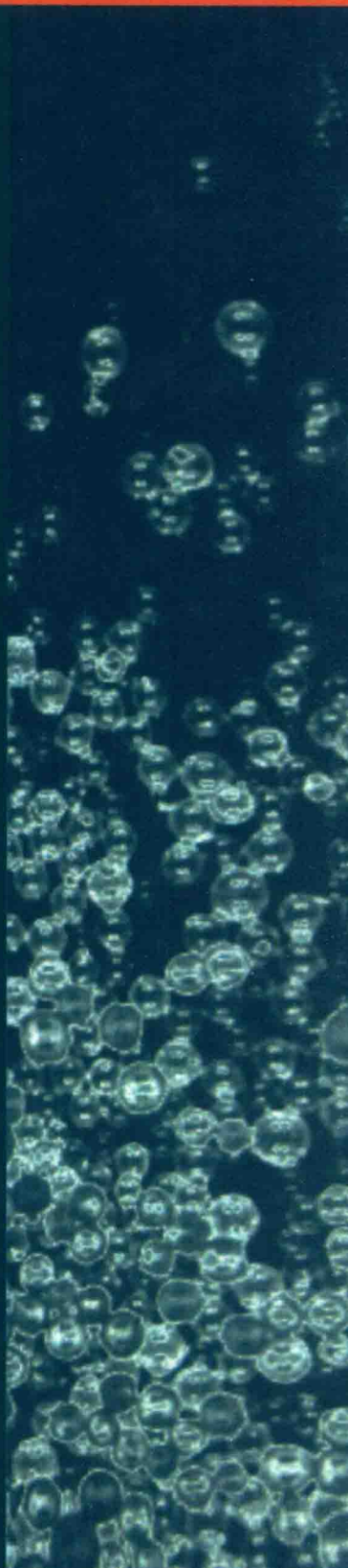


ENZYMATIC REACTIONS IN ORGANIC MEDIA

Edited by
A.M.P. Koskinen
and
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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 catalyzed 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	1
A.M.P. KOSKINEN	
References	7
2 Modes of using enzymes in organic media	9
P. ADLERCREUTZ	
2.1 Introduction	9
2.2 Choice of solvent	10
2.3 Effects of water	13
2.3.1 Quantification of water	14
2.3.2 Reactions at controlled water activity	14
2.3.3 Quantification of water in micro-emulsions	15
2.4 Solid enzyme preparations	15
2.4.1 Lyophilized enzyme powders and enzyme crystals	15
2.4.2 Enzymes immobilized on supports	17
2.5 Solubilized enzyme preparations	26
2.5.1 Covalently modified enzymes soluble in organic media	26
2.5.2 Non-covalent complexes soluble in organic media	30
2.5.3 Enzymes in micro-emulsions	32
2.5.4 Other surfactant-containing systems	34
2.6 Other non-conventional reaction media	35
2.7 Comparisons between different modes of using enzymes in organic media	36
References	37
3 Fundamentals of non-aqueous enzymology	43
Z. YANG and A.J. RUSSELL	
3.1 Introduction	43
3.2 Structural integrity	43
3.3 Mechanistic integrity	46
3.4 Water	49
3.4.1 Effect of water on enzyme activity	49
3.4.2 Effect of water on protein mobility	50
3.4.3 Relationship between enzyme activity and protein mobility	50
3.4.4 Water activity	51
3.4.5 Effect of water activity on enzyme activity	53
3.5 Solvent	54
3.5.1 Effect of solvent on the water associated with the enzyme	54
3.5.2 Effect of solvent on the enzyme	55
3.5.3 Effects of solvent on substrates and products	57

