Studies Towards Combined Chemo-Biocatalytic Reactions In Water

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Submitted Towards A PhD

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To my parents and Angela.

"Paths that lead to the most profound destinations, to moments of illumination or change, have nothing to do with actual travel, but rather negotiate a mental geography."

- *from Life During Wartime by Lucius Shepard*

"From a certain point onward there is no longer any turning back. That is the point that must be reached."

- *Kafka*

Declaration

I, Alexander Byron Jones, hereby state that the following is entirely my own work and has not been submitted for any other degree or examination.

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Abstract

In recent years green chemistry has been increasingly applied to industrial syntheses. One key area of growth is the use of biocatalysts to perform reactions selectively in non-organic media. However, there is currently no set development process for the discovery and evolution of enzymes to be applied in these reactions. A process has been suggested by Hailes et al, part of which is the development of chemical reactions that work in combination with the biocatalysts.[1] The interface between traditional chemistry and biocatalysis has yet to be fully explored, particularly in relation to the potential degree of interaction between the two synthetic techniques.

The development of a chemical reaction that can be used in one-pot with a biotransformation is explored within this thesis. The synthesis of a standard to calibrate an assay of the transketolase selectivity was performed first. This allowed the stereochemistry of the starting material of the reductive amination being developed to be determined.

Next a reductive amination reaction that proceeded in water and in the presence of transketolase was discovered and then optimized using cyclohexanone as a test substrate. This reaction was developed so that it could offer an alternative to a similar transformation performed by a transaminase enzyme, specifically with respect to substrate and stereoselectivity.

This optimized reaction was then applied to an intermediate (1,3-dihydroxy-pentan-2-one), synthesized both using standard organocatalytic techniques and a biotransformation using transketolase, to produce 2-benzylamino-pentane-1,3-diol. The reaction was seen to

be diastereoselective and an alternative transfer hydrogenation reaction that displayed the opposite selectivity was also investigated. This complimentary pair of reactions meant that all four diastereoisomers of 2-benzylamino-pentane-1,3-diol could potentially be synthesized.

This work illustrated that the development of chemical reactions towards one-pot cascade reactions with biocatalysts is possible.

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Abbreviations

L-AAO - L-amino acid oxidase

AcOH - acetic acid

AIBN - azobisisobutyronitrile

Alk - alkyl

Ar - aryl

aq - aqueous

atm - atmosphere

BDPP - 2,4-bis(diphenylphosphino)pentane

BER - borohydride exchange resin

BETP - Biological Effluent Treatment Plant

BiCE - Biocatalysis-Chemistry-Engineering Interface

BINOL - 1,1‘-Bi-2-naphthol

Bn - benzyl

BPB - *N*-(2-Benzoylphenyl)-1-(benzyl)pyrrolidine-2-carboxamide

bpy - 2,2‘-bipyridine

CAN - ceric ammonium nitrate

cat - catalyst

cod - cyclo-octa-1,5-diene

conc. - concentration

CTAB - cetyl trimethylammonium bromide

d - day

DCE - 1,2-dichloroethane

DMAP - 4-dimethylaminopyridine

DMF - dimethyl formamide

DMSO - dimethylsulfoxide

DPBB - 4,4-diphenyl-3-butenyl bromide

dppp - diphenyl-1-pyrenylphosphine

DS - dodecyl sulfate

E.C. - European Community

e.e. - enantiomeric excess

FDA - Food and Drug Administration

GC - gas chromatography

h - hours

H-bond - hydrogen bond

HPLC - high performance liquid chromatography

LAH - lithium aluminium hydride

LASC - Lewis acid combined with surfactant

min(s) - minute(s)

mol. - molecular

MS - mass spectrometry

NBS - *N*-bromo succinimide

NDMBA - N,N‘-dimethylbarbituric acid

NMR - nuclear magnetic resonance

PCC - pyridinium chloro chromate

PEG - poly ethylene glycol

Ph - phenyl

Phos - phosphate

pic - 2-picoline

PKD - 1,3-dihydroxypentan-2-one

PMP - pyridoxamine-5‘-phosphate

ps - pico second

PTSA - *para*-Toluene Sulfonic Acid

pyr - pyridine

quant. - quantitative

rac - racemic

RedAm - reductive amination

r.t./rt - room temperature

SAMP - (*S*)-1-amino-2-methoxymethylpyrrolidine

SDS - sodium dodecyl sulfate

t - tonnes

TAm - transaminase

TBDMS - *tert*-butyl dimethylsilyl chloride

TBDPS - *tert*-butyl diphenylsilyl chloride

TEG - tetra ethylene glycol

TFA - trifluoroacetic acid

THF - tetrahydrofuran

TK - transketolase

TLC - thin layer chromatography

TMS - trimethylsilyl

ThDP - thiamine diphosphate

tppts - tris(3-sulfophenyl)phosphine

TsCYDN - *N*-toluenesulfonyl-(*1R,2R*)-(-)-1,2-diaminocyclohexane

U - enzyme activity unit (amount of enzyme that will catalyse the transformation of one mole of substance per second)

UDP - uridine diphosphate

UTP - uridine triphosphate

UV - ultraviolet

VOC - volatile organic compound

WT - wild type

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Introduction

1.1 General introduction

The environmental impact of chemistry, and in particular of large scale chemical processes, is of increasing global concern and the field of green chemistry has been developed to respond to this. The strategies employed in green chemistry are based upon the principle of reducing the risk involved in performing chemical reactions, by reducing their inherent hazardousness.[1,2] This is contrary to the traditional view of chemists and chemical engineers, that the risk should be reduced by reducing people's exposure to the hazards of individual chemicals. This traditional approach works to reduce the risks involved, but means that hazardous chemicals are still used and will need to be handled at some point in the life cycle of the reaction.[2] It also means that human error can have serious consequences, and if an accident were to occur, then it might have a large impact on the environment. The idea of reducing the inherent risks in chemistry by reducing the potential hazards is summed up in the most widely accepted definition of green chemistry:

> "the design, development and implementation of chemical processes and products to reduce or eliminate substances hazardous to human health and the environment"[2]

Anastas and Warner subsequently expanded this statement to highlight 12 ways this can be achieved (Figure 1.1 on page 3).[3,4]

At a later date Anastas and Zimmermann developed the 12 principles of green engineering, which allow process engineers to evaluate the environmental impact of their work and provide guidance on improving it.[2] These principles make it possible to examine the green credentials of processes, based upon their own merits and not just on the reactions being undertaken. Both sets of green principles are best summed up in the mnemonic created by Poliakoff *et al* which is shown in Table 1.1.[5]

Industry is implementing as many changes as possible, based on these principles, and there are two main impetuses for this. The first and most powerful driving force is economics, with the financial benefits of applying the green chemical principles mainly brought about by minimizing the energy and chemicals used in a process, as well as reducing the waste stream.[6] This is important because the profit margins in chemical manufacture are often small, mostly due to the escalating cost of developing new drugs, the highly competitive nature of the business and limited patent lives.[7,8] However, these economic arguments will not cause the overnight transformation of the chemical industry to a more environmentally friendly practice. Synthetic routes to molecules in most parts of the chemical industry are highly regulated, needing to be proved safe, as well as giving a consistent quality of

1 **Prevention**
It is better to prevent waste than to treat or clean up waste after it has been created

2 **Atom economy**
Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product

3 **Less hazardous chemical syntheses**
Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment

4 **Designing safer chemicals**
Chemical products should be designed to effect their desired function while minimizing their toxicity

5 **Safer solvents and auxiliaries**
The use of auxiliary substances (e.g. solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used

6 **Design for energy efficiency**
Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure

7 **Use of renewable feedstocks**
A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable

8 **Reduce derivatives**
Unnecessary derivatization (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste

9 **Catalysis**
Catalytic reagents (as selective as possible) are superior to stoichiometric reagents

10 **Design for degradation**
Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment

11 **Real-time analysis for pollution prevention**
Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances

12 **Inherently safer chemistry for accident prevention**
Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires

Figure 1.1: The 12 principles of green chemistry[3]
Anastas PT, Warner JC. Green Chemistry: Theory and Practice, Oxford University Press: New York, 1998, p.30. By permission of Oxford University Press

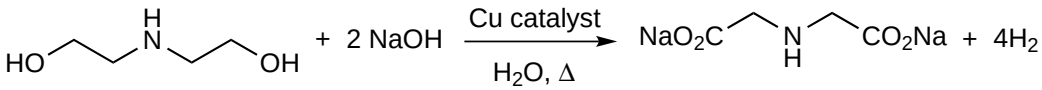
12 Principles of Green Chemistry	12 Principles of Green Engineering
Prevent wastes	**I**nherently non-hazardous and safe
Renewable materials	**M**inimize material diversity
Omit derivatization steps	**P**revention instead of treatment
Degradable chemical products	**R**enewable material and energy inputs
Use safe synthetic methods	**O**utput-led design
Catalytic reagents	**V**ery simple
Temperature, pressure ambient	**E**fficient use of mass, energy, space and time
In-process monitoring	**M**eet the need
Very few auxiliary substances	**E**asy to separate by design
E-factor, maximize feed in product	**N**etworks for exchange of local mass and energy
Low toxicity of chemical products	**T**est the life cycle of the design
Yes its safe	**S**ustainability throughout product life cycle

Table 1.1: Mnemonic for the 12 principles of green chemistry and the 12 principles of green engineering[5]
Tang S, Bourne R, Smith R, Poliakoff M. *Green Chemistry* 2008; **10**: 268–269. By permission of the Royal Society of Chemistry

product. This is particularly the case with the pharmaceutical industry where the molecules synthesized are to be used in medicines, and so each batch needs to be identical in order to provide identical therapeutic outcomes. The complex nature of some of the molecules, which often need to be made as a single enantiomer, means that the pharmaceutical industry has the highest, and therefore worst, ratio of mass of chemical feedstock required to synthesize each kilogram of product.[3] Therefore, where the effect of applying green chemical principles would cause the greatest benefit, the application is stifled. This regulation does not encourage changes within the industry unless there is a large enough economic incentive, which will more than cover the costs incurred in changing the process. Furthermore current government policy does not reward companies for improving their practices, but rather punishes those who pollute and break the law.[2] The level of pollution at which companies are punished is defined in national and international environmental regulations and these do not encourage companies to improve processes far beyond these levels.[2] The stan-

dards are increased incrementally but the chemical industry tends to oppose these changes, as they could find themselves liable for fines and punishments once a regulatory limit has been changed, as well as having to potentially shut down profitable plants.[3]

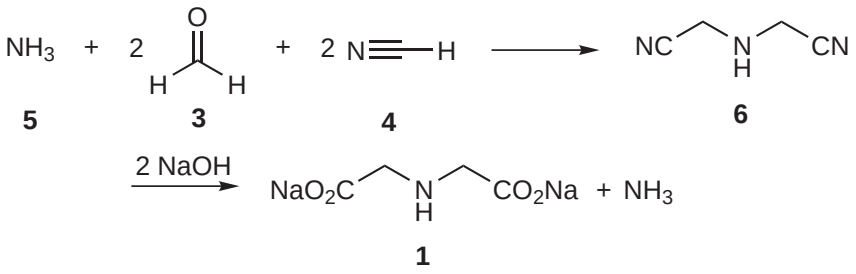
The second major driving force for change to greener chemistry within the chemical industry is that while it has helped deliver a much higher standard of living, for example developing and providing many modern materials and life saving medicines, its practice is often viewed with suspicion and hostility.[9] This is not helped by the growing call for environmentally friendly products and lower reliance on oil, one of the major feedstocks for the chemical industry. This encourages people to demand 'natural' products and so synthetic materials are considered less environmentally friendly, often without proof. The impact of this is that the chemical industry has only a 26% approval rating amongst young adults, similar to both the nuclear energy and tobacco industries.[10] This suspicion means that people do not take claims by the chemical industry seriously, in particular that it is working for the greater good.[11] In order to combat this, detailed and verifiable examples need to be given that dispel any negative ideas.[11] One way of doing this is to display a drive towards green chemical principles, which demonstrate a positive commitment to efficiency and environmental stewardship.



2 **1**

Scheme 1.1: The modified synthesis of disodium iminodiacetate (**1**) for which Monsanto won a Presidential Green Challenge Award in 1996[12]

An example of how this is being done is the Presidential Green Chemistry Challenge Awards: companies receive awards for green developments, allowing them to give a clear example of how they are making their processes more environmentally friendly.[13] One of these awards was given to Monsanto in 1996 for modifying the synthesis of disodium iminodiacetate (DSIDA, **1**) from a stoichiometric synthesis to a catalytic dehydrogenation of diethanolamine (**2**, Scheme 1.1).[12] This change in the synthesis produced both less waste and a higher yield, as well as removing several hazardous chemicals from the original industrial synthesis (Scheme 1.2).[12–14] In the original Strecker synthesis of DSIDA (**1**), formaldehyde (**3**) and hydrogen cyanide (**4**) were reacted with ammonia (**5**) to form **6**, which was then converted to DSIDA (**1**) using sodium hydroxide.[12] The new synthetic route meant that DSIDA could be produced without using either formaldehyde or hydrogen cyanide, both of which are very hazardous, making the process much safer. This also has the added benefit that the product requires very little purification; the catalyst is removed from the mixture by filtration, and the by-product is gaseous and so does not need to be physically removed in a separate purification step. As diethanolamine is a key intermediate in the synthesis of *Roundup*®, a herbicide produced on a very large scale, these changes had a very large, positive environmental impact.[13]



Scheme 1.2: The original synthesis of disodium iminodiacetate by Monsanto[12]

However, there are concerns that many green methods reported in the chemical literature will have no useful application in industry.[15] This is because the chemical industry is primarily motivated by economic factors and corporate image, and the easiest way to make a process greener is to use atom economy.[15] In fact the need to make reactions cleaner, safer and more atom economic are already factored into most process decisions due to these monetary concerns.[7] Atom economy makes the process cheaper as fewer raw materials need to be purchased, and also means that the waste stream will be smaller and potentially contain fewer hazardous chemicals making it both cheaper and easier to dispose of. Clark performed an interesting analysis of the cost of waste from the chemical industry, and a modified version showing only the direct economic impacts upon industry is shown in Figure 1.2.[16] This figure does not include the indirect economic impacts of waste generation, which come from increased raw material costs due to depletion of natural resources, and increased costs as a result of additional legislation, which would include the increased costs of developing new technologies to allow the company to stay in business.[16] Indeed environmental laws, which strictly limit emission levels for many of the more harmful substances used, are one of the main motivations for industry to make itself greener.[16] These laws mean that the chemical industry has to contain and safely dispose of these regulated substances if they are used. This can be very costly, as these chemicals have to be fully contained during use, and stripped from the waste stream for safe disposal once the process is complete.[16]

The need to make chemical reactions inherently safer is one of the key motivators in both green chemistry and the chemical industry. In industry this is evident from its highly regulated nature, which makes companies prove that each new process is safe, as well as abiding by a strict health and safety code. All of this means that the chemical industry can already be considered to be embracing key ideas from green chemistry.

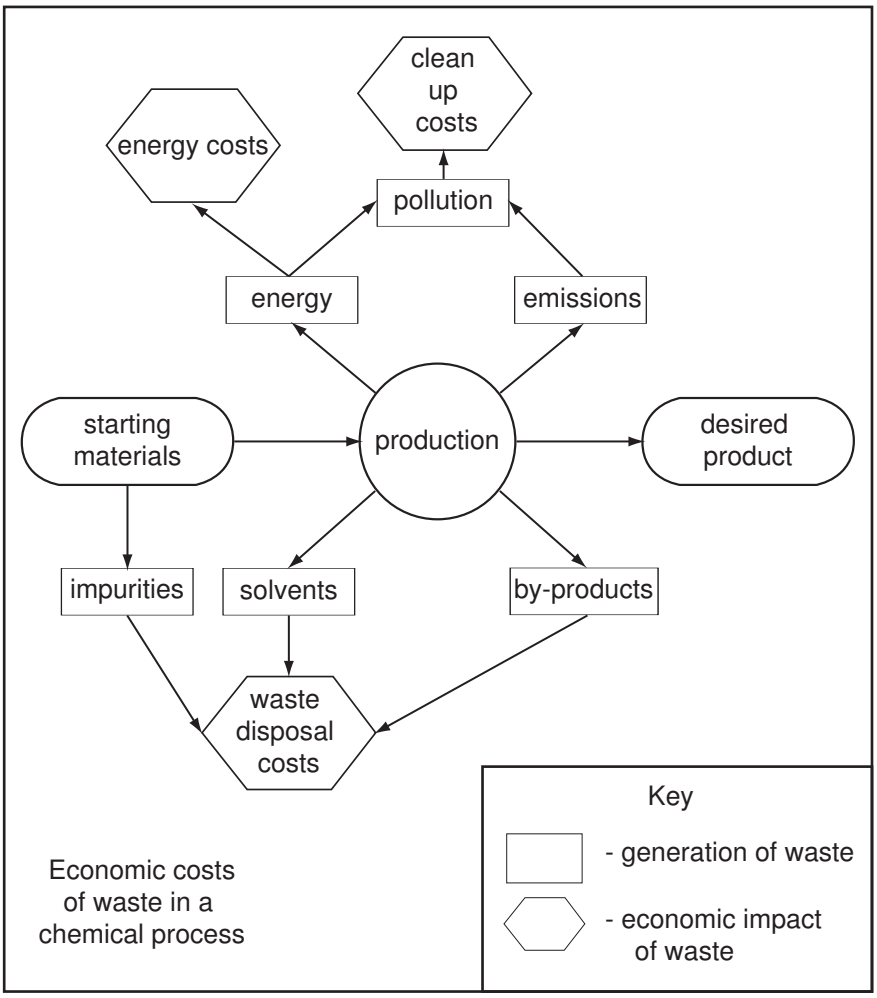
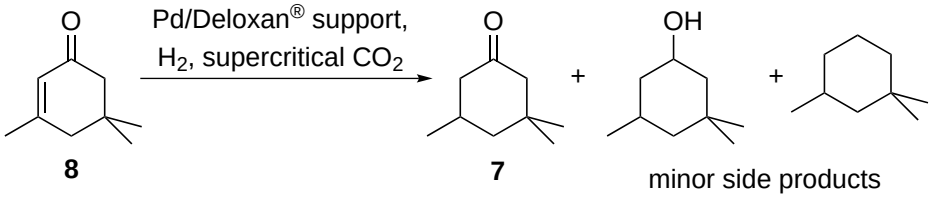


Figure 1.2: Sources of waste that have an economic impact on the chemical industry[16] Clark JH. *Green Chemistry* 1999; **1**: 1–8. By permission of the Royal Society of Chemistry

Atom economy can often be implemented by the chemical industry without the need to build major new infrastructure, which makes it preferential to the development and use of new methods, technologies and techniques that are unproven at scale.[17] In addition, as some of the green processes currently in development rely on new technology that has not been used on a process scale before, any new infrastructure that is built will be unlikely to work at optimal efficiency, as well as requiring the development of new designs for both

the equipment being used and the overall plan, as well as the development of new health and safety procedures. All of these factors make the building of new green chemical plants more expensive. One example of the development of new technology at a process scale is the multi-purpose plant built in collaboration between Thomas Swan and Co. Ltd and the chemistry department of the University of Nottingham.[18] This plant was built to explore the possible use of supercritical carbon dioxide as a solvent in chemical manufacture, at the scale of a thousand tonnes per year.[18] The plant started production of trimethylcyclohexanone (**7**) in 2002, producing the product by the hydrogenation of isophorone (**8**, Scheme 1.3) as a continuous flow process, but it is currently not in use as the method proved to be too costly when compared with the existing method of manufacture, even though the product was significantly purer.[18]

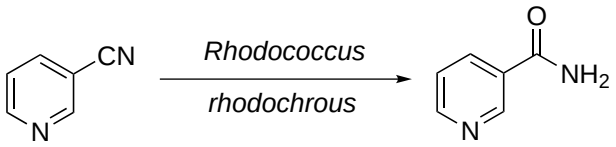


Scheme 1.3: The industrial synthesis of trimethylcyclohexanone in supercritical carbon dioxide[18]

Finally, it is important that any benefits from the application of green chemistry should not be gained at the expense of synthetic efficiency. This is because even a small decrease in yield, catalyst turnover or reaction selectivity can lead to substantial increases in both cost and waste generation.[19] This is of particular importance in the pharmaceutical industry where the waste generated during the production of pharmaceuticals can be greater than 100 kg/kg product.[20] This large ratio of product to waste generation has led to an increased urgency in creating greener and more economically competitive processes.[2]

1.2 Chemocatalysis versus biocatalysis

One of the most important ways of reducing the amount of waste generated by a chemical process is the use of catalysis, using both chemical and bio-catalysts. Biocatalysts have proven to be very effective as they are environmentally benign, as well as highly efficient. In the year 2000 there were over 300 procedures involving biocatalysis being used by industry, as well as 130 biocatalytic processes being sold commercially.[21–23] For example the hydrolysis of nicotinonitrile (**9**) to nicotinamide (**10**) using *Rhodococcus rhodochrous*, is carried out commercially on a scale of 4000 tonnes/year (Scheme 1.4).[23–29] Nicotinamide is the less toxic form of niacin (Vitamin B3) and is what niacin is converted to *in vivo*. One of its many uses is in the treatment of skin conditions, for example acne.[24]

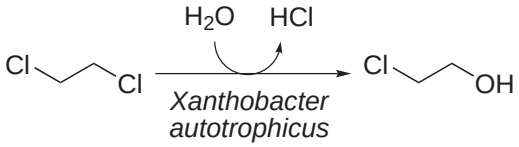


9 **10**

Scheme 1.4: The industrial synthesis of nicotinamide, which is prepared on a scale of 4000 tonnes/year[24]

The typical rate of acceleration provided by a biocatalyst in a chemical process is 10^8–10^{10}, with some reactions having up to 10^{17}-fold rate acceleration, which is much greater than that provided by chemical catalysts.[24,30] Another benefit is that while a chemical catalyst will typically be used at a concentration of 0.1–1.0 mol%, a biocatalyst will generally be used at 10^{-3}–10^{-4} mol% in order to carry out a reaction at a reasonable rate.

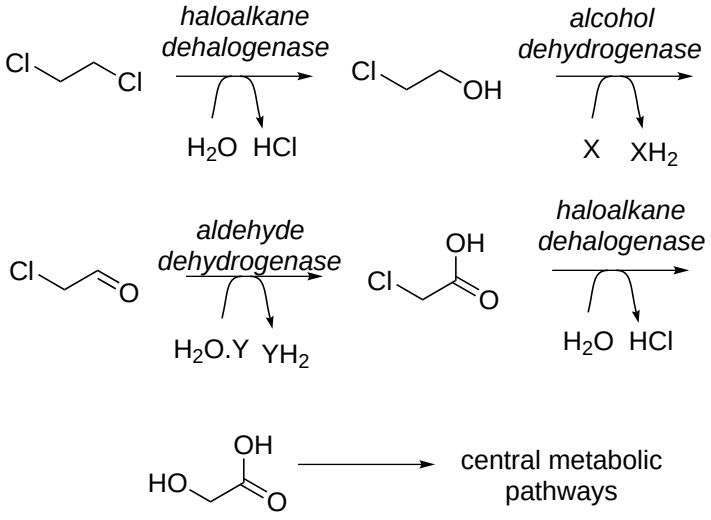
Biocatalysts are more environmentally benign than chemical catalysts: they come from a sustainable source; are fully biodegradable; and can act under mild conditions, typically pH 5–8 and 20–40 °C. This has the additional advantage that substrates that might not be compatible with traditional methods, can be stable under these milder conditions and not undergo undesired side reactions, such as decomposition, isomerization, racemization and rearrangements. One example where chemocatalysis fails but biocatalysis succeeds is the hydrolysis of haloalkanes by haloalkane dehydrogenase (Scheme 1.5).[31,32]



Scheme 1.5: Hydrolysis of 1,2-dichloroethane by *Xanthobacter autotrophicus*[32]

One strain of *Xanthobacter autotrophicus* has been shown to be able to use halogenated short-chain hydrocarbons and halogenated carboxylic acids as its sole source of carbon for growth. The organism produces two different dehalogenases which enable it to perform this extraordinary feat, one specific for halogenated alkanes and the other for halogenated carboxylic acids. The metabolic pathway within the bacteria uses the two different enzymes in order to convert haloalkanes into compounds that can participate in its central metabolic pathways (Scheme 1.6).[33]

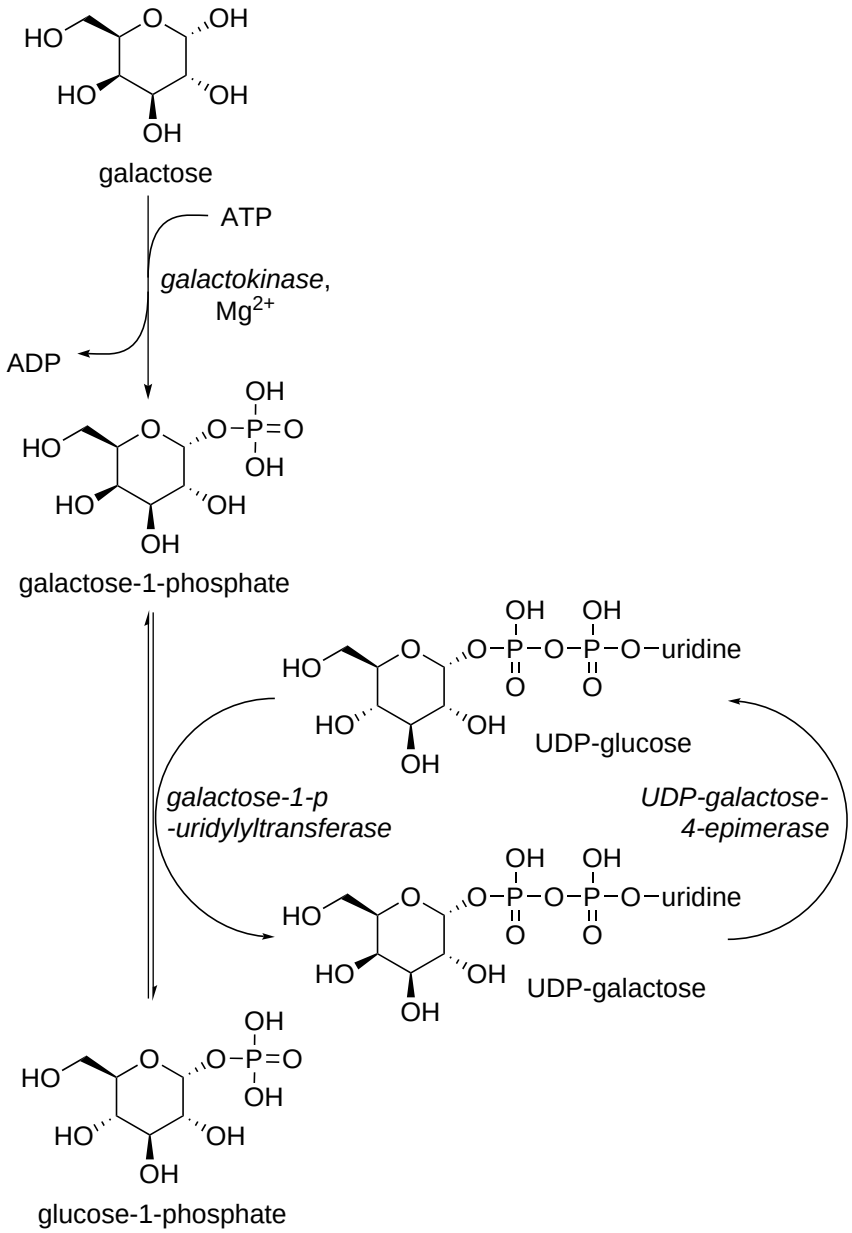
Enzymes that can be used to catalyse the same reaction can be found in many organisms, often acting under a range of conditions. This means that there will often be a variety of enzymes to choose from for each reaction that one might want to carry out, allowing enzymes that act under similar sets of conditions to be identified, possibly in several dif-



Scheme 1.6: Incorporation of 1,2-dichloroethane into the central metabolic pathways by *Xanthobacter autotrophicus*[33]
X and Y are not defined in the paper

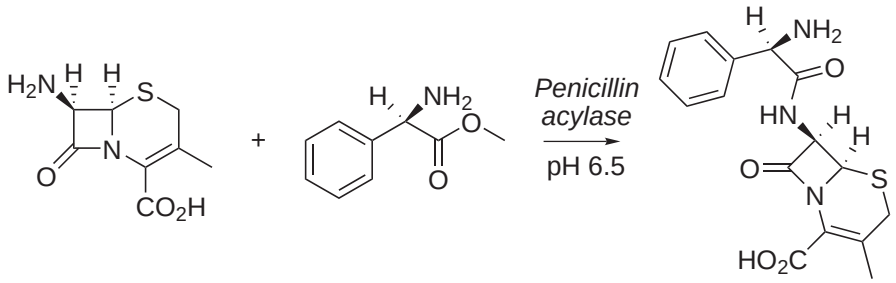
ferent organisms, and used together. Multi-step, sequential or parallel reactions are therefore feasible using multi-enzyme systems to simplify the reaction processes or allow cascades of reactions to be carried out in the same flask. Ideally these syntheses would mimic pre-existing biosynthetic pathways (*e.g.* the Leloir pathway which converts galactose to glucose, Scheme 1.7) as this would mean that a pre-existing set of reactions from nature could be exploited.[24,34,35] In these cascades of reactions, unstable intermediates need not be isolated, unfavourable equilibria can be shifted towards the desired product and the final product extracted continually.

Both traditional organic synthesis and biocatalysis have limitations which influence their use. For example, the sometimes harsh conditions used in organic synthesis can damage other functionality within the molecule, which can necessitate the use of multiple protection-deprotection strategies. These are not very atom efficient and on occasion



Scheme 1.7: The conversion of galactose to glucose (The Leloir pathway)[24, 34, 35]
ATP=adenosine triphosphate; ADP=adenosine diphosphate; UDP=uridine diphosphate

can lead to dead ends where protecting groups cannot be cleaved. However, if biocatalysis is to be used then the required biocatalytic toolbox and method may not be readily available. Nevertheless, their use can remove the need for multiple protection and deprotection steps. An example of this is the commercial enzymatic synthesis of the β-lactam antibiotic cephalexin (**11**) by Chemferm. This enzymatic synthesis reduced the total number of steps involved from 10 to six by removing the need for several protection/deprotection steps.[36] In the final step a selective coupling reaction is performed biocatalytically using *Penicillin acylase* in the presence of a number of similarly reactive groups (Scheme 1.8), thus removing the need for protection strategies.[36]



11

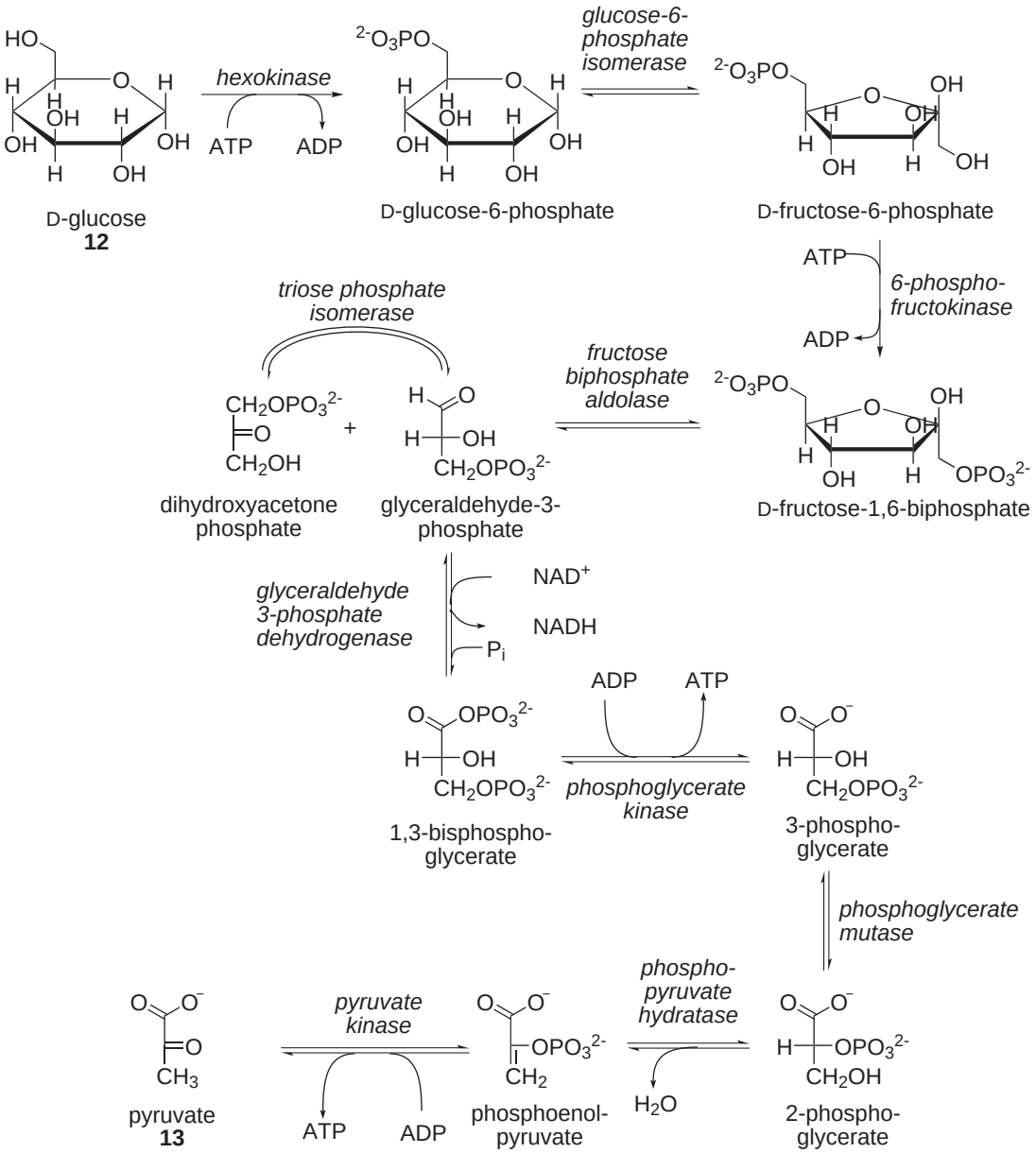
Scheme 1.8: The final highly selective biocatalytic step in the Chemferm synthesis of cephalexin (**11**)[36]

As both chemo- and biocatalytic methods become more widespread in chemical manufacture, there has been a gradual disappearance of traditional barriers between homogenous, heterogenous and bio-catalysis. This has led many to desire the full integration of a number of catalytic steps into a one-pot, multi-step catalytic cascade, mimicking the biosynthetic pathways seen in nature.[37] For example metabolic pathways in living cells involve a series of biocatalytic steps carried out as part of a multi-enzyme cascade, without the need for the separation of intermediates.[38] One example of a multi-enzyme cascade in nature

is glycolysis (Scheme 1.9), during which D-glucose (**12**) is metabolised to pyruvate (**13**). This cascade involves 10 different reactions catalysed by enzymes, that result in an overall release of energy. The free energy produced during this process is converted into high energy compounds that can then be used in other pathways. It results in the production of two molecules of adenosine triphosphate (ATP) and two molecules of nicotinamide adenine dinucleotide hydride (NADH). The pyruvate product is then used in several different pathways within the cell, including conversion to acetyl coenzyme A when oxygen is present, this can then participate in the Krebs (citric acid) cycle during which it is converted to alanine. The pathway is regulated by several feedback mechanisms and is dependent upon the amounts of ATP, NADH and the starting materials for the cycle already present in the cell.

Combining multi-step syntheses in a single cascade, in the manner pioneered by nature, is the ultimate aim of many green chemical technologies.[39] This is because these interconnected series of reactions involve fewer unit operations; less solvent and reactor volume; shorter cycle times; higher volumetric and space yields; and less waste. This provides substantial environmental and economic benefits, as well as the possibility of driving the reaction equilibria throughout the entire series of reactions towards products, avoiding the need for excess reagents.

Bio- and chemocatalysts often complement each other. Transition metal based catalysts are very versatile for oxidations and reductions, which are often difficult to perform with enzymes, as difficult cofactor regeneration makes the reactions very complicated. Meanwhile enzymes readily perform hydrolytic reactions and their reverse, which often produce large amounts of waste when performed chemically.[40] One of the major challenges encountered during the development of these cascades is that the reagents and conditions for the



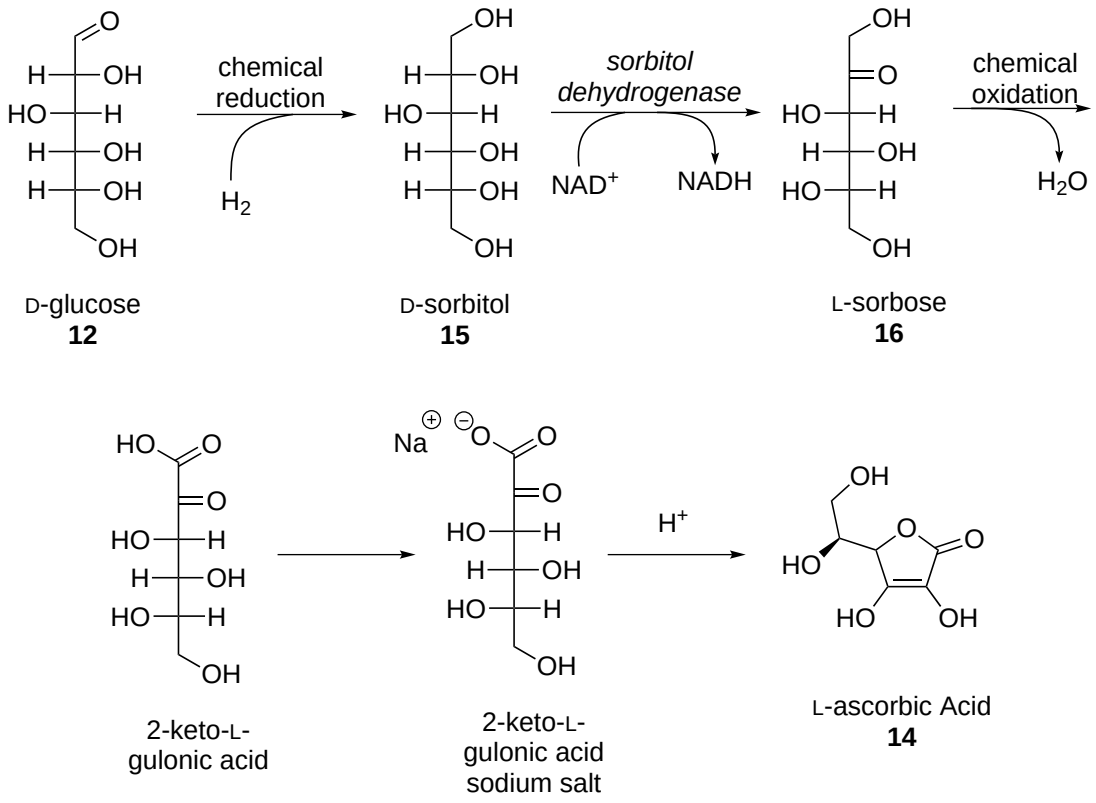
Scheme 1.9: Glycolysis[38]

ATP=adenosine triphosphate; ADP=adenosine diphosphate; NAD^+=nicotinamide adenine dinucleotide; NADH=nicotinamide adenine dinucleotide hydride; P_i=phosphate group

different modes of catalysis are often incompatible. Methods have already been developed to overcome this incompatibility if it is present, one of which is reaction compartmentalization. Reagents can be immobilized in a number of ways, including attachment to polymer beads or surfaces within the reaction vessel.[41,42] Immobilization is particularly successful when used in flow reactors, where reactants are fixed in place and the substrate flows through each reactor in sequence. Immobilization is particularly suited to organic chemistry as it traditionally has a compartmentalized philosophy. This is one of the reasons why the development of cascades reactions is difficult, and where using biocatalysts provides a distinct advantage. This is because biocatalysts, unlike chemical catalysts make use of enzymes, this means that there is often a high degree of compatibility between the reactions, as they often have to perform reactions at similar temperatures, pressures and pHs.[43] Therefore the development of a series of chemical reactions that all occur under similar conditions to those used in biocatalysis would be advantageous and would allow for the development of a toolbox of chemical reactions that could be combined in one pot. This approach has been highlighted by Barry Sharpless and his Click chemistry approach.[44] By choosing reaction conditions that are the same as those required by enzymes a high degree of cross compatibility would be introduced and the possibility of a very flexible synthetic system put in place .

The combination of chemical and biological methods in synthesis is growing in popularity, but is still quite a limited field. One of the earliest reported examples of the use of biocatalysis were Pasteur's microbial resolution experiments using the fungus *Penicillium glaucum* on racemic tartaric acid in 1858.[45–47] The first reported example of the use of combined biocatalysis and organic synthesis was the 1934 Reichstein–Grussner synthesis of L-ascorbic acid (vitamin C; **14**; Scheme 1.10).[48] This is used as an antioxidant in the

food industry, as well as in many vitamin supplements. About 80,000 tonnes a year are produced and the industrial synthesis involves a combinations of enzymatic and chemical steps. The Reichstein–Grussner synthesis, upon which many current syntheses are still involves a single enzymatic dehydrogenation step, in which D-sorbitol (**15**) is converted into L-sorbose (**16**), amidst several chemical steps (Scheme 1.10). A more recent commercial synthesis of L-ascorbic acid involves a two-stage fermentation. The first step converting D-glucose to 2,5-diketo-D-gluconic acid, which is then further reduced to 2-keto-gluonic acid by a second enzyme. This can then be converted to L-ascorbic acid chemically.



Scheme 1.10: The Reichstein-Grussner synthesis of L-ascorbic acid (vitamin C)[48]
NAD^+=nicotinamide adenine dinucleotide; NADH=nicotinamide adenine dinucleotide hydride

Recent developments in recombinant DNA technology mean that all biocatalysts are

cheaper to develop and use, meaning that there is now widespread interest in creating new routes to lower value chemicals using biocatalysts, as well as renewed interest in biocatalysis in general.[1,49–52] As biocatalysis becomes more ubiquitous, it has expanded in several different directions, which include:[53]

- Application of multiple enzymes, sequentially or in parallel (either *in vivo* or *in vitro*) in synthesis
- Identification and characterization of new enzymes to match industrial (process) conditions and increase substrate repertoire
- Integration of enzymatic conversions with chemical catalysis and synthetic steps

The current major applications of biocatalysis are the performance of racemic resolutions and asymmetric synthesis. Both of these involve the creation of optically pure molecules, and so interest in these techniques in high in the pharmaceutical and agrochemical sectors.[54] The highly regio-, stereo- and enantioselective properties of enzymes, even on non-natural substrates, make them ideal for use by pharmaceutical companies. This is because the Food and Drug Administration (FDA) requires that any non-therapeutic isomer of a drug is shown to be non-toxic if it is to be present as part of the formulation, this has resulted in 35% of all pharmaceutical intermediates produced in 2000 being optically pure, with the number expected to rise to 70% by 2010.[8,55,56] Nevertheless, the target for the application of biocatalysis as part of commercial syntheses is very demanding, with a minimum requirement of product purity with a 98% enantiomeric excess (e.e.).[57,58] In order to meet this target a lot of, sometimes expensive, development work is often required. therefore, the step in the drug development process that is a preferential target for the incorporation of biocatalysis is the initial scale up process, when several kilograms of the drug

need to be synthesized for testing (Figure 1.3).[1]

At this stage of the drug development process it is important that the molecule is accessible at this scale for testing purposes. This means that if biocatalysis is to be used, there needs to be an efficient and effective set of procedures in place that process chemists could follow to optimize the reactions. This would ideally allow for several iterations of rapid development and screening with constant feedback into the system. One model for biocatalysis implementation during this early process development stage has been suggested by Hailes *et al*, and a modified version of this model is shown in Figure 1.4.[1] Many of the development steps in both the initial screening step and the catalyst improvement cycle could be automated once a suitable assay had been developed. This means that the most likely bottleneck in the process would be assay development. Once the procedures were in place and the process repeated for several different projects, it would be expected that a large enzyme library would be produced that could then be used in the development of other process syntheses. This would hopefully speed up the initial screening and improvement/evolution stages as many subtly different enzymes would be present.

This proposed sequence should allow a biocatalytic step to be developed at a similar rate to conventional chemical steps. This is very important if biocatalysis is to compete with established synthetic methods, as rapid initial development is necessary to generate large quantities of the lead compounds for clinical trials. During this development phase, it is common for there to be multiple routes to the target compound are being developed simultaneously, so if one were to hit a dead end there would be several back up syntheses already in place. This is, therefore, the optimal time for biocatalysis to be implemented as it would allow the process chemist to evaluate the biocatalytic steps within the overall

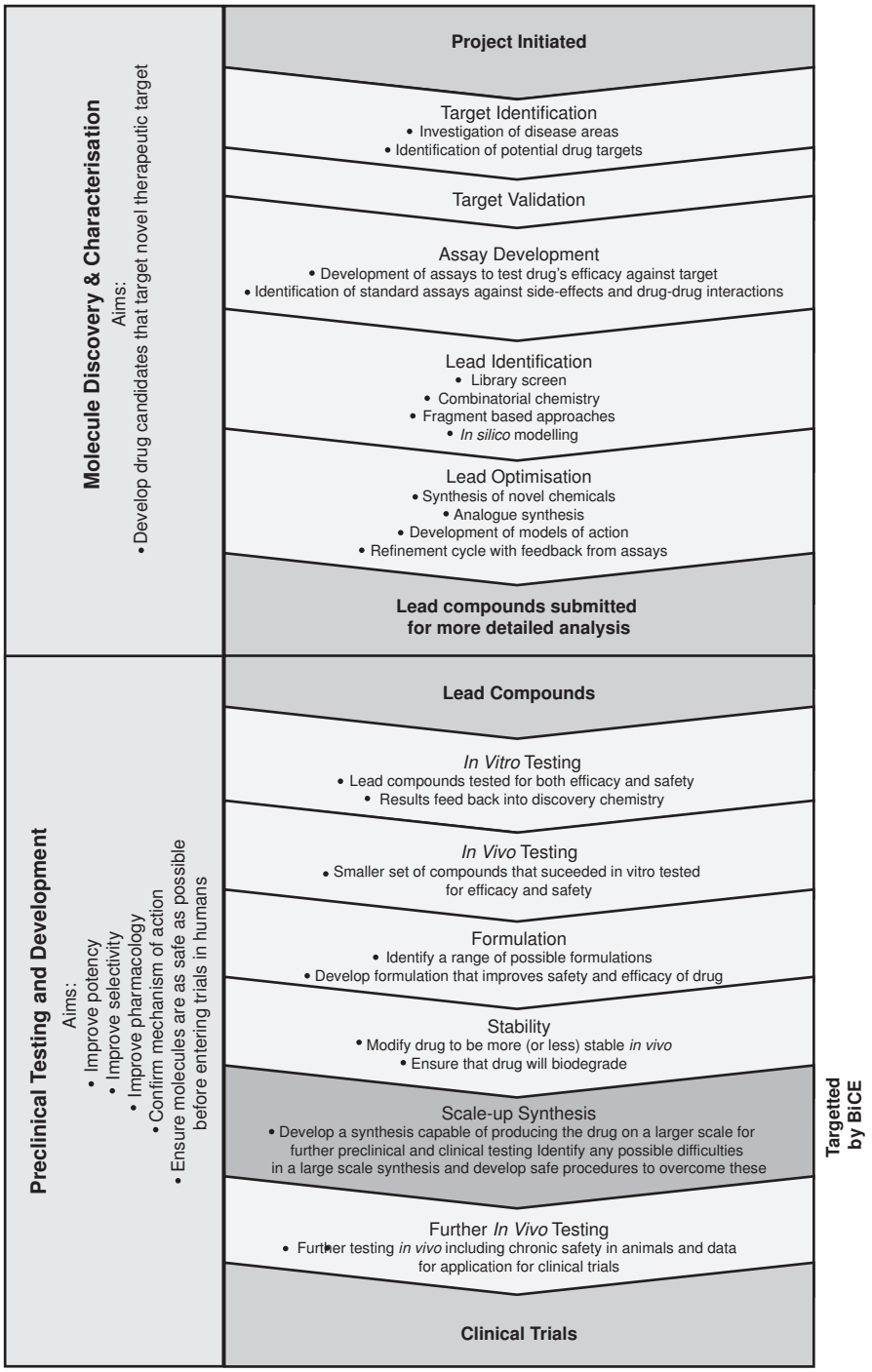


Figure 1.3: A scheme showing the drug development process, with the step being targeted as the point at which the proposed biocatalytic development cycle (Figure 1.4) would be implemented highlighted[1]

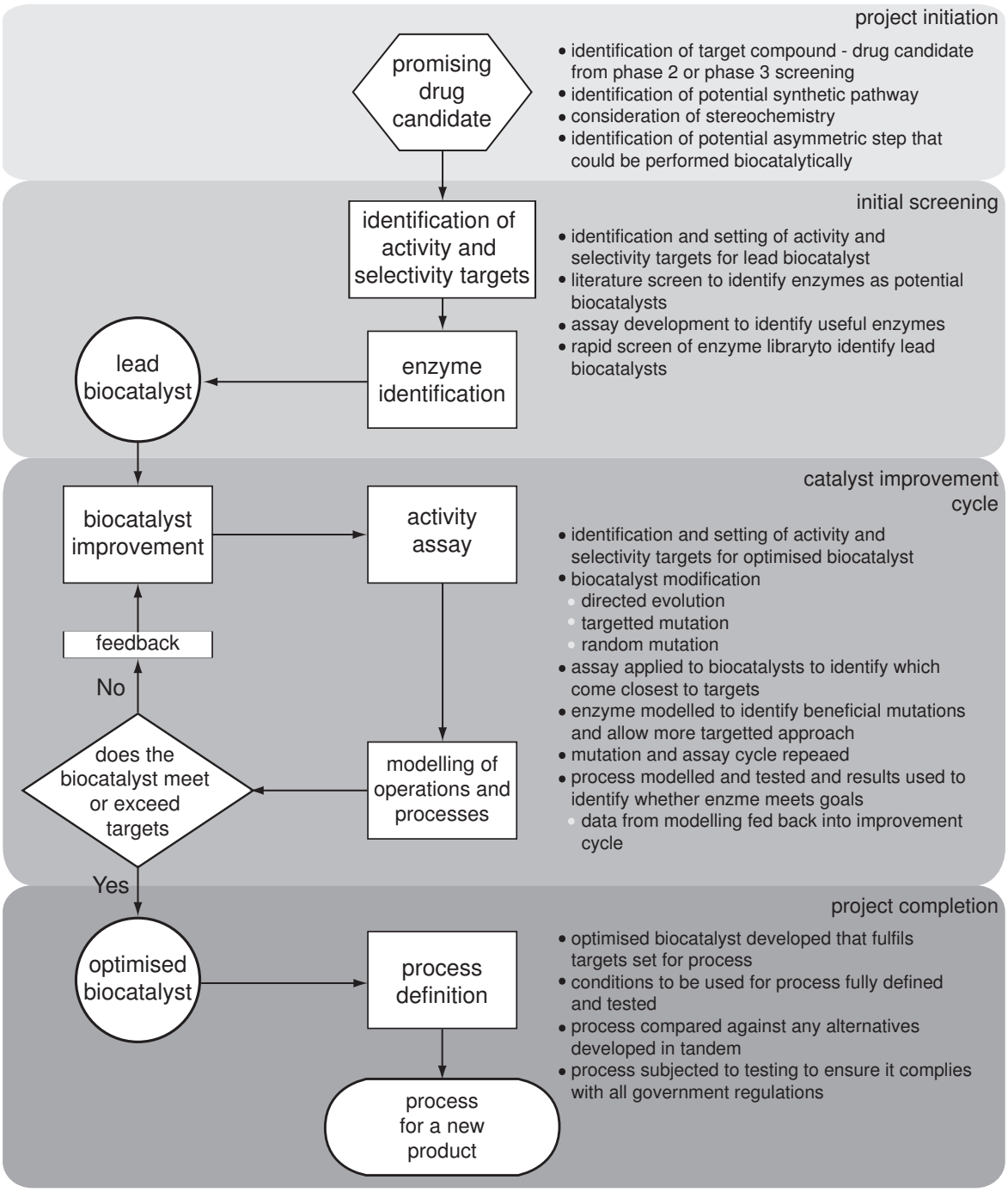
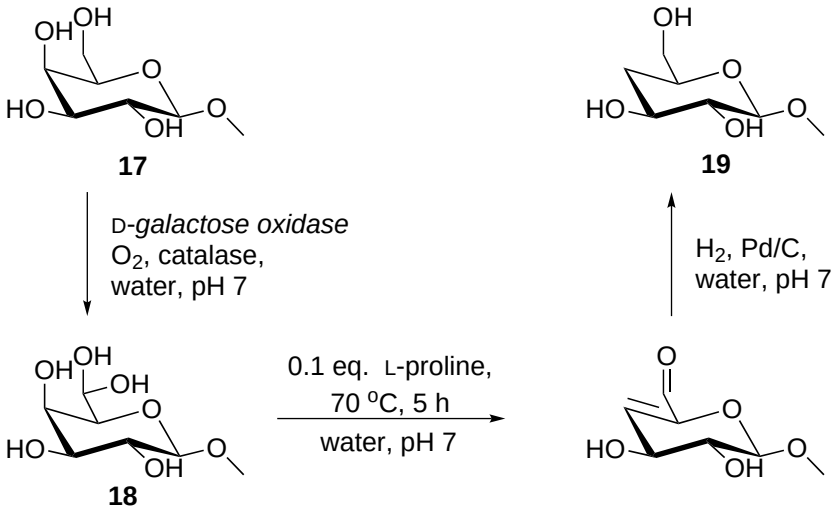


Figure 1.4: A suggested route for the effective development of a biocatalytic reaction in a process context[1]

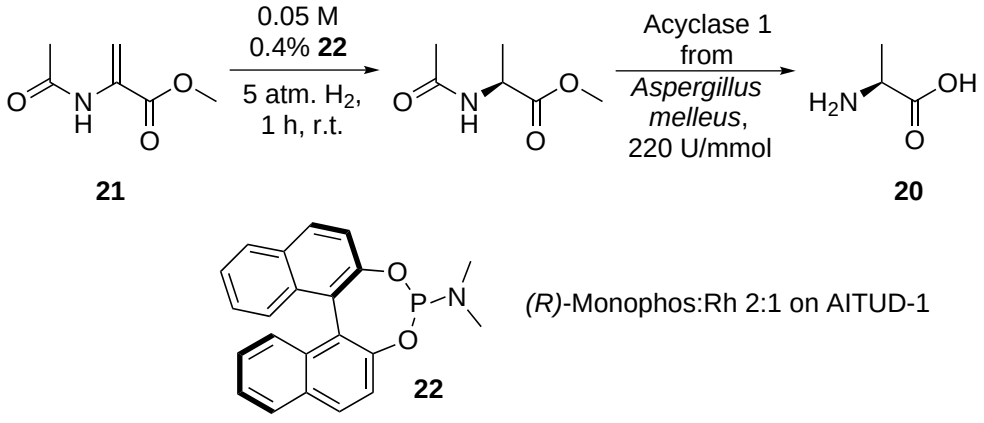
optimized synthetic route. The most important factors to consider when evaluating biocatalysis at this stage are whether the desired product is formed and if the required reaction concentrations are viable for neighbouring steps. However, initially, in the synthesis of a complex drug molecule only a few key steps that would be difficult to perform chemically would be considered for biocatalytic alternatives. Due to the highly selective and mild nature of biocatalysis it is considered best if this step can occur near the end of the synthesis, as this means that fewer transformations that might alter the e.e. of the material will be performed. Carrying out the biocatalysis late in the synthesis can cause problems, as any biological materials present (*e.g.* nucleic acids) would need to be completely removed from the reaction product. This is because they could participate in subsequent chemical steps leading to undesired side-products, as well as tainting the pharmaceutical.

There are two distinct ways that the reaction sequence can be manipulated to allow for the integration of neighbouring reactions. Firstly, the reactions can be separated physically, with an intermediate purification and clean up between each reaction step. Otherwise the neighbouring transformations can be altered to better match one another, thus avoiding purification and allowing for a one-pot operation.

Several examples of the use of both chemo- and biocatalysis integrated in a single synthesis have been reported. One example is the three-step catalytic cascade shown in Scheme 1.11.[59] In the first step *D-galactose oxidase* catalyses the selective oxidation of the primary hydroxyl group of the methyl ether of galactose (**17**) to the corresponding hydrated aldehyde (**18**). This is then followed by an L-proline catalysed elimination of water and sunsequent catalytic hydrogenation, affording the deoxy sugar (**19**).

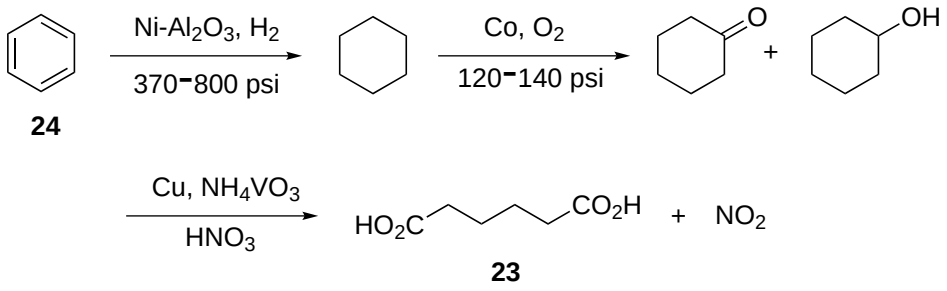


Scheme 1.11: The construction of the methyl ether of a galactose deoxy sugar (**19**), using a three step catalytic cascade[59]



Scheme 1.12: Chemoenzymatic amino acid synthesis[60,61]

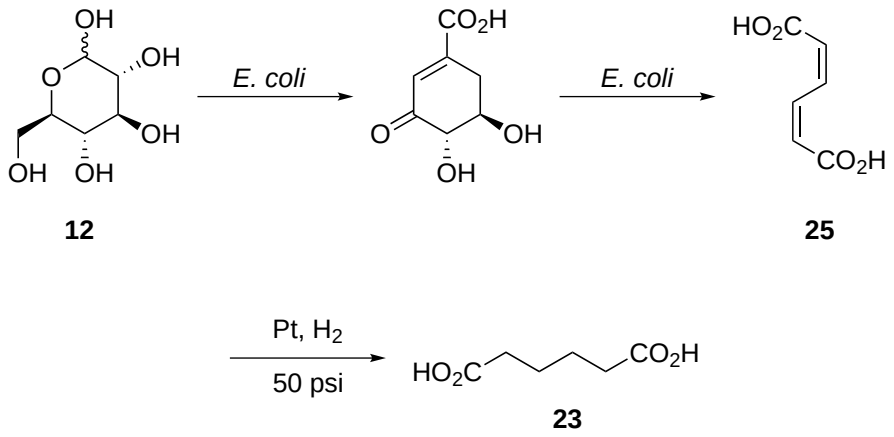
Another example of a synthesis involving both chemo- and biocatalysis in adjacent steps is shown in Scheme 1.12. The first step of the synthesis is an asymmetric hydrogenation performed with a solid-supported chiral rhodium catalyst (supported on AITUD-1, which is a mesoporous aluminosilicate). This is followed by an enzymatic hydrolysis of the product of the hydrogenation reaction, using Acylase 1 from *Aspergillus melleus*, in a one-pot cascade process in water (Scheme 1.12).[60,61]



Scheme 1.13: Original industrial synthesis of adipic acid[12]

This method of performing synthesis with combined chemo- and biocatalysis is not confoned to academic chemistry and a good example of its use in industry is the redesign of the synthesis of adipic acid (**23**).[49] Industrially this is used on a very large scale, primarily for the production of Nylon 6,6. The traditional synthesis of this commodity chemical (Scheme 1.13) starts with benzene (**24**), a known carcinogen and highly regulated compound. This is first reduced and then oxidized at extremely high pressures, due to the unfavourable nature of the reactions. This means that the synthesis has high energy costs as well as requiring a lot of health and safety regulation. The large scale synthesis using this process also results in the production of large quantities of nitrogen dioxide which are normally vented into the atmosphere and contribute as much as 2% of the total amount of nitrogen dioxide released into the atmosphere by man every year.[49] It is therefore unlikely

that this process will be allowed to be used indefinitely, as tighter environmental laws will inevitably be introduced.



Scheme 1.14: Draths' and Frost's modified synthesis of adipic acid[49]

Draths and Frost developed a synthesis of adipic acid, that used enzymatic transformations for the majority of steps,by manipulating the shikimic acid pathway in *Escherichia coli* (Scheme 1.14).[49] This synthesis starts from D-glucose (**12**), a renewable feedstock, which is converted by the *E. coli* into *cis,cis*-muconic acid (**25**). This material can then be reduced using platinum and hydrogen at 50 psi to give adipic acid (**23**).[49] This is a significantly lower pressure than was needed in the original synthesis where extremely forcing conditions were needed, first to dearomatize the benzene and then to perform the unfavourable oxidation of the cyclohexane, and its conversion to adipic acid. This means that as well as using raw materials from a renewable source, all of the steps are catalytic and a much lower energy cost is incurred during the process.

There are several crucial points that have to be considered if biocatalysts are to be used in industrial processes. Despite being very effective on their natural substrates, on

non-natural substrates the reaction rate can decrease severely. In industry the majority of reactions will be carried out on non-natural substrates, which can mean that in a cascade reaction the biocatalytic step has a much lower rate than neighbouring chemical steps, which can lead to a bottleneck with reagents accumulating in excess. This can cause problems by causing unfavourable reaction equilibria for the earlier reactions, as well as potentially increasing the likelihood of undesired side reactions. Secondly the stability of the enzyme has to be considered. Even though enzymes have been evolved in labs to withstand operational (process) conditions, a high concentration of reagents from chemical steps, whether active in the reaction or not, can often damage the enzyme. Finally the reaction medium must be considered. Enzymes usually operate in water, but have also been developed to work in organic and biphasic (organic–aqueous) media, as well as more novel media like supercritical fluids and ionic liquids.[1,24,50–52] Chemistry conversely has traditionally been performed in organic solvents, but recently there has been a surge in the development of reactions in alternative reaction media, including water.[62] This has increased the likelihood of finding compatible reactions, and has also highlighted the possibility of developing neighbouring reactions that have very similar conditions.

A good starting point for developing integrated chemo-biocatalytic reaction sequences is to consider whether the chemical reaction can be carried out in the same medium as the enzyme. This will typically be water and so the properties of the reacting substrates must also be taken into consideration. If the reagents or intermediates can react with water, or a potentially useful catalyst is water-sensitive, the approach could fail. An assessment must also be made to establish whether the chemical substrate might poison the biocatalyst, and whether the concentrations and other reaction conditions required by the biocatalyst will be compatible with the surrounding chemical reactions. All of these factors will dictate

whether the reaction can feasibly be carried out in a one-pot fashion, or whether the reactions will have to be performed sequentially.[53] If the chemical reactions are found to be incompatible with the biocatalyst, and a one-pot reaction is still desired, then there are three possibilities for further development. Either the chemistry or the biocatalyst could be modified, or both. This is because the use of integrated chemical and enzymatic transformations will place a much greater burden on the compatibility requirements of the enzyme, as a much broader range of chemical environments have to be anticipated.

With the current state of the art, it is cheaper to modify the chemistry for most reaction sequences, as this is still where there is a lot more expertise. At this stage the reaction parameters (*e.g.* substrate concentration) must be established as this will impact on all further development. The other major decision that will have to be made at this stage is at what point in the reaction sequence chirality is to be introduced into the molecule. If the key steps involve a chemical conversion followed by an enantioselective biocatalytic conversion (Figure 1.5), should the process be developed so that the chemistry is optimized for enantioselectivity or should the enzyme step be modified to accept only one isomer?[53] The latter option is less atom-efficient, but would be faster to develop, so if speed is a necessity then a compromise will have to be reached. If a chemical step is to follow a biocatalytic step then the biocatalytic step will usually be enantioselective to some degree, but the chemical step might not be. In this case it is often preferential to develop an enantioselective chemical reaction, but once more if speed is important then it would be possible to go to the racemate and resolve it in some way. This might be through a further enzymatic step using kinetic resolution or a more traditional technique.

The other integration strategy that is available is the modification of the biocatalyst so

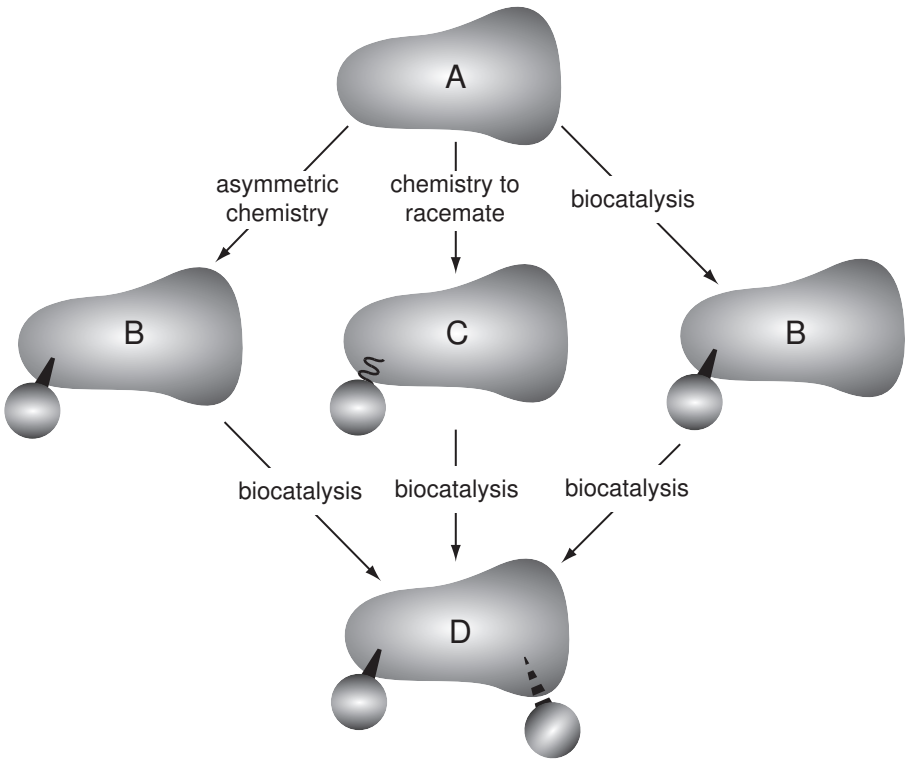


Figure 1.5: Alternative chemo-enzymatic routes to an optically pure product, containing two chiral centres[53]

A=starting material; B=intermediate with one defined chiral centre; C=racemic intermediate; D=product with two well defined chiral centres; Adapted from Hailes HC, Dalby PA, Woodley JM. *Journal Of Chemical Technology and Biotechnology* 2007; **82**(12): 1063–1066. By permission of John Wiley and Sons

that the conditions it requires better match those of neighbouring steps. This is often more difficult and time consuming than altering the chemistry, due to the need to develop assays to monitor the properties of the evolved enzymes, as well the evolution of the enzymes which can be difficult to control. If this is to happen then there are two important considerations for the biocatalyst. If possible its rate must be at least that of the chemical reaction, and the enzyme must be tolerant of the conditions of the chemical reaction. There have been three types of improvement reported that help the integration of enzymes with chemical reactions. Firstly, the enzyme can be engineered against product inhibition so it can produce product at a higher concentration.[63] Secondly improved organic solvent tolerance can be developed, this is useful because many compounds of industrial interest have minimal solubility in water, and so in order to work the enzyme has to be able to be used in the presence of organic solvent. This can be achieved by solubilizing the enzyme, which can be done by modifying it with polyethylene glycol, fatty acids or surfactants, however, this is not always successful and most enzymes will still be inactivated by high concentrations of organic solvents. Finally the enzymes can be improved by making them more tolerant of reactive groups on substrates (*e.g.* aldehydes) or reactive side products (*e.g.* H_2O_2) that can potentially react with and inhibit the enzyme's activity. All three improvements can be brought about by directed evolution, however, the desired reaction conditions need to be known in advance, so that the enzymes can be assayed against them.[53]

The overall goal of these improvements is to obtain a set of near perfect reactions that occur under very similar conditions, which include reactions performed with both chemo- and biocatalysis. With this in mind the Biocatalysis-Chemistry-Engineering Interface (BiCE) programme was set up with the aim of developing a process that would enable the rapid expansion of the range of substrates that could be used with the transketo-

lase enzyme. This was coupled with the secondary goal of producing a cascade of reactions to enable the synthesis of the amino-diol synthon, which is present in many biologically interesting compounds, including sphingosines and the antibiotic triamphenicol (Figure 1.6).[1]

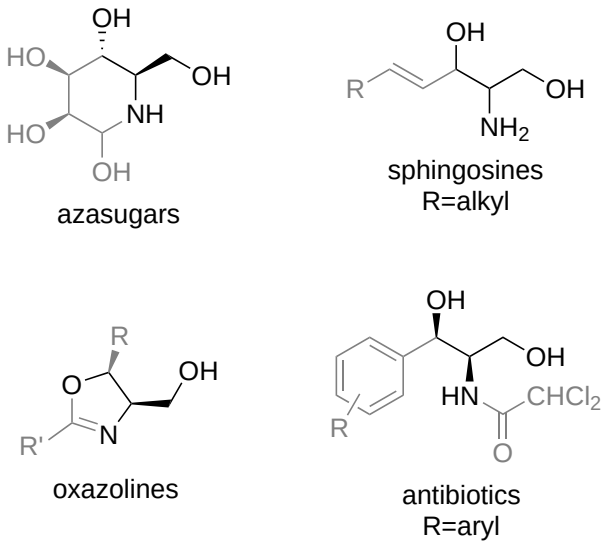
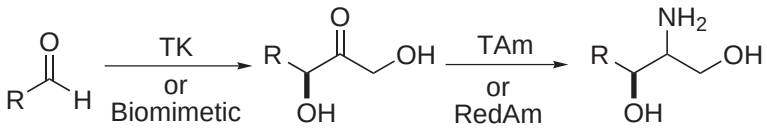


Figure 1.6: Examples of compounds containing the amino-diol synthon[1]

Several different possible methods to carry out this cascade were investigated. It could be performed as a linked two enzyme system; a linked enzyme and chemical route; or a pair of linked chemical reactions (Scheme 1.15).

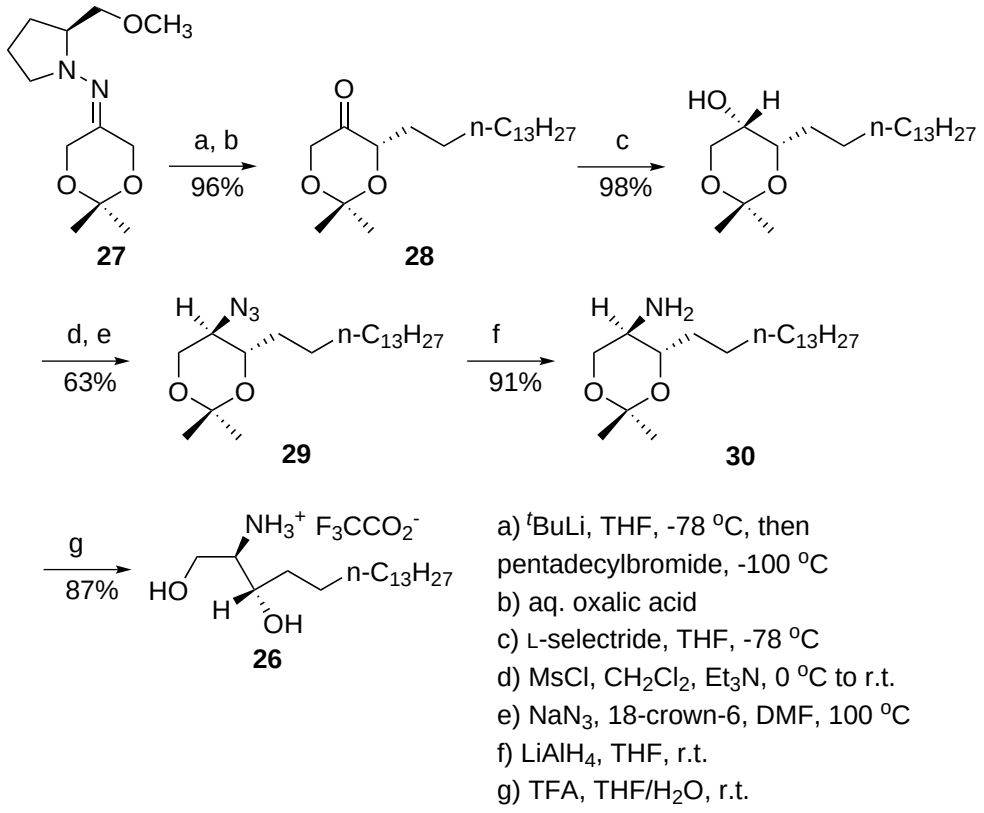


Scheme 1.15: Reaction pathway to the amino-diol synthon
TK=transketolase; TAm=transaminase; RedAm=reductive amination; Biomimietic=biomimetic chemical transformation

2-Amino-1,3-diols are not straightforward to synthesize in an enantiospecific manner

using traditional chemical methods. The methods reported in the literature involve multiple steps and the use of a chiral starting material, chiral auxiliary or chiral chemocatalyst to impose the correct stereochemistry on the final product. One example of this is the synthesis of a series of 2-amino-1,3-diols and D-*erythro*-sphinganine (**26**) by Enders and Muller-Huwen (Scheme 1.16).[64] The strategy gives the amino-diol, **26**, in very good diastereomeric and enantiomeric excess, in a 47% overall yield over seven steps. The starting hydrazone, **27**, is made in three steps with an overall yield of 49% and incorporates a chiral auxiliary, that has to be synthesized from a chiral pool starting material.[65] The hydrazone, **27**, is initially deprotonated and coupled to 1-bromopentadecane before the chiral auxiliary is selectively removed using oxalic acid to give the ketone, **28**. This is then reduced and an azide functional group introduced using sodium azide to give **29**. The azide, **29**, is in turn reduced to give the amine, **30**, before the diol functional group is finally deprotected using trifluoroacetic acid (TFA) to complete the synthesis of the amino-diol, **26**. If the synthesis of the chiral auxiliary is not included, the synthesis of amino-diol, **26**, gives an overall yield of 23% over 10 sequential steps.

The synthesis has a high reagent cost, as well as taking a large number of man hours. This combined with the air- and water-sensitive nature of some of the reagents make the synthesis unattractive from an industrial perspective. There is also limited scope when existing methods are used, as it is very hard to incorporate an aromatic ring adjacent to one of the hydroxyl groups. If these products could be made enantioselectively in two steps with high yield, then this would help open up an area of the chiral pool that is currently largely inaccessible on a large scale.



Scheme 1.16: Synthesis of D-*erythro*-sphinganine by Enders and Muller-Huwen[64]
TFA=trifluoroacetic acid

2

Chemistry in Water

The role solvents play in organic synthesis is very important but often overlooked.[66,67] The effect of the solvent is often considered to be purely physical, helping solubilize reactants but playing no active role in the reaction. There are, however, occasions when the solvent participates in the reaction, for example by hydrogen bonding interactions. When this occurs, the reaction mechanism, rate or even selectivity can be altered, as certain intermediates are either stabilized or activated. Chemistry has traditionally been performed in organic solvents which usually do not participate in the reaction and are present solely to homogenize it. Nevertheless, the role the solvent plays in the reaction has come to the forefront of organic chemistry, with the emergence of green chemistry as a priority. This has led to a move away from volatile organic compounds (VOCs) and the development and reevaluation of alternative reaction solvents.[68] These include ionic liquids, supercritical fluids, fluorous solvents, water and inexpensive natural products (*e.g.* ethanol).[69–71]

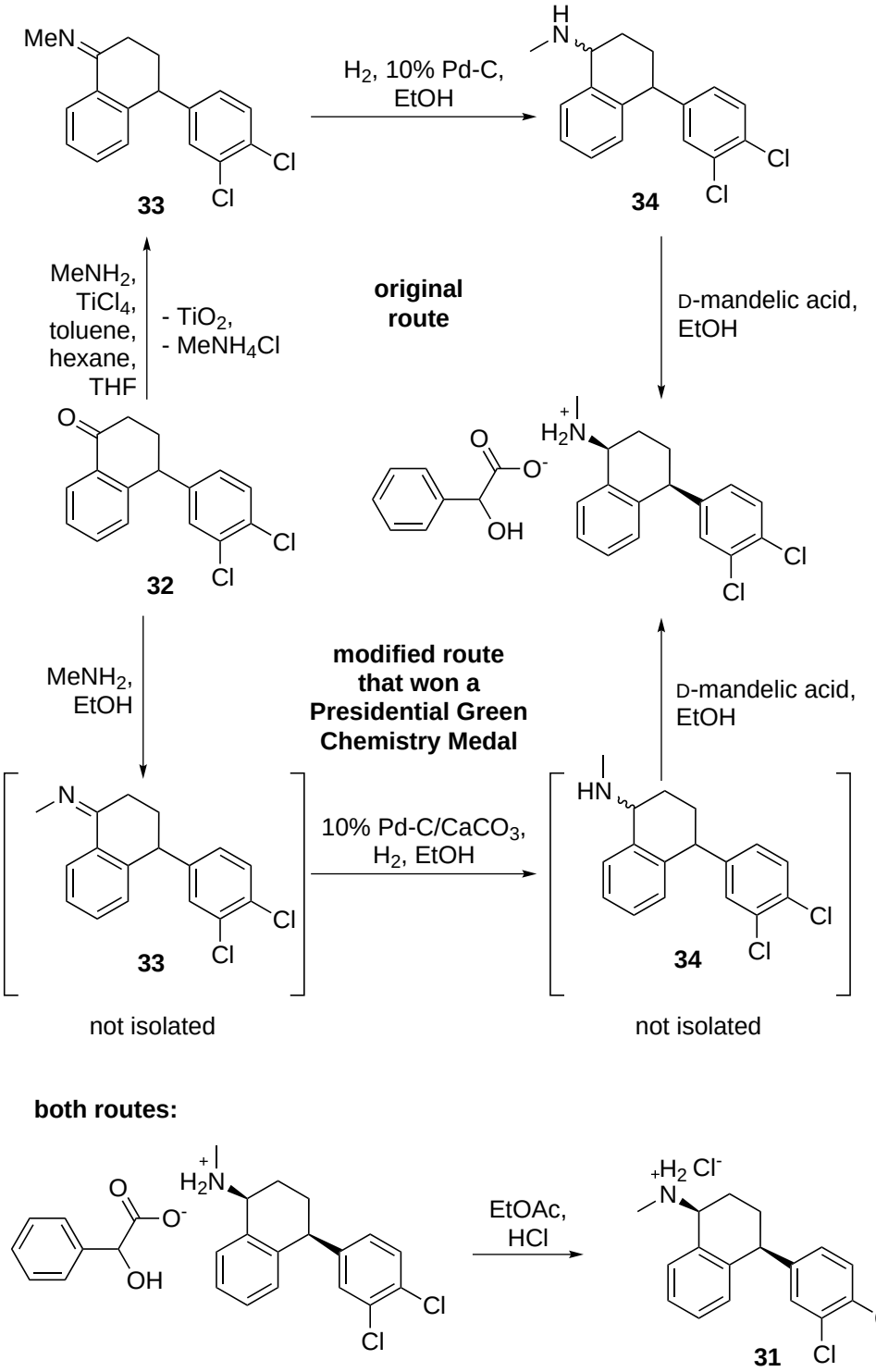
This re-evaluation of solvent use is important, as solvents make up the majority of

chemicals (80–90%) used by the pharmaceutical and fine chemical industries, and current recovery efficiencies are typically between 50 and 80%.[72,73] Industry has already started to redesign syntheses to reduce the amount of solvent used. An excellent example of this is the redesign of the sertraline (**31**) manufacturing process (Scheme 2.1), for which Pfizer received a Presidential Green Chemistry Challenge Award in 2002.[74,75] Amongst other improvements, a three step sequence from the ketone **32** involving an indirect reductive amination, fractional crystallization and resolution were streamlined to give a one-pot reaction sequence using ethanol as the only solvent, thus avoiding the isolation and purification of reaction intermediates (**33** and **34**). This eliminated the need to use, distil and recover four solvents (methylene chloride, tetrahydrofuran [THF], toluene and hexane) employed in the original process.

