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Asymmetric bioreduction of α,β-unsaturated nitriles and ketones

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ABSTRACT

Two applications for the asymmetric reduction of activated alkenes employing isolated enoate reductases are reported. A series of α,β-unsaturated nitriles were shown to be converted to the optically active nitrile products in high yields and excellent enantioselectivities (up to 99% ee). In addition, the reduction of 2,3-disubstituted cyclopentenones was shown to provide almost exclusively *trans*-2,3-disubstituted cyclopentanones in high yield and enantiopurity (94% ee).

1. Introduction

The development of asymmetric bioreductive routes to enantiomerically pure compounds has emerged in recent years to become a key component in the synthesis of increasingly complex pharmaceutical drugs, which often contain multiple stereocenters.[1] Bioreductions are of great industrial significance, because the enzyme catalysts are highly chemo-, regio-, and stereoselective, preventing the formation of side products and avoiding protection/deprotection steps. The processes have low energy requirements because of their ambient temperature and pressure conditions. Enzymes are renewable resources and also biodegradable, thus presenting a valuable tool for more environmentally friendly industrial transformations.[2]

The selective reduction of carbon–carbon double bonds provides access to molecules with an α- and possibly an additional β-stereocenter, depending on the substitution pattern.[3] Many advances in the catalytic asymmetric hydrogenation of activated alkenes have been reported using either chiral rhodium or ruthenium phosphines or enoate reductase enzymes.[3–7] The reduction mechanism for enoate reductases proceeds according to an *anti*-addition of one hydride from the flavin cofactor, and one hydrogen from the solvent. This stereo-complementary approach to homogeneous chemical catalysts, which generally transfers hydrogen to the olefin in *syn* fashion, provides access to chiral intermediates with a *trans*-configuration.[3,5–8]

We were interested in evaluating bioreduction in systems where the substitution patterns of the carbon–carbon double bond had not previously been investigated. In particular, enantiopure nitriles are important synthetic precursors for the preparation of pharmacologically important building blocks.[9] Furthermore, nitriles can easily be converted to carboxylic acids, amines, or aldehydes. Some examples of metal catalyzed hydrogenation exist, but the enantioselective carbon–carbon bond reduction of conjugated nitriles remains a challenge due to their inherent low reactivity.[10] For example, the reduction of γ-cyano-α,β-unsaturated ketones by a yeast species is known, but only product mixtures of the saturated keto nitrile and the corresponding saturated alcohol were obtained due to the presence of ketone reductases in the whole cells.[11] Smallridge has described the reduction of 2-phenyl-2-propenenitrile to (*R*)-phenylpropanenitrile in high enantiopurity by employing baker's yeast in petroleum ether.[12] Herein, we report the asymmetric bioreduction of a series of α,β-unsaturated nitriles **1**–**4** and **9** employing a library of commercially available enoate reductases.[13]

We also investigated the enzymatic carbon–carbon double bond reduction of α,β-disubstituted cyclopentenones. While reductions of cyclic enones are known, the substituents are confined to protons, methyl, and ethyl groups.[6,7,14] We describe a biocatalytic 1,4-reduction of 2,3-disubstituted cyclopenten-3-ones **11** and **12** as the asymmetric step in the synthesis of 1,2,3-trisubstituted cyclopentanes, which provide useful pharmaceutical intermediates.[15a]

2. Results and discussion

2.1. Biocatalytic reduction of α,β-unsaturated nitriles 1–4 and 9

A series of *para*-substituted phenyl butenenitriles **1**–**4** were prepared according to a literature protocol and were fully characterized by NMR spectroscopy.[16] Phenyl butenenitriles **1**–**4** were obtained in the *Z*-configuration as determined by NOE experiments.[17] Screening of compounds **1**–**4** was performed with commercially available enoate reductases in the presence of excess NAD(P)H (Fig. 1).[18] The enzymes showed the enantioselective reduction of all phenyl butenenitriles **1**–**4**, giving the corresponding saturated products **5**–**8** in good yield and excellent

