First simple and mild synthesis of 2-alkylbenzimidazoles involving a supported enzymatic catalyst

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A new enzymatic synthesis is described for 2-alkyl-benzimidazoles a technologically important class of compounds. It is a one-step synthesis which involves a supported enzyme (Lipozyme®) and is carried out in very mild conditions in hydrocarbon solvents to give 2-alkyl-benzimidazoles in good yields, starting from the free acids. Preliminary results indicate that the reaction has a potential to produce new molecules using sensitive or conjugated acids.

Introduction

It was shown earlier that Lipozyme®, a *Mucor miehie* lipase, immobilized on a weak anionic ion exchange resin, could act as an acyl-transferase in various organic solvents.[1–4] In fact, lipase could form amides directly starting from an amine and an acid solubilized in an organic medium. This field is still very active presently,[5–9] and a biocatalytic solvent free reaction was even recently described.[10] It was then interesting to test aromatic amines such as *o*-phenylene-diamine which were expected to lead to 2-alkyl-benzimidazoles as the latter compounds are in demand in various technological domains (see below).

Initially the formation of 2-alkyl-benzimidazoles was involved in the characterization of linear alkyl acids derivatives, or of natural aldoses. Later, benzimidazoles were recognized to display a significant activity against several viruses. Similarly, antiviral activity was also demonstrated for 2-alkyl substituted benzimidazoles.[11] Another interesting property of 2-substituted benzimidazoles was shown to be their metal chelating potential. Furthermore, since 2-alkyl-benzimidazoles displayed photoluminescence and acid–base properties,[12] they became important molecular subunits in the conception of photopolymers. As a result of all these interesting characteristic properties, numerous syntheses of benzimidazoles were described.[13–17]

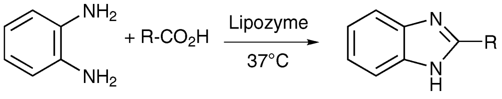
The simplest preparation methods involved the condensation of *o*-di-amino aromatic compounds with aldehydes to form the corresponding imines followed by a cyclization step.[13] However, due to the presence of many secondary products, the yields in the desired benzimidazoles were very low. Another path involved condensing the same amines with acid chlorides to form amides that were cyclized.[18] Here also, the yield in the desired 2-alkyl-benzimidazoles was low. In a different synthesis, the condensation with free acids resulted in the one-step formation of 2 alkyl-benzimidazoles with overall high yields. Even if this was an actual improvement over previous syntheses, the conditions and eventually the equipment needed to carry out that synthesis were not adapted to the laboratory scale (high pressure or water in super-critical conditions). Moreover the need to use a large excess of the free acids set limits to this synthetic path, particularly in the case of expensive or unstable acids (poly-unsaturated acids for instance).

Many other synthetic paths, some of them even leading to bis- or tetra-benzimidazoles, were explored and most of them were reviewed in a paper by Tandon *et al.*[17] The common feature of all these syntheses is that a high temperature is needed which precludes the use of labile compounds.

The present work reports the first direct enzymatic synthesis of 2-alkyl-subtituted benzimidazoles. The one-pot reaction is carried out in very mild conditions (37 °C), in hydrocarbon solvents. The acid, which is used in stoichiometric excess, is unharmed and is easily recycled. To take advantage of these mild conditions, the reaction was also tested with two conjugated acids in the presence of the same supported enzyme and lead to the expected totally conjugated molecules. Even if the yields were low, the prospect of an extension of the enzymatic route to work with very sensitive polyunsaturated acids is favourable since the enzyme actually used here is not adapted to these substrates but only to long chain fatty acids.

In the patent of Graille *et al.*,[1] it was shown that Lipozyme® is able to form N-substituted fatty acid amides. The only example described therein was the reaction of palmitic acid with *o*-phenylene-diamine. In the present work, two key parameters of the reaction, the nature of the solvent and the length of the alkyl chain of the acid (Scheme 1), were analyzed in detail.

In the first part of the study, the reaction of palmitic acid was tested with a variety of solvents (THF, Toluene, $CHCl_3$, DMSO, *t*-amylic alcohol, *n*-alkanes). Hydrocarbons turned out to be the only ones leading to the expected product,



1 a-e

Scheme 1 **1a**: R = n-$C_{17}H_{35}$, **1b**: R = n-$C_{15}H_{31}$, **1c**: R = n-$C_{11}H_{23}$, **1d**: R = n-C_9H_{19}, **1e**: R = n-C_5H_{11}.

