Exploiting activated esters in novel chemoselective amide formation reactions

by

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Declaration

I declare that while registered as a candidate for the research degree, I have not been a registered candidate or enrolled student for another award of the University or other academic or professional institution

Samridhi Lal

Signature 02.04.2014

Abstract

Detailed within this thesis are the synthetic studies performed on the preparation of lipase catalysed activated esters and their subsequent use in the synthesis of pharmaceutically relevant amides. It also includes the application of activated esters in chemoselective amide formation reactions.

The introduction summarizes the significance and use of activating agents in lipase catalysed reactions over the classical methods of amides synthesis. Additionally, a detailed review on some currently available chemoselective *N*-acylating agents along with their applications is also mentioned. A separate chapter is given to the application of related amides in the designing of biological probes to ultimately detect enzymes which are over-expressed in disease. The discussion begins with the lipase-catalysed synthesis of acetone oxime activated esters and their role in the one-pot synthesis of acetanilides. Further discussion continues with the synthesis of activated esters from ethylene glycol and haloalcohols. The use of a lipase in the synthesis of glycol esters prevented the formation of dimers which indicates its potential usefulness in the future. Unfortunately, these activated esters could not be applied to a synthesis of amides in the same manner as acetone oxime. Thereafter the section focuses on the investigation and synthesis a novel chemoselective *N*-acylating agent and its subsequent applications in the synthesis of pharmaceutically important amides, in particular, those involving chemoselective reactions between anilines and *N*-substituted anilines. Additionally, the chemoselective agent was also used in preparation of pharmaceutically important compounds. To round off the thesis the application of one of the target compounds-benzanilide-was used to develop and synthesise a novel biological probe and to scope out its potential for application in the detection of enzyme-controlled disease. The preliminary utility of the probe was demonstrated using HPLC and NMR.

The thesis concludes with the experimental encompassing experimental details, spectroscopic and analytical analysis of all the compounds described.

Table of Contents

Acknowledgements

In the name of God, who is most beneficial and merciful each individual creature on this earth is created by God to fulfil the particular role. Whatever I have achieved in life is through his help and expression of his will. His grace in me showed some outstanding teachers, colleagues, friends and family members. Whenever, I pay my tribute to those noble people I am praising his glory.

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Abbreviations

AAEM	acetoacetoxy ethyl methacrylate
ABPs	activity-based probes
BACE1	β-Site amyloid-β peptide cleaving enzyme 1
CAL-A	*Candida antarctica* lipase - A
CAL-B	*Candida antarctica* lipase - B
CIEEL	chemically initiated electron exchange luminescence
CMC	Comprehensive Medicinal Chemistry
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DHA	docosahexanoic acid
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
EDAC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EGFR	epidermal growth factor receptor
EPA	eicosapentaenoic acid
FDA	Food and Drug Administration
FRET	Forster resonance energy transfer
HIV	Human immunodeficiency virus
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	hydroxybenzotriazole
IR	infra-red
IUPAC	International Union of Pure and Applied Chemistry
JNK	c-Jun N-terminal kinases
MMP	matrix metalloproteinase
MTBE	methyl-*tert*-butyl ether
NIS	*N*-iodosuccinimide
NMDA	*N*-methyl-D-aspartate
NMR	nuclear magnetic resonance
PDGF	platalet-derived factor
PEG	polyethylene glycol

PTKs	protein tyrosine kinases
PTPs	protein tyrosine phosphatases
TGF-β	transforming growth factor β
THF	tetrahydrofuran
TLC	thin layer chromatography

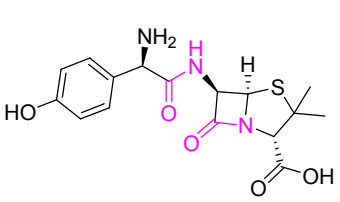
CHAPTER 1- INTRODUCTION

Chapter 1:
INTRODUCTION

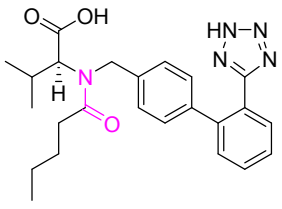
1.1 *N*-acylation reactions in the synthesis of amides

N-acylation refers to the formation of an amide bond. A simple example is the aminolysis reaction, which involves the interaction of an ester with an amine. Depending on the method and the desired product, these reactions involve a wide variety of reagents to facilitate the formation of the amide bond.[1] The methods of synthesis of amides can be broadly classified into chemical and enzymatic. For example, chemical synthesis of amides commonly involves use of acid chlorides,[1] dicyclohexylcarbodiimide,[2] benzotriazoles[3] and anhydrides.[4] On the other hand, enzyme catalysed methods may involve use of acetylated esters, e.g., acetone oxime[5-8] and haloalcohols,[9, 10] along with a variety of hydrolase enzymes.

Amide functionality is found in many important drugs, chemicals, proteins and biomolecules. Out of 6454 molecules of Comprehensive Medicinal Chemistry (CMC) database, 1752 molecules contain carboxamide functionality.[11-13] Moreover, in the year 2003, the sale of amide containing drug exceeded to $1 Bn. Some notable examples of carboxamide containing drugs are the β-lactam and cephalosporin antibiotics amoxicillin (**a**), and cephalexin respectively, the antihypertensive agent valsartan (**b**), the anticancer agent paclitaxel (**c**) and methotrexate and the anti-emetic metoclopramide.[11]



a



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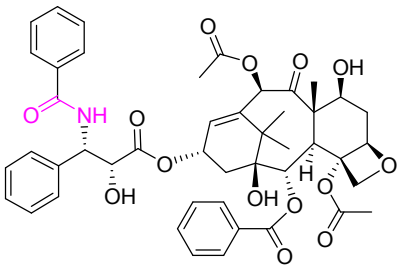
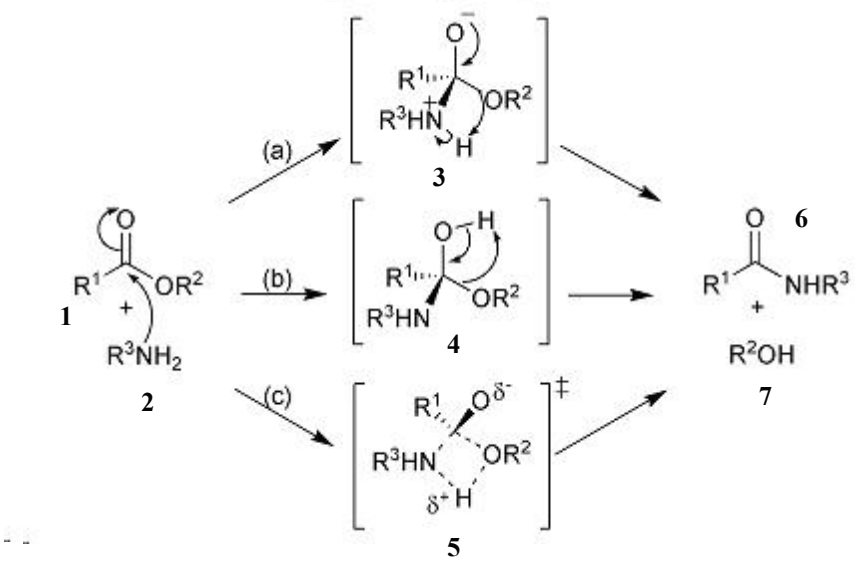


c

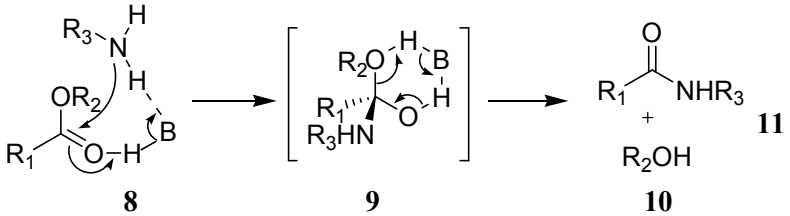
The presence of the carboxamide group in a large proportion of drugs could be correlated to its stable and neutral nature along with both hydrogen bond donating and accepting properties.

One of the basic examples of amide forming reaction is the aminolysis reaction involving the interaction of carbonyl group with a nucleophile. From various kinetic and theoretical studies on aminolysis reactions, it has been concluded that amide bond formation, (Scheme 1.1) involves three possible pathways: (a) a stepwise mechanism through zwitterionic intermediates (**3**); (b) a stepwise path through neutral intermediates (**4**); and (c) a concerted pathway (Scheme 1.1).[14, 15]



Scheme 1.1 General pathways of aminolysis, a) stepwise mechanism through zwitterionic intermediates; (b) stepwise through neutral intermediates and (c) concerted pathway.[14]

Theoretical studies have shown that due to a low energy barrier in the base catalysed stepwise pathway (b) occurring via formation of neutral intermediates (**9**), it is the most preferred pathway of aminolysis reactions. In this pathway (Scheme 1.2), the



R_1, R_2, R_3 = any group

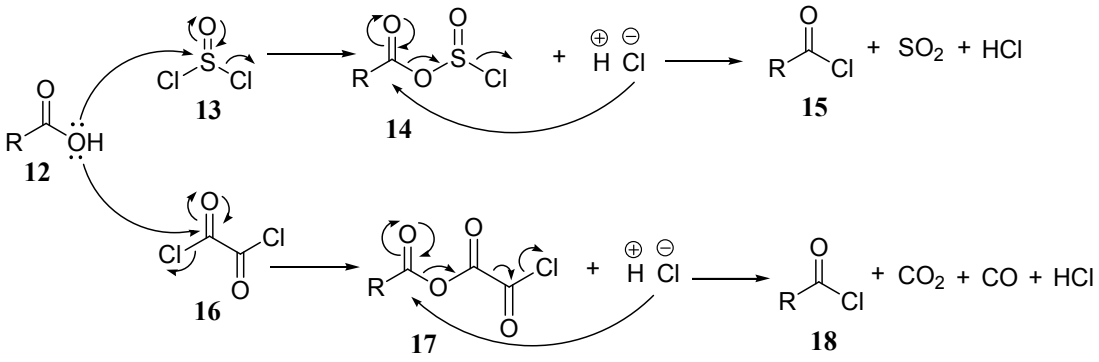
Scheme 1.2 Base catalysed neutral stepwise mechanism.[14]

first step involves an interaction of base (B) with the nucleophile and partial negative carbonyl group of the ester, resulting in the formation of a neutral tetrahedral intermediate (**9**). The base then interacts with the hydroxyl and the oxygen of the leaving group (**9**). Here, a proton is transferred from the nucleophilic amine to the alcohol leaving group in presence of the base acting as a proton shuttle, resulting in formation of amide (**11**) along with the alcohol molecule (**10**).

In the year 2007, the American Chemical Society Green Chemistry Institute (including members from key pharmaceutical industries worldwide) voted ‘‘amide formation avoiding poor atom economy reagents’’ as the topmost and major challenge for synthetic chemists.[16] Although new methods, such as boronic acid catalyzed reactions are being reported, there is still a need for the development of new C-N bond forming methods.[16]

1.1.1 Chemical *N*-acylation

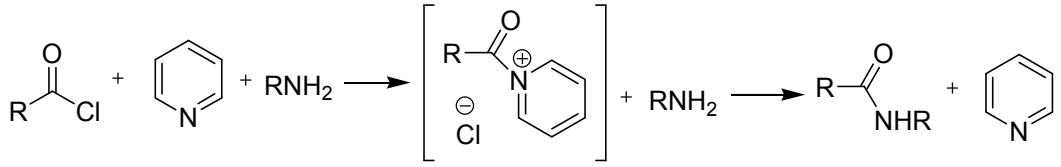
The representative methods applied for *N*-acylation reactions[1,15,17] mostly involve the use of acyl halides where acids (**12**) are activated to acyl halides (**15** and **18**), which further react with amines to form the corresponding amides. Commonly used activating agents to generate the acid chloride are thionyl chloride (**13**),[18] oxalyl chloride (**16**),[1] phosphorous trichloride[1] and phosphorous pentachloride.[1] A general mechanism for the activation of acids (**12**) using thionyl (**13**) or oxalyl chloride (**16**) is illustrated in Scheme 1.3:



Scheme 1.3 Mechanism of formation of acyl chlorides using thionyl or oxalyl chloride.[1]

For amide bond generation, acyl chlorides (**15**) are allowed to react with the desired amine (**21**) in presence of an additional base such as pyridine (**20**) to get the desired

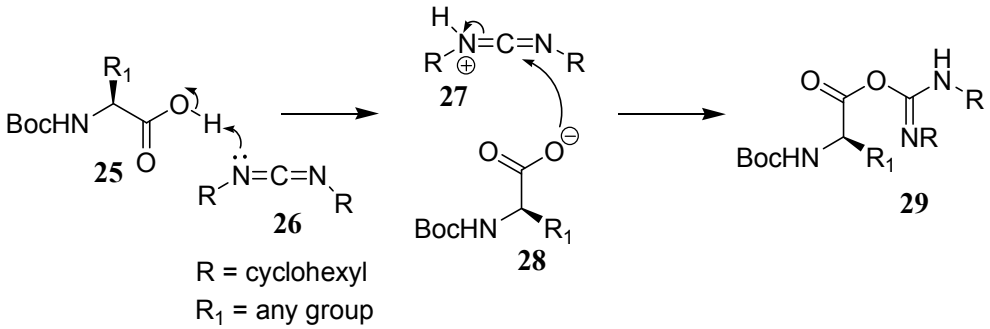
N-acylated product (**23**) (Scheme 1.4). However, these activating agents are highly corrosive and produce hydrochloric acid in the reaction along with the production of sulphur dioxide, carbon dioxide and carbon monoxide in case of thionyl chloride and oxalyl chloride respectively. All these by-products make the reaction conditions undesirable and also raise concerns over health and safety, especially on a large scale. Alternatively, activated esters can be synthesised using activating or coupling agents such as, dicyclohexylcarbodiimide (DCC),[2, 19] hydroxybenzotriazole (HOBt)[2, 20-22] and 1-hydroxy-7-azabenzotriazole (HOAt).[23]



19 **20** **21** **22** **23** **24**

Scheme 1.4 Synthesis of amides in presence of pyridine as base.[1]

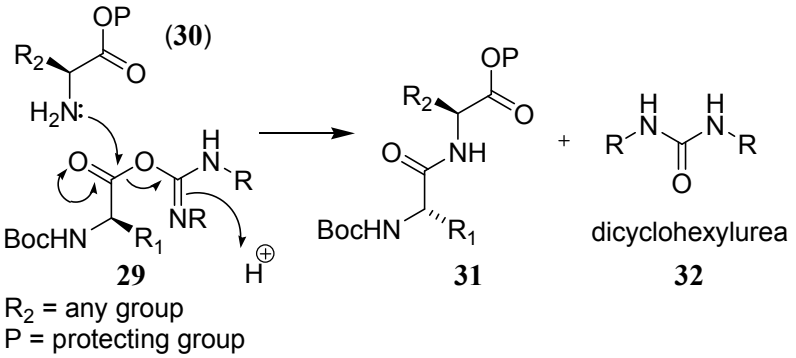
DCC-HOBt is commonly used in the coupling of amino acids used in peptides synthesis where dicyclohexylcarbodiimide (**26**) is used in the activation of amino acids (**25**).



25 **26** **27** **28** **29**

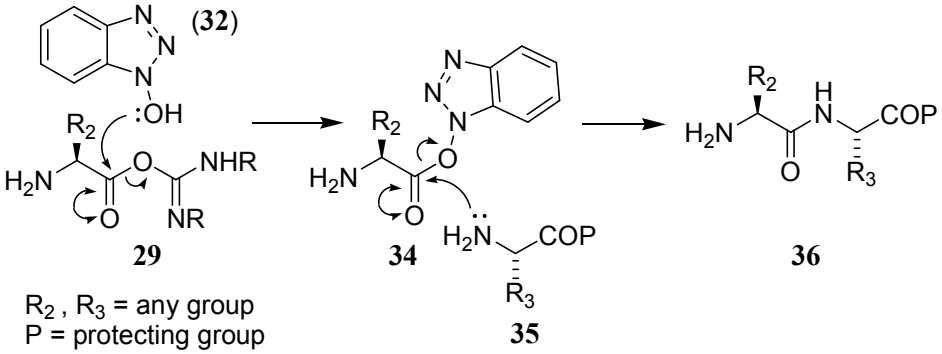
Scheme 1.5 Synthesis of activated ester using dicyclohexylcarbodiimide.[2]

In a typical reaction (Scheme 1.5), dicyclohexylcarbodiimide (**26**) undergoes protonation by a Boc-protected amino acid (**25**) generating a carboxylate anion (**28**) and protonated dicyclohexylcarbodiimide (**27**). After which the protonated dicyclohexylcarbodiimide (**27**) undergoes nucleophilic attack by the carboxylate anion (**28**) and forms the activated ester (**29**). This ester is activated because it releases stable urea (**32**) as a leaving group on undergoing substitution with any nucleophile (**30**) (Scheme 1.6).



Scheme 1.6 Synthesis of a dipeptide and release of dicyclohexylurea.[2]

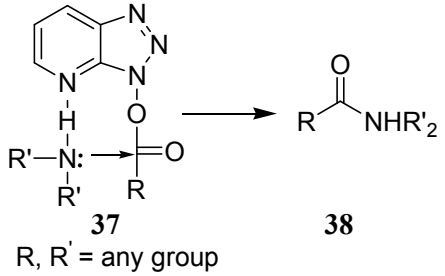
Unfortunately, problems arise when the activated ester (**29**) undergoes racemization at the time of nucleophilic attack by the amino group of the attacking amino acid ester (**30**). Therefore, in order to overcome this racemization (at the chiral centre created by R_1 in **29**) hydroxybenzotriazole (HOBt) is added to the reaction mixture (Scheme 1.7). The hydroxybenzotriazole (**32**) attacks on the activated ester (**29**) forming a new intermediate (**34**) which, by undergoing nucleophilic attack by the amino acid, releases the dipeptide product (**36**) without any racemization.



Scheme 1.7 Reaction of benzotriazole with the activated ester.[2]

However, this method has various disadvantages such as, dicyclohexylcarbodiimide is an allergen and requires a careful handling. Also, hydroxybenzotriazole has been reported to exhibit explosive properties.[24,25] Moreover, these are not preferred reagents from an industrial point of view for *N*-acylation of bulky or sterically hindered amines and atom economy too. Towards the synthesis of hindered amides, some coupling agents based on benzotriazoles have been reported to be more competent than benzotriazole itself. For example, 1-hydroxy-7-azabenzotriazole (HOAt) and additives based on this are reported to be more efficient than benzotriazole (HOBt) with less chances of epimerisation.[23,26] 1-Hydroxy-7-

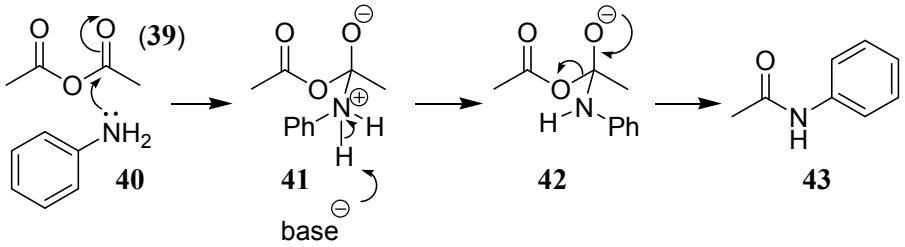
azabenzotriazole when used in the formation of the activated ester has shown better yield of hindered amides over benzotriazole. This increase in efficiency has been correlated to the neighbouring group effect (Scheme 1.8) at the time of aminolysis by the nitrogen of the pyridine ring of the 1-hydroxy-7-azabenzotriazole (**37**) which assists in the amide bond formation (**38**).[23]



Scheme 1.8 Neighbouring group effect produced by 1-hydroxy-7-azabenzotriazole.[23]

Due to the ease of availability and very good leaving ability, benzotriazoles are used in the synthesis of amides, but being inherently bulky in nature, they are not very efficient for synthesis of bulky amides.

Another classical technique to form the amide bond is the use of anhydrides. In a typical reaction (Scheme 1.9) the carbonyl group of the anhydride (**39**) undergoes nucleophilic attack by the amine (**40**) forming an unstable tetrahedral intermediate (**41**), of which when the proton is abstracted by a base and subsequent leaving of the acetate group from **42**, forms the desired amide product (**43**). However, anhydrides are highly reactive and corrosive and therefore not a preferred route for the synthesis of amides on a large scale.



Scheme 1.9 Synthesis of amide from anyhydride.[1]

1.1.2 Lipases in *N*-acylation

To overcome the use of corrosive reagents and the problems of the generation of hazardous by-products in *N*-acylation reactions, these days' green and eco-friendly procedures are in demand combining the following features:[27]

i) the procedure should not require purification of intermediates at every step in a multi-step synthetic process.

ii) preferably, the method should be catalytic involving an appropriate combination of solvents and reagents, giving the required product without any selectivity or stereochemical problems.

iii) ideally, in case of multistep synthesis, the steps should be concerted, with mutually compatible chemical or enzymatic reactions.

iv) the method should be applicable at industrial scale incorporating procedures with no undesired side reactions or by-products.

Ideally, the synthesis of compounds should be like the chemistry of living cells, which occur without any need of recovery or purification of intermediates. Classical laboratory methods require several steps to isolate and purify intermediates to use in further steps. These in turn generate lot of by-products and waste. On the other hand, in living cells, there is no need of purification. All reactions are enzyme catalysed and produce only the desired product which is immediately used in subsequent steps, making the process clean and free from by-products. Moreover, the enzymes being substrate specific omit any possibility of side reactions which are often seen in chemical synthesis.[27]

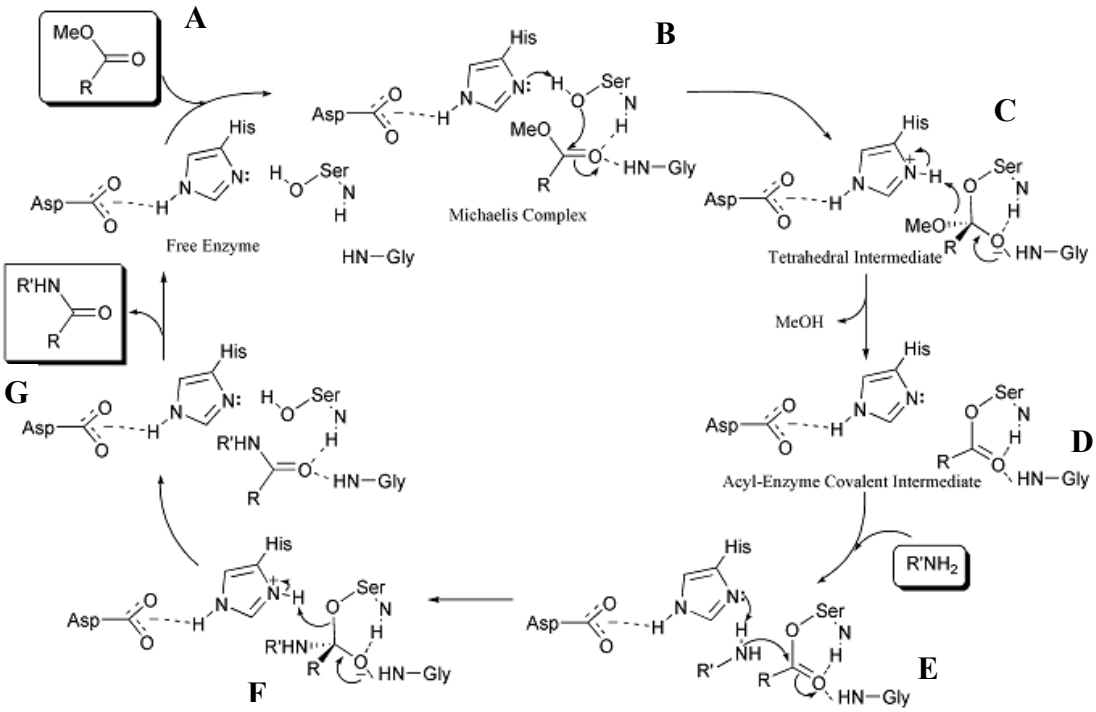
Analysing the advantages of enzyme catalysed reactions, nowadays enzymes, mainly lipases, are being used for *N*-acylation of amines.[14] Lipases[28] have been reported in various esterification reactions,[29] regioselective,[30,31] chemoselective[32] and enantioselective amidations[33, 34] and desymmetrization reactions.[35]

The use of enzymes is advantageous as they are used as a catalyst and can be regenerated after the completion of reaction. Moreover, enzymatic reactions can be carried out at ambient conditions without the use of harsh reaction conditions.

Additionally, the enzymes are substrate specific and leave a low impact on the environment.[36] All these features make enzymes attractive reagents over toxic chemicals, such as acid chlorides and anhydrides, to carry out *N*-acylation.

1.1.2.1 Mechanism of aminolysis by *Candida antarctica* lipase enzyme

Candida antarctica lipase belongs to the hydrolase family (EC 3.1.1.3) of enzymes.[37] Its active site contains a catalytic triad of serine, aspartic acid and histidine residues (Scheme 1.10).[14]



Scheme 1.10 Schematic representation of the mechanism of aminolysis by lipase enzymes.[14]

As the substrate (**A**) (for example, an ester) reaches at the active site, its acyl group gets accepted by serine 184 which is prior activated by abstraction of a proton by the histidine 366/aspartic acid 334 pair (**B**). This reaction forms the first tetrahedral intermediate (**C**) which is further stabilized by electrostatic interactions with the NH of glycine 193 and serine 195 making the oxyaninon hole. This oxyanion interaction stabilizes the negatively charged carbonyl oxygen. In the next step, the first tetrahedral intermediate collapses and, forms an acyl-enzyme covalent intermediate (**D**) after the release of proton from histidine 366 which further goes with the alcohol

product. The formed acyl-enzyme intermediate then reacts with the amine nucleophile and a second tetrahedral intermediate is formed (**E**), stabilized by the oxyanion hole interactions.

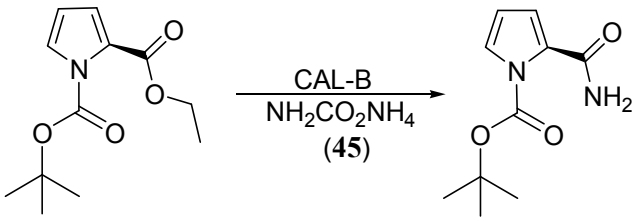
Finally this second intermediate collapses as a result of release of a proton from histidine 366 again (**F**) and subsequently forms the amide product (**G**) along with the release of the free enzyme.[38, 39]

1.1.2.2 Contributions of *Candida antarctica* lipases in the synthesis of amides

Candida antarctica lipase - A and B have provided an immense contribution in the synthesis of amides.[40-43] Some notable contributions are following:

(a) Preparation of the intermediate of the dipeptidyl peptidase IV inhibitor, saxagliptin

The direct aminolysis of the ester (**44)** was very difficult due to side reactions and racemisation of the amide product. Also, use of coupling reagents such as 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholiniumchloride, reduced the product yield. However, CAL-B was able to successfully catalyse the synthesis of amide (**46**) from (**44**) using ammonium carbamate (**45**) (Scheme 1.11).



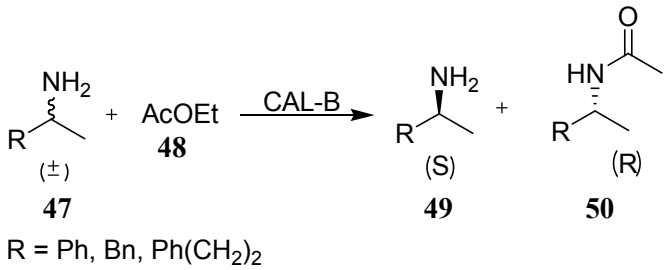
44 **46**

Scheme 1.11 CAL-B catalysed formation of a saxagliptin intermediate.[44]

The 71% yield of the amide (**46**) was an improvement in comparison to the yields obtained through chemical routes which ranged between 57-64%.[44] Additionally, the enzyme also obviated the occurrence of side reactions and racemisation of the amide product. The suggested reason for this chemoselective amidation is attributed to the position of the functionality at the active site which is aligned nicely for selective amidation.

(b) Enantioselective resolution of pharmacologically important amines

Here a range of amines including amphetamine and mexilitine (as well as their corresponding amides) were synthesised employing CAL-B with ethyl acetate (**48**) as both acyl donor and solvent (Scheme 1.12).[33]



Scheme 1.12 CAL-B catalysed resolution of amines.[33]

In most of the cases the (*R*)-amide enantiomer was the major product indicating that the enzyme followed Kazlauskas' rule (Figure 1.1).[30]

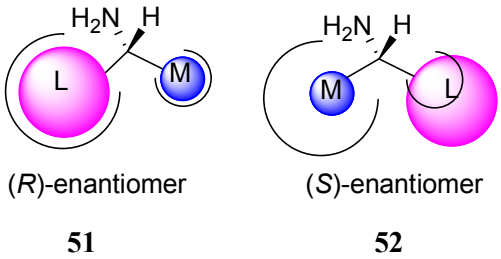


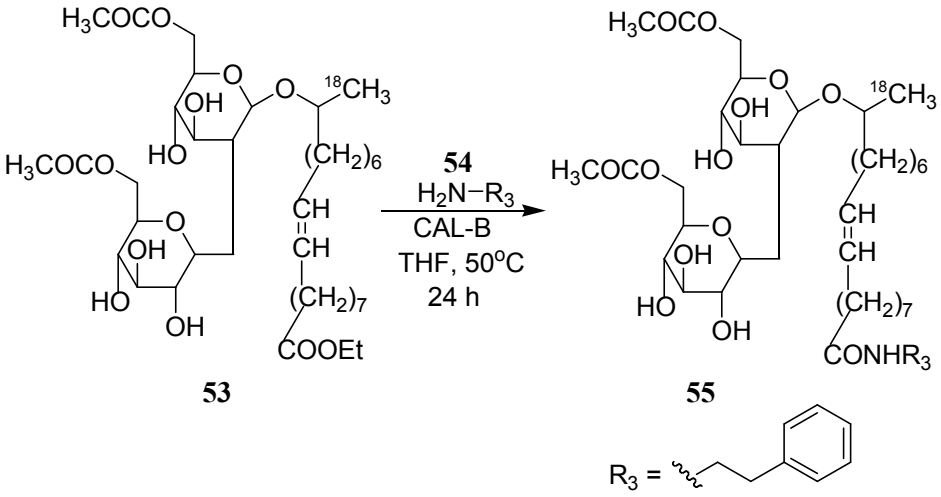
Figure 1.1 Accommodation of substrates in the pocket of enzyme.[33]

In case of *R*-enantiomer (**51**), its medium size group fits properly in the small pocket of the enzyme and large group in the bigger pocket containing the active site (Figure 1.1). This fitting orientates the amino group in such a way that it undergoes acylation which is not feasible in the case of *S*-enantiomer (**52**).

(c) Chemoselective amidation of sophorolipids

Sophorolipids and their derivatives, by altering the immune system, have shown a promising efficacy in the treatment of cancer, autoimmune disorders and angiogenesis. Apart from this, they are also used in the recovery of enhanced oils, cosmetics, detergents and germicidal preparations. But the generation of amide functionality in these glycolipids by chemical methods has failed.

Enzymatic methodology involving amine (**54**) and CAL-B successfully catalysed the amidation of **53** at 50°C in 24h, and gave **55** selectively (Scheme 1.13).



Scheme 1.13 CAL-B catalysed chemoselective amidation of sophorolipids.[45]

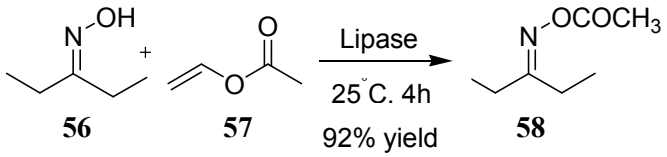
The use of an enzyme here is remarkable because of the hydrolytic susceptibility of acetal linkages of these sophorolipids which link the glucose residues with the 17-hydroxy oleic acid chain. At high temperatures or treatment with harsh reagents the acetal moieties hydrolyse resulting in loss of integrity of the glycolipid. Additionally, CAL-B selectively catalysed the amidation of the ester group of the oleic acid chain rather than catalysing nucleophilic attacks on the carbonyl carbons in **53** (Scheme 1.13). The reason of this chemoselective amidation is attributed to the position of the functionality reaching at the active site for selective amidation.[45]

1.1.3 Use of activating agents in lipase catalysed reactions

In the lipase catalysed synthesis of amides and transesterification reactions, various activating agents are also reported which, by increasing the reactivity of the ester substrate, facilitate the reaction. Some representative agents used, in addition to the traditional vinyl acetate are acetone oxime,[5, 6, 43, 46-49] trifluoroethanol[9, 10, 50-53] and ethylene glycol.[54-56]

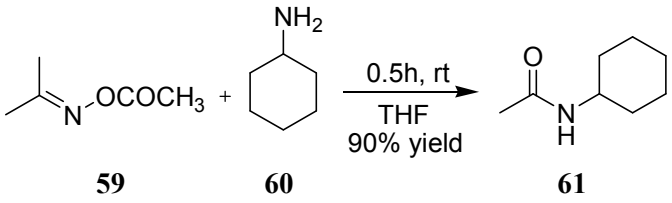
In 1990, Gotor and Menendez reported first application of acetone oxime in lipase catalysed transesterification reactions in the synthesis of oxime esters.[5] In a typical experiment (Scheme 1.13) when the reaction of oxime (**56**) was carried out with vinyl

acetate (**57**) in presence of lipase Amano P in diisopropyl ether as a solvent, the resulting oxime ester product (**58**) was obtained in 92% yield.



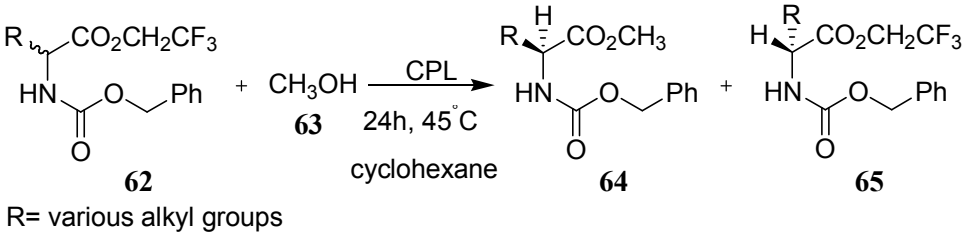
Scheme 1.13 Lipase catalysed synthesis of oxime ester.[5]

In 1991, Gotor *et al*., further reported the lipase catalysed synthesis of amides using oxime activated esters (Scheme 1.14).[6] In an experiment when the reaction of pre-synthesised oxime activated ester (**59**) from lipase was performed with the amine (**60**), it gave the amide product (**61**) in 90% yield.



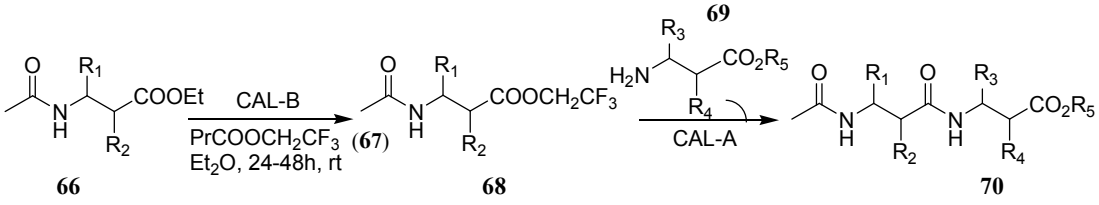
Scheme 1.14 Synthesis of amide from oxime ester.[6]

Among haloalcohols used to activate esters for enzyme-catalysed reactions, trifluoroethanol and trichloroethanol are reported in various lipase catalysed reactions. For example, trifluoroesters are reported in transesterification reactions catalysed by *Carica papaya* lipase to obtain L-methyl esters from racemic *N*-protected amino acids (Scheme 1.15).[57]



Scheme 1.15 CPL enantioselective transesterification of racemic *N*-protected amino acids.[57]

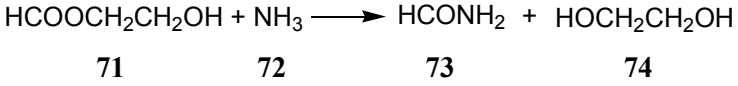
Here, previously prepared *N*-protected DL-amino acid 2,2,2-trifluoroethyl esters (**62**) were subjected to enzyme catalysed enantioselective transesterification using methanol (**63**). In this study, a range of alkyl groups (R) such as methyl, ethyl, isobutyl, pentyl and cyclohexyl attached on **62**were tested and in all the cases studied the conversion percentage (**62** to **64**) was in between 44 to 56%. This demonstrated the efficiency of these activated esters in enantioselective transesterification reactions which was otherwise not possible with *Carica papaya* lipase when other non-activated esters were used. Apart from individual amino acids, halogenated esters have also been used in chemoenzymatic synthesis of peptides. For example, use of 2,2,2-trifluoroethyl butanoate (**67**) in the synthesis of dipeptides (Scheme 1.16).



Scheme 1.16 Peptide synthesis using two-step lipase catalysed approach.[58]

In this methodology first the amino ester (**66**) was activated as 2,2,2-trifluoroethyl ester (**68**) using 2,2,2-trifluoroethyl butanoate (**67**) in presence of CAL-B which was then subsequently employed to acylate the next amino ester (**69**) in the presence of CAL-A to obtain the desired dipeptide product (**70**).

In addition to acetone oxime and haloalcohols, ethylene glycol has also been reported in the activation of esters. In 2007 Bayryamov *et al*., reported that the *syn*-vicinal hydroxyl-group assists in the amide formation by stabilizing the intermediate (Scheme 1.17).[54]



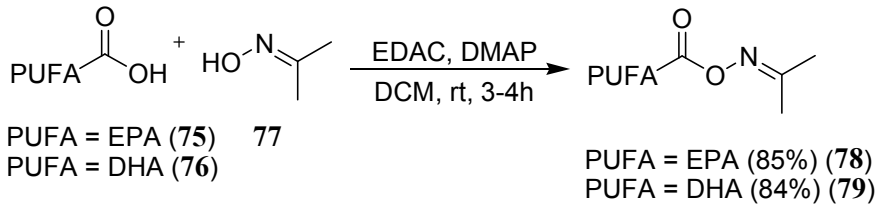
Scheme 1.17 Synthesis of amide using 2-hydroyethyl formate.[56]

Here, when the reaction of 2-hydroxyethyl formate (**71**) was carried out with ammonia (**72**), it gave the formamide (**73**) product along with the ethylene glycol (**74**). From mechanistic studies it was shown that the *syn*-hydroxy group accepts the hydrogen bond from ammonia and donates to the carbonyl oxygen, thus acting like a

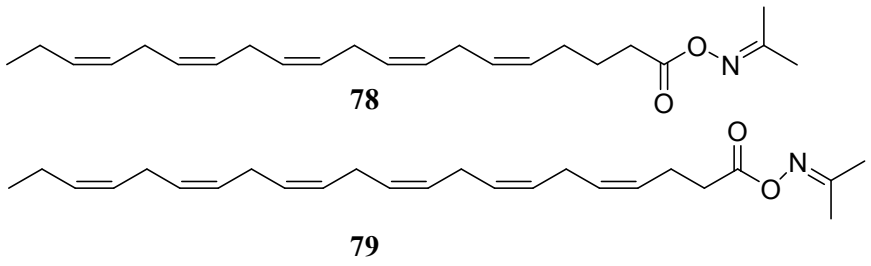
bridge providing linear proton transfer geometry. But the reaction was reported only with small nucleophiles such as ammonia and not with, more synthetically useful amines.[56]

1.1.3.1 Applications of acetone oxime in the synthesis of oxime esters

Among the above discussed activating agents used in biocatalysis, acetone oxime has shown its utility in many valuable reactions.[49, 59-63] One pharmaceutically important application is the incorporation of polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) in 1,3-diacylglycerol in the making of lipid based prodrugs.[60] These long chain eicosapentaenoic and docosahexanoic fatty acids found in fish oils are reported to exhibit cardioprotective,[64, 65] anticancer,[66, 67] and anti-inflammatory properties.[68, 69] They are also required for normal brain and cognitive functions.[70, 71] They are also useful agents in lowering triglycerides level. For instance, a prescription drug Omacor containing ethyl esters of EPA and DHA is registered for the treatment of hypertriglyceridemia along with secondary prevention of post-myocardial infarction.[72] Recently, in 2012, Magnusson and Haraldsson reported the use of acetone oxime in the preparation of EPA and DHA prodrugs.[60] They incorporated these long chain eicosapentaenoic and docosahexanoic fatty acids in 1,3-diacylglycerol. Diacylglycerol is used in pharmaceutical preparations especially in prodrugs to increase the bioavailability of the drug following oral administration.[73-76] In this study, the acids (**75** and **76**) were first activated (**78** and **79**) (Figure 1.2) with acetone oxime (**77**) using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) in the presence of 4-dimethylaminopyridine (Scheme1.18).



Scheme 1.18 Activation of EPA and DHA using acetone oxime.[60]

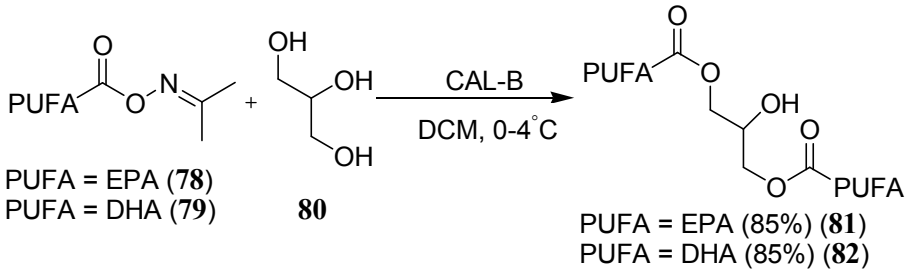


78

79

Figure 1.2 Acetoneoxime esters of EPA (**75**) and DHA (**76**).[60]

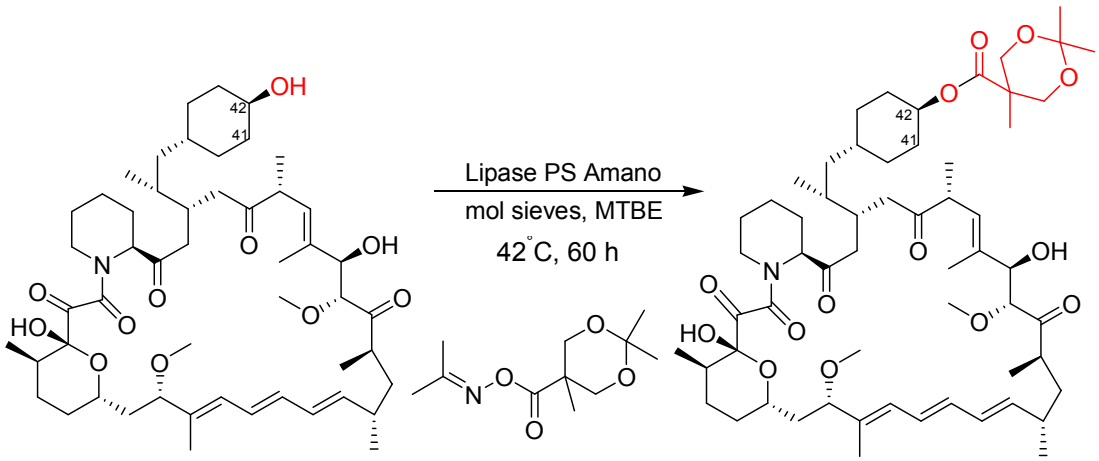
Thereafter, these oxime activated esters (**78** and **79**) were subjected to transesterification reaction with glycerol (**80**) in presence of CAL-B (Scheme 1.19) to obtain the prodrugs (**81** and **82**).



Scheme 1.19 Transesterification of EPA and DHA oxime esters using CAL-B.

The use of CAL-B provided excellent regioselectivity and achieved acylation at position 1 and 3 of diacyglycerol which was otherwise carried out with toxic chemicals usually avoided in these prodrugs' preparation. For comparison with acetone oxime, the activation of EPA and DHA was also carried out with 2,2,2-trifluoroethanol but the CAL-B catalysed reaction rate was very low compared to that of oxime activated esters (**78** and **79**) and therefore the reactions of trifluoro-activated esters were abandoned.

Another important use of acetone oxime was in the regioselective lipase catalysed acylation of antiproloferative 41-desmethoxyrapamycin (**83**) which was otherwise not feasible with the use of standard chemical acylation methods.[61]

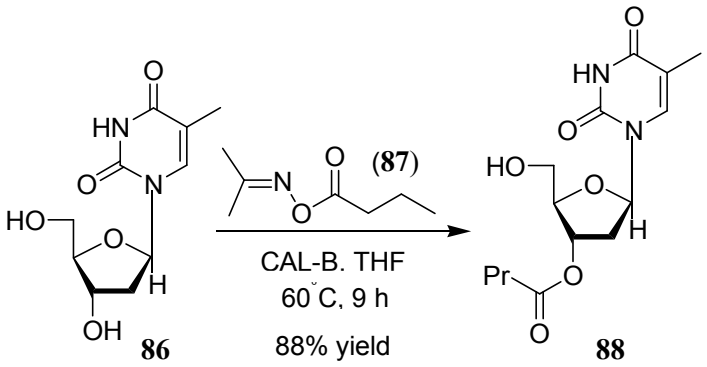


83 **84** **85**

Scheme 1.20 Regioselective acylation of 41-desmethoxyrapamycin.[61]

In this study a range of oxime esters were tested in the acylation at position 42 of the 41-desmethoxyrapamycin. In one of the representative reaction (Scheme 1.20) when the reaction of **83** was carried with butanedione-monooxime (**84**) in presence of lipase enzyme, it gave the acylated product (**85**) in 95% yield.[61]

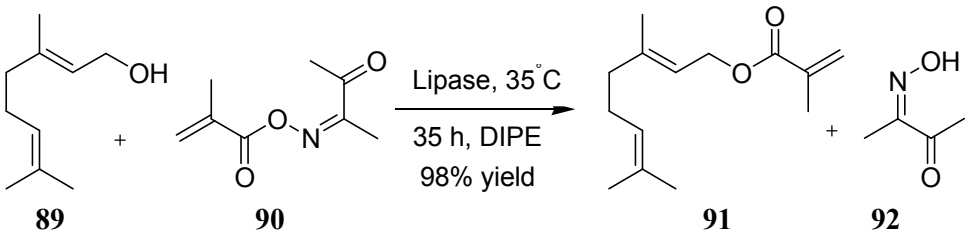
Other than drug compounds, oxime esters are reported in the regioselective acylation in nucleosides and sugars (Scheme 1.21).[77]



86 **88**

Scheme 1.21 Acylation of nucleoside **86** using oxime ester **87**and CAL-B.[77]

When the reaction of nucleoside **86** was carried out with the oxime ester **87**, it achieved the acylation in a regioselective manner and gave the product (**88**) in 88% yield. The reason for this regioselective acylation could be attributed to the steric bulk created by the base around the hydroxy group at position 3 which prevented the fitting of the substrate inside the active site of CAL-B and subsequent reaction with the oxime ester (**87**). Oxime esters have also been reported in the synthesis of esters of terpenes. Terpene esters (for example, geraniol ester) are commonly used for



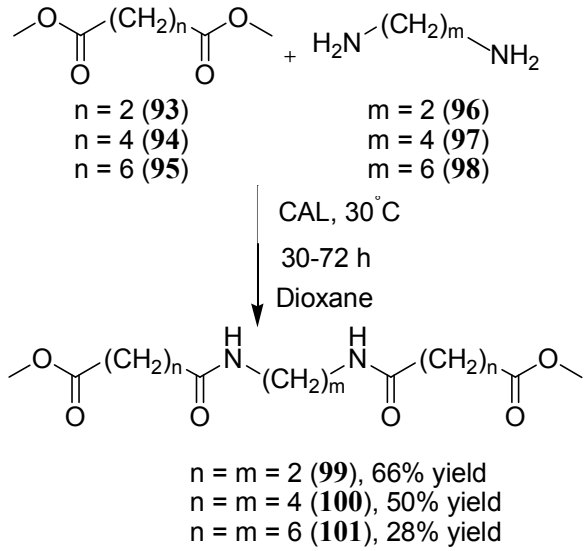
Scheme 1.22 Synthesis of geraniol ester from oxime ester.[59]

fragrance and flavour in food, beverages and perfumes. Due to their use in food products their synthesis is often preferred through a biocatalytic route rather than a chemical one. In this context, in 2002 Athawale *et al*., reported the synthesis of geraniol ester using acetone oxime in presence of *Candia rugosa* lipase (Scheme 1.22).[59] Here, pre-synthesised oxime ester (**90**)[62] by this group using *Candida cylindracia* lipase was subjected to transesterification using geraniol (**89**) in presence of *Candia rugosa* lipase taking diisopropyl ether as solvent to get the geraniol ester product (**91**) along with the oxime (**92**) in excellent 98% yield.[59]

1.1.4 Limitations of CAL-B catalysed amidation

1.1.4.1 Synthesis of bulky amines

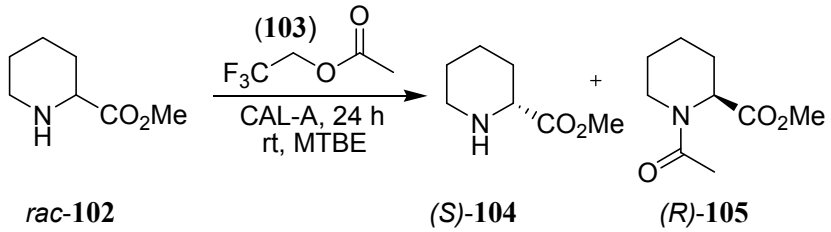
Although having an immense contribution in the synthesis of pharmaceutically important amides, the *N*-acylation of bulky amines using lipases is still elusive.[78, 79]



Scheme 1.23 Lipase catalysed aminolysis of diesters.[79]

For example, in the CAL-B catalysed reaction of non-activated diesters (**93** to **95**) with diamines (**96** to **98**), the yield of amide products (**99** to **101**) dropped down from 70% to 28% as the chain of the diester increased from 2 to 6 carbons (Scheme 1.23).[79] This decrease in the yield could be correlated to the limited size of the enzyme's substrate binding pocket which in the case of CAL-B is elliptical and funnel shaped with dimensions 9.5 x 4.5 Å.[80]

So far, there are very few examples in which enzymatic acylation of secondary or hindered amines have been carried out. For example, the resolution of racemic pipecolic acid derivative, methyl pipecolinate (**102**), was achieved via *Candida antarctica* A (CAL-A) catalysed acylation of hindered secondary nitrogen (Scheme 1.24).[81] This reaction was otherwise not possible with CAL-B and other common enzymes used in acylation reactions.



