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|  | REVIEW |

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Enzyme-catalyzed Synthesis of Secondary and Tertiary Amides

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| **Abstract**  The synthesis of the amide bond between an amine and a carboxylic acid is one of the most significant reactions in industrial pharmaceutical synthesis. Despite the apparent simplicity of synthetic methods for amide bond formation, many of these are disadvantaged by their requirement for toxic or hazardous reagents for the activation of the acid component, or poor atom economy resulting from the need for stoichiometric amounts of coupling reagents. In this context, biocatalysis has emerged as an alternative catalytic method for amide bond formation, presenting the advantages of both environmentally benign reagents and conditions and also atom economy. | In this review we detail developments in the enzyme-catalyzed preparation of secondary amides for the synthesis of pharmaceutical-type molecules and review the applications of hydrolases, such as lipases and penicillin acylases, to these reactions. We also summarise the activity of ATP-dependent enzymes for amide bond formation and assess their potential for the preparative synthesis of amides from carboxylic acids and amines in aqueous media.  **Keywords:** Biocatalysis, Amides, Lipase, Penicillin Acylase, NRPS, Amide Bond Synthetase |

1 Introduction

The synthesis of the amide bond is one of the most significant reactions in pharmaceutical chemistry, as amides feature in many of the world’s most prescribed small-molecule pharmaceuticals, including, for example, tyrosine kinase inhibitors such as imatinib **1**, dolutegravir (anti-HIV) **2**, ivacaftor (cystic fibrosis) **3** and teriflunamide (multiple sclerosis) **4**, but also of course the beta-lactam antibiotics **5** (**Scheme 1**). Indeed, it is thought that anything up to 16% of all reactions in medicinal synthetic chemistry laboratories are amide bond forming reactions that couple carboxylic acids, or their derivatives, with amines, to form the product amides. [1,2] Reagents for the formation of amide bonds are of course well-known;[2-4] typically the carboxylic acid moiety is activated for coupling through the formation of, for example, an acyl chloride, or a coupling agent, such as EDC, DCC or T3P, which must be added in stoichiometric amounts, to create an activated acid that may be attacked by the amine nucleophile. The issues raised by even these simple synthetic procedures - the use of hazardous reagents and the generation of considerable amounts of waste - mean that amide bond formation has become an issue of significant focus from the perspective of sustainable chemistry,[5] and is still considered to be one of the top reactions for the development of sustainable chemical alternatives by an industrial panel of synthetic chemists.[6] Given the green chemistry credentials of biocatalytic methods, it is no surprise that enzymatic catalysts are being studied increasingly for their suitability for the formation of amide bonds of relevance to the pharmaceutical, cosmetic and agrochemical industries.



**Scheme 1**. Some examples of pharmaceutical amides.

While the formation of the amide bond is of course common in Nature, within the ribosome for the formation of peptides, the synthesis of small molecule secondary amides, with structures that echo those of commonly prescribed pharmaceutical amides, is achieved using a number of different enzyme types and biochemical mechanisms.[7-10] These have included simple hydrolase enzymes, including predominantly lipases, but also proteases and acylases, which use amines as nucleophiles in the aminolysis of esters. There are also penicillin acylases, which catalyze the formation and hydrolysis of amide bonds in the synthesis of

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semisynthetic penicillins. There is also an increasing interest in recruiting ATP-dependent amide ligation mechanisms from microbial secondary metabolism for the synthesis of pharmaceutical-like secondary amides. However, given the relative simplicity of the competing conventional chemistry, the recruitment of these enzymes to the synthesis of pharmaceutically relevant secondary amides remains a challenge. In this review, we summarise and contrast the enzymatic reactions that have been investigated for the *in vitro* synthesis of secondary and tertiary amides, with an emphasis on advances that look to further the adoption of these systems for the preparative synthesis of molecules of pharmaceutical relevance.

**2. Aminolysis of Esters by Lipases**

Lipases have evolved to catalyze the hydrolysis of the carboxylester in triacylglycerides, but have found extensive application in the hydrolytic resolution or desymmetrisation of racemic or prochiral esters respectively, and also for the selective acylation of alcohols in organic solvents.[11-13] Lipases also catalyze the conversion of esters to amides, using amines as the nucleophile for the breakdown of the acyl enzyme intermediate that is at the core of the enzyme mechanism – a process called aminolysis. Using the aminolysis reaction, lipases have been extensively applied to the simple resolution, or dynamic kinetic resolution, of racemic amines through acylation using simple acyl donors such as vinyl acetate.[11-13] However, in this review we will focus on the application of lipases not to the resolution of chiral amines but rather to the synthesis of amide targets, including reactions that form more complex pharmaceutical intermediates or products.

*Porcine pancreatic lipase (PPL)*

Early experiments by Zaks and Klibanov demonstrated that commercially available lipase enzymes, such as porcine pancreatic lipase (PPL), would catalyze the aminolysis of methylbutyrate with *n-*butylamine, in a number of anhydrous organic solvents including hexane, acetone and tetrahydrofuran.[14] The group subsequently showed that PPL would also catalyze the formation of peptide bonds by coupling *N-*acyl amino acid esters such as *N-*Ac-L-Phe-OEt with L-Leucine to form the dipeptides in up to 86% yield in toluene.[15] The application of lipases and proteases to the formation of amide (peptide) bonds in di- and oligopeptides constitutes a major area of research and will not be covered further here, but readers are referred to recent reviews of the topic.[16,17] Following these early reports, Gotor and co-workers established that PPL also catalyzed the enantioselective acylation of 2-aminobutan-1-ol with ethyl acetate, to give a mixture of the (*R*)-amide and the (*S*)-amidoester, each with 95% e.e. By contrast, 1-aminopropan-2-ol was acylated almost exclusively at the amine nitrogen.[18]

*Candida cylindracea lipase (CCL)*

Gotor and co-workers showed that microbial enzymes, such as the lipase from *Candida cylindracea* (CCL), would also catalyze the aminolysis of simple chiral esters, such as ethyl-2-chloropropionate **6**, to form chiral amides with high enantioselectivity (**Scheme 2**).[19,20] These reactions resulted in yields of between 26% and 85% of the (*S*)-amides in hexane, with the best e.e*.*s of 92% and 95% achieved when using **7** and *n-*butylamine **9** as the amine donor respectively.[18] This work was extended to include the CCL-catalyzed aminolysis of ethyl-2-chloropropionate with chiral amines, such as 2-aminoheptane and 1-phenylethylamine, to form amides with two chiral centres. In one example, the aminolysis of ethyl-2-chloropropionate **6** with1-phenylethylamine **11** gave the (2*S*,1’*R*) and (2*R*,1’*S*) amide diastereomers in a 15% yield overall, with a ratio of 1:3, and with 46% and 95% e.e. respectively, using hexane as the solvent (**Scheme 2**).[21]



**Scheme 2**. Resolution of ethyl-2-chloropropionate using the lipase from *Candida cylindracea* (CCL).[18-21]

In later work, CCL was used to catalyze the aminolysis of racemic ethyl-2-bromopropionate with alkylamines including *n*-butylamine and dodecylamine, to give (*S*)-amide products with e.e*.*s of 90% and 64% respectively,[22] although the reaction with benzylamine was not selective. CCL also catalyzed the aminolysis of ethyl-2-chloropropionate with ethylenediamine to form the (*S*,*S*)-diamide in 50% yield with 100% d.e. and 88% e.e. in tetrachloromethane. By contrast, the protease subtilisin gave predominantly the (*R*,*R*)-isomer.[23] Much later, the group of Kostić showed that the activity and enantioselectivity of CCL could be substantially enhanced if the enzyme was encapsulated in sol-gel silica glass.[24] In one example, the aminolysis of ethyl-2-chloropropionate with benzylamine gave the (*S*)-amide product with 43% yield and 97% e.e. using the encapsulated lipase, against 4.5% yield and 62% e.e. for the free enzyme.

Gotor and co-workers subsequently showed that CCL would also catalyze the aminolysis of ethyl propiolate **13** with aromatic amines, such as aniline **14**, to give the propargyl amides **15** in up to 85% yield, also using tetrachloromethane as the solvent (**Scheme 3**).[25,26]



**Scheme 3**. Aminolysis of ethyl propiolate by aniline using the lipase from *Candida cylindracea* (CCL).[25,26]

*Candida antarctica Lipase B (CAL or CAL-B)*

Following these early advances using PPL and CCL, the lipase from *Candida antarctica –* ‘CAL’ in earlier publications, or CAL-B - the lipase most often deployed in biocatalytic reactions, has been the enzyme of choice for many aminolysis processes. Gotor and co-workers showed that methyl acrylic esters could be transformed to amides, with *n*-butylamine and benzylamine as amine donors, in between 40% and 88% yield using CAL-B in tetrahydrofuran as the solvent.[26] CAL-B also catalyzed the enantioselective aminolysis of acrylic esters when chiral amines were used. In one example, methylmethacrylate **16** was transformed with racemic 2-aminobutane **17** to give a 27% yield of the (*R*)-amide **18** with 95% e.e. (**Scheme 4**).[25,26]



**Scheme 4**. Enantioselective aminolysis of an acrylic ester using a lipase from *Candida antarctica* (‘CAL’).[25,26]

‘CAL’ was applied successfully to the aminolysis of ethyl-3-hydroxybutyrate with *n*-butylamine, allylamine and benzylamine.[27,28] In the last case, a 45% conversion to the (*R*)-amide was achieved, with a product *e.e.* of >99%. These amides could be converted to valuable chiral amino alcohol products through subsequent reduction with lithium aluminium hydride. CAL-B also catalyzed the aminolysis of -furyl and -phenyl esters and structurally-related ethylenic esters with high conversion and enantioselectivity.[29] Hence furyl ester **19** was coupled to allyl-, *n-*butyl or benzylamine to form amide products **20, 21** and **23** in 91%, 83% and 87% yield, respectively (**Scheme 5**).



