ORGANIC LETTERS

2009
Vol. 11, No. 14
3052−3055

Lipase-Catalyzed Highly Enantioselective Kinetic Resolution of Boron-Containing Chiral Alcohols

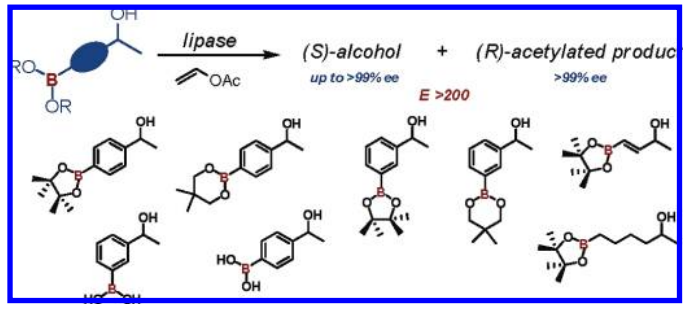
Leandro H. Andrade* and Thiago Barcellos

Institute of Chemistry, University of São Paulo, Av. Prof. Lineu Prestes, 748, CEP 05508-900, São Paulo, SP, Brazil

leandroh@iq.usp.br

Received May 18, 2009

ABSTRACT



The first application of enzymes as catalysts to obtain optically pure boron compounds is described. The kinetic resolution of boron-containing chiral alcohols via enantioselective transesterification catalyzed by lipases was studied. Aromatic, allylic, and aliphatic secondary alcohols containing a boronate ester or boronic acid group were resolved by lipase from *Candida antartica* (CALB), and excellent *E* values (*E* > 200) and high enantiomeric excesses (up to >99%) of both remaining substrates and acetylated product were obtained.

The chemistry of boron-containing compounds is an area of growing interest, mainly due to the wide application of these compounds in the formation of new carbon−carbon bonds.[1,2] Both chiral and achiral boron compounds are useful building blocks in organic synthesis and can be prepared by several synthetic methodologies.[3] Beside the synthetic application, organoboron compounds have found important biological applications, such as new agents for cancer therapy[4] and boron neutron capture therapy (BNCT) agents for tumor-specific radiation therapy.[5]

Despite the importance of chiral boron compounds, to our knowledge, no enzymatic methods have been developed to synthesize these compounds. The lack of biocatalytic studies can be related to the well-known enzyme inhibition potential of boron compounds.[6] However, a survey of the literature reveals that boron compounds inhibit enzymes in a reversible way. Thus, it encouraged us to start studies related to the application of enzymes in enantioselective reactions of organoboron compounds. Herein, we report the first study involving the kinetic resolution (KR) of boron-containing

(1) For reviews concerning Suzuki-Miyaura reaction, see: (a) Martin, R.; Buchwald, S. L. *Acc. Chem. Rev.* **2008**, *41*, 1461. (b) Bellina, F.; Carpita, A.; Rossi, R. *Synthesis* **2004**, *15*, 2419. (c) Kotha, S.; Lahiri, K.; Kashinath, D. *Tetrahedron* **2002**, *58*, 9633. (d) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.

(2) Potassium organotrifluoroborates in palladium-catalyzed cross-coupling reactions: (a) Darses, S.; Genet, J.-P. *Chem. Rev.* **2008**, *108*, 288. (b) Molander, G. A.; Ellis, N. *Acc. Chem. Rev.* **2007**, *40*, 275. (c) Molander, G. A.; Figueroa, R. *Aldrichim. Acta* **2005**, *38*, 49. (d) Stefani, H. A.; Cella, R.; Vieira, A. S. *Tetrahedron* **2007**, *63*, 3623.

(3) Hall, D. G. *Boronic Acids: Preparation, Aplications in Organic Synthesis and Medicine*; Wiley-VCH: Weinheim, Germany, 2005.

