The Concept of Docking and Protecting Groups in Biohydroxylation

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Abstract: The hydroxylation of unactivated carbon atoms employing methods developed in the realms of classical organic chemistry is difficult to achieve and the processes available lack the degree of chemo-, regio- and enantioselectivity required for organic synthesis. To improve this situation, the concept of docking/protecting groups should enable the organic chemist to employ biohydroxylation as an easy tool for preparative work. Similar to the common practice of using protective groups in organic chemistry, a docking/protecting (d/p) group is introduced first, then the biotransformation is performed, and finally the d/p group is removed. The aim of this concept is not only to avoid time consuming microorganism screening methods, but also to improve hydroxylation position predictability, prevent undesired side reactions, aid substrate detection, and product recovery. This approach is successfully applied to carboxylic acids, ketones, aldehydes, and alcohols.

Keywords: biohydroxylation · biotransformations · chiral auxiliaries · enantioselective synthesis · stereoselective synthesis

Introduction

The idea of using microorganisms to hydroxylate unactivated carbon atoms of organic compounds is not new in organic synthesis. First reviews have been published many decades ago.[1–3] Although the yields for these conversions are often quite low compared with those found for chemical reactions, many microbial hydroxylations have been investigated and have also been applied on an industrial scale.[4–8] The reason for this is that the chemical methods available to introduce hydroxyl groups chemo- and regioselectively into organic compounds[9, 10] are, at present, inferior to those employing microorganisms. In addition, a huge gain in material value can be obtained by this reaction because with an easy access to enantiopure hydroxylated compounds, important in their own right, useful intermediates for further syntheses are attainable.

Therefore, why do we believe it is important to introduce a new concept into the relatively old area of biohydroxylation? One problem is certainly the low predictability of the outcome of hydroxylations on new and not yet investigated substrates.

The hydroxylation of steroids can be predicted to a certain extent by the analysis of the results obtained from a large number of conversions carried out mainly in the fifties and sixties.[1, 11] For other classes of compounds a few models have also been published for particular microorganisms in order to explain the behavior of these biocatalysts in hydroxylation reactions.[12–19]

We were looking for a more general approach which would allow the transformation of different substrate classes by only a limited number of microorganisms. Organic chemists are trained to categorize compounds in terms of their functional groups present such as alcohols, ketones, carboxylic acids. In doing so, we are able to predict reactions which can be carried out with them. Consequently, we thought that it would be advantageous to apply this way of thinking to the area of biohydroxylation in order to find the most suitable microorganisms to hydroxylate, for example, alcohols, ketones, or acids with predictable selectivity.

However, it quickly became clear, that these compounds are often not useful for biohydroxylation as they are and should be protected or derivatised. For example, ketones are often reduced in the fermentation broth and the polarity of carboxylic acids sometimes hampers isolation by conventional separation techniques. The idea of using protecting groups is of course not new in organic synthesis, but has been relatively rarely employed in biocatalysis.[20–22]

An additional feature of using protecting groups, apart from the ability to prevent undesired side reactions, would be to achieve a better recognition of the substrate by the hydroxylating enzymes. It had already been shown by several groups, especially using the fungus *Beauveria bassiana* ATCC 7159, that the hydroxylation of certain substrates occurs at a certain distance from, for example, a benzamide or carbamate group.[18–32] This and other observations led to the development of substrate models for the hydroxylation by this fungus. For this kind of behavior the term docking group has been used by us because results suggest that substrate fixation

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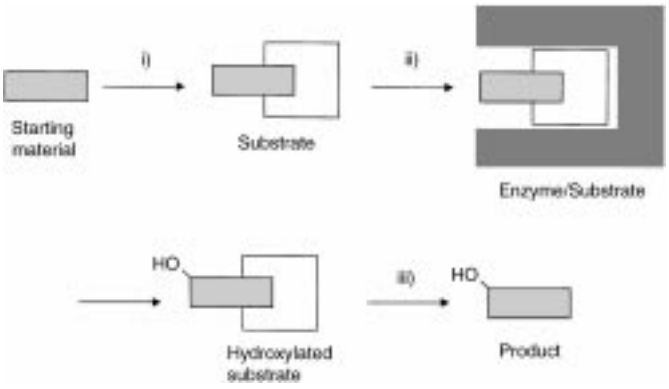
in the enzyme active site is facilitated by the functional group in question, leading to a very specific hydroxylation pattern. However, one has to be aware that generally several hydroxylating systems can be present in a microorganism, as demonstrated recently for *Beauveria bassiana*.[33] Yet, for the hydroxylation of a defined range of substrates, this docking group concept plays an important role.

