# Transaminase Triggered Aza-Michael Approach for the Enantioselective Synthesis of Chiral Alkaloids

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# Abstract

This thesis focuses on the development of new biocatalytic strategies as a contemporary solution to synthetic design. Here we have put to use the unique regio-, stereo- and/or chemoselectivity offered by biocatalysts to develop synthetically attractive routes to enantiopure materials.

The first chapter discusses the development of a transaminase triggered aza-Michael cascade towards the synthesis of enantioenriched 2,6-disubstituted piperidines in good yield with >99% *e.e.* and >99% *d.e*. This methodology utilises a favourable spontaneous intramolecular aza-Michael reaction (IMAMR) to drive the reversible enzymatic transformation towards the formation of cyclic products, thus removing the need for additional approaches to displace the reaction equilibrium towards product formation. The alkaloid, (-)-pinidinone, was synthesised in three steps on a 0.5 g scale and a range of analogues was also successfully prepared to demonstrate the scope of the reaction. The reversible transamination reaction in combination with the thermodynamically favourable IMAMR, forming a stable cyclic product, results in a regioselective transamination of (3*E*)-dec-3-ene-2,8-dione. This inspired us to develop an amino donor and acceptor substrate that was successfully transaminated to form pinidinone with no external source of amine.

The second chapter discusses the synthesis of novel bis-conjugated enones and their subsequent transamination to provide bicyclic alkaloids via double aza-Michael additions. However, under the tested reaction conditions, the TA reaction resulted in complete decomposition of all but two of the tested substrates. 1-Methyldecahydropyrrolo[1,2-a]quinolin-5(1H)-one was produced as three isomers

whose relative stereochemistry was assigned by NMR. Interestingly, transamination of (2*E*)-1-(cyclohex-1-en-1-yl)oct-2-ene-1,7-dione provided 1-(cyclohex-1-en-1-yl)-2-[(2*S*,6*S*)-6-methylpiperidin-2-yl]ethanone as the major product and an inseparable mixture of 1-methyldodecahydro-6*H*-pyrido[1,2-*a*]quinolin-6-one isomers as the minor product. Initiating the second IMAMR of 1-(cyclohex-1-en-1-yl)-2-[(2*S*,6*S*)-6-methylpiperidin-2-yl]ethanone was attempted. The epimerisation of 1-methyldecahydropyrrolo[1,2-a]quinolin-5(1H)-one and 1-methyldodecahydro-6*H*-pyrido[1,2-*a*]quinolin-6-one proved unproductive.

The third chapter investigates the synthesis of 2-alkyl-3-(4-oxopentyl)cyclohex-2-en-1-one and their subsequent use in a TA-IMAMR cascade towards the pragmatic synthesis of the natural product histrionicotoxin (HTX) and its derivatives. A Baylis-Hillman reaction was optimised for the insertion of the α-alkyl substituent into the cyclohexanone scaffolds. TA conditions were found to convert the unsubstituted scaffold to provide (2*S*)-2-methyl-1-azaspiro[5.5]undecan-8-one as a 1:1 mixture of diastereoisomers. The optimal epimerisation conditions found provided a 3:1 mixture of diastereoisomers, however, isolation of the compounds proved unsuccessful. Reduction of the carbonyl to provide the core HTX structure was tested. This provided an inseparable mixture of products. Under the biocatalytic reaction conditions, the IMAMR of 2-ethyl-3-(4-oxopentyl)cyclohex-2-en-1-one provided 3-[(4*S*)-4-aminopentyl]-2-ethylcyclohex-2-en-1-one as the major product. This is due to the (2*S*)-7-ethyl-2-methyl-1-azaspiro[5.5]undecan-8-one being an unfavourable product, which is in agreement with current literature.

# Acknowledgements

I would first like to thank Dr Elaine O'Reilly for providing me with the opportunity to undertake my PhD, her subsequent supervision, and for making the past three years not only successful, but enjoyable. Herein I also wish to thank Dr Vittorio Caprio and Dr Beatriz Maciá, for sharing their wealth of knowledge and experience, and for supporting me in all my endeavours. I would further like to take this opportunity to wish their new families all the happiness in the world.

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# Abbreviations

| | |
|---|---|
| $[\alpha]_D$ | Specific rotation, specific activity |
| API | Active pharmaceutical ingredients |
| ARA | Asymmetric reductive amination |
| COD | 1,5-Cyclooctadiene |
| *d.e.* | Diastereomeric excess |
| *d.r.* | Diastereomeric ratio |
| DMF | Dimethyl formamide |
| DMSO | Dimethyl sulfoxide |
| *e.e.* | Enantiomeric excess |
| FDA | Food and Drug Administration |
| GABA | γ-Aminobutyric acid |
| GC | Gas chromatography |
| HEPES | 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid |
| HPLC | High performance liquid chromatography |
| HTX | Histrionicotoxin |
| HWE | Horner-Wadsworth-Emmons |
| IMAMR | Intramolecular aza-Michael reaction |

| | |
|---|---|
| IPA | Isopropylamine |
| MAO | Monoamine oxidase |
| PLP | Pyridoxal-5’-phosphate |
| PMP | Pyridoxalamine-5’-phosphate |
| NMR | Nuclear magnetic resonance |
| TA | Transaminase |
| TBAI | Tetrabutylammonium iodide |
| TFE | 2,2,2-trifluoroethanol |
| THF | Tetrahydrofuran |
| Tol | Toluene |

# 1. Introduction

## 1.1 Alkaloids

The alkaloids are a group of secondary metabolites that contain one or more nitrogen atoms, often incorporated into a (hetero)cyclic system(s). Originally, they were categorised as occurring predominantly in plant species, however it is now known that they are ubiquitous to all terrestrial and marine dwelling organisms. Their diverse structures and biological properties have fascinated both chemists and biologists, which has led to alkaloids being one of the most intensely investigated class of natural products.[1,2]

Humans have exploited the biological activity of alkaloids for centuries.[1] This includes their application as traditional medicines, toxins and the modern day substances of abuse: for instance, the well-known alkaloids cocaine and nicotine (Figure 1). Natural products are unique in the roles they play in biological systems, in which evolution has designed them to bind selectively to proteins such as enzymes and receptors. Nature has provided chemists with a catalogue of inherently selective scaffolds, which often prove to be leads for potential drug candidates.[3,4] In general, nitrogen heterocycles are invaluable to the pharmaceutical industry, appearing in 59% of FDA approved unique small-molecule drugs.[5]

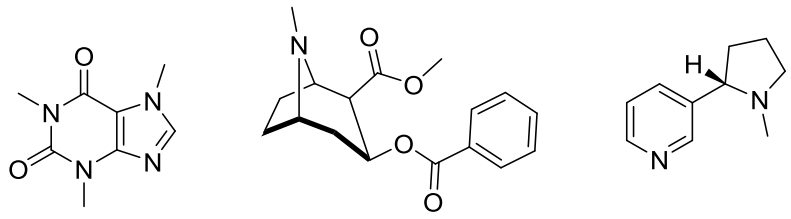


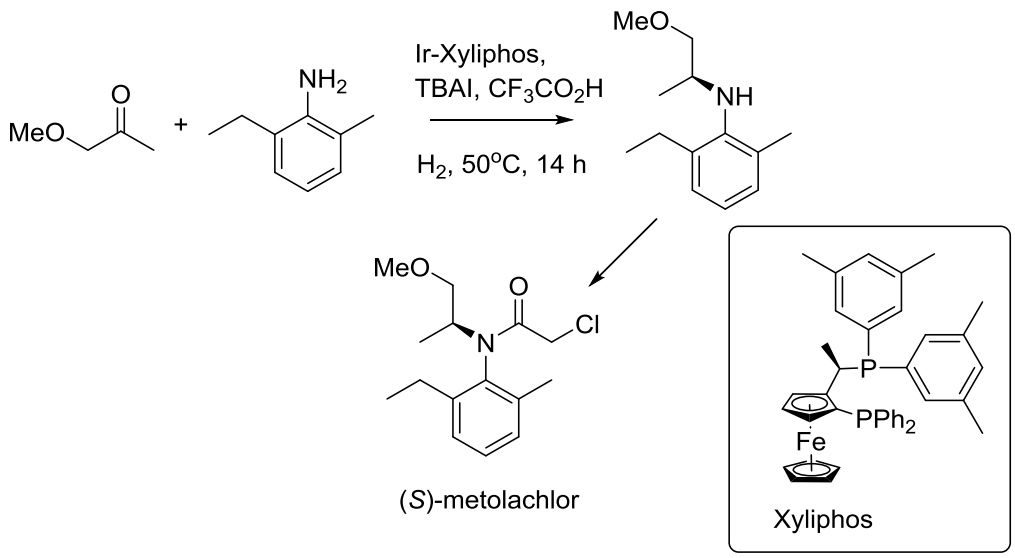

*Figure 1: Common natural alkaloids*

In order to perform biological testing of alkaloid natural products, they must be isolated in a reasonable quantity. Extraction from the original source is often deemed complicated, expensive and can be prohibited due to the protection of an endangered species. Biosynthetic pathways to natural products can be derived from identifiable amino acids, however, producing these reactions in a laboratory with enzymes is far from trivial. Therefore, there is a clear need for the development of new synthetic methods to access alkaloid natural products and their derivatives in an accessible manner. One of the greatest challenges in total synthesis is to prepare a natural product target stereoselectively whilst being applicable on a large scale.[6] To this end, organic chemists must endeavour to explore novel asymmetric methodologies, for the synthesis of chiral amines, which are the most straightforward precursors for these valuable *N*-containing heterocycles.

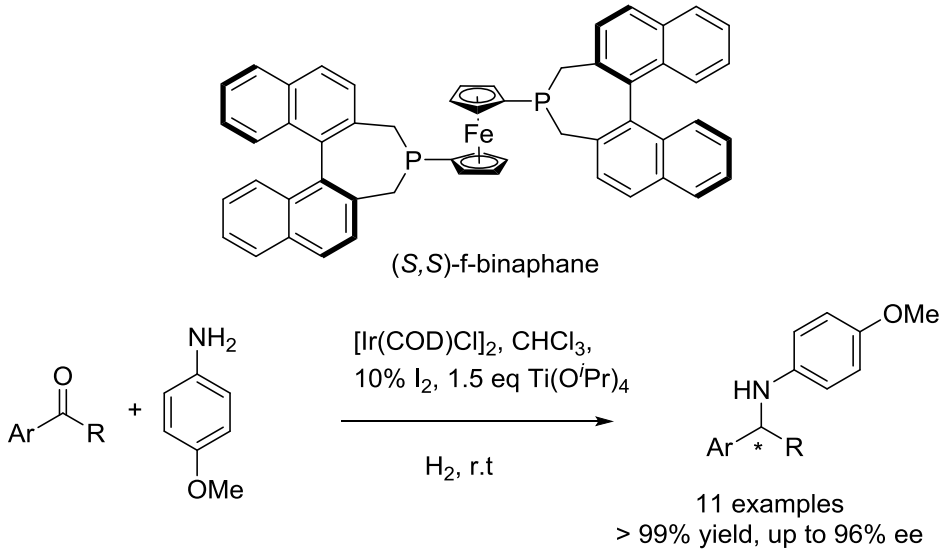
## 1.2 Asymmetric synthesis of amines via reductive amination

An effective method for installing chiral amine functionality is via asymmetric reductive amination (ARA) of a carbonyl group. This procedure involves the *in situ* formation of an imine and the use of a chemoselective catalyst that can selectively reduce the imine to afford the desired amine, whilst circumventing any potential carbonyl reduction.[7]

The hydrogenation of an imine intermediate utilising transition metal catalysts and chiral ligands offers a potential route for performing an ARA, however, the amine products can often lead to catalyst poisoning, producing certain limitations.[8] Blaser *et al.* were the first to demonstrate the use of an enantioselective reductive amination for the synthesis of (*S*)-metolachlor (Scheme 1).[9] This methodology utilises an iridium catalyst in the presence of a chiral diphosphine ferrocene ligand, xyliphos. Zhang *et al.* expanded the scope of the iridium catalysed hydrogenation of imines by using (*S,S*)-binaphane as the ligand.[10] The addition of the Lewis acid titanium isopropyloxide accelerates the formation of the imine, which results in complete conversion to the desired chiral amines with ≤ 96% *e.e.* (Scheme 2).




*Scheme 1: Synthesis of (S)-metolachlor* via *asymmetric reductive amination*

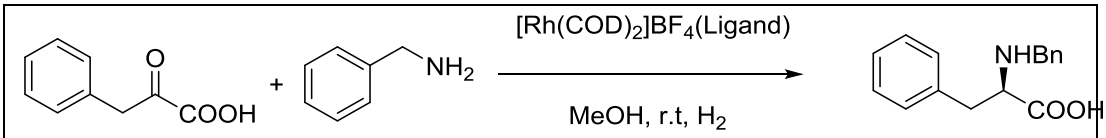


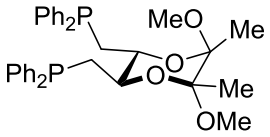
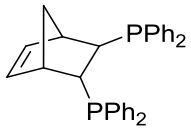
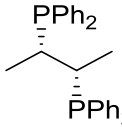
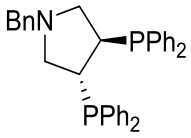

*Scheme 2: Ir catalysed reductive aminations by Zhang*[10]

Moving away from iridium, in 2000, Borner *et al.* demonstrated that a chiral rhodium (I) catalyst can perform an ARA reaction, diverging from aniline as the amine source to the more reactive benzylamine.[11] Their initial results demonstrated moderate selectivity between the imine and carbonyl hydrogenation reaction, however, the exchange of the achiral ligands dppb and dpoe to a chiral ligand allows for an ARA

reaction (Table 1, entry 1). Borner *et al.* then developed a high-throughput screening method, testing 96 chiral phosphorus based ligands, to optimise the rhodium catalysed asymmetric reductive amination procedure.[12] The best results are achieved with diphenylphosphino ligands (Table 1, entry 2-4), due to the proposed formation of five-membered chelate intermediates.

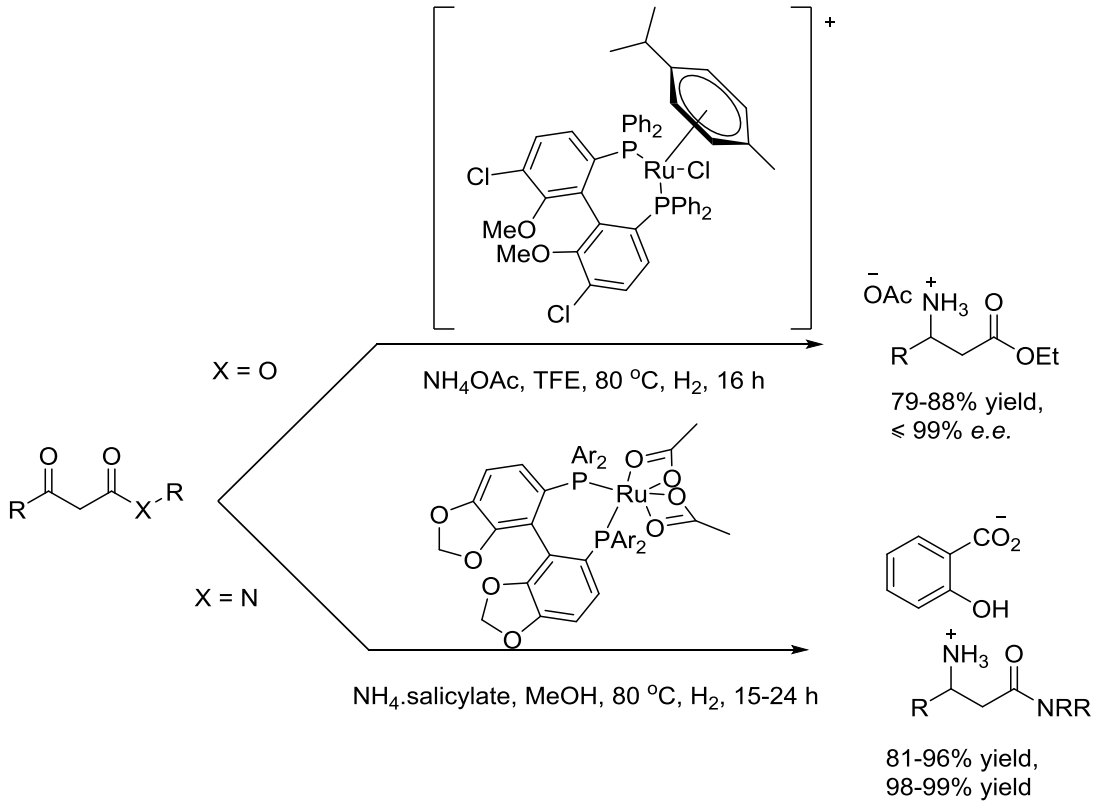
*Table 1: Rhodium catalysed reductive amination developed by Borner*[11,12]




| Entry | Ligand | Yield (%) | *e.e.* (%) |
|---|---|---|---|
| 1 |  | 59 | 38 (*R*) |
| 2 |  | 99 | 95 (*S*) |
| 3 |  | 99 | 91 (*R*) |
| 4 |  | 98 | 92 (*S*) |

Inspired by work carried out at Merck on the chemoselective hydrogenation of unprotected enamines, in the presence of an amide/ester group,[13]

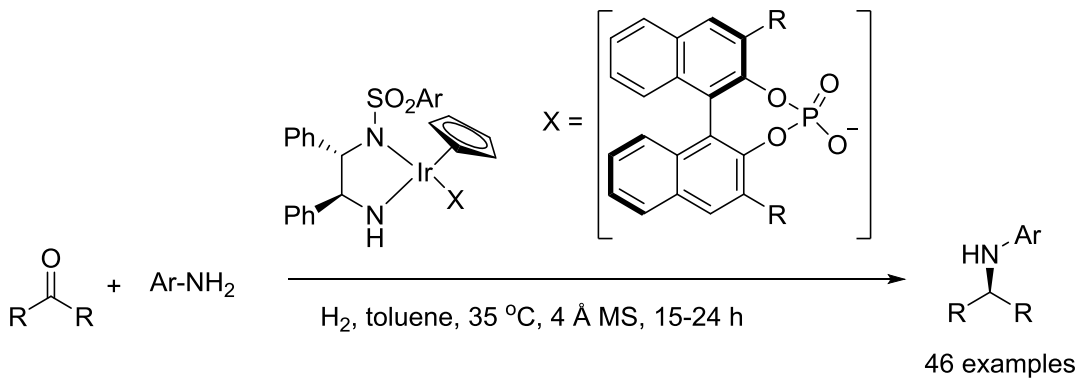
Bunlaksananusorn *et al.* developed a ruthenium catalysed ARA of β-keto esters.[14] The use of a diphenylphosphino ligand results in excellent chemo- and stereoselectivity, providing chiral β-amino esters in excellent *e.e.* and good yield (Scheme 3). Later, Merck reported the chemoselective ruthenium catalysed ARA of β-keto amides, using ammonium salicylate as the ammonia source (Scheme 3).[15]




*Scheme 3: Chemoselective ARA of dicarbonyl substrates by Bunlaksananusorn*[14]

Inspired by the organocatalytic ARA promoted via a cooperative catalyst system of Hantzsch esters and a chiral phosphoric acid described by MacMillan *et al.*[16] and List *et al.*[17] (*Scheme 7*, Scheme 8), Xiao *et al.* developed a metal-Brønsted acid equivalent.[18] The use of a diamine-ligated iridium catalysts and a phosphoric acid provides excellent yield and selectivity in the hydrogenation of acyclic imines across

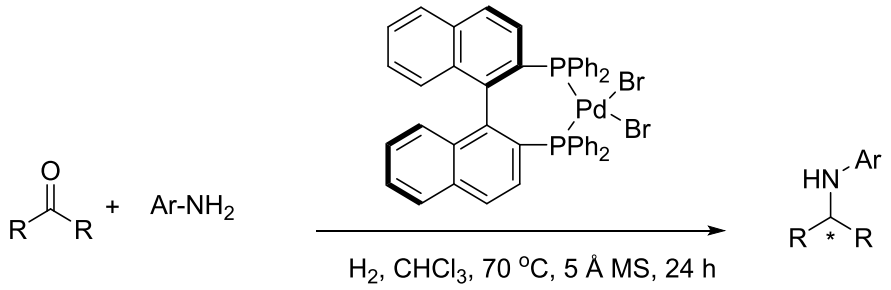
a broad range of substrates. A mechanistic study of this reaction concluded that the formation of noncovalent interactions, provides a highly ordered active catalytic species, which allows the selective transfer of the hydride ion.[19] The direct ARA of carbonyl compounds was then described;[20] where the phosphoric acid has a dual catalytic effect, catalysing both the *in situ* imine formation and its selective reduction. The process provides excellent yields and selectivity for a broad range of aromatic and aliphatic carbonyl substrates (Scheme 4).



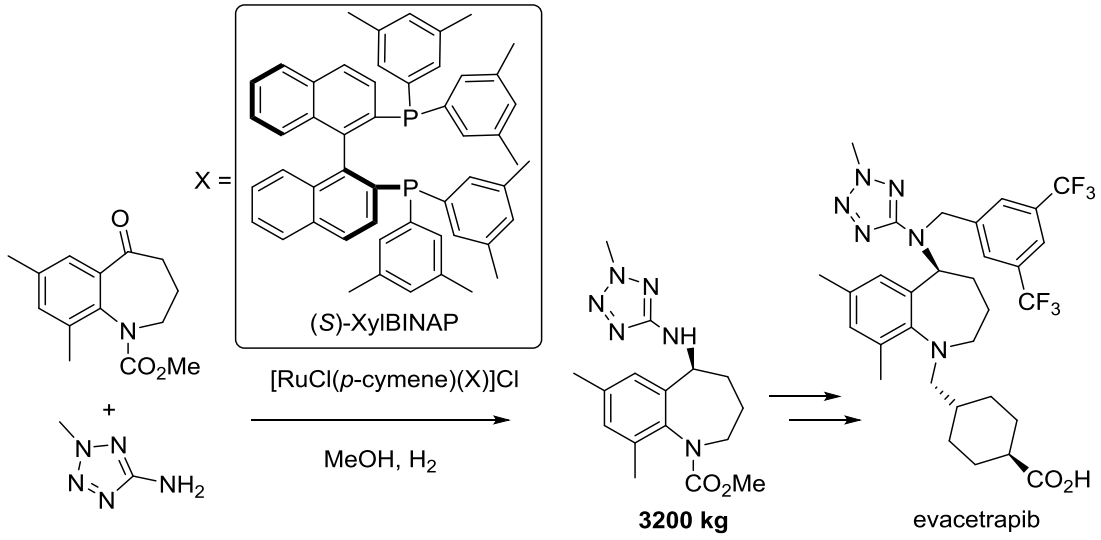

*Scheme 4: Cooperative metal-Brønsted acid catalysed ARA*

Finally, Rubio-Pérez *et al.* employed a palladium species as a catalyst for the ARA of ketones (Scheme 5).[21] In contrast to previous reports, the reaction proceeds with low selectivity for aromatic ketones, but aliphatic ketones provide very good yields and enantioselectivites. Although a wide range of methods have been developed to effect by catalytic hydrogenation, the majority of the highlighted processes are ineffective or poorly suitable for large-scale preparations. Many of the existing methods are superseded by an indirect reductive amination (where the imine species must be pre-formed and previously isolated), to circumvent complicated reactions and sophisticated metal complexes. To challenge this, Changi *et al.* demonstrated

that an ARA can be utilised as an industrial process, for the synthesis of the cholesteryl ester transfer protein inhibitor evacetrapib (Scheme 6). Their report encompasses extensive optimisation via testing of the reaction parameters and the development of mathematical models.[22]



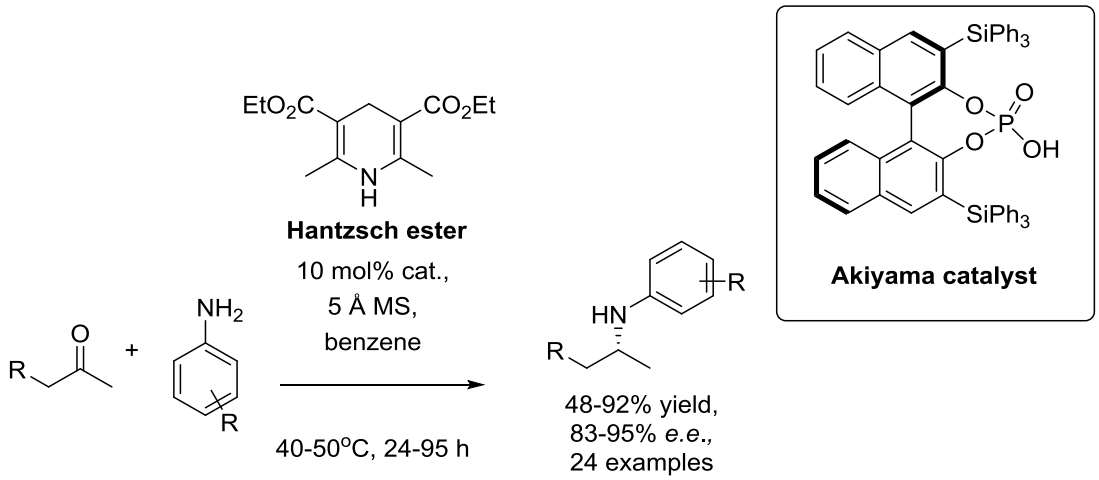

*Scheme 5: Palladium catalysed ARA*




*Scheme 6: The utilisation of an ARA for the synthesis of evacetrapib*

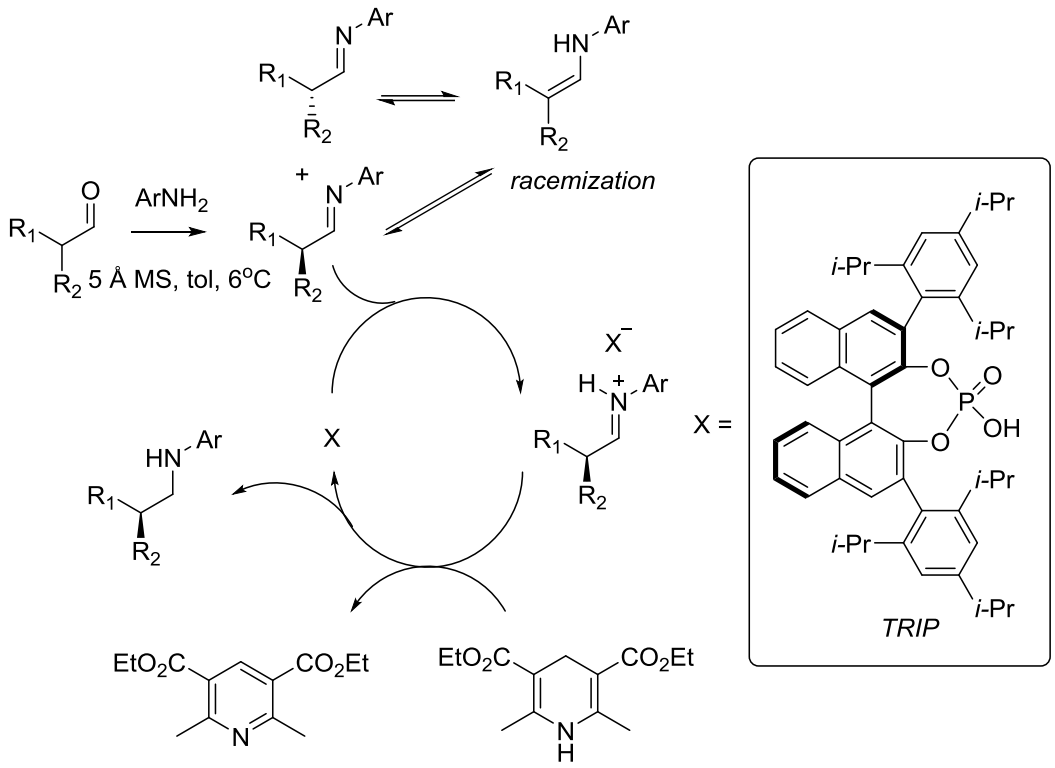
An alternative process that avoids the use of expensive and precious metals is the organocatalytic ARA. This was first achieved by MacMillan *et al.*. Their report highlights the use of a Hantzsch ester in combination with a modified Akiyama chiral

hydrogen-bonding catalyst to provide chiral amines from the corresponding ketones (*Scheme 7*).[16] The formation of water arising from imine condensation has an adverse effect on the overall process, hindering the overall imine formation and the hydride reduction step. Therefore, the incorporation of 5 Å sieves is deemed vital for attaining practical yields and selectivities.



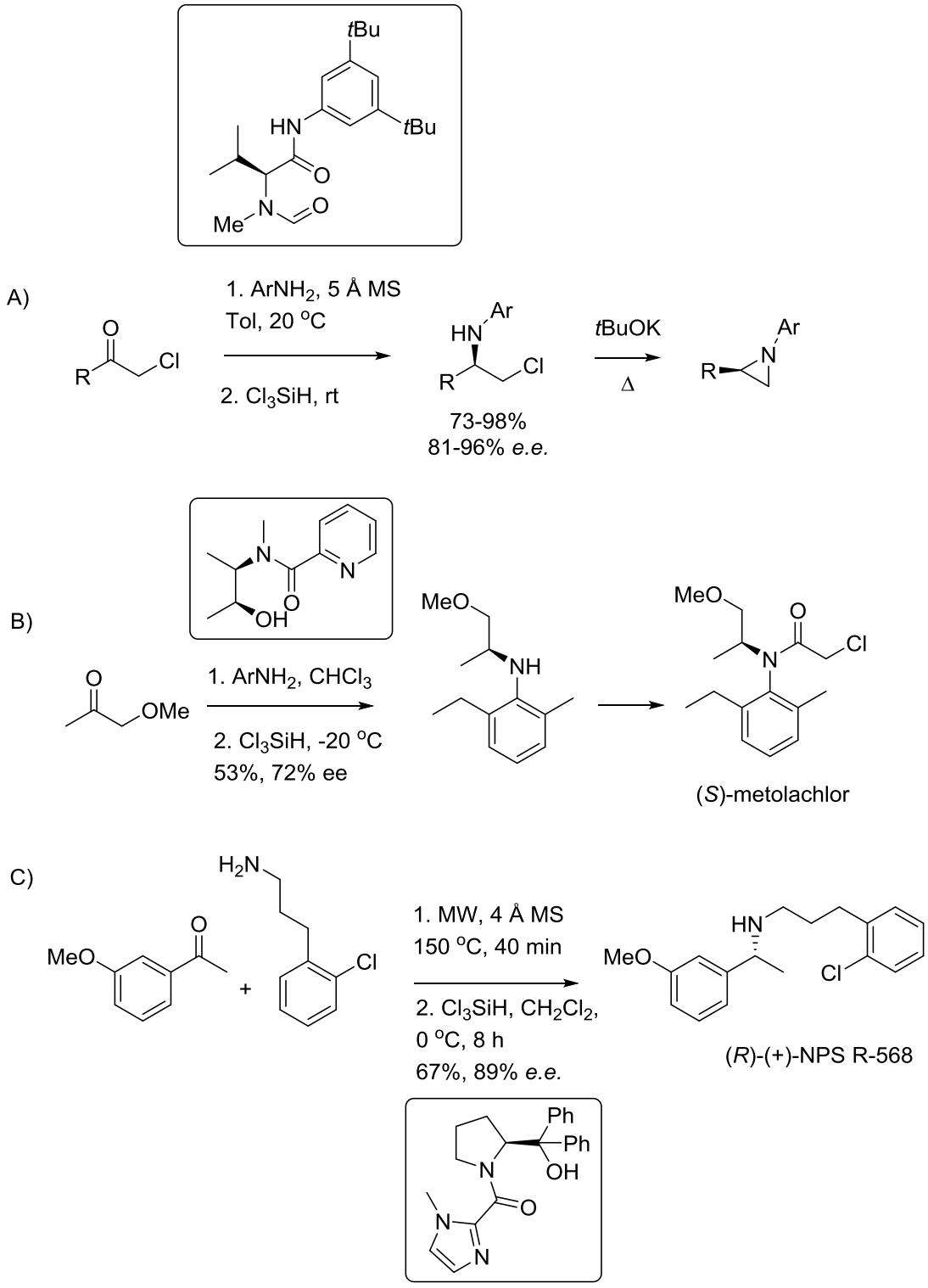

*Scheme 7: Organocatalytic ARA of ketones by MacMillan*[16]

In a similar fashion, List *et al.* developed a procedure for the organocatalytic ARA of aldehydes.[17] Using a chiral phosphoric acid as the catalyst, racemic α-branched aldehydes - in the presence of *p*-anisidine and a Hantzsch ester - afford β-branched secondary amines in excellent yields and enantioselectivities via an efficient dynamic kinetic resolution (Scheme 8).




