Pergamon

Tetrahedron Letters, Vol. 35, No. 47, pp. 8743-8746, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01950-9

Enantiomerically Enriched α-Vinyl Amino Acids Via Lipase-Mediated "Reverse Transesterification."

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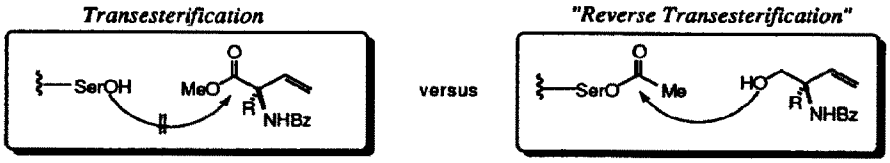
Abstract: Reduction of protected α-vinyl amino acids produces "neopentyl" alcohols that may be enriched in the L-antipode by lipase-mediated acylation with vinyl acetate. Subsequent deacetylation, oxidation and hydrolysis yields enantiomerically enriched L-α-vinyl amino acids.

α-Vinyl amino acids are known to inhibit pyridoxal phosphate-dependent enzymes, and in particular, amino acid decarboxylases.[1] They are also potential precursors to new, α-branched α-amino acids as building blocks for *de novo* peptide design.[2] For both applications, enantiomerically pure α-vinyl amino acids are desirable. Although there are several syntheses of optically enriched vinylglycine,[3] to our knowledge, the only asymmetric synthetic routes to higher α-vinyl amino acids are Seebach's self-reproduction of chirality approach (for α-vinylalanine,[4a] α-vinylbutyrine[4a] and α-vinylphenylalanine[4b]) and the Hegedus-Ojima chiral β-lactam-based approach (for α-vinylalanine).[4c] Resolution methodologies have been limited to classical resolution procedures, requiring up to 7 recrystallizations (for α-vinylalanine),[5] and one elegant HPLC procedure which employs a reverse phase column and a chiral mobile phase (for α-vinylornithine).[6]

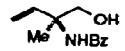
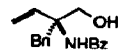
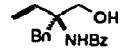
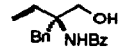
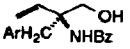
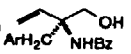
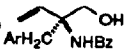
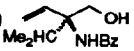
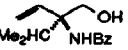
Having recently developed a convenient synthesis of racemic α-vinyl amino acids from the corresponding amino acids,[7] we set out to explore the possibility of enzymatically resolving these. Although enzymatic resolution procedures are known for many amino acid derivatives, including examples of α-methyl amino acids,[8] we are aware of no previous reports of the formal kinetic resolution of α-vinyl amino acids. Since our vinylation procedure affords the racemic α-vinyl amino acids protected as the N-benzoyl methyl esters,[7] we initially attempted to enzymatically transesterify these in *n*-butanol with a variety of acyl transferases (PPL, PLE, chymotrypsin and lipases PS-30, GC-4, AY-30 and L-10). No reaction was observed in any case.[9]

We reasoned that we may have been placing too high a steric demand on these acyl transferases and decided to reduce these esters to the corresponding alcohols (using $NaBH_4$ or $LiAlH_4$) in order to attempt "reverse transesterfications." In other words, roles would be reversed, and the enzyme would now act as acyl donor, rather than acceptor. The substrate, in turn, would act as acyl acceptor, rather than donor. Consequently, the relevant enzymatic serine oxygen would be two atoms more distant from the sterically encumbered α-carbon of the substrate in the transition state for transesterification:



In the event, although PPL, PLE and lipase L-10 still failed as catalysts, a number of lipases did prove capable of catalyzing the acylation of these "neopentyl" alcohols when suspended in vinyl acetate as both solvent and acetyl donor. The results are tabulated in Table 1.[10]