In addition to these features, this docking and protecting group ("d/p group") should also change the properties of the substrates to facilitate detection and to ease handling of the products. The general principle of the employment of the docking/protecting group concept is shown in Scheme 1.



Scheme 1. The general principle of d/p-groups. i) introduction of the d/p group; ii) microbial conversion; iii) deprotection.

For the evaluation of this strategy the choice of the d/p groups is of fundamental importance. In our experiments, several derivatives were selected (at least three per compound class) and a screening program was undertaken to find the suitable d/p groups. The following derivatives were chosen for further investigation:

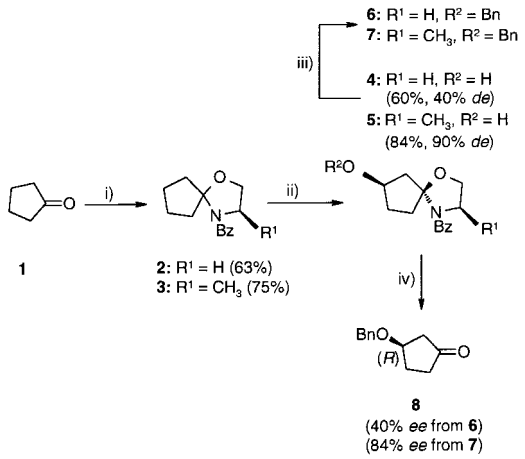
- *N*-benzoylated spirooxazolidines for the hydroxylation of aldehydes and ketones;
- benzoxazoles for the hydroxylation of carboxylic acids;
- isosaccharine derivatives for the hydroxylation of alcohols.

Discussion

Hydroxylation of ketones: Ketones converted into *N*-benzoylated oxazolidines were hydroxylated by a number of bacteria and fungi in this screening program. In particular, the well known[34] fungus *Beauveria bassiana* ATCC 7159 turned out to be especially interesting.

The d/p group approach for ketones[35, 36] is demonstrated in Scheme 2 for cyclopentanone **1**. Derivative **2** was easily prepared from this ketone in a two-step, one-pot reaction sequence. Hydroxylation with *Beauveria bassiana* furnished product **4** in good isolated yield. In order to avoid elimination, **4** was benzylated to give **6** prior to the d/p group removal step. Subjecting **6** to mildly acidic conditions gave the *R*-configured ketone **8**.

However, the optical purity of ketone **8** was far from being satisfactory for synthetic purposes since the enantiomeric excess measured was only 40%. Therefore, the use of chiral

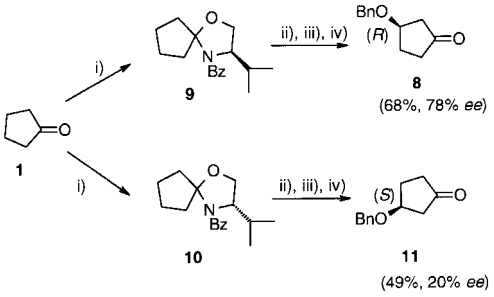


Scheme 2. The d/p group concept as applied to ketones. i) K_2CO_3, CH_2Cl_2 for **3**: (*R*)-2-amino-1-propanol, for **2**: aminoethanol, BzCl; ii) *Beauveria bassiana* ATCC 7159; iii) BnBr, NaH, DMF, THF; iv) IR 120, CH_3CN.

auxiliaries[37, 38] was examined. Although rarely applied in the field of biocatalysis,[39, 40] chiral auxiliaries are one of the methods of choice in synthetic organic chemistry to achieve stereoselectivity in a given transformation. Accordingly, instead of employing achiral aminoethanol, (*R*)-2-amino-1-propanol was used in the synthetic sequence to give spirooxazolidine **3**. As can be seen from Scheme 2, this derivative furnished hydroxylated product **5** (major isomer shown) not only in higher *de* (90% compared with 40%), but in a considerably improved yield even after isolation (84% compared with 60%). In accordance with previous findings, d/p group removal afforded the *R*-configured ketone **8**.

