EurJOC European Journal of Organic Chemistry

SHORT COMMUNICATION

DOI: 10.1002/ejoc.201200689

First Total Syntheses of Oresbiusins A and B, Their Antipodes, and Racemates: Configuration Revision and Anti-HIV Activity

Jih Ru Hwu,*[a,b] Tirumala G. Varadaraju,[a] Ibrahim S. Abd-Elazem,[c] and Ru Chih C. Huang*[c]

Keywords: Chirality / Biological activity / Configuration determination / Natural products / Total synthesis

The first total syntheses of oresbiusin A in the (+)-, (–)-, and (±)-forms were accomplished in five steps with overall yields of ca. 70 %. The key intermediates with optical activity were generated through a Sharpless asymmetric dihydroxylation reaction. These efforts allowed us to establish the absolute configuration of this natural product with dextrorotary and a 2*S* configuration. In addition, the total syntheses of both enantiomers of oresbiusin B along with its racemate were accomplished in six steps with overall yields of 58 %. Among these compounds, the unnatural oresbiusin A with a levorotary rotation was found to possess dose dependent anti-HIV-1 activity in H9 cells, whereas the other compounds were inactive.

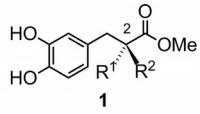
Introduction

Found in open dry rocky areas of Sichuan and Yunnan, China, the dried plant of *Isodom oresbius* has been used in traditional Chinese folk medicine for years. It is often used in the treatment of blood clots in internal organs of the body.[1] In 1996, Huang and co-workers[2] reported the isolation of oresbiusins A (**1a**) and B (**2a**) from the same resource. By possessing a common α-hydroxy ester moiety, their absolute configuration was unfortunately not assigned. In 2010, Zhu and co-workers[3] disclosed that oresbiusin A (**1b**) isolated from the *Ranunculus chinensis* was a light yellow gum with $[\alpha]_D^{22}$ = +7.9 (*c* = 0.410, MeOH). It was assigned as the (2*R*) enantiomer. In 2009, Synder and Kontes[4] published their synthesis of enantiomerically enriched (*R*)-oresbiusin B derivatives from naturally occurring (*R*)-rosmarinic acid. They also utilized its benzylated species in the total syntheses of polyhydroxylated complex natural products.

[a] Department of Chemistry and Frontier Research Center on Fundamental and Applied Sciences of Matters
National Tsing Hua University
Hsinchu, Taiwan, 30013 Republic of China
Fax: +886-3-572-1594
E-mail: jrhwu@mx.nthu.edu.tw
Homepage: http://chem-en.web.nthu.edu.tw/files/13-1117-33468.php
[b] Department of Chemistry
National Central University
Jhongli City, Taiwan 32001 Republic of China
[c] Department of Biology
The Johns Hopkins University
3400 North Charles Street, Baltimore, MD 21218, USA
Fax: +1-410-516-5213
E-mail: rhuang@jhu.edu
Homepage: http://www.bio.jhu.edu/Faculty/Huang/Default.html

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201200689.

We noticed that several optically pure caffeic acid derivatives isolated from the aqueous extracts of *Salvia miltiorrhiza* (Dan-shen) possess an α-hydroxy or α-acetoxy moiety as those in oresbiusins A (**1**) and B (**2**).[5,6] These caffeic acids exhibit various biological activities of importance, including anti-ischemia reperfusion, antithrombosis, antihypertension, antioxidation, antitumor,[6] and antihuman immunodeficiency virus (HIV).[7] On the other hand, α-hydroxy acids and esters are frequently encountered as parts of biologically active compounds, such as methyl 4-hydroxyphenyl lactate, ragaglitazar, tesaglitazar, and their analogues.[8,9] In these compounds, the stereochemistry of the hydroxy functional group is essential to the anticancer and antidiabetic activities.[8,9]

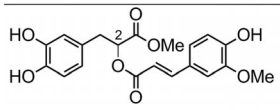
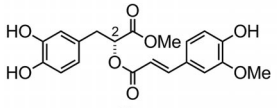
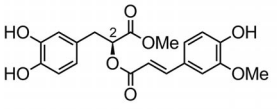


