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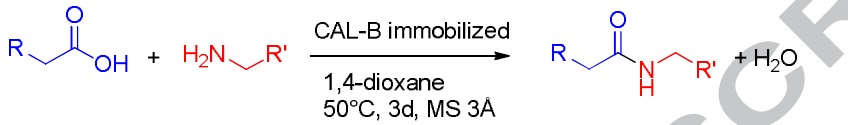

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Lipase-Catalyzed Amidation of Carboxylic Acid and Amines

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ABSTRACT

The amidation reaction is of a very particular interest, especially in the pharmaceutical industry and always requires the activation of the acid with a large excess of reactants. Therefore, a large amount of waste is generated. In order to reduce the environmental impact of such reaction, we have developed enzymatic amidation conditions which are compatible with a wide range of amines and acids, in particular with the biologically relevant lipoic acid. Water is the only by-product generated during this reaction thus a very high atom economy is obtained. In addition, we have shown that the lipase can be recovered and reused several times without a significant loss of activity.

1. Introduction

The amide bond is one of the most widespread bonds since it is the core structure of peptides and proteins. Nature assembles enzymatically the free aminoacids in the ribosome. However, synthetic chemists have devised the use of alternative methods since the reaction of a free carboxylic acid and a free amine (as in the case of free aminoacids) will solely results in the formation of the ammonium carboxylate salt, obtained from the acid-base reaction, unless elevated temperature is applied.[1]

For peptides and proteins formation, the use of protected aminoacids and a rather expensive activator or the immobilization on a resin, allowing the use of large excess of reagents, are key parameters for a successful synthesis and are routinely applied.[2] However, the amide bond formation avoiding poor atom economy or large excess of reagents has been identified as a priority by the ACS Green Chemistry Institute and by pharmaceutical companies.[3] Therefore, new sustainable methods for the formation of amide bonds are of high concern. In the recent years several methods have been developed for the acylation of amines with free carboxylic acids.[1] Probably one of the most versatile methods rely on the use of boronic acids which transiently generates an activated acid.[4] These Lewis acids, which activity can be finely tuned by modifying the substituents at the boron atom, usually have a high functional group tolerance and are stable in the presence of water. However, the water generated in the course of the reaction has to be removed and usually elevated temperatures are required to achieve good yields, which conditions can also lead to racemization.

Transition metal catalysts, especially elements from group IV (Ti, Zr, Hf), have been used on several occurrence since the simultaneous seminal work of Williams and Adolfsson.[5,1c] The same drawbacks (water removal and high temperature) can also be pointed out. Very recently, diphenylsilane has been used stoichiometrically for the direct coupling of carboxylic acid and amines in refluxing acetonitrile.[6]

Enzymes, especially lipases, have been used for a long time to perform esterification reactions using the alcohol as solvent to draw the equilibrium towards the formation of the ester. When both a primary or secondary amine and an alcohol (which is typically used as solvent) are present in the reaction medium, the ester formed is ultimately converted to the corresponding amide.[7] The direct conversion of a carboxylic acid and an amine remains scarce in the literature,[8] or limited to specific substrates such as the formation of primary amides from various sources of ammonia.[9]

2. Discussion

We wish to report here a simple and convenient method for the direct amidation of free carboxylic acids and amines without the transient formation of an active ester, generating therefore water as the sole, non-toxic, by-product.

2.1. Solvent Screening

We initially started our investigations of the lipase catalyzed amidation reaction in various solvents with octanoic acid and benzyl amine.[10] Immobilized *Candida Antarctica* Lipase B (CAL-B) was chosen for its ability to perform several kinds of

organic reaction and for its compatibility with organic solvents.[11] The reaction was carried out at 50°C with molecular sieves to avoid the reversible hydrolysis reaction. For all the reactions described throughout this paper a control reaction without lipase was performed in order to assess the lipase amidation activity under various conditions. It is well-known that the solvent can have a dramatic influence on the outcome of a biocatalyzed reaction.[12] Indeed, the solvent should be able to solubilize the reactants as well as the product and stabilize the intermediates involved, but it can also change the conformation of the active site or drain out the water molecules from it, thus modifying the catalytic properties of the enzymes.

Although lipases are known to perform better in apolar solvents,[13] we have screened usual organic solvents such as toluene or well tolerated ethereal solvent like 1,4-dioxane, MTBE, diglyme and cyclopentyl methyl ether CPME[14] in order to ensure solubilization of the reactants or the product and to avoid heterogeneous conditions.