After this encouraging result, we turned our attention to the possibility of obtaining the *S*-configured ketone by the simple choice of d/p group. Indeed, after examining a range of differently substituted d/p groups,[36] we discovered that while compound **9** furnished *R*-configured ketone **8**, derivative **10** afforded the *S*-configured ketone **11** (Scheme 3). Although the yield of *S*-enantiomer **11** is lower than that for the *R*-enantiomer **8** and the *ee* for **11** is only 20% as compared to 78% for the product obtained from enantiomer **9**, these results indicate a possible change in the enantioselectivity from *R* to *S* depending on the choice of the d/p group.

Spirooxazolidines have also been used as d/p groups for several other cycloalkanones, bicyclic ketones and methyl cyloalkylketones.[41]



Scheme 3. Influence on end product configuration by choice of d/p group. i) (*R*) or (*S*)-2-amino-3-methylbutan-1-ol, K_2CO_3, CH_2Cl_2, BzCl; ii) *Beauveria bassiana* ATCC 7159; iii) BnBr, NaH, DMF, THF; iv) IR 120, CH_3CN.

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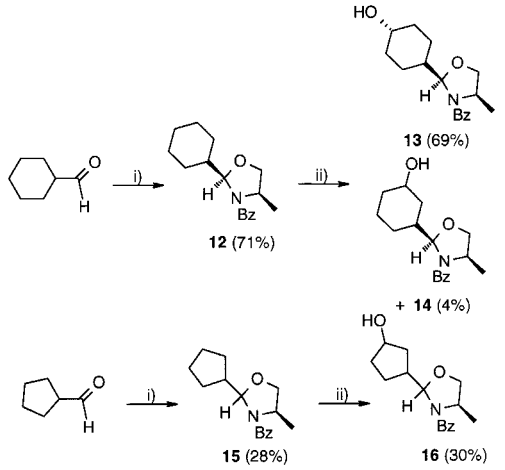
Hydroxylation of aldehydes: The extension of the d/p group concept to the hydroxylation of aldehydes was also realised by derivatisation into *N*-benzoylated oxazolidines, employing chiral d/p groups and *Beauveria bassiana* ATCC 7159. In a simple two-step, one-pot reaction, oxazolidines **12** and **15**, for example, could be prepared in good yields as a mixture of easily separable diastereoisomers. For the hydroxylation step, only one diastereoisomer for both **12** and **15** was used as shown in Scheme 4. The hydroxylation proceeded with a

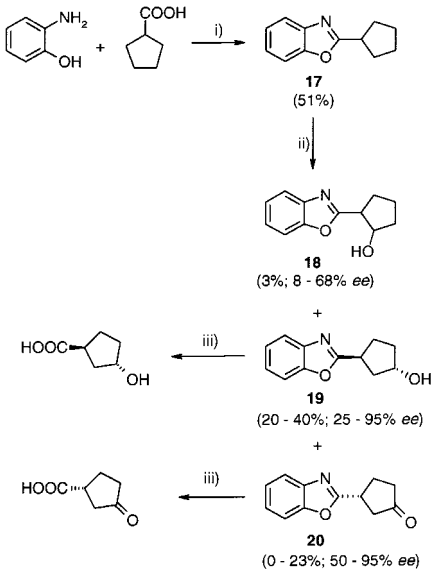


Scheme 4. The d/p group concept as applied to aldehydes. i) (*R*)-2-amino-1-propanol, K_2CO_3, CH_2Cl_2, BzCl; ii) *Beauveria bassiana* ATCC 7159.

lower regioselectivity as compared to cycloalkanones. While substrate **12** yielded a mixture of 4-OH (**13**) and 3-OH (**14**) products, compound **16** was obtained from **15** as an inseparable mixture of four compounds. The low hydroxylation regioselectivity might be due to the increased conformational flexibility of these substrates; this would be in accordance to observations made with benzoxazole substrates.[42] Removal of the d/p group could be smoothly effected under mildly acidic conditions (IR 120, CH_3CN) to afford the expected hydroxylated aldehyde.