1

	R^1	R^2	Configuration	Note	Prepared with	Synthetic product [α]
a	H (or OH)	OH (or H)	–	isolated by Huang (1996)	OsO_4, NMO	0°
b	OH	H	*R*	proposed by Zou (2010)	AD-mix-α	–7.8°
c	H	OH	*S*	natural (revised, 2012)	AD-mix-β	+9.1°

We have been working on a project devoted to the development of natural products and their derivatives with activity against HIV. We have found that the activity can be improved significantly by converting the naturally occurring nordihydroguaiaretic acid (i.e., NDGA) into its tetramethylated derivative.[10,11] Furthermore, several heterocycle-

containing NDGA derivatives with good aqueous solubility and stability have been synthesized that exhibit appealing anti-HIV activity.[12] Recently, we have also developed perglycosylated NDGA as anticancer agents.[13]

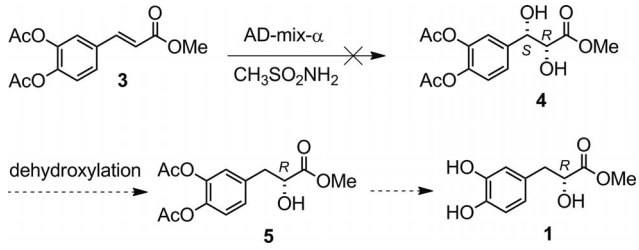
	Configuration	Note	Prepared with	Synthetic product [α]
 **2a**	–	isolated by Huang (1996)	OsO_4, NMO	0°
 **2b**	*R*	synthetic (2012)	AD-mix-α	+21.0°
 **2c**	*S*	synthetic (2012)	AD-mix-β	–29.3°

Our long-term interests in the development of new anti-HIV agents promoted us to investigate the syntheses of compounds in the family of oresbiusins. To the best of our knowledge, no total syntheses of oresbiusins A and B have been reported to date. Here we describe our success on the first total syntheses of all optical isomers of oresbiusins A (**1b** and **1c**) and B (**2b** and **2c**) in highly enantiomeric excess (> 99%*ee*) as well as their racemates (i.e., **1a** and **2a**). We also discovered that the absolute stereochemistry of the natural oresbiusin A should be revised from the (2*R*)- to the (2*S*)-configuration (i.e., **1c**). Among the newly synthesized six oresbiusins A and B, the oresbiusin A (**1b**) was found to exhibit the biological activity against HIV-1 replication.

Results and Discussion

For the total syntheses of optically active oresbiusins A and B, our first stage was to obtain a common asymmetric lactate intermediate, such as compound **5** (Scheme 1). We envisioned that Sharpless asymmetric dihydroxylation[14] of α,β-unsaturated ester **3** would fulfil our need of introducing the stereogenic centers. Subsequently, selective dehydroxylation at the benzylic position of 1,2-diol **4** would afford α-hydroxy ester **5**. We selected the acetyl protecting group in phenol derivative **3** because (1) it can be easily removed in the final step to complete the total synthesis, (2) acetylated analogues could serve as candidates for the identification of an inhibitor-binding site of HIV-1 integrase through affinity acetylation,[15] and (3) several acetylated Dan-shen derivatives with closely related moieties in our target molecules show antimyocardial ischemia activity.[16]

We applied AD-mix-α[14] to the asymmetric dihydroxylation of (3,4-diacetoxy)cinnamate (**3**)[17] in the presence of methanesulfonamide at 0 °C. After a reaction time of 24 h and longer, only the starting material was recovered. When this reaction was performed at elevated temperatures (e.g., 40 °C) for 24 h, the starting material decomposed. Changing the solvent to acetone or water along with increasing the amount of AD-mix-α also met with failure.