*Scheme 8: Asymmetric reductive amination of aldehydes by List*

A Lewis basic formamide (derived from *N*-methylvaline), in combination with trichlorosilane, has also been utilised in an efficient organocatalytic reductive amination of α-chloroketones, for the synthesis of chiral aziridines.[23] Trichlorosilane is employed to selectively catalyse the reduction of the prochiral *N*-aryl ketimines (Scheme 9A). Benaglia *et al.* investigated this methodology further and reported 29 chiral Lewis bases that provide good catalytic activity in this process.[24] Using their most selective catalyst the synthesis of (*S*)-metachlor was achieved in comparable selectivity and yield to the iridium catalysed methodology by Blaser *et al.* (Scheme 9B). Jones *et al.* reported that pre-formed imines, synthesised from aliphatic amines via microwave irradiation, can subsequently be reduced with a chiral Lewis base and trichlorosilane. This methodology has been applied to the synthesis of the calcimimetic drug, (*R*)-(+)-NPS R-568 (Scheme 9C).[25]

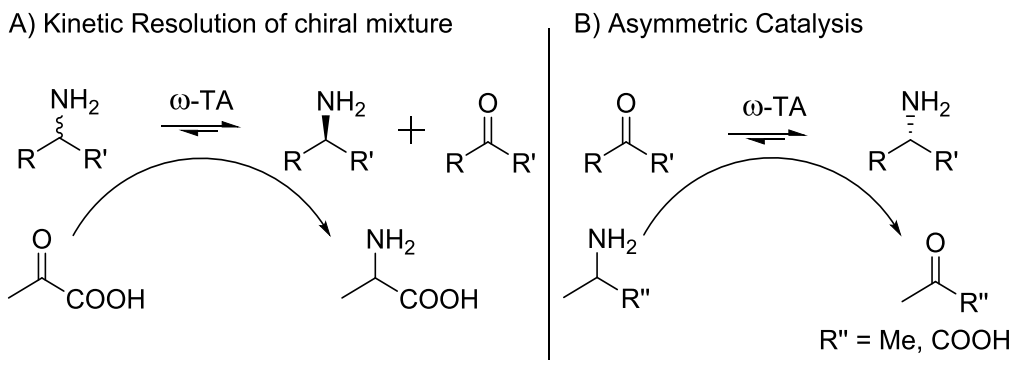



*Scheme 9: Organocatalysed reductive amination with trichlorosilane*

## 1.3 ω-Transaminases in synthesis: Biocatalytic reductive amination

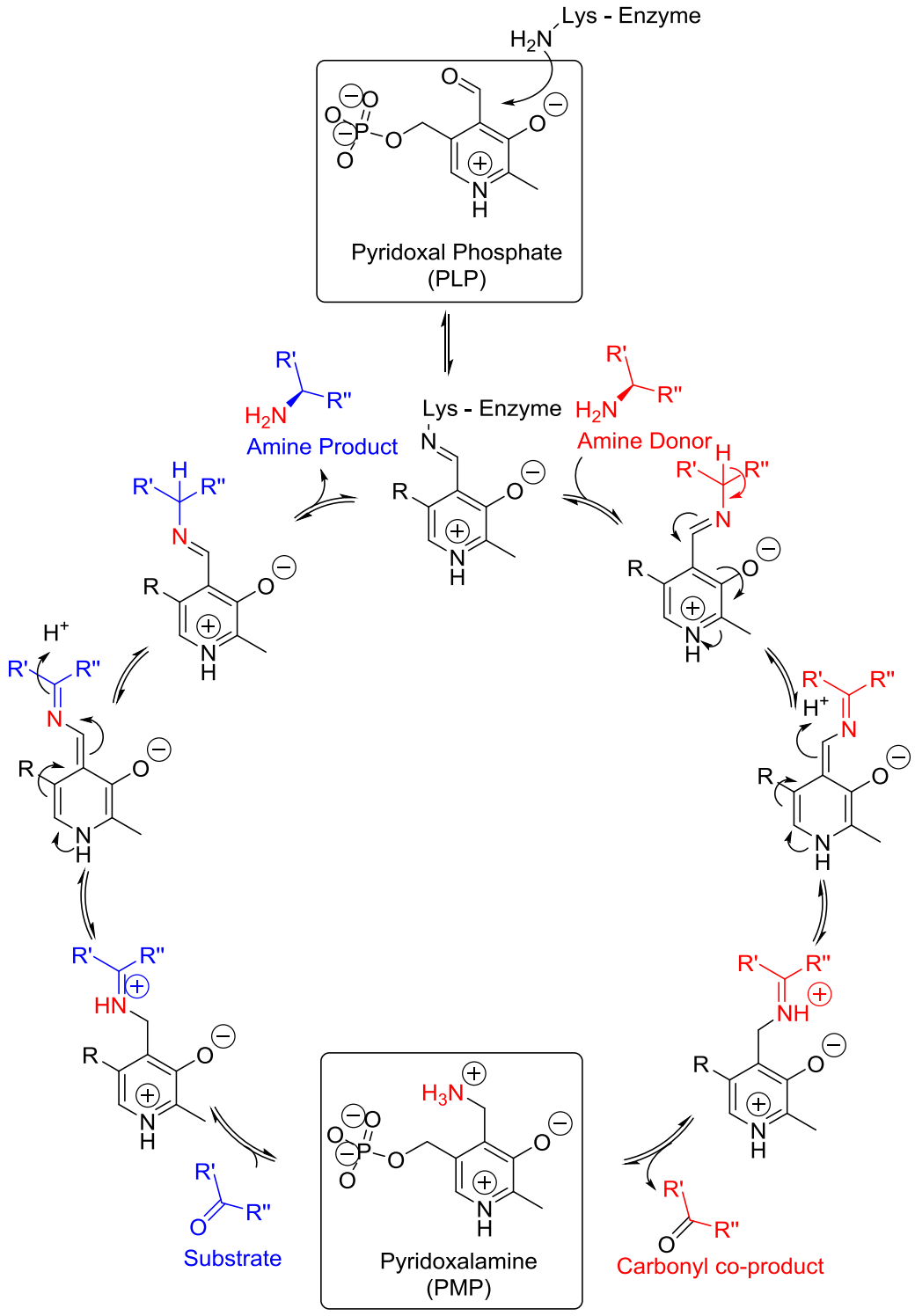
Recent publications have demonstrated the use of biocatalysis to insert chiral amine functionality, supplementing the existing organo- and transition metal-based catalytic methodologies.[26] Biocatalysts can perform a number of transformations with levels of regio-, stereo- and/or chemoselectivity that current chemo-catalysts cannot achieve. This has led to the concept of "biocatalytic retrosynthesis",[27] which provides a variety of new tools to approach certain synthetic disconnections and functional group interconversions that were previously implausible.

In particular, transaminase (TA) enzymes are of significant interest due to their ability to catalyse the reversible reaction between a prochiral carbonyl compound (amino acceptor) and an amine source (amino donor), in the presence of its cofactor pyridoxal-5'-phosphate (PLP), to form the desired chiral amine with both high stereo- and regioselectivity. The use of the α-TA family as catalysts for the synthesis of chiral α-amino acids, is a well establish process, however ω-TA have only recently been highlighted for their ability to also catalyse reactions with ketone and aldehyde functionalities.[28] Celgene was the pioneering biopharmaceutical company to report the use of (*R*)- and (*S*)- selective ω-TA for the production of chiral amines via both a kinetic resolution of a racemic amine mixture (Scheme 10A) and the enantioselective synthesis of amines from prochiral ketones (Scheme 10B).[29]




*Scheme 10: Transaminase utility in the production of chiral amines*

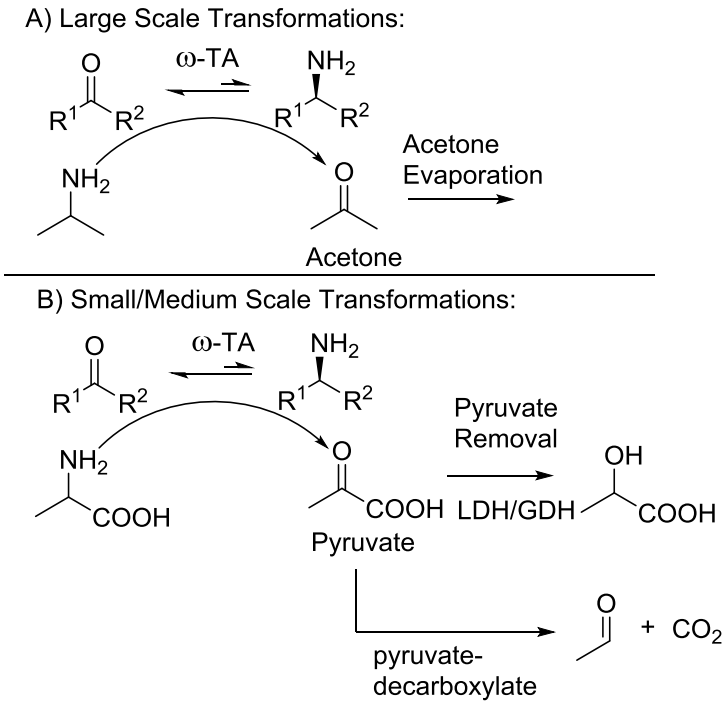
The reaction proceeds via the cofactor PLP that undergoes a reversible prototropic tautomerization followed by hydrolysis with an amino group to form the intermediate pyridoxalamine (PMP), effectively acting as a transport for the amine group and electrons between amine donor and acceptor (Scheme 11).




*Scheme 11: The reaction mechanism of the cofactor PLP with amines and carbonyls within a ω-TA active site.*

A major obstacle in the use of TAs as catalysts for the production of chiral amines is that the reaction thermodynamics are often unfavourable. However, troublesome

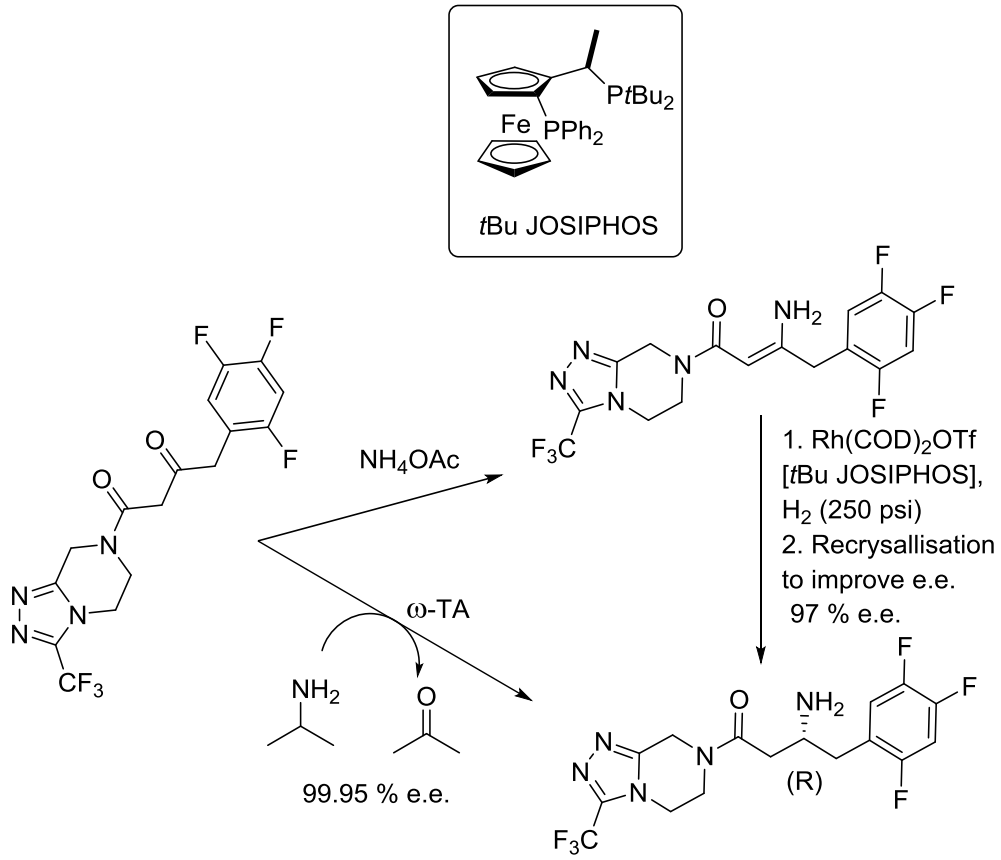
reaction equilibria can be overcome by, for example, the removal of the carbonyl co-product formed upon transamination. Thus, in large scale reactions, the use of an excess of isopropylamine as amine donor, and *in-situ* evaporation of acetone co-product, drives the equilibrium towards the desired chiral amine product. Industrially, this approach is very effective for some substrates, although technically challenging, as the concentration of isopropyl amine has to be closely controlled (typically under 1 M) to prevent adverse effects on the TA (Scheme 12A).[30] An alternative approach to displace the equilibrium towards the desired amine, which is commonly applied for small-scale reactions, consists of the use of L-alanine as amine donor in combination with a pyruvate removal co-enzyme (Scheme 12B). Although this provides an elegant way to drive reaction equilibrium, coenzymes are often expensive and the possibility of them causing undesired side reactions hinders their use in large-scale reactions.[31,32]




*Scheme 12: A) In situ acetone removal technique, B) Pyruvate removal system*

The recently reported synthesis of the anti-diabetic sitagliptin demonstrates how biocatalysis can now compete with contemporary chemocatalytic procedures.[33] The traditional synthesis of sitaglipin, reported by Merk Pharmaceuticals in 2009, consists of the hydrogenation of the enamine derivative from the dicarbonyl intermediate (*Scheme 13*). A rhodium catalyst with a bulky Josiphos diphosphine ligand, under high hydrogen pressure, allows the formation of the desired chiral amine in 97% *e.e.* after recrystallisation.

In contrast, the biocatalytic strategy developed by Codexis and Merck (*Scheme 13*) involves the use of an engineered enzyme (produced after 27 rounds of mutagenesis from a wild-type TA) that was capable of accepting the bulky/bulky-substituted dicarbonyl.[34] This biotransformation route provides a more economical and safer approach by removing the need for an expensive high-pressure hydrogen reactor and toxic and expensive rhodium catalyst and ligand. The biocatalytic methodology provides an approximate 10-13% increased overall yield, 53% increase in productivity (kg $L^{-1}$) and a 19% decrease in total waste. This remarkable outcome received the Presidential Green Chemistry Award in 2010, and in 2012, the Food and Drug Administration (FDA) approved the biocatalytic synthesis of sitagliptin.



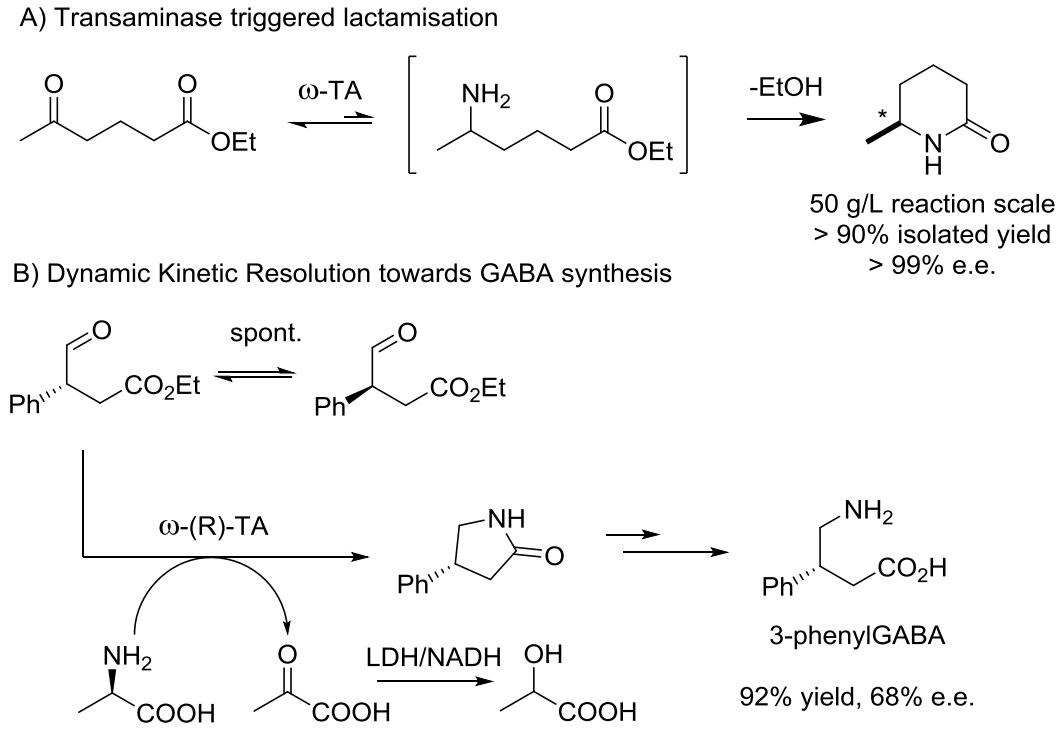

*Scheme 13: Chemocatalytic vs enzymatic synthesis of sitagliptin*

## 1.4 TA applications for the synthesis of chiral alkaloids

Turner was the first to report the incorporation of TA enzymes as part of a cascade process, utilising a keto-ester system where, upon transamination, spontaneous lactamisation occurs, allowing access to a variety of chiral lactam products in excellent *e.e.* (*Scheme 14*A).[35] The irreversable spontaneous cyclisation provides an equillibrium drive towards the formation of the desired product, negating the need for a pyruvate removal system. Additionally, TA enzymes can often be inhibited by high concentration of starting materials and reaction products, which can occupy the enzyme's active site, reducing the catalytic turnover and, therefore, hampering the implementation of these procedures on a preparative/industrial scale. However, the spontaneous cyclisation of the amine product helps to overcome this obstacle,

making the methodology suitable on a 50 g/L scale, therefore deemed suitable as an industrial process.

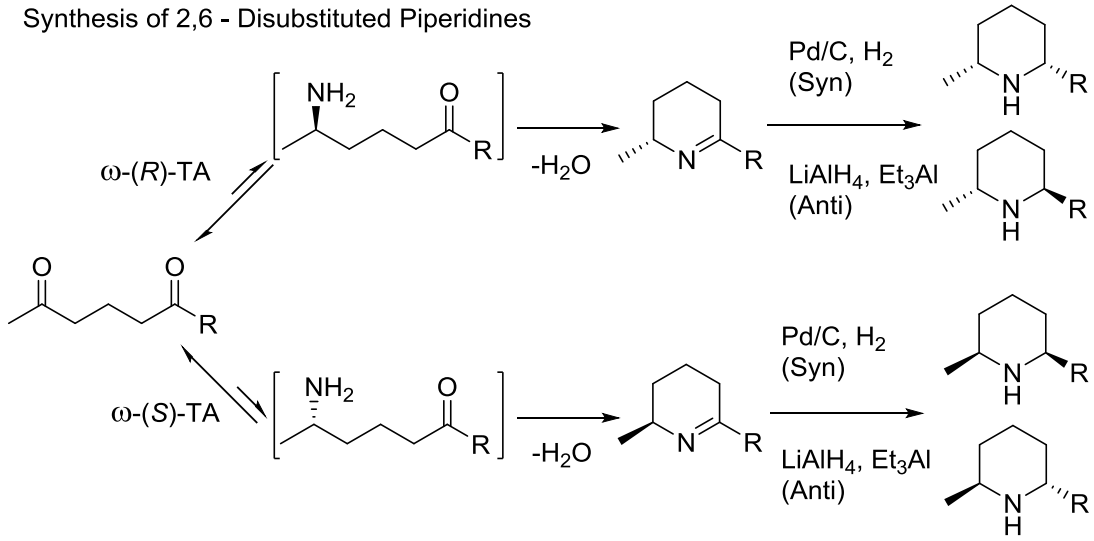
This methodology was then elegantly applied to access a range of biological relevant molecules, a collection of γ-aminobutyric acid (GABA) derivatives, in a dynamic kinetic resolution (*Scheme 14*B). When Kroutil subjected 4-oxo-3-phenylbutyric acid ethyl ester to an (*R*)-selective ω-TA, the enzyme displayed a preferential selectivity towards one of the enantiomers to undergo transamination and subsequent cyclisation.[36] As the substrate readily racemised, over a period of time, all of the starting material was converted and a good *e.e.* was observed in all the GABA products (*Scheme 14*B).




*Scheme 14: Spontaneous lactamisation towards chiral products*

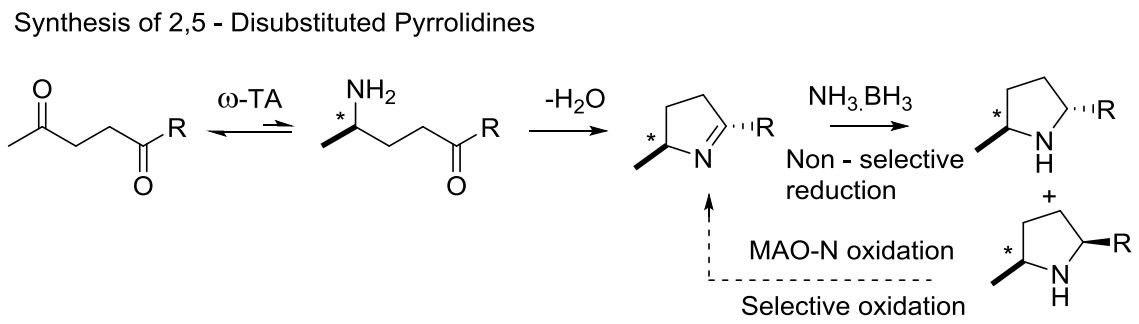
The ensuing example highlights the excellent regioselectivity of ω-TA enzymes (Scheme 15).[37] This two step synthesis of 2,6-disubstituted piperidines, developed

by Kroutil, involves the selective conversion of a single ketone group in a number of diketone substrates. Exposure of these substrates to either an (*R*) or (*S*) – selective ω-TA converts the least hindered keto site, with complete selectivity, into the corresponding chiral amine intermediates, providing the corresponding enantiopure cyclic imines upon spontaneous cyclisation. These imines can then be isolated and selectively reduced in a *syn* or *anti* manner to all four diastereomers of the desired 2,6-disubstituted piperidines (Scheme 15).




*Scheme 15: Two-pot TA method for the synthesis of piperidines*

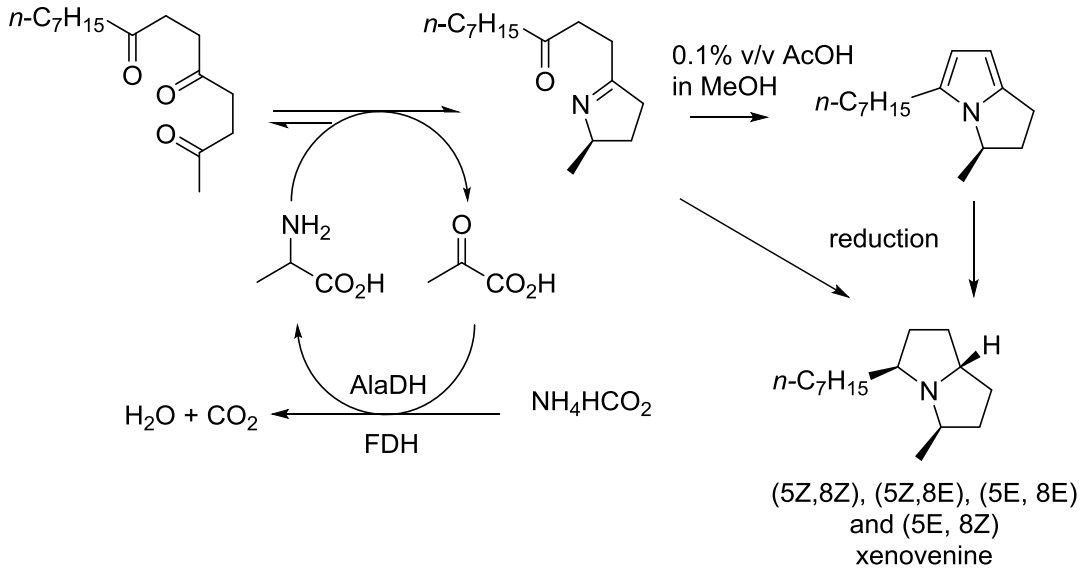
Turner *et al.* have also reported an elegant multi enzyme one-pot reductive system, utilising a selective monoamine oxidase (MAO) in combination with a non-selective ammonia borane reduction system, to generate chiral pyrrolidines with excellent enantio- and diastereoselectivity (>94% *e.e.* and >98% *d.e.*).[38] The report highlights how a multienzyme cascade can work in complete synergy, having no adverse effect on the regio- and stereoselectivity of the corresponding enzyme (Scheme 16).




*Scheme 16: One-pot TA method for the synthesis of pyrrolidines*

The most recent report on the topic, highlights both the excellent regioselectivity and the ability for enzymes to accept bulky and complex unnatural substrates (Scheme 17).[39] The triketone substrate undergoes selective monoamination and subsequent condensation to form the expected enantioriched pyrroline. Aromatisation of the pyrroline with catalytic acetic acid provides the corresponding pyrrole. Again, the selective reduction of either the pyrroline or the pyrrole systems provides the

corresponding *syn* or *anti* products, allowing access to four isomers of the natural product xenovenine, a venom isolated from ants and amphibians.

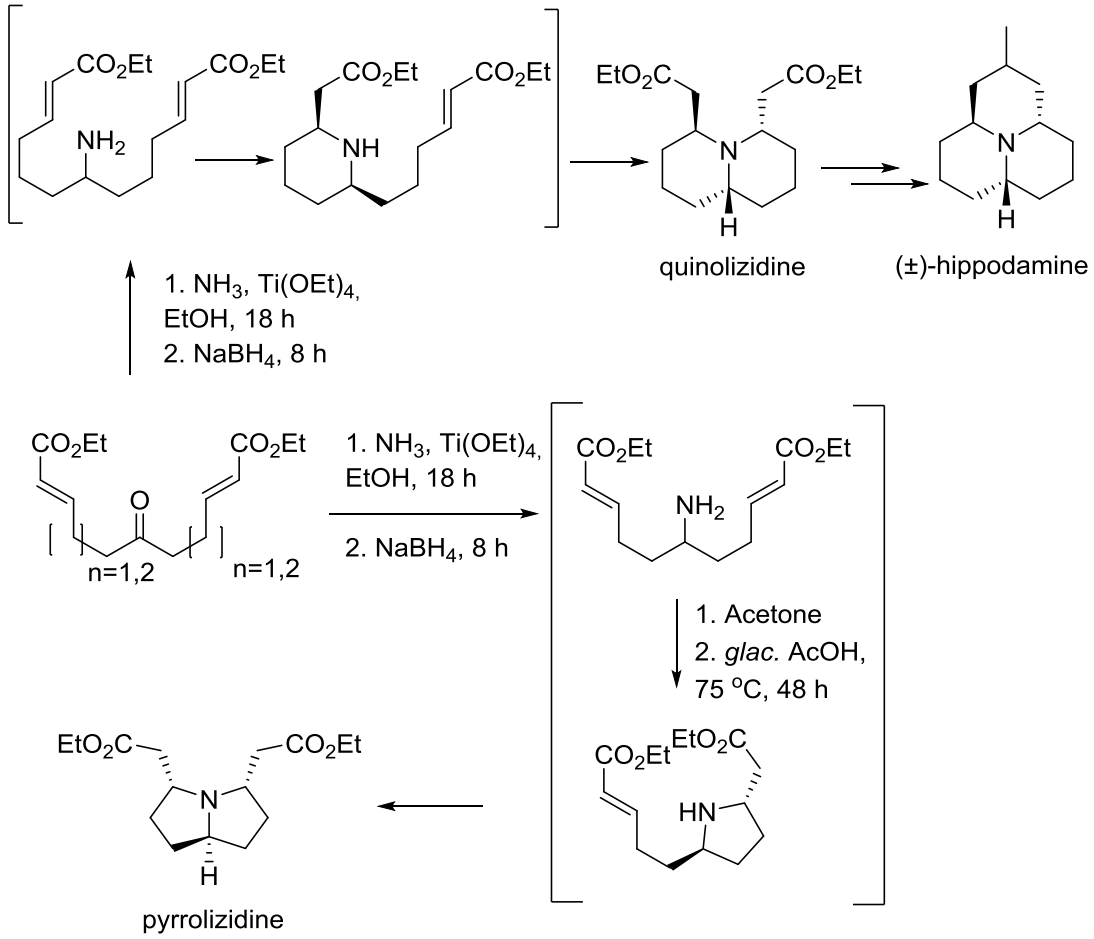



*Scheme 17: Bio-catalysed synthesis of xenovenine*

## 1.5 Intramolecular aza-Michael reaction (IMAMR)

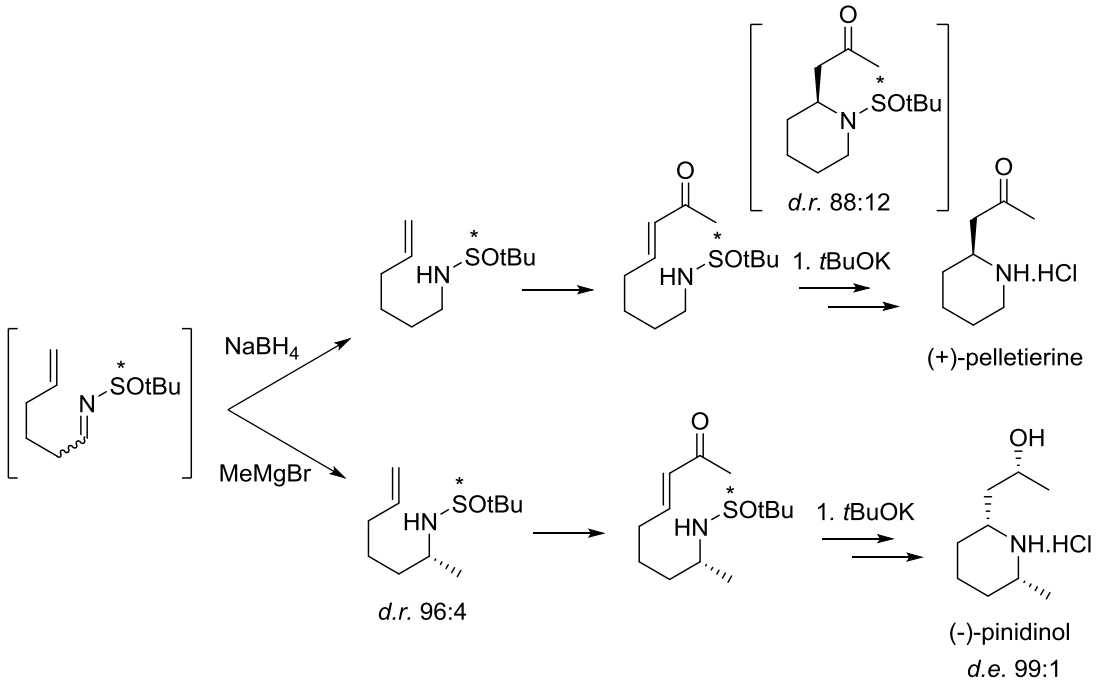
The intramolecular aza-Michael reaction (IMAMR) is one of the most commonly used methods for generating chiral alkaloids.[40] This class of reaction involves the arrangement of a nitrogen nucleophile within close proximity to an electron deficient double bond. Until this point, there have been no reports demonstrating the use of a biocatalyst to insert amine functionality followed by an IMAMR. Previous traditional synthetic methods for performing an IMAMR rely on the synthesis of appropriate precursors, where either a deprotection of the nitrogen or the activation of the α,β-unsaturated system promotes the formation from the desired carbon-nitrogen bond. To induce an enantioselective addition, a chiral catalyst or a chiral nitrogen nucleophile can be employed.[41]

As an example of a traditional method using a non-selective reduction, Stockman *et al.* established a fascinating tandem double intramolecular Michael reaction methodology towards the synthesis of an assortment of quinolizidine[42] and pyrrolizidine alkaloids (Scheme 17).[43] Interestingly, the IMAMR proceeded with relative stereocontrol. For the quinolizidine series, a 2,6-*cis*-configuration is the thermodynamic intermediate, due to the large side chains preferring the equatorial positions. The second addition, again controlled by thermodynamics, arranges the system into a 4,6-*trans*-configuration, due to the *cis*-configuration suffering of steric clashes. The pyrrolizidine series however, proceeds through a 2,5-*trans*-intermediate that allows the formation of *cis*- products.



