Tetrahedron Letters 57 (2016) 603–606

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Highly stereoselective kinetic resolution of α-allenic alcohols: an enzymatic approach

Wenhua Li [a], Zuming Lin [a], Long Chen [a], Xuechao Tian [b], Yan Wang [a], Sha-Hua Huang [a,b,*], Ran Hong [a,*]

[a] CAS Key Laboratory of Synthetic Chemistry of Natural Substances, Shanghai Institute of Organic Chemistry (CAS), Shanghai 200032, China
[b] School of Chemical and Environmental Engineering, Shanghai Institute of Technology, Shanghai 201418, China

ARTICLE INFO

Article history:
Received 2 December 2015
Revised 23 December 2015
Accepted 24 December 2015
Available online 25 December 2015

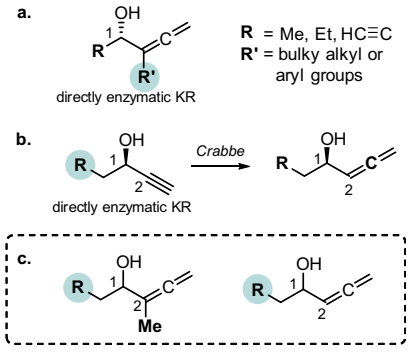
Keywords:
Kinetic resolution
Enzyme
α-Allenic alcohol
High enantioselectivity
Suzuki–Miyaura coupling

ABSTRACT

A highly efficient lipase AK-catalyzed direct kinetic resolution of a variety of α-allenic alcohols was developed. With the complementary to previous studies, the current reaction system is effective on a broad range of substituents (R^1) at C(1), such as alkyl, aryl, alkenyl, and alkynyl groups. The Jones–Burgess empirical model was modified to interpret the reversed selectivity during the acetylation of secondary alcohol. The methyl group at C(2) of allenic alcohols implied a small structural adjustment in the catalytic triad of lipase AK, representing a potential direction for future site-directed mutagenesis.

Biotransformation has emerged into the chemical arsenal for organic synthesis due to the advantages of high stereoselectivity and relatively simple operations.[1] In particular, enzymatic transesterification and its reverse transformation have found a wide application in numerous syntheses of natural products and pharmaceutically interesting compounds.[2] With the innovative site-directed mutagenesis approach in the past two decades, biotransformation has been expanded into the field of *unnatural* reactions which was conventionally considered as a formidable challenge for natural enzymes.[3] While new and unnatural chemical reactions with enzymes are enrolled into the frontier of biotechnology, defining a new substrate scope remains a useful approach since many enzymes and mutants are commercialized and some of them have been immobilized for the practical use both in academia and industry.[4]

During our study on the development of a non-aldol approach to construct chiral polyol units in biologically significant polyketides,[5] an optical α-allenic alcohol was chosen as the suitable starting material due to its multiple functionalizable sites.[6] Enzymatic kinetic resolution (EKR) of α-allenic alcohols by Novozym 435 resulted in several useful chiral building blocks (Scheme 1a).[7] A variety of groups can be tolerated at C(2) albeit that substituents at C(1) were limited to simple alkyls (methyl, ethyl, or ethynyl). This problem was partially solved when the combination of enzymatic resolution of propargylic alcohols[8] and subsequent Crabbé homologation or S_N2' substitution was developed[8f] (Scheme 1b). However, the products bearing a methyl group at C(2) (Scheme 1c) were not feasible from these protocols. Our initial approach on asymmetric cycloisomerization achieved a limited success.[9]



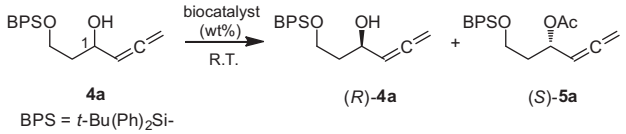
Scheme 1. Enzymatic approaches to chiral α-allenic alcohols.

