Daucus carota L. mediated bioreduction of prochiral ketones

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Stereoselective reductions of ketones to secondary alcohols are of the utmost importance in organic synthesis. Very high selectivities are observed with traditional reducing agents, mainly based on boron or transition metals, complexed with chiral ligands. Bioreductions mediated by intact cells from cut plants, vegetables and fruits are attractive alternatives and could facilitate transition towards a more biobased economy. This emerging area highlights the recent results obtained in the aqueous bioreduction of prochiral ketones using carrot roots. The applications of this methodology to asymmetric protonation, dynamic kinetic resolution and the synthesis of biologically relevant targets are presented.

Asymmetric reduction of prochiral ketones is an essential transformation in organic synthesis, owing to the prevalence of secondary alcohols in naturally and biologically active compounds. Enantioselective borane or metal-mediated reduction processes are among the most efficient strategies to prepare chiral secondary alcohols. An excellent π-facial selectivity can usually be obtained by the fine tuning of the steric and electronic properties of the reducing complex. Moreover, the predictable stereochemical outcome has contributed to the success of these methods within the organic synthetic community.

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In the context of developing green and sustainable chemical processes as part of the transition towards a more biobased[1] economy, biotechnologies are attractive alternatives. The role of biotechnologies for the synthesis of bulky and fine organic chemical substances is increasing considerably. A 2001 OECD report estimates that 15 million tons of chemical specialities had been produced *via* biocatalytic processes since 1998.[2] A recent market study predicts that in 2010, 60% of the fine chemical products will be prepared by a biotechnology method (16% in 2001).[3] Biochemical reductions performed in aqueous media have traditionally involved isolated alcohol dehydrogenase (ADH) coupled with a reduced nicotinamide cofactor (NADH or NADPH).[4] The main advantages of these biocatalytic methods are the mild reaction conditions used associated with a high stereoselectivity, a broad substrate specificity and an easy recovery of the product and disposal of the biological material. A limitation to these bioreductions is the necessity to recycle the oxidized cofactor during

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Pierre van de Weghe (left) and Nicolas Blanchard (right)

Pierre van de Weghe (born 1969, left) studied at the Université de Paris-Sud (Orsay, France) and obtained his Doctorat en chimie organique in 1995 from the Université de Paris-Sud under the guidance of Prof. H. B. Kagan and Dr J. Collin, working on the use of samarium iodides as catalysts. After postdoctoral work at the University of Stuttgart (Germany) with V. Jäger as an Alexander von Humboldt fellow, he joined the group of Prof. J. Eustache in 1997 as Chargé de Recherche (CNRS). His research is focused on new methodologies for total synthesis of biological active compounds via *the Ring Closing Metathesis reaction. Recipient of CNRS bronze medal in 2004.*

the reaction.[5] Two modes of recycling were designed, involving a single-enzyme or a coupled-enzyme system. Conceptually, the single-enzyme system performs the redox transformation of the couple oxidized cofactor/cosubstrate during the reaction, and is therefore more convenient. The price of nicotinamide cofactors associated with the necessity of their recycling has impeded their development on a larger scale in the organic synthetic community. Another limitation concerns the steric requirement of the carbonyl derivative. Sterically demanding substrates are badly, if not at all, recognized by traditional ADH.[4a]

Bioreductions mediated by growing or immobilized plant cell cultures have been known for several decades.[6] Recently, the use of functionally intact cells ("whole plant cells") obtained directly from cut portions of plants has emerged. Highly chemo- and stereoselective reductions were observed for endogenous substrates as well as synthetic organic compounds in general. The use of whole plant cells has many advantages. First of all, a large array of taxonomically different plants is available at very low cost from local markets. The whole cells also ensure the recycling of the oxidized cofactors. Separation of the product from the reaction mixture is very easily done by filtration/centrifugation and the remaining biodegradable material could be disposed or reused (*vide infra*). Moreover, the stereochemical outcome of the bioreduction can be predicted based on a model proposed by Prelog in 1964 for the reduction of prochiral ketones by the micro-organism *Curvularia falcata* (Fig. 1).[7] The hydride delivery occurs to the *re* face of the carbonyl function from the reduced nicotinamide cofactor when the large group L and the small group S substitute the carbonyl as shown in Fig. 1.[8] Anti-Prelog selectivity was sometimes observed depending on the steric and electronic nature of the substrate.[9]