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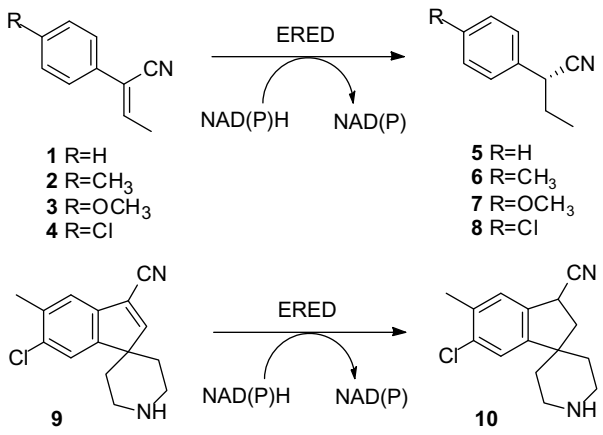

Figure 1. Asymmetric reduction of α,β-unsaturated nitriles **1**–**4**, **9** using enoate reductases (ERED). The absolute configuration of compound **10** is not specified.

enantioselectivities up to 99% ee (Table 1).[19] All enzymes consistently displayed the same facial selectivity giving the (*R*)-(+)-enantiomer. This was established for compound **5** as determined by comparison of the specific rotation of **5** from ERED112 to the known (*S*)-(−)-phenyl butyronitrile.[20] Given the structural similarity of each of the substrates, we speculate that the (*R*)-(+)-enantiomers of **6**–**8** are also formed based on the enzyme mechanism likely directing the reduction of analogous compounds with the same facial selectivity when compared to **5**.

Encouraged by the promising results from the *para*-substituted phenyl butenenitriles **1**–**4**, we examined the bioreduction of a more complex pharmaceutical building block **9**. Substrate **9** was well accepted by five enzymes (Table 1, entries 3, 4, and 6–8) and proceeded to give ~85% conversion with enantioselectivities up to 98% ee observed for compound **10** (Table 1).[21] The absolute configuration of **10** was not determined with absolute certainty. The reaction likely follows the same facial selectivity as shown for compound **5**, but the sterically more demanding substitution pattern could influence the enzyme's stereopreference as examples for a switch in stereopreference for an enoate reductase isozymes that have been reported.[22]

The present results demonstrate the applicability of enoate reductases for the reduction of more complex α,β-unsaturated nitriles.

Table 2
Screening results for enzymatic reduction of substrates **11** and **12**[a]

Entry	Enzyme[b]	**13**		**14**	
		% Conversion[c]	% ee *trans*[d]	% Conversion[c]	% ee *trans*[e]
1	ERED101	98	85 (1*R*,2*R*)	100	98 (1*R*,2*R*)
2	ERED102	98	92 (1*R*,2*R*)	100	98 (1*R*,2*R*)
3	ERED103	98	93 (1*R*,2*R*)	100	98 (1*R*,2*R*)
4	ERED104	95	93 (1*R*,2*R*)	100	98 (1*R*,2*R*)
5	ERED105	100	93 (1*R*,2*R*)	100	98 (1*R*,2*R*)
6	ERED106	100	92 (1*R*,2*R*)	100	98 (1*R*,2*R*)
7	ERED107	63	93 (1*R*,2*R*)	100	98 (1*R*,2*R*)
8	ERED108	97	93 (1*R*,2*R*)	100	98 (1*R*,2*R*)
9	ERED109	100	93 (1*R*,2*R*)	100	98 (1*R*,2*R*)
10	ERED110	100	93 (1*R*,2*R*)	100	98 (1*R*,2*R*)
11	ERED111	100	91 (1*R*,2*R*)	100	98 (1*R*,2*R*)
12	ERED112	100	92 (1*R*,2*R*)	100	98 (1*R*,2*R*)
13	ERED113	56	93 (1*R*,2*R*)	52	98 (1*R*,2*R*)
14	ERED114	100	93 (1*R*,2*R*)	100	98 (1*R*,2*R*)

[a] 0.5 mg **11** or **12** per reaction in 0.5 ml 0.1 M KH_2PO_4 pH 7.0, 5 vol % DMSO, 5 mg lyophilized enzyme preparation (ERED101-116, Biocatalytics, Inc.), 5 mg NAD(P)H. 30 °C, 3 h.
[b] ERED = enoate reductase.
[c] mol % conversion determined by HPLC analysis. ERED115, 116 gave <15% conversion.
[d] Stereochemical assignments made based on comparison to Ref. 15.
[e] Stereochemical assignment made based on results for analogous **13**.

