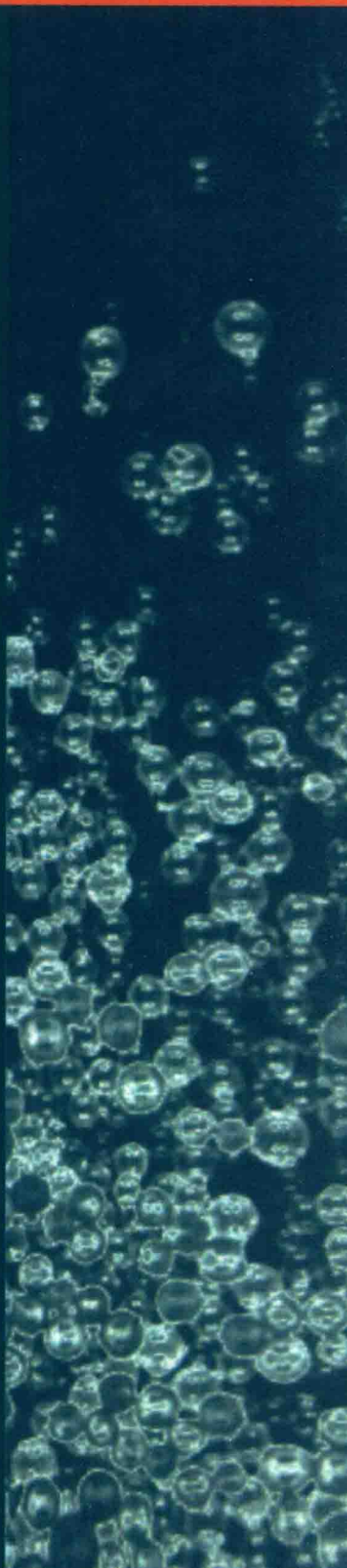
ENZYMATIC REACTIONS IN ORGANIC MEDIA

Edited by
A.M.P. Koskinen
and
A.M. Klibanov



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Enzymatic Reactions in Organic Media

Preface

The outlook of organic synthesis has changed many times during its tractable history. The initial focus on the synthesis of substances typical of living matter, exemplified by the first examples of organic chemistry through the synthesis of urea from inorganic substances by Liebig, was accepted as the birth of organic chemistry, and thus also of organic synthesis. Although the early developments in organic synthesis closely followed the pursuit of molecules typical in nature, towards the end of the 19th century, societal pressures placed higher demands on chemical methods appropriate for the emerging age of industrialization. This led to vast amounts of information being generated through the discovery of synthetic reactions, spectroscopic techniques and reaction mechanisms.

The basic organic functional group transformations were discovered and improved during the early part of this century. Reaction mechanisms were elucidated at a growing pace, and extremely powerful spectroscopic tools, such as infrared, nuclear magnetic resonance and mass spectrometry were introduced as everyday tools for a practising organic chemist. By the 1950s, many practitioners were ready to agree that almost every molecule could be synthesized. Some difficult stereochemical problems were exceptions; for example Woodward concluded that erythromycin was a "hopelessly complex target". This frustration led to a hectic phase of development of new and increasingly more ingenious protecting group strategies and functional group transformations, and also saw the emergence of asymmetric synthesis.

The last two decades have brought about a stunning wealth of new asymmetric reactions, initially as stoichiometrically controlled processes, where the chiral information is introduced from natural sources through chiral pool molecules (internal asymmetric induction), or through the use of chiral auxiliaries (relayed asymmetric induction). These rapid changes in synthesis technology have placed more and more emphasis on the development of economical routes of introducing the chiral information into the synthetic products. Currently, it is accepted that catalytic asymmetric reactions are the most economical to perform in terms of atom and chiral economy; the chiral information of the catalyst is transferred in a most efficient way from the catalyst to the substrate (external asymmetric induction). Synthetic chemistry is thus trying to mimic one of the pretexts of enzymatic transformations.

