JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Synthesis of Robalzotan, Ebalzotan, and Rotigotine Precursors via the Stereoselective Multienzymatic Cascade Reduction of α,β-Unsaturated Aldehydes

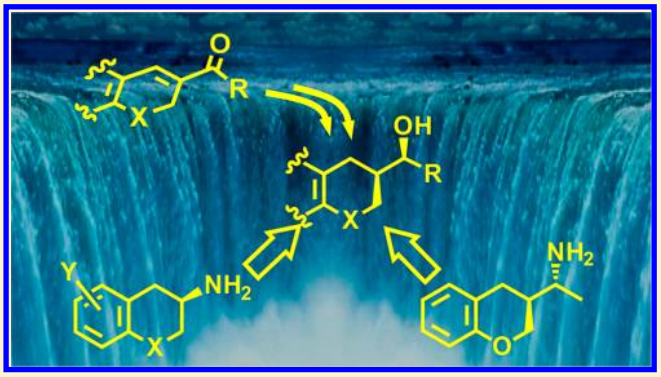
Elisabetta Brenna,[†] Francesco G. Gatti,*[,†] Luciana Malpezzi,[†] Daniela Monti,[‡] Fabio Parmeggiani,[†] and Alessandro Sacchetti*[,†]

[†]Dipartimento di Chimica, Materiali ed Ingegneria Chimica "G. Natta", Politecnico di Milano, P.zza Leonardo da Vinci 32, 20133 Milano, Italy

[‡]Istituto di Chimica del Riconoscimento Molecolare, CNR, Via Mario Bianco 9, 20131 Milano, Italy

Supporting Information

ABSTRACT: A stereoselective synthesis of bicyclic primary or secondary amines, based on tetralin or chroman structural moieties, is reported. These amines are precursors of important active pharmaceutical ingredients such as rotigotine (Neupro), robalzotan, and ebalzotan. The key step is based on a multienzymatic reduction of an α,β-unsaturated aldehyde or ketone to give the saturated primary or secondary alcohol, in a high yield and with a high ee. The catalytic system consists of the combination of an ene-reductase (ER; i.e., OYE2 or OYE3 belonging to the Old Yellow Enzyme family) with an alcohol dehydrogenase (ADH), applying the in situ substrate feeding product removal technology. By this system the formation of the allylic alcohol side product and the racemization of the chirally unstable α-substituted aldehyde intermediate are minimized. The primary alcohols were elaborated via a Curtius rearrangement. The combination of OYE2 with a Prelog or an anti-Prelog ADH allowed the preparation of the secondary alcohols with ee > 99% and de > 87%. The absolute configuration of the primary amines was unambiguously assigned by comparison with authentic samples. The stereochemistry of secondary alcohols was assigned by X-ray crystal structure and NMR analysis of Mosher esters.



INTRODUCTION

In the last few decades, the stereochemically well-defined chiral bicyclic amines **I** and **II**, based on tetralin or chroman carbon backbones, have become very popular in the pharmaceutical industry. Indeed, they are precious precursors of several biologically active molecules with a very large spectrum of possible applications, spanning from the treatment of depression and anxiety up to Parkinson's disease and as antibacterial agents (Figure 1). Despite the fact that a large number of biological assays have proven a strong relationship between their activity and a preferential stereochemistry, most of the stereoselective syntheses of **I** and **II** are based on chemical resolutions. This kind of strategy, even if it is extremely reliable, is intrinsically inefficient because at least half of the starting material has to be discarded,[1] in contrast with the more efficient catalytic asymmetric methodologies.

Among all the derivatives of **I** and **II**, the (*R*)-aminochromans **1** and **2** (Figure 2) have gained a particular interest in medicinal chemistry, since they are key intermediates of many antidepressant drug candidates such as robalzotan[2] and ebalzotan[3] and the DBH inhibitors chromanylimidazolethiones.[4] The (*S*)-tetralin **3** is another important synthon as well, because it is used for the production of rotigotine (Neupro), a drug indicated for the treatment of Parkinson's disease.[5] In addition to these relevant targets, also the 1*R*,3′*S* diastereoisomer of amine **4** (key precursor of chromanyl analogues of NK-1 tachykinin receptor antagonist[6] recently developed by Eli Lilly) was taken into consideration as a part of our ongoing research program, which is devoted to the syntheses of biologically active molecules by means of biocatalytic methodologies.[7,8]

So far, amine **2** and its derivatives have been prepared by starting from the chiral pool, using Garner's aldehyde[9] or by means of a radical cyclization on an L-serine derivative.[10] However, although they ensure a high optical purity of products, both routes suffer from a long synthetic sequence and a low overall yield. Recently we described the synthesis of **2** by means of an enzymatic resolution of its 3-aryl-2-nitropropanol precursor, but the ee remained unsatisfactory; another biocatalytic approach based on the microbial reduction of an α,β-unsaturated nitrostyrene was disappointing as well.[11]

Received: February 13, 2013
Published: April 23, 2013

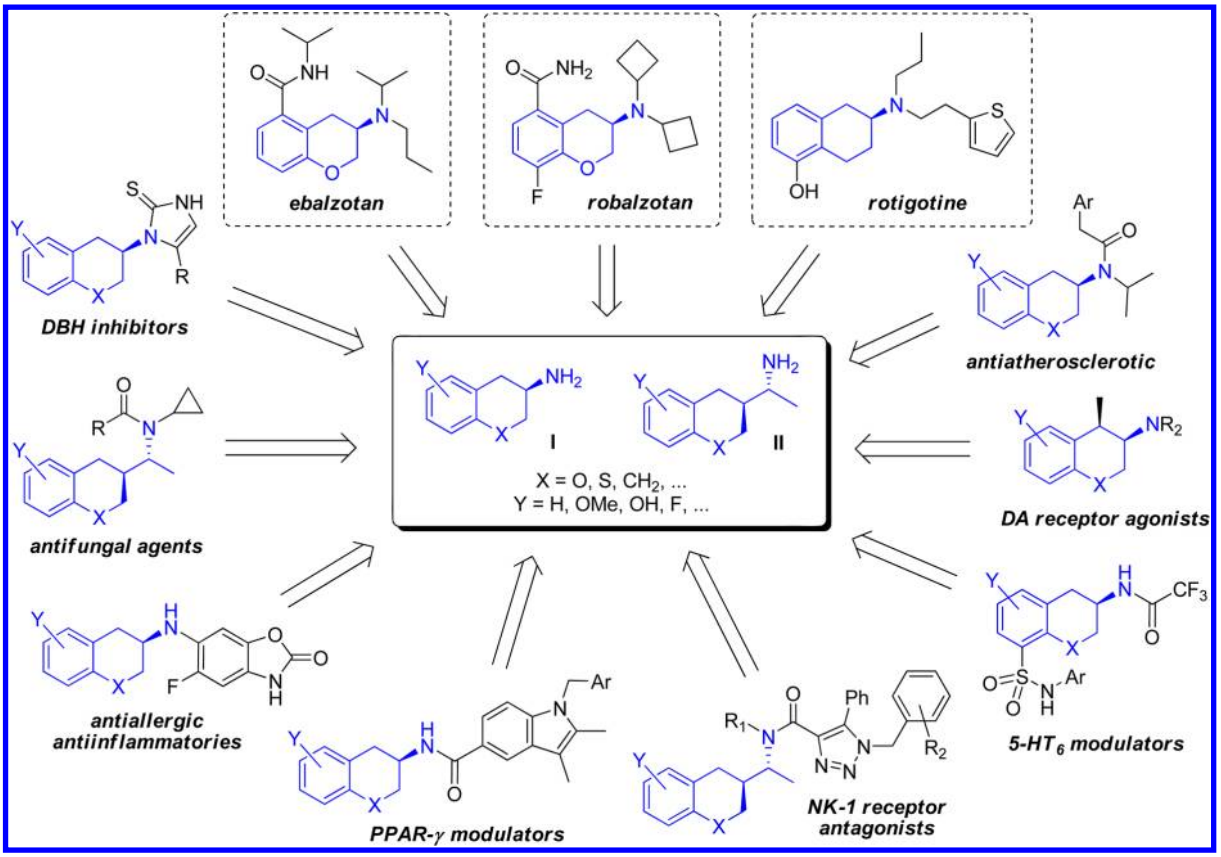


Figure 1. Biologically active compounds based on amines **I** or **II**.

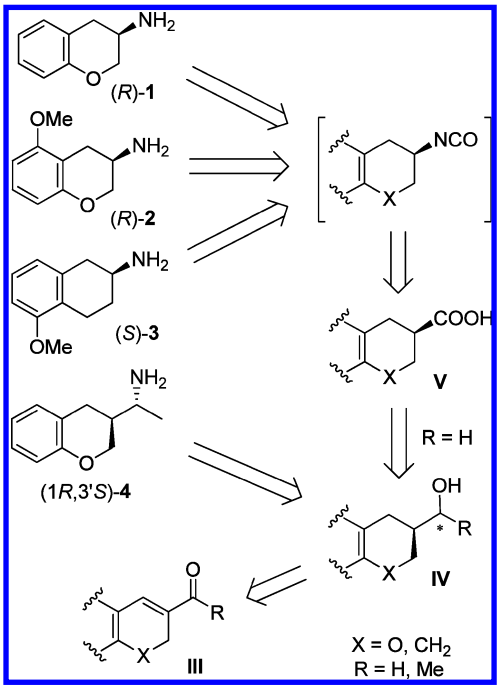


Figure 2. Retrosynthesis of amines **1**–**4**.

The asymmetric hydrogenation of chroman-3-enamides in the presence of a Ru–Synphos catalyst has been exploited for the preparation of several chiral 3-aminochromans.[12] However, despite the fact that an excellent enantioselection was obtained with several substrates, for the acetamide of **2** a poor ee of 34% was achieved, whereas the opposite enantiomer of **1** was prepared with an excellent ee = 96%.

For amine **3** just one preparation has been reported; it consists of the elaboration of (1*R*,2*R*)-1-hydroxy-2-carboxyethyltetralin, which was obtained by microbial reduction of the 2-carboxyethyl-1-tetralone precursor with an excellent de and optical purity.[13] However, with the substantial improvement of asymmetric catalytic methodologies, synthetic strategies based on the removal of stereogenic centers are progressively becoming less appealing.

To the best of our knowledge, no asymmetric synthesis of amine **4** has ever been reported. Conversely, several tetralin analogues have been prepared with quite good ee's and de's, but exclusively by chemical resolutions of racemic amines by means of resolving agents such as (+)-tartaric or (+)-mandelic acids.[6] The efficient enzymatic resolution of *N*-hydroxyl-*O*-methylamines was explored as well, but that for the tetralin analogue of **4** proceeded with an unacceptably low conversion.[14]

Therefore, current synthetic methods for the preparation of optically pure amines **I** and **II** show significant limitations for different reasons. In the search for alternative strategies, the introduction of biocatalysis into asymmetric synthesis,[15] especially in the pharmaceutical chemistry, would be highly desirable and should be encouraged.[16]

With this background, in the following we report a new stereoselective synthesis of amines **1**–**4** by means of biocatalytic methodologies.

dx.doi.org/10.1021/jo4003097 | J. Org. Chem. 2013, 78, 4811–4822

RESULTS AND DISCUSSION

Our retrosynthetic plan relies on the enantiospecific reduction of the C=C double bond of α,β-unsaturated aldehyde/ketone **III** to give primary or secondary alcohols **IV** (Figure 2). Concerning targets **1**−**3**, the amino group is meant to be introduced by Curtius rearrangement of the carboxylic acid precursor **V** via isocyanate intermediate. Instead, for the more complex amine **4** the second stereocenter is created after an enantioselective reduction of the carbonyl group of **III** to give the corresponding secondary alcohol **IV**; the latter should be easily transformed into **4** after few simple functional group manipulations.