Hydroxylation of carboxylic acids: The main problems associated with the biohydroxylation of carboxylic acids are sometimes poor detectability and high polarity leading to difficulties in monitoring the conversion and separation of the hydroxylated products from the fermentation broth. However, protecting the acid with 2-aminophenol to give a benzoxazole proved to be a viable alternative. These benzoxazoles are not hydrolyzed in the fermentation mixture, are UV active which allows detection by both TLC and HPLC, and are much less polar compared with the parent compounds.

Upon conversion of several substituted benzoxazoles it turned out that substrates prepared from cyclic carboxylic acids gave better results than the long-chain carboxylic acids; the latter gave a mixture of several products and, in the end, complete metabolization of these substrates was observed. As already mentioned, results for a number of cyclic substrates were rather promising; as an example the conversion of 2-cyclopentylbenz-1,3-oxazole (**17**) is shown in Scheme 5.



Scheme 5. The d/p group concept as applied to carboxylic acids. i) polyphosphate ethyl ester, CH_2Cl_2; ii) *Cunninghamella blakesleeana* DSM 1906; iii) $ZnCl_2$, $MeOH/H_2O$, HCl.

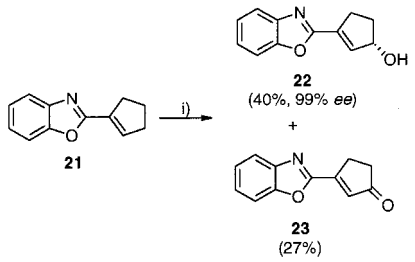
Microbial hydroxylation of 2-cyclopentylbenzoxazole with *Cunninghamella blakesleeana* DSM 1906 or *Bacillus megaterium* DSM 32 gave three products **18**, **19**, and **20** in varying yields depending on the fermentation time chosen.[35, 42–45] It was found that hydroxylation usually took place *anti* to the benzoxazole moiety but with sometimes less pronounced regioselectivity. This led to diastereomerically pure products with enantiomeric excesses in the range of 30–50% *ee*. The concomitant microbial oxidation of the main alcohol **19** to the corresponding ketone was beneficial because this transformation, presumably by an alcohol dehydrogenase, was more selective than the hydroxylation itself thus leaving only one enantiomer behind. By careful adjustment of the fermentation conditions, we were therefore able to increase the optical purity of the alcohol **19** to over 90%.[46–48]

In addition, a combination of microbial hydroxylation and lipase-catalyzed resolution permitted the synthesis of optically pure alcohols or esters. *Pseudomonas sp.* lipase was generally the best catalyst for acylations or hydrolyses. Ketone **20** itself could also be easily recrystallized which led to an *ee* $\geq$ 95%.[49]

With these simple biocatalytic and chemical operations almost optically pure compounds can be prepared in a multigram scale from fermentation broths.

In the series of unsaturated substrates the situation was even better since usually only one alcohol and the corresponding ketone was formed and the *ee* of the alcohol was very often close to 90%. An example is shown in Scheme 6 with 2-(cyclohex-1-enyl)benz-1,3-oxazole (**21**).[50]

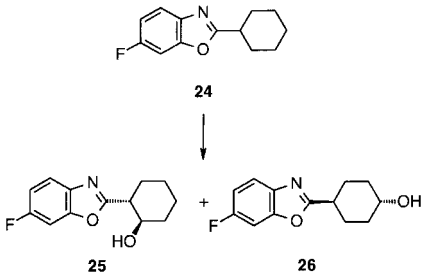
Carboxylic acids protected with fluorine-containing d/p-groups: In microbial hydroxylations the use of whole cell systems has the disadvantage that these systems are capable of performing a wide range of different reactions which, in turn, can lead to undesirable side reactions and products. Consequently, a detailed knowledge of all compounds formed in a typical fermentation and the time course of product formation



Scheme 6. Microbial hydroxylation of 2-(cyclohex-1-enyl)benz-1,3-oxazole. i) *Cunninghamella blakesleeana* DSM 1906.

is advisable. For this we used fluorinated d/p-groups. Fluorine atoms can easily be traced by ^{19}F NMR spectroscopy because of the very high selectivity (due to its large chemical shift range) and, more importantly, sensitivity of ^{19}F to NMR detection, which is comparable to protons.