Water is a well established solvent for chemistry, its use being reported as far back as 1931 for Diels–Alder reactions.[76] Nevertheless, even though its use has attracted more research, applications and concepts, it has not attracted the attention of many of the other new solvents being developed for green chemistry. This may be because it is established, and therefore not seen as ground breaking; or because intellectual property surrounding it is harder to control; or it might be that chemists have been trained to think that water is often detrimental to reactions and so non-aqueous solvents are best.

2.1 Why use water as a solvent?

Within the framework of green chemistry there are several issues that influence the choice of solvent. It should be relatively non-toxic and non-hazardous (*e.g.* not inflammable or



Scheme 2.1: A section of the original and modified industrial syntheses of sertraline[75]

corrosive) and should also be easily contained (*i.e.* not released into the environment). This is often difficult, as the removal of residual solvent is usually achieved by evaporation or distillation, and so the most commonly used solvents are volatile. Spillage and evaporation inevitably lead to atmospheric pollution, a major environmental issue of global proportions, and worker exposure to toxic and hazardous VOCs, which is a serious health issue. As a result of this, solvent use is being subjected to close scrutiny and increasingly stringent environmental legislation (*e.g.* the FDA guidelines).[77] As well as this, many chlorinated hydrocarbon solvents have already been banned or are likely to be banned in the near future.[39]

Volatile organic compounds are not the only solvents that have problems associated with their use. The polar aprotic solvents (*e.g.* dimethylformamide [DMF], dimethyl sulfoxide [DMSO]), the solvents of choice for many nucleophilic substitutions, have high boiling points and so are not easily removed by distillation. Instead they are separated by washing with water, with which they are miscible, and this leads to the formation of contaminated aqueous waste. Therefore the problem with many solvents is not in their use, but in the inefficiencies associated with their containment, recovery and reuse.

Often it is said that water is an environmentally friendly and safe solvent, and that the problems that arise from the use of organic solvents can be avoided by using it. Water makes a good choice for a solvent as it does not need to be synthesized and a delivery infrastructure is already in place, which cuts down the environmental footprint of its use as a solvent. Water is also non-toxic, non-flammable, abundantly available and inexpensive, all of which are important factors in solvent choice. The advantages and disadvantages of water as a solvent are shown in Table 2.1.[68]

Advantages	Disadvantages
• Dissolves at least small quantities of many compounds	• Generally poor for non-polar substrates
• May be separated from most organics	• Purification may be energy demanding
• Non-toxic, non-flammable and safe to handle	• Decomposition of water-sensitive compounds
• Very low cost	• Energy costs high
• Sustainable and safe to the environment	• May need purification
• Non-flammable	• Large heat of evaporation
• Non-toxic	• Difficult to detect in case of leakage
• Odourless	• Hard to collect in case of spills
• Good separation with many organic compounds	• No incineration or bleed streams
• Inexpensive	
• Unique fluid properties	
• Stabilization of certain organometallic complexes	

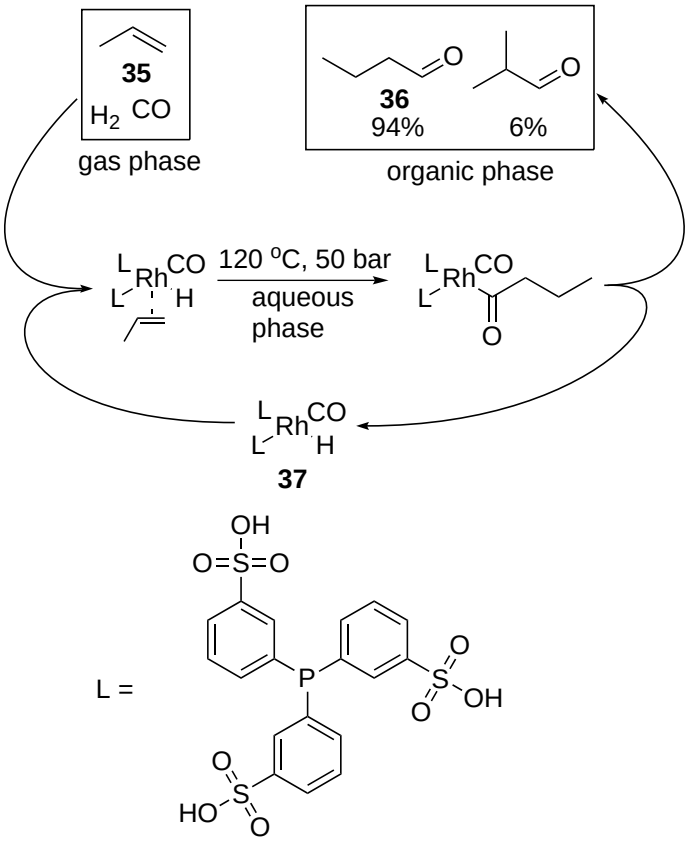
Table 2.1: The advantages and disadvantages of water as a solvent[68]

A lot of these advantages only hold true for pure water and it is when reactions are carried out in, or in the presence of, water, that complications arise. This is because there is then a waste-water stream contaminated by organic compounds which can be difficult and costly to dispose of. Strict regulations govern how waste-water streams from chemical plants may be released into the environment, including the Water Framework Directive, the most substantial piece of water legislation produced by the European Commission.[78] As a result of this, water is only a truly green solvent if it can be discharged directly into a biological effluent treatment plant (BETP).[79] The problem of removing all traces of organic compounds from the water is often made much worse at the end of the reaction when further organics are used in product work up protocols. Often the volume of organic solvent used in the work up exceeds the total volume of water used in the reaction by factors of up

to 30-fold. The aqueous waste must be stripped under vacuum, incinerated or treated with activated charcoal which itself must then be incinerated. As a result of this, in many cases a comparative analysis will conclude that an organic solvent based process is cheaper and ultimately more environmentally sound than a water-based organic reaction.[79]

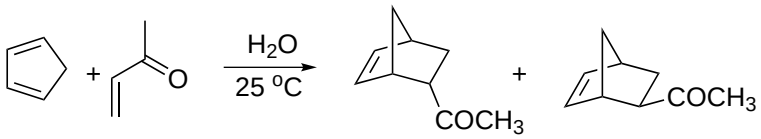
This analysis of solvents in terms of atom economy and green chemistry has led to the idea that 'the best solvent is no solvent'. Nevertheless, if a solvent is needed then water has a lot to recommend it. Owing to its highly polar character, one can expect novel reactions and selectivities, in particular for organic catalysis in water. The most important feature of water is often thought to be its three-dimensional hydrogen-bond network, in particular its hydrophobic interactions. These originate from the tendency of water molecules to find the most favourable arrangement of hydrogen bonds around the solute. This will apply to the reactants, products and the activated complex or intermediate, as the hydrogen bond network is highly dynamic, with water molecule reorientation times of about 2 picoseconds and times to move one molecular distance of about 7 picoseconds.[80] The polarity, acidity and entropy of water also have important roles to play in the ultimate influence that water exerts on organic reactions. The majority of biological processes are conducted in water, and so the chemistry of living organisms is dependent upon this combination of crucial properties. The varied behaviours that these properties can impart make water an excellent candidate as a solvent, or co-solvent, even before environmental benefits are considered.[79] Nevertheless, even though some reactions are accelerated when performed in an aqueous environment, others are inhibited.[62] As well as this, many functional groups in organic molecules may react with water and as organic molecules are generally non-polar, they may be hydrophobic and insoluble in water.[81]

Water also allows for the recovery and recycling of catalysts by phase separation.[82,83] These aqueous biphasic systems are an attractive method that have found broad application in industry. An example of this is the Ruhrchemie/Rhone–Poulenc process for the hydroformylation of propylene (**35**) to *n*-butanal (**36**) which employs a water soluble rhodium(I) complex of trisulfonated triphenylphosphine (tris(3-sulfophenyl)phosphine, tppts; **37**) as the catalyst (Scheme 2.2). This process has annual production rates of about 800,000 tonnes/year.[84,85]



Scheme 2.2: The Ruhrchemie/Rhone–Poulenc process[84,85]
L=tppts

2.2 Reactions in water



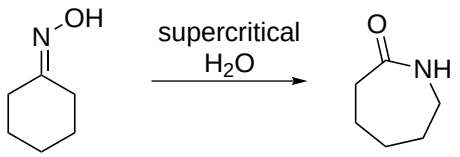
38 **39** **40**

Scheme 2.3: An example of a Diels–Alder reaction carried out in water[86]

Before the 1980s, water was an unlikely choice of reaction solvent, however, in 1980 Breslow and co-workers reported that Diels–Alder reactions could be greatly accelerated by carrying them out in water.[86,87] It was observed that the Diels–Alder reaction of cyclopentadiene (**38**) with butenone (**39**) in water to give **40** (Scheme 2.3) was more than 700-times faster than the same reaction in isooctane.[86] The Diels–Alder reaction had always been considered to be weakly sensitive to solvent effects, because of the small changes in charge observed during the activation process. Indeed it was these small solvent effects that were used to support the hypothesis of the concerted nature of Diels–Alder reactions. This meant that aspects of the aqueous medium would have to be invoked to explain the large rate enhancements seen in water. It is now generally accepted that enforced hydrophobic interactions, and hydrogen bond stabilization of the activated complex are responsible for the aqueous rate acceleration.[88]

Once it had been seen that water could provide enhanced reactivity, many new reactions performed in an aqueous environment were developed, some even involving water sensitive compounds.[89] Often the change in reactivity was attributed to hydrophobic effects, where the reactants have low solubility in water and so rates and selectivities are enhanced. This idea is key to 'on water' chemistry, which is discussed later in the chapter. Another influ-

ence on the reactivity is the low solubility of oxygen in water, which can allow air-sensitive transition-metal chemistry to be carried out without the need for an inert atmosphere. The use of water as a solvent can also allow the elimination of, sometimes harsh, protection and deprotection steps, as polar water-soluble compounds can be used without the need for derivatization.[85] Also in the case of water soluble homogenous catalysts, the catalyst does not have to be separated from the reactants and products if an aqueous biphasic solvent mixture is used. This allows for easy catalyst recovery and recycling, with the water acting as a supporting fluid rather than a true solvent. Finally, another exciting aspect of water as a reaction medium is that it exists in different states with very different properties. These have been used to good effect with reactions carried out in ice and supercritical water.[90–92] For example the Beckmann rearrangement of cyclohexanone oxime (**41**) into ϵ-caprolactam (**42**) occurs readily in supercritical water.[92]



41 **42**

Scheme 2.4: The Beckmann rearrangement of cyclohexanone oxime into ϵ-caprolactam in supercritical water[92]

2.2.1 'On water' chemistry

Breslow *et al* showed that for the Diels–Alder reaction, vigorous stirring could increase the rate of reaction in suspension.[93,94] These reactions also preferentially gave the *endo* product in excess of 95%, the selectivity increasing as increasing amounts of insoluble cyclopentadiene were used. The substrates were not fully soluble in water and in discussing

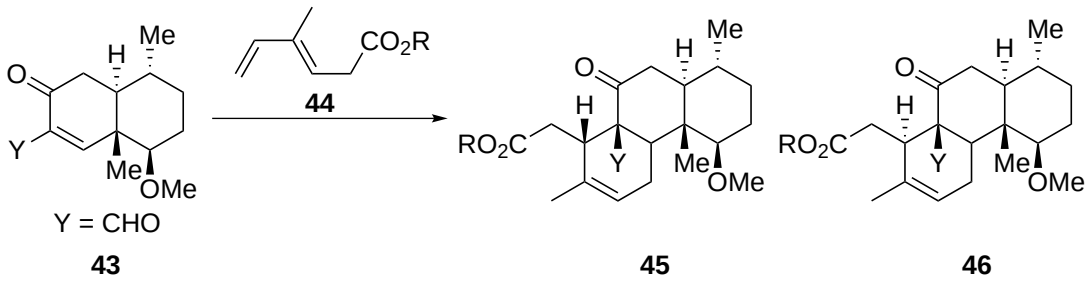
R	Solvent	Diene conc. (M)	Time (h)	Yield (%)	endo (45)/exo (46)
Et	benzene	1	288	52	0.9
Et	-	-	144	69	1.3
Et	H_2O	1	168	82	1.3
Na	H_2O	1	8	83	2.0
Na	H_2O	2	5	100	3.0

Table 2.2: Some of the results from Grieco's research into the aqueous Diels–Alder reaction[95]

these results Breslow said:

> "... these observations made the use of water in suspensions more practical than requiring true solutions of insoluble compounds"[93]

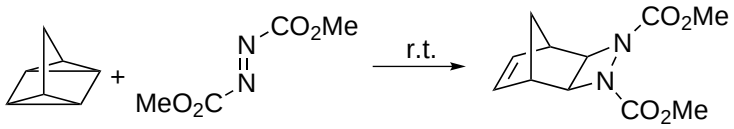
Another important result from Diels–Alder studies came from Grieco *et al* when they were investigating the synthesis of the quassinoid family of natural products.[95] A dramatic solvent effect was observed on the rate and *endo* selectivity of the cycloaddition of enone, **43**, to diene, **44**, to give diastereoisomers **45** and **46**. The best results were obtained when the dienophile and sodium salt of the diene carboxylic acid were combined in aqueous suspension at room temperature (Scheme 2.5 and Table 2.2).



Scheme 2.5: Grieco's study on the aqueous Diels–Alder reaction[95]

These and other earlier reactions interested Sharpless and led to his investigations into an 'on water' approach to chemistry.[96–100] In this, uni- and bi-molecular reactions are

greatly accelerated when carried out in vigorously stirred aqueous suspension. The reactants initially float on the surface of the water, and the product is often produced in a pure state either under or on the water, thus allowing for it to be easily isolated by phase separation or filtration. One of the first examples was the $2\sigma + 2\sigma + 2\pi$ cycloaddition of quadricyclane (**47**) to dimethyl azodicarboxylate (**48**) to yield 1,2-diazetidine (**49**) as the single product (Scheme 2.6). The reaction 'on water' takes only 10 minutes at 23 °C, compared to 48 hours for the neat reaction, 18 hours for the reaction in methanol and more than 120 hours for the same reaction in toluene, all at ambient temperature.



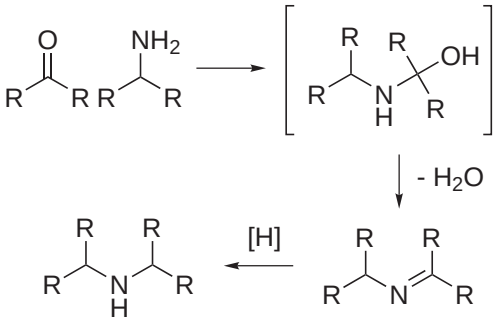
47 **48** **49**

Scheme 2.6: $2\sigma + 2\sigma + 2\pi$ cycloaddition of quadricyclane, **47**, to azodicaboxylate, **48**, in water[100]

2.3 Reductive amination

Reductive amination involves the conversion of a carbonyl group, typically an aldehyde or ketone, to an amine *via* the imine.[101] It is also referred to as reductive alkylation of the amine. It is a very important reaction as it provides access to amines, which are found throughout nature, as well as in medicine.[102–104] It involves the initial formation of the carbinol amine, which subsequently dehydrates to give the imine. This imine is then reduced to the desired amine as shown in Scheme 2.7.

Reductive amination can be performed in two distinct ways, either directly or indirectly,



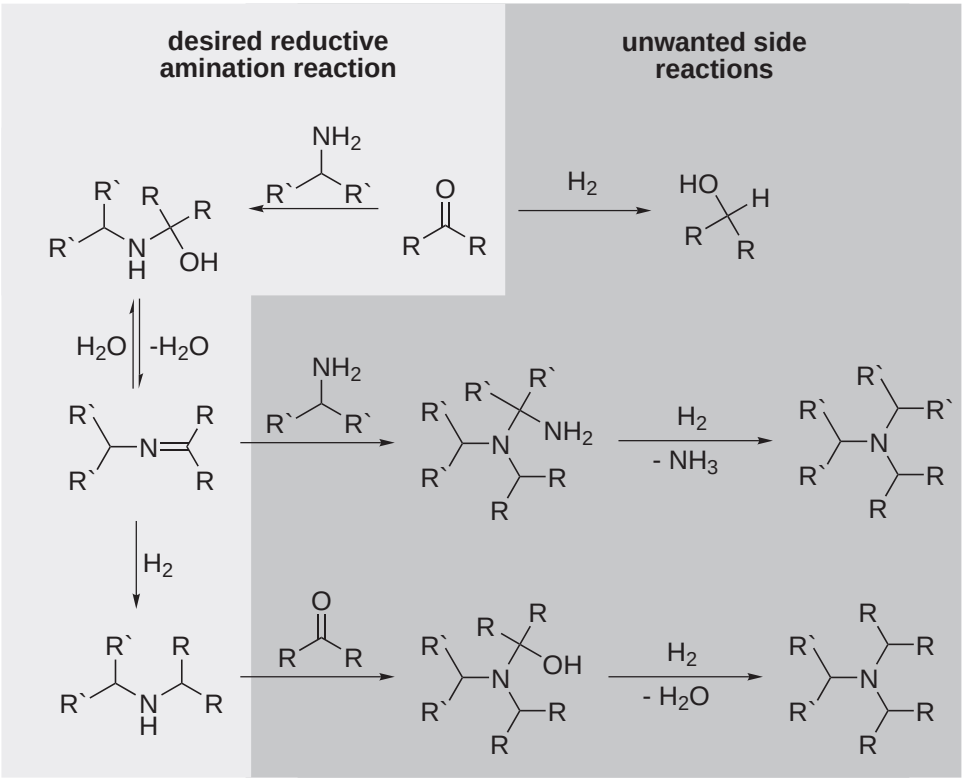
Scheme 2.7: Reductive amination

with the choice of which to use usually dependent upon the stability of the imine.

2.3.1 Indirect reductive amination

During indirect reductive amination, the reaction is performed as two distinct steps, with the isolation of the intermediate imine.[105,106] This provides the advantages that you can get higher yields and a purer product. This is because there are many possible side reactions to reductive amination, as shown in Scheme 2.8.[107]

The most common side reaction is the multiple addition of carbonyl to amine. These additions are very difficult to prevent, with both primary and secondary amines able to form tertiary amines. Thus by isolating the imine, which cannot react further, the product is more likely to be of a higher purity. However, it is not always possible to carry out reductive aminations indirectly as the imine can be unstable. As well as this the additional purification step will add to the time, cost and environmental impact of the reaction.



Scheme 2.8: Reductive amination and its possible side reactions[107]

2.3.2 Direct reductive amination

This involves the reduction of an imine that is formed *in situ*, making this a one-pot reaction.[105] As the imine is not isolated, this reaction is ideal for use when the imine is unstable. However, as all the reactants are present together, the reaction can undergo all of the side reactions shown in Scheme 2.8. The side reactions that result in multiple additions can be minimized by using a low equivalence of the amine and possibly slow addition of the reactant amine over time.

Reductive amination can be considered as comprising two separate reactions, the imine formation and the reduction. This allows them to be treated as two independent problems to be tackled.

2.3.3 Imine formation

This involves the addition of the amine to the carbonyl compound, and the subsequent dehydration of the intermediate. Imine formation is affected by the basicity and steric hindrance of the amine used, with amines whose conjugate acids have higher pK_as typically giving better imine formation, which is, however, tempered by the steric hindrance of the molecule which can reduce reduction selectivity.[108] The groups surrounding the carbonyl group also have a major impact on its reactivity. This means that some reductive amination techniques will only work if specific functionality (*e.g.* an aldehyde) is present. The reductive amination of aldehydes is limited by the steric and electronic factors of both the aldehyde and the amine. Formaldehyde and aliphatic aldehydes are particularly reactive and will form dialkylated products with primary amines. Ketones typically do not bisalkylate, and aliphatic

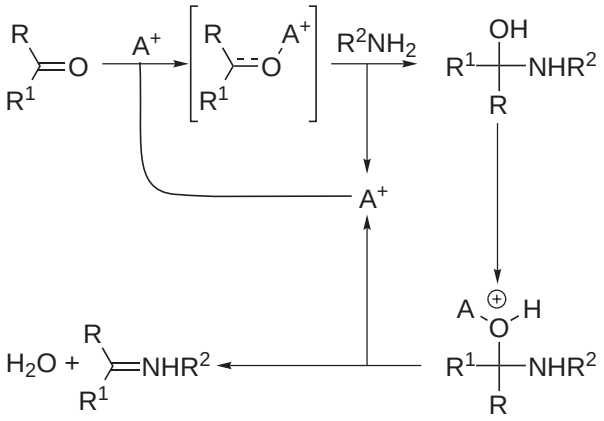
and cyclic ketones are generally excellent substrates for reductive amination. As well as this, α-keto acids can be utilized to provide α-amino acids.

Several methods have been employed to drive the imine formation to completion by removing water from the reaction. The most common approach is to heat the reaction at reflux temperature, with a Dean–Stark trap fitted.[109] This helps shift the reaction equilibrium towards formation of the imine, thus improving the yield. Another approach that has gained a lot of popularity is the use of activated 4Å molecular sieves.[110,111] This is thought to help promote the reaction by providing a large surface upon which the reaction can occur, as well as removing any water that is formed. Imine formation can also be promoted by having an excess of the amine in the reaction mixture. This, however, leads to the problem of multiple additions if a direct reductive amination reaction is being used.

When carrying out direct reductive amination reactions, stirring the amine and the carbonyl compound together before carrying out the reduction can also increase the yield.[105,112] This is because it allows the imine to preform before any reduction occurs. If this is combined with a method of dehydration then it can reduce the amount of alcohol formed by making the reaction *pseudo*-indirect. However, as the imine formation step is an equilibrium this pre-stirring does not eliminate side reactions altogether, and some investigators have found that pre-stirring either has a negative impact, or no impact at all, on the reductive amination.[105,107]

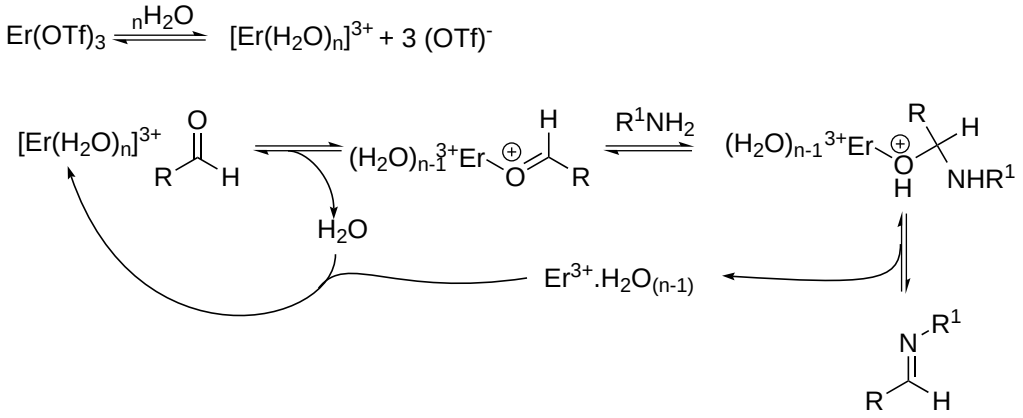
Lewis acids have also been used frequently to catalyse the formation of the imine, either by removing water from the reaction mixture, or by promoting the formation of the imine by activating the alcohol of the carbinol amine.[113] This makes loss of water easier, and

acids have also been used to help form the imine for exactly the same reason (Scheme 2.9). They have been used so frequently that it is often assumed that acid has to be present for reductive amination to occur.[114,115] However, this is not the case as reductive amination can also occur in basic medium, and the use of acidic conditions can also favour reduction of the carbonyl species to the alcohol.[116] Imines are more basic than carbonyl compounds, and this can be exploited to achieve preferential protonation and therefore reduction.



Scheme 2.9: Acid catalysis of reductive amination[113]
A^+=H^+ or Lewis acid

In recent years metal triflates have been introduced as mild and efficient Lewis acid catalysts for reductive amination.[117] The rare earth metal triflates are of particular interest because of their water stability.[118,119] Kobayashi reported that only the hydrated cations of these metals are able to act as Lewis acids, as they show little activity under anhydrous conditions.[117,120,121] During the fast water exchange experienced by the hydrated cations, carbonyl compounds can be activated by coordinating to the metal cation.[117] Therefore, water is required in the reaction environment to partially hydrate the cation, thus enabling the catalysis (Scheme 2.10).



Scheme 2.10: Activation of erbium triflate with water[117]

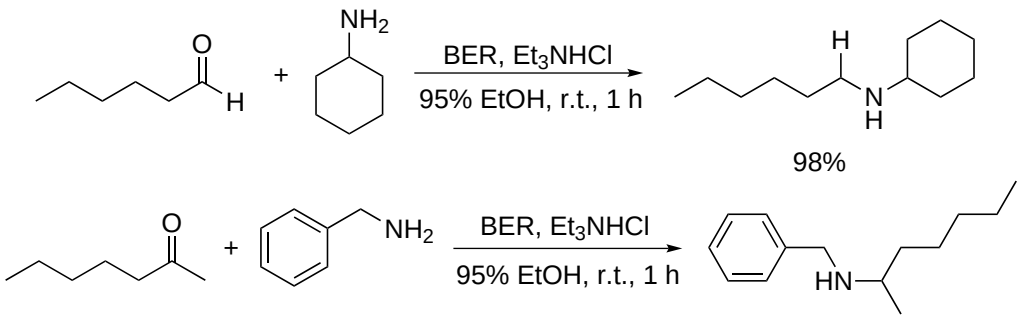
Additives other than Lewis acids have also been used to promote the reaction. These include sugars, which are thought to promote the reaction by stabilizing intermediates through hydrogen bonding, and unreactive amines and their salts, these are used to capture the product after a single addition and prevent side reactions.[107,122–124]

2.3.4 Reduction

Reduction is a frequently used reaction in organic synthesis that has been widely studied, including with respect to reductive amination. A variety of reducing agents have been used to perform reductive amination reactions including sodium borohydride, sodium cyanoborohydride and palladium on carbon with hydrogen with good yields.[105,125–127] Sodium borohydride generally does not require acidic conditions as it readily reduces imines. However, it is not selective for the reduction of imines over aldehydes or ketones, so it is essential to ensure imine formation is complete prior to reduction. Sodium cyanoborohydride on the other hand is selective for the reduction of the imine functional group over the reduction

of aldehyde or ketone functional groups because the cyanoborohydride ligand is electron withdrawing, which decreases the hydride reactivity. However, slightly acidic conditions, pH 5.0–7.0, are required, for this selectivity to work.[128] Amine boranes provide a non-toxic alternative to sodium cyanoborohydride and are highly tunable. This is because their reducing capabilities are affected by the basicity and steric character of the amine ligand. Bulky, weakly basic amines (*e.g. N,N*-diethylaniline) impart electrophilic properties, whilst small strongly basic amines (*e.g.* ammonia) impart reducing character and so can reduce the carbonyl group. Borane pyridine is generally selective forthe reduction of imines, but no reductive amination is usually observed at neutral pH, and glacial acetic acid, or acetic acid with a protic solvent are typically used to get the reaction to proceed.[129] Other hydrides that can be used for reductive amination include lithium aluminium hydride, sodium triacetoxyborohydride and lithium borohydride.[130–132] Palladium on carbon can be used as a heterogenous catalyst for direct reductive amination and allows for direct transfer of hydrogen to the carbinolamine whilst the borohydrides go *via* a large complex that involves the imine.[122] All of these variations make choosing the reducing agent the most important choice when performing direct reductive amination, but less important for indirect reductive amination. This is because it is desirable for the reducing agent to selectively reduce the imine over the carbonyl group.

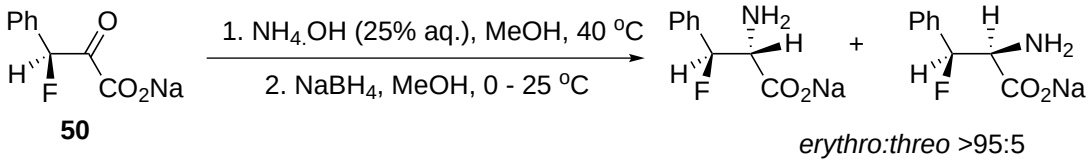
Solid supported reducing agents have been used, including borohydride exchange resin, pyridine borane on alumina and sodium borohydride on clay support amongst others.[133–137] Borohydride exchange resin, for example, has been used to perform reductive amination on both aliphatic aldehydes and ketones in good yield (Scheme 2.11).[133–137] This provides the advantage that the reducing agent will not contaminate the product, as well as allowing the possibility of using a continuous flow system.



Scheme 2.11: Reductive amination using borohydride exchange resin (BER)[133]

2.3.5 Asymmetric reductive amination

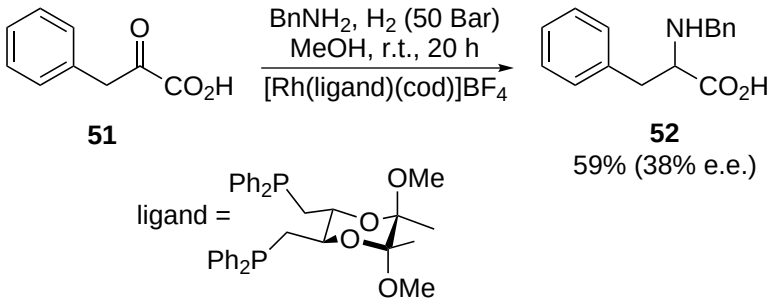
As already stated, enantiomerically pure chiral amines are valuable synthetic intermediates, especially for pharmaceuticals. Traditionally chiral amines have been obtained by resolution procedures, kinetic resolution of a racemate by an enzyme, or crystallization of a single diastereomer using a chiral acid.[138] However, there is a growing need to develop systems that can deliver product in 100% yield and 100% e.e. These methods include the use of transaminases, as well as several more traditional chemical methods.



Scheme 2.12: Reductive amination with stereocontrol[105]

In the past it was observed that if acyclic ketones were used in reductive amination modest levels of stereocontrol were normally achieved. However, there are some exceptions,

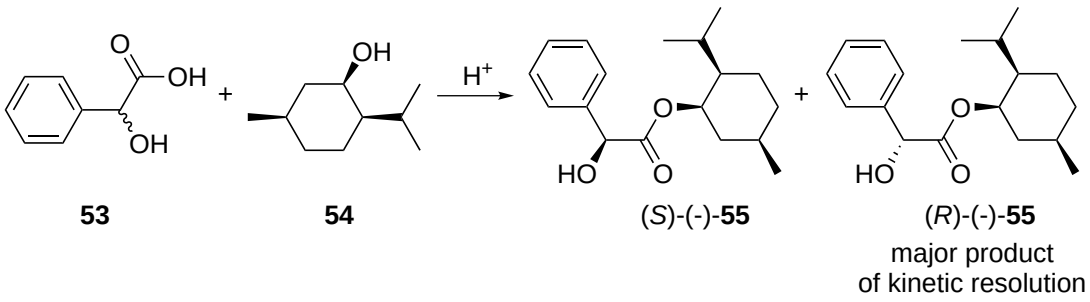
one is shown in Scheme 2.12, where the carboxylic acid anion in **50** directs the amine addition giving good stereocontrol.[105] Recently there has been a lot of research into asymmetric reductive amination, and it has been achieved using chiral ligands with metal catalysed reduction, and to a lesser extent by using chiral additives to direct the reaction.[139–142] One of the first asymmetric reductive aminations was carried out by Tararov *et al* and is shown in Scheme 2.13.[108] In this reaction the chiral rhodium catalyst directs the stereoselectivity of the reaction converting ketone **51** to secondary amine **52** in 59% yield and 38% e.e.[108]



Scheme 2.13: One of the first asymmetric reductive amination reactions[108]
cod=cyclooctadiene

If chemoenzymatic methods are going to work successfully, this is going to be one of the major barriers to their success. One way round this lack of stereoselectivity in many reactions is to use deracemization techniques, for example kinetic resolution in which two enantiomers of a compound exhibit different reaction rates in a chemical reaction and this leads to an excess of the less reactive enantiomer. The reaction is then halted prior to completion, otherwise the excess disappears as the slower reacting enantiomer continues to react after the faster has finished reacting. This leaves a reaction mixture enriched with the slower reacting enantiomer, which can then be purified. Often a reversible reaction is chosen and the product is converted back to the starting materials to give a mixture enriched

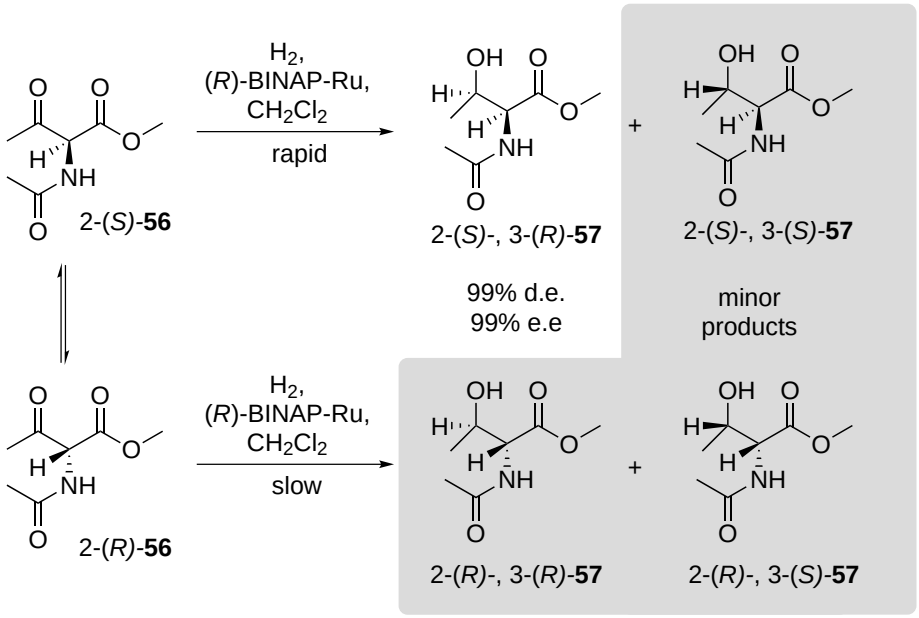
with the faster reacting enantiomer. This process was first observed in 1899 by Marckwald and McKenzie who observed that in the esterification reaction of racemic mandelic acid, **53**, with optically active (–)-menthol, **54**, the (*R*)-enantiomer of mandelic acid reacts faster giving the ester, (*R*)-(–)-**55**, leaving the mixture enriched with (*S*)-mandelic acid (Scheme 2.14).[143,144]



Scheme 2.14: The first observation of a kinetic resolution[143,144]

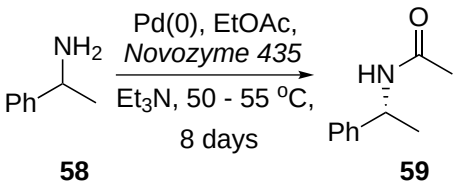
This idea was extended to give dynamic kinetic resolution, which tackles the major drawbacks of kinetic resolution. These are that the maximum possible conversion of the reaction is 50%, and also that the product must be separated out. In dynamic kinetic resolution it is possible to get 100% conversion to the desired enantiomer, as both of the enantiomers of the reactant are in equilibrium, therefore the faster reacting enantiomer is constantly replenished at the expense of the slower reacting enantiomer. An example of this from the research of Noyori (Scheme 2.15) involves the conversion of the enantiomers of **56** through a common enol intermediate to gives a single enantiomer of **57** with 99% diastereomeric excess (d.e.) and 99% e.e.[144,145]

This was applied to amines and the first dynamic kinetic resolution of an amine was demonstrated by Reetz and Schimossek in 1996.[146] This used a palladium(0) species to



Scheme 2.15: An example of a dynamic kinetic resolution[144,145]
BINAP=2,2'-bis(diphenylphosphino)-1,1'-binaphthyl

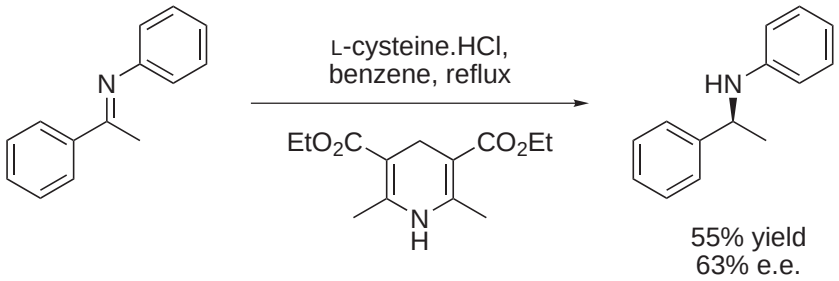
racemize amine **58**, *via* the imine. The enzyme *Novozyme 435* then selectively reacted only one enantiomer of the amine to give a single enantiomer of amide **59** in high yield and e.e. (Scheme 2.16).



Scheme 2.16: The first dynamic kinetic resolution of an amine[146]

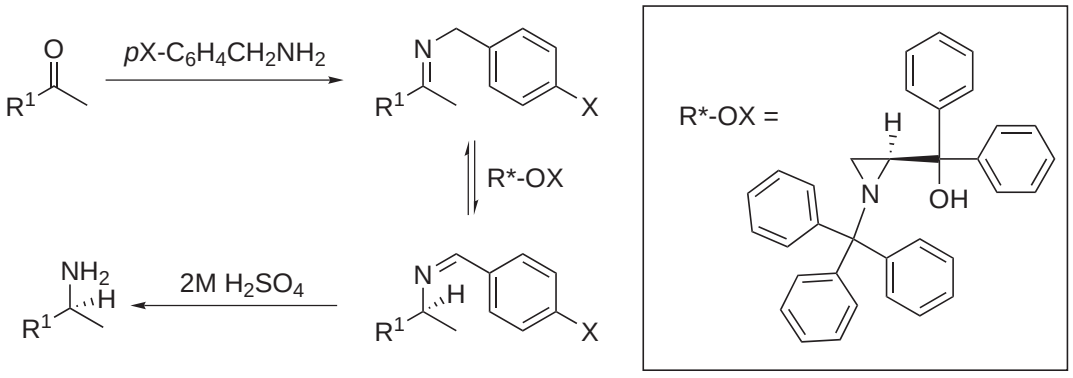
This approach has since been applied as part of one of the first asymmetric reductive aminations of aldehydes.[139,147] This reaction uses an organocatalyst together with a bulky Hantzsch ester to direct hydride addition to only one face of the imine intermedi-

ate(Scheme 2.17).[147] It is important in this reaction to remove as much water as possible from the reaction, otherwise the enantioselectivity of the reaction drops.[139]



Scheme 2.17: The first enantioselective reductive amination reaction on an aldehyde[147]

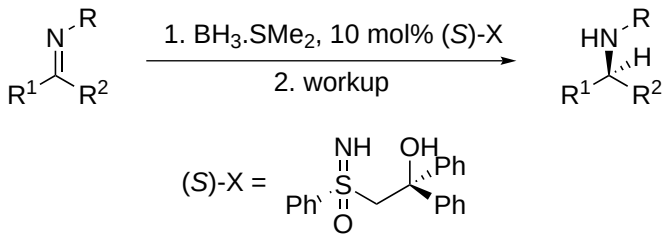
Asymmetric catalytic synthesis of chiral amines has also been performed using a chiral base catalyst (Scheme 2.18).[148] In this reaction a *para*-substituted benzylamine is reacted with a ketone, and the base catalyst then isomerizes the imine to form a single isomer of the amine with excellent stereocontrol. The imine is subsequently broken down leaving the amine as a single enantiomer, and a substituted benzaldehyde.[148]



Scheme 2.18: Asymmetric transamination reaction using a chiral base catalyst[148]

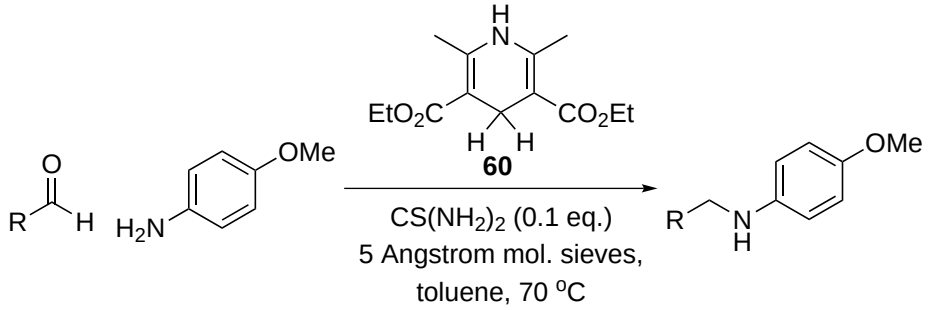
Enantioselective borane reduction of imines has also been shown to be possible, catal-

ysed by β-hydroxy sulfoximine.[149] This can give optically active primary and secondary amines with enantioselectivities of up to 72% e.e. (Scheme 2.19).[149] It was seen that the N-substituent on the ketimine had a major influence on the asymmetric induction. However, this method was only effective with indirect reductive amination methods.[149] It is also possible to use chiral reducing agents, like Alpine-Borane®.[150]



Scheme 2.19: Asymmetric reductive amination reaction using borane and an asymmetric β-hydroxy sulfoximine catalyst[149]

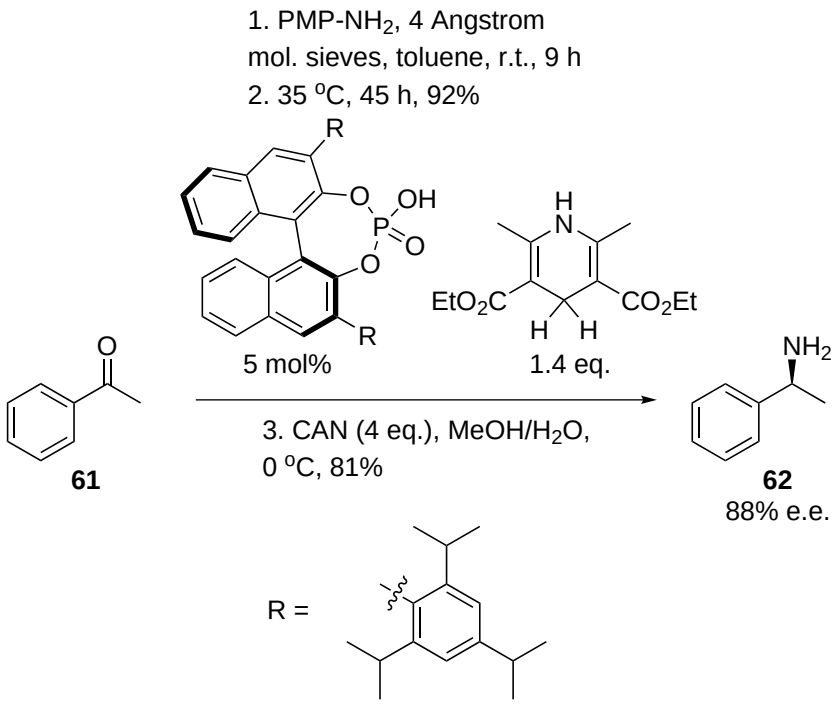
2.3.6 Organocatalytic reductive amination



Scheme 2.20: Organocatalytic reductive amination[151]
mol.=molecular

Reductive amination of aldehydes has been carried out in an organocatalytic fashion with thiourea as a hydrogen bond donor and a Hantzsch ester, **60**, acting as a hydride source

(Scheme 2.20).[151,152] This is still under development and has yet to be made to work in a protic solvent.

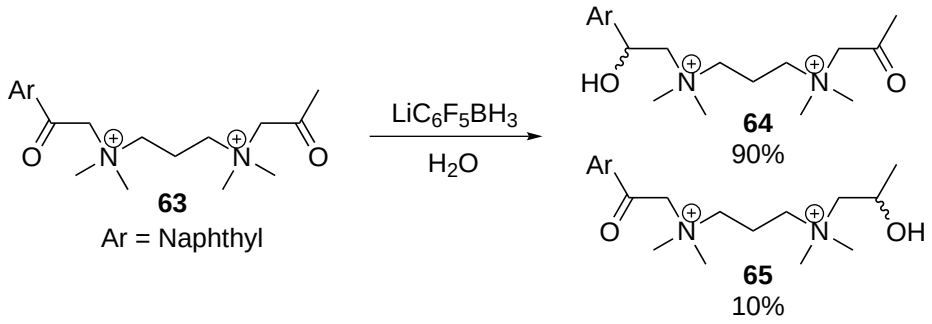


Scheme 2.21: Asymmetric organocatalytic reductive amination[153]
PMP=*para*-methoxyphenylamine; mol.=molecular; CAN=ceric ammonium nitrate

This organocatalytic reductive amination has also been carried out in an asymmetric fashion using acetophenone, **61**.[153] This can react with *para*-methoxyphenylamine (PMP-NH_2) to give a pro-chiral imine, that can then be reduced to give the product in up to 88% e.e. (Scheme 2.21).

2.4 Reductions in water

Hydrogen has poor solubility in water, which has a high dielectric constant. Recent studies by Blackmond have indicated that the kinetics of mass transfer for the gaseous reactant, as influenced by agitation speed, is important for achieving high selectivity, including high enantioselectivity.[154] The majority of reducing agents are incompatible with water, however, sodium and lithium borohydride are frequently used in water to reduce aldehydes and ketones.[155,156] When lithium borohydride is modified to the more hydrophobic reducing agent lithium pentafluorophenyl borohydride ($LiC_6F_5BH_3$), it can be used to perform hydrophobically directed selective reduction of ketones in water.[157,158] For example, the quaternized β-keto diamine, **63**, can be reduced by $LiC_6F_5BH_3$ in water almost exclusively at the naphthyl ketone to give alcohol **64**, rather than at the methyl ketone to give alcohol **65** (90:10, Scheme 2.22).[157,158] This is attributed to hydrophobic directing effects, as when methanol is used as the solvent, the reaction gives equal amounts of reduction at the naphthyl and methyl ketones (45:55).[157,158]

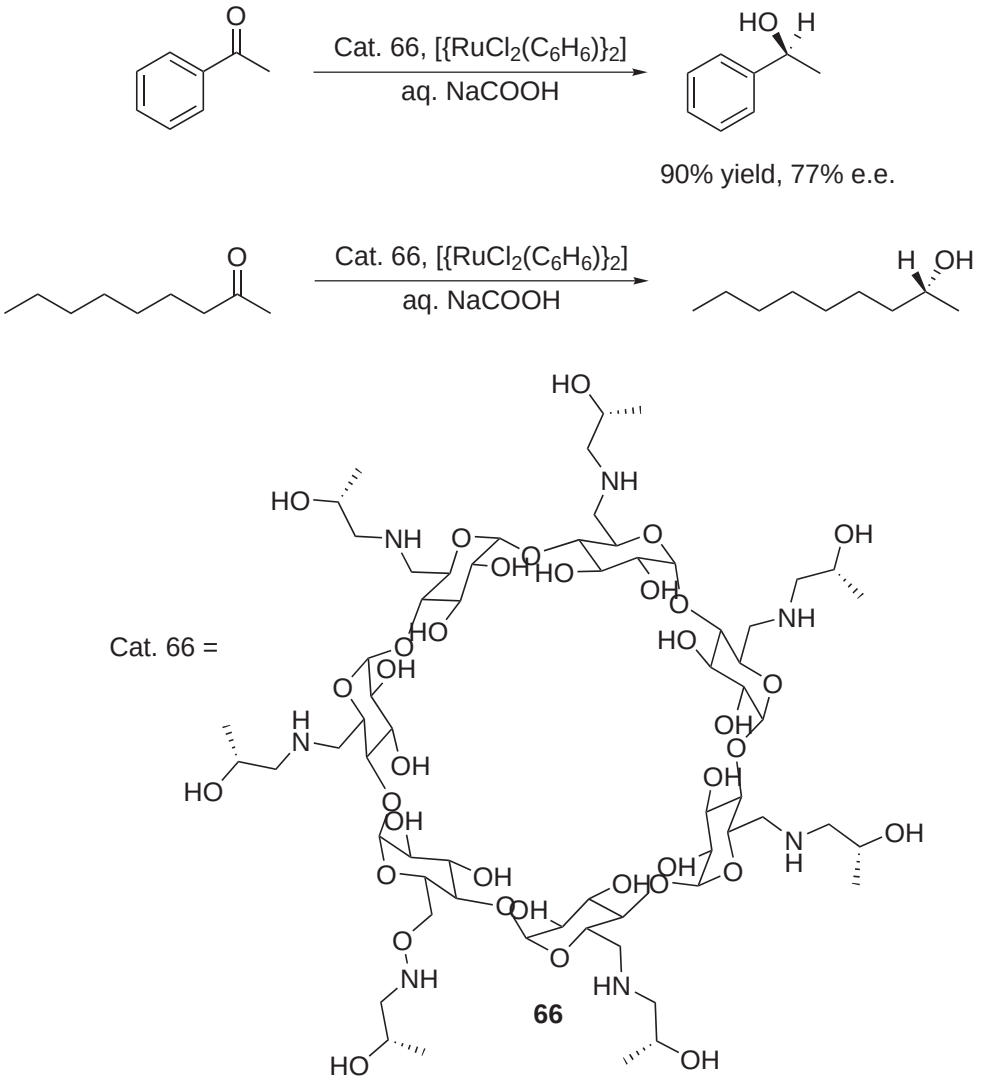


Scheme 2.22: Hydrophobically directed selective reduction of ketones in water[157,158]

From the perspective of large scale manufacture, the poor recovery of homogenous catalyst from the reaction medium and contamination of the product with metal salts, has often

hindered catalyst use in many practical industrial applications.[159,160] By taking advantage of the solubility of the catalysts in water, decanting off the organic phase containing only the reactants and products, and not the catalyst, would allow for practical catalyst recovery and reuse.[161] The use of water can also give higher rates (especially for hydrogen activation by ruthenium(II) complexes) and selectivities for several reactions.[162] However, these advantages are counterbalanced by the disadvantages, such as the high heat capacity, heat of evaporation and reactivity of water that can cause difficulties in the separation and purification of water soluble products. Despite this, several highly successful processes have been developed, including the Ruhrchemie/Rhone–Poulenc hydroformylation (Scheme 2.2) and the Rhone–Poulenc aldehyde hydrogenation.[163–165] Another application for reactions in water is the selective functionalization of water soluble biomolecules, *e.g.* polypeptides, oligonucleotides and oligosaccharides, thus allowing protection and deprotection steps to be avoided.[85]

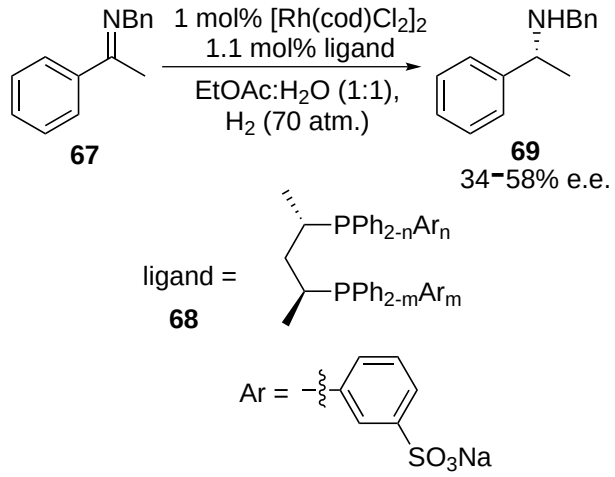
Ligand modification to endow water solubility has been the most widely utilized approach when attempting to immobilize homogenous catalysts in the aqueous phase. For example a water soluble ruthenium complex of β-cyclodextrin modified amino alcohols, **66**, serves as a supramolecular catalyst for the selective reduction of ketones. In the presence of sodium formate, the ruthenium-based catalyst reduces both conjugated and non-activated unconjugated ketones to the corresponding alcohols with good yields and enantioselectivities in water (Scheme 2.23).[166] Water often plays a fundamental role in coordination chemistry and many metal complexes can bind water to fill their coordination sphere, which can influence their reactivity.



Scheme 2.23: Enantioselective reduction of ketones in water[166]
Cat.=catalyst

2.4.1 Imine reduction

The asymmetric hydrogenation of pro-chiral imines has been less systematically investigated than that of the alkene or carbonyl bond. Asymmetric hydrogenation of the imine bond in water was first examined by Sinou and coworkers in 1989.[167] They prepared a mixture of mono-, di- and tri-sulfonated derivatives of the 2,4-bis(diphenylphosphino)pentane ligand, and tested it on the hydrogenation of an imine, **67**, derived from acetophenone, using a catalyst generated *in situ* from $[Rh(cod)Cl_2]_2$ and their ligand, **68**, in a biphasic mixture of ethyl acetate and water. This gave the secondary amine **69** in 34–58% e.e (Scheme 2.24).[167]



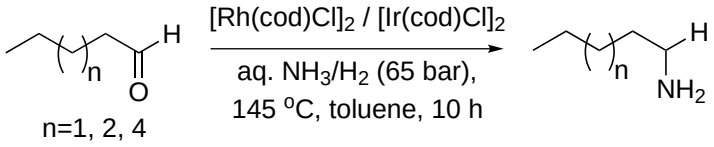
Scheme 2.24: The first asymmetric hydrogenation of an imine[167]
cod=cyclooctadiene

Although only moderate enantioselectivity was observed (34–58% e.e.) and a hydrogen pressure of 70 bar was needed, this was the first example of asymmetric hydrogenation of an imine in water.[167] Later Devries *et al* confirmed that the enantioselective catalyst for the imine reduction was the monosulfonated ligand.[168–170] Using this monosulfonated ligand as an inseparable mixture of diastereoisomers (chirality on phosphorus) under the

same conditions as Sinou *et al*, the same ketimine was reduced with excellent conversion (100%) and enantioselectivity (94%). They also found that the bisulfonated ligand gave almost racemic product. Other imine reductions include the rhodium/iridium bimetallic hydroformylation/hydrogenation of terminal alkenes in the presence of ammonia.[171] This reaction involves the *in situ* formation of a primary amine. Up to 91% selectivity for primary amines was achieved in a medium containing rhodium and iridium sources and tppts. However, the reduction of imines in aqueous medium remains a largely unsolved problem in spite of several other efforts.[172–174]

2.5 Reductive amination in water

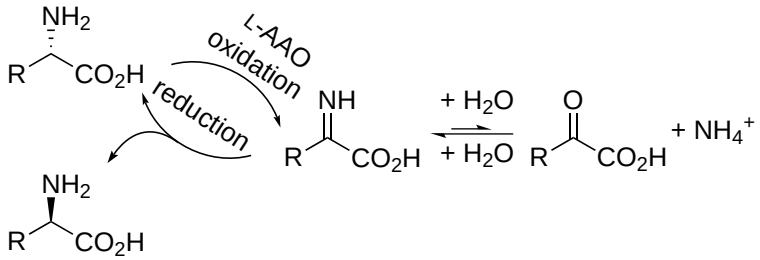
Until recently, no one had attempted to perform reductive amination in pure water. However, there was evidence that it was possible from reactions involving aqueous ammonia as the nitrogen source (Scheme 2.25).[175] One of the reasons that the reaction has not been extensively explored is that imine formation involves the loss of water, and so is unfavourable if the reaction is performed in an aqueous environment, since the reaction is an equilibrium.



Scheme 2.25: Reductive amination with aqueous ammonia as the amine source[175]
cod=cyclooctadiene

This unfavourable equilibrium suggests that reductive amination would fail it was performed in water. However, there are examples of both catalytic and stoichiometric methods

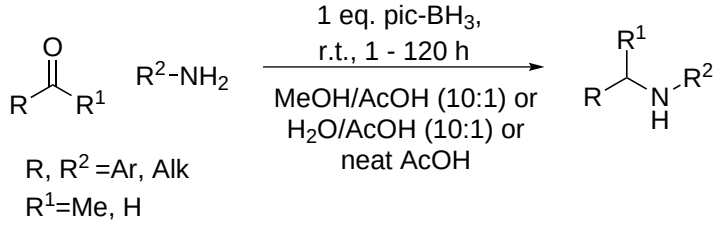
of reductive amination being performed in water. In 2002 Turner and Beard published an article on the deracemization and stereoinversion of α-amino acids using D-amino acid oxidase and hydride reducing agents, which was followed in the same year by a second report on a similar system (Scheme 2.26).[176,177] The first publication with Beard used sodium cyanoborohydride as the reducing agent, whilst the second paper used a range of amine boranes. These latter reagents are preferable as they are relatively water stable, as well as fairly non-toxic. The best result was found with ammonia borane, in conjunction with a large excess of ammonium ions.[177] This is probably because it makes the loss of ammonia very unfavourable, forcing the equilibrium to the imine. Pyridine borane was also tested and shown to be highly active in the same system.[177]



Scheme 2.26: Deracemization of DL-amino acids using L-amino acid oxidase (L-AAO)[176,177]

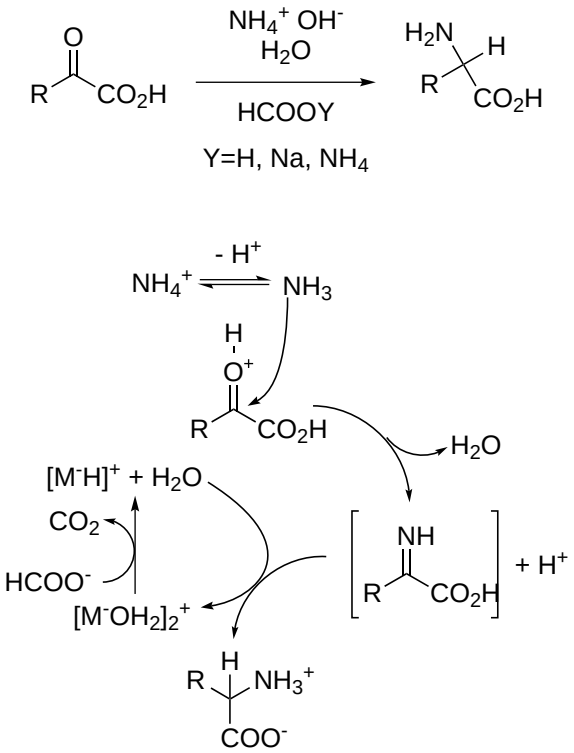
In 2004 Sato *et al* published a paper on the use of α-picoline borane as a reducing agent for the reductive amination of aldehydes and ketones under several conditions, including in water mixtures and neat.[178] All of the conditions used acetic acid as an acid catalyst and so the neat reactions can be considered to be very concentrated with acetic acid as the solvent. The overall conditions used in their reaction are shown in Scheme 2.27.[178]

The third example of reductive amination in water was first reported in 2001 by Ogo *et*



Scheme 2.27: Reductive amination using α-picoline-borane[178]
pic-BH_3=picoline borane

al, and a much improved system was published in 2004, and was the first to be metal catalysed in water.[179,180] It involves the reductive amination of α-keto acids with an acid stable iridium hydride complex, using either aqueous ammonia and formic acid, sodium formate or ammonium formate, the latter acting as both a hydrogen donor ($HCOO^-$) and an amine source (NH_4^+). They found that the reaction worked best when $[Cp^*Ir^{III}(bpy)H]_2(SO_4)$ was used as the catalyst, and that it was highly pH dependent, working best at pH 5.0.[180] At any pH below 4.5, the reaction also gave the α-hydroxy carboxylic acid. The overall mechanism for the reaction is shown in Scheme 2.28.[180] This work has been repeated, as it was found to be a good method for the modification of lysine.[181–183] This is partly because the catalyst is selective for aldehydes, and so any ketones present are left unreacted.[180]

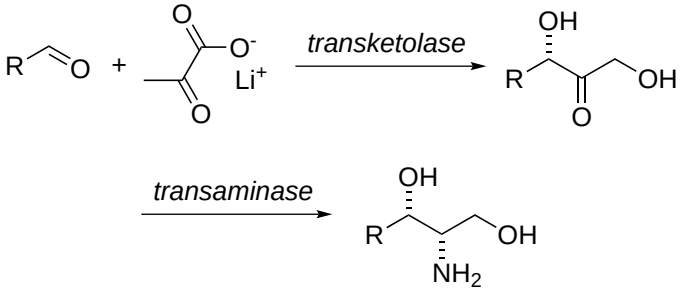


Scheme 2.28: Reductive amination using the catalyst $[Cp^{*}Ir^{III}(bpy)H]_2(SO_4)$ in water[180]
bpy=2,2'-bipyridine

3

Synthesis of 1,3-Ketodiol Analogues

In Chapter 1 a route was outlined for the effective development of biocatalysis for use in a process context (Figure 1.4 on page 22).[1] In order to test this suggested development scheme, the synthesis of 1,3-amino-diols using a two enzyme biocatalytic cascade was proposed (Scheme 3.1). As this synthesis contained two different enzymes it should provide a tougher test of the proposed development route than a synthesis containing only a single biocatalytic step.



Scheme 3.1: The proposed route to 1,3-amino-diols *via* a two enzyme cascade[53]

This product was chosen because the amino-diol functional group is present in many

natural products that demonstrate biological activity.[1] As a specific product had to be targeted, the amino-diols shown in Figure 3.1 were chosen. They were selected because they would present two different molecular architectures to the active site of the enzymes being used to perform the biocatalysis.

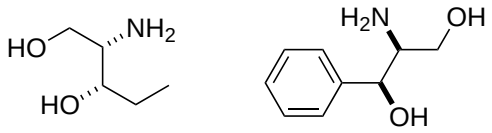
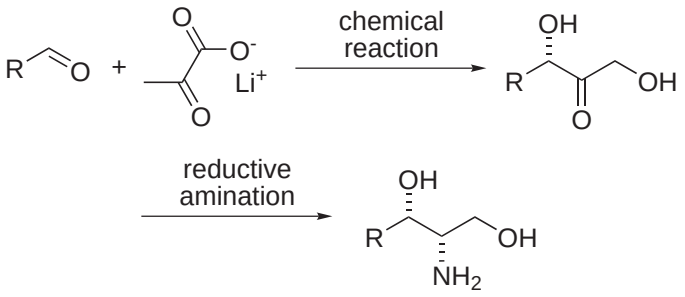


Figure 3.1: The proposed 1,3-amino-diol products of the biocatalytic cascade[53]

In the initial screening section of the development, the first step is the analysis of chemical and biocatalytic alternatives for the proposed reaction sequence (Figure 3.2).

The section of the process that was examined, during the research programme that is described in this thesis, was the analysis of potential chemical routes and their parallel development with the biocatalytic options. As two biocatalysts were proposed, there were two possible alternative chemical routes to be examined (Scheme 3.2).



Scheme 3.2: The two possible alternative chemical reactions[1]

The first step that was proposed, using transketolase, did not have any parallels in the

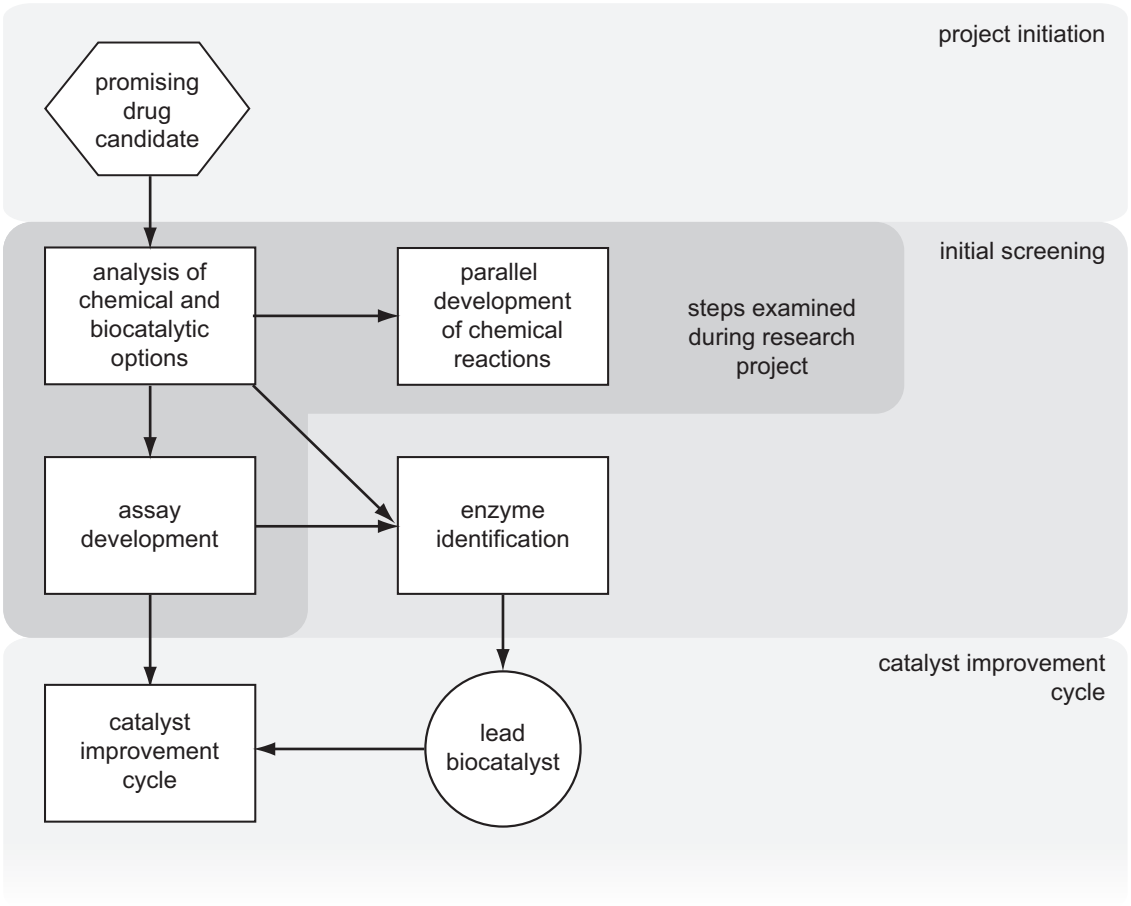


Figure 3.2: The initial screening section of the process development of a biocatalytic synthesis[1]

chemical literature, it was, therefore, decided that this reaction would not be examined first. This was because the second proposed reaction was chemically identical to reductive amination, of which there are a number of examples reported in the literature. Therefore one of the key goals of the research programme was to develop a reductive amination method that could be performed in a one-pot reaction with transketolase. The transketolase was being modified, using directed evolution methods, by other members of the the BiCE research group with the aim of both enhancing and reversing its stereoselectivity, as well as increasing the range of substrates it would accept, including aromatic aldehydes. This and the other steps shown in Figure 3.3 (see also Figure 1.4 on page 22) would all be examined by the BiCE project.

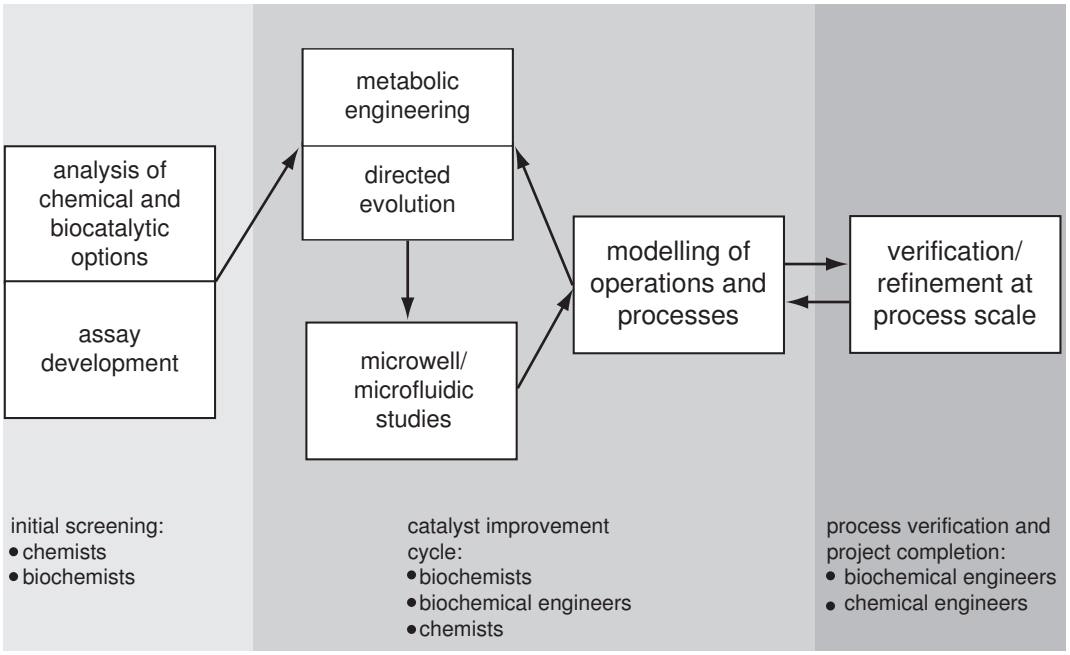
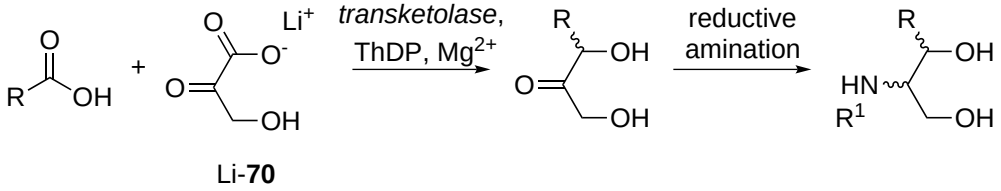


Figure 3.3: All of the different sections of the BiCE research programme[1]

The aliphatic aldehyde used in early directed evolution studies was propanal, which when used in conjunction with transketolase and lithium hydroxypyruvate (Li-HPA, Li-

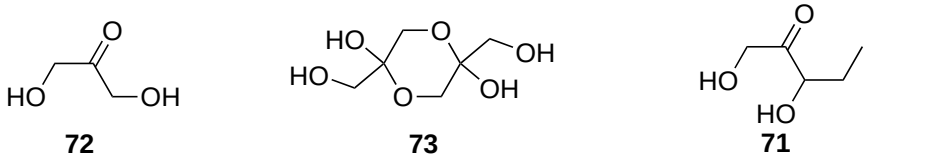
70) gave 1,3-dihydroxypentan-2-one, **71**. This α,α'-dihydroxy ketone was to be used in the reductive amination (Figure 3.4), however, as at the start of the research programme there was no short synthetic route to such 1,3-dihydroxy ketones, and so an analogue was required, upon which to develop the reductive amination methods.[64] As the choice of analogue would influence the reaction methods explored, it was expedient to choose one that had a good structural similarity to the 1,3-dihydroxy ketones, and this work is described below.



Li-**70**

Figure 3.4: The overall reaction sequence examined
ThDP=thiamine diphosphate

In the directed evolution studies, the stereoselectivity of the enzyme was monitored using a gas chromatography–mass spectrometry (GC–MS) assay. However, there was no standard against which to define the absolute stereochemistry (*R* or *S*) of the product; it was possible to conclude that one enantiomer was being produced in excess, but not which enantiomer. A standard to calibrate the assay was therefore needed, and it was proposed that this was synthesized using Enders' (*S*)-1-amino-2-methoxymethylpyrrolidine (SAMP) chiral auxiliary method.



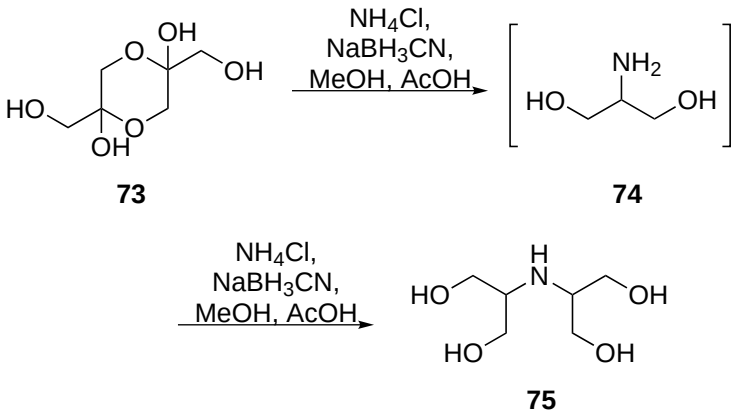
72 **73** **71**

1,3-dihydroxyacetone 1,3-dihydroxyacetone dimer 1,3-dihydroxypentan-2-one

Figure 3.5: 1,3-Dihydroxyacetone as a model substrate[105,178]

3.1 Finding a 1,3-dihydroxyacetone analogue

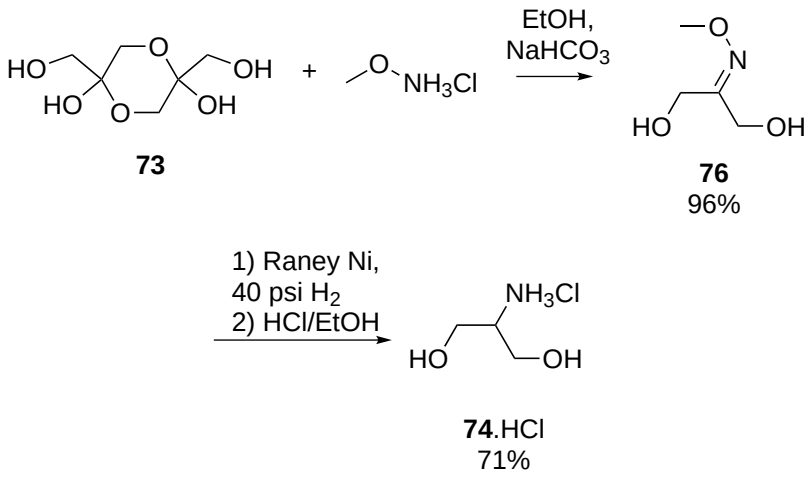
1,3-Dihydroxyacetone (**72**, Figure 3.5) was initially chosen as a good model substrate upon which to develop the reductive amination. This exists as a stable dimer (**73**), however, there was some literature precedent for its use in reductive aminations.[184–187] These reports describe strategies towards 2-amino-1,3-propane diol, **74**, a key intermediate in the synthesis of the radiocontrast agent Iopamidol. Scott *et al* demonstrated reductive amination upon it using ammonium salts (*e.g.* ammonium chloride) and sodium cyanoborohydride in methanol, with catalytic acetic acid (Scheme 3.3). These selectively gave the di-addition product, **75**, in good yield, with no mono-addition product formed.[185]



73 **74** **75**

Scheme 3.3: Reductive amination of 1,3-dihydroxyacetone dimer using sodium cyanoborohydride and ammonium chloride in methanol[185]

Klix *et al* suggest a number of different reductive amination reagents that can be used to perform the mono-addition reaction, including anhydrous liquid ammonia and a hydrogenation catalyst under very high pressures (up to 1450 psi).[186] They state that this is not normally a viable process, and suggest several alternative strategies. The first method involved reacting the dimer, **73**, with an alkoxyamine or benzylamine to form an alkoxyimino intermediate, **76**, that was then reduced at lower pressures to give amino diol **74** as the hydrochloride salt in yields of up to 71% (Scheme 3.4).[186]



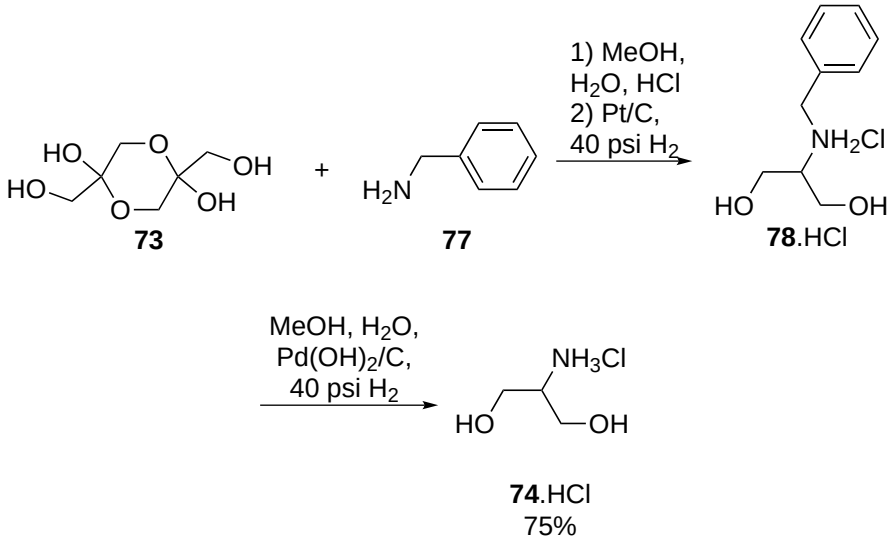
73

76
96%

74.HCl
71%

Scheme 3.4: Reductive amination of 1,3-dihydroxyacetone dimer *via* an alkoxyimino intermediate[186]

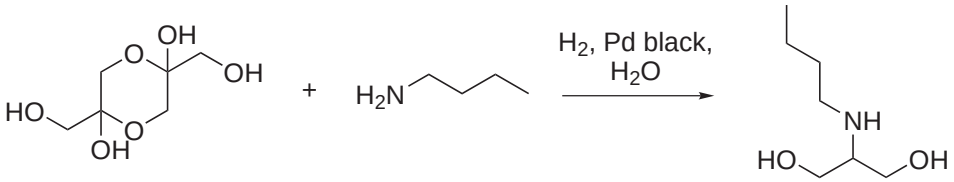
Klix *et al* also synthesized 2-amino-1,3-propane diol, **74**, by the direct reductive amination of 1,3-dihydroxy acetone, **73**, using benzylamine, **77**, and hydrogenation using a platinum on carbon catalyst to give the secondary amine salt, **78**.HCl.[186] The benzylamine group can then be cleaved to give 2-amino-1,3-propane diol, **74**, in 75% yield as shown in Scheme 3.5.[186]



Scheme 3.5: Reductive amination of 1,3-dihydroxyacetone dimer *via* a benzylamine intermediate[186]

Merckle *et al* used a similar approach, carrying out a direct reductive amination with *n*-butylamine, **79**, in water using palladium black as the hydrogenation catalyst, as shown in Scheme 3.6.[184] All of these reports suggested that the 1,3-dihydroxyacetone dimer, **73**, exists in a dynamic equilibrium with its monomer, **72**, and that this monomer could then participate in the reductive amination. If the amine source to be used was ammonia or one of its salts, then very forcing conditions, *i.e.* a high concentration of ammonia and high pressure hydrogenation, had to be used, otherwise the only product seen was *bis*-(1,3-dihydroxy-isopropyl)amine, **75**.[185,186] This is because the 1,3-dihydroxyacetone monomer reacts faster with primary amines than with ammonia. In order to avoid this, the dimer has to first be reacted with a primary amine, *e.g.* *n*-butylamine, **79**, or similar in order to form the secondary amine which cannot over react. This can then be converted to the desired 2-amino-1,3-propane diol, **80**, in good yield.[186]

With these reactions in mind, a series of reductive amination methods, initially using

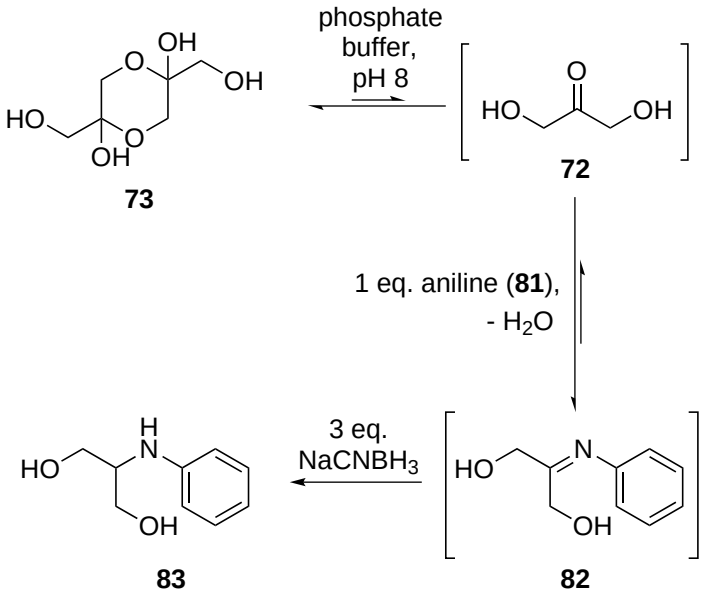


73 **79** **80**

Scheme 3.6: Reductive amination with the 1,3-dihydroxyacetone dimer[184]

aniline, **81**, as the amine source, were carried out. Aniline was chosen as it had been shown to be effective in reductive amination reactions in protic solvent, often giving the highest yields.[178] For example in acidic methanol Sato *et al* saw a 95% yield for the reductive amination of benzaldehyde with aniline using picoline borane as the reducing agent, compared with a 76% yield for the same reaction when benzylamine was used instead of aniline.[178] This may be due to conjugation of the imine with the aromatic ring, as in **82**, providing stabilization. Another benefit of using aniline in the test reactions was that both it and the reductive amination product it formed were be ultraviolet (UV) active, allowing the reaction to be monitored more easily using thin layer chromatography (TLC) or high performance liquid chromatography (HPLC).