Accordingly, the C=C double bond biocatalyzed reduction of activated olefins seems to fit particularly well with our retrosynthetic plan; indeed, this biotransformation has been largely exploited for the synthesis of many valuable chiral products,[8b,c,17] quite often with a high stereoselectivity, under mild reaction conditions and with minimal generation of waste. However, the nature of the products is strictly related to the type of biocatalyst applied, so that the following compounds are obtained: (i) chiral aldehydes or ketones with overexpressed and/or purified recombinant ene-reductases (ERs),[18] in which the C=C double bond reduction is dominant over other catalytic activities (Figure 3), and (ii) more elaborated and

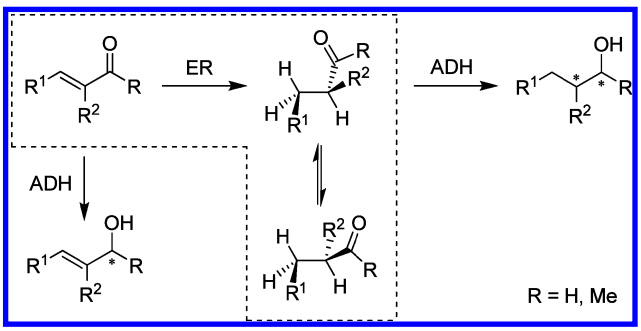


Figure 3. Typical chemical path of α,β-unsaturated aldehyde/ketone reductions, with isolated enzymes or microorganisms.

complex products in all the cases where the ER activity is not isolated but is combined with other enzymatic activities, often in a cascade process. For instance, in the resting cells of baker's yeast (BY),[19] ERs (specifically the old yellow enzymes OYE2 and OYE3) work in tandem with alcohol dehydrogenases (ADHs),[20] which catalyze the reduction of the carbonyl group to give the saturated primary or secondary alcohols (Figure 3). ADH-catalyzed competitive CO reduction, to give the side product allylic alcohol, is more evident for enals than for enones.

The synthetic plan adopted for the preparation of the α,β-unsaturated substrates **5a**−**8a** is depicted in Scheme 1. The domino oxa-Michael aldol condensation between salicylaldehyde or 6-methoxysalicylaldehyde[11] and acrolein or methyl vinyl ketone followed by an in situ elimination reaction gave a straightforward and very efficient access to the α,β-unsaturated aldehydes **5a** and **6a** and to the enone **7a** on a multigram scale (Scheme 1a). In contrast, the synthesis of aldehyde **8a** was more troublesome (Scheme 1b). A first approach was based on the Shapiro reaction: tosyl hydrazone **9**, easily prepared from the commercially available 5-methoxy-2-tetralone, was treated with *n*-BuLi in the presence of TMEDA to give the corresponding alkenyllithium intermediate that was then quenched using DMF as electrophile to give **8** together with its regioisomer **10** (4/6 ratio by GC-MS) in a modest yield of 32%. Unfortunately, both purification and separation of the two regioisomers by chromatography failed. The use of formaldehyde as electrophile and other reaction conditions also did not provide any significant improvement.[21]

Alternatively, the strategy based on the elimination of the mesylate leaving group of the cyanohydrin derivative **12** produced the α,β-unsaturated nitrile **13** with a high regioselectivity. Thus, sodium cyanide addition to the tetralone, in the presence of TMSCl, gave the corresponding *O*-protected cyanohydrin **11** in a quantitative yield.[22] The latter was converted into **12** after acid cleavage of the *O*-silyl group followed by treatment with MsCl in pyridine in an overall yield of 78%. The elimination reaction needed some optimization; indeed, when **12** was treated with pyridine at 90 °C, together with α,β-unsaturated nitrile **12**, also its regioisomer (83/17 ratio by ^{1}H NMR) and the naphthalene derivative were produced. However, if the reaction was carried out at 60 °C, both the regioselectivity and chemoselectivity improved substantially, affording **13** together with a lower amount of its regioisomer (93/7). The pure isomer **13** was obtained by crystallization in cold *n*-hexane/*i*-Pr_2O (9/1). Lastly, the cyano group was reduced with DIBAL-H affording the desired aldehyde **8a** in a 75% yield after column chromatography purification.

If on one hand the use of purified ERs has allowed significant improvements, among which the complete chemoselectivity and the high conversions are undoubtedly the most important, on the other hand it has introduced the problem of the relatively low chemical stability of the products. This is particularly relevant in the case of the optically pure α-substituted aldehydes, which can spontaneously racemize under the typical biotransformation conditions,[23] therefore, even at neutral pH (Figure 3).

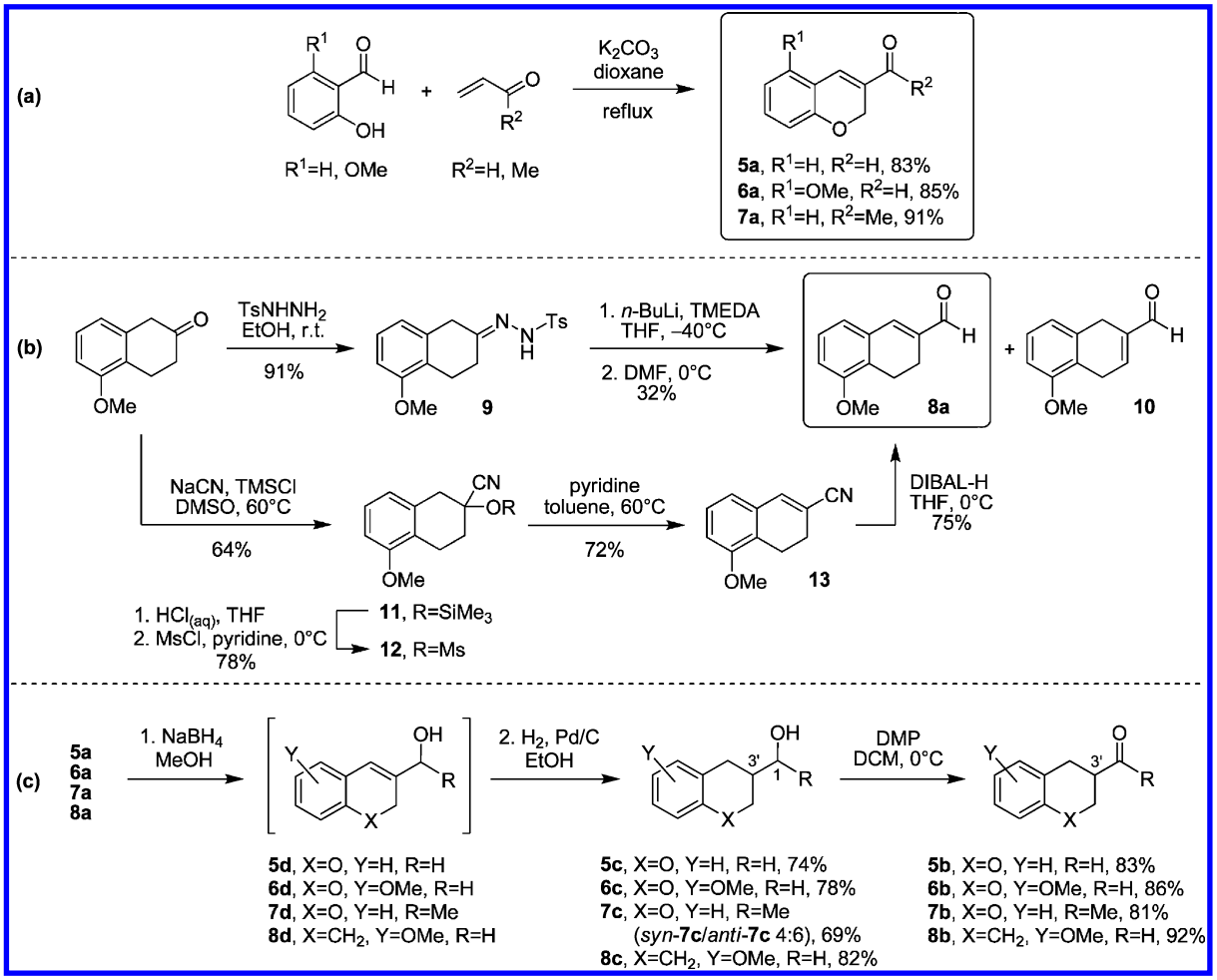
It has been demonstrated that the loss of optical purity by racemization during the reaction can be minimized using aqueous−organic biphasic solvent systems[17b,c] or applying the in situ substrate feeding product removal (SFPR) technology.[23,24] However, when the substrate **6a** was submitted to our standard reduction protocol[25b] in the presence of OYE2 and using the SFPR technology, aldehyde (*S*)-**6b** was obtained in an excellent yield but with an unacceptably low optical purity (ee = 32%, by chiral GC). The progress curve (Figure 4) clearly shows that the ee observed at the end of the biotransformation is not due to poor stereoselectivity of OYE2 but instead to a concomitant racemization process.

Very recently, we found a solution to this problem by combining the in situ SFPR technology with a multienzymatic catalytic system comprising an ER and an ADH.[25] Indeed, by this approach, if the unstable saturated aldehyde is immediately reduced to the more stable alcohol, the detrimental racemization should be suppressed. However, in this case, the ADH should reduce exclusively the CO of saturated aldehyde; otherwise, the undesired allylic alcohol would be produced as well.

In this work the concept was further investigated by testing a set of commercially available ADHs (recombinant and/or purified) from different sources. The reductions were performed on an approximately equimolar mixture of the unsaturated carbonyl compound and its corresponding saturated compound, i.e., **5a**/**5b**, **6a**/**6b**, **7a**/**7b**, and **8a**/**8b**, under the same reaction conditions usually adopted for OYE-

dx.doi.org/10.1021/jo4003097 | J. Org. Chem. 2013, 78, 4811−4822

Scheme 1. Synthesis of Substrates 5a−8a and Reference Racemic Materials 5b−8b and 5c−8c



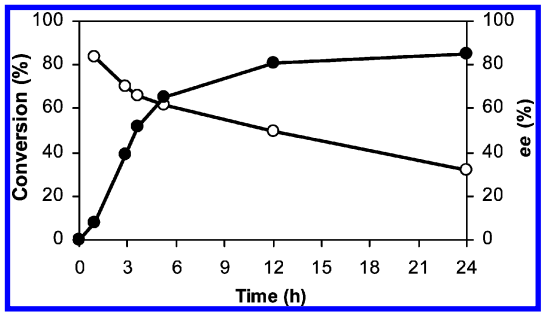


Figure 4. Progress curve of the biotransformation of **6a** into (*S*)-**6b** by OYE2 (conversion (●), ee (○)). Experimental conditions: 10 g L^{-1} of substrate (loaded on 10 g L^{-1} resin), 150 μg mL^{-1} of OYE2, 4 U mL^{-1} of GDH, 4 equiv of glucose, 0.1 mM $NADP^+$, 50 mM KP_i buffer pH 7.0, 30 °C, 160 rpm.

catalyzed reductions. The saturated carbonyl compounds **5b−8b** and the racemic alcohols **5c−8c** were prepared according to Scheme 1c. For the regeneration of the NAD(P)H cofactors, a glucose dehydrogenase (GDH) was employed, adding glucose as the sacrificial substrate. In Table 1 we report the conversion of the saturated aldehyde and the selectivity of ADHs evaluated as ratios between the amount of saturated and allylic alcohols.

First, we observed that all the screened ADHs reduce the unconjugated carbonyl group better than the conjugated one. In certain cases, this preferential outcome leads to the exclusive formation of the saturated alcohol, as for the mixtures **6a**/**6b** with DRADH and **8a**/**8b** with HLADH. Moreover, the enzyme concentration can play a crucial role in the selectivity, as shown for instance in the reduction of **8a**/**8b**: with READH the saturated aldehyde **8a** was completely consumed but also a small amount of allylic alcohol was formed (92/8), whereas with one-fifth of the same ADH almost no allylic alcohol was detected (99/1), but at the expense of a lower conversion of **8a** (61%).

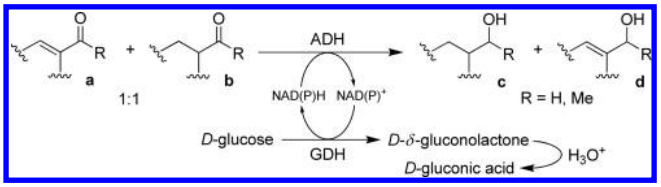
Concerning ketones **7a**/**7b**, in addition to the selectivity toward the formation of the saturated alcohol, i.e. **7d**, in certain cases the CO reduction of **7b** was also influenced by the configuration of the stereocenter C(3′), giving rise to a nonequimolar mixture of two possible diastereoisomers. For instance, with HLADH the alcohols *syn*-**7c** and *anti*-**7c** were produced in a ratio of 61/39. In any event, none of the screened ADHs reached a diastereoselectivity level sufficiently high to foresee a possible enzymatic resolution of racemic **7b**.

Finally, we tested the combination of selected ADHs and ERs on substrates **5a−8a** in coupled reactions (Table 2). The choice of the ADH was based on the best compromise between selectivity and conversion, to achieve not only an exclusive but also a fast reduction of the saturated aldehyde, thus preventing undesired racemization.