2.2. Biocatalytic reduction of 2,3-disubstituted cyclopenten-3-ones 11 and 12[15a,23]

Having established that the biocatalytic reduction of α,β-unsaturated nitriles provided a means of preparing chiral nitriles in excellent yield and enantioselectivity, we next examined the same library of isolated enzymes against 2,3-disubstituted cyclopentenones **11** and **12** in the presence of excess NAD(P)H.[18] These substrates are particularly attractive for enoate reductases since the alkene is conjugated to two electron-withdrawing carboxyl groups. Most of the enzymes gave complete 1,4-reduction of the doubly activated cyclopentenones **11** and **12** as shown in Table 2. In each case, no detectable amount of 1,2-reduction of the ketone was observed because of the excellent chemo- and regio-specificity of the enoate reductases. The use of isolated enzymes avoids any interfering competitive ketone reduction by ketone reductases present in some whole cell systems.[3,14] Therefore, this bioreduction provides a valuable tool for the 1,4-reduction of α,β-unsaturated carbonyl compounds. The corresponding cyclopentanones **13** and **14** were obtained in a *trans*-configuration as the major product (**13**: ~94%

Table 1
Screening results for enzymatic reduction of substrates **1**–**4** and **9**[a]

Entry	Enzyme[b]	**1**	**5**	**2**	**6**	**3**	**7**	**4**	**8**	**9**	**10**
		% Conversion[c]	% ee	% Conversion[c]	% ee	% Conversion[c]	% ee	% Conversion[c]	% ee	% Conversion[c]	% ee
1	ERED101	73	98 (*R*)	76	99	100	97	100	96		
2	ERED102	3		10	92	42	98	25	88		
3	ERED103	2		13	90	37	91	27	92	83	98
4	ERED104	2		5		9		5		85	94
5	ERED105	65	98 (*R*)	15	99	22	92	33	82	54	98
6	ERED106	1		5		9		13	70	86	98
7	ERED107	63	94 (*R*)	10	85	32	93	35	87	83	98
8	ERED108	3		4		5		2		85	98
9	ERED109	100	99 (*R*)	63	94	90	90	88	74	55	98
10	ERED110	100	99 (*R*)	32	88	55	98	55	70	37	98
11	ERED111	24	98 (*R*)	25	92	86	98	67	89		
12	ERED112	100	98 (*R*)	99	97	97	98	96	89		
13	ERED114	80	97 (*R*)	36	93	79	98	72	87		

[a] 0.5 mg substrate **1**–**4** and **9** per reaction in 0.5 ml 0.1 M KH_2PO_4 pH 7.0, 5 vol % DMSO, 5 mg lyophilized enzyme preparation (ERED101-116, Biocatalytics, Inc.), 5 mg NAD(P)H. 30 °C, 3 h.
[b] ERED = enoate reductase.
[c] mol % conversion determined by HPLC analysis.

trans, **14**: ~92% *trans*) which was determined by NMR spectroscopy (NOE).[24,25] This is consistent with the proposed mechanism by Massey and Karplus, whereby *anti*-addition of H_2 is observed and the thermodynamically most favored configuration is isolated.[3c,d,7] Good enantioselectivity was observed for **13** with 93% ee for the (1*R*,2*R*)-enantiomer. Product **13** was converted to the known 2-(4-fluorophenyl)-3-hydroxycyclopentane-1-carboxylic acid in order to unequivably establish the absolute configuration of **13**.[15] The larger ester group of substrate **12** increased the enantioselectivity of the enzymes yielding **14** in 98% ee (1*R*,2*R*). The stereochemical assignment for **14** was based on analogous results obtained for the bioreduction of **13** (Fig. 2).