Different plants have been studied for their alcohol dehydrogenase activity, for example celeriac (*Apium graveolens* L.),[9] horseradish (*Amroracia lapatifolia* Gilib.),[9] arracacha roots (*A. xanthorriza*),[10] carrot (*Daucus carota* L.). The latter showed the broadest substrate scope and the highest enantioselectivity. This highlight is intended to introduce the reader to the high synthetic potential of *Daucus carota* L. (DC) mediated reduction of prochiral ketones through the presentation of recent literature results. The advantages and limitations of DC mediated bioreductions will be discussed in light of results obtained using other reduction strategies.

Daucus carota mediated reductions of representative aromatic, heteroaromatic and aliphatic ketones are presented in Table 1.† For comparison, results obtained with Baker's yeast (*Saccharomyces cerevisiae*)[11] and borane or metal-mediated reductions (Fig. 2) are included when available.

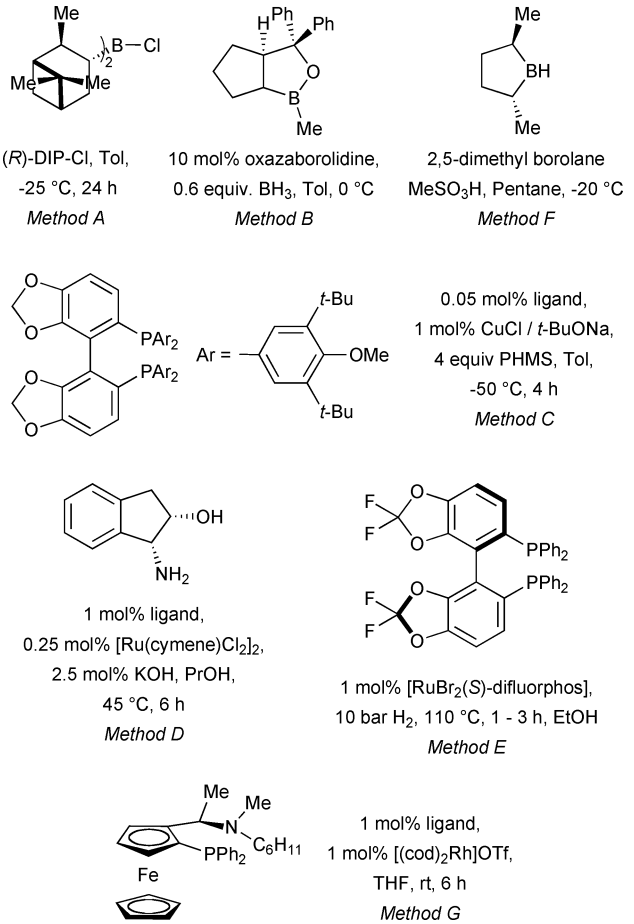


Fig. 2 Chemical reducing agents.

† Typical reduction of ketones with *D. carota* roots:[12] Carrots were obtained from a local market. The external layer was removed and the rest was cut into small thin pieces (1 cm long slice). Ketones (100 mg) were added to a suspension of freshly cut carrot root (10 g) in 70 mL of water and the reaction mixtures were incubated in an orbital shaker at room temperature. The suspension was then filtered off and the carrot root was washed three times with water. Filtrates were extracted with ethyl acetate, organic layers were dried on sodium sulfate, filtered and evaporated. The crude products were purified by flash chromatography.