**Scheme 5**. Aminolysis of furyl esters using a lipase from *Candida antarctica* (‘CAL’).

Furthermore, aminolysis of **19** using racemic amines, such as 1-phenylethylamine, gave (*R*)-amide products with up to >95% e.e. In addition, CAL-B catalyzed the aminolysis of racemic 2-methylbutyrate with benzylamine to give the (*R*)-amide with 78% e.e., although reactions with alkylamines were not as enantioselective.[22] The enzyme was also employed in the preparation of -keto amides such as **25** and **27** with yields of up to 98% (**Scheme 6**), although the rate of reaction was dependent upon the structure of the substrate ester.[30] For example, an 89% yield in the aminolysis of ethyl 3-oxobutyrate **24** with benzylamine **22** was achieved in 18 h, but 93 h were required to achieve a yield of 73% in the reaction of ethyl 3-oxo-3-phenylpropionate **26** with the same amine.



**Scheme 6**. Aminolysis of -ketoesters using CAL-B.[30]

The aminolysis of ethyl 3-oxobutyrate with the chiral amines 2-aminohexane or 1-phenylethylamine gave the chiral (*R*)-amide products with >97% e.e. in each case.[30] Gotor and co-workers later showed that racemic methyl 3-hydroxypentanoate was resolved by aminolysis using CAL-B with benzylamine as the nucleophile to give the (*R*)-amide with an *E* value of 42, threefold higher than when using ammonia as the amine donor.[31] This was attributed to the different binding modes of the enantiomeric tetrahedral intermediates in the active site, when using either ammonia or benzylamine, with greater restrictions on the benzylamine intermediate leading to greater enantiodiscrimination.

CAL-B also proved effective in the aminolysis of CBz-glutamic acid diesters, with 100% selectivity for formation of the -amide products.[32] Hence **28** was transformed to **30** and **31** (**Scheme 7**) with 94% and 66% yield when *n*-pentylamine or benzylamine were employed as the amine donor in diisopropyl ether as solvent. Interestingly, when Cbz-D-glutamate diester was used as the substrate, and with *n*-pentylamine as the amine donor, the selectivity was for the -amide over the -amide in a ratio of 69:14. In addition, the use of racemic 1-phenylethylamine as the amine donor resulted in enantiomerically enriched amide products with (*R*)-selectivity. CAL-B was also applied to the aminolysis of pyroglutamic acid derivatives by Conde and co-workers.[33] Hence ethyl- and benzyl pyroglutamic acid esters **32** and **34** were converted to amides using either *n*-pentylamine or cyclohexylamine as the donor to give amide products **33** and **36** with quantitative conversion, albeit with poor selectivity (**Scheme 7**). However, the use of 1-phenylethylamine as the amine donor revealed selectivity in the formation of chiral amides, with the (*S*,*R*)-product diastereomer produced preferentially, with an *E* value of 100 reported for the reaction.



**Scheme 7**. Aminolysis of pyroglutamic acid derivatives using CAL-B.[32,33]

CAL-B was also used for the acylation of -aminonitriles, in which *N*-acylamino nitriles were the amine donor in an amidation reaction.[34] Hence, the enzyme catalyzed the enantioselective acylation of 2-amino-2-phenylacetonitrile with ethyl phenylacetate in isopropyl ether, to give the amide product in 47% yield and 100% e.e. Interestingly, when ethyl acetate was used as the acyl donor, the amide product was obtained with only 10% e.e. This was attributed to racemisation of the tetrahedral oxyanion formed when the aminonitrile nucleophile attacks the acyl enzyme intermediate formed by the reaction of the catalytic serine with the ester substrate.

CAL-B-catalyzed aminolysis was also applied to the resolution of chiral biaryl esters of interest for their application as chiral ligands in transition metal catalysis.[35] Further investigation of the alkyl length within the ester showed that, whereas shorter esters were not successfully transformed, **37** was successfully converted to its amide using 3-aminopropionitrile **38** as the amine donor to give the residual (*S*)-ester in 25% yield and with 98% e.e., and the (*R*)-amide in 72% yield and with 49% e.e. (**Scheme 8**).[36]



**Scheme 8**. Aminolysis of chiral biaryl esters using CAL-B.[35,36]

More recently, CAL-B has been used to catalyze the aminolysis of a range of methyl esters, including methyl *p*-methoxy dihydrocinnamate and *N-*Cbzamino acid methyl esters, with propargyl amine **42** in *tert*-butyl methyl ether as solvent, in order to furnish propargyl amides for functionalisation using click chemistry. In one example methyl ester **43** was converted to the amide **44** in 87% yield (**Scheme 9**).[37] The addition of benzyl azide and copper oxide to the reaction, following aminolysis, gave the 1,2,3-triazole product **45** in 83% overall yield.



**Scheme 9**. Synthesis of propargyl amides using CAL-B.

In a wide-ranging study of lipases and reaction conditions, Soledad de Castro and Sinisterra Gago showed that enzymes including CAL-B were most effective in aminolysis reactions using racemic 1-phenylethylamine as the amine donor in small volumes, with low (or no) added water in hydrophobic solvents. This was thought to be because CAL-B exists in a hydrated form on its resin support.[38] Hence the enzyme was applied to the aminolysis of racemic 2-methyl butyrate with benzylamine, giving the (*R*)-amide in 88% yield and with 77% e.e in a mixture of 97:3 ethyl acetate with 3% Tris HCl buffer at pH 9.0.

*Pseudomonas (Burkholderia*) *cepacia* lipase (PCL)

Adamczyk and Grote showed that another microbial lipase, from *Pseudomonas* (now *Burkholderia*) *cepacia* (PCL, Amano PS-30 lipase) catalyzed the aminolysis of benzyl esters with a range of amine donors to form a range of benzyl amides. Thus, the aminolysis of benzyl acetate with 1-phenylethylamine in isopropyl ether gave an 88% yield of the amide after 168 h reaction time, and benzyl valerate **46** was converted to products **48** using 2-aminomethyl pyridine **47** in 51% yield (**Scheme 10**).[39] When dibenzyl ester substrates were used,only monoamidation products were obtained; thus the reaction of dibenzylmalonate **49** with tyramine **50** gave only product **51** in 78% yield after 168 h.

The same group also applied PCL to the synthesis of -hydroxyamides from -hydroxyesters.[40] Hence, -hydroxy benzyl esters such as (*R*)- or (*S*)-benzyl lactate were converted to -hydroxyamides using either benzylamine or 2-phenylethylamine with up to >99% conversion. In the latter case, racemic benzyl lactate was converted to a mixture of the (*S*)-amide and the residual (*R*)-ester with 82% and 80% e.e. respectively.



**Scheme 10**. Aminolysis of benzyl esters catalyzed by lipase from *Pseudomonas cepacia.*[39]

*Pseudomonas stutzeri* lipase (PSL)

A screen of lipases for aminolysis activity by researchers in Delft, in collaboration with Pfizer, identified a lipase, PSL, from *Pseudomonas stutzeri,* that displayed good activity for the aminolysis of methyl esters of benzoic acid, cyclohexanecarboxylic acid and 2-alkylated phenylpropionic acids with a range of aromatic, alicyclic and aromatic amines.[41] PSL proved to be a superior catalyst to CAL-B for the aminolysis of methyl-2-phenylpropionate with secondary amines including piperidine. Using the aminolysis of methyl 3-phenylpropionate with cyclohexylamine as a model, it was shown that the addition of molecular sieves substantially improved the efficiency of aminolysis, removing both water and methanol, which were responsible for hydrolysis of the substrate ester and enzyme inhibition respectively, from the reaction mixture.

Recently Li and co-workers have described the aminolysis activity of an intracellular lipase SpL from *Sphingomonas* sp. HXN-200. [42] SpL catalysed the aminolysis of simple methyl esters including **52**, **54** and **56** to form amides **53**, **55** and **57** in up to 99% yield in partially hydrated hexane and included the product of the weak aniline nucleophile **14**, which gave anilide **58** in 85% yield (**Scheme 11**).Moreover the aminolysis of racemic esters such as **59** resulted in the formation of chiral amide products such as **60** in up to 99% e.e. Interestingly SpL also catalysed the amidation of free carboxylic acids; indeed the amidation of phenylpropionic acid with benzylamine as the nucleophile was approximately 2-fold faster than for the equivalent ester substrate. As the lipase is intracellular, wet cell preparations containing the enzyme could be used for preparative amidations of carboxylic acid substrates. In this mode, SpL catalysed the coupling of phenylpropionic acid to benzylamine to give the amide in 94% yield with just a small excess of the amine nucleophile.



**Scheme 11**. Aminolysis of esters catalyzed by the intracellular lipase SpL from *Sphingomonas* sp. HXN-200.[42]

**3. Amidation of Carboxylic Acids by Lipases**

One feature associated with the lipase-catalyzed aminolysis of esters, in common with many abiotic methods for coupling carboxylic acids and amines, is the the activation of the acid, in these cases through ester formation. However, as as has already been shown with the example of the lipase SpL above,[42] lipases can also under some circumstances catalyze the amidation of free carboxylic acids. Following a report in which Sheldon and co-workers used CAL-B, first for the formation of an ester from octanoic acid, followed by ammonolysis to form the amide product,[43] Baldessari and Mangone showed that this strategy could also be applied to the synthesis of secondary amides.[44] Incubation of C3-C18 carboxylic acids with, for example, CAL-B and ethanol, gave the esters in quantitative yield, however introduction of an amine, such as *n-*propylamine, resulted in quantitative yields of the relevant amide. The strategy was applied to a 20 g–scale synthesis of tetrahydro-*N*-[3-(methylamino)-propyl]-2-furancarboxamide **64**, a precursor of the pharmaceutical amide alfuzosin. CAL-B catalysed the formation of ethyl ester **62** from 2-tetrahydrofuric acid **61** with ethanol as alcohol donor and solvent, followed by aminolysis with *N*-methyl-1,3-propanediamine **62**, to give the product in 69% yield from the acid (**Scheme 12**).