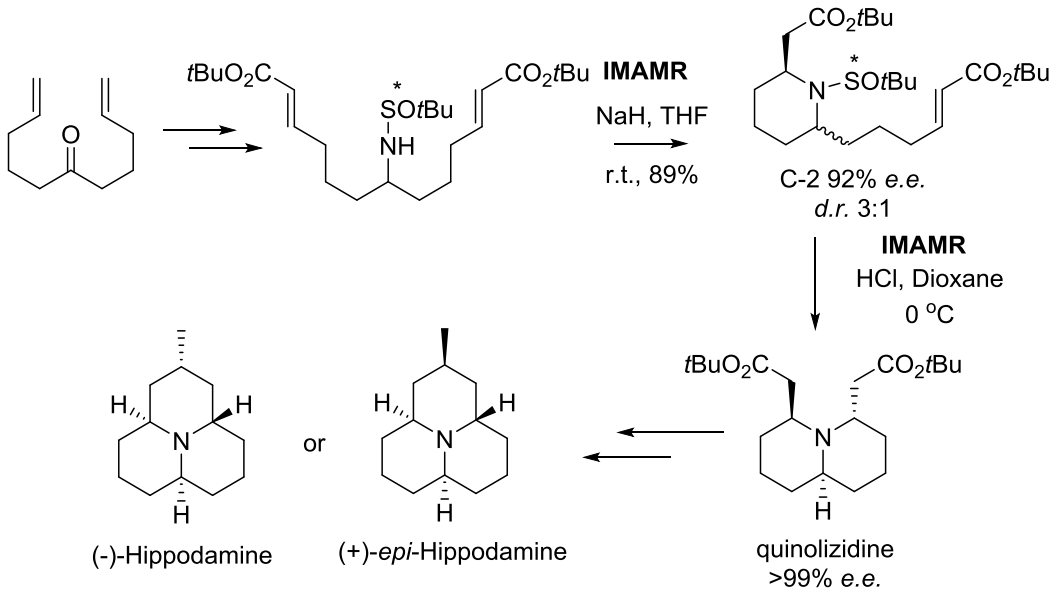

*Scheme 17: Tandem reductive amination/double IMAMR*

The enantioselective synthesis of (+)-pelletierine and (–)-pinidinol has been achieved via an asymmetric IMAMR utilising chiral *N*-sulfinyl amines as the nitrogen nucleophile (Scheme 18).[44] In this report, del Pozo *et al.* describe the use of sufinyl chiral auxiliaries. For the synthesis of (–)-pinidinol, the *N*-sulfinyl amine allows a stereoselective addition of the Grignard.[45] In the presence of potassium *tert*-butoxide at –40 °C, the IMAMR proceeds with good selectivity, providing a novel route to chiral piperidines.



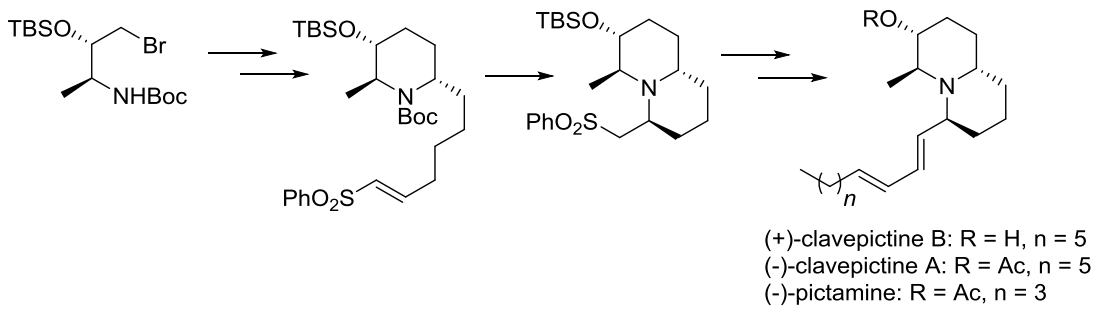

*Scheme 18: N-sulfinyl chiral auxiliary for the synthesis of chiral piperidines*

Fustero *et al.* have shown, in the synthesis of (±)-hippodamine, that the *N*-sulfinyl chiral auxiliary can be used in a similar fashion to Stockman *et al.* (Scheme 17). The *N*-sulfinyl chiral auxiliary allows the first IMAMR to proceed selectively with 92% *e.e.* Separation of the diastereoisomers by flash column chromatography, followed by the removal of the *N*-sulfinyl group, allows the second IMAMR to occur, resulting in the formation of the enantiopure quinolizidine (Scheme 19). Subsequent transformations allow access to enantiopure (–)-hippodamine and (+)-*epi*-hippodamine.[46]



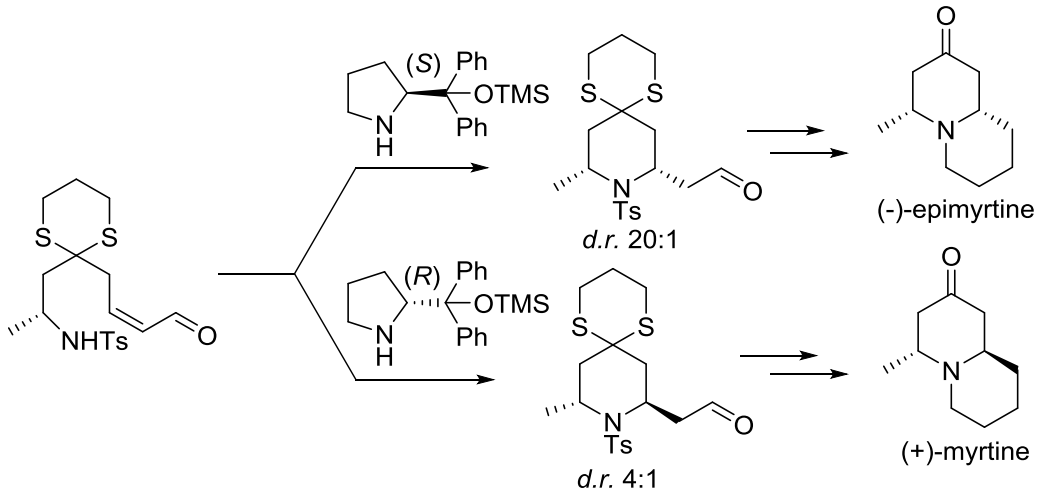

*Scheme 19: Double IMAMR utilising an N-sulfinyl chiral auxiliary*

The use of readily available chiral starting materials is also a useful way to perform a diastereoselective IMAMR. In this way, Ma *et al.* have synthesised three natural products from the protected chiral amino alcohol (Scheme 20).[47] The report highlights that the stereo outcome of the IMAMR was greatly determined by the substituents on the piperidine ring.




*Scheme 20: Diastereoselective IMAMR of a functionalised piperidine ring*

Hong *et al.* have shown that Jørgensen's pyrrolidine is able to catalyse the IMAMR (Scheme 21).[48] The (*S*)-pyrrolidine catalyst provides the *cis* product in a 20:1 *d.r.,* due to the transition state having a complementary alignment, with the bulky pyrrolidine substituent pointing away from the tosylate group. In contrast, the (*R*)-pyrrolidine catalyst points towards the tosylate group, producing an unfavourable transition state, which results in a drop to a 4:1 *d.r.* (Scheme 21). However, this organocatalysed IMAMR allows the access to both (–)-epimyrtine and (+)-myrtine.




*Scheme 21: Jørgensen's pyrrolidine catalysts for the diastereoselective IMAMR*

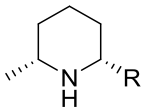
# 2. Biocatalytic Preparation of Chiral Piperidines

The research described in this chapter has been published: J. Ryan, M. Siauciulis, A. Gomm, B. Macia, E. O'Reilly, V. Caprio, *J. Am. Chem. Soc.* **2016**, *138*, 15798-15800.

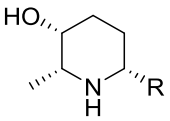
## 2.1 Introduction

The piperidine, 6-membered nitrogen saturated heterocycle, is arguably one of the most observed scaffolds in natural products and is occurring more and more in potential drug candidates.[5] A recent report[49] highlighted that a simple survey of the word "piperidine" on the SciFinder search engine produced 93,984 references in October 2015 and 97,972 references in January 2017. The increase in almost 4,000 additional publications over two years proves how much attention this basic heterocycle has received. A search on the chemical abstracts service corroborates the above analysis by identifying piperidine as the most common heterocycle.[50]

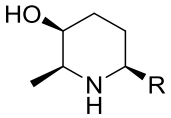
Of particular interest to our group are the piperidine-based natural products that contain a 2,6-*cis*-disubstituted pattern; lupetidine,[51] dihydropinidine,[52] solenopsin A,[51] cassine,[53] spectaline,[54] pinidinol and pinidinone[55] are examples of 2,6-disubstituted piperidines bearing a 2-methyl substituent and exhibit antifeedant properties (Scheme 22).



Lupetidine: R = Me
Dihydropinidine: R = $C_3H_7$
Solenopsin A: R = $C_{11}H_{23}$
Pinidinol: R = $CH_2CH(OH)CH_3$



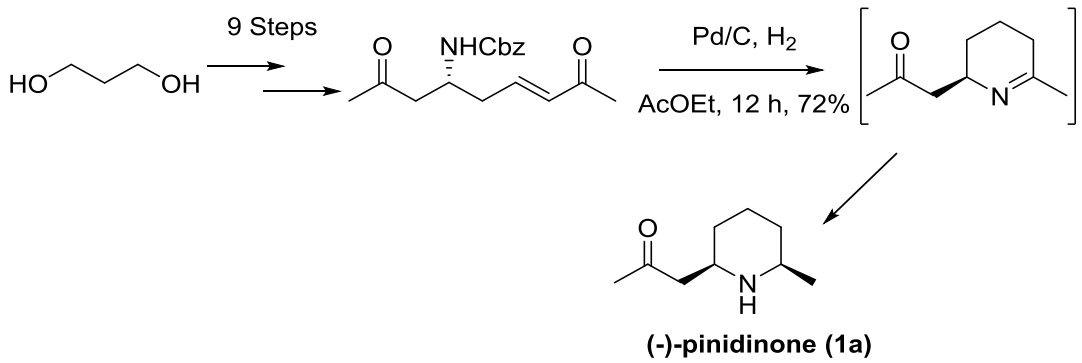
Cassine: R = $C_{10}CH_{20}COCH_3$
Deoxocassine: R = $C_{12}CH_{25}$



Spectaline: R = $C_{12}CH_{24}COCH_3$

*Scheme 22: Examples of natural products containing a 2,6-disubstituted piperidine motif*

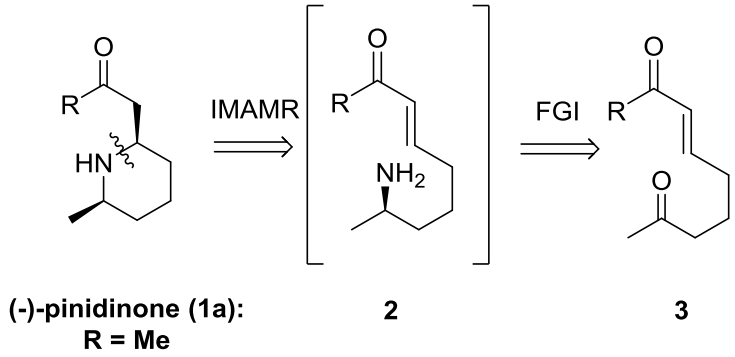
Amongst the naturally occurring 2,6-disubstituted piperidines, the alkaloid (–)-pinidinone (**1a**) is found in pine and spruce trees indigenous to the Americas.[55] It can also be extracted from the Australian ladybug beetle (*Cryptolaemus montrouzieri*)[56] and the Mexican bean beetle (*Epilachna varivestis*).[57] It is a defensive alkaloid, adversely affecting predators upon consumption. Its asymmetric synthesis has been achieved through several routes, which often involve complex and long protecting/de-protecting strategies (approx. average of 10 steps) with poor overall yields (see example in Scheme 23).[58–63]




*Scheme 23: Asymmetric synthesis of (–)-pinidinone in 10 linear steps*[62]

## 2.2 Aims and objectives

Herein we propose a novel methodology utilising TA enzymes to insert a chiral amine functionality that will then undergo an IMAMR as summarised in the retrosynthesis of (–)-pinidinone (**1a**) (R = Me) (Scheme 24). This methodology will allow access to both enantiomers of pinidinone (**1a**) and a number of non-natural 2,6-disubstituted piperidine derivatives.

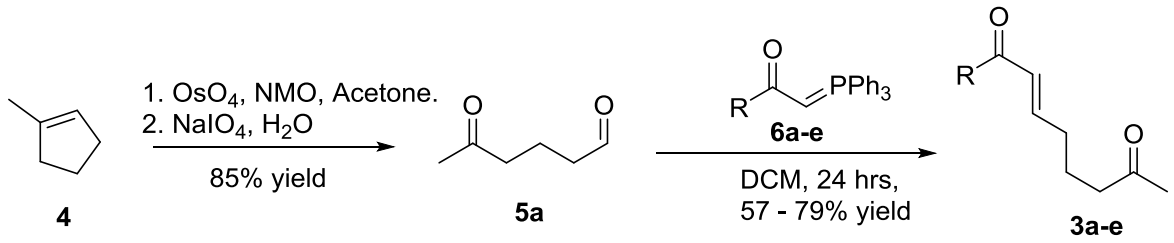



*Scheme 24: Biocatalytic retrosynthetic analysis of (–)-pinidinone*

To achieve this, a number of keto-enone substrates **3** where R comprises a broad range of functionalities are synthesised. Optimisation of a key biocatalytic transformation step of **3** utilising industrially available ω-TA enzymes are tested. The final products are fully characterised to determine their absolute stereochemistry.

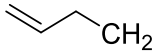
## 2.3 Substrate synthesis: Ketoenones **3a-e**

Synthesis of a few of our desired keto-enone substrates **3** have previously been described by the oxidative ring opening of 1-methylcyclopentene (**4**) by ozone.[64] Looking for an alternative procedure that avoided the ozonolysis reaction, we performed the oxidative ring opening of **4** using osmium tetroxide and sodium periodate (Lemieux–Johnson oxidation),[65] which provided 5-oxo-hexanal (**5**) in 85% yield, with no purification required (Scheme 25). Next, the Wittig olefination of **5** with a range of phosphorus ylids **6a-e**, provided the target ketoenones **3a-e** in good yield (Table 2).



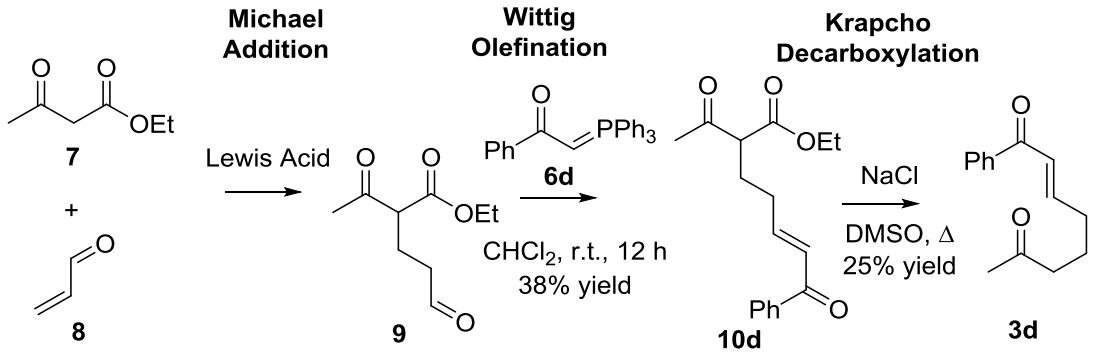

*Scheme 25: Synthesis of target ketoenone substrates **3a-e***

*Table 2: Wittig olefination reaction of **5a***

| Entry | R | Product | Yield (%)[a] |
|---|---|---|---|
| 1 | Me | **3a** | 79 |
| 2 | *t*Bu | **3b** | 58[b] |
| 3 |  | **3c** | 57 |
| 4 | Ph | **3d** | 76 |
| 5 | OEt | **3e** | 66 |

[a] Isolated yield after flash chromatography. [b] Reaction carried out under reflux.

An ideal synthesis would avoid the use of the expensive and toxic osmium catalyst. Therefore, we endeavoured to synthesise the keto-enone **3a-e** in a sustainable manner, based on the commercially available reagent ethyl acetoacetate (**7**) and acrolein (**8**) (Scheme 26). We envisaged that, in the presence of an appropriate catalyst, the two reagents would undergo a Michael addition to provide the Michael adduct **9**. Torregiani *et al.* have reported a solvent free reaction using a cerium catalyst to produce **9** in 95% yield.[66] In a different study, Sanjay et al. reported the Michael addition reaction of **7** and **8** to provide compound **9** in 95% yield, using neutral alumina as catalyst.[67] However, both the methodologies have been reported on a 1 mmol scale, and the scaling up to a 10 mmol scale proved inadequate in both cases (Table 3, entry 1 & 2). Increasing the amount of neutral alumina to 4 eq provides a conversion of 77% (Table 3, entry 3). A screening of different amounts of basic alumina as catalyst provided lower conversions in all cases (Table 3, entry 4-6).




*Scheme 26: Alternative synthetic route to ketoenone **3d***

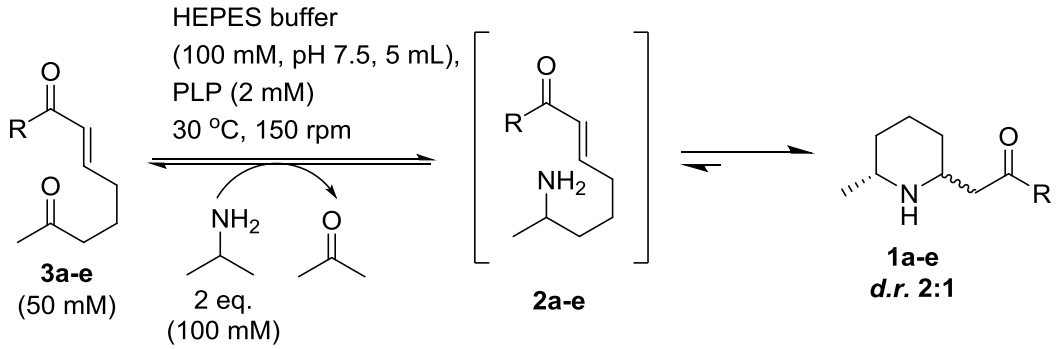
*Table 3: Base catalysed Michael addition of acrolein (**8**) and ethylacetoacetate (**7**)[a]*

| Entry | Catalyst | Catalyst (eq.) | Time (min) | % Conversion[b] |
|---|---|---|---|---|
| 1 | Cerium Heptahydrate | 0.2 | 24 h | n.d |
| 2 | Neutral Alumina | 0.2 | 10 | 10 |
| 3 | Neutral Alumina | 0.4 | 10 | 77 |
| 4 | Basic Alumina | 0.2 | 10 | n.d |
| 5 | Basic Alumina | 0.4 | 10 | 33 |
| 6 | Basic Alumina | 0.4 | 30 | 20 |
| [a] **7** (1.0 eq), **8** (1.1 eq), 0 °C, neat. [b] Conversion determined by $^1$H NMR | | | | |

The Wittig olefination of the crude mixture **9** with **6d**, in $CHCl_3$ as solvent at room temperature, provided compound **10d** in 38% yield after 12 h (Scheme 26). The final step of this synthetic strategy consisted on the Krapcho decarboxylation[68] of compound **10d**, which provided the target compound **3d** in 25% yield (Scheme 26). Overall, this route proved troubling and unviable on a synthetically useful scale due to low yields.

## 2.4 Transamination of ketoenones **3a-e** and epimerisation of resulting 2,6-disubstituted piperidines

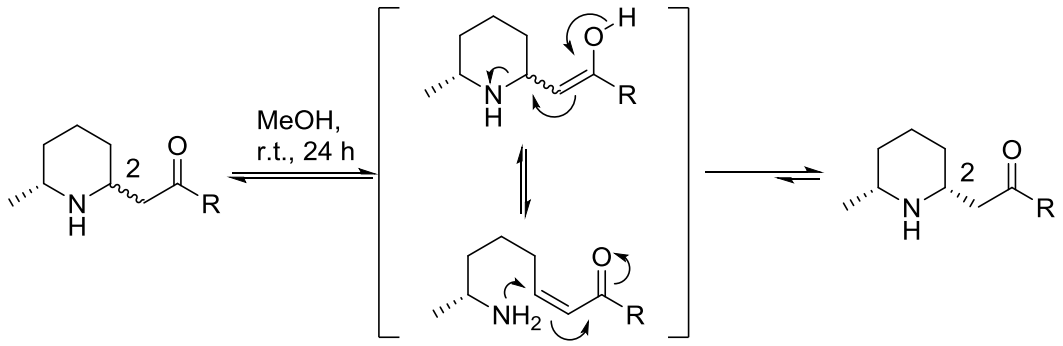
The work of Turner *et al.* outlined in *Scheme 16* utilises two commercially available ω-TA biocatalysts from Codexis (ATA-(*S*)-113 and ATA-(*R*)-117), which having complementary selectivity, have proved to be apt catalysts for the transamination reaction of dicarbonyl substrates.[38] We decided to start our investigations using this set of enzymes. Initial amine donor screening displayed exceptional conversions with IPA and, after optimisation across our substrate scope, two equivalents of the amine donor were found to be optimal, although in some cases only one equivalent of IPA was required (Table 5, entry 1a). This methodology seems to contradict the known reversibility of TA reactions, as discussed in chapter 1.3. We attribute the excellent conversions with low quantities of amine donor to the favourable IMAMR, which provides a thermodynamic sink by driving the reaction towards the formation of the corresponding cyclic products.




*Scheme 27: Transamination of keto-enones **3a-e***

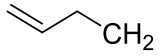
Under our optimised biocatalytic reaction conditions, the IMAMR provided approximately a 2:1 ratio in favour of the *cis*-configuration over the *trans*-isomer for

all the substrates. Isolation of the *trans*-isomer by column chromatography proved unsuccessful for all the substrates, as the C-2 of the piperidine ring readily epimerises on silica gel. Therefore, following previous reports,[58] dissolving the corresponding crude reaction mixtures in methanol, allowed the 2,6-disubstituted piperidines **1a-e** to undergo a retro-Michael addition and subsequent IMAMR, resulting in the exclusive formation of the *cis*-isomer, which is approximately 3.8 kcal $mol^{-1}$ more stable than the *trans*-piperidine (Scheme 28).[69] The relative stereochemistry of all the synthesised piperidines was confirmed by 2D-NMR experiments. For example, the COSY spectrum of (–)-pinidinone showed no coupling between $H_A$ and $H_B$ (Figure 2A) while its NOESY spectrum revealed a clear coupling between $H_A$ and $H_B$, indicating an interaction across space between those protons (*i.e. cis* configuration) (Figure 2B).

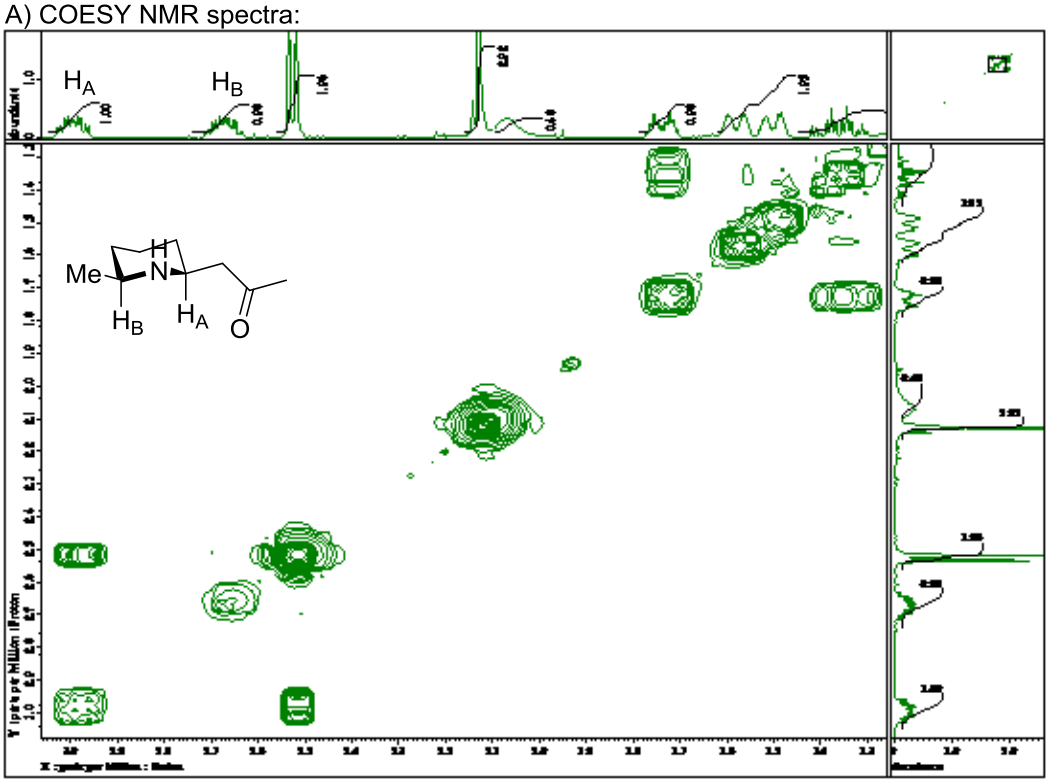



*Scheme 28: Epimerisation of carbon 2 in piperidines **1a-e**.*

*Table 4: Epimerisation conditions of carbon 2 in piperidines **1a-e***

| **Entry** | **R** | **Product** | **Temp. (°C)** | **Solvent** | **Time (h)** |
|---|---|---|---|---|---|
| 1 | Me | **1a** | r.t. | MeOH | 48 |
| 2 | *t*Bu | **1b** | 65 | MeOH | 24 |
| 3 |  | **1c** | r.t. | MeOH | 48 |
| 4 | Ph | **1d** | r.t. | MeOH | 48 |
| 5 | EtO | **1e** | 80 | EtOH | 24 |

A) COESY NMR spectra:




B) NOESY NMR spectra:

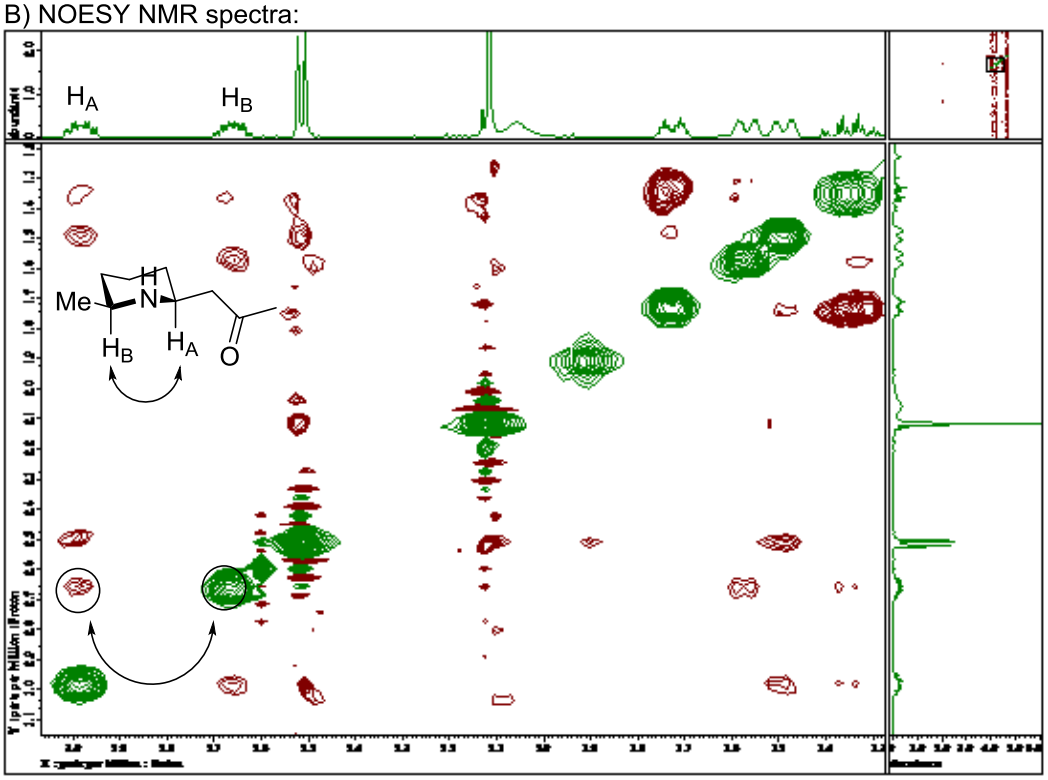
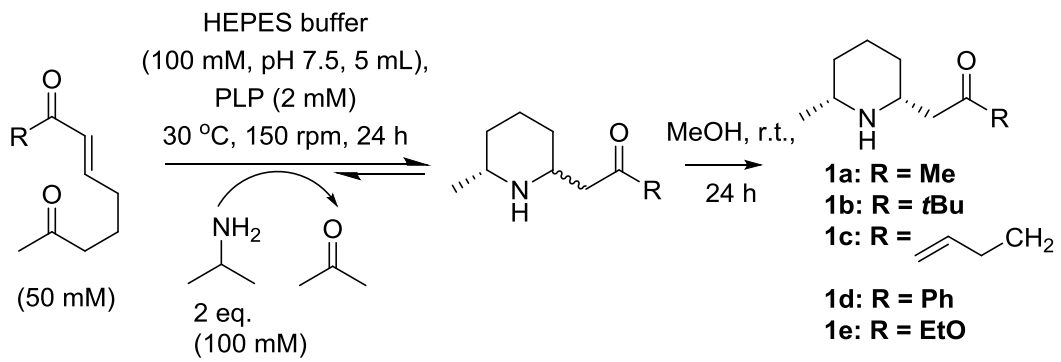



*Figure 2: COESY/NOESY analysis for the determination of stereochemistry of pinidinone* ***1a***

Utilising the optimised conditions, with ATA113 and ATA117, pinidinone (**1a**) was isolated in good yield (Table 5, entry 1 and 2). We then tested a range of non-natural equivalents of pinidinone to demonstrate the scope of the methodology. The bulky *t*Bu compound **3b** was readily converted with ATA113 (Table 5, entry 3); however, with ATA117 an extended reaction time was required (Table 5, entry 4). An extended alkyne chain **3c**, offering the opportunity of further functionalisation, again provided excellent isolated yields (Table 5, entry 5 and 6). The aromatic equivalent **3d** was insoluble in the aqueous reaction medium, however, as long as the enzyme readily converts the substrate, full conversion of starting material can be achieved. ATA113 provided full conversion after 24 h, indicating **3d** is an ideal substrate for this enzyme (Table 5, entry 7). In contrast, ATA117 only provided a 50% conversion of substrate, which shows that **3d** is not as readily accepted for this enzyme (Table 5, entry 8). To demonstrate further functional groups that can be tolerated by this methodology, the ester **3e** was examined. This was smoothly converted with the optimised reaction conditions (Table 5, entry 9 and 10). To highlight the synthetic utility of the developed methodology, we decided to scale up the reaction for the natural product (–)-pinidinone **1a**. The scaling up of the reaction by seven fold allowed the synthesis of 0.48 g of (–)-pinidinone **1a** with no adverse effects on the conversion, yield, *e.e.* or *d.e.* of the reaction.