A clear example of this concept is offered by the reduction of **6a**: in the presence of OYE2 and HLADH, (*S*)-**6c** was obtained with an excellent ee of 99% and in a high yield of 88%,[25b] whereas by replacing HLADH with DRADH, which, with

dx.doi.org/10.1021/jo4003097 | J. Org. Chem. 2013, 78, 4811−4822

Table 1. Selectivity of a Panel of Commercially Available ADHs[a]



	substrate **5a**/**5b**		substrate **6a**/**6b**		substrate **8a**/**8b**		substrate **7a**/**7b**		
alcohol dehydrogenase	conversn **5b** (%)[b]	selec[c]	conversn **6b** (%)[b]	selec[c]	conversn **8b** (%)[b]	selec[c]	conversn **7b** (%)[b]	selec[c]	de[d] (%)
C. parapsilosis (CPADH)	98	67/33	80	83/17	94	92/8	**49**	**94/6**	24
R. erythropolis (READH)	>99	69/31	64	80/20	99	92/8	>99	69/31	−3
horse liver (HLADH)	**>99**	**98/2**	**81**	**95/5**	**84**	**99/1**	39	94/6	22
baker's yeast (BYADH)	41	83/17	5	42/58	2	99/1	16	74/26	0
P. lavamentivorans (PLADH)	74	91/9	85	92/8	>99	76/24	>99	59/41	4
D. radiodurans (DRADH)	66	97/3	48	99/1	47	96/4	**87**	**92/8**	−9
L. kefir (LKADH)	58	94/6	58	74/26	86	62/38	>99	61/39	2
T. brockii (TBADH)	6	75/25	56	60/40	65	93/7	4	86/14	3
ketoreductase (KRED)							**>99**	**58/42**	0

[a]Experimental conditions: 5 g L^{-1} of substrate (~1:1 mixture, loaded on 5 g L^{-1} of resin), 100 μg mL^{-1} of ADH, 4 U mL^{-1} of GDH, 4 equiv of glucose, 0.1 mM NAD(P)$^+$, 50 mM KPi buffer pH 7.0, 30 °C, 160 rpm, reaction time 12 h. [b]Conversion of **b** to **c** by GC. [c]Defined as the ratio between saturated alcohol **c** and allylic alcohol **d**, by GC. [d]Defined as the ratio between *syn*-**7c** and *anti*-**7c**, by ^{1}H NMR.

respect to the former, ensured a better selectivity on this substrate (99/1 vs. 95/5, Table 1), the ee of the alcohol decreased to 86%. This is likely due to the fact that DRADH reduces **6b** more slowly than HLADH (conversion 48% vs 81%; see Table 1) and thus the racemization becomes a competitive process. This type of behavior was also observed in the case of the reduction of **8a**, in which between READH and HLADH the latter provided the best results in terms of conversion and selectivity (ee = 99%).

The reduction of **5a** with OYE2 and HLADH gave the alcohol (*S*)-**5c** with a good ee of 91%, and by substituting OYE2 with OYE3 the ee was slightly worse (ee = 88%). Assuming that the observed ee's are the fruit of the stereoselectivity of OYEs, these results confirm a trend already seen for the reduction of **6a**, in which OYE2 affords an ee better than that achieved with OYE3.[25b]

The ER-catalyzed reduction of α,β-unsaturated ketones such as **7a** is definitely less problematic, since it is well-known that enantiomerically enriched ketones are less prone to racemize than their aldehyde counterparts. This implies that the ADH does not have to be added simultaneously with the ER, and therefore, provided that a quantitative conversion of the α,β-unsaturated ketone into the saturated one is ensured, the chemoselectivity is no longer a critical parameter.

Both OYE2 and OYE3 afforded (*R*)-**7b** in almost quantitative yields, but the best enantioselectivity was achieved with OYE2 (98% vs 94% ee). Thus, the reduction of **7a** was carried out by a one-pot sequential addition of OYE2 and a pro-(*S*) or a pro-(*R*) ADH under the same experimental conditions adopted for the aldehydes. In the first case, either CPADH or HLADH produced mainly the syn diastereoisomer, i.e. (1*S*,3′*R*)-**7c**, with an excellent ee = 99% and discrete de's of 87% and 80%, respectively, but the reduction with HLADH was not quantitative (61% vs 97%, Table 2). Instead, KRED furnished the anti diastereoisomer (1*R*,3′*R*)-**7c** with de = 89% and once again with a high optical purity (99% ee). To stress the efficiency of this multienzymatic catalytic system, we also tested the reduction with baker's yeast, which afforded a complex mixture composed of the starting material **7a**, the saturated ketone (*R*)-**7b**, and the two diastereoisomers of alcohol **7c**, i.e. (1*S*,3*R*′)-**7c** and (1*R*,3*R*′)-**7c**, in a ratio of 77/23, together with other side products, among which the allylic alcohol was present in a negligible amount.

As shown for the reduction of aldehydes, the integration of both biocatalytic transformations in a cascade system[26] would be particularly appealing. Thus, we tested the reduction of **7a** by simultaneous addition of OYE2 and DRADH, which previously provided the best selectivity toward the formation of **7c**. However, in this case the alcohol **7c** was obtained with a lower diastereoselectivity (de = 76%). This might be explained by the fact that DRADH reduces slightly more the ketone **7b** with an *S* absolute configuration (de = −9%, Table 1), whereas OYE2 generates mainly (*R*)-**7b**; therefore, their combination is stereochemically mismatched, giving rise to a lower diastereoselectivity.

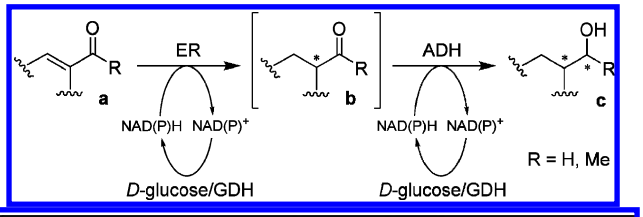
Finally, the alcohols **5c**−**8c** were produced in high yields on a preparative scale using the best combination of ER and ADH and applying always the SFPR technology: for the reduction of aldehydes **5a**, **6a**, and **8a** the cascade catalytic system was adopted, whereas for the ketone **7a** the one-pot sequential approach was chosen.

The *de* of each diastereoisomer of alcohol **7c** was then improved by crystallization in cold *n*-hexane (de = 99% for either (1*S*,3′*R*)-**7c** or (1*R*,3′*R*)-**7c**). It must be mentioned that the same procedure performed on a mixture of diastereoisomers of **7c** obtained from BY (de = 54%) was unsuccessful, and any attempt to enrich the de by column chromatography failed as well.

The analysis of the X-ray structure of (1*S*,3′*R*)-**7c** allowed us to assign the relative stereochemistry (see the Supporting Information, Figure S6), whereas, in the absence of a significant anomalous scattering, the attempt to determine the absolute configuration by refinement of the Flack parameter[27] led to an inconclusive value. The absolute stereochemistry was initially predicted on the basis of the following considerations: (i) substrate **7a** likely binds to the catalytically active site of OYE2 with the same orientation of the aldehydes, and consequently the newly created C(3′) stereocenter should be *R*; (ii) it is

dx.doi.org/10.1021/jo4003097 | J. Org. Chem. 2013, 78, 4811−4822

Table 2. Cascade Synthesis of Alcohols 5c−8c[g]



Subs.	Catalytic system[a]	Conv.[b] (%)	ee[c] (%)	Product
5a	**OYE2+HLADH**	95	91	(*S*)-**5c**
	OYE3+HLADH	89	88	
6a	OYE2+DRADH	61	86	(*S*)-**6c**
	OYE2+HLADH[d]	88	99	
8a	OYE2+READH[e]	54	85	(*S*)-**8c**
	OYE2+HLADH[d]	94	99	
7a	**OYE2; CPADH**	**97** *de*=**87**[f]	**99**	(1*S*,3′*R*)-**7c**
	OYE2; HLADH	61 *de*=80[f]	99	
	OYE2+DRADH	93 *de*=76[f]	99	
	BY	31 *de*=54[f]	96	
7a	**OYE2; KRED**	**94** *de*=**89**[f]	**99**	(1*R*,3′*R*)-**7c**

[a]A plus sign indicates a cascade system, and a semicolon indicates a one-pot sequential system. [b]By GC on the crude reaction mixture, except for BY where the products have been isolated. [c]By chiral HPLC. [d]Reference 25b. [e]20 μg mL^{-1} of READH. [f]By ^{1}H NMR. [g]Experimental conditions: 5 g L^{-1} of substrate (loaded on 15 g L^{-1} of resin), 150 μg mL^{-1} of OYE, 100 μg mL^{-1} of ADH, 4 U mL^{-1} of GDH, 4 equiv of glucose, 0.1 mM NAD(P)$^+$, 50 mM KPi buffer pH 7.0, 30 °C, 160 rpm, reaction time 12 h.

known that the stereochemistry of baker's yeast mediated reduction of ketones bearing a methyl group and a large substituent, such as **7b**, very often satisfies the Prelog rule,[20,28] according to which the absolute configuration of the C(1) stereocenter of the alcohol would be *S*. Altogether, these configurations are in agreement with the relative stereochemistry. Then, ^{1}H and ^{13}C NMR spectroscopic analysis of the (*S*)-MTPA esters[29] of *rac*-**7c** and of the optically pure **7c** obtained from the sequential reduction with OYE2 and CPADH confirmed our initial assignment of the absolute configuration: i.e., (1*S*,3′*R*)-**7c**. Indeed, both ^{1}H and ^{13}C chemical shifts of the methyl group of (*S*)-MTPA-(1*S*,3′*R*)-**7c** are upfield with respect to those of the Mosher derivative of its antipode alcohol ($\Delta\delta$ = −0.07 and −0.28 ppm for ^{1}H and ^{13}C NMR, respectively; see the Supporting Information, Figure S9), whereas the CH_2O signals resonated downfield ($\Delta\delta$ = +0.10 and +0.14 ppm for ^{1}H and ^{13}C NMR, respectively; see the Supporting Information, Figure S10).

Finally, the synthetic sequence for the preparation of amines **1**−**4**, from the optically pure precursors **5c**−**8c**, is illustrated in Scheme 2. First, alcohol (*S*)-**5c** was oxidized[30] with BAIB and catalytic TEMPO in a biphasic solvent system (CH_2Cl_2/H_2O, 3/1), to give the carboxylic acid (*R*)-**5e** in a yield of 94% without any significant loss of optical purity (vide infra). Then, by treatment with diphenylphosphoryl azide (DPPA) and Et_3N in refluxing toluene, followed by addition of benzyl alcohol,[31] acid (*R*)-**5e** was converted to the *N*-Cbz-protected amine (*R*)-**5f** in 84% yield after column chromatography.

As an alternative to the Curtius rearrangement, we also considered the Hoffman degradation route via the primary amide of (*R*)-**5e**; however, using the standard conditions (Br_2, NaOH in MeOH) together with the formation of **1** a considerable amount of bromo derivatives were produced as well. This result was ascribed to the presence of electron-donating groups on the phenyl ring, which favor the aromatic electrophilic substitution reaction.

Finally, deprotection of the amino function by catalytic hydrogenolysis of the Cbz group afforded the target compound (*R*)-**1** in 96% yield after a simple acid−base workup. Moreover, since the specific optical rotatory power value of the hydrochloride salt (*R*)-**1**·HCl matched quite well with that reported in the literature ($[\alpha]_D^{25}$ = +60.2° (*c* 1.6, MeOH), lit.[4b] $[\alpha]_D^{25}$ = +61.3° (*c* 0.5, MeOH, ee > 99%)), the initial stereochemistry was completely retained during the entire sequence.

The same route was applied to alcohols **6c** and **8c**, affording amines (*R*)-**2** ($[\alpha]_D^{25}$ = −13.3° (*c* 1.5, $CHCl_3$) lit.[9] $[\alpha]_D^{25}$ = −13.7° (*c* 1.0, $CHCl_3$)) and (*S*)-**3** ((*S*)-**3**·HCl $[\alpha]_D^{25}$ = −60.3° (*c* 1.3, MeOH) lit.[9] $[\alpha]_D^{25}$ = −61.0° (*c* 2.3, MeOH)) with similar yields.