**Scheme 12**. Synthesis of tetrahydro-*N*-[3-(methylamino)-propyl]-2-furancarboxamide using CAL-B catalyzed aminolysis.[44]

More recently, the same group has applied an equivalent approach to the synthesis of the endocannabinoid anandamide [*N*-(2-hydroxyethyl)arachidonoylamide].[45] CAL-B first catalyzed the formation of ethyl linolenate from linolenic acid and ethanol in hexane at 55°C. Following completion of this reaction, ethanolamine was added to the same pot to give the target amide product in 92% overall yield. Some branched alkanolamines were also tolerated, giving yields in the range of 60-87%.

A further strategy for converting carboxylic acids to amides in one-pot was presented by Irimescu and Kato, who showed that acylation of 1-phenylethylamine and 2-phenyl-1-propylamine could be achieved in either a no-solvent system, or in an ionic liquid under reduced pressure, which enabled removal of the water generated during the condensation reaction.[46] Hence, the amidation of dodecanoic acid with 1-phenylethylamine was achieved with an *E* value of >500 in a no-solvent system in a vacuum. Additionally, 4-pentenoic acid was amidated with 1-phenylethylamine in the ionic liquid [bmim]PF6, also with an *E*-value of >400, although conversion was low at 8 %. Castillo and co-workers showed that caproic acid was efficiently amidated using 1-phenylethylamine in 2-methyl-2-butanol as the solvent, yielding the (*R*)-amide with 49.5% conversion and >99% e.e.[47] A further extension to this approach was reported by Lubin-Germain and co-workers, who showed that CAL-B catalyzed the coupling of equimolar amounts of octanoic acid and benzylamine in up to 94% yield in 1,4-dioxane, or 92% in toluene at 50°C, in the presence of molecular sieves, in order to prevent the reverse hydrolysis reaction.[48] Further amines, including allylamine and *n*-propylamine were also employed, giving yields of 60% and 83% respectively. The amidation of the antioxidant (*R*)-lipoic acid with benzylamine was achieved with a yield of 87%.

Rahman and co-workers employed Lipozyme TL IM for the synthesis of the COX enzyme inhibitor *N*-*trans*-feruloyl tyramine, from ferulic acid and tyramine, added in a 6: 1 ratio, to give a 93.5% yield of the product when acetonitrile was used as the solvent.[49] Similarly, Singh and co-workers showed that CAL-B catalyzed the amidation of a range of phenolic acids, such as cinnamic acid, 4-hydroxy cinnamic acid and ferulic acid, with alkyl amines ranging from *N-*propylamine to *N-*hexadecylamine.[50] These reactions were operated ina solvent free system at between 60 and 90 °C under reduced pressure to give amide products in between 76% and 84% yield.

**4. Enzyme-catalysed formation of lactam rings**

The enzyme-catalyzed cyclisation of amino esters or amino acids can lead to the formation of amide bonds to give lactam products. The first demonstration of this enzymatic reaction was by Abell and co-workers, who showed that PPL catalyzed the cyclisation of pre-formed isopropyl amino esters such as **65** and **67** (**Scheme 13**) to give lactams **66** and **68** with up to 80% conversion using *tert*-amyl alcohol as the solvent.[51] The cyclisation of chiral **69** by subtilisin was catalyzed enantioselectively, although with an e.e. of only 23%. PPL also catalyzed the formation of bislactams from diesters and diamines in dichloromethane or chloroform in 35-45% yield.



**Scheme 13**. Formation of lactams catalyzed by porcine pancreatic lipase (PPL) and subtilisin.[57]

In later work, the transformation of 6-aminocaproic acid to -caprolactam by CAL-B demonstrated that lipases could catalyse the cyclisation of aminocarboxylic acids to form lactams.[52] Hence 4-, 5-, and 6-aminocaproic acid were converted to lactams with 30%, 70% and 83% yield. 8-aminocaproic acid was converted to a mixture of dimeric and trimeric lactams in a 3:2 ratio. CAL-B was later applied to the cyclisation of fluorinated diester **71** to give the fluorolactam **72** in 43% isolated yield and with 99% e.e. on a 10 g scale (**Scheme 14**).[53]



**Scheme 14**. Enantioselective synthesis of a fluorolactam using CAL-B catalyzed intramolecular aminolysis.[53]

The amino esters that serve as precursors to lactam formation can be generated by the action of either transaminase[54,55] or reductive aminase[56,57] enzymes on - or -keto esters. Kroutil and co-workers demonstrated that an (*R*)-selective transaminase ATA-117 catalyzed the amination of 4-oxo-3-phenylbutyric acid ethyl ester **73** (**Scheme 15**), which spontaneously racemised under reaction conditions,[58] permitting the synthesis of (*R*)-4-phenylpyrrolidin-2-one **74**, a precursor of 3-phenyl gamma amino butyric acid, in 93% yield with 61% e.e.



**Scheme 15**. Amination of 4-oxo-3-phenylbutyric acid ethyl ester by transaminase ATA-1117.[58]

A similar transformation was exploited by Chung and co-workers at Merck to convert aldehyde **75** to lactam **76** in 86% yield and with >99% e.e. on a 35 g scale using ATA-301, as part of a proposed synthesis of the poly(ADP-ribose)polymerase (PARP) inhibitor niraparib (**Scheme 16**).[59] Similarly, Truppo and co-workers used both (*S*)- and (*R*)-selective transaminases ATA-113 and ATA-117 for the amination of ethyl 4-acetylbutyrate **77** to give 6-methyl-2-piperidinone **78**, each with >99% yield and >99% e.e. (**Scheme 16**).[60]

Gotor-Fernández and co-workers recently demonstrated that a generic approach to lactams from - or -keto esters was achievable through screening a range of commercially available transaminases.[61] Hence ethyl levulinate could be converted to either (*R*)- or (*S*)-5-methylpyrrolidin-2-one using, for example, TA-P2-B01 or ArS-TA respectively, with 87% and 92% yield and with >99% e.e. in each case. If ethyl-5-hexanoate were used as the substrate,



**Scheme 16**. Synthesis of lactams using transaminase-catalyzed amination.[59,60]

enantiomers of 6-methylpiperidin-2-one were obtained with similar selectivity. The screen also discovered TAs that catalysed the lactamization of methyl 4-oxo-4-phenylbutanoates and 5-aryl-5-oxopentanoates to give aryl-substituted lactams, again with high conversions and e.e.s., although conversions were lower in the latter case. 100 mg scale reactions were performed using 100 mM isopropylamine as the amine donor and acetonitrile as co-solvent, to give isolated yields of between 66-89% with excellent stereoselectivity. Ethyl levulinate also served as the substrate for the reductive aminase (RedAm) enzyme from *Aspergillus oryzae*, which catalysed the coupling of the ester with either propargylamine or cyclopropylamine to form the imine intermediates, followed by NADPH-dependent reduction and spontaneous cyclization to give the (*R*)-lactam products with 97% and 82% e.e. respectively.[62]

**5. Amidation reactions catalyzed by Pencillin Acylases (PACs)**

Pencillin Acylases (PACs) catalyze the hydrolysis of penicillin amides such as penicillin G **79** to give 6-aminopenicillanic acids **80** derivatives and phenylacetic acid **81** and are exploited industrially for this hydrolysis reaction (**Scheme 17**).[63,64] However, they also catalyze the amidation of 6-APA with alternate acyl donors, such as D-phenylglycine side chains, to give semi-synthetic penicillins).[63,64]



**Scheme 17**. Reversible hydrolysis of penicillin amides by Penicillin Acylases (PAC).

In early studies, Bauer and co-workers first demonstrated the use of PACs in the amidation direction, with the synthesis of penicillin G **79** from 6-APA **80** and phenylacetic acid **81**. This was achieved under thermodynamic control, using a pH of 4.5 to increase the amount of acid in solution, and high concentrations of water miscible organic solvents.[65] Cole then studied the substrate tolerance of PACs, investigating the coupling of 6-APA, glycine and hydroxylamine as amino components,with carboxylic acids, amides, esters and *N*-acyl glycine derivatives.[66] Hence it was determined that the preferred acyl group was phenylacetyl, although hydroxylation on the benzene ring was tolerated, with a preference for (*p*-hydroxyphenyl)acetic acid. Aliphatic acids *n*-valeric, *n*-hexanoic, *n*-hexenoic, *n*-heptanoic and 2-thienylacetic acid were reported to be reasonably well accepted when coupled with 6-APA, a phenomenon attributed to their similarity in size with the phenylacetyl compounds. Luisi and co-workers also noted that the structure of the acyl acceptor is limited to small derivatisations of phenylacetic acid. However, within this study a reasonable tolerance towards the structure of the amino component was demonstrated, with a number of amino acids, dipeptides and tripeptide coupled to phenylacetic acid.[67] The coupling of methyl esters of serine, methionine and tyrosine with phenylacetic acid gave yields ranging from 20-77%, however, free amino acids were not substrates for the coupling reaction.

