A new enzymatic synthesis of blockbuster drug intermediates - Five-membered cyclic γ-amino acid and γ-lactam enantiomers

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A very efficient new enzymatic method was developed for the synthesis of cyclic γ-lactam and γ-amino acid enantiomers, intermediates for drugs with a prominent turnover (*e.g.* abacavir and carbovir), through the CAL-B-catalyzed enantioselective (*E* > 200) hydrolysis of the corresponding activated and inactivated racemic γ-lactams with H_2O in *i*Pr_2O.

Introduction

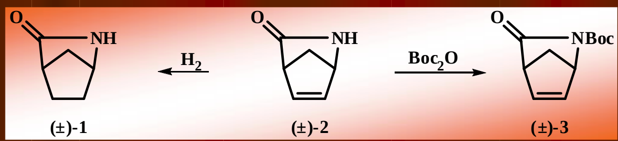
(1*S*,4*R*)-4-Aminocyclopent-2-ene-1-carbocyclic acid is a key intermediate used in one of the syntheses of purine and pyrimidine carbonucleosides with significant antiviral activity (*e.g.* abacavir and carbovir)[1-3], through known methods, for example by reduction[4] and a subsequent coupling reaction[5]. Abacavir is a selective and potent reverse transcriptase inhibitor for the treatment of human immunodeficiency virus and hepatitis B virus infections in adults and children.

The antipode γ-amino acid enantiomer is also used as starting material for substances exerting valuable biological activity[6]. Further, the saturated analogue 3-aminocyclopentane-1-carboxylic acid is a starting material applied in the search for biologically active compounds[7].

Results and discussion

A. Syntheses of (±)-1 and (±)-3

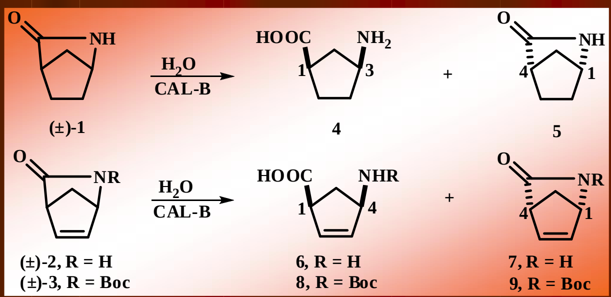
The γ-lactam (±)-1 was prepared from the commercially available 2-azabicyclo-[2.2.1]hept-5-en-3-one (Vince lactam) [(±)-2] by catalytic transfer hydrogenation in the presence of the hydrogen donor cyclohexene, while the reaction of (±)-2 with di-*t*-butyl dicarbonate resulted in the desired *N*-Boc-protected γ-lactam (±)-3 (Scheme 1).



Scheme 1.

B. Lipase-catalyzed enantioselective ring cleavage of (±)-1 - (±)-3

On the basis of the results on the enantioselective hydrolyses of carbocyclic β-lactams[8] and β-amino esters[9] by using lipase B from *Candida antarctica* in an organic solvent, we started preliminary experiments in *i*Pr_2O with enzyme screening (Scheme 2, Table 1)[10]. Lipolase was chosen as the enzyme for further studies and for the gram-scale resolution.



Scheme 2.

Table 1. Conversion and enantioselectivity on hydrolysis of (±)-2[a]

Entry	Time	Enzyme (30 mg mL^{-1})	*T* (°C)	H_2O (equiv)	Conv (%)	*ees*[b] (%)	ee_p[c] (%)	*E*
1	140 min	Lipolase	60	1	42	72	> 99	> 200
2	140 min	Lipolase	60	0.5	50	> 99	> 99	> 200
3	140 min	Lipolase	60	-	50	> 99	> 99	> 200
4	140 min	Lipolase	60	2	36	55	> 99	> 200
5	140 min	Lipolase	60	5	24	32	> 99	> 200
6	140 min	Lipolase	60	10	11	12	> 99	> 200
7	17 h	Lipolase	30	0.5	43	74	> 99	> 200
8	50 min	Lipolase	45	0.5	11	12	> 99	> 200
9	50 min	Lipolase	70	0.5	29	40	> 99	> 200
10	50 min	Lipolase	80	0.5	48	86	> 99	> 200
11	140 min	Chiraz L-2	60	1	41	68	> 99	> 200
12	140 min	Novozym 435	60	1	40	65	> 99	> 200
13	64 h	Chiraz L-5[d]	60	1	15	17	> 99	> 200
14	64 h	PPL	45	1	20	25	> 99	> 200
15	64 h	Lipase PS[d]	45	1	10	11	> 99	> 200
16	64 h	Lipase AK[d]	45	1	14	16	> 99	> 200

[a]0.05 M substrate, 1 mL *i*-Pr_2O. [b]According to GC. [c]According to GC after double derivatization. [d]Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

The catalytic activity of the tested Lipolase was affected by the amount of added H_2O to the reaction mixture (Table 1, entries 2-6). The shortest reaction time (the time needed to reach 50% conversion) was obtained for 0.5 equiv. of added H_2O (Table 1, entry 2).

Next, we analysed the effects of temperature on the enantioselectivity and reaction rate. When the reaction was performed at 45 °C (Table 1, entry 8), the reaction rate increased; on further increase on the temperature (to 70 °C or 80 °C), considerably faster reactions were observed (Table 1, entries 9 and 10), without a drop in enantioselectivty.