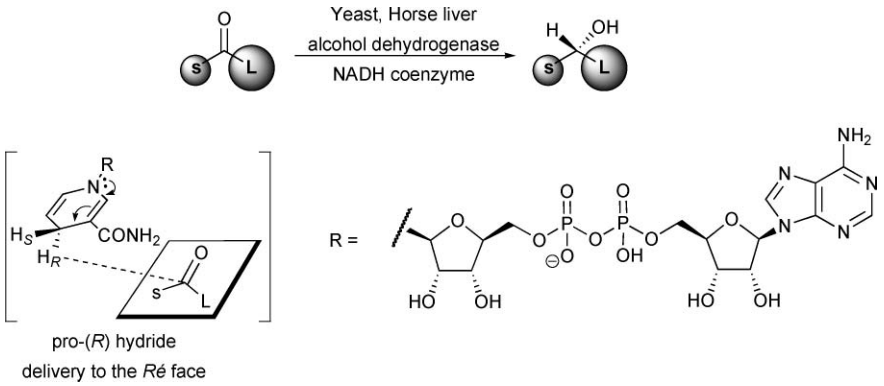
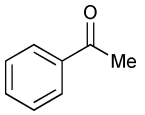
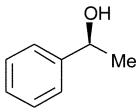
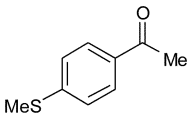
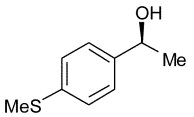
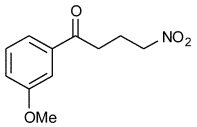
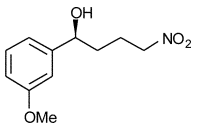
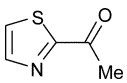
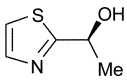
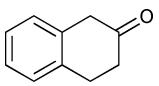
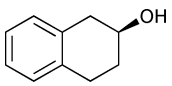
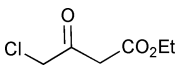
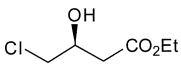
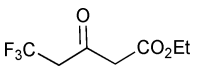
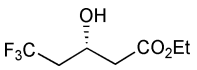
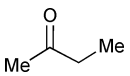
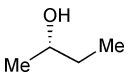
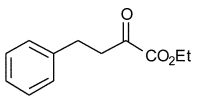
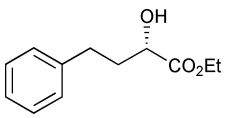


Fig. 1 Prelog model for bioreduction of prochiral ketones.[7]

Table 1 Reduction of representative prochiral ketones by *Daucus carota*, Baker's yeast and chemical reducing agents[a]

Entry	Substrate	Product	Conditions	B/S[b]	Abs. Conf.	Yield (%)	Ee (%)	Ref.
1			*Daucus carota*	11	*S*	73–100	92–100	12,13
			Baker's yeast	3	*S*	90	100	21
			Method A	—	*S*	72	98	22
2			*Daucus carota*	55	*S*	95[c]	>99	14
			Helminthosporium	—	*S*	9	76	23
			Method B	—	*R*	98	99	24
3			*Daucus carota*	11	*S*	100[c]	>99	15
			Baker's yeast	155	*S*	67[c]	76	25
			—	—	—	—	—	—
4			*Daucus carota*	4.4	*S*	60	94	16
			Baker's yeast	—	—	—	—	—
			Method C	—	*R*	97	90	26
5			*Daucus carota*	11	*S*	58	95	12
			Baker's yeast	100	*S*	85	29	17
			Method D	—	*S*	85	81	27
6			*Daucus carota*	11	*S*	50	90	12
			Baker's yeast	27	*S*	n.d.	55	28
			Method E	—	*R*	100[c]	97	29
7			*Daucus carota*	11	*R*	72	78	12
			Baker's yeast	9	*R*	35	62	30
			Method E	—	*S*	100[c]	70	29
8			*Daucus carota*	11	*S*	38	87	12
			Baker's yeast	190	*S*	30	67	31
			G. candidum	17	*S*	73	94	19
			Method F	—	*R*	75	79	32
9			*Daucus carota*	100	*S*	80	99	20
			Baker's yeast		*S*	15	32	33
			Method G	—	*R*	>95[c]	92.4	34

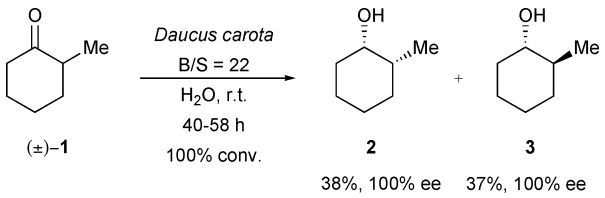
[a] Chemical reducing agents as shown in Fig. 2, methods A–G. [b] Biocatalyst to substrate ratio (dry weight). [c] Conversion.