Therefore we prepared 6-fluorobenzoxazoles and were pleased that the biohydroxylation proceeded analogously to the non-fluorinated counterparts (Scheme 7). The products



Scheme 7. Microbial hydroxylation of 2-cyclohexyl-6-fluorobenz-1,3-oxazole with *Bacillus megaterium* DSM 32.

obtained had different chemical ^{19}F chemical shifts and were therefore detectable in an NMR spectrum directly from the fermentation mixture. Therefore, fermentation extracts could easily be examined by using ^{19}F NMR spectroscopy without the need to separate these compounds. An example of this approach is shown in Figure 1 in which substrate **24** as well as products **25** and **26** can be easily detected.[51]

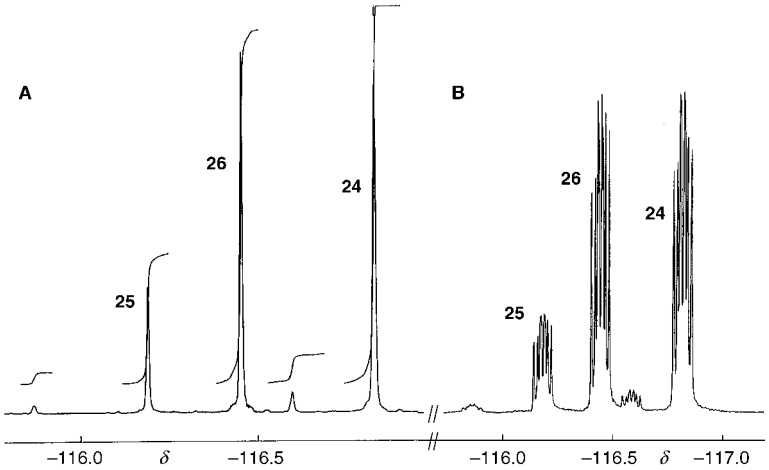
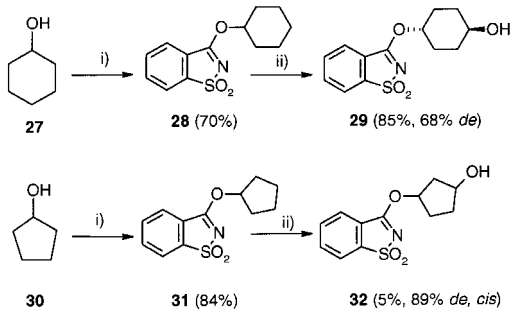


Figure 1. ^{19}F NMR spectrum of a fermentation extract of 2-cyclohexyl-6-fluorobenz-1,3-oxazole with *Bacillus megaterium* DSM 32 dissolved in $CDCl_3$. **A** is the proton decoupled and **B** the coupled spectrum. 16 Scans were accumulated.

Hydroxylation of alcohols: In the form of isosaccharine derivatives, such as **28** and **31**, alcohols **27** and **30** could be successfully hydroxylated to give **29** and **32**. After d/p group removal under basic conditions ($NaOCH_3$, CH_3OH) the corresponding diol was obtained.[35] A range of alcohols was hydroxylated in this manner. It should also be noted at this point that under the conditions employed in this work the underivatised alcohols were either oxidised to the corresponding ketone, completely metabolised, or remained unchanged. Products of direct hydroxylation could not be detected in the fermentation broth (Scheme 8).



Scheme 8. The d/p group concept as applied to alcohols. i) pyridine, 3-chloro-1,2-benzisothiazole-1,1-dioxide, CH_2Cl_2; ii) *Cunninghamella blakesleana* DSM 1906.