We have also screened greener or safer alternatives (MeTHF, propylene carbonate, PEG200) and the ionic liquid BMP(NTf_2).[15] These different types of solvents allow us to screen various polarities and proticities thus selecting the best solvent. In most of the solvents the yields were very satisfactory (Table 1: entries 1-7), above 80%, which proves the versatility of this enzyme in organic solvents. Interestingly, the reaction performed in Butyl Methyl Pyrrolidinium Triflimide (BMP(NTf_2)) only afford the desired compound in 28 % yield. This result is rather surprising since CAL-B has been for amidation with ammonia in ionic liquids,[16] or used in a Baeyer-Villiger oxidation in BMP(NTf_2)[17] with good results.

In order to rationalize the influence of the solvent we have initially examined the dielectric constant (ε, Table 1) and the dipole moment (μ, Table) of these solventsIt turns out that the solvents with a dielectric constant below 12.5 (except pyridine) afford yields above 80 %. A similar trend can be drawn with the dipole moment, which best yields are obtained with the less polar solvents (< 2 Debye). We have then examined the solvatochromic parameters of these solvents and another trend can be drawn. Yields over 80 % are achieved with solvents having hydrogen-bond acidity (α) of 0 (pyridine excluded) whereas the hydrogen-bond basicity (β) doesn't seem to have an influence. However the polarity/polarizability parameter (π^*) needs to remain below 0.65. Similar observation has been made for the E_T^N parameter which has to be under 0.25 to give the best yields (*t*-BuOH excluded).

Considering other criterions such as the ability to dissolve a wider range of substrates or the price we have decided to use the 1,4-dioxane as the solvent of choice despite its relative toxicity. However less acute solvents can be used albeit with a slight decrease in yield.

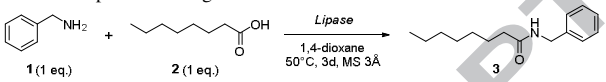
As the reactivity of the lipase is known to be reversible we have also explored the influence of the water amount in these conditions (Table 1: entries 14, 15). Anhydrous conditions are not mandatory since only a small decrease in yield is observed when molecular sieve is absent. However, the presence of larger amounts of water (10 % v/v) inhibits partially the conversion.

2.2. Lipase Screening

Since lipases are specific for either a transformation or for some substrates, we have then investigated the role of the biocatalyst on the amidation reaction in the conditions determined previously.

It turns out that the lipases from *Candida Rugosa, Aspergillus Niger*, *Pseudomonas Cepacia*, *Mucor Javanicus* or the Amano Lipase were almost inactive (Table 2, Entry 1-5). The case of *Pseudomonas Cepacia* is rather surprising since this lipase was used for the synthesis of amides (including *N*-benzyloctylamide **3**) by amidation of the corresponding benzyl esters.[18] More interestingly, the free *Candida Antarctica* Lipase B afforded the desired product in a very low yield (Table 2, Entry 6) compared to the immobilized one. If the role of the acrylic resin cannot be excluded by specific interactions or a specific conformation of the lipase, the immobilization itself does not seem responsible for the yield since the Amano Lipase PS is immobilized on diatomite and was inefficient for the amidation reaction.

Table 1: Lipase screening



Entry	Lipase	Yield
1	*Candida Rugosa* lipase type VII	2%
2	*Aspergillus Niger*Lipase	5%
3	Amano Lipase PS	5%
4	*Pseudomonas Cepacia*Lipase	5%
5	*Mucor Javanicus* lipase	3%
6	*Candida Antarctica* Lipase B free	9%
7	*Candida Antarctica* Lipase B immobilized	94%

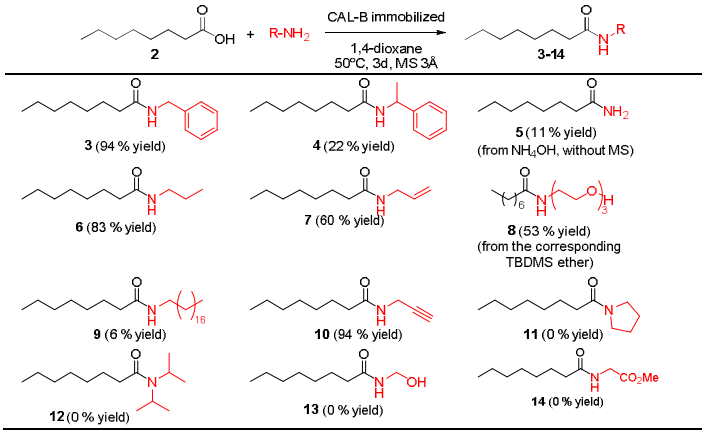
2.3. Exemplification

Having in hand the optimized conditions for the amidation reaction we then undertook a screening of both carboxylic acid and amine in order to define the scope of this reaction.