Table 1

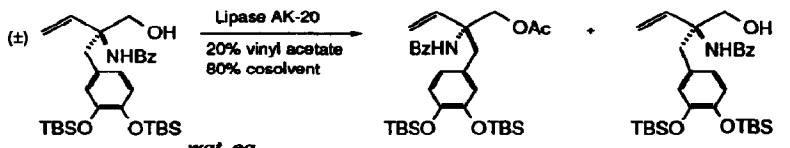
Side Chain		Benzamidoalcohol	Lipase	Time	Wgt. Equiv. Enz.	Yield Acetate	enant. excess	Yield Recovered Alcohol	enant. excess
Ala	(1)	(±) 	AY-30	5 days	10	38%	34%	62%	10%
	(2)	(±) 	AK-20	10 h	10	73%	n.d.	27%	[64%]
Phe	(3)	(±) 	AY-30	10 days	10	10%	34%	90%	n.d.
	(4)	(±) 	GC-4	10 days	10	15%	46%	85%	n.d.
	(5)	(±) 	PS-30	10 days	10	41%	58%	59%	n.d.
	(6)	(±) 	AK-20	14 h	10	26%	[62%]	72%	n.d.
DOPA	(7)*	(±) 	GC-4	20 days	10	27%	42%	73%	n.d.
	(8)*	(±) 	PS-30	14 days	7	25%	26%	75%	n.d.
	(9)*	(±) 	AK-20	2 days	10	30%	[52%]	69%	n.d.
Val	(10)	(±) 	GC-4	7 days	10	25%	[76%]	75%	n.d.
	(11)	(±) 	AK-20	4 days	10	41%	34%	59%	n.d.

* Note: Ar = 3,4-bis(t-butyldimethyl)silyloxyphenyl; n.d. = not determined. Expts. were run on ca. 100 mg scales in neat vinyl acetate. All lipases were from Amano: GC-4 = Geotrichum candidum; PS-30 = Pseudomonas cepacia; AY-30 = Candida rugosa; AK-20 = unspecified Pseudomonas species.

A number of trends are apparent from these results. Firstly, acyl transfer was quite slow for the side chains derived from phenylalanine, DOPA and valine, yet fast for the alanine side chain. Indeed, only for this latter alcohol could the acetylation reaction readily be driven beyond 50% conversion (entry 2). Secondly, although four lipases were found to act on these substrates, AY-30 did not give useful enantioselectivity and clear preferences were apparent for the other three. Both PS-30 and AK-20 exhibited greater enantioselectivity with smaller R groups (Phe as opposed to DOPA or Val) whereas GC-4 displayed maximal enantiodiscrimination with the highly hindered, β-branched R group of valine (entry 10). Only in this latter case did the level of enantiomeric enrichment (88:12 ratio), after a single acylation cycle, approach practically useful levels .

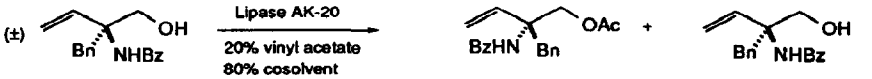
Lipase AK-20 appeared to be the most enantioselective catalyst for all other substrates. We chose, therefore, to focus on this enzyme and to examine the effects, if any, of organic cosolvents upon the enantioselectivity of this lipase. The results for the DOPA, phenylalanine and alanine side chains are collected in Tables 2, 3 and 4, respectively. Interestingly, a relatively significant solvent effect was observed. For all three substrates, enantiomeric excesses varied by 15-20%, dependent only on the choice of organic cosolvent. Best results were generally obtained with water-saturated benzene as cosolvent.