One aspect of this d/p group concept is depicted in Scheme 9 in which the *R*-configured alcohol **33** was employed. After conversion to substrate **34**, the diastereomeric product **35** was afforded after exposure to either *Cunninghamella blakesleana* DSM 1906 or *Mortierella alpina* ATCC 8979. Removal of the d/p-group under basic conditions furnished diol **36** (80% yield, *C.b.* 52% *de*, *M.a.* 72% *de*). The configuration of the newly formed center of chirality bearing the hydroxyl group has not yet been assigned. Depending on the fungus used, the "other" diastereoisomer was obtained from this sequence. Reversal of hydroxyl group configuration upon changing the kind of microorganism has been previously observed with benzoxazoles.[47]

Outlook

It is clear that the use of biohydroxylation as a means to introduce hydroxyl groups into unactivated carbons in simple organic molecules still has a long way to go with respect to such factors as predictabilty, ease of use, presence of undesired side reactions, and unsufficient product yields as well as product enantio- and diastereomeric purities. However, considering what can be achieved with classical organic chemistry in this field and comparing that with what microorganisms can do, indicates that the latter certainly is a valuable tool after all. We hope that a step in the right direction is the concept of docking/protecting groups which will help aid the organic chemist to reach future goals.

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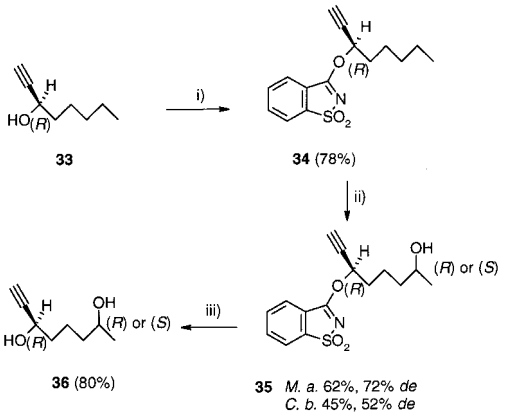

Scheme 9. Reversal of hydroxyl group configuration upon changing the microorganism employed. i) pyridine, 3-chloro-1,2-benzisothiazole-1,1-dioxide, CH_2Cl_2; ii) *C.b.* (*Cunninghamella blakesleana*) DSM1906 or *M.a.* (*Mortierella alpina*) ATCC 8979; iii) $NaOCH_3$, CH_3OH.