2.3.1.1. Amine screening

We thus focused our attention on the screening of different amines using octanoic acid and CAL-B at 50°C in 1,4-dioxane. The amidation reaction was stopped for all the amines after 3 days and the yield was checked after washing off the starting materials with basic and acidic aqueous solutions and the purity of the product was assessed by NMR analysis.

Table 3. Amines screening



We noticed that the amidation is efficient (Table 3) with aliphatic (propyl 83% yield, compound **6** or benzyl 94% yield, compound **3**) amines and tolerates also functionalized amines such allylic (60% yield, compound **7**), propargylic (94%, compound **10**) or bearing a functionalization such as a silyl ether protecting group. However, this protecting group is partially deprotected during the acidic washing step (as observed by ^{1}H-NMR). Therefore, the compound **8** is obtained in a good overall yield, after full deprotection of the TBDMS ether using standard TBAF procedure. The low yield observed in the case of octadecylamine (6%, compound **9**) is explained by the very low solubility of the product impeding the extraction step. Steric hindrance at the carbon bearing the amine function leads to a dramatic decrease in the lipase activity (22%, compound **4**). Moreover CAL-B was inefficient with secondary amines (compounds **11** and **12**) as well as hydrophilic amines (compound **8**). In the case of aminoethanol no conversion was observed, whereas in the case of glycine methyl ester no product

Table 1. Solvent screening

Entry	Solvent	Yield	ε	μ (D)	π*	α	β	E_T^N
1	1,4-dioxane	94%	2,21	0,45	0.55	0	0.37	0.164
2	Toluene	92%	2,38	0,31	0.54	0	0.11	0.099
3	MeTHF	89%	7,53	1,38	0.48	0	0.45	0.179
4	CPME	89%	4,76	1,27	0.42	0	0.53	-
5	t-BuOH	83%	12,5	1,66	0.41	0.68	0.93	0.389
6	MTBE	83%	2,6	1,32	-	0	-	0.124
7	Diglyme	80%	7,3	1,92	0.64	0	-	0.244
8	Propylene Carbonate	73%	65,5	4,94	0.83	0	0.4	0.472
9	Acetone	33%	20,7	2,69	0.71	0.08	0.48	0.355
10	Acetonitrile	23%	37,5	3,44	0.75	0.19	0.31	0.460
11	$BMP(NTf)_2$	28%	-	-	0.95	0.41	0.723	0.540
12	PEG 200	50%	18,35	-	-	-	-	-
13	Pyridine	4%	12,4	2,37	0.87	0	0.64	0.302
14	1,4-dioxane*	87%						
15	1,4-dioxane/H_2O (10 :1)*	25%						

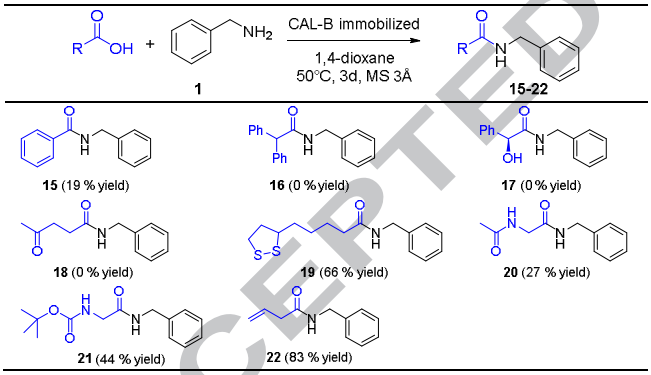
* No MS 3Å was used

was observed probably due to self-oligomerization (amidation of the methyl ester).