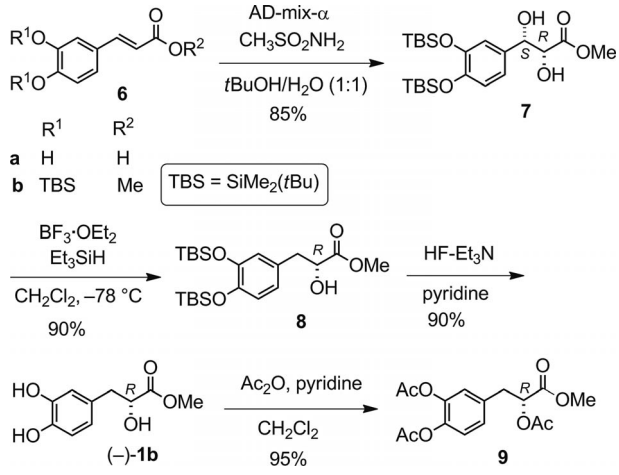


Scheme 1. Initial attempts at the asymmetric synthesis of acetylated lactate **5**.

The components in the reagent AD-mix-α include potassium osmate [$K_2OsO_2(OH)_4$], $(DHQ)_2$-PHAL, $K_3Fe(CN)_6$, and K_2CO_3.[14] Serving as a nonvolatile Os source, potassium osmate reacts with an inorganic co-oxidant $K_3Fe(CN)_6$ to generate OsO_4 in situ.[18] Because OsO_4 is electrophilic,[19] the rate of its osmylation of electron-deficient olefins (e.g., RR′C=C–CO_2R′′) could be very low. However, some unsaturated esters still give satisfactory reaction rates at room temperature under the standard asymmetric dihydroxylation conditions. We believe that difficulties in the reaction of AD-mix-α with substrate **3** came from the additional two acetyl groups attached to the styrene moiety. With its electron-withdrawing nature,[20] the acetyl groups decreased the electron density of the C=C bond in α,β-unsaturated ester **3** and, thus, impeded its reaction with OsO_4.

To circumvent the problem of osmylation, we considered optically active silylated lactate **8** as an ideal common intermediate in the asymmetric total syntheses of oresbiusins A and B (Scheme 2). The *tert*-butyldimethylsiloxyl [i.e., –OSiMe_2(*t*Bu)] moiety therein possesses electron-donating capability. Therefore, it could serve our purpose of introducing chirality onto α,β-unsaturated ester **6b** by using AD-mix-α or AD-mix-β. AD-mix-α differs from AD-mix-β by containing the chiral ligand $(DHQD)_2$-PHAL instead of $(DHQ)_2$-PHAL (DHQD: dihydroquinine; DHQ: dihydroquinidine; PHAL: phthalazine).

Our synthesis commenced with caffeic acid (**6a**), which can be converted into 3,4-bis(*tert*-butyldimethylsiloxy)-cinnamate (**6b**)[21] in two steps by Duynstee's procedure (Scheme 2). Asymmetric dihydroxylation of cinnamate **6b**[21] with AD-mix-α[14] (1.40 g/1.00 mmol) in the presence of methanesulfonamide (1.0 equiv.) afforded diol (2*R*,3*S*)-**7** in 85% yield with 99%*ee* and $[\alpha]_D^{26}$ = –2.2. The stereogenic centers were assigned on the basis of establish methods reported by various research groups.[18,22] Moreover, Sharpless asymmetric dihydroxylation[14] of diversely substituted cinnamates in the presence of AD-mix-α has been reported to proceed smoothly to afford a diol with the absolute configuration as 2*R*,3*S* at the two newly generated stereogenic centers.[18,22] Then, we dehydroxylated optically active diol **7** by using Et_3SiH[23] (1.1 equiv.) in the presence of CF_3COOH at different temperatures. The optimal yield we obtained was only 20% (Table 1, entries 1–3). Replace-



Scheme 2. Asymmetric total synthesis of (*R*)-oresbiusin A (**1**).

ment of CF_3COOH with $BF_3{\cdot}OEt_2$[24] allowed us to improve the yield to 90% by performing the dehydroxylation reaction at –78 °C over 2.0 h (Table 1, entry 5). White solids of desired product **8** (m.p. 46–47 °C) displayed positive specific rotation with $[\alpha]_D^{26}$ = +7.0 and its enantiomeric excess was determined to be >99% by HPLC analysis with a Chiralcel OD column. Finally, treatment of (+)-**8** with HF–Et_3N in pyridine[21] at room temperature gave target **1b** in 90% yield as a pale yellow gum. Its ^1H NMR spectrum, ^{13}C NMR spectrum, and HRMS were in full agreement with those of the naturally isolated oresbiusin A (**1a**).[2]

Table 1. Reductive dehydroxylation of optically pure diol **7**.