3.6	Kinetics and thermodynamics of non-aqueous enzyme-catalyzed processes	57
3.6.1	Models	57
3.6.2	Apparent kinetic constants versus individual rate constants	59
3.6.3	Transition state stabilization	61
3.6.4	Substrate solvation	62
3.7	Concluding remarks	64
	References	65
4	New enzymatic properties in organic media	70
	A. ZAKS	
4.1	Introduction	70
4.2	Specificity of enzymes in non-aqueous media	71
4.2.1	Substrate specificity	71
4.2.2	Stereoselectivity	75
4.2.3	Regioselectivity	80
4.2.4	Chemoselectivity	80
4.3	Thermal stability of enzymes in non-aqueous media	81
4.3.1	Mechanistic considerations	81
4.3.2	Hydration and thermal stability	83
4.3.3	Mechanism of protein inactivation in organic solvents	85
4.3.4	Stabilization of enzymes in organic solvents	86
4.4	Conclusions	88
	References	89
5	Enzymatic resolutions of alcohols, esters, and nitrogen-containing compounds	94
	C.J. SIH, G. GIRDAUKAS, C.-S. CHEN and J.C. SIH	
5.1	Introduction	94
5.2	Quantitative aspects of enantioselective biocatalytic reactions	94
5.3	Stereochemical recognition of lipases	98
5.4	Working models for predicting stereoselectivity	101
5.5	Molecular and submolecular heterogeneity of <i>Candida rugosa</i> lipase	103
5.6	Sequential biocatalytic kinetic resolutions	105
5.6.1	Asymmetric catalysis of meso/prochiral diesters (or diols)	106
5.6.2	Consecutive kinetic resolution of racemic axially disymmetric substrate	107
5.6.3	Coupled kinetic resolution of racemic monofunctional alcohols in organic media	107
5.7	Acyl donors and acceptors	109
5.8	Solvent and enzyme enantioselectivity	110
5.9	General behaviour of enzymes in organic solvents	112
5.10	Resolution of alcohols in organic solvents	113
5.10.1	Resolution of diols and polyols	114
5.10.2	Lipase resolution of allylic and propargyl alcohols	118
5.10.3	Lipase-mediated transesterification versus deacylation for resolution of racemic alcohols	121
5.11	Resolution of acids and esters in organic solvents	124
5.12	Lipase-mediated synthesis of nitrogen-containing compounds (amines, amides, amino acids, nitriles)	128
5.13	Conclusion	133
	References	134

6 Regioselectivity of hydrolases in organic media 140

S. RIVA

6.1	Introduction	140
6.2	Enzymatic acylation of polyhydroxylated compounds	140
6.2.1	Carbohydrates	140
6.2.2	Natural glycosides	146
6.2.3	Other polyols	152
6.2.4	Esterification with different acyl moieties	160
6.2.5	Miscellaneous	161
6.3	Enzymatic hydrolysis of peracylated polyhydroxylated compounds	162
6.4	Scaled-up procedures	163
6.5	Closing remarks	165
	References	166

7 Hydrolase-catalysed asymmetric and other transformations of synthetic interest 170

L.T. KANERVA

7.1	Hydrolases	170
7.2	Lipases versus esterases: mechanistic models	172
7.3	Principles of enzymatic kinetic resolutions	174
7.3.1	Quantitative analysis of irreversible kinetic resolution	176
7.3.2	Quantitative analysis of reversible kinetic resolution	180
7.3.3	Methods of irreversible hydrolase-catalysed resolutions	181
7.3.4	Possibilities affecting enantioselectivity	187
7.4	Practical resolution of racemic mixtures: transesterification	191
7.4.1	Resolution of primary alcohols	192
7.4.2	Resolution of secondary alcohols	194
7.4.3	Acylation of diols and resolution of other polyfunctional compounds	201
7.4.4	Resolution of tertiary alcohols	205
7.4.5	Resolution of amines and thiols	205
7.4.6	Resolution of carboxylic acids through transesterification	206
7.4.7	Resolution of hydroperoxides	207
7.5	Resolution of racemates without a chiral carbon centre and ferrocene-containing substrates	208
7.6	Hydrolases in other transformations	210
	References	213

8 Peptide synthesis 224

H. KITAGUCHI

8.1	General aspects of protease-catalyzed peptide synthesis	224
8.2	Thermodynamically controlled synthesis	225
8.2.1	Addition of water-miscible solvents	226
8.2.2	Biphasic systems	229
8.2.3	Use of organic media containing a low content of water	232
8.3	Kinetically controlled synthesis	234
8.3.1	Addition of water-miscible solvents	236
8.3.2	Use of anhydrous organic solvents	238
	References	242