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Table 1 Solvent effect on the yield in the synthesis of 2-pentadecyl-benzimidazole (**1b**, R = n-$C_{15}H_{31}$)

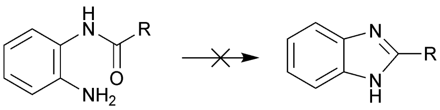
Solvent	Cyclohexane	*n*-Hexane	*n*-Pentane	Others
Yield[a]	60%	50%	50%	≈0%

[a] Yields expressed as the percentage of converted *o*-phenylene-diamine.

2-pentadecyl-benzimidazole (**1b**) (Table 1), the yields being negligible for the other solvents. Furthermore, **1b** was the only product formed.

The present results could be explained on the basis of the solubility of both the fatty acids and the 2-alkylbenzimidazoles formed. As *o*-phenylene-diamine is almost insoluble in hydrocarbons, it remains at the interface between the organic phase and the water associated to the active site of the enzyme. On the contrary, the 2-alkylbenzimidazole formed is very soluble in hydrocarbons and diffuses rapidly away from the active site. So the reaction is displaced towards the synthesis of the 2-alkylbenzimidazole. It should be noted that this is in agreement with earlier observations made in the enzymatic synthesis of glycerides for which a strong effect of the nature of the solvent was reported.[3,19] In this reaction, benzimidazoles are the sole compounds formed in the reaction. However, a first step of the reaction could be expected to lead to a monoamide. In this respect, Roeder *et al.*[20,21] noticed that following heating, in the absence of any enzyme, one could isolate **1b** from 2-amino-*N*-pentadecyl-benzamide **2** (Scheme 2). To check the possible formation of the latter as an intermediate in our reaction, its chemical synthesis was carried out. However, in the reaction conditions defined above with Lipozyme®, with or without palmitic acid added, **2** remained unchanged. The previous observations favour the formation of 2-alkylbenzimidazole at the enzymatic site, probably as a result of the attack of the acetal formed in the acylation step of the active site.

Lipozyme® is known to display a group selectivity (or typoselectivity) towards long chain alkyl acids (from C_{10} to C_{14}).[22,23] For that reason, in the second part of the present study, our synthesis protocol was applied to a set of acids for which the length of the acid alkyl chain was progressively increased. As seen in Fig. 1, the best results were obtained for long chain acids (**1a**, **1b**, **1c**) containing, respectively 18, 16 and 12 carbon atoms, whereas the yield decreased quickly for the shorter ones (**1d**, **1e**,) and was null for those containing less than 6 carbon atoms. Those results are consistent with the known lipase group selectivity, whereby the enzyme recognizes long alkyl chains and is acylated by them before being attacked by the amine. Since no side products were obtained in all these syntheses, the acid added in excess to the reaction medium could be easily recycled. This feature combined to the mild conditions used for the reaction were good reasons to test the synthesis using unsaturated or conjugated acids in anticipation of future work with fragile polyenic acids.



2 R = $CH_3(CH_2)_{14}$ 1b

Scheme 2 The mono-amide does not react with Lipozyme.

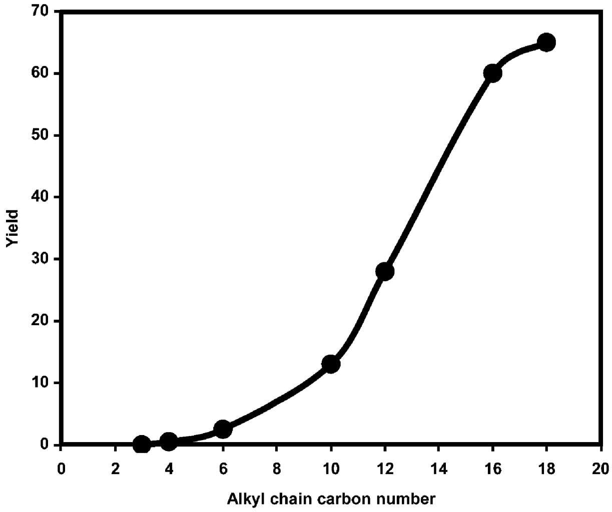


Fig. 1 Effect of alkyl chain length on the 2-alkylbenzimidazoles yield.