Entry	Acid (equiv.)	Et_3SiH [equiv.]	*T* [°C]	*t* [h]	Yield [%]
1	CF_3COOH (5.0)	1.1	r.t.	12	0
2	CF_3COOH (5.0)	1.1	0	4.0	0
3	CF_3COOH (5.0)	1.1	–78	2.0	20
4	$BF_3{\cdot}OEt_2$ (2.0)	2.0	0	5.0	45
5	$BF_3{\cdot}OEt_2$ (2.0)	2.0	–78	2.0	90

To our surprise, synthetic target **1b** displayed a levorotary specific rotation with $[\alpha]_D^{20}$ = –7.8 (*c* = 0.600, MeOH), which did not match that of isolated natural **1c** with $[\alpha]_D^{22}$ = +7.9 (*c* = 0.410, MeOH).[25] Recently, Dong et al.[16] reported triacetylate **9** with $[\alpha]_D^{20}$ = +3.6 (*c* = 1.0, $CHCl_3$). Therefore, we acetylated triol **1b** with acetic anhydride in pyridine to produce **9** (95% yield) for comparison, and we found that $[\alpha]_D^{21}$ = +4.8 (*c* = 1.0, $CHCl_3$), which is consistent with that reported by Dong et al. To this stage, we are certain about the 2*R* configuration of triol **1b**.

Furthermore, we decided to synthesize the antipode of **1b** to reconfirm the optical rotation of oresbiusin A. We repeated the aforementioned synthetic strategy for the total synthesis of (2*S*)-oresbiusin A (**1c**) except that AD-mix-β[14] was replaced by AD-mix-α. Accordingly, enantiomerically enriched diol (+)-(2*S*,3*R*)-**7** and dehydroxylated (–)-(2*S*)-methyl lactate (**8**) were generated with >99% *ee*. Finally, desilylation of lactate **8** produced (+)-(2*S*)-oresbiusin A (**1c**)[25] in 95% yield with $[\alpha]_D^{21}$ = +9.1, of which the spectroscopic data were fully consistent with those of **1a** and **1b**.

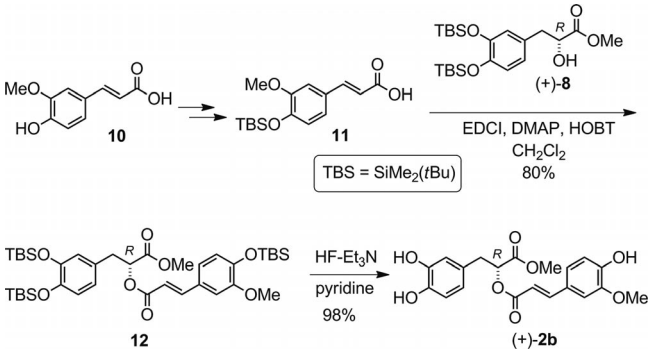
Both compounds **1b**[3] and **1c** are pale yellow gums that turned to foam under low pressure. Accordingly, we were unable to obtain the crystal structures of **1b** and **1c** to establish their absolute configuration. Nevertheless, the absolute configuration of **1b** was confirmed by comparison of the specific rotation of synthetic **9** with the data reported by Dong et al.[16]

Zou and co-workers[3] assigned isolated oresbiusin A with dextrorotary as the (2*R*)-enantiomer (i.e., **1b**). We adopted the well-established Sharpless asymmetric dihydroxylation method with the reagent AD-mix-β to generate the compound of the same skeleton with dextrorotary as well. Nevertheless, many reports unanimously indicate that asymmetric dihydroxylation on α,β-unsaturated esters with AD-mix-β affords the products with 2*S* configuration. *Accordingly, we believe that the configuration at C2 should be revised from R to S for naturally occurring oresbiusin A.*

In addition to (+)- and (–)-oresbiusin A, we prepared (±)-oresbiusin A for anti-HIV assay. All of the synthetic steps were the same as those described in Scheme 2 except that AD-mix-α was replaced by osmium tetroxide[26] and *N*-methylmorpholine *N*-oxide (NMO).