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[1] W. Charney, H. L. Herzog, *Microbial Transformations of Steroids*, Academic Press, New York, **1967**.
[2] G. S. Fonken, R. A. Johnson, *Chemical Oxidations with Microorganisms*, Marcel Dekker, New York, **1972**.
[3] K. Kieslich, *Microbial Transformations of Non-Steroid Cyclic Compounds*, Thieme, Stuttgart, **1976**.
[4] R. A. Sheldon, *Chirotechnology*: *Industrial Synthesis of Optically Active Compounds*, Marcel Dekker, New York, **1993**.
[5] V. Lamare, R. Furstoss, *Tetrahedron* **1990**, *46*, 4109–4132.
[6] H. L. Holland, *Organic Synthesis with Oxidative Enzymes*, VCH, USA, **1992**.
[7] R. Azerad, *Chimia* **1993**, *47*, 93–96.
[8] R. Azerad, *Bull. Soc. Chim. Fr.* **1995**, 17–51.
[9] D. H. R. Barton, *Tetrahedron* **1998**, *54*, 5805–5817.
[10] For recent examples of the chemical hydroxylation of saturated carbon atoms see: a) K. Matsunaka, T. Iwahama, S. Sakaguchi, Y. Ishii, *Tetrahedron Lett.* **1999**, *40*, 2165–2168; b) G. Asensio, R. Mello, M. E. Gonzalez-Nunez, G. Castellano, J. Corral, *Angew. Chem.* **1996**, *108*, 196–198; *Angew. Chem. Int. Ed. Engl.* **1996**, *108*, 217–218; c) L. C. Kao, A. Sen, *J. Chem. Soc. Chem. Commun.* **1991**, 1242–1243.
[11] H. L. Holland in *Biotechnology-Biotransformations*, *Vol. 8a* (Eds.: D. R. Kelly, H.-J. Rehm, G. Reed, A. Pühler, P. Stadler), 2nd ed., VCH, Weinheim, **1998**, pp. 475–533.
[12] A. Hammoumi, G. Revial, J. D'Angelo, J. P. Girault, R. Azerad, *Tetrahedron Lett.* **1991**, *32*, 651–654.
[13] H. L. Holland, *Catalysis Today*, **1994**, *22*, 427–440.
[14] J. D. Fourneron, A. Archelas, R. Furstoss, *J. Org. Chem.* **1989**, *54*, 2478–2483.
[15] R. Furstoss, A. Archelas, J. D. Fourneron, B. Vigne in *Enzymes as Catalysts in Organic Synthesis* (Ed.: M. P. Schneider), Reidel, Dortrecht, **1986**, pp. 361–370.
[16] R. Furstoss, A. Archelas, B. Waegell, J. Le Petit, L. Deveze, *Tetrahedron Lett.* **1980**, *21*, 451–454.
[17] H. L. Holland, *Adv. Appl. Microbiol.* **1997**, *44*, 125–165.
[18] S. Pietz, D. Wolker, G. Haufe, *Tetrahedron* **1997**, *53*, 17067–17078.
[19] H. G. Davies, M. J. Dawson, G. C. Lawrence, J. Mayall, D. Noble, S. M. Roberts, M. K. Turner, W. F. Wall, *Tetrahedron Lett.* **1986**, *27*, 1089–1092.
[20] R. Furstoss, A. Archelas, J. D. Fourneron, B. Vigne, *Organic Synthesis*: *an interdisciplinary challenge*, IUPAC (Eds.: J. Streith, H. Prinzbach, G. Schill), Blackwell, Oxford, **1985**, pp. 215–226.
[21] H. L. Holland, F. M. Brown, B. G. Larsen, M. Zabic, *Tetrahedron Asymmetry* **1995**, *6*, 1569–1574.
[22] a) E. Sundby, R. Azerad, T. Anthonsen, *Biotechnol. Lett.* **1998**, *20*, 337–340; b) M. H. Ansari, T. Kusumoto, T. Hiyama, *Tetrahedron Lett.* **1993**, *34*, 8271–8274.
[23] B. Vigne, A. Archelas, R. Furstoss, *Tetrahedron* **1991**, *47*, 1447–1458.
[24] A. Archelas, J. D. Fourneron, R. Furstoss, *J. Org. Chem.* **1988**, *53*, 1797–1799.
[25] A. Archelas, J. D. Fourneron, R. Furstoss, *Tetrahedron Lett.* **1988**, *29*, 6611–6614.
[26] B. Vigne, A. Archelas, J. D. Fourneron, R. Furstoss, *New J. Chem.* **1987**, *11*, 297–298.
[27] R. A. Johnson, M. E. Herr, H. C. Murray, W. C. Krueger, L. M. Pschigoda, D. J. Duchamp, *J. Org. Chem.* **1992**, *57*, 7212–7216.