Initially sodium cyanoborohydride was used as the reducing agent, using the reaction conditions described by Turner *et al* (Scheme 3.7), with the sole difference that an amine source, aniline, was added. In the research carried out by Turner and his group the amine was generated *in situ* from the deracemization of the amino acid.[176] The reaction was performed in potassium phosphate buffer (pH 8) using two equivalents of aniline, **81**, and six equivalents of sodium cyanoborohydride for each equivalent of 1,3-dihydroxyacetone dimer, **73**. No reaction was observed and so it was repeated, with the pH monitored and maintained at pH 8.0, using glacial acetic acid, but still no reaction was observed. There-



Scheme 3.7: An example of a reductive amination reaction attempted on the 1,3-dihydroxyacetone dimer using aniline as the amine source[176]

fore a series of reactions were performed, varying the reducing agent (sodium borohydride, sodium cyanoborohydride, sodium triacteoxyborohydride, pyridine borane, ammonia borane, trimethylamine borane, ^{t}butylamine borane and lithium borohydride), solvent (glacial acetic acid, ethanol, methanol, chloroform, dichloromethane, ethyl acetate, pentane, hexane, acetonitrile, toluene, THF, 1,2-dichloroethane, water and diethyl ether), temperature, reactant concentrations, methods of water removal (Dean–Stark and molecular sieves) and acid catalysts (glacial acetic acid, $AlCl_3$), but in all cases product **83** was not observed.[105] Several different amine sources were also tested (aniline, ammonium chloride, ammonium hydroxide, ammonium formate, ammonium acetate). None of the desired product (**83**) was formed, however, some of the reactions gave the double addition product (characterized by ^{1}H nuclear magnetic resonance [NMR] spectroscopy) in low yields (<5%), which were formed as previously described by Baxter *et al* (Scheme 3.3).[105] As a di-addition product was not desired, this indicated that primary amines would not be suitable if 1,3-

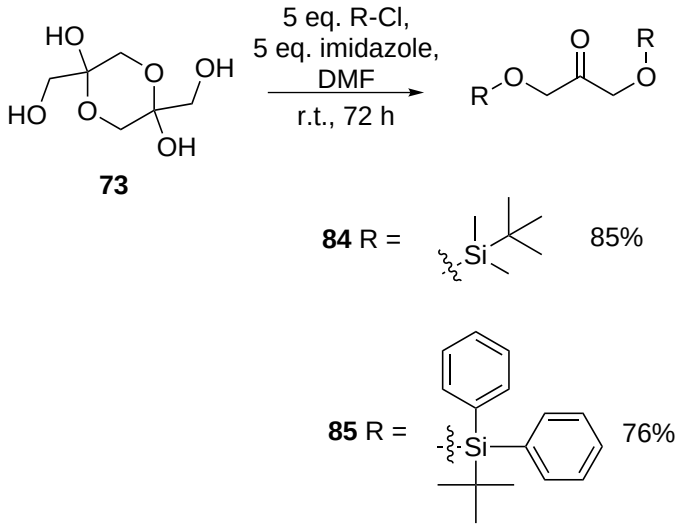
dihydroxyacetone was to be used, and suggested that secondary amines would be more suitable amine sources. The examples of reductive amination reactions with secondary amines in the literature were all performed using heterogenous catalysis, frequently in water. One concern of using heterogenous catalysis was that it might also reduce functional groups on the enzyme (*e.g.* disulfide bridges), thus deactivating it, while homogenous catalysts tend to be weaker reducing agents and should not affect the enzyme to the same extent. Therefore, no heterogenous reductions were attempted.

It was concluded that the use of dimeric 1,3-dihydroxyacetone, **73**, as the 1,3-dihydroxy pentan-2-one, **71**, analogue was not effective and that protection of 1,3-dihydroxyacetone, **72**, to exclude dimer formation would give a more effective substrate for the development of the reductive amination reaction.

3.2 Silyl protected 1,3-dihydroxyacetone analogues

Upon examination of the literature, there were a number of examples of silyl protected 1,3-dihydroxy acetone analogues that would be suitable.[188–190] The first analogue to be synthesized was the *tert*-butyl dimethyl silyl (*t*BDMS) protected species, **84**. The Corey method involving *t*BDMS triflate with 2,6-lutidine as the base was explored, however, the reaction failed.[191,192] Upon further examination of the literature the conditions more typically used were excess *t*BDMS chloride and imidazole, as shown in Scheme 3.8.[189]

This method worked well, giving the *t*BDMS protected species, **84**, in 85% yield, a slightly lower yield than that reported in the literature of 93%.[188–190] This slightly lower

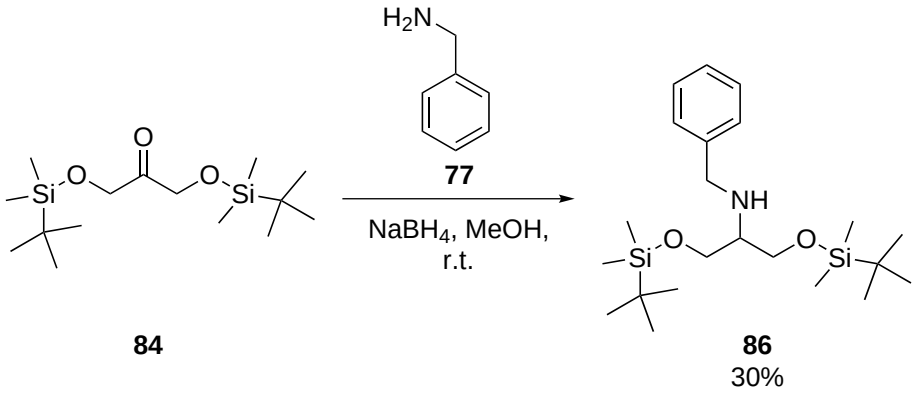


Scheme 3.8: Silyl protection of 1,3-dihydroxyacetone using imidazole[184,188–190]

yield might be because an older batch of *t*BDMS chloride was used, that may have degraded to some extent. Several other silyl protected analogues of 1,3-dihydroxyacetone were also prepared with varying stability and size. The *tert*-butyl diphenyl silyl (*t*BDPS) analogue, **85**, was synthesized in 76% yield, however, the trimethyl silyl (TMS) analogue was not successfully prepared. Upon examination of the literature, even though it had been synthesized three times, the protected species was not isolated, rather existing as part of a mixture with other silylated species.[193]

3.2.1 Reductive amination of silyl analogues

To ensure that the silyl protected species, **84** and **85**, were suitable test substrates, reductive amination (Scheme 3.9) was performed upon them using conventional methods. The *t*BDMS protected analogue, **84**, was used as it could be synthesized in the highest yield. Selected examples of reaction conditions explored are shown in Table 3.1. The product,



Scheme 3.9: Reductive amination of tBDMS protected 1,3-dihydroxyacetone

Entry	Conditions	Yield (%)
1	$NaBH_4$ and benzylamine in methanol at room temperature with pre-stirring	30
2	$NaCNBH_3$ and benzylamine in methanol at pH 6.0 at room temperature with pre-stirring	31
3	$NaBH(OAc)_3$ and benzylamine in DCE at room temperature with pre-stirring	29
4	$NaBH(OAc)_3$ and benzylamine in DCE at pH 6.0 at room temperature with pre-stirring	45
5	Pyridine borane and benzylamine in methanol at room temperature with pre-stirring	36

Table 3.1: Reductive amination of tBDMS protected 1,3-dihydroxyacetone, **84**[105]
DCE=1,2-dichloroethene

86, was only formed in moderate yield, the highest yield obtained was 45% (entry 4 in Table 3.1). This yield was low, possibly because the reactions were only carried out for 1 hour. This length of time was chosen as the reaction is generally rapid, however, the reactions may not have gone to completion.[178,194] In order to see if this was the case, the reaction ought to be monitored by either liquid chromatography-mass spectrometry (LCMS) or TLC, in order, to monitor the consumption of the starting materials, and only worked up when the reaction has been seen to go to completion. However, the reactions performed with short time scales established that it was possible to perform reductive amination reactions upon **84**.

3.2.2 Cyclic protected analogues

It was envisaged that protection of 1,3-dihydroxy ketones as cyclic species would mean that subsequent reductive amination reactions would be directed by the shape of the molecule, providing selectivity for a single isomer. Facial selectivity in the reductive amination of the benzylidene acetal of 1,3-dihydroxyacetone, **87**, has been reported in the literature.[195–197] This adopts the least sterically hindered conformation prior to reduction of the imine (**88**, chair conformation, with the phenyl group equatorial) and preferentially gives one stereoisomer of the product.[195–197] This selectivity occurs because the nucleophile can attack either equatorially or axially. Equatorial attack by the reducing agent is sterically unhindered, but torsional strain caused by the attacking group eclipsing the ring protons will make attck from this direction less favourable, whereas axial attack is sterically hindered, but there is no torsional strain. As the hydride nucleophile is small, the steric hindrance will have little effect, meaning that the torsional strain is the main cause of selectivity, and

so **89** should form preferentially (Figure 3.6).[196,197]

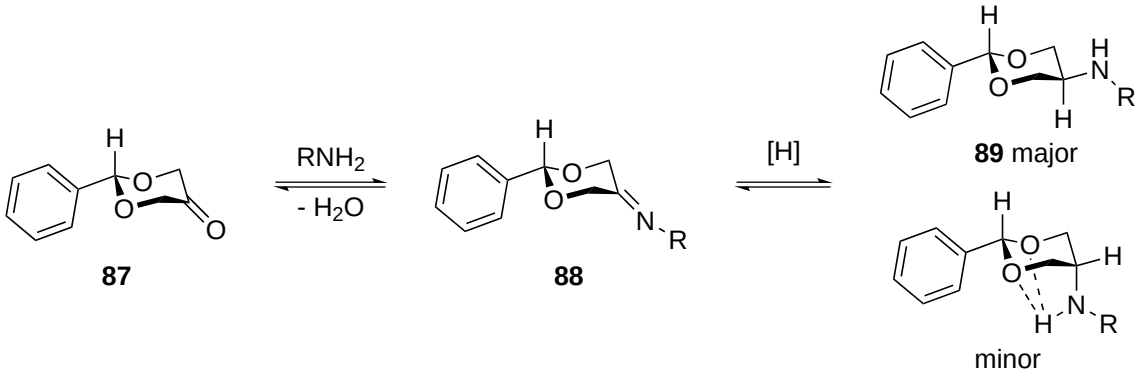


Figure 3.6: The outcome of reductive amination upon 2-phenyl-1,3-dioxan-5-one[196,197]

Cyclic analogues were initially prepared using silyl diprotecting reagents. The synthesis was attempted using dichlorodiphenyl-silane, dichlorodiethyl-silane and di-*tert*-butyldichloro-silane, with imidazole as the base, in anhydrous DMF. However, the only reagent that reacted as desired was di-*tert*-butyldichloro-silane which gave the diprotected species, **90**, in 37% yield (Scheme 3.7).[184,188–190] This limited reactivity with the diprotecting agents to form the cyclic species is probably caused by the presence of the 1,3-dihydroxyacetone dimer, **73**. This meant that the molecules were close together after the first protection and due to steric effects it was easier for the diprotecting agents to attack a second molecule of 1,3-dihydroxypentanone rather than perform a second attack on the first molecule. These polymeric protected species reduced the yield of the cyclic protected species massively.

In summary, three silyl protected 1,3-dihydroxyacetone analogues were prepared and the tBDMS protected species, **84**, was reductively aminated selectively. A cyclic analogue, **90**, was prepared in 37% yield, but no unsymmetrical cyclic silyl-protected analogues could be prepared to test whether they confered diastereoselectivity to the reductive amination re-

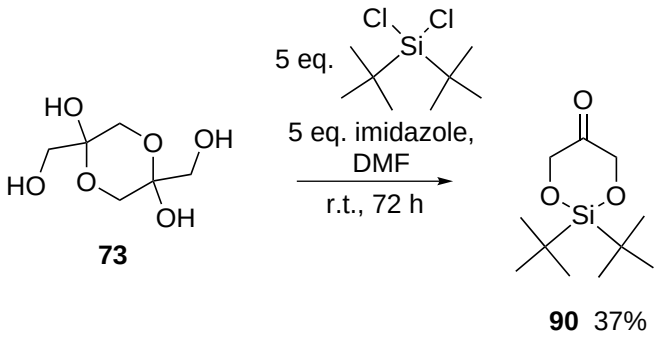


Figure 3.7: Synthesis of 2,2-di-*tert*-butyl-1,3,2-dioxasilinan-5-one[184,188–190]

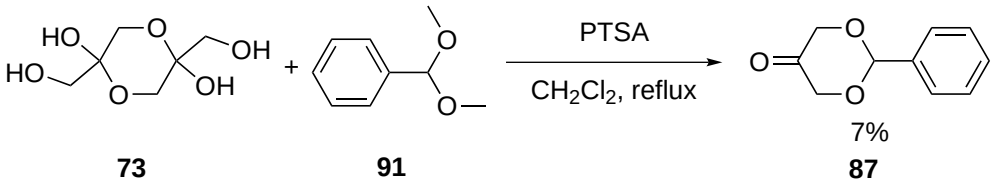
action. We therefore turned our attention to acetal protecting strategies, in particular benzyl acetal protected species.

3.3 Acetal protected 1,3-dihydroxyacetone analogues

Cyclic analogues of 1,3-dihydroxyacetone were desirable as they would restrict the conformation of the reactant, which could be useful in enhancing reaction stereoselectivity as discussed by Jochims *et al.*[196,197] In this paper acetal protection provided this selectivity, and so this benzylidene acetal was targeted for synthesis. The synthesis was initially attempted using benzaldehyde dimethyl acetal.[191] Acetal protecting groups would be less bulky than similar silyl systems, which should make them more atom economic and sterically less hindered towards reductive amination. They can also be cleaved using different reaction conditions to the silyl analogues, and so would be stable under different conditions, which would enable a wider set of reaction conditions to be explored.

The 1,3-dihydroxyacetone dimer, **73**, was reacted with 2,2-dimethoxypropane or benzaldehyde dimethyl acetal, and an acid catalyst (*para* toluene sulfonic acid [PTSA], cam-

phor sulfonic acid [CSA] or zinc chloride) in several solvents (including benzene, dichloromethane, acetic acid and carbon tetrachloride) and at a range of different temperatures.[191,198] The only procedure performed successfully, used benzaldehyde dimethyl acetal, **91**, and PTSA in refluxing dichloromethane (Scheme 3.10).[199] The product, **87**, was isolated in 7% yield by Kugelrohr distillation, as column chromatography and standard distillation techniques (including the use of a Vigreux condenser) failed to separate the product from the starting materials and side-products. This low yield was probably caused by difficulty in breaking the dimer apart, as had been observed when the silyl protections had been explored.



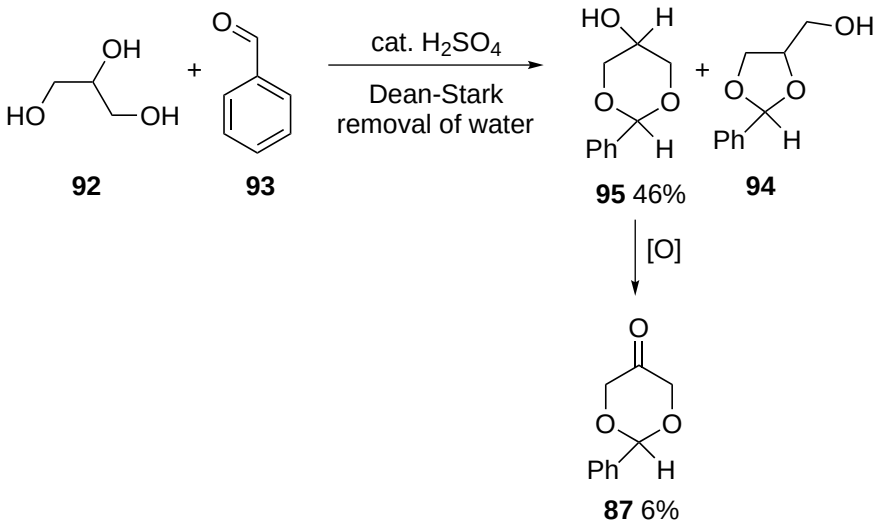
73 **91** **87**

Scheme 3.10: Synthesis of benzylidene protected 1,3-dihydroxyacetone[191,198]
PTSA=*para* toluene sulfonic acid

The synthesis of a range of acetal protected 1,3-dihydroxyacetone analogues, including benzylidene protected analogues, has been described in the literature from glycerol, paraformaldehyde or *tris*(hydroxymethyl)amino methane, all of which were multi-step syntheses.[195,200–202] The literature also highlighted that:

> "The reason for dihydroxyacetone not having found more extensive synthetic use may be ascribed to the fact that the desired acetals and ketals have not been generally available. Synthesis of *e.g.* 1,3-di-O-benzylidene dihydroxyacetone in reasonable yield has not yet appeared in the literature."[200]

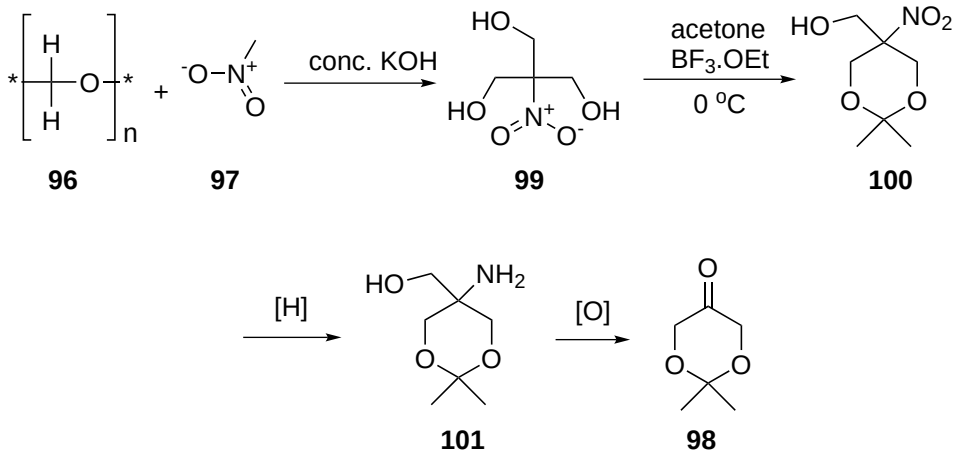
This theme is continued in other papers discussing this synthesis, with the major difficulty being the lack of repeatability of the syntheses.[195,201,202] One of these syntheses of 2-phenyl-l,3-dioxan-5-one, **87**, from the literature, starting from glycerol, **92**, (Scheme 3.11) was explored.[200] Glycerol, **92**, was first reacted with benzaldehyde, **93**, giving both the five-, **94**, and six-membered, **95**, ring benzaldehyde acetals. The six-membered ring containing species, **95**, was then purified out and oxidized to give 2-phenyl-1,3-dioxan-5-one, **87**.[200]



Scheme 3.11: Synthesis of benzylidene diacetal protected 1,3-dihydroxyacetone[200]
cat.=catalytic

The reaction between glycerol, **92**, and benzaldehyde, **93**, worked well, giving the six-membered ring containing species, **95**, in an isolated yield of 46%. This was slightly lower than the 53% yield reported in the literature, and was probably a consequence of material losses during purification. However, the literature method for performing the oxidation, using a slurry of ruthenium dioxide in sodium hypochlorite worked poorly giving very low

yields (1%) of **87**, much lower than the highest literature yield of 76%.[200] This low yield might be explained by poor slurrying of the starting materials for the oxidizing agent. This would mean that the intermediate that actually performs the oxidation would not be formed and so an extremely poor yield was obtained. As the oxidation had not worked, it was then attempted using a range of oxidizing reagents including the Jones reagent, tetrapropylammonium perruthenate (TPAP), pyridinium chlorochromate (PCC) and sodium hypochlorite with glacial acetic acid, but no product (**87**) was formed.[203–205] The oxidation reaction was successfully performed using Dess–Martin periodinane in refluxing dichloromethane giving **87**, but only in 6% yield. This suggested that very specific oxidation conditions were required as the ruthenium method is catalytic, it is likely that the ruthenium also participates in the reaction the reaction by activating the molecule for oxidation. The overall yield from both steps was 3% which was less than that obtained from the direct protection of 1,3-dihydroxyacetone dimer, and so this route was not explored further.

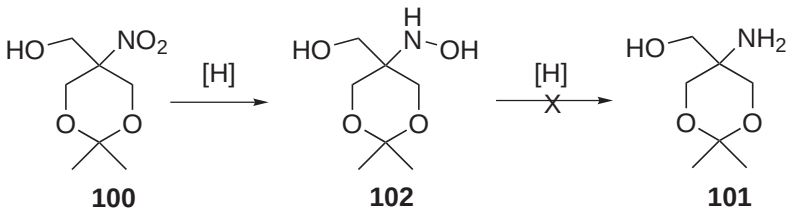


Scheme 3.12: Synthesis of acetonide protected 1,3-dihydroxyacetone[201]

A different literature synthesis developed by Enders *et al*, as part of their synthesis of a range of 1,3-dihydroxy ketones, was therefore attempted.[201] This route used paraformalde-

hyde, **96**, and nitromethane, **97**, as the starting materials (Scheme 3.12) and gave the acetonide, **98**, rather than the benzylidene protected species, **87**. This would not provide any directing effects due to its small, symmetrical nature, but if successful a similar approach to give the benzylidene protected species could be tried. The first step involved the reaction of paraformaldehyde, **96**, and nitromethane, **97**, under strongly basic conditions to give 2-(hydroxymethyl)-2-nitropropane-1,3-diol, **99**, in 89% yield, which was greater than the 73% yield reported in the literature.[201] This higher obtained yield might be due to the reaction being performed on a smaller scale than that described in the literature, which would allow for more efficient heat transfer, and could possibly encourage the reaction. This was then reacted with acetone under Lewis acidic conditions to give nitro species **100** in 59% yield, which was slightly less than the literature yield of 66%. The lower yield could have come about because the reactants were not fresh, and could have become slightly damp which would destroy some of the water sensitive Lewis acid. The reduction of the nitro species, **100**, to the corresponding amine, **101**, was described in the literature using Raney-Ni in methanol at 70 °C and 100 atmospheres hydrogen.[201] The reaction could not be performed under these conditions as such high pressure hydrogenation equipment was not available for our use, instead the possibility of performing the reaction at lower pressures was investigated. This reduction was attempted with an array of different reducing agents under a variety of different conditions (some examples are shown in Table 3.2). The only product observed was the intermediate hydroxylamine, **102**, (Scheme 3.13), as confirmed by analysis using NMR and mass spectrometry (MS). This indicated that the extremely forcing conditions used in the literature were essential for this reaction to work.

When the oxidation of hydroxylamine **102** (from entry 6 in Table 3.2) was attempted using sodium metaperiodate, the desired ketone product, **98**, was observed with a maximum

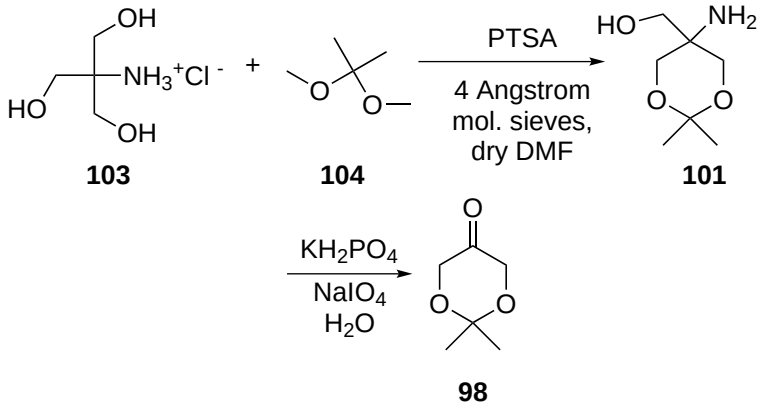


Scheme 3.13: Reduction of the nitro group on (2,2-dimethyl-5-nitro-1,3-dioxan-5-yl)methanol (**100**)

Entry	Catalyst	Hydrogen Source	Solvent	Yield of **102** (% conversion)
1	10% Pd/C	H_2	methanol	75
2	10% Pd/C	H_2	ethanol	0
3	10% Pd/C*	H_2	methanol	70
4	PtO_2	H_2	methanol	50
5	Raney Ni	H_2	methanol	50
6	20% $Pd(OH)_2$/C	H_2	methanol	90**
7	20% $Pd(OH)_2$/C	H_2	ethanol	0
8	10% Pd/C	$H_2CO_2NH_4$	methanol	0
9	10% Pd/C	$H_2CO_2NH_4$	THF/methanol	50**
10	10% Pd/C	cyclohexene	ethanol	0
11	20% $Pd(OH)_2$/C	$H_2CO_2NH_4$	THF/methanol	100
12	20% $Pd(OH)_2$/C	H_2CO_2H	2.5 M NaOH(aq)	0
13	Raney Ni	H_2CO_2H	methanol	50
14	PtO_2	H_2	triethylene glycol	0
15	$SnCl_2$	–	ethanol	45
16	10% Pd/C	$NaBH_4$	THF	75
17	10% Pd/C	$NaBH_4$	THF	50
18	20% Pd/C	$LiBH_4$	THF	50
19	20% $Pd(OH)_2$/C	$NaBH_4$	THF	85

Table 3.2: Reduction attempts on 5-hydroxymethyl-2,2-dimethyl-5-nitro-1,3-dioxane, **100**. The reaction was tested on 1 mmol of material and unless otherwise stated was carried out at room temperature in 25 mL of solvent for 16 hours.[127,206] The yield of 5-hydroxymethyl-2,2-dimethyl-5-nitro-1,3-dioxane was estimated from the NMR spectrum of the product
*=Degussa type carbon; **=100% after 7 days

yield of 10%. This might have been formed if some of the amine, **101**, was present, or from the oxidation of the hydroxylamine, **102**, to the ketone, **98**, as has been suggested in the literature.[195] However, this reaction was not reliable, and when repeated on other batches of material from the same reduction much lower yields were seen. Additionally, when the oxidation was attempted upon the products of repeated reduction reactions the ketone, **98**, was not formed. This suggested that the hydroxylamine, **102**, was not oxidized, rather that some of the reduction reactions formed small quantities of the amine, **101**, even at lower pressures.



Scheme 3.14: Synthesis of 2,2-dimethyl-1,3-dioxan-5-one from tris-hydrixymethyl-aminomethane hydrochloride and 2,2-dimethoxypropane[202]
PTSA=*para* toluene sulfonic acid; mol.=molecular

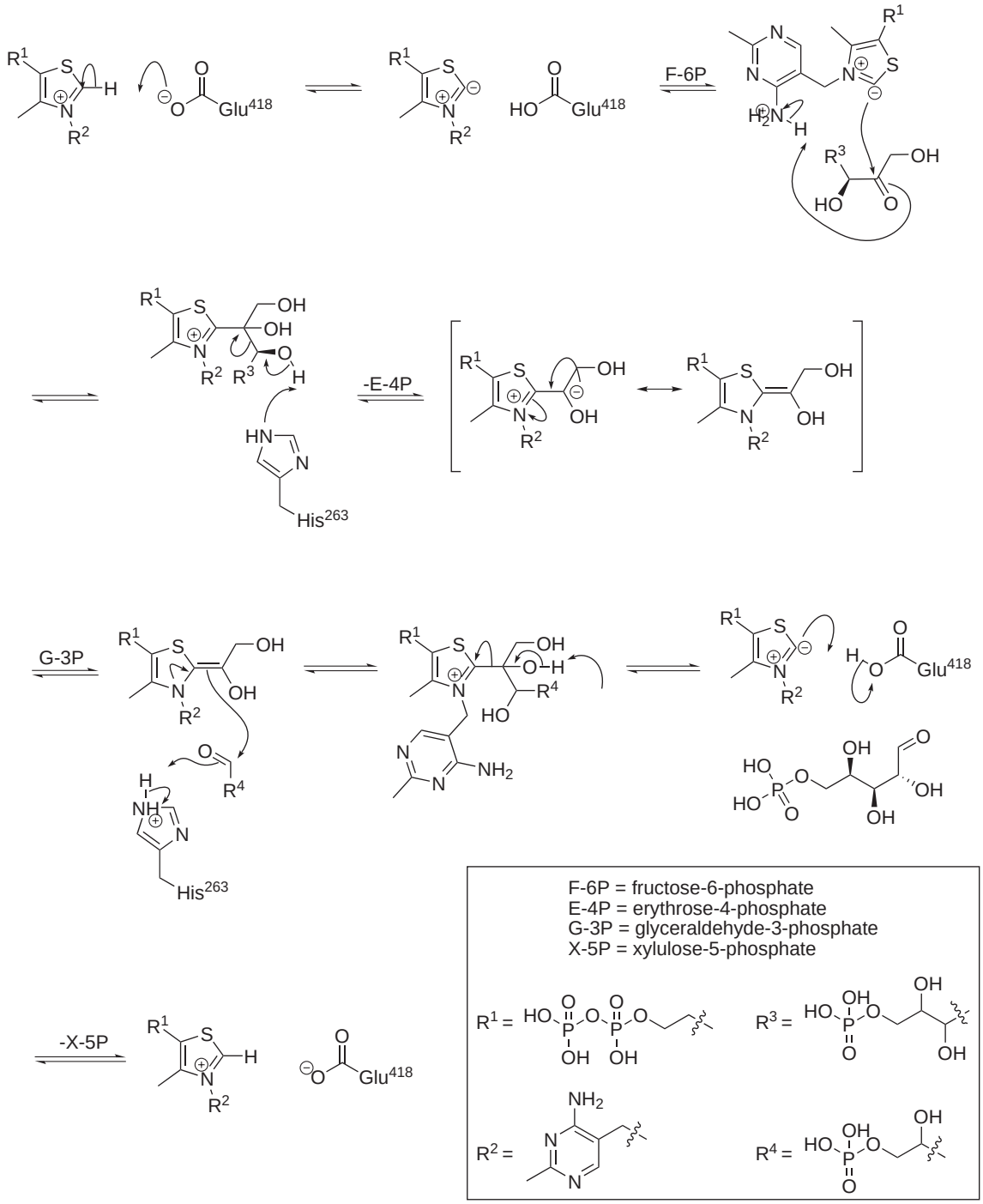
As reduction of the nitro compound, **100**, to the alcohol proved unsuccessful, an alternative route to the acetal protected species, **98**, was investigated. This literature route, reported by Forbes *et al*, also went *via* (5-amino-2,2-dimethyl-1,3-dioxan-5-yl)methanol, **101**, (Scheme 3.14).[202] Tris-hydroxymethyl-aminomethane hydrochloride, **103**, was reacted with 2,2-dimethoxypropane, **104**, to give alcohol **101** in 40% yield when fresh anhydrous DMF and 4Å molecular sieves were used. This reaction was reproducible, but

the yield was significantly lower than the 86% reported in the literature. However, as the starting materials were cheap and the reaction could be easily performed on a large scale the reaction was not optimized any further.[202] Alcohol **101** was then oxidized using sodium metaperiodate to give the desired acetonide protected 1,3-diol, **98**, in 22% yield. This yield was poor compared with the 90% reported in the literature, but once again no optimization was attempted.[202]

Synthesis of the benzylidene protected species, **87**, was also attempted using this route but low yields, no greater than 1% were obtained. The protection was not explored any further at this time because an alternative route to 1,3-dihydroxypentan-2-one, **71**, was discovered by members of the BiCE group.[207] This synthesis involved a single step from easily available starting materials, and the 1,3-dihydroxypentan-2-one, **71**, produced using this reaction could then be benzylidene protected directly. The acetonide protected 1,3-diol, **98** was, however, used in the enantioselective synthesis of (*S*)-1,3-dihydroxypentan-2-one, *S*-**71**, as described by Enders *et al*.[65] This was then used as a standard for a GC–MS-assay to determine the stereochemistry of the product from the transketolase biotransformations being examined by the BiCE group.

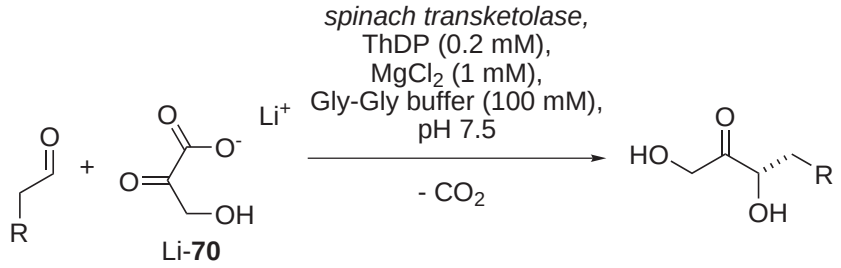
3.4 Synthesis of (*S*)-1,3-dihydroxypentan-2-one

Transketolase (EC 2.2.1.1.) is an important enzyme that is used to perform stereospecific and stereoselective carbon–carbon bond formation.[208–221] It is found in the pentose phosphate pathway in animals and the Calvin cycle within photosynthesis. In the pentose phosphate pathway, transketolase catalyses the thiamine diphosphate (ThDP) mediated trans-



Scheme 3.15: Mechanism of the transketolase catalysed carbon–carbon bond formation in yeast (*S. cerevisiae*)[34]

fer of a two carbon fragment from D-xylulose-5-phosphate to D-ribose-5-phosphate which forms sedoheptulose-7-phosphate. The other product of this reaction is glyceraldehyde-3-phosphate, which is formed from D- xylulose-5-phosphate. It also catalyses the conversion of erythulose-4-phosphate to fructose-6-phosphate in a similar manner. The full pathway is shown in Scheme 3.15.[34] This transfer is fully reversible unless β-hydroxypyruvate is used as the donor, as this renders the reaction irreversible (Scheme 3.16).[222]



Scheme 3.16: The reaction of a variety of non-α-hydroxyaldehydes with Li-**70** can be performed using spinach transketolase[223]
ThDP=thiamine diphosphate

Transketolase is popular for use in industry as it accepts a large variety of substrates and forms carbon–carbon bonds in an enantioselective manner. It is usually used with α-hydroxy aldehyde acceptors and hydroxypyruvic acid, **70**, (both the free base and Li salt) with which it stereospecifically forms the α-(*R*)-hydroxyaldehyde.[213–221,224] Non-α-hydroxylated aldehydes tend not to be used in the reaction as the relative rate of reaction is much lower with them, typically 5–35% of the rate of α-hydroxylated species.[219–221] This reaction with non-α-hydroxylated species was of particular interest to members of the BiCE research group, especially the reaction with the non-α-hydroxy-aldehyde propanal to form 1,3-dihydroxypentan-2-one, a small molecule that is difficult to synthesize chemically. With this goal, the directed evolution of transketolase was investigated by members

of the BiCE group, directed towards the enhancement of the enzyme's specificity and activity towards propanal.[225,226]

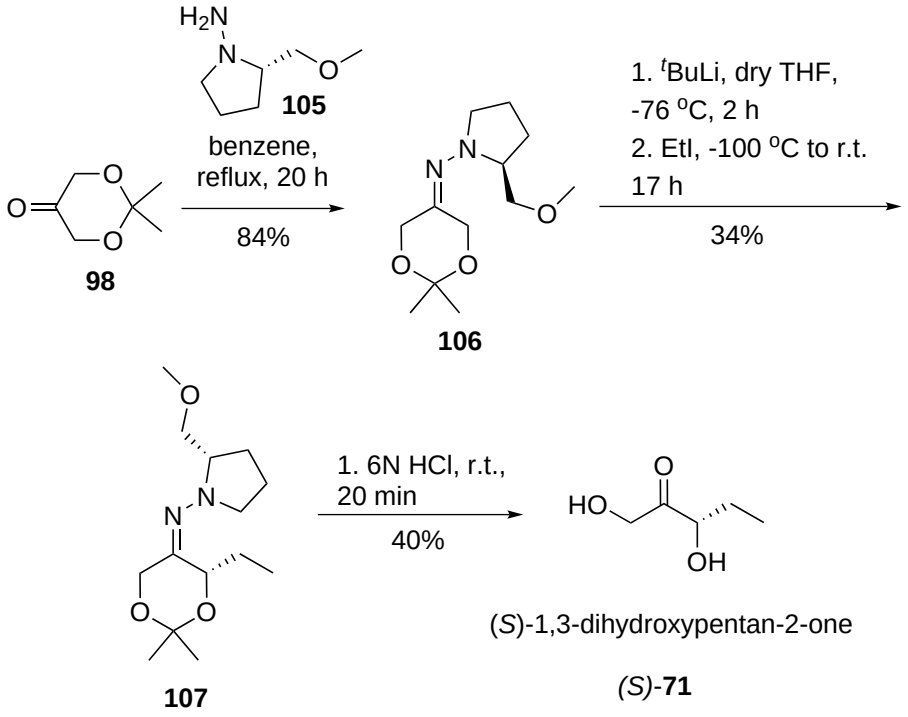
In the literature there are a number of examples where the enantioselectivity of the carbon–carbon bond forming reaction of transketolase with α-hydroxyaldehyde acceptors had been examined.[213–218] The determination of the enantioselectivity was accomplished by comparing the products formed with standards of known enantiopurity, and in general the products were formed in high enantioselectivities. However, only one piece of work looking at the reaction with non-α-hydroxyaldehyde acceptors had been carried out, by Dalmas and Demuynck.[219,223] They found that when spinach tranketolase was used, e.e.s of 60–76% were obtained with with non-α-hydroxyaldehyde acceptors as shown in Scheme 3.16 and Table 3.3.[223]

R	e.e. (%)
OCH_3	60
SCH_3	64
CH_2OH	>95
CH_2SCH_3	76

Table 3.3: The reaction of a variety of non-α-hydroxyaldehydes with Li-**70** catalysed by spinach transketolase, as shown in Scheme 3.16[223]

The major enantiomer was the (*S*)-enantiomer in all of these cases, and so it was anticipated that the major enantiomer formed by the *E. coli* transketolase would also be the (*S*)-enantiomer.[223] The synthesis of *(S)*-1,3-dihydroxypentan-2-one, (*S*)-**71**, was therefore carried out to create a standard against which to compare the stereoselectivity of transketolase. The acetonide species, **98**, synthesized when examining possible cyclic acetal protecting groups, was the starting material used in the synthesis.[65,201] This synthesis used the

SAMP-hydrazone method developed by Enders *et al* to provide the selectivity. First SAMP, **105**, was coupled to the acetonide, **98**, to give a SAMP-hydrazone, **106**). The SAMP group can then direct the attack of an alkyl halide onto the deprotonated hydrazone to give **107**. This could then be fully deprotected to give an enantiomerically pure sample of *(S)*-1,3-dihydroxypentan-2-one, (*S*)-**71**.[227,228] The overall synthesis using SAMP purchased from Aldrich is shown in Scheme 3.17. The SAMP was purchased rather than synthesized as large quantities were not needed and this would save time during the synthesis.

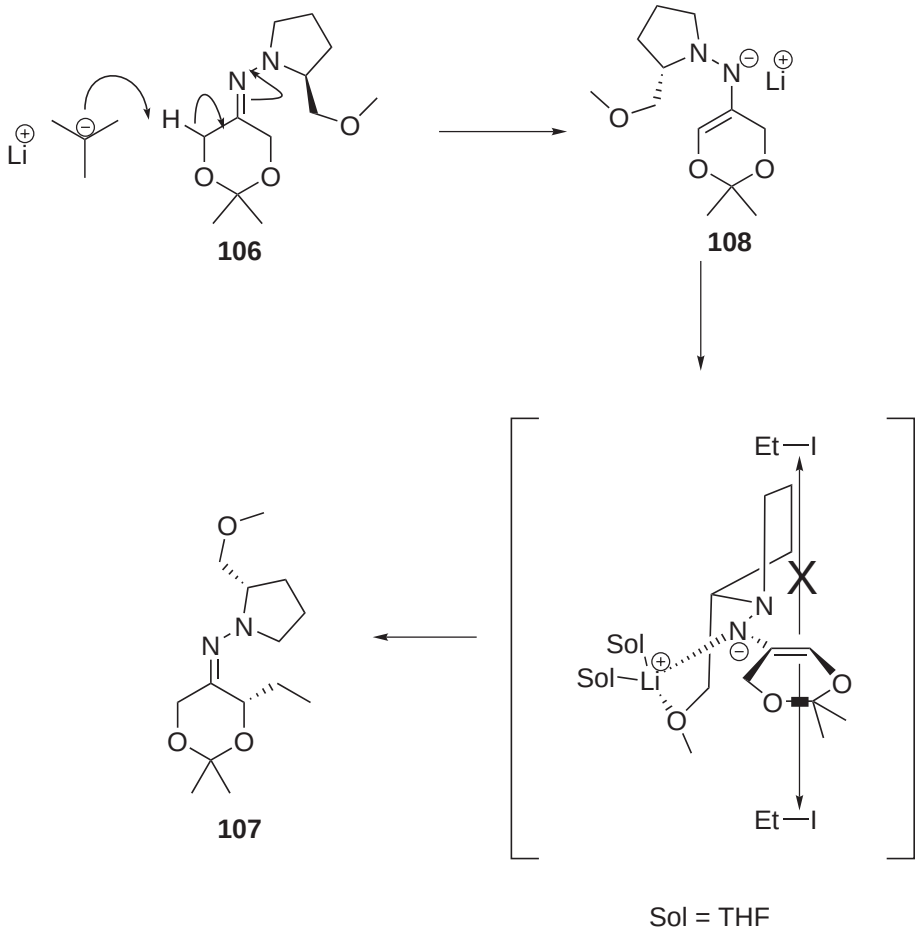


Scheme 3.17: Synthesis of (*S*)-1,3-dihydroxypentan-2-one using Enders' SAMP-hydrazone method[65,201]
SAMP=(*S*)-(–)-1-amino-2-(methoxymethyl)pyrrolidine

2,2-Dimethyl-1,3-dioxan-5-one, **98**, was coupled to the SAMP chiral auxiliary, **105**, to give the SAMP-hydrazone, **106**, in 84% yield, by mixing them together in refluxing ben-

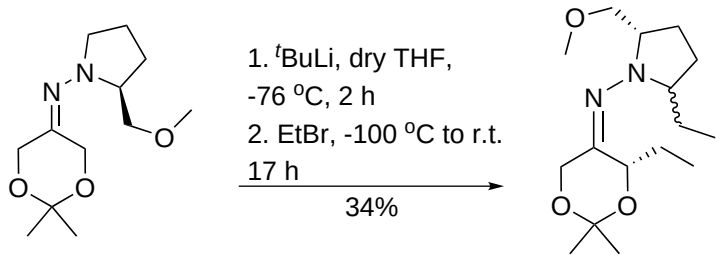
zene. This was a similar yield to the 90% obtained in the literature.[65] SAMP-hydrazone, **106**, was then converted to the aza-enolate, **108**, by lithiating the material with ^{t}BuLi. This adopts the *E* isomer conformation around the C=C bond, with the *Z* isomer conformation dominant around the C-N bond, as it is more stable than the three other possible isomers.[229] Ethyl iodide was then added and it was attacked by the nucleophile that has been formed, this attack coming from the sterically more accessible side of the complex. The stereospecificity of the reaction comes about because the alkyl lithium species, formed when the lithiation occurs, adopts a *pseudo*-six-membered ring structure as shown in Scheme 3.18. The yields obtained were significantly lower than those in the literature, as a 34% yield for the alkylation to give **107** was obtained, compared with the 58% yield seen in the literature for this step. This probably came about because we were working on a significantly smaller scale than that described in the literature.

A dialkylated species, **109**, was also observed as a product from the reaction, this was proposed to have the structure shown in Scheme 3.19. The stereoselectivity of the second alkylation could not be determined as little material was isolated. The second alkylation is not mentioned in the literature, however, the low reported yield of 58% for the desired alkylation product could be because of this.[65] Consequently the reaction conditions were modified in an attempt to avoid the formation of **109**, and by using iodoethane instead of bromoethane the second alkylation was suppressed, with the yield of **107** remaining 34%. Due to the problems associated with handling ^{t}BuLi, the use of other lithiating agents in the reaction was also investigated. The reaction worked with ^{s}BuLi, though with a decreased yield of 23%, and the reaction did not proceed at all when ^{n}BuLi was used. When ^{s}BuLi was used with bromoethane the dialkylated product was not seen, however, even though the reaction could be performed with ^{s}BuLi, ^{t}BuLi was the favoured reagent for the reaction



Scheme 3.18: Facial selectivity during alkyl halide addition[229,230]
Sol=solvent

as it gave a significantly higher yield.

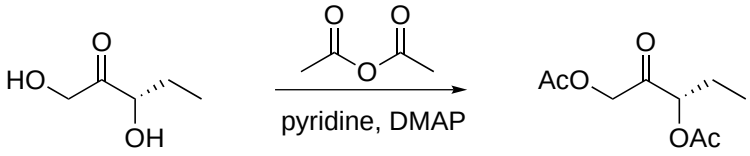


106 **109**

Scheme 3.19: Dialkylation during the (*S*)-1,3-dihydroxypentan-2-one synthesis

Once the alkylation had been carried out, removal of both the chiral auxiliary and the acetal protecting group was investigated. It was possible to remove the auxiliary selectively, to give acetonide protected **71**, in 45% yield by using a mild acidic work up involving aqueous oxalic acid.[231] However, to access the desired product, (*S*)-**71**, it was more efficient to remove both protecting groups simultaneously under stronger acidic conditions. This gave the desired product, (*S*)-**71**, in 40% yield. This was much lower than the 86% yield obtained in the literature, however, once again this is likely to be because the reaction was performed on the microscale unlike in the literature where a multigram synthesis was reported.[65] The optical rotation of the product was then determined to confirm that the *S*-enantiomer had been formed.

Once (*S*)-1,3-dihydroxypentan-2-one, *(S)*-**71**, had been prepared, it was diacetylated for application in a GC–MS assay which had been developed to determine the relative stereochemistry of product, 1,3-dihydroxypentan-2-one, **71**, from the transketolase reactions. This acetylation was required to get the sample to 'fly' in the mass spectrometer. The diacetylation was initially attempted using acetic anhydride and pyridine, however, the



(S)-**71** *3-(S)*-**110**

Scheme 3.20: (*S*)-1,3-Diacetoxypentan-2-one synthesis[232]
DMAP=4-dimethylaminopyridine

reaction did not proceed.[233] 4-Dimethylaminopyridine (DMAP) was, therefore, added to catalyse the reaction, and it proceeded in quantitative yield to 3-*(S)*-**110** (Scheme 3.20).[232] The optical rotation was measured to confirm that the acetylating conditions had not racemized the product and it was seen that it had not. This confirmed that the GC–MS assay would give accurate results as the derivatization would not affect the product. The GC–MS assay developed to determine the e.e. of the 1,3-dihydroxypentan-2-one produced using transketolase was then applied to (*S*)-1,3-dihydroxypentan-2-one prepared *via* the chiral auxiliary. The GC trace obtained confirmed 3-*(S)*-**110** was formed with an e.e. > 95% *via* the SAMP route. This GC trace was then compared with a trace from the product prepared using wild type *E. coli* transketolase (Figure 3.8). This indicated that **110** from the transketolase reaction, performed by the members of the BiCE research group carrying out the directed evolution work, was formed in 58% e.e. ([*S*]-isomer major).[222] This result was comparable with previous studies using spinach transketolase and similar aldehyde acceptors.[219,223]

This work helped to demonstrate that an assay to analyse the biocatalysis products could feasibly be developed rapidly during the initial work of modelling and analysing the biocatalytic reaction. This was necessary as the assay needed to be in place before the mutation and biocatalysis improvement cycle could be initiated. This, therefore, confirmed that the initial sections of the process development route shown in Figure 1.4 on page 22 were valid

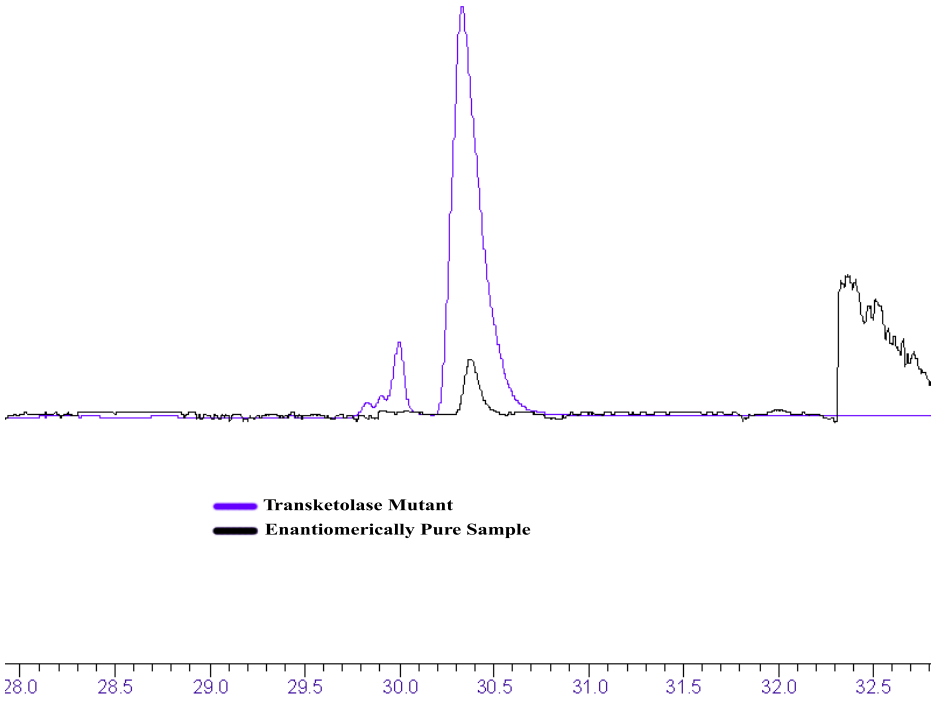


Figure 3.8: The GC trace obtained form the GC–MS assay performed on chemically synthesized 3-(*S*)-1,3-diacetoxypentan-2-one, 3-(*S*)-**110**, compared with the trace obtained from the acetylated product, **110**, of an enzymatic synthesis of the same molecule. The GC–MS assay was performed on a β-Dex 225 chiral column (Supelco, 30 m; 0.25 mm). 1 mL of sample was injected with the carrier gas, helium (15 psi). The sample was injected at 250 °C with the oven at 60 °C. The oven temperature was then increased 3 °C/min. The detector was at 300 °C and was a flame ionized detector

in principle.

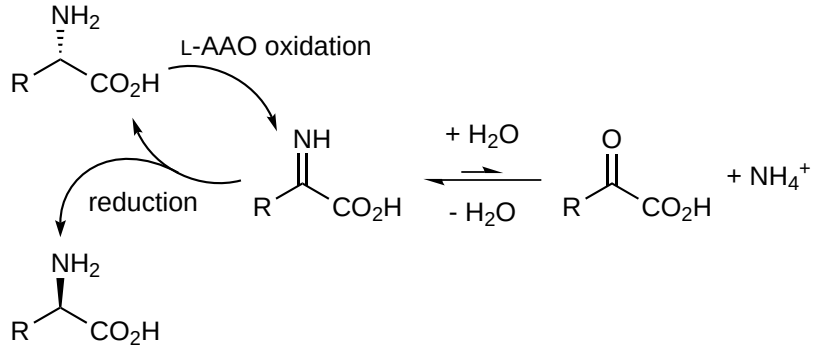
4

Development of Reductive Amination in Water

4.1 Reaction discovery

Cyclohexanone was chosen as the test substrate upon which to develop the reductive amination reaction as it was commercially available and structurally similar to the acetonide, **98**. The reaction was to be used in sequence with a biocatalytic step and would need to work well under the mild conditions necessary for the enzyme to function. This meant that the reaction would ideally need to be performed in water at a pH of about 7 and at 25–37 °C. As well as this, the reaction being developed needed, if possible, to be both stereoselective and enantioselective. There were three literature examples of reductive amination being performed under similar conditions to these, and these provided a starting point from which to develop the reaction. These included a pair of papers from Turner's research group in which an enzyme based deracemization to form D-amino acids in high enantiomeric excess

was performed (Scheme 4.1).[176,177]

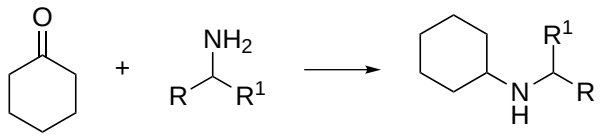


Scheme 4.1: Overall deracemisation reaction developed by the Turner research group including the reductive amination step[176,177]
L-AAO=L-amino acid oxidase

In the papers from the Turner group, DL-α-amino acids were deracemized using dynamic kinetic resolution, to give D-amino acids in high enantiomeric excess.[176,177] This resolution was performed by selectively oxidizing the L-amino acid with an L-amino acid oxidase from *Proteus myxofaciens*, followed by a non-stereoselective reduction. The oxidation gave an achiral intermediate, and after several repetitions of this sequence the desired D-amino acid was present in yields of up to 90% and e.e.s of up to 99%.[176,177] Amine-boranes were chosen as the reducing agents as they are stable in water at neutral or basic pH.[234,235] They also offer a wide range of reducing capabilities and selectivity, because their reducing ability is dependent upon the amine species present. This is because the reducing ability of the amine-boranes is dependent upon the stability of the boron–nitrogen bond. If there is incomplete charge transfer then the bond will be weak, resulting in a stronger reducing agent. This is dependent upon two factors, the electronegativity of the amine group and also steric effects, this is because strongly basic, unhindered amines impart a lot more reducing character than weakly basic, hindered amines.[116,235] For example ammonia borane, is a much stronger reducing agent than tri-*tert*-butylamine borane, **112**, due to its stronger

basicity imparting greater electronegativity to the boron atom.[116,235] This widely variable reducing character means that amine-boranes can show a wide range of reactivities, which means that the amine can often be tuned to make the reducing power of the agent suitable for the reaction being performed.[234]

In their study, Turner *et al* found that the reducing agent concentration had to be high enough to ensure the immediate reduction of the intermediate α-imino acid.[176,177] This was to guarantee that none of the intermediate was hydrolysed to the α-keto acid. A high concentration of ammonium formate was also used to help prevent this hydrolysis. This reaction provided a starting point for our research, as the reaction was similar to the one envisioned at the start of the project, as an imine would be formed and chemically reduced in the same pot as a biocatalytic reaction. To ensure that cyclohexanone, **115**, would react as required and also that it could be purified, reductive amination was performed upon it using several well established reductive amination methods (Scheme 4.2 and Table 4.1).[178,234]



115

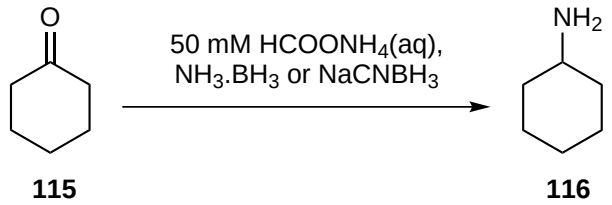
Scheme 4.2: Reductive amination of cyclohexanone

These reactions showed that cyclohexanone could be reductively aminated using a range of solvents including water-based mixtures, and the product isolated. For example, entry 4 in Table 4.1 compared favourably to the literature where a 94% yield was obtained.[178] The reactions using benzylamine in polar solvents were significantly lower yielding (*e.g.* entry 5 in Table 4.1), suggesting that the solvent interacts with the reaction unfavourably in

Entry	Reducing agent	Amine	Solvent	Isolated yield (%)
1	$NaBH_4$	benzylamine	methanol	41
2	$NaCNBH_3$	benzylamine	methanol	46
3	$NaBH(OAc)_3$	benzylamine	1,2-DCE	81
4	picoline borane	aniline	H_2O/AcOH (10:1)	85
5	picoline borane	benzylamine	H_2O/AcOH (10:1)	15

Table 4.1: Reductive amination of cyclohexanone using literature based reductive amination methods
DCE=1,2-dichloroethene

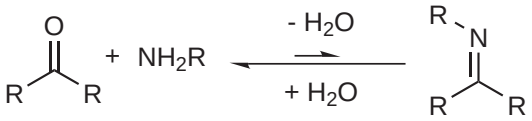
these cases. Reductive amination upon cyclohexanone, **115**, was then performed using the two sets of optimized conditions developed by Turner *et al* with the hope of forming the primary amine, **116** (Scheme 4.3).[176,177]



Scheme 4.3: Reductive amination attempt on cyclohexanone using the optimized conditions from Turner *et al*[176,177]

Amine **116** was not formed with either set of reaction conditions, as reductive amination did not occur. In order to ensure that the reaction was not substrate specific it was repeated using a variety of substrates including *t*BDMS and *t*BDPS protected 1,3-dihydroxyacetone, **84** and **85**, ethyl pyruvate, methyl pyruvate and benzaldehyde. However, the reaction never gave the amine (**116**) instead giving solely the reduction product, cyclohexanol. Therefore, the full range of conditions used by Turner *et al* were tested upon all the substrates. As well as this, the conditions were varied widely, varied including the reducing agent (ammonia borane, pyridine borane, sodium borohydride, sodium cyanoborohydride, lithium boro-

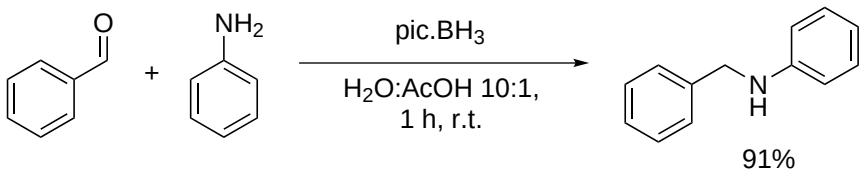
hydride, picoline borane, dimethylamine borane, morpholine borane and *tert*-butylamine borane), ammonia source (ammonium hydroxide, ammonium acetate, ammonium formate and ammonium chloride), reaction concentrations and temperatures (room temperature–60 °C). None of the reactions gave the desired amine product, but rather only the alcohol from reduction of the carbonyl group, suggesting that the imine was not being formed. This is probably because the imine is less likely to form in water as it involves the loss of one molecule of water (Scheme 4.4). This would make the reaction in water thermodynamically unfavourable and therefore drive the equilibrium away from the imine and towards the starting carbonyl and ammonia. In addition to this, imines are usually unstable in water as they are easily hydrolysed, which also suggests that the rate determining step in water will be the imine formation.[114,236]



Scheme 4.4: Imine formation in water

Turner *et al* mention that they attempted the reductive amination using ammonia borane upon 4-methyl-2-oxo pentanoic acid, with the hope of forming D-leucine.[177] However, the reaction failed suggesting that the imine was not hydrolysed to the carbonyl group during their biocatalytic deracemization. This is most probably because of the large excess of ammonium ions present, combined with the low concentration of imine present in the reaction mixture at any one time due to the rapidity of the chemical reduction step compared with the biocatalytic step.[24] Later papers from Turner *et al* do not include the hydrolysis in the reaction scheme, suggesting that they think it is a very minor side reaction. There was another literature example of reductive amination being performed in an aqueous environment

from the Sato group, an example of which is shown in Scheme 4.5.[178]



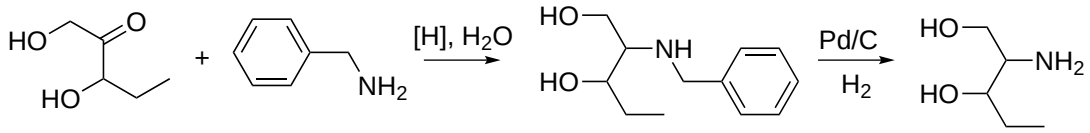
Scheme 4.5: An example of a reductive amination reaction performed in the presence of water from the work by Sato *et al*[178]

Entry	Carbonyl	Amine	MeOH:H_2O (10:1) (%)	H_2O:AcOH (10:1) (%)	AcOH (%)
1	benzaldehyde	aniline	95	91	99
2	benzaldehyde	octylamine	72	71	-
3	hexanal	aniline	82	73	77*
4	isopropylacetone	aniline	95	90	-
5	isopropylacetone	benzylamine	80	51	63
6	cyclohexanone	benzylamine	73	-	78

Table 4.2: Reductive amination using picoline borane performed by Sato *et al*[178]
*=reaction performed with no acetic acid as solvent

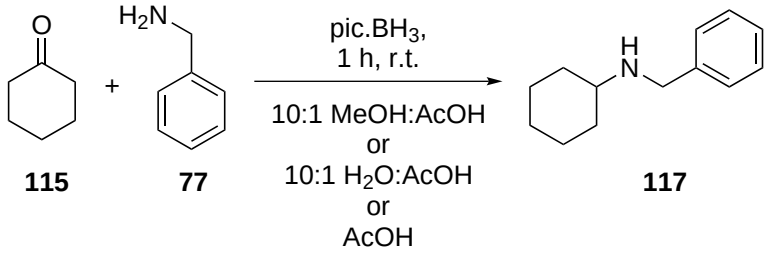
In this paper Sato *et al* performed reductive amination upon a variety of ketones and aldehydes, including cyclohexanone and benzaldehyde, using a range of primary and secondary amines. The reaction was initially performed in wet methanol, and having confirmed that the reaction proceeded in the presence of water they used a 10:1 water:acetic acid mix as the solvent, and observed that the reaction still gave high yields (Table 4.2). α-Picoline borane was used as the reducing agent as it is a commercially available crystalline solid that is more stable than pyridine borane, and so is easier to store and handle. Apart from the choice of reducing agent, the most important difference between this work and that of Turner *et al* was that both primary and secondary amines were used as the amine source, rather than ammonia, so that secondary or tertiary amines were obtained as the products.

This meant that a deprotection step would be required to access the desired primary amines (Scheme 4.6).



Scheme 4.6: The full synthesis if a secondary amine product is formed, including the deprotection

The reaction conditions described by Sato *et al* had already been tested, as the reductive amination of cyclohexanone, **115**, with aniline in a 10:1 water:acetic acid mixture as solvent had been performed during the initial examination of the feasabliity of reductive amination in water (entry 4 in Table 4.1). This reaction had given a yield of 85%, close to the literature yield of 94%.[178] The same reaction conditions were used to perform the reductive amination with benzylamine, as this would be a better option in the full synthesis as the benzylamine group could be easily cleaved.



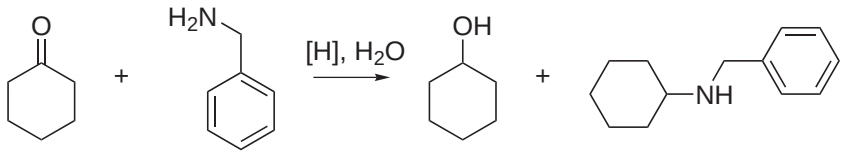
Scheme 4.7: The reductive amination of cyclohexanone using benzylamine and the full range of conditions reported by Sato *et al*[178]

The Sato group carried out the reductive amination reaction on cyclohexanone, **115**, with benzylamine, **77**, in wet methanol and glacial acetic acid (entry 6 in Table 4.2).[178]

This gave secondary amine **117** in 73% yield in 10:1 methanol:acetic acid and 78% yield in glacial acetic acid. When the reaction was performed in 10:1 water:acetic acid, **117** was obtained in a purified yield of 15%. This low yield was due to the predominance of the reduction of cyclohexanone to cyclohexanol, an unwanted side reaction also observed by Sato *et al* for the reactions in water.[178] They suggested that the acid catalyst was necessary to increase the yield, and it was also seen that the choice of amine dramatically affected the amount of product formed. These test reactions had demonstrated that the reaction was feasible, working on several different substrates, providing a starting point for the investigations.

4.2 Reaction development and optimization

4.2.1 Reaction screening using an NMR-based assay



115 **77** **118** **117**

Scheme 4.8: Model system of reductive amination reaction used with the NMR-based assay

As a result of successfully repeating the work described in the literature, it was decided to start the development work from the reaction reported by Sato *et al*, and the model reaction explored is shown in Scheme 4.8.[178] A rapid screening technique was considered the best way to develop the reaction, as it would allow a large number of reaction conditions to be examined. This initial large screen could then be used to develop the reaction based

upon the best results obtained. Therefore, an assay needed to be developed to aid reaction development. Initially the possibility of creating an assay using NMR spectroscopy was explored. This was because the NMR spectrometer had an auto-sampler allowing for automation, and would also hopefully allow the yield to be calculated from a 10 mg sample. This was attempted by using a deuterated solvent containing TMS as a standard against which to compare the relative integrations of the cyclohexanone, **115**, cyclohexanol, **118** and the secondary amine product, **117**, in a sample of known concentration. However, this did not give reproducible data and so the ratio of cyclohexanol, **118**, produced relative to the desired amine product, **117** (Scheme 4.8) was examined, as this data had been shown to be reproducible. This information could be used to help optimize the reaction, as the major side product of the reductive amination was the alcohol from the reduction of the carbonyl group (Figure 4.1).

The reaction could be optimized to some degree by trying to minimize the formation of the unwanted alcohol side product. This would potentially allow a purer product to be produced in higher yields, as no starting material would be wasted in undesired reactions. The amount of reduction that occurs is greatly influenced by the reducing agent used and its selectivity for the imine over the carbonyl.[236] A selection of reducing agents were, therefore, screened to see which gave the best selectivity (Table 4.3).

The data showed that sodium cyanoborohydride gave the best selectivity. This was not unexpected as it is often used to perform reductive amination (the Borch reaction/reduction), due to its high selectivity for imines.[126] This selectivity is due to the reduced activity of the borohydride group caused by stabilization of the boron-hydrogen bond, which makes it a weaker reducing agent. This makes it more selective towards imines, which have a

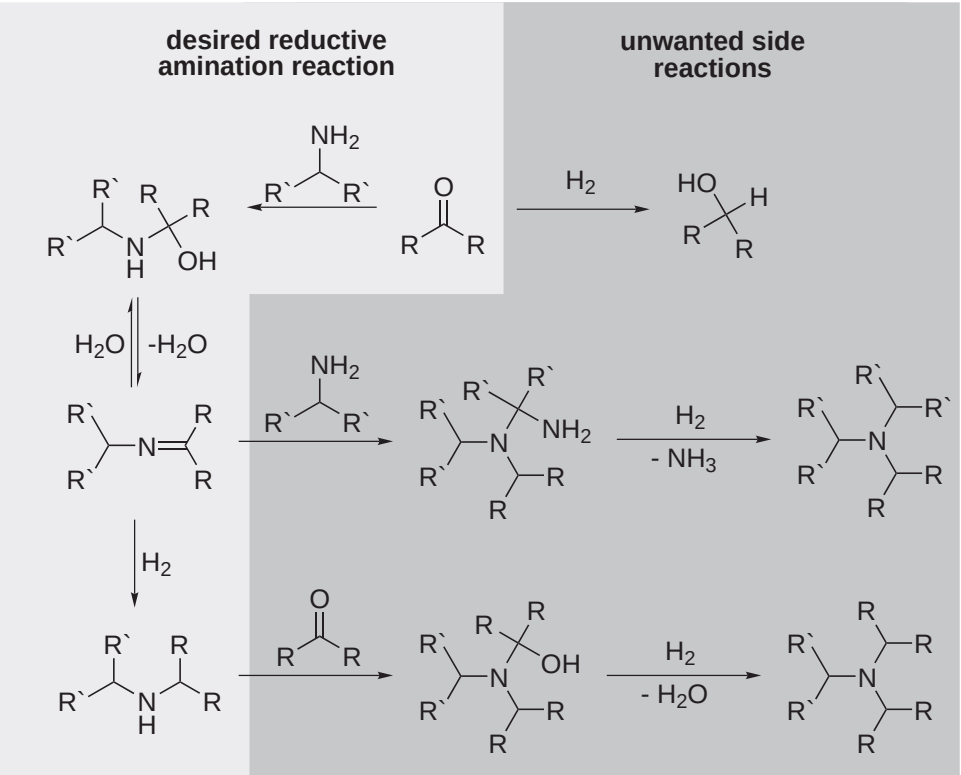


Figure 4.1: The possible side reactions in reductive amination[107]

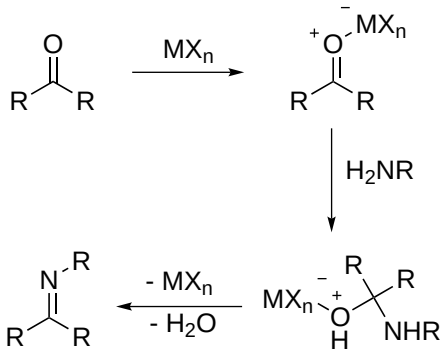
Reducing agent	Ratio (**117/118**)
$NaBH_4$	0.9
$NaCNBH_3$	8.3
4-methylmorpholine borane	1.9
pyridine borane	2.7
picoline borane	1.1
ammonia borane	0.8
$LiBH_4$	1.1
2,6-lutidine borane	3.0
benzylamine borane	0.6
tert-butylamine borane	0.5
dimethylamine borane	1.9

Table 4.3: Varying the reducing agent for the reductive amination of cyclohexanone in water. The reaction was performed on 2 mmol of cyclohexanone using one equivalent of benzylamine and one equivalent of reducing agent in 2 mL H_2O, the reaction was stirred for 1 hour at room temperature and the pH was not controlled. The ratio of compound **117/118** has been calculated from the integrations displayed on the spectrum. The average ratio from several reactions is reported

lower average bond dissociation enthalpy than carbonyl bonds (C=N 147 kcal/mol; aldehyde C=O 177 kcal/mol; ketone C=O 178 kcal/mol) as they are weaker bonds.[237] However, as this reaction was being developed for application in a one-pot system with an enzyme, sodium cyanoborohydride would not be the ideal choice of reducing agent due to the toxicity of both itself and the side products of the reaction.[177,238] Turner *et al* had initially used sodium cyanoborohydride for the enzymatic deracemization they were developing, but chose not to continue working with it for this very reason, preferring to use amine boranes. Amine boranes were found to be the next best reagents for the reaction, giving a range of ratios of product, **117**, to alcohol, **118**, from 0.5 to 3.0, indicating a range of selectivities. They are also water stable as highlighted by both Sato and Turner.[177,178] Pyridine borane was chosen for further investigation in the reaction development due to its commercial availability and ratio of amine to alcohol products of 2.7. The reaction using pyridine

borane was then tested with a range of additives in order to explore how they affected the substrate selectivity.

The first additives tested were Lewis acids, as they are known to catalyse reductive amination reactions. The suggested mechanism for this catalysis (Scheme 4.9) involves Lewis acid activation of the carbonyl functionality enabling formation of the aminol, and also subsequently aiding in the dehydration step by activating the imine towards nucleophilic attack. This complexation, which polarizes the bond, means that the imine formation is also more easily reversed.[236,238] This reverse reaction is usually avoided by removing water from the reaction mixture.[236,238]



Scheme 4.9: Lewis acid catalysis of reductive amination[238]

Surfactants were also examined, as they can greatly reduce the surface tension of water when used at very low concentration.[236] Also, once they are present in sufficiently high concentration (above the critical micelle concentration, CMC) they form micelles (Figure 4.2). In micelles all of the hydrophobic sections of the molecules are approximately aligned and all the hydrophilic sections interact with water.[236]

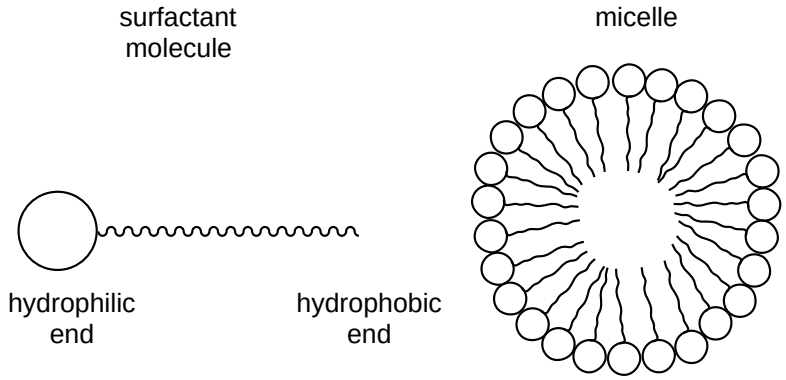
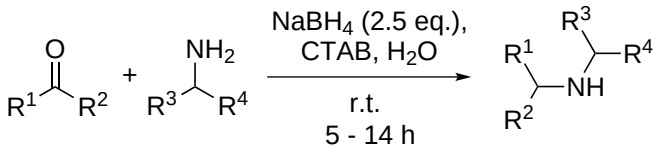


Figure 4.2: Surfactants and micelles

This can increase the reactivity of the substrates in several ways, firstly by helping to solubilize substrates that are water-insoluble or scarcely soluble.[239,240] They do this by forming a coating on the surface of the material, with the hydrophobic ends gathered at the surface of the material and the hydrophilic ends pointing out from it. The hydrophilic end groups can then associate with the surrounding water, dragging the compound into solution. As the insoluble particles decrease in size, the emulsified droplet becomes the same size as the micelles. At this point the substrate will be fully dissolved, and the solution will be clear. During this process the reactants are concentrated within the small volumes of the emulsified droplets, bringing them into much closer contact, thus, enabling them to react more easily.[241,242] This interaction with the hydrophobic tails of the micelles can also orient the substrates to improve reaction selectivity.[243–249] The counter ions of the surfactants can affect reactivity by stabilizing substrates, intermediates or products, as well as modifying the properties of the reacting species (*e.g.* dissociation constants).[250–252] In the literature surfactants have been shown to alter the reaction rate, mechanism and regio- and stereochemistry of reactions, with rate enhancements in the order of 10^6-fold being reported.[253–256] Reductive amination reactions have been performed using sodium borohydride in a concentrated aqueous solution of cetyl trimethyl ammonium bromide (CTAB).