*Table 5: Substrate screening of **3a-e***

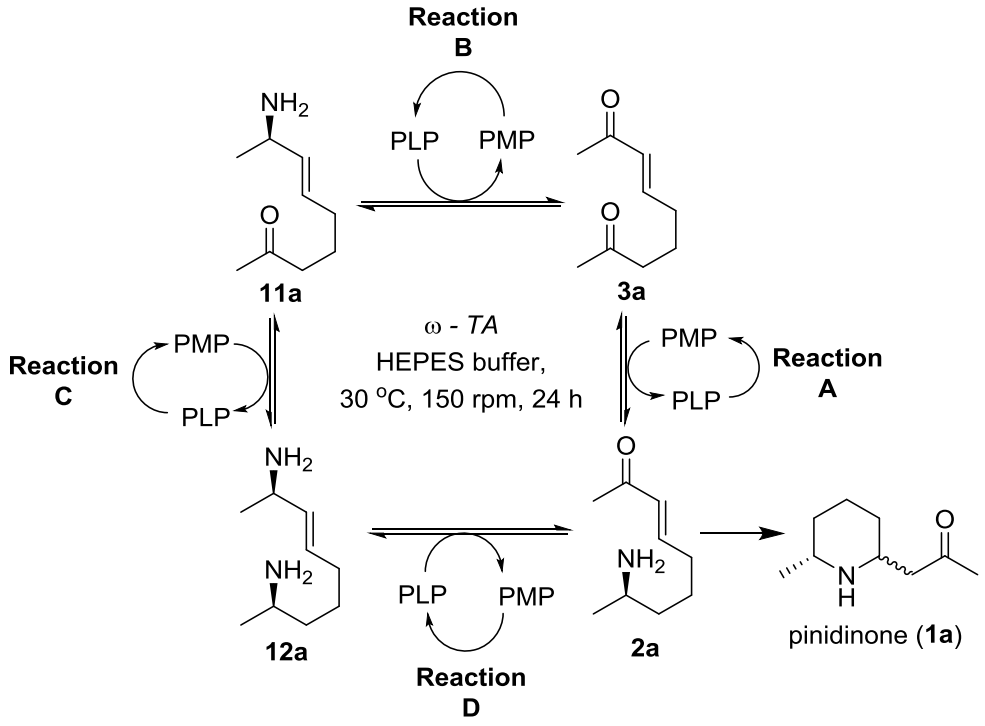



| Entry | R | ω-TA | Conv (%)[a] | ee (%)[b] | de (%)[c] | Yield (%)[d] |
|---|---|---|---|---|---|---|
| **1** | **1a** | ATA113 | >99 | >99 | >99 | 91 (*S,S*) |
| **2** | **1a** | ATA117 | >99 | >99 | >99 | 90 (*R,R*) |
| **3** | **1b** | ATA113 | >99[e] | >99 | >99[f] | 78 (*S,S*)[g] |
| **4** | **1b** | ATA117 | >99[e] | >99 | >99[f] | 88 (*R,R*)[g] |
| **5** | **1c** | ATA113 | >99 | >99 | >99 | 92 (*S,S*)[g] |
| **6** | **1c**[h] | ATA117 | >99[e] | >99 | >99 | 90 (*R,R*)[g] |
| **7** | **1d** | ATA113 | >99 | >99 | >99 | 76 (*S,S*)[g] |
| **8** | **1d**[h] | ATA117 | 50[e] | >99 | >99 | 44 (*R,R*)[g] |
| **9** | **1e** | ATA113 | >99 | >99 | >99[i] | 72 (*S.S*)[g] |
| **10** | **1e** | ATA117 | >99 | >99 | >99[i] | 70 (*R,R*)[g] |

[a] Conversion determined by $^1$H NMR after 24 h. [b] *ee* determined by chiral GC or HPLC. [c] *de* determined by NMR after the epimerization step. [d] Isolated yield after flash chromatography. [e] Conversion after 48 h. [f] Epimerization was carried out at 65 °C for 24 h. [g] Configuration assigned by analogy with **1a** and in agreement with NOESY experiments. [h] 4 eq of isopropylamine were used. [i] Epimerization was carried out in EtOH at 80 °C for 24 h.

When considering regioselectivity issues in the assayed commercial enzymes, it is worth paying special attention to the biocatalysed TA reaction of substrate **3a**, which displays a clear preference between the two present methyl ketones. The enzymes are able to accept readily both of the carbonyls (Scheme 29, reaction A or B), however, only pinidinone (**1a**) is observed (Scheme 29, reaction A). We propose that reaction B does occur to from **11a**, however, this subsequently undergoes a further transamination to either reform the diketone **3a** (reaction B) or afford diamine **12a** (reaction C). Eventually, reaction A or D will occur to form **2a** and the spontaneous IMAMR will drive the reaction towards the formation of product **1a**. To prove our hypothesis we decided to synthesise the conjugated aminoketone substrate **11a** and subject it to a TA reaction with no external source of amine (Scheme 29).[I] This provided full conversion of the starting material to pinidinone (**1a**). To our knowledge, this is also the first example of an internal amine source, as the only amine source, being transported across a molecular framework in a TA reaction resulting in the first donorless transamination process.

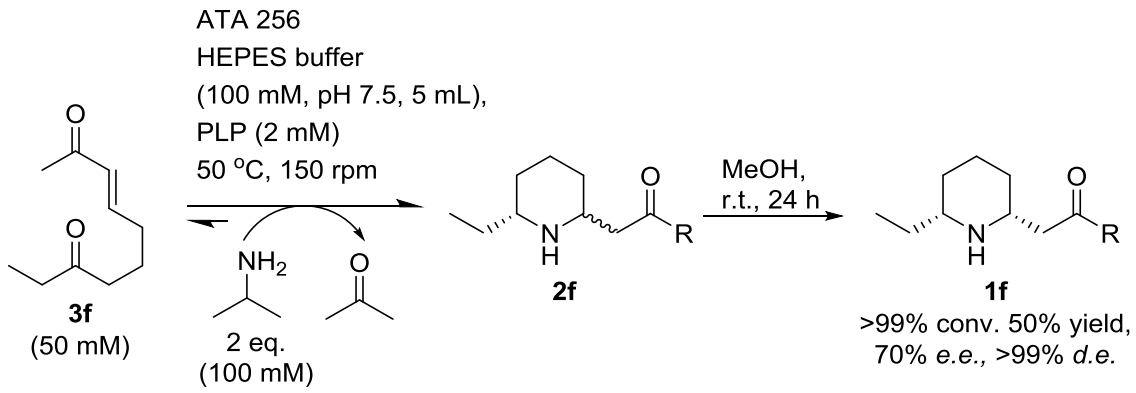
[I] This work by Andrew Gomm and Mindaugas Šiaučiulis was carried out at the University of Nottingham under the supervision of Dr E. O'Reilly




*Scheme 29: Proposed transamination of diketone **3a** and ketoamine **11a** to pinidinone (**1a**)*

After identifying that the IMAMR induced a regio-control of the reaction, we were interested to see if we could potentially reverse the regioselectivity of the TA reaction in favour of a bulkier ketone than a methyl ketone. Synthesis of the ethylketo-methylenone **3f** was achieved utilising 1-ethylcyclopentene and the appropriate Wittig reagent **6a** following the original procedure. Initial studies on the biocatalysed transamination showed poor conversion when ATA-(*S*)-113 was employed as catalyst at room temperature or 30 °C. Fortunately, the use of the Codexis enzyme ATA-(*S*)-256 at 50 °C, in the presence of two equivalent of IPA, allowed full conversion of all the starting material (Scheme 30). Identification of the Michael cyclic product **1f** demonstrates a clear example of the IMAMR controlling the regioselectivity of the reaction. Piperidine **1f** was obtained in 70% *e.e.*, lower enantioselectivity in

comparison to the methyl ketoenones **3a**-**e**; we attribute this to the fact that the commercially available enzyme ATA256 is heavily engineered towards methylketones and that a higher enantioselectivity of the reaction could be achieved with an enzyme with a bulky-bulky accepting active site.




*Scheme 30: Transamination of an ethylketone in the presence of a methylketone*

## 2.5 Conclusion

In conclusion, we have developed an extremely efficient biocatalytic aza-Michael strategy for the enantioselective synthesis of 2,6-disubstituted piperidines from prochiral ketoenones, with excellent conversion, isolated yield, *e.e.* and *d.e.* Our approach reveals that tethering a ω-TA biocatalysed amination reaction with the strong thermodynamic driving force of an IMAMR, allows the use of very low amounts of amine donor (sometimes stoichiometric amounts of IPA are enough to reach full conversion) and therefore bypassing the need of a supplementary equilibrium driving method. In addition, we have utilised the IMAMR to influence the regioselectivity of the TA reaction and demonstrated the first example of an amine functionality being transported across a molecular framework to form the desired

product. This work significantly expands the scope of ω-TA methodology in total synthesis and offers the biocatalytic retrosynthetic route for the synthesis of more complex alkaloid scaffolds.

## 2.6 Experimental

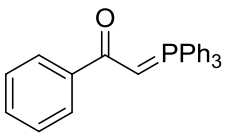
**General Methods and Materials**

**General**: NMR spectra were recorded on a JEOL ECS 400 NMR spectrometer ($^{1}$H 400 MHz, and $^{13}$C 100 MHz). The chemical shifts were recorded in ppm with the residual $CHCl_3$ signal referenced to 7.26 ppm and 77.00 ppm for $^{1}$H and $^{13}$C respectively. Coupling constants (*J*) are reported in Hz, are corrected and refer to the apparent peak multiplicities. Thin layer chromatography was performed on Alfa Aesar silica gel 60 F254 plates. Flash column chromatography was performed on silica gel (60 Å, 230-400 mesh). GC-MS spectra were recorded on a HP 5973, HP-5MS (30 m × 0.25 mm × 0.25 µm), Helium carrier gas, flow 1 mL/min. Infrared spectra were recorded using a Thermo Nicolet 380 FT-IR. GC-FID analysis was performed on Agilent 6850 equipped with a CP CHIRASIL-DEX CB (25 m × 0.25 mm) DF = 0.25 column. HPLC analysis was performed on an Agilent 1100 series equipped with a Lux Amylose-1 chiral column from Phenomenex. All racemic standards were prepared by mixing equimolar quantities of enantiomerically pure biotransformation products.

**Materials**: Commercially available reagents, purchased from Sigma Aldrich or Acros, were used throughout without further purification. Anhydrous THF, $CH_2Cl_2$, diethyl ether and toluene were obtained from a Pure Solvent apparatus. Commercially available transaminases, ATA113 and ATA117 were purchased from Codexis in the

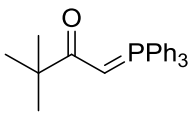
form of lyophilised cell extract. All biotransformations were carried out in HEPES buffer (100 mM, pH 7.5) at 30 ºC.

**1-phenyl-2-(triphenylphosphanylidene)ethanone**[65]




To a solution of triphenylphosphine (7.9 g, 30.14 mmol) in $CH_2Cl_2$ (20 mL), 2-bromoacetophenone (5 g, 25.12 mmol) was added and the mixture was stirred for 24 h. Diethyl ether (100 mL) was then added and stirred for a further hour. The resulting precipitate was suspended in a mixture of water/methanol (100 mL, 1:1) and stirred for 1 h. Next, aqueous NaOH (2 M) was added until pH 7-8 was reached and the mixture was stirred for 5 h at r.t. Methanol was then removed under reduced pressure and the aqueous layer was extracted with $CH_2Cl_2$ (3 x 20 mL). The combined organic extracts were then dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to provide the title compound as a white solid (9.03 g, 94% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.02-7.91 (m, 2H), 7.78-7.65 (m, 6H), 7.60-7.42 (m, 9H), 7.40-7.28 (m, 3H), 4.43 (d, *J* = 24.7 Hz, 1H). In accordance with literature data.

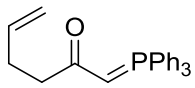
**3,3-dimethyl-1-(triphenylphosphoranylidene)-2-butanone**[70]




To a solution of triphenylphosphine (9.7 g, 37 mmol) in toluene (50 mL), 1-bromo-3,3-dimethylbutan-2-one (5 mL, 37 mmol) was added and the mixture was refluxed for 4 h. The solid precipitate was filtered and washed with diethyl ether (3 × 100 mL). The phosphonium salt was then dissolved in the minimal amount of water/$CH_2Cl_2$ (1.5:1) and NaOH (2M, 100 mL) was added and the mixture stirred for 2 h. The reaction mixture was extracted with $CH_2Cl_2$ (3 × 100 mL)

and washed with brine (300 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to provide the title compound as a beige solid (10.58 g, 79% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.68-7.57 (m, 6H), 7.56-7.47 (m, 3H), 7.47-7.38 (m, 6H), 3.77 (d, *J* = 27.0 Hz, 1H), 1.20 (s, 9H). In accordance with literature data.

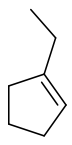
**1-(triphenylphosphoranylidene)-5-hexen-2-one**[71]



To a solution of 1-(triphenylphosphoranylidene)-2-propanone (1.00 g, 3.14 mmol) in dry THF (50 mL), *n*-BuLi (1.9 mL, 2.5 M in hexanes) was added at −60°C and the mixture was stirred for 30 min. Excess of allylbromide (1.9 mL, 22.0 mmol) was then added and the reaction left to warm to room temperature slowly. The reaction mixture was quenched with water (100 mL) and extracted was $CH_2Cl_2$ (3 × 100 mL), washed with brine (300 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to provide the title compound as a yellow oil (990 mg, 88% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.68-7.59 (m, 6H), 7.59-7.51 (m, 3H), 7.49-7.41 (m, 6H), 5.99-5.83 (m, 1H), 5.11-4.92 (m, 2H), 3.71 (d, *J* = 26.6 Hz, 1H), 2.48-2.36 (m, 4H). In accordance with literature data.

**Preparation of conjugated substrates 3a-f**

**1-ethylcyclopentene**[72]



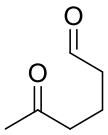
Ethyl magnesium bromide (1.2 eq, 48 mmol, 16 mL, 3 M in $Et_2O$) was added to cyclopentanone (1 eq, 40 mmol, 3.54 mL) in $Et_2O$ (40 mL) under nitrogen at 0 °C. The mixture was then allowed to warm to room temperature and stirred for a further 2 h. The reaction mixture was then carefully quenched with cold water followed by sat. $NH_4$.Cl. The organic phase was separated and the aqueous layer

extracted with $Et_2O$ (2 × 40 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to provide the crude 1-ethylcyclopentanol (3.90 g, 86% yield). To this crude material, 85% wt. phosphoric acid (0.05 eq, 1.71 mmol, 0.20 mL) was added and the mixture was heated to 115 °C. The product was distilled off as it formed and the biphasic solution was separated to provide crude 1-ethylcyclopentene as a colourless oil (1.84 g, 48% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 5.35-5.29 (1H, m, 2-H), 2.35-2.26 (2H, m, 3-H), 2.26-2.19 (2H, m, 5-H), 2.11-2.02 (2H, m, 1'-H), 1.91-1.80 (2H, m, 4-H), 1.04 (3H, t, *J* = 7.3 Hz, 2'-H). In accordance with literature data.

**General Procedure for Oxidative Ring Opening of 1-methyl- and 1-ethylcyclopentene**

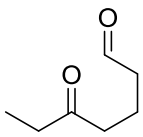
The corresponding 1-alkylcyclopentene (1 eq, 16.6 mmol) and $OsO_4$ (4% w.t/v *t*BuOH, 0.01 eq, 0.17 mmol, 1.06 mL) were added to a solution of *N*-methylmorpholine *N*-oxide (2 eq, 33.2 mmol, 3.89 g) in acetone (20 mL). The mixture was stirred at room temperature for 4 h and then quenched with sat. $Na_2SO_3$ solution (80 mL) and extracted with $CH_2Cl_2$ (3 × 60 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The crude material was dissolved in water (20 mL) and sodium periodate (2 eq, 33.2 mmol, 7.10 g) was added. The resulting mixture was stirred for 1 h at room temperature and then diluted with water (60 mL) and extracted with $CH_2Cl_2$ (3 x 30 mL). Next, the combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to provide the desired aldehyde.

**5-oxo-hexanal (5a)**[73]



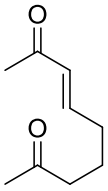
Colourless oil (460 mg, 85% yield). FTIR (neat) $V_{max}$: 2941, 2729, 1709, 1369, 1162 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 9.75 (1H, s, 1-H), 2.55-2.45 (4H, m, 2,4-H), 2.13 (3H, s, 6-H), 1.93-1.83 (2H, m, 3-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.0 (CO, C-5), 201.9 (CHO, C-1), 42.9 ($CH_2$, C-4), 42.2 ($CH_2$, C-2), 29.9 ($CH_3$, C-6), 15.9 ($CH_2$, C-3); Calculated $C_6H_{11}O_2^+$ $[M+H]^+$: 115.0754, found: 115.0758. In accordance with literature data.

**5-oxoheptanal**[74]



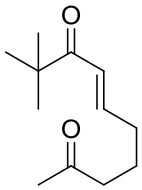
Pale yellow oil (850 mg, 40% yield). $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 9.76-9.75 (1H, m, 1-H), 2.51-2.44 (4H, m, 2,4-H), 2.41 (2H, q, *J* = 7.3 Hz, 6-H), 1.90 (2H, m, 3-H), 1.04 (3H, t, *J* = 7.3 Hz, 7-H).

**(3*E*)-non-3-ene-2,8-dione (3a)**[75]



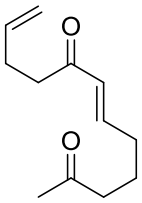
Colourless oil (367 mg, 79% yield). R.f. 0.25 (EtOAc/hexane 1:1); FTIR (neat) $V_{max}$: 3004 2938, 1712, 1670, 1626, 1358 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 6.75 (1H, dt, *J* = 16.0, 6.9 Hz, 4-H), 6.06 (1H, d, *J* = 16.0 Hz, 3-H), 2.50-2.43 (2H, m, 7-H), 2.28-2.18 (5H, m, 1,5-H), 2.13 (3H, s, 9-H), 1.81-1.70 (2H, m, 6-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.1 (CO, C-8), 198.5 (CO, C-2), 147.2 (CH, C-4), 131.7 (CH, C-3), 42.6 ($CH_2$, C-7), 31.6 ($CH_2$, C-5), 30.0 ($CH_3$, C-9), 26.9 ($CH_3$, C-1), 21.8 ($CH_2$, C-6); LC-MS (m/z): Calculated $C_9H_{14}O_2Na^+$ $[M+Na]^+$: 177.0886, found: 177.0870. In accordance with literature data.

**(6*E*)-9,9-dimethyldec-6-ene-2,8-dione (3b)**[75]



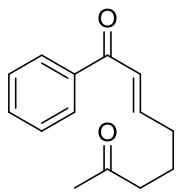
The synthesis of **3b** was carried out according the general procedure for Wittig reactions, but in this case, the reaction mixture was heated to reflux for 24 h. Colourless oil (537 mg, 58% yield). R.f. 0.48 (EtOAc/hexane 1:1); FTIR (neat) $V_{max}$: 2967, 1714, 1687, 1622, 1365, 1077, 983 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 6.94-6.82 (1H, m, 6-H), 6.50 (1H, d, *J* = 15.1 Hz, 7-H), 2.46 (2H, t, *J* = 7.3 Hz, 3-H), 2.25-2.20 (2H, m, 5-H), 2.14 (3H, s, 1-H), 1.80-1.70 (2H, m, 4-H), 1.14 (9H, s, 10-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.3 (CO, C-8), 204.2 (CO, C-2), 146.2 (CH, C-6), 124.7 (CH, C-7), 42.8 (C, C-9), 42.6 ($CH_2$, C-3), 31.5 ($CH_2$, C-5), 30.0 ($CH_3$, C-1), 26.1 ($CH_3$, C-10), 22.0 ($CH_2$, C-4); LC-MS (m/z): Calculated $C_{12}H_{21}O_2^+$ $[M+H]^+$: 197.1536, found: 197.1544. In accordance with literature data.

**(6*E*)-dodeca-6,11-diene-2,8-dione (3c)**



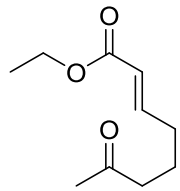
Pale yellow oil (308 mg, 57% yield). R.f. 0.45 (EtOAc/hexane 1:1); FTIR (neat) $V_{max}$: 3078, 2935, 1713, 1668, 1628, 1360, 980, 912 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 6.78 (1H, dt, *J* = 16.0, 6.9 Hz, 6-H), 6.20-5.99 (1H, m, 7-H), 5.94-5.62 (1H, m, 11-H), 5.06-4.94 (2H, m, 12-H), 2.63 (2H, t, *J* = 7.3 Hz, 9-H), 2.45 (2H, t, *J* = 7.3 Hz, 3-H), 2.38-2.31 (2H, m, 10-H), 2.25-2.18 (2H, m, 5-H), 2.13 (3H, s, 1-H), 1.79-1.68 (2H, m, 4-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.1 (CO, C-2), 199.5 (CO, C-8), 146.2 (CH, C-6), 137.2 (CH, C-11), 130.7 (CH, C-7), 115.1 ($CH_2$, C-12), 42.5 ($CH_2$, C-3), 39.1 ($CH_2$, C-9), 31.6 ($CH_2$, C-5), 30.0 ($CH_3$, C-1), 28.0 ($CH_2$, C-10), 21.8 ($CH_2$, C-4); LC-MS (m/z): Calculated $C_{12}H_{19}O_2^+$ $[M+H]^+$: 195.1380, found: 195.1382.

**(2*E*)-1-phenyloct-2-ene-1,7-dione (3d)**[75]



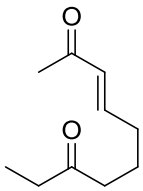
White solid (328 mg, 76% yield). R.f. 0.42 (EtOAc/hexane 1:1); M.p. 68 – 71 °C; FTIR (neat) $V_{max}$: 3055, 3000, 2955, 2934, 1709, 1663, 1616, 1356, 1244, 982, 699 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 7.96-7.90 (2H, m, 2'-H), 7.60-7.53 (1H, m, 4'-H), 7.51-7.44 (2H, m, 3'-H), 7.03 (1H, dt, *J* = 15.5, 6.8 Hz, 3-H), 6.90 (1H, d, *J* = 15.5, Hz, 2-H), 2.51 (2H, t, *J* = 7.3 Hz, 6-H), 2.37-2.31 (2H, m, 4-H), 2.15 (3H, s, 8-H), 1.87-1.78 (2H, m, 5-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.2 (CO, C-7), 190.7 (CO, C-1), 148.6 (CH, C-3), 137.8 (C, C-1'), 132.7 (CH C-4'), 128.5 (2CH, C-2',3'), 126.4 (CH, C-2), 42.6 ($CH_2$, C-6), 31.9 ($CH_2$, C-4), 30.0 ($CH_3$, C-8), 22.0 ($CH_2$, C-5); LC-MS (m/z): Calculated $C_{14}H_{17}O_2^+$ $[M+H]^+$: 217.1223, found: 217.1221. In accordance with literature data.

**ethyl (2*E*)-7-oxooct-2-enoate (3e)**[73]



Colourless oil (171 mg, 66% yield). R.f. 0.41 (EtOAc/hexane 1:1); FTIR (neat) $V_{max}$: 2939, 1709, 1653, 1266, 1178, 1148 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 6.96-6.85 (1H, dt, *J* = 15.6, 7.0 Hz, 3-H), 5.82 (1H. d, *J* = 15.6 Hz, 2-H), 4.18 (2H, q, *J* = 7.2 Hz, 1'-H), 2.45 (2H, t, *J* = 7.3 Hz, 6-H), 2.23-2.17 (2H, m, 4-H), 2.13 (3H, s, 8-H), 1.79-1.69 (2H, m, 5-H), 1.28 (3H, t, *J* = 7.2 Hz, 2'-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.2 (CO, C-7), 166.5 (CO, C-1), 148.0 (CH, C-3), 122.0 (CH, C-2), 60.2 ($CH_2$, C-1'), 42.6 ($CH_2$, C-6), 31.3 ($CH_2$, C-4), 30.0 ($CH_3$, C-8), 21.8 ($CH_2$, C-5), 14.2 ($CH_3$, C-2'); LC-MS (m/z): Calculated $C_{10}H_{17}O_3^+$ $[M+H]^+$: 185.1172, found: 185.1147. In accordance with literature data.

**(3*E*)-dec-3-ene-2,8-dione (3f)**



The synthesis of **3f** was carried out according the general procedure for Wittig reactions, but in this case, the reaction mixture was heated to reflux for 24 h in THF. Pale yellow oil (770 mg, 69% yield). R.f. 0.37 (EtOAc/hexane 1:1); FTIR (neat) $V_{max}$: 2976, 2938, 1711, 1698, 1672, 1626, 1458, 1415, 1360, 1253, 976 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 6.75 (1H, dt, *J* = 16.0, 6.9 Hz, 4-H), 6.06 (1H, d, *J* = 16.0 Hz, 3-H), 2.48-2.36 (4H, m, 7,9-H), 2.28-2.18 (2H, m, 5-H), 2.23 (3H, s, 1-H), 1.82-1.71 (2H, m, 6-H), 1.04 (3H, t, *J* = 7.3 Hz, 10-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 210.8 (CO, C-8), 198.6 (CO, C-2), 147.3 (CH, C-4), 131.7 (CH, C-3), 41.2 ($CH_2$, C-7), 36.0 ($CH_2$, C-9), 31.7 ($CH_2$, C-5), 26.9 ($CH_3$, C-1), 21.9 ($CH_2$, C-6), 7.8 ($CH_3$, C-10); LC-MS (m/z): Calculated $C_{10}H_{17}O_2^+$ $[M+H]^+$: 169.1223, found: 169.1220.

**Biotransformations**

**General procedure for the preparation of compounds 1a-e**

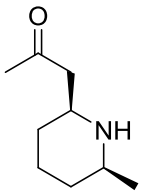
Commercially available (*S*)-selective ATA113 or (*R*)-selective ATA117 (25 mg) was rehydrated in HEPES buffer (4.5 mL, 100 mM, pH 7.5) containing PLP (2.00 mM) and isopropylamine (0.5 mmol, 2 eq). The pH of the mixture was adjusted to 7.5, using aq HCl solution (1 M), and the total volume of the reaction was adjusted to 5 mL by addition of HEPES buffer. The ketoenone substrate **3a-e** was added (50 mM, 0.5 mL from a 500 mM stock solution in DMSO) and the reaction mixture incubated at 30 $^{o}C$, 150 rpm for 24-48 h. The reaction was monitored by GC. After completion, the pH of the supernatant was adjusted to 12 using aq NaOH solution (4 M) and the resulting

mixture was extracted with $Et_2O$ (3 × 10 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure and analysed by GC. The crude mixture was dissolved in an alcoholic solvent (5 mL) and stirred at the stated temperature for 24-48 h. The crude material was purified by column chromatography on silica gel (gradient from $CH_2Cl_2$ to 15% MeOH:$CH_2Cl_2$) to provide pure compounds **1a-e**.

**General procedure for the preparation of compound 1f**

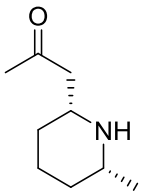
Commercially available (*S*)-selective ATA256 or (*R*)-selective ATA025 (25 mg) was rehydrated in HEPES buffer (4.5 mL, 100 mM, pH 7.5) containing PLP (2.00 mM) and isopropylamine (4 eq, 86 uL, 200 mM). The pH of the mixture was adjusted to 7.5, using aq HCl solution (1 M), and the total volume of the reaction was adjusted to 4.5 mL by addition of HEPES buffer. The ketoenone substrate **3f** was added (50 mM, 0.5 mL from a 500 mM stock solution in DMSO) and the reaction mixture incubated at 50 $^oC$, 150 rpm for 48 h. The reaction was monitored by GC-MS. After completion, the pH of the supernatant was adjusted to 12 using aq NaOH solution (4 M) and the resulting mixture was extracted with EtOAc (4 × 20 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure and analysed by GC. The crude mixture was dissolved in an alcoholic solvent (5 mL) and stirred at the stated temperature for 24-48 h. The crude material was purified by column chromatography on silica gel (gradient from $CH_2Cl_2$ to 15% MeOH:$CH_2Cl_2$) to provide pure compound **1f**.

**(*S*,*S*)-(1-[6-methylpiperidin-2-yl]propan-2-one); (*S*,*S*)-(+)-pinidinone (1a)**[9]



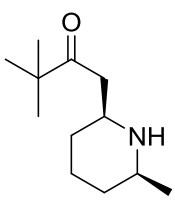
Yellow oil (36 mg, 93% yield, 99% *ee*). FTIR (neat) $V_{max}$: 2927, 2859, 1706, 1360, 1159, 734, 552 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 3.06-2.96 (m, 1H), 2.74-2.63 (m, 1H), 2.54 (d, *J* = 6.4 Hz, 2H), 2.13 (s, 3H), 2.00 (br, s, 1H), 1.75 (m, 1H), 1.64-1.55 (m, 1H), 1.55-1.47 (m, 1H), 1.43-1.21 (m, 2H), 1.15-0.97 (m, 2H), 1.04 (d, *J* = 6.4 Hz, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.5, 52.3, 52.0, 50.6, 33.8, 31.8, 30.6, 24.6, 23.0; LC-MS (m/z): Calculated $C_9H_{18}NO^+$ $[M+H]^+$: 156.1383, found: 156.1385. $[\alpha]_D^{24}$ = +26 (*c* 3.9, MeOH) {$^{Lit.}[\alpha]_D^{24}$ = +25 (*c* 0.4, MeOH)}. *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 105 °C, P = 6 psi, retention times: $t_r$(*S*,*S*) = 26.2 min (only enantiomer), $t_r$(*R*,*R*) = 24.1 min (not observed). In accordance with literature data.