Acetophenone is the most frequently encountered aromatic ketone in bio- or metal mediated reduction studies. *Daucus carota* roots performed very well. The corresponding (*S*) alcohol is obtained in 73–100% yield with 92–100% enantiomeric excess (Table 1, entry 1).[12,13] Excellent enantioselectivity was also obtained using fennel (*Foeniculum vulgare*) and marrow (*Cucurbita pepo*) albeit with low yields (37 and 10% respectively).[13] No reduction was observed with aubergine (*Solanum melongena*), cucumber (*Cucumis sativus*), white and red onion (*Allium cepa*), garlic (*Allium sativum*) and radish (*Raphanus sativus*).[13]

Para-substituted acetophenones in general are reduced by *Daucus carota* with high enantioselectivity, independently of the electronic nature of the *para*-substituent (halo, nitro, methoxy, hydroxy; ee = 91–96%) albeit at different rates (*vide infra*).[12] *Meta* and *para*-substituted aromatic thioethers are compatible with *Daucus carota* mediated reduction as can be seen from Table 1, entry 2.[14] The corresponding benzyl alcohol is produced as a single enantiomer. Aliphatic nitro moieties are also well tolerated even though side products arising from Nef reactions have been reported (Table 1, entry 3).[15] Heteroaromatic ketones are good substrates (Table 1, entry 4), 94% ee is obtained for the reduction of 1-(1,3-thiazol-2-yl)ethanone to the corresponding (*S*) alcohol.[16] 2-Tetralone is known to be reduced with low enantioselectivity by Baker's yeast (Table 1, entry 5, 29% ee).[17] In contrast, DC mediated bioreduction led to the (*S*) alcohol in 58% yield and 95% ee.[12] *D. Carota* reduction of β-ketoesters compares favourably with the reduction mediated by Baker's yeast (Table 1, entries 6 and 7) both in terms of yield and enantioselection.[12] An anti-Prelog selectivity is observed for entry 7, possessing a trifluoromethyl substituant. In comparison, the corresponding trichloromethyl group led to a Prelog-like selectivity (51%, 88% ee, not shown).[12] A challenging substrate for asymmetric reduction is 2-butanone (Table 1, entry 8).[18] The slight difference in steric requirement of the alkyl groups flanking the carbonyl function renders the

selection of one enantiotopic face difficult. However, *Daucus carota* is able to discriminate between these two faces and (*S*) butan-2-ol is obtained with an impressive 87% ee.[12] The only other efficient biocatalytic method employs non commercially available *G. candidum* IFO 4597 with NAD^+ as a coenzyme and 2-propanol as an additive.[19] α-Ketoesters are also reduced with an impressive level of enantioselectivity (Table 1, entry 9), although at the expense of longer reaction times and higher B/S ratios.[20] As a general trend, *Daucus carota* mediated bioreductions necessitate lower B/S ratios and are more enantioselective than Baker's yeast reductions.

Chemical reducing agents (Fig. 2) perform very well in terms of enantioselectivity. However a lower enantiomeric excess is generally obtained compared to the bioreduction process (Table 1, entries 1 to 9).[35] The main advantages of the asymmetric chemical reducing agents are shorter reaction times and possible access to the two enantiomers of the chiral ligand, thus allowing straightforward synthesis of both enantiomers of the desired product.

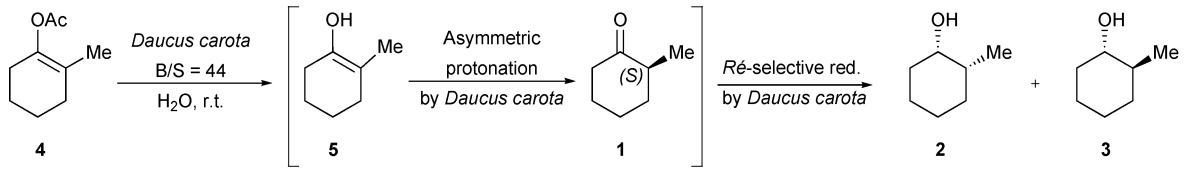


Scheme 1 Diastereoselective reduction of (±)-**1**.[36]

Racemic α-substituted ketones have been evaluated in *Daucus carota* mediated bioreductions. No specific match/mismatch effect from the adjacent stereocenter have been observed, as can be seen from the reduction of 2-methylcyclohexanone (±)-**1** (Scheme 1).[36] At 100% conversion, an equal amount of enantiopure cyclohexanols **2** and **3** were recovered in 75% yield. Reduction occurs only from the *re*-face of the prochiral ketone, independently of the configuration of the α-methyl-substituted stereocenter.