The amine (1*R*,3′*S*)-**4** was prepared by a three-step sequence: formation of the tosyl derivative of alcohol (1*S*,3′*R*)-**7c**, which after nucleophilic substitution with NaN_3 in DMF gave the azide (1*R*,3′*S*)-**14** in an overall yield of 65%; then the latter was cleanly reduced to amine (1*R*,3′*S*)-**4** in a quantitative yield by hydrogenation with a catalytic amount of Pd/C, without any significant loss of optical purity, since the initial de = 99% was preserved.

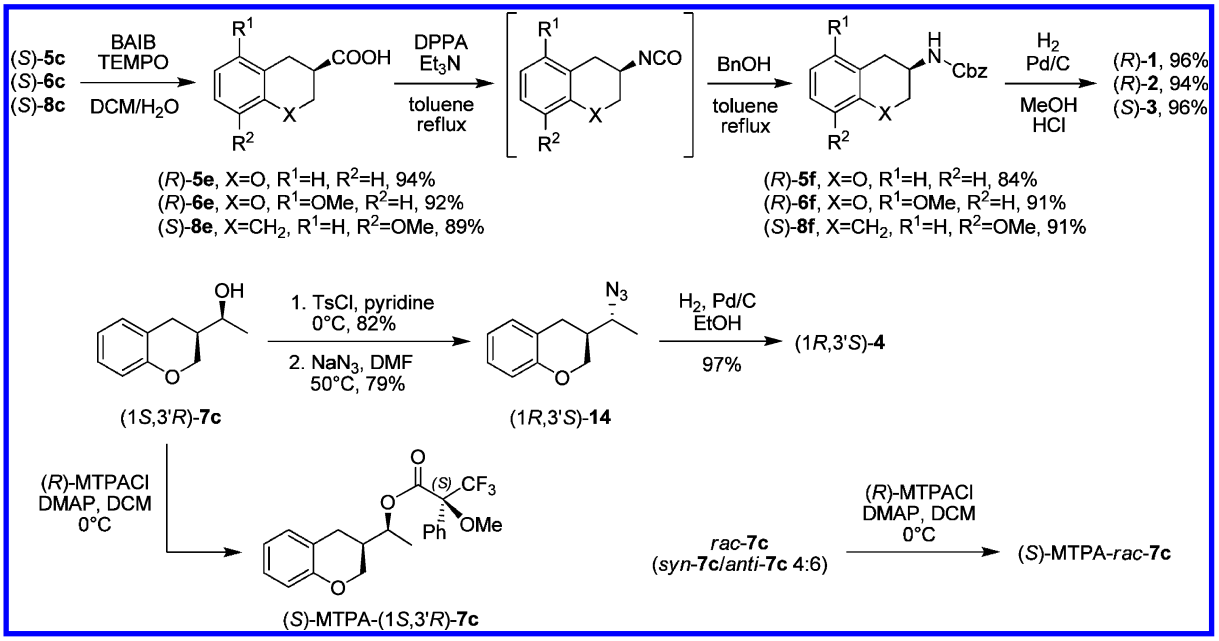
■ CONCLUSION

In summary, we have described a new synthetic route for the preparation of amines **1**−**4**, including a key biocatalytic step based on selected ERs and ADHs. In the case of α-substituted aldehydes, the addition of an ADH allows us to minimize the detrimental racemization process, preserving the optical purity of the chirally unstable aldehyde intermediate generated during the highly enantioselective reductive step of the C=C double bond.

Moreover, for the reduction of ketone **7a** the selection of a Prelog or an anti-Prelog ADH together with OYE2 gave access to (1*S*,3′*R*)-**7c** and (1*R*,3′*R*)-**7c**, respectively, with a discrete diastereoselectivity and with an excellent optical purity (ee = 99%). However, in this case, the one-pot sequential addition of the enzymes was preferred to the cascade system, since the intermediate ketones are typically less unstable. Finally, due to the different nature of all substrates taken into consideration in this work, we are quite confident that our multienzymatic catalytic system together with the synthetic strategy adopted should be broadly applicable to the enantioselective synthesis of other important amines **I** and **II**.

dx.doi.org/10.1021/jo4003097 | J. Org. Chem. 2013, 78, 4811−4822

Scheme 2. Synthesis of Amines 1−4 from Alcohols 5c−8c



EXPERIMENTAL SECTION

General Methods. Chemicals and solvents were purchased from suppliers and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a 250, 400, or 500 MHz spectrometer at room temperature, using TMS as an internal standard for ^{1}H and $CDCl_3$ for ^{13}C; chemical shifts δ are expressed in ppm relative to TMS. The GC-MS analyses of the α,β-unsaturated **5a**, **6a**, and **8a**, saturated aldehydes **5b**, **6b**, and **8b**, saturated alcohols **5c**, **6c**, and **8c**, and allylic alcohols **5d**, **6d**, and **8d** were performed on a HP-5MS column (30 m × 0.25 mm × 0.25 μm). Method: 60 °C (1 min)/6 °C min^{-1}/150 °C (1 min)/12 °C min^{-1}/280 °C (5 min). The GC-MS analyses of compounds **7a−d** were performed on a DB-5ht column (15 m × 0.25 mm × 0.10 μm) and flame ionization detector. Method: 80 °C (1 min)/0.8 °C min^{-1}/105 °C (0 min)/30 °C min^{-1}/220 °C (3 min). The enantiomeric excess values of alcohols **5c**, **6c**, and **8c** were determined by chiral HPLC analysis with a Chiralcel OD column and UV detector (210 nm): mobile phase *n*-hexane/*i*-PrOH 97/3, flow rate 0.6 mL min^{-1}. The enantiomeric excess values of ketone **7b** and of alcohol **7c** were determined by chiral HPLC equipped with a Lux 5u Cellulose-3 column and UV detector (210 nm): mobile phase *n*-hexane/*i*-PrOH 97/3; flow rate 0.6 mL min^{-1}. High-resolution MS spectra were recorded with an FT-ICR (Fourier transform ion cyclotron resonance) instrument, equipped with an ESI source. Optical rotations were measured at 589 nm and are given in deg cm^3 g^{-1} dm^{-1}. X-ray diffraction data were collected on a diffractometer using Mo Kα radiation (λ = 0.71073 Å). CIF files containing crystallographic data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request (CCDC reference file 918584). TLC analyses were performed on precoated silica gel 60 F_{254} plates, and spots were visualized either by UV light (254 nm) or by spraying with phosphomolybdic acid reagent. All chromatographic separations were carried out on silica gel columns (230−400 mesh). Protein concentrations were determined according to Bradford, using bovine serum albumine (BSA) as a standard.

Enzymes and Strains. OYE2-3 from *Saccharomyces cerevisiae* and GDH from *Bacillus megaterium* were overexpressed in *E. coli* BL21 (DE3) strains harboring a specific plasmid, according to standard molecular biology techniques as described in ref 23. CPADH from *Candida parapsilosis* and READH from *Rhodococcus erythropolis* were purchased from Jülich. HLADH from horse liver, BYADH from baker's yeast, PLADH from *Parvibaculum lavamentivorans*, DRADH from *Deinococcus radiodurans*, LKADH from *Lactobacillus kefir*, TBADH from *Thermoanaerobium brockii*, and KRED (ketoreductase) from an unspecified source were purchased from Sigma-Aldrich. Fresh baker's yeast from Lesaffre Italia was employed.

General Procedure for the Synthesis of 5a−7a. To a mechanically stirred solution of salicylaldehyde or 6-methoxysalicylaldehyde (0.133 mol), in 1,4-dioxane (200 mL), was added portionwise K_2CO_3 (22.0 g, 0.160 mol) and then acrolein (10.5 mL, 0.160 mol) or methyl vinyl ketone (13.3 mL, 0.160 mol); the reaction mixture was refluxed for 4 h. After cooling, water was slowly added (70 mL). The solid was removed by vacuum filtration, and the solution was concentrated under reduced pressure and then extracted with EtOAc (3 × 200 mL). The organic phase was washed with an aqueous solution of H_3PO_4 (5%, 200 mL) and then with brine (200 mL). The organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure. Purification by column chromatography afforded the products (EtOAc/*n*-hexane, 10/90).

*2H-Chromene-3-carbaldehyde (**5a**):* 17.61 g, 83% yield, yellow wax; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.51 (s, 1H), 7.25 (dt, J = 7.5, 1.9 Hz, 1H), 7.18 (d, J = 0.9 Hz, 1H), 7.16 (dd, J = 7.5, 1.9 Hz, 1H), 6.92 (dt, J = 7.5, 0.9 Hz, 1H), 6.83 (d, J = 8.5 Hz, 1H), 4.98 (d, J = 1.4 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.1, 155.5, 140.4, 132.6, 131.1, 128.8, 121.4, 120.0, 115.9, 62.7; HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{10}H_9O_2^+$ 161.0597, found 161.0599; GC t_r 18.01 min.

*5-Methoxy-2H-chromene-3-carbaldehyde (**6a**):* 21.45 g, 85% yield, yellow solid, mp 51−58 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.48 (s, 1H), 7.56 (s, 1H), 7.18 (t, J = 8.3 Hz, 1H), 6.44 (d, J = 8.3 Hz, 1H), 6.41 (d, J = 8.3 Hz, 1H), 4.92 (s, 2H), 3.83 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.4, 157.0, 156.6, 136.4, 133.4, 129.4, 110.4, 108.8, 103.3, 62.5, 55.5; HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{11}H_{11}O_3^+$ 191.0703, found 191.0706; GC t_r 22.33 min.

*1-(2H-Chromen-3-yl)ethanone (**7a**):* 16.42 g, 71% yield, colorless solid crystals, mp 48−50 °C (Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (s, 1H), 7.14 (td, J = 7.7, 1.5 Hz, 1H), 7.06 (dd, J = 7.7, 1.8 Hz, 1H), 6.81−6.86 (m, 1H), 6.75 (d, J = 8.1 Hz, 1H), 4.89 (s, 2H), 2.27 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.5, 155.5, 133.7, 132.3, 130.6, 129.2, 121.7, 120.7, 116.2, 64.2, 24.8; HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{11}H_{11}O_2^+$ 175.0754, found 175.0747; GC t_r 26.40 min.

Synthesis of 5-Methoxy-3,4-dihydronaphthalene-2-carbaldehyde (8a). *Method A (via Shapiro Reaction). N′-(5-Methoxy-3,4-dihydronaphthalen-2(1H)-ylidene)-4-methylbenzenesulfonohydrazide (**9**).* To a slurry of tosylhydrazine (3.8 g, 20.6 mmol) in EtOH (10 mL) was added a solution of 5-methoxy-2-tetralone (4.0 g, 22.7 mmol) in EtOH (45 mL). The mixture was stirred for 24 h and then filtered. The solid was washed with EtOH (2 × 20 mL) and dried to afford **9** as

dx.doi.org/10.1021/jo4003097 | J. Org. Chem. 2013, 78, 4811−4822

a mixture of two isomers: 6.5 g, 91% yield, yellow powder, mp 155−162 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 1/1 mixture of two isomers) δ 7.83 (d, J = 8.0 Hz, 2 H, isomer A), 7.79 (d, J = 8.0 Hz, 2H, isomer B), 7.24−7.27 (m, 4H, isomer A + B), 7.07−7.01 (m, J = 8,4 Hz, 2H isomer A + B), 6.58−6.68 (m, 4H, isomer A + B), 3.72 (s, 3H, isomer A), 3.71 (s, 3H, isomer B), 3.45 (s, 2 H, isomer A), 3.42 (s, 2 H, isomer B), 2.80 (t, J = 7.0 Hz, 2H, isomer A), 2.74 (t, J = 7.0 Hz, 2H, isomer B), 2.43 (t, J = 7.0 Hz, 2H, isomer A), 2.35 (s, 3H, isomer A), 2.34 (s, 3H, isomer B), 2.29 (t, J = 7.0 Hz, 3H, isomer B); ^{13}C NMR (101 MHz, $CDCl_3$, 1/1 mixture of two isomers) δ 159.6 and 159.4 (isomer A + B), 156.5 and 155.9 (isomer A + B), 144.1 and 144.0 (isomer A + B), 136.2 (isomer A), 135.4 and 135.3 (isomer A + B), 133.1 (isomer B), 129.6 (2C, isomer A + B), 128.0 and 127.9 (2C, isomer A + B), 127.2 and 127.1 (isomer A + B), 125.6 and 125.4 (isomer A + B), 120.8 and 119.9 (isomer A + B), 108.4 and 108.0 (isomer A + B), 55.4 and 55.3 (isomer A + B), 38.0 (isomer A), 31.5 (isomer B), 30.5 (isomer A), 25.3 (isomer B), 21.7 and 21.6 (isomer A + B), 21.5 (isomer A), 19.5 (isomer B); HRMS (ESI) m/z $(M + H)^+$ calcd for $C_{18}H_{21}N_2O_3S^+$ 345.1267, found 345.1271.