As a particular interest on lipase AK-catalyzed reaction, Burgess and co-workers rationalized the enzymatic kinetic resolution of secondary alcohols.[10] Despite the fitness of secondary alcohols in these empirical models, the state-of-art of various allenic alcohols remains to be scholarly appreciated. We intended to design a new

* Corresponding authors.
E-mail addresses: shahua@sit.edu.cn (S.-H. Huang), rhong@sioc.ac.cn (R. Hong).

http://dx.doi.org/10.1016/j.tetlet.2015.12.098
0040-4039/

Table 1
Enzymatic kinetic resolution of α-allenic alcohol **4a**[a]



4a (*R*)-**4a** (*S*)-**5a**

BPS = *t*-Bu(Ph)$_2$Si-

Entry	Biocatalyst	Time (h)	C[c] (%)	(*R*)-**4a** ee%[d]	(*S*)-**5a** ee%[d]	*E*
1	PPL	162[b]	<1	—	—	—
2	PLE	162	<1	—	—	—
3	PS	262	42.7	73.9	99.4	741
4	Novozym 435	162[b]	48.3	43.2	44.2	4
5	CAL-A	262	53.8	99.8	86.4	92
6	CAL-B	162[b]	21.5	13.5	49.7	3
7	CRL	162[b]	13.0	5.7	19.7	2
8	AK	197	50.2	99.6	99.0	1630
9	AK (CH_3CN)	144	37.5	59.1	98.8	303
10	AK (Et_2O)	144	38.9	62.7	99.1	423
11	AK (toluene)	144	45.5	82.7	99.3	741
12	AK (hexane)	72	50.7	98.2	98.8	785
13	AK (octane)	72	50.5	97.6	98.6	632

[a] Standard reaction conditions: biocatalyst (amount: 100 Units, 1–20 mg), alcohol **4a** (0.1 mmol), vinyl acetate (8.0 equiv), solvent (1.0 mL), 23 °C, (^{t}BuOMe for entries 1–8; other solvents indicated in parentheses from entries 9–13). Enantiomeric ratio (E) was calculated according to the equation in Ref 11.

[b] The reaction temperature was 37 °C for 162 h.

[c] *C* (conversion) = 100 × (ee_s/(ee_s + ee_p)), ee_s for the recovered starting material, ee_p for the resulting acetate.

[d] Enantiomeric excess (ee) was determined by chiral HPLC. PPL: porcine pancreatic lipase; PLE: pig liver esterase; PS: *Pseudomonas cepacia* lipase; CAL-A: *Candida antarctica* lipase A; CAL-B: *Candida antarctica* lipase B; CRL: *Candida rugosa* lipase.

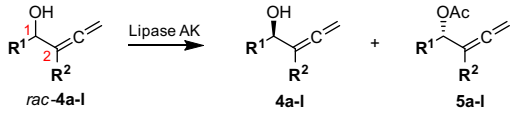
type of substrates to explore EKR, which is compatible with the use of other asymmetric syntheses without a complete new design of catalysis. From this approach, an allene group is considered as a medium group and orientates toward the medium pocket (M) while a variety of substituents may reside in the hydrophobic pocket (L). If this assumption does work, we may readily introduce bulky functional groups in the hydrophobic pocket to expand the scope of enzymatic kinetic resolution in organic synthesis.

As a model substrate, 1-alkyl allenic alcohol **4a** was subjected to the desired kinetic resolution. Several commercially available lipases were examined (Table 1) and lipase AK (*Pseudomonas fluorescens*) was identified as a superior candidate to match the criteria in terms of the reaction rate, isolated yield, and enantioselectivity of products (enantiomeric ratio $E > 1000$)[11] (entry 8). Although Novozym 435 was very efficient for the kinetic resolution of several α-allenic alcohols as in Ma and Li's report,[7b] it was found less effective on a bulky alkyl substituent at C(1) in **4a** (entry 4, $E = 4$). Lipase PS (*Pseudomonas cepacia* lipase) was found to have a close activity to lipase AK (entry 3, $E = 741$) and would be an alternative catalyst in the future application. Polar solvents (CH_3CN and diethyl ether in entries 9 and 10, respectively) have shown less efficiency than nonpolar solvents (toluene, hexane, and octane) in terms of selectivity and reaction time (entries 11–13).[12] With the consideration of solubility in hydrocarbon solvents, ^{t}BuOMe was then chosen as the reaction media for the optimal conditions (entry 8).