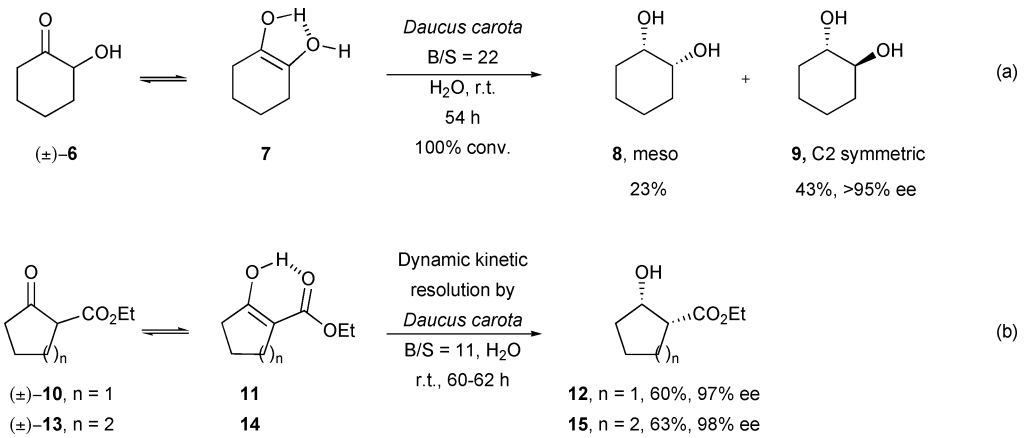
The α-substituted ketone **1** can also be obtained by hydrolysis of the corresponding enol acetate **4**, followed by asymmetric protonation of enol **5** (Scheme 2).[13] Among a large array of plants, results obtained with *Daucus carota* roots were the most promising. After two hours, ketone **1** was obtained in 89% yield with 45% ee (in favor of the (*S*)-enantiomer). As in Scheme 1, a diastereospecific reduction of the resultant enantioenriched cyclohexanone occurred, exclusively from the *re*-face. After 24 h at room temperature, cyclohexanol **3** was obtained in 75% yield with 100% ee thanks to this domino sequence. Stereoselective synthesis of the (*S*)-configured ketone **1** was also possible, using *Oxalis tuberosa* tuber (72 h, 44%, 99% ee). Reduction of the resulting prochiral carbonyl group occurred in a highly diastereoselective fashion from the *si*-face, leading to *cis*-alcohol **2** in 56% yield and 100% ee.

When the adjacent substituent is an hydroxy group, an equilibrium is established between the hydroxyketone and the corresponding enolic form **7**, thus constantly epimerizing the α-stereocenter of the substrate (Scheme 3, eqn a).[36a] Submitted to bioreduction with *Daucus carota*, the (*S*)-enantiomer is converted faster than the (*R*)-enantiomer, leading to a 2 : 1 ratio of