The use of activated phenylacetic acids, in the form of amides, such as D-phenylglycine amide (D-PGA), methyl ester (D-PGM), or thioester derivatives, can permit the use of a broader range acyl acceptors in PAC-catalysed amidations. For example, phenethicillin was synthesised by coupling a highly activated thioester of α-phenoxypropionic acid with 6-APA.[66] In addition, cephalexin was produced from 7-aminodesacetoxycephalosporanic acid (7-ADCA) and D-(−)-PGA by Bruggink.[68,69] However, these kinetically controlled transformations suffer from the use of an excess of the amino component and also the unproductive hydrolysis of the acyl-enzyme intermediate, yielding D-phenylglycine. In addition, extra synthetic steps are required to synthesise the activated acids. To address these issues, Sheldon and co-workers developed a process using a mixture of D-PGA **82** and D-PGM **83** as acyl donors in the synthesis of ampicillin **85**.[70] Waste D-phenylglycine **84** resulting from the non-productive hydrolysis of D-PGA **82** was converted to D-PGM-HCl using methanol-HCl, recycling the acyl donor and resulting in a more atom efficient process (**Scheme 18**).



**Scheme 18**. Process for recycling D-PG **83** to achieve a more atom efficient synthesis of ampicillin **84.**[70]

Subsequently Sheldon also demonstrated a two-step, one-pot enzymatic synthesis of cephalexin from D-phenylglycine nitrile (D-PG-CN) and 7-aminodesacetoxycephalosporanic acid (7-ADCA) using a nitrile hydratase coupled with Penicillin G acylase.[71] The D-phenylglycine nitrile appeared to selectively inhibit the PAC in the hydrolysis direction, leading to a threefold increase in the synthesis/hydrolysis (S/H) ratio. The nitrile hydratase, from *Rhodococcus rhodochrous*, was used to convert D-PG-CN to the activated intermediate, D-PGA, which then underwent PAC-catalysed acylation of 7-ADCA, to give cephalexin in 79% yield, with a S/H ratio of 7.7.

Another enzyme cascade was employed by Guisán and co-workers for the synthesis of the antibiotic cefazolin.[72] D-amino acid oxidase (DAO) catalyzed the oxidative deamination of the -aminoadipate moiety of Cephalosporin C **86** to give the -ketoadipate. Spontaneous decarboxylation, resulted in the glutaryl analog, which was then deacylated by glutaryl acylase (GA) to obtain 7-aminocephalosporanic acid (7-ACA) **87** in 97% yield. Penicillin G acylase (PGA) then catalysed the coupling of 7-ACA with tetrazolylacetic acid methyl ester (TZAM) **88**, to give the acetoxy intermediate **89** in 98% yield (**Scheme 19**). Displacement of the 3’-acetoxy group with 2-mercapto-5-methylthiadiazole (MMTD) gave the final product cefazolin in 70% yield.



**Scheme 19**. Enzyme cascade for the production of an intermediate in cefazolin synthesis.[72]

Examples of enantioselective amidation reactions by PACs are limited, as in most cases enantiomerically pure precursors, such as D-PGA or D-PGM, are used in the coupling reactions. However, there are a small number of cases in which the starting materials are racemic. Zmijewski Jr. and co-workers showed that the selective acylation of the (2*R*,3*S*)-isomer from a *cis*-racemic azetidone amino precursor with methylphenoxyacetate as the acyl donor, catalyzed by Penicillin G acylase, could be used in a synthesis of loracarbef, a carbacephalosporin antibiotic.[73] The amide was obtained in 45% yield and with 100% e.e., with the reaction driven in the synthetic direction by the precipitation of the product. For an example in which the acyl donor was chiral, Fuganti and co-workers explored the use of chiral mandelic acid esters in the PAC-catalysed synthesis of diastereoisomerically pure cefamandole nafamate, a cephalosporin antibiotic.[74] The reaction of L-methyl mandelate with 7-ADCA gave L-cefamandole in 35% yield, whereas the reaction with the D-enantiomer gave only 8% of cefamandole. In contrast, D-methyl-*O*-formyl methyl mandelate gave a yield of 38% of cefamandole nafate, whereas no reaction was observed with the L-enantiomer.

A surprisingly broad amidation substrate specificity was described for a pencillin V acylase (PVA) from *Streptomyces mobaraensis*, by Nakanishi and co-workers.[75] *Sm*-PVA catalysed the synthesis of penicillin V, penicillin K, dihydropenicillin F, and deacetoxycephalosporin V phenylacetate methyl ester and the relevant amino acceptors in yields of 66%, 91%, 90% and 69% yield respectively. However, the synthesis of a number of capsaicin amides, using vanillylamine as the nucleophile and methyl *n*-octanoate, methyl laurate, or methyl myristate as acyl donors, was also achieved, in 7.5%, 23% and 22% yield respectively. Furthermore, various *N*-lauroyl-amino acids and *N*-lauroyl peptides could be synthesized using the enzyme. All amino acid methyl esters reacted with methyl laurate to give the *N*-lauroyl amino acid products.

In a more unusual application of a PAC, Pencillin G acylase was applied to the synthesis of enantiopure diketopiperazines by Sheldon and co-workers.[76] The immobilised enzyme (Assemblase 7500®) catalyzed the coupling of D-PGA with amino acids such as L-norvaline, L-norleucine and L-homocysteine to give dipeptides in between 32% and 52% yields. Treatment with thionyl chloride in methanol, to give the methyl esters, followed by reaction with sodium hydroxide gave the enantiopure ketopiperazines, the latter in between 62 and 63% yield from the dipeptides.

**6. Amide Bond Formations Catalyzed by Other Hydrolases**

Although most hydrolase-based aminolyses have been achieved using lipases or PACs, other studies have revealed additional enzymes with potent aminolytic activities, and these may offer complementary opportunities for amide synthesis. For example, in early studies that used PPL as a catalyst, Klibanov and co-workers also showed that the acylation of amines could be achieved with high enantioselectivity by the protease subtilisin, using triflurorethylbutyrate as the acyl donor in anhydrous 3-methyl-3-pentanol as the solvent.[77] Hence 1-phenylethylamine and 1-(1-naphthyl)ethylamine were transformed to their (*S*)-butyramides with 85% and 98% e.e., although alkylamines such as 2-aminoheptane and 1,3-dimethylbutylamine were converted with lower selectivities of 63% and 59% respectively.

Acyl transferases[78] have also been applied in preparative amidation reactions. In an effort to discover enzymes that catalyse amidations in an aqueous environment, an acyltransferase from *Mycobacterium smegmatis* (*Ms*AcT) was shown to catalyze the formation of *N*-acyl amines in phosphate buffer at pH 8.0.[79] (*E*)-cinnamyl amine was successfully converted to amides using 20 equivalents of ethyl, isopropyl and paranitrophenyl acetate in buffer respectively, although vinyl acetate was the superior acyl donor, giving a 92% yield of the amide product. *N-*formylation was also possible, but yields were lower when *N*-butyryl amide was the donor. Moreover, *Ms*AcT catalyzed the transamidation of (*E*)-cinnamyl amine with 20 equivalents of acetamide in aqueous buffer to give 57% conversion to the acetamide. In another extension to the use of *Ms*AcT, Berglund and co-workers constructed a transaminase-acyl transferase cascade for the one-pot conversion of aldehydes or ketones to secondary amides.[80] In a model reaction, a transaminase from *Silicibacter pomeroyi* (*Sp*-ATA) was incubated with benzaldehyde and L-alanine as the ammonia donor, in order to give benzylamine, which was then converted to *N*-benzylacetamide using methylmethoxyacetate as the acyl donor and *Ms*AcT as the acyl transfer catalyst in the same pot. Conversions of up to 92% were achieved for the synthesis of *N*-benzyl-2-methoxyacetamide following reaction optimisation, however, *Ms*AcT was shown to be poorly enantioselective for chiral amine intermediates in the attempted construction of an asymmetric cascade reaction.

More recently, a *C*-acyl transferase PpATaseCH from *Pseudomonas protegens* was shown to catalyse the *N-*acetamidation of a range of aniline structures including **90**, **93** and **95** based on *m*-hydroxyaniline, also in an aqueous buffer, with yields of up to 96% when 1.5 equivalents of phenylacetate **91** were used as the acyl donor (**Scheme 20**).[81] Neither benzylamine nor 1-octylamine were accepted as substrates.



**Scheme 20**. *N*-acetamidation of anilines using a *C*-acyl transferase PpATaseCH from *Pseudomonas protegens.*[81]

**7. ATP-dependent Systems**

The desire for amide-bond formation reactions that could be applied in aqueous media has led to researchers to assess the potential of natural aminolysis reactions for the synthesis of amides.[7-10] In nature, as in chemical synthesis, the activation of carboxylic acids is required for amidation reactions, and this is typically achieved through activation *via* either a phosphorylation or adenylation reaction at the expense of adenosine triphosphate (ATP), to give an acyl phosphate or acyl adenylate respectively. In the case of acyl phosphates, the intermediate is then attacked by an amine to form an amide in relevant ‘ATP-grasp’ enzymes. In the case of adenylate formation, in for example, non-ribosomal peptide synthases (NRPSs), this is typically catalysed by an adenylation domain (A-domain). The acid substrate is then transferred from the adenylate to the phosphopantetheinyl (Ppant) group of an acyl carrier protein (ACP) where a second intermediate, a thioester, is formed, which then undergoes the attack of an amine to form the amide. In non-ribosomal peptide synthases (NRPSs), the amide-bond formation reaction is catalysed by a condensation domain. A chemical process equivalent to this ‘adenylation-thiolation-condensation’ sequence also occurs in some systems where an acid is converted to an adenylate, thence a Coenzyme A thioester, prior to aminolysis. Alternatively, in a small group of amide bond ligases that includes NRPS-independent siderophore (NIS) enzymes and other enzymes from microbial antibiotic biosynthetic pathways, the adenylate is attacked directly by an amine nucleophile to form an amide from a carboxylic acid and an amine within one polypeptide chain and without the involvement of a thioester intermediate. In the following summary, we will focus on those examples of ATP-dependent amide bond formations which have been recruited to the preparative formation of non-native amide products, or for which the natural activity suggests potential for application in preparative biocatalysis in the future.