[28] R. A. Johnson, M. E. Herr, H. C. Murray, C. G. Chidester, F. Han, *J. Org. Chem.* **1992**, *57*, 7209–7212.
[29] R. A. Johnson, M. E. Herr, H. C. Murray, G. S. Fonken, *J. Org. Chem.* **1968**, *33*, 3217–3221.
[30] R. A. Johnson, H. C. Murray, L. M. Reineke, G. S. Fonken, *J. Org. Chem.* **1969**, *34*, 2279–2284.
[31] R. A. Johnson, M. E. Herr, H. C. Murray, G. S. Fonken, *J. Org. Chem.* **1970**, *35*, 622–626.
[32] G. S. Fonken, M. E. Herr, H. C. Murray, L. M. Reineke, *J. Am. Chem. Soc.* **1967**, *89*, 672–675.
[33] H. L. Holland, T. A. Morris, P. J. Nava, M. Zabic, *Tetrahedron* **1999**, *55*, 7441–7460.
[34] G. J. Grogan, H. L. Holland, *J. Mol. Catal. B*: *Enzym.* **2000**, *9*, 1–32.
[35] G. Braunegg, A. de Raadt, S. Feichtenhofer, H. Griengl, I. Kopper, A. Lehmann, H. Weber, *Angew. Chem.* **1999**, *111*, 2946–2949; *Angew. Chem. Int. Ed.* **1999**, *38*, 2763–2765.
[36] A. de Raadt, B. Fetz, H. Griengl, M. F. Klingler, I. Kopper, B. Krenn, D. F. Münzer, R. G. Ott, P. Plachota, H. Weber, G. Braunegg, W. Mosler, R. Saf, *Eur. J. Org. Chem.*, in press.
[37] J. Seyden-Penne, *Chiral Auxiliaries and Ligands in Asymmetric Synthesis*, Wiley, New York, **1995**.
[38] M. Nogradi, *Stereoselective Synthesis*, VCH, Weinheim, **1995**.
[39] C. G. Rabiller, K. Königsberger, K. Faber, H. Griengl, *Tetrahedron* **1990**, *46*, 4231–4240.
[40] E. Holmberg, E. Dahlen, T. Norin, K. Hult, *Biocatalysis* **1991**, *4*, 305–312.
[41] A. de Raadt, H. Griengl, M. F. Klingler, unpublished results.
[42] A. de Raadt, H. Griengl, M. Petsch, P. Plachota, N. Schoo, H. Weber, G. Braunegg, I. Kopper, M. Kreiner, A. Zeiser, K. Kieslich, *Tetrahedron*: *Asymmetry* **1996**, *7*, 467–472.
[43] A. de Raadt, H. Griengl, M. Petsch, P. Plachota, N. Schoo, H. Weber, G. Braunegg, I. Kopper, M. Kreiner, A. Zeiser, *Tetrahedron*: *Asymmetry* **1996**, *7*, 473–490.
[44] A. de Raadt, H. Griengl, M. Petsch, P. Plachota, N. Schoo, H. Weber, G. Braunegg, I. Kopper, M. Kreiner, A. Zeiser, *Tetrahedron*: *Asymmetry* **1996**, *7*, 491–496.
[45] H. Griengl, A. de Raadt, A. Hickel, D. V. Johnson, M. Schmidt, H. Weber, *Proceedings of the Chiral Europe '96*, *Symposium* **1996**, 59–66.
[46] M. Kreiner, G. Braunegg, A. de Raadt, H. Griengl, I. Kopper, M. Petsch, P. Plachota, N. Schoo, H. Weber, A. Zeiser, *Appl. Environ. Microbiol.* **1996**, *62*, 2603–2609.
[47] M. Kreiner, G. Braunegg, A. de Raadt, H. Griengl, P. Plachota, H. Weber, *Food Technol. Biotechnol.* **1997**, *35*, 5–11.
[48] M. Kreiner, G. Braunegg, A. de Raadt, H. Griengl, I. Kopper, O. Sukcharoen, H. Weber, *Food Technol. Biotechnol.* **1997**, *35*, 99–106.
[49] A. de Raadt, H. Griengl, M. Petsch, P. Plachota, H. Weber, G. Braunegg, I. Kopper, M. Kreiner, A. Zeiser, "*Preparation of Enantiomerically Pure Hydroxylated Benzoxazoles via a Combination of Microbial Hydroxylation*, *Enzymatic Resolution and Crystallization Methods*", 8th International Symposium on Chiral Discrimination, June 30–July 3, **1996**, Edinburgh, Scotland.
[50] S. Feichtenhofer, H. Weber, H. Griengl, A. de Raadt, M. F. Klingler, *J. Mol. Catal. B*, in press.
[51] H. Weber, G. Braunegg, A. de Raadt, S. Feichtenhofer, H. Griengl, K. Lübke, M. F. Klingler, M. Kreiner, A. Lehmann, *J. Mol. Catal. B* **1998**, *5*, 191–198.