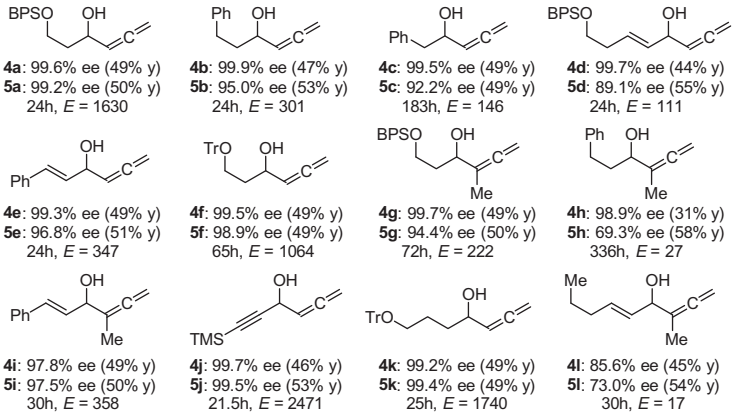
Encouraged by the optimized enzymatic kinetic resolution event, we thus explored the substrate scope, particularly those bearing functional groups at C(1) which could be applied in future polyketide synthesis.[5] The current reaction system offers an excellent stereoselectivity outcome and a broad substrate scope with alkyl substituents at C(1) as well as alkenyl chains (**4a–f**, Table 2). The more encouraging C(2)-methylated allenic alcohols are also tolerated and a good resolution was obtained (**4g–i**). The less sterically bulky substrate **4h** bearing a flexible side chain displayed a lower selectivity ($E = 27$). Since methylated allenic alcohols cannot be prepared directly from propargylic alcohol through the Crabbé homologation,[13] the generality of enzymatic resolution of mono-substituted and 1,1-disubstituted allenes (R^2 = H or Me) offers a powerful approach to access such synthetically useful building blocks.

The current protocol was also applicable for C(1)-aryl substituted allenic alcohols (Table 3), which was more challenging for

Table 2
Enzymatic kinetic resolution of alkyl and alkenyl substituted α-allenic alcohols **4a–l**[a,b14]



rac-**4a-l** **4a-l** **5a-l**



4a: 99.6% ee (49% y)
5a: 99.2% ee (50% y)
24h, *E* = 1630

4b: 99.9% ee (47% y)
5b: 95.0% ee (53% y)
24h, *E* = 301

4c: 99.5% ee (49% y)
5c: 92.2% ee (49% y)
183h, *E* = 146

4d: 99.7% ee (44% y)
5d: 89.1% ee (55% y)
24h, *E* = 111

4e: 99.3% ee (49% y)
5e: 96.8% ee (51% y)
24h, *E* = 347

4f: 99.5% ee (49% y)
5f: 98.9% ee (49% y)
65h, *E* = 1064

4g: 99.7% ee (49% y)
5g: 94.4% ee (50% y)
72h, *E* = 222

4h: 98.9% ee (31% y)
5h: 69.3% ee (58% y)
336h, *E* = 27

4i: 97.8% ee (49% y)
5i: 97.5% ee (50% y)
30h, *E* = 358

4j: 99.7% ee (46% y)
5j: 99.5% ee (53% y)
21.5h, *E* = 2471

4k: 99.2% ee (49% y)
5k: 99.4% ee (49% y)
25h, *E* = 1740

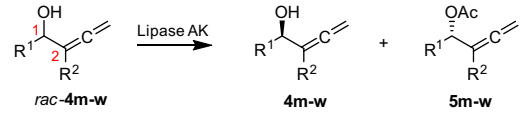
4l: 85.6% ee (45% y)
5l: 73.0% ee (54% y)
30h, *E* = 17

[a] Reaction conditions: allenic alcohol **4** (0.5 mmol), lipase AK (25 mg), vinyl acetate (8.0 equiv), ^{t}BuOMe (5.0 mL), 30 °C; enantiomeric excess (ee) was determined by chiral HPLC; isolated yield in parentheses; enantiomeric ratio (*E*) was calculated according to the equation in Ref. 11.

