Tetrahedron Letters 54 (2013) 1185–1188

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Candida antarctica lipase A and *Pseudomonas stutzeri* lipase as a pair of stereocomplementary enzymes for the resolution of 1,2-diarylethanols and 1,2-diarylethanamines

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ARTICLE INFO

Article history:
Received 21 September 2012
Revised 28 November 2012
Accepted 30 November 2012
Available online 7 December 2012

Keywords:
Enzymatic catalysis
Lipase
Resolution
Alcohol
Amine

ABSTRACT

Candida antarctica lipase A (CALA) and *Pseudomonas stutzeri* lipase (PSL) displayed opposite enantioselectivities in the acylation of 1,2-diphenylethanol and 1,2-diphenylethanamine. CALA was (*S*)-selective while PSL was (*R*)-selective. In addition, fourteen different 1,2-diarylethanols were tested as the substrates of CALA. It was found that most of them were accepted by CALA with high enantioselectivity. The DKR of five representative substrates by the combination of CALA and a ruthenium-based racemization catalyst provided good yields (82–91%) and acceptable enantiopurities (87–94% ee).

Among enzymes, lipases have been most frequently employed as catalysts for enantioselective transformations in organic synthesis.[1] They accept a broad range of substrates and often display good enantioselectivity. They are particularly useful in the kinetic resolution (KR) of racemic alcohols and amines. The enzymatic KRs provide two separated enantiomers with 50% theoretical maximum yield for each of them. The maximum yield can be improved up to 100% if the enzymatic KR is coupled with in situ racemization of substrate to transform all of the substrates to single enantiomeric products. This process is called dynamic kinetic resolution (DKR).[2] In contrast to KR, DKR provides only one type of enantiomer (*R* or *S*) as the product depending on the stereospecificity of enzyme employed. Accordingly, a pair of stereocomplementary enzymes should be available to prepare both (*R*)- and (*S*)-products via DKR.[3,4] We herein report that *Candida antarctica* lipase A (CALA) and *Pseudomonas stutzeri* lipase (PSL) are a pair of stereocomplementary enzymes for the resolution of 1,2-diarylethanols and 1,2-diarylethanamines.[5]

It has been known that most lipases have the same stereospecificity. In the transesterification of simple secondary alcohols, for example, lipases accept the enantiomer (**1**) shown in Figure 1 selectively if the substrate has one small and one relatively larger substituent at the hydroxymethine center.[6] According to the empirical rule, lipases accept (*R*)-enantiomer (**2**) selectively in the transesterification of 1-phenylethanol. Interestingly, subtilisin Carlsberg (SC, *Bacillus licheniformis*), a proteolytic enzyme, has been known to accept (*S*)-enantiomer selectively in this case.[7] The empirical rule, however, is hardly applicable to sterically more demanding secondary alcohols which have two similarly large substituents at the hydroxymethine center. As such a class of secondary alcohols, 1,2-diarylethanols **3a**–**o** have one phenyl and one benzyl at the hydroxymethine center (Fig. 2). In this case, it is difficult to tell which is the larger of two in terms of enzymatic enantioselectivity. In this work, we indeed found that two lipases, PSL and CALA, were opposite in the enantiopreference toward 1,2-diarylethanols. PSL was (*R*)-selective so that it appears to recognize the phenyl group as the larger substituent. In contrast to this, CALA was (*S*)-selective.

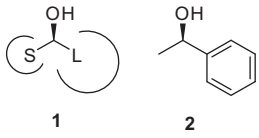

Figure 1. Faster-reacting enantiomer in the lipase-catalyzed transesterification of secondary alcohols.

The stereopreferences of PSL and CALA were explored with the transesterification of 1,2-diphenylethanol (**3a**). The CALA-catalyzed transesterification reaction was performed in the presence of trifluoroethyl butyrate (TFEB) as an acyl donor in acetonitrile[8]

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0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.11.147