Scheme 1.24 CAL-A catalysed resolution of methyl pipecolinate.[81]

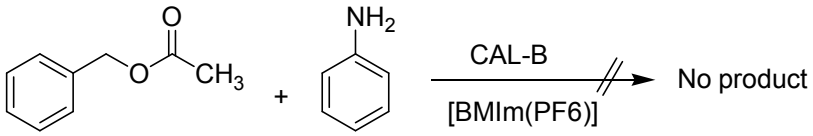
In a representative reaction, when the reaction of **102** was carried out with 2,2,2-trifluoroethyl acetate (**103**) in presence of CAL-A, it gave (*S*)-**104** in greater than 99% yield. The feasibility of the reaction could be correlated to the larger active site pocket of CAL-A over CAL-B which might have accommodated the racemic **102** well inside the active site. Other contributing factors may include the cyclic structure of the nucleophile amine, where the carbon atoms were tightly held back leaving the nucleophilic nitrogen less hindered. Additionally, activation of the ester (**103**) with trifluoroethanol made the carbonyl carbon more electrophilic and thus increasing the reaction rate.

In addition to the use of wild-type enzymes in organic synthesis, and the inherent limitations of substrate scope, improvements in the selectivity and efficiency of biocatalysts, can be achieved by being modified at a molecular level using techniques such as rational protein design and directed evolution, techniques which have been

utilised to prepare synthetically and industrially useful fine chemicals where traditional enzymes have failed.[82-86]

1.1.4.2 The need for a green *N*-acylation route in the synthesis of acetanilides

Though the utility of CAL-B in *N*-acylation reactions is well documented,its use in the*N*-acylation of aniline is still elusive.[34,87] To date, there is only one report on the attempted CAL-B catalysed reaction of benzyl acetate (**106**) with aniline (**107**), in an ionic liquid; a reaction which did not give the corresponding acetanilide (Scheme 1.25).[88]



106 **107**

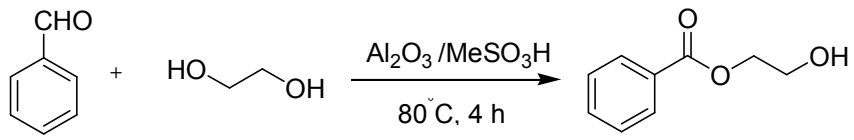
Scheme 1.25 Attempted CAL-B catalysed reaction of benzyl acetate and aniline.[88]

Acetanilides are known for their use as chemical hybridising agents.[89] They are also reported as antiparasitic agents against *Trichomonas vaginalis* which causes trichomoniasis in humans.[90] Currently, a single 2g-oral dose of metronidazole is the only FDA-approved treatment available. However, 5% of all the reported cases are resistant to the drug and the percentage is still increasing. The only way to increase the efficiency remains to increase the dose which leads to resistance with the drug and causes various side-effects such as constipation and neurotoxicity.[91]

Considering these limitations therefore, in the presented study a detailed synthesis of amides from anilines and bulky amines was performed combining both chemical as well as enzymatic reactions in a single pot.

Synthesis of glycol esters

Glycol based activated esters are reported to assist in amide bond formation via the *syn*-vicinal hydroxyl-group of ethylene glycol.[54]The synthesis of glycol monoesters has received attention because of their use as intermediates in the synthesis of cross-linking agents for polyesters,[92] fungicides[92] and sex pheromones of lepidoptera.[93-95] But the preparation of glycol esters suffers with a major problem of formation of the diester along with the monoester thus requiring a tedious separation procedure.[96] Most of the reported methods for the synthesis of glycol esters involve use of heavy-metal oxidants such as $KMnO_4$,[97] CrO_3[98] or expensive catalysts like ruthenium[99] and rhodium.[100] In 2002, Sharghi and Sarvari reported (Scheme 1.26) a selective methodology for direct conversion of aromatic aldehydes to glycol monoesters using glycols with Al_2O_3 and $MeSO_3H$.[101]



108 **109** **110**

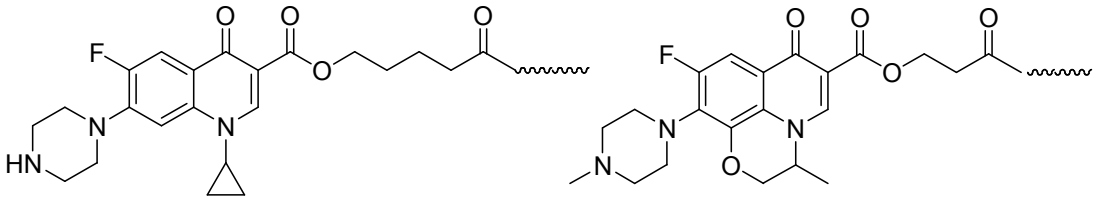
Scheme 1.26 Reaction of benzaldehyde with ethylene glycol.[101]

The study was performed with a range of aromatic aldehydes and glycols. In one of the reactions (Scheme 1.26) when the reaction of benzaldehyde (**108**) and ethylene glycol (**109**) was performed withAl_2O_3 and $MeSO_3H$, it gave the corresponding ester product (**110**) in 80% yield.

1.2.1 Applications of ethylene glycol

Ethylene glycol is also an important intermediate in the synthesis of aliphatic polyesters which are widely used in the preparation of biodegradable drug-polymer conjugates.[102-104] Some reported drug- polyester conjugates involving utilization of glycol and its derivatives are ciprofloxacin-polylactide or poly (ε-caprolactone) conjugates (**111**)[105] and ofloxacin-polyethylene conjugate (**112**) (Figure 1.2).[106] The polymerization reactions for the synthesis of these polymers (for example, polyethylene) are carried out using many chemical initiators or metal ions, which are often hard to separate and remain in the finished polymer. Therefore, polymers which are employed in making medical implants and drug-delivery systems, such as drug-

polymer conjugates, are often synthesized in the presence of enzymes which can replace toxic and expensive catalysts and polymerization initiators.[107-109]

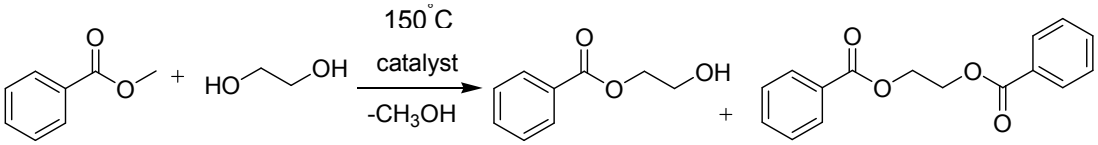


111 Ciprofloxacin-polyester conjugate

112 Ofloxacin-polyethylene conjugate

Figure 1.2 Drug-polymer conjugates.[105, 106]

Other than the use in the synthesis of biopolymers, ethylene glycol is commonly used in the synthesis of polymers extensively used in the making of fibres used in the manufacturing of clothes and plastic items. But the synthesis procedures require high temperatures and presence of minerals such as, sodium and cesium zeolites. Also, the reaction product is often mixed with the dimer as well. Therefore, in this field to improve the synthesis and reduce the amount of dimer, various studies have been reported. For example, in one of the studies it has been shown that under controlled reaction conditions of temperature and amount of substrate and specially designed zeolite catalysts, the amount of dimer (**116**) formed can be controlled (Scheme 1.27).



113 **114** **115** **116**

Scheme 1.27 Synthesis of 2-hydroxyethyl benzoate.[110]

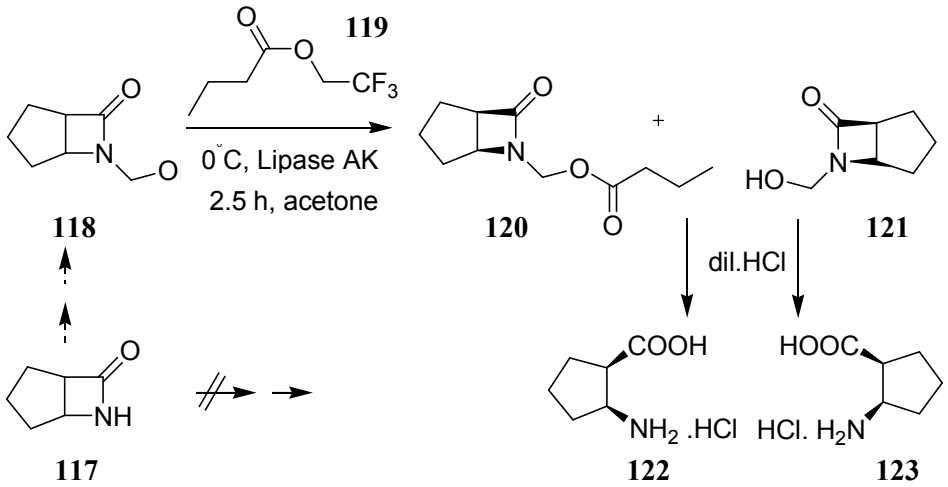
In this study various zeolites were used to synthesise glycol monomer (**115**) from methyl benzoate (**113**) and ethylene glycol (**114**). It was concluded that sodium based zeolites can reduce the formation of dimer formed in the reaction. But still the route needs lot of improvement in the preparation of these zeolites.[110]

1.3 Significance and implications of halogenated alcohols in enzyme catalysed reactions

Halogenated alcohols impart high acidity to the hydrogen atom of a proximal hydroxy group. For example, due to the electron withdrawing nature of CF_3 the pKa of the hydroxyl hydrogen in CF_3CH_2OH is 12.4 compare to ethanol.[111, 112] Halogenated esters are commonly used activated esters in enzyme catalysed reactions. Some important applications of trifluoro- and trichloroesters in enzymatic reactions are in the preparation and resolution of amino acid precursors,[51, 57] the synthesis of peptides[113, 114] and in the resolution of other pharmaceutically relevant molecules.[115]

1.3.1 Synthesis of β-amino carboxylic acids and esters

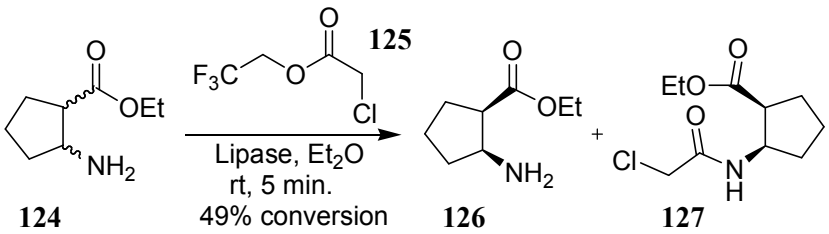
Among amino acids, alicyclic β-aminocarboxylic acids are valuable intermediates employed in the synthesis of various compounds with significant pharmacological activity.[51] They can also be introduced into biologically active peptides to enhance their activity.[116-119] An example of such an amino acid is cispentacin (**122**) which has potential antifungal properties.[120-123] Unfortunately, its synthesis was complex and tedious and therefore to simplify the route, Cosmos *et al*., in 1996, reported an improved synthesis employing a halogenated activated ester, trifluoroethyl butyrate ester (**119**) (Scheme 1.28).[51]



Scheme 1.28 Chemoenzymatic synthesis of 2-aminocarboxylic acid.[51]

Here, a resolution of **118** was performed by carrying out an asymmetric acylation of the hydroxy group in presence of *Pseudomonas* lipase AK using chemically prepared 2,2,2-trifluoroethyl butyrate (**119**) as the acyl donor. After which, treatment with dilute acid gave the desired acids (**122**, **123**). This was otherwise not possible when the route was performed from β-lactam starting material (**117**) through enzymatic ring opening. The reason behind the use of trifluoroalkyl esters as acyl donors is to make the reaction irreversible because the 2,2,2-trifluoroethanol released is a very weak nucleophile and can not attack the ester product formed.

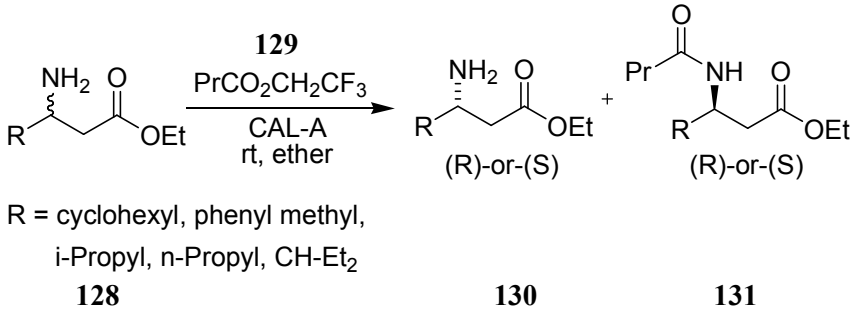
In another study, a range of chemically synthesised halogenated activated esters were used as acyl donors for the resolution of alicyclic β-aminocarboxylic esters, which were subsequently hydrolysed to get optically pure amino acids.[50] For example, in one reaction, 2,2,2-trifluoroethyl chloroacetate (**125**) was used for the enantioselective acylation of the amino group of the aminoester **124** (Scheme 1.29) which gave 49% of *N*-acylated product (**127**) (1*S*,2*R*) and 51% free aminoester (**126**) (1*R*,2*S*).



Scheme 1.29 Enantioselective acylation using 2,2,2-trifluoroethyl chloroacteate.[50]

In this study a range of acyl donors from 2,2,2-trifluoroethyl acetate to 2,2,2-trifluoroethyl hexanoate were tested on β-aminocarboxylic esters using SP 526 from *Candida antarctica* lipase PS and *Pseudomonas cepacia* and it was found that in the case of lipase PS the reactivity and enantioselectivity decreases with increasing chain length from acetate to hexanoate in the acyl donor. On the other hand, both parameters increase in case of SP 526 on increasing the chain length. Additionally, it was also found that increasing the hydrophobicity of the acyl-enzyme intermediate is unfavourable in the reactions catalysed by PS but is favourable for SP 526 catalysed reactions. Among all of the acyl donors studied, 2,2,2-trifluoroethyl chloroacetate (**125**) gave best results with lipase PS where both the acyl and alkyl moieties of the ester are activated.

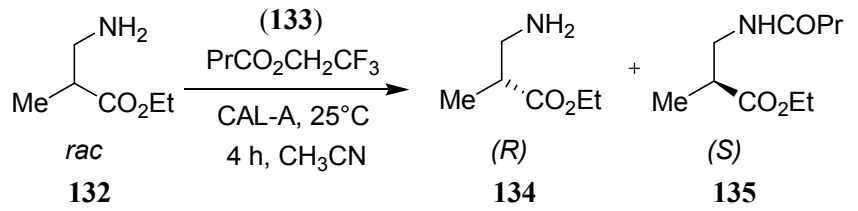
Alkyl activated esters have also been used as the acyl donor for the synthesis of enantiopure β-amino esters (**130**) from their corresponding racemic esters (**128**) (Scheme 1.30) using CAL-A.[9]



Scheme 1.30 CAL-A catalysed acylation of β-amino esters.[9]

In a comparative analysis between the use of activated and non-activated esters, in one of the examples (Scheme 1.30), it was shown that the activated ester 2,2,2-trifluoroethyl butanoate (**129**) works equally well independent of the size of the R group (= cyclohexyl or phenyl methyl, ethyl n-propyl, *i*-propyl, CH-Et_2) and the conversion (**128** to **130**) is nearly 50% in all of the cases. The reaction worked well on gram-scale as well. On the other hand when un-activated ester butyl butanoate ($PrCO_2Bu$) was used in the study, it was not effective when the R group was bulky (CH-Et_2 and cyclohexyl) and the conversion percentage of **128** to **130** was only 33 and 23% respectively. This demonstrates an advantage of using activated alkyl esters in enzyme catalysed resolutions.

In 2002, Solymar *et al*., reported the first enzyme catalysed resolution of α-substituted racemic analogues of β-amino esters (Scheme 1.31) and their subsequent conversion to the *N*-alkyl esters.[10]



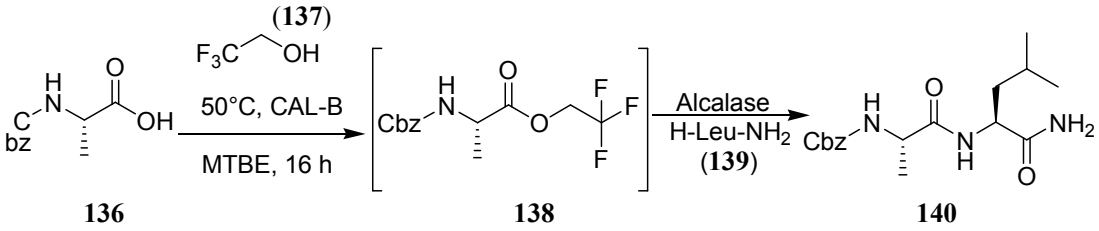
Scheme 1.31 CAL-A catalysed resolution of racemic α-substituted β-amino esters.[10]

Here, chemically prepared 2,2,2-trifluoroethyl butanoate (**133**) was used for acylation in the presence of CAL-A for the resolution of racemic **132** to obtain β-amino ester

(**134**) in 97% e.e. which was further converted to the *N*-alkyl ester with retention of configuration.

Synthesis of peptides

Apart from amino acids, in the past few decades, many peptide based drugs and therapeutic compounds have been introduced. Several peptides have been reported for their use as additives or ingredients in nutritional and cosmetic preparations.[124-127] Despite this importance and demand, their bulk scale production remains complicated, time consuming and expensive.[128] Some common synthetic approaches are chemical and chemo-enzymatic peptide synthesis.[129] The chemical synthesis involves use of wasteful and expensive coupling reagents. In comparison to this, the chemo-enzymatic synthesis is cost-effective and feasible up to the synthesis of medium size peptides.[58, 130] However, problems arise when the acyl donors are sterically hindered. In these cases, sometimes activated esters are employed such as trifluoroethyl[114] and carboxyamidomethyl.[131] In 2011, Nuijens *et al*., reported the use of trifluoroethanol (**137**) for the synthesis of peptides employing a two-enzyme-one-pot approach (Scheme 1.32).[113] In one of the experiments involving the synthesis of leucine based peptide the methodology gave 87% yield of the desired product (**140**).

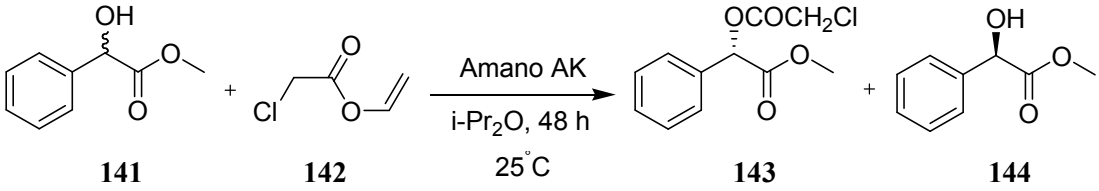


Scheme 1.32 Peptide synthesis using a two-enzyme-one-pot approach.[113]

The method involved first the conversion of *N*-protected amino acid (**136**) to its activated ester (**138**) using CAL-B and 2,2,2-trifluoroethanol (**137**) and then coupling it with leucine (**139**) in the presence of Alcalase to obtain the desired dipeptide product (**140**). The method is clean and straightforward. Moreover, it proves the importance of a trifluoro-activated ester in the synthesis of hindered peptides which was otherwise not feasible when unactivated esters were used.

Utility of halogenated esters in the synthesis of drugs and drug intermediates

Apart from amino acids and peptides, halogenated esters have also been used as acyl donors in the resolution of mandelic acid derivatives (Scheme 1.33) which have been

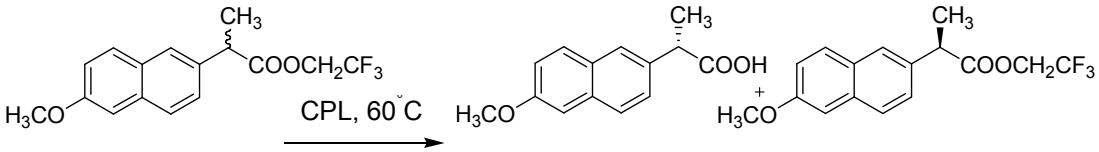


141 **142** **143** **144**

Scheme 1.33 Resolution of mandelic acid esters using vinyl chloroacteate.[133]

used as an important starting material used in the synthesis of various drug molecules.[132, 133] In this study *Pseudomonas sp.* (Amano AK) catalysed the transesterification reaction of **141** with vinyl chloroacteate (**142**) used as the acyl donor and gave the desired product(*S*)-**144** in 33% e.e.

In the field of resolution of drug molecules, trifluoroesters have been used in the resolution of racemic naproxen (**145**) ester to obtain pure (*S*)-naproxen (**146**) which is an important anti-inflammatory agent.[52, 53, 115] In a typical experiment (Scheme 1.34)[52] the (*R*,*S*)-naproxen ester (**145**) was synthesised using 2,2,2-trifluoroethanol and racemic naproxen. After which it was hydrolysed using *Carica papaya* lipase to obtain the (*S*)-naproxen (**146**).



(R, S)-naproxen ester (S)-naproxen (R)-naproxen ester

145 **146** **147**

Scheme 1.34 Hydrolysis of racemic naproxen ester using CPL.[52]

1.4 Chemoselectivity and its significance in organic synthesis

Most organic molecules contain more than one functional group with varying selectivity as to where and how each group will react. These differences in reactivities could be addressed by their selectivity. For example, chemoselectivity, which indicates which functional group, will react first. It plays a crucial role in enzyme catalysed reactions such as chemoselective amidation of sophorolipids discussed in Scheme 1.13.

According to the International Union of Pure and Applied Chemistry (IUPAC) system, chemoselectivity is the ‘‘preferential reaction of a chemical reagent with one of two or more functional groups’’.[134] This selectivity can be exploited in many ways such as:

i) chemoselective reactions in the synthesis of complex molecules. For example, chemoselective reductions or C-N bond formation in natural products

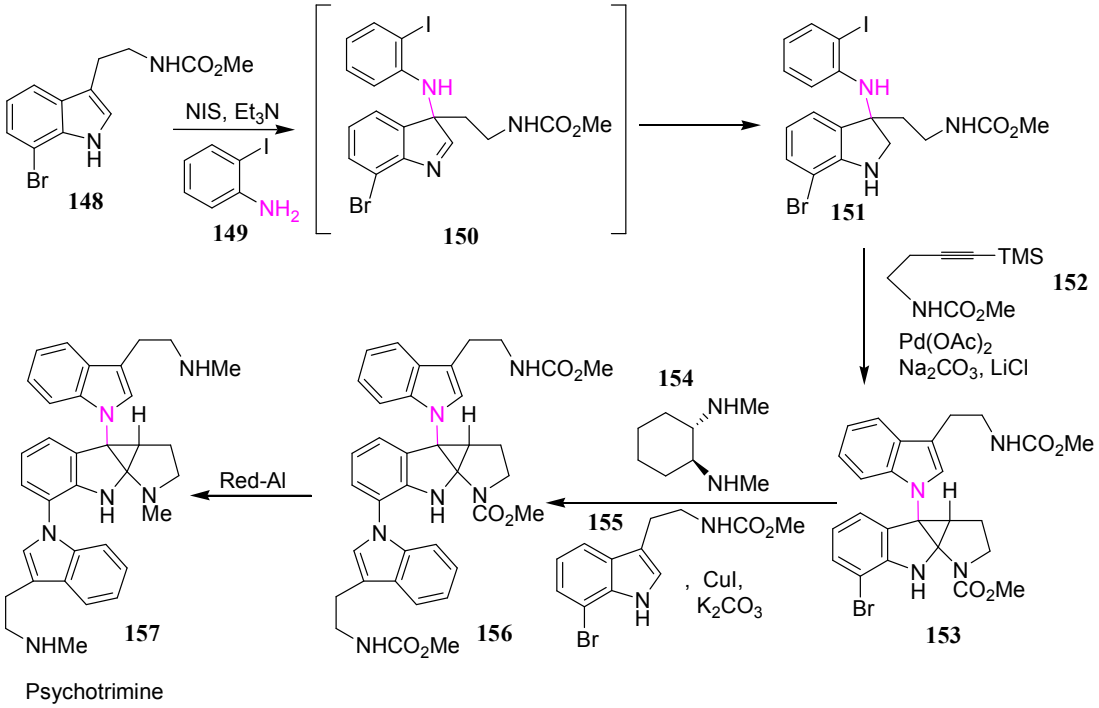
ii) chemoselective acylation of functional groups. Such as, amino or hydroxyl groups

iii) separation of primary and secondary amines during their bulk scale production

iv) scavenging of reactants to control the reaction

v) sequestration of undesired reaction products

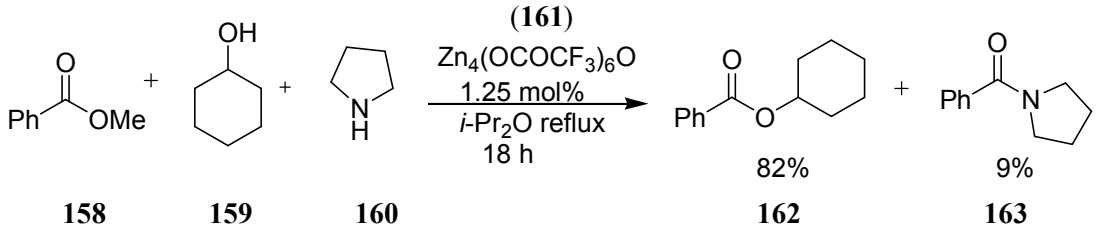
Chemoselectivity plays a crucial role in minimizing the number of synthetic steps and increasing the percentage yield of bond forming events. For example, in the year 2008, Takayama*et al*.,[135] developed a gram scale synthesis (Scheme 1.35) of a complex molecule psychotrimine, employing a novel chemoselective method of oxidative C-N bond formation (**148** to **151**) between indoles and anilines,[136] which was otherwise impossible due to dimerization of the tryptamine or indole ring to favour C-C bond formation (Scheme 1.15) .[137-139] The route starts with the C-N bond formation (**151**) proceeding via *N*-iodoaniline intermediate (**150**) to give **151**. Further, Larockindole synthesis forms the indole core (**151** to **153**) which, when subjected to a chemoselective Buchwald-Golberg-Ullmann coupling (**153** to **156**) furnished the psychotrimine frame (**156**). Finally, reduction of carbamates (**156**) with Red-Al furnished the psychotrimine (**157**) in around 45% yield, which is, to date, the maximum yield recorded in just four steps.



Scheme 1.35 A new method of synthesis of psychotrimine.[135]

Reported chemoselective acylating agents and their applications

Due to the high nucleophilicty of amino and hydroxy groups, difficulty arises when there is need of selective *O*-acylation; in the presence of *N*-nucleophiles although lipases are reported to catalyse selective *O*-acylation in the presence of primary alkyl amines.[140] For example, in the preparation of aminoesters, the only common non-enzymatic route is the laborious selective protection-deprotection of the amino group.[140, 141] But this multistep transformation reduces the atom-economy and increases the waste of the reaction, thus increasing its environmental impact.[142] To improve this, recently Ohshima *et al*., reported a novel enzyme-like chemoselective *O*-acylation of alcohols in the presence of primary and secondary amines, catalysed by a tetranuclear zinc cluster (Scheme 1.36).[143]

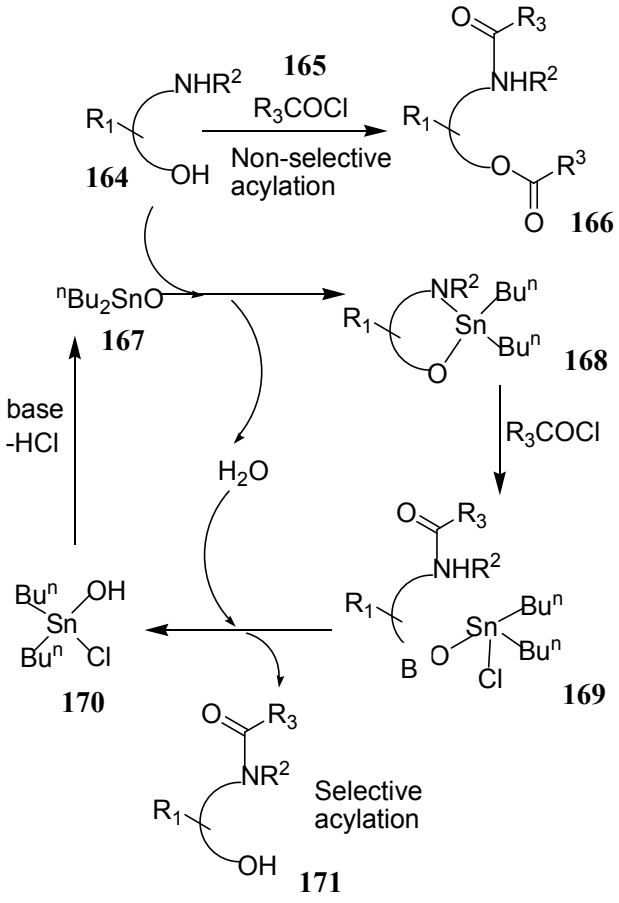


Scheme 1.36 Chemoselective acylation of alcohols.[143]

For example, in one reaction when the reaction of methyl benzoate (**158**) was carried out with cyclohexanol (**159**) and pyrrolidine (**160**) in the presence of zinc cluster (**161**), the yield of the desired ester product (**162**) was 82% whereas the yield of the amide (**163**) was only 9%. It was suggested that the zinc cluster having high oxophilicity carried out the reaction in a manner analogous to that of lipase.

Similar to the importance of *O*-acylation is the chemoselective *N*-acylation which has some very important applications in synthesis of drug molecules. The problem arises when the *N*-acylation procedure requires the protection of other sensitive groups such as hydroxyl groups, reducing the atom economy and increasing the waste. This use of protecting group indicates the incapacity to control the chemoselectivity in these reactions. Thus, there is a need of chemoselective reagents in order to improve the atom economy.[144]

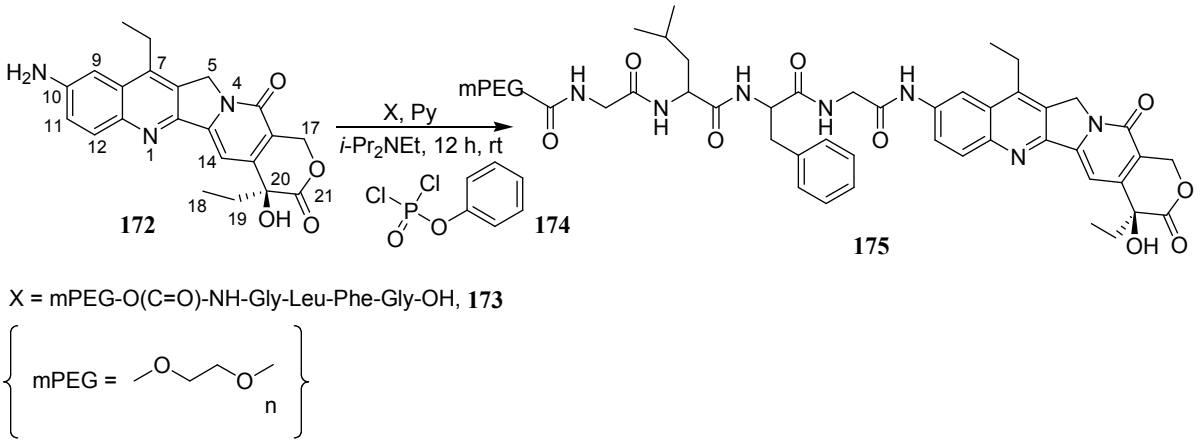
In the year 1996, Takayama *et al*., reported a novel reagent dibutyltin oxide (**167**) for the selective *N*-acylation in amino alcohols.[145] The acylation reaction of amino



Scheme 1.37 Selective *N*-acylation of alcohols in presence of dibutyltin oxide.[135]

alcohol (**164**) was non-selective (**166**) when the reaction was carried out without dibutyltin oxide (**167**) (Scheme 1.37). But when the reaction of **164** was performed in presence of dibutyltin oxide, in the first step it gave the *N*,*O*-dibutylstannylene acetal (**168**) and water. Further, when the acid chloride (**165**) was added it reacted with *N*,*O*-dibutylstannylene acetal (**168**) and afforded the intermediate **169** which by reacting with the molecule gave the *N*-monoacylated product (**171**) along with the intermediate **170**. If the reaction is carried out in the presence of a base, the reaction generates a catalytic cycle where the dibutyltin oxide (**167**) acts as a catalyst and regenerates in the reaction mixture.

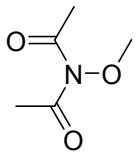
Occasionally, in the synthesis of drug molecules chemoselective acylation becomes difficult even if the one sensitive group is hindered. One such example is the case of



Scheme 1.38 Pegylated camptothecin analogue.[146]

N-acylation of 10-amino-7-ethyl camptothecin (**172**) (Scheme 1.38). In 2004, Guiotto *et al*., reported the first poly(ethylene glycol) conjugate of 10-amino-7-ethyl camptothecin (**175**) in which the *N*-acylation was carried out using phenyl dichlorophosphate (**174**).[146] On P388 murine leukemia cell lines the conjugate was equipotent to the camptothecin analogue CPT-11 which is already in clinical use. Here, in **172**, despite the hindered location of the C20-OH group, the selective *N*-acylation with poly(ethylene glycol) at 10-position was not possible, and with standard coupling methods such as DCC/HOBt it gave a low yield mixture of 20-*O*-monoPEGylated with 10 and 20-diPEGylated derivatives. But when the reaction was carried out using phenyl dichlorophosphate (**174**), a coupling reagent employed in esterification of alcohols, gave **175** without any PEGylation at 20-position.[146]

In 1990, Kikugawa *et al*., reported a novel chemoselective acetylating agent, *N*-methoxy diacetamide (**176**), particularly useful in *N*-acetylation of less hindered amino groups in the presence of secondary amines or alcohols.[147]



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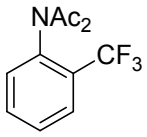

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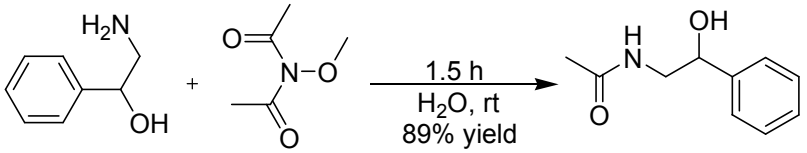
Figure 1.3 Chemoselective agent *N*-methoxy diacetamide and 2-trifluoromethyl-*N*,*N*-diacetylaniline.[147, 148]

In a typical experiment, when a reaction of **176** was carried out with 2-amino-1-phenylethanol (**178**), it gave only one *N*-acetylated product (**180**) (Scheme 1.39) without any *O*-acylation being observed.

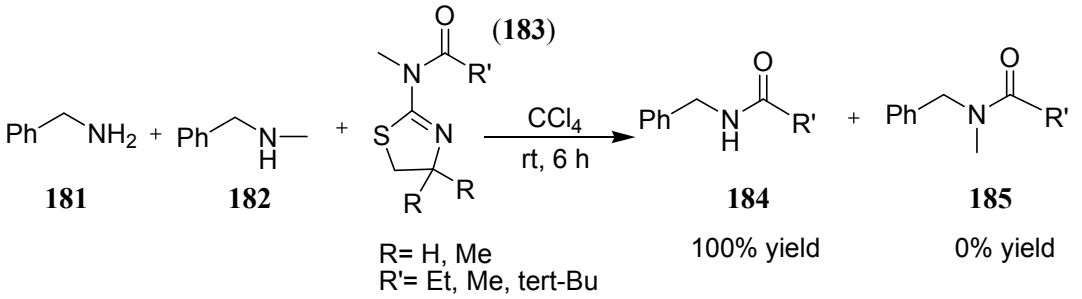


178 **179** **180**

Scheme 1.39 Chemoselective acetylation of 2-amino-1-phenylethanol.[147]

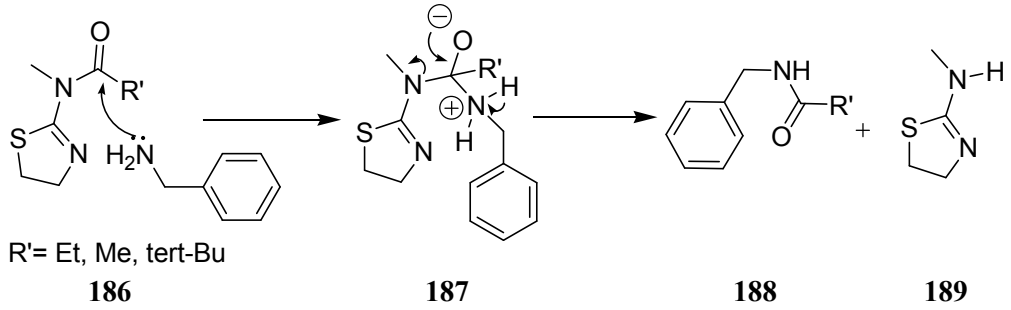
Another reported acetylating agent is, 2-trifluoromethyl-*N*,*N*-diacetylaniline (**177**), suitable for the effective acetylation of benzylamine and *N*-benzylamine in the mixture of secondary amines at room temperature and the acetylation of aniline at refluxing wide range of solvents.[148]

In 2002, Kim and Yang reported the sulfur containing compound, *N*-acyl 2-methylamino-2-thiazoline (**183**), as a chemoselective acylating agent (Scheme 1.40).[149]



Scheme 1.40 Reaction of *N*-acyl 2-methylamino-2-thiazoline with amines.[149]

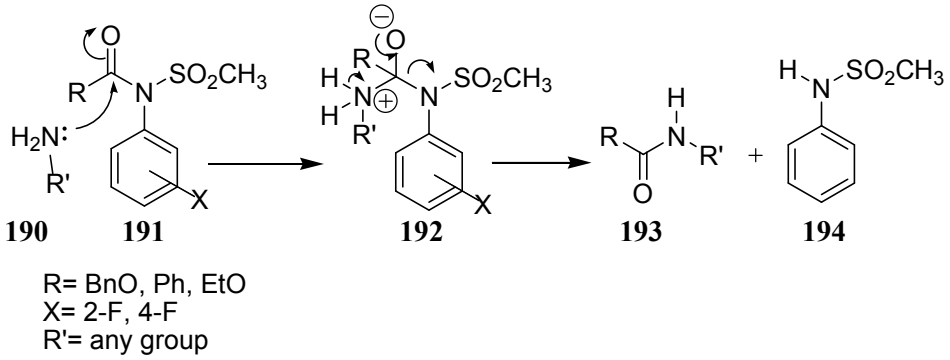
It is useful for acylation of primary amines (**181**) in the presence of secondary amines (**182**) and for the less hindered amine among two different primary amines. In a competition reaction of benzylamine (**181**) with *N*-methyl benzylamine (**182**) in presence of **183**, compound **184** was formed in 100% yield demonstrating the selectivity of **183** for primary amines over secondary amines.



Scheme 1.41 Mechanism of chemoselective acylation by *N*-acyl 2-methylamino-2-thiazoline.[149]

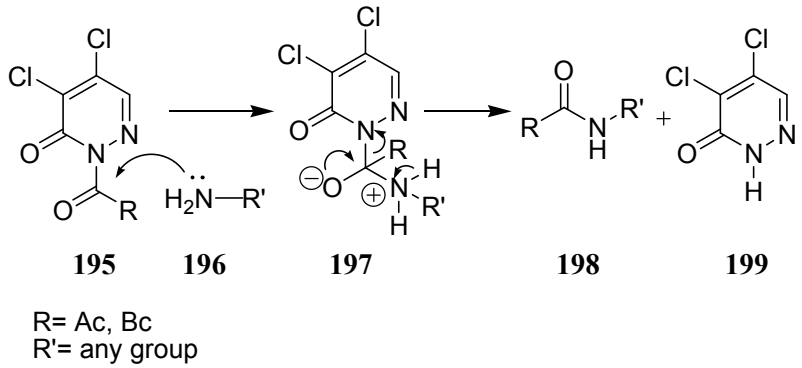
The reagent works well on various benzylamines as well as cyclic and acyclic amines (Scheme 1.41).

Other chemoselective *N*-acylating agents reported are mesylaniline agents (**191**)[150] and 2-acyl-4,5-dichloropyridazin-3-ones (**195**).[151] The mesylaniline agents (**191**) work well on less hindered amino groups in a molecule containing both hindered and less hindered amines (Scheme 1.42). Also, it is stable in air and easy to handle. In case of 2-acyl-4,5-dichloropyridazin-3-ones, they show good chemoselectivity for primary amino groups and work well on amines containing hydroxy and carboxyl groups. At the end of the reaction **199** is recovered and can be reused (Scheme 1.43).



190 **191** **192** **193** **194**

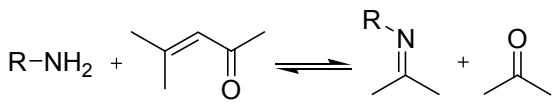
Scheme 1.42 Mechanism of acylation by mesylaniline agents.[150]



195 **196** **197** **198** **199**

Scheme 1.43 Mechanism of acylation by 2-acyl-4,5-dichloropyridazin-3-ones.[151]

For the separation of primary amines from secondary amines during their bulk scale production, a reported method involves the use of mesityl oxide (**201**). It is particularly useful in the separation of amines with close boiling points and other chemical and physical properties such as, piperidine (B.P. 106°C.) and n-amyl amine (B.P. 104°C.). The mesityl oxide reacts (**201**) with the primary amine (**200**) and forms an imine adduct (**202**) along with acetone (**203**) as a by-product which is distilled from the reaction; the imine-adduct is hydrolysed to recover the primary amine starting material (Scheme 1.44).[152]

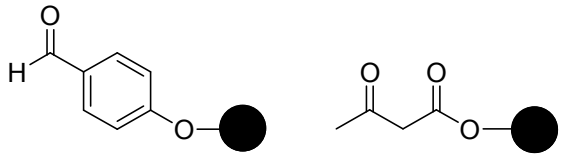


200 **201** **202** **203**

Scheme 1.44 Reaction of a primary amine with mesityl oxide.[152]

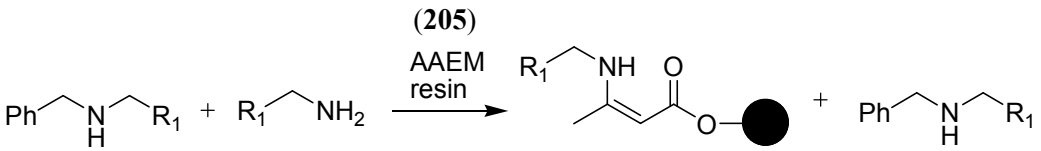
Chemoselectivity has also proved useful in controlling the outcome of other reactions, not just acylations. One such example is in the reductive alkylation of primary amines with aldehyde and ketones, which often ends up in over

alkylation.[153-155] It is particularly desirable for the chemical library synthesis of secondary and tertiary amines. In library synthesis, traditional chromatographic and distillation methods are not applicable.[156, 157] Because secondary amines serve as a valued pharmacophore in many biologically active compounds, there is a pressing need to develop methods which help in selective removal of primary amines in the presence of secondary amines. In this area many resins[158] are reported such as benzaldehyde (**204**)[159, 160] and acetoacetoxy ethyl methacrylate resin (AAEM) (**205**).[161]The acetoacetoxy ethyl methacrylate resin (**205**) selectively removes benzylamine (**207**) from its mixture with secondary amines (**206**), and is particularly useful in solution phase library synthesis (Scheme 1.45).



204 **205**

Figure 1.4 Benzaldehydeand acetoacetoxy ethyl methacrylate (AAEM) resins.[161]



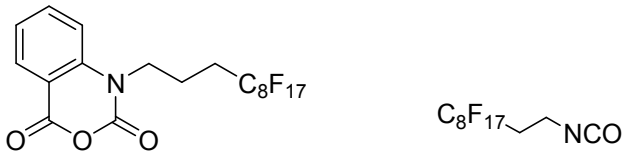
206 **207** **208** **206**

R^1= Ph, Bn, 2-Furyl

Scheme 1.45 Selective removal of benzylamine with AAEM resin.[161]

After the completion of reaction, the resin is filtered off and the pure secondary amine is obtained.[161]

Among some other scavengers, fluorine based compounds (**209** and **210**) have also been reported as useful in scavenging a wide range of primary amines, including



209 **210**

Figure 1.5 Fluorine based scavengers.[162]

benzylamine, and secondary amines such as tetrahydro-isoquinolines (Figure 1.5).[162] Another strategy reported in 1997 by Parlow *et al.*,[163] is the use of sequestration enabling reagents (Figure 1.6) such as hexafluoroisopropyl oxalate (**211**) which reacts with unreacted amines and forms a hemi-amide which is then subsequently sequestrated (**213**) by the scavenger-supported amine (**212**) added in the reaction mixture. At the end of the reaction, the scavenger and hemi-amide are removed from the product by a simple filtration.[163]

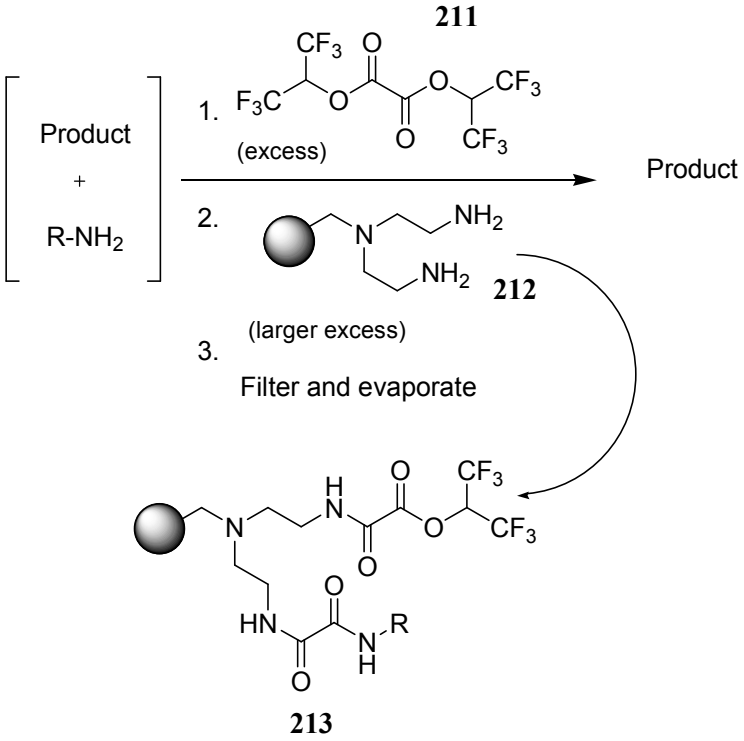


Figure 1.6 Sequestration of un-reacted amine with hexafluoroisopropyl oxalate.[163]

In addition to this methodology, another technique is impurity annihilation.[164] In this strategy, two scavengers are required in excess, but in this technique both are soluble in the crude reaction mixture containing the amine (Figure 1.7).[164]

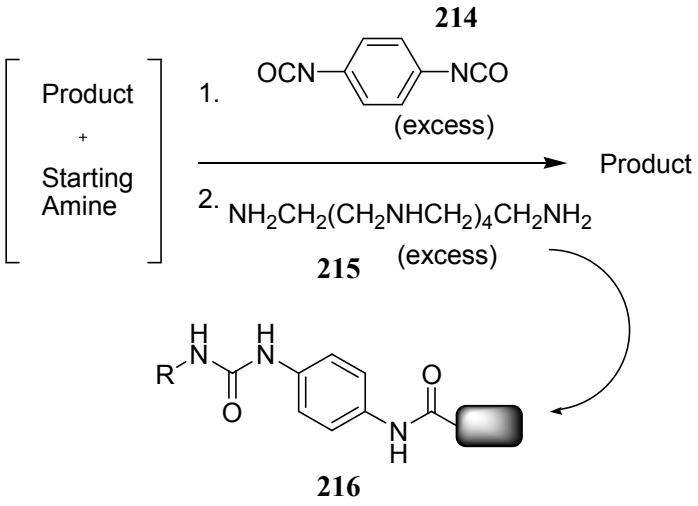


Figure 1.7 Impurity annihilation using scavengers.[164]

The first scavenger benzene-1,4-di-isocyanate (**214**) reacts with the amine impurity and forms an intermediate which further reacts with the second scavenger tetraethylenehexamine (**215**) and forms a precipitate (**216**) which is removed by filtration.[164]

Concluding all of the above discussed chemoselective reaction strategies, most of them require either the use of specially designed reagents which are not applicable on large scale reactions or require expensive polymeric supports which makes the process costly. Additionally, many reported sequestration enabling and impurity annihilation reagents are not very effective and carry a risk of producing un-wanted side products. Moreover, very few agents are reported to scavenge the relatively unreactive aniline family of amines from reaction mixtures. Thus, there is a need for developing a safe and broadly applicable chemoselective *N*-acylating reagent with an ability to differentiate between NH_2 and NH sites in a cost effective manner, particularly with aniline and its derivatives.

CHAPTER 2- DISCUSSION

Chapter 2:

DISCUSSION

2.1 Hypothesis and aims of the work

Taking together all the utilities and limitations of chemical and biocatalytic *N*-acylation, we hoped to develop a proof of principle *N*-acylation reaction of anilines and bulky amines for which lipase mediated *N*-acylations were not reported.