*5-Methoxy-3,4-dihydronaphthalene-2-carbaldehyde (**8a**).* Hydrazone **3b** (2.41 g, 6.97 mmol) in THF (50 mL) was cooled to −40 °C. TMEDA (2.2 mL) and *n*-BuLi (6.2 mL, 2.5 M hexane solution, 15.15 mmol) were added dropwise to the reaction mixture, which was stirred at −40 °C for 1 h. Then the mixture was warmed to 0 °C and N_2 evolution took place. After 30 min DMF (1.6 mL, 21 mmol) was added. The reaction mixture was stirred for 12 h at 0 °C. The reaction mixture was then treated with water (20 mL) and warmed to room temperature. The reaction mixture was diluted with Et_2O (30 mL), and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 30 mL). The combined organic layers were dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography (EtOAc/*n*-hexane, 10/90) to afford an inseparable mixture of **8a** and its regioisomer **10** (4;6): 420 mg, 32% yield.

*Method B (via Cyanohydrin Formation). 5-Methoxy-2-(trimethylsilyloxy)-1,2,3,4-tetrahydronaphthalene-2-carbonitrile (**11**).* To a solution of NaCN (4.92 g, 0.1 mol) in dry DMSO (50 mL) at 60 °C was added 5-methoxy-2-tetralone (8.81 g, 0.05 mol). The mixture was stirred for 15 min at 60 °C, and then TMSCl (7.5 mL, 0.06 mol) was added dropwise. After 15 min the reaction mixture was cooled to room temperature and diluted with water. The solution was extracted with ether (3 × 30 mL). The collected organic phases were washed with brine (2 × 30 mL). After the extract was dried with Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified by chromatography (EtOAc/*n*-hexane, 5/95) to give the TMS derivative **11** (8.8 g, 64% yield, oil): ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.15 (t, J = 7.9 Hz, 1 H), 6.71 (t, J = 8.4 Hz, 2 H), 3.84 (s, 3 H), 3.32 (d, J = 16.5 Hz, 1 H), 3.11 (d, J = 15.6 Hz, 1 H), 2.80−3.04 (m, 2 H), 2.22−2.34 (m, 1 H), 2.03−2.17 (m, 1 H), 0.29 (s, 9 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 157.0, 132.6, 126.9, 123.0, 121.6, 121.1, 107.9, 68.4, 55.2, 42.9, 35.4, 20.8, 1.4; HRMS (ESI) m/z $(M + H)^+$ calcd for $C_{15}H_{22}NO_2Si^+$ 276.1414, found 276.1418.

*2-Cyano-5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl methanesulfonate (**12**).* To a solution of **11** (8.0 g, 0.029 mol) in THF (50 mL), an aqueous HCl solution (3 N, 20 mL) was added. The mixture was stirred for 3 h and then diluted with water (30 mL). The two layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The organic layers were then dried with anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure to yield 2-hydroxy-5-methoxy-1,2,3,4-tetrahydronaphthalene-2-carbonitrile, which was used in the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (t, J = 7.8 Hz, 1H), 6.72 (d, J = 7.8 Hz, 1H), 6.69 (d, J = 7.8 Hz, 1H), 3.82 (s, 3H), 3.33 (d, J = 16.4 Hz, 1H), 3.10 (d, J = 16.4 Hz, 1H), 2.83−3.00 (m, 2H), 2.59 (br. s., 1H), 2.23−2.31 (m, 1H), 2.11−2.20 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.1, 131.9, 127.1, 122.8, 121.6, 121.2, 108.2, 67.3, 55.3, 41.3, 33.8, 20.3; HRMS (EI) calcd for $C_{12}H_{14}NO_2^+$ 204.1019, found 204.1014). To a stirred solution of the crude cyanohydrin in CH_2Cl_2 (50 mL) were added pyridine (10 mL) and MsCl (2.47 mL, 0.032 mol). The mixture was stirred overnight and then was diluted with CH_2Cl_2 (40 mL). The solution was washed with aqueous HCl solution (1 N, 40 mL), and the organic phase was separated and dried over Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography (EtOAc/*n*-hexane, 10/90) of crude afforded compound **13**: 10.95 g, 78% yield after two steps; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (t, J = 7.8 Hz, 1H), 6.73 (d, J = 7.8 Hz, 1H), 6.69 (d, J = 7.8 Hz, 1H), 3.82 (s, 3H), 3.58 (d, J = 17.1 Hz, 1H), 3.49 (d, J = 17.1 Hz, 1H), 3.17 (s, 3H), 2.91−3.06 (m, 2H), 2.45−2.57 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.0, 130.4, 127.5, 126.9, 122.1, 121.0, 120.9, 108.5, 55.3, 40.3, 40.2, 33.1, 20.3; HRMS (ESI) m/z $(M + H)^+$ calcd for $C_{13}H_{16}NO_4S^+$ 282.0795, found 282.0791.

*5-Methoxy-3,4-dihydronaphthalene-2-carbonitrile (**13**).* Compound **12** (10.0 g, 0.035 mol) was dissolved in pyridine (20 mL) and then heated at 60 °C for 12 h. After completion of the reaction, the solvent was removed under reduced pressure. The crude product was then dissolved in EtOAc (30 mL) and washed with aqueous HCl solution (1 N, 3 × 20 mL). The organic phase was separated and dried with Na_2SO_4. After evaporation of the solvent the crude product was purified by column chromatography (EtOAc/*n*-hexane, 10/90) to afford the product **13** and its regioisomer (93/7). Further crystallization from cold *n*-hexane/Et_2O (9/1) afforded **13** as a single isomer: 4.7 g, 72% yield, yellow-brown powder, mp 62−65 °C (hexane/Et_2O, 9/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (t, J = 8.2 Hz, 1 H) 7.15 (m, 1H), 6.90 (d, J = 8.2 Hz, 1 H), 6.79 (d, J = 7.4 Hz, 1 H), 3.84−3.87 (m, 3 H), 2.91 (t, J = 8.6 Hz, 2 H), 2.52 (td, J = 8.6, 1.5 Hz, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.3, 141.6, 132.0, 127.4, 123.3, 120.6, 119.7, 112.8, 109.8, 55.6, 24.2, 19.1; HRMS (ESI) m/z $(M + H)^+$ calcd for $C_{12}H_{12}NO^+$ 186.0913, found 186.0907.

*5-Methoxy-3,4-dihydronaphthalene-2-carbaldehyde (**8a**).* A solution of DIBAL-H in toluene (1.5 M, 20 mL) was added dropwise to a solution of **13** (4.50 g, 24 mmol) in THF (60 mL) at 0 °C. The reaction mixture was slowly warmed to room temperature and stirred for an additional 5 h before being quenched with saturated aqueous Rochelle salt (20 mL). The resulting mixture was partitioned between aqueous HCl (2 N, 20 mL) and EtOAc (40 mL). The organic layer was washed with aqueous saturated $NaHCO_3$ (2 × 40 mL). The organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure. Purification by column chromatography (EtOAc/*n*-hexane, 10/90) afforded the product **8a**: 3.38 g, 75% yield, oil which after prolonged standing turned into a solid wax; ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.68 (s, 1H), 7.19−7.27 (m, 2H), 6.95−6.92 (m, 2H), 3.87 (s, 3H), 2.89 (t, J = 8.9 Hz, 2H), 2.55 (t, J = 8.9 Hz, 2H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 192.8, 156.5, 145.5, 139.3, 133.1, 127.2, 126.1, 121.5, 113.0, 55.6, 19.4, 18.7; HRMS (ESI) m/z $(M + H)^+$ calcd for $C_{12}H_{13}O_2^+$ 189.0910, found 189.0916; GC t_r 22.67 min.

General Procedure for the Synthesis of Compounds 5c−8c. To a solution of the unsaturated carbonyl precursor **5a−8a** (10.0 mmol) in MeOH (50 mL) was added $NaBH_4$ (850 mg) in one portion at 0 °C. After completion of the reaction (by TLC and GC monitoring: t_r 20.25 min for **5d**, t_r 23.25 min for **6d**, t_r 29.28 min for **7d**, t_r 23.15 min for **8d**) the solution was acidified with aqueous HCl (1 N, 30 mL), the solvent was evaporated under reduced pressure, and the residue was neutralized with aqueous saturated $NaHCO_3$. The solution was extracted with EtOAc (3 × 30 mL), and the organic phases were dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the allylic alcohols **5d−8d**, which were submitted to the next step without further purification. Thus, **5d−8d** were dissolved in EtOH (10 mL) and 10% Pd/C (100 mg) was added. The mixture was stirred under a H_2 atmosphere overnight. The solution was filtered and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography (EtOAc/*n*-hexane, 10/90) to afford the alcohols **5c−8c**.

*Chroman-3-ylmethanol (**5c**):* 1.22 g, 74% yield, yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (t, J = 2.2 Hz, 1H), 6.88 (dd, J = 1.9, 0.2 Hz, 1H), 6.64−6.73 (m, 2H), 4.18 (dt, J = 2.6, 0.7 Hz, 1H), 3.86 (dd, J = 2.6, 1.9 Hz, 1H), 3.55−3.41 (m, 3H), 2.76 (dd, J = 3.9, 1.4 Hz, 1H), 2.49 (dd, J = 4.0, 2.0 Hz, 1H), 2.10−2.23 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.2, 129.7, 126.9, 120.8, 120.2, 116.2, 67.3,

dx.doi.org/10.1021/jo4003097 | J. Org. Chem. 2013, 78, 4811−4822

62.7, 34.5, 27.1; HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{10}H_{13}O_2^+$ 165.0910, found 165.0906; GC t_r 19.36 min.

*(5-Methoxychroman-3-yl)methanol (**6c**):* 1.51 g, 78% yield, oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (t, J = 8.2, Hz, 1H), 6.48 (d, J = 8.2 Hz, 1H), 6.42 (d, J = 8.2 Hz, 1H), 4.27 (ddd, J = 10.7, 3.1, 1.4 Hz, 1H), 3.95 (dd, J = 10.7, 7.7 Hz, 1H), 3.81 (s, 3H), 3.73 (dd, J = 10.8, 5.8 Hz, 1H), 3.64 (dd, J = 10.8, 7.8 Hz, 1H), 2.79 (dd, J = 17.1, 6.0 Hz, 1H), 2.38 (dd, J = 17.1, 7.8 Hz, 1H), 2.29−2.19 (m, 1H), 1.62 (br s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.2, 155.4, 126.9, 110.2, 109.4, 102.0, 67.3, 63.6, 55.4, 34.4, 21.9; HRMS (ESI) m/z (M + H)$^+$ calcd for 195.1016, found 195.1020; GC t_r 22.74 min.

*1-(Chroman-3-yl)ethanol (**7c**):* 1.22 g, 69% yield, wax; the product was obtained as an inseparable mixture of syn/anti (4/6) isomers (vide infra for spectra of isolated isomers); GC t_r 26.77 min for *anti*-**7c** and 27.06 min for *syn*-**7c**.

*5-Methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)methanol (**8c**):* 1.57 g, 82% yield, oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 (t, J = 8.0 Hz, 1 H), 6.74 (d, J = 8.0 Hz, 1 H), 6.68 (d, J = 8.0 Hz, 1 H), 3.84 (s, 3 H), 3.64 (d, J = 6.6 Hz, 2 H), 2.85−2.96 (m, 2 H), 2.48−2.61 (m, 2 H), 2.01−2.09 (m, 1 H), 1.90−2.00 (m, 1 H), 1.65−1.75 (br s, 1 H), 1.37−1.47 (m, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.6, 137.6, 126.3, 125.9, 121.8, 107.3, 68.0, 55.6, 37.0, 32.9, 25.8, 22.9; HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{12}H_{17}O_2^+$ 193.1223, found 193.1217; GC t_r 21.65 min.

General Procedure for the Preparation of Compounds 5b−8b. To a solution of the alcohols **5c−8c** (1.0 mmol) in CH_2Cl_2 (20 mL) was added Dess−Martin periodinane (508 mg, 1.2 mmol). After completion of the reaction by TLC monitoring, saturated aqueous Na_2SO_3 (8 mL) was added and the mixture stirred for 30 min. The organic layer was separated, and the aqueous phase was extracted with EtOAc (3 × 30 mL). The collected organic phases were then washed with saturated aqueous $NaHCO_3$, separated, and dried with Na_2SO_4. The solvent was evaporated under reduced pressure, and the crude residue was purified by column chromatography (EtOAc/*n*-hexane, 10/90) to afford the products **5b−8b**.