**(*R*,*R*)- 1-[6-methylpiperidin-2-yl]propan-2-one); (*R*,*R*)-(-)-pinidinone (1a)**[10]



Yellow oil (35 mg, 91% yield, 99% *ee*). $[\alpha]_D^{24}$ = –25 (*c* 2.4, MeOH) {$^{Lit.}[\alpha]_D^{24}$ = –4 (*c* 3.5, MeOH)}. *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 105 °C, P = 6 psi, retention times: $t_r$(*S*,*S*) = 26.2 min (not observed), $t_r$(*R*,*R*) = 24.1 min (only enantiomer). In accordance with literature data.

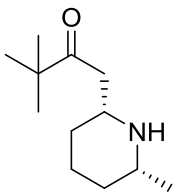
**3,3-dimethyl-1-[(2*S*,6*S*)-6-methylpiperidin-2-yl]butan-2-one (1b)**



Yellow oil (38 mg, 78% yield, 99% *ee*). FTIR (neat) $V_{max}$: 2962, 2972, 1700, 1466, 1366, 1062, 732, 570 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 3.01-2.90 (m, 1H), 2.73-2.62 (m, 1H), 2.61-2.48 (m, 2H), 1.77-1.66 (m,

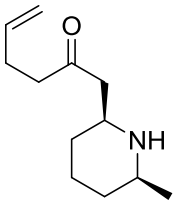
1H), 1.60-1.44 (m, 2H), 1.43-1.28 (m, 1H), 1.18-0.98 (m, 2H), 1.09 (s, 9H), 1.03 (d, *J* = 6.4 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 215.9, 52.6, 52.2, 44.1, 43.4, 33.6, 31.6, 26.3, 24.5, 22.8; LC-MS (m/z): Calculated $C_{12}H_{24}NO^+$ $[M+H]^+$: 198.1852, found: 198.1850. $[\alpha]_D^{24}$ = +15 (*c* 2.6, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 110 °C, P = 6 psi, retention times: $t_r$(*S*,*S*) = 47.4 min (only enantiomer), $t_r$(*R*,*R*) = 44.8 min (not observed).

**3,3-dimethyl-1-[(2*R*,6*R*)-6-methylpiperidin-2-yl]butan-2-one (1b)**



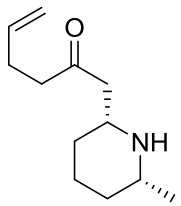

Yellow oil (43 mg, 88% yield, 99% *ee*). $[\alpha]_D^{24}$ = −16 (*c* 3.8, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 110 °C, P = 6 psi, retention times: $t_r$(*S*,*S*) = 47.4 min (not observed), $t_r$(*R*,*R*) = 44.8 min (only enantiomer).

**1-[(2*S*,6*S*)-6-methylpiperidin-2-yl]hex-5-en-2-one (1c)**



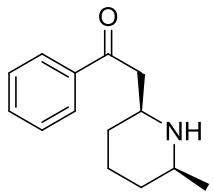

Yellow oil (45 mg, 92% yield, 99% *ee*). FTIR (neat) $V_{max}$: 2925, 1709, 1440, 1373, 1318, 1128, 995, 911, 747, 535 $cm^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 5.83-5.67 (m, 1H), 5.05-4.87 (m, 2H), 3.04-2.89 (m, 1H), 2.69-2.59 (m, 1H), 2.51-2.40 (m, 4H), 2.31-2.19 (m, 3H), 1.76-1.66 (m, 1H), 1.60-1.42 (m, 2H), 1.41-1.25 (m, 1H), 1.12-0.92 (m, 2H), 1.00 (d, *J* = 6.4 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 209.8, 136.9, 115.2, 52.3, 52.0, 49.6, 42.3, 33.7, 31.8, 27.5, 24.5, 22.9; LC-MS (m/z): Calculated $C_{12}H_{22}NO^+$ $[M+H]^+$: 196.1696, found: 196.1689. $[\alpha]_D^{24}$ = +28 (*c* 2.1, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 125 °C, P = 6 psi, retention times: $t_r$(*S*,*S*) = 43.2 min (only enantiomer), $t_r$(*R*,*R*) = 40.6 min (not observed).

**1-[(2*R*,6*R*)-6-methylpiperidin-2-yl]hex-5-en-2-one (1c)**



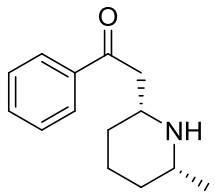
The synthesis of (*R*,*R*)-**1c** was carried out according to the general procedure for biotransformations using ATA117 and 4 equivalent of isopropylamine. Yellow oil (44 mg, 90% yield, 99% *ee*). $[\alpha]_D^{24} = -27$ (*c* 4.4, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 125 °C, P = 6 psi, retention times: $t_r$(*S*,*S*) = 43.2 min (not observed), $t_r$(*R*,*R*) = 40.6 min (only enantiomer).

**2-[(2*S*,6*S*)-6-methylpiperidin-2-yl]-1-phenylethanone (1d)**



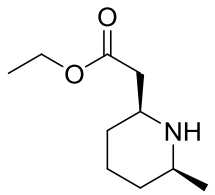
Yellow oil (41 mg, 76% yield, 99% *ee*). FTIR (neat) $V_{max}$: 2926, 1680, 1448, 1223, 751, 731, 689, 572 $cm^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): δ 7.97-7.89 (m, 2H), 7.59-7.51 (m, 1H), 7.48-7.40 (m, 2H), 3.26-3.15 (m, 1H), 3.11-3.03 (m, 2H), 2.82-2.69 (m, 1H), 2.55 (br s, 1H), 1.83-1.72 (m, 1H), 1.68-1.55 (m, 2H), 1.49-1.34 (m, 1H), 1.30-1.17 (m, 1H), 1.17-1.02 (m, 1H), 1.07 (d, *J* = 6.4 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.5, 136.9, 133.2, 128.6, 128.0, 52.8, 52.2, 45.4, 33.8, 32.0, 24.6, 22.9; LC-MS (m/z): Calculated $C_{14}H_{20}NO^+$ $[M+H]^+$: 218.1539, found: 218.1543. $[\alpha]_D^{24}$ = +20 (*c* 3.0, MeOH). *Ee* determination by chiral HPLC analysis, Lux Amylose-1 column, flow = 0.5 mL/min, hexane/2-propanol 9:1, retention times: $t_r$(*S*,*S*) = 11.8 min (only enantiomer), $t_r$(*R*,*R*) = 27.0 min (not observed).

**2-[(2*R*,6*R*)-6-methylpiperidin-2-yl]-1-phenylethanone (1d)**



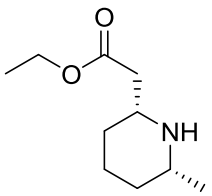
The synthesis of (*R*,*R*)-**1d** was carried out according the general procedure for biotransformations using ATA117 enzyme and 4 equivalents of isopropylamine. Yellow oil (24 mg, 44% yield, 99% *ee*). $[\alpha]_D^{24} = -21$ (*c* 1.0, MeOH). *Ee* determination by chiral HPLC analysis, lux amylose-1 column, flow = 0.5 mL/min, 90% hexane: 10% 2-propanol, retention times: $t_r$(*S*,*S*) = 11.8 min (not observed), $t_r$(*R*,*R*) = 27.0 min (only enantiomer).

**ethyl [(2*S*,6*S*)-6-methylpiperidin-2-yl]acetate (1e)**



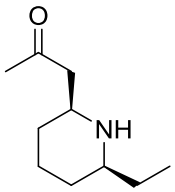
Yellow oil (33 mg, 72% yield, 99% *ee*). FTIR (neat) $V_{max}$: 2927, 1729, 1288, 1188, 115, 1096, 1027, 773, 557 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 4.12 (q, *J* = 7.1 Hz, 2H), 3.01-2.90 (m, 1H), 2.74-2.61 (m, 1H), 2.44-2.32 (m, 2H), 2.06 (br s, 1H), 1.80-1.70 (m, 1H), 1.63-1.53 (m, 2H), 1.44-1.29 (m, 1H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.16-0.96 (m, 2H), 1.05 (d, *J* = 6.4 Hz, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 172.5, 60.3, 53.4, 52.2, 41.5, 33.8, 31.9, 24.5, 22.9, 14.2; LC-MS (m/z): Calculated $C_{10}H_{20}NO_2^+$ $[M+H]^+$: 186.1489, found: 186.1502. $[\alpha]_D^{24} = +21$ (*c* 3.8, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 110 °C, P = 6 psi, retention times: $t_r$(*S*,*S*) = 34.0 min (only enantiomer), $t_r$(*R*,*R*) = 32.0 min (not observed).

**ethyl [(2*R*,6*R*)-6-methylpiperidin-2-yl]acetate (1e)**



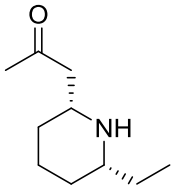
Yellow oil (32 mg, 70% yield, 99% *ee*). $[\alpha]_D^{24}$ = –20 (*c* 3.0, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 110 °C, P = 6 psi, retention times: $t_r$(*S*,*S*) = 34.0 min (not observed), $t_r$(*R*,*R*) = 32.0 min (only enantiomer).

**(*S*,*S*)-(1-[6-ethylpiperidin-2-yl]propan-2-one) (1f)**



Yellow oil (19 mg, 45% yield, 70% *ee*). FTIR (neat) $V_{max}$: 3326, 2927, 2855, 1709, 1458, 1359, 1155, 1111, 963 $cm^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): δ 3.03-2.88 (m, 1H), 2.57-2.49 (m, 2H), 2.47-2.35 (m, 1H), 2.11 (s, 3H), 1.79-1.69 (m, 1H), 1.69-1.60 (m, 1H), 1.54-1.45 (m, 1H), 1.41-1.24 (m, 3H), 1.16-0.90 (m, 2H), 0.87 (t, *J* = 7.6 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 208.5, 58.3, 52.4, 50.3, 32.1, 31.5, 30.6, 29.9, 24.4, 10.3; LC-MS (m/z): Calculated $C_{10}H_{20}NO^+$ $[M+H]^+$: 170.1538, found: 170.1539. $[\alpha]_D^{24}$ = +18 (*c* 3.4, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 110 °C, P = 6 psi, retention times: $t_r$(*S*,*S*) = 29.4 min (85%), $t_r$(*R*,*R*) = 27.4 min (15%).

**(*R*,*R*)-(1-[6-ethylpiperidin-2-yl]propan-2-one) (1f)**



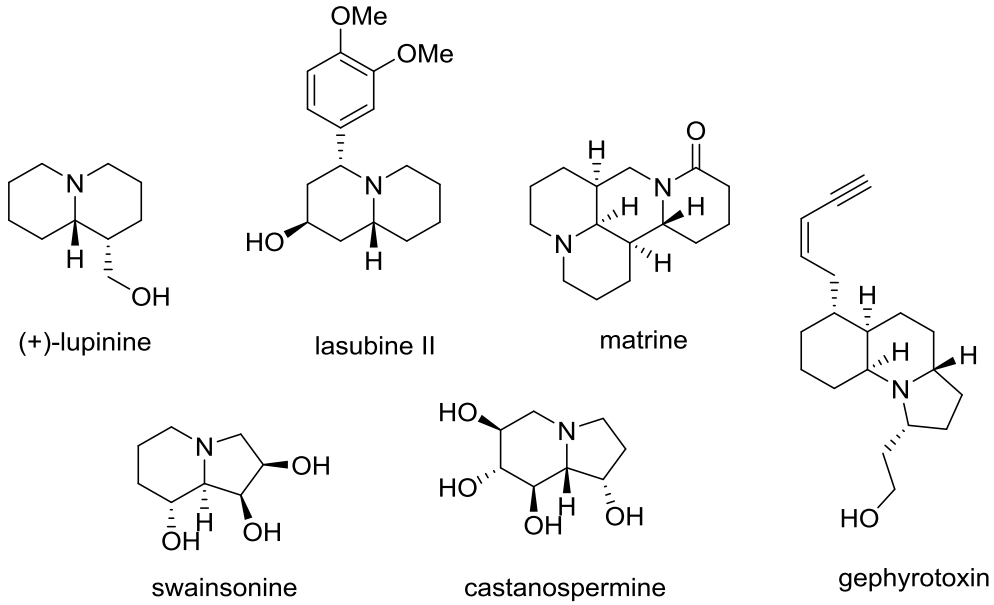
Yellow oil (1 mg, 2% yield, 99% *ee*). $[\alpha]_D^{24}$ = –60 (*c* 0.3, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 110 °C, P = 6 psi, retention times: $t_r$(*S*,*S*) = 29.4 min (not observed), $t_r$(*R*,*R*) = 27.4 min (only enantiomer).

# 3. Investigation into TA Triggered Double-IMAMR

## 3.1 Introduction

The quinolizidine and indolizidine alkaloids are members of a series of azabicyclic compounds, frequently found in natural products, which have extensively been reviewed.[76] They have received great interest from organic synthetic chemists due to their diverse and interesting 3D structure and their corresponding biological activity (Scheme 31). The array of biological activities includes antitumor activity (martine,[77] swainsonine[78]), antiviral properties (castanospermine[79]) and neuroactivity (gephyrotoxin[80]), amongst others.

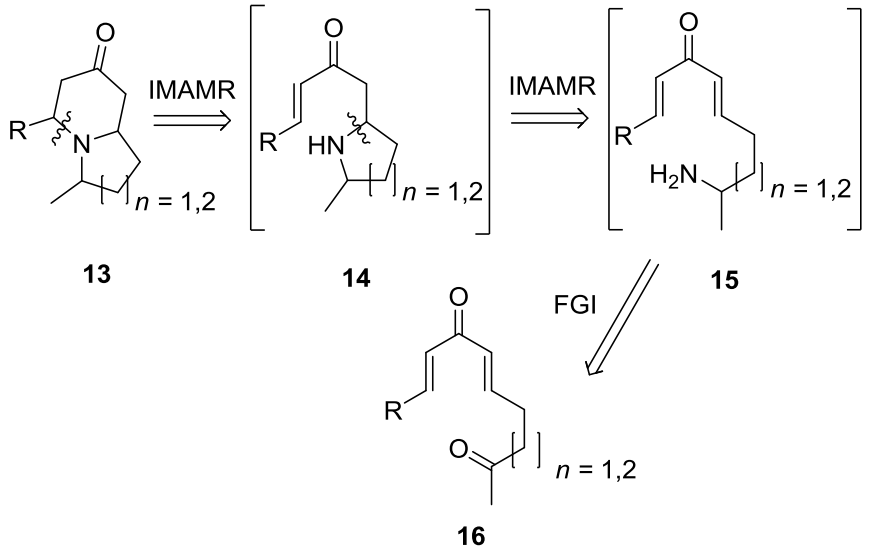
The Colombian tropical frog *Dendrobates histrionicus* has been an abundant source of alkaloids with intriguing molecular structures and biological activities, such as gephyrotoxin (first isolated in 1977 by Daly and co-workers, Scheme 31).[81] Gephyrotoxin derivatives are muscarinic antagonists and thought to be non-toxic. Thus, their total synthesis has received much interest.[82–86]




*Scheme 31: Structures of some quinolizidine and indolizidine natural alkaloids*

## 3.2 Aims and objectives

Herein we explore the scope of the TA triggered IMAMR to include substrates that have the potential to undergo a double IMAMR for the synthesis of quinolizidine and indolizidine natural products or their core structures. To do this, we synthesised a number of compounds, similar to the previously prepared **3a-e**, that contain a second conjugated double bond (Scheme 32), generating bis-enone systems that resemble Nazarov cyclisation precursors.



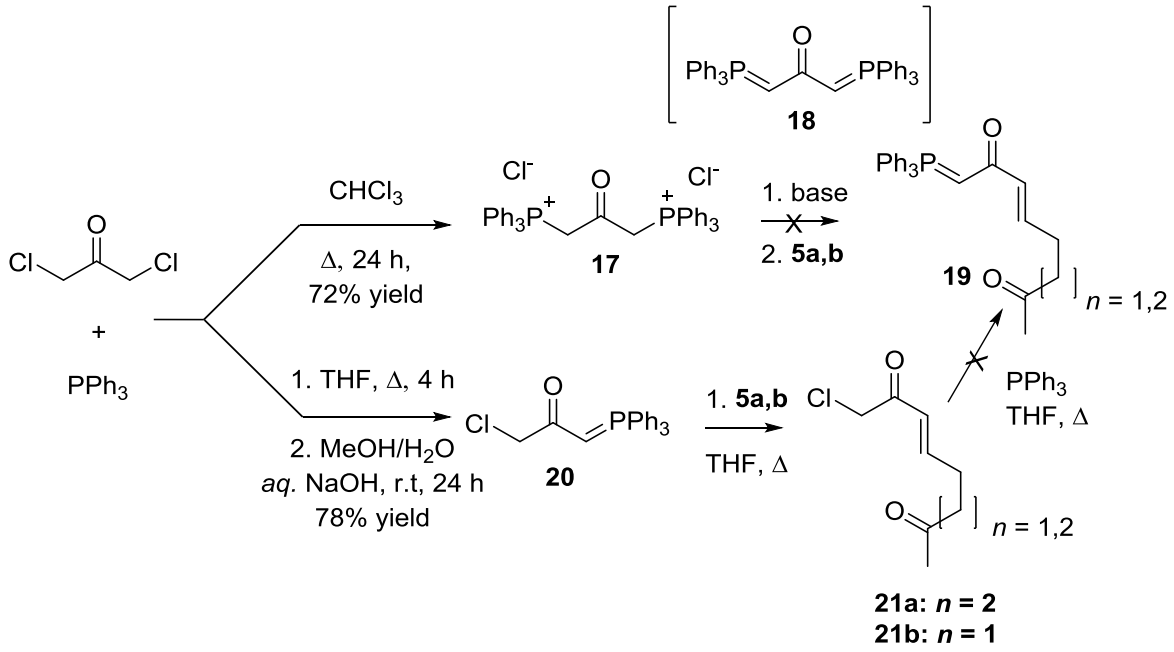

*Scheme 32: Retrosynthetic analysis of quinolizidine and indolizidine* via *double IMAMR*

## 3.3 Substrate synthesis

Danishefsky *et al.* have reported the use of acetone-bis-1,3-triphenylphosphonium chloride (**17**) as precursor for the *in-situ* preparation of the acetone bisphosphorane ylid **18** in the synthesis of (+)-isomigrastatin.[87] The acetone-bis-1,3-triphenylphosphonium chloride (**17**) can be readily prepared from dichloroacetone and two equivalents of triphenylphosphine in $CHCl_3$, under reflux (Scheme 33).[88] Unfortunately, the treatment of **17** with *t*-BuOK, in order to obtain the desired ylid **18**, followed by the addition of ketoaldehydes **5a,b** did not provide the desired Wittig olefination products **19** (Scheme 33). Further screening of bases (*n*-BuLi, LiHMDS and $K_2CO_3$) were also unsuccessful in this olefination reaction.

We also attempted a different approach towards the synthesis of **19.** Dichloroacetone in THF, in the presence of a single equivalent of triphenylphosphine under reflux conditions provided α-chloroketo ylid **20** in good yield (78%).[89].

Compound **20** was then reacted with the ketoaldehydes **5a,b** to provide novel ketoenones **21a,b** in 39 and 31% yield, respectively. The reaction of the α-chloro ketoenones **21a,b** with $PPh_3$ in THF was unsuccessful at either room temperature or under reflux conditions. However, although the targeted phosphorus enone **19** was not achieved, compounds **21a,b** are themselves, interesting substrates to study in a biocatalysed transamination reaction.

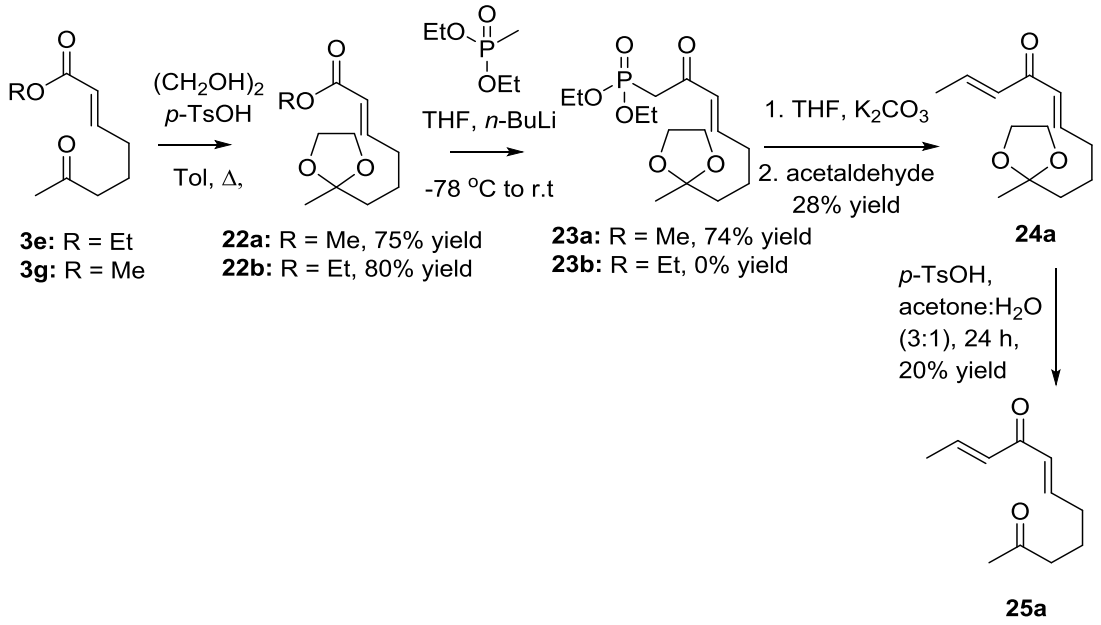



*Scheme 33: Attempted synthesis of phosphorus enone **19** from dichloroacetone*

Danishefsky's *et al.* report on the bisphosphorane **18** highlights the difficulties in the purification of the crude reaction when this reagent is employed. Therefore, they modified their synthetic route to utilise the corresponding Horner-Wadsworth-Emmons (HWE) reagent.[87] We also applied this strategy, based on the addition of the anion of methyl diethylphosphonate to a suitable ester precursor, and developed

both a linear and a convergent route to the bis-conjugated enones **25a** (Scheme 34) and **25b** (Scheme 35).

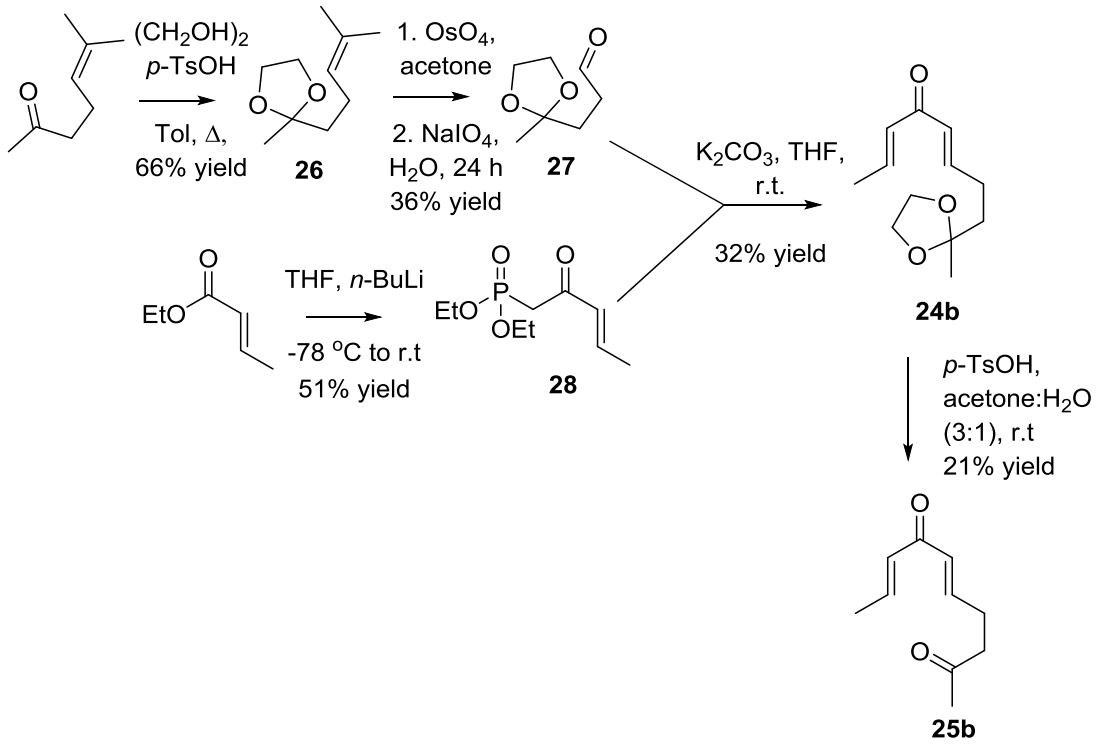
The initially employed linear route involved the selective protection of keto-esters **3e,g**, which provided ketals **22a,b** in 80 and 70% yield, respectively (Scheme 34). The nucleophilic attack of the deprotonated methyl phosphonate to the ester moiety of compounds **22a,b** only proceeded for the methyl ester substrate (R = Me, **22a**), providing **23a** in 74% yield. The deprotonation of **23a** with $K_2CO_3$ in THF followed by reaction with acetaldehyde provided **24a** in 28% yield, which upon deprotection with catalytic *p*-TsOH in a mixture of acetone:$H_2O$ (3:1), provided our target ketodienone **25a** in 20% yield**.**




Scheme 34: Direct synthetic route utilising a HWE to dienone-ketone **25a**

The convergent synthetic route towards **25b** involved the use of the corresponding HWE reagent **28** (Scheme 35). The reaction of ethyl crotonate with deprotonated diethyl methylphosphonate provided diethyl [(3*E*)-2-oxopent-3-en-1-yl]phosphonate

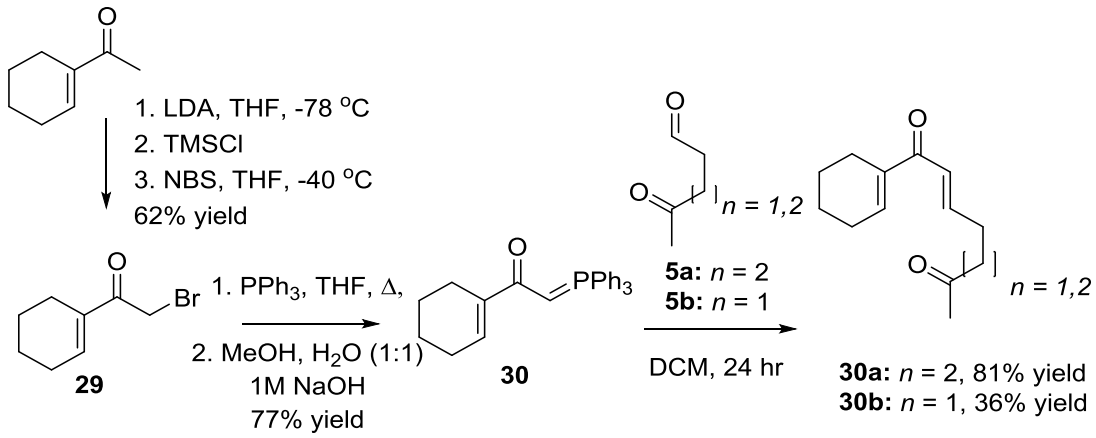
(**28**) in 51% yield.[90] The ketal aldehyde **27** was readily prepared by protection of 6-methyl-5-hepten-2-one with ethylene glycol under acidic conditions, to provide **26**, followed by oxidative cleavage of the double bond (following the same method used for the preparation of compound **5a**) providing compound **27** in 36% yield.[91] Subsequent Wittig reaction of **27** and **28** provided compound **24b** in 32% yield. Later, deprotection under the previously optimised reaction conditions, resulted in the formation of ketodienone **25b** in 21% yield.




*Scheme 35: Convergent synthetic route to dienone-ketone **25b***

A novel 1-cyclohex-1-enyl-2-(triphenylphosphanylidene)ethanone (**30**) ylid reagent has also been prepared, as a useful reagent towards the synthesis of two substrates containing bis-conjugated enones (Scheme 36). Iwasawa *et al.* report the α-bromination of 1-cyclohexenylmethylketone via enolate trapping with TMSCl and

subsequent reaction with NBS, to provide **29** in 62% yield.[92] The reaction of bromoketone **29** with triphenylphosphine, in THF under reflux conditions, generated the corresponding phosphonium salt, which was subsequently deprotonated with NaOH in methanol to provide the stable novel phosphorus ylid **30**. Reaction of **30** with ketoaldehydes **5a,b** in $CHCl_3$ at r.t., provided compounds **30a,b** in 81 and 36% yield, respectively, after purification by flash chromatography.



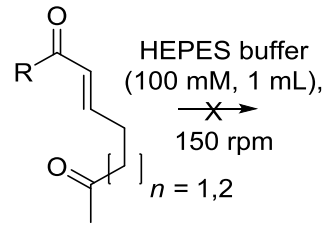

*Scheme 36: Novel 1-cyclohex-1-enyl-2-(triphenylphosphanylidene)ethanone reagent for the synthesis of bis-conjugated enones **30a,b***

## 3.4 Transamination of substrates **21a,b, 25a,b and 30a,b**

The transamination reaction of compounds **21a,b** and **25a,b** was evaluated in the presence of four different Codexis enzymes (Table 6). Using the previously optimised reaction conditions (see section 2.4 of this thesis), at the optimal working temperature for each enzyme (see Table 6), no conversion into the desired amine product was observed (Table 6, entries 1-4). Instead, complete decomposition of the starting material took place after a reaction time of 24 h. An excess of IPA was also employed in the reaction with ATA117 at 30 °C (Table 6, entry 5), but no effect on

the outcome of the reaction was observed. The reaction with ATA 256 at 50 °C was attempted at pH 7.0 and 9.0 (Table 6, entry 6 and 7), but no conversion into the desired amine product was observed in any case. The control experiment where no enzyme was present also resulted in the complete decomposition of starting materials (Table 6, entry 8).

*Table 6: Screening of transamination conditions for compounds **21a,b** and **25a,b**[a,b,c]*




**21a**, n = 2, R = $CH_2Cl$
**21b**, n = 1, R = $CH_2Cl$
**25a**, n = 2, R = $CHCHCH_3$
**25b**, n = 1, R = $CHCHCH_3$

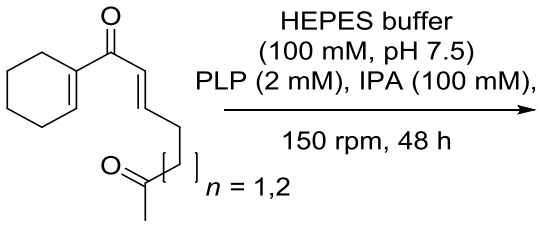
| Entry | ω-TA | Temp (°C) | pH | IPA (eq) | Conv. (%) |
|---|---|---|---|---|---|
| 1 | ATA113 | 30 | 7.5 | 2 | decomp |
| 2 | ATA117 | 30 | 7.5 | 2 | decomp |
| 3 | ATA256 | 50 | 7.5 | 2 | decomp |
| 4 | ATA025 | 50 | 7.5 | 2 | decomp |
| 5 | ATA117 | 30 | 7.5 | 20 | decomp |
| 6 | ATA256 | 50 | 7.0 | 2 | decomp |
| 7 | ATA256 | 50 | 9.0 | 2 | decomp |
| 8 | - | 30 | 7.5 | 2 | decomp |

[a] Reaction conditions: (i) ω-TA (5 mg/mL), substrate (50 mM), PLP (2 mM), HEPES buffer (1 mL, 100 mM), 150 rpm, 24 h. [b] Complete

decomposition of the starting material was observed for all entries. [c] Reactions monitored by GC-MS.