2.1.1 Working hypothesis: Can activated esters formed by an enzyme-catalysed reaction be converted to amides with the amide bond forming reaction occurring outside of the enzymes' active site and the activating agent be catalytically recycled?

2.1.2 Aims of the work

The study was intended to develop a proof-of-principle for the synthesis of amides through a dual catalytic cycle, with the following aims:

1. To design and develop an enzymatic route for the synthesis of activated esters.

2. To convert these activated esters into amides with low reactive anilines and bulky amines.

3. To develop a one-pot synthesis of amides by combining step 1 and 2.

4. To perform the one-pot synthesis with catalytic amounts of activating agent to prove the regeneration of the catalyst in the reaction mixture.

5. To perform the background reactions of the one pot synthesis reactions including reactions of amines with the unactivated ester along with CAL-B alone catalysed amidation.

6. To purify and characterize the compounds using ^{1}H, ^{13}C NMR, mass spectrometry and IR.

It was decided to synthesise various activated esters using CAL-B and then subsequently perform its reaction with the amines to get the corresponding amide product. Thus, a one-pot method could be developed to synthesise a range of amides

using various esters and activating agents such as acetone oxime, ethylene glycol, trifluoroethanol, trichloroethanol and tribromoethanol.

It was presumed that the CAL-B catalysed first step will occur inside the active site of the enzyme and will produce the activated ester (**219**) from the ester substrate (**217**) and activating agent (**218**). In the second step, the newly formed activated ester (**219**) will react with the amine (**220**) outside the active site of the enzyme and give the amide product (**221**) whilst regenerating the activating agent (**218**) which will enter the cycle again to form the activated ester. Thus, the cycle will continue as long as the starting materials remain in the reaction mixture and at the end of the reaction only amide product will be formed. In this way the proposed methodology will be green and environment friendly, producing minimal waste or harmful or toxic by-products.

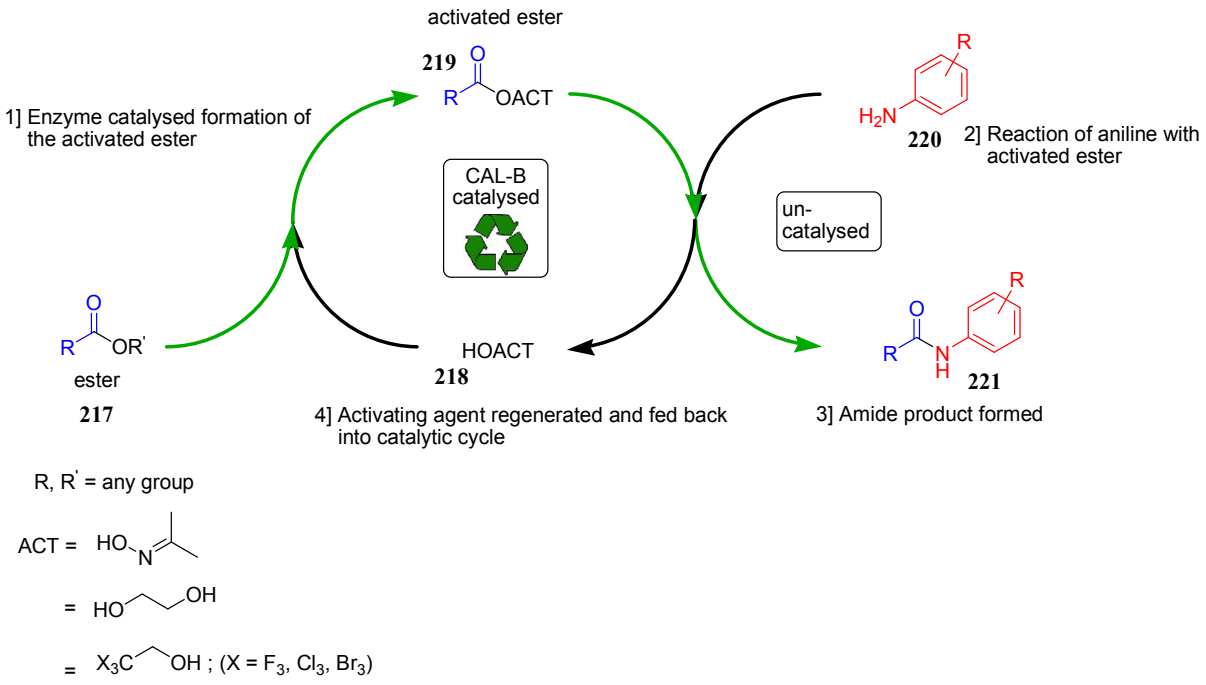


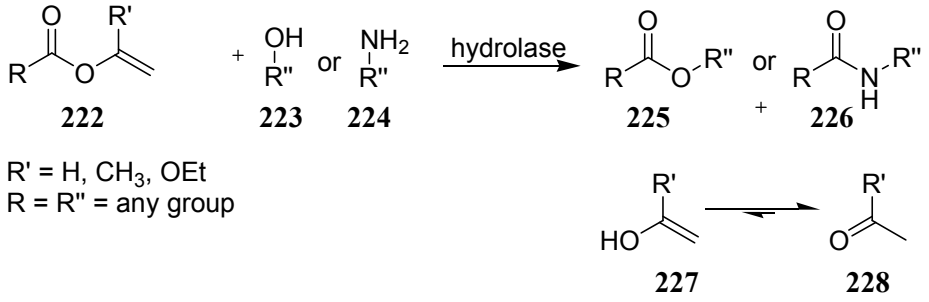
Figure 2.1 Catalytic cycle in the synthesis of amides.

Thus, the biocatalytic cycle will be beneficial in the synthesis of amides over classical methods involving use of corrosive acid chlorides and anhydrides which produce toxic by-products and make the process environment unfriendly. Additionally, amines, too big for the CAL-B active site, could be acylated by this method (**220** to **221**).

2.2 Synthesis of an acetone oxime activated ester and its use in the synthesis of acetanilides

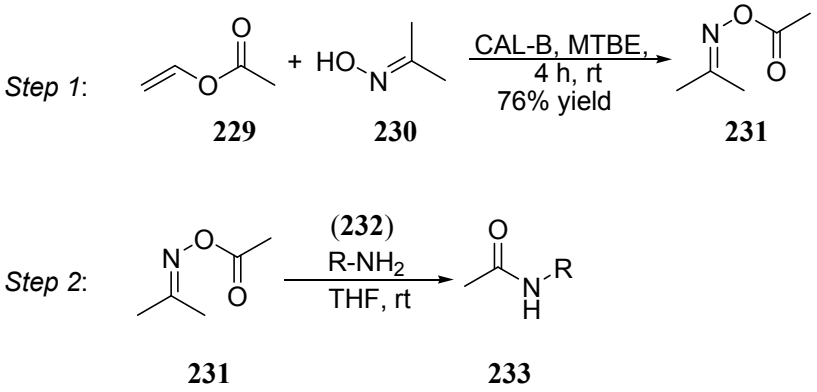
On the basis of previous discussions (Section 1.1 and 2.1) about the limitations of chemical and biocatalytic *N*-acylation, we hoped to develop a proof of principle *N*-acylation reaction of anilines and bulky amines for which lipase mediated *N*-acylations were not reported. It was envisaged that activated esters could be formed by an enzyme-catalysed reaction and subsequently be converted to amides with the amide bond forming reaction occurring outside of the enzymes' active site.

Initially it was decided to attempt the synthesis of the activated ester using CAL-B. For this, vinyl acetate was taken as the starting ester and acetone oxime was taken as the ester activating agent.[5, 7, 14, 43, 46, 47] In enzyme catalysed acylation reactions vinyl acetate (**222**, R= CH_3, R' = H) is known as an irreversible acyl donor in which the enol leaving group (**227**) readily tautomerises into its keto form (**228**) and thus the reaction mixture remains free from the nucleophile and becomes irreversible (Scheme 2.1).[165]



Scheme 2.1 Enzyme catalysed acylation using irreversible acyl donors.[165]

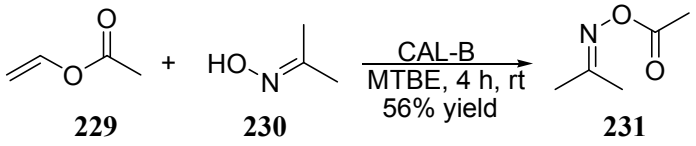
For ester activation, acetone oxime was chosen since they are known as quasi-irreversible acyl donors, although some cases of reversibility have been reported. In the reaction of quasi-irreversible acyl donors, the released nucleophile is not reactive enough to undergo nucleophilic attack on the ester product.[36] Also, oxime esters (**231**) have been reported in the synthesis of various amides (**233**) at room temperature (Scheme 2.2).[5, 6, 165, 166] Here, when the reaction of vinyl acetate (**229**) is carried out with acetone oxime (**230**) in presence of CAL-B, it gives the oxime ester product (**231**). Further reaction of this oxime activated ester (**231**) with amines (**232**) gives the corresponding amide product (**233**).



Scheme 2.2 Enzyme catalysed synthesis of amides from oxime ester.[6]

The reaction works well and is often clean and could be carried out at room temperature (Scheme 2.2).

Based on such precedent, a scheme was designed involving the CAL-B catalysed generation of an acetone oxime activated ester (**231**) as the first step with the expectation that amide (**233**, Scheme 2.4) formation would occur in second step via the *in-situ* generated activated ester. To start with, initially, the acetone oxime activated ester (**231**) was synthesised from vinyl acetate (**229**) and acetone oxime (**230**) (Scheme 2.3, Procedure 2.1). This reaction was performed to confirm the above discussed lipase catalysed synthesis of oxime activated ester from vinyl acetate and oxime (Scheme 2.2) would actually occur.

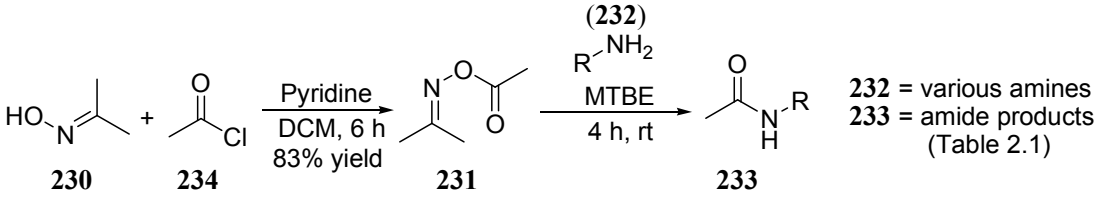


Scheme 2.3 Synthesis of CAL-B catalysed oxime ester.

For this, a reaction of vinyl acetate (**229**) and acetone oxime (**230**) in presence of CAL-B was carried out at room temperature for 4 hours using methyl *tert*-butyl ether (MTBE) as a solvent. The reaction gave 56% yield of the acetone oxime activated ester (**231**) with amines. After which, to check the reactivity of the proposed acetone oxime activated ester (**231**) it was synthesised chemically, on a multi-gram scale, in order to save enough material (Scheme 2.4, Procedure 2.2) and a list of reactions (Control 1, Table 2.1, Procedure 2.3) were carried out with a range of anilines and bulky amines (**232**) to yield the desired amide product (**233**). For this, a reaction of

acetone oxime (**230**) was carried out with acetyl chloride (**234**) in presence of pyridine base in DCM for 6 hours.

To check the reactivity of the obtained acetone oxime activated ester (**231**), it was subsequently allowed to react with various amines (**232**) to yield the corresponding amide products (**233**).



Scheme 2.4 Synthesis of amides from oxime ester, **231**.

After confirming the yield of amide products obtained (Scheme 2.4), a one-pot synthesis (combining step 1 and 2 in Scheme 2.3 and 2.4) of amide (control 3, Table 2.1, Procedure 2.4) with *in-situ* generation of activated ester was carried out with those amines that proved successful in the reaction with the acetone oxime activated ester to obtain the desired amide product (Figure 2.2, Scheme 2.5).

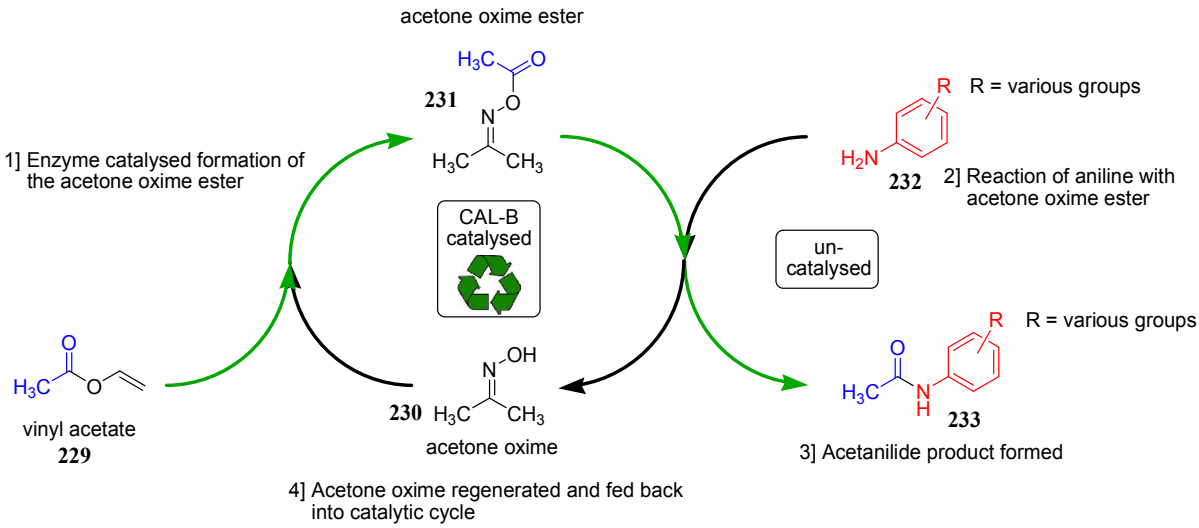
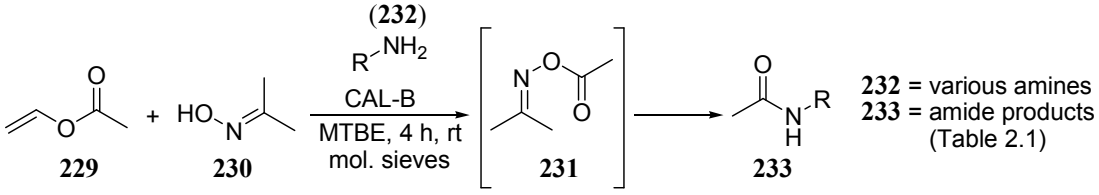


Figure 2.2 Catalytic cycle in the synthesis of amides.[167]

In the one-pot synthesis the first step is catalysed by CAL-B generating the acetone oxime activated ester (*in-situ*) which further undergoes nucleophilic attack by an amine to yield the corresponding amide product. This nucleophilic addition occurring

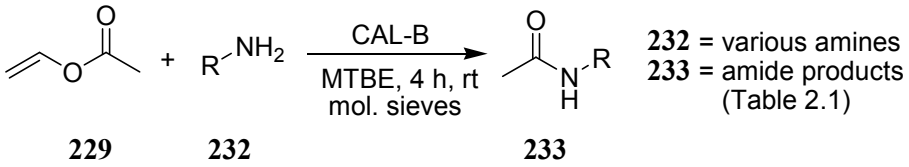
outside the enzyme's active site, can overcome the problem of the limited active site space (9.5x 4.5Å) of CAL-B,[80] which can not incorporate bulky substrates inside it. Additionally, the acetone oxime released can be re-used in the next cycle. Thus, it was presumed that the reaction of vinyl acetate (**229**) and acetone oxime (**230**) will be catalysed by CAL-B to give the acetone oxime activated ester (**231**) which will further react with the amine (**232**) to give the corresponding amide product (**233**) along with the regeneration of the acetone oxime (**230**).

To carry out the one-pot synthesis of amides, reactions of vinyl acetate (**229**), acetone oxime (**230**) and amine (**232**) were performed in one pot for 4 hours in presence of CAL-B and 4Å molecular sieves taking MTBE as a solvent (Scheme 2.5, Procedure 2.4). Molecular sieves were used to prevent the hydrolysis of the ester substrate.



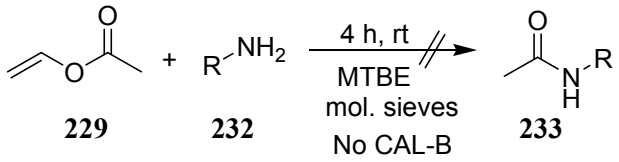
Scheme 2.5 One-pot synthesis of amides.

Alongside the one-pot synthesis of amides (control 3), a comparative study was also performed to compare the yield of amides directly catalysed by the lipase itself (Control 2, Table 2.1, Procedure 2.5), without any ester activation (Scheme 2.6, Procedure 2.5), over the one pot reaction involving the *in-situ* generation of the activated ester (Scheme 2.5).



Scheme 2.6 CAL-B catalysed amidation.

For this, a list of reactions (Scheme 2.6) were carried out with vinyl acetate (**229**) and various anilines and bulky amines (**232**) in presence of CAL-B and 4Å molecular sieves to obtain the desired amide products (**233**) and the results are listed in Table 2.1, Control 2.

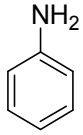
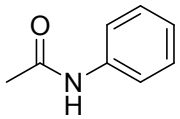
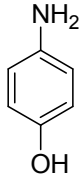
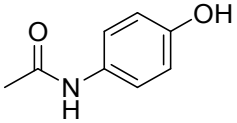
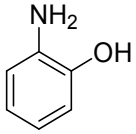
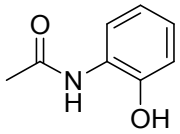


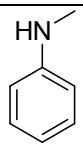
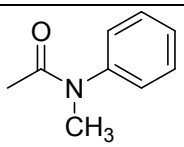
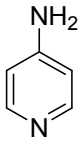
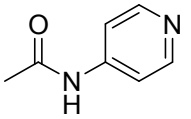
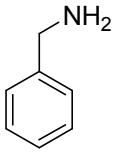
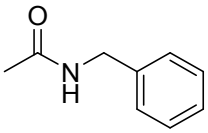
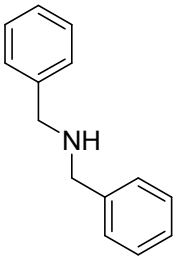
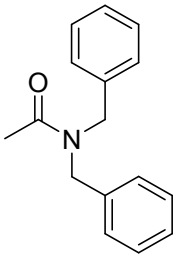
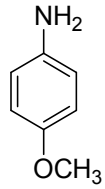
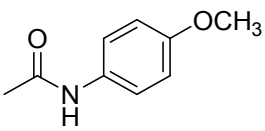
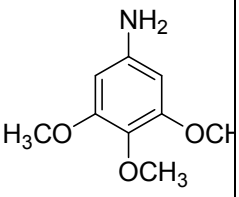
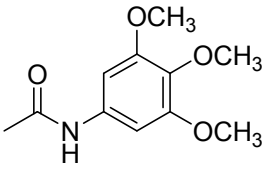
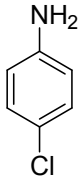
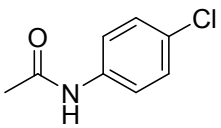
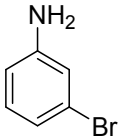
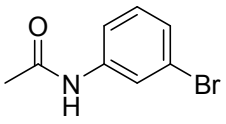
229 **232** **233**

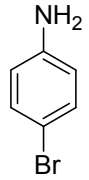
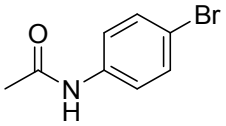
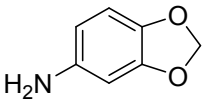
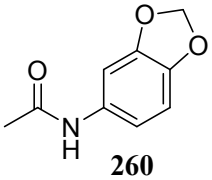
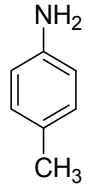
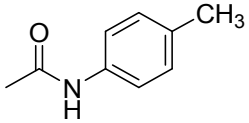
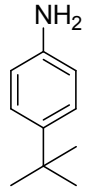
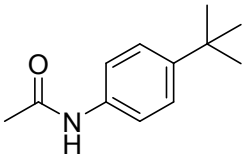
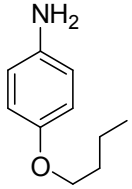
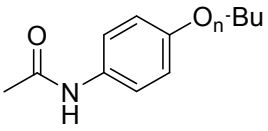
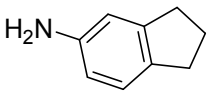
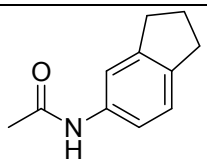
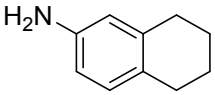
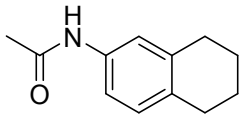
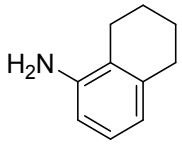
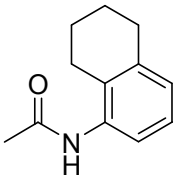
Scheme 2.7 Reaction of non-activated ester vinyl acetate with amine.

Additionally, background reactions of the non-activated ester vinyl acetate (**229**) and the amines (**232**) without CAL-B were also performed which did not yield the *N*-acylated product (confirmed by crude ^{1}H NMR of the reaction mixture) (Scheme 2.7, Procedure 2.6). The reaction of the non-activated ester vinyl acetate and the amines proved the utility of using activated ester in the synthesis of amides (Scheme 2.5), where the products were obtained in good yields.

Up to this point a stoichiometric amount of acetone oxime was used in combination with catalytic CAL-B (Control 2 and 3). However, in principle, since it is recycled in the reaction, it could also be reduced to catalytic amounts without a detrimental effect on the yield of the amide product. As such, it was reduced to 0.1mol. eq. and its reaction with the amines under the conditions of one-pot synthesis examined (Table 2.1). The results in Table 2.1 indicate a significantly better yield of amides are produced when a one pot-synthesis is performed, using acetone oxime to activate the vinyl acetate (see Increase c/b column in Table 2.1).

Entry	Amine (**232**)	Products (**233**)	Control 1[a] Yield (%)[d]	Control 2[b] Yield (%)[d]	One-pot[c] Yield (%)[d]	Increase (c/b)	Catalytic acetone oxime[e] Yield (%)[d]
1	 **235**	 **236**	52	13	84	**6.5**	35
2	 **237**	 **238**	54[f]	<1%	72	**>72**	nr
3	 **239**	 **240**	Traces	n.d.	n.d.	-	n.d.

4	 **241**	 **242**	nr	n.d.	n.d.	-	n.d.
5	 **243**	 **244**	nr	n.d.	n.d.	-	n.d.
6	 **245**	 **246**	50	42	62	**1.5**	n.d.
7	 **247**	 **248**	Traces	6	n.d.	-	n.d.
8	 **249**	 **250**	75	23	69	**3**	72
9	 **251**	 **252**	nr	n.d.	n.d.	-	n.d.
10	 **253**	 **254**	Traces	n.d.	n.d.	-	n.d.
11	 **255**	 **256**	11	7	45	**6.4**	24

12	 **257**	 **258**	46	7	48	**6.9**	33
13	 **259**	 **260**	40	43	64	**1.5**	40
14	 **261**	 **262**	88	55	90	**1.64**	52
15	 **263**	 **264**	88	11	41	**3.7**	27
16	 **265**	 **266**	86	7	41	**5.9**	29
17	 **267**	 **268**	83	18	65	**3.6**	35
18	 **269**	 **270**	84	35	54	**1.54**	41
19	 **271**	 **272**	nr	n.d.	n.d.	-	n.d.

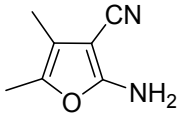
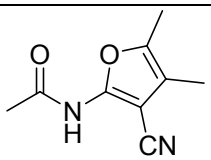
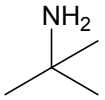
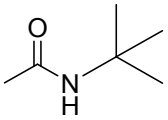
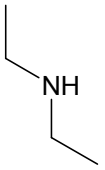
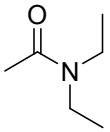
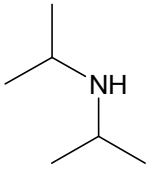
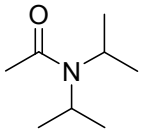
20	 **273**	 **274**	nr	n.d.	n.d.	-	n.d.
21	 **275**	 **276**	nr	n.d.	n.d.	-	n.d.
22	 **277**	 **278**	nr	n.d.	n.d.	-	n.d.
23	 **279**	 **280**	nr	n.d.	n.d.	-	n.d.

Table 2.1 Amides synthesised by activated ester and CAL-B.

a Control 1: **231** (1 equiv.), **232** (1 equiv.), 4Å molecular sieves (1g) in MTBE (10ml), 4 h. **b** Control 2:vinyl acetate (**229**) (2 equiv.), **232** (1 equiv.), 4Å molecular sieves (1g), CAL-B (1.5 g, 0.00263g/mol of ester) in MTBE (10 ml), 4 h. **c** One-pot synthesis: vinyl acetate (**229**) (2 equiv.), **230** (1 equiv.), 4Å molecular sieves (1g), **232** (1 equiv.), CAL-B (1.5g, 0.00263g/mol of ester) in MTBE (15ml), 4 h. **d** Isolated yield of pure material. **e** Catalytic acetone oxime: as one-pot synthesis method but with **230** (0.1 equiv. instead of 1 equiv,). **f** Yield based on crude NMR. n.d.: not determined.

Presumably the activated ester increases the electrophilicity of the carbonyl carbon causing it to be more reactive towards nucleophilic attack by the amine. Furthermore, the intermediate formed after the nucleophilic attack of the amine readily releases the stable anion along with the amide product.

Starting with aniline (entry 1, Table 2.1), it reacted well in the one-pot synthesis (Control 3, 84%) compared to the CAL-B catalysed amidation (Control 2, 13%). To date, there is only a single report, on the use of CAL-B to synthesise *N*-phenyl acetamide from aniline which failed to give the amide product.[88] This report also confirmed the result of Control 2 where the CAL-B catalysed amidation gave only 13% yield of *N*-phenyl acetamide (Table 2.1). However, in our study, we showed that aniline reacts well when vinyl acetate was activated as the acetone oxime ester (Control 1, 52%). Here, the 52% yield obtained in 4h, is comparable to the reported

84% yield of *N*-phenyl acetamide obtained by Fernandez *et al*.,[6] however, in that case the reaction of the activated ester and aniline was carried out in tetrahydrofuran (THF) for 24h. This difference in yield could possibly be due to solvent used because it has been shown that the nucleophilicty of amines can be affected by solvent.[168] For example anilines although being less nucleophilic than alkyl amines, are more reactive in acetonitrile solvent when compared to alkyl amines. The reason of this greater reactivity than alkyl amines is reported to be dependent on the polarity of the solvents.[168]

To date, the reported methods for the synthesis of *N*-phenyl acetamide, involve heating the reagents from 2-8 h upto 70°C.[169, 170] To prove the importance of acetone oxime in the one-pot method, a reaction of vinyl acetate (1 eq.), aniline (1 eq.) and CAL-B was carried out in MTBE, without addition of acetone oxime. After 4h, there was no amide formation in the reaction mixture, confirmed by ^{1}H NMR. After NMR, one equivalent of acetone oxime was added to the same reaction mixture and the reaction was further continued for 4 h. After which the NMR was taken again which showed the formation of acetanilide had occurred along with the activated ester. The purified product gave 35% yield of the amide product. The low yield obtained could be due to continuous stirring for 8h, causing denaturation of the enzyme. Nevertheless, the experiment demonstrated the importance of acetone oxime in this protocol.

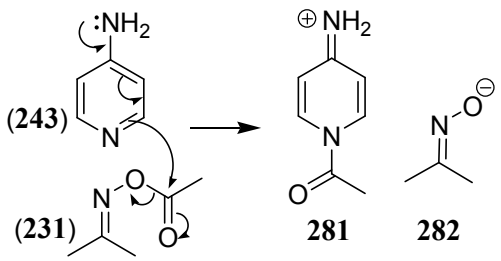
The presence of the hydroxyl group at the 4-position (entry 2) also reacted well in the one-pot synthesis (Control 3, 48%) compared to the CAL-B catalysed amidation (Control 2) which gave negligible yield of the amide product. Whilst it is hypothesised that the electron donating hydroxyl group would increase the nucleophilicty of the amine, its yield was less than with aniline, suggesting that factors other than resonance affect the reactivity of this amine. In stark contrast, when the hydroxyl group is in the 2-position (entry 3), negligible yield of the amide product was obtained (Control 1), presumably either due to steric hindrance of the hydroxyl group preventing its reaction with the activated ester, or inter- or intramolecular hydrogen bonding taking place in 2-aminophenol, preventing reaction;[171] therefore, further reactions of Control 2 and one-pot synthesis were not carried out with this amine.

Gratifyingly, the presence of a methoxy or butoxy group at position-4 in the ring (entry 8 and 16 respectively) also reacted well in the one-pot synthesis (69% and 41% yield) compared to the CAL-B catalysed amidation (Control 2, 23% and 7%), suggesting that further functionalization is tolerated at this position. However, in the one-pot reaction, the yield of the amide product decreased on increasing the number of carbon atoms from one (entry 8, 69%) to four (entry 16, 41% yield).

In the case of 4-aminophenol (entry 2) and 4-methoxyaniline (entry 8) the one-pot reaction gave significantly better yield compared to the CAL-B catalysed amidation (entry 2 and 8, Control 2).

Increasing the number of methoxy groups from one (entry 8) to three (entry 9) should also increase the basicity of the amine, and thus reaction yield, however, surprisingly, 3,4,5-trimethoxyaniline (entry 9) did not give the amide product upon reaction with the activated ester (Control 1). This might be correlated to its bulky structure hampering its reaction with the activated ester, and is possibly a limitation of the method.

Unsurprisingly, the presence of a methyl group on nitrogen (i.e. a secondary amine) failed to give any product upon reaction with the activated ester (entry 4, Control 1). Presumably, the increased steric hindrance of the secondary amine overrides the increase in nucleophilicity gained by the extra alkyl group. This result is a stark contrast to the yield of amide obtained from aniline where there is no substituent on the amino group (entry 1, Control 1, 52%). Furthermore, the presence of nitrogen (entry 5, Control 1) in the ring also did not give any product. This could be correlated to the delocalisation of nitrogen's electrons ($-NH_2$) into the ring causing a decrease in the electron density on the nitrogen atom and thus a decrease in nucleophilicity. However, the similarity to 4-dimethylaminopyridine (DMAP) in this case means that the sp^2-nitrogen (**243**) in the ring may have reacted with the activated ester (**231**) (Scheme 2.8) giving intermediate **281**, but this compound was not observed experimentally, where only activated ester was present in the crude ^1H NMR of the reaction.



Scheme 2.8 Potential reaction of 4-aminopyridine with activated ester **231**.

The inclusion of halogen atoms seems to have an effect on yield. For example, the reaction of 4-chloroaniline (entry 10, Control 1) with the activated ester gave negligible yield, indicating the dominance of an electron withdrawing inductive effect, compared to electron donating resonance in the molecule making the amine less reactive than aniline itself.[172]

However, the presence of the less electronegative bromine in 3-bromoaniline gave a better yield of the corresponding amide in the one-pot synthesis, that is, 45% (entry 11), compared to the CAL-B catalysed amidation (Control 2, 7%) suggesting that the electronic effects are less dominating here, although the yield is reduced compared to aniline. Furthermore, presence of bromine at position-4 in 4-bromoaniline (entry 12), gives a similar yield (one-pot synthesis, 48%) compared to that obtained from 3-bromoaniline (one-pot method, 45%). Despite this similarity in the yields of the one-pot reaction, the yields of their reaction with the activated ester differ by up to 4-fold (11% for 3-bromoaniline and 46% for 4-bromaniline). This variation in yield could be attributed to their basicity. At position-3, the inductive effect of bromine is more dominant than its resonance contribution, in comparison to its effects at position-4 in 4-bromoaniline. Here, the electron donating resonance is dominant over electron withdrawing inductive effect. The inverse relationship of the distance of the bromine from the amine with its reactivity means that the yield of the amide from the more nucleophilic 4-bromoaniline is higher.[172]

The presence of two oxygen atoms in 3,5-methylenedioxyaniline (entry 13) also gave an increased yield (one-pot synthesis, 64%) compared to the CAL-B catalysed amidation without acetone oxime (Control 2, 43%). This improved yield could be correlated to the electron donating resonance effect increasing the basicity of the amine. The 64% yield obtained with the one-pot synthesis is satisfactory compared to other reported methods giving 97-98% yield, which involve either use of acetic

anhydride or heating of the reaction mixture.[173, 174] However, in the present study, the amide was obtained at ambient conditions with use of green reagents and environmentally friendly lipase enzyme. This one-pot yield is comparable to the one-pot yield of the amide obtained from 4-methoxyaniline (entry 8, Control 3, 69%). Presumably, in both cases the oxygen atoms are contributing to resonance, causing an increase in the nucleophilicty of the amine's nitrogen atom. However, increasing the bulk around the nitrogen atom (entry 9) completely inhibits the formation of the amide product in our hands.

In the presence of a methyl group (entry 14), which pushes the electrons towards the ring via its electron donating inductive effect, the activated ester (Control 1) and one-pot synthesis worked well. Presence of a *tert*-butyl group (entry 15) gave a better yield of the corresponding amide, that is, 88% and 41% (Control 1 and one-pot synthesis respectively) over lipase catalysed amidation (Control 2) which gave only 11% of the amide product. This is an excellent result which highlights the potential of this method for preparing bulky amides, which cannot be catalysed by the enzyme alone,[78, 79] since it is proposed that the amidation step takes place outside of the enzymes active site.

Other more hindered amines, such as, 5-aminoindane (entry 17) reacted well with the activated ester in the one-pot reaction (one-pot synthesis, 65%), in comparison to the CAL-B catalysed amidation (Control 2, 18%). Thus, again the catalytic amidation indicated the limited size of the enzyme's active site. Additionally, the proposed methodology involving *in-situ* generation of the activated ester, demonstrated an improved way to synthesise the amide of 5-aminoindane which is reported to be an intermediate used in the synthesis of tricyclic 1,2,4-triazine oxides, used in cancer treatments.[175] Its related amine (entry 18) also gave good yield in the one-pot reaction (one-pot synthesis, 54%). However the isomeric amine did not react with the activated ester (entry 19). This could be due to the steric hindrance of created by adjacent methylene group preventing attack on the electrophilic carbonyl of the activated ester.

Other amines, such as, 2-amino-4,5-dimethyl-3-furancarbonitrile (entry 20, Control 1) did not react with the activated ester indicating that delocalisation of electrons from nitrogen onto the cyano group may be having an effect. In case of benzylamine,

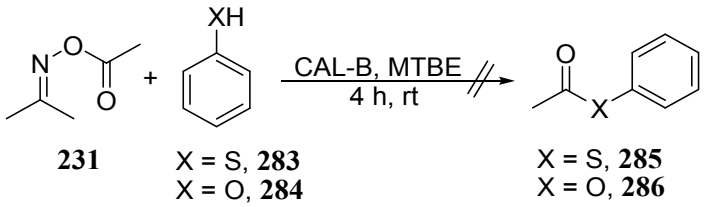
the one-pot yield was better (entry 6, one-pot synthesis, 62%), compared to the CAL-B catalysed amidation (Control 2, 42%). However, the presence of two benzyl rings (entry 7) completely inhibited the amide formation (Control 1). Its enzyme catalysed amidation also gave a very low yield (Control 2, 6%) possibly indicating the limited active site area of CAL-B being unable to accommodate this bulky amine. More basic bulky acyclic amines (entry 21-23) did not react with the activated ester at all, presumably due to their increased steric hindrance.

2.2.1 Synthesis of acetanilides using a catalytic amount of acetone oxime

After obtaining the above discussed results, where acetone oxime was used in equimolar amounts with the amine and ester substrate, it was decided to do all the one-pot reactions with 0.5 equivalent of acetone oxime in order to prove that the activating agent acts as a catalyst and regenerates in the reaction mixture. Therefore keeping all reaction conditions the same, as were used in the one pot-synthesis (see one-pot synthesis[c], Table 2.1), when acetone oxime was used in catalytic amounts, the formation of the desired amide product occurred in the majority of the reactions (see Catalytic acetone oxime[e], Table 2.1, Procedure 2.7) proving that the acetone oxime can be recycled in the reaction. This novel aspect of the proposed methodology, that the activating agent acetone oxime is regenerated in the reaction mixture, means that at the end of the reaction only the desired amide product is formed with minimal generation of any toxic or harmful by-products.[167]

2.2.2 Comparison of the nucleophilicty of amines with oxygen and sulphur nucleophiles

After completion of the one-pot synthesis of acetanilides from amines it was decided to compare the nucleophilicty of the amines with other nucleophiles in order to expand the reaction scope. As such, reactions of acetone oxime activated ester (**231**) were also carried out with thiophenol (**283**) and phenol (**284**).



Scheme 2.9 Reaction of activated ester with thiophenol and phenol.

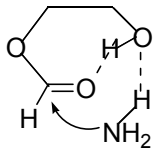
To test these new substrates, a reaction of the acetone oxime activated ester (**231**) was carried out separately with thiophenol (**283**) and phenol (**284**) in presence of CAL-B taking MTBE as a solvent. Unfortunately, these reactions did not yield the desired products (**285**, **286**), indicating their poor nucleophilicty over their nitrogen-based aniline analogues (Scheme 2.9).

In summary, the obtained results demonstrated that anilines, which are reported to be less reactive towards CAL-B catalysed *N*-acylation, could be acylated well with the proposed methodology since the amide bond formation seems to occur outside the enzyme's active site. Also, since the reaction is independent of the size of the active site it could be useful in the synthesis of bulky amides.[167]

2.3 Novel methodology for the synthesis of glycol activated esters

After the successful synthesis of acetanilides through our developed one-pot method and highlighting the importance of ester activation, it was decided to further expand the substrate scope of the enzyme catalysed reactions and to synthesise some pharmaceutically important esters using additional activating agents such as glycol based esters.[56]

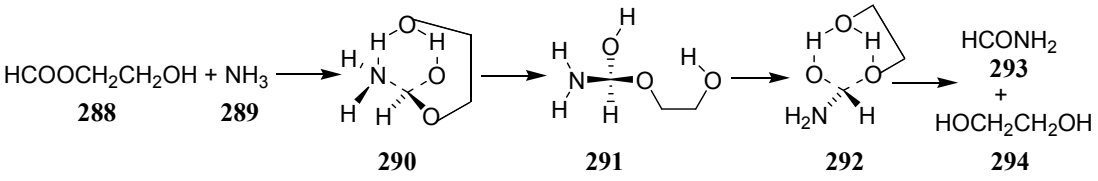
It is reported that the *syn*-vicinal hydroxyl-group of ethylene glycol assists in the amide formation by stabilizing the intermediate.[54] In this field, in the year 2007, Bayryamov *et al*., first reported the intramolecular aminolysis reactions catalysed by a vicinal hydroxyl group in a ribosome model depicting the role of the 3'- terminal adenosine 2'-OH of the peptidyl tRNA in the ribosome peptidyl transfer.[56] In computational studies it was shown that the hydroxyl group assists in forming a favourable conformation to undergo nucleophilic attack by the amino group (Figure 2.3).



287

Figure 2.3 Computational model of intramolecular aminolysis reaction catalysed by a vicinal hydroxyl group.[56]

After their suggested hypothesis, this group reported more details on the proton shuttling role of the vicinal hydroxyl group of the ester between the ester (**288**) and the amine (**289**) (Scheme 2.10).[54, 55] Here, the *syn*-2-OH assists in proton transfer during the aminolysis of the ester.

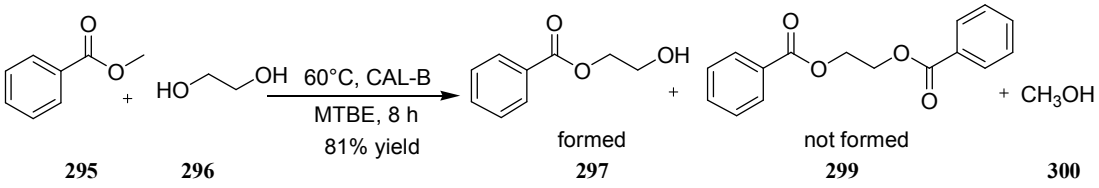


Scheme 2.10 Computational model of proton shuttling by a vicinal hydroxyl group.[54]

The hydroxyl group helps in forming a favorable and more stable transition state. It accepts the hydrogen bond from the amine (ammonia) and donates it to the carbonyl oxygen, thus acting like a bridge providing linear proton transfer geometry (**290** to **292**). The hydroxyl group acts as a proton shuttle and decreases the activation energy of the reaction by 16 kcal/mol and accelerates the reaction by around 10^9-fold.[55] At the end of the reaction the amide product (**293**) forms along with the ethylene glycol (**294**).

Analyzing the above discussed assistance of a proximal hydroxyl group in amide formation reactions, it was decided to carry out a one-pot synthesis of amides (as discussed in section 2.1.2, see Figure 2.1 catalytic cycle in the synthesis of amides) from activated esters using ethylene glycol as an ester activating agent. It was presumed that in the first step CAL-B would catalyze the formation of ethylene glycol activated ester which in the second step will react with the amine (as shown above, Figure 2.3) and give the corresponding amide product along with the regeneration of ethylene glycol. It was believed that the use of ethylene glycol would be useful in carrying out the synthesis on a large scale due to its less toxic nature.

To start with, a simple benzoate ester, methyl benzoate, was chosen for the study as it is commonly used in industry. Initially, a reaction of methyl benzoate (**295**) and ethylene glycol (**296**) was carried out with 1.5g of CAL-B giving 81% product (**297**) yield (Scheme 2.11, Procedure 2.8) with no dimer formation (**299**).



Scheme 2.11 Reaction of methyl benzoate with ethylene glycol.

While studying these reactions with ethylene glycol it was found that such reactions with benzoate esters, when carried out at high temperature, give unwanted dimer (**299**) also along with the monomer as the reaction product (Scheme 1.27).[110] The removal of the dimer is tedious and makes the process costly. As such, to prevent this dimer formation various sodium based zeolites have been used. Unfortunately, these zeolites require pre-preparation, making the process costly and time consuming as

well. Compare such work with the reaction in Scheme 2.11 where the reaction was carried out employing CAL-B for transesterification and only monomer (**297**) product was formed with 100% selectivity with 81% yield. This is a novel advantage of using CAL-B over the use of zeolites which require previous preparation and tedious purification techniques of the products. Also, in this reaction, the product can be easily isolated from the crude mixture as the glycol sticks to the silica at the time of chromatography. Additionally, the trans-esterification does not require any harsh reagents and the enzyme reaction is clean compared to chemical ones. The route could potentially also be applied for synthesis of glycol esters required in the synthesis of polyesters,[92] fungicides[92] and pheromones[93, 94] (see Section 1.2).

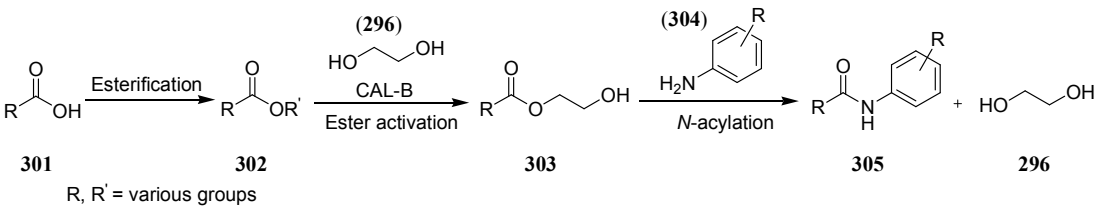
Being able to control the formation of the monoester product exclusively from benzoate ester (Scheme 2.11), it was decided to work on the synthesis of glycol esters. Also, so far, enzymes are not known to catalyse the synthesis of glycol esters from benzoate esters and ethylene glycol. Therefore it was decided to use CAL-B in the synthesis of glycol esters here to improve upon the reported methods.

In this context, a novel route has been applied to synthesise glycol esters with minimum formation of dimer in the reaction. Additionally, the synthesised glycol esters being activated were also tried to synthesise some amides.

In Scheme 2.11, the selectivity for the monomer product was 100% even after reducing the enzyme level to 0.5g. But with 0.5g of CAL-B, the product yield (**297**) dropped down to 38% which could be correlated to the reversibility of the reaction due to release of methanol from the ester substrate undergoing nucleophilic attack again on the ester product (**297**). It is already proven that the reaction reversibility in CAL-B catalysed transesterification reactions is due to release of alcohol from the ester substrate.[176]

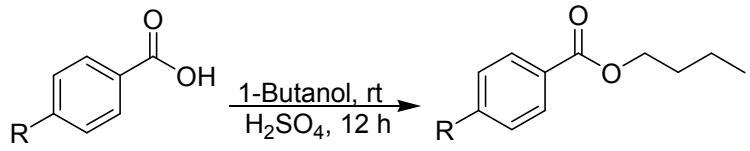
Nevertheless, the yield can be improved by increasing the amount of enzyme. In order to study the reactions in detail, it was decided to synthesize a range of esters (**302**) with different functionalities. It was proposed that the electron withdrawing or donating groups would affect the reactivity of the carbonyl carbon in the esters and therefore the yield of the glycol activated ester product (**303**). It was decided to synthesise amides (**305**) applying our one-pot methodology (Figure 2.1 catalytic cycle in the synthesis of amides). For this it was planned first to perform the reaction

of activated ester (**303**) and amines (**304**), and if they were successful, then continue with the one-pot synthesis of amides along with CAL-B catalysed background reactions of the non-activated ester (**302**) with amines (**304**) in addition to the reaction of the non-activated esters with amines without CAL-B (Scheme 2.12).



Scheme 2.12 General scheme of synthesis of amides from ethylene glycol activated esters.

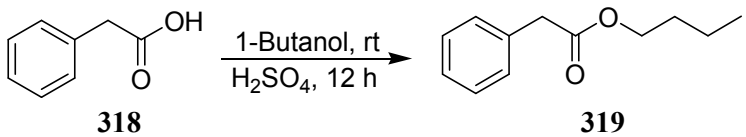
Additionally, it was also decided to study the reaction using butanoate esters, as it is known that the released butanol, being bigger than methanol, is less able to undergo nucleophilic attack on the ester product and will therefore, reduce the rate of reversibility.[176] For this, a range of esters (**312-317**, **319**) with electron donating and withdrawing groups were synthesized from their corresponding acid (**306-311**, **318**) using Fischer's esterification procedure (Scheme 2.13 and 2.14, Procedure 2.9).



R = H (**306**), CH_3 (**307**), OCH_3 (**308**), NH_2 (**309**), Br (**310**), NO_2 (**311**)

R = H (**312**), CH_3 (**313**), OCH_3 (**314**), NH_2 (**315**), Br (**316**), NO_2 (**317**)

Scheme 2.13 Synthesis of esters using Fischer's esterification procedure.



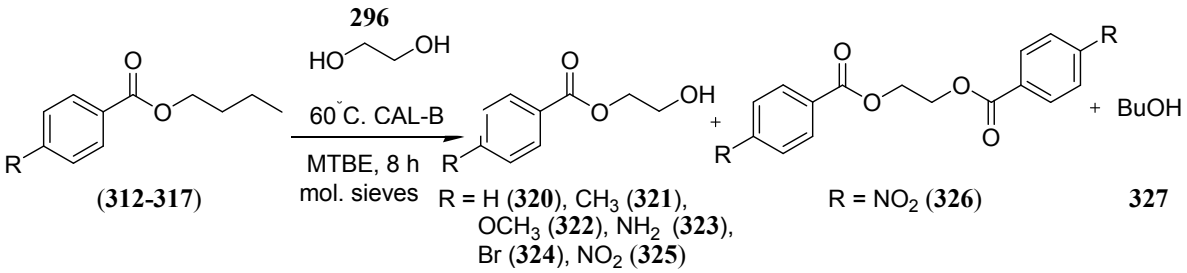
Scheme 2.14 Synthesis of ester using Fischer's esterification procedure.

Here phenyl acetic acid (**318**) (Scheme 2.14) was also chosen to synthesise the ester (**319**) because it was believed that the presence of the methylene unit between the ring and the carbonyl carbon in the ester would preclude delocalization of electrons

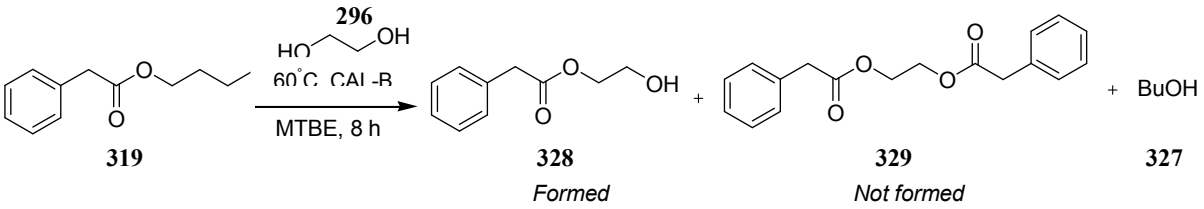
from the ring and therefore would increase the reactivity of the carbonyl carbon and thus increase the rate of formation of the glycol activated ester on its reaction with ethylene glycol. Also, it would increase the possibility of amide formation on reaction with amines (**304**) due to the increased electrophilicity of the carbonyl carbon, in addition to the assistance in amide (**305**) formation by the hydroxyl group of the glycol present in the activated ester (**303**) as discussed in Figure 2.3.

Additionally, it was also decided to do a comparative study on the percentage of monomer and dimer formation as discussed in Scheme 2.11. This could be an important application of the novel methodology for the synthesis of glycol esters using CAL-B because a reaction without any formation of dimer at the end is a major challenge in the synthesis of glycol monomers.[110]

From the esters synthesised in Scheme 2.13 and 2.14, the corresponding glycol activated esters were synthesised (Scheme 2.15 and 2.16, Procedure 2.10) in the presence of CAL-B with varying equivalents of ester and ethylene glycol (see Table 2.2).



