.1 M phosphate buffer (100mL), pH 6.50 (CC) or pH 7.00 (P) at 25°C, substrate 25 mmol; enzyme 0.50 g; [b] isolated yield; [c] c = 2, CH_2Cl_2; [d] assigned by hydrogenation of the products and comparison of the values of optical rotation with literature data (see also table 2), [e] determined by ^1H- and ^{19}F-NMR spectra of the respective MTPA esters; [f] Enantiomeric ratio. $E = \ln[1-c(1+ee_p)]/\ln[1-c(1-ee_p)]$, p = product; [g] obtained from the NMR spectra of the MTPA esters of the respective alcohols, which were formed by methanolysis; [h] $E = \ln[1-c(1+ee_s)]/\ln[1-c(1-ee_s)]$, s = substrate;

Table 2. Results of the enzymatic resolution of butyrates **8a, 8b, 9a, 9b, 11** with lipases CC and P, resp.

Although several commercially available hydrolytic enzymes have been tested[28], only lipases from *Candida cylindracea* (CC) and *Pseudomonas fluorescens* (P) gave satisfactory results. Of these, P showed the better enantiodifferentiation together with acceptable rates and degrees of conversion, especially for substrates *rac*-**8a,b** but gave no conversion at all with *rac*-**9a,b**. CC acted comparably to P, somewhat less active and with lower enantiodifferentiation.

Surprisingly, the 2-azidophenylpropanates **9a** and **9b** could not be resolved with P, whereas the 3-azidophenylpropanates **8a** and **8b** were converted by both CC and P, respectively. An explanation for this could be, that lipase P needs two activating groups vicinal to the acyl group to be hydrolyzed, as given in substrates **8a** and **8b**. There, both the azido- and the carboxyl-group are attached vicinal to this center. In substrates **9a** and **9b**, one of the two neighbouring groups is phenyl, which does not activate the position to be hydrolyzed as much. The results of the resolutions together with *e.e.*s are shown in table 2.

The optical yields were determined by ^{1}H- and ^{19}F-NMR of the respective (*S*)-MTPA-esters. The absolute configurations were assigned after hydrogenation of the enantiomerically pure products to phenylserine- and phenylisoserine esters, and comparison of the values of optical rotation with literature data, or, if no data were given, assigned by "lanthanide induced shift"-(LIS)-experiments of the (*S*)-MTPA-esters with Eu(fod)$_3$[29]. Table 3 shows the results obtained in the hydrogenations of the hydroxyazidoesters **6a** and **6b** together with **14a** and **14b**

Enzyme catalyzed esterification[30] of **6b** with acetic anhydride and lipase P in benzene was attempted, but showed similiar results to the hydrolyses, only with lower *e.e.* and conversion rate.

	Hydrogenated products (phenylserines and -isoserines)				
educt	$[\alpha]_D^{20}$ [a]	product config.[b]	ester $[\alpha]_D^{20}$ [c]	acid $[\alpha]_D^{20}$	assigned by[d]
(+)-14a	+97.8	2*R*,3*S*	−12.1	–	literature[31] ($[\alpha]_D^{20} = -13.1$ [CH_3OH])[e]
(−)-14a	−98.2	2*S*,3*R*	+12.2	–	literature[31] ($[\alpha]_D^{20} = +15.6$ [CH_3OH])[e]
(+)-14b	+98.6	2*S*,3*S*	+34.1	–	literature[32] ($[\alpha]_D^{20} = +2.2$ [$CHCl_3$])[e]
(−)-14b	−98.0	2*R*,3*R*	−33.8	–	–
(−)-6a	−132.9	2*S*,3*R*	+29.7	+14.6	literature[15a] ($[\alpha]_D^{20} = +14.6$ [6N HCl])[f]
(+)-6a	+133.5	2*R*,3*S*	−30.0	−14.8	literature[15a] ($[\alpha]_D^{20} = -14.6$ [6N HCl])[f]
(+)-6b	+125.0	2*S*,3*S*	+20.0	−6.3	–
(−)-6b	−125.1	2*R*,3*R*	−20.1	+6.4	–