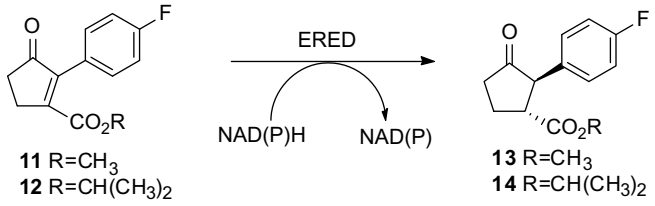

Figure 2. Asymmetric reduction of α,β-unsaturated ketones **11** and **12** using enoate reductases.

We then focused on scalability and increasing the cost-efficiency of two example reactions compared to the screening conditions. ERED112 was chosen to demonstrate process development on the reduction of **1** and ERED114 was selected as catalyst for the reduction of **11**. The first goal was accomplished by lowering the substrate to catalyst ratio by weight from 1:10 in the screen to 1:1 for both processes. Reduction of doubly activated **11** was complete within a 3 h time period similar to the screening reactions, to provide **13** in similar yield and ee. The conversion of **1** was complete within 18 h which is still a feasible reaction time for scale-up.[26] An in situ NAD(P)H cofactor recycling system employing a sodium phosphite/phosphite dehydrogenase system was added to reduce the cost of the expensive cofactor.[27] This cofactor regeneration system offers the advantage of a consistent pH throughout the reaction in contrast to common glucose/glucose dehydrogenase systems where pH control is required.[28] The improved bioreductions of compounds **1** and **11** were successfully demonstrated on a 200 mg scale, providing scope for efficient and scalable processes for the asymmetric reduction of conjugated olefins with commercially available enoate reductases.

3. Conclusion

Two applications of the asymmetric carbon–carbon double bond reduction using commercially available enoate reductase enzymes are reported. The reduction of a series of α,β-unsaturated nitriles was demonstrated in good yield and enantioselectivity. This approach for the synthesis of optically active nitrile compounds was also successfully applied to a pharmaceutical intermediate. The enzymatic reduction of 2,3-disubstituted cyclopentenones yielded valuable *trans*-cyclopentanone cores in high diastereo- and enantioselectivities which demonstrates the scope for these enzymes in the construction of more complex substrates. The described bioreductions of both α,β-unsaturated nitriles and ketones provide access to highly functionalized compounds which may be elaborated to more structurally intriguing pharmacophores. The complete scope and depth of the these bioreductions is still under active investigation and the results will be published in due course.

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13. ERED101 to ERED116 were purchased from Biocatalytics, Inc., Pasadena, CA.
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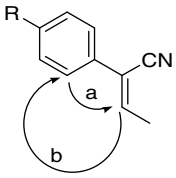
16. Pepin, Y.; Nazemi, H.; Payette, D. *Can. J. Chem.* **1978**, *56*, 41–45. *2-Phenyl-but-2-enenitrile* **1**. Light yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53 (m, 2H), 7.42–7.34 (om, 3H), 6.91 (q, J = 7.2, 1H), 2.23 (d, J = 7.2, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.04, 133.48, 129.09, 128.98, 125.70, 117.27, 116.65, 17.96. *2-(4-Methyl)-phenyl-but-2-enenitrile* **2**. Yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41 (m, 2H), 7.20 (m, 2H), 6.84 (q, J = 7.2, 1H), 2.37 (s, 3H), 2.20 (d, J = 7.2, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 140.90, 138.99, 130.67, 129.73, 125.53, 117.06, 116.76, 21.25, 17.84. *2-(4-Methoxy)-phenyl-but-2-enenitrile* **3**. Yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40 (m, 2H), 6.86 (m, 2H), 6.71 (q, J = 7.2, 1H), 3.78 (s, 3H), 2.14 (d, J = 7.2, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.22, 139.75, 126.93, 126.08, 116.84, 116.59, 114.44, 55.50, 17.79. *2-(4-Chloro)-phenyl-but-2-enenitrile* **4**. Light yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44 (m, 2H), 7.35 (m, 2H), 6.88 (q, J = 7.2, 1H), 2.21 (d, J = 7.2, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.58, 134.86, 131.88, 129.59, 126.87, 116.19, 116.12, 17.94.
17. Observed NOE