Scheme 2.15 CAL-B catalysed synthesis of glycol esters.

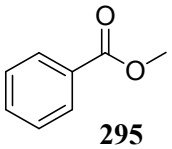
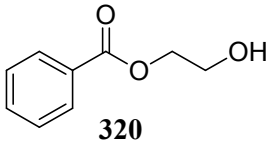
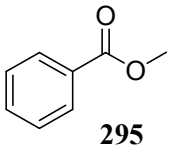
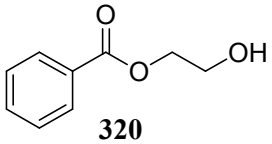
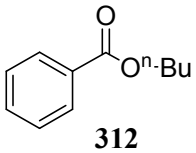
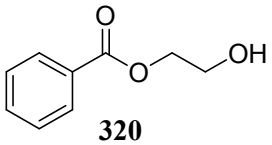
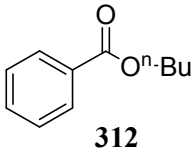
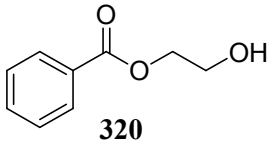
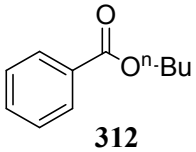
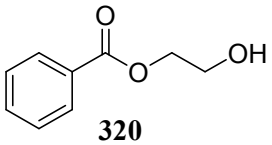


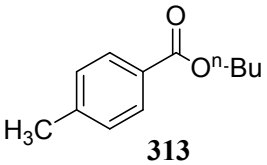
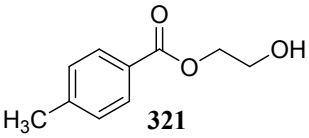
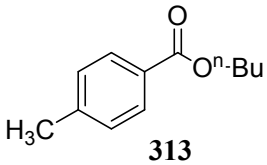
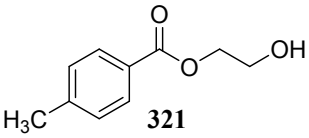
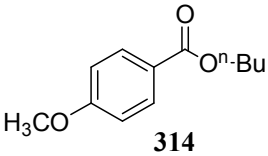
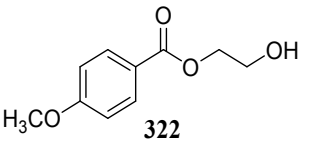
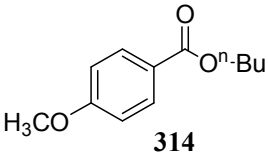
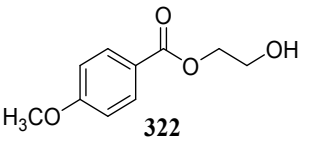
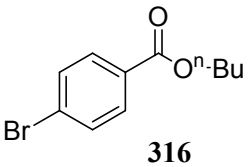
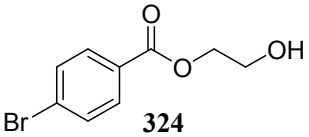
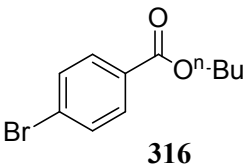
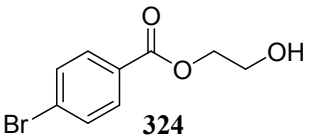
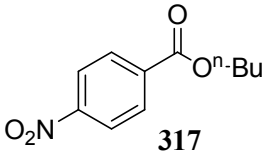
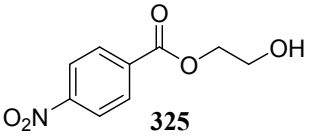
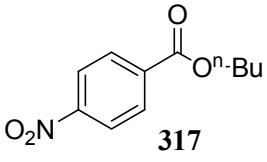
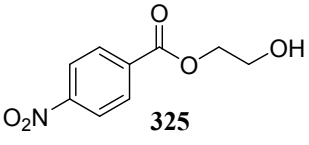
Scheme 2.16 CAL-B catalysed synthesis of glycol esters.

For this, a range of reactions of esters (**312-317, 319**) were performed with ethylene glycol (**296**) in presence of CAL-B at 60°C using MTBE as a solvent and the results

are tabulated in Table 2.2. In all but one of the cases (Scheme 2.15) no dimer was formed, except in the case of ester **317** which gave dimer (**326**), albeit in small amount, along with the glycol monomer product (**325**). Similarly in Scheme 2.16, no dimer (**329**) was formed in the CAL-B catalysed transesterification of **319** to get the glycol monomer product (**328**).

The results obtained (Table 2.2) from scheme 2.15 and 2.16 showed in all cases except the nitro-ester (entry 12), there was no yield of the dimer making the route useful for the synthesis of glycol esters without the use of zeolites. This is an advantage over other reported methods for the synthesis of glycol esters. Also, the enzyme catalysed reaction is green and inherently safe. In case of with methyl benzoate (**295**, entry 1), the amount of CAL-B used affected the product yield but the selectivity for the monomer remained unaffected.

Entry	Ester	CAL-B (g)	Product	Product conversion (%) yield[a]	Selectivity (%) (Monomer: Dimer)[b]
1[c]	 **295**	1.5	 **320**	81	100
2[c]	 **295**	0.5	 **320**	33	100
3[d]	 **312**	0.75	 **320**	44	100
4[d]	 **312**	0.5	 **320**	38	100
5[c]	 **312**	0.5	 **320**	34	100

6[d]	 **313**	0.5	 **321**	39	100
7[c]	 **313**	0.5	 **321**	33	100
8[d]	 **314**	0.5	 **322**	21	100
9[c]	 **314**	0.5	 **322**	29	100
10[d]	 **316**	0.5	 **324**	21	100
11[c]	 **316**	0.5	 **324**	19	100
12[d]	 **317**	0.5	 **325**	34	98:2
13[c]	 **317**	0.5	 **325**	32	100

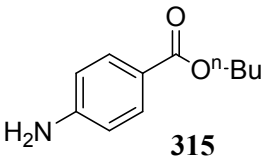
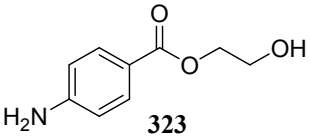
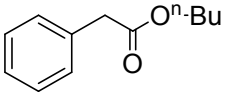
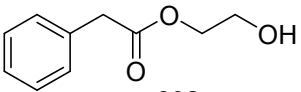
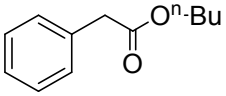
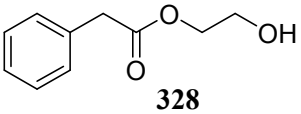
14[c]	 **315**	0.5	 **323**	nr	n.d.
15[d]	 **319**	0.5	 **328**	28	100
16[c]	 **319**	0.5	 **328**	14	100

Table 2.2 CAL-B catalysed synthesis of glycol activated esters.

a: isolated yields. **b**: confirmed from crude NMR of the reaction mixture and isolated yields of the products. **c**: Conditions (Entry 1, 2, 5, 7, 9, 11, 13, 14, 16): **ester** (1 equiv.), **ethylene glycol** (1 equiv.), CAL-B (varying amounts mentioned in the text, Table 2.2), molecular sieves (1g) in MTBE (10ml) 60°C, 8 h. **d**: Conditions (Entry 3, 4, 6, 8, 10, 12, 15): **ester** (1 equiv.), **ethylene glycol** (2 equiv.), CAL-B (varying amounts mentioned in the text, Table 2.2), molecular sieves (1g) in MTBE (10ml) 60°C, 8 h. nr: no reaction. n.d : not determined.

When the amount of CAL-B was 1.5g, the product (**320**) yield was 81%. Keeping all the reaction conditions same, except for the amount of enzyme which was reduced to 0.5g, the yield of **320** dropped to 33% (entry 2). Nevertheless the selectivity for the monomer remained 100% without any dimer formation. With butyl esters (entry 3, 4 ad 5), the yield of the desired glycol ester product (**320**) was not affected as much with 0.75g or 0.5g of CAL-B. At 0.75g of CAL-B with butyl benzoate and ethylene glycol (1:2 equivalents) the product yield was 44% (entry 3) which further reduced to 38% (**320**) when CAL-B was reduced to 0.5g, keeping butyl benzoate and ethylene glycol were in a ratio of 1:2 equivalents. Furthermore, keeping the amount of CAL-B at 0.5g but reducing the amount of ethylene glycol to 1 equivalent (entry 5) did not affect the product yield much (**320**, 34% yield) or the selectivity percentage of the monomer over dimer. This indicated that the amount of CAL-B remains the decisive factor in the formation of the product. Also, compared to methyl benzoate ester (**295**), when the chain length of the ester was increased to the butyl ester (**312**), i.e., from one carbon to four carbons, the product yield did not increase significantly at 0.75g or 0.5g. This indicated that the butanol released from the butyl ester substrate in the reaction mixture is able to undergo nucleophilic attack on the glycol ester product

resulting in reaction reversibility similar to that observed in lipase catalysed transesterification reactions of methyl esters.[176]

Esters with electron donating groups methyl and methoxy (entry 6 to 9) at 4-position, gave varying yields of the desired glycol ester product. In case of entry 6 when the ester substrate (**313**) and ethylene glycol (**296**) were used in 1:2 equivalents, and the amount of CAL-B was 0.5g the yield of the desired glycol monomer product was 39% (**321**, entry 6) with 100% monomer selectivity. But when the substrates' ratio was reduced to 1:1 (entry 7) the product yield reduced to 33% (**321**, entry 7) but the selectivity for the monomer (**321**) remained at 100% with no dimer formation. The low yield, in addition to reaction reversibility, could be correlated to the electron donating inductive and resonance effects making the carbonyl carbon less electrophilic. But again, from the monomer selectivity point of view, the conversion to monomer was pleasingly 100%. In case of a methoxy group at position 4 (entry 8 and 9) the yield of the desired glycol monoester product was 32% (**322**, entry 8) and 29% (**322**, entry 9) at 1:2 and 1:1 equivalents of ester substrate and ethylene glycol respectively. These low product yields indicate the dominance of electron donating effect of methoxy reducing the electrophilicity of the carbonyl carbon of the ester substrate (**314**). But again the selectivity for the monomer over dimer was 100%.

The presence of bromine at position 4 (entry 10 and 11) reduced the yield of the desired product indicating the dominance of electron donating resonance over electron withdrawing inductive effect as seen with methoxy group at position 4 in entries 8 and 9. With a 1:2 ratio of ester substrate and ethylene glycol, the product yield was 21% (**324**, entry 10) which further reduced to 19% with equimolar ratio of substrates (**324**, entry 11).

The presence of the electron withdrawing nitro group at position 4 (entry 12 and 13) did not contribute much in improving the yield of the desired product (34%, entry 12 and 32%, entry 13) indicating the dominance of reaction reversibility due to the released butanol over electron withdrawing resonance effect of the nitro group. Interestingly, the latter reaction also gave a dimer **326** (2% isolated yield) when the ratio of ester substrate and ethylene glycol was 1:2 (entry 12) indicating the high reactivity of the ester due to the electron withdrawing nitro group (Figure 2.4).

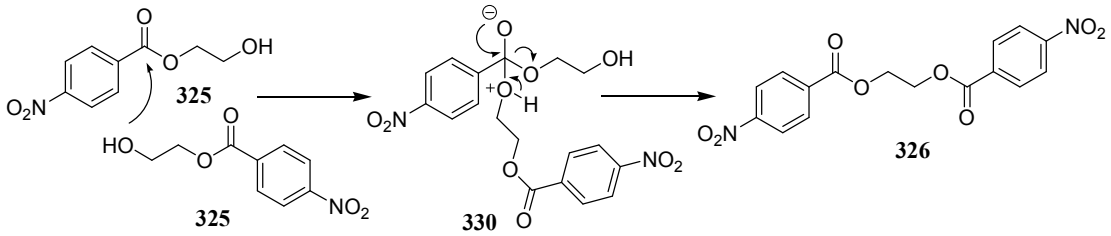


Figure 2.4 Mechanism of formation of dimer.

But when the concentration of ethylene glycol was reduced, and an equimolar ratio (entry 13) of ester substrate (**317**) and ethylene glycol (**296**) was used, no dimer product was formed in the reaction.

In the case of a 4-amino group at 4-position (entry 14), no desired product (**323**) was obtained indicating the very low reactivity of the ester towards nucleophilic attack.

In the case of butyl-2-phenylacetate (entry 15 and 16), the product yield (**328**) was 28% with 0.5g of CAL-B and 1:2 ratio of ester and ethylene glycol (entry 15). The product further reduced to 14% when equimolar ratio of substrates was used in the reaction (entry 16). It was expected that the presence of a methylene unit between the ring and the carbonyl carbon would inhibit the electron delocalization from the ring and increase the possibility of the nucleophilic attack on the carbonyl carbon. Additionally, the butanol released being much bigger in size compared to methanol released (entry 1 and 2) in the reaction mixture, will be less able to undergo nucleophilic attack on the ester product and thus the reaction reversibility may be reduced. Unfortunately, these changes did not help in increasing the product's yield. But more importantly there was also no dimer formation in the reaction which is an advantage of the proposed route using CAL-B to synthesise glycol esters without formation of dimer.

Overall, in all cases the selectivity of monomer was ~100% which could potentially be utilized in making the glycol monomers without any generation of dimer product in the reaction mixtures. Additionally, the methodology is safe and can be applied to the synthesis of glycol esters used in the synthesis of polymers employed in the synthesis of drug-polymer conjugates or other biomedical applications.[92-94]

2.3.1 Reaction of glycol esters with amines and alcohol

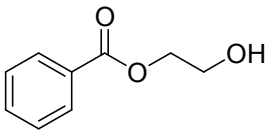
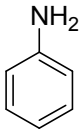
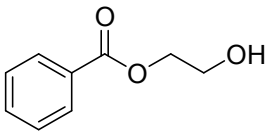
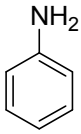
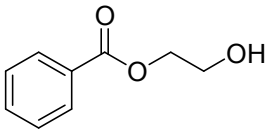
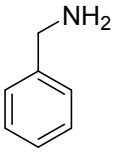
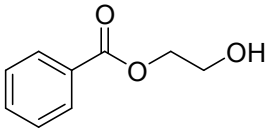
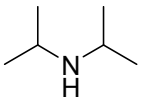
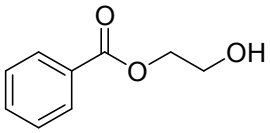
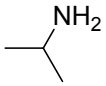
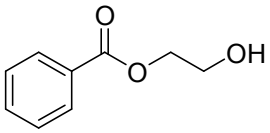
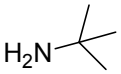
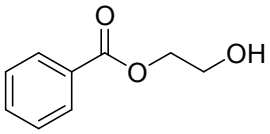
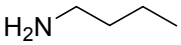
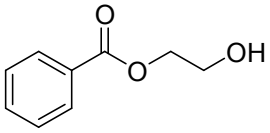
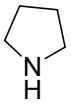
After synthesizing the ethylene glycol activated esters by a CAL-B catalyzed transesterification reaction, it was decided to perform the reaction of these esters with anilines in an attempt to get the corresponding amide products (see Figure 2.1) as discussed in Section 2.1.

Despite the low yields of the glycol activated esters (see Table 2.2) it was believed that in the one-pot synthesis of amides the activated ester is formed as part of the catalytic cycle (see Figure 2.1, Section 2.1) and so the formation of the amide product would still occur as long as the reaction cycle continues. Investigating this aspect of the catalytic cycle, along with hydroxyl group assistance in aminolysis reactions,[54-56] a reaction (Scheme 2.17) of glycol ester (**320**) was carried out with aniline (**235**) at 60°C for 8 hours to check the reactivity of the ester with aniline to hopefully obtain the amide product.

320 **235** **331** **296**

Scheme 2.17 Reaction of 2-hydroxyethyl benzoate with aniline.

Unfortunately, the reaction did not give the desired benzanilide product (**331**). This failure could be due to the low reactivity of the ester (**320**) towards nucleophiles. Also, aniline itself is not highly nucleophilic due to delocalization of the lone pair on nitrogen onto the ring. Additionally, the assistance of the hydroxy group of the ester in the amide bond formation had not appeared to have helped due to the bulky size of both the ester and amine. This statement could be supported by the reports of hydroxyl group assistance in amide formation which included very small and more nucleophilic ammonia compared to other amines such as aniline used in this study (Scheme 2.17).[54-56] After this, a further set of reactions was carried out to try and obtain the feasibility of the route (Table 2.3).

Entry	Ester	Nucleophile	Solvent/Additive at 60°C	Product
1	 **320**	 **235**	MTBE (with or without Zn-triflate)/ DCM/ toluene	nr
2	 **320**	 **235**	MTBE and $CaCl_2$	nr
3	 **320**	 **245**	MTBE and $CaCl_2$	Traces
4	 **320**	 **279**	MTBE	nr
5	 **320**	 **332**	MTBE	nr
6	 **320**	 **275**	MTBE	nr
7	 **320**	 **333**	MTBE, $CaCl_2$	nr
8	 **320**	 **334**	MTBE	nr

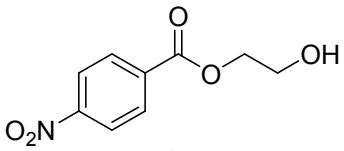
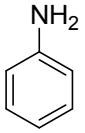
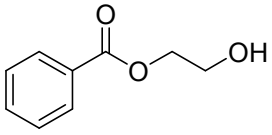
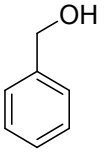
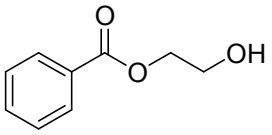
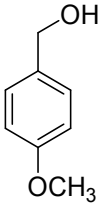
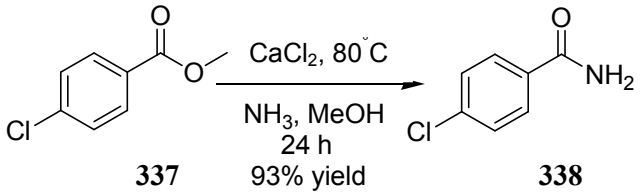
9	 **325**	 **235**	MTBE	nr
10	 **320**	 **335**	MTBE, DCM	nr
11	 **320**	 **336**	MTBE	nr

Table 2.3 Reactions of glycol esters with different nucleophiles in varying conditions. nr: no reaction.

In entry 1, the reaction of glycol activated ester (**320**) and aniline (**235**) was carried out in different solvents including dichloromethane (DCM) and toluene. It was supposed that MTBE might be hindering the reaction by forming hydrogen bonds with the hydroxy of the glycol ester substrate. Unfortunately, none of the reactions gave the desired amide product. In a repeated attempt, a Lewis-acid catalyst was included in the reaction to increase the elcetrophilicty of the carbonyl carbon of the ester substrate (**320**). For this, zinc-triflate was added in the reaction mixture, but unfortunately it also did not yield the desired benzanilide product, despite being a reported catalyst for amidation and esterification reactions.[177]

In a repeated attempt, an alternative additive was added to the reaction mixture (entry 2). For this, a reaction of the glycol ester (**320**) and aniline (**235**) was carried out in presence of calcium chloride (entry 2), since calcium and magnesium chloride are reported to facilitate amide bond formation (Scheme 2.18).[178] In one of the experiments, when the reaction of methyl 4-chlorobenzoate (**337**) was performed with ammonia in presence of calcium chloride at 80°C for 24 hours it gave the desired amide product (**338**) in 93% yield.



Scheme 2.18 Amidation of ester in presence of calcium chloride.[178]

It was presumed that the calcium ion coordinates with the carbonyl group of the ester, increases its electrophilicity, and predisposes it to the nucleophilic attack. But when calcium chloride was added to the reaction mixture of ester and aniline here, no desired amide product was obtained (entry 2, Table 2.3).

After such lack of desired results in the screen it was decided to use a more basic nucleophile than aniline (for example, benzylamine, pKa 9.3) since it was thought that the reaction might not be working because of the low nucleophilicty of aniline due to the delocalization of the nitrogen's lone pair (Figure 2.5).[172]

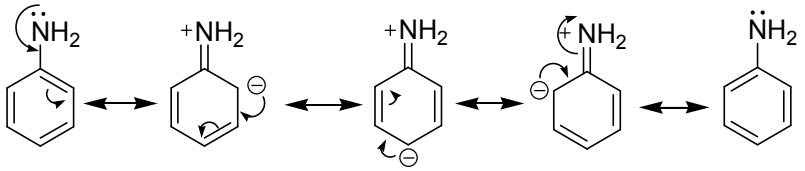


Figure 2.5 Electrons delocalisation in aniline.

As before, it was envisaged that with benzylamine the methylene unit would increase reactivity by inhibiting the delocalization of electrons between the benzene ring and the nitrogen. Therefore, in order to test this, a reaction of glycol ester (**320**) was carried out with benzylamine (**245**), which gave only traces of the desired amide product (entry 3). Analyzing these results it was decided to carry out the reactions of aliphatic amines with the ester substrate (**320**). It was believed that aliphatic amines being more nucleophilic than aniline and benzylamine would react with the ester. Therefore, a reaction of diisopropylamine (pKa 11.05) was carried out with the ester (**320**) (entry 4).

Similarly, reactions of **320** were also performed with isopropylamine (pKa 10.63) and *tert*-butylamine (pKa 10.45) (**275**) (entry 5 and 6 respectively). Unfortunately, none of the reactions gave the desired amide products. The reason for this might be due to the bulk of the alkyl groups around the nucleophilic nitrogen hindering the

nucleophilic attack on the carbonyl carbon of the substrate. Therefore, a straight chain amine n-butylamine (**333**) was reacted with the glycol activated ester (**320**) in presence of calcium chloride as an additive (entry 7). Unfortunately, this reaction also did not yield the desired amide product. After which, it was decided to use pyrrolidine (pKa 11.27) in which, due to its cyclic structure, the carbon atoms are tightly held back leaving the amine in the front and making it more nucleophilic over acyclic amines such as *tert*-butylamine, where the alkyl groups create hindrance around the nitrogen. Unfortunately, as previously, no desired product was obtained between the reaction of pyrrolidine (**334**) and the ester (**320**) (entry 8).

Evaluating the above discussed results, it was decided to replace the glycol ester substrate with a more reactive ester as it was presumed that the nitro group, by withdrawing electrons from the ring, will increase the electrophilicity of the carbonyl group (Figure 2.6).

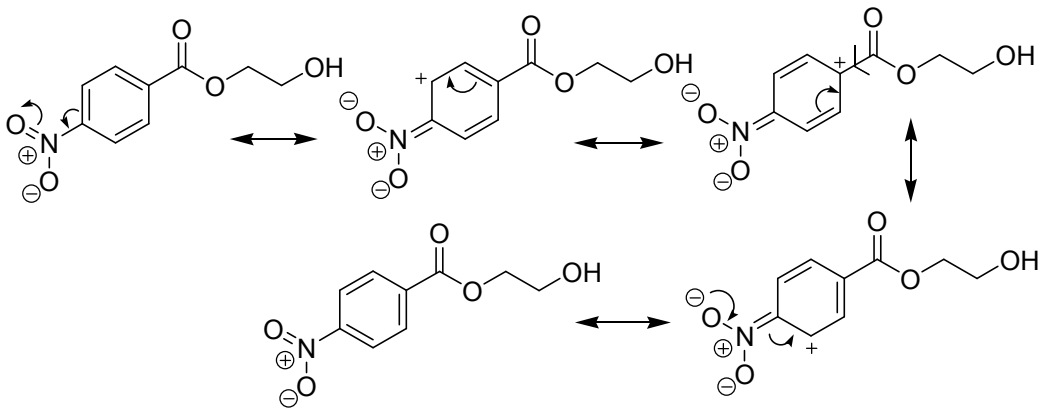


Figure 2.6 Electron delocalisation in 2-hydroxyethyl 4-nitrobenzoate.

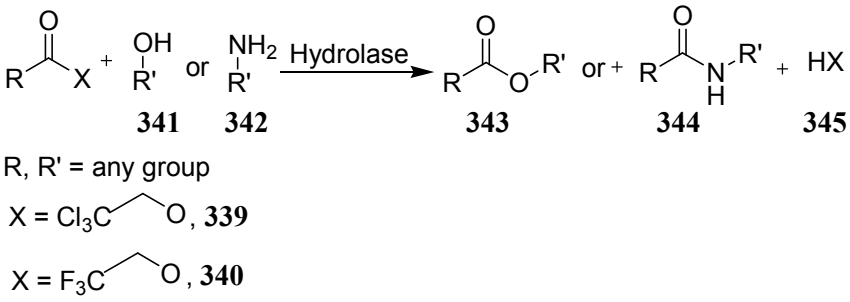
For this, a reaction of 2-hydroxyethyl-4-nitrobenzoate (**325**, entry 9) was carried out with aniline (**235**), but unfortunately, again it also did not yield the desired amide product.

Due to the low reactivity of the amines with these esters it was decided to replace amines with nucleophilic alcohols such as benzyl alcohol. It was presumed that the methylene unit between the ring and the hydroxy group will prevent the delocalization of electrons on the ring and thus will increase the nucleophilicty of the hydroxy group. To test this, a reaction of benzyl alcohol (**335**) was performed with the glycol ester (**320**) in MTBE (entry 10). But this did not yield the desired ester

product. The reason for this was believed to be that the MTBE might be forming hydrogen bonds with the alcohol and thus preventing nucleophilic attack. Therefore, in order to confirm this, a reaction of benzyl alcohol (**335**) was performed with the glycol ester (**320**) in DCM as solvent (entry 10). But again, this reaction did not yield the desired ester product, which indicated that the ester substrate (**320**) itself is not activated enough to undergo nucleophilic attack. In continuation, a reaction of 4-methoxy benzyl alcohol (**336**, entry 11, Table 2.5) was carried out with the glycol ester (**320**) but unfortunately, it also did not yield the desired product and therefore the use of glycol activated ester in the one-pot synthesis of amides was abandoned. The reason for the absence of any product formation might be due to the strongly electronegative oxygen preventing nucleophilic attack on the carbonyl carbon of the ester.

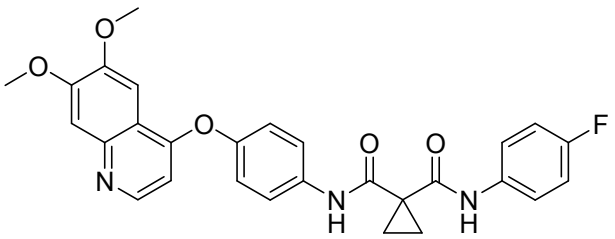
2.4 Synthesis of halogenated activated esters

After analysing the low reactivity of ethylene glycol activated esters towards amidation reactions, it was decided to study the reactions of some other activating agents known to have been used in enzyme catalysed reactions (Section 1.3). For this, it was decided to work on the reactions of trifluoroethanol, trichloroethanol and tribromoethanol. These halogen based activating agents are highly reactive due to presence of electron withdrawing fluorine, chlorine and bromine atoms.[165] Moreover, in enzyme catalyzed reactions these halogen based activating agents are known as quasi-irreversible acylating agents (Scheme 2.19) and thus make the reactions irreversible. Here the released nucleophile is not reactive enough to undergo nucleophilic attack again on the ester product thus shifting the reaction equilibrium forward.[165]



Scheme 2.19 Quasi-irreversible acylating agents.[165]

As mentioned in Section 1.3 it is useful for the activated ester (i.e., halogenated esters) of the enzyme substrates to be prepared chemically beforehand. Therefore, in order to make the synthesis more convenient, it was decided to make the activated esters using haloalcohols using CAL-B. Additionally, after using vinyl acetate esters[5,6] in the one-pot synthesis of amides[167] it was also decided to attempt the one-pot synthesis of amides using alkyl esters by their activation using CAL-B. It was supposed that the introduction of a trifluoro group in the ester will increase the electrophilicity of the carbonyl carbon making it more susceptible to nucleophilic attack. Additionally, it was also planned to apply the one-pot method and perform a synthesis of a tyrosine kinase inhibitor drug cabozantinib prescribed in medullary thyroid cancer.



Cabozantinib

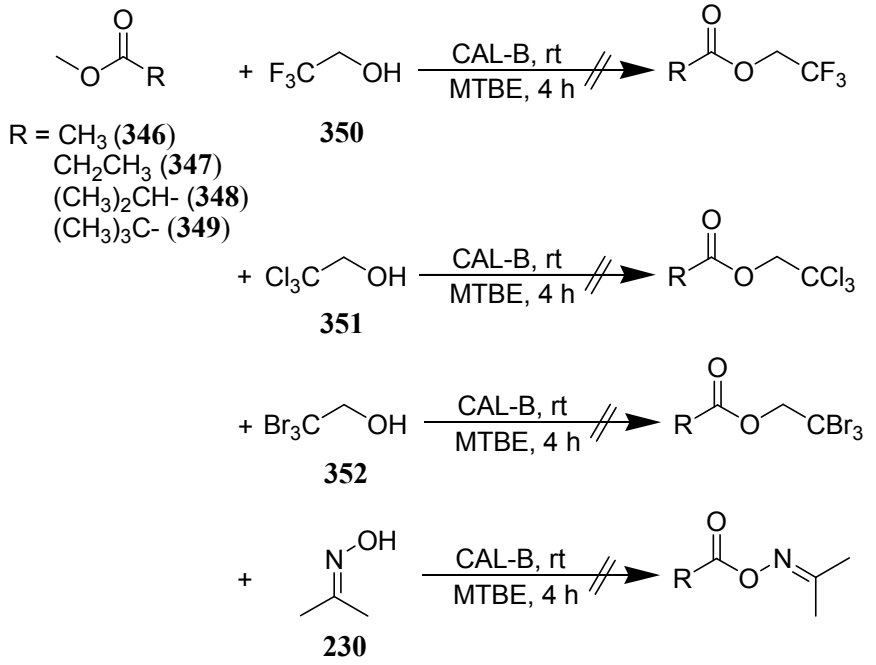
To start with, a range of alkyl esters were screened and their activation reaction was performed using trifluoroethanol, trichloroethanol and tribromoethanol. Additionally, acetone oxime was also included in the study to determine its reactivity with alkyl esters (Scheme 2.21). So far acetone oxime had only been used for the activation of vinyl acetate, ester giving good yields of amide products in the one-pot synthesis (Figure 2.2, Section 2.2). Therefore, in light of this it was envisaged to carry out the activation of alkyl esters also using acetone oxime as an activating agent. Also, to compare the reactivity of these oxime activated alkyl esters with that of halogenated activated alkyl esters.

2.4.1 Activation reactions of alkyl esters

On the basis of the above discussion, a set of alkyl esters were chosen to perform the reaction with various activating agents. Initially, the reactions were carried out with methyl acetate (**346**) an ester, with minimal hindrance around the carbonyl carbon. Keeping the reaction conditions the same for all the reactions, the reactions of methyl acetate (**346**) were performed with trifluoroethanol (**350**), trichloroethanol (**351**), tribromoethanol (**352**) and acetone oxime (**230**) in presence of 1.5g of CAL-B at room temperature for 4 hours using MTBE as a solvent (Scheme 2.20). Unfortunately, no product was formed in any of the reactions, which could be correlated to the reaction reversibility due to release of methanol from the ester, which is further supported by the reported reversibility in the enzyme catalysed reactions due to released nucleophiles such as methanol.[165, 176]

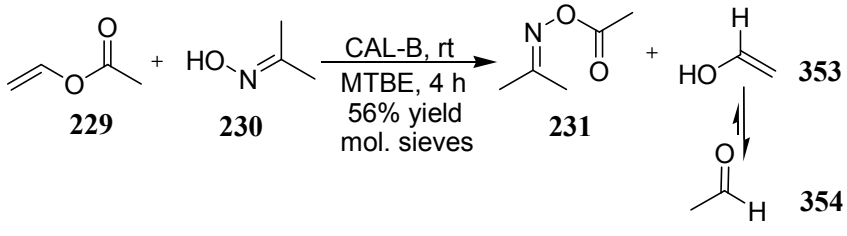
Moving on to study other esters, it was supposed that the nucleophilic attack by the released nucleophile on the activated ester product will be low if it is hindered. Therefore to test this hypothesis, a range of reactions of other esters including methyl propionate (**347**), methyl isobutyrate (**348**) and methyl trimethyl acetate (**349**) were carried out with trifluoroethanol (**350**), trichloroethanol (**351**) and tribromoethanol

(**352**) and acetone oxime (**230**). Unfortunately, as before, none of the reactions gave the desired product, indicating the low reactivity of the starting esters. Also, in the reported literature, lipase catalysed reactions for the introduction of a trifluro group in aromatic esters or acids are typically carried out for long reaction times ranging from minimum 15 hours upto 15 days at temperatures greater than 50°C (see Scheme 1.32).[113]



Scheme 2.20 Attempted CAL-B catalysed synthesis of activated esters from alkyl esters.

The reaction of alkyl esters with acetone oxime (**230**) also did not yield the desired product (Scheme 2.20) presumably due to low reactivity of the starting esters. The low reactivity of these alkyl esters with acetone oxime activating agent could be compared with the reaction of vinyl acetate and acetone oxime studied earlier (Scheme 2.21) in which the yield of the oxime activated ester product was 56%.



Scheme 2.21 CAL-B catalysed synthesis of an oxime ester from vinyl acetate.[165, 167]

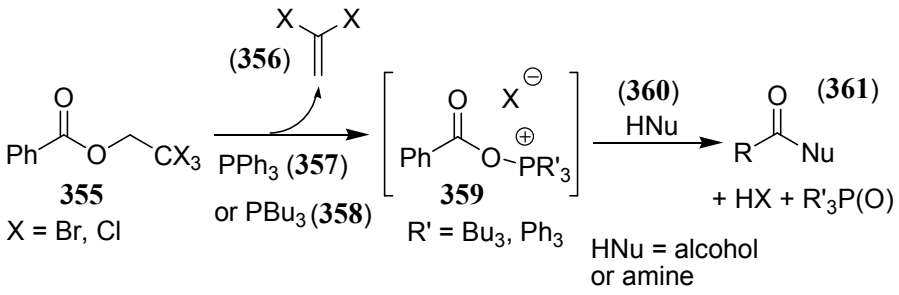
Vinyl acetate, which is an irreversible acyl donor, acylates the oxime and releases the acetaldehyde (**354**) and thus the reaction becomes free from nucleophile and becomes irreversible (Scheme 2.21).

Analysing the results of alkyl esters with haloalcohols (Scheme 2.20) it was decided to move back on the CAL-B catalysed reactions of benzoate esters with activating agents. Benzoate esters worked well in the CAL-B catalysed transesterification reactions when ethylene glycol was taken for ester activation (see Scheme 2.15 and 2.16, Section 2.3). Therefore it was decided to perform the CAL-B catalysed reactions of benzoate esters with other activating agents in the hope of extending the catalytic method developed so far.

2.5 Synthesis of aromatic activated esters and their applications in chemoselective reactions

After performing a series of reactions of the one-pot synthesis of acetanilides in the presence of CAL-B, via generation of acetone oxime activated ester from vinyl acetate (Section 2.2), it was decided to synthesize activated esters of aromatic esters using a different activating agent. After an initial investigation it was found that ethylene glycol could be used for activation of esters. Therefore, it was decided to synthesize a glycol activated ester and subsequently react it with a list of anilines to get the corresponding amides (Section 1.2 and 2.3). To carry out the synthesis of the glycol activated ester, the scheme was initiated with a simple benzoate ester, methyl benzoate. Its CAL-B catalysed reaction with ethylene glycol gave good yield of the activated ester but unfortunately the ester did not react with aniline under different conditions (Section 2.1). Nonetheless, a novel route to the synthesis of glycol esters was found, which could show utility in the synthesis of glycol monoesters for various applications. In light of this, various glycol activated esters were synthesized from substituted benzoate esters and a comparative study was performed on the formation of glycol monoester and dimer reaction products (Table 2.2). After this scheme, it was decided to move on some other activating agents like trifluoroethanol, trichloroethanol and tribromoethanol and prepare their activated ester using CAL-B. But unfortunately none of them worked.

In an effort to fully explore all possible options it was decided to prepare alternative aromatic activated esters utilising CAL-B and react them with anilines to get their corresponding amides, using the one-pot methodology applied in the synthesis of acetanilides (Section 2.2).[167]

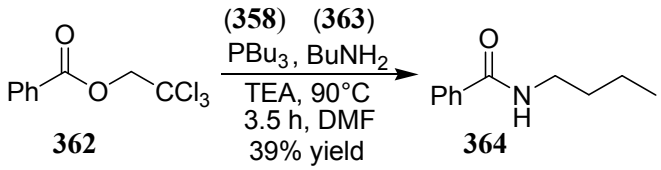


Scheme 2.22 Amidation or transesterification of trihaloethyl esters using phosphorus compounds.[179, 180]

As with the alkyl esters (Section 2.4.1), initial screening it was decided to test trifluoroethanol, trichloroethanol and tribromoethanol again for ester activation. Here, methyl benzoate was used because it is commonly used in industries and labs for the synthesis of various pharmacophores and chemicals.[31, 179-183] Apart from this, the 2,2,2-tribromoethyl and 2,2,2-trichloroethyl benzoate esters (**355**) are also reported in the synthesis of amides in presence of phosphorous reagents (Scheme 2.22).[184, 185]

These trihaloesters (**355**) undergo nucleophilic attack by phosphorous reagents (such as triphenyl phosphine (**357**) and tributyl phosphine (**358**)) and form acyloxyphosphonium intermediate (**359**) *in situ* along with the release of 1,1-dihaloethylene gas (**356**). These electrophilic salts in turn activate the nucleophile (**360**), such as an amine or alcohol, and release the amide or ester product (**361**) along with the triphenyl or tributyl phosphine oxide.

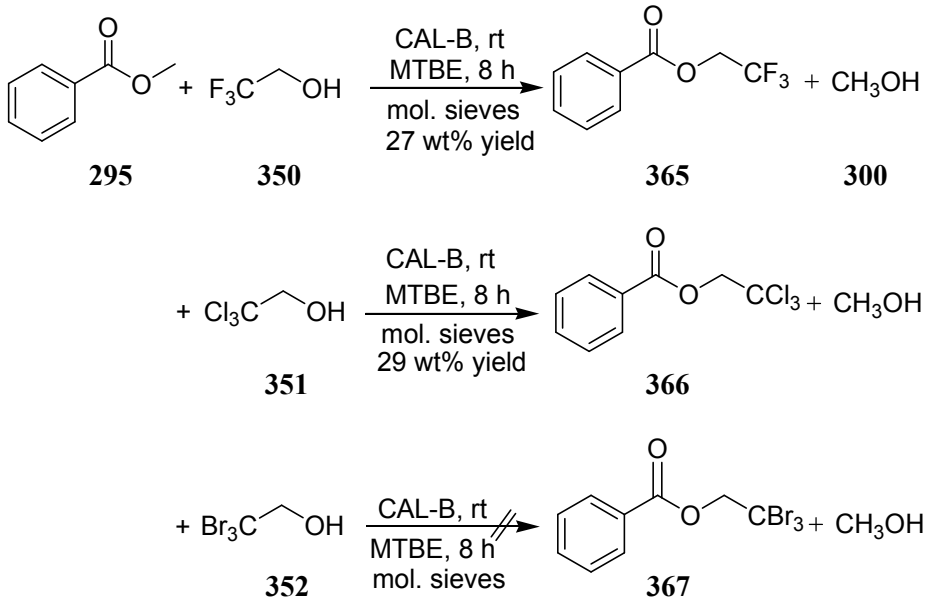
In one representative reaction, when the reaction of 2,2,2-trichloroethyl benzoate (**362**) was performed with tributyl phosphine (**358**) in the presence of butylamine (**363**) and triethylamine in dimethyl formamide for 3.5 hours at 90°C gave the amide product (**364**) in 39% yield. This is a direct single step method for the conversion of trihaloesters to their corresponding amides and esters. [179-181]



Scheme 2.23 Amidation of trichloroester (**362**) in the presence of tributyl phosphine (**358**).[181]

In light of this a set of reactions of methyl benzoate with trifluoroethanol, trichloroethanol and tribromoethanol were carried out in presence of 1.5g of CAL-B and 4 Å molecular sieves (Scheme 2.24) in the hope of being able to form amides from the products in a manner similar to that in Scheme 2.23. In the results obtained (Scheme 2.24), the yield of the activated esters (**365** and **366**) obtained from trifluoroethanol (**350**) and trichloroethanol (**351**) was 27 wt. % and 29 wt. % (calculated from crude ^{1}H NMR) respectively. However, the products were not separable from their corresponding ester starting materials using column

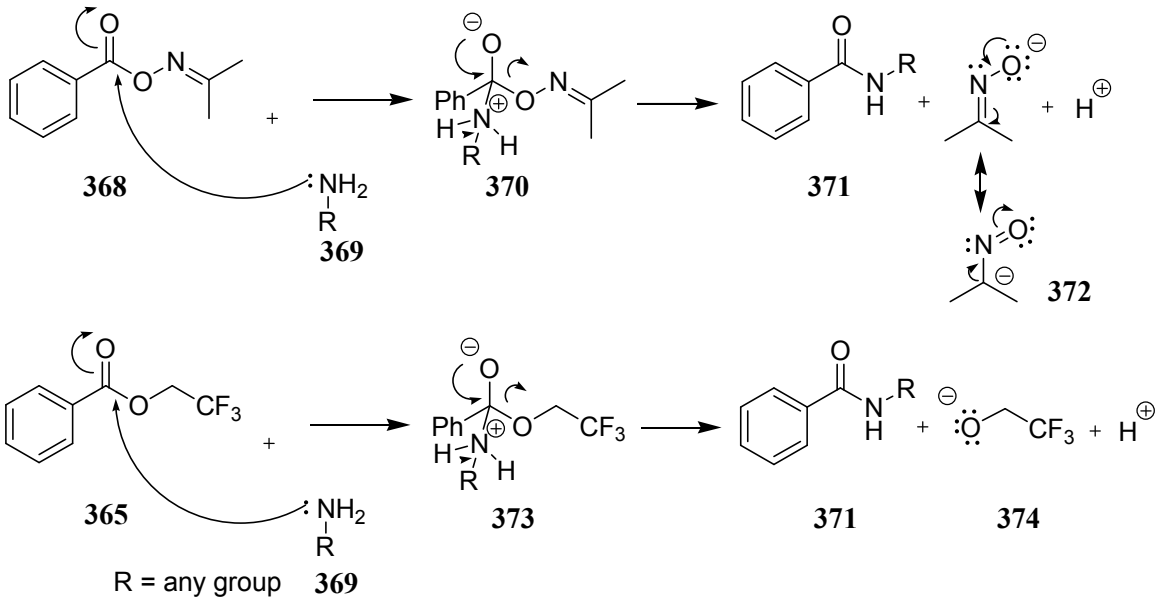
chromatography. Additionally, removal of trichloroethanol was difficult because of its high boiling point (151°C). In the reaction with tribromoethanol (**352**), no yield of the desired product (**367**) was obtained which could possibly be correlated to the bulky size of the bromine atoms which might be hindering accommodation of the ester product (**367**) inside the active site of CAL-B.



Scheme 2.24 CAL-B catalysed synthesis of activated esters from methyl benzoate.

The results suggested that haloalcohols are not very effective in CAL-B catalysed transesterification reactions and release methanol which is, reported of undergoing nucleophilic attack on the newly formed ester product resulting in lowering the product yield.[176] Additionally, in the reported[179, 180] study on the use of of 2,2,2-tribromoethyl and 2,2,2-trichloroethyl benzoate esters in the synthesis of amides, anilines were not included in the study which are also known to have low reactivity in CAL-B catalysed amidation reactions. The amines studied were more nucleophilic than aniline for example, butylamine and diethylamine (with pKa 10.59 and 10.98 respectively compared with aniline of 4.87).[172] Also, the study, itself used phosphine additives to generate the highly electrophilic phosphonium intermediate to react it with the amine and did not directly convert the trihalogenated ester into the amide product.[179, 180] Furthermore, the esters used in the study were 2,2,2-tribromoethyl and 2,2,2-trichloroethyl benzoate, which in our case were not feasible because, firstly, there was no product (**367**) formed in the reaction of methyl benzoate and 2,2,2-tribromorethanol. Secondly, in case of 2,2,2-trichloroethyl benzoate (**366**) the yield

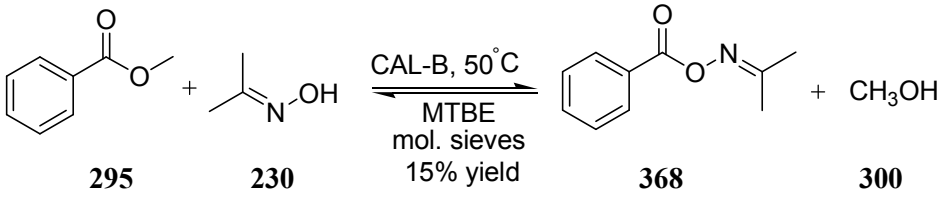
was only 29 wt % and the ester product was inseparable from the starting ester. Also, removal of 2,2,2-trichloroethanol is also difficult making the process poor. As a result it was decided to stick with the synthesis of acetone oxime esters as they have demonstrated their good reactivity in the synthesis of acetanilides (Section 2.2).[167] Also, from a theoretical point of view, it could be argued that if a reaction of trifluoro-activated ester and acetone oxime activated ester is performed with an amine (**369**) to get the corresponding amide (**371**) (Scheme 2.25), the reaction of oxime ester (**368**) should be faster than that of the halogenated activated ester (**365**) because, in case of oxime ester **368**, the released oxime anion is resonance stabilized (**372**) and therefore the reaction will be faster towards completion.



Scheme 2.25 Mechanism of amide formation from activated esters.

In case of trifluoroester **365**, there is no resonance stabilization in the released anion (**374**). Although there is stability via the inductive effect there is no electron delocalization like in the oxime anion (**372**), and therefore **374** should be less stable in comparison to the oxime anion **372** and will make the reaction slower. Therefore, it was decided to continue with acetone oxime for the synthesis of activated esters from methyl benzoate.

To this end, a reaction of methyl benzoate and acetone oxime was carried out in the presence of CAL-B (Scheme 2.26) to test the hypothesis.



295 **230** **368** **300**

Scheme 2.26 CAL-B catalysed synthesis of an oxime ester from methyl benzoate.

The reaction showed reversibility due to the released methanol which, as seen previously, undergoes nucleophilic attack on the activated ester (**368**) leading to the formation of methyl benzoate (**295**) again. The reaction's reversibility was confirmed by the change in intensity of the methyl benzoate peak at δ 3.87 in the crude ^{1}H NMR of the reaction mixture when the reaction was followed at 8, 12, 16, 24 and 32h. When the reaction was carried out at 50°C, the yield of the activated ester was around 10% after 8h which further increased to around 15% after 12h. However, after 16h and longer, no product was detected in the crude ^{1}H NMR. The reaction was carried out with various equivalents of the ester and oxime at 40, 50 and 60°C, but under all conditions, it showed reversibility. The low product yield could also be related to the low reactivity of the carbonyl carbon due to electron delocalization from the ring (Figure 2.7).

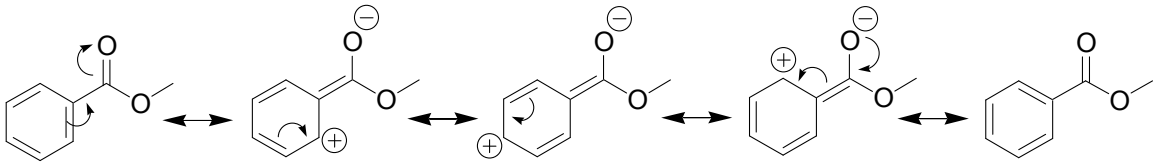
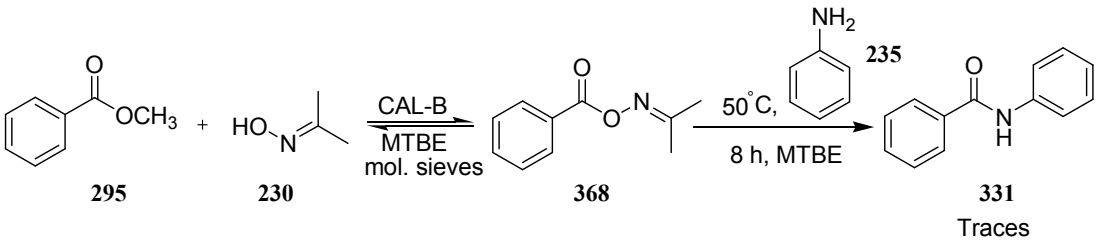


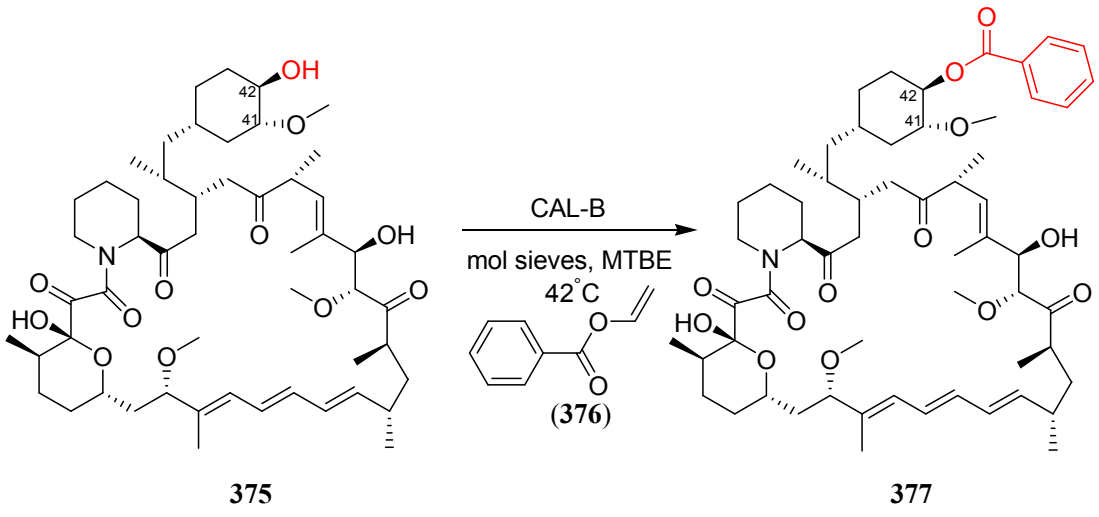
Figure 2.7 Electron delocalization in methyl benzoate.

