Pergamon

Tetrahedron Letters, Vol. 37, No. 35, pp. 6287-6290, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01378-0

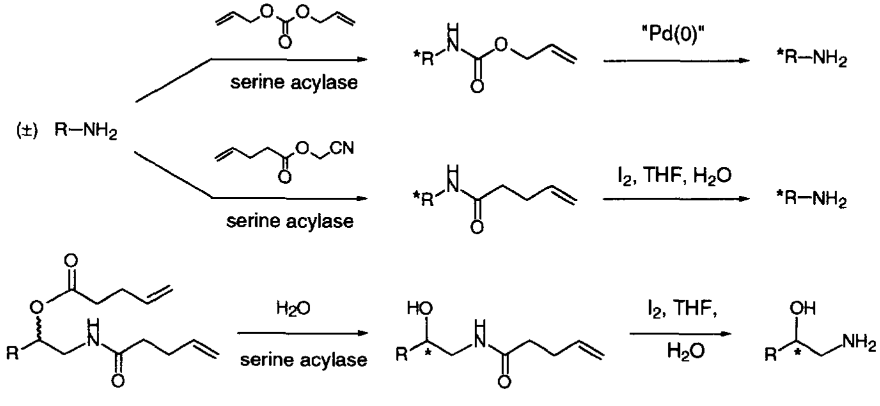
Enzymatic Resolution of Amines and Amino Alcohols Using Pent-4-enoyl Derivatives

Shuichi Takayama, Wilna J. Moree and Chi-Huey Wong*

Department of Chemistry, The Scripps Research Institute,
10666 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.

Abstract: *Racemic amines and amino alcohols were enzymatically resolved by enantioselective acylation using cyanomethyl pent-4-enoate or by enantioselective enzymatic hydrolysis of the dipent-4-enoyl derivatives of amino alcohols to afford chiral pent-4-enamides which can be deacylated chemically to their chiral amines under mild conditions.* Copyright © 1996 Elsevier Science Ltd

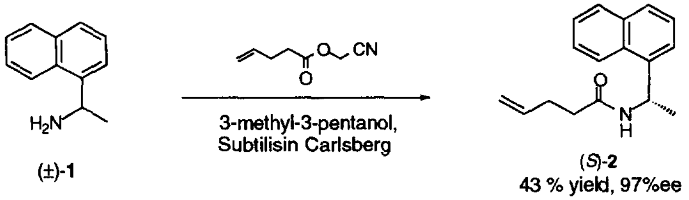
Serine acylases have been utilized extensively for the stereo- and regioselective acylation/deacylation of alcohols,[1] whereas enzymatic acylation/deacylation of amines has seen only limited use.[2] Several factors make the selective enzymatic acylation/deacylation of amines less appealing compared to that of alcohols. One of the limiting factors is that unlike esters, the deacylation of amides is generally difficult to perform either chemically or enzymatically. This precludes use of selective enzymatic deacylation of amides and restricts further manipulation of the chiral amides obtained by enzymatic acylation. Recently, we introduced diallyl carbonate as a useful enzymatic acylating reagent for amines (Scheme 1).[2a] The resulting allyl carbamates can be readily deprotected under mild conditions to give the free amines or converted to methylated amines. During our work with allyl carbonates, we noticed that the structurally similar pent-4-enoyl group could also be used as an amine-protecting group that can be cleaved under mild conditions using iodine and water.[3] In this work, we introduce the use of pent-4-enoyl derivatives in the enantioselective enzymatic acylation/deacylation of amines and amino alcohols (Scheme 1). The pent-4-enoyl group is suitable for enzymatic resolution of amines not only because it



Scheme 1. Use of diallyl carbonate and pent-4-enoyl derivatives in the enzymatic resolution of amines

