Integrated Heterogeneous Catalysis and Biocatalysis for Sustainable Synthesis

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Declaration

I, Alice Dunbabin confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.
Abstract

The development of sustainable methods with applications in synthetic organic chemistry has been investigated widely in recent years. Catalytic processes often provide sustainable strategies with high efficiency, high selectivity and low environmental impact in terms of energy consumption and waste production. In this project, heterogeneous catalysis and biocatalysis have been investigated separately and combined into a two-step process.

Gold nanoparticle catalysts supported on inert bulk materials have been applied in the oxidation of alcohol feedstocks as an alternative to stoichiometric oxidation techniques. Synthesis of a range of gold nanoparticle catalysts has been undertaken with variation in support materials and preparation methods. These catalysts have been tested in the oxidation of the benchmark substrate benzyl alcohol, and the substrate scope also extended to include secondary alcohols. The low gold loading and reusability of these heterogeneous catalysts coupled with the use of water as the solvent has provided a sustainable oxidation method for primary and secondary alcohols.

Transaminases are enzymes which catalyse the transfer of an amino group to the carbonyl group of an aldehyde or ketone. Screening of transaminases from the UCL transaminase library was undertaken to identify enzymes for application in this project. These enzymes have been applied in the synthesis of furfurylamines from furfurals, in a one-step biocatalytic reaction under mild conditions on a preparative scale. The transaminases were also applied in the synthesis of chiral amines from ketone precursors, with high yields and stereoselectivities achieved.

Heterogeneous catalysis and biocatalysis have been coupled together into a novel two-step cascade to produce chiral amines from secondary alcohol feedstocks. The oxidation of secondary alcohols using gold nanoparticle catalysts was followed by the transamination of the ketone intermediates. This process was conducted in one pot, with water as the solvent and no isolation or purification of the ketone intermediate.
Impact Statement

The work described in this thesis is directed towards the broad goal of identifying new green practices in chemistry, for application in academic laboratories or the chemical industry. The use of transaminase enzymes in the synthesis of amines provides an environmentally benign technique, applicable in many areas of organic chemistry. The impact of this work was demonstrated through publication in the journal 'Green Chemistry', detailing the application of biocatalysis to the upgrading of biomass-derived furfural products. The biocatalytic synthesis of furfurylamines was demonstrated on a preparative scale, with good to excellent yields, showing the potential of this synthetic route inside and outside academia as biocatalytic processes are increasingly being applied in the chemical and pharmaceutical industries.

The investigation of gold nanoparticle oxidation catalysis has potential impact in the fields of organic, inorganic and materials chemistry. Further investigation is required into the general applicability of the heterogeneous catalysts prepared in this work, but advances have been made in understanding the impact of the preparation method on catalytic activity. This gold nanoparticle catalysis could provide an alternative to stoichiometric, toxic oxidation reagents. Heterogeneous catalysis is a promising method for use in the chemical and pharmaceutical industries due to the easily removable and reusable nature of the solid catalysts.

Overall this work contributes to the drive for more sustainable, ‘green’ chemical processes, aiming to reduce the impact of chemical waste on the environment and reduce energy consumption.
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List of Abbreviations

2-MeTHF – 2-methyltetrahydrofuran

α,ω-DTA – α,ω-diamine transaminases

AADH – amino acid dehydrogenase

ABTS – 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid

ADH – alcohol dehydrogenase

AlaDH – alanine dehydrogenase

ATA – aminotransferase

AuNPore – nanoporous gold skeleton material

AZADO – 2-azaadamantane-N-oxyl

BLAST – basic local alignment search tool

Boc – tert-butyloxycarbonyl

BSTFA – N,O-bistrifluoroacetamide

CAL-B – Candida antarctica lipase B

Cbz – carboxybenzyl

DCE – dichloroethane

de – diastereomeric excess

DMC – dimethyl carbonate

DMF – dimethylformamide

DMI – dimethylisosorbide

DMSO – dimethylsulfoxide

DoE – Design of Experiment

DP – deposition-precipitation

E. coli – Escherichia coli

ee – enantiomeric excess
eq. – equivalents
ER – ene reductase
EXAFS – extended X-ray absorption fine spectra
F – filtration
FAD – flavin adenine dinucleotide
FDCA – furandicarboxylic acid
FDH – formate dehydrogenase
GABA – gamma-aminobutyric acid
GC – gas chromatography
GDH – glucose dehydrogenase
GOase – glucose oxidase
h – hour(s)
HDP – homogeneous deposition-precipitation
HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMF – 5-hydroxymethylfurfural
HMFCA – 5-hydroxymethylfurancarboxylic acid
HMFO – hydroxymethylfurfural oxidase
HPLC – high performance liquid chromatography
HRP – horseradish peroxidase
HT – hydrotalcite
ic – isomeric content
ICC – immobilised capillary column
IPA – isopropylamine
IPTG – isopropyl β-D-1-thiogalactopyranoside
LCAO – long chain alcohol oxidase
LCMS – liquid chromatography mass spectrometry
LDH – lactate dehydrogenase

$m$ – meta
MAO – monoamine oxidase
MBA – α-methylbenzylamine
MS – molecular sieves
MTBE – methyl tert-butyl ether
NADH – nicotinamide adenine dinucleotide
NADPH – nicotinamide adenine dinucleotide phosphate
NCS – norcoclarine synthase
NMR – nuclear magnetic resonance

$o$ – ortho
OD – optical density
OYE – old yellow enzyme

$p$ – para
PaoABC – periplasmic aldehyde oxidase
PDC – pyruvate decarboxylase
Pi – phosphate buffer
PI-CB – polymer incarcerated and carbon black stabilised nanoclusters
PLP – pyridoxal-5’-phosphate
PMO – periodic mesoporous organosilica
PMP – pyridoxamine-5’-phosphate
PVP – poly(N-vinyl-2-pyrrolidine)
RE – rotary evaporation

rt – room temperature
SDS-PAGE – sodium dodecyl sulfate polyacrylamide gel electrophoresis
SIL-g-G – supramolecular ionic liquid grafted graphene
SMO – smoothened receptor
TAm – transaminase
TAME – tert-amyl methyl ether
TBHP – tert-butyl hydroperoxide
TEM – transmission electron microscopy
TEMPO – 2,2,6,6-tetramethylpiperidine-N-oxyl
TFA – trifluoroacetic acid
ThDP – thiamine diphosphate
THF – tetrahydrofuran
THPC – tetrakis(hydroxymethyl)phosphonium chloride
TMS – trimethylsilyl
wt% – weight percent
XANES – X-ray absorption near edge structure
XRD – X-ray diffraction
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1 Introduction

1.1 Sustainability in organic synthesis

The drive to increase the sustainability of organic chemistry processes has been increasing in recent years. This has included strategies to reduce consumption of fossil fuels, limit the release of hazardous waste into the environment and make laboratory practices safer. The twelve principles of green chemistry developed by Anastas and Warner in 1998 have acted as a guide for chemists to implement green processes and practices across synthetic chemistry, process chemistry and chemical engineering.\(^1\)\(^,\)\(^2\) These principles aim to conserve energy, increase the use of sustainable feedstocks and reduce the production of hazardous material and waste.

The move towards greener processes promotes the use of green solvents, which have been ranked taking into account health and safety impacts on humans and the environment. Assessments of solvent flammability, bioaccumulation and toxicity towards humans and aquatic life were among the criteria in the CHEM21 solvent guide ranking system.\(^3\) Generally, halogenated solvents are regarded as problematic and hazardous, and water and alcohols such as isopropanol and ethanol are recommended for use. Less commonly known solvents such as tert-amyl methyl ether (TAME) and dimethyl carbonate were also recommended and are gaining prominence as alternative solvents.\(^3\)

The use of biocatalysis in organic synthesis clearly fits within the green chemistry agenda, due to the mild, aqueous conditions, ambient temperatures and low levels of toxic waste produced. Enzyme catalysis is often more cost effective and less harmful to the environment than using precious or transition metal catalysts, while retaining high yields and stereoselectivities, as recently demonstrated in the industrial biocatalytic synthesis of Sitagliptin using a transaminase.\(^4\)

The use of supported metal nanoparticle catalysis can also be described as sustainable due to the reusability of the catalysts together with the low metal loadings usually required. The catalysts are also easily recoverable by simple filtration, leading to less waste.
1.1.1 Chemical feedstocks from biomass derived sources

A key focus of sustainable and green chemistry is the use of feedstocks derived from biomass and waste streams for conversion into useful higher value products. In 2004, the US Department of Energy developed a list of ‘Top 10’ platform chemicals from carbohydrate biomass to be used as building blocks for organic synthesis.\(^5\) These ‘Top 10’ chemicals were revisited in a 2010 review describing the advances in the use of these chemicals, and a new table of chemical opportunities from biomass derived carbohydrates was reported (Table 1). These platform chemicals included ethanol, furans 1-3, glycerol based compounds 4-6, isoprene 7, and lactic, succinic, 3-hydroxypropanoic and levulinic acids 8-11, as well as sugar alcohols xylitol 12 and sorbitol 13.\(^6\)

**Top chemical opportunities from biorefinery carbohydrates (2010)**

<table>
<thead>
<tr>
<th>Type</th>
<th>Compound</th>
<th>Structure</th>
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</thead>
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<tr>
<td>Alcohols</td>
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<tr>
<td></td>
<td>HMF</td>
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</tr>
<tr>
<td></td>
<td>FDCA</td>
<td><img src="#" alt="FDCA structure" /></td>
</tr>
<tr>
<td>Glycerol and derivatives</td>
<td>Glycerol</td>
<td><img src="#" alt="Glycerol structure" /></td>
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<tr>
<td></td>
<td>Glycerol carbonate</td>
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<td></td>
<td>Epichlorohydrin</td>
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<tr>
<td>Biohydrocarbons</td>
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Organic acids

<table>
<thead>
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<tr>
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<tr>
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<tr>
<td>3-Hydroxypropanoic acid</td>
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</tr>
<tr>
<td>Levulinic acid</td>
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</table>

Sugar alcohols

<table>
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<tr>
<th></th>
<th>Chemical Structure</th>
</tr>
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<tbody>
<tr>
<td>Xylitol</td>
<td><img src="image" alt="Xylitol" /></td>
</tr>
<tr>
<td>Sorbitol</td>
<td><img src="image" alt="Sorbitol" /></td>
</tr>
</tbody>
</table>

Table 1 – Top chemical opportunities from biorefinery carbohydrates.\(^6\)

Chemical feedstocks can be derived from various food waste sources including potato, coffee, sugar and corn waste, and converted into biopolymers. Different waste products can provide access to varying chemical feedstocks. While waste products from foods such as potato and corn provide mainly carbohydrates, citrus peels can provide access to many terpene products.\(^7\)

The transformations of furan compounds furfural 1 and 5-hydroxymethylfurfural (HMF) 2 are discussed in Section 1.1.2. The oxidation of glycerol 4 has been reported using supported gold nanoparticle catalysis, which is discussed in Section 1.2.

Levulinic acid 11 can be derived from cellulose and hemicellulose through hydrolysis and dehydration using acid treatment. This platform chemical can then be used to access valuable chemicals (Scheme 1). Hydrogenation of levulinic acid with hydrogen to form γ-valerolactone 14 has been reported with a Ru(acac)\(_3\) catalyst with phosphine ligand, giving >99% yield.\(^8\) This transformation has also

---

\(^6\) Section 1.1.2

\(^7\) Section 1.2

\(^8\) Scheme 1
been reported with supported Ru and Pt catalysts, with a 97% yield achieved with Ru/C.\(^9\) The further hydrogenation of γ-valerolactone 14 with hydrogen has been reported with Ru catalysts, and depending on the additive used 1,4-pentanediol 15 and 2-methyltetrahydrofuran (2-MeTHF) 16 could be accessed. A yield of 95% of 1,4-pentanediol 15 was achieved with a Ru-triphos complex, and in the presence of an acidic ionic liquid, 2-MeTHF 16 was formed in 92% yield. Both γ-valerolactone 15 and 2-MeTHF 16 can be used as sustainably sourced solvents.\(^8\)

\[
\begin{align*}
\text{cellulose} & \quad \text{hemicellulose} \quad \xrightarrow{H^+} \quad \text{levulinic acid 11} \\
i. & \quad \gamma\text{-valerolactone 14} \\
ii. & \quad 2\text{-methyl THF 16} \\
iii. & \quad 1,4\text{-pentanediol 15}
\end{align*}
\]

Scheme 1 – Transformations of platform chemical levulinic acid 11 using Ru catalysts with phosphine ligands. i-iii. Ru(acac)\(_3\) (0.1 mol%), ligand (1 mol%), additive (1 mol%), 18 h, 160 °C, \(H_2\) (10 MPa). i. \(P(n\text{Oct})_3\) ligand, \(\text{NH}_4\text{PF}_6\) additive, >99% 14. ii. Triphos ligand, no additive, 95% 15. iii. Triphos ligand, acidic ionic liquid and \(\text{NH}_4\text{PF}_6\) additives, 92% 16. Yields were determined by GC.\(^8\)

The reductive amination of a levulinic acid 11 has also been reported using a carbon supported FeNi alloy heterogeneous catalyst (Scheme 2). This transformation provided access to pyrrolidine 17 after the spontaneous cyclisation of the amine intermediate, with 92% conversion and 91% selectivity, although this required hydrogen pressure of 50 bar using an H-cube continuous reactor. The selectivity could be increased further to 99% by changing the solvent to 2-methylfuran and increasing the hydrogen pressure to 85 bar.\(^10\) This transformation could also be undertaken in a stereoselective manner using transaminase enzymes, and this is discussed in Section 1.3.2.
Scheme 2 – Reductive amination of levulinic acid **11** in a flow system. Selectivity for desired pyrrolidine product **17** over by-product ethyl phenethylamine, determined by GC analysis.\(^{10}\)

Sorbitol **13** is an attractive platform chemical due to its high number of chiral centres. The stereochemistry of this compound can be conserved and used to form complex structures such as isosorbides. Sorbitol **13** can be derived from starch cellulose via glucose using supported Ru catalysts such as Ru/C, with excellent yields of >99%.\(^{11}\) Sorbitol **13** can then be converted into isosorbide **18** via sorbitan **19** using a sulfated copper oxide (CuSO) catalyst, with 100% conversion and 68% selectivity for isosorbide **18** (Scheme 3).\(^{12}\) A green synthesis of dimethylisosorbide (DMI) **20** has been reported using dimethyl carbonate (DMC) as the solvent, giving 98% yield of DMI **20** in the presence of sodium methoxide.\(^{13}\) DMI **20** has applications as an alternative high boiling solvent to DMSO and DMF.\(^{11}\)

Scheme 3 – Synthesis of isosorbides from sorbitol **13**.\(^{11}\) i. CuSO-650 catalyst, flow conditions, N\(_2\) carrier gas, 4 h, 200 °C, 100% conversion, 68% selectivity for **18**, determined by HPLC.\(^{12}\) ii. NaOMe, dimethyl carbonate (DMC), N\(_2\) atmosphere, 20 h, 90 °C, 98% isolated yield of **20**.\(^{13}\)
1.1.2 Furan based chemical feedstocks
Transformations of platform chemicals such as furfural 1 and HMF 2 (Figure 1) have been investigated in this project.

![Furan and HMF structures](image)

**Figure 1** – Furfural 1 and 5-hydroxymethylfurfural 2 synthesised from biomass sources.

Target products from the upgrading of furfural and derivatives include furfurylamines and furandicarboxylic acid (FDCA) 3. Furfurylamines are of interest as building blocks for pharmaceuticals,14 and furfurylamines such as 2,5-bis(aminomethyl)furan 21 have been used as hardeners for epoxy resins.15 Furfurylamines and FDCA both have applications as monomers for renewable biopolymer production (Scheme 4).16,17 Indeed, FDCA 3 has been reported in the production of polymeric furanoate esters 22 as replacements for terephthalic or isophthalic acids.18

![Polymerisation scheme](image)

**Scheme 4** – Polymerisation of FDCA 3 and furfurylamine derivatives.16 Polymers prepared by melt, solution or interfacial polymerisation.

Access to furfurylamines has been reported using reductive amination techniques. A one-pot two-step reductive amination approach was applied to bio-based furfurals through imine formation followed by reduction with NaBH₄ (Scheme 5). Mild conditions were required due to the sensitivity of the furan ring, 6
and yields of 77-99% were achieved with a range of aromatic and aliphatic amines.\textsuperscript{19}

\[
\begin{align*}
\text{HO} & \quad \text{O} & \quad \text{O} & \quad \text{H} \\
\text{i. RNH}_2 & \quad \text{ii. NaBH}_4 & \quad \text{H}_2\text{O}, \text{MeOH} & \quad \text{or EtOH} \\
& & \quad 2-7 \text{ h}, \text{rt} & \quad \text{HO} & \quad \text{O} & \quad \text{NHR} \\
& & & & \quad \text{R = Bu; 99\%} & \quad \text{R = CH}_2\text{CH}_2\text{Ph; 97\%} & \quad \text{R = } ^{1}\text{Pr; 88\%} & \quad \text{R = } ^{9}\text{Bu; 99\%} & \quad \text{R = Bn; 92\%}
\end{align*}
\]

Scheme 5 – One pot, two-step reductive amination of HMF 2. Isolated yields shown.\textsuperscript{19}

The transformation of biomass products into amines has been reported using a number of supported metal heterogeneous catalysts, with the main routes described using reductive amination or hydrogen borrowing techniques.\textsuperscript{20} Recently, the reductive amination of furfurals to primary furfurylamines with supported Pd, Ru, Rh and Pt catalysts and ammonia was reported by Kawanami et al. (Scheme 6). The best catalyst for the transformation was found to be Rh/Al\textsubscript{2}O\textsubscript{3}, which had the highest selectivity for the desired furfurylamine product. The metals exhibited different selectivities for the by-products, with Pd catalysts resulting in the formation of mainly disubstituted product 23, and Pt catalysts favouring the direct hydrogenation to furfuryl alcohol 24 with some ring hydrogenated products 25 also observed. Rh/Al\textsubscript{2}O\textsubscript{3} was taken forward for reaction development and more substrates were tested including 5-methylfurfural 26 and HMF 2. The Rh/Al\textsubscript{2}O\textsubscript{3} catalyst was also reusable, with the yield of furfurylamine only dropping below 90% on the fifth cycle of use.\textsuperscript{21}

\[
\begin{align*}
\text{R} & \quad \text{O} & \quad \text{O} & \quad \text{H} \\
& & & \quad \text{H}_2 (2 \text{ MPa}) & \quad \text{aq. NH}_3, 2 \text{ h} \\
& \quad \text{Rh/Al}_2\text{O}_3 & & & \quad \text{R} & \quad \text{O} & \quad \text{NH}_2 \\
\text{Compound} & \quad \text{R} & \quad \text{Conversion} & \quad \text{Selectivity} \\
1 & \text{H} & \text{100\%} & \text{92\%} \\
26 & \text{Me} & \text{100\%} & \text{91\%} \\
2 & \text{CH}_2\text{OH} & \text{100\%} & \text{86\%} \\
\end{align*}
\]

Scheme 6 – Reductive amination of furfurals with a supported Rh catalyst. Conversions determined by GC analysis.\textsuperscript{21}

The reductive amination of biomass with a renewable nitrogen source has also been reported using a carbon supported FeNi alloy catalyst (Scheme 7). The process utilised nitrogen donors such as aminopropanol and alanine under
hydrogen pressure using an H-cube continuous reactor. The use of amino acids such as alanine as a nitrogen donor was investigated in the amination of furfural and HMF, and although problems were encountered with competitive hydrogenation of the furan ring, yields of 50-77% of 27 were achieved.\textsuperscript{10}

\begin{center}
\begin{equation}
\begin{array}{c}
\text{HO} - \text{O} - \text{O} - \text{H} \\
\text{L-alanine}
\end{array}
\begin{array}{c}
\text{FeNi/C}
\end{array}
\begin{array}{c}
\text{EtOH, 125 °C} \\
\text{H}_2 (10 \text{ bar})
\end{array}
\begin{array}{c}
\text{HO} - \text{O} - \text{HN} - \text{ONa}
\end{array}
\end{equation}
\end{center}

Scheme 7 – Reductive amination of HMF 2 using alanine as the amino donor with a carbon supported FeNi alloy catalyst in a flow system. Yield determined by NMR spectroscopy.\textsuperscript{10}

The direct amination of 2,5-bis(aminomethyl)furan 21 from 2,5-diformylfuran 28 has been reported using Raney-Ni (Scheme 8).\textsuperscript{22} Although this process provided access to the primary amine product 21, the formation of dimer and trimer by-products was also observed. A variety of Raney-Ni catalysts were tested and the best result was achieved with acid-treated Raney-Ni at 10 bar H\textsubscript{2} pressure with a yield of 43%.\textsuperscript{22}

\begin{center}
\begin{equation}
\begin{array}{c}
\text{HO} - \text{O} - \text{O} - \text{H} \\
\text{Raney-Ni}
\end{array}
\begin{array}{c}
\text{NH}_3, \text{H}_2 \\
\text{THF:H}_2\text{O} \text{, } 120 \text{ °C, } 6 \text{ h}
\end{array}
\begin{array}{c}
\text{H}_2\text{N} - \text{O} - \text{NH}_2
\end{array}
\end{equation}
\end{center}

Scheme 8 – Reductive amination of 2,5-diformylfuran 28 with Raney-Ni. Yield determined by GC analysis.\textsuperscript{22}

Attempts to transform biomass derived feedstocks into useful products using sustainable heterogeneous catalysts described above are promising. However, efforts to reduce the use of toxic reagents such as NaBH\textsubscript{4}, have given rise to methodologies requiring H\textsubscript{2}, often at elevated pressures. Furthermore, although nickel is an earth abundant metal, Raney-Ni can also be dangerous to work with due to its pyrophoric nature. These problems can be overcome by using flow chemistry, where hydrogen pressure can be used safely and the risks are lowered, but this can also be expensive to implement. Despite these drawbacks, the use of aqueous solutions or green solvents such as ethanol, and the reusability of catalysts such as Rh/Al\textsubscript{2}O\textsubscript{3} allow access to furfurylamines in a sustainable manner.
The oxidation of HMF 2 to FDCA 3 has been reported using both heterogeneous catalysis and biocatalysis. However, as this reaction requires the oxidation of two functional groups, an alcohol and an aldehyde, reports of single enzymatic approaches are rare. An FAD-dependent oxidase was found to be active towards HMF 2, and was named HMF oxidase (HMFO) (Scheme 9). An excellent yield of 95% of FDCA 3 was achieved at ambient temperature and pressure. The enzyme was also found to be active on substituted benzyl alcohols.23,24

![Scheme 9 – Oxidation of HMF 2 to FDCA 3 with FAD-dependent HMF oxidase. Yield determined by HPLC analysis.24](image)

This transformation has been reported with a galactose oxidase (GOase) and a periplasmic aldehyde oxidase (PaoABC) in a two-enzyme cascade, in the presence of catalase and horseradish peroxidase (HRP) to remove H₂O₂ (Scheme 10). The process was shown to give higher yields of the desired product when the enzymes were added sequentially rather than together. The one-pot procedure gave 97% yield of FDCA 3, but when the enzymes were added sequentially this increased to >99%. Over 99% conversions of other benzylic, cinnamyl and aliphatic primary alcohols to the corresponding carboxylic acids were also achieved using this technique.25

![Scheme 10 – Two-enzyme cascade for the oxidation of HMF 2. Yield determined by HPLC analysis.25](image)

This transformation was also extended to a continuous flow process with immobilised PaoABC which could be recycled 14 times without loss of activity.26

The upgrading of bio-based furfural and derivatives has been investigated in amination and oxidation processes to form useful products. Heterogeneous catalysis and biocatalysis have been used for these processes, and a focus on
green chemistry is evident in this field. However, work is still needed to overcome some of the challenges of selectivity and toxicity, through more investigations into sustainable transformations of renewable feedstocks.

1.2 Supported gold nanoparticle catalysts

Gold nanoparticle catalysts can provide an effective alternative to stoichiometric oxidants in organic synthesis, such as permanganates and chromates, which are usually hazardous, toxic and expensive. These supported catalysts can act as greener, more sustainable reagents in catalytic processes. Supported gold catalysts have been used for a variety of reactions including the functionalisation of unreactive starting materials, in the oxidation of methane, cyclohexane, toluene, CO, sugars and glycerol, and the epoxidation of alkanes and styrenes.\textsuperscript{27}

Gold nanoparticles deposited on oxide supports were initially found to be catalytically active for oxidation reactions such as oxygen insertion, epoxidation and alcohol oxidation in the late 1980s.\textsuperscript{28} These catalysts exhibited high stability due to the low loading of gold onto the support, and high functional group tolerance due to the mild reaction conditions required, often solvent free and using air as the oxidant. The catalysts were highly selective, providing the desired oxidation state in the product, and also regioselectively oxidised one hydroxy group on a diol or sugar.\textsuperscript{29,30}

The synthesis of supported gold nanoparticle catalysts has been investigated as the preparation method can have a large effect on catalytic activity (Table 2). Variations in preparation method centre around the use of additives, pH control, temperature and method of water removal.
<table>
<thead>
<tr>
<th>Method</th>
<th>Reagents</th>
<th>Additives</th>
<th>pH</th>
<th>Temp</th>
<th>Water removal</th>
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<tbody>
<tr>
<td>Co-precipitation&lt;sup&gt;31&lt;/sup&gt;</td>
<td>Support HAuCl₄</td>
<td>Tetraethyl orthosilicate, Ammonia</td>
<td>10</td>
<td>rt</td>
<td>Filtration</td>
</tr>
<tr>
<td>Impregnation&lt;sup&gt;31,32&lt;/sup&gt;</td>
<td>Support HAuCl₄</td>
<td>None</td>
<td>Not controlled</td>
<td>rt</td>
<td>Evaporation</td>
</tr>
<tr>
<td>Colloidal immobilization&lt;sup&gt;33&lt;/sup&gt;</td>
<td>Support HAuCl₄</td>
<td>THPC, NaOH</td>
<td>Not controlled</td>
<td>rt</td>
<td>Filtration</td>
</tr>
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<td>Deposition-Precipitation&lt;sup&gt;34&lt;/sup&gt;</td>
<td>Support HAuCl₄</td>
<td>NaOH</td>
<td>10</td>
<td>70 °C</td>
<td>Filtration</td>
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<td>Homogeneous Deposition-Precipitation&lt;sup&gt;34&lt;/sup&gt;</td>
<td>Support HAuCl₄</td>
<td>Urea</td>
<td>Not controlled</td>
<td>95 °C</td>
<td>Filtration</td>
</tr>
</tbody>
</table>

Table 2 – Preparation methods of supported gold nanoparticle catalysts. THPC: tetrakis(hydroxymethyl)phosphonium chloride. rt: room temperature.

The co-precipitation method was reported by Haruta et al. using tetraethylorthosilicate and ammonia as additives, high pH and water removed by filtration to prepare supported gold nanoparticle catalysts for the oxidation of CO (Scheme 11). Prior to this report, Mn, Cu, Pt and Pd catalysts had been reported for this transformation but had issues with water tolerance and required reaction temperatures above 100 °C. The oxidation of CO in the presence of Au/Fe₂O₃ could proceed with high efficiency at temperatures of 30 °C, 0 °C and -70 °C.<sup>35</sup>

\[
\text{CO} + \text{Au/Fe}_2\text{O}_3, \text{O}_2 \text{ (air)} \xrightarrow{30 \degree \text{C, 0 \degree \text{C}, -70 \degree \text{C}}} \text{CO}_2
\]

Scheme 11 – Oxidation of CO gas at low temperatures. At 30 °C and 0 °C, catalytic efficiency was 100% after 167 h. At -70 °C, catalytic efficiency was 100% for 8 min and decreased over time to 50% after 80 min.<sup>35</sup>

The impregnation method was also used to prepare Au/Fe₂O₃ with no additives, no pH control, and water removal by evaporation. The impregnation catalysts were only active at temperatures over 100 °C.<sup>35</sup> The impregnation method has also been reported to give larger gold crystallites and a lower gold dispersion level than the co-precipitated catalysts.<sup>35</sup> Colloidal gold catalysts were prepared...
by immobilization, using tetrakis(hydroxymethyl)phosphonium chloride (THPC) and sodium hydroxide as additives, with no pH control and removal of water by filtration. These catalysts were used in the oxidation of benzyl alcohol to study the effect of particle size on catalytic activity, which is discussed in Section 1.2.1.

Following Haruta’s work, other supports were reported for potential use in CO oxidations, and another method of gold deposition became popular, known as deposition-precipitation (DP), that was found to produce more active catalysts. In the DP method the metal precursor H\(_{2}\)AuCl\(_4\) is added to an aqueous solution of the support, and the gold is precipitated as a hydroxide by raising the pH with the addition of aqueous sodium hydroxide solution. This gives small sized gold nanoparticles, but not all of the gold in solution is deposited onto the support, giving rise to variation in the properties of the catalysts. The DP method was later improved by adding urea rather than sodium hydroxide to precipitate the gold, and this gave a complete and more uniform gold dispersion onto the support. The method using urea became known as homogeneous deposition-precipitation (HDP).

In 1998, the DP method was used to prepare novel gold catalysts with carbon as a support, which were used to oxidise vicinal diols to \(\alpha\)-hydroxy carboxylates under mild conditions, with oxygen as the oxidant (Scheme 12). The gold catalyst provided complete selectivity for lactic acid formation at a lower catalyst loading than the carbon supported Pt and Pd catalysts also tested. The selective oxidation of ethane- and propane-1,2-diols and glycolic acid, using gold nanoparticles supported on alumina and carbon was also reported.

\[ \text{O}_2 \text{ (3 bar) as the oxidant in the presence of NaOH. Catalysts prepared by deposition precipitation. Selectivity for lactic acid 8 reported. Conversion and selectivity determined by HPLC.} \]

In 2002, the Hutchings group explored the oxidation of platform chemical glycerol with Au/C nanoparticle catalysts. Selectivity was a priority for this transformation as glycerol has a complicated oxidation pathway with many potential oxidation pathways.
products (Scheme 13). The Au/C catalysts could give up to 100% selectivity for glyceric acid 30, with moderate to high conversions of glycerol. When varying the pressure of oxygen and catalyst loading, the main by-products observed were glyceraldehyde 31 and tartronic acid 32.\textsuperscript{29}

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {glycerol 4 \textsuperscript{56\% conversion}};
\node (b) at (2,0) {glyceraldehyde 31};
\node (c) at (4,0) {glyceric acid 30 \textsuperscript{100\% selectivity}};
\node (d) at (6,0) {tartronic acid 32};
\node (e) at (0,-2) {dihydroxyacetone};
\node (f) at (2,-2) {hydroxypyruvic acid};
\node (g) at (4,-2) {mesoxalic acid};
\node (h) at (6,-2) {oxalic acid};
\draw[->, thick] (a) -- (b);
\draw[->, thick] (b) -- (c);
\draw[->, thick] (c) -- (d);
\draw[->, thick] (a) -- (e);
\draw[->, thick] (e) -- (f);
\draw[->, thick] (f) -- (g);
\draw[->, thick] (g) -- (h);
\end{tikzpicture}
\end{center}

Scheme 13 – Oxidation pathway of glycerol 4. Reaction conditions: Au/C catalyst, O\textsubscript{2} (3 bar) H\textsubscript{2}O, NaOH, 60 °C, autoclave, 3 h. Conversion and selectivity determined by HPLC.\textsuperscript{29}

Gas phase oxidations of a range of aliphatic primary and secondary alcohols catalysed by gold nanoparticles were reported in 2003 by the Rossi group (Scheme 14). The oxidation was performed on alcohols including 3-pentanol 33, 1-butanol 34, and prop-2-en-1-ol 35. High conversions of starting materials were reported, with high selectivity to the desired ketone and aldehyde products 36 and 37. 97% selectivity for methacrolein 38 was achieved in the case of prop-2-en-1-ol 35, with no unwanted oxidation taking place at the alkene centre.\textsuperscript{38}

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {33};
\node (b) at (2,0) {36};
\node (c) at (0,-2) {34};
\node (d) at (2,-2) {37};
\node (e) at (0,-4) {35};
\node (f) at (2,-4) {38};
\draw[->, thick] (a) -- (b) node[above,midway] {Au/SiO\textsubscript{2}} node[below,midway] {air, 150 °C} node[below] {97\% conversion 100\% selectivity};
\draw[->, thick] (c) -- (d) node[above,midway] {Au/SiO\textsubscript{2}} node[below,midway] {air, 250 °C} node[below] {51\% conversion 100\% selectivity};
\draw[->, thick] (e) -- (f) node[above,midway] {Au/SiO\textsubscript{2}} node[below,midway] {air, 250 °C} node[below] {42\% conversion 97\% selectivity};
\end{tikzpicture}
\end{center}

Scheme 14 – Gas phase oxidation of alcohols 33-35 with an Au/SiO\textsubscript{2} (1 wt\%) catalyst and flowing air as the oxidant. Conversion of starting material and selectivity to desired carbonyl derivatives determined by GC.\textsuperscript{38}
1.2.1 Benzyl alcohol oxidation

The oxidation of benzyl alcohol 39 has become widely used as benchmark reaction for supported gold nanoparticle catalysts. The aldehyde product benzaldehyde 40 has applications in the pharmaceutical, dye, and agricultural industries. A highly active catalytic, chlorine-free route to this product would be valuable, as benzaldehyde is commercially produced via the hydrolysis of benza chloride leading to traces of chlorine in the product, or alternatively through the poorly selective oxidation of toluene. Benzyl alcohol 39 can be oxidised to the desired product benzaldehyde using oxidants such as air or peroxide, and catalysed by gold nanoparticle catalysts. Under these conditions, benzaldehyde 40 can be over-oxidised to benzoic acid 41, and react further to form benzyl benzoate 42 (Scheme 15).

In 2005, Choudhary et al. reported the solvent-free oxidation of benzyl alcohol 39 to benzaldehyde 40 using molecular oxygen as the oxidant, with a wide variety of supports, with MgO, U₃O₈, Al₂O₃ and ZrO₂ supports showing good activity and product selectivity (Table 3). No benzoic acid side product 41 was reported, but the formation of benzyl benzoate 42 was noted, and it was hypothesised that the benzoic acid was being formed but was immediately reacting to form the ester product. The oxidation was also described as a “totally clean process” as no organic solvent was used, and the oxidant, air, forms no unwanted side products.
Catalyst | wt% | Conversion of benzyl alcohol 39 (%) | Benzaldehyde 40 yield (%)
--- | --- | --- | ---
Au/MgO | 7.5 | 51 | 44
Au/U₃O₈ | 8.0 | 53 | 50
Au/Fe₂O₃ | 6.1 | 16 | 16
Au/ZrO₂ | 3.0 | 51 | 44

Table 3 – Oxidation of benzyl alcohol 39 in the presence of O₂ (1.5 bar) and supported gold nanoparticle catalysts. Catalysts prepared by homogeneous deposition precipitation. Conversion of benzyl alcohol and yield of benzaldehyde determined by GC.⁴⁰

Hutchings et al. also investigated the oxidation of benzyl alcohol 39 using gold on silica and titania supports, and reported no benzoic acid product 41 in the reaction mixture. It was suggested that the by-product benzyl benzoate 42 was formed directly by the reaction of benzyl alcohol 39 with benzoic acid 41. Another possible route to benzyl benzoate is the Bronsted acid catalysed formation of hemiacetal 43 which could then be oxidised to the ester (Scheme 16).³¹

![Scheme 16 – Oxidation pathway of benzyl alcohol 39 via hemiacetal 43, with Au/SiO₂ (0.7 wt%) catalyst and O₂ (2 bar) as the oxidant at 100 °C.³¹](image)

Bimetallic Au-Pd/TiO₂ nanoparticle catalysts were reported for the oxidation of benzyl alcohol 39, and were compared with Au/TiO₂ and Pd/TiO₂ (Table 4).⁴¹ The Au-Pd/TiO₂ catalyst showed increased conversion, selectivity for benzaldehyde 40 and turnover number compared to Au/TiO₂ and Pd/TiO₂ catalysts. In the presence of Au/TiO₂ acetal by-product 43 was observed, and with Pd/TiO₂ toluene and benzene were noted as side products, explaining the low selectivity. With Au-Pd/TiO₂ the only by-product was benzyl benzoate 43, and the selectivity for benzaldehyde production was above 96%.⁴¹
Catalyst | Conversion of benzyl alcohol 39 (%) | Selectivity for benzaldehyde 40 (%)
--- | --- | ---
Au-Pd/TiO₂ | 75 | 92
Au/TiO₂ | 15 | 64
Pd/TiO₂ | 60 | 54

Table 4 – Oxidation of benzyl alcohol 39 by Au-Pd, Au and Pd catalysts with TiO₂ supports, with O₂ (2 bar). Catalysts prepared by impregnation. Conversion and selectivity determined by GC.⁴¹

It was generally agreed that the method of deposition of gold had a significant effect on the gold nanoparticle activity, and studies have also been carried out into the effect of particle size. According to Haider et al., the optimum average nanoparticle size is 6.9 nm, regardless of the support used.³³ In this case, CeO₂ and TiO₂ were used as supports in the oxidation of benzyl alcohol 39 by molecular oxygen, and the catalysts were prepared by colloidal immobilization. The choice of solvent was explored, using toluene, mesitylene and supercritical CO₂. The highest conversion and selectivity was in supercritical CO₂ rather than conventional organic solvents.³³ However, supercritical CO₂ required an autoclave and highly elevated pressures, limiting the applicability of this process.

Choudhary et al. reported the use of tert-butyl hydroperoxide (TBHP) as the oxidant in the oxidation of benzyl alcohol 39, to compare with conversions using molecular oxygen.³⁴ Although molecular oxygen is a desirable oxidant as it forms only water as a by-product, problems can be encountered with the transfer of gaseous oxygen into the liquid media of the reaction. Using a liquid oxidant such as TBHP can increase yields due to its enhanced solubility and proximity to the reactants and catalyst. A range of supports were investigated, and Au/MgO gave the highest conversion of 100%, with 70% selectivity for benzaldehyde 40, and the only by-product was benzyl benzoate 42.³² The second best catalyst reported was Au/Al₂O₃ which gave a 71% conversion with 65% selectivity for benzaldehyde 40.³²

The influence of the preparation technique on catalytic activity was also investigated, with a comparison of Au/MgO prepared by DP, HDP and impregnation methods (Table 5).³²
Catalyst Preparation Conversion of benzyl alcohol (39 %) Selectivity for benzaldehyde (40 %)

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Preparation</th>
<th>Conversion</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au/MgO</td>
<td>DP</td>
<td>97</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>HDP</td>
<td>100</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Impregnation</td>
<td>44</td>
<td>75</td>
</tr>
<tr>
<td>Au/Al₂O₃</td>
<td>HDP</td>
<td>71</td>
<td>65</td>
</tr>
<tr>
<td>Au/CaO</td>
<td>HDP</td>
<td>41</td>
<td>86</td>
</tr>
</tbody>
</table>

Table 5 – Results of the oxidation of benzyl alcohol with supported Au catalysts. DP: deposition-precipitation, HDP: homogeneous deposition-precipitation. Conversion and selectivity determined by GC.³²

The HDP prepared catalyst gave a 10% increase in selectivity over the DP catalyst but only a 3% increase in conversion, and impregnation gave a significantly lower conversion. The average gold nanoparticle size was 9 nm for Au/MgO and 4.1 nm for Au/Al₂O₃, and all of the catalysts were prepared at 8 wt% of gold. It was concluded that Au/MgO was a good catalyst for the conversion of benzyl alcohol 39 to benzaldehyde 40 with good selectivity, using TBHP as the oxidant, and they reported an increase in conversion from their previous reactions using molecular oxygen as the oxidant.³²,⁴⁰ Au/MgO catalysts were also reported for the epoxidation of styrenes, and Au/U₃O₈ and Au/TiO₂ were highlighted as other interesting catalysts for the oxidation of benzyl alcohol 39. In gold loading experiments, it was generally found that as the gold loading increased, conversions increased but the selectivity for benzaldehyde 40 decreased.³⁴,³⁹ There have been many reports of benzyl alcohol oxidation with supported gold catalysts, and it has become a benchmark reaction for testing the effects of catalyst preparation and support material on the catalytic activity. This has also been summarised in many reviews.²⁷,²⁸,⁴²
1.2.2 Extension of alcohol oxidation substrate scope

Although the oxidation of benzyl alcohol 39 has been widely reported for testing the activity of supported gold nanoparticle catalysts, some extension of the substrate scope has also been investigated.