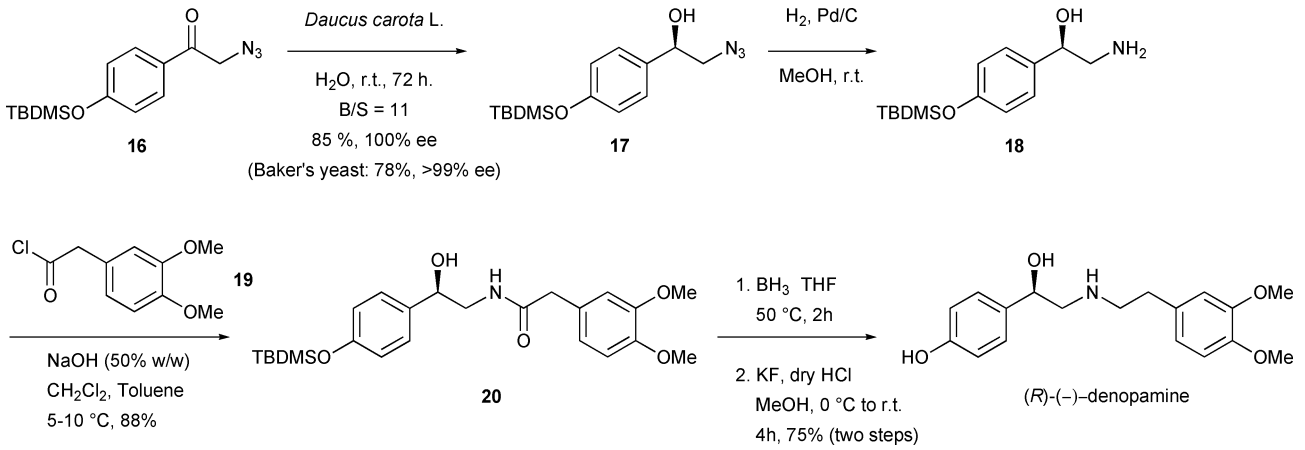


2h	89%, 45% ee *(S)*	-	5%, ee n.d.
24h	14%, 100% ee *(R)*	11%, ee n.d.	**75%, 100% ee**
72h (*Oxalis tuberosa*)	44%, 99% ee *(S)*	**56%, 100% ee**	-

Scheme 2 Asymmetric protonation by *D. carota* coupled with diastereoselective reduction.[13]



Scheme 3 Dynamic kinetic resolution of (±)-2-hydroxycyclohexanone **6** and β-ketoesters **10** and **13**.[12,36]



Scheme 4 Synthesis of (R)-(−) denopamine using a *D. carota* mediated reduction of azidoketone **16**.[38]

C2-symmetric : *meso* cyclohexan-1,2-diols **9** and **8**. A more efficient dynamic kinetic resolution[36b] system was studied by Yadav *et al.* with cyclic β-ketoesters **10** and **13** (Scheme 3, eqn b).[12] Reduction of the (*R*)-enantiomer is considerably faster than reduction of the (*S*) one, leading to the β-hydroxyester **12** and **15** in 60–63% yield and 97–98% ee. Hydride delivery occurs from the *re*-face of the carbonyl group, following Prelog's rule.[8]

These easy-to-perform asymmetric DC bioreductions are accompanied by several limitations, concerning both the conversion and the stereoselectivity. Long reaction times and large biocatalyst to substrate (B/S) ratios are sometimes required since the organic substrate might modify or disrupt the cellular system, thus impeding a synthetically useful conversion. A drop in conversion is usually observed when the recovered biomaterial is subjected to a second reduction reaction.[12] Recent efforts to overcome this limitation have been reported by Chênevert in 2005 using *Daucus carota* highly branched roots, obtained by natural genetic transformation.[16] The corresponding intact cells showed a higher biochemical stability allowing six consecutive reuses in the reduction of acetophenone without erosion of the enantioselectivity (>98%). Moreover, the B/S ratio could be lowered to 4.4, a very low figure compared to Baker's yeast mediated reductions. Substrate specificity is also more pronounced with *Daucus carota* than with Baker's yeast. *Para*-substitution of acetophenone with an electron donating group can slow down dramatically the reduction rate, highlighting the sensitivity of bioreduction kinetics to electronic effects.[15,37] Moreover, *ortho*-substituted acetophenones are badly recognized and poor conversions are generally obtained.[14,37] On the stereoselectivity standpoint, two main limitations have appeared. The Prelog-like selectivity usually observed in these bioreductions could be problematic if the other absolute configuration is required. Moreover, several oxidoreductases of *Daucus carota* might come into play, with their own intrinsic selectivities, thus complicating the prediction of the stereochemical outcome of the reduction reaction.[8]

To date, few applications of *Daucus carota* mediated reductions to the synthesis of biologically active compounds have been disclosed. Yadav[38] reported a short synthesis of (*R*)-(−) denopamine,[39] a selective β_1-adrenergic agonist which stimulates alveolar fluid clearance, using a *Daucus carota* reduction of α-azidoketone **16** as a key step (Scheme 4). The corresponding benzylic alcohol **17** is obtained as a unique enantiomer in good yield. Hydrogenation reaction followed by acylation with acyl chloride **19** led to amide **20** in 88% yield. Two subsequent steps afforded (*R*)-(−) denopamine in enantiopure form.

Conclusions

Intact cells from cut portions of plants can mediate useful asymmetric transformations. For example, *Daucus carota* L. bioreduction of prochiral ketones offers new possibilities to the synthetic organic chemist in terms of simplicity and efficiency. This emerging methodology could also simplify environmental issues raised by the traditional use of borane or metal-mediated asymmetric reduction reactions. Future work in this area should be devoted to the current limitations concerning conversion and stereoselectivity. A better understanding of the underlying biochemical mechanism would also be of interest.