Table 2. Conversion and enantioselectivity on hydrolysis of (±)-2[a]

Several solvents were tested to study the solvent effect in the Lipolase-cataly-sed hydrolysis of (±)-2 at 60 °C (Table 2).

T	Solvent	Conv (%)	ee_s[b] (%)	ee_p[c] (%)	*E*
36 h	Me_2CO	*no reaction*			
36 h	1,4-dioxane	22	28	> 99	>> 200
36 h	THF	7	7	> 99	>> 200
50 min	Et_2O	18	21	> 99	>> 200
50 min	*t*-BuOMe	35	54	> 99	>> 200
36 h	toluene	33	49	> 99	>> 200
50 min	*n*-hexane	34	51	> 99	>> 200
36 h	CH_2Cl_2	20	24	> 99	>> 200

[a]0.05 M substrate in the solvent tested, 30 mg mL^{-1} Lipolase and 0.5 equiv. of H_2O, at 60 °C. [b]According to GC. [c]According to GC after double derivatization.

When we analysed the reusability of the enzyme: the hydrolysis of (±)-2 was tested with Lipolase that had already been used in 1, 2 or 3 cycles (data not showed), the catalytic activities of the tested Lipolase were progressively slightly lowered, though the enantioselectivity was apparently not affected.

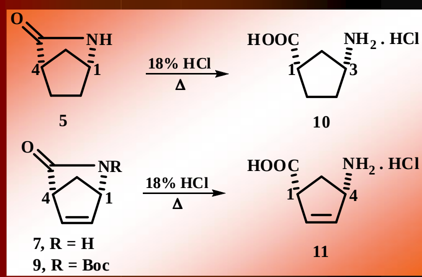
On the basis of the preliminary results, the gram-scale resolutions of (±)-1 – (±)-3 were performed with 0.5 equiv. of H_2O in the presence of Lipolase in *i*Pr_2O at 30 ºC for (±)-3 and at 65 ºC for (±)-1 and (±)-2. The products were characterized by a very good enantiomeric excess at close to 50% conversion. The results are reported in Table 4.

Table 3. Lipolase-catalysed preparative-scale hydrolyses of (±)-1 - (±)-3[a]

			γ-Amino acid (4, 6 and 8)				γ-Lactam (5, 7 and 9)			
T (h)	Conv (%)	*E*	Yield (%)	Isomer	*ee*[b] (%)	$[\alpha]_D^{25}$ (H_2O)	Yield (%)	Isomer	*ee*[c] (%)	$[\alpha]_D^{25}$ ($CHCl_3$)
91	50	> 200	42	1*R*,3*S*	98	-10.6[d]	47	1*R*,4*S*	>99	+158[e]
4	50	> 200	48	1*S*,4*R*	>99	-243[f]	46	1*S*,4*R*	>99	+549[g]
18	50	> 200	44	1*S*,4*R*	96	-40.8[h]	44	1*S*,4*R*	97	+187[i]

...ed by GC after double derivatization. [c]According to GC. [d]c = 0.35. [e]c = 0.45. [f]c = 0.34. [g]c = 0.26. [h]c = 0.25. [i]c = 0.28.

The transformations involving the hydrolysis of γ-lactams 5, 7 and 9 with 18% aqueous HCl resulted in the enantiomers of γ-amino acid hydrochlorides 10 and 11 (Scheme 3).



Scheme 3.

Conclusions

The CAL-B-catalysed highly enantioselective (*E* > 200) ring cleavage of activated and inactivated γ-lactams with added H_2O (0.5 equiv.) in *i*Pr_2O at 30 ºC or 60 ºC afforded γ-amino acid and γ-lactam enantiomers (*ee* ≥ 96%), blockbuster intermediates (chemical yields ≥ 42%).

References

1 J. Weiss, N. Weis, N. Ketabi-Kiyanvash, C. H. Storch, G. Caroline, W. E. Haefeli, *Eur. J. Pharm.* 2008, *579*, 104.
2 M. A. Wainberg, J. L. Martinez-Cajas, B. G. Brenner, *Fut. HIV Ther.* 2007, *1*, 291.
3 N. Venhoff, B. Setzer, K. Melkaoui, U. A. Walker, *Antivir. Ther.* 2007, *12*, 1075.
4 S. M. Daluge, M. T. Martin, B. R. Sickles, D. A. Livingston *Nucleos. Nucleot. Nucl.* 2000, *19*, 297.
5 M. Ferrero, V. Gotor, *Chem. Rev.* 2000, *100*, 4319.
6 D. E. Levy, M. Bao, D. B. Cherbavaz, J. E Tominson, D. M. Sedlock, C. J. Homcy, R. M. Scarborough, *J. Med. Chem.* 2003, *46*, 2177.
7 C. Evans, R. McCague, S. M. Roberts, A. G. Sutherland, *J. Chem. Soc., Perkin Trans 1* 1991, 656.
8 E. Forró, F. Fülöp, *Org. Lett.* 2003, *5*, 1209.
9 E. Forró; F. Fülöp, *Chem. Eur. J.* 2007, *22*, 6397.
10 E. Forró; F. Fülöp, *Eur. J. Org. Chem.* 2008, *31*, 5263.