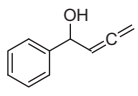
[b] For substrates **4g**, **4h**, **4i**, and **4l**, the reaction was performed at 37 °C with lipase AK (250 mg).

TMS = trimethylsilyl; Tr = triphenylmethyl.

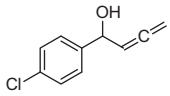
Table 3
Enzymatic kinetic resolution of aryl substituted α-allenic alcohols **4m–w**[a,b]



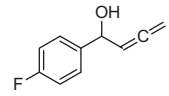
rac-**4m-w** **4m-w** **5m-w**



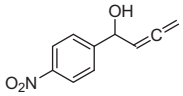
4m: 98.3% ee (45% y)
5m: 90.9% ee (54% y)
19h, *E* = 99



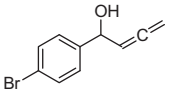
4n: 99.2% ee (47% y)
5n: 94.0% ee (53% y)
44h, *E* = 177



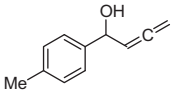
4o: >99.9% ee (49% y)
5o: 99.5% ee (50% y)
41h, *E* = 3031



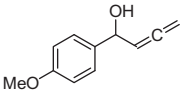
4p: >99.9% ee (49% y)
5p: 97.1% ee (51% y)
46h, *E* = 568



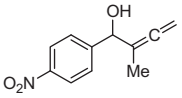
4q: 99.6% ee (46% y)
5q: 93.0% ee (53% y)
58h, *E* = 169



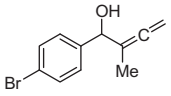
4r: 95.2% ee (47% y)
5r: 88.1% ee (52% y)
59h, *E* = 59



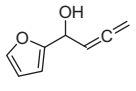
4s: 98.9% ee (34% y)
5s: 69.9% ee (58% y)
71h, *E* = 28



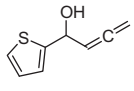
4t: 97.3% ee (48% y)
5t: 88.5% ee (49% y)
67h, *E* = 70



4u: 99.1% ee (38% y)
5u: 71.2% ee (61% y)
131h, *E* = 30



4v: 95.1% ee (7% y)
5v: 60.9% ee (60% y)
16.5h, *E* = 15

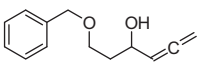


4w: 99.2% ee (34% y)
5w: 53.8% ee (64% y)
26h, *E* = 16

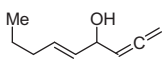
[a] Reaction conditions: allenic alcohol **4** (0.5 mmol), lipase AK (25 mg), vinyl acetate (8.0 equiv), ^{t}BuOMe (5.0 mL), room temperature (23 °C); enantiomeric excess (ee) was determined by chiral HPLC; isolated yield in parentheses; enantiomeric ratio (*E*) was calculated according to the equation in Ref. 11.
[b] For substrates **4t** and **4u**, the reaction was performed at 37 °C with lipase AK (250 mg).

other enzymatic resolution. Both electron-donating and electron-deficient groups were tolerated on the arene moiety to deliver the recycled alcohols **4m–w** and the corresponding acetates **5m–w** in excellent selectivities (*E* > 25). The *para*-fluorinated substrate **4o** gave the best result among all the substrates executed (*E* = 3031). Hetereoaryl substituents (**4v** and **4w**) demonstrated to be not good substances (*E* = 15–16) probably due to their interfering with amino acid residues by a possible hydrogen bonding interaction.[15] Such phenomenon might be severe when an intramolecular hydrogen bonding interaction in **4x** (Scheme 2) disturbs the active site to differentiate a particular enantiomer (*E* = 4) while the resolution of **4f** bearing a bulky protecting group at the primary alcohol resulted in an excellent selectivity (*E* = 1064).