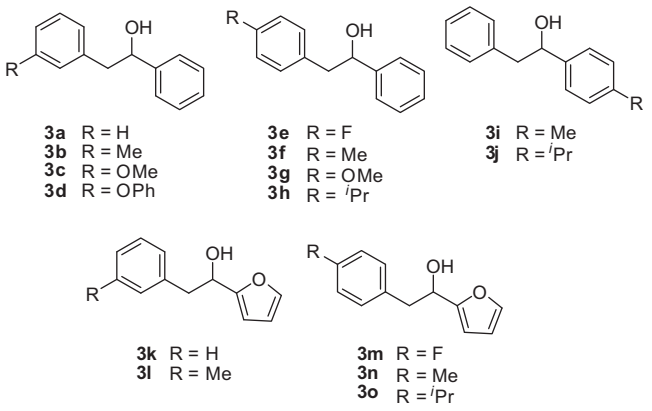


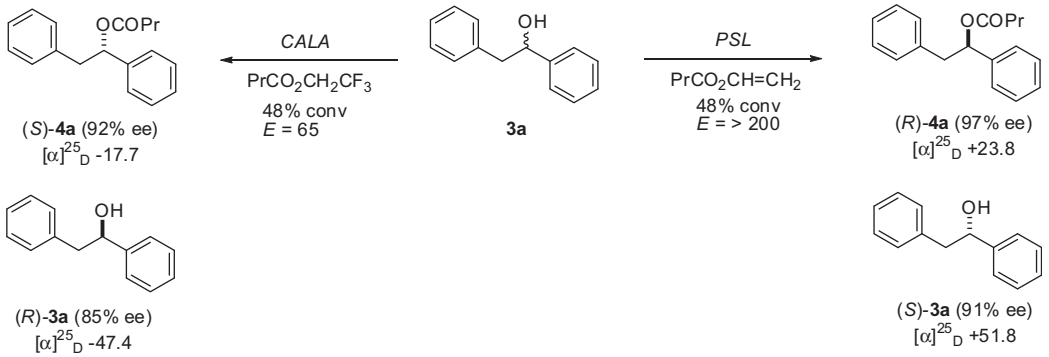
Figure 2. 1,2-Diarylethanols.

while the PSL-catalyzed reaction was carried out using vinyl butyrate (VB) as an acyl donor in toluene (Scheme 1).[9] After the reactions had been carried out to 48% conversion, acylated products and unreacted substrates were separated to determine their enantiopurities and optical rotations.[8,9] It was found that the signs of optical rotations for acylated products and recovered substrates were opposite between two enzymatic reactions, indicating that CALA is (*S*)-selective because the (*R*)–selectivity of PSL was previously reported.[3d]

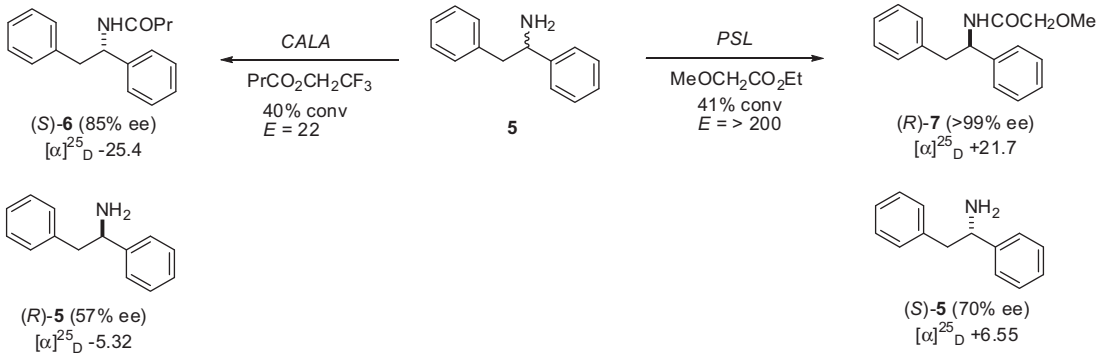
The stereocomplementary relationship between PSL and CALA was also found in the acylation of 1,2-diphenylethanamine (**5**) which is isosteric to 1,2-diphenylethanol. The CALA-catalyzed acylation of **5** was best performed in the presence of trifluoroethyl butyrate (TFEB) in acetonitrile (Scheme 2).[10] In contrast to this, the PSL-catalyzed acylation of **5** proceeded smoothly in the presence of ethyl methoxyacetate (EMA) in toluene (Scheme 2).[11] The optical rotations of unreacted substrates from both reactions, which were isolated at 40–41% conversion, gave opposite signs, thus indicating that the stereopreferences of PSL and CALA were opposite as well. It is noted that the selectivity of PSL was higher (E = >200) than that of CALA (E = 22).

Previously, we reported that PSL accepted a wide range of 1,2-diarylethanols with good enantioselectivity.[3d] For comparison in substrate scope and stereospecificity between CALA and PSL, we tested fourteen 1,2-diarylethanols (**3b**–**o**) as the additional substrates of CALA in this work. Most of them reacted smoothly with good to excellent enantioselectivity but two, **3i** and **3j**, were poorly reactive, indicating that the substitution on 1-phenyl ring reduces the reactivity significantly (Table 1). This is compared to the broader specificity of PSL which tolerated the substitution on the 1-phenyl ring well.[3d] It is particularly noted that in general a substitution on the 2-phenyl ring and the replacement of 1-phenyl group by a smaller ring (furyl) enhanced the enantioselectivity (compare **3a** with **3b**–**d** or **3e**–**h**, and also **3a**,**b**,**e**,**f**,**h** with **3k**–**o**, respectively).