In the resonance structures above, the carbonyl carbon is less electrophilic due to high electron density generated by the delocalization of electrons from the ring. To further confirm the reaction reversibility, a reaction of **368** was performed with methanol at 50°C which after 12 h gave methyl benzoate and acetone oxime (confirmed from crude ^{1}H NMR of the reaction mixture). Understanding this reversibility and to force the equilibrium forward towards product formation, a one-pot synthesis was carried out with aniline (**235**) in presence of 4Å molecular sieves, but again only traces of the desired amide product (**331**) was obtained in the crude ^{1}H NMR (Scheme 2.27).



Scheme 2.27 One-pot synthesis of benzanilide.

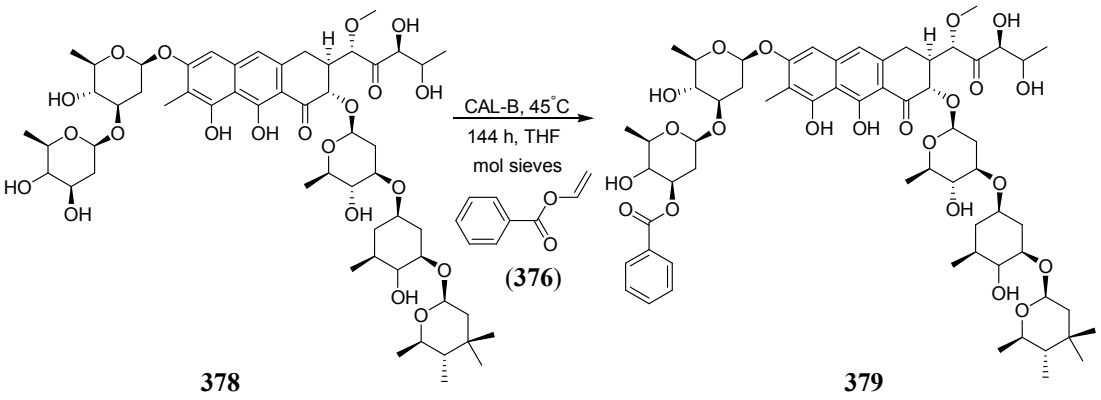
Due to the reversibility of the reaction, due to the released methanol, it was decided to carry out the reaction with vinyl benzoate (**376**) ester in place of methyl benzoate (**295**). In Section 2.2, when the reaction of vinyl acetate (**229**) and acetone oxime (**230**) was performed in the presence of CAL-B, it gave 56% yield of the oxime ester product (**231**). Here, vinyl acetate, being an irreversible acyl donor, acylated the oxime and released the acetaldehyde (**354**) and thus the reaction became free from nucleophile and become irreversible (Scheme 2.21). Therefore, it was presumed that the use of vinyl benzoate will also prevent the reaction reversibility occurring, as seen with methyl benzoate ester (Scheme 2.26). Vinyl benzoate has commonly been used in lipase catalysed acylation reactions.[182-186] For example, vinyl benzoate (**376**) has been used in the regioselective acylation of the antitumour drug rapamycin (**375**) to obtain **377** (Scheme 2.28).



Scheme 2.28 CAL-B catalysed regioselective acylation of rapamycin.[183]

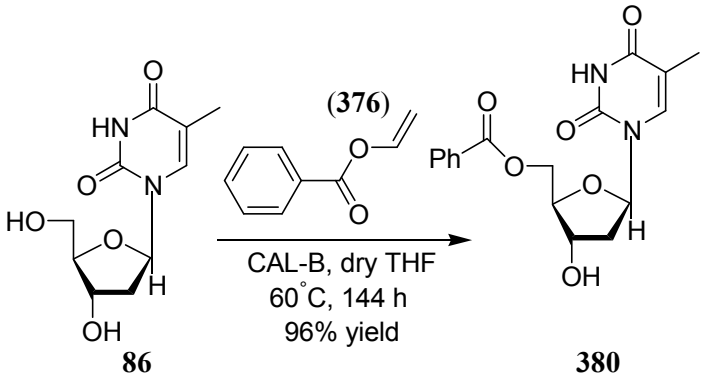
Similarly, it has also been used in the regioselective acylation of aurolic acid derivatives which possess antineoplastic activity.[187] For example, mithramycin A (an aurolic acid derivative) which blocks the expression of cell proliferation and

transforming growth factor β (TGF- β) in tumour cells.[188] The drug has also been used in novel antiangiogenic combination therapies, such as in combination with betulinic acid, which is used as an antiangiogenic therapy in pancreatic cancer.[189, 190] In one of the studies vinyl benzoate (**376**) was used for regioselective acylation of a novel anticancer aurolic acid derivative **378** to obtain acylated product **379** (Scheme 2.29).



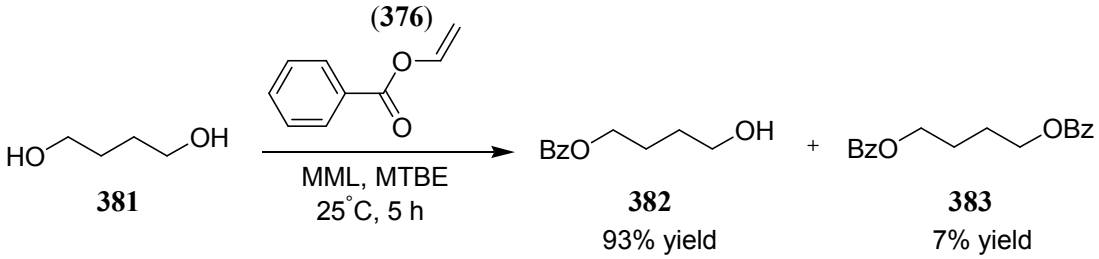
Scheme 2.28 CAL-B catalysed regioselective acylation of aurolic acid derivative.[184]

Vinyl benzoate has also been used in the acylation of nucleosides without using any harsh chemical reagents, such as acid chlorides. For example, in one particular study various parameters, including the amount of nucleoside substrate, CAL-B and vinyl benzoate, temperature and reaction time, were studied for the regioselective acylation of the substrate.[182] In one of the reactions the yield of the acylated product (**380**) was 96% when the ratio of substrate (**86**) and vinyl benzoate (**376**) was 1:5 equivalents and the reaction was performed in THF at 60°C for 144 hours (Scheme 2.29).[182]



Scheme 2.29 CAL-B catalysed regioselective acylation of nucleoside **86**.[182]

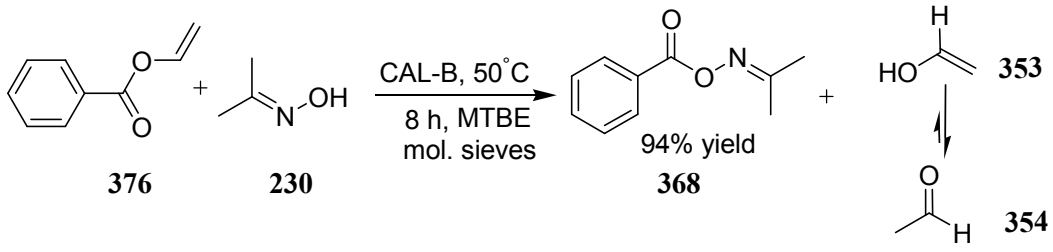
Apart from applications in the synthesis and modification of biological compounds, vinyl benzoate has also been reported in a lipase selective mono-benzoylation of hydroxy groups in 1,4-diols which is more suitable over chemical methods,[191, 192] which often give a mixture of acylated products and require use of catalysts such as, cerium trichloride.[197, 193] Due to the importance of selective acylation of hydroxy compounds with benzoic group, owing to its greater stability and ease of substitution on the ring, a suitable route is required in organic synthesis. In this field, Ciuffreda *et al*., first reported the use of vinyl benzoate for the selective benzoylation of butane-1,4-diol (**381**) (Scheme 2.30).[185]



Scheme 2.30 MML catalysed esterification of butane-1,4-diol **381**.[185]

Here, when the reaction of **381** was performed with vinyl benzoate (**376**) in MTBE at 25°C for 5 hours in the presence of the lipase obtained from *Mucor miehei* (MML), the monobenzoylated product **382** was obtained in 93% yield while the dibenzoylated product (**383**) was obtained in only 7%.[185]

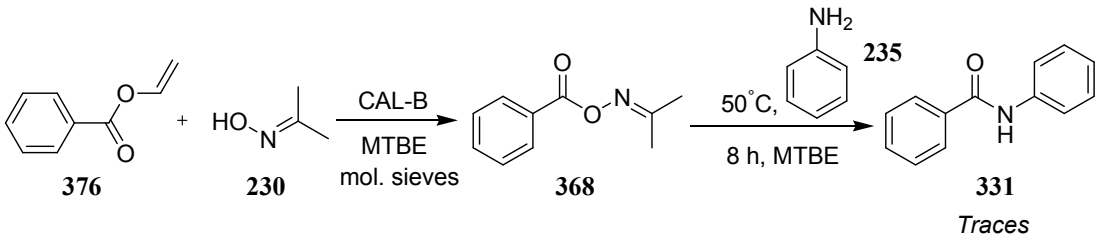
The ease of such lipase catalysed transesterification reactions with vinyl benzoate, led to a study between vinyl benzoate (**376**) and acetone oxime (**230**). The reaction was performed in the presence of CAL-B and 4Å molecular sieves which gave 94% yield of the desired activated ester (**368**) product (Scheme 2.31, Procedure 2.11).



Scheme 2.31 CAL-B catalysed synthesis of oxime ester from vinyl benzoate.

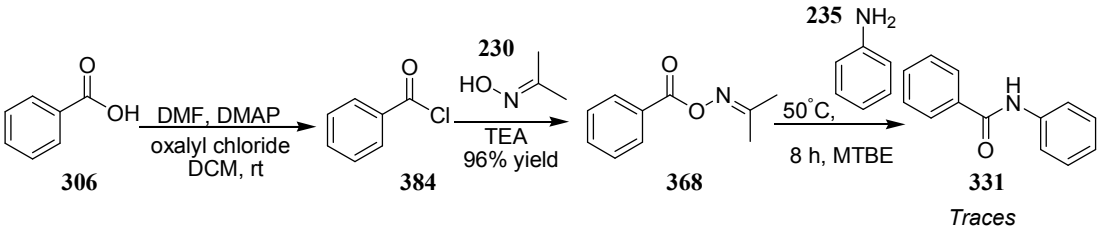
The 94% yield of the activated ester (**368**) highlighted the benefits of irreversible acylation by the vinyl ester **376** due to the released acetaldehyde (**354**). Additionally,

it also indicated that the lipase can catalyse the formation of activated ester. Therefore, in order to continue it was decided to replace the methyl benzoate ester (**295**) with vinyl benzoate and perform a one-pot synthesis of benzamide (**331**) using aniline in presence of CAL-B, a reaction which had been deemed not feasible in Scheme 2.27 when methyl benzoate was used as the starting ester.



Scheme 2.32 One-pot synthesis of benzanilide using vinyl benzoate.

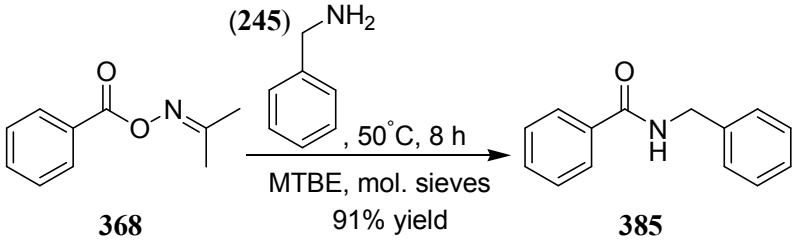
For this, a reaction of vinyl benzoate (**376**), acetone oxime (**230**) and aniline (**235**) was carried out for 8 hours in one-pot in the presence of CAL-B and 4Å molecular sieves in MTBE as solvent (Scheme 2.32). Unfortunately, the reaction only gave the desired amide (**331**) in trace amounts which indicates that either step 1, step 2 or both are not working efficiently. Therefore, in order to confirm the feasibility of the amide forming second step it was decided to synthesise the activated ester (**368**) chemically and react it chemically with aniline (**235**).



Scheme 2.33 Attempted reaction of oxime ester **368** with aniline.

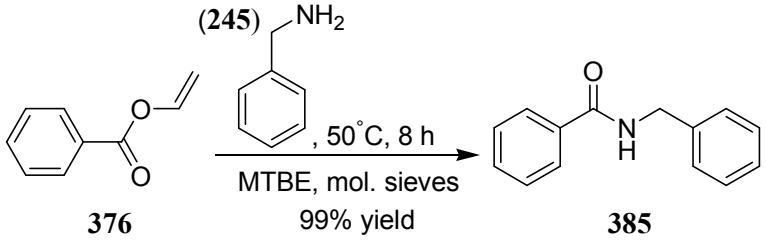
For this, a reaction of benzoic acid (**306**) was performed with dimethyl formamide (DMF), 4-dimethylaminopyridine (DMAP), oxalyl chloride and acetone oxime (**230**) in DCM (Scheme 2.33). The reaction gave 96% yield of the activated ester **368** (Procedure 2.12). Taking this activated ester (**368**) a reaction was subsequently performed with aniline (**235**) at 50°C for 8 hours in MTBE. Unfortunately, it gave only traces (confirmed from ^{1}H NMR of the reaction mixture) of the desired amide product (**331**), which could be correlated to the low reactivity of the activated ester due to electron pushing from the benzene ring making the carbonyl carbon less

electrophilic and reducing its reactivity towards nucleophiles. Thus, this chemical reaction confirmed that the activated ester (**368**) itself is not reactive enough to undergo reaction with aniline (Scheme 2.32, Step 2). Therefore, it was decided to carry out the one-pot synthesis of amides using vinyl benzoate ester (Scheme 2.31) and the more nucleophilic benzylamine (**245**). Before performing the one-pot synthesis of amides using vinyl benzoate, acetone oxime and benzylamine in presence of CAL-B, initially, a reaction of ester **368** was performed with benzylamine (**245**) to check their reactivity (Scheme 2.34, Procedure 2.13). Gratifyingly; the reaction gave 91% yield of the amide product (**385**).



Scheme 2.34 Reaction of oxime ester with benzylamine.

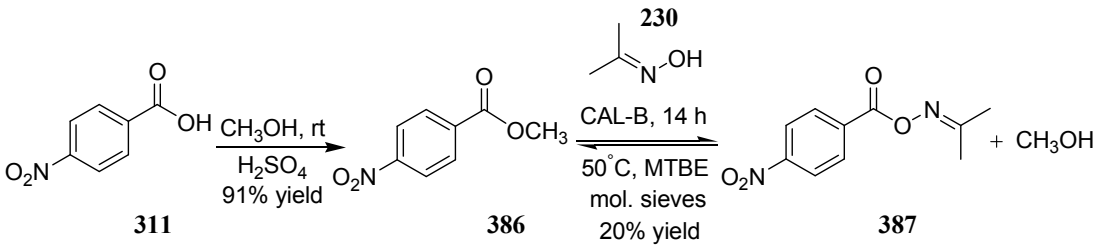
To further continue, a reaction of vinyl benzoate (**376**) was performed with benzylamine to check the background reaction. Unfortunately, the reaction gave around 99% product yield at both 50°C and room temperature (Scheme 2.35). Therefore, seeing the high background reaction due to high reactivity of vinyl benzoate, the one-pot synthesis of amides using vinyl benzoate was abandoned.



Scheme 2.35 Reaction of vinyl benzoate with benzylamine.

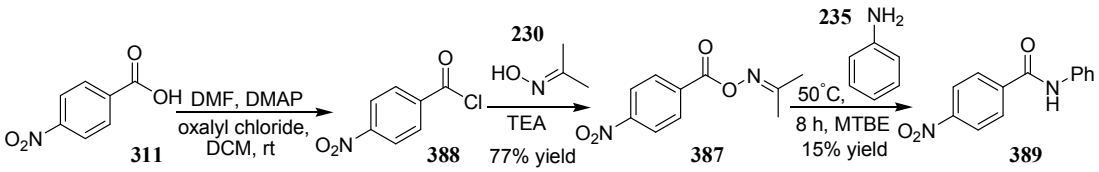
After these results it was decided to shift back to the methyl benzoate ester again and try to increase the reactivity of the carbonyl carbon. In a repeated effort to increase the electrophilicity of **368** and to force the equilibrium to product formation, it was decided to use the nitro-substituted ester. It was assumed that the nitro group will withdraw electrons from the ring and increase the electrophilicity of the carbonyl

carbon and thus may assist in increasing the yield of the amide. To test this hypothesis, a reaction of methyl 4-nitrobenzoate (**311**), prepared from 4-nitrobenzoic acid (**311**) (Procedure 2.14), was carried out with acetone oxime (**230**) in the presence of CAL-B and 4 Å molecular sieves in MTBE at 50°C for 8 hours (Scheme 2.36).



Scheme 2.36 Synthesis of oxime ester **387** from 4-nitrobenzoic acid.

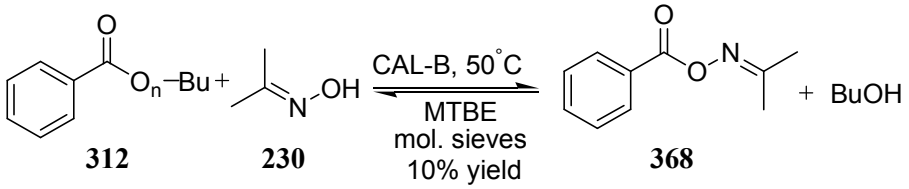
Unfortunately, this reaction also showed reversibility and gave only ~20% yield (calculated from crude ^{1}H NMR) of the activated ester (**387**) after 14h. As such, it was decided to do the reaction of the activated ester and aniline chemically to synthesize the amide product and check its reactivity with aniline. For this a reaction of 4-nitrobenzoic acid (**311**) was performed with DMF, DMAP, oxalyl chloride and acetone oxime (**230**) in DCM (Scheme 2.37). The reaction gave 77% yield of the activated ester **387** (Procedure 2.15). Taking this activated ester (**387**) a reaction was performed with aniline (**235**) at 50°C for 8 hours in MTBE but it only formed around 15% of amide **389** (calculated from crude ^{1}H NMR), and therefore, due to such poor reactivities in these compounds, the reaction series was abandoned (Scheme 2.37).



Scheme 2.37 Chemical synthesis of 4-nitro-*N*-phenylbenzamide.

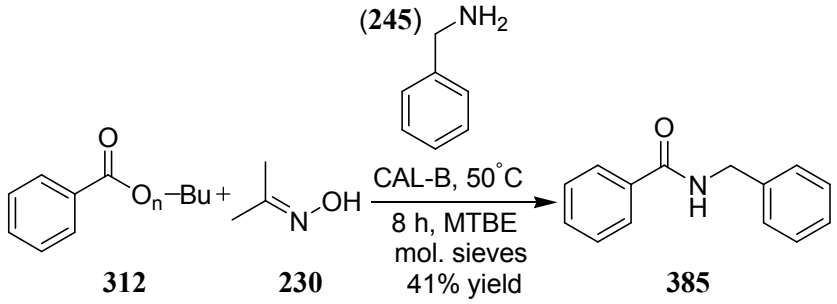
Due to the reversibility of the CAL-B catalysed reactions of methyl benzoate and 4-nitro methyl benzoate with acetone oxime, it was decided to synthesize the activated ester from an ester with a longer alkyl chain, such as butyl benzoate. The butyl esters synthesised earlier (Scheme 2.13 and 2.14) gave good yields of the glycol activated esters when the reactions were performed with ethylene glycol in presence of CAL-B

(2.15 and 2.16, Table 2.2). Therefore it was believed that CAL-B would also catalyse the reaction of the butyl ester with acetone oxime and give the desired activated ester. Also, it was believed that the released butanol, being bigger than methanol, would be less able to undergo nucleophilic attack on the ester product and would therefore, reduce the rate of reversibility seen with methyl benzoate ester.[176] For this study a reaction of pre-synthesized butyl benzoate (Scheme 2.13) was performed with acetone oxime in presence of CAL-B. Unfortunately, the reaction gave ~10% yield (calculated from crude ^{1}H NMR) of the activated ester product (**368**) (Scheme 2.38).



Scheme 2.38 CAL-B catalysed synthesis of an oxime ester from methyl benzoate.

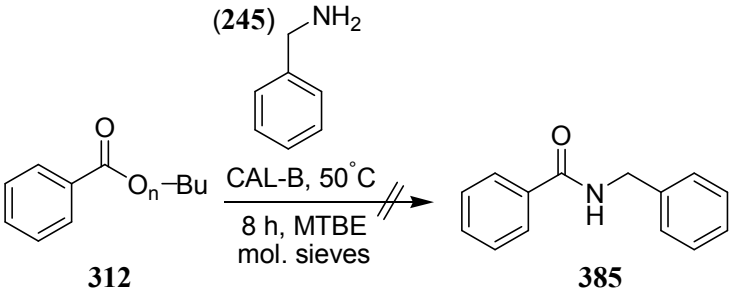
The low yield of the activated ester **368** indicated that the released butanol is still capable of undergoing nucleophilic attack on the ester product (**368**).[176] Nonetheless to further study the reaction scheme with amines it was decided to perform a one-pot synthesis of the ester **312** with benzylamine. It was not performed with aniline because the activated ester **368** gave no amide product when its reaction was carried out with aniline (Scheme 2.33). For the one-pot synthesis a reaction of butyl benzoate



Scheme 2.39 One-pot synthesis of *N*-benzyl benzamide **385**.

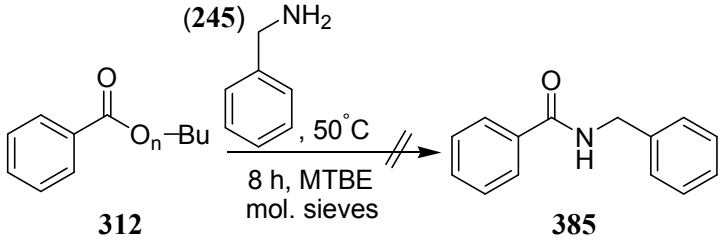
(**312**), benzylamine (**245**), acetone oxime (**230**) and CAL-B was performed at 50°C for 8 hours, giving 41% yield of the amide product (**385**) (Scheme 2.39). In light of this it was decided to compare the yield of **385** with that of CAL-B catalysed amidation. For this, a reaction of butyl benzoate and benzylamine was carried out

with CAL-B in MTBE at 50°C for 8 hours. This time, a negligible product yield in the crude ^{1}H NMR of the reaction mixture (Scheme 2.40), was obtained.



Scheme 2.40 CAL-B catalysed synthesis of *N*-benzyl benzamide **385**.

A background reaction of the unactivated ester butyl benzoate (**312**) and benzylamine (**245**) was also performed to see the reactivity and compare with the yield amide **385** synthesised by one-pot synthesis using acetone oxime as ester activating agent. No product was obtained in this reaction (Scheme 2.41).



Scheme 2.41 Reaction of butyl benzoate and benzylamine.

Due to the formation of the amide in the one-pot synthesis (Scheme 2.39), it was decided to carry out a one-pot synthesis using previously synthesised (Scheme 2.13) butyl esters and to study the effect of substituents on the yield of amide products; the results are tabulated in Table 2.4. The yield of amides obtained from the one-pot synthesis (Control 1, Table 2.4, Procedure 2.16) was compared with that of CAL-B catalysed (Control 2, Table 2.4, Procedure 2.17) and the reaction between non-activated ester amine (Control 3, Table 2.4, Procedure 2.18).

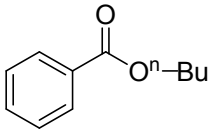
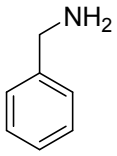
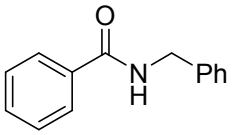
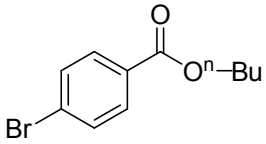
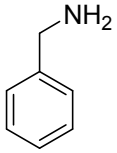
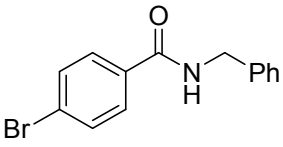
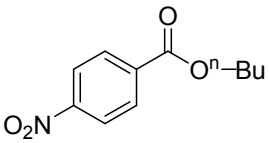
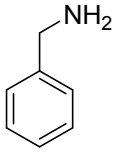
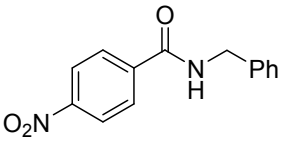
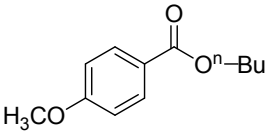
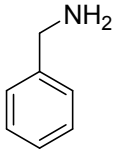
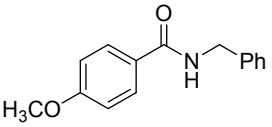
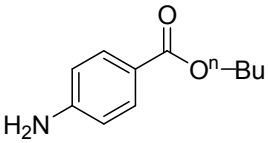
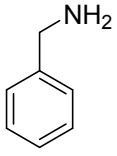
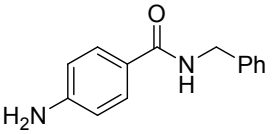
Entry	Ester	Amine	Product	Control 1[a] Yield (%)[d]	Control 2[b] Yield (%)[d]	Control 3[c] Yield (%)[d]
1	 **312**	 **245**	 **385**	41	Traces	nr
2	 **316**	 **245**	 **390**	30	26	nr
3	 **317**	 **245**	 **391**	42	42	nr
4	 **314**	 **245**	 **392**	nr	nr	nr
5	 **315**	 **245**	 **393**	nr	nr	nr

Table 2.4 Amides synthesised by activated ester and CAL-B.
a Control 1: ester (1 equiv.), amine (**245**) (1 equiv.), acetone oxime (1 equiv.), CAL-B (0.5g), 4Å molecular sieves (1g) in MTBE (1ml), 8 h at 50°C. **b** Control 2:ester (1 equiv.), amine (1 equiv.), CAL-B (0.5g), 4Å molecular sieves (1g) in MTBE (10ml), 8 h at 50°C. **c** Control 3: ester (1 equiv.), amine (1 equiv.), 4Å molecular sieves (1g) in MTBE (10ml), 8 h at 50°C. **d** Isolated yield of pure material. nr.: no reaction.

In entry 1 the yield of amide product (**385**) was good compared to CAL-B catalysed reaction which gave no product. This demonstrated the importance of the ester activation using acetone oxime. But the low product yield indicated the dominance of reaction reversibility due to released butanol (see Scheme 2.38).[176]

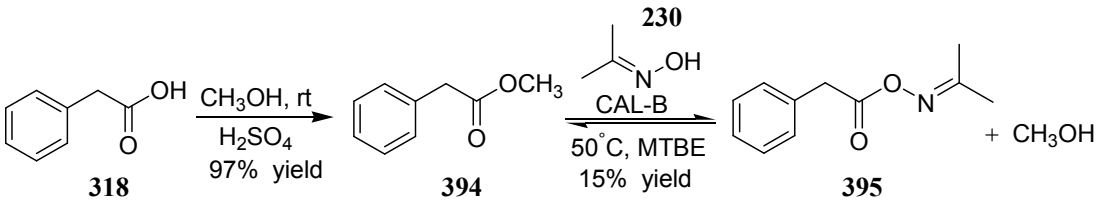
In entry 2 the yield of amide product (30%, **390**) was not much higher than the CAL-B catalysed amidation (26%, **390**). Also, the 30% yield from one-pot synthesis was less than that in entry 1 (41%, **385**). This could presumably be due to the presence of bromine group on the ester substrate (**316**) reducing the electrophilicity of the carbonyl carbon by electron donating resonance effect. This could be further confirmed by the 42% amide product yield (entry 3) from one-pot synthesis when nitro group was present at the ring of the ester substrate (**317**). But unfortunately, CAL-B catalysed amidation also gave 42% of the amide product (**391**) which might be due to increased reactivity of the ester substrate. Additionally, in both entries 2 and 3 the low product yields (Control 1 and 2) again indicated the dominance of reaction reversibility due to released butanol.

In entry 4 and 5 when methoxy (**314**) and amino (**315**) group were present at position-4 respectively, no amide products were obtained from one-pot synthesis and CAL-B catalysed amidation. In case of entry 4, presence of methoxy group at position 4 might be reducing the electrophilicity of the ester substrate (**314**) or the oxime activated ester formed *in-situ* via electron donating resonance effect. In case of entry 5, amino group at position 4 presumably reduced the electrophilicity of the carbonyl carbon by electron donating resonance effect resulting in absence of reaction with benzylamine. Also, the absence of product could be correlated to the reversibility of the reaction studied in Scheme 2.38.

Comparing the CAL-B catalysed background reactions (control 2, entry 2 and 3, Table 2.4) and the amide product yields obtained from one-pot synthesis using acetone oxime as ester activating agent (Control 1, entry 2 and 3) it appears as though the reaction is dominated by the CAL-B alone catalysed reactions, and so the scheme was abandoned.

Due to the reversibility of the CAL-B catalysed reactions of methyl benzoate, 4-nitro methyl benzoate and butyl benzoate with acetone oxime, it was decided to synthesise the activated ester with a methylene unit between the ring and the carbonyl carbon,

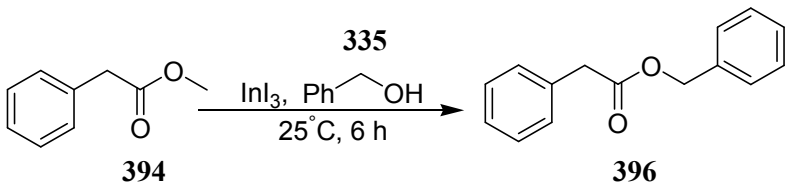
which would negate the electronic effects of the ring, thus making the ester more reactive. As such, a reaction of methyl 2-phenylacetate (**394**) was prepared (Procedure 2.19) from phenyl acetic acid (**318**) and a reaction was carried out with acetone oxime (**230**) in the presence of CAL-B (Scheme 2.42).



Scheme 2.42 Synthesis of oxime ester from 2-phenylacetic acid.

Unfortunately, this reaction also showed reversibility due to the released methanol in the reaction mixture, which was confirmed by the integration of the peak of the methyl 2-phenylacetate at δ 3.67 in the crude ^{1}H NMR of the reaction mixture. The reaction was followed at 8, 12, 16, 24 and 32 h. When the reaction was carried out at 50°C, the yield of the activated ester was around 10% after 8h which further increased to around 20% after 12 h. But after 16h no product was detected in the crude ^{1}H NMR. The reaction was carried out with various equivalents of the ester and oxime at 40, 50 and 60°C, but under all conditions, it showed reversibility as before. Increasing the temperature to 60°C and the addition of triethylamine to the reaction mixture of the ester, oxime, with or without CAL-B, did not give the desired product. It was envisaged that triethylamine, having higher pKa, (10.78) than acetone oxime (10.5) would abstract the proton from acetone oxime and generate the anion which would undergo the nucleophilic attack on the ester substrate and form the activated ester product (**395**) but this also did not work. The preparation was also carried out in the presence of potassium hydroxide at 70°C, without the enzyme, believing that it would also abstract the proton from acetone oxime and facilitate the reaction in forming the activated ester product, but again no ester was obtained.

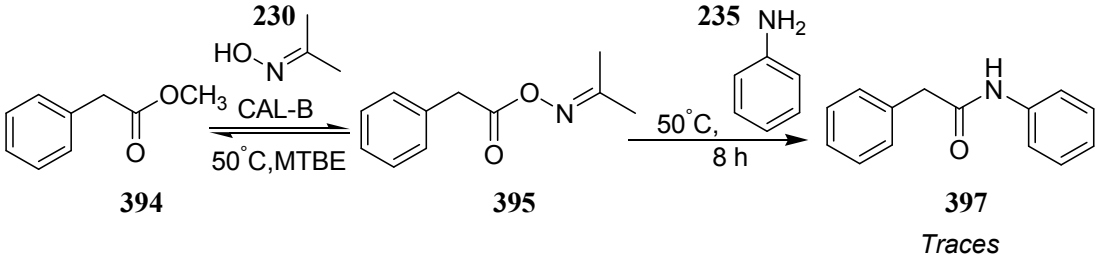
In a repeated effort, the addition of a Lewis acid catalyst, indium triiodide, was used in the reaction of methyl 2-phenylacetate (**394**) and acetone oxime (**230**), but again it



Scheme 2.43 Transesterification of esters using indium triiodide.[195]

did not give any product, despite indium triiodide being known to catalyse the transesterification of esters (Scheme 2.43) and in the acylation of alcohols and amines.[194-196]

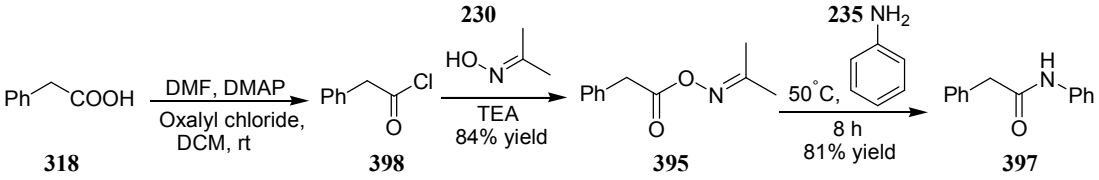
Lastly, in an effort to force the equilibrium forward towards product formation, a one-pot synthesis was also carried out with aniline, but unfortunately, it only produced traces of product in the crude ^{1}H NMR (Scheme 2.44).



Scheme 2.44 One-pot synthesis of amides.

Removing the enzyme and changing the solvent from MTBE to toluene, and increasing the temperature to 110°C also did not impart any effect in the ester product formation.

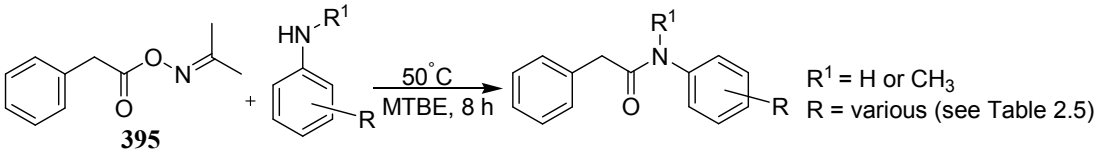
Thereafter, it was decided to check the reactivity of the activated ester **395** with aniline (**235**) directly. It was suspected that, due to the low yield of the ester (**395**) in the CAL-B catalysed reaction, a consequence of the reversibly of the reaction by the released methanol, **395** is not forming and therefore not reacting with the aniline. Therefore, in order to determine this, an activated ester (**395**) from 2-phenylacetic acid (**318**) was synthesized chemically and its reaction was carried out with aniline (as a model substrate), Scheme 2.45, Procedure 2.20. The synthesis of **395** was successfully carried out, as performed earlier, and upon isolation it was subjected to a reaction with aniline (Scheme 2.45).



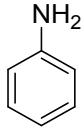
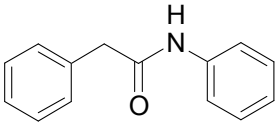
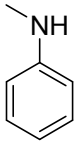
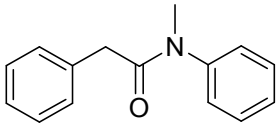
Scheme 2.45 Chemical synthesis of amide **397** from the oxime ester of 2-phenylacetic acid.

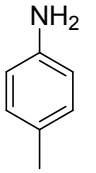
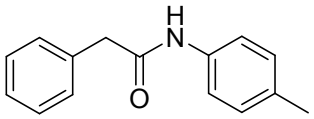
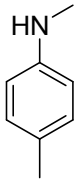
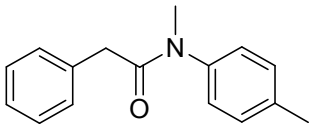
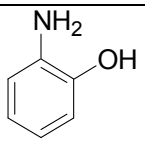
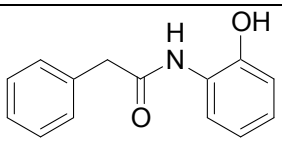
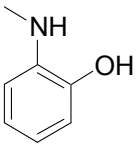
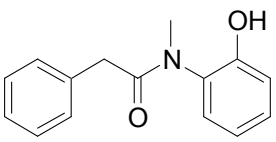
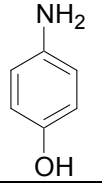
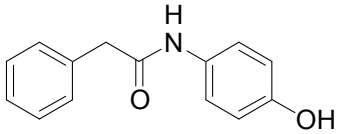
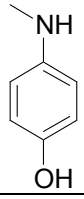
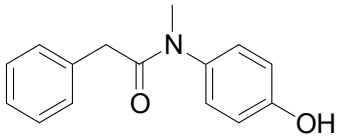
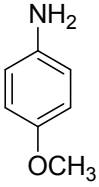
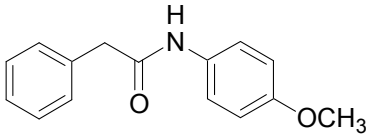
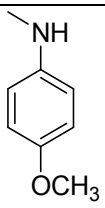
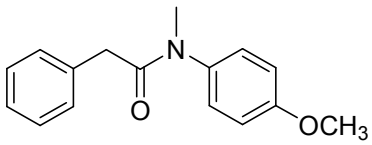
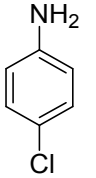
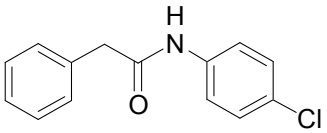
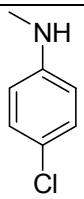
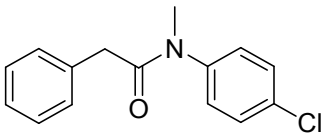
The successful formation of amide (**397**) confirmed the reason it was not possible in Scheme 2.44 was likely due to the reversibility of the CAL-B catalysed reaction in the synthesis of activated ester (**395**). Delighted from the good yield of the amide product (**397**), it was decided to try and broaden the scope of the applications of the activated ester **395** in the hope that it would react with various anilines to give the corresponding amide products, even though it could not be synthesised enzymatically.

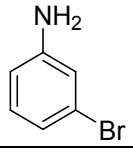
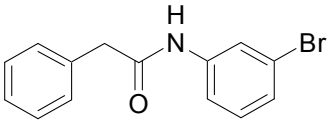
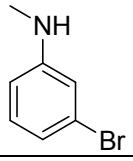
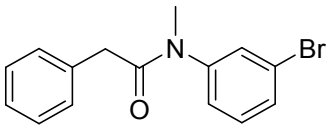
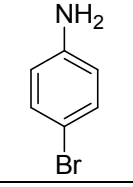
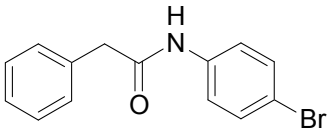
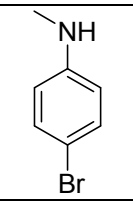
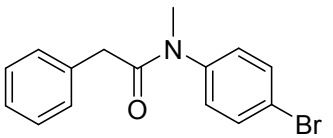
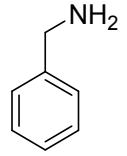
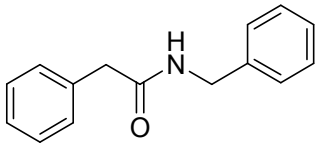
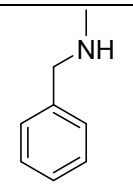
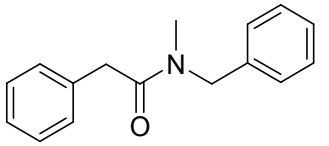
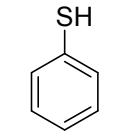
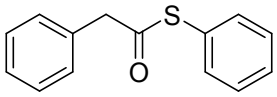
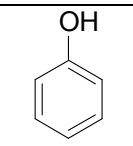
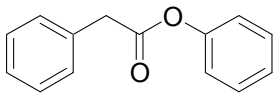
Thereafter, a series of reactions with anilines were carried out with the activated ester **395** to yield the corresponding amide product (Scheme 2.46, Procedure 2.21) and the results are depicted in Table 2.5.



Scheme 2.46 General scheme for the synthesis of amides from oxime ester **395**.

Entry	Amine	Amine number	Product	Compound number	Isolated yield (%)
1		**235**		**397**	81
2		**241**		**407**	Traces

3		**261**		**408**	100
4		**399**		**409**	Traces
5		**239**		**410**	nr
6		**400**		**411**	nr
7		**237**		**412**	Traces
8		**401**		**413**	nr
9		**249**		**414**	100
10		**402**		**415**	nr
11		**253**		**416**	92
12		**403**		**417**	Traces

13		**255**		**418**	40
14		**404**		**419**	nr
15		**257**		**420**	83
16		**405**		**421**	nr
17		**245**		**422**	100
18		**406**		**423**	13
19		**283**		**424**	nr
20		**284**		**396**	nr

Reagents and conditions: **395** (1 eq.), amine (1 eq.), MTBE (1M), 50°C for 8 hours; nr = no reaction.

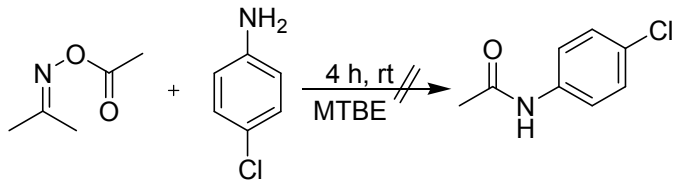
Table 2.5 Amides synthesised by activated ester and amines.

The 81% yield of the amide product from the reaction of aniline (**235**) and **395** (entry 1, Table 2.5) could be attributed to the increased reactivity of the activated ester due to the presence of the methylene unit which inhibits the conjugation between the benzene ring and the carbonyl carbon.

Anilines substituted with electron donating groups (entry 3 and 9, Table 2.5) gave 100% yield of the desired amide product which could be attributed to the electron

donating inductive (4-methyl aniline, **261**) or resonance effect (4-methoxy aniline, **249**) of the substituted group making the amino-group more nucleophilic, in addition to the increased reactivity of the activated ester.

Anilines with a bromine at the 3 or 4-position (entry 13 and 15, Table 2.5) gave different yields indicating the dominance of electron withdrawing inductive effect of 3-bromo aniline (entry 13, 40% yield) over electron donating resonance which was dominant at the 4-postion (entry 15, 83% yield). Surprisingly, 4-chloroaniline (**253**, entry 11) also gave 92% amide product which suggests a higher reactivity of the oxime ester along with the dominance of electron donating effect of the small chloro group when compared to the bromo-substituted anilines. It is noteworthy to mention that, no amide product (**254**) was obtained when the reaction of the highly reactive oxime ester, **231** (entry 10, Table 2.1) was carried out with 4-chloroaniline (**253**), Section 2.2.



231 **253** **254**

Scheme 2.47 Attempted reaction of oxime ester **231**with 4-chloroaniline.

Thus, this remarkable reactivity of the reagent is an advantage over many other reported agents which do not react with anilines. Moreover, the reagent could potentially be used in the removal of primary anilines in the presence of secondary anilines, with high selectivity. As mentioned in Section 1.4 the selective removal of primary anilines is often required in library synthesis, and so compound **395** could be used because of its selectivity for primary anilines over secondary anilines. It can be seen (Table 2.5) that in none of the reactions did it give the amide product when its reaction was performed with secondary anilines.

However, an unexpected result, wherein only a trace amount (entry 7, Table 2.5) of the desired amide product was obtained, occurred in the reaction of 4-hydroxy aniline with the activated ester. Whilst it is hypothesized that the electron donating hydroxyl group would increase the nucleophilicty of the amine, its yield was less than with aniline, suggesting that factors other than resonance affect the reactivity of this

amine. Similarly, when the hydroxyl group is in the 2-position (entry 5, Table 2.5), negligible yield of the amide product was obtained, presumably either due to steric hindrance of the hydroxyl group preventing its reaction with the activated ester, or inter- or intramolecular hydrogen bonding taking place in 2-amino phenol (**239**), preventing reaction.[171]

Overall, with anilines, the activated ester showed excellent chemoselectivity for primary anilines over secondary anilines, such that no significant amount of amide product was obtained with all the corresponding secondary anilines studied.

Following on from the aniline work, it was envisaged that benzylamine, (**245**, Scheme 2.48) with a methylene unit between the ring and the amine group inhibiting the electron delocalization, would be more reactive compared to the anilines initially studied. Study on the synthesis of **245** (entry 17, Table 2.5) is also significant since it is known that amides, such as **425**, possess important biological activity. For example, derivatives are known to behave as: novel haemoglobin oxygen-affinity decreasing agents;[197] non-natural modulators of quorum sensing in *Pseudomonas aeruginosa*;[198] biphenylacetamide-derived inhibitors of β-Site amyloid-β peptide cleaving enzyme 1 (BACE1);[199] and an intermediate in the synthesis of a RAD51 inhibitor that disrupts homologous recombination.[200] In particular, analogue **425** gave good inhibitory activity towards β-site amyloid-β peptide cleaving enzyme 1 (BACE1) (Figure 2.8).

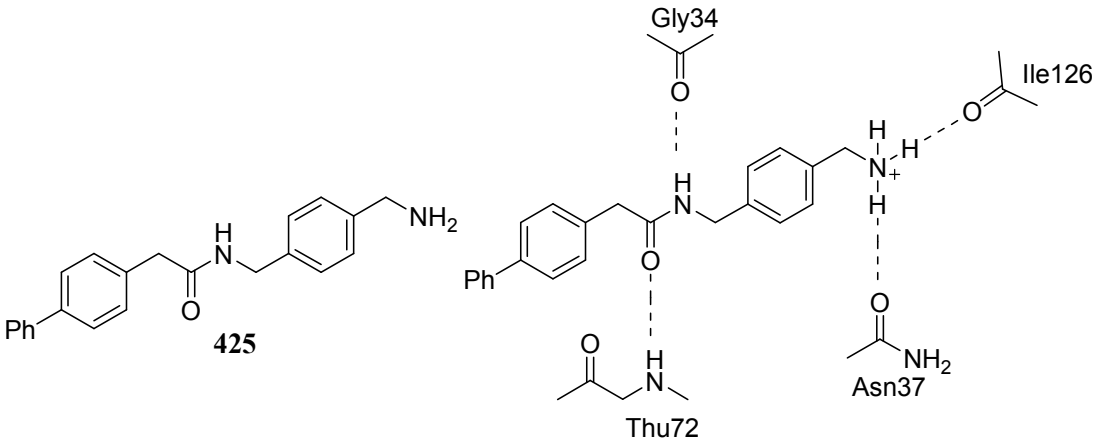
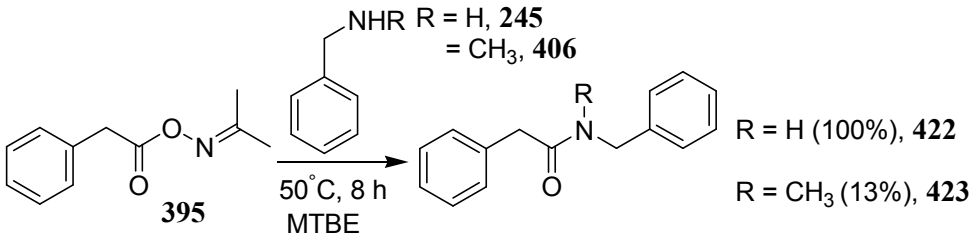


Figure 2.8 BACE1 inhibitor (**425**) interactions with BACE1.[199]

Its hydrophobic rings fit well in the enzyme's binding pocket (PDB 1M4H) which is further supported by hydrogen bonding and ion-dipole interactions. The compound is under study for the treatment of Alzheimer's disease for which currently there is no effective treatment with the only drugs available in the market being *N*-methyl-D-aspartate (NMDA) receptor antagonists and cholinesterase inhibitors which provide only symptomatic relief.

As expected, benzylamine with its higher pKa (9.34) compared to aniline (pKa 4.87)[172] gave 100% yield of the desired amide product (entry 17, Table 2.5). This yield indicated the high nucleophilicty of the nitrogen due to absence of electron delocalisation on the ring like in aniline which gave 81% yield of the amide product



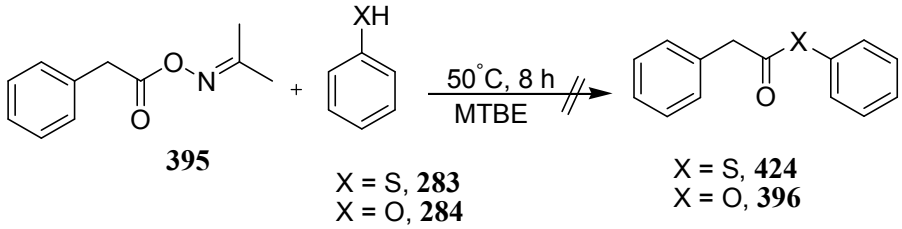
Scheme 2.48 Reaction of oxime ester with benzylamines.

(entry 1, Table 2.5) on reaction with the oxime ester **395**. Conversely, a reaction of the activated ester **395** with *N*-methyl benzylamine (**406**), only gave 13% yield of the desired amide product (**423**) as conformational isomers (rotamers), demonstrating the dominance of steric factors on the nucleophilicty of the amine (entry 18, Table 2.5). Their formation was confirmed from the crude ^{1}H NMR of the reaction mixture. It is reported that conformational isomers which interconvert with a speed not more than 1000 s^{-1} at 25°C can be detected in the NMR. [201, 202] Thus the rotamers were detected by the presence of two peaks for the methyl group at δ 3.67 and 2.97 in the ^{1}H NMR.