Regarding the biocatalysed transamination reaction of compounds **30a,b**, our initial screening, with ATA113 and ATA117 Codexis enzymes, showed that, under the previously optimised conditions at 30 °C (see chapter 2.4), no reaction took place (Table 7, entry 1,2) and all the starting material could be recovered at the end of the reaction. However, the use of ATA025 and ATA256 at higher temperature (50 °C), provided full conversion of the starting material (Table 7, entry 3,4).

*Table 7: Screening of transamination conditions for compounds **30a,b**[a,b]*

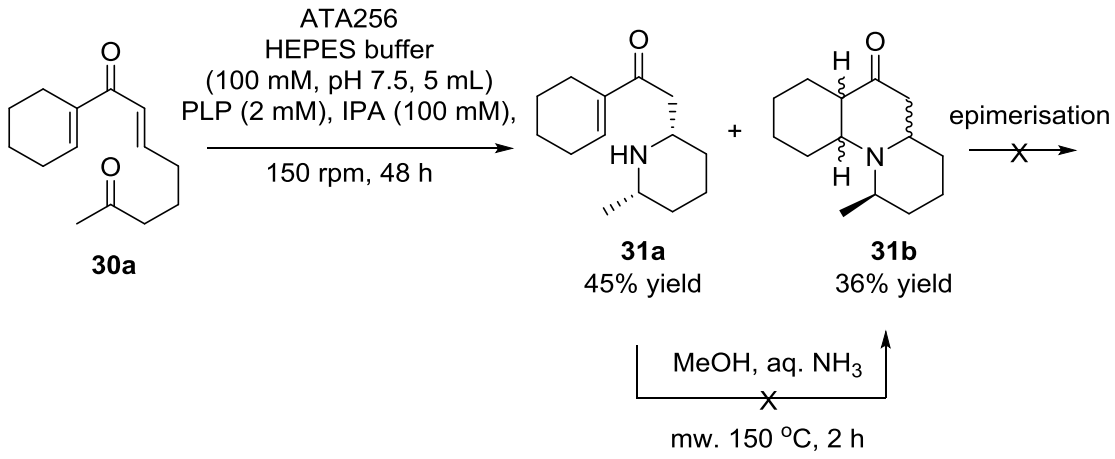



**30a**, *n* = 2
**30b**, *n* = 1

| Entry | ω-TA | Temp (°C) | Conv. of **30a and 30b** (%) |
|---|---|---|---|
| 1 | ATA113 | 30 | 0 |
| 2 | ATA117 | 30 | 0 |
| 3 | ATA256 | 50 | >99% |
| 4 | ATA025 | 50 | >99% |

[a] Reaction conditions: (i) ω-TA (5 mg/mL), substrate (50 mM), PLP (2 mM), HEPES buffer (5 mL, 100 mM, pH 7.5), isopropylamine (100 mM), 150 rpm, 24 h. [b] Reactions monitored by GC-MS.

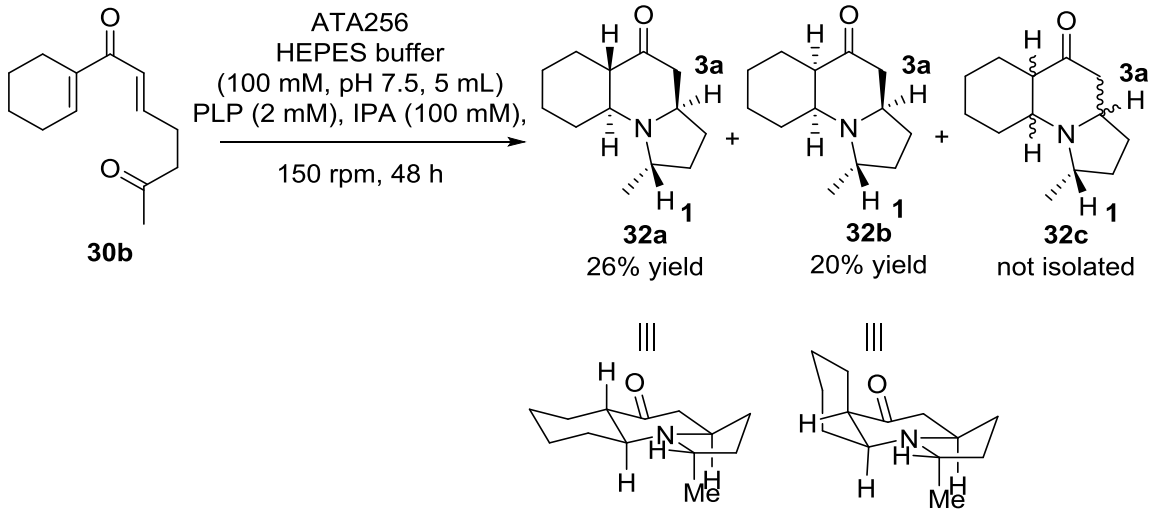
The transamination reaction of **30a** provided five compounds, with the major product, arising from single IMAMR being isolated as compound **31a** in 45% yield and **31b,** the double IMAMR product as an inseparable mixture of stereoisomers (Scheme 37). Dissolving compound **31b** and/or **31a** in MeOH (at r.t or reflux) resulted in no epimerisation in any capacity. Previous work on tetrasubstituted quinolizidines,[93] similar to compound **31a**, by Pill *et al.,* describes that heating in MeOH and aq. $NH_3$ in a sealed vessel, catalyses an IMAMR in this kind of system. Our attempts to induce the second IMAMR in **31a** in MeOH and aq $NH_3$ and heating in a microwave reactor resulted in product degradation. We also explored the possibility of epimerising the mixture of **31a,b** isomers under these conditions, which again, resulted in degradation of the products (Scheme 38).




*Scheme 37: Transamination of compound **30a***

In comparison, the biocatalysed transamination of compound **30b** with ATA256 at 50 $^{o}$C, produced three stereoisomers all rising from double aza-Michael addition, with

compound **32a** being the major product (approx. 62% calculated yield based on $^{1}$H-NMR analysis, by integration of 1-H and 3a-H, Scheme 38). However, upon flash column chromatography on silica gel, compound **32a** epimerised to a 1:1:1 mixture of compounds **32a-c**. Purification by flash chromatography allowed the isolation of compounds **32a** and **32b** in 26 and 20% yield, respectively (Scheme 38).




*Scheme 38: Transamination of compound **30b** into three stereoisomers **32a-c***

The relative stereochemistry of compound **32a** was confirmed by 2D-NMR experiments. In the NOESY spectrum (Figure 3A), we observe a coupling between the Me group and $H_B$ (*J*1) indicating a *trans*-configuration in the pyrrolidine ring. For $H_c$, we see a coupling with the Me group and $H_b$ (*J*2 and 3), however, it does not couple to $H_d$. Therefore, we can assign the absolute configuration of **32a** as *S,R,S,S* (Figure 3A).

The relative stereochemistry of compound **32b** was also confirmed by 2D-NMR experiments. The corresponding NOESY spectrum, showed a coupling between the

Me group and $H_B$ (*J*1), indicating a *trans*-configuration in the pyrrolidine ring. For $H_c$ we see a coupling with the Me group (*J*2), however, this time we do not see a coupling to $H_b$. Finally, between $H_c$ and $H_d$ we see a coupling showing a *cis*-fused cyclohexane/ piperidin-4-one configuration, leading to the assignment of the absolute configuration of **32b** as *S,R,R,S* (Figure 3B).

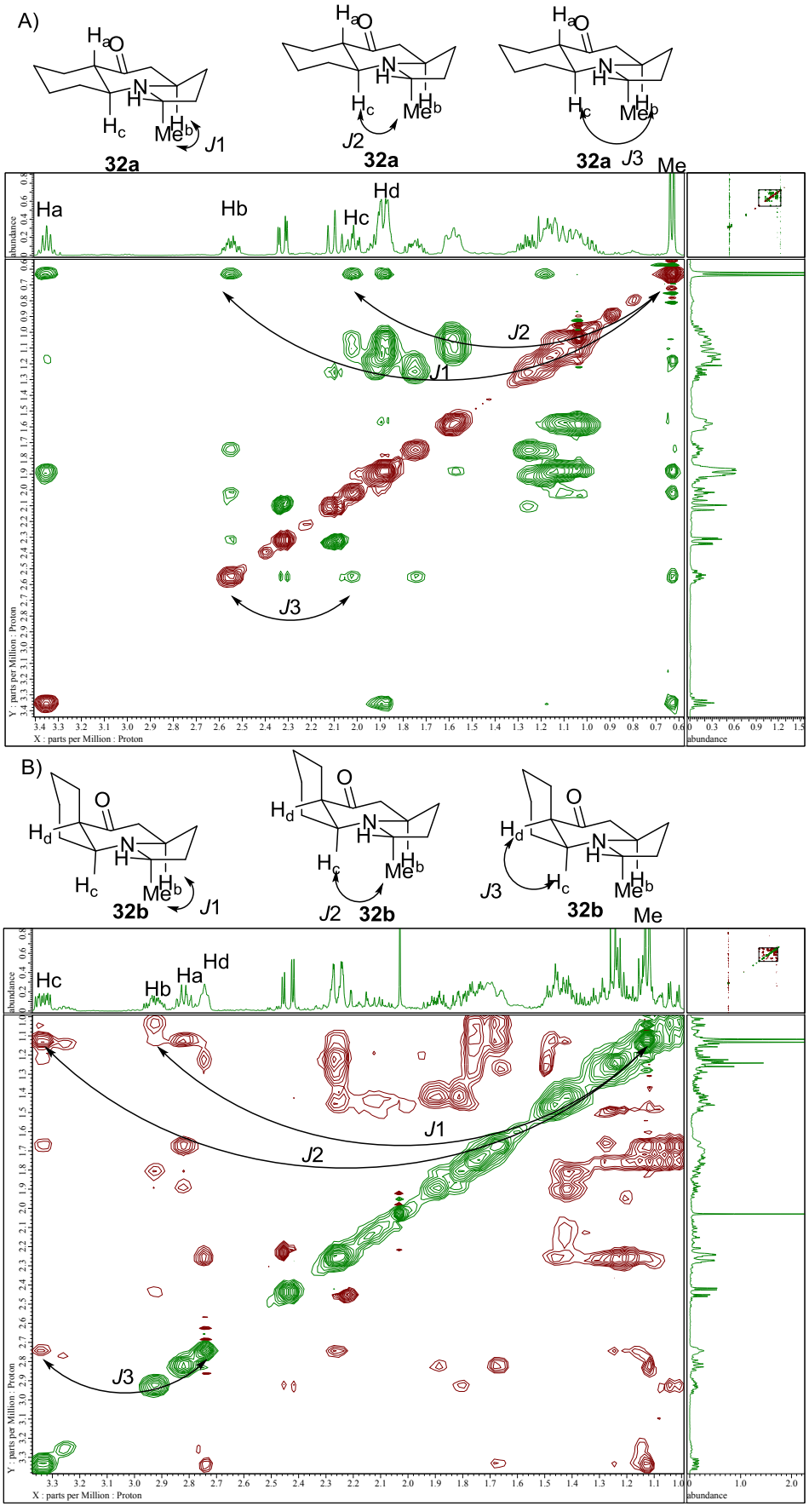



*Figure 3: A) NOESY analysis of isomers **32a** B) NOESY analysis of isomers **32b***

## 3.5 Conclusion and future work

In conclusion, two novel α-chloroenones **21a,b** have been synthesised. However, attempted transamination of these substrates resulted in complete decomposition of starting materials. We have also developed both direct and convergent methodologies for the synthesis of diconjugated substrates **25a,b**, resembling Nazarov precursors, utilising a HWE intermediate. Subsequent exposure of these diconjugated substrates, to the previously optimised TA-IMAMR conditions, resulted in complete decomposition of the starting materials in the reaction media.

A novel phosphorus yild **30** was synthesised and employed to provide diconjugated enones **30a,b**. These proved to be interesting substrates for a biocatalytic transamination, undergoing complete conversion under newly optimised conditions with ATA256, providing assorted products. Compound **30a** preferentially underwent a mono-IMAMR providing compound *cis*-**31a** as the major product. An unsuccessful attempt was made to epimerise and/or catalyse the second potential IMAMR. Therefore, compound **31a** has been here reported as the only isolated compound. Compound **30b** exclusively underwent a double IMAMR cascade providing compound **32a** as the major isomer (approx. 62% calculated yield based on $^{1}$H-NMR) and a mixture of **32b,c**. Epimerisation attempts of **32a-c** resulted in a 1:1:1 mixture of isomers. Silica gel column chromatography resulted in the epimerisation of **32a-c** and therefore **32a** was isolated in a 26% yield and **32b** in a 20% yield. The absolute configurations of **32a,b** were determined by NOESY experiments.

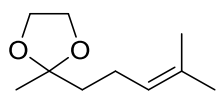
Other methods of catalysing the IMAMR of **31a** should be examined. If successful, a secondary precaution could include the selective reduction of the carbonyl group to

prevent epimerisation and/or a retro Michael reaction occurring. Any future attempt at the isolation of the compound **32a-c** should also include the selective reduction of the carbonyl group to prevent epimerisation, and therefore, increasing the isolated yield of the reduced **32a**, which would contain five stereogenic centres in good yield from a prochiral substrate. Finally, other α,β-substituted enones (similar to **30a,b**) could be investigated as substrates for transamination to see if the scope of the double-IMAMR can be expanded.

## 3.6 Experimental

**For general methods and materials, see experimental section 2.6.**

**2-methyl-2-(4-methylpent-3-en-1-yl)-1,3-dioxolane (26)**[94]



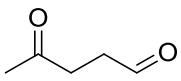
To 6-methyl-5-hepten-2-one (2.95 mL**,** 20 mmol) in toluene (20 mL), ethylene glycol (4.46 mL, 80 mmol) and *p*-TsOH (76 mg, 4.0 mmol) were added and the mixture was refluxed for a period of 6 h in a Dean-Stark apparatus, to remove water from the reaction mixture. The reaction mixture was then concentrated to remove the toluene and the resulting residue was diluted with $Et_2O$ (100 mL) and washed successively with sat. $NaHCO_3$ solution, water and brine (2 × 50 mL each). The organic layer was then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to provide the title compound as a colourless oil (2.25 g, 66% yield). R.f. 0.31 ($Et_2O$/Hexane 1:9); $^1$H NMR (400 MHz, $CDCl_3$) δ 5.10 (1H, s, 3'-H), 3.99-3.87 (4H, m, 1,3-H), 2.14-1.99 (2H, m, 2'-H), 1.73-1.58 (8H, m, 1',5',1'''-H), 1.32 (3H, s, 1''-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 131.6 (C, C-4'), 124.0 (CH, C-3'), 109.9

(C, C-2), 64.6 ($CH_2$, C-1,3), 39.1 ($CH_2$, C-1'), 25.7 ($CH_3$), 23.8 ($CH_3$), 22.7 ($CH_2$, C-2'), 17.6 ($CH_3$).

**General Procedure for the oxidative cleavage of 6-methyl-5-hepten-2-one and 2-methyl-2-(4-methylpent-3-en-1-yl)-1,3-dioxolane**

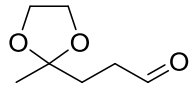
The corresponding 6-methyl-5-hepten-2-one or 2-methyl-2-(4-methylpent-3-en-1-yl)-1,3-dioxolane (**26**) (1 eq, 16.6 mmol) and $OsO_4$ (4% w.t/v *t*BuOH, 0.01 eq, 0.17 mmol, 1.06 mL) were added to a solution of *N*-methylmorpholine *N*-oxide (2 eq, 33.2 mmol, 3.89 g) in acetone (20 mL). The mixture was stirred at room temperature for 4 h and then quenched with sat. aq. $Na_2SO_3$ solution (80 mL) and extracted with $CH_2Cl_2$ (3 × 60 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The crude material was dissolved in water (20 mL) and sodium periodate (2 eq, 33.2 mmol, 7.10 g) was added. The resulting mixture was stirred for 1 h at room temperature and then diluted with water (60 mL) and extracted with $CH_2Cl_2$ (3 x 30 mL). Next, the combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to provide **5b** and **27**, which were used in the next step without further purification.

**4-oxopentanal (5b)**[95]



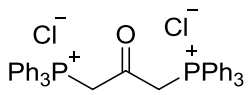
Colourless oil (3.85 g, 77 % yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 9.78 (1H, s, 1-H), 2.74 (4H, s, 2,3-H), 2.19 (3H, s, 5-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 206.4 (CO, C-4), 200.1 (CHO, C-1), 37.4 ($CH_2$, C-2), 35.4 ($CH_2$, C-3), 29.7 ($CH_3$, C-5).

**3-(2-methyl-1,3-dioxolan-2-yl)propanal (27)**[96]



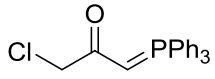
Pale yellow oil (1.03 g, 36% yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 9.71 (1H, s, 3'-H), 3.98-3.83 (4H, m, 1,3-H), 2.51-2.42 (2H, m, 2'-H), 2.06 (2H, t, J = 7.1 Hz, 1'-H), 1.33 (3H, s, 1''-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 202.1 (CO, C-3'), 109.1 (C, C-2), 64.7 ($CH_2$, C-1,3), 38.4 ($CH_2$, C-2'), 31.8 ($CH_2$, C-1'), 24.1 ($CH_3$, C-1'').

**2-oxotrimethylene-1,3-bis(triphenylphosphonium) chloride (17)**[88]



To 1,3-dichloroacetone (1.0 eq, 23.6 mmol, 3.00 g) in $CHCl_3$ (100 mL) was added triphenylphosphine (2.2 eq, 47.2 mmol, 13.64 g) and the mixture stirred under reflux for 24 h. The resulting solid was collected by filtration and washed with $Et_2O$ to provide the title compound as a white solid (11.07 g, 72% yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.86 (12H, m), 7.66 (6H, m), 7.54 (m, 12H), 6.89 (4H, d, *J* = 11.0 Hz, 1,3-H).

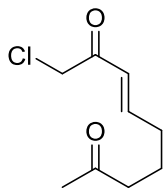
**1-chloro-3-(triphenylphosphoranylidene)acetone (20)**[89]



To 1,3-dichloroacetone (1.0 eq, 23.6 mmol, 3.00 g) in THF (100 mL) was added triphenylphosphine (1.1 eq, 26.0 mmol, 6.82 g) and the mixture stirred under reflux for 4 h. The resulting solid was collected by filtration, washed with $Et_2O$ and then redissolved in the minimal volume of MeOH/$H_2O$ (1:1). NaOH (2 M) was added until pH 8 was reached and the mixture was allowed to stir at room temperature for 24 h. The MeOH was then removed by vacuum filtration and then the aqueous layer was extracted with $CH_2Cl_2$ (3 x 50 mL). The organic layers were combined and dried over $MgSO_4$, filtered and concentrated under reduced pressure to provide the title compound as a pale orange solid. (6.52 g, 78% yield). $^{1}$H

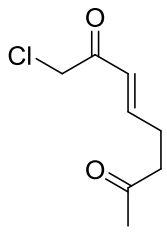
NMR (400 MHz, $CDCl_3$) δ 7.69-7.61 (6H, m), 7.60-7.52 (3H, m, 4'-H), 7.51-7.44 (6H, m), 4.28 (1H, d, *J* = 23.8 Hz, 3-H), 4.02 (2H, s, 1-H);

**(3*E*)-1-chloronon-3-ene-2,8-dione (21a)**



To 1-chloro-3-(triphenylphosphoranylidene)acetone (2.0 eq, 8.42 mmol, 2.97 g) in $CHCl_3$ (20 mL) was added ketoaldehyde **5a** (1.0 eq, 4.21 mmol, 422 mg) and the mixture stirred under refluxed for 24 h. The reaction mixture was concentrated under reduced pressure and purified by column chromatography on silica gel to provide the title compound as a yellow oil (175 mg, 39% yield). R.f. 0.39 (EtOAc/$CHCl_3$ 1:9); FTIR (neat) $V_{max}$: 2928, 1708, 1694, 1626, 1359, 1160, 901, 774 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.01-6.90 (1H, m, 4-H), 6.36-6.27 (1H, m, 3-H), 4.20 (2H, s, 1-H), 2.50-2.43 (2H, m, 7-H), 2.31-2.22 (2H, m, 5-H), 2.14 (3H, s, 9-H), 1.82-1.71 (2H, m, 6-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 207.9 (CO, C-8), 191.0 (CO, C-2), 149.4 (CH, C-4), 126.5 (CH, C-3), 47.0 ($CH_2$, C-1), 42.4 ($CH_2$, C-7), 31.7 ($CH_2$, C-5), 30.0 ($CH_3$, C-9), 21.6 ($CH_2$, C-6); LC-MS (m/z): Calculated $C_9H_{14}ClO_2^+$ $[M+H]^+$: 189.0677, found: 189.0661.

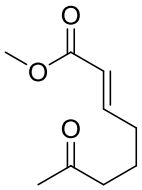
**(3*E*)-1-chlorooct-3-ene-2,7-dione (21b)**



To 1-chloro-3-(triphenylphosphoranylidene)acetone (2.0 eq, 8.42 mmol, 2.97 g) in $CHCl_3$ (20 mL) was added ketoaldehyde **5b** (1.0 eq, 4.21 mmol, 422 mg) and the mixture stirred under reflux for 24 h. The reaction mixture was concentrated under reduced pressure and purified by column chromatography on silica gel to provide the title compound as a yellow oil (228 mg, 31% yield). R.f. 0.33 (EtOAc/$CHCl_3$ 1:9); FTIR (neat) $V_{max}$: 2927, 1709, 1628, 1404,

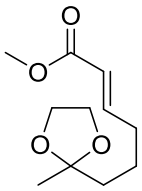
1359, 1193, 1160, 979, 772 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 7.00-6.90 (1H, m, 4-H), 6.35-6.27 (1H, m, 3-H), 4.19 (2H, s, 1-H), 2.66-2.60 (2H, m, 6-H), 2.55-2.47 (2H, m, 5-H), 2.16 (3H, s, 8-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 206.5 (CO, C-7), 190.9 (CO, C-2), 148.4 (CH, C-4), 126.5 (CH, C-3), 47.0 ($CH_2$, C-1), 41.2 ($CH_2$, C-6), 29.9 ($CH_3$, C-8), 26.3 ($CH_2$, C-5); LC-MS (m/z): Calculated $C_8H_{12}ClO_2^+$ $[M+H]^+$: 175.0520, found: 175.0515.

**methyl (2*E*)-7-oxooct-2-enoate (3g)**[97]



Compound **3g** was prepared following the same procedure for compounds **3a-e.** Colourless oil (1.01 g, 68% yield). R.f. 0.42 (EtOAc/hexane 1:1); $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 6.91 (1H, dt, J = 15.6, 6.9 Hz, 3-H), 5.85-5.80 (1H, m, 2-H), 3.72 (3H, s, 1'-H), 2.45 (2H, t, J = 7.1 Hz, 6-H), 2.24-2.18 (2H, m, 4-H), 2.13 (3H, s, 8-H), 1.78-1.69 (2H, m, 5-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.1 (CO, C-7), 166.9 (CO, C-1), 148.3 (CH, C-3), 121.6 (CH, C-2), 51.4 ($CH_2$, C-1'), 42.5 ($CH_2$, C-6), 31.3 ($CH_2$, C-4), 30.0 ($CH_3$, C-8), 21.8 ($CH_2$, C-5).

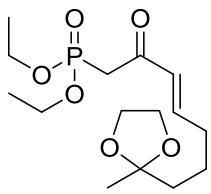
**methyl (2*E*)-6-(2-methyl-1,3-dioxolan-2-yl)hex-2-enoate (22a)**



To compound **3g** (1.0 eq, 5.29 mmol, 0.90 g) in toluene (50 mL) and *p*-TsOH (0.10 eq, 0.53 mmol, 101 mg) and ethylene glycol (3.0 eq, 15.87 mmol, 0.89 mL) were added and the mixture was refluxed for a period of 6 h in a Dean-Stark apparatus, to remove water from the reaction mixture. The reaction mixture was then concentrated to remove the toluene and the resulting residue was diluted with $Et_2O$ (100 mL) and washed successively with sat. $NaHCO_3$ solution, water and brine (2 × 50 mL each). The organic layer was then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified

by column chromatography on silica gel to provide the title compound as a colourless oil (760 mg, 75% yield). R.f. 0.31 (EtOAc/hexane 1:1); FTIR (neat) $V_{max}$: 2984, 2949, 2876, 1720, 1656, 1436, 1376, 1270, 1197, 1171, 1039, 853 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 6.95 (1H, dt, J = 15.7, 7.1 Hz, 3-H), 5.87-5.76 (1H, m, 2-H), 3.97-3.87 (4H, m, 1''-H), 3.72 (3H, s, 1'-H), 2.26-2.17 (2H, m, 4-H), 1.68-1.62 (2H, m, 6-H), 1.60-1.52 (2H, m, 5-H), 1.30 (3H, s, 8-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 167.1 (CO, C-1), 149.2 (CH, C-3), 121.1 (CH, C-2), 109.8 (CO, C-7), 64.7 ($CH_2$, C-1''), 51.4 ($CH_2$, C-1'), 38.5 ($CH_2$, C-6), 32.2 ($CH_2$, C-4), 23.8 ($CH_3$, C-8), 22.4 ($CH_2$, C-5); LC-MS (m/z): Calculated $C_{11}H_{19}O_4^+$ $[M+H]^+$: 215.1278, found: 215.1272.

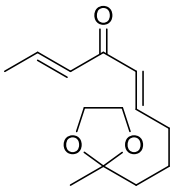
**diethyl [(3E)-7-(2-methyl-1,3-dioxolan-2-yl)-2-oxohept-3-en-1-yl]phosphonate (23a)**



To methyl diethylphosphonate (1.0 eq, 1.78 mmol, 0.26 mL) in THF (10 mL) at –78 °C was added *n*-BuLi (1.2 eq, 2.13 mmol, 0.79 mL) and the mixture stirred for 15 mins. Compound **22b** (1.0 eq, 1.78 mmol, 381 mg) in a solution of THF (2 mL) was then added slowly and the mixture allowed to stir for 12 h. The reaction mixture was then quenched with sat. $NH_4Cl$ (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by vacuum distillation and then eluted through a silica pad with MeOH/DCM (1:9) to provide the title compound as a pale yellow oil (384 mg, 74% yield). FTIR (neat) $V_{max}$: 2981, 2939, 1730, 1665, 1625, 1376, 1247, 1018, 961, 856, 799 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 6.97-6.86 (m, 1H), 6.27-6.18 (m, 1H), 4.19-4.00 (m, 4H), 3.99-3.85 (m, 4H), 3.19 (2H, d, *J* = 22.9 Hz, 1-H), 2.30-2.21

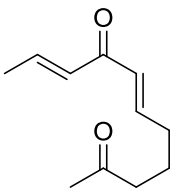
(2H, m, 5-H), 1.74-1.53 (4H, m, 6,7-H), 1.37-1.27 (9H, m); LC-MS (m/z): Calculated $C_{15}H_{28}O_6P^+$ $[M+H]^+$: 335.1618, found: 335.1616.

**(2*E*,5*E*)-9-(2-methyl-1,3-dioxolan-2-yl)nona-2,5-dien-4-one (24a)**



To compound **23b** (1.0 eq, 3.11 mmol, 904 mg) dissolved in dry THF (10 mL) at 0 °C was added $K_2CO_3$ (5.0 eq, 15.57 mmol, 2.15 mg) and the mixture stirred for 15 mins. Acetaldehyde (10 eq, 31.14 mmol, 1.74 mL) was then added and the mixture stirred for a further 12 h. The crude reaction mixture was filtered, concentrated under reduced pressure and purified by column chromatography on silica gel to provide the title compound as a colourless oil (195 mg, 28% yield). R.f. 0.40 (EtOAc/hexane 1:1); FTIR (neat) $V_{max}$: 2982, 2941, 2877, 1717, 1666, 1636, 1614, 1443, 1375, 1295, 1204, 1060, 976, 862 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.01-6.78 (2H, m, 2,6-H), 6.43-6.22 (2H, m, 3,5-H), 4.03-3.82 (4H, m, 1',3'-H), 2.29-2.19 (2H, m, 7-H), 1.91 (3H, dd, *J* = 6.9, 1.4 Hz, 1-H), 1.70-1.62 (2H, m, 9-H), 1.62-1.52 (2H, m, 8-H), 1.30 (3H, s, 1''-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 189.4 (CO, C-4), 147.3 (CH, C-6), 143.0 (CH, C-2), 130.2 (CH, C-3), 128.8 (CH, C-5), 109.8 (C, C-2'), 64.6 ($2CH_2$, C-1',3'), 38.6 ($CH_2$, C-9), 32.6 ($CH_2$, C-7), 23.8 ($CH_3$, C-1''), 22.5 ($CH_2$, C-8), 18.4 ($CH_3$, C-1); LC-MS (m/z): Calculated $C_{13}H_{21}O_3^+$ $[M+H]^+$: 225.1485, found: 225.1479.

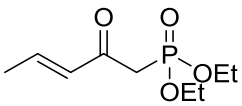
**(6*E*,9*E*)-undeca-6,9-diene-2,8-dione (25a)**



To **24a** (1.0 eq, 0.83 mmol, 150 mg) dissolved in acetone:$H_2O$ (3:1, 8 mL) was added *p*-TsOH (0.05 eq, 0.042 mmol, 8.0 mg) and the mixture stirred for 24 h. The reaction mixture was diluted with brine (10 mL)

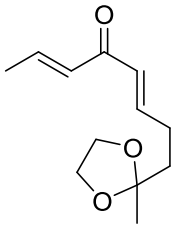
and extracted with $CH_2Cl_2$ (3 x 10 mL). The combined organic layers were then washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to provide the title compound as a colourless oil (30 mg, 20% yield). R.f. 0.31 (EtOAc/hexane 1:1); FTIR (neat) $V_{max}$: 2937, 1711, 1665, 1636, 1612, 1440, 1357, 1294, 1204, 1161, 979, 746 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 6.96-6.78 (2H, m, 6,10-H), 6.38-6.26 (2H, m, 7,9-H), 2.45 (2H, t, *J* = 7.3 Hz, 3-H), 2.29-2.18 (2H, m, 5-H), 2.12 (3H, s, 1-H), 1.91 (3H, dd, *J* = 6.9, 1.8 Hz, 11-H), 1.81-1.68 (2H, m, 4-H); LC-MS (m/z): Calculated $C_{10}H_{15}O_2^+$ $[M+H]^+$: 181.1223, found: 181.1217

**diethyl [(3*E*)-2-oxopent-3-en-1-yl]phosphonate (28)**[90]



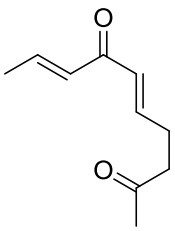

To diethyl methylphosphonate (1.0 eq, 20 mmol, 2.92 mL) in THF (80 mL) at −78 °C was added *n*-BuLi (1.2 eq, 24 mmol, 2.5 M, 9.60 mL) and the mixture stirred for 30 min. Ethyl crotonate (1.2 eq, 24 mmol, 2.98 mL) in THF (10 mL) was then added slowly and the mixture stirred for 24 h at −78 °C. The reaction mixture was then quenched with sat. $NH_4Cl$ and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by vacuum distillation to provide the title compound as a yellow oil (2.23 g, 51% yield). $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.06-6.86 (1H, m, 4-H), 6.34-6.16 (1H, m, 3-H), 4.26-3.96 (4H, m, 1'-H), 3.19 (2H, d, *J* = 22.9 Hz, 1-H), 1.97-1.90 (3H, m, 5-H), 1.36-1.27 (6H, m, 2'-H).