(4) For boron compounds as proteasome inhibitors, see: (a) Borissenko, L.; Groll, M. *Chem. Rev.* **2007**, *107*, 687. (b) Dorsey, B. D. *J. Med. Chem.* **2008**, *51*, 106. For full reference, see Supporting Information.

(5) (a) Barth, R. F.; Coderre, J. A.; Vicente, M. G.; Blue, T. E. *Clin. Cancer Res.* **2005**, *11*, 3987. (b) Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F.-G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. *Chem. Rev.* **1998**, *98*, 1515.

(6) Yang, W.; Gao, X.; Wang, B. *Med. Chem. Res.* **2003**, *23*, 346.

10.1021/ol901091f CCC: $40.75
Published on Web 06/24/2009

chiral alcohols via enantioselective transesterification catalyzed by lipases. Beside kinetic resolution using lipases,[7] chiral alcohols can also be prepared by ketone reduction mediated by alcohols dehydrogenases.[8] However, in this work, we explored the effect of different groups attached at the chiral center and the nature of the boron species in the lipase-catalyzed enantioselective transesterification. In this way, aromatic, allylic and aliphatic secondary alcohols containing a boronate ester or boronic acid group were selected as substrates (Figure 1).

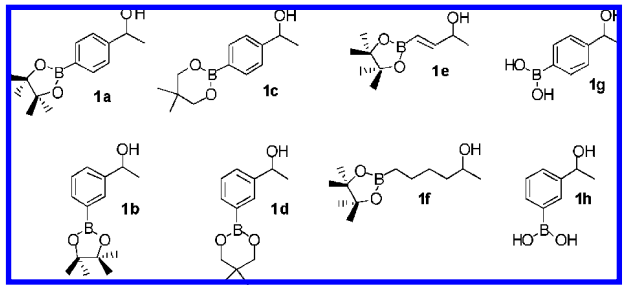


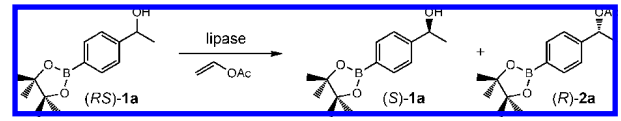
Figure 1. Boron-containing chiral alcohols.

As the selected boron compounds are not commercially available, they were prepared using different methods.[9] In summary, the benzylic alcohols **1a**−**d**, **g**, **h** containing a boronate group were prepared from 4- and 3-acetyl phenylboronic acid as starting material. Meanwhile, the allylic **1e** and aliphatic secondary alcohols **1f** were prepared from their precursor alkyne and alkene, respectively, by hydroboration reaction.

Having in hand all the racemic organoboron compounds **1a**−**h**, we selected several lipases to be used as biocatalysts for the enantioselective transesterification (acylation) of the hydroxyl group attached at the chiral center. For the lipase screening, we chose vinyl acetate as the acyl donor, hexane as solvent and the compound (*RS*)-**1a** as the substrate model (Table 1).

The lipases from *A. niger*, *C. cylindracea*, *C. rugosa*, *M. javanicus*, porcine pancreas and *P. camemberti* showed very low (≤3%) or no activity with the substrate (*RS*)-**1a**. (Table 1, entries 1, 3−7). However, we observed that the kinetic resolution mediated by lipases from *C. antarctica* (CAL-B),[10] *P. cepacia* [immobilized on ceramic (Amano PS-CII) or diatomite (Amano PS-DI), and nonimmobilized (Amano PS)] and *P. fluorescens* showed excellent enantiomeric ratios[11] (*E* value, $E > 200$) and enantiomeric excesses for