Corma and coworkers investigated the application of gold nanoparticle catalysts in the oxidation of allylic alcohols to aldehydes. These substrates have the potential for overoxidation at the alkene position, and therefore require selective oxidation catalysts. The formation of cinnamaldehyde 44, (S)-(+) -carvone 45 and octenones 46 and 47 through oxidation was reported with Au/CeO$_2$ and Au/TiO$_2$ in comparison to bimetallic Au-Pd catalysts using oxygen at temperatures greater than 100 °C (Scheme 17). The Au/CeO$_2$ catalyst gave excellent conversions and selectivities for the primary and secondary allylic alcohols tested, and selectivities were much higher when Au/CeO$_2$ was used compared with Au-Pd/CeO$_2$ and Au-Pd/TiO$_2$. For example, in the formation of 1-octen-3-one 46, >99% conversion was achieved with all three catalysts, but selectivity for the desired product was 26% with Au-Pd/CeO$_2$ and 40% with Au-Pd/TiO$_2$. This was due to isomerisation of the starting material, double bond hydrogenation and polymerisation in the presence of Pd. The carvone and octenone products 45-47 are of interest in the flavour and fragrance chemistry.

The Choudhary group reported the oxidation of a range of primary alcohols in the presence of a Au/MgO (HDP) catalyst and molecular oxygen, again at temperatures greater than 100 °C. Benzyl alcohol 39 and $p$-methoxybenzyl
alcohol 48, and non-benzyllic alcohols 2-phenylethanol 49, 3-phenyl-1-propanol 50 and 3-(4-methoxyphenyl)-1-propanol 51 were tested (Table 6). Selectivity for the corresponding aldehydes was high, with some ester by-products also reported. Benzyl alcohol 39 and 4-methoxybenzyl alcohol 48 were the best substrates with 55% and 52% conversion of the alcohol respectively, and both substrates gave 95% selectivity for the aldehyde product. Non-benzyllic alcohol 2-phenylethanol 49 had a lower conversion of 46%, and a lower aldehyde selectivity of 61%. 3-Phenyl-1-propanol 50 and 3-(4-methoxyphenyl)-1-propanol 51 also had low conversions of 42% and 30% respectively. However, these had higher aldehyde selectivities of 86% and 79% compared to 2-phenylethanol 49. Overall, non-benzyllic substrates showed lower conversion and lower aldehyde selectivity than the benzyl alcohols tested.

The group suggested that any acid formed immediately reacted with unreacted starting alcohol to form the corresponding ester. When pure benzaldehyde 40 was subjected to the reaction conditions, 95% of benzoic acid 41 was recovered. The oxidation of 4-methoxybenzaldehyde 52 and phenylacetaldehyde 53 gave the corresponding acids in 89% and 80% yields respectively. It was thought that when these acids were formed in the presence of primary alcohols in the previous reactions, ester products were formed quickly leaving no acid products.
Table 7 – Oxidation of aldehydes in the presence of Au/MgO (8 wt%) with O$_2$ (0.95 bar). Catalyst prepared by homogeneous deposition-precipitation.$^{44}$

The oxidative esterification of cinnamyl alcohol $^{54}$ was reported along with a range of primary alcohols, with Au/CeO$_2$ and Au/ZrO$_2$ catalysts and O$_2$ as the oxidant (Scheme 18). Benzyl alcohol $^{39}$ and cinnamyl alcohol $^{54}$ gave excellent conversions and selectivities of over 95%. Heterocyclic alcohol 2-pyridinemethanol $^{55}$ had a lower conversion of 51% with 82% selectivity for the ester. Furfuryl alcohol was also tested, but had a very low conversion of 6%. The substrates were also all tested with ethanol as the esterification partner, and this reaction generally gave slightly lower conversions of 70% for benzyl alcohol $^{39}$ and 92% for cinnamyl alcohol $^{54}$. In the case of 2-pyridinemethanol $^{55}$, a higher conversion of 68% was achieved with ethanol, but selectivity was lower at 62%.$^{45}$

Scheme 18 – Oxidative esterification of primary alcohols the presence of Au/CeO$_2$ (3 wt%) and O$_2$ (1 bar) in MeOH with Cs$_2$CO$_3$ additive (10 mol%) at 25 °C for 3 h. Selectivity for desired ester product shown. Conversion and selectivity determined by GC. $^{45}$
The oxidative esterification of substituted benzyl alcohols and aliphatic primary alcohols with methanol has also been reported using Au/MgO under 2 bar pressure of oxygen. Methoxy, methyl and hydroxy substituted benzyl alcohols showed high conversions of 81-99% and selectivities for the ester products of 80-99%, with $p$-trifluoromethyl benzyl alcohol giving a lower conversion of just 12%. Aliphatic alcohol 1-octanol gave an excellent conversion of 89% with a selectivity of 98% for desired product methyl octanoate.\(^{46}\)

The oxidation of a range of primary and secondary alcohols was also reported with O\(_2\) and Au/MgO (Table 8). After initial reaction optimisation with benzyl alcohol\(^\text{39}\), the conditions were extended to include various natural monoterpenic alcohols, giving ketone products which are important in flavour and fragrance chemistry. An excellent conversion of 97% was achieved with benzylic secondary alcohol 1-phenyl-1-propanol, with desired ketone 1-phenyl-1-propanone 56 the sole product with 100% selectivity. Carvone 45 was also the only product in the oxidation of carveol, with 86% conversion. A lower selectivity for isopulegone 57 of 80% was observed, with a low conversion of 34%. While menthone 58 was the sole product in the oxidation of menthol, conversion was very low at 12%. However, the oxidation of isoborneol was very successful, with 98% conversion and 100% selectivity for camphor 59.\(^{47}\)

<table>
<thead>
<tr>
<th>Major product</th>
<th>Temperature (°C)</th>
<th>Conversion (%)</th>
<th>Selectivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>130</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>45</td>
<td>120</td>
<td>86</td>
<td>100</td>
</tr>
<tr>
<td>57</td>
<td>120</td>
<td>34</td>
<td>80</td>
</tr>
<tr>
<td>58</td>
<td>130</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>59</td>
<td>110</td>
<td>98</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 8 – Oxidation of secondary alcohols with Au/MgO (2.6 wt%) and O\(_2\) (10 bar) in methanol. Selectivity for desired product shown. Conversion and selectivity determined by GC.\(^{47}\)
Gold nanoparticles supported on a mixture of Al$_2$O$_3$ and CeO$_2$ have been tested in the oxidation of long chain fatty alcohol 1-tetradecanol under 1 atm oxygen. Moderate conversions were achieved with high selectivity for the aldehyde product, and some ester by-products formed.$^{48}$ Furthermore, Au/Al$_2$O$_3$ has been identified as a good catalyst for the oxidation of arabinose 60 to arabinoic acid 61 under flowing oxygen. At pH 9 in water at 60 °C, 99% conversion and 99% selectivity for arabinoic acid was achieved after 200 min.$^{49}$

Scheme 19 – Oxidation of arabinose 60 with commercially available Au/Al$_2$O$_3$ (1 wt%) and flowing O$_2$ for 200 min. Conversion and selectivity determined by HPLC.$^{49}$

1.2.3 Recent extensions of oxidation substrate scope

This section details extensions to the substrate scope of the gold nanoparticle catalysed oxidation reported during the course of this project.

In 2016, the oxidation of cinnamyl alcohol 54 was reported with a bimetallic Au-Pd/TiO$_2$ catalyst in a flow reactor. The main products of this transformation were the desired oxidised product cinnamaldehyde 44, reduced product 3-phenyl-1-propanol 50 and hydrogenolysis product trans-β-methylstyrene 62 (Scheme 20). The conversion of cinnamyl alcohol was greatest (58%) at 120 °C, with selectivity for cinnamaldehyde 44 around 60% after 1 h. 3-Phenyl-1-propanol 50 was present in around 10% yield and trans-β-methylstyrene 62 was formed in less than 5% yield.$^{50}$

Scheme 20 – Oxidation of cinnamyl alcohol 54 with bimetallic Au-Pd/TiO$_2$ under flowing oxygen.$^{50}$
The oxidation of a range of secondary benzylic alcohols has also been reported with aqueous TBHP oxidant with no catalysts. Using 6-10 equivalents of TBHP, excellent yields of substituted acetophenones 63-64 and benzophenones 65-66 were achieved as well as cyclic systems 67-69.\(^{51}\)

![Scheme 21 – Metal free oxidation of secondary benzylic alcohols with aqueous TBHP (6-10 eq.). Isolated yields shown.\(^{51}\)](image)

This work highlights the significant potential background reactions in the oxidation of benzylic alcohols, and the need for control reactions when testing supported gold nanoparticle catalysts on these systems. In some earlier literature reports, these background reactions were not highlighted leading to uncertainty around the benefits of the catalysts.

In 2017, gold nanoparticles supported on Al\(_2\)O\(_3\), TiO\(_2\) and ZnO were tested on a range of primary alcohols and some secondary allylic alcohols, in the presence of a catalytic amount of H\(_2\)O\(_2\) or TBHP and 1 atm oxygen (Scheme 22). The substrates included substituted benzylic and allylic primary alcohols with good to excellent yields with Au/Al\(_2\)O\(_3\). Notably, two secondary alcohols were included in this work, forming ketones cyclohexen-1-one 70 and 3,5,5-trimethylcyclohexen-1-one 71 with excellent conversions, although the volatility of 70 caused isolation difficulties. The reaction was also tested in a continuous process, with excellent yields also achieved under flow conditions.\(^{52}\)
Scheme 22 – Oxidation of primary and secondary alcohols with O\textsubscript{2} (1 bar) in the presence of commercially available Au/Al\textsubscript{2}O\textsubscript{3} and TBHP. Isolated yields shown.\textsuperscript{52}

The oxidation of glucose to gluconic acid has also recently been reported with Au/Al\textsubscript{2}O\textsubscript{3} and O\textsubscript{2}, giving 80% conversion and over 90% selectivity for gluconic acid.\textsuperscript{53}

1.2.4 Other applications of supported gold nanoparticle catalysis

Gold nanoparticle catalysts have been applied to the alkylation of amines with alcohols. In 2012, a commercially available Au/TiO\textsubscript{2} catalyst (1 wt %) was used for the alkylation of aniline with benzyl alcohol (Scheme 23). The alcohol was first oxidised to the aldehyde, then condensed with aniline to form imine 72, which was then reduced to the corresponding amine 73. It was thought that the reduction was performed by a hydride species adsorbed to the metal during the reaction.\textsuperscript{54}

Scheme 23 – Alkylation of amines by benzyl alcohol 39 with a commercially available Au/TiO\textsubscript{2} (1 wt%) catalyst, O\textsubscript{2} (50 bar), and the reduction performed by a gold-adsorbed hydride species.\textsuperscript{54}

The reaction was performed in a continuous flow reactor, and gave high yields for a broad range of aromatic and aliphatic alcohols and amines, including application to the synthesis of the anti-Parkinson’s drug Piribedil 74 (Scheme 24).\textsuperscript{54}
Scheme 24 - Synthesis of Piribedil 74 using commercially available Au/TiO$_2$ catalyst and O$_2$ (50 bar). Isolated yield shown.$^{54}$

Another route to secondary amines was reported using Au/Al$_2$O$_3$ as the catalyst in the reductive aminations of aldehydes with nitroarenes (Scheme 25). The nitroarene 75 was reduced to the aniline 76 in situ and reductive amination then took place with an aldehyde. High yields of amine products 77 and 78 were reported using Au/Al$_2$O$_3$ in toluene at high temperature in a flow system under hydrogen pressure. The reaction was also successful when using $p$-methyl nitrobenzene as the nitroarene starting material.$^{55}$

Scheme 25 – Reductive amination of aldehydes with nitroarenes. Yields determined by GC analysis.$^{55}$

Gold nanoparticle catalysts supported on a range of materials have also been reported in the catalysis of Suzuki-Miyaura couplings. Au/MgO was again found to be the best catalyst, giving excellent yields of biaryl products 79-81 (Scheme 26). Other metal oxide supported catalysts Au/CaO, Au/BaO and Au/SrO also gave excellent yields of 88-89% in the model reaction between iodobenzene and phenylboronic acid.$^{56}$
The synthesis of propargylic amines in a three-component reaction catalysed by supported gold nanoparticles has been reported (Scheme 27). A range of supports were tested including TiO$_2$, SiO$_2$ and Fe$_2$O$_3$, but CeO$_2$ and ZrO$_2$ were found to be the best for this application. Propargylic amine 82 was formed in quantitative yield, and the procedure was applied to a range of aldehyde, amine and alkyne partners with mostly excellent yields.\textsuperscript{57}

The supported gold nanoparticle oxidation of HMF 2 in the presence of base has been investigated using a range of supports. A report in 2008 used NaOMe as the base with an Au/TiO$_2$ catalyst giving furan-2,5-dimethylcarboxylate 83 in 98%, with an O$_2$ pressure of 4 bar in an autoclave (Scheme 28). The pressure was required as at room temperature and 1 bar of O$_2$, the main product was 5-hydroxymethyl methylfuroate 84.\textsuperscript{58}

---

**Scheme 26** – Au/MgO (7.5 wt%) catalysed Suzuki-Miyaura coupling of aryl halides with phenylboronic acids. Catalyst prepared by homogeneous deposition-precipitation. Isolated yields shown.\textsuperscript{56}

**Scheme 27** – Au/CeO$_2$ catalysed three-component synthesis of 82. Catalyst prepared by deposition-precipitation. Isolated yield shown.\textsuperscript{57}
27

Scheme 28 – Oxidation of HMF in the presence of NaOMe and commercially available Au/TiO₂ (1 wt%), with O₂ (1 bar).⁵⁸

The oxidative esterification of HMF 2 was later reported in the absence of base, with O₂ as the oxidant. Au/CeO₂ was found to be the most selective catalyst for this reaction, giving >99% conversion and >99% selectivity for furan-2,5-dimethylcarboxylate 83. Au/TiO₂, Au/Fe₂O₃ and Au/C catalysts were also tested, all giving conversions over 90% but with low selectivities for the desired product 83, with the major product instead found as 5-hydroxymethyl methylfuroate 84.⁵⁹

This transformation was also reported with Au/TiO₂ and sodium hydroxide as the base in a large excess, at a much lower temperature of 30 °C and 20 bar oxygen pressure, giving FDCA 3 in 71% yield after 18 h.⁶⁰ Analysing the result of the control reaction in the absence of O₂, HMF 2 was found to disproportionate via the Cannizzaro reaction to 2,5-bis(hydroxymethyl)furan 85 and 5-hydroxymethylfurancarboxylic acid (HMFCA) 86 (Scheme 29).⁶¹

![Scheme 29 – Disproportionation of HMF 2 via the Cannizzaro reaction. Isolated yields shown.⁶¹](image)

The formation of FDCA 3 from HMF 2 was also reported in a base-free procedure, with Au/HT (hydrotalcite (HT) is a mixture of Mg and Al), Au/MgO, Au/Al₂O₃, Au/SiO₂ and Au/C tested, under O₂ flow in water (Table 9). Au/HT and Au/MgO showed the highest conversions of HMF 2, but Au/HT had a much better selectivity for 3, with Au/MgO producing mainly HMFCA 86. Au/Al₂O₃ produced more 3 than 86, but overall the conversion was much lower at 35%. In the presence of Au/C, conversion of HMF 2 was again low at 28%, and 86 was produced in 6% compared to 1% of 3. Au/SiO₂ showed no conversion of HMF 2 with no detection of any products.⁶²
The presence of 5-formyl-2-furancarboxylic acid intermediate was also observed.\textsuperscript{62}

Gold nanoparticle catalysts have also been used in reductive processes including hydrogenations and reductive aminations. The hydrogenation of aqueous or ethanolic benzaldehyde \textsuperscript{40} has been achieved using Au/Al\textsubscript{2}O\textsubscript{3} with hydrogen under flow conditions with 100\% selectivity for benzyl alcohol \textsuperscript{39,63}

In summary, the synthesis and applications of gold nanoparticle catalysts supported on a range of materials have been widely reported. Initially, activity was assessed through the oxidation of benzyl alcohol \textsuperscript{39}, but more recently extensions to the substrate scope have been investigated. Many gold nanoparticle catalysed oxidations of primary alcohols have been described, and some examples of secondary alcohols have also been reported, although these are more limited in terms of substrates and catalysts used.\textsuperscript{45,52} These heterogeneous catalysts have also found diverse applications in the synthesis of amines, C-C bond formation, and the valorisation of biomass materials.\textsuperscript{54,56,62}

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Conversion of HMF 2 (%)</th>
<th>Yield of FDCA 3 (%)</th>
<th>Yield of HMFCA 86 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au/HT</td>
<td>&gt;99</td>
<td>&gt;99</td>
<td>0</td>
</tr>
<tr>
<td>Au/MgO</td>
<td>&gt;99</td>
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</tr>
<tr>
<td>Au/Al\textsubscript{2}O\textsubscript{3}</td>
<td>35</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>Au/C</td>
<td>28</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 9 – Oxidation of HMF 2 in the presence of supported Au catalysts (2 wt\%) under flowing O\textsubscript{2}. Catalysts prepared by deposition-precipitation. Conversions and yields determined by HPLC.
1.3 Transaminase enzymes

Single isomer chiral compounds are important in the pharmaceutical industry, as many drug molecules have multiple chiral centres, due to the three-dimensional nature of many drug interactions with proteins and enzyme sites in the body. Traditional synthetic routes to asymmetric compounds are often multi-step, include complex and challenging chemistry and are overall very low-yielding, with poor atom efficiencies. Biocatalysis has been explored as an alternative to traditional organic synthesis to generate enantiopure compounds, and has many advantages. Biocatalysis is the use of the natural function of enzymes to catalyse chemical reactions. Enzymes accept a range of substrates in nature, and can be manipulated to accept non-natural substrates through reaction and protein engineering. Enzymes possess a range of other advantages, exhibiting extremely high stereo-, regio- and chemoselectivity and reacting under mild aqueous conditions, which reduces costs and is more environmentally sustainable.64

Chiral amines are an important functionality in pharmaceuticals and natural products, and can be produced chemically through the use of chiral auxiliaries,65 chiral organocatalysts,66 and the combination of metal catalysts and chiral ligands, such as rhodium catalysed asymmetric hydrogenation.67 Possible enzymes for the production of chiral amines include amino acid dehydrogenases (AADHs) and transaminases (TAs) (or aminotransferases (ATAs)).68 AADHs have been explored for the production of chiral amines, but these enzymes require redox cofactor recycling which can be expensive.69

Transaminases are enzymes which catalyse the transfer of an amino group to the carbonyl group of an acceptor such as an aldehyde, ketone or ketoacid. The enzymes require a catalytic amount of co-factor pyridoxal-5-phosphate (PLP) and can perform the reaction in a stereoselective manner. Transaminases are grouped into six classes based on sequence alignment and evolutionary relatedness, for example class IV contains D-aspartate-D-glutamate transaminases and class VI consists of the sugar transaminases. Class III transaminases have a broader substrate scope and accept a wide range of aldehydes and ketones as amino acceptors.70 Transaminases can also be labelled by their positional specificity, as α-, β-, γ- or ω-transaminases. Examples of transaminases from different classes and varying positional specificities are shown in Table 10. The α-transaminases catalyse the transfer of an amino group
to and from the α-position of a carboxylic acid, commonly with amino acids such as alanine and serine as amine donors and α-ketoacid acceptors such as pyruvate 87 and α-ketoglutarate 88. The β-transaminases use β-amino acids as the amine donors such as β-alanine and β-homoleucine 89. The γ-transaminases are also known as 4-aminobutyrate transaminases (GABA transaminases) as they use GABA as the amino donor with a range of aldehyde and ketone amino acceptors. The ω-transaminases have a wide substrate scope and can accept a range of amine donors and acceptors. Accepted donors include amines such as phenethylamine 90 as well as amino acids such as L-ornithine, and many aldehydes and ketones are accepted along with the ketoacids shown.\(^\text{70}\) As the ω-transaminases showed a relaxed substrate specificity, their application in organic chemistry through biocatalysis has been explored in recent years.

<table>
<thead>
<tr>
<th>Transaminase</th>
<th>Main donor</th>
<th>Main acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
<td><strong>Class</strong></td>
<td><strong>Positional specificity</strong></td>
</tr>
<tr>
<td>Alanine TAm</td>
<td>I/II</td>
<td>α</td>
</tr>
<tr>
<td>D-Alanine TAm</td>
<td>VI</td>
<td>α</td>
</tr>
<tr>
<td>β-Amino carboxylic acid TAm</td>
<td>III</td>
<td>β</td>
</tr>
<tr>
<td>GABA TAm</td>
<td>III</td>
<td>γ</td>
</tr>
<tr>
<td>ω-Amino acid TAm</td>
<td>III</td>
<td>ω</td>
</tr>
<tr>
<td>Ornithine TAm</td>
<td>III</td>
<td>ω</td>
</tr>
</tbody>
</table>

Table 10 – Examples of transaminases of different classes and positional specificities with their main amino donors and acceptors.\(^\text{70}\)
Transaminases are PLP cofactor dependent enzymes which catalyse the transfer of an amino group from an amine donor to an amino acceptor with a carbonyl moiety, through the ping-pong bi-bi mechanism (Scheme 30).

The mechanism can be thought of as two half-reactions, the first being the binding of the amine donor to PLP, and the amination of PLP to pyridoxamine-5-phosphate (PMP), releasing the corresponding keto product of the donor. In the second half-reaction, the amino group is transferred from PMP to the amine acceptor and the transamination is complete. The stereoselectivity is provided by the active site of the enzyme and its interactions with the donor, acceptor, and PLP.\(^{68,69,71}\)

Transaminases can be used to produce chiral amines through two main approaches, kinetic resolution and asymmetric synthesis (Scheme 31).\(^{69}\) Kinetic resolution is the process of using a transaminase to selectively convert one enantiomer of a racemic amine mixture to the corresponding carbonyl compound, leaving a pure sample of the desired chiral amine. In this process, the yield of the desired enantiomer can only reach a maximum of 50%, as the undesired
enantiomer is removed from the racemate. Although this approach is straightforward, it can also have disadvantages, including the inhibition of the enzyme by the ketone product and donor and a maximum of 50% of the desired amine product.

The direct asymmetric synthesis of chiral amines from prochiral ketones using a stereoselective transaminase is a desirable method as the starting material can be quantitatively converted into one enantiomer of a chiral amine. However, this approach can also be adversely affected by the equilibrium of the reaction being unfavourable towards the desired amine product. The problem of the equilibrium can be combated in several different ways, including co-product removal, co-product recycling and use of specific amine donors. Methods of shifting the unfavourable transaminase equilibrium using amine donors are discussed further in Section 1.3.3.

A common amine donor in the synthesis of chiral amines using transaminases is alanine, due to its low cost, high abundance and solubility in water. Therefore, many techniques have been developed to remove the co-product pyruvate. The addition of multiple enzymes to remove pyruvate have been developed, including lactate dehydrogenase (LDH), glucose dehydrogenase (GDH) and pyruvate decarboxylase (PDC) (Scheme 32). The removal of pyruvate has been developed using LDH to reduce pyruvate to lactate. The LDH enzyme requires NADH as a co-factor, which can be expensive to use. This co-factor can be recycled using GDH with the addition of glucose, reducing the cost associated with NADH. The removal of pyruvate co-product generated from the amine donor alanine has been investigated using LDH in a whole cell approach, as LDH is produced \textit{in situ} by \textit{E. coli}. In this case, the equilibrium was successfully
shifted to the desired chiral amine product, but the whole cell approach was affected by problems including large amounts of cells being required and the amine product being toxic to the cell. Another method for pyruvate removal is by decarboxylation with a PDC, forming acetaldehyde and carbon dioxide. This also faces problems as the acetaldehyde product is aminated by the transaminase, which is detrimental to the desired process.

Scheme 32 – Transaminase reaction using alanine as the amine donor, showing different techniques for the removal of pyruvate co-product. LDH: lactate dehydrogenase, GDH: glucose dehydrogenase, PDC: pyruvate decarboxylase, NADH: nicotinamide adenine dinucleotide.

Recycling of pyruvate back into alanine has been investigated using an amino acid dehydrogenase (AADH) in the presence of NADH cofactor, with recycling of NADH by formate dehydrogenase (FDH) or GDH as previously shown. This strategy was successful but only with high concentrations of alanine.
Scheme 33 – Transaminase reaction using alanine as the amine donor, showing recycling of pyruvate co-product. AADH: amino acid dehydrogenase, NAD(P)H: nicotinamide adenine dinucleotide (phosphate), FDH: formate dehydrogenase.\textsuperscript{69,76}

Both the AADH and LDH processes require the use of NADH as a cofactor, which is expensive, although the recycling of pyruvate is a desirable process in terms of sustainability.\textsuperscript{73}

A full deracemisation process has been designed by combining a kinetic resolution with asymmetric synthesis. This deracemisation technique involved the kinetic resolution of a racemic amine with an (S)-transaminase, followed by conversion of the ketone product using a transaminase of the opposite stereoselectivity (Scheme 34). An (S)-selective transaminase from \textit{Polaromonas} sp. (Ps-TAm) was selected for the first step, with \(\alpha\)-ketoglutaric acid \textsuperscript{88} chosen from a panel of keto-acids and esters tested. The second step was performed with D-alanine as the amine donor and a transaminase from \textit{Mycobacterium vanbaalenii} (Mv-TAm). An LDH system was used for pyruvate removal, with NADH recycled using a GDH. Amine product (S)-\textsuperscript{91} was produced with 99% conversion and in >99% ee.\textsuperscript{74} This process can in theory produce 100% of the desired amine, however two transaminases must be employed rather than one.\textsuperscript{69,77}
Scheme 34 – Deracemisation of racemic amines using two transaminases and LDH pyruvate recycling. (S)-TAm: Ps-TAm, (R)-TAm: Mv-TAm. Conversion and ee determined by HPLC.74

1.3.1 Selected transaminases

In the late 1990s, Shin et al. reported the asymmetric synthesis of chiral amines with the ω-transaminase Vibrio fluvialis JS17 (Vf-TAm), and proposed a binding model involving two pockets, one large and one small.75,78 A transaminase from Chromobacterium violaceum (Cv-TAm) was discovered in 2007 through its 38% sequence homology to Vf-TAm using a BLAST search.68 Cv-TAm was found to be (S)-selective and have high activities towards aromatic amines such as (S)-MBA and (S)-aminindane, with no reactivity detected when using (R)-MBA, matching the (S)-selectivity of Vf-TAm. Other accepted donors included 1-methyl-3-phenylpropylamine, benzylamine and L-alanine. The amino acceptor scope was tested with a range of substrates including ketoacids, aldehydes and ketones using (S)-MBA as the amine donor (Table 11). Pyruvate 87 was an excellent amino acceptor in this reaction, with quantitative conversion reached at equilibrium. Hydroxypyruvate 92 gave a good conversion of 65%, and glyoxylate 93 was another excellent substrate with quantitative conversion at equilibrium. Aliphatic aldehyde butanal 37 was accepted with a good conversion of 74-78%, and aromatic benzaldehyde 40 showed 90% conversion. Phenylacetaldehyde 53 was also accepted with 68% conversion. Conjugated aldehyde cinnamaldehyde 44 and substituted aromatic aldehyde vanillin 94 were both accepted with high initial rates and high conversions. Cyclohexanone 95 was the best accepted ketone with an increased initial rate compared to Vf-TAm. All initial rates were higher with Cv-TAm than with Vf-TAm, with very large increases observed with hydroxypyruvate 92 and glyoxylate 93. Cv-TAm showed a very broad substrate scope with increased activity, making it a useful enzyme for many transaminase reactions and applications of biocatalysis.68
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Conversion at equilibrium (%)</th>
<th>Relative initial rate Cv-TAm (%)</th>
<th>Relative initial rate Vf-TAm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>95-100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>92</td>
<td>65</td>
<td>144.1</td>
<td>29.2</td>
</tr>
<tr>
<td>93</td>
<td>95-100</td>
<td>176.2</td>
<td>60.2</td>
</tr>
<tr>
<td>37</td>
<td>74-78</td>
<td>122.9</td>
<td>113.5</td>
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<tr>
<td>40</td>
<td>90</td>
<td>91.2</td>
<td>72.8</td>
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<tr>
<td>53</td>
<td>68</td>
<td>107.0</td>
<td>68.5</td>
</tr>
<tr>
<td>44</td>
<td>&gt;60*</td>
<td>79.7</td>
<td>31.5</td>
</tr>
<tr>
<td>94</td>
<td>&gt;60*</td>
<td>61.7</td>
<td>n.d</td>
</tr>
<tr>
<td>95</td>
<td>n.d.</td>
<td>29.2</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Table 11 – Substrate scope of Cv-TAm compared to Vf-TAm. Conversion and initial rates based on (S)-MBA depletion determined by HPLC. Initial rates calculated after 3 min reaction time. Equilibrium reached at 2-3 h. 10 mM substrate concentration and 10 mM (S)-MBA. *Reactions not run to equilibrium.
In 2010, the first application of transaminase catalysis in an industrial setting was reported by Savile et al., for the synthesis of anti-diabetic drug Sitagliptin 96 (Scheme 35). Commercially available transaminases were found to be inactive towards the pro-sitagliptin ketone 97. Using docking studies of commercial (R)-selective transaminase ATA-117, it was suggested that the bulky ketone substrate would be unable to bind in the active site due to steric interference in the small binding pocket and unfavourable interactions in the large binding pocket. Enzyme mutagenesis was undertaken to improve the active site interactions of the prositagliptin ketone, resulting in a 75-fold increase in activity after two rounds of mutation. The enzyme then needed to be improved further taking into account process conditions. Due to the low solubility of the substrate in aqueous medium, tolerance of high concentrations of DMSO was required, as well as high concentrations of IPA to shift the equilibrium. High temperatures of above 40 °C and an excellent ee of >99.9% were also required. After 11 further rounds of mutation, a variant was found which provided Sitagliptin in 92% yield with >99.95% ee, and was tolerant to DMSO, IPA and elevated temperatures. This resulted in a 13% increase in overall yield and a 19% reduction in waste compared to the previously developed rhodium catalysed process.

Scheme 35 – Synthesis of Sitagliptin with a variant of ATA-117 and isopropylamine donor. The reaction was performed with 97 (200 g/L), DMSO (50%), IPA (4–10 eq.) enzyme (3 wt%) in triethanolamine buffer pH 8.5 (200 mM) at 45 °C for 24 h, with in situ acetone removal under reduced pressure.

The group went on to show activity for other products which had been difficult to access both chemically and enzymatically, which were now feasible with these enzymes (Scheme 36).
Scheme 36 – Synthesis of chiral amines with variants of ATA-117 and isopropylamine as the amine donor. The reactions were performed in triethanolamine buffer pH 8.5 (100 mM), at 60 °C for 24 h. Conversions were determined by GC and isolated yields are also shown.\(^4\)

Due to its tolerance of a very bulky substrate in the synthesis of Sitagliptin \(^97\), and its tolerance of co-solvents and IPA, ArRMut11 was used in the synthesis of 17-\(\alpha\)-amino steroids (Figure 2). In the traditional chemical synthesis of 17-amino steroids such as these, reductive amination and deprotection result in the \(\beta\)-epimer at the amine position. The use of transaminases provided access to the \(\alpha\)-epimers such as steroid \(^98\) which exhibits potent activity as a sulfatase inhibitor. DMF (35 vol%) was used as a co-solvent due to the insolubility of the steroid starting materials. The process provided access to the \(\alpha\)-epimers of these biologically active steroidal derivatives in high yields and stereoselectivities on a preparative scale.\(^79\)

Figure 2 – Steroids synthesised using ArRMut11, with IPA as the amine donor. Reactions were performed on 0.17 mmol (50 mg) substrate scale (8.5 mM) with IPA (120 eq.), DMF co-solvent, pH 10, 45 °C. Isolated yields shown.\(^79\)

The majority of the transaminases identified before 2010 were \((S)\)-selective, and a new approach was developed to address the lack of \((R)\)-selective
transaminases available. Mv-TAm from *Mycobacterium vanbaalenii* was identified during this search for (R)-selective transaminases. A strategy was developed where key residues and motifs in the amino acid sequence of known transaminases were identified to allow predictions of substrate tolerance and enantiopreference using sequence data. Existing databases and libraries were then searched *in silico* for the desired sequences, and 17 (R)-selective transaminases were identified using the technique. These transaminases were overexpressed in *E. coli*, and included Mv-TAm. Mv-TAm was tested with a range of aromatic and aliphatic ketone substrates and excellent ee values were achieved with all substrates (Table 12). D-Alanine was used as the amine donor with a LDH/GDH pyruvate removal system. Aliphatic ketones were well accepted achieving good conversions of 69% with 2-heptanone 99 and 68% with 4-methyl-2-pentanone 100. Excellent conversions of 89% with benzylacetone 101 and 85% with vanillin ketone 102 were achieved.

![Diagram of Mv-TAm reaction]

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Substrate 99" /></td>
<td>69</td>
<td>&gt;99</td>
</tr>
<tr>
<td><img src="image2.png" alt="Substrate 100" /></td>
<td>68</td>
<td>&gt;99</td>
</tr>
<tr>
<td><img src="image3.png" alt="Substrate 101" /></td>
<td>89</td>
<td>&gt;99</td>
</tr>
<tr>
<td><img src="image4.png" alt="Substrate 102" /></td>
<td>85</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

Table 12 – Transaminase reactions with Mv-TAm, D-alanine, LDH pyruvate removal, GDH NADH recycling system. Conversion determined by HPLC and ee determined by GC.
Cyclohexanone was also reported as an accepted substrate of Mv-TAm with a range of amine donors. 50% conversion was achieved with alanine and an AlaDH/FDH pyruvate removal system, and 39% conversion was reported with IPA as the amine donor.\(^82\)

Mv-TAm has also been reported in the kinetic resolution of substituted methylbenzylamines (Figure 3), showing tolerance to bromo, fluoro and methyl substituents at the para- and meta positions of the phenyl ring.\(^83\)

![Figure 3 – Products from the kinetic resolution of substituted methylbenzylamines with Mv-TAm and pyruvate. Conversion yields and ee values determined by HPLC.\(^83\)](image)

A range of transaminases are also commercially available in the form of screening kits developed by Codexis.\(^84\) These transaminases are usually referred to as amine transaminases (ATA) followed by a product code, for example ATA-117. These enzymes are widely used in the literature and have been used to develop many synthetic strategies and transaminase applications.

### 1.3.2 Synthesis of pharmaceutically relevant molecules

A transaminase mediated kinetic resolution has been applied to a synthetic route from rac-\(103\) to \((R)-4\)-phenylpyrrolidin-2-one 104 (Scheme 37), providing access to cyclic analogues of GABA derivatives, which are of pharmacological interest.\(^85\)

![Scheme 37 – Synthesis of GABA derivatives using ATA-117 and LDH pyruvate recycling.\(^85\)](image)
The synthesis of cyclic compounds via transamination followed by spontaneous cyclisation was also reported as a route to piperidone structures which are present in many natural products. \((R)\)- and \((S)\)-selective transaminases ATA-117 and ATA-113 were used to form 6-methyl-2-piperidone \(105\) in over 90% isolated yield with over 99% ee (Scheme 38).\(^{86}\)

\[
\text{O} \quad \begin{array}{c}
\text{OEt} \\
\text{H} \\
\text{NH}_2 \
\text{O}
\end{array}
\xrightarrow{\text{ATA-117 or ATA-113}}
\begin{array}{c}
\text{H} \\
\text{NH}_2 \\
\text{O}
\end{array}
\xrightarrow{\text{imine}}
\begin{array}{c}
\text{H} \\
\text{O}
\end{array}
\]

>90% yield  >99% ee

Scheme 38 – Synthesis of 6-methyl-2-piperidone \(105\) with ATA-117 or ATA-113 with isopropylamine donor. The reaction was performed in KPi pH 9.5 (100 mM) at 20 °C on a 50 mL scale.\(^{86}\)

The piperidone structure is present in many pharmaceuticals including a potent potential chemotherapeutic drug \(106\) reported by Pfizer in 2012.\(^{87}\) The synthesis of the promising drug molecule was found to be unscalable and low yielding, and the molecule became a target for a simultaneous transamination and dynamic kinetic resolution (Scheme 39). Due to the racemisation of compound \(107\) in solution, the \(\alpha\)-position of the piperidone structure underwent dynamic kinetic resolution in the presence of the transaminase, with the ketone simultaneously undergoing transamination. The intermediate \(108\) was then directly reacted with imidazole \(109\) to form the desired product \(106\), which was recrystallised resulting in >98% product purity.\(^{88}\)

\[
\begin{array}{c}
\text{O} \\
\text{H} \\
\text{N} \\
\text{N} \\
\text{H} \\
\text{N} \\
\text{H}
\end{array}
\xrightarrow{\text{ATA-036, } ^1\text{PrNH}_2, \text{PLP}}
\begin{array}{c}
\text{H} \\
\text{N} \\
\text{N} \\
\text{H} \\
\text{N}
\end{array}
\xrightarrow{\text{imidazole}}
\begin{array}{c}
\text{H} \\
\text{N} \\
\text{N}
\end{array}
\]

85% assay,  >10:1 anti:syn,  >99% ee

Scheme 39 – Transaminase mediated synthesis of a smoothened receptor (SMO) inhibitor.\(^{88}\)
The synthesis of the anti-Parkinson’s and Alzheimer’s drug (S)-Rivastigmine 110 was reported using a novel transaminase from *Paracoccus denitrificans* (Pd-TAm) (Scheme 40). This synthetic pathway gave the target drug molecule in 66% yield over 4 steps, which was an improvement on the previous synthetic routes.\(^8^9\)

\[
\begin{align*}
\text{HO-} & \quad \text{NaH} \\
\text{THF, 5 h, 89\%} & \quad \text{Pd-TAm, alanine} \\
\text{O} & \quad \text{PLP, KPi pH 7} \\
\text{N} & \quad \text{LDH/GDH} \\
\text{(S)-Rivastigmine 110} & \quad 99\% \text{ ee (S)} \\
\end{align*}
\]

Scheme 40 – Synthesis of (S)-Rivastigmine 19 using Pd-TAm.\(^8^9\)

Transaminases can also be used in the kinetic resolution of neighbouring chiral centres, such as in the kinetic resolution of 2-phenylpropanal derivatives.\(^9^0\) In this process, racemic 2-phenylpropanal and derivatives were aminated with transaminases to give the β-chiral 2-phenylpropylamine products 111 (Scheme 41). Usually, kinetic resolutions have a maximum of 50% yield, but as the starting material racemised in solution, the yield of desired chiral product could reach 100% in principle. Experimentally, conversions of up to 99% were achieved for *ortho*-, *meta*- and *para*-methyl and methoxy substituted aromatic aldehydes, and on a preparative scale isolated yields were between 56% and 86% for (*R*)-selective *Pseudomonas putida* (Pp1-TAm) and 50% for (*S*)-selective *Hyphomonas neptunium* (Hn-TAm).\(^9^0\)
Scheme 41 – Kinetic resolution of 2-phenylpropanal derivatives on preparative scale with Pp1-TAm (Pseudomonas putida) and Hn-TAm (Hyphomonas neptunium), with L- or D-alanine as the amine donor.\textsuperscript{90}

Cyclic ketones were also investigated as transaminase substrates to define two chiral centres in a one-step dynamic asymmetric transamination, using Cv-TAm, Pp-TAm (Pseudomonas putida PP\_3718) and ArRMut11 (Scheme 42). The transaminases readily accepted IPA as the amine donor which helped to displace the unfavourable equilibrium. It was found that as the reaction time increased, conversion increased but cis:trans ratio decreased, however the ee values for the new amine stereocentre formed remained excellent throughout.\textsuperscript{82}

Scheme 42 – Transaminase reaction of methylcyclohexanone. ee refers to the new amine stereocentre formed.\textsuperscript{82}

The synthesis of chiral 2,5-disubstituted pyrrolidine product 112 was investigated using an enzyme cascade with transaminases and monoamine oxidases. The 1,4-diketone 113 underwent amination by an (S)- or (R)-selective transaminase, and the amine product spontaneously cyclised to give the pyrrolidine products (S)- and (R)-114 with excellent yields and ee values (Scheme 43). The next step in the cascade was formation of another chiral centre in the pyrrolidine ring using a monoamine oxidase enzyme (MAO) to give (2S,5R)- and (2R,5R)-112. The
synthesis of \((2S,5R)-112\) was also performed on a preparative scale in one pot, giving an isolated yield of 82% with >99% de.\(^{91}\)

Scheme 43 – Synthesis of pyrrolidines with ATA-117 and ATA-113 and spontaneous cyclisation.\(^{91}\)

1.3.3 Shifting the equilibrium with amine donors

Alanine has been a commonly used amine donor for transaminase reactions in the literature, but alternative amine donors have recently been developed to help shift the unfavourable equilibrium of the reaction. Isopropylamine is a useful amine donor as it and its co-product acetone are both volatile, making work-up procedures simpler. Isopropylamine is also very low cost, and due to its solubility in water many equivalents can be used to drive the equilibrium. Acetone removal through heat and reduced pressure can also be used to shift the equilibrium during the reaction.