**(2*E*,5*E*)-8-(2-methyl-1,3-dioxolan-2-yl)octa-2,5-dien-4-one (24b)**



To a solution of **28** (2.0 eq, 2.0 mmol, 304 mg) and $K_2CO_3$ (5.0 eq, 5.0 mmol, 691 mg) in dry THF (20 mL) was added **27** (1.0 eq, 1.0 mmol 144 mg) and the mixture stirred at 0 °C for 24 h. The reaction mixture was then filtered, concentrated under reduced pressure and the residue was purified by column chromatography on silica gel to provide the title compound as a colourless oil (68 mg, 32% yield). FTIR (neat) $V_{max}$: 2936, 1713, 1665, 1376, 1163, 1042, 973, 861 731 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 6.98-6.82 (2H, m, 2,6-H), 6.38-6.27 (2H, m, 3,5-H), 3.99-3.85 (4H, m, 1',3'-H), 2.38-2.28 (2H, m, 7-H), 1.91 (3H, dd, *J* = 6.9, 1.4 Hz, 1'-H), 1.85-1.77 (2H, m, 8-H), 1.33 (3H, s, 1''-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 189.4 (CO, C-4), 147.4 (CH, C-6), 143.0 (CH, C-2), 130.2 (CH, C-3), 128.4 (CH, C-5), 109.4 (C, C-2'), 64.7 ($2CH_2$, C-1',3'), 37.4 ($CH_2$, C-8), 27.2 ($CH_2$, C-7), 24.0 ($CH_3$, C-1''), 18.4 ($CH_3$, C-1); LC-MS (m/z): Calculated $C_{12}H_{19}O_3^+$ $[M+H]^+$: 211.1329, found: 211.1326.

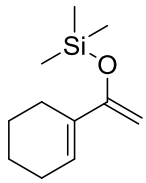
**(5*E*,8*E*)-deca-5,8-diene-2,7-dione (25b)**



To a solution of **24b** (1 eq, 0.32 mmol, 68 mg) in acetone:$H_2O$ (3:1, 4 mL) was added *p*-TsOH (0.05 eq, 0.016 mmol, 3 mg) and the mixture was stirred at r.t. for 12 h. The resulting mixture was diluted with brine (5 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were then washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel to provide the title compound as a colourless oil (11 mg, 21% yield). R.f. 0.28 (EtOAc/hexane 1:1); FTIR (neat) $V_{max}$: 2913, 1712, 1665, 1637,

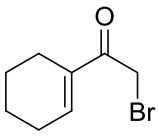
1613, 1441, 1358, 1288, 1161, 977, 868 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 7.00-6.73 (2H, m, 5,9-H), 6.40-6.22 (2H, m, 6,8-H), 2.65-2.56 (2H, m, 3-H), 2.52-2.44 (2H, m, 4-H), 2.15 (3H, s, 1-H), 1.90 (3H, dd, *J* = 6.9, 1.4 Hz, 10-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 206.9 (CO, C-2), 189.1 (CO, C-7), 145.4 (CH, C-5), 143.3 (CH, C-9), 130.2 (CH, C-8), 129.1 (CH, C-6), 41.6 ($CH_2$, C-3), 29.9 ($CH_3$, C-1), 26.3 ($CH_2$, C-4), 18.4 ($CH_3$, C-10); LC-MS (m/z): Calculated $C_{10}H_{15}O_2^+$ $[M+H]^+$: 167.1067, found: 161.1060

**1-(1-trimethylsilyloxy)vinyl-1-cyclohexene**[98]



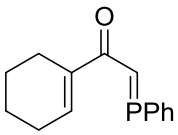

To a solution of diisopropylamine (4.23 mL, 30 mmol) in dry THF (20 mL) at −78 °C was added *n*-BuLi (12 mL, 2.5 M, 30 mmol) and the mixture was allowed to warm to −40 °C and stirred for 20 mins. The reaction was then cooled to −78 °C and 1-acetylcyclohex-1-ene (2.57 mL, 20 mmol) in dry THF (5 mL) was added dropwise and was stirred for 1 h. Trimethylsilyl chloride (3.81 mL, 30 mmol) was then added and the mixture was allowed to warm to r,t, overnight. The THF was removed under reduced pressure and the residue diluted with hexanes. The mixture was then filtered quickly through a sintered funnel. The residue was concentrated under reduced pressure to provide the crude title compound as a colourless oil. $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 6.19 (1H, m, 2'-H), 4.27 (2H, d, J = 64.1 Hz, 2-H), 2.12 (4H, m, 3',6'-H), 1.71-1.53 (4H, m, 4',5'-H), 0.22 (9H, s, 1''-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 156.5 (C, C-1), 133.0 (C, C-1'), 125.4 (CH, C-2'), 89.7 ($CH_2$, C-2), 25.4 ($CH_2$), 24.9 ($CH_2$), 22.7 ($CH_2$), 22.1 ($CH_2$), 0.0 ($CH_3$, C-1'').

**1-bromo-2-(cyclohex-1-enyl)ethan-2-one (29)**[99]



*N*-bromosuccinimide (3.56 g, 20 mmol) was added to a THF solution (30 mL) of the crude silyl enol ether at –40 °C and the mixture was stirred overnight at the same temperature. The mixture was then poured into sat. aq. $Na_2S_2O_3$ solution (60 mL) and the aqueous layer extracted with EtOAc (3 x 30 mL). The combined organic extracts were then washed with brine (2 × 60 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel (EtOAc 1:9 Hexane) to provide the title compound as an orange oil (2.53 g, 62% yield). R.f 0.31 (EtOAc 1:9 Hexane); $^1$H NMR (400 MHz, $CDCl_3$): δ 6.99 (1H, m, 2'-H), 4.17 (2H, s, 1-H), 2.39-2.18 (4H, m, 3',6'-H), 1.74-1.54 (4H, m, 4',5'-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.2 (C, C-2), 142.9 (CH, C-2'), 136.9 (C, C-1'), 30.0 ($CH_2$, C-1), 26.3 ($CH_2$), 23.2 ($CH_2$), 21.7 ($CH_2$), 21.3 ($CH_2$).

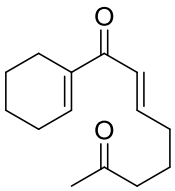
**1-cyclohex-1-enyl-2-(triphenylphosphanylidene)ethanone (30)**



To 1-bromo-2-(cyclohex-1-enyl)ethan-2-one (1.0 eq, 10 mmol, 2.04 g) in THF (20 mL) was added triphenylphosphine (1.0 eq, 10 mmol, 2.62 g) and heated to reflux for 4 h. The reaction was allowed to cool to r.t. and the precipitate was collected by filtration. The solid was then suspended in MeOH:$H_2O$ (1:1, 50 mL) and NaOH solution (1 M) was added until the mixture became slightly basic and stirred for 24 h. The MeOH was removed under reduced pressure and the aqueous solution was extracted with $CHCl_3$ (3 x 30 mL). The combined organic extracts were then washed with brine (30 mL) and dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The crude solid was recrystallised in $EtO_2$ to provide the title compound as a beige solid (2.96 g, 77% yield). FTIR (neat) $V_{max}$: 2923,

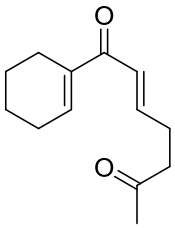
1511, 1480, 1433, 1385, 1157, 1103, 889, 714, 690 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 7.70-7.62 (6H, m, 2''-H), 7.56-7.49 (3H, m, 4''-H), 7.48-7.41 (6H, m, 3''-H), 6.68-6.59 (1H, m, 2'-H), 3.95 (1H, d, *J* = 26.4 Hz, 2-H), 2.40-2.36 (2H, m), 2.16-2.13 (2H, m), 1.69-1.63 (2H, m), 1.60-1.54 (2H, m); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 187.0 (CO, C-1), 138.8 (C, d, *J* = 20 Hz, C-1'), 133.1 (CH), 133.0 (CH), 131.8 (CH, C-4''), 131.8 (CH), 128.8 (CH, C-2'), 128.7 (CH), 127.4 (C, d, *J* = 90.6 Hz, C-1''), 49.7 (CH, d, *J* = 110.6 Hz, C-2), 25.7 ($CH_2$), 25.6 ($CH_2$), 22.9 ($CH_2$), 22.1 ($CH_2$); $^{31}P$ NMR (161 MHz, $CDCl_3$) δ 16.9 (s); LC-MS (m/z): Calculated $C_{26}H_{26}OP^+$ $[M+H]^+$: 385.1721, found: 385.1716.

**(2*E*)-1-(cyclohex-1-en-1-yl)oct-2-ene-1,7-dione (30a)**



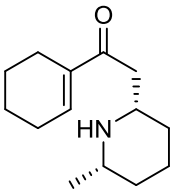
Compound **30a** was prepared following the same procedure for compounds **3a-e.** Yellow oil (1.78 g, 81% yield). R.f 0.42 (EtOAc 1:1 Hexane); FTIR (neat) $V_{max}$: 2932, 2860, 1712, 1659, 1633, 1612, 1422, 1354, 1285, 1202, 1157, 979, 732 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 6.89 (1H, m, 2'-H), 6.85-6.75 (1H, m, 3-H), 6.65 (1H, m, 2-H), 2.50-2.42 (2H, m, 6-H), 2.31-2.19 (6H, m, 4,3',6'-H), 2.13 (3H, s, 8-H), 1.81-1.70 (2H, m, 5-H), 1.68-1.57 (4H, m, 4',5'-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.3 (CO, C-7), 191.1 (CO, C-1), 145.5 (CH, C-3), 140.2 (CH, C-2'), 139.8 (C, C-1'), 125.4 (CH, C-2), 42.7 ($CH_2$, C-6), 31.7 ($CH_2$, C-4), 30.0 ($CH_3$, C-8), 26.1 ($CH_2$), 23.4 ($CH_2$), 22.1 ($CH_2$, C-5), 21.9 ($CH_2$), 21.6 ($CH_2$); LC-MS (m/z): Calculated $C_{14}H_{21}O_2^+$ $[M+H]^+$: 221.1542, found: 221.1536.

**(2*E*)-1-(cyclohex-1-en-1-yl)hept-2-ene-1,6-dione (30b)**



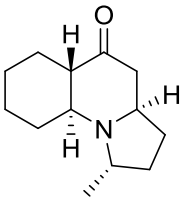
Compound **30b** was prepared following the same procedure for compounds **3a-e** where **5b** is the appropriate aldehyde**.** Yellow oil (750 mg, 36% yield). R.f 0.39 (EtOAc 1:1 Hexane); FTIR (neat) $V_{max}$: 2930, 2860, 1713, 1659, 1633, 1612, 1422, 1364, 1204, 1160, 976, 921, 794 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 6.88 (1H, s, 2'-H), 6.77 (1H, dt, *J* = 15.3, 6.6 Hz, 3-H), 6.65 (1H, d, *J* = 15.3 Hz, 2-H), 2.61 (2H, t, *J* = 7.1 Hz, 5-H), 2.53-2.43 (2H, m, 4-H), 2.29-2.22 (4H, m, 3',6'-H), 2.15 (3H, s, 7-H), 1.66-1.58 (4H, m, 4',5'-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 207.1 (CO, C-6), 191.1 (CO, C-1), 144.4 (CH, C-3), 140.4 (CH, C-2'), 139.7 (C, C-1'), 125.6 (CH, C-2), 41.8 ($CH_2$, C-5), 30.0 ($CH_3$, C-7), 26.4 ($CH_2$, C-4), 23.4 ($CH_2$), 22.1 ($CH_2$), 21.9 ($CH_2$), 21.6 ($CH_2$); LC-MS (m/z): Calculated $C_{13}H_{19}O_2^+$ $[M+H]^+$: 207.1385, found: 207.1380.

**1-(cyclohex-1-en-1-yl)-2-[(2*S*,6*S*)-6-methylpiperidin-2-yl]ethanone (31a)**



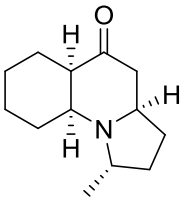
Compound **31a** was prepared following the same procedure for compounds **1f.** Colourless oil (25mg, 45% yield). FTIR (neat) $V_{max}$: 2952, 2856, 1740, 1660, 1637, 1435, 1375, 1318, 1284, 1193, 1163, 1122, 921, 773, 731 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 6.97-6.82 (1H, m, 2'-H), 3.13-2.93 (1H, m, 2''-H), 2.85 (1H, br s, 1''-H), 2.77-2.71 (2H, m, 2-H), 2.70-2.61 (1H, m, 6''-H), 2.30-2.09 (4H, m), 1.79-1.67 (1H, m), 1.65-1.48 (6H, m), 1.45-1.29 (1H, m), 1.04 (3H, d, *J* = 6.4 Hz, 1'''-H), 1.27-0.99 (2H, m); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 200.5 (CO, C-1), 140.6 (CH, C-2'), 139.4 (C, C-1'), 53.1 (CH, C-2''), 52.2 (CH, C-6''), 43.6 ($CH_2$, C-2), 33.6 ($CH_2$), 31.8 ($CH_2$), 26.0 ($CH_2$), 24.5 ($CH_2$), 22.9 ($CH_2$), 22.7 ($CH_3$, C-1'''), 21.8 ($CH_2$), 21.4 ($CH_2$); LC-MS (m/z): Calculated $C_{14}H_{24}NO^+$ $[M+H]^+$: 222.1852, found: 222.1860.

**(1*S*,3a*R*,5a*S*,9a*S*)-1-methyldecahydropyrrolo[1,2-*a*]quinolin-5(1*H*)-one (32a)**



Compound **32a** was prepared following the same procedure for compounds **1f.** Colourless oil (13 mg, 26% yield). $^{1}$H NMR (400 MHz, *o*-$C_6D_4Cl_2$) δ 3.48-3.37 (1H, m, 1-H), 2.68-2.55 (1H, m, 3a-H), 2.39 (1H, dd, *J* = 12.5, 3.2 Hz, 4-H), 2.16 (t, *J* = 12.5 Hz, 4-H), 2.09 (1H, td, *J* = 9.8, 3.1 Hz, 9a-H), 1.99-1.91 (3H, m, 5a-H), 1.87-1.75 (1H, m), 1.71-1.59 (2H, m), 1.38-1.02 (7H, m), 0.70 (3H, d, *J* = 6.9 Hz, 1'-H); $^{13}$C NMR (100 MHz, *o*-$C_6D_4Cl_2$) δ 208.3 (CO, C-5), 60.6 (CH, C-9a), 58.5 (CH, C-3a), 53.7 (CH, C-5a), 52.3 (CH, C-1), 48.1 ($CH_2$, C-4), 31.8 ($CH_2$), 30.3 ($CH_2$), 30.0 ($CH_2$), 25.3 ($CH_2$), 24.8 ($CH_2$), 24.0 ($CH_2$), 13.0 ($CH_3$, C-1').

**(1*S*,3a*R*,5a*R*,9a*S*)-1-methyldecahydropyrrolo[1,2-*a*]quinolin-5(1*H*)-one (32b)**



Compound **32b** was prepared following the same procedure for compounds **1f.** Colourless oil (10 mg, 20% yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.39-3.31 (1H, m, 9a-H), 2.99-2.89 (1H, m, 3a-H), 2.88-2.78 (1H, m, 1-H), 2.78-2.73 (1H, m, 5a-H), 2.45 (1H, dd, *J* = 14.0, 3.4 Hz, 4-H), 2.32-2.23 (2H, m, 4-H), 1.98-1.64 (5H, m), 1.53-1.36 (3H, m), 1.14 (3H, d, *J* = 6.0 Hz, 1'-H), 1.35-0.96 (3H, m); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 210.9 (CO, C-5), 56.7 (CH, C-3a), 55.6 (CH, C-9a), 52.4 (CH, C-1), 49.6 (CH, C-5a), 47.9 ($CH_2$, C-4), 31.1 ($CH_2$), 29.6 ($CH_2$), 25.0 ($CH_2$), 24.9 ($CH_2$), 22.4 ($CH_2$), 19.6 ($CH_2$), 18.5 ($CH_3$, C-1').

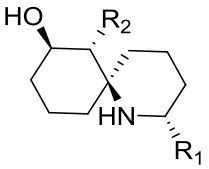
# 4. Synthesis of Perhydrohistrionicotoxin Analogues

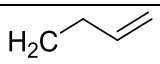
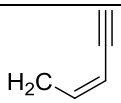
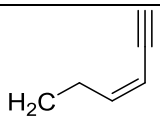
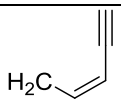
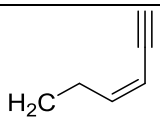
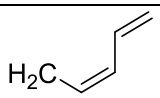
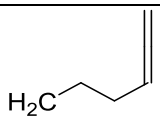
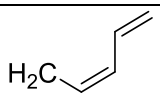
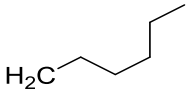
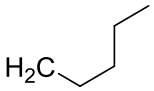
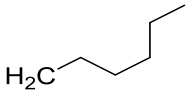
## 4.1 Introduction

When considering the design of potential novel drug candidates, the affinity towards the specific drug-binding site is a key factor.[100] Spiro-fused bicyclic ring systems containing quaternary $sp^3$ carbons provide rigid 3D-motifs capable of enhanced selectivity in drug binding.[101] Interestingly, not many general synthetic methods towards the synthesis of 2-spiropiperidines have been developed to date.[102]

Histrionicotoxins (HTXs) (**33**) are naturally occurring alkaloids, isolated from the skin of Colombian frogs (*Dendrobates histrionicus*) that contain a 2-spiropiperidine motif (Table 8).[103] Due to its unique structure and potent biological activity, as a very selective non-competitive antagonist of the nicotinic acetylcholine receptor,[104–106] numerous research groups have developed imaginative routes towards this molecule and related derivatives.[107–113]

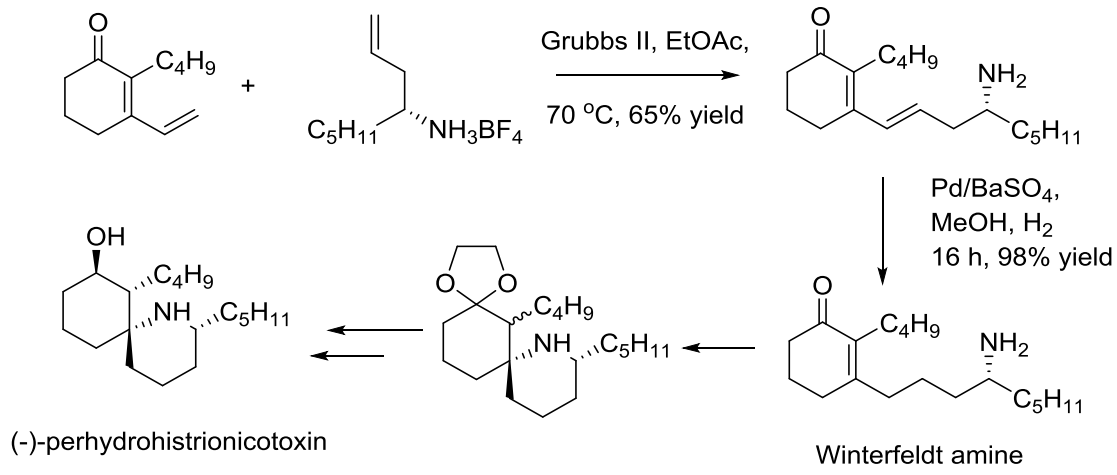
*Table 8: Structures of Histrionicotoxin alkaloids*




| **(HTX)** | **$R_1$** | **$R_2$** |
|---|---|---|
| 259A |  |  |
| 283A[113] |  |  |
| 285B |  |  |
| 287A |  |  |
| perhydro[114] |  |  |
| 7-debutyl-perhydro |  | H |

The implementation of an IMAMR towards the synthesis of HTX products has previously been explored. Winterfeldt *et al.* synthesised perhydrohistrionicotoxin via a key IMAMR reaction. However, the reaction was not spontaneous and required the formation of a ketal to force the IMAMR (Scheme 39).[115] Godleski *et al.* then reported a Lewis acid-catalysed IMAMR of analogous non-cyclised amine intermediates.[116] Most recently, Robinson *et al.* have reported a shorter synthetic

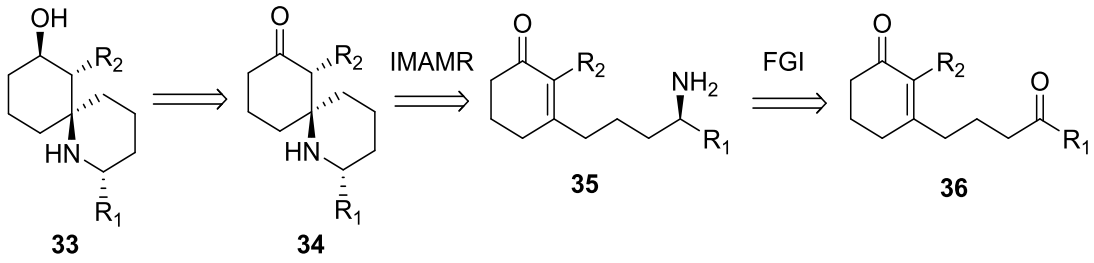
route to the Winterfeldt amine via a cross-metathesis–hydrogenation during a formal synthesis of (-)-perhydrohistrionicotoxin. During this study, it was confirmed that the ketal formation is optimal for inducing the IMAMR (Scheme 39).[114]




*Scheme 39: Reported synthesis of (-)-perhydrohistrionicotoxin by Robinson et al.*[114]

## 4.2 Aims and objectives

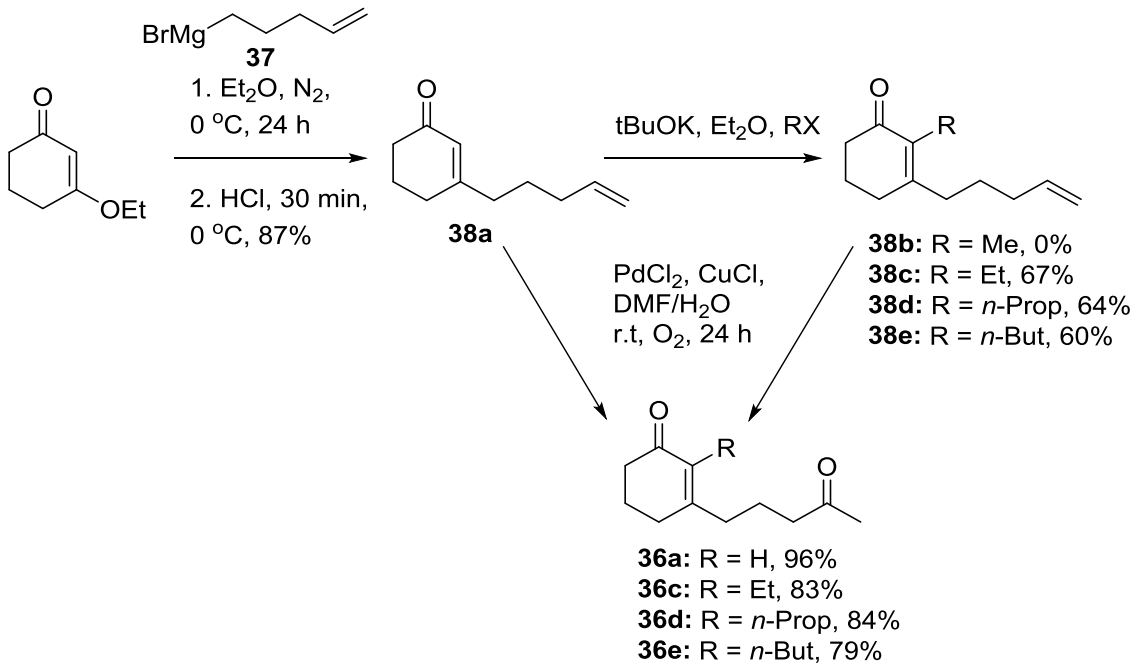
Herein we explore the TA-triggered IMAMR towards the synthesis of HTX derivatives. The proposed retrosynthetic analysis of the core HTX structure involves the oxidation of the chiral alcohol **33** followed by a retro-IMAMR to the comparable Winterfeldt amine **35** (Scheme 40). A synthetic route to compound **36** is planned and optimised. Then, investigation of the biocatalysed transamination reaction of **36** and their subsequent epimerisation are examined.




*Scheme 40: Retrosynthetic analysis of the core structure of HTX*

## 4.3 Synthesis of keto-cyclohexanones **36a-e**

Suemune *et al.* reported the synthesis of compound **36a** (R=H) in a two-step synthesis (Scheme 41).[117] This synthetic route consists of the addition of the Grignard reagent **37** onto commercially available 3-ethoxy-2-cyclohexenone. Selective Wacker oxidation of the terminal alkene in **38a** provides the ketoenone **36a**. We applied the same strategy to generate the desired histrionicotoxin precursor **36a-e.** The addition of the Grignard reagent **37** to 3-ethoxy-2-cyclohexenone produced target compound **38a** in 87% yield. For the Wacker oxidation, Suemune *et al.* reported the use of 0.5 eq of $PdCl_2$ and 4.0 eq of CuCl as co-catalyst, which provides a 76% yield.[117] In comparison, we found that, when 0.1 eq of $PdCl_2$ and 2 eq of the CuCl, the reaction proceeded with full conversion and provided the target compound **36a** in an increased 96% yield.




*Scheme 41: Synthetic route to keto-cyclohexenones **36a-e***

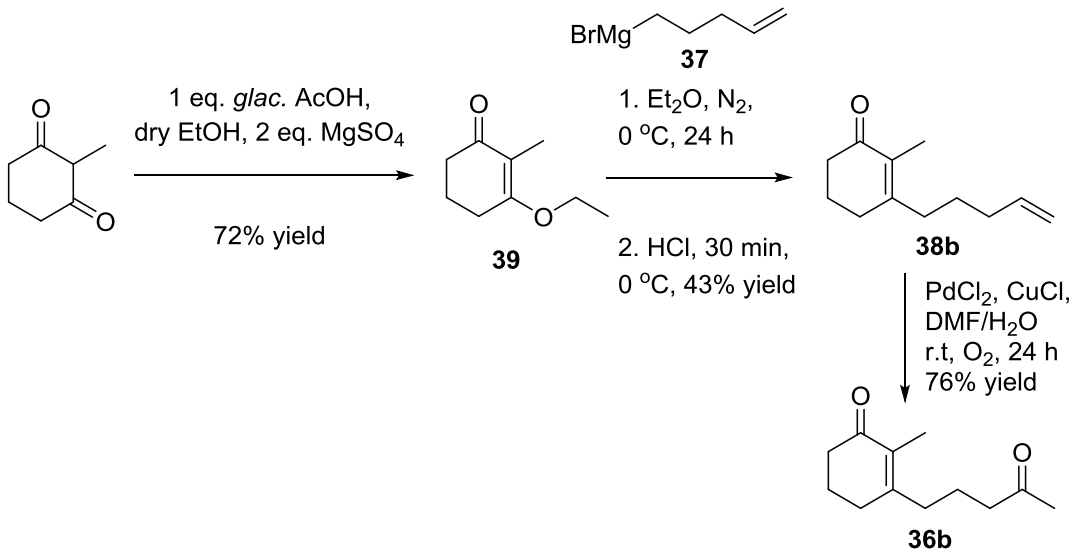
A Baylis-Hillman reaction was employed to insert alkyl groups as substituents onto the α-position of compound **38a**. The alkylation reaction of **38a** with MeI, using potassium *tert*-butoxide as base in THF, at 0 °C,[118] was unsuccessful (Table 9, entry 1); however, the use of bromoethane as electrophile, under the same reaction conditions, provided **38c** in a modest 33% yield (Table 9, entry 2). When the reaction with bromoethane was carried out at room temperature, an increased 56% yield of **38c** was obtained (Table 9, entry 3). At this optimal temperature, a change in the solvent of the reaction to diethyl ether provided **38c** in 67% yield and a drastic decrease in side product formation, allowing for easier purification (Table 9, entry 4). The alkylation of **38a** with bromopropane and bromobutane provided comparable yields for the synthesis of **38d,e** (Table 9, entry 5 and 6). Wacker oxidation of

compounds **38c-e** utilising the previously optimised conditions developed for the oxidation of **38a** provided **36c-e** in good yield (Scheme 41).

*Table 9: Baylis-Hillman Reaction Optimisation*[a]

| **Entry** | **RX** | **Solvent** | **Temp (°C)** | **Yield (%)**[b] |
|---|---|---|---|---|
| 1 | MeI | THF | 0 | n.d |
| 2 | EtBr | THF | 0 | 33 |
| 3 | EtBr | THF | 25 | 56 |
| 4 | EtBr | $Et_2O$ | 25 | 67 |
| 5 | *n*-PrBr | $Et_2O$ | 25 | 64 |
| 6 | *n*-BuBr | $Et_2O$ | 25 | 60 |
| [a] 1.5 eq *t*BuOK, 1.2 eq RX, 1 h. [b] Isolated yield after flash chromatography | | | | |

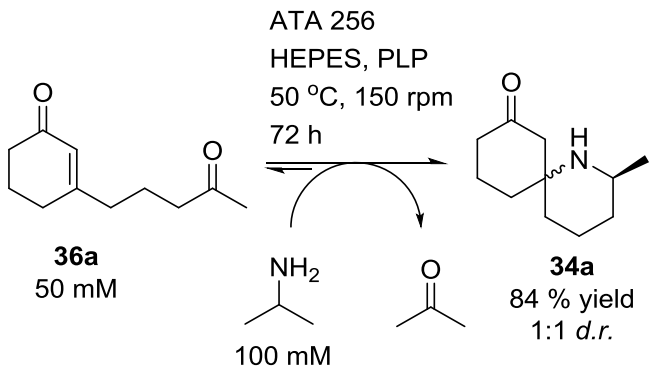
An alternative synthetic approach for the methyl substituted cyclohexanone **38b** was developed, due to the Baylis-Hillman reaction proving ineffective with iodomethane. Thus, the commercially available 2-methyl-cyclohexan-1,3-dione was converted to **39** in the presence of acid, in 72% yield (Scheme 42). The addition of **37** to compound **39** provided **38b** in 43% yield. Subsequent Wacker oxidation reaction of **38b**, under the previously optimised conditions for the synthesis of **38a,c-e**, provided our target compound **36b** in 76% yield (24% overall yield from starting material 2-methyl-cyclohexan-1,3-dione).




Scheme 42: Synthesis of 2-methyl-3-(4-oxopentyl)cyclohex-2-en-1-one (**36b**)

## 4.4 Transamination of substrates **36a,c**

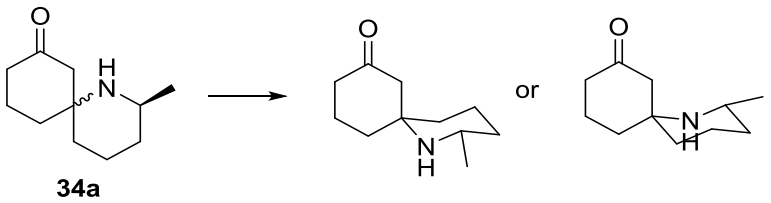
The transamination reaction of the unsubstituted keto enone **36a**, under the conditions developed in section 2.4 (ATA113, PLP, HEPES, 2 eq IPA, 30 °C, 24 h) resulted in no conversion. We believe that the IMAMR at a disubstituted β-position of the enone proceeds via a higher activation energy due to steric hindrance, hampering the equilibrium drive of our methodology. The use of the Codexis enzyme ATA 256 instead of ATA 113, allows the use of higher temperatures of the reaction (50 °C), which could potentially facilitate the desired IMAMR. Thus, the reaction of **36a** with ATA 256 at 50 °C, in the presence of two equivalent of IPA, provided, after three days, full conversion of starting material into the desired spirocyclic compound **34a** as a mixture of diastereomers (Scheme 43).