Table 1. Screening of Lipases for the Kinetic Resolution of (*RS*)-**1a**[a]



		ee (%)			
entry	lipase source	(*S*)-**1a**[b]	(*R*)-**2a**[c]	*c* (%)[d]	*E*[e]
1	*Aspergillus niger* (Amano A)	3	>99	3	>200
2	*Candida antarctica* (CAL-B, Novozym 435)	>99	>99	50	>200
3	*Candida cylindracea* (Fluka)	–	–	–	–
4	*Candida rugosa* (Amano, type VII)	–	–	–	–
5	*Mucor javanicus* (Amano M)	2	>99	2	>200
6	*Penicillium camemberti* (Amano G)	3	>99	3	>200
7	Porcine pancreas (Sigma, type II)	–	–	–	–
8	*Pseudomonas cepacia* (Amano PS-DI)	>99	>99	50	>200
9	*Pseudomonas cepacia* (Amano PS-CII)	>99	>99	50	>200
10	*Pseudomonas fluorescens* (Amano AK)	40	>99	29	>200
11	*Pseudomonas cepacia* (Amano PS)	11	>99	10	>200
12	*Pseudomonas* sp. (Sigma-Aldrich)	6	>99	6	>200

[a] General conditions: Substrate (0.2 mmol), lipase (40 mg), vinyl acetate (0.6 mmol), *n*-hexane (10 mL), 30 °C, 150 rpm, 6 h. (−) = no reaction. [b] Determined by chiral HPLC analysis. [c] Determined by chiral GC analysis. [d] Conversion: $c = ee_S/(ee_S + ee_P)$. [e] $E = \{\ln[ee_P(1 - ee_S)]/(ee_P + ee_S)\}/\{\ln[ee_P(1 + ee_S)]/(ee_P + ee_S)\}$.

(*R*)-**2a** (>99%). For all these enzymes, the KR occurred by the enantioselective acylation of the (*R*)-enantiomer from (*RS*)-**1a**.

CAL-B was selected as the enzyme to be applied in the following studies. This decision was also based on the wide application of CAL-B as biocatalyst for kinetic resolution and dynamic kinetic resolution of amines[12] and alcohols.[13] Besides, CAL-B is less expensive than the lipases from *Pseudomonas* (Amano).

KR of (*RS*)-**1a** was performed by using different lipase concentrations (0.005, 0.01, 0.02, 0.03, and 0.04 g) and the same amount of the substrate (0.2 mmol; Table 2, entries 1−5).

In this study, we found that for the perfect kinetic resolution of the (*RS*)-**1a** (0.2 mmol), the required amount of CAL-B was 0.02 g (Table 2, entry 3). In addition, as expected for the same reaction time (6 h), lower enzyme amounts than 0.02 g led to a small decrease in conversion, but excellent *ee* for the product (*R*)-**2a** (>99%) and excellent *E* value ($E > 200$, Table 2, entries 4 and 5). Unfortunately,

(7) (a) Reviews Gotor-Fernández, V.; Brieva, R.; Gotor, V. *J. Mol. Catal. B: Enzym.* **2006**, *40*, 111. (b) Ghanem, A. *Tetrahedron* **2007**, *63*, 1721.

(8) (a) Faber, K. *Biotransformations in Organic Chemistry*, 5th ed.; Spring-Verlag: Berlin, Germany, 2004. (b) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994. (c) De Wildeman, S. M. A.; Sonke, T.; Schoemaker, H. E.; May, O. *Acc. Chem. Res.* **2007**, *40*, 1260. (d) Matsuda, T.; Yamanaka, R.; Nakamura, K. *Tetrahedron: Asymmetry* **2009**, *20*, 513.

(9) The synthesis and characterization for all compounds are reported in Supporting Information.

(10) Immobilized and commercially available as Novozym 435.

Table 2. Evaluation of CAL-B Amount and Reaction Time for Kinetic Resolution of (*RS*)-**1a**[a]

entry	CAL-B (mg)	time (h)	*ee* (%) (*S*)-**1a**[b]	*ee* (%) (*R*)-**2a**[c]	*c* (%)[d]	*E*[e]
1	40	6	>99	>99	50	>200
2	30	6	>99	>99	50	>200
3	20	6	>99	>99	50	>200
4	10	6	95	>99	49	>200
5	5	6	75	>99	43	>200
6	20	4	92	>99	48	>200
7	20	2	70	>99	41	>200
10	20	0.5	24	>99	20	>200