9 Productivity of enzymatic catalysis in non-aqueous media: New developments	244
E.N. VULFSON, I. GILL and D.B. SARNEY	
9.1 Introduction	244
9.2 Enzymatic solvent-free synthesis	245
9.3 Enzymatic catalysis in eutectic mixtures	254
9.4 Design and implementation of continuous bioreactors	260
9.5 Conclusions	263
References	263
10 Large-scale enzymatic conversions in non-aqueous media	266
R.A. SHELDON	
10.1 Introduction	266
10.2 General aspects	266
10.2.1 Why enzymatic processes?	266
10.2.2 Why non-aqueous media?	267
10.2.3 Factors influencing industrial utility	268
10.2.4 The industrial enzyme market	270
10.2.5 Whole cells versus isolated enzymes	271
10.3 Modification of oils and fats	272
10.3.1 Lipases in the oleochemical industry	272
10.3.2 Structured lipids for nutrition	274
10.3.3 Fatty acid amides via enzymatic ammoniolysis	275
10.3.4 Bioesters as ingredients of personal care products	275
10.4 Regioselective acylation of carbohydrates and steroids	276
10.4.1 Carbohydrate-based surfactants by lipase-catalyzed acylation of glucose and its derivatives	276
10.4.2 Sucrose fatty acid esters by enzymatic acylation	278
10.4.3 Selective acylation of steroids	280
10.5 Flavors and fragrances	280
10.5.1 Esters of terpene alcohols	281
10.5.2 Chiral alcohols, carboxylic acids, esters and lactones	282
10.6 Optically active pharmaceuticals and pesticides	284
10.6.1 Lipases in the synthesis of chiral C ₃ synthons for beta blockers	285
10.6.2 Enzymatic synthesis of chiral glycidic esters	286
10.6.3 α -Arylpropionic and α -aryloxypropionic acids	288
10.6.4 ACE-Inhibitor intermediates	290
10.6.5 Lipases in the synthesis of pyrethroid intermediates	291
10.6.6 Enzymatic ester ammoniolysis	291
10.6.7 Enzymatic hydrocyanation in organic media	294
10.7 Enzymatic polymer synthesis	297
10.7.1 Polyester synthesis	298
10.7.2 Peroxidase-catalyzed synthesis of polyphenols	298
10.7.3 Enzymatic synthesis of specialty monomers	299
10.8 Concluding remarks and future prospects	301
References	302

CONTENTS

xiii

**Epilogue: Prospects and challenges of biocatalysis
in organic media** **308**

A.M. KLIBANOV

References 310

Index **311**

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

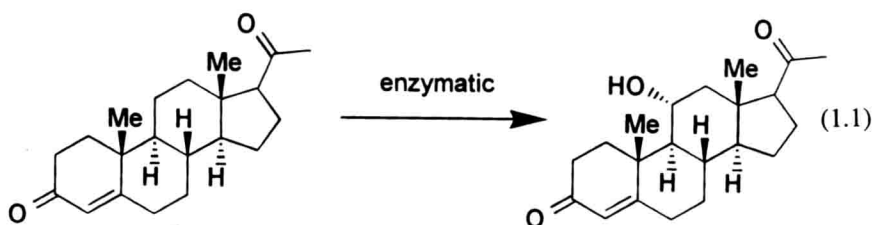
Table 1.1 Worldwide drug market for 25 top-selling prescription drugs

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

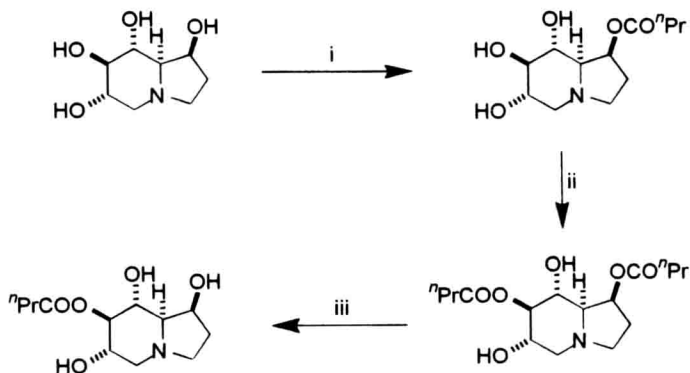
From Stinson (ref. 2).

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 11 α -progesterone could be transformed into cortisone in nine steps, allowing the synthesis of cortisone in only 14 steps from diosgenin (equation 1.1) (6).