This gave the amine product in purified yields of 84–97% (Scheme 4.10 and Table 4.4).[256] In these reactions chemoselectivity was observed for imine reduction using both aryl and alkyl aldehydes and ketones. The authors suggested that this chemoselectivity comes about because the reaction occurs within a micelle.[256]



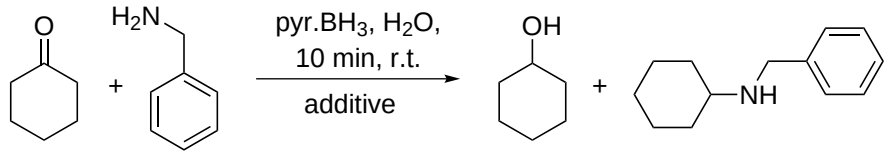
Scheme 4.10: Chemoselective reductive amination reaction using surfactants to form micelles and enhance the reaction[256]
CTAB=cetyl trimethyl ammonium bromide

Carbonyl	Amine	Product	Yield (%)
benzaldehyde	aniline	*N*-phenyl-benzylamine	92
butanal	aniline	*N*-butyl-aniline	89
acetophenone	aniline	*N*-(1-phenylethyl)-aniline	90
benzaldehyde	*N*-methyl-aniline	*N*-benzyl-*N*-methylaniline	94

Table 4.4: Chemoselective reductive amination reaction using surfactants to form micelles and enhance the reaction[256]

Chaotropic, or salting-in and salting-out, agents were also tested, as these can also be used to help solubilize reagents and reactants.[87,94,257] At low concentrations salts can stabilize charged groups in a molecule by acting as counter ions, encouraging the molecule into solution and enhancing its solubility. However, as the concentration is increased further the salt interacts more with the water, at the expense of its interactions with the molecule, rendering the molecule less soluble again. This technique is often used to recover proteins from aqueous solution, as it enables them to be solubilized and subsequently extracted. This salting-out effect occurs because a large increase in surface tension is created by salting-out

agents, such as lithium chloride, and the increased surface tension drives molecules out of the water matrix. This has also been used to good effect for carrying out reactions 'on water'.[100] All of the additives screened in the reductive amination reaction (Scheme 4.11) are shown in Table 4.5.



115 **77** **118** **117**

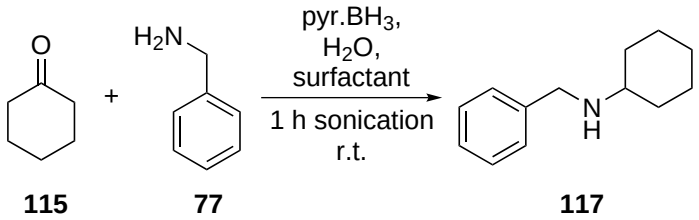
Scheme 4.11: Reductive amination reaction with additives used with the NMR-based assay

The results indicated that the best additives for the reaction were the water-stable Lewis acids. Both the anion and the cation of the Lewis acid affect the reaction, with the anion having a much larger effect on the selectivity. Triflate salts offered the best reaction selectivity, with the exception of polystyrene bound scandium triflate which gave a very poor result, most likely due to poor swelling of the polystyrene beads in the water. The chaotropic agents had little effect on the selectivity of the reaction. The addition of surfactants reversed the selectivity of the reactants somewhat (entries 8, 9 and 10 in Table 4.5). This might have been caused by micelles separating the reagents by selectively encapsulating some, thus making them less likely to interact. The reduced selectivity might also be due to problems extracting the substrate and product at the end of the reaction for analysis. This is suggested because many of the surfactants tested made product extraction difficult due to the formation of emulsions. Although solutions were saturated with sodium chloride during extraction, to force the product into the organic phase, this also displaced the surfactant which led to masking of product signals in the NMR spectra. Therefore, only the reactions containing surfactants where it was possible to obtain reproducible data are

Entry	Additive	Ratio (**117/118**)
1	-	2.7
2	$InCl_3$	6.3
3	$Sc(OTf)_3$	9.9
4	$Yb(OTf)_3$	10.0
5	$Gd(OTf)_3$	10.0
6	$YbCl_3.6H_2O$	2.3
7	$Ds(OTf)_3$	9.7
8	PB-$Sc(OTf)_3$	1.3
9	SDS	2.6
10	CTAB	1.7
11	triethylene glycol	1.8
12	glycerol	1.0
13	β-cyclodextrin	1.2
14	sucrose	2.2
15	fructose	3.8
16	LiOH	2.9
17	guanidine HCl	2.1
18	LiCl	2.0

Table 4.5: Reductive amination of cyclohexanone in water with pyridine borane and various additives. The ratios are averaged over a minimum of three repeat experiments. The reaction was performed on 2 mmol of cyclohexanone using one equivalent of benzylamine, 10 mol% of the additive and one equivalent of reducing agent in 2 mL H_2O, the reaction was stirred for 1 hour at room temperature and the pH was not controlled
PB=polystyrene bound molecule

included in Table 4.5. The reaction was also tested with ultrasonic mixing, as this can encourage solubilization and micelle formation with surfactants. The results with ultrasonic mixing (Scheme 4.12) are shown in Table 4.6.



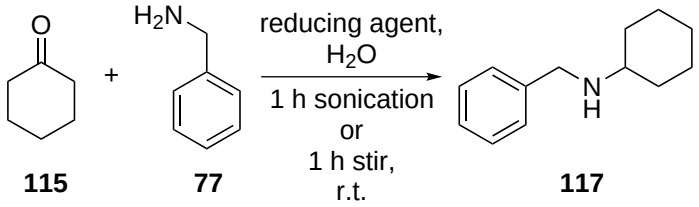
Scheme 4.12: Reductive amination reaction with ultrasonic mixing that was tested

Entry	Surfactant	Ratio (**117/118**)
1	SDS	2.5
2	CTAB	2.5
3	triethylene glycol	2.6
4	$Yb(DS)_3$	11.3
5	LiCl	1.5
6	$Me_4N^+Cl^-$	1.2

Table 4.6: Reductive amination of cyclohexanone with benzylamine using pyridine borane in water with various surfactants and sonication. The ratios are averaged over a minimum of three repeat experiments. The reaction was performed on 2 mmol of cyclohexanone using one equivalent of benzylamine, 10 mol% of the surfactant and one equivalent of reducing agent in 2 mL H_2O, the reaction was sonicated for 1 hour at room temperature and the pH was not controlled

The reaction selectivity for the formation of the desired secondary amine increased when the reaction was performed with either CTAB or triethylene glycol (TEG), but decreased when sodium dodecyl sulfate (SDS) was used. However, the best result obtained when using sonication was for ytterbium dodecyl sulfate ($Yb(DS)_3$), which is the same molecule as SDS except it has an ytterbium as its counter ion rather than sodium. This is a Lewis acid combined surfactant (LASC) and provides the benefits of both Lewis acid catal-

ysis and the presence of a surfactant. This might explain why it gave a much larger increase in selectivity compared with the other surfactants, as the Lewis acid also helped catalyse the reaction as seen previously (entry 4 in Table 4.5). Sonication conditions were also tested with salting-in and salting-out agents, because this should provide improved mixing, and would indicate whether the selectivities seen were the result of the reaction occurring 'on water'. This is because the sonication should provide enhanced mixing throughout the solution, including at the surface. This should ensure that if the reactants are floating on the water surface they are drawn into the solution and react there. The selectivity decreased when sonication was employed, indicating that the stirred reaction probably did occur 'on water'. Since mixed results were observed, and to test whether sonication alone had an effect on the reaction, or if the improved selectivity observed was due to the surfactant additives, several reductive amination reactions were performed without additives but using a variety of reducing agents, with sonication rather than stirring. The results for the reaction shown in Sceme 4.13 are shown in Table 4.7.



Scheme 4.13: Reductive amination reaction tested without additives but with either stirring or ultrasonic mixing

All of the reactants tested, except sodium cyanoborohydride, displayed an increased selectivity for the desired product when sonication was used. This improvement might be because of several factors. Firstly improved mixing might have occurred, which would improve the yield as the surface area of the reactants would be larger, giving a greater like-

Conditions	Ratio from stirred reaction (**117/118**)	Ratio from sonicated reaction (**117/118**)
$NaBH_4$	0.9	1.0
$NaCNBH_3$	8.3	1.6
pyridine borane	2.7	2.9
picoline borane	1.1	3.5

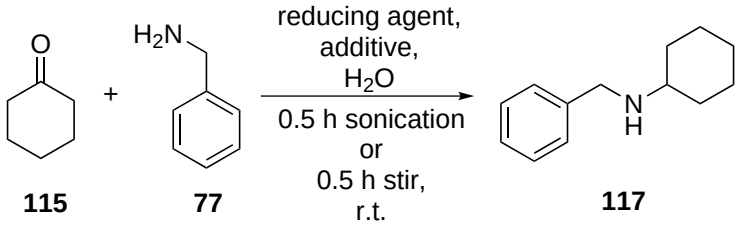
Table 4.7: Varying the reducing agent for reductive amination of cyclohexanone in water with sonication. The ratios are averaged over a minimum of three repeat experiments. The reaction was performed on 2 mmol of cyclohexanone using one equivalent of benzylamine and one equivalent of reducing agent in 2 mL H_2O, the reaction was stirred or sonicated for 1 hour at room temperature and the pH was not controlled

lihood of interaction. Another possible reason could be that sonication results in cavitation, in which bubbles are formed and subsequently collapse giving out a very localized burst of energy as heat.[258] This can also produce radicals due to the high temperatures and pressures experienced locally, which might then provide a novel reaction pathway.[258] However, the moderate increase in selectivity observed with sodium borohydride and pyridine borane suggested that in these reactions the sonication was causing more efficient mixing. Nevertheless, the much larger increase in selectivity with picoline borane suggests that under certain circumstances the reactivity might be being aided by sonication.

4.2.2 Isolated yields

As proton NMR spectroscopic analysis had not been able to be used to provide data with which to calculate reaction yields, the selectivity data that had been obtained was used to select the most promising sets of reaction conditions. These reactions were then repeated so that purified yields of desired product, **117**, from these reactions could be obtained. These would then act as a starting point for the optimization of the reaction. The initial set of con-

ditions selected for the reductive amination of cyclohexanone with benzylamine in water were a concentration of 0.5 mol/L with 30 minutes stirring at room temperature. Sodium cyanoborohydride, pyridine borane and picoline borane all exhibited good selectivity and so were tested, as was sodium borohydride to act as a control. The reactions were also performed with Lewis acids, as they had been shown to be the most useful additives. The isolated yields from these reactions (Scheme 4.14) are shown in Table 4.8.



Scheme 4.14: Test reductive amination reaction used for optimization with isolated yields

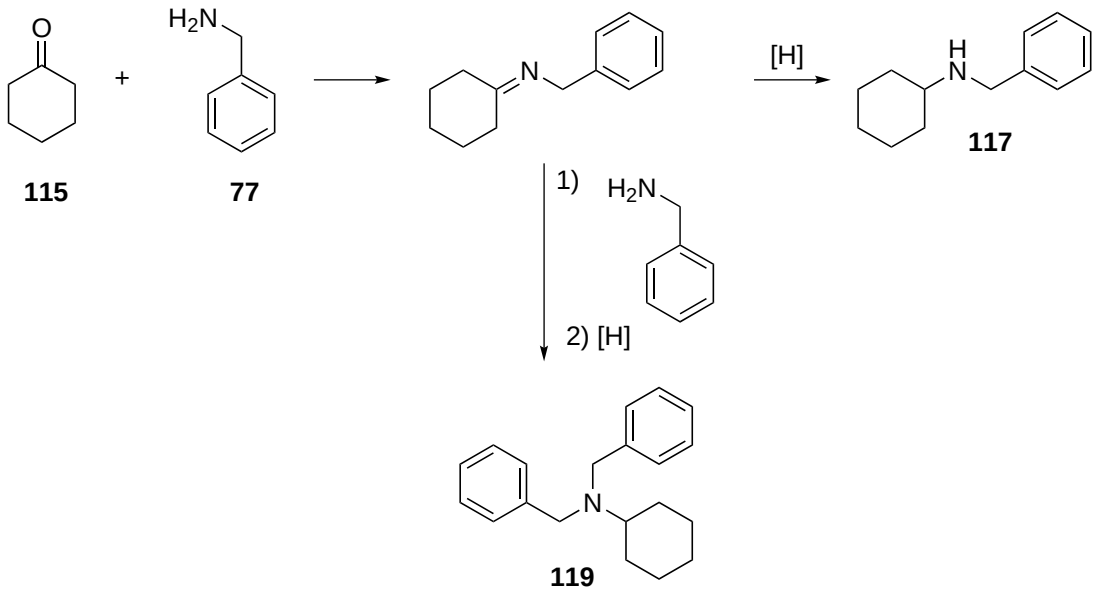
The isolated yield data, however, showed a lack of consistency (*cf.* entry 8 in Table 4.8). This was because the desired mono-addition product, **117**, co-eluted with one of the side products, **119** (Scheme 4.15).[107]

There was no indication in the literature of this being a problem with reductive amination reactions, so purification was attempted using a range of methods. A variety of different column conditions, including column packing materials were also explored, both normal phase and reverse phase. However, the separation was not successful with any of the column conditions tested. Preparative TLC, proved to be unsuccessful due to streaking. The use of scavenger resins was explored with several different resins being prepared by coupling the Merrifield resin to hydroxybenzaldehydes (*e.g.* **120** in Scheme 4.16).[259] To test the effectiveness of these, the extraction of the desired product, **117**, from a 1:1:1 mix-

Entry	Reducing Agent	Additive	Isolated yield for stirred reaction (%)	Isolated yield for sonicated reaction (%)
1	$NaBH_4$	-	6	23
2	$NaBH_4$	$Sc(OTf)_3$	0	0
3	$NaCNBH_3$	-	75	42
4	pyridine borane	-	20	17
5	pyridine borane	$Gd(OTf)_3$	38	15
6	pyridine borane	$InCl_3$	14	27
7	pyridine borane	$Sc(OTf)_3$	24	45
8	pyridine borane	$Yb(OTf)_3$	28/56*	48/61*
9	pyridine borane	$YbCl_3.6H_2O$	37	17
10	picoline borane	-	5	15

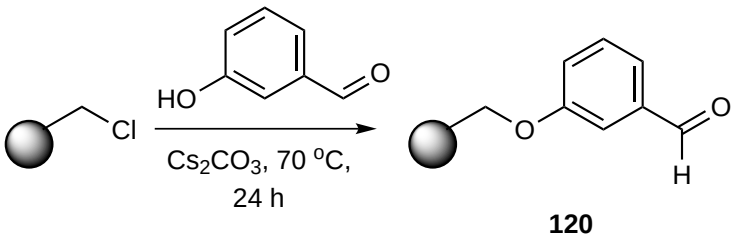
Table 4.8: Examples of isolated yields of **117** from reactions using a variety of reductive aminations. The results show the isolated yield of the mono-addition product of the reaction with 2 mmol of cyclohexanone, one equivalent of benzylamine, 10 mol% of the additive and one equivalent of reducing agent in 2 mL H_2O. The reaction was stirred or sonicated for 0.5 hours at room temperature and the pH was not controlled. The crude mixture was purified by flash silica column chromatography

*=two yields are shown, to indicate the lack of consistency observed



Scheme 4.15: Formation of the side product that partially co-eluted with the desired reductive amination product[107]

ture with benzylamine, **77**, and cyclohexanone, **115**, was attempted. The resin did not give reliable results as the benzylamine was also scavenged by the resin.

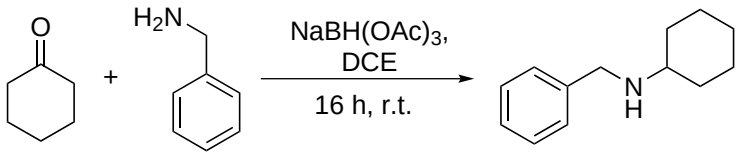


120

Scheme 4.16: Synthesis of one of the scavenger resins[259]

4.2.3 HPLC screening

As consistent purified yields were difficult to obtain, a high-performance liquid chromatography (HPLC) assay was explored.[260] The presence of an easily detected UV chromophore in one of the starting materials, **77**, and the product, **117**, was ideal as it allowed the system to readily detect these. However, the consumption of cyclohexanone, **115**, and the concomitant production of cyclohexanol, **118**, could not be monitored in the system as these systems do not contain readily detected UV chromophores.



115 **77** **117**
63%

Scheme 4.17: Reductive amination reaction used to synthesize sample for assay calibration
DCE=1,2-dichloroethene

A pure sample of the mono-addition product, **117**, was synthesized using existing chemistry in non-aqueous solvent and used to calibrate the assay.[105] This reaction was performed using sodium triacetoxyborohydride in 1,2-dichloroethane with overnight stirring, and the product was obtained in 81% yield (Scheme 4.17). A separation method was then developed, using this pure material as a standard, upon a reverse phase HPLC column, eluted with an isocratic mixture of 15% acetonitrile in water, containing 0.1% trifluoroacetic acid. The assay took 15 minutes and the products were identified using a UV detector at 254 nm. A calibration curve was then produced using the pure sample to enable yields to be calculated, and this calibration was regularly repeated to ensure that the results were consistent. The calibration curve gave a linear fit, and its line equation was used to calculate the concentration of product present in the samples. As the HPLC was automated the assay enabled us to rapidly screen a wide range of reaction conditions, which could be repeated to ensure that the assay was consistent. This was performed by repeating each reaction at least three times non-sequentially. As previously it had been impossible to examine the reaction yields, looking instead at the reaction selectivity, all of the previously examined conditions were re-evaluated. An example of the HPLC calibration is shown in Figure 4.3 and the data for this calibration is in Table 4.9. The HPLC was calibrated before each set of runs.

Reducing agent

The first variable to be screened was the choice of reducing agent, which was examined at both 30 and 40 °C to see if the reducing agent was affected by temperature. This might be caused by a number of effects, for example improved dissolution of the reducing agent. The results of the reactions (Scheme 4.18) are shown in Table 4.10.

	mg/mL	mol injected (x 10^{-8})	area
1	0.0503	0.266	8064
2	0.1005	0.531	13211
3	0.2010	1.063	28188
4	0.3015	1.594	40731
5	0.4020	2.125	59205
6	0.5025	2.657	70499
7	0.6030	3.188	84909
8	0.7035	3.719	104955
9	0.8040	4.251	124119
10	0.9045	4.782	133835
11	1.0050	5.313	151111

Table 4.9: HPLC calibration curve

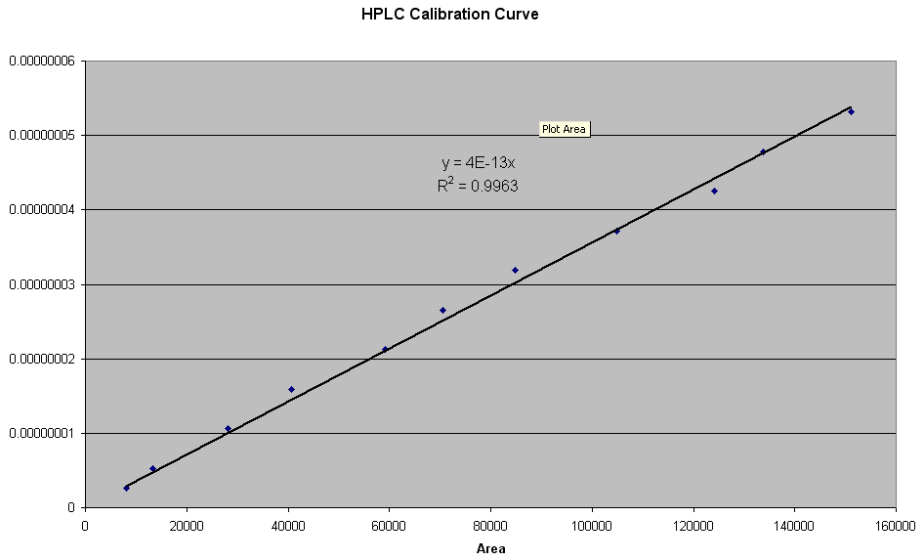
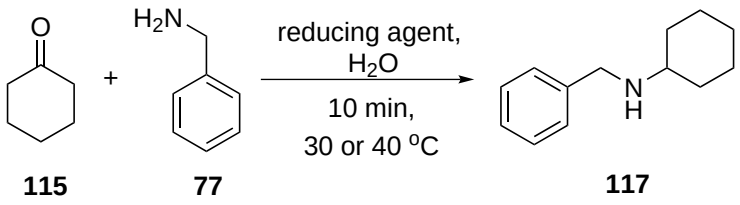


Figure 4.3: HPLC calibration curve



Scheme 4.18: Reductive amination reaction optimized using the HPLC assay

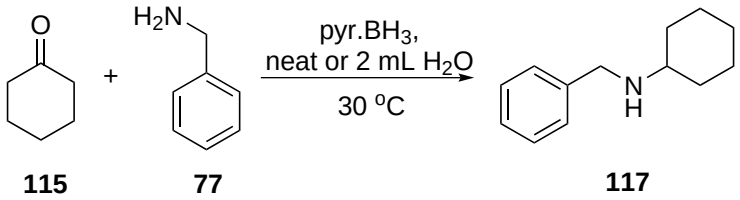
Entry	Reducing agent	Yield at 30 °C (%)	Yield at 40 °C (%)
1	pyridine borane	60	35
2	$NaBH_4$	17	9
3	$NaCNBH_3$	67	36
4	ammonia borane	2	2
5	4-methylmorpholine borane	n.d.	51
6	*tert*-butylamine borane	8	8
7	picoline borane	48	27
8	$LiBH_4$	20	32

Table 4.10: Product yields were determined by HPLC. The reaction was performed with various reducing agents at 30 °C and 40 °C. The reaction was carried out using 1 mmol of benzylamine and 1 mmol of the reducing agent with 1 mmol cyclohexanone. It was stirred for 10 minutes in 2 mL water at the desired temperature
n.d.=not determined

The results obtained from the initial HPLC screening matched the results from the NMR assay well, with the reagents that gave the best selectivity for imine reduction, to give **117**, giving the highest calculated yields. Anomalous results were obtained for 4-methylmorpholine borane (entry 5 in Table 4.10), because the HPLC assay had been developed using pyridine borane and the peak for 4-methylmorpholine overlapped with the product peak. This result was therefore discounted and 4-methylmorpholine borane not explored any further. Sodium cyanoborohydride gave the highest yields, however, due to the previously discussed problems with toxicities it was decided to optimize the reaction further using pyridine borane, which gave the second highest yield. Pyridine borane was also chosen as it is easy to handle, relatively stable and commercially available in large quantities at a consistent quality.

Reaction time

The effect of varying the reaction time was investigated by performing the reaction over progressively longer reaction times until the yield plateaued. These reactions were performed both neat (*i.e.* with no water present as solvent) as well as in water. If the reaction occurred 'on water' (*i.e.* the reaction mixture was concentrated upon the surface of the water) the reaction outcome in water should be the same as that under neat conditions. The results for the reaction shown in Scheme 4.19 are shown in Table 4.11 and are plotted in Figure 4.4.



Scheme 4.19: Reductive amination reaction investigated with varying reaction times, using the HPLC assay

Time (min)	Yield – reaction in water (%)	Yield – neat reaction (%)
2	32	24
4	59	23
6	58	26
8	58	28
10	60	28
20	61	37
30	52	47
40	59	52
50	40	52
60	60	50

Table 4.11: Product yields were determined by HPLC. The reaction was performed both neat and in 2 mL of water. The reaction was carried out using 1 mmol of benzylamine and 1 mmol of pyridine borane with 1 mmol cyclohexanone and stirred at 30 °C for the required length of time

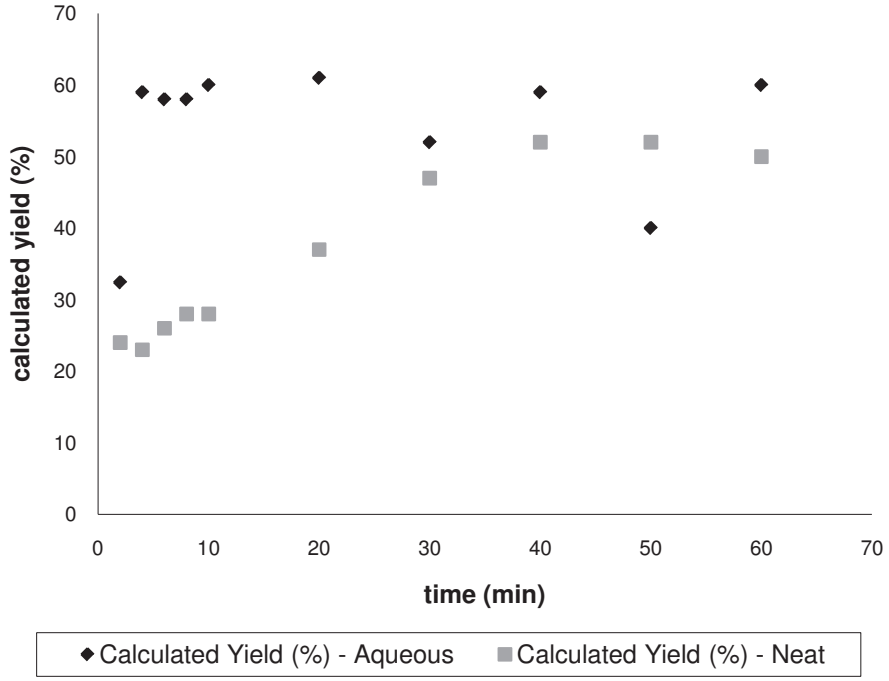
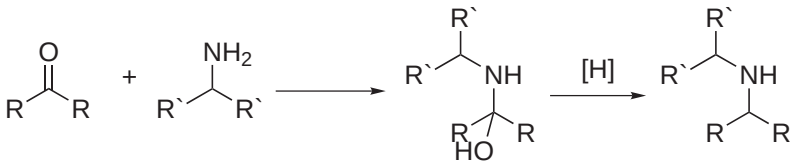


Figure 4.4: Plot of the yields determined by HPLC for reactions carried out with varying reaction times. The reaction was performed both neat and in 2 mL of water. The reaction was carried out using 1 mmol of benzylamine and 1 mmol of pyridine borane with 1 mmol cyclohexanone. It was stirred at 30 °C

When the reaction was performed neat, the yield steadily increased and started to plateau after 30 minutes. According to collision theory the frequency of the molecules' collisions will decrease as the concentration of the reactant decreases. Applying this to the reaction in water, the rate would be predicted to be slower initially, as the reactants are more dilute, but that the final yield of **117** would be similar to that of the neat reaction. This was not the case, as the reaction occurs much more rapidly in water plateauing after 4 minutes. The yield of **117** obtained for the reaction in water was also higher, with an ultimate yield of about 60% in water compared with 50% for the neat reaction. This suggests that the reaction is not occurring 'on water' as if this was the case then a similar reaction rate to that of the neat reaction would be obtained, rather than the much greater reaction rate observed at the start of the reaction. This suggests that the water is not acting solely as a passive support or as a solubilizing agent, but rather is taking an active role in the reaction. It might be possibly catalysing the reaction through the hydrogen bond network present in the bulk water, which could be stabilizing intermediates or activating reaction participants. Another possible explanation for the increased yield and reaction rate in water is that the water enables the reaction to go *via* a different mechanism (*e.g.* Scheme 4.20). This alternative reaction mechanism might occur because the large amount of water present in the environment makes the loss of water from the carbinolamine unfavourable. This would mean that the imine is not formed prior to reduction and it is the carbinolamine that is reduced. This possibility has been suggested before as part of the general mechanism for reductive amination in more traditional solvents.[107]

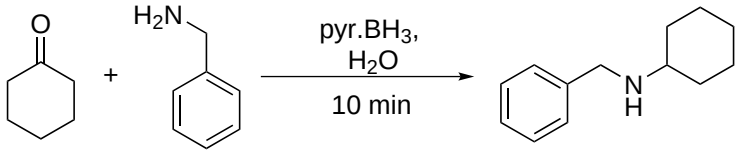
A reaction time of 10 minutes was selected for reactions in water, as even though the reaction plateaued at 4 minutes it was felt that 10 minutes would ensure that the reaction had reached completion.



Scheme 4.20: Possible reductive amination pathway in water

Reaction temperature

The initial test reactions suggested that increasing the temperature decreased the yield. Taking this as a starting point, a range of temperatures were investigated, including those both above and below 30 °C, and the results for the reaction (Scheme 4.21) are shown in Table 4.12 and Figure 4.5.



Scheme 4.21: Reductive amination reaction investigated with varying reaction temperature, using the HPLC assay

Analysis of the data in Table 4.12 indicated that the optimal reaction temperature was 25 °C. Heating the reaction lowered the yields, probably because it encouraged side-reactions, especially the reduction of the ketone. This was confirmed by an increase in the amount of cyclohexanol observed at higher temperatures. This optimal temperature of 25 °C was close to the operational temperature of the enzyme, which is approximately 30 °C, and so this temperature would not be a barrier to the development of a one-pot reaction.

Temperature (°C)	Yield (%)
15	66
20	70
25	76
30	60
35	51
40	35
45	25
50	29
60	9
70	22

Table 4.12: Product yields were determined by HPLC. The reaction was performed using 1 mmol of benzylamine and 1 mmol of pyridine borane with 1 mmol cyclohexanone in 2 mL water. The reaction was stirred for 10 minutes at a range of temperatures

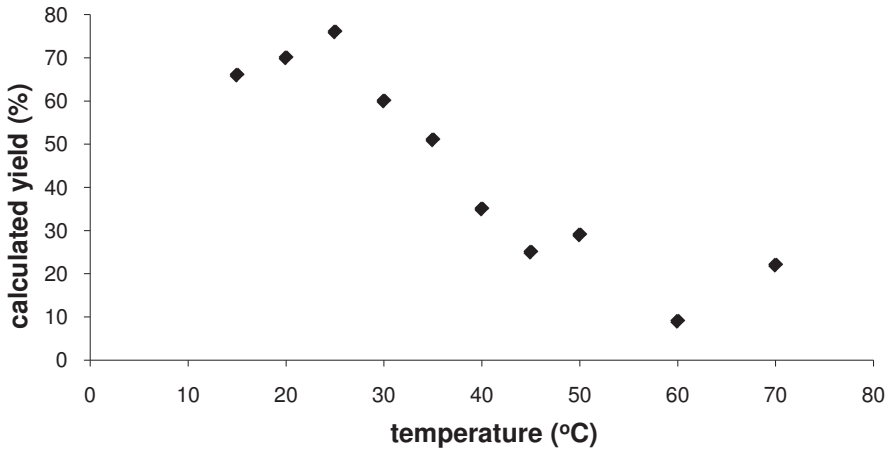


Figure 4.5: Plot of reaction yields for the reductive amination reaction performed at various reaction temperatures. Product yields were determined by HPLC. The reaction was performed on 1 mmol of cyclohexanone, using 1 mmol of benzylamine and 1 mmol of pyridine borane in 2 mL H_2O. The reaction was stirred for 10 minutes at the temperature indicated

Reaction pH

It has been reported in the literature that imine formation is disfavoured below pH 4, because the amine is protonated and will be unlikely to react with the ketone to form the carbinolamine.[114,236] The reaction is also disfavoured above pH 6 because the dehydration step will be slow as this reaction is acid catalysed (Scheme 4.6 and Figure 4.7).

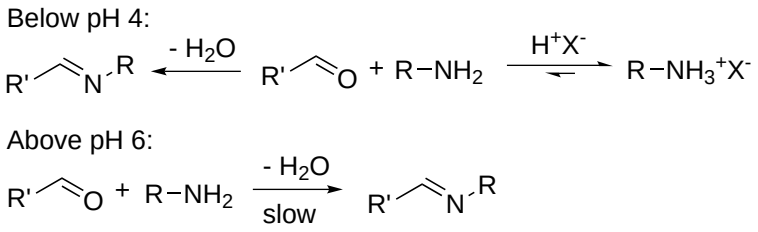


Figure 4.6: The reductive amination reaction is influenced by the pH of its environment[236]

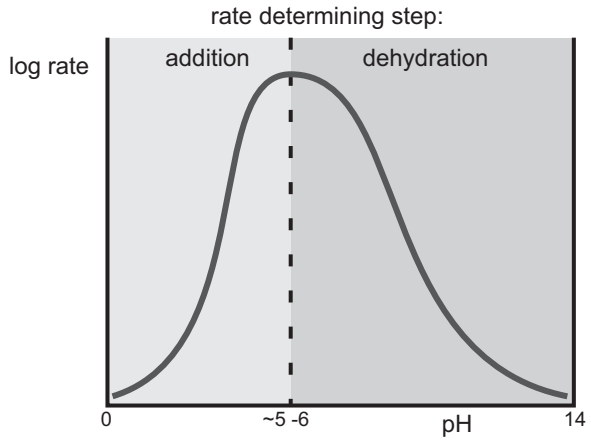


Figure 4.7: Variation of rate of imine formation with pH[236]
Adapted from Clayden J, Greeves N, Warren S, Wothers P. Organic Chemistry, First Edition, Oxford University Press: Oxford, 2001, p.350. By permission of Oxford University Press

The pH of the test reactions was found to be typically 11.4, which placed it within a pH

bracket that would encourage lower rates according to the literature, as acid catalysis could not occur. Again this suggested that a novel reaction might be occurring, with water acting as a proton donor, or possibly even an alternative base catalysed mechanism occurring (Scheme 4.8).

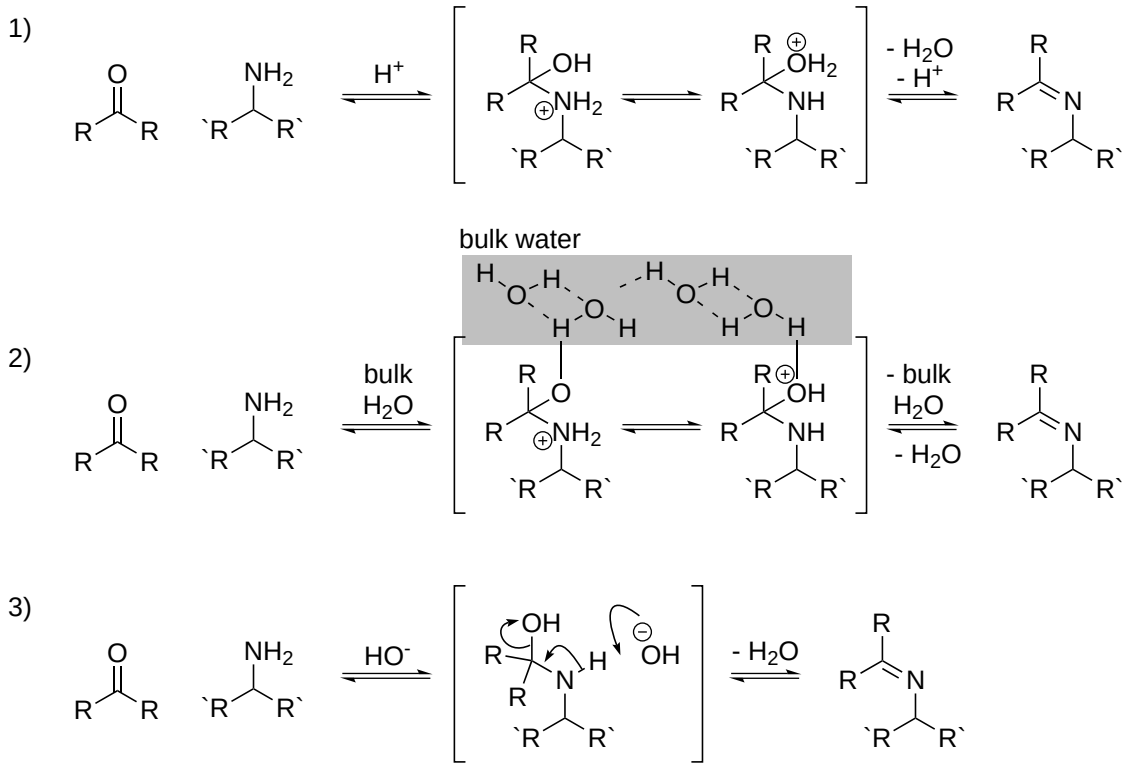


Figure 4.8: Possible mechanisms for the imine formation in water. 1 = acid catalysis; 2 = catalysis by H-bond donation into bulk water; 3 = base catalysis

In order to see whether the reaction worked under basic conditions only in water due to hydrogen-bonding to the bulk water (2 in Scheme 4.8), or whether there was a more general base catalysed mechanism (3 in Scheme 4.8), the reaction was performed in several non-polar solvents, including 1,2-dichloroethane, chloroform and ethyl acetate, at basic pH. All of the reactions proceeded but in low yields (typically 10–27%), indicating that there is a

more general base catalysed route for imine formation, or that the reductive amination does not go *via* the imine, but rather *via* the direct reduction of the carbinolamine (Scheme 4.9).

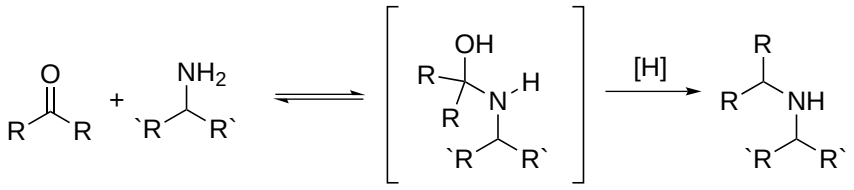
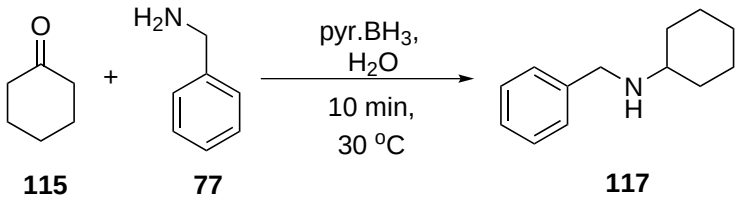


Figure 4.9: Possible alternative reductive amination route to the amine without the imine being formed

Alkaline reductive amination reactions have been reported in the literature for the formation of primary amines, but these tend to be under forcing conditions, for example in ammonia.[261] However, the reaction being investigated is more facile than this and so would be expected to work under less forcing conditions. Having confirmed that the reaction did not need to be performed at acidic pH, a series of reductive aminations at different pHs from pH 5.0–11.5 were performed for the reaction shown in Scheme 4.22. The results of which are shown in Table 4.13 and Figure 4.10.



Scheme 4.22: Reductive amination reaction investigated with varying reaction pH, using the HPLC assay

The data indicated that the optimal pH for the reaction would be 7.5. This is slightly higher than the pH that is generally considered to be optimal for reductive aminations, pH

pH	Yield (%)
5.0	33
6.0	46
6.5	58
7.0	74
7.5	75
11.4	60

Table 4.13: Product yields were determined by HPLC. The reaction was performed using 1 mmol of benzylamine and 1 mmol of pyridine borane, with 1 mmol cyclohexanone in 2 mL water. It was stirred for 10 minutes at 30 °C. The pH of the reaction was monitored using a pH meter and adjusted back to the desired pH with either 1M NaOH(aq) or 37% HCl in water, thus maintaining a constant pH

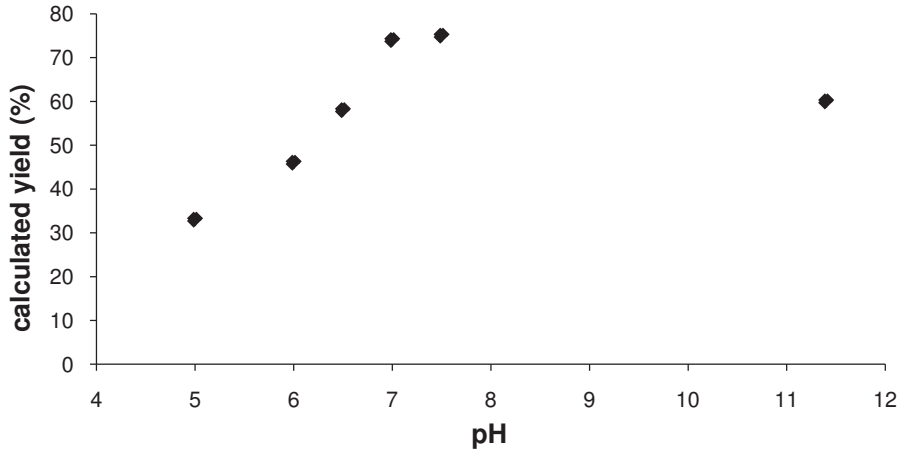


Figure 4.10: Plot of reaction yields for the reductive amination reaction performed at various pHs. Product yields were determined by HPLC. The reaction was performed on 1 mmol of cyclohexanone, using 1 mmol of benzylamine and 1 mmol of pyridine borane in 2 mL H_2O. The reaction was stirred for 10 minutes at 30 °C. The pH of the reaction was monitored using a pH meter and adjusted back to the desired pH with either 1M NaOH(aq) or 37% HCl in water, thus maintaining a constant pH

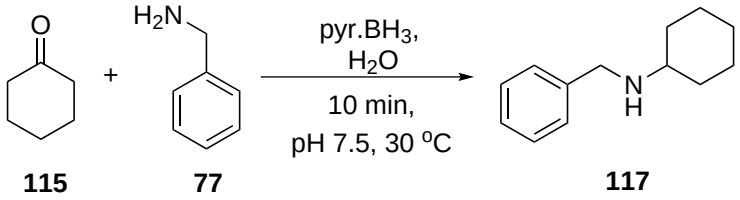
5–6 (Figure 4.7). This suggested that formation of the protonated amine has much more impact on the rate than acid catalysis. The stability of reaction intermediates and hence their likelihood of being reduced is related to the stability of the carbinolamine, which is directly related to the pK_a of the constituent nitrogen.[262] The pK_a of the carbinolamine (using SPARC online calculator) was calculated to be 9.43, which suggests that the carbinolamine will be more stable at alkaline pH. This might explain the slower decrease in yield at alkaline pH as the intermediate is more likely to be present, but the decreasing number of free protons in solution might be reducing the yield.[263] The rapid decrease in yield as the reaction mixture became more acidic was probably due to an increase in the reduction of the ketone to the alcohol, which was observed in the NMR spectroscopic analysis of crude reaction mixtures. This reduction needs free protons to help form the hydroxyl group, which could explain this trend. Another possibility is that at acidic pH benzylamine is deactivated by protonation.

The optimized pH of the reaction was the same as that at which transketolase operates, pH 7.5. This suggested that a one-pot strategy would be possible, as the pH and temperatures of the reaction are compatible. One potential problem could be that the reactants in either reaction might deactivate the others, which would necessitate reactant separation and the use of membrane systems.

Nitrogen donor

The more expensive substrate in the reaction was the ketone, as it was to be formed in the biotransformation, and so the number of equivalents of amine was optimized. This was because the amount of amine present might affect the number of additions that occur, as

well as the rate of addition and hence reaction selectivity. The mono-addition of benzylamine was required as this would make the reaction more economic, as well as making the product easier to isolate and deprotect. The reaction was initially attempted with excess amine (4–10 equivalents), but these gave poor yields (7%) of the mono-addition product, **117**, probably because the amine can also attack the imine to give a di-addition product (Scheme 4.1 on page 109). The reaction (Scheme 4.23) was, therefore, explored with fewer equivalents of benzylamine, and the results are shown in Table 4.14 and Figure 4.11. The yields were calculated based upon the amount of ketone used.



Scheme 4.23: Reductive amination reaction investigated with varying concentrations of benzylamine, using the HPLC assay

Equivalents of benzylamine	Yield (%)
0.5	28
0.7	67
0.8	70
0.9	73
1.0	60
1.1	54
1.2	51

Table 4.14: Product yields of **117**, based upon the amount of ketone used, were determined by HPLC. The reaction was performed using the required amount of benzylamine, 1 mmol of pyridine borane with 1 mmol cyclohexanone in 2 mL water at an initial pH of 7.5. It was stirred for 10 minutes at 30 °C

The optimum number of equivalents of benzylamine was found to be 0.9, as it lowered

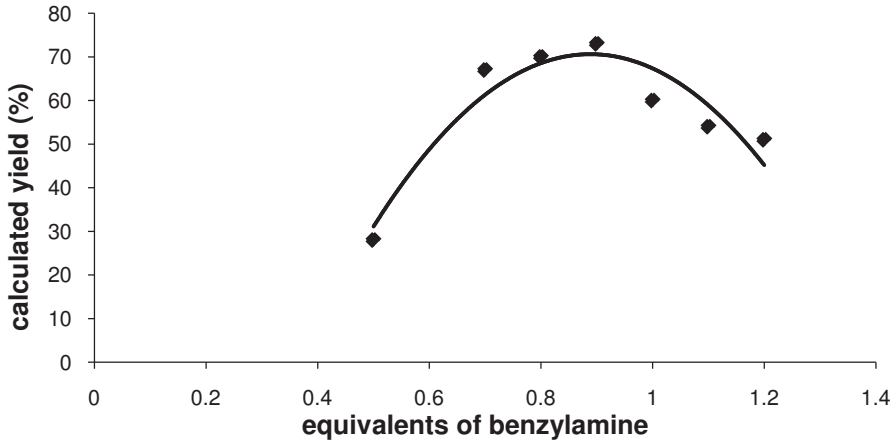


Figure 4.11: Plot of reaction yields for the reductive amination reaction performed at various amine concentrations. Product yields of **117**, based upon the amount of ketone used, were determined by HPLC. The reaction was performed using the required amount of benzylamine, 1 mmol of pyridine borane with 1 mmol cyclohexanone in 2 mL water at an initial pH of 7.5. It was stirred for 10 minutes at 30 °C

the amount of product lost to side reactions. Even though the ketone may be the most expensive reactant, fewer equivalents of the amine group gave a higher yield and cleaner reaction. In addition any unreacted ketone could be purified and recycled back into the reaction.

Reducing agent

In the previous series of reactions three hydrogen equivalents (one molar equivalence) of reducing agent had been used to carry out the reduction, however, this is not economic as only one reduction is performed. Therefore the number of reducing equivalents present was varied, using both fewer and greater hydrogen equivalents (Table 4.15 and Figure 4.12).

The use of fewer equivalents of reducing agent increased the yield, which was probably because this lower concentration allowed more time for imine formation to occur, as the

Molar equivalents of reducing agent	Yield (%)
0.33	68
0.66	63
1.00	60
2.00	34
3.00	21
4.00	22
5.00	16

Table 4.15: Product yields of **117** determined by HPLC. The reaction was performed using 1 mmol of benzylamine, 1 mmol cyclohexanone and the required amount of pyridine borane in 2 mL water at an initial pH of 7.5. It was stirred for 10 minutes at 30 °C

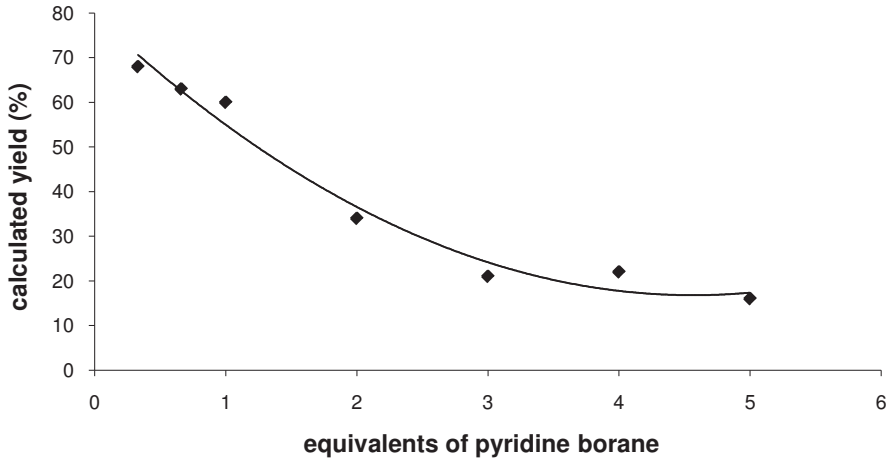
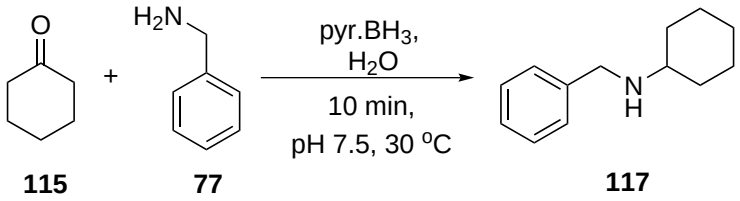


Figure 4.12: Plot of reaction yields for the reductive amination reaction performed at various amine concentrations. Product yields of **117** determined by HPLC. The reaction was performed using 1 mmol of benzylamine, 1 mmol cyclohexanone and the required amount of pyridine borane in 2 mL water at an initial pH of 7.5. It was stirred for 10 minutes at 30 °C

ketone would not be reduced so rapidly and so the formation of the alcohol side product was diminished. The product yield rapidly dropped after more than one molar equivalent was used and so this was selected for use in the reaction.

Reaction concentration

The final reaction variable that was optimized was the reaction concentration, with respect to the ketone. This was an important variable as the biotransformation is typically performed at concentrations of 150–500 mM. The chemical reaction was performed neat and at concentrations from 0.20 to 2.04 mol/L in order to optimize it. The results of the test reaction shown in Scheme 4.24 are shown in Table 4.16.



Scheme 4.24: Reductive amination reaction investigated with varying reaction concentration, using the HPLC assay

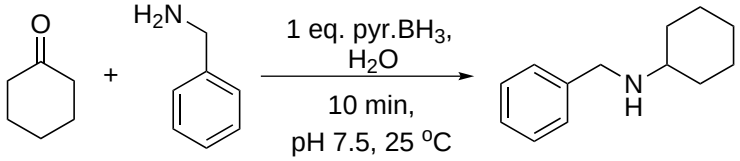
The reaction concentration did not affect the reaction yield, and the neat reaction gave a much lower yield, agreeing with the earlier results (Table 4.11). However, as the yield did not vary with concentration, as long as some water was present. This suggested that the reaction was occurring at the surface, with water playing an important catalytic role. The reaction occurring at the water/air interface is significant as it means that in a one-pot reaction with the enzyme it will be a lot easier to perform as it forms a second discrete reaction location, distinct from that in which the enzyme operates. This reaction compartmentaliza-

Cyclohexanone concentration (mol/L)	Yield (%)
neat	28
2.04	57
1.02	61
0.51	60
0.34	60
0.25	59
0.20	60

Table 4.16: Product yields of **117** determined by HPLC. The reaction was performed using 1 mmol of benzylamine, 1 mmol of pyridine borane and the required amount of cyclohexanone in 2 mL water at an initial pH of 7.5. It was stirred for 10 minutes at 30 °C

tion should mean that any biological material that contaminates the main volume of water will interact minimally with the chemical reactants, preventing either from deactivating the other. The only possible difficulty will be if the product of the biotransformation is highly water soluble and remains in the bulk water, rather than migrating to the surface, preventing it from interacting with the reductive amination reagents.

4.2.4 Optimized reaction conditions



Scheme 4.25: The optimized reductive amination reaction from the HPLC assay

The optimal reaction conditions found, were:

- Sodium cyanoborohydride, 2,6-lutidine borane and pyridine borane were the best reducing agents. Pyridine borane was chosen due to its lower toxicity and commercial

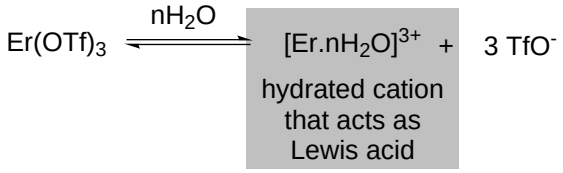
availability

- Water must be present – but the concentration seems unimportant
- Optimal temperature was 25 °C
- Optimal pH was 7.0–8.0
- Optimal reaction period was 10 minutes
- Optimal number of equivalents of benzylamine was 0.9
- Optimal amount of reducing agent was 0.33 equivalents, however, 1.0 equivalent will be used so as to ensure that if it becomes deactivated by the biological material from the biocatalysis then there will be enough left to perform the reductive amination reaction

4.2.5 Reaction additives

Once the reaction had been optimized, the addition of various additives was investigated, as this had been effective at increasing both the selectivity and yield in earlier studies.

Lewis acids



Scheme 4.26: Conversion of rare earth metal Lewis acids to their hydrated cations in water[118]

During earlier reaction development, the addition of Lewis acids to the reaction resulted in a large increase in yield. Recently Lewis acids that are water stable have been researched and developed, in particular by Kobayashi.[118,119] These Lewis acids are based upon rare earth metals, like scandium and ytterbium, and include both the triflate and the chloride salts. Kobayashi postulated that these Lewis acids needed to have hydrated cations in order to function as catalysts (Scheme 4.26).[118] The results of our studies are shown in Table 4.17.

Entry	Lewis acid	Calculated Yield (%)
1	-	60
2	$YbCl_3$	57
3	$Yb(OTf)_3$	75
4	$Yb(DS)_3$	77
5	PyB–$Sc(OTf)_3$	62
6	$Sc(OTf)_3$	57
7	$In(DS)_3$	41
8	$InCl_3$	38
9	$Gd(OTf)_3$	35
10	$Dy(OTf)_3$	43

Table 4.17: Product yields of **117** determined by HPLC. The reaction was performed using 0.9 mmol of benzylamine, 1 mmol of pyridine borane, 1 mmol of cyclohexanone and 0.2 mmol of Lewis acid in 2 mL water at an initial pH of 7.5. It was stirred for 10 minutes at 25 °C
PyB=polymer bound molecule

Lewis acids with different counter ions were examined, as their different dissociation constants should mean that there is a variation in the amount of the hydrated cation present in solution and, therefore, a variation in the amount of catalysis. If this was the case then the triflate (entry 3 in Table 4.17), being a better leaving group, should give higher yields than the chloride (entry 2 in Table 4.17) which is less likely to dissociate. This was what was observed, with the Lewis acids with chloride counter ions giving lower yields. A similar effect was observed by Srinivasan *et al.* who observed that the less nucleophilic (and

slightly softer) triflate counter ions were superior to the chloride species at catalysing reactions in water.[264] The use of a LASC (entry 7 in Table 4.17) as a catalyst also increased the yield, by about the same amount as the triflate. These are molecules that contain both a surfactant group and a Lewis acidic cation, thus imparting the properties of both Lewis acid and surfactant to the same molecule.

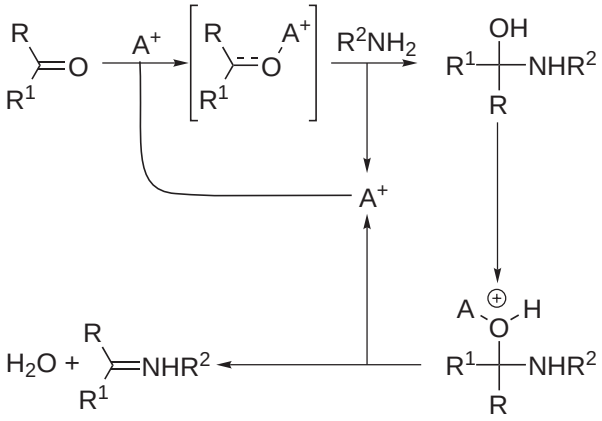
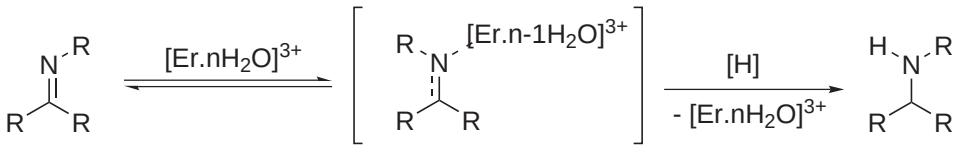


Figure 4.13: Lewis acid catalysis of reductive amination[113]
A^+=H^+ or Lewis acid

The addition of a Lewis acid gave the greatest increase in yield when they contained ytterbium and to a lesser extent scandium, probably because it activated the ketone carbonyl to attack by the amine, as well as assisting in the loss of water (Figure 4.13). The other Lewis acids tested (indium, gadolinium and dysprosium) had a negative impact upon the yield, probably because the different metal cations have different affinities for different functional groups and so complex preferentially to different groups. A similar selectivity was seen by Kobayashi *et al* who saw that ytterbium was strongly aldimine selective, scandium weakly aldimine selective and gadolinium aldehyde selective.[265] This was also reported by Srinivasan *et al* who saw that scandium and ytterbium Lewis acids were strongly

imine selective, whilst gadolinium and dysprosium ones were only moderately selective.[264] This agrees with our results where ytterbium appears to activate the imine, and gadolinium, dysprosium and indium activate the ketone. This suggested that the imine was probably formed during the reaction as it is being complexed by the Lewis acid and activated for reduction as shown in Scheme 4.27.

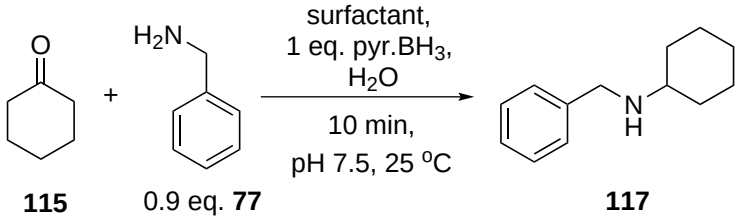


Scheme 4.27: Lewis acid activation of imine in water[265]

Surfactants

Having noted that the LASC provided the greatest increase in reaction yield, other surfactants were examined as additives. The reaction was performed with a variety of polyethylene glycols (PEGs; PEG-600, PEG-600 diacid, TEG, PEG-4000, PEG-6000, PEG ethyl ether) and a variety of the Brij surfactants (35, 76 and 98). Most of the PEG additives had little effect on the reaction, warranting no further investigation, and the Brij molecules retarded the reaction significantly and so were dismissed. The reactions were performed at a range of concentrations, as it was essential that the concentration was above the critical micelle concentration. For example, the concentration of TEG had some effect on the yield obtained, with the yield eventually plateauing at 78% at about 10 mol% TEG and this concentration proved to be the same for most of the other surfactants. The reactions (Scheme 4.28) with surfactants were performed with both stirring and sonication, as sonication should promote the formation of the micelles, hopefully promoting the reaction

(Table 4.18).[239]



Scheme 4.28: The optimized reductive amination reaction investigated with varying surfactants

Surfactant	Yield – stirred reaction (%)	Yield – sonicated reaction (%)
TEG	78	75
CTAB	78	82
SDS	77	81
$Yb(DS)_3$	77	80

Table 4.18: Product yields of **117** determined by HPLC. The reaction was performed using 0.9 mmol of benzylamine, 1 mmol of pyridine borane, 1 mmol of cyclohexanone and 10 mol% of surfactant in 2 mL water at an initial pH of 7.5. It was stirred or sonicated for 10 minutes at 25 °C

A small increase in yield was observed with all surfactants and this might be because the reaction is occurring within micelles. However, it is more likely that the effect is caused by the surfactant molecules helping to solubilize the reactants. This occurs because they disrupt the bulk structures of the water, much like with phase transfer catalysis, allowing any reactants at the surface to better interact with the reactants that are dissolved in the water, thus encouraging the reaction.

Sonication

The data from the sonicated experiments performed with surfactants suggested that sonication might slightly improve reaction yields. This agreed with the earlier experiments screened using an NMR assay that suggested that sonication discouraged the reduction of the carbonyl species to some extent. The reductive amination was performed with sonication but no additives to see if this was the case. The improved yield of **117** from sonication was not observed for most of the reducing agents tested, with just sodium cyanoborohydride and pyridine borane displaying an increased yield. Representative examples are shown in Table 4.19.

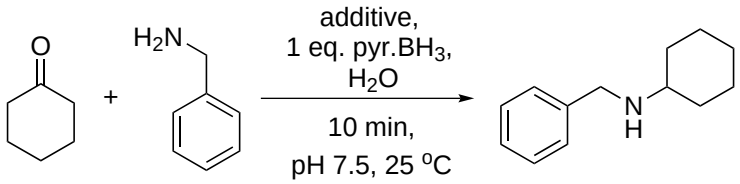
Reducing agent	Yield – stirred reaction (%)	Yield – sonicated reaction (%)
pyridine borane	60	72
picoline borane	48	36

Table 4.19: Product yields of **117** determined by HPLC. The reaction was performed using 0.9 mmol of benzylamine, 1 mmol of reducing agent and 1 mmol of cyclohexanone in 2 mL water at an initial pH of 7.5. It was stirred or sonicated for 10 minutes at 25 °C

The mixed results obtained with sonication suggested that it was not a different reaction pathway caused by sonication, but rather improved mixing that caused the increase in yield. Further repeat reactions with additives were performed but sonication was seen to provide only a very small benefit to the reaction and as it would be difficult to scale was not explored further.

Other additives

Other types of additives that have been successfully applied to this type of chemistry previously were highlighted in Chapter 1. Of these, chaotropic agents, which modify the surface tension and also to some extent the bulk properties of water, had not yet been examined. The results of reactions (Scheme 4.29) with these and other additives are shown in Table 4.20.



Scheme 4.29: Optimized reductive amination reaction investigated with various additives

Entry	Additive	Calculated yield (%)
1	-	60
2	LiOH	60
3	KOH	60
4	guanidine HCl	54
5	β-cyclodextrin	72
6	LiCl	80
7	LiCl*	73
8	LiCl**	73
9	Me_4NCl	76
10	Me_4NCl*	81

Table 4.20: Product yields of **117** determined by HPLC. The reaction was performed using 0.2 mmol of the additive, 0.9 mmol of benzylamine, 1 mmol of reducing agent and 1 mmol of cyclohexanone in 2 mL water at an initial pH of 7.5. It was stirred (or sonicated - where indicated) for 10 minutes at 25 °C

*=reaction sonicated rather than stirred; **=added during work up

The addition of base was examined with the hope that ions produced in solution might help solubilize the reactants and reagents and, therefore, bring them more into contact

with one another. However, these did not affect the yield in any significant manner. β-Cyclodextrin did increase the yield of the reaction and this is probably because it has a large number of pendant hydroxyl groups which can interact with water through hydrogen-bonding, thus breaking up the bulk structure of water and allowing the solubilization of reactants. Alternatively, it might be causing the salt of the amine reactant to form, making it less likely to react. However, this is unlikely because tetramethyl ammonium chloride (a salting-in reagent, entries 9 and 10 in Table 4.20) causes an increased yield to be observed. Lithium chloride (a salting-out reagent, entries 6 and 7 in Table 4.20) also gave an increase in yield, perhaps due to disruption of the bulk properties of the water rather than salt formation. However, there was one other possibility, which was that the additives were helping to drive the product into the organic phase during the work up. To see if this was the case, the reaction was performed with the addition of lithium chloride at the end of the reaction, immediately prior to work-up (entry 8 in Table 4.20). This gave an improved yield, but not as high a yield increase as when the additive could participate in the reaction, highlighting that the improved yield is on;y partly the result of better product extraction. The increase in yield was the same as when $Yb(DS)_3$ was used, and when the reaction was performed with both $Yb(DS)_3$ and lithium chloride present no extra reactivity was observed. It was, therefore, decided that lithium chloride should be added at the end of the reaction to help product extraction.

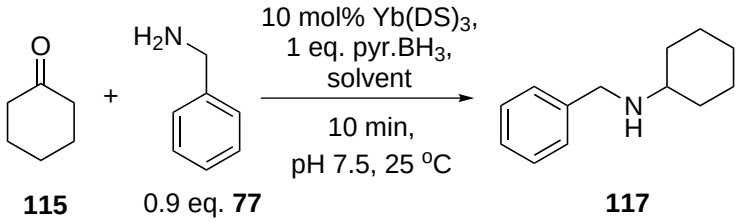
The final additive to be investigated was glycerol, and this was examined as both an additive and as a solvent, as it is commonly used as an alternative 'green' solvent. There was no significant increase in yield when glycerol was present at a concentration of 10 mol%. However, when it was used as the solvent, a much improved yield was observed, higher than the best yield in water. For comparison purposes the reaction was also performed in

Solvent	Yield (%)
water	60
water with 10 mol% glycerol	62
glycerol	92
triethylene glycol	60

Table 4.21: Product yields of **117** determined by HPLC. The reaction was performed using 0.9 mmol of benzylamine, 1 mmol of reducing agent and 1 mmol of cyclohexanone in 2 mL of the indicated solvent. It was stirred for 10 minutes at 25 °C

TEG, another 'green' solvent. This gave an identical yield to when the reaction was carried out in water, which suggested that the glycerol may be interacting with the reactants to increase the yield. This might also explain the increased yield when β-cyclodextrin was used as an additive, as both reagents contain several hydroxy groups that might interact in the reaction. Bulk water takes on a regular lattice structure which provides an excellent lowest energy conformation, and is more likely to bond with other water molecules than other molecules. Glycerol, however, cannot form a regular lattice of hydrogen-bonds and so should be more likely to hydrogen-bond to the participants in the reaction, helping to catalyse the reaction.

4.2.6 Optimized reaction



Scheme 4.30: Fully optimized reductive amination reaction

Having explored a wide range of possible conditions, the optimal conditions identi-

fied were: one equivalent of pyridine borane; 0.9 equivalents of benzylamine; 10 mol% $Yb(DS)_3$; 10 minutes of stirring; pH 7.5; a concentraion of 0.5 mol/L; 25 °C; LiCl added just before work-up. When these conditions were applied to the reductive amination of cyclohexanone in water, chloroform and deuterium oxide the results shown in Table 4.22 were observed. The reaction was also tested in several other solvents.

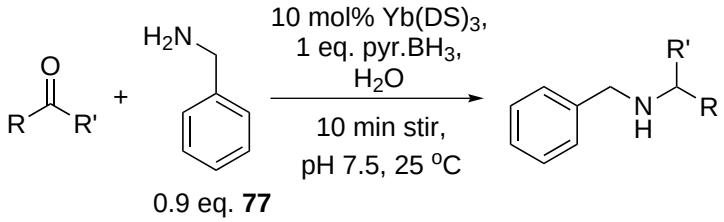
Solvent	Yield (%)
H_2O	81
$CHCl_3$	76
D_2O	75

Table 4.22: Product yields of **117** determined by HPLC. The reaction was performed using 0.9 mmol of benzylamine, 1 mmol of reducing agent, 10 mol% $Yb(DS)_3$ and 1 mmol of cyclohexanone in 2 mL of the indicated solvent at an initial pH of 7.5. It was stirred for 10 minutes at 25 °C

The reaction conditions appeared to be fairly robust with only a slight decrease in the yield observed when other solvents were used. The fact that the yield decreased when performed in deuterium oxide suggests that hydrogen bonding does play a role within the reaction, as it might be the result of the kinetic isotope effect. This would suggest that water is participating in the rate determining step of the reaction, but further studies would need to be performed to confirm this.

4.3 Application of the optimized reaction in water

The optimized reaction conditions were applied to a variety of different aldehydes and ketones (Scheme 4.31) and the results are shown in Table 4.23.



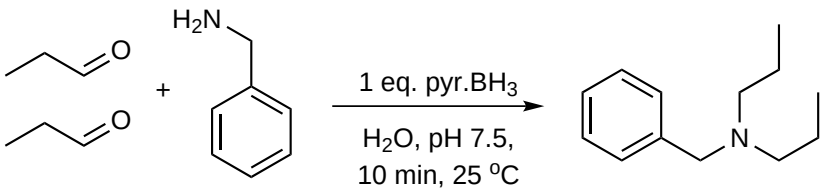
Scheme 4.31: Reductive amination reaction used in the reactions in water

Substrate	Yield (%)
cyclohexanone	24
acetophenone	26
benzophenone	0
methyl pyruvate	21
ethyl pyruvate	31
benzaldehyde	45
propanal	19*
octanal	22*

Table 4.23: Isolated yields for reductive amination reactions performed on various compounds. The reaction was performed using 1.0 mmol of the carbonyl compound, 0.9 mmol of benzylamine, 10 mol% $Yb(DS)_3$ and 1.0 mmol of pyridine borane in 2 mL of H_2O. The reaction was stirred for 10 minutes at 25 °C and pH 7.5

*=di-addition of aldehyde to amine

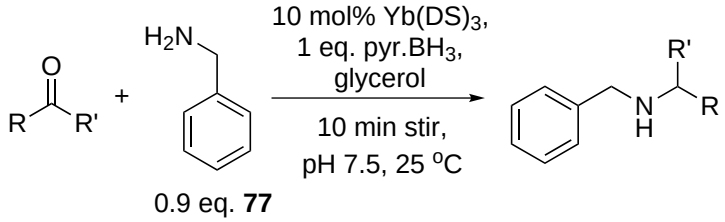
The yields obtained were calculated with respect to the carbonyl compound, however, as stated previously the maximum possible yield is 90%, as only 0.9 equivalents of benzylamine were used. The reasons for the lower yields are two-fold. Isolated yields are presented and some of the products were difficult to purify, for example the product of the reaction with cyclohexanone as discussed earlier. Secondly, a large amount of di-addition of aldehyde to amine was noted (see Scheme 4.32). This di-addition was different to the small amount of di-addition seen before, as it resulted from the addition of two equivalents of aldehyde to each molecule of amine and was not observed for the more hindered ketones. Nevertheless since the reaction is to be used with the 2-keto-1,3-diol product of the enzyme reaction, this was not examined further. In addition, reactions were performed for 10 minutes which may not be optimal for these substrates and cause a decreased yield to be obtained.



Scheme 4.32: Di-addition of aldehyde to amine observed with less hindered ketones and aldehydes

4.4 Application of the reaction in glycerol

When optimizing the reaction in water, it was found that one of the best reaction conditions was neat glycerol as the reaction solvent (Scheme 4.33). These reaction conditions were therefore also tested on a series of substrates. The results are shown in Table 4.24.



Scheme 4.33: Reductive amination reaction used in the reactions in glycerol

Substrate	Yield (%)
cyclohexanone	17
acetophenone	21
benzophenone	0
methyl pyruvate	27
ethyl pyruvate	19
benzaldehyde	24
propanal	27*
octanal	11*

Table 4.24: Isolated yields of reductive amination performed on various compounds. The reaction was performed using 1.0 mmol of the carbonyl compound, 0.9 mmol of benzylamine, 10 mol% $Yb(DS)_3$ and 1.0 mmol of pyridine borane in 2 mL of glycerol. The reaction was stirred for 10 minutes at 25 °C and pH 7.5

*=di-addition of aldehyde to amine

Much lower yields were observed than when the product was not isolated and the yields were mostly lower than the same reaction performed in water. This was probably due to a similar difficulty to the one encountered when obtaining isolated yields for the reactions in water.

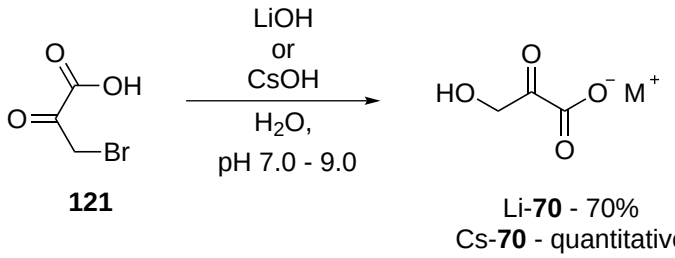
5

Application of the Reductive Amination Reaction in Water to Enzymatically Synthesized Ketodiol

5.1 Synthesis of 1,3-dihydroxypentan-2-one

The reductive amination reaction with water as a solvent had been developed upon a test substrate. It was, therefore, essential to synthesize the actual substrate, 1,3-dihydroxypentan-2-one, **71**, to ensure that the reaction would proceed with the product of the biocatalytic step. This would also enable the development of an isolation method for the desired product, as product isolation and extraction were found to be the most difficult aspects of the synthesis involving cyclohexanone. Therefore, in order to further reaction development 1,3-dihydroxypentan-2-one, **71** needed to be synthesized.

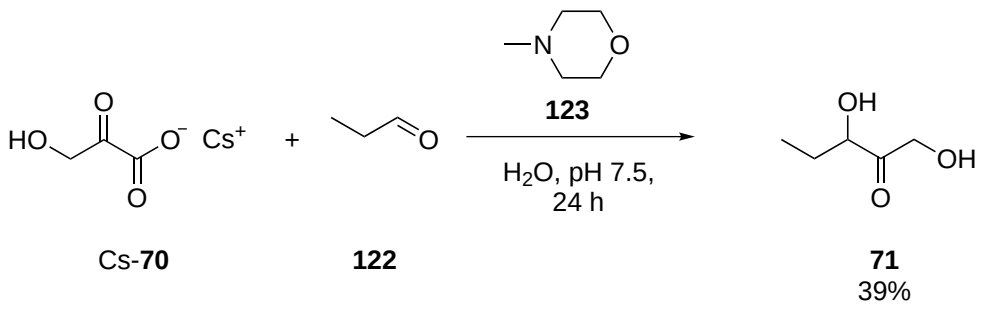
As previously mentioned, at the start of the research programme this was a difficult synthesis, hence the use of the test substrate. However, during the course of the research a facile synthesis of α-α'-dihydroxyketones was discovered.[207] This synthesis was discovered when α-α'-dihydroxyketones were observed in control reactions for the biocatalytic step, where no enzyme was present. The initial reactions involved reacting aldehydes with lithium hydroxypyruvate, to donate the ketol group, in aqueous 3-(N-morpholino)propane sulfonic acid (MOPS) buffer at pH 7.0.[207] The lithium hydroxypyruvate for these reactions was prepared as described in the literature by titrating bromopyruvic acid with two equivalents of lithium hydroxide in water, ensuring the pH remained below 9.0 (Scheme 5.1).[266] Li-**70** was obtained in 70% yield, higher than the 49% obtained in the literature.[266] This improvement was most likely due to using high purity starting materials and monitoring the pH during the neutralization.



Scheme 5.1: The synthesis of Group 1 hydroxypyruvate salts[207,266]

Additional work by members of the BiCE research group indicated that the reaction readily proceeded with other Group 1 salts of hydroxypyruvate. As a consequence of this work the caesium salt was subsequently used, as it could be made in higher yields and with greater purity.[207] The caesium salt, Cs-**70**, was synthesized in quantitative yield, much higher than the 70% yield obtained for the formation of the lithium salt. Using the synthesis developed within the research group, 1,3-dihydroxypentan-2-one, **71**, was synthesized by

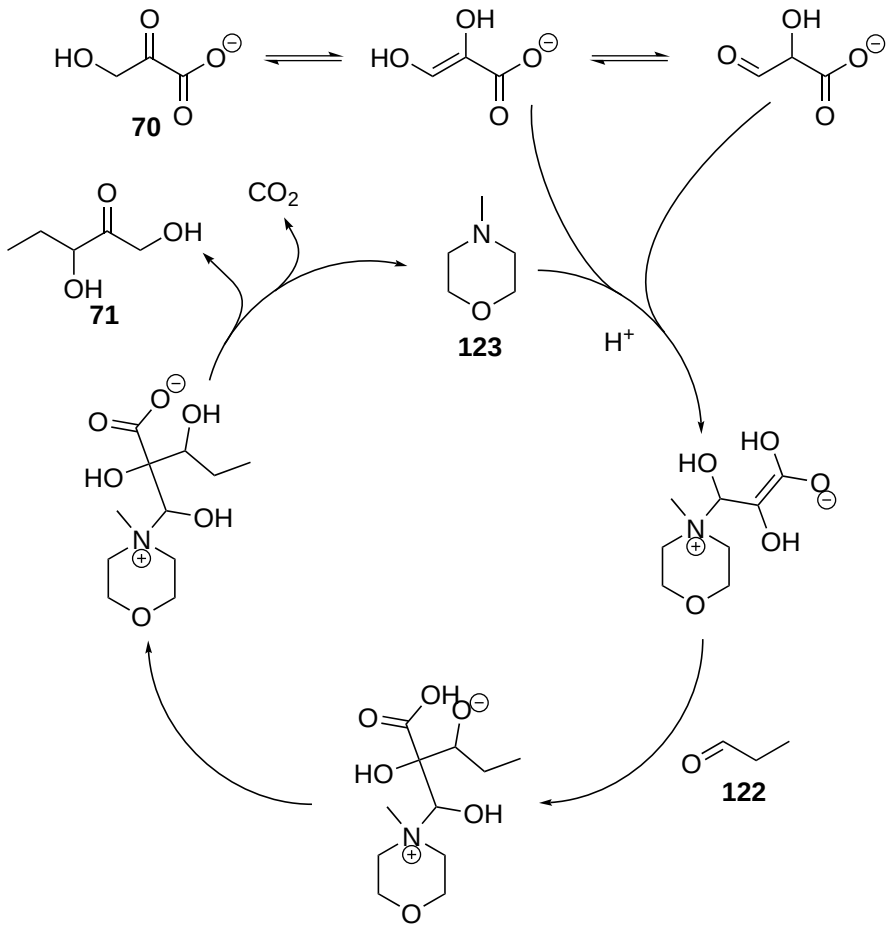
reacting the hydroxypyruvate salt, Cs-**70** with propanal, **122**, and *N*-methyl morpholine, **123**, in water at pH 7.5 (Scheme 5.2). This reaction proceeded, and **71** was obtained in 39% yield when the caesium salt was used, compared with yields of 25–30% when the lithium salt was used. The yields obtained were comparable with the literature yield for this reaction of 35%.[207] The major difficulty encountered during the synthesis was extracting 1,3-dihydroxypentan-2-one, **71**, formed during the reaction from the water, due to the hydrophilic nature of the molecule.



Scheme 5.2: The one-step chemical synthesis of 1,3-dihydroxypentan-2-one, **71**[207]

In order to see whether the *N*-methyl morpholine in the reaction was catalytic or whether it was acting only as a base, the reaction was tested with other amine containing species.[207] The results of these reactions indicated that the amine was not acting solely as a base, as no product formation was observed for several of the bases tested. Instead it was postulated that particular structural elements (*i.e.* a tertiary amine in a cyclic system) were necessary to promote the reaction, suggesting a catalytic mode of action. A suggested mechanism for this catalysis is shown in Scheme 5.3.[207]

This reaction provided a simple one-pot route to the desired ketodiols and 1,3-dihydroxypentan-2-one, **71**, synthesized using this route was then used to further develop the reductive

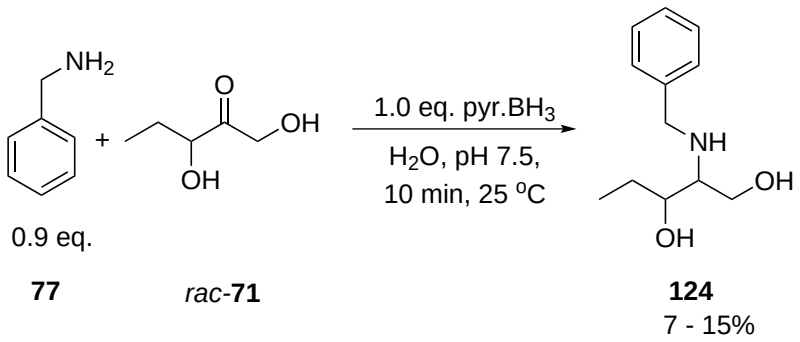


Scheme 5.3: The base catalysed mechanism for the synthesis of 1,3-dihydroxypentan-2-one in water proposed by Smith *et al*[207]

amination reaction.