*Chroman-3-carbaldehyde (**5b**):* 134 mg, 83% yield, oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (d, J = 0.8 Hz, 1H), 7.08−7.15 (m, 2H), 6.90 (td, J = 7.4, 1.2 Hz, 1H), 6.83 (d, J = 8.6 Hz, 1H), 4.41 (ddd, J = 11.3, 3.5, 1.2 Hz, 1H), 4.34 (ddd, J = 11.3, 6.7, 0.8 Hz, 1H), 3.12 (dd, J = 16.4, 7.4 Hz, 1H), 3.02 (dd, J = 16.4, 5.9 Hz, 1H), 2.90−2.98 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.6, 154.4, 129.8, 127.6, 121.0, 119.6, 116.9, 64.6, 45.2, 24.5; HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{10}H_{11}O_2^+$ 163.0754, found 163.0751; GC t_r 17.02 min.

*5-Methoxychroman-3-carbaldehyde (**6b**):* 165 mg, 86% yield, oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.83 (s, 1H), 7.07 (t, J = 8.5 Hz, 1H), 6.48 (d, J = 8.5 Hz, 1H), 6.46 (d, J = 8.5 Hz, 1H), 4.36 (dd, J = 10.8, 2.8 Hz, 1H), 4.30 (dd, J = 10.8, 6.1 Hz, 1H), 3.84 (s, 3 H), 3.82 (m, 1H), 2.88−2.99 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.5, 158.4, 155.5, 127.7, 109.8, 109.3, 102.7, 64.6, 55.8, 45.0, 19.7; HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{11}H_{13}O_3^+$ 193.0859, found 193.0855; GC t_r 21.25 min.

*1-(Chroman-3-yl)ethanone (**7b**):* 142 mg, 81% yield, oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06−7.12 (m, 2H), 6.87 (td, J = 7.4, 1.2 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 4.40 (ddd, J = 10.8, 2.8, 2.6 Hz, 1H), 4.04 (ddd, J = 10.8, 7.5, 1.4 Hz, 1H), 2.88−3.04 (m, 3H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.9, 154.5, 130.1, 127.8, 121.1, 120.8, 117.0, 66.7, 46.1, 28.9, 27.5; HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{11}H_{13}O_2^+$ 177.0910, found 177.0907; GC t_r 22.02 min.

*5-Methoxy-1,2,3,4-tetrahydronaphthalene-2-carbaldehyde (**8b**):* 175 mg, 92% yield, oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (d, J = 1.2 Hz, 1H), 7.03 (t, J = 7.6 Hz, 1H), 6.69 (d, J = 7.4 Hz, 1H), 6.60 (d, J = 7.8 Hz, 1H), 3.74 (s, 3H), 2.78−2.92 (m, 3H), 2.47−2.64 (m, 2H), 2.11−2.22 (m, 1H), 1.60−1.73 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.9, 157.2, 135.6, 126.3, 124.9, 121.4, 107.3, 55.2, 46.6, 28.7, 22.7, 22.0; HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{12}H_{15}O_2^+$ 191.1067, found 191.1073; GC t_r 21.65 min.

General Procedure for the Screening-Scale OYE-Mediated Reductions. The substrates **5a−8a** (5 mg) adsorbed on XAD 1180 resin (15 mg) were added to a KP_i buffer solution (1.0 mL, 50 mM, pH 7.0) containing glucose (4 equiv with respect to **5a−8a**), NADP$^+$ (0.1 mM), GDH (4 U mL^{-1}), and an OYE (150 μg mL^{-1}). The mixture was stirred for 12 h in an orbital shaker (160 rpm, 30 °C). The solution was decanted, and both the resin and the aqueous phase were extracted with EtOAc (500 μL each), centrifuging after each extraction (15000g, 1.5 min). The combined organic solutions were dried over Na_2SO_4 for further analysis.

General Procedure for the Screening-Scale ADH-Mediated Reductions. The same procedure as for OYE-mediated reduction was followed, adding an ADH (100 μg mL^{-1}) instead of the OYE and adding also NAD$^+$ (0.1 mM) to the reaction mixture. In this case, instead of a single starting material, a ∼1/1 mixture of the unsaturated (**5a−8a**) and the racemic saturated (**5b−8b**) substrates was loaded on the resin (according to Table 1). The crude mixtures were analyzed by GC.

General Procedure for the Screening-Scale Multienzymatic Cascade Reductions. The same procedure as for OYE-mediated reduction was followed, adding also an ADH (100 μg mL^{-1}) and NAD$^+$ (0.1 mM) to the reaction mixture (according to Table 2). The crude mixtures were analyzed by GC.

General Procedure for the Preparative Scale Biotransformations. For all of the substrates a similar protocol was followed (either as a cascade system or as a one-pot sequential system) on a larger scale, employing the OYE and the ADH which provided the best conversion and/or ee.

Method A (Multienzymatic Cascade System). The substrate **5a**, **6a**, or **8a** (1.25 mmol) adsorbed on resin (750 mg) was added to a KP_i buffer solution (100 mL, 50 mM, pH 7.0) containing OYE2 (150 μg mL^{-1}), HLADH (100 μg mL^{-1}), GDH (400 U), glucose (5 mmol, 900 mg), NAD$^+$ (10 μmol, 6.6 mg), and NADP$^+$ (10 μmol, 7.4 mg). The reaction mixture was stirred in an orbital shaker (160 rpm, 30 °C) and monitored by GC until complete conversion, with further addition of aliquots of OYE2, HLADH, and GDH if necessary. The solution was decanted, and both the resin and the aqueous phase were extracted with EtOAc (2 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude materials were submitted to column chromatographic purification (EtOAc/*n*-hexane 10/90).

Bioreduction of **5a** with OYE2 and HLADH gave (*S*)-**5c**: 184 mg, 90% isolated yield, 91% ee by HPLC (t_R(*R*) = 34.6 min, t_R(*S*) = 37.5 min); $[\alpha]^{25}_D$ = −19.6 (*c* 1.2, $CHCl_3$), lit.[32] $[\alpha]^{25}_D$ = −19.0 (*c* 0.3, $CHCl_3$).

Bioreduction of **6a** with OYE2 and HLADH gave (*S*)-**6c**: 201 mg, 83% isolated yield, 99% ee by HPLC (t_R(*S*) = 39.2 min, t_R(*R*) = 42.9 min); $[\alpha]_D$ = −6.9 (*c* 1.2, $CHCl_3$), lit.[25b] $[\alpha]_D$ = −6.2 (*c* 1.17, $CHCl_3$).

Bioreduction of **8a** with OYE2 and HLADH gave (*S*)-**8c**: 214 mg, 89% isolated yield, 99% ee by HPLC (t_R(*R*) = 22.8 min, t_R(*S*) = 23.6 min); $[\alpha]_D$ = −42.2 (*c* 1.5, $CHCl_3$).

Method B (Multienzymatic One-Pot Sequential System). Substrate **7a** (1.25 mmol, 220 mg) adsorbed on resin (750 mg) was added to a KP_i buffer solution (100 mL, 50 mM, pH 7.0) containing OYE2 (150 μg mL^{-1}), GDH (400 U), glucose (5 mmol, 900 mg), and NADP$^+$ (10 μmol, 7.4 mg). The reaction mixture was stirred in an orbital shaker (160 rpm, 30 °C) and monitored by GC until complete conversion, and then the required ADH (100 μg mL^{-1}) and NAD$^+$ (10 μmol, 6.6 mg) were added in one portion. The stirring was continued until complete conversion, with further addition of aliquots of ADH and GDH if necessary. The mixture was then processed as described in Method A. The products **7c** were then recrystallized from cold *n*-hexane in order to improve the *de*.

Bioreduction of **7a** with OYE2 and no ADH gave (*R*)-**7b**: 211 mg, 96% isolated yield, 99% ee by HPLC (t_R(*S*) = 29.1 min, t_R(*R*) = 30.7 min), $[\alpha]_D$ = −39.1 (*c* 1.5, $CHCl_3$).

Bioreduction of **7a** with OYE2 and CPADH gave (1*S*,3′*R*)-**7c**: 158 mg, 71% isolated yield after recrystallization, colorless solid, mp 48−51 °C, 99% ee by HPLC (t_R(1*S*,3′*R*) = 28.5 min, t_R(1*R*,3′*S*) = 30.2 min, t_R(1*R*,3′*R*) = 31.3 min, t_R(1*S*,3′*S*) = 36.0 min); $[\alpha]_D$ = −50.1 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (t, J = 8.1 Hz, 1H), 7.02 (d, J = 7.3 Hz, 1H), 6.77−6.85 (m, 2H), 4.42 (ddd, J = 10.8, 3.1, 1.8 Hz, 1H), 3.95 (dd, J = 10.8, 8.8 Hz, 1H), 3.72 (quin, J = 6.6 Hz, 1H), 2.78 (dd, J = 15.9, 5.1 Hz, 1H), 2.57 (dd, J = 15.9, 9.4 Hz, 1H), 1.94−

dx.doi.org/10.1021/jo4003097 | J. Org. Chem. 2013, 78, 4811−4822

2.04 (m, 1H), 1.47 (s, 1H), 1.30 (d, J = 6.2 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.1, 130.2, 127.6, 121.5, 120.7, 116.8, 68.8, 68.0, 40.2, 28.3, 21.8; HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{11}H_{15}O_2^+$ 179.1067, found 179.1072.

Bioreduction of **7a** with OYE2 and KRED gave (1*R*,3′*R*)-**7c**: 163 mg, 73% isolated yield after recrystallization, colorless powder, mp <45 °C, 99% ee by HPLC (t_R reported above); $[\alpha]_D$ = −50.6 (c 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08−7.18 (m, 2H), 6.90 (td, J = 7.6, 1.4 Hz, 1H), 6.85 (d, J = 8.6 Hz, 1H), 4.23 (ddd, J = 11.0, 3.4, 1.3 Hz, 1H), 3.94 (dd, J = 11.0, 9.2 Hz, 1H), 3.85 (quin, J = 6.4, 1H), 2.85 (dd, J = 16.2, 6.4, 1H), 2.76 (dd, J = 16.2, 11.0 Hz, 1H), 1.90−2.02 (m, 1H), 1.22 (d, J = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.7, 130.1, 127.2, 121.6, 120.4, 116.4, 68.2, 68.1, 39.6, 26.4, 21.3; HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{11}H_{15}O_2^+$ 179.1067, found 179.1070.

Baker's Yeast Mediated Reduction of Ketone 7a. To a mechanically stirred mixture of commercial baker's yeast (250 g) in tap water (2.0 L) at 30 °C was added a solution of glucose (100 g) in water (200 mL). After 1 h the substrate **7a** (3.0 g, 17.2 mmol) adsorbed on XAD 1180 resin (60 g) was added in one portion. Vigorous stirring was continued for 5 days. After 24 h more baker's yeast (250 g) and glucose (100 g) were added. Then, the mixture was filtered on a sintered glass funnel (porosity 0, >165 μm) and the aqueous phase was extracted again with more resin (50 g). The combined resin crops were washed with acetone (20 mL) and EtOAc (3 × 50 mL). The organic solution was washed with brine (2 × 20 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give a brownish oil, which was submitted to column chromatographic purification (EtOAc/*n*-hexane, 10/90), affording residual **7a** (180 mg, 6% yield), ketone (*R*)-**7b** (1.27 g, 42% yield, colorless oil), and a mixture of saturated alcohol **7c** (800 mg, 26% yield, diastereomeric ratio 77/23 *syn*-**7c**/*anti*-**7c**).

General Procedure for the Preparation of Acids (*R*)-5e, (*R*)-6e, and (*S*)-8e. To stirred solutions of alcohols (*S*)-**5c**, (*S*)-**6c**, and (*R*)-**8c** (1.0 mmol) in CH_2Cl_2/H_2O (2/1, 6 mL) were added TEMPO (46 mg, 0.3 mmol) and BAIB (640 mg, 2.0 mmol). After completion of the reaction, the mixture was quenched with saturated aqueous Na_2SO_3 solution (5 mL). Then, the mixture was washed with saturated aqueous $NaHCO_3$ (2 × 10 mL). The combined aqueous phase was washed with EtOAc (5 mL) and acidified with HCl (1 M, 10 mL). The aqueous solution was washed with EtOAc (3 × 20 mL). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to give the corresponding acid without further purification.