It is no wonder that chemists have turned to the nature-evolved enzymes from which to learn and utilize these efficient transformations. Although the subject

area of enzyme reactions is still rather unfamiliar territory to the average practitioner of organic synthesis, we firmly believe that such processes and transformations do provide methods which are not easily achieved by classical organic transformations. Enzyme engineering through site-directed mutagenesis will provide additional avenues for refining the enzymes' specificities. Solvent engineering, as described in this book, will provide a very powerful tool to fine-tune enzymatic properties to suit particular needs in synthesis.

Transition metal cayalyzed processes have recently proved to be very efficient, with phenomenal catalytic turnover numbers. Similarly, catalytic antibodies are beginning to provide tailor-made catalysts capable of performing arduous catalytic reactions in an enzyme-like fashion. Combining these technologies with enzymatic transformations will provide the practitioner of synthesis with an armoury unprecedented in the history of chemistry. For example, the synthesis of complex oligosaccharides in a few synthetic transformations, without the need for protecting groups, is one of the dreams coming true at this very moment. It remains to be seen how far one can take this happy marriage between the different fields of chemistry and natural sciences.

It has been a joy to compile this volume, and although the schedule has at times been rather stringent, the contributors have done an excellent job in conveying the most important facets of each topic. It has been a pleasure working with the authors, without whose diligent and painstaking efforts this volume would not have emerged. We also would like to thank the publishers for their help in the practical matters of this task.

A.M.P. Koskinen
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October 1995

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Contents

1 Enzymes in organic solvents: meeting the challenges

A.M.P. KOSKINEN

Enantiopure compounds have undoubtedly gained a central role in the development of modern chemical technology. This is most evident from the changes in the drug market, where single-enantiomer drugs currently occupy a share of some 35 billion US dollars (1). Two-thirds of the world market of the top 25 selling drug wholesale final forms is covered by enantiopure compounds, and the sales are steadily growing at an average annual rate of nearly 7% (see Table 1.1) (2). At the same time, the sales of racemic drugs are diminishing by nearly 30% per year, currently covering only some 1.4 billion dollars. The quest for more efficient, more specifically targeted drugs will place a growing demand on enantiopure materials. The rapid pace of development is evident from the fact that of the 95 best selling drugs in the USA in 1994, 36 single-isomer drugs were new chemical entities approved between 1990 and 1993.

Similar trends can be seen in other specialty and consumer chemical sectors. Environmental issues and the demand for sustainable technology require new chemical materials which are environmentally benign, can be produced without extra burden on the ecosystem, and are truly biodegradable. Polymers, detergents, consumer products, and industrial chemicals all fall prey to these new requirements.

Enzymatic transformations have long been known to be highly stereoselective, but their wider utilization has been slowed down by the commonly held opinion that the substrate-specificity of enzymes cannot be altered easily. Despite some early non-productive suggestions that the course of enzymatic reactions could be reversed, no serious efforts towards this direction were made until the early 1980s, when Alexander Klibanov showed that enzymes do indeed function in

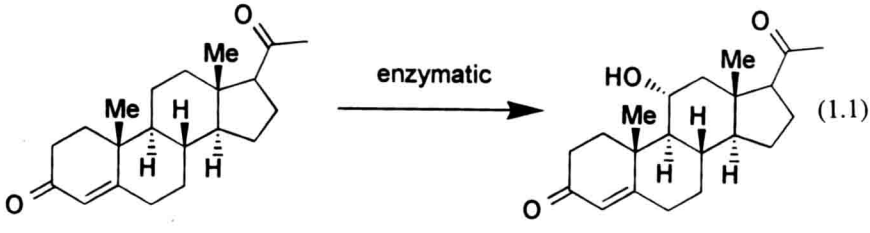
Table 1.1 Worldwide drug market for 25 top-selling prescription drugs

Drug category	Sales, $ billion				% Average annual change
	1993	1994	1995	1996	
Achiral	8.4	9.5	9.8	10.2	7.0
Racemic	3.9	3.0	2.2	1.4	–29.5
Enantiomeric	22.1	23.0	24.8	26.7	6.6

From Stinson (ref. 2).