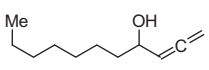
To further rationalize the structure–activity relationship of lipase AK on the resolution of α-allenic alcohols, more substrates bearing a longer carbon chain at C(1) were examined (Scheme 2). A significant loss in selectivity (*E* = 8 and 6 for **4y** and **4z**, respectively) was found with the comparison of similar compounds **4d** and **4f** (Table 2). This deterioration might reside on the closer size between two groups around the secondary alcohol. The lower selectivity of *meta*-chlorinated **4aa** may be attributed to the possible interaction of chlorine with the amino residue in the large hydrophobic pocket (L). On the basis of aforementioned structural features, we assumed that lipase AK may have a deeper medium pocket (M) than that suggested in Novozym 435 (Fig. 1). The hydrophobic pocket (L) of lipase AK can tolerate a bigger size of functional groups, such as in **4a**, **4d**, **4f**, **4g**, and **4k**, that were not realized in previous Letters.[7a,16,17]



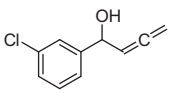
4x: 99.0% ee (10% y)
5x: 11% ee (89% y)
89h, *E* = 4



4y: 95.5% ee (29% y)
5y: 40.0% ee (70% y)
17.5h, *E* = 8



4z: 95.5% ee (29% y)
5z: 39% ee (70% y)
17.5h, *E* = 6



4aa: 98.1% ee (36% y)
5aa: 49.0% ee (63% y)
162h, *E* = 12

Scheme 2. Other substrates for EKR.

To illustrate the utility of the corresponding chiral allenic alcohols, we turned to carry out several transformations to access key intermediates bearing a β-amino alcohol for potentially interesting bioactivity (Scheme 3). A gram-scale enzymatic kinetic resolution of **4a** was realized to give sufficient amount (2.6 g) of (−)-**4a** in >99% ee. Following the Friesen procedure,[18] (−)-**4a** was converted to vinyl iodide **6** (*trans*/*cis* = 9/1) in 89% yield in 2 steps. Although the removal of the carbonyl group was usually undertaken with basic conditions, the facile elimination of vinyl iodide to alkyne[19] rendered the deprotection challenging. After several experiments, we were pleased to find that hydrolysis proceeded smoothly under acid conditions to afford the requisite amino alcohol **7**. The following Suzuki–Miyaura coupling of vinyl iodide also confirmed the sensitivity of such structural motif. Gratifyingly, after optimization, $Pd(dppf)Cl_2$ was effective to promote this reaction with different borane reagents.[20] With the following removal of silyl group, a potentially useful structural variant of **8** and **9** were isolated in good yields, respectively.

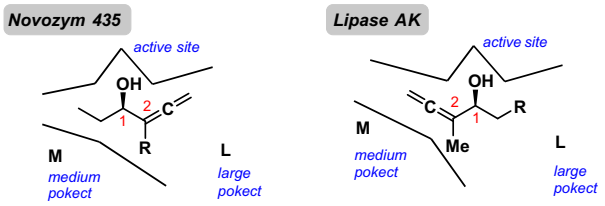
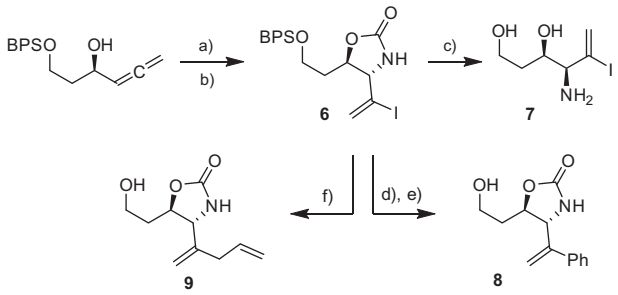


Figure 1. Stereoselectivity rational of lipase AK and Novozym 435 (the enatiomer shown reacts faster with lipase).