[a] c = 2, CH_2Cl_2; [b] All hydrogenations were performed in methanol with Pd/C 5% at ambient pressure and room temperature; [c] c = 2, methanol; [d] independently checked by LIS-experiments; [e] literature data of the respective esters; [f] data of the respective acids;

Table 3. Results of the hydrogenation of optically pure hydroxy azido esters

CONCLUSION

Hydroxyazidophenylpropanoates constitute excellent substrates for the enzymatic resolution with lipases. They show very good conversion rates together with excellent *e.e.*s, and thus allow facile synthesis of enantiomerically pure ß-phenylserines and -isoserines. In contrary N-acylderivatives of ß-phenylserines and -isoserines are not suitable for the enzymatic resolution with lipases. Attempts to apply our methodology to the synthesis of other products with biological interest are in progress.

EXPERIMENTAL

Melting points are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter in CH_2Cl_2 solution. NMR spectra were recorded on a Bruker MSL 300 at 300 MHz (^{1}H), 75.47 MHz (^{13}C), and 282.7 MHz (^{19}F). Chemical shifts are given in p.p.m. downfield relative from TMS or $CFCl_3$ as internal standard. s = singlet, d = doublet, t = triplett, m = multiplett, and br = broad signal. Numbering of carbon atoms in NMR data (see tables 4 and 5): prime (') denotes carbons in the butyric acid part, double prime (") denotes corbons in the butyramide residue. HPLC was performed on a Merck-Hitachi instrument with column LiChrosorb RP-18 (250-4), eluent water/acetonitril : 9/1 and UV detection (254 nm). Reactions were monitored by TLC using silica gel Merck 60F_{254} plates; purifications of products and separations of esters and alcohols after the enzymatic conversions were performed on silica gel Merck 60 with mixtures of ethyl acetate and light petroleum as mobile

phase. All esters were purified by bulb-to-bulb distillation prior to enzymatic hydrolysis. All new products showed correct elemental analyses.

All commercially available compounds were used as received. Crude enzyme preparations were employed without further purification. Lipase P (30 U/mg) is a product from Amano Pharamaceutical Co., Candida cylindracea lipase (12 U/mg) was purchased from Sigma (1 U is able to liberate 1 μmol fatty acid/min at pH 7.0 and 25°C). All enzymatic hydrolyses were performed with a Schott TR 156 pH-stat.

Hydrogenation of **methyl phenylpropinoate (3a)** to **(*Z*)-methyl cinnamate (1b)**: A mixture of methyl phenylpropinoate (0.50 g, 3.12 mmol), palladium on bariumsulfate (5%, 5 mg) and pyridine (5 μl) in methanol (5 ml) was hydrogenated at ambient pressure until the calculated amount of hydrogen was consumed (1 h). Then the catalyst was filtered off, the filtrate evaporated in vacuo and the residue purified by silica gel filtration (97%). The purity was determined by ^{1}H-NMR (integration of the vinyl protons).

Preparation of ***trans*-methyl phenylglycidate (4b)**: A solution of (*E*)-methyl cinnamate (8.00 g, 49.3 mmol) in dichloromethane was treated with m-chloroperbenzoic acid (18.5 g, 59.2 mmol, 55%) and refluxed for 48 hours. The reaction mixture was cooled with ice, filtered, extracted with saturated aqueous $NaHSO_3$, aqueous $NaHCO_3$ and brine. The resulting organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. Vacuum distillation afforded *trans*-methyl phenylglycidate (6.10 g, 69%) as a colourless oil with a small amount of methyl cinnamate. b.p. = 112 - 114°C (0.8 mm Hg) [lit.[33] bp = 100-110°C (0.6 mm Hg); n_D^{20} = 1.5300]. This product was used without further purification.

Preparation of Bromohydrins **5a** and **5b**: These products were prepared according to literature methods[23]. NMR- data are given in table 4 and 5.

***erythro*-2-bromo-3-hydroxy-3-phenyl-ethylpropanoate (5a)**: m.p. = 71 - 73°C (40%) [lit.[23] 72-3°C] .

***threo*-2-bromo-3-hydroxy-3-phenyl-methylpropanoate (5b)**: colourless oil (35%)

Preparation of the racemic azidohydroxyesters **6a** and **6b**[15c]: A solution of *cis*-ethyl phenylglycidate (2 g, 10.4 mmol) in 80 % aqueous ethanol (30 ml) was treated with NaN_3 (0.95 g, 14.6 mmol) and NH_4Cl (0.78 g, 14.6 mmol). This mixture was refluxedovernight, poured into water (100 ml), extracted three times with ether (50 ml) and dried over Na_2SO_4. The crude product was purified by column-chromatography (ethyl acetate/light petroleum 1:5). Results and NMR- data are given in table 4 and 5.