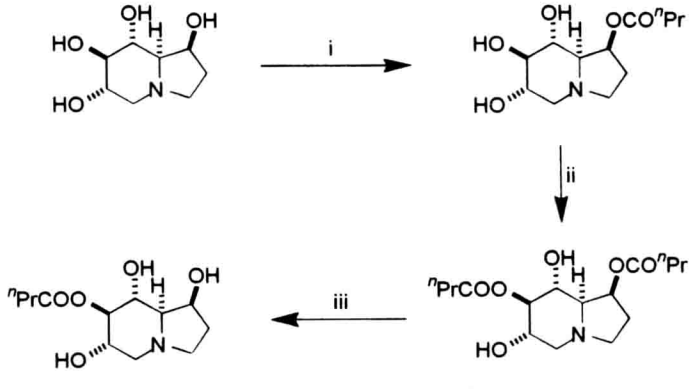
organic solvents (3), and through this modification one can alter the course the enzymatic reactions take (4). The seminal observations soon led to a fast-growing interest in the possibilities of such a powerful new technology, and only a decade later one can find numerous commercial applications.

The first industrially applied microbiological fermentation, paving the way to the development of the subject area of the present treatise, was the introduction of the 11-hydroxy group into a progesterone nucleus, achieved by the Upjohn Company in 1951 (5). Thus 11α-progesterone could be transformed into cortisone in nine steps, allowing the synthesis of cortisone in only 14 steps from diosgenin (equation 1.1) (6).



(1.1)

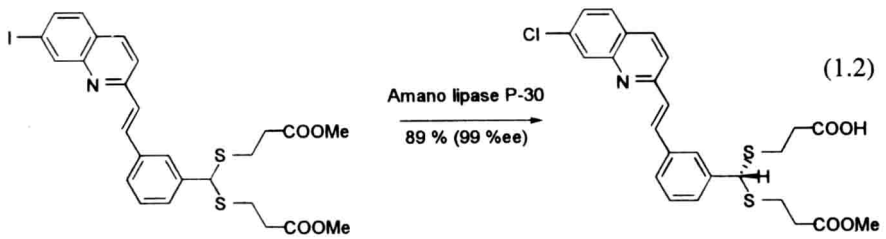
Recent industrial applications can be illustrated by the following two examples. The synthesis of a highly effective HIV-inhibitor, 7-butyroyl-castanospermine, has been achieved through a selective transformation of the 7-hydroxy group into its butyroyl ester by a sequence of three consecutive enzymatic steps (Scheme 1.1). Castanospermine is first transformed into the 1-butyroyl ester with transesterification with subtilisin under non-aqueous conditions. The monoester is converted into the 1,7-dibutyroyl ester with a lipase



Scheme 1.1 Reagents: (i) Subtilisin, *n*-$PrCOOCH_2CCl_3$, pyridine, 92 h, 84%; (ii) Lipase CV, *n*-$PrCOOCH_2CCl_3$, THF, 72%; (iii) Subtilisin, phosphate buffer, pH 6.0, 64%.

in tetrahydrofuran (THF), and finally the 1-butyroyl ester is cleaved with subtilisin (7, 8).

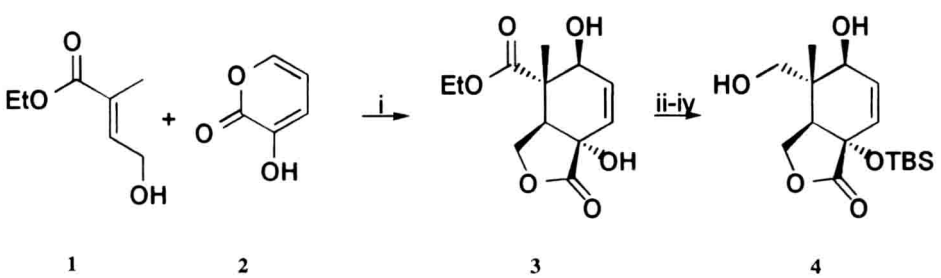
The Merck synthesis of a leukotriene D_4 antagonist developed for the treatment of asthma, relies on the use of the 'meso trick' (equation 1.2). Treatment of the diester with a lipase releases only one of the acid groups from its methyl ester protecting functions, despite the fact that the chiral center is rather distant from the two enantiotropic functional groups (9).