New amine donors have been identified to help shift the unfavourable equilibrium of the transaminase reaction, and involve the spontaneous rearrangement or tautomerisation of the ketone by-product, resulting in equilibrium displacement.

In 2013, shifting of the unfavourable transaminase equilibrium using an amine donor, 3-aminocyclohexa-1,5-dienecarboxylic acid \(115\) (Scheme 44) was reported.\(^{92}\) The ketone product of this amine donor spontaneously tautomerises to 3-hydroxybenzoic acid \(116\), driving the equilibrium towards the desired chiral amine product. The approach gave quantitative conversions and >99% ee values for aromatic amines using Cv-TAm, and commercially available enzymes ATA-113 and ATA-117.\(^{92}\) However, this technique is limited by the cost of the amine donor, making the process not feasible for large scale reactions.
A sustainable process was investigated recently using bio-based diamines such as putrescine 117, cadaverine 118 and spermidine 119 as amine donors in the transaminase reaction. These amines were found to be incompatible with most \( \omega \)-transaminases, but have been accepted by \( \alpha,\omega \)-diamine transaminases (\( \alpha,\omega \)-DTAs) in the past, although only with pyruvate as the amino acceptor. In order to combine the substrate scope of \( \omega \)-transaminases with the \( \alpha,\omega \)-DTAs, a putrescine transaminase gene spuC was identified, the sequence similarity with well known \( \omega \)-TAs was compared, and multiple spuC genes selected for testing based on sequence alignment. Excellent conversions and ee values were obtained with 1.5 and 5 equivalents of amine donor with Pp-SpuC, and the substrate scope investigated with a range of aromatic and aliphatic ketones. This class of transaminases offer a sustainable process for shifting the equilibrium allowing access to pharmaceutically relevant chiral amines.

These techniques can provide not only an equilibrium shift resulting in increased yields, but a visual representation of activity, allowing high throughput screening and an alternative to HPLC analysis.
In 2014, a colorimetric assay to assess enzyme activity was reported which also helped to drive the equilibrium towards the desired products (Scheme 46). The commercially available xylylenediamine donor 120 was converted to the cyclic imine 121, which spontaneously tautomerised to the more stable aromatic isoindole 122, shifting the equilibrium towards the products while also polymerising to give a black precipitate. This allowed high throughput transaminase assays to be undertaken giving a clear indication of activity. The technique was tested on a range of aromatic and aliphatic ketones using commercially available (S)-selective ATA-113 which gave conversions of over 99% for most of the substrates selected. However, this technique suffered problems when being used for high throughput assays, as a level of black precipitate can be formed in background reactions, reducing the sensitivity of the assay.

![Scheme 46 – Colorimetric assay for the detection of transaminase activity, using xylylenediamine 120 as the amine donor with ATA-113. The reaction was performed in HEPES buffer at pH 7.5 and 30 °C.](image)

2-(4-Nitrophenyl)ethan-1-amine 123 has also been reported as the amine donor for a sensitive colorimetric assay where a red coloration is formed through reaction of the aldehyde by-product 124 with excess amine donor followed by tautomerisation to give 125 (Scheme 47). The assay was found to be highly sensitive, could be used quantitatively to estimate conversions of up to 30%, and was successfully used with several enzymes including Cv-TAm and ArRMut11. This assay provided an alternative to time consuming HPLC analysis and allowed the use of high throughput screening to identify active enzymes from large libraries.
Scheme 47 – Colorimetric assay for the detection of transaminase activity, using 2-(4-nitrophenyl)ethan-1-amine 123 as the amine donor.\(^{96}\)

Two more amine donors were tested for this application, cyano substituted 126 and cyclic nitro analogue 127 (Figure 4). Compound 126 did provide a colour change but formed a yellow precipitate. As PLP is required for the reaction and is also yellow, this donor was not applicable in transaminase screening. Cyclic compound 127 formed a black precipitate, and could therefore be used for screening transaminases which accept cyclic substrates. This method was also shown to be applicable in solid-phase high throughput screening of colonies to identify active transaminases.\(^{96}\)

Figure 4 – Amine donors tested in the development of colorimetric assays.\(^{96}\)

The use of but-2-ene-1,4-diamine 128 was also reported to shift the equilibrium in a similar process to xylylenediamine 120. Cis-128 was accepted by the commercially available transaminases from Codexis, including (S)-selective TA-P1-A06 and TA-P1-G06 and (R)-selective ATA-033 and ATA-025. High conversions of 79-99% were achieved with a range of ketones including substituted acetophenone derivatives, acetylpyridines, phenylacetone and benzylacetone with excellent ee values of >99%, but the ee values were slightly lower at 82-88% with aliphatic ketones. The reaction was performed on a preparative scale with α-bromoacetophenone 129 and ATA-033, giving (R)-130 in 83% isolated yield and >99% ee. Trans-128 was also tested and moderate
conversions were achieved, and this lower reactivity could be due to the isomerisation required for cyclisation.97

![Scheme 48 – Shifting the equilibrium with cis-but-2-ene-1,4-diamine 128. Isolated yield shown.97](image)

1.3.4 Transaminase applications in cascades

In 2012, the conversion of benzylic and cinnamic primary alcohols to amines was reported in a one-pot, multi-enzyme cascade. The first enzymatic step was the oxidation of the primary alcohol to the corresponding aldehyde, followed by conversion to the amine by a transaminase (Scheme 49). The galactose oxidase enzyme (GOase) from Fusarium was previously reported to oxidise primary alcohols using oxygen and catalytic copper, provided by copper sulfate. Removal of hydrogen peroxide was performed by a catalase or a horseradish peroxidase (HRP) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) system.

The enzymes used for the transaminase step were Vf-TAm and Paracoccus denitrificans (Pd-TAm), with alanine as the amine donor and a pyruvate recycling system involving an AlaDH, with NADH recycling by FDH or GDH systems. This cascade was optimised using benzyl alcohol 39 as the starting material, achieving >99% conversion with HRP/ABTS, Vf-TAm, NH₄Cl as the ammonia source and GDH for NADH recycling.98
A range of substituted benzyl alcohols were also tested, and finally cinnamyl alcohol 54 was found to be accepted by Pd-TAm to form cinnamylamine 131. This enzyme retained the E/Z geometry of the alkene, and this showed the potential applicability of the system in the synthesis of the antifungal agent naftifine 132 (Scheme 50).  

Scheme 49 – Oxidation-transamination cascade for the amination of alcohols. GOase: galactose oxidase, HRP: horseradish peroxidase, ABTS: 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), AlaDH: alanine dehydrogenase, FDH: formate dehydrogenase, GDH: glucose dehydrogenase.  

Scheme 50 – Formation of cinnamylamine 131 using galactose oxidase and Pd-TAm cascade, showing its application in the synthesis of antifungal agent naftifine 132. Yield of 131 determined by GC. Isolated yield of 132 shown from pure 131. GOase: galactose oxidase, HRP: horseradish peroxidase, ABTS: 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), AlaDH: alanine dehydrogenase, GDH: glucose dehydrogenase, MS: molecular sieves.  

Alcohol oxidases were also used in a five-enzyme cascade for the transformation of aliphatic alcohols to their corresponding primary amines (Scheme 51). Long chain alcohol oxidases (LCAO) were employed for the oxidation of fatty chain alcohols with FAD recycling under oxygen (2 bar), with any peroxide formed
removed by a catalase. A transaminase was then used to convert the aldehydes to primary amines, with alanine as the amine donor, AlaDH pyruvate recycling and GDH NADH recycling systems in the presence of ammonium chloride and glucose. This complex one-pot, five-enzyme cascade gave excellent yields of >99% of aliphatic substrate 133 and 98% of ω-chloro-1-amine 134. The presence of a nitrile group resulted in a lower yield of 42% of 135, and the system was not tolerant of free amine, hydroxy or thiol substituents on the aliphatic chain.99

Scheme 51 – Long chain alcohol oxidase (LCAO) and transaminase cascade. The reaction was performed in one pot in sodium Pi pH 10 at room temperature. Conversions to desired amine determined by GC analysis.99

Transaminases have also been reported in combination with alcohol dehydrogenases (ADH) to access amines from primary and secondary alcohols. Due to the required cofactors and recycling of NADH, a network of co-factor recycling was developed (Scheme 52). As alanine was used as the amine donor, pyruvate removal was required to displace the equilibrium, and the ADH required NADH recycling. A LDH enzyme was used for both of these purposes, and the resulting amines 136 and 137 were produced with high yields and ee values. The network was therefore a redox-neutral process, with the NADH produced in the ADH reaction recycled by the LDH while also removing the pyruvate co-product of the transaminase reaction. This meant that the reaction only required catalytic amounts of co-factors NAD+ and PLP.100 Another cascade for the production of chiral amines from alcohols has been reported using an amine dehydrogenase in place of the transaminase, together with an ADH.101
Scheme 52 – Alcohol dehydrogenase and transaminase cascade with lactate dehydrogenase for the removal of pyruvate and recycling of NADH. The reaction was performed in one pot in KPi pH 7.5 at 30 °C. Yields were determined by GC analysis.\textsuperscript{100}

Commercial transaminases ATA-113 and ATA-025 have also been used in a cascade with ene reductases belonging to the Old Yellow Enzyme (OYE) family. Ene reductases are enzymes which catalyse the reduction of an alkene in a stereoselective manner. These enzymes reduce only activated alkenes such as α,β-unsaturated ketones, for example 3-methyl-2-cyclohexenone \textsuperscript{138} and 1-(2\textit{H}-chromen-3-yl)ethan-1-one \textsuperscript{139} (Scheme 53). The combination of these two enzymes provided access to diastereomERICally enriched compounds \textsuperscript{140} and \textsuperscript{141} in a one-pot procedure. The OYE enzymes required NADPH as a co-factor, and a GDH system was used for NADPH production and recycling. Commercially available (S)- and (R)-selective transaminases from Codexis were used with IPA as the amine donor. Using combinations of ene reductases with transaminases of opposing stereoselectivity, conversions of 78-99\% were achieved with de values ranging from 86-98\%.\textsuperscript{102}
A three-step enzymatic cascade has been recently reported using a carboligase, transaminase and Pictet-Spenglerase enzymes to form isoquinoline product 142. The first step was the carboligation of 3-hydroxybenzaldehyde with acetaldehyde produced from the decarboxylation of pyruvate. The enzyme used was a thiamine diphosphate (ThDP) dependent acetohydroxy acid synthase from *E. coli* which catalysed both the decarboxylation of pyruvate and the carboligation to form hydroxyketone 143. This intermediate was formed in 95% yield and >99% ee. The transaminase used for the next step was Cv-TAm with IPA as the amine donor, producing the (S)-amine centre of the intermediate 144 in 91% yield and with 97% isomeric content (ic). The third step was an enzyme-catalysed Pictet-Spengler reaction, performed by a norcoclaurine synthase from *Thalictrum flavum*. Norcoclaurine synthase (NCS) enzymes catalyse the condensation of aldehydes or ketones with aryl amines and the subsequent ring closure to form tetrahydroisoquinoline products in a stereoselective manner. This three-enzyme cascade provided tetrahydroisoquinoline product (1S,3S,4R)-142 with an excellent yield of 88% over three steps, and a total isomeric content of 97%.
Scheme 54 – Carboligase, transaminase and Pictet-Spenglerase cascade reaction forming isoquinoline product 142. Carboligase acetohydroxy acid synthase from *E. coli* used with MgCl$_2$, ThDP and FAD in HEPES buffer pH 7.5, 30 °C. Cv-TAm used with PLP and IPA (10 eq.) at 30 °C. Pictet-Spenglerase norcoclaurine synthase variant from *Thalictrum flavum* used at 50 °C.\textsuperscript{103}

1.3.5 Immobilisation of transaminases

The use of transaminases in conditions other than buffer solution have been explored to extend their application in synthetic chemistry. In 2014 transaminases were shown to be active in an organic solvent, methyl-tert-butyl ether (MTBE), if saturated with water, for the synthesis of valinol 145 (Scheme 55). The reaction in organic solvent achieved higher conversions than in aqueous media using a transaminase from *Arthrobacter sp.* (ArR-TAm) where 95% conversion was obtained in MTBE compared with 85% conversion in aqueous buffer, although the two reactions used different amine donors. The enzyme was also able to be recycled after the reaction in organic solvent, and the activity and ee were retained for several 24 h cycles, with 73% conversion and >99% ee in the sixth cycle.\textsuperscript{104}

Scheme 55 – ArR-TAm used in organic solvent methyl tert-butyl ether with isopropylamine donor in the synthesis of valinol 145.\textsuperscript{104}

Following this work, transaminases were immobilised on methacrylate beads for use in flow chemistry, using MTBE as a solvent to prevent the leaching of PLP (Scheme 56). This work was the first example of cofactor-dependent enzymes
being used on immobilised systems for flow chemistry, as the transaminase and PLP were anchored on the polymer resin. Using an *Arthrobacter sp.* transaminase (ArR-TAm), and isopropylamine as the donor, α-alkoxy and α-aryloxy acetones were asymmetrically aminated with conversions of between 85 and 95% and >99% ee.\textsuperscript{105}

In summary, transaminases have been used extensively in the production of chiral amines of high value and pharmacological interest. They provide an alternative to traditional organic synthesis with a stereoselective route to chiral amine products. This is currently a major area of interest in the scientific community. Transaminases can also give rise to a number of challenges, such as the unfavourable equilibrium and difficulties with substrate acceptance, but these can be overcome with the choice of amine donor, cofactors and enzyme engineering to provide a highly selective and sustainable route to chiral amines.
1.4 Integrated heterogeneous catalysis and biocatalysis

The coupling of heterogeneous catalysis and biocatalysis has had some investigation in the literature, including oxidation steps using supported nanoparticle catalysts and radical catalysts such as (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO), in combination with enzymes such as transaminases and alcohol dehydrogenases.

In 2014, the use of Pd nanoparticles to catalyse aerobic oxidations of primary alcohols was coupled with a transaminase to access amine products (Scheme 57). One of the substrates tested was vanillyl alcohol 146, which can be accessed from the renewable feedstock lignin. The best catalyst for the transformation was found to be a Pd nanoparticle catalyst supported on meso-cellular foam and controlled pore glass, with Pd(0)/C also able to catalyse the transformation. The one-pot two-step process gave high conversions of 87% of vanillyl alcohol 146 and 84% of cinnamyl alcohol 54 to the desired amines, with no isolation of the aldehyde intermediate. The Pd catalysed oxidation step could be replaced with an alcohol dehydrogenase to form a two-enzyme cascade, also giving a high conversion of 61% with vanillyl alcohol.

Another transformation was reported in a reductive amination/amidation sequence using the same supported Pd nanoparticle catalysts and lipase CAL-B (Scheme 58). Commercially available lipase CAL-B immobilised on a macroporous resin was used to catalyse the amidation step, and the reductive...
amination was catalysed by Pd nanoparticles with ammonium formate as the amine donor. Both steps were performed in toluene giving excellent yields with a range of aldehydes and carboxylic acids. The cascade was extended to include the previously reported Pd catalysed aerobic oxidation from benzyl alcohol \(39\) to benzaldehyde \(40\) as the first step, giving a 49% yield of nonivamide \(147\) over the three steps in one pot.\(^{107}\)

\[
\begin{align*}
\text{Acyl} & \quad [\text{Pd}, \text{HCO}_2\text{NH}_4] \\
\text{PhMe, 80 °C} & \quad 2.5-3.5 \text{ h}
\end{align*}
\]

\[
\begin{align*}
\text{R} & \quad \text{O} \\
\text{H} & \quad \text{[Pd], Lipase, MS 4 Å, PhMe, 80 °C, 36 h}
\end{align*}
\]

\[
\begin{align*}
\text{R} & \quad \text{O} \\
\text{H} & \quad \text{[Pd], Lipase, MS 4 Å, PhMe, 80 °C, 36 h}
\end{align*}
\]

\[
\begin{align*}
\text{R} & = \text{vanillyl, R’ = nPentyl; 147 74%} \\
\text{R} & = \text{furyl, R’ = nHexyl; 62%} \\
\text{R} & = \text{nPentyl, R’ = CH}_2\text{CCPh; 70%}
\end{align*}
\]

Scheme 58 – Pd nanoparticle catalysed reductive amination followed by lipase catalysed amidation in one pot. Isolated yields shown.\(^{107}\)

Transaminases have also been used in chemoenzymatic cascades with oxidative laccase enzymes, which require a chemical mediator such as TEMPO to extend the substrate scope of these laccases beyond their natural substrate phenol. In 2017, a system using a laccase from \textit{Trametes versicolor} and TEMPO, coupled with a transaminase was reported for the transformation of racemic alcohols to chiral amines (Scheme 59). A range of substituted benzylic secondary alcohols were tested for this purpose with compounds bearing halogen, methoxy and trifluoromethyl substituents, all giving excellent yields of methylbenzylamines \(130\) and \(148-150\). Excellent ee values were also obtained with commercially available \((S)\)- and \((R)\)-selective transaminases and IPA as the amine donor. This one-pot, two-step process required the presence of both buffer and organic solvent MTBE, large excesses of TEMPO and IPA, and only benzylic alcohols were tested.\(^{108}\)
Scheme 59 – Chemoenzymatic cascade using laccase, TEMPO and transaminases. Transaminases used were ATA-P1-A06 for (S)-130, ATA-025 for (R)-130 and (R)-150, ATA-033 for (R)-148 and ATA-254 for (S)-149. Yields determined by GC analysis.108

The process was also performed on a preparative scale using diamine 128 as the amine donor instead of IPA. A yield of 91% of (R)-151 was obtained on a 100 mg scale, with >99% ee (Scheme 60).108

Scheme 60 – Laccase and transaminase cascade on a preparative 100 mg scale in a one-pot two-step procedure. Isolated yield shown.108

In 2017, the use of another radical mediator 2-azaadamantyl N-oxyl (AZADO) was reported with NaOCl as the oxidant, followed by a transaminase step to access the corresponding amines in a hybrid organo-biocatalytic process (Scheme 61). Amine products 136 and 152-154 were formed with excellent yields and ee values, with commercial (S)- and (R)-selective transaminases, low loadings of organocatalyst AZADO, and the presence of α,α,α-trifluorotoluene and DMSO as cosolvents.109
Scheme 61 – Organo-biocatalytic cascade using 2-azaadamantyl N-oxyl (AZADO), NaOCl and transaminases. Transaminases used were ATA-P1-A06 for (S)-136, ArR-TAm for (R)-136, ATA-303 for 152, ATA-P2-B01 for (R)-153, ArS-TAm (Arthrobacter sp.) for (S)-153, and ATA-033 for (R)-154. Yields determined by HPLC or GC analysis.\textsuperscript{109}

These processes show the potential for the incorporation of heterogeneous catalysis and organocatalysis into biocatalytic cascades to access complex enantoienriched compounds from sustainable sources.
1.5 Aims of the project

The aim of this project was to bring together heterogeneous catalysis and biocatalysis to create new synthetic routes to high value, small molecule targets from sustainable feedstocks, along with applying the principles of green chemistry. Gold nanoparticle catalysts were investigated for the oxidation of alcohols to aldehydes and ketones, with a focus on increasing the scope of these reactions. Transaminase enzymes were employed to provide mild, one-step routes to amines, and these transformations were combined into a two-step process (Scheme 62).

Gold nanoparticle catalysis is an attractive catalytic technique as high conversions and selectivity can be achieved with low gold loading, meaning the catalysts are relatively inexpensive to synthesise. An oxidant is required for these transformations, and various oxidants have been used in the literature, such as molecular oxygen and TBHP. Molecular oxygen produces no waste products, but working with oxygen can introduce issues related to safety, and problems with the incorporation of gaseous oxygen into liquid media. TBHP is simple to use as it is in liquid form, and once quenched the only waste product is t-butanol which can be easily removed from the desired product.

Transaminases provide a highly stereoselective route to chiral amines through a one-step transformation under mild, aqueous conditions. Recently, interest in
transaminases for the transformation of aldehydes to primary amines has also been increasing as the lack of toxic waste, expensive catalysts and high temperatures is attractive to the pharmaceutical industry. The synthesis of primary amines can be challenging by traditional chemistry routes, and it has been shown in this work that transaminases can provide effective alternative route to both achiral and chiral amines.

The combination of these two catalytic processes is unprecedented in the literature and provides a novel route from sustainable alcohol feedstocks to valuable amines. In the future, these processes could also be applied in a flow setting as use of the two steps have been separately reported in flow.\textsuperscript{54,105}
2 Gold nanoparticle catalysed oxidation of benzyl alcohol

2.1 Aims

Supported gold nanoparticles have been used in the literature as catalysts in the oxidation of benzyl alcohol, as discussed in Section 1.2.1. This section describes the synthesis of gold nanoparticles supported on various materials using the deposition-precipitation (DP) method with subsequent testing in the oxidation of benzyl alcohol, with a view to understanding the effects of variations in gold loading, support material and calcination temperature. The best catalysts were then taken forward for testing in the oxidation of secondary alcohols (Section 3).

2.2 Catalyst synthesis

Gold nanoparticle catalysts have been synthesised using the DP method, as reported by Choudhary et al. for use in the oxidation of benzyl alcohol, discussed in Section 1.2.1. The supports investigated were TiO$_2$ (anatase), SiO$_2$ nanospheres (500 nm), Al$_2$O$_3$ (corundum) and MgO. In the literature, TiO$_2$, MgO and Al$_2$O$_3$ supported gold catalysts have been reported to have high activity and selectivity in the oxidation of benzyl alcohol, highlighting these materials as good initial catalyst supports to investigate (Table 13).

\[
\begin{array}{|c|c|c|c|}
\hline
\text{Catalyst} & \text{wt\%} & \text{Conversion of benzyl alcohol 39 (\%)} & \text{Selectivity for benzaldehyde 40 (\%)} \\
\hline
\text{Au/MgO DP}^{32} & 8 & 97 & 62 \\
\hline
\text{Au/TiO}_2 \text{ DP}^{39} & 0.4 & 32 & 98 \\
\hline
\text{Au/TiO}_2 \text{ HDP}^{39} & 4 & 63 & 79 \\
\hline
\text{Au/Al}_2\text{O}_3 \text{ HDP}^{32} & 8 & 71 & 65 \\
\hline
\end{array}
\]

Table 13 – Literature data on the use of Au nanoparticle catalysts in the oxidation of benzyl alcohol. Conversions of benzyl alcohol were determined by GC, with selectivity for benzaldehyde over benzyl benzoate quoted. DP: deposition-precipitation method, HDP: homogeneous deposition-precipitation method.
A 97% conversion of benzyl alcohol 39 was reported with Au/MgO (8 wt%, DP preparation), with a 62% selectivity for benzaldehyde 40 and the main by-product was benzyl benzoate 42.32 Au/TiO₂ prepared by the DP method at a much lower gold loading showed a lower conversion of 32% but a much higher selectivity of 98% for benzaldehyde 40.39 Higher gold loading was achieved using the homogeneous deposition-precipitation (HDP) method, which increased the conversion of benzyl alcohol 39 to 63% but lowered the selectivity for benzaldehyde 40 to 79%.39 An Au/Al₂O₃ (8 wt%) catalyst also prepared by the HDP method achieved a higher conversion of 71% but a lower selectivity of 65% for benzaldehyde 40.32 The DP synthesised catalysts generally gave lower conversions but higher selectivities, compared to HDP prepared catalysts. In addition, SiO₂ has been used as a support in oxidations of benzyl alcohol and other primary alcohols in its standard form, but not in nanosphere form. For example, the oxidation of benzyl alcohol 39 with O₂ as the oxidant in the presence of Au/SiO₂ gave 75% conversion and 98% selectivity for benzaldehyde 40 at a very high reaction temperature of 280 °C.38

Here, the DP method was used to prepare catalysts using 1 wt% of the gold precursor HAuCl₄ and these were washed and dried. The catalysts were then subjected to calcination, a process where the powders are heated to high temperatures (300-500 °C) to remove any volatile compounds and moisture. The catalysts were pink and purple in colour suggesting the deposition of gold nanoparticles onto the supports, which were colourless prior to the deposition (Scheme 63).

![Scheme 63 – Deposition of gold nanoparticles onto supports showing the change in colour from white to pink and purple.](image)

The three catalysts were analysed by TEM, and the pictures (taken before calcination) show the TiO₂ particles as heterogeneous rounded particles, and the
SiO$_2$ particles as uniform spheres of 500 nm (Figure 5). The gold nanoparticles are the darker spots covering the surface of the larger rounded particles of TiO$_2$ and SiO$_2$. The structure of MgO is heterogeneous, as it is made up of sheet- and shard-like crystals, and the gold nanoparticles can be seen distributed around the material. The pictures show the gold nanoparticles as heterogeneous in size, between 5 nm and 20 nm in diameter.

**Figure 5** – Transmission Electron Microscopy (TEM) pictures of gold nanoparticles (Au NP) deposited on TiO$_2$ (A & B), SiO$_2$ nanospheres (C & D) and MgO (E & F).
After testing of the 1 wt% catalysts, discussed in Section 2.4, catalysts with higher gold loading were prepared. Gold nanoparticle catalysts were prepared at 4 wt% and 10 wt% with TiO$_2$, and 10 wt% with MgO and Al$_2$O$_3$ using the original DP method with varying calcination temperatures of 300 °C, 400 °C and 500 °C. Al$_2$O$_3$ was used for the 10 wt% loading rather than SiO$_2$ nanospheres as the 1 wt% SiO$_2$ catalysts showed very poor activity.

During the synthesis of these catalysts, NaOH was added to raise the pH following the standard DP procedure. It was found that when using a basic support such as MgO, no base addition was required as the pH of MgO in water was sufficiently high. An MSci student under my supervision, Patricia Flemming, investigated the preparation of 10 wt% MgO using different techniques, preparing the catalysts with and without NaOH and varying the separating and drying techniques of the materials (Figure 6).

![Figure 6 – Modifications to the original DP catalyst preparation method.](image)

In the initial catalyst synthesis using the DP method, the solid was removed by centrifugation, but this approach was now varied and filtration and rotary evaporation were used to remove the water. The 10 wt% catalysts were also calcined in air at 300 °C and 500 °C to compare activity with calcination temperature. After these investigations, water was removed by filtration in the synthesis of future 10 wt% Au/MgO and Au/Al$_2$O$_3$ catalysts, and they were calcined at 500 °C as this led to more active catalysts. The effects of preparation method and calcination temperature on the activity of the catalysts are discussed in Section 2.5.
All of the catalysts were also characterised by powder X-ray diffraction (XRD).\(^1\)

In the diffraction pattern of the Au/MgO 1 wt% (DP) catalyst, MgO peaks could be seen but no gold peaks were visible due to the low loading of gold. With the higher gold loaded 10 wt% Au/MgO catalyst the gold peaks were visible in the diffraction pattern (Figure 7). The data showed a correlation between the experimental diffraction pattern of the Au/MgO 10 wt% catalyst with literature data for the MgO peaks (red) and metallic gold peaks (blue). In this diffraction pattern, the pattern was dominated by the bulk material MgO, with peaks at 2\(\theta\) values of 16.7, 19.4, 27.5, 32.3, 34.0 and 39.3. The gold peaks were much smaller, at 2\(\theta\) values of 17.3, 20.0, 28.5 and 33.5.

![Diagram of X-ray diffraction pattern](image)

**Figure 7 – Experimental powder X-ray diffraction pattern of the Au/MgO 10 wt% catalyst. Standard X-ray diffraction patterns of MgO\(^{10}\) are shown in red and Au\(^{11}\) in blue.**

In the diffraction pattern of Au/TiO\(_2\) 10 wt% (Figure 8), the reference pattern for TiO\(_2\) correlated with the experimental pattern, shown with the red peaks. Gold peaks shown in blue at 2\(\theta\) values of 17.3, 20.0, 28.5 and 33.5 were detected but were very low intensity. A diffraction pattern of an Au/TiO\(_2\) catalyst (0.2-0.5 wt%) has been reported previously with visible gold peaks.\(^{39}\)

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\(^1\) XRD run by Dr. Ian Godfrey.
The diffraction pattern of the Au/Al₂O₃ 10 wt% catalyst (Figure 9) clearly correlated with the literature Al₂O₃ pattern, but no gold peaks were visible. In the literature, no visible gold peaks were observed in the diffraction pattern of an Au/meso-γ-Al₂O₃ catalyst and this was suggested to be due to the small size and highly dispersed nature of the nanoparticles.¹¹³

In summary, gold nanoparticles were deposited on MgO, Al₂O₃, TiO₂ and SiO₂ at different gold loadings between 1 wt% and 10 wt%. Aspects of the DP synthesis method including water removal, presence of NaOH and calcination temperature were varied to understand the effects of these factors on the oxidation on catalyst performance (see Section 2.5). The catalysts were analysed by transmission electron microscopy (TEM), showing the dispersed nature of the nanoparticles, as well as their heterogeneous sizes. Powder XRD was also used to analyse the catalysts, showing low levels of gold due to the high dispersion of the
nanoparticles. Work is ongoing in the group on analysis by EXAFS (extended X-ray absorption fine structure) and XANES (X-ray absorption near edge structure) to determine the chemical state of the metallic gold nanoparticles.

2.3 Analytical methods for the benzyl alcohol oxidation reaction

The benzyl alcohol oxidation reaction was analysed by GC and the products quantified using an internal standard dodecane, as reported in the literature.\textsuperscript{54,115} The oxidation of benzyl alcohol 39 (Scheme 64) produces the desired product benzaldehyde 40 but can also result in overoxidation to benzoic acid 41 and further reaction to form benzyl benzoate 42. As benzoic acid 41 is not detectable by GC, the products were derivatised with a trimethylsilyl (TMS) group using standard protocols to give TMS-benzyl alcohol 155 and TMS-benzoic acid 156.\textsuperscript{116,117}

Scheme 64 – Oxidation of benzyl alcohol with Au catalysts and tert-butyl hydroperoxide (TBHP) as the oxidant followed by derivatisation of the products with TMSCl.\textsuperscript{116}

A method was developed which allowed benzyl alcohol 39, benzaldehyde 40 and benzyl benzoate 42 to be separated by GC along with the internal standard, dodecane, and the solvent, decane. An example GC trace is shown in Figure 10.
Figure 10 – GC trace showing TBHP (3.7 min), decane (3.8 min), benzaldehyde 40 (6.4 min), dodecane (7.4 min) and benzyl alcohol 39 (8.9 min). Benzyl benzoate 42 eluted at 13.8 min. GC analytical method A used.

To observe benzoic acid, the reaction mixture was silylated giving rise to TMS-benzoic acid and TMS-benzyl alcohol products (Scheme 64). These products were also analysed by GC with respect to the internal standard, dodecane, and eluted at 3.5 min (155) and 8.8 min (156) (Figure 11).

Figure 11 – GC trace showing TBHP (2.9 min), TMS-benzyl alcohol 155 (3.5 min), benzaldehyde 40 (6.6 min), dodecane (7.7 min) and TMS-benzoic acid 156 (8.8 min). GC analytical method E used.
2.4 Oxidation of benzyl alcohol with 1 wt% catalysts

The oxidation of benzyl alcohol is the benchmark reaction used to test gold nanoparticle catalytic activity in the literature, using molecular oxygen or tert-butyl hydroperoxide (TBHP) as the oxidant. The synthesised gold nanoparticle catalysts were tested first on the oxidation of benzyl alcohol using TBHP as the oxidant in decane. The oxidation of benzyl alcohol by TBHP in the absence of a catalyst has been reported, and therefore the background reaction was monitored in parallel. Recently, the oxidation of a range of benzylic secondary alcohols by aqueous TBHP with no catalyst has been reported in the literature, as discussed in Section 1.2.3, with excellent yields achieved using 6-10 equivalents of TBHP. This highlights the importance of monitoring the background oxidation of benzyl alcohol in these systems, although in many reports this has not been thoroughly reported. However, there are advantages to using gold nanoparticle catalysts in these oxidations, as the number of equivalents of TBHP can be lowered leading to less waste. Also, the oxidations performed by TBHP were all with benzylic alcohols, and when using less readily oxidisable substrates the catalysts are required to gain reasonable yields (see Section 3).

The oxidation of benzyl alcohol 39 was tested with Au/MgO, Au/TiO\(_2\) and Au/SiO\(_2\) 1 wt% catalysts over a period of 4 h and 24 h. For 24 h reactions, the yield of benzaldehyde 40 was seen to decrease over time due to overoxidation to benzoic acid 41, and the presence of benzyl benzoate 42 was also observed. Shorter reaction times were therefore preferable to limit the amount of further reaction and formation of unwanted acid and ester by-products. The formation of benzaldehyde 40 and depletion of benzyl alcohol 39 over time are shown in Figure 12 and Figure 13.

The background reaction with no catalyst produced benzaldehyde in 20% yield after 2 h, and benzyl alcohol 39 conversion was around 30%. The reaction with Au/TiO\(_2\) (1 wt%) was monitored for 2 h, and reached the same yield of benzaldehyde 40 and conversion of 39 as the background reaction. This showed that Au/TiO\(_2\) (1 wt%) was not catalysing the reaction. The Au/SiO\(_2\) (1 wt%) catalyst also matched the negative control in terms of formation of 40, with a yield of 20%. However, the conversion of 39 was higher with this catalyst at 51% after 4 h. The highest yield of 33% of 40 was observed with Au/MgO (1 wt%) after 2 h,
after which it began to decrease. The conversion of 39 was also highest with this catalyst, with a 75% conversion achieved after 4 h.

Figure 12 – Benzaldehyde formation over time in the presence of different 1 wt% Au catalysts, with TBHP oxidant in decane. Reaction conditions (procedure A): benzyl alcohol (3.0 mmol), TBHP (2.3 mmol), Au catalyst (0.03 mol%), decane, 95 °C. Benzaldehyde yields were determined by GC using dodecane as an internal standard.

Figure 13 – Benzyl alcohol depletion over time in the presence of different 1 wt% Au catalysts, with TBHP in decane. Reaction conditions (procedure A): benzyl alcohol (3.0 mmol), TBHP (2.3 mmol), Au catalyst (0.03 mol%), decane, 95 °C. Conversions of benzyl alcohol were determined by GC using dodecane as an internal standard.
Analysis of the silylated reaction mixtures with Au/SiO$_2$ and Au/MgO catalysts showed the formation of benzoic acid 156 (Table 14). In the absence of gold catalyst, a 10% yield of 156 was observed. In the presence of Au/SiO$_2$ and Au/MgO catalysts, yields of 17% and 16% were observed respectively. The presence of 156 showed that the benzaldehyde product was reacting further under the reaction conditions, decreasing the yield.

Table 14 – Formation of benzoic acid in the oxidation of benzyl alcohol with gold catalysts (1 wt%). Reaction conditions (procedure A): benzyl alcohol (3.0 mmol), TBHP (2.3 mmol), Au catalyst (0.03 mol%), decane, 95 °C. Yields of benzoic acid were determined by GC using dodecane as an internal standard, after silylation of the reaction mixture with TMSCl.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Benzoic acid 156 yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au/SiO$_2$ (1 wt%)</td>
<td>17</td>
</tr>
<tr>
<td>Au/MgO (1 wt%)</td>
<td>16</td>
</tr>
<tr>
<td>No catalyst</td>
<td>10</td>
</tr>
</tbody>
</table>

The Au/MgO (1 wt%) catalyst was identified as the most promising with high conversion, however the yield of desired benzaldehyde product was low due to the formation of benzoic acid. This could be combated in a flow reactor setting, where the contact time between the reaction mixture and the catalyst can be lowered, reducing the potential for overoxidation to benzoic acid.

Further control reactions were performed to investigate the oxidation reaction and improve the selectivity for benzaldehyde over benzoic acid. Performing the reaction under argon decreased the formation of benzoic acid to negligible quantities with and without catalyst. Unfortunately, this also diminished the overall reactivity, leading to a lowering of the alcohol conversion and benzaldehyde yield.
Figure 14 – Benzyl alcohol depletion over time under argon in the presence of TBHP oxidant and Au catalyst. Reaction conditions (procedure B): benzyl alcohol (5 mmol), TBHP (8 mmol), 1 wt\% Au/MgO (0.02 mol\%), decane, argon atmosphere, 95 °C. Benzyl alcohol conversions were determined by GC using dodecane as an internal standard.

Compared to a conversion of 75\% in the presence of Au/MgO in air, the conversion under argon was only 30\%. The yield of 40 also fell from 25\% to 18\% after 4 h. The removal of air was clearly detrimental to the overall activity, showing that oxygen in the air may have been contributing to the oxidation process as well as TBHP. Another control reaction, heating benzyl alcohol in decane under air in the absence of TBHP, resulted in the formation of 40 in 10\% yield after 24 h, highlighting the high reactivity at the benzylic centre. However, no benzaldehyde was observed after 4 h, the standard time period used for the TBHP reactions.

A commercial Au/TiO$_2$ 1 wt\% catalyst was also tested for comparison purposes, as its activity in the oxidation and alkylation of benzyl alcohols in a flow reactor has been reported in the literature [1.2.4].\textsuperscript{54} The catalyst was tested in the oxidation of benzyl alcohol 39 in decane (Figure 15).

![Graph showing benzyl alcohol depletion over time under argon](image-url)
Figure 15 – Benzaldehyde formation over time in the presence of commercial Aurolite Au/TiO₂ 1 wt% catalyst, with TBHP oxidant in decane. Reaction conditions (procedure A): benzyl alcohol (3 mmol), TBHP (2.3 mmol), Aurolite 1 wt% Au/TiO₂ (0.03 mol%), decane, 95 °C. Benzaldehyde yields were determined by GC using dodecane as an internal standard.

A 66% yield of 40 was achieved after 4 h, well above the negative control. The commercial 1 wt% Au/TiO₂ catalyst was clearly more active than the synthesised 1 wt% catalysts, and due to this, an increase in gold loading was investigated to try to increase activity further. The lower reactivity of the synthesised 1 wt% catalysts could be due to the synthetic process, due to small differences in the preparation methods. The average particle size of the commercial catalyst is reported as 2-3 nm, whereas the synthesised nanoparticles were between 5 nm and 20 nm.
2.5 Oxidation of benzyl alcohol using catalysts with higher gold loading

The oxidation of benzyl alcohol 39 was tested with 10 wt% Au/MgO catalysts which were prepared by the filtration and rotary evaporation methods and calcined at different temperatures (Figure 16, Figure 17).\(^2\) The highest yield of 68% of benzaldehyde 40 was achieved after 1 h using the 10 wt% catalyst prepared using the filtration method and calcined at 500 °C. After this time, the yield of 40 fell as further oxidation took place, leaving just 20% of 40 remaining after 6 h. This trend was also observed with the 10 wt% catalyst prepared by filtration and calcined at 300 °C, giving a similar yield of 63% after 1 h. The conversion of 39 was high with these two catalysts, with less than 10% of 39 remaining after 2 h, falling to below 5% after 6 h.

The catalysts prepared by the rotary evaporation method performed much less well. The catalyst calcined at 500 °C showed a much slower increase in benzaldehyde yield, with 29% of 40 observed after 3 h, and the yield of 40 then stayed the same before decreasing after 5 h. Conversion of 39 with this catalyst was also slower, reaching 63% conversion after 6 h. The catalyst calcined at 300 °C showed the same trend, with a slightly lower maximum yield of 26%, and a lower conversion of 48% after 6 h.

The background reaction without the catalyst reached a 14% yield of 40 after 1 h, remaining constant until it decreased to 10% after 5 h. The conversion of 39 was low, with 59% of 39 still remaining after 6 h.

\(^2\) Experiments conducted and analysed by Patricia Flemming (MSci student)
Figure 16 – Benzaldehyde formation in the presence of Au/MgO catalysts prepared using different water removal techniques: filtration (F) and rotary evaporation (RE), and different calcination temperatures, 300 °C and 500 °C, with TBHP as oxidant in decane. Reaction conditions (procedure B): benzyl alcohol (5 mmol), TBHP (8 mmol), 10 wt% Au/MgO (0.2 mol%), decane, 95 °C. Benzyl alcohol conversions were determined by GC using dodecane as an internal standard.