This result could be compared to the reaction of *N*-methylaniline (**241**) and the activated ester **395**, where the amide product was obtained in traces (entry 2, table 2.5), indicating the dominance of steric factor in *N*-methylaniline compared to the greater nucleophilicty in *N*-methyl benzylamine (**423**).

To further explore the range of nucleophiles suitable for the reaction, it was decided to study if any reaction occurred between the activated ester **395** with *O*- and *S*-based nucleophiles. For this, its reactions were carried out with thiophenol (**283**) and phenol

(**284**) (Scheme 2.49). Unfortunately, these reactions did not give the desired product in either case.

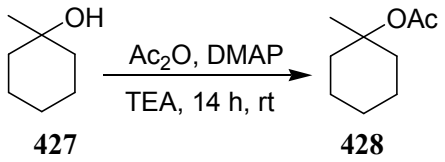


Scheme 2.49 Attempted reaction of oxime ester **395** with phenol or thiophenol.

This demonstrated the chemoselectivity of the activated ester for primary anilines over hydroxyl and thiol groups.

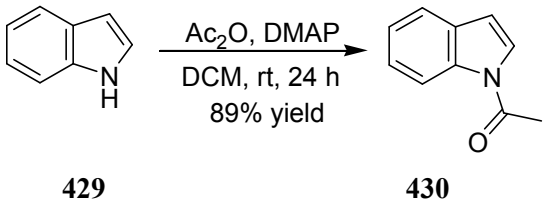
2.5.1 Addition of 4-Dimethylaminopyridine (DMAP)

4-Dimethylaminopyridine (**426**) is a reported catalyst for acylation of alcohols and amines. For example, in Scheme 2.50, the yield of **428** was only 5% when its reaction was carried out in acetic anhydride and pyridine at room temperature. But use of 4-dimethylaminopyridine gave 86% yield of the desired product.[203]



Scheme 2.50 Acylation of alcohols in presence of 4-dimethylaminopyridine.[203]

Similarly, in a case of *N*-acylation, the acylation of indole (**429**) using 4-dimethylaminopyridine results in 89% yield whereas the yield of **430** was half without 4-dimethylaminopyridine (Scheme 2.51).



Scheme 2.51 Acylation of indole in the presence of 4-dimethylaminopyridine.[204]

4-Dimethylaminopyridine acts as a catalyst and generates a reactive intermediate which further reacts with the nucleophile and forms the acylated product (Figure 2.9).

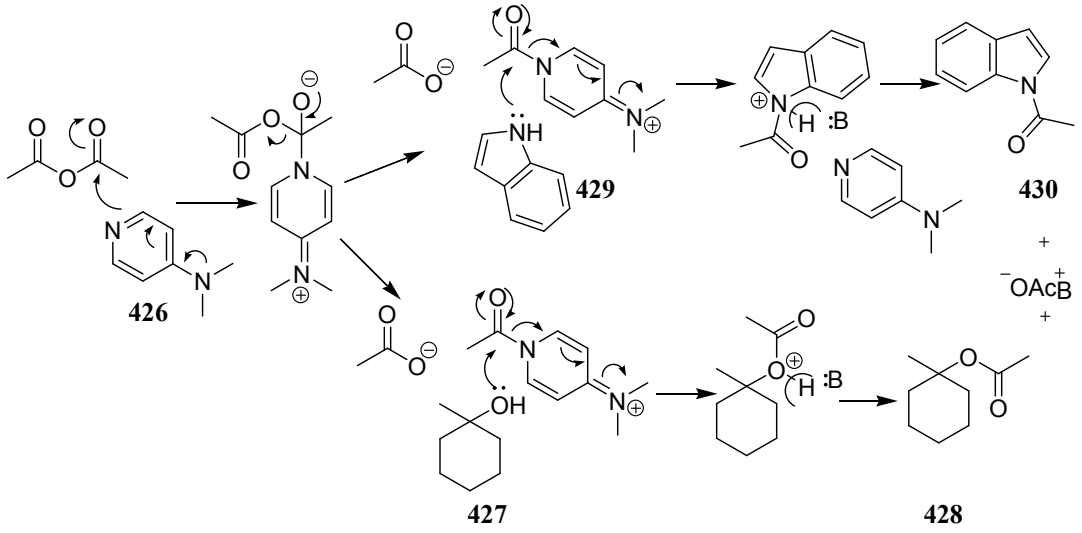


Figure 2.9 Mechanism of 4-dimethylaminopyridine mediated acylation of an alcohol and indole.[203, 204]

Analysing the results of the reaction of *N*-methyl aniline (entry 2, Table 2.5), it was decided to add 4-dimethylaminopyridine catalyst in its reaction with the activated ester **395**. It was envisaged that it would promote the reaction by acting as an activating agent for the activated ester (Figure 2.10).[203-205]

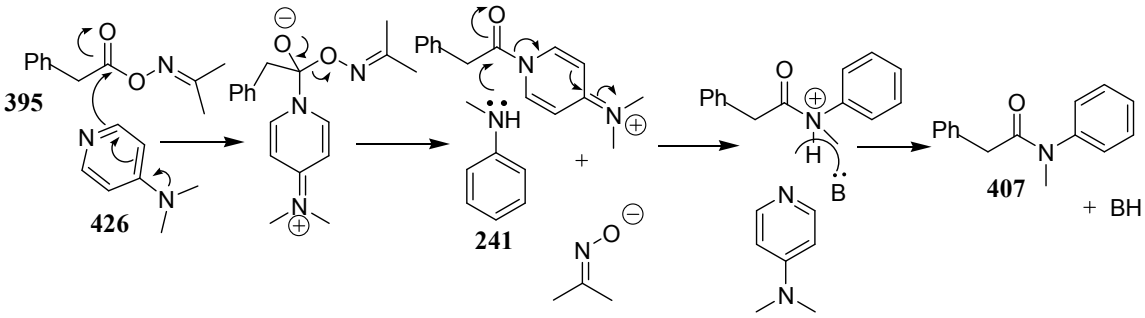
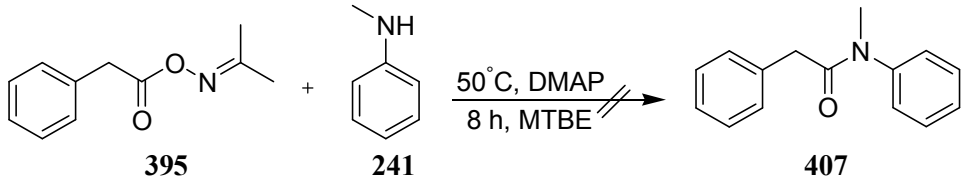


Figure 2.10 Proposed mechanism of 4-dimethylaminopyridine mediated acylation.

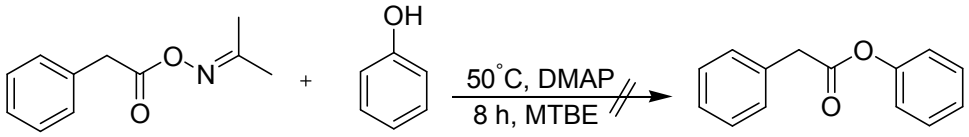
To test such a hypothesis, a reaction of *N*-methylaniline (**241**) with the activated ester **395** was carried out in the presence of 4-dimethylaminopyridine (Scheme 2.52).



Scheme 2.52 Attempted reaction of oxime ester **395** with *N*-methylaniline in presence of 4-dimethylaminopyridine.

Unfortunately, this also did not induce the desired product formation indicating the absence of formation of the intermediate (Figure 2.10) between the activated ester (**395**) and 4-dimethylaminopyridine (**426**) possibly due to the low reactivity of the ester **395**.

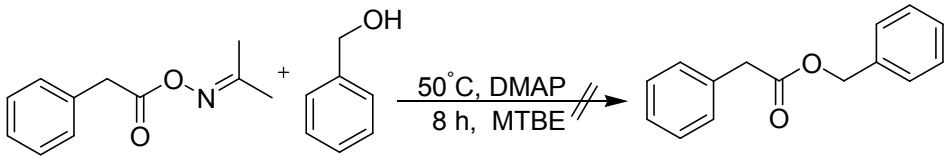
In a similar manner, it was decided to study the reactivity of ester **395** with other nucleophiles, such as phenol, in the presence of 4-dimethylaminopyridine (Scheme 2.53).



395 **284** **396**

Scheme 2.53 Reaction of oxime ester with phenol in presence of 4-dimethylaminopyridine.

However, this also did not give the desired ester product **396** which could be correlated to the very low nucleophilicity of phenol, having a pKa of 9.95. Oxygen being highly electronegative holds the electron pairs strongly contributing to the low reactivity of phenol with activated ester **395**. In a repeated attempt, the more basic nucleophile benzyl alcohol (pKa 15.4) was also carried out in place of phenol, but this also failed (Scheme 2.54).



395 **335** **431**

Scheme 2.54 Reaction of oxime ester with benzyl alcohol in presence of 4-dimethylaminopyridine.

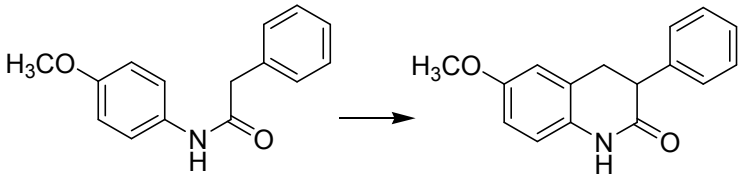
Added together, the results obtained confirmed the chemoselectivity of the activated ester for primary amines over secondary, hydroxy and thiol groups. Prior to this work there had been no reports of an oxime based chemoselective acylating agent selective for anilines in high yield. As such, the reagent could be particularly useful in solution phase library synthesis involving benzylamines or anilines due to the cleanliness and selectivity of the reactions. It would be noteworthy to mention that, in the field of

solution phase library synthesis, very few effective benzylamine scavengers are reported. Those known include a benzaldehyde resin and an acetoacetoxy ethyl methacrylate resin, which are very costly.

2.5.2 Applications of methodology

a) Improved N-acylation route

The *N*-acylation of amines with this oxime ester is a new way to synthesize intermediates which are used in the preparation of anticancer agents. For example, compound **433**, the main intermediate employed in the synthesis of 3-aryl-2-quinolone derivatives has been synthesized from compound **432** by others (Figure 2.11). These quinolone derivatives significantly reduced the migration of cancerous cells when tested on MCF-7 human breast cancer cell lines.[206] Additionally, these antimigratory compounds were more effective when used with either doxorubicin or etopside. The starting material of this intermediate was synthesized using a standard acid chloride method and gave 92% yield of the amide product.[206]

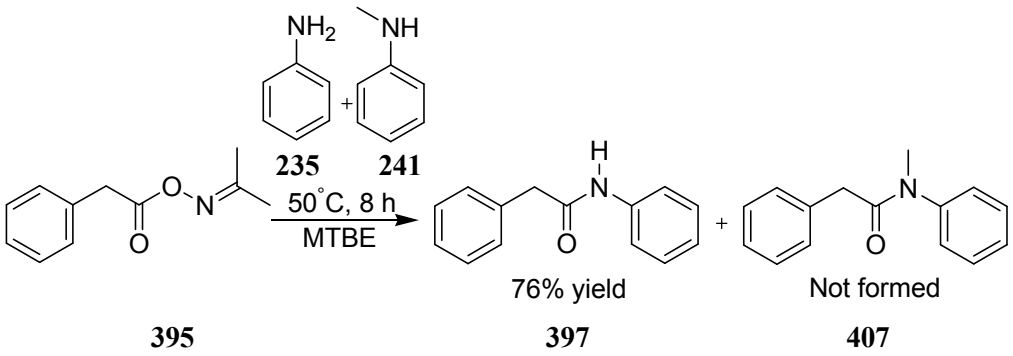


432 **433**

Figure 2.11 Intermediate used in the synthesis of anticancer agents.[206]

However, when the activated ester (**395**) developed herein was used in this work, the yield of the amide product was 100% (Table 2.5, entry 9). Moreover, the reaction was clean and efficient. Thus, this method could be employed in the synthesis of important amide bond containing molecules which are otherwise synthesized using less desirable methods.

Analysing the importance of the activated ester **395**, it was decided to further validate its chemoselectivity for primary nitrogen. For this, a competition reaction of aniline (**235**) and *N*-methylaniline (**241**) was carried out which gave only one acylated product (**397**), confirmed from the crude ^{1}H NMR and upon isolation of the reaction product (Scheme 2.55, Procedure 2.22).

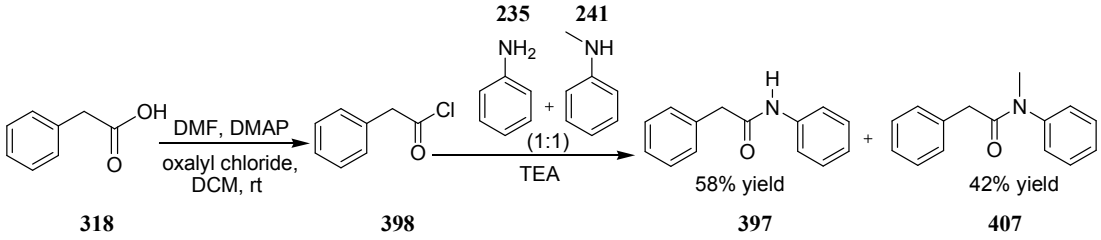


Scheme 2.55 Competition reaction between aniline and *N*-methyl aniline using the activated ester **395**.

This demonstrated the high selectivity of the ester and indicated that it could be added to a mixture of primary and secondary anilines for selective removal of the primary one. Thus, the ester can potentially be effectively used in the scavenging reactions of amines, particularly primary anilines.

b) Selectivity over non-selective acid chloride method

As seen earlier in Section 1.1, acid chloride mediated acylations are non-selective, and when they are carried out on the compounds containing both primary and secondary amines there is a need for the protection of the acylation-sensitive groups. Therefore, to further show the advantage of ester (**395**) over acid chlorides, a competition reaction was carried out with the corresponding acid chloride **398**, which this time gave both expected *N*-acylated products (Scheme 2.56, Procedure 2.23).

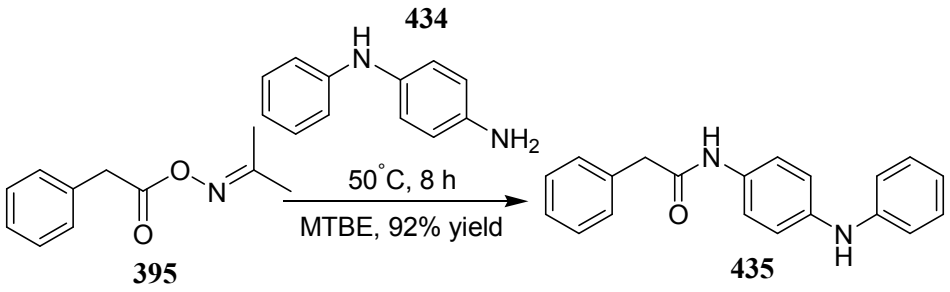


Scheme 2.56 Competition reaction of aniline with *N*-methyl aniline.

This demonstrated the chemoselective nature of the activated ester over the non-selective nature of commonly used acid chlorides for acylation reactions.

c) Derivatization of pharmaceutically significant pharmacophores

A great deal of pharmaceutically relevant molecules contain primary or secondary nitrogen.[11, 207] Therefore, to further broaden the scope on bigger and more drug-like compounds, a reaction of activated ester (**395**) was carried out with *N*-phenyl-*p*-phenylenediamine (**434**) (Scheme 2.57, Procedure 2.24), the acylation occurred only at the primary position which was confirmed by X-ray crystallography (Figure 2.12, Appendix).



Scheme 2.57 Synthesis of 2-phenyl-*N*-(4-(phenylamino)phenyl)acetamide.

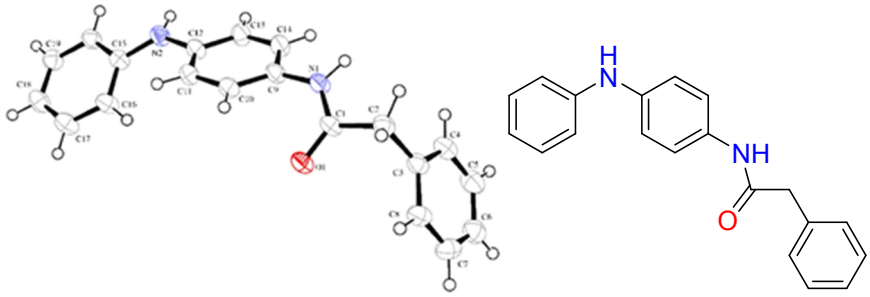


Figure 2.12 Crystal structure of 2-phenyl-*N*-(4-(phenylamino)phenyl)acetamide (**435**).

N-Phenyl-*p*-phenylenediamine (**434**) is an important intermediate used in the synthesis of anti-depressants,[208] tyrosine kinase inhibitors,[209] antivirulents[210] and platelet-derived growth factor (PDGF) receptor phosphorylation inhibitors.[211] Additionally, it has also been used in the synthesis of biodegradable hydrogels.[212] The selective acylation observed here at the primary nitrogen could be utilized in further derivatization of the molecule which in turn could be useful in further enhancing the utility of the molecule. Because C-N bond forming reactions serve as an important pillar in the synthesis of pharmacophores and biologically active compounds, there is an urgent need to develop procedures, especially in amide bond synthesis, which are elegant and clean.

Keeping all these key points in mind, it was decided to study the applicability of the ester on the derivatization of some other important drug intermediates. After careful consideration, the important drug intermediates given below, containing both primary as well as secondary amines, were considered (Figure 2.13):

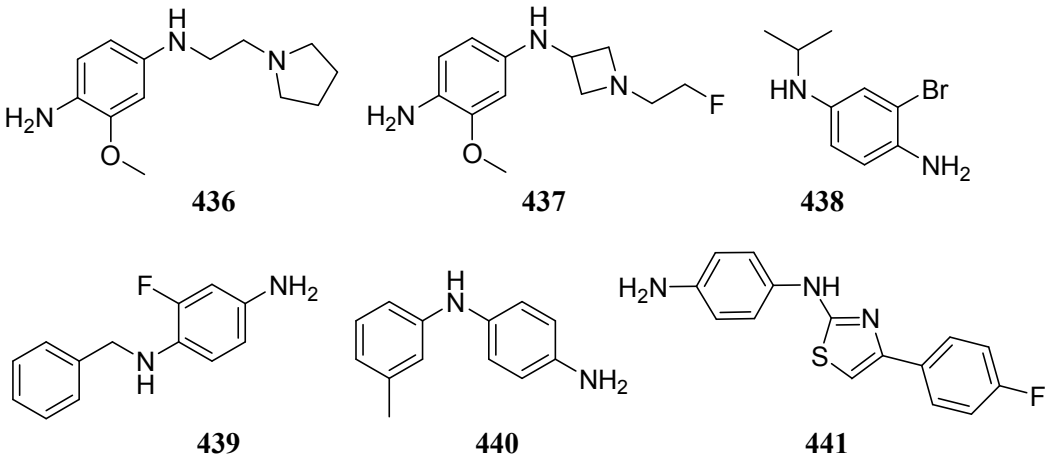
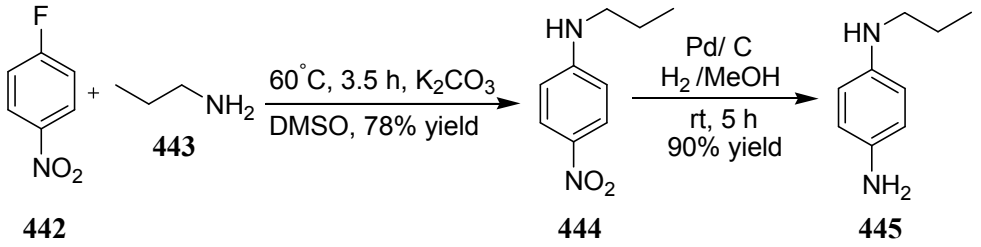
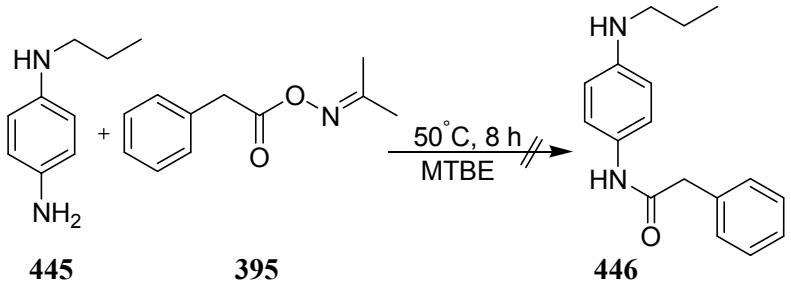


Figure 2.13 Drug intermediates containing primary and secondary anilines.

Compound **436** has been used in the synthesis of many pharmaceutical compositions.[213] Compound **437** and **438**, have been used in the preparation of novel epidermal growth factor receptor (EGFR)[214] and c-Jun N-terminal kinases (JNK) inhibitors[215] respectively which are currently in patent. Similarly, compound **439** has been used in the preparation of antibacterials.[216] Compound **440** has been used in the synthesis of materials used in the synthesis of anticancer agents[217] and compound **441** and its derivatives are lipoxygenase inhibitors currently in patent.[218] Keeping these utilities in mind it was envisaged to synthesize some significant intermediates containing both primary and secondary amine groups which could be used in the synthesis of pharmacologically active compounds and, subsequently study the chemoselectivity of the prepared activated ester **395** on them. In light of this, 4-amino-*N*-propylamine (**445**) was synthesized (Scheme 2.58, Procedure 2.25 and 2.26) and reacted with the prepared activated ester (**395**). Unexpectedly no product was obtained when the reaction was performed with **445** at 50°C for 8 hours (Scheme 2.59)

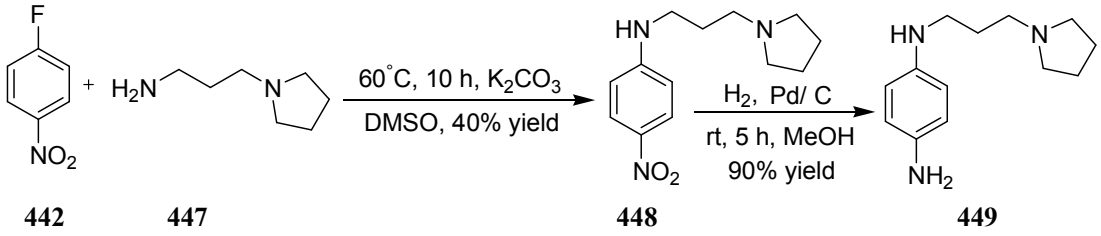


Scheme 2.58 Synthesis of N^1-propylbenzene-1,4-diamine.

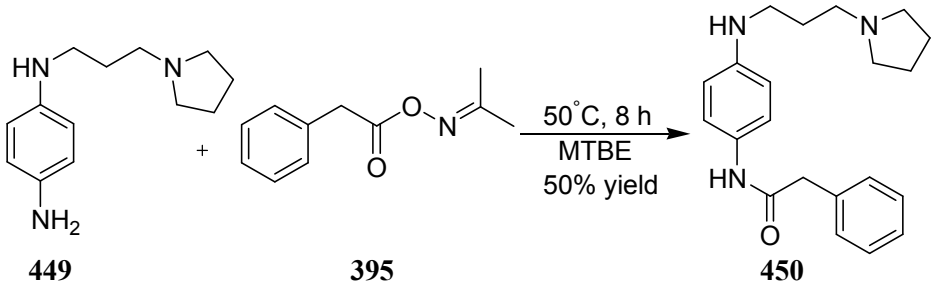


Scheme 2.59 Synthesis of 2-phenyl-*N*-(4-(propylamino)phenyl)acetamide.

In a repeated effort, another amine (**449**) was synthesized (Procedure 2.27 and 2.28) by a similar method (Scheme 2.60) and a reaction was performed with the activated ester (Scheme 2.61, Procedure 2.29).



Scheme 2.60 Synthesis of *N*1-(3-(pyrrolidin-1-yl)propyl)benzene-1,4-diamine.



Scheme 2.61 Synthesis of 2-phenyl-*N*-(4-(3-(pyrrolidin-1-yl) propylamino) phenyl) acetamide.

The amide product **450** obtained was confirmed only from the crude ^{1}H NMR but could not be isolated from the reaction mixture after silica gel column chromatography, presumably due to its high polarity. Nevertheless, the yield calculated from its crude ^{1}H NMR was 50%. It was also confirmed by mass spectrometry. From these results it could be concluded that the activated ester **395** can be effectively applied on simple molecules to generate the amide bond cleanly and safely; however, the route needs optimization in the case of complicated drug-like molecules.

CHAPTER 3- PROBE DESIGNING

Chapter 3:

BENZANILIDE BASED PROBE

3.1 Applications of a benzanilide based probe in the detection of disease-specific enzymes: CAL-B as a model

3.1.1 Hydrolysis reactions of lipases

It is known that lipases, besides catalysing the formation of amides, also catalyse the hydrolysis of lipid esters. A general mechanism of hydrolysis catalysed by esterases is given below (Figure 3.1).

In this reaction, the ester as substrate enters the active site of the enzyme, it undergoes nucleophilic attack, assisted by the hydroxy group of the amino acid present at the active site, leading to release of the alcohol molecule from the ester substrate. In the next step, this acyl-enzyme complex undergoes nucleophilic attack by a water molecule leading to release of a carboxylic acid product and a free active site ready to accommodate the next substrate molecule.

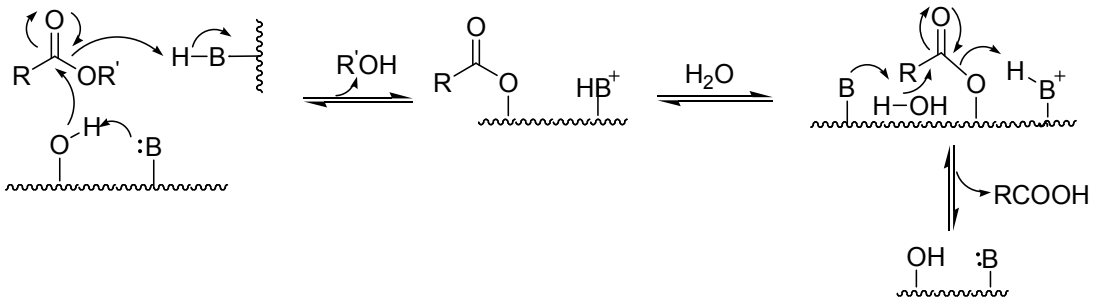


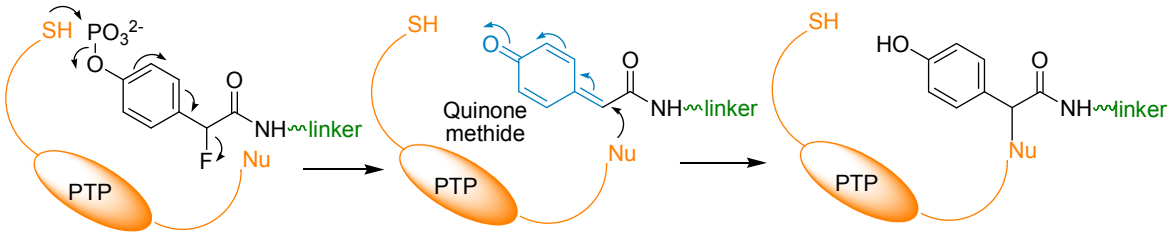
Figure 3.1 Ester hydrolysis by esterases.[219]

3.1.1.1 Importance of such enzymatic reactions and its clinical applications

Enzymes are key regulators in signal transduction pathways of the body and any aberrancy in their level implicates various disease conditions such as cancers and metabolic or autoimmune disorders.[220-224] Being of such significance many probes or diagnostic tools have been synthesised to detect the expression of enzymes in clinical conditions.[225-228]

The basic principle behind designing such probes is the synthesis of enzyme sensitive molecules which when undergo an enzyme-catalysed transformation, release the product. If the probes are tagged with special (for example, fluorescent) molecules they can be easily detected by various methods such as electrochemical assays, fluorescence imaging, mass spectrometry and voltammetry and therefore assist in detection of diseases.

Some important reported probes are activity-based probes,[229, 230] ferrocene-peptide probes[231] and self-cleavable chemiluminscent probes.[232] Activity-based probes were first reported in 1999 by Lo and colleagues.[233, 234] These are reported to detect the expression level and post-translational modifications of the enzymes in diseased conditions which is otherwise very difficult to achieve using conventional genetic or proteomic tools. An activity-based probe reported for the detection of over-expressed protein tyrosine phosphatases (PTPs) in cancers is given below (Figure 3.2).[235, 236] These protein tyrosine phosphatases (PTPs), by performing the hydrolytic removal of phosphates of tyrosine residues in phosphoproteins regulate the phosphorylation performed by the protein tyrosine kinases (PTKs) and thus maintain these phosphorylation-dephosphorylation reactions in various physiological processes such as cell growth, metabolism and apoptosis. Any irregulation in the phosphorylation-dephosphorylation cycle leads to various diseases; for example, excessive protein tyrosine phosphorylation is among one of the contributing factor in the development of cancers.[220] Whereas reduced phosphorylation is related to type-2 diabetes.[224] Thus, early detection in the level of these enzymes is crucial in the diagnosis and cure of several diseases which are otherwise incurable in late stages.



451 **452** **453**

Figure 3.2 Mechanism of PTP trapping by activity-based probes.[234, 235]

The activity-based probes (ABPs, Figure 3.2) consist of a 'warhead' and a 'latent trapping device' attached to a linker containing the reporter group. Initially, the probe

is activated by the enzyme. Here, the activity-based probe containing 4-fluoromethylphenylphosphate as a warhead undergoes nucleophilic attack (**451**) by the thiol of the cysteine present at the active site of the enzyme leading to the elimination of the fluorine along with the hydrolysis of the phosphate, and subsequently generating the reactive quinone methide moiety, called a protein tyrosine phosphatase-activated probe complex (**452**). This quinone methide then undergoes nucleophilic addition through an attack by a nucleophile residue present near the active site of the enzyme and forms the ABP-PTP complex (**453**) which can be detected by electrophoretic, fluorescence or mass spectrometric technique depending upon the nature of the functional groups present in the reporter group attached to the linker.[235]

Other than activity-based probes, a novel class of electrochemical biosensors based on modified electrodes has been reported for monitoring the activity and action of enzymes.[237-242] A notable one was reported by Liu *et al*., in 2006.[243] This was the first feasible and simple electrochemical biosensor system for the detection of matrix metalloproteinase MMP7. Here, a protease-specific ferrocene-labelled peptide segment was attached to an electrode. In presence of metalloproteinase MMP7 the peptide was cleaved causing a decrease in the electrochemical signal flowing in the ferrocene-peptide-electrode system which was detected by electrochemical voltammetry method. On a similar theme, in 2010, Adjemian *et al*., reported a

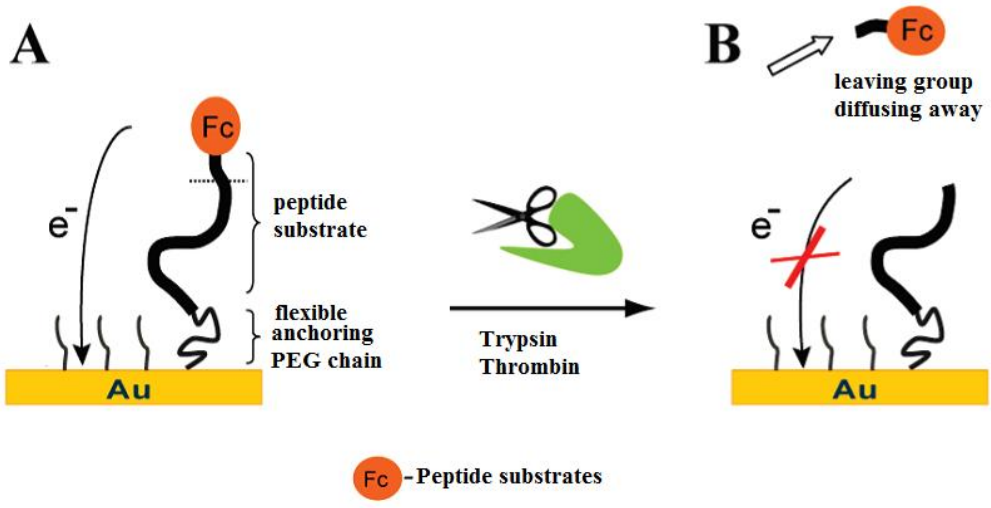


Figure 3.3 Electrochemical system for the detection of proteases.[231]

ferrocene-labelled peptide grafted on gold electrodes via a flexile polyethylene glycol linker. Here, the peptide, was sensitive to trypsin and α-thrombin enzymes (Figure 3.3).[231] In this system the peptide was attached with polyethylene glycol (PEG) chain to prevent the unwanted adsorption of the peptide chain on the gold electrode. Also, polyethylene glycol being flexible orients the chain in such a manner where it is easily accessible and accommodated in the active site of the enzyme. Initially, the current flow in the gold electrode due to ferrocene is detected in the voltammogram (**A**). But when the system is added in to the enzyme solution the flow reduces and eventually stops due to cleavage of the peptide chain (**B**). In the detection the amount or level of the enzyme is proportional to the reduction in the current flow. Because of this detection pattern, these biosensors are also known as 'signal off' type of sensors.

A different class of probe reported for the detection of proteases are the self-cleavable chemiluminscent probes (Figure 3.4) based on a dioxetane containing molecule.[232] In this system the enzyme sensitive peptide is attached to a high energy molecule (1,2-dioxetane) through a cleavable linker. As the probe system is added in to the enzyme solution the amide bond is cleaved and the 1,2-dioxetane is released along with the linker.

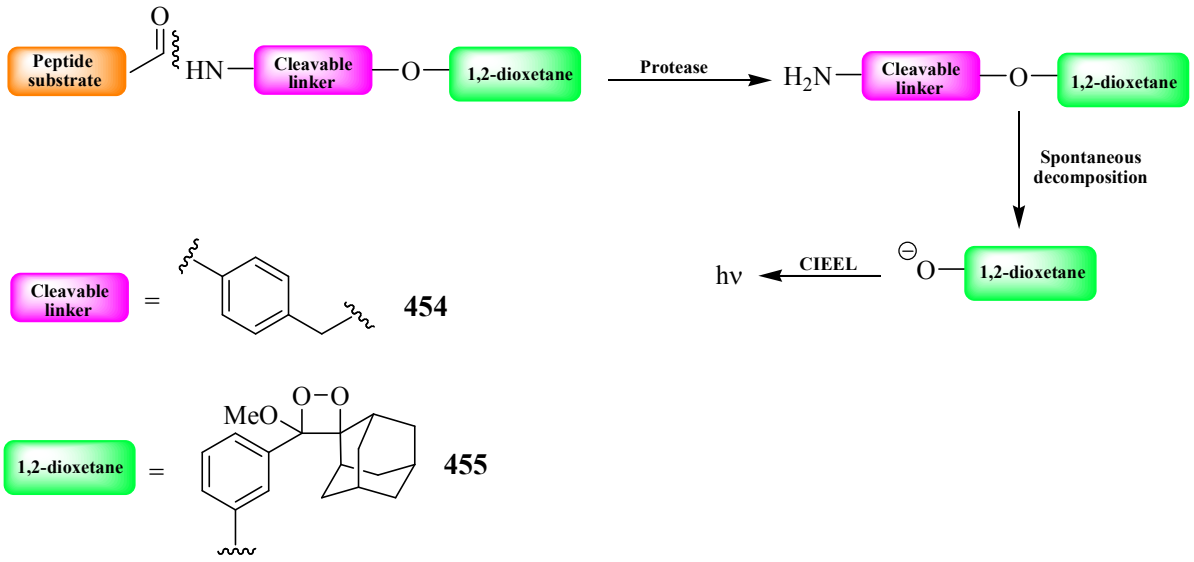


Figure 3.4 Chemiluminscent substrate activation by proteases.[232]

This system, being highly unstable decomposes spontaneously and releases the reactive dioxetane anion which undergoes chemically initiated electron exchange luminescence (CIEEL) where electrons go in excited state and on returning back to

their ground state release energy in the form of phosphorescence and emit light. This type of probe containing 1,2-dioxetane has also been reported in the detection of other enzymes such as alkaline phosphatase and acetylcholine esterase.[232]

In many biosensors the magnitude of the intensity signal depends on the efficiency of the energy transfer between the non-biological donors and acceptors (such as pyrene rings) attached to the molecules.

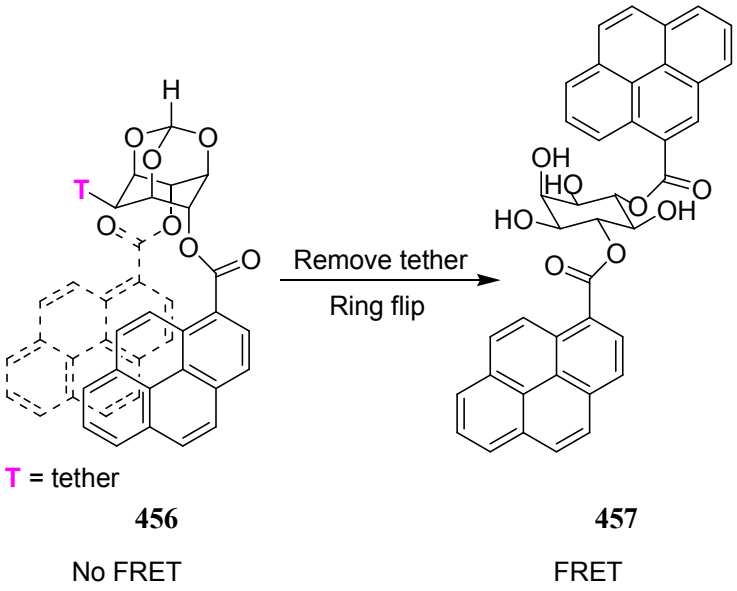


Figure 3.5 Flipping of axial to equatorial conformation in *myo*-inositol-pyrene derivative.[244]

This efficiency is known as Forster resonance energy transfer (FRET) and decreases on reducing the distance between the donors and acceptors groups. FRET is usually effective when the distance between the donors and acceptors groups is 10-100 Å. On this principle, in 2008, Kadirvel *et al*., reported on the fluorescence pattern of exciplex and excimer based *myo*-inositol-pyrene based molecules.[244] For example, when the compound **456** (Figure 3.5) was locked into an unstable penta-axial ester confirmation, the pyrene rings were in close proximity and due to π-π stacking between them they formed an excimer (excited state dimer) causing quenching of fluorescence. But removal of the tether group (**T**) flips the system into a more stable penta-equatorial chair conformation (**457**) causing loss of π-π stacking between the pyrene rings which ultimately resulted in blue fluorescence due to Forster resonance energy transfer (FRET) which was high compared to the excimer fluorescence. FRET

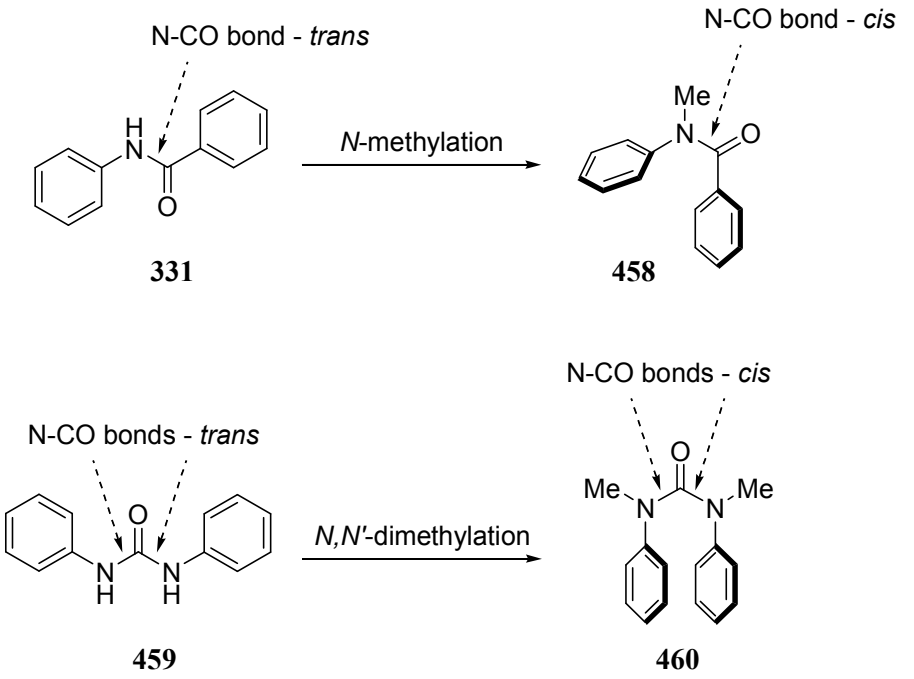
based probes have shown utility in some key areas of research such as protein-ligand binding interactions,[245] detection of proteases[246] and HIV diagnosis.[241]

Analysing such systems it was decided to synthesise a probe and lock it into a less stable conformation which after undergoing CAL-B catalysed hydrolysis flips back into its stable conformation. Here, it was decided to develop a proof of principle showing the conformational flip in a molecule and its utility in making of biological probes.

3.2 Synthesis of a benzanilide based probe with an application of CAL-B

After developing an important method for the CAL-B catalysed transesterification reactions to prepare *N*-acylated anilines, it was decided to study the hydrolytic reaction of CAL-B applied to some applications based on the discussion in Section 2.6.

Initially it was studied that the *N*-unsubstituted diarylureas and benzanilides exhibit a distinct difference in three-dimensional shape or conformation.[247-251] For instance, it has been reported that upon *N*-methylation, benzanilide undergoes an N-CO bond rotation resulting in the preferred cis-conformation predominating,[249, 252] whereas *N,N'*-diphenylurea changes from the trans, trans-conformation to the *cis*-conformation upon *N,N'*-dimethylation, as shown in Scheme 3.1.[247, 251]



Scheme 3.1 Conformational change induced in aromatic amides (top) and diarylureas (bottom) upon *N*-methylation.

Such conformational controls by substitution on nitrogen has been studied in number of applications such as for modifying the activity of synthetic retinoids,[253] the synthesis of anticancer agents[254] and for the development of fluorescent sensors to identify alkylating agents.[228] All these studies indicate that the addition of a substituent on the nitrogen can control the conformation of the resulting N-CO bond where the *N*-substituted product adopts a *cis*-orientation of their aromatic rings (Scheme 3.1). On the basis of this evidence it was decided to synthesize a *N*-substituted benzanilide molecule which upon removal of any enzyme cleavable group attached to the nitrogen atom there is a ring flip back to its *trans* conformation. It was envisaged that the introduction of an enzyme-cleavable group on the benzanilide nitrogen would enable us to exploit its potential in the development of diagnostic probes to detect disease-specific enzymes. For this, it was decided to take CAL-B as a model enzyme for the hydrolytic removal of the *N*-substituent.

3.2.1 Probe designing

(a) CAL-B active site geometry

All lipases are able to hydrolyse fatty acid esters of C4 to C16 carbon atoms, and some may accommodate even up to C22 carbons.[80] In the inactive state, the active

site of lipases is covered by a lid made of two α-helices. When the substrate approaches, this lid moves making the active site ready (Figure 3.6).

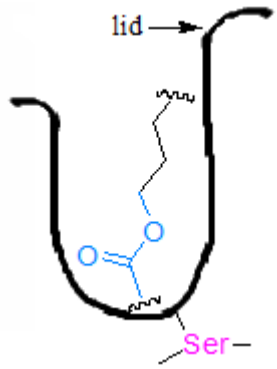


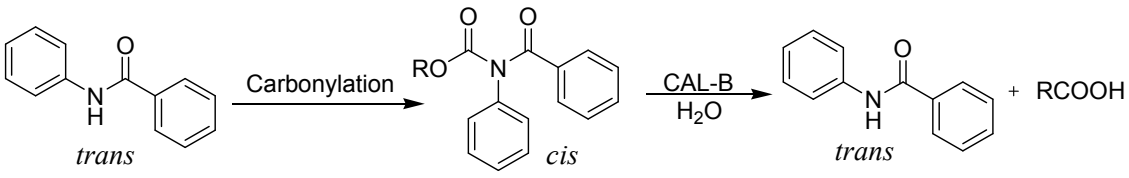
Figure 3.6 Shape of CAL-B binding site (front view).[80]

This funnel shaped binding site is ~9.5 x 4.5 Å with left side wall up to 6 Å high. The lid comprising right side wall is of ~10.5 Å.[80]

Due to the above parameters it was decided to synthesise a long ester chain as the model enzyme cleavable group in order to see of it could be used to control the conformation in benzanilide.[80, 255]

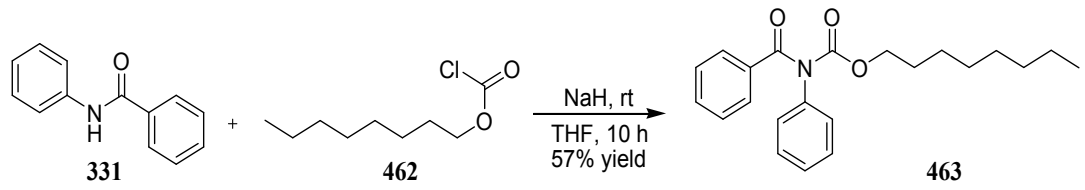
(b) Probe conformation

It was decided to use benzanilide as a model system to study the conformational flip from the unstable to the stable form by utilizing enzymatic hydrolysis. To lock the benzanilide into its unstable *cis* conformation,[249, 252] it was decided to carbonylate the



Scheme 3.2 Enzyme-catalysed conformational change of benzanilide.

nitrogen which after undergoing CAL-B catalysed hydrolysis, would revert it back into the more stable *trans* conformation (Scheme 3.2). For this, a reaction of benzanilide (**331**) and octylchloroformate (**462**) was performed to get the carbamate product (**463**) in which benzanilide was locked into a *cis*-conformation (Scheme 3.3, Procedure 3.1).

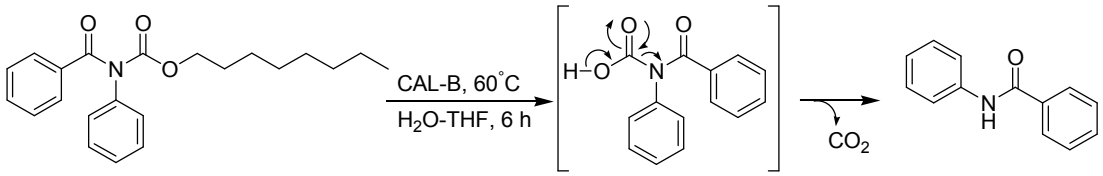


331 462 463

Scheme 3.3 Introduction of a potential CAL-B cleavable group on benzanilide **331**.

3.2.2 Hydrolysis of the substrate

It was presumed that the substrate (**463**) locked in the *cis*-conformation will revert back to the *trans* conformation (**331**) after enzymatic hydrolysis (Scheme 3.4).



463 464 331

Scheme 3.4 CAL-B catalysed hydrolysis of the substrate.

To test this hypothesis, a hydrolysis reaction of the substrate (**463**) was carried out in water- tetrahydrofuran in the presence of CAL-B (100 wt. %), and monitored by HPLC with aliquots taken at every hour for 7 hours (Scheme 3.4, Procedure 3.2). In the hydrolytic reaction, the ester group of **463** is hydrolysed releasing the unstable carbamic acid derivative (**464**) which subsequently releases carbon dioxide and releases the benzanilide (**331**) in its *trans* configuration. The results obtained from HPLC (Table 3.1, Figure 3.7) showed that the substrate undergoes hydrolysis, and

Entry 1	Time (h)	Substrate (%)	Benzanilide (%)
0	0	100	0
1	1	80	16
2	2	68	29
3	3	55	41
4	4	54	43
5	5	39	57
6	6	14	81

Table 3.1 Percentage of substrate and benzanilide after hydrolysis.

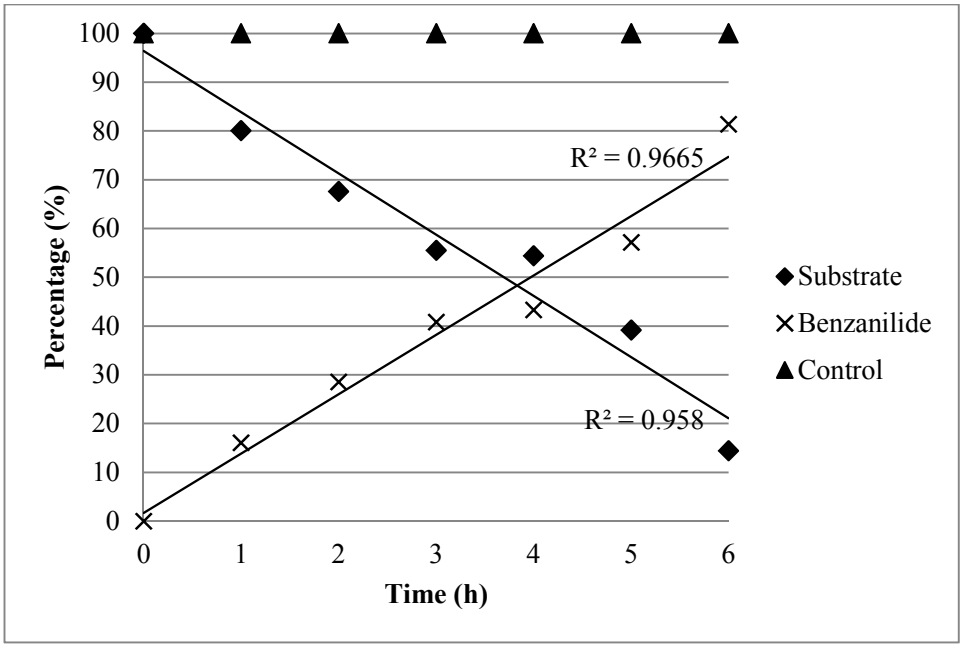
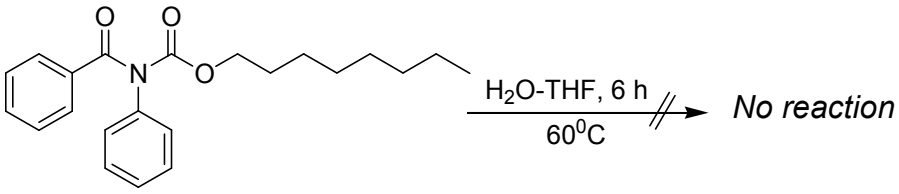


Figure 3.7 Percentage of substrate and benzanilide after 6 hours.

after 6 hours the percentage of the benzanilide (**331**) reached 81%. Initially after 3 hours, the conversion of benzanilide was 41% which after 6 hours gradually increased to 81% (Table 3.1). The graph demonstrates that the concentration of the substrate gradually reduced to 14% after 6 hours (Figure 3.7). To further ensure that the reaction was being enzyme-catalysed, a background reaction (Scheme 3.5, Procedure 3.3) was also carried out with the substrate and solvents but without CAL-B. The reaction gave negligible amount of the benzanilide product, indicating that CAL-B is hydrolysing the substrate and not the water alone.