*ATP Grasp Enzymes*

ATP-grasp enzymes can catalyse the ATP-dependent coupling of a carboxylic acid with an amine, *via* an activated acylphosphate intermediate (**Scheme 21**) and have roles in a wide range of biochemical processes.[82,83]



**Scheme 21**. General scheme for amidation reactions catalysed by ATP-grasp enzymes.

A variety of enzymes within this family has been described including D-ala-D-ala ligase[84] biotin carboxylases (BCases),[85] glutathione synthetase[86] and enzymes coupling amino acids in the synthesis of bacterial reserve polymer compounds such as cyanophycin.[87] From the perspective of biocatalytic potential, enzymes that catalyse the synthesis of dipeptide metabolites, such as the L-amino acid ligases (Lals)[88] have perhaps the greatest relevance, and have in some cases been mutated for altered catalytic properties.

BacD (YwfE) is a Lal that catalyzes the ligation of the epoxycyclohexanone amino acid L-anticapsin **97** with L-alanine **98** to form the antibiotic bacilysin **99** (**Scheme 22**).[89] Tsuda and co-workers showed that a single mutation of tryptophan residue, Trp332, to alanine, altered the substrate specificity of YwfE, permitting the coupling of larger amino acids such as L-Phe to the form an L-Phe-L-Phe dipeptide.[90]



**Scheme 22**. Synthesis of bacilysin by the L-amino acid ligase BacD.[89]

Arai and co-workers have applied TabS, a Lal from *Pseudomonas syringae*, involved in the biosynthesis of the dipeptide phytotoxin tabtoxin, to the synthesis of a range of functional peptides.[91] The recombinant enzyme demonstrated the broadest substrate specificity of any known Lal, accepting 136 of 231 combinations of amino acid substrates, including L-pipecolic acid, hydroxyl-L-proline, and β-alanine, when applied to dipeptide synthesis. A number of functional peptides were synthesised, including L-arginyl-L-phenylalanine and L-leucyl-L-isoleucine in 62% and 77% yield and which are reported to have antihypertensive and antidepressive properties, respectively. Further structure-guided mutation of TabS resulted in double mutant S85T/H294D, which displayed improved activity with respect to the synthesis of the salt taste enhancing dipeptide Pro-Gly.[92]

Despite the potentially broad specificity of these enzymes for amino acid coupling, to our knowledge, no ATP-grasp enzymes have yet been exploited for the coupling of non-amino acid substrates for the formation of more hydrophobic pharmaceutical-like amides, although the successful modification of their substrate specificity using protein engineering suggests a wider potential that has not yet been explored.

*Adenylation domains, including NRPSs and Carboxylic Acid Reductases (CARs)*

The adenylation-thiolation-condensation cascade is observed in biosynthetic pathways towards a host of amide natural products, including those synthesised by a number of non-ribosomal peptide synthases (NRPS),[93] including some bacterial siderophores such as mycobactin[94] and vibriobactin (**Scheme 23**).[95,96]In the biosynthesis of vibriobactin in *Vibrio cholerae*, for example, 2,3-dihydroxybenzoic acid (DHB) **100** is first adenylated by the enzyme VibE.[95] The adenylate DHB-OAMP **101** is transferred to the acyl carrier protein VibH, giving a phosphopantotheinyl ester DHB-S-Ppant **102** and AMP as products. In the case of vibriobactin, the thioester is then coupled to the amine norspermidine **103** (NSPD) by the amide synthetase VibH, to give DHB-NSPD as the amide product **104**, a biosynthetic precursor of the siderophore. The chemical route from acid to amide is therefore complex, requiring three chemical reactions and two intermediates in addition to three separate enzymatic activities, which do not sometimes form part of one complex.



**Scheme 23**. Production of an amide precursor in the biosynthesis of vibriobactin.[95]

This complexity has militated against the recruitment of these systems for preparative biocatalytic amide bond formation *in vitro*, however, recent advances suggest they have potential for these applications, especially where systems can be engineered to operate in whole-cells.[93,97,98]

However, researchers have shown that that standalone adenylation domains (A-domains) of NRPS-type systems can perform both adenylation and amidation reactions *in vitro* without the assistance of other domains. In one example, Maruyama and co-workers showed that the adenylase rORF19, which participates in the biosynthesis of streptothricin antibiotics in *Streptomyces*, autonomously catalyses both the adenylation of -L-lysine, followed by its amidation with a -L-lysine 4-mer to extend the polylysyl moiety that will form part of the antibiotic molecule.[99] These observations have stimulated research into the possible application of standalone adenylation domains for the *in vitro* synthesis of amide compounds, simply through the addition of amine nucleophiles to reactions containing the adenylase, ATP and a carboxylic acid substrate. Abe and co-workers showed that DhbE, the A-domain that adenylates DHB **100** in the biosynthesis of the siderophore bacillibactiin in *Bacillus subtilis*, catalysed the synthesis of *N*-DHB-L-cysteine **106** in the presence of DHB, L-cysteine **105** and ATP (**Scheme 24**).[100]



**Scheme 24**. Synthesis of *N*-DHB-L-cysteine using the adenylating enzyme DhbE.[100]

Although the ratio of cysteine to DHB in the model reaction was 40:1, correlation between the consumption of ATP and the evolution of the amide product suggested that DhbE was responsible for catalyzing both the adenylation reaction and also the amide coupling. In addition, 2- and 3-hydroxybenzoic acid, as well as benzoic acid, yielded amide products using the same system, as well as pentanoic, heptanoic and octanoic acid, although at lower levels of conversion. In this example it was suggested that DhbE first catalysed the synthesis of the *S*-acyl-L-cysteine, followed by *S- to N*-acyl transfer, and could therefore only be achieved using cysteine as the amine donor. The same group subsequently demonstrated that isolated ‘internal’ adenylation domains of the NRPS responsible for bacillibactin biosynthesis, DhbFA1 and DhbFA2, catalysed both the adenylation and amide ligation of L-cysteine with glycine and L-threonine respectively to form *N*-glycyl-L-cysteine and *N*-threonyl-L-cysteine, using an equivalent chemical mechanism.[101]

Kino and co-workers established an assay for quantifying the activity of A-domains for the synthesis of amide bonds, based on their ability to catalyse the amide ligation reaction between amino acids and hydroxylamine, to form hydroxamates, which could be detected by UV assay upon complexation with Fe3+ ions.[102] The system was validated using the adenylation-amidation activity of the A-domains of tyrocidin synthase (TycA-A), and surfactin and bacitracin synthetase, and revealed a surpringly broad substrate specificity for TycA-A that included L-Leu, L-Val and L-Met in addition to the native L-Trp substrate and its amide coupling to L-proline. The same group subsequently demonstrated that TycA-A could be employed for the amidation of L-Trp and some halogenated analogs using simple amines including methylamine, -alanine and piperidine, although no reaction was observed with trimethylamine.[103] The efficiency of amidation was reported to be improved when a 20-fold excess of amine was employed.

Marchetti and co-workers applied the adenylating enzyme TamA, from the pathway to the biosynthesis of tambjamine YP1, to the adenylation of a number of carboxylic acids, with the objective of synthesising *N-*acyl amides.[104] Incubation of TamA with alinear C2-C16 acids, ammonia and ATP gave a series of primary amide products. Further investigation of the amine scope of the reaction using dodecanoic acid with ethanolamine and benzylamine gave secondary amide products, but a study with separate enantiomers of histidine as the amine partner suggested that the amidation of the intermediate adenylate was not enantioselective, indicating that this second half-reaction may not be enzyme-catalyzed.

Carboxylic acid reductases (CARs), such as the enzymes from *Nocardia iowensis* and *Segniliparus rugosus*catalyse the reduction of carboxylic acid substrates.[105] This is accomplished in a mode similar to that observed in NRPSs, with adenylation by an A-domain, followed by transfer to a Ppant arm on an acyl carrier protein, followed by delivery of hydride from NADPH in a terminal reductase domain. Following the determination of CAR structures,[106]Flitsch and co-workers have investigated the potential of CARs for amide bind formation, through interruption of the adenylation-thiolation-reduction cascade with an amine nucleophile, and in the absence of the cofactor NADPH.[107] Although no amide production was observed for the reaction between cinnamic acid and 100 equivalents of ammonia at pH 7, at pH 9, up to 25% conversion of acid to amide was observed. Furthermore, benzoic acid was coupled to methylamine and piperidine with conversions of 25% and 8% respectively. The utility of the catalyst was demonstrated in a synthesis of the anticonvulsant compound ilepcimide **109** to give a 19% isolated yield from a system containing carboxylic acid **107**, and 19 equivalents of piperidine **108** (**Scheme 25**).



**Scheme 25**. Synthesis of ilepcimide using the adenylation domain of a carboxylic acid reductase (CAR).[107]

Amidation products were still observed when either a mutant CAR lacking the serine residue responsible for covalent attachment of PPant to the ACP, or a truncated AR that lacked the reductase domain, were employed. This suggested that amidation resulted from attack of the amine at the adenylate intermediate, although the involvement of the adenylation domain in active catalysis of the amidation reaction was still not clear.

Further research has drawn inspiration from an alternative biochemical mode of carboxylic acid activation and amidation for the construction of a library of complementary amide bond synthesis systems, albeit in whole cells. In these systems, which are observed, for example both in the metabolism of xenobiotics in fungal detoxification[108] and microbial secondary metabolite biosynthetic pathways, the acid is first adenylated, then converted to a Coenzyme A thioester via the action of an ATP-dependent Coenzyme A ligase (CL), followed by amidation catalysed by an *N*-acyl transferase enzyme (NAT). For example, in the biosynthesis of thiomarinol C **113** in *Pseudoalteromonas*, marinolic acid C **110** is converted to an adenylate and thence a Coenzyme A thioester **111** by the CL TmlU (**Scheme 26**).[109] Amide bond formation with the amine holothin **112** is then catalysed by the NAT enzyme HolE.