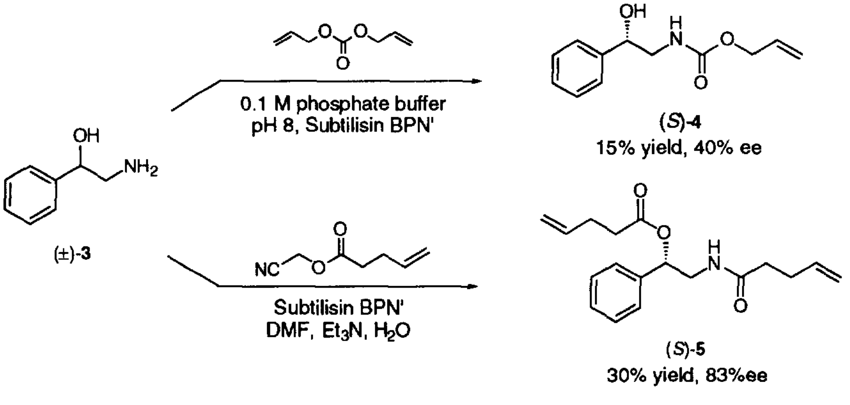
can be easily deprotected, but also because it is similar to pentanoates which are known to be especially good substrates for many serine acylases.[4]

For enzymatic acylations of amines and amino alcohols we prepared cyanomethyl pent-4-enoate, a novel activated ester which is readily prepared from the acid in one step.[5] This ester complements diallyl carbonate in enzymatic acylation reactions due to its activated nature and subsequent ability to acylate not only amines but alcohols as well. This allows a convenient double acylation in the case of amino alcohols. Cyanomethyl pent-4-enoate also has an advantage over activated unsymmetrical carbonates in that the acyl group transferred by the enzyme is unambiguously the pent-4-enoyl group.[2d, 6]



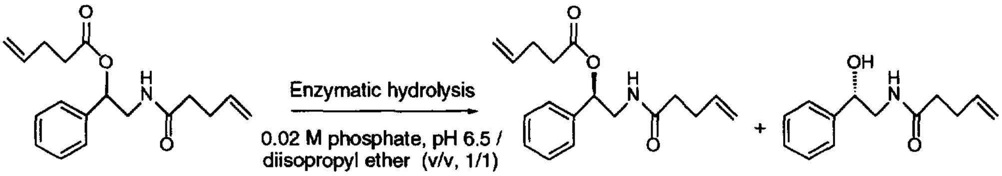
Scheme 2. Enantioselective enzymatic acylation of 1-(1-naphthyl)ethylamine[7]

The results of the enzymatic acylations are shown in Schemes 2 and 3. Enantioselective acylation of simple amines worked well. Thus, 1-(1-naphthyl)ethylamine (**1**) was acylated to give the pent-4-enamide **2** in 43% yield and 97% ee using Subtilisin Carlsberg as the biocatalyst and 3-methyl-3-pentanol as the solvent (Scheme 2). Activated esters are known to undergo spontaneous aminolysis with amines under a variety of conditions. 3-Methyl-3-pentanol was chosen as the solvent because it supressed the non-enzymatic background aminolysis which was observed in other solvents such as THF, CH_2Cl_2, DMF and *t*-butyl alcohol.



Scheme 3. Enantioselective enzymatic acylation of 2-amino-1-phenylethanol[8]

In the enzymatic acylation of 2-amino-1-phenylethanol (**3**), fast acylation of the primary amine was followed by slower but enantioselective acylation of the alcohol to give chiral amide ester **5** (Scheme 3). Enzymatic acylation of the secondary hydroxyl group using cyanomethyl pent-4-enoate provided much higher enantioselectivity compared to the enzymatic acylation of the primary amine using diallyl carbonate[9]. Reaction conditions, such as solvent and base, had a large effect on the reaction rate and selectivity of this cyanomethyl pent-4-enoate reaction. Though CH_2Cl_2, $CHCl_3$, CH_3CN gave faster reactions, DMF gave the highest enantioselectivity (other solvents screened: *t*-butyl alcohol, 3-methyl-3-pentanol, DMSO, THF). The addition of Et_3N[10] greatly enhanced the reaction rate in DMF as well as in other solvents for Subtilisin BPN'.