463

Scheme 3.5 Attempted hydrolysis of the substrate in water and tetrahydrofuran.

Obtaining the above results, it was decided to perform the hydrolysis reaction of the substrate (**463**) at various concentrations of CAL-B from 25 to 100 wt. % for 6 hours (Table 3.2, Procedure 3.4). to determine the minimum amount of CAL-B ‘‘detectable’’ by the probe.

Entry 1	CAL-B (wt. %)	Substrate (%)	Benzanilide (%)
1	10	79	21
2	25	68	32
3	50	65	35
4	75	64	36
5	100	29	71

Table 3.2 Percentage of substrate and benzanilide after 6 hours at different wt.% of CAL-B.

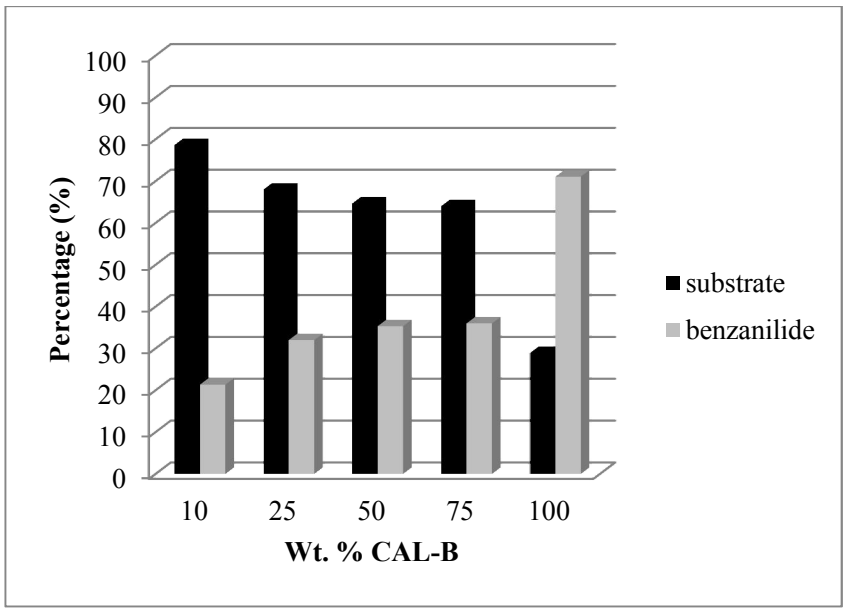


Figure 3.8 Percentage of substrate and benzanilide at different wt. % of CAL-B after 6 hours.

The graph plotted from Table 3.2 (Figure 3.8) demonstrates that the substrate undergoes hydrolysis and forms benzanilide even at lower concentrations of the enzyme. There is not much difference in the amount of benzanilide obtained when the amount of CAL-B was 50 and 75 wt. % (35 and 36% respectively). After 6 hours the amount of hydrolysis product benzanilide was 71% when CAL-B used was 100 wt. %.

The results obtained suggest that a benzanilide based system, comprising an enzyme sensitive group and fluorescence groups which work on Forster resonance energy

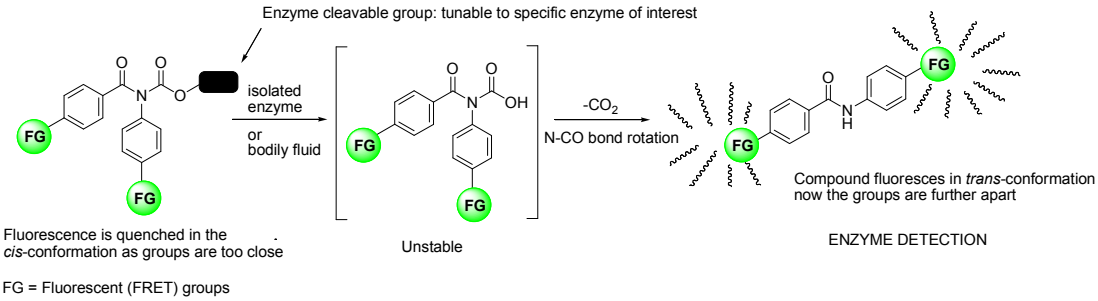


Figure 3.9 Designing of a benzanilide based probe.

transfer (FRET) could be designed as a probe for enzymes involved in various clinical conditions. In probe (**465**) the benzanilide system is locked in a *cis* conformation where the fluorescence groups are close to each other and therefore, the Forster resonance energy transfer will be low causing quenching of the fluorescence (Figure 3.9). But as the system undergoes reaction with the enzyme (**466**) and releases the benzanilide in the *trans* conformation (**467**), the fluorescence groups being distant will fluoresce and therefore the over-expressed enzyme could be easily detected. The results also demonstrate that the substrate undergoes hydrolysis and forms benzanilide even at lower concentrations (10 wt %) of the enzyme.

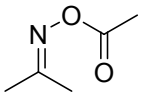
CHAPTER 4- EXPERIMENTAL

Chapter 4:

EXPERIMENTAL

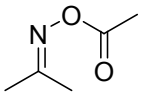
Commercially available reagents were used as received without purification. Analytical thin layer chromatography (TLC) was performed with plastic-backed TLC plates coated with silica G/UV_{254}, in a variety of solvents. The plates were visualised by UV light (254 nm). Flash column chromatography was conducted with Davisil silica 60Å (40-63 μm) under bellows pressure. Low resolution mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX using electron spray ionisation (ESI). High resolution mass spectra were obtained from EPSRC national mass spectrometry facility, Swansea University. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 250 (250 MHz) and 300 (300 MHz) spectrometer. All chemical shifts (δ) are quoted in parts per million (ppm) relative to a calibration reference of the residual protic solvent; $CHCl_3$ (δ_H 7.26, s) or DMSO (δ_H 2.54, s) was used as the internal standard in ^{1}H NMR spectra, and ^{13}C NMR shifts were referenced using $CDCl_3$ (δ_C 77.16, t) with broad band decoupling and the *J* values are measured in Hertz. Infrared spectra were recorded using a Perkin-Elmer 881 infrared spectrometer. Melting points were measured using a Stuart SMP20 apparatus and are uncorrected.

2.1 Procedure for the synthesis of activated ester 231 using CAL-B enzyme[5]



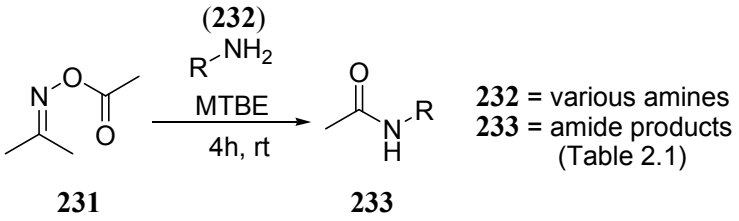
To a solution of vinyl acetate **229** (0.36ml, 3.96mmoles) in MTBE (10ml) was added acetone oxime **230** (0.14g, 1.98mmoles), CAL-B (1.5g, 0.00263g/mol of ester) and 4 Å molecular sieves (1g). The resulting suspension was stirred at room temperature for 4 hours. After which, it was filtered and the solvent was evaporated. The obtained activated ester (**231**) was purified by flash chromatography to yield the pure activated ester as colourless liquid (0.12g, 56%); R_f (50% EtOAc/Petrol): 0.53.^{1}H NMR ($CDCl_3$, 250MHz): δ_H 1.97(s, 3H), 2.02 (s, 3H), 2.13 (s, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ_C 16.83, 19.49, 21.84, 163.61, 168.58; MS (ESI^+, *m*/*z*): 115 ($[M]^+$, 100%); IR (neat, cm^{-1}): ν_{max} 876 (alkyl stretching), 1754 (C=O stretching).

2.2 General procedure for the synthesis of activated ester 231 using acid chloride 234[5]



To a solution of acetone oxime **230** (5.1g, 70mmoles) in DCM (50ml) was added pyridine (5.6ml, 70mmoles) and acetyl chloride **234** (5ml, 70mmoles). The resulting solution was stirred in an ice bath for 6 hours. After which, it was filtered and evaporated. The crude reaction product was purified by flash chromatography to yield the activated ester (**231**) as colourless liquid (4.02g, 50%). For characterization see Procedure **2.1**.

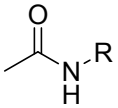
2.3 General procedure for the synthesis of amides - Control 1 (Reaction of activated ester with amine)



231 **233**

To a solution of activated ester **231** (0.2g, 1.73mmoles) in MTBE (0.1M) was added amine **232** (1.73mmoles) and 4Å molecular sieves (1g). The resulting solution was stirred at room temperature for 4 hours. After which, it was filtered and evaporated. The crude *N*-acylated product (**233**) was purified by flash chromatography to yield the pure amide.

2.4 General procedure for the synthesis of amides (One-pot synthesis[c] of amide with *in-situ* generation of activated ester)

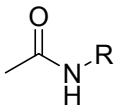


233 = amide products (See Table 2.1)

To a solution of vinyl acetate **229** (0.36ml, 3.96mmoles) in MTBE (0.1M) was added acetone oxime **230** (0.14g, 1.98mmoles), CAL-B (1.5g, 0.00263g/mol of ester), amine **232** (1.98mmoles) and 4Å molecular sieves (1g). The resulting suspension was stirred at room temperature for 4 hours. After which, it was filtered and evaporated.

The crude *N*-acylated product (**233**) was purified by flash chromatography to yield the pure amide.

2.5 General procedure for the synthesis of amides - Control 2 (CAL-B catalysed amidation)



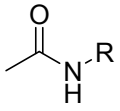
233 = amide products (See Table 2.1)

To a solution of vinyl acetate **229** (0.36ml, 3.96mmoles) in MTBE (0.1M) was added amine **232** (1.98mmoles), CAL-B (1.5g, 0.00263g/mol of ester) and 4Å molecular sieves (1g). The resulting suspension was stirred at room temperature for 4 hours. After which, it was filtered and evaporated. The crude *N*-acylated product (**233**) was purified by flash chromatography to yield the pure amide.

2.6 General procedure for the synthesis of amides (Reaction of non-activated ester with amine)

To a solution of vinyl acetate **229** (0.36ml, 3.96 mmoles) in MTBE (0.1M) was added amine **232** (1.98mmoles) and 4Å molecular sieves (1g). The resulting solution was stirred at room temperature for 4 hours. After which, it was filtered and evaporated. The absence of *N*-acylated product (**233**) was confirmed by the ^{1}H NMR of the crude reaction mixture.

2.7 General procedure for the synthesis of amides with catalytic amount of acetone oxime (One-pot synthesis of amide with *in-situ* generation of activated ester using catalytic amount of acetone oxime)



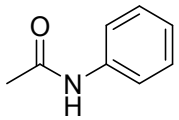
233 = amide products (See Table 2.1)

To a solution of vinyl acetate **229** (0.36ml, 3.96mmoles) in MTBE (0.1M) was added acetone oxime **230** (0.014g, 0.198mmoles), CAL-B (1.5g, 0.00263g/mol of ester), amine **232** (1.98mmoles) and 4Å molecular sieves (1g). The resulting suspension was stirred at room temperature for 4 hours. After which, it was filtered and evaporated.

The crude *N*-acylated product (**233**) was purified by flash chromatography to yield the pure amide.

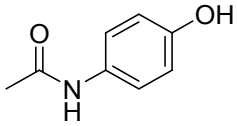
Characterizations of *N*-acylated products obtained from the one-pot method (Control 3; Procedure 2.4):

***N*-phenylacetamide, 236** [6]



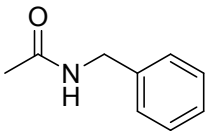
White crystals (0.12g, 84%, eluted at 50% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.27. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.18 (s, 3H), 7.10 (t, 1H, *J*=7.5Hz), 7.32 (t, 2H, *J*=7.5Hz), 7.50 (d, 2H, *J*=6.0Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 24.49, 120.19, 124.32, 128.95, 138.10, 169.21; MS (ESI$^+$, *m*/*z*): 135 ([M]$^+$, 34%), 93 (100); IR (neat, cm^{-1}): ν_{max} 1556 (C=O stretching), 1698 (NH bending), 3136, 3546 (Ar ring), 3614 (NH stretching); M.p.92-94°C (EtOAc/Petrol).

***N*-(4-hydroxyphenyl)acetamide, 238** [256]



Off white crystals (0.14g, 48%, eluted at 60% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.20. ^{1}H NMR (DMSO, 300MHz): δ_H 2.01 (s, 3H), 6.70 (d, 2H, *J*=9.0Hz), 7.34 (d, 2H, *J*=9.0Hz); ^{13}C NMR (DMSO, 75MHz): δ_C 23.79, 115.50, 121.33, 131.39, 153.66, 168.26; MS (ESI$^+$, *m*/*z*): 151 ([M]$^+$, 33%), 109 (100); IR (neat, cm^{-1}): ν_{max} 1656 C=O stretching), (1640 (NH bending), 3100 (Ar ring); M.p.166-168°C (EtOAc/Petrol).

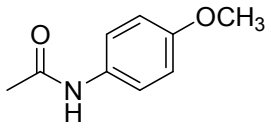
***N*-benzylacetamide, 246** [257]



Off white crystals (0.18g, 62%, eluted at 60% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.21. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.03 (s, 3H), 4.43 (d, 2H, *J*=6.0Hz), 7.28-7.34

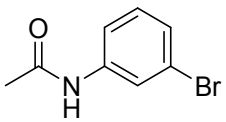
(m, 5H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 23.07, 43.57, 127.38, 127.74, 128.61, 138.34, 170.35; MS (ESI$^+$, *m*/*z*):149 ($[M]^+$, 82%), 106 (99), 91 (30); IR (neat, cm^{-1}): ν_{max} 1646 (C=O stretching), 1545 (NH bending), 2926, 3289 (Ar ring); M.p.55-57°C (EtOAc/Petrol).

_N_-(4-methoxyphenyl)acetamide, 250 [174]



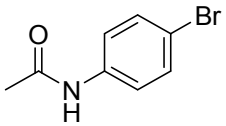
Off white crystals (0.22g, 69%, eluted at 40% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.15. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.16 (s, 3H), 3.79 (s, 3H), 6.85 (d, 2H, *J*=9.0Hz), 7.38 (d, 2H, *J*=9.0Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 24.35, 55.57, 114.03, 122.08, 131.09, 156.49, 168.73; MS (ESI$^+$, *m*/*z*): 164.91 ($[M-H]^+$, 34%), 107.82 (99), 122.89 (65); IR (neat, cm^{-1}): ν_{max} 1509(C=O stretching), 1556, 1604, 1644 (NH bending); M.p.138-140°C (EtOAc/Petrol).

_N_-(3-bromophenyl)acetamide, 256 [258]



Off white crystals (0.19g, 45%, eluted at 25% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.28. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.18 (s, 3H), 7.15-7.22 (m, 3H), 7.4 (d, 1H, *J*=6Hz), 7.76 (s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 24.70, 118.45, 122.74, 122.94, 127.43, 130.40, 139.29, 168.70; MS (ESI$^+$, *m*/*z*): 213 ($[M-H]^+$, 24%), 215 ($[M+H]^+$, 23%), 171 (99), 173 (93), 92 (56); IR (neat, cm^{-1}): ν_{max} 1662 (C=O stretching), 1589 (NH bending), 3292 (NH stretching); M.p. 84-86°C (EtOAc/Petrol).

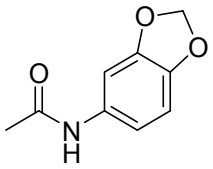
_N_-(4-bromophenyl)acetamide, 258 [259]



Brown crystals (0.20g, 48%, eluted at 20% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.24. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.17 (s, 3H), 7.47 (s, 4H); ^{13}C NMR ($CDCl_3$,

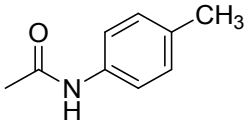
75 MHz): δ_C 24.73, 117.13, 121.72, 132.10, 137.10, 168.47; MS (ESI$^+$, *m*/*z*): 215 ([M+H]$^+$, 25%), 171 (99), 173 (92); IR (neat, cm^{-1}): ν_{max} 1586 (C=O stretching), 1598 (NH bending), 3400 (NH stretching); M.p.166-168°C (EtOAc/Petrol).

_N_-(benzo[*d*][1,3]dioxol-5-yl)acetamide, 260 [175]



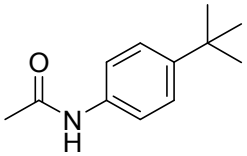
Brown crystals (0.22g, 64%, eluted at 50% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.26. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.14 (s, 3H), 5.94 (s, 2H), 6.71-6.78 (m, 2H), 7.20 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 24.38, 101.32, 103.14, 108.31, 113.42, 132.44, 144.31, 147.99, 168.89; MS (ESI$^+$, *m*/*z*): 179 ([M]$^+$, 48%), 137 (99), 136 (30); IR (neat, cm^{-1}): ν_{max} 1543 (C=O stretching),1635 (NH bending), 3307 (NH stretching); M.p.140-142°C (EtOAc/Petrol).

_N_-*p*-tolylacetamide, 262 [259]



Brown crystals (0.26g, 90%, eluted at 40% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.33. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.16 (s, 3H), 2.30 (s, 3H), 7.10 (d, 2H, *J*=9.0 Hz), 7.37 (d, 2H, *J*=9.0Hz); ^{13}C NMR ($CDCl_3$, 75MHz): 20.9, 24.55, 120.2, 129.5, 134.0, 135.5, 168.7; MS (ESI$^+$, *m*/*z*): 149 ([M]$^+$, 50%), 106 (98), 107 (100); IR (neat, cm^{-1}): ν_{max} 1660 (C=O stretching), 1548 (NH bending), 3289 (NH stretching); M.p.141-143°C (EtOAc/Petrol).

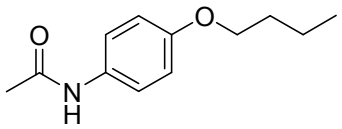
_N_-(4-*tert*-butylphenyl)acetamide, 264 [260]



Brown crystals (0.15g, 41%, eluted at 35% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.28. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 1.30 (s, 9H), 2.17 (s, 3H), 7.32-7.42 (m, 4H);

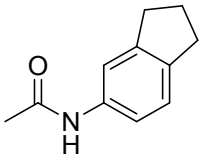
^{13}C NMR ($CDCl_3$, 75MHz): δ_C 24.49, 31.44, 34.33, 120.01, 125.80, 135.43, 147.27, 169.00; MS (ESI$^+$, *m*/*z*): 191 ([M]$^+$, 27%), 134 (100), 176 (66); IR (neat, cm^{-1}): ν_{max} 1664 (C=O stretching), 1541(NH bending), 3251 (NH stretching), 1375 (*tert*-butyl); M.p.181-183°C (EtOAc/Petrol).

_N_-(4-(butyloxy)phenyl)acetamide, 266 [167]



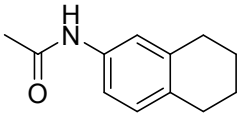
White crystals (0.17g, 41% eluted at 30% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.20. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 0.96 (t, 3H, *J*=7.5Hz), 1.44-1.54 (m, 2H), 1.70-1.79 (m, 2H), 2.15 (s, 3H), 3.93 (t, 2H, *J*=6.0Hz), 6.84 (d, 2H, *J*=9.0Hz), 7.08 (s, 1H), 7.36 (d, 2H, *J*=9.0Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 13.93, 19.29, 24.23, 31.37, 67.97, 114.68, 122.10, 131.01, 156.06, 168.74; MS (ESI$^+$, *m*/*z*): 207 ([M]$^+$, 24%), 109 (100); IR (neat, cm^{-1}): ν_{max} 1657 (C=O stretching), 1658 (NH bending), 3305 (NH stretching), 1368 (alkyl chain); M.p.119-121°C (EtOAc/Petrol).

_N_-(2, 3-dihydro-1H-inden-5-yl)acetamide, 268 [175]



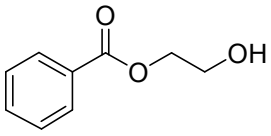
Light yellow crystals (0.22g, 65%, eluted at 30% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.32. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.01-2.11 (m, 2H), 2.16 (s, 3H), 2.83-2.91 (q, 4H, *J*=9.0Hz), 7.14 (s, 2H), 7.44 (s, 1H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 23.92, 25.67, 32.19, 32.81, 116.81, 118.50, 124.09, 136.26, 139.89, 144.82, 169.34; MS (ESI$^+$, *m*/*z*): 175 ([M]$^+$, 46%), 176 ([M+H]$^+$, 55%), 132 (100); IR (neat, cm^{-1}): ν_{max} 1656 (C=O stretching), 1538 (NH bending), 3276 (NH stretching); M.p.113-115°C (EtOAc/Petrol).

_N_-(5,6,7,8-tetrahydronaphthalen-2-yl)acetamide, 270 [261]



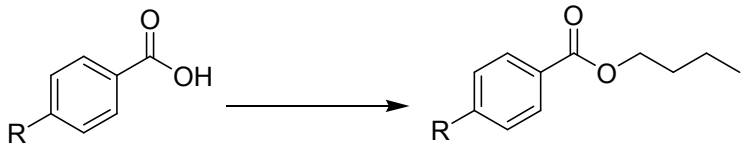
Light brown crystals (0.2g, 54%, eluted at 25% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.31. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 1.79 (s, 4H), 2.16 (s, 3H), 2.73 (s, 4H), 7.00 (d, 1H, *J*=9.0Hz), 7.19 (d, 1H, *J*=9.0Hz), 7.29 (s, 1H, *J*=7.3Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 23.1, 23.3, 24.3, 28.9, 29.5, 117.9, 120.9, 129.3, 133.2, 135.4, 137.6, 169.1; MS (ESI$^+$, *m/z*): 189 ($[M]^+$, 60%), 147 (100), 119 (83); IR (neat, cm^{-1}): ν_{max} 1654 (C=O stretching), 1537 (NH bending), 3244 (NH stretching); M.p.109-111°C (EtOAc/Petrol).

2.8 Procedure for the synthesis of glycol ester 297 from methyl benzoate (295) and ethylene glycol (296) [101]



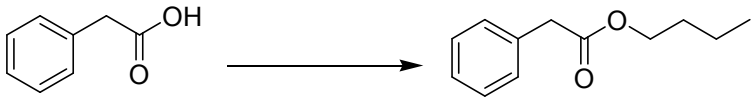
To a solution of methyl benzoate (0.25ml, 1.98mmoles) in MTBE (10ml) was added ethylene glycol (0.22ml, 3.96mmoles) and CAL-B (1.5g). The resulting suspension was stirred at 60°C for 8 hours. After which, the solvent was evaporated and the crude mixture was purified by flash chromatography to yield the pure glycol ester as colourless oil (0.25g, 80%, eluted at 70% EtOAc/Petrol), R_f (35% EtOAc/Petrol): 0.20. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.05 (s, 1H), 3.96 (t, 2H, *J*=6.0Hz), 4.46 (t, 2H, *J*=6.0Hz), 7.44 (t, 2H, *J*=6.0Hz), 7.57 (t, 1H, *J*=6.0Hz), 8.05 (d, 2H, *J*=9.0Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 61.20, 66.65, 128.45, 129.72, 129.85, 133.24, 167.07; MS (ESI$^+$, *m/z*): 123 $[(M-C_2H_5O+2H)^+$, 55%), 105 $[(M-C_2H_5O_2)^+$, 100%); IR (neat, cm^{-1}): ν_{max} 1715 (C=O stretching), 3424 (OH stretching).

2.9 General procedure for the synthesis of butanoate esters



R = CH_3 (**307**), OCH_3 (**308**), NH_2 (**309**), Br (**310**), NO_2 (**311**)

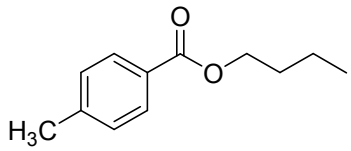
R = CH_3 (**313**), OCH_3 (**314**), NH_2 (**315**), Br (**316**), NO_2 (**317**)



318 **319**

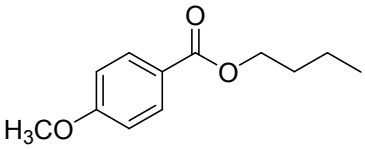
To a solution of the acid (5g) in 1-butanol (2M) was added concentrated sulfuric acid (6 drops). The resulting suspension was stirred at room temperature for 12 hours. After which, sat. aq. $NaHCO_3$ (50ml) was added and the product was extracted with ethyl acetate (30ml) and washed with brine (30ml). The organic layer was dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The crude product was purified by flash chromatography.

Butyl 4-methylbenzoate, 313 [262]



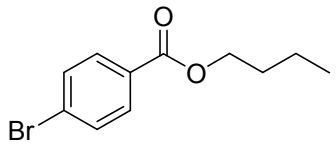
Colourless oil (2.4g, 34%, eluted at 50% EtOAc/Petrol), R_f (35% EtOAc/Petrol): 0.92. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 0.97 (t, 3H, *J*=7.9Hz), 1.47 (tq, 2H, *J*=7.4Hz), 1.74 (quin, 2H, *J*=6.0Hz), 2.39 (s, 3H), 4.30 (t, 2H, *J*=6.5Hz), 7.22 (d, 2H, *J*=8.0Hz), 7.93 (d, 2H, *J*=8.0Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 13.85, 19.36, 21.70, 30.86, 64.69, 127.82, 129.07, 129.61, 143.45, 166.81; MS (ESI$^+$, *m*/*z*): 192 ([M]$^+$, 100%); IR (neat, cm^{-1}): ν_{max} 690 (Ar ring), 1432 (chain CH_2 bending), 1714 (C=O stretching), 2959 (Ar ring).

Butyl 4-methoxybenzoate, 314 [263]



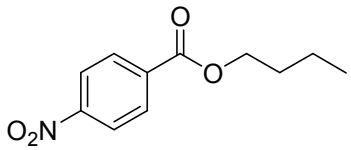
Colourless oil (2.4g, 35%, eluted at 50% EtOAc/Petrol), R_f (10% EtOAc/Petrol): 0.81. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 0.95 (t, 3H, *J*=7.3Hz), 1.38-1.50 (m, 2H), 1.66-1.76 (m, 2H), 3.81 (s, 3H), 4.26 (t, 2H, *J*=6.57Hz), 6.8 (d, 2H, *J*=8.8Hz), 7.97 (d, 2H, *J*=8.8Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 13.82, 19.33, 30.86, 55.39, 64.58, 113.55, 122.95, 131.53, 163.25, 166.43; MS (ESI$^+$, *m*/*z*): 208 ([M]$^+$, 7%), 152 [M+H$^+$- C_4H_9, 100%); IR (neat, cm^{-1}): ν_{max} 696 (Ar ring), 1103 (C-O stretching), 1461 (chain CH_2 bending), 1708 (C=O stretching), 2958 (Ar ring).

Butyl 4-bromobenzoate, 316 [264]



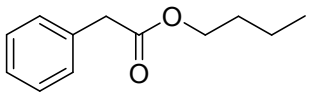
Colourless oil (5.3g, 84%, eluted at 50% EtOAc/Petrol), R_f (10% EtOAc/Petrol): 0.84. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 0.97 (t, 3H, *J*=7.3Hz), 1.46 (tq, 2H, *J*=6.2Hz), 1.74 (quin, 2H, *J*=7.5Hz), 7.56 (d, 2H, *J*=8.5Hz), 7.89 (d, 2H, *J*=8.5Hz). ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 13.89, 19.38, 30.82, 65.24, 128.01, 129.49, 131.19, 131.77, 166.06. MS (ESI$^+$, *m*/*z*): 202 [($M^{81}Br$)+H^+- C_4H_9, 73%], 200 [($M^{79}Br$)+H^+- C_4H_9, 69%], 185 [($M^{81}Br$)+H^+- C_4H_9, 90%], 183 [($M^{79}Br$)+H^+- C_4H_9, 100%]; IR (neat, cm^{-1}): ν_{max} 707 (C-Br),1717 (C=O stretching).

Butyl 4-nitrobenzoate, 317 [264]



Yellow solid (4.9g, 76%, eluted at 50% EtOAc/Petrol), R_f (35% EtOAc/Petrol): 0.83. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 0.97 (t, 3H, *J*=7.4Hz), 1.46 (tq, 2H, *J*=6.2Hz), 1.76 (quin, 2H, *J*=5.6Hz), 4.36 (t, 2H, *J*=6.6Hz), 8.22 (d, 2H, *J*=9.0Hz), 8.22 (d, 2H, *J*=9.0Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 14.50, 19.31, 30.70, 65.89, 123.59, 130.73, 135.93, 150.51, 164.82; MS (ESI$^+$, *m*/*z*): 150 [$(M-C_4H_9O)^+$, 100%], 168 (43); IR (neat, cm^{-1}): ν_{max} 712 (Ar ring), 1273 (N=O sym stretch), 1467 (chain CH_2 bending), 1711 (C=O stretching); M.p. 36-38°C (EtOAc/Petrol).

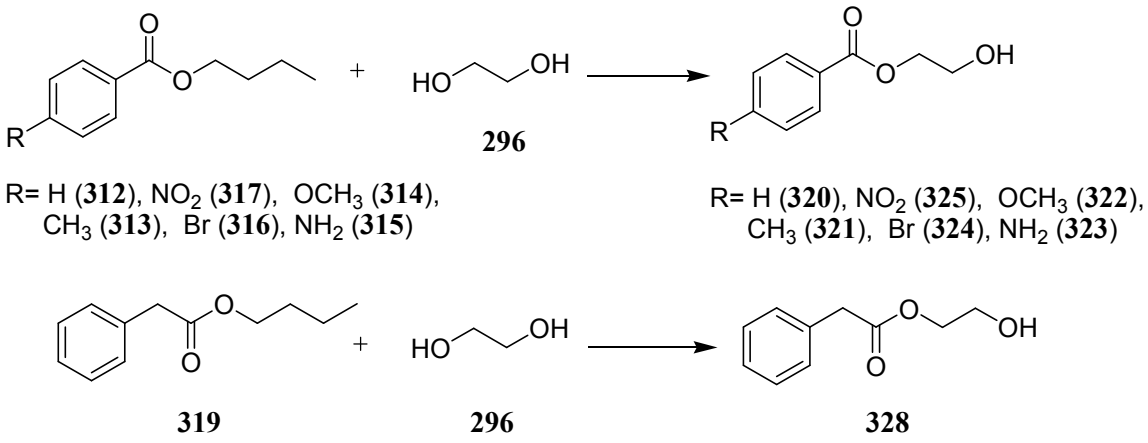
Butyl 2-phenylacetate, 319 [265]



Colourless oil (6.1g, 87%, eluted at 50% EtOAc/Petrol), R_f (35% EtOAc/Petrol): 0.65. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 0.90 (t, 3H, *J*=8.1Hz), 1.34 (tq, 2H, *J*=6.2Hz), 1.59 (quin, 2H, *J*=5.6Hz), 3.61 (s, 2H), 4.09 (t, 2H, *J*=6.1Hz), 7.29-7.35 (m, 5H); ^{13}C

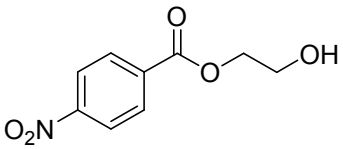
NMR ($CDCl_3$, 75MHz): δ_C 13.77, 19.15, 30.67, 41.53, 64.82, 127.08, 128.59, 129.31, 134.26, 171.76; MS (ESI$^+$, *m/z*): 192 ([M]$^+$, 100%); IR (neat, cm^{-1}): ν_{max} 696 (Ar ring), 1455 (chain CH_2 bending), 1732 (C=O stretching), 2959 (Ar ring).

2.10 General procedure for the synthesis of glycol esters from butanoate esters and ethylene glycol



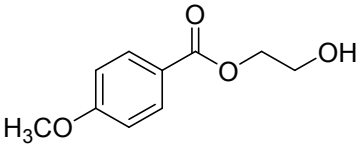
To a solution of butanoate ester (1.98mmoles) in MTBE (0.1M) was added ethylene glycol (1.98 or 3.96mmoles) and CAL-B (0.5g, 0.0039g/mol of ester). The resulting suspension was stirred at 60°C for 8 hours. After which, the enzyme was filtered off and the solvent was evaporated. The crude mixture was purified by flash chromatography to yield the pure glycol ester.

2-Hydroxyethyl 4-nitrobenzoate, 325 [266]



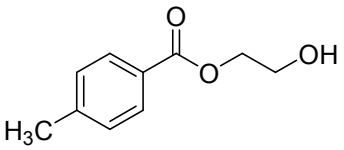
White crystals (0.13g, 32%, eluted at 25% EtOAc/Petrol), R_f (35% EtOAc/Petrol): 0.12. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.27 (br s, 1H), 3.98 (s, 2H), 4.49 (s, 2H), 8.19-8.28 (m, 4H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 61.04, 67.42, 123.64, 130.90, 135.33, 150.62, 165.07; MS (ESI$^+$, *m/z*): 211 ([M]$^+$, 11%); 168 [(M-C_2H_5O+ 2H)$^+$, 100%], 150 [(M-$C_2H_5O_2$)$^+$, 74%]; IR (neat, cm^{-1}): ν_{max} 713 (Ar ring),1270 (N=O sym stretch), 1715 (C=O stretching), 2952 (O-H stretching); M.p.79-81°C (EtOAc/Petrol).

2-Hydroxyethyl 4-methoxybenzoate, 322 [101]



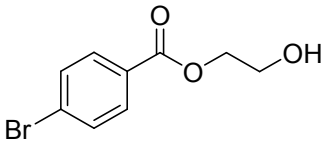
Colourless oil (0.11g, 29%, eluted at 90% EtOAc/Petrol), R_f (35% EtOAc/Petrol): 0.16. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 3.83 (s, 3H), 3.91 (t, 2H, *J*=6.0Hz), 4.40 (t, 2H, *J*=6.0Hz), 6.88 (d, 2H, *J*=9.0Hz), 7.98 (d, 2H, *J*=9.0Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 54.3, 60.2, 65.5, 114.1, 123.2, 128.8, 165.1, 169.2; MS (ESI$^+$, *m*/*z*): 196 ([M] $^+$, 10%), 135 [(M-C_2H_5O+2H)$^+$, 100%]; IR (neat, cm^{-1}): ν_{max} 1421 (chain CH_2 bending), 1709 (C=O stretching), 3409 (O-H stretching).

2-Hydroxyethyl 4-methylbenzoate, 321 [101]



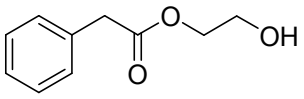
Colourless oil (0.12g, 33%, eluted at 45% EtOAc/Petrol), R_f (35% EtOAc/Petrol): 0.3. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.41 (s, 3H), 3.94 (t, 2H, *J*=6.0Hz), 4.44 (t, 2H, *J*=6.0Hz), 7.23 (d, 2H, *J*=9.0Hz), 7.94 (d, 2H, *J*=9.0Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 21.87, 61.58, 66.66, 127.15, 129.24, 129.82, 144.05, 167.18; MS (ESI$^+$, *m*/*z*): 181 [(M+H)$^+$, 100%]; IR (neat, cm^{-1}): ν_{max} 1710 (C=O stretching), 3054 (O-H stretching).

2-Hydroxyethyl 4-bromobenzoate, **324** [101]



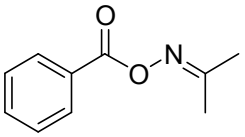
Yellow oil (0.09g, 19%, eluted at 45% EtOAc/Petrol), R_f (35% EtOAc/Petrol): 0.3. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.76 (br s, 1H), 3.93 (t, 2H, *J*=4.4Hz), 4.42 (t, 2H, *J*=4.4Hz), 7.54 (d, 2H, *J*=8.7Hz), 7.87 (d, 2H, *J*=8.7Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 61.19, 66.87, 128.42, 128.74, 131.26, 131.82, 166.31; MS (ESI$^+$, *m*/*z*): 203 [(M^{81}Br-C_2H_5O+2H)$^+$, 40%], 201 [(M^{79}Br-C_2H_5O+2H)$^+$, 40%]; IR (neat, cm^{-1}): ν_{max}1715 (C=O stretching), 2951(O-H stretching).

2-Hydroxyethyl 2-phenylacetate, 328 [267]



Colourless oil (0.05g, 14%, eluted at 40% EtOAc/Petrol), R_f (35% EtOAc/Petrol): 0.18. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.04 (s, 1H), 3.66 (s, 2H), 3.77 (t, 2H, *J*=6.0Hz), 4.20 (t, 2H, *J*=6.0Hz), 7.24-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 41.26, 61.09, 66.51, 127.29, 128.71, 129.30, 133.84, 172.09; MS (ESI$^+$, *m*/*z*): 180 ([M] $^+$, 100%); IR (neat, cm^{-1}): ν_{max} 730 (Ar ring), 1453 (CH_2 bending), 1725 (C=O stretching), 2953 (O-H stretching).

2.11 Procedure for the synthesis of the activated ester 368 from vinyl benzoate (375) [268]



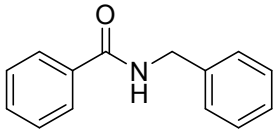
To a solution of vinyl benzoate (0.54ml, 3.96mmoles) in methyl-*tert*-butyl ether (15 ml) was added acetone oxime (0.14g, 1.98mmoles), CAL-B (0.5g) and 4Å molecular sieves. The resulting solution was stirred at 50°C for 8 hours, after which, the solvent was evaporated and the crude mixture was purified by flash chromatography to yield the pure activated ester as colourless oil (0.33g, 94%); R_f (35% EtOAc/Petrol): 0.34. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.01 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 7.47-8.10. (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 16.79, 21.78, 128.13, 130.11, 131.23, 133.10, 162.20, 166.65; MS (ESI$^+$, m/z): 177 ([M]$^+$, 100%); IR (neat, cm^{-1}): ν_{max} 1750 (C=O stretching), 3025 (Ar ring).

2.12 Procedure for the synthesis of the activated ester 368 using acid chloride 384

To a solution of benzoic acid (2.2g, 14.68mmoles) in dichloromethane (60ml) was added DMF (6 drops) and oxalyl chloride (1.24ml, 14.68mmoles). The resulting solution was stirred at room temperature for 12 hours. After which, 4-dimethylaminopyridine (0.17g, 1.46mmoles), acetone oxime (1.06g, 14.68mmoles) and triethylamine (3.06ml, 22mmoles) were added. The resulting mixture was stirred at room temperature for 8 hours. The solvent was evaporated and the crude mixture

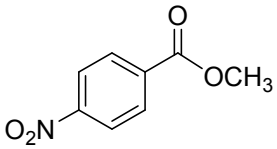
purified by flash chromatography to yield the pure activated ester as a colourless oil (2.3g, 92%). (Characterization of the isolated product was identical to activated ester, **368** prepared above).

2.13 Procedure for the synthesis of *N*-benzylbenzamide 385 from activated ester (368) [269]



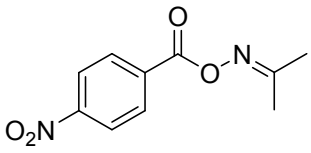
To a solution of activated ester **368** (0.2g, 1.12mmoles) in MTBE (10ml) was added benzylamine (0.12ml, 1.12mmoles) and 4Å molecular sieves. The resulting solution was stirred at 50°C for 8 hours, after which, the solvent was evaporated and the crude mixture was purified by flash chromatography to yield the pure amide product as a white solid (0.21g, 91%); R_f (40% EtOAc/Petrol): 0.35. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 4.53 (d, 2H, *J*=5.7Hz), 6.73 (s, 1H), 7.19-7.44 (m, 8H), 7.72 (d, 2H, *J*=8.2Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 44.01, 127.08, 127.49, 127.82, 128.54, 128.71, 131.52, 134.33, 138.32, 167.56; MS (ESI$^+$, *m*/*z*): 211 ($[M]^+$, 62%), 212 ($[M+H]^+$, 9%); IR (neat, cm^{-1}): ν_{max} 1635 (C=O stretching), 3285 (NH stretching); M.p.100-102°C (EtOAc/Petrol).

2.14 Preparation of methyl 4-nitrobenzoate, 386 [270]



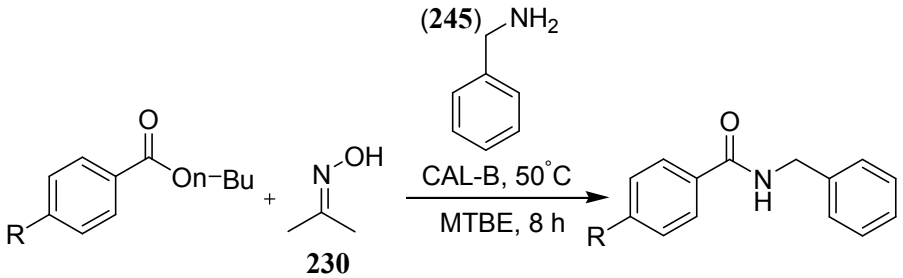
To a solution of 4-nitrobenzoic acid (1.6g, 10mmoles) in methanol (20ml) was added concentrated H_2SO_4 (6-7 drops). The resulting suspension was stirred at room temperature for 7 hours, after which, the solvent was evaporated. The crude ester was used as obtained as a colourless solid (1.64g, 91%); R_f (45% EtOAc/Petrol): 0.36. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H, 3.99 (s, 3H), 8.22 (d, 3H, *J*= 9.0Hz), 8.29 (d, 2H, *J*= 9.0Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 52.8, 123.5, 130.7, 135.4, 150.5, 165.3; MS (ESI$^+$, m/z): 181 ($[M]^+$, 90%), 164 (50); IR (neat, cm^{-1}): ν_{max} 1358, 1530 (NO_2 stretching), 1719 (C=O stretching), 2940 (Ar ring)); M.p.94-96°C (EtOAc/Petrol).

2.15 Procedure for the synthesis of activated ester 387 using 4-nitrobenzoic acid (311)



To a solution of 4-nitrobenzoic acid (2g, 12 mmoles) in dichloromethane (60ml) was added dimethylformamide (6 drops) and oxalyl chloride (1.01ml, 12mmoles). The resulting solution was stirred at room temperature for 12 hours. After which, added 4-dimethylaminopyridine (0.14g, 1.2mmoles), acetone oxime (0.87g, 12mmoles) and triethylamine (2.5ml, 18mmoles) were added. The resulting mixture was stirred at room temperature for 8 hours. The solvent was evaporated and the crude mixture purified by flash chromatography to yield the pure activated ester as yellow solid (2.04g, 77%); R_f (50% EtOAc/Petrol): 0.55. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.12 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 8.24 (d, 2H, *J*=8.6Hz), 8.24 (d, 2H, *J*=8.6Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 17.35 (CH_3), 22.10 (CH_3), 123.73 (CH), 130.71 (CH), 134.74, 150.62, 162.12, 165.80; MS (ESI$^+$, *m*/*z*): 222.9 ($[M]^+$, 100%), 195 (55) HRMS: Found 223.0714 $[M+H]^+$; $C_{10}H_{10}N_2O_4$ requires 223.0713; IR (neat, cm^{-1}): ν_{max} 1280, 1521 (NO_2 stretching), 1739 (C=O stretching), 3072 (Ar ring); M.p.146-148°C (EtOAc/Petrol).

2.16 General procedure for the synthesis of amides - Control 1 (One-pot synthesis of amide with *in-situ* generation of activated ester)



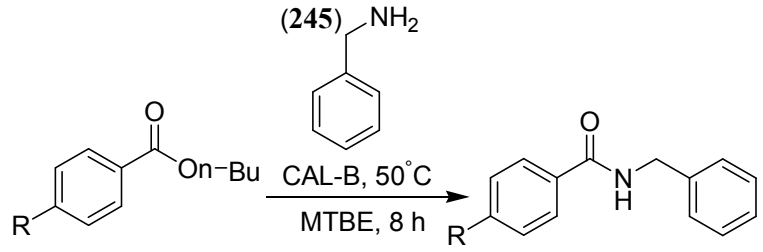
R = H (**312**), Br (**316**), NO_2 (**317**), OCH_3 (**314**), NH_2 (**315**)

R = H (**385**), Br (**390**), NO_2 (**391**), OCH_3 (**392**), NH_2 (**393**)

To a solution of ester (1.98mmoles) in MTBE (0.1M) was added acetone oxime (0.14g, 1.98mmoles), CAL-B (0.5g, 0.00396g/mol of ester), benzylamine (0.21ml, 1.98mmoles) and 4Å molecular sieves (1g). The resulting suspension was stirred at

50°C for 8 hours. After which, it was filtered and evaporated. The crude *N*-acylated product was purified by flash chromatography to yield the pure amide.

2.17 General procedure for the synthesis of amides - Control 2 (CAL-B catalysed amidation)

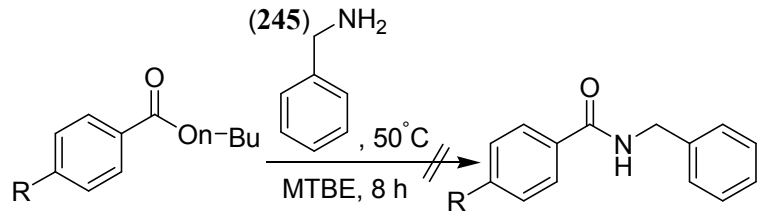


R = H (**312**), Br (**316**), NO_2 (**317**), OCH_3 (**314**), NH_2 (**315**)

R = H (**385**), Br (**390**), NO_2 (**391**), OCH_3 (**392**), NH_2 (**393**)

To a solution of ester (1.98mmoles) in MTBE (0.1M) was added benzylamine (0.21ml, 1.98mmoles), CAL-B (0.5g, 0.00396g/mol of ester) and 4Å molecular sieves (1g). The resulting suspension was stirred at 50°C for 8 hours. After which, it was filtered and evaporated. The crude *N*-acylated product was purified by flash chromatography to yield the pure amide.

2.18 General procedure for the synthesis of amides - Control 3 (Reaction of non-activated ester with amine)



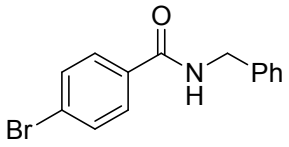
R = H (**312**), Br (**316**), NO_2 (**317**), OCH_3 (**314**), NH_2 (**315**)

R = H (**385**), Br (**390**), NO_2 (**391**), OCH_3 (**392**), NH_2 (**393**)

To a solution of ester (1.98mmoles) in MTBE (0.1M) was added benzylamine (0.21ml, 1.98mmoles) and 4 Å molecular sieves (1g). The resulting solution was stirred at 50°C for 8 hours. After which, it was filtered and evaporated. The absence of *N*-acylated product was confirmed by the ^{1}H NMR of the crude reaction mixture.

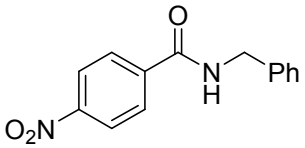
Characterizations of *N*-acylated products obtained from the one-pot method (Control 1; Procedure 2.16):

_N_-benzyl-4-bromobenzamide, 390 [271]



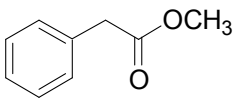
White solid (0.17g, 30%, eluted at 35% EtOAc/Petrol); R_f (35% EtOAc/Petrol): 0.45. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 4.61 (d, 2H, *J*=5.4Hz), 6.50 (s, 1H), 7.29-7.38 (m, 5H), 7.54 (d, 2H, *J*=8.4Hz), 7.64 (d, 2H, *J*=8.4Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 44.33, 126.36, 127.85, 128.05, 128.71, 128.95, 131.92, 133.24, 137.99, 166.51; MS (ESI$^+$, *m*/*z*): 291 ([M+H]$^+$, 45%), 183 (95); IR (neat, cm^{-1}): ν_{max} 699 (Ar ring), 1636 (C=O stretching), 3306 (NH stretching); M.p.120-122°C (EtOAc/Petrol).

_N_-benzyl-4-nitrobenzamide, 391 [272]



White solid (0.21g, 42%, eluted at 20% EtOAc/Petrol); R_f (35% EtOAc/Petrol): 0.30. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 4.62 (d, 2H, *J*=5.6Hz), 6.77 (s, 1H), 7.29-7.36 (m, 5H), 7.92 (d, 2H, *J*=8.8Hz), 8.2 (d, 2H, *J*=8.8Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 44.50, 123.89, 128.00, 128.32, 128.99, 129.11, 137.53, 139.97, 149.63, 165.10; MS (ESI$^+$, *m*/*z*): 256 ([M]$^+$, 100%); IR (neat, cm^{-1}): ν_{max} 622 (Ar ring), 1281 (N=O sym stretch), 3277 (NH stretching); M.p.140-142°C (EtOAc/Petrol).