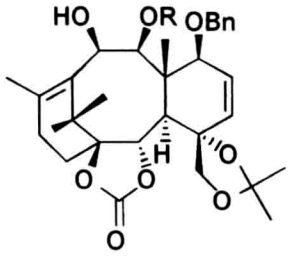
(1.2)

In the course of the total synthesis of taxol, a promising anti-cancer drug, Nicolaou *et al.* developed an expeditious synthesis of the functionalized C-ring precursor utilizing a boronic acid tethered Diels–Alder reaction between the hydroxyester **1** and 3-hydroxypyrone **2** (Scheme 1.2) (10, 11). The Diels–Alder product **3** was then converted to the corresponding primary alcohol **4** through a sequence of protection, reduction and deprotection steps in high overall yield. Due to the nature of the initial Diels–Alder reaction, the C-ring precursor **4** was obtained in a racemic form.

The synthesis requires a resolution step, which in the original synthetic sequence was performed at a relatively late stage in the synthesis, only after the construction of the tricyclic taxoid skeleton (compound **5**). The resolution, with the associated derivatization and cleavage steps, brought about a major material loss and thus reduced the overall appeal of the synthesis.



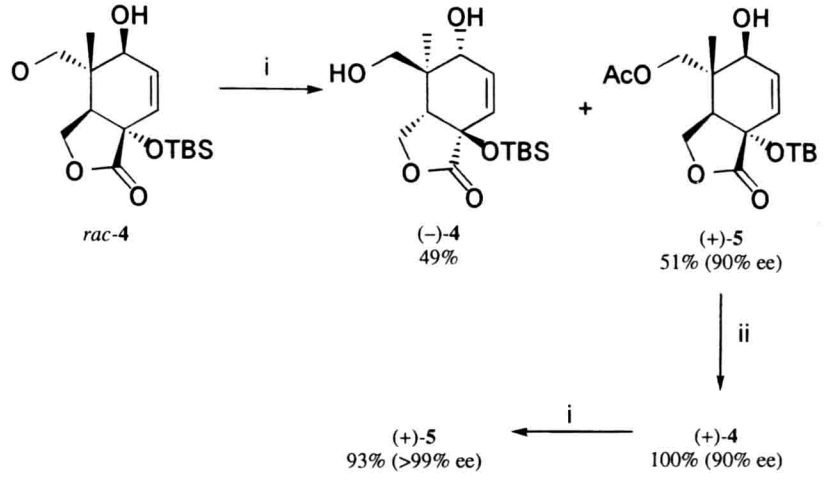
Scheme 1.2 Reagents: (i) $PhB(OH)_2$, PhH, 90°C, then 2,2-dimethyl-1,3-propanediol, 61%; (ii) TBSOTf, 2,6-lutidine, DMAP, CH_2Cl_2, 0°C, 4 h, 95%; (iii) $LiAlH_4$, Et_2O, 0°C, 1 h, 94%; (iv) CSA, MeOH, CH_2Cl_2, 0°C, 1 h, 90%.



R = camphanoyl

5

Johnson's laboratory at Wayne State University has met with considerable success in the development of enzymatic transesterifications of sterically encumbered neopentyl alcohols (12), and this method was tested for the particular problem at hand (13). Thus, the racemic intermediate **4** was subjected to two consecutive enzymatic kinetic resolution steps with recombinant *Candida antarctica* lipase B (SP-435) in isopropenyl acetate–hexane (Scheme 1.3). The enzyme was produced, after transfer of the genetic coding, by *Aspergillus oryzae*, and immobilized by adsorption on an acrylic resin. Treatment of the racemic alcohol rac-**4** at 50°C in isopropenyl acetate–hexane (1:2.5) for 24 h gave the monoacetate (90%ee) in 51% yield. Separation, and treatment with Hünig's base gave the enantiomeric alcohol (+)-**4**, whose enantiopurity could be enhanced by resubjection to the same enzymatic transesterification conditions



Scheme 1.3 Reagents: (i) SP-435, isopentenyl acetate–hexane (1 : 2.5), 50°C, 24 h; (ii) i-Pr_2NEt, MeOH, 25°C.