Figure 17 – Benzyl alcohol depletion in the presence of Au/MgO catalysts prepared using different water removal techniques: filtration (F) and rotary evaporation (RE), and different calcination temperatures, 300 °C and 500 °C, with TBHP as oxidant in decane. Reaction conditions (procedure B): benzyl alcohol (5 mmol), TBHP (8 mmol), 10 wt% Au/MgO (0.2 mol%), decane, 95 °C. Benzyl alcohol conversions were determined by GC using dodecane as an internal standard. 0 h time point taken when reaction reached temperature, >100% starting material due to experimental error.
The increase in gold loading of the Au/MgO catalysts from 1% to 10% clearly increased the yield of \( 40 \) and conversion of \( 39 \) overall. Catalysts prepared using the filtration method were much more active than those prepared by the rotary evaporation method, giving higher yields and conversions with shorter reaction times. This could be attributed to the fact that when water is removed using a using a rotary evaporator, only the water will be removed leaving behind residues from the gold precursor H\( \text{AuCl}_4 \). A comparison of the activity of catalysts prepared by HDP, DP and ‘wet impregnation’ techniques discussed in Section 1.2 has been reported in the literature.\(^{32}\) In the wet impregnation technique, water was removed by heating on a water bath, similar to but at a higher temperature than removing water on a rotary evaporator. The wet impregnation catalysts were found to have the lowest activity of the tested catalysts, correlating with our results.

Varying the calcination temperature did not have a large effect on the catalytic activity, but catalysts calcined at 500 °C showed slightly higher activity than catalysts calcined at 300 °C. In the literature, calcination temperature has been varied in the preparation of Au/MgO catalysts and the effects on benzaldehyde yield investigated. Catalysts were calcined at 200 °C and 400 °C, and the oxidation of benzyl alcohol performed with TBHP as the oxidant. The conversion of \( 39 \) was high (90-100%) with both catalysts, but the selectivity was highest at 90% with the 200 °C calcined catalyst. With the 400 °C calcined catalyst, complete conversion of \( 39 \) was achieved, with a 75% selectivity for benzaldehyde. These literature results showed that Au/MgO catalysts are active when calcined at 200 °C or 400 °C, and our results also showed that high catalytic activity can also be achieved with calcination temperatures of 300 °C and 500 °C.

\[
\text{Ph}^\text{39}\text{OH} \underset{\text{Au/MgO (8 wt%)}}{\xrightarrow{\text{TBHP (1.5 eq.)}}} \text{Ph}^\text{40}\text{H} + \text{Ph}^\text{42}\text{O}^\text{Ph}
\]

<table>
<thead>
<tr>
<th>Catalyst calcination temperature (°C)</th>
<th>Conversion of benzyl alcohol 39 (%)</th>
<th>Selectivity for benzaldehyde 40 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>91</td>
<td>90</td>
</tr>
<tr>
<td>400</td>
<td>100</td>
<td>75</td>
</tr>
</tbody>
</table>

Table 15 – The effect of calcination temperature on the conversion of benzyl alcohol and selectivity for benzyl alcohol. Conversions and selectivities for benzaldehyde (over benzyl benzoate) were determined by GC.\(^{32}\)
Gold catalysts with varying support materials were then synthesised with higher gold loading, using the filtration DP preparation method and 400-500 °C calcination temperature. Two Au/TiO$_2$ catalysts were synthesised at 4 wt% and 10 wt%, and one Au/Al$_2$O$_3$ at 10 wt%, and these catalysts were tested with the oxidation of benzyl alcohol. The results of the Au/Al$_2$O$_3$ catalyst are shown in Figure 18. The Au/Al$_2$O$_3$ 10 wt% catalyst showed a different reaction profile to Au/MgO, with a 30% yield of 40 observed after 2 h followed by a decrease in yield to 23% after 4 h. This suggested that in the presence of Au/Al$_2$O$_3$, more overoxidation and side reactions occurred. Overall, the catalyst did not provide a significantly higher yield of 40 than the reaction containing no catalyst.

The Au/TiO$_2$ catalysts are shown in Figure 19. The reaction conditions used in this case contained less than 1 equivalent of TBHP, but the catalysts still showed a higher activity than the no catalyst control. The Au/TiO$_2$ 4 wt% catalyst gave the highest yield of 40 of 37% after 6 h, and also showed the fastest formation of 40 (23%) after 1 h. Increasing the loading to 10 wt% for this catalyst had little impact on the benzaldehyde yield, also reaching 36% after 6 h.

Figure 18 – Benzaldehyde formation over time in the presence of Au/Al$_2$O$_3$ (10 wt%) with TBHP as the oxidant in decane. Reaction conditions (procedure B): benzyl alcohol (5 mmol), TBHP (8 mmol), Au/Al$_2$O$_3$ (0.2 mol%), decane, 95 °C. Yields of benzaldehyde were determined by GC using dodecane as an internal standard.
However, the initial rate of formation was lower with the 10 wt% catalyst, with a 17% yield after 1 h compared to 23% with the 4 wt% catalyst.

Figure 19 – Benzaldehyde formation over time in the presence of higher loaded Au catalysts. Reaction conditions (procedure A): benzyl alcohol (3 mmol), TBHP (2.3 mmol), Au catalyst (0.3 mol%), decane, 95 °C. Benzaldehyde yields were determined by GC using dodecane as an internal standard.

Overall, the Au/MgO catalysts were found to give the highest yields of 40 of almost 70% after 1 h, compared to 30-40% with Au/TiO₂ and Au/Al₂O₃. Although TiO₂ and Al₂O₃ supported catalysts gave yields of 40 above the negative control, these 10 wt% catalysts were comparable with 1 wt% Au/MgO, showing the large effect the support material has on catalytic activity. The effects of support material on catalytic activity have been reported, but the cause of any differences in activity are not well understood. Different support materials interact differently with the gold nanoparticles under the preparation conditions, giving rise to variations in gold species within the catalyst, with Au(0) and Au(III) species reported.34,118
2.6 Summary

Supported gold nanoparticle catalysts were investigated for the oxidation of benzyl alcohol 39, as it is a benchmark reaction in the literature for this type of catalysis. Gold nanoparticles were deposited on a range of supports using DP and wet impregnation methods. It was shown that the synthesised 1 wt% catalysts were poorly selective for the desired product, benzaldehyde 40, and the main by-product observed was benzoic acid 41. A commercial 1 wt% Au/TiO₂ catalyst was tested for comparison and gave higher yields of 40, possibly due to the smaller average particle size of the nanoparticles.

![Benzyl Alcohol Oxidation Reaction](image)

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Water removal</th>
<th>Calcination (°C)</th>
<th>Yield 40 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au/MgO</td>
<td>F</td>
<td>500</td>
<td>68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Au/MgO</td>
<td>RE</td>
<td>500</td>
<td>22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Au/MgO</td>
<td>F</td>
<td>300</td>
<td>63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Au/Al₂O₃</td>
<td>F</td>
<td>500</td>
<td>30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Au/TiO₂</td>
<td>F</td>
<td>500</td>
<td>36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 16 – Summary of benzyl alcohol oxidation results with varying catalyst synthesis and calcination temperatures. <sup>a</sup>Reaction conditions: alcohol (5 mmol), TBHP (8 mmol), Au catalyst (0.2 mol%), decane, 95 °C. <sup>b</sup>Reaction conditions: alcohol (3 mmol), TBHP (2.3 mmol), Au catalyst (0.3 mol%), decane, 95 °C. All Au catalysts prepared at 10 wt%. Water removal methods filtration (F) and rotary evaporation (RE) were used. Yields of benzaldehyde were determined by GC using dodecane as an internal standard.

When the loading was increased to 10 wt%, the reaction conversion and selectivity for 40 increased for Au/MgO. Activity was highest with catalysts calcined at 500 °C, and water removal during catalyst preparation using the filtration method gave much better catalytic activity than with the rotary evaporation method (Table 16). Although Au/TiO₂ showed some improvement with an increase in loading to 4 wt% and 10 wt%, this catalyst and the 10 wt% Au/Al₂O₃ catalyst gave low yields of 40 compared to Au/MgO, showing the applicability of MgO as a support material for this process. Control reactions were undertaken to assess the production of 40 in the absence of catalyst. Under the conditions shown above, yields of 14% and 21% of 40 were observed in reactions...
containing oxidant but no catalyst. With the best performing catalysts, increases in yield of 54% and 49% were achieved over the control reaction.
3 Oxidation of secondary alcohols

3.1 Aims

In this section, the work reported on gold nanoparticle catalysis was extended to a selection of secondary alcohols. As the substrate scope for these catalysts has only recently begun to be explored in the literature, a small set of substrates were chosen and tested with the best catalysts identified in the benzyl alcohol work (Section 2). As discussed in Section 1.2.3, recent reports in 2012 and 2017 include the oxidation of two cyclohexen-1-ol derivatives in a batch and flow setting with an Au/Al₂O₃ catalyst, and the oxidation of terpene derived cyclic alcohols with Au/MgO (Figure 20). The oxidation of cinnamyl alcohol 54 has been reported with Au/Al₂O₃, Au/CeO₂ and Au-Pd/TiO₂ catalysts during the course of this PhD.⁴⁵,⁵⁰,⁵²

![Literature substrates](image)

**Figure 20** – Compounds reported in the literature used in gold nanoparticle catalysed oxidations,⁴⁵,⁴⁷,⁵⁰,⁵² and compounds explored in this work.
In this work, Au/MgO and Au/Al$_2$O$_3$ catalysts were used in the oxidation of a selection of non-benzylic secondary alcohols (Figure 20). Aryl aliphatic alcohols 157 and 158 were chosen as examples of non-benzylic alcohols which oxidise less readily, while retaining the presence of a phenyl ring. These more challenging substrates would probe the limitations of the catalytic system. 4-Phenyl-3-buten-2-ol 159 was selected for testing to probe the selectivity of the catalytic system for oxidation of an alcohol over an alkene moiety. This substrate was also expected to be more readily oxidised than 157 and 158 due to the conjugated system. Propargylic alcohol 160 would also provide insights into the functional group tolerance of an alkyne. Aliphatic substrate 161 was selected as an example of a substrate not containing a phenyl ring, and therefore displaying no conjugation.

As discussed in Section 1.2, Au/MgO catalysts have been used in catalytic oxidations of CO, benzyl alcohol, cyclohexane and glycerol, as well as some secondary alcohols.$^{32,119–121}$ Au/Al$_2$O$_3$ has been used in the oxidation of cyclohexane and CO, in addition to benzylic and allylic alcohols.$^{48,113,122}$ Au/MgO and Au/Al$_2$O$_3$ have also both been used in the catalysis of reductive processes and hydrogenations.$^{63,123}$

3.2 Analysis

The oxidation reactions of secondary alcohols were initially analysed by GC, but as the solvent was changed from decane to water, the use of an organic internal standard became more unreliable. Indeed, when the reaction was conducted in water, the materials were not fully dissolved and the reaction mixture was an emulsion, so removing aliquots was not possible. Therefore, in reactions using water as a solvent, the reaction mixture was extracted into ethyl acetate before analysis by GC. NMR spectroscopy was also used to confirm the oxidation products formed.
3.3 Oxidation of 1-phenyl-2-propanol 157

Secondary alcohol 1-phenyl-2-propanol 157 (Scheme 65) was chosen as a substrate for oxidation, as this would be less readily oxidisable than benzyl alcohol 39, and would extend the scope of the gold nanoparticle catalysts.

Scheme 65 – Oxidation of 1-phenyl-2-propanol 157 with TBHP and Au/MgO. Reaction conditions: 1 mmol alcohol (1 mmol), TBHP (9 mmol), decane, 95 °C, Au catalyst (1 mol%).

The production of phenylacetone 162 with Au/MgO 1 wt% and 10 wt% catalysts, and the reaction containing oxidant but no catalyst is shown in Figure 21.

Figure 21 – Oxidation of 1-phenyl-2-propanol 157 with TBHP in decane. Reaction conditions: alcohol (1 mmol), TBHP (9 mmol), decane, 95 °C, Au catalyst (1 mol%). Yields of phenylacetone 162 were determined by GC using dodecane as an internal standard.

The use of Au/MgO 1 wt% gave a slightly higher yield of phenylacetone 162 after 4 h compared to the no catalyst control, after which time formation of the product stopped. After 24 h, the yield of 162 was the same in the presence of 1 wt% catalyst and without catalyst. An improvement in yield was observed with Au/MgO 10 wt% up to 4 h, reaching 32% compared with 21% for Au/MgO 1 wt% and 18% with no catalyst. However, after 24 h the yield of 162 had decreased to 12%. This suggested that the product 162 could be reacting further under the reaction conditions.
conditions. Indeed, an investigation of starting material depletion by GC analysis of alcohol 157 showed total consumption after 24 h. The drop in yield could be caused by competing reactions such as aldol condensations due to the acidity of the benzylic protons. For these reasons, work was continued with another secondary alcohol.

3.4 Oxidation of 4-phenyl-2-butanol 158

4-Phenyl-2-butanol 158 was chosen as a substrate as both it and the product, benzylacetone 101, are less susceptible to aldol condensations and other side reactions.

In the literature, no reports on the oxidation of this substrate with Au/MgO or Au/Al₂O₃ have been found. The use of gold nanoparticle catalysts has been applied to 158, but required complex support materials, with O₂ or air as the oxidant and additives were also required (Table 17). Gold nanoparticles or nanoclusters ‘incarcerated’ in polystyrene/carbon supports have been described by Kobayashi et al. with O₂ (1 atm) and K₂CO₃ as an additive at ambient temperatures, giving 95% yield of benzylacetone 101. Gold nanoclusters stabilised by a water-soluble polymer poly(N-vinyl-2-pyrrolidine) (Au:PVP) have also been reported using K₂CO₃ as the additive and air as the oxidant, with a 93% yield reported. The oxidation of this substrate with an Au/AlO(OH) catalyst has been achieved with 95% isolated yield after 8 h, using CsCO₃ as the additive and O₂ as the oxidant. Bimetallic Au-Pd and Au-Pt nanoparticle catalysts have also been applied to this transformation, again with high yields. Although all of these methods achieved excellent yields of the desired product 101, the main drawback of these techniques is the need for complex support materials, rather than cheap bulk materials such as MgO and Al₂O₃. These methodologies also required basic additives and in some cases the use of toxic cosolvent trifluorotoluene.
In this work, Au/MgO, Au/TiO₂ and Au/Al₂O₃ catalysts were used in the oxidation of 4-phenyl-2-butanol 158, using TBHP as the oxidant and decane or water as the solvent (Scheme 66).

The Au/MgO catalysts prepared with 1 wt% and 10 wt% gold loading were utilised in this reaction. As discussed in Section 2.4, background oxidation reactions can be minimised by conducting the reaction under argon, but this negatively impacts on the overall yield of the reaction. The 1 wt% and 10 wt% Au/MgO catalyst reactions were conducted under argon, and although the yields were affected by the argon atmosphere (7-15%), differences in catalytic activity were still observed (Figure 22). After these findings, argon was no longer used for oxidations due to the low yields observed. The reaction yield with no catalyst present was very low, due to the less reactive non-benzylic alcohol centre. A small increase in yield was observed with Au/MgO 1 wt%, but the initial rate of reaction slowed after 2 h. The Au/MgO 10 wt% catalyst showed a similar reactivity to the lower loaded catalyst.
up to 2 h, but an increase in yield was observed after 4 h giving a final yield of 15%. For this reason, investigations were continued with 10 wt% catalysts.

![Diagram of oxidation reaction](image)

**Figure 22** – Oxidation of 4-phenyl-2-butanol 158 in the presence of Au/MgO catalysts with 1 wt% and 10 wt% gold loading, and TBHP in decane under argon. Reaction conditions (Procedure A): substrate (3 mmol), 2.6 eq. TBHP (8 mmol), decane, 95 °C, Au catalyst (0.3 mol%). Yields of benzylacetone 101 were determined by GC using dodecane as an internal standard.

The substrate was tested with 10 wt% Au/TiO2, Au/Al2O3 and Au/MgO catalysts, and these results are shown in Figure 23. Overall, yields were higher in the presence of air than under argon. The oxidation with no catalyst reached 13% after 3 h, after which time the production of benzylacetone 101 plateaued remaining at 14% after 6 h. With Au/MgO, 101 was produced at the same rate as the negative control up to 3 h, but then showed a slight increase in yield to 18% after 6 h. The best catalyst was Au/Al2O3, with increased 101 formation after 2 h compared to the other catalysts and the no catalyst control. This catalyst achieved 23% yield after 6 h. The Au/TiO2 reaction was monitored up to 3 h, and showed no activity over the negative control. The reactions were also monitored after 24 h, with the no catalyst control reaching 27%, Au/MgO 30% and Al2O3 32%. The yield of 101 with Au/TiO2 remained the same as the negative control. No problems were encountered with loss of 101 over time, confirming that this system was less susceptible to side reactions than 1-phenyl-2-propanol 157 discussed previously.
Figure 23 – Oxidation of 4-phenyl-2-butanol 158 with TBHP in decane with 10 wt% catalysts. Reaction conditions (procedure B): substrate (0.3 mmol), TBHP (1.2 mmol), decane, 95 °C, Au catalyst (1 mol%). Yields of benzylacetone 101 determined by GC using dodecane as an internal standard. Reactions were performed in duplicate with standard deviations below 10%.

Catalysts Au/Al₂O₃ and Au/MgO were taken forward for investigation using water as a solvent, as it is more sustainable and made work up procedures more straightforward than when using high boiling decane. Initial analysis by GC showed the formation of benzylacetone 101, but these results could not be quantified due to the poor solubility of the materials in water, making aliquot removal inaccurate. The reactions were therefore stopped, extracted into organic solvent and analysed by NMR spectroscopy after 24 h. Only starting material and the desired ketone were detected, but problems with mass recovery were encountered. GC analysis proved suitable for monitoring the reactions when the products were extracted into ethyl acetate. The results of the time-point reactions in water are shown in Figure 24. The reaction profile was very different in water than in decane; the control reaction without catalyst produced no 101, whereas in decane yields of up to 25% were observed. There was also a large difference between the catalysts, with Au/MgO giving a 45% yield after 4 h, while with Au/Al₂O₃ the yield was below 10% at all time points. NMR spectroscopy of the Au/MgO reaction products confirmed the presence of benzylacetone 101 after 4 h, along with starting material, although overall mass recovery was only at 60%.
Figure 24 – Oxidation of 4-phenyl-2-butanol 158 over time. Reaction conditions: alcohol (0.3 mmol), TBHP (1.2 mmol), water, 95 °C, Au catalyst (1 mol%). Reaction mixtures were extracted into ethyl acetate and analysed by GC using dodecane as a standard. Reactions were performed in duplicate.

The oxidation of this substrate proceeded cleanly with minimal impurities observed by GC, and the main species by GC and NMR analysis were the starting material and product.

Scheme 67 – Oxidation of 4-phenyl-2-butanol 158 by TBHP in the presence of Au/MgO (10 wt%) catalyst.

When comparing water and decane as solvents for this reaction, it was found that the reaction proceeded much more cleanly in water than in decane. The GC trace of the reaction in water after 4 h (Figure 25) showed oxidant TBHP, internal standard dodecane, starting material and product, with few impurities. The GC trace of the reaction in decane (Figure 26) contained many more impurities after just 2 h.
Figure 25 – GC trace showing the oxidation of 4-phenyl-2-butanol 158 (13.3 min) to benzylacetone 101 (12.7 min) in water after 4 h. Reaction conditions: alcohol (0.3 mmol), TBHP (1.2 mmol), water, 95 °C, Au/MgO (10 wt%) catalyst (1 mol%). Dodecane (0.1 eq.) was added on extraction into ethyl acetate. GC analytical method A was used.

Figure 26 – GC trace showing the oxidation of 4-phenyl-2-butanol 158 (13.3 min) to benzylacetone 101 (12.7 min) in decane after 2 h. Reaction conditions: alcohol (0.3 mmol), dodecane (0.03 mmol), TBHP (1.2 mmol), decane, 95 °C, Au/MgO (10 wt%) catalyst (1 mol%). GC analytical method A was used.

The successful, clean oxidation of this substrate shows the increased scope and potential of supported gold nanoparticle catalysts in oxidations of secondary non-benzylic alcohols. The literature reports of gold catalysed oxidations for this substrate included the use of polymer supports and basic additives. Depositing gold nanoparticles on readily available bulk materials provides a simpler route involving less complex organic or inorganic synthesis than the reported techniques.
3.5 Extension of the substrate scope

The extension of the substrate scope of this oxidation system was investigated with three more substrates with varying functionalities: allylic alcohol 4-phenyl-3-buten-2-ol \textbf{159}, propargylic alcohol 4-phenyl-3-butyn-2-ol \textbf{160} and alkyl alcohol 2-heptanol \textbf{161} (Figure 27).

Figure 27 – Substrates for oxidations by TBHP in the presence of supported gold catalysts.

The oxidation of alkene \textbf{159} has been reported in the literature with various metal catalysts, some involving complex stabilising ligands and expensive or toxic transition metals such as Rh and Cr\textsuperscript{129,130}. Gold nanoparticle catalysts have been used for this substrate with O\textsubscript{2} as the oxidant, but required complex support materials (Table 18). As with the oxidation of 4-phenyl-2-butanol \textbf{158}, Au-PI (polymer incarcerated) catalysts have been used for the oxidation of allylic alcohol \textbf{159}. Other support materials included mesoporous organosilica, supramolecular ionic liquid grafted graphene and monolithic nanoporous gold skeleton material. The microencapsulation and immobilisation of Au nanoparticles was also reported for use in a flow system. These processes all resulted in excellent yields of 93-99%, but required complex support materials, and in some cases the use of toxic solvents.
<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Oxidant</th>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>Solvent</th>
<th>Additive</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au-PI/CB&lt;sup&gt;125&lt;/sup&gt;</td>
<td>O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>30</td>
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<td>99&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 18 – Literature reports of the gold nanoparticle catalysed oxidation of 159. <sup>a</sup>Determined by GC. <sup>b</sup>Determined by 1H NMR with internal standard. <sup>c</sup>Isolated yield. Au-PI/CB: Polymer incarcerated and carbon black stabilised Au nanoclusters. AuNPore: Nanoporous gold skeleton material. PMO: Periodic mesoporous organosilica. SIL-g-G: Supramolecular ionic liquid grafted graphene. Au-ICC: Au-immobilised capillary column, under flow conditions. DCE: dichloroethane.

Reports of the metal catalysed oxidation of propargylic alcohol 160 are limited to a Fe/TEMPO system<sup>135</sup> and a vanadium catalysed oxidation requiring a quinolinate ligand.<sup>136</sup> The metal catalysed oxidation of 2-heptanol has been reported with Cu and Rh complexes.<sup>137,138</sup>

The oxidations of these substrates were tested in water with TBHP as the oxidant, and analysed by GC after extraction into ethyl acetate. The oxidation of alkene 159 to 4-phenyl-3-buten-2-one 163 was found to be extremely rapid with Au/MgO (Figure 28).
Figure 28 – Oxidation of 4-phenyl-3-buten-2-ol 159 over time. Reaction conditions: alcohol (0.3 mmol), TBHP (1.2 mmol), water, 95 °C, Au (10 wt%) catalyst (1 mol%). Reaction mixtures were extracted into ethyl acetate and analysed by GC using dodecane as a standard. Reactions were performed in duplicate.

With Au/MgO, a 77% product yield was observed after just 15 min. After this time the yield of 163 decreased, suggesting the product was reacting further. This could be due to epoxidation of the alkene by the excess TBHP, as four equivalents were used, and could easily be combated by stopping the reaction after 15 min. A moderate yield of 30% was achieved with Au/Al₂O₃ after 15 min, and this yield decreased slightly before increasing again, finishing with a yield of 46% after 6 h. The background reactivity of this substrate was low, reaching a maximum of 21% product yield after 4 h.

The reactivity of the Au/MgO catalysed system was further investigated using one equivalent of TBHP to observe the formation of the product. The reaction did proceed more slowly, with the yield rising from 22% after 15 min to 45% after 1 h (Figure 29). The presence of the desired ketone product 4-phenyl-3-buten-2-one 163 was also confirmed by NMR spectroscopy.
Figure 29 – Oxidation of 4-phenyl-3-buten-2-ol 159 over time. Reaction conditions: alcohol (0.3 mmol), TBHP (0.3 mmol), water, 95 °C, Au/MgO (10 wt%) catalyst (1 mol%). Reaction mixtures were extracted into ethyl acetate and analysed by GC using dodecane as a standard. Reactions were performed in duplicate.

The best catalyst for this reaction was the Au/MgO catalyst, with a 77% yield achieved after 15 min. By stopping the reaction after this time, the product could be extracted and analysed by NMR spectroscopy, confirming the presence of the desired ketone product 163.

The gold catalysts were also tested in the oxidation of propargylic alcohol 160 (Scheme 68).

Both the starting material and product of this reaction caused problems with analysis by GC. The standard sample of ketone 164, although clean by NMR, showed two peaks by GC analysis. It is thought that this was due to the instability of the compound under the flame ionization conditions. It appeared that after 15 min of reaction time, no starting material remained. However, when analysed by NMR spectroscopy, the presence of starting material and product was confirmed, but this was also difficult to quantify due to the volatility of the ketone product.
Although the yield of ketone 164 was not quantified, its presence was confirmed by NMR spectroscopy showing the oxidation of this substrate was occurring. Due to the problems with analysis, substrate 160 was not explored further.

The GC data for 2-heptanol 161 showed large experimental errors for some time points (Figure 30). However, the reaction with no catalyst clearly showed minimal formation of ketone 99 of less than 5%. Despite the large analytical errors, the data clearly showed that both Au/MgO and Au/Al₂O₃ successfully catalysed this reaction achieving yields of around 20% after 4 h.

![Chemical reaction diagram](image)

**Figure 30** – Oxidation of 2-heptanol 161 over time. Reaction conditions: alcohol (0.3 mmol), TBHP (1.2 mmol), water, 95 °C, Au (10 wt%) catalyst (1 mol%). Reaction mixtures were extracted into ethyl acetate and analysed by GC using dodecane as a standard. Reactions were performed in duplicate.

Further investigation of this substrate including earlier time points and an improved analytical procedure would provide more understanding of the oxidation of this substrate and the stability of the materials over time. The oxidations of 2-heptanol 161 and propargylic alcohol 160 both require more investigation, but show potential for the rapid, clean transformation of diverse substrates. Although problems were encountered with the analysis of some reaction mixtures using 160 and 161, all reactions in water were much cleaner than reactions in decane, with fewer side products observed by GC and NMR spectroscopy. Indeed,
analysis of the reaction mixtures typically showed only the presence of starting material and desired product (Figure 31).

Figure 31 – GC trace showing the oxidation of 2-heptanol 161 to 2-heptanone 99. Reaction conditions: alcohol (0.3 mmol), TBHP (1.2 mmol), water, 95 ºC, Au/Al₂O₃ (10 wt%) catalyst (1 mol%). Dodecane (0.1 eq.) was added on extraction into ethyl acetate. GC analytical method C was used.

3.6 Catalyst properties

To probe the applicability of the Au/MgO catalyst in sustainable chemistry the recycling of the catalyst was also tested. The reusability of the Au/MgO catalyst was explored using 4-phenyl-2-butanol 158 and 4-phenyl-3-buten-2-ol 159 (Figure 32), as these were the highest yielding oxidations observed. The catalyst was used in water under standard conditions, separated from the reaction mixture by centrifugation, washed with water and dried (50 ºC) before being used again.

The yield of benzylacetone 101 remained similar in all three cycles of the catalyst. The initial yield of 30% rose to 40% during the second use of the catalyst, and then fell to 26% on the third use. These variations in yield could be due to the error associated with the extraction of products before analysis, but show that overall only a small loss in yield was observed after three uses. A 75% yield of 4-phenyl-3-buten-2-one 163 was observed after the first use, and this remained high giving 73% product yield during the second use. The yield then decreased to 48% on the third use of the catalyst.
Gold leaching experiments were undertaken to understand the type of catalysis observed. True heterogeneous catalysis takes place on the surface of the catalyst, but gold catalysts have been reported to leach into solution and perform homogeneous catalysis with the reagents in solution.\textsuperscript{139} To explore this possibility, gold leaching experiments were undertaken in the oxidation of 4-phenyl-2-butanol \textbf{158}. The reaction was set up under standard conditions, and the gold catalyst was removed from the reaction via hot filtration after 2 h. If the catalysis was homogeneous in nature, the oxidation would continue after the removal of the solid catalyst. When the reaction mixture was analysed after 4 h, no further oxidation had taken place after removal of the catalyst, suggesting the catalysis was heterogeneous in nature.
3.7 Summary

The application of supported gold nanoparticle catalysts has been successfully achieved in the oxidation of non-benzylic secondary alcohols such as 4-phenyl-2-butanol 158 and 4-phenyl-3-buten-2-ol 159, giving rise to moderate to high yields and short reaction times. Propargylic alcohol 160 was oxidised using the gold catalysts, but difficulties with quantification of the results were encountered. 2-Heptanol 161 showed low to moderate conversions in the presence of Au catalysts, but crucially these conversions were higher than the no catalyst control. Formation of all of the ketone products were confirmed by GC and NMR spectroscopy. The results of the substrate scope investigations are summarised in Table 19.

![Reaction Scheme]

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalyst</th>
<th>Reaction time</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /> 101</td>
<td>Au/MgO (10 wt%)</td>
<td>4 h</td>
<td>45%</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /> 163</td>
<td>Au/MgO (10 wt%)</td>
<td>15 min</td>
<td>77%</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /> 164</td>
<td>Au/MgO (10 wt%)</td>
<td>15 min</td>
<td>n.d.</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /> 99</td>
<td>Au/MgO (10 wt%)</td>
<td>30 min</td>
<td>10-30%</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /> 99</td>
<td>Au/Al2O3 (10 wt%)</td>
<td>30 min</td>
<td>10-30%</td>
</tr>
</tbody>
</table>

Table 19 – Oxidations of secondary alcohols to ketones with Au (10 wt%) catalysts and aqueous TBHP as the oxidant. Reaction conditions: alcohol (0.3 mmol), TBHP (1.2 mmol), water, 95 °C, Au (10 wt%) catalyst (1 mol%). Yields were determined by GC using dodecane as a standard.
Au/MgO was consistently the best catalyst for these reactions, with Au/Al₂O₃ giving generally lower yields, although still above the no catalyst control. Water was used as a solvent, and after quenching of the peroxide with sodium sulfite, the only by-product was tert-butanol. The reusability of the Au/MgO catalyst is promising for use in green chemistry, but the high reaction temperatures and low yields require more investigation and reaction optimisation.
4 Transaminase catalysed synthesis of furfurylamines

4.1 Introduction

Transformations of chemicals derived from waste products have been gaining prominence, with platform chemicals such as furfural and HMF being investigated in catalytic processes involving both biocatalysis and heterogeneous catalysis, discussed in Section 1.1.2.\textsuperscript{21,25} In this section, the use of transaminases in the transformation of furfurals to furfurylamines was investigated.\textsuperscript{140}

Furfural 1 and HMF 2 (Scheme 69) were initially selected for screening against transaminases to form furfurylamines 165 and 166. As these compounds can be derived from waste products and can be considered ‘green’ chemicals, there is currently substantial interest in investigating their transformations to higher value chemicals and useful building blocks.\textsuperscript{141}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{scheme69}
\caption{Transaminase catalysed synthesis of furfurylamines 165 and 166.\textsuperscript{140}}
\end{figure}

These platform chemicals can be produced from agricultural waste feedstocks such as corn starch, rice waste and sugar beet pulp, through acid catalysed processes via hexose and pentose sugars (Scheme 70).\textsuperscript{16} The transformation of sugars to HMF and furfural has been reported with many homogeneous acid catalysts such as p-toluenesulfonic acid, sulfuric acid and hydrochloric acid. The use of catalytic acidic resins such as Amberlyst and Dowex ion exchange resins has also been investigated.\textsuperscript{142}
Scheme 70 – Acid catalysed routes to HMF 2 and furfural 1 from cellulose and hemicellulose. 

Furfurylamines, produced via amination of furfurals, are compounds with diverse applications. Biopolymers containing furfurylamines are a growing area of interest as they provide a renewable alternative to conventional polymers and plastics (Figure 33). Furfurylamine motifs appear in some pharmaceutical agents such as the diuretic drug Furosemide 167. 5-Aminomethylfurancarboxylic acid 168 has recently been identified as a potential GABA inhibitor, and could be produced in one step from 5-formyl-2-furoic acid in a transaminase catalysed process. 

A range of commercially available furfural analogues 26, 28 and 169-174 were included alongside furfural and HMF in the transaminase screening (Figure 34), to probe the different electronic and steric effects on transaminase activity. Screening of 3-, 4- and 5-bromofurfurals 169-171 would give an insight into the effect of steric bulk at different positions on the furan ring. Additionally, the bromine groups would provide a synthetic handle for further functionalisation of the amine products. 5-Chlorofurfural 172 and 5-methylfurfural 26 were chosen as examples of electron donating and withdrawing groups respectively.
5-Formyl-2-furoic acid 173 would provide access to GABA inhibitor 166 and 2,5-diformylfuran 28 was of interest as two aldehyde centres would be available for transamination. 2-Acetyluran 174 was included as an example of a ketone, which could provide access to both (S)- and (R)-enantiomers of the amine product.

![Chemical structures](image)

Figure 34 – Furfural substrates for transaminase screening.

Traditional organic syntheses of the desired furfurylamine products were problematic due to the sensitivity of the furan ring. The chemical routes also generally involved chlorinated solvents, toxic materials and are typically low yielding. These chemical routes are discussed in more detail in Section 4.4.

The synthesis of furfurylamines by reductive amination techniques has been discussed in Section 1.1.2, and biocatalytic synthesis of these compounds has been limited in the literature, with one report of a kinetic resolution in 2015 and a patent of the transaminase catalysed transformation of HMF 2 in 2012. The kinetic resolution of rac-1-(2-furyl)ethylamine 175 was reported using (S)- and (R)-selective commercial transaminases and a lipase (Scheme 71).
Two transaminases of opposing selectivity were found from a small commercial library, and provided \((S)\)- and \((R)\)-175 with excellent ee values. An alternative route was investigated using a lipase from *Candida antarctica* in an acylative kinetic resolution. This provided both \((S)\)-175 and acetamide \((R)\)-175 with excellent ee values.\(^{144}\) A patent was also found describing the application of Vf-TAm in the amination of HMF 2.\(^{145}\) The limited reports in this area made the transaminase catalysed synthesis of a range of furfurylamines an interesting and valuable application of these enzymes.

### 4.2 Enzyme preparation

The enzymes discussed in this section were prepared by overexpression in *E. coli* using IPTG, and the presence of the desired proteins verified by SDS-PAGE analysis. Protein concentrations were analysed by Bradford assay, with final enzyme concentrations of 0.6-1.1 mg/mL Cv-TAm, 0.4-0.8 mg/mL ArRMut11 and 0.25-0.5 mg/mL Mv-TAm used in assays. The enzymes were used as crude lysates and tolerated the presence of 2-10% final concentration of DMSO in the reaction media to aid solubility of some substrates.
4.3 Initial screening

Three transaminases were selected for screening from the UCL transaminase toolbox. The discovery and substrate scope of these transaminases was discussed in detail earlier (Section 1.3.1). As Cv-TAm had previously shown high activity with aromatic aldehydes, this transaminase was selected for testing with furfurals. ArRMut11 was selected for screening against furfurals due to its high IPA and DMSO tolerance. Mv-TAm has mainly been used in the transformation of ketones in the literature, and it was selected for screening with furfurals to extend its substrate scope.

An initial screen of the selected furfural substrates and transaminases was undertaken using a sensitive colorimetric assay developed at UCL, as discussed in Section 1.3.3 (Scheme 72).

Scheme 72 – Colorimetric assay using 2-(4-nitrophenyl)ethan-1-amine 123 as the amine donor forming red precipitate 125.

When amine donor 2-(4-nitrophenyl)ethan-1-amine 123 is converted to the aldehyde byproduct, this can react with another molecule of 123 forming an intermediate which tautomerises to form a red precipitate. The results of the colorimetric assay are shown in Table 20. Benzaldehyde 40 was included as a positive control.
The substituted aldehyde substrates showed excellent activity with all three of the selected enzymes, and the strongest colourations were with Cv-TAm, suggesting high product yields. Cv-TAm showed good activity with all of the aldehyde substrates, with the strongest colouration visible with furfural 1, HMF 2, 5-methylfurfural 26 and carboxylic acid 173 (visually discernible by eye). Bromofurfurals 169-171, 5-chlorofurfural 172 and dialdehyde 28 were also accepted, but very little red precipitate was formed with ketone 174. ArRMut11 showed lower colouration overall, but was active with furfural 1, HMF 2, bromofurfurals 169-171 and 5-methylfurfural 26. Lower activity was observed with 5-chlorofurfural 172, carboxylic acid 173 and dialdehyde 28, and no activity was observed with ketone 174. Mv-TAm had high activity with furfural 1, HMF 2 and 5-methylfurfural 26. Activity was also visible with bromofurfurals 169-171, 5-chlorofurfural 172, carboxylic acid 173 and dialdehyde 28. Some red precipitate was observed with ketone 174, showing higher activity than with the other enzymes.

The colorimetric assay showed high activity of all three enzymes with furfural 1 and HMF 2 and variation in activity of the other furfural derivatives. Ketone 174 was not well accepted in this assay, but some activity was observed with Mv-TAm.

Table 20 – Results of the colorimetric assay with three enzymes and 11 substrates. Reaction conditions: substrate (10 mM), 123 (25 mM), PLP (0.4 mM), KPi pH 7.5 (100 mM), crude cell lysate (0.4 mg/mL). Red/orange colouration shows transaminase activity.
The next step in the screening of transaminases with selected furfural substrates was the use of a quantitative assay using (S)- or (R)-α-methylbenzylamine (MBA) as the amine donor. Ketone by-product acetophenone 176 was detected by HPLC at 254 nm (Scheme 73), allowing indirect yields to be calculated.

Scheme 73 – Transaminase reaction with (S)- or (R)-α-methylbenzylamine (MBA) as the amine donor.

This assay was developed to provide a fast and simple way to determine transaminase activity. By testing enzymes with both enantiomers of MBA, enantiopreference of enzymes can easily be determined by detection of acetophenone. Acetophenone can be detected with high sensitivity, and therefore reaction kinetics can also be investigated using this technique.146

As the enzymes used in the screening were already characterised, MBA screening was undertaken using the enantiomer corresponding to the established stereoselectivity. As Cv-TAm is (S)-selective, (S)-MBA was used, and (R)-MBA was used for (R)-selective ArRMut11 and Mv-TAm. The results of the screening are shown in Figure 35, again including benzaldehyde 40 as a positive control. Reactions were carried out in triplicate and showed good consistency between the repeats, as shown by the error bars.
Figure 35 – Results of MBA screening. Conversion based on acetophenone formation. Reaction conditions: substrate (5 mM), (S)- or (R)-MBA (25 mM), PLP (1 mM), KPi pH 7.5 (100 mM), crude cell lysate (0.4 mg/mL). *Yield based on formation of diamine.

As expected from the initial colorimetric screen, furfural 1 and HMF 2 were converted in excellent yields of 76 and 79% with Cv-TAm, above the yield of benzaldehyde, the positive control. With bromofurfurals 169-171, Cv-TAm activity fell slightly from 57% with 3-bromofurfural 169 to 48% with 4-bromofurfural 170, but decreased further to 35% with 5-bromofurfural 171. Conversions were also low at 17% for 5-chlorofurfural 172 and 30% for 5-methylfurfural 26. This suggests that steric bulk or lipophilic groups at the C-5 position are not well tolerated by Cv-TAm, possibly due to unfavourable interactions in the active site. Interestingly, with a carboxylic acid group at the C-5 position, activity was higher with 54% conversion. The negative effect of steric bulk could be counteracted in this case with favourable electrostatic interactions from the carboxylate group.

As dialdehyde 28 could be aminated twice by the enzyme, this resulted in over 100% yield of acetophenone for this substrate (Scheme 74).
Scheme 74 – Double amination of dialdehyde 28 with transaminases to form diamine 21.

To simplify this data, HPLC yields of diamine product are shown in Figure 35, which are discussed further in Section 4.4. A high yield of 70% of diamine product bis(aminomethyl)furan 21 was achieved with Cv-TAm. Ketone substrate 174 was converted in 17% yield, which was higher than expected from the colorimetric screen.

Cv-TAm has a wide substrate scope reported in the literature, and its high activity in the amination of aromatic aldehydes such as benzaldehyde, cinnamaldehyde and vanillin has been highlighted. The high activity of Cv-TAm with aromatic aldehydes is further demonstrated in the successful amination of furfurals.