*(R)-Chroman-3-carboxylic acid ((R)-**5e**):* 165 mg, 94% yield, yellow solid, mp 125−128 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.53 (br s, 1H), 6.98−7.07 (m, 2H), 6.82 (td, J = 8.1, 0.8 Hz, 1H), 6.77 (d, J = 8.1 Hz, 1H), 4.36 (d, J = 10.6 Hz, 1H), 4.06−4.15 (m, 1H), 2.93−3.07 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.1, 154.3, 130.0, 128.0, 121.2, 120.3, 117.1, 66.4, 38.6, 27.5; $[\alpha]^{25}_D$ +29.5 (c 1.3, $CHCl_3$); HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{10}H_{11}O_3^+$ 179.0703, found 179.0707.

*(R)-5-Methoxychroman-3-carboxylic acid ((R)-**6e**):* 172 mg, 92% yield, yellow solid, mp 171−173 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.1−9.3 (s br, 1H), 7.08 (t, J = 8.2, Hz, 1H), 6.50 (dd, J = 8.2, 0.8 Hz, 1H), 6.45 (dd, J = 8.2, 0.8 Hz, 1H), 4.41 (ddd, J = 10.7, 3.2, 1.7 Hz, 1H), 4.13 (dd, J = 10.7, 8.2 Hz, 1H), 3.83 (s, 3H), 3.04 (m, 2H), 2.89 (dd, J = 18.5, 10.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.5, 158.0, 154.8, 127.2, 109.4, 102.3, 65.7, 55.4, 38.0, 29.6, 22.0; $[\alpha]^{25}_D$ −14.9 (c 1.2, $CHCl_3$); HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{11}H_{13}O_4^+$ 209.0808, found 209.0811.

*(S)-5-Methoxy-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid ((S)-**8e**):* 181 mg, 89% yield, colorless solid, mp 149−151 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14−10.14 (s, br, 1H), 7.08 (t, J = 7.9 Hz, 1H), 6.72 (d, J = 7.8 Hz, 1H), 6.67 (d, J = 8.1 Hz, 1H), 3.80 (s, 3H), 2.98−3.05 (m, 1H), 2.89−2.98 (m, 1H), 2.68−2.77 (m, 1H), 2.59 (ddd, J = 17.6, 11.1, 6.2 Hz, 1H), 2.22−2.31 (m, 1H), 1.76−1.90 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.9, 157.1, 135.9, 126.1, 124.5, 121.1, 107.1, 55.0, 39.4, 31.4, 25.3, 22.3; $[\alpha]^{25}_D$ −19.3 (c 1.6, $CHCl_3$); HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{12}H_{15}O_3^+$ 207.1016, found 207.1010.

General Procedure for the Preparation of (*R*)-5f, (*R*)-6f, and (*S*)-8f. To solutions of the carboxylic acids (*R*)-**5e**, (*R*)-**6e**, and (*S*)-**8e** (0.87 mmol) in toluene (3 mL) were added DPPA (234 μL, 1.1 mmol) and Et_3N (151 μL, 1.1 mmol). The reaction mixture was refluxed for 3 h, and then benzyl alcohol (224 μL, 2.2 mmol) was added. After 2 h the reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residual oil was purified by chromatography (EtOAc/*n*-hexane, 5/95) to yield the *N*-Cbz-protected amine.

*(R)-Benzyl chroman-3-ylcarbamate ((R)-**5f**):* 207 mg, 84% yield, oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24−7.38 (m, 5H), 7.10 (td, J = 7.7, 0.8 Hz, 1H), 7.01 (d, J = 7.3 Hz, 1H), 6.87 (dt, J = 7.6, 1.4 Hz, 1H), 6.82 (dd, J = 8.1, 1.1 Hz, 1H), 5.09 (br. s., 3H), 4.23 (br s, 1H), 4.05−4.14 (m, 2H), 3.10 (dd, J = 16.5, 5.0 Hz, 1H), 2.75 (d, J = 16.5 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.7, 153.9, 136.3, 130.4, 128.5, 128.2, 128.1, 127.8, 121.2, 119.1, 116.8, 68.2, 66.9, 44.0, 31.1; $[\alpha]^{25}_D$ +16.3 (c 1.2, $CHCl_3$); HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{17}H_{18}NO_3^+$ 284.1281, found 284.1289.

*(R)-Benzyl 5-methoxychroman-3-ylcarbamate ((R)-**6f**):* 248 mg, 91% yield, oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26−7.36 (m, 5 H), 7.06 (t, J = 8.3 Hz, 1 H), 6.48 (d, J = 8.4 Hz, 1 H), 6.43 (d, J = 8.1 Hz, 1 H), 5.02−5.16 (m, 3 H), 4.23 (br. s., 1 H), 4.02−4.11 (m, 2 H), 3.78 (s, 3 H), 2.88 (dd, J = 17.4, 5.6 Hz, 1 H), 2.71 (br. d, J = 17.4 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.2, 149.5, 149.4, 146.4, 131.1, 123.2, 122.8, 122.1, 104.1, 103.1, 97.3, 62.5, 61.5, 50.1, 38.3, 20.7; $[\alpha]^{25}_D$ +46.4 (c 1, $CHCl_3$); HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{18}H_{20}NO_4^+$ 314.1387, found 314.1381.

*(S)-Benzyl 5-methoxy-1,2,3,4-tetrahydronaphthalen-2-ylcarbamate ((S)-**8f**):* 246 mg, 91% yield, oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30−7.40 (m, 5H), 7.10 (t, J = 8.0 Hz, 1H), 6.68 (d, J = 8.0 Hz, 2H), 5.11 (br s, 2H), 4.75−4.89 (m, 1H), 4.01−4.11 (m, 1H), 3.79−3.86 (m, 3H), 3.11 (dd, J = 16.2, 4.4 Hz, 1H), 2.59−2.88 (m, 3H), 2.12−2.00 (m, 1H), 1.85−1.73 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.4, 151.0, 136.3, 134.9, 128.2, 127.8, 126.3, 126.1, 124.0, 121.3, 107.0, 66.3, 54.9, 46.0, 35.6, 28.0, 20.6; $[\alpha]^{25}_D$ +27.2 (c 1, $CHCl_3$); HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{19}H_{22}NO_3^+$ 312.1594, found 312.1600.

General Procedure for the Synthesis of Amines (*R*)-1, (*R*)-2, and (*S*)-3. Compounds (*R*)-**5f**, (*R*)-**6f**, or (*S*)-**8f** (0.7 mmol) were dissolved in MeOH (2 mL), and then 10% Pd/C (5 mg) and a few drops of concentrated aqueous HCl were added. The mixture was stirred under an H_2 atmosphere for 12 h. Then the mixture was filtered and the solvent was removed under reduced pressure to give the amine without further purification.

*(R)-Chroman-3-amine ((R)-**1**):* 127 mg, 98% yield, oil; ^{1}H NMR (HCl salt, 400 MHz, DMSO-d_6) δ 8.74 (s, 3H), 7.08−7.16 (m, 2H), 6.89 (t, J = 7.6 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 4.27 (d, J = 11.2 Hz, 1H), 4.12−4.21 (m, 1H), 3.68 (br s, 1H), 3.17 (dd, J = 16.9, 4.1 Hz, 1H), 2.96 (dd, J = 16.9, 6.1 Hz, 1H); ^{13}C NMR (HCl salt, 101 MHz, DMSO-d_6) δ 153.4, 130.0, 127.5, 121.1, 118.7, 116.5, 65.4, 43.3, 28.0; $[\alpha]^{25}_D$ +60.2 (c 1.6, MeOH); HRMS (ESI) m/z (M + H)$^+$ calcd for $C_9H_{12}NO^+$ 150.0913, found 150.0916.

*(R)-5-Methoxy-3-aminochroman ((R)-**2**):* 118 mg, 94% yield, oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (t, J = 8.3 Hz, 1H), 6.40 (d, J = 8.4 Hz, 1H), 6.34 (dd, J = 8.1, 0.8 Hz, 1H), 3.99 (ddd, J = 10.4, 2.9, 1.5 Hz, 1H), 3.72 (s, 3H), 3.67 (ddd, J = 10.4, 7.1, 1.3 Hz, 1H), 3.22 (tdd, J = 7.0, 7.0, 5.6, 3.1 Hz, 1H), 2.86 (ddd, J = 16.8, 5.6, 1.4 Hz, 1H), 2.32 (dd, J = 16.9, 6.9 Hz, 1H), 1.59 (br s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.3, 154.7, 127.0, 109.4, 109.1, 102.1, 70.9, 55.3, 43.7, 29.2; $[\alpha]^{25}_D$ −13.3 (c 1.5, $CHCl_3$); HRMS (ESI) m/z (M + H)$^+$ calcd for $C_{10}H_{14}NO_2^+$ 180.1019, found 180.1022.

*(S)-5-Methoxy-2-amino-1,2,3,4-tetrahydronaphthalene ((S)-**3**):* 143 mg, 96% yield, oil; ^{1}H NMR (HCl salt, 400 MHz, DMSO-d_6) δ 8.48 (br s, 3H), 7.10 (t, J = 8.1 Hz, 1H), 6.77 (d, J = 8.1 Hz, 1H), 6.69 (d, J = 7.3 Hz, 1H), 3.75 (s, 3H), 3.32 (br s, 1H), 3.05 (dd, J = 16.1, 3.7 Hz, 1H), 2.79−2.85 (m, 2H), 2.41−2.60 (m, 2H), 2.15 (br d, J = 10.3 Hz, 1H), 1.64−1.79 (m, 1H); ^{13}C NMR (HCl salt, 101 MHz, DMSO-d_6) δ 157.2, 134.5, 127.2, 123.7, 121.4, 108.3, 55.7, 46.8, 33.4,

dx.doi.org/10.1021/jo4003097 | J. Org. Chem. 2013, 78, 4811−4822

26.7, 21.8; $[\alpha]^{25}_{D}$ −60.3 (*c* 1.3, MeOH); HRMS (ESI) *m*/*z* (M + H)$^+$ calcd for $C_{11}H_{16}NO^+$ 178.1226, found 178.1220.

(*S*)-3-((*R*)-1-Azidoethyl)chroman ((1*R*,3′*S*)-14). To a stirred solution of (1*S*,3′*R*)-**7c** (85 mg, 0.48 mmol) in CH_2Cl_2 (5 mL) and pyridine (2 mL) was added TsCl (91 mg, 0.48 mmol). The mixture was stirred overnight and then was diluted with CH_2Cl_2 (10 mL). The solution was washed with aqueous HCl (1 N, 10 mL), and the organic phase was dried with Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography (EtOAc/*n*-hexane, 10/90) afforded the intermediate tosylate: 130 mg, 82% yield, yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 7.8 Hz, 2H), 7.08 (td, *J* = 8.3, 1.5 Hz, 1H), 7.01 (d, *J* = 7.3 Hz, 1H), 6.84 (td, *J* = 7.3, 1.5 Hz, 1H), 6.75 (dd, *J* = 8.3, 1.0 Hz, 1H), 4.60 (dq, *J* = 7.2, 6.2 Hz, 1H), 4.22 (ddd, *J* = 10.8, 2.9, 1.9 Hz, 1H), 3.67 (dd, *J* = 10.8, 9.3 Hz, 1H), 2.76 (dd, *J* = 16.6, 1.5 Hz, 1H), 2.57 (dd, *J* = 16.1, 9.8 Hz, 1H), 2.47 (s, 3H), 2.18−2.27 (m, 1H), 1.41 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.3, 144.8, 134.0, 129.8 (2C), 129.7, 127.7 (2C), 127.5, 120.5, 120.1, 116.5, 79.6, 66.5, 37.9, 27.3, 21.6, 18.8. The tosylate (110 mg, 0.33 mmol) was dissolved in DMF (5 mL), and NaN_3 (33 mg, 0.5 mmol) was added. The reaction mixture was stirred at 60 °C for 8 h, and then after cooling water (10 mL) was added. The mixture was extracted with Et_2O (3 × 20 mL). The combined organic phases were washed with brine (2 × 10 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the crude product by column chromatography (EtOAc/*n*-hexane, 5/95) afforded (1*R*,3′*S*)-**14**: 53 mg, 79% yield, yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.01−7.14 (m, 2H), 6.85 (t, *J* = 7.2 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 4.19 (dt, *J* = 10.8, 2.4 Hz, 1H), 3.94 (dd, *J* = 10.8, 8.4 Hz, 1H), 3.55 (quint, *J* = 6.4 Hz, 1H), 2.89 (dd, *J* = 16.5, 5.2 Hz, 1H), 2.77 (dd, *J* = 16.5, 8.4 Hz, 1H), 2.05 (dtq, *J* = 12.4, 6.0, 2.8 Hz, 1H), 1.40 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.5, 130.1, 127.3, 120.8, 120.6, 116.5, 67.7, 58.3, 37.9, 27.3, 16.9; HRMS (ESI) *m*/*z* (M + H)$^+$ calcd for $C_{11}H_{14}N_3O^+$ 204.1131, found 204.1136; $[\alpha]^{25}_{D}$ −48.2 (*c* 2.3, $CHCl_3$).