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References

1 OECD report *Biotechnology for Sustainable Growth and Development*, 2004, http://www.oecd.org/dataoecd/43/2/33784888.PDF.
2 OECD report *The Application of biotechnology to Industrial Sustainability*, 2001, http://www.oecd.org/sti/biotechnology.
3 G. Festel, J. Knöll, H. Götz and H. Zinke, *Chem.-Ing.-Tech.*, 2004, **76**, 307–312.
4 (*a*) W. Kroutil, H. Mang, K. Edegger and K. Faber, *Curr. Opin. Chem. Biol.*, 2004, **8**, 120–126; (*b*) K. Nakamura, R. Yamanaka, T. Matsuda and T. Harada, *Tetrahedron: Asymmetry*, 2003, **14**, 2659–2681; (*c*) S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 2000, 611–633.
5 Z. Shaked and G. M. Whitesides, *J. Am. Chem. Soc.*, 1980, **102**, 7104–7105.
6 K. Ishihara, H. Hamada, T. Hirata and N. Nakajima, *J. Mol. Catal. B: Enzym.*, 2003, **23**, 145–170.

7 V. Prelog, *Pure Appl. Chem.*, 1964, **9**, 119–130; E. G. Weinhold, A. Glasfeld, A. D. Ellington and S. A. Benner, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 8420–8424. It should be noted that the two heterotopic hydrogen atoms could be transferred depending on the alcohol dehydrogenase:; K. A. Reynolds and K. A. Holland, *Chem. Soc. Rev.*, 1997, **26**, 337–343 and references cited therein.

8 In intact cells, several oxidoreductases can compete for a substrate. Numerous parameters have to be considered: apparent kinetic constants (v_{max} and K), number, relative concentration and compartmentalization of enzymes, effective co-enzyme concentration, permeability factor. Thus, the Prelog rule has to be carefully used in whole cell bioreduction of prochiral ketones; *cf.* C. J. Sih and C.-S. Chen, *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 570–578.

9 W. K. Maczka and A. Mironowicz, *Tetrahedron: Asymmetry*, 2002, **13**, 2299–2302.

10 L. H. Andrade, R. S. Utsunomiya, A. T. Omori, A. L. M. Porto and J. V. Comasseto, *J. Mol. Catal. B: Enzym.*, 2006, **38**, 84–90.

11 T. Hometani, H. Yoshii and R. Matsuno, *J. Mol. Catal. B: Enzym.*, 1996, **1**, 45–52; E. Santaniello, P. Ferraboschi, P. Grisenti and A. Manzocchi, *Chem. Rev.*, 1992, **92**, 1071–1140; R. Czuk and B. I. Glänzer, *Chem. Rev.*, 1991, **91**, 49–97.

12 J. S. Yadav, S. Nanda, P. Thirupathi Reddy and A. Bhaskar Rao, *J. Org. Chem.*, 2002, **67**, 3900–3903.

13 R. Bruni, G. Fantin, A. Medici, P. Pedrini and G. Sacchetti, *Tetrahedron Lett.*, 2002, **43**, 3377–3379.

14 J. V. Comasseto, A. T. Omori, A. L. M. Porto and L. H. Andrade, *Tetrahedron Lett.*, 2004, **45**, 473–476.

15 D. Scarpi, E. G. Occhiato and A. Guarna, *Tetrahedron: Asymmetry*, 2005, **16**, 1479–1483.

16 D. Caron, A. P. Coughlan, M. Simard, J. Bernier, Y. Piché and R. Chênevert, *Biotechnol. Lett.*, 2005, **27**, 713–716.

17 P. Manitto, G. Speranza, D. Monti, G. Fontana and E. Panosetti, *Tetrahedron*, 1995, **51**, 11531–11546.

18 G.-J. Shen, Y.-F. Wang, C. Bradshaw and C.-H. Wong, *J. Chem. Soc., Chem. Commun.*, 1990, 677–679; C. Zheng and R. S. Phillips, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1083–1084; K. Nakamura, M. Fujii and Y. Ida, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3205–3211.