Our second objective was to perform an asymmetric total synthesis of oresbiusin B (**2**). To the best of our knowledge, its absolute configuration has never been given. Therefore, we decided to obtain molecules of its skeleton with dextrorotary and levorotary specific rotations as well as the racemic form. As shown in Scheme 3, our synthesis started with ferulic acid (**10**), which can be converted into silylated intermediate **11** by following the Snyder's method.[4] Esterification of cinnamic acid **11** with (+)-(*R*)-methyl lactate **8** in the presence of 4-dimethylaminopyridine (DMAP), 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDCI), and 1-hydroxybenzotriazole (HOBt) provided trisilylated intermediate (+)-(*R*)-**12** as a yellow gum in 80% yield with >99% *ee* and $[\alpha]_D^{19}$ = +43.0. This compound exhibited two singlets between 3.70 and 3.81 ppm in its ^1H NMR spectrum for the six protons resulting from the methoxy and methyl ester groups. In its ^{13}C NMR spectrum, resonance occurred at 166.39 and 170.40 ppm for the α-carboxy and the α,β-unsaturated ester sp^2 carbon atoms, respectively. Moreover, its exact mass was detected as 730.3742, which well agreed to the theoretical value of 730.3752 for $C_{38}H_{74}O_8Si_3$. Finally, treatment of (+)-(*R*)-**12** with HF–Et_3N in pyridine[21] gave (+)-(*R*)-oresbiusin B (**2b**) in 98% yield as a yellow gum with $[\alpha]_D^{19}$ = +21.0. Its ^1H NMR, ^{13}C NMR, and HRMS data are consistent with those of the naturally isolated oresbiusin B (**2**) reported by Huang and co-workers.[2]

Furthermore, the enantiomer of (+)-(*R*)-**2b** was synthesized through the same steps shown in Scheme 3 except that (–)-(*S*)-methyl lactate **8** was used to replace its antipode (+)-(*R*)-**8**. As the result, a yellow gum of (–)-(*S*)-oresbiusin B (**2c**) with $[\alpha]_D^{21}$ = –29.3 was generated with success. Moreover, we utilized the aforementioned synthetic strategy and intermediate (±)-methyl lactate **8** to accomplish a total synthesis of (±)-oresbiusin B (**2a**).



Scheme 3. Asymmetric total synthesis of (+)-(*R*)-oresbiusin B (**2**).

Investigation on the activities of the newly synthesized six oresbiusins A and B against HIV-1_{RTMF} infection was performed in H9 cells. We measured their viral replication in the absence and presence of compounds **1a**–**c** and **2a**–**c** at concentrations of 0, 1.25, 2.5, 5.0, 10, 20, 40, and 80 μM by using the HIV-1 p24 antigen ELISA after 8 d as described by Huang et al.[7] The optically active isomers of oresbiusin A (**1b** and **1c**) showed striking differences in their anti-HIV activities. Only (*R*)-**1b** showed dose-dependent anti-HIV activity, whereas both (*S*)-**1c** and racemate **1a** were inactive (Figure 1). Meanwhile, **2a**–**c** were found to be inactive.

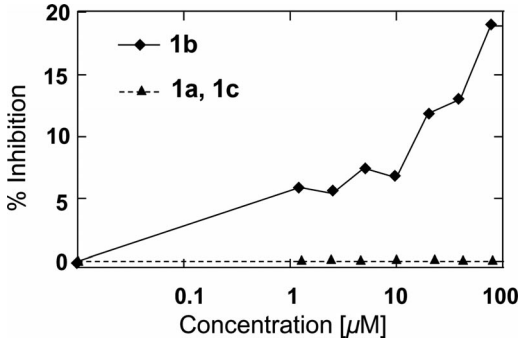


Figure 1. Effect of compounds **1a**–**c** on HIV-1 replication in H9 cells. HIV-1_{RTMF} (AZT-resistant virus) was treated with increasing concentrations (0, 1.25, 2.5, 5.0, 10, 20, 40, and 80 μM) of **1a**–**c** compounds. Viral replication in the absence and presence of **1a**–**c** was measured by using HIV-1 p24 antigen ELISA after 8 d of infection.