ArRMut11 showed lower yields overall than Cv-TAm, which was expected from the colorimetric screen. Furfural 1 and HMF 2 were converted in 62% and 54% yields respectively, comparable to the 54% yield positive control benzaldehyde. As seen with Cv-TAm, as the bromo group was moved from C-3 in 169 to C-4 in 170 the yield fell from 59% to 46%. Low yields of 16% with 5-bromofurfural 171 and 14% with 5-chlorofurfural 172 were observed, showing the low tolerance of ArRMut11 to halogen groups at the C-5 position. However, 5-methylfurfural 26 had a high conversion of 60%, suggesting that electronic effects at the C-5 position were more important than steric effects. ArRMut11 showed negligible conversions of below 10% with carboxylic acid 173 and ketone 174, and dialdehyde 28 was converted to diamine 21 in a moderate 57% yield.

The lower overall yields with ArRMut11 could be attributed to the fact that this enzyme was designed to tolerate a very bulky substrate in the synthesis of Sitagliptin (see Section 1.3.1). Due to its high steric tolerance, this enzyme has since been used in the amination of very bulky steroids and challenging cyclic ketones. As the active site can accommodate sterically demanding substrates, the interactions with smaller substrates such as furfurals may be reduced.
Mv-TAm again showed high conversions of 70% with furfural 1 and HMF 2, above the positive control benzaldehyde 40. Mv-TAm was tolerant of bromo groups at the C-3 and C-4 positions with conversions of 53% and 54%, and showed an excellent conversion of 82% with 5-bromofurfural 171. Mv-TAm also had good conversions of 48% with 5-chlorofurfural 172 and 79% with 5-methylfurfural 26, showing its tolerance to electron withdrawing and donating groups as well as steric bulk at the C-5 position. Carboxylic acid 173 was converted in 35% yield and dialdehyde 28 in 56% yield. Ketone 174 was accepted with a 32% conversion by Mv-TAm, which was the highest yield of the three transaminases for this substrate.

Mv-TAm has previously been reported in the kinetic resolution of substituted methylbenzylamines (Section 1.3.1), with bromo, fluoro and methyl substituents at the para- and meta-positions of the phenyl ring. The successful kinetic resolution of these substrates shows the tolerance of the Mv-TAm active site to these substituents, and can be compared to the high tolerance of Mv-TAm to C-3, C-4 and C-5 substituted furfurals with bromo, chloro and methyl groups.

These results highlighted furfural 1 and HMF 2 as excellent substrates for all three transaminases, with conversions exceeding that of the positive control, benzaldehyde 40. Tolerance of steric bulk at the C-5 position was investigated using 3-, 4- and 5-bromofurfurals 169-171. For Cv-TAm and ArRMut11, activity fell as the bromo group moved from C-3 to C-4 and C-5. Mv-TAm showed the opposite trend, with 5-bromofurfural 171 showing the highest conversion of the bromofurfurals. The presence of chloro, methyl and carboxylic acid groups at the C-5 position was also tested, with 5-methylfurfural 26 better accepted than 5-chlorofurfural 172 by all three enzymes, and the carboxylic acid 173 tolerated by Cv-TAm and Mv-TAm. Yields of diamine 21 were above 50% for all three enzymes, showing the ability of the transaminases to perform a double amination. Overall, the activity of aldehyde substrates was much higher than ketone 174, which was accepted by Cv-TAm and Mv-TAm with low conversions.
4.4 Reaction development and amine synthesis

The unfavourable equilibrium of the transaminase reaction can be overcome by multiple techniques including coproduct removal with additional enzyme systems,72 using selected amine donors to displace the equilibrium through polymerisation,95,97 and using large excesses of amine donors such as isopropylamine (IPA),4 as discussed in Section 1.3.3.

Reaction development was undertaken with selected substrates from the initial screen, using IPA as the amine donor. This donor was investigated as it is inexpensive, and the quantity of amine used can be increased to shift the equilibrium of the transaminase reaction (Scheme 75). Additionally, IPA and its ketone by-product, acetone 176, are both volatile making isolation of products simpler on a preparative scale, avoiding the need for purification by column chromatography.

Scheme 75 – Transaminase reaction with isopropylamine as the amine donor.

ArRMut11 was developed to accept IPA as a donor, and Cv-TAm and Mv-TAm have also been successfully used with IPA, in the amination of cyclic ketones.4,82 Furfural 1, HMF 2, acid 173, dialdehyde 28 and ketone 174 were selected for reaction optimisation using IPA as a donor, due to their high activities and potentially interesting applications of their furfurylamine products. Furfurylamine 165 and 1-(2-furan)ethylamine 175 were commercially available, but furfurylamine products 166, 21 and 168 were unavailable so these compounds were synthesised chemically for use as HPLC standards.
In the literature, the synthesis of substituted furfurylamines 166, 21 and 168 is mostly achieved by reductive amination techniques. 166 has been synthesised using a Co MOF catalyst, Raney Ni and supported Pd and Rh catalysts, as discussed in Section 1.1.2. These procedures are summarised in Table 20.

![Figure 36 – Desired furfurylamine products.](image)

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Solvent</th>
<th>Temp (°C)</th>
<th>H₂ (bar)</th>
<th>NH₃</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raney Ni¹⁴⁷</td>
<td>MeOH</td>
<td>65-75</td>
<td>100</td>
<td>liquid</td>
<td>72% isolated</td>
</tr>
<tr>
<td>Rh/Al₂O₃²¹</td>
<td>-</td>
<td>80</td>
<td>20</td>
<td>aq. solution</td>
<td>100% conversion 86% selectivity</td>
</tr>
<tr>
<td>Co NPs¹⁴⁸</td>
<td>¹BuOH</td>
<td>120</td>
<td>40</td>
<td>5-7 bar</td>
<td>89% isolated</td>
</tr>
</tbody>
</table>

Table 21 – Reductive amination of HMF 2 in the literature, using various catalysts, ammonia and H₂. CO NPs: CO-DABCO-TPA@C-800 nanoparticle catalyst.

Although these approaches were high yielding, the disadvantages of using dangerous and expensive catalysts, high temperatures and hydrogen pressure prevented the use of these techniques. Another report of the synthesis of 166 from furfurylamine 165 was found, reacting acetaldehyde with 165 in the presence of HCl.¹⁴⁹ This route was not attempted due to the very low yield reported.
The synthesis of 21 has also been reported using reductive amination techniques involving NH₃, H₂ and catalysts such as Raney Ni and supported Ru nanoparticles. The procedure using Raney Ni resulted in a low yield of 33%, and although the Ru procedure had an excellent yield of 93% over two steps, it required the use of two different Ru catalysts, Ru/Nb₂O₃ and Ru-Xantphos. Again, these procedures also required elevated hydrogen pressures.

Reported syntheses of amino acid 168 involved the transformation of 5-chloromethylfurfural 177 via oxidation and amination. A patented report used a gold catalysed oxidation procedure followed by treatment with ammonia. Another route was reported using Jones oxidation followed by azide formation (Scheme 77).

This route involved oxidation and esterification of the aldehyde group, followed by azide formation and reduction using PPh₃. The amine was then Boc protected, and the ester and amine finally deprotected using LiOH and TFA to give the amino acid TFA salt in 68% overall yield.
The synthesis of secondary furfurylamines was reported using various amine partners to form imines, followed by reduction with NaBH₄ (Scheme 78).\textsuperscript{19}

![Scheme 78 - Literature synthesis of 178 via reductive amination of HMF 2 with benzylamine.\textsuperscript{19}]

This procedure was replicated using benzylamine, to form the benzyl protected hydroxymethylfurfurylamine product 178 in 40% yield (Scheme 79).

![Scheme 79 - Attempted synthesis of 166 via benzyl protected intermediate.]

Removal of the benzyl group was then attempted by hydrogenation with H₂ (balloon pressure) and a Pd/C catalyst,\textsuperscript{64} but this reaction was unsuccessful, producing a complex mixture of products. Imine formation was also attempted with \textit{t}-butyl carbamate (BocNH₂) using stoichiometric borate reagent B(OCH₂CF₃),\textsuperscript{154} followed by reduction. This intermediate 179 could then be deprotected to form 166 (Scheme 80).

![Scheme 80 - Attempted synthesis of 166 via Boc protected intermediate 179.]

The presence of the imine intermediate was observed by NMR, but after addition of NaBH₄ no amine product could be isolated. Reductive amination was then attempted using ammonium formate and sodium cyanoborohydride (Scheme 81).
Scheme 81 – Attempted synthesis of 166 with ammonium acetate/sodium cyanoborohydride system, resulting in formation of trimer 180.

NMR analysis of the product seemed promising, but MS analysis showed that the trimer 180 had been formed rather than the desired primary amine product. This strategy was also attempted on 2,5-diformylfuran 28 to form the diamine product 21, but no furan product was observed by NMR. The low mass recovery and formation of unwanted trimer product 180 made this procedure unfeasible, and therefore another route was attempted.

A multi-step route was established based on the published route involving azide formation and reduction discussed previously. Bis(hydroxymethyl)furan 85 and 5-hydroxymethylfurancarboxylic acid 86 were required as starting materials, and were synthesised by reduction of commercially available compounds (Scheme 82).

Scheme 82 – Reduction of commercially available compounds with NaBH₄ to form 5-hydroxymethylfurancarboxylic acid 86 and bis(hydroxymethyl)furan 85.

Tosylation of HMF 2 and bis(hydroxymethyl)furan 85 was attempted but was unsuccessful, so chlorination of the furan substrates was investigated using concentrated HCl to give chloromethylfurans 177, 181 and 182 (Scheme 83).
Scheme 83 – Chlorination of 2, 86 and 85 with concentrated HCl to form 5-chloromethylfurfural 177, 5-chloromethyl-2-furancarboxylic acid 181 and bis(chloromethyl)furan 182.

With the chloro substituted furan compounds in hand, the next steps of the strategy were investigated. The chloro compounds would first be displaced by an azide group, then reduced with PPh3. These steps would be attempted without purification or isolation of the intermediates, so Boc protection was required to simplify isolation and purification of the final amine. After purification, the Boc group would be removed using HCl to give the desired primary amines 166, 21 and 168 (Scheme 84).

Scheme 84 – General scheme showing amine synthesis via azide formation, reduction, Boc protection and deprotection.

Synthesis of 166 was attempted using this strategy, but initially problems with polymerisation were encountered. This was thought to be due to the presence of the reactive aldehyde group, so this was protected using an acetal (Scheme 85).

Scheme 85 – Synthesis of Boc-5-aminomethylfurfural 183 via azide formation and reduction.
Boc-5-aminomethylfurfural 183 was successfully isolated and purified using this synthetic strategy, with a 36% yield over 5 steps. Reduction of the aldehyde group with NaBH₄ to form 179 was successful, but the final deprotection of the Boc amine resulted in polymerisation and degradation of the product (Scheme 86).

\[
\begin{align*}
183 \xrightarrow{\text{NaBH}_4, \text{MeOH}} 179 \xrightarrow{\text{HCl, MeOH}} 166 \\
\end{align*}
\]

Scheme 86 – Attempted reduction and deprotection of 183 to form 166.

Protected amine 183 could also provide access to the singly aminated byproduct in the transamination of dialdehyde 28. Deprotection of the Boc group with acid provided 5-aminomethylfurfural 184 (Scheme 87), but this product rapidly decomposed forming an insoluble orange compound.

\[
\begin{align*}
183 \xrightarrow{\text{HCl, MeOH}} 184 \\
\end{align*}
\]

Scheme 87 – Deprotection of 183 with HCl to form 5-aminomethylfurfural 184.

Bis(chloromethyl)furan 182 was subjected to the azide formation, reduction and Boc protection conditions, giving Boc-bis(aminomethyl)furan 185 in 39% yield over three steps (Scheme 88). The Boc protected diamine was then successfully deprotected to the bis(aminomethyl)furan hydrochloric salt 21.HCl in 34% overall yield. This compound was then used as an HPLC standard.

\[
\begin{align*}
182 \xrightarrow{\text{i. NaN₃, ii. PPh₃, iii. Boc₂O}} 185 \xrightarrow{\text{HCl}} 21 \text{HCl} \quad 34\% \text{ overall yield from 182} \\
\end{align*}
\]

Scheme 88 – Synthesis of bis(aminomethyl)furan hydrochloric salt 21.HCl from 182.

Boc-5-aminomethylfurancarboxylic acid 186 was synthesised using the same strategy, and was isolated and purified in 71% yield over three steps. Upon
deprotection with HCl, desired amino acid product 168.HCl was isolated in 62% overall yield, and this compound was also used as an analytical standard.

Scheme 89 – Synthesis of 5-aminomethylfurancarboxylic acid hydrochloric salt 168.HCl from 181.

Reduction of amino acid 168 was attempted as another route to the final desired amine 166. The azide intermediates 187 and 188 were also isolated and reduced as alternative routes (Scheme 90).

Scheme 90 – Attempted reduction of amino and azido acids to form hydroxymethylfurfurylamine 166. Reduction using BH₃.THF (2 eq.) with 168 and 187 resulted in the recovery of starting material. Reduction using LiAlH₄ (2 eq., in dry THF under argon) with 187 resulted in the recovery of starting material. Reduction of 188 with NaBH₄ (1.5 eq., in MeOH) was successful but the addition of PPh₃ (2 eq., in MeOH) resulted in trace amounts of product.

Reduction of the amino acid product 168 was attempted with BH₃.THF but only starting material was recovered from this reaction. Azide acid 187 reduction was attempted with LiAlH₄ and BH₃.THF, again resulting in recovery of starting material. The aldehyde group of azide aldehyde 188 was successfully reduced with NaBH₄, but when PPh₃ was added to reduce the azide, only trace amounts of furan product were recovered. The scale up of the biocatalytic reaction using
Cv-TAm and IPA was therefore the only successful route to 166 (Section 4.4), and a sample from the preparative scale reaction was used as an HPLC standard.

The chemical routes to the furfurylamine products used here, as well as others reported in the literature were typically low yielding, multi-step, and required the use of metal catalysts eg. ruthenium$^{151,155,156}$ and nickel.$^{157}$ The one-step biocatalytic route to these compounds highlights the benefits of green processes to access pharmaceutically relevant products, as well as some of the drawbacks of traditional synthetic approaches.

Using commercial samples and synthesised compounds, HPLC calibration curves were prepared using a reverse phase column with a gradient of acetonitrile and water containing 0.1% trifluoroacetic acid.

The reaction conditions used for the MBA assay were changed when using IPA as the amine donor. To shift the unfavourable equilibrium of the transaminase reaction, the quantity of amine donor used was increased from 5 to 10 equivalents, and the temperature was increased. Initially, when tested on the positive control benzaldehyde 40, the higher IPA concentration caused the pH to increase and reduced enzyme activity. To avoid a high pH, stock solutions of IPA were prepared at pH 8, and therefore the pH of the buffer was changed to pH 8 from pH 7.5. As these conditions were hoped to be used on a preparative scale, the substrate concentration was increased from 5 mM to 10 mM, to probe whether the enzymes would tolerate elevated substrate concentrations. The effect of increasing the substrate concentration from 5 mM to 10 mM using 10 equivalents of IPA was investigated using furfural 1 as a substrate, monitoring furfurylamine 165 formation by HPLC (Figure 37).
Figure 37 – Effect of substrate concentration of yield of furfurylamine. Reaction conditions: furfural (5 or 10 mM), IPA (50 or 100 mM), PLP (1 mM), KPi pH 8 (100 mM), 35 °C, 24 h. Reactions were performed in triplicate and furfurylamine yield was determined by HPLC.

The yield of furfurylamine was the same with Cv-TAm and Mv-TAm, showing the enzymes were tolerant of an increase in furfural concentration, whereas with ArRMut11, the yield fell by around 10%.

These conditions were taken forward for testing on furfural, HMF, carboxylic acid 173 and dialdehyde 28, initially on a 200 µL scale, as with the MBA assay performed previously. As calibration curves of these products had been established, product yields were obtained with both MBA and IPA as amine donors, and a comparison is shown in Table 22.
<table>
<thead>
<tr>
<th>TAm</th>
<th>165</th>
<th>166</th>
<th>168</th>
<th>21</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MBA</td>
<td>IPA</td>
<td>MBA</td>
<td>IPA</td>
</tr>
<tr>
<td>Cv-TAm</td>
<td>80</td>
<td>92</td>
<td>75</td>
<td>89</td>
</tr>
<tr>
<td>ArRMut11</td>
<td>59</td>
<td>34</td>
<td>69</td>
<td>44</td>
</tr>
<tr>
<td>Mv-TAm</td>
<td>75</td>
<td>78</td>
<td>53</td>
<td>66</td>
</tr>
</tbody>
</table>

Table 22 - Yields for transaminase catalysed reactions producing 165, 166, 168 and 21 using MBA and IPA as amine donors. Reactions were performed in triplicate and conversions were determined using HPLC against product standards. Reaction conditions (MBA): substrate (5 mM), (S)- or (R)-MBA (25 mM), KPi pH 7.5 (100 mM), PLP (1 mM), 30 °C, 24 h. Reaction conditions (IPA): substrate (10 mM), IPA (100 mM), KPi pH 8 (100 mM), PLP (1 mM), 35 °C, 24 h.

Cv-TAm tolerated the IPA reaction conditions well, showing yield increases from 80% to 92% for furfurylamine 165 and 75% to 89% for 166. In the case of amino acid 168, the yield increased from 47% to 88%, showing the potential of this substrate for further reactions. A decrease in yield was observed with diamine 21 from 70% to 48%, which could be in part due to a higher rate of polymerisation of the monoamine product caused by the elevated temperature used.

Although ArRMut11 is known to tolerate IPA well, decreases in yields were observed for all substrates with this enzyme. The lowering in yield could be due to the increase in substrate concentration, as shown in Figure 37. As ArRMut11 has been used previously for very bulky substrates, the small size of the furfural substrates could also partly explain the low activity.

Mv-TAm performed well with IPA, showing increases in yields from 75% to 78% for furfurylamine 165 and 53% to 66% for 166. A large increase in yield from 32% to 59% was observed with amino acid 168. Diamine 21 was again formed in a 10% lower yield than with (R)-MBA, showing that this substrate performed better with (S)- or (R)-MBA as a donor with all three enzymes.

As formation of diamine 21 was not as efficient with IPA as the donor, another technique was applied to this reaction. As the dialdehyde substrate 28 required two amination reactions to form the desired product and not the unstable monoamine intermediate, twice the concentration of enzyme was used in the
reaction with IPA as the donor (Scheme 91). This resulted in an increase in yield with all three enzymes, reaching a yield of 60% with Cv-TAm.

Scheme 91 – Transaminase catalysed synthesis of 21 with the enzyme concentration doubled from 0.6 to 1.2 mg/mL (Cv-TAm), 0.4 to 0.8 mg/mL (ArRMut11) and 0.3 to 0.6 mg/mL (Mv-TAm). Reaction conditions: substrate (10 mM), IPA (100 mM), KPi pH 8 (100 mM), PLP (1 mM), 35 °C, 24 h. Yields of 21 were determined by HPLC.

The use of IPA to displace the equilibrium together with an increased temperature had a positive effect on yields with Cv-TAm and Mv-TAm. Overall, the highest yields obtained were with Cv-TAm producing furfurylamine 165 in 92% yield, 166 in 89% yield and 168 in 88% yield. Yields of diamine 21 were highest with Cv-TAm and (S)-MBA.

Ketone substrate 2-acetylfuran 174 displayed very different activity when tested under the IPA conditions compared to (S)- or (R)-MBA (Table 23). Yields of 1-(2-furan)ethylamine 175 fell significantly when using IPA instead of (S)- or (R)-MBA, from 14% to 2% with Cv-TAm and from 11% to 2% with ArRMut11. Mv-TAm provided the highest yield of 44% when using (R)-MBA, but this also fell to 3% when using IPA. This shows the ketone substrate to be a more difficult amination substrate than the aldehyde substrates discussed previously, as out of three amine donors and three enzymes tested, the only successful conditions to form 1-(2-furan)ethylamine 175 required Mv-TAm and (R)-MBA.
Table 23 – Transaminase catalysed synthesis of 1-(2-furan)ethylamine 175 using IPA or (S)- or (R)-MBA as the amine donor. Reactions were performed in triplicate with standard deviations <5%. Product yields were determined using HPLC against a commercial sample of rac-175. Reaction conditions (MBA): substrate (5 mM), (S)- or (R)-MBA (25 mM), KPi pH 7.5 (100 mM), PLP (1 mM), 30 °C, 24 h. Reaction conditions (IPA): substrate (10 mM), IPA (100 mM), KPi pH 8 (100 mM), PLP (1 mM), 35 °C, 24 h.

The reaction using Mv-TAm and (R)-MBA was scaled up to 800 µL in order to obtain ee values. A commercial sample of racemic 175 was used to develop an analytical method using normal phase chiral HPLC. The racemic amine was found to separate without derivatisation, but when reaction mixtures were injected the peaks were too small to accurately measure. The peak area could be maximised by increasing the absorbance of the amine by derivatisation with groups containing chromophores. The racemic amine was derivatised with a carbobenzyloxy (Cbz) group. Acetamide formation was also tested, as this could improve the peak shape compared to the unprotected primary amine. Cbz-175 provided the best separation and absorbance, so this method was used on reaction mixtures.
Scheme 92 – Derivatisation of rac-175 with Cbz (carboxybenzyl) and acetyl groups.

The large excess of (R)-MBA dominated the spectrum, but an ee value of 78% of (R)-Cbz-175 was calculated (Scheme 93), although the accuracy of this value may be low due to the poor peak shape and quality of the spectrum. The absolute configuration of the centre was assigned based on the reported stereoselectivity of this transaminase.\textsuperscript{158}

An ee value for the (S)-enantiomer formed with Cv-TAm was not determined as the yield of this reaction was too low to accurately measure the product peaks.
4.5 Preparative scale reactions

Excellent yields of furfurylamine products 165, 166 and 168 were achieved on a 200 µL scale with Cv-TAm and IPA as discussed previously, and these reactions were then performed on a preparative scale. The effect of increased substrate concentration was investigated on a 200 µL scale with furfural as the model substrate, together with changes in enzyme loading and IPA equivalents (Table 24). The reaction temperature was also increased from 35 °C to 37 °C.

![Chemical reaction diagram]

<table>
<thead>
<tr>
<th>[Substrate] (mM)</th>
<th>Crude cell lysate (mM)</th>
<th>IPA equivalents</th>
<th>Yield 165 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>20</td>
<td>10</td>
<td>85</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>10</td>
<td>52</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>10</td>
<td>78</td>
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<tr>
<td>40</td>
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<td>7.5</td>
<td>55</td>
</tr>
<tr>
<td>40</td>
<td>40</td>
<td>5</td>
<td>68</td>
</tr>
</tbody>
</table>

Table 24 – Effect of changing substrate concentration, enzyme loading and IPA equivalents on the transaminase catalysed synthesis of furfurylamine 165. Yields were determined by HPLC.

An increased substrate concentration from 10 mM to 20 mM resulted in a drop in yield from 85% to 52% when using the same amount of enzyme, but when the enzyme was doubled to match the increased substrate, a yield of 78% was obtained. Increasing the substrate concentration to 40 mM also gave a lower yield of 55% with the initial enzyme loading, but increased to 68% when more enzyme was added. Although a good yield was achieved with 40 mM substrate concentration, an excellent yield was obtained at 20 mM substrate concentration and therefore these conditions were used for the preparative scale reactions.

Preparative scale reactions were performed with 1 mmol substrate at 20 mM concentration, with 200 mM IPA and 50 mL total volume, for 24 h at 37 °C. The yields of the preparative scale reactions determined by HPLC are shown in Table 25.
Table 25 – Transaminase catalysed synthesis of furfurylamines on a preparative scale, with conversion yields determined using HPLC analysis against product standards. *Product isolated via Boc protection and deprotection to give amino acid hydrochloric salt 168.HCl. Reaction conditions: 1 mmol substrate (20 mM), IPA (200 mM), PLP (1 mM), KPi pH 8 (100 mM), 37 °C, 24 h, total reaction volume 50 mL. The reaction mixture contained DMSO (4%) in the case of 168 to aid solubility of the starting material.

An excellent yield of 83% of furfurylamine 165 was achieved on the 50 mL scale. This product was not isolated as issues were encountered with volatility of the product, and it is also a commercially available compound.

A good yield of 58% of 166 was obtained using HPLC analysis, and this product was isolated. IPA and acetone were removed by evaporation under reduced pressure, and an acid-base work up procedure was used to remove any starting material under acidic conditions, followed by extraction of the free amine under basic conditions. Pure amine 166 was extracted out of solution into ethyl acetate with no derivatisation or purification required.

A moderate yield of 40% was observed by HPLC of amino acid 168, which could have been caused by substrate inhibition as the 20 mM substrate loading had not been tested on a small scale for this reaction. This yield could be increased by reaction optimisation as an excellent yield of 88% was achieved on a 200 µL scale with lower substrate concentration. The insolubility of the carboxylic acid starting material in water or buffer solutions could also have affected the yield on a large scale, with 4% DMSO added to aid solubility. This product was also isolated but due to its solubility in water and the presence of both acid and amine groups, it could not be directly extracted out of the reaction mixture. After removal of the aqueous reaction media, amino acid 168 was Boc protected in methanol. With the amine protected, an acid-base work up could be used to remove starting...
material under basic conditions, and Boc-\textbf{168} was then extracted into ethyl acetate under mildly acidic conditions. The solvent was removed and the amino acid deprotected using HCl, to give the amino acid hydrochloride salt \textbf{168.HCl} in 31\% yield, with no need for column chromatography.

Three furfurylamine products were synthesised on a 1 mmol preparative scale, using IPA. Amine donor IPA and keto by-product acetone could be removed by evaporation after the reaction, and amines \textbf{166} and \textbf{168} were isolated with no purification required. As previous chemical syntheses discussed in Section 4.4 were unsuccessful in preparing \textbf{166}, the product from the biocatalytic reaction was used as an analytical standard for HPLC. This one-step synthesis of pure amine \textbf{166} shows the benefits of biocatalysis compared with traditional organic synthesis. Amino acid \textbf{168} was also of interest as it has been recently identified as a GABA inhibitor, as it is a cyclic analogue of GABA.\textsuperscript{143} Comparing this mild, one-step procedure to the synthetic route involving toxic and air sensitive reagents and solvents such as sodium azide and DMF, chlorinated solvents such as CH\textsubscript{2}Cl\textsubscript{2} and column chromatography, clearly highlights the benefits of using transaminase enzymes as synthetic tools.

\textbf{4.6 Summary}

In this section, transaminase enzymes have been identified as a mild and scalable route to access furfurylamines. Furfurylamines have diverse applications as pharmaceutical agents and biopolymers, and are difficult to access using traditional synthetic methods due to the sensitivity of the furan ring. Three transaminases have been shown to accept a range of furfural derivatives, using two amine donors, 2-(4-nitrophenyl)ethan-1-amine \textbf{123} and (\textbf{S})- or (\textbf{R})-MBA. Yields ranged from 6\% to 82\%, with every aldehyde substrate achieving around or over 50\% conversion yield with at least one transaminase. Furfural \textbf{1} and HMF \textbf{2} outperformed the positive control, benzaldehyde \textbf{40}, with all three enzymes.
Selected substrates including one ketone were taken forward for reaction optimisation investigating the use of IPA as the amine donor, to drive the reaction equilibrium towards the desired amine product. Amines 21 and 175 were formed in higher yields with (S)- or (R)-MBA than with IPA as the amine donor (Figure 38). A 70% yield of 21 was achieved with Cv-TAm with (S)-MBA compared to 60% with IPA. (R)-175 was formed in 54% yield when using Mv-TAm with (R)-MBA, but achieved just 3% yield with IPA. An ee value of 78% was obtained by chiral HPLC for this compound, compared to a racemic commercially available sample.

Other selected substrates furfural 1, HMF 2 and carboxylic acid 173 performed better with IPA as the amine donor, with yields increasing with Cv-TAm and Mv-TAm. Good to excellent yields were achieved of 88-92% with Cv-TAm and 59-78% with Mv-TAm with these three substrates. Reactions with IPA and Cv-TAm were then tested on a preparative scale, giving a high HPLC yield of 83% of furfurylamine at elevated substrate concentration and temperature. 5-Hydroxymethylfurfurylamine 166 was isolated in 54% through simple extraction into organic solvent, with no need for column chromatography. Amino acid 168 was also isolated via Boc protection and deprotection in a 31% yield.
The use of transaminases in the synthesis of these furfurylamine products has clear benefits over the traditional synthetic routes discussed in Section 4.4. The mild, aqueous reaction conditions, environmentally benign biocatalyst and one-step nature provide a high yielding, sustainable synthesis of furfurylamines on a small and preparative scale.
5 Two-step synthesis of chiral amines from alcohols

5.1 Introduction

The aim of the work described in this section was to use heterogeneous catalysis and biocatalysis methods in tandem to access chiral amines from alcohol feedstocks. This was investigated using an oxidation step catalysed by supported gold nanoparticles, and a transamination step using stereoselective transaminases.

![Scheme 95 – Two-step synthesis of chiral amines using supported gold nanoparticle catalysed oxidation and stereoselective transamination.](image)

Although the coupling of these two specific reactions is unprecedented, integrated heterogeneous catalysis and biocatalysis is gaining prominence in the literature, as discussed in Section 1.4. Initially, transaminases were identified for the transformation of the ketone intermediates using the screening techniques discussed in Section 4. The compatibility of the two reactions was then investigated using the model transaminase substrate benzaldehyde 40, to probe the tolerance of the enzymes to the presence of peroxide and catalyst from the oxidation step. The steps were then combined into a two-step process as a proof of concept on small and medium scales.

5.2 Enzyme preparation

The enzymes discussed in this section were prepared by overexpression in *E. coli* using IPTG, and the presence of the desired proteins verified by SDS-PAGE analysis. Protein concentrations were analysed by Bradford assay, with final enzyme concentrations of 0.4-1 mg/mL used in assays. The enzymes were used as clarified cell lysates and tolerated the presence of 2-10% final concentration of DMSO in the reaction media to aid solubility of some substrates.

5.3 Transaminase screening with oxidation products

Secondary alcohol oxidation substrates were identified as previously discussed in Section 3, and were successfully converted into ketone intermediates using
supported gold nanoparticle catalysed oxidations. For these ketones, transaminases had to be identified for the second step of the process. The use of transaminases with benzylacetone 101 has been described in the literature (Table 26). High conversions and stereoselectivities have been reported with (R)-selective transaminases from *Arthrobacter* (ArR-TAm), *Aspergillus terreus* (At-TAm) and *Hyphomonas neptunium* (Hn-TAm) with alanine and LDH pyruvate removal in situ.\(^{159}\) Commercial transaminases from Codexis were reported using these reaction conditions, but a low conversion of 15% was observed with ATA-114, although the ee was excellent. ATA-103 gave a higher conversion of 44% but a drop in ee value to 87% was observed.\(^{73}\) A low conversion of 30% but a high ee value of >99% was reported with a purified (S)-selective transaminase from *Burkholderia vietnamiensis* (Bv-TAm) using IPA as the donor.\(^{160}\) An (S)-selective enzyme from *Pseudomonas fluorescens* (Pf-TAm) was reported in a hydrogen borrowing system with alanine as the donor with a high conversion of 86% and 92% ee.\(^{161}\) Also, ArRMut11 was reported to give 93% conversion and 81% ee when used with IPA.\(^{159}\) This substrate has also been applied in 2015 in a flow system with immobilised transaminases.\(^{162}\)

![Diagram](image)

<table>
<thead>
<tr>
<th>Transaminase</th>
<th>Amine donor</th>
<th>Conversion (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArR-TAm(^{159})</td>
<td>Alanine (LDH)</td>
<td>82</td>
<td>&gt;99 (R)</td>
</tr>
<tr>
<td>At-TAm(^{159})</td>
<td>Alanine (LDH)</td>
<td>87</td>
<td>&gt;99 (R)</td>
</tr>
<tr>
<td>Hn-TAm(^{159})</td>
<td>Alanine (LDH)</td>
<td>94</td>
<td>&gt;99 (R)</td>
</tr>
<tr>
<td>ATA-114(^{73})</td>
<td>Alanine (LDH)</td>
<td>15</td>
<td>&gt;99 (S)</td>
</tr>
<tr>
<td>ATA-103(^{73})</td>
<td>Alanine (LDH)</td>
<td>44</td>
<td>87 (S)</td>
</tr>
<tr>
<td>Bv-TAm(^{160})</td>
<td>IPA</td>
<td>30</td>
<td>&gt;99 (S)</td>
</tr>
<tr>
<td>Pf-TAm(^{161})</td>
<td>Alanine</td>
<td>86</td>
<td>92 (S)</td>
</tr>
<tr>
<td>ArRMut11(^{159})</td>
<td>IPA</td>
<td>93</td>
<td>81 (R)</td>
</tr>
</tbody>
</table>

Table 26 – Transaminase catalysed transformations of 101 to 136. ArR-TAm (*Arthrobacter*), At-TAm (*Aspergillus terreus*), Hn-TAm (*Hyphomonas neptunium*), ATA-114 & ATA-103 (Codexis), Bv-TAm (*Burkholderia vietnamiensis*), Pf-TAm (*Pseudomonas fluorescens*), ArRMut11 (*Arthrobacter variant*).
Alkyne substrate 4-phenyl-3-butyn-2-one 164 has been previously used with (S)-selective Cv-TAm and (R)-selective Ar-R and At-TAm, with alanine as the donor and a recycling system involving ammonium formate, FDH and AlaDH, with 99% conversion and >99% ee.163

Building upon these reports, the selected ketones were tested against a range of transaminases to identify potential enzymes for the two-step synthesis of chiral amines. Fifteen transaminases from the UCL transaminase toolbox were tested against the standard amine acceptor benzaldehyde 40, together with the substrates of interest benzylacetone 101, 4-phenyl-3-buten-2-one 163, 4-phenyl-3-butyn-2-one 164 and 2-heptanone 99 using the colorimetric assay discussed previously (Figure 40).96

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16</td>
</tr>
<tr>
<td>101</td>
<td></td>
</tr>
<tr>
<td>163</td>
<td></td>
</tr>
<tr>
<td>164</td>
<td></td>
</tr>
<tr>
<td>99</td>
<td></td>
</tr>
<tr>
<td>No substrate</td>
<td></td>
</tr>
</tbody>
</table>


Benzaldehyde 40 showed the strongest colouration with Cv-TAm (Chromobacterium violaceum), Pp958-TAm and Pp959-TAm (Pseudomonas putida), Rr1017-TAm (Rhodospirillum rubrum) and Rs1019-TAm (Rhodobacter
sphaeroides). Activity was also observed with Sa807-TAm (Streptomyces avermilitis), Kp904-TAm (Klebsiella pneumoniae), Bs906-TAm (Bacillus subtilis), Mv-TAm (Mycobacterium vanbaalenii) and ArRMut11 (Arthrobacter sp. variant). No activity was observed with Sa805-TAm, Bs960-TAm, Bs961-TAm, Vf-TAm (Vibrio fluvialis) and Kp1006-TAm. The lack of activity with some of these enzymes could be due to incompatibility with the substrate or amine donor, or problems with the enzyme expression. For example, problems with enzyme expression were likely to be the cause of the low activity with Vf-TAm, which has been reported to accept benzaldehyde as a substrate. These problems were not investigated further as other active enzymes were identified for use with the substrates of interest.

Overall, the other substrates showed less activity than benzaldehyde with the fifteen transaminases. Benzylacetone 101 showed activity with Cv-TAm, Pp958-TAm, Pp959-TAm, Rs1019-TAm and ArRMut11. Alkene 163 had much less colouration but showed some activity with Cv-TAm, Rs1019-TAm and Sa807-TAm. Alkyne 164 had a high background reaction and showed low activity with Cv-TAm, Pp958-TAm, Pp959-TAm, Rs1019-TAm, Mv-TAm and ArRMut11. 2-Heptanone 99 had very low activity with Cv-TAm and Pp959-TAm.

Although activity was generally low, seven transaminases were found to have activity with the substrates and these were taken forward for testing with (S)- or (R)-MBA as the amine donor. Conversion yields were calculated by observing formation of the by-product acetophenone by HPLC, and the results are shown in Table 27. Benzylacetone 101 achieved over 60% conversion with Cv-TAm and 58% with ArRMut11. Moderate yields of 32% with Rs1019-TAm and 29% with Pp958-TAm were achieved, and a low yield of 8% was observed with Pp959-TAm. Alkene 163 was lower yielding overall, with Cv-TAm, ArRMut11 and Mv-TAm performing best giving 12-14% yields of acetophenone, and Rs1019-TAm and Sa807-TAm giving yields of below 5%. Low yields were also observed with alkyne 164, with ArRMut11 and Mv-TAm giving yields of 14% and 15%, and Cv-TAm, Pp958-TAm, Pp959-TAm and Rs1019-TAm giving less than 5% conversion yield. 2-Heptanone 99 was the lowest yielding substrate, with yields of below 10% with Cv-TAm, Pp959-TAm and ArRMut11. As Cv-TAm, ArRMut11 and Mv-TAm gave the highest yields of the enzymes tested, these were taken forward for reaction development.
Table 27 – (S)- or (R)-MBA screening of substrates with selected transaminases. (S)-MBA used with Cv-TAm, Pp958-TAm, Pp959-TAm, Rs1019-TAm and Sa807-TAm. (R)-MBA used with ArRMut11 and Mv-TAm. Reaction conditions: substrate (5 mM), (S)- or (R)-MBA (25 mM), KPi pH 7.5 (100 mM), PLP (1 mM), clarified cell lysate (0.4 mg/mL). Reactions were performed in triplicate. Conversions were based on the formation of acetophenone by HPLC.

The loading of enzyme used in these reactions was 0.4 mg/mL, and this was increased to 1 mg/mL to increase reaction yields. This strategy had some success, and the results are shown in Figure 41. Benzylacetone 101 was the most readily accepted substrate, with a 67% yield achieved with Cv-TAm, 51% with ArRMut11 and 28% with Mv-TAm. Alkene 163 showed reductions in yields with Cv-TAm and ArRMut11 to below 10%, but an increase in yield to 23% with Mv-TAm. Yields also remained low for alkyne 164 with Cv-TAm and ArRMut11, but increased with Mv-TAm to 20%. 2-Heptanone 99 showed increased yields with all three enzymes, achieving 15% with Cv-TAm, 20% with ArRMut11 and 26% with Mv-TAm.
5.4 Transaminase reaction development with benzylacetone 101

As benzylacetone was the best accepted substrate by the transaminases, and was produced in moderate yields in the oxidation reaction, this substrate was further investigated with the transaminases (Scheme 96). The desired amine product 1-methyl-3-phenylpropylamine 136 was also commercially available, and could be detected by HPLC using a reverse phase column.

Scheme 96 – Transaminase reaction with benzylacetone 101 to form 136.

The use of IPA as the amine donor, as discussed in Section 4, was investigated with 101. The substrate concentration and IPA equivalents were varied, and the results are shown in Figure 42. Yields of 136 were lower overall when using IPA as the amine donor compared to (S)- or (R)-MBA. Yields were higher when using 10 equivalents of IPA instead of 5, shown by an increase from 29% to 43% with
Cv-TAm with a 10 mM substrate concentration. This trend was also observed with Pp958-TAm and Pp959-TAm, and to a lesser extent with Rs1019-TAm and ArRMut11. No difference in yield was observed when the substrate concentration was increased from 10 mM to 20 mM with all of the enzymes tested, although higher yields of over 60% were observed with a 5 mM substrate concentration with (S)-MBA and Cv-TAm. Lower yields were observed when using 50 mM substrate concentration, but this could also be due to the lower equivalents of IPA used. Overall, with a 10 mM 101 concentration and 5 equivalents of IPA, Cv-TAm gave the highest yield of 43%, Pp958-TAm and Pp959-TAm gave moderate yields of 32% and 36%, and Rs1019-TAm and ArRMut11 were lower yielding at 20%.

As higher IPA equivalents enhanced the yield of 136, the reaction was also investigated with 25 and 50 equivalents of IPA at 10 mM substrate concentration. This resulted in a lowering of yields with all five enzymes, with a maximum of 15% achieved with Cv-TAm.

The effect of pH on activity was probed by varying the pH between 8 and 12, and the results are shown in Figure 43. The highest yields of 136 were achieved when the reaction was conducted at pH 8, with a 44% yield observed with Cv-TAm, correlating with previous results (Figure 42). Cv-TAm was tolerant of higher pH conditions.
values, with the yield dropping to 38% at pH 9 and 33% at pH 10. At pH 11 the yield decreased to 11%, and activity was completely lost at pH 12. The Pp958-TAm was much less tolerant of high pH values, giving a yield of 21% at pH 8 and negligible yields at pH 9-12. The Pp959-TAm showed higher activity at pH 8-10, and yields dropped to below 5% at pH 11 and 12. The Rs1019-TAm was active at pH 8 with a 16% product yield, dropping to 7% and 5% at pH 9 and 10, and no activity was observed at pH 11 and 12. ArRMut11 is known to tolerate higher pH values, and showed similar yields of between 9% and 16% for all pH values.