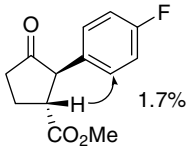


R:	%NOE:	
H	a: 0.5%	b: 1.1%
CH_3	a: 1.0·%	
CH_3O	a: 1.2%	b: 2.4%
Cl	a: 1.7%	b: 0.9%

18. *General screening conditions for the enoate reductases library with excess NAD(P)H.* NAD(P)(H) was purchased from Biocatalytics, Inc., Pasadena, CA. 5 mg lyophilized enzyme preparation was dissolved in buffer (0.4 ml 0.1 M KH_2PO_4, pH 7) and combined with a solution of NADPH (5 mg, 6 μmol) in

KH_2PO_4 buffer (0.037 ml, 0.5 M, pH 7) and a solution of NADH (5 mg, 7 μmol) in KH_2PO_4 buffer (0.0.37 ml, 0.5 M, pH 7). A solution of appropriate substrates **1**–**4**, **9**, **11**, **12** (0.5 mg) in DMSO (0.025 ml) was added. The reaction was incubated at 30 °C for 3 h.

19. *Racemic 2-phenyl-butyronitrile* was obtained from Aldrich. Racemic standards for **6**–**8** were prepared according to Profitt, J. A.; Watt, D. S.; Corey, E. J. *J. Org. Chem*., **1975**, *40*, 127. *2-(4-Methyl)-phenyl-butyronitrile* **6**. Colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.20 (m, 4H), 3.70 (t, J = 7.2, 1H), 2.35 (s, 3H), 1.93 (m, 2H), 1.07 (t, J = 7.2, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.00, 132.95, 129.86, 127.37, 121.11, 38.75, 29.40, 21.23, 11.66. *2-(4-Methoxy)-phenyl-butyronitrile* **7**. Yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.24 (m, 2H), 6.91 (m, 2H), 3.82 (s, 3H), 3.69 (t, J = 7.2, 1H), 1.92 (m, 2H), 1.07 (t, J = 7.2, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.48, 128.58, 127.55, 121.18, 114.57, 55.52, 38.29, 29.40, 11.60. *2-(4-Chloro)-phenyl-butyronitrile* **8**. Light yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37 (m, 2H), 7.28 (m, 2H), 3.73 (t, J = 7.2, 1H), 1.98–1.90 (m, 2H), 1.08 (t, J = 7.2, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 134.43, 130.03, 129.41, 128.85, 120.46, 38.52, 29.30, 11.54. Conversions of **1**–**4** and the enantiomeric excesses of **5**–**8** were measured by HPLC chromatography: Agilent HPLC system, tandem Zorbax SB C18 (4.6 × 50 mm, 3.5 μm)-Chiralpak OJ-RH (4.6 × 150 mm, 5 μm), isocratic 40/60 MeCN/water (0.1% H_3PO_4), 1 ml/min, rt, 210 nm, 35 min. 17.1 min (**1**), 14.1 min (**5**, *R*), 16.1 min (**5**, *S*); 30.6 min (**2**), 22.4 min, 26.6 min (**6**); 20.5 min (**3**), 13.9 min, 15.3 min (**7**); 30.4 min (**4**), 23.6 min, 25.1 min (**8**).
20. (a) Compound **5**: $[\alpha]_D^{20} = +26.1$ (*c* 7.08, methanol).; (b) Shibata, S.; Matsushita, H.; Kaneko, H.; Noguchi, M.; Saburi, M.; Yoshikawa, S. *Agric. Biol. Chem* **1982**, *46*, 1271–1275; (c) Cram, D. J.; Haberfield, P. *J. Am. Chem. Soc.* **1961**, *83*, 2354–2366.