Table 2 *Solvent Effects: DOPA Side Chain*



cosolvent	wgt. eq. lipase	time	yield acetate	yield alcohol	e.e. acetate
1) wet cyclohexene	5	1 week	28%	70%	52%
2) dry THF	5	1 week	50%	46%	62%
3) wet benzene	5	1 week	32%	64%	67%

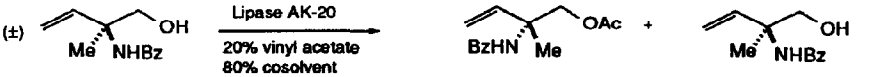
Table 3 *Solvent Effects: Phenylalanine Side Chain*



cosolvent	wgt. eq. lipase	time	yield acetate	yield alcohol	e.e. acetate
1) wet i-Pr_2O	2	28 h	21%	77%	52%
2) wet CH_2Cl_2	2	3 d	24%	74%	40%
3) dry THF	2	28 h	26%	74%	60%
4) wet hexane	2	4 d	46%	52%	61%
5) wet benzene (1st cycle)	2	4 d	50%	48%	60%
6) wet benzene (2nd cycle)	5	2 d	42%	55%	86%

Indeed, we are pleased to report that highly optically enriched L-α-vinylphenylalanine (86% ee) and L-α-vinylalanine (98% ee) may be obtained by the simple expedient of running two cycles of the lipase AK-mediated acetylation in wet benzene (Tables 3 and 4). These enzymatic acylations are easily run on gram scales.

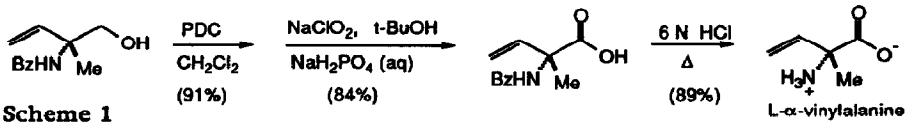
Table 4 *Solvent Effects: Alanine Side Chain*



cosolvent	wgt. eq. lipase	time	yield acetate	yield alcohol	e.e. acetate
1) wet hexane	1	1.5 d	48%	50%	67%
2) wet benzene (1st cycle)	0.5	1.5 d	48%	50%	80%
3) wet benzene (2nd cycle)	0.5	2.5 d	43%	53%	98%

In both cases, the enzymatically-derived acetates were deacetylated quantitatively with K_2CO_3 in MeOH, after each cycle. Oxidation to the aldehydes,[11] and thence to the acids,[12] followed by acidic hydrolysis,[7] provided the free α-vinyl amino acids (Scheme 1). Comparison of optical rotations with literature values

demonstrated that lipase AK preferentially acetylates the L-antipode (*R*-configuration) for both the α-vinylalanine- and the α-vinylphenylalanine-derived alcohols.[13]



Scheme 1

Acknowledgment.

Financial support from the University of Nebraska-Lincoln Research Council is gratefully acknowledged. J. A. P. was a participant in the University of Nebraska-Lincoln Summer Undergraduate Research Program. We thank Koichi Suzuki of Amano for providing us with a sample of Lipase AK-20.

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10. All enzymatic reactions were carried out by suspending the enzyme in a solution of the substrate, and shaking at 35°C. All yields are isolated yields of chromatographically purified compounds. All enantiomeric excesses were determined by integration of the ^{1}H NMR spectra of the corresponding Mosher esters (from *(S)*-2-trifluoromethyl-2-methoxyphenylacetyl chloride, after deacetylation).
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13. For α-vinylphenylalanine: $[\alpha]_D^{rt}$ (observed for 86% ee) = +14.8° (*c* = 1.3, MeOH) [lit.: -16.6° (*c* = 1.0, MeOH) (ref 4b: D-antipode)]. For α-vinylalanine: $[\alpha]_D^{rt}$ (observed) = -35.2° (*c* = 0.60, H_2O) [lit.: (i) -31.0° (*c* = 0.73, H_2O) (ref 4a: L-antipode); (ii) +33.0° (*c* = 0.61, H_2O) (ref 5b: D-antipode); (iii) +36.1° (*c* = 0.50, H_2O) (ref 4c: D-antipode)].

(*Received in USA* 1 *September* 1994; *revised* 20 *September* 1994; *accepted* 26 *September* 1994)