Figure 43 – Transaminase reaction of benzylacetone 101 with varying pH. Reaction conditions: substrate (10 mM), IPA (100 mM), PLP (1 mM), KPi (100 mM), clarified cell lysate (0.4 mg/mL). Yields of 136 were determined by HPLC. Reactions were performed in triplicate.

These results showed that the maximum yield of 1-methyl-3-phenylpropylamine was achieved when using 10 equivalents of amine donor at pH 8, and that increasing the substrate concentration to 20 mM did not adversely affect the yield. Yields of 136 were compared using MBA and IPA as the amine donor, and the results are shown in Table 28. With Cv-TAm, a higher yield of 57% of 136 was observed with (S)-MBA as the donor compared with 43% for IPA. The Rs1019-TAm had a higher yield with (S)-MBA of 26% compared to 20% with IPA, and Mv-TAm had a significantly higher yield of 14% with (R)-MBA, compared to just 5% with IPA. The Pp958-TAm showed a slight increase in yield when using 136
IPA instead of (S)-MBA, from 28% to 32%. With ArRMut11, a slight increase in yield from 15% to 19% was observed when replacing (R)-MBA with IPA. Overall, Cv-TAm, Rs1019-TAm and Mv-TAm all performed better with (S)- or (R)-MBA than with IPA, and with Pp958-TAm and ArRMut11 only small increases in yield were achieved with IPA. Although IPA is a low cost amine donor, further reactions with substrate 101 were conducted with (S)- or (R)-MBA as the amine donor due to the increased yields.

<table>
<thead>
<tr>
<th>Amine donor</th>
<th>1-Methyl-3-phenylpropylamine 136 yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cv-TAm</td>
</tr>
<tr>
<td>MBA</td>
<td>57</td>
</tr>
<tr>
<td>IPA</td>
<td>43</td>
</tr>
</tbody>
</table>

Table 28 – Transaminase reaction of 101 with (S)- or (R)-MBA or IPA as the amine donor. Reaction conditions (MBA): substrate (5 mM), (S)- or (R)-MBA (25 mM), PLP (1 mM), KPi pH 7.5 (100 mM), clarified cell lysate (0.5 mg/mL). Reaction conditions (IPA): substrate (10 mM), IPA (100 mM), PLP (1 mM), KPi pH 8 (100 mM), clarified cell lysate (0.5 mg/mL). Yields of 136 were determined by HPLC. Reactions were performed in triplicate with standard deviations below 10%.

The screening and reaction development of the transaminase reactions were performed on a 200 µL scale. The reactions were increased in scale, from 200 µL to 10-25 mL reaction volume, using MBA as the donor and monitoring the production of desired amine product 136 again by HPLC. Increasing the substrate concentration from 5 mM to 10 mM was also investigated, and the results are shown in Figure 44. For Cv-TAm, a high yield of 76% was achieved at a 5 mM substrate concentration and 10 mL reaction volume, with a decrease to 60% yield when increasing the substrate concentration to 10 mM. A large decrease was observed when increasing the reaction volume from 10 mL to 25 mL, giving a 21% yield. With ArRMut11, a high yield of 82% was achieved at a 5 mM substrate concentration and 10 mL reaction volume, and only a slight decrease to 79% was observed when increasing the substrate concentration to 10 mM. When the reaction volume was increased to 25 mL with ArRMut11, the yield dropped to 51%. Generally, increased yields were observed with Cv-TAm and ArRMut11 on the larger scales compared to the initial 200 µL scale. This could be due to the increased accuracy when preparing larger scale reactions.
Figure 44 – Transaminase reactions of 101 on varying scales with Cv-TAm and ArRMut11. Reaction conditions: Substrate concentration and final volume as indicated, (S)- or (R)-MBA (5 eq.), PLP (1 mM), KPi pH 7.5 (100 mM), clarified cell lysate (1 mg/mL). Yields of 136 were determined by HPLC.

The ee values for these reactions were calculated using chiral HPLC of the acetylated products against an acetylated standard of commercially available 136. In the literature, ee values for amine 136 have been determined using chiral GC and chiral HPLC of the free amines, after derivatisation with Marfey’s reagent and through acetylation. When analysed by chiral HPLC, the acetylated amine was found to give sharper peaks than the free amine, so this procedure was used to determine the ee. Excellent ee values of 94% for Cv-TAm and >99% for ArRMut11 were achieved (Scheme 97).
Scheme 97 – Transaminase catalysed synthesis of (S)- and (R)-136 followed by acetylation. Yields of 136 were determined by HPLC and ee values of N-acetyl-136 were determined by chiral HPLC.

In the literature report of the transaminase catalysed synthesis of (R)-136 with ArRMut11 (see Table 26), 93% yield was achieved with 81% ee, which was determined by chiral GC analysis of the free amine. Comparatively a slightly lower yield of 82% was found for this enzyme, however an improved ee value of >99% was achieved.

5.5 Transaminase reaction development with 4-phenyl-3-buten-2-one

An analytical standard of 4-phenyl-3-buten-2-amine was required to quantify the product yield and ee of this reaction by HPLC, as this compound was not commercially available. In the literature, reports of the preparation of this compound include amination with ammonia in the presence of titanium isopropoxide followed by reduction (Scheme 98). NMR spectroscopy showed the formation of a trace amount of new alkene product, but this could not be successfully isolated. The reaction was repeated with fresh titanium isopropoxide for a longer reaction time, but this was also unsuccessful, and the reason for the failure of this strategy is unclear.
Scheme 98 – Synthesis of 4-phenyl-3-buten-2-amine 189 with ammonia, titanium isopropoxide and NaBH₄.¹⁶⁴

A synthetic route to 189 from propargylic alcohol 160 has been reported which proceeded via reduction of the alkyne, mesylation, azide formation and reduction to give the primary amine in 14% yield (Scheme 99).¹⁶⁵

Scheme 99 – Synthesis of 189 from propargylic alcohol 160 via reduction of the alkyne, mesylation, azide formation and reduction.¹⁶⁵

As 4-phenyl-3-buten-2-ol 159 had already been synthesised for the oxidation step (Section 3.5) the synthetic route was applied to 159. The amine product 189 was successfully isolated with a 4% yield over three steps, providing a sample of the desired amine for analytical HPLC purposes.

Scheme 100 – Synthesis of 4-phenyl-3-buten-2-amine 189 via azide formation and reduction.

When the amine was analysed by HPLC, problems were encountered due to insolubility and decomposition of the product. An accurate calibration curve could not be produced and yields of 189 could not be analysed quantitively in the transaminase reactions. However, the retention time of the product was determined and the presence of the desired amine product was therefore confirmed qualitatively by HPLC and LCMS. The synthesised racemic standard
was successfully acetylated and analysed by chiral HPLC, allowing ee values to be calculated.

Due to the problems with quantification of the product yields, the transaminase reaction of alkene 163 was scaled up using (S)- or (R)-MBA as the amine donor, and yields determined by HPLC analysis of the by-product, acetophenone. The results of this are shown in Figure 45. A low yield of 2% was observed with Cv-TAm on the initial 200 µL scale, but this increased to 12% when the reaction volume was increased to 10 mL. When the substrate concentration was increased to 10 mM, the yield dropped again to 4% at 10 mL and 1% at 25 mL. With Mv-TAm, the yields were higher, with 23% on the 200 µL scale, increasing to 32% on the 10 mL scale. The yield dropped slightly to 27% when the substrate concentration was increased to 10 mM with a 10 mL reaction volume, and to 24% when the reaction volume was increased to 25 mL.

![Diagram](image)

**Figure 45 – Transaminase reactions of 4-phenyl-3-buten-2-one 163 on varying scales with Cv-TAm and Mv-TAm.** Reaction conditions: Substrate concentration and final volume as indicated, (S)- or (R)-MBA (5 eq.), PLP (1 mM), KPi pH 7.5 (100 mM), clarified cell lysate (1 mg/mL). Conversions were based on the formation of acetophenone determined by HPLC. Reactions were performed in triplicate.
The ee values of the product 189 were calculated from the acetylated products by chiral HPLC analysis with respect to the acetylated racemic standard. In the literature, the ee value of amine 189 has been determined by chiral HPLC of the acetyl derivatised amine. Excellent ee values of 96% (S) with Cv-TAm and >99% (R) with Mv-TAm were achieved in the transaminase reaction of 163 (Scheme 101). The amine configuration was assigned following the reported selectivity of the transaminases.

Scheme 101 – Transaminase catalysed synthesis of (S)- and (R)-189 followed by acetylation. Yields of 189 were determined by HPLC and ee values of N-acetyl-189 were determined by chiral HPLC.

Overall, benzylacetone 101 and 4-phenyl-3-buten-2-one 163 were accepted by the transaminases on a small and medium scale using (S)- or (R)-MBA as the amine donor. The results are summarised in Table 29. Access to (S)- and (R)-enantiomers of the amine products was achieved using transaminases of opposing stereoselectivity.
<table>
<thead>
<tr>
<th>Product</th>
<th>TAm</th>
<th>Yield (%)</th>
<th>ee (%)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>136</td>
<td>Cv-TAm</td>
<td>76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94 (S)</td>
</tr>
<tr>
<td></td>
<td>ArRMut11</td>
<td>82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;99 (R)</td>
</tr>
<tr>
<td>189</td>
<td>Cv-TAm</td>
<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96 (S)</td>
</tr>
<tr>
<td></td>
<td>Mv-TAm</td>
<td>32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;99 (R)</td>
</tr>
</tbody>
</table>

Table 29 – Yields and ee values of the transaminase catalysed synthesis of 136 and 189. <sup>a</sup>Yields of 136 were determined by HPLC. <sup>b</sup>Yields of 189 were based on the formation of acetophenone determined by HPLC. <sup>c</sup>ee values were determined by chiral HPLC of the acetylated products.

Reaction conditions: 10 mL total reaction volume, substrate (5 mM), (S)- or (R)-MBA (5 eq.), PLP (1 mM), KPi pH 7.5 (100 mM), clarified cell lysate (1 mg/mL).

High yields and ee values were achieved for 1-methyl-3-phenylpropylamine 136 with Cv-TAm and ArRMut11. Alkene 189 was produced in lower yields, but high enantioselectivities were still achieved with Cv-TAm and Mv-TAm.

5.6 Studies into the compatibility of transaminases with peroxide quenching agents

For the development of a two-step reaction, the compatibility of the two reaction conditions was then investigated. The oxidation reaction was conducted in water at 95 °C and 50-150 mM substrate concentration, containing TBHP and the gold nanoparticle catalyst. As the optimal conditions for the transaminases were 30-40 °C and 5-10 mM substrate concentration, the reaction mixture would have to be cooled and diluted before addition of the enzyme, and DMSO added to solubilise the ketone intermediate in water. It was not known how the gold catalyst would interact with the enzyme, and it was thought that the presence of a peroxide could adversely affect the enzyme performance. Previously, for the work up of the oxidation step, the peroxide was quenched with sodium sulfite to form tert-butanol. Other peroxide quenching agents in the literature include sodium thiosulfate, sodium hypochlorite and catalase.51,167

After the oxidation, the reaction mixture was quenched with sodium sulfite, the gold catalyst either removed by centrifugation or left in the mixture, and the enzyme reaction was then tested on a 200 µL scale with IPA as the amine donor.
Although the yields were generally low (below 10%), there was no difference in the yield with and without the gold catalyst with both the Cv-TAm and ArRMut11 enzymes. The effect of the quenching agent was investigated using a model system where aqueous TBHP was quenched before a well-accepted transaminase substrate, benzaldehyde 40, was added and the transaminase reaction run on a small scale (Scheme 102).

A control reaction with no TBHP and a reaction with no quenching agent added were also included and the results are shown in Figure 46. Under standard conditions with no TBHP or quenching agent, an 80% yield of benzylamine 190 was achieved with Cv-TAm. When using sodium sulfite as the quenching agent this decreased to 65%, and to 54% with sodium thiosulfate. A high yield of 67% was observed with sodium hypochlorite, but a higher yield of 75% was achieved with no quenching agent present. This trend was also observed with Mv-TAm and ArRMut11, with Mv-TAm giving 52% yield under standard conditions and 48% in the presence of TBHP, and ArRMut11 giving 70% under standard conditions and 58% in the presence of TBHP. The final concentration of TBHP in the enzyme reaction after dilution was 20 mM, and this only affected the yields by around 5% for Cv-TAm and Mv-TAm, and slightly higher for ArRMut11. The quenching agents tested adversely affected the benzylamine 190 yields with all three enzymes. These results showed that after the oxidation step, the reaction mixture only had to be cooled and diluted before continuing with the transaminase step.
Figure 46 – Transaminase reaction of benzaldehyde 40 following quenching of TBHP by various quenching agents. Standard conditions were without TBHP or quenching agent. Reaction conditions: substrate (10 mM), TBHP (4 eq.), quenching agent (4 eq.), PLP (1 mM), KPi pH 7.5 (100 mM), IPA (100 mM). Yields of 190 were determined by HPLC. Reactions were performed in triplicate.

5.7 Two-step reaction development

The oxidation and transaminase reaction steps were then combined into a two-step process. As the initial aim was to make a one-pot, two-step reaction, we aimed to manipulate the reaction mixture as little as possible between the two steps. Removal of the gold catalyst by centrifugation was investigated as described above, but the presence of the gold catalyst had a minimal effect on the yields. Investigations into the quenching of the peroxide were discussed in the previous section, the outcome of which was that no reaction quench was required. The first step was carried out on a standard 0.3 mmol scale, and after the oxidation, the mixture was cooled, diluted and aliquoted out into a 96 well plate for transaminase screening on a 200 µL scale.

Initially, the two-step synthesis of benzylamine 190 from benzyl alcohol 39 was tested (Scheme 103). The oxidation of benzyl alcohol was discussed in Section 2 where decane was used as a solvent. The two-step procedure required water as a solvent for the oxidation reaction, and the yield of benzaldehyde 40 reached a maximum of 17% after 2 h with Au/Al₂O₃. The background reaction with no catalyst reached a 3% yield, and in the presence of Au/MgO, no 40 was observed. As the transaminase step could be achieved in over 70% yield, the two steps
were combined on a 200 µL scale, using IPA as the amine donor for the transaminase step. The two-step process produced 190 in a 9% yield with Cv-TAm, 8% yield with ArRMut11 and 9% yield with Mv-TAm, providing an initial proof of concept.

Scheme 103 – Two-step transformation of benzyl alcohol 39 into benzylamine 190. The oxidation step was performed with a 10 wt% Au/Al₂O₃ catalyst (1 mol% Au) and TBHP (4 eq.) in H₂O with 39 (0.3 mmol). The oxidation was followed by dilution and addition of transaminase Cv-TAm, Mv-TAm or ArRMut11, PLP (1 mM) and IPA (10 eq.) in KPi (100 mM) with 10 mM substrate concentration and 200 µL total reaction volume. Yields of 190 were determined by HPLC. Transaminase reactions were performed in triplicate.

The two-step procedure was then performed on secondary alcohol 4-phenyl-2-butanol 158. With the first step on a 0.3 mmol scale and the second step on a 200 µL scale, 1-methyl-3-phenylpropylamine 136 was formed in 24% yield with Cv-TAm and 14% with ArRMut11. The transaminase step was then performed on a larger scale after the oxidation step. The results are shown in Table 30. With Cv-TAm, a decrease in yield of the amine 136 was observed when the reaction volume was increased from 200 µL to 5 mL, from 24% to 12%, with the substrate concentration for the transaminase step remaining constant. Increasing the reaction volume from 5 mL to 10 mL resulted in a similar yield of 11%, but when the substrate concentration was increased to 10 mM, yields fell to 4% at 5 mL and 7% at a 10 mL reaction volume. With ArRMut11, a dramatic loss in yield was observed when the reaction volume was increased from 200 µL to 5 mL, from 14% to 3%. A higher yield of 9% was achieved when the substrate concentration was increased to 10 mM with a reaction volume of 25 mL.
Table 30 – Increasing scale of one-pot two-step oxidation transamination reaction sequence. Yields of 136 were determined by HPLC. 5 eq. (S)-MBA used with Cv-TAm and 5 eq. (R)-MBA used with ArRMut11. The substrate concentration and reaction volume are shown for the transaminase step. After the oxidation step, the reaction mixture was cooled and diluted with KPi before addition of the transaminase and amine donor.

The two-step reaction sequence was also applied to the alkene substrate 4-phenyl-3-buten-2-ol 159 on small and medium scales, using (S)- or (R)-MBA as the amine donor (Table 31). The formation of the amine product 189 was not monitored directly due to problems with stability (Section 5.5). The yields of acetophenone were calculated by HPLC giving 17% with Cv-TAm and 17% with Mv-TAm on the initial 200 µL scale. With Cv-TAm, a decrease in yield was observed when increasing the reaction volume from 200 µL to 10 mL from 17% to 5%. The reaction with Mv-TAm gave similar yields of 17% and 15% when the reaction volume was increased from 200 µL to 10 mL. When the reaction volume was increased to 25 mL, a 10% yield was achieved.
Enzyme Oxidation scale (mmol) Transaminase scale (mmol) Substrate concentration (mM) Reaction volume (mL) Yield (%)
--- --- --- --- ---
Cv-TAm 0.3 0.001 5 0.2 17
0.05 0.05 10 5 5
Mv-TAm 0.3 0.001 5 0.2 17
0.05 0.05 5 10 15
0.125 0.125 5 25 10

Table 31 – Increasing scale of one-pot two-step oxidation transamination reaction sequence. Yields of 189 were based on the formation of acetophenone determined by HPLC. 5 eq. (S)-MBA used with Cv-TAm and 5 eq. (R)-MBA used with Mv-TAm. The substrate concentration and reaction volume are shown for the transaminase step. After the oxidation step, the reaction mixture was cooled and diluted with KPi before addition of the transaminase and amine donor.

Generally, lower yields were observed with increased scales of the two-step reaction sequence with 158 and 159. As the transaminase reaction was found to be high yielding with all three transaminases (see Sections 5.4 and 5.5), the reason for the lower yields was thought to be the presence of the gold nanoparticle catalyst. The effect of the catalyst’s presence in the transaminase reaction was previously found to be negligible, however this was only tested on a small scale (200 µL). As the scale was increased, the presence of the catalyst could have more of an effect. Further work into the compatibility of the two reaction conditions is required on a large scale to increase the yields. More investigation into the use of different amine donors could also help with the scalability and yield of the two-step process.

The two-step procedure was therefore most successful on a small scale with 4-phenyl-2-butanol 158 and 4-phenyl-3-buten-2-ol 159 using (S)- or (R)-MBA as the amine donors. The results of the one- and two-step transformations of 158 are summarised in Table 32. Yields of 45% for the oxidation of 158 and 67% and 51% for the amination of intermediate 101 on a 200 µL scale were presented earlier in Sections 3.4 and 5.4. For the two-step reactions, a 24% overall yield of 136 was achieved with Cv-TAm and a 14% yield with ArRMut11. However, when
considering the one-step reaction yields, a maximum of 30% yield overall was expected for Cv-TAm, and 23% overall for ArRMut11. The ee values for the reaction products were calculated by chiral HPLC analysis of the acetylated products as discussed previously. With Cv-TAm, a decrease in ee from 94% to 63% was observed when the two-step process was applied. The enantioselectivity with ArRMut11 remained at >99% in the two-step process. The absolute configuration of these amines was based on the reported selectivity of each transaminase, which corresponded with the enantiomer of MBA used.\textsuperscript{4,68}

\[
\begin{align*}
\text{Oxidation} & \quad \text{Transaminase step (200 µL)} & \quad \text{Two-step process} \\
\text{Yield (\%)} & \quad \text{TAm} & \quad \text{Yield (\%)} & \quad \text{ee (\%)} & \quad \text{Yield (\%)} & \quad \text{ee (\%)} \\
45 & \quad \text{Cv-TAm} & \quad 67 & \quad 94 (S) & \quad 24 & \quad 63 (S) \\
& \quad \text{ArRMut11} & \quad 51 & \quad >99 (R) & \quad 14 & \quad >99 (R)
\end{align*}
\]

Table 32 – Two-step reaction sequence applied to 158, with one-step oxidation and transamination yields and ee values shown. For the two-step process, the oxidation step was conducted on a 0.3 mmol scale, and the transaminase step on a 0.001 mmol scale (200 µL). Transaminase and two-step yields were determined by formation of 136 determined by HPLC. ee values were determined by chiral HPLC of the acetylated products.

The results of the one- and two-step transformations of 159 are summarised in Table 33. An oxidation yield of 77% and transaminase yields of 2-23% were presented previously in Sections 3.5 and 5.5. Overall yields of 17% were observed with both Cv-TAm and Mv-TAm. With Cv-TAm, the combined maximum yield of the one-step processes was exceeded and with Mv-TAm, a maximum yield of 18% was expected. The ee values were determined by chiral HPLC analysis of the acetylated products. With Cv-TAm, a decrease in ee was again observed in the two-step process, from 96% to 80%, but the ee value with Mv-TAm remained excellent at >99%. The amine configuration was based on the reported selectivity of the transaminases.\textsuperscript{68,80}
Table 33 – Two-step reaction sequence applied to 159, with one-step oxidation and transamination yields and ee values shown. For the two-step process, the oxidation step was conducted on a 0.3 mmol scale, and the transaminase step on a 0.001 mmol scale (200 µL). Transaminase and two-step yields of 189 were based on formation of acetophenone determined by HPLC. ee values were determined by chiral HPLC of the acetylated products.

Further investigations are needed to understand the changes in yield and stereoselectivities between the one- and two-step processes described. Increased yields were observed in the two-step process with Cv-TAm and ArRMut11, which could be caused by changes in the enzyme activity between experiments. Generally, the combination of the two steps did not adversely affect the yields, with both steps operating with the same efficiency as in the one-step transformations. A decrease in ee value was consistently observed with Cv-TAm in the two-step sequence, suggesting that the presence of the gold catalyst or the peroxide could be inhibiting the stereoselectivity of the enzyme. The desired amine product could also be affected by the presence of excess peroxide, with the potential for oxidation to form an imine.

Scheme 104 – Two step synthesis of 4-phenyl-3-butyln-2-amine 191.

The one-step transamination of 4-phenyl-3-butyln-2-one 164 resulted in very low conversions with all three enzymes, however, the two-step approach was tested on 4-phenyl-3-butyln-2-ol 160 (Scheme 104). Although the yields calculated by acetophenone formation were low (<5%), a new peak was visible in the HPLC
trace (Figure 47) and the presence of 4-phenyl-3-butyln-2-amine 191 was confirmed using LCMS.

![HPLC trace](image)

**Figure 47** – HPLC trace showing formation of propargylic amine 191 in the two-step reaction sequence (procedure A) from 160. Oxidation reaction conditions: 160 (0.3 mmol), TBHP (4 eq.), Au/MgO (10 wt%) catalyst (1 mol%), H2O, 95 °C, 15 min. Transaminase reaction conditions: total volume 200 µL, substrate (5 mM), (R)-MBA (25 mM), KPi pH 7.5 (100 mM), PLP (1 mM), Mv-TAm clarified cell lysate (1 mg/mL). HPLC analytical method A was used.

Although the yield of the amine was poor, it confirmed the production of ketone during the oxidation step and if coupled with higher yielding transaminases, 160 could be another promising substrate.
5.8 Summary

Transaminase activity was investigated for the ketone products of the oxidations discussed in Section 3, and activity was observed with Cv-TAm, Mv-TAm and ArRMut11, giving access to the (S)- and (R)-enantiomers of amine products 1-methyl-3-phenylpropylamine 136 and 4-phenyl-3-buten-2-amine 189. The transaminase step was then combined with the oxidation step in a two-step reaction sequence, with no isolation or purification of the ketone intermediate.

Investigations into the compatibility of the two steps concluded that the use of quenching agents was detrimental to transaminase activity, while the presence of remaining peroxide in solution had a negligible effect on yield. The presence of the supported gold catalyst was also well tolerated by the enzymes. These conditions resulted in the formation of (S)-136 in 24% yield with Cv-TAm and (R)-189 in 17% yield with Mv-TAm over two steps. A high ee value was obtained with Mv-TAm, but the stereoselectivity of Cv-TAm appeared to be hindered by the two-step reaction conditions (Scheme 105).

![Scheme 105 – Two-step transformations of 4-phenyl-2-butanol 158 and 4-phenyl-3-buten-2-ol 159.](image)

The supported gold nanoparticle catalysed oxidation of new substrates along with the coupling of these two steps in a novel two-step process has been achieved. Although the yields reported were for small scale transformations, the process is well suited to application in a flow chemistry setting. Flow chemistry could lower substrate overoxidation issues and side reactions by lowering the amount of time the substrate and product are exposed to the catalyst. The yield of the reaction could also potentially be increased by reaction recycling.
6 Conclusions and Future Work

In summary, the oxidation of a range of substrates including benzyl alcohol and five secondary alcohols was achieved with supported gold nanoparticle catalysts. Investigations into the effect of support material, level of gold loading, calcination temperature and preparation technique on catalytic activity were undertaken. Gold nanoparticle catalysts supported on MgO, Al₂O₃, TiO₂ and SiO₂ were synthesised and their activity evaluated in the oxidation of benzyl alcohol. The optimal catalysts were prepared at 10 wt% gold loading, with a 500 °C calcination temperature and water removal by filtration, with MgO identified as the best support giving the highest yields of benzaldehyde 40 in the oxidation of benzyl alcohol 39 (Scheme 106).

Scheme 106 – Oxidation of benzyl alcohol 39 with Au/MgO 10 wt% catalysts with TBHP (1.6 eq.) in decane at 95 °C. Yields were determined by GC using dodecane as an internal standard.

The use of gold nanoparticle catalysts in oxidations was extended to include non-benzylic secondary alcohols. More sustainable reaction conditions were developed using water as the solvent instead of decane, and high reactivity was found with allylic alcohol 4-phenyl-3-buten-2-ol 159 (Scheme 107). As the reaction of allylic alcohol 159 was very rapid with a 77% yield of ketone 163 achieved after 15 min, this substrate could potentially tolerate a lower reaction temperature, making the reaction conditions more sustainable. A moderate yield of benzylacetone 101 was achieved. The gold catalyst Au/MgO was also reused three times, with a minimal decrease in the yield of 101. Although issues arose with the analysis of ketones 164 and 99, some oxidation was found to occur. Investigation into the optimal reaction conditions and analytical techniques for monitoring of propargylic alcohol 160 and 2-heptanol 161 oxidations are required for the future use of the gold catalysts.
Scheme 107 – Ketone products from the oxidation of secondary alcohols with Au/MgO (10 wt%) and TBHP (4 eq.) in water at 95 °C. Yields were determined by GC using dodecane as a standard.

Extensions to the substrate scope of oxidations catalysed by supported gold nanoparticles is ongoing, and more work is needed on the effect of the support material on catalytic activity for specific substrates. An interesting application of the gold nanoparticle catalysed oxidation could be the oxidation of HMF 2 to 2,5-furandicarboxylic acid 3, as this is another valuable platform chemical.

Transaminases have been identified as excellent catalysts for the production of furfurylamines from bio-derived furfural sources. Screening was undertaken with a range of furfural derivatives and three enzymes, using a colorimetric assay developed in our group, and showing high activity. The results were quantified using MBA as the donor, and selected substrates were taken forward for reaction development using IPA as the donor. Excellent yields were achieved on a small scale for the synthesis of furfurylamine 165, 5-hydroxymethylfurfurylamine 166, 2,5-bis(aminomethyl)furan 21 and 5-aminomethyl-2-furandicarboxylic acid 168 (Scheme 108). The biocatalytic syntheses of 165, 166 and 168 were performed on a preparative scale, giving a high yield of 165 by HPLC, and isolated yields of 54% of 166 and 31% of 168. This transformation provides a mild, sustainable route to furfurylamines which have useful applications as pharmaceutical intermediates and renewable biopolymers.
Scheme 108 – Synthesis of furfurylamines with transaminases on varying scales. Small (200 µL) and large (50 mL) scale reaction yields were determined by HPLC, unless stated isolated. Reaction conditions: substrate (10-20 mM), IPA (10 eq.), PLP (1 mM), KPi pH 8 (100 mM), 35 °C, 24 h.

Further work could be undertaken on the synthesis of furfurylamines, through optimisation of larger scale reactions to increase yields and show applicability on an industrial scale. 5-Aminomethyl-2-furancarboxylic acid 168 was particularly difficult to isolate, and the recovery of this product could be improved through the use of catch and release resins.

The gold nanoparticle catalysed oxidation reactions were combined with a biocatalytic transformation to access chiral amines in a novel two-step procedure. For this, (S)- and (R)-selective transaminases were identified for the transformation of benzylacetone 101 and 4-phenyl-3-buten-2-one 163 (Scheme 109) on a medium scale with 10 mL reaction volume (0.05 mmol). A high yield of 76% of (S)-136 was achieved with Cv-TAm in 94% ee. A lower yield of 12% of (S)-189 was obtained with the same enzyme, but the ee remained high at 96%. Yields of 82% of (R)-136 and 32% of (R)-189 were achieved with ArRMut11 and Mv-TAm respectively, both with excellent ee values of >99%.
Scheme 109 – Transaminase catalysed synthesis of (S)- and (R)-enantiomers of amines 136 and 189 with (S)- or (R)-MBA as the amine donor. Yields of 136 were determined by HPLC. Yields of 189 were based on yields of acetophenone determined by HPLC. Reaction conditions: substrate (5 mM), (S)- or (R)-MBA (25 mM), PLP (1 mM), KPi pH 7.5 (100 mM), total reaction volume of 10 mL. ee values were determined by chiral HPLC.

Preliminary investigations into the combination of the Au/MgO (10 wt%) catalysed oxidation procedure with the transaminase step was then achieved. It was found that the enzymes could tolerate the presence of excess TBHP and the gold catalyst with a minimal decrease in yield. The two-step process was explored using 4-phenyl-2-butanol 158 and 4-phenyl-3-buten-2-ol 159, giving access to both enantiomers of the amine products 1-methyl-3-phenylpropylamine 136 and 4-phenyl-3-buten-2-amine 189 (Scheme 110). Overall yields of 24% of (S)-136 and 14% of (R)-136 were achieved from 158 over two steps, with Cv-TAm and ArRMut11 respectively. An excellent ee of >99% was achieved with ArRMut11, but the ee value with Cv-TAm decreased to 63% in the two-step reaction. Yields of 17% of (S)-189 and 17% of (R)-189 were achieved with Cv-TAm and Mv-TAm respectively. An excellent ee of >99% was again obtained for the (R)-enantiomer, but with Cv-TAm the ee decreased again to 80%.
Scheme 110 – Two-step transformation of two secondary alcohols to the corresponding chiral amines. Yields of 136 were determined by HPLC. Yields of 189 were based on yields of acetophenone determined by HPLC. ee values were determined by chiral HPLC. The oxidation step was performed on a 0.3 mmol scale, then diluted and the transaminase step performed on a 200 µL scale (0.025 mmol) in triplicate.

This work is a proof of principle of a sustainable, novel process, with a reusable low-loaded gold nanoparticle catalyst and renewable transaminase biocatalyst, with water as the solvent. No isolation or purification of the intermediate was performed, and the combined yields of the amine products over two steps show the potential for the combination of the oxidation and transamination processes.

The two-step process developed using oxidation and transamination reactions to access chiral amines would be a good candidate for use in a flow chemistry setting. The use of supported gold nanoparticle catalysts and transaminase enzymes have both been reported separately in flow.\textsuperscript{54,105} Issues arising from the decomposition or side reactions of the ketone intermediate under the oxidation conditions could be combated by reduced exposure to the catalyst. The yields may also be increased by recycling of the reactants. Immobilisation of transaminases allows them to be reused, lowering the amount of waste further, which has been previously reported.\textsuperscript{105,162}
7 Experimental

7.1 General experimental

Reactions were carried out in oven dried glassware. Column chromatography was carried out using BDH (40-60 μm) silica gel. Thin layer chromatography was carried out using Merck Keiselgel aluminium-backed silica gel plates and compounds visualised by exposure to UV light, potassium permanganate or ninhydrin stain. Infrared spectra were carried out neat on a Perkin-Elmer spectrum 100 FTIR spectrometer. Sterilisation of media was carried out in a Priorclave autoclave at 121 °C for 30 min. Enzymes and reactions were incubated in a New Brunswick Scientific Innova 44 incubator shaker. Cell lysates were sonicated using a Branson Sonifier 150 for 15 s pulses at an output of 15 watts. Assays were mixed using a BIOER Mixing Block MB-102. The centrifuges used were an Eppendorf centrifuge 5415R, Philip Harris 2-5 Sigma centrifuge and Beckman Coulter Avanti J26 XP centrifuge. Catalysts were calcined using a Carbolite furnace. TEM images were obtained using a JEOL 100 kV Transmission Electron Microscope at the Materials Chemistry Centre, UCL Department of Chemistry. X-ray diffraction data was collected at ambient temperature on a STOE STADI P with a Mythen 1K detector. Melting points were collected by Electrothermal IA9000 Series melting point apparatus. $^1$H and $^{13}$C NMR spectra were recorded at 298 K using Bruker AMX300, Bruker AMX400, Bruker Avance 500, and Bruker Avance 600 spectrometers. Chemical shifts are recorded in ppm, coupling constants $J$ in Hz, and IR peaks in cm$^{-1}$. Mass spectra were recorded on Thermoscientific Trace 1310 Gas Chromatograph connected to Thermoscientific ISQ Single Quadrupole MS, and VG70-SE MS at the UCL Mass Spectrometry Service, Department of Chemistry. GC analysis was carried out on an Agilent 7820A GC System with a flame ionization detector. HPLC analysis was carried out on an Agilent series 1100 HPLC and an Agilent 1260 Infinity HPLC with UV detection.
7.2 Transaminase expression and preparation

Selected TAm glycerol stocks from the UCL TAm library were used to inoculate 2TY broth containing kanamycin (50 µg/mL) and incubated at 37 °C for 16-18 h. This pre-inoculum was then used to inoculate a larger culture containing the same antibiotic which was incubated at 37 °C for 3 h until an OD$_{600}$ of 0.5-0.7 was reached. Enzyme expression was induced by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) (1 mM), and the temperature was reduced to 30 °C for 5 h or 25 °C for 18 h. Cells were harvested by centrifugation (8000 rpm, 20 min) and the pellet suspended in potassium phosphate buffer (100 mM, pH 7.5) containing PLP (1 mM) and freeze dried. The freeze dried cells were used fresh or stored at -20 °C for up to six months. To prepare cell crude extract freeze dried cells (25 mg) were suspended in potassium phosphate buffer (1 mL, 100 mM, pH 7.5), lysed by sonication on ice (10 s on and 10 s off for 5 cycles) and used as a crude lysate (10% v/v) or centrifuged (30000 rpm, 10 min) for use as clarified cell lysate.

Transaminases as referred to in report:

<table>
<thead>
<tr>
<th>Transaminase</th>
<th>Microorganism</th>
<th>PQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cv-TAm$^{58}$</td>
<td>Chromobacterium violaceum</td>
<td>801</td>
</tr>
<tr>
<td>ArRMut11$^4$</td>
<td>Arthrobacter sp.</td>
<td>AS</td>
</tr>
<tr>
<td>Mv-TAm$^{80}$</td>
<td>Mycobacterium vanbaalenii</td>
<td>Mb</td>
</tr>
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<td>Sa805-TAm</td>
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</tr>
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<td>Sa807-TAm</td>
<td>Streptomyces avermitilis</td>
<td>807</td>
</tr>
<tr>
<td>Kp904-TAm</td>
<td>Klebsiella pneumoniae</td>
<td>904</td>
</tr>
<tr>
<td>Bs906-TAm</td>
<td>Bacillus subtilis</td>
<td>906</td>
</tr>
<tr>
<td>Pp958-TAm</td>
<td>Pseudomonas putida</td>
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<td>Klebsiella pneumoniae</td>
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<td>Rhodospirillum rubrum</td>
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<tr>
<td>Rs1019-TAm</td>
<td>Rhodobacter sphaeroides</td>
<td>1019</td>
</tr>
</tbody>
</table>
TAm concentrations were determined by SDS-PAGE densitometry:³ densitometry of samples electrophoresed on a NuPAGE 10% Bis-Tris gel (Novex) was used. The Coomassie stained gel was imaged using LabWork image software to calculate the TAm band density. A range of concentrations of commercial BSA was run in each SDS-PAGE gel and used to calculate a standard curve based on integrated optical density for calibration of the enzyme concentration.

Lane 1-5: increasing amount of BSA (1, 1.6, 2, 2.4, 3.2, 3.6 µg)

Lane 6: Protein ladder

Lane 7-9: Cv-TAm, Mv-TAm, ArRMut11 (10 µg total protein)

Total protein was determined using a standard Bradford assay. TAm concentrations in assays discussed in Section 4 were determined as 0.6 mg/mL for Cv-TAm, 0.4 mg/mL for ArRMut11 and 0.3 mg/mL for Mv-TAm. TAm concentrations in assays discussed in Section 5 using clarified cell lysates were 0.4 mg/mL, 0.5 mg/mL or 1 mg/mL.

³ Performed by Dr. Fabiana Subrizi
7.3 Analytical methods

Analytical GC

GC analysis was performed using an Agilent 7820A GC System with flame ionization detector at 300 °C, a temperature gradient and an injection volume of 1 µL.

Column A: Supelco Beta Dex 225 capillary GC column (30 m x 250 µm x 0.25 µm).

Column B: Agilent J&W capillary GC column (60 °C – 325 °C, 30 m x 320 µm x 0.25 µm).

Method A (Column A): Initial temperature 90 °C, 1 min hold, ramp 5 °C/min to 150 °C, ramp 20 °C/min to 210 °C, 2 min hold. Retention times: benzaldehyde 40 6.4 min, dodecane 7.4 min, benzyl alcohol 39 8.9 min, benzylacetone 101 12.7 min, 4-phenyl-2-butanol 158 13.3 min, benzyl benzoate 42 13.8 min.

Method B (Column A): Initial temperature 100 °C, 1 min hold, ramp 5 °C/min to 210 °C, 2 min hold. Retention times: dodecane 5 min, 4-phenyl-3-buten-2-one 163 9.4 min, 4-phenyl-3-buten-2-ol 159 9.5 min.

Method C (Column A): Initial temperature 90 °C, 1 min hold, ramp 5 °C/min to 105 °C, ramp 20 °C/min to 210 °C, 2 min hold. Retention times: 2-heptanone 99 4.3 min, 2-heptanol 161 4.4 min, dodecane 6.3 min.

Method D (Column B): Initial temperature 60 °C, 1 min hold, ramp 5 °C/min to 100 °C, ramp 20 °C/min to 220 °C, 3 min hold. Retention times: TMS-benzyl alcohol 155 3.5 min, benzaldehyde 40 6.6 min, dodecane 7.7 min, TMS-benzoic acid 156 8.8 min.

Method E (Column B): Initial temperature 60 °C, 1 min hold, ramp 5 °C/min to 80 °C, ramp 0.5 °C/min to 83 °C, ramp 20 °C/min to 220 °C, 3 min hold. Retention times: dodecane 8.5 min, 1-phenyl-2-propanol 157 6.3 min, phenylacetone 162 6.1 min.
Analytical HPLC

Analysis of the reactions were performed using an Agilent 1260 Infinity HPLC with an Ace 5 C18 column 150 x 4.6 mm. Elution was carried out at 1 mL/min with a linear gradient of acetonitrile/H$_2$O containing 0.1% TFA, with detection at 250 or 210 nm, injection volume of 10 µL and column temperature of 30 °C.

Method A: Linear gradient 15 – 72% acetonitrile over 15 min with detection at 250 nm. Retention times: acetophenone 11 min, 5-aminomethylfuran carboxylic acid 168 1.9 min, 4-phenyl-3-buten-2-amine 189 6.1 min, 4-phenyl-3-butyn-2-amine 191 5.8 min.

Method B: Linear gradient 5 – 95% acetonitrile over 14 min with detection at 210 nm. Retention times: furfurylamine 165 2.9 min, 2,5-bisaminomethylfuran 21 2.2 min.

Method C: Linear gradient 15 – 72% acetonitrile over 15 min with detection at 210 nm. Benzylamine 190 3.7 min, 5-hydroxymethylfurfurylamine 166 2.6 min, 1-(2-furan)ethylamine 175 2.9 min, 1-methyl-3-phenylpropylamine 136 6.1 min.