2.3.1.2. Carboxylic acid screening

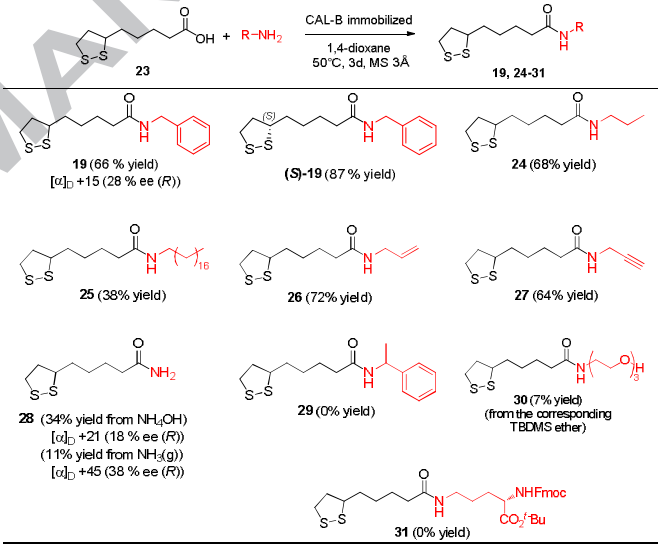
We then explored the scope concerning the other partner of the reaction by testing different carboxylic acids. The amidation reactions were carried out in 1,4-dioxane at 50°C during 3 days in the presence of CAL-B. We heve noted that the lipase was less tolerant with the carboxylic acids since no reaction occurred with diphenylethanoic acid, hydroxyphenyl ethanoic acid or 4-oxopentanoic acid (compounds **16**, **17** and **18**). Nevertheless we succeeded to obtain the amide compounds with benzoic acid, with a low yield explained by solubility problems (compound **15**, 19%) and *N*-protected glycine (compounds **20** and **21**: 27% and 44% respectively). The reaction was very efficient with but-3-enoic acid (compound **22**, 83%).

Table 4: Acids screening



One of our best result was obtained using lipoic acid as substrate (compound **19**, 66%). This constitutes an interesting opportunity to further explore the reactivity of this specific acid as the amidation of lipoic acid provides compounds with unique antioxidant and cytoprotective properties.[19] Furthermore, lipoylated peptides have been recently described as potential immunological probes for the diagnosis of primary biliary cirrhosis.[20] Similarly to the use of octanoic acid (Table 5), best results were obtained with aliphatic amines such as propyl (68%, compound **24**), benzyl (66%, compound **19**), allyl (72%, compound **26**) or propargyl (64%, compound **27**). Interestingly, the octadecylamide of lipoic acid **25** is obtained in a better yield than the compound **9** owing to a better solubility of the product. Lipoamide **28** can be prepared with different ammonia donors. Aqueous ammonia solution yields the product **28** with a low yield of 34 % explained by the large amount of water present. Dry ammonia solution, obtained by bubbling gaseous ammonia in 1,4-dioxane, gives an unexpectedly worse yield. The amidation of lipoic acid with 2-(2-(2-*tert*-butyldimethyl-silyloxyethoxy)ethoxy)ethylamine gives the desired product with partial deprotection. The compound **30** can then be isolated after TBAF treatment, with a low overall yield due to solubility problems (of both the TBDMS ether intermediate and the compound **31**). Finally, amidation of the side-chain of a suitably protected L-lysine has been carried out in order to prepare a building block for the peptide synthesis.[20] Unfortunately, all the attempts to prepare compound **31** failed.

Table 5. Lipoic acid amidation



Lipoic acid is also an interesting substrate for lipase-catalzyed reactions owing to its chiral center. Indeed, lipases have a chiral active pocket which can discriminate a racemic mixture. Therefore, we have measured the optical rotation of some of the amides obtained previously (Table 5). The optical rotation of the amide **19** obtained from the lipase-catalyzed reaction between racemic lipoic acid and benzyl amine has been found to be +15. The major enantiomer has been attributed to be (*R*) configured (with an enantiomeric excess of 28 %) after having recorded that the pure (*S*)-lipoamide **29** has an optical rotation of −54. This proves that the lipase is able to partially discriminate the 2 enantiomers of lipoic acid even though the chiral center is rather remote from the reactive site. This behavior has also been observed for the lipoamide **29**. Using aqueous ammonia solution, the enantiomeric excess remains moderate whatever the reaction time (16 % *ee* after 1 day and 18 % *ee* after 3 days). Although the yields obtained with dry ammonia solution in 1,4-dioxane are significantly lower (11 % yield), the enantiomeric excess is improved, reaching 38 % *ee* after 3 days. Under these conditions, the reaction can favorably be stopped after only 1 day (14 % yield, 42 % *ee*).