Conclusions

The first asymmetric total syntheses of both enantiomers of oresbiusin A (i.e., **1b** and **1c**) as well as racemate **1a** were completed in five steps with ca. 70% overall yields from commercially available caffeic acid. Sharpless asymmetric dihydroxylation was utilized to the construct the stereogenic centers with excellent enantioselectivity (>99%*e*). The outcome allowed us to establish the absolute stereoconfiguration of oresbiusin A: this natural product was dextrorotary and should be the (2*S*) enantiomer. In addition, an efficient, convergent route was developed for the total syntheses of (+)-, (–)-, and (±)-oresbiusin B. By starting with caffeic acid, the total syntheses included six steps with ca. 58% overall yields. Among the six compounds in the family of oresbiusins, unnatural (–)-(*R*)-**1b** exhibited activity against HIV-1 replication.

Supporting Information (see footnote on the first page of this article): Synthetic procedures, ^{1}H NMR and ^{13}C NMR spectroscopic data, HPLC chromatograms, and biological data for new compounds.

Acknowledgments

We thank the National Science Council (Grant No. 99-2113M-007-008-MY3) and Ministry of Education, Republic of China (Grant No. P690-C25-2407) for support of this research.

[1] H. Hao, S. Handong, Z. Shouxun, *Phytochemistry* **1996**, *42*, 1247–1248 and references cited therein.
[2] H. Huang, H. D. Sun, M. S. Wang, S. X. Zhao, *J. Nat. Prod.* **1996**, *59*, 1079–1080.
[3] Y. Zou, C. Tan, B. Wang, S. Jiang, D. Zhu, *Chem. Nat. Compd.* **2010**, *46*, 19–21.
[4] S. A. Snyder, F. Kontes, *J. Am. Chem. Soc.* **2009**, *131*, 1745–1752.
[5] R. S. Xu, *Dan-Shen (Salvia miltiorrhiza) – Its Biology and Applications*, Science press (in Chinese), Beijing, **1990**.
[6] For biological activities, see: a) R. W. Jiang, K. M. Lau, P. M. Hon, T. C. W. Mak, K. S. Woo, K. P. Fung, *Curr. Med. Chem.* **2005**, *12*, 237–246; b) A. Watzke, S. J. O'Malley, R. G. Bergman, J. A. Ellman, *J. Nat. Prod.* **2006**, *69*, 1231–1233.
[7] I. S. Abd-Elazem, H. S. Chen, R. B. Bates, R. C. Huang, *Antiviral Res.* **2002**, *55*, 91–106.
[8] N. J. Lawrence, S. Brown, *Tetrahedron* **2002**, *58*, 613–619 and references cited therein.
[9] E. J. Hicken, Ph. D. Thesis, Brigham Young University, **2005**.
[10] J. R. Hwu, W. N. Tseng, J. Gnabre, P. Giza, R. C. Huang, *J. Med. Chem.* **1998**, *41*, 2994–3000.
[11] H. Chen, L. Teng, J. N. Li, R. Park, D. E. Mold, J. Gnabre, J. R. Hwu, W. N. Tseng, R. C. Huang, *J. Med. Chem.* **1998**, *41*, 3001–3007.
[12] J. R. Hwu, M. H. Hsu, R. C. Huang, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1884–1888.
[13] J. R. Hwu, C.-I. Hsu, M. H. Hsu, Y. C. Liang, R. C. Huang, Y. C. Lee, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 380–382.
[14] K. B. Sharpless, W. Amberg, Y. L. Bennani, G. A. Crispino, J. Hartung, K. S. Jeong, H. L. Kwong, K. Morikawa, Z. M. Wang, D. Xu, X. L. Zang, *J. Org. Chem.* **1992**, *57*, 2768–2771.
[15] N. Shkriabai, S. S. Patil, S. Hess, S. R. Budihas, R. Craigie Jr., T. R. Burke, S. J. L. Grice, M. Kvaratskhelia, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 6894–6899.
[16] C. Dong, Y. Wang, Y. Z. Zhu, *Bioorg. Med. Chem.* **2009**, *17*, 3499–3507.
[17] T. Garnelis, C. M. Athanassopoulos, D. Papaioannou, I. M. Eggleston, A. H. Fairlamb, *Chem. Lett.* **2005**, *34*, 264–265.
[18] H. C. Kolb, M. S. Vannieuwenhze, K. B. Sharpless, *Chem. Rev.* **1994**, *94*, 2483–2547.
[19] a) Y. L. Bennani, K. B. Sharpless, *Tetrahedron Lett.* **1993**, *34*, 2079–2082; b) P. J. Walsh, K. B. Sharpless, *Synlett* **1993**, 605–607.
[20] J. March, *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 4th ed., Wiley, New York, **1992**, ch. 9, pp. 273–275.
[21] H. I. Duynstee, M. C. de Koning, H. Ovaa, G. A. van der Marel, J. H. van Boom, *Eur. J. Org. Chem.* **1999**, 2623–2632.
[22] For representative works, see: a) Z. M. Wang, H. C. Kolb, K. B. Sharpless, *J. Org. Chem.* **1994**, *59*, 5104–5105; b) S. K.