*Scheme 43: Transamination of **36a** for the synthesis of compound **34a***

The diastereomeric ratio (*d.r.*) of spirocycle **34a** was determined to be 1:1 by analysis of the $^{1}$H NMR spectrum and GC-MS. Traditional epimerisation methods performed on these substrates were tested with limited success.[116] Stirring in MeOH at room temperature or at reflux, as previously described for 2,6-disubstituted piperidines (section 2.4), provided no change in *d.e.* and induced the degradation of spirocycle **34a** (Table 10, entry 1-2). The addition of sodium methoxide in methanol at room temperature had no effect in the diastereomeric ratio of the crude mixture (Table 10, entry 3-4). Changing the solvent to $CH_3Cl$ or toluene yielded no epimerisation when sodium methoxide was employed at either room temperature or reflux conditions (Table 10, entries 5-8). Next, we examined two solid phase catalysts, silica gel and basic alumina, which provided no epimerisation (Table 10, entries 9 and 12). Fortunately, neutral alumina, at room temperature, provided a 3:1 mixture of diastereomers **34a** (Table 10, entry 10). When the reaction with neutral alumina was carried out at higher temperature (50 °C) the diastereomeric ratio did not improve further than the previous 3:1 ratio (Table 10, entry 11).

*Table 10: Epimerisation of spirocycle **34a***



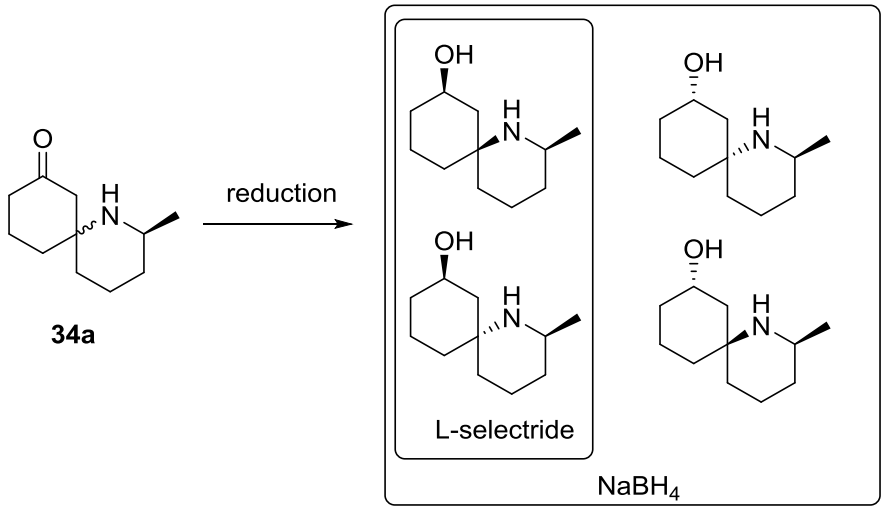

**34a**

| Entry | Solvent | Additive[a] | Temp (°C) | *d.r.*[b,c] |
|---|---|---|---|---|
| 1 | MeOH | - | 25 | 1:1 |
| 2 | MeOH | - | 65 | 1:1 |
| 3 | MeOH | NaOMe | 25 | 1:1 |
| 4 | MeOH | NaOMe | 65 | 1:1 |
| 5 | $CH_3Cl$ | NaOMe | 25 | 1:1 |
| 6 | $CH_3Cl$ | NaOMe | 40 | 1:1 |
| 7 | Tol | NaOMe | 25 | 1:1 |
| 8 | Tol | NaOMe | 110 | 1:1 |
| 9 | - | Silica gel | 25 | 1:1 |
| 10 | - | Neutral alumina | 25 | 3:1 |
| 11 | - | Neutral alumina | 50 | 3:1 |
| 12 | - | Basic alumina | 25 | 1:1 |

[a] 2 eq. of additive. [b] *D.r*. determined by GC-MS. [c] *D.r* tested every 12 h until no change was observed.

Attempted separation of the 3:1 mixture of diastereomers **34a** on silica gel proved unsuccessful, as epimerisation (via a retro Michael reaction) occurred. For this reason, we attempted the reduction of the 3:1 mixture of diastereomers **34a** with

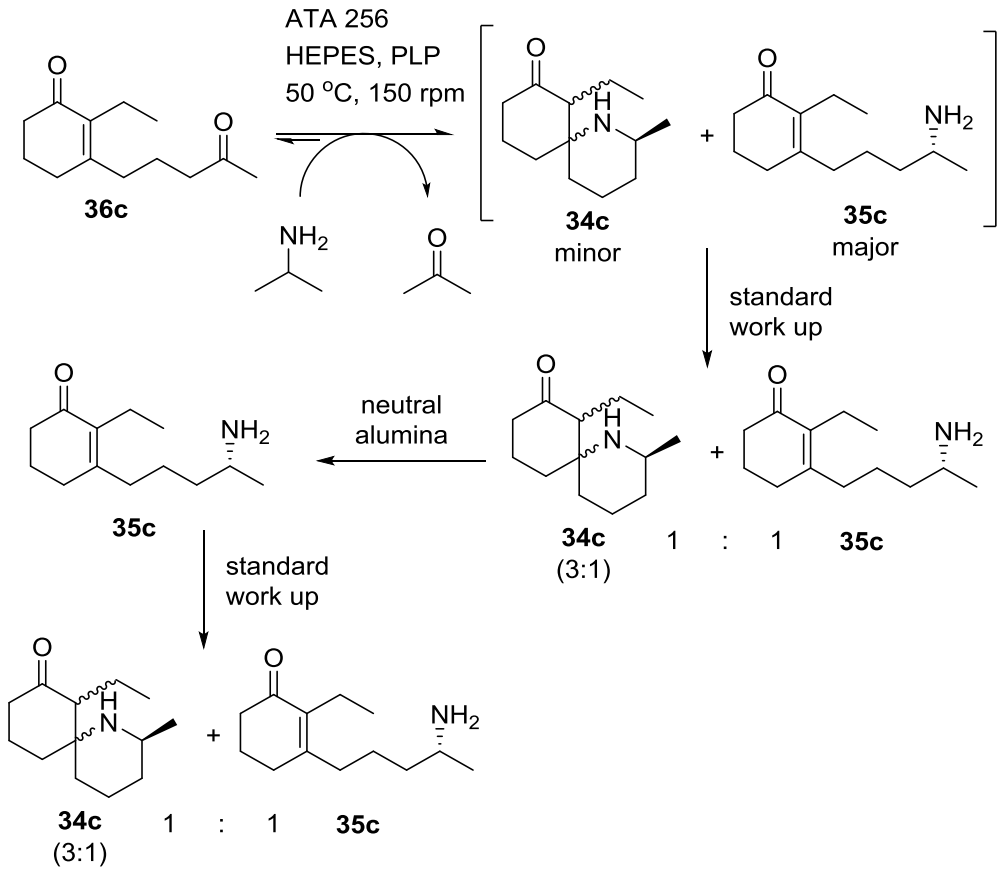
$NaBH_4$ and L-selectride. Although the reduction of the crude **34a** (3:1 diastereomeric mixture) proceeded with full conversion to HTX **33a**, separation of the potential four diastereomers by column chromatography was unsuccessful. Magnus *et al.* had reported that, when employing the bulky reducing agent L-selectride, the reduction proceeds with good selectivity.[119] In our case, this method provided two major isomers (identified by $^1$H NMR) (Scheme 44). In contrast, the use of the non-bulky (and therefore less selective) $NaBH_4$ provided a 3:3:1:1 mixture of **33a**.




*Scheme 44: Reduction of **34a** with the reducing agents $NaBH_4$ and L-selectride*

Investigations into the transamination reaction of the α-ethyl ketocyclohexanone **36c** were also carried out. Unfortunately, the optimised conditions for the unsubstituted ketocyclohexanone **36a** (ATA256, 50 $^o$C, 72 h) proved to be ineffective. After two days, only two minor peaks representing the corresponding cyclised product **34c** were present in the GC-MS analysis of a crude sample. In addition, an assumed non-cyclised product **35c** was identified in the GC-MS chromatogram of the sample (Scheme 45). A standard work up of the reaction mixture (extraction with

EtOAc and concentration under reduced pressure) shifted the reaction towards the cyclised product **34c**, providing a 3:1 mixture of isomers **34c**, and non-cyclised **35c**. The epimerisation of this 3:1 mixture of isomers **34c** and **35c** was attempted utilising neutral alumina. However, these conditions promoted a retro-Michael reaction and the reformation of non-cyclised product **35c** as the only product. Standard work up of the non-cyclised **35c** provides the 1:1 mixture of **35c** and **34c** (3:1 *d.r.*). Isolation of products **34c** and/or **35c** on silica gel proved unsuccessful.




*Scheme 45: Transamination of keto cyclohexanone **36c** and epimerisation of **34c** and **35c***

Preliminary work on the transamination of **36b,d** and **e** revealed similar behaviour to substrate **36c**, with low conversions towards the desired spirocyclic systems **34b,d**

and **e**. In addition, we observed that, as the alkyl chain length at the $\alpha$ position of the enone increased, the aza-Michael cyclisation became less favourable and the conversion of the reaction decreased.

## 4.5 Conclusion and future work

In conclusion, we have developed a practical methodology towards the synthesis of 2-alkyl-3-(4-oxopentyl)cyclohex-2-en-1-one substrates **36a-e**, by means of an optimised Baylis-Hillman reaction. The transamination reaction for the unsubstituted ketocyclohexanone **36a** proceeded well, which is ascribed to the Michael product **34a** being stable in the reaction media. In this case, the IMAMR provided the expected thermodynamic driving force towards the formation of the desired cyclic product requiring no additional drive. Epimerisation of the diastereomeric mixture yielded a 3:1 *d.r.* of isomers, however, purification/separation of the two diastereomers proved unsuccessful. The selective reduction of the carbonyl offers a potential route to isolating spirocyclic compound **33a**, but this reaction needs optimisation. Preparative (chiral) HPLC could also be employed for the separation of isomers.

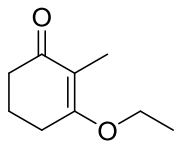
The transamination of 2-ethyl-3-(4-oxopentyl)cyclohex-2-en-1-one **36c** under the previously optimised reaction conditions proved unsuccessful. This can be explained by the steric clash of the alkyl substituent with the piperidine ring, which induces a retro-Michael reaction, negating the possibility of the IMAMR driving the equilibrium towards the formation of the desired product **34c**. The use of an excess of amine donor was also examined, however, it had negligible effect on the reaction equilibrium. We believe that future attempts at the transamination of 2-alkyl-3-(4-

oxopentyl)cyclohex-2-en-1-one **36b-e** should include an external method of driving the reaction equilibrium towards the formation of product (e.g. IPA removal system, *o*-xylylenediamine as the amine donor or use of a flow reactor). In addition, isolation of the Winterfeldt amines **34b-e**[113] could offer a competitive route to non-natural derivatives of perhydrohistrionicotoxin.

## 4.6 Experimental

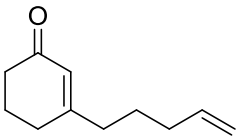
**For general methods and materials, see experimental section 2.6.**

**3-ethoxy-2-methylcyclohex-2-en-1-one (39)**[120]



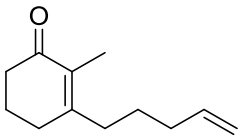
To 2-methyl-1,3-cyclohexanedione (1.0 eq, 30.52 mmol, 3.85 g) dissolved in ethanol was added glacial acetic acid (1.0 eq, 30.52 mmol, 1.75 mL) and $MgSO_4$ (5.0 eq, 152.6 mmol, 18.36 g) and the mixture left to stir for 24 h at room temperature. The crude product was filtered, concentrated under reduced pressure and purified by column chromatography on silica gel ($Et_2O$) to provide the title compound as a yellow oil (3.37 g, 72% yield). R.f 0.26 ($Et_2O$); FTIR (neat) $\nu_{max}$ ($cm^{-1}$): 3411, 2938, 1732, 1647, 1617, 1197, 1116; $^1H$ NMR (400 MHz, $CDCl_3$): δ 4.05 (2H, q, *J* = 7.2 Hz, 1''-H), 2.54-2.52 (2H, m, 4-H), 2.34-2.31 (2H, m, 6-H), 1.99-1.96 (2H, m, 5-H), 1.68 (3H, s, 1'-H), 1.37-1.33 (3H, t, *J* = 5.3 Hz, 2''-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 199.1 (CO, C-1), 171.6 (C, C-3), 115.0 (C, C-2), 63.5 ($CH_2$, C-1''), 36.2 ($CH_2$, C-6), 25.3 ($CH_2$, C-4), 21.0 ($CH_2$, C-5), 15.3 ($CH_3$, C-2''), 7.4 ($CH_2$, C-1'); LC-MS (m/z): calculated $C_9H_{15}O_2$ $[M+H]^+$: 155.1067, found: 155.0994.

**3-(4-Pentenyl)cyclohex-2-en-1-one (38a)**[117]



To a solution of Grignard reagent prepared from 5-bromo-1-pentene (1.3 eq, 26 mmol, 3.08 mL) and magnesium metal (1.2 eq, 24 mmol, 564 mg) in diethyl ether (25 mL) was added 3-ethoxy-2-cyclohexenone (1 eq, 20 mmol, 2.91 mL) in diethyl ether (20 mL) at room temperature and stirred for 30 min. The reaction mixture was quenched with sat. $NH_4Cl$ solution (50 mL). The aqueous mixture was extracted with EtOAc (3 x 20 mL), the organic extracts were combined, washed with brine (50 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel (EtOAc 1:9 hexane) to provide the title compound as a colourless oil (2.84 g, 87% yield). R.f 0.38 (EtOAc 3:7 Hexane); FTIR (neat) $V_{max}$: 2930, 1665, 1624, 1428, 1251, 909, 888 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 5.87 (1H, s, 2-H), 5.84-5.71 (1H, m, 4'-H), 5.05-4.95 (2H, m, 5'-H), 2.35 (2H, t, J = 6.6 Hz, 6-H), 2.28 (2H, t, J = 6.0 Hz, 4-H), 2.21 (2H, t, J = 7.8 Hz, 1'-H), 2.11-2.03 (2H, m, 3'-H), 2.02-1.94 (2H, m, 5-H), 1.64-1.55 (2H, m, 2'-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 199.9 (CO, C-1), 166.2 (C, C-3), 137.8 (CH, C-4'), 125.8 (CH, C-2), 115.3 ($CH_2$, C-5'), 37.3 ($2CH_2$, C-6,1'), 33.2 ($CH_2$, C-3'), 29.7 ($CH_2$, C-4), 26.0 ($CH_2$, C-2'), 22.7 ($CH_2$, C-5); LC-MS (m/z): calculated $C_{11}H_{17}O$ $[M+H]^+$: 165.1279, found: 165.1273.

**2-methyl-3-(pent-4-en-1-yl)cyclohex-2-en-1-one (38b)**[121]



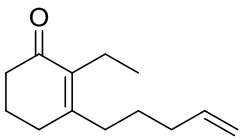
Compound **38b** was prepared following the same procedure for compounds **38a**, starting from compound **39**. The resulting crude material was purified by column chromatography ($EtO_2$ 4:6 hexane) to provide a yellow oil (845 mg, 43% yield); $R_f$ 0.31 (4:6 $EtO_2$ 4:6 Hexane); FTIR (neat) $\nu_{max}$ ($cm^{-1}$):

3310, 3076, 2930, 2866, 1673, 1627, 1191, 1083, 911; $^{1}$H NMR (400 MHz, $CDCl_3$): δ 5.91-5.69 (1H, m, 4''-H), 5.12-4.90 (2H, m, 5''-H), 2.37 (2H, t, *J* = 6.8 Hz, 4-H), 2.35-2.29 (2H, m, 6-H), 2.27-2.20 (2H, m, 1''-H), 2.14-2.03 (2H, m, 3''-H), 1.97-1.86 (2H, m, 5-H), 1.75 (3H, s, 1'-H), 1.65-1.45 (2H, m, 2''-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.5 (CO, C-1), 158.8 (C, C-3), 138.0 (CH, C-4''), 130.9 (C, C-2), 115.2 ($CH_2$, C-5''), 37.7 ($CH_2$, C-4), 34.7 ($CH_2$, C-1''), 33.7 ($CH_2$, C-3''), 30.8 ($CH_2$, C-6), 26.6 ($CH_2$, C-2''), 22.5 ($CH_2$, C-5), 10.6 ($CH_3$, C-1'); LC-MS (m/z): calculated $C_{12}H_{19}O$ $[M+H]^+$: 179.1430, found: 179.1432.

**General Procedure for the alkylation of 38a**

To 3-(4-pentenyl)cyclohex-2-en-1-one (**38a**) (1 eq, 3.0 mmol, 493 mg) in dry $Et_2O$ (12 mL) was added *t*BuOK (1.5 eq, 4.5 mmol, 505 mg) and the mixture stirred for 15 min. The appropriate alkyl halide (1.2 eq, 3.6 mmol) was then added dropwise and the reaction stirred for 1 h. The reaction mixture was quenched with sat. $NH_4Cl$ solution (20 mL) and extracted with EtOAc (3 x 20 mL), the organic extracts were combined, washed with brine (50 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel (EtOAc 1:9 hexane) to provide the title compounds **38c-e**.

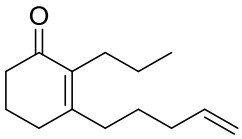
**2-ethyl-3-(pent-4-en-1-yl)cyclohex-2-en-1-one (38c)**



Yellow oil (510 mg, 67% yield). R.f 0.33 ($Et_2O$ 3:7 hexane); FTIR (neat) $V_{max}$: 2931, 2868, 1660, 1620, 1456, 1431, 1362, 909 $cm^{-1}$; $^{1}$H NMR (400 MHz, $CDCl_3$): δ 5.94-5.68 (1H, m, 4''-H), 5.14-4.86 (2H, m, 5''-H), 2.41-2.20 (8H, m, 4, 6, 1', 1''-H), 2.17-2.04 (2H, m, 3''-H), 1.97-1.85 (2H, m, 5-H), 1.62-1.50

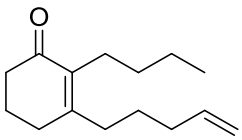
(2H, m, 2''-H), 0.93 (3H, t, J = 7.6 Hz, 2'-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.1 (CO, C-1), 158.4 (C, C-3), 138.0 (CH, C-4''), 137.0 (C, C-2), 115.2 ($CH_2$, C-5''), 38.1 ($CH_2$, C-6), 34.2 ($CH_2$, C-1''), 33.8 ($CH_2$,,C-3''), 30.6 ($CH_2$, C-4), 27.2 ($CH_2$, C-2''), 22.6 ($CH_2$, C-5), 18.3 ($CH_2$, C-1'), 14.2 ($CH_3$, C-2'); LC-MS (m/z): calculated $C_{13}H_{21}O$ $[M+H]^+$: 193.1587, found: 193.1587.

**3-(pent-4-en-1-yl)-2-propylcyclohex-2-en-1-one (38d)**



Yellow oil (264 mg, 64% yield). R.f 0.32 ($EtO_2$ 3:7 hexane); FTIR (neat) $V_{max}$: 2956, 2930, 2867, 1661, 1619, 1456, 1365, 1188, 1108, 910 $cm^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): δ 5.92-5.69 (1H, m, 4''-H), 5.13-4.91 (2H, m, 5''-H), 2.39-2.36 (2H, t, *J* = 6.6 Hz, 6-H), 2.31 (2H, t, *J* = 6.0 Hz, 4-H), 2.27-2.18 (4H, m, 1',1''-H), 2.14-2.05 (2H, m, 3''-H), 1.95-1.85 (2H, m, 5-H), 1.61-1.49 (2H, m, 2''-H), 1.42-1.20 (2H, m, 2'-H), 0.89 (3H, t, *J* = 7.3 Hz, 3'-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 2; $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.2 (CO, C-1), 158.7 (C, C-3), 138.0 (CH, C-4''), 135.6 (C, C-2), 115.2 ($CH_2$, C-5''), 38.1 ($CH_2$, C-6), 34.4 ($CH_2$, C-1''), 33.8 ($CH_2$, C-3''), 30.6 ($CH_2$, C-4), 27.2 ($CH_2$,C-2''), 27.1 ($CH_2$, C-1'), 22.9 ($CH_2$, C-2'), 22.6 ($CH_2$, C-5), 14.3 ($CH_3$, C-3'); LC-MS (m/z): calculated $C_{14}H_{23}O$ $[M+H]^+$: 207.1749, found: 207.1744.

**2-butyl-3-(pent-4-en-1-yl)cyclohex-2-en-1-one (38e)**



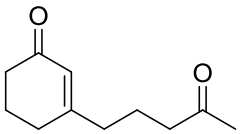
Yellow oil (525 mg, 60% yield). R.f 0.35 ($Et_2O$ 3:7 hexane); FTIR (neat) $V_{max}$: 2954, 2930, 2861, 1663, 1456, 1356, 1187, 910 $cm^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 5.87-5.75 (1H, m, 4''-H), 5.09-4.97 (2H, m, 5''-H), 2.36 (2H, t, *J* = 6.6 Hz, 6-H), 2.33-2.28 (2H, m, 4-H), 2.26-2.22 (4H, m, 2',1''-H), 2.13-2.08 (2H, m, 3''-H), 1.96-1.86 (2H, m, 5-H), 1.62-1.50 (2H, m, 2''-H), 1.38-1.22 (4H, m, 1',3'-

H), 0.89 (3H, t, *J* = 7.1 Hz, 4'-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 199.2 (CO, C-1), 158.5 (C, C-3), 138.0 (CH, C-4''), 135.8 (C, C-2), 115.2 ($CH_2$, C-5''), 38.1 ($CH_2$, C-6), 34.3 ($CH_2$, C-1''), 33.8 ($CH_2$, C-3''), 31.9 ($CH_2$, C-1'), 30.6 ($CH_2$, C-4), 27.2 ($CH_2$, C-2''), 24.9 ($CH_2$, C-2'), 23.0 ($CH_2$, C-3'), 22.6 ($CH_2$, C-5), 14.0 ($CH_3$, C-4'); LC-MS (m/z): calculated $C_{15}H_{25}O$ $[M+H]^+$: 221.1905, found: 221.1900.

**General Procedure for Wacker Oxidation of (38a-e)**

To $PdCl_2$ (50 mg, 0.60 mmol) and CuCl (1.19 g, 12.0 mmol) in DMF (20 mL) and water (5 mL) that had been stirred under an $O_2$ atmosphere for 1 h was added a solution of **38a-e** (985 mg, 6.00 mmol) in DMF (5 mL) drop wise and the mixture stirred for 30 mins. The reaction mixture was quenched with $NH_4Cl$ (20 mL) and ammonium hydroxide solution (20 mL) and extracted with diethyl ether (3 x 40 mL). The combined organic extracts were then washed with brine (2 x 60 mL) and dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel (EtOAc 3:7 hexane) to provide the isolated compounds **36a-e**.

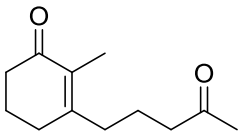
**3-(4-oxopentyl)cyclohex-2-en-1-one (36a)**[117]



Yellow oil (527 mg, 96% yield). R.f 0.18 (EtOAc 1:1 hexane); FTIR (neat) $V_{max}$ 2928, 1712, 1662, 1623, 1367, 1252, 887, 729 $cm^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): δ 5.83 (1H, s, 2-H), 2.44 (2H, t, *J* = 7.1 Hz, 3'-H), 2.33 (2H, t, J = 6.6 Hz, 6-H), 2.26 (2H, t, J = 6.2 Hz, 4-H), 2.19 (2H, t, *J* = 7.6 Hz, 1'-H), 2.12 (3H, s, 5'-H), 2.00-1.91 (2H, m, 5-H), 1.81-1.72 (2H, m, 2'-H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 208.0 (CO, C-4'), 199.8 (CO, C-1), 165.4 (C, C-3), 125.9 (CH, C-2), 42.5 ($CH_2$, C-3'), 37.2

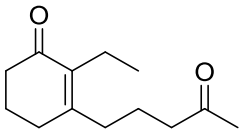
($CH_2$, C-6), 37.1 ($CH_2$, C-1'), 30.0 ($CH_3$, C-5'), 29.4 ($CH_2$, C-4), 22.6 ($CH_2$, C-5), 20.5 ($CH_2$, C-2'); LC-MS (m/z): calculated $C_{11}H_{17}O_2$ $[M+H]^+$: 181.1229, found: 181.1220.

**2-methyl-3-(4-oxopentyl)cyclohex-2-en-1-one (36b)**



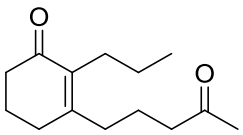
Yellow oil (703 mg, 76% yield); FTIR (neat) $\nu_{max}$ ($cm^{-1}$): 3406, 2941, 1712, 1658, 1624, 1410, 1357, 1170, 1085; $^1H$ NMR (400 MHz, $CDCl_3$): δ 2.46 (2H, t, *J* = 7.1 Hz, 3''-H), 2.37 (2H, t, *J* = 6.6 Hz, 4-H), 2.34-2.28 (2H, m, 6-H), 2.22 (2H, t, *J* = 8.0 Hz, 1''H), 2.14 (3H, s, 5''-H), 1.96-1.86 (2H, m, 5-H), 1.75 (3H, s, 1'-H), 1.82-1.67 (2H, m, 2''-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.1 (CO, C-4''), 199.5 (CO, C-1), 157.9 (C, C-3), 131.3 (C, C-2), 42.9 ($CH_2$, C-3''), 37.7 ($CH_2$, C-4), 34.3 ($CH_2$, C-1''), 30.6 ($CH_2$, C-6), 30.0 ($CH_3$, C-5''), 22.4 ($CH_2$, C-6), 21.2 ($CH_2$, C-2''), 10.6 ($CH_3$, C-1'); LC-MS (m/z): calculated $C_{12}H_{19}O_2$ $[M+H]^+$: 195.1380, found: 195.1384.

**2-ethyl-3-(4-oxopentyl)cyclohex-2-en-1-one (36c)**



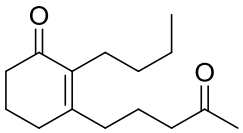
Yellow oil (458 mg, 83% yield). R.f 0.34 (EtOAc 1:1 hexane); FTIR (neat) $V_{max}$ 2933, 2871, 1713, 1657, 1620, 1362, 1168, 1102 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 2.48 (2H, t, *J* = 7.1 Hz, 3''-H), 2.39-2.19 (8H, m, 4,6,1',1''-H), 2.15 (3H, s, 5''-H), 1.96-1.85 (2H, m, 5-H), 1.80-1.67 (2H, m, 2''-H), 0.92 (3H, t, *J* = 7.6 Hz, 2'-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.1 (CO, C-4''), 199.0 (CO, C-1), 157.4 (C, C-3), 137.4 (C, C-2), 43.1 ($CH_2$, C-3''), 38.1 ($CH_2$, C-6), 33.9 ($CH_2$, C-1''), 30.4 ($CH_2$, C-4), 30.0 ($CH_3$, C-5''), 22.5 ($CH_2$, C-5), 21.7 ($CH_2$, C-2''), 18.3 ($CH_2$, C-1'), 14.2 ($CH_3$, C-2'); LC-MS (m/z): calculated $C_{13}H_{21}O_2$ $[M+H]^+$: 209.1536, found: 209.1536.

**3-(4-oxopentyl)-2-propylcyclohex-2-en-1-one (36d)**



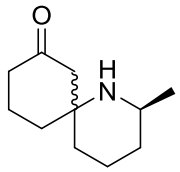
Yellow oil (239 mg, 84% yield). R.f 0.38 (EtOAc 1:1 hexane); FTIR (neat) $V_{max}$ 2956, 2869, 1713, 1658, 1619, 1364, 1169, 1108, 731 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 2.48 (2H, t, *J* = 7.2 Hz, 3''-H), 2.36 (2H, m, 6-H), 2.32 (2H, m, 4-H), 2.26-2.18 (4H, m, 1',1''-H), 2.15 (3H, s, 5''-H), 1.94-1.87 (2H, m, 5-H), 1.77-1.70 (2H, m, 2''-H), 1.33-1.25 (2H, m, 2'-H), 0.89 (3H, t, *J* = 7.2 Hz, 3'-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.1 (CO, C-4''), 199.2 (CO, C-1), 157.7 (C, C-3), 136.0 (C, C-2), 43.1 ($CH_2$, C-3''), 38.1 ($CH_2$, C-6), 34.0 ($CH_2$, C-1''), 30.4 ($CH_2$, C-4), 30.0 ($CH_3$, C-5''), 27.1 ($CH_2$, C-1'), 22.9 ($CH_2$, C-2'), 22.5 ($CH_2$, C-5), 21.7 ($CH_2$, C-2''), 14.3 ($CH_3$, C-3'); LC-MS (m/z): calculated $C_{14}H_{23}O_2$ $[M+H]^+$: 223.1698, found: 223.1693.

**2-butyl-3-(4-oxopentyl)cyclohex-2-en-1-one (36e)**



Orange oil (447 mg, 79% yield). R.f 0.41 (EtOAc 1:1 hexane); FTIR (neat) $V_{max}$ 2953, 2930, 2869, 1713, 1660, 1364, 1164, 1112, 947 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 2.47 (2H, t, *J* = 7.1 Hz, 3''-H), 2.36 (2H, t, *J* = 6.9 Hz, 6-H), 2.31 (2H, t, *J* = 6.0 Hz, 4-H), 2.28-2.18 (4H, m, 1',1''-H), 2.15 (3H, s, 5''-H), 1.93-1.87 (2H, m, 5-H), 1.79-1.65 (2H, m, 2''-H), 1.35-1.20 (4H, m, 2',3'H), 0.88 (3H, t, *J* = 7.1 Hz, 4'-H); $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 208.0 (CO, C-4''), 199.2 (CO, C-1), 157.5 (C, C-3), 136.2 (C, C-2), 43.1 ($CH_2$, C-3''), 38.1 ($CH_2$, C-6), 34.0 ($CH_2$, C-1''), 31.9 ($CH_2$, C-1'), 30.4 ($CH_2$, C-4), 30.0 ($CH_3$, C-5''), 24.9 ($CH_2$, C-2'), 22.9 ($CH_2$, C-3'), 22.5 ($CH_2$, C-5), 21.7 ($CH_2$, C-2''), 14.0 ($CH_3$, C-4'); LC-MS (m/z): calculated $C_{15}H_{25}O_2$ $[M+H]^+$: 237.1855, found: 237.1849.

**(2*S*)-2-methyl-1-azaspiro[5.5]undecan-8-one (34a)**



Compound **34a** was prepared following the same procedure for compounds **1f.** Pale yellow oil (38 mg, 84% yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 2.91-2.78 (0.5H, m, 2-H), 2.78-2.64 (0.5H, m, 2-H), 2.54 (1H, s), 2.39-2.16 (3H, m), 2.16-1.79 (2H, m), 1.78-1.69 (1H, m), 1.69-1.46 (m, 6H), 1.28-0.77 (m, 6H) $^{13}$C NMR (400 MHz, $CDCl_3$) δ 211.1 (CO, C-8), 57.1, 56.9, 56.8, 56.7, 48.4, 45.7, 45.5, 41.2, 41.0, 40.0, 36.1, 35.6, 35.3, 34.5, 34.4, 30.1, 23.1, 21.0, 20.7, 20.6, 20.2, 20.0.

# 5. References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