(±)-5 (*R*)-5 (*S*)-6

	(*R*)-5	(*S*)-6
Subtilisin BPN'	50% yield, 91-96% ee	50% yield
Lipase PS-30	49 % yield, 94% ee	49% yield

Scheme 4. Enantioselective enzymatic deacylation of 2-amino-1-phenylethanol[11]

Though enzymatic hydrolysis of the pent-4-enamide is difficult and not useful for asymmetrization, enzymatic monohydrolysis of the pent-4-enoate ester in *N*, *O*-dipent-4-enoyl derivative (**5**), prepared from the corresponding amino alcohol using pent-4-enoic anhydride[3] or pent-4-enoic acid/DCC/DMAP,[12] provided excellent stereoselectivity (Scheme 4). The amide bond was not cleaved by lipase nor subtilisin BPN' under these conditions.

In summary, we have described methods for the efficient resolution of amines and amino alcohols through enzymatic acylations using cyanomethyl pent-4-enoate and through the selective enzymatic monohydrolysis of the *N*, *O*-dipent-4-enoyl derivative of an amino alcohol. The obtained chiral pent-4-enamides can be easily converted to their chiral amines under mild conditions.[3]

Acknowledgment: We thank NIH (GM 44154), Sandoz for financial support, Dr. Lei Qiao for assistance with lipase reactions and Dr. Mahn-Joo Kim, Dr. Francisco Morís-Varas for helpful discussions.

Notes and references:

1. (a) Schoffers, E.; Golebiowski, A.; Johnson, C. R. *Tetrahedron* **1996**, *52*, 3769. (b) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, **1994** and references cited therein.
2. (a) Orsat, B.; Alper, P. B.; Moree, W. J.; Mak, C.-P.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 712. (b) Pozo, M.; Gotor, V. *Tetrahedron* **1993**, *49*, 10725. (c) Gotor, V.; Menéndez, E.; Mouloungui, Z.; Gasset, A. *J. Chem. Soc., Chem. Commun.* **1993**, 2453. (d) Pozo, M.; Pulido, R.; Gotor, V. *Tetrahedron* **1992**, *48*, 6477. (e) Fernández, S.; Brieva, R.; Rebolledo, F.; Gotor, V. *J. Chem. Soc., Chem. Commun.* **1992**, 2885. (f) Kitaguchi, H.; Fitspatrick, P. A.; Huber, J. E.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 3094.
3. Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Org. Chem.* **1995**, *60*, 7920.

4. Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200.
5. **Cyanomethyl pent-4-enoate**: A mixture of pent-4-enoic acid (5 g, 50 mmol), triethylamine (7.6 g, 75 mmol) and chloroacetonitrile (11.4 g, 150 mmol) were stirred at 70°C for 4 h. The mixture was then cooled and dissolved in EtOAc. The organic layer was washed with H_2O, sat. $NaHCO_3$, H_2O, 1 N HCl, H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was distilled (bp 96°C/4 mmHg) to give the title compound (6.5 g, 94%). For other example of cyanomethyl ester synthesis, see: West, J. B.; Hennen, W. J.; Lalonde, J. L.; Bibbs, J. A.; Zhong, Z.; Meyer, E. F. J.; Wong, C.-H. *J. Am. Chem. Soc.* **1990**, *112*, 5313.
6. Morís, F.; Gotor, V. *Tetrahedron*, **1992**, *48*, 9869.
7. **Acylation of 1-(1-naphthyl)ethylamine (1) with cyanomethyl pent-4-enoate:** A mixture of the amine (64 mg, 0.37 mmol), cyanomethyl pent-4-enoate (117 mg, 0.85 mmol), 3-methyl-3-pentanol (1.0 mL), and Subtilisin Carlsberg (50 mg, 700 U at the begining and an additional 25 mg, 350 U after 30 h) was stirred at 36°C for 69 h. The reaction was worked up by adding EtOAc and filtering the reaction mixture through celite. The filtrate was washed with 1N HCl, H_2O, sat. $NaHCO_3$ solution, and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Addition of hexane/Et_2O (1/1) caused the title compound to crystallize (41 mg, 43%). The enantiomeric excess was determined to be 97% ee ($[\alpha]_D^{24}=$ -66^o, $CHCl_3$, c= 0.21) by comparison of the optical rotation with the (*R*)-amide ($[\alpha]_D^{24}= +67^o$, $CHCl_3$, c= 0.22) obtained chemically from authentic (*R*)-amine (98% ee).
8. **Acylation of 2-amino-1-phenylethanol (3) with cyanomethyl pent-4-enoate:** A mixture of the amino alcohol (22 mg, 0.16 mmol), cyanomethyl pent-4-enoate (0.05 mL), DMF (0.2 mL), H_2O (0.005 mL), triethylamine (0.005 mL) and subtilisin BPN' (5 mg, 50U) was stirred at 37°C for 36 h. The reaction was worked up by adding EtOAc and filtering the reaction mixture through celite. The filtrate was washed with sat. $NaHCO_3$ solution, then with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give an oil. Much of the pent-4-enamide alcohol (8.5 mg) was crystallized out at this stage by addition of diisopropyl ether. The mother liquor was chromatographed (SiO_2, hexane/EtOAc, 9/1-1/1) to give the dipent-4-enoyl product (13 mg, 27%) and some more of the pent-4-enamide alcohol (total 17 mg, 50 %). The enantiomeric excess of the dipent-4-enoyl product was determined to be 83% ee by HPLC analysis (CHIRALPAK AD, hexane/IPA 95/5, v/v, 1.0 ml/min).
9. With diallyl carbonate the highest ee observed was 40% (15% yield). At higher pH, *N*-acylation was quantitative but the ee was 0% due to spontaneous non-enzymatic reaction. Acylation of alcohols were not observed when diallyl carbonate was the acyl donor.
10. For addition of Et_3N to lipase reactions, see: Theil, F.; Ballschuh, S.; Schick, H.; Haupt, M.; Hafner, B.; Schwarz, S. *Synthesis* **1988**, 540.
11. **Hydrolysis of *N*, *O*-dipent-4-enoyl-2-amino-1-phenylethanol (5):** A mixture of the *N*-(pent-4-enoyl)-2-amino-1-phenylethyl pent-4-enoate (303 mg, 1 mmol), 0.02 M pH 6.5 phosphate buffer (1 mL), diisopropyl ether (1 mL) and subtilisin BPN' (10 mg, 100U) was stirred at room temperature for 36 h, keeping the pH to 6.5 with a pH stat (0.5 N NaOH). After addition of 0.5 equivalent of base, the reaction was worked-up by separating the organic layer and extracting the aqueous layer with EtOAc. The combined organic layers were washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Addition of diisopropyl ether and crystallization afforded 94 mg of the hydrolyzed product. The mother liquor was chromatographed to afford the unhydrolyzed dipent-4-enoyl compound (154 mg, 50%) and another 29 mg of pent-4-enamide alcohol (total 113 mg, 50%). The enantiomeric excess of the unhydrolyzed dipent-4-enoyl compound was determined to be 91% ee by HPLC analysis (CHIRALPAK AD, hexane/IPA 95/5, v/v, 1 ml/min).
12. For a similar example, see: van Boom, J. H.; Burgers, P. M. J. *Tetrahedron Lett.* **1976**, *17*, 4875.

(*Received in USA* 31 *May* 1996; *revised* 1 *July* 1996; *accepted* 3 *July* 1996)