**Scheme 26**. Biosynthesis of thiomarinol C in *Pseudoalteromonas*.[109]

In an effort to explore the versatility of these systems to create biocatalyst for amide bond formation, Lovelock and co-workers expressed a library of CLs in *E. coli* and the isolated enzymes were then screened for activity against a panel of carboxylic acid substrates, unearthing complementary substrate profiles in which enzymes with activity towards phenylacetic acids, heteroaryl acids and aliphatic acids were discovered.[110] A second library of NATs was assembled, and further screening using four amine and one CoA ester substrate was performed, again revealing complementary substrate profiles for the aminolysis of different CoA ester structures. In one instance, tyramine hydroxycinnamyl transferases displayed promiscuity towards a number of both amine and CoA ester substrates. To demonstrate the utility of the approach, a strain of *E.* *coli* was constructed that expressed both 4-chlorobenzoate CoA ligase (CBL) from *Alcaligines* sp. and a serotonin hydroxycinnamoyl transferase from *Capsicum annuum* (66CaAT). The former catalysed the transformation of 6-chloronicotinic acid **114** to CoA thioester **115**, which was subsequently coupled to neopentylamine **116** to give a 74% yield of amide **117**, an intermediate in the synthesis of the kinase inhibitor losmapimod (**Scheme 27**).



**Scheme 27**. Synthesis of an amide precursor of losmapimod using a Co-A ligase coupled with an *N*-acyl transferase.[110]

The CL-NAT cascade has also been applied *in vitro* by Andexer and coworkers.[111] In order to enable these systems *in vitro*,the issue of recycling of the requisite ATP cofactor of the CL must be considered. For the ligation of acetate and *para*-amino benzoate, as one example of a model system, the ATP required for the CL reaction was regenerated from AMP and polyphosphate using polyphosphate kinases PPK2-I and PPK2-II, which favour ADP and AMP as substrates respectively. The overall amidation system, which consisted of an acetate-CoA ligase, an arylamine–*N*-acetyltransferase, plus the recycling system, gave a conversion of 50-65% to the acetyl *para-amino* benzoic acid product. Benzoylation reactions were also possible, achieved by substituting a benzoate-CoA ligase for the acetate-CoA enzyme.

*Amide Bond Ligases or Amide Bond Synthetases*

Further classes of amide bond forming enzymes exploit the adenylation-based mode of carboxylic acid activation, but then also actively catalyze amidation, without further assistance other proteins, nor a thiolation step, and within one polypeptide chain. These amide bond ligases or synthetases or ligases, are most often found in bacterial biosynthetic pathways leading to secondary metabolites, however, their complementary substrate specificities, and the wealth of unexplored homologs in the databases, suggest that these enzymes may be considered as potential biocatalysts for more diverse amide bond formation reactions.

In addition to the NRPS-mediated synthesis of siderophores described above, some ‘NRPS-independent siderophore’ (NIS) pathways exploit a single enzyme for the activation of citric acids, followed by aminolysis of the adenylate to form the amide product.[112] For example, in the synthesis of achromobactin in *Pseudomonas syringae*, the enzyme AcsC couples *O-*citrylethanolamine **118** to 2,4-diaminobutyrate **119** to form diaminobutyryl-citryl-ethanolamine **120** (**Scheme 28**).[113]



**Scheme 28.** Synthesis of diaminobutyryl-citryl-ethanolamine from acide and amine precursors catalyzd by the enzyme AcsC.[113]

In *Bacillus anthracis*, the enzyme AsbB catalyzes the ATP-dependent ligation of spermidine with *N*8-citrylspermidine or N1-(3,4-dihydroxbenzoyl)-N8-citryl-spermidine to form amide intermediates in the biosynthesis of petrobactin.[114] The limitation of these enzymes’ activity to citric acid derivatives suggests less scope for wider application to amide syntheses at this point.

Examples with further substrate specificity present in the bacterial production of antibiotics. Heide and co-workers characterised the activity of CouL (originally CumG), which catalyzes adenylation of 3‐methylpyrrole‐2,4‐dicarboxylic acid **122** and its amidation with a 3‐amino‐4,7‐dihydroxy‐8‐methyl coumarin **121** to form amide **123** in the biosynthesis of the aminocoumarin antibiotic coumermycin A1 (**Scheme 29**).[115] Although activity towards most tested acids was poor, 20% and 36% residual activity was reported towards 4-hydroxybenzoic acid and 3-dimethylallyl-4-hydroxybenzoic acid respectively.[116] Moreover, CouL was shown to catalyse a further adenylation/amidation of the second carboxylic acid moiety to give the diamide product. With a similar activity, NovL catalyzes the adenylation/amidation of 3-dimethylallyl-4-hydroxybenzoic acid **124** with **121** to form novobiocic acid **125** as part of the biosynthetic pathway to novobiocin in *Streptomyces spheroidis*[117] (**Scheme 29**), however the substrate specificity of this enzyme was not as wide as that of CouL, with only 3-methyl-4-aminobenzoate (5% relative activity) and 3-geranyl-4-hydroxybenzoate (26%) transformed significantly. In addition, Walsh and co-workers have described the activity of amide bond ligase SimL in the biosynthesis of simocyclinone D.[118] In this instance, SimL catalyzes the adenylation/amidation of the Δ2,4,6,8 tetraene C10 diacid **126**, again with **121** to form the amide product **127** (**Scheme 29**). SimL was also able



**Scheme 29**. Examples of amide-bond formations catalysed by amide bond synthetases.

to accept C6 and C8 polyenoates, *trans*-retinoic acid, and also decanoic acid, among other saturated acyl chains, as carboxylic acid substrates.

A further amide bond synthetase, McbA, was reported to be active in the biosynthesis of the marinacarboline antibiotics by Ju and co-workers.[119] As part of this pathway, beta-carboline acid **128** is coupled to 2-phenylethylamine **129** to form amide **130** via an adenylate intermediate and catalyzed by the single protein (**Scheme 29**).[120] An initial analysis of the substrate specificity suggested that while methyl, methoxy, hydroxyl and bromo-substituted analogs of 1-phenylethylamine were accepted, simpler amines, including aniline, 4-aminobenzoic acid and ammonia were not. The activity of McbA perimitted its application to the preparative synthesis of marinacarboline analogs on milligram scale with yields of up to 93%. The specificity of McbA for the carboxylic acid substrate was investigated by Petchey and co-workers,[121] who discovered that the enzyme has a surprisingly broad specificity in this instance, which included a number of bicylic heteroaryl compounds and even benzoic acid. A number of preparative amidation reactions was performed using the isolated enzyme, and giving yields of products in up to 85%. In addition the structure of McbA was determined, revealing a large hydrophobic active site suitable for accommodating a range of aryl acid substrates, and suggesting a mechanism of adenylation and amidation reminiscent of other adenylase enzymes.[122]

**8. Concluding Remarks**

Nature has evolved a large and diverse reservoir of biochemical tools for the ligation of carboxylic acids and amines to form amides. The utility and potential of these systems for scaleable applications varies greatly dependent upon the nature and mechanism of these enzymes, and to what extent these are compatible with the envisaged application. For simple, scaleable reactions, clearly the application of lipases and other hydrolases is most advanced, with process intensification straightforward, and, for simple amidations such as the acylation of amines, already established as industrial processes. However, from the perspective of coupling synthetically interesting acid and amine partners, the substrate specificity of lipases, and their requirements for the esterificaton of most substrates, limit their applicability. However, the discovery and application of intracellular lipases such as SpL, by Li and coworkers,[42] suggest new avenues for exploration in lipase diversity and selectivity. The application of penicillin acylases to industrial-scale versions of their native reactions is again of course established, but the application of PACs to other amidations is again limited by the substrate specificity of the enzymes to largely phenylacetic acids. The greatest diversity of substrate tolerance within large classes of natural enzymes resides perhaps in the ATP-dependent enzymes, and the substrates and products of these reactions begin to resemble the bulky amide products that are features of pharmaceutical amides. These enzymes also present advantages of amide formation in aqueous solution directly from acid and amine partners, without further derivatisation. However, these enzymes are clearly underdeveloped from a process perspective: ATP-grasp enzymes are mostly restricted to coupling amino acid substrates, and NISs for citrate and related compounds, however, protein engineering may be employed to expand the activity of these enzymes; systems dependent on adenylation and CoA ligation display broad substrate tolerance, but introduce an additional reaction step, although this can be addressed through the enginering of whole cells.[110] Even the simplest ATP-dependent systems – the amide bond ligases or synthetases, require the addition of stoichimetric amounts of this cofactor. However, a reurgent interest in ATP recycling methods for biocatalytic application[111,123,124] suggest that these enzymes offer further opportunities worthy of exploration in the future.

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References

[1]. J. S. Carey, D. Laffan, C. Thomson, M. T. Williams, *Org. Biomol. Chem.* **2006**, *4*, 2337-2347.

[2]. J. R. Dunetz, J. Magano, G. A. Weisenburger, *Org. Process Res. Dev.* **2016**, *20*, 140-177.

[3]. E. Valeur, M. Bradley, *Chem. Soc. Rev.* **2009**, *38*, 606-631.

[4] R. M. de Figueiredo, J.-S. Suppo, J.-M. Campagne, *Chem. Rev.* **2016**, *116*, 12029-12122.

[5]. M. T. Sabatini, L. T. Boulton, H. F. Sneddon, T. D. Sheppard, *Nat. Catal.* **2019**, *2*, 10-17.