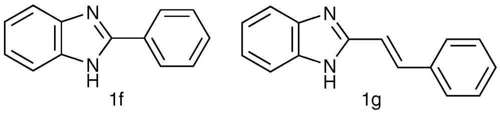
In these preliminary tests carried out with the same supported enzyme, benzoic acid and cinnamic acid were tested and lead to sole products 2-phenyl-benzimidazole **1f** and 2-styryl-benzimidazole **1g**, respectively (Scheme 3). These compounds were identical to those obtained in non enzymatic reactions.[15,24] and they were fluorescent as were all the long-chain compounds. The yields for **1f** and **1g** were low as expected, since the lipase used here was naturally adapted to react with long chain fatty acids, and it did so even in the present experimental conditions. The challenge is now left to the authors to find an enzyme that lend itself to the reaction in higher yield with these sensitive acids.

In conclusion, this work describes a simple one step protocol to obtain 2-alkyl-benzimidazoles from fatty acids in mild conditions, at 37 °C. This first direct enzymatic synthesis in the series, which uses the cheap and commercially available Lipozyme® reagent, is naturally best adapted to saturated long chain acids. The excess in acid is easily recovered unharmed and recycled. So it is expected that lipases of a different typoselectivity should lead to interesting 2-alkylbenzimidazoles derived from other fragile acids, such as unsaturated acids for instance, as the latter cannot be reacted using existing protocols that are too harsh. Interesting work is now being carried along these lines.

Experimental

Enzymatic synthesis of the 2-alkyl-benzimidazoles 1a to 1e

To a solution of acid (**1a–e**, 10 mmol) and *o*-phenylene-diamine (1 mmol) in cyclohexane (30 mL) was added 400 mg



1f 1g

Scheme 3 2-Phenyl-benzimidazole **1f** and 2-styryl-benzimidazole **1g**.

Lipozyme®. The reaction was allowed to proceed for 3 days at 37 °C. The reactional mixture was taken up in ethyl ether (200 mL) and filtered to eliminate Lipozyme® and the unreacted diamine. The filtrate was washed first with ethanolic soda (1 N NaOH in an EtOH:H_2O (1 : 5, *v*/*v*) mixture), then with water and finally dried on sodium sulfate. After removing the solvent under vacuum, the resulting solids were recrystallized in EtOH to give white crystals. Butyric and acetic acids did not react.

2-Heptadecyl-benzimidazole (1a). Yield 233 mg, 65%. Mp. 93–94 °C (lit.[25] mp 93.5–94.5 °C). IR (KBr, cm^{-1}): 2920, 2850, 1475, 1450, 1410, 1275, 750, 740. ^{1}H NMR (250 MHz,$CDCl_3$): δ 10.1 (s, 1H, NH), 7.6 (m, 2H, ArH), 7.2 (t, J = 3.18 et 5.94 Hz, 2H, ArH), 2.9 (t, J = 7.72 Hz, 2H, CH_2–Ar), 1.85 (m, 2H, CH_2–CH_2Ar), 1.3 (m, 24H, $(CH_2)_n$), 0.88 ppm (t, J = 3H, CH_3). FAB-MS: m/z 357 (M^+ + 1), 356 (M^+) 145, 132.

2-Pentadecyl-benzimidazole (1b). Yield 254 mg, 60%. Mp. 94–96 °C (lit.[25] mp 96.5–97 °C). R_f: 0.5 (Et_2O-petroleum ether, 8 : 2, (*v*/*v*)). IR (KBr, cm^{-1}): 2900, 2850, 1500, 1475, 1410, 750, 74; ^{1}H NMR (250 Mhz, DMSO-*d6*): δ 12.1 (s, 1H, NH), 7.5 (m, 2H, ArH), 7.1 (m, 2H, ArH), 2.7 (t, 2H, CH_2–Ar), 1.7 (m, 2H, CH_2–CH_2Ar), 1.3 (m, 24H, $(CH_2)_n$), 0.88 ppm (t, 3H, CH_3). FAB-MS: m/z 329 (M^+ + 1), 328 (M^+) 145, 132.

2-Undecyl-benzimidazole (1c). Yield (77 mg) 28%. Mp. 106–107 °C (lit.[25] Mp. 107.5 °C). IR (KBr, cm^{-1}): 2950, 2850, 1475, 1450, 1410, 1275, 750, 740. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 10.1 (s, 1H, NH), 7.6 (m, 2H, ArH), 7.2 (t, J = 3.18 et 5.94 Hz, 2H, ArH), 2.9 (t, J = 7.72 Hz, 2H, CH_2–Ar), 1.82 (m, 2H, CH_2–CH_2Ar), 1.3 (m, 24H, $(CH_2)_n$), 0.86 ppm (t, J = 3H, CH_3). FAB-MS: m/z 273 (M^+ + 1), 272 (M^+), 145, 132.