Chiral HPLC

Chiral analysis was performed using a HP Series 1100 HPLC with a Chiralcel OD column (4.6 mm x 250 mm) or a Chiralpak AD-H column (5 µm particle size, 4.6 mm x 250 mm). Elution was carried out at the specified flow rate with an isocratic flow of isopropanol/hexane with UV detection, injection volume of 10 µL and the column temperature was not controlled.

*N*-Cbz-1-(2-furan)ethylamine Cbz-175: 4% i-PrOH:hexane (isocratic), 250 nm, 0.5 mL/min, OD column. Retention times (S)-isomer 28 min, (R)-isomer 35 min.

*N*-Acetyl-1-methyl-3-phenylpropylamine N-acetyl-136 with (S)-N-Acetyl-MBA: 4% i-PrOH:hexane (isocratic), 214 nm, 0.8 mL/min, AD-H column. Retention times (R)-isomer 18 min, (S)-isomer 20 min, (S)-MBA 23 min.

*N*-Acetyl-1-methyl-3-phenylpropylamine N-acetyl-136 with (R)-N-Acetyl-MBA: 4% i-PrOH:hexane (isocratic), 214 nm, 0.5 mL/min, AD-H column. Retention times (R)-MBA 19 min, (R)-isomer 24 min, (S)-isomer 27 min.
N-Acetyl-4-phenyl-3-buten-2-amine acetyl-189: 4% i-PrOH:hexane (isocratic), 214 nm, 0.8 mL/min, AD-H column. Retention times (R)-MBA 18 min, (R)-isomer 21 min, (S)-MBA 23 min, (S)-isomer 28 min.

**Powder X-ray Diffraction Analysis**

Data was collected at ambient temperature on a STOE STADI P with a Mythen 1K detector. Samples were measured in foil mode in a Debye-Scherrer geometry using Kα1-Mo radiation. XRD patterns were obtained in 0.5° steps with 10 s/step measuring time over a range of 2 to 40°.

**7.4 General Procedures**

**Preparation of 1 wt% Au catalysts**

The catalyst support (TiO₂ (anatase)/SiO₂ (500 nm nanospheres)/MgO) (1 g) and HAuCl₄·3H₂O (0.04 M, 1.5 mL, 1 wt%) were stirred in distilled water (48.5 mL) and NaOH (0.1 M) was added dropwise until the solution reached pH 8. The mixture was heated to 80 °C for 1 h, left to stand for 16 h, centrifuged (3000 rpm, 30 min), washed with water and centrifuged again. The catalyst was then dried in an oven at 100 °C and calcined in air at 400 °C for 4 h.

**Preparation of 10 wt% Au catalysts**

The catalyst support (TiO₂ (anatase)/MgO/Al₂O₃ (corundum)) (1 g) and HAuCl₄·3H₂O (0.04 M in H₂O, 15 mL, 10 wt%) were stirred in distilled water (35 mL) and NaOH (1 M) was added dropwise until the solution reached pH 8. The mixture was heated to 80 °C for 1 h, left to stand for 16 h, filtered and washed with water under vacuum. The catalyst was then dried in an oven at 100 °C and calcined in air at 500 °C for 4 h.

**Procedures for the gold catalysed oxidations of benzyl alcohol 39 in decane**

**Procedure A**

Benzyl alcohol (0.31 mL, 3.00 mmol), dodecane (internal standard, 0.23 mL, 1.00 mmol) and TBHP (5-6 M in decane, 0.46 mL, 2.30 mmol) were stirred in a carousel tube. Gold nanoparticle catalyst (20 mg) was added and the mixture heated at 95 °C. Aliquots were taken every hour, any solids removed by
centrifugation (30000 rpm, 4 min), the solution diluted with ethyl acetate and analysed by GC using method A.

**Procedure B**

Benzyl alcohol (0.52 mL, 5.00 mmol), dodecane (internal standard, 0.23 mL, 1.00 mmol) and TBHP (5-6 M in decane, 1.47 mL, 8.00 mmol) were stirred in a carousel tube. Gold nanoparticle catalyst (20 mg) was added and the mixture heated at 95 °C. Aliquots were taken every hour, any solids removed by centrifugation (30000 rpm, 4 min), the solution diluted with ethyl acetate and analysed by GC using method A.

**Procedure for the silylation of oxidation products**

Samples were removed from the reaction for silylation, the solids removed by centrifugation (30000 rpm, 4 min), the solution diluted with pyridine and BSTFA/TMSCl (100 µL) added. The mixture was shaken at 400 rpm for 16 h, further diluted and analysed by GC using method D.

**Oxidation of 1-phenyl-2-propanol 157 to 1-phenyl-2-propanone 162 in decane**

1-Phenyl-2-propanol 157 (140 µL, 1.00 mmol), dodecane (internal standard, 45 µL, 0.20 mmol) and TBHP (5-6 M in decane, 1.82 mL, 9.10 mmol) were stirred in a carousel tube. Gold nanoparticle catalyst (20 mg) was added and the mixture heated at 95 °C. Aliquots were taken every hour, any solids removed by centrifugation (30000 rpm, 4 min), the solution diluted with ethyl acetate and analysed by GC using Method D.

**Oxidation of 4-phenyl-2-butanol 158 to benzylacetone 101 in decane**

**Procedure A**

4-Phenyl-2-butanol 158 (0.46 mL, 2.97 mmol), dodecane (68 µL, 0.30 mmol) and TBHP (5-6 M in decane, 1.47 mL, 8.00 mmol) were stirred in a carousel tube under argon. Au/MgO (10 wt%) catalyst (50 mg, 0.3 mol% Au) was added and the mixture heated at 95 °C. Aliquots were taken, any solids were removed by centrifugation (30000 rpm, 4 min), the solution was diluted with ethyl acetate and analysed by GC using Method A.
Procedure B

4-Phenyl-2-butanol 158 (46 µL, 0.30 mmol), dodecane (6.8 µL, 0.030 mmol) and TBHP (5-6 M in decane, 0.25 mL, 1.20 mmol) were stirred in a carousel tube with decane (1.55 mL). Gold nanoparticle catalyst (10 wt%) (5 mg, 1 mol% Au) was added and the mixture heated at 95 °C. Aliquots were taken, any solids were removed by centrifugation (30000 rpm, 4 min), the solution was diluted with ethyl acetate and analysed by GC using Method A.

General procedure for secondary alcohol oxidations in water

The alcohol (0.30 mmol), TBHP (70% solution in water, 0.15 mL, 1.20 mmol) and supported Au catalyst (5 mg, 1 mol% Au) were stirred in water (1.85 mL) at 95 °C. For each time point, the reaction mixture was extracted into ethyl acetate (4 mL) containing dodecane (internal standard, 0.1 equivalents), and the ethyl acetate was centrifuged and diluted for injection into the GC. This procedure was using in the oxidations of 4-phenyl-2-butanol 158, 4-phenyl-3-buten-2-ol 159, 4-phenyl-3-butyn-2-ol 160 and 2-heptanol 161.

Oxidation of 4-phenyl-3-buten-2-one 159 with TBHP (1 equivalent)

4-Phenyl-3-buten-2-one 159 (44 mg, 0.30 mmol), TBHP (70% solution in water, 37.5 mL, 0.30 mmol) and Au/MgO (10 wt%) catalyst (5 mg, 1 mol% Au) were stirred in water (2 mL) at 95 °C. For each time point, the reaction mixture was extracted into ethyl acetate (4 mL) containing dodecane (internal standard, 0.1 eq.), and the ethyl acetate was centrifuged and diluted for injection into the GC.

Reusing catalyst experiments

The general procedure for the oxidation of secondary alcohols was followed with 4-phenyl-2-butanol 158 (46 µL, 0.30 mmol, 4 h) or 4-phenyl-3-buten-2-one 159 (44 mg, 0.3 mmol, 15 min) and Au/MgO (10 wt%) (5 mg, 1 mol% Au). After the reaction time specified, the reaction mixture was extracted into ethyl acetate. The aqueous layer containing the catalyst was centrifuged at 4000 rpm for 15 min. The water was decanted and the catalyst washed with water (10 mL) and centrifuged again. The water was decanted and the catalyst dried at 50 °C overnight. This procedure was repeated 3 times.
Gold leaching experiments

The general procedure for the oxidation of secondary alcohols was followed using 4-phenyl-2-butanol 158 (46 µL, 0.30 mmol) and Au/MgO (10 wt%) catalyst (5 mg, 1 mol% Au). After 2 h, the reaction mixture was filtered through a syringe filter while still hot and the filtrate heated for another 2 h. After this time the reaction mixture was extracted into ethyl acetate and analysed by GC using Method A.

Colorimetric screening96

The assay was performed in 96 well-plate with a total volume of 200 µL containing 2-(4-nitrophenyl)ethan-1-amine hydrochloride 123 (25 mM) as amine donor, substrate (10 mM), PLP (0.2 mM) and potassium phosphate buffer (pH 7.5, 100 mM). The reaction was started by the addition of crude cell lysate (20 µL) or clarified cell lysate (0.4 mg/mL) and incubated at 30 °C and 500 rpm for 24 h. Two negative controls were also performed, one without amine acceptor and another without enzyme. An orange/red colouration indicated TAm activity.

MBA screening

The assay was performed in an Eppendorf tube (200 µL total volume) containing (R)- or (S)-MBA (25 mM), PLP (1 mM), potassium phosphate buffer (pH 7.5, 100 mM), substrate (5 mM) and crude cell lysate (20 µL) or clarified cell lysate (0.4-1 mg/mL). After incubation at 30 °C and 300 rpm for 24 h, the reaction was stopped by addition 10 µL of 10% trifluoroacetic acid (TFA) in water (10 µL). Denatured protein was removed by centrifugation (4 °C, 3000 rpm, 4 min) and the supernatant diluted and analysed by HPLC.

Isopropylamine assay

The assay was performed in an Eppendorf tube (200 µL total volume) containing isopropylamine (pH 8, 100 mM), PLP (1 mM), potassium phosphate buffer (pH 8, 100 mM), substrate (10 mM) and the crude cell lysate (20 µL) or clarified cell lysate (0.4-1 mg/mL). After incubation at 35 °C and 300 rpm for 24 h, the reaction was stopped by addition of 10% TFA in water (10 µL). Denatured protein was removed by centrifugation (4 °C, 3000 rpm, 4 min) and the supernatant diluted and analysed by HPLC.
Derivatisation of 175 with CBz for ee determination

The assay was performed (800 µL total volume) containing (R)-MBA (25 mM, 0.02 mmol), PLP (1 mM), KPi (100 mM, pH 7.5), 2-acetylfuran 174 (5 mM, 0.004 mmol) and crude cell lysate (80 µL). After incubation at 30 °C and 300 rpm for 24 h, the reaction was stopped by addition of 10% trifluoroacetic acid (TFA) in water (40 µL). Denatured protein was removed by centrifugation (4 °C, 30000 rpm, 4 min), the supernatant extracted with diethyl ether (1 mL) and the solvent evaporated. The residue was dissolved in THF (1 mL), 2 M NaOH (50 µL) and benzyl chloroformate (100 µL, 0.70 mmol) were added and the reaction shaken at room temperature for 16 h. Solvents were removed under reduced pressure and the residue dissolved in water (500 µL), extracted with diethyl ether (1 mL) and the solvent evaporated. The product was dissolved in EtOH (100 µL) and analysed by chiral HPLC.

IPA assay with varying substrate and IPA concentrations

The assay was performed in a 96-well plate with each well containing potassium phosphate buffer (100 mM, pH 8) and PLP (1 mM). At a substrate concentration of 10 mM, isopropylamine was added at 50 mM or 100 mM. At a substrate concentration of 20 mM, isopropylamine was added at 100 mM or 200 mM. At a substrate concentration of 50 mM, isopropylamine was added at 200 mM. The reaction was started by addition of crude cell lysate (20-40 µL) or clarified cell lysate (0.4 mg/mL). After incubation at 37 °C for 24 h, the reaction was stopped by addition of 10% TFA in water (10 µL). Denatured protein was removed by centrifugation (30000 rpm, 4 min) and the supernatant diluted and analysed by HPLC.

Preparative scale biocatalytic reactions with IPA

The transaminase reaction was scaled up (50 mL) with substrate (20 mM), isopropylamine (200 mM, pH 8), potassium phosphate buffer (100 mM, pH 8), PLP (1 mM) and Cv-TAm crude cell lysate (10 mL). The reaction was incubated at 37 °C and 200 rpm for 24 h.
IPA assay with varying pH values

The general procedure for the isopropylamine assay was followed. The pH of the potassium phosphate buffer and isopropylamine solutions were varied from pH 9 to pH 12.

Larger scale biocatalytic reactions with MBA

The reactions were performed in falcon tubes (10 mL or 25 mL total volume) containing (S)- or (R)-MBA (5 eq.), potassium phosphate buffer (100 mM, pH 7.5), PLP (1 mM), substrate (5 mM or 10 mM) and clarified cell lysate (1 mg/mL). After incubation at 30 °C for 24 h, the reaction was stopped by the addition of TFA (1% v/v). Denatured protein was removed by centrifugation (4000 rpm, 20 min) and the supernatant diluted and analysed by HPLC.

Acetylation procedure for chiral HPLC analysis of 136 and 189

The reaction mixture was extracted into ethyl acetate (10 mL) and the solvent removed under reduced pressure. The residue was dissolved in ethyl acetate (0.5 mL), acetic anhydride (100 µL) and K$_2$CO$_3$ (100 mg) were added and the mixture shaken for 30 min. Water (1 mL) was added, and the ethyl acetate was extracted, dried with Na$_2$SO$_4$ and the solvent evaporated. The residue was dissolved in i-PrOH/hexane (1:9) and analysed by chiral HPLC.

Two-step reaction sequence with and without gold nanoparticle catalyst

4-Phenyl-2-butanol 158 (0.15 mL, 0.97 mmol), TBHP (70% in water, 0.5 mL, 4.00 mmol) and water (1.35 mL) were stirred in a carousel tube. Au/Al$_2$O$_3$ (10 wt%) catalyst (17 mg, 1 mol% Au) was added and the mixture heated at 95 °C for 24 h. The reaction was stopped by addition of NaSO$_3$ (630 mg, 5.00 mmol), diluted with water (7 mL) and DMSO (1 mL) and this solution used as the substrate stock solution (100 mM) for the transaminase reaction. Half of this stock solution was centrifuged (30000 rpm, 10 min) to remove the solid gold catalyst. The general procedure for the IPA assay was used with and without the presence of the gold catalyst, and the formation of 136 was analysed by HPLC using method C.
IPA assay in the presence of TBHP and quenching agents

TBHP (0.50 mL, 4.00 mmol) was dissolved in water (2 mL) and the quenching agent (4 mmol) was added. Water (14 mL), DMSO (4 mL) and benzaldehyde 40 (100 µL, 1.00 mmol) were added and this solution was used as the substrate stock solution (50 mM). The general procedure for the IPA assay was used. Benzylamine 190 formation was analysed by HPLC using method C.

Procedure for the oxidation of benzyl alcohol in water

Benzyl alcohol (31 µL, 0.30 mmol), TBHP (70% in water, 0.15 mL, 1.20 mmol) and water (1.85 mL) were stirred in a carousel tube. Gold nanoparticle catalyst (5 mg, 1 mol% Au) was added and the mixture heated at 95 °C. The reaction was monitored over 4 h, and for each time point, the reaction mixture was extracted into ethyl acetate (4 mL) containing dodecane (internal standard, 0.1 eq.), centrifuged, diluted and analysed by GC using method A.

General procedures for the two-step reaction sequence for secondary alcohols

Procedure A (transaminase scale 200 µL):

The general procedure for the oxidation of secondary alcohols was followed with alcohol (0.30 mmol), TBHP (70% solution in water, 0.15 mL, 0.20 mmol) and gold catalyst (10 wt%, 5 mg, 1 mol% Au) in water (1.85 mL) at 95 °C. The reaction was stirred for the specified time and then allowed to cool to room temperature. Water (3 mL) and DMSO (1 mL) were added and this solution was used as the substrate stock solution (50 mM).

The transaminase assay was performed in a 96 well plate (200 µL per well) adding the substrate stock solution from the oxidation reaction (20 µL, 5 mM final substrate concentration) to each well containing (R)- or (S)-MBA (25 mM), PLP (1 mM), potassium phosphate buffer (pH 7.5, 100 mM), and clarified cell lysate (1 mg/mL). After incubation at 30 °C and 300 rpm for 24 h, the reaction was stopped by addition 10 µL of 10% trifluoroacetic acid (TFA) in water (10 µL). Denatured protein was removed by centrifugation (4 °C, 3000 rpm, 4 min) and the supernatant diluted and analysed by HPLC.
Procedure B (transaminase scale 5-25 mL):

The oxidation step was performed in a carousel tube containing alcohol (50 mM or 100 mM), TBHP (70% solution in water, 200 mM) and gold catalyst (10 wt%, 1 mol% Au) in water at 95 °C. The reaction was stirred for the specified time and then allowed to cool to room temperature. To this solution was added (S)- or (R)-MBA (25 mM or 50 mM), PLP (1 mM), potassium phosphate buffer (pH 7.5, 100 mM), DMSO (10% v/v), clarified cell lysate (1 mg/mL) and water to reach a substrate concentration of 5 mM or 10 mM. This reaction mixture was then incubated at 30 °C and 200 rpm for 24 h after which the reaction was stopped by addition of trifluoroacetic acid (1% v/v). Denatured protein was removed by centrifugation (4 °C, 4000 rpm, 20 min) and the supernatant diluted and analysed by HPLC.
7.5 Compound synthesis

**Benzylacetone 101**

The general procedure for the oxidation of secondary alcohols in water was followed with 4-phenyl-2-butanol (0.46 µL, 0.30 mmol) and the Au/MgO (10 wt%) catalyst for 4 h. The reaction was quenched with sat. NaSO₃ (5 mL) and the product extracted with ethyl acetate (3 x 5 mL). The solvent was removed under reduced pressure and analyzed by NMR spectroscopy. ¹H NMR (CDCl₃; 400 MHz) 2.14 (3H, s, CH₃), 2.76 (2H, m, CH₂), 2.90 (2H, m, CH₂), 7.26 (5H, m, Ph). The characterization was in accordance with the literature.

**4-Phenyl-3-buten-2-ol 159**

NaBH₄ (597 mg, 15.8 mmol) was added in portions to 4-phenyl-3-buten-2-one (1.46 g, 10.0 mmol) in methanol (30 mL) at 0 °C. After 2 h the reaction was quenched with water (20 mL), and the methanol removed under reduced pressure. The water was then extracted with ethyl acetate (3 x 100 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure to give 159 as a colourless oil (1.27 g, 85%). ν<sub>max</sub> (film) 3326, 2967, 1491 cm⁻¹; ¹H NMR (CDCl₃; 600 MHz) 1.38 (3H, d, J 6.3 Hz, CH₃), 4.50 (1H, quint, J 6.3 Hz, CHO), 6.27 (1H, dd, J 15.9, 6.3 Hz, C=CH), 6.57 (1H, d, J 15.9 Hz, CHPh) 7.23-7.27 (1H, m, Ph), 7.32 (2H, t, 7.3 Hz, Ph), 7.38 (2H, d, 7.3 Hz, Ph); ¹³C NMR (CDCl₃; 150 MHz) 23.5 (CH₃), 69.1 (CHOH), 126.6 (Ph), 127.8 (Ph), 128.7 (Ph), 129.5 (PhCH=CH), 133.7 (PhCH=CH), 136.8 (Ph); m/z (EI) 148 ([M⁺, 65%) 133 ([M⁺-CH₃, 55), 105 (100), 77 (45).
4-Phenyl-3-buten-2-one 163\(^{170}\)

\[
\begin{align*}
\end{align*}
\]

The general procedure for the oxidation of secondary alcohols in water was followed with 4-phenyl-3-buten-2-ol (44 mg, 0.30 mmol) and the Au/MgO (10 wt%) catalyst for 15 min. The reaction was quenched with sat. NaSO\(_3\) (5 mL) and the product extracted with ethyl acetate (3 x 5 mL). The solvent was removed under reduced pressure and analysed by NMR spectroscopy. \(^1\)H NMR (CDCl\(_3\); 600 MHz) 2.40 (3H, s, CH\(_3\)), 6.73 (1H, d, \(J\) 16.3, PhCH=CH\(_2\)), 7.38-7.41 (3H, m, Ph), 7.51-7.56 (3H, m, Ph, PhCH=CH). The characterisation was in accordance with the literature.\(^{170}\)

4-Phenyl-3-butyne-2-ol 160\(^{171}\)

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\begin{align*}
\end{align*}
\]

To phenylacetylene (0.66 mL, 6.01 mmol) in dry THF (8 mL) under argon was added \(n\)-BuLi (2.5 M in hexane; 3.60 mL, 9.00 mmol) at -78 °C. The reaction was warmed to 0 °C and acetaldehyde (0.36 mL, 6.46 mmol) was added and the reaction stirred for 2 h. The reaction was quenched with sat. NH\(_4\)Cl (10 mL) and the THF removed under reduced pressure. The aqueous residue was extracted with ethyl acetate (3 x 50 mL), washed with sat. NaCl (50 mL), dried (Na\(_2\)SO\(_4\)) and the solvent removed under reduced pressure. The product was purified by column chromatography (ethyl acetate/petroleum ether (40-60), 1:3) to give 4-phenyl-3-butyne-2-ol 160 as a yellow oil (616 mg, 70%). \(\nu_{\text{max}}\) (film) 3316, 2977, 2250, 1441 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\); 600 MHz) 1.57 (3H, d, \(J\) 6.6 Hz, CH\(_3\)), 4.76 (1H, q, \(J\) 6.6 Hz, CH\(_2\)), 7.31-7.32 (3H, m, Ph), 7.42-7.44 (2H, m, Ph); \(^{13}\)C NMR (CDCl\(_3\); 150 MHz) 24.5 (CH\(_3\)), 59.0 (CHOH), 84.1 (C=C), 91.0 (C=C), 122.7 (Ph), 128.4 (Ph), 128.5 (Ph), 131.8 (Ph); \(m/z\) (EI) 146 ([M]\(^+\), 20%), 145 ([M-H]\(^+\), 20), 131 ([M]\(^+\)-CH\(_3\), 50), 129 ([M]\(^+\)-OH, 15), 84 (95), 77 ([Ph]\(^+\), 15), 67 ([M-H]\(^+\)-Ph, 100).
4-Phenyl-3-butyn-2-one 164\textsuperscript{172}

![4-Phenyl-3-butyn-2-one](image)

The general procedure for the oxidation of secondary alcohols in water was followed with 4-phenyl-3-butyn-2-ol (44 mg, 0.30 mmol) and the Au/Al\textsubscript{2}O\textsubscript{3} (10 wt\%) catalyst for 15 min. The reaction was quenched with sat. NaSO\textsubscript{3} (5 mL) and the product extracted with ethyl acetate (3 x 5 mL). The solvent was removed under reduced pressure and analysed by NMR spectroscopy. \textsuperscript{1}H NMR (CDOl\textsubscript{3}; 600 MHz) 2.46 (3H, s, CH\textsubscript{3}), 7.39 (2H, t \( J = 7.6 \) Hz, Ph), 7.45 (1H, t \( J = 7.6 \) Hz, Ph), 7.57 (2H, m, Ph), \textsuperscript{13}C NMR (CDCl\textsubscript{3}; 150 MHz) 32.9 (CH\textsubscript{3}), 88.4 (C≡C), 90.5 (C≡C), 120.0 (Ph), 128.4 (Ph), 128.8 (Ph), 130.9 (Ph), 184.9 (CO). The characterisation was in accordance with the literature.\textsuperscript{172}

2-Heptanone 99\textsuperscript{172}

![2-Heptanone](image)

The general procedure for the oxidation of secondary alcohols in water was followed with 2-heptanol (42 \( \mu \)L, 0.30 mmol) and the Au/Al\textsubscript{2}O\textsubscript{3} (10 wt\%) catalyst for 4 h. The reaction was quenched with sat. NaSO\textsubscript{3} (5 mL) and the product extracted with ethyl acetate (3 x 5 mL). The solvent was removed under reduced pressure and analysed by NMR spectroscopy. \textsuperscript{1}H NMR (CDCl\textsubscript{3}; 600 MHz) 0.89 (5H, m, CH\textsubscript{3}CH\textsubscript{2}), 1.31 (2H, m, CH\textsubscript{2}), 1.57 (2H, m, CH\textsubscript{2}), 2.14 (3H, s, COCH\textsubscript{3}), 2.42 (2H, t, CH\textsubscript{2}CO). The characterisation was in accordance with the literature.\textsuperscript{172}

(5-(Benzylaminomethyl)furan-2-yl)methanol 178\textsuperscript{19}

![5-(Benzylaminomethyl)furan-2-yl)methanol](image)

5-HMF (145 mg, 1.15 mmol) and benzylamine (0.13 mL, 1.19 mmol) were stirred in water (5 mL) at rt for 5 h. Sodium borohydride (65 mg, 1.72 mmol) was added and the reaction stirred at 35 \textdegree C for 16 h. The product was extracted with diethyl ether (3 x 30 mL), dried (MgSO\textsubscript{4}) and the solvent removed under reduced
pressure. The product was purified by column chromatography (MeOH/CH₂Cl₂ 4:96) to give (5-(benzylaminomethyl)furan-2-yl)methanol 178 as a colourless oil (104 mg, 45%). \( \nu_{\text{max}} \) (neat) 3296 br, 3060, 3025, 2840, 1559 cm\(^{-1}\); \(^1\)H NMR (CDCl₃; 300 MHz) 3.05 (2H, br s, OH, NH) 3.71 (2H, s, CC₃H₂NH), 3.74 (2H, s, NHCH₂Ph), 4.47 (CH₂OH) 6.10 (1H, d, J 3.0 Hz, 4-H), 6.14 (1H, d, J 3.0 Hz, 3-H), 7.24–7.34 (5H, m, Ph); \(^{13}\)C NMR (CDCl₃;75 MHz) 45.3 (CH₂NH), 52.8 (CH₃Ph), 57.1 (CH₂OH), 108.2 (C-4), 108.3 (C-3), 127.3 (Ph), 128.5 (Ph), 128.6 (Ph), 139.5 (Ph), 153.2 (C-2), 154.3 (C-5); \( m/z \) (ESI) 218 ([M+H]⁺), 100%, 111 (M⁺-NHCH₂Ph, 40).

**Tris(5-hydroxymethylfurfuryl)amine 180\(^{173}\)**

![Chemical structure](image)

5-HMF (126 mg, 1.00 mmol) was dissolved in methanol (3 mL) and ammonium acetate (0.77 g, 10.0 mmol) added. Sodium cyanoborohydride (189 mg, 3.01 mmol) was added in portions and the reaction heated to 60 °C for 16 h. The methanol was removed under reduced pressure and the residue dissolved in CH₂Cl₂:MeOH (9:1, 10 mL). Sat. NaHCO₃ (20 mL) was added and the product extracted with CH₂Cl₂ (3 x 30 mL), washed with brine (20 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure to give tris(5-hydroxymethylfurfuryl)amine 180 (17 mg, 15%) as a colourless oil. \( \nu_{\text{max}} \) (neat) 3267 br, 2919, 2848, 1558, 1360 cm\(^{-1}\); \(^1\)H NMR (CDCl₃; 600 MHz) 3.63 (2H, s, CH₂N), 4.53 (2H, s, CH₂OH), 6.12-6.17 (2H, m, 3-H, 4-H); \(^{13}\)C NMR (CDCl₃; 150 MHz) 49.9 (CH₂N), 57.3 (CH₂OH), 108.2 (C-4), 110.1 (C-3), 151.4 (C-2), 154.1 (C-5); \( m/z \) (EI) 347 ([M⁺], 10%), 111 ([M⁺-C₁₂H₁₄NO₄], 100).
5-Hydroxymethyl-2-furancarboxylic acid 86\textsuperscript{174}

![Chemical structure](image)

5-Formyl-2-furancarboxylic acid (140 mg, 1.00 mmol) was dissolved in methanol (5 mL) and cooled in an ice bath. NaBH\textsubscript{4} (57 mg, 1.51 mmol) was added in portions and the reaction stirred for 4 h. After this time, the reaction was quenched with sat. NaCl (5 mL) and the methanol removed under reduced pressure. The aqueous residue was acidified and extracted with ethyl acetate (3 x 30 mL), washed with sat. NaCl solution (2 x 30 mL), dried (Na\textsubscript{2}SO\textsubscript{4}) and the solvent removed under reduced pressure to give 5-hydroxymethyl-2-furancarboxylic acid 86 as a colourless solid (115 mg, 81%). M.p. 157-158 °C (EtOAc), lit. 163-164 °C;\textsuperscript{174} \nu\textsubscript{max} (neat) 3236, 2414 br, 1650, 1595 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (MeOH-d\textsubscript{4}; 500 MHz) 4.56 (2H, s, C\textsubscript{H}\textsubscript{2}OH), 6.45 (1H, d, J 3.4 Hz, 4-H), 7.15 (1H, d, J 3.4 Hz, 3-H);
\textsuperscript{13}C NMR (MeOH-d\textsubscript{4}; 125 MHz) 57.5 (CH\textsubscript{2}OH), 110.2 (C-4), 119.9 (C-3), 145.7 (C-2), 160.7 (C-5), 161.8 (CO); m/z (El) 142.1 ([M+\textsuperscript{+}] 28%), 123 (22), 97 (100), 69 (50).

2,5-Bis(hydroxymethyl)furan 85\textsuperscript{175}

![Chemical structure](image)

NaBH\textsubscript{4} (170 mg, 4.50 mmol) was added in portions to 5-HMF (378 mg, 3.00 mmol) in methanol (10 mL) at 0 °C. The reaction was stirred for 4 h, brine (5 mL) was added and the methanol removed under reduced pressure. The aqueous residue was extracted with ethyl acetate (3 x 30 mL), washed with brine (2 x 30 mL), dried (Na\textsubscript{2}SO\textsubscript{4}) and the solvent removed under reduced pressure to give 2,5-bis(hydroxymethyl)furan 85\textsuperscript{175} as a colourless oil (296 mg, 77%). \nu\textsubscript{max} (neat) 3281, 2924, 2866, 1631 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) 2.45 (2H, br s, 2 x OH), 4.56 (4H, s, 2 x CH\textsubscript{2}OH), 6.22 (2H, s, 3-H, 4-H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}; 150 MHz) 57.5 (CH\textsubscript{2}OH), 108.7 (C-3, C-4), 154.2 (C-2, C-5); m/z (Cl): 256 ([2M\textsuperscript{+}], 100%), 191 (38), 173 (15), 146 ([MH + NH\textsubscript{3}]\textsuperscript{+}, 35), 128 (5).
5-Chloromethylfurfural 177[176,177]

![Chemical Structure](url)

5-HMF (252 mg, 2.00 mmol) was dissolved in CH₂Cl₂ (10 mL) and conc. HCl (37%, 5 mL) was added. The reaction was stirred at room temperature for 24 h. Water (10 mL) was added and the reaction was extracted with CH₂Cl₂ (3 x 30 mL), washed with sat. NaCl solution (2 x 30 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure to give 5-chloromethylfurfural 177 as a yellow oil (228 mg, 79%). ν_max 2834, 1670 cm⁻¹; ¹H NMR (CDCl₃; 600 MHz) 4.60 (2H, s, CH₂Cl), 6.58 (1H, d, J 3.6, 4-H), 7.20 (1H, d, J 3.6, 3-H), 9.62 (1H, s, CHO); ¹³C NMR (CDCl₃; 150 MHz) 36.7 (CH₂Cl), 112.1 (C-4), 122.0 (C-3), 153.0 (C-2), 156.2 (C-5), 177.9 (C=O); m/z (EI) 146 ([M₃⁷Cl]⁺, 20%), 144 ([M₃⁵Cl]⁺, 35), 109 ([M-Cl]⁺, 100).

5-Chloromethyl-2-furancarboxylic acid 181

![Chemical Structure](url)

5-Hydroxymethyl-2-furancarboxylic acid (91 mg, 0.64 mmol) was stirred in CH₂Cl₂ (6 mL) and conc. HCl (37%, 2 mL) was added. The reaction was stirred at room temperature for 24 h. Water (10 mL) was added and the reaction was extracted with CH₂Cl₂ (3 x 15 mL), washed with sat. NaCl solution (2 x 15 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure to give 5-chloromethyl-2-furancarboxylic acid 181 as a colourless solid (45 mg, 44%). M. p. 123-124 °C (CH₂Cl₂); ν_max (neat) 3212, 2929, 2800 br, 1675, 1590 cm⁻¹; ¹H NMR (MeOH-d₄; 600 MHz) 4.70 (2H, s, CH₂Cl), 6.59 (1H, d, J 3.5, 4-H), 7.15 (1H, d, J 3.5, 3-H); ¹³C NMR (CDCl₃; 150 MHz) 36.6 (CH₂Cl), 111.8 (C-4), 121.1 (C-3), 144.0 (C-2), 155.5 (C-5), 163.1(C=O); m/z (EI) 162 ([M₃⁷Cl]⁺, 6%), 160 ([M₃⁵Cl]⁺, 16), 125 (100), 79 (33); HRMS (FTMS) found [M-H] 158.9858; C₆H₄₃⁵ClO₃ requires 158.9854.
Boc-5-aminomethylfurfural 183

![Boc-5-aminomethylfurfural](image)

5-Chloromethylfurfural (467 mg, 3.23 mmol) was dissolved in n-butanol (10 mL) and conc. HCl (37%, 25 µL) added. The mixture was stirred for 5 min and the solvent removed under reduced pressure. The residue was dissolved in dry DMF (9 mL) under argon, sodium azide (624 mg, 9.60 mmol) added and the reaction heated to 65 °C for 3 h. The DMF was removed under reduced pressure and the residue dissolved in methanol (15 mL). PPh₃ (1.70 g, 6.48 mmol) was added and the reaction stirred at room temperature. After 2 h, Boc₂O (1.40 g, 6.41 mmol) was added and the reaction stirred for 16 h. The methanol was removed under reduced pressure, sat. NaHCO₃ (50 mL) added and the product extracted with ethyl acetate (3 x 50 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure. The residue was stirred in dil. HCl (1 M, 10 mL), and the product extracted with ethyl acetate (3 x 30 mL). Boc-5-aminomethylfurfural 183 was purified by column chromatography (ethyl acetate/petroleum ether (40-60), 1:4) to give a colourless oil (259 mg, 36%). ν_max (neat) 3122, 2927, 2836, 2093, 1671, 1518 cm⁻¹; ¹H NMR (CDCl₃; 600 MHz) 1.40 (9H, s, t-Bu), 4.34 (2H, d J 5.9 Hz), 5.28 (1H, br s, NH), 6.41 (1H, d J 3.3 Hz, 4-H), 7.16 (1H, d J 3.3 Hz, 3-H), 9.52 (1H, s, CHO); ¹³C NMR (CDCl₃; 150 MHz) 28.3 (t-Bu), 38.0 (t-Bu), 79.6 (CH₂NH), 109.9 (C-4), 123.2 (C-3), 155.7 (C-2), 156.6 (C=O ester), 159.3 (C-5), 177.5 (C=O); m/z (ES⁺) 225 ([M]+, 5%).

2,5-Bis(aminomethyl)furan 21

![2,5-Bis(aminomethyl)furan](image)

To 2,5-bis(hydroxymethyl)furan 85 (256 mg, 2.03 mmol) in CH₂Cl₂ (6 mL), conc. HCl (37%; 2 mL) was added. The reaction was stirred at room temperature for 16 h. Water (10 mL) was added and the product was extracted with CH₂Cl₂ (3 x 15 mL), washed with brine (2 x 15 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure to give 2,5-bis(chloromethyl)furan 182 as a brown oil.
(82 mg, 25%) which was taken directly through to the next step. \(^1\)H NMR (CDCl\(_3\), 600 MHz) 4.58 (4H, s, 2 x CH\(_2\)Cl), 6.34 (2H, s, 3-H, 4-H); \(^13\)C NMR (CDCl\(_3\), 150 MHz) 37.4 (CH\(_2\)Cl\(_2\)), 110.9 (C-3, C-4), 151.1 (C-2, C-5).

Sodium azide (130 mg, 2.00 mmol) was added to 2,5-bis(chloromethyl)furan 182 (83 mg, 0.50 mmol) in dry DMF (8 mL) under argon, and the reaction was heated to 65 °C for 16 h. The DMF was removed under reduced pressure and the residue was dissolved in methanol (8 mL). PPh\(_3\) (525 mg, 2.00 mmol) was added and the reaction was stirred at room temperature. After 2 h, Boc\(_2\)O (436 mg, 2.00 mmol) was added and the reaction was stirred for 16 h. The methanol was removed under reduced pressure, sat. NaHCO\(_3\) added (30 mL) and the product was extracted with ethyl acetate (3 x 30 mL), dried (Na\(_2\)SO\(_4\)) and the solvent removed under reduced pressure. 2,5-Bis(boc-aminomethyl)furan 185 was purified by column chromatography (ethyl acetate/petroleum ether (40-60), 1:4) to give a brown solid (63 mg, 39%). It was then directly deprotected by stirring in a 1:1 mixture of methanol and 4M HCl (6 mL) for 1 h, and the solvent removed under reduced pressure to give 2,5-bis(aminomethyl)furan hydrochloric salt 21.HCl\(^{150}\) as a brown solid (34 mg, 87%, 34% from 182). M. p. 240 °C (decomp.; H\(_2\)O); \(\nu_{\text{max}}\) (neat) 3090, 2851 br, 1596 cm\(^{-1}\); \(^1\)H NMR (MeOH-d\(_4\); 600 MHz) 4.21 (4H, s, 2 x C\(_2\)H\(_2\)NH\(_2\)), 6.61 (2H, s, 3-H, 4-H); \(^13\)C NMR (MeOH-d\(_4\); 150 MHz) 36.8 (CH\(_2\)NH\(_2\)), 113.2 (C-3, C-4), 149.6 (C-2, C-5). \(m/z\) (EI) 126 ([M]\(^+\), 30%), 96 (100); HRMS (EI) found [M]\(^+\) 126.0788; C\(_6\)H\(_{10}\)N\(_2\)O requires 126.0788.

5-Aminomethyl-2-furancarboxylic acid 168\(^{143}\)

5-Chloromethyl-2-furancarboxylic acid 181 (44 mg, 0.27 mmol) was dissolved in dry DMF (5 mL) under argon. NaN\(_3\) (53 mg, 0.82 mmol) was added and the reaction heated to 65 °C for 16 h. The DMF was removed under reduced pressure and the residue dissolved in methanol (8 mL). PPh\(_3\) (212 mg, 0.808 mmol) was added and the reaction was stirred at room temperature. After 3 h Boc\(_2\)O (118 mg, 0.541 mmol) was added to the reaction mixture and stirred for 16 h. The methanol was removed under reduced pressure, sat. NaHCO\(_3\) added (30 mL) and the side products removed with ethyl acetate (3 x 30 mL). The aqueous layer
was then acidified and extracted with ethyl acetate (3 x 30 mL), the extracts dried (Na₂SO₄) and the solvent removed under reduced pressure. The product was then stirred in a 1:1 mixture of methanol and 4M HCl (6 mL) for 2 h, and the solvents removed under reduced pressure to give \textbf{168.HCl}\textsuperscript{143} as a colourless solid (29 mg, 60%). M.p. 250 °C (decomp.; H₂O); \(\nu_{\text{max}}\) (neat) 3150, 3011, 2791 br, 1685, 1586 cm\(^{-1}\); \(^1\text{H} \text{NMR} \) (MeOH-d₄; 600 MHz) 4.26 (2H, s, CH₂NH₂), 6.71 (1H, d, \(J = 3.5, 4\)-H), 7.22 (1H, d, \(J = 3.5, 3\)-H); \(^{13}\text{C} \text{NMR} \) (MeOH-d₄; 150 MHz) 36.8 (CH₂NH₂), 113.8 (C-4), 119.9 (C-3), 147.4 (C-2), 152.1 (C-5), 161.2 (C=O); HRMS (Cl) found [M + H]\(^+\) 142.0499; C₆H₇NO₃ requires 142.0499.