Preparation of the butyrates **7a,b , 8a,b, 11, 15** and other esters **8c-g**: The esters were prepared by standard methods[16] and purified by column chromatography. Results and NMR- data are given in table 4 and 5.

Preparation of the 2-azido-3-butyroxy-3-phenylpropionic esters **9a,b** from the respective bromo derivatives **7a,b** was carried out by literature methods[23]. Results and NMR- data are given in table 4 and 5.

Preparation of ***threo*-ß-phenylserine ethyl ester hydrochloride (10)**[24]: A solution of glycine ethyl ester hydrochloride (3 g, 21.5 mmol), benzaldehyde (4.6 g, 43.0 mmol) and triethylamine (2.2 g, 21.7 mmol) in dry methanol (15 ml) was stirred for 3 days. The reaction mixture was saturated with gaseous hydrogen chloride, the white precipitate filtered off, and recrystallized from methanol (0.95 g, 18%). m.p. = 183-4°C; [lit.[34] 180-182°C]. NMR- data are given in table 4 and 5.

Preparation of ***erythro*-ß-phenylisoserineamide (12)**[12a]: A solution of *trans*-ethyl phenylglycidate (5.56 g, 28.9 mmol) in dry methanol saturated with gaseous ammonia was stirred for 35 h. Evaporation of the solvent and purification of the residue by column chromatography (ethyl acetate/light petroleum 2:1) afforded **12** (4.01 g , 77 %). mp = 183-185°C; [lit.[12a] 188-191°C].

Enzymatic resolution of racemic azidoesters **8a** and **8b** as well as **9a** and **9b** with lipases: The following procedure is representative:

To a vigorously stirred solution of lipase CC (200 mg) in phosphate buffer (0.1 *M*, pH = 6.50, 100 ml) was added **8b** (1.00g, 3.43 mmol) in one portion. The pH was kept constant by addition of 1 *N* NaOH from an autoburette (pH 7.00 for P, pH 6.50 for