21. Limanto, J.; Shultz, S. C.; Dorner, B.; Desmond, R. A.; Devine, P. N.; Krska, S. W. *J. Org. Chem.* **2008,** *73*, 1639–1642. *Compounds* **9** and **10** were synthesized from the corresponding *N*-Boc derivatives. Removal of the BOC group was performed under acidic conditions (BF_3·Et_2O in toluene) and standard aqueous work-up followed by crystallization from *tert*-butyl methyl ether. Compound **9**: Yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.57 (s, 1H), 7.39 (s, 1H), 7.36 (s, 1H), 3.21 (dt, J = 12.8, 3.6, 2H), 2.95 (td, J = 12.8, 2.8, 2H), 2.43 (s, 3H), 2.02 (ddd, J = 13.6, 12.0, 4.0, 2H), 1.41 (m, 2H). ^{13}C NMR ($CDCl_3$, 100MHz) δ 154.39, 149.69, 136.64, 136.01, 134.14, 123.54, 123.02, 114.79, 114.47, 53.72, 44.34, 33.44, 20.39. Compound: **10**: Yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 (s, 1H), 7.22 (s, 1H), 4.07 (t, J = 8.0, 1H), 3.09 (m, 2H), 2.80 (td, J = 12.8, 2.8, 2H), 2.65 (dd, J = 12.8, 8.0, 1H), 2.38 (s, 3H), 2.28 (ddd, J = 12.8, 8.0, 1.2, 1H), 1.94 (td, J = 12.8, 4.0, 1H), 1.71–1.63 (om, 3H), 1.44 (dq, J = 13.2, 2.8, 1H). ^{13}C NMR ($CDCl_3$, 100MHz) δ142.92, 136.04, 135.30, 134.92, 126.86, 124.09, 121.11, 46.82, 43.78, 43.51, 40.68, 38.14, 36.94, 31.82, 20.21. Conversion of **9** was determined on an Agilent HPLC system using a Zorbax SB C18 column (4.6 × 75 mm, 3.5 μm) at a gradient from 2/98 MeCN/water (0.1% H_3PO_4) held for 1 min to 100/0 over 8 min at 1.5 ml/min, rt, 215 nm. 5.6 min (**9**), 5.3 min (**10**). Enantiomeric excess of **10** was determined with a Berger SFC system employing a Chiralpak ADH column (250 × 4.6 mm), isocratic 10% methanol (25 mM *iso*-butylamine)/CO_2 @ 1.5 ml/min, 200 bar, 215 nm, 35 °C, 20 min. 13.6 min, 14.9 min (**10**).
22. Hall, M.; Stueckler, C.; Ehammer, H.; Pointner, E.; Oberdorfer, G.; Gruber, K.; Hauer, B.; Stuermer, R.; Kroutil, W.; Macheroux, P.; Faber, K. *Adv. Synth. Catal.* **2008**, *350*, 411–418.
23. Compound **12** was prepared according to **11** described in Ref. 14. **12**: Yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 (m, 2H), 7.07 (m, 2H), 5.10 (septet, J = 6.4, 1H), 2.92 (m, 2H), 2.64 (m, 2H), 1.17 (d, J = 6.4, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 207.57, 165.47, 163.15 (d, J_{CF} = 249.0 Hz), 157.92, 144.91, 131.19 (d, J_{CF} = 8.0 Hz), 126.47 (d, J_{CF} = 3.6 Hz), 115.11 (d, J_{CF} = 21.7 Hz), 69.64, 34.62, 21.17, 21.66.
24. Observed NOE