Scheme 3. Synthesis of β-amino alcohols: (a) $OCNCOCCl_3$, CH_2Cl_2, 96%; (b) I_2, K_2CO_3, THF, *dr* 9/1, 93%; (c) HCl (6 N), MeOH, 88%; (d) $PhB(OH)_2$, $Pd(dppf)Cl_2$ (10 mol %), THF/H_2O (4/1), NaOH, 91%; (e) TBAF, THF, 81%; (f) AllylB(pin), $Pd(dppf)Cl_2$ (10 mol %); TBAF, THF, 50% for 2 steps.

In summary, we have developed an efficient lipase AK-catalyzed direct kinetic resolution of α-allenic alcohols bearing a variety of alkyl, aryl, alkenyl, and alkynyl substituents at C(1), which is complementary to previous methods to prepare highly enantiomeric pure chiral building blocks.[21,22] The substituent pattern around the secondary alcohol was rationalized by a refined Jones–Burgess model of lipase AK. Further mechanistic insights related to the protein structure of lipase AK and the site-directed mutagenesis to tolerate more challenging substrates in the context of application in natural product synthesis are currently underway in our laboratory.

Acknowledgments

Financial supports from the National Natural Science Foundation of China (21402121 to S.H. Huang and 21472223 to R Hong), the National Basic Research Program of China (2011CB710800 to R. Hong), and the Shanghai Institute of Technology (XTCX2015-16 to S.H. Huang). S.H. Huang thanks the partial support by the Open Funding Project of the State Key Laboratory of Bioreactor Engineering at ECUST. We thank Professor Huilei Yu (ECUST) and Professor Jia-Hai Zhou (SIOC) for invaluable discussions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.12.098.