[a] General conditions: Substrate (0.2 mmol), vinyl acetate (0.6 mmol), *n*-hexane (10 mL), 30 °C, 150 rpm. [b] Determined by chiral HPLC analysis. [c] Determined by chiral GC analysis. [d] Conversion: $c = ee_S/(ee_S + ee_P)$. [e] $E = \{\ln[ee_P(1 - ee_S)]/(ee_P + ee_S)\}/\{\ln[ee_P(1 + ee_S)]/(ee_P + ee_S)\}$.

a decrease in conversion was observed when the KR was performed with the same amount of CAL-B (0.02 g) and shorter reaction times than 6 h (0.5, 2, and 4 h; Table 2). However, the enantiomeric excess for the product (*R*)-**2a** was >99% and $E > 200$.

Encouraged by these excellent results, we employed the CAL-B in the kinetic resolution of different boron compounds (*RS*)-**1b**−**f** (Table 3). We applied the best reaction

Table 3. Evaluation of the Kinetic Resolution of (*RS*)-**1b**−**f** by CAL-B-Catalyzed Enantioselective Transesterification[a]

entry	substrate	time (h)	*ee* (%) (*S*)-**1**[b]	*ee* (%) (*R*)-**2**[c]	*c* (%)[d]	*E*[e]
1	(*RS*)-**1b**	6	>99	>99	50	>200
2	(*RS*)-**1c**	6	>99	>99	50	>200
3	(*RS*)-**1d**	6	>99	>99	50	>200
4	(*RS*)-**1e**	6	82	>99	45	>200
5		8	92	>99	48	>200
6		10	96	>99	49	>200
7		12	99	>99	50	>200
8	(*RS*)-**1f**	6	77	>99	44	>200
9		8	86	>99	46	>200
10		10	93	>99	48	>200
11		12	97	>99	49	>200
12		14	>99	>99	50	>200

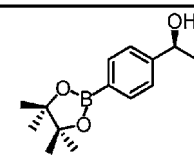
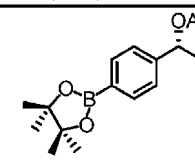
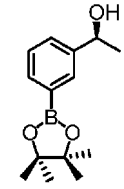
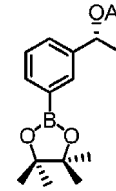
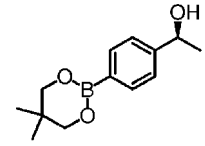
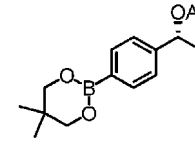
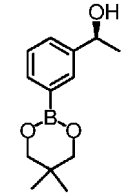
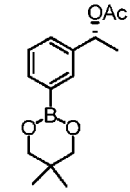
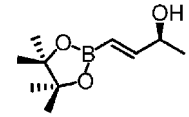
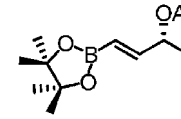
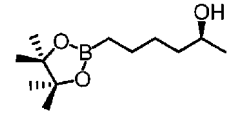
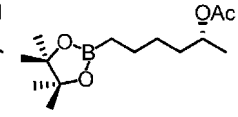
[a] General conditions: Substrate (0.2 mmol), lipase (20 mg), vinyl acetate (0.6 mmol), *n*-hexane (10 mL), 30 °C, 150 rpm. [b] Determined by chiral HPLC analysis. [c] Determined by chiral GC analysis. [d] Conversion: $c = ee_S/(ee_S + ee_P)$. [e] $E = \{\ln[ee_P(1 - ee_S)]/(ee_P + ee_S)\}/\{\ln[ee_P(1 + ee_S)]/(ee_P + ee_S)\}$.

condition described previously for KR of (*RS*)-**1a** to all the boron compounds **1b**−**f** in a small scale reaction.