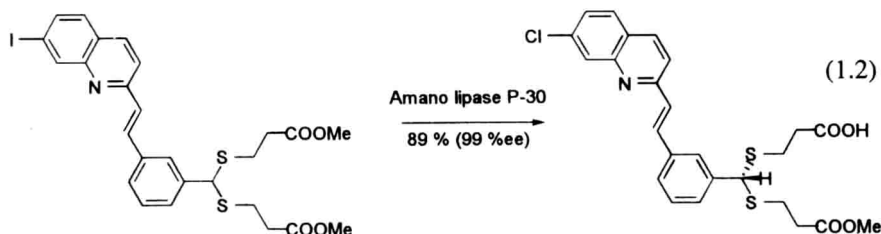
Recent industrial applications can be illustrated by the following two examples. The synthesis of a highly effective HIV-inhibitor, 7-butyroyl-castanospermine, has been achieved through a selective transformation of the 7-hydroxy group into its butyroyl ester by a sequence of three consecutive enzymatic 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 -PrCOOCH₂CCl₃, pyridine, 92 h, 84%; (ii) Lipase CV, n -PrCOOCH₂CCl₃, THF, 72%; (iii) Subtilisin, phosphate buffer, pH 6.0, 64%.

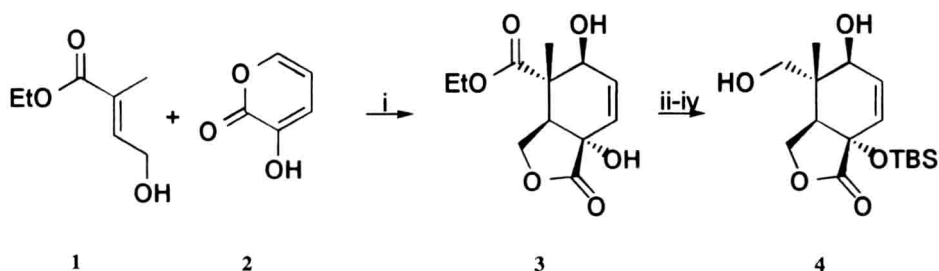
in tetrahydrofuran (THF), and finally the 1-butyroyl ester is cleaved with subtilisin (7, 8).

The Merck synthesis of a leukotriene D₄ 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).

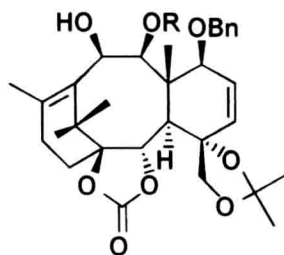


In the course of the total synthesis of taxol, a promising anti-cancer drug, Nicolaou *et al.* developed an expeditious synthesis of the functionalized C-ring precursor utilizing a boronic acid tethered Diels–Alder reaction between the hydroxyester **1** and 3-hydroxypyrene **2** (Scheme 1.2) (10, 11). 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.



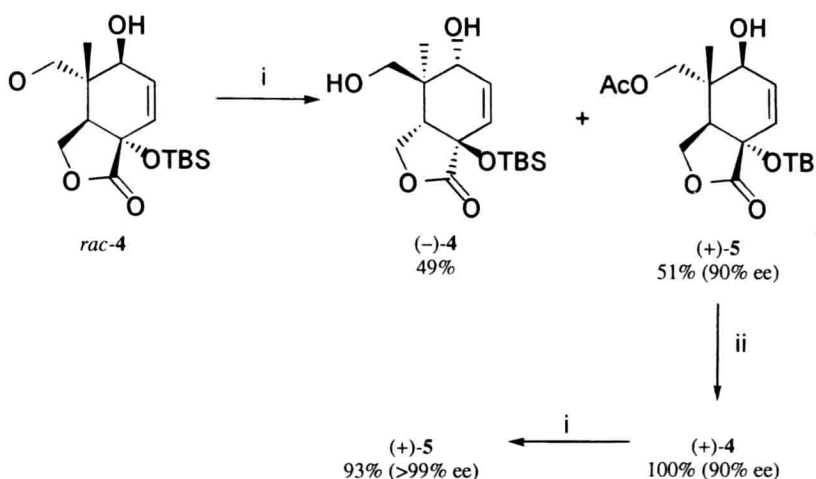
Scheme 1.2 Reagents: (i) PhB(OH)_2 , PhH , 90°C , then 2,2-dimethyl-1,3-propanediol, 61%; (ii) TBSOTf , 2,6-lutidine, DMAP, CH_2Cl_2 , 0°C , 4 h, 95%; (iii) LiAlH_4 , Et_2O , 0°C , 1 h, 94%; (iv) CSA, MeOH , CH_2Cl_2 , 0°C , 1 h, 90%.



$R = \text{camphanoyl}$

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) $i\text{-Pr}_2\text{NEt}$, MeOH, 25°C.