References and notes

1. Selected monographs and reviews: (a) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon Press, Elsevier: Oxford, 1994; (b) Kazlauskas, R. J.; Bornscheuer, U. T. *Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations*, 2nd ed.; Wiley-VCH: Weinheim, 2005; (c) *Enzyme Catalysis in Organic Synthesis*; Karlheinz, D., Harald, G., Oliver, M., Eds., 3rd ed.; Wiley-VCH: Weinheim, 2012. vols. 1–3; For a very recent review: (d) Friedrich, S.; Hahn, F. *Tetrahedron* **2015**, *71*, 1473.
2. Otte, K. B.; Hauer, B. *Curr. Opin. Biotechnol.* **2015**, *35*, 16.
3. For a very recent perspective, see: Prier, C. K.; Arnold, F. H. *J. Am. Chem. Soc.* **2015**. http://dx.doi.org/10.1021/jacs.5b09348.
4. (a) Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolts, M.; Witholt, B. *Nature* **2001**, *409*, 258; (b) Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K. *Nature* **2012**, *485*, 185.
5. Yang, L.; He, G.; Yin, R.; Zhu, L.; Wang, X.; Hong, R. *Angew. Chem., Int. Ed.* **2014**, *53*, 11600.
6. Selected monographs and reviews: (a) *Modern Allene Chemistry*; Krause, N., Hashimi, A. S. K., Eds.; Wiley-VCH: Weinheim, Germany, 2004; (b) Deng, Y.-Q.; Gu, Z.-H.; Ma, S.-M. *Chin. J. Org. Chem.* **2006**, *26*, 1468; (c) Yu, S.; Ma, S. *Angew. Chem., Int. Ed.* **2012**, *51*, 3074.
7. (a) Burgess, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1990**, *112*, 7434; (b) Xu, D.; Li, Z.; Ma, S. *Chem. Eur. J.* **2002**, *8*, 5012; (c) Xu, D.; Li, Z.; Ma, S. *Tetrahedron: Asymmetry* **2003**, *14*, 3657; (d) Xu, D.; Li, Z.; Ma, S. *Chin. J. Chem.* **2004**, *22*, 310; (e) Asikainen, M.; Krause, N. *Adv. Synth. Catal.* **2009**, *351*, 2305; (f) Deska, J.; Bäckvall, J.-E. *Org. Biomol. Chem.* **2009**, *7*, 3379; (g) Sapu, Manzuna; Bäckvall, C.; Deska, J.-E. *Angew. Chem., Int. Ed.* **2011**, *50*, 9731; (h) Sapu, Manzuna; Deska, C. *J. Org. Biomol. Chem.* **2013**, *11*, 1376.
8. Enzymatic KR of propargylic alcohols: (a) Glänzer, B. I.; Faber, K.; Griengl, H. *Tetrahedron* **1987**, *43*, 5791; (b) Treihou, M.; Fauve, A.; Pougny, J.-K.; Prome, J.-C.; Veschambre, H. *J. Org. Chem.* **1992**, *57*, 3203; (c) Waldinger, C.; Schneider, M.; Botta, M.; Corelli, F.; Summa, V. *Tetrahedron: Asymmetry* **1996**, *7*, 1485; (d) Nakamura, K.; Takenaka, K.; Ohno, A. *Tetrahedron: Asymmetry* **1998**, *9*, 4429; (e) Xu, D.; Li, Z.; Ma, S. *Tetrahedron Lett.* **2003**, *44*, 6343; (f) Li, J.; Kong, W.; Fu, C.; Ma, S. *J. Org. Chem.* **2009**, *74*, 5104.
9. Wang, Y.; Zheng, K.; Hong, R. *J. Am. Chem. Soc.* **2012**, *134*, 4096.
10. (a) Toone, E. J.; Werth, M. J.; Jones, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 4946; (b) Burgess, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1991**, *113*, 6129.
11. Enantiomeric ratio for enzymatic catalysis, $E = \ln[(1 - C)(1 - ees)]/\ln[(1 - C)(1 + ees)]$, *C* for the conversion, ees for the recovered starting material. See: Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.
12. (a) Carrea, G.; Ottolina, G.; Riva, S. *Trends Biotechnol.* **1995**, *13*, 63; (b) Huang, S.-H.; Li, W.; Chen, L.; Xu, J.; Hong, R. *Bioresour. Bioprocess* **2015**, *2*, 10.
13. Crabbé, P.; André, D.; Fillion, H. *Tetrahedron Lett.* **1979**, *20*, 893.
14. The amount of all biocatalysts for screening was 100 Units. For instance, the activity of immobilized lipase AK is 20,000 Units/g. That means only 5 mg of lipase AK was used for optimization in Table 1. However, for the C(1)-methylated allenic alcohols in Table 2, 250 mg of lipase AK (5000 Units) was used for 0.5 mmol of substrate to complete the reaction in a reasonable time (3 days for most of substrates). See the Supporting information for details.
15. Compound **4v** was unstable on silica gel column even the neutralization was taken before the chromatography. Based on the enantioselectivity, conversion, and isolated yield of the ester **5v**, *E* for **4v** was close to **4w**.
16. The absolute configuration of the recovered alcohols **4a** and **4g** were determined to be (*R*) by Mosher's ester. For the determination of absolute configuration, see the Supporting information for details.
17. Burgess and co-workers also found the reversed stereoselectivity when the sizes of two groups were inversed during the lipase AK-catalyzed kinetic resolution, see Ref. 7a.
18. (a) Friesen, R. W. *Tetrahedron Lett.* **1990**, *30*, 4249; (b) Friesen, R. W.; Kolaczewska, A. E. *J. Org. Chem.* **1991**, *56*, 4888.
19. For instance, sodium hydroxide resulted in elimination of iodine to generate a terminal alkyne as major product (>30% yield).
20. (a) Hayashi, T.; Konishi, M.; Kobori, Y.; Kumada, M.; Higuchi, T.; Hirotsu, K. *J. Am. Chem. Soc.* **1984**, *106*, 158; (b) Brown, J. M.; Guiry, P. J. *Inorg. Chim. Acta* **1994**, *220*, 249.
21. An alternative method to access syn-β-amino alcohols from secondary alcohols via palladium-catalyzed allylic C–H amination: Fraunhoffer, K. J.; White, M. C. *J. Am. Chem. Soc.* **2007**, *129*, 7274.
22. The recycled lipase AK was subjected to the second run of the resolution of *rac*-**4b** to deliver the desired products without a considerable deterioration of enantioselectivity (94.6% ee for (*S*)-**4b**, 89.3% ee for (*R*)-**5b**, *E* = 65).