2-Nonyl-benzimidazole (1d). Yield (32 mg) 13%. Mp. 126–127 °C (lit.[25] Mp. 127–127.5 °C). IR (KBr, cm^{-1}): 2950, 2850, 1475, 1450, 1410, 1275, 750, 740. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 10.1 (s, 1H, NH), 7.6 (m, 2H, ArH), 7.2 (t, J = 3.18 et 5.94 Hz, 2H, ArH), 2.9 (t, J = 7.72 Hz, 2H, CH_2–Ar), 1.82 (m, 2H, CH_2–CH_2Ar), 1.3 (m, 24H, $(CH_2)_n$), 0.86 ppm (t, J = 3H, CH_3). FAB-MS: m/z 245 (M^+ + 1), 244 (M^+), 145, 132.

2-Pentyl-benzimidazole (1e). Yield (4.7 mg) 2.5%. Mp. 160 °C (lit.[25] mp 163.0–163.5 °C). IR (KBr, cm^{-1}): 2950, 2850, 1475, 1450, 1410, 1275, 750, 740. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 10.1 (s, 1H, NH), 7.6 (m, 2H, ArH), 7.2 (t, J = 3.18 et 5.94 Hz, 2H, ArH), 2.9 (t, J = 7.72 Hz, 2H, CH_2–Ar), 1.82 (m, 2H, CH_2–CH_2Ar), 1.3 (m, 24H, $(CH_2)_n$), 0.86 ppm (t, J = 3H, CH_3). EI-MS (70 eV): m/z 188 (M^+), 159, 145, 132.

Chemical synthesis 1-*N*-(palmitoyl)-amino-2-benzene (2)

To a solution of 800 mg of 1,2-diamino-benzene (7.4 mmol) in CH_2Cl_2 (25 mL) was added 1 g (3.6 mmol) of palmitoyl chloride, drop wise and under stirring. After the addition was completed, the solution was heated to reflux (50 °C) for 20 h. The solvent was removed and the amide and any acid left were extracted from the raw reaction product with Et_2O (500 mL). After further extraction with a 1 M NaOH solution containing EtOH (10%, *v*/*v*), the organic layer was dried on sodium sulfate. Removal of Et_2O under vacuum left a solid that was re-crystallized. Mp. 106 °C. R_f: 0.5 (Et_2O-petroleum-ether 8 : 2 (*v*/*v*)). IR ($CHCl_3$, cm^{-1}): 3600, 3400, 2925, 2860, 1670, 1450. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 8.3 (s, 1H, NH), 7.5 (m, 2H, ArH), 7.0 (m, 2H, ArH), 2.3 (t, J = 7.5 Hz, 2H, CH_2–CO), 1.7–1.55 (m, 2H, CH_2–CH_2–CO), 1.26 (m, 24H, $(CH_2)_n$), 0.88 ppm (t, J = 6.5H, CH_3). FAB-MS: m/z 347 (M^+ + 17), 330 (M^+), 154.

2-Phenyl-benzimidazole (1f). Orthophenylenediamine (1.08 g, 10 mM), benzoic acid (1.22 g, 10 mM), lipozyme (1 g) and toluene (200 mL) were added to a 100 mL flask. The flask was stoppered and shaken for 60 h at 250 rpm in a bath kept at 45 °C. The solid mixture obtained was then filtered, and thoroughly washed with cyclohexane. The organic phase was evaporated under vacuum and the resulting solid was dissolved in chloroform (200 mL). The solution was washed first with a 1 M sodium hydroxide solution and then with water until neutrality (pH 7). The organic phase was then dried over anhydrous sodium sulfate and chloroform was evaporated under vacuum. The crude residue was chromatographied on a silica gel column and 120 mg of the expected solid **1f** are obtained. Yield 6%, Mp. 290 °C (lit.[24] 290 °C).

2-Styryl-benzimidazole (1g). Orthophenylenediamine (108 mg, 1 mM), cinnamic acid (592 mg, 4 mM), lipozyme (500 mg), *t*-amylic alcohol, (20 mL) and water (20 mL) were added to a 200 mL flask and mixed together. The flask was stoppered and shaken for 6 h at 250 rpm in a 37 °C water bath. The mixture is then diluted with ethyl acetate (200 mL), washed first with a 1 M sodium hydroxide solution and then with water until neutrality. The organic phase was dried over anhydrous sodium sulfate and evaporated under vacuum. The crude residue was chromatographied on a silica gel column and 22 mg of the solid **1g** were collected. Yield 10%. Mp. 200 °C (lit.[26] 202 °C).

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