substrate[a]	yield	mp/n$^{D}_{20}$	C-1	C-2	C-3	C-i	C-o	C-m	C-p	C-1'	C-2'	C-3'	C-4'	OMe	OEt	others
5a	40	71-3°C	169.5	48.2	75.4	139.4	128.9	128.7	127.2	-	-	-	-	-	62.5, 13.9	
5b	35	20-2°C	169.1	52.6	74.0	138.6	128.9	128.8	126.9	-	-	-	-	53.2	-	
6a	60	1.5317	172.4	74.1	67.4	135.7	129.0	128.9	128.0	-	-	-	-	-	62.4, 14.0	
6b	65	1.5361	171.9	73.9	67.4	134.7	128.9	128.7	127.9	-	-	-	-	52.6	-	
7a	90	1.5010	167.8	46.8	75.5	136.4	129.3	128.7	128.1	171.4	36.2	18.5	13.7	-	62.4, 14.1	
7b	89	1.5052	167.6	48.2	74.8	136.3	129.4	128.9	127.6	172.0	36.3	18.6	13.8	53.1	-	
8a	91	1.4990	167.7	74.6	65.3	134.6	128.9	128.7	127.4	172.2	35.6	18.1	13.3	-	62.5, 13.9	
8b	91	1.5037	167.7	73.7	64.8	134.4	129.0	128.6	127.9	172.0	35.5	18.1	13.3	52.3	-	
8c	90	1.5119	167.8	74.2	65.0	134.5	129.2	128.9	128.0	169.6	20.5	-	-	52.6	-	
8d	89	1.5257	166.9	75.3	64.8	133.9	129.4	129.0	128.2	166.3	40.4	-	-	52.7	-	
8e	90	1.5068	167.9	73.9	65.1	134.7	129.2	128.9	128.1	175.7	33.9	16.7	16.7	52.6	-	
8f	81	1.5163	167.8	73.8	65.1	134.7	129.1	128.8	128.1	174.5	-	-	-	52.4	-	42.7 (C-2'), 28.7 (C3'), 25.8 (C-4'), 25.3 (C-5')
8g	81	1.4880	168.0	74.0	65.1	135.6	129.3	128.9	128.1	172.1	34.0	24.9	29.4	52.6	-	29.0 (C-5'), 31.8 (C-6'), 22.7 (C-7'), 14.1 (C-8')
9a	31	1.5006	167.8	66.0	75.2	136.4	129.1	128.9	126.9	172.1	36.3	18.6	13.8	-	62.5, 14.2	
9b	44	1.5078	168.0	65.5	74.5	135.7	129.3	128.6	127.5	172.0	38.3	18.5	13.7	52.7	-	
10[b]	18	183-4°C	169.5	59.6	72.0	139.2	130.2	130.0	127.1	-	-	-	-	-	64.7, 14.3	
11	42	39-41°C	169.4	58.1	74.8	136.1	128.7	128.6	126.8	172.5	36.3	18.4	13.8	-	61.9, 14.2	173.0 (NHCO), 38.6 (C-2"), 19.2 (C-2"), 14.2 (C-4")
12[c]	77	183-5°C	168.2	75.0	58.2	136.7	128.9	128.8	126.2	-	-	-	-	-		
13	12	133-5°C	172.8	74.6	54.5	137.6	128.8	128.2	127.4	173.1	36.0	18.4	13.6	-	-	173.1 (NHCO), 38.8 (C-2"), 19.2 (C-3"), 13.6 (C-4")
14a	45	1.5328	168.5	67.3	74.1	139.3	128.0	126.3	125.9	-	-	-	-	-	61.6, 13.3	
14b	43	1.5351	168.5	66.5	73.6	138.9	127.8	126.3	125.9	-	-	-	-	52.7	-	
15	71	41-3°C	169.8	59.0	74.9	139.6	128.2	127.9	126.1	-	-	-	-	-	61.8, 14.0	174.5 (NHCO), 38.2 (C-2"), 19.0 (C-3"), 13.7 (C-4")

[a] all spectra were recorded in $CDCl_3$, exept where noted; [b] D_2O; [c] DMSO-d_6

Table 4. Yields, physical and ^{13}C-NMR data of compounds **5 - 15**.

substrate[a]	H-2	J	H-3	J	Ph	OCH_2	CH_3	OCH_3	CH_2-2'	CH_2-3'	CH_3-4'	others
1b	5.98	12.6	6.97	12.6	7.37	-	-	3.73	-	-	-	
5b	4.45	6.8	5.07	6.8	7.34	-	-	3.65	-	-	-	3.26 (OH)
6a	4.89	3.1	4.40	3.1	7.40	4.23	1.24	-	-	-	-	3.14 (OH)
6b	4.80	4.4	4.44	4.4	7.27	-	-	3.59	-	-	-	3.34 (OH)
7a	4.50	10.2	6.13	10.2	7.38	4.28	1.31	-	2.26	1.59	0 89	
7b	4.51	9.1	6.13	9.1	7.31	-	-	3.46	2.29	1.61	0.87	
8a	5.26	4.8	5.09	4.8	7.37	4.21	1.17	-	2.34	1.62	0.88	
8b	5.36	5.8	4.97	5.8	7.37	-	-	3.67	2.33	1.61	0.88	
8c	5.27	5.7	4.90	5.7	7.30	-	-	3.60	-	-	-	2.01 ($COCH_3$)
8d	5.04	5.4	5.42	5.4	7.39	-	-	3.70	-	-	-	4.12 ($COCH_2Cl$)
8e	4.98	6.0	5.34	6.0	7.34	-	-	3.67	-	-	-	2.60 (CH), 1.11 (CH_3)
8f	4.89	5.8	5.27	5.8	7.26	-	-	3.54	-	-	-	2.27 (CH), 1.05-1.93 (5xCH_2)
8g	5.03	5.9	5.42	5.9	7.42	-	-	3.76	2.41	-	-	1.63(CH_2)1.32 (4xCH_2), 0 94 (CH_3)
9a	3.92	4.8	6.20	4.8	7.31	4.12	1.14	-	2.31	1.60	0.86	
9b	4.35	6.6	6.14	6.6	7.38	-	-	3.78	2.37	1.66	0.94	
10[b]	4.49	2.0	5.41	2.0	7.47	4.20	1.13	-	-	-	-	
11	5.02	4.7	6.25	4.7	7.24	4.06	1.14	-	2 29	1.56	0.89	6.02, J = 9.4Hz (NH), 2.07 (CH2-2"), 1.49 (CH2-3"), 0.76 (CH3-4")
13	5.47	7.3	5.53	7.3	7.29	-	-	-	2 26	1.62	0.87	6.38 (NH2), 6.25, J = 7.5 Hz, 2.19 (CH2-1.62 (CH2-3"), 0.87 (CH3-4")
14a	3.90	4.8	5.09	4.8	7.30	4.08	1.15	-	-	-	-	3.51, J = 3.6Hz (OH)
14b	4.02	7.3	4.34	7.3	7.30	-	-	3.79	-	-	-	3.65, J = 4.3Hz (OH)
15	4.87	3.6	5.11	3.6	7.19	4.03	1.08	-	-	-	-	6.46, J = 7.5Hz (NH), 2 09 (CH2-2"), 1.51 (CH2-3"), 0.78 (CH3-4")