5.2 Reductive amination upon racemic 1,3-dihydroxy pentan-2-one

The *rac*-**71** synthesized chemically was used in the reductive amination method developed upon the test compound, cyclohexanone (Scheme 5.4). The reductive amination was performed upon *rac*-**71** using benzylamine, **77**, and pyridine borane in water. This was to ensure that the reaction would work upon the same product as that produced by the biotransformation.



Scheme 5.4: Test of the optimized reductive amination reaction upon 1,3-dihydroxypentan-2-one. The reaction was performed using 0.9 equivalents of benzylamine and one equivalent of pyridine borane in 2 mL H_2O (at a reaction concentration of 0.5 mol/L). The reaction was stirred for 10 minutes at 25 °C and pH 7.5. The pH of the reaction was monitored using a pH meter and adjusted to the desired pH with either 1M NaOH(aq) or 37% HCl in water, thus maintaining a constant pH

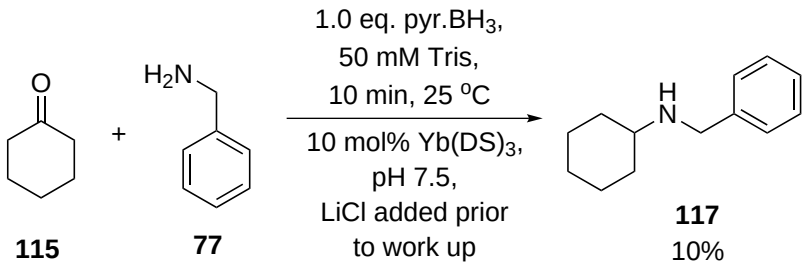
The reaction proceeded, but gave very poor isolated yields of secondary amine **124** (7%, Scheme 5.4) probably due to the hydrophilic nature of the product, which made extraction

from the aqueous phase into the organic phase difficult. This had also been encountered earlier in the reaction development and so was not unexpected. In order to improve the yield, the aqueous reaction mixture was extracted with dichloromethane rather than ethyl acetate, and the number of extractions was increased from two to 10. This improved the isolated yield to 15%. At this stage, an assay to determine the stereoselectivity of the reaction was not available, the reaction being performed both to ensure that the reaction proceeded on the substrate and to provide a compound with which to develop the column conditions for the HPLC assay.

5.3 Reductive amination in Tris buffer

The enzymatic transformation that had been developed by other members of the BiCE research group took place in a buffered solution at pH 7.0. Tris buffer is a solution of 2-amino-2-hydroxymethyl-propane-1,3-diol, and as this contains a primary amine group there was the possibility that it might also undergo reductive amination. This would mean that a competing side reaction could occur that might prevent the reaction being used in a one-pot manner. In order to find out if this was the case, the reductive amination of cyclohexanone using the optimized conditions was performed in 50 mM Tris buffer rather than deionized water (Scheme 5.5).

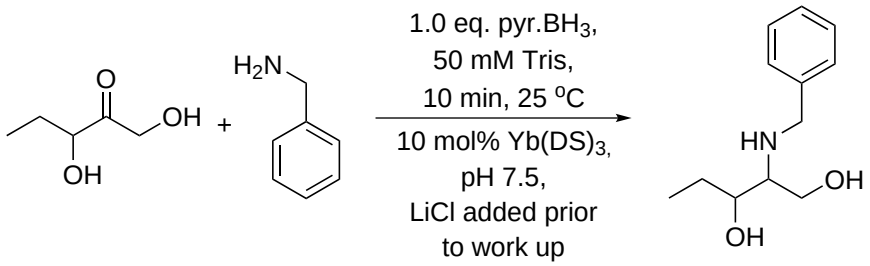
The reaction proceeded and gave an isolated yield of 10%, significantly lower than the 25% yield obtained for the same reaction in water. None of the reductive amination product with 2-amino-2-hydroxymethyl-propane-1,3-diol was observed which suggested that this is not a competing side reaction. However, it is possible that the yield is lowered because



Scheme 5.5: Reductive amination reaction of cyclohexanone with benzylamine in Tris buffer. The reaction was performed using 0.9 equivalents of benzylamine and one equivalent of pyridine borane in 2 mL 50 mM aqueous Tris buffer (at a reaction concentration of 0.5 mol/L). The reaction was stirred for 10 minutes at 25 °C and pH 7.0. The pH of the reaction was monitored using a pH meter and adjusted to the desired pH with either 1M NaOH(aq) or 37% HCl in water, thus maintaining a constant pH

the ketone is forming the imine with the primary amine in the Tris buffer, which is not then reduced. This would lower the amount of ketone available to react with the benzylamine thus lowering the yield. The other possibility is that the Tris buffer is making the product more difficult to isolate as the solution remains closer to acidic pH than in the unbuffered reactions, and so a higher proportion of the product molecules exist as the amine salt making them more hydrophilic. Having seen that the reductive amination reaction worked, but with greatly reduced yields in the buffered solution, the same reaction was performed upon *rac*-**71** (Scheme 5.6).

The reaction proceeded but again with a low yield of 11%. The decrease in yield was not as great as that seen with cyclohexanone and suggests that it is probably caused by the Tris buffer interfering with the extraction. This is because the extraction procedure for the reaction with cyclohexanone was not altered but the modified extraction procedure (10 washes with dichloromethane) was used for the reaction with *rac*-**71**. This modified extraction procedure is not environmentally friendly as it uses a chlorinated solvent in much



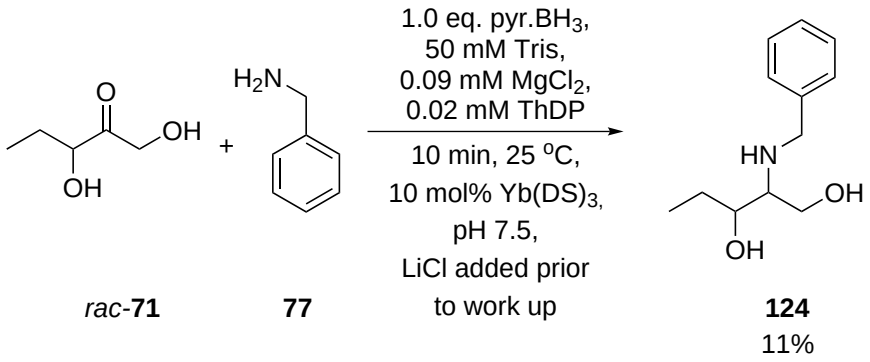
rac-**71** **77** **124**
11%

Scheme 5.6: Reductive amination of *rac*-**71** with benzylamine in Tris buffer. The reaction was performed using 0.9 equivalents of benzylamine and one equivalent of pyridine borane in 2 mL 50 mM aqueous Tris buffer (at a reaction concentration of 0.5 mol/L). The reaction was stirred for 10 minutes at 25 °C and pH 7.0. The pH of the reaction was monitored using a pH meter and adjusted to the desired pH with either 1M NaOH(aq) or 37% HCl in water, thus maintaining a constant pH

greater quantities than the aqueous solvent used for the reaction. It also adds greatly to the expense of the reaction, and so if the reaction is to be used on a larger scale then a much improved extraction procedure would be needed. This problem was also encountered when the reaction was performed with two enzymes in sequence, and so other members of the BiCE research group have been looking into alternative extraction techniques. The product of the second biotransformation is different from the product when a chemical reductive amination is used, as a primary amine present in the product. Isolation of this compound (2-amino-1-hydroxy pentan-3-ol) has been achieved using acid functionalized columns (*e.g.* SCX-2). This method should also be applicable to the secondary amine present in the molecule produced by the chemical synthesis, however, if this is not the case then the product could be debenzylated prior to purification. These techniques were not used for these reactions, however, they should allow for an improvement in both the reaction yield and also its green chemistry credentials.

5.4 Reductive amination in sequence with biotransformation

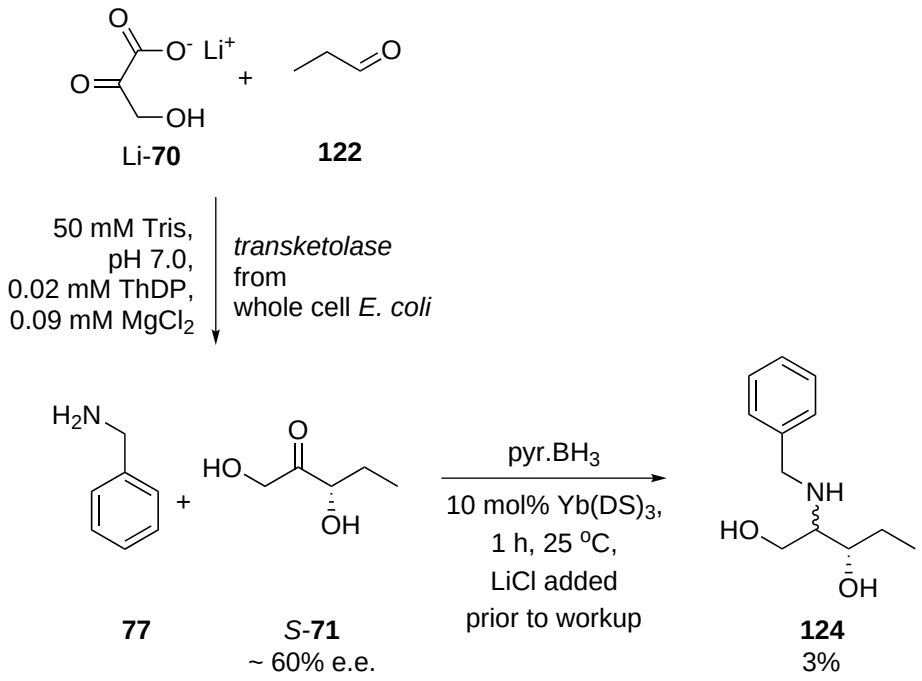
Having observed that the reaction would proceed in Tris buffer, the next step in the reaction development process was to see if the cofactors used for the biotransformation would interfere with the reductive amination. The reaction was therefore performed in 50 mM Tris buffer in the presence of magnesium chloride and thiamine diphosphate (Scheme 5.7).



Scheme 5.7: Reductive amination of *rac*-**71** with benzylamine in Tris buffer. The reaction was performed using 0.9 equivalents of benzylamine, 0.02 mM thiamine diphosphate, 0.09 mM magnesium chloride and one equivalent of pyridine borane in 2 mL 50 mM aqueous Tris buffer (at a reaction concentration of 0.5mol/L). The reaction was stirred for 10 minutes at 25 °C and pH 7.0. The pH of the reaction was monitored using a pH meter and adjusted to the desired pH with either 1M NaOH(aq) or 37% HCl in water, thus maintaining a constant pH

The reductive amination reaction proceeded in 10% yield, which was comparable with the yield obtained when the reaction was performed in Tris buffer without the addition of the cofactors. As this reaction had proceeded it was then applied in sequence with the biotransformation. The biotransformation was performed using whole cell wild-type *E. coli* transketolase (supplied by Dr Martina Micheletti) in 50 mM Tris buffer at pH 7.0 with

propanal, magnesium chloride, thiamine diphosphate and lithium hydroxypyruvate. The biotransformation was performed using the whole cells rather than a lysate, as this meant that there would be less free material in the reaction mixture that might interfere with the reaction and subsequent product extraction. The reaction sequence carried out is shown in Scheme 5.8.



Scheme 5.8: One-pot combined biocatalytic and chemical synthesis of 2-(benzylamino)pentane-1,3-diol. The biocatalytic step was performed with wild-type transketolase from whole cell *E. coli* and the reductive amination performed in water

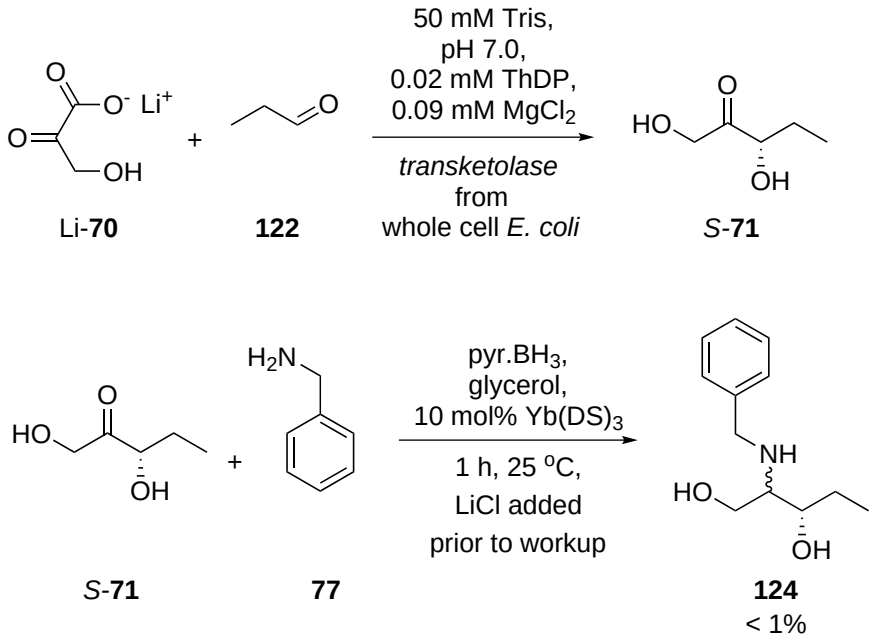
As the reactions were carried out in sequence, the biotransformation was performed first and was monitored by TLC to ensure that (*S*)-1,3-dihydroxypentan-2-one, *S*-**71**, was produced prior to the reductive amination. After 48 hours the reaction was observed to have produced 1,3-dihydroxypentan-2-one, as indicated by both TLC and ^{1}H NMR spectroscopy. At this point $Yb(DS)_3$, benzylamine and pyridine borane were added directly to

the biocatalytic reaction mixture and stirred at 25 °C for 1 hour. The reaction was then extracted with dichloromethane, as before, and purified to give 2-(benzylamino)pentane-1,3-diol, **124**, in 3% isolated yield over both steps. Even though this is a low yield it was considered to be reasonable as the normal yield for the biotransformation with wild-type *E. coli* transketolase is between 5 and 35%, and the highest yield for the reductive amination in Tris buffer was 11%. One of the reasons for this low yield was almost certainly that the product does not fully partition into the organic solvent used in the extraction. The method of isolating the 2-(benzylamino)pentane-1,3-diol therefore needs to be improved, however, at this stage the aim was solely to establish that a one-pot method was viable.

5.5 Reductive amination of 1,3-dihydroxypentan-2-one, produced enzymatically, in glycerol

The other reductive amination reaction developed in the previous chapter was performed in glycerol, and so could not be performed in a sequential one-pot manner like the reaction in water. As the enzyme had not been developed to work in glycerol the synthesis was performed sequentially, as two separate reactions with an intermediate extraction (Scheme 5.9).

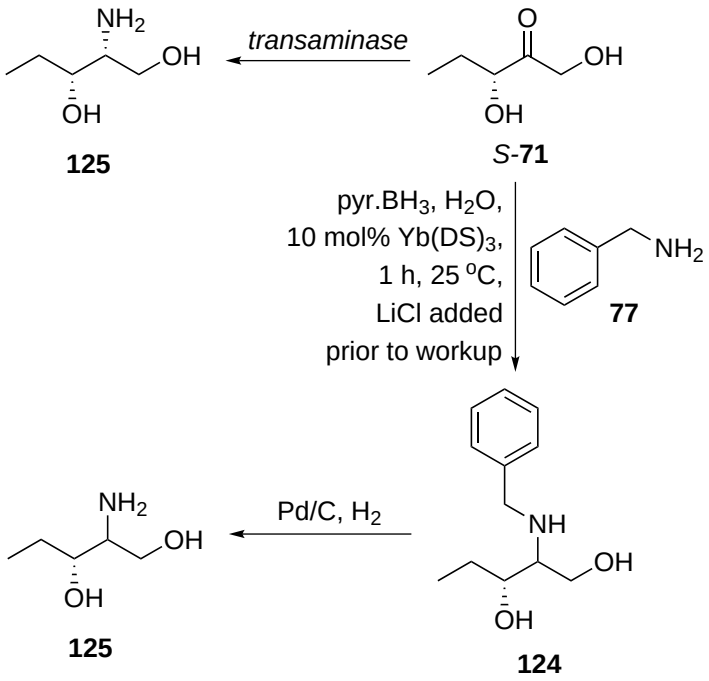
The biotransformation was once again monitored using TLC, and after 50 hours the biotransformation was extracted using dichloromethane in a similar manner to the final product extraction. The optimized reductive amination reaction in glycerol was then applied to the residue left when the combined dichloromethane washes were concentrated *in vacuo*. The



Scheme 5.9: One pot combined biocatalytic and chemical synthesis of 2-(benzylamino)pentane-1,3-diol. The biocatalytic step was performed with wild-type transketolase from whole cell *E. coli* and the reductive amination performed in glycerol

product gave an overall yield over both steps of 1%, which was much lower than when the optimized reductive amination reaction in water was applied in a one-pot manner. This suggests that the isolation of *S*-**71** from the biotransformation reaction mixture is much more difficult than the isolation of **124**. This was probably due to the hydrophilicity of the molecule and the fact that salting agents were not used to enhance the extraction of **71** as were used for the extraction of **124**. As this reaction could not be performed in a one pot manner, as was intended for the reaction being developed, and gave a lower yield – the reaction was not followed-up any further.

5.6 Assay development



Scheme 5.10: Two possible routes to **125**, one using biocatalysis and the other chemical reductive amination

The reductive amination reaction was being developed in parallel with a biotransformation that would give the same product, **125** (Scheme 5.10). Both of the reactions were being developed simultaneously with the aim of offering greater reaction flexibility, the chemical reaction hopefully being complementary to the biotransformation using transaminase. The aim was to develop a chemical reductive amination reaction that was stereoselective, because if the reaction could be performed in a stereoselective manner, then when used in conjunction with the enantioselective biocatalytic first step, the second stereocentre in the molecule would also be stereochemically defined. This would mean that it might offer the same stereoselectivity as the biotransformation and could then be developed to offer a similar or higher yield, or that it would give the opposite stereoselectivity.

Once it had been established that the one-pot sequential reaction would work (Scheme 5.8), then the next goal of the research programme was to examine whether the reductive amination reaction could be performed in either a diaseteroselective or enantioselective manner. In order to determine the stereoselectivity of the reaction an assay needed to be developed. As an HPLC assay had proved to be useful before it was decided to use a similar assay, with the intention that if the reaction was shown to be stereoselective then the assay could be modified to use a chiral HPLC column to differentiate between the enantiomers. In order to develop the assay to assess the diastereoselectivity of the chemical reductive amination method, the four possible diastereoisomers of **124** (Figure 5.1) had to be synthesized.

If the biotransformation using transketolase was stereospecific, then when the reductive amination reaction in water that had been developed was applied to its product, it could only give two of the four possible diastereoisomers of **124**, unless racemization at the α-position of the α,α'-dihydroxy ketone has occurred. This is because the chemical reductive

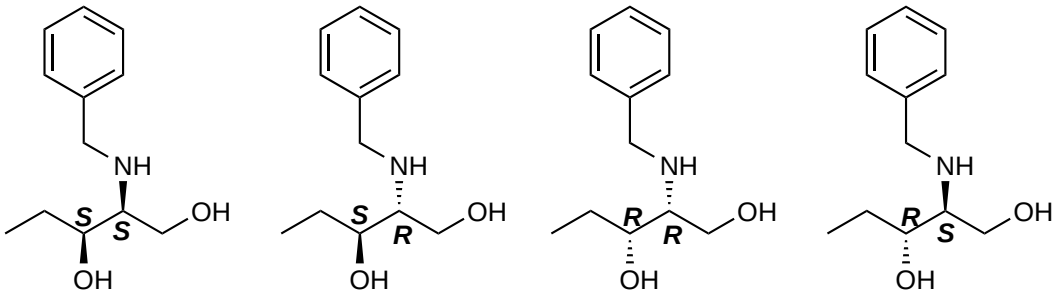
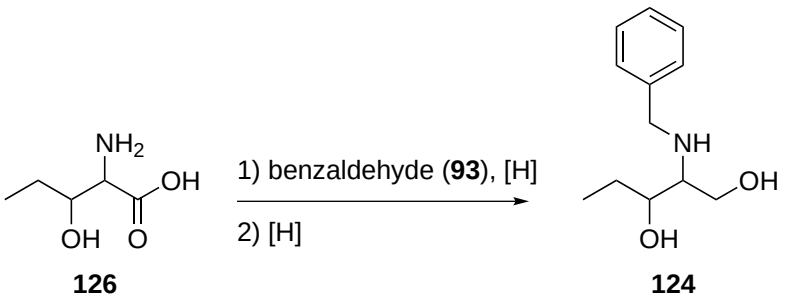


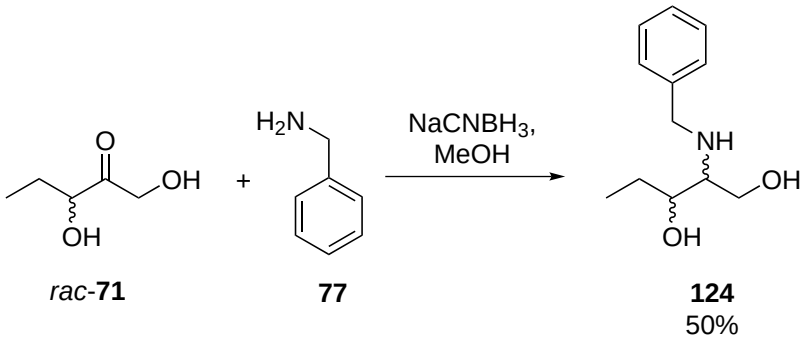
Figure 5.1: Isomers of 2-(benzylamino)pentane-1,3-diol, **124**

amination can give both the *syn*- and the *anti*-products. The consequence of this is that if the biocatalytic step is fully selective for the *S*-isomer then only products **124a** and **124b** are possible, whilst if it is fully selective for the *R*-isomer then only products **124c** and **124d** are possible. In order to ensure that all four possible isomers could be prepared as standards for the assay, two different methods were selected. One method that gave the *syn* product selectively and the other that gave the *anti* product. In the literature there were no reported diastereoselective syntheses of **124**, however, there were several diastereoselective syntheses of the equivalent amino acid, which could be reduced to give 2-(benzylamino)pentane-1,3-diols, **124** (Scheme 5.11).



Scheme 5.11: Synthesis of 2-(benzylamino)pentane-1,3-diol, **124**, from β-hydroxy norvaline, **126**

In order to develop the assay, **124** was synthesized by performing reductive amination upon *rac*-**71**. Initially this was carried out using the optimized method in water, so that a range of different extraction methods could be tested in an attempt to optimize the extraction. However, as more **124** was needed for the assay development it was synthesized by performing reductive amination upon *rac*-**71** using sodium cyanoborohydride in methanol as this gave a much higher yield of 50% (Scheme 5.12). This meant that the assay could be developed in parallel with the synthesis of the standards.



rac-**71** **77** **124**
50%

Scheme 5.12: Synthesis of 2-(benzylamino)pentane-1,3-diol, **124** from *rac*-**71** using benzylamine and sodium cyanoborohydride in methanol

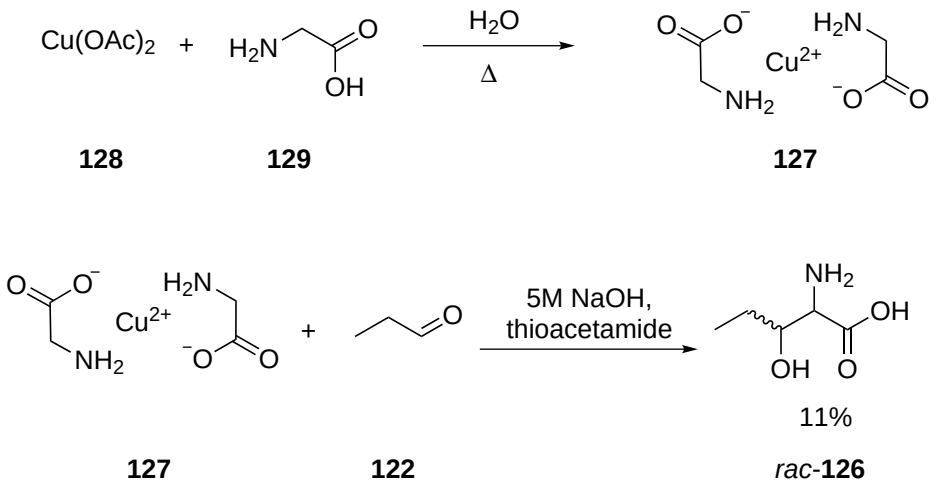
The HPLC assay was developed using a reverse phase HPLC column, eluted with an isocratic mixture of 15% acetonitrile in water, containing 0.1% trifluoroacetic acid. The assay took 30 minutes and products were identified using a UV detector at 254 nm.

5.6.1 Synthesis of racemic β-hydroxy norvaline

The planned stereoselective syntheses of 2-(benzylamino)-pentane-1,3-diol involved the synthesis of β-hydroxy norvaline, **126**, which would subsequently be reductively alkylated

to give the desired product. As the reductive alkylation had not been performed upon **126** before, and the stereo-controlled routes were multi-step, hence making the product from them more valuable, it was decided that a short synthesis of racemic **126** should also be undertaken. The route selected to *rac*-**126** had significant literature precedent and would provide material upon which to develop the reductive alkylation step in two steps from low cost starting materials (Scheme 5.13).[267–271] In the first step *cis-bis*(glycinato)copper(II) complex, **127**, was synthesized by reacting copper (II) acetate, **128**, with glycine, **129**, in water.[267,269] This gave the copper complex in near quantitative yield which was then coupled to propanal, **122**, under basic conditions. After this the copper was decomplexed from the molecule using thioacetamide, giving racemic β-hydroxy norvaline, *rac*-**126**, in only 11% yield.[268] This yield was much lower than the 90% yield reported in the literature and this might be because old reagents were used in the synthesis and the impurities present in them might have adversely affected the reaction yield.[268] However, the reaction optimization was not investigated as the starting materials were available, at low cost, on a large scale and sufficient *rac*-**126** could be synthesized for the development of the reductive alkylation reaction.

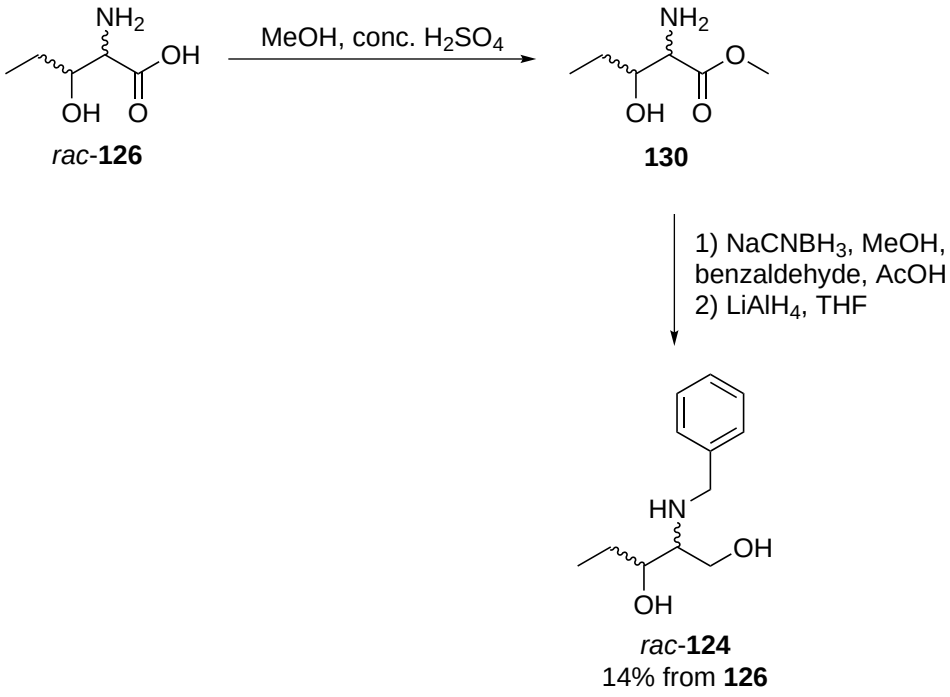
Initially the conversion of *rac*-**126** to its methyl ester, **130** was investigated using TMS-diazomethane. This was because it was thought that the mild reaction conditions associated with this reagent should prevent any racemization at the α-position. This would mean that it could be applied to the products of the stereoselective syntheses without negatively affecting the diastereostereomeric ratio of the final product. The reaction was attempted under a range of conditions (including a variety of pHs and gentle heating), however the methyl ester was not generated. The methyl ester was, therefore, produced by stirring *rac*-**126** in acidic methanol. The methyl ester, **130**, was not isolated at this point as the TLC of the



Scheme 5.13: Racemic synthesis of β-hydroxy norvaline[268]

reaction mixture indicated that the starting materials had been fully consumed. Instead the reaction mixture was neutralized with aqueous sodium hydrogen carbonate and extracted from the aqueous layer using dichloromethane. The combined organic extracts were then concentrated *in vacuo* and the residue taken up in methanol. Benzaldehyde, glacial acetic acid and sodium cyanoborohydride were then added to this mixture and the reductive alkylation monitored using TLC. When the reaction appeared to have gone to completion, the mixture was once again neutralized and extracted with dichloromethane. The combined extarcts were then dried and the mixture concentrated *in vacuo*, before being dissolved in dry THF and reduced using lithium aluminium hydride to give racemic 2-(benzylamino)-pentane-1,3-diol, *rac*-**124**.

Overall, 2-(benzylamino)-pentane-1,3-diol, *rac*-**124**, was synthesized from β-hydroxy norvaline, *rac*-**126**, in 14% yield (Scheme 5.14). The low yield was most probably due to the hydrophilic nature of each intermediate product as well as the final product. Each time the mixture was washed with aqueous base and re-extracted a significant proportion of the



rac-**126** **130** *rac*-**124**
14% from **126**

Scheme 5.14: Conversion of racemic β-hydroxy norvaline to 2-(benzylamino)pentane-1,3-diol

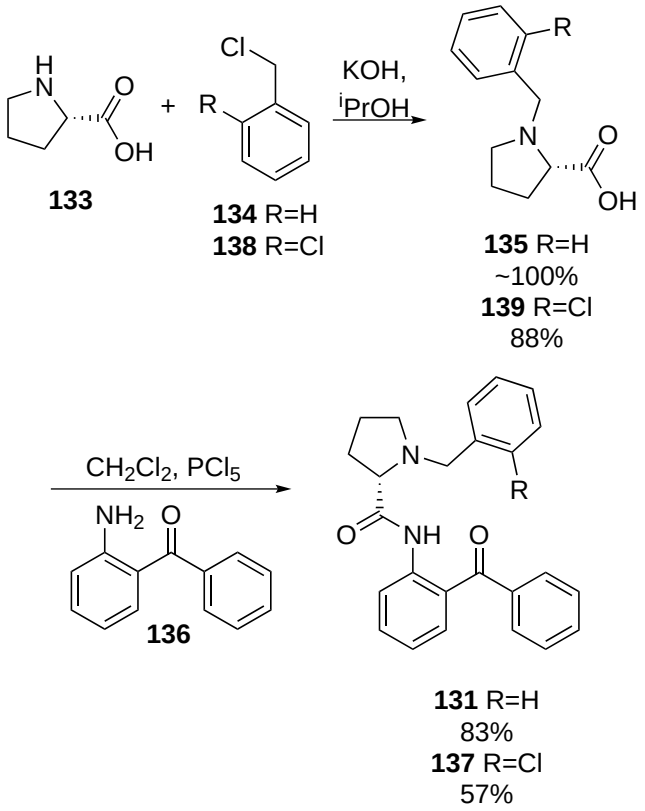
product from each step would probably have been lost, resulting in a significant lowering of the yield, as was observed. One way to improve the yield would be to perform each step without the use of an aqueous base to neutralize the reaction, thus eliminating the need to extract the product from water. This would be possible if ammonia in methanol was used to neutralize each step. The salts formed during the neutralization would all hopefully precipitate out of the reaction mixture, allowing the mixture to be filtered and concentrated before the next step was performed. The product was analysed using the HPLC assay developed using the product of the reductive amination reaction, **124**, using *rac*-**71** from the chemical synthesis (Scheme 5.12). The *syn:anti* ratio was later established as 10:7 (once the diastereomeric products had been analysed and the peaks assigned).

5.6.2 Synthesis of the *syn*-diastereoisomer

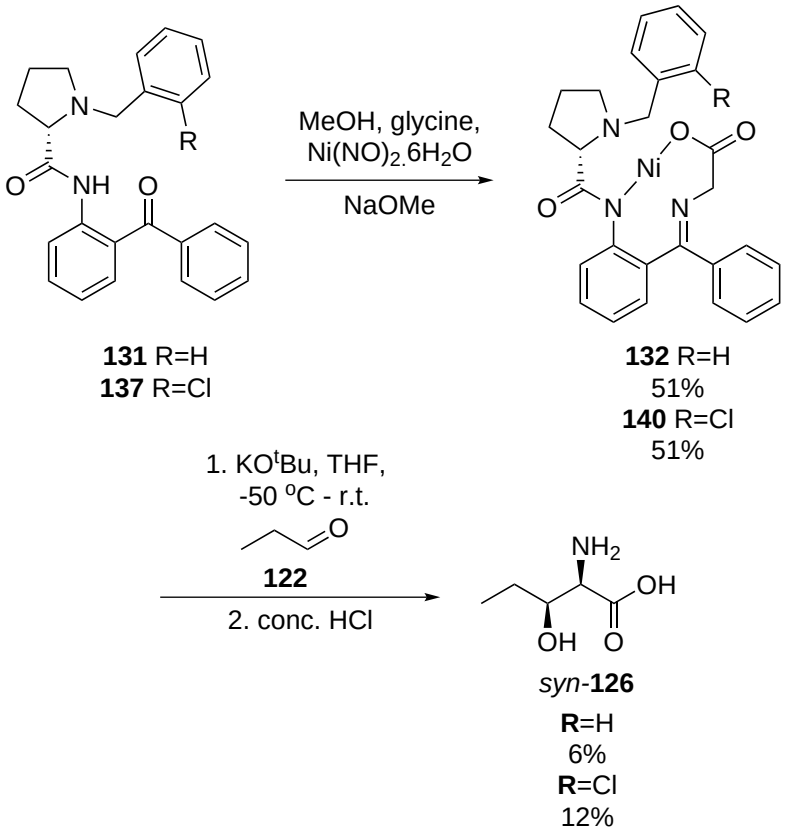
The *syn*-selective synthesis of β-hydroxy norvaline, *syn*-**126**, was performed using a chiral nickel(II)-adduct based method, as it had good literature precedent.[272,273] This method had been developed by Belokon *et al* as an enantioselective way to synthesize amino acids.[272] This synthetis would allow both *syn* enantiomers, **124a** and **124c** to be synthesized separately by using different adducts made using opposite enantiomers of proline. The approach involved synthesizing the adduct, *(R)*- or *(S)*-2-[*N*-(*N*-benzylprolyl)amino]-benzophenone (BPB, **131**) to which glycine and nickel nitrate were added.[272–276] The aldehydes were coupled onto the nickel-glycine-BPB complex, **132**, using an aldol reaction, before the nickel complex was broken up under strongly acidic conditions to give *syn*-**126**. The chiral nickel(II) complex directs the aldol reaction, ensuring exclusively *syn*-addition, and the desired enantiomer can be obtained by carrying out the reaction with either the (*R*) or (*S*)

isomer of the auxiliary, **131**. The synthesis of (*S*)-BPB, (*S*)-**131**, is shown in Scheme 5.15. The first step of the synthesis involved reacting (*S*)-proline, (*S*)-**133**, with benzyl chloride, **134**, under basic conditions, to give **135** in near quantitative yield. This was then converted to the acid chloride using phosphorus pentachloride, and directly coupled to 2-amino-benzophenone, **136**, to give (*S*)-**131** in an 83% isolated yield. Both of these yields were higher than those in the literature, 89% for the synthesis of **135** and 81% for the synthesis of **131**.[277] This was most probably because the literature synthesis was performed on a much larger scale and the superior heating control achievable when the reaction is performed upon a smaller scale gave better results. The literature reported that a better *syn*:*anti* selectivity could be obtained if the 2-chloro derivative of (*S*)-BPB, **137**, was used. This was synthesized in a similar manner to (*S*)-BPB, as shown in Scheme 5.15.[276]

The synthesis of the chlorinated analogue of (*S*)-BPB, **137**, was the same as the synthesis of (*S*)-BPB, **131**, except that 2-chlorobenzyl chloride, **138**, was used instead of benzyl chloride, **134**, in the first step. All of the steps for the synthesis of the chlorinated analogue of (*S*)-BPB, **137**, proved to be lower yielding than the similar reactions to make (*S*)-BPB. The lowest yielding step was the coupling of **139** to 2-aminobenzophenone, **136**, (57% yield) to give **137** which was also much more difficult to purify by recrystallization. This meant that the synthesis was much slower, as the recrystallization took over 100 hours rather than the 24 hours required for **131**. This might have been because the chlorine atom attached to the aromatic ring alters the shape of the entire molecule, making it less planar and so making it harder to pack together in a crystalline solid. As a result of this lengthened synthesis and the much lower yields, the formation of *syn*-**126** was initially performed using (*S*)-BPB to direct the aldol reaction (Scheme 5.16).



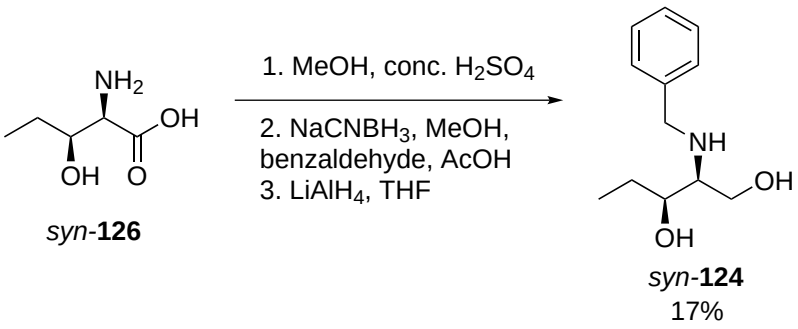
Scheme 5.15: Synthesis of (*S*)-BPB and 2-chloro-(*S*)-BPB[272–276]



Scheme 5.16: Synthesis of the *syn*-β-hydroxy norvaline using *S*-BPB to direct the aldol reaction[276]

The (*S*)-BPB, **131**, was reacted with glycine and nickel(II) nitrate hexahydrate under basic conditions to give the (*S*)-BPB nickel complex, **132**, in 51% yield (Scheme 5.16).[276] This was notably lower than in the literature where a 91% yield was obtained, the decreased yield might have been caused by the use of old chemicals to perform the reaction as these could have contained impurities that might have had a negative impact upon the reaction. Coupling of the nickel complex, **132**, to propanal, **122**, under basic conditions had been described in the literature using sodium methoxide. However, this gave very low yields (5%) of the desired product, again these low yields might have been caused by the use of old reagents that had degraded in the presence of moisture from the air to give methanol and sodium hydroxide. Hydroxide might not have been a suitable base for the deprotonation of propanal. The use of other bases to catalyse the reaction was, therefore, examined and it was found that potassium *tert*-butoxide would catalyse the reaction but with a significantly lower overall yield for this step, giving 6% compared with the yield of 55-96% seen in the literature. This yield might have been greatly improved by using fresh sodium methoxide as the base catalyst. The nickel complex was then broken apart under acidic conditions to form β-hydroxy-norvaline, **126**. The product was purified by cation exchange chromatography using Dowex 50 X 8 resin, with the compound being loaded onto the column and then washed off using 2 M aqueous ammonium hydroxide solution. This extraction was inefficient and partly responsible for the low yield of 6%, this purification step was, therefore, improved for the synthesis using **137** (Scheme 5.16) by increasing the amount of resin used, so that more of the product was captured. This gave an increased yield of β-hydroxy norvaline, **126**, of 12% which was still low and in all probability this low yield was due to the fact that butoxide was used as the base instead of methoxide. Another possible way to improve the yield for this step would be to use 2M ammonia in methanol rather than aqueous ammonium hydroxide. This would mean that the product would not need to be

extracted from the aqueous washings but rather the mixture could be concentrated *in vacuo* to remove both the solvent and the ammonia leaving the product behind. This would be likely to increase the yield as the molecule is hydrophilic and so difficult to extract from water.



syn-**126**

syn-**124**
17%

Scheme 5.17: Synthesis of *syn*-2-(benzylamino)-pentane-1,3-diol, *syn*-**124**[272]

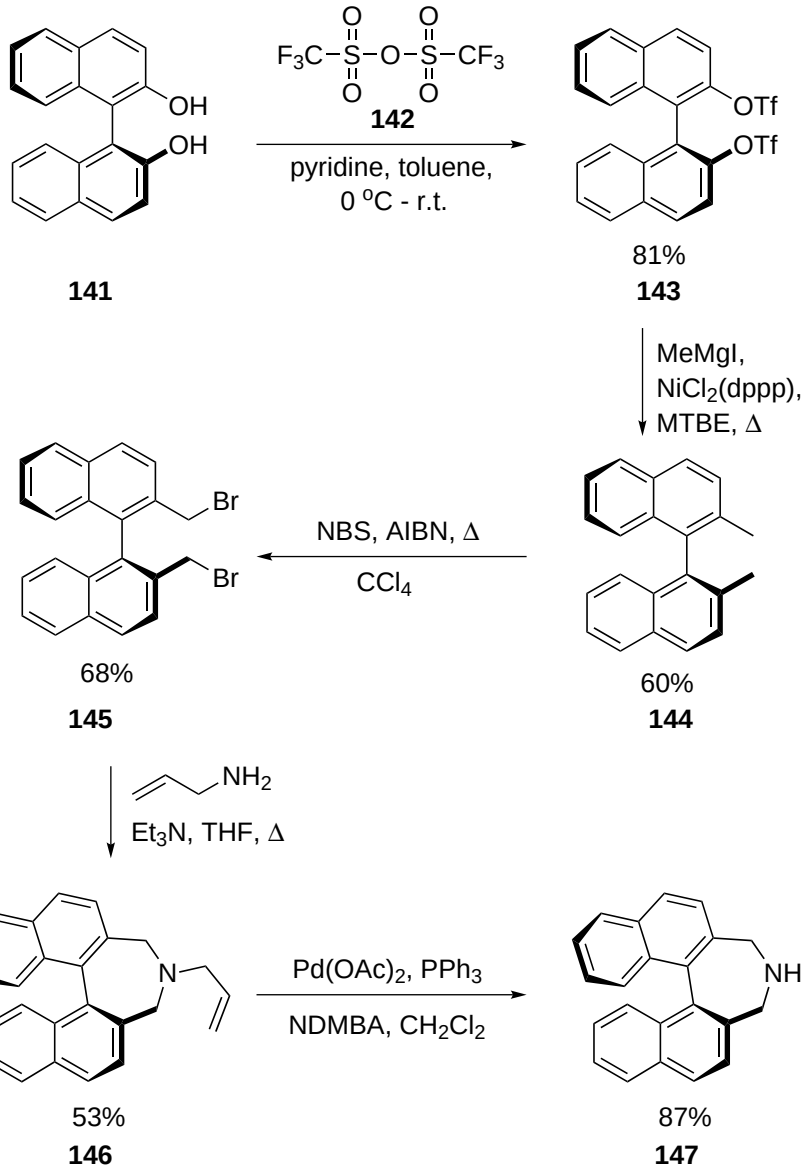
Syn-β-hydroxy norvaline, *syn*-**126** was then converted to the methyl ester, reductively alkylated and reduced, using the method developed upon the racemate, to give *syn*-**126** in 17% yield (Scheme 5.17). The low yield could be improved by optimizing the extraction and purification steps as discussed earlier, so that fewer aqueous extractions were performed. When *syn*-**126** made using **131** was subjected to the HPLC assay that had been developed, a 2:1 *syn:anti* ratio was observed. This was significantly lower than in the literature which suggested that only the *syn* enantiomers were made.[272] This reduced selectivity might have been due to the aldehyde used, as it was not used in the literature, and as it is a small molecule might bring about lowered selectivity.[272] Another possibility is that the selectivity is partly dependent upon the base used to perform the aldol reaction and by using *tert*-butoxide, which is a larger base, the enantioselectivity of the reaction was negatively affected. The product from the synthesis using the 2-chloro-(*S*)-BPB, **137**, adduct gave almost exclusively the *syn*-enantiomer which was similar to the literature results.[276] At this

point the HPLC assay could only determine between the *syn* and *anti* diastereomers as a chiral HPLC column was not being used, the e.e.s could, therefore, not be compared with the literature. Also, the opposite enantiomer was not prepared at this point as the different enantiomers would not be distiguishable on the HPLC trace.

5.6.3 Synthesis of the *anti*-diastereoisomer

A different method was used to synthesize the *anti*-diastereoisomer as a single isomer. This involved using a chiral 1,1'-binaphthalene-2,2'-diol (BINOL)-based phase transfer catalyst developed by Ooi *et al* to induce the required chirality into the β-hydroxy norvaline.[278,279] In the literature β-hydroxy norvaline was not synthesized, the literature synthesis being directed towards (*S*)-2-amino-3-(3,4-dihydroxyphenyl) propanoic acid (L-DOPA), which meant that it had to be adapted slightly.[278] As with the synthesis of the *syn* diastereoisomer, this method was selected as it provided access to each isomer separately and had significant literature precedent.[278,279] The different *anti* isomers could be accessed by using the catalyst based upon either (*S*)- or (*R*)-BINOL.[278,279] As (*S*)-BINOL is considerably cheaper to purchase, the catalyst based upon it was synthesized.

The first step of the synthesis involved the activation of the alcohol functional groups in (*S*)-BINOL, (*S*)-**141**, as the triflates. This was performed using trifluoromethanesulfonic anhydride, **142**, and pyridine to give triflate protected BINOL, **143**, in 81% yield. This was lower than the 97% yield observed for similar reactions in the literature and might have been caused by the reagents being damp, reducing their effectiveness. Another reason might have been that the base used to catalyse the reaction in the literature was triethy-



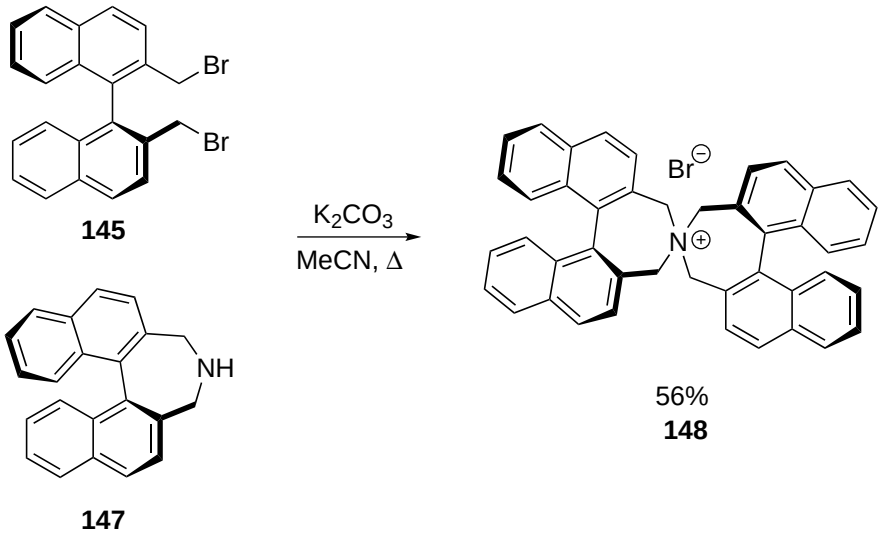
Scheme 5.18: Synthesis of the monomers, **145** and **147** that are reacted together to form the chiral phase transfer catalyst[278]

MTBE=methyl *tert*-butyl ether; NBS=*N*-bromosuccinimide; AIBN=2,2'-azobis(isobutyronitrile); NDMBA=*N*,*N*'-dimethylbarbituric acid

lamine and this might have resulted in higher yields.[278] Triflate protected BINOL, **143**, was then converted to the dimethylated compound, **144**, using a nickel catalysed Grignard reaction. In this reaction **143** was reacted with methyl magnesium iodide and a nickel chloride catalyst in methyl *tert*-butyl ether to give the dimethylated compound, **144**, in 60% yield, this yield was significantly lower than the near quantitative yield obtained in the literature.[278] This lower yield might have been because the literature system reported was not the most efficient, and a slight variation in which a different solvent and temperature were used gave the quantitative yield. Another possible cause of the low yields could be that the reaction was warmed which encouraged side reactions. The dimethyl species, **144**, was then dibrominated using *N*-bromosuccinimide (NBS) and 2,2'-azobis(isobutyronitrile) (AIBN) in carbon tetrachloride to give the dibromo compound, **145**. This reaction, however, did not initially proceed in yields greater than 1%. After attempting the reaction at a variety of temperatures and in various solvents, as well as trying a different radical initiator (1,1'-azobis[cyclohexanecarbonitrile]). It was discovered that the reaction was not working because the NBS being used was too pure, having been recrystallized to make it easier to handle. This meant that there was insufficient bromine impurity present to initiate the reaction. This problem was solved by mixing together the recrystallized NBS in a 2:1 ratio with the original batch of impure NBS. Once this challenge had been overcome, the synthesis proceeded well giving the dibromomethyl compound, **145**, in 68% yield which was higher than the 62% yield obtained in the literature.[278]

The dibromo compound, **145**, was then divided into two portions, one of which was reserved to be used later in the synthetic sequence. The other portion of **145** was heated with allylamine in THF, with triethylamine, to give tertiary amine **146** in 53% yield. This was lower than the 96% yield seen in the literature where triethylamine was not added to

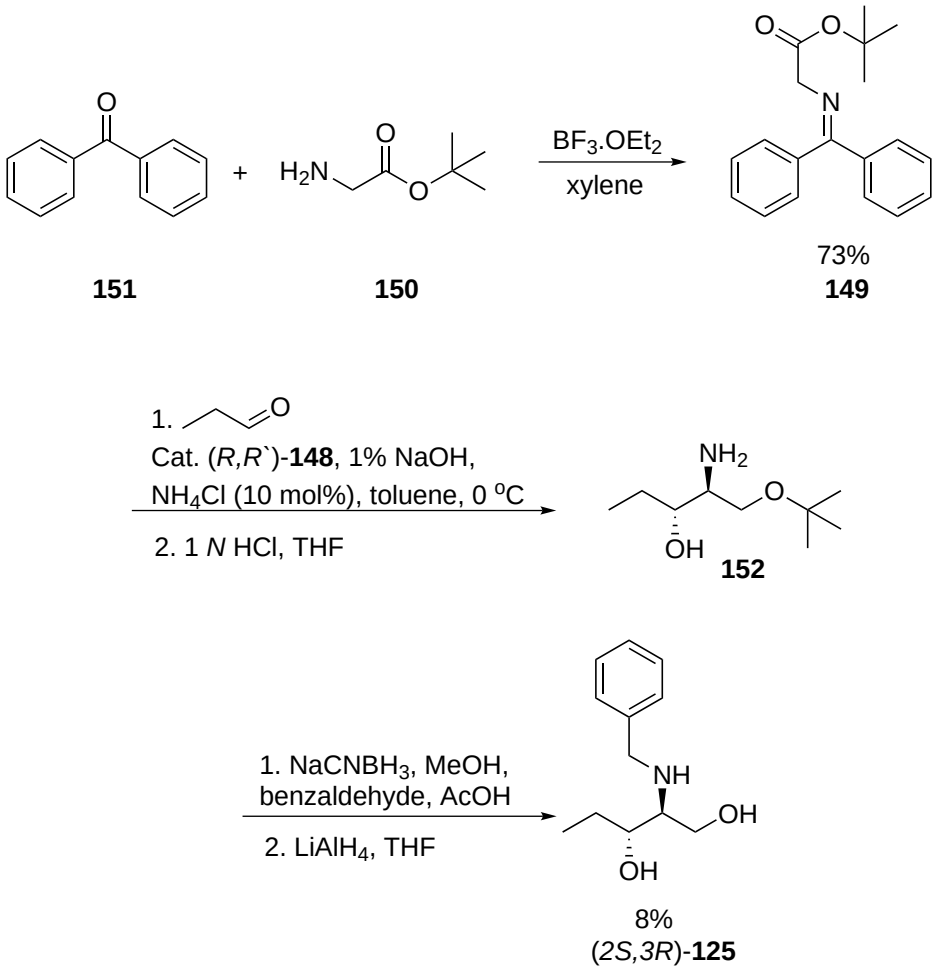
the reaction, and this might have been why the yield was different, or it could have been because the reaction was performed on a small scale (0.5 g) compared to the literature (4.4 g), and this might have meant that the product was harder to purify as a higher proportion was lost.[278] The tertiary amine, **146**, was then used in a Tsuji–Trost reaction, using palladium acetate and triphenylphosphine with *N*,*N*'-dimethylbarbituric acid in dichloromethane, to give the secondary amine **147** in 87% yield (Scheme 5.18), close to that seen in the literature.



Scheme 5.19: Synthesis of the dimer for the chiral phase transfer catalyst[278]

The reserved portion of the dibromo compound, **145**, was then coupled to the secondary amine, **147**, under basic conditions to give the desired catalyst, **148**, as the bromide salt, in 56% yield, once again close to that obtained in the literature (53%; Scheme 5.19).[278] This catalyst, **148**, was subsequently used to catalyse the asymmetric aldol coupling of propanal, **122**, to the benzophenone imine of glycine *tert*-butyl ester, **149**. **149** had been synthesized in 73% yield by reacting glycine *tert*-butyl ester, **150**, with benzophenone, **151**, in xylene

using boron trifluoride diethyl etherate (Scheme 5.20).



Scheme 5.20: Synthesis of the *anti*-2-(benzylamino)-pentane-1,3-diol
Cat.=catalytic

When the catalytic aldol step was performed using catalyst **148** the *anti* diastereomer of the *tert*-butyl ester of β-hydroxy norvaline, **152**, was obtained. This was because catalyst **148** directed the addition of propanal to the least hindered face. This meant that only the reductive amination reaction and ensuing reduction needed to be carried out on the molecule, as the acid was already activated, as the ester, for reduction. These were carried

out as previously described in the syntheses of both *syn*- and *rac*-**124** (Scheme 5.20) to give (*2R,3R*)-2-benzylamino-pentan-1,3-diol, **124d**, in 8% yield. Once again this reaction sequence was low yielding due to the hydrophilic nature of the product. Compound **124d** was subjected to the HPLC assay and shown to be a mixture of the a *syn* and *anti* compounds in a ratio of 1:10.

5.7 HPLC assay results

Once the reverse phase HPLC assay conditions had been developed for the separation of compound **124** and the *anti* and *syn* peaks identified, the aqueous chemical reductive amination reaction was investigated with it. The assay was tested using the products of the reductive amination reaction upon *rac*-**71**, produced by the chemical reaction, as well as **71** produced using transketolase. The results are shown in Figure 5.2.

The results indicated that both the optimized reductive amination reaction in water and in glycerol were selective for the *anti*-diastereoisomer. This was unexpected, even though there are a number of other chemical reactions in water where a single isomer predominates because the reaction is hydrophobically directed. This occurs because hydrophobic sections of the molecule tend to pack together to keep out water, as is seen with surfactant molecules. One example of this is the benzoin reaction when performed in water (Scheme 5.21). In this case there is a partial overlap of the phenyl rings caused by a hydrophobic interaction between them. This results in a faster reaction rate in water and also means that in the transition state one arrangement is favoured over all the others making the reaction at least partly stereoselective.[87] Many similar reactions in water have been investigated by Breslow

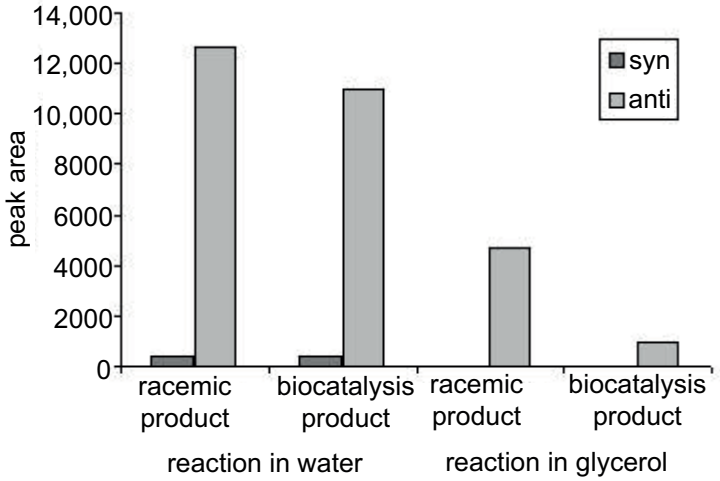
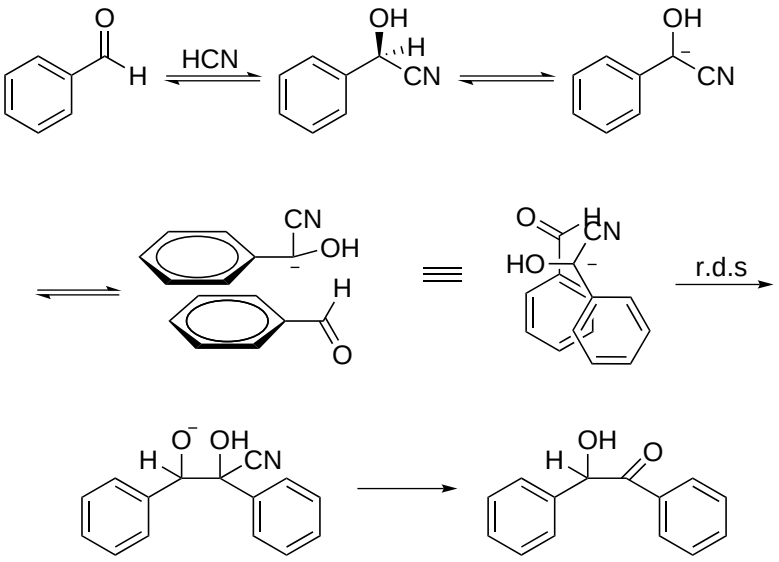


Figure 5.2: HPLC assay results for the product (**124**) of the optimized reductive amination reactions upon 1,3-dihydroxypentan-2-one, **71**, produced both chemically (racemic) and with transketolase (biocatalytic product)

and his research group, and this effect is fairly common.[87] This might also explain the rate increase shown when the reaction is performed in water.

Another possibility is that the Lewis acid used to catalyse the reaction is forming a complex with the intermediate. This might then direct the facial addition of either the amine group to form the imine, or the reduction of the imine to form the amine.[280–283] An example from the literature is the enhanced facial selectivity for the reductive amination reaction of ketones with (*S*)-α-methyl benzylamine seen by Nugent *et al*.[280] These reactions were only selective when ytterbium acetate was added to the reaction using Raney nickel and molecular hydrogen (Scheme 5.22).[280] In the reaction shown in Scheme 5.22 an 87% d.e., with an excess of the *trans* isomer, was observed when ytterbium acetate was present.[280] In the paper it is suggested that *in situ* ketimine isomerization occurs, mediated by the ytterbium acetate (Scheme 5.22).[280] This idea was confirmed by the d.e. observed when ytterbium

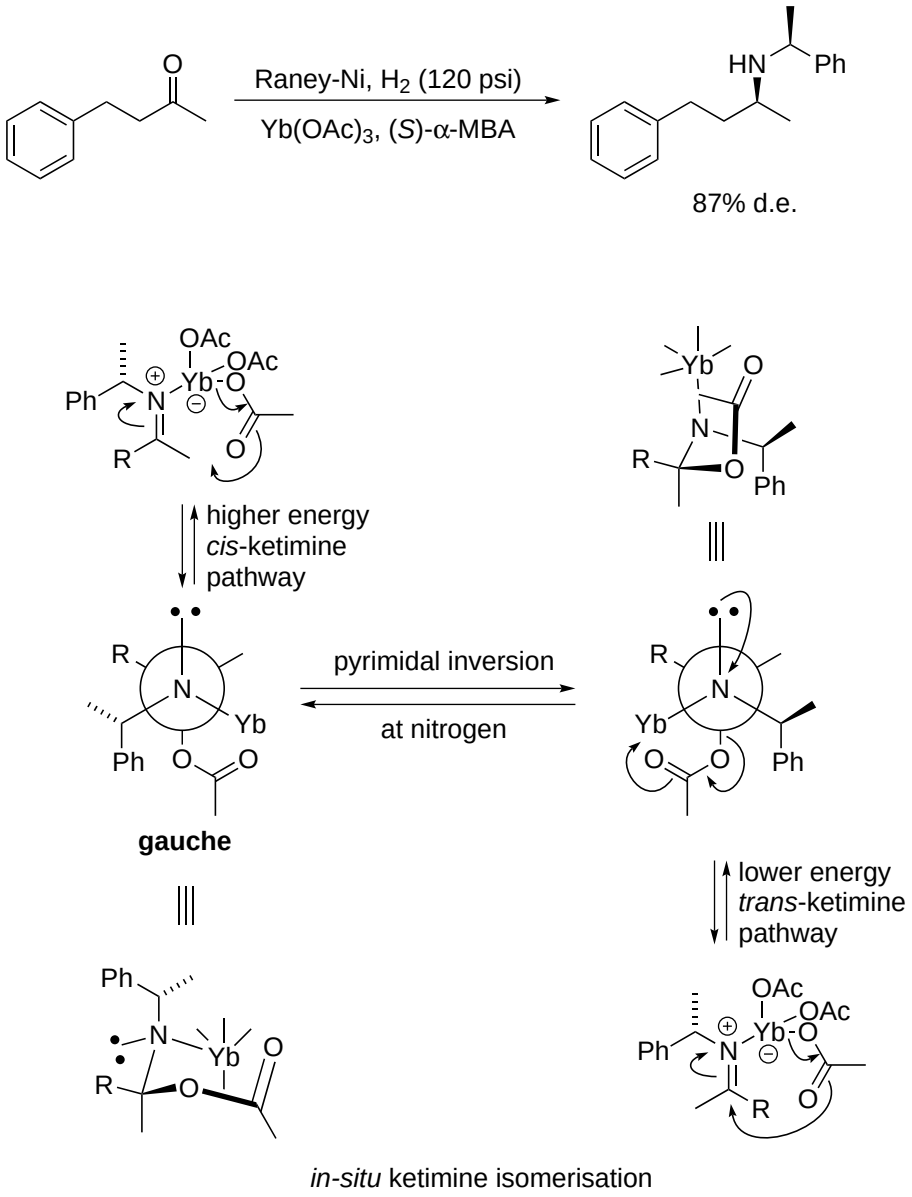


Scheme 5.21: Hydrophobic direction of a Benzoin condensation in water.[87]
r.d.s.=rate determining step

acetate was not added (72%), and also when the reaction was catalysed by titanium isopropoxide (67%) rather than ytterbium acetate.[280] As well as this the free energy of the intermediates backs up the idea.

However, the simplest explanation for the *anti*-selectivity comes from the application of the Felkin–Anh rules. Using a computer model (MOPAC - PM3 energy minimization based upon heat of formation, gradient norm and Cosmo solvation in water) the preferred conformation was investigated. This was calculated starting from the *S*-stereoselective biotransformation, and the results of energy minimization for both the *E* and *Z* isomers are shown in Figure 5.3.

As the *E* isomer had a larger calculated heat of formation, the model used for the Felkin–Anh predictions used this isomer as shown in Figure 5.4. The hydride then attacks on the



Scheme 5.22: Enhanced facial selectivity in reductive amination reactions using ytterbium acetate, Raney nickel, molecular hydrogen and (*S*)-α-methyl benzylamine ([*S*]-α-MBA)[280]

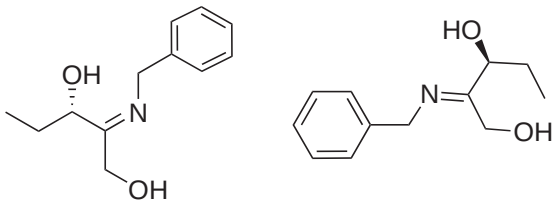


Figure 5.3: Minimized energy conformations of *cis* and *trans* isomers of imine. Energy minimized in Chem3D using MOPAC – PM3 energy minimization based upon: heat of formation; gradient norm; and Cosmo solvation in water

least hindered side at the Burgi-Dunitz angle (107°). This gives the *anti* product, which is the predominant product observed experimentally. The fact that the presence of different cations does not significantly affect the selectivity suggests that it is not this that causes the effect, and lends weight to the explanation using the Felkin–Anh model.

During the development of the HPLC assay, problems were encountered when attempting to generate the calibration curve for compound **124**, as it was found that there was too much variability in the HPLC peak areas obtained. Due to time constraints it was not possible to spend more time modifying the assay to get more consistent peak areas. Nevertheless, even though the peak areas varied widely the ratios obtained for *syn*-**124** and *anti*-**124** did not, and so it was decided to use the assay solely to determine the diastereomeric ratio. This would enable us to investigate how modifying various conditions affected the selectivity of the reaction, therefore, only the diastereoselectivity of the products is discussed and not the yields.

Having seen that the reaction that had been developed using cyclohexanone as a substrate was selective for the *anti*-diastereoisomer when using *rac*-**71**, the work focussed on

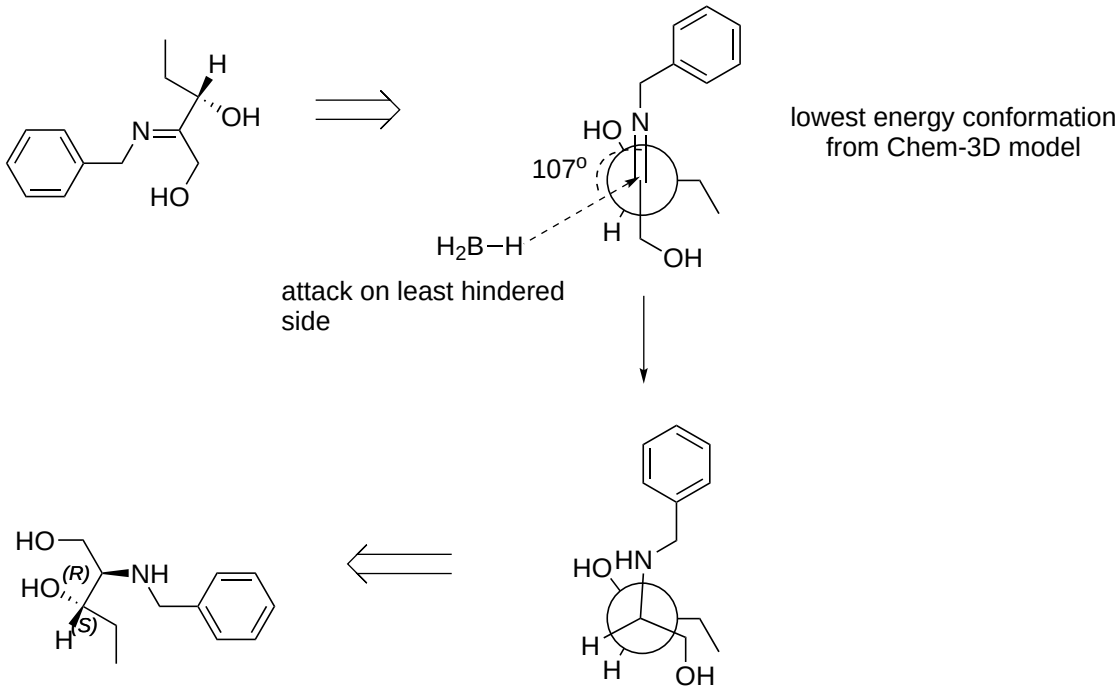
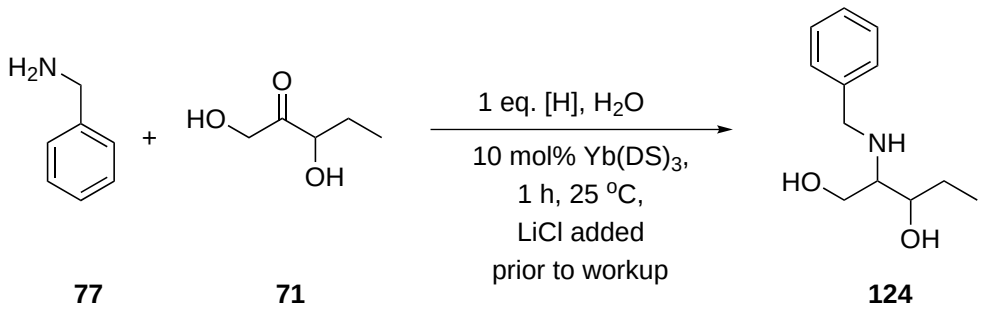


Figure 5.4: *Anti*-selectivity as predicted by the Felkin–Anh rules

testing different reagents in order to establish how each component of the reaction mixture affected the selectivity and whether the opposite diastereomer could be obtained.

5.7.1 Varying the reducing agent



Scheme 5.23: Reaction optimized by varying the reducing agent

In order to investigate whether the reducing agent was responsible to any degree for the diastereoselectivity observed for the reductive amination reaction, a variety of reducing agents were tested with *rac*-**71**, under the same conditions developed for the aqueous reductive amination of cyclohexanone (Scheme 5.23). The results of this investigation are shown in Table 5.1 and Figure 5.5.

Entry	Reducing agent	*syn:anti* ratio
1	borohydride on solid support	1:4
2	cyanoborohydride on solid support	1:3
3	picoline borane	0:1
4	pyridine borane	1:22
5	ammonia borane	1:14
6	sodium triacetoxyborohydride	1:18
7	sodium cyanoborohydride	1:5
8	lithium borohydride	1:11
9	morpholine borane	1:1
10	*tert*-butylamine borane	1:0
11	dimethylamine borane	1:0

Table 5.1: HPLC assay results for the conversion of *rac*-**71** to **124** with a variety of reducing agents

From the table it can be seen that the reduction is typically selective, to varying degrees, for the *anti*-diastereoisomer. This indicates that the reducing agent plays a key role in determining the selectivity of the reductive amination reaction. The only reducing agents that do not show this selectivity are *tert*-butylamine borane, dimethylamine borane and morpholine borane (Entries 9, 10 and 11 in Table 5.1). These all demonstrated very little reductive amination, as was expected from earlier results when using cyclohexanone. These very low yields might be the reason that the opposite selectivity was observed, as very little material was available to be subjected to the assay. This meant that there was a wide margin for error

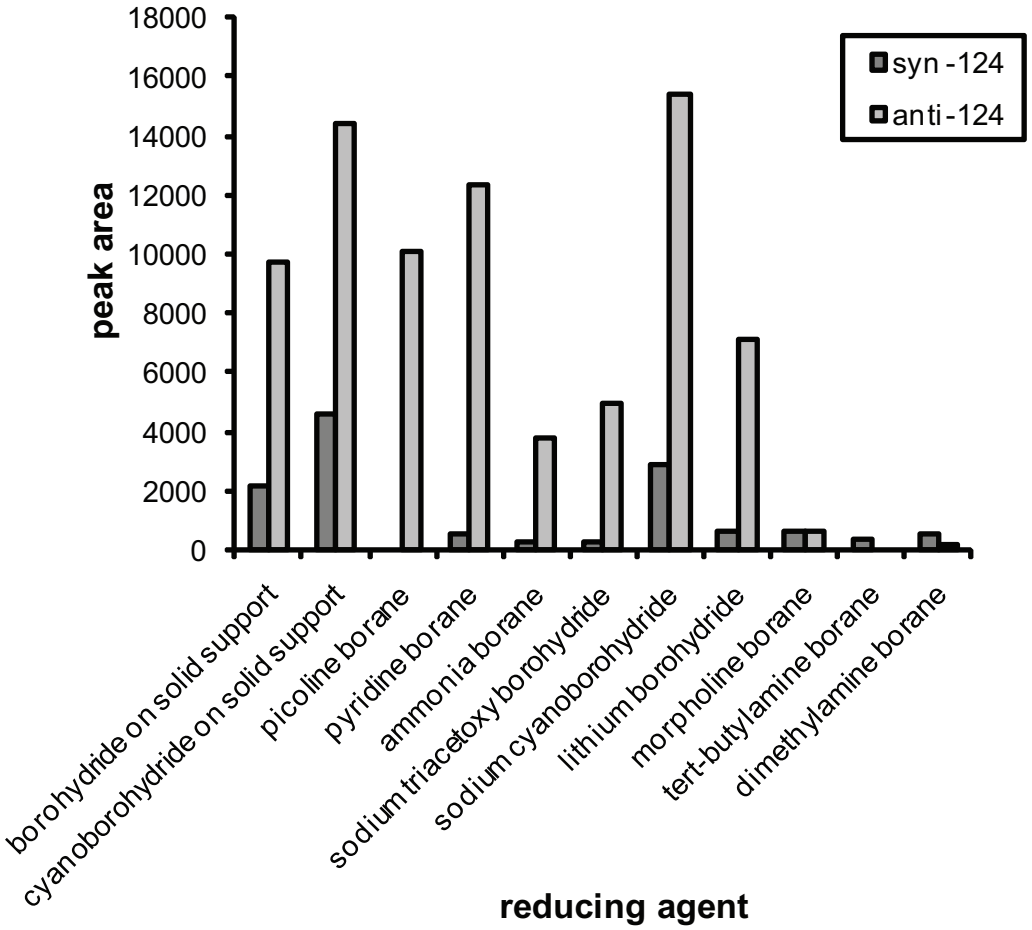
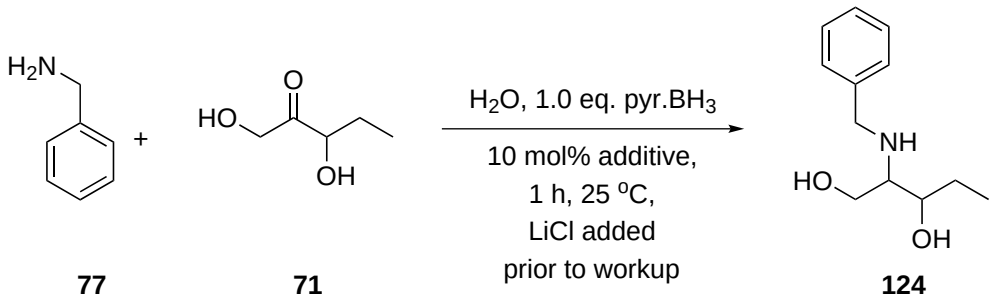


Figure 5.5: HPLC assay results for the conversion of *rac*-**71** to **124** with a variety of reducing agents using the conditions indicated in Scheme 5.23

in the results and so this inversion of selectivity was ignored, as it was likely that it was an artefact and the low yields meant that they did not warrant further investigation. However, ammonia borane (entry 5 in Table 5.1) which also showed very little activity when used upon cyclohexanone appeared to be a lot more active when used in the reductive amination reaction with *rac*-**71**. This could indicate that the imine formed from **71** is more stable than that formed from cyclohexanone. Nevertheless the increase did not impact upon its use in the reaction, as the selectivity data indicated that it was not as good as the reaction with either pyridine or picoline borane (entries 3 and 4 in Table 5.1). In terms of diastereoselectivity, the best reducing agent was picoline borane (entry 3 in Table 5.1) which did not produce any of the *syn* isomer, however, pyridine borane (entry 4 in Table 5.1) was also very selective (22:1, *syn:anti*) and when peak areas were taken into consideration it appeared to give a better conversion than picoline borane. Sodium cyanoborohydride on the other hand appeared to give a good reaction conversion, as indicated by the combined peak areas for both diastereoisomers, but gave poor selectivity with an *anti*:*syn* ratio of 5:1. The best selectivity provided by a borohydride reducing reagent was with sodium triacetoxyborohydride, which is a larger reagent, suggesting that at least for the borohydride reagents it might be steric bulk that provides selectivity. Even though steric bulk appeared to be an important factor, the selectivity decreased when the reagent was attached to a solid support with an *anti*:*syn* ratio of 3:1 observed for polystyrene-bound cyanoborohydride. This might have been caused by the extremely large steric bulk of the solid support, which means that the comparative difference in steric bulk for each face became relatively small. The hydride group will attack from the least hindered side, but as the size of the hydride group increases, the molecule will be able to determine between the less and more hindered faces to a lesser extent. This will mean that more attack will occur on the more-hindered face and the diastereoselectivity of the reaction will decrease.

5.7.2 Addition of additives

The effect of additives upon the reductive amination of *rac*-**71** with benzylamine, using pyridine borane, was investigated by adding a range of additives to the reaction (Scheme 5.24, Table 5.2 and Figure 5.6). The formation of **124** remained selective for the *anti*-diastereoisomer with all of the additives tested, however, varying the additive affected the selectivity slightly, with some additives enhancing the selectivity to a greater or lesser extent.