[6] M. C. Bryan, P. J. Dunn, D. Entwistle, F. Gallou, S. G. Koenig, J. D. Hayler, M. R. Hickey, S. Hughes, M. E. Kopach, G. Moine, P. Richardson, F. Roschangar, A. Steven, F. J. Weiberth, *Green Chem.* **2018**, *20*, 5082-5103.

[7] A. Goswami, S. G. Van Lanen, *Mol. Biosyst.* **2015**, *11*, 338-353.

[8] J. Pitzer, K. Steiner, *J. Biotechnol.* **2016**, *235*, 32-46.

[9] B. M. Dorr, D. E. Fuerst, *Curr. Opin. Chem. Biol.* **2018**, *43*, 127-133.

[10] J.W. Schmidberger, L.J. Hepworth, A.P. Green, S.L. Flitsch in *Science of synthesis*, *Biocatalysis in Organic Synthesis* Vol 1. (Eds K. Faber, W-D. Fessner, N.J. Turner). Thieme, Stuttgart **2015**, pp 329-373.

[11] V. Gotor, *Bioorg. Med. Chem.* **1999**, *7*, 2189-2197.

[12] V. Gotor-Fernández, E. Busto, V. Gotor, *Adv. Synth. Catal.* **2006**, *348*, 797-812.

[13] R. N. Lima, C. S. dos Anjos, E. V. M. Orozco, A. L. M. Porto, *Mol. Catal.* **2019**, *466*, 75-105.

[14] A. Zaks, A. M. Klibanov, *Proc. Nat. Acad. Sci.* **1985**, *82*, 3192-3196.

[15] A. L. Margolin, A. M. Klibanov, *J. Am. Chem Soc.* **1987**, *109*, 3802-3804.

[16] F. Guzmán, S. Barberis, A. Illanes, *Electron. J. Biotechnol*. **2007**, *10*, 279-314.

[17] K. Yazawa, K. Numata, *Molecules*, **2014**, *19*, 13755-13774.

[18] V. Gotor, R. Brieva, F. Rebolledo, *Chem. Commun.* **1988**, 957-958.

[19] V. Gotor, R. Brieva, F. Rebolledo, *Tetrahedron Lett.* **1988**, *29*, 6973-6974.

[20] V. Gotor, R. Brieva, C. González, F. Rebolledo, *Tetrahedron* **1991**, *47*, 9207-9214.

[21] R. Brieva, F. Rebolledo, V. Gotor, *Chem. Commun.* **1990**, 1386-1387.

[22] M. Quirós, V. M. Sánchez, R. Brieva, F. Rebolledo, V. Gotor, *Tetrahedron: Asymmetry* **1993**, *4*, 1105-1112.

[23] V. Gotor, M. J. García, F. Rebolledo, *Tetrahedron: Asymmetry* **1990**, *1*, 277-278.

[24] J. D. Badjić, E. N. Kadnikova, N. M. Kostić, *Org. Lett.* **2001**, *3*, 2025-2028.

[25] F. Rebolledo, R. Brieva, V. Gotor, *Tetrahedron Lett.* **1989**, *30*, 5345-5346.

[26] S. Puertas, R. Brieva, F. Rebolledo, V. Gotor, *Tetrahedron* **1993**, *49*, 4007-4014.

[27] M. J. García, F. Rebolledo, V. Gotor, *Tetrahedron: Asymmetry* **1992**, *3*, 1519-1522.

[28] M. J. Garćia, F. Rebolledo, V. Gotor, *Tetrahedron: Asymmetry* **1993**, *4*, 2199-2210.

[29] V. Gotor, E. Menéndez, Z. Mouloungui, A. Gaset, *J. Chem. Soc. Perkin Trans. 1* **1993**, 2453-2456.

[30] M. J. García, F. Rebolledo, V. Gotor, *Tetrahedron* **1994**, *50*, 6935-6940.

[31] E. García-Urdiales, N. Ríos-Lombardía, J. Mangas-Sánchez, V. Gotor-Fernández, V. Gotor, *ChemBioChem* **2009**, *10*, 1830-1838.

[32] C. Chamorro, R. González-Muñiz, S. Conde, *Tetrahedron: Asymmetry* **1995**, *6*, 2343-2352.

[33] S. Conde, P. López-Serrano, A. Martı́nez, *J. Mol. Catal. B-Enzym.* **1999**, *7*, 299-306.

[34] P. López-Serrano, J. A. Jongejan, F. van Rantwijk, R. A. Sheldon, *Tetrahedron: Asymmetry* **2001**, *12*, 219-228.

[35] N. Aoyagi, T. Izumi, *Tetrahedron Lett.* **2002**, *43*, 5529-5531.

[36] N. Aoyagi, S. Kawauchi, T. Izumi, *Tetrahedron Lett.* **2003**, *44*, 5609-5612.

[37] S. Hassan, R. Tschersich, T. J. J. Müller, *Tetrahedron Lett.* **2013**, *54*, 4641-4644.

[38] M. S. de Castro, J. Sinisterra Gago, *Tetrahedron* **1998**, *54*, 2877-2892.

[39] M. Adamczyk, J. Grote, *Tetrahedron Lett.* **1996**, *37*, 7913-7916.

[40] M. Adamczyk, J. Grote, S. Rege, *Tetrahedron: Asymmetry* **1997**, *8*, 2509-2512.

[41] S. van Pelt, R. L. M. Teeuwen, M. H. A. Janssen, R. A. Sheldon, P. J. Dunn, R. M. Howard, R. Kumar, I. Martínez, J. W. Wong, *Green Chem.* **2011**, *13*, 1791-1798.

[42] S. Zeng, J. Liu, S. Anankanbil, M. Chen, Z. Guo, J. P. Adams, R. Snajdrova, Z. Li, *ACS Catal.* **2018**, *8*, 8856-8865.

[43] M. C. de Zoete, A. C. K.-v. Dalen, F. van Rantwijk, R. A. Sheldon, *Chem. Commun.* **1993**, 1831-1832.

[44] A. Baldessari, C. P. Mangone, *J. Mol. Catal. B-Enzym.* **2001**, *11*, 335-341.

[45] P. G. Quintana, G. García Liñares, S. N. Chanquia, R. M. Gorojod, M. L. Kotler, A. Baldessari, *Eur. J. Org. Chem.* **2016**, *2016*, 518-528.

[46] R. Irimescu, K. Kato, *Tetrahedron Lett.* **2004**, *45*, 523-525.

[47] A. Torres-Gavilán, J. Escalante, I. Regla, A. López-Munguía, E. Castillo, *Tetrahedron: Asymmetry* **2007**, *18*, 2621-2624.

[48] D. Manova, F. Gallier, L. Tak-Tak, L. Yotava, N. Lubin-Germain, *Tetrahedron Lett.* **2018**, *59*, 2086-2090.

[49] M. A. Alrub, M. Basri, E. A. Malek, S. A. Alang Ahmad, A. B. Salleh, M. B. Abdul Rahman, *Biocatal. Biotransform.* **2012**, *30*, 385-390.

[50] P. Kaushik, N. A. Shakil, J. Kumar, B. B. Singh, *Syn. Commun.* **2015**, *45*, 569-577.

[51] A. L. Gutman, E. Meyer, X. Yue, C. Abell, *Tetrahedron Lett.* **1992**, *33*, 3943-3946.

[52] E. Stavila, K. Loos, *Tetrahedron Lett.* **2013**, *54*, 370-372.

[53] N. J. Willis, C. A. Fisher, C. M. Alder, A. Harsanyi, L. Shukla, J. P. Adams, G. Sandford, *Green Chem.* **2016**, *18*, 1313-1318.

[54] M. Fuchs, J. E. Farnberger, W. Kroutil, *Eur. J. Org. Chem.* **2015**, *2015*, 6965-6982.

[55] F. Guo, P. Berglund, *Green Chem.* **2017**, *19*, 333-360.

[56] M. Sharma, J. Mangas-Sanchez, N. J. Turner, G. Grogan, *Adv. Synth. Catal.* **2017**, *359*, 2011-2025.

[57] G. Grogan, *Curr. Opin. Chem. Biol.* **2018**, *43*, 15-22.

[58] D. Koszelewski, D. Clay, K. Faber, W. Kroutil, *J. Mol. Catal. B-Enzym.* **2009**, *60*, 191-194.

[59] C. K. Chung, P. G. Bulger, B. Kosjek, K. M. Belyk, N. Rivera, M. E. Scott, G. R. Humphrey, J. Limanto, D. C. Bachert, K. M. Emerson, *Org. Process Res. Dev.* **2014**, *18*, 215-227.

[60] M. D. Truppo, J. D. Rozzell, N. J. Turner, *Org. Process Res. Dev.* **2010**, *14*, 234-237.

[61] Á. Mourelle-Insua, L. A. Zampieri, I. Lavandera, V. Gotor-Fernández, *Adv. Synth. Catal.* **2018**, *360*, 686-695.

[62] G. A. Aleku, S. P. France, H. Man, J. Mangas-Sanchez, S. L. Montgomery, M. Sharma, F. Leipold, S. Hussain, G. Grogan, N. J. Turner, *Nat. Chem.* **2017**, *9*, 961-969.

[63] M. Arroyo, I. de la Mata, C. Acebal, M. Pilar Castillón, *Appl. Microbiol. Biotechnol.* **2003**, *60*, 507-514.

[64] H. Marešová, M. Plačková, M. Grulich, P. Kyslík, *Appl. Microbiol. Biotechnol.* **2014**, *98*, 2867-2879.

[65] W. Kaufman, K. Bauer, *Naturwissenschaften* **1960**, *47*, 474–475.

[66] M. Cole, *Biochem. J.* **1969**, *115*, 747–756.