Fortunately, for all compounds employed, the CAL-B catalyzed the acylation of the (*R*)-enantiomer with excellent enantioselectivity ($E > 200$). The product (*R*)-ester **2b**−**f** and the remaining (*S*)-alcohol **1b**−**f** showed excellent enantiomeric excess (>99%). The kinetic resolution of boron-containing benzylic alcohols **1b**−**d** were efficiently achieved within 6 h. However, the boron-containing allylic **1e** and aliphatic **1f** alcohols required 12 and 14 h for efficient resolution.

After establishment of the appropriate condition for the kinetic resolutions of **1a**−**f**, the reactions were again performed, but in a higher scale to obtain both enantiomers separately (Table 4).

Table 4. Kinetic Resolution of Boron-Containing Secondary Alcohols (*RS*)-**1b**−**f** Mediated by CAL-B[a]

entry	substrate	isolated yield % (*ee* %)[b]	
1	(*RS*)-**1a**	 (*S*)-**1a**, 44 (>99)	 (*R*)-**2a**, 43 (>99)
2	(*RS*)-**1b**	 (*S*)-**1b**, 45 (>99)	 (*R*)-**2b**, 46 (>99)
3	(*RS*)-**1c**	 (*S*)-**1c**, 39 (>99)	 (*R*)-**2c**, 42 (>99)
4	(*RS*)-**1d**	 (*S*)-**1d**, 35 (>99)	 (*R*)-**2d**, 40 (>99)
5	(*RS*)-**1e**[c]	 (*S*)-**1e**, 45 (>99)	 (*R*)-**2e**, 43 (>99)
6	(*RS*)-**1f**[d]	 (*S*)-**1f**, 45 (>99)	 (*R*)-**2f**, 47 (>99)

[a] General conditions: Substrate (1.5 mmol), CAL-B (0.150 g), vinyl acetate (4.5 mmol), *n*-hexane (150 mL), 30 °C, 150 rpm, 6 h. [b] Determined by chiral HPLC or GC analysis (see Supporting Information). [c] Reaction time 12 h. [d] Reaction time 14 h.

As can be seen in Table 4, the kinetic resolution of all boron compounds in a large scale reaction afforded the

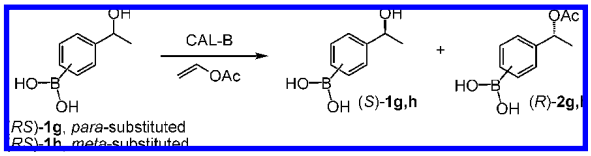
(11) (a) Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294. (b) Straathof, A. J. J.; Jongejan, J. A. *Enzyme Microb. Technol.* **1997**, *21*, 559.

alcohols (*S*)-**1a**−**f** and the ester (*R*)-**2a**−**f** in excellent enantiomeric excesses (>99%), and with high isolated yields (up to 47%).

The stereochemistry of the resolved boron-containing chiral alcohols and their acetates is in accordance with Kazlauskas' rule.[14] The absolute configuration for the compounds **1a**−**f** and **2a**−**f** was attributed after some chemical transformations to a known chiral compound.[15]

In view of the wide application of boronic acid in coupling reaction,[1,3] we decided to study the kinetic resolution of the boronic acids-containing secondary alcohol **1g**, **h** using CAL-B at different reaction times (8−48 h). Fortunately, by using these compounds, we observed the corresponding ester **2g**, **h** with excellent enantioselectivity (*ee* > 99%, *E* > 200, Table 5).