[a] all spectra were recorded in $CDCl_3$, exept where noted; [b] D_2O

Table 5. ^{1}H-NMR data of all racemic compounds.

CC). The enzymatic hydrolysis was monitored by a plot of % conversion (measured by the consumption of NaOH) versus time. After 40% conversion (for **8b**: 37 %, 6h) the reaction mixture was extracted with CH_2Cl_2 (3 x 100 ml), the organic phases evaporated in vacuo, and the residue separated by column chromatography, which yielded (+)-alcohol (0.25 g, 33 %, $[\alpha]_D^{20}$ = 86.5 [c = 2,CH_2Cl_2]) and (−)-ester (0.56 g, 56 %, $[\alpha]_D^{20}$ = −38.2 [c = 2,CH_2Cl_2]). The ester was hydrolyzed up to nearly 60 % conversion in the same way as described above. Subsequent workup and chromatography gave (−)-ester (0.36 g, 36 %, $[\alpha]_D^{20}$ = −66.3 [c = 2,CH_2Cl_2]). Chemical hydrolysis (catalytic amounts of NaOMe in dry methanol, 97%) of this ester gave (-)-alcohol (0 26g, 35 %, $[\alpha]_D^{20}$ = − 79.0 [c = 2,CH_2Cl_2]).

For products with excellent enantiodifferentiation , the second enzymatic hydrolysis was not necessary (*e.g.* hydrolysis of **8b** practically ceased at 50 % conversion with lipase P). All the other enzymatic resolutions were done in the same way. Results including e.e.s are shown in table 2.

Enzymatic esterification of hydroxy azido ester **6b** with lipase and acetic anhydride[31]:

A mixture of *erythro*-3-azido-2-hydroxy-3-phenyl-propionic acid methyl ester **6b** (1.00 g, 4.52 mmol), acetic anhydride (0.46 g, 4.52 mmol) and lipase P (60 mg) in benzene (20 ml) was stirred at room temperature. After 72 h the conversion was about 30 % (determined by HPLC), the yellow reaction mixture was filtered through silica gel, washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4 and the solvent was evaporated in vacuo. Chromatographic separation (light petroleum/ethyl acetate 5:1) yielded ester (+)-**8c** (230 mg, 19 %, $[\alpha]_D^{20}$ = +81.6 [c = 2;CH_2Cl_2], *e.e.* = 80 %) and alcohol (-)-**6b** (500 mg, 50 %, $[\alpha]_D^{20}$ = -16.8 [c = 2;CH_2Cl_2]).

Hydrogenation of hydroxyazidoesters **6a** and **6b** as well as **14a** and **14b** : A solution of the hydroxyazidoester (170 mg, 0.77 mmol) in methanol was treated with palladium on carbon (5%, 20 mg) and hydrogenated at ambient pressure. When no more educt was detectable by TLC, the catalyst was filtered off and the solvent evaporated in vacuo. Results are shown in table 3.

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