[67] A. Pessina, P. Lüthi, P. L. Luisi, J. Prenosil, Y. Zhang, *Helv. Chim. Acta* **1988**, *71*, 631–641.

[68] A. Bruggink, *CHIMIA,* **1996**, *50*, 431–432.

[69] A. Bruggink, E. C. Roos, E. de Vroom, *Org. Process Res. Devel.* **1998**, *2*, 128-133.

[70] L. M. van Langen, E. de Vroom, F. van Rantwijk, R. A. Sheldon, *Green Chem.* **2001**, *3*, 316-319.

[71] M. A. Wegman, L. M. van Langen, F. van Rantwijk, R. A. Sheldon, *Biotechnol. Bioeng.* **2002**, *79*, 356-361.

[72] R. Fernández-Lafuente, J. Guisán, M. Pregnolato, M. Terreni, *Tetrahedron Lett.* **1997**, *38*, 4693-4696.

[73] M. J. Zmijewski, B. S. Briggs, A. R. Thompson, I. G. Wright, *Tetrahedron Lett.* **1991**, *32*, 1621-1622.

[74] C. Fuganti, C. M. Rosella, R. Rigoni, S. Servi, A.

Tagliani, M. Terreni, *Biotechnol. Lett.* **1992**, *14*, 543–546.

[75] M. Koreishi, K. Tani, Y. Ise, H. Imanaka, K. Imamura, K. Nakanishi, *Biosci. Biotechnol. Biochem.* **2007**, *71*, 1582–1586.

[76] P. C. Pereira, I. W. C. E. Arends, R. A. Sheldon, *Tetrahedron Lett.* **2014**, *55*, 4991-4993.

[77] H. Kitaguchi, P. A. Fitzpatrick, J. E. Huber, A. M. Klibanov *J. Am. Chem. Soc.* **1989**, *111*, 3094-3095.

[78] A. Röttig, A. Steinbüchel, *Microbiol. Mol. Biol. Rev.* **2013**, *77*, 277-321.

[79] M. L. Contente, A. Pinto, F. Molinari, F. Paradisi, *Adv. Synth. Catal.* **2018**, *360*, 4814-4819.

[80] H. Land, P. Hendil-Forssell, M. Martinelle, P. Berglund, *Catal. Sci. Technol.* **2016**, *6*, 2897-2900.

[81] A. Żądło-Dobrowolska, N. G. Schmidt, W. Kroutil, *Chem. Commun.* **2018**, *54*, 3387-3390.

[82] M. Y. Galperin, E. V. Koonin, *Protein Sci.* **1997**, *6*, 2639–2643.

[83] M. V. Fawaz, M. E. Topper, S. M. Firestine, *Bioorg. Chem.* **2011**, *39*, 185-191.

[84] C. Fan, P. C. Moews, C. T. Walsh, J.R. Knox, *Science* **1994**, *266*, 439-443.

[85] L. Tong, *Cell. Mol. Life Sci.* **2013**, *70*, 863-891.

[86] A. Dinescu, T. R. Cundari, V. S. Bhansali, J.-L. Luo, M. E. Anderson, *J. Biol. Chem.* **2004**, *279*, 22412-22421.

[87] H. Berg, K. Ziegler, K. Piotukh, K. Baier, W. Lockau, R. Volkmer-Engert, *Eur. J. Biochem.* **2000**, *267*, 5561-5570.

[88] T. Arai, K. Kino, *Biosci. Biotechnol. Biochem.* **2010**, *74*, 1572-1577.

[89] K. Tabata, H. Ikeda, S.-i. Hashimoto, *J. Bacteriol.* **2005**, *187*, 5195-5202.

[90] T. Tsuda, M. Asami, Y. Koguchi, S. Kojima, *Biochemistry* **2014**, *53*, 2650–2660.

[91] T. Arai, Y. Arimura, S. Ishikura, K. Kino, *Appl. Environ. Microbiol.* **2013**, *79*, 5023–5029.

[92] H. Kino, S. Nakajima, T. Arai, K. Kino, *J. Biosci. Bioeng.* **2016**, *122*, 155-159.

[93] M. Winn, J. K. Fyans, Y. Zhuo, J. Micklefield, *Nat. Prod. Rep.* **2016**, *33*, 317-347.

[94] M. D. McMahon, J. S. Rush, M. G. Thomas, *J. Bacteriol.* **2012**, *194*, 2809-2818.

[95] T. A. Keating, C. G. Marshall, C. T. Walsh, *Biochemistry* **2000**, *39*, 15513-15521.

[96] E. E. Wyckoff, S. L. Smith, S. M. Payne, *J. Bacteriol.* **2001**, *183*, 1830.

[97] G. J. Williams, *Curr. Opin. Struct. Biol.* **2013**, *23*, 603-612.

[98] K. A. J. Bozhüyük, F. Fleischhacker, A. Linck, F. Wesche, A. Tietze, C.-P. Niesert, H. B. Bode, *Nat. Chem.* **2017**, *10*, 275-281.

[99] C. Maruyama, J. Toyoda, Y. Kato, M. Izumikawa, M. Takagi, K. Shin-ya, H. Katano, T. Utagawa, Y. Hamano, *Nat. Chem. Biol.* **2012**, *8*, 791-797.

[100] T. Abe, Y. Hashimoto, S. Sugimoto, K. Kobayashi, T. Kumano, M. Kobayashi, *J. Antibiot.* **2016**, *70*, 435-442.

[101] T. Abe, K. Kobayashi, S. Kawamura, T. Sakaguchi, K. Shiiba, M. Kobayashi, *J. Gen. Appl. Microbiol.* **2019**, *65*, 1-10.

[102] R. Hara, R. Suzuki, K. Kino, *Anal. Biochem.* **2015**, *477*, 89-91.

[103] R. Hara, K. Hirai, S. Suzuki, K. Kino, *Sci. Rep.* **2018**, *8*, 2950.

[104] P. M. Marchetti, S. M. Richardson, N. M. Kariem, D. J. Campopiano, *MedChemComm* **2019**, doi 10.1039/c9md00063a.

[105] D. Gahloth, M. S. Dunstan, D. Quaglia, E. Klumbys, M. P. Lockhart-Cairns, A. M. Hill, S. R. Derrington, N. S. Scrutton, N. J. Turner, D. Leys, *Nat. Chem. Biol.* **2017**, *13*, 975.

[106] M. Winkler, *Curr. Opin. Chem. Biol.* **2018**, *43*, 23-29.

[107] A. J. L. Wood, N. J. Weise, J. D. Frampton, M. S. Dunstan, M. A. Hollas, S. R. Derrington, R. C. Lloyd, D. Quaglia, F. Parmeggiani, D. Leys, N. J. Turner, S. L. Flitsch, *Angew. Chem. Int. Ed.* **2017**, *56*, 14498-14501.

[108] E. P. Karagianni, E. Kontomina, B. Davis, B. Kotseli, T. Tsirka, V. Garefalaki, E. Sim, A. E. Glenn, S. Boukouvala, *Sci. Rep.* **2015**, *5*, 12900.

[109] Z. D. Dunn, W. J. Wever, N. J. Economou, A. A. Bowers, B. Li, *Angew. Chem. Int. Ed.* **2015**, *54*, 5137-5141.

[110] H. K. Philpott, P. J. Thomas, D. Tew, D. E. Fuerst, S. L. Lovelock, *Green Chem.* **2018**, *20*, 3426-3431.

[111] S. Mordhorst, A. Maurer, D. Popadić, J. Brech, J. N. Andexer, *ChemCatChem* **2017**, *9*, 4164-4168.

[112] C. S. Carroll, M. M. Moore, *Crit. Rev. Biochem. Mol. Biol.* **2018**, *53*, 356-381.

[113] A. D. Berti, M. G. Thomas, *J. Bacteriol.* **2009**, *191*, 4594-4604.

[114] D. Oves-Costales, N. Kadi, M. J. Fogg, L. Song, K. S. Wilson, G. L. Challis, *Chem. Commun.* **2008**, 4034-4036.

[115] Z.-X. Wang, S.-M. Li, L. Heide, *Antimicrob. Agents Chemother.* **2000**, *44*, 3040-3048.

[116] E. Schmutz, M. Steffensky, J. Schmidt, A. Porzel, S.-M. Li, L. Heide, *Eur. J. Biochem.* **2003**, *270*, 4413-4419.

[117] M. Steffensky, S.-M. Li, L. Heide, *J. Biol. Chem.* **2000**, *275*, 21754-21760.

[118] M. Pacholec, C. L. Freel Meyers, M. Oberthür, D. Kahne, C. T. Walsh, *Biochemistry* **2005**, *44*, 4949-4956.

[119] Q. Chen, C. Ji, Y. Song, H. Huang, J. Ma, X. Tian and J. Ju, *Angew. Chem. Int. Ed.* **2013**, *16*, 9980-9984.

[120] C. Ji, Q. Chen, Q. Li, H. Huang, Y. Song, J. Ma, J. Ju, *Tetrahedron Lett.*, **2014**, *55*, 4901-4904.

[121] M. Petchey, A. Cuetos, B. Rowlinson, S. Dannevald, A. Frese, P. W. Sutton, S. Lovelock, R. C. Lloyd, I. J. S. Fairlamb, G. Grogan, *Angew. Chem. Int. Ed.* **2018**, *130*, 11584-11788.

[122] A. M. Gulick, *ACS Chem. Biol.* **2009**, *4*, 811-827.

[123] J. N. Andexer, M. Richter, *ChemBioChem* **2015**, *16*, 380-386.

[124] G. A. Strohmeier, I. C. Eiteljörg, A. Schwarz, M. Winkler, *Chem. Eur. J.* **2019**, *25*, 6119-6123.

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| Enzyme-catalyzed Synthesis of Secondary and Tertiary Amides | |  |  |
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