19 T. Matsuda, Y. Nakajima, T. Harada and K. Nakamura, *Tetrahedron: Asymmetry*, 2002, **13**, 971–974.

20 A. Chadha, M. Manohar, T. Soundararajan and T. S. Lokeswari, *Tetrahedron: Asymmetry*, 1996, **7**, 1571–1572.

21 G. Fantin, M. Fogagnolo, M. E. Guerzoni, A. Medici, P. Pedrini and S. Poli, *J. Org. Chem.*, 1994, **59**, 924–925.

22 J. Chandrasekharan, P. V. Ramachandran and H. C. Brown, *J. Org. Chem.*, 1985, **50**, 5446–5448.

23 H. L. Holland, M. J. Bornmann and G. Lakshmaiah, *J. Mol. Catal. B: Enzym.*, 1996, **1**, 97–102.

24 J. Xu, T. Wei and Q. Zhang, *J. Org. Chem.*, 2004, **69**, 6860–6866.

25 A. Guarna, E. G. Occhiato, L. M. Spinetti, M. E. Vallechi and D. Scarpi, *Tetrahedron*, 1995, **51**, 1775–1788.

26 B. H. Lipshutz, A. Lower and K. Noson, *Org. Lett.*, 2002, **4**, 4045–4048.

27 M. J. Palmer, J. A. Kenny, T. Walsgrove, A. M. Kawamoto and M. Wills, *J. Chem. Soc., Perkin Trans. 1*, 2002, 416–427.

28 B.-N. Zhou, A. S. Gopalan, F. VanMiddlesworth, W.-R. Shieh and C. J. Sih, *J. Am. Chem. Soc.*, 1983, **105**, 5925–5926.

29 S. Jeulin, S. Duprat de Paule, V. Ratovelomanana-Vidal, J.-P. Genêt, N. Champion and P. Dellis, *Angew. Chem., Int. Ed.*, 2004, **43**, 320–325.

30 M. Bertau, M. Bürli, E. Hungerbühler and P. Wagner, *Tetrahedron: Asymmetry*, 2001, **12**, 2103–2107.

31 R. MacLeod, H. Prosser, L. Fikentscher, J. Lanyi and H. S. Mosher, *Biochemistry*, 1964, **3**, 838–846.

32 T. Imai, T. Tamura, A. Yamamuro, T. Sato, T. A. Wollman, R. M. Kennedy and S. Masamune, *J. Am. Chem. Soc.*, 1986, **108**, 7402–7404.

33 A. Matsuyama, T. Nikaido and Y. Kobayashi, Daicel Chemical Industries, Ltd., Japan, PCT. Int. Appl. 1989, WO 8907648.

34 N. W. Boaz, E. B. MacKenzie, S. D. Debenham, S. E. Large and J. A. Ponasik, *J. Org. Chem.*, 2005, **70**, 1872–1880.

35 T. C. Rosen and T. Dauβmann, *Chim. Oggi*, 2004, (Suppl.), 43–45.

36 (*a*) F. Baldassarre, G. Bertoni, C. Chiappe and F. Marioni, *J. Mol. Catal. B: Enzym.*, 2000, **11**, 55–58; (*b*) For a review on dynamic kinetic resolution, see: H. Pellissier, *Tetrahedron*, 2003, **59**, 8291–8327.

37 W. K. Maczka and A. Mironowicz, *Tetrahedron: Asymmetry*, 2004, **15**, 1965–1967. For cases where electronic effects does not seem to play a role, see: B. Baskar, S. Ganesh, T. S. Lokeswari and A. Chadha, *J. Mol. Catal. B: Enzym.*, 2004, **27**, 13–17.

38 J. S. Yadav, P. Thirupathi Reddy, S. Nanda and A. Bhaskar Rao, *Tetrahedron: Asymmetry*, 2001, **12**, 3381–3385.

39 T. Sakuma, C. Tuchihara, M. Ishigaki, K. Osanai, Y. Nambu, H. Toga, K. Takahashi, N. Ohya, T. Kurihara and M. A. Matthay, *J. Appl. Physiol.*, 2001, **90**, 10–16. Total synthesis:; A. Kamal, A. A. Shaik, M. Sandbhor and M. S. Malik, *Tetrahedron: Asymmetry*, 2004, **15**, 3939–3944 and references cited therein.