Das, G. Panda, *Tetrahedron* **2008**, *64*, 4162–4173; c) C. Xiong, W. Wang, V. J. Hurby, *J. Org. Chem.* **2002**, *67*, 3514–3517; d) R. Ramon, M. Alonso, A. Riera, *Tetrahedron: Asymmetry* **2007**, *18*, 2797–2802; e) Y. N. Zhang, S. L. Zhang, L. Ma, Y. Zhang, X. Shen, W. Wang, L. H. Hu, *Adv. Synth. Catal.* **2008**, *350*, 2373–2379; f) A. R. Jagdale, R. S. Reddy, A. Sudalai, *Org. Lett.* **2009**, *11*, 803–806; g) J. E. Green, D. M. Bender, S. Jackson, M. J. O'Donnell, J. R. McCarthy, *Org. Lett.* **2009**, *11*, 807–810; h) A. Schmauder, L. D. Sibley, M. E. Maier, *Chem. Eur. J.* **2010**, *16*, 4328–4336.

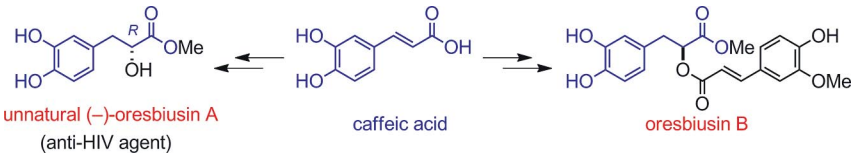
[23] M. Nakajima, K. Tomioka, K. Koga, *Tetrahedron* **1993**, *49*, 10807–10816.

[24] K. Li, K. Vanka, W. H. Thompson, J. A. Tunge, *Org. Lett.* **2006**, *8*, 4711–4714.

[25] The specific rotation for naturally occurring oresbiusin A was reported as $[a]_D^{22}$ = +7.9 (*c* = 0.410, MeOH). See ref.[2]

[26] P. Dupau, R. Epple, A. A. Thomas, V. V. Fokin, K. B. Sharpless, *Adv. Synth. Catal.* **2002**, *344*, 421–433.

Received: May 22, 2012
Published Online: ■



The first total synthesis of oresbiusin A was accomplished, and the key intermediate with optical activity was generated through a Sharpless asymmetric dihydroxylation reaction. The absolute configuration of this natural product was determined. The total syntheses of oresbiusin B and its racemate were also accomplished, and their anti-HIV-1 activities were investigated.

Total Synthesis

Keywords: Chirality / Biological activity / Configuration determination / Natural products / Total synthesis