Scheme 5.24: Reductive amination reaction converting *rac*-**71** to **124** using various additives

The rare earth metal Lewis acids gave the best selectivity, however, even when they were not present reaction selectivity was still observed for the *anti*-isomer. This provided more evidence that the selectivity was due to either hydrophobic effects or Felkin–Anh rules of attack, rather than Lewis acid complexation. It appeared that the Lewis acid enhances the yield of the reaction rather than the selectivity, and so was still a useful addition to the reaction for this reason. It was also seen that ytterbium triflate gave better selectivity than the ytterbium dodecyl sulphate complex (Entries 1 and 2 in Table 5.2). This suggested that the triflates could be used in optimized reactions, however, if product extraction and isola-

Entry	Additive	*syn:anti* ratio
1	ytterbium triflate	0:1
2	ytterbium dodecyl sulfate	1:13
3	β-cyclodextrin	1:2
4	gadolinium triflate	0:1
5	scandium triflate	1:18
6	polystyrene-bound scandium triflate	1:10
7	dysprosium triflate	1:105
8	indium chloride	1:11
9	L-proline	0:1
10	lithium chloride	1:71

Table 5.2: HPLC assay results for the conversion of *rac*-**71** to **124** with a variety of additives, as shown in Scheme 5.24

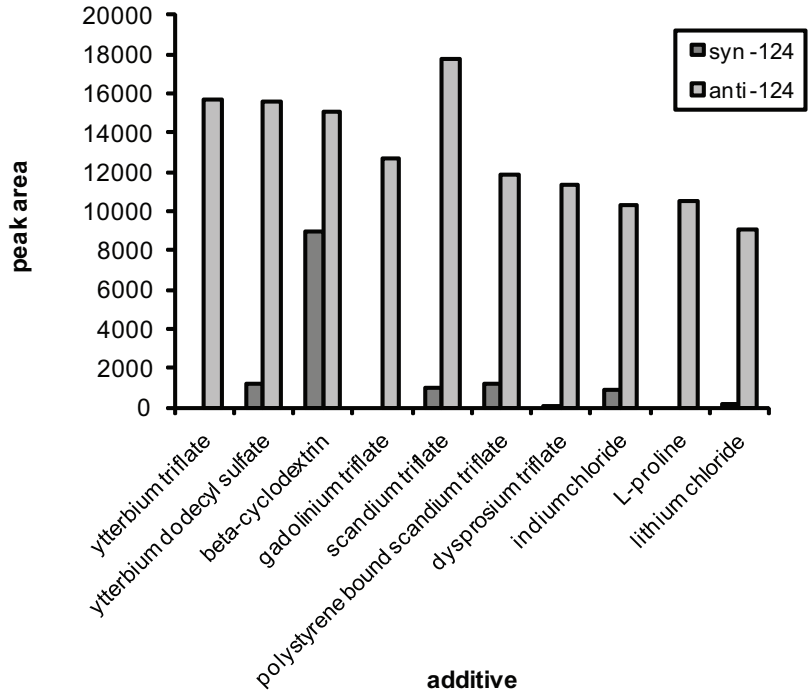


Figure 5.6: HPLC assay results for the reductive amination reaction converting *rac*-**71** to **124** using a variety of additives, as shown in Scheme 5.24

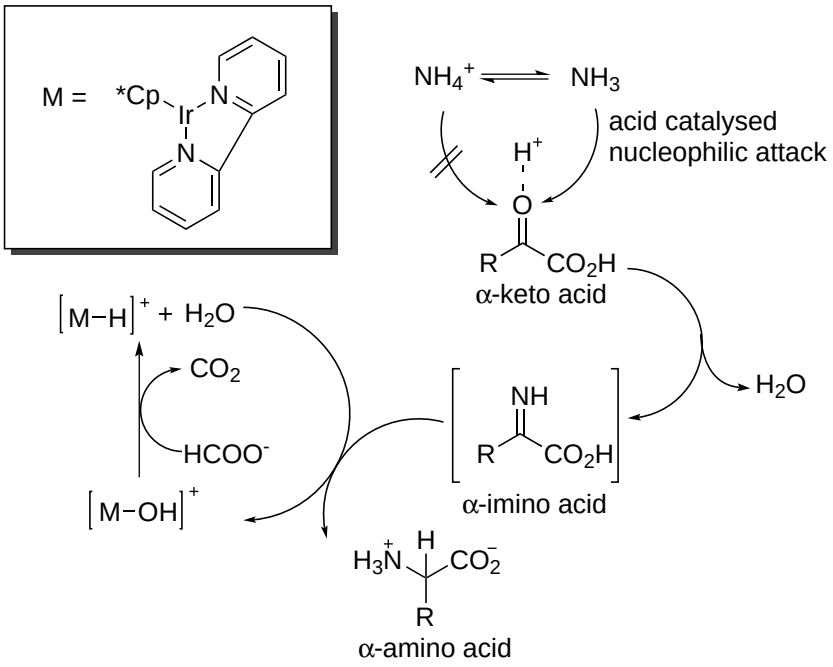
tion remained difficult then the LASC should remain a good choice as it was chosen in the test optimization because it helps drive the product into the organic layer to aid extraction.

It appeared that the reaction conditions that had been developed upon cyclohexanone were giving very good results. This combined with the fact that the optimization would take a lot of time to give relatively small changes in yields and selectivity it was decided to examine alternative strategies that might offer a different selectivity.

5.8 Development of reductive amination using catalytic asymmetric transfer hydrogenation

As one diastereoisomer consistently predominated when the stoichiometric reduction method, that had been developed, was used, the reductive amination reaction was also attempted using transfer hydrogenation. This was because by performing the reduction catalytically it should be possible to test a variety of already existing catalysts that had been shown to provide selectivity in other reactions. This would provide the best chance of identifying a catalyst that offered the opposite selectivity to the stoichiometric reaction and would also mean that the reaction might be able to be made fully enantioselective. This route was examined because a lot of work has been published in the literature on catalytic asymmetric reduction reactions. As well as providing good reaction selectivity, the catalyst should also be recyclable as it remains in the aqueous phase after the organic extraction step. This should mean that the catalyst can be easily recycled increasing the atom efficiency of the reaction, as well as potentially reducing the reaction costs.

Ogo *et al* have previously reported that the catalytic reductive amination of α-keto acids can be achieved using iridium based catalysts.[179,180] The suggested reaction mechanism for the process is shown in Scheme 5.25.



Scheme 5.25: Reductive amination of α-keto acids using iridium catalysts[179,180]

Ogo *et al* performed reductive amination, using ammonia, upon a range of α-keto acids to give α-amino acids. One example from his work was the conversion of pyruvic acid to alanine using their iridium catalyst and ammonium formate in 96% yield.[180] In order to test the catalytic reaction and see whether it could also be applied more generally to reductive amination reactions it was performed on several substrates, and the products isolated. These reactions were carried out by reacting pentamethylcyclopentadienyl iridium(III) chloride dimer ($[Cp^*IrCl_2]_2$) with the *N*-toluenesulfonyl-(*1R,2R*)-1,2-diaminocyclohexane ((*R,R*)-

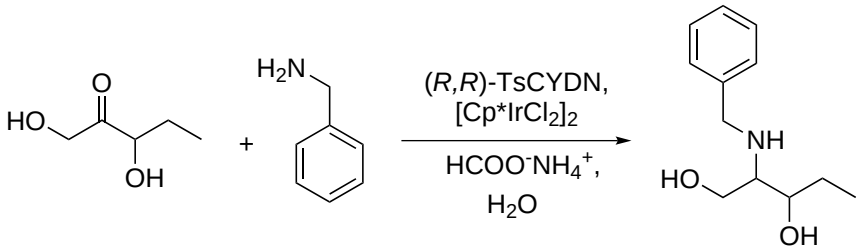
TsCYDN) ligand in un-degassed water at 40 °C for 1 hour in the open air.[180] Ammonium formate, the amine and the aldehyde or ketone were then introduced and the mixture stirred for a further 1 hour. The mixture was then extracted into ethyl acetate and purified by silica column chromatography. The results are shown in Table 5.3.

Substrate	Amine	Isolated yield (%)
benzaldehyde	aniline	57
benzophenone	aniline	24
cyclohexanone	benzylamine	26

Table 5.3: Test reactions for iridium transfer hydrogenation. Pentamethylcyclopentadienyl iridium(III) chloride dimer was stirred with the *N*-toluenesulfonyl-(*1R,2R*)-1,2-diaminocyclohexane ligand in un-degassed water at 40 °C for 1 hour in the open air. Ammonium formate, the amine and the aldehyde or ketone were then introduced and the mixture stirred for a further 1 hour at 40 °C[180]

The yields obtained for this reaction were higher than those obtained from the stoichiometric reduction. This was particularly noticeable for the reductive amination of benzophenone that had not worked when performed using the stoichiometric method. This suggested that the reaction selectivity was different from the previously optimized reaction and worth investigating. The reaction was, therefore, partially optimized in order to see whether the yield could be increased. As the reaction was catalytic the only variables that were examined were the reaction time and temperature. As there was now a simple chemical synthesis of **71** the reaction was optimized upon this, rather than upon an alternative substrate as was used for the optimization of the stoichiometric reaction. The reaction was monitored using ^{1}H NMR spectroscopy to follow the consumption of the starting material. The results obtained for the reaction using **71** (Scheme 5.26) are shown in Table 5.4.

Having investigated a range of reaction temperatures the optimal temperature was ob-



71 **77** **124**

Scheme 5.26: Transfer hydrogenation reductive amination reaction performed upon 1,3-dihydroxypentan-2-one

Temperature (°C)	Ratio (**124**/**77**)
25	0.00
30	0.05
40	0.29
50	0.11
60	0.07
80	0.00
100	0.00

Table 5.4: Optimization of temperature of asymmetric transfer reductive amination in water. Pentamethylcyclopentadienyl iridium(III) chloride dimer was stirred with the *N*-toluenesulfonyl-(*1R,2R*)-1,2-diaminocyclohexane ligand in un-degassed water at 40 °C for 1 hour in the open air. Ammonium formate, benzylamine and *rac*-**71** were then introduced and the mixture stirred for a further 1 hour at the temperature indicated

served to be 40 °C, which is the same as that noted for the literature reactions.[179,180] The other variable to be optimized was the reaction time, using the same reaction conditions (Scheme 5.26) and the results are shown in Table 5.5.

Time (h)	Ratio (**124**/**77**)
1	0.29
20	0.32
50	1.03
60	4.03
70	Complete

Table 5.5: Optimization of reaction time of asymmetric transfer reductive amination in water. Pentamethylcyclopentadienyl iridium(III) chloride dimer was stirred with the *N*-toluenesulfonyl-(*1R,2R*)-1,2-diaminocyclohexane ligand in un-degassed water at 40 °C for 1 hour in the open air. Ammonium formate, benzylamine and *rac*-**71** were then introduced and the mixture stirred for the amount of time indicated at 40 °C

From Table 5.5 it can be seen that the reaction was complete after 70 hours. In order to obtain an isolated yield, the reaction was performed using cyclohexanone and benzylamine, as this was easier to extract and purify as it is less hydrophilic than **71**. An isolated yield of *N*-benzyl-cyclohexylamine (**117**) of 45% was obtained, this low yield was probably in part due to material being lost during the product extraction. This isolated yield was, nevertheless, much better than those of the stoichiometric reduction. This may be because the long reaction time enabled the reaction to fully reach completion, which may not have been possible for the stoichiometric reactions. This is because they were performed using a predefined set of reaction conditions based upon the trial reaction, which included a predefined short reaction time. Another possible explanation for the increase in yield could be that the reaction mixture contains fewer components, in particular fewer amines, which might make the separation simpler. The best way to determine which of these reasons is correct would be to repeat the earlier reactions using the stoichiometric conditions, follow-

ing the reactions by HPLC and only stopping the reactions when they reached completion. However, we did not have enough time to investigate this idea further.

The catalytic transfer hydrogenation reaction had been shown to be versatile, working on a wider range of ketones and aldehydes than the stoichiometric reaction, as well as working on 1,3-dihdroxypentan-2-one, **71**, and so the reactions diastereoselectivity was investigated using the HPLC assay. This was to see if the reaction exhibited a different diastereoselectivity, as well as its slightly different substrate selectivity, to the stoichiometric reduction. The optimized reaction was, therefore, performed upon *rac*-**71** and analysed using the HPLC assay. As well as the tppts ligand originally used, several other phosphine ligands that were water stable (Dave-Phos, X-Phos and S-Phos, Figure 5.7) were tested, to see whether these would affect the stereoselectivity of the reaction. The results for the reaction with the different ligands are shown in Figure 5.8.

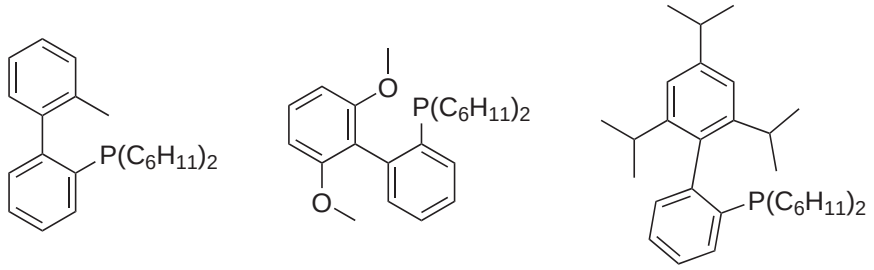


Figure 5.7: Other ligands tested with the catalytic transfer hydrogenation procedure. Pentamethylcyclopentadienyl iridium(III) chloride dimer was stirred with the ligand in undegassed water at 40 °C for 1 hour in the open air. Ammonium formate, benzylamine and *rac*-**71** were then introduced and the mixture stirred for a further 1 hour at 40 °C

The transfer hydrogenation method worked well with all of the ligands and furnished

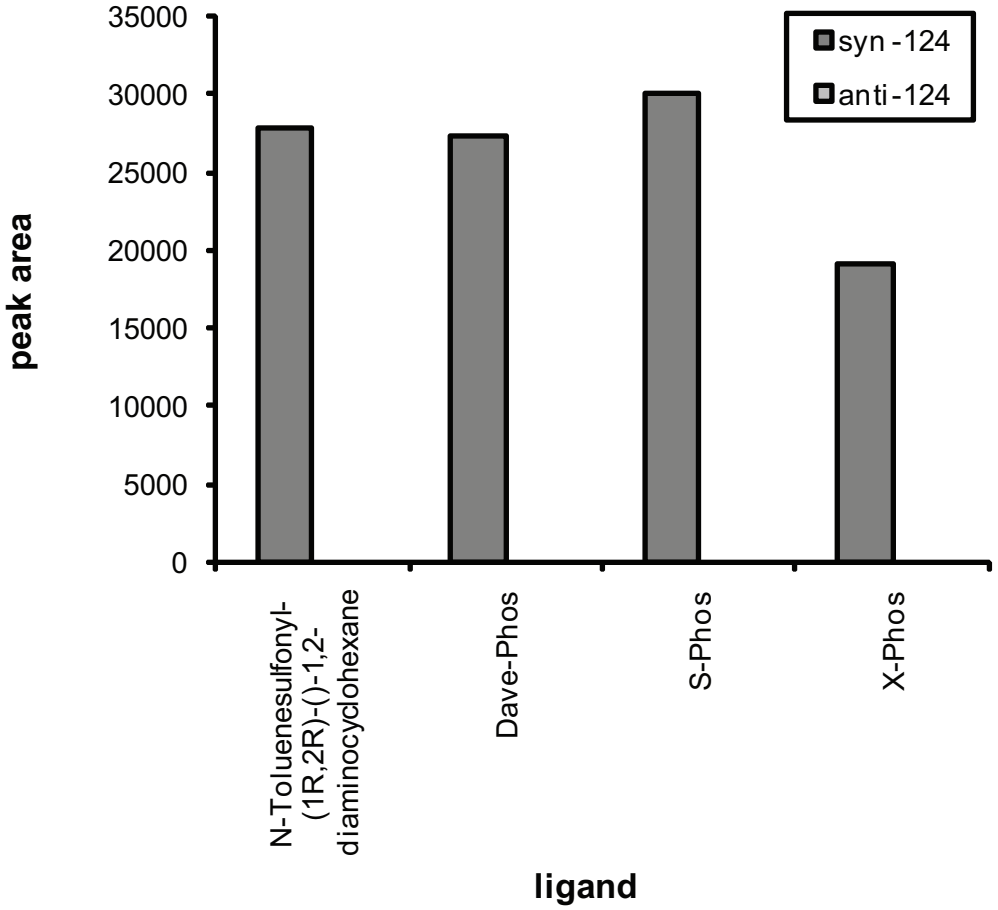
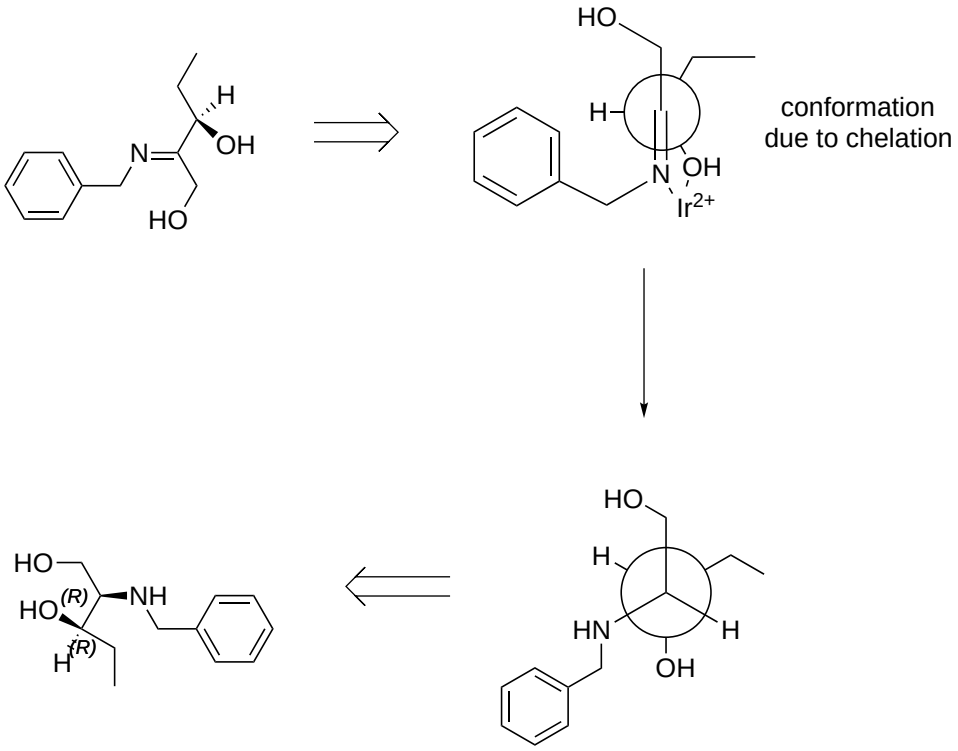


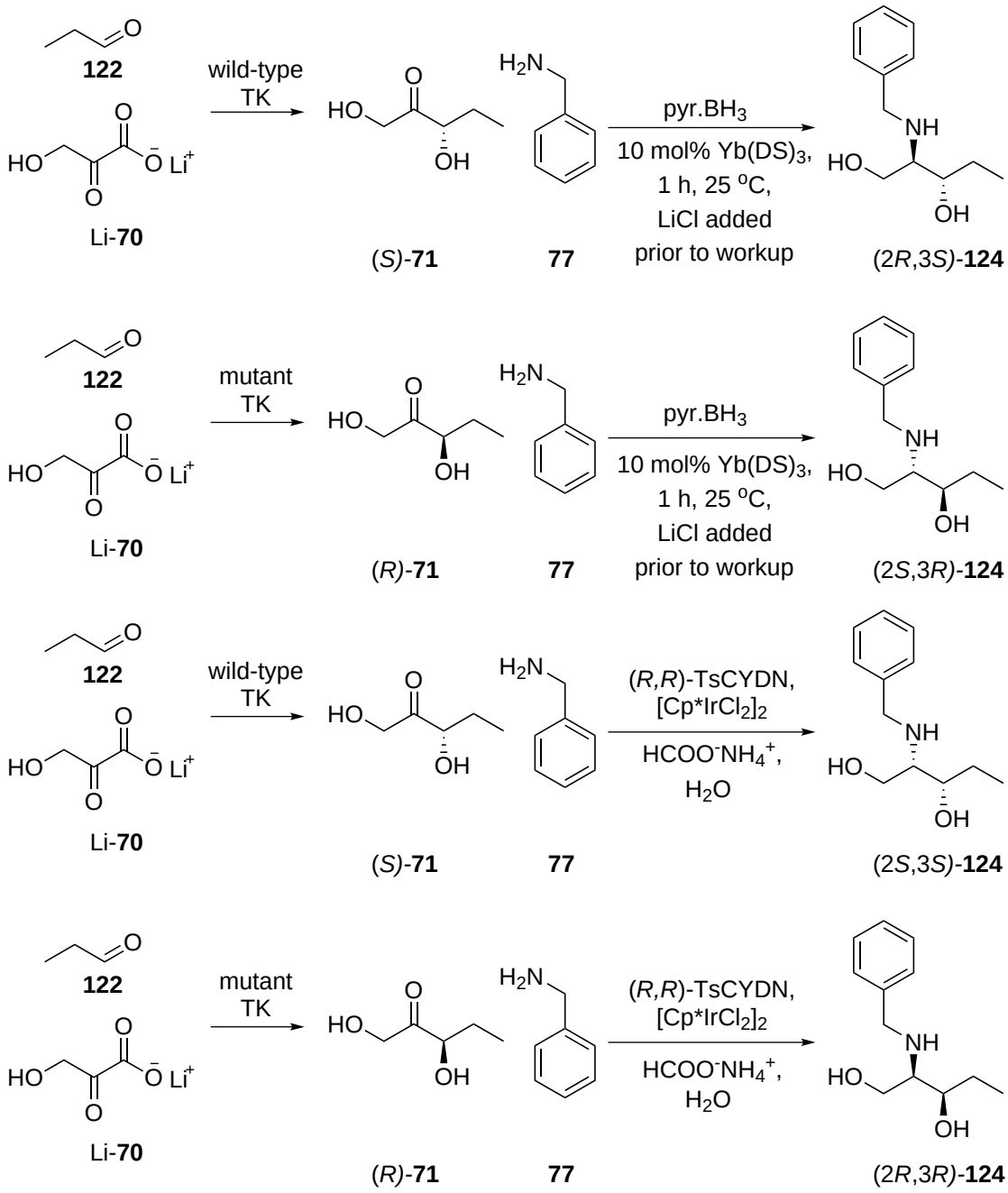
Figure 5.8: HPLC assay results for transfer hydrogenation with various ligands on *rac*-**71**. Pentamethylcyclopentadienyl iridium(III) chloride dimer was stirred with the ligand in undegassed water at 40 °C for 1 hour in the open air. Ammonium formate, benzylamine and *rac*-**71** were then introduced and the mixture stirred for a further 1 hour at 40 °C

124 solely as the *syn* stereoisomer with all of the ligands. *S*-Phos was found to be the best ligand for the reaction as it appeared to give a slightly increased yield according to the HPLC data, whilst maintaining exclusively *syn* selectivity. However, all of the ligands gave the same selectivity and all but X-Phos displayed similar activity, it would, therefore, be useful to repeat these reactions, obtaining an isolated yield and reanalysing the diastereoselectivity to make certain that the results are reliable, as well as providing more data as to which ligand works best. The *syn* diastereoselectivity might be explained using the Felkin–Anh model. This is because it is possible for the iridium to chelate with the imine intermediate, changing its preferred, lowest energy conformation, as shown in Scheme 5.27. This complexation might also allow for inversion of the stereocentre as was seen in a similar system by Nugent *et al*.[280]



Scheme 5.27: *Syn*-selectivity explained by the Felkin–Anh model

It is also possible that the iridium could chelate to the two hydroxyl groups in the molecule which could also confer *syn*-selectivity to the reaction. Overall these results were encouraging, as this was the opposite selectivity to that observed with the stoichiometric reduction method. This means that the two different diastereomers **124a** and **124b** could be obtained selectively from a single enantiomer of (*S*)-**71**. A mutant transketolase enzyme that gives the opposite enantiomer of **71** to the wild type enzyme ((*R*)-**71** rather than (*S*)-**71**) has been developed using directed evolution by other members of the BiCE research group, which means that with these two enzymes and the pair of complementary reductive amination reactions in water, all four stereoisomers of **124** are available selectively *via* a combined chemoenzymatic approach (Scheme 5.28).[222] However, these combined reaction sequences are currently untested and this along with further development of the transfer hydrogenation reaction need to be performed. However, a lack of time meant that these could not be carried out.



Scheme 5.28: Possible enantioselective synthesis of all four enantiomers of 2-benzylamino-pentane-1,3-diol, **124**

TK=transketolase; TsCYDN=*N*-toluenesulfonyl-(1*S*,2*S*)-1,2-diaminocyclohexane

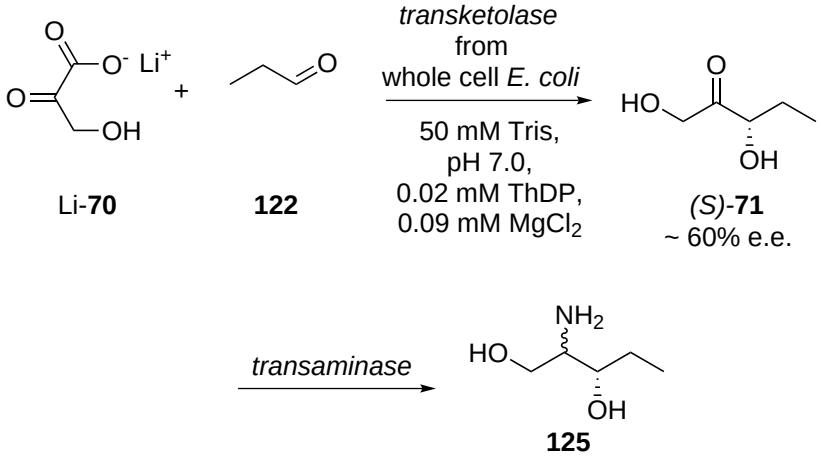
6

Conclusions and Further Work

6.1 Conclusions

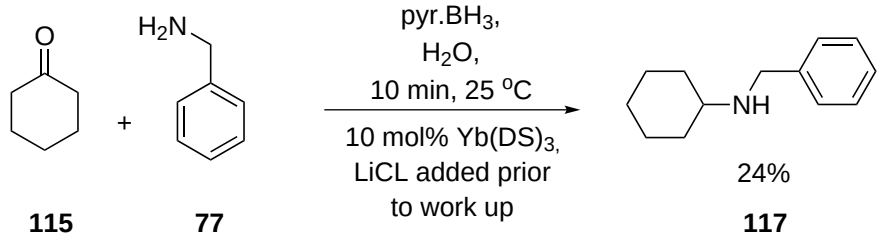
The aim of this research programme was to examine one aspect of a proposed biocatalyst development route for process engineers.[1] The project examined the development of a chemical route to a product, that was also being targeted, simultaneously, by a biocatalytic synthesis. The enzymatic synthesis in the model system examined involved a two reaction cascade that would be performed in one pot. The proposed reaction is shown in Scheme 6.1 and involves the conversion of pyruvate to a 1,3-dihydroxyketone using transketolase and an aldehyde, followed by an amination reaction performed using transaminase.[224]

Upon examining the proposed synthesis it was decided that the transaminase step would be more easily targeted for the concurrent development of a chemical reaction. This was because an analogous chemical reaction, reductive amination (alkylation), already existed, which had been shown to work in water.[178] At the start of the research the intermedi-



Scheme 6.1: Synthesis of aminodiol **125** using a two enzyme cascade[224]
ThDP=thiamine diphosphate

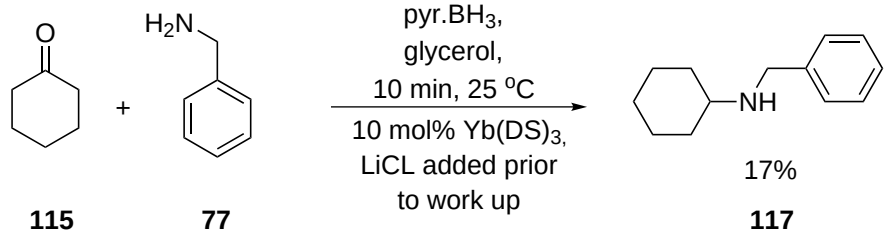
ate ketodiol, **71**, in the synthesis of aminodiol **125** could not be easily prepared and so a model system was used to develop the reaction. Cyclohexanone was chosen as the substrate upon which to develop the reaction, and an HPLC assay developed to allow rapid reaction screening and optimization. Once a reaction that worked in water had been discovered, the reaction was quickly optimized and the results of this optimization are shown in Scheme 6.2.



Scheme 6.2: Application of the optimized reductive amination reaction to cyclohexanone

The reaction was then applied to a selection of aldehydes and ketones and isolated yields of 19–45% were obtained. A similar reaction in glycerol was also developed (Scheme 6.3)

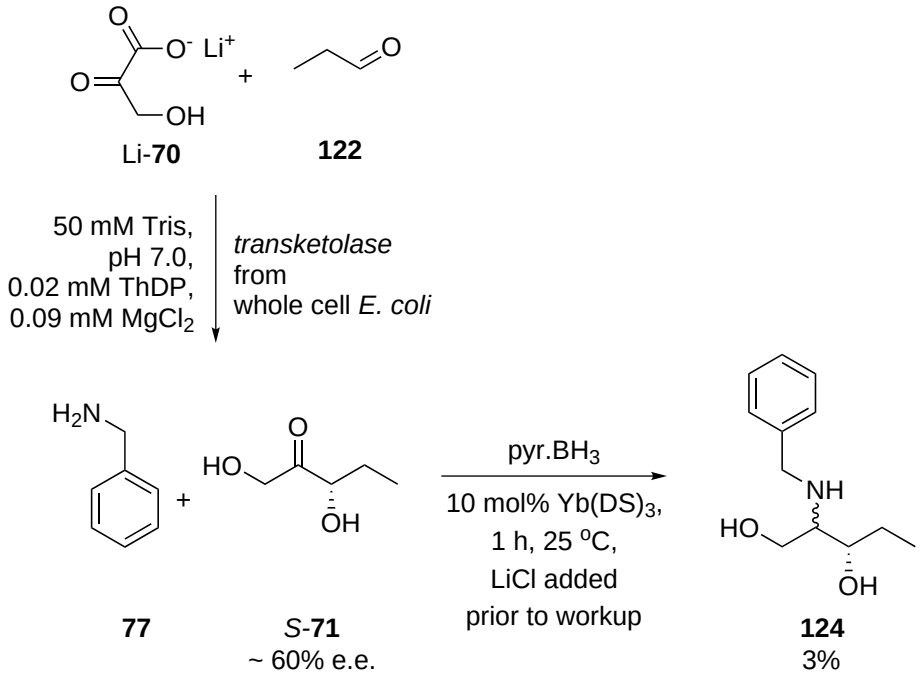
and gave isolated yields of 11–27%.



Scheme 6.3: Application of the optimized reductive amination reaction in glycerol to cyclohexanone

The reaction in glycerol was not developed any further, as the solvent was not compatible with the biotransformation carried out with transketolase. The reaction in water was tested upon racemic 1,3-dihydroxypentan-2-one, *rac*-**71**, in Tris buffered solution identical to that used in the biotransformation. When it was observed that the reaction still proceeded (10% yield) the reaction was performed in a one-pot sequential reaction with the biotransformation catalysed by transketolase (Scheme 6.4).

The reaction performed in a one-pot sequential manner was low yielding (3%) because of the difficulties encountered in the isolation and purification of the product. The reaction was not developed further at this point as the reaction had been shown to work as desired. The stoichiometric reductive amination reaction still needs to be optimized further, as the isolation and purification of the product are not ideal and this is currently the major failing of the reaction. This is also where the majority of the chemicals are used in the synthesis, as a massive excess of solvent is required to fully extract the product from the aqueous reaction mixture. This difficulty is caused by the polar nature of the molecule which makes it very hydrophilic. Until this extraction method is improved upon, either using alternative

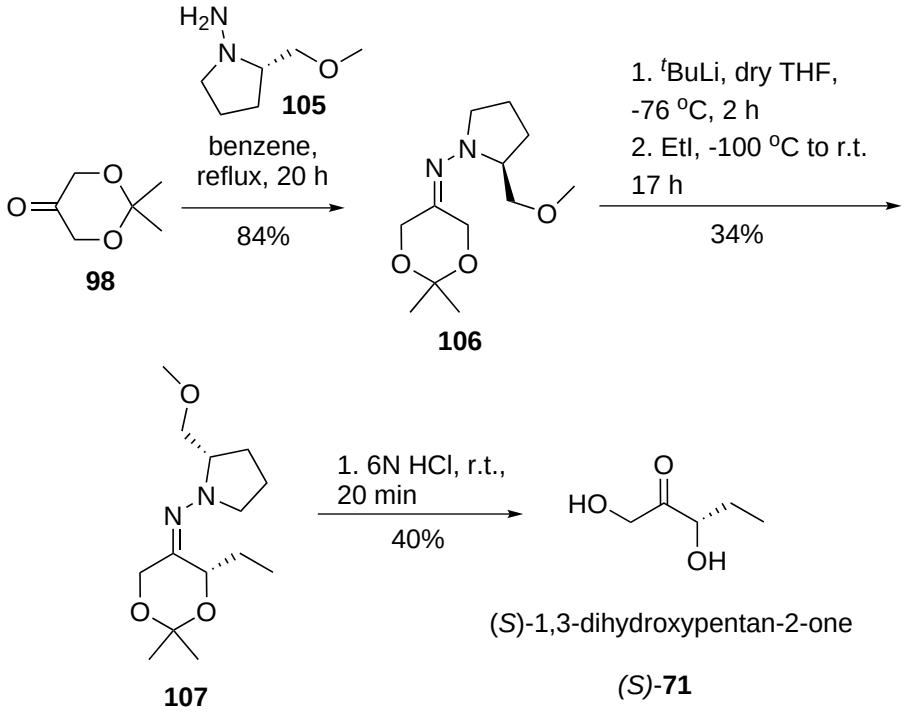


Scheme 6.4: One pot combined biocatalytic and chemical synthesis of 2-(benzylamino)pentane-1,3-diol

green solvents (*e.g.* supercritical CO_2) or a chromatographic method that works with water as the solvent (*e.g.* reverse phase chromatography) the reaction cannot be considered to be 'green'. In fact the reaction is a lot less environmentally benign than if it was performed in an organic solvent, where the extraction is simplified. Other investigators from the BiCE research group are already working on improving the isolation of 2-aminopentane-1,3-diol, **125**, that is produced by a two enzyme cascade (Scheme 6.1). This isolation is being developed using affinity chromatography. Isolute SCX-2 cartridges, which are packed with propylsulfonic acid functionalized silica, are being used, as they are acid functionalized and so can capture the basic amine functionality present in the molecule. The dual enzymatic cascade reaction gives a simpler product mixture than the cascade involving the biotransformation and the chemical reductive amination, which contains benzylamine and pyridine (from the pyridine borane) as well as the desired product. However, it should be possible to isolate only the desired product as it has a different pK_a to benzylamine and pyridine and so should associate differently to the column. This same method could then also be applied to the catalytic transfer hydrogenation reaction whose product is equally hard to isolate. However, as this reaction only uses one other amine (benzylamine) the capture should be simplified.

The next part of the reaction that needed to be explored was whether the chemical reaction could be performed to give a single enantiomer of aminodiol **125**. In order to calculate the selectivity of the reaction it was essential to know the enantiomeric purity of the starting ketodiol **71** produced by the transketolase biotransformation. The absolute stereochemistry of the wild-type transketolase enzyme was determined by the asymmetric synthesis of one of the enantiomers of the product. This was synthesized using Enders' SAMP-hydrazone chiral auxiliary to direct the stereochemistry of the product, and the synthesis is shown in

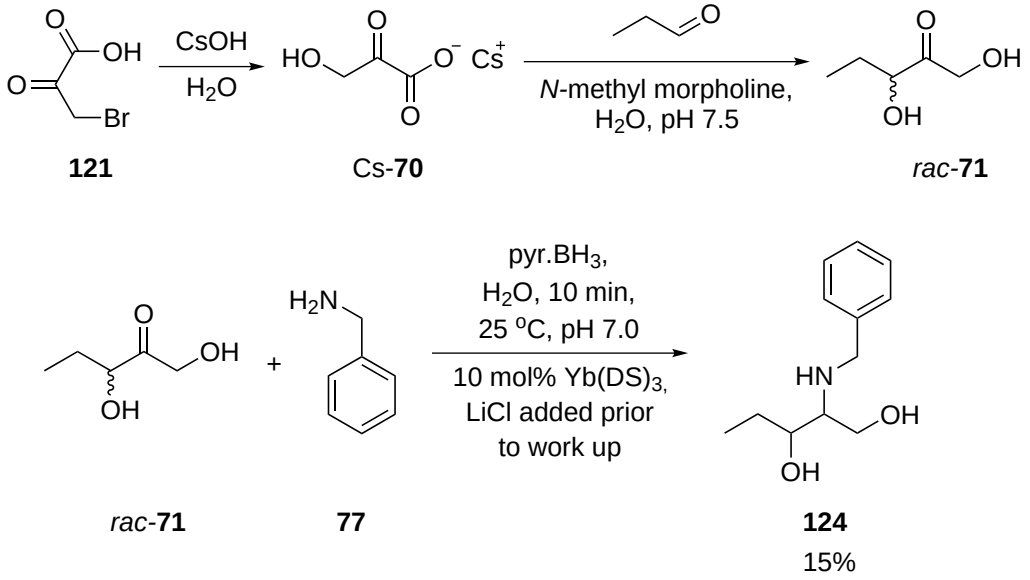
Scheme 6.5.[65,201] The product was subsequently diacetylated and subjected to a GC assay. When propanal and lithium hydroxy pyruvate were used as the substrates, the wild-type transketolase was seen to be *S*-selective (58% e.e.), by the GC–MS assay.[222]



Scheme 6.5: Synthesis of (*S*)-1,3-dihydroxypentan-2-one for use in a GC–MS assay[65,201]

During the course of the research a chemical synthesis of *rac*-**71** was discovered by other members of the BiCE research group.[207] This meant that the selectivity of the reductive amination reaction upon ketodiol **71** could be explored more easily, as the starting material for the reaction was much easier to obtain. As the starting material was racemic it was felt that it would be best to initially explore the diastereoselectivity of the reductive amination reaction. This was because a similar assay to that used for the initial optimization could be used rather than having to develop a new chiral assay. This assay was developed using the purified product from the reductive amination reaction. This was generated

by reacting *rac*-**71** synthesized chemically with benzylamine, **77**, and sodium triacetoxyborohydride in 1,2-dichloroethane. By using this well established method a better yield of the pure product was available upon which to develop the assay. This assay was then tested upon aminodiol **124** synthesized using the optimized reaction in water, as shown in Scheme 6.6.[207]



Scheme 6.6: Optimized reductive amination reaction performed upon *rac*-**71** synthesized chemically

In order to calibrate the HPLC assay, both the *syn*- and *anti*-diastereoisomers of 2-benzylamino-pentane-1,3-diol, **124**, were individually synthesized so that their HPLC peaks could be identified. The *syn*-enantiomer was synthesized using a nickel complex of *(S)*-BPB, **131**, to provide the required diastereoselectivity (Scheme 6.7).[273] The synthesis of the *trans*-isomer used a phase transfer catalyst based upon BINOL to give the opposite diastereoselectivity (Scheme 6.7).[279] These different diastereomers of β-hydroxy-norvaline were then converted to the desired product, **124** (Scheme 6.7). The product of the reductive

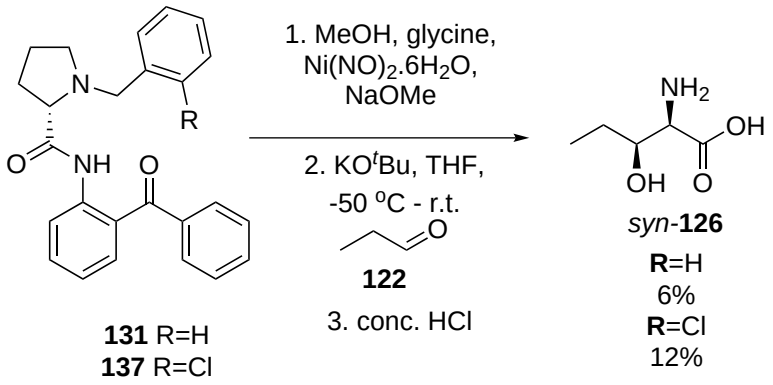
amination reaction in water (Scheme 6.6) was subjected to the assay and the reaction found to be highly *anti*-selective with selectivities up to 22:1 when pyridine borane was used as the reducing agent for the reductive amination of *rac*-**71** with benzylamine, **77**, in water. This selectivity could be increased by adding rare earth metal Lewis acid catalyst, for example dysprosium triflate which gave selectivities according to the HPLC assay of 105:1 *anti:syn*.

In order to discover whether a different stereoselectivity could be obtained by using a different method of reduction, a catalytic transfer hydrogenation method was also investigated. The reaction (Scheme 6.8) was found to proceed in water giving isolated yields of 26–57 %. The reaction was also analysed by the HPLC assay and only the *syn*-diastereomer was detected. The selectivity and yield were improved by using other ligands, for example *S*-Phos.

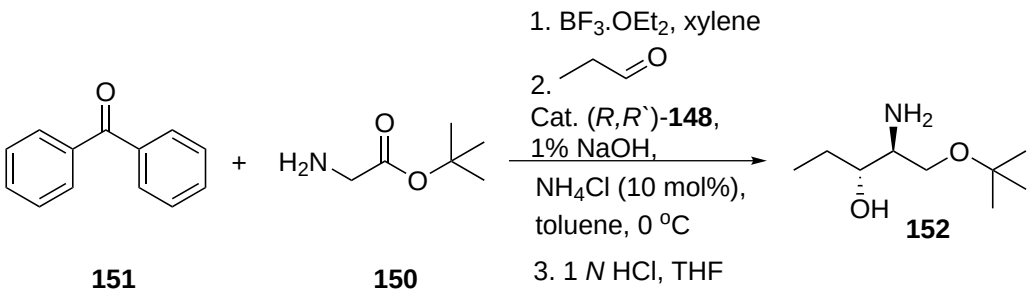
6.2 Further work

The diastereoselectivity of the reaction needs to be explored further and independently confirmed. This is of particular importance for the catalytic transfer hydrogenation reactions, as this work was performed near the end of the research programme and this meant that there was insufficient time to fully verify the results. It is possible that the HPLC detection of only the *syn* product might be a result of a systematic error, caused by part of the procedure used or an artifact from within within the HPLC machine. This could easily be checked by repeating this work. The stoichiometric reductive amination work was repeated during the research programme. and so these results are more reliable. Nevertheless it

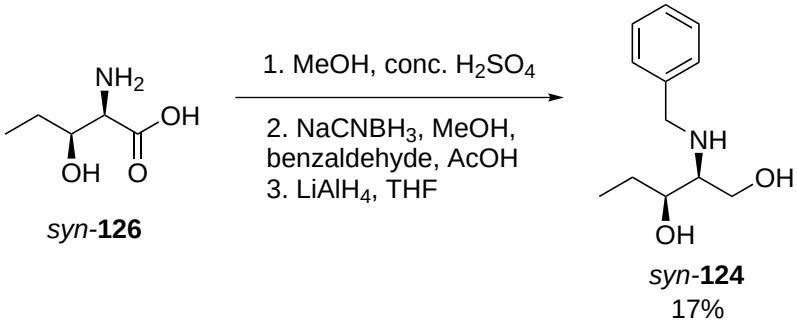
Synthesis of *syn*-β-hydroxy norvaline:



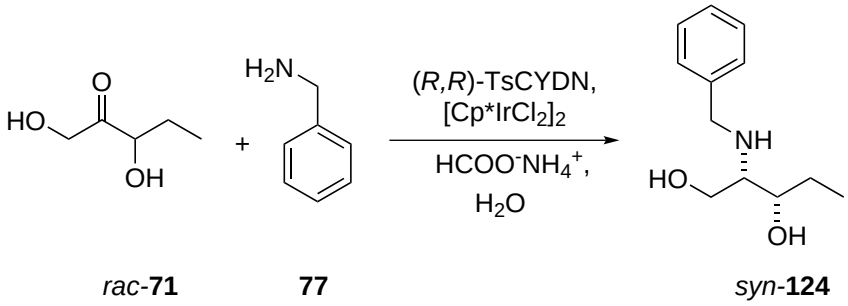
Synthesis of ^{t}butyl ester of *trans*-β-hydroxy norvaline:



Conversion of β-hydroxy norvaline to **124**:



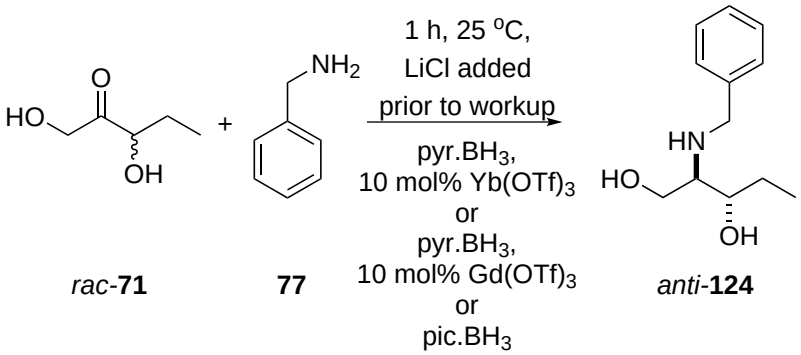
Scheme 6.7: Reactions towards the opposite diastereomers of **124** for use in an assay[276,279]



Scheme 6.8: Transfer hydrogenation reductive amination of 1,3-dihydroxypentan-2-one

would be useful to explore this reaction further to see whether the *anti*-selectivity observed can be improved. This is because a diastereospecific reaction would be more useful as the product obtained would be a single enantiomer and so no resolution would be needed to isolate each enantiomer. This possibility is suggested by some of the results of the *syn:anti* screen of the stoichiometric method. In this HPLC screen only the *anti* diastereomer was observed with three of the sets of conditions used, shown in Scheme 6.9. These results need to be verified by repeating the reactions and the HPLC analysis, and if they are seen to be specific for the *anti* diastereoisomer then these reactions need to be optimized further to ensure that the best yields are obtained. As the metal ion of the Lewis acid appears to have the largest effect on the *anti* selectivity this could be further examined and a wider range of Lewis acids, containing different metal species, could be explored. Another way to improve the diastereoselectivity of the reaction might be to use a more hindered amine than benzylamine as the amine source, for example 1-methyl-aminomethyl naphthalene or 3,5-bis-(trifluoromethyl)-benzylamine. These would make the reaction product and intermediates more sterically hindered and maybe direct the configuration of the product.

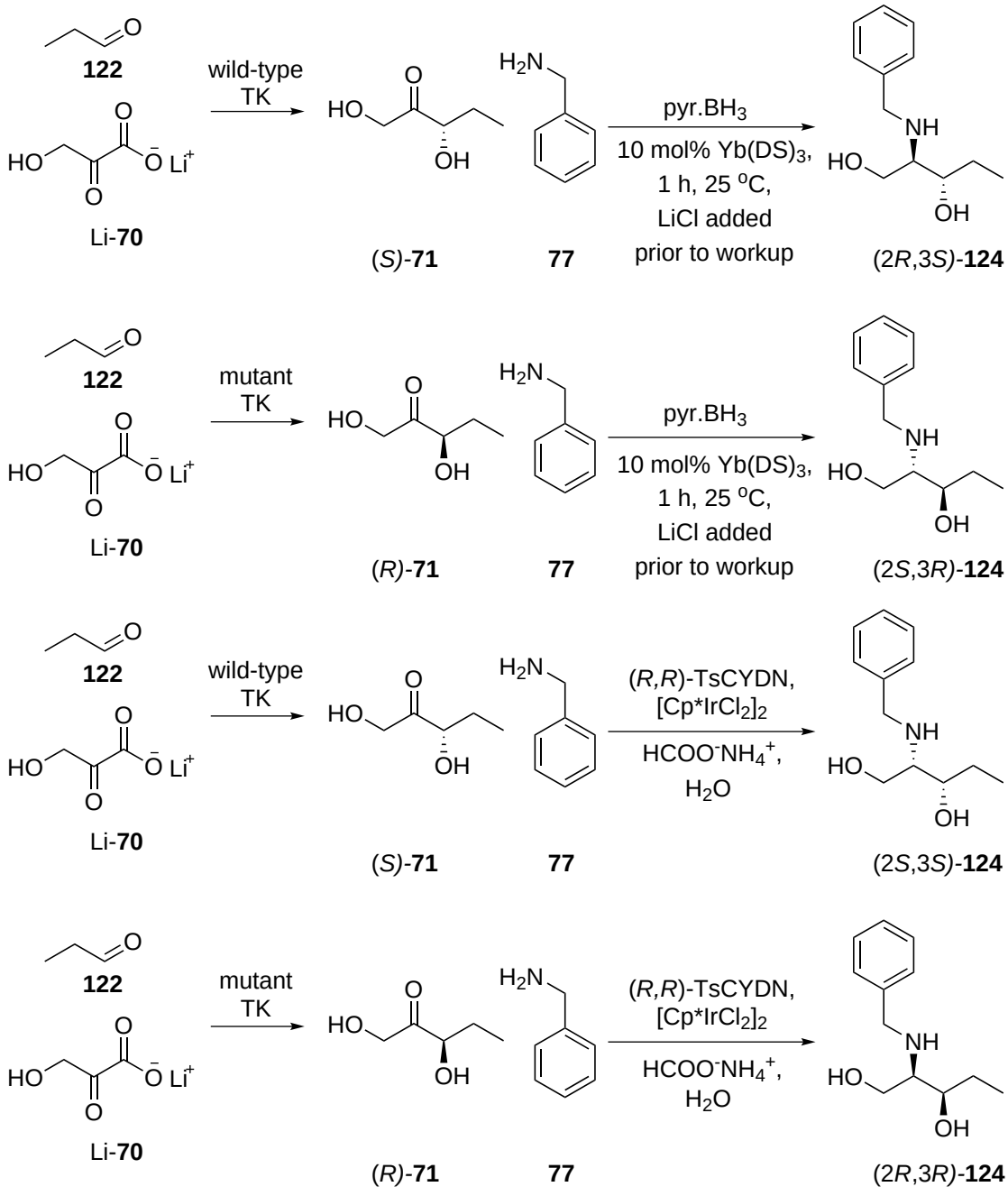
The aim of the research project had been to develop a reductive amination reaction that proceeded in water and would be compatible with a whole cell biotransformation,



Scheme 6.9: Synthesis of exclusively *anti*-**124**, as detected by HPLC, using reductive amination in water[224]

performed by a transketolase enzyme. Two reductive amination reactions in water were successfully developed. This meant that it would be possible to selectively synthesize all four stereoisomers of the product. This is because the biotransformation is enantioselective and the reductive amination is diastereoselective. Both the enantioselectivity of the biotransformation and the diastereoselectivity of the reductive amination reaction are known and so the major enantiomer of the product that is formed can be deduced (Scheme 6.10).

This demonstrated that the idea of developing chemistry to be applied in tandem with biocatalytic reactions was possible. However, the slowest part of the development was designing suitable assays that gave accurate data for the optimization of the reaction. Having seen the success of the HPLC assay developed for the optimization of the reductive amination reaction, and as automated analytical techniques (*e.g.* LCMS and HPLC with autosamplers) are becoming more widespread in chemical research, it might be possible to speed up this process. This is because a range of different conditions for each method can be tested automatically and the best methods chosen from this rapid automated screen. There is also potential for using automated purification techniques to speed up the process because if the same reaction is being carried out repeatedly, exactly the same column conditions can



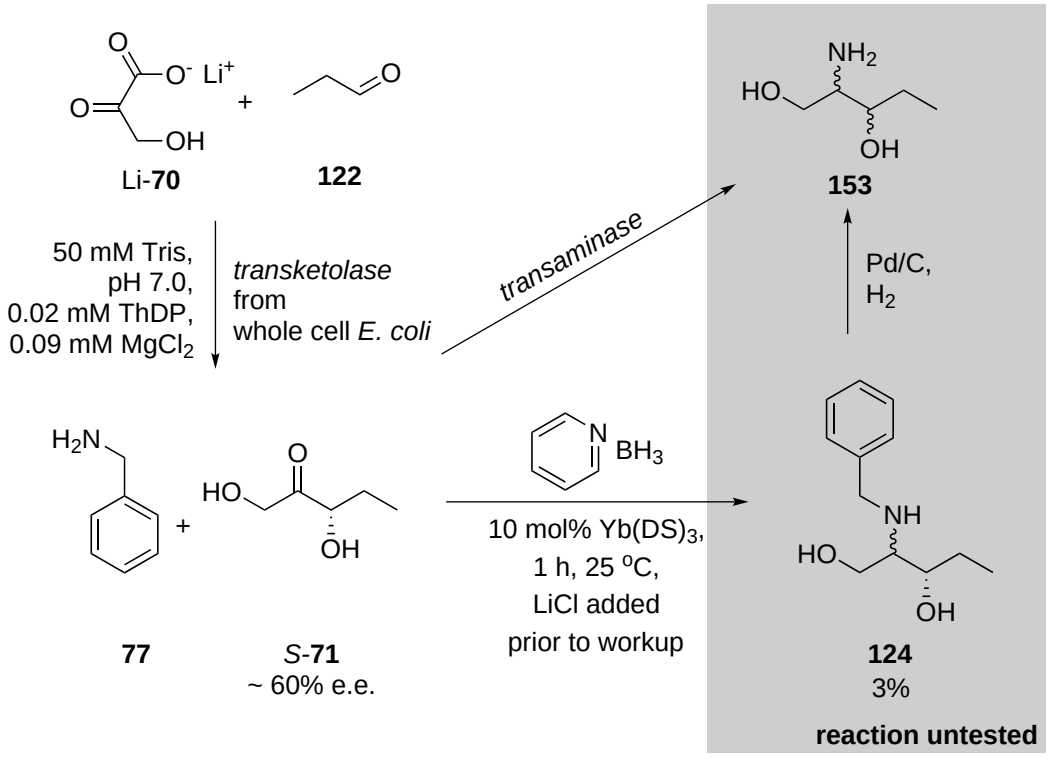
Scheme 6.10: Possible enantioselective synthesis of all 4 stereoisomers of 2-benzylamino-pentane-1,3-diol

be used for each reaction. If small scale reactions are being performed then with the small columns available for these systems a rapid turnaround can be achieved. These systems often have automated collecting systems based upon detecting chromophores which means that if a suitable system is chosen then the product can be detected and isolated more easily. As many different column types are available with these systems this would also be widely applicable. This more automated approach uses much fewer man hours for the optimization but needs to be tested to see whether it makes the process more cost effective and also whether it would allow several reactions to be optimized simultaneously. This might be accomplished by using computer based design of experiment techniques. These enable several different reaction variables to be compared simultaneously in an efficient manner, and would hopefully allow the development to be accelerated. As this technique varies several different conditions simultaneously, unlike in the development performed in this thesis, it would be beneficial to repeat the optimization using this technique. This would indicate whether the final optimized reaction was different from the optimized reaction discussed in the thesis. If it was seen that it gave a much improved reaction then this process could be applied to the discovery of other reactions to show that the ideas are applicable to other reactions and not specific to the reductive amination in water.

Once the diastereoselective reactions had been fully optimized then the complete reaction sequence to obtain the same product as the dual enzymatic cascade needs to be performed (Scheme 6.11). The first two reactions in this sequence have been carried out as a sequential one-pot reaction; however, the third reaction has not been tested. Initially the cleavage of the benzylamine group would need to be carried out upon the isolated aminodiol **124**. However, if the reaction was successful then it might be possible to include the *N*-debenzylation reaction in the reaction cascade and this possibility needs to be explored.

It is possible to carry out hydrogenations using Pd/C in water and if this can be used for this *N*-debenzylation then this cascade might be feasible.[284]

Other members of the BiCE research group have managed to both improve and reverse the selectivity of the transketolase enzyme, when it is used with propanal as its substrate.[222] These modified transketolases can then be used in combination with the optimized reductive amination reactions and the *N*-debenzylation to synthesize the four possible stereoisomers of **124**. This would enable the diastereoselectivities of the chemical reactions to be verified further as well as proving the syntheses as proposed in the thesis.



Scheme 6.11: Full synthesis

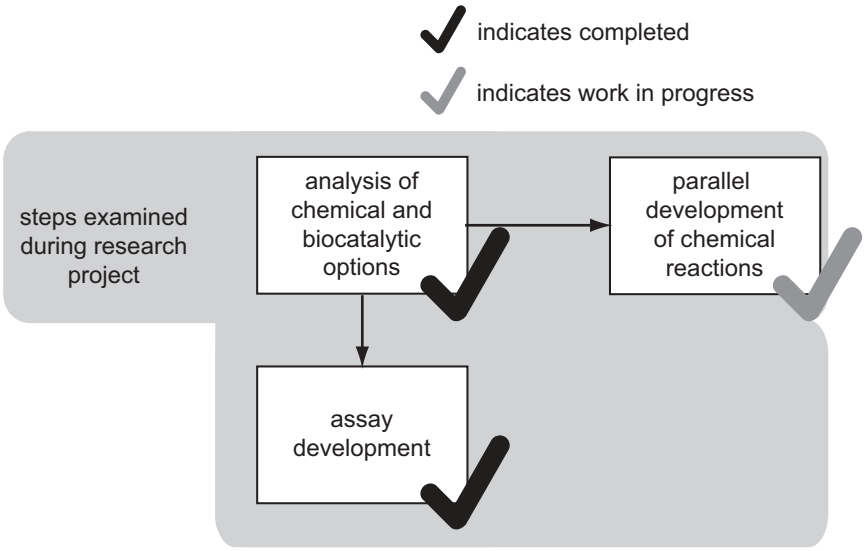
When the synthesis of the four possible isomers of aminodiol **124** had been completed,

then further reactions to make analogues would be useful to demonstrate that the reaction could be applied more generally. These analogues should contain a wide range of functionalities so that the scope of the reaction could be determined, and also how the presence of different functional groups affected the selectivity of the reaction, as this would provide more evidence to allow the mechanism of the selectivity to be determined. If the reaction was found to work in the presence of a wide range of functional groups then it would be useful to search the literature for other reactions that work under similar conditions. These reactions could then be developed and optimized to enhance their compatibility both with the chemical reductive amination reactions and the biotransformations. This would enable a set of reactions to be developed that could be linked together with minimal intermediate purification. As these reactions would be compatible with enzymatic biotransformations this would hopefully enable more complex molecules to be made stereoselectively.

Furthermore, as the research programme had initially set out to find a reaction that would be viable for use in industrial syntheses, once the reaction has been developed and optimized further, so that the problems highlighted earlier are solved, the reaction needs to be progressively scaled up. This would highlight any problems that might result from carrying out the reaction at larger scale, for example if there were any negative effects brought about by thermal transfers or if side-reactions started to dominate as the scales changed.[285] If these difficulties were seen then it might be possible to use a continual flow reactor. These have been successfully used with both chemical reactions and biotransformations and have the advantage of allowing continual reaction and purification.[36]

6.3 Summary

Although there is still much work left to do, this research project has shown that it is possible to optimize a combined biocatalytic and chemical route towards a single product. In summary, a reaction that could be performed in one pot with a biotransformation was developed and tested. This reaction was shown to be diastereoselective and a second reaction with the opposite selectivity was found. Both of the reactions still need further development work carried out upon them, as the benzylamine group has yet to be cleaved to give the desired product. This means that currently the synthesis of the desired product cannot be considered as complete, and the final debenzylation reaction should be performed. It is also necessary to investigate whether an improved method of product extraction and isolation can be discovered, as the methods used during this research project were generally unsatisfactory due to the large quantities of chlorinated solvent used. As the assays were developed and performed using a single machine by a single operator it would be advisable to duplicate the reactions that give the best selectivity and confirm the results. This could then be used to launch an investigation into the chirality of the products obtained when the reaction is performed on the products of both the chemical and biocatalytic 1,3-dihydroxyketone products.



completed work:

- exploration of chemical literature to identify chemical reactions that delivered the same product as the biotransformation
- chemical synthesis of standard for enzyme selectivity assay
- development of an assay to perform rapid optimisation of the chemical reaction
- reductive amination that works in one-pot with transketolase discovered, developed and tested
- development of an assay to investigate diastereoselectivity of reductive amination
- initial discovery work and a small amount of optimisation performed on second reductive amination reaction in water with opposite diastereoselectivity

incomplete work:

- confirmation of diastereoselectivities of reductive amination reactions
- chiral synthesis of four stereoisomers of 2-benzylamino-pentane-1,3-diol
- development of an assay to explore if chiral molecules can be produced using reductive amination either on racemic starting material or product from transketolase biotransformation
- synthesis of 2-amino-pentane-1,3-diol using coupled biotransformation and chemical reactions
- development of isolation and purification techniques for reductive amination products
- synthesis of a range of aminodiols to demonstrate versatility of synthetic methods

Figure 6.1: Thesis outcomes and work that still needs to be carried out

7

Experimental

7.1 General experimental methods

Unless otherwise stated, solvents and reagents were reagent grade from commercial suppliers and used without further purification. Anhydrous solvents were obtained using a column system (activated alumina), except for anhydrous DMF which was purchased from Sigma–Aldrich. All moisture-sensitive reactions were performed under an argon atmosphere using oven dried glassware. Reactions were monitored by TLC on Kieselgel 60 F_{254} plates with detection by UV, ninhydrin (ninhydrin (0.30 g) dissolved in *n*-butanol (100 mL) and acetic acid (3 mL) added), potassium permanganate (potassium permanganate (3.00 g) and potassium carbonate (20.00 g) dissolved in 5% aqueous sodium hydroxide (5 mL) and water (300 mL)), vanillin (Vanillin (15.00 g) dissolved in ethanol (250 mL) and concentrated sulfuric acid (2.5 mL) slowly added) and phosphomolybdic acid (Phosphomolybic acid (12.00 g) dissolved in ethanol (250 mL)) stains. Flash column chromatography was carried out using silica gel (particle size 40–63 μm).[286] ^{1}H NMR and ^{13}C NMR spectra were recorded in the

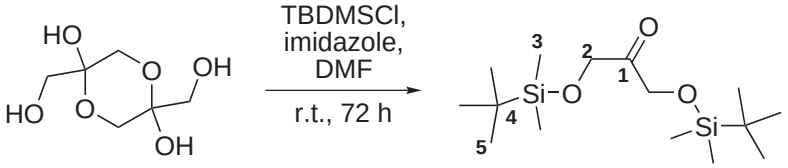
solvent specified and at the field indicated using Bruker AMX300 MHz and AMX 500MHz machines. Coupling constants are measured in Hertz (Hz) and unless otherwise specified, NMR spectra were recorded at 298 K. C NMR assignments were all based upon typical shifts, unless otherwise stated when the specific assignment method will be stated. Mass spectra were recorded using a Thermo Finnigan MAT900XP spectrometer and a Fisons VG70-SE spectrometer. Infrared spectra were recorded on a Shimadzu FTIR-8700 infrared spectrometer and were all performed neat. Optical rotations were recorded on an Optical Activity Limited PolAAR2000 polarimeter at 589 nm, quoted in deg cm^2 g^{-1} and conc (c) in g/100 mL. HPLC experiment were performed with a Varian Star chromatography workstation equipped with two Varian ProStar Model 210 HPLC pumps, a Varian ProStar Model 410 HPLC Autosampler, a Varian ProStar Model 320 HPLC UV-Vis Detector and a Uniflows DG-2410 Degasys. The column used was a Supelco Discovery® Bio Wide Pore C18-10 Column and all solvents used were HPLC grade and had 0.1% trifluoroacetic acid added.

7.2 Chapter 3: Synthesis of 1,3-ketodiol analogues

7.2.1 General silylation procedure

1,3-Dihydroxyacetone dimer, **73**, (5.00 g, 27.8 mmol) and imidazole (10.00 g, 146.9 mmol) were dissolved in DMF (100 mL). The required silyl chloride (5 eq.) was added and the mixture stirred at room temperature with monitoring by TLC (ethyl acetate:hexane, 1:15) until product formation was complete. Water (100 mL) and hexane (300 mL) were added and the organic layer extracted. The organic layer was washed with saturated sodium chloride solution (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified using flash silica column chromatography.[190]

1,3-*Bis*-(*tert*-butyldimethylsilyloxy)propan-2-one (84)



73 **84**

86%

The general silylation procedure was used with TBDMSCl (20.6 g, 136.7 mmol) and the mixture stirred for 72 hours. The flash silica column chromatography was performed using ethyl acetate:hexane, 1:15 to give 1,3-*bis*-(*tert*-butyldimethylsilyloxy)propan-2-one, **84**, (11.21 g, 47.8 mmol, 86%) as a yellow oil.[190,287]

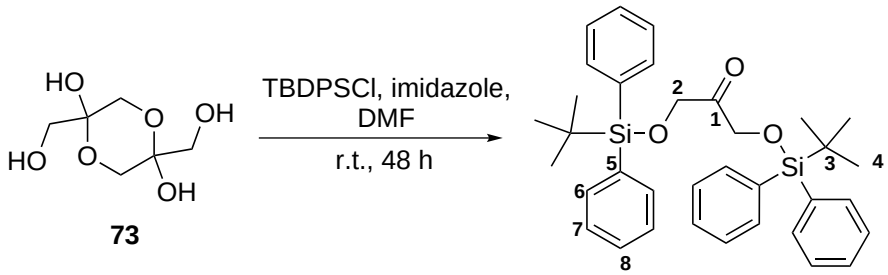
δ_H (300 MHz; $CDCl_3$): 4.38 (4H, s, C^2H_2), 0.89 (18H, s, C^5H_3), 0.06 (12H, s, C^3H_3);

δ_C (75 MHz; $CDCl_3$): 208.6 (C^1), 67.9 (C^2), 25.7 (C^5), 18.3 (C^4), -5.6 (C^3);

m/z (+ESI) found $[MNa]^+$ 341.19370. $C_{15}H_{31}NaO_3Si_2$ requires $[MNa]^+$ 341.19442;

ν_{max}/cm^{-1} 1720 (C=O).

1,3-*Bis*-(*tert*-butyldiphenylsilyloxy)propan-2-one (85)



73 **85**

84%

The general silylation procedure was used with TBDPSCl (7.63 g, 27.8 mmol) and the mixture stirred for 48 hours. The flash silica column chromatography was performed using dichloromethane to give 1,3-*bis*-(*tert*-butyldiphenylsilyloxy)propan-2-one, **85**, (4.56 g, 9.4 mmol, 84%) as a pale yellow oil.[288,289]

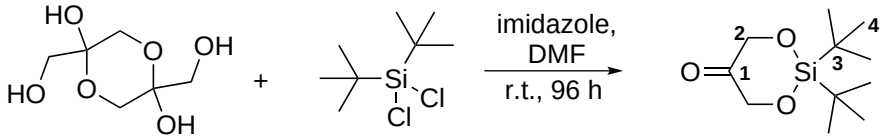
δ_H (300 MHz; $CDCl_3$): 7.72 (8H, m, C^6H), 7.47 (12H, m, C^7H, C^8H), 4.62 (4H, s, C^2H_2), 1.16 (18H, s, C^4H_3);

δ_C (75 MHz; $CDCl_3$): 207.3 (C^1), 135.6 (C^6), 132.7 (C^5), 129.8 (C^8), 127.6 (C^7), 68.7 (C^2), 26.8 (C^4), 19.4 (C^3);

m/z (+ESI) found $[MNa]^+$ 589.58 (100%)

ν_{max}/cm^{-1} 3297 (Aromatic C-H), 1705 (CO).

2,2-Di-*tert*-butyl-[1,3,2]dioxasilinan-5-one (90)



73 **90** 18%

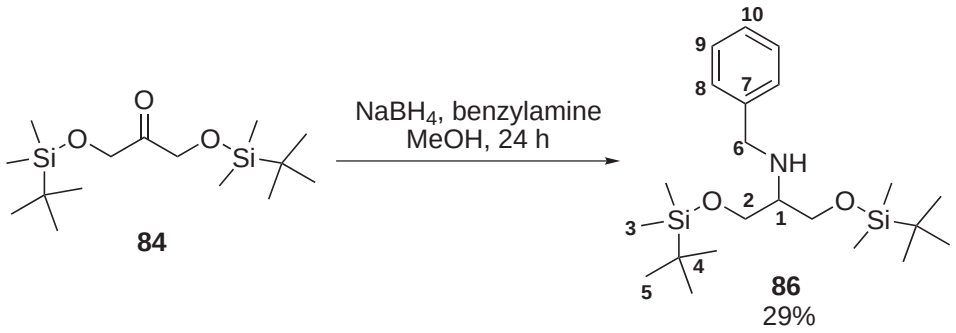
The general silylation procedure was used with di-*tert*-butyldichlorosilane (5.00 g, 23.4 mmol) and activated 3Å molecular sieves and the mixture stirred for 96 hours. The flash silica column chromatography was performed using dichloromethane) to give 2,2-di-*tert*-butyl-[1,3,2] dioxasilinan-5-one, **90**, (0.47 g, 2.04 mmol, 18%) as a pale yellow oil.

δ_H (300 MHz; $CDCl_3$): 4.35 (4H, s, C^2H_2), 1.07 (18H, s, C^4H_3);

δ_C (75 MHz; $CDCl_3$): 211.2 (C^1), 71.6 (C^2), 27.1 (C^4), 21.8 (C^3);

m/z (+ CI-Methane) found $[MH]^+$ 231.1419. $C_{11}H_{23}O_3Si$ requires $[MH]^+$ 231.1417;

7.2.2 2-Benzylamino-1,3-*bis*(*tert*-butyl-dimethyl-silanyloxy) propane (86)



A solution of 1,3-*bis*-(*tert*-butyldimethylsilyloxy)propan-2-one, **84**, (1.09 g, 3.4 mmol) and benzylamine, **77** (0.73 g, 0.76 mL, 6.8 mmol) in methanol (10 mL) was stirred for 1 hour at room temperature Then $NaBH_4$ (0.39 g, 10.3 mmol) was added and the mixture stirred for a further 24 hours at room temperature, with monitoring by TLC (ethyl acetate:hexane, 1:4). The solvent was then removed under reduced pressure and the residue dissolved in water (10 mL). The aqueous solution was extracted with dichloromethane (3 x 20 mL) and the combined organic extracts washed with saturated sodium chloride solution (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 1:4) to give pure 2-benzylamino-1,3-*bis*(*tert*-butyl-dimethyl-silanyloxy)propane, **86**, (0.41 g, 1.0 mmol, 29%) as an orange oil.

δ_H (300 MHz; $CDCl_3$): 7.35–7.20 (5H, m, Ar-H), 3.88 (2H, s, C^6H_2), 3.59 (4H, d, J=5.7 Hz, C^2H_2), 2.75 (1H, qn, J=5.7 Hz, C^1H), 1.93 (1H, br s, NH), 0.87 (18H, s, C^5H_3), 0.05 (12H, s, C^3H_3);

δ_C (75 MHz; $CDCl_3$): 140.8, 128.4, 128.0, 126.8, 62.4 (C^2), 60.2 (C^1), 51.8 (C^6), 25.9 (C^5), 18.3 (C^4), -5.8 (C^3);

m/z (EI) found $[M]^+$ 409.2815. $C_{22}H_{43}NO_2Si_2$ requires $[M]^+$ 409.2832;

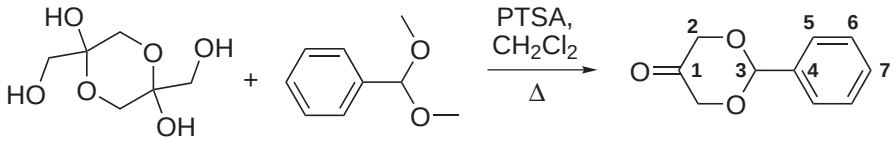
ν_{max}/cm^{-1} 3336 br (NH), 2924 (Aromatic C-H), 1496 (Aromatic C=C).

Other syntheses mentioned identical to method on page 228 except for the conditions shown in table 7.1.

Reducing agent	Solvent	pH	Yield
$NaCNBH_3$	methanol	6.0	0.43 g (31%)
$NaBH(OAc)_3$	1,2-dichloroethane		0.40 g (29%)
$NaBH(OAc)_3$	1,2-dichloroethane	6.0	0.54 g (45%)
pyridine borane	methanol		0.50 g (36%)

Table 7.1: Various syntheses of 2-Benzylamino-1,3-*bis*(*tert*-butyl-dimethyl-silanyloxy)propane, **86** – pH was controlled using phosphate buffer

7.2.3 2-Phenyl-1,3-dioxan-5-one (87)



73 **91** **87** 7%

1,3-Dihydroxyacetone dimer, **73**, (1.00 g, 5.6 mmol) and benzaldehyde dimethyl acetal, **91**, (3.31 g, 3.3 mL, 21.7 mmol) were dissolved in dichloromethane (20 mL). *p*-Toluene sulfonic acid (1 crystal) was added and the reaction heated at reflux with stirring for 24 hours. The solution was then washed with saturated sodium hydrogen carbonate solution (3 x 25 mL), saturated sodium chloride solution (25 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by Kugelrohr distillation to give 2-phenyl-1,3-dioxan-5-one, **87**, (0.07 g, 0.4 mmol, 7%) as a colourless crystalline solid.[200,290]

δ_H (300 MHz; $CDCl_3$): 7.51–7.27 (5H, m, Ar-CH), 5.40 (1H, s, C^3H), 4.27 (2H, d, J=12.9 Hz C^2H), 3.71 (2H, d, J=12.9 Hz C^2H);

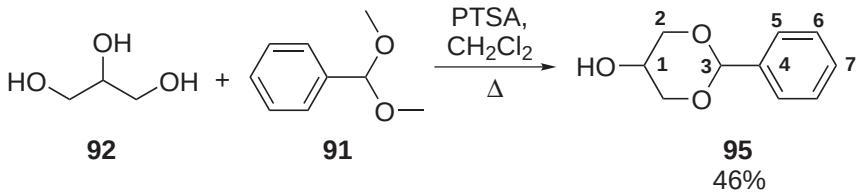
δ_C (75 MHz; $CDCl_3$): 192.6 (C^1), 134.3, 129.5, 128.9, 128.0, 103.1 (C^3), 69.8 (C^2);

m/z (+ CI-Methane) 180 (M(^{13}C)+H, 0.6%), 179 (M+H, 6), 123 (M-CH_2COCH_2, 58), 107 (M-CH_2COCH_2O, 100), 91 (M-$C_6H_5CH_2$, 41); Found (+HRES) $[MH]^+$ 179.07053. $C_{10}H_{10}O_3$ requires $[MH]^+$ 179.07082;

mp 73 °C (lit. 69–71 °C[290]);

ν_{max}/cm^{-1} 2920 (Aromatic C-H), 1738 (CO), 1246 (C-O-C), 1163 (C-O-C).

7.2.4 2-Phenyl-1,3-dioxan-5-ol (95)



92 **91** **95** 46%

Glycerol, **92**, (1.00 g, 10.8 mmol) and benzaldehyde dimethyl acetal, **91**, (3.31 g, 21.7 mmol) were dissolved in dichloromethane (20 mL). *p*-Toluene sulfonic acid (1 crystal) was added and the reaction stirred for 3 hours at reflux. The solution was washed with saturated sodium hydrogen carbonate solution (3 x 25 mL), saturated sodium chloride solution (25 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was taken up in dry diethyl ether (5 mL) and placed in a freezer to crystallize out the product. 2-Phenyl-1,3-dioxan-5-ol, **95**, (0.92 g, 5.3 mmol, 46%) was isolated by suction filtration as a colourless crystalline solid.[200,291,292]

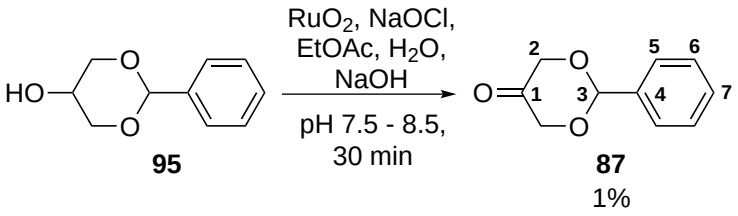
δ_H (300 MHz; $CDCl_3$): 7.47–7.23 (5H, m, C_6H_5), 5.56 (1H, s, C^3H), 4.28–4.01 (4H, m, C^2H_2), 3.66 (1H, m, C^1H), 2.20 (1H, br s, O*H*);

δ_C (75 MHz; $CDCl_3$): 143.4 (C^4), 128.4 (C^6), 125.9 (C^5, C^7), 102.7 (C^3), 72.3 (C^2), 64.5 (C^1);

mp 82 °C (lit. 83–84 °C[292]);

m/z (EI) found $[M]^+$ 180.0790. $C_{10}H_{12}O_3$ requires $[M]^+$ 180.0786.

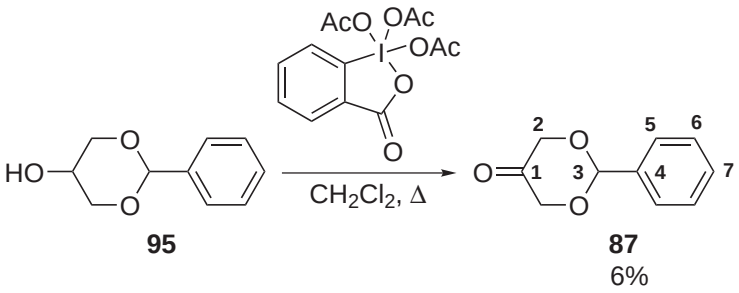
7.2.5 2-Phenyl-1,3-dioxan-5-one (87)



2-Phenyl-1,3-dioxan-5-ol, **95**, (1.00 g, 5.5 mmol) in ethyl acetate (5.6 mL) and water (22.2 mL) was added to a slurry of oxidized ruthenium dioxide hydrate (14.1 mg [the oxidation had been carried out by stirring it with sodium hypochlorite solution (12%) for 1 hour in water (0.56 mL)]). Sodium hypochlorite solution (12%) was then added to the stirred reaction at a rate of 0.30–0.35 mL.min^{-1}, with the pH being maintained at 7.5–8.5 by the addition of a 20% aqueous sodium hydroxide solution. This was continued for 30 minutes and the mixture extracted with ethyl acetate (5 x 10 mL). The combined organic extracts were stirred with cellulose (1.00 g), filtered and dried ($MgSO_4$), before being concentrated under reduced pressure. The residue was purified by Kugelrohr distillation to give 2-phenyl-1,3-dioxan-5-one, **87**, (0.01 g, 0.1 mmol, 1%).[200]

The characterization data was the same as that described on page 230

7.2.6 2-Phenyl-1,3-dioxan-5-one (87)

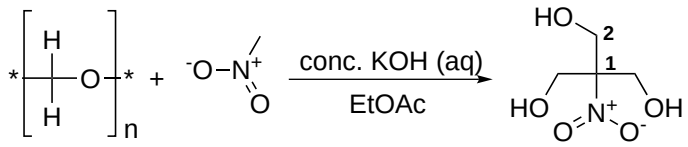


95 **87** 6%

2-Phenyl-1,3-dioxan-5-ol, **95**, (0.17 g, 0.9 mmol) in dry dichloromethane (5 mL) was added dropwise to a suspension of Dess-Martin periodinane (1.19 g, 2.8 mmol) in anhydrous dichloromethane (5 mL). The resulting suspension was stirred for 15 hours at reflux, cooled to room temperature and then quenched with saturated sodium thiosulphate solution (5 mL). The solution was extracted with diethyl ether (15 mL) and ethyl acetate (2 x 15 mL) and the combined organic extracts washed with saturated sodium hydrogen carbonate solution (5 mL), saturated sodium chloride solution (15 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by Kugelrohr distillation to give 2-phenyl-1,3-dioxan-5-one, **87**, (0.01 g, 0.06 mmol, 6%).[200]

The characterization data was the same as that described on page 230

7.2.7 2-(Hydroxymethyl)-2-nitropropane-1,3-diol (99)



96 **97** **99**

89%

Saturated aqueous potassium hydroxide solution (10 drops) was added to a suspension of nitro-methane, **97**, (61.00 g, 1.0 mol) and paraformaldehyde, **96**, (30.00 g) in ethyl acetate (500 mL). The suspension was then heated until the paraformaldehyde dissolved completely. Ethyl acetate (400 mL) was removed under reduced pressure and chloroform (100 mL) and chloroform:ethyl acetate (2:3; 1 L) were added and a white precipitate formed. The mixture was heated and the solution concentrated to 200 mL and placed in a fridge overnight for the product to precipitate out. The product was collected *via* suction filtration and the precipitate washed with chloroform (100 mL). The mother liquor was then concentrated under reduced pressure and chloroform (100 mL) and ethyl acetate (100 mL) added to the residue which was left to precipitate overnight in a fridge. The precipitate was collected by suction filtration and the combined precipitates air-dried overnight. This gave 2-(hydroxymethyl)-2-nitropropane-1,3-diol, **99**, as a colourless crystaline solid (134.50 g, 0.9 mol, 89%).[293]

δ_H (300 MHz; Acetone-D_6): 4.31 (3H, br s, O*H*), 4.00 (6H, br s, C^2H_2);

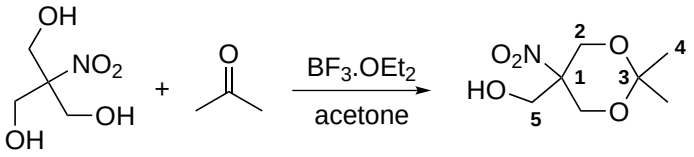
δ_C (75 MHz; Acetone-D_6): 95.8 (C^1), 60.8 (C^2);

m/z (+ CI-Methane) found $[MH]^+$ 152.05592. $C_4H_10NO_5$ requires $[MH]^+$ 152.04807;

mp 162 °C (lit. 158–159 °C[293]);

ν_{max}/cm^{-1} 3350 br (OH), 1541 (NO_2), 1463 (OH).