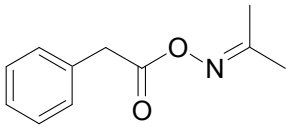
2.19 Preparation of methyl 2-phenylacetate, 394 [273]



To a solution of phenyl acetic acid (5g, 37mmoles) in methanol (20ml) was added concentrated H_2SO_4 (6-7 drops). The resulting suspension was stirred at room temperature for 7 hours, after which, the solvent was evaporated. The crude ester was used as obtained as a colourless oil (5g, 88%); R_f (35% EtOAc/Petrol): 0.33. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 3.62 (s, 2H, CH_2), 3.67 (s, 3H, CH_3), 7.23-7.34 (m, 5H,

Ar-H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 41.17, 51.99, 127.10, 128.58, 129.25, 134.00, 172.00; MS (ESI^+, m/z): 150 ($[M]^+$, 46%), 91 (100); IR (neat, cm^{-1}): ν_{max} 1434 (CH_2 bending), 1734 (C=O stretching), 2952 (Ar ring).

2.20 Procedure for the synthesis of activated ester 395 using acid chloride from 318 [274]

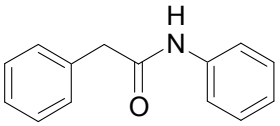


To a solution of phenyl acetic acid (2g, 14.68mmoles) in dichloromethane (60ml) was added dimethylformamide (6 drops) and oxalyl chloride (1.24ml, 14.68mmoles). The resulting solution was stirred at room temperature for 12 hours. After which, 4-dimethylaminopyridine (0.17g, 1.46mmoles), acetone oxime (1.06g, 14.6 mmoles) and triethylamine (3.06ml, 22mmoles) were added. The resulting mixture was stirred at room temperature for 8 hours. The solvent was evaporated and the crude mixture purified by flash chromatography to yield the pure activated ester as colourless oil (2.6g, 94%); R_f (50% EtOAc/Petrol): 0.52. ^1H NMR ($CDCl_3$, 300MHz): δ_H 1.78 (s, 3H, CH_3), 1.89 (s, 3H, CH_3), 3.61 (s, 2H, CH_2), 7.21-7.27 (m, 5H, Ar-H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 16.76 (CH_3), 21.75 (CH_3), 40.14 (CH_2), 127.03 (CH_2), 128.45 (CH_2), 129.11 (CH_2), 133.37 (CH), 164.24 (CH), 168.65; MS (ESI^+, *m/z*): 191 ($[M]^+$, 60%); IR (neat, cm^{-1}): ν_{max} 1753 (C=O stretching), 1431 (CH_2 bending), 3031 (Ar ring).

2.21 General procedure for the synthesis of amides from anilines, benzylamine and *N*-methyl benzylamine

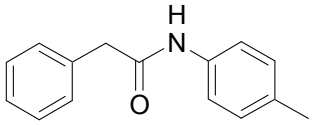
To a solution of the oxime ester of methyl 2-phenylacetate **395** (1eq.) in MTBE (1M) was added the amine (1eq.). The resulting solution was stirred at 50°C for 8 hours. After which, the suspension was evaporated and the crude *N*-acylated product was purified by flash chromatography (silica gel) to yield the pure amide.

2,*N*-Diphenyl-acetamide, 397 [275]



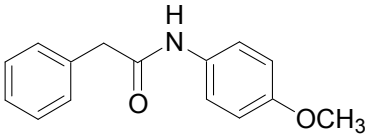
White crystals (0.17g, 81%, eluted at 35% EtOAc/Petrol), R_f (40% EtOAc/Petrol): 0.52. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 3.75 (s, 2H), 7.02 (br s, 1H), 7.08 (t, 2H, *J*=6.9Hz), 7.28-7.41 (m, 8H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 45.05, 119.89, 124.61, 127.88, 129.09, 129.43, 129.71, 134.52, 137.68, 169.14; MS (ESI$^+$, *m*/*z*): 212 ([M+H]$^+$, 22%), 93 (90); IR (neat, cm^{-1}): ν_{max} 1439 (CH_2 bending), 1655 (C=O stretching), 3254 (NH stretching), 3635 (Ar ring); M.p.120-122°C (EtOAc/Petrol).

2-Phenyl-*N*-p-tolyl-acetamide, 408 [276]



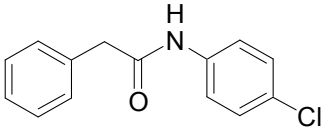
White crystals (0.22g, 100%, eluted at 50% EtOAc/Petrol), R_f (40% EtOAc/Petrol): 0.51. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 2.28 (s, 3H), 3.73 (s, 2H), 6.99 (br s, 1H), 7.07 (d, 2H, *J*=8.2Hz), 7.25-7.42 (m, 7H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 20.95, 44.72, 120.10, 127.60, 129.19, 129.46, 129.57, 134.12, 134.69, 135.20, 169.28; MS (ESI$^+$, *m*/*z*): 225 ([M] $^+$, 100%); IR (neat, cm^{-1}): ν_{max} 1651 (C=O stretching), 3288 (NH stretching); M.p.134-136°C (EtOAc/Petrol).

***N*-(4-Methoxy-phenyl)-2-phenyl-acetamide, 414** [277]



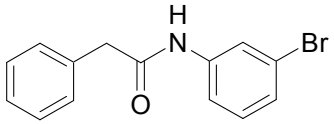
White solid (0.25g, 100%, eluted at 50% EtOAc/Petrol), R_f (35% EtOAc/Petrol): 0.37. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 3.73 (s, 2H), 3.76 (s, 3H), 6.81 (d, 2H, *J*=8.9Hz), 6.95 (br s, 1H), 7.29-7.43 (m, 7H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 44.61, 55.50, 114.08, 121.96, 127.60, 129.19, 129.58, 130.85, 134.75, 156.54, 169.27; MS (ESI$^+$, *m*/*z*): 241 ([M] $^+$, 60%), 242 (10); IR (neat, cm^{-1}): ν_{max} 1508 (C=O stretching), 3287 (NH stretching); M.p.125-127°C (eluted at 50% EtOAc/Petrol).

***N*-(4-Chloro-phenyl)-2-phenyl-acetamide, 416** [277]



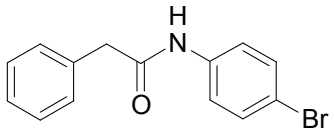
White solid (0.23g, 92%, eluted at 25% EtOAc/Petrol), R_f (35% EtOAc/Petrol): 0.67. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 3.74 (s, 2H), 7.02 (br s, 1H), 7.23 (d, 1H, *J*=8.9Hz), 7.31-7.44 (m, 8H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 44.96, 121.12, 127.99, 129.08, 129.50, 129.56, 129.69, 134.23, 136.24, 169.27; MS (ESI$^+$, *m*/*z*): ^{35}Cl ($[M]^+_{245}$, 20%), ^{37}Cl ($[M]^+_{245+2}$, 7%); IR (neat, cm^{-1}): ν_{max} 1522 (C=O stretching), 3274 (NH stretching), 3027 (Ar ring); M.p.162-164°C (EtOAc/Petrol).

_N_-(3-Bromo-phenyl)-2-phenyl-acetamide, 418 [278]



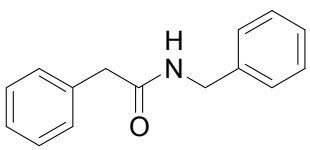
White solid (0.12g, 40%, eluted at 28% EtOAc/Petrol), R_f (40% EtOAc/Petrol): 0.46. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 3.74 (s, 2H), 6.99 (br s, 1H), 7.10-7.44 (m, 9H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 44.53, 118.58, 122.52, 123.00, 127.42, 127.66, 129.16, 129.45, 130.23, 134.26, 139.05, 169.84; MS (ESI$^+$, *m*/*z*): ^{79}Br ($[M]^+_{289}$, 54%), ^{81}Br ($[M]^+_{289+2}$, 43%); IR (neat, cm^{-1}): ν_{max} 1472 (CH_2 bending), 1653 (C=O stretching), 3256 (NH stretching), 3029 (Ar ring); M.p.108-110°C (EtOAc/Petrol).

_N_-(4-Bromo-phenyl)-2-phenyl-acetamide, 420 [279]



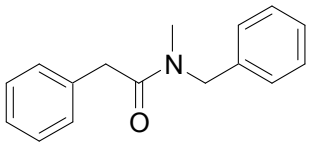
White crystals (0.25g, 83%, eluted at 45% EtOAc/Petrol), R_f (40% EtOAc/Petrol): 0.52. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 3.73 (s, 2H), 7.02 (br s, 1H), 7.29-7.43 (m, 9H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 44.87, 117.13, 121.48, 127.90, 129.40, 129.62, 131.98, 134.23, 136.79, 169.28; MS (ESI$^+$, *m*/*z*): ^{79}Br ($[M]^+_{289}$, 18%), ^{81}Br ($[M]^+_{289+2}$, 16%); IR (neat, cm^{-1}): ν_{max} 1487 (CH_2 bending), 1658 (C=O stretching), 3274 (NH stretching), 3026 (Ar ring); M.p.184-186°C (EtOAc/Petrol).

_N_-Benzyl-2-phenyl-acetamide, 422 [275]



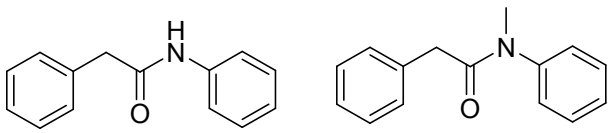
White crystals (0.23g, 100%, eluted at 40% EtOAc/Petrol), R_f (50% EtOAc/Petrol): 0.41. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 3.63 (s, 2H), 4.41 (d, 2H, *J*=5.7Hz), 5.67 (br s, 1H) 7.16-7.37 (m, 10H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 43.47, 43.99, 127.40, 127.57, 127.60, 128.80, 129.22, 129.61, 134.84, 138.22, 170.97; MS (ESI$^+$, *m*/*z*): 225 ([M]$^+$, 34%); IR (neat, cm^{-1}): ν_{max} 1636 (C=O stretching), 3030 (Ar ring), 3284 NH stretching); M.p.118-120°C (EtOAc/Petrol).

_N_-Benzyl-_N_-methyl-2-phenyl-acetamide, 423 [277]



White crystals (0.03g, 13%, eluted at 38% EtOAc/Petrol), R_f (38% EtOAc/Petrol): 0.70. ^{1}H NMR ($CDCl_3$, 300MHz): major rotamer: δ_H 2.92 (s, 3H), 3.80 (s, 2H), 4.63 (s, 2H) 7.27-7.32 (m, 10H); minor rotamer: δ 2.97 (s, 3H), 3.77 (s, 2H), 4.54 (s, 2H), 7.11 (d, 2H, *J*=6.9Hz), 7.27-7.32 (m, 8H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 34.06, 35.26, 40.94, 41.28, 51.01, 53.70, 126.43, 126.86, 126.90, 127.42, 127.71, 128.12, 128.63, 128.76, 128.84, 128.89, 128.98, 135.01, 135.15, 136.53, 137.34, 171.20, 171.54; MS (ESI$^+$, *m*/*z*): 239 ([M]$^+$, 100%); IR (neat, cm^{-1}): ν_{max} 1639 (C=O stretching), 3027 (Ar ring), 2922 (NH stretching); M.p. 66-68°C (EtOAc/Petrol).

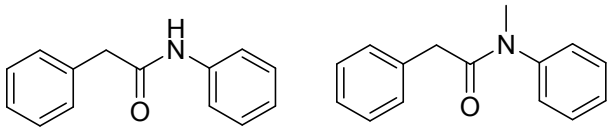
2.22 Competition reaction of aniline with *N*-methyl aniline using the activated ester of methyl 2-phenylacetate, 395



397 **407**

To a solution of oxime ester **395**, (0.2g, 1.04 mmoles) in MTBE (1 ml) was added *N*-methyl aniline (0.11ml, 1.04mmoles) and aniline (0.09ml, 1.04mmoles) pre-dissolved separately in MTBE. The resulting solution was stirred at 50°C for 8 hours, after which the suspension was evaporated and the crude *N*-acylated product was purified by flash chromatography to yield the pure amide **(397)** in 76% yield. (Characterization of the isolated product was identical to 2,*N*-diphenyl-acetamide, **397** prepared above). None of the *N*-methylated amide **(407)** was obtained in the crude NMR or isolated by column chromatography.

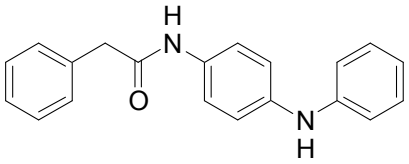
2.23 Competition reaction between aniline and *N*-methyl aniline with the corresponding acid chloride, 398



397 **407**

To a solution of phenyl acetic acid (2g, 14.68mmoles) in dichloromethane (60ml) was added dimethylformamide (6 drops) and oxalyl chloride (1.24ml, 14.68mmoles). The resulting solution was stirred at room temperature for 12 hours and 4-dimethylaminopyridine (0.17g, 1.46mmoles), aniline (0.67ml, 7.34mmoles) and *N*-Methyl aniline (0.80ml, 7.34mmoles) (both amines were pre-dissolved separately in dichloromethane) and triethylamine (3.06ml, 22mmoles) were added. The resulting mixture was stirred at room temperature for 8 hours. The solvent was evaporated and the crude mixture was purified by flash chromatography (eluted at 45% EtOAc/Petrol) to yield the mixture of pure amides as a dark green solid (2.18g, 58% of **397** and 42% **407**; calculated from the pure NMR of the mixture. R_f (35% EtOAc/Petrol): 0.65. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 3.32 (s, 3H), 3.52 (s, 2H), 3.66 (s, 2H), 7.12-7.55 (m, 20H), 8.43 (br s, NH); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 37.54, 40.78, 44.10, 119.97, 123.93, 126.54, 126.96, 127.38, 127.95, 128.23, 128.62, 129.21, 129.66, 134.99, 135.05, 135.19, 138.19, 138.25; MS (ESI$^+$, m/z): 211 ([M]$^+$, 100%), 147 (45); IR (neat, cm^{-1}): ν_{max} 1651, 1596 (C=O stretching), 3253 (NH stretching), 3059, 3024 (Ar ring); M.p.140-142°C (EtOAc/Petrol).

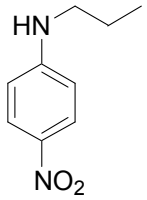
2.24 Preparation of 2-phenyl-*N*-(4-phenylamino-phenyl)-acetamide, 435



To a solution of the oxime ester (**395**) (0.2g, 1.04mmoles) in MTBE (1ml) was added *N*-phenyl-*p*-phenylenediamine (0.19g, 1.04mmoles). The resulting solution was stirred at 50°C for 8 hours. After which the solvent was evaporated and the crude product was purified by flash chromatography (eluted at 58% EtOAc/Petrol) to yield the pure amide as white crystals (0.29g, 92%, R_f (60% EtOAc/Petrol): 0.62. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 3.64 (s, 2H), 5.54 (br s, 1H), 6.79 (t, 1H, *J*=8.0Hz), 6.89 (d,

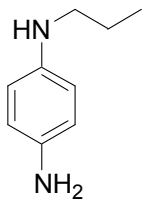
4H, *J*=6.7Hz), 7.11-7.33 (m, 9H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 44.66 (CH_2), 117.20 (CH), 118.85 (CH), 120.75 (CH), 121.75 (CH), 127.70 (CH), 129.28 (CH), 129.44 (CH), 129.64 (CH), 131.28 (CH), 134.68 (CH), 139.80 (CH), 143.50 (CH), 169.24 (C=O); MS (ESI$^+$, *m*/*z*): 301 ([M]$^+$, 34%), 191 (100); IR (neat, cm^{-1}): ν_{max} 1517 (C=O stretching), 3245 (NH stretching), 3096 (Ar ring); M.p.137-139°C (EtOAc/Petrol).

2.25 Preparation of (4-nitro-phenyl)-propyl-amine, 444 [280]



To a solution of 1-fluoro-4-nitrobenzene (0.22g, 2.08mmoles) in DMSO (20ml) was added propylamine (0.68g, 2.08mmoles) and potassium carbonate (0.71g, 5.2 mmoles). The resulting mixture was stirred at 60°C for 3.5 hours. After which, 1M HCl (50ml) was added and the product was extracted with ethyl acetate (30ml) and washed with brine (30ml). The organic layer was dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The crude product was purified by flash chromatography (eluted at 20% EtOAc/Petrol) to yield the pure product as a yellow solid (0.29g, 78%), R_f (35% EtOAc/Petrol): 0.53. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 0.97 (t, 3H, *J*=6.8Hz), 1.64 (br s, 2H, *J*=6.8Hz), 3.12 (br s, 2H), 4.87 (br s, 1H), 6.49 (d, 2H, *J*=8.8Hz), 8.02 (d, 2H, *J*=8.8Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 11.45, 22.25, 45.07, 110.87, 126.46, 137.21, 253.81; MS (ESI$^+$, *m*/*z*): 180 ([M]$^+$, 30%), 151 (100); IR (neat, cm^{-1}): ν_{max} 1285, 1600 (NO_2 stretching), 3336 (NH stretching), 2959 (Ar ring); M.p.63-65°C (EtOAc/Petrol).

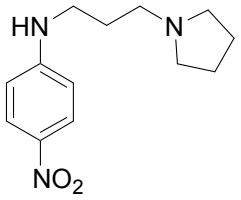
2.26 Preparation of *N*-propyl-benzene-1,4-diamine, 445 [281]



To a solution of (4-nitro-phenyl)-propyl-amine (0.20g, 1.10mmoles) in methanol (11 ml) was added 10% Pd-C (0.02g). The resulting suspension was stirred under a balloon of hydrogen for 5 hours. After which, the reaction mixture was filtered and

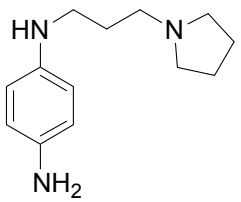
the solvent was evaporated. The crude product was purified by flash chromatography (eluted at 35% EtOAc/Petrol) to yield the pure product as sticky dark pink oil (0.15g, 90%), R_f (50% EtOAc/Petrol): 0.11. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 0.98 (t, 3H, *J*=7.4Hz), 1.55-1.67 (m, 2H), 3.01 (t, 2H, *J*=7.1Hz), 3.24 (br s, 3H), 6.50-6.62 (m, 4H); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 11.82 (CH_3), 22.99 (CH_2), 47.16 (CH_2), 114.63 (CH), 117.06 (CH), 137.64, 141.88; MS (ESI$^+$, *m*/*z*): 150 ([M] $^+$, 50%), 121 (100) HRMS: Found 151.1226 $[M+H]^+$; $C_9H_{14}N_2$ requires 151.1230; IR (neat, cm^{-1}): ν_{max} 3329 (NH stretching), 2870 (Ar ring), 819 (alkyl chain).

2.27 Preparation of (4-nitro-phenyl)-(3-pyrrolidin-1-yl-propyl)-amine, 448 [282]



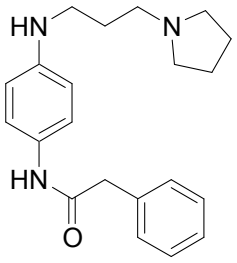
To a solution of 1-fluoro-4-nitrobenzene (0.57g, 5.45mmoles) in DMSO (20ml) was added 1-(3-aminopropyl) pyrrolidine (0.7g, 5.45mmoles) and potassium carbonate (1.8g, 13.62mmoles). The resulting mixture was stirred at 60°C for 10 hours. After which, 1M HCl (50ml) was added and the product was extracted with ethyl acetate (30ml) and washed with brine (30ml). The organic layer was dried ($MgSO_4$) and the solvent was evaporated *in vacuo*. The crude product was purified by flash chromatography (eluted at 90% MeOH/DCM) to yield the pure product as a yellow solid (0.54g, 40%). R_f (35% EtOAc/Petrol): 0.10. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 1.8 (m, 6H), 2.53 (m, 4H), 2.65 (t, 2H, *J*=6.0Hz), 3.29 (d, 2H, *J*=4.7Hz), 6.44 (d, 2H, *J*=8.37Hz), 6.50 (br s, 1H), 8.06 (d, 2H, *J*=8.37Hz); ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 23.69, 26.79, 43.73, 54.24, 55.19, 110.75, 110.85, 126.65, 154.07; MS (ESI$^+$, *m*/*z*): 250 ($[M+H]^+$, 90%), 169 (70); IR (neat, cm^{-1}): ν_{max} 1281, 1593 (NO_2 stretching), 3235 (NH stretching), 2811 (Ar ring); M.p.61-63°C.

2.28 Preparation of *N*-(3-pyrrolidin-1-yl-propyl)-benzene-1,4-diamine, 449 [282]



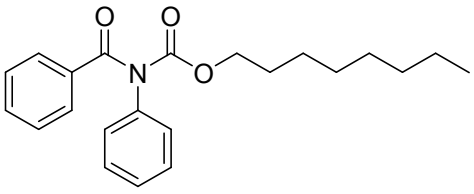
To a solution of (4-nitro-phenyl)-(3-pyrrolidin-1-yl-propyl)-amine (0.54g, 2.17 mmoles) in methanol (22ml) was added 10% Pd-C (0.054g). The resulting suspension was stirred under a balloon of hydrogen for 5 hours. After which, the reaction mixture was filtered and the solvent was evaporated. The crude product was purified by flash chromatography (eluted in acetone) to yield the pure product as sticky brown oil (0.16g, 34%). R_f (35% EtOAc/Petrol): 0.10. ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 23.23, 28.31, 43.98, 53.99, 54.54, 114.27, 116.69, 137.38, 141.57; MS (ESI$^+$, *m*/*z*): 220 ([M+H]$^+$, 100%); IR (neat, cm^{-1}): ν_{max} 3320 (NH stretching), 1045 (alkyl chain).

2.29 Preparation of 2-phenyl-*N*-[4-(3-pyrrolidin-1-yl-propylamino)-phenyl]-acetamide, 450



To a solution of the oxime ester (**395**) (0.03g, 0.20 mmoles) in MTBE (1ml) was added *N*-(3-pyrrolidin-1-yl-propyl)-benzene-1,4-diamine, **449** (0.22g, 0.10mmoles). The resulting solution was stirred at 50°C for 8 hours. After which, the solvent was evaporated and a violet sticky oil was obtained. Yield calculated from the NMR of the crude reaction mixture: 71%. MS (ESI$^+$, m/z): 338 ([M+H]$^+$, 100%).

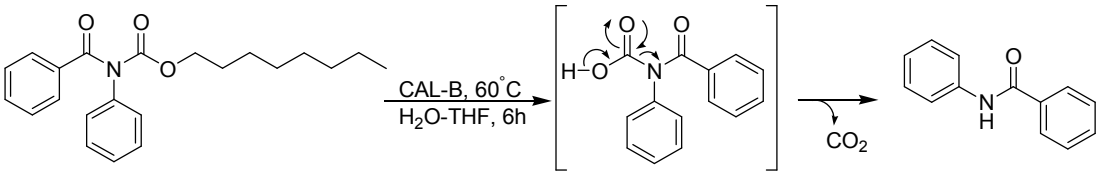
3.1 Preparation of octyl benzoyl(phenyl)carbamate, 463



To a solution of benzanilide (1g, 5.08 mmoles) in THF (50ml) was added sodium hydride (60% in oil, 0.4g, 10.16mmoles) and octyl chloroformate (1.5ml, 7.6mmoles). The resulting suspension was stirred at room temperature for 10 hours, After which, 1M HCl (50ml) was added and the product was extracted with ethyl

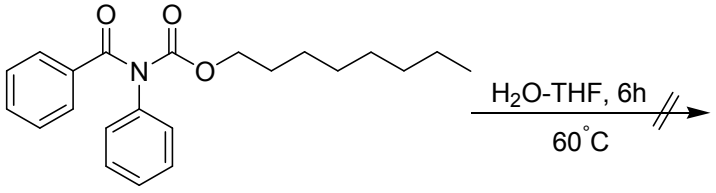
acetate (30ml) and washed with brine (30ml). The organic layer was dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The crude product was purified by flash chromatography (eluted at 20% EtOAc/Petrol) to yield the pure product as colourless oil (0.98g, 57%, eluted at 7% EtOAc/Petrol), R_f (10% EtOAc/Petrol): 0.15. ^{1}H NMR ($CDCl_3$, 300MHz): δ_H 0.86 (t, 3H, *J*=7.2Hz), 1.15-1.39 (m, 12H), 4.02 (t, 2H, *J*=6.5Hz), 7.23-7.50 (m, 8H), 7.71 (d, 2H, *J*=8.1Hz). ^{13}C NMR ($CDCl_3$, 75MHz): δ_C 14.15 (CH_3), 22.66 (CH_2), 25.53 (CH_2), 28.20 (CH_2), 29.08 (CH_2), 31.73 (CH_2), 67.41 (CH_2), 127.98 (CH), 128.26 (CH), 128.35 (CH), 129.26 (CH), 131.97 (CH), 136.10, 138.81, 154.85, 172.36; MS (ESI$^+$, *m*/*z*): 354 ($[M+H]^+$, 25%), 306 (20) HRMS: Found 354.2064 $[M+H]^+$; $C_{22}H_{27}NO_3$ requires 354.2064; IR (neat, cm^{-1}): ν_{max} 1689, 1737 (C=O stretching), 2924 (Ar ring).

3.2 CAL-B catalysed hydrolysis of octyl benzoyl(phenyl)carbamate, 463



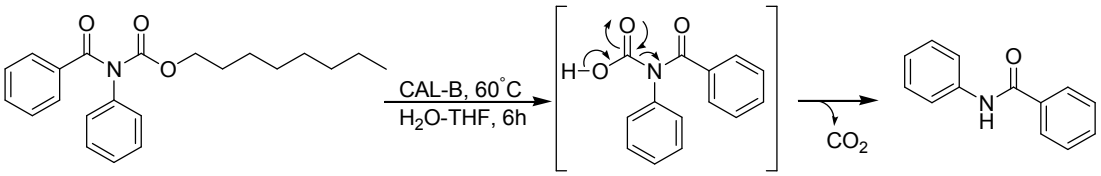
To a solution of octyl benzoyl(phenyl)carbamate (0.053g, 0.15mmoles) in THF (1.24ml) and distilled water (0.76ml) was added CAL-B (0.053g, 100 wt.%). The resulting suspension was stirred at 60°C for 6 hours, after which seven aliquots (100 µl each; t=0 to 6h) were taken from the reaction mixture and analysed using HPLC. The samples were passed through a C-18 column with mobile phase, consisting of 65:35 isopropanol: water. The column was equilibrated at 40°C at a flow rate of 1.0 ml/min. The volume of injection was 100µl. The area % in the chromatogram was taken as the amount of substrate left and the amount of benzanilide product obtained, in the reaction. Also, the chromatogram of benzanilide obtained was similar to the chromatogram of pure benzanilide.

3.3 Background reaction of octyl benzoyl(phenyl)carbamate, 463 with water and tetrahydrofuran



To a solution of octyl benzoyl(phenyl)carbamate (0.053g, 0.15mmoles) in THF (1.24ml) was added distilled water (0.76ml). The resulting solution was stirred at 60°C for 6 hours, after which seven aliquots (100 µl each; t=0 to 6h) were taken from the reaction mixture and analysed using HPLC. The samples were passed through a C-18 column with mobile phase, consisting of 65:35 isopropanol: water. The column was equilibrated at 40°C at a flow rate of 1.0 ml/min. The volume of injection was 100µl.

3.4 CAL-B catalysed hydrolysis of octyl benzoyl(phenyl)carbamate, 463 with varying amounts of the enzyme



Six solutions containing octyl benzoyl(phenyl)carbamate (0.053g, 0.15mmoles) in THF (1.24ml) and distilled water (0.76ml) were prepared. After which, in each sample added 0.053g (100 wt. %), 0.039g (75 wt. %), 0.026g (50 wt. %), 0.0132g (25 wt. %), 0.0053g (10 wt. %) of CAL-B respectively. The resulting suspensions were stirred at 60°C for 6 hours. From each reaction mixture one aliquot (100 µl; t=0 to 6h) was taken and analysed using HPLC. The samples were passed through a C-18 column with mobile phase consisting of 65:35 isopropanol: water. The column was equilibrated at 40°C at a flow rate of 1.0 ml/min. The volume of injection was 100µl. The area % in the chromatogram was taken as the amount of substrate left and the amount of benzanilide product obtained, in the reaction.

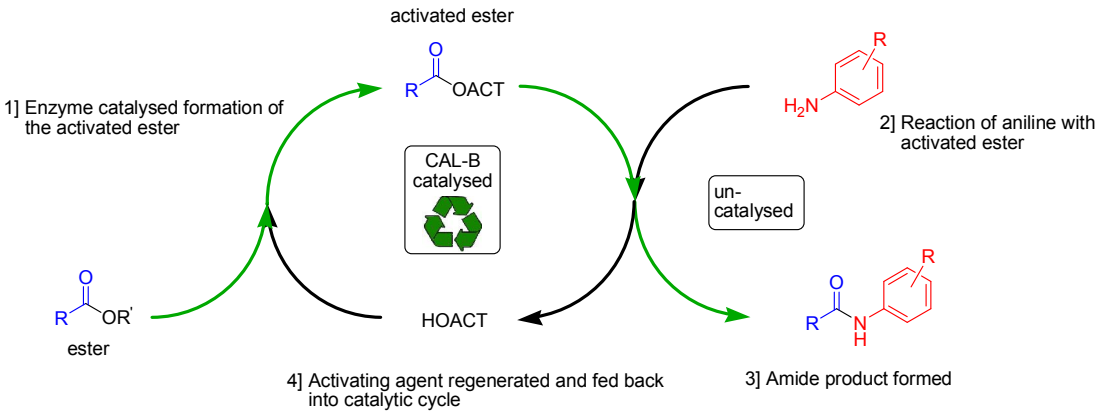
CHAPTER 5- CONCLUSIONS

Chapter 5:

CONCLUSIONS AND FUTURE WORK

5.1 Conclusion

In conclusion, a novel one-pot method for the *N*-acetylation of anilines, in addition to some more bulky amines, has been developed. Instead of using toxic and corrosive chemical reagents, CAL-B (a lipase), and a mild activating agent, were utilized to synthesise amides from their corresponding amines, via a catalytic cycle which generates the active acetylating agent *in situ*.



The results obtained proved the utility of the hypothesised ester activation method in the synthesis of amides, especially those based on anilines, which are notoriously difficult to directly acetylate enzymatically. In addition to vinyl acetate, other esters including methyl benzoate, phenyl benzoate and butyl benzoate were also explored to try and expand the synthetic utility of the reaction for the preparation of amides via the one-pot protocol. Unfortunately, only the vinyl acetate seemed to work in the developed method.

Similarly, to further try and expand the scope of the reaction, other activating agents, including: trifluoroethanol, trichloroethanol and tribromoethanol were also employed with a range of esters, but again, poor results were obtained. Furthermore, a series of small alkyl esters were also studied, in the hope of applying the newly developed method to the synthesis of a known drug (for example, cabozantinib) using the one-pot methodology and acetone oxime. Unfortunately, due to unsuccessful results in these reactions, further study of them was abandoned as well.

Ethylene glycol was employed for the first time in the activation of benzoate esters using CAL-B. The method developed avoided the formation of dimers, demonstrating the practicality of the CAL-B catalysed route in the synthesis of mono-esters of ethylene glycol. To our chagrin, however, the glycol esters were not reactive enough to be used to synthesise amides from anilines, as was shown with acetone oxime.

Success was achieved with the development of a novel chemoselective acylating agent for the synthesis of pharmaceutically relevant amides in good yield. The acylating agent being stable and reactive with a range of anilines could be beneficial over standard agents such as acid chlorides which are corrosive and toxic. Additionally, the acylating agent was also used in the synthesis of some biological compounds. However, the route needs more optimization and standardization.

The work also included the CAL-B catalysed hydrolysis of a specially designed carbamate, to one of the target compounds – benzanilide – which is currently being developed as a novel biological probe.

5.2 Future work

Whilst serious attempts have been made to optimise the method developed, with respect to acyl donor, starting ester and amine, limited success was achieved in places. Further optimisation in order to make the process more industrially applicable, could however, be achieved through the use of an enzyme screen, or directed evolution of CAL-B, in order to find better enzymes to catalyse the formation of the activated species *in* situ; thereby, selectively developing the method to problems with real application. One such target would be to optimize the method towards the synthesis of cabozantinib. For example, the quinoline ether and the anilines used in the synthesis of cabozantinib could be synthesised, and employed in our method, making a potentially scalable and green synthesis of this anticancer agent, but only if the current limitations can be overcome. Nevertheless, the proof-of-principle of the hypothesis has been developed and the project is now primed to take forward, optimise and exploit.

The probe work is currently being further expanded as a diagnostic tool by using fluorescent tagging. Ultimately, the probe will be tested on biological systems to hopefully detect enzymes which are over-expressed as disease.

CHAPTER 6- REFERENCES

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APPENDIX

UK National Crystallography Service

Table 1. Crystal data and structure refinement.

Identification code	**2013ncs0269 / SL4-131-407**	
Empirical formula	$C_{20}H_{18}N_2O$	
Formula weight	302.36	
Temperature	100(2) K	
Wavelength	0.71075 Å	
Crystal system	Orthorhombic	
Space group	*Aba*2	
Unit cell dimensions	a = 15.376(4) Å	α = 90°
	b = 24.615(8) Å	β = 90°
	c = 8.371(3) Å	γ = 90°
Volume	3168.3(17) Å^3	
Z	8	
Density (calculated)	1.268 Mg / m^3	
Absorption coefficient	0.079 mm^{-1}	
F(000)	1280	
Crystal	Plate; colourless	
Crystal size	0.150 × 0.100 × 0.010 mm^3	
θ range for data collection	3.124 – 27.478°	
Index ranges	$-19 \le h \le 16$, $-30 \le k \le 31$, $-10 \le l \le 10$	
Reflections collected	16624	
Independent reflections	3616 [R_{int} = 0.0497]	
Completeness to θ = 25.242°	99.7 %	
Absorption correction	Semi–empirical from equivalents	
Max. and min. transmission	1.000 and 0.829	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3616 / 3 / 217	
Goodness-of-fit on F^2	1.083	
Final R indices [$F^2 > 2\sigma(F^2)$]	*R1* = 0.0632, *wR2* = 0.1579	
R indices (all data)	*R1* = 0.0735, *wR2* = 0.1710	
Absolute structure parameter	–0.1(7)	
Extinction coefficient	0.0106(17)	
Largest diff. peak and hole	0.244 and –0.235 e Å^{-3}	

Diffractometer: *Rigaku AFC12* goniometer equipped with an enhanced sensitivity (HG) *Saturn724+* detector mounted at the window of an *FR-E+ SuperBright* molybdenum rotating anode generator with VHF *Varimax* optics (70μm focus). **Cell determination and data collection**: *CrystalClear-SM Expert 3.1 b25* (Rigaku, 2012). **Data reduction, cell refinement and absorption correction**: *CrystalClear-SM Expert 3.1 b 25* (Rigaku, 2012).
Structure solution: *SHELXS97* (G Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122.).
Structure refinement: *SHELXL-2012* (Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122). **Graphics:** *ORTEP3 for Windows* (L. J. Farrugia, J. Appl. Crystallogr. 1997, 30, 565

Special details:
The two hydrogen atoms boned to nitrogen were restrained to a distance of 0.91(2) Å, All other hydrogen atoms were fixed using a standard riding model.

Table 2. Atomic coordinates [$\times 10^4$], equivalent isotropic displacement parameters [$Å^2 \times 10^3$] and site occupancy factors. U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Atom	*x*	*y*	*z*	U_{eq}	*S.o.f.*
C1	8094(2)	6629(2)	3914(4)	31(1)	1
C2	8486(3)	6234(2)	2735(5)	34(1)	1
C3	8355(2)	5654(2)	3271(5)	32(1)	1
C4	7622(3)	5369(2)	2795(5)	40(1)	1
C5	7477(3)	4844(2)	3356(6)	45(1)	1
C6	8066(3)	4601(2)	4370(5)	43(1)	1
C7	8798(3)	4882(2)	4832(5)	41(1)	1
C8	8944(3)	5406(2)	4291(5)	36(1)	1
C9	6905(2)	7304(2)	4206(4)	32(1)	1
C10	7276(2)	7678(1)	5244(5)	32(1)	1
C11	6769(2)	8075(2)	5963(5)	32(1)	1
C12	5875(2)	8097(2)	5692(5)	33(1)	1
C13	5511(3)	7729(2)	4620(5)	37(1)	1
C14	6024(3)	7342(2)	3883(5)	36(1)	1
C15	5371(2)	8729(2)	7888(5)	32(1)	1
C16	5989(3)	8600(2)	9038(5)	39(1)	1
C17	5976(3)	8861(2)	10506(5)	43(1)	1
C18	5349(3)	9243(2)	10872(5)	41(1)	1
C19	4715(3)	9357(2)	9746(5)	37(1)	1
C20	4728(2)	9108(2)	8272(5)	35(1)	1
N1	7397(2)	6904(1)	3384(4)	32(1)	1
N2	5321(2)	8488(1)	6368(4)	35(1)	1
O1	8399(2)	6682(1)	5274(3)	34(1)	1

Table 3. Bond lengths [Å] and angles [°].

C1–O1	1.238(4)	C11–C12	1.394(5)
C1–N1	1.342(5)	C11–H11	0.9500
C1–C2	1.511(5)	C12–C13	1.394(5)
C2–C3	1.511(5)	C12–N2	1.406(5)
C2–H2A	0.9900	C13–C14	1.381(5)
C2–H2B	0.9900	C13–H13	0.9500
C3–C4	1.386(5)	C14–H14	0.9500
C3–C8	1.387(5)	C15–C16	1.390(6)
C4–C5	1.392(6)	C15–C20	1.397(5)
C4–H4	0.9500	C15–N2	1.405(5)
C5–C6	1.377(6)	C16–C17	1.387(6)
C5–H5	0.9500	C16–H16	0.9500
C6–C7	1.376(6)	C17–C18	1.383(6)
C6–H6	0.9500	C17–H17	0.9500
C7–C8	1.386(6)	C18–C19	1.385(6)
C7–H7	0.9500	C18–H18	0.9500
C8–H8	0.9500	C19–C20	1.377(6)
C9–C14	1.385(5)	C19–H19	0.9500
C9–C10	1.389(5)	C20–H20	0.9500
C9–N1	1.420(5)	N1–H1N	0.91(3)
C10–C11	1.387(5)	N2–H2N	0.93(3)
C10–H10	0.9500		
O1–C1–N1	123.6(3)	C7–C6–C5	119.6(4)
O1–C1–C2	121.1(3)	C7–C6–H6	120.2
N1–C1–C2	115.3(3)	C5–C6–H6	120.2
C3–C2–C1	111.2(3)	C6–C7–C8	120.5(4)
C3–C2–H2A	109.4	C6–C7–H7	119.7
C1–C2–H2A	109.4	C8–C7–H7	119.7
C3–C2–H2B	109.4	C7–C8–C3	120.3(4)
C1–C2–H2B	109.4	C7–C8–H8	119.9
H2A–C2–H2B	108.0	C3–C8–H8	119.9
C4–C3–C8	119.1(4)	C14–C9–C10	118.6(3)
C4–C3–C2	120.1(3)	C14–C9–N1	118.3(3)
C8–C3–C2	120.8(3)	C10–C9–N1	123.0(3)
C3–C4–C5	120.2(4)	C11–C10–C9	120.6(3)
C3–C4–H4	119.9	C11–C10–H10	119.7
C5–C4–H4	119.9	C9–C10–H10	119.7
C6–C5–C4	120.3(4)	C10–C11–C12	120.7(3)
C6–C5–H5	119.8	C10–C11–H11	119.7
C4–C5–H5	119.8	C12–C11–H11	119.7

C13–C12–C11	118.4(3)
C13–C12–N2	117.5(3)
C11–C12–N2	124.0(3)
C14–C13–C12	120.4(4)
C14–C13–H13	119.8
C12–C13–H13	119.8
C13–C14–C9	121.2(4)
C13–C14–H14	119.4
C9–C14–H14	119.4
C16–C15–C20	118.5(4)
C16–C15–N2	124.6(3)
C20–C15–N2	116.8(3)
C17–C16–C15	119.9(4)
C17–C16–H16	120.1
C15–C16–H16	120.1
C18–C17–C16	121.5(4)
C18–C17–H17	119.3
C16–C17–H17	119.3
C17–C18–C19	118.5(4)
C17–C18–H18	120.8
C19–C18–H18	120.8
C20–C19–C18	120.7(4)
C20–C19–H19	119.7
C18–C19–H19	119.7
C19–C20–C15	120.9(4)
C19–C20–H20	119.6
C15–C20–H20	119.6
C1–N1–C9	127.9(3)
C1–N1–H1N	117(3)
C9–N1–H1N	115(3)
C12–N2–C15	128.3(3)
C12–N2–H2N	113(4)
C15–N2–H2N	115(4)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters [$Å^2 \times 10^3$]. The anisotropic displacement

factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + \cdots + 2\ h\ k\ a^*\ b^*\ U^{12}]$.

Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C1	30(2)	41(2)	22(2)	–1(1)	0(1)	–1(2)
C2	34(2)	41(2)	27(2)	–1(2)	4(2)	3(2)
C3	30(2)	42(2)	25(2)	–2(2)	2(2)	4(2)
C4	39(2)	50(2)	31(2)	–2(2)	–4(2)	–2(2)
C5	49(2)	48(2)	37(2)	–1(2)	–3(2)	–9(2)
C6	50(3)	46(2)	34(2)	–1(2)	5(2)	0(2)
C7	37(2)	49(2)	37(2)	9(2)	7(2)	12(2)
C8	29(2)	50(2)	29(2)	2(2)	1(2)	6(2)
C9	33(2)	39(2)	24(2)	–3(1)	1(2)	2(2)
C10	30(2)	38(2)	27(2)	0(1)	1(1)	–1(1)
C11	34(2)	36(2)	27(2)	–4(1)	1(2)	0(2)
C12	34(2)	38(2)	27(2)	–3(2)	1(2)	0(2)
C13	32(2)	41(2)	37(2)	–8(2)	–2(2)	3(2)
C14	34(2)	41(2)	34(2)	–7(2)	–3(2)	2(2)
C15	28(2)	39(2)	29(2)	0(2)	2(2)	–3(1)
C16	35(2)	53(2)	29(2)	–1(2)	–2(2)	6(2)
C17	35(2)	62(3)	32(2)	–2(2)	0(2)	0(2)
C18	39(2)	53(2)	30(2)	–7(2)	5(2)	–4(2)
C19	39(2)	40(2)	33(2)	–1(2)	6(2)	–1(2)
C20	37(2)	40(2)	29(2)	–2(2)	3(2)	2(2)
N1	31(2)	42(2)	24(1)	–4(1)	–2(1)	4(1)
N2	33(2)	41(2)	30(2)	–6(1)	–2(1)	6(1)
O1	33(1)	45(1)	24(1)	–3(1)	–2(1)	2(1)

Table 5. Hydrogen coordinates [× 10^4] and isotropic displacement parameters [$Å^2$ × 10^3].

Atom	*x*	*y*	*z*	U_{eq}	*S.o.f.*
H2A	8212	6286	1676	41	1
H2B	9116	6308	2626	41	1
H4	7217	5532	2084	48	1
H5	6969	4652	3037	54	1
H6	7967	4243	4747	52	1
H7	9206	4715	5528	49	1
H8	9451	5596	4621	44	1
H10	7881	7663	5463	38	1
H11	7034	8335	6646	39	1
H13	4906	7744	4394	44	1
H14	5767	7097	3142	43	1
H16	6419	8333	8819	47	1
H17	6408	8775	11276	51	1
H18	5353	9425	11874	49	1
H19	4266	9608	9992	45	1
H20	4294	9196	7507	42	1
H1N	7180(30)	6801(19)	2410(40)	52(14)	1
H2N	4770(20)	8480(20)	5920(70)	67(17)	1

Table 6. Torsion angles [°].

O1–C1–C2–C3	67.2(5)
N1–C1–C2–C3	–111.9(4)
C1–C2–C3–C4	90.6(4)
C1–C2–C3–C8	–86.9(4)
C8–C3–C4–C5	0.9(6)
C2–C3–C4–C5	–176.6(4)
C3–C4–C5–C6	–0.9(7)
C4–C5–C6–C7	0.3(7)
C5–C6–C7–C8	0.3(7)
C6–C7–C8–C3	–0.2(6)
C4–C3–C8–C7	–0.4(6)
C2–C3–C8–C7	177.1(4)
C14–C9–C10–C11	1.2(6)
N1–C9–C10–C11	177.2(4)
C9–C10–C11–C12	1.8(6)
C10–C11–C12–C13	–3.3(6)
C10–C11–C12–N2	–179.6(4)
C11–C12–C13–C14	1.9(6)

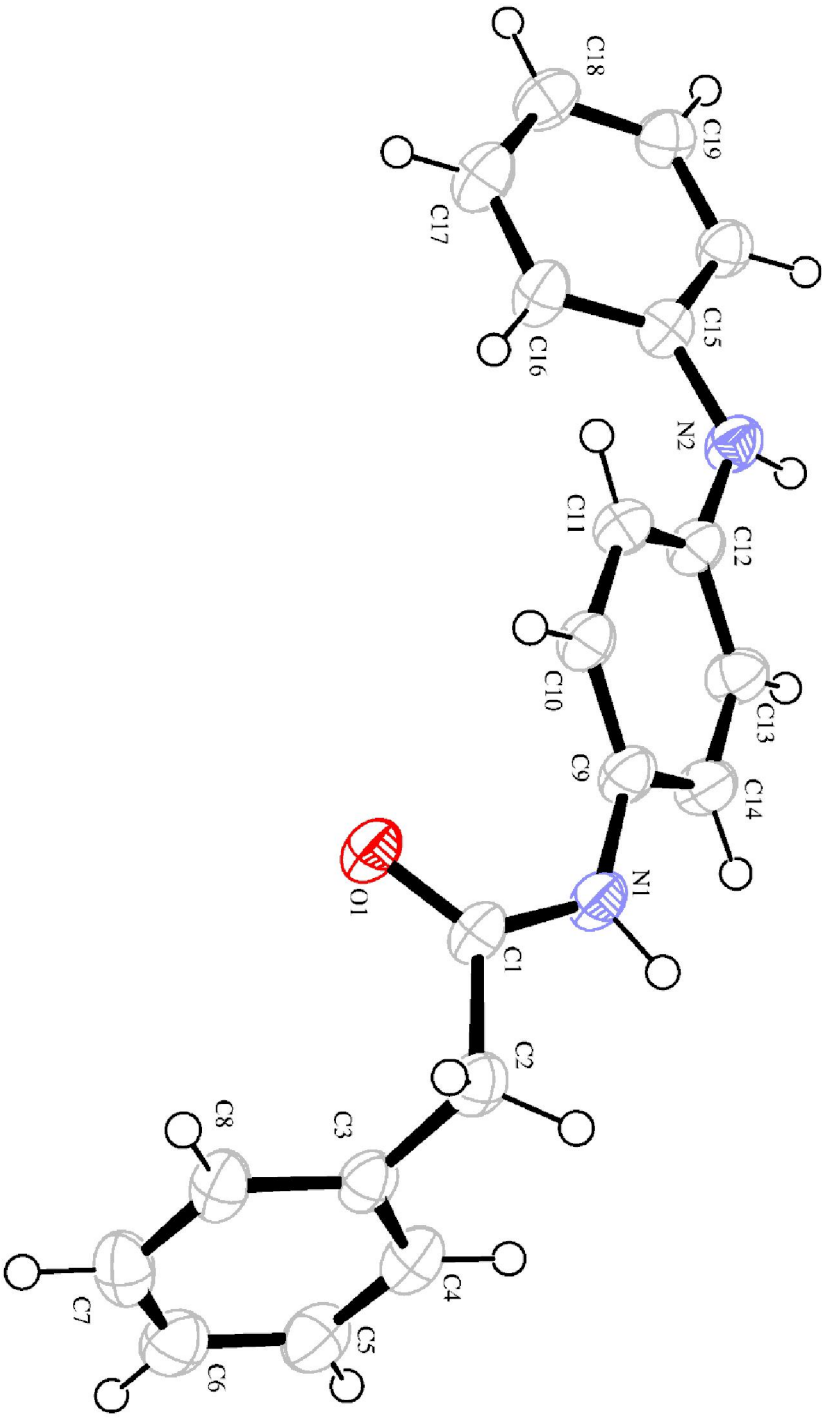
N2–C12–C13–C14	178.4(4)
C12–C13–C14–C9	1.0(6)
C10–C9–C14–C13	–2.6(6)
N1–C9–C14–C13	–178.8(4)
C20–C15–C16–C17	–2.5(6)
N2–C15–C16–C17	–179.6(4)
C15–C16–C17–C18	1.2(6)
C16–C17–C18–C19	1.2(6)
C17–C18–C19–C20	–2.4(6)
C18–C19–C20–C15	1.1(6)
C16–C15–C20–C19	1.3(6)
N2–C15–C20–C19	178.7(4)
O1–C1–N1–C9	2.0(6)
C2–C1–N1–C9	–179.0(3)
C14–C9–N1–C1	–147.8(4)
C10–C9–N1–C1	36.1(6)
C13–C12–N2–C15	148.5(4)
C11–C12–N2–C15	–35.1(6)
C16–C15–N2–C12	–2.4(6)
C20–C15–N2–C12	–179.6(4)

Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen bonds [Å and °].

D–H···*A*	*d*(*D*–H)	*d*(H···*A*)	*d*(*D*···*A*)	∠(*D*H*A*)
N1–H1N···O1^{i}	0.91(3)	2.02(3)	2.929(4)	171(4)
N2–H2N···O1^{ii}	0.93(3)	2.21(3)	3.122(4)	167(5)

Symmetry transformations used to generate equivalent atoms:
(i) –x+3/2,y+0,z–1/2 (ii) x–1/2,–y+3/2,z


C18
C19
C17
C15
C16
N2
C11
C12
C10
C13
C9
C14
O1
N1
C1
C2
C8
C3
C4
C7
C5
C6