(*R*)-1-((*S*)-Chroman-3-yl)ethanamine ((1*R*,3′*S*)-4). (1*R*,3′*S*)-**14** (30 mg, 0.15 mmol) was dissolved in EtOAc (5 mL), and then 10% Pd/C (5 mg) was added. The mixture was stirred under an H_2 atmosphere for 12 h. The mixture was filtered, and the solvent was removed under reduced pressure to give (1*R*,3′*S*)-**4** without further purification: 25 mg, 97% yield, yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04−7.08 (m, 2H), 6.84 (t, *J* = 7.2 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 4.27 (ddd, *J* = 10.8, 3.2, 1.2 Hz, 1H), 3.89 (dd, *J* = 10.8, 8.8 Hz, 1H), 2.97 (quin, *J* = 6.4 Hz, 1H), 2.89 (ddd, *J* = 16.4, 5.6, 2.0 Hz, 1H), 2.73 (dd, *J* = 16.4, 9.6 Hz, 1H), 1.86−1.96 (m, 1H), 1.20 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.9, 130.3, 127.5, 122.7, 120.7, 116.7, 68.9, 48.3, 40.0, 27.9, 21.9; HRMS (ESI) *m*/*z* (M + H)$^+$ calcd for $C_{11}H_{16}NO^+$ 178.1226, found 178.1222; $[\alpha]^{25}_{D}$ −7.1 (*c* 1, $CHCl_3$).

Preparation of the Mosher Derivatives. The Mosher esters were prepared by adding a solution of the alcohol ((1*S*,3′*R*)-**7c** or *rac*-**7c**, 10 mg) in CH_2Cl_2 (1 mL) to an ice-cold solution of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid chloride in CH_2Cl_2 (2 mL) in the presence of DMAP (20 mg) under a N_2 atmosphere. The acid chloride was prepared from (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid according to a previously reported procedure.[33] After 12 h the reaction mixture was quenched with few drops of water and the solvent was removed under reduced pressure. The crude material was submitted to column chromatographic purification (EtOAc/*n*-hexane, 5/95) to give the corresponding Mosher ester. The ^{1}H, ^{13}C, and ^{19}F spectra of selected signals are reported in the Supporting Information (Figures S8−S11).

■ ASSOCIATED CONTENT

Supporting Information

Text, tables, and figures giving the procedure for the overexpression and purification of the enzymes, HPLC chromatograms, ^{1}H and ^{13}C NMR spectra, X-ray structure and crystallographic data for *syn*-**7c**, NMR spectra of MTPA derivatives of *syn*-**7c**, and computational details for (*S*)-MTPA-(1*S*,3′*R*)-**7c**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: francesco.gatti@polimi.it (F.G.G.); alessandro.sacchetti@polimi.it (A.S.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Prof. Claudio Fuganti (Politecnico di Milano) is gratefully acknowledged for fruitful discussions and for providing a sample of (*S*)-**3**·HCl. We thank Dr. Martin Hedberg (AstraZeneca, UK) for the gift of a sample of (*R*)-**2**. Prof. Sven Panke and Christian Femmer (ETH-Zürich, Department of Biosystems Science and Engineering, Basel, CH) are kindly acknowledged for the help and advice given in the preparation of plasmids and strains. We are particularly grateful to the undergraduate students Marco Rigamonti, Francesco Rossi, and Ernesto Mandarino, who contributed to the preliminary phase of this work. This research has been was supported by EU project INTENANT (Grant FP7-NMP2-SL2008-214129).

■ REFERENCES

(1) (a) Cusack, N. J.; Peck, J. V. *Drugs Future* **1993**, *18*, 1005. (b) Federsel, H.-J.; Sveno, A. *Process Chem. Pharm. Ind.* **2008**, 111.

(2) Evenden, J. L.; Hammarberg, E. M.; Hansson, H. S.; Hellberg, S. E.; Johansson, L. G.; Lundkvist, J. R. M.; Ross, S. B.; Sohn, D. D.; Thorberg, S. O. U.S. Patent 5,616,610, 1997.

(3) Federsel, H.-J. *Org. Process Res. Dev.* **2000**, *4*, 362−369.

(4) (a) Beliaev, A.; Learmonth, D. A.; Soares-da-Silva, P. *J. Med. Chem.* **2006**, *49*, 1191−1197. (b) Brisander, M.; Caldirola, P.; Johansson, A. M.; Hackell, U. *J. Org. Chem.* **1998**, *63*, 5362−5376.

(5) Horn, A. S. U.S. Patent US 4885308 A 19891205, 1989.

(6) Eli Lilly and Company, Patent WO2005/821 A1, 2005.

(7) Brenna, E.; Fuganti, C.; Gatti, F. G.; Serra, S. *Chem. Rev.* **2011**, *111*, 4036−4072.

(8) (a) Brenna, E.; Fuganti, C.; Gatti, F. G.; Parmeggiani, F. *Tetrahedron: Asymmetry* **2009**, *20*, 2594−2599. (b) Brenna, E.; Fuganti, C.; Gatti, F. G.; Parmeggiani, F. *Tetrahedron: Asymmetry* **2009**, *20*, 2694−2698. (c) Serra, S.; Gatti, F. G.; Fuganti, C. *Eur. J. Org. Chem.* **2008**, 1031−1037.

(9) Usse, S.; Pave, G.; Guillamet, G.; Viaud-Massuard, M. C. *Tetrahedron: Asymmetry* **2001**, *12*, 1689−1694.

(10) Pave, G.; Usse-Versluys, S.; Viaud-Massuard, M. C.; Guillamet, G. *Org. Lett.* **2003**, *5*, 4253−4256.

(11) Fuganti, C.; Sacchetti, A. *J. Mol. Catal. B: Enzym.* **2010**, *66*, 276−284.

(12) Tahar Ayad, Z. W.; Ratovelomanana-Vidal, V. *Org. Lett.* **2011**, *13*, 3782−3785.

(13) Buisson, D.; Cecchi, R.; Laffitte, J.-A.; Guzzi, U.; Azerad, R. *Tetrahedron Lett.* **1994**, *35*, 3091−3094.

(14) Eve, T. S. C.; Wells, A.; Turner, N. J. *Chem. Commun.* **2007**, 1530−1531.

(15) (a) Faber, K. *Biotransformations in Organic Chemistry*, 6th ed.; Springer-Verlag: Berlin, 2011. (b) Clouthier, C. M.; Pelletier, J. N. *Chem. Soc. Rev.* **2012**, *41*, 1585−1605. (c) Hall, M.; Bommarius, A. S. *Chem. Rev.* **2011**, *111*, 4088−4110.

(16) (a) Pollard, D. J.; Woodley, J. M. *Trends Biotechnol.* **2006**, *25*, 66−73. (b) Constable, D. J. C.; Dunn, P. J.; Hayler, J. D.; Humphrey, G. R.; Leazer, J. L.; Linderman, R. J.; Lorenz, K.; Manley, J.; Pearlman, B. A.; Wells, A.; Zaks, A.; Zhang, T. Y. *Green Chem.* **2007**, *9*, 411−420.

(17) (a) Korpak, M.; Pietruszka, J. *Adv. Synth. Catal.* **2011**, *353*, 1420−1424. (b) Stueckler, C.; Mueller, N. J.; Winkler, C. K.; Glueck, S. M.; Gruber, K.; Steinkellner, G.; Faber, K. *Dalton Trans.* **2010**, *39*,

8472−8476. (c) Yanto, Y.; Winkler, C. K.; Lohr, S.; Hall, M.; Faber, K.; Bommarius, A. S. *Org. Lett.* **2011**, *13*, 2540−2543. (d) Bougioukou, D. J; Walton, A. Z.; Stewart, J. D. *Chem. Commun.* **2010**, *46*, 8558−8560. (e) Walton, A. Z.; Conerly, W. C.; Pompeu, Y.; Sullivan, B.; Stewart, J. D. *ACS Catal.* **2011**, 989−993. (f) Kosjek, B.; Fleitz, F. J.; Dormer, P. G.; Kuethe, J. T.; Devine, P. N. *Tetrahedron: Asymmetry* **2008**, *19*, 1403−1406. (g) Brenna, E.; Gatti, F. G.; Manfredi, A.; Monti, D.; Parmeggiani, F. *Eur. J. Org. Chem.* **2011**, 4015−4022. (h) Brenna, E.; Gatti, F. G.; Manfredi, A.; Parmeggiani, F. *Org. Process Res. Dev.* **2012**, *16*, 262−268.

(18) (a) Toogood, H.; Gardiner, J. M.; Scrutton, N. S. *ChemCatChem* **2010**, *2*, 892−914. (b) Winkler, C. K.; Tasnádi, G.; Clay, D.; Hall, M.; Faber, K. *J. Biotechnol.* **2012**, *162*, 381−389. (c) Stuermer, R.; Hauer, B.; Hall, M.; Faber, K. *Curr. Opin. Chem. Biol.* **2007**, *11*, 203−213.

(19) (a) Servi, S. *Synthesis* **1990**, 1−25. (b) Csuk, R.; Glaenzer, B. I. *Chem. Rev.* **1991**, *91*, 49−97.

(20) Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659−2681.

(21) Chamberlin, A. R.; Bloom, S. H. *Org. React.* **1990**, *39*, 1−84.

(22) (a) Yoneda, R.; Santo, K.; Harusawa, S.; Kurihara, T. *Synthesis* **1986**, 1054−1055. (b) Cabirol, F. L.; Lim, A. E. C.; Hanefeld, U.; Sheldon, R. A.; Lyapkalo, I. M. *J. Org. Chem.* **2008**, *72*, 2446−2449.

(23) Bechtold, M.; Brenna, E.; Femmer, C.; Gatti, F. G.; Panke, S.; Parmeggiani, F.; Sacchetti, A. *Org. Process Res. Dev.* **2012**, *16*, 269−276.

(24) (a) Vicenzi, J. T.; Zmijewski, M. J.; Reinhard, M. R.; Landen, B. E.; Muth, W. L.; Marler, P. G. *Enzyme Microb. Technol.* **1997**, *20*, 494−499. (b) Anderson, B. A.; Hansen, M. M.; Harkness, A. R.; Henry, C. L.; Vicenzi, J. T.; Zmijewski, M. J. *J. Am. Chem. Soc.* **1995**, *117*, 12358−12359. (c) D'Arrigo, P.; Fuganti, C.; Pedrocchi Fantoni, G.; Servi, S. *Tetrahedron* **1998**, *54*, 15017−15026.

(25) (a) Brenna, E.; Gatti, F. G.; Monti, D.; Parmeggiani, F.; Sacchetti, A. *ChemCatChem* **2012**, *4*, 653−659. (b) Brenna, E.; Gatti, F. G.; Monti, D.; Parmeggiani, F.; Sacchetti, A. *Chem. Commun.* **2012**, *48*, 79−81.

(26) (a) Ricca, E.; Brucher, E.; Schrittwieser, J. H. *Adv. Synth. Catal.* **2011**, *353*, 2239−2262.

(27) Flack, H. D. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *39*, 876−881.

(28) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119−130.

(29) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.

(30) Epp, J. B.; Widlanski, T. S. *J. Org. Chem.* **1999**, *64*, 293−295.

(31) Kim, D.; Weinreb, S. M. *J. Org. Chem.* **1978**, *43*, 125−131.

(32) Broggini, G.; Folcio, F.; Sardone, N.; Sonzogni, M.; Zecchi, G. *Tetrahedron: Asymmetry* **1996**, *7*, 797−806.

(33) Pontius, A.; Krick, A.; Kehraus, S.; Koenig, G. M.; Brun, R. *J. Nat. Prod.* **2008**, *71*, 1579−1584.