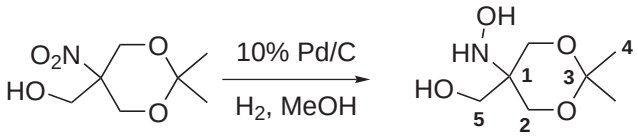
7.2.8 (2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)methanol (100)



To 2-(hydroxymethyl)-2-nitropropane-1,3-diol, **99**, (110.00 g, 0.7 mol) in acetone (160 mL), boron trifluoride etherate (100.80 g, 90 mL, 0.7 mol) was added. After 5 minutes the product had crystallized out and the reaction mixture was poured onto a mixture of ice and saturated sodium hydrogen carbonate solution (1.0 L) with the evolution of CO_2. The mixture was stirred for 15 minutes and the product collected by suction filtration. The product was washed with cold water (100 mL) and dissolved in dichloromethane/diethyl ether (3:2,2.5 L). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure to give (2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)methanol, **100**, (79.60 g, 0.4 mol, 59%) as a crystalline yellow solid.[294]

δ_H (300 MHz; $CDCl_3$): 4.37 (2H, d, J=12.7 Hz, C^2H), 4.05 (2H, d, J=12.7 Hz, C^2H), 4.06 (2H, s, C^5H_2), 1.43 (3H, s, C^4H_3), 1.41 (3H, s, C^4H_3);
δ_C (75 MHz; Acetone-D_6): 99.6 (C^3), 88.2 (C^5), 64.1 (C^1), 62.2 (C^2), 26.2 (C^4), 21.2 (C^4);
m/z (+ CI-Methane) found $[MH]^+$ 192.0874. $C_7H_{14}NO_5$ requires $[MH]^+$ 192.0794;
mp 137 °C (lit. 134–135 °C[294]);
ν_{max}/cm^{-1} 3422 (OH), 1531 (NO_2).

7.2.9 (5-(Hydroxyamino)-2,2-dimethyl-1,3-dioxan-5-yl)methanol (102)



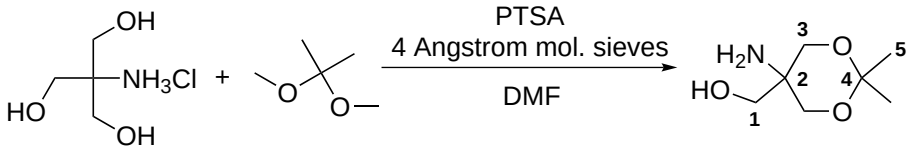
100 **102**
75%

A suspension of (2,2-Dimethyl-5-nitro-1,3-dioxan-5-yl)methanol, **100**, (0.38 g, 2.0 mmol) and 10% Pd/C (0.10 g) in methanol (10 mL) was stirred under H_2 at room temperature After 24 hours the mixture was filtered through Celite^{TM} and the solvent removed under reduced pressure. The residue was dissolved in chloroform (20 mL) and washed with saturated sodium chloride solution (3 x 15 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give (5-(hydroxyamino)-2,2-dimethyl-1,3-dioxan-5-yl)methanol, **102**, (0.26 g, 1.5 mmol, 75%) as a colourless solid.[195]

δ_H (300 MHz; $CDCl_3$): 3.79 (2H, d, J=6.3Hz, C^2H), 3.63 (2H, d, J=6.3 Hz, C^2H), 3.42 (2H, s, C^5H_2), 1.44 (3H, s, C^4H_3), 1.39 (3 H, s, C^4H_3);
δ_C (75 MHz; $CDCl_3$): 98.5 (C^3), 67.0 (C^2), 64.6 (C^5), 50.2 (C^1), 25.1 (C^4), 21.9 (C^4);
m/z (+ CI-Methane) found $[MH]^+$ 178.1086. $C_7H_{16}NO_4$ requires $[MH]^+$ 178.1079;
mp 142 °C (lit. 139–140 °C[195]);
ν_{max}/cm^{-1} 3298 br (OH).

Other syntheses mentioned identical to method on page 236 except for the conditions shown in table 3.2 on page 87.

7.2.10 (5-Amino-2,2-dimethyl-1,3-dioxan-5-yl)methanol (101)



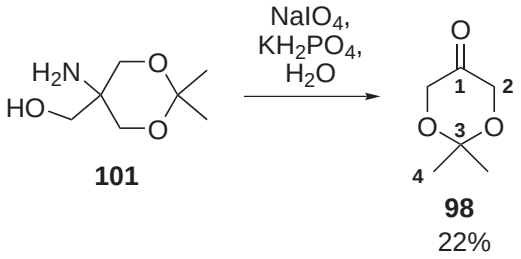
103 **104** **101**

40%

To a solution of *tris*(hydroxymethyl)aminomethane hydrochloride, **103**, (20.00 g, 126.9 mmol) in anhydrous DMF (140 mL) and activated 4Å molecular sieves was added *p*-toluene sulfonic acid monohydrate (1.80 g, 9.5 mmol) followed by 2,2-dimethoxypropane, **104**, (14.37 g, 16.9 mL, 137.9 mmol) in one portion. The resulting clear and colourless solution was stirred overnight, Et_3N (1.0 mL) was added and allowed to stir for an additional 10 min. The mixture was then concentrated *in vacuo* and treated with Et_3N (13.7 mL) and ethyl acetate (500 mL). The white precipitate formed was removed *via* suction filtration, the filtrate concentrated under reduced pressure and purified by Kugelrohr distillation to afford (5-amino-2,2-dimethyl-1,3-dioxan-5-yl)methanol, **101**, (8.14 g, 50.0 mmol, 40%) as a white microcrystalline solid.[202]

δ_H (300 MHz; $CDCl_3$): 3.78 (2H, d, J=11.7 Hz, C^3H), 3.52 (2H, d, J=11.7 Hz, C^3H), 3.48 (2H, s, C^1H_2), 2.13 (3H, br s, NH_2, OH), 1.43 (3H, s, C^5H_3), 1.40 (3H, s, C^5H_3);
δ_C (75 MHz; $CDCl_3$): 98.4 (C^4), 67.2 (C^1), 64.8 (C^3), 50.2 (C^2), 25.0 (C^5), 22.1(C^5);
mp 116 °C (lit. 117–118 °C[290]);
m/z (+ CI-Methane) found $[MH]^+$ 162.9152. $C_7H_{16}NO_3$ requires $[MH]^+$ 162.9150.

7.2.11 2,2-Dimethyl-1,3-dioxan-5-one (98)



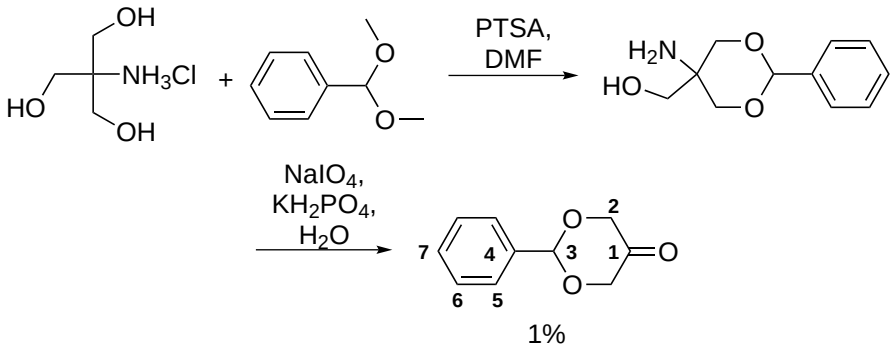
To a cold (5 °C) solution containing 5-amino-5-hydroxymethyl-2,2-dimethyl-1,3-dioxane, **101**, (14.66 g, 90.9 mmol) and potassium dihydrogen phosphate (12.38 g, 91.0 mmol) in water (300 mL) was added dropwise, *via* an addition funnel, a solution of sodium metaperiodate (19.50 g, 91.2 mmol) in water (265 mL). Upon completion, ca. 3 hours, the mixture was stirred for an additional hour at 5 °C and then 15 hours at room temperature Sodium thiosulphate (14.38 g, 91.0 mmol) was added, and the resulting solution was stirred for approximately 15 min then extracted with dichloromethane (15 x 75 mL). The combined organic phases were dried ($MgSO_4$), filtered, concentrated under reduced pressure, and purified by Kugelrohr distillation to afford 2,2-dimethyl-5-oxo-1,3-dioxane, **98**, (2.7 g, 22%) as a clear and colourless oil.[202]

δ_H (300 MHz; $CDCl_3$): 4.15 (4H, s, C^2H_2), 1.45 (6H, s, C^4H_3);

δ_C (75 MHz; $CDCl_3$): 207.9 (C^1), 100.0 (C^3), 66.7 (C^2), 23.4 (C^4);

m/z (+ EI) found $[M]^+$ 130.0576. $C_6H_{10}O_3$ requires $[M]^+$ 130.0629;

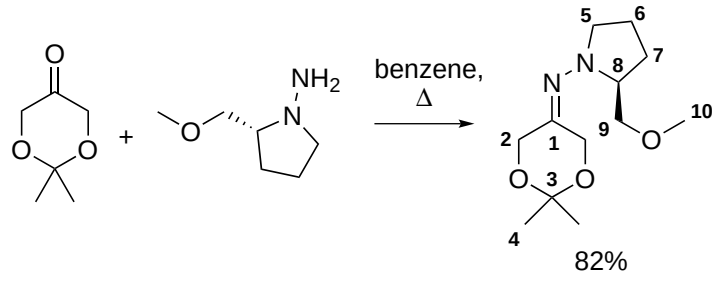
7.2.12 2-Phenyl-1,3-dioxan-5-one (87)



To a solution of *tris*(hydroxymethyl)aminomethane hydrochloride, **103**, (20.00 g, 126.9 mmol) in anhydrous DMF (140 mL) and activated 4Å molecular sieves was added *p*-toluene sulfonic acid monohydrate (1.80 g, 9.5 mmol) followed by benzaldehyde dimethyl acetal, **91**, (21.00 g, 20.8 mL, 138.0 mmol) in one portion. The resulting clear and colourless solution was stirred overnight at which time Et_3N (1.0 mL) was added and the reaction stirred for an additional 10 min. The mixture was then concentrated under reduced pressure and treated with Et_3N (13.7 mL) and ethyl acetate (500 mL). The white precipitate formed was removed *via* suction filtration and the filtrate concentrated under reduced pressure. To a cold (5 °C) solution containing the residue and potassium dihydrogen phosphate (12.38 g, 91.0 mmol) in water (300 mL) was added dropwise, *via* addition funnel, a solution of sodium metaperiodate (19.50 g, 91.2 mmol) in water (265 mL). Upon completion, ca. 3 hours, the mixture was stirred for an additional hour at 5 °C and then 15 hours at room temperature Sodium thiosulphate (14.38 g, 91.0 mmol) was added, and the resulting solution was stirred for approximately 15 min at which time it was extracted with dichloromethane (15 x 75 mL). The combined organic phases were dried ($MgSO_4$), filtered, concentrated *in vacuo*, and purified by Kugelrohr distillation to afford 2-phenyl-1,3-dioxan-5-one, **87**, (0.3 g, 1%) as a clear and colourless oil.[200]

The characterization data was the same as that described on page 230

7.2.13 *N*-((*S*)-2-(Methoxymethyl)pyrrolidin-1-yl)-2,2-dimethyl-1,3-dioxan-5-imine (106)



In a flask equipped with a reflux condenser, 2,2-dimethyl-1,3-dioxan-5-one, **98**, (0.12 g, 0.9 mmol) and (*S*)-1-amino-2-methoxymethylpyrrolidine (SAMP, **105**) (0.17 g, 1.3 mmol) in benzene (10 mL) were heated at reflux for 20 hours. After cooling diethyl ether (30 mL) was added and the mixture washed with H_2O (2 x 5 mL). The organic layer was dried ($MgSO_4$) and the solvent removed under reduced pressure. The crude hydrazone was purified by Kugelrohr distillation (82–84 °C, 0.05 Torr) to afford *N*-((*S*)-2-(methoxymethyl)pyrrolidin-1-yl)-2,2-dimethyl-1,3-dioxan-5-imine, **106**, (0.18 g, 0.8 mmol, 84%) as a red oil.[65]

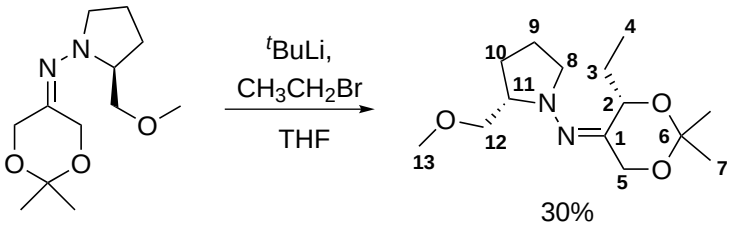
δ_H (300 MHz; $CDCl_3$): 4.21–4.60 (4H, m, C^2H_2), 3.28 (3H, s, $C^{10}H_3$), 2.97–3.34 (4H, m, C^5H, C^8H, C^9H_2), 2.41 (1H, q, J=9.0 Hz, C^5H), 1.50–1.97 (4H, m, C^6H_2, C^7H_2), 1.34 (3H, s, C^4H_3), 1.31(3H, s, C^4H_3);

δ_C (75 MHz; $CDCl_3$): 160.0 (C^1), 99.8 (C^3), 75.3 (C^9), 66.6 (C^8), 62.5 (C^2), 60.2 (C^2), 59.1 (C^{10}), 55.3 (C^5), 24.6 (C^7), 24.4 (C^4), 23.1 (C^4), 22.6(C^6);

m/z (+ EI) found $[M]^+$ 242 (100%);

ν_{max}/cm^{-1} 1667 (CN).

7.2.14 (*Z*)-4-Ethyl-*N*-((*S*)-2-(methoxymethyl)pyrrolidin-1-yl)-2,2-di-methyl-1,3-dioxan-5-imine (107)



A dry, argon flushed, 3 necked round-bottom flask, equipped with a magnetic stirring bar, had *N*-((*S*)-2-(methoxymethyl)pyrrolidin-1-yl)-2,2-dimethyl-1,3-dioxan-5-imine, **106**, (0.05 g, 0.2 mmol) and anhydrous THF (10 mL) added. Then, ^{t}BuLi (0.14 mL, 15% in n-pentane) was added dropwise by syringe at -78 °C. After stirring for 2 hours at this temperature, the mixture was cooled to -100 °C and bromoethane (0.03 g, 0.02 mL, 0.2 mmol) was added slowly. After further stirring for 2 hours at -100 °C, the mixture was allowed to warm to room temperature over 15 hours. The mixture was quenched with pH 7 buffer solution (citric acid-phosphate buffer, 2 mL) and diluted with diethyl ether (80 mL). The organic layer was washed with pH 7 buffer solution (citric acid-phosphate buffer, 10 mL) and saturated sodium chloride solution (2 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The monoalkylated SAMP-hydrazone obtained was purified by flash silica column chromatography (eluent: *n*-pentane:diethyl ether, 5:1) to afford (*Z*)-4-ethyl-*N*-((*S*)-2-(methoxymethyl)pyrrolidin-1-yl)-2,2-dimethyl-1,3-dioxan-5-imine, **107**, (17 mg, 0.06 mmol, 34%) as a colourless oil.[65]

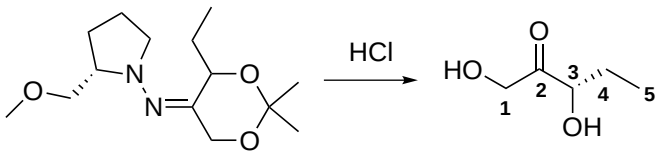
δ_H (300 MHz; $CDCl_3$): 4.50 (1H, d, J=15.6 Hz, C^5H), 4.25 (1H, m, C^2H), 4.14 (1H, d, J=15.6 Hz, C^5H), 3.45–2.98 (4H, m, $C^{12}H_2$, $C^{11}H$, C^8H), 3.34 (3H, s, $C^{13}H_3$), 2.40 (1H, dt, J=7.6 Hz, 8.4 Hz, C^8H), 2.08–1.50 (6H, m, C^9H_2, $C^{10}H_2$, C^3H_2), 1.39 (6H, s, C^7H_3), 0.97

(3H, t, J=7.2 Hz, C^4H_3);

δ_C (75 MHz; $CDCl_3$): 160.4 (C^1), 99.7 (C^6), 75.8 (C^{12}), 72.3 (C^2), 66.6 (C^3), 64.1 (C^{13}), 59.1 (C^{11}), 53.3 (C^8), 30.9 (C^7), 26.8 (C^9), 24.3 (C^5), 22.9 (C^{10}), 9.4 (C^4);

m/z (+ CI-Methane) found $[MH]^+$ 271.2010. $C_{14}H_{26}N_2O_3$ requires $[MH]^+$ 271.2021.

7.2.15 (*S*)-1,3-Dihydroxypentan-2-one ((*S*)-71)



41%

(*Z*)-4-Ethyl-*N*-((*S*)-2-(methoxymethyl)pyrrolidin-1-yl)-2,2-dimethyl-1,3-dioxan-5-imine, **107**, (0.10 g, 0.4 mmol) was dissolved in *n*-pentane (2 mL) and treated with 6*N* HCl (0.4 mL). The mixture was vigorously stirred and the reaction monitored by TLC. After 20 min the mixture was extracted with diethyl ether (3 x 20 mL), washed with saturated sodium chloride solution (20 mL) and dried ($MgSO_4$). The crude product was purified by flash silica column chromatography (eluent: *n*-pentane:diethyl ether, 1:2) to give pure (*S*)-1,3-dihydroxypentan-2-one, (*S*)-**71**), (0.018 g, 0.2 mmol, 40%) as a colourless oil.[65]

δ_H (300 MHz; $CDCl_3$): 4.70 (1H, d, J=19.0 Hz, C*H*HOH), 4.59 (1H, d, J=19.0 Hz, C*H*HOH), 4.24 (1H, t, J=4.4 Hz, C*H*OH), 1.99–1.54 (3H, m, OH, CH_2CH_3), 1.01 (3H, t, J=6.5 Hz, CH_3);

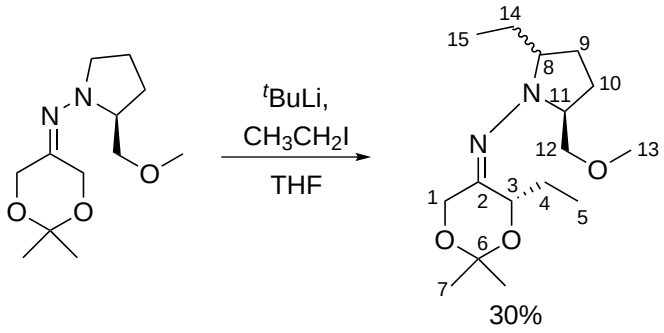
δ_C (75 MHz; $CDCl_3$): 212.5 (C^2), 76.0 (C^3), 65.6 (C^1), 27.1 (C^4), 9.0 (C^5);

m/z (+ CI-Methane) 120 (M(^{13}C)+H, 5%), 119 (M+H, 100); Found (+HRES) $[M+H]^+$ 119.0710. $C_5H_{11}O_3$ requires $[M+H]^+$ 119.0708;

ν_{max}/cm^{-1} 3277 br (OH), 1717 (CO);

Optical Rotation $[\alpha]^{25}{}_D$=-44.5 (*c* 3.1, H_2O).

7.2.16 (*Z*, 4*S*)-4-Ethyl-*N*-((*S*)-2-ethyl-5-(methoxymethyl)pyrrolidin-1-yl) -2,2-dimethyl-1,3-dioxan-5-imine (109)

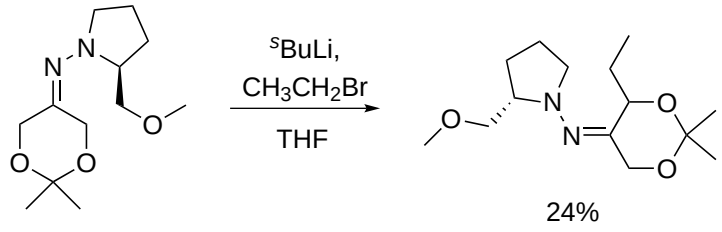


A dry, argon flushed, 3 necked round-bottom flask, containing a magnetic stirring bar, had *N*-((*S*)-2-(methoxymethyl)pyrrolidin-1-yl)-2,2-dimethyl-1,3-dioxan-5-imine, **106**, (0.05 g, 0.2 mmol) and anhydrous THF (10 mL) added. Then ^{t}BuLi (0.14 mL, 0.2 mmol, 15% in *n*-pentane) was added dropwise by syringe at -78 °C. After stirring for 2 hours at this temperature, the mixture was cooled to -100 °C and iodoethane (0.03 g, 0.02 mL, 0.2 mmol) was added slowly. After further stirring for 2 hours at -100 °C, the mixture was allowed to warm to room temperature over 15 hours. The mixture was quenched with pH 7 buffer solution (citric acid-phosphate buffer, 2 mL) and diluted with diethyl ether (80 mL). The organic layer was washed with pH 7 buffer solution (citric acid-phosphate buffer, 10 mL) and saturated sodium chloride solution (2 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The dialkylated SAMP-hydrazone

obtained was purified by flash silica column chromatography (eluent: *n*-pentane:diethyl ether, 5:1) to afford (*Z*,4*S*)-4-ethyl-*N*-((*S*)-2-ethyl-5-(methoxymethyl)pyrrolidin-1-yl)-2,2-dimethyl-1,3-dioxan-5-imine, **109**, (20 mg, 0.2 mmol, 34 %) as a colourless oil.[65]

δ_H (300 MHz; $CDCl_3$): 4.72 (1H, d, *J*=11.6 Hz, C^1HH), 4.24 (1H, br d, *J*=12.7 Hz, C^3H), 4.10 (1H, dd, *J*=2.6 Hz, 11.6 Hz, C^1HH), 3.45–2.89 (4H, m, $C^{12}H_2$, C^8H, $C^{11}H$), 3.33 (3H, s, $C^{13}H_3$), 2.03–1.47 (8H, m, C^9H_2, $C^{10}H_2$, $C^{14}H_2$, C^4H_2), 1.41 (3H, s, C^7H_3), 1.37 (3H, s, C^7H_3), 0.91 (6H, t, *J*=7.3 Hz, C^5H_3, $C^{15}H_3$);
δ_C (75 MHz; $CDCl_3$): 160.4 (C^2), 99.7 (C^6), 75.8 (C^{12}), 72.3 (C^3), 66.6 (C^7), 64.1 (C^{13}), 59.1 (C^{11}), 53.3 (C^8), 26.8 (C^7), 24.3 (C^9), 22.9 (C^{10}), 21.5 (C^{14}), 21.1 (C^4), 9.4 (C^5), 9.2 (C^{15});
m/z (+ EI) found $[M]^+$ 298 (100%).

7.2.17 (*Z*)-4-Ethyl-*N*-((*S*)-2-(methoxymethyl)pyrrolidin-1-yl)-2,2-dimethyl-1,3-dioxan-5-imine (107)

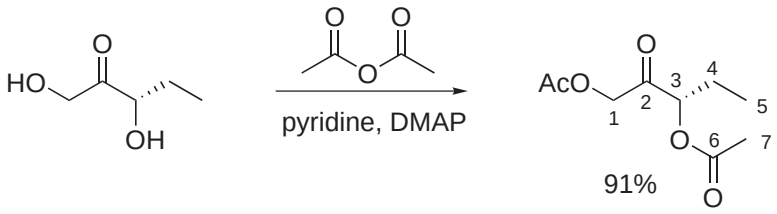


A dry, argon flushed, 3 necked round-bottom flask, equipped with a magnetic stirring bar, had *N*-((*S*)-2-(methoxymethyl)pyrrolidin-1-yl)-2,2-dimethyl-1,3-dioxan-5-imine, **106**, (0.0446 g, 0.2 mmol) and anhydrous THF (10 mL) added. Then, ^{s}BuLi (0.14 mL, 15% in n-pentane) was added dropwise by syringe at -78 °C. After stirring for 2 hours at this tem-

perature, the mixture was cooled to -100 °C and bromoethane (0.03 g, 0.02 mL, 0.2 mmol) was added slowly. After further stirring for 2 hours at -100 °C, the mixture was allowed to warm to room temperature over 15 hours. The mixture was quenched with pH 7 buffer solution (citric acid-phosphate buffer, 2 mL) and diluted with diethyl ether (80 mL). The organic layer was washed with pH 7 buffer solution (citric acid-phosphate buffer, 10 mL) and saturated sodium chloride solution (2 x 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The monoalkylated SAMP-hydrazone obtained was purified by flash silica column chromatography (eluent: *n*-pentane:diethyl ether, 5:1) to afford (*Z*)-4-Ethyl-*N*-((*S*)-2-(methoxymethyl)pyrrolidin-1-yl)-2,2-dimethyl-1,3-dioxan-5-imine, **107**, (12 mg, 0.04 mmol, 23%) as a colourless oil.[65]

The characterization data was the same as that described on page 242

7.2.18 (*S*)-1,3-Diacetoxypentan-2-one (3-(*S*)-110)



(*S*)-1,3-Dihydroxypentan-2-one, (*S*)-**71**, (0.02 g, 0.2 mmol) was dissolved in pyridine (0.1 mL) and acetic anhydride (0.015 g, 0.2 mmol) and DMAP (0.02 g, 0.2 mmol) were added and the mixture stirred overnight at room temperature The solution was concentrated under reduced pressure and partitioned between ethyl acetate and saturated sodium bicarbonate solution. The crude product was purified by column chromatography (eluent: diethyl

ether:hexane, 2:3) to give (*S*)-1,3-diacetoxypentan-2-one, (3-(*S*)-**110**), (28 mg, 0.2 mmol, 100%) as a colourless oil.[232,233]

δ_H (300 MHz; $CDCl_3$): 5.05 (1H, dd, *J*=7.5 Hz, 5.0 Hz, C^3H), 4.89 (1H, d, *J*=12.3 Hz, C^1HH), 4.83 (1H, d, *J*=12.3 Hz, C^1HH), 2.19 (3H, s, C^7H_3), 2.15 (3H, s, C^7H_3), 1.91–1.73 (2H, m, C^4H_2), 0.97 (3H, t, *J*=7.4 Hz, C^5H_3);

δ_C (75 MHz; $CDCl_3$): 200.8 (C^2), 170.4 (C^6), 170.1 (C^6), 77.5 (C^3), 67.8 (C^1), 24.1 (C^7), 20.5 (C^7), 20.4 (C^4), 9.2 (C^5);

m/z (+ CI-Methane) found $[MH]^+$ 203.0915. $C_9H_{15}O_5$ requires $[M]^+$ 203.0919;

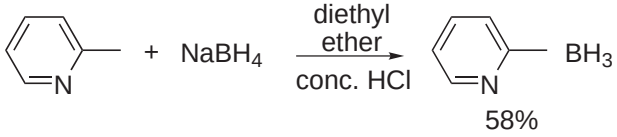
Optical Rotation $[\alpha]^{25}_D$=-23.0 (*c* 1.2, H_2O).

7.3 Chapter 4: Development of reductive amination in water

7.3.1 General amine borane synthesis procedure

Amine (50.7 mmol) in diethyl ether (25 mL) was cooled in an ice bath and the mixture acidified by dropwise addition of ice-cooled concentrated HCl. Once the solution was acidic according to Litmus paper the solvent was removed under reduced pressure. The residue was then redissolved in THF (100 mL) and $NaBH_4$ (4.04 g, 106.8 mmol) was slowly added with stirring. Once addition was complete the reaction was stirred for 24 hours and then the mixture purified by suction filtration. The solvent was removed under reduced pressure to give the desired amine borane.[296]

2-Picoline borane



The general amine borane synthesis procedure was followed using 2-Picoline (4.72 g, 5 mL, 50.7 mmol). This gave 2-picoline borane (3.18 g, 29.5 mmol, 58%) as an off white solid.[296]

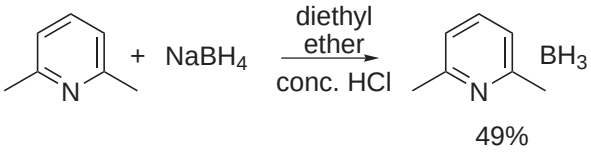
δ_H (300 MHz; $CDCl_3$): 8.69 (1H, d, J=7.8 Hz), 7.81 (1H, t, J=7.8 Hz), 7.36 (1H, d, J=7.8 Hz), 7.28 (1H, t, J=7.8 Hz), 2.71 (3H, s, CH_3);
δ_C (75 MHz; $CDCl_3$): 157.7, 148.7, 139.6, 126.9, 122.5, 22.6 (CH_3);

m/z (+ CI-Methane) 95 (M(^{13}C)-BH_3+H, 7%), 94 (M-BH_3+H, 100); Found (+HRES) [MH-BH_3]$^+$ 94.0650. C_6H_8N requires [MH-BH_3]$^+$ 94.0656;

mp 45 °C (lit. 45–46 °C[296]);

ν_{max}/cm^{-1} 3389 br (C-H), 1618 (C=C Ring), 1571 (C=C ring).

2,6-Lutidine borane



49%

The general amine borane synthesis procedure was followed using 2,6-Lutidine (5.0 g, 5 mL, 46.7 mmol). This gave 2,6-lutidine borane (2.75 g, 22.7 mmol, 49%) as a white solid.[297,298]

δ_H (300 MHz; $CDCl_3$): 7.63 (1H, t, *J*=8.0 Hz), 7.23 (2H, d, *J*=8.0 Hz), 2.79 (6H, s, CH_3);

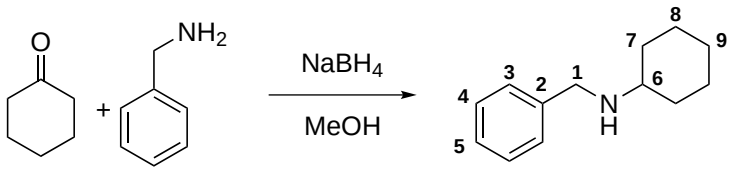
δ_C (75 MHz; $CDCl_3$): 158.5, 138.1, 124.7, 25.3 (CH_3);

m/z (+ CI-Methane) 109 (M(^{13}C)-BH_3+H, 7%), 108 (M-BH_3+H, 100); Found (+HRES) [MH-BH_3]$^+$ 108.0818. $C_7H_{10}N$ requires [MH-BH_3]$^+$ 108.0813;

mp 108 °C (lit. 110–111 °C[299]);

ν_{max}/cm^{-1} 3210 br (CH), 1651 (C=C Ring).

7.3.2 *N*-Benzylcyclohexylamine (117)



115 **77** **117**
41%

Cyclohexanone, **115**, (0.10 g, 0.1 mL, 1 mmol), benzylamine, **77**, (0.11 g, 0.11 mL, 1 mmol) and $NaBH_4$ (0.04 g, 10 mmol) were stirred in methanol (2 mL) for 16 hours at room temperature The mixture was basified with saturated ammonium hydroxide solution(2.5 mL). The mixture was extracted with ethyl acetate (2 x 25 mL) and the combined organic extracts washed with saturated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 3:1) to give *N*-benzylcyclohexylamine, **117**, (0.08 g, 0.4 mmol, 41%) as a pale brown oil.[194]

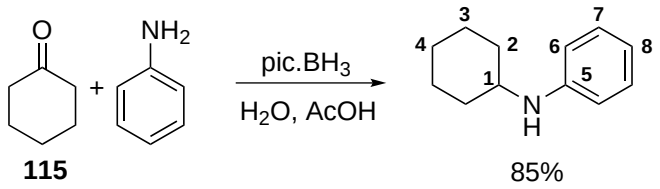
δ_H (300 MHz; $CDCl_3$): 7.47–7.13 (5H, m, Ar C*H*), 3.81 (2H, s, C^1H_2), 2.53–2.31 (1H, m), 2.01–1.87 (2H, m), 1.79–1.64 (2H, m), 1.61–1.47 (2H, m), 1.37–1.11 (4H, m);
δ_C (75 MHz; $CDCl_3$): 141.0 (C^2), 128.4 (C^4), 128.1 (C^3), 126.8 (C^5), 56.2 (C^6), 51.1 (C^1), 33.6 (C^7), 26.2 (C^9), 25.0 (C^8);
m/z (+ EI) found $[M]^+$ 189.1458. $C_{13}H_{19}N$ requires $[M]^+$ 189.1517;
ν_{max}/cm^{-1} 3027 (Aromatic C-H), 1604 (C=C ring).

Other syntheses mentioned identical to method on page 250 except for the conditions shown in table 7.2 on page 251.

Reducing agent	Solvent	Yield
$NaCNBH_3$	methanol	0.09 g (0.5 mmol, 46%)
$NaBH(OAc)_3$	1,2-dichloroethane	0.15 g (0.8 mmol, 81%)

Table 7.2: Reagents for various reductive aminations of cyclohexanone with benzylamine

7.3.3 *N*-Cyclohexylaniline



115 85%

Cyclohexanone, **115**, (0.20 g, 0.2 mL, 2.0 mmol), aniline (0.19 g, 0.2 mL, 2.0 mmol) and picoline borane (0.22 g, 2.1 mmol) were stirred for 2 hours at room temperature in H_2O:glacial acetic acid (10:1, 5.5 mL). 10% Aqueous sodium hydrogen carbonate solution (20 mL) was added and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with saturated sodium chloride solution (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was then purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 1:3) to give *N*-cyclohexylaniline (0.30 g, 1.7 mmol, 85%) as a yellow oil.[194]

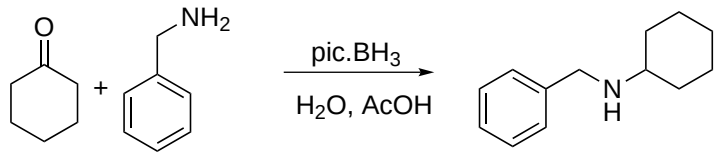
δ_H (300 MHz; $CDCl_3$): 7.26–7.17 (2H, m), 6.74–6.61 (3H, m), 3.52 (1H, br s, NH), 3.37–3.18 (1H, m, C^1H), 2.10 (2H, m, C^3HH), 1.80 (2H, m, C^3HH), 1.83–1.76 (2H, m, C^4H_2), 1.46–1.16 (4H, m, C^2H_2);

δ_C (75 MHz; $CDCl_3$): 147.5 (C^5), 129.3 (C^7), 116.9 (C^6), 113.2 (C^8), 51.7 (C^1), 33.6 (C^2), 26.0 (C^4), 25.1 (C^3);

m/z (+ CI-Methane) 177 (M(^{13}C)+H, 13%), 176 (M+H, 100); Found (+HRES) ($[MH]^+$), 176.1437. $C_{12}H_{18}N$ requires 176.1439;

ν_{max}/cm^{-1} 3403 br (NH), 3051 (Aromatic C-H), 1600 (Aromatic C=C), 1501 (Aromatic C=C).

7.3.4 *N*-Benzylcyclohexylamine (117)

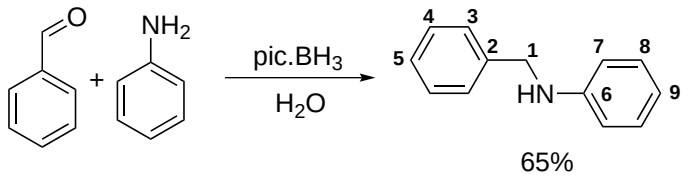


115 **77** **117**
15%

Cyclohexanone, **115**, (0.20 g, 0.2 mL, 2.0 mmol), benzylamine, **77**, (0.22 g, 0.2 mL, 2.0 mmol) and picoline borane (0.22 g, 2.1 mmol) were stirred for 2 hours at room temperature in H_2O:glacial acetic acid (10:1, 5.5 mL). 10% Aqueous sodium hydrogen carbonate solution (20 mL) was added and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with saturated sodium chloride solution (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was then purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 1:2) to give *N*-benzylcyclohexylamine, **117**, (0.06 g, 0.3 mmol, 15%) an a pale brown oil.[194]

The characterization data was the same as that described on page 250

7.3.5 *N*-Benzylaniline



65%

Benzaldehyde (0.32 g, 0.30 mL, 3.0 mmol), aniline (0.28 g, 0.30 mL, 3.0 mmol) and picoline borane (0.32 g, 3.0 mmol) were stirred in water (6 mL) at 40 °C and the reaction monitored by TLC (ethyl acetate:hexane, 1:6). After 1 hour 10% aqueous sodium hydrogen carbonate solution (20 mL) was added and the aqueous solution extracted with ethyl acetate (2 x 30 mL). The combined organic extracts were washed with saturated sodium chloride solution (15 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 1:6) to afford *N*-benzylaniline (0.36 g, 2.0 mmol, 65%) as an orange crystalline solid.[300]

δ_H (300 MHz; $CDCl_3$): 7.48–7.40 (5H, m, Ar-*H*), 7.27 (2H, t, *J*=6.5 Hz), 6.82 (1H, t, *J*=6.5 Hz), 6.72 (2H, d, *J*=6.5 Hz), 4.40 (2H, s, C^1H_2), 4.09 (1H, br s, NH);

δ_C (75 MHz; $CDCl_3$): 148.3 (C^6), 139.6 (C^2), 129.4, 128.7, 127.6, 127.3, 117.7, 113.0, 48.4 (C^1);

m/z (+ EI) found $[M]^+$ 183.1046. $C_{13}H_{13}N$ requires $[M]^+$ 183.1048;

mp 38 °C (lit. 37–38 °C[301]);

ν_{max}/cm^{-1} 3364 br (NH), 2917 (Aromatic C-H), 1634 (Aromatic C=C), 1456 (Aromatic C=C).

Other syntheses mentioned identical to method on page 254 except for the conditions shown in table 7.3 on page 255.

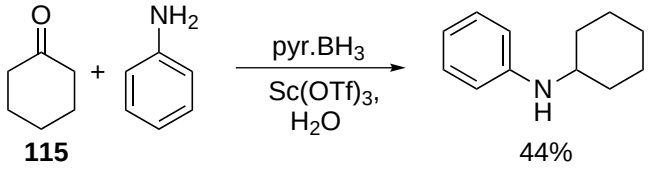
Reducing agent	Additive	Yield
pyridine borane	methanol co-solvent	0.44 g (2.4 mmol, 79%)
pyridine borane	$Sc(OTf)_3$ (10 mol%)	0.43 g (2.3 mmol, 77%)

Table 7.3: Reagents for various reductive aminations of benzaldehyde with aniline

7.3.6 General procedure for reductive amination with scandium triflate in warm water

Ketone/aledehyde, amine, scandium triflate (0.15 g, 10 mol%) and pyridine borane (0.28 g, 3.0 mmol) were stirred in water (20 mL) at 40 °C and the reaction followed by TLC (ethyl acetate:hexane, 1:6). After reaction was observed to be complete 10% aqueous sodium hydrogen carbonate solution (6 mL) was added and the aqueous solution extracted with ethyl acetate (2 x 30 mL). The combined organic extracts were washed with saturated sodium chloride solution (15 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography.

7.3.7 *N*-Cyclohexylaniline

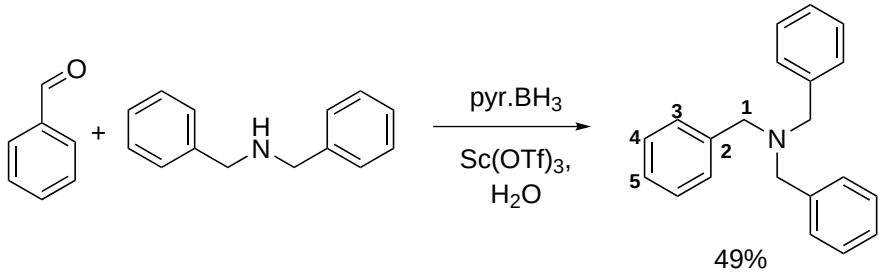


115 44%

The general procedure on page 256 was used with cyclohexanone (0.29 g, 0.3 mL, 3.0 mmol) and aniline (0.28 g, 0.3 mL, 3.0 mmol). The reaction took 1h and the chromatography was performed with ethyl acetate:hexane, 1:6 to afford *N*-cyclohexylaniline (0.23 g, 1.3 mmol, 44%) as a pale brown oil.[194]

The characterization data was the same as that described on page 252

7.3.8 Tribenzylamine



The general procedure on page 256 was used with benzaldehyde (0.32 g, 0.3 mL, 3.0 mmol) and dibenzylamine (0.59 g, 0.6 mL, 3.0 mmol). The reaction took 2h and the chromatography was performed with ethyl acetate:hexane, 1:6 to afford tribenzylamine (0.42 g, 1.5 mmol, 49%) as a pale off-white solid.[302]

δ_H (300 MHz; $CDCl_3$): 7.41 (6H, d, J=7.0 Hz, C^3H), 7.31 (6H, t, J=7.0 Hz, C^4H), 7.06 (3H, t, J=7.0 Hz, C^5H), 3.56 (6H, s, C^1H_2);

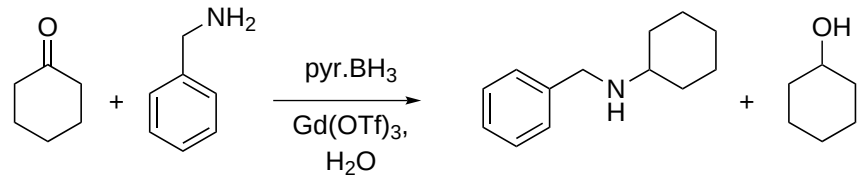
δ_C (75 MHz; $CDCl_3$): 139.7 (C^2), 128.7, 128.2, 126.9, 57.9 (C^1);

m/z (Positive CI-Methane) found $[MH]^+$ 288.1754. $C_{21}H_{22}N$ requires $[MH]^+$ 288.1752;

mp 93 °C (lit. 91–93 °C[302]);

ν_{max}/cm^{-1} 1603 (Aromatic C=C), 1453 (Aromatic C=C).

7.3.9 Typical conditions for NMR ratio experiments



Pyridine borane (0.19 g, 2.0 mmol) was added to a stirred mixture of cyclohexanone, **115**, (0.20 g, 0.2 mL, 2.0 mmol), benzylamine, **77**, (0.21 g, 0.2 mL, 2.0 mmol) and gadolinium triflate (0.12 g, 10 mol%) in H_2O (4 mL). The mixture was stirred for 1 hour at room temperature and 10% aqueous sodium hydrogen carbonate (5 mL) added. The mixture was extracted with ethyl acetate (3 x 20 mL) and the combined organic extracts washed with saturated sodium chloride solution (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in $CDCl_3$ and analysed by 300 MHz NMR.

The peaks compared are the C*H*OH peak at 3.52 for cyclohexanol and the CH_2NH peak at 3.82 for *N*-benzylcyclohexylamine, **117**. These are shown in Figure 7.1 on page 259.

The other reactions that were analysed by NMR spectroscopy were performed in a similar manner with the modified conditions highlighted in the chepter text.

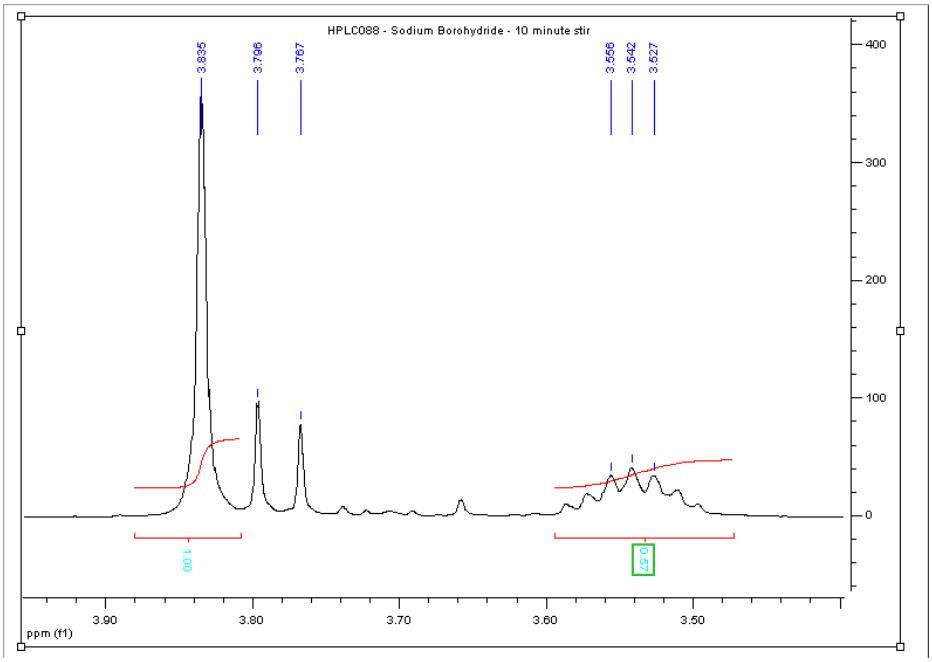
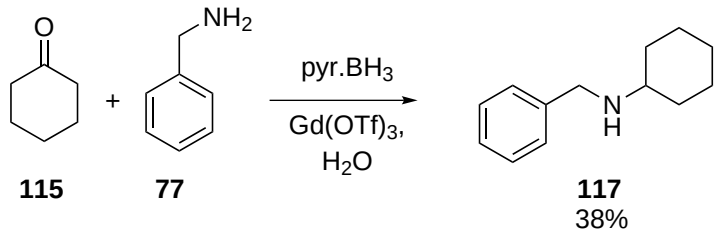


Figure 7.1: Example of peaks examined during NMR analysis

7.3.10 Typical conditions for purified yields



115 **77** **117**
38%

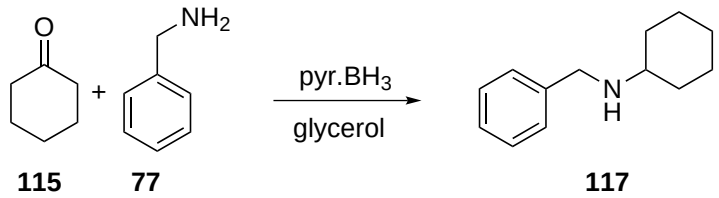
Cyclohexanone, **115**, (0.20 g, 0.2 mL, 2.0 mmol), benzylamine, **77**, (0.21 g, 0.2 mL, 2.0 mmol), gadolinium triflate (0.12 g, 10 mol%) and pyridine borane (0.19 g, 2.0 mmol) were stirred for 10 min at room temperature in H_2O(4 mL). 10% Aqueous sodium hydrogen carbonate solution (20 mL) was added to the mixture, which was then extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with saturated sodium chloride solution (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was then purified by flash silica column chromatography (eluent: ethyl

acetate:hexane, 1:2) to give *N*-benzylcyclohexanamine, **117**, (0.15 g, 0.8 mmol, 38%).[194]

The characterization data was the same as that described on page 250

The other reactions that were analysed by obtaining a purified yield were performed in a similar manner with the modified conditions highlighted in the chepter text.

7.3.11 Typical conditions for calculated yields by HPLC



Cyclohexanone, **115**, (0.20 g, 0.2 mL, 2.0 mmol), benzylamine, **77**, (0.21 g, 0.2 mL, 2.0 mmol) and pyridine borane (0.19 g, 2.0 mmol) were stirred for 10 min at 25 °C in glycerol (4 mL). 10% Aqueous sodium hydrogen carbonate solution (20 mL) was added and the mixture extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with saturated sodium chloride solution (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. A portion of the residue was dissolved in acetonitrile:H_2O (15:85) to make a 1 mg/mL solution. This was then injected into an HPLC machine running a 15% acetonitrile in water mixture with 0.1% trifluoroacetic acid over 15 minutes on a reverse phase column, monitored at 254 nm. The product came off of the column at about 13.5 minutes. The area of this peak was then compared to a calibration curve to find the concentration of the product in the mixture. From this and the crude yield a calculated yield could be obtained. An example calculation is shown below, for this reaction.

Maximum Yield=0.002 mol

Crude Weight=0.4627 g

Retention time=13.358 min

Peak area=79021

mol injected (worked out from calibration curve): $\frac{79021}{2x10^{12}} = 3.95105x10^{-8}$

Estimated mol=mol in 1 g x crude weight=0.00183

Yield=91%

The other reactions that were analysed with the HPLC assay were performed in a similar manner with the modified conditions highlighted in the chepter text.

7.3.12 General method for the preparation of Lewis acid-surfactant-combined catalysts (LASCs)

To sodium dodecylsulfate (SDS, 3.4 g, 11.56 mmol) in H_2O (100 ml) was added the chloride of the desired cation (3.85 mmol) in H_2O (20 ml) at room temperature, a white precipitates appeared immediately, and the mixture was stirred for 10 min. The white solid was collected by filtration, washed with water (5 x 50 mL), and dried (0.1 mm Hg/20 °C, 20 hours) to afford the LASC as a white solid.[121]

Ytterbium(III) dodecyl sulfate ($Yb(DS)_3$)

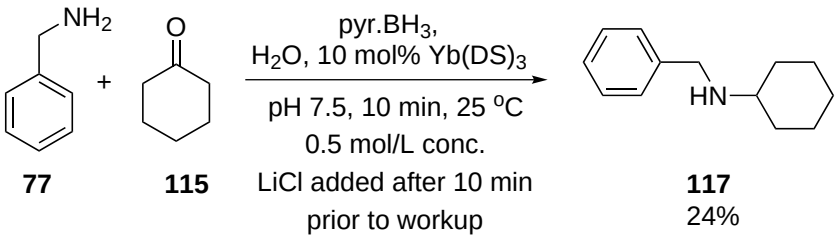
$YbCl_3.6H_2O$ (1.0 g, 3.85 mmol) was used to afford $Yb(DS)_3$ (2.17 g, 2.24 mmol, 58%) as a white solid.[121]

δ_H (500 MHz; D_2O): 4.35–4.24 (br m, 6H), 2.11–1.95 (br m, 6H), 1.89–1.34 (br m, 54H), 1.26–0.95 (br m, 9H);
δ_C (125 MHz; D_2O): 70.3, 32.8, 30.7, 30.5, 30.33, 29.8, 26.3, 23.4, 14.6; ν_{max}/cm^{-1} 2920 (C-H).

7.3.13 General method for the optimized reductive amination reaction in water

Cyclohexanone, **115**, (1 eq.) was added to a stirred solution of amine (0.85 eq.), $Yb(DS)_3$ (1 eq.) and pyridine borane (1 eq.) in H_2O at pH 7.5 and 25 °C. The mixture was stirred for 10 minutes and saturated aqueous sodium hydrogen carbonate solution (10 mL) and lithium chloride monohydrate (0.5 g) added. The mixture was extracted with ethyl acetate (2 x 40 mL) and the combined organic extracts washed with saturated sodium chloride solution (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography to give the product.

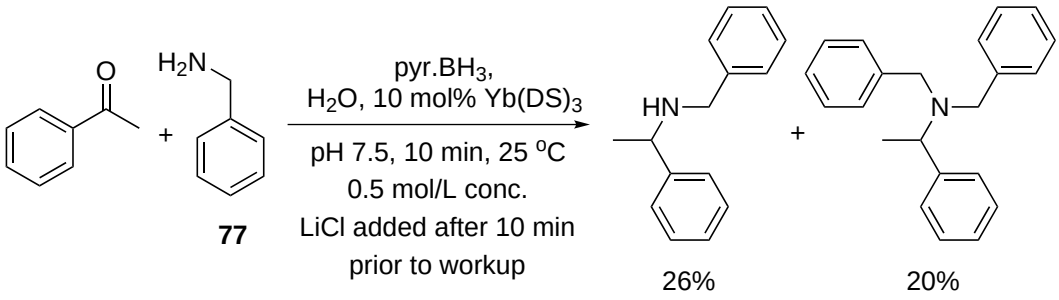
***N*-Benzylcyclohexylamine (117)**



Cyclohexanone, **115**, (0.40 g, 0.4 mL, 4.1 mmol) was added to a stirred solution of benzylamine, **77**, (0.38 g, 0.4 mL, 3.5 mmol), $Yb(DS)_3$ (0.40 g, 10 mol%) and pyridine borane (0.38 g, 4.1 mmol) in H_2O (8 mL) following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 2:1) to give *N*-benzylcyclohexylamine, **117**, (0.19 g, 1.0 mmol, 24%) as a pale brown oil.[194]

The characterization data was the same as that described on page 250

_N_-Benzyl-1-phenylethylmine and (_N,N_)-dibenzyl-1-phenylethylmine



Acetophenone (0.24 g, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol), $Yb(DS)_3$ (0.20 g, 10 mol%) and pyridine borane (0.19 g, 2.0 mmol) in H_2O (4 mL) following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 2:1) to give *N*-benzyl-1-phenylethylamine (0.11 g, 0.5 mmol, 26%) and *N,N*-dibenzyl-1-phenylethanamine (0.12 g, 0.4 mmol, 20%) both as off-white microcrystalline solids.[303,304]

N-benzyl-1-phenylethylamine

δ_H (300 MHz; $CDCl_3$): 7.53–7.02 (10H, m, Ar C*H*), 4.88 (1H, q, *J*=6.5 Hz, C*H*), 3.80 (2H, q, *J*=6.6 Hz, CH_2NH), 1.48 (3H, d, *J*=6.5 Hz, CH_3);

δ_C (75 MHz; $CDCl_3$): 128.5, 128.2, 126.9, 126.7, 125.4, 57.5 (*C*HNH), 51.6 (*C*H_2), 25.3 (*C*H_3);

m/z (+ CI-Methane) 211 (M^+, 17%), 120 (M-C_7H_7, 19), 106 (M-C_8H_9, 45), 91 (M-$C_8H_{10}N$, 100); Found (+HRES) ($[M]^+$), 211.1356. $C_{15}H_{17}N$ requires $[MH]^+$ 211.1361;

mp 260 °C (lit. 249–252 °C[305,306]);

ν_{max}/cm^{-1} 3324 br (NH), 1603 (Aromatic C=C), 1451 (Aromatic C=C).

N,N-dibenzyl-1-phenylethanamine

δ_H (300 MHz; $CDCl_3$): 7.62–7.07 (15H, m, Ar C*H*), 4.88 (1H, q, *J*=6.4 Hz, C*H*), 3.61 (4H, q, *J*=8.0 Hz, CH_2NH), 1.27 (3H, d, *J*=5.9 Hz, CH_3);

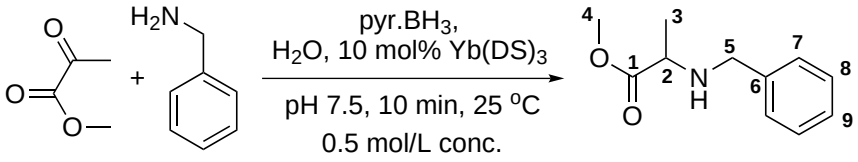
δ_C (75 MHz; $CDCl_3$): 128.5, 128.2, 126.9, 126.7, 125.4, 70.3 (*C*HNH), 57.6 (2 x *C*H_2), 24.5 (*C*H_3);

m/z (+ CI-Methane) found $[MH]^+$ 301.1812. $C_{22}H_{23}N$ requires $[MH]^+$ 301.1830;

mp 61 °C (lit. 58 °C[307]);

ν_{max}/cm^{-1} 1681 (Aromatic C=C), 1449 (Aromatic C=C).

Methyl 2-(benzylamino)propanoate



Methyl pyruvate (0.20 g, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol), $Yb(DS)_3$ (0.20 g, 10 mol%) and pyridine borane (0.19 g, 2.0 mmol) in H_2O (4 mL) following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 2:1) to give methyl 2-(benzylamino)propanoate (0.08 g, 0.4 mmol, 21%) as a pale oil.[308]

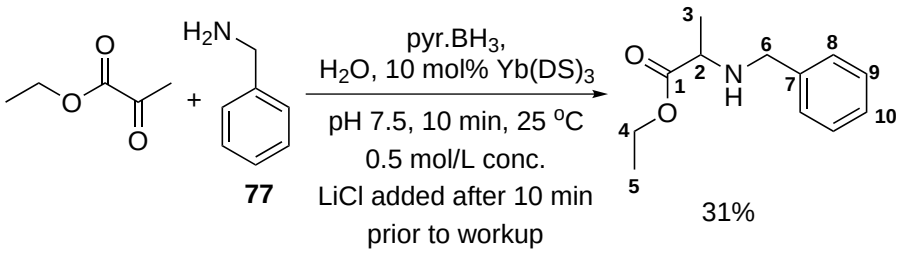
δ_H (300 MHz; $CDCl_3$): 7.48–7.26 (5H, m, Ar-C*H*), 3.70 (2 x 1H, d, *J*=6.6 Hz, C^5HH), 3.69 (3H, s, C^4H_3), 3.38 (1H, q, *J*=7.0 Hz, C^2H, 1.31 (3H, d, *J*=7.0 Hz, C^3H_3);

δ_C (75 MHz; $CDCl_3$): 176.2 (C^1), 139.7 (C^6), 128.4 (C^8), 128.3 (C^7), 127.1 (C^9), 55.9 (C^2), 52.0 (C^4), 51.8 (C^5), 19.1 (C^3);

m/z (+ CI-Methane) 195 (M(^{13}C)+H, 3%), 194 (M+H, 21), 178 (M-CH_3, 8), 134 (M-$C_2H_3O_2$, 44), 106 (M-$C_4H_7O_2$, 9), 91 (M-$C_4H_8NO_2$, 100); Found (+HRES) ($[MH]^+$), 194.1182. $C_{11}H_{16}NO_2$ requires $[MH]^+$ 194.1181;

ν_{max}/cm^{-1} 1681 (Aromatic C=C), 1449 (Aromatic C=C).

Ethyl 2-(benzylamino)propanoate



Ethyl pyruvate (0.23 g, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol), $Yb(DS)_3$ (0.20 g, 10 mol%) and pyridine borane (0.19 g, 2.0 mmol) in H_2O (4 mL) following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 2:1) to give ethyl 2-(benzylamino)propanoate (0.13 g, 0.6 mmol, 31%) as a pale oil.[194]

δ_H (300 MHz; $CDCl_3$): 7.45–7.11 (5H, m, Ar-C*H*), 4.18 (2H, q, *J*=7.2 Hz, C^4H_2), 3.66 (2 x 1H, d, *J*=9.1 Hz, C^6H_2), 3.37 (1H, q, *J*=7.0 Hz, C^2H), 1.29 (6H, m, C^3H_3, C^5H_3);

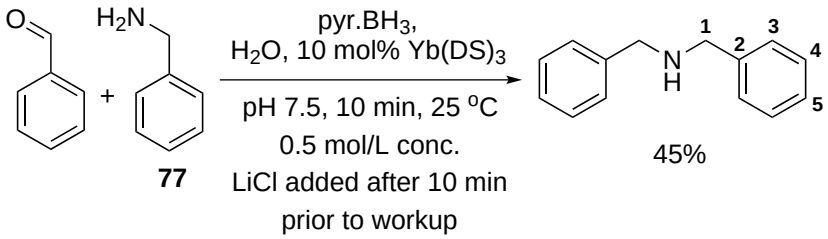
δ_C (75 MHz; $CDCl_3$): 175.7 (C^1), 139.7 (C^7), 128.4 (C^9), 128.3 (C^8), 127.1 (C^{10}), 60.7 (C^2), 56.0 (C^4), 52.0 (C^6), 19.1 (C^5), 14.3 (C^3);

m/z (+ CI-Methane) 208 (M+H, 44%), 178 (M-C_2H_5, 13), 134 (M-$C_3H_5O_2$, 25), 106 (M-

$C_5H_9O_2$, 14), 91 (M-$C_5H_{10}NO_2$, 100); Found (+HRES) ([MH]$^+$), 208.1340. $C_{12}H_{18}NO_2$ requires [MH]$^+$ 208.1338;

ν_{max}/cm^{-1} 3029 br (NH), 1661 (CO), 1495 (Aromatic C=C), 1453 (Aromatic C=C).

Dibenzylamine



Benzaldehyde, **93**, (0.21 g, 0.2 mL, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol), Yb(DS)$_3$ (0.20 g, 10 mol%) and pyridine borane (0.19 g, 2.0 mmol) in H_2O (4 mL) following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 2:1) to give dibenzylamine (0.18 g, 0.9 mmol, 45%) as a pale yellow oil.[194]

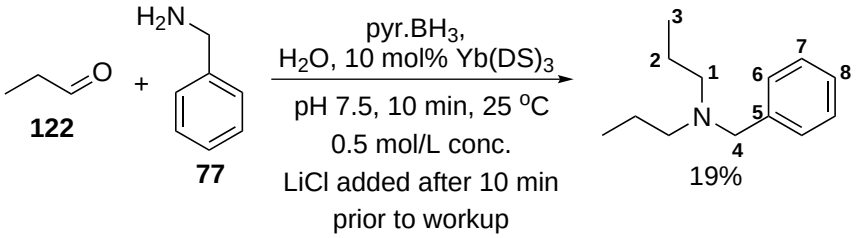
δ_H (300 MHz; $CDCl_3$): 7.43 (4H, d, J=8.1 Hz, C^3H), 7.36 (4H, t, J=8.1 Hz, C^4H), 7.28 (2H, d, J=8.1 Hz, C^5H), 3.60 (s, 4H, C^1H_2);

δ_C (75 MHz; $CDCl_3$): 139.7 (C^2), 128.8 (C^4), 128.3 (C^3), 126.9 (C^5), 58.0 (C^1);

m/z (-EI) 197 (M(^{13}C)-H, 3%), 196 (M-H, 23), 91 (M-C_7H_9N, 100); Found (+HRES) [M-H] 196.1132. $C_{14}H_{14}N$ requires [M-H] 196.1132;

ν_{max}/cm^{-1} 3026 (NH), 1602 (Aromatic C=C), 1451 (Aromatic C=C).

N-Benzyl-*N*-dipropylamine



Propanal, **122**, (0.12 g, 0.2 mL, 2.1 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol), $Yb(DS)_3$ (0.20 g, 10 mol%) and pyridine borane (0.19 g, 2.0 mmol) in H_2O (4 mL) following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 2:1) to give *N*-benzyl-*N*-dipropylamine (0.08 g, 0.4 mmol, 19%) as a yellow oil.[178]

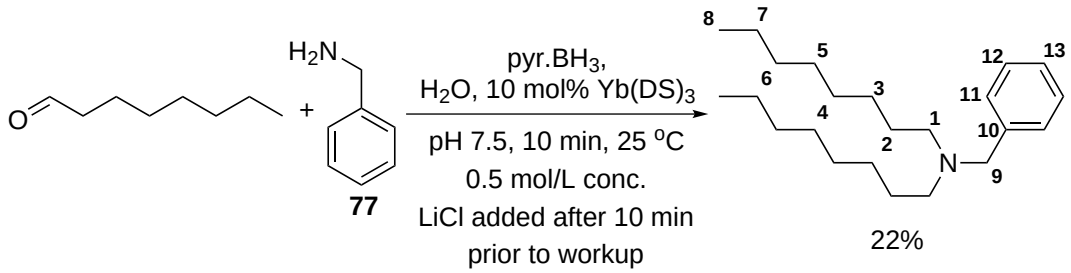
δ_H (300 MHz; $CDCl_3$): 7.35–7.13 (5H, m, Ar-C*H*), 3.56 (2H, s, C^4H_2), 2.38 (4H, t, *J*=7.4 Hz, C^1H_2), 1.49 (4H, sx, *J*=7.4 Hz, C^2H_2), 0.87 (6H, t, *J*=7.4 Hz, C^3H_3);

δ_C (75 MHz; $CDCl_3$): 140.4 (C^5), 128.8 (C^6), 128.0 (C^7), 126.6 (C^8), 58.7 (C^4), 55.9 (C^1), 20.2 (C^2), 11.9 (C^3);

m/z (+ CI-Methane) found $[M]^+$ 191.1692. $C_{13}H_{21}N$ requires $[M]^+$ 191.1674;

ν_{max}/cm^{-1} 3392 br (NH), 2962 (Aromatic C-H), 1627 (Aromatic C=C), 1495 (Aromatic C=C).

_N_-Benzyl-dioctylamine



Octanal (0.26 g, 0.3 mL, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol), $Yb(DS)_3$ (0.20 g, 10 mol%) and pyridine borane (0.19 g, 2.0 mmol) in H_2O (4 mL) following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 2:1) to give *N*-benzyl-dioctylamine (0.15 g, 0.5 mmol, 22%) as a clear oil.[302]

δ_H (300 MHz; $CDCl_3$): 7.42–7.21 (5H, m, Ar-*H*), 3.55 (2H, s, C^9H_2), 2.39 (4H, t, *J*=7.6 Hz, C^1H_2), 1.68–1.50 (4H, br m, C^2H_2), 1.35–1.15 (20 H, br s, CH_2), 0.88(6H, t, *J*=7.0 Hz, C^8H_3);

δ_C (75 MHz; $CDCl_3$): 141.2 (C^{10}), 128.9 (C^{11}), 128.0 (C^{12}), 126.6 (C^{13}), 58.6 (C^1), 53.8, 31.9, 29.6, 29.3, 27.5, 27.0, 22.7, 14.1 (C^8);

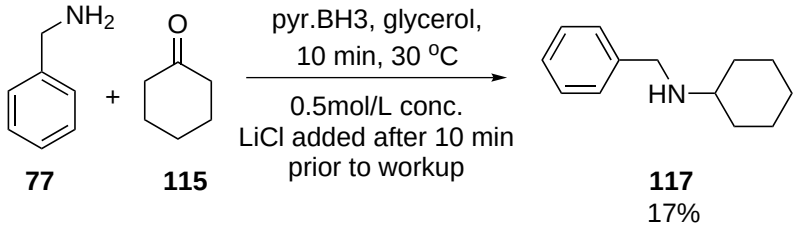
m/z (+ CI-Methane) found 331 (M^+, 100%), 219 (M-C_8H_{17}, 19); Found (+HRES) $[M]^+$ 331.3238. $C_{23}H_{41}N$ requires $[M]^+$ 331.3239;

ν_{max}/cm^{-1} 2916 (C-H), 2854 (N-H).

7.3.14 General procedure for reactions in glycerol

Cyclohexanone, **115**, (1 eq.) was added to a stirred solution of benzylamine, **77** (0.85 eq.) and pyridine borane (1 eq.) in glycerol at 30 °C. The mixture was stirred for 10 minutes and saturated aqueous sodium hydrogen carbonate solution (10 mL) and lithium chloride monohydrate (0.5 g) added. The mixture was extracted with ethyl acetate (2 x 40 mL) and the combined organic extracts washed with saturated sodium chloride solution (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography to give the product.

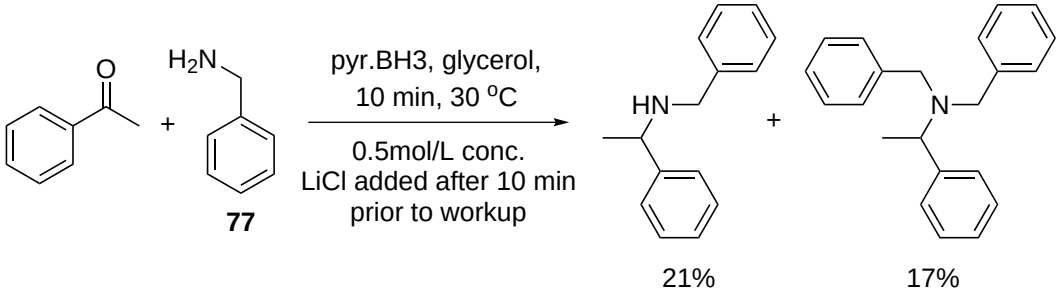
_N_-Benzylcyclohexanamine



Cyclohexanone, **115**, (0.40 g, 0.4 mL, 4.1 mmol) was added to a stirred solution of benzylamine, **77**, (0.38 g, 0.4 mL, 3.5 mmol) and pyridine borane (0.38 g, 4.1 mmol) in glycerol (8 mL)following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 3:1) to give *N*-benzylcyclohexanamine, **117**, (0.13 g, 0.7 mmol, 17%) as a pale brown oil.[194]

The characterization data was the same as that described on page 250

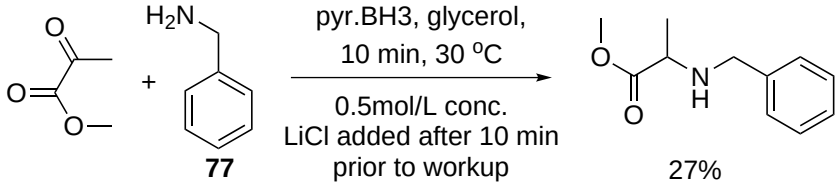
_N_-Benzyl-1-phenyl-ethylamine and _N,N_-dibenzyl-1-phenyl-ethylamine



Acetophenone (0.24 g, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol) and pyridine borane (0.19 g, 2.0 mmol) in glycerol (4 mL) following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 5:1) to give *N*-benzyl-1-phenylethylamine (0.09 g, 0.4 mmol, 21%) and *N,N*-dibenzyl-1-phenylethylamine (0.10 g, 0.3 mmol, 17%) both as off-white microcrystalline solids.[303,304]

The characterization data was the same as that described on page 265

Methyl 2-(benzylamino)propanoate

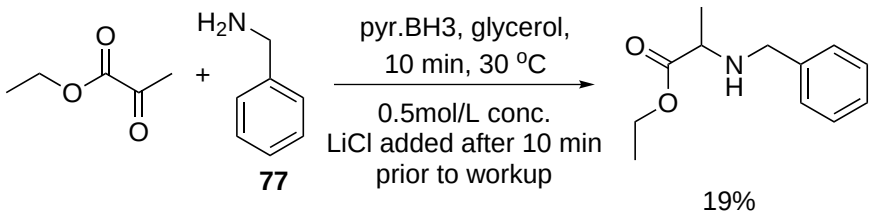


Methyl pyruvate (0.20 g, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol) and pyridine borane (0.19 g, 2.0 mmol) in glycerol (4 mL)

following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 5:1) to give methyl 2-(benzylamino)propanoate (0.10 g, 0.5 mmol, 27%) as a pale yellow oil.[308]

The characterization data was the same as that described on page 266

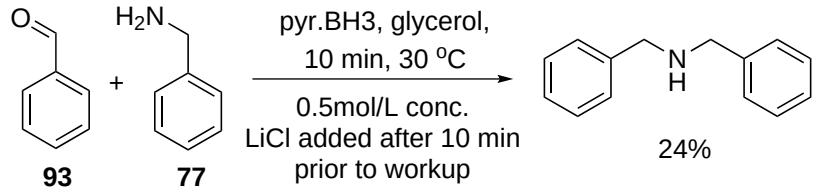
Ethyl 2-(benzylamino)propanoate



Ethyl pyruvate (0.23 g, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol) and pyridine borane (0.19 g, 2.0 mmol) in glycerol (4 mL) following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 5:1) to give ethyl 2-(benzylamino)propanoate (0.08 g, 0.4 mmol, 19%) as a pale brown oil.[194]

The characterization data was the same as that described on page 267

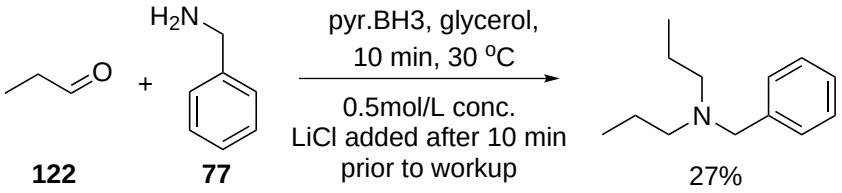
Dibenzylamine



Benzaldehyde, **93**, (0.21 g, 0.2 mL, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol) and pyridine borane (0.19 g, 2.0 mmol) in glycerol (4 mL) following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 5:1) to give dibenzylamine (0.09 g, 0.5 mmol, 24%) as a pale brown oil.[194]

The characterization data was the same as that described on page 268

***N*-Benzyl-dipropylamine**

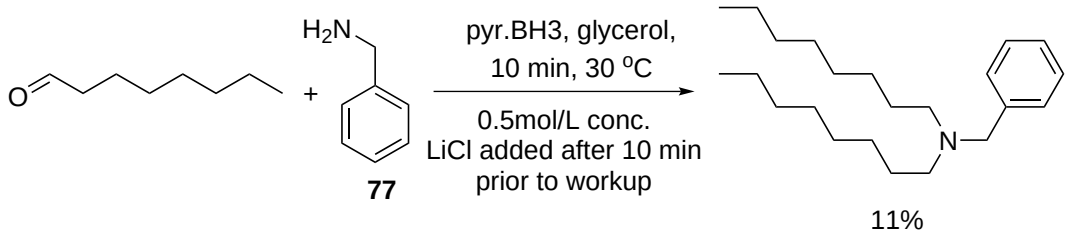


Propanal, **122**, (0.12 g, 0.2 mL, 2.1 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol) and pyridine borane (0.19 g, 2.0 mmol) in glycerol (4 mL) following the general procedure. The residue was purified by flash silica column chromatography eluent: ethyl acetate:hexane, 3:1) to give *N*-benzyl-dipropylamine (0.11 g, 0.6

mmol, 27%) as a pale yellow oil.[178]

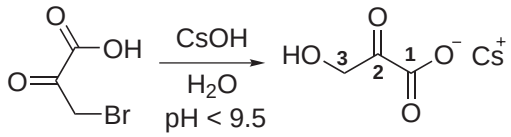
The characterization data was the same as that described on page 269

7.3.15 *N*-Benzyl-dioctylamine



Octanal (0.26 g, 0.3 mL, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.19 g, 0.2 mL, 1.8 mmol) and pyridine borane (0.19 g, 2.0 mmol) in glycerol (4 mL) following the general procedure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 5:1) to give *N*-benzyl-dioctylamine (0.07 g, 0.2 mmol, 11%) as a clear oil.[302]

The characterization data was the same as that described on page 270



121 Cs-**70**

7.4 Chapter 5: Application of the reductive amination reaction in water to enzymatically synthesized ketodiol

7.4.1 Caesium hydroxypyruvate (Cs-70)

Bromopyruvic Acid, **121**, (15.00 g, 90.4 mmol) was dissolved in water (100 mL) and 3M CsOH (25.00 g, 166.8 mmol in 50 mL) was added at such a rate that the pH did not exceed 9.5 whilst stirring. Glacial acetic acid was then added dropwise to adjust the solution pH to 5.0, the mixture concentrated under reduced pressure to approximately 20 mL final volume and left to crystallize overnight. The crude product was collected by suction filtration and washed with ethanol. The solid was then suspended in ethanol (50 mL) at 40 °C for 30 min. An off white powder was collected by suction filtration and washed with further ethanol to give caesium hydroxypyruvate, Cs-**70**, as an off white solid in quantitative yield.[207]

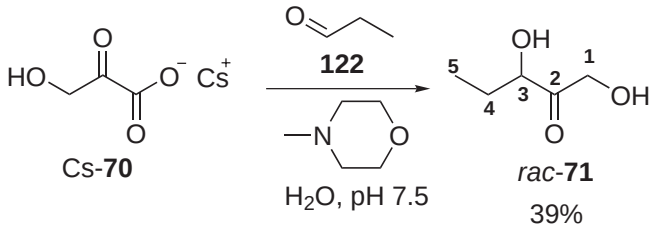
δ_H (300 MHz; $CDCl_3$): 4.99 (2H, br s, C^3H_2);

δ_C (75 MHz; $CDCl_3$): 172.1 (C^1), 161.4 (C^2) 64.2 (C^3);

m/z (+ EI) found $[M]^+$ 235.88 (100%);

ν_{max}/cm^{-1} 3391 br (OH), 1595 br (CO), 1381 (COO^-).