As the stereocomplementary procedure to the PSL-catalyzed DKR,[3d] CALA-catalyzed DKR was explored with five 1,2-diarylethanols **3a**,**e**–**h** (Table 2). Ru complex **8** was chosen as the racemization catalyst. The Ru-catalyzed racemization of **3a** in acetonitrile turned out to be so sluggish. Accordingly, acetonitrile was replaced by toluene for DKR to promote the racemization at the cost of enzymatic activity and enantioselectivity. The DKR reactions were performed with solutions containing substrate (0.1 mmol), **8** (4 mol %), CALA (60 mg), TFEB (1.5 equiv), and K_2CO_3 (1 equiv) in toluene at room temperature for 48 h.[14] Three portions of enzymes (3 × 20 mg) were added periodically at t = 0, 12, and 36 h because the enzymatic activity decreased gradually with time. All the DKR reactions provided good yields (82–91%) with acceptable enantiopurities (87–94% ee).



Scheme 1. Lipase-catalyzed acylation of 1,2-diphenylethanol.



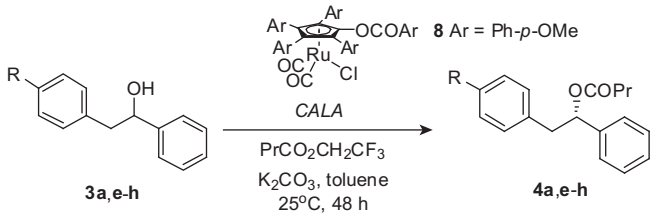
Scheme 2. Lipase-catalyzed acylation of 1,2-diphenylethanamine.

Table 1
CALA-catalyzed transesterification of 1,2-diarylethanols (**3b–o**)[a]

Substrate	Product	ee_s (%)	ee_p (%)	Conv (%)	E[b]	Substrate	Product	ee_s (%)	ee_p (%)	Conv (%)	E[b]
3b	**4b**	91	95	49	128	**3i**	**4i**	nd[c]	nd[c]	10[d]	nd[c]
3c	**4c**	91	96	49	156	**3j**	**4j**	nd[c]	nd[c]	3[d]	nd[c]
3d	**4d**	95	95	50	133	**3k**	**4k**	>99	86	54	80
3e	**4e**	98	91	52	97	**3l**	**4l**	>99	95	51	227
3f	**4f**	92	96	49	168	**3m**	**4m**	>99	90	53	117
3g	**4g**	94	96	49	197	**3n**	**4n**	>99	99	50	>400
3h	**4h**	94	96	49	187	**3o**	**4o**	>99	96	51	271

[a] The values of optical rotation of unreacted substrates and acylated products are described in Refs. 12,13.
[b] $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$ with $c = ee_s/(ee_s+ee_p)$.
[c] Not determined.
[d] Determined by ^{1}H NMR.

Table 2
DKR of 1,2-diarylethanols with CALA



Entry	Substrate	Product	Yield[a] (%)	ee[b] (%)
1	**3a**	**4a**	90	89
2	**3e**	**4e**	90	87
3	**3f**	**4f**	91	91
4	**3g**	**4g**	82	93
5	**3h**	**4g**	84	94

[a] Determined by H NMR spectroscopy.
[b] Determined by HPLC using a chiral column (Whelk-O1, Merck).

In summary, we have demonstrated that CALA and PSL are stereocomplementary in the acylation reactions of 1,2-diphenylethanol and 1,2-diphenylethanamine: CALA was (*S*)-selective but PSL was (*R*)-selective. CALA accepted 1,2-diarylethanols with good to high enantioselectivity but its substrate scope was relatively narrower than that of PSL reported previously. The DKR of 1,2-diarylethanols by the combination of CALA with a ruthenium-based racemization catalyst was successful. Further applications of CALA and PSL to dynamic kinetic resolution are in progress at this laboratory.

Acknowledgment

Authors are grateful to the National Research Foundation of Korea for its generous support of their works (2009-0074357 and 2012R1A1A2006595).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.11.147.