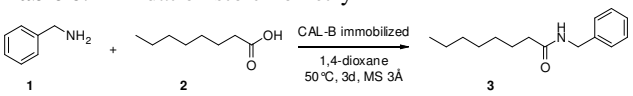
2.4. Sustainability

The amidation reaction usually requires an excess of one reactant and thus generates large amounts of wastes. Our methodology intends to respect the sustainability criterion and we have decided to set the reaction conditions in order to limit the wastes generated or to be able to recover the biocatalyst.

2.4.1. Stoichiometry

Therefore, the stoichiometric ratio of carboxylic acid and amine was initially defined for the optimization of the reaction conditions (Table 6). However, we have still checked the influence of an excess of either the amine or the carboxylic acid with immobilized CAL-B in 1,4-dioxane. In both cases, the yields obtained were significantly lower than the yield of the stoichiometric ratio.

Table 6. Amidation stoichiometry



Eq.	Eq.	Yield
1	1	94 %
5	1	62 %
1	5	75 %

This observation can be quantified by measuring the atom economy of this reaction. The atom economy is one the 12 principles of green chemistry[21] and has been defined as the amount of atoms from the reactants and the reagents that are present in the product. In our conditions, AE has been calculated to be 0.93, which is close to the value of an ideal reaction (AE = 1). This direct amidation reaction can be compared with other classical amide synthesis. The use of acyl chlorides (**32**) also requires a base such as triethylamine (**33**) thus reducing the atom economy.[22] The aminolysis of an ester (**34**) gives an even worse atom economy due to the ester itself and the need of an excess of amine.[18]

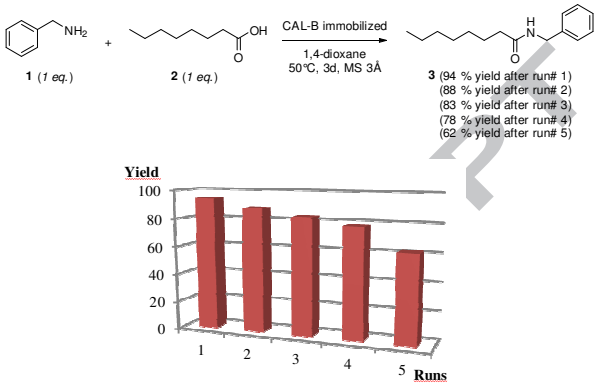
Scheme 1. Atom economy

2.4.2. Recycling

The next parameter we were interested in was the recyclability of the biocatalyst (Scheme 2). The reactions were performed in the optimized conditions as described above (50 mg of lipase, 1,4-dioxane, 50°C, 3 days). After the first run, the immobilized lipase was filtered off and reused for the next reaction with only a slight decrease in yield (from 94 % to 88 % yield). The activity of the lipase remains stable until the run #4 (78 % yield). The overall decrease in yield, which is significant in the 5th run (62 % yield), is imputed to the loss of the biocatalyst in each filtration step. Indeed, a loss of 15 mg of lipase has been observed after the 4th run. The 5th experiment was performed using only 25 mg, which is half of the amount required in the optimized conditions, hence explaining the drop in yield.

3. Conclusion

including lipoic acid. However, as often observed with lipases the scope of substrates is limited by steric constraints. In addition, we have studied the solvent dependence, by means of different parameters, and demonstrated that the lipase can be efficiently recovered and reuse for several runs.



Scheme 2. Lipase recycling

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Supplementary data

Supplementary data (experimental data, NMR spectra) associated with this article can be found in the online version.

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10. Typical procedure: in a screw-capped tube are successively introduced the amine (0.915 mmol, 1 eq), the acid (0.915 mmol, 1 eq) in a suitable solvent (usually 1,4-dioxane, 2 mL). The 3Å molecular sieve (50 mg) and the immobilized CAL-B (50 mg) are finally introduced. The reaction mixture is heated to 50°C for 3 days. After cooling, the solvent is evaporated the crude residue is partionned between water and EtOAc. The aqueous layer is extracted with EtOAc (2 x 2 mL) and the combined organic phases are washed with 3 M HCl (2 x 2 mL) and 10 % aqueous K_2CO_3 (2 x 2 mL). The solvent is finally evaporated under reduced pressure giving the pure amide (assessed by NMR).

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Enzymatic amidation reaction
No activation needed
Wide range of substrates
Lipoîc acid amide derivatives