25. *trans*-**13**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.11 (m, 2H), 7.02 (m, 2H), 3.70 (dd, J = 11.5, 1.2, 1H), 3.69 (s, 3H), 3.21 (td, J = 11.5, 6.4, 1H), 2.64 (m, 1H), 2.45 (m, 1H), 2.38 (ddd, J = 19.4, 10.3, 8.7, 1H), 2.10 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 214.70, 174.09, 162.32 (d, J_{CF} = 245.7), 132.44 (d, J_{CF} = 3.2), 130.61 (d, J_{CF} = 8.0), 115.86 (d, J_{CF} = 21.7), 58.11, 52.42, 49.44, 37.87, 25.03. *trans*-**14**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.11 (m, 2H), 7.01 (m, 2H), 5.00 (septet, J = 6.4, 1H), 3.68 (d, J = 12.0, 1H), 3.14 (m, 1H), 2.62 (m, 1H), 2.44 (m, 1H), 2.36 (m, 1H), 2.09 (m, 1H), 1.21 (d, J = 6.4, 3H), 1.13 (d, J = 6.4, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 214.87, 173.08, 162.28 (d, J_{CF} = 245.7 Hz), 132.50 (d, J_{CF} = 3.6 Hz), 130.25 (d, J_{CF} = 8.0 Hz), 115.75 (d, J_{CF} = 21.7 Hz), 68.79, 58.27, 49.85, 37.82, 24.85, 21.87. Racemic standards **13** and **14** were prepared via reduction of **11** and **12** with H_2 (1 mol equiv) and wet Pt/C in methanol. Conversion of **11** and **12** was determined on an Agilent HPLC system using a Zorbax Extend C18 column (4.6 × 50 mm, 3.5 μm) at a gradient from 20/80 MeCN/water (0.1% H_3PO_4) to 40/60 MeCN/water (0.1% H_3PO_4) over 4 min to 90/10 over 1 min, held for 2 min, 1 ml/min, rt, 215 nm. 5.5 min (**11**), 5.3 min (**13** *trans*), 5.1 min (**13** *cis*); 5.3 min (**12**), 5.2 min (**14** *trans*), 5.0 min (**14** *cis*). Enantiomeric excess of **13** and **14** was determined with a Berger SFC system employing a Chiralpak ADH (250 × 4.6 mm), 4% methanol/CO_2 for 4 min, ramp to 40% methanol/CO_2 over 18 min, hold for 3 min, 1.5 ml/min, 200 bar, 215 nm, 35 °C. 5.3 min, 6.2 min 1*R*,2*R* (**13**); 4.4 min, 4.8 min (**14**). Alternatively, conversion of **11** and enantiomeric excess of **13** can be measured using an Agilent HPLC system equipped with tandem Zorbax Extend C18 (4.6 × 50 mm, 3.5 μm)-Chiralpak AD-RH (4.6 × 150 mm, 5 μm) at 40/60 MeCN/water (0.1% H_3PO_4) held for 5 min, ramp to 90/10 MeCN/water (0.1% H_3PO_4) over 8 min, held for 2 min, 1 ml/min, rt, 215 nm. 9.3 min (**11**), 9.1 min, 9.6 min (**13**).
26. A solution of 250 mg ERED114 (ERED112) in l8 ml 0.3 M $Na_2(PHO_3)$ pH 7 was added to a solution of 125 mg NADP and 125 mg PDH101 (phosphite dehydrogenase, Biocatalytics, Inc.) in 220 ml 0.3 M $Na_2(PHO_3)$ pH 7. To the solution was added 250 mg of **11** (**1**) in 12 ml DMSO. The reaction was aged at 30 °C for 3 h (18 h). After extraction with 200 ml MTBE, the organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to give 198 mg of **13** (176 mg **5**).
27. (a) Relyea, H. A.; van der Donk, W. A. *Bioorg. Chem.* **2005**, *33*, 171–189; (b) Johannes, T. W.; Woodyer, R. D.; Zhao, H. *Appl. Environ. Microbiol.* **2005**, *71*, 5728–5734; (c) Woodyer, R.; van der Donk, W. A.; Zhao, H. *Comb. Chem. High Throughput Screening* **2006**, *9*, 237–245; (d) Johannes, T. W.; Woodyer, R. D.; Zhao, H. *Biotechnol. Bioeng.* **2006**, *96*, 18–26.
28. (a) Pollard, D.; Truppo, M.; Pollard, J.; Chen, C.; Moore, J. *Tetrahedron: Asymmetry* **2006**, *17*, 554–559; (b) Truppo, M. D.; Pollard, D.; Devine, P. *Organic Lett.* **2007**, *9*, 335–338.