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8. CALA (trade name, Novozym-735) was immobilized on Celite before use as follows. Lyophilized CALA (10 mg) and sucrose (6 mg) was dissolved in water (10 mL), followed by the addition of celite (84 mg). The resulting mixture was stirred for 30 min and water was removed under reduced pressure. The residue was dried under vacuum for 1 day and then used in acylation. The acylation of **3a** with CALA was performed with a solution containing **3a** (0.2 mmol), CALA on celite (8 mg), and TFEB (3 equiv) in acetonitrile (2 mL) at room temperature for 24 h. (*R*)-**3a**: $[\alpha]_D^{25}$ −47.4 (*c* 1.0, EtOH, 85% ee) (lit.[3d] $[\alpha]_D^{25}$ −53.8 (*c* 1.0, EtOH, 98% ee). (*S*)-**4a**: $[\alpha]_D^{25}$ −17.7 (*c* 1.0, $CHCl_3$, 92% ee).
9. PSL was used as received. The acylation of **3a** with PSL was performed with a solution containing **3a** (0.2 mmol), PSL (24 mg), and VB (3 equiv) in toluene (0.7 mL) at room temperature for 24 h. (*S*)-**3a**: $[\alpha]_D^{25}$ +51.8 (*c* 1.0, EtOH, 91% ee) (lit. $[\alpha]_D^{20}$ +47.6 (*c* 0.50, EtOH, 94% ee; Xie, J.-B.; Xie, J.-H.; Liu, X.-Y.; Zhang, Q.-Q.; Zhou, Q.-L. *Chem. Asian J.* **2011**, *6*, 899–908). (*R*)-**4a**: $[\alpha]_D^{25}$ +23.8 (*c* 1.0, $CHCl_3$, 97% ee).
10. The acylation of **5** with CALA was performed with a solution containing **5** (0.2 mmol), CALA on celite (8 mg), and TFEB (3 equiv) in acetonitrile (2 mL) at room temperature for 24 h. (*R*)-**5**: $[\alpha]_D^{25}$ −5.32 (*c* 2.0, $CHCl_3$, 57% ee) (lit. $[\alpha]_D$ −10.1 (*c* 1.96, $CHCl_3$, 91% ee; Eddine, J. J.; Cherqaoui, M. *Tetrahedron: Asymmetry* **1995**, *6*, 1225–1228). (*S*)-**6**: $[\alpha]_D^{25}$ −25.4 (*c* 2.0, $CHCl_3$, 85% ee).

11. The acylation of **5** with PSL was performed with a solution containing **5** (0.2 mmol), PSL (40 mg), and EMA (2 equiv) in toluene (2 mL) at room temperature for 24 h. (*S*)-**5**: $[\alpha]_D^{25}$ +6.55 (*c* 2.0, $CHCl_3$, 70% ee) (lit. $[\alpha]_D^{20}$ +10.7 (*c* 1.05, $CHCl_3$, >99% ee; Berger, M. L.; Schweifer, A.; Rebernik, P.; Hammerschmidt, F. *Bioorg. Med. Chem.* **2009**, *17*, 3456–3462). (*R*)-**6**: $[\alpha]_D^{25}$ +21.7 (*c* 1.4, $CHCl_3$, >99% ee).
12. $[\alpha]_D^{25}$ (*c* 1.0, EtOH) for recovered substrates: **3b**, −33.9; **3c**, −31.7; **3d**, −50.9; **3e**, −34.9; **3f**, −50.0; **3g**, −41.5; **3h**, −43.2; **3k**, +8.18; **3l**, +7.29; **3m**, +6.98; **3n**, +8.24; **3o**, +8.08
13. $[\alpha]_D^{25}$ (*c* 1.0, $CHCl_3$) for acylated products: **4b**, −16.2; **4c**, −17.3; **4d**, −2.0; **4e**, −24.0; **4f**, −16.6; **4g**, −12.5; **4h**, −15.7; **4k**, −70.4; **4l**, −80.4; **4m**, −80.4 **4n**, −74.8; **4o**, −69.7.
14. The procedure for the DKR of **3a** is described as the representative. Alcohol **3a** (19.8 mg, 0.1 mmol), **8** (3.4 mg, 4 mol %), and K_2CO_3 (13.8 mg, 0.1 mmol) were added in a Schlenk flask and dried for 1 h. And then, dry and degassed toluene (0.2 M, 0.5 mL) was added under argon atmosphere and stirred at 60 °C for 30 min. The color of solution was changed from yellow to brown. CALA (20 mg) and trifluoroethyl butyrate (30 μL, 0.2 mmol) was added and the resultant solution was stirred at 25 °C under argon atmosphere. A portion of fresh enzyme (20 mg) was added once after 12 and one more after 36 h. The reaction was stopped after 48 h. The solution was filtered through a celite-pad and then analyzed by ^{1}H NMR spectroscopy and HPLC using a chiral column (Whelk-O1, Merck).