Table 5. Enzymatic Kinetic Resolution of 4- and 3-(1-hydroxyethyl)-phenylboronic acid (*RS*)-**1g**,**h**[a]



			ee (%)			
entry	substrate	time (h)	(*S*)-**1**[b]	(*R*)-**2**[c]	*c* (%)[d]	*E*[e]
1	(*RS*)-**1g**	6	56	>99	36	>200
2		12	58	>99	37	>200
3		24	71	>99	42	>200
4		48	91	>99	48	>200
5	(*RS*)-**1h**	24	3	>99	3	>200
6		48	5	>99	5	>200

[a] General conditions: Substrate (0.2 mmol), lipase (40 mg), vinyl acetate (0.6 mmol), *n*-hexane/THF (4:1, 10 mL), 30 °C, 150 rpm. [b] Determined by chiral HPLC analysis after esterification reaction of the boronic acid group with pinacol. [c] Determined by chiral GC analysis after esterification reaction of the boronic acid group with pinacol. [d] Conversion: $c = ee_S/(ee_S + ee_P)$. [e] $E = \{\ln[ee_P(1 - ee_S)]/(ee_P + ee_S)\}/\{\ln[ee_P(1 + ee_S)]/(ee_P + ee_S)\}$.

As we can see from the results with boronic acids **1g**, **h**, the enantioselectivity of the lipase CAL-B was not affected by the presence of the boronic acid group. The (*R*)-enantiomers were acetylated in excellent *ee*. On the other hand, to achieve the almost complete kinetic resolution of the (*RS*)-**1g**, the reaction time was higher than the corresponding boronic ester **1a** (48 h for **1g** and 6 h for **1a**). Meanwhile, the kinetic resolution of (*RS*)-**1h** gave the product (*R*)-**2h** with 5% conversion after 48 h, but with an excellent *E*-value (*E* > 200). In comparison to the results with the boronic ester **1b** in which the conversion was maximun (50%) after 6 h reaction, we postulate that the low conversion for (*RS*)-**1h** is related to a lipase inhibition. Moreover, some authors reported that the boronic acid group attached to an organic compound exhibited the potential for greater enzyme inhibition than the corresponding boronic ester.[6,16] To obtain more information about these results, further studies regarding enzymatic inhibition caused by the boronic acid group from compounds **1g**, **h** are in progress in our lab.

In conclusion, we have demonstrated a new strategy to prepare optically active boron-containing chiral alcohols using kinetic resolution catalyzed by lipases. This is the first report of the application of enzymes as catalysts to obtain optically pure boron compounds. We expect that this enzymatic protocol will prove to be useful in the synthesis of chiral boron-containing organic compounds.

Acknowledgment. We thank Professor Gary Molander (University of Pennsylvania) for helpful discussions, and FAPESP (State of São Paulo Research Foundation) and CNPq (National Counsel of Technological and Scientific Development) for financial support.

Supporting Information Available: Complete experimental procedures, characterization data (^{1}H, ^{13}C, and ^{11}B NMR, IR and mass spectrometry) for all compounds and enantiomeric purity data (chiral CG and HPLC). This material is available free of charge via the Internet at http://pubs.acs.org.

OL901091F

(12) Reviews: (a) Ahn, Y.; Ko, S.-B.; Kim, M.-J.; Park, J. *Coord. Chem. Rev.* **2008**, *252*, 647. (b) Gotor-Fernández, V.; Busto, E.; Gotor, V. *Adv. Synth. Catal.* **2006**, *348*, 797.

(13) (a) Examples: reference 12a. (b) Marr, A. C.; Pollock, C. L.; Saunders, G. C. *Organometallics* **2007**, *26*, 3283. (c) Martin-Matute, B.; Edin, M.; Bogár, K.; Kaynak, F. B.; Bäckvall, J.-E. *J. Am. Chem. Soc.* **2005**, *127*, 8817. (d) Magnusson, A. O.; Rotticci-Mulder, J. C.; Santagostino, A.; Hult, K. *ChemBioChem* **2005**, *6*, 1051.

(14) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656.

(15) For example: To assign the absolute configuration of boronate esters **1a** and **1b** we performed their Suzuki-Miyaura cross-coupling reaction with bromobenzene. The resulting biaryl alcohol derivatives were obtained with retention of configuration. For more detailed information, see Supporting Information.

(16) Ebdrup, S.; Jacobsen, P.; Farrington, A. D.; Vedso, P. *Bioorg. Med. Chem.* **2005**, *13*, 2305.