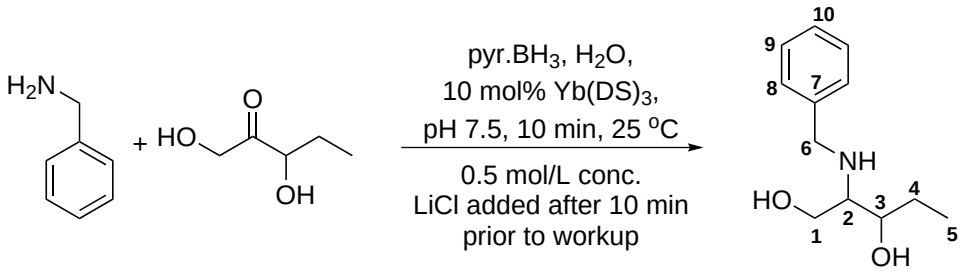
7.4.2 *rac*-1,3-Dihydroxypentan-2-one (*rac*-71)



To a stirred solution of caesium hydroxypyruvate, Cs-**70**, (2.12 g, 9.0 mmol) in water (180 mL) at room temperature *N*-Mmethylmorpholine (0.91 g, 1.0 mL, 9.0 mmol) was added and the solution was adjusted to pH 7.5 using glacial acetic acid. Freshly distilled propanal, **122**, (0.52 g, 0.7 mL, 9.0 mmol) was added and the mixture stirred at room temperature for 24 hours. The solvent was removed under reduced pressure, the product adsorbed onto silica from aqueous solution and purified by flash silica column chromatography (eluent: ethyl acetate : petroleum ether (40-60), 4:1) to give racemic 1,3-dihydroxypentan-2-one, *rac*-**71**, (0.41 g, 3.5 mmol, 39%) as a clear oil.[207]

δ_H (300 MHz; $CDCl_3$): 4.38 (2 x 1H, d, J=12 Hz, C^1HH), 4.24 (1H, t, J=4 Hz, C^3H), 1.93–1.72 (1H, br m, OH), 1.67 (2H, spt, J=6.5 Hz, C^4H_2), 1.01 (3H, t, J=6.5 Hz, C^5H_3);
δ_C (75 MHz; $CDCl_3$): 212.5 (C^2), 76.0 (C^3), 65.6 (C^1), 27.1 (C^4), 9.0 (C^5);
m/z (+ CI-Methane) found $[MH]^+$ 119.0710. $C_5H_{11}O_3$ requires $[MH]^+$ 119.0708;
ν_{max}/cm^{-1} 3277 br (OH), 1717 (CO).

7.4.3 2-(Benzylamino)pentane-1,3-diol (*rac*-124)

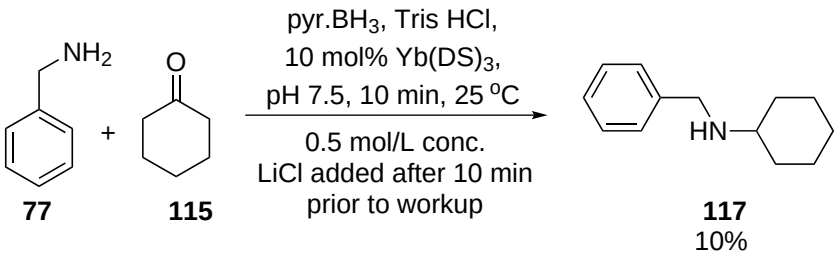


77 *rac*-**71** *rac*-**124**
7%

Racemic 1,3-dihydroxypentan-2-one, *rac*-**71**, (0.24 g, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.21 g, 0.2 mL, 2.0 mmol), $Yb(DS)_3$ (0.20 g, 10 mol%) and pyridine borane (0.19 g, 2.0 mmol) in H_2O (4 mL) at pH 7.5 and 25 °C. The mixture was stirred for 10 minutes and saturated aqueous sodium hydrogen carbonate solution (5 mL) and lithium chloride monohydrate (0.5 g) added. The mixture was extracted with ethyl acetate (2 x 20 mL) and the combined organic extracts washed with saturated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: methanol:ethyl acetate, 1:9) to give 2-(benzylamino)pentane-1,3-diol, *rac*-**124**, (0.03 g, 0.1 mmol, 7%) as an orange oil.

δ_H (300 MHz; $CDCl_3$): 7.61–7.30 (5H, m, C_6H_5), 3.95–3.64 (4H, m, C^1H_2, C^6H_2), 3.58 (1H, m, C^3H), 2.89 (1H, m, C^2H), 1.19 (2H, m, C^4H_2), 0.98 (3H, t, J=7.4 Hz, C^5H_3);
δ_C (75 MHz; $CDCl_3$): 138.1 (C^7), 128.6 (C^9), 128.3 (C^8), 127.3 (C^{10}), 72.9 (C^3), 61.4 (C^2), 60.3 (C^1), 51.3 (C^6), 26.5 (C^4), 10.7 (C^5);
m/z (+ CI-Methane) 211 (M(^{13}C)+H, 13%), 210 (M+H, 100), 108 (M-$C_5H_{10}O_2$; Found (+HRES) ($[MH]^+$) 210.1491. $C_{12}H_{20}NO_2$ requires $[MH]^+$ 210.1494.
ν_{max}/cm^{-1} 3344 br (NH), 2876 (Aromatic C-H), 1495 (Aromatic C=C).

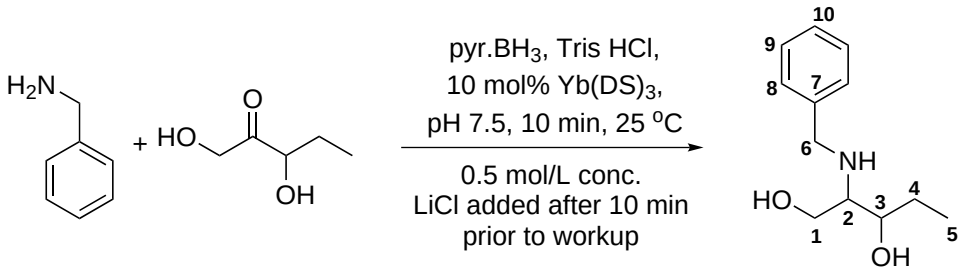
7.4.4 *N*-Benzylcyclohexylamine (117)



Cyclohexanone, **115**, (0.20 g, 0.2 mL, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.21 g, 0.2 mL, 2.0 mmol), $Yb(DS)_3$ (0.20 g, 10 mol%) and pyridine borane (0.19 g, 2.0 mmol) in *tris* HCl_{aq} (4 mL) at pH 7.5 and 25 °C. The mixture was stirred for 20 minutes and saturated aqueous sodium hydrogen carbonate solution (5 mL) and lithium chloride monohydrate (0.5 g) added. The mixture was extracted with ethyl acetate (2 x 20 mL) and the combined organic extracts washed with saturated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 2:1) to give *N*-benzylcyclohexylamine, **117**, (0.04 g, 0.2 mmol, 10 %) as a yellow oil.[194]

The characterization data was the same as that described on page 250

7.4.5 2-(Benzylamino)pentane-1,3-diol (*rac*-124)



77 *rac*-**71** *rac*-**124**
11%

1,3-Dihydroxypentan-2-one, *rac*-**71**, (0.24 g, 2.0 mmol) was added to a stirred solution of benzylamine, **77**, (0.21 g, 0.2 mL, 2.0 mmol), $Yb(DS)_3$ (0.20 g, 10 mol%) and pyridine borane (0.19 g, 2.0 mmol) in *tris* HCl_{aq} (4 mL) at pH 7.5 and 25 °C. The mixture was stirred for 10 minutes and saturated aqueous sodium hydrogen carbonate solution (5 mL) and lithium chloride monohydrate (0.5 g) added. The mixture was extracted with ethyl acetate (2 x 20 mL) and the combined organic extracts washed with saturated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: methanol:ethyl acetate, 1:9) to give 2-(benzylamino)pentane-1,3-diol, *rac*-**124**, (0.05 g, 0.2 mmol, 11%) as an orange oil.

The characterization data was the same as that described on page 278

7.4.6 (*S*)-1,3-Dihydroxypentan-2-one (*S*-71)

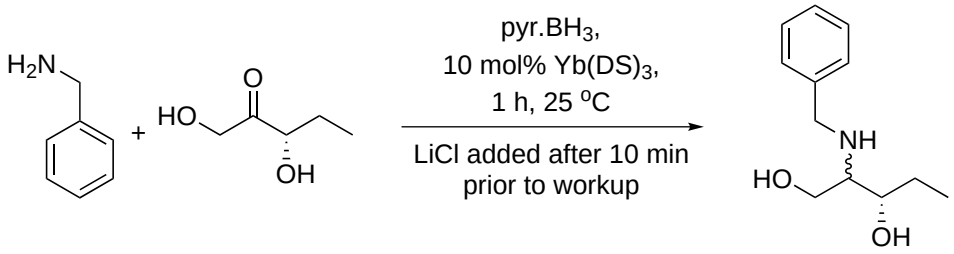
Cofactors solution ThDP (0.008 g, 0.02 mmol), $MgCl_2$ (0.009 g, 0.09 mmol) and *tris* (0.061 g, 0.39 mmol) in H_2O (10 mL) to make a 50 mM solution adjusted to pH 7.0 with dilute aqueous hydrochloric acid.

Substrates solution Propanal (0.029 g, 0.04 mL, 0.50 mmol), LiHPA (0.055 g, 0.50 mmol) and *tris* (0.061 g, 0.39 mmol) in H_2O (10 mL) to make a 50 mM solution adjusted to pH 7.0 with dilute aqueous hydrochloric acid.

Reaction Whole cell sample (*E. coli*) (10 mL) poured into 50 mL RB flask and cofactors solution (10 mL) added and stirred at 30 °C for 20 min. The substrate solution (6 mL) was then added and the mixture stirred at 30 °C for a further 48 hours. The solution was used for reductive amination without any purification.

The characterization data was the same as that described on page 277

7.4.7 (*S*)-2-(Benzylamino)pentane-1,3-diol (124)



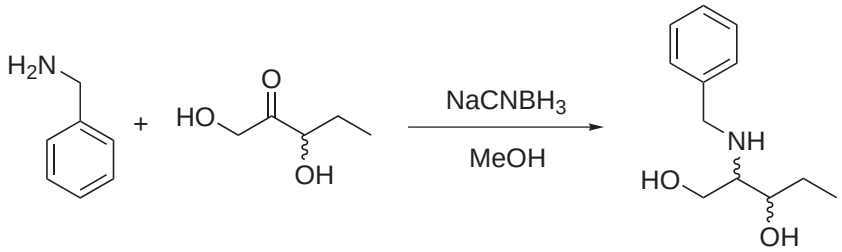
77 (*S*)-**71** **124** 3 %

To the solution of (*S*)-1,3-dihydroxy-pentan-2-one, *S*-**71**, (13 mL) from whole cell biotransformation on page 281) was added benzylamine, **77**, (0.12 g, 0.12 mL, 1.12 mmol), $Yb(DS)_3$ (0.10 g) and pyr BH_3 (0.1 g, 1.0 mmol). The mixture was then stirred for 1 hour at 25 °C. The mixture was then partitioned between aqueous sodium hydrogen carbonate solution (100 mL) and dichloromethane (120 mL) with vigorous stirring. The aqueous layer was extracted with dichloromethane (2 x 100 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: methanol:ethyl acetate, 1:9) to give 2-(benzylamino)pentane-1,3-diol, **124**, (3.12 mg, 0.015 mmol, 3%).

Optical Rotation $[\alpha]^{25}{}_D$=-27.1 (*c* 0.85,$CHCl_3$).

All other characterization data was the same as that described on page 278.

7.4.8 2-(Benzylamino)pentane-1,3-diol (124)

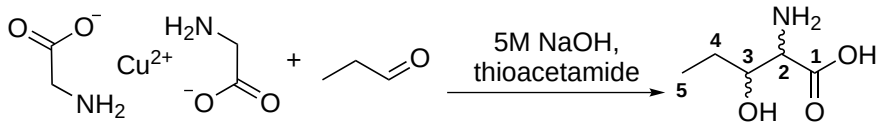


77 *rac*-**70** *rac*-**124**
50%

1,3-Dihydroxypentan-2-one, *rac*-**71**, (0.34 g, 2.9 mmol) was added to a stirred solution of benzylamine, **77**, (0.61 g, 0.6 mL, 5.7 mmol) and $NaCNBH_3$ (0.54 g, 8.6 mmol) in methanol (10 mL). The pH was adjusted to 6.0 with glacial acetic acid and stirred overnight at room temperature The residue was concentrated under reduced pressure and partitioned between saturated aqueous sodium hydrogen carbonate solution (100 mL) and DCM (100 mL). The mixture was separated and the aqueous layer washed with dichloromethane (2 x 100 mL). The combined organic extracts were washed with saturated sodium chloride solution (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: methanol:ethyl acetate, 1:9) to give 2-(benzylamino)pentane-1,3-diol, **124**, (0.30 g, 1.4 mmol, 50%) as a pale red oil.

The characterization data was the same as that described on page 278.

7.4.9 Racemic 3-hydroxy-norvaline (*rac*-126)



127 **122** *rac*-**126**
11%

Copper glycinate, **127**, (1.06 g, 5 mmol) was dissolved in 5M sodium hydroxide solution under argon at 4 °C. propanal (0.87 g, 1.1 mL, 15 mmol) was added dropwise and the mixture stirred for a further 4 hours. The copper hydroxynorvalinate complex was then disproportionated by adding thioacetamide (0.75 g, 10 mmol) and precipitated copper sulphide was then removed by filtration through cellulose and celite. The solution was treated with activated charcoal, and then extracted with $CHCl_3$ (10 x 50 mL) and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by cation exchange chromatography (Dowex 50 X 8 200 mesh; elution by 2M NH_4OH) to give 3-hydroxy-norvaline, *rac*-**126**, (0.15 g, 1.1 mmol, 11%).[267–271]

δ_H (300 MHz; D_2O): 3.68–3.51 (1H, m, C^3H), 2.61–2.45 (1H, m, C^2H), 1.41–1.31 (2H, m, C^4H_2), 0.86 (3H, t, J=4.5 Hz, C^5H_3);

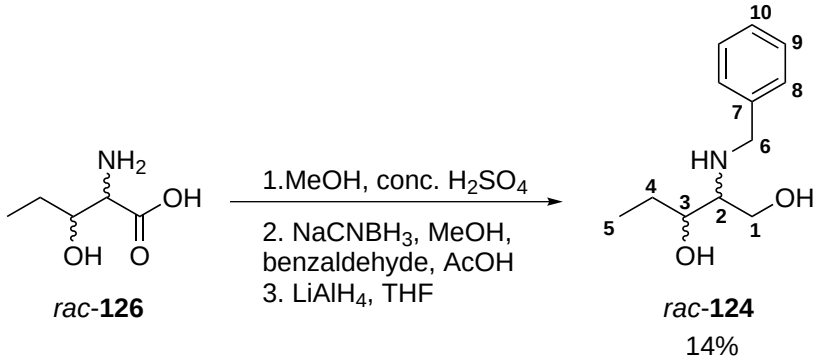
δ_C (75 MHz; D_2O): 160.7 (C^1), 48.2 (C^3), 26.4 (C^2), 13.8 (C^4), 9.6 (C^5);

m/z (+ CI-Methane) 134 (M(^{13}C+H), 5%), 133 (M+H, 100); Found (+HRES) $[M]^+$ 133.0738. $C_5H_{11}NO_3$ requires $[M]^+$ 133.0738;

mp 214–218 °C (lit. 214–215 °C[267]);

ν_{max}/cm^{-1} 3200 br (N-H, O-H), 1700 (C=O).

7.4.10 2-(Benzylamino)pentane-1,3-diol (*rac*-124)



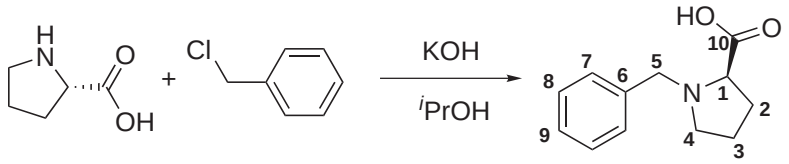
rac-**126** *rac*-**124**
14%

3-Hydroxy-norvaline, *rac*-**126**, (0.15 g, 1.1 mmol) was dissolved in methanol (10 mL) at 0 °C and concentrated sulfuric acid (5 mL) added dropwise. The mixture was stirred at reflux for 15 hours and then neutralized with a saturated aqueous sodium hydrogen carbonate solution. The mixture was then extracted with dichloromethane (3 x 100 mL) and the combined organic extracts washed with saturated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was then dissolved in methanol (20 mL) and freshly distilled benzaldehyde (1.43 g, 1.4 mL, 13.5 mmol) added to the mixture. Glacial acetic acid (2.0 mL) was then added to the reaction and stirred for 1 hour. Sodium cyanoborohydride (0.85 g, 13.5 mmol) was added in one portion and the mixture stirred at room temperature for 15 hours. The mixture was then neutralized with a saturated aqueous sodium hydrogen carbonate solution and extracted with dichloromethane (3 x 100 mL). The combined organic extracts washed with saturated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was dissolved in anhydrous THF (20 mL), cooled to 0 °C and $LiAlH_4$ (0.50 g, 13.5 mmol) added. The reaction was warmed to room temperature and the reaction stirred for 15 hours. Sodium sulfate decahydrate (6.50 g, 20.3 mmol) was added in one portion and the reaction stirred for 1 hour at room temperature H_2O (20 mL) was then

added and the mixture extracted with dichloromethane (10 x 100 mL) and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: methanol:ethyl acetate, 1:9) to give pure 2-(benzylamino)pentane-1,3-diol, *rac*-**124**, (0.03 g, 0.2 mmol, 14%) an a pale orange oil.[126]

The characterization data was the same as that described on page 278.

7.4.11 (*S*)-1-Benzylpyrrolidine-2-carboxylic acid (135)



133 **134** **135**
~ 100%

(*S*)-Proline, **133**, (10.20 g, 88.6 mmol) was added to a solution of KOH (15.12 g, 269.5 mmol) in ^{i}PrOH (40 mL), and the mixture was stirred at 40–50 °C until the compound completely dissolved. The solution was then cooled to 0 °C. After that, freshly distilled benzyl chloride, **134**, (15.00 g, 118.5 mmol) was added dropwise over a period of 30 min, and the mixture was stirred for an additional 15 hours at room temperature The reaction was monitored by TLC ($CHCl_3$:ethanol, 1 : 1). Then the reaction mixture was neutralized with 6 M HCl to pH 5–6, diluted with $CHCl_3$ (25 mL), and allowed to stand for 4 hours. The precipitated salts were filtered off and washed with $CHCl_3$, the filtrate was concentrated, and the residue was treated with Me_2CO. The precipitate was filtered off, washed with ^{i}PrOH, and dried with P_2O_5 to give (*S*)-1-benzylpyrrolidine-2-carboxylic acid, **135**, as a brown solid in quantitative yield.[273,309]

δ_H (300 MHz; $CDCl_3$): 7.53–7.33 (5H, m, Ar-CH), 4.36 (2H, dd, *J*=13.1 Hz, 5.2 Hz, C^5H_2), 3.92 (1H, t, *J*=6.6 Hz, C^1H), 3.78–3.65 (1H, m, C^4H), 3.00 (1H, dd, *J*=3.2 Hz, 8.6 Hz, C^4H_2), 2.36–2.14 (2H, m, C^2H_2), 2.03–1.84 (2H, m, C^3H_2);

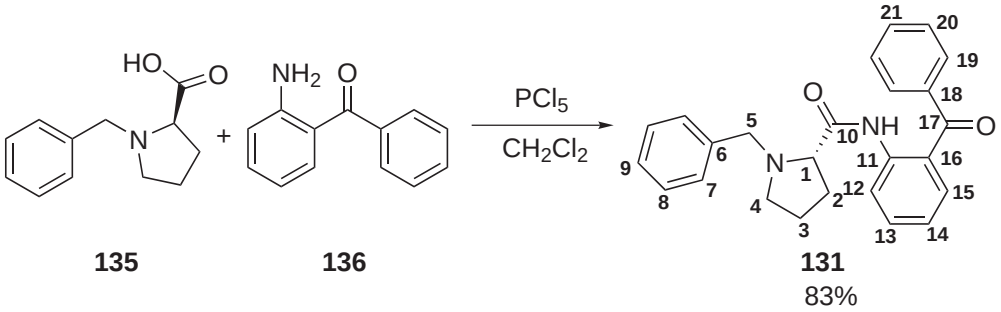
δ_C (75 MHz; $CDCl_3$): 173.4 (C^{10}), 139.8, 129.0, 127.5, 126.7, 71.6 (C^1), 67.1 (C^5), 56.1 (C^4), 53.7 (C^2), 47.6 (C^3);

m/z (+ EI) found $[M]^+$ 205 (100 %);

mp 169 °C (lit. 167 °C[309]);

ν_{max}/cm^{-1} 2986 (O-H), 1671 (C=O), 1498 (C-C).

7.4.12 (*S*)-*N*-(2-Benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide (131)



Phosphorus pentachloride (6.90 g, 33.1 mmol) was added at 0 °C to a solution of (*S*)-1-benzyl-pyrrolidine-2-carboxylic acid, **135**) (9.00 g, 43.8 mmol) in dichloromethane (10 mL) and the mixture stirred for 10 min. 2-Aminobenzophenone, **136**, (6.51 g, 33.0 mmol) was then added slowly and the mixture stirred at room temperature for 15 hours. The reaction mixture was then concentrated under reduced pressure and treated successively with acetone (20 mL) and water. The crystals formed were dissolved in concentrated HCl (5 mL) and diluted with H_2O and the precipitate of the pure hydrochloride salt filtered, washed with water (5 mL) and dried in the air. This gave (*S*)-*N*-(2-Benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide, **131**, (10.4 g, 27.1 mmol, 83 %) as a crystalline orange solid.[273]

δ_H (300 MHz; $CDCl_3$): 7.62–7.31 (13H, m, Ar-CH), 6.93 (1H, d, *J*=7.9 Hz, C^9H), 4.39 (1H, d, *J*=12.8 Hz, C^4HH), 4.17–3.98 (2H, m, C^4HH, C^1H), 3.69–3.55 (1H, m, C^5HH), 3.21 (1H, q, *J*=7.4 Hz, C^5HH), 2.07–1.93 (2H, m, C^3H_2), 1.73 (1H, td, *J*=6.8 Hz, 16.0 Hz,

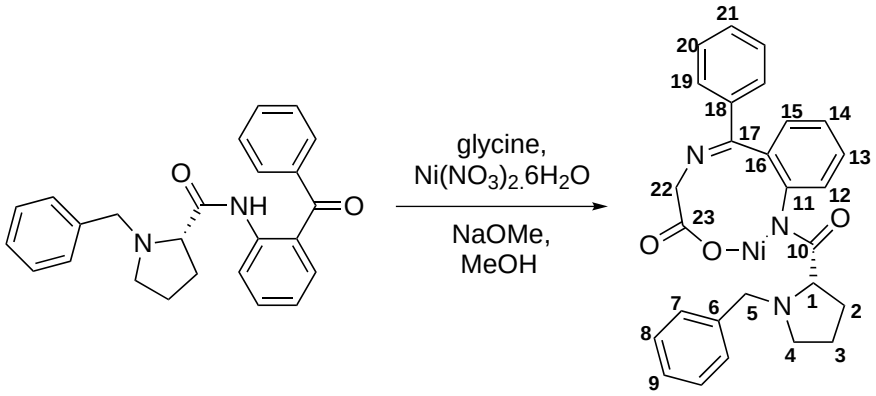
C^2HH), 0.97 (1H, td, *J*=6.8Hz, 16.0 Hz, C^2HH);

m/z (+ EI) found $[M]^+$ 384.1838. $C_{25}H_{24}N_2O_2$ requires $[M]^+$ 384.1838;

mp 113 °C (lit. 114 °C[272]);

ν_{max}/cm^{-1} 2953 (N-H), 1661 (C=O), 1606 (Aromatic C=C), 1485 (Aromatic C=C).

7.4.13 (*S*)-(2-[1-(Benzyl)pyrrolidine-2-carboxamide]phenyl-phenyl -methylene)-glycinato-*N,N',N'',O*-nickel(II) (132)



131 **132** 51%

A solution of 25% NaOMe in MeOH (50 mL) was added to a solution containing (*S*)-*N*-(2-Benzoylphenyl)-1-benzyl-pyrrolidine-2-carboxamide, **131**, (4.89 g, 11.7 mmol), glycine (1.50 g, 20.0 mmol) and $Ni(NO_3)_2.6H_2O$ (5.82 g, 25.4 mmol) in MeOH (15 mL). The mixture was stirred for 2 hours at 60 °C. After the mixture had cooled it was neutralized with glacial acetic acid to pH 5-6 and diluted with water. The precipitate was filtered off and recrystallized from hexane/$CHCl_3$ to give (*S*)-(2-[1-(benzyl)pyrrolidine-2-carboxamide]phenyl-phenylmethylene)-glycinato-*N,N',N'',O*-nickel(II), **132**, (3.18 g, 6.0 mmol, 51 %) as a crystalline red solid.[273]

δ_H (300 MHz; $CDCl_3$): 8.44 (1H, d, *J*=8.3 Hz), 8.07 (2H, d, *J*=8.3 Hz), 7.48–7.29 (5H, m), 7.25 (2H, t, *J*=8.3 Hz), 7.15 (1H, t, *J*=7.7 Hz), 7.06 (1H, t, *J*=7.7 Hz), 6.76 (1H, t, *J*=7.7 Hz), 6.63 (1H, t, *J*=7.7 Hz), 4.39 (2H, d, *J*=12.6 Hz, $C^{22}H_2$), 3.56 (2H, d, *J*=8.0 Hz, C^4H_2), 2.57–2.44 (2H, m, C^5H_2), 2.40 (1H, t, *J*=8.7 Hz, C^1H), 2.23–2.02 (4H, m, C^2H_2, C^3H_2);

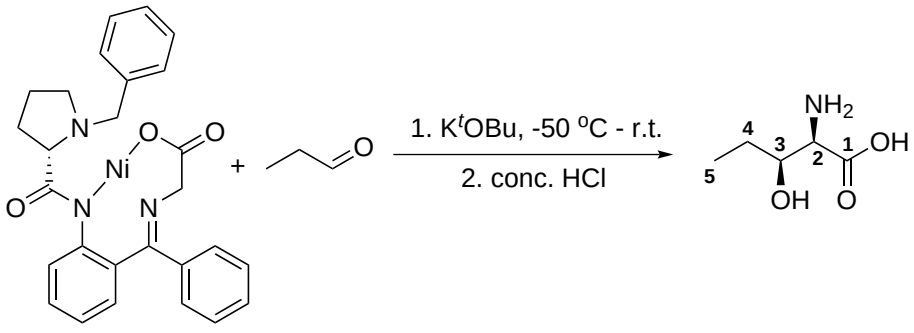
δ_C (75 MHz; $CDCl_3$): 181.5 (C^{23}), 177.7 (C^{10}), 171.8 (C^9), 142.4, 133.2, 132.2, 131.7, 129.8, 129.7, 129.4, 129.1, 128.9, 126.3, 125.7, 125.2, 124.3, 121.0, 70.1 (C^1), 63.3 (C^4), 61.2 (C^5), 57.7 (C^{22}), 30.7 (C^2), 23.7 (C^3);

m/z (+ CI-Methane) found $[MH]^+$ 498.1318. $C_{27}H_{24}ClN_3NiO_3$ requires $[MH]^+$ 498.1328;

mp 207 °C (lit. 209–213 °C[273]);

ν_{max}/cm^{-1} 3333 (N-H), 2924 (Aromatic C-H), 1630 br (C=O), 1589 (Aromatic C=C), 1471 (Aromatic C=C).

7.4.14 (*2R,3S*)-3-Hydroxy-norvaline (*syn*-126)



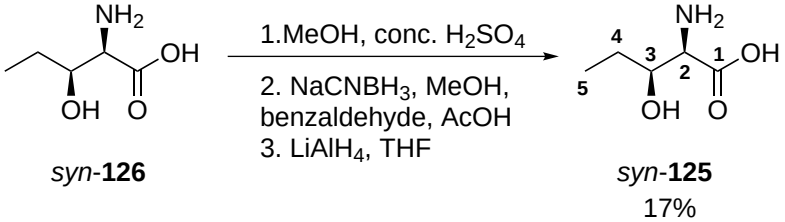
132 **122** *syn*-**126**
6%

(*S*)-(2-[1-(benzyl)pyrrolidine-2-carboxamide]phenyl-phenylmethylene)-glycinato-*N,N',N'',O*-nickel(II), **132**, (0.18 g, 0.4 mmol) was added to a mixture of potasium ^{t}butoxide (0.80

g,7.1 mmol) in anhydrous THF (50 mL) at -50 °C. The reaction was stirred for 15 min. and freshly distilled propanal, **122**, (0.81 g, 1.0 mL, 13.9 mmol) added dropwise. The mixture was warmed to room temperature and stirred for 40 min, and then cooled to 0 °C and quenched by adding glacial acetic acid (5 mL) in H_2O (100 mL). The product was extracted with $CHCl_3$ (10 x 50 mL) and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in methanol (60 mL) and 2 M HCl (40 mL) added. The mixture was stirred at reflux for 3 hours, and then cooled to room temperature and evaporated under reduced pressure. H_2O (30 mL) was added and the pH adjusted to pH 9–10 using conc ammonium solution. The mixture was extracted with $CHCl_3$ (10 x 50 mL) and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by cation exchange chromatography (Dowex 50 X 8 200 mesh; elution by 2M NH_4OH) to give 3-hydroxy-norvaline, *syn*-**126**, (28.9 mg, 0.02 mmol, 6%) as an off-white solid.[267]

δ_H (300 MHz; D_2O): 3.55 (1H, m, C^3H), 2.58 (1H, m, C^2H), 1.37 (2H, m, C^4H_2), 0.86 (3H, t, J=4.5 Hz, C^5H_3);
All other characterization data was the same as that described on page 284.

7.4.15 (*2R,3S*)-2-(Benzylamino)pentane-1,3-diol (*syn*-125)



syn-**126**

syn-**125**
17%

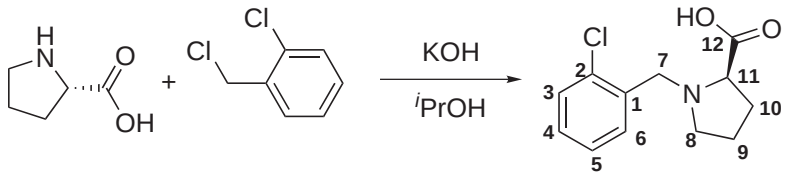
3-Hydroxy-norvaline, *syn*-**126**, (28.9 mg, 0.02 mmol) was dissolved in methanol (2 mL) at 0 °C and concentrated sulfuric acid (1 mL) added dropwise. The mixture was stirred at reflux for 15 hours and then neutralized with sodium hydrogen carbonate. The mixture was then extracted with dichloromethane (3 x 100 mL) and the combined organic extracts washed with saturated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was then dissolved in methanol (20 mL) and freshly distilled benzaldehyde (0.14 g, 0.14 mL, 1.4 mmol) added to the mixture. Glacial acetic acid (2.0 mL) was then added to the reaction and stirred for 1 hour. Sodium cyanoborohydride (0.09 g, 1.4 mmol) was added in one portion and the mixture stirred at room temperature for 15 hours. The mixture was then neutralized with sodium hydrogen carbonate and extracted with dichloromethane (3 x 100 mL). The combined organic extracts washed with saturated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was dissolved in anhydrous THF (20 mL), cooled to 0 °C and $LiAlH_4$ (0.05 g, 1.4 mmol) added. The reaction was warmed to room temperature and the reaction stirred for 15 hours. Sodium sulfate decahydrate (0.65 g, 2.0 mmol) was added in one portion and the reaction stirred for 1 hour at room temperature H2O (20 mL) was then added and the mixture extracted with dichloromethane (10 x 100 mL) and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: methanol:ethyl acetate, 1:9) to give pure 2-(benzylamino)pentane-1,3-diol, *syn*-**125**, (0.08 mg, 0.004 mmol, 17%) as a pale yellow oil.[126]

δ_H (300 MHz; $CDCl_3$): 7.61–7.30 (5H, m, C_6H_5), 3.95–3.64 (4H, m, C^1H_2, C^6H_2), 3.57–3.43 (1H, m, C^3H), 2.98–2.79 (1H, m, C^2H), 1.26–1.13 (2H, m, C^4H_2), 0.98 (3H, t, J=7.4 Hz, C^5H_3);

All other characterization data was the same as that described on page 278.

HPLC analysis indicated that the product was formed selectively as the *syn* diastereomer (71:50, *syn:anti*).

7.4.16 (*S*)-1-(2-Chlorobenzyl)pyrrolidine-2-carboxylic acid (139)



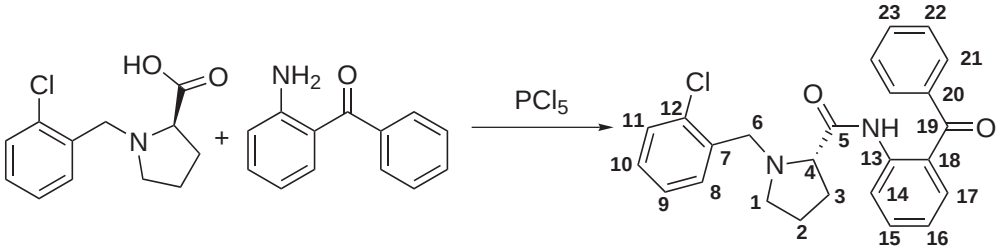
133 **138** **139**
88%

(*S*)-Proline, **133**, (10.20 g, 88.6 mmol) was added to a solution of KOH (15.12 g, 269.5 mmol) in Pr^{i}OH (40 mL), and the mixture was stirred at 40–50 °C until the compound completely dissolved. The solution was then cooled to 0 °C and freshly distilled 2-chlorobenzyl chloride, **138**, (15.00 g, 93.15 mmol) was added dropwise over a period of 30 min, and the mixture was stirred for an additional 15 hours at room temperature The reaction was monitored by TLC ($CHCl_3$:ethanol, 1:1). Then the reaction mixture was neutralized with 6 M HCl to pH 5–6, diluted with $CHCl_3$ (25 mL) and allowed to stand for 4 hours. The precipitated salts were filtered off and washed with $CHCl_3$. The filtrate was concentrated, and the residue was treated with Me_2CO. The precipitate was filtered off, washed with Pr*i*OH, and dried with P_2O_5 to give (*S*)-1-(2-chlorobenzyl)pyrrolidine-2-carboxylic acid, **139**, (18.75 g, 78.2 mmol, 88%) as a yellow solid.[273]

δ_H (300 MHz; $CDCl_3$): 7.48–7.21 (4H, m, Ar H), 4.65 (2H, s, C^7H_2), 4.27 (1H, t, *J*=7.07 Hz, $C^{11}H$), 3.72 (1H, q, *J*=6.4 Hz, C^8HH), 3.15 (1H, q, *J*=6.4 Hz, C^8HH), 2.53 (1H, sx, *J*=3.7 Hz, $C^{10}HH$), 2.35 (1H, sx, *J*=6.2 Hz, $C^{10}HH$), 2.08 (2H, sx, *J*=3.8 Hz, C^9H_2)
δ_C (75 MHz; $CDCl_3$): 170.8, 135.1, 130.1, 128.3, 127.8, 76.6, 66.9. 28.5, 22.6;
m/z (+ EI) found $[M]^+$ 239.0653. $C_{12}H_{14}ClNO_2$ requires $[M]^+$ 239.0713;
mp 160 °C (lit. 160–162 °C[276]);

ν_{max}/cm^{-1} 2925 (O-H), 1721 (C=O), 1444 (C-C);

7.4.17 (*S*)-*N*-(2-Benzoylphenyl)-1-(2-chlorobenzyl)-pyrrolidine-2-carboxamide (137)



139 **136** **137**
57%

Phosphorus pentachloride (6.90 g, 33.1 mmol) was added at 0 °C to a solution of (*S*)-1-(2-chlorobenzyl)-pyrrolidine-2-carboxylic acid, **139**, (9.00 g, 37.5 mmol) in dichloromethane (10 mL) and the mixture stirred for 10 min. 2-Aminobenzophenone, **136**, (6.51 g, 33.0 mmol) was then added slowly and the mixture stirred at room temperature for 15 hours. The reaction mixture was then concentrated under reduced pressure and treated successively with acetone (20 mL) and water. The crystals formed were dissolved in conc HCl (5 mL) and diluted with H_2O and the precipitate of the pure hydrochloride salt filtered, washed with water (5 mL) and dried in the air to give pure (*S*)-*N*-(2-Benzoylphenyl)-1-(2-chlorobenzyl)pyrrolidine-2-carboxamide, **137**, (7.50 g, 17.9 mmol, 57%).[273]

δ_H (300 MHz; $CDCl_3$): 7.72–7.58 (8H, m, Ar), 7.31–7.25 (3H, m, Ar), 6.98 (2H, d, *J*=8.0 Hz, $C^{21}H$), 4.42 (2H, d, *J*=13.2 Hz, C^6H_2), 3.75–3.63 (1H, m, NC*H*), 2.22–1.86 (4H, m, C^1H_2, C^3*H*H, C^2*H*H), 1.79–1.71 (1H, m, C^2H*H*), 1.13–0.97 (1H, m, C^3H*H*);

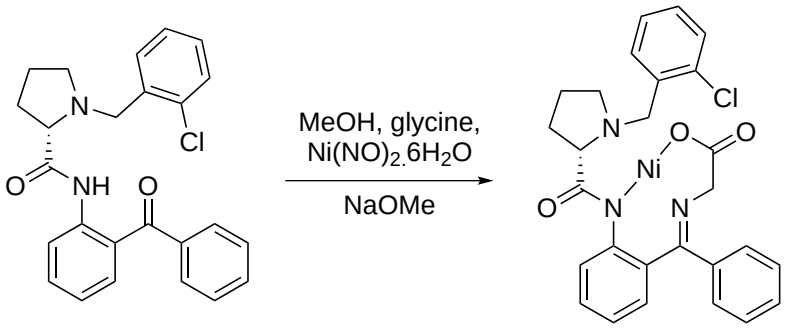
δ_C (75 MHz; $CDCl_3$): 199.1, 168.5, 151.5, 151.0, 140.2, 137.5, 134.6, 134.0, 133.5, 131.5, 129.8, 129.1, 128.5, 128.1, 127.0, 126.3, 121.8, 118.1, 117.0, 115.5, 71.2, 54.3, 32.0, 23.1, 21.8;

m/z (+ CI-Methane) found $[MH]^+$ 419.1500. $C_{22}H_{23}ClN_2O_2$ requires $[MH]^+$ 419.1526; *m/z* (ESI) found [M + Na] 441.1369. $C_{22}H_{23}ClN_2O_2$ requires [M + Na] 441.1346;

mp 201–205 °C (lit. 203–205 °C[276]);

ν_{max}/cm^{-1} 2921 (N-H), 1662 (C=O), 1578 (Aromatic C=C), 1447 (Aromatic C=C).

7.4.18 (*S*)-(2-[1-(2-Chlorobenzyl)pyrrolidine-2-carboxamide]phenyl-phenylmethylene)- glycinato-*N,N',N'',O*-nickel(II) (140)



137 **140** 51%

A solution of 25% NaOMe in MeOH (50 mL) was added to a solution containing (*S*)-*N*-(2-Benzoyl phenyl)-1-(2-chlorobenzyl)pyrrolidine-2-carboxamide, **137**, (4.89 g, 11.7 mmol), glycine (1.50 g, 20.0 mmol) and $Ni(NO_3).6H_2O$ (5.82 g, 25.4 mmol) in MeOH (15 mL). The mixture was stirred for 2 hours at 60 °C. After the mixture had cooled it was neutralized with glacial acetic acid to pH 5–6 and diluted with water. The precipitate was filtered off and recrystallized from hexane/$CHCl_3$ to give (*S*)-(2-[1-(2-Chlorobenzyl)pyrrolidine-2-

carboxamide]phenyl-phenylmethylene)-glycinato-*N,N',N'',O*-nickel(II), **140**, (3.18 g, 6.0 mmol, 51%) as a yellow crystalline solid.[273]

δ_H (300 MHz; $CDCl_3$): 7.71–7.56 (8H, m, Ar), 7.34–7.26 (3H, m, Ar), 6.98 (2H, d, *J*=8.0 Hz, $C^{21}H$), 4.42 (2H, d, *J*=13.2 Hz, C^6H_2), 4.16 (1H, t, $C^{24}HH$), 3.73–3.63 (1H, m, NC*H*), 3.32 (1H, t, $C^{24}HH$), 2.19–1.83 (4H, m, C^1H_2, C^3HH, C^2HH), 1.79–1.66 (1H, m, C^2HH), 1.07–0.98 (1H, m, C^3HH);

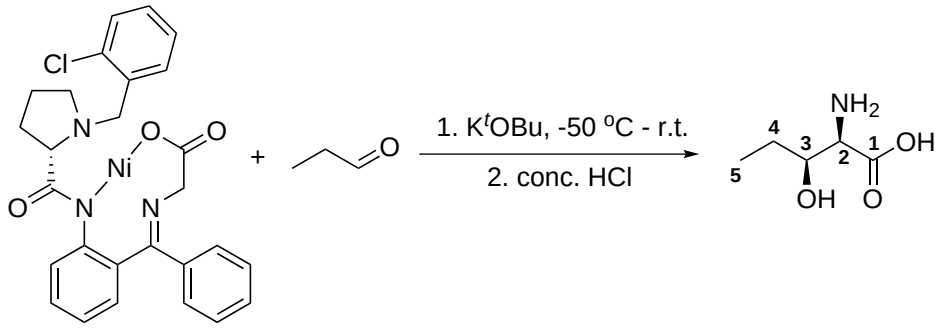
δ_C (75 MHz; $CDCl_3$): 168.5, 166.9, 151.5, 136.8, 134.0, 133.7, 133.4, 131.5, 130.1, 129.8, 129.1, 128.9, 128.5, 128.3, 128.1, 127.8, 127.0, 126.9, 126.3, 125.3, 121.8, 71.2, 55.2, 54.3, 32.0, 23.1, 21.5;

m/z (+ CI-Methane) found $[MH]^+$ 532.0933. $C_{27}H_{24}ClN_3NiO_3$ requires $[MH]^+$ 532.0938;

mp 190 °C (lit. 186–188 °C[276]);

ν_{max}/cm^{-1} 3336 br (N-H), 2923 (Aromatic C-H), 1614 br (C=O), 1578 (Aromatic C=C), 1471 (Aromatic C=C).

7.4.19 (*2R,3S*)3-Hydroxy-norvaline (*syn*-126)



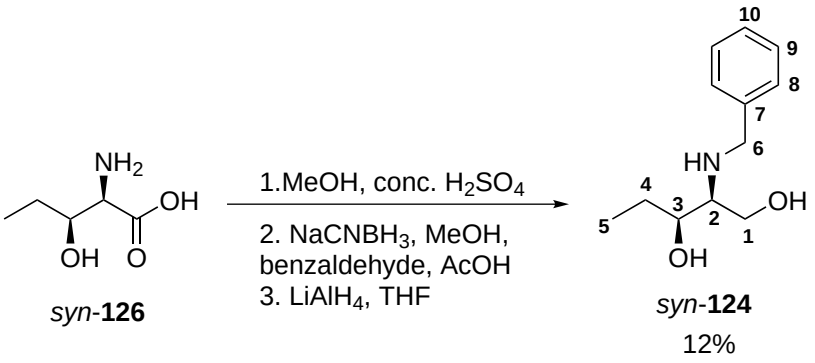
140 **122** *syn*-**126** 12%

(*S*)-(2-[1-(2-Chlorobenzyl)pyrrolidine-2-carboxamide]phenyl-phenylmethylene)-glycinato-*N,N',N'',O*-nickel(II), **140**, (1.00 g, 1.9 mmol) was added to a mixture of potasium ^{t}butoxide (0.80 g,7.1 mmol) in anhydrous THF (50 mL) at -50 °C. The reaction was stirred for 15 min. and freshly distilled propanal, **122**, (0.81 g, 1.0 mL, 13.9 mmol) added dropwise. The mixture was warmed to room temperature and stirred for 40 min, and then cooled to 0 °C and quenched by adding glacial acetic acid (5 mL) in H_2O (100 mL). The product was extracted with $CHCl_3$ (10 x 50 mL) and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in methanol (60 mL) and 2 M HCl (40 mL) added. The mixture was stirred at reflux for 3 hours, and then cooled to room temperature and evaporated under reduced pressure. H_2O (30 mL) was added and the pH adjusted to pH 9–10 using conc ammonium solution. The mixture was extracted with $CHCl_3$ (10 x 50 mL) and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by cation exchange chromatography (Dowex 50 X 8 200 mesh; elution by 2M NH_4OH) to give 3-hydroxy-norvaline, *syn*-**126**, (0.03 g, 0.2 mmol, 12%) as an off-white solid.[267]

The characterization data was the same as that described on page 290.

7.4.20 (*2R,3S*)-2-(Benzylamino)pentane-1,3-diol (*syn*-124)

3-Hydroxy-norvaline, *syn*-**126**, (0.03 g, 0.2 mmol) was dissolved in methanol (10 mL) at 0 °C and concentrated sulfuric acid (5 mL) added dropwise. The mixture was stirred at reflux for 15 hours and then neutralized with sodium hydrogen carbonate. The mixture was then extracted with dichloromethane (3 x 100 mL) and the combined organic extracts washed



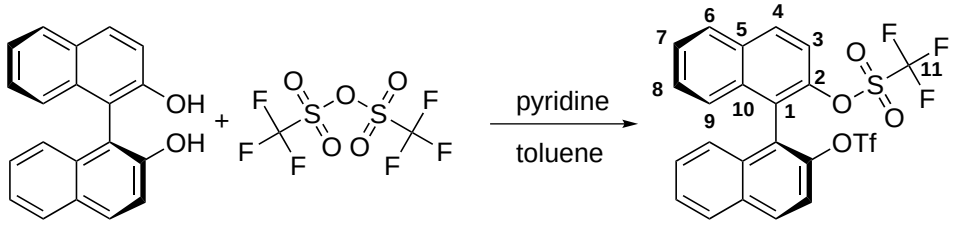
syn-**126** *syn*-**124** 12%

with saturated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was then dissolved in methanol (20 mL) and freshly distilled benzaldehyde (1.43 g, 1.4 mL, 13.5 mmol) added to the mixture. Glacial acetic acid (2.0 mL) was then added to the reaction and stirred for 1 hour. Sodium cyanoborohydride (0.85 g, 13.5 mmol) was added in one portion and the mixture stirred at room temperature for 15 hours. The mixture was then neutralized with sodium hydrogen carbonate and extracted with dichloromethane (3 x 100 mL). The combined organic extracts washed with saturated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was dissolved in anhydrous THF (20 mL), cooled to 0 °C and $LiAlH_4$ (0.50 g, 13.5 mmol) added. The reaction was warmed to room temperature and the reaction stirred for 15 hours. Sodium sulfate decahydrate (6.50 g, 20.3 mmol) was added in one portion and the reaction stirred for 1 hour at room temperature H2O (20 mL) was then added and the mixture extracted with dichloromethane (10 x 100 mL) and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: methanol:ethyl acetate, 1:9) to give pure 2-(benzylamino)pentane-1,3-diol, *syn*-**124**, (5.02 mg, 0.03 mmol, 12%) as a pale yellow oil.[126,278,279]

The characterization data was the same as that described on page 292.

HPLC analysis indicated that the product was formed selectively as the *syn* diastereomer (48:25, *syn:anti*).

7.4.21 (*S*)-1,1'-Bi-2-naphthol-*bis*(trifluoromethanesulfonate) (143)



141 **142** **143**
81%

Under an inert atmosphere of argon, trifluoromethanesulfonic anhydride, **142**, (4.93 g, 5.0 mL, 17.5 mmol) was added dropwise to a stirred and ice cooled solution of (*S*)-1,1'-bi-2-naphthol, **141**, (2.00 g, 7.0 mmol) and pyridine (2.21 g, 2.3 mL, 27.9 mmol) in toluene (14 mL) over 40 min at 2 °C–9 °C. After addition was complete, the cooling bath was removed and the mixture stirred at room temperature for 3 hours. Toluene (10 mL), H_2O (10 mL) and 35% aqueous HCl (3 mL) were added in sequence at room temperature The layers were separated and the toluene layer washed with H_2O (2 x 10 mL) and saturated sodium chloride solution (10 mL). The toluene solution was dried ($MgSO_4$) and concentrated under reduced pressure to give crude (*S*)-1,1'-bi-2-naphthol *bis*(trifluoromethanesulfonate), **143**, (3.13 g, 5.7 mmol, 81%) as a pink solid.[310]

δ_H (300 MHz; $CDCl_3$): 8.20 (2H, d, *J*=9.3 Hz), 8.07 (2H, d, *J*=8.4 Hz), 7.75 (2H, d, *J*=9.3 Hz), 7.65 (2H, ddd, *J*=1.2 Hz, 6.8 Hz, 8.4 Hz), 7.48 (2H, ddd, *J*=1.2 Hz, 6.8 Hz, 8.4 Hz), 7.37–7.27 (m, 2H);

δ_C (75 MHz; $CDCl_3$): 145.6, 133.3, 132.5, 132.1, 129.2, 128.5, 128.4, 128.1, 127.4, 126.8, 125.4 (q *J*=270 Hz);

m/z (+ EI) 551 (M(^{13}C), 24%), 550 (M, 100), 417 (M-CO_2F_3S, 34), 284 (M-$C_2O_4F_6S_2$, 57), 142 (M-$C_{12}H_6O_4F_6S_2$, 8); Found (+HRES) $[M]^+$ 549.9987. $C_{22}H_{12}F_6O_6S_2$ requires $[M]^+$

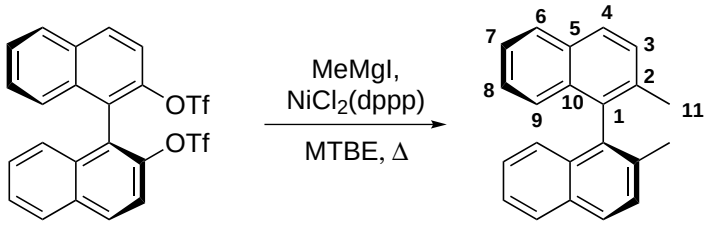
549.9980;

ν_{max}/cm^{-1} 3066 (Aromatic C-H), 1623 (Aromatic C=C), 1403 (Aromatic C=C);

mp 73 °C (lit. 64–74 °C[310]);

Optical Rotation $[\alpha]^{25}{}_D$=+166.6 (*c* 0.85, $CHCl_3$).

7.4.22 (*R*)-2,2'-Dimethyl-1,1'-binaphthyl (144)



143 **144** 60%

Under an atmosphere of argon, a solution of MeI (0.78 g, 0.4 mL, 5.5 mmol) in *tert*-butyl methyl ether (MTBE, 0.8 mL) was added dropwise to a stirred suspension of Mg turnings (132 mg, 5.5 mmol) in MTBE (1.4 mL), that had been stirring previously overnight. The mixture was allowed to stir at room temperature until formation of the Grignard reagent was complete. MTBE (1 mL) and $NiCl_2$(dppp) (50 mg, 0.09 mmol) were added in sequence. Then a solution of crude (*S*)-1,1'-Bi-2-naphthol *bis*(trifluoromethanesulfonate), **143**, (1.00 g, 1.8 mmol) in MTBE (4 mL) was added and the mixture stirred and heated under reflux (55 °C) for 45 min. The mixture was allowed to cool to room temperature and toluene (6 mL) added. The mixture was poured into ice-chilled water (10 mL) and 35% aqueous HCl (1.0 mL) added. The layers were separated and the organic layer washed with H_2O (2 x 6 mL) and saturated sodium chloride solution (6 mL). The organic solution was dried ($MgSO_4$), concentrated under reduced pressure and the crude product purified by flash sil-

ica column chromatography (eluent: hexane) to give (*R*)-2,2'-Dimethyl-1,1'-binaphthyl, **144**, (0.31 g, 1.1 mmol, 60 %) as a colourless crystalline solid.[310]

δ_H (300 MHz; $CDCl_3$): 7.89 (4H, dd, *J*=4.5 Hz, 8.1 Hz), 7.52 (2H, d, *J*=7.5 Hz), 7.47–7.34 (2H, m), 7.29–7.11 (2H, m), 7.05 (2H, d, *J*=8.1 Hz), 2.04 (6H, s, $C^{11}H_3$);

δ_C (75 MHz; $CDCl_3$): 134.3, 132.8, 132.2, 128.7, 127.9, 127.4, 127.1, 126.1, 125.6, 124.9, 20.1 (C^{11});

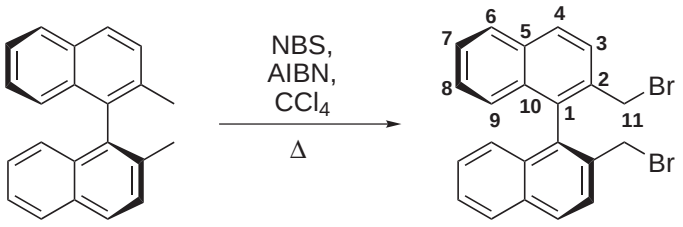
m/z (+ EI) 283 (M(^{13}C), 24%), 282 (M, 100), 267 (M-CH_3, 41), 252 (M-C_2H_6, 23); Found (+HRES) $[M]^+$ 282.1394. $C_{22}H_{18}$ requires $[M]^+$ 282.1408;

ν_{max}/cm^{-1} 3054 (Aromatic C-H), 1591 (Aromatic C=C), 1507 (Aromatic C=C);

mp 80 °C (lit. 77–79 °C[310]);

Optical Rotation $[\alpha]^{25}_D$=+155.2 (*c* 0.25, $CHCl_3$).

7.4.23 (*S*)-2,2'-*Bis*(bromomethyl)-1,1'-binaphthyl (145)



144 **145** 68%

To a suspension of (*R*)-2,2'-Dimethyl-1,1'-binaphthyl, **144**, (1.00 g, 3.5 mmol) in CCl_4 (7.0 mL) was added *N*-bromosuccinimide (NBS, 1.39 g, 7.8 mmol) followed by 2,2'-azobisisobutyronitrile (AIBN, 0.03 g, 0.2 mmol) at room temperature The mixture was stirred and heated at reflux for 2 hours, during which time the reaction's progress was

monitored by TLC (ethyl acetate:hexane, 1:10). On consumption of the starting material the mixture was allowed to cool to room temperature and ethyl acetate (2.5 mL) added with stirring. The mixture was poured into H_2O (14.5 mL). The biphasic mixture was stirred for no longer than an hour, after which time crystals had ceased to precipitate. Crystalline product was collected by vacuum filtration and air-dried overnight, to obtain (*S*)-2,2'-*bis*(bromomethyl)-1,1'-binaphthyl, **145**, (1.06 g, 2.4 mmol, 68 %) as a crystalline solid.[310]

δ_H (500 MHz; $CDCl_3$): 8.03 (2H, d, *J*=8.6 Hz), 7.93 (2H, d, *J*=8.1 Hz), 7.77 (2H, d, *J*=8.6 Hz), 7.49 (2H, t, *J*=7.0 Hz), 7.27 (2H, t, *J*=6.9 Hz), 7.08 (2H, d, *J*=8.4 Hz), 4.26 (4H, s, $C^{1}1H_2$);

δ_C (75 MHz; $CDCl_3$): 134.2, 134.1, 133.3, 132.5, 129.4, 128.0, 127.8, 127.4, 127.2, 126.8, 32.7 (C^{11});

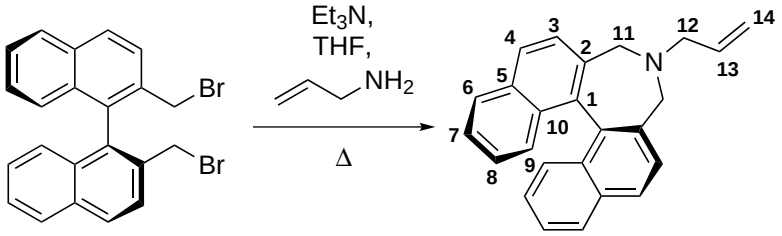
m/z (+ CI-Methane) 442 ($M^{81}Br_2$, 19%), 440 ($M^{81}Br^{79}Br$, 38) 439 (M(^{13}C), 8), 438 ($M^{79}Br_2$, 19), 359 (M-Br, 69), 280 (M-Br_2, 50), 266 (M-CH_3Br_2, 100), 252 (M-$C_2H_6Br_2$, 16); Found (+HRES) $[M]^+$ 437.9619. $C_{22}H_{16}Br_2$ requires $[M]^+$ 437.9619;

ν_{max}/cm^{-1} 3055 (Aromatic C-H), 1595 (Aromatic C=C), 1508 (Aromatic C=C);

mp 175 °C (lit. 178–181 °C[310]);

Optical Rotation $[\alpha]^{25}{}_D$=+161.0 (*c* 0.50, $CHCl_3$).

7.4.24 (*S*)-4-Allyl-4,5-dihydro-3*H*-dinaphtho[2,1-c:1,2-e]azepine (146)



145 **146** 53%

Under an atmosphere of argon, allylamine (0.15 g, 0.2 mL, 2.6 mmol) was added to a stirred solution of (*S*)-2,2'-*bis*(bromomethyl)-1,1'-binaphthyl, **145**, (0.5 g, 1.1 mmol) and Et_3N (0.29 g, 0.4 mL, 2.9 mmol) in THF (10 mL) at room temperature The mixture was stirred and heated at 55 °C for 3 hours and the completion of the reaction confirmed by TLC (ethyl acetate:hexane, 1:4; R_f=0.56 for starting material; R_f=0.11 for product). Toluene (10 mL) was added to the mixture followed by 1M aqueous sodium hydroxide solution (10 mL). The layers were then separated and the organic layer washed with H_2O (3 x 10 mL) and saturated sodium chloride solution (10 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was suspended in acetone (1 mL), rinsed with stirring and the precipitated crystals collected by suction filtration and air-dried overnight to give (*S*)-4-allyl-4,5-dihydro-3*H*-dinaphtho[2,1-c:1,2-e]azepine, **146**, (0.36 g, 1.1 mmol, 94%) as a crystalline solid.[310]

δ_H (500 MHz; $CDCl_3$): 7.98 (4H, d, *J*=8.1 Hz, C^6H, C^9H), 7.56 (2H, d, *J*=14.5 Hz, C^4H), 7.53 (2H, d, *J*=14.5 Hz, C^3H), 7.47 (2H, d, *J*=14.5Hz, C^8H), 7.27 (2H, t, *J*=6.8 Hz, C^7H), 6.07–5.82 (1H, m, $C^{13}H$), 5.33 (1H, t, *J*=10.1 Hz, $C^{14}HH$), 5.28 (1H, d, *J*=10.2 Hz, $C^{14}HH$), 3.74 (2H, d, *J*=6.0 Hz, $C^{11}HH$), 3.21–3.03 (4H, m, $C^{11}HH$, $C^{12}H_2$);

δ_C (75 MHz; $CDCl_3$): 136.9, 135.4, 134.1, 133.6, 131.8, 128.6, 128.1, 127.7, 126.1, 125.8, 119.3 (C^{13}), 117.8 (C^{14}), 58.7 (C^{11}), 55.1 (C^{12});

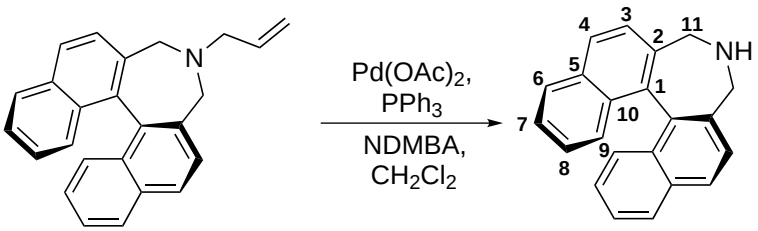
m/z (+ CI-Methane) 337 (M(^{13}C)+H, 27%), 336 (M+H, 100), 296 (M-C_3H_5, 7), 281 (M-C_3H_5N, 70), 268 (M-C_4H_5N, 91), 252 (M-C_5H_11N, 28); Found (+HRES) $[MH]^+$ 336.1757. $C_{25}H_{22}N$ requires $[MH]^+$ 336.1752;

ν_{max}/cm^{-1} 3049 (Aromatic C-H), 1642 (C=C) 1594 (Aromatic C=C), 1508 (Aromatic C=C);

mp 180 °C (lit. 177–178 °C[310]);

Optical Rotation $[\alpha]^{25}{}_D$=+192.4 (*c* 0.25, $CHCl_3$).

7.4.25 (*S*)-4,5-Dihydro-3*H*-4-azacyclohepta[2,1-*a*;3,4-*a'*] dinaphthalene (147)



146 **147** 87%

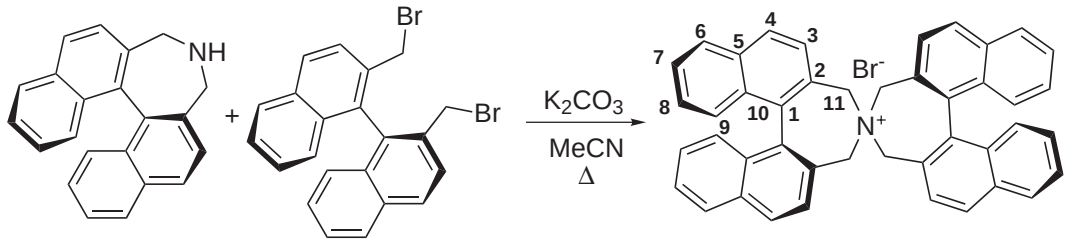
A mixture of (*S*)-4-allyl-4,5-dihydro-3*H*-dinaphtho[2,1-c:1,2-e]azepine, **146**, (0.50 g, 1.5 mmol) *N,N*-dimethylbarbituric acid (NDMBA) (0.60 g, 3.8 mmol), $Pd(OAc)_2$ (10 mg, 3 mol%) and triphenylphosphine (35 mg, 0.1 mmol) in anhydrous, degassed dichloromethane (10 mL) was heated to 35 °C and stirred overnight under an argon atmosphere. After cooling, dichloromethane was removed under reduced pressure and replaced by benzene (10 mL). The mixture was then washed with sat. aqueous sodium hydrogen carbonate solution

(2 x 10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: methanol:dichloromethane, 1:10) to give (*S*)-4,5-dihydro-3*H*-4-azacyclohepta[2,1-*a*;3,4-*a'*]dinaphthalene, **147**, (0.38 g, 1.3 mmol 87%) as a crystalline solid.[278]

δ_H (300 MHz; $CDCl_3$): 7.98 (2H, d, *J*=8.3 Hz), 7.95 (2H, d, *J*=8.3 Hz), 7.58 (2H, d, *J*=8.3 Hz), 7.45 (4H, d, *J*=8.3 Hz), 7.27 (2H, d, *J*=8.3 Hz), 3.89 (2H, d, *J*=11.0 Hz, $C^{11}H_2$), 3.55 (2H, d, *J*=11.0 Hz, $C^{11}H_2$);
δ_C (75 MHz; $CDCl_3$): 135.1, 133.7, 133.4, 131.5, 129.2, 128.4, 127.4, 127.2, 126.1, 125.8, 50.5 (C^11);
m/z (FAB) 296 (M(^{13}C), 24%), 295 (M, 100), 280 (M-CH_3N, 25), 268 (M-C_2H_5N, 15); Found (+HRES) $[M]^+$ 295.1357. $C_{22}H_{17}N$ requires $[M]^+$ 295.1361;
ν_{max}/cm^{-1} 3363 (N-H), 3053 (Aromatic C-H), 1571 (Aromatic C=C), 1509 (Aromatic C=C);
mp 74–79 °C (lit. 147–149 °C[311]);
Optical Rotation $[\alpha]^{25}{}_D$=+189.2 (*c* 0.15, $CHCl_3$).

7.4.26 (*S,S'*)-3,3',5,5'-Tetrahydro-4,4'-spirobi[4*H*-di-naphtho[2,1-*c*:1',2'-*e*]azepinium] bromide (148)

A mixture of (*S*)-4,5-Dihydro-3*H*-4-azacyclohepta[2,1-*a*;3,4-*a'*]dinaphthalene, **147**, (0.20 g, 0.7 mmol), (*S*)-2,2'-*bis*(bromomethyl)-1,1'-binaphthyl, **145**, (0.35 g, 0.8 mmol), and K_2CO_3 (0.15 g, 1.1 mmol) in acetonitrile (10 mL) was heated to reflux, and stirring was maintained for 15 hours. The resulting mixture was poured into water and extracted with dichloromethane. The organic extracts were dried (Na_2SO_4) and concentrated under re-



147 **145** **148**
56%

duced pressure. The residue was purified by flash silica column chromatography (eluent: MeOH:dichloromethane, 1:30) to furnish (*S,S'*)-3,3',5,5'-Tetrahydro-4,4'-spirobi[4*H*-di-naphtho[2,1

-*c*:1',2'-*e*]azepinium] bromide, **148**, (0.25 g, 0.4 mmol, 56%) as a crystalline red solid.[278]

δ_H (300 MHz; $CDCl_3$): 8.35 (4H, d, *J*=8.3 Hz), 8.12 (4H, d, *J*=8.3 Hz), 8.08 (4H, d, *J*=8.3 Hz), 7.60 (4H, t, *J*=6.7 Hz), 7.41 (4H, d, *J*=8.3 Hz), 7.37 (4H, d, *J*=6.7 Hz), 4.45 (4H, d, *J*=13.2 Hz, $C^{11}HH$), 3.87 (4H, d, *J*=13.2 Hz, $C^{11}HH$);

δ_C (75 MHz; $CDCl_3$): 136.8, 134.7, 131.6, 131.3, 128.9, 128.0, 127.8, 127.7, 127.6, 125.5, 60.9 (C^{11});

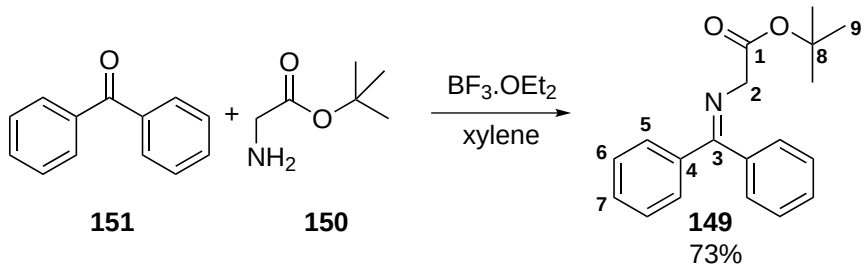
m/z (+ CI-Methane) 575 (M(^{13}C), 48%), 574 (M, 100); Found (+HRES) $[M]^+$ 574.2532. $C_{44}H_{32}N$ requires $[M]^+$ 574.2529;

ν_{max}/cm^{-1} 3414 (N^+), 2957 (Aromatic C-H), 1671 ((N^+)), 1579 (N^+);

mp 269 °C (lit. 278 °C[278]);

Optical Rotation $[\alpha]^{25}{}_D$=+201.2 (*c* 0.37, $CHCl_3$).

7.4.27 2-[*N*-(Diphenylmethylene)amino]ethanoic acid *tert*-butyl ester (149)



151 **150** **149** 73%

Glycine *tert*-butyl ester, **150**, (5.0 g, 38.1 mmol) and benzophenone, **151**, (13.89 g, 76.2 mmol) were added to xylene (50 mL). Boron trifluoride etherate (15 drops) was added and the mixture stirred at reflux overnight. The xylene was then removed under reduced pressure and the crude product purified by Kugelrohr distillation (200 °C/2 mm Hg) to give 2-[*N*-(Diphenylmethylene)amino]ethanoic acid *tert*-butyl ester, **149**, (8.22 g, 27.8 mmol, 73%) as a microcrystalline solid.[312]

δ_H (300 MHz; $CDCl_3$): 7.76 (4H, d, J=7.7 Hz, C^5H), 7.53 (2H, t, J=7.7 Hz, C^7H), 7.4 (4H, t, J=7.7 Hz, C^6), 4.11 (2H, s, C^2H_2), 1.46 (9H, s, C^9H_3);

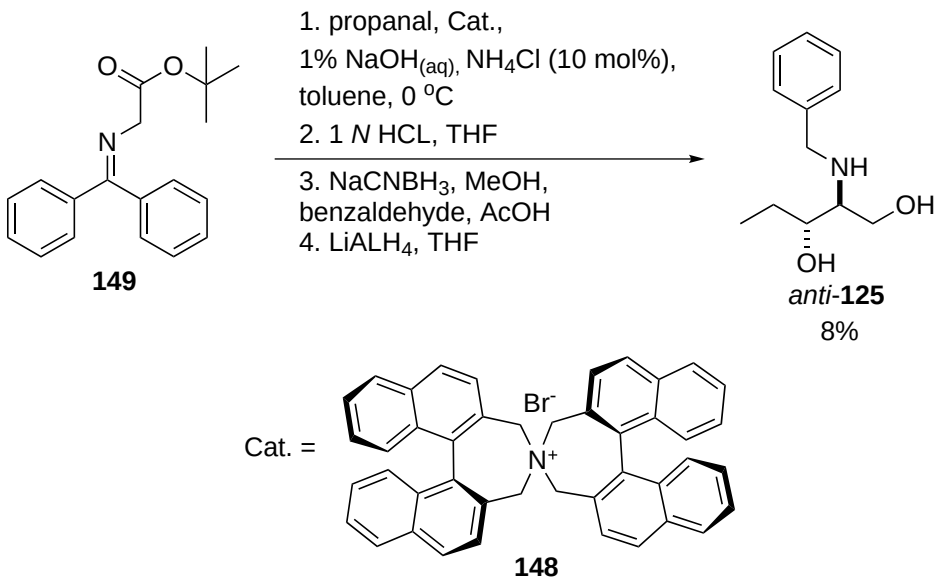
δ_C (75 MHz; $CDCl_3$): 170.4 (C^1), 168.9 (C^3), 136.2, 131.4, 128.9, 127.8, 81.0(C^8), 56.4 (C^2), 28.2 (C^9);

m/z (+ CI-Methane) 297 (M(^{13}C+H), 21%), 296 (M+H, 100); Found (+HRES) $[MH]^+$ 296.1642 $C_{19}H_{22}NO_2$ requires $[MH]^+$ 296.1651;

mp 113 °C (lit. 111–112 °C[312]);

ν_{max}/cm^{-1} 3060 (Aromatic C-H), 1598 (Aromatic C=C), 1657 (C=O).

7.4.28 (*2S, 3R*)-2-(Benzylamino)pentane-1,3-diol (*anti*-125)



2-[*N*-(Diphenylmethylene)amino]ethanoic acid *tert*-butyl ester, **149**, (2.00 g, 6.7 mmol) and (*S,S'*)-3,3',5,5'-Tetrahydro-4,4'-spirobi[4*H*-di-naphtho[2,1 -*c*:1',2'-*e*]azepinium] bromide, **148**, (40 mg) were disolved in toluene (20 mL) and 1% aqueous sodium hydroxide solution (20 mL) was added dropwise at 0 °C, under argon atmosphere. Propanal (0.78 g, 1.0 mL, 13.5 mmol) was then introduced dropwise and the mixture stirred at 0 °C for 3 hours. Saturated ammonium chloride solution (100 mL) and diethyl ether (200 mL) were then added and the ethereal layer separated, washed with saturated sodium chloride solution (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was then dissolved in THF (25 mL) and 1 *N* HCl (10.0 mL) added at 0 °C. After the mixture had stirred for 2 hours the THF was removed under reduced pressure and the resulting aqueous solution washed with diethyl ether (3 x 100 mL) and neutralized with sodium hydrogen carbonate. The mixture was then extracted with dichloromethane (3 x 100 mL) and the combined organic extracts washed with satu-

rated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was then dissolved in methanol (20 mL) and freshly distilled benzaldehyde (1.43 g, 1.4 mL, 13.5 mmol) added to the mixture. Glacial acetic acid (2.0 mL) was then added to the reaction and stirred for 1 hour. Sodium cyanoborohydride (0.85 g, 13.5 mmol) was added in one portion and the mixture stirred at room temperature for 15 hours. The mixture was then neutralized with sodium hydrogen carbonate and extracted with dichloromethane (3 x 100 mL). The combined organic extracts washed with saturated sodium chloride solution (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was dissolved in anhydrous THF (20 mL), cooled to 0 °C and $LiAlH_4$ (0.50 g, 13.5 mmol) added. The reaction was warmed to room temperature and the reaction stirred for 15 hours. Sodium sulfate decahydrate (6.50 g, 20.3 mmol) was added in one portion and the reaction stirred for 1 hour at room temperature H2O (20 mL) was then added and the mixture extracted with dichloromethane (10 x 100 mL) and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash silica column chromatography (eluent: methanol:ethyl acetate, 1:9) to give pure 2-(benzylamino)pentane-1,3-diol, *anti*-**125**, (0.11 g, 0.5 mmol, 8%) as an orange oil.[126,278]

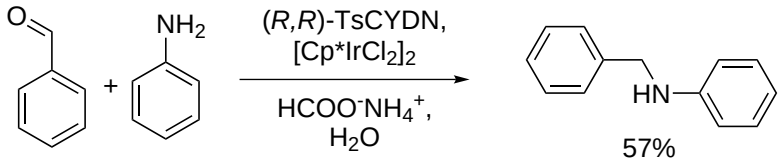
The characterization data was the same as that described on page 278.

HPLC analysis indicated that the product was formed selectively as the *anti* diastereomer (1:30, *syn:anti*). No optical rotation was obtained, the assignment is based upon the literature reference.

7.4.29 General procedure for reductive amination by transfer hydrogenation

N-Toluenesulfonyl-(*1R,2R*)-1,2-diaminocyclohexane (0.013 g, 0.05 mmol) was reacted with pentamethylcyclopentadienyl iridium(III) chloride dimer (0.024 g, 0.03 mmol) in undegassed water (6 mL) at 40 °C for 1 hour in the open air. The resulting suspension was then used without further purification in the reaction. The reaction was initiated by introducing ammonium formate (0.9 g, 14.3 mmol), the aldehyde/ketone (3.0 mmol) and the amine (3.0 mmol) and stirred at 40 °C and the reaction followed by TLC (ethyl acetate:hexane, 1:6). After the reaction had gone to completion, 10% aqueous sodium hydrogen carbonate solution (20 mL) was added and the aqueous solution extracted with ethyl acetate (2 x 30 mL). The combined organic extracts were washed with saturated sodium chloride solution (15 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by flash silica column chromatography to afford the product.[278]

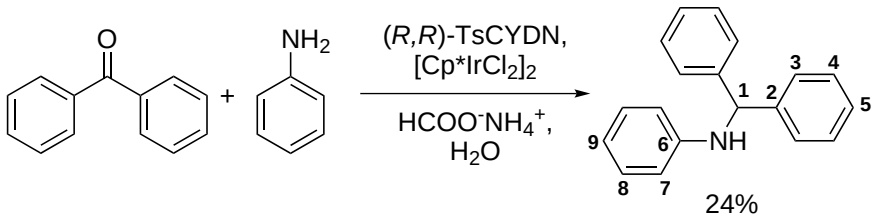
_N_-Benzylaniline



The general procedure was followed using benzaldehyde (0.32 g, 0.3 mL, 3.0 mmol) and aniline (0.28 g, 0.3 mL, 3.0 mmol) with stirring for 20 hours. The crude product was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 1:6) to afford *N*-benzylaniline (0.31 g, 1.7 mmol, 57%) as an off-white solid.[300]

The characterization data was the same as that described on page 254

N-Benzhydrylaniline



The general procedure was followed using benzophenone (0.50 g, 0.5 mL, 2.7 mmol) and aniline (0.28 g, 0.3 mL, 3.0 mmol) with stirring for 20 hours. The crude product was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 1:6) to afford *N*-benzhydrylaniline (0.17 g, 0.7 mmol, 24%) as a yellow solid.[313]

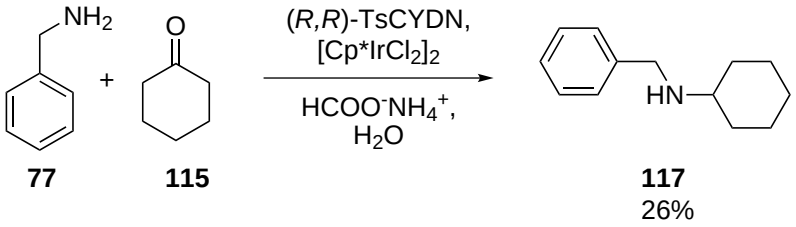
δ_H (300 MHz; $CDCl_3$): 7.54–7.35 (5H, Ar-H), 7.23 (4H, t, J=7.0 Hz, C^4H) 6.85 (2H, t, J=7.0 Hz, C^5H), 6.72 (4H, d, J=7.0 Hz, C^3H), 5.26 (1H, s, C^1H);

δ_C (75 MHz; $CDCl_3$): 146.5, 144.2, 129.4, 128.6, 127.6, 126.7, 118.7, 115.3, 76.2 (C^1);

mp 55 °C (lit. 56–58 °C[314]);

m/z (+ CI-Methane) found $[M]^+$ 259.1261. $C_{19}H_{17}N$ requires $[M]^+$ 259.1361.

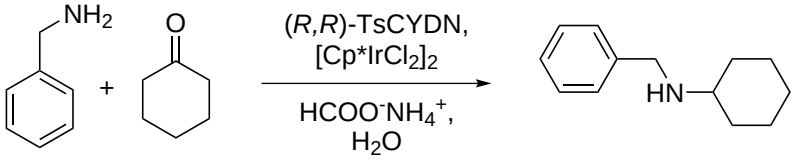
N-Benzylcyclohexylamine (117)



The general procedure was followed using cyclohexanone, **115**, (0.30 g, 0.3 mL, 3.1 mmol) and benzylamine, **77**, (0.32 g, 0.3 mL, 3.0 mmol) with stirring for 20 hours. The crude product was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 1:6) to afford *N*-benzylcyclohexylamine, **117**, (0.15 g, 0.8 mmol, 26%) as a pale brown oil.[194]

The characterization data was the same as that described on page 250

Example of an optimization reaction

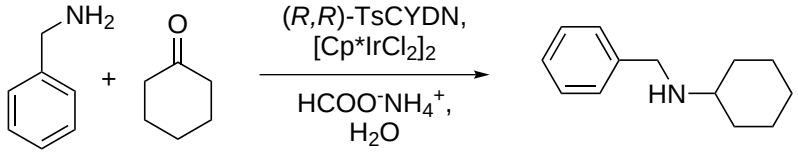


Scheme 7.1: N-Benzylcyclohexanamine

The general procedure was followed using cyclohexanone, **115**, (0.30 g, 0.3 mL, 3.1 mmol) and benzylamine, **77**, (0.32 g, 0.3 mL, 3.0 mmol). The crude product was analysed by NMR. The peak at around 3.85 was **117** and the peak at 3.80 was **77**. These were then

compared so that the ratio is **Peak area 117/Peak area 77**.[194]

N-Benzylcyclohexylamine (117)



77 **115** **117**
45%

The general procedure was followed using cyclohexanone, **115**, (0.30 g, 0.3 mL, 3.1 mmol) and benzylamine, **77**, (0.32 g, 0.3 mL, 3.0 mmol) with stirring for 70 hours. The crude product was purified by flash silica column chromatography (eluent: ethyl acetate:hexane, 1:6) to afford *N*-benzylcyclohexylamine, **117**, (0.26 g, 1.4 mmol, 45%) as a pale brown oil.[194]

The characterization data was the same as that described on page 250

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