\textbf{5-Azidomethylfuran-2-carboxylic acid 187\textsuperscript{179,180}}

\begin{center}
\includegraphics[width=0.5\textwidth]{5-Azidomethylfuran-2-carboxylic_acid.png}
\end{center}

5-Chloromethylfuran-carboxylic acid \textbf{181} (119 mg, 0.74 mmol) was dissolved in dry DMF (10 mL) under argon, sodium azide (144 mg, 2.22 mmol) added and the reaction heated to 65 °C for 16 h. The DMF was removed under reduced pressure and water (30 mL) was added. Sat. NaHCO₃ (10 mL) was added and the side products removed with ethyl acetate (2 x 30 mL). The aqueous layer was then acidified and extracted with ethyl acetate (3 x 30 mL), the extracts dried (Na₂SO₄) and the solvent removed under reduced pressure to give \textbf{187\textsuperscript{179}} as a colourless oil (91 mg, 88%), which was used without purification. \(\nu_{\text{max}}\) (neat) 2922 br, 2668, 2103, 1694 cm\(^{-1}\); \(^1\text{H} \text{NMR} \) (MeOH-d₄; 600 MHz) 4.43 (2H, s, CH₂N₃), 6.57 (1H, d, \(J = 3.5\) Hz, 4-H), 7.17 (1H, d, \(J = 3.5\) Hz, 3-H); \(^{13}\text{C} \text{NMR} \) (MeOH-d₄; 150 MHz) 47.6 (CH₂N₃), 112.2 (C-4), 119.8 (C-3), 146.7 (C-5), 155.3 (C-2), 161.4 (C=O); m/z (EI) 167 ([M]\(^+\), 45%), 125 ([M]\(^+\)-N₃, 100), 84 (30), 67 (40).

\textbf{5-Azidomethylfurfural 188\textsuperscript{181}}

\begin{center}
\includegraphics[width=0.5\textwidth]{5-Azidomethylfurfural.png}
\end{center}

5-Chloromethyl furfural (204 mg, 1.41 mmol) was dissolved in dry DMF (5 mL) under argon, sodium azide (273 mg, 4.20 mmol) added and the reaction heated to 65 °C for 16 h. The DMF was removed under reduced pressure and water
(30 mL) was added. The product was extracted with ethyl acetate (3 x 30 mL), washed with brine (30 mL), dried (Na$_2$SO$_4$) and the solvent removed under reduced pressure. 5-Azidomethylfurfural $^{188}$ was purified by column chromatography (ethyl acetate/petroleum ether (40-60), 1:9) to give a colourless oil (30 mg, 14%). $\nu_{max}$ (neat) 2750, 2094, 1673, 1518 cm$^{-1}$; $^1$H NMR (CDCl$_3$; 600 MHz) 4.42 (2H, s, CH$_2$N$_3$), 6.55 (1H, d, $J$ 3.6 Hz, 4-H), 7.22 (1H, d, $J$ 3.6 Hz, 3-H), 9.64 (1H, s, CHO); $^{13}$C NMR (CDCl$_3$; 150 MHz) 47.1 (CH$_2$N$_3$), 111.6 (C-4), 121.9 (C-3), 153.1 (C-5), 155.5 (C-2), 177.8 (C=O); $m/z$ (ESI) 174.1 ([M+Na]$^+$, 100%), 146 (50), 129 (40).

**Furfurylamine 165**

\[
\begin{align*}
\text{O} \\
\text{NH}_2
\end{align*}
\]

Furfural 1 (83 µL, 1.00 mmol) was subjected to the preparative scale reaction conditions with IPA, and after removal of the denatured protein by centrifugation (4000 rpm, 4 °C, 20 min), was analysed by HPLC to give the product in 83% yield.

**5-Hydroxymethylfurfurylamine 166$^{149}$**

\[
\begin{align*}
\text{OH} \\
\text{O} \\
\text{NH}_2
\end{align*}
\]

5-HMF (126 mg, 1.00 mmol) was subjected to the preparative scale biocatalytic reaction conditions, quenched with MeOH (100 mL) and the denatured protein removed by centrifugation (4000 rpm, 4 °C, 20 min). Volatile organics were removed under reduced pressure and the remaining aqueous solution extracted with ethyl acetate (3 x 50 mL) to remove any remaining starting material. The pH was changed to pH 10 by addition of 2 M NaOH and the aqueous layer extracted with ethyl acetate (10 x 30 mL) and dried (Na$_2$SO$_4$). The solvent was removed under reduced pressure to give 166$^{149}$ as a yellow oil (product yield 58% by HPLC; isolated yield 69 mg, 54%). $\nu_{max}$ (film) 3288, 2926, 1650 cm$^{-1}$; $^1$H NMR (MeOH-$d_4$; 600 MHz) 3.76 (2H, s, CH$_2$NH$_2$), 4.47 (2H, s, CH$_2$OH), 6.17 (1H, d, $J$ 3.2 Hz, 3-H), 6.22 (1H, d, $J$ 3.2 Hz, 4-H); $^{13}$C NMR (MeOH-$d_4$; 150 MHz) 39.2 (CH$_2$NH$_2$), 57.4 (CH$_2$OH), 107.7 (C-3), 109.2 (C-4), 155.3 (C-5), 156.2 (C-2); $m/z$ (EI) 127 ([M]$^+$, 20%), 96 (100).
5-Aminomethyl-2-furancarboxylic acid HCl salt 168.HCl

![Structure Image]

5-Formyl-2-furancarboxylic acid (140 mg, 1.00 mmol) was subjected to the preparative scale reaction conditions containing DMSO (4%) to aid solubility. After 24 h, the reaction was quenched with MeOH (100 mL) and the denatured protein removed by centrifugation (4000 rpm, 4 °C, 20 min). The solution was evaporated to dryness under reduced pressure and the residue dissolved in MeOH (15 mL). To this solution was added Boc₂O (874 mg, 4.00 mmol) and the reaction stirred at room temperature for 3 h. Methanol was removed under reduced pressure and the residue dissolved in water (20 mL) and extracted with ethyl acetate (2 x 30 mL) to remove side products. The aqueous solution was acidified and extracted with ethyl acetate (3 x 30 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure to give Boc-168. This was directly deprotected in a 1:1 mixture of methanol and 4M HCl (6 mL) for 2 h, and the solvent removed under reduced pressure to give 168.HCl (55 mg, 31%). The characterisation data was identical to 168 synthesised above.

4-Phenyl-3-butyne-2-one 164

![Structure Image]

4-Phenyl-3-butyne-2-ol (145 µL, 1.00 mmol) and TBHP (70% in water, 0.50 mL, 4.00 mmol) were stirred in water (1.35 mL) at 95 °C for 4 h. Saturated Na₂CO₃ was added and the aqueous layer extracted with diethyl ether (3 x 10 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure. The product was purified by column chromatography (diethyl ether/petroleum ether (40-60) 1:50) to give 164 as a yellow oil (19 mg, 13%). ν max (film) 2922, 2200, 1669, 1488 cm⁻¹; ¹H NMR (CDCl₃; 600 MHz) 2.46 (3H, s, CH₃), 7.39 (2H, t, J 7.6 Hz, Ph) 7.46 (1H, t, J 7.6 Hz, Ph), 7.57-7.58 (m, 2H, Ph); ¹³C NMR (CDCl₃; 150 MHz) 32.9 (CH₃), 88.4 (C≡C), 90.5 (C≡C), 120.0 (Ph), 128.0 (Ph), 128.8 (Ph), 130.9 (Ph), 182
184.8 (C=O). m/z (El) 144 ([M]+, 10%), 145 ([M+H]+, 40), 131 (100), 129 ([M]-CH₃, 40), 103 (60), 77 ([Ph]+, 40).

4-Phenyl-3-buten-2-amine 189¹⁶⁵,¹⁸³

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Benzylamine 190 (two-step synthesis)

\[
\text{\includegraphics[width=0.2\textwidth]{benzylamine.png}}
\]

The general procedure for the two-step reaction sequence (Procedure A) was performed with benzyl alcohol (31 \(\mu\)L, 0.30 mmol), Au/Al\(_2\)O\(_3\) (10 wt\%) (5 mg, 1 mol\% Au) for 2 h. After this time the reaction was allowed to cool to room temperature, water (4 mL) and DMSO (1 mL) were added and this solution was used as the substrate stock solution (50 mM). The IPA assay conditions were then applied with Cv-TAm, Mv-TAm and ArRMut11, in triplicate. Yields of benzylamine 190 were determined by HPLC using Method C.

1-Methyl-3-phenylpropylamine 136 (two-step synthesis example)

\[
\text{\includegraphics[width=0.2\textwidth]{1-methyl-3-phenylpropylamine.png}}
\]

The general procedure for the two-step reaction sequence (procedure B) was used with 4-phenyl-2-butanol 158 (38 \(\mu\)L, 0.25 mmol), TBHP (70\% solution in water, 140 \(\mu\)L, 1.00 mmol) and Au/MgO (10 wt\%) catalyst (2.5 mg, 1 mol\% Au) in water (2.5 mL) at 95 °C for 4 h. After cooling, this solution was combined with DMSO (4\%), PLP (1 mM), KPi pH 7.5 (100 mM), (S)- or (R)-MBA, water and clarified cell lysate (1 mg/mL) to a total volume of 25 mL. Yields of 136 were determined by HPLC using Method C.

4-Phenyl-3-buten-2-amine 189 (two-step synthesis example)

\[
\text{\includegraphics[width=0.2\textwidth]{4-phenyl-3-buten-2-amine.png}}
\]

The general procedure for the two-step reaction sequence (procedure B) was used with 4-phenyl-3-buten-2-ol 159 (7 mg, 0.05 mmol), TBHP (70\% solution in water, 28 \(\mu\)L, 0.2 mmol) and Au/MgO (10 wt\%) catalyst (0.5 mg, 1 mol\% Au) were stirred in water (1 mL) at 95 °C for 4 h. After cooling, this solution was combined with DMSO (4\%), PLP (1 mM), KPi pH 7.5 (100 mM), (S)- or (R)-MBA (5 eq.), water and clarified cell lysate (1 mg/mL), to a total volume of 10 mL. Yields of acetophenone were determined by HPLC using Method A.
8 References


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9 Appendix

9.1 GC Traces

Benzyl alcohol 39 (8.8 min), dodecane (7.4 min), GC method A:

Benzaldehyde 40 (6.3 min), dodecane (7.4 min), GC method A:

TMS-benzoic acid 156 (8.8 min), dodecane (6.9 min) GC method D:
TMS-benzyl alcohol 155 (3.9 min), dodecane (6.7 min), GC method D:

Phenylacetone 162 (6.1 min), dodecane (8.5 min), GC method E:

4-Phenyl-2-butanol 158 (13.3 min), dodecane (7.4 min), GC method A:
Benzylacetone **101** (12.7 min), dodecane (7.4 min), GC method A:

4-Phenyl-3-buten-2-ol **159** (9.4 min), dodecane (5.0 min), GC method B:

4-Phenyl-3-buten-2-one **163** (9.5 min), dodecane (5.0 min), GC method B:
2-Heptanone 99 (4.3 min), dodecane (6.3 min), GC method C:
9.2 HPLC Traces

Acetophenone, HPLC method A

Benzylamine 190, HPLC method C

Furfurylamine 165, HPLC method B
2,5-Bis(aminomethyl)furan 21, HPLC method B

5-Aminomethyl-2-furancarboxylic acid 168, HPLC method A

1-(2-Furan)ethylamine 175, HPLC method C

1-Methyl-3-phenylpropylamine 136, HPLC method C
4-Phenyl-3-buten-2-amine 189, HPLC method A

4-Phenyl-3-buten-2-amine 189 after decomposition

4-Phenyl-3-buten-2-amine 189 from two-step reaction sequence
4-Phenyl-3-butyne-2-amine 191 from transaminase reaction, HPLC method A

4-Phenyl-3-butyne-2-amine 191 from two-step reaction sequence
9.3 Chiral HPLC Traces

Rac-\(N\)-Cbz-175 (28 min and 35 min)

(R)-\(N\)-Cbz-175 (35 min)

Rac-\(N\)-Acetyl-136 (0.8 mL/min, 18 min and 20 min)

(S)-\(N\)-Acetyl-136 from transaminase (0.8 mL/min, 20 min)
(S)-N-Acetyl-136 from two-step process (0.8 mL/min, 20 min)

Rac-N-Acetyl-136 (0.5 mL/min, 24 min and 27 min)

(R)-N-Acetyl-136 from transaminase (0.5 mL/min, 24 min)

(R)-N-Acetyl-136 from two-step process (0.5 mL/min, 24 min)
Rac-N-Acetyl-189 (21 min and 28 min)

(S)-N-Acetyl-189 from transaminase (28 min)

(S)-N-Acetyl-189 from two-step process (28 min)

(R)-N-Acetyl-189 from transaminase (21 min)
(R)-N-Acetyl-189 from transaminase (21 min)
9.4 Publication
Green Chemistry

PAPER

Furfurylamines from biomass: transaminase catalysed upgrading of furfurals†

Alice Dunbabin, a Fabiana Subrizi, a John M. Ward,b Tom D. Shepparda and Helen C. Hailes a

Furfural is recognised as an attractive platform molecule for the production of solvents, plastics, resins and fuel additives. Furfurylamines have many applications as monomers in biopolymer synthesis and for the preparation of pharmacologically active compounds, although preparation via traditional synthetic routes is not straightforward due to by-product formation and sensitivity of the furan ring to reductive conditions. In this work transaminases (TAmS) have been investigated as a mild sustainable method for the amination of furfural and derivatives to access furfurylamines. Preliminary screening with a recently reported colorimetric assay highlighted that a range of furfurals were readily accepted by several transaminases and the use of different amine donors was then investigated. Multistep synthetic routes were required to synthesise furfurylamine derivatives for use as analytical standards, highlighting the benefits of using a one step biocatalytic route. To demonstrate the potential of using TAmS for the production of furfurals, the amination of selected compounds was then investigated on a preparative scale.

Introduction

Furfurals have attracted significant interest in recent years as renewable feedstocks for the production of biofuels and chemicals.1 In particular, furfural 1a and 5-hydroxymethylfurfural (5-HMF) 2a are valuable platform chemicals, prepared via the acid-catalysed dehydration of pentoses and hexoses obtained by the hydrolysis of cellulose biomass waste including cornstalks, corncobs and rice waste.2 Due to improved catalytic processes furfurals are becoming more available and their production has been integrated into a biorefinery context.3 Indeed, renewable 5-HMF is being manufactured commercially.4 As a consequence, several processes have been developed for the conversion of furfurals into a number of valuable chemicals and fuels such as furfuryl alcohols, THFs, furfurylamine 1b, 1,5-pentanediol and functionalised aromatics.3,5 Among these, the production of furfurylamines by the selective reductive amination of furfurals has received interest due to many potential applications, including as intermediates in the synthesis of pharma-ceuticals such as antiseptic agents, antihypertensives, and diuretics (e.g. Furosemide).6 The 5-HMF 2a derived amine 2b also has potential as a curing agent in epoxy resins. Selective synthesis of these primary amines from carbonyl compounds is still challenging. Indeed, the use of traditional synthetic routes is not straightforward due to the sensitivity of the furan ring to reductive conditions and the tendency to form secondary or tertiary amine by-products.7 Moreover, the waste generated from using such reducing agents has to be considered especially within the green chemistry agenda.

Transaminase (TAmS) enzymes have been investigated in recent years for the transformation of pro-chiral ketones and aldehydes into the corresponding chiral secondary, or primary amines.8 These transferases can provide a sustainable high yielding, selective route to amines under mild aqueous conditions. The use of TAmS with furfural analogues in the literature has surprisingly received very little attention with only two previous reports: a kinetic resolution of racemic 1-(2-furfuryl)ethylamine using commercially available TAmS;9 use of 5-HMF 2a in an enzyme cascade incorporating the Vibrio fluvialis TAm with alanine dehydrogenase to shift the TAm equilibrium.10

Here we describe the use of TAmS for the amination of furfural derivatives 1a–9a and ketone 10a to access amines 1b–10b (Scheme 1). High conversions were observed with a range of substrates. The amination of selected compounds was then
investigated for the production of value-added chemicals on a preparative scale.

**Results and discussion**

**Initial substrate screening**

Ten furan-based substrates were screened including furfural 1a, 5-HMF 2a, halogenated furfurals 3a–6a, 5-methylfurfural 7a, 5-formyl-2-furan carboxylic acid 8a, 2,5-furandicarboxaldehyde 9a, and 2-acetyl furan 10a (Fig. 1). TAm s selected for screening included the (S)-o-TAm *Chromobacterium violaceum* DSM30191 (CV-TAm), which has been used with a range of cyclic and aromatic substrates and demonstrated tolerance towards the low cost amine donor isopropylamine (IPA) 11.11–14 The (R)-selective TAm *Arthrobacter* sp. variant ArRmut115 and *Mycobacterium vanbaalenii* (Mv-TAm),16 were also selected due to their complementary enantiomericities compared to CV-TAm for use with the ketone 10a. In addition, ArRmut11 has been used with a wide range of substrates and also has good tolerance towards IPA 11 as an amine donor.15–19

Initial screening for the conversion of aldehydes and ketone 1a–10a, was carried out using crude cell extract and our recently reported rapid and sensitive TAm colorimetric assay (Scheme 2).20 It uses an inexpensive amine donor 12 which forms the corresponding aldehyde shown when a TAm reaction occurs: the aldehyde generated can then react with the major amine present (12) to form an imine which tautomerizes to give a red precipitate, indicating that the TAm reaction has occurred.20 The results of the colorimetric assay are shown in Fig. 2 against benzaldehyde (-) as a positive control. When using CV-TAm a strong coloration was observed with all aldehyde substrates. Notably, furfural 1a, 5-HMF 2a and acid 8a were readily converted showing a deep red coloration. In addition, aldehydes were accepted by both ArRmut11 and Mv-TAm while ketone 10a showed little coloration with all TAm s perhaps due to imine formation with 12.

Activity of the TAm s towards 1a–10a (with benzaldehyde as a positive control) was also confirmed using either (S)- or (R)-α-methylbenzylamine (MBA) 13 as amine donors, depending on the selectivity of the enzyme (Fig. 3). Notably furfural 1a and 5-HMF 2a were readily accepted by all three enzymes and in comparable conversions to benzaldehyde with CV-TAm and Mv-TAm (see Fig. 3 caption). Also, the 3- and 4-bromo derivatives 3a and 4a gave 50–60% conversions with all the selected transaminases. Within this series, the 5-substituted furfurals 5a and 7a were particularly well accepted by Mv-TAm (82% and 79% conversions respectively), while significantly lower conversions were achieved with 5a and both CV-TAm and ArRmut11 and 7a with CV-TAm. However, the less polar 5-methylfurfural 7a showed a 60% conversion with ArRmut11.
mines 2b, 8b, and 9b were not readily available commercially but required as analytical standards they were synthesised (Scheme 3). As the furan ring is sensitive to reductive conditions, and traditional reductive amination routes resulted in mixtures of di- and trisubstituted amines, a multi-step route for the preparation of 9b and 8b was established through chlorination to 14 and 15 and azide formation followed by reduction with triphenylphosphine (PPh₃). Derivatisation of the amine with tert-butoxycarbonyl anhydride (Boc₂O) was necessary to enable more straightforward product isolation and purification from side-products. The amine standards were then used as chemical standards to determine TAm reaction yields by HPLC.

Efforts to prepare 5-hydroxymethylfurfurylamine 2b starting from 5-HMF 2a following a similar strategy were unsuccessful. Moreover, attempts to reduce the carboxylic acid 8b or the corresponding azide with a range of reducing agents including LiAlH₄ and BH₃·THF resulted in complex mixtures of products that could not be purified. Instead, an enzymatic reaction using CV-TAm and IPA as amine donor was the most efficient method of preparing 2b, which was isolated after scale-up (see below) and used as a standard.

It is worth noting that procedures reported to afford 2b are typically very low yielding (4% yield) or use unsustainable metal-based catalysts. This example, together with the generally low yields of the multi-step synthesis, emphasises problems with such traditional synthetic approaches and the benefits of a one step biocatalytic route to compounds such as the amino acid 8b.

Reaction product yields when using amine donors 11 and 13 are shown in Table 1 using typical reaction conditions previously utilised in such TAm reactions. For IPA 11 (10 equiv.) higher yields were generally observed than when using MBA 13 (5 equiv.), highlighting the benefits of using excess amine donor and possibly the slightly higher reaction temperature. Yields of up to 92% were obtained for furylurea 1b with CV-TAm, but again lower yields (34%) were observed when using ArRMut11. The same trend was observed using 2a, which was readily accepted by CV-TAm and Mv-TAm, but showed a lower product yield with ArRMut11. Notably, with CV-TAm the amino acid 8b was produced in 88% yield with IPA, compared to 47% with MBA, and again ArRMut11 gave 8b in very low yield. For the dialdehyde 9a, when using IPA, yields of the diame 9b were slightly lower than for MBA. However, since a double transamination was required the use of twice as much enzyme was explored with IPA 11. Yields of the diame increased up to 60% for CV-TAm (Table 1). It was not possible to determine the yield of the mono-aminated product since it was unstable during synthetic investigations, forming complex polymerised mixtures via imine formation: this may also account for why the TAm yields observed did not increase markedly when using twice as much enzyme with 9a. The product yields for 2-acetylfuran 10a with MBA were confirmed using a commercial sample of 10b and HPLC, again highlighting Mv-TAm (54%) as the most productive transaminase, giving (R)-10b in 78% ee (absolute stereochemistry based on
the reported stereoselectivity of this TAm). The reaction with CV-TAm was lower yielding so the ee was not investigated.

Preparative scale reaction

Furfurylamines are valuable biomass-derived products and direct syntheses were then investigated on a preparative scale using CV-TAm, due to its high yields using IPA 11 as the amine donor (Table 1), together with aldehydes 1a, 2a, and 8a. The reactions were performed on a 200 mL scale (in 50 mL) for 24 h after which no starting materials remained. HPLC analysis established product yields of 83%, 58%, and 40% for 1b, 2b, and 6b respectively. The lower yields for 2b and 6b may have been due to some product inhibition at higher substrate concentrations. Furfurylamine 1b was not isolated as it is commercially available. As mentioned above, 2b was required as a product standard for analytical purposes in this work and was readily generated using this one-step enzymatic route from 5-HMF 2a. The product was isolated in 54% yield, highlighting the potential and benefits of this synthetic strategy compared to traditional chemical approaches. The preparative scale reaction using acid 8a was also of particular interest as amino acid 8b is a cyclic analogue of γ-aminobutyric acid (GABA) and has activity as an inhibitor of GABA aminotransferase, with $K_m$ values similar to GABA.23 Moreover, 8b belongs to the class of furanoid sugar amino acids which has been incorporated into novel anticancer peptides.24 The product 8b of the biotransformation reaction was successfully isolated as Boc-8b for ease of isolation purposes, and directly deprotected to give 8b in 31% overall isolated yield and high purity.

Table 1 Yields for transaminase catalysed reactions producing 1b, 2b, 8b, 9b and 10b using MBA 13 and IPA 11 as amine donors
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<th>MBA 13</th>
<th>IPA 11</th>
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<tr>
<td>CV-TAm</td>
<td>80%</td>
<td>92%</td>
<td>75%</td>
<td>89%</td>
<td>47%</td>
<td>88%</td>
<td>70%</td>
<td>48% (60%)</td>
<td>18% [ee n.d.]</td>
<td></td>
</tr>
<tr>
<td>ArRMut11</td>
<td>59%</td>
<td>34%</td>
<td>69%</td>
<td>44%</td>
<td>4%</td>
<td>3%</td>
<td>57%</td>
<td>45% (53%)</td>
<td>1% [ee n.d.]</td>
<td></td>
</tr>
<tr>
<td>Mo-TAm</td>
<td>75%</td>
<td>78%</td>
<td>53%</td>
<td>66%</td>
<td>32%</td>
<td>59%</td>
<td>56%</td>
<td>46% (52%)</td>
<td>54% [78% ee (R)]</td>
<td></td>
</tr>
</tbody>
</table>

*Reactions were performed in triplicate and conversions were determined using HPLC against product standards. Assays were performed on a 200 μL total reaction volume containing MBA 11 (25 mM) or IPA 11 (100 mM), PLP (1 mM), potassium phosphate buffer (100 mM, pH 7.5 for MBA and pH 8 for IPA), amine acceptor (5 mM when MBA was used or 10 mM when IPA was used) and crude cell lysate (20 μL) at 30 °C with MBA and 35 °C with IPA, for 24 h. (S)-11 or (R)-11 was used depending on enzyme selectivity. Yields obtained using double the amount of TA m enzyme. IPA was used as an amine donor except for compound 10b, ee determined using chiral HPLC; n.d. not determined.

Experimental

General

All chemicals were obtained from commercial suppliers and used as received unless otherwise stated. Thin layer chromatography (TLC) analysis was performed on Merck Kieselgel precoated aluminium-backed silica gel plates and compounds visualised by exposure to UV light, potassium permanganate or ninhydrin stains. Flash column chromatography was carried out using silica gel (particle size 40–60 μm). NMR: 1H and 13C NMR spectra were recorded at 298 K at the field indicated using Bruker spectrometers AMX400, Avance 500, and Bruker Avance III 600. Coupling constants (J) are measured in Hertz (Hz) and multiplicities for 1H NMR couplings are shown as s (singlet), d (doublet), and m (multiplet). Chemical shifts (in ppm) are given relative to tetramethylsilane and referenced to residual protonated solvent. Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer. Mass spectrometry analyses were performed at the UCL Chemistry Mass Spectrometry Facility using a Finnigan MAT 900 XP mass spectrometer and the EPSRC UK National Mass Spectrometry Facility.
Facility at Swansea University using a Thermo scientific LTQ Orbitraltap XL. Melting points were recorded on an Electrothermal IA9000 Series melting point apparatus and are uncorrected.

Analytical HPLC

Analyses of the reactions were performed using an Agilent 1260 Infinity HPLC with an Acc 5 C18 column (150 × 4.6 mm). Elution was carried out at 1 mL min⁻¹ with a linear gradient of acetonitrile/H₂O containing 0.1% TFA, with detection at 250 or 210 nm, injection volume of 10 µL and column temperature of 30 °C.

Transaminase expression and cell crude extract preparation

Selected TAm glycerol stocks of Chromobacterium violaceum DSM30191 T (CV-TAm), Arthrobacter sp. variant ArRMut1 T and Mycobacterium vanbaalenii (Mv-TAm), from the UCL TAm library were used to inoculate 2YT broth (5 mL) containing kanamycin (50 µg mL⁻¹) and incubated at 37 °C for 16–18 h. This pre-inoculum was then used to inoculate a larger culture (500 mL) containing the same antibiotic which was incubated at 37 °C for 3 h until an OD₆₀₀ of 0.5–0.7 was reached. Enzyme expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside (1 mM), and the temperature was reduced to 30 °C. After 5 h cells were harvested by centrifugation (8000 rpm, 20 min) and the pellet suspended in potassium phosphate buffer (100 mM, pH 7.5) containing pyridoxal-5-phosphate (PLP) (1 mM) and freeze dried. The freeze-dried cells were used fresh or stored at −20 °C for up to six months. To prepare crude cell extract, freeze-dried cells (25 mg) were suspended in potassium phosphate buffer (1 mL, 100 mM, pH 7.5), lysed by sonication on ice (10 s on and 10 s off for 5 cycles) and used as a crude lysate (10% v/v).

TAm concentrations were determined by SDS-PAGE densitometry: densitometry of samples electrophoresed on a NuPAGE 10% Bis-Tris gel (Novex) was used. The Coomassie stained gel was imaged using LabWork image software to calculate the TAm band density. A range of concentrations of commercial BSA was run in each SDS-PAGE gel (ESI Fig. 1f), and used to calculate a standard curve based on integrated optical density for calibration of the enzyme concentration. Total protein was determined using a standard Bradford assay. TAm concentrations in the crude lysates were determined as 5.7 mg mL⁻¹ CV-TAm, 4.0 mg mL⁻¹ ArRMut11 and 2.5 mg mL⁻¹ Mv-TAm.

Colorimetric screening

The assay was performed in a 96 well-plate with a total volume of 200 µL containing 2-(4-nitrophenyl)ethan-1-amino hydrochloride (25 mM) as amine donor, amine acceptor (10 mM), PLP (0.2 mM) and potassium phosphate buffer (100 mM, pH 7.5). The reaction was started by the addition of E. coli crude cell extract (20 µL) containing the overexpressed TAm and the reaction was incubated at 30 °C and 500 rpm for 24 h. Two negative controls were also performed, one without amine acceptor and another without enzyme. An orange/red coloration indicated that the TAm was active towards the selected furfurals (Fig. 2).

MBA (13) screening

The assay was performed in an Eppendorf tube (200 µL total volume) containing (R)- or (S)-MBA (13) (25 mM), PLP (1 mM), potassium phosphate buffer (100 mM, pH 7.5), amine acceptor (5 mM) and crude cell lysate (20 µL). After incubation at 30 °C and 300 rpm for 24 h, the reaction was stopped by the addition of 10% trifluoroacetic acid (TFA) in water (10 µL). Denatured protein was removed by centrifugation (4 °C, 3000 rpm, 4 min) and the supernatant diluted and analysed by analytical HPLC.

Isopropylamine (11) assay

The assay was performed in an Eppendorf tube containing isopropylamine (11) (100 mM, pH 8), PLP (1 mM), potassium phosphate buffer (100 mM, pH 8), amine acceptor (10 mM) and crude cell lysate (20 µL). After incubation at 35 °C and 300 rpm for 24 h, the reaction was stopped by the addition of 10% TFA in water (10 µL). Denatured protein was removed by centrifugation (4 °C, 3000 rpm, 4 min) and the supernatant diluted and analysed by analytical HPLC.

5-Hydroxymethyl-2-furancarboxylic acid

To 5-formyl-2-furancarboxylic acid (8a (140 mg, 1.00 mmol) in methanol (5 mL) at 0 °C, NaBH₄ (57 mg, 1.55 mmol) was added in portions and the reaction was stirred for 4 h. The reaction was quenched with brine (5 mL) and the methanol removed under reduced pressure. The aqueous residue was acidified and extracted with ethyl acetate (3 × 30 mL), washed with brine (2 × 30 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure to give 5-hydroxymethyl-2-furancarboxylic acid as a colourless solid (115 mg, 81%). M.p. 157–158 °C (EIOAc), lit. 163–164 °C,48 νmax (film) 3236, 2414 br, 1650, 1595 cm⁻¹; 1H NMR (MeOD-d₄; 500 MHz) 4.56 (2H, s, CH₂OH), 6.45 (1H, d, δ 3.4 Hz, 4-H), 7.15 (1H, d, δ 3.4 Hz, 3-H), 13C NMR (MeOD-d₄; 125 MHz) 57.5, 110.2, 120.0, 145.7, 160.7, 161.8, m/z (EI) 142 ([M⁺]⁺, 28%), 123 (22), 97 (100), 69 (50).

5-Chloromethyl-2-furancarboxylic acid (15)

To 5-hydroxymethyl-2-furancarboxylic acid (91 mg, 0.64 mmol) in CH₂Cl₂ (6 mL), conc. HCl (37%, 2 mL) was added. The reaction was stirred at room temperature for 24 h. Water (10 mL) was added and the reaction was extracted with CH₂Cl₂ (3 × 15 mL), washed with brine (2 × 15 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure to give 15 as a colourless solid (45 mg, 44%). M.p. 123–124 °C (CH₂Cl₂); νmax (film) 3121, 2929, 2800 br, 1675, 1590 cm⁻¹; 1H NMR (MeOD-d₄; 600 MHz) 4.70 (2H, s, CH₂Cl), 6.59 (1H, d, δ 3.5 Hz, 3-H), 7.15 (1H, d, δ 3.5 Hz, 4-H); 13C NMR (CDCl₃; 125 MHz) 36.6, 111.8, 121.1, 144.0, 155.5, 163.1; m/z MS (EI) 162 ([M⁺CHO]⁺, 6%), 160 ([M⁺Cl]⁺), 16), 125 (100), 79 (33); HRMS (FTMS) found [M – H]⁺ 158.9858; C₆H₆ClO requires 158.9854.
5-Aminomethyl-2-furancarboxylic acid (8b)
Sodium azide (51 mg, 0.82 mmol) was added to 15 (44 mg, 0.27 mmol) in dry DMF (5 mL) under argon, and the reaction was heated at 65 °C for 16 h. The DMF was removed under reduced pressure and the residue dissolved in methanol (8 mL). PPh₃ (212 mg, 0.809 mmol) was then added and the reaction was stirred at room temperature. After 3 h Boc₂O (118 mg, 0.541 mmol) was added and the reaction was stirred for 16 h. The methanol was removed under reduced pressure, sat. NaHCO₃ was added (30 mL) and the product was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were dried (Na₂SO₄) and the solvent removed under reduced pressure. 2,5-Bis(Boc-aminomethyl) furan was purified by column chromatography (ethyl acetate/petroleum ether (40-60), 1:4) to give a brown solid (63 mg, 39%). It was then directly depolymerized by stirring in a 1:1 mixture of methanol and 4 M HCl (6 mL) for 2 h, and the solvent removed under reduced pressure to give 5-aminomethyl-2-furancarboxylic acid hydrochloride salt 8b HCl (32 mg, 60%), as a colourless solid. M.p. 250 °C (decomp.); H₂O; vmax (film) 3150, 3011, 2791 br, 1685, 1586 cm⁻¹; 1H NMR (MeOD-d₄; 600 MHz) 4.26 (2H, s, CH₂N), 6.71 (1H, d, J = 3.5 Hz, 4-H), 7.22 (1H, d, J = 3.5 Hz, 3-H), 13C NMR (MeOD-d₄; 600 MHz) 36.8, 113.8, 119.9, 147.4, 152.1, 161.2; HRMS (Cl) found [M + H]+ 142.0499; C₇H₆N₂O₃ requires 142.0499.

2,5-Bis(hydroxymethyl)furan
NaBH₄ (170 mg, 4.49 mmol) was added in portions to 5-HMF 2a (378 mg, 3.00 mmol) in methanol (10 mL) at 0 °C. The reaction was stirred for 4 h, (5 mL) was added and the methanol was removed under reduced pressure. The aqueous residue was extracted with ethyl acetate (3 × 30 mL), washed with brine (2 × 30 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure to give 2,5-bis(hydroxymethyl)furan (9b) as a colourless oil (296 mg, 77%). vmax (film) 3281, 2924, 2866, 1631 cm⁻¹; 1H NMR (CDCl₃; 400 MHz) 2.45 (2H, br s, 2 × OH), 4.56 (4H, s, 2 × CH₂OH), 6.22 (2H, s, 3-H, 4-H); 13C NMR (CDCl₃; 150 MHz) 57.5, 108.7, 154.2; m/z (Cl) 256 ([M+H]+, 100%), 191 (38), 173 (15), 146 ([MH + NH]⁺ 35), 128 (5).

2,5-Bis(aminoethyl)furan hydrochloride salt (9b)
To 2,5-bis(hydroxymethyl)furan (256 mg, 2.00 mmol) in CH₂Cl₂ (6 mL), conc. HCl (37%; 2 mL) was added. The reaction was stirred at room temperature for 16 h. Water (10 mL) was added and the product was extracted with CH₂Cl₂ (3 × 15 mL), washed with brine (2 × 15 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure to give 14 (82 mg, 25%) which was taken directly through to the next step. 1H NMR (CDCl₃; 600 MHz) 4.58 (4H, s, CH₂Cl), 6.34 (2H, s, 3-H, 4-H); 13C NMR (CDCl₃; 150 MHz) 57.4, 110.9, 151.1.

Sodium azide (130 mg, 2.00 mmol) was added to 14 (83 mg, 0.50 mmol) in dry DMF (8 mL) under argon, and the reaction was heated at 65 °C for 16 h. The DMF was removed under reduced pressure and the residue was dissolved in methanol (8 mL). PPh₃ (525 mg, 2.00 mmol) was added and the reaction was stirred at room temperature. After 2 h Boc₂O (416 mg, 2.00 mmol) was added and the reaction was stirred for 16 h. The methanol was removed under reduced pressure, sat. NaHCO₃, added (30 mL) and the product was extracted with ethyl acetate (3 × 30 mL), dried (Na₂SO₄) and the solvent removed under reduced pressure. 2,5-Bis(Boc-aminomethyl) furan was purified by column chromatography (ethyl acetate/petroleum ether (40-60), 1:4) to give a brown solid (63 mg, 39%). It was then directly depolymerized by stirring in a 1:1 mixture of methanol and 4 M HCl (6 mL) for 2 h, and the solvent removed under reduced pressure to give 9b HCl (32 mg, 34% from 14). M.p. 240 °C (decomp.); H₂O; vmax (film) 3090, 2831 br, 1596 cm⁻¹; 1H NMR (MeOD-d₄; 600 MHz) 4.21 (4H, s, 2 × CH₂NH₂), 6.61 (2H, s, 1-H, 4-H); 13C NMR (MeOD-d₄; 150 MHz) 36.8, 113.2, 149.6; m/z (EI) 126 ([M]+, 30%), 96 (100); HRMS (EI) found [M]+ 126.0788; C₇H₆N₂O requires 126.0788.

1-Furan-2-ethylamine ee determination for Mv-Tam
The assay was performed (800 µL total volume) containing (R)-13 (25 µM), PLP (1 mM), potassium phosphate buffer (100 mM, pH 7.5), amine acceptor (5 mM) and crude cell lysate (80 µL). After incubation at 30 °C and 300 rpm for 24 h, the reaction was stopped by the addition of 10% trifluoroacetic acid (TFA) in water (40 µL). Denatured protein was removed by centrifugation (4 °C, 3000 rpm, 4 min), the supernatant extracted with diethyl ether (1 mL) and the solvent evaporated. The residue was dissolved in THF (1 mL), 2 M NaOH (50 µL) and benzyl chloroformate (100 µL) were added and the reaction shaken at room temperature for 16 h. Solvents were removed under reduced pressure and the residue dissolved in water (500 µL), extracted with diethyl ether (1 mL) and the solvent evaporated. The product was dissolved in EtOH (100 µL) and analysed by chiral HPLC to give the ee (78% (R)-isomer 27.5 min, (S)-isomer 35.1 min).

Preparative scale biocatalytic reactions
The TAM reaction was scaled up (50 mL) with substrate (20 mM), isopropylamine (200 mM, pH 8), potassium phosphate buffer (100 mM, pH 8), PLP (1 mM) and CV-Tam crude cell lysate (10 mL). The reaction was incubated at 37 °C and 200 rpm for 24 h.

Furfurylamine (1b)
Furfural 1a (83 µL, 1.0 mmol) was subjected to the preparative scale reaction conditions, and after removal of the denatured protein by centrifugation (4000 rpm, 4 °C, 20 min) was analysed by HPLC, to give the product in 83% yield.

5-Hydroxymethylfurfurylamine (2b)
5-HMF 2a (126 mg, 1.00 mmol) was subjected to the preparative scale biocatalytic reaction conditions, quenched with MeOH (100 mL) and the denatured protein removed by centrifugation (4000 rpm, 4 °C, 20 min). Volatile organics were removed under reduced pressure and the remaining aqueous
solution extracted with ethyl acetate (3 × 50 mL) to remove any
remaining starting material. The pH was changed to pH 10 by
addition of 2 M NaOH and the aqueous layer extracted with
ethyl acetate (10 × 30 mL) and dried (Na2SO4). The solvent
was removed under reduced pressure to give 2b as a yellow oil
(product yield 58% by HPLC; isolated yield 69 mg, 54%). vmax
(film) 3288, 2926, 1650 cm−1; 1H NMR (MeOH-d4; 600 MHz)
1.76 (2H, s, CH2NH2), 4.47 (2H, s, CH2OH), 6.17 (1H, d J = 3.2
Hz, 3-H), 6.22 (1H, d, J = 3.2 Hz, 4-H); 13CNMR (MeOH-d4;
150 MHz) 39.3, 37.4, 107.7, 109.3, 153.5, 156.2; m/z MS (El) 137
([M]+, 20%), 96 (100).

5-Aminomethyl-2-furanacarboxylic acid (8b)

5-Formyl-2-furanacarboxylic acid 8a (140 mg, 1.00 mmol) was
subjected to the preparative scale reaction conditions, quenched
with MeOH (100 mL) and the denatured protein removed by
centrifugation (4000 rpm, 4 °C, 20 min). The solution was
evaporated to dryness under reduced pressure and the residue
isolated in MeOH (15 mL) (product yield by HPLC 40%). To
this solution was added Boc2O (874 mg, 4.00 mmol) and the reaction
was stirred at room temperature for 3 h. Methanol was removed
under reduced pressure and the residue dissolved in water
(20 mL) and extracted with ethyl acetate (2 × 30 mL) to
remove side products. The aqueous solution was acidified and extracted
with ethyl acetate (3 × 30 mL), dried (Na2SO4) and the solvent
removed under reduced pressure to give Boc-8b. This was
directly deprotected in a 1:1 mixture of methanol and 4 M HCl
(6 mL) for 2 h, and the solvent removed under reduced pressure
to give 5-aminomethyl-2-furanacarboxylic acid hydrochloride salt
8b-HCl (55 mg, 31%). The characterisation data was